DISSERTATION

Titel der Dissertation

"DNA Intercalating Compounds with a Tetra- or Pentacyclic Nucleus: Design and Biological Evaluation"

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1   INTRODUCTION

1. 1   Epidemiology of Cancer[1]

The World Health Organisation (WHO) describes cancer as "a generic term for a large group of diseases that can affect any part of the body." One feature of cancer is the rapid creation of abnormal cells that grow beyond their boundaries, their invasion to adjoining parts of the body and spreading to other organs, a process which is called metastasising. Metastases are the main cause of death from cancer. Benign tumours do not spread into other organs and are therefore not called cancer.[2]

With approximately 14 million new cases and over 8.2 million cancer related deaths worldwide, it is one of the leading causes of mortality and morbidity in 2012.

It is expected that the number of new cases will rise by about 70 % in the next 20 years.

The most five diagnosed types of cancer in men (2012) were lung, prostate, colorectal, stomach and liver cancer, whereas in women breast, colorectal, lung, cervix and stomach cancer are dominant.

About one third of all deaths of cancer can be attributed to five dietary and behavioural risks. These are smoking, alcohol abuse, low intake of fruit and vegetables, low physical activity and a high body mass index (BMI). Among these, the most important risk factor is the use of tobacco, which causes 20 % of global cancer deaths and about 70 % of global lung cancer deaths.[1]

In low- and middle developed countries viral infections such as HBV/HCV and HPV are responsible for up to 20 % of cancer deaths.[21]

In Africa, Asia and Central and South America occur more than 60 % of the annual new cases of cancer and over 70 % of the world's cancer deaths.

It is estimated that annual cancer cases will rise from 14 million in 2012 to 22 in the next 20 years.

1. 2   The Classification of Cancer

There are over 100 types of cancer known in humans affecting virtually every organ or tissue. They are described by their primary site or the location (topographic) where they have first developed (lung, brain, etc.), and based on the histological type (morphologic), because all organs or body parts are composed of different types of cells. The international standard for the classification and nomenclature of histologies is the International Classification of Diseases for Oncology, Third Edition (ICD-O-3)[3]. These types include

- carcinoma, which is derived from epithelial cells; the most common cancers are carcinoma, for example many breast, lung, prostate, colon and lung cancers
- sarcoma, if a cancer originates from connective tissue, for example bone, cartilage, fat; each of which develops from cells originating from mesenchymal cells;
- lymphoma and leukemia are derived from blood forming cells;
- germ cell tumours which are derived from pluripotent cells, for example seminoma originating from the testicle or dysgerminoma from the ovary;
- blastoma which are cancers from embryonic tissue or immature precursor cells and are more common in children than in adults.

In cancer nomenclature the suffix -carcinoma, -sarcoma or -blastoma is used with the Greek or the Latin word for the source as the root, for example "hepatocarcinoma" as cancer originating from ma-

lignant epithelial cells of the liver parenchym.

Benign tumours are usually nomenclated with organ name as the root and the suffix -oma. Confusingly, some types of malignant cancer use the -noma suffix, such as melanoma or seminoma.

Some types of cancer are named after their morphology under a microscope, such as giant cell carcinoma or small-cell carcinoma.

1. 3 Causes of Cancer

90 to 95 % of the cancer cases are due to environmental factors, only 5 to 10 % can be attributed to inherited gene mutations.[4] In this context, the term environmental is by cancer researchers not used to merely describe pollution of air or water but also behavioural, economic and lifestyle factors.[5]

The environmental factors contributing to cancer deaths include tobacco (25 to 30 %), physical inactivity, diet and obesity (30 to 35 %), radiation (up to 10 %), infection diseases (15 to 20 %), stress and environmental pollutants.[4]

1. 3. 1 Chemicals

Chemicals which have been linked to specific types of cancers are called carcinogens, Tobacco smoke contains over fifty known carcinogens, including polycyclic aromatic hydrocarbon and nitrosamines.[6] Smoking tobacco causes 90 % of lung cancers[7] and is responsible for one third of all cancer deaths in the developed world[8] and for about 20 % worldwide[6]. Cancer in the larynx, neck, head, esophagus, stomach, kidney, bladder, and pancreas are also related to tobacco use.[6]

The International Agency for Research on Cancer (IARC) classifies alcoholic beverages as a group 1 carcinogen (carcinogenic to humans) and as a cause of female breast cancer, as well as larynx, pharynx, esophagus, liver (hepatocellular carcinoma), oral cavity and colorectal cancer. It is also a probable cause of pancreatic cancer.[9]

Alcohol intake is responsible for 3.6 % of all cancer cases and 3.5% of global cancer deaths[10] and for 10 % of cancers in males and 3 % in females in Western Europe, especially cancer of the liver and the digestive tract.[11]

Chemical exposure at work is believed to cause 2 to 20 % of all cancer cases. In 1996, the Harvard Center for Cancer Prevention (HCCP) classified 32 substances or industries as carcinogenic in humans. Recently, 28 agents have been considered as definite occupational carcinogens in human, 27 as probable occupational carcinogens and 113 as possible occupational carcinogens." [12]

1. 3. 2 Diet and Exercise

The risk of cancer is significantly influenced by dietary factors, which can have both an increasing or reducing effect. 30 to 35 % of cancer deaths may be related to diet, lack of physical activity and obesity.[4] The most important dietary cause of cancer is overnutrition; obesity and consumption of alcohol are accepted causes of cancer.[13] Consequently, many dietary recommendations have been proposed

but only few are supported by scientific evidence.[13]

Some specific foods are associated with specific cancers. Studies have demonstrated that the consumption of red or processed meat is linked to an increased risk of breast cancer, colon cancer,[14] prostate cancer[15] and pancreatic cancer. At least partially responsible are carcinogens which are formed at high temperatures during the cooking process.[16,17]

The food contaminate Aflatoxin B1 is known to cause liver cancer[18], whereas drinking coffee reduces the risk.[19] Dietary habits in different countries partially explain the differences in cancer incidence. The fundamental link between cancer and diet is suggested by the observation that immigrant communities often develop the cancer risk of their new country, often within one generation.[20]

1. 3. 3 Infection

Infectious diseases are responsible for 16.1 % of cancers worldwide, varying in different regions with 32.7 % in Sub-Saharan Africa to only 3.3 % in Australia and New Zealand.[21] The main infectious agents causing cancer are viruses, called oncoviruses, but *Mycobacterium* and other bacteria or parasites are also responsible.

These oncoviruses include human papillomavirus which causes cervical carcinoma, Epstein-Barr virus, responsible for B-cell lymphoproliferative disease and nasopharyngeal carcinoma, Kaposi's sarcoma herpesvirus causing Kaposi's Sarcoma and primary effusion lymphomas, hepatitis B and C viruses causing hepatocellular carcinoma, and Human T-cell leukemia virus-1.

The most prominent example for bacterial infection that may increase the risk of cancer is *Helicobacter pylori*, responsible for inducing gastric carcinoma.[22, 23, 24]

One example for a cancer-related parasite is *Schistosoma haematobium*, causing bladder cancer, probably due to chronic inflammation triggered by the worm's eggs.[25]

1. 3. 4 Radiation

Ionising and non-ionising UV radiation exposure are responsible for up to 10 % of invasive cancers.[4] From the non-invasive cancers the majority are non-melanoma skin cancers which are caused by ultraviolet radiation, mostly from UVB waves of sunlight. Long-term exposure to sunlight can lead to melanoma.[27] Radon gas and medical imaging are the main sources for ionising radiation, especially computed tomography is a widespread source of ionising radiation in the modern world and the cancerogenic effects are unclear.[26] Although ionising radiation is not particularly strong it can be a potent source of cancer in combination with other cancerogenic agents such as smoking tobacco. Ionising radiation is also used to treat cancers, but can also form secondary neoplasms as a consequence.[28]

The world health organisation has described non-ionising radio frequency radiation from power lines, mobile phones and similar sources as possible risk for the development of cancer.

1. 3. 5   Heredity

The majority of all cancers are sporadic, only 3 to 10 % of annual diagnosed cancers are triggered by an inherited genetic mutation. For example, mutations in the genes BRCA1 and BRCA2 are associated with a risk of more than 75 % to develop breast and ovarian cancer.[29]

1. 3. 6   Hormones

The development of certain cancers can be triggered by hormones that are promoting cell proliferation such as insulin-like growth factors.[30] Especially sex-related cancers such as prostate, ovary, testis and breast cancer, but also thyroid or bone cancer can be attributed to hormones.[30] Elevated levels of estrogen and progesterone for example in daughters of women with breast cancer may explain their higher risk of developing breast cancer, even without breast-cancer genes.

For example, the daughters of mothers who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene.[30] Similarly, men with African ancestors have a higher rate of prostate cancer due to significantly higher testosterone level compared to men with European ancestors whereas the low levels of testosterone-activating androstanediol glucuronide in men with asian ancestors reflect the low rates of prostate cancer.[30]

1. 4   Carcinogenesis - A Multistep Model

The development of cancer, called carcinogenesis is a multistage process that transforms a normal cell to a malignant cell. This is characterised by changes at the genetic and epigenetic level altering the cellular program to undergo uncontrolled cell division. Under physiological conditions of healthy cells and tissues proliferation and programmed cell death (apoptosis) are in balance. This process is sustained by a series of a complex regulation machinery which is eventually damaged by mutations and epimutations in the DNA, in particular in DNA repair genes and genes involved in cell growth, cell division or apoptosis. Genes that govern cell growth are called proto-oncogenes, which become to oncogenes when their function is disabled by mutation, whereas tumour suppressor genes and related proteins suppress mitosis and cell growth. Mutations in these genes cause a deregulation of signalling pathways and eventually tumour cells start to divide uncontrollably.

The development of cancer can be divided into four conceptual stages: initiation, promotion, malignant conversion, and tumour progression. Malignant conversion of benign hyperplastic cells to a malignant state is a prerequisite for carcinogenesis, invasion and metastasis are the result of further genetic and epigenetic changes.[30, 31, 32] The study of this process in humans relies merely on information from occupational exposures to chemical carcinogens and lifestyle. Age-dependent cancer cases have demonstrated that the rate of tumour development is proportional to the sixth power of time, suggesting that at least four to six independent steps are necessary.[34]

1. 4. 1   Tumour Initiation

The early concept of tumour initiation indicated an irreversible genetic damage as first step in chemical carcinogenesis, but recent data from molecular studies also indicate that epigenetic changes as another early event are involved. These are caused by DNA methylation of promoter regions of genes that are capable of silencing tumour-suppressor genes. The accumulation of mutations can only occur in cells that proliferate and survive the lifetime of the affected organism. According to Knudson's hypothesis,[36] mutations in both tumour suppressor genes and proto-oncogenes are required to transform a cell into a cancer cell. Only when enough tumour suppressor genes are deactivated or damaged and proto-oncogenes have mutated into oncogenes cell proliferation gets out of control.

1. 4. 2   Tumour Promotion

The term tumour promotion describes the selective clonal expansion of initiated cells. The high accumulation rate of mutations is proportional to rate of cell divisions or at least the rate of stem cell replacement and promotes the clonal expansion of initiated cells, thus producing a great number of cells which are prone to further genetic mutations and malignant conversion.[31, 35]
Tumour promoters are not carcinogenic alone, nonmutagenic and often capable of mediating their effect without metabolic activation. After exposure to an initiatior, tumour promotors are capable of reducing the latency period for tumour formation, or increasing the number of tumours formed in that tissue. In addition, in conjunction with a dose of an initiator, that is too low to be carcinogenic alone the promoter can induce tumour formation. Agents capable of triggering initiation and promotion are called complete carcinogens, such as benzo[a]pyrene.

1. 4. 3   Malignant Conversion

The transformation of a preneoplastic cell into a cell with malignant phenotype is called malignant conversion, a process that requires further genetic changes, again in connection with tumour suppressor genes and activation of proto-oncogenes. These further genetic changes are to some extent the result from errors in DNA synthesis.[37] The relatively low probability of malignant conversion can be increased substantially by the exposure of preneoplastic cells to DNA-damaging agents.[38] The total dose of a tumour promoter is not as significant as frequently repeated administrations and in case it is discontinued before the malignant transformation has occurred the benign lesion may regress.

1. 4. 4   Tumour Progression

Tumour progression is characterised by the expression of malignancy and a tendency to develop more aggressive properties over time. A prominent example is the tendency for genomic instability on either the nucleotide or the chromosomal level and uncontrolled growth.[39] This results in further genetic and epigenetic changes, again including activation of proto-oncogenes and inactivation of tumour suppressor genes. Also, a tumour can develop the ability to secrete proteases that allow invasion beyond its primary location, a process referred to as metastasis. Although a certain scheduling of these mutational incidents is suggested, merely the accumulation of genetic changes appears to be the determining factor, not the stage of carcinogenesis in which they occur. [39, 40]

1. 5   The Hallmarks of Cancer[41]

In 2000, cancer researchers Douglas Hanahan and Robert Weinberg have published an artice[41] in the Journal "Cell" in which the complex mechanism of multistep carcinogenesis is simplified to six common traits ("hallmarks") that govern the transformation of normal cells to malignant cells. 11 years later[42] two further hallmarks ("deregulated metabolism" and "avoiding immune destruction") were added, which are labelled "emerging hallmarks", since they are not yet fully validated. In addition, two capabilities of neoplasia are described which facilitate the acquisition of both core and emerging hallmarks. These are "genomic instability" and thus mutability which endows cancer cells with genetic alterations that drive tumour progression and "inflammation" by innate immune cells can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumour-promoting consequences of inflammatory responses.[42]

1. 5. 1   Self-Sufficiency In Growth Signals[41]

In order to grow and divide, normal cells need external stimuli *via* growth factors which are transmitted through receptors in the cell membrane. If these signals are missing they don't grow. In contrast, cancer cells don't need that stimulation, they can generate their own growth signals, possess overexpressed receptors or have mutated receptors that transmit signals without growth factors. Glioblastoma cells, for example, can produce their own platelet-derived growth factor (PDGF) whereas the epidermal growth factor receptor (EGF-R/erbB) is overexpressed in brain, stomach, and breast cancers; in stomach and breast cancers HER2/neu receptor is overexpressed.

1. 5. 2   Insensitivity To Anti-Growth Signals[41]

In the same manner normal cells receive growth signals to divide and grow they are kept under control by growth inhibitors of the surrounding environment, on the surface of neighbouring cells and in the extracellular matrix. These inhibitors influence the cell cycle clock by interrupting mitosis. Cancer cells are generally resistant to growth-preventing signals from their neighbours.

Eventually, the growth inhibitor signals are transmitted *via* the retinoblastoma protein (pRB), which prevents the G1 to S phase transition. If pRB is damaged by a mutation or by interference from human papilloma virus, cells can divide uncontrollably, leading to cervical cancer.

1. 5. 3   Evading Apoptosis[41]

Apoptosis is the term of programmed cell death (cell suicide) in the event they become irreparably damaged. It is characteristic for cancer cells to be able to overcome this mechanism.

The apoptotic machinery can be divided into sensors, which monitor the cell for abnormal behavior, and effectors, which cause apoptosis. Sensors include IGF-1/IGF-2 and their receptors and IL-3 and its receptor. The effectors include FAS ligand and its receptor, and TNF-α and its receptor.

The p53 tumour suppressor protein is an important trigger of apoptosis and acts on DNA damage. If p53 is mutated, which is the case in over 50 % of cancers, this regulation mechanism fails.

1. 5. 4   Limitless Replicative Potential[41]

Normal cells have a limited number of divisions before they die. This number is called the Hayflick limit, which is about 60 to 70 doublings before the cell reaches the state of senescence. Cancer cells, on the other hand, have to overcome this limit and acquire immortality, that means the capability of limitless growth and cell division. However, these immortalised cells have damaged chromosomes which may become cancerous. The counter for cell doublings is the telomere, a sequence of repetitive nucleotides at the tips of each chromatide, of which some are lost during each cell cycle. Many cancers involve the upregulation of telomerase, the enzyme that replenishes nucleotides of the telomere.

1. 5. 5   Sustained Angiogenesis[41]

Angiogenesis is the term used for the formation of new blood vessels. Cancer cells are able to start this process, ensuring to receive a sufficient amount of oxygen and nutrients. Initially cancer cells lack that capability, limiting their further growth (usually about 1 to 2 square mm).[44] This process, which is governed by inducers and inhibitors is vital for cancer cells to expand (for example cervix, breast and melanoma tumours). Among the inducers are vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factor (FGF-1 and FGF-2), which bind to transmembrane tyrosine kinase receptors expressed on endothelial cells. An inhibitor is thrombospondin-1 which is regulated by p53, thus angiogenesis can be triggered by loss of p53. Drugs targeting the inhibition of angiogenesis in tumours are a promising research field, under investigation and is tightly connected with the capability of metastasis.

1. 5. 6   Tissue Invasion and Metastasis[41]

The probably most dangerous ability a cancer cell aquires is to spread from its place of origin to invade the surrounding tissue and into distant organs or body parts. This capability is referred to as metastasis and is a complex process comprised of a series of steps which involve the mimicking of normal cell-cell interactions through cell adhesion molecules (CAMs) and integrins. Matrixdegrading proteases are also vital to facilitate the invasion into the new tissue. Moreover, the body's natural defense mechanisms, such as the expression of E-cadherin which transmits antigrowth signals must be overcome by cancer cells to grow. The probably most critical event required for successful metastasis is angiogenesis, the process of forming new blood vessels which is why this process is under investigation.[45]

1. 5. 7 Deregulated Metabolism[42]

Cancer cells can reprogram their energy metabolism to use primarily glycolysis for energy production instead of oxidative phosphorylation in the mitochondria. This is seemingly counterintuitive as the cancer cell has to compensate for the 18-fold lower efficiency of ATP production. This is achieved in part by upregulating glucose transporters to increase the glucose import into the cytoplasm. On the other hand, increased glycolysis allows diversion of glycolytic intermediates into diverse biosynthetic pathways, including those producing amino acids and nucleosides. This facilitates the biosynthesis of organelles and other macromolecules required for new cells. Since an altered energy metabolism is as common as other cancer-associated hallmarks of cancer the designation as emerging hallmark seems appropriate.

1. 5. 8 Evading the Immune System[42]

A still unresolved issue related tumour formation involves the role of the immune system. Cancer cells seem to be able to avoid detection of the immune system or evade eradication by disabling components of the immune system which were designed to eliminate them. This might be modulated by secretion of TGF-β or other immunosuppressive factors. In studies, genetically engineered immuno-deficient mice were assessed for the development of tumours. Indeed, tumours grew more frequently and/or faster than in the immunocompetent control group. In addition, human patients with colon and ovarian tumours that are infiltrated with NK cells and CTLs have a better prognosis than patients lacking those cells. On the other hand, epidemiological data does not show an increased incidence of cancer in immunosuppressed people, which might be attributed to a residual defense capability mediated through NK and other innate immune cells. Yet, the scientific demonstrations of antitumour immunity as barrier against tumourigenesis is still rudimentary and needs further confirmation.

1. 6   Therapeutic Concepts - Mechanism-based Targeting[42]
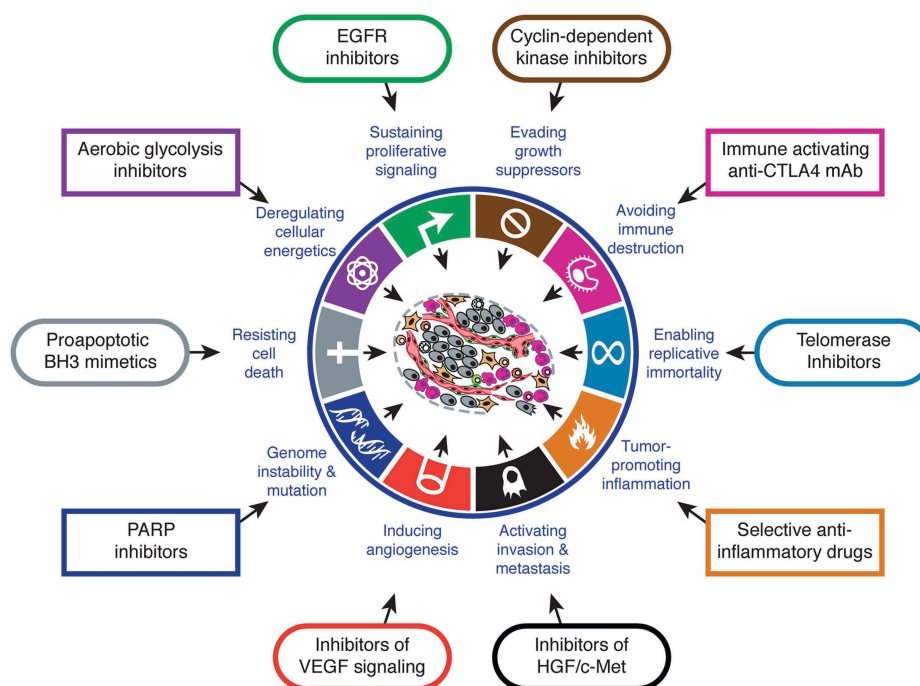
Figure 1. *Therapeutic Targeting of the Hallmarks of Cancer[42]*

With the introduction of mechanism-based targeting myriads of therapies to treat cancer have evolved reflecting the description of the hallmark principles. The rapidly growing armamentarium of cancer therapeutics can be categorised according to their effects on a hallmark capability and their efficacy demonstrates the validity of that concept (***Figure 1***).

Most of the hallmark-targeting cancer drugs developed to date have been directed toward specific molecular targets which in theory should provide inhibitory activity while having fewer off-target effects, leading to reduced toxicity. This is not the case, a circumstance that can be interpreted by the fact that each hallmark capability has redundant signalling pathways which results in only a partial inhibition of a capability instead of a complete shutdown, leaving behind some cells with residual function. They or their progeny can eventually adapt by mutation of epigenetic reprogramming or remodelling of their microenvironment which causes relapses in clinical therapy.

The emerging of adaptive resistance should be prevented by therapeutically adressing all of the supporting pathways of a certain hallmark. Instead, recent discoveries suggest that cancer cells might be able to shift their dependence from one particular capability to another, which can be regarded as completely different form of acquired drug resistance. This was found in antiangiogenic therapies where tumours adapted from a dependence on angiogenesis to increased invasiveness and metastasis which resulted in invasion to surrounding tissue where preestablished blood vessels could restore the oxygen supply of the tumour.[46, 47, 48]

Such observations suggest that future anti-cancer drugs and treatment will benefit from incorporating the concept of hallmark capabilities and of the supporting biochemical pathways in each of them. In particular, "we can envisage that selective cotargeting of multiple core and emerging hallmark capabilities and enabling characteristics in mechanism-guided combinations will result in more effective and durable therapies for human cancer."[42]

1. 7 Therapeutic Concepts - Intercalation and Topoisomerase Inhibition

Despite the latest advances in targeting hallmark capabilities as anticancer agents with varying success and the first steps in immunotherapy there are still many small molecules with cytotoxic properties in clinical use and there is still need of compounds with an optimised side effect profile. The small molecules comprise several classes of cytotoxic compounds: antimetabolites, alkylating agents, microtubule inhibitors, intercalating agents and topoisomerase inhibitors.

1. 7. 1 Intercalation

The concept of intercalation was first described by Leonard Lerman[49] in 1961 for the acridine Proflavin. Intercalation is the term for the insertion of virtually any polyaromatic structure between the base pairs of the DNA with the right steric properties. Based on his research a number of structural requirements for molecules were proposed by Moore et al.[50] to optimise intercalation. A system should be polyaromatic with 3 to 4 rings and coplanar, with a width of 3-4 Å and a length of 6-8 Å. The surface area should have approximately 28 Å^2. Intercalative binding is primarily driven by charge-transfer-complexes, dipole-dipole- and electrostatic interactions. The redox activity of the *para*-conjugated quinone ring is also beneficial for the radical cleavage of DNA, as shown in doxorubicin, daunorubicin or mitoxantrone. A positive charge is generally needed for activity, which is usually provided by a protonable or quaternised aromatic nitrogen of the chromophore.[51, 52] A very large number of chemically heterogenous compounds has been screened and positively identified as DNA intercalating compounds, of which many show cytotoxic activity. It could be demonstrated that the cytotoxic effects were primarily caused by inhibiting the topoisomerase enzymes by formation of ternary complexes with DNA, called the "ternary cleavable complex". Many DNA intercalators are now recognised as a class of topoisomerase inhibitors.

1. 7. 2 Topoisomerase Inhibitors - Modes of Action and Cardiomyopathy[53, 54, 55]

Topoisomerase enzymes are necessary during replication and transcription where they regulate the overwinding and underwinding of the DNA. This is achieved by forming a DNA-topoisomerase-complex, which is referred to as "cleavable complex" which temporarily introduces DNA single strand (topoisomerase I, TOP1) or double strand breaks (topoisomerase II, TOP2). After successful replication or transcription the strands are reannealed and the enzyme dissociates from the DNA.

Human DNA topoisomerase I (TOP1) is an essential enzyme that relaxes DNA supercoiling during replication and transcription by generating DNA single-strand breaks and religates the cleaved strand to reestablish intact duplex DNA. TOP1 is target for the treatment of human cancers. Camptothecin derivative topotecan and irinotecan belong to the most effective anticancer agents in clinical practice. Topotecan is approved for the treatment of ovarian and lung cancer, irinotecan for the treatment of colon cancer.

Clinical limitations of the camptothecin derivatives include spontaneous inactivation to a lactone form in blood, overexpressing membrane transporters resulting in resistance of cancer cells, rapid

reversal of the trapped cleavable complex after drug removal, requiring prolonged infusions, and dose-limiting side effects of neutropenia and diarrhea.

To overcome these limitations, non-camptothecin indenoisoquinoline derivatives as inhibitors of TOP1 were developed which are chemically stable in blood, more effective as anti-cancer compounds in animal models, inhibitors of TOP1 cleavable complexes at distinct sites and not substrates of membrane transporters. Based on these characteristics, the indenoisoquinoline derivatives indotecan and indimitecan are currently under evaluation in a Phase I clinical trial for patients with relapsed solid tumours and lymphomas.

Topoisomerase II has two closely related isoforms, designated α and β, in vertebrate species. TOP2α and TOP2β share 70 % amino acid sequence identity, but are encoded by separate genes and have different molecular masses (170 kDa and 180 kDa, respectively). The α isoform is the type II topoisomerase originally described in mammalian species and mainly represents the enzyme that was characterised as topoisomerase II in early studies.

Drugs targeting TOP2 can be divided into TOP2 poisons and TOP2 catalytic inhibitors. TOP2 poisons can be further subdivided into intercalating and non-intercalating poisons. The intercalators include doxorubicin and other anthracyclines, mitoxantrone and other compounds that are not currently in clinical use, such as ellipticine and amonafide. They share only the ability to intercalate into the DNA, but there is no apparent chemical similarity that would explain their ability to trap TOP2. Non-intercalating TOP2 poisons include teniposide and etoposide, as well as fluoroquinolones, a class of widely used oral broad-spectrum antibiotics, which are mainly affecting prokaryotic topoisomerases. Non-intercalating TOP2 poisons don't interact tightly with the DNA, hence it has been suggested that protein–drug interactions are responsible for trapping TOP2 covalent complexes.
The biochemical mechanism by which TOP2 poisons exert their cytotoxic effects seem to be multifaceted, however their role as a topoisomerase II poison has been well established.[55]

Unlike catalytic inhibitors of topoisomerase II, which block the catalytic function of the TOP2 enzyme, the drugs poison the enzyme by increasing the steady-state concentration of its cleavage complexes. This converts TOP2 into a physiological toxin inducing double-stranded DNA breaks triggering numerous mutagenic and cytotoxic events, such as deletions, insertions, and illegitimate recombination.
"Due to their mechanism of action, these drugs are considerably more lethal to cells that contain high levels of topoisomerase II and are undergoing high rates of DNA replication. Since the concentration of the enzyme is usually elevated in rapidly proliferating or transformed cells, clinically aggressive cancers appear to be most responsive to these drugs. Although both isoforms of topoisomerase II probably interact with anticancer drugs in human cells, several lines of evidence suggest that the α-isoform of the enzyme is the primary cytotoxic target of these agents."[55] Associated with this mode of action seems to be the cardiotoxicity which is inherent to the anthracyclins such as doxorubicin and the anthracenedione derivative mitoxantrone. This side effect is generally attributed to the generation of free radicals by the anthracyclines. This is usually explained with the quinone moiety in ring B, which can undergo a reduction to its hydroquinone form *via* an intermediate semiquinone which subsequently reduces oxygen into reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals. ROS can exert cellular damage *via* DNA, cell membrane, and other intracellular macromolecules.[56]
The relevance of ROS regarding antitumour effects and cardiomyopathy is still heavily discussed, as it

is uncertain if the generated amounts of free radicals at clinically relevant drug concentrations could be responsible for anthracycline toxicity to the tumour and to cardiac tissue. The application of antioxidants such as α-tocopherol, probucol, melatonin, N-acetylcysteine to counteract cardiotoxicity was promising in preclinical models, but the outcome in clinical studies was not convincing.[57, 58]

In this context, lipid peroxidation of cell membranes through free-radical production and their antitumour effects is doubtful.

Instead, an alternative molecular mechanism was described recently by Zhang and co-workers,[59] which could - if confirmed in future investigations - broaden our molecular understanding about TOP2-inhibitors and their associated therapeutic disavantages.

Of both isoforms TOP2α and TOP2β the latter is the only isoform expressed in mammalian cardiomyocytes, whereas proliferating cells, such as cancer cells preferentially express TOP2α.

The authors demonstrated in a mouse model with cardiomyocyte specific deletion of TOP2β that "in the presence of TOP2β, doxorubicin activates the DNA response and apoptosis pathways and triggers a marked alteration in the transcriptome that selectively affects oxidative phosphorylation and mitochondrial biogenesis in cardiomyocytes."[59] These results suggest that redox cycling of doxorubicin is not solely responsible for doxorubicin-induced cardiotoxicity, but implicate that TOP2β is the essential driver of doxorubicin-induced cardiotoxicity.[59] Once confirmed, these findings could have the potential to incorporate new aspects in cancer therapy. Novel selective TOP2α inhibitors without cardiotoxic properties or selective TOP2β protecting molecules could be designed.

1. 7. 3   Mitoxantrone

Bearing the cardiotoxic properties of doxorubicin in mind hundreds of anthracyclines have been synthesised in hope to overcome these side effects but only daunorubicin, doxorubicin, epirubicin, idarubicin, aclarubicin, pirarubicin and valrubicin earned clinical approval.[60] These second generation anthracyclins have altered pharmacokinetic and pharmacodynamic profiles as well as improved efficacy, but the cardiomyopathy is still an unresolved issue. Another disadvantage is the complex chemical structure which makes synthesis difficult.

Originally designed as stable dye, mitoxantrone can be regarded a simplified analogue of anthracyclines with a planar anthracenedione nucleus. The non-planar ring was removed to form a tricylic system with two identical side chains (*Figure 2*). Structure-activity-relationship (SAR) studies revealed the importance of one hydroxyl and one carbonyl group. The nitrogen-containing side chains is also important for the activity as it stabilises the ring in between base pairs by intercalating with the negatively charged phosphate backbone of the DNA.[63]
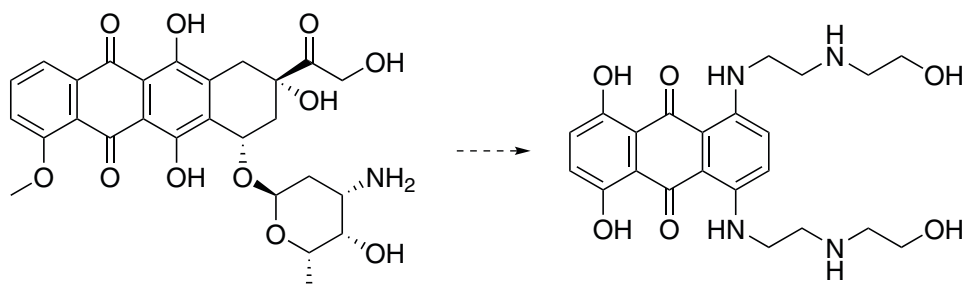
Figure 2. Simplification of Doxorubicin

Mitoxantrone is used in the treatment of metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma. In combination with prednisone it is approved as a second-line treatment for metastatic hormone-refractory prostate cancer. Mitoxantrone also inhibits T-cell, B-cell, and macrophage proliferation, and is indicated for reducing neurologic disability and relapse frequency in patients with secondary progressive multiple sclerosis (SPMS), progressive relapsing MS, or worsening relapsing-remitting MS (RRMS).[62]

In addition to the topoisomerase-based mechanism there is one which was discovered first in doxorubicin as well as daunorubicin, finding stable covalent DNA adducts in the presence of formaldehyde as illustrated in *Figure 3*.
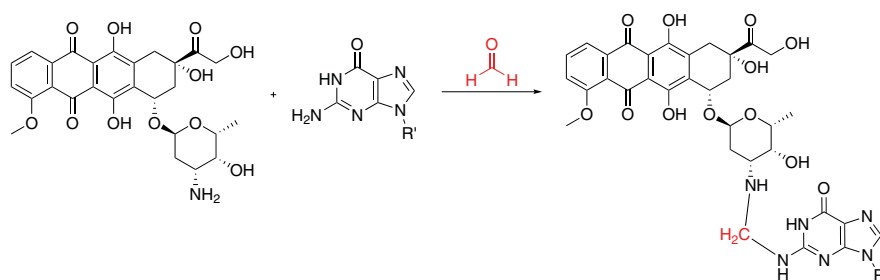
Figure 3. *Doxorubicin-DNA Adduct Formation*

Later, this mechanism was also discovered in mitoxantrone and pixantrone.
Formaldehyde activates mitoxantrone to produce the adducts which stabilise DNA by forming interstrand crosslinks (*Figure 4*).[64, 65]
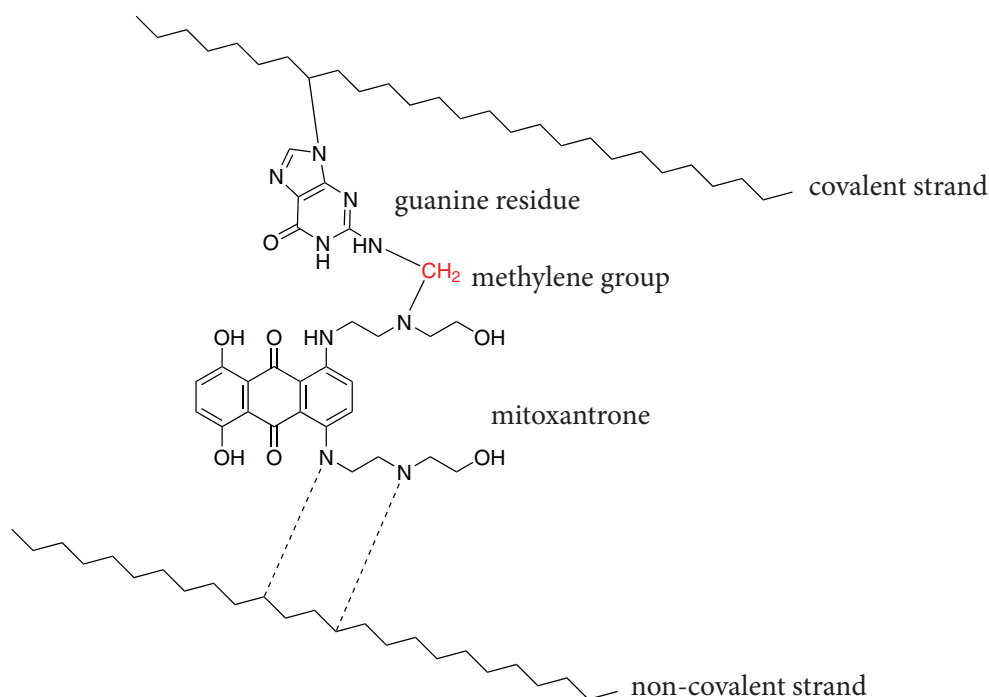
Figure 4. *Probable structure of formaldehyde-activated mitoxantrone-DNA adducts.*
The drug is attached covalently to only one strand with sufficient local stabilisation deriving from the intercalated drug plus hydrogen bonding (the localisation of the presumed hydrogen bonds has not yet been clarified) to the second DNA strand (non-covalent), similar to that of doxorubicin-DNA adducts)[64]

This may be biologically relevant since cells of myeloid origin have elevated levels of formaldehyde and mitoxantrone is effective against myeloid tumours.[65] Inflammation sites of solid tumours attract neutrophils releasing hydrogen peroxide which oxidises the biologically available polyamines, such as spermine to generate formaldehyde upon respiratory burst.[65, 66] This appears to be a Fenton reaction and is precedented by the Cu(II)/EDTA-catalysed oxidation of Tris to formaldehyde.[67, 68] This provides an increased level of formaldehyde at the site of these solid tumours and may also result in activation of mitoxantrone. It could also be demonstrated that the crosslink formation is concentration dependent from both formaldehyde and mitoxantrone. The same correlation could be shown for pixantrone.[69] Similarly, synthetic doxorubicin-formaldehyde-derivatives showed increased cytotoxic activity, presumably because they do not require drug-induced intracellular production of formaldehyde.

1. 7. 4   Pixantrone[69]

Pixantrone was prepared as second-generation group of anthracenediones with the aim to develop compounds with better therapeutic efficacy and reduced side-effects. The anthraquinone nucleus of mitoxantrone was retained, and the 5,8-dihydroxy phenylene ring implicated in its cardiotoxicity was substitued by a pyridine moiety. Krapcho et al. rationalised that these nitrogen atoms may provide basic sites or improved hydrogen bonding, therefore providing the analogues with a potentially greater affinity for DNA and topoisomerase II. Moreover, the side chain was truncated to an aminoethyl group as a primary amino function (*Figure 5 on page 15*).

Pixantrone emerged as the most promising drug candidate and demonstrated superior anti-leukemic activity in mice over a wide, well tolerated range of doses compared with mitoxantrone. Further studies showed that pixantrone has a wide spectrum of anti-tumour activity, and a marked efficacy against lymphomas and leukemias. Histopathological evaluation of the heart tissue in these studies revealed that pixantrone induced no detectable delayed cardiotoxicity. These key findings prompted the entry of pixantrone into clinical trials for further development. In 2012, pixantrone was approved by the EMA in the treatment of relapsed or refractory aggressive NHL

The mechanism of action is like in mitoxantrone probably multimodal. Pixantrone interacts with DNA *via* intercalation and as topoisomerase II poison. Mitoxantrone and pixantrone share close structural similarity, nevertheless the primary amino group of pixantrone in each of its side chains is more susceptible to formaldehyde activation and consequently formaldehyde-activated pixantrone reacts far more efficiently with DNA in vitro to generate covalent drug-DNA adducts than mitoxantrone and also exhibits greater stability than the corresponding mitoxantrone species. This may also be important since an extended pixantrone–DNA adduct half-life may maximise cytotoxicity and enable an enhanced disruption of critical cellular processes such as DNA transcription and replication.

2   AIMS OF THE THESIS

The aim of this research was to modify and expand the core structure which was previously synthesised at the Department of Drug and Natural Product Synthesis to further enhance its cytotoxic potential.

The rationale for the development of novel cytotoxic compounds was based on the works of Krapcho and Menta[61] who have synthesised a number of aza-bioisosteric chemotypes related to mitoxantrone that lack the 5,8-dihydroxy substitution pattern and have shown that in these chemotypes the positioning of the nitrogen atom exerts a profound effect on the expressed antitumour activity. This modification led to the development of the topoisomerase II inhibitor BBR 2778, which showed antitumour activity, but lacks cardiotoxicity. It is now known by its international non-proprietary name pixantrone dimaleate, and in 2012 it received conditional marketing authorisation in the European Union (Pixuvri®) as monotherapy to treat adult patients with relapsed or refractory aggressive Non-Hodgkin B-Cell lymphomas. However, pixantrone lacks the wide range of therapeutic use compared to doxorubicin and mitoxantrone. The goal to develop a potent anticancer agent lacking the cardiotoxicity while retaining the broad therapeutic spectrum of mitoxantrone could not be achieved yet.
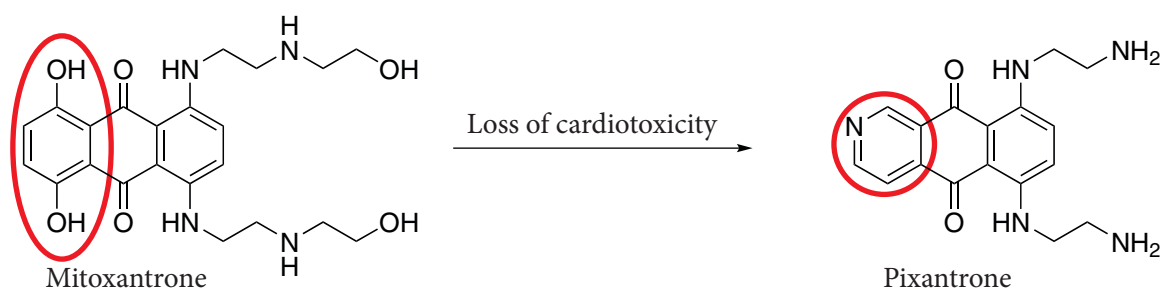
Figure 5. *Bioisosteric Modification of Mitoxantrone*

In this context, in cooperation with Aeterna-Zentaris our department[70-78] subjected the tricyclic skeleton of pixantrone to bioisosteric replacement of the diamino substituted benzene ring with a pyrrole unit. Extensive variations on pyrrole, as well as pyridine, pyrazine and pyrimidine annulation were performed. Furthermore, different basic amino and DNA alkylating side chains were linked to the pyrrole nitrogen (***Figure 6 on page 16***).
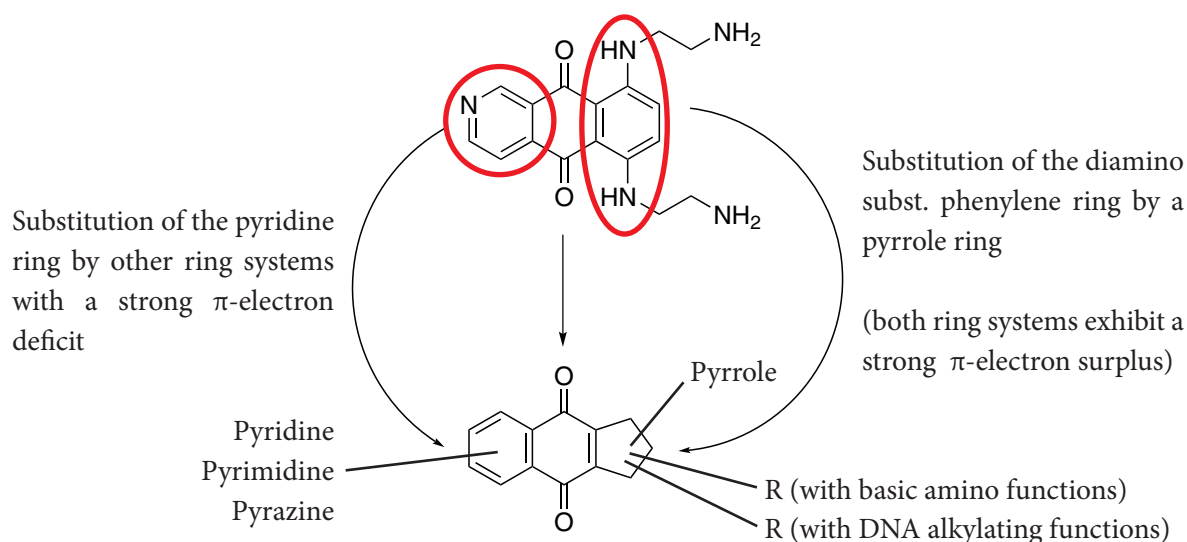
Figure 6. Modification of Pixantrone

In the following, the tricyclic nucleus was expanded by one benzene ring into a pyrido-annulated carbazoledione of the ellipticine quinone type which is known to possess cytotoxic properties (***Figure 7***). A regioisomer of ellipticine quinone endowed with the same side chains as its tricyclic precursors was prepared.
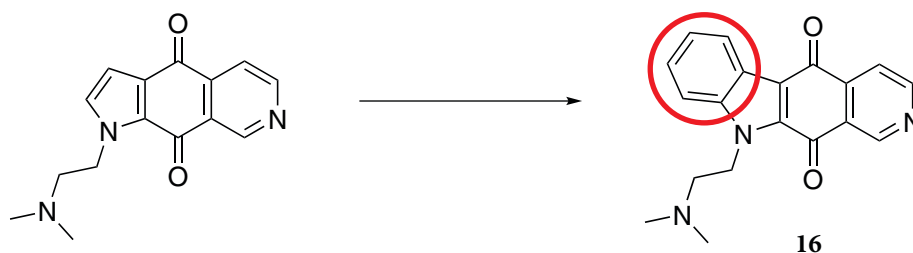
Figure 7. Ring Expansion to Regioisomeric Ellipticine Quinone

The synthesised compound library was tested by Aeterna-Zentaris against a panel of standard cancer cell lines using a XTT antiproliferative assay revealing that the ellipticine quinone isomer endowed with a *N,N*-dimethylaminoethyl side chain (**16**) was the most cytotoxic compound so far (***Table 1***).

Cell Line	Tissue	IC$_{50}$ (µg/mL)
Kb/HeLa	Cervix (HeLa contaminant)	0.35
NCI-H460	Lung (pleural effusion)	0.12
SF-268	Brain (astrocytoma)	0.27
SKOV-3	Ovary (ascites)	0.33
RKO-p27	Colon	0.18

Table 1. Biological Evaluation of 16

The aim of this thesis was to use compound **16** as a scaffold for further investigations. We were interested in modifying the heterocyclic system as well as expanding the core to pentacyclic isomers of the known anticarcinogenic natural compounds calothrixin A and B[80] linked to the same basic side chain which has demonstrated to be the most promising so far (**Figure 8**).

Pyridine
Pyrazine
Pyrimidine
Pyridazine

Ring expansion
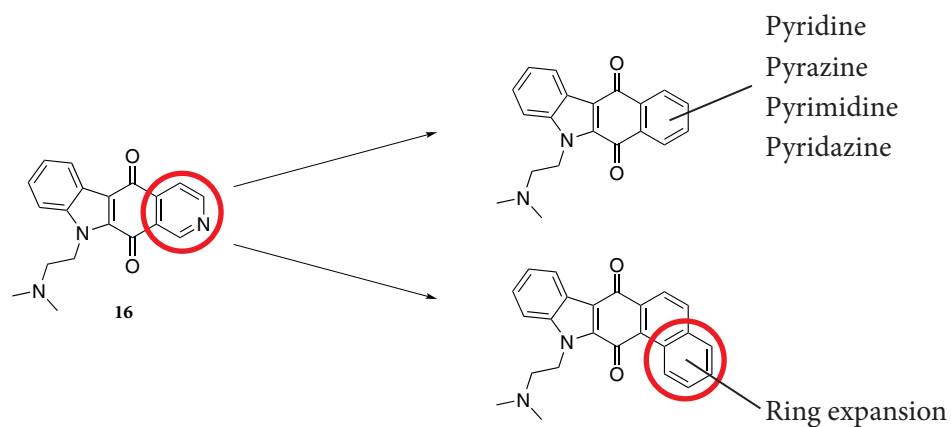
Figure 8. *Structural Modification of* **16**

Thus, we were interested in expanding our library by the following target compounds and evaluating their cytotoxic potential (**Figure 9**). In the following, the most active candidates from the antiproliferation assays should serve as scaffolds for further side chain modifications.
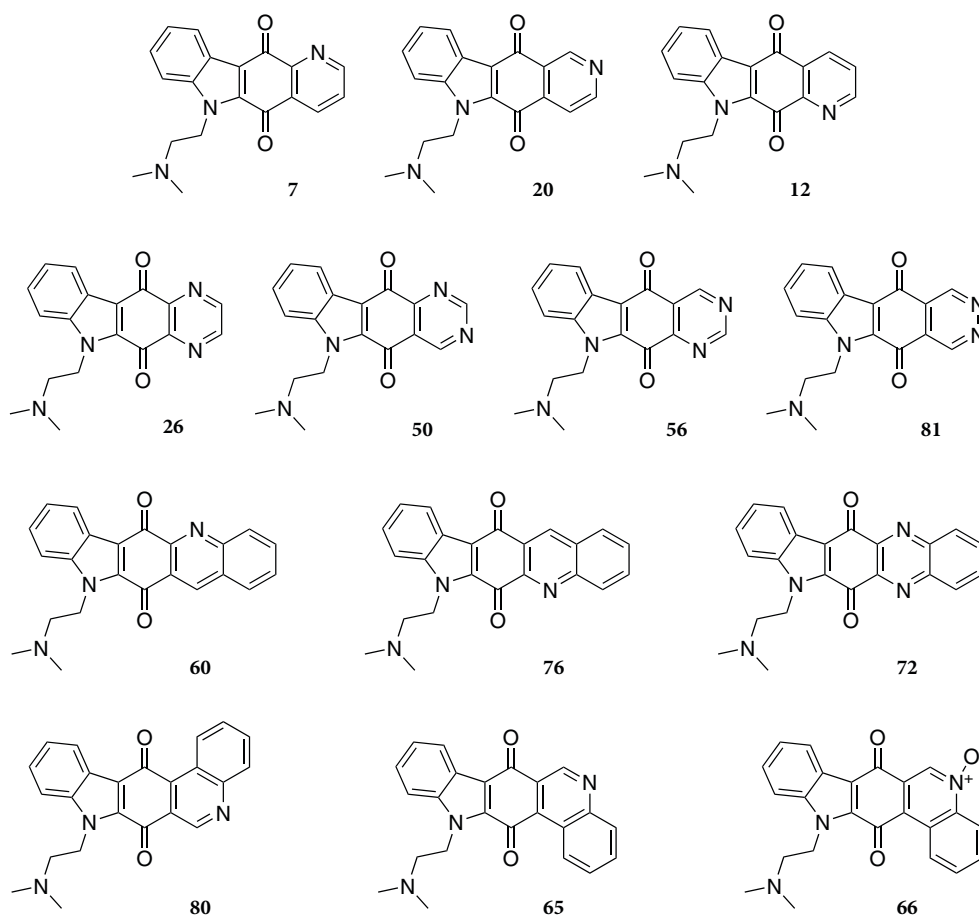
Figure 9. *Target Compounds*

3 RESULTS AND DISCUSSION

3. 1 Synthesis of 6-[2-(dimethylamino)ethyl]-5*H*-pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione (7)

The construction of the 5*H*-pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione ring system **6** was achieved by the known procedure of Ashcroft et al.[84,85] in three steps. The synthesis of **7** required in total seven steps which is outlined in ***Scheme 1***.
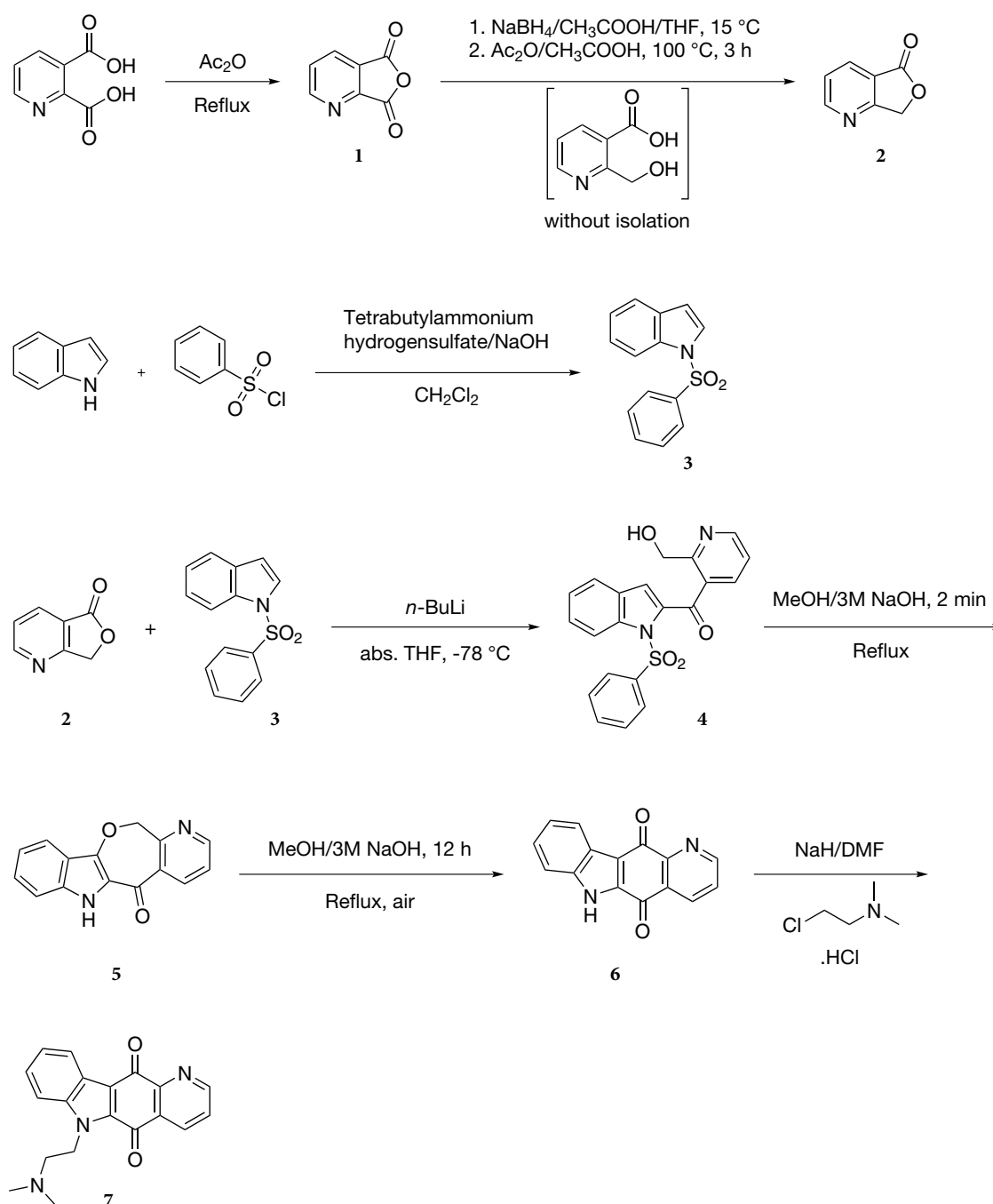
Scheme 1. *Synthesis of* ***7***

3. 1. 1   Synthesis of Furo[3,4-*b*]pyridin-5,7-dione (1)

1
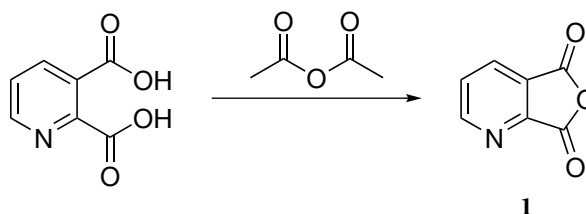
The synthesis of commercially available **1** was achieved by a simple condensation reaction of pyridine-2,3-dicarboxylic acid in the presence of acetic anhydride which was carried out according to the procedure by Vitry et al.[82] by refluxing pyridine-2,3-dicarboxylic acid in acetic anhydride for 3 h and purification by sublimation to give **1** as white crystals in 75 % yield. The identity was confirmed by NMR spectroscopy and was in accordance with literature (***compare 6. 2. 1 on page 83***).

3. 1. 2   One-Pot-Synthesis of Furo[3,4-*b*]pyridin-5(7)-one (2)[81]

1. NaBH$_4$/CH$_3$COOH/THF, 15 °C
2. Ac$_2$O/CH$_3$COOH, 100 °C, 3 h

1

without isolation

2
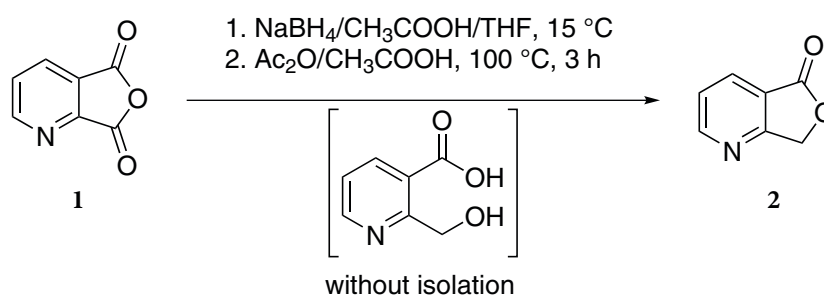
Key intermediate **2** was prepared by a more recent procedure described by Inoue et al.[81] which comprises two steps from pyridine-2,3-dicarboxylic acid or one step from commercially available furo[3,4-*b*]pyridin-5,7-dione (**1**), respectively, and is thus favourable to the 4-step synthesis by Ashcroft et al.[79] which results in lower overall yields and also employs a reduction step using highly inflammable LiAlH$_4$.

The reaction involves the regioselective reduction of anhydride **1** with NaBH$_4$ in the presence of acetic acid in dry THF. The authors suggest that in the presence of the additive acetic acid the pyridine nucleus of **1** might be partially protonated or chelated and the 7-carbonyl group would be more activated for an attack by the hydride ion in comparison with the 5-carbonyl group, which would preferentially produce intermediate 2-(hydroxymethyl)nicotinic acid. The lactonisation thereof was executed without isolation by treatment with acetic anhydride. After the aqueous workup and extraction with dichloromethane, the pure product could be obtained by recrystallisation from isopropanol, affording long, pale brown needles. Some discolouration and little of the undesired isomer could be removed by purification using column chromatography (silica gel, ethyl acetate) affording a colourless crystalline product. The identity of the product was confirmed by NMR spectroscopy which is in accordance with literature[81] (***compare 6. 2. 2 on page 84***).

3. 1. 3   Synthesis of 1-(phenylsulfonyl)-1*H*-indole (3)
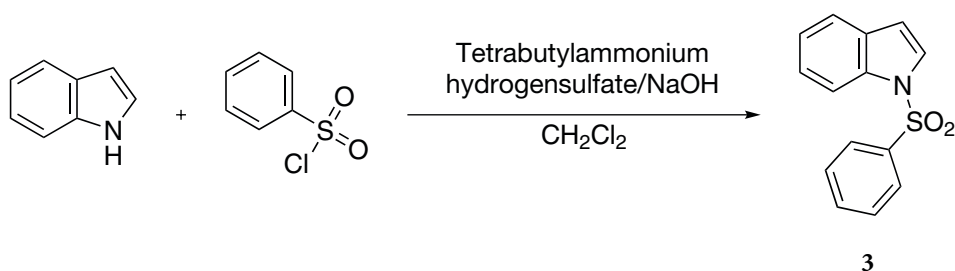
Building block **3** was prepared according to the procedure described by Conway et al.[83] Indole was added to to an ice-cold mixture of powdered NaOH and tetra-*n*-butylammonium hydrogen sulfate in dry dichloromethane under argon in one portion, followed by a solution of benzenesulfonyl chloride in dry dichloromethane. After 2 h of vigourous stirring the obtained thick orange oil was recrystallised twice from hot methanol to afford white needles in 75 % yield. The identity of the product was confirmed by NMR spectroscopy (*compare 6. 2. 3 on page 85*).

3. 1. 4   Synthesis of [2-(hydroxymethyl)-3-pyridinyl][1-(phenylsulfonyl)-1*H*-indol-2-yl]methanone (4)[84]
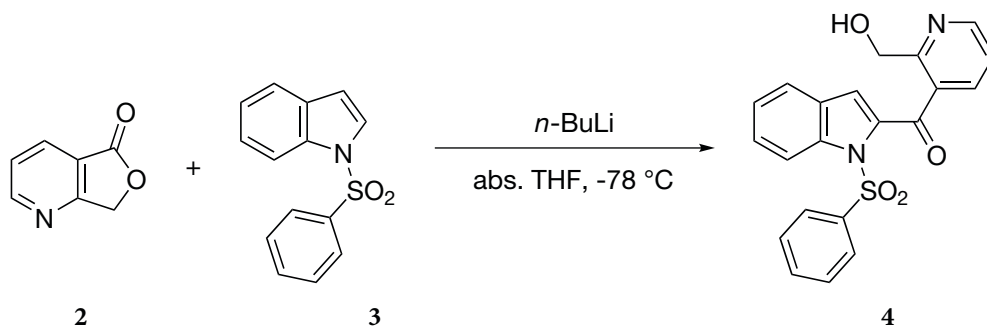
The condensation of 2-lithiated synthon **3** and lactone **2** gave the desired ketone **4**. The lithiation was conveniently effected with commercial *n*-BuLi initially at -78 °C and then at room temperature. The condensation yielded 21.8 % of the desired intermediate (after purification) which could be used without purification in the following cyclisation step. Yet, the product was purified by column chromatography for NMR spectroscopic purposes and melting point determination. The successful reaction was indicated in the ¹H spectra by the absence of the H-2 proton of the indole, and the emerging of the methylene singlet counting two protons at 5.12 ppm and a broad singlet at 4.93 ppm, which can be attributed to the hydroxy group showing no correlation to a carbon atom in the HSQC spectrum. Moreover, the ¹³C NMR clearly indicates the introduction of the carbonyl-C at 193.1 ppm. HRMS analysis is in agreement with the calculated mass (*compare 6. 2. 4 on page 86*).

3. 1. 5 Synthesis of 6*H*-pyrido[3',2':5,6]oxepino[3,2-*b*]indol-5(12*H*)-one (5)[84]
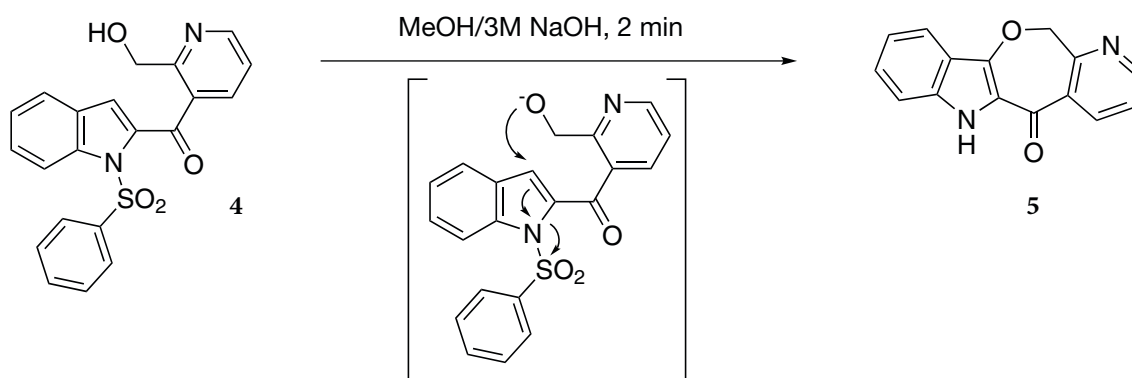
Ketone **4** was transformed into the corresponding pyrido-oxepino-indole **5** by intramolecular indole-β-nucleophilic substitution. This reaction was performed in refluxing aqueous methanolic NaOH. The reaction time of not exceeding 2-3 minutes is essential for good yields, longer reaction time deteriorates the results, as the initial product disappears. The successful ring-formation was indicated in the ^{1}H spectrum by the absence of the indole H-3 proton an the OH-proton of **4**, also the aromatic signals of the protecting group have disappeared and the broad singlet at 11.39 ppm can be assigned to the indole NH proton. The ^{13}C spectrum is also consistent with these results as are the 2D-NMR experiments. These findings are also in agreement with HRMS analysis (***compare 6. 2. 5 on page 87***).

3. 1. 6 Synthesis of 5*H*-pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione (6)[85]

Pyrido-oxepino-indole **5** was subsequently transformed into quinone **6** under refluxing aqueous methanolic NaOH with the passage of air. Literature suggests that "the quinone-forming process to work best/at all when the methylene hydrogens in the oxepino-indole are acidified (either by a fused pyridine or quinoline ring or by an aromatic nitro-group)."[85] The formed anion seems to be in equilibrium with the benzene oxide which is trapped by oxygen giving the product **6** at the oxidation level of the quinone (***Figure 10***).
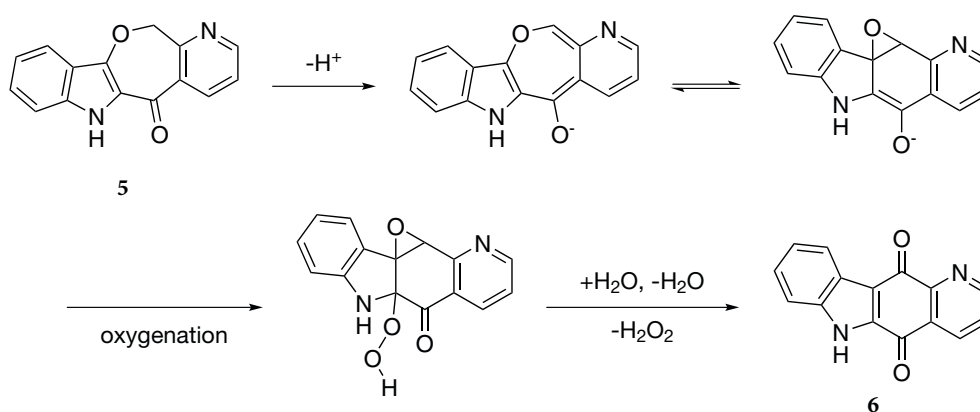

Figure 10. *Suggested Quinone-forming Process*

The mixture was poured into water and the reddish-brown precipitate was filtered off, dried and purified by sublimation to afford 95 % of **6** as yellow crystals. The identity of the desired compound was elucidated by NMR spectroscopy and HRMS analysis (***compare 6. 2. 6 on page 88).*** In particular, the carbazole NH proton gives a broad singlet at around 12 ppm in the ^{1}H spectrum which was found to be characteristic for all nuclei of these series, as are the two quinone carbons at about 178 ppm to 180 ppm.

3. 1. 7 Synthesis of 6-[2-(dimethylamino)ethyl]-5*H*-pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione (7)

6   NaH/DMF   **7**
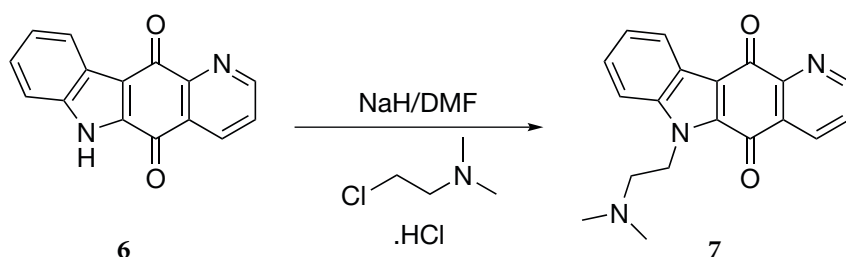
The alkylation of compound **6** is a S_N2 reaction which was carried out by a standard procedure described in countless publications by forming the pyrido-carbazole anion using sodium hydride followed by substitution of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride in dry DMF. The product was extracted with ethyl acetate and purified using column chromatography to yield 26 % of **7** as orange-brown crystals. Structure elucidation *via* NMR spectroscopy showed two N-methyl groups giving a singlet at 2.41 ppm and two pseudo-triplets at 4.84 ppm for the methylene group attached to the carbazole N and at 2.78 ppm for the methylene group adjacent to the dimethylamino group. The absence of the carbazole NH singlet and the spatial proximity of the methylene signals to H-7 demonstrated *via* NOE experiment are indicative for the desired substitution pattern. HRMS analysis supported the results of the NMR experiments (***compare 6. 2. 7 on page 89)***.

3. 2 Synthesis of 10-[2-(dimethylamino)ethyl]-5*H*-pyrido[2,3-*b*]carbazole-5,11(10*H*)-dione (12)

The synthesis of **12** in analogy to the methodology for **16** described by Ashcroft et al.[85] would have required the preparation of the corresponding isomeric lactone as necessary builing block in 3 steps[79] employing the double esterfication of pyridine-2,3-dicarboxylic acid followed by reduction to the diol with LiAlH$_4$ and finally oxidation with MnO$_2$ in chloroform (***Scheme 2 on page 23***).
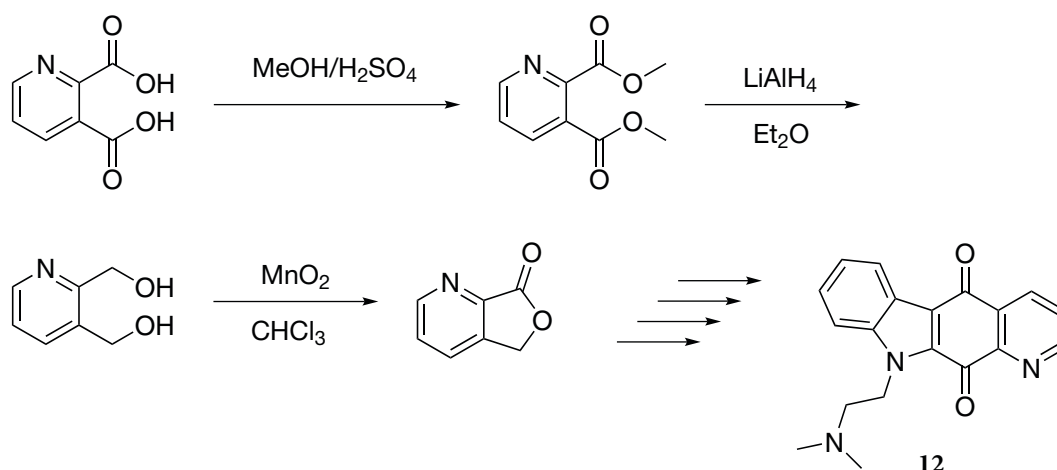
Scheme 2. *Discarded Synthesis of* **12**

Additional four steps for the construction of the final compound in analogy to compound **7** makes this synthetic pathway unattractive and was dismissed in favour of a method developed by Ketcha and Gribble[86], which was successfully used for the construction of **16** by Leber[70].

This strategy should employ a Friedel-Crafts C-3 acylation of 1-(phenylsulfonyl)-1*H*-indole with carboxylic acid anhydrides or acid chlorides in the presence of aluminium chloride to give 3-acyl-1-(phenylsulfonyl)indoles in good yields. The ketoester would then be prepared by Fischer esterification of the carbocylic acid with ethanol in presence of *p*-TsOH over 5 days followed by the ring-formation step with LHMDS with in situ N-deprotection. The synthesis of compound **12** should be achieved in analogy to this methodology as depicted in **Scheme 3**.
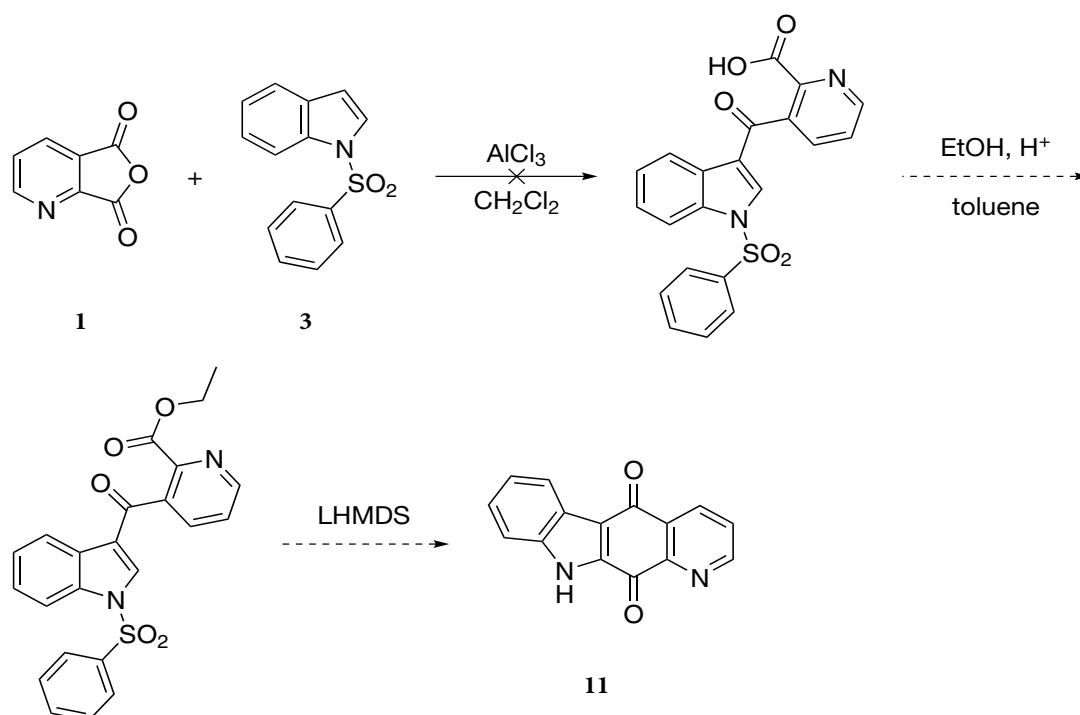
Scheme 3. *Attempted Synthesis of* **11** via Friedel-Crafts Acylation

1-(phenylsulfonyl)-1*H*-indole (**3**) was stirred with furo[3,4-*b*]pyridine-5,7-dione (**1**) in the presence of aluminium chloride in dry dichloromethane at room temperature for two hours. After the workup procedure NMR spectroscopy revealed that the desired product was not formed, but anhydride **1**

was hydrolysed presumably during the aqueous workup and only pyridine-2,3-dicarboxylic acid and 1-(phenylsulfonyl)-1*H*-indole (**3**) could be recovered. Several attempts to modify the reaction conditions with varying amounts of aluminium chloride and/or reaction times did not succeed either.

Following a literature survey of similar compounds we abandoned the Friedel-Crafts-acylation strategy in favour of an *ortho* metallation strategy applied for the construction of the calothrixins by Watanabe and Snieckus[88] and further developed by Kelly et al.[89]

The well-established directed *ortho* metallation[90] strategy to construct the tetracyclic nucleus should be applied, starting from key compounds 1-(methoxymethyl)-1*H*-indole-3-carbaldehyde (**8**) and *N,N*-diethylpyridine-2-carboxamide (**9**). Although this method is unattractive in terms of overall yield compared to other strategies, it is highly convergent and the building blocks **8** and **9** are easily prepared in good yields.

Since the reaction conditions reported by Watanabe and Snieckus[88] failed in the construction of the calothrixins, the optimised reaction conditions of Kelly et al.[89] were applied. The same methodology was later applied for the preparation of calothrixin analogues. After the construction of the MOM-protected nucleus the protecting group would be removed and the side chain should be linked to the carbazole nitrogen (*Scheme 4*).
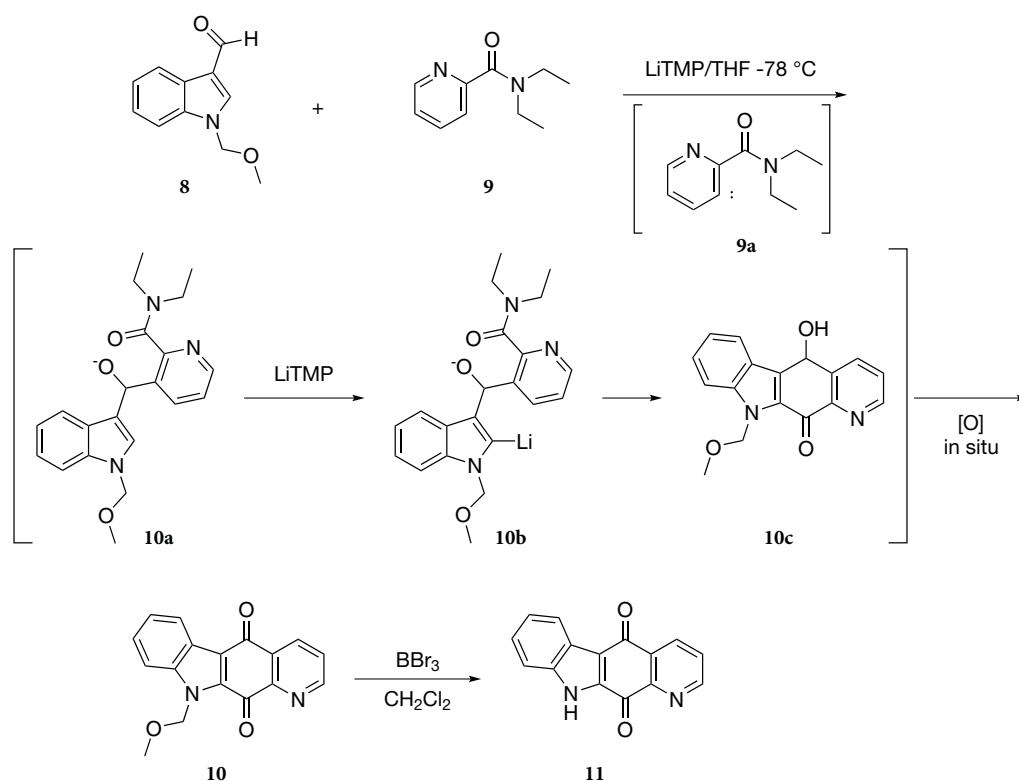
Scheme 4. Directed Ortho Metallation

3. 2. 1   Synthesis of 1-(methoxymethyl)-1*H*-indole-3-carbaldehyde (8)
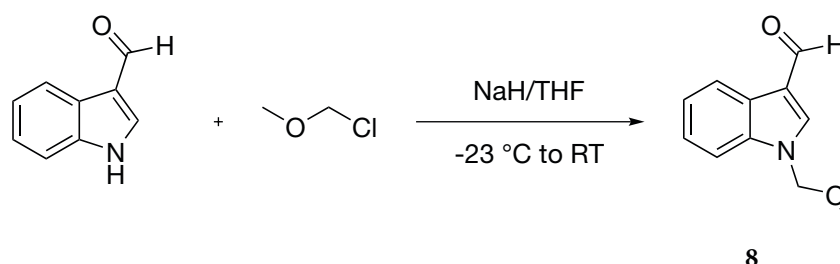
The method of Comins et al.[91] was used. A solution of 1*H*-indole-3-carbaldehyde in dry THF was added to a cooled suspension of sodium hydride in dry THF. The indole proton is abstracted by sodium hydride giving the indole anion and the N-methoxymethyl protecting group (N-MOM) was introduced in a nucleophilic substitution (S_N2) reaction by slowly adding chloro(methoxy)methane into the reaction vessel. After warming to room temperature and stirring for 30 min the reaction mixture was poured into cold aqueous 5 % $NaHCO_3$ solution, extracted with diethyl ether, and evaporated in vacuo. The resulting crude product was purified by balltube distillation or recrystallisation from ethyl acetate/light petroleum (1:1, v/v) to afford beige crystals in good yields. Structure eludication *via* NMR spectroscopy confirmed the identity of the product (***compare 6. 2. 8 on page 90***).

3. 2. 2   Synthesis of *N,N*-Diethylpyridine-2-carboxamide (9)
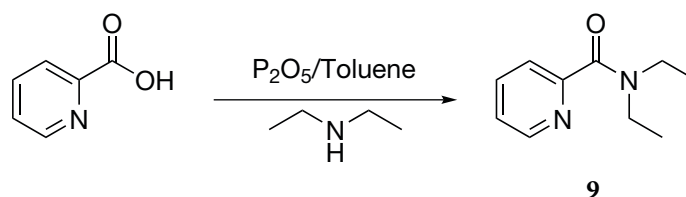

The method of Swain et al.[92] was used to prepare the amide. It employs a simple condensation reaction of a carboxylic acid and an amine with the strong dehydrating agent phosphorous pentoxide. Pyridine-2-carboxylic acid and diethylamine were refluxed in dry toluene in presence of excess phosphorous pentoxide for 4 hours. The product was extracted with toluene/diethyl ether, dried, and the solvent was removed in vacuo. The brown residue was purified by balltube distillation or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to give the title compound as yellow oil in good yields. A benefit of this method is the simple reaction setup and the proceeding of the reaction requires little attention.

Therefore, this method was extended to the following diethylcarboxamides which were prepared accordingly from the corresponding carboxylic acids to give yellow to orange oils in 64 to 84 % yields.
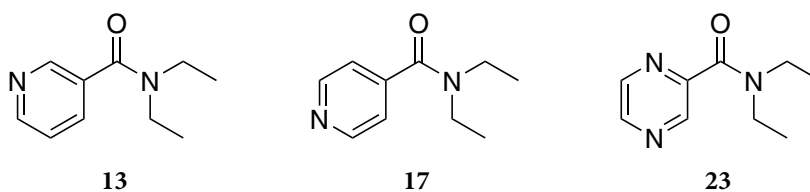
The identity of all compounds was verified *via* NMR spectroscopy (***compare 6. 2. 9 on page 91, 6. 2. 13 on page 95, 6. 2. 18 on page 100 and 6. 2. 24 on page 106***).

3. 2. 3 Synthesis of 10-(methoxymethyl)-5*H*-pyrido[2,3-*b*]carbazole-5,11(10*H*)-dione (10)

8   **9**   *n*-BuLi/TMP / THF, -78 °C to RT   **10**
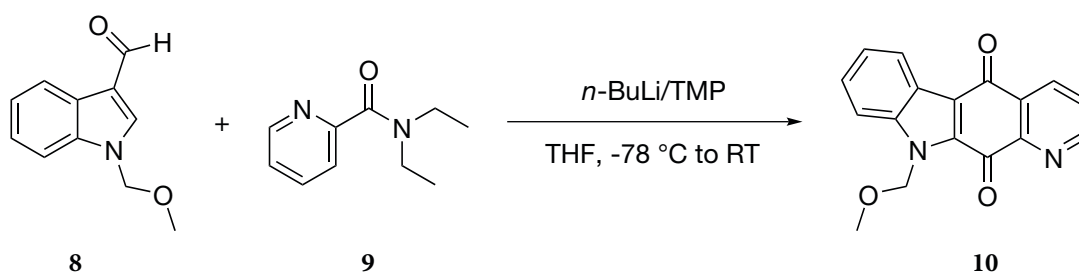
The building blocks **8** and **9** were coupled in a one-pot operation to give **10** directly. None of the putative intermediates were isolated, but the choice of LiTMP as base was crucial. Although Snieckus and Watanabe successfully constructed 6-(methoxymethyl)-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (**18**) using *sec*-BuLi as base, Kelly et al. failed in the construction of the calothrixin system. *Tert*-BuLi and hexamethyldisilazide were equally useless resulting in no product. The more basic LDA produced only small amounts. The even more basic LiTMP proved to be most effective.

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine to a cold (–70 °C) solution of *n*-BuLi in dry THF, under argon, and then stirring the mixture at –5 to 0 °C for 1 hour. The temperature was then reduced to –78 °C and a solution of the diethylamide **9** and aldehyde **8** in dry THF was added dropwise in such a rate, that the temperature did not exceed -60 °C. The reaction solution turned orange. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h). During this time the solution turned dark. The time allowed to reach room temperature was crucial for acceptable product yield. Prolonged stirring in the cold as well as stirring overnight generated undesired side products.

The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to furnish an orange residue which was purified by column chromatography, eluting with ethyl acetate/light petroleum (4:6, v/v) giving the desired product in 33 % yield as an amorphous orange solid which could be further purified by recrystallisation from ethyl acetate.

After our first attempt of reacting **8** with **9** by adding the electrophile 30 min after lithiating the amide, NMR structure elucidation revealed that the amide had undergone a dimerisation process to give *N*,*N*-diethyl-3-(2-pyridinylcarbonyl)-2-pyridinecarboxamide (*Figure 11 on page 27*). This is a recurring problem in lithiations with -CONEt$_2$[93], which was overcome by an in situ trapping technique by adding substrate and electrophile simultaneously to the LiTMP solution.
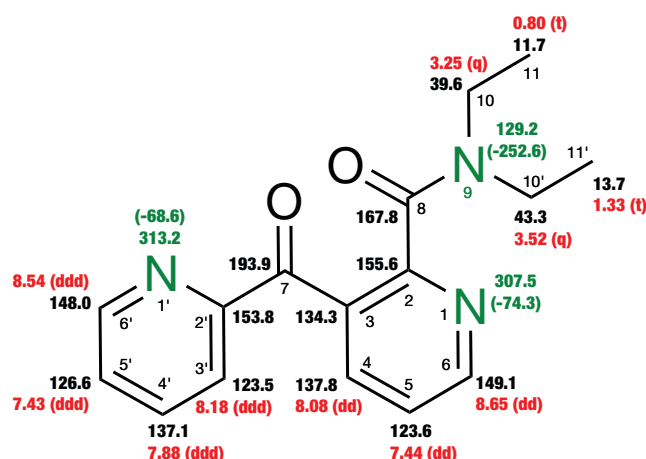
Figure 11. *Dimerisation Product of* **9**

¹H NMR (500 MHz, CDCl₃) δ 8.65 (dd, *J* = 4.9, 1.7 Hz, H-6, 1H), 8.54 (ddd, *J* = 4.8, 1.7, 0.9 Hz, H-6', 1H), 8.18 (ddd, *J* = 7.9, 1.2 Hz, 0.9 Hz, H-3', 1H), 8.08 (dd, *J* = 7.8, 1.7 Hz, H-4, 1H), 7.88 (ddd, *J* = 7.9, 7.5, 1.7 Hz, H-4', 1H), 7.44 (dd, *J* = 7.7, 4.9 Hz, H-5, 1H), 7.43 (ddd, *J* = 7.5, 4.8, 1.2 Hz, H-5', 1H), 3.52 (q, *J* = 7.0 Hz, H-10', 2H), 3.25 (q, *J* = 7.1 Hz, H-10, 2H), 1.33 (t, *J* = 7.1 Hz, H-11', 3H), 0.80 (t, *J* = 7.1 Hz, H-11, 3H).

¹³C NMR (125 MHz, CDCl₃) δ 193.9 (C-7), 167.8 (C-8), 155.6 (C-2), 153.8 (C-2'), 149.1 (C-6), 148.0 (C-6'), 137.8 (C-4), 137.1 (C-4'), 134.3 (C-3), 126.6 (C-5'), 123.6 (C-5), 123.5 (C-3'), 43.3 (C-10'), 39.6 (C-10), 13.7 (C-11'), 11.6 (C-11).

¹⁵N NMR (50 MHz, CDCl₃) δ -68.6 (N-1'), -74.3 (N-1), -252.6 (N-9).

We could demonstrate that this procedure could be extended to the synthesis of the intermediary MOM-protected isomeric pyridocarbazole-diones **14**, **18** and the pyrazinocarbazole-dione **24**, from which we could access the desired basic substituted derivatives in two steps. Nevertheless, the construction of compound **24** was more difficult, yields were comparably low (approximately 10 %) generating unwanted tarry side products which made the purification process challenging. These findings were not surprising and are usually explained with the preferential reactivity of the electrophiles with the nitrogen atoms and the very low reactivity of these heterocycles towards electrophilic substitution on the carbon atoms. The electron-withdrawing effect of the additional nitrogen ring atom decreases the energy level of the LUMO of the pyrazines making them more reactive towards nucleophiles than pyridines. Although the lithiated species is stabilised by the electron-withdrawing effect of the ring nitrogen atom, it is, however, destabilised by electronic repulsion between the carbanion and the lone pair of the adjacent nitrogen.[94]
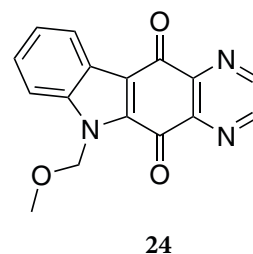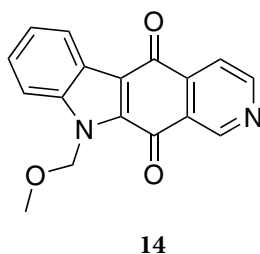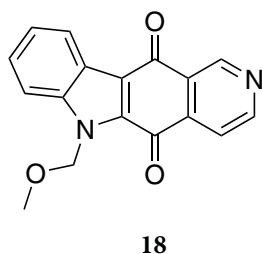

The identity of all MOM-pyridocarbazole-diones was confirmed by NMR structure elucidations studies and HRMS (*compare 6. 2. 10 on page 92, 6. 2. 14 on page 96, 6. 2. 19 on page 101 and 6. 2. 25 on page 107*). In particular, in all instances the MOM protection group can be identified in the ¹H NMR spectrum by the methyl singlet at approximately 3.40 ppm and its methylene singlet at approxi-

mately 6.20 ppm. In the ^{13}C spectrum the two carbonyl groups can be observed at approximately 180 ppm. In case of MOM-protected pyrazine derivative **24**, an unambiguous NMR spectroscopic signal assignment of the fused pyrazine unit was not possible *via* HMBC experiment, which is also valid for all other pyrazine derivatives.

### 3. 2. 4 General Procedure for the Synthesis of 11, 15, 19 and 35 by BBr₃ Effected MOM Cleavage

The cleavage of the MOM protecting group was accomplished according to literature procedure employed by Bernardo et al.[87] by reacting the educts with 1.2 eq. of BBr₃ in dry dichloromethane at 0 °C and stirring at room temperature for 1 h (*Scheme 5*). The reaction mixture was quenched with saturated NaHCO₃ solution and stirred for 1 h at 60 °C. The product was collected by suck filtration. The filter cake was washed with water and remaining starting material could be removed by washing with dichloromethane which is an excellent solvent for the starting material, but not for the product. The filter cake was recrystallised from ethyl acetate to afford the unprotected nucleus in 75 to 95 % yield. The successful deprotection step was verified by NMR investigation and HRMS. The ^{1}H spectra show the absence of the methyl and the methylene protons of the MOM group, and a broad singlet of the newly formed carbazole NH proton at approximately 13.20 ppm can be observed. Moreover, in case of **11** (the first compound derived from this approach) a NOESY experiment was performed, showing the spatial proximity of the carbazole NH and the adjacent H-9 proton (*compare 6. 2. 11 on page 93, 6. 2. 15 on page 97, 6. 2. 20 on page 102 and 6. 2. 26 on page 108*).
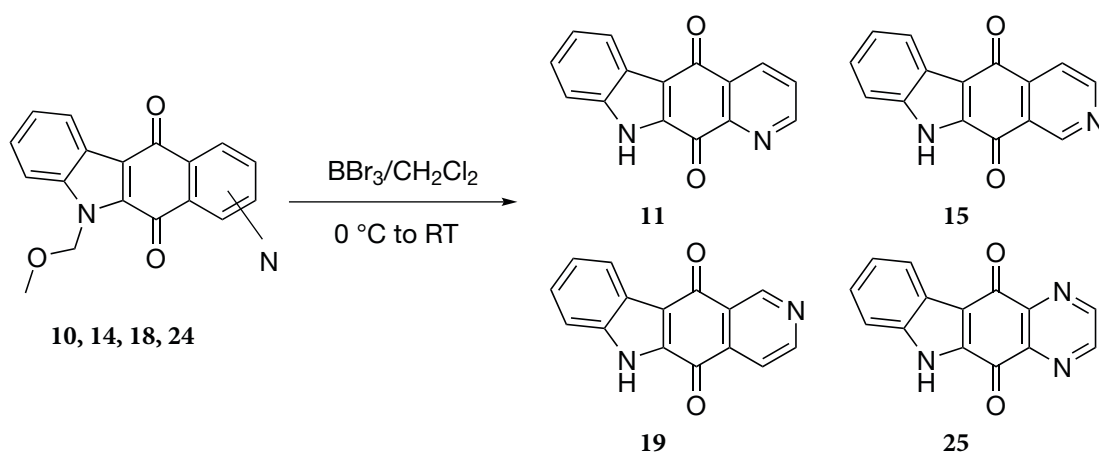
Scheme 5. *BBr₃ Effected MOM Cleavage*

3. 2. 5   General Procedure of Sodium Hydride Effected Alkylation of 11, 15, 19 and 25

The functionalisation of the nuclei to give the title compounds was carried out in analogy to compound **7** (*compare 3. 1. 7 on page 22*) as outlined in *Scheme 6*. In all cases except for compound **26** the purification step of the desired compounds involved column chromatography eluting with ethyl atetate/light petroleum (2:1, v/v), followed by ethyl acetate/methanol (8:2, v/v) and recrystallisation from ethyl acetate to give orange or brown crystals. Compound **26** was purified eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate. NMR experiments showed a consistent set of signals for the side chains in the ^{1}H spectra with very similar shifts and comparable peak patterns relating to compound **7**. HRMS analyses are in agreement with the NMR spectroscopic identification.
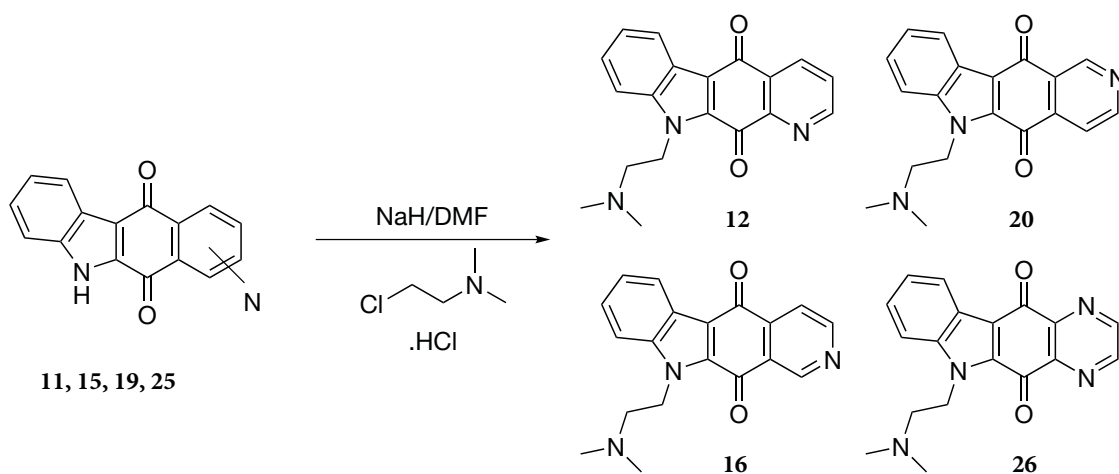

Scheme 6. *Sodium Hydride Effected Alkylation*

3.3 Synthesis of 12-[2-(dimethylamino)ethyl]-7*H*-indolo[3,2-*j*]phen-anthridine-7,13(12*H*)-dione (65)

We were also interested in the synthesis of isomeric calothrixin analogues functionalised with basic amino side chains and the evaluation of their cytotoxic potential in vitro.

The tetracyclic pyridocarbazole system should be expanded by benzo-annulation into pentacyclic quinoline-annulated carbazoledione systems to give calothrixin isomers. Then different basic side chains should be linked to the skeleton. This step is consistent with previous structure modification steps and in-vitro evaluation. The decision was supported by the known cytotoxic properties of the calothrixin skeleton against certain cancer cell lines as well as their inhibitory effect on the growth of plasmodium falciparum.[80]

Some of the isomeric calothrixin analogues were constructed by directed *ortho* metallation following the method of Kelly et al.[89] (*Scheme 7*), which was also successfully adapted for the synthesis of some tetracyclic compounds, as described in the previous chapters.
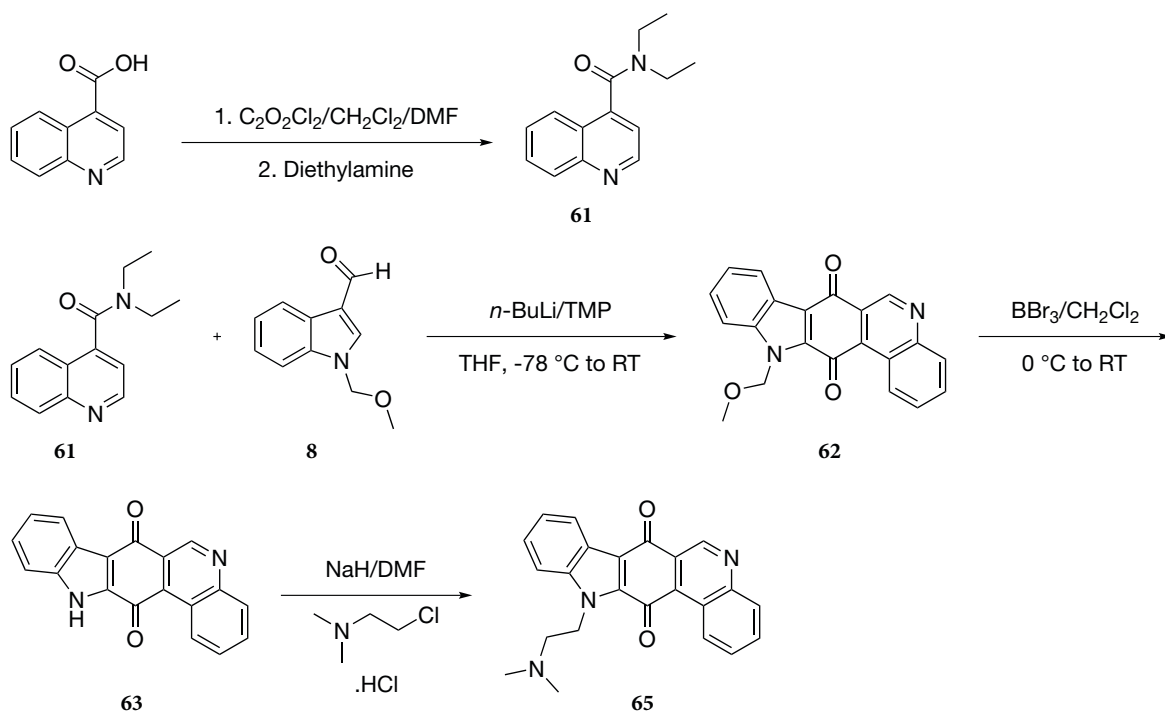

Scheme 7. *Synthesis of Calothrixin B Derivative* **65**

3. 3. 1 Synthesis of *N,N*-diethylquinoline-4-carboxamide (61)

61
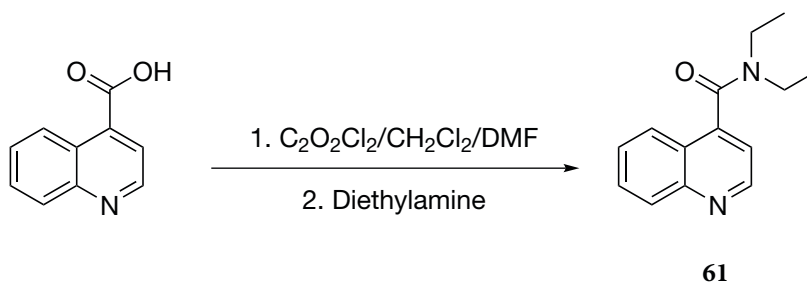
The known method of Gilman and Spatz[95] was modified. Oxalyl chloride was used instead of phosphoryl chloride for the formation of the intermediate acid chloride which resulted in shorter reaction time and better product yield. Quinoline-4-carboxylic acid was treated under cooling with oxalyl chloride in presence of catalytic amounts of DMF to form an intermediate acid chloride which reacts in an S_N2t type reaction with *N,N*-diethylamine to give the title compound (**61**) which was obtained in good yield as yellow-orange oil after extraction and purification by column chromatography eluting with ethyl acetate/light petroleum (4:1, v/v). The identity of the product was confirmed by NMR spectroscopy (***compare 6. 2. 63 on page 145***).

3. 3. 2 Synthesis of 12-(methoxymethyl)-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (62)

61   **8**   **62**
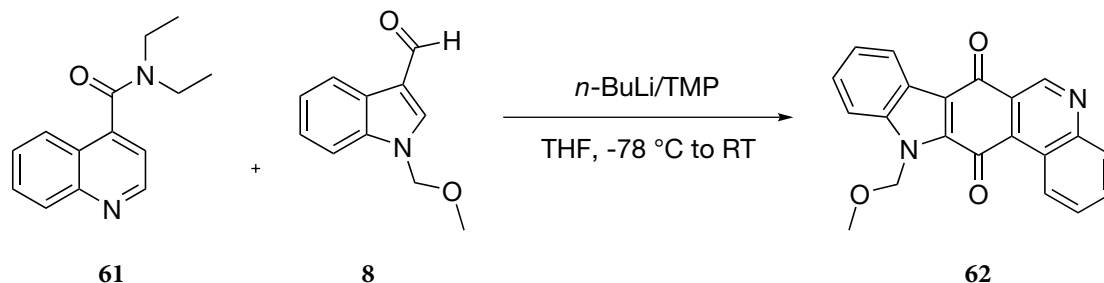
The construction of compound **62** was performed in analogy to **10**, **14**, **18** and **24** except for the trapping technique applied during the reaction. A dimerisation process occurring during the ring formation process of the tetracyclic nuclei required an in situ trapping of substrate and electrophile to give the desired compounds. Following the publication for constructing the calothrixin B skeleton this was unnecessary.

The D*o*M reaction was accomplished by adding a solution of the diethylamide **61** and after 15 minutes of stirring a solution of aldehyde **8** to a prepared solution of LiTMP at -78 °C. After allowing to reach room temperature over 2 h, the reaction mixture was quenched with water and after extraction with ethyl acetate and purification by column chromatography eluting with ethyl acetate/light petroleum 4:6 (v/v) the title compound was obtained as an amorphous orange-red solid. The structure of the title compound was confirmed *via* 2D-NMR technique and HRMS and is in agreement with literature.[87]

3. 3. 3   Synthesis of 7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (63)

62   **63**
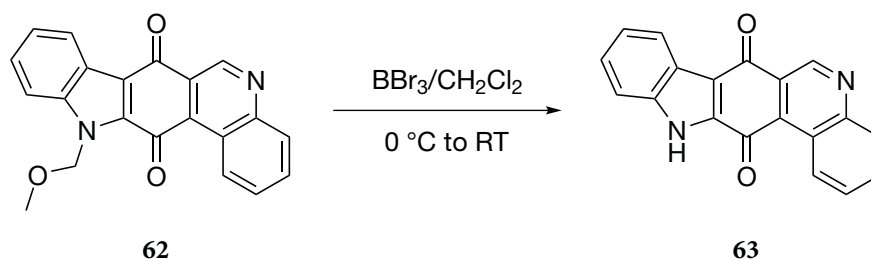
The deprotection of **62** was realised by the same procedure as described for compounds **11**, **15**, **19**, and **25** (*compare 3. 2. 4 on page 28).* The detailed procedure as well as NMR spectroscopic assignment is described in chapter *6. 2. 65 on page 147*.

3. 3. 4   Synthesis of 12-[2-(dimethylamino)ethyl]-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (65)

63   **65**
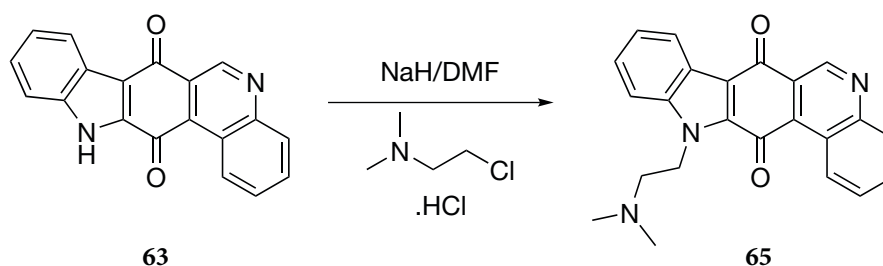
The alkylation of **63** was performed in analogy to **6** by treating the calothrixin B framework with sodium hydride in dry DMF and subsequent reaction with 2-chloro-*N,N*-dimethylethanamine hydrochloride in a S_N2 type reaction to give **65** as red-orange crystals in 39.5 % yield. The detailed procedure as well as NMR spectroscopic assignment is referenced in chapter *6. 2. 67 on page 149*.

3. 4   Synthesis of 12-[2-(dimethylamino)ethyl]-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione 5-oxide (66)

63   **64**   **66**
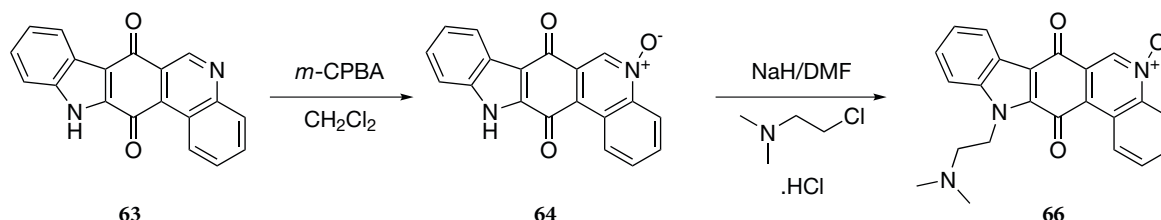
The synthesis of the title compound was achieved by following the procedure of Kelly et al.[89] which involved *N*-oxidation of calothrixin B (**63**) with 3-chloro perbenzoic acid in dichloromethane and alkylation of the resulting product. As would be expected upon switching from **65** to the corresponding calothrixin-*N*-oxide **66** a shift of δ to lower frequency of N-5 from -53.7 ppm to -88.3 ppm was

apparent, although higher than in the transition from pyridine to pyridine-*N*-oxide (approximately 23 ppm). This shift can be explained by resonance structures which describe both π-electron donor- and acceptor functions of the *N*-oxide moiety.[139]

In the ^{13}C NMR spectra of **64** and **66**, the expected shifts to lower frequencies for the carbons in *ortho* and *para* positions to N-5 (C-6: -15.7/-15.5 ppm, C-4a: -8.2/-8.0 ppm, C-13a: -10.7/-10.4 ppm) can be observed (relative to the corresponding shifts in the parent compounds **63** and **65**). This can be explained by resonance structures which indicate an increase in electron density in these positions.[140]

A detailed NMR spectroscopic characterisation of **64** and **66** is in chapter ***6. 2. 66 on page 148*** and ***6. 2. 68 on page 150***, respectively.

3. 5 Synthesis of 8-[2-(dimethylamino)ethyl]-7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione (80)[142]

The synthesis of the isomeric calothrixin derivative **80** should be achieved in analogy to **65** as outlined in ***Scheme 8***.[142] It was uncertain which position of the quinoline system would be attacked preferentially by the electrophile. The successful cyclisation step would depend on a lithiation at position 4. A literature review by Snieckus[90] suggested that the electrophile would indeed preferentially attack position 4 over position 2. In the following, Quinoline-3-carboxylic acid was converted into its corresponding *ortho* directing diethylamide derivative *via* acid chloride by treatment with oxalyl chloride in dry dichloromethane and a catalytic amount of DMF followed by reacting with diethylamine. The resulting diethylamide was subjected to the *ortho* lithiation strategy with **8** to give the corresponding MOM-protected pentacyclic product.

After the BBr₃ effected MOM cleavage the indole nitrogen was alkylated with 2-chloro-*N*,*N*-dimethylethanamine hydrochloride to give **80** as orange-red crystals. The identity of the title compound was confirmed by NMR spectroscopy and HRMS in chapter ***6. 2. 82 on page 164***.
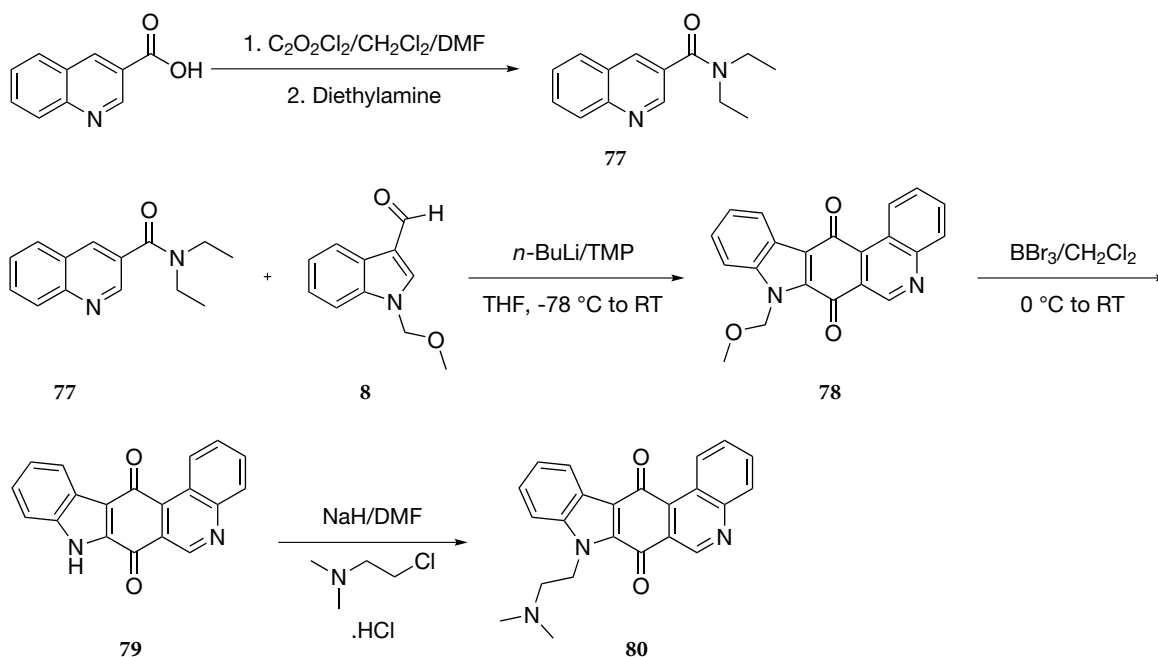
Scheme 8. *Synthesis of Isomeric Calothrixin B Derivative **80***

3. 6 Synthesis of 7-[2-(dimethylamino)ethyl]-6*H*-indolo[3,2-*b*]acridine-6,12(7*H*)-dione (76)

The synthesis of **76** was the third of four pentacyclic isomers based on a quinoline system. In this case, the lithiation of the **73** would clearly occur at position 3. The resulting system was the linear annulated indolo[3,2-*b*]acridine system bearing the quinoline nitrogen at position 5. The procedure was performed in analogy to compounds **65** and **80** as depicted in *Scheme 9*. The detailed spectroscopic characterisation of the title compound can be found in chapter *6. 2. 78 on page 160*.
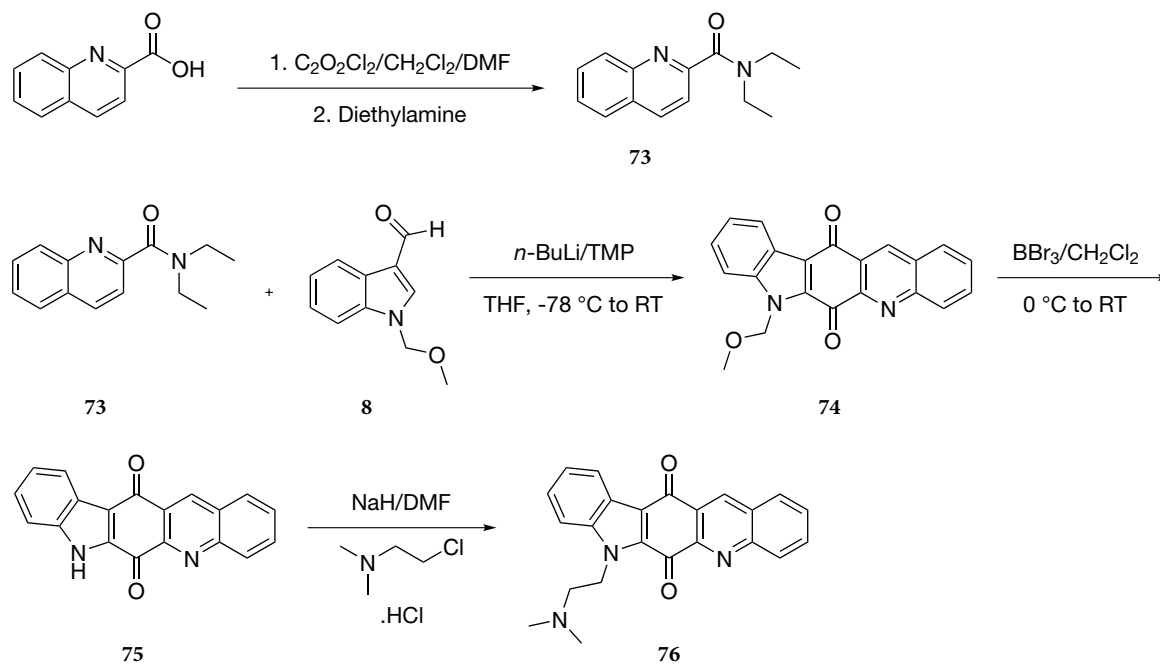
Scheme 9. *Synthesis of Isomeric Calothrixin B Derivative 76*

3. 7 Synthesis of 11-[2-(dimethylamino)ethyl]-6*H*-indolo[2,3-*b*]acri-dine-6,12(11*H*)-dione (60)

Compound **60** could not be realised by employing an *ortho* lithiation strategy but should be accessible *via* the same route as compound **7** (***compare 3. 1 on page 18)***. Lactone **57** was accessible in 71 % yield by reacting commercially available 2-aminobenzaldehyde with tetronic acid in ethanol,[96] and was then reacted with **3** to give a keto-alcohol which was subjected to an intramolecular β-nucleophilic substitution without isolation.[85] Refluxing the resulting intermediate **58** for 6 days in methanolic NaOH converted the oxepino-indole into its quinone **59** which was finally connected to the side chain using the usual procedure (***Scheme 10)***.
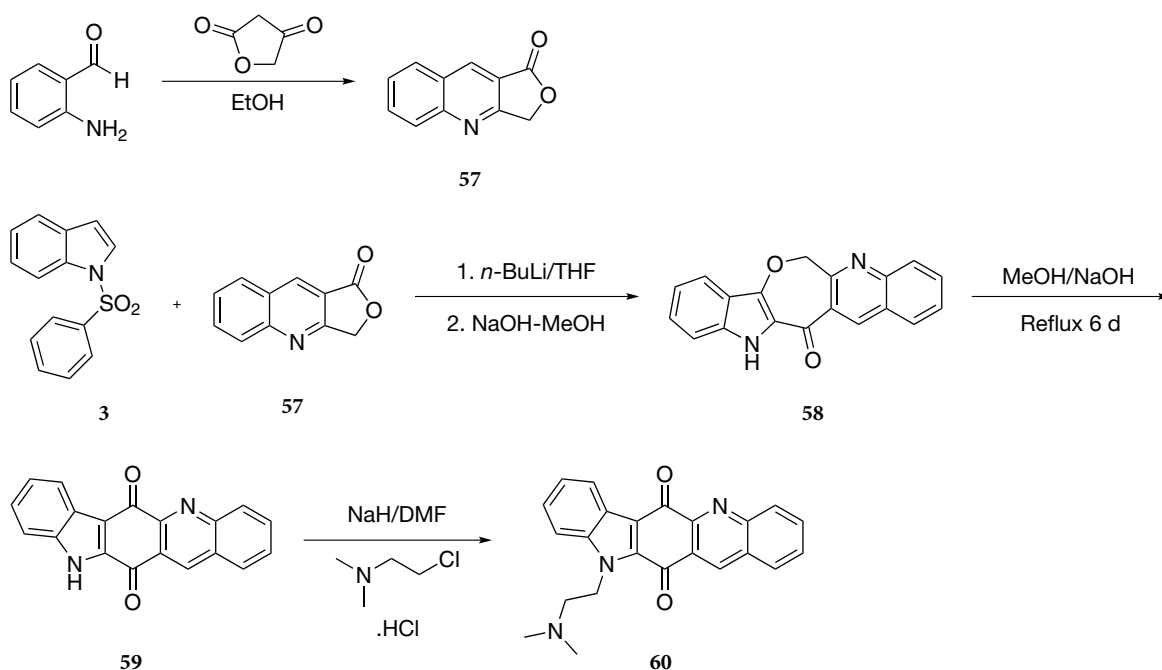
Scheme 10. *Synthesis of Isomeric Calothrixin B Derivative **60***

3. 8 Biological Evaluation - Viability Assay of the First Set of Compounds

Since the synthesis of the pyrimidine and pyridazine derivatives could not be accomplished in time viability assays of the the first series of 9 compounds were performed using both EZ4U® XTT assay and a Neutral Red assay for comparison (a detailed protocol of the assays is referenced in chapter *5. 4 on page 80)*.

Two different viability assays targeting different cell organelles were performed to assess the antiproliferative activity of these compounds. Neutral Red measures lysosomal activity to assess viability, whereas XTT targets mitochondrial enzymes. Judged by structural properties an intercalating effect on the DNA and the topoisomerase enzymes was expected, which should result in a quantifiable antiproliferative effect. The rationale for using two different assays was to minimise the chance of flawed test results caused by unknown effects of the test compounds on the targeted organelle. Influences on two different cellular targets by compounds of similar structure on the other hand is unlikely.

The first set of compounds consisted of 9 structures sharing a basic *N,N*-dimethylaminoethyl side chain linked to the carbazole nitrogen of 4 ellipticine quinone and 5 calothrixin analogues, respectively. In previously synthesised compounds of our research group this side chain had exerted the highest cytotoxic effect.[70]

The previously synthesised and biologically evaluated compound **16** was used for comparison with the new compounds, mitoxantrone dihydrochloride served as reference compound.

The cells were cultivated on 96-well plates and exposed to increasing concentrations of each compound for 24 h. The results of at least 3 independent experiments with Neutral Red in at least quadruplicate was used to calculate IC_{50} values. For comparison, at least 1 experiment in quadruplicate using the XTT assay was performed. (*compare 3. 19 on page 68*).

IC_{50} values for all compounds were established in SW480 cells (colon carcinoma). All IC_{50} values were calculated with GraphPad Prism® 6 using a non-linear regession model (variable slope, 4 parameters). The expressed values are μM.

As would be expected, a comparable outcome of both assays could be demonstrated on all cell lines with all tested compounds. Exemplarily, comparative dose-response-curves of the most active compounds **20** and **26** against SW480 cells are depicted in *Figure 12 on page 38 and Figure 13 on page 38*. Since the XTT assay is easier to handle and more sensitive, the second set of compounds, consisting of 17 compounds was performed using XTT only.

Highly cytotoxic tetracyclic derivatives **20** and **26** and one also highly active representative of the pentacyclic series (calothrixin A derivative **66**) were also tested against A549 (lung carcinoma), Hep3B (liver cell carcinoma), HTB65 (melanoma) and U373 (glioblastoma) cells (*Table 2 on page 37*).

The skeletons of **20** and **26** were used as lead structures for further side chain modifications.

Our efforts to open a synthetic pathway to the pyrimidine and the pyridazine nuclei were continued.

Com- pound	SW480 (Colon) [95%-CI]	A549 (Lung) [95%-CI]	HEP3B (Liver) [95%-CI]	U373 (Brain) [95%-CI]	HTB65 (Skin) [95%-CI]
7	2.69 [1.13 - 6.41] *3.68* *[2.10 - 6.43]*	-	-	-	-
12	2.07 [1.16 - 3.69] *2.76* *[1.49 - 5.11]*	-	-	-	-
16	0.796 [0.696 - 0.911] *1.41* *[1.00 - 1.97]*	-	-	-	-
20	0.519 [0.499 - 0.539] *0.924* *[0.708 - 1.21]*	0.514 [0.485 - 0.544] *0.194* *[0.153 - 0.246]*	0.627 [0.562 - 0.700] *0.584* *[0.506 - 0.673]*	0.961 [0.804 - 1.15] *1.12* *[0.643 - 1.94]*	0.582 [0.543 - 0.623] *0.452* *[0.382 - 0.536]*
26	0.492 [0.351 - 0.688] *0.389* *[0.278 - 0.544]*	1.06 [0.971 - 1.16] *0.436* *[0.378 - 0.503]*	0.371 [0.327 - 0.419] *0.452* *[0.409 - 0.499]*	0.645 [0.465 - 0.894] *0.717* *[0.592 - 0.869]*	0.225 [0.204 - 0.248] *0.336* *[0.318 - 0.355]*
60	>10 *> 10*	-	-	-	-
65	1.34 [1.11 to 1.62] *2.01* *[0.789 - 5.10]*	-	-	-	-
66	0.772 [0.704 - 0.846] *1.00* *[0.688 - 1.46]*	1.04 [0.927 - 1.16] *0.907* *[0.819 - 1.01]*	0.861 [0.765 - 0.969] *0.629* *[0.556 - 0.711]*	0.794 [0.549 - 1.15] *1.21* *[0.904 - 1.62]*	0.569 [0.541 - 0.598] *0.902* *[0.831 - 0.980]*
76	>10 *>10*	-	-	-	-
80	1.02 [0.941 - 1.11] *1.64* *[1.30 - 2.09]*	-	-	-	-

Table 2. *Antiproliferative XTT (EZ4U®) and Neutral Red Assays.*
IC$_{50}$ values are expressed in µM. Values of Neutral Red assay are expressed in Red Italic. Numbers in square brackets represent IC$_{50}$ values in the 95 % confidence interval.
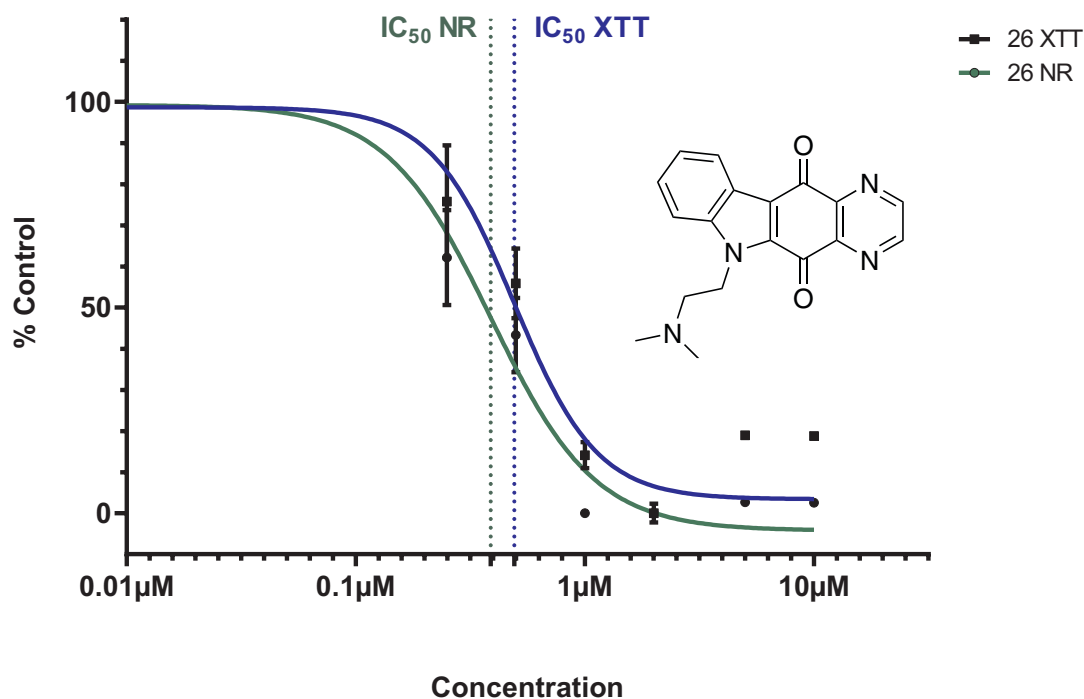
Figure 12. *Dose-Response Curve of Compound **26** with Neutral Red and XTT Assay.*
Dotted lines indicate the respective IC$_{50}$ value. Error bars represent ± standard error mean of at least 3 independent experiments in quadruplicate (NR) or at least 2 experiments in quadruplicate (XTT).
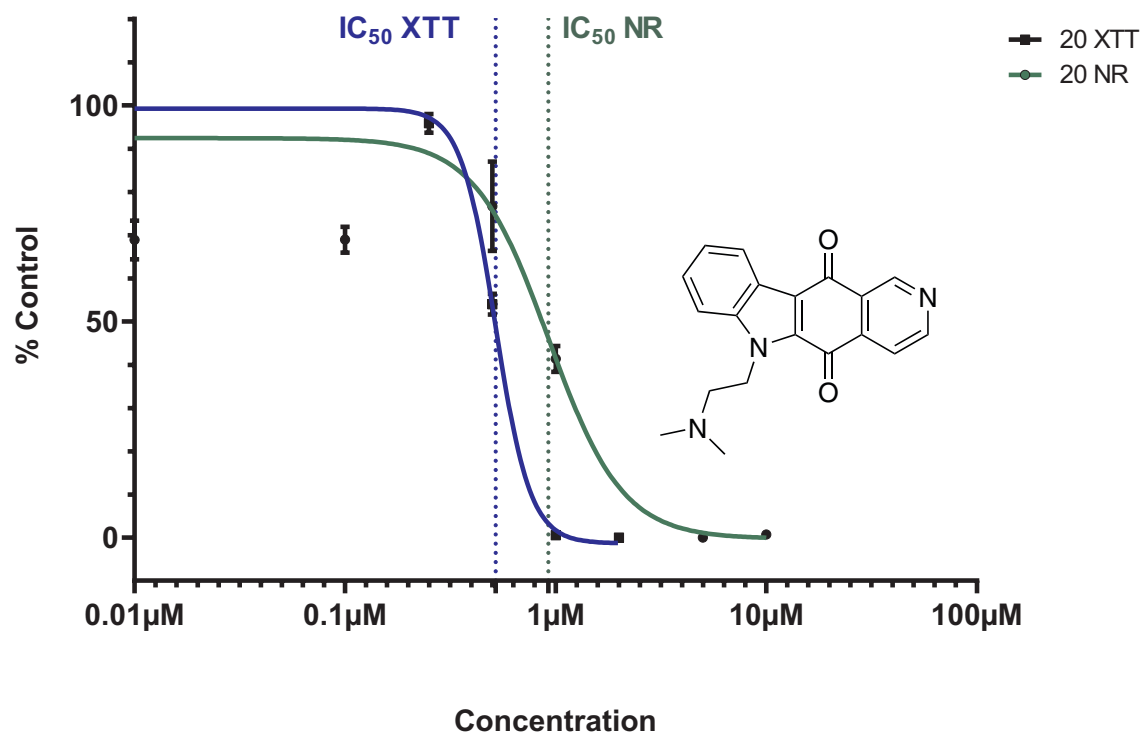
Figure 13. *Dose-Response Curve of Compound **20** with Neutral Red and XTT Assay.*
Dotted lines indicate the respective IC$_{50}$ value. Error bars represent ± standard error mean (SEM) of at least 2 independent experiments in quadruplicate (NR) or at least 1 experiment in quadruplicate (XTT).

3. 9   General Alkylation Procedure of 1*H*-Indole-3-carbaldehyde

After the biological evaluation of the first series of compounds had been completed one aim was to modify the substitution pattern of the most active compounds to further enhance their activity. In this context we were able to optimise the synthetic strategy by avoiding the MOM-protection/deprotection step. This was achieved by linking the desired side chain to the indole unit prior to the cyclisation step. The substituted aldehydes could be prepared by the standard alkylation procedure using sodium hydride and the respective side chain in DMF affording the desired products in good yields as yellow or orange oils or as beige low melting solids (*Scheme 11*). The detailed procedure and NMR spectroscopic assignments are referenced in the experimental part. Compared with the MOM-protected compounds yields were a bit lower, nevertheless we favoured this method saving one step.
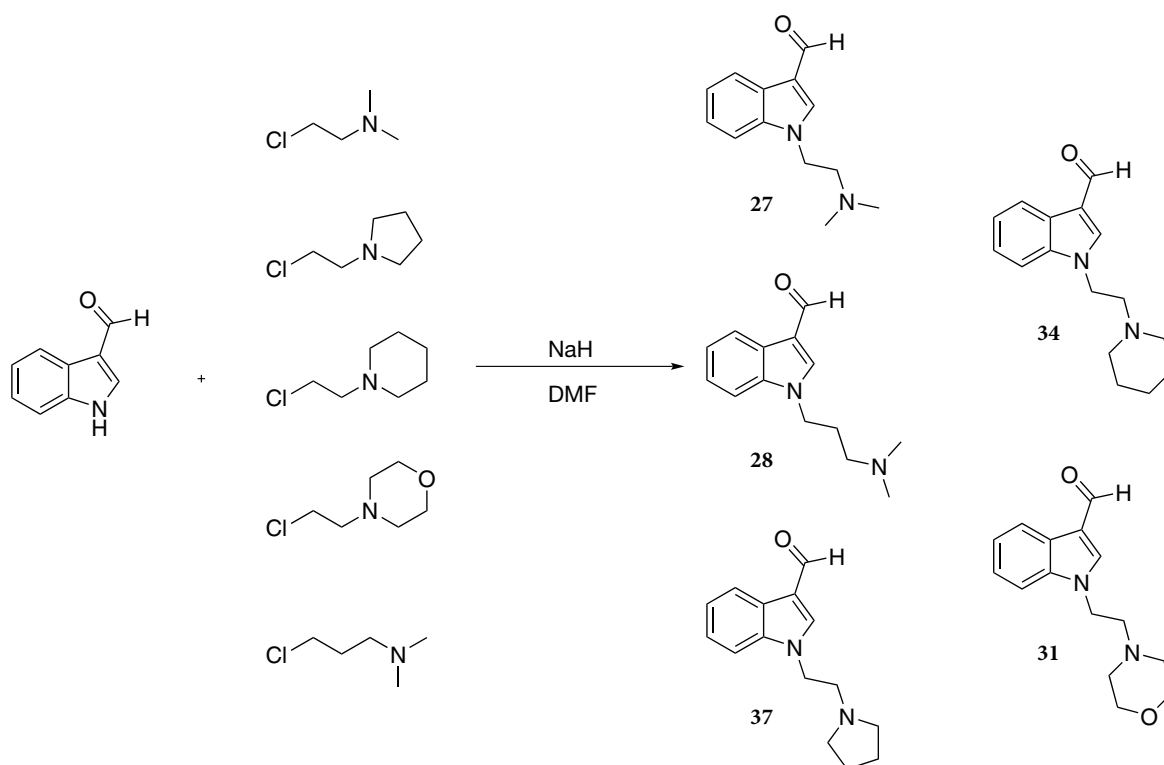
Scheme 11. *General Alkylation Procedure*

3. 10 Synthesis of 5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (19) and 5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (25) with Alternate Substitution Patterns

Having prepared the respective substituted 1*H*-indole-3-carbaldehyde the cyclisation step was performed using the the usual procedure (*Scheme 12*) to afford the desired compounds which were purified using column chromatography to afford orange to brown crystals. The prepared compounds are depicted in the scheme below. The detailed procedure, yield and spectroscopic characterisation for each compound is referenced in the experimental part. Employing this methodology also enabled us to shorten the route for the already prepared compounds **20** and **26**.
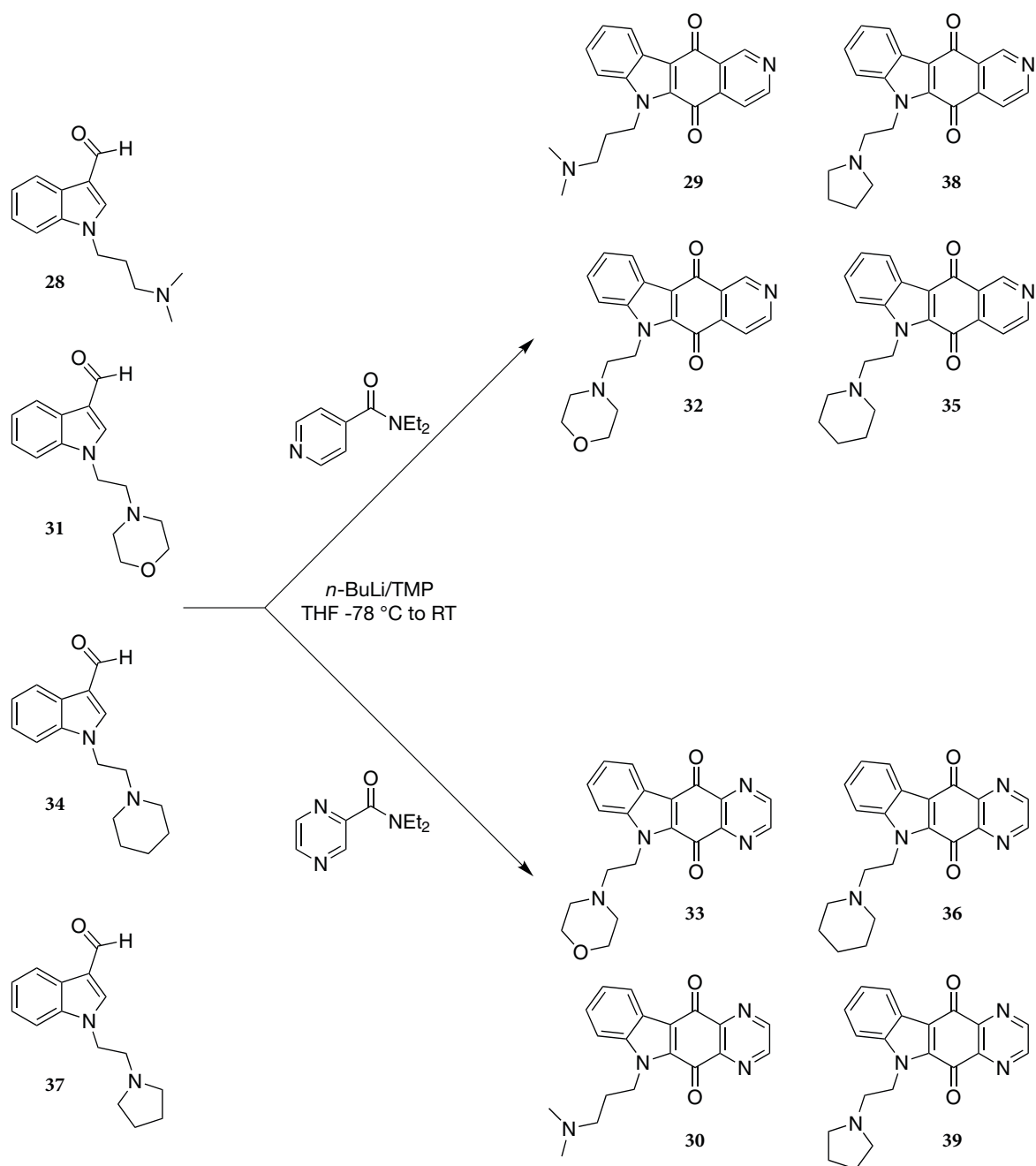
Scheme 12. General Procedure of the Cyclisation Step

3. 11 Synthesis of 5-[2-(dimethylamino)ethyl]-5*H*-indolo[2,3-*b*]phen-azine-6,13-dione (72)

The viability assay of the first set of compounds showed high activity of the tetracylic pyrazine derivative **26** against SW480 cells (IC_{50} = 0.492 μM), but low activity of the pentacyclic derivatives **60** and **76** (IC_{50} >10 μM). Hence we were interested in the activity of the pentacyclic pyrazine derivative **72**. The synthetic pathway is depicted in *Scheme 13*.

The synthesis was accomplished by the preparation of amide **69** in three steps and cyclisation with functionalised aldehyde **27**. The pentacyclic framework was prepared in the same way by cyclisation with MOM-protected aldehyde **8** and subsequent BBr₃ effected MOM cleavage of **70** to afford **71**.
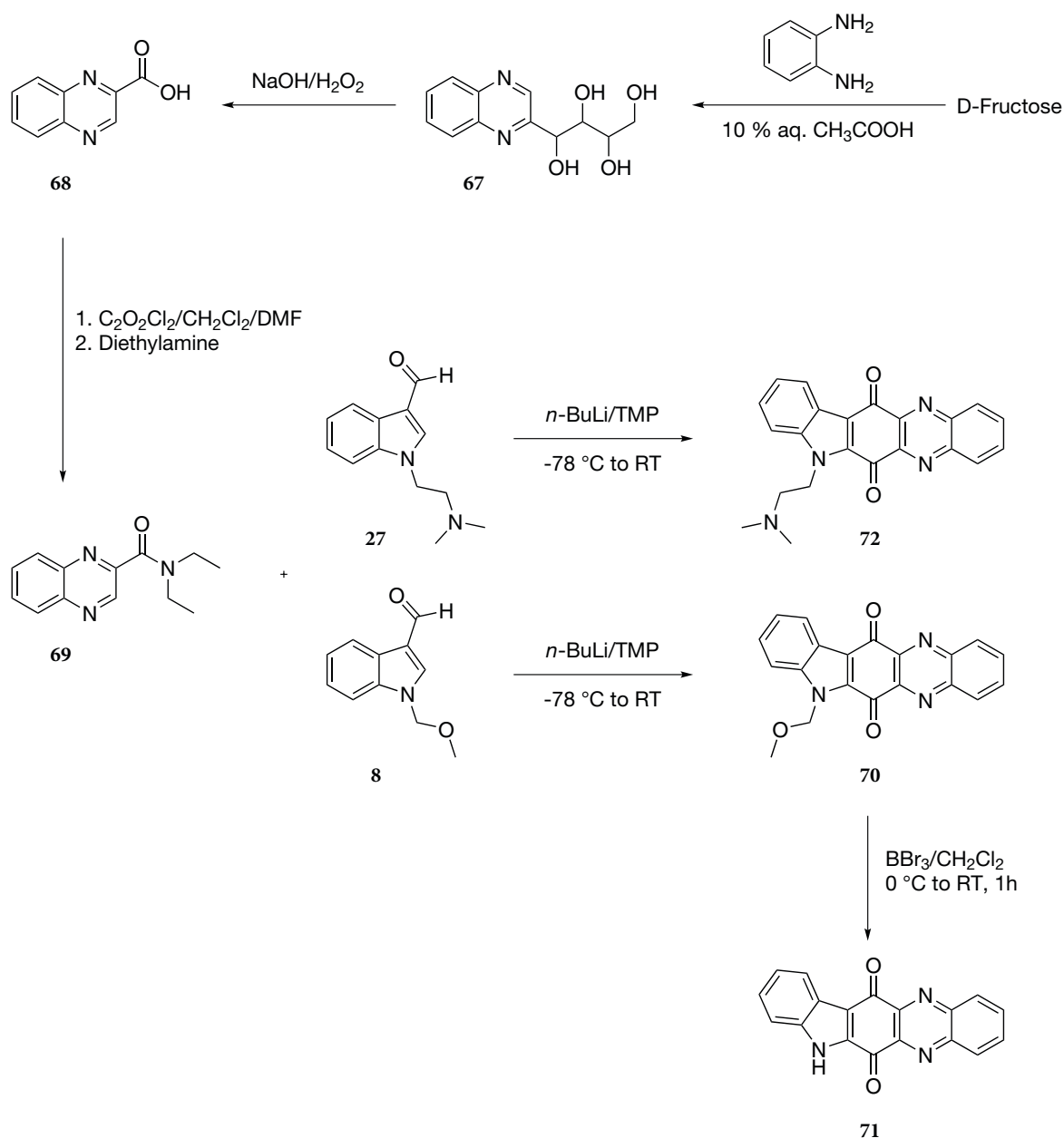
Scheme 13. Synthesis of 71

Building block **69** was prepared from D-fructose in three steps.[132, 95] D-Fructose was subjected to condensation with 1,2-benzenediamine under acidic conditions, followed by oxidation with H_2O_2/ NaOH. The amidation of the carboxylic acid function was achieved following the routine procedure *via* intermediary acid chloride and reacting with diethylamine. The identity of all intermediates and the target compound was confirmed *via* NMR spectroscopy and HRMS and are referenced in the experimental section.

3. 12 Synthesis of 6-[2-(dimethylamino)ethyl]-5*H*-pyrido[4,3-*b*]car-bazole-5,11(6*H*)-dione 2-oxide (22)

The biological evaluation revealed the significantly higher activity of calothrixin A derivative **66** endowed with *N*-oxide function than calothrixin B derivative **65** lacking thereof. This prompted us to investigate if its corresponding tetracyclic counterpart would also benefit from a pyridine *N*-oxide moiety. Thus, we synthesised **22** in analogy to **66** by reacting derivative **19** with *m*-CPBA to afford *N*-oxide **21** in over 90 % yield, followed by alkylation with 2-chloro-*N,N*-dimethylethanamine hydrochloride to give the target compound as orange crystals (**Scheme 14**). As expected a shift of δ to lower frequency of N-2 from -51.0 ppm to -76.9 ppm (for compound **21**) and from 55.0 ppm to 77.7 ppm (for compound **22**) was apparent, running completely parallel to the pyridine → pyridine *N*-oxide transition (approximately 23 ppm).[139] As in the ^{13}C NMR spectra of *N*-oxides **64** and **66**, the expected shift to lower frequency for the carbons in *ortho* and *para* positions to N-2 (C-1: -11.5/-11.5 ppm, C-3: -12.8/-12.6 ppm, C-4a: -11.0/-10.7 ppm) could be observed (relative to the corresponding shifts in the parent compounds **19** and **20**). A full NMR and HRMS spectroscopic analysis of **21** and **22** is referenced in chapters *6. 2. 22 on page 104 and 6. 2. 23 on page 105*.
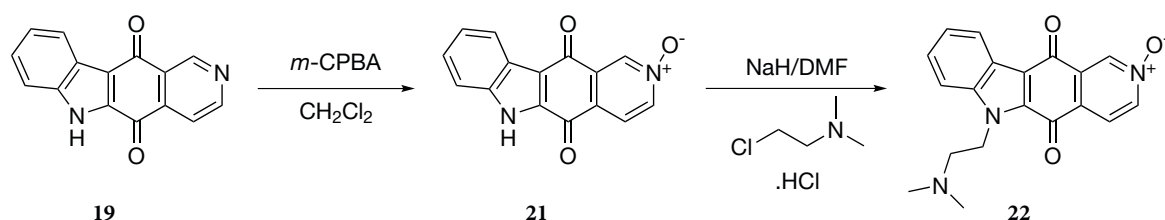
Scheme 14. *Synthesis of N-oxide derivative 22*

3. 13 Synthesis of 6-{2-[(2-hydroxyethyl)amino]ethyl}-5*H*-pyri-do[4,3-*b*]carbazole-5,11(6*H*)-dione (43)

Among the basic substitution pattern we were particularly interested in evaluating the effect of the 2-(2-hydroxyethylamino)-ethyl group which is an essential motif of our lead compound mitoxantrone. The free amino and hydroxy group of the side chain prohibits a simple alkylation preceding the metallation step. Instead, we envisaged the introduction of a chloroethyl group and subsequent S_N2 reaction in an alkaline milieu with 2-aminoethanol (**Scheme 15 on page 43**).
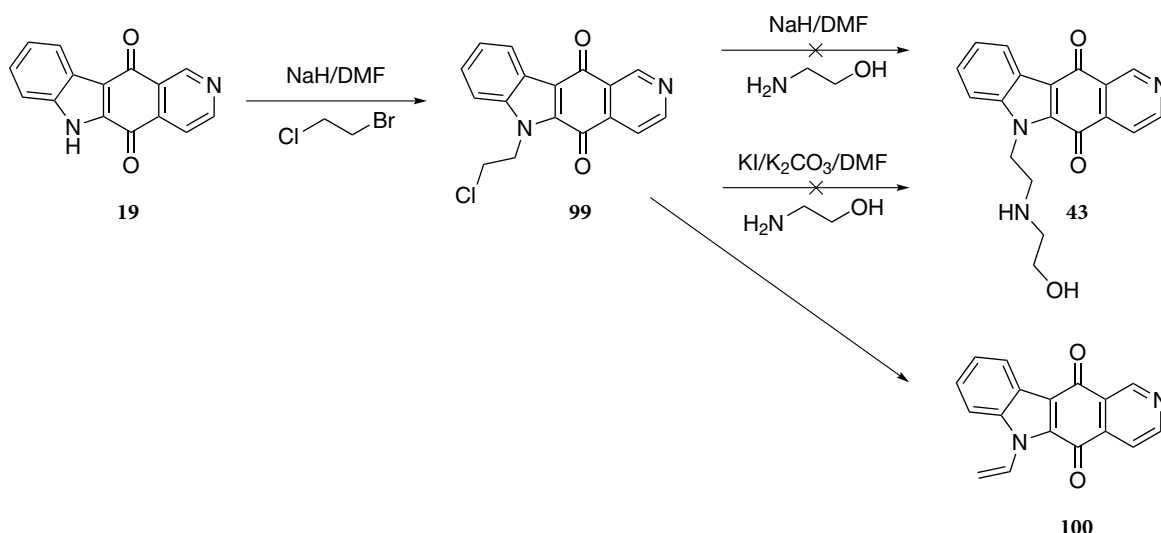
Scheme 15. *Synthesis of Olefine* **100**

This should be achieved by employing the routine procedure in DMF with sodium hydride and 1-bromo-2-chloroethane under moderate heating. The intermediary compound **99** was obtained in good yield as orange crystals, m.p. 235-237 °C. The identity was confirmed by NMR spectroscopy showing a consistent signal set for the ring system and two triplets indicating the successful introduction of the chloroethyl group. This finding was also confirmed by HRMS.
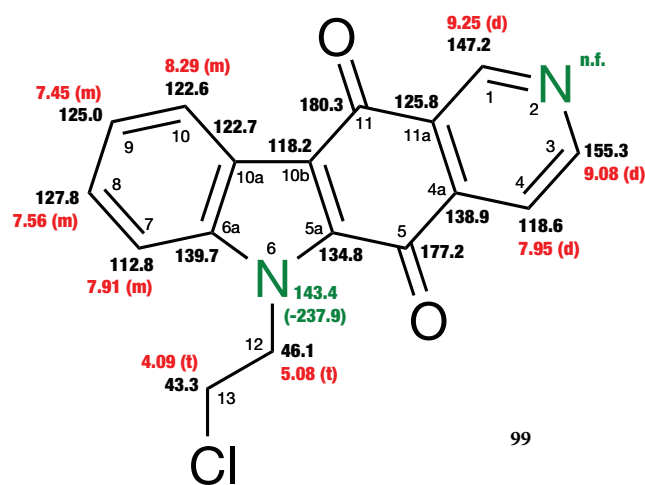
¹H NMR (400 MHz, DMSO-*d₆*) δ 9.25 (d, *J* = 0.7 Hz, H-1, 1H), 9.08 (d, *J* = 5.0 Hz, H-3, 1H), 8.29 (m, H-10, 1H), 7.95 (dd, *J* = 5.0 Hz, 0.7 Hz, 1H), 7.91 (m, H-7, 1H), 7.56 (m, H-8, 1H), 7.45 (m, H-9, 1H), 5.08 (t, *J* = 6.0 Hz, H-12, 2H), 4.09 (t, *J* = 6.0 Hz, H-13, 2H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 180.3 (C-11), 177.2 (C-5), 155.3 (C-3), 147.2 (C-1), 139.7 (C-6a), 138.9 (C-4a), 134.8 (C-5a), 127.8 (C-8), 125.8 (C-11a), 125.0 (C-9), 122.7 (C-10a), 122.6 (C-10), 118.6 (C-4), 118.2 (C-10b), 112.8 (C-7), 46.1 (C-12), 43.3 (C-13).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -237.9 (N-6), not found (N-2).

Intermediate **99** was then reacted with 2-aminoethanol and sodium hydride in DMF to substitute the chlorine of the side chain. However, the isolated product was not the desired target compound but was elucidated as 6-vinyl-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (***Figure 14 on page 44***). Evidently, the basic reaction conditions led to HCl elimination affording olefine **100** as orange crystals, m.p. 210-212 °C. The same phenomenon occurred on treating **99** with the less basic K₂CO₃ and KI as catalyst in DMF. This reaction might be favoured due to a conjugational effect of the vinyl group to the aromatic system.
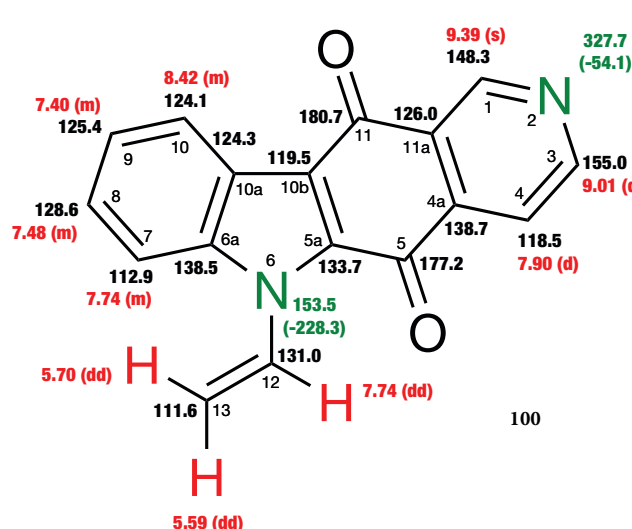
¹H NMR (400 MHz, CDCl₃) δ 9.39 (s, H-1, 1H), 9.01 (d, J = 5.0 Hz, H-3, 1H), 8.42 (m, H-10, 1H), 7.90 (d, J = 5 Hz, H-4, 1H), 7.74 (dd, J = 15.9, 8.8 Hz, H-12, 1H), 7.74 (m, H-7, 1H), 7.48 (m, H-8, 1H), 7.40 (m, H-9, 1H), 5.70 (dd, J = 15.9, 1.0 Hz, H-13, 1H), 5.59 (dd, J = 8.8, 1.0 Hz, H-13, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 180.7 (C-11), 177.2 (C-5), 155.0 (C-3), 148.3 (C-1), 138.7 (C-4a), 138.5 (C-6a), 133.7 (C-5a), 131.0 (C-12), 128.6 (C-8), 126.0 (C-11a), 125.4 (C-9), 124.3 (C-10a), 124.1 (C-10), 119.5 (C-10b), 118.5 (C-4), 112.9 (C-7), 111.6 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -54.1 (N-2), -228.3 (N-6).

Figure 14. NMR Assignment of Olefine 100

3. 13. 1 Synthesis of 3-(2-chloroethyl)-1,3-oxazolidin-2-one (41)
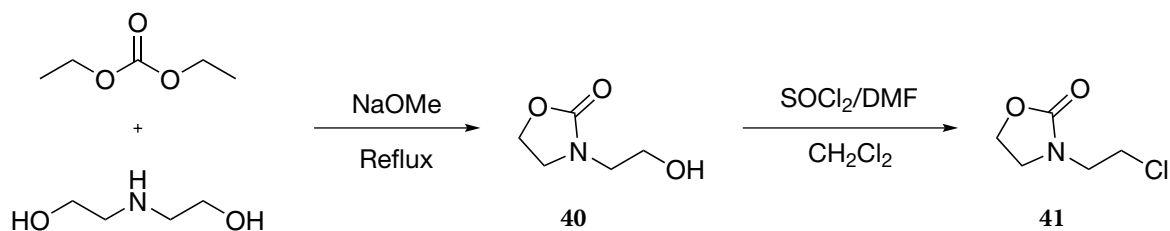
Finally we introduced the side chain masked as carbamate with subsequent alkaline hydrolysis. This procedure was performed according to the procedure by Leepasert.[130] For that intention building block 3-(2-hydroxyethyl)-1,3-oxazolidin-2-one (**40**) had to be prepared which was achieved by reacting 2,2'-iminodiethanol with diethyl carbonate and sodium methoxide[131] to afford intermediate **40** in almost quantitative yield. Subsequent treatment with thionylchloride in dry dichloromethane and a small amount of absolute DMF converted the alcohol into desired halide **41**.[131] NMR spectroscopic information of **40** and **41** is according to literature and referenced in the experimental section in chapters ***6. 2. 41 on page 123*** and ***6. 2. 42 on page 124***.

3. 13. 2 Synthesis of 6-[2-(2-oxo-1,3-oxazolidin-3-yl)ethyl]-5*H*-pyrido[4,3-*b*] carbazole-5,11(6*H*)-dione (42)

19   **41**   **42**
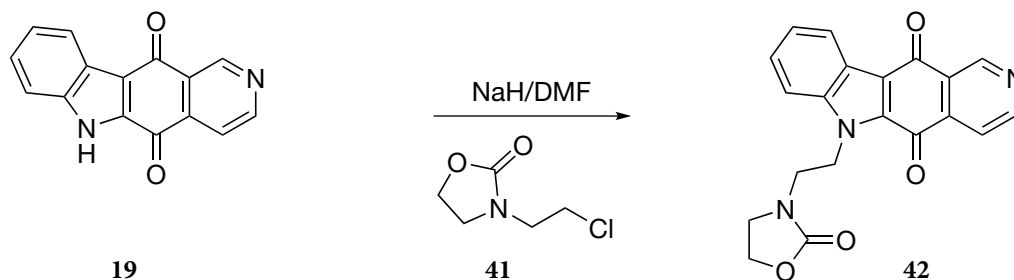
In the following compound **19** was treated with sodium hydride in dry DMF in the presence of **41**. Surprisingly, heating at 70 °C for 6 d was necessary to obtain the target compound in good yields as bright red needles.

¹H NMR spectrum of **42** shows pseudo-triplets at 3.32 ppm and 4.09 ppm which are assigned to the methylene protons of the oxazolidine ring whereas the triplets at 4.92 ppm (J = 6.3 Hz, 2H) and 3.75 ppm (J = 6.3 Hz, 2 H) are attributed to the side chain (***compare 6. 2. 43 on page 125***).

Since the carbamate moiety is susceptible to enzymatic hydrolysis compound **42** should act as precursor thus releasing active compound **43**. In order to test this hypothesis both compounds should be evaluated in the viability assays to compare their potency. However, it turned out, that precursor **42** was considerably weaker after 24 h treatment on SW480 cells. Prolonged treatment for 48 h and 72 h showed no significant improvement of cytotoxic activity.

3. 13. 3 Alkaline Carbamate Hydrolysis of 42
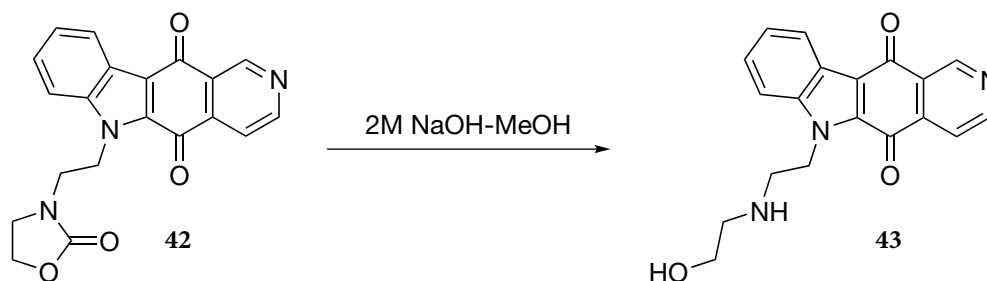
The carbamate hydrolysis was performed under harsh conditions by refluxing **42** in methanolic 2M NaOH furnishing only 11 % of **43.** Employing only 0.5 M NaOH required longer reaction times to convert the starting material, thus having the same detrimental effect in terms of yield. Moreover, the purification process of **43** was tedious due to the high polarity of the attached side chain which forced us to apply RP column chromatography to purify the product. The identity of **43** was confirmed by NMR and HRMS analysis (***compare 6. 2. 44 on page 126***).

3. 14 Synthesis of 6-[2-(2-oxo-1,3-oxazolidin-3-yl)ethyl]-5*H*-pyra-zino[2,3-*b*]carbazole-5,11(6*H*)-dione (44)

The synthesis of the carbamate bearing pyrazine derivative **44** was accomplished in analogy to **42**. The NMR spectroscopic characterisation is referenced in chapter *6. 2. 45 on page 127.*
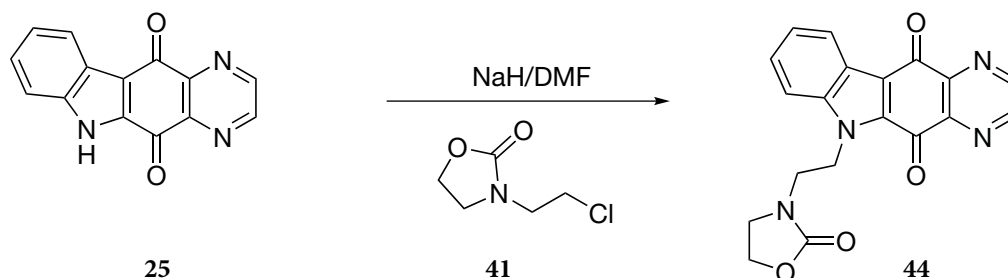
Due to the poor yields of **43** and the associated difficulties in the purification process we refrained from the carbamate hydrolysis in the case of **44**. Instead, we postponed our efforts awaiting the results from the biological tests.

The successful introduction of a mesyl group and the substitution with aziridine in acceptable yields during our ongoing research prompted us to explore this methodology further with the prospect of opening a more practical approach to the 2-(2-hydroxyethylamino)-ethyl substituted pyrazine derivative. This needs to be investigated in future works.
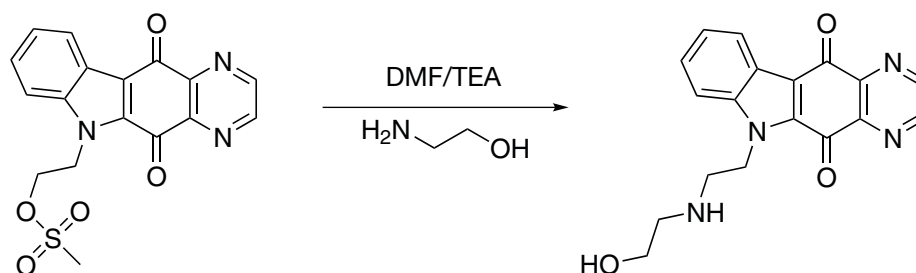
3. 15 Attempted Synthesis of the Isomeric Pyrimidocarbazolediones

For the construction of the first of two possible pyrimidine-annulated carbazoledion derivatives we envisaged the construction *via* the proven *ortho* lithiation strategy. Already available MOM-protected indole-3-carbaldehyde **8** should be cyclised with amide **87** to give the MOM-protected framework which should then be converted into the desired compound in two steps as depicted in *Scheme 16 on page 47*.

3. 15. 1 Attempted Synthesis *via* Directed *Ortho* Lithiation
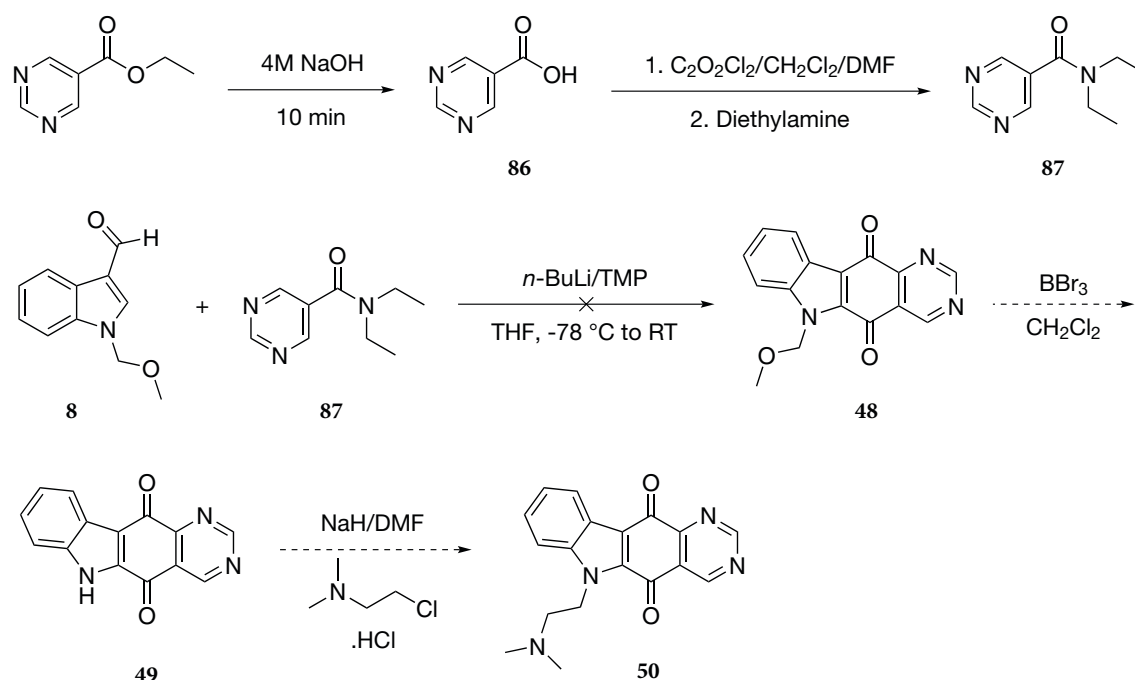
Scheme 16. *Attempted Synthesis of **50** via Directed Ortho Lithiation*

Amide **87** was prepared starting from commercially available ethyl 5-pyrimidinecarboxylate which was subjected to alkaline ester hydrolysis using 4M NaOH following the procedure of Maier and co-workers.[97] The free carboxylic acid could be obtained in over 80 % yield and was used in the next step without purification. The modified procedure by Gilman and Spatz[95] was used to convert **86** in its corresponding amide **87** *via* S_N2t reaction by treatment with oxalyl chloride and diethylamine in dichloromethane yielding 67 % as a yellow oil. The identity of the product was confirmed *via* NMR spectroscopy.
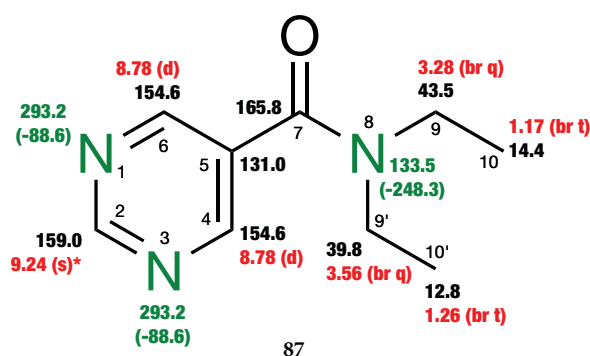
^{1}H NMR (400 MHz, CDCl₃) δ 9.24 (d after Gauss multiplication, *J* = 0.7 Hz, H-2, 1H), 8.78 (d, *J* = 0.7 Hz, H-4/6, 2H), 3.56 (br q, H-9', 2H), 3.28 (br q, H-9, 2H), 1.26 (br t, H-10', 3H), 1.17 (br t, H-10, 3H).

^{13}C NMR (100 MHz, CDCl₃) δ 165.8 (C-7), 159.0 (C-2), 154.6 (C-4/6), 131.0 (C-5), 43.5 (C-9), 39.8 (C-9'), 14.4 (C-10), 12.8 (C-10').

^{15}N NMR (40 MHz, CDCl₃) δ -88.6 (N-1/3), -248.3 (N-8).

Amide **87** should then be reacted with MOM-protected aldehyde **8** in a *ortho* directed metallation reaction. Unfortunately, after the usual workup procedure and purification by column chromatography only the starting materials could be recovered along with many tarry side products. Adding an additional eq. LiTMP didn't furnish the desired product. Reacting the educts at -100 °C in a bath of liquid nitrogen/ethanol was also not successful. Subsequently, this route was abandoned in favour of the following 7-step synthesis as depicted in *Scheme 17*.

3. 15. 2 Attempted Synthesis *via* Palladium-mediated Biaryl Coupling Reaction[141, 143]
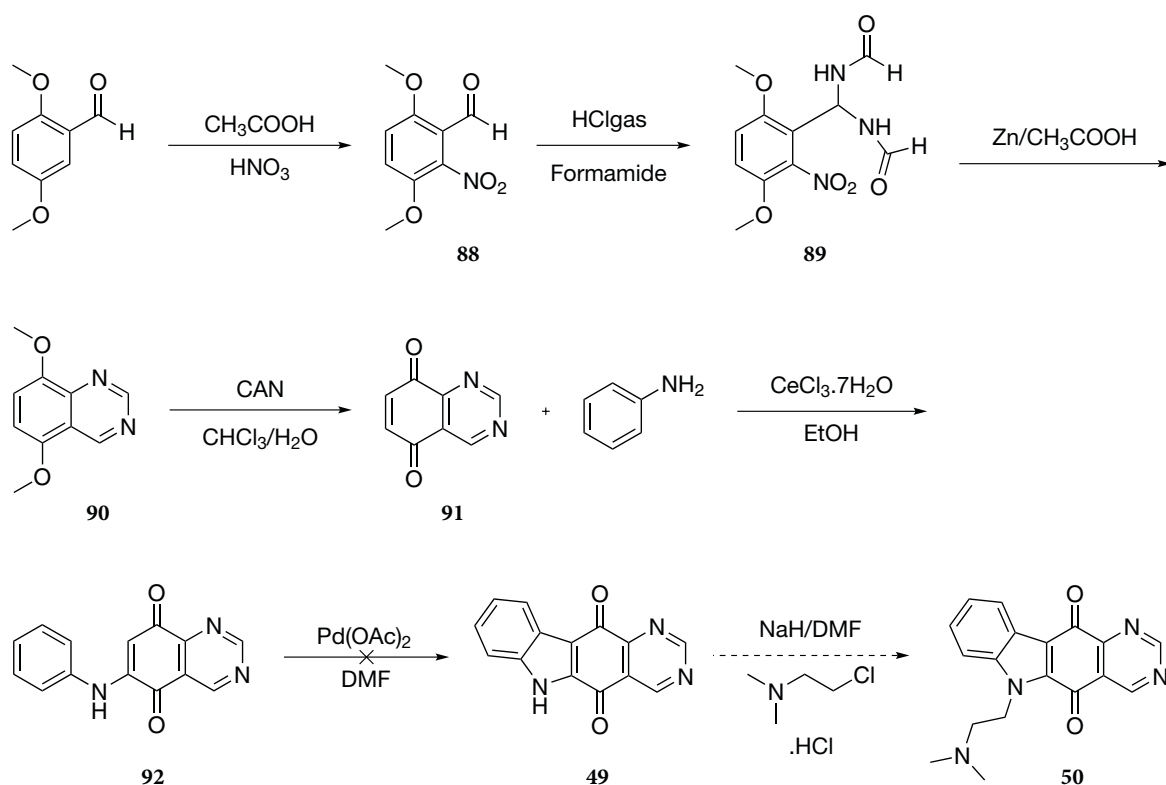
Scheme 17. Palladium-mediated Biaryl Coupling

In contrast to our previous strategy, the requisite quinone system should be constructed first which should allow us to effect the coupling of aniline; the subsequent ring-closure step should be realised in a palladium(II) acetate mediated biaryl C-C coupling reaction to afford the desired nucleus.
The methods of Shen et al.[98] and Groszek et al.[99] were adapted to prepare the 2-nitrobenzaldehyde derivative **88** by treating a solution of 2,5-dimethoxybenzaldehyde in glacial acetic acid at 0 °C with nitric acid (1 to 1.5 mol-eq.). After the workup procedure and rerystallisation from dichloromethane the desired 2-nitro isomer could be obtained as yellow needles, m.p. 163-165 °C. The mother liquor containing additional product could be separated from the undesired 4-nitro isomer by column chromatography eluting with ethyl acetate/hexane (1:1, v/v). Both isomers could be discriminated *via* NMR spectroscopy. In particular, for the 2-nitro isomer the aromatic protons H-4 and H-5 can be observed as two doublets at 7.28 ppm and 7.11 ppm (J = 9.3 Hz), in contrast to the protons H-2 and H-5 of the 4-nitro derivative which give two singlets. Moreover, the spatial proximity of the methoxy protons (H-8 and H-9) to the adjacent aromatic protons H-4 and H-5 is apparent in the NOE spectrum.

Structure 88 (NMR annotations):
3.86 (s) · 57.2 · 8
NOE
7.28 (d) · 120.0
144.5
6 · 1 · 116.1 · 7 · 186.0
H · 10.35 (s)
5
114.1 · 4 · 2 · 138.6
3
7.11 (d) · 155.3 · NO₂
NOE
56.7 · 9
3.94 (s)
88
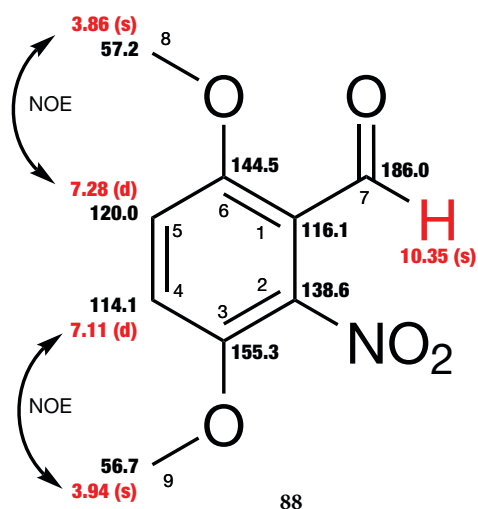
¹H NMR (400 MHz, CDCl₃) δ 10.35 (s, H-7, 1H), 7.28 (d, *J* = 9.3 Hz, H-5, 1H), 7.11 (d, *J* = 9.3 Hz, H-4, 1H), 3.94 (s, H-9, 3H), 3.86 (s, H-8, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 186.0 (C-7), 155.3 (C-3), 144.5 (C-6), 138.6 (C-2), 120.0 (C-5), 116.1 (C-1), 114.1 (C-4), 57.2 (C-8), 56.7 (C-9).

Following the procedure of Shaikh et al.[100] 3,6-dimethoxy-2-nitrobenzaldehyde was converted into its respective aminal **89** under stirring in formamide in the presence of dry HCl gas.

Structure 89 (NMR annotations):
3.86 (s) · 56.7 · 8
8.67 (d) · 10 · 160.2 · 11
HN · H · 7.91 (dd)
150.8 · 48.5 · H · 6.77 (t)
7.26 (s) · 114.3 · 6 · 1 · 119.6 · 9
NH · 8.67 (d)
5 · 10'
113.9 · 4 · 2 · 139.6 · 11' · H · 7.91 (dd)
7.26 (s) · 3 · 160.2
143.6 · NO₂ · O
57.0 · 7
3.80 (s)
89
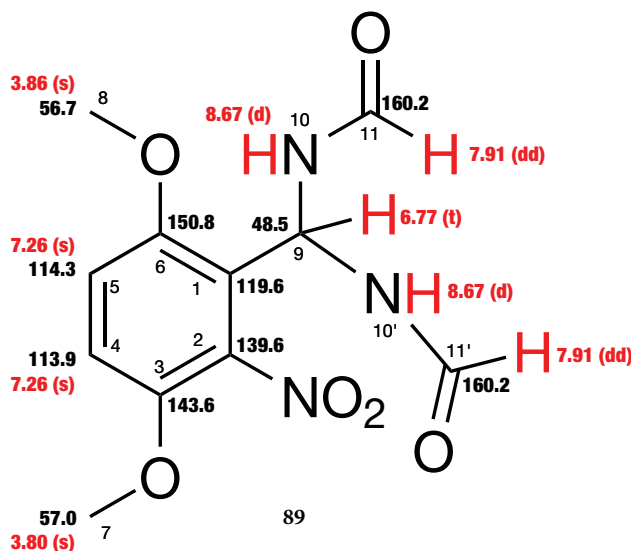
¹H NMR (400 MHz, DMSO-*d₆*) δ 8.67 (d, *J* = 8.1 Hz, H-10/10', 2H), 7.91 (dd, *J* = 1.4, 0.9 Hz, H-11/11', 2H), 7.26 (s, H-4/5, 2H), 6.77 (t, *J* = 8.1 Hz, H-9, 1H), 3.86 (s, H-8, 3H), 3.80 (s, H-7, 3H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 160.2 (C-11/11'), 150.8 (C-6), 143.6 (C-3), 139.6 (C-2), 119.6 (C-1), 114.3 (C-5), 113.9 (C-4), 57.0 (C-7), 56.7 (C-8), 48.5 (C-9).

Subsequently, crude intermediate **89** was subjected to reductive cyclisation with Zn metal in glacial acetic acid to give 5,8-dimethoxyquinazoline (**90**) as pale yellow crystals, m.p. 116-117 °C. ¹H NMR data is in agreement with literature[100] and was amended by ¹³C and ¹⁵N NMR spectra.

90 should be converted into its respective quinone by oxidative demethylation with ceric ammonium nitrate (CAN) in a mixture of acetonitril/water, described by Potts et al.[101] in 1986. However, following this procedure we couldn't reproduce their results. The product could only be obtained in 3 % yield in unsatisfying grade of purity. Adjusting the reaction parameters by using different eq. of CAN, varying reaction times and/or temperature did not have any impact on the results. Most interestingly, in two later publications[102, 103] a different research group has prepared **90** *via* oxidation of the intermediate 5,8-quinazolinediol employing Fremy's Salt (disodium nitrosodisulfonate) or K₂Cr₂O₇ as oxidising agents. This implicates the insertion of an additional reaction step, because 5,8-quinazolinediol in turn had to be prepared from **90** by heating with AlCl₃.
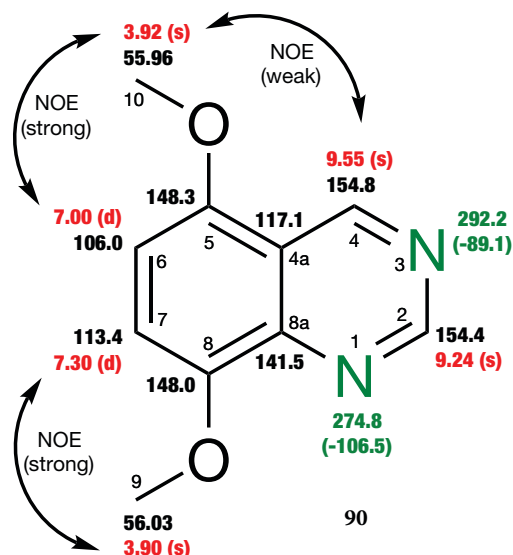
¹H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, H-4, 1H), 9.24 (s, H-2, 1H), 7.30 (d, J = 8.7 Hz, H-7, 1H), 7.00 (d, J = 8.7 Hz, H-6, 1H), 3.92 (s, H-10, 3H), 3.90 (s, H-9, 3H).

¹³C NMR (100 MHz, DMSO-d_6) δ 154.8 (C-4), 154.4 (C-2), 148.2 (C-5), 147.9 (C-8), 141.5 (C-8a), 117.1 (C-4a), 113.4 (C-7), 106.0 (C-6), 56.03 (C-9), 55.96 (C-10).

¹⁵N NMR (40 MHz, DMSO-d_6) δ -89.1 (N-3), -106.5 (N-1).

This step could be circumvented by preparing **91** following a modified procedure[104] by Ubeda et al.[105] which involved oxidative demethylation of **90** with CAN in a biphasic mixture of chloroform/water (1:2, v/v). Chloroform was substituted with dichloromethane due to its lower carcinogenic potential. After stirring **90** in a mixture of dichloromethane/water (1:2, v/v) for 1 h at room temperature in the presence of three eq. CAN the organic layer was collected and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water and the solvent was evaporated to afford **91** without further purification as brown crystals of high purity in 80 to 90 % yield, m.p. 113-115 °C.
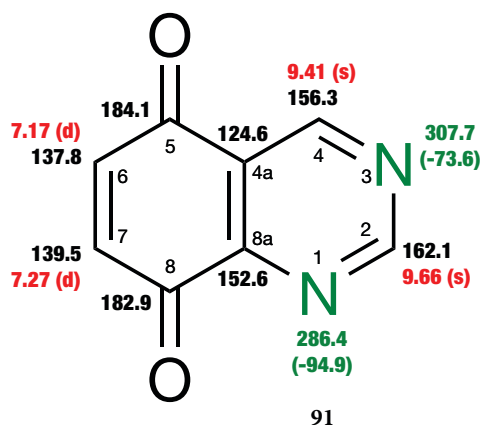
¹H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, H-2, 1H), 9.41 (s, H-4, 1H), 7.27 (d, J = 10.5 Hz, H-7, 1H), 7.17 (d, J = 10.5 Hz, H-6, 1H).

¹³C NMR (100 MHz, DMSO-d_6) δ 184.1 (C-5), 182.9 (C-8), 162.1 (C-2), 156.3 (C-4), 152.6 (C-8a), 139.5 (C-7), 137.8 (C-6), 124.6 (C-4a).

¹⁵N NMR (40 MHz, DMSO-d_6) δ -73.6 (N-3), -94.4 (N-1).

A regioselective addition[102, 104] of aniline to the prepared quinone at position 6 was achieved by stirring **91** in ethanol in presence of the lewis acid cerium(III)chloride heptahydrate at room temperature for 24 h. The reaction was quenched by pouring onto ice-cold water that was acidified with 3 % acetic acid. The product was collected by suck-filtration and purified by column chromatography eluting with dichloromethane followed by column chromatography eluting with ethyl acetate/light petroleum (8:2, v/v) to give 34 % of 6-anilino-5,8-quinazolinedione (**92**) as purple crystals, m.p. 209-211 °C, which should be subjected to a palladium catalysed biaryl coupling reaction.[143]

The palladium acetate promoted cyclodehydrogenation procedure was based on the works of Sridharan et al.,[107] Knoelker et al.,[110], Choi et al.,[109] and Liegault et al.[108] using catalytic amounts of palladium for the synthesis of various carbazole-based compounds.
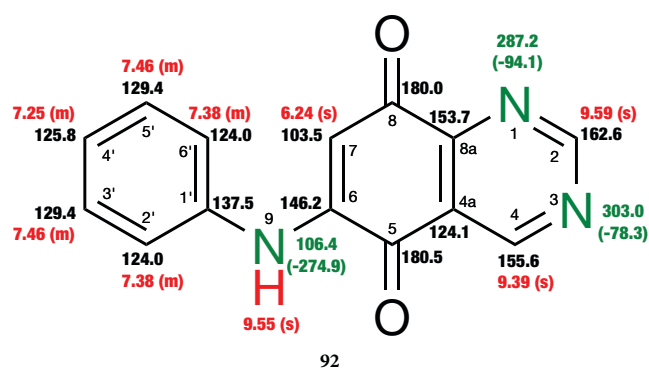
¹H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, H-2, 1H), 9.55 (s, H-9, 1H), 9.39 (s, H-4, 1H), 7.46 (m, H-3'/5', 2H), 7.38 (m, H-2', 1H), 7.25 (m, H-4', 1H), 6.24 (s, H-7, 1H).

¹³C NMR (100 MHz, DMSO-d_6) δ 180.5 (C-5), 180.0 (C-8), 162.6 (C-2), 155.6 (C-4), 153.7 (C-8a), 146.2 (C-6), 137.5 (C-1'), 129.4 (C-3'/5'), 125.8 (C-4'), 124.1 (C-4a), 124.0 (C-2'/6'), 103.5 (C-7).

¹⁵N NMR (40 MHz, DMSO-d_6) δ -78.3 (N-3), -94.1 (N-1), -274.9 (N-9).

First, a microwave-assisted approach[107] was attempted by treating **92** with Pd(OAc)₂ (0.4 eq.) and Cu(OAc)₂ (2 eq.) as co-oxidant in DMF in a microwave reactor (100 W, 130 °C, 60 min). Unfortunately, the reaction was unsuccessful and the starting material was decomposed. Shorter reaction times or prolonged reaction times at reduced microwave power had no influence on the result. In any case either the starting material was destroyed or no reaction at all could be observed.
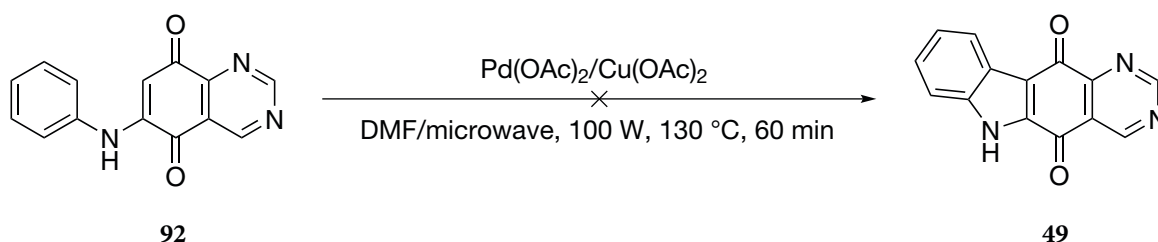
Next we chose a conventional reaction setup following a procedure by Norcott and co-workers.[111] **92** was refluxed with Pd(OAc)₂ (1 eq.) and Cu(OAc)₂ (1 eq.) in glacial acetic acid. The reaction process was monitored by TLC. However, the reaction was unsuccessful, leading to the decomposition of the educt. By lowering the temperature no reaction was observed.
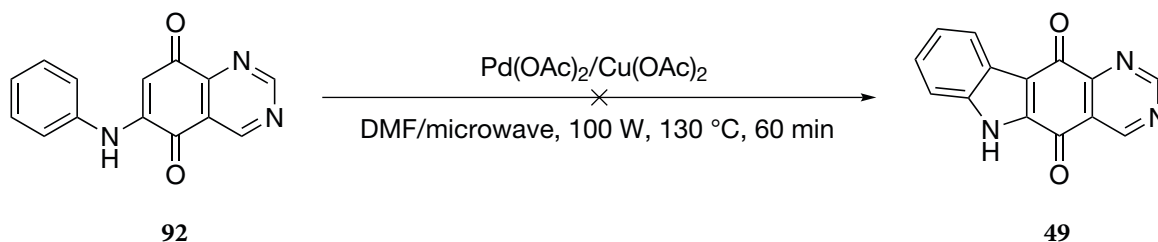
Liegault et al.[108] reported the intramolecular Pd(II)-catalysed oxidative biaryl synthesis under air with substituting acetic acid with 2,2-dimethylpropionic acid (pivalic acid, PivOH), which gives the prospect of not only increasing the reactivity but also improving the selectivity for the formation of the carbazole product. Previous work in their group has also demonstrated that PivOH can play an important role as a co-catalyst in Pd(0)-catalysed benzene arylation.[112] Schultz et al.[113] have also demonstrated the efficiency of the pivaloyl moiety for palladium-catalysed aerobic oxidation of alcohols. In both cases, the pivalate ligand is proposed to play a key role during the C-H bond cleavage.

Moreover, in contrast to many reactions performed in glacial acetic acid, little secondary oxidative byproduct can be detected in the crude reaction mixtures with PivOH.

6-anilino-5,8-quinazolinedione (**92**) was heated at 60 °C with Pd(OAc)$_2$, Cu(OAc)$_2$ and K$_2$CO$_3$ in PivOH under air for 12 h. TLC monitoring showed no reaction progress which prompted us to increase the temperature to 90 °C. Still, after continued stirring of 2 d no reaction progress could be observed.
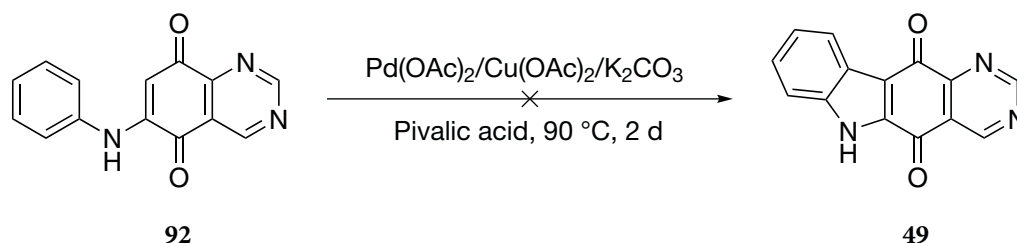
Consequently, this route was abandoned in favour of the pathway outlined in *scheme 18*, used by Bernardo et al.[114] as synthetic pathway for calothrixin B.

3. 15. 3   Attempted Synthesis *via* Cadogan Reaction[141]

Since there are many reports on the synthesis of carbazoles using the Cadogan reaction[116, 117] we envisaged that an appropriate Cadogan precursor will allow access to the pyrimido[5,4-*b*]carbazoledione skeleton (*Scheme 18*). Hence, the nitro derivative should be synthesised using nitrophenylboronic acid in a Suzuki coupling reaction. The nitrophenylquinazoline should then be cyclisised to the target system in a microwave assisted Cadogan reaction which refers to a deoxygenation of aromatic nitro-compounds by triethyl phosphite and subsequent cyclisation of the resulting intermediate nitrene.[118]
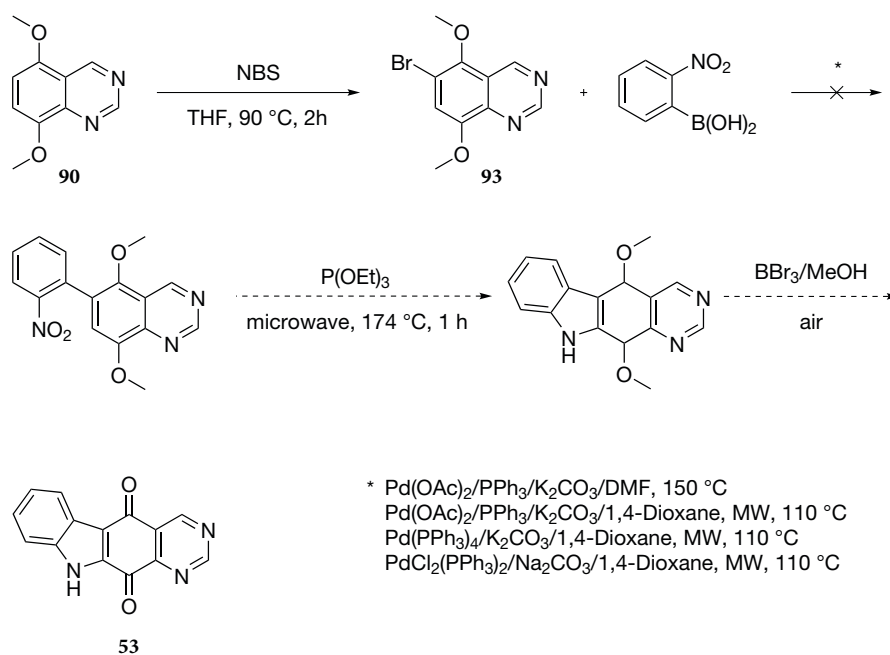
Scheme 18. Attempted Synthesis of 53 via Cadogan Reaction

6-Bromo derivative **93** was furnished by refluxing **90** with *N*-bromosuccinimide (2 eq.) in dry THF for 2 h following a procedure described by Song and co-workers.[119] Under these conditions only the 6-bromo derivative could be obtained as brown crystals, m.p. 125-127 °C, which could be determined by NMR spectroscopy and HRMS. In particular, a correlation between the H-7 proton and the -OCH₃ protons at C-8 can be observed in the NOESY experiment, whereas the -OCH₃ protons at C-5 indicate the spatial proximity only to the pyrimidine H-4 proton.
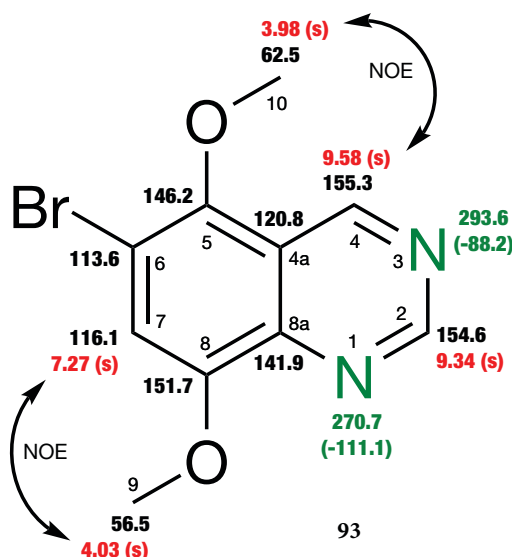
¹H NMR (400 MHz, CDCl₃) δ 9.58 (s, H-4, 1H), 9.34 (s, H-2, 1H), 7.27 (s, H-7, 1H), 4.03 (s, H-9, 3H), 3.98 (s, H-10, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 155.3 (C-4), 154.6 (C-2), 151.7 (C-8), 146.2 (C-5), 141.9 (C-8a), 120.8 (C-4a), 116.1 (C-7), 113.6 (C-6), 62.5 (C-10), 56.5 (C-9).

¹⁵N NMR (40 MHz, CDCl₃) δ -88.2 (N-3), -111.1 (N-1).

The 6-bromo derivative should be reacted with (2-nitrophenyl)boronic acid in a Suzuki coupling to yield the required Cadogan precursor. The reaction was carried out following the method of Bernardo et al.[114] by heating **93** with Pd(OAC)₂, PPh₃ and K₂CO₃ in DMF at 150 °C for 16 h. After the workup procedure and NMR spectroscopic investigation it became evident that the reaction failed and only starting material **93** and the debrominated precursor **90** could be recovered.
The reaction was repeated in the microwave reactor using 1,4-dioxane as solvent and heating only to 110 °C at 150 W, but no conversion into the desired compound could be observed.
Then we followed the procedure developed by Prabakaran et al.[115] in a microwave assisted ligand-free Suzuki coupling using PdCl₂(PPh₃)₂ and Na₂CO₃ as base in 1,4-dioxane. This reactions also failed and only led to debromination of the starting material. Replacing PdCl₂(PPh₃)₂ with Pd(PPh₃)₄ as catalyst was also unsuccessful.

In the following attempt we used the less reactive 6-chloro derivative with the boronic acid assuming it wouldn't lead to a dehalogenation. The 6-chloro derivative could be prepared in analogy to **93** with *N*-chlorosuccinimide in dry THF. When reacting it with (2-nitrophenyl)boronic acid no dechlorination could be observed, neither could any other reaction. PdCl₂(PPh₃)₂ or Pd(PPh₃)₄ served as palladium catalysts, Na₂CO₃ or K₂CO₃ was used as base, and different solvents were used (DMF or 1,4-dioxane). All reactions were heated by conventional means, but in all setups only the starting material could be recovered. As a consequence this route was discarded in favour to the one outlined in ***Scheme 19 on page 54***.
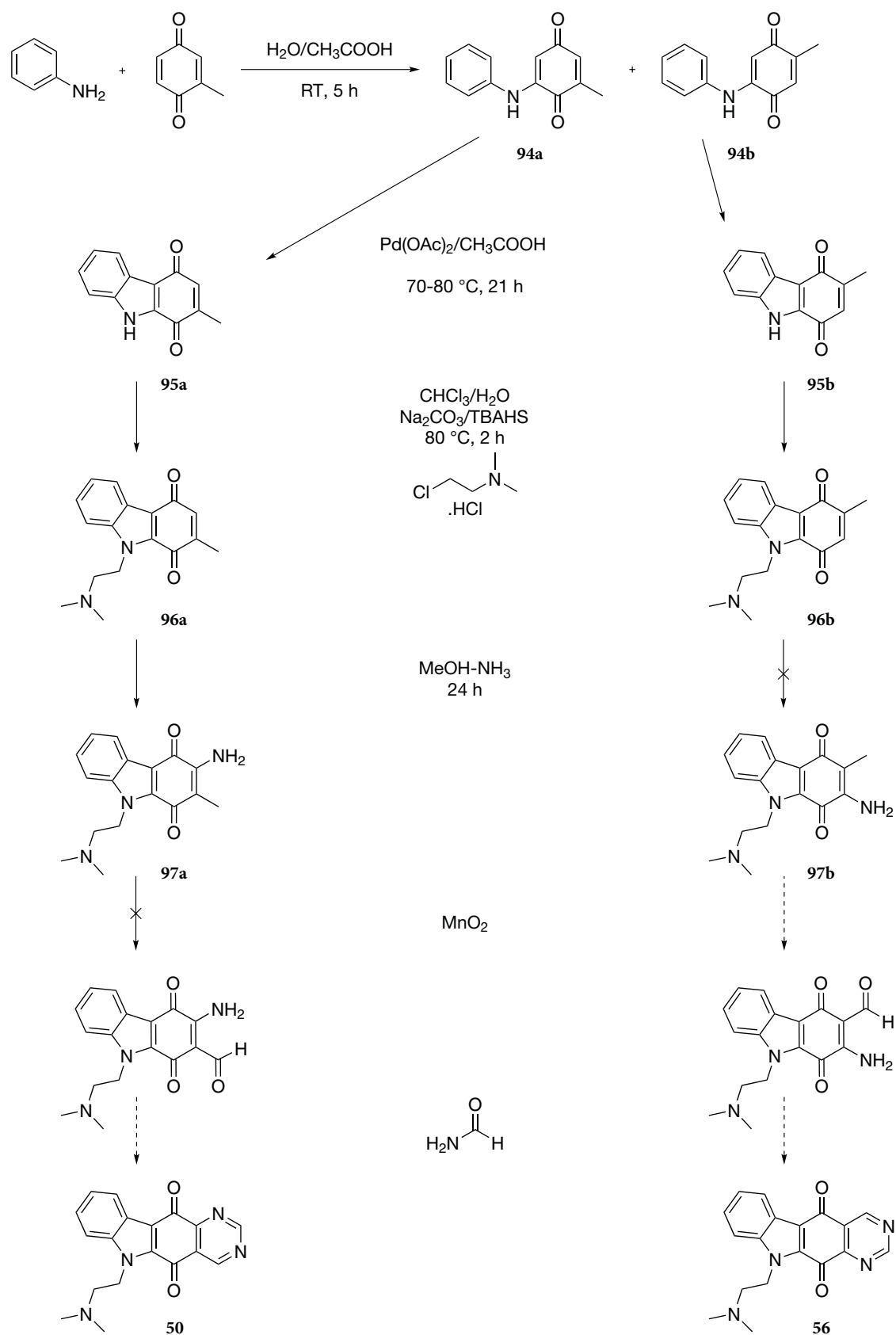
Scheme 19. *Attempted Synthesis of 50 and 56 via Condensation Reaction*

3. 15. 4   Attempted Synthesis *via* Condensation Reaction[141]

This strategy employed the construction of a carbazole skeleton followed by condensation to form the pyrimidine unit. Hence it was necessary to introduce both amino and aldehyde function in positions 5 and 6 of the quinone system. The first step of this route comprised a non-regioselective addition of aniline to 2-methyl-1,4-benzoquinone, in which both isomers should be accessible. This procedure was described by Yogo et al.[120] and was based on the works of Jacini.[121] Aniline was added to 2-methyl-1,4-benzoquinone in a mixture of acetic acid/water under stirring at 50 °C to furnish both isomers **94a,** m.p. 138-140 °C and **94b,** m.p. 144-145 °C as violet crystals in a ratio of 1:1.7 (31 % and 53 %, respectively). The ^{1}H and ^{13}C NMR spectra of both isomers show very similar signal sets, which made it impossible to discriminate between them based on 1D spectra. Only 2D-NMR techniques such as HMBC allowed an unambiguous signal assignment for both compounds.

^{1}H NMR (400 MHz, CDCl₃) δ 7.37 (m, H-3'/5', 2H), 7.31 (br s, H-8, 1H), 7.19 (m, H-2'/6', 2H), 7.17 (m, H-4', 1H), 6.51 (dq, J = 2.5, 1.6 Hz, H-5, 1H), 6.12 (d, J = 2.5 Hz, H-3, 1H), 2.07 (d, J = 1.6 Hz, H-7, 3H).

^{13}C NMR (100 MHz, CDCl₃) δ 186.8 (C-4), 184.4 (C-1), 143.0 (C-2), 141.2 (C-6), 137.6 (C-1'), 135.9 (C-5), 129.6 (C-3'/5'), 125.3 (C-4'), 122.1 (C-2'/6'), 100.7 (C-3), 15.5 (C-7).

^{15}N NMR (40 MHz, CDCl₃) δ -285.2 (N-8).

^{1}H NMR (400 MHz, CDCl₃) δ 7.38 (m, H-3'/5', 2H), 7.29 (br s, H-8, 1H), 7.19 (m, H-2'/6', 2H), 7.17 (m, H-4', 1H), 6.55 (q, J = 1.6 Hz, 1H), 6.16 (s, H-3, 1H), 2.08 (d, J = 1.6 Hz, H-7, 3H).

^{13}C NMR (100 MHz, CDCl₃) δ 186.6 (C-4), 183.7 (C-1), 149.6 (C-5), 142.9 (C-2), 137.5 (C-1'), 129.6 (C-3'/5'), 129.2 (C-6), 125.3 (C-4'), 122.0 (C-2'/6'), 100.9 (C-3), 16.4 (C-7).

^{15}N NMR (40 MHz, CDCl₃) δ -285.1 (N-8).

The ring closure was realised by a palladium promoted oxidative biaryl coupling reaction following the procedure of Yogo et al.[120] **94a** was dissolved in glacial acetic acid and refluxed under argon atmosphere in the presence of equimolar amounts of Pd(OAc)₂. After the workup procedure yields were comparably low, furnishing only 20 % of **95a**.

The procedure of Liegault et al.[108] which was described before gave the prospect of higher yields by using pivalic acid (PivOH) instead of glacial acetic acid. **94a** and **94b**, respectively were suspended in PivOH, Pd(OAc)$_2$ (10 mol-%) and K$_2$CO$_3$ (10 mol-%) were added and the resulting mixture was heated at 110 °C for 14 h under air. However, after the workup and purification procedure yields had not significantly improved. Consequently, we evaluated the benefits of Cu(OAc)$_2$ as co-catalyst to reoxidise Pd(0) to Pd(II)[122]. After several reaction setups employing 50 mol-% Pd(OAc)$_2$ and stirring at 70 °C for 21 h yields could be raised to 47 % for **95a** and 60 % for **95b**. However, the additive Cu(Oac)$_2$ seemed to have no influence in terms of yield. Instead, the amount of glacial acetic acid used seemed to be relevant. 28 mL solvent per mmol starting material resulted in the highest yields.[141]

94a/94b were stirred with Pd(OAc)$_2$ (10 mol-%) in glacial acetic acid at 70 °C for 21 h under air. After removal of the solvent under reduced pressure, the products were purified by column chromatography eluting with dichloromethane followed by recrystallisation from ethyl acetate to give red crystals of **95a**, m.p. 237-239 °C (yielding 47 %) or **95b**, m.p. 240-242 °C (yielding 60 %).

95a
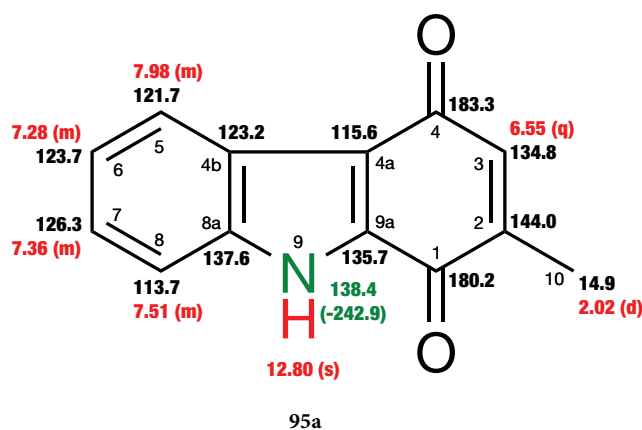
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (s, H-9, 1H), 7.98 (m, H-5, 1H), 7.51 (m, H-8, 1H), 7.36 (m, H-7, 1H), 7.28 (m, H-6, 1H), 6.55 (q, J = 1.6 Hz, H-3, 1H), 2.02 (d, J = 1.6 Hz, H-10, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3 (C-4), 180.2 (C-1), 144.0 (C-2), 137.6 (C-8a), 135.7 (C-9a), 134.8 (C-3), 126.3 (C-7), 123.7 (C-6), 123.2 (C-4b), 121.7 (C-5), 115.6 (C-4a), 113.7 (C-8), 14.9 (C-10).

^{15}N NMR (40 MHz, DMSO-d_6) δ -242.9 (N-9).

95b
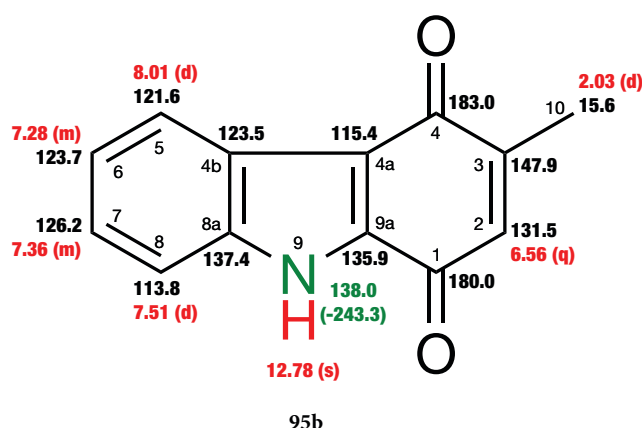
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, H-9, 1H), 8.01 (d, J = 8.0 Hz, H-5, 1H), 7.51 (d, J = 8.2 Hz, H-8, 1H), 7.36 (m, H-7, 1H), 7.28 (m, H-6, 1H), 6.56 (q, J = 1.6 Hz, H-2, 1H), 2.03 (d, J = 1.6 Hz, H-10, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0 (C-4), 180.0 (C-1), 147.9 (C-3), 137.4 (C-8a), 135.9 (C-9a), 131.5 (C-2), 126.2 (C-7), 123.7 (C-6), 123.5 (C-4b), 121.6 (C-5), 115.4 (C-4a), 113.8 (C-8), 15.6 (C-10).

^{15}N NMR (40 MHz, DMSO-d_6) δ -243.3 (N-9).

The next step comprised the functionalisation of the carbazole nitrogen of **95a** and **95b** following the procedure of Luo et al.[123] which was - in contrast to previous alkylations employing sodium hydride - carried out in a biphasic mixture of chloroform/water in the presence of the phase-transfer catalyst tetrabutylammonium hydrogensulfate, Na_2CO_3 and 2-chloro-*N*,*N*-dimethylethanamine hydrochloride. This mixture was vigorously stirred under reflux for 2 h. The reaction smoothly converted the starting material of both isomers into the desired compounds in good yields. The crude products could be easily isolated using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) yielding 91 % of **96a**, m.p. 109-11 °C and 67 % of **96b**, m.p. 95-97 °C, both as red crystals. The successful reaction was readily confirmed in the ^1H spectra, showing the absence of the carbazole proton. Instead, the introduced side chain shows two pseudo-triplets for H-11 (4.68 ppm) and H-12 (2.69 ppm) and a singlet for the six protons of the N-methyl groups at 2.37 ppm for **96a** and 2.36 ppm for **96b**, respectively.

^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (m, H-5, 1H), 7.45 (m, H-8, 1H), 7.41 (m, H-7, 1H), 7.33 (m, H-6, 1H), 6.41 (q, *J* = 1.6, H-2, 1H), 4.68 (m, H-11, 2H), 2.69 (m, H-12, 2H), 2.36 (s, H-14/14', 6H), 2.12 (d, *J* = 1.6 Hz, H-10, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 183.5 (C-4), 181.1 (C-1), 147.5 (C-3), 138.6 (C-8a), 133.5 (C-9a), 132.6 (C-2), 126.7 (C-7), 124.4 (C-6), 123.8 (C-4b), 123.1 (C-5), 117.0 (C-4a), 110.9 (C-8), 58.3 (C-12), 45.6 (C-14/14'), 42.9 (C-11), 15.7 (C-10).

^{15}N NMR (40 MHz, $CDCl_3$) δ -240.5 (N-9), -358.4 (N-13).

^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (m, H-5, 1H), 7.44 (m, H-8, 1H), 7.41 (m, H-7, 1H), 7.32 (m, H-6, 1H), 6.48 (q, *J* = 1.6 Hz, 1H), 4.68 (m, H-11, 2H), 2.69 (m, H-12, 2H), 2.37 (s, H-14/14', 6H), 2.12 (d, *J* = 1.6 Hz, H-10, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 183.6 (C-4), 181.2 (C-1), 144.6 (C-2), 138.8 (C-8a), 134.7 (C-3), 133.3 (C-9a), 126.8 (C-7), 124.3 (C-6), 123.3 (C-4b), 123.2 (C-5), 117.3 (C-4a), 110.8 (C-8), 58.4 (C-12), 45.7 (C-14/14'), 43.0 (C-11), 15.6 (C-10).

^{15}N NMR (40 MHz, $CDCl_3$) δ -240.3 (N-9), -358.3 (N-13).

The amination of **96a** and **96b** should be performed using the same methodology with both isomers. **96a** could be converted into the amino-derivative **97a** by two different methods with varying success.[141] Josey et al.[124] employed NaN_3 in an acidic milieu for the synthesis of 2-amino-3-methyl-1,4-naphthoquinone, a route which could be adapted for the construction of **97a**, but failed in the synthesis of regiosiomer **97b**.

96a was dissolved in methanol and treated with a solution of NaN_3 in water, which was adjusted to pH 3-4 by hydrochloric acid. After stirring at 50 °C for 5 h the reaction mixture was quenched with water, extracted with ethyl acetate and purified with column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford **97a** in 15 % yield.

The successful reaction could be confirmed by NMR spectroscopy. The quartet at 6.48 ppm of the proton in position 3 is absent and a broad singlet counting two protons appears at 5.09, indicative for exchangeable protons of the amino group. Moreover, in the ^{15}N HMBC spectrum the introduced amino function is indicated by a correlation to the methyl protons H-10. HRMS analysis is also in agreement with the calculated mass.
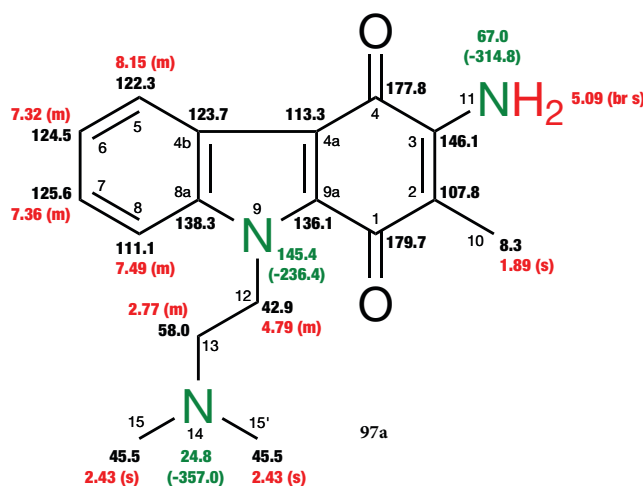
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15 (m, H-5, 1H), 7.49 (m, H-8, 1H), 7.36 (m, H-7, 1H), 7.32 (m, H-6, 1H), 5.09 (br s, H-11, 2H), 4.79 (m, H-12, 2H), 2.77 (m, H-13, 2H), 2.43 (s, H-15/15', 6H), 1.89 (s, H-10, 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 179.7 (C-1), 177.8 (C-4), 146.1 (C-3), 138.3 (C-8a), 136.1 (C-9a), 125.6 (C-7), 124.5 (C-6), 123.7 (C-4b), 122.3 (C-5), 113.3 (C-4a), 111.1 (C-8), 107.8 (C-2), 58.0 (C-13), 45.5 (C-15/15'), 42.9 (C-12), 8.3 (C-10).

^{15}N NMR (40 MHz, CDCl$_3$) δ -236.4 (N-9), -314.8 (N-11), -357.0 (N-14).

The reaction was repeated for the amination of **96b** in a modified procedure by Adams et al.[126] who used NH_4Cl solution as acidifying agent. **96b** was dissolved in methanol and treated with NaN_3 and NH_4Cl and refluxed for 4 h. After the extraction with dichloromethane and column chromatography **97b** could not be isolated.

In order to raise yields of **97a** and possibly open access to **97b** we investigated the route of Allen et al.[125] who employed a solution of methanol saturated with dry ammonia for the construction of 6-amino-1-ethyl-2-methyl-4,7-dioxo-3-indolecarboxaldehyde.

We modified this procedure[141] by dissolving **96a** in a solution of 7M ammonia in methanol and stirring for 12 h at room temperature in a closed reaction vessel. After evaporation of the volatiles the crude product was purified using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford **97a** as dark green solid, m.p. 167-169 °C in excellent 81 % yield. Unfortunately, this methodology failed in the construction of **97b**.

Since the outcome of the final two steps was uncertain we postponed our efforts on the amination of **96b** and focused on the final two steps of the synthesis of isomer **a**.

A prerequisite for the condensation of the pyrimidine ring to the existing framework with formamide was the presence of an aldehyde function in position 10. This should be realised by oxidation of the methyl group at position 2 with an excess of activated MnO_2. This strategy was applied by Takayanagi and co-workers[127] for the oxidation of Mitomycin C into its formyl derivative. This strategy seemed to be promising because of the structural similarity of Mitomycin C to **97a** (*Figure 15*).

Mitomycin   **97a**

*Figure 15. Structural Similarity of Mitomycin and **97a***

97a was dissolved in ethyl acetate and treated with two eq. of activated MnO_2. The resulting suspension was vigorously stirred. TLC monitoring revealed that the starting material had not reacted. Two additional eq. of MnO_2 were added and stirring was continued. After a total of 4 hours with no noticeable effect the reaction mixture was heated at 70 °C for 12 h. After the workup procedure and NMR spectroscopic investigation the desired compound could not be determined. In the following setup the solvent was changed to dichloromethane and the reaction mixture stirred at room temperature for 2.5 days, showing no effect, only the starting material could be recovered. Changing the solvent to toluene and heating to 70 °C for 24 h in presence of 10 eq. MnO_2 also was uneffective.[141]

A literature survey revealed that oxidation reactions of methyl groups to aldehydes is in most cases achieved by using SeO_2 as oxidant in 1,4-dioxane. The procedure of Goswami et al.[128] was used. **97a** was dissolved in 1,4-dioxane and treated with an equimolar amount of SeO_2. The mixture was refluxed for 4 h after which TLC monitoring showed the complete consumption of the starting material. However, after the workup procedure the desired product could not be isolated. Repeating the setup and stirring at room temperature also consumed the starting material, but the desired product could not be isolated.[141]

With the prospect of countless oxidation methods we abandoned this route and returned to a Friedel-Crafts hydroxyalkylation based approach. The strategy is based on the recent works of Ramkumar and co-workers[129] as outlined in *Scheme 20 on page 60*.

3. 16 Synthesis of the Isomeric Pyrimidocarbazolediones *via* Friedel-Crafts Hydroxyalkylation with Subsequent *Ortho* Metallation[129, 143]

The latest strategy comprised the coupling of 1*H*-indole-2-carboxylate in position 3 with pyrimidine-5-carbaldehyde *via* Friedel-Crafts hydroxyalkation leading to **51** followed by conversion of the alcohol moiety into ketone **52** with IBX. The ring formation should then be achieved in a metallation step using LiTMP.
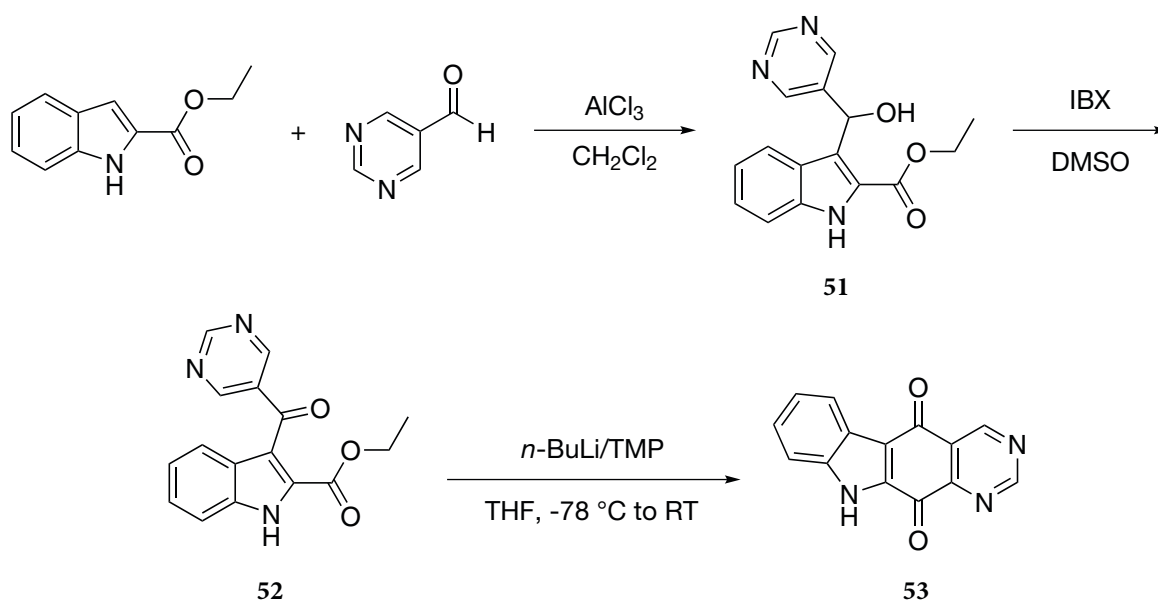
Scheme 20. *Synthesis of **53** via Friedel-Crafts Hydroxyalkylation*

3. 16. 1 Synthesis of Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1*H*-indole-2-carboxylate (51)
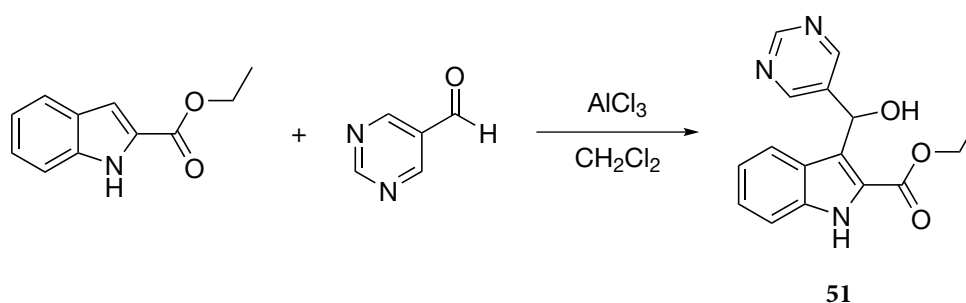
Commercially available building blocks ethyl 1*H*-indole-2-carboxylate and pyrimidine-5-carbaldehyde were reacted in a Friedel-Crafts hydroxylalkylation[129, 143] in dry dichloromethane to furnish **51** in 35 % yield as white crystals. It should be noted that pyrimidine-5-carbaldehyde is hygroscopic and had to be properly dried before usage, otherwise yields were comparably low. Intermediate **51** was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate/light petroleum. The structure was elucidated *via* NMR spectroscopy and HRMS. The coupling product shows a consistent signal set in the ¹H NMR spectrum. In particular, the pyrimidine protons give two singlets at 9.03 ppm (H-2') and 8.85 ppm (H-4'/6')

in the expected ratio of 1:2. Moreover, the proton of the newly formed hydroxy group (at 6.85 ppm) along with the coupling adjacent proton H-8 (at 6.30 ppm) give doublets in both cases (J = 3.9 Hz). Due to the chiral C-8 atom the stereotopic methylene protons of the ester function exhibit a complex coupling pattern (***compare 6. 2. 52 on page 134)***.

3. 16. 2 Synthesis of Ethyl 3-(5-pyrimidinylcarbonyl)-1*H*-indole-2-carboxylate (52)

51   **52**
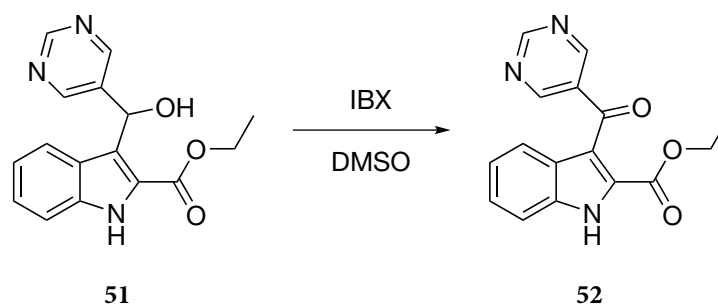
Compound **51** was smoothly transformed into its respective ketone **52** by oxidation with 2-iodoxy-benzoic acid (IBX).[129] The title compound was afforded in excellent 84 % yield. The successful reaction was confirmed *via* NMR and HRMS. The ¹H spectrum reveals the absence of both OH group and the C-8 proton. In the ¹³C APT spectrum the carbinol C-8 at 64.3 ppm has disappeared and the newly formed C=O bond can be clearly identified at 189 ppm. The full spectroscopic characterisation is referenced in chapter ***6. 2. 53 on page 135***.

3. 16. 3 Synthesis of 5*H*-pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (53)

52   **53**
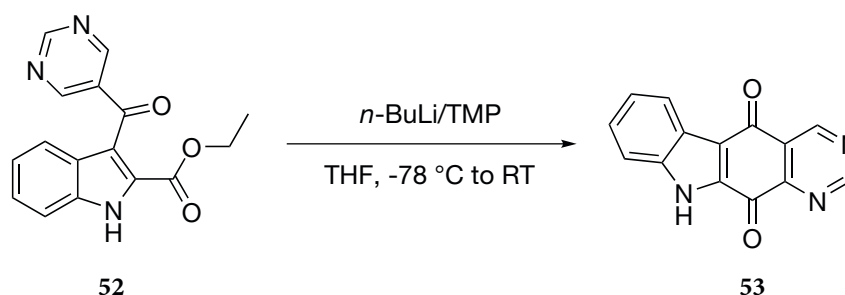
The ring formation to obtain compound **53** was achieved by directed *ortho* metallation[129, 143] using 5 eq. LiTMP in THF. The title compound was afforded in only 5 % yield and the purification process using column chromatography was tedious due to the poor solubility in most organic solvents and the similar R_f of **53** and unreacted **52**. A simple recrystallisation process was also out of question because unreacted **52** crystallised along with the product. Therefore, we envisaged a more practical approach with the alkylation preceding the cyclisation step. The alkylated nucleus would be easier to purify using column chromatography due its good solubility in the eluent. Moreover, the solid product would be easy to separate from the oily starting material by recrystallisation.

The structure was elucidated *via* HRMS and NMR spectroscopy where the absence of the H-6' pyrimidine proton and the methyl and methylene protons of the ester group indicate the successful ring formation. This can be also observed in the ¹³C spectrum with a shift to higher frequency of the ester

carboxyl from 160.1 ppm to the newly formed quinone C-11 at 175.4 ppm as well as a shift to lower frequency of the ketone carbonyl from 189.0 ppm to 178.8 ppm of the second quinone C-5 which is in good agreement with the assignments made for the other compounds of this series and the correlations obtained from the ^{13}C and ^{15}N HMBC spectrum (*compare 6. 2. 54 on page 136*).

3. 16. 4 Synthesis of Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1*H*-indole-2-carboxylate (54)
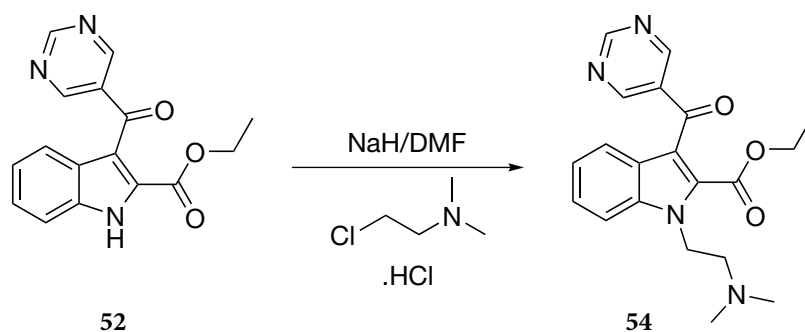
The alkylation of **52** was achieved using the routine alkylation procedure employing sodium hydride in DMF. The title compound **54** was afforded in 49 % yield as a yellow oil and was elucidated *via* NMR spectroscopy and HRMS (*compare 6. 2. 55 on page 137*).

3. 16. 5 Synthesis of 10-[2-(dimethylamino)ethyl]-5*H*-pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (56)
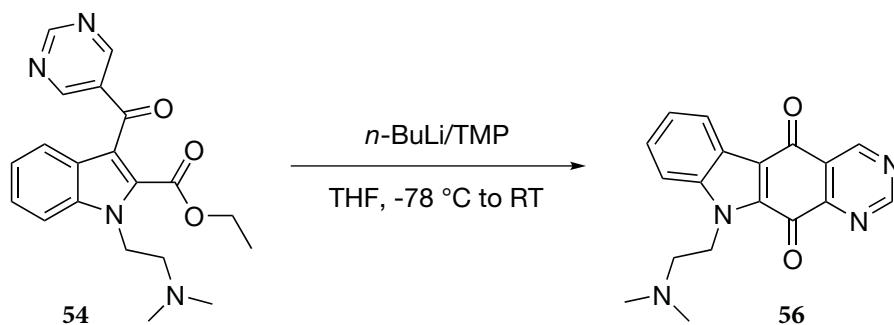
Compound **54** underwent cyclisation in analogy to **52** to obtain compound **56** as red needles in 7.5 % yield, which is 50 % higher than of compound **53**, possibly due to the protected indole-nitrogen or a reduced loss during the purification procedure. As usual, HRMS and a full NMR spectroscopic signal assignment were performed (*compare 6. 2. 57 on page 139*).

Since the *ortho* lithiation procedure using the ester was successful we were interested to investigate if ester and the aldehyde would undergo the cyclisation process without adding the pyrimidine aldehyde to position 3 of the indole. It was conceivable that indole ester and the pyrimidine aldehyde would react by *ortho* lithiation and in situ oxidation following the same methodology as indole-3-carbaldehyde with the pyridine or quinoline amides. If successful, the synthesis of the target compound could be reduced from four steps to only two.

3. 16. 6 Synthesis of Ethyl 1-[2-(dimethylamino)ethyl]-1*H*-indole-2-carboxylate (55)
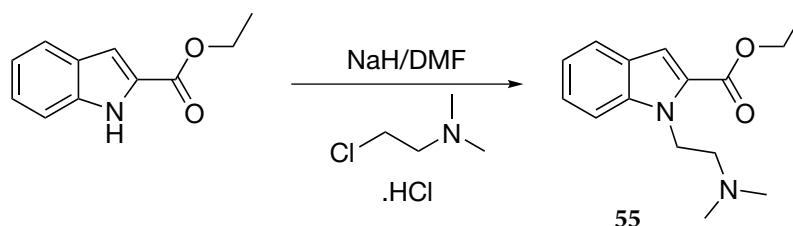
To avoid the tedious purification of the naked tetracycle we decided to attach the side chain to the indole ester before carrying out the ring formation step. Synthon **55** was prepared in the usual manner to give the title compound in 70 % yield as colourless oil. The full spectroscopic characterisation is in chapter **6. 2. 56 on page 138**.

3. 16. 7 Alternative Synthesis of 10-[2-(dimethylamino)ethyl]-5*H*-pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (56)
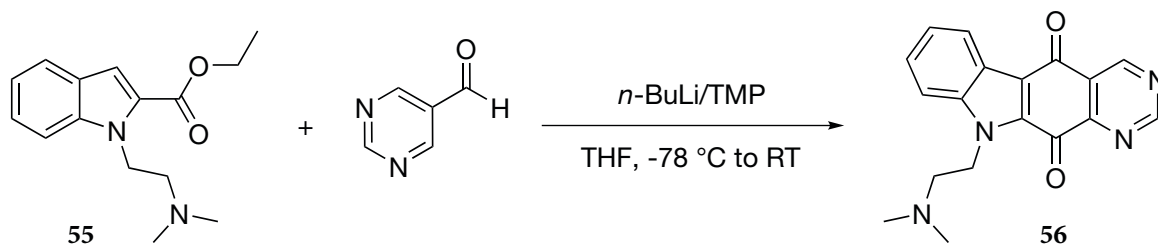
The ring formation step underwent as hoped following the usual procedure by *ortho* lithiation employing an in situ trapping technique. The building blocks reacted as planned and no dimerisation by-product of either synthon could be obtained, only some starting material was recovered. Yields were low at 3.6 % but this route and the preparation of the synthons was very convenient. NMR spectroscopy confirmed that this compound was identical to the compound synthesised earlier.

3. 17 Synthesis of 6-[2-(dimethylamino)ethyl]-5*H*-pyrimido[5,4-*b*] carbazole-5,11(6*H*)-dione (50)
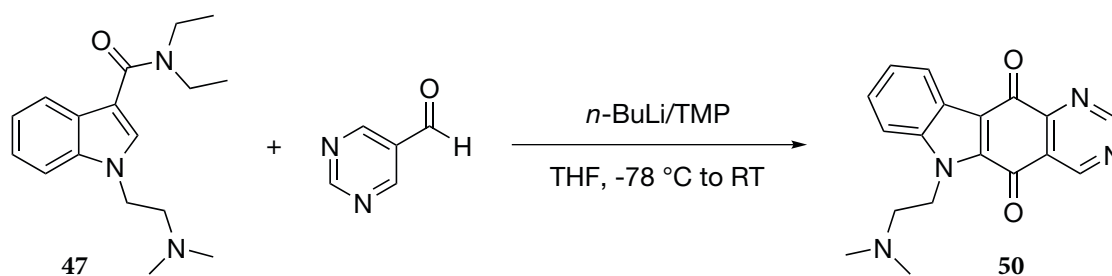
With the successful preparation of **56** we were interested also in the preparation of the second py-rimidine isomer under similar conditions using the same aldehyde and subject it to the cyclisation step with amide **47**. Position 2 of the indole should in theory be lithiated more easily than position 3, thus giving better yields. Indeed, the reaction proceeded as expected and afforded **50** in 7.5 % yield as red needles. We considered this route elegant since it opened access to both tetracyclic pyrimidine isomers using the same pyrimidine-5-carbaldehyde. The structure was elucidated by NMR spectroscopy and verified by HRMS. The unambiguous discrimination of isomers **50** and **56** was possible by 2D-NMR technique HMBC (*compare 6. 2. 51 on page 133*).

3. 17. 1   Synthesis of 5*H*-pyrimido[5,4-*b*]carbazole-5,11(6*H*)-dione (49)

In order to synthesise and spectroscopically characterise the mere skeleton, we referred to the known procedure by preparing the MOM-protected framework and cleavage of the protecting group after the ring formation process with BBr₃ as outlined in *Scheme 21*.
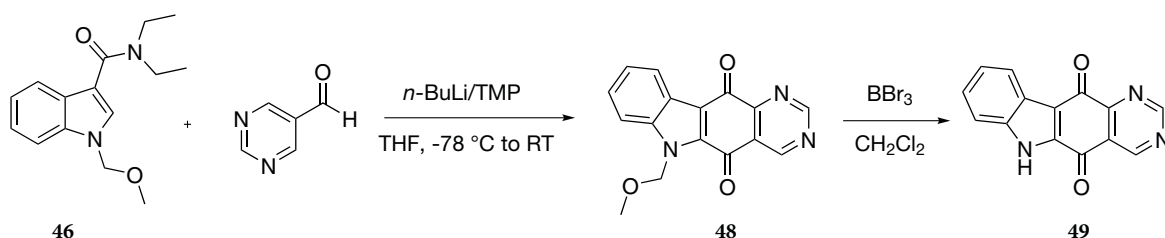
Scheme 21. Synthesis of 49

3. 17. 2   Synthesis of *N,N*-diethyl-1*H*-indole-3-carboxamide (45), *N,N*-diethyl-1-(methoxymethyl)-1*H*-indole-3-carboxamide (46) and 1-[2-(dimethylamino)ethyl]-*N,N*-diethyl-1*H*-indole-3-carboxamide (47)
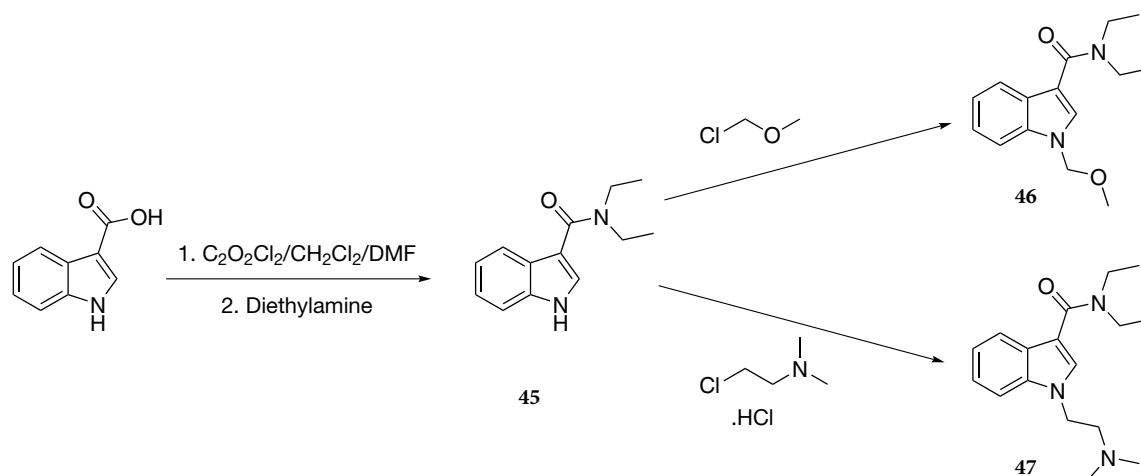
The requisite builiding blocks **46** and **47** were prepared accordingly by alkylation with the corre-sponding side chain starting from **45**, which in turn was prepared in analogy to compound **61** in the modified procedure of Gilman and Spatz[95] in a one-pot reaction from commercially available

1*H*-indole-3-carboxylic acid. The detailed procedure and full spectroscopic characterisation of all intermediates are in chapters *6. 2. 46 on page 128, 6. 2. 47 on page 129* and *6. 2. 48 on page 130*.

Considering the ease of both methods we attempted to prepare compound **7** which was the first compound of this series starting from the available ester **55**. If this method had succeeded the synthesis could have been considerably optimised reducing it by five steps (*compare 3. 1 on page 18*). Suprisingly, both building blocks didn't react as expected, but formed compound **98**, which is a dimerisation product of **55** as illustrated in *Figure 16.* Compound **98** was isolated using column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by recrystallisation from ethyl acetate to furnish orange crystals in 9 % yield, m.p. 203-205 °C.
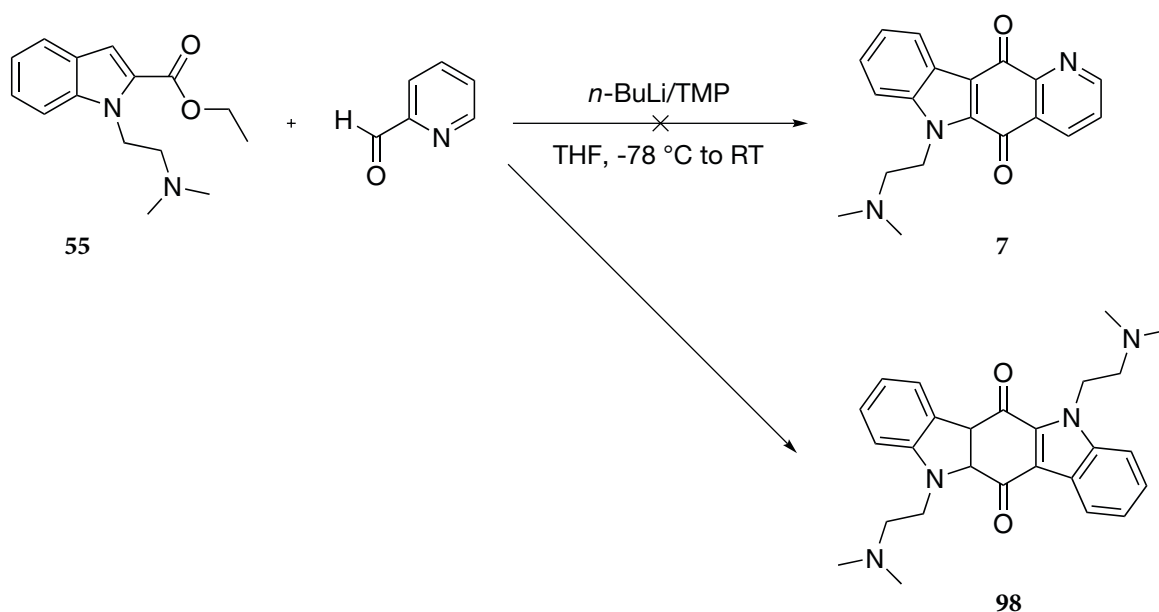
Figure 16. Dimerisation of 55

The ¹H spectrum shows the absence of ester group and indole proton H-3 while retaining the remaining indole protons and the side chain which is indicating a dimerisation product of **55**. This evidence could be confirmed in the ¹³C spectrum showing signals of one half of the molecule only, with the characteristic quinone carbons giving a single peak, which reflects the symmetry of the molecule (*Figure 17 on page 66*). HRMS analysis confirmed the calculated mass.

RESULTS AND DISCUSSION

¹H NMR (400 MHz, CDCl₃) δ 8.30 (m, H-1/7, 2H), 7.39 (m, H-4/10, 2H), 7.33 (m, H-3/9, 2H), 7.32 (m, H-2/8, 2H), 4.73 (m, H-13/13‘, 4H), 2.73 (m, H-14/14‘, 4H), 2.38 (s, H-16/16‘/16“/16‘“, 12H).

¹³C NMR (100 MHz, CDCl₃) δ 177.2 (C-6/12), 138.8 (C-4a/10a), 135.9 (C-5a/11a), 125.7 (C-3/9), 124.3 (C-2/8), 124.2 (C-6b/12b), 122.8 (C-1/7), 117.2 (C-6a/12a), 111.0 (C-4/10), 58.5 (C-14/14‘), 45.8 (C-16/16‘/16“, 16‘“), 43.2 (C-13/13‘).

¹⁵N NMR (40 MHz, CDCl₃) δ -237.5 (N-5/11), -357.5 (N-15/15‘).

Figure 17. NMR Signal Assignment of 98

3. 18 Synthesis of 6-[2-(dimethylamino)ethyl]-5H-pyridazino[4,5-b] carbazole-5,11(6H)-dione (81)

With the successful construction of **50** we were confident that the same procedure would be applicable to the construction of the missing pyridazine derivative. Commercially available, but expensive pyridazine-4-carbaldehyde underwent the cyclisation in the usual procedure by metallating **47** and subsequent trapping with the aldehyde. After the purification procedure the yield was acceptable furnishing 6.2 % of **81** as red needles. The structure was elucidated *via* NMR and HRMS. However, an unambiguous NMR spectroscopic signal assignment of C-4a and C-11a was not possible *via* HMBC experiment because correlations of C-4a and C-11a to adjacent pyridazine protons H-1 and H-4 are apparent, and can therefore not be discriminated. Moreover, the spectrum lacks correlation of C-4a/C-11a to protons of the carbazole system. The invariable part of **81** exhibits a consistent set of signals, which is in complete agreement to other compounds of the tetracyclic series. A full spectroscopic assignment is referenced in the experimental section (**6. 2. 83 on page 165**).

66

3. 18. 1 Synthesis of 5*H*-pyridazino[4,5-*b*]carbazole-5,11(6*H*)-dione (83)
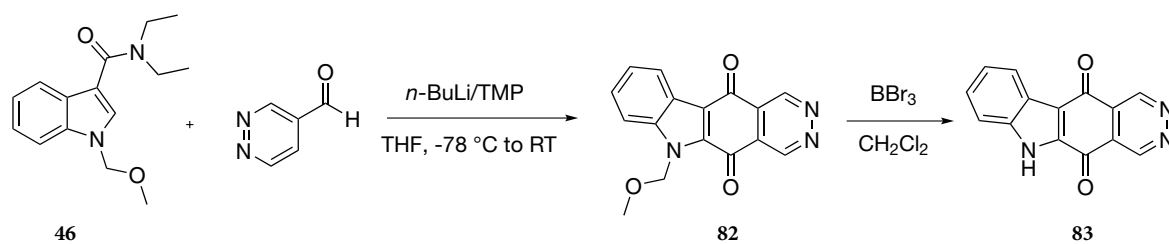
The construction of the naked pyridazine skeleton was achieved in analogy to **49** *via* the MOM-derivative **82** and subsequent MOM cleavage. The detailed procedure and spectroscopic reference is in chapters *6. 2. 84 on page 166* and *6. 2. 85 on page 167.*

3. 19 Biological Evaluation - Viability Assay of the Second Set of Compounds, FACS Analysis and Conclusion

The second set of our compound library was tested against SW480 cells using the EZ4U® XTT assay (*Figure 18*). They also exhibited the highest cytotoxic properties of all compounds tested.
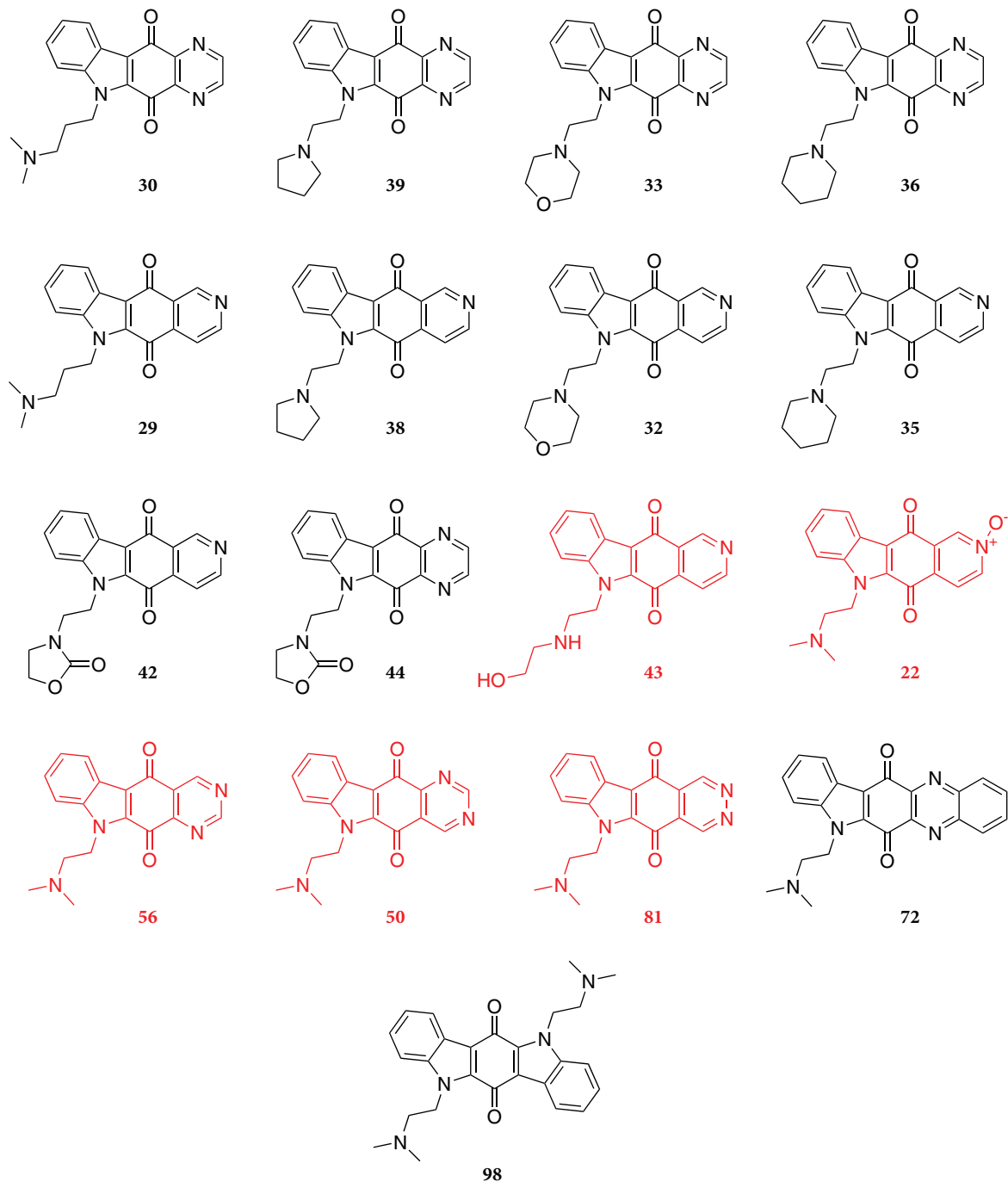
Figure 18. Second Set of Compounds.

*The most cytotoxic compounds are depicted in red. Compounds of the first set are in **Figure 19 on page 69).***
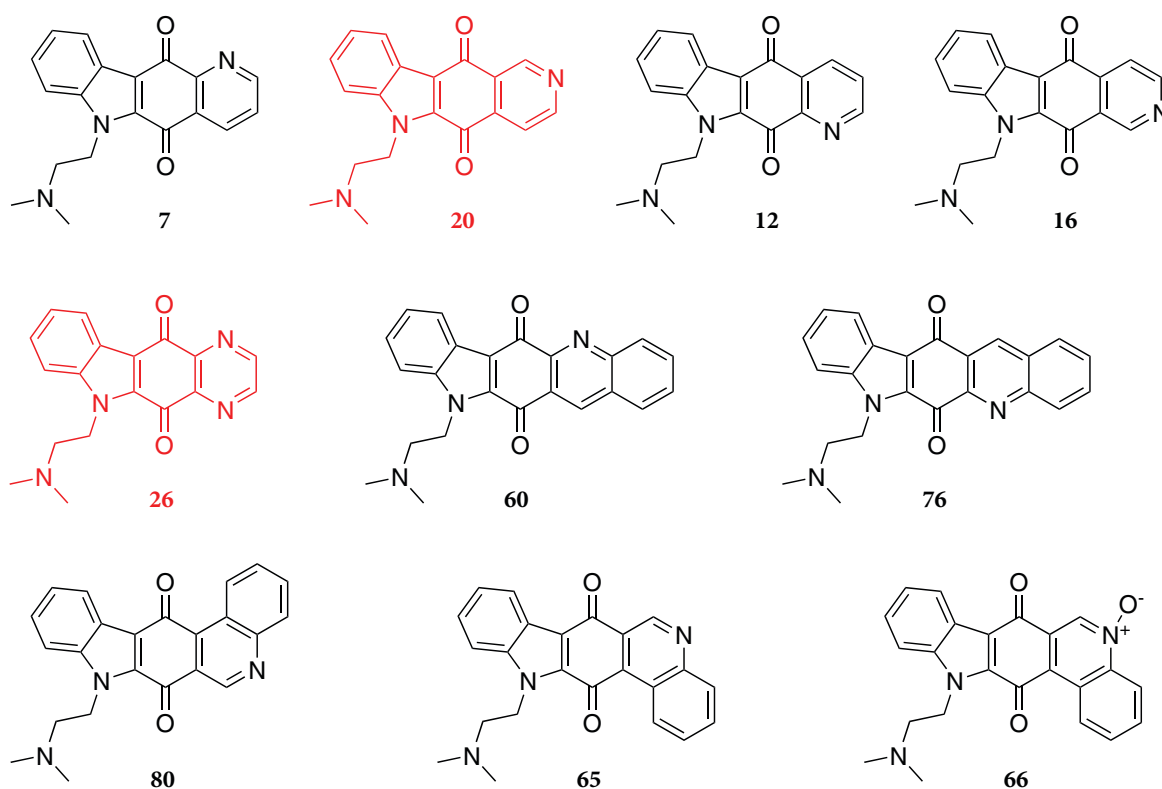
Figure 19. *First Set of Synthesised and Tested Compounds.*
The most cytotoxic compounds are depicted in red.

3. 19. 1 Discussion of the Biological Evaluation of the Tetracyclic Compounds

Viability assays revealed that all tested compounds are highly cytotoxic, but tetracyclic pyridazine derivative **81** showed an exceptional IC_{50} value of approximately 19 nM against SW480 cells (**Table 3 on page 70**).

Similarly, both pyrimidine derivatives **50** and **56** showed excellent activity at about $IC_{50} = 0.2$ μM (**Table 3 on page 70 and Figure 21 on page 71**).

Based on the observation that the N-oxid bearing calothrixin A derivative **66** showed almost two-fold increased activity compared with calothrixin B derivative **65**, we synthesised **22** as tetracyclic *N*-oxide analogue of compound **20**. As expected **22** showed a more than six-fold increased activity ($IC_{50} = 84$ nM) compared with non-*N*-oxide species **20** (**Table 3 on page 70)**.

Derivative **43** which shares the same side chain motif as mitoxantrone enhanced the activity significantly (**Table 3 on page 70**). This might be attributed to the secondary amino function which allows the formation of DNA adducts with cellular formaldehyde.

All other basic substituents do not show a consistent pattern of improvement on the pyridine- or the pyrazine nucleus. On the contrary, their contribution to the overall activity appears to be completely random and lies in a range of an IC_{50} value of approximately 0.5 to 1.5 μM. Endowed with the same substituent in some cases the pyridine derivative shows higher activity and in others the pyrazine derivative.

On the other hand, the second nitrogen atom of the heterocycle and the polar *N*-oxide moiety seems to be highly beneficial in terms of activity. Especially the pyridazine unit seems to be contributing

very much, presumably due to the strong dipole momentum of the two adjacent nitrogens in connection with the carbonyl groups of the quinone.

Compound	SW480 (Colon) [95%-CI]	Compound	SW480 (Colon) [95%-CI]
7	**2.69 [1.13 - 6.41]**	42	0.878 [0.650 - 1.18]
12	**2.07 [1.16 - 3.69]**	43	0.387 [0.355 - 0.421]
16	**0.796 [0.696 - 0.911]**	44	4.56 [4.05 - 5.12]
20	**0.519 [0.499 - 0.539]**	50	0.202 [0.164 - 0.247]
22	0.0845 [0.0788 - 0.0906]	56	0.169 [0.151 - 0.189]
26	**0.498 [0.363 - 0.684]**	**60**	**> 10**
29	0.354 [0.317 - 0.394]	**65**	**1.34 [1.11 to 1.62]**
30	0.672 [0.625 - 0.723]	**66**	**0.772 [0.704 - 0.846]**
32	1.54 [1.23 - 1.95]	72	0.732 [0.591 - 0.906]
33	1.08 [0.891 - 1.30]	**76**	**> 10**
35	0.733 [0.670 - 0.803]	**80**	**1.02 [0.941 - 1.11]**
36	0.376 [0.361 - 0.391]	81	0.0185 [0.00879 - 0.0391]
38	0.400 [0.378 - 0.423]	98	14.1 [12.6 - 15.7]
39	0.560 [0.528 - 0.594]		

Table 3. *Antiproliferative XTT (EZ4U®) Assay of the Second Set of Compounds against SW480 cells. IC_{50} values are expressed in μM. Error bars represent ± standard error mean (SEM) of at least 3 independent experiments in quadruplicate. Numbers in square brackets represent IC_{50} values in the 95 % confidence interval. Results from the first set against SW480 cells are printed in red.*

Surprisingly, there was a big difference in the activity of the carbamate bearing compounds. Pyrazine derivative **44** showed an IC_{50} of only 4.56 μM whereas the IC_{50} value of **42** is 0.88 μM. Since the carbamate unit should be susceptible for enzymatic hydrolysis we repeated viability assays after 48 h and 72 h in order to detect any delayed increase in activity. However, no significant improvement could be detected (***Table 4 and Figure 20 on page 71***).

Compound (treatment)	SW480 (Colon) [95%-CI]	Compound (treatment)	SW480 (Colon) [95%-CI]
42 (24 h)	0.878 [0.650 - 1.18]	44 (24 h)	4.56 [4.05 - 5.12]
42 (48 h)	0.914 [0.838 - 0.996]	44 (48 h)	4.21 [2.54 - 6.97]
42 (72 h)	0.907 [0.813 - 1.01]	44 (72 h)	3.29 [2.35-4.62]

Table 4. *Antiproliferative XTT (EZ4U®) Assay of Carbamate Derivatives **42** and **44**. Cells were treated for 24 h, 48 h and 72 h with compounds and viability was measured. IC_{50} values are expressed in μM. Numbers in square brackets represent IC_{50} values in the 95 % confidence interval.*
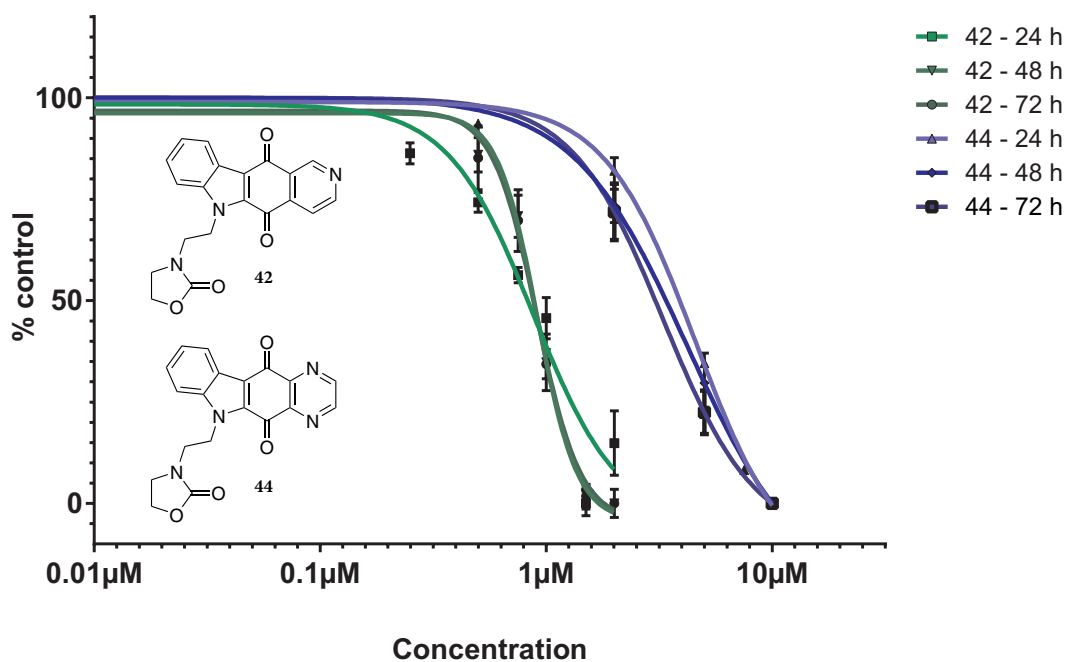
Figure 20. *Dose-Response Curve of Compounds **42** and **44** (XTT Assay).*
Cells were treated with compounds for 24, 48 and 72 h and viability was measured. Error bars represent ± standard error mean (SEM) of at least 2 independent experiments in quadruplicate.
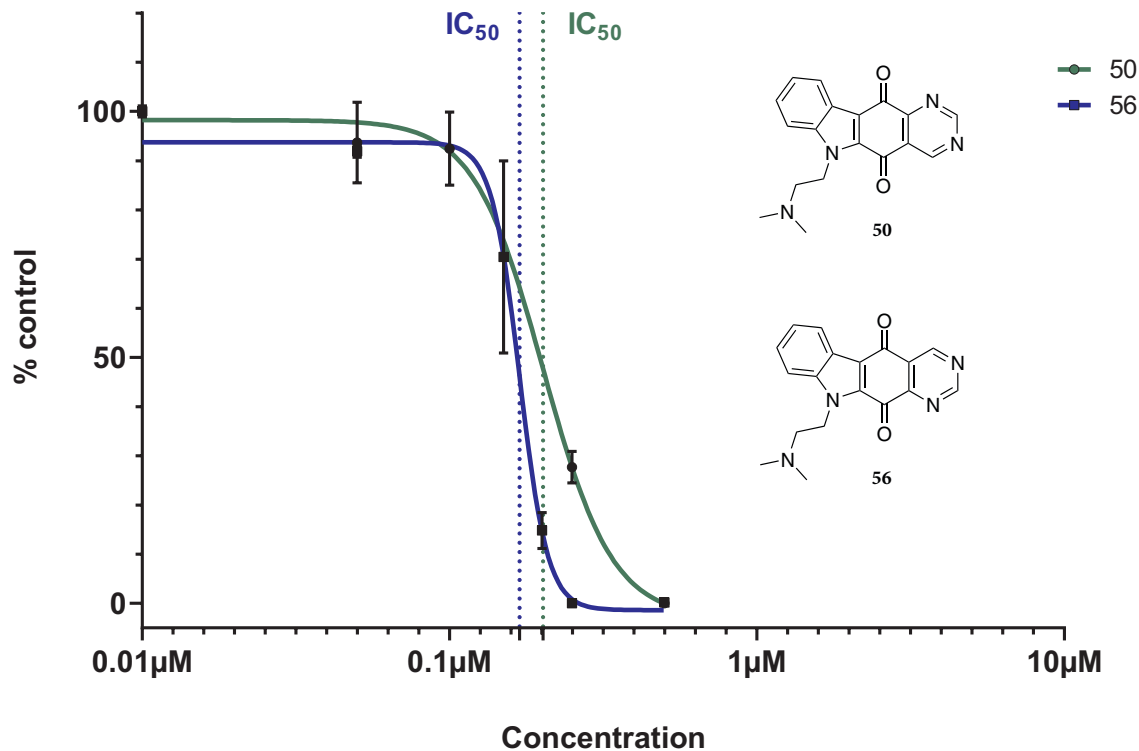
Figure 21. *Dose-Response Curve of Compounds **50** and **56** (XTT Assay).*
Dotted line indicate the respective IC_{50} value. Error bars represent ± standard error mean (SEM) of at least 3 independent experiments in quadruplicate.
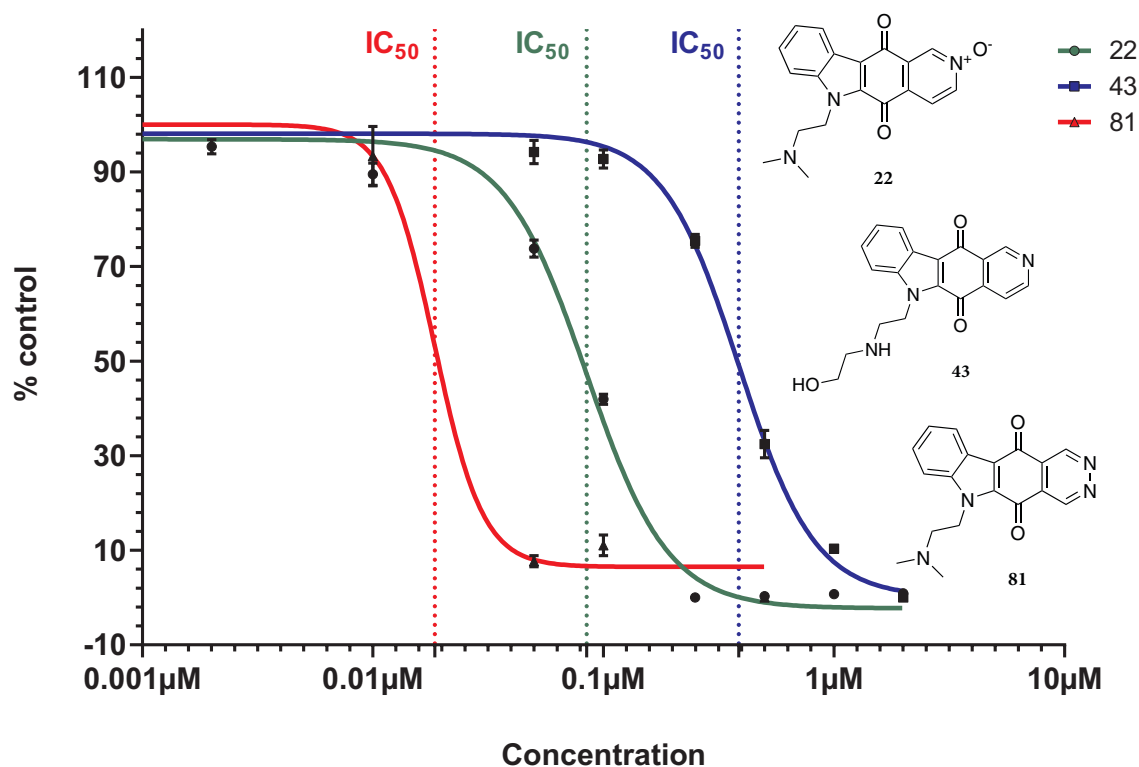
Figure 22. *Dose-Response Curve of Compounds **22**, **43** and **81** (XTT Assay).*
Dotted line indicate the respective IC_{50} value. Error bars represent ± standard error mean (SEM) of at least 3 independent experiments in quadruplicate.

In future works, the two remaining isomeric pyridazine derivatives are of major interest as well as several *N*-oxide derivatives (***Figure 23 on page 73***).

According to our previous optimisation strategy, the best structures resulting from biological testings should then again be modified with the mitoxantrone or even a pixantrone side chain motif.
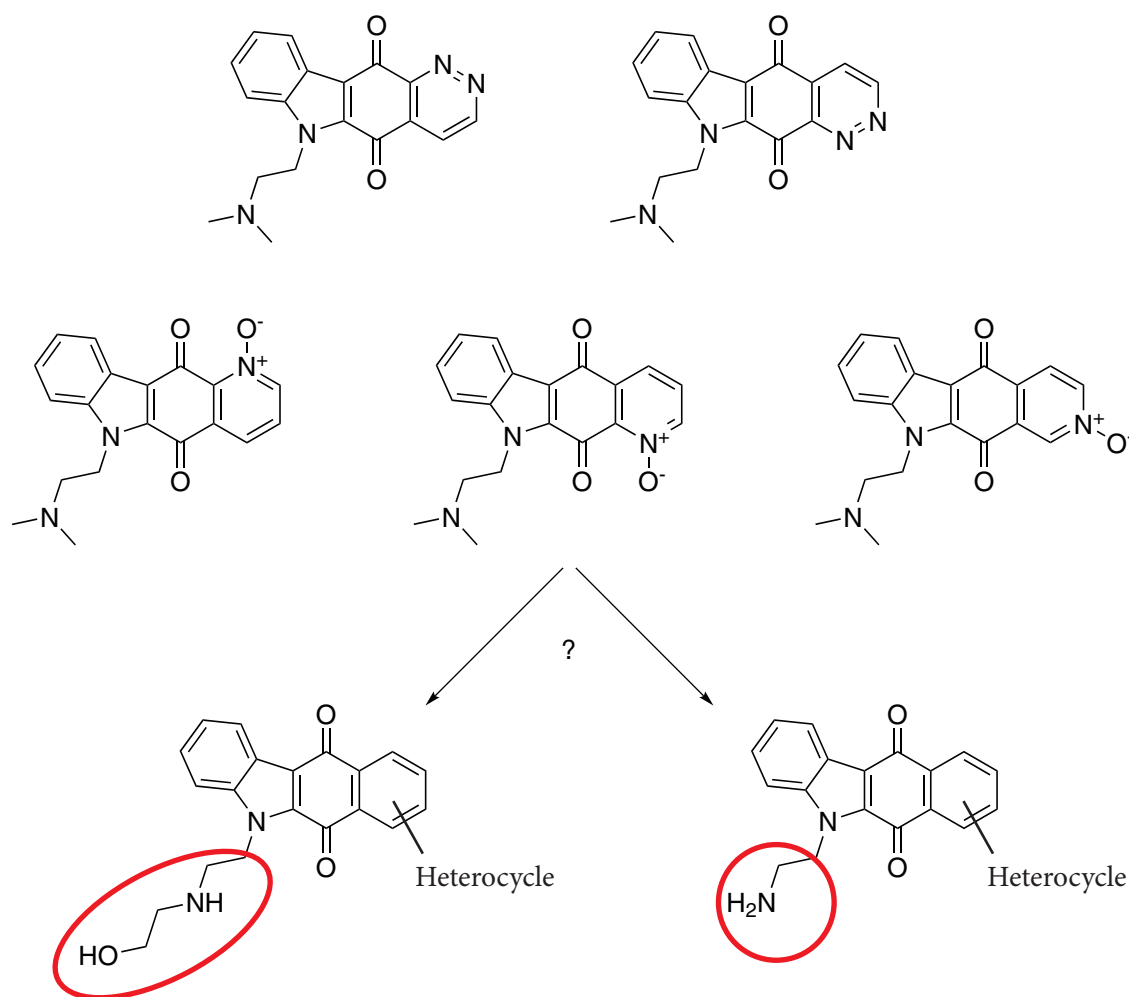
Figure 23. *Future Structures of Interest with a Tetracyclic Nucleus*

3. 19. 2 Discussion of the Biological Evaluation of the Pentacyclic Compounds

Pentacyclic pyrazine derivative **72**, which is a benzoannulated analogue of the highly active compound **26** (IC$_{50}$ = 0.50 µM) exhibits a slightly decreased activity (IC$_{50}$ = 0.73 µM) compared with **26**. This is consistent with the observation that all pentacyclic (benzoannulated) derivatives showed at least slightly decreased activity compared with their tetracyclic counterparts, Interestingly, benzoannulated analogues of **7** and **12**, (compounds **60** and **76)** showed only weak activity (no activity at 10 µM).Nevertheless, the mere cytotoxic potential is not a paramount feature of a successful anticancer agent, which is why the synthesis of other pentacyclic structures will be pursued; isoquinoline based calothrixin analogues are of still of interest, as well as *N*-oxide derivatives thereof and the *N*-oxide derivative of synthesised compound **80** (***Figure 24 on page 74***)

Compound **98** which contains two basic side chains attached to a symmetric planar framework, was synthesised by coincidence, and showed only moderate activity against SW480 cells (IC$_{50}$ = 14.1 µM).
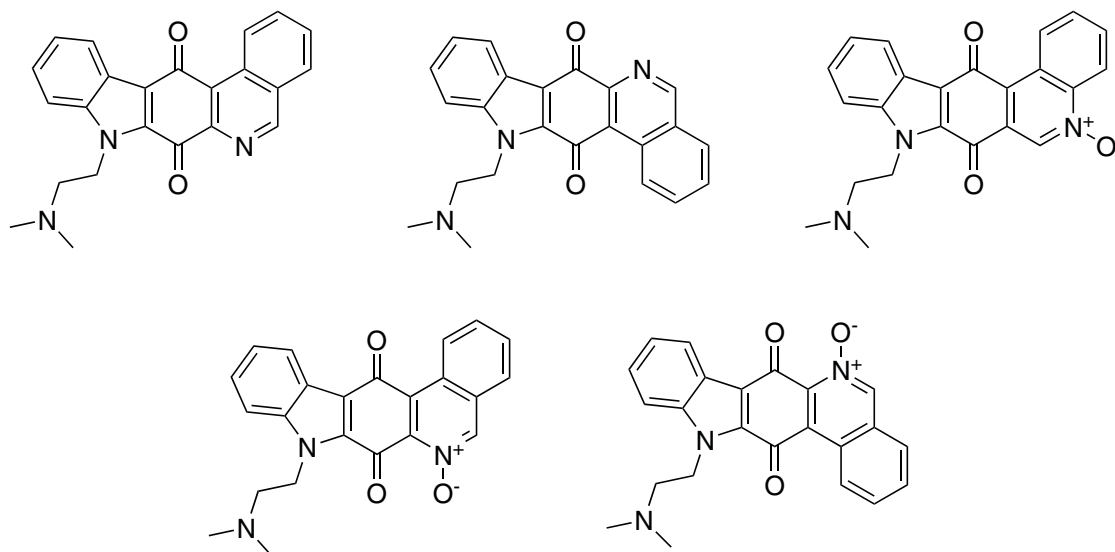
Figure 24. *Future Structures of Interest with a Pentacyclic Nucleus.*

3. 19. 3   Cell Cycle (FACS®) Analysis[133] of Compounds 20, 22, 26 and 43
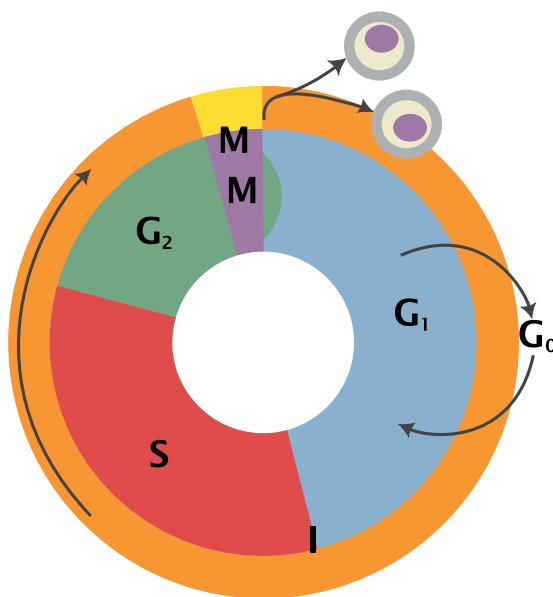
Figure 25. *Schematic Representation of the Cell Cycle (by Richard Wheeler, Zephyris. 2006)*

The synthesis and duplication of nuclear DNA before division, and mitosis, the process of cellular division itself are the two most obvious features of the cell cycle. These two components of the cell cycle are usually termed "S phase" and "M phase". Later, a temporal gap between mitosis and the onset of DNA synthesis was discovered, and another gap between the completion of DNA synthesis and the onset of mitosis. In consequence, these gaps were termed G1 and G2, respectively (**Figure 25**).[133] When cells are not in the preparation process for cell division, they remain in the G1 phase of the cell cycle, which is why the G1 phase is numerically the most predominant phase. Non-proliferating cells can enter a quiescent state from the G1 phase for a long period of time or indefinitely which is often the case in fully differentiated cells, such as neurons or muscle cells. This is phase is termed G0. Some

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cells can reenter the G1 phase, which is the case in liver, stomach or kidney cells.[133]

The G1 phase is characterised as a synthetic phase in which cell organelles, cytoplasma, RNA, replication enzymes and other molecules are produced before DNA replication. In the G2 phase, DNA damage of the preceding cell cycle phases is being repaired, and DNA structure is being reorganised which must take place before the DNA can be divided equally between daughters during mitosis.

FACS®, a registered trademark of Becton Dickinson (BD) standing for "fluorescence activated cell sorting", has become a generic term in cell biology which employs flow cytometry to distinguish cells in different phases of the cell cycle. Cells are lysed and the extracted nuclei are stained with propidium iodide, an intercalating dye, which stains DNA in a stoichiometric manner (the amount of stain is directly proportional to the amount of DNA in the cell).

As the DNA content of cells duplicates during the S phase of the cell cycle, the relative amount of cells in the G0 phase and G1 phase (before S phase), in the S phase, and in the G2 phase and M phase (after S phase) can be determined, as the fluorescence of cells in the G2/M phase will be twice as high as that of cells in the G0/G1 phase.[133]

Cell cycle analysis of highly cytotoxic compounds **20** and **26** of the first series and **22** and **43** of the second series was performed (*Figure 26 on page 76*). After treatment of SW480 cells with compounds **26**, **22**, **43** at their respective IC_{50} and IC_{25} concentrations for 24 h, cells were arrested in G2/M phase, whereas compound **20** was causing an S-phase arrest. These results were not surprising and as similar to other compounds of our library. Similar reports are also reported for mitoxantrone[134, 135], which is known to cause a dose-dependent S-phase and G2/M-arrest. Still, the underlying mechanism needs further investigation.
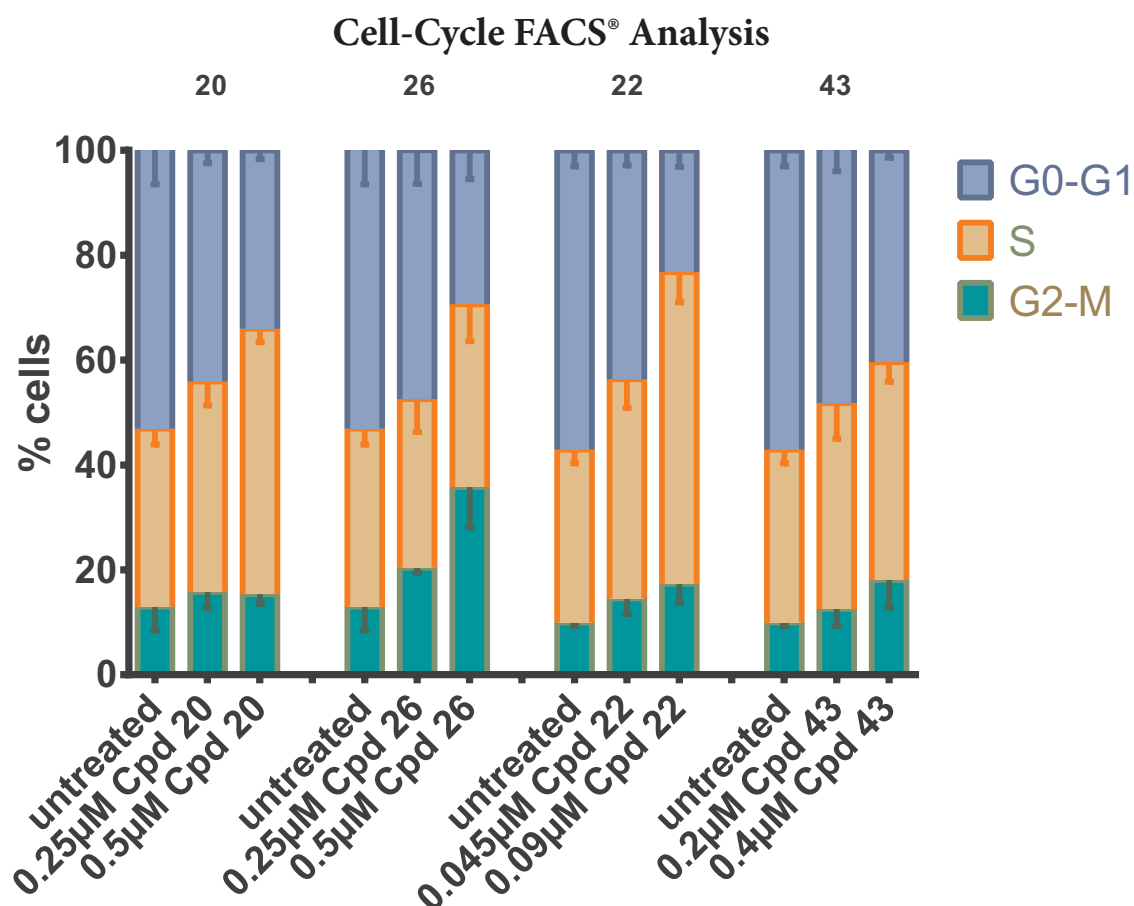
Figure 26. *Cell Cycle FACS® Analysis.*
Compounds **20** *(P < 0.0001),* **22** *(P < 0.0005),* **26** *(P < 0.0001) and* **43** *(P< 0.0016). Calculated P Values of One-Way ANOVA are expressed in round brackets.*

4   CONCLUSION

A series of substituted tetracyclic ellipticine quinone and pentacyclic calothrixin analogues has been synthesised and tested for antiproliferative activity.

Tetracyclic compounds **22**, **43**, **50**, **56** and **81** showed the highest cytotoxic activity, particularly pyridazine derivative **81**.

Ring expansion by one benzoannulation which leads to calothrixin isomers could not further improve cytotoxic activity.

Of all substituents the 2-[(2-Hydroxyethyl)amino]ethyl side chain was the most effective, presumably because of its capability to form formaldehyde-DNA adducts with its secondary amino function as could be demonstrated for mitoxantrone.

If this motif can improve the activity of other nuclei will be investigated in future works.

5   EXPERIMENTAL PART - CELL CULTURE

5. 1   Instrumentation And Materials Used For Cell Culture

Petri Dishes (10 cm and 6 cm):	Sarstedt
24-well plates and 96-well plates:	Sarstedt
Penicillin/Streptomycin (Penstrep):	PAA
Fetal Calf Serum (FCS):	Sigma-Aldrich
RPMI-1640 Medium 10x:	Sigma-Aldrich
Trypsin/PBS 10x (for use diluted 1:10 in PBS):	PAA
Eagles Minimal Essential Medium (MEM):	Sigma-Aldrich
Bovine Serum Albumin (BSA):	Sigma-Aldrich
Phosphate Buffered Saline (PBS):	Sigma-Aldrich
DMSO:	Sigma-Aldrich
PBS/EDTA (10mM EDTA in PBS):	Merck
Neutral Red	Merck
EZ4U® Cell Proliferation & Cytotoxicity Assay:	Biomedica

Plate Reader:	TECAN Infinite® 200 PRO
Flow Cytometer:	BD Sciences FACSCalibur®

5. 2   Cell Lines

Viability assays were performed on 5 different standard cell lines, consisting of SW480, Hep3B, A549, U373 MG and HTB-65 which were obtained from the American Type Culture Collection (ATCC®).

5. 2. 1   SW480 (ATCC® CCl-228™)[136]

SW480 (ATCC® CCl-228™) cells were established from a primary adenocarcinoma of the colon from a 50-year-old Caucasian male. The chromosome number is hypotriploid. This line has a mutation in codon 12 of the ras proto-oncogene, and can be used as a positive control for PCR assays of mutation in this codon. It has an elevated level of p53 protein and is positive for expression of c-myc, K-ras, H-ras, N-ras, myb, sis and fos oncogene. The cells have been reported to produce GM-CSF. SW480 cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.

100 µm
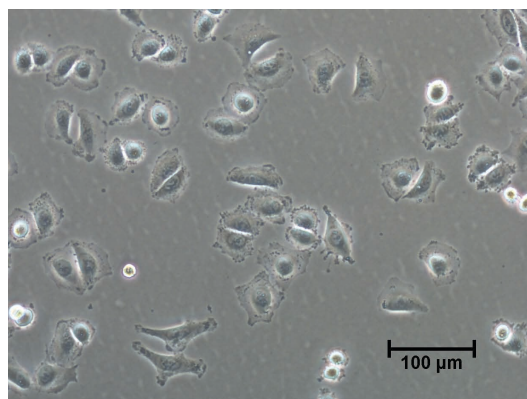
5. 2. 2 Hep3B (ATCC® HB-8064™)[136]

Hep3B (ATCC® HB-8064™) is a human hepatoma derived cell line from an 8-year-old black male. This line contains an integrated hepatitis B virus genome. Hep3B cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.
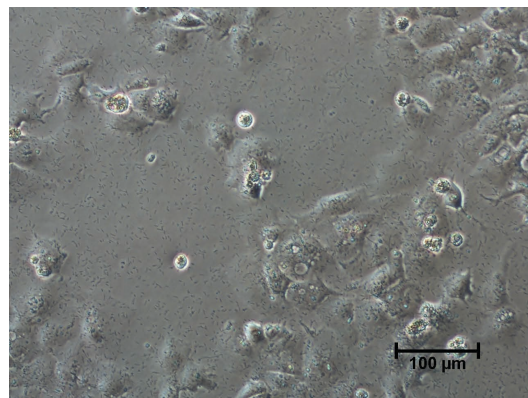
5. 2. 3 A549 (ATCC® CCL-185™)[136]

A549 (ATCC® CCL-185™) was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. It is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24 % of cells. The cells are positive for keratin by immunoperoxidase staining. Cells with 64 (22 %), 65, and 67 chromosome counts also occurred at relatively high frequencies. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40 % of 50 cells analysed. Cytogenetic information is based on initial seed stock at ATCC®. Cytogenetic instability has been reported in the literature for some cell lines. A549 cells are cultivated in MEM containing 10 % FCS and 4 % Pen-Strep.
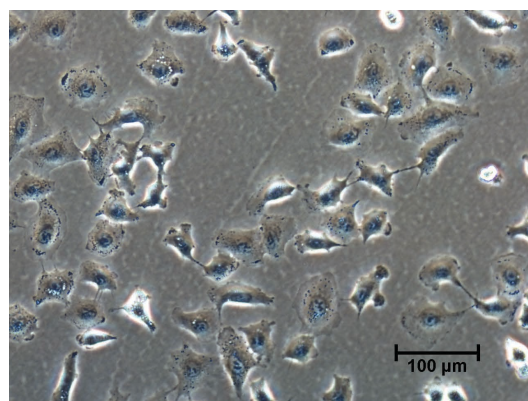
5. 2. 4 U373 MG (ATCC® HTB-17™)[136]

U373 MG (ATCC® HTB-17™) is a human glioblastoma astrocytoma cell line derived from a malignant tumour by explant technique. The ATCC® reported that their stock of U373 MG had been shown to have differing genetic properties to stock from the originator's laboratory. (STR)-PCR-profiling, morphological and immunological analysis has revealed that U373 MG is identical to U251 MG. U373 MG cells are grown in MEM containing 10 % FCS, 1 % non-essential amino acids, and 1 mM sodium pyruvate.
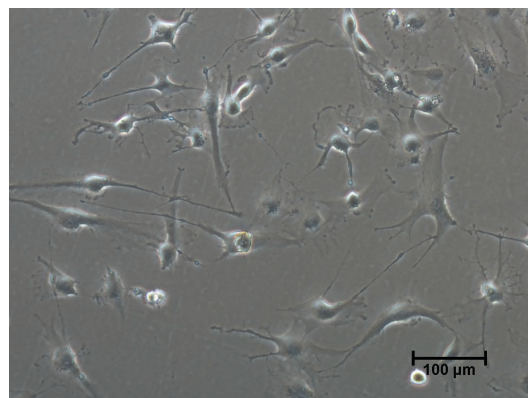
5. 2. 5   MeWo (ATCC® HTB-65™)[136]

MeWo (ATCC® HTB-65™) is a malignant melano-
ma cell line which was initiated by Y. Kodera and M.
Bean in 1974 derived from lymph node tissue of a
78-year-old caucasian male. HTB-65 cells are cultiva-
ted in RPMI-1640 medium containing 10 % FCS and
4% Pen-Strep.
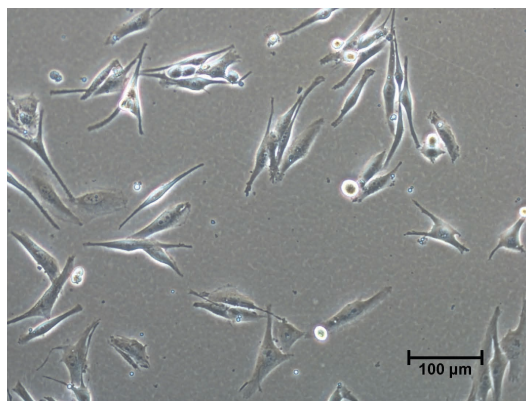
5. 3   Passaging of Cells

SW480, Hep3B, A-549, U-373 MG HTB-65 cells were passaged when they reached a confluence of
about 90 %. Culture medium was removed and the cells were washed with 10 mL of PBS/EDTA. After
removal of PBS/EDTA cells were trypsinised by adding 600 µL of trypsin/PBS and incubating for 5-10
min at 37 °C. The detached cells were taken up in 10 mL culture medium and passaged into new 10
cm petri dishes (PD). All cell lines were usually split by a ratio of 1:5 to 1:10.

5. 4   Cell Viability Assays

5. 4. 1   XTT Cell Proliferation & Cytotoxicity Assay – EZ4U®

The non-radioactive cell proliferation and cytotoxicity assay EZ4U® is based on the capability of living cells to reduce slightly coloured or uncoloured tetrazolium salts in the mitochondria into intensely coloured formazan derivatives. This water soluble formazan is secreted into the culture medium and can be measured with a standard colorimetric reader.[137]

Cells were seeded at a density of $1x10^4$ cells (SW480, Hep3B and A549 cell lines) or $6x10^3$ cells (U373 MG, HTB-65) per well into 96-well plates and after 24 hour treatment with active compounds in increasing concentrations, the supernatant was replaced with 100 μl of freshly prepared EZ4U® solution. The cells were incubated at 37 °C for 3 hours and the absorbance was measured at 450 nm with 620 nm as reference in a microplate reader Tecan Infinite® 200 Pro. Absorbance from a substrate blank in serum free medium without cells was subtracted from all other values.

EZ4U® Solution

Dissolve 1 vial SUB (substrate) in 2.5 mL prewarmed (37 °C) ACT (activator) solution. This procedure yields a yellow solution which was added to serum free medium (1:10, v/v).

5. 4. 2   Neutral Red Assay

This colorimetric assay is based on the ability of viable cells to incorporate and bind Neutral Red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride). Viable cells incorporate neutral red into their lysosomes. As cells begin to die, their ability to incorporate neutral red diminishes. Thus, loss of Neutral red uptake corresponds to loss of cell viability.[138]

Cells were seeded at a density of $1x10^4$ cells (SW480, Hep3B and A549 cell lines) or $6x10^3$ cells (U373 MG and HTB-65 cell lines) per well into 96-well plates and treated with active compounds in increasing concentrations for 24 h.

The supernatant from each well was removed and 200 μl Neutral red solution was added in each well. After incubation at 37 °C for 2 h, the Neutral red solution was removed and the cells were washed with 200 μL PBS. Then, the dye was extracted by adding 100 μL of a mixture of 70 % ethanol + 1 % acetic acid and shaking gently for 5 minutes at room temperature on a titer-plate shaker. The absorbance was measured at 562 nm with 620 nm as reference in a microplate reader Tecan Infinite® 200 Pro.

Neutral Red Solution:

Dissolve 50 μg/mL Neutral Red in serum free medium (SFM) at 37 °C for 1 hour followed by filtering..

5. 5   Cell Cycle Distribution (FACS®)

Cells were seeded at a density of 1 x 10⁶ cells per 6 cm PD. Cells were treated with active compounds for 24 h at 37 °C. The supernatant of treated cells was removed and the cells were washed with 3 mL PBS/EDTA. Cells were trypsinised by adding 250 µL trypsin/EDTA and incubation at 37 °C for 5-10 minutes. 3 mL MEM containing 10 % FCS was added and the cells were passaged into a 15 mL Falcon® tube. After centrifugation at 1200 rpm for 5 minutes the cell pellet was resuspended in 1 mL PBS and transferred into a 1.5 mL tube. Cells were lysed by adding 1 mL of nuclear isolation buffer, mixed well and incubated for 5 minutes on ice. Nuclei were prepared and separated using a syringe. Nuclei were collected by centrifugation for 5 minutes at 4 °C at 2000 rpm, resuspended in 0.5 mL propidium iodide staining solution and transferred into light protected FACS vials. Cell cycle was measured using a BD Sciences FACSCalibur®.

Nuclear Isolation Buffer:

10.5 g citric acid and 0.5 g Tween 20 are added to to 100 mL aqua bidest.

RNAse Stock Solution:

Dissolve 10 mg RNAse A in 10 mL 1x PBS. Incubate for 15 min at 100 °C in heating block. Put on ice immediately and store at -20 °C.

Propidium Iodide Stock Solution:

Dissolve 5 mg propidium iodide in 10 mL 1x PBS. Store protected from light (wrap in aluminium foil).

Propidium Iodide Staining Solution: 0.5 mL necessary for 1 sample

Mix 0.05 mL RNAse stock solution and 0.05 mL propidium iodide stock solution with 5 mL 1x PBS.

6 EXPERIMENTAL PART - SYNTHESIS

6. 1 Instrumentation and Chemicals

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 293 K (400.23 MHz for ^{1}H, 100.65 MHz for ^{13}C, 40.56 MHz for ^{15}N) or on a Bruker Avance 500 spectrometer at 293 K (500.13 MHz for ^{1}H, 125.77 MHz for ^{13}C, 50.68 MHz for ^{15}N). The center of the residual undeuterated solvent signal was used as an internal standard, which was related to TMS with δ 7.26 ppm (^{1}H in CDCl$_3$), δ 2.49 ppm (^{1}H in DMSO-d_6), δ 77.0 ppm (^{13}C in CDCl$_3$), and δ 39.5 ppm (^{13}C in DMSO-d_6). ^{15}N NMR spectra were referenced against external nitromethane with a 'directly' detecting broadband observe probe (BBFO). Digital resolutions were 0.25 Hz/data point in the ^{1}H spectra and 0.4 Hz/data point in the ^{13}C NMR spectra. Assignments of signals was carried out by the combined application of standard NMR spectroscopic techniques such as ^{1}H coupled ^{13}C-NMR spectra, APT, HSQC, HMBC, COSY, and NOESY spectroscopy. Chemical shift values (δ) are reported in ppm, coupling constants (J) in Hz.

HRESIMS spectra were obtained on a Bruker maXis HD Qq-TOF mass spectrometer. Samples were dissolved in MeOH (1-100 µg/mL) and directly infused into the ESI source at a flow rate of 3 µL/min with a syringe pump. The ESI ion source was operated as follows: capillary voltage: 1.5 to 4.0 kV (individually optimised), nebuliser: 0.4 bar (N$_2$), dry gas flow: 4 L/min (N$_2$), and dry temperature: 200 °C. Mass spectra were recorded in the range of m/z 50 – 1550 in the positive-ion mode. For one sample, an APCI source was used instead of ESI, with comparable parameter settings. The sum formulas were determined using Bruker Compass DataAnalysis 4.2 based on the mass accuracy (Δm/z $\leq$ 2 ppm) and isotopic pattern matching (SmartFormula algorithm).

Microwave reactions were performed with a CEM Discover® Labmate microwave reactor.

For TLC, Merck Millipore aluminum sheets pre-coated with Silica gel 60 F254 were used and developed under UV light. For column chromatography, Silica gel 60 (63-200 µm) or LiChroprep® RP-18 (40-63 µm) by Merck Millipore was used.

Melting points were determined on a Reichert–Kofler® hot-stage microscope and are uncorrected. Systematic names were generated with ACD/Name® according to the IUPAC recommendations.

All chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, Alfa Aesar, TCI Europe N.V. and VWR except of pyridazine-4-carbaldehyde (purchased from Activate Scientific) and pyrimidine-5-carbaldehyde (purchased from Frontier Scientific).

6. 2 Synthesis

6. 2. 1 Furo[3,4-*b*]pyridin-5,7-dione (1)
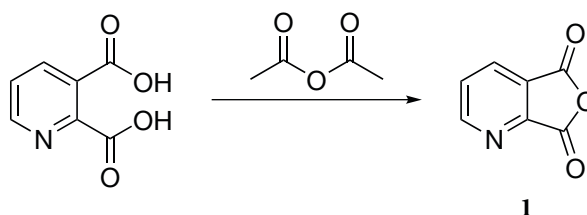
Pyridine-2,3-dicarboxylic acid (10 g, 0.06 mol) was dissolved in freshly distilled acetic anhydride (30 mL, 0.32 mol) and the solution was refluxed for 2 h. The excess of acetic anhydride was removed in vacuo (70 °C at 20 mbar). The wet brown residue was then transferred in a recystallising dish and completely dried under reduced pressure in a desiccator overnight, ground and purified by sublimation (120 °C) to afford 6.7 g (75.0 %) of **1** as white crystals, m.p. 135-136 °C.
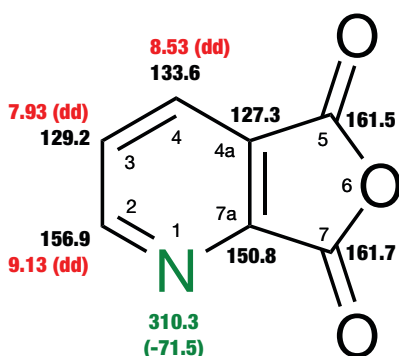
¹H NMR (400 MHz, DMSO-*d₆*) δ 9.13 (dd, *J* = 4.9, 1.5 Hz, H-2, 1H), 8.53 (dd, *J* = 7.8, 1.5 Hz, H-4, 1H), 7.93 (dd, *J* = 7.8, 4.9 Hz, H-3, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 161.7 (C-7), 161.5 (C-5), 156.9 (C-2), 150.8 (C-7a), 133.6 (C-4), 129.1 (C-3), 127.3 (C-4a).

¹⁵N NMR (40 MHz, DMSO-*d₆*) -71.5 (N-1).

6. 2. 2 Furo[3,4-*b*]pyridin-5(7)-one (2)
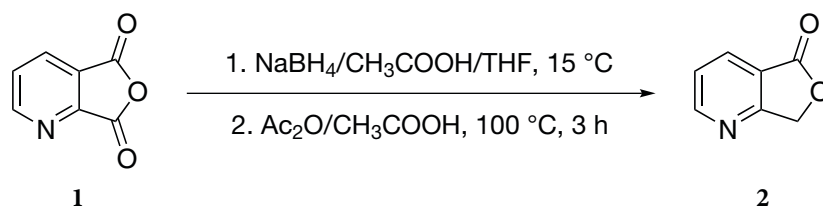
To a solution of **1** (5.0 g, 0.2 mol) in dry THF (40 mL) was added NaBH$_4$ (1.25 g, 0.03 mol) at 15 °C under argon atmosphere, then CH$_3$COOH (4.0 g, 3.3 mL) was added dropwise along with evolving H$_2$ gas at 15 °C under argon. After stirring at 15 °C for 4 h under argon, the mixture was concentrated in vacuo. CH$_3$COOH (14 mL) and acetic anhydride (14 mL) were added to the residue and the solution was stirred at 100 °C for 3 h. The mixture was concentrated in vacuo and to the residue were added H$_2$O (40 mL) and NaCl (7 g). The water layer was extracted with CH$_2$Cl$_2$ (2 x 40 mL). The combined organic layers were dried over Na$_2$SO$_4$ and the solvents removed in vacuo. The residue was purified by column chromatography eluting with ethyl acetate to afford 1.76 g (38.0 %) of **2** as colourless crystals, m.p. 152-154 °C. Alternatively, **2** can be purified sufficiently by recrystallising twice from isopropranol to give beige needles.
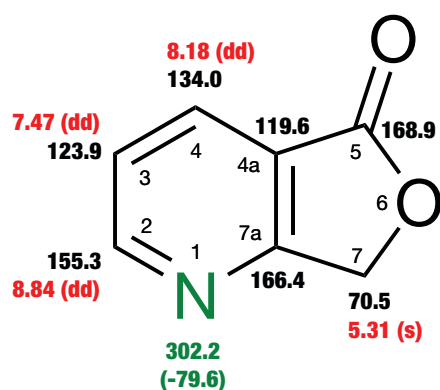
¹H NMR (400 MHz, CDCl₃) δ 8.84 (dd, *J* = 4.9, 1.6 Hz, H-2, 1H), 8.18 (dd, *J* = 7.8, 1.6 Hz, H-4, 1H), 7.47 (dd, *J* = 7.8, 4.9, H-3, 1H), 5.31 (s, H-7, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 168.9 (C-5), 166.4 (C-7a), 155.3 (C-2), 134.0 (C-4), 123.9 (C-3), 119.6 (C-4a), 70.5 (C-7).

¹⁵N NMR (40 MHz, CDCl₃) δ -79.6 (N-1).

6. 2. 3   1-(Phenylsulfonyl)-1*H*-indole (3)

3
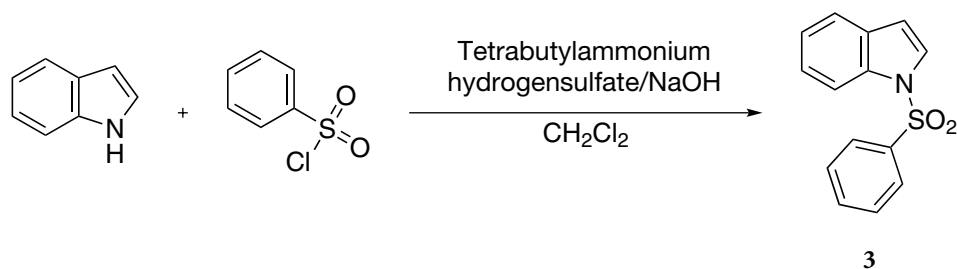
To an ice-cold mixture of powdered NaOH (50.0 g, 1.25 mol) and tetra-n-butylammonium hydrogen sulfate (3.50 g, 0.10 mol) in dry CH_2Cl_2 (500 mL) under argon was added indole (46.75 g, 0.40 mol) in one portion, followed by a solution of benzenesulfonyl chloride (63.8 mL, 0.50 mol) in CH_2Cl_2 (300 mL) at such a rate as to keep the internal temperature below 20 °C. The mixture was then vigorously stirred at room temperature for 2 h, filtered, and evaporated in vacuo to produce a thick orange oil. Trituration with methanol afforded 77 g (74.9 %) of **3** as colourless crystals, m.p. 77-79 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (m, H-2‘/6‘, 2H), 7.95 (m, H-7, 1H), 7.81 (d, *J* = 3.7 Hz, H-2, 1H), 7.64 (m, H-4‘, 1H), 7.59 (m, H-4, 1H), 7.55 (m, H-3‘/5‘, 2H), 7.33 (m, H-6, 1H), 7.23 (m, H-5, 1H), 6.84 (dd, *J* = 3.7, 0.8 Hz, H-3, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 137.0 (C-1‘), 134.5 (C-4‘), 134.1 (C-7a), 130.4 (C-3a), 129.7 (C-3‘/5‘), 126.9 (C-2), 126.6 (C-2‘/6‘), 124.7 (C-6), 123.5 (C-5), 121.6 (C-4), 113.0 (C-7), 109.5 (C-3).

^{15}N NMR (40 MHz, DMSO-d_6) δ -207.9 (N-1).
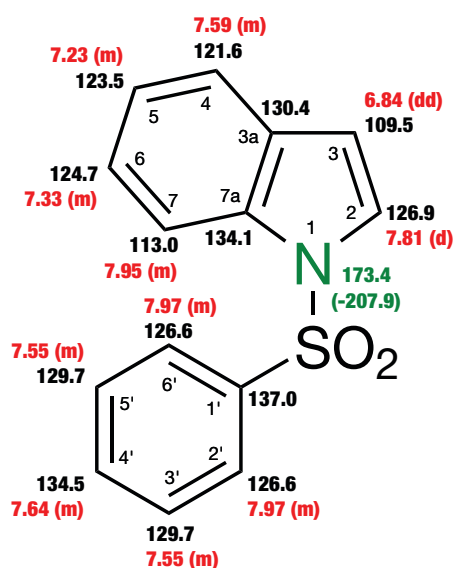
6. 2. 4   [2-(Hydroxymethyl)-3-pyridinyl][1-(phenylsulfonyl)-1*H*-indol-2-yl] methanone (4)
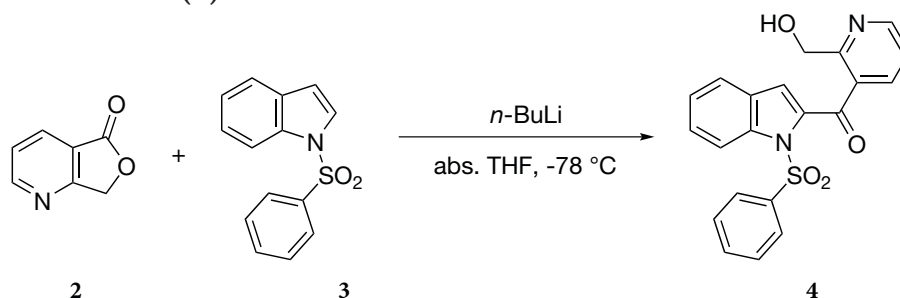
To a solution of **2** (4.0 g, 15.5 mol) in dry THF (200 mL) at -78 °C was added *n*-BuLi (9.8 mL of 1.6M-solution in n-hexanes) in one portion. The mixture was allowed to warm to room temperature during 1 h, and then warmed to 40 °C for 5 min. After recooling to -78 °C furo[3,4-*b*]pyridin-5(7*H*)-one **3** (2.0 g, 14.8 mmol) in dry THF (200 mL) was added rapidly in one portion. The cooling bath was removed and the mixture allowed to warm to room temperature, then brought to 40 °C for 5 min. The solvent was then removed in vacuo at 20 °C. The residue was partitioned between water (60 mL) and Et$_2$O (4 x 200 mL) and the product extracted from the organic layer with 2M HCl. Basification of the aqueous extract with solid K$_2$CO$_3$, extraction with CH$_2$Cl$_2$, and evaporation of the solvent under reduced pressure affords 2 g (34 %) of **4** as orange-red amorphous solid which can be used without purification in the next step. After purification with column chromatography using ethyl acetate as eluent the ketone appears as colourless irregular prisms (1.27 g, 21.8 %), m.p. 139-140 °C.

HRMS: m/z calculated for C$_{21}$H$_{17}$N$_2$O$_4$S ([M+H]$^+$): 393.0904; found: 393.0909.
HRMS: m/z calculated for C$_{21}$H$_{16}$N$_2$NaO$_4$S ([M+Na]$^+$): 415.0723; found: 415.0727.
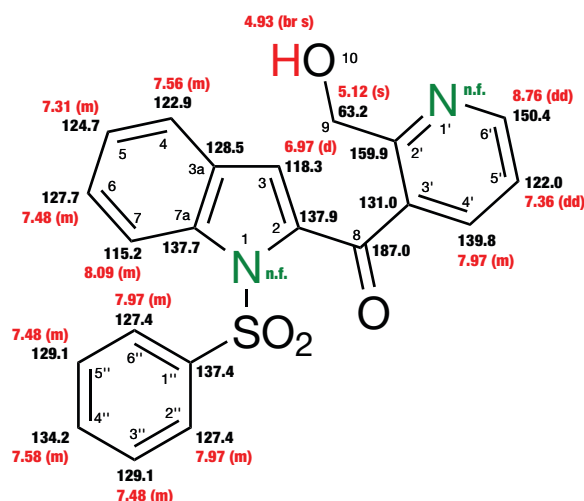
¹H NMR (400 MHz, CDCl₃) δ 8.76 (dd, *J* = 4.9, 1.6 Hz, H-6‘, 1H), 8.09 (m, H-7, 1H), 7.97 (m, H-4‘/2“/6“, 3H), 7.58 (m, H-4“, 1H), 7.56 (m, H-4, 1H), 7.48 (m, H-6‘/3“/5“, 3H), 7.36 (dd, *J* = 7.8, 4.9 Hz, H-5“, 1H), 7.31 (m, H-5, 1H), 6.97 (d, *J* = 0.7 Hz, H-3, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 187.0 (C-8), 159.9 (C-2‘), 150.4 (C-6‘), 139.8 (C-4‘), 137.9 (C-2), 137.7 (C-7a), 137.4 (C-1“), 134.2 (C-4“), 131.0 (C-3‘), 129.1 (C-5“/3“), 128.5 (C-3a), 127.7 (C-6), 127.4 (C-6“/2“), 124.7 (C-5), 122.9 (C-4), 122.0 (C-5‘), 118.3 (C-3), 115.2 (C-7), 63.2 (C-9).

¹⁵N NMR (40 MHz, CDCl₃) δ not found (N-1/1‘).

6. 2. 5   6*H*-Pyrido[3‘,2‘:5,6]oxepino[3,2-*b*]indol-5(12*H*)-one (5)
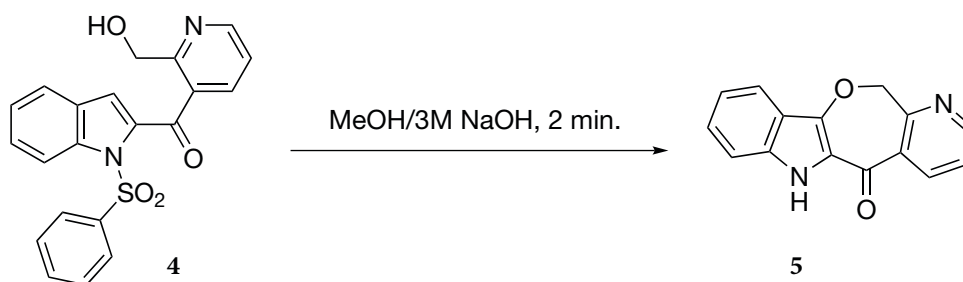
Ketone **4** (2.0 g, 0.005 mol) was dissolved in methanol (200 mL) under reflux. Aqueous 3M NaOH (100 mL) was added in one portion to the colourless solution which turned red instantly. The mixture was refluxed for further 2-5 minutes. Prolonged heating must be avoided. The red mixture was poured into ice water (1500 mL) and the yellow precipitate was collected by filtration. The filtrate was extracted with CH_2Cl_2 (3 x) to extract additional product from the aqueous layer. The collected crystals and the CH_2Cl_2 extract were purified by column chromatography using ethyl acetate as eluent affording 420 mg of **5** (32.9 % of unpurified compound 4) as bright yellow crystals, m.p. 195-197 °C. Compound **5** is LIGHT SENSITIVE and should be stored accordingly.

HRMS: m/z calculated for $C_{15}H_{11}N_2O_2$ ([M+H]⁺): 251.0815; found: 251.0811.
HRMS: m/z calculated for $C_{15}H_{10}N_2NaO_2$ ([M+Na]⁺): 273.0634; found: 273.0632.
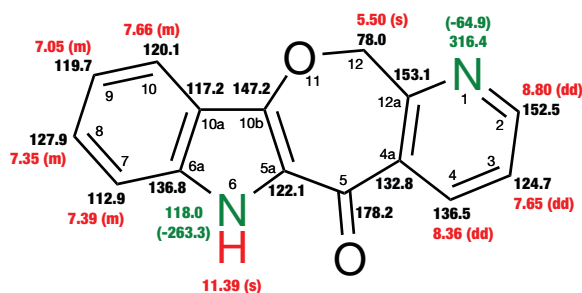
¹H NMR (400 MHz, DMSO-*d₆*) δ 11.39 (s, H-6, 1H), 8.80 (dd, *J* = 4.8, 1.8 Hz, H-2, 1H), 8.36 (dd, *J* = 7.9, 1.8 Hz, H-4, 1H), 7.66 (m, H-10, 1H), 7.65 (dd, *J* = 7.9, 4.8 Hz, H-3, 1H), 7.39 (m, H-7, 1H), 7.35 (m, H-8, 1H), 7.05 (m, H-9, 1H), 5.50 (s, H-12, 2H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 178.2 (C-5), 153.1 (C-12a), 152.5 (C-2), 147.2 (C-10b), 136.8 (C-6a), 136.5 (C-4), 132.8 (C-4a), 127.9 (C-8), 124.7 (C-3), 122.1 (C-5a), 120.1 (C-10), 119.7 (C-9), 117.2 (C-10a), 112.9 (C-7), 78.0 (C-12).

¹⁵N NMR (40 MHz, DMSO-*d₆*) -64.9 (N-1), -263.3 (N-6).

6.2.6   5*H*-Pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione (6)
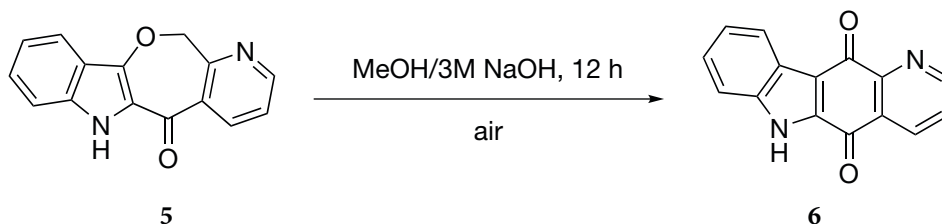
Oxepinoindole **5** (420 mg, 1.7 mmol) from the previous step was refluxed in methanol (120 mL) and 3M NaOH (60 mL) for 12 h with passage of air. The mixture was poured into water (1500 mL) and the reddish-brown precipitate was filtered off and dried in a desiccator in vacuo. The crude product was purified by sublimation (200 °C at < 0.01 Torr) to afford 395 mg (95.0 %) of **6** as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{15}H_9N_2O_2$ ([M+H]$^+$): 249.0659; found: 249.0660.
HRMS: m/z calculated for $C_{15}H_8N_2NaO_2$ ([M+Na]$^+$): 271.0478; found: 271.0481.
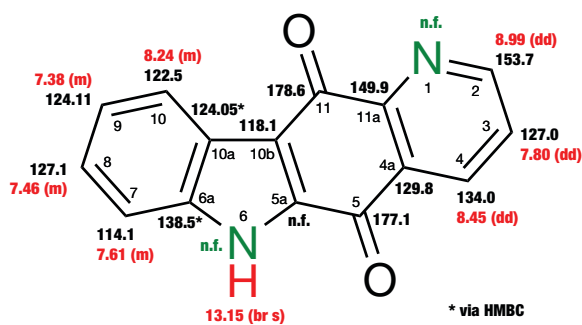
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.15 (br s, H-6, 1H), 8.99 (dd, *J* = 4.7, 1.7 Hz, H-2, 1H), 8.45 (dd, *J* = 7.8, 1.7 Hz, H-4, 1H), 8.24 (m, H-10, 1H), 7.80 (dd, *J* = 7.8, 4.7 Hz, H-3, 1H), 7.61 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.38 (m, H-9, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 178.6 (C-11), 177.1 (C-5), 153.7 (C-2), 149.9 (C-11a), 138.5 (C-6a, via HMBC), 134.0 (C-4), 129.8 (C-4a), 127.1 (C-8), 127.0 (C-3), 124.11 (C-9), 124.05 (C-10, via HMBC), 122.5 (C-10), 118.1 (C-10b), 114.1 (C-7), not found (C-5a).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ not found (N1/6).

6. 2. 7 6-[2-(Dimethylamino)ethyl]-5*H*-pyrido[3,2-*b*]carbazole-5,11(6*H*)-dione (7)

NaH (380 mg of a 60 % dispersion in mineral oil, 9.5 mmol) was suspended in dry DMF (13 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **6** (396 mg, 1.6 mmol) in dry DMF (24 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (460 mg, 3.19 mmol) in dry DMF (20 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 134 mg (26.3 %) of **7** as orange-brown crystals, m.p. 187-189 °C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_2$ ([M+H]$^+$): 320.1394; found: 320.1395.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_2$ ([M+Na]$^+$): 342.1213; found: 342.1215.

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.01 (dd, *J* = 4.7, 1.7 Hz, H-2, 1H), 8.54 (m, H-10, 1H), 8.49 (dd, *J* = 7.8, 1.7 Hz, H-4, 1H), 7.62 (dd, *J* = 7.8, 4.7 Hz, H-3, 1H), 7.51 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.42 (m, H-9, 1H), 4.83 (m, H-12, 2H), 2.75 (m, H-13, 2H), 2.38 (s, H-15/15', 6H).

**^{13}C NMR (100 MHz, CDCl$_3$)** δ 179.1 (C-11), 177.7 (C-5), 154.0 (C-2), 149.8 (C-11a), 139.7 (C-6a), 134.5 (C-4), 134.2 (C-5a), 130.3 (C-4a), 127.9 (C-8), 126.5 (C-3), 124.9 (C-9), 124.4 (C-10), 124.2 (C-10a), 119.8 (C-10b), 111.0 (C-7), 58.6 (C-13), 45.9 (C-15/15'), 43.6 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -71.7 (N-1), -238.4 (N-6), -358.0 (N-14).

6. 2. 8   1-(Methoxymethyl)-1*H*-indole-3-carbaldehyde (8)

NaH (60 % dispersion in mineral oil) (1.26 g, 31.5 mmol) was suspended in dry THF (20 mL). The mixture was cooled to -23 °C and 1*H*-indole-3-carbaldehyde (4.35 g, 30 mmol) in dry THF (70 mL) was added dropwise. After warming to room temperature and stirring for 30 min, the solution was cooled to -23 °C and chloro(methoxy)methane (2.50 mL, 33 mmol) was added, and the mixture was allowed to warm to ro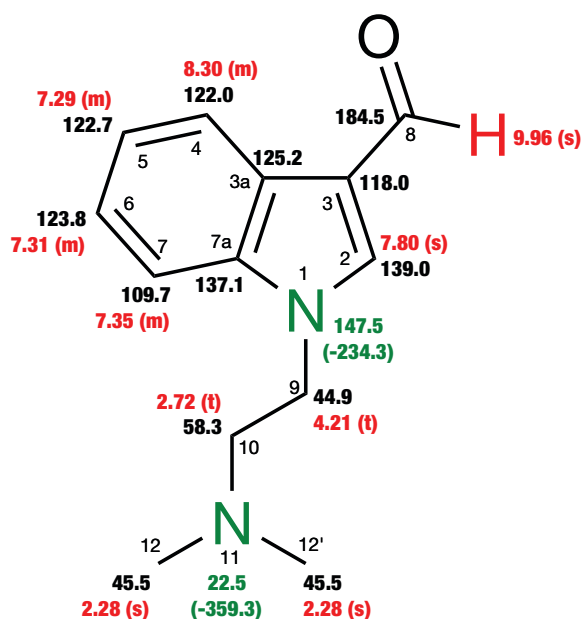om temperature. The mixture was poured into a vigorously stirred cold 5 % NaHCO₃ solution (30 mL) and extracted with diethyl ether. The combined organic layers were washed with brine and dried over MgSO₄. The mixture was filtered and concentrated in vacuo to give a solid. The crude product was purified by recrystallisation from ethyl acetate/light petroleum (1:1, v/v) to afford 4 g (70.5 %) of **8** as beige crystals, m.p. 77-78 °C.

¹H NMR (400 MHz, CDCl₃) δ 9.96 (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.72 (s, H-2, 1H), 7.47 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 5.40 (s, H-9, 2H), 3.23 (s, H-10, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 184.7 (C-8), 138.5 (C-2), 136.9 (C-7a), 125.2 (C-3a), 124.2 (C-6), 123.1 (C-5), 121.7 (C-4), 118.6 (C-3), 110.5 (C-7), 77.9 (C-9), 56.0 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -228.6 (N-1).
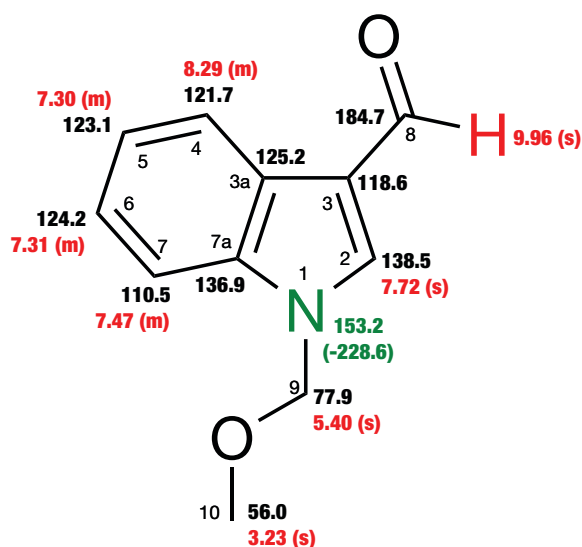
6. 2. 9   *N,N*-Diethylpyridine-2-carboxamide (9)
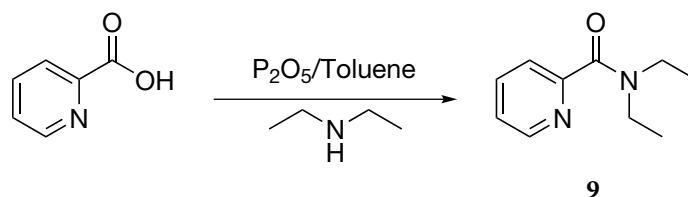
To a solution of pyridine-2-carboxylic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). Then the mixture was refluxed for 4 h, cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over Na_2SO_4 and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 102-110 °C at < 1 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 20 g (69.0 %) of a viscous yellow oil.
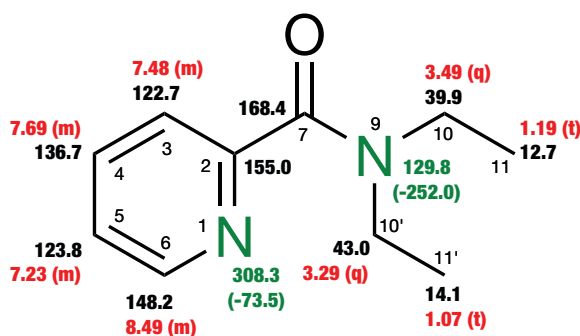
¹H NMR (400 MHz, CDCl₃) δ 8.49 (m, H-6, 1H), 7.69 (m, H-4, 1H), 7.48 (m, H-3, 1H), 7.23 (m, H-5, 1H), 3.49 (q, *J* = 7.1 Hz, H-10, 2H), 3.29 (q, *J* = 7.1 Hz, H-10', 2H), 1.19 (t, *J* = 7.1 Hz, H-11, 3H), 1.07 (t, *J* = 7.1 Hz, H-11', 3H).

¹³C NMR (100 MHz, CDCl₃) δ 168.4 (C-7), 155.0 (C-2), 148.2 (C-6), 136.7 (C-4), 123.8 (C-5), 122.7 (C-3), 43.0 (C-10'), 39.9 (C-10), 14.1 (C-11'), 12.7 (C-11).

¹⁵N NMR (40 MHz, CDCl₃) δ -73.5 (N-1), -252.0 (N-9).

6. 2. 10   10-(Methoxymethyl)-5*H*-pyrido[2,3-*b*]carbazole-5,11(10*H*)-dione (10)
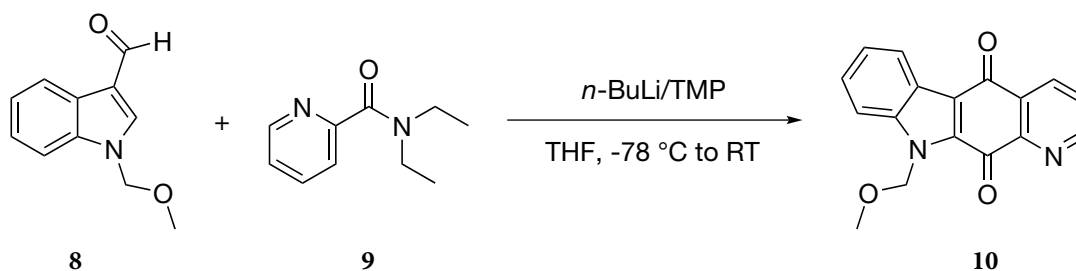
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidi-ne (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of **9** (1.00 g, 5.61 mmol, 1 eq.) and **8** (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish an orange residue. The residue was purified by recrystallisation from ethyl acetate to give 579 mg (35.3 %) of **10** as orange needles, m.p. 196-198 °C.

HRMS: m/z calculated for C$_{17}$H$_{13}$N$_2$O$_3$ ([M+H]$^+$): 293.0921; found: 293.0918.
HRMS: m/z calculated for C$_{17}$H$_{12}$N$_2$NaO$_3$ ([M+Na]$^+$): 315.0740; found: 315.0739.
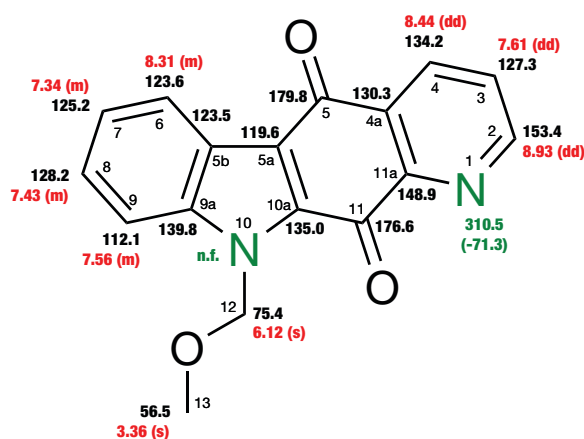
¹H NMR (400 MHz, CDCl₃) δ 8.93 (dd, *J* = 4.7, 1.7 Hz, H-2, 1H), 8.44 (dd, *J* = 7.8, 1.7 Hz, H-4, 1H), 8.31 (m, H-6, 1H), 7.61 (dd, *J* = 7.8, 4.7 Hz, H-3, 1H), 7.56 (m, H-9, , 1H), 7.43 (m, H-8, 1H), 7.34 (m, H-7, 1H), 6.12 (s, H-12, 2H), 3.36 (s, H-13, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 179.8 (C-5), 176.6 (C-11), 153.4 (C-2), 148.9 (C-11a), 139.8 (C-9a), 135.0 (C-10a), 134.2 (C-4), 130.3 (C-4a), 128.2 (C-8), 127.3 (C-3), 125.2 (C-7), 123.6 (C-6), 123.5 (C-5b), 119.6 (C-5a), 112.1 (C-9), 75.3 (C-12), 56.5 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -71.3 (N-1), not found (N-10).

6. 2. 11   5*H*-Pyrido[2,3-*b*]carbazole-5,11(10*H*)-dione (11)
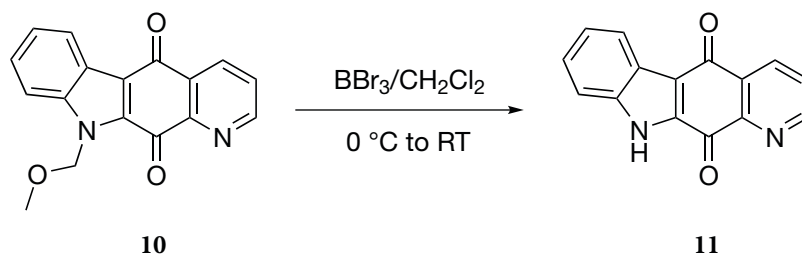
To an ice-cold solution of **10** (547 mg, 1.87 mmol) in dry CH_2Cl_2 (120 mL) a solution of BBr_3 (1M in CH_2Cl_2, 2.24 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (120 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. Recrystallisation from ethyl acetate afforded 542 mg (95.0 %) of **11** as orange crystals, m.p. 317-319 °C.

HRMS: m/z calculated for $C_{15}H_9N_2O_2$ ([M+H]$^+$): 249.0659; found: 249.0659.
HRMS: m/z calculated for $C_{15}H_8N_2NaO_2$ ([M+Na]$^+$): 271.0478; found: 271.0479.
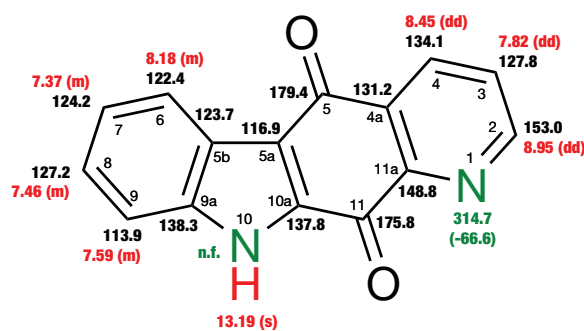
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, H-10, 1H), 8.95 (dd, *J* = 4.7, 1.7 Hz, H-2, 1H), 8.45 (dd, *J* = 7.8, 1.7 Hz, H-4, 1H), 8.18 (m, H-6, 1H), 7.82 (dd, *J* = 7.8, 4.7 Hz, H-3, 1H), 7.59 (m, H-9, 1H), 7.46 (m, H-8, 1H), 7.37 (m, H-7, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 179.4 (C-5), 175.8 (C-11), 153.0 (C-2), 148.8 (C-11a), 138.3 (C-9a), 137.8 (C-10a), 134.1 (C-4), 131.2 (C-4a), 127.8 (C-3), 127.2 (C-8), 124.2 (C-7), 123.7 (C-5b), 122.4 (C-6), 116.9 (C-5a), 113.9 (C-9).

^{15}N NMR (40 MHz, DMSO-d_6) δ -66.6 (N-1), not found (N-10).

6. 2. 12 10-[2-(Dimethylamino)ethyl]-5*H*-pyrido[2,3-*b*]carbazole-5,11(10*H*)-dione (12)
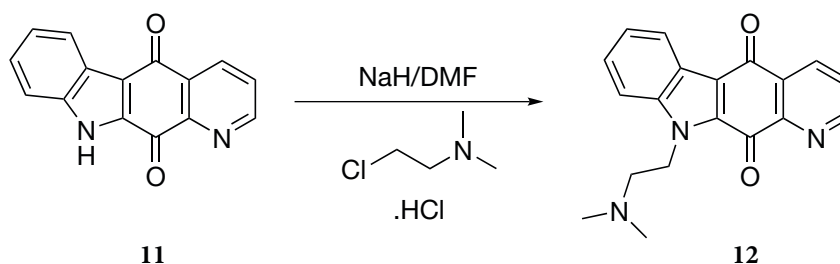
NaH (528 mg of a 60 % dispersion in mineral oil, 13.2 mmol) was suspended in dry DMF (18 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **11** (547 mg, 2.2 mmol) in dry DMF (24 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N,N*-dimethylethanamine hydrochloride (634 mg, 4.4 mmol) in dry DMF (30 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (350 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 240 mg (29.4 %) of **12** as orange-brown crystals, m.p. 136-138 °C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_2$ ([M+H]$^+$): 320.1394; found: 320.1396.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_2$ ([M+Na]$^+$): 342.1213; found: 342.1214.
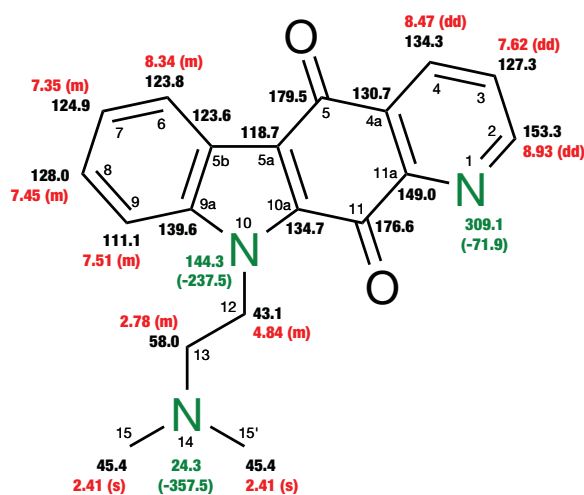
¹H NMR (500 MHz, CDCl₃) δ 8.93 (dd, *J* = 4.6, 1.7 Hz, H-2, 1H), 8.47 (dd, *J* = 7.8, 1.6 Hz, H-4, 1H), 8.34 (m, H-6, 1H), 7.62 (dd, *J* = 7.8, 4.6 Hz, H-3, 1H), 7.51 (m, H-9, 1H), 7.45 (m, H-8, 1H), 7.35 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.78 (m, H-13, 2H), 2.41 (s, H-15/15ʻ, 6H).

¹³C NMR (125 MHz, CDCl₃) δ 179.5 (C-5), 176.6 (C-11), 153.3 (C-2), 149.0 (C-11a), 139.6 (C-9a), 134.7 (C-10a), 134.3 (C-4), 130.7 (C-4a), 128.0 (C-8), 127.3 (C-3), 124.9 (C-7), 123.8 (C-6), 123.6 (C-5b), 118.7 (C-5a), 111.1 (C-9), 58.0 (C-13), 45.4 (C-15ʻ/15), 43.1 (C-12).

¹⁵N NMR (50 MHz, CDCl₃) δ -71.9 (N-1), -237.5 (N-10), -357.5 (N-14).

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6. 2. 13 *N,N*-Diethylnicotinamide (13)
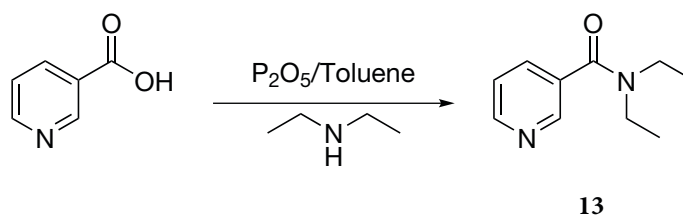
To a solution of nicotinic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). Then the mixture was refluxed for 4 h, cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1/1, v/v). The combined organic layers were dried over Na_2SO_4 and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 115 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 30 g (84.2 %) of **13** as viscous yellow oil.
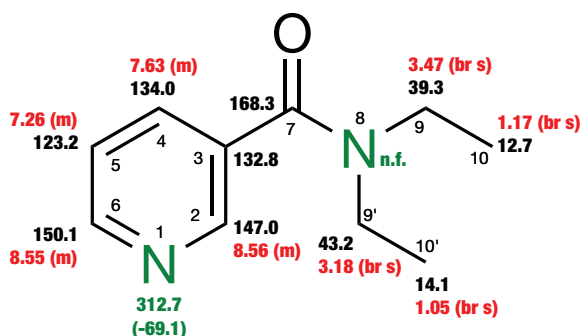
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.55 (m, H-6, 1H), 7.63 (m, H-4, 1H), 7.26 (m, H-5, 1H), 3.47 (br s, H-9, 2H), 3.18 (br s, H-9', 2H), 1.17 (br s, H-10, 3H), 1.05 (br s, H-10', 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 168.3 (C-7), 150.1 (C-6), 147.0 (C-2), 134.0 (C-4), 132.8 (C-3), 123.2 (C-5), 43.2 (C-9'), 39.3 (C-9), 14.1 (C-10'), 12.7 (C-10).

^{15}N NMR (40 MHz, CDCl$_3$) δ -69.1 (N-1), not found (N-8).

6. 2. 14 10-(Methoxymethyl)-5*H*-pyrido[3,4-*b*]carbazole-5,11(10*H*)-dione (14)
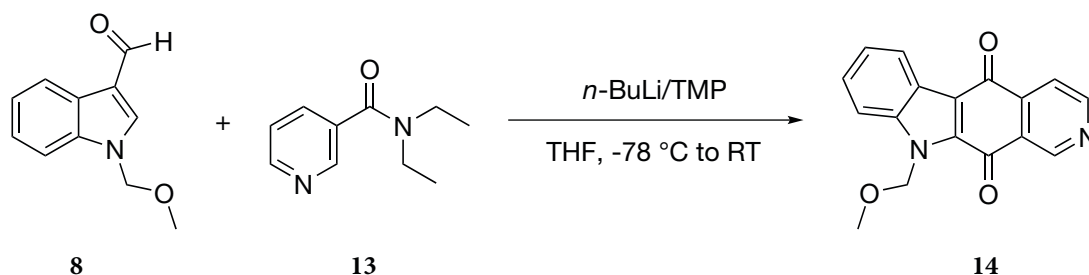
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of **13** (1.00 g, 5.61 mmol, 1 eq.) and **8** (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a dark orange residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 526 mg (32.1 %) of **14** as orange needles, m.p. 178-180 °C.

HRMS: m/z calculated for $C_{17}H_{12}N_2NaO_3$ ([M+Na]$^+$): 315.0740; found: 315.0736.
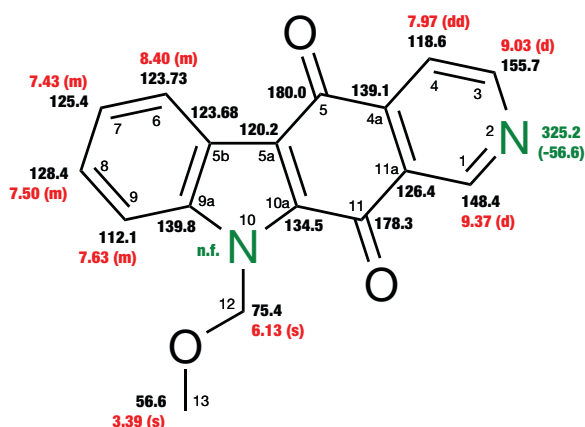
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.37 (d, *J* = 0.7 Hz, H-4, 1H), 9.03 (d, *J* = 4.9 Hz, H-2, 1H), 8.40 (m, H-10, 1H), 7.97 (dd, *J* = 4.9, 0.7 Hz, H-1, 1H), 7.63 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.43 (m, H-9, 1H), 6.13 (s, H-12, 2H), 3.39 (s, H-13, 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 180.0 (C-5), 178.3 (C-11), 155.7 (C-3), 148.4 (C-1), 139.8 (C-9a), 139.1 (C-4a), 134.5 (C-10a), 128.4 (C-8), 126.4 (C-11a), 125.4 (C-7), 123.73 (C-6), 123.68 (C-5b), 120.2 (C-5a), 118.6 (C-4), 112.1 (C-9), 75.4 (C-12), 56.6 (C-13).

^{15}N NMR (40 MHz, CDCl$_3$) δ -56.6 (N-2), not found (N-10).

6. 2. 15   5*H*-Pyrido[3,4-*b*]carbazole-5,11(10*H*)-dione (15)
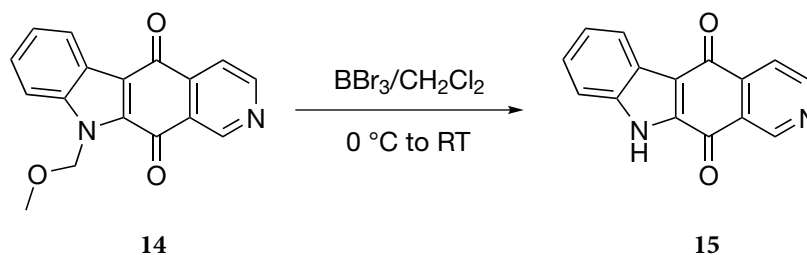
To an ice-cold solution of **14** (223 mg, 0.76 mmol) in dry CH_2Cl_2 (40 mL) a solution of BBr_3 (1M in CH_2Cl_2, 0.92 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (40 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. Recrystallisation from ethyl acetate afforded 150 mg (79.5 %) of **15** as orange crystals, m.p. 318-320 °C.

HRMS: m/z calculated for $C_{15}H_9N_2O_2$ ([M+H]$^+$): 249.0659; found: 249.0655.
HRMS: m/z calculated for $C_{15}H_8N_2NaO_2$ ([M+Na]$^+$): 271.0478; found: 271.0474.
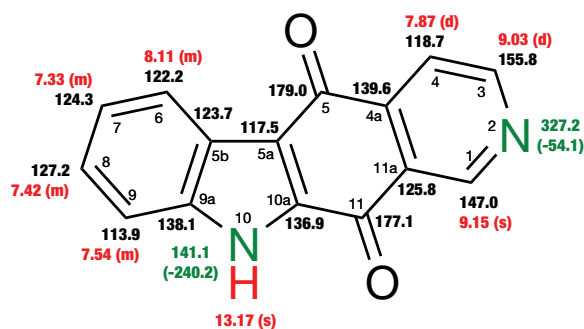
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.17 (s, H-10, 1H), 9.15 (s, H-1, 1H), 9.03 (d, *J* = 4.9 Hz, H-3, 1H), 8.11 (m, H-6, 1H), 7.87 (d, *J* = 4.9 Hz, H-4, 1H), 7.54 (m, H-9, 1H), 7.42 (m, H-8, 1H), 7.33 (m, H-7, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 179.0 (C-5), 177.1 (C-11), 155.8 (C-3), 147.0 (C-1), 139.6 (C-4a), 138.1 (C-9a), 136.9 (C-10a), 127.2 (C-8), 125.8 (C-11a), 124.3 (C-7), 123.7 (C-5b), 122.2 (C-6), 118.7 (C-4), 117.5 (C-5a), 113.9 (C-9).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -54.1 (N-2), -240.2 (N-10).

6. 2. 16 10-[2-(Dimethylamino)ethyl]-5*H*-pyrido[3,4-*b*]carbazole-5,11(10*H*)-dione (16)

15   **16**
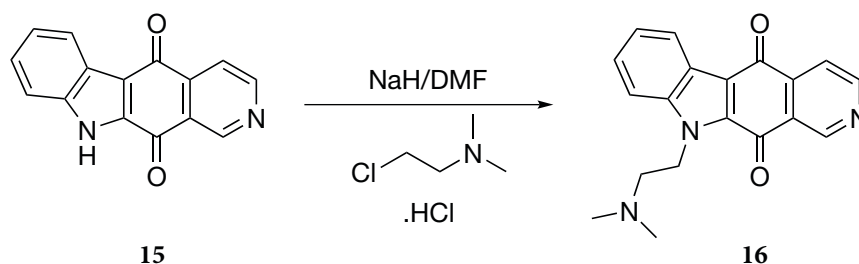
NaH (296 mg of a 60 % dispersion in mineral oil, 7.2 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **15** (463 mg, 1.9 mmol) in dry DMF (27 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N,N*-dimethylethanamine hydrochloride (533 mg, 3.7 mmol) in dry DMF (22 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 260 mg (43.6 %) of **16** as orange crystals m.p. 149-151°C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_2$ ([M+H]$^+$): 320.1394; found: 320.1393.
**HRMS:** m/z calculated for $C_{19}H_{17}N_3NaO_2$ ([M+Na]$^+$): 342.1213; found: 342.1211.

¹H NMR (500 MHz, CDCl₃) δ 9.37 (d, *J* = 0.6 Hz, H-1, 1H), 9.02 (d, *J* = 4.9 Hz, H-3, 1H), 8.40 (m, H-6, 1H), 7.98 (dd, *J* = 4.9, 0.7 Hz, H-4, 1H), 7.53 (m, H-9, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.77 (m, H-13, 2H), 2.40 (s, H-15/15', 6H).

¹³C NMR (125 MHz, CDCl₃) δ 179.6 (C-5), 178.1 (C-11), 155.5 (C-3), 148.3 (C-1), 139.5 (C-9a/4a), 134.3 (C-10a), 128.0 (C-8), 126.5 (C-11a), 125.1 (C-7), 123.9 (C-6), 123.8 (C-5b), 119.2 (C-5a), 118.6 (C-4), 111.2 (C-9), 58.5 (C-13), 45.7 (C-15'/ 15), 43.5 (C-12).

**¹⁵N NMR (50 MHz, CDCl₃)** δ -57.9 (N-2), -237.2 (N-10), -358.5 (N-14).

6. 2. 17 10-[2-(Dimethylamino)ethyl]-5*H*-pyrido[3,4-*b*]carbazole-5,11(10*H*)-dione (16)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **27** (1.21 g, 5.61 mmol, 1 eq.) and **17** (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a orange-brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to to obtain 618 mg (34.5 %) of **16** as orange crystals, m.p. 149-151°C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_2$ ([M+H]$^+$): 320.1394; found: 320.1393.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_2$ ([M+Na]$^+$): 342.1213; found: 342.1211.

**¹H NMR (500 MHz, CDCl₃)** δ 9.37 (d, *J* = 0.6 Hz, H-1, 1H), 9.02 (d, *J* = 4.9 Hz, H-3, 1H), 8.40 (m, H-6, 1H), 7.98 (dd, *J* = 4.9, 0.7 Hz, H-4, 1H), 7.53 (m, H-9, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-7, 1H), 4.84 (m, H-12, 2H), 2.77 (m, H-13, 2H), 2.40 (s, H-15/15', 6H).

¹³C NMR (125 MHz, CDCl₃) δ 179.6 (C-5), 178.1 (C-11), 155.5 (C-3), 148.3 (C-1), 139.5 (C-9a/4a), 134.3 (C-10a), 128.0 (C-8), 126.5 (C-11a), 125.1 (C-7), 123.9 (C-6), 123.8 (C-5b), 119.2 (C-5a), 118.6 (C-4), 111.2 (C-9), 58.5 (C-13), 45.7 (C-15'/ 15), 43.5 (C-12).

¹⁵N NMR (50 MHz, CDCl₃) δ -57.9 (N-2), -237.2 (N-10), -358.5 (N-14).

6. 2. 18   *N,N*-Diethylisonicotinamide (17)
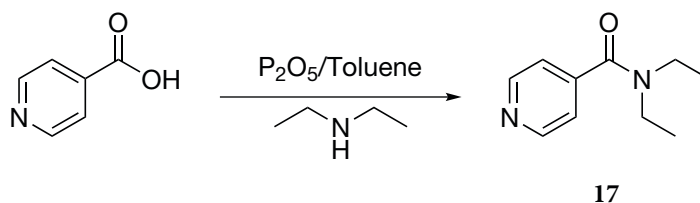
To a solution of isonicotinic acid (24.5 g, 0.2 mol) and diethylamine (74 mL, 0.7 mol) in dry toluene (100 mL) was added phosphoric anhydride (31.2 g, 0.22 mol) in portions (exothermic). The mixture was refluxed for 4 h, and then cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over Na_2SO_4 and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 105 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 28.2 g (78.5 %) of **17** as viscous orange oil.
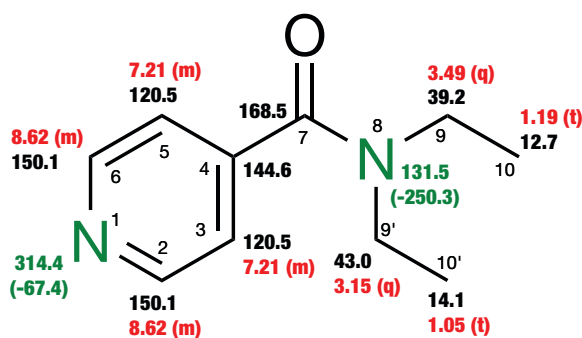
¹H NMR (400 MHz, CDCl₃) δ 8.62 (m, H-2/6, 2H), 7.21 (m, H-3/5, 2H), 3.49 (q, *J* = 7.1 Hz, H-9, 2H), 3.15 (q, *J* = 7.0 Hz, H-9', 2H), 1.19 (t, *J* = 7.1 Hz, H-10, 3H), 1.05 (t, *J* = 7.1 Hz, H-10', 3H).

¹³C NMR (100 MHz, CDCl₃) δ 168.5 (C-7), 150.1 (C-2/6), 144.6 (C-4), 120.5 (C-3/5), 43.0 (C-9'), 39.2 (C-9), 14.1 (C-10'), 12.7 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -67.4 (N-1), -250.3 (N-8).

6. 2. 19   6-(Methoxymethyl)-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (18)
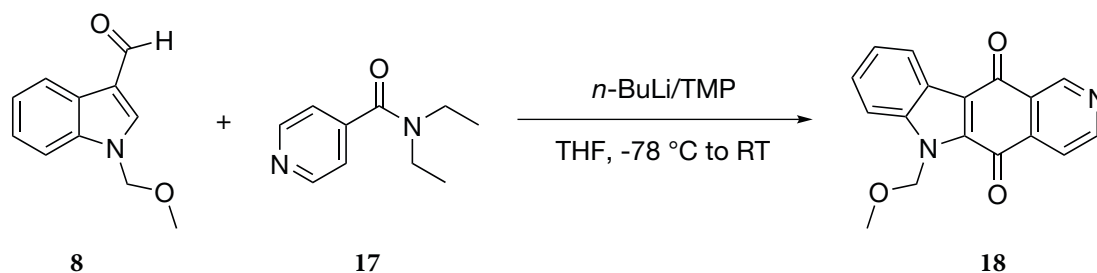
8   **17**   **18**

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **17** (1.00 g, 5.61 mmol, 1 eq.) and **8** (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a dark orange residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 540 mg (32.9 %) of **18** as orange needles, m.p. 199-201 °C.

HRMS: m/z calculated for $C_{17}H_{12}N_2NaO_3$ ([M+Na]$^+$): 315.0740; found: 315.0740.
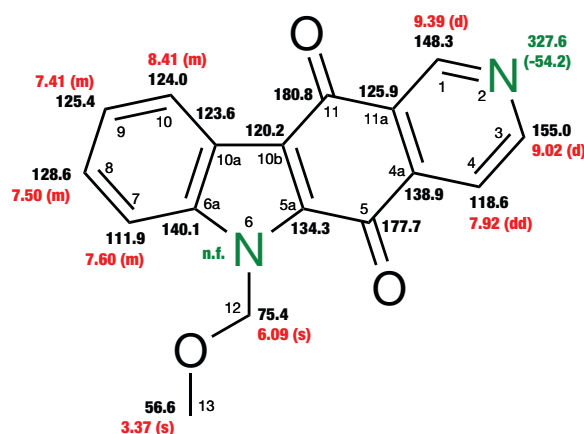
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.39 (d, *J* = 0.7 Hz, H-1, 1H), 9.02 (d, *J* = 5.0 Hz, H-3, 1H), 8.41 (m, H-10, 1H), 7.92 (dd, *J* = 5.0, 0.8 Hz, H-4, 1H), 7.60 (m, H-7, 1H), 7.50 (m, H-8, 1H), 7.41 (m, H-9, 1H), 6.09 (s, H-12, 2H), 3.37 (s, H-13, 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 180.8 (C-11), 177.7 (C-5), 155.0 (C-3), 148.3 (C-1), 140.1 (C-6a), 138.9 (C-4a), 134.3 (C-5a), 128.6 (C-8), 125.9 (C-11a), 125.4 (C-9), 124.0 (C-10), 123.6 (C-10a), 120.2 (C-10b), 118.6 (C-4), 111.9 (C-7), 75.4 (C-12), 56.6 (C-13).

^{15}N NMR (40 MHz, CDCl$_3$) δ -54.2 (N-2), not found (N-6).

6. 2. 20   5*H*-Pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (19)
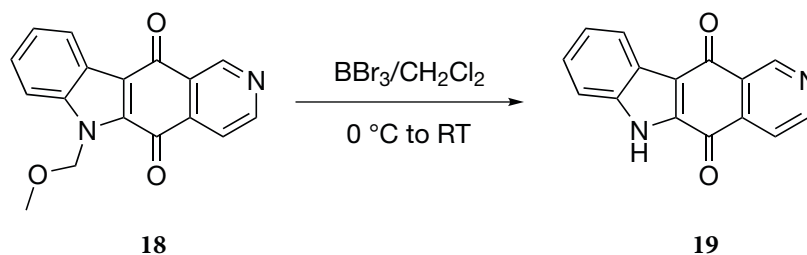
To an ice-cold solution of **18** (540 mg, 1.85 mmol) in dry CH_2Cl_2 (120 mL) a solution of BBr_3 (1M in CH_2Cl_2, 2.24 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (120 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. Recrystallisation from ethyl acetate afforded 365 mg (79.6 %) of **19** as orange crystals, m.p. 345-347 °C.

HRMS: m/z calculated for $C_{15}H_9N_2O_2$ ([M+H]$^+$): 249.0659; found: 249.0656.
HRMS: m/z calculated for $C_{15}H_8N_2NaO_2$ ([M+Na]$^+$): 271.0478; found: 271.0477.
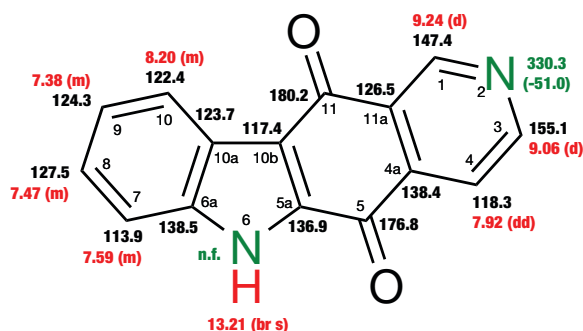
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.21 (s, 1H), 9.24 (d, *J* = 0.7 Hz, H-1, 1H), 9.06 (d, *J* = 4.9 Hz, H-3, 1H), 8.20 (m, H-10, 1H), 7.92 (dd, *J* = 4.9, 0.8 Hz, H-4, 1H), 7.59 (m, H-7, 1H), 7.47 (m, H-8, 1H), 7.38 (m, H-9, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 180.2 (C-11), 176.8 (C-5), 155.1 (C-3), 147.4 (C-1), 138.5 (C-6a), 138.4 (C-4a), 136.9 (C-5a), 127.5 (C-8), 126.5 (C-11a), 124.3 (C-9), 123.7 (C-10a), 122.4 (C-10), 118.3 (C-4), 117.4 (C-10b), 113.9 (C-7).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -51.0 (N-2), not found (N-6).

6. 2. 21   6-[2-(Dimethylamino)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (20)

NaH (288 mg of a 60 % dispersion in mineral oil, 7.2 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **19** (446 mg, 1.8 mmol) in dry DMF (26 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (513 mg, 3.6 mmol) in dry DMF (22 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (280 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (8:2, v/v) to obtain 170 mg (29.6 %) of **20** as orange crystals, m.p. 157-159 °C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_2$ ([M+H]$^+$): 320.1394; found: 320.1394.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_2$ ([M+Na]$^+$): 342.1213; found: 342.1213.

^{1}H NMR (500 MHz, CDCl$_3$) δ 9.40 (d, *J* = 0.7 Hz, H-1, 1H), 9.01 (d, *J* = 4.9 Hz, H-3, 1H), 8.42 (m, H-10, 1H), 7.92 (dd, *J* = 4.9, 0.8 Hz, H-4, 1H), 7.49 (m, H-7, 1H), 7.48 (m, H-8, 1H), 7.40 (m, H-9, 1H), 4.80 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.37 (s, H-15/15', 6H).

^{13}C NMR (125 MHz, CDCl$_3$) δ 180.5 (C-11), 177.6 (C-5), 154.8 (C-3), 148.3 (C-1), 139.8 (C-6a), 139.0 (C-4a), 134.2 (C-5a), 128.2 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.5 (C-4), 111.1 (C-7), 58.5 (C-13), 45.8 (C-15'/15), 43.5 (C-12).

^{15}N NMR (50 MHz, CDCl$_3$) δ -55.0 (N-2), -237.2 (N-6), -358.7 (N-14).

6. 2. 22 5*H*-Pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione 2-oxide (21)

19   **21**

To a suspension of **19** (400 mg, 1.61 mmol) in CH_2Cl_2 (500 mL), was added in one portion *m*-CPBA (57-86 % peracid, 2.1 g, excess) in CH_2Cl_2 (250 mL). The mixture was refluxed overnight. The resulting orange mixture was washed with a saturated $NaHCO_3$ solution (3 x 15 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3 x) and the combined organic layers were dried over Na_2SO_4 and evaporated to give an orange-red solid which was purified by column chromatography eluting with ethyl acetate to afford 410 mg (96.3 %) of **21** as orange-red crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{15}H_9N_2O_3$ ([M+H]$^+$): 265.0608; found: 265.0607.
HRMS: m/z calculated for $C_{15}H_8N_2NaO_3$ ([M+Na]$^+$): 287.0427; found: 287.0427.
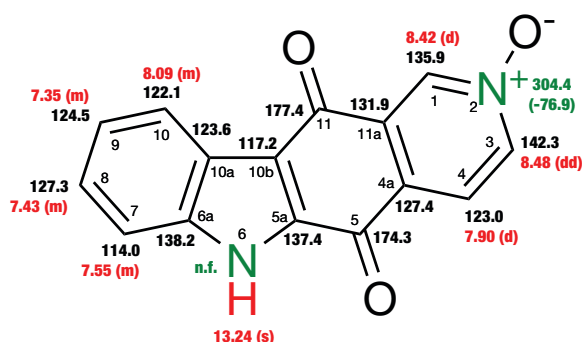
^{1}H NMR (400 MHz, DMSO-*d_6*) δ 13.24 (s, H-6, 1H), 8.48 (dd, *J* = 6.6, 1.9 Hz, H-3, 1H), 8.42 (d, *J* = 1.9 Hz, H-1, 1H), 8.09 (m, H-10, 1H), 7.90 (d, *J* = 6.6, H-4, 1H), 7.55 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.35 (m, H-9, 6H).

^{13}C NMR (100 MHz, DMSO-*d_6*) δ 177.4 (C-11), 174.3 (C-5), 142.3 (C-3), 138.2 (C-6a), 137.4 (C-5a), 135.9 (C-1), 131.9 (C-11a), 127.4 (C-4a), 127.3 (C-8), 124.5 (C-9), 123.6 (C-10a), 123.0 (C-4), 122.1 (C-10), 117.2 (C-10b), 114.0 (C-7).

^{15}N NMR (40 MHz, DMSO-*d_6*) δ -76.9 (N-2), not found (N-6).

6. 2. 23   6-[2-(Dimethylamino)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione 2-oxide (22)

21   NaH/DMF   **22**
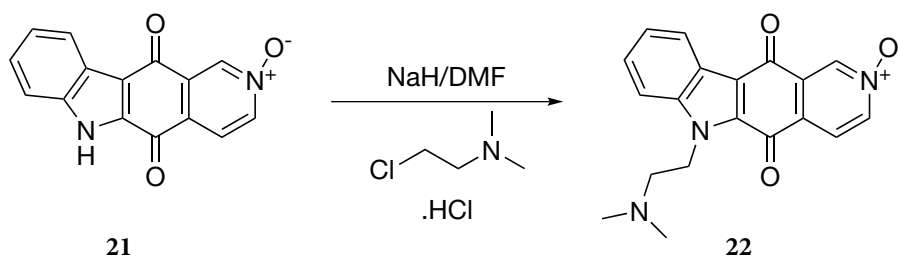
NaH (242 mg of a 60 % dispersion in mineral oil, 6.0 mmol) was suspended in dry DMF (15 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **21** (400 mg, 1.51 mmol) in dry DMF (25 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N,N*-dimethylethanamine hydrochloride (435 mg, 3.0 mmol) in dry DMF (25 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/light petroleum (2:1, v/v) followed by ethyl acetate/methanol (9:1, v/v) to obtain 131 mg (26.0 %) of **22** as orange crystals, m.p. 154-156 °C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_3$ ([M+H]$^+$): 336.1343; found: 336.1345.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_3$ ([M+Na]$^+$): 358.1162; found: 358.1166.
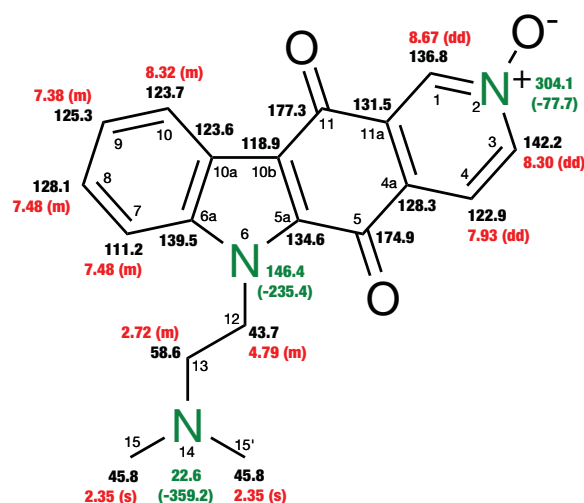
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.67 (dd, *J* = 1.9, 0.5 Hz, H-1, 1H), 8.32 (m, H-10, 1H), 8.30 (dd, *J* = 6.7, 1.9 Hz, H-3, 1H), 7.93 (dd, *J* = 6.7, 0.5 Hz, H-4, 1H), 7.48 (m, H-7/8, 2H), 7.38 (m, H-9, 1H), 4.79 (m, H-12, 2H), 2.72 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 177.3 (C-11), 174.8 (C-5), 142.2 (C-3), 139.5 (C-6a), 136.8 (C-1), 134.6 (C-5a), 131.5 (C-11a), 128.3 (C-4a), 128.1 (C-8), 125.3 (C-9), 123.7 (C-10), 123.6 (C-10a), 122.9 (C-4), 118.9 (C-10b), 111.2 (C-7), 58.6 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -77.7 (N-2), -235.4 (N-6), -359.2 (N-14).

6. 2. 24 *N,N*-Diethyl-2-pyrazinecarboxamide (23)

23
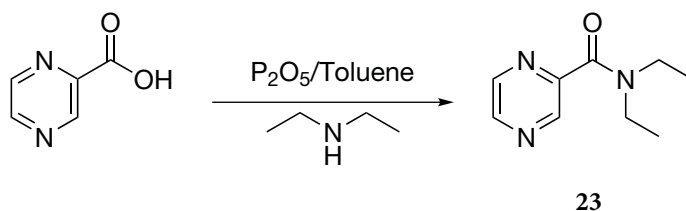
To a solution of 2-pyrazinecarboxylic acid (6 g, 48.3 mmol) and diethylamine (17.6 mL, 0.17 mol) in dry toluene (250 mL) was added phosphoric anhydride (7.54 g, 0.53 mol) in portions (exothermic). The mixture was refluxed for 4 h, and then cooled in an ice-bath while an excess of aqueous 10 % NaOH was added. The toluene layer was separated, and the aqueous layer was thoroughly extracted with a mixture of ether and toluene (1:1, v/v). The combined organic layers were dried over Na_2SO_4 and the solvents removed in vacuo. The dark brown, oily residue was purified by ball-tube distillation (b.p. 110 °C at < 0.5 Torr) or by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) affording 5.56 g (64.2 %) of **23** as viscous yellow oil.
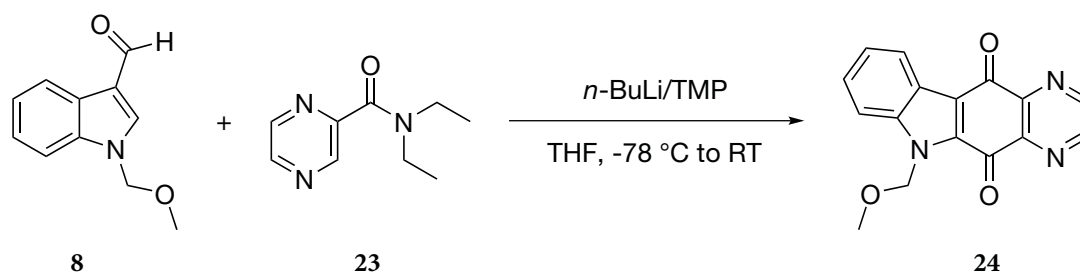
¹H NMR (400 MHz, CDCl₃) δ 8.81 (d, *J* = 1.5 Hz, H-3, 1H), 8.53 (d, *J* = 2.6 Hz, H-5, 1H), 8.46 (dd, *J* = 2.6, 1.5 Hz, H-6, 1H), 3.50 (q, *J* = 7.1 Hz, H-10, 2H), 3.32 (q, *J* = 7.1 Hz, H-10ʻ, 2H), 1.19 (t, *J* = 7.1 Hz, H-11, 3H), 1.12 (t, *J* = 7.1 Hz, H-11ʻ, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 165.9 (C-7), 150.2 (C-2), 144.8 (C-5), 144.7 (C-3), 142.4 (C-6), 43.1 (C-9ʻ), 40.3 (C-9), 14.2 (C-10ʻ), 12.6 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -55.4 (N-1), -57.2 (N-4), -250.0 (N-8).

6. 2. 25   6-(Methoxymethyl)-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (24)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **23** (1.01 g, 5.61 mmol, 1 eq.) and **8** (1.06 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (1:1, v/v) followed by recrystallisation from ethyl acetate to give 180 mg (10.9 %) of **24** as orange crystals, m.p. 250-252 °C.

HRMS: m/z calculated for $C_{16}H_{11}N_3NaO_3$ ([M+Na]$^+$): 316.0693; found: 316.0691.
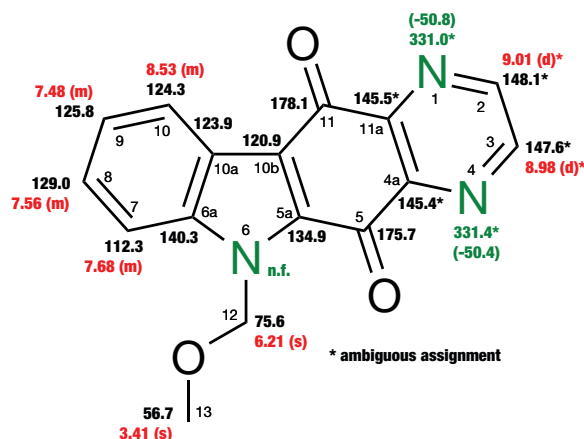
^{1}H NMR (500 MHz, CDCl$_3$) δ 9.01 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.98 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.53 (m, H-10, 1H), 7.68 (m, H-7, 1H), 7.56 (m, H-8, 1H), 7.48 (m, H-9, 1H), 6.21 (s, H-12, 2H), 3.41 (s, H-13, 3H).

^{13}C NMR (125 MHz, CDCl$_3$) δ 178.1 (C-11), 175.7 (C-5), 148.1 (C-2*/3*), 147.6 (C-2*/3*), 145.5 (C-4a*/11a*), 145.4 (C-4a*/11a*), 140.3 (C-6a), 134.9 (C-5a), 129.0 (C-8), 125.8 (C-9), 124.3 (C-10), 123.9 (C-10a), 120.9 (C-10b), 112.3 (C-7), 75.6 (C-12), 56.7 (C-13).

^{15}N NMR (40 MHz, CDCl$_3$) δ -50.4 (N-1*/4*), -50.8 (N-1*/N-4*), not found (N-6).

6. 2. 26   5*H*-Pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (25)
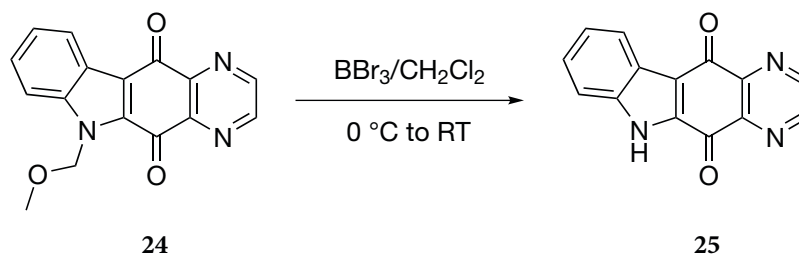
To an ice-cold solution of **24** (500 mg, 1.70 mmol) in dry CH_2Cl_2 (150 mL) a solution of BBr_3 (1M in CH_2Cl_2, 2.1 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (150 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. Recrystallisation from ethyl acetate afforded 320 mg (75.3 %) of **25** as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{14}H_7N_3NaO_2$ ([M+Na]$^+$): 272.0430; found: 272.0431.
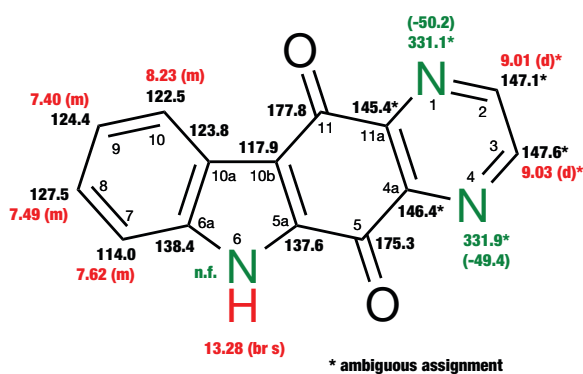
^{1}H NMR (400 MHz, DMSO-*d₆*) δ 13.28 (br s, H-10, 1H), 9.03 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 9.01 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.23 (m, H-10, 1H), 7.62 (m, H-7, 1H), 7.49 (m, H-8, 1H), 7.40 (m, H-9, 1H).

^{13}C NMR (100 MHz, DMSO-*d₆*) δ 177.8 (C-11), 175.3 (C-5), 147.6 (C-3*/2*), 147.1 (C-2*/3*), 146.4 (C-4a*/11a*), 145.4 (C-4a*/11a*), 138.4 (C-6a), 137.6 (C-5a), 127.5 (C-8), 124.4 (C-9), 123.8 (C-10a), 122.5 (C-10), 117.9 (C-10b), 114.0 (C-7).

^{15}N NMR (40 MHz, DMSO-*d₆*) δ -50.2 (N-1), -49.4 (N-4), not found (N-6).

6. 2. 27 6-[2-(Dimethylamino)ethyl]-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-di-one (26)
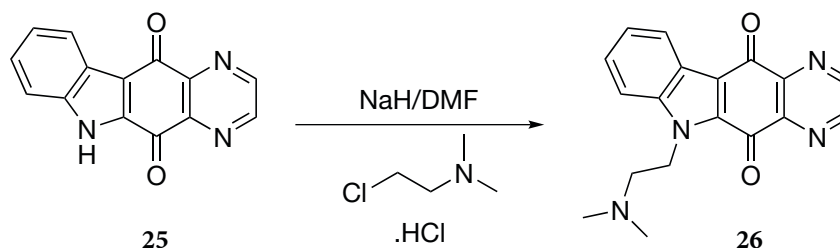
NaH (206 mg of a 60 % dispersion in mineral oil, 5.14 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **25** (320 mg, 1.28 mmol) in dry DMF (30 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (369 mg, 2.56 mmol) in dry DMF (25 mL) was added dropwise and the mixture was stirred at 75 °C overnight. After cooling down the reaction mixture was quenched with water (250 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to obtain 105 mg (25.4 %) of **26** as orange crystals, m.p. 213-215 °C.

HRMS: m/z calculated for $C_{18}H_{17}N_4O_2$ ([M+H]⁺): 321.1346; found: 321.1347.
HRMS: m/z calculated for $C_{18}H_{16}N_4NaO_2$ ([M+Na]⁺): 343.1165; found: 343.1168.
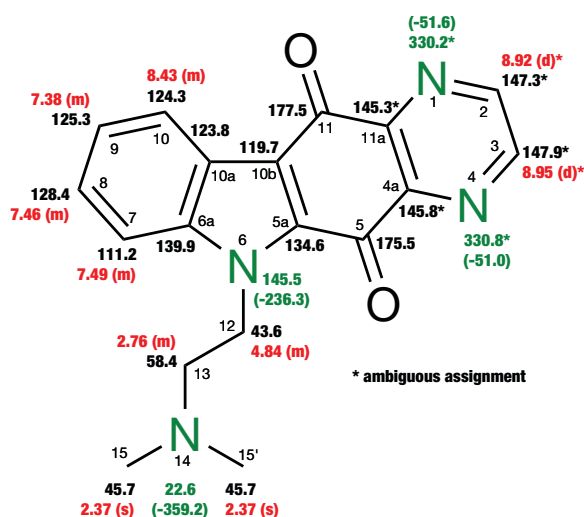
¹H NMR (400 MHz, CDCl₃) δ 8.95 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.92 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.43 (m, H-10, 1H), 7.49 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.38 (m, H-9, 1H), 4.84 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.37 (s, H-15/15', 6H).

¹³C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.5 (C-5), 147.9 (C-2*/3*), 147.3 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 134.6 (C-5a), 128.4 (C-8), 125.3 (C-9), 124.3 (C-10), 123.8 (C-10a), 119.7 (C-10b), 111.2 (C-7), 58.4 (C-13), 45.7 (C-15'/15), 43.6 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -51.6 (N-1*/4*), -51.0 (N-1*/4*), -236.3 (N-6), -359.2 (N-14).

109

6. 2. 28 1-[2-(Dimethylamino)ethyl]-1*H*-indole-3-carbaldehyde (27)
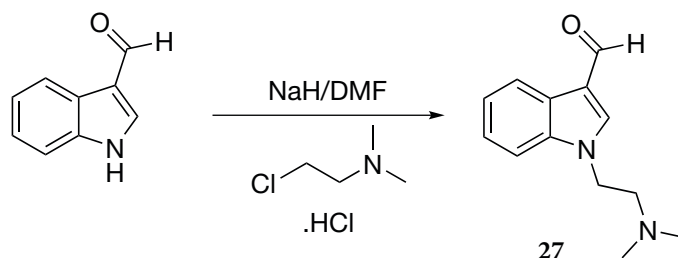
NaH (8.27 g of a 60 % dispersion in mineral oil, 206.7 mmol) was suspended in dry DMF (50 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1*H*-Indole-3-carbaldehyde (10 g, 68.9 mmol) in dry DMF (200 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 2-chloro-*N,N*-dimethylethanamine hydrochloride (14.9 g, 103.4 mmol) was added in portions and the mixture was stirred at 75 °C overnight. After cooling the reaction mixture was quenched with water (500 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to afford 11.45 g (76.8 %) of **27** as brown oil.

HRMS: m/z calculated for $C_{13}H_{17}N_2O$ ([M+H]$^+$): 217.1335; found: 217.1339.
HRMS: m/z calculated for $C_{13}H_{16}N_2NaO$ ([M+Na]$^+$): 239.1155; found: 239.1160.

¹H NMR (400 MHz, CDCl₃) δ 9.96 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.80 (s, H-2, 1H), 7.35 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.29 (m, H-5, 1H), 4.21 (t, *J* = 6.6 Hz, H-9, 2H), 2.72 (t, *J* = 6.6 Hz, H-10, 2H), 2.28 (s, H-12/12‛, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 184.5 (C-8), 139.0 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.8 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.7 (C-7), 58.3 (C-10), 45.5 (C-12/12‛), 44.9 (C-9).

¹⁵N NMR (40 MHz, CDCl₃) δ -234.3 (N-1), -359.3 (N-11).

6. 2. 29 1-[3-(Dimethylamino)propyl]-1*H*-indole-3-carbaldehyde (28)
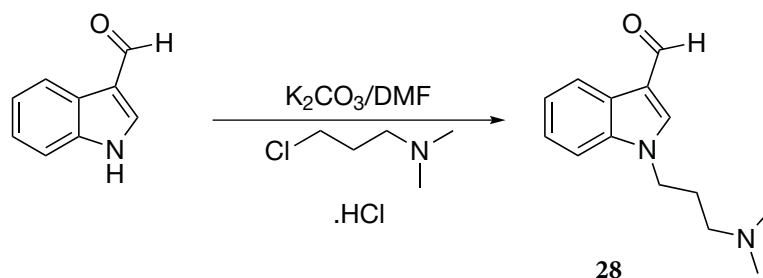
1*H*-Indole-3-carbaldehyde (5.0 g, 34.4 mmol), anhydrous K_2CO_3 (14.3 g, 103.3 mmol) and *N,N*-dimethylpropan-1-amine hydrochloride (6.53 g, 41.3 mmol) were dissolved in DMF (100 mL) and stirred at 100 °C for 24 h. After cooling down the reaction mixture was diluted with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by ethyl acetate/methanol/triethylamine (7:2:1, v/v) to afford 5.58 g (70.3 %) of **28** as viscous dark orange oil.

¹H NMR (400 MHz, CDCl₃) δ 9.96 (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.77 (s, H-2, 1H), 7.41 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 4.27 (t, *J* = 6.8 Hz, H-9, 2H), 2.30 (t, *J* = 6.8 Hz, H-11, 2H), 2.26 (s, H-13/13', 6H), 2.05 (m, H-10, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 184.5 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.3 (C-3a), 123.9 (C-6), 122.8 (C-5), 122.0 (C-4), 118.0 (C-3), 110.1 (C-7), 55.6 (C-11), 45.0 (C-13'/13), 44.5 (C-9), 27.1 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -232.1 (N-1), -356.5 (N-12).
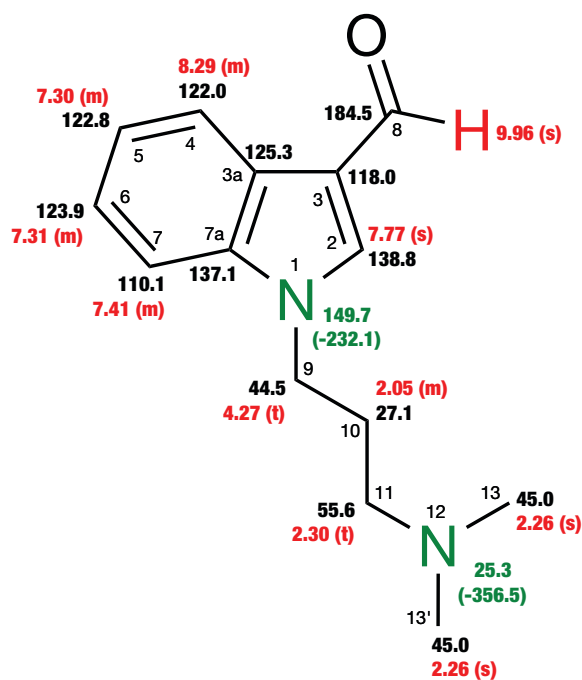
6. 2. 30 6-[3-(Dimethylamino)propyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-di-one (29)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **28** (1.29 g, 5.61 mmol, 1 eq.) and **17** (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a orange-brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to give 178 mg (9.5 %) of **29** as orange crystals, m.p. 133-135 °C.

HRMS: m/z calculated for $C_{20}H_{20}N_3O_2$ ([M+H]$^+$): 334.1550; found: 334.1549.
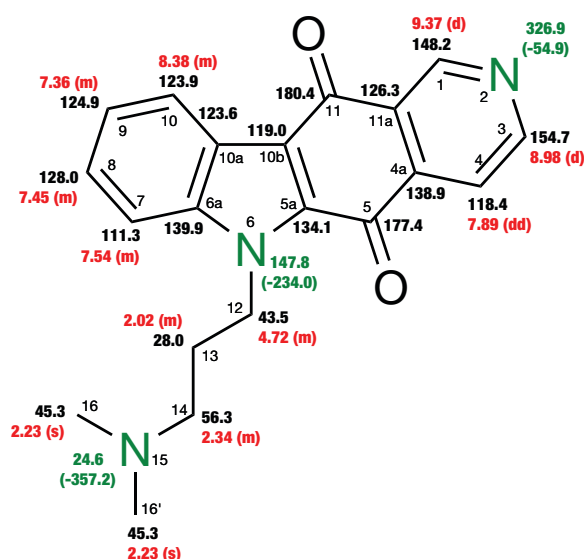
¹H NMR (400 MHz, CDCl₃) δ 9.37 (d, *J* = 0.7 Hz, H-1, 1H), 8.98 (d, *J* = 5.0 Hz, H-3, 1H), 8.38 (m, H-10, 1H), 7.89 (dd, *J* = 5.0, 0.7 Hz, H-4, 1H), 7.54 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.36 (m, H-9, 1H), 4.72 (m, H-12, 2H), 2.34 (m, H-14, 2H), 2.23 (s, H-16/16', 6H), 2.02 (m, H-13, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C-11), 177.4 (C-5), 154.7 (C-3), 148.2 (C-1), 139.9 (C-6a), 138.9 (C-4a), 134.1 (C-5a), 128.0 (C-8), 126.3 (C-11a), 124.9 (C-9), 123.9 (C-10), 123.6 (C-10a), 119.0 (C-10b), 118.4 (C-4), 111.3 (C-7), 56.3 (C-14), 45.3 (C-16'/16), 43.5 (C-12), 28.0 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ -54.9 (N-2), -234.0 (N-6), -357.2 (N-15).

### 6.2.31 6-[3-(Dimethylamino)propyl]-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (30)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of **28** (1.29 g, 5.61 mmol, 1 eq.) and **23** (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to give 160 mg (8.5 %) of **30** as orange crystals, m.p. 171-173 °C.

HRMS: m/z calculated for $C_{19}H_{19}N_4O_2$ ([M+H]$^+$): 335.1503; found: 335.1506.
HRMS: m/z calculated for $C_{19}H_{18}N_4NaO_2$ ([M+Na]$^+$): 357.1322; found: 357.1326.

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.95 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.92 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.42 (m, H-10, 1H), 7.54 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.36 (m, H-9, 1H), 4.76 (m, H-12, 2H), 2.34 (t, *J* = 6.7 Hz, H-14, 2H), 2.20 (s, H-16/16', 6H) 2.03 (m, H-13, 2H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 177.5 (C-11), 175.3 (C-5), 147.8 (C-2*/3*), 147.2 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.0 (C-6a), 134.6 (C-5a), 128.2 (C-8), 125.3 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.6 (C-10b), 111.4 (C-7), 56.3 (C-14), 45.3 (C-16/16'), 43.7 (C-12), 27.9 (C-13).

^{15}N NMR (40 MHz, CDCl$_3$) δ -51.1 (N1*/4*), -51.7 (N1*/4*), -232.0 (N-6), -357.6 (N-15).

6. 2. 32   1-[2-(Morpholin-4-yl)ethyl]-1*H*-indole-3-carbaldehyde (31)
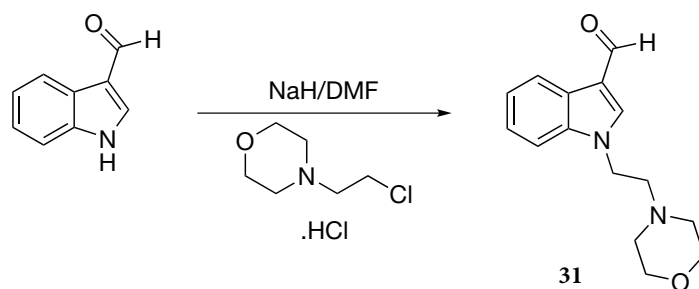
NaH (5.5 g of a 60 % dispersion in mineral oil, 137.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1*H*-Indole-3-carbaldehyde (5.0 g, 34.4 mmol) in dry DMF (50 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 4-(2-chloroethyl)morpholine hydrochloride (9.6 g, 51.6 mmol) was added in portions and the mixture was stirred at 90 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product as dark oil which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to afford 5.6 g (62.9 %) of **31** as brown solid, m.p. 63-64 °C.
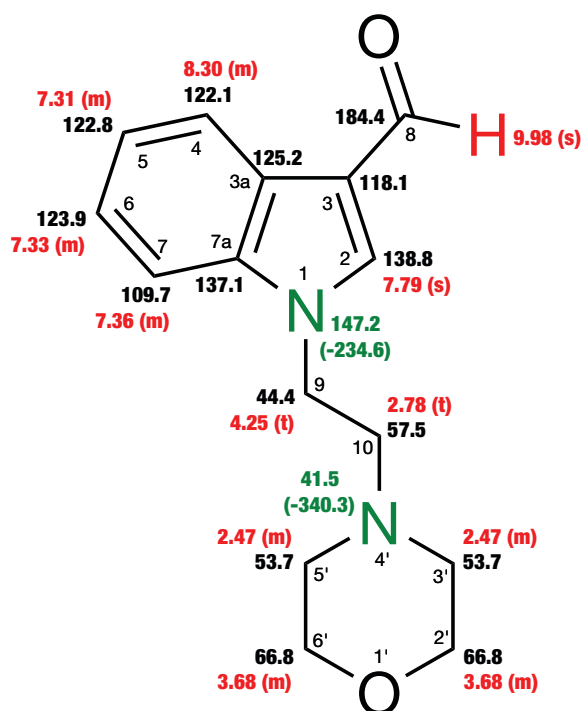
¹H NMR (400 MHz, CDCl₃) δ 9.98 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.79 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.33 (m, H-6, 1H), 7.31 (m, H-5, 1H), 4.25 (t, *J* = 6.4 Hz, H-9, 2H), 3.68 (m, H-2'/6', 4H), 2.78 (t, *J* = 6.4 Hz, H-10, 2H), 2.47 (m, H-3'/5', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 184.4 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.9 (C-6), 122.8 (C-5), 122.1 (C-4), 118.1 (C-3), 109.7 (C-7), 66.8 (C-2'/6'), 57.5 (C-10), 53.7 (C-3'/5'), 44.4 (C-9).

**¹⁵N NMR (40 MHz, CDCl₃)** δ -234.6 (N-1), -340.3 (N-4').

6. 2. 33 6-[2-(Morpholin-4-yl)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (32)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **31** (1.45 g, 5.61 mmol, 1 eq.) and **17** (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a brown oily residue that was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to give 280 mg (9.5 %) of **32** as orange crystals, m.p. 189-191 °C.

HRMS: m/z calculated for $C_{21}H_{20}N_3O_3$ ([M+H]$^+$): 362.1499; found: 362.1496.

**HRMS:** m/z calculated for $C_{21}H_{19}N_3NaO_3$ ([M+Na]$^+$): 384.1319; found: 384.1317.
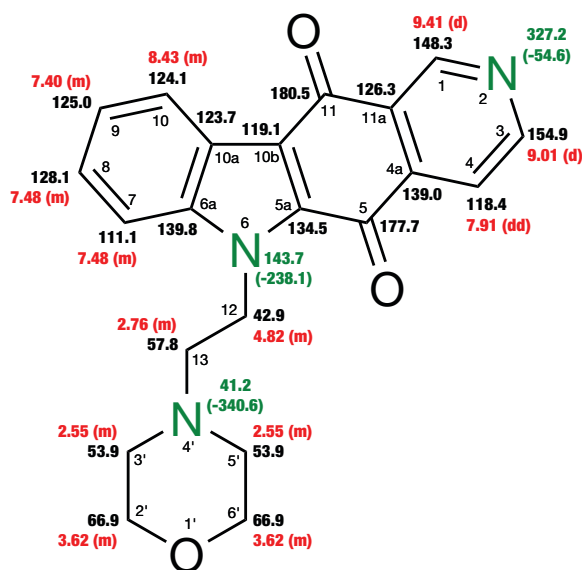
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.41 (d, *J* = 0.8 Hz, H-1, 1H), 9.01 (d, *J* = 5.0 Hz, H-3, 1H), 8.43 (m, H-10, 1H), 7.91 (dd, *J* = 5.0, 0.8 Hz, H-4, 1H), 7.48 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.82 (m, H-12, 2H), 3.62 (m, H-2'/6', 4H), 2.76 (m, H-13, 2H), 2.55 (m, H-3'/5', 4H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 180.5 (C-11), 177.7 (C-5), 154.9 (C-3), 148.3 (C-1), 139.8 (C-6a), 139.0 (C-4a), 134.5 (C-5a), 128.1 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.1 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.4 (C-4), 111.1 (C-7), 66.9 (C-2'/6'), 57.8 (C-13), 53.9 (C-3'/5'), 42.9 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -54.6 (N-2), -238.1 (N-6), -340.6 (N-4').

6. 2. 34 6-[2-(Morpholin-4-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (33)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of n-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **31** (1.45 g, 5.61 mmol, 1 eq.) and **23** (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown oily residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (8:2, v/v) to give 42 mg (2.1 %) of **33** as orange crystals, m.p. 180-182 °C.

HRMS: m/z calculated for $C_{20}H_{19}N_4O_3$ ([M+H]$^+$): 363.1452; found: 363.1450.
HRMS: m/z calculated for $C_{20}H_{18}N_4NaO_3$ ([M+Na]$^+$): 385.1271; found: 385.1272.
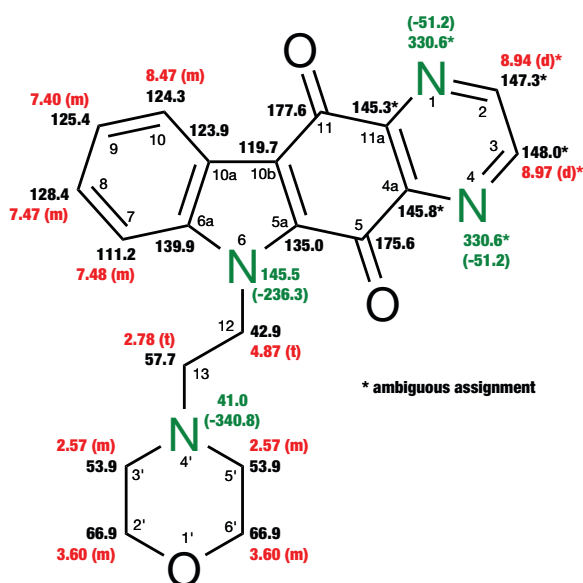
^{1}H NMR (400 MHz, CDCl₃) δ 8.97 (d, J = 2.3 Hz, H-2*/3*, 1H), 8.94 (d, J = 2.2 Hz, H-2*/3*, 1H), 8.47 (m, H-10, 1H), 7.48 (m, H-7, 1H), 7.47 (m, H-8, 1H), 7.40 (m, H-9, 1H), 4.87 (t, J = 6.9 Hz, H-12, 2H), 3.60 (m, H-2'/6', 4H), 2.78 (t, J = 6.9 Hz, H-13, 2H), 2.57 (m, H-3'/5', 4H).

^{13}C NMR (100 MHz, CDCl₃) δ 177.6 (C-11), 175.6 (C-5), 148.0 (C-2*/3*), 147.3 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 135.0 (C-5a), 128.4 (C-8), 125.4 (C-9), 124.3 (C-10), 123.9 (C-10a), 119.7 (C-10b), 111.2 (C-7), 66.9 (C-2'/6'), 57.7 (C-13), 53.9 (C-3'/5'), 42.9 (C-12).

^{15}N NMR (40 MHz, CDCl₃) δ -51.2 (N-1/4), -236.3 (N-6), -340.8 (N-4').

6. 2. 35   1-[2-(Piperidin-1-yl)ethyl]-1*H*-indole-3-carbaldehyde (34)
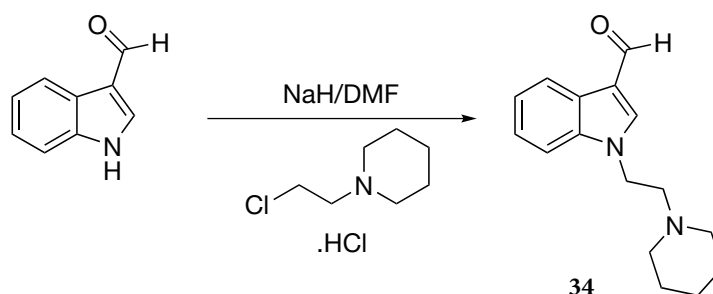
NaH (5.5 g of a 60 % dispersion in mineral oil, 137.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of 1*H*-Indole-3-carbaldehyde (5.0 g, 34.4 mmol) in dry DMF (50 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 1-(2-chloroethyl)piperidine hydrochloride (9.5 g, 51.6 mmol) was added in portions and the mixture was stirred at 90-100 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product as oily residue which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to afford 8.1 g (91.7 %) of **34** as beige solid, m.p. 80-81 °C.

HRMS: m/z calculated for $C_{16}H_{21}N_2O$ ([M+H]$^+$): 257.1648; found: 257.1652.
HRMS: m/z calculated for $C_{16}H_{20}N_2NaO$ ([M+Na]$^+$): 279.1468; found: 279.1469.
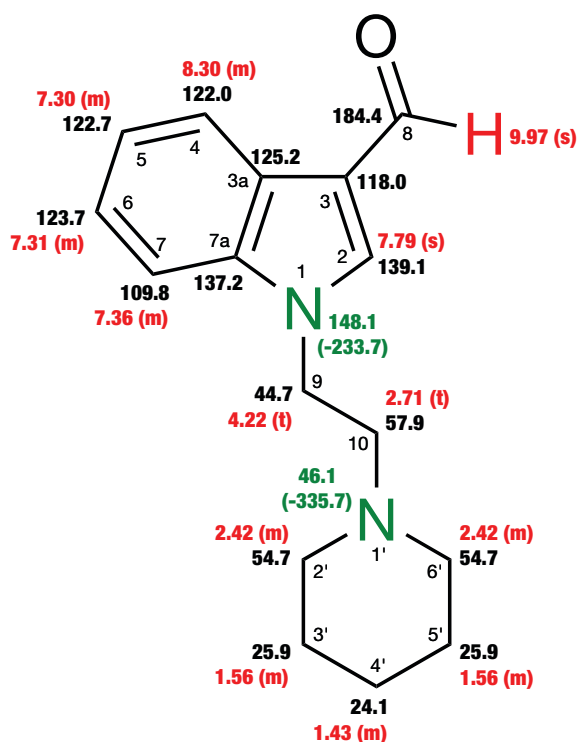
¹H NMR (400 MHz, CDCl₃) δ 9.97 (s, H-8, 1H), 8.30 (m, H-4, 1H), 7.79 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.31 (m, H-6, 1H), 7.30 (m, H-5, 1H), 4.22 (t, *J* = 6.7 Hz, H-9, 2H), 2.71 (t, *J* = 6.7 Hz, H-10, 2H), 2.42 (m, H-2'/6', 4H), 1.56 (m, H-3'/5', 4H), 1.43 (m, H-4', 2H).

¹³C NMR (100 MHz, CDCl₃) δ 184.4 (C-8), 139.1 (C-2), 137.2 (C-7a), 125.2 (C-3a), 123.7 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.8 (C-7), 57.9 (C-10), 54.7 (C-2'/6'), 44.7 (C-9), 25.9 (C-3'/5'), 24.1 (C-4').

¹⁵N NMR (40 MHz, CDCl₃) δ -233.7 (N-1), -335.7 (N-1').

6. 2. 36 6-[2-(Piperidin-1-yl)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (35)
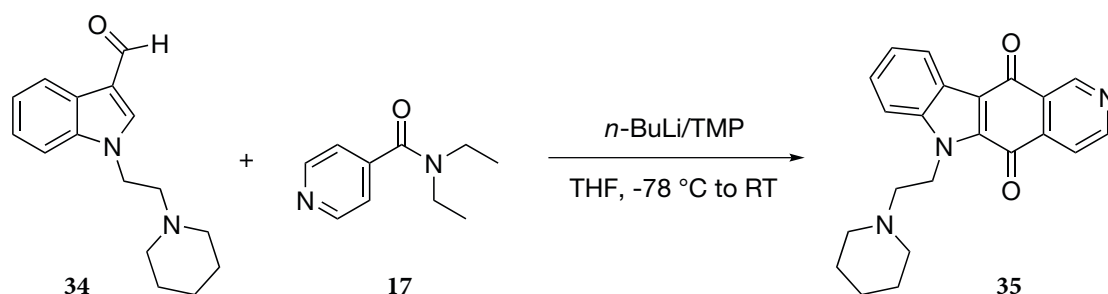
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **34** (1.01 g, 3.93 mmol, 0.7 eq.) and **17** (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 429 mg (30.4 %) of **35** as orange crystals, m.p. 168-170 °C.

HRMS: m/z calculated for $C_{22}H_{22}N_3O_2$ ([M+H]$^+$): 360.1707; found: 360.1707.
HRMS: m/z calculated for $C_{22}H_{21}N_3NaO_2$ ([M+Na]$^+$): 382.1526; found: 382.1525.
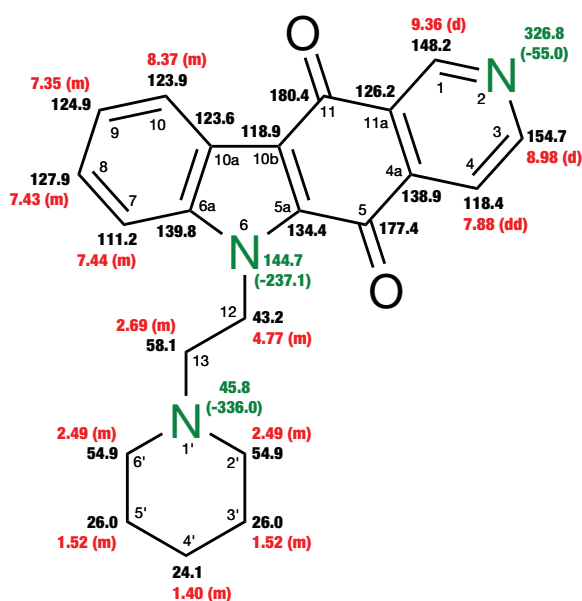
¹H NMR (400 MHz, CDCl₃) δ 9.36 (d, *J* = 0.8 Hz, H-1, 1H), 8.98 (d, *J* = 4.9 Hz, H-3, 1H), 8.37 (m, H-10, 1H), 7.88 (dd, *J* = 4.9, 0.8 Hz, H-4, 1H), 7.44 (m, H-7, 1H), 7.43 (m, H-8, 1H), 7.35 (m, H-9, 1H), 4.77 (m, H-12, 2H), 2.69 (m, H-13, 2H), 2.49 (m, H-2'/6', 4H), 1.52 (m, H-3'/5', 4H), 1.40 (m, H-4', 2H).

¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C-11), 177.4 (C-5), 154.7 (C-3), 148.2 (C-1), 139.8 (C-6a), 138.9 (C-4a), 134.4 (C-5a), 127.9 (C-8), 126.2 (C-11a), 124.9 (C-9), 123.9 (C-10), 123.6 (C-10a), 118.9 (C-10b), 118.4 (C-4), 111.2 (C-7), 58.1 (C-13), 54.9 (C-2'/6'), 43.2 (C-12), 26.0 (C-3'/5'), 24.1 (C-4').

¹⁵N NMR (100 MHz, CDCl₃) δ -55.0 (N-2), -237.1 (N-6), -336.0 (N-1').

6. 2. 37 6-[2-(Piperidin-1-yl)ethyl]-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (36)
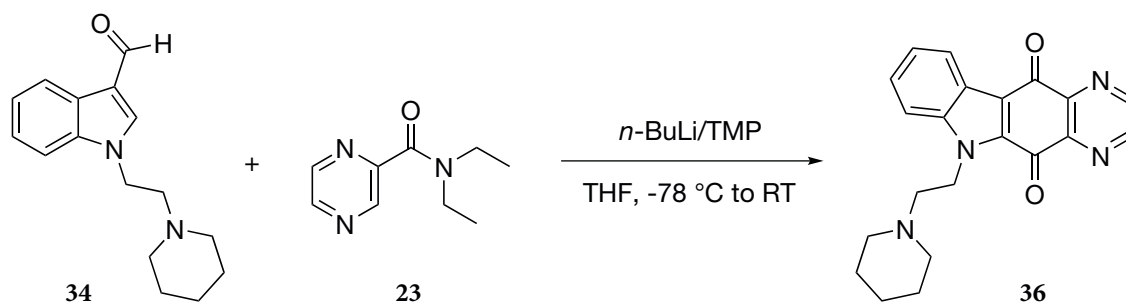
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **34** (1.44 g, 5.61 mmol, 1 eq.) and **23** (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) and recrystallisation from ethyl acetate to afford 180 mg (8.9 %) of **36** as orange crystals, m.p. 127-129 °C.

HRMS: m/z calculated for $C_{21}H_{21}N_4O_2$ ([M+H]$^+$): 361.1659; found: 361.1658.
HRMS: m/z calculated for $C_{21}H_{20}N_4NaO_2$ ([M+Na]$^+$): 383.1478; found: 383.1479.
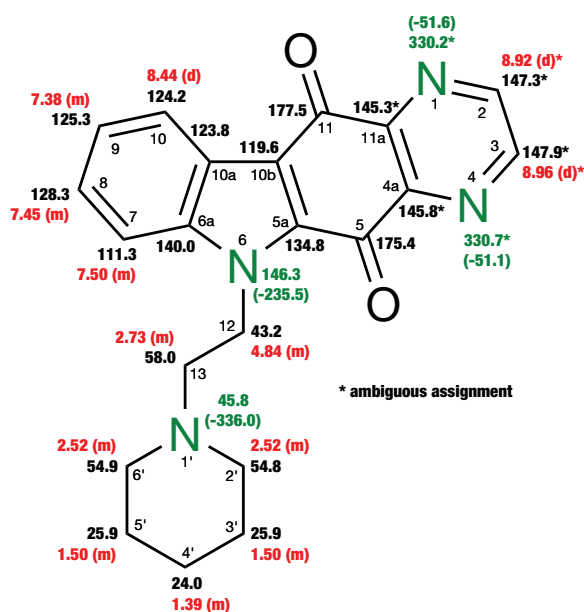
¹H NMR (400 MHz, CDCl₃) δ 8.96 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.92 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.44 (d, *J* = 8.0 Hz, H-10, 1H), 7.50 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.38 (m, H-9, 1H), 4.84 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.52 (m, H-2'/6', 4H), 1.50 (m, H-3'/5', 4H), 1.39 (m, H-4', 2H).

¹³C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.4 (C-5), 147.9 (C-2*/3*), 147.2 (C-2*/3*), 145.8 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.0 (C-6a), 134.8 (C-5a), 128.3 (C-8), 125.3 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.6 (C-10b), 111.3 (C-7), 58.0 (C-13), 54.8 (C-2'/6'), 43.2 (C-12), 25.9 (C-3'/5'), 24.0 (C-4').

¹⁵N NMR (40 MHz, CDCl₃) δ -51.1 (N-1*/4*), -51.6 (N-1*/4*), -235.5 (N-6), -336.0 (N-1').

6. 2. 38   1-[2-(Pyrrolidin-1-yl)ethyl]-1*H*-indole-3-carbaldehyde (37)
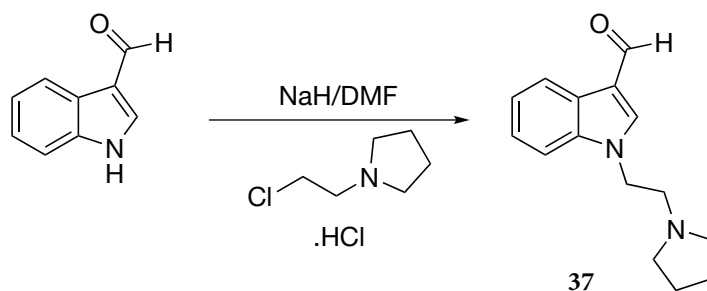
1*H*-Indole-3-carbaldehyde (5.0 g, 34.4 mmol), anhydrous K_2CO_3 (14.3 g, 103.5 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (8.79 g, 51.7 mmol) were dissolved in DMF (100 mL) and stirred at 90 °C for 24 h. After cooling down the reaction mixture was diluted with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to afford 7.3 g (70.3 %) of **28** as beige solid, m.p. 73-75 °C.

HRMS: m/z calculated for $C_{15}H_{19}N_2O$ ([M+H]$^+$): 243.1492; found: 243.1495.
HRMS: m/z calculated for $C_{15}H_{18}N_2NaO$ ([M+Na]$^+$): 265.1311; found: 265.1313.
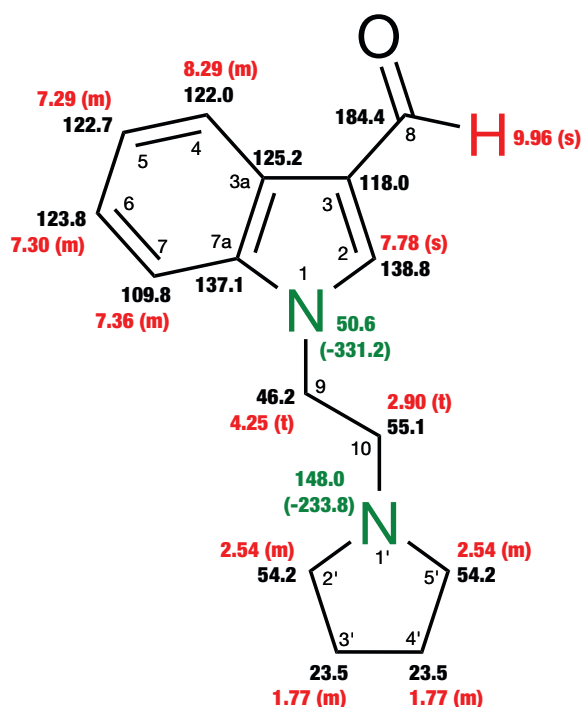
¹H NMR (400 MHz, CDCl₃) δ 9.96 (s, H-8, 1H), 8.29 (m, H-4, 1H), 7.78 (s, H-2, 1H), 7.36 (m, H-7, 1H), 7.30 (m, H-6, 1H), 7.29 (m, H-5, 1H), 4.25 (t, *J* = 6.9 Hz, H-9, 2H), 2.90 (t, *J* = 6.9 Hz, H-10, 2H), 2.54 (m, H-2'/5', 4H), 1.77 (m, H-3'/4', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 184.4 (C-8), 138.8 (C-2), 137.1 (C-7a), 125.2 (C-3a), 123.8 (C-6), 122.7 (C-5), 122.0 (C-4), 118.0 (C-3), 109.8 (C-7), 55.1 (C-10), 54.2 (C-2'/5'), 46.2 (C-9), 23.5 (C-3'/4').

¹⁵N NMR (40 MHz, CDCl₃) δ -233.8 (N-1'), -331.2 (N-1).

6. 2. 39   6-[2-(1-Pyrrolidinyl)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (38)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **37** (1.36 g, 5.61 mmol, 1 eq.) and **17** (1.0 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na$_2$SO$_4$ and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 296 mg (15.3 %) of **38** as orange-brown crystals, m.p. 125-127 °C.

HRMS: m/z calculated for C$_{21}$H$_{20}$N$_3$O$_2$ ([M+H]$^+$): 346.1550; found: 346.1546.
HRMS: m/z calculated for C$_{21}$H$_{19}$N$_3$NaO$_2$ ([M+Na]$^+$): 368.1369; found: 368.1363.
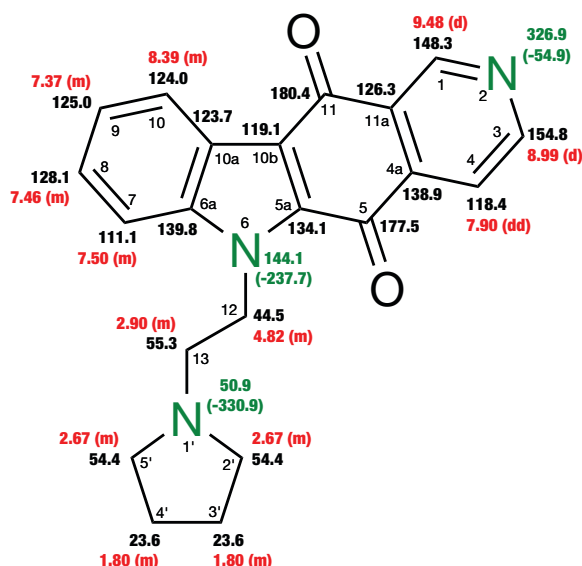
¹H NMR (400 MHz, CDCl₃) δ 9.38 (d, *J* = 0.8 Hz, H-1, 1H), 8.99 (d, *J* = 5.0 Hz, H-3, 1H), 8.39 (m, H-10, 1H), 7.90 (dd, *J* = 5.0, 0.8 Hz, H-4, 1H), 7.50 (m, H-7, 1H), 7.46 (m, H-8, 1H), 7.37 (m, H-9, 1H), 4.82 (m, H-12, 2H), 2.90 (m, H-13, 2H), 2.67 (m, H-2'/5', 4H), 1.80 (m, H-3'/4', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C-11), 177.5 (C-5), 154.8 (C-3), 148.3 (C-1), 139.8 (C-6a), 138.9 (C-4a), 134.1 (C-5a), 128.1 (C-8), 126.3 (C-11a), 125.0 (C-9), 124.0 (C-10), 123.7 (C-10a), 119.1 (C-10b), 118.4 (C-4), 111.1 (C-7), 55.3 (C-13), 54.4 (C-2'/5'), 44.5 (C-12), 23.6 (C-3'/4').

¹⁵N NMR (40 MHz, CDCl₃) δ -54.9 (N-2), -237.7 (N-6), -330.9 (N-1').

6. 2. 40 6-[2-(Pyrrolidin-1-yl)ethyl]-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (39)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **37** (1.36 g, 5.61 mmol, 1 eq.) and **23** (1.01 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (8:2, v/v) to give 39 mg (2.0 %) of **39** as orange crystals, m.p. 176-177 °C.

HRMS: m/z calculated for $C_{20}H_{19}N_4O_2$ ([M+H]⁺): 347.1503; found: 347.1504.
HRMS: m/z calculated for $C_{20}H_{18}N_4NaO_2$ ([M+Na]⁺): 369.1322; found: 369.1325.
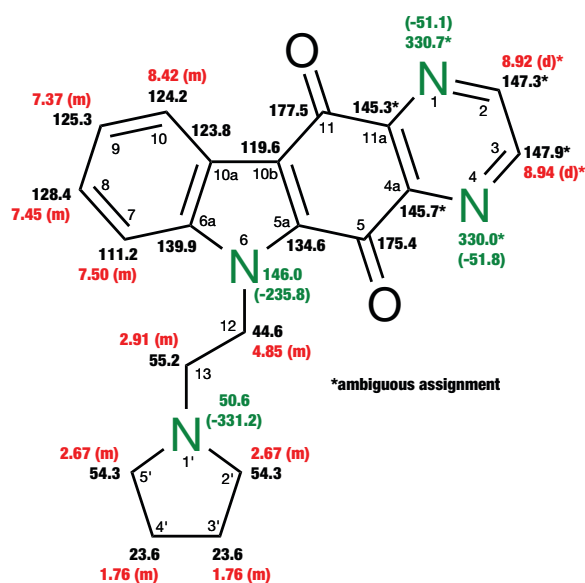
¹H NMR (400 MHz, CDCl₃) δ 8.94 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.92 (d, *J* = 2.2 Hz, H-2*/3*, 1H), 8.42 (m, H-10, 1H), 7.50 (m, H-7, 1H), 7.45 (m, H-8, 1H), 7.37 (m, H-9, 1H), 4.85 (m, H-12, 2H), 2.91 (m, H-13, 2H), 2.67 (m, H-2'/5', 4H), 1.76 (m, H-3'/4', 4H).

¹³C NMR (100 MHz, CDCl₃) δ 177.5 (C-11), 175.4 (C-5), 147.9 (C-2*/3*), 147.3 (C-2*/3*), 145.7 (C-4a*/11a*), 145.3 (C-4a*/11a*), 139.9 (C-6a), 134.6 (C-5a), 128.4 (C-8), 125.3 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.6 (C-10b), 111.2 (C-7), 55.2 (C-13), 54.3 (C-2'/5'), 44.6 (C-12), 23.6 (C-3'/4').

¹⁵N NMR (40 MHz, CDCl₃) δ -51.1 (N-1*/4*), -51.8 (N-1*/4*), -235.8 (N-6), -331.2 (N-1').

6. 2. 41   3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (40)
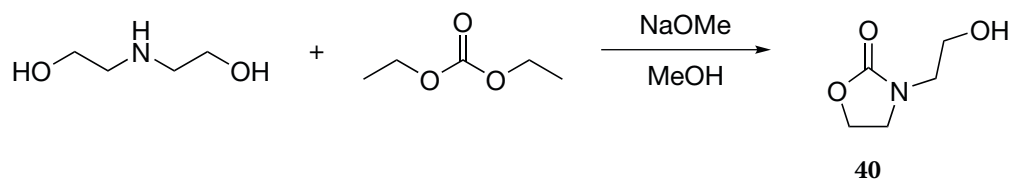
To a solution of diethanolamine (31 g, 29.4 mmol) and diethyl carbonate (44.0 mL, 36.3 mmol) was added methoxide (0.14 g, 2.6 mmol) in one portion and the resulting mixture was refluxed for 3 h. Ethanol formed in the reaction was removed under reduced pressure to give 38 g (98 %) of the crude product **40** as colourless oil which was used for the following reaction step. A small amount was purified by ball-tube distillation (b.p. 150-155 °C at 0.3 Torr) for NMR spectroscopic purposes.

^{1}H NMR (400 MHz, CDCl$_3$) δ 4.28 (m, H-5, 2H), 3.68 (m, H-7, 2H), 3.64 (m, H-4, 2H), 3.31 (m, H-6, 2H), not found (H-8).

^{13}C NMR (100 MHz, CDCl$_3$) δ 159.2 (C-2), 62.1 (C-5), 59.8 (C-7), 46.5 (C-6), 45.3 (C-4).

^{15}N NMR (40 MHz, CDCl$_3$) δ -201.5 (N-3).
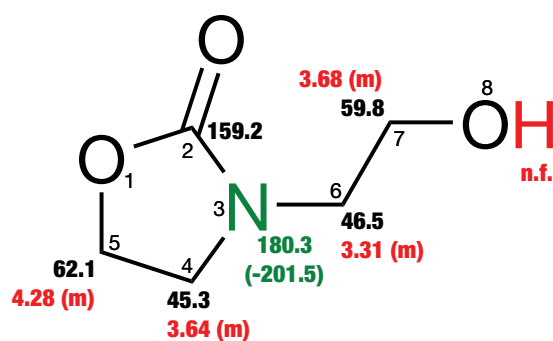
6. 2. 42   3-(2-Chloroethyl)-1,3-oxazolidin-2-one (41)

40   **41**
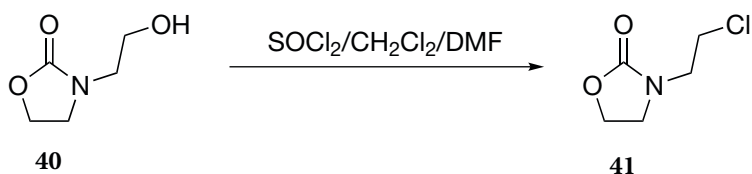
To a solution of alcohol **40** (30 g, 228.8 mmol) in dry CH₂Cl₂ (100 mL) were slowly added thionyl chloride (29.9 g, 18.3 mL, 251.6 mmol) and 3 drops of dry DMF. The mixture was refluxed for 4 h. To the cooled reaction mixture was carefully added saturated Na₂CO₃ solution (250 mL). The organic layer was collected and the aqueous layer was extracted with CH₂Cl₂ (3 x). The combined organic layers were washed with small amounts of water and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was distilled (b.p. 110 °C at 0.1 Torr) to obtain 27.4 g (80 %) of **41** as a pale yellow oil.
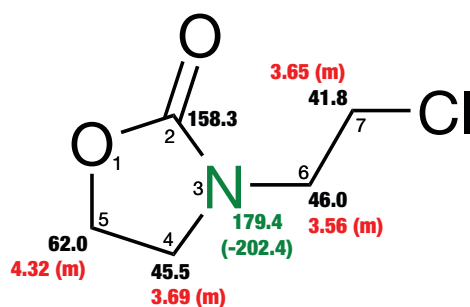
¹H NMR (400 MHz, CDCl₃) δ 4.32 (m, H-5, 2H), 3.69 (m, H-4, 2H), 3.65 (m, H-7, 2H), 3.56 (m, H-6, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 158.3 (C-2), 62.0 (C-5), 46.0 (C-6), 45.5 (C-4), 41.8 (C-7).

¹⁵N NMR (40 MHz, CDCl₃) δ -202.4 (N-3).

6. 2. 43 6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (42)
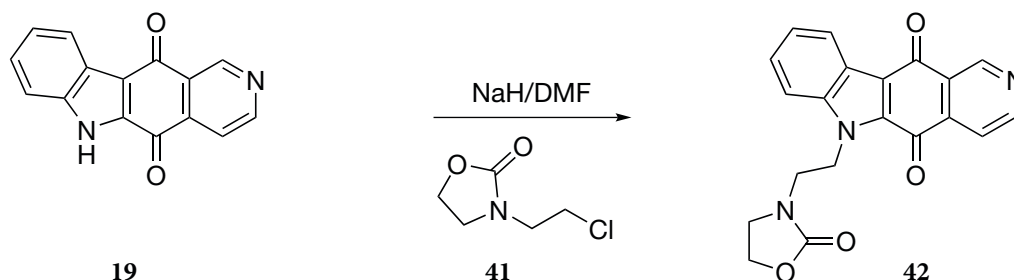
NaH (36 mg of a 60 % dispersion in mineral oil, 0.89 mmol) was suspended in dry DMF (5 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **19** (147 mg, 0.59 mmol) in dry DMF (10 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of **41** (179 mg, 1.2 mmol) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 75 °C for 6 d. After cooling down the reaction mixture was quenched with water (30 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate to obtain 124 mg (57.9 %) of **42** as bright red needles, m.p. 224-226 °C.

HRMS: m/z calculated for $C_{20}H_{16}N_3O_4$ ([M+H]$^+$): 362.1135; found: 362.1135.
HRMS: m/z calculated for $C_{20}H_{15}N_3NaO_4$ ([M+Na]$^+$): 384.0955; found: 384.0955.
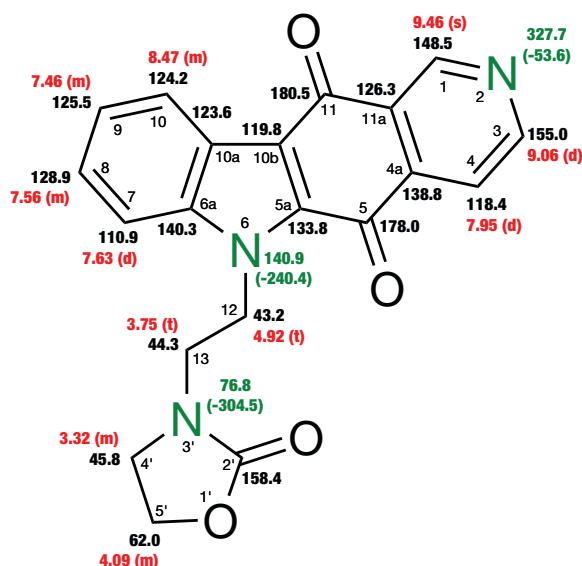
^{1}H NMR (400 MHz, CDCl₃) δ 9.46 (s, H-1, 1H), 9.06 (d, *J* = 5.0 Hz, H-3, 1H), 8.47 (d, *J* = 8.0 Hz, H-10, 1H), 7.95 (d, *J* = 5.0 Hz, H-4, 1H), 7.63 (d, *J* = 8.4 Hz, H-7, 1H), 7.56 (m, H-8, 1H), 7.46 (m, H-9, 1H), 4.92 (t, *J* = 6.3 Hz, H-12, 2H), 4.09 (m, H-5', 2H), 3.75 (t, *J* = 6.3 Hz, H-13, 2H), 3.32 (m, H-4', 2H).

^{13}C NMR (100 MHz, CDCl₃) δ 180.5 (C-11), 178.0 (C-5), 158.4 (C-2'), 155.0 (C-3), 148.5 (C-1), 140.3 (C-6a), 138.8 (C-4a), 133.8 (C-5a), 128.9 (C-8), 126.3 (C-11a), 125.5 (C-9), 124.2 (C-10), 123.6 (C-10a), 119.8 (C-10b), 118.4 (C-4), 110.9 (C-7), 62.0 (C-5'), 45.8 (C-4'), 44.3 (C-13), 43.2 (C-12).

^{15}N NMR (40 MHz, CDCl₃) δ -53.6 (N-2), -240.4 (N-6), -304.5 (N-3').

6. 2. 44 6-{2-[(2-Hydroxyethyl)amino]ethyl}-5*H*-pyrido[4,3-*b*]carbazole-5,11(6*H*)-dione (43)
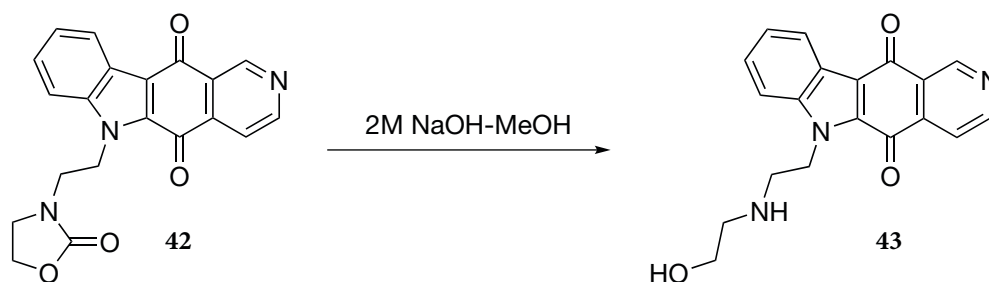
Carbamate **42** (218 mg, 0.60 mmol) was dissolved in 2N NaOH in methanol (50 mL) and refluxed (approximately 4 h) until no starting material could be detected by TLC (ethyl acetate). The cooled solution was neutralised with 6M HCl. Precipitated NaCl was removed by filtration through a Buchner funnel. The filter cake was washed with small amounts of cold ethanol and the filtrate was concentrated in vacuo. The residue was basified with saturated Na_2CO_3 solution and then the water evaporated in vacuo. The crude product was taken up in acetonitrile and filtered to remove the inorganic salts, the filtrate was concentrated in vacuo and the residue subjected to RP-chromatography eluting with methanol/water (6:4, v/v) to afford 23 mg (11.3 %) of **43** as brown crystals, m.p. 100-102 °C.

HRMS: m/z calculated for $C_{19}H_{18}N_3O_3$ ([M+H]$^+$): 336.1343; found: 336.1347.
HRMS: m/z calculated for $C_{19}H_{17}N_3NaO_3$ ([M+Na]$^+$): 358.1162; found: 358.1165.
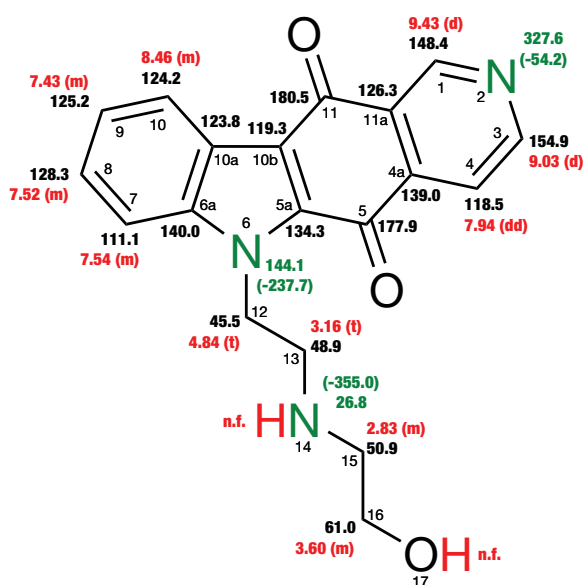
^{1}H NMR (400 MHz, CDCl₃) δ 9.43 (d, *J* = 0.8 Hz, H-1, 1H), 9.03 (d, *J* = 5.0 Hz, H-3, 1H), 8.46 (m, H-10, 1H), 7.94 (dd, *J* = 5.0, 0.8 Hz, H-4, 1H), 7.54 (m, H-7, 1H), 7.52 (m, H-8, 1H), 7.43 (m, H-9, 1H), 4.84 (t, *J* = 6.5 Hz, H-12, 2H), 3.60 (m, H-16, 2H), 3.16 (t, *J* = 6.5 Hz, H-13, 2H), 2.83 (m, H-15, 2H), not found (H-14/17).

^{13}C NMR (100 MHz, CDCl₃) δ 180.5 (C-11), 177.9 (C-5), 154.9 (C-3), 148.4 (C-1), 140.0 (C-6a), 139.0 (C-4a), 134.3 (C-5a), 128.3 (C-8), 126.3 (C-11a), 125.2 (C-9), 124.2 (C-10), 123.8 (C-10a), 119.3 (C-10b), 118.5 (C-4), 111.1 (C-7), 61.0 (C-16), 50.9 (C-15), 48.9 (C-13), 45.5 (C-12).

^{15}N NMR (40 MHz, CDCl₃) δ -54.2 (N-2), -237.7 (N-6), -355.0 (N-14).

6. 2. 45   6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5*H*-pyrazino[2,3-*b*]carbazole-5,11(6*H*)-dione (44)
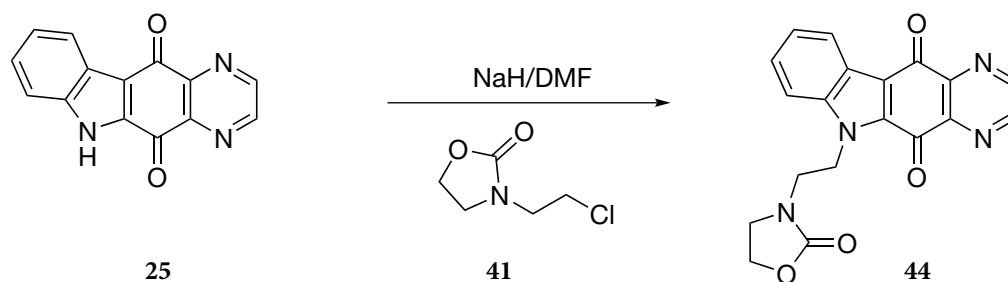
NaH (147 mg of a 60 % dispersion in mineral oil, 3.68 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **25** (612 mg, 2.46 mmol) in dry DMF (30 mL) was added dropwise. The cooling bath was removed and after stirring for further 30 min a solution of **41** (735 mg, 4.91 mmol) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 75 °C for 3 d. After cooling down the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was subjected to column chromatography eluting with ethyl acetate to obtain 253 mg (28.4 %) of **44** as orange crystals, m.p. 288-290 °C.

HRMS: m/z calculated for $C_{19}H_{15}N_4O_4$ ([M+H]$^+$): 363.1088; found: 363.1087.
HRMS: m/z calculated for $C_{19}H_{14}N_4NaO_4$ ([M+Na]$^+$): 385.0907; found: 385.0908.
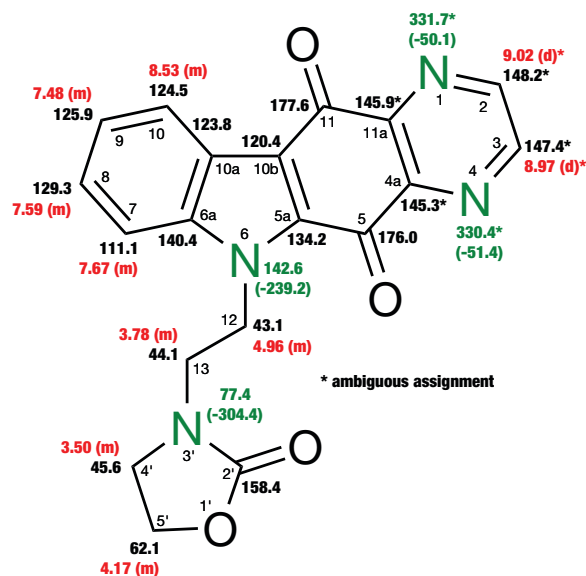
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.02 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.97 (d, *J* = 2.3 Hz, H-2*/3*, 1H), 8.53 (m, H-10, 1H), 7.67 (m, H-7, 1H), 7.59 (m, H-8, 1H), 7.48 (m, H-9, 1H), 4.96 (m, H-12, 2H), 4.17 (m, H-5', 2H), 3.78 (m, H-13, 2H), 3.50 (m, H-4', 2H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 177.6 (C-11), 176.0 (C-5), 158.4 (C-2'), 148.2 (C-2*/3*), 147.4 (C-2*/3*), 145.9 (C-4a*/11a*), 145.3 (C-4a*/11a*), 140.4 (C-6a), 134.2 (C-5a), 129.3 (C-8), 125.9 (C-9), 124.5 (C-10), 123.8 (C-10a), 120.4 (C-10b), 111.1 (C-7), 62.1 (C-5'), 45.6 (C-4'), 44.1 (C-13), 43.1 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -50.1 (N-1*/4*), -51.4 (N-1*/4*), -239.2 (N-6), -304.4 (N-3').

6. 2. 46   *N,N*-Diethyl-1*H*-indole-3-carboxamide (45)
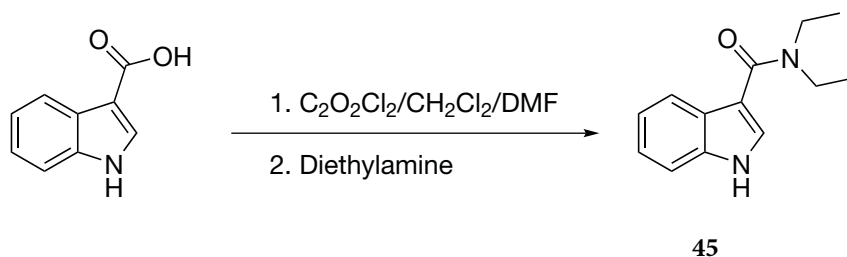
To a solution of 1*H*-Indole-3-carboxylic acid (25 g, 155.13 mmol) in dry CH₂Cl₂ (500 mL) were slowly added oxalyl chloride (18.6 mL, 209.7 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO₂ was completely gone (approximately 1h). The mixture was ice-cooled, then diethylamine (40.5 mL, 387.8 mmol) was added slowly. The reaction mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na₂CO₃ solution (400 mL). The organic layer was collected and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with water, dried over Na₂SO₄ and the solvent was removed in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate as eluent to obtain 18.12 g (54.0 %) of **45** as colourless crystals, m.p. 151-152 °C.


¹H NMR (400 MHz, DMSO-*d₆*) δ 11.45 (br s, H-1, 1H), 7.69 (m, H-4, 1H), 7.58 (s, H-2, 1H), 7.41 (m, H-7, 1H), 7.13 (m, H-6, 1H), 7.06 (m, H-5, 1H), 3.46 (q, *J* = 7.1 Hz, H-10/10', 4H), 1.14 (t, *J* = 7.1 Hz, H-11/11', 6H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 165.6 (C-8), 135.5 (C-7a), 126.5 (C-3a), 125.8 (C-2), 121.7 (C-6), 120.3 (C-4), 119.8 (C-5), 111.7 (C-7), 110.4 (C-3), 40.8 (C-10/10'), 13.8 (C-11/11').

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -244.1 (N-1), not found (N-9).
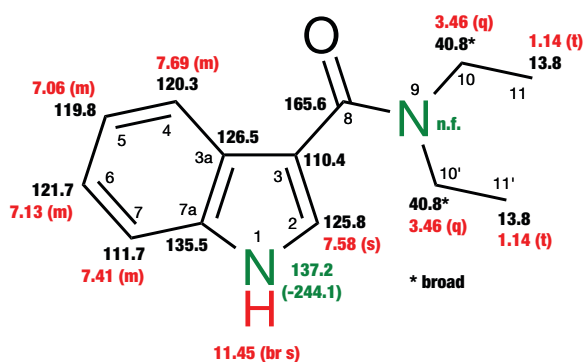
6. 2. 47   *N,N*-Diethyl-1-(methoxymethyl)-1*H*-indole-3-carboxamide (46)

45   **46**
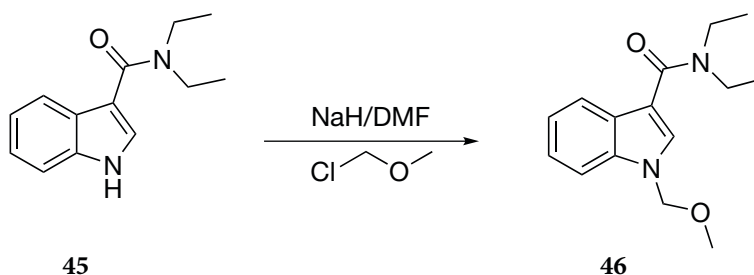
NaH (444 mg of a 60 % dispersion in mineral oil, 11.09 mmol) was suspended in dry DMF (10 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **45** (2.0 g, 9.24 mmol) in dry DMF (30 mL) was added. The cooling bath was removed and after stirring for further 60 min the mixture was cooled to -10 °C and chloro(methoxy)methane (0.967 mg, 931µL, 12.01 mmol) was added slowly. The mixture was allowed to reach room temperature and was stirred for 2 h. The reaction was quenched by pouring onto a cold saturated NaHCO₃ solution (100 mL) and the product was extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and the solvent was removed in vacuo to give the crude product as yellowish oil which was purified by trituration under heating with ethyl acetate/light petroleum (1:1, v/v) followed by cooling to afford 1.38 g (58.0 %) of **46** as colourless crystals, m.p. 76-77 °C.
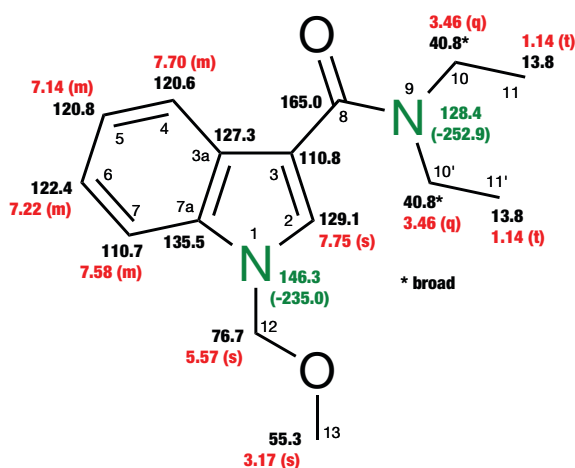
¹H NMR (400 MHz, DMSO-*d₆*) δ 7.75 (s, H-2, 1H), 7.70 (m, H-4, 1H), 7.58 (m, H-7, 1H), 7.22 (m, H-6, 1H), 7.14 (m, H-5, 1H), 5.57 (s, H-12, 2H), 3.46 (q, *J* = 7.1 Hz, H-10/10', 4H), 3.17 (s, H-13, 3H), 1.14 (t, *J* = 7.1 Hz, H-11/11', 6H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 165.0 (C-8), 135.5 (C-7a), 129.1 (C-2), 127.3 (C-3a), 122.4 (C-6), 120.8 (C-5), 120.6 (C-4), 110.8 (C-3), 110.7 (C-7), 76.7 (C-12), 55.3 (C-13), 40.8 (C-10/10'), 13.8 (C-11/11').

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -235.0 (N-1), -252.9 (N-9).

6. 2. 48 1-[2-(Dimethylamino)ethyl]-*N*,*N*-diethyl-1*H*-indole-3-carboxamide (47)
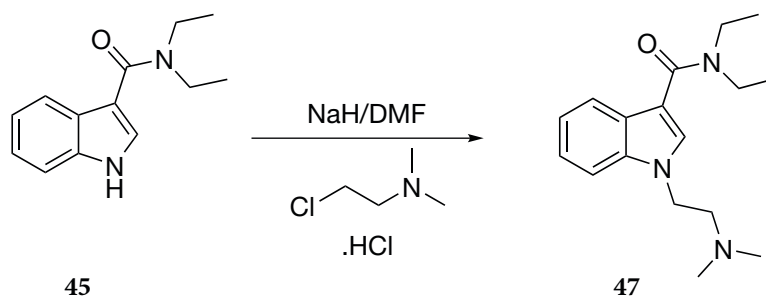
NaH (5.825 g of a 60 % dispersion in mineral oil, 145.6 mmol) was suspended in dry DMF (30 mL). The mixture was cooled down to 0 °C to -10 °C and a solution of **45** (9.0 g, 41.6 mmol) in dry DMF (70 mL) was added. The cooling bath was removed and after stirring for further 60 min solid 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (11.98 g, 83.2 mmol) was added in portions and the mixture was stirred at 90-100 °C overnight. After cooling the reaction mixture was quenched with water (300 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate followed by ethyl acetate/methanol (9:1, v/v) to afford 7.71 g (64.5 %) of **47** as yellow oil.

HRMS: m/z calculated for $C_{17}H_{26}N_3O$ ([M+H]$^+$): 288.2070; found: 288.2072.
HRMS: m/z calculated for $C_{17}H_{25}N_3NaO$ ([M+Na]$^+$): 310.1890; found: 310.1892.
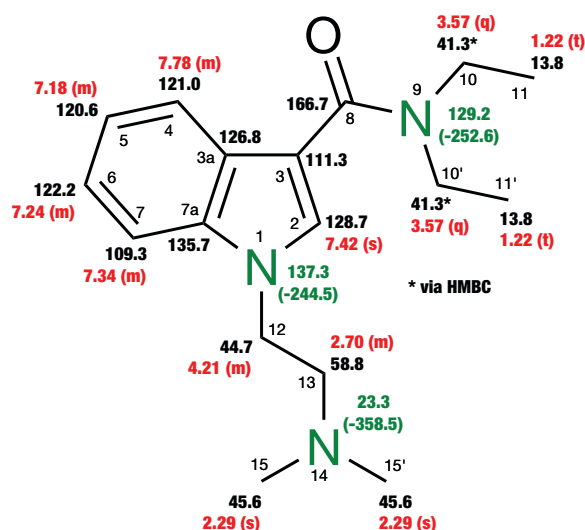
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.78 (m, H-4, 1H), 7.42 (s, H-2, 1H), 7.34 (m, H-7, 1H), 7.24 (m, H-6, 1H), 7.18 (m, H-5, 1H), 4.21 (m, H-12, 2H), 3.57 (q, *J* = 7.1 Hz, H-10/10', 4H), 2.70 (m, H-13, 2H), 2.29 (s, H-15/15', 6H), 1.22 (t, *J* = 7.1 Hz, H-11/11', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 166.7 (C-8), 135.7 (C-7a), 128.7 (C-2), 126.8 (C-3a), 122.2 (C-6), 121.0 (C-4), 120.6 (C-5), 111.3 (C-3), 109.3 (C-7), 58.8 (C-13), 45.6 (C-15/15'), 44.7 (C-12), 41.3 (C-10/10', via HMBC), 13.8 (C-11/11').

^{15}N NMR (40 MHz, CDCl$_3$) δ -244.5 (N-1), -252.6 (N-9), -358.5 (N-14).

6. 2. 49　6-(Methoxymethyl)-5*H*-pyrimido[5,4-*b*]carbazole-5,11(6*H*)-dione (48)
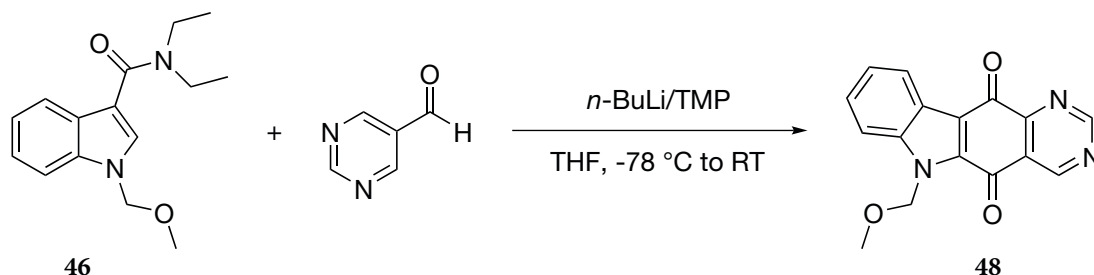
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **46** (1.46 g, 5.61 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (30 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a dark orange residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate to give 150 mg (9.1 %) of **48** as bright orange crystals, m.p. 238-240 °C.

HRMS: m/z calculated for $C_{16}H_{12}N_3O_3$ ([M+H]$^+$): 294.0873; found: 294.0873.
HRMS: m/z calculated for $C_{16}H_{11}N_3NaO_3$ ([M+Na]$^+$): 316.0693; found: 316.0696.

^{1}H NMR (400 MHz, CDCl$_3$) δ 9.65 (s, H-2, 1H), 9.54 (s, H-4, 1H), 8.50 (m, H-10, 1H), 7.66 (m, H-7, 1H), 7.55 (m, H-8, 1H), 7.47 (m, H-9, 1H), 6.14 (s, H-12, 2H), 3.40 (s, H-13, 3H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 178.3 (C-11), 176.6 (C-5), 162.8 (C-2), 156.8 (C-4), 154.8 (C-11a), 140.1 (C-6a), 133.8 (C-5a), 129.0 (C-8), 125.9 (C-9), 125.5 (C-4a), 124.2 (C-10), 123.8 (C-10a), 121.1 (C-10b), 112.1 (C-7), 75.6 (C-12), 56.7 (C-13).

^{15}N NMR (40 MHz, CDCl$_3$) δ -77.1 (N-3), -95.7 (N-1), not found (N-6).
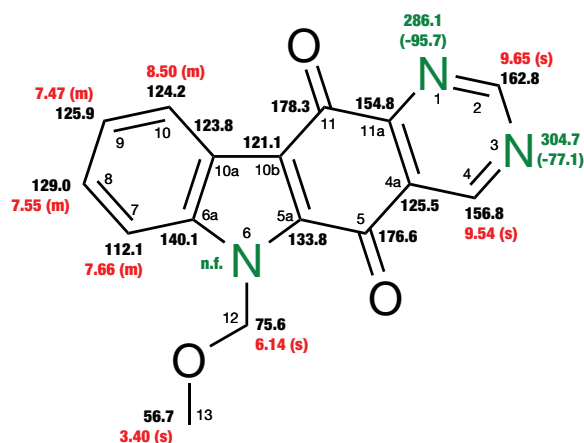
6. 2. 50   5*H*-Pyrimido[5,4-*b*]carbazole-5,11(6*H*)-dione (49)

48 → **49**
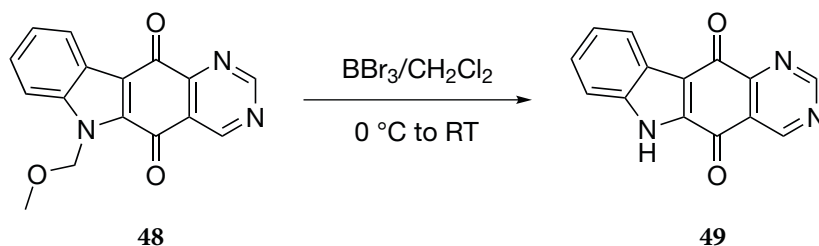
To an ice-cold solution of **48** (138 mg, 0.47 mmol) in dry CH_2Cl_2 (15 mL) a solution of BBr_3 (1M in CH_2Cl_2, 0.56 mL) was added dropwise. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (15 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. The filter cake was subjected to column chromatography eluting with ethyl acetate/methanol (8:2, v/v) followed by recrystallisation from methanol afforded 51 mg (43.5 %) of **49** as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{14}H_8N_3O_2$ ([M+H]$^+$): 250.0611; found: 250.0610.
HRMS: m/z calculated for $C_{14}H_7N_3NaO_2$ ([M+Na]$^+$): 272.0430; found: 272.0433.
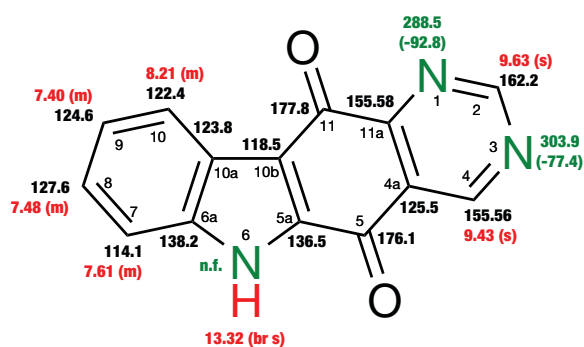
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (br s, H-6, 1H), 9.63 (s, H-2, 1H), 9.43 (s, H-4, 1H), 8.21 (m, H-10, 1H), 7.61 (m, H-7, 1H), 7.48 (m, H-8, 1H), 7.40 (m, H-9, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 177.8 (C-11), 176.1 (C-5), 162.2 (C-2), 155.58 (C-11a), 155.56 (C-4), 138.2 (C-6a), 136.5 (C-5a), 127.6 (C-8), 125.5 (C-4a), 124.6 (C-9), 123.8 (C-10a), 122.4 (C-10), 118.5 (C-10b), 114.1 (C-7).

^{15}N NMR (40 MHz, DMSO-d_6) δ -77.4 (N-3), -92.8 (N-1).

6. 2. 51   6-[2-(Dimethylamino)ethyl]-5*H*-pyrimido[5,4-*b*]carbazole-5,11(6*H*)-dione (50)
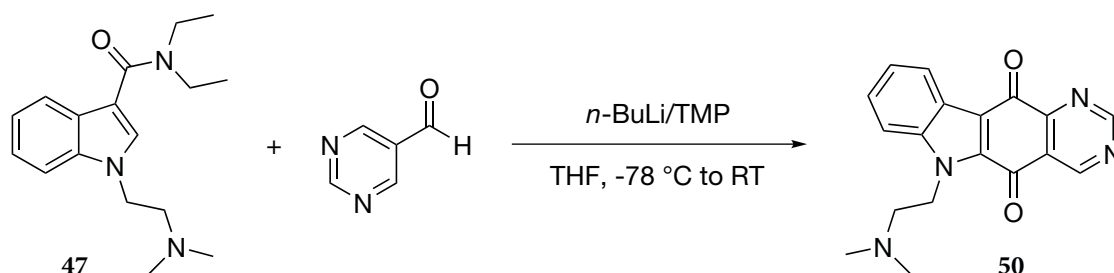
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **47** (1.61 g, 5.61 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (30 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue, which was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) followed by column chromatography eluting with CH_2Cl_2/methanol (9:1, v/v) and recrystallisation from ethyl acetate to give 135 mg (7.5 %) of **50** as red needles, m.p. 195-197 °C.

HRMS: m/z calculated for $C_{18}H_{17}N_4O_2$ ([M+H]$^+$): 321.1346; found: 321.1350.
HRMS: m/z calculated for $C_{18}H_{16}N_4NaO_2$ ([M+Na]$^+$): 343.1165; found: 343.1167.
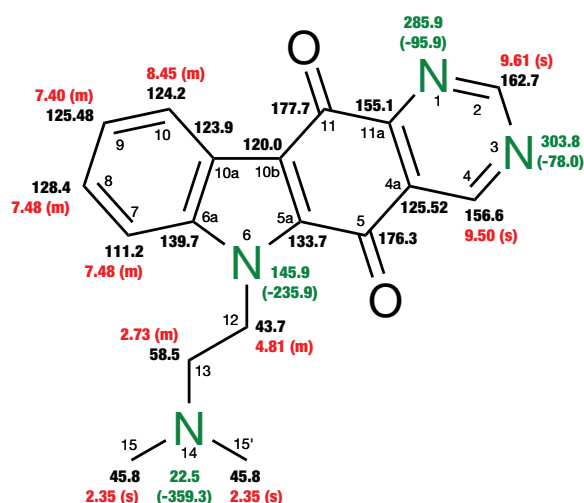
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.61 (s, H-2, 1H), 9.50 (s, H-4, 1H), 8.45 (m, H-10, 1H), 7.48 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.81 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 177.7 (C-11), 176.3 (C-5), 162.7 (C-2), 156.6 (C-4), 155.1 (C-11a), 139.7 (C-6a), 133.7 (C-5a), 128.4 (C-8), 125.52 (C-4a), 125.48 (C-9), 124.2 (C-10), 123.9 (C-10a), 120.0 (C-10b), 111.2 (C-7), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -78.0 (N-3), -95.9 (N-1), -235.9 (N-6), -359.3 (N-14).

6. 2. 52 Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1*H*-indole-2-carboxylate (51)
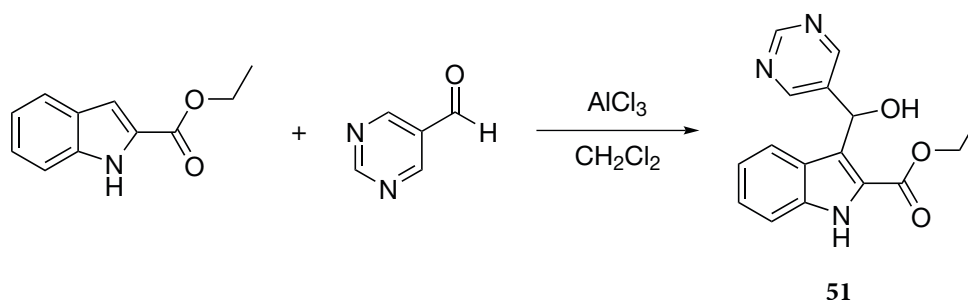
To a solution of ethyl 1*H*-indole-2-carboxylate (8.76 g, 46.3 mmol) in dry CH_2Cl_2 (250 mL) was added anhydrous $AlCl_3$ (6.79 g, 50.9 mmol), and the mixture was stirred at room temperature for 30 min under argon. A solution of pyrimidine-5-carbaldehyde (hygroscopic, must therefore be dried properly in a desiccator before use) (5 g, 46.3 mmol) in dry CH_2Cl_2 (600 mL) was added rapidly via dropping funnel to the mixture at 0 °C, brought back to room temperature and stirred for 2–4 h. The completion of the reaction was monitored by TLC (ethyl acetate). The mixture was then poured on ice water (300 mL), the organic layer was collected and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate/light petroleum to afford 4.86 g (35.3 %) of **51** as colourless crystals, m.p. 216-218 °C.

HRMS: m/z calculated for $C_{16}H_{16}N_3O_3$ ([M+H]$^+$): 298.1186; found: 298.1186.
HRMS: m/z calculated for $C_{16}H_{15}N_3NaO_3$ ([M+Na]$^+$): 320.1006; found: 320.1007.
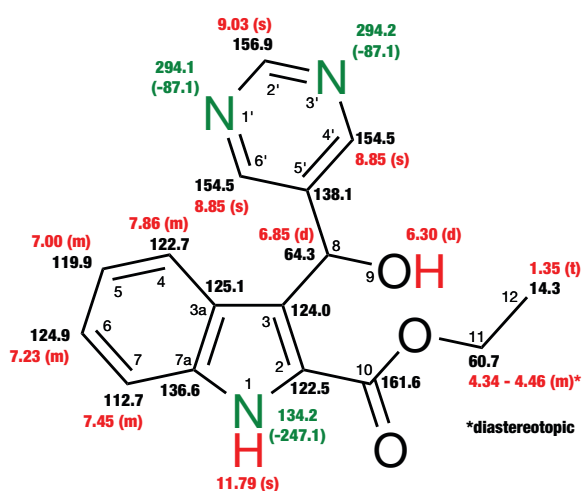
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (s, H-1, 1H), 9.03 (s, H-2', 1H), 8.85 (s, H-4'/6', 2H), 7.86 (m, H-4, 1H), 7.45 (m, H-7, 1H), 7.23 (m, H-6, 1H), 7.00 (m, H-5, 1H), 6.85 (d, *J* = 3.9 Hz, H-8, 1H), 6.30 (d, *J* = 3.9 Hz, H-9, 1H), 4.34-4.46 (m, H-11, 2H), 1.35 (t, *J* = 7.1 Hz, H-12, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 (C-10), 156.9 (C-2'), 154.5 (C-4'/6'), 138.1 (C-5'), 136.6 (C-7a), 125.1 (C-3a), 124.9 (C-6), 124.0 (C-3), 122.7 (C-4), 122.5 (C-2), 119.9 (C-5), 112.7 (C-7), 64.3 (C-8), 60.7 (C-11), 14.3 (C-12).

^{15}N NMR (40 MHz, DMSO-d_6) δ -87.1 (N-1'/3'), -247.1 (N-1).

6. 2. 53   Ethyl 3-(5-pyrimidinylcarbonyl)-1*H*-indole-2-carboxylate (52)
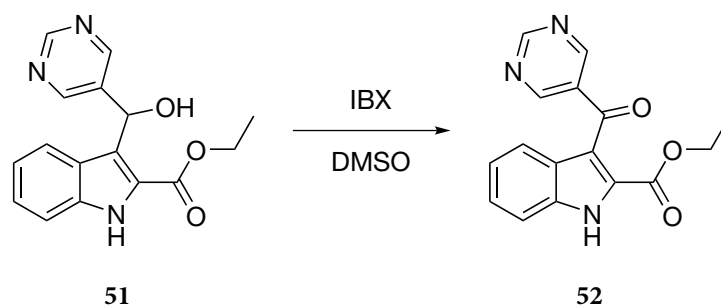
To a solution of **51** (195 mg, 0.66 mmol) in DMSO (10 mL) was added 2-iodoxybenzoic acid (IBX stabilised, containing 45 wt. % IBX, 492.8 mg, 0.79 mmol). The reaction mixture was stirred at room temperature for 2-4 h, the reaction progress was monitored by TLC (ethyl acetate). The reaction mixture was quenched with ice-cold water (75 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) to afford 163 mg of **52** (84.2 %) as colourless crystals, m.p. 224-226 °C.

HRMS: m/z calculated for $C_{16}H_{14}N_3O_3$ ([M+H]$^+$): 296.1030; found: 296.1030.
HRMS: m/z calculated for $C_{16}H_{13}N_3NaO_3$ ([M+Na]$^+$): 318.0849; found: 318.0851.
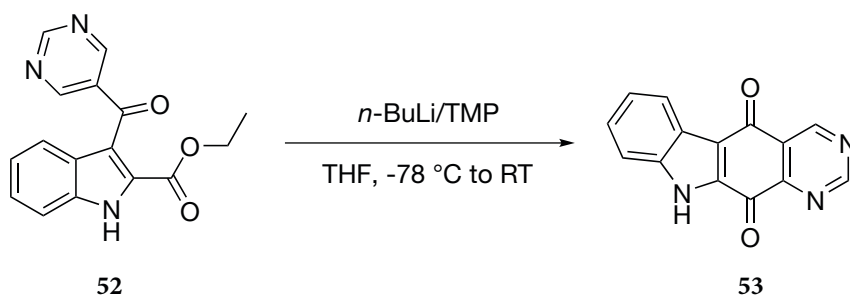
¹H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, H-1, 1H), 9.38 (s, H-2', 1H), 9.09 (s, H-4'/6', 2H), 7.82 (m, H-4, 1H), 7.60 (m, H-7, 1H), 7.39 (m, H-6, 1H), 7.25 (m, H-5, 1H), 4.01 (q, *J* = 7.1 Hz, H-10, 2H), 0.90 (t, *J* = 7.1 Hz, H-11, 3H).

¹³C NMR (100 MHz, DMSO-d_6) δ 189.0 (C-8), 160.4 (C-2'), 160.1 (C-9), 156.7 (C-4'/6'), 136.0 (C-7a), 133.1 (C-5'), 129.0 (C-2), 126.8 (C-3a), 125.7 (C-6), 122.6 (C-5), 121.3 (C-4), 116.6 (C-3), 113.1 (C-7), 61.3 (C-10), 13.4 (C-11).

**¹⁵N NMR (40 MHz, DMSO-d_6)** δ -87.2 (N-1'/3'), not found (N-1).

6. 2. 54 5*H*-Pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (53)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (1.89 mL, 11.22 mmol) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 4.49 mL, 11.22 mmol) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **52** (1.66 g, 5.61 mmol) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (30 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/ light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to give 74 mg (5.3 %) of **53** as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{14}H_8N_3O_2$ ([M+H]+): 250.0611; found: 250.0612.
HRMS: m/z calculated for $C_{14}H_7N_3NaO_2$ ([M+Na]+): 272.0430; found: 272.0432.
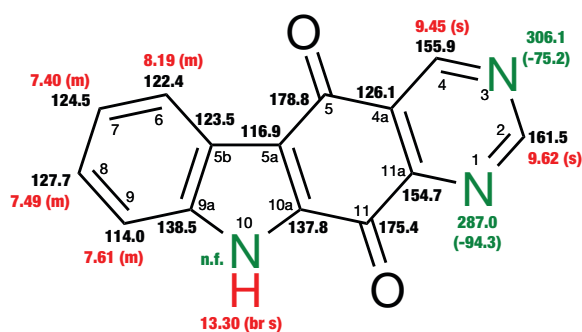
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.30 (br s, H-10, 1H), 9.62 (s, H-2, 1H), 9.45 (s, H-4, 1H), 8.19 (m, H-6, 1H), 7.61 (m, H-9, 1H), 7.49 (m, H-8, 1H), 7.40 (m, H-7, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 178.8 (C-5), 175.4 (C-11), 161.5 (C-2), 155.9 (C-4), 154.7 (C-11a), 138.5 (C-9a), 137.8 (C-10a), 127.7 (C-8), 126.1 (C-4a), 124.5 (C-7), 123.5 (C-5b), 122.4 (C-6), 116.9 (C-5a), 114.0 (C-9).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -75.2 (N-3), -94.3 (N-1), not found (N-10).

6. 2. 55 Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1*H*-indole-2-carboxylate (54)
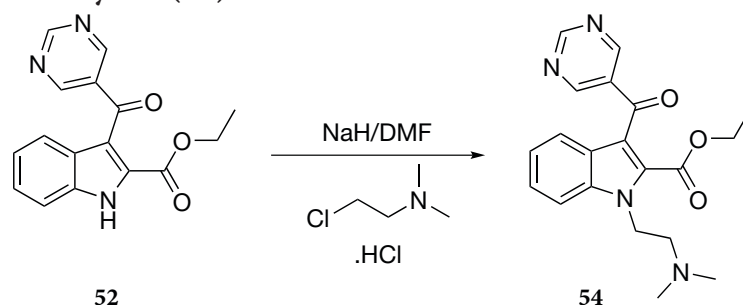
To an ice-cooled solution of **52** (500 mg, 1.69 mmol) in dry DMF (60 mL) was first added NaH (237 mg of a 60 % dispersion in mineral oil, 5.92 mmol), then solid 2-chloro-*N,N*-dimethylethanamine hydrochloride (487.8 mg, 3.39 mmol) and the mixture was stirred at 80 °C overnight. After cooling the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate/methanol (95:5, v/v) to afford 305 mg (49.1 %) of **54** as colourless oil.

HRMS: m/z calculated for $C_{20}H_{23}N_4O_3$ ([M+H]+): 367.1765; found: 367.1770.
HRMS: m/z calculated for $C_{20}H_{22}N_4NaO_3$ ([M+Na]+): 389.1584; found: 389.1589.
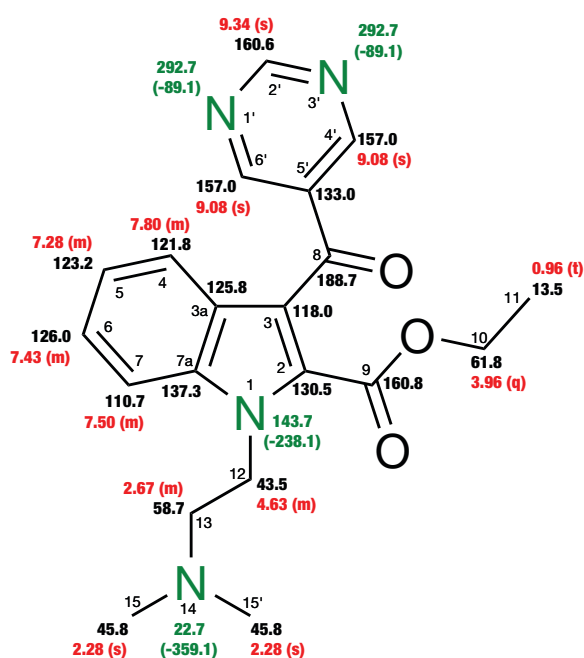
1H NMR (400 MHz, CDCl₃) δ 9.34 (s, H-2', 1H), 9.08 (s, H-4'/6', 2H), 7.80 (m, H-4, 1H), 7.50 (m, H-7, 1H), 7.43 (m, H-6, 1H), 7.28 (m, H-5, 1H), 4.63 (m, H-12, 2H), 3.96 (q, *J* = 7.2 Hz, H-10, 2H), 2.67 (m, H-13, 2H), 2.28 (s, H-15/15', 6H), 0.96 (t, *J* = 7.2 Hz, H-11, 3H).

13C NMR (100 MHz, CDCl₃) δ 188.7 (C-8), 160.8 (C-9), 160.6 (C-2'), 157.0 (C-4'/6'), 137.3 (C-7a), 133.0 (C-5'), 130.5 (C-2), 126.0 (C-6), 125.8 (C-3a), 123.2 (C-5), 121.8 (C-4), 118.0 (C-3), 110.7 (C-7), 61.8 (C-10), 58.7 (C-13), 45.8 (C-15/15'), 43.5 (C-12), 13.5 (C-11).

15N NMR (40 MHz, CDCl₃) δ -89.1 (N-1'/3'), -238.1 (N-1), -359.1 (N-14).

6. 2. 56 Ethyl 1-[2-(dimethylamino)ethyl]-1*H*-indole-2-carboxylate (55)
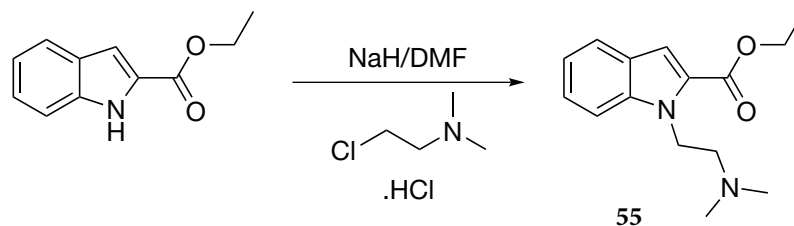
To an ice-cooled solution of ethyl 1*H*-indole-2-carboxylate (10 g, 52.9 mmol) in dry DMF (150 mL) was first added NaH (7.41 g of a 60 % dispersion in mineral oil, 185.2 mmol), then solid 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (15.24 g, 105.8 mmol) and the mixture was stirred at 60 °C for 4 h. After cooling the reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo to give the crude product which was purified by column chromatography eluting with ethyl acetate to afford 9.59 g (69.6 %) of **55** as yellow oil.
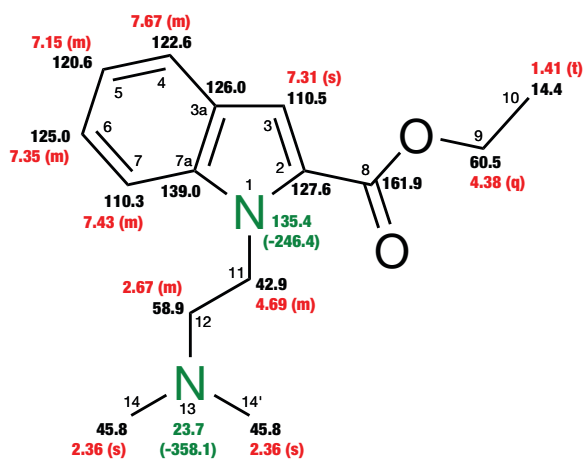
¹H NMR (400 MHz, CDCl₃) δ 7.67 (m, H-4, 1H), 7.43 (m, H-7, 1H), 7.35 (m, H-6, 1H), 7.31 (s, H-3, 1H), 7.15 (m, H-5, 1H), 4.69 (m, H-11, 2H), 4.38 (q, *J* = 7.1 Hz, H-9, 2H), 2.67 (m, H-12, 2H), 2.36 (s, H-14/14ʻ, 6H), 1.41 (t, *J* = 7.1 Hz, H-10, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 161.9 (C-8), 139.0 (C-7a), 127.6 (C-2), 126.0 (C-3a), 125.0 (C-6), 122.6 (C-4), 120.6 (C-5), 110.5 (C-3), 110.3 (C-7), 60.5 (C-9), 58.9 (C-12), 45.8 (C-14/14ʻ), 42.9 (C-11), 14.4 (C-10).

¹⁵N NMR (40 MHz, CDCl₃) δ -246.4 (N-1), -358.1 (N-13).

6. 2. 57   10-[2-(Dimethylamino)ethyl]-5*H*-pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (56)
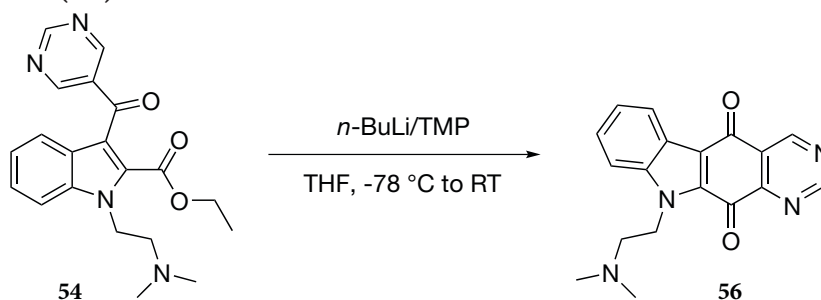
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (0.83 mL, 4.84 mmol) dropwise (over less than 1 min) to a cold (−70 °C) solution of *n*-BuLi (2.5M in hexanes, 3.87 mL, 9.68 mmol) in dry THF (20 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of **54** (355 mg, 0.96 mmol) in dry THF (10 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (30 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to give 23 mg (7.4 %) of **56** as red needles, m.p. 180-182 °C.

HRMS: m/z calculated for $C_{18}H_{17}N_4O_2$ ([M+H]$^+$): 321.1346; found: 321.1349.
HRMS: m/z calculated for $C_{18}H_{16}N_4NaO_2$ ([M+Na]$^+$): 343.1165; found: 343.1170.
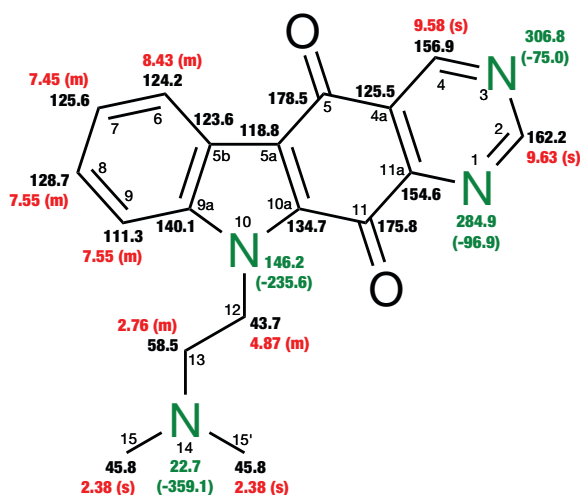
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.63 (s, H-2, 1H), 9.58 (s, H-4, 1H), 8.43 (m, H-6, 1H), 7.55 (m, H-8/9, 2H), 7.45 (m, H-7, 1H), 4.87 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.38 (s, H15/15', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 178.5 (C-5), 175.8 (C-11), 162.2 (C-2), 156.9 (C-4), 154.6 (C-11a), 140.1 (C-9a), 134.7 (C-10a), 128.7 (C-8), 125.6 (C-7), 125.5 (C-4a), 124.2 (C-6), 123.6 (C-5b), 111.3 (C-9), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -359.1 (N-14), -235.6 (N-10), -96.9 (N-1), -75.0 (N-3).

6. 2. 58 10-[2-(Dimethylamino)ethyl]-5*H*-pyrimido[4,5-*b*]carbazole-5,11(10*H*)-dione (56)
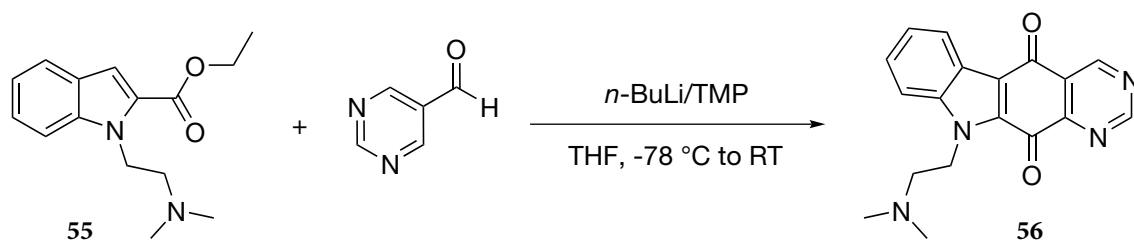
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **55** (1.46 g, 5.61 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After stirring the mixture at -75 °C for 15 min a solution of pyrimidine-5-carbaldehyde (606 mg, 5.61 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) to afford 65 mg (3.6 %) of **56** as red needles, m.p. 180-182 °C.

HRMS: m/z calculated for $C_{18}H_{17}N_4O_2$ ([M+H]$^+$): 321.1346; found: 321.1349.
HRMS: m/z calculated for $C_{18}H_{16}N_4NaO_2$ ([M+Na]$^+$): 343.1165; found: 343.1170.
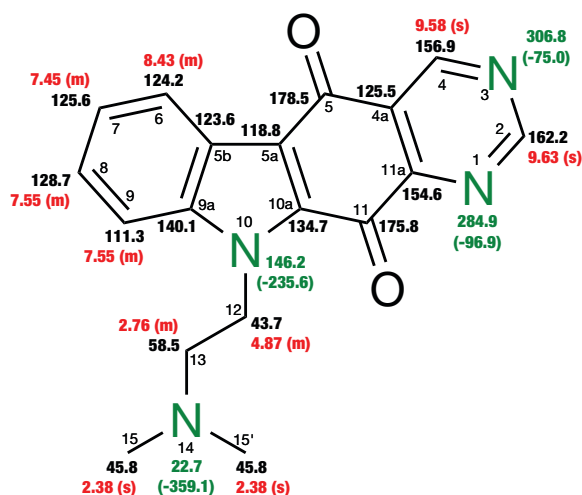
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.63 (s, H-2, 1H), 9.58 (s, H-4, 1H), 8.43 (m, H-6, 1H), 7.55 (m, H-8/9, 2H), 7.45 (m, H-7, 1H), 4.87 (m, H-12, 2H), 2.76 (m, H-13, 2H), 2.38 (s, H15/15', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 178.5 (C-5), 175.8 (C-11), 162.2 (C-2), 156.9 (C-4), 154.6 (C-11a), 140.1 (C-9a), 134.7 (C-10a), 128.7 (C-8), 125.6 (C-7), 125.5 (C-4a), 124.2 (C-6), 123.6 (C-5b), 111.3 (C-9), 58.5 (C-13), 45.8 (C-15/15'), 43.7 (C-12).

^{15}N NMR (40 MHz, CDCl$_3$) δ -359.1 (N-14), -235.6 (N-10), -96.9 (N-1), -75.0 (N-3).

6. 2. 59   Furo[3,4-*b*]quinolin-1(3*H*)-one (57)
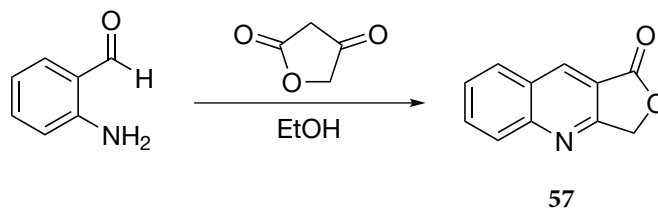
2-aminobenzaldehyde (1.05 g, 8.7 mmol) and tetronic acid (0.87 g, 8.7 mmol) were dissolve in dry ethanol (12 mL). The mixture was stirred at room temperature for 5 h. The precipitate was filtered and recrystallised from dry ethanol to afford **57** as pale yellow crystals (1.15 g, 71.0 %), m.p. 215-217 °C.
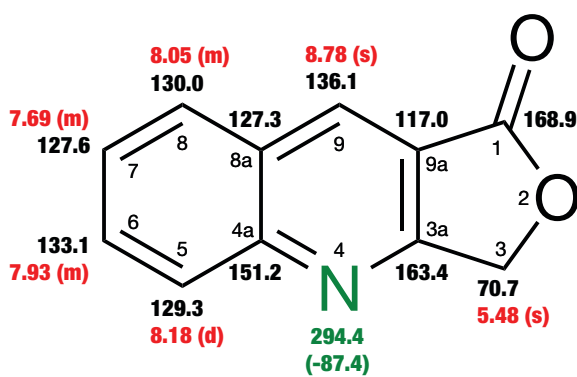
¹H NMR (400 MHz, CDCl₃) δ 8.78 (s, H-9, 1H), 8.18 (d, *J* = 8.6 Hz, H-5, 1H), 8.05 (m, H-8, 1H), 7.93 (m, H-6, 1H), 7.69 (m, H-7, 1H), 5.48 (s, H-3, 2H).

¹³C NMR (100 MHz, CDCl₃) δ 168.8 (C-1), 163.4 (C-3a), 151.2 (C-4a), 136.1 (C-9), 133.1 (C-6), 130.0 (C-8), 129.3 (C-5), 127.6 (C-7), 127.3 (C-8a), 117.0 (C-9a), 70.7 (C-3).

¹⁵N NMR (40 MHz, CDCl₃) δ -87.4 (N-4).

6. 2. 60   6*H*-Indolo[2‘,3‘:6,7]oxepino[3,4-*b*]quinolin-13(12*H*)-one (58)
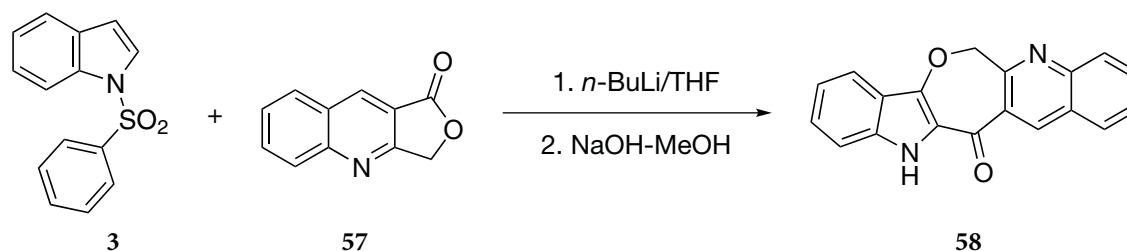
To a solution of **3** (6.0 g, 23.3 mmol) in dry THF (200 mL) at -78 °C was slowly added *n*-BuLi (1.6 M in hexane, 15.2 mL). The mixture was allowed to reach room temperature for 1 h then warmed to 40 °C for 5 min. The mixture was cooled to -78 °C, then lactone **57** (3 g, 16.2 mmol) in dry THF (250 mL) was added in one portion. The ice bath was removed and the mixture was allowed to reach room temperature then brought to 40 °C for 5 min. The solvent was removed in vacuo. Water (100 mL) was added to the residue and extracted with diethyl ether (4 x 150 mL). The combined etheric layers were extracted with 2N hydrochloric acid (3 x), then the aqueous layer was basified using solid K_2CO_3. Extraction with CH_2Cl_2 and evaporation gave a brown foam (1.2 g) which was dissolved in a 3:1 (v/v) mixture of methanol (120 mL) and 3M NaOH (40 mL). The resulting mixture was refluxed for 10 minutes. The yellow precipitate was filtered from the cooled solution, washed with water and recrystallised from methanol, affording 600 mg (12.3 %) of **58** as yellow flakes, m.p. 290-292 °C.

HRMS: m/z calculated for $C_{19}H_{13}N_2O_2$ ([M+H]$^+$): 301.0972; found: 301.0974.
HRMS: m/z calculated for $C_{19}H_{12}N_2NaO_2$ ([M+Na]$^+$): 323.0791; found: 323.0794.
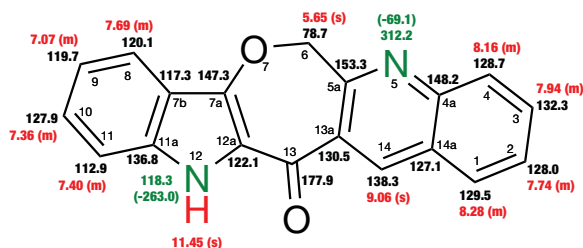
^{1}H NMR (400 MHz, DMSO-*d₆*) δ 11.45 (s, H-12, 1H), 9.06 (s, H-14, 1H), 8.28 (m, H-1, 1H), 8.16 (m, H-4, 1H), 7.94 (m, H-3, 1H), 7.74 (m, H-2, 1H), 7.69 (m, H-8, 1H), 7.40 (m, H-11, 1H), 7.36 (m, H-10, 1H), 7.07 (m, H-9, 1H), 5.65 (s, H-6, 2H).

^{13}C NMR (100 MHz, DMSO-*d₆*) δ 177.9 (C-13), 153.3 (C-5a), 148.2 (C-4a), 147.3 (C-7a), 138.3 (C-14), 136.8 (C-11a), 132.3 (C-3), 130.5 (C-13a), 129.5 (C-1), 128.7 (C-4), 128.0 (C-2), 127.9 (C-10), 127.1 (C-14a), 122.1 (12a), 120.1 (C-8), 119.7 (C-9), 117.3 (C-7b), 112.9 (C-11), 78.7 (C-6).

^{15}N NMR (40 MHz, DMSO-*d₆*) δ -69.1 (N-5), -263.0 (N-12).

6. 2. 61   6*H*-Indolo[2,3-*b*]acridine-6,12(11*H*)-dione (59)

58   **59**
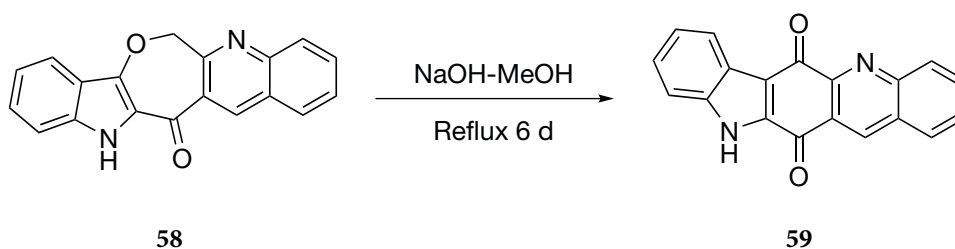
Oxepinoindole **58** (300 mg, 1.00 mmol) dissolved in methanol (400 mL) and 3M NaOH (80 mL) was refluxed for 7 days. Then most of the solvent was evaporated and the reaction mixture poured into cold water. The precipitate was collected by filtration, purified by column chromatography eluting with ethyl acetate and recrystallising from ethyl acetate to afford 120 mg (40.2 %) of **59** as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{19}H_{11}N_2O_2$ ([M+H]$^+$): 299.0815; found: 299.0816.
HRMS: m/z calculated for $C_{19}H_{10}N_2NaO_2$ ([M+Na]$^+$): 321.0634; found: 321.0638.
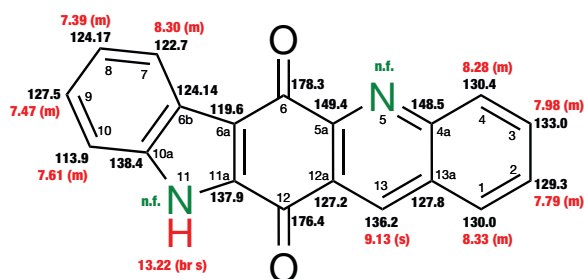
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.22 (br s, H-11, 1H), 9.13 (s, H-13, 1H), 8.33 (m, H-1, 1H), 8.30 (m, H-7, 1H), 8.28 (m, H-4, 1H), 7.98 (m, H-3, 1H), 7.79 (m, H-2, 1H), 7.61 (m, H-10, 1H), 7.47 (m, H-9, 1H), 7.39 (m, H-8, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 178.3 (C-6), 176.4 (C-12), 149.4 (C-5a), 148.5 (C-4a), 138.4 (C-10a), 137.9 (C-11a), 136.2 (C-13), 133.0 (C-3), 130.4 (C-4), 130.0 (C-1), 129.3 (C-2), 127.8 (C-13a), 127.5 (C-9), 127.2 (C-12a), 124.17 (C-8), 124.14 (C-6b), 122.7 (C-7), 119.6 (C-6a), 113.9 (C-10).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ not found (N-5/11).

6. 2. 62   11-[2-(Dimethylamino)ethyl]-6*H*-indolo[2,3-*b*]acridine-6,12(11*H*)-dione (60)
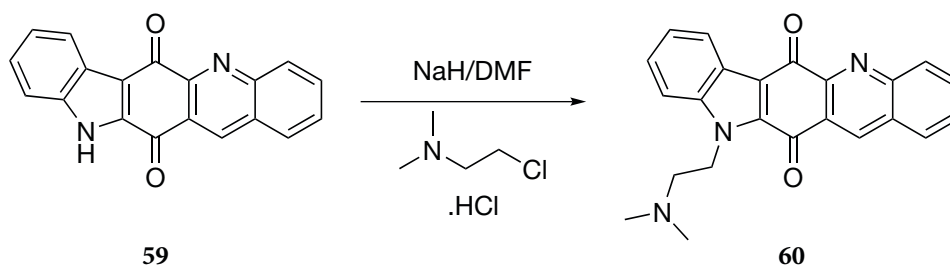
A solution of **59** (92.5 mg, 0.3 mmol) in dry DMF (8 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (40 mg, 0.93 mmol, 60 % in paraffin) in dry DMF (4 mL). After 30 min of stirring 2-chloro-*N,N*-dimethylethanamine hydrochloride (69 mg, 0.46 mmol) in DMF (6 mL) was added slowly and the reaction mixture was stirred at 75 °C for 4 h. After quenching with 30 mL of water the mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with CH_2Cl_2/methanol (95:5, v/v) affording compound **60** (50 mg, 43.8 %) as yellow flakes, m.p. 165-167 °C.

HRMS: m/z calculated for $C_{23}H_{20}N_3O_2$ ([M+H]$^+$): 370.1550; found: 370.1545.
HRMS: m/z calculated for $C_{23}H_{19}N_3NaO_2$ ([M+Na]$^+$): 392.1369; found: 392.1368.
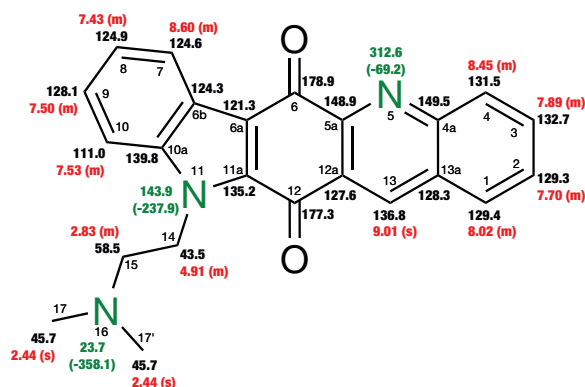
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.01 (s, H-13, 1H), 8.60 (m, H-7, 1H), 8.45 (m, H-4, 1H), 8.02 (m, H-1, 1H), 7.89 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.53 (m, H-10, 1H), 7.50 (m, H-9, 1H), 7.43 (m, H-8, 1H), 4.91 (m, H-14, 2H), 2.83 (m, H-15, 2H), 2.44 (s, H-17/17', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 178.9 (C-6), 177.3 (C-12), 149.5 (C-4a), 148.9 (C-5a), 139.8 (C-10a), 136.8 (C-13), 135.2 (C-11a), 132.7 (C-3), 131.5 (C-4), 129.4 (C-1), 129.3 (C-2), 128.3 (C-13a), 128.1 (C-9), 127.6 (C-12a), 124.9 (C-8), 124.6 (C-7), 124.3 (C-6b), 121.3 (C-6a), 111.0 (C-10), 58.5 (C-15), 45.7 (C-17/17'), 43.5 (C-14).

^{15}N NMR (40 MHz, CDCl$_3$) δ -69.2 (N-5), -237.9 (N-11), -358.1 (N-16).

6. 2. 63 *N,N*-Diethylquinoline-4-carboxamide (61)
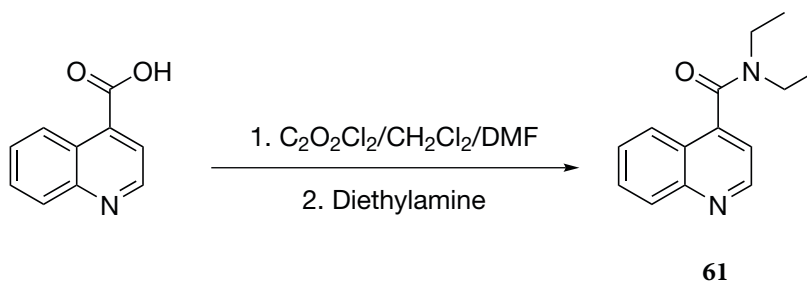
To a solution of quinoline-4-carboxylic acid (5.07 g, 29.3 mmol) in dry CH_2Cl_2 (75 mL) were slowly added oxalyl chloride (3.4 mL, 39.6 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO_2 was completely gone (approximately 1 h). The mixture was ice-cooled then diethylamine (7.6 mL, 73.4 mmol) was added slowly. The reaction mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na_2CO_3 solution (50 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with ethyl acetate/light petroleum (4:1, v/v) to afford 6.52 g (97.5 %) of **61** as yellow oil.
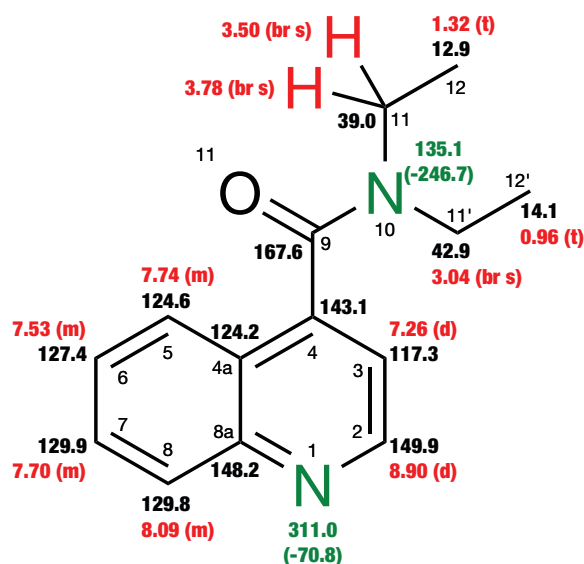
^{1}H NMR (400 MHz, CDCl₃) δ 8.90 (d, *J* = 4.3 Hz, H-2, 1H), 8.09 (m, H-8, 1H), 7.74 (m, H-5, 1H), 7.70 (m, H-7, 1H), 7.53 (m, H-6, 1H), 7.26 (d, *J* = 4.3 Hz, H-3, 1H), 3.78 (br s, H-11, 1H), 3.50 (br s, H-11, 1H), 3.04 (br s, H-11', 2H), 1.32 (t, *J* = 7.1 Hz, H-12, 3H), 0.96 (t, *J* = 7.1 Hz, H-12', 3H).

^{13}C NMR (100 MHz, CDCl₃) δ 167.6 (C-9), 149.9 (C-2), 148.2 (C-8a), 143.1 (C-4), 129.9 (C-7), 129.8 (C-8), 127.4 (C-6), 124.6 (C-5), 124.2 (C-4a), 117.3 (C-3), 42.9 (C-11'), 39.0 (C-11), 14.1 (C-12'), 12.9 (C-12).

^{15}N NMR (40 MHz, CDCl₃) δ -70.8 (N-1), -246.7 (N-10).

6.2.64 12-(Methoxymethyl)-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (62)
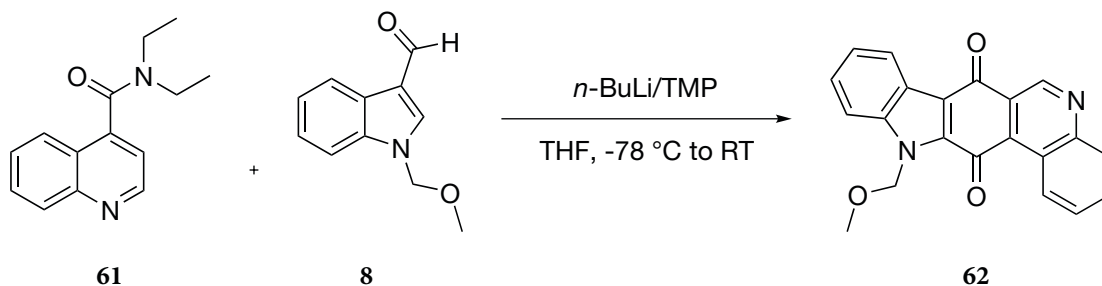
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.6 mL, 21.3 mmol) to a cold (-78 °C) solution of *n*-BuLi (2.5 M in hexane, 8.5 mL, 21.3 mmol) in dry THF (60 mL), under Argon atmosphere. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **61** (1.20 g, 5.26 mmol) in dry THF (30 mL) was added dropwise over 10 min. The mixture was stirred at -78 °C for a total of 30 min and then a solution of aldehyde **8** (400 mg, 2.11 mmol) in dry THF (18 mL) was added over 5 min. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with CH_2Cl_2 (3 x 70 mL) The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a brown residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to afford 200 mg (27.6 %) of **62** as orange crystals, m.p. 236-237 °C.

HRMS: m/z calculated for $C_{21}H_{15}N_2O_3$ ([M+H]+): 343.1077; found: 343.1081.
HRMS: m/z calculated for $C_{21}H_{14}N_2NaO_3$ ([M+Na]+): 365.0897; found: 365.0901.
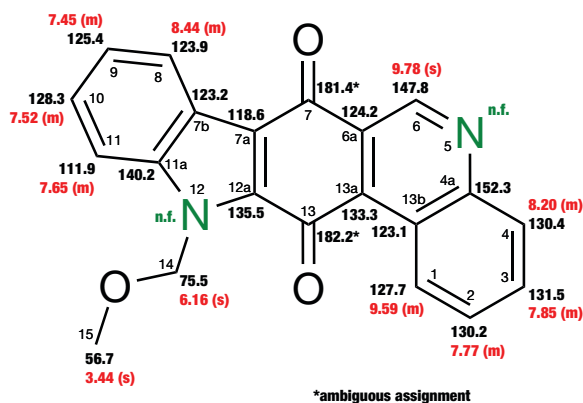
1H NMR (400 MHz, CDCl₃) δ 9.78 (s, H-6, 1H), 9.59 (m, H-1, 1H), 8.44 (m, H-8, 1H), 8.20 (m, H-4, 1H), 7.85 (m, H-3, 1H), 7.77 (m, H-2, 1H), 7.65 (m, H-11, 1H), 7.52 (m, H-10, 1H), 7.45 (m, H-9, 1H), 6.16 (s, H-14, 2H), 3.44 (s, H-15, 3H).

13C NMR (100 MHz, CDCl₃) δ 182.2 (C-7*/13*), 181.4 (C-7*/13*), 152.3 (C-4a), 147.8 (C-6), 140.2 (C-11a), 135.5 (C-12a), 133.3 (C-13a), 131.5 (C-3), 130.4 (C-4), 130.2 (C-2), 128.3 (C-10), 127.7 (C-1), 125.4 (C-9), 124.2 (C-6a), 123.9 (C-8), 123.2 (C-7b), 123.1 (C-13b), 118.6 (C-7a), 111.9 (C-11), 75.5 (C-14), 56.7 (C-15).

15N NMR (40 MHz, CDCl₃) δ not found (N-5/12).

6. 2. 65   7*H*-Indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (63)

62 → **63**
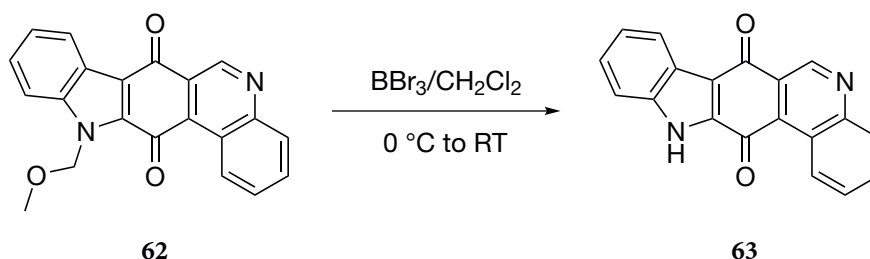
To an ice-cold solution of **62** (620 mg, 1.81 mmol) in dry CH_2Cl_2 (150 mL) a solution of BBr_3 (1 M in CH_2Cl_2, 2.26 mL) was added dropwise. The solution was allowed to reach room temperature and continued stirring for a total of 1 h. The mixture was quenched with saturated $NaHCO_3$ solution (120 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. Recrystallisation from ethyl acetate afforded 500 mg (92.6 %) of **63** as orange crystals, m.p. 273-275 °C.

HRMS: m/z calculated for $C_{19}H_{11}N_2O_2$ ([M+H]$^+$): 299.0815; found: 299.0814.
HRMS: m/z calculated for $C_{19}H_{10}N_2NaO_2$ ([M+Na]$^+$): 321.0634; found: 321.0634.

*ambiguous assignment
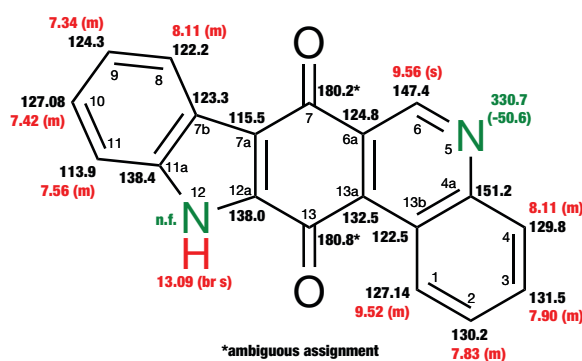
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.09 (br s, H-12, 1H), 9.56 (s, H-6, 1H), 9.52 (m, H-1, 1H), 8.11 (m, H-4/8, 2H), 7.90 (m, H-3, 1H), 7.83 (m, H-2, 1H), 7.56 (m, H-11, 1H), 7.42 (m, H-10, 1H), 7.34 (m, H-9, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 180.8 (C-7*/13*), 180.2 (C-7*/13*), 151.2 (C-4a), 147.4 (C-6), 138.4 (C-11a), 138.0 (C-12a), 132.5 (C-13a), 131.5 (C-3), 130.2 (C-2), 129.8 (C-4), 127.14 (C-1), 127.08 (C-10), 124.8 (C-6a), 124.3 (C-9), 123.3 (C-7b), 122.5 (C-13b), 122.2 (C-8), 115.5 (C-7a), 113.9 (C-11).

^{15}N NMR (40 MHz, DMSO-d_6) δ -50.6 (N-5), not found (N-12).

6. 2. 66   7*H*-Indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione 5-oxide (64)

63   **64**
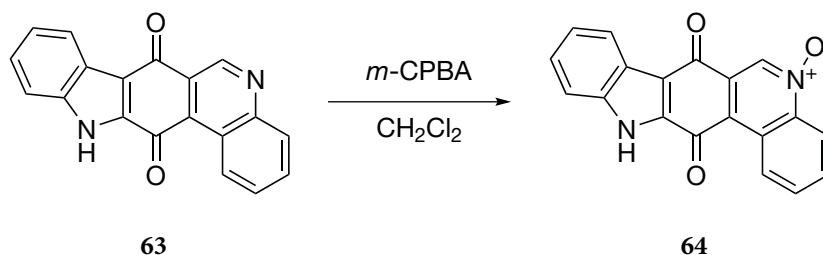
To a suspension of **63** (88 mg, 0.3 mmol) in CH_2Cl_2 (50 mL), was added in one portion *m*-CPBA (57-86 % peracid, 400 mg, excess) in CH_2Cl_2 (125 mL). The mixture was refluxed until no starting material could be detected by TLC (ethyl acetate/light petroleum 1:1, v/v) (approx. 14 h). The resulting mixture was diluted with 375 mL of CH_2Cl_2, which was washed with saturated $NaHCO_3$ solution (3 x 15 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3 x). The combined organic layers were dried over Na_2SO_4 and evaporated to give an orange-red solid. The product was purified by column chromatography eluting with ethyl acetate to give 89 mg (96.3 %) of **64** as amorphous orange-red solid, m.p. 284-286 °C.

HRMS: m/z calculated for $C_{19}H_{11}N_2O_3$ ([M+H]$^+$): 315.0764; found: 315.0767.
HRMS: m/z calculated for $C_{19}H_{10}N_2NaO_3$ ([M+Na]$^+$): 337.0584; found: 337.0585.
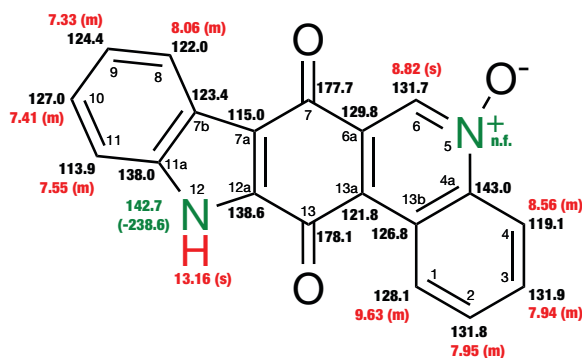
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.16 (s, H-12, 1H), 9.63 (m, H-1, 1H), 8.82 (s, H-6, 1H), 8.56 (m, H-4, 1H), 8.06 (m, H-8, 1H), 7.95 (m, H-2, 1H), 7.94 (m, H-3, 1H), 7.55 (m, H-11, 1H), 7.41 (m, H-10, 1H), 7.33 (m, H-9, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 178.1 (C-13), 177.7 (C-7), 143.0 (C-4a), 138.6 (C-12a), 138.0 (C-11a), 131.9 (C-3), 131.8 (C-2), 131.7 (C-6), 129.8 (C-6a), 128.1 (C-1), 127.0 (C-10), 126.8 (C-13b), 124.4 (C-9), 123.4 (C-7b), 122.0 (C-8), 121.8 (C-13a), 119.1 (C-4), 115.0 (C-7a), 113.9 (C-11).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -238.6 (N-12), not found (N-5).

6. 2. 67 12-[2-(Dimethylamino)ethyl]-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione (65)

63   **65**
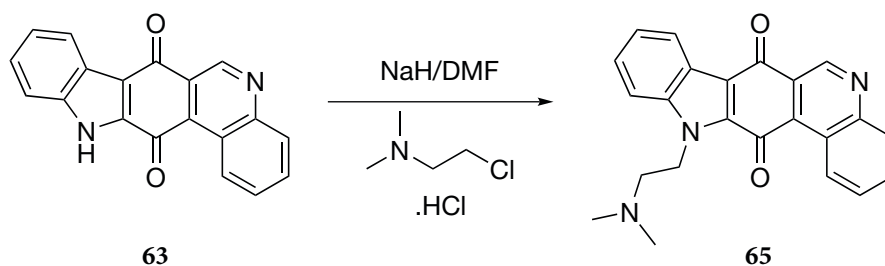
A solution of **63** (450 mg, 1.51 mmol) in dry DMF (20 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (160 mg, 4.00 mmol, 60 % in paraffin) in dry DMF (15 mL). After 30 min of stirring 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (330 mg, 2.27 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with 50 mL of water the mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by ethyl acetate/methanol (95:5, v/v) to afford **65** (200 mg, 39.5 %) as orange-brown crystals, m.p. 208-209 °C.

HRMS: m/z calculated for $C_{23}H_{20}N_3O_2$ ([M+H]$^+$): 370.1550; found: 370.1551.
HRMS: m/z calculated for $C_{23}H_{19}N_3NaO_2$ ([M+Na]$^+$): 392.1369; found: 392.1371.

*ambiguous assignment
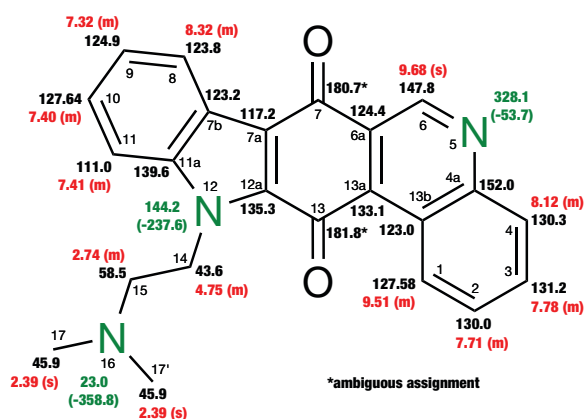
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.68 (s, H-6, 1H), 9.51 (m, H-1, 1H), 8.32 (m, H-8, 1H), 8.12 (m, H-4, 1H), 7.78 (m, H-3, 1H), 7.71 (m, H-2, 1H), 7.41 (m, H-11, 1H), 7.40 (m, H-10, 1H), 7.32 (m, H-9, 1H), 4.75 (m, H-14, 2H), 2.74 (m, H-15, 2H), 2.39 (s, H-17/17', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 181.8 (C-7*/13*), 180.7 (C-7*/13*), 152.0 (C-4a), 147.8 (C-6), 139.6 (C-11a), 135.3 (C-12a), 133.1 (C-13a), 131.2 (C-3), 130.3 (C-4), 130.0 (C-2), 127.64 (C-10), 127.58 (C-1), 124.9 (C-9), 124.4 (C-6a), 123.8 (C-8), 123.2 (C-7b), 123.0 (C-13b), 117.2 (C-7a), 111.0 (C-11), 58.5 (C-15), 45.9 (C-17/17'), 43.6 (C-14).

^{15}N NMR (40 MHz, CDCl$_3$) δ -53.7 (N-5), -237.6 (N-12), -358.8 (N-16).

6.2.68 12-[2-(Dimethylamino)ethyl]-7*H*-indolo[3,2-*j*]phenanthridine-7,13(12*H*)-dione 5-oxide (66)
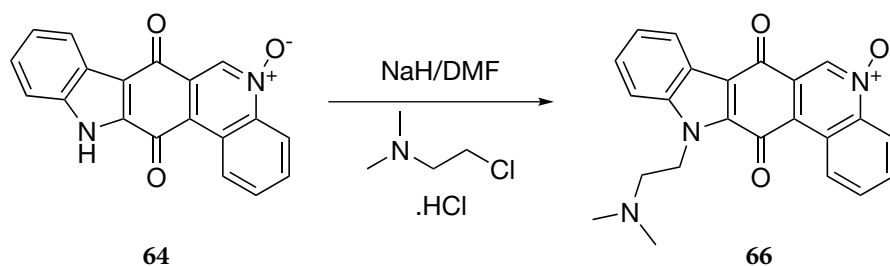
A solution of **64** (89 mg, 0.28 mmol) in dry DMF (7 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (30 mg, 0.73 mmol, 60 % in paraffin) in dry DMF (2 mL). After 30 min of stirring 2-chloro-*N,N*-dimethylethanamine hydrochloride (61 mg, 0.42 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 4 h. After quenching with 50 mL of water the mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) to ethyl acetate/methanol (95:5, v/v) affording compound **66** (23 mg, 21.1 %) as red crystals, m.p. 204-205 °C.

HRMS: m/z calculated for $C_{23}H_{20}N_3O_3$ ([M+H]$^+$): 386.1499; found: 386.1491.
HRMS: m/z calculated for $C_{23}H_{19}N_3NaO_3$ ([M+Na]$^+$): 408.1319; found: 408.1311.
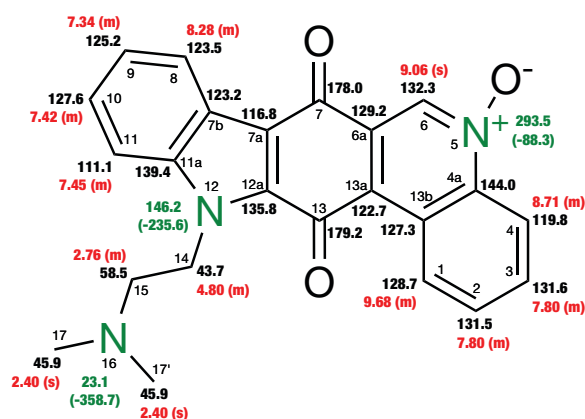
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.68 (m, H-1, 1H), 9.06 (s, H-6, 1H), 8.71 (m, H-4, 1H), 8.28 (m, H-8, 1H), 7.80 (m, H-2/3, 2H), 7.45 (m, H-11, 1H), 7.42 (m, H-10, 1H), 7.34 (m, H-9, 1H), 4.80 (m, H-14, 2H), 2.76 (m, H-15, 2H), 2.40 (s, H-17/17', 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 179.2 (C-13), 178.0 (C-7), 144.0 (C-4a), 139.4 (C-11a), 135.8 (C-12a), 132.3 (C-6), 131.6 (C-3), 131.5 (C-2), 129.2 (C-6a), 128.7 (C-1), 127.6 (C-10), 127.3 (C-13b), 125.2 (C-9), 123.5 (C-8), 123.2 (C-7b), 122.7 (C-13a), 119.8 (C-4), 116.8 (C-7a), 111.1 (C-11), 58.5 (C-15), 45.9 (C-17/17'), 43.7 (C-14).

^{15}N NMR (40 MHz, CDCl$_3$) δ -88.3 (N-5), -235.6 (N-12), -358.7 (N-16).

6. 2. 69   1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (67)
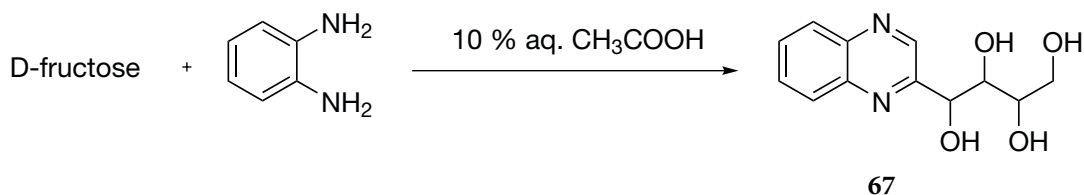
Benzene-1,2-diamine (50.0 g, 0.46 mol) was placed in round bottom flask then aqueous acetic acid (225 mL, 10 % solution) was added and the mixture was stirred for 30 min. Then D-Fructose (83 g, 0.46 mol) was added portionwise over 10 min. The mixture was heated to 80 °C and stirred for 18 h. The reaction was cooled to -2 °C for 4 h. The brown solids were collected by filtration, washed with water (2 x 100 mL) and recrystallised twice from ethanol to afford 38.5 g (33.5 %) of **67** as beige crystals, m.p. 194-196 °C.
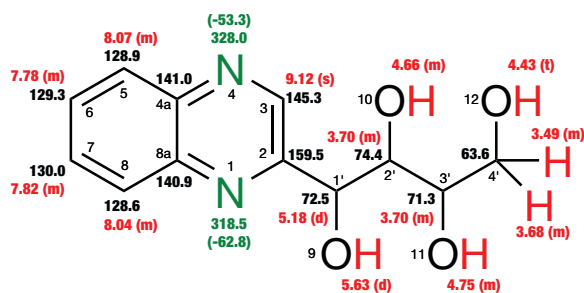
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, H-3, 1H), 8.07 (m, H-5, 1H), 8.04 (m, H-8, 1H), 7.82 (m, H-7, 1H), 7.78 (m, H-6, 1H), 5.63 (d, J = 6.2 Hz, H-9, 1H), 5.18 (d, J = 6.2 Hz, H-1', 1H), 4.75 (m, H-11, 1H), 4.66 (m, H-10, 1H), 4.43 (t, J = 5.5 Hz, H-12, 1H), 3.70 (m, H-2'/3', 2H), 3.68 (m, H-4', 1H), 3.49 (m, H-4', 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 159.5 (C-2), 145.3 (C-3), 141.0 (C-4a), 140.9 (C-8a), 130.0 (C-7), 129.3 (C-6), 128.9 (C-5), 128.6 (C-8), 74.4 (C-2'), 72.5 (C-1'), 71.3 (C-3'), 63.6 (C-4').

^{15}N NMR (40 MHz, DMSO-d_6) δ -53.3 (N-4), -62.8 (N-1).

6. 2. 70   2-Quinoxalinecarboxylic acid (68)
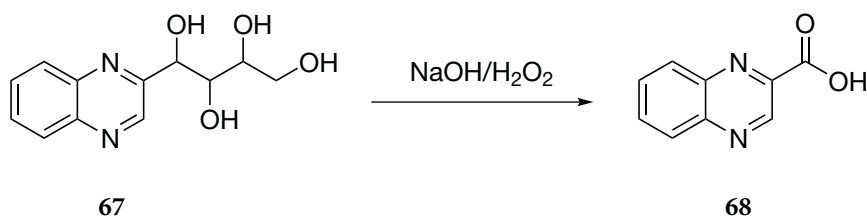
67 (38.5 g, 0.15 mol) was added portionwise into a solution of NaOH (93.2 g, 2.33 mol) in water (945 mL) then stirred for 45 min. A 30 % solution of H_2O_2 (72 mL) was added to the reaction mixture over 10 min. The mixture was slowly heated to 60 °C over a period 3 h. Then the remainder of the 30 % H_2O_2 solution (62.75 mL) was added slowly over 20 min (maximum temperature reached 91 °C) and stirring was continued for further 30 min. The reaction mixture was then refluxed for 15 min. The reaction was cooled to 80 °C, and the liquid was decanted away from the tar-like material. The tar-like material was discarded and the separated liquid was allowed to cool to room temperature. Then the solution was adjusted to pH 2 with concentrated hydrochloric acid. The mixture was allowed to stand overnight at room temperature. The pale brown solids were collected by filtration and washed with water (2 x 50 mL) and dried in a desiccator in vacuo. 17.3 g (50.3 %) of **68** were obtained as off-white solid, m.p. 206-207 °C.
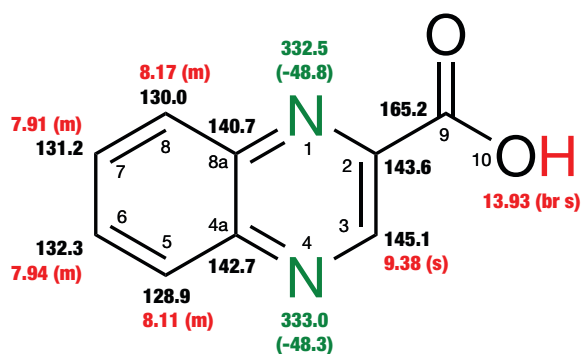
¹H NMR (400 MHz, DMSO-d_6) δ 13.93 (s, H-10, 1H), 9.38 (s, H-3, 1H), 8.17 (m, H-8, 1H), 8.11 (m, H-5, 1H), 7.94 (m, H-6, 1H), 7.91 (m, H-7, 1H).

¹³C NMR (100 MHz, DMSO-d_6) δ 165.2 (C-9), 145.1 (C-3), 143.6 (C-2), 142.7 (C-4a), 140.7 (C-8a), 132.3 (C-6), 131.2 (C-7), 130.0 (C-8), 128.9 (C-5).

¹⁵N NMR (40 MHz, DMSO-d_6) δ -48.3 (N-4), -48.8 (N-1).

6. 2. 71 *N,N*-Diethylquinoxaline-2-carboxamide (69)
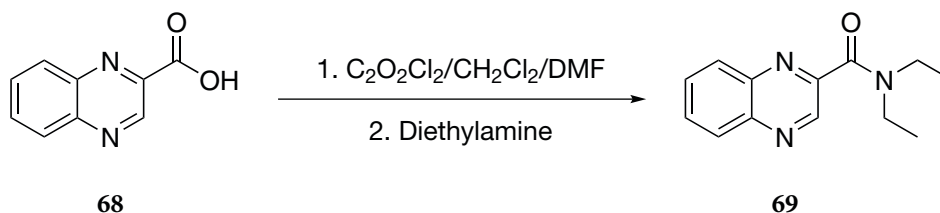
To a solution of **68** (15.0 g, 86.1 mmol) in dry CH_2Cl_2 (225 mL) were added slowly oxalyl chloride (8.1 mL, 94.7 mmol) and 3 drops of dry DMF. The mixture was stirred at room temperature until the formed CO_2 was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (27 mL, 258.4 mmol) was added slowly. The mixture was allowed to to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na_2CO_3 solution (100 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate as eluent to give 18.5 g (94.0 %) of **69** as red-brown oil.
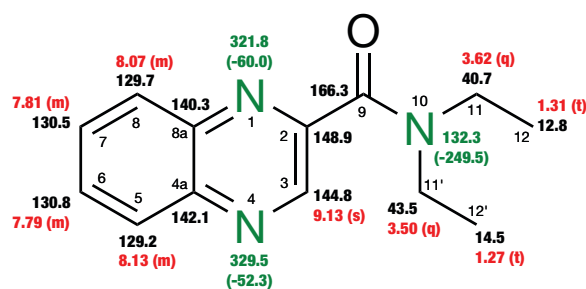
¹H NMR (400 MHz, CDCl₃) δ 9.13 (s, H-3, 1H), 8.13 (m, H-5, 1H), 8.07 (m, H-8, 1H), 7.81(m, H-7, 1H), 7.79 (m, H-6, 1H), 3.62 (q, *J* = 7.1 Hz, H-11, 2H), 3.50 (q, *J* = 7.1 Hz, H-11', 2H), 1.31 (t, *J* = 7.1 Hz, H-12, 3H), 1.27 (t, *J* = 7.1 Hz, H-12', 3H).

¹³C NMR (100 MHz, CDCl₃) δ 166.3 (9), 148.9 (2), 144.8 (3), 142.1 (4a), 140.3 (8a), 130.8 (6), 130.5 (7), 129.7 (8), 129.2 (5), 43.5 (11), 40.7 (11'), 14.5 (12), 12.8 (12').

¹⁵N NMR (40 MHz, CDCl₃) δ -52.3 (N-4), -60.0 (N-1), -249.5 (N-10).

6. 2. 72   5-(Methoxymethyl)-5*H*-indolo[2,3-*b*]phenazine-6,13-dione (70)
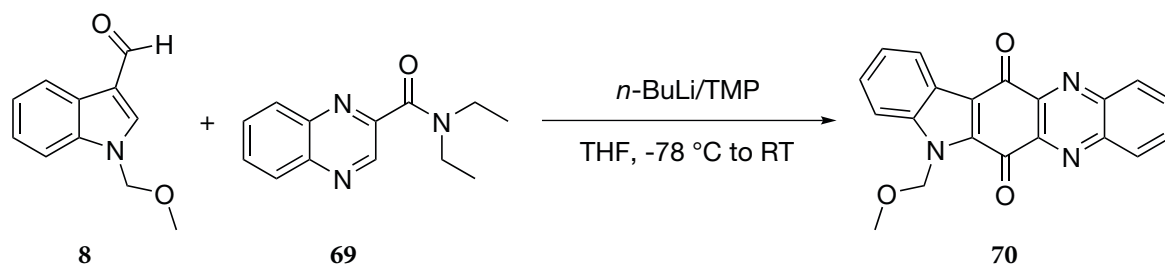
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **8** (1.06 g, 5.61 mmol, 1 eq.) and **69** (1.29 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a dark residue. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (6:4, v/v) followed by recrystallisation from ethyl acetate to give 145 mg (10.9 %) of **70** as yellow flakes, m.p. 265-267 °C.

HRMS: m/z calculated for $C_{20}H_{13}N_3NaO_3$ ([M+Na]^+): 366.0849; found: 366.0849.
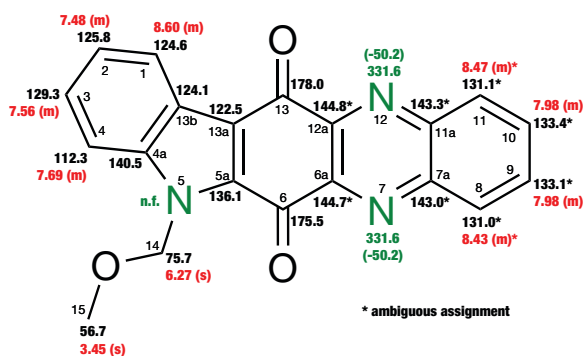
¹H NMR (400 MHz, CDCl₃) δ 8.60 (m, H-1, 1H), 8.47 (m, H-8*/11*, 1H), 8.43 (m, H-8*/11*, 1H), 7.98 (m, H-9/10, 2H), 7.69 (m, H-4, 1H), 7.56 (m, H-3, 1H), 7.48 (m, H-2, 1H), 6.27 (s, H-14, 2H), 3.45 (s, H-15, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 178.0 (C-13), 175.5 (C-6), 144.8 (C-6a*/12a*), 144.7 (C-6a*/12a*), 143.3 (C-7a*/11a*), 143.0 (C-7a*/11a*), 140.5 (C-4a), 136.1 (C-5a), 133.4 (C-9*/10*), 133.1 (C-9*/10*), 131.1 (C-8*/11*), 131.0 (C-8*/11*), 129.3 (C-3), 125.8 (C-2), 124.6 (C-1), 124.1 (C-13b), 122.5 (C-13a), 112.3 (C-4), 75.7 (C-14), 56.7 (C-15).

¹⁵N NMR (40 MHz, CDCl₃) δ -50.2 (N-7/12), not found (N-5).

6. 2. 73 5*H*-Indolo[2,3-*b*]phenazine-6,13-dione (71)
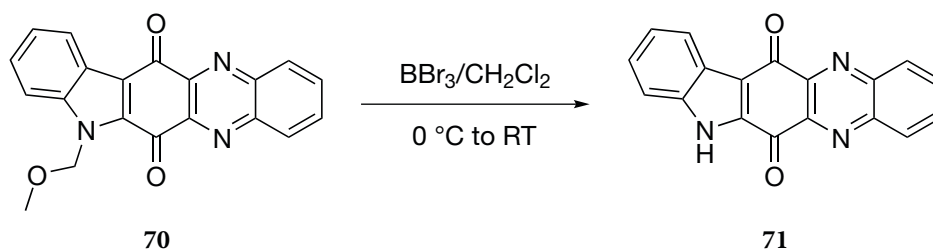
To an ice-cold solution of **70** (145 mg, 0.42 mmol) in dry CH_2Cl_2 (15 mL) a solution of BBr_3 (1M in CH_2Cl_2, 0.56 mL) was added slowly. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (15 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. The filter cake was purified by recrystallisation from ethyl acetate to afford 52 mg (41.3 %) of **71** as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{18}H_9N_3NaO_2$ ([M+Na]$^+$): 322.0587; found: 322.0589.
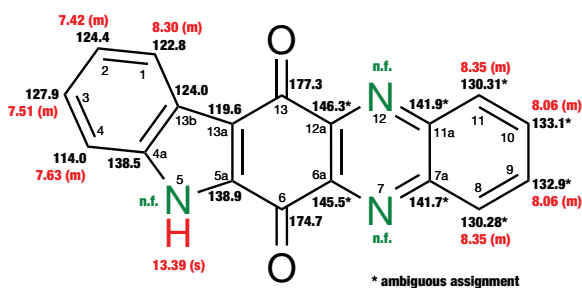
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.39 (s, H-5, 1H), 8.35 (m, H-8/11, 2H), 8.30 (m, H-1, 1H), 8.06 (m, H-9/10, 2H), 7.63 (m, H-4, 1H), 7.51 (m, H-3, 1H), 7.42 (m, H-2, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3 (C-13), 174.7 (C-6), 146.3 (C-6a*/12a*), 145.5 (C-6a*/12a*), 141.9 (C-7a*/11a*), 141.7 (C-7a*/11a*), 138.9 (C-5a), 138.5 (C-4a), 133.1 (C-9*/10*), 132.9 (C-9*/10*), 130.31 (C-8*/11*), 130.28 (C-8*/11*), 127.9 (C-3), 124.4 (C-2), 124.0 (C-13b), 122.8 (C-1), 119.6 (C-13a), 114.0 (C-4).

^{15}N NMR (40 MHz, DMSO-d_6) δ not found (N-5/7/12).

6. 2. 74   5-[2-(Dimethylamino)ethyl]-5*H*-indolo[2,3-*b*]phenazine-6,13-dione (72)

A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.80 mL, 22.44 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (−70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.98 mL, 22.44 mmol, 4 eq.) in dry THF (80 mL) under argon. Then the mixture was allowed to reach −5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to −78 °C and a solution of **27** (1.21 g, 5.61 mmol, 1 eq.) and **69** (1.29 g, 5.61 mmol, 1 eq.) in dry THF (80 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) followed by recrystallisation from ethyl acetate to afford 152 mg (7.3 %) of **72** as yellow crystals, m.p. 248-250 °C.

HRMS: m/z calculated for $C_{22}H_{19}N_4O_2$ ([M+H]⁺): 371.1503; found: 371.1502.
HRMS: m/z calculated for $C_{22}H_{18}N_4NaO_2$ ([M+Na]⁺): 393.1322; found: 393.1323.

¹H NMR (400 MHz, CDCl₃) δ 8.43 (m, H-1, 1H), 8.37 (m, H-8*/11*, 1H), 8.36 (m, H-8*/11*, 1H), 7.91 (m, H-9/10, 2H), 7.42 (m, H-4, 1H), 7.36 (m, H-3, 1H), 7.30 (m, H-2, 1H), 4.85 (m, H-14, 2H), 2.78 (m, H-15, 2H), 2.38 (s, H-17/17ʻ, 6H).

¹³C NMR (100 MHz, CDCl₃) δ 177.2 (C-13), 175.0 (C-6), 144.9 (C-6a*/12a*), 144.6 (C-6a*/12a*), 143.1 (C-7a*/11a*), 142.8 (C-7a*/11a*), 140.0 (C-4a), 135.7 (C-5a), 133.1 (C-9*/10*), 132.8 (C-9*/10*), 130.9 (C-8*/11*), 130.8 (C-8*/11*), 128.6 (C-3), 125.2 (C-2), 124.5 (C-1), 123.9 (C-13b), 121.2 (C-13a), 111.0 (C-4), 58.3 (C-15), 45.7 (C-17/17ʻ), 43.6 (C-14).

¹⁵N NMR (40 MHz, CDCl₃) δ -50.2 (N-7/12), -235.8 (N-5), -358.8 (N-16).

6. 2. 75 *N,N*-Diethylquinoline-2-carboxamide (73)
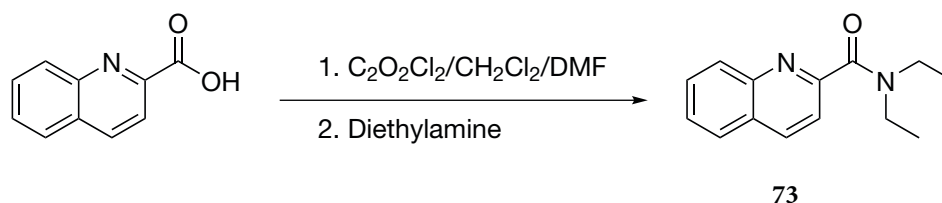
To a solution of quinoline-2-carboxylic acid (10.00 g, 57.8 mmol) in dry CH_2Cl_2 (150 mL) were slowly added oxalyl chloride (6.0 mL, 69.8 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO_2 was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (15.0 mL, 0.14 mol) was added slowly. The mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na_2CO_3 solution (100 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography eluting with ethyl acetate/light petrolum (8:2, v/v) to afford 12.50 g (95.4 %) of **73** as yellow oil.
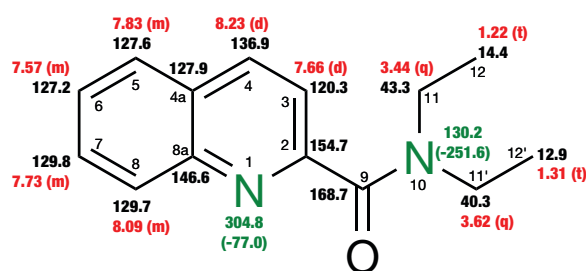
¹H NMR (400 MHz, CDCl₃) δ 8.23 (d, *J* = 8.5, H-4, 1H), 8.09 (m, H-8, 1H), 7.83 (m, H-5, 1H), 7.73 (m, H-7, 1H), 7.66 (d, *J* = 8.5 Hz, H-3, 1H), 7.57 (m, H-6, 1H), 3.62 (q, *J* = 7.1 Hz, H-11', 2H), 3.44 (q, *J* = 7.1 Hz, H-11, 2H), 1.31 (t, *J* = 7.1 Hz, H-12', 3H), 1.22 (t, *J* = 7.1 Hz, H-12, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 168.7 (C-9), 154.7 (C-2), 146.6 (C-8a), 136.9 (C-4), 129.8 (C-7), 129.7 (C-8), 127.9 (C-4a), 127.6 (C-5), 127.2 (C-6), 120.3 (C-3), 43.3 (C-11), 40.3 (C-11'), 14.4 (C-12), 12.9 (C-12').

¹⁵N NMR (40 MHz, CDCl₃) δ -77.0 (N-1), -251.6 (N-10).

6. 2. 76   7-(Methoxymethyl)-6*H*-indolo[3,2-*b*]acridine-6,12(7*H*)-dione (74)
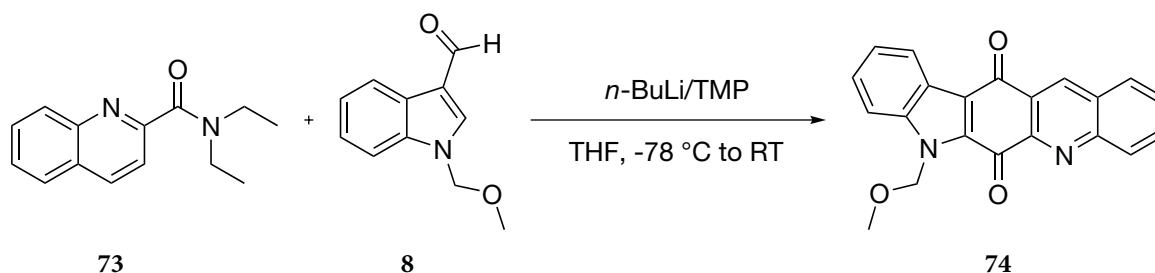
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperi-dine (3.6 mL, 21.3 mmol) dropwise (over less than 1 min) to a cold (-70 °C) solution of *n*-BuLi (2.5M in hexanes, 8.5 mL, 21.3 mmol) in dry THF (60 mL) under argon. Then the mixture was allowed to reach –5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **73** (1.20 g, 5.26 mmol) in dry THF (30 mL) was added dropwise over 10 min. The mixture was stirred at -78 °C for further 20 min and then a solution of aldehyde **8** (0.40 g, 2.11 mmol) in dry THF (18 mL) was added over 5 min. The flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with CH_2Cl_2 (3 x 70 mL) The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a yellowish residue which was purified by column chromatography eluting with ethyl acetate followed by recrystallisation from ethyl acetate. 0.37 g (21.0 %) of **74** were obtained as yellow flakes, m.p. 285-287 °C.

HRMS: m/z calculated for $C_{21}H_{15}N_2O_3$ ([M+H]$^+$): 343.1077; found: 343.1080.
HRMS: m/z calculated for $C_{21}H_{14}N_2NaO_3$ ([M+Na]$^+$): 365.0897; found: 365.0900.
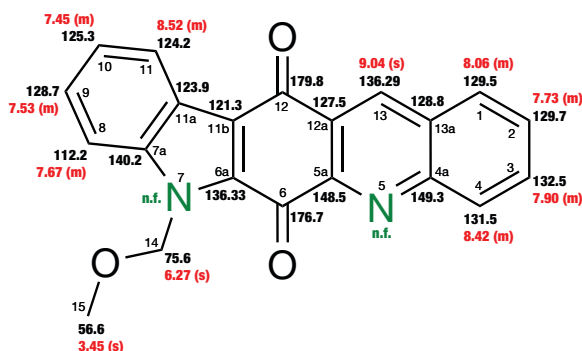
^{1}H NMR (400 MHz, CDCl$_3$) δ 9.04 (s, H-13, 1H), 8.52 (m, H-11, 1H), 8.42 (m, H-4, 1H), 8.06 (m, H-1, 1H), 7.90 (m, H-3, 1H), 7.73 (m, H-2, 1H), 7.67 (m, H-8, 1H), 7.53 (m, H-9, 1H), 7.45 (m, H-10, 1H), 6.27 (s, H-14, 1H), 3.45 (s, H-15, 1H).

^{13}C NMR (100 MHz, CDCl$_3$) δ 179.8 (C-12), 176.7 (C-6), 149.3 (C-4a), 148.5 (C-5a), 140.2 (C-7a), 136.33 (C-6a), 136.29 (C-13), 132.5 (C-3), 131.5 (C-4), 129.7 (C-2), 129.5 (C-1), 128.8 (C-13a), 128.7 (C-9), 127.5 (C-12a), 125.3 (C-10), 124.2 (C-11), 123.9 (C-11a), 121.3 (C-11b), 112.2 (C-8), 75.6 (C-14), 56.6 (C-15).

^{15}N NMR (40 MHz, CDCl$_3$) δ not found (N-5/7).

6. 2. 77   6*H*-Indolo[3,2-*b*]acridine-6,12(7*H*)-dione (75)

74   **75**
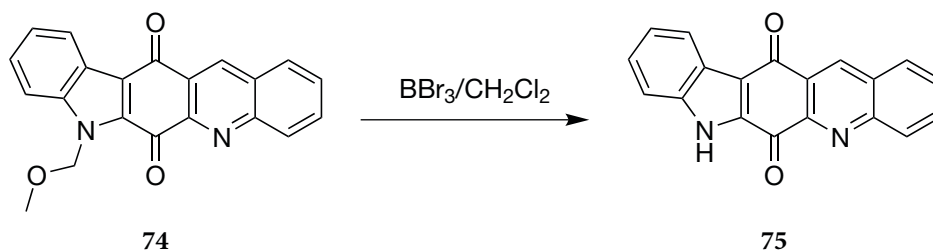
To a cold solution of **74** (410 mg, 1.20 mmol) in dry CH$_2$Cl$_2$ (120 mL) at 0 °C a solution of BBr$_3$ (1M in CH$_2$Cl$_2$, 1.50 mL) was added slowly. The solution was allowed to reach room temperature and continued stirring for 1 h. The mixture was quenched with saturated NaHCO$_3$ solution (100 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH$_2$Cl$_2$. Recrystallisation from ethyl acetate afforded 350 mg (98 %) of **75** as yellow crystals, m.p. >350 °C.

HRMS: m/z calculated for C$_{19}$H$_{10}$N$_2$NaO$_2$ ([M+Na]$^+$): 321.0634; found: 321.0635.
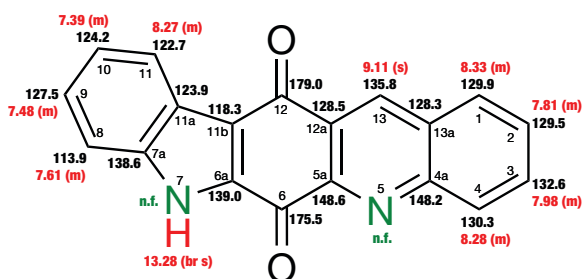
^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 13.28 (br s, H-7, 1H), 9.11 (s, H-13, 1H), 8.33 (m, H-1, 1H), 8.28 (m, H-4, 1H), 8.27 (m, H-11, 1H), 7.98 (m, H-3, 1H), 7.81 (m, H-2, 1H), 7.61 (m, H-8, 1H), 7.48 (m, H-9, 1H), 7.39 (m, H-10, 1H).

^{13}C NMR (100 MHz, DMSO-*d$_6$*) δ 179.0 (C-12), 175.5 (C-6), 148.6 (C-5a), 148.2 (C-4a), 139.0 (C-6a), 138.6 (C-7a), 135.8 (C-13), 132.6 (C-3), 130.3 (C-4), 129.9 (C-1), 129.5 (C-2), 128.5 (C-12a), 128.3 (C-13a), 127.5 (C-9), 124.2 (C-10), 123.9 (C-11a), 122.7 (C-11), 118.3 (C-11b), 113.9 (C-8).

^{15}N NMR (40 MHz, DMSO-*d$_6$*) δ not found (N-5/7).

6. 2. 78   7-[2-(Dimethylamino)ethyl]-6*H*-indolo[3,2-*b*]acridine-6,12(7*H*)-dione (76)

75 → **76**
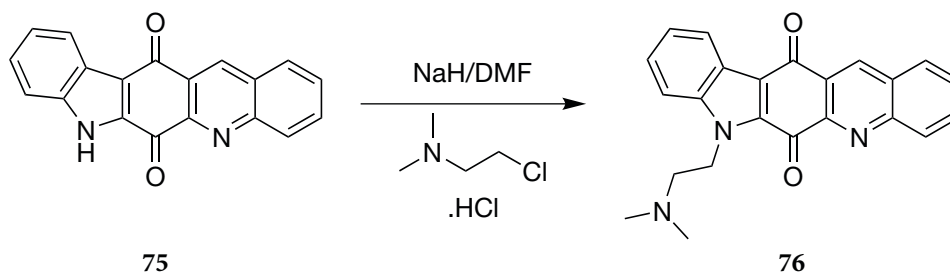
A solution of **75** (300 mg, 1.01 mmol) in dry DMF (20 mL) was added dropwise to a cooled (-5 to -10 °C) suspension of NaH (140 mg, 3.50 mmol, 60 % in paraffin) in dry DMF (10 mL). After 30 min of stirring 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (290 mg, 2.02 mmol) in DMF (15 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with water (50 mL) the mixture was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with CH_2Cl_2/methanol (95:5, v/v) affording compound **76** (100 mg, 26.9 %) as yellow flakes, m.p. 240-242 °C.

HRMS: m/z calculated for $C_{23}H_{20}N_3O_2$ ([M+H]$^+$): 370.1550; found: 370.1548.
HRMS: m/z calculated for $C_{23}H_{19}N_3NaO_2$ ([M+Na]$^+$): 392.1369; found: 392.1369.
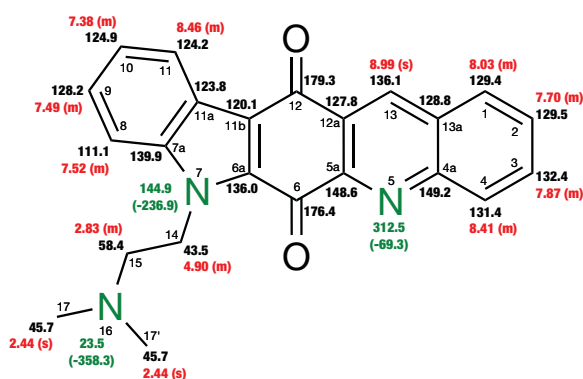
¹H NMR (500 MHz, CDCl₃) δ 8.99 (s, H-13, 1H), 8.46 (m, H-11, 1H), 8.41 (m, H-4, 1H), 8.03 (m, H-1, 1H), 7.87 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.52 (m, H-8, 1H), 7.49 (m, H-9, 1H), 7.38 (m, H-10, 1H), 4.90 (m, H-14, 2H), 2.83 (m, H-15, 2H), 2.44 (s, H-17/17', 6H).

¹³C NMR (125 MHz, CDCl₃) δ 179.3 (C-12), 176.4 (C-6), 149.2 (C-4a), 148.6 (C-5a), 139.9 (C-7a), 136.1 (C-13), 136.0 (C-6a), 132.4 (C-3), 131.4 (C-4), 129.5 (C-2), 129.4 (C-1), 128.8 (C-13a), 128.2 (C-9), 127.8 (C-12a), 124.9 (C-10), 124.2 (C-11), 123.8 (C-11a), 120.1 (C-11b), 111.1 (C-8), 58.4 (C-15), 45.7 (C-17, 17'), 43.5 (C-14).

¹⁵N NMR (50 MHz, CDCl₃) δ -69.3 (N-5) -236.9 (N-7), -358.3 (N-16).

6. 2. 79   *N,N*-Diethylquinoline-3-carboxamide (77)

1. $C_2O_2Cl_2/CH_2Cl_2/DMF$

2. Diethylamine

77
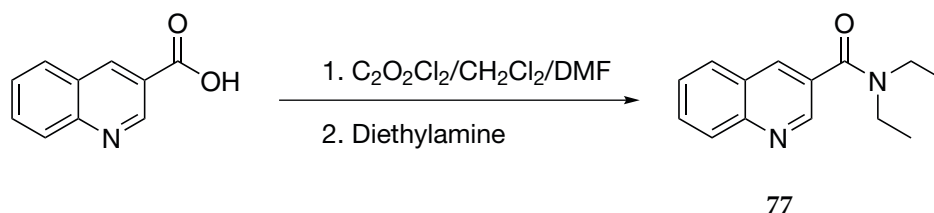
To a solution of quinoline-3-carboxylic acid (1.0 g, 5.78 mmol) in dry CH_2Cl_2 (30 mL) were slowly added oxalyl chloride (0.65 mL, 6.93 mmol) and 3 drops of dry DMF. The mixture was continuously stirred at room temperature until the formed CO_2 was completely gone (approximately 1 h). The mixture was ice-cooled, then diethylamine (5.0 mL, 47.9 mmol) was added slowly. The mixture was allowed to reach room temperature and stirred for further 30 min. The reaction was quenched by carefully adding saturated Na_2CO_3 solution (50 mL). The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with ethyl acetate/light petroleum (8:2, v/v) to obtain 1.05 g (85.0 %) of **77** as yellow oil.
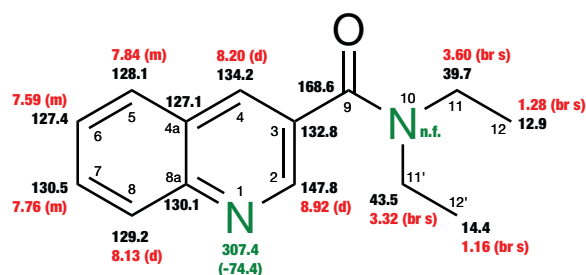
¹H NMR (400 MHz, CDCl₃) δ 8.92 (d, *J* = 2.1 Hz, H-2, 1H), 8.20 (d, *J* = 2.1, H-4, 1H), 8.13 (d, *J* = 8.4 Hz, H-8, 1H), 7.84 (m, H-5, 1H), 7.76 (m, H-7, 1H), 7.59 (m, H-6, 1H), 3.60 (br s, H-11, 2H), 3.32 (br s, H-11', 2H), 1.28 (br s, H-12, 3H), 1.16 (br s, H-12', 3H).

¹³C NMR (100 MHz, CDCl₃) δ 168.6 (C-9), 147.8 (C-2), 134.2 (C-4), 130.5 (C-7), 130.1 (C-8a), 129.2 (C-8), 128.1 (C-5), 127.4 (C-6), 127.1 (C-4a), 43.5 (C-11'), 39.7 (C-11), 14.3 (C-12'), 12.9 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ -74.4 (N-1), not found (N-10).

6. 2. 80   8-(Methoxymethyl)-7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione (78)
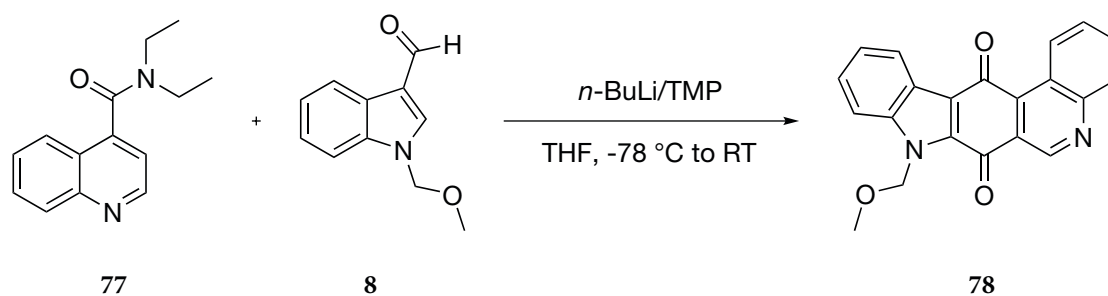
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.2 mL, 17.89 mmol) dropwise (over less than 1 min) to a cold (-70 °C) solution of *n*-BuLi (2.5 M in hexane, 7.2 mL, 17.89 mmol) in dry THF (65 mL), under argon. then allowing the mixture to –5 °C to 0 °C over 30 min and further stirring at this temperature for 30 min. The mixture was then recooled to –78 °C and a solution of **77** (1.02 g, 4.47 mmol) and **8** (350 mg, 1.85 mmol) in dry THF (30 mL) was added dropwise over 10 min. After stirring at this temperature for 40 min the flask was then taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to furnish a brown residue which was purified by column chromatography eluting with ethyl acetate/light petroleum (4:6, v/v) followed by recrystallisation from ethyl acetate to afford 304 mg (47.9 %) of **78** as orange crystals, m.p. 228 °C.

HRMS: m/z calculated for $C_{21}H_{15}N_2O_3$ ([M+H]$^+$): 343.1077; found: 343.1078.
HRMS: m/z calculated for $C_{21}H_{14}N_2NaO_3$ ([M+Na]$^+$): 365.0897; found: 365.0896.
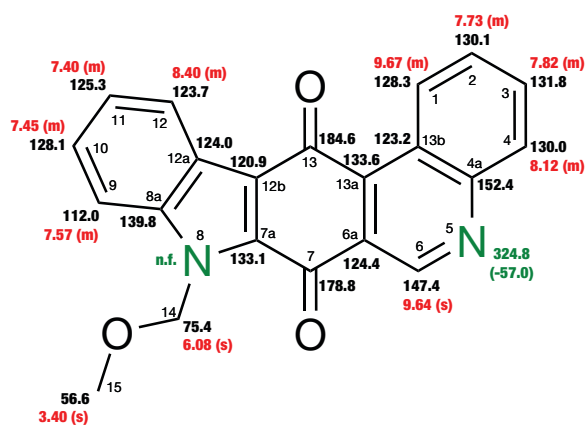
^{1}H NMR (500 MHz, CDCl$_3$) δ 9.67 (m, H-1, 1H), 9.64 (s, H-6, 1H), 8.40 (m, H-12, 1H), 8.12 (m, H-4, 1H), 7.82 (m, H-3, 1H), 7.73 (m, H-2, 1H), 7.57 (m, H-9, 1H), 7.45 (m, H-10, 1H), 7.40 (m, H-11, 1H), 6.08 (s, H-14, 2H), 3.40 (s, H-15, 3H).

^{13}C NMR (125 MHz, CDCl$_3$) δ 184.6 (C-13), 178.8 (C-7), 152.4 (C-4a), 147.4 (C-6), 139.8 (C-8a), 133.6 (C-13a), 133.1 (C-7a), 131.8 (C-3), 130.1 (C-2), 130.0 (C-4), 128.3 (C-1), 128.1 (C-10), 125.3 (C-11), 124.4 (C-6a), 124.0 (C-12a), 123.7 (C-12), 123.2 (C-13b), 120.9 (C-12b), 112.0 (C-9), 75.4 (C-14), 56.6 (C-15).

^{15}N NMR (50 MHz, CDCl$_3$) δ -57.0 (N-5), not found (N-8).

6. 2. 81   7*H*-Indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione (79)
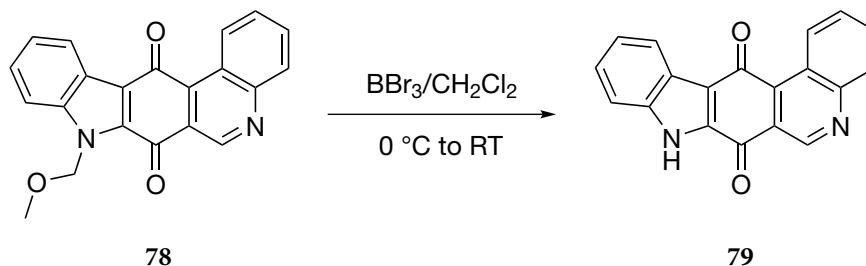
To an ice-cold solution of **78** (124 mg, 0.36 mmol) in dry CH₂Cl₂ (25 mL) a solution of BBr₃ (1M in CH₂Cl₂, 0.43 mL, 0.43 mmol) was added dropwise. The solution was allowed to reach room temperature and continued stirring for a total of 1 h. The mixture was quenched with saturated NaHCO₃ solution (25 mL) and vigorously stirred for 1 h at 60 °C. The cooled mixture was filtrated, the filter cake was washed with water and cold CH₂Cl₂. Recrystallisation from acetone afforded 75.6 mg (70.0 %) of **79** as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{19}H_{11}N_2O_2$ ([M+H]⁺): 299.0815; found: 299.0815.
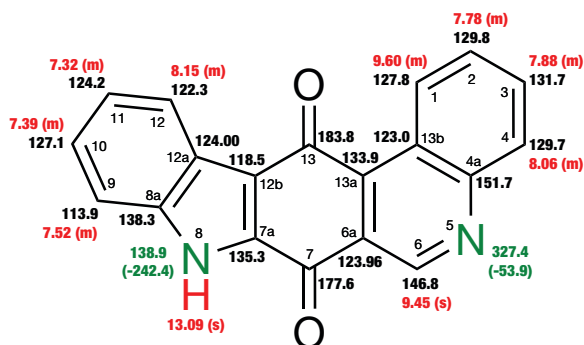
¹H NMR (400 MHz, DMSO-*d₆*) δ 13.09 (s, H-8, 1H), 9.60 (m, H-1, 1H), 9.45 (s, H-6, 1H), 8.15 (m, H-12, 1H), 8.06 (m, H-4, 1H), 7.88 (m, H-3, 1H), 7.78 (m, H-2, 1H), 7.52 (m, H-9, 1H), 7.39 (m, H-10, 1H), 7.32 (m, H-11, 1H).

¹³C NMR (100 MHz, DMSO-*d₆*) δ 183.8 (C-13), 177.6 (C-7), 151.7 (C-4a), 146.8 (C-6), 138.3 (C-8a), 135.3 (C-7a), 133.9 (C-13a), 131.7 (C-3), 129.8 (C-2), 129.7 (C-4), 127.8 (C-1), 127.1 (C-10), 124.2 (C-11), 124.0 (C-12a), 123.96 (C-6a), 123.0 (C-13b), 122.3 (C-12), 118.5 (C-12b), 113.9 (C-9).

¹⁵N NMR (40 MHz, DMSO-*d₆*) δ -53.9 (N-5), -242.4 (N-8).

6. 2. 82 8-[2-(Dimethylamino)ethyl]-7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione (80)
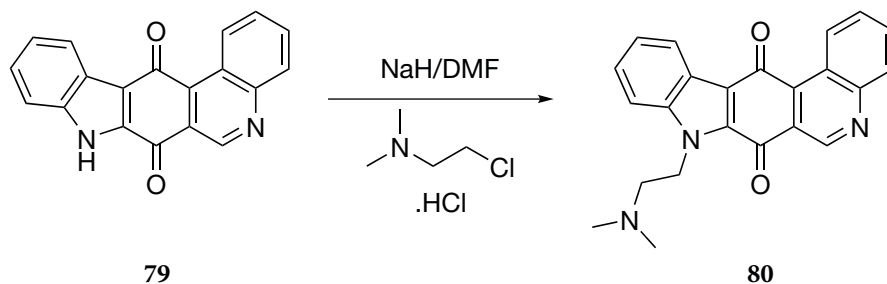
79   **80**

A solution of **79** (800 mg, 2.68 mmol) in dry DMF (50 mL) was added dropwise to a cooled (-10 to 0 °C) suspension of NaH (430 mg, 10.75 mmol, 60 % in paraffin) in dry DMF (20 mL). After 30 min of stirring 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (580 mg, 4.03 mmol) in DMF (20 mL) was added slowly and the reaction mixture was stirred at 75 °C for 5 h. After quenching with water (150 mL) the mixture was extracted with ethyl acetate and the combined organic layers were dried over Na₂SO₄. The solvent was removed under reduced pressure to give a crude product which was purified by column chromatography eluting with ethyl acetate/methanol (95:5, v/v) to ethyl acetate/methanol/triethylamine (93:5:2, v/v) affording compound **80** (320 mg, 32.3 %) as red crystals, m.p. 168-172 °C.

HRMS: m/z calculated for $C_{23}H_{20}N_3O_2$ ([M+H]⁺): 370.1550; found: 370.1555.
HRMS: m/z calculated for $C_{23}H_{19}N_3NaO_2$ ([M+Na]⁺): 392.1369; found: 392.1374.
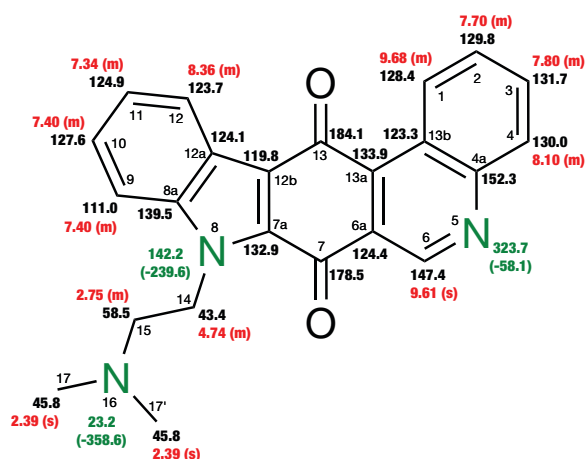
¹H NMR (500 MHz, CDCl₃) δ 9.68 (m, H-1, 1H), 9.61 (s, H-6, 1H), 8.36 (m, H-12, 1H), 8.10 (m, H-4, 1H), 7.80 (m, H-3, 1H), 7.70 (m, H-2, 1H), 7.40 (m, H-9/10, 2H), 7.34 (m, H-11, 1H), 4.74 (m, H-14, 2H), 2.75 (m, H-15, 2H), 2.39 (s, H-17/17', 6H).

¹³C NMR (125 MHz, CDCl₃) δ 184.1 (C-13), 178.5 (C-7), 152.3 (C-4a), 147.4 (C-6), 139.5 (C-8a), 133.9 (C-13a), 132.9 (C-7a), 131.7 (C-3), 130.0 (C-4), 129.8 (C-2), 128.4 (C-1), 127.6 (C-10), 124.9 (C-11), 124.4 (C-6a), 124.1 (C-12a), 123.7 (C-12), 123.3 (C-13b), 119.8 (C-12b), 111.0 (C-9), 58.4 (C-15), 45.8 (C-17/17'), 43.4 (C-14).

¹⁵N NMR (50 MHz, CDCl₃) δ -58.1 (N-5), -239.6 (N-8), -358.6 (N-16).

6. 2. 83 6-[2-(Dimethylamino)ethyl]-5*H*-pyridazino[4,5-*b*]carbazole-5,11(6*H*)-dione (81)
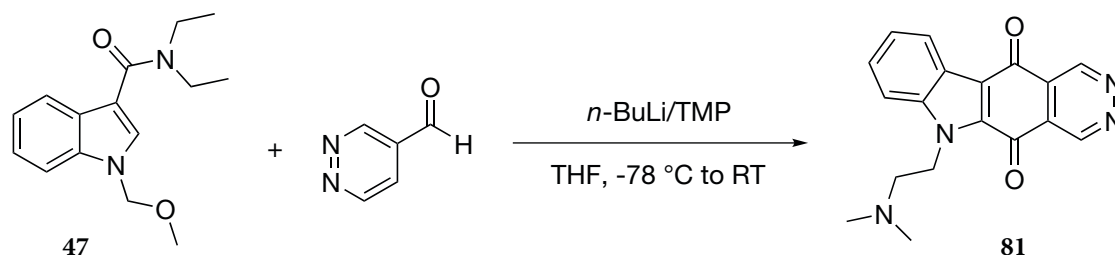
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.15 mL, 18.48 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 7.39 mL, 18.48 mmol, 4 eq.) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **47** (1.33 g, 4.62 mmol, 1 eq.) in dry THF (20 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyridazine-4-carbaldehyde (500 mg, 4.62 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate, dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue. The residue was purified by column chromatography eluting with ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate to afford 90 mg (6.1 %) of **81** as red needles, m.p. 204-206 °C.

HRMS: m/z calculated for $C_{18}H_{17}N_4O_2$ ([M+H]$^+$): 321.1346; found: 321.1348.
HRMS: m/z calculated for $C_{18}H_{16}N_4NaO_2$ ([M+Na]$^+$): 343.1165; found: 343.1166.
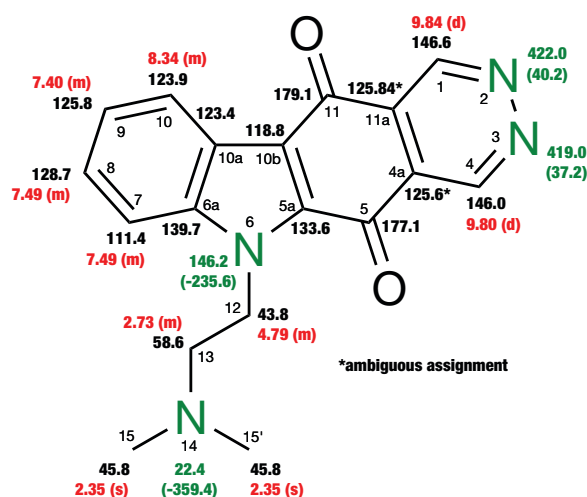
¹H NMR (400 MHz, CDCl₃) δ 9.84 (d, *J* = 1.2 Hz, H-1, 1H), 9.80 (d, *J* = 1.2 Hz, H-4, 1H), 8.34 (m, H-10, 1H), 7.49 (m, H-7/8, 2H), 7.40 (m, H-9, 1H), 4.79 (m, H-12, 2H), 2.73 (m, H-13, 2H), 2.35 (s, H-15/15', 6H).

¹³C NMR (100 MHz, CDCl₃) δ 179.1 (C-11), 177.1 (C-5), 146.6 (C-1), 146.0 (C-4), 139.7 (C-6a), 133.6 (C-5a), 128.7 (C-8), 125.84 (C-4a*/11a*), 125.8 (C-9), 125.6 (C-4a*/11a*), 123.9 (C-10), 123.4 (C-10a), 118.8 (C-10b), 111.4 (C-7), 58.6 (C-13), 45.8 (C-15/15'), 43.8 (C-12).

¹⁵N NMR (40 MHz, CDCl₃) δ 40.2 (N-2), 37.2 (N-3), -235.6 (N-6), -359.4 (N-14).

165

6. 2. 84   6-(Methoxymethyl)-5*H*-pyridazino[4,5-*b*]carbazole-5,11(6*H*)-dione (82)
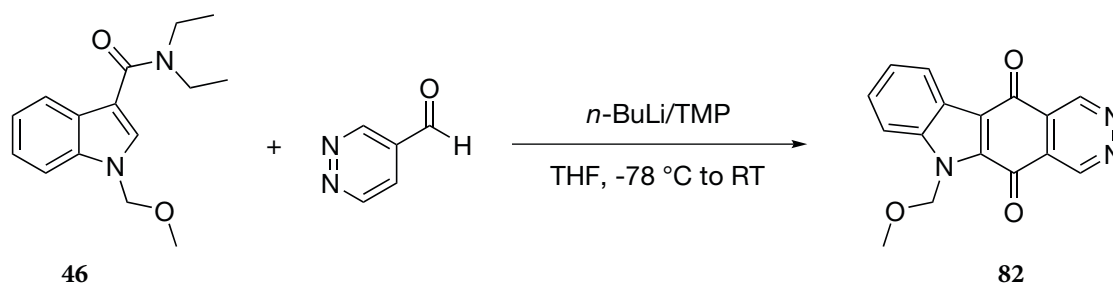
A lithium 2,2,6,6-tetramethylpiperidide solution was prepared by adding 2,2,6,6-tetramethylpiperidine (3.15 mL, 18.48 mmol, 4 eq.) dropwise (over less than 1 min) to a cold (–70 °C) solution of *n*-BuLi (2.5M in hexanes, 7.39 mL, 18.48 mmol, 4 eq.) in dry THF (60 mL) under argon. The mixture was allowed to reach -5 °C to 0 °C over 30 min and was stirred at this temperature for further 30 min. The mixture was then recooled to –78 °C and a solution of **46** (1.20 g, 4.62 mmol, 1 eq.) in dry THF (40 mL) was added dropwise over 10 min. After stirring the mixture at -75 °C for 30 min a solution of pyridazine-4-carbaldehyde (500 mg, 4.62 mmol, 1 eq.) in dry THF (25 mL) was added dropwise in such a rate as to keep the internal temperature below -65 °C. After the addition had been completed, the flask was taken out of the cold bath and left standing just above the level of the bath liquid to slowly reach room temperature (about 2 h), then quenched with water (50 mL). The aqueous layer was extracted with ethyl acetate and dried over Na_2SO_4 and the solvent was evaporated to furnish a brown residue which was purified by column chromatography eluting with ethyl acetate, followed by ethyl acetate/methanol (9:1, v/v) and recrystallisation from ethyl acetate to afford 55 mg (4.1 %) of **82** as red needles, m.p. 237-239 °C.

HRMS: m/z calculated for $C_{16}H_{12}N_3O_3$ ([M+H]⁺): 294.0873; found: 294.0874.
HRMS: m/z calculated for $C_{16}H_{11}N_3NaO_3$ ([M+Na]⁺): 316.0693; found: 316.0695.
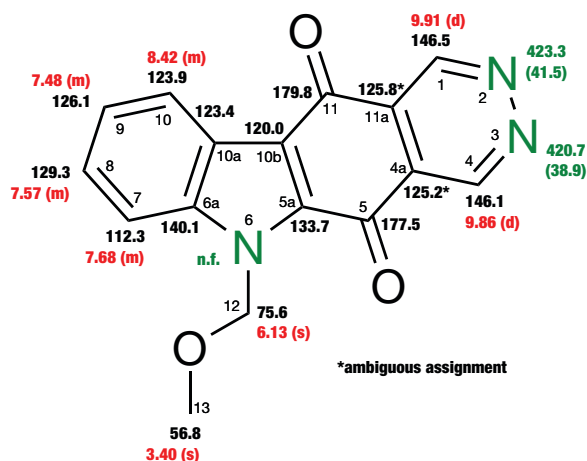
¹H NMR (400 MHz, CDCl₃) δ 9.91 (d, *J* = 1.2 Hz, H-1, 1H), 9.86 (d, *J* = 1.2 Hz, H-4, 1H), 8.42 (m, H-10, 1H), 7.68 (m, H-7, 1H), 7.57 (m, H-8, 1H), 7.48 (m, H-9, 1H), 6.13 (s, H-12, 2H), 3.40 (s, H-13, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 179.8 (C-11), 177.5 (C-5), 146.5 (C-1), 146.1 (C-4), 140.1 (C-6a), 133.7 (C-5a), 129.3 (C-8), 126.1 (C-9), 125.8 (C-4a*/11a*), 125.2 (C-4a*/11a*), 123.9 (C-10), 123.4 (C-10a), 120.0 (C-10b), 112.3 (C-7), 75.6 (C-12), 56.8 (C-13).

¹⁵N NMR (40 MHz, CDCl₃) δ 41.5 (N-2), 38.9 (N-3).

6. 2. 85   5*H*-Pyridazino[4,5-*b*]carbazole-5,11(6*H*)-dione (83)

82 → **83**
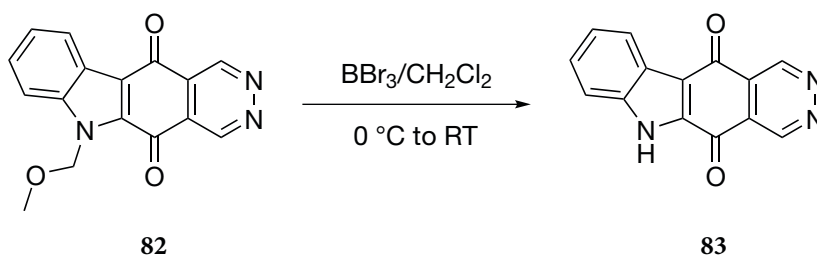
To an ice-cold solution of **82** (14 mg, 0.048 mmol) in dry CH_2Cl_2 (5 mL) a solution of BBr_3 (1M in CH_2Cl_2, 57 µL) was added slowly. The solution was allowed to reach room temperature and stirred for a total of 1 h. To the solution was added saturated $NaHCO_3$ solution (5 mL) and the mixture was stirred vigorously at 60 °C for 1 h. The cooled mixture was filtrated, the filter cake was washed with water and cold CH_2Cl_2. The filter residue was purified by column chromatography eluting with ethyl acetate to afford 4 mg (33.6 %) of **83** as orange crystals, m.p. >350 °C.

HRMS: m/z calculated for $C_{14}H_7N_3NaO_2$ ([M+Na]$^+$): 272.0430; found: 272.0434.

*ambiguous assignment
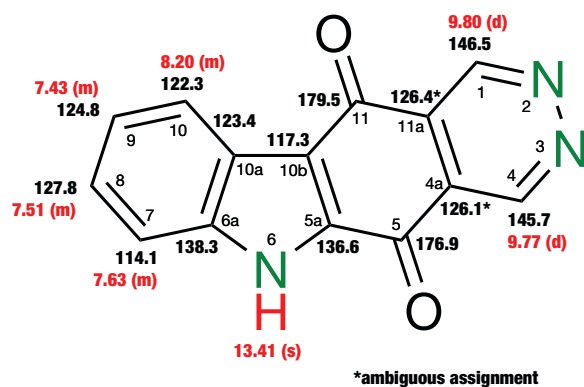
^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 13.41 (s, 1H), 9.80 (d, *J* = 1.2 Hz, H-1, 1H), 9.77 (d, *J* = 1.2 Hz, H-4, 1H), 8.20 (m, H-10, 1H), 7.63 (m, H-7, 1H), 7.51 (m, H-8, 1H), 7.43 (m, H-9, 1H).

^{13}C NMR (100 MHz, DMSO-*d$_6$*) δ 179.5 (C-11), 176.9 (C-5), 146.5 (C-1), 145.7 (C-4), 138.3 (C-6a), 136.6 (C-5a), 127.8 (C-8), 126.4 (C-4a*/11a*), 126.1 (C-4a*/11a*), 124.8 (C-9), 123.4 (C-10a), 122.3 (C-10), 117.3 (C-10b), 114.1 (C-7).

^{15}N NMR (40 MHz, DMSO-*d$_6$*) not measured.

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8   LIST OF ABBREVIATIONS AND PHYSICAL UNITS

Å	ångström (a unit of length, 0.1 nm)
ANOVA	analysis of variance
APCI	atmospheric pressure chemical ionisation
APT	attached proton test
aqua bidest.	double-distilled water
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
BMI	body mass index
BSA	bovine serum albumine
$C_2O_2Cl_2$	oxalyl chloride
CAM	cell adhesion molecule
CAN	ceric ammonium nitrate
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
CPD	composite pulse decoupling
CTL	cytotoxic T lymphocyte
$Cu(OAc)_2$	copper(II) acetate
d	doublet *or* day
dd	doublet of doublets
ddd	doublet of doublet of doublets
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
D*o*M	directed *ortho* metallation
dq	doublet of quartets
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EMA	European Medicines Agency
eq.	equivalent
ESI	electrospray ionisation
EtOH	ethanol
FACS®	fluorescence activated cell sorting
FCS	fetal calf serum
FGF	fibroblast growth factor
h	hour
HMBC	heteronuclear multiple bond correlation
HOESY	heteronuclear Nuclear Overhauser Effect spectroscopy
HOMO	highest occupied molecular orbital
HRESIMS	high-resolution electrospray ionisation mass spectrometry
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
IBX	2-iodoxybenzoic acid
IC_{50}	half maximal inhibitory concentration

IUPAC	International Union of Pure and Applied Chemistry
IGF	insulin-like growth factor
J	coupling constant
LDA	lithium diisopropylamide
LHMDS	lithium hexamethyldisilazide (lithium bis(trimethylsilyl)amide)
LiTMP	lithium tetramethylpiperidide
LUMO	lowest unoccupied molecular orbital
m	multiplet
M	molar
m/z	a value of mass divided by charge
M^+	molecular ion
m-CPBA	*meta*-chloroperoxybenzoic acid
MEM	Minimum Essential Medium
MeOH	methanol
mg	milligram
MHz	megahertz
min	minute(s)
mL	millilitre
mmol	millimole
MOM	methoxymethyl protecting group
m.p.	melting point
MS	mass spectrometry
MW	microwave
NBS	*N*-Bromosuccinimide
NaOMe	sodium methoxide
n-BuLi	butyllithium
NCI	National Cancer Institute
NHL	non-Hodgkin lymphoma
NK	Natural killer cells
NMR	nuclear magnetic resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect spectroscopy
NR	Neutral Red (3-Amino-7-dimethylamino-2-methylphenazine hydrochloride)
PBS	phosphate buffered saline
$Pd(OAc)_2$	palladium(II) acetate
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium(0)
$PdCl_2(PPh_3)_2$	bis(triphenylphosphine)palladium(II) dichloride
PDGF	platelet-derived growth factor
PivOH	pivalic acid (IUPAC: dimethylpropanoic acid)
$P(OEt)_3$	triethyl phosphite
PPh_3	triphenylphosphine
ppm	parts per million
p-TsOH	*para*-toluene sulfonic acid
q	quartet
RNA	ribonucleic acid
ROS	reactive oxygen species
RP	reversed phase

rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
RT	room temperature
s	singlet
sec-BuLi	*sec*-Butyllithium
SFM	serum free medium
t	triplet
TBAHS	tetrabutylammonium hydrogensulfate
TEA	triethylamine
tert-BuLi	*tert*-Butyllithium
TGF	transforming growth factor
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidine
TMS	tetramethylsilane
TNF	tumor necrosis factor
TOP	topoisomerase
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
v/v	volume to volume ratio
VEGF	vascular endothelial growth factor
WHO	World Health Organisation
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide
°C	degree centigrade
δ	chemical shift

9 SPECTROSCOPIC INFORMATION

Furo[3,4-b]pyridine-5,7-dione (PROTON)

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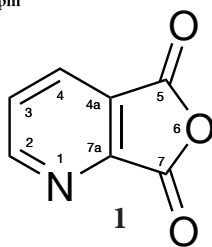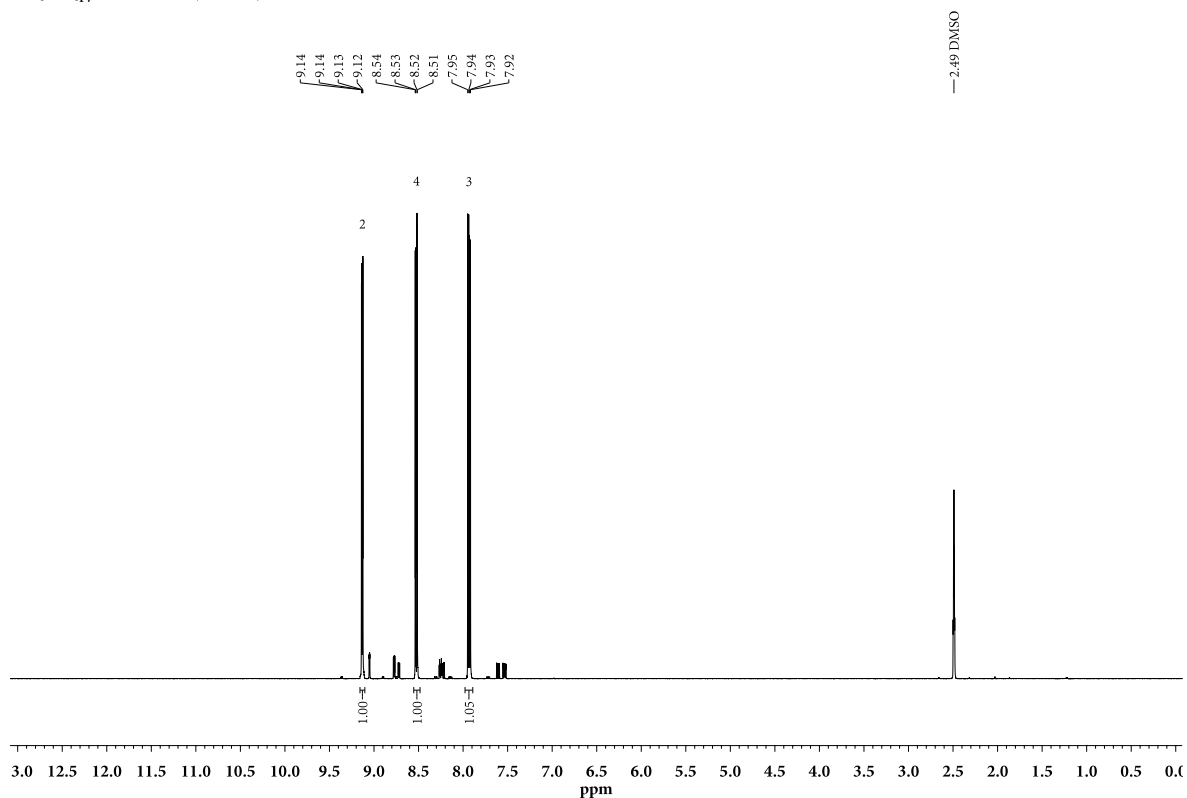
Furo[3,4-b]pyridine-5,7-dione (PROTON)

Furo[3,4-b]pyridine-5,7-dione (C13 CPD)

Furo[3,4-b]pyridine-5,7-dione (C13 CPD)

Furo[3,4-b]pyridine-5,7-dione (HSQC)
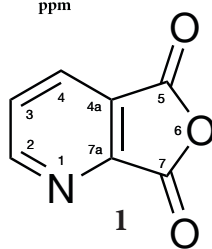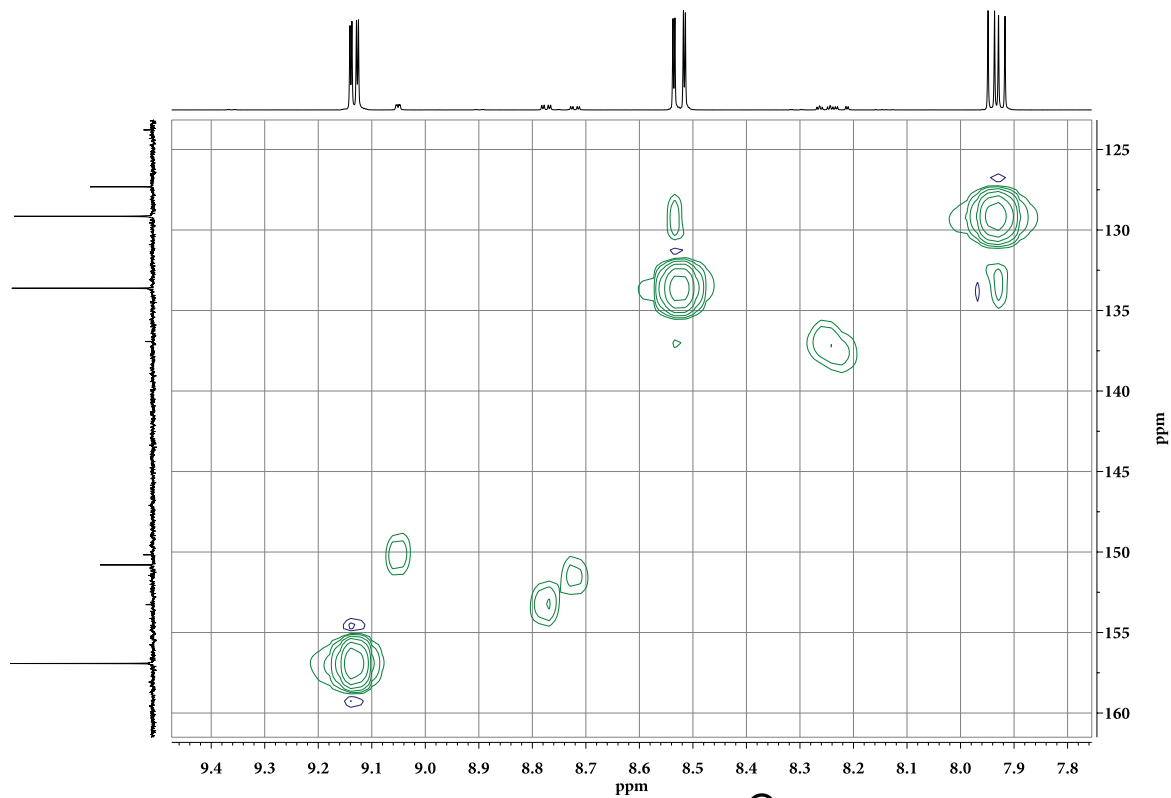
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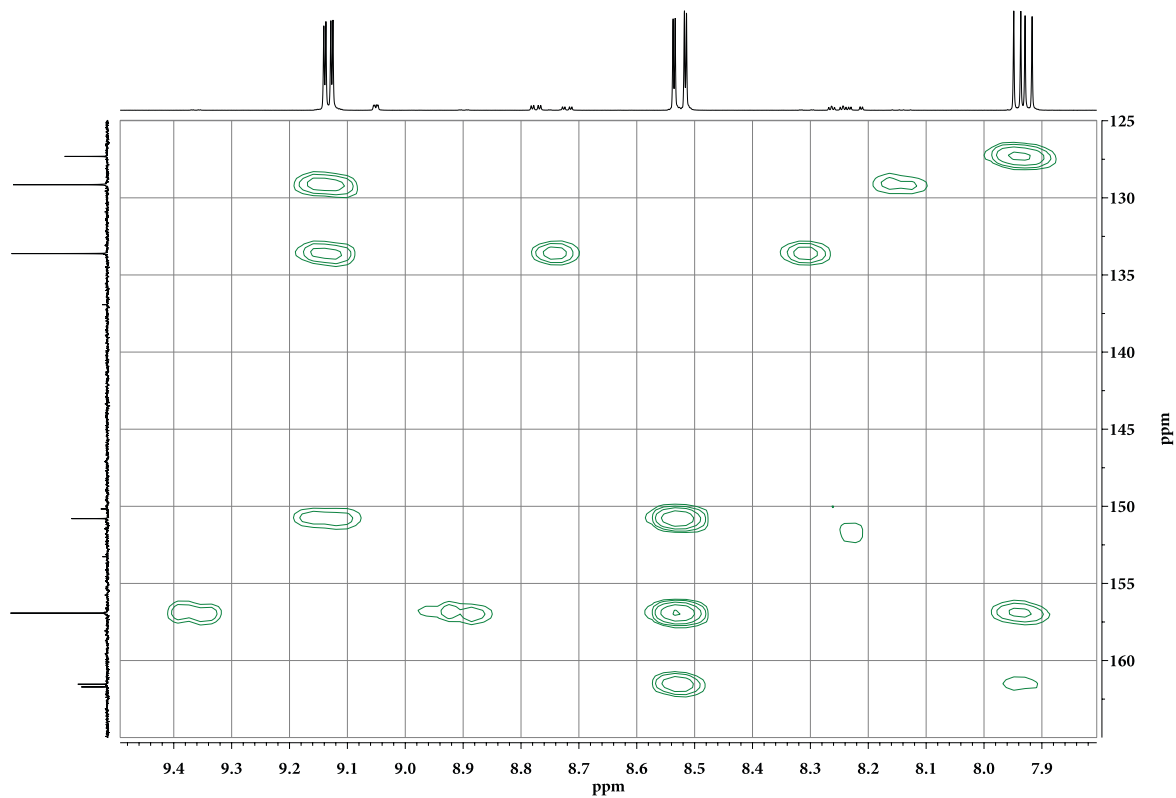
Furo[3,4-b]pyridine-5,7-dione (15N HMBC)

Furo[3,4-b]pyridin-5(7H)-one (PROTON)
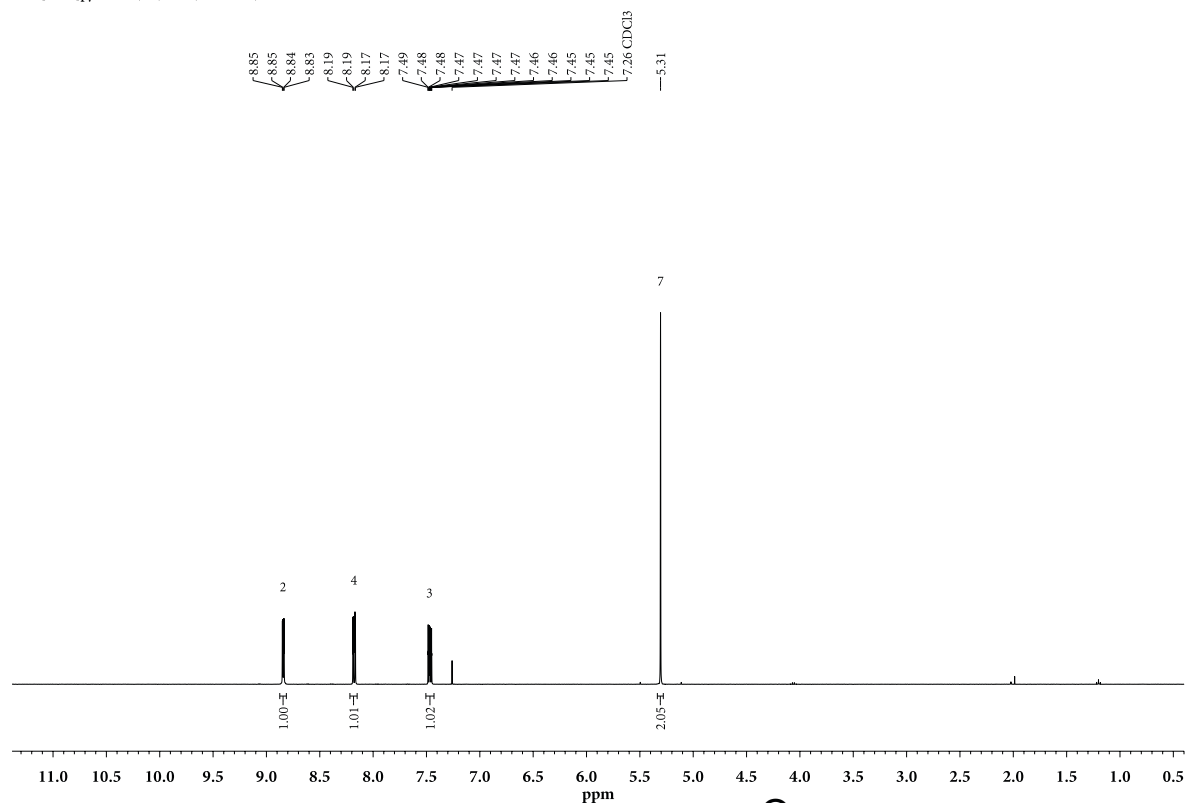
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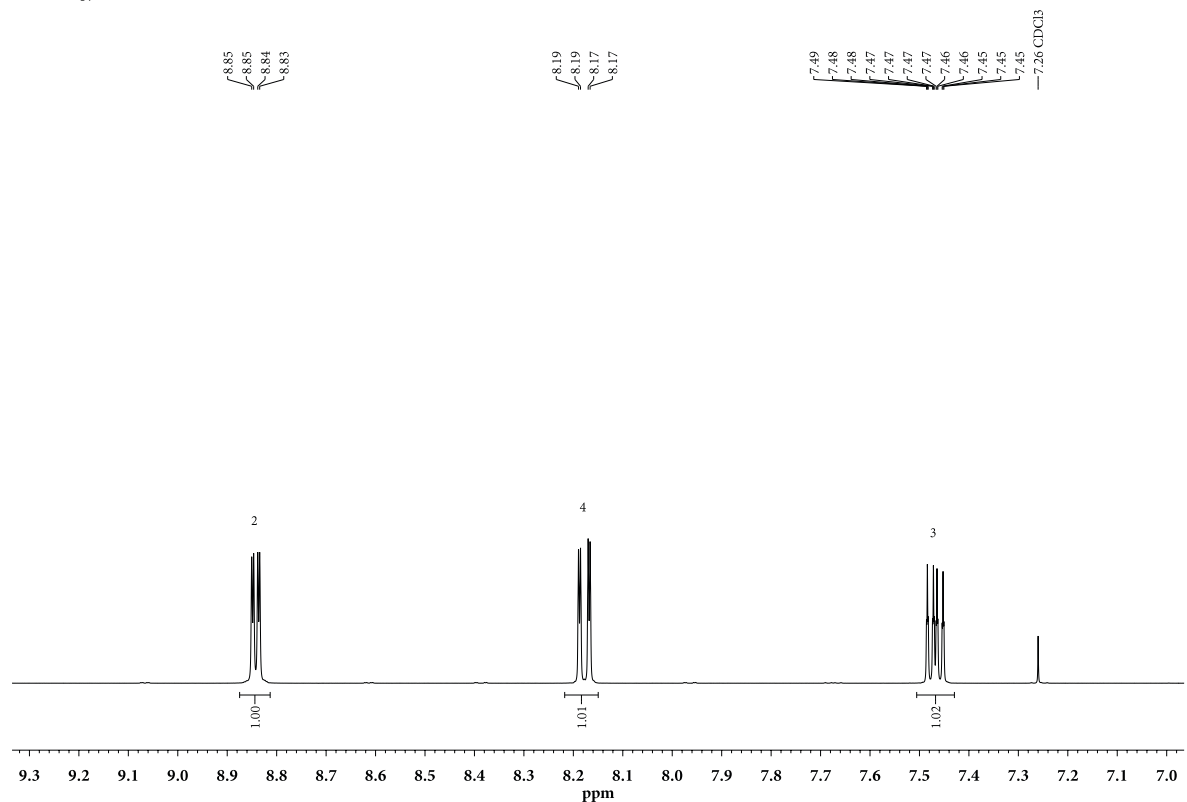
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168.93
166.43
155.27
133.96
123.89
119.60
77.00 CDCl3
70.46

2

4

3

5 7a

4a

7

180   170   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0
ppm

2

Furo[3,4-b]pyridin-5(7H)-one (HSQC)

Furo[3,4-b]pyridin-5(7H)-one (HMBC)

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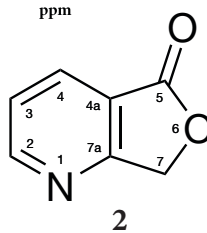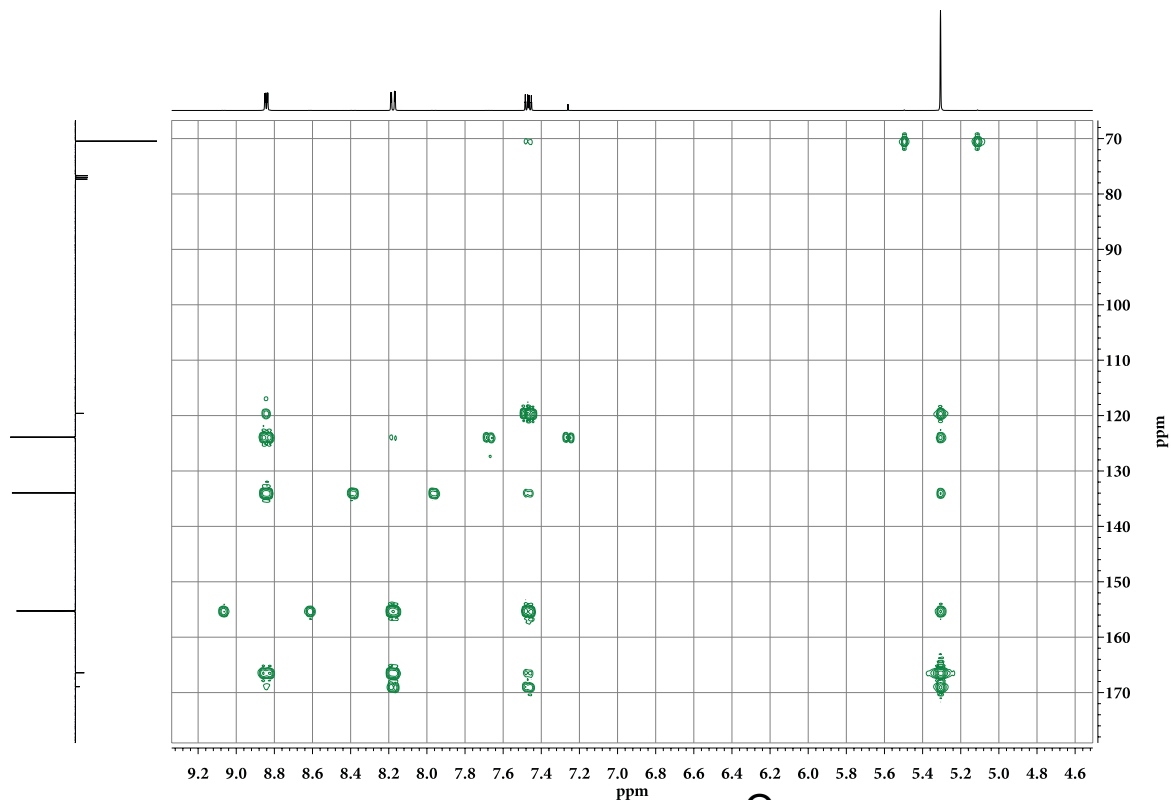
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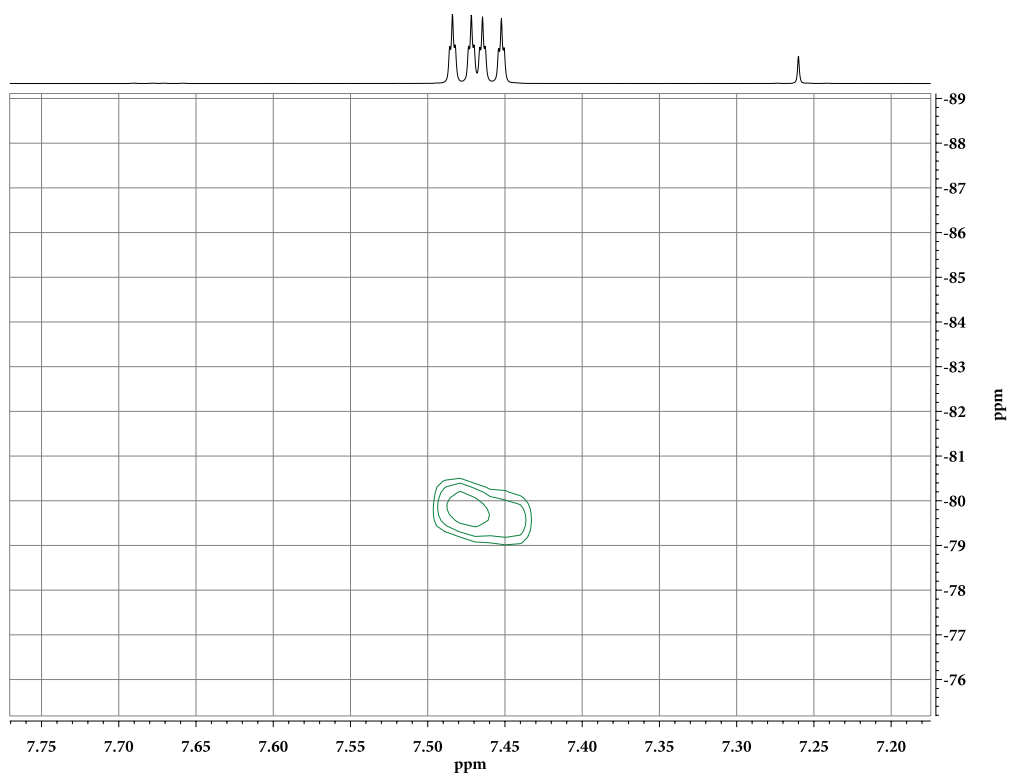
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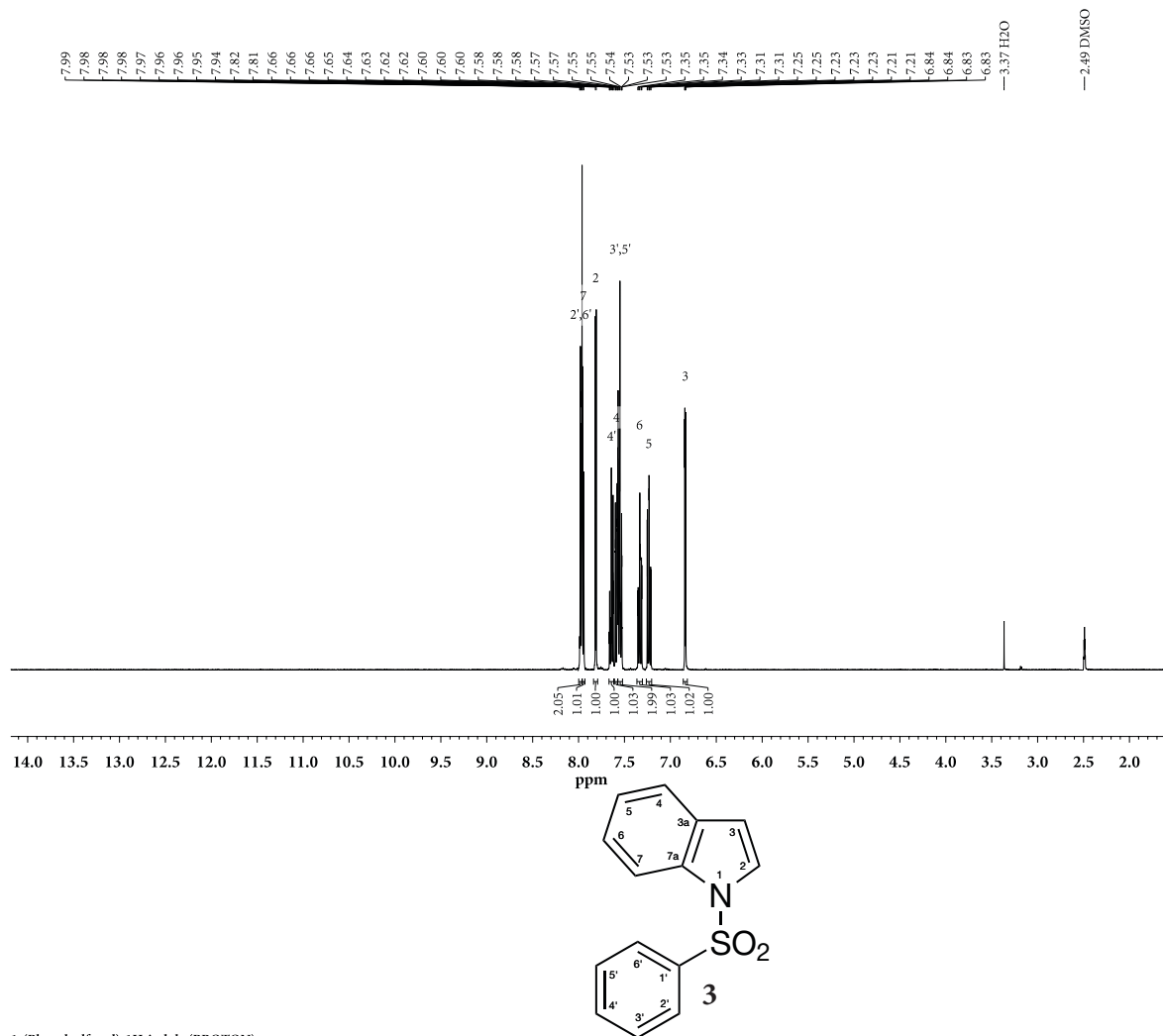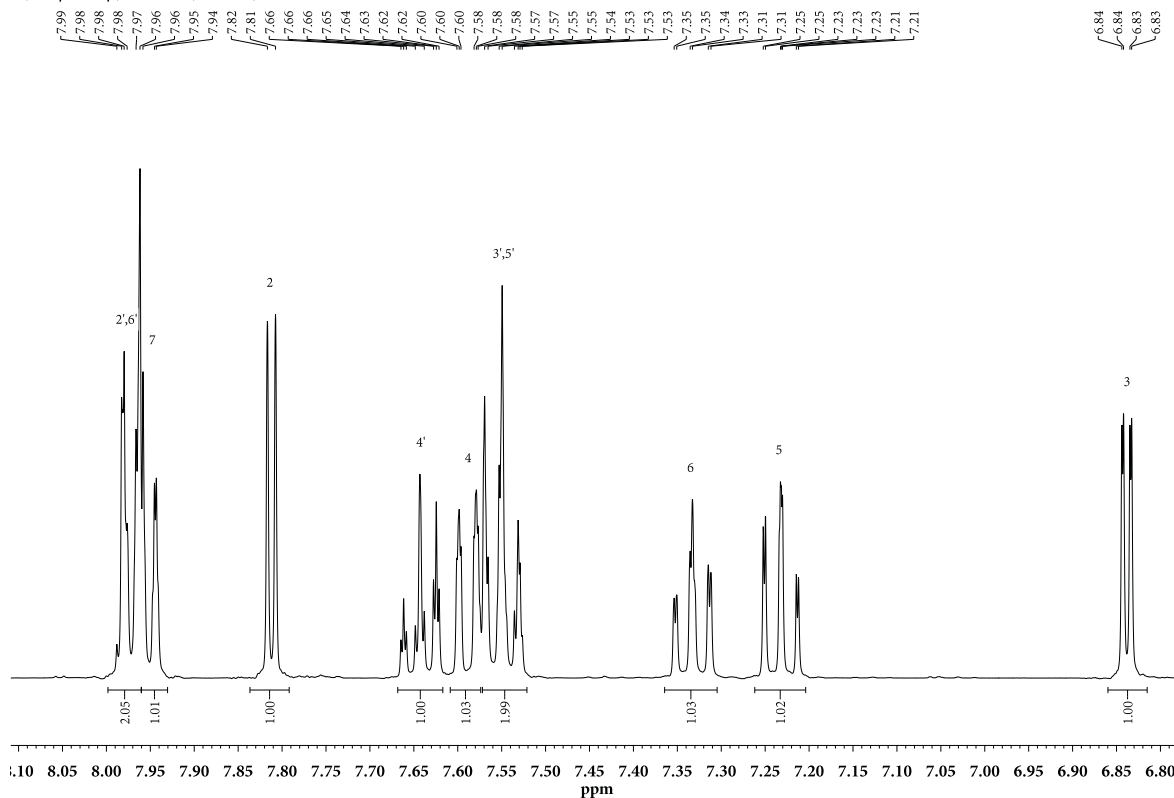
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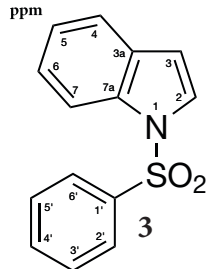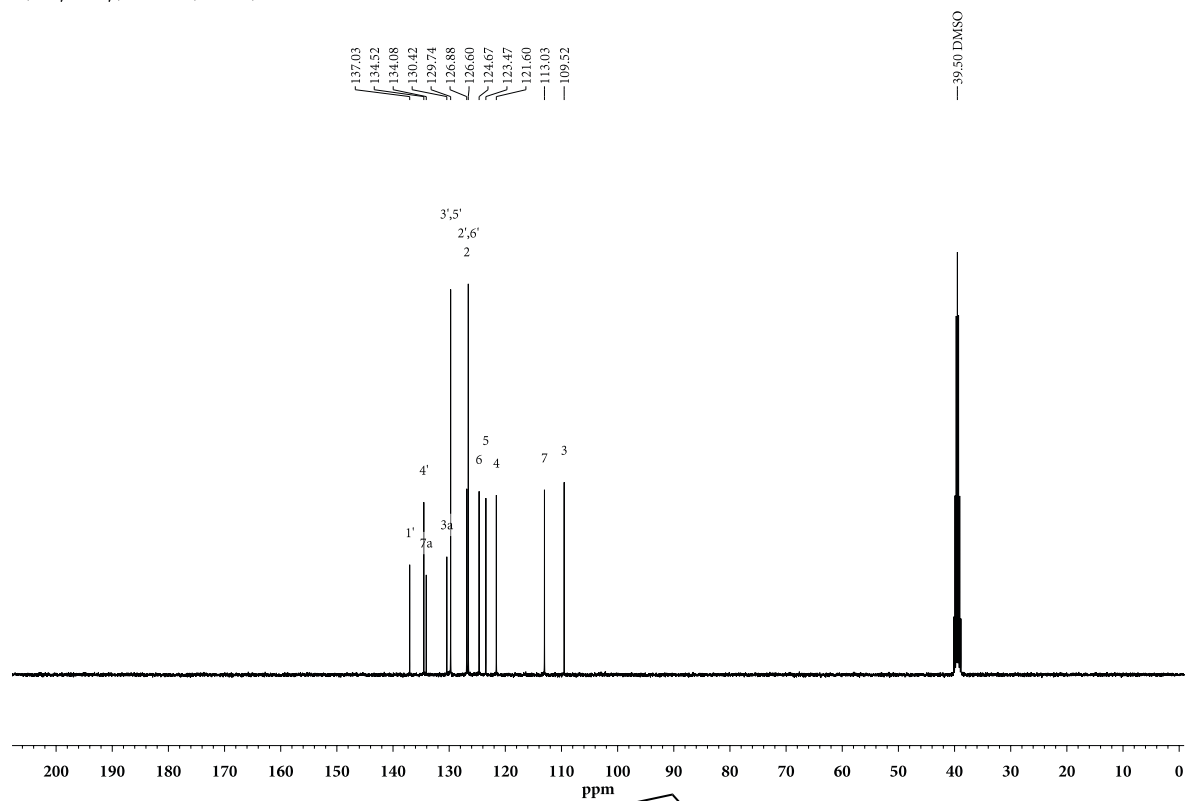
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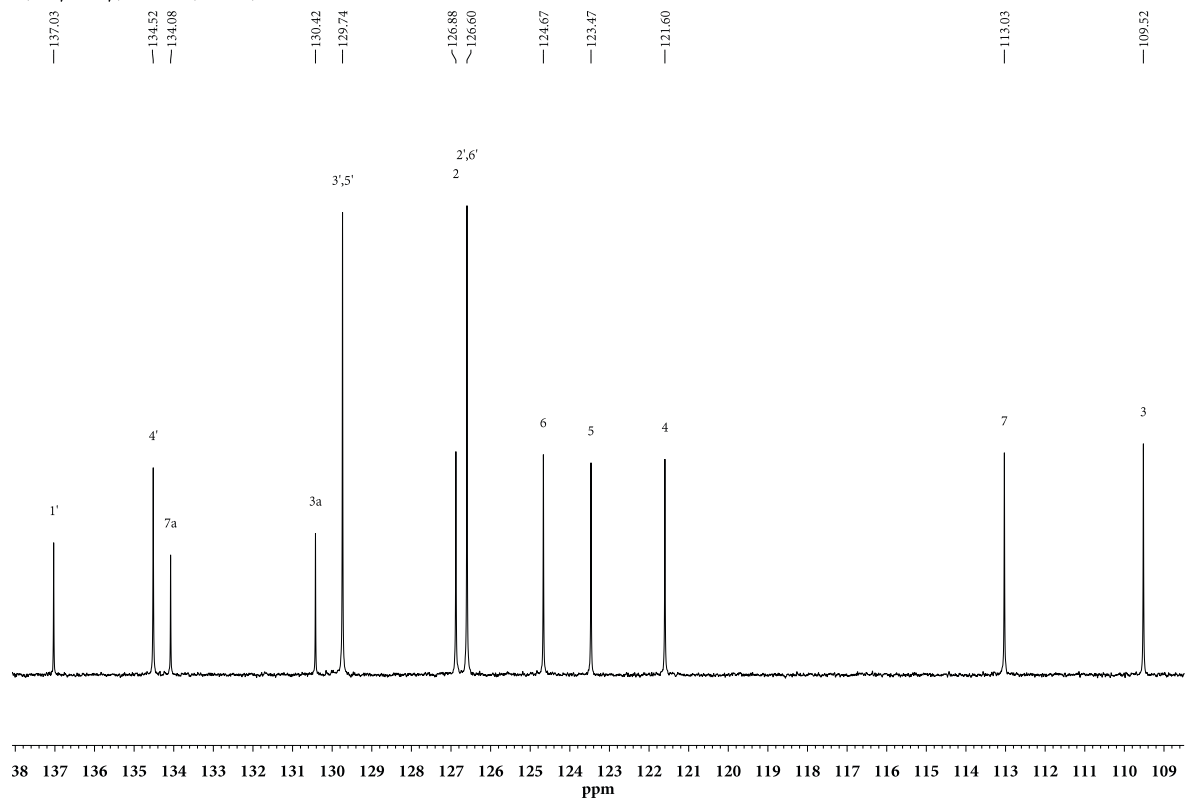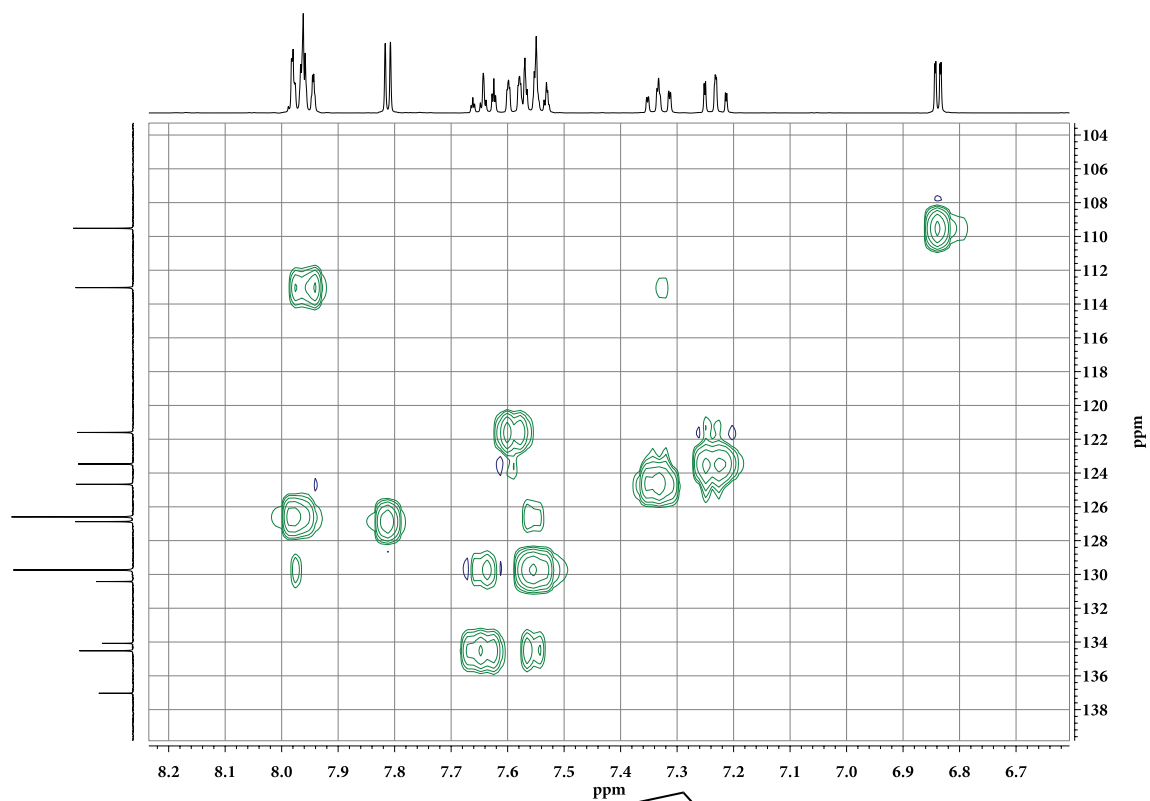
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1-(Phenylsulfonyl)-1H-indole (HMBC)
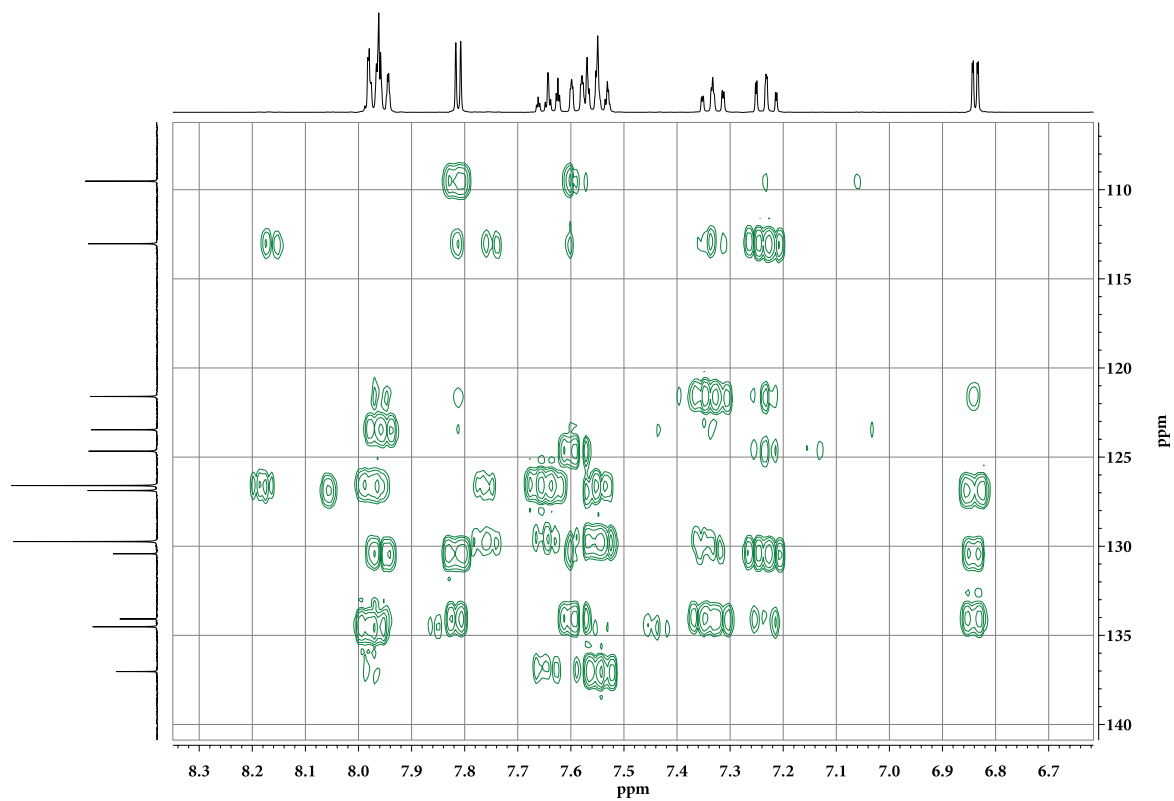
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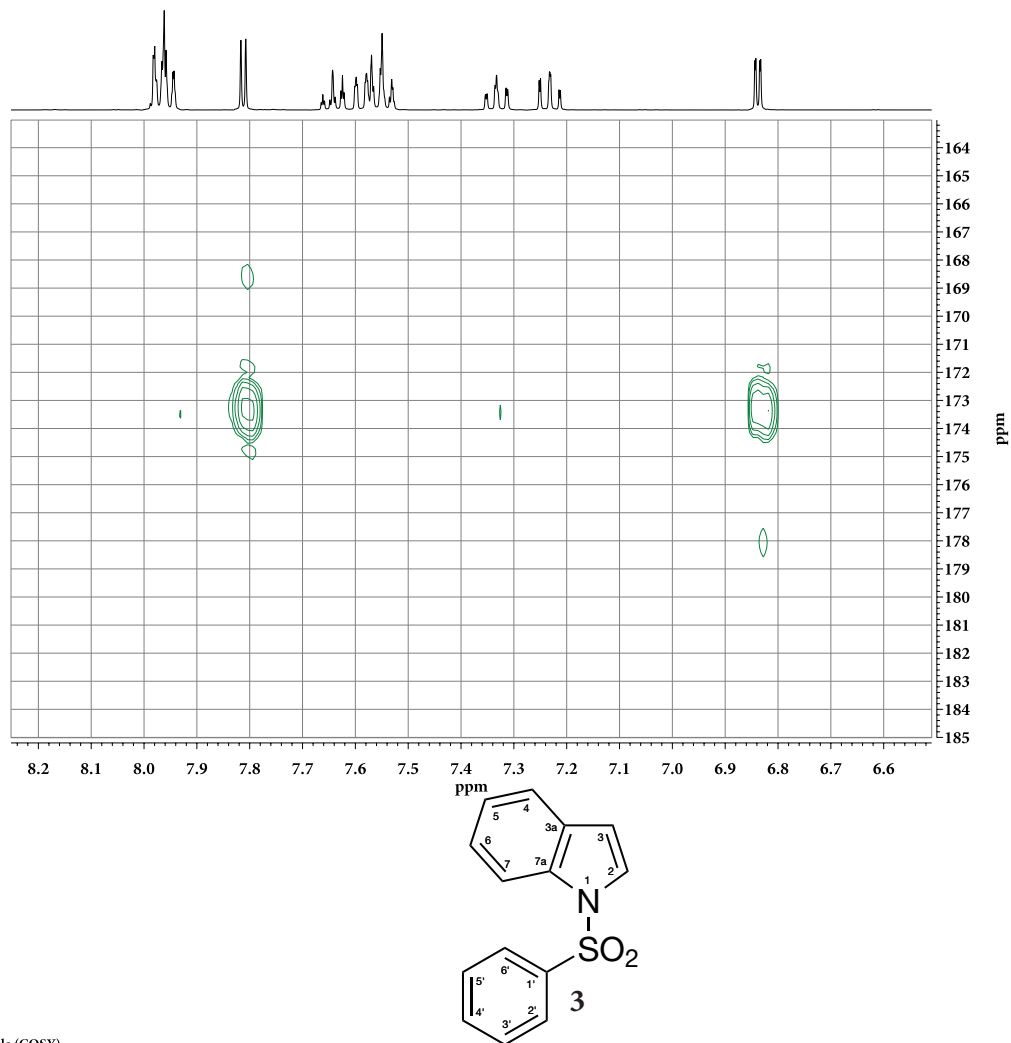
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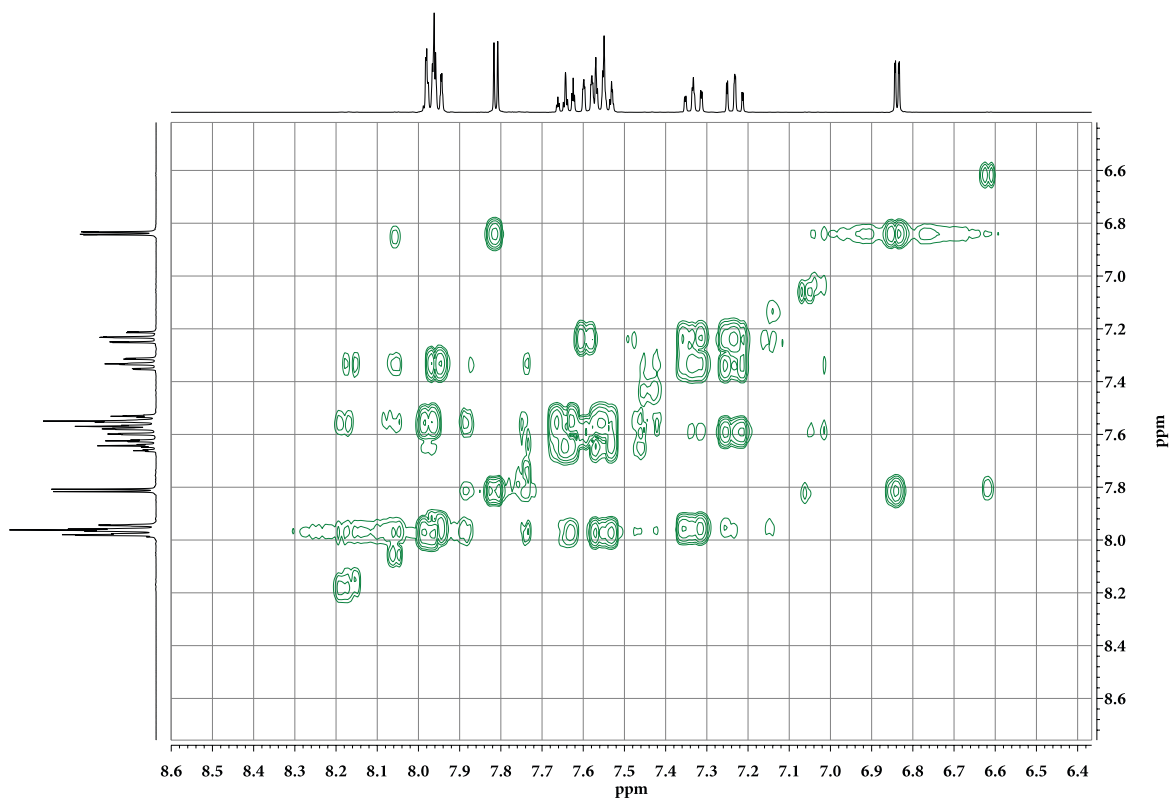
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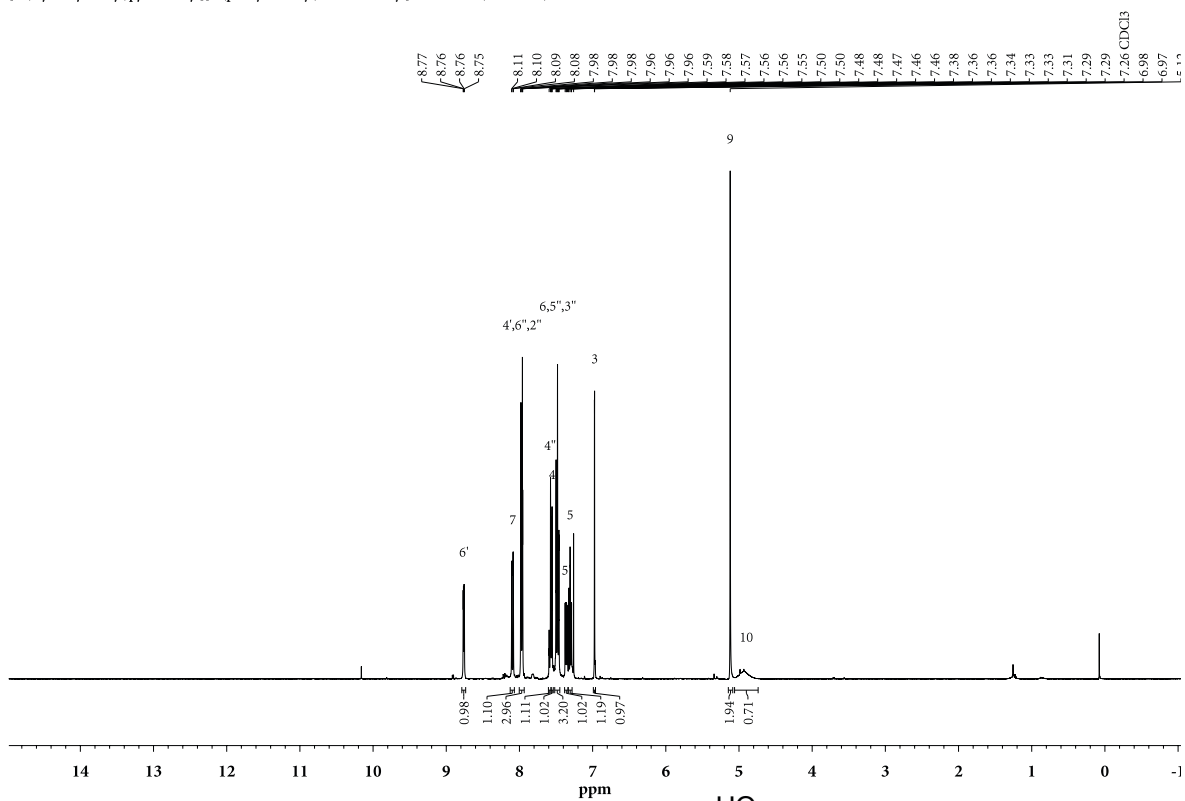
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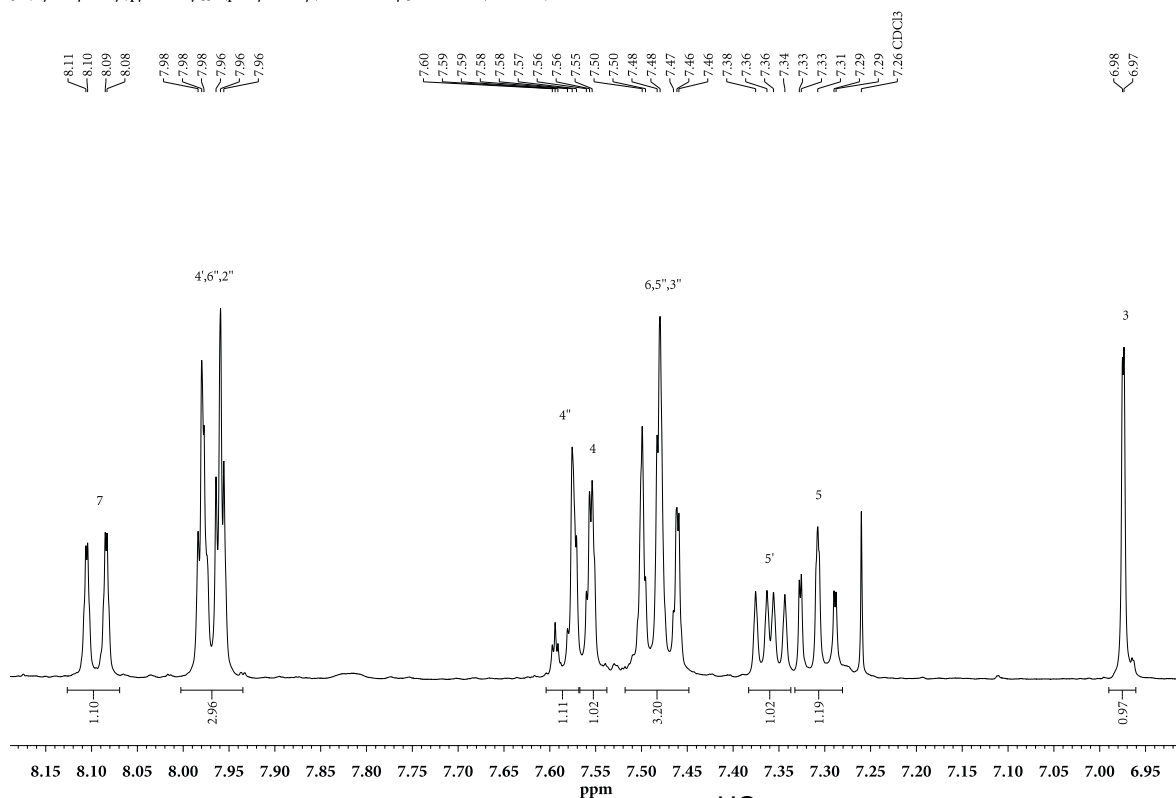
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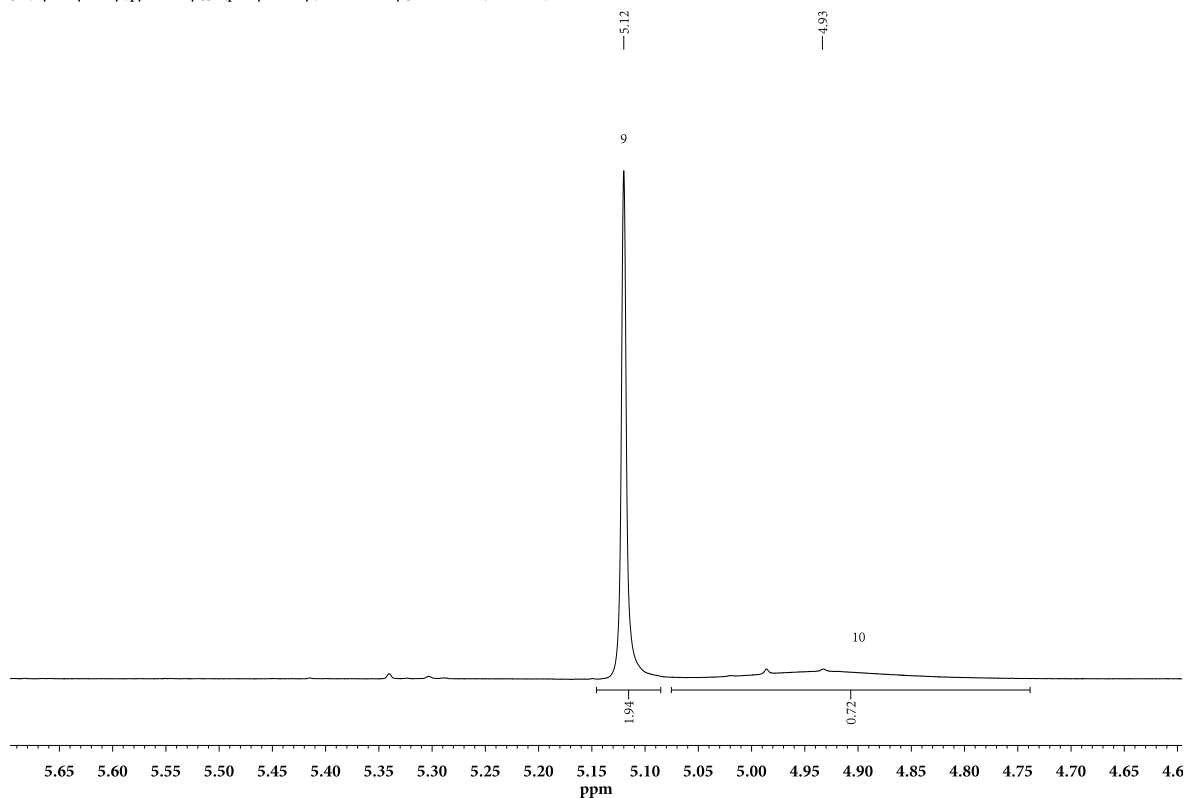
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[2-(Hydroxymethyl)pyridin-3-yl][1-(phenylsulfonyl)-1H-indol-2-yl]methanone (HSQC)
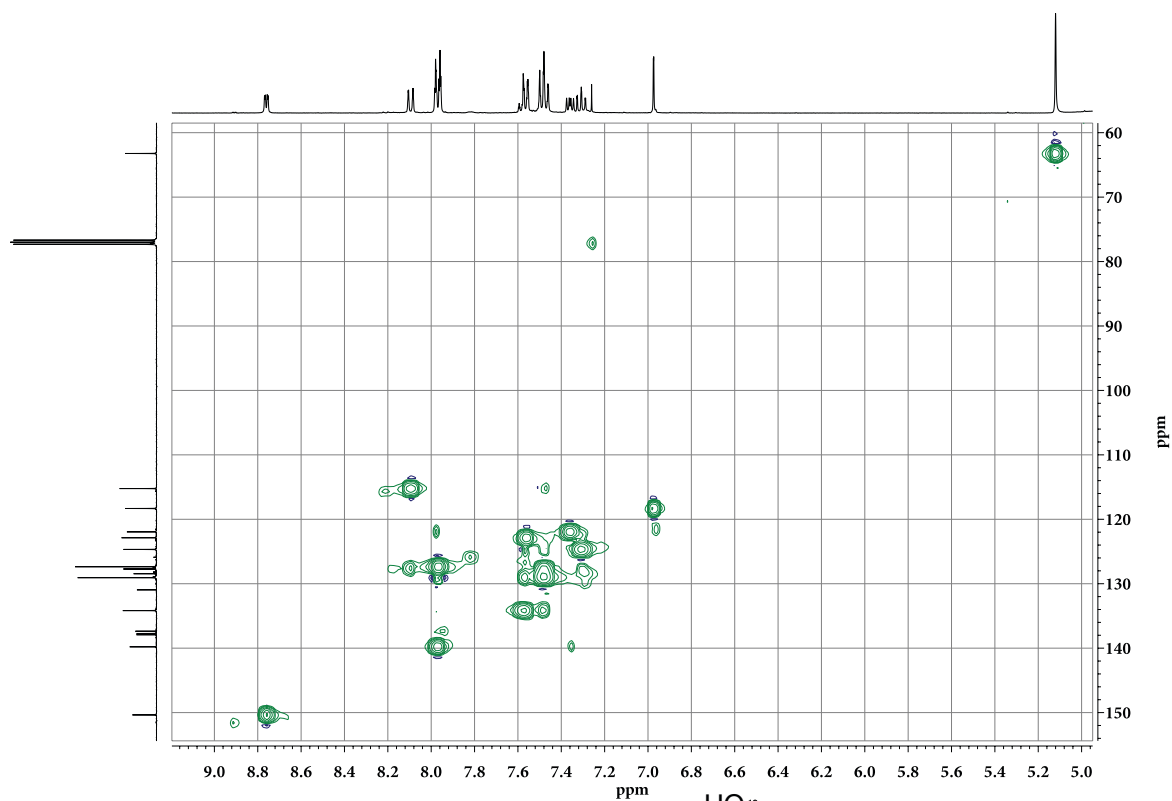
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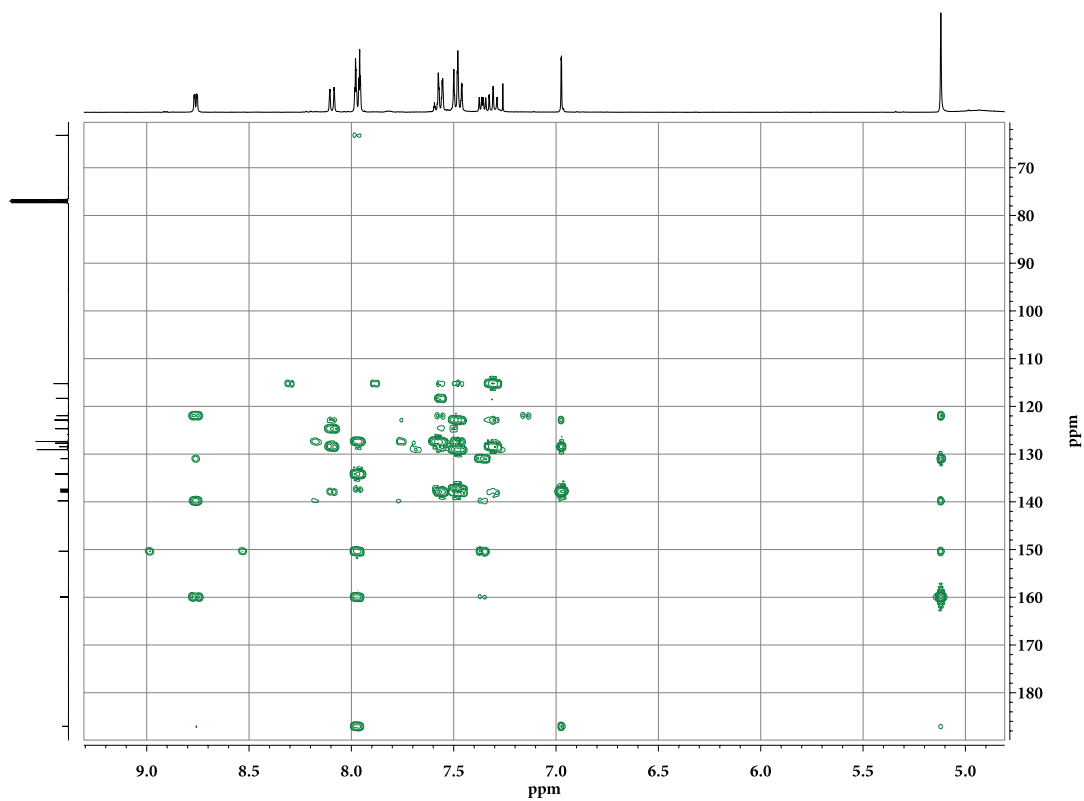
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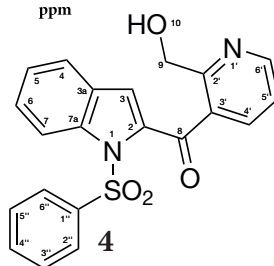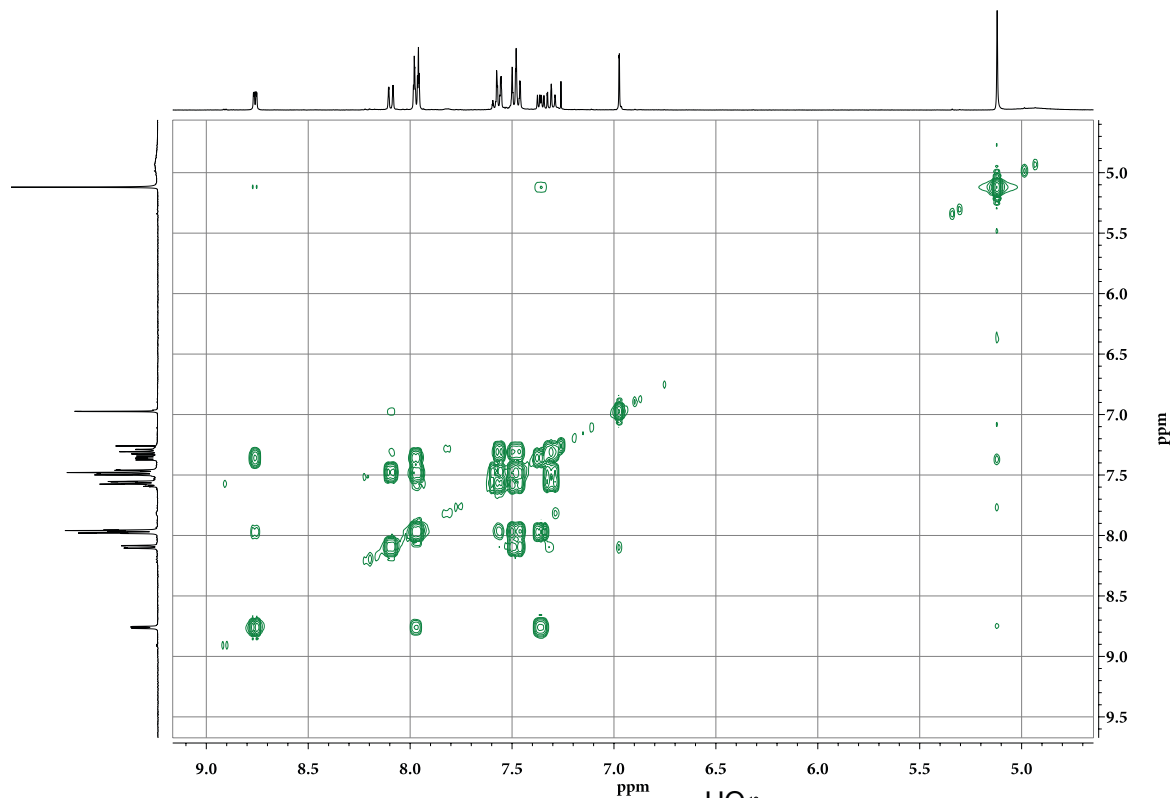
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Generic Display Report

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Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 11/17/2014 2:10:14 PM

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Sample Name	0.1 mg/mL in MeOH
Comment	

		Operator	MZ
		Instrument	maXis HD 1820881.21300

Acquisition Parameter

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Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
393.0909	1	C14H9N12O3	393.0915	1.6	20.3	1	72.87	16.5	even	ok
	2	C21H17N2O4S	393.0904	-1.4	20.6	2	100.00	14.5	even	ok
	3	C22H13N6S	393.0917	2.0	27.7	3	74.77	19.5	even	ok
	4	C14H17N8O2S2	393.0910	0.4	35.7	4	83.71	10.5	even	ok
	5	C29H13O2	393.0910	0.3	52.6	5	39.25	23.5	even	ok
	6	C14H25N4OS4	393.0906	-0.8	70.8	6	31.92	4.5	even	ok
415.0727	1	C21H16N2NaO4S	415.0723	-1.0	15.5	1	100.00	14.5	even	ok
	2	C29H12NaO2	415.0730	0.6	45.2	2	39.01	23.5	even	ok
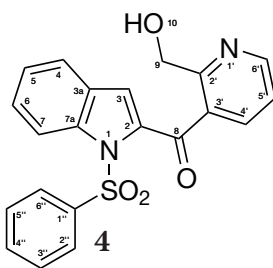
4

6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (PROTON)

6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (PROTON)

6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (C13 CPD)

6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (HSQC)

SPECTROSCOPIC INFORMATION

6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (HMBC)
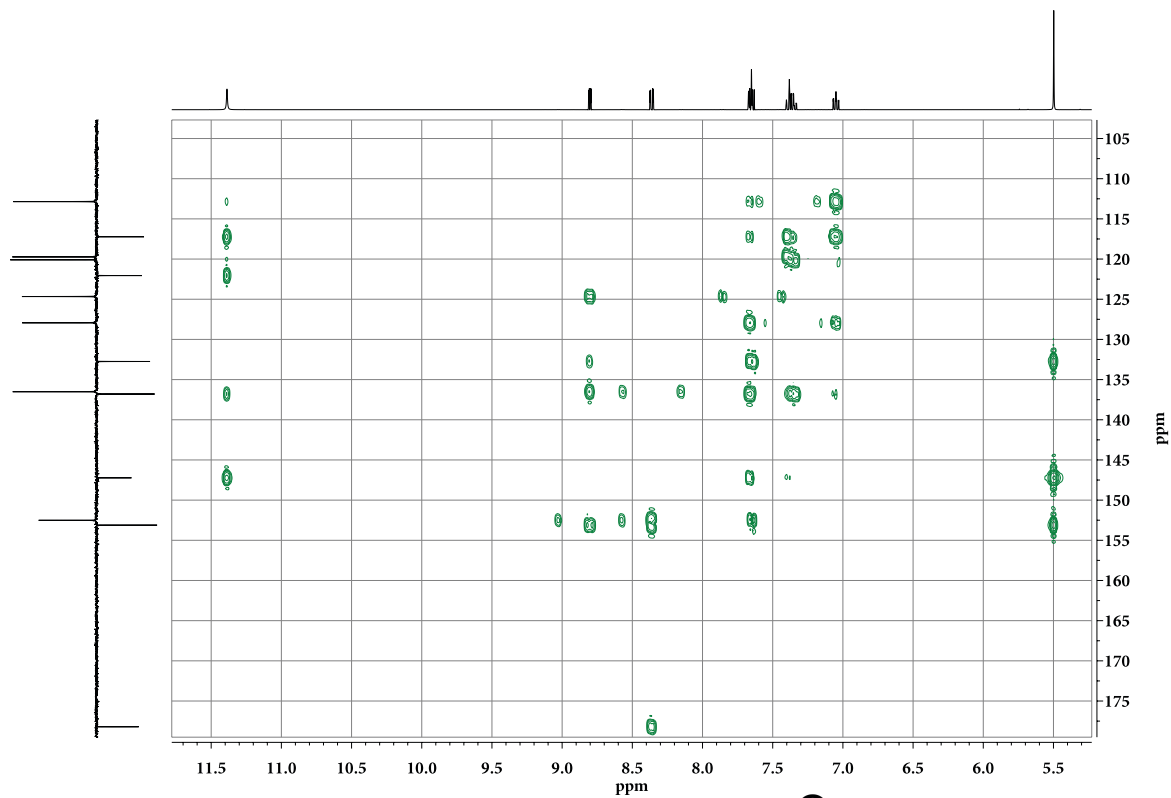
6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (15N HMBC)
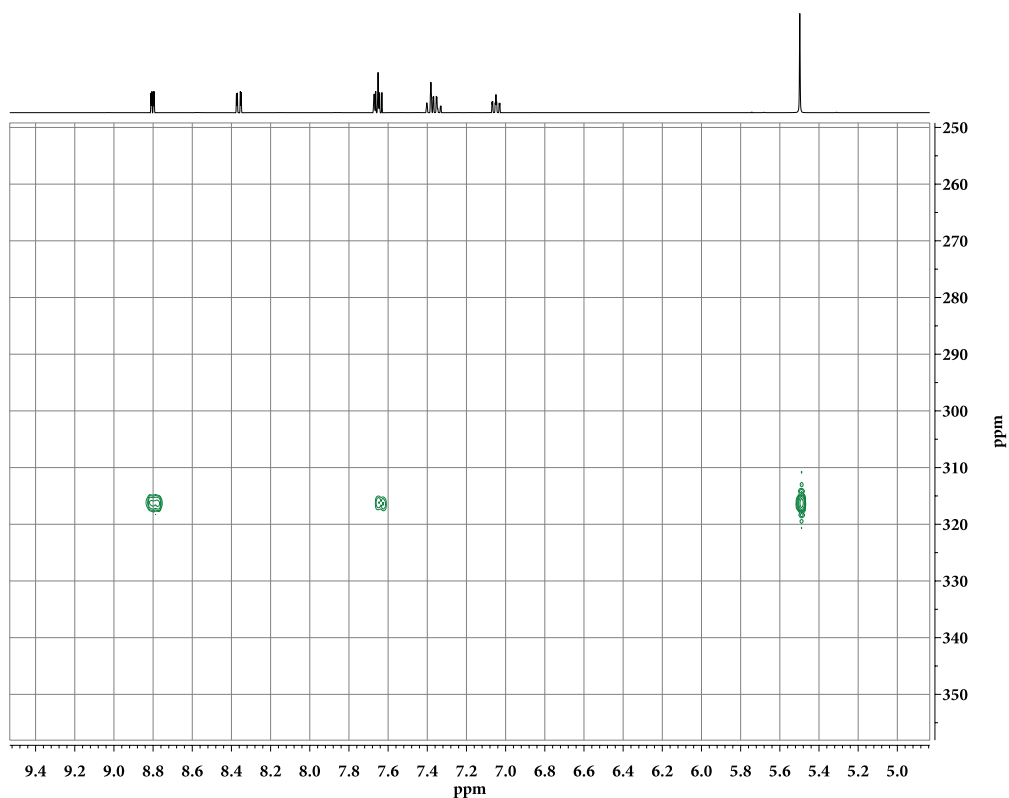
6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (15N HSQC)
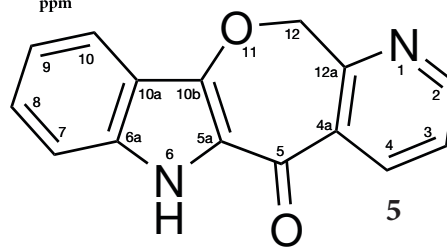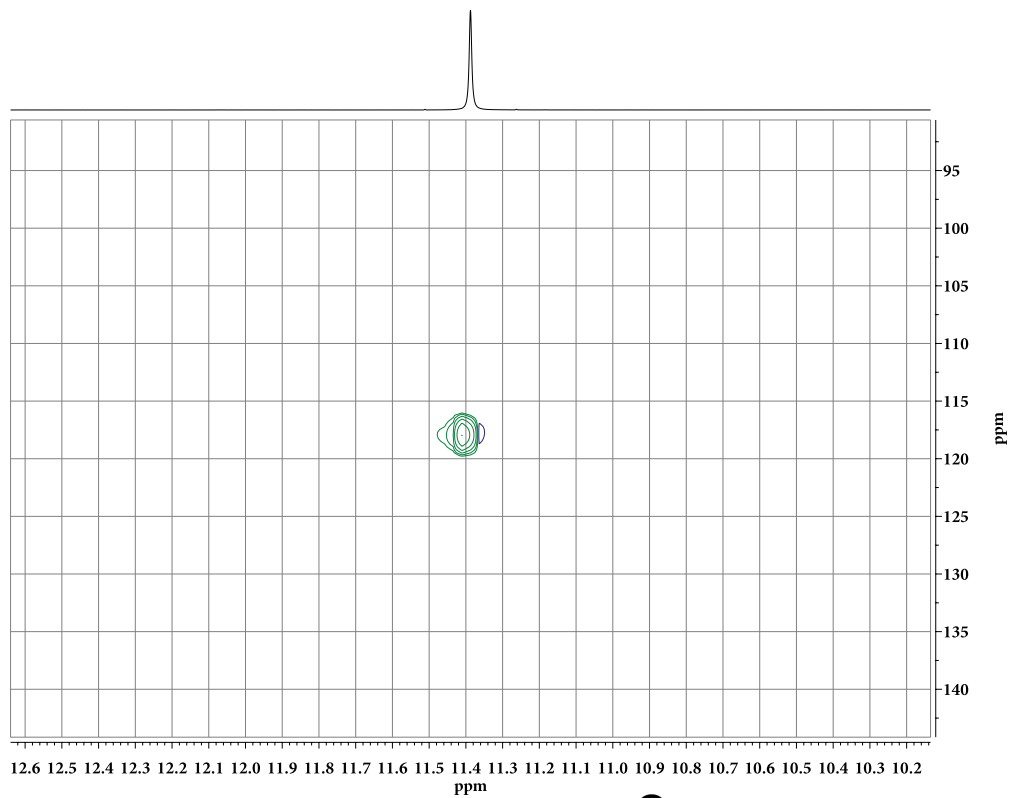
6H-Pyrido[3',2':5,6]oxepino[3,2-b]indol-5(12H)-one (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 2:57:24 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN140_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Intens. x10^4

+MS, 0.0-3.9min #1-223

273.0632

249.0655 250.2138 252.2293 253.2635 255.2791 258.2788 261.1305 264.2295 268.2745 271.3112 274.0663 275.0700

x10^4

$C_{15}H_{10}N_2NaO_2$, 273.0634

1+
273.0634

1+
274.0666

$C_{15}H_{11}N_2O_2$, 251.0815

1+
251.0815

1+
252.0846

250 255 260 265 270 275 m/z

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/17/2014 2:57:24 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN140_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
251.0811	1	C15H11N2O2	251.0815	1.6	n.a.	1	100.00	11.5	even	ok
273.0632	1	C15H10N2NaO2	273.0634	0.9	7.4	1	100.00	11.5	even	ok

5

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

—13.15

6

1.02

13.75 13.70 13.65 13.60 13.55 13.50 13.45 13.40 13.35 13.30 13.25 13.20 13.15 13.10 13.05 13.00 12.95 12.90 12.85 12.80 12.75 12.70 12.65
ppm

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

9.00
8.99
8.99
8.98

8.46
8.46
8.44
8.44

8.25
8.25
8.24
8.23
8.23
8.22

2

4

10

1.00

1.03

1.04

9.00 8.95 8.90 8.85 8.80 8.75 8.70 8.65 8.60 8.55 8.50 8.45 8.40 8.35 8.30 8.25 8.20
ppm

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)
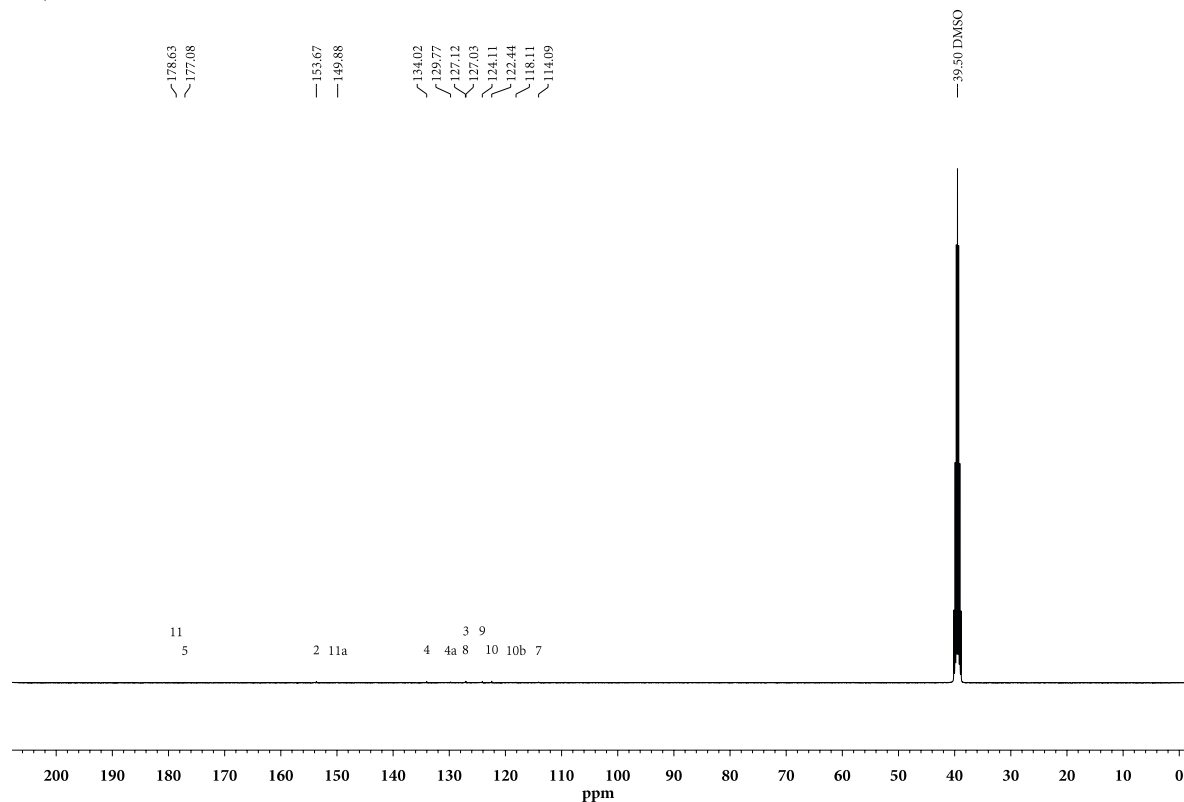
5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (C13 CPD)

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (C13 CPD)
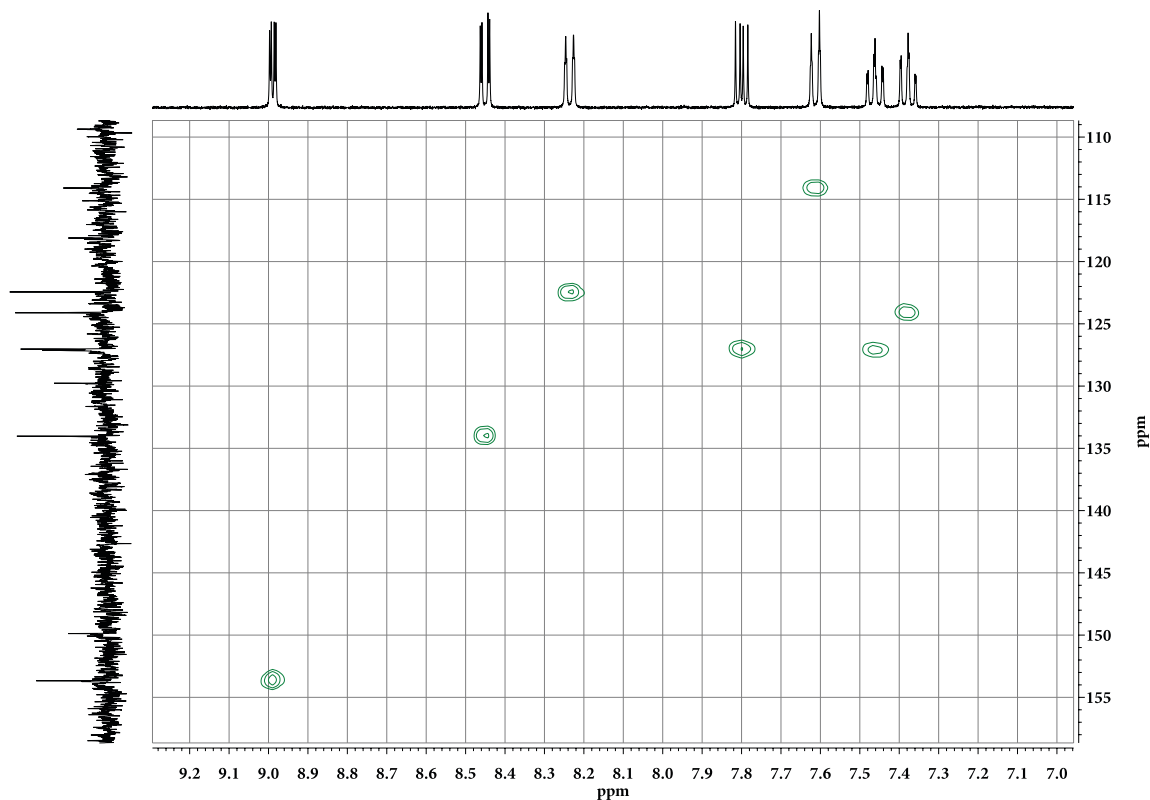
5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (HSQC)

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (HMBC)

5H-Pyrido[3,2-b]carbazole-5,11(6H)-dione (15N HMBC)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN036_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 9:47:51 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.9min #1-106

$C_{15}H_9N_2O_2$, 249.0659

$C_{15}H_8N_2NaO_2$, 271.0478

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/10/2014 9:47:51 AM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN036_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 0.01 mg/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
249.0660	1	C15H9N2O2	249.0659	-0.8	11.2	1	100.00	12.5	even	ok
271.0481	1	C15H8N2NaO2	271.0478	-1.3	6.2	1	100.00	12.5	even	ok

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

—7.64
—7.63
—7.62
—7.61

—7.53
7.51
7.51
7.50
7.49
7.47
7.47

7.43
7.43
7.42
7.41
7.41
7.40
7.39

7

3

8

9

1.20

2.13

1.16

680   7.660   7.640   7.620   7.600   7.580   7.560   7.540   7.520   7.500   7.480   7.460   7.440   7.420   7.400   7.380   7.360

ppm

7

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (PROTON)

4.85
4.83
4.82

2.77
2.75
2.73

—2.38

15',15

12

13

2.14

2.04

6.00

5.40   5.20   5.00   4.80   4.60   4.40   4.20   4.00   3.80   3.60   3.40   3.20   3.00   2.80   2.60   2.40   2.20   2.00   1.80   1.60

ppm

213

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (HSQC)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (HMBC)

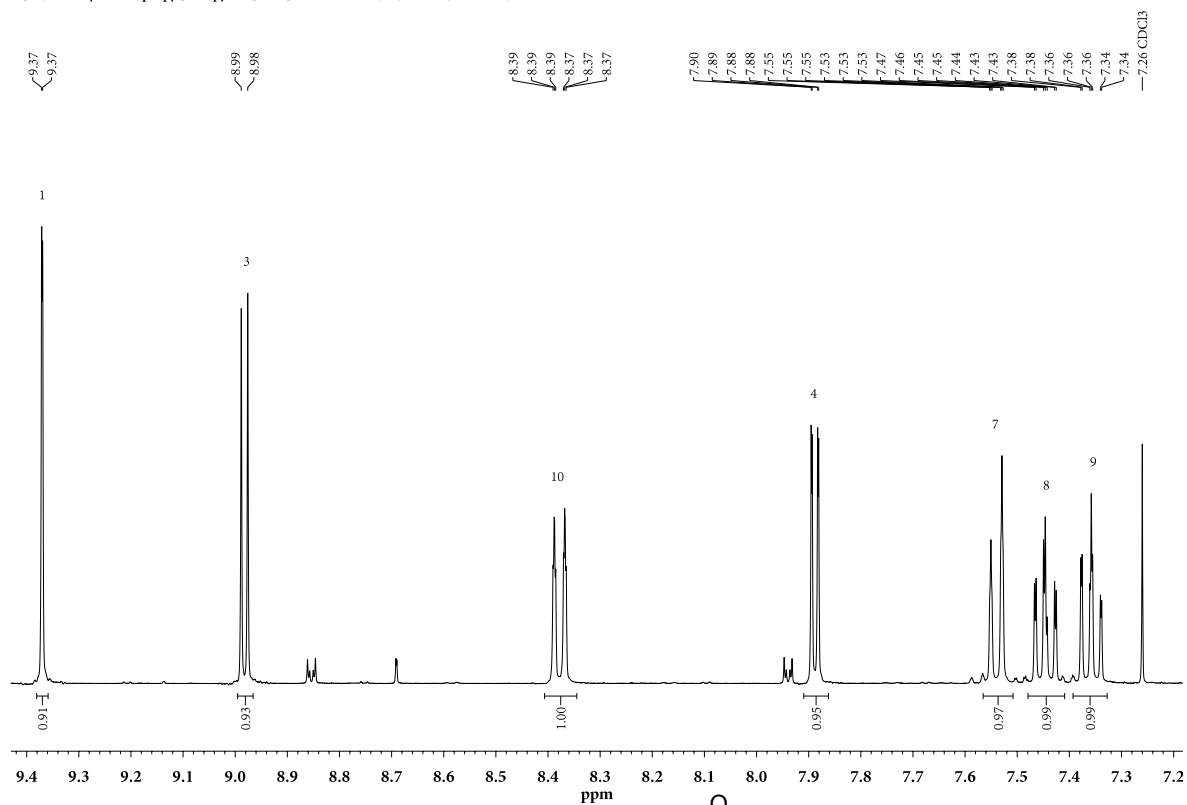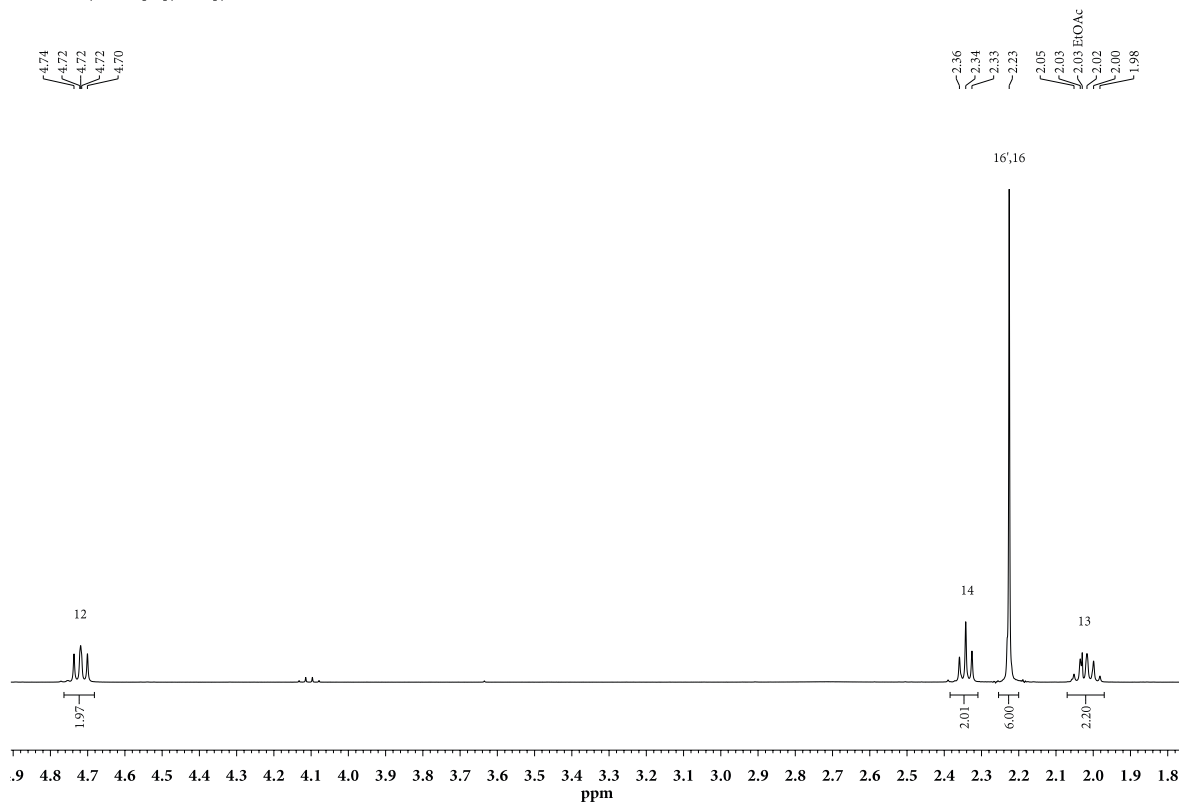6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (15N HMBC)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (COSY)

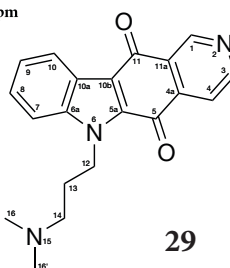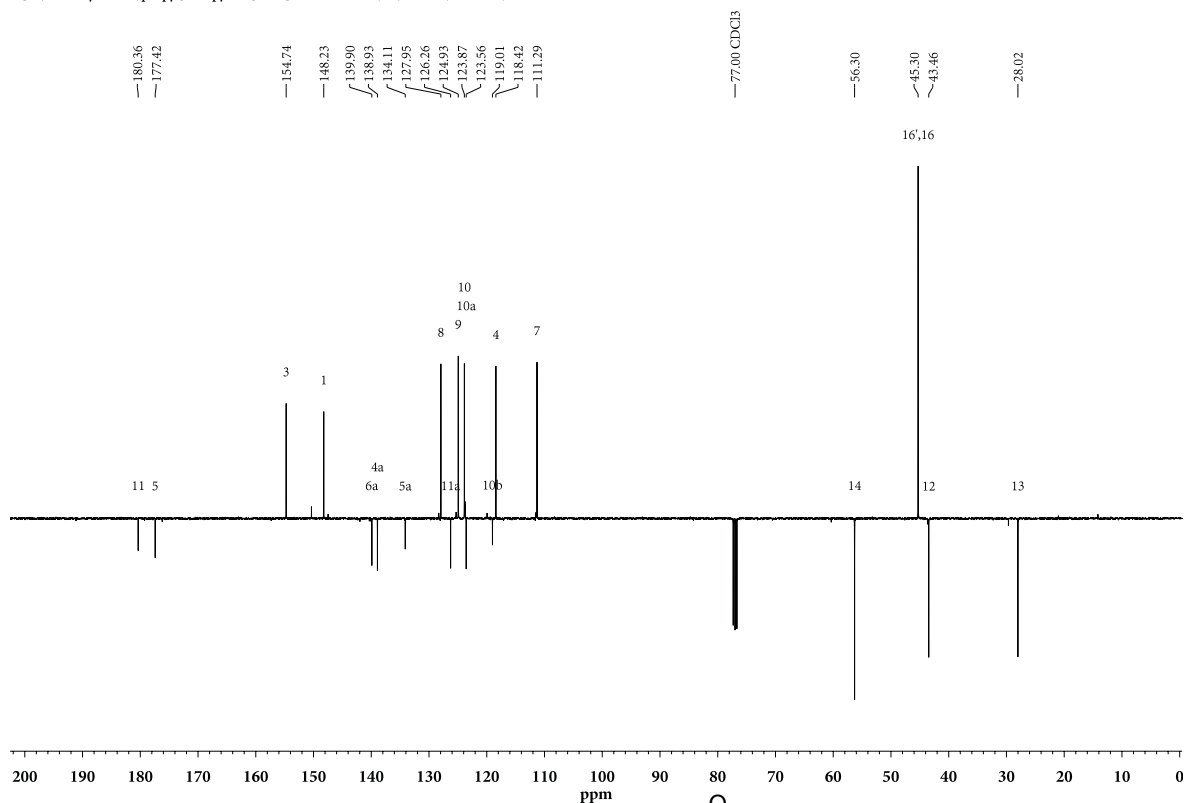6-[2-(Dimethylamino)ethyl]-5H-pyrido[3,2-b]carbazole-5,11(6H)-dione (NOESY)

7

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN050_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 9:57:42 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 9:57:42 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN050_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
320.1395	1	C19H18N3O2	320.1394	-0.5	3.5	1	100.00	12.5	even	ok
342.1215	1	C19H17N3NaO2	342.1213	-0.7	7.1	1	100.00	12.5	even	ok
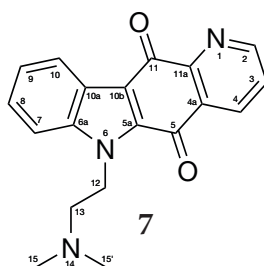
1-(Methoxymethyl)-1H-indole-3-carbaldehyde (PROTON)
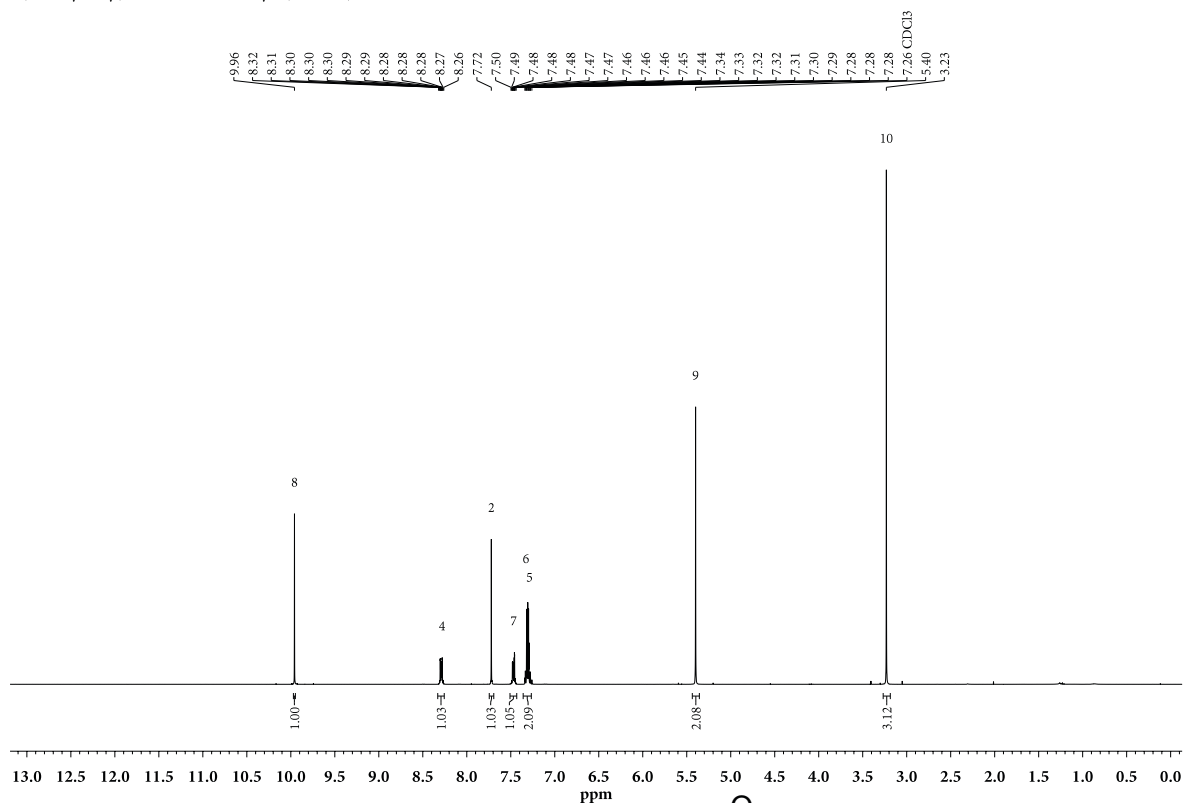
1-(Methoxymethyl)-1H-indole-3-carbaldehyde (PROTON)
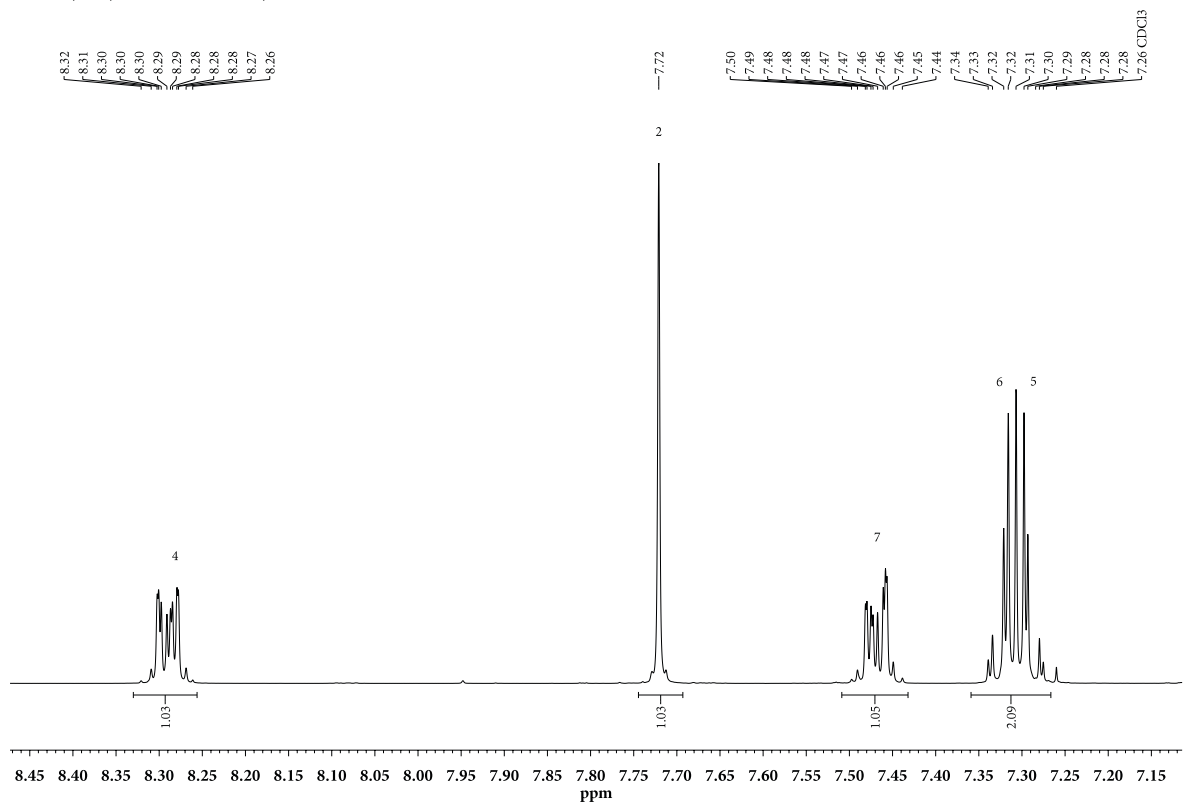
1-(Methoxymethyl)-1H-indole-3-carbaldehyde (C13 CPD)

1-(Methoxymethyl)-1H-indole-3-carbaldehyde (HSQC)

1-(Methoxymethyl)-1H-indole-3-carbaldehyde (HMBC)

8
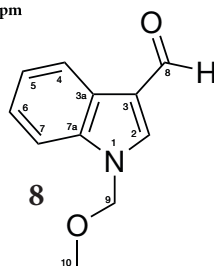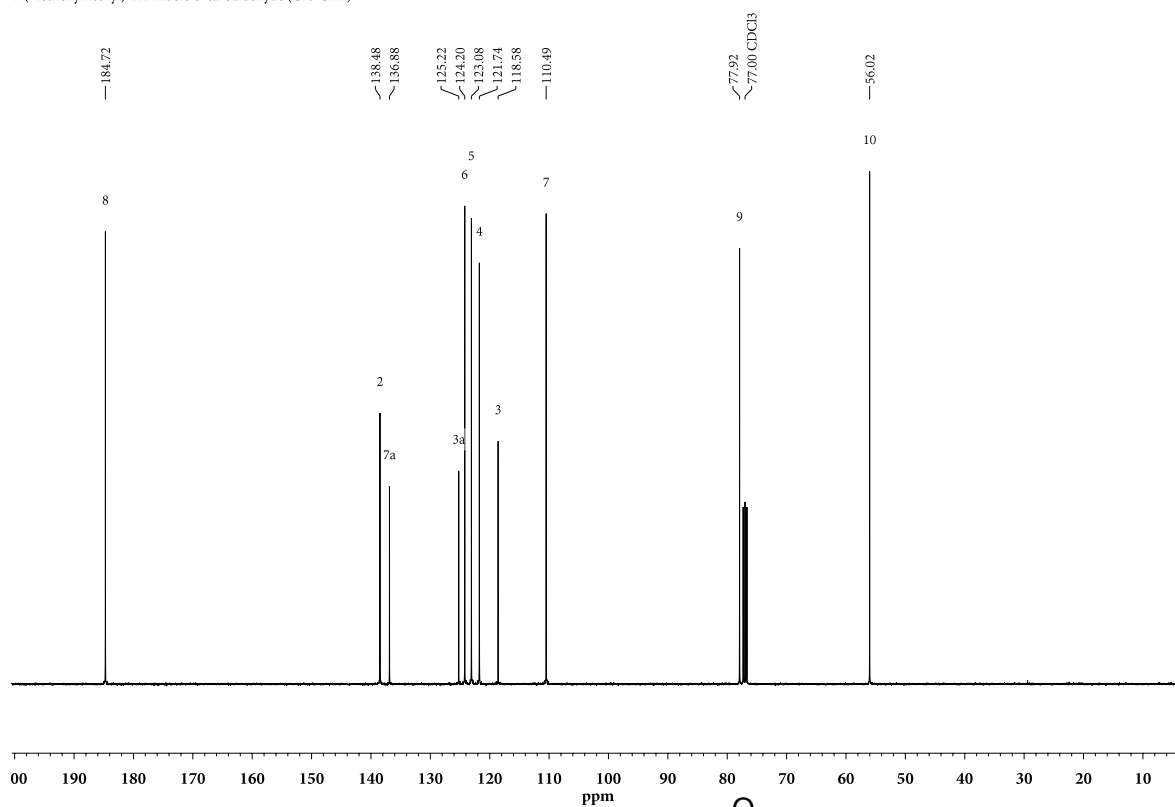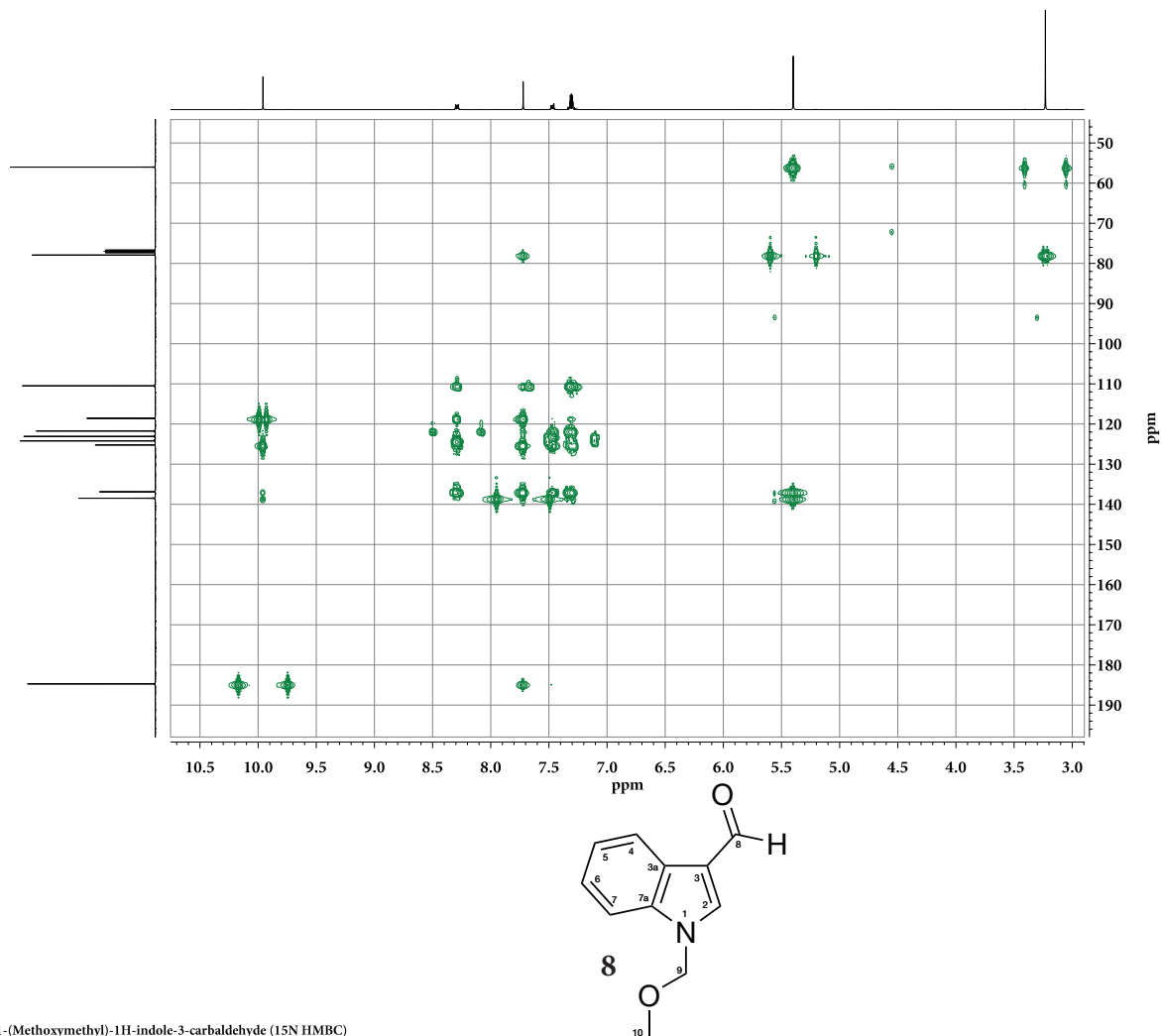
1-(Methoxymethyl)-1H-indole-3-carbaldehyde (15N HMBC)

1-(Methoxymethyl)-1H-indole-3-carbaldehyde (NOESY)

8

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

7.71
7.71
7.71
7.70
7.69
7.69
7.69
7.67
7.67
7.67
7.67

7.49
7.49
7.49
7.47
7.47
7.47

7.26 CDCl3
7.25
7.25
7.25
7.24
7.24
7.24
7.23
7.23
7.23
7.23
7.22
7.22
7.21

4

3

5

1.02   1.01   1.01

7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.22 7.20
ppm

N,N-Diethyl-2-pyridinecarboxamide (PROTON)

3.51
3.50
3.48
3.46
3.32
3.30
3.28
3.27

1.21
1.19
1.17
1.08
1.07
1.05

11   11'

10   10'

2.09   2.08   3.18   3.14

.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8
ppm

225

N,N-Diethyl-2-pyridinecarboxamide (C13 CPD)

N,N-Diethyl-2-pyridinecarboxamide (HSQC)

N,N-Diethyl-2-pyridinecarboxamide (HMBC)

N,N-Diethyl-2-pyridinecarboxamide (15N HMBC)
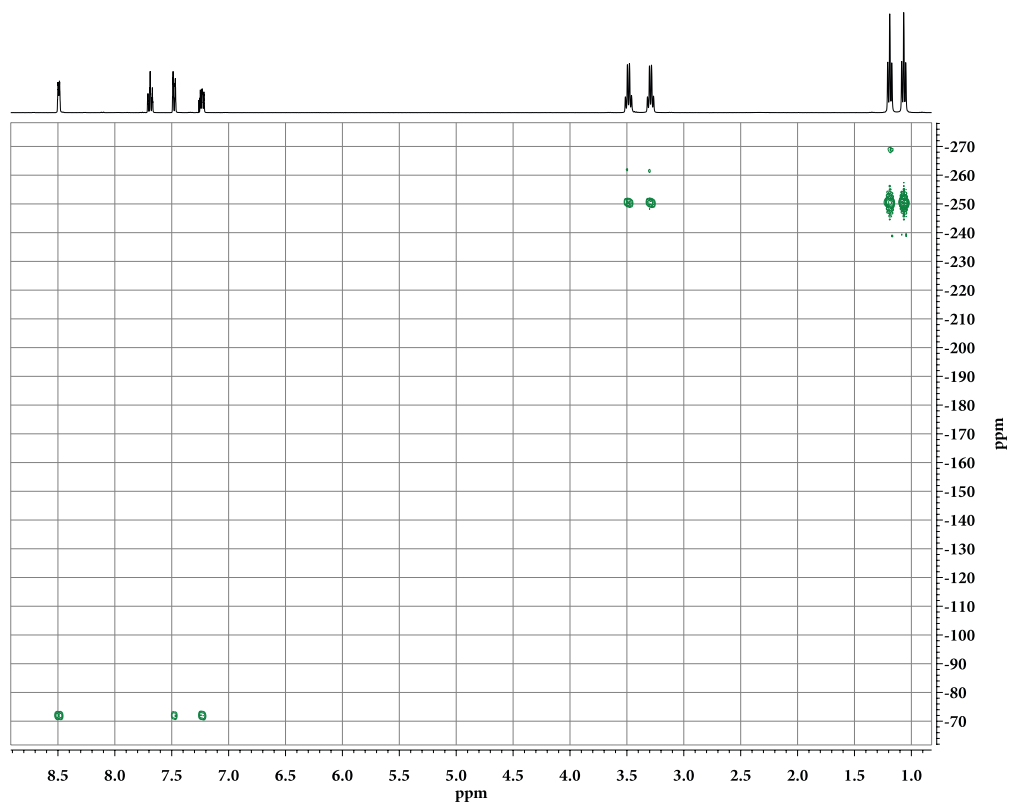
10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)
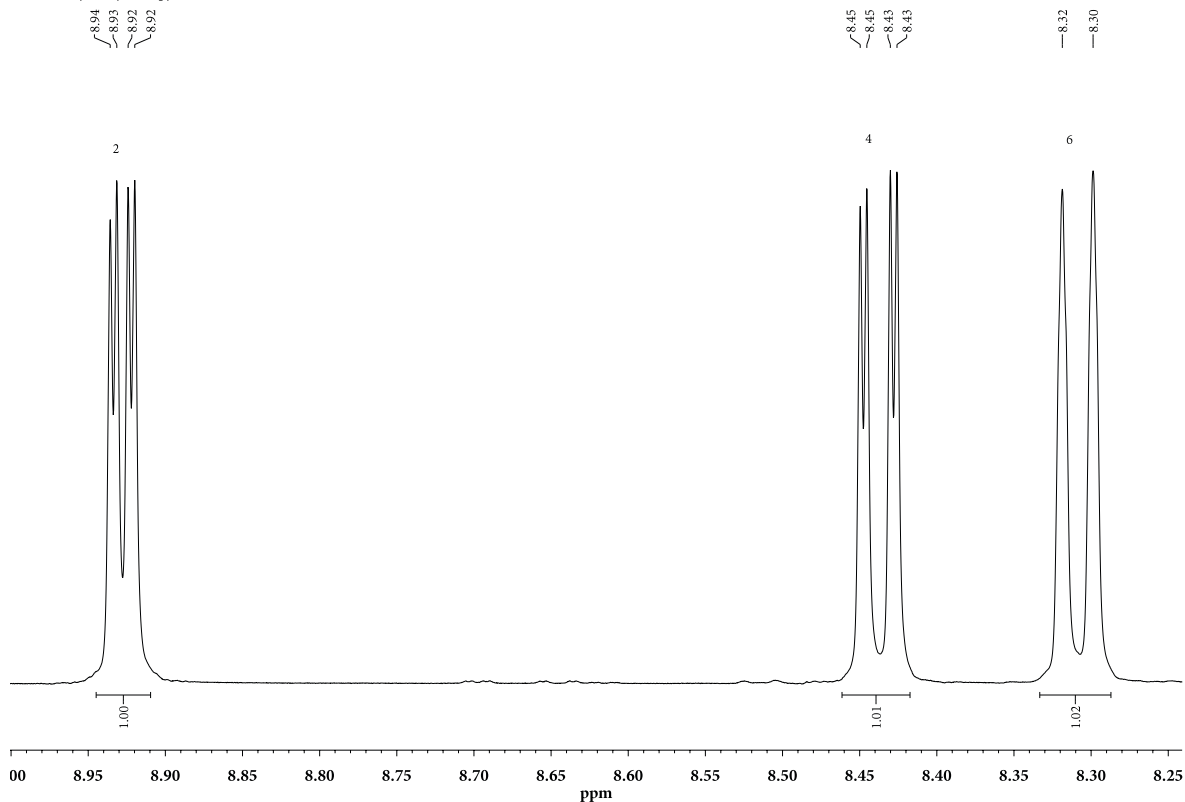
10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 APT)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 APT)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (HSQC)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (HMBC)
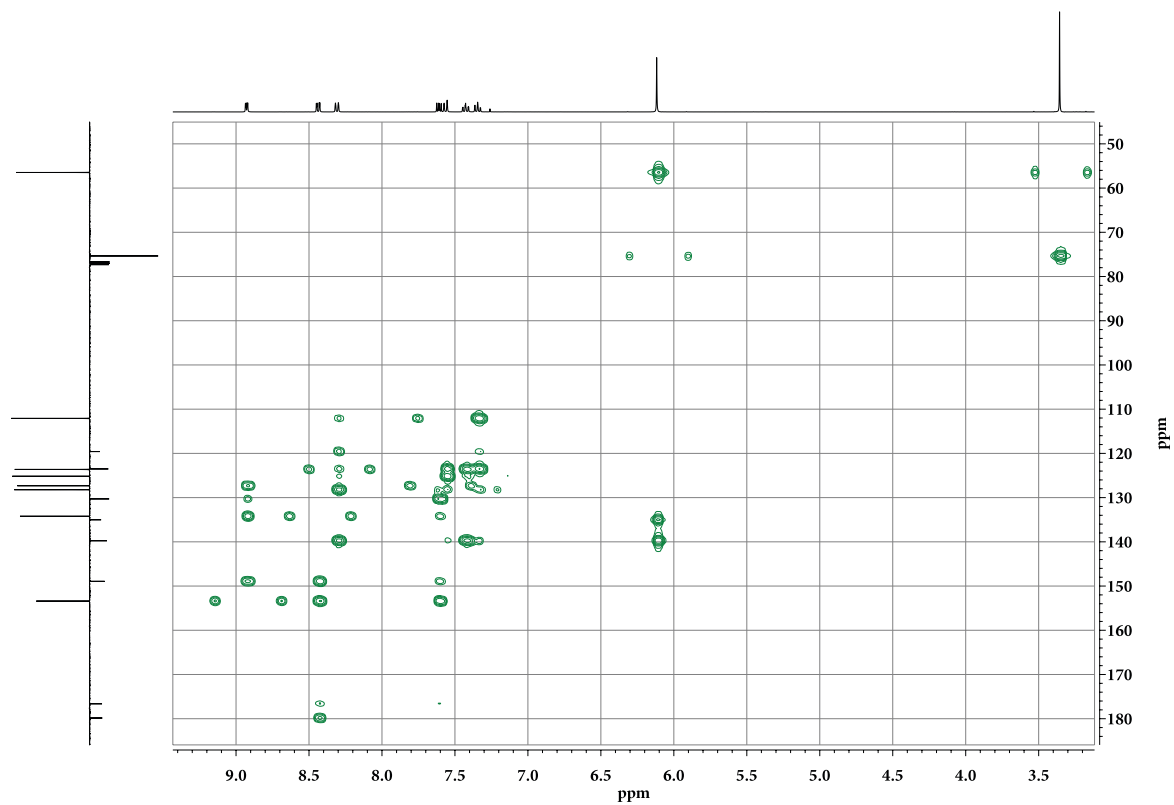
10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (COSY)

10-(Methoxymethyl)-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (15N HMBC)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN052_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:13:49 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.6min #1-90

315.0739

304.2610

302.2453

305.2642

316.0770

293.0918 296.3058 297.3091 300.2296 303.2486 310.2714 317.3240

$C_{17}H_{13}N_2O_3$, 293.0921

1+ 293.0921

1+ 294.0952

1+ 295.0978

$C_{17}H_{12}N_2NaO_3$, 315.0740

1+ 315.0740

1+ 316.0772

1+ 317.0797

10

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN052_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:13:49 AM
Method	DI_mz_50-1550.m		
Sample Name	approx. 0.01 mg/mL in MeOH	Operator	MZ
Comment		Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar		
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C		
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min		
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste		
		Set Corona	0 nA	Set APCI Heater	0 °C		

+MS, 0.0-1.6min #1-90

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
293.0918	1	C17H13N2O3	293.0921	0.8	13.8	1	100.00	12.5	even	ok
315.0739	1	C17H12N2NaO3	315.0740	0.3	8.0	1	100.00	12.5	even	ok
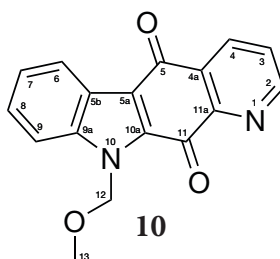
10
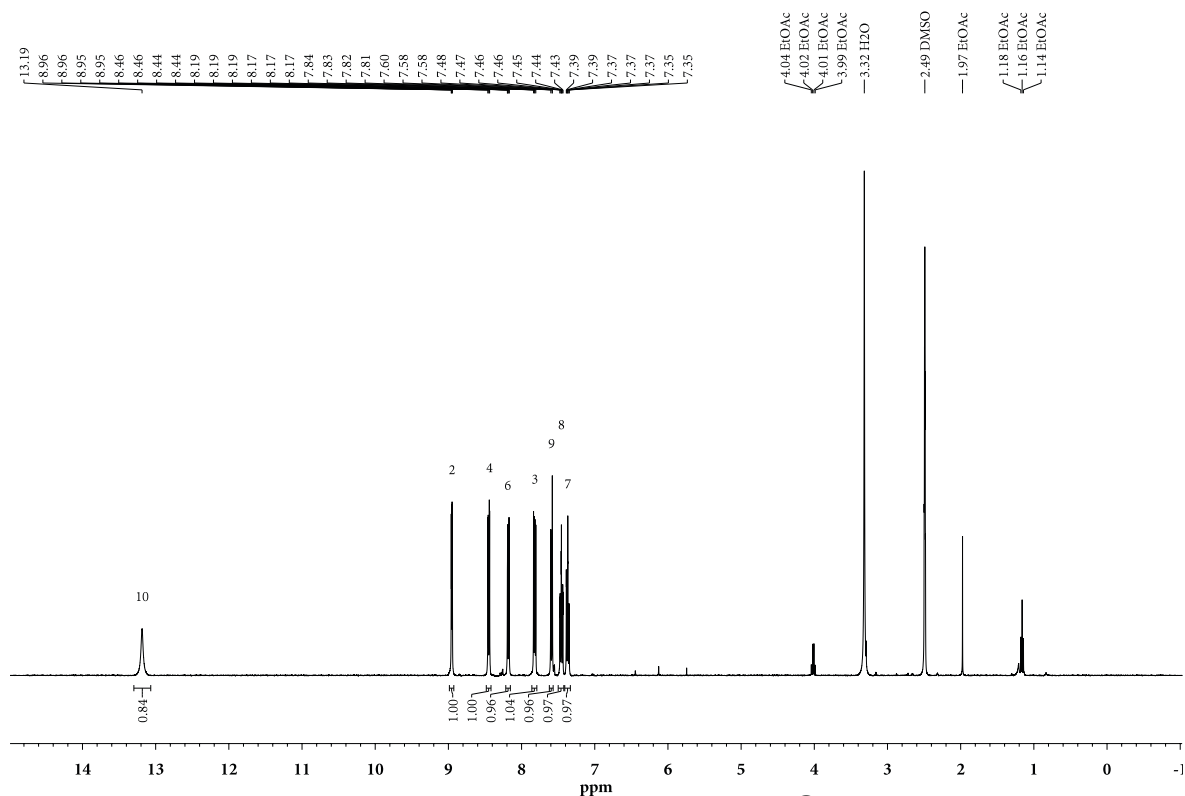
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)
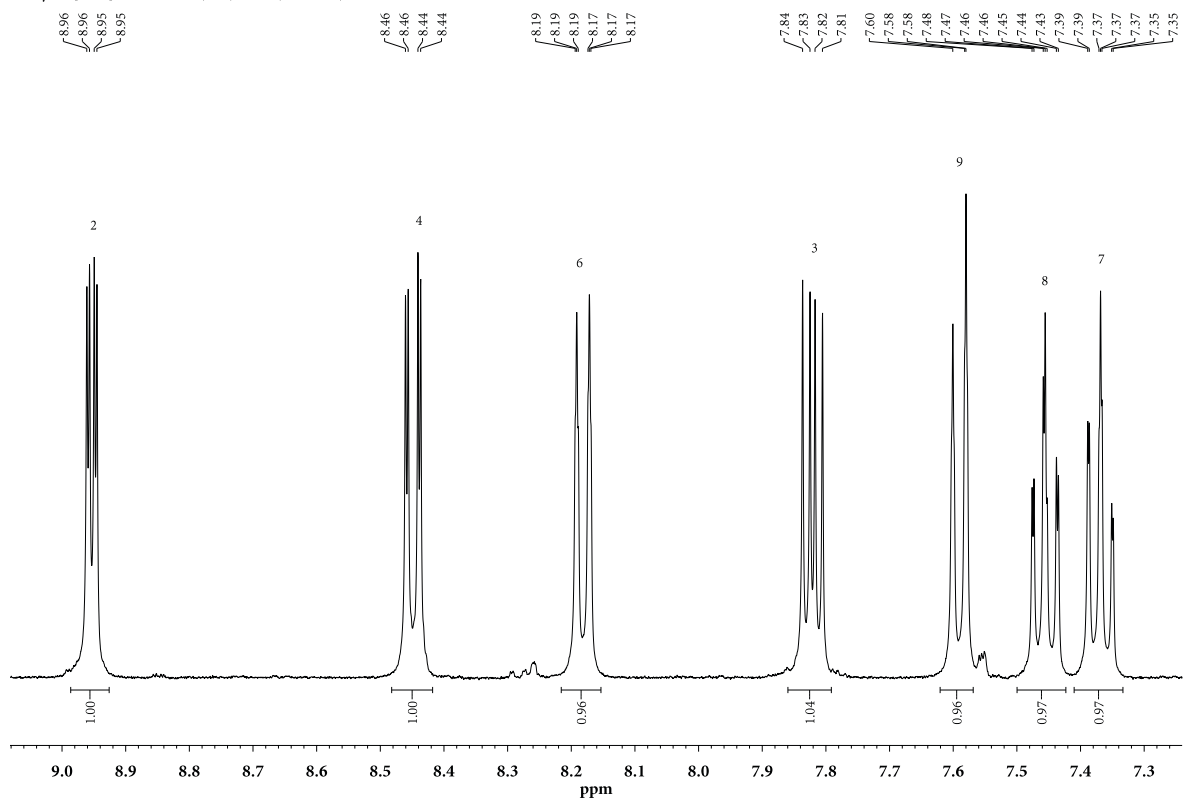
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)
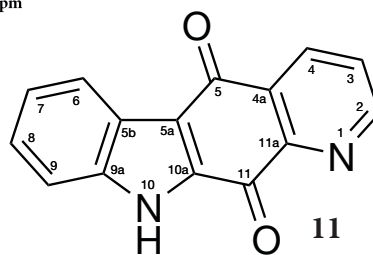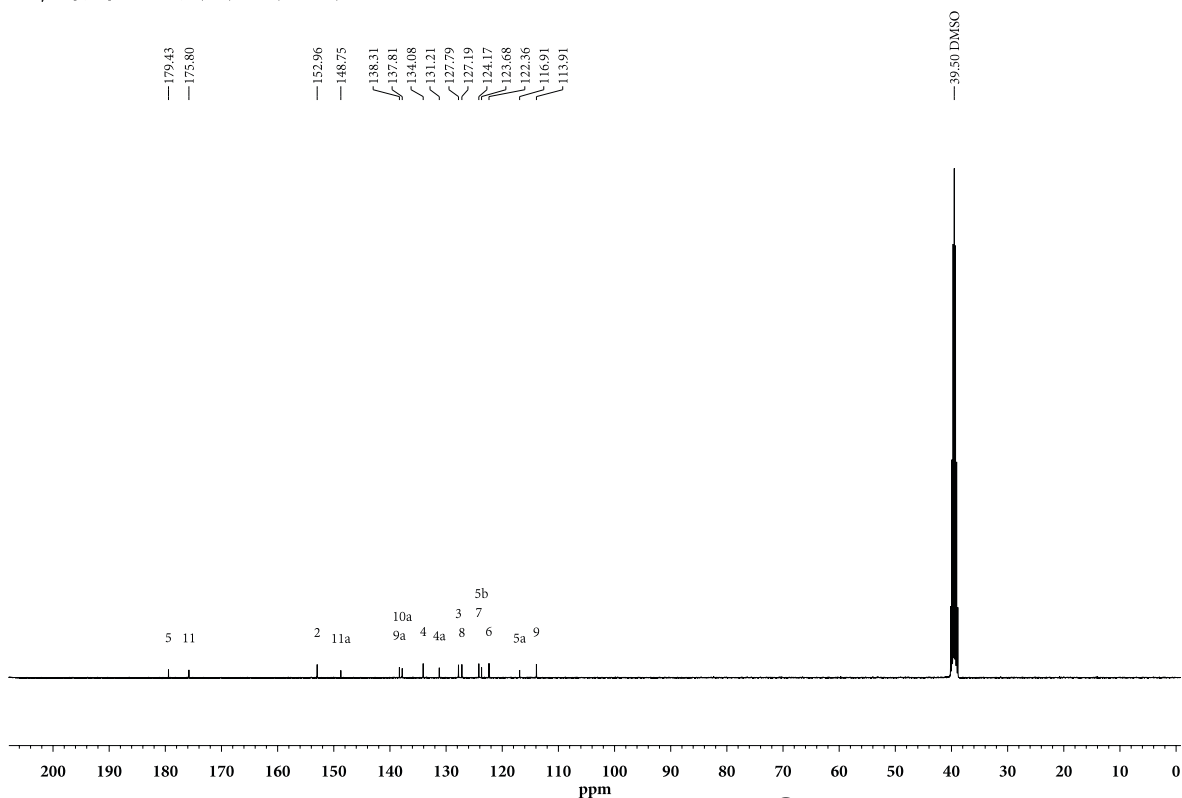
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 CPD)
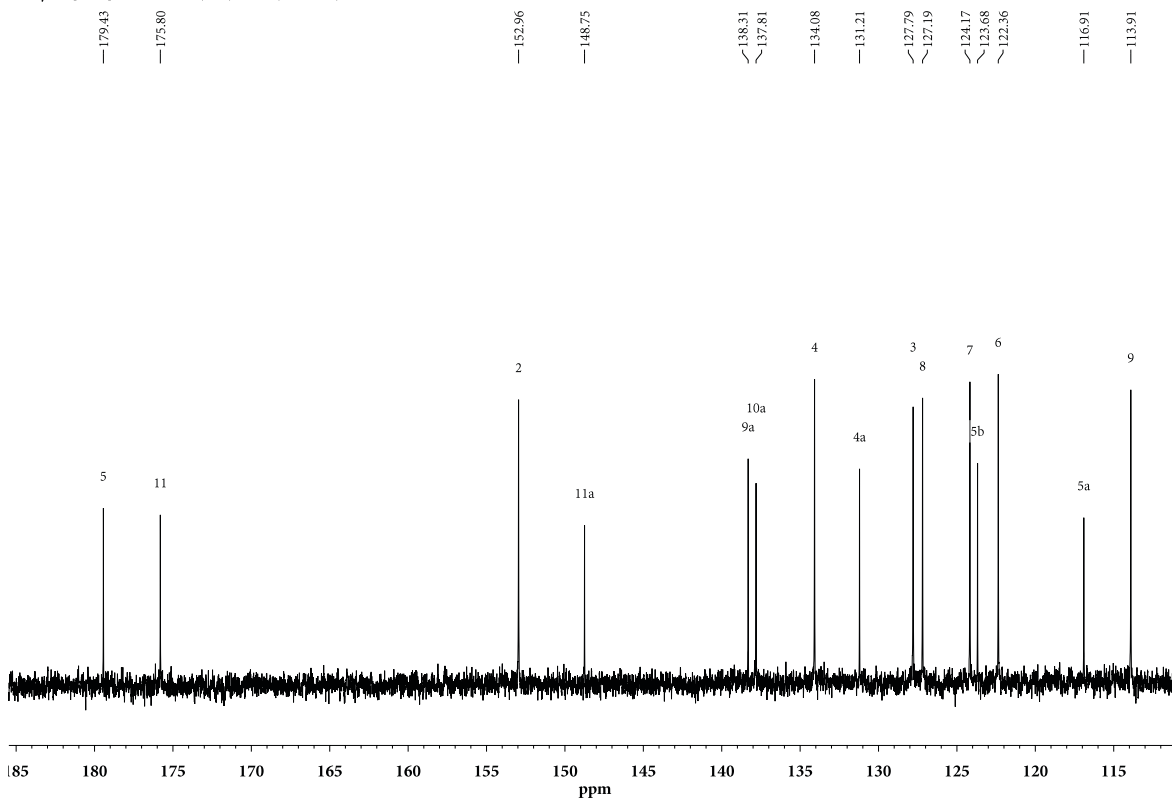
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 CPD)

5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (HSQC)
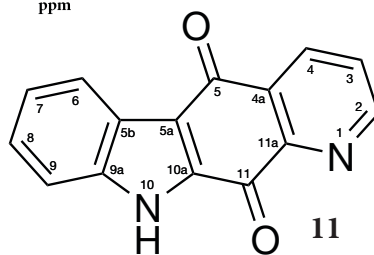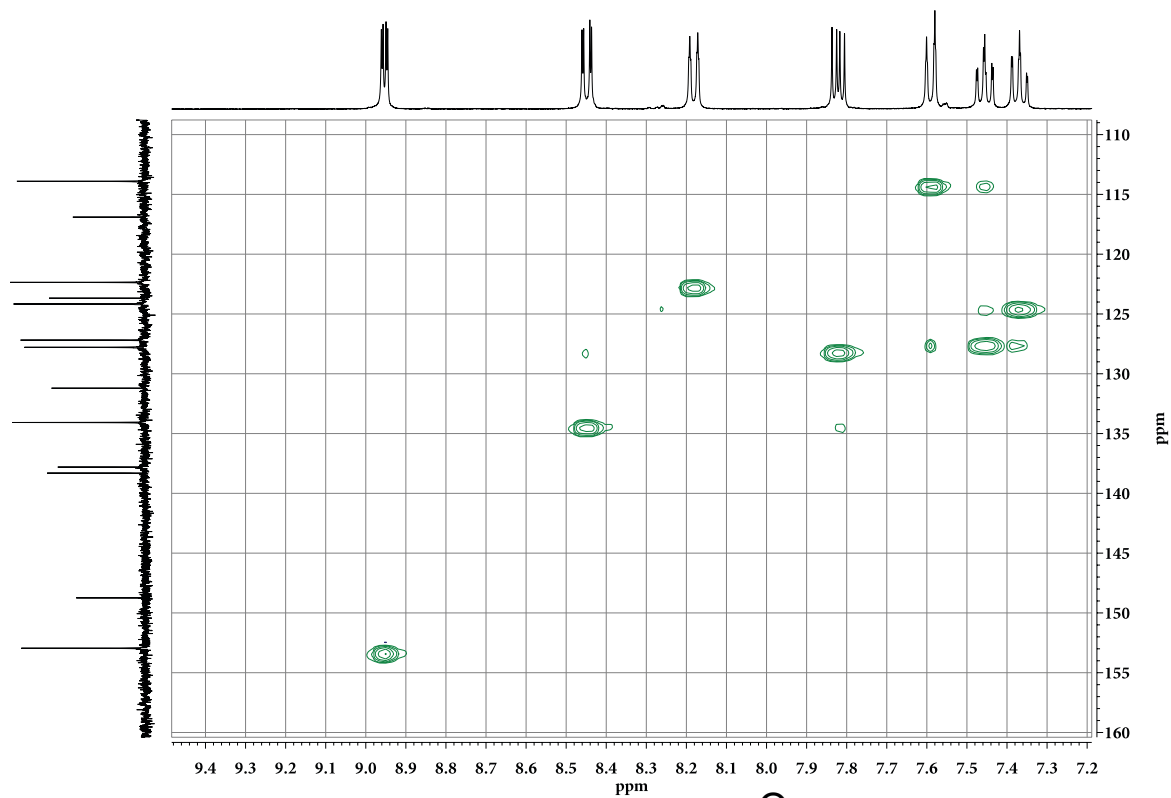
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (HMBC)
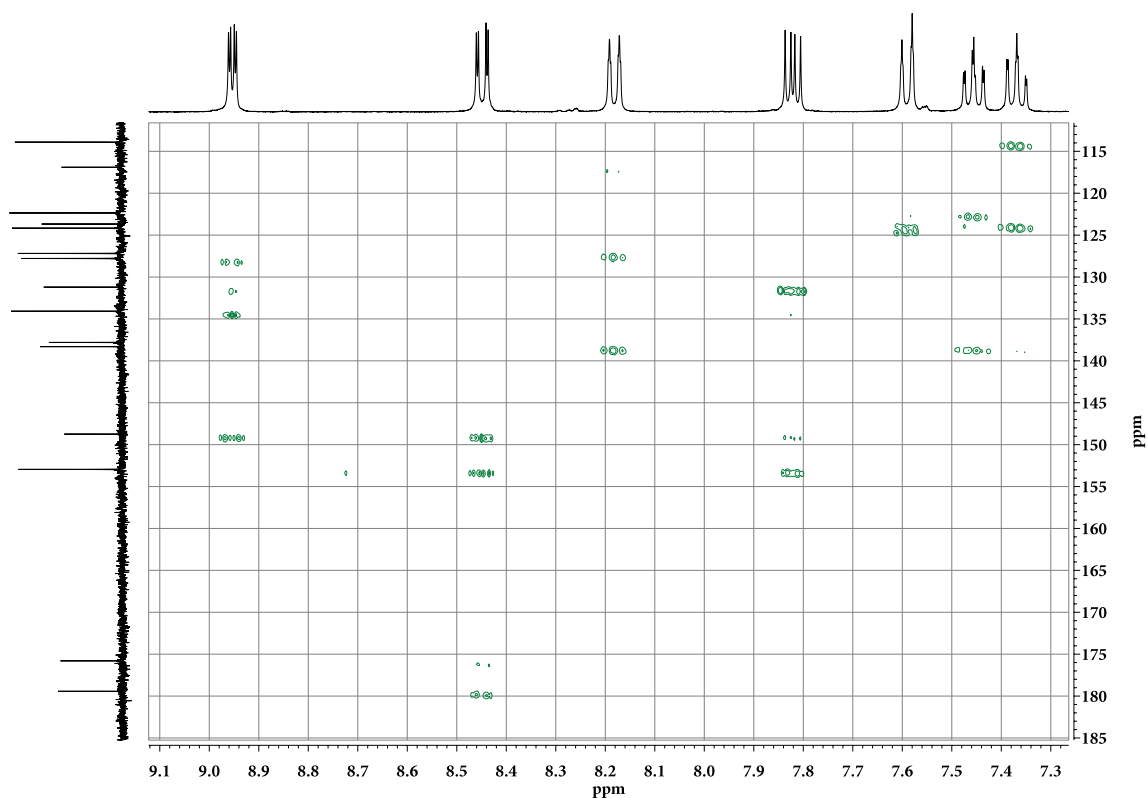
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (15N HMBC)
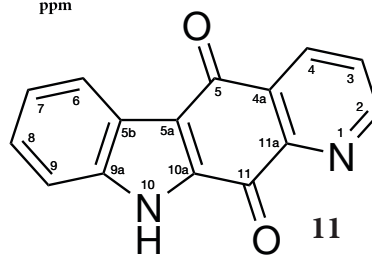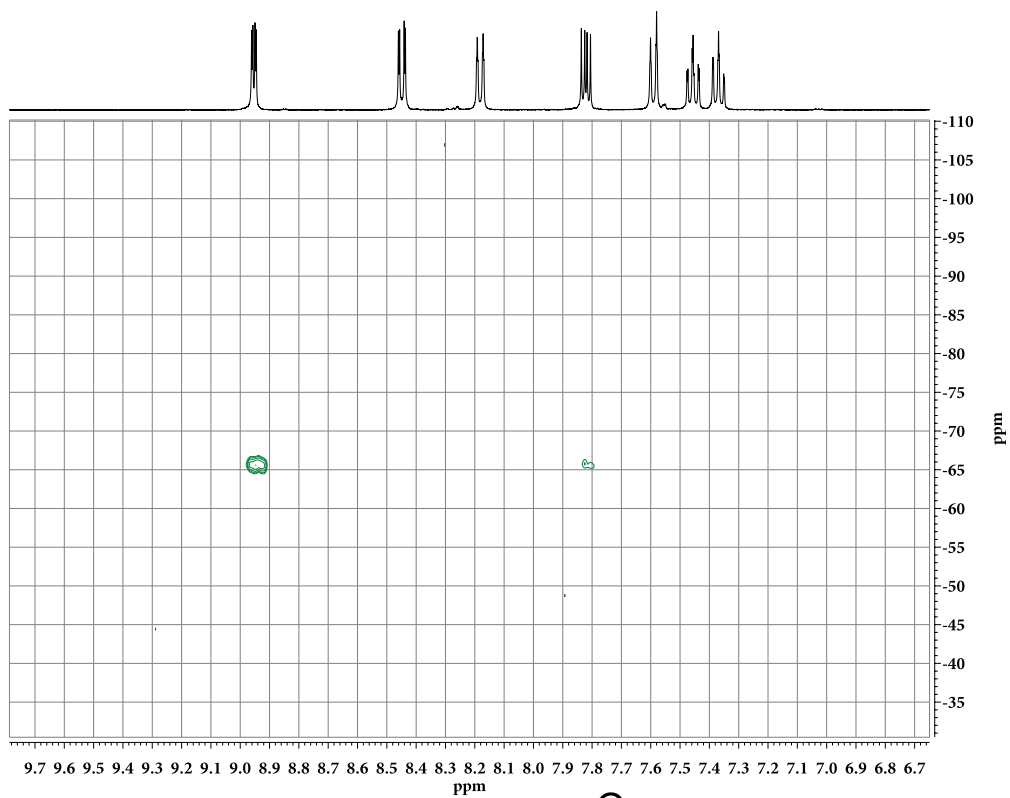
5H-Pyrido[2,3-b]carbazole-5,11(10H)-dione (15N HSQC)
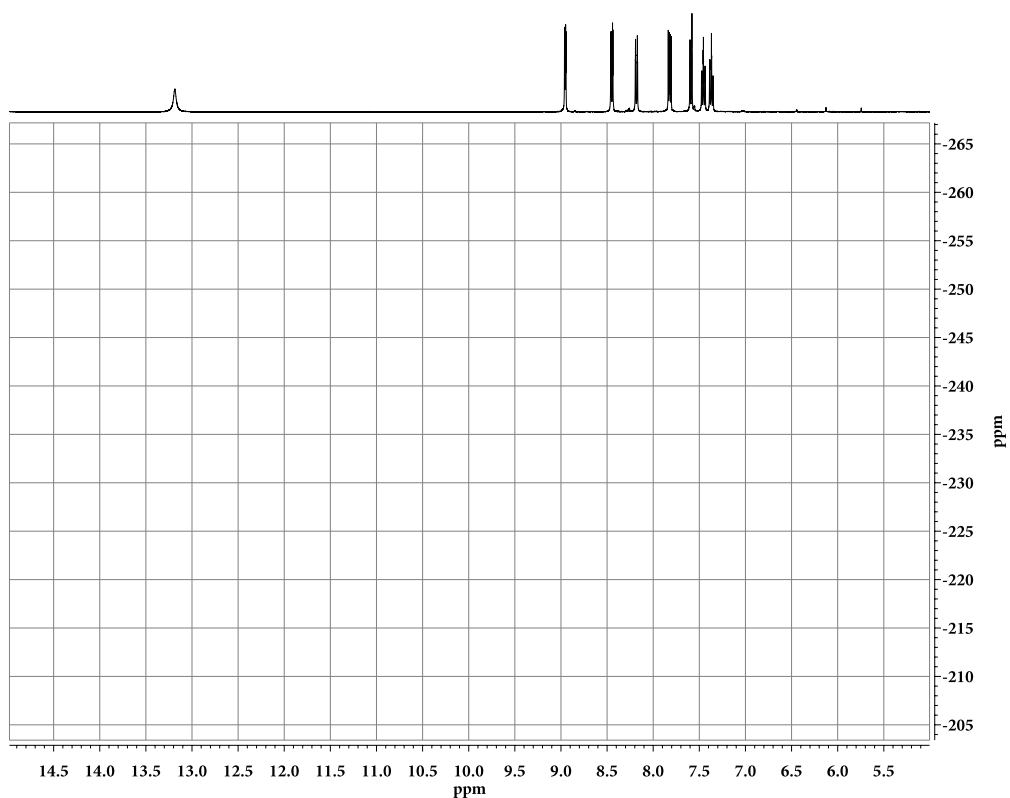
Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN085_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:59:12 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Intens.
x10⁴

+MS, 0.0-1.6min #1-88

271.0479

272.0509

250.2141   253.2639   255.2794   261.1308   264.2299   268.2749

$C_{15}H_9N_2O_2$, 249.0659

1+
249.0659

1+
250.0690

1+
251.0716

$C_{15}H_8N_2NaO_2$, 271.0478

1+
271.0478

1+
272.0509

1+
273.0535

11

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 10:59:12 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN085_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
249.0659	1	C15H9N2O2	249.0659	-0.3	10.8	1	100.00	12.5	even	ok
271.0479	1	C15H8N2NaO2	271.0478	-0.4	2.2	1	100.00	12.5	even	ok
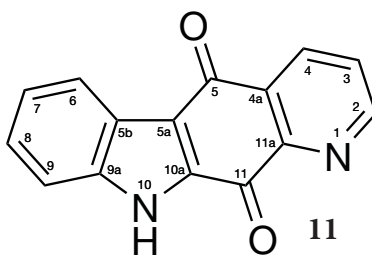
11

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

12

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

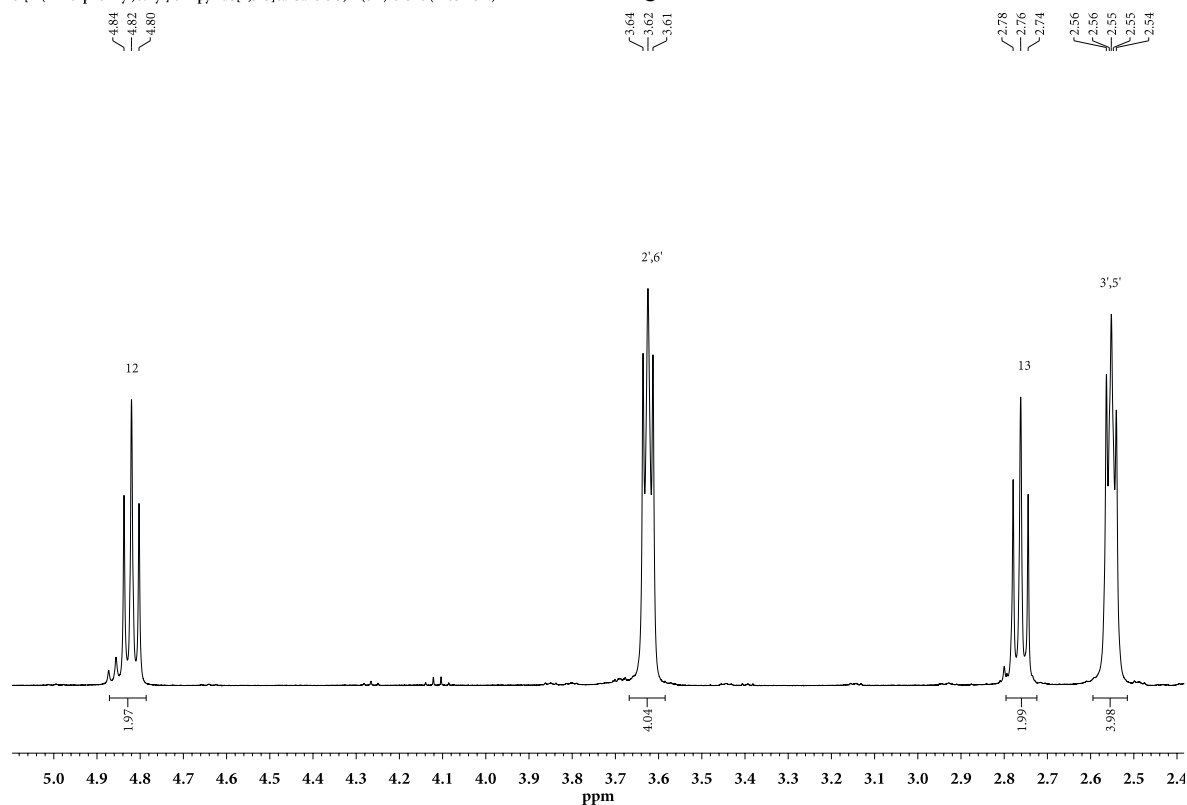10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

7.63
7.62
7.62
7.61
7.52
7.50
7.47
7.45
7.44
7.39
7.37
7.35
7.34
7.26 CDCl3

3
9
8
7

1.04   1.01   0.97   1.06

78 7.76 7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.22
ppm

12

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (PROTON)

4.86
4.84
4.83
2.79
2.78
2.76
2.41

15',15

12
13

1.91   2.03   5.81

5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2
ppm

243

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 APT)

12
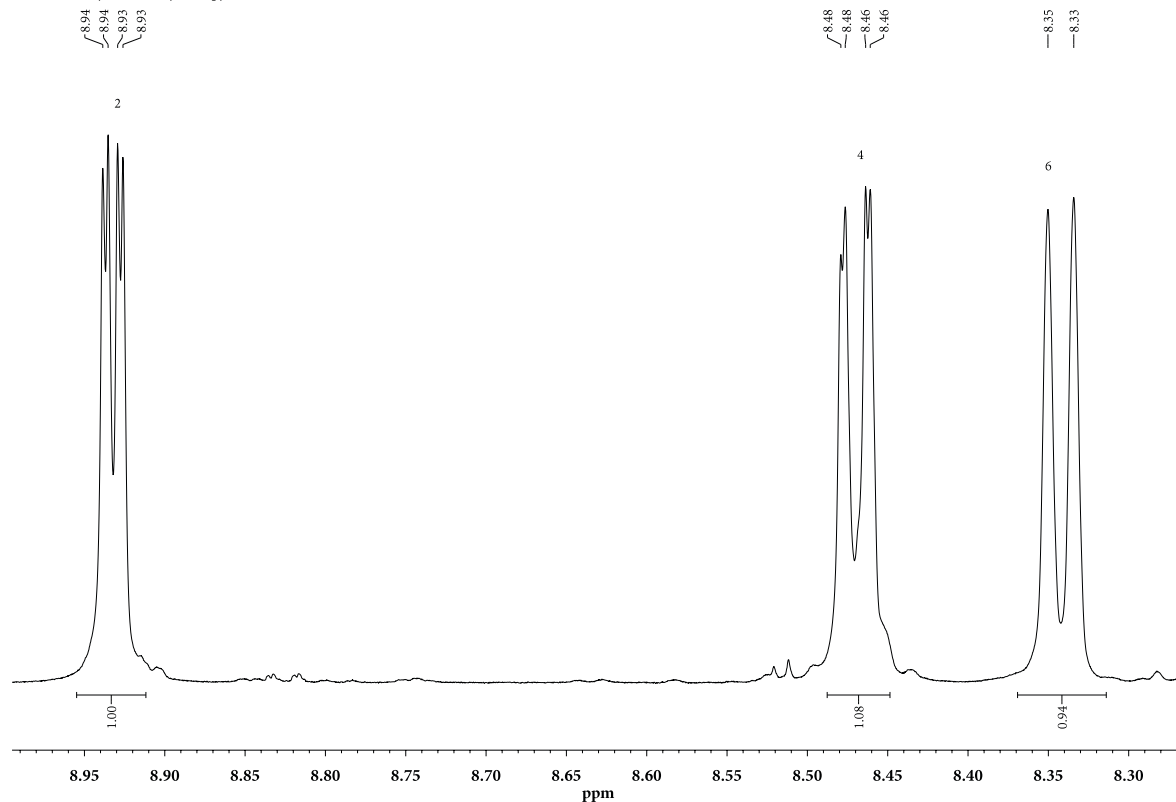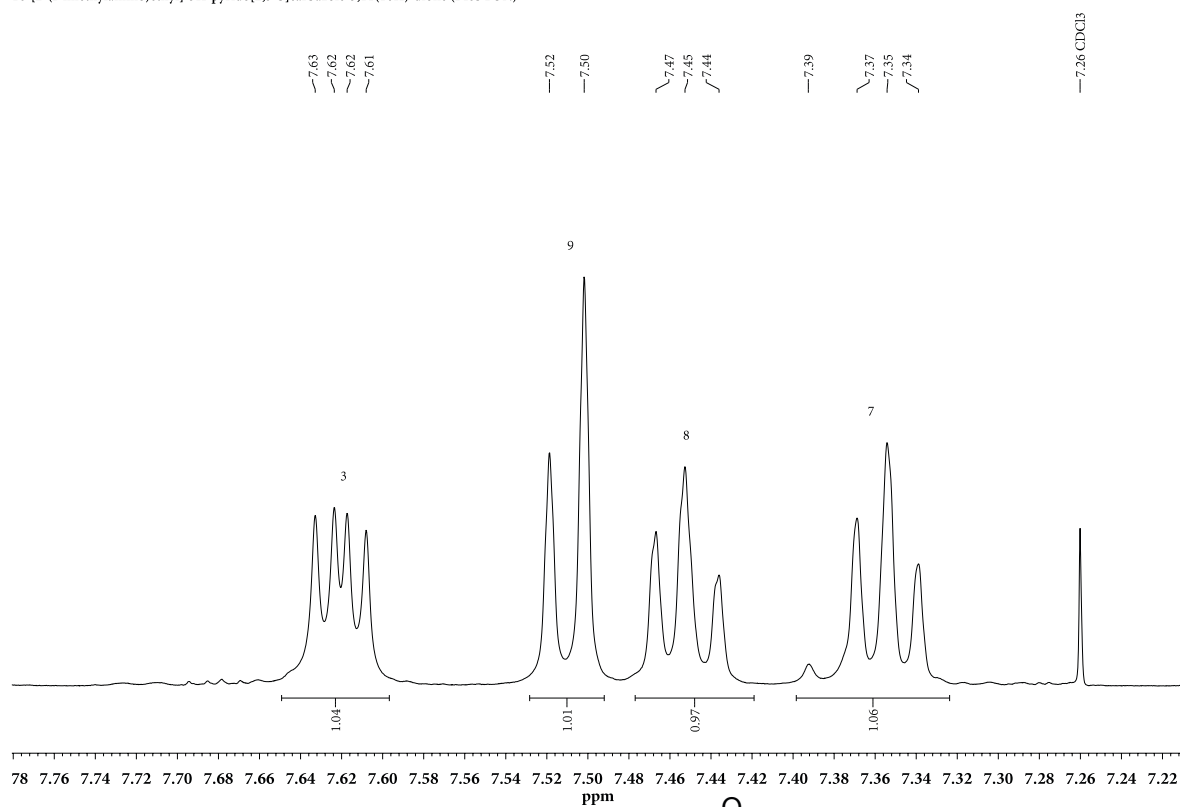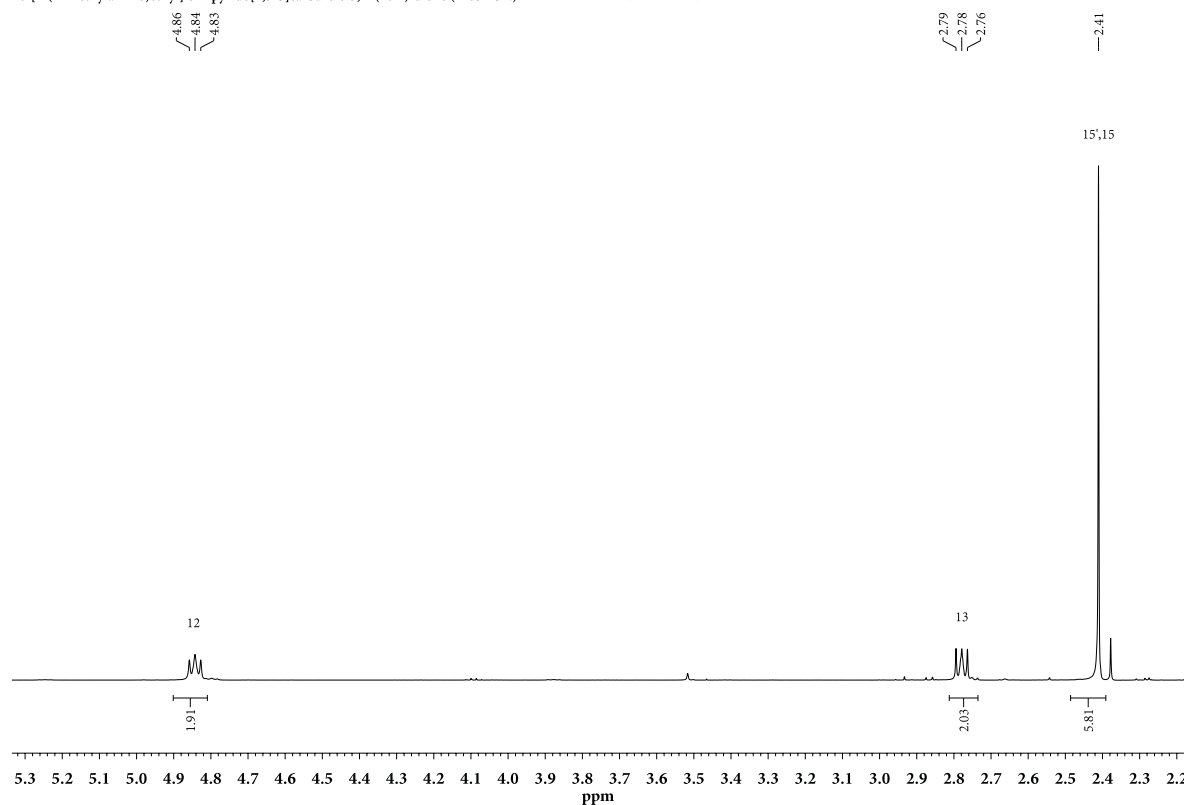
10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (C13 APT)
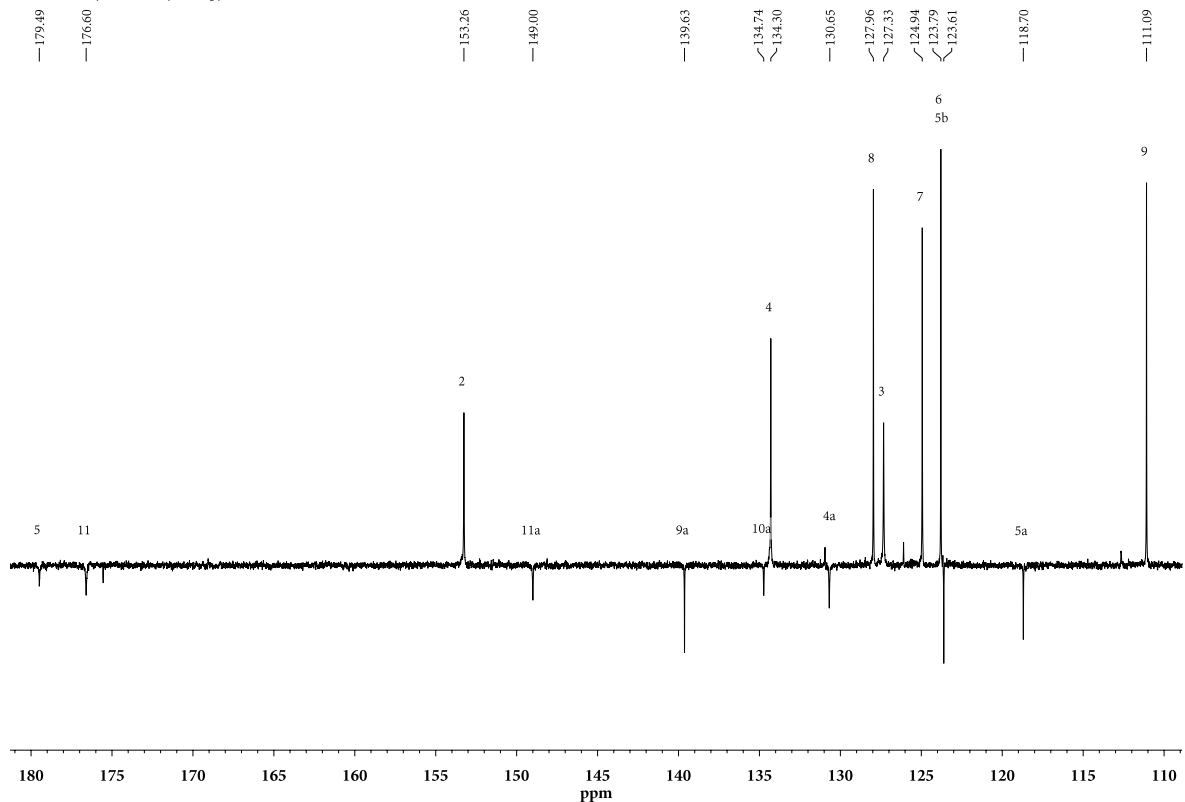
10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (HSQC)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (HMBC)
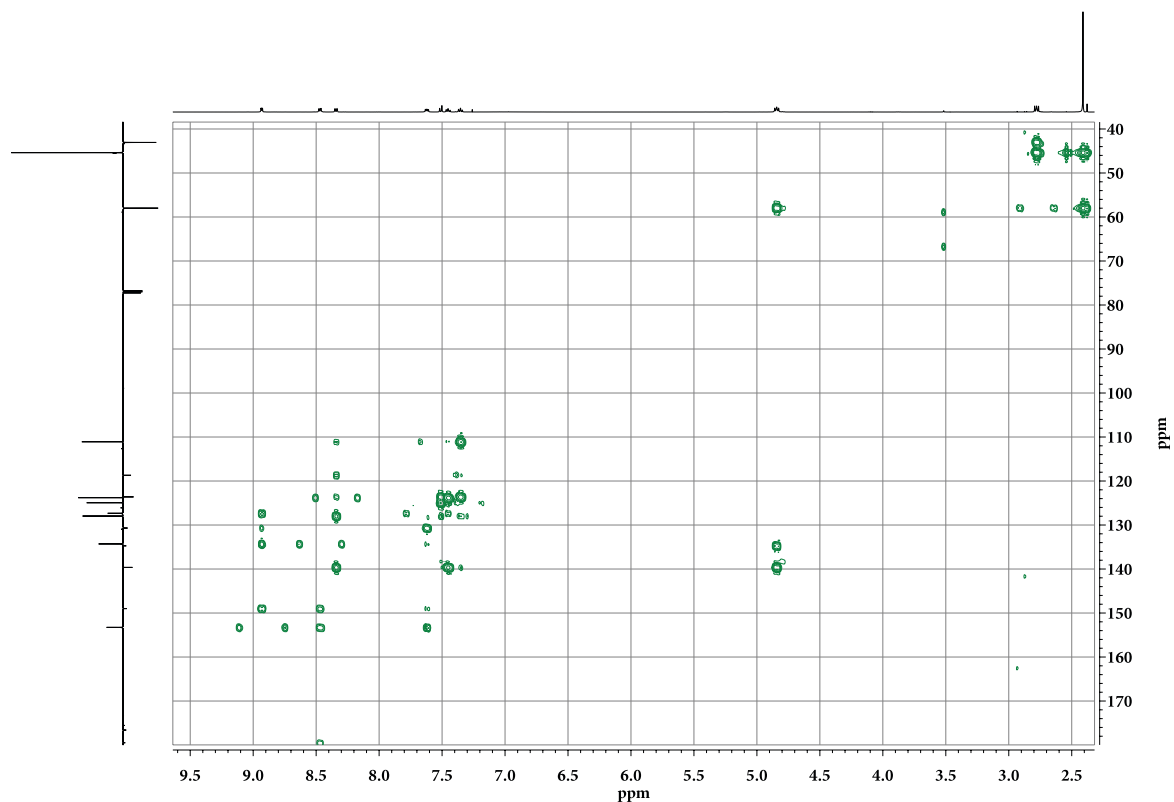
12

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (15N HMBC)

12
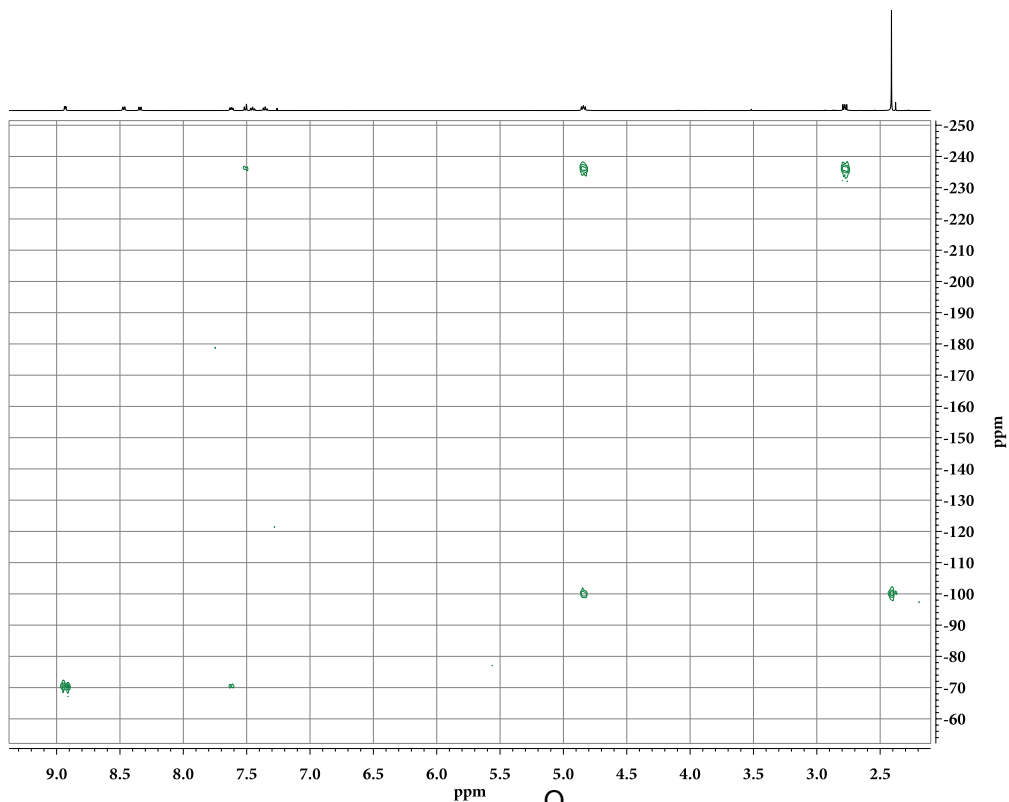
10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (15N HMBC)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[2,3-b]carbazole-5,11(10H)-dione (NOESY)

12

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 10:05:09 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN051_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN051_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 0.01 mg/mL in MeOH
Comment	

Acquisition Date	11/10/2014 10:05:09 AM
Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

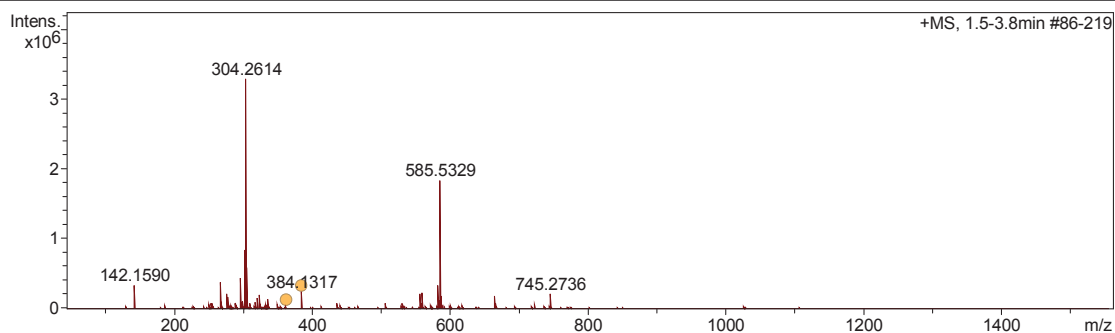|					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.2min #1-123

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
320.1396	1	C19H18N3O2	320.1394	-0.8	9.4	1	100.00	12.5	even	ok
342.1214	1	C19H17N3NaO2	342.1213	-0.4	10.3	1	100.00	12.5	even	ok

12

N,N-Diethylnicotinamide (PROTON)

13
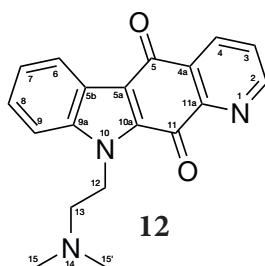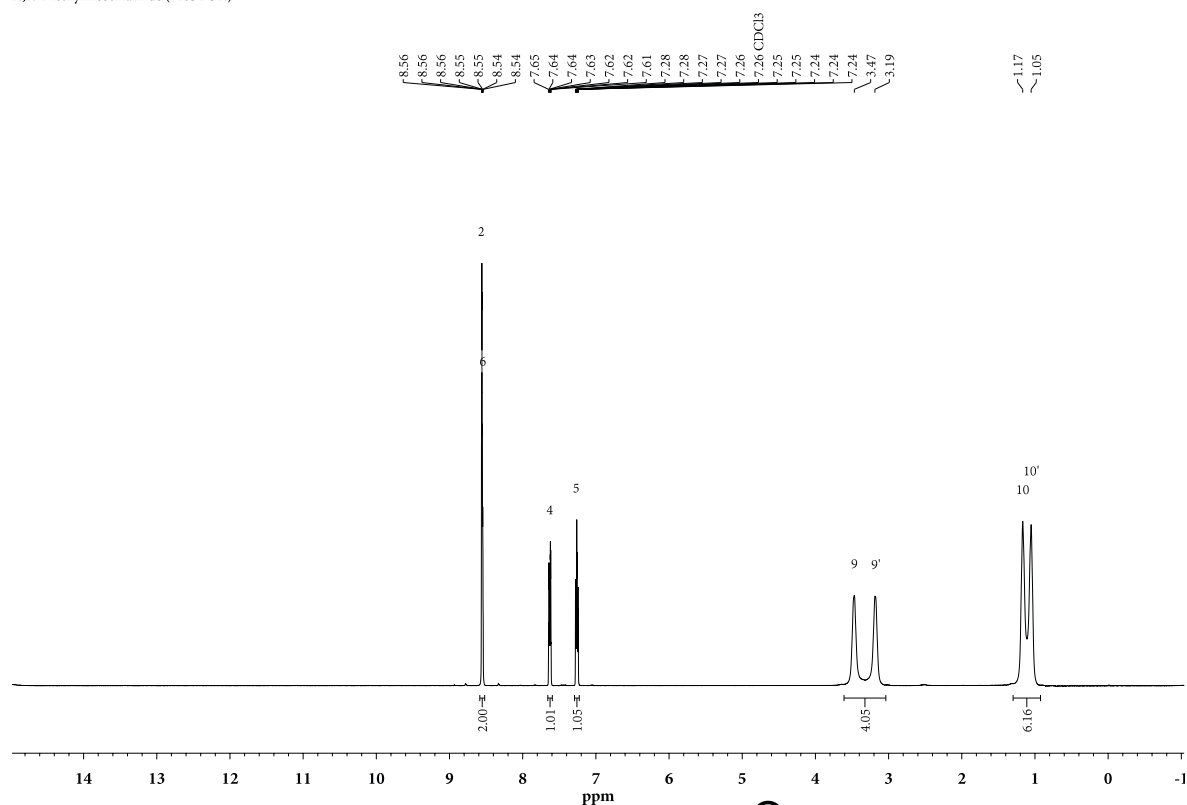
N,N-Diethylnicotinamide (PROTON)

N,N-Diethylnicotinamide (C13 CPD)
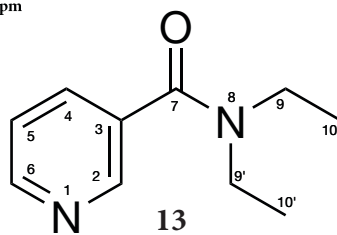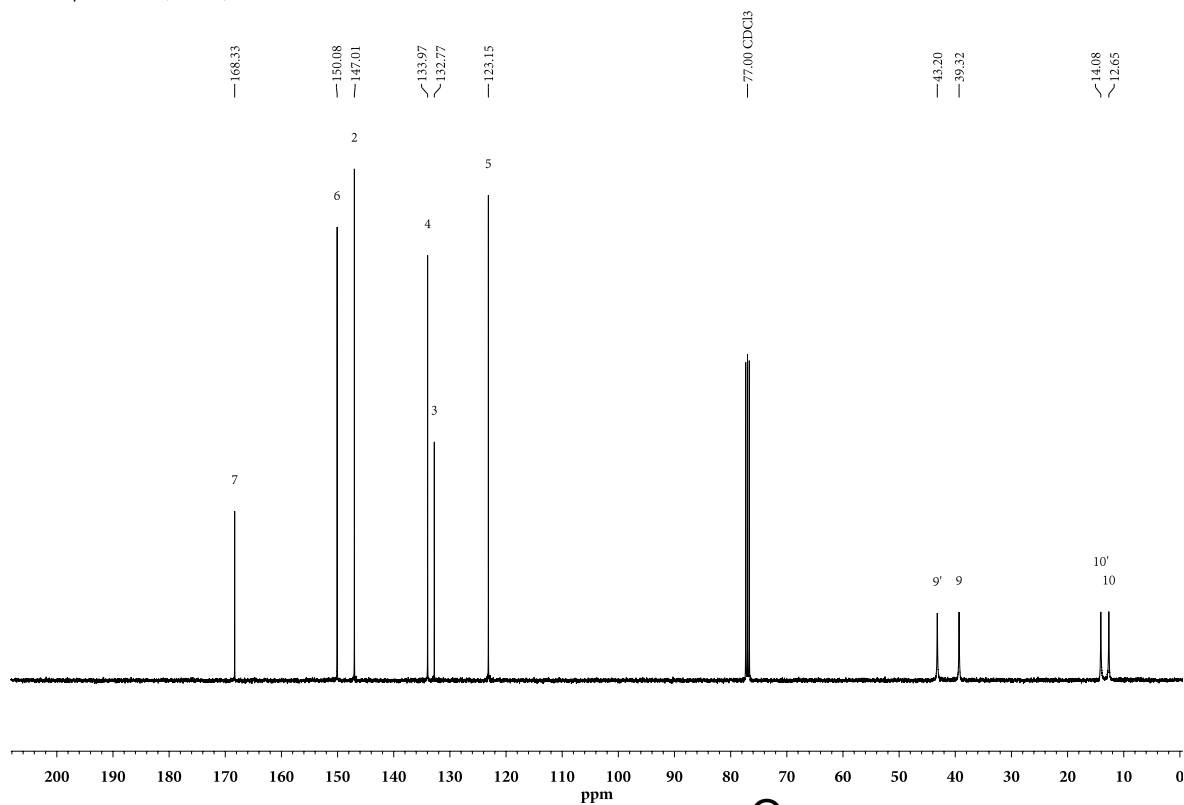
N,N-Diethylnicotinamide (HSQC)
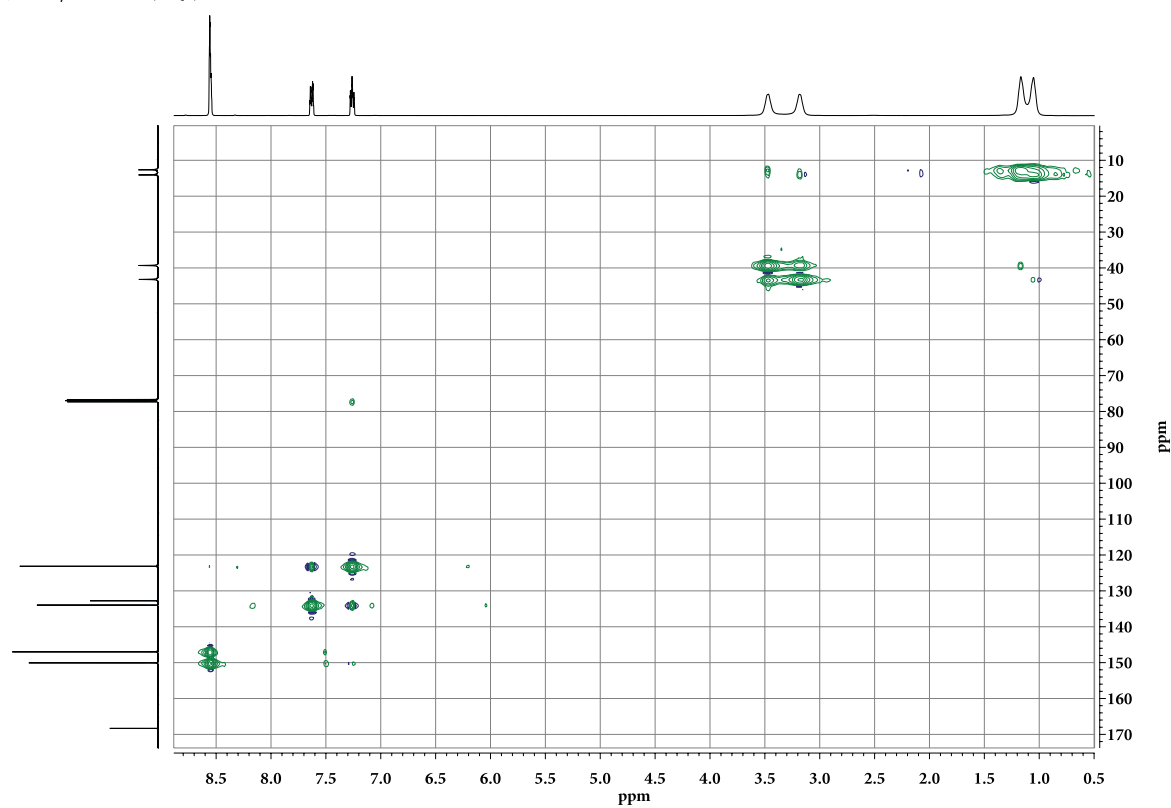
N,N-Diethylnicotinamide (HMBC)

N,N-Diethylnicotinamide (15N HMBC)

N,N-Diethylnicotinamide (NOESY)

253

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

254

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 APT)

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 APT)

255
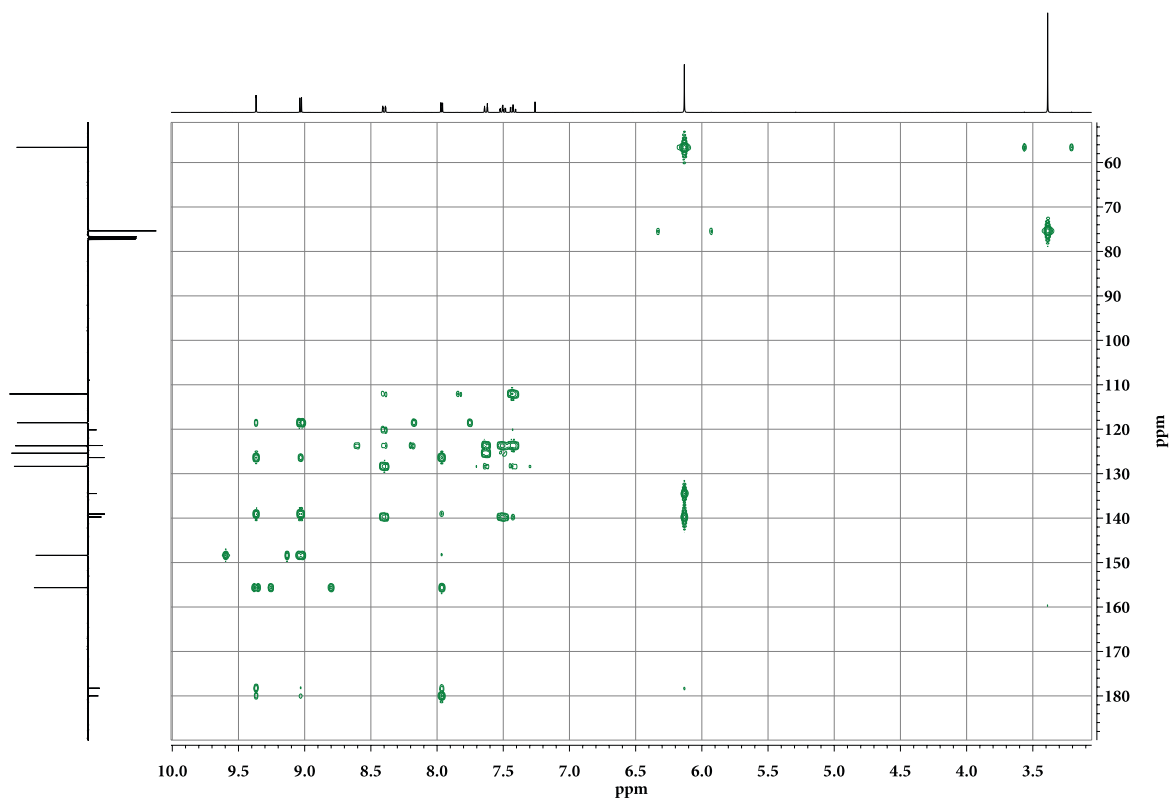
10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (15N HMBC)

10-(Methoxymethyl)-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (COSY)

257

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN054_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:29:37 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 10:29:37 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN054_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
315.0736	1	C17H12N2NaO3	315.0740	1.3	1.8	1	100.00	12.5	even	ok
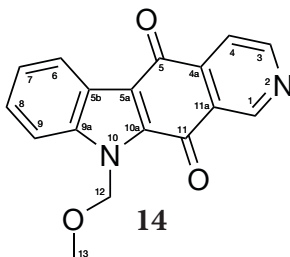
14
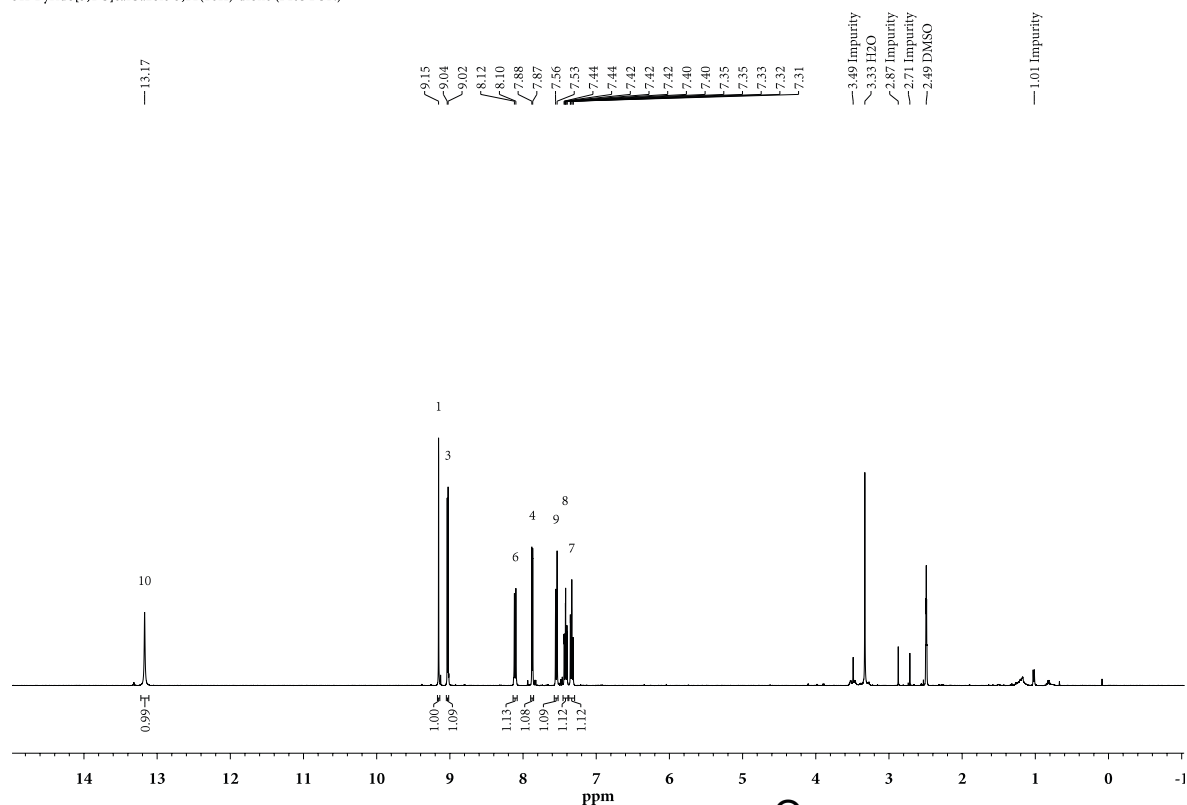
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)
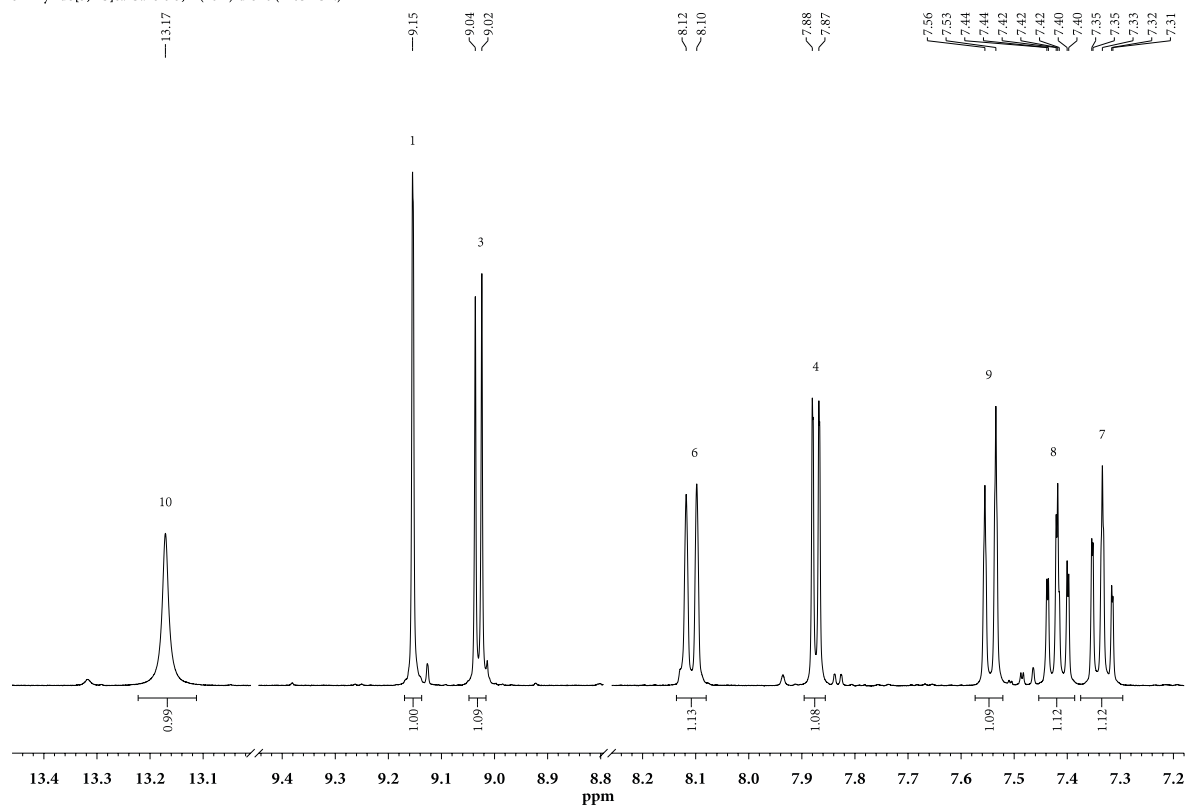
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)
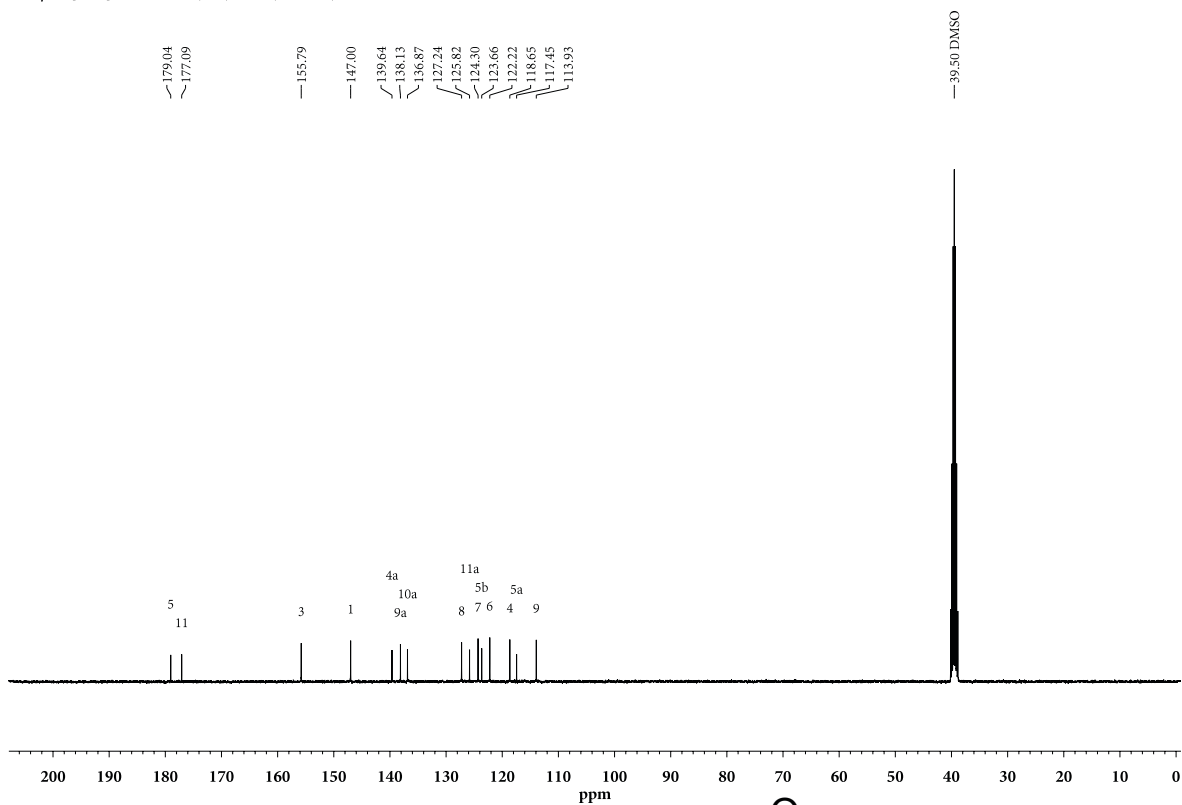
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 CPD)
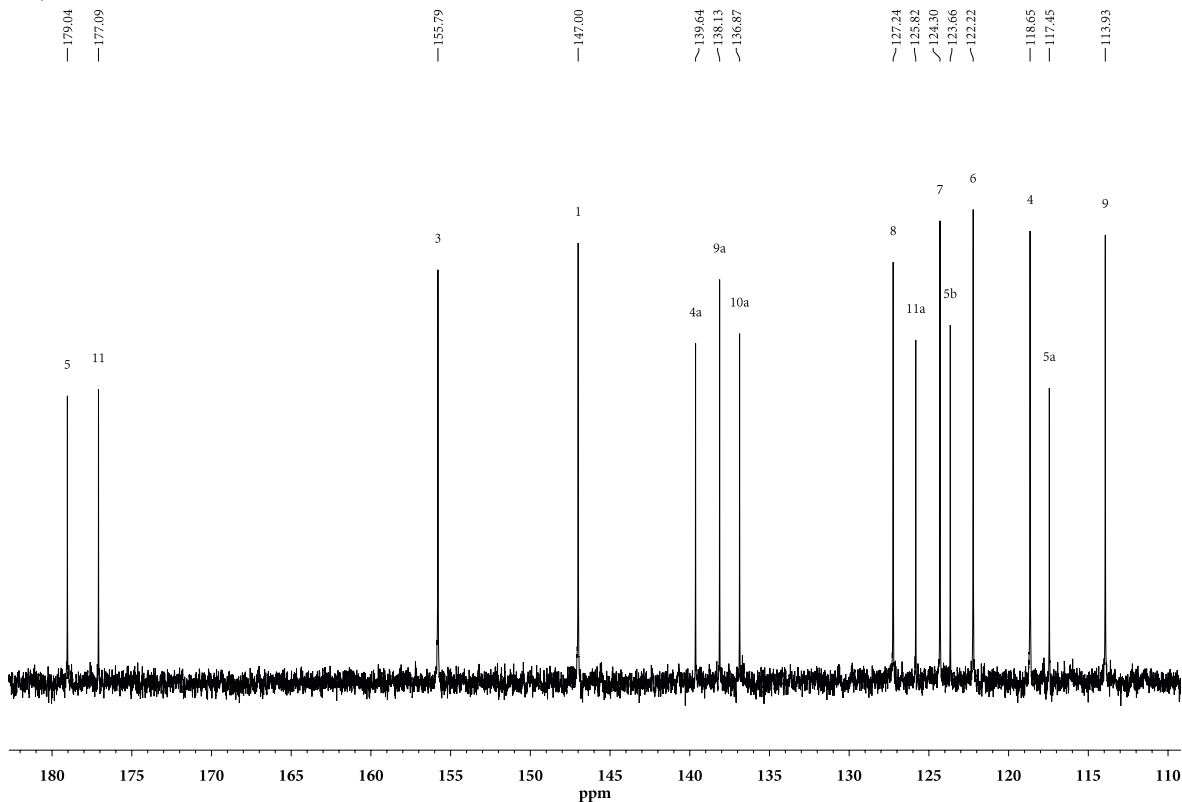
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 CPD)

5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (HSQC)
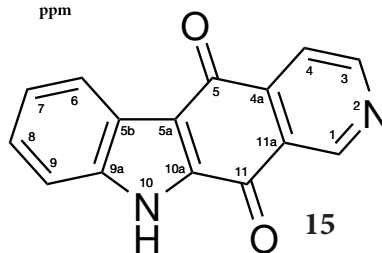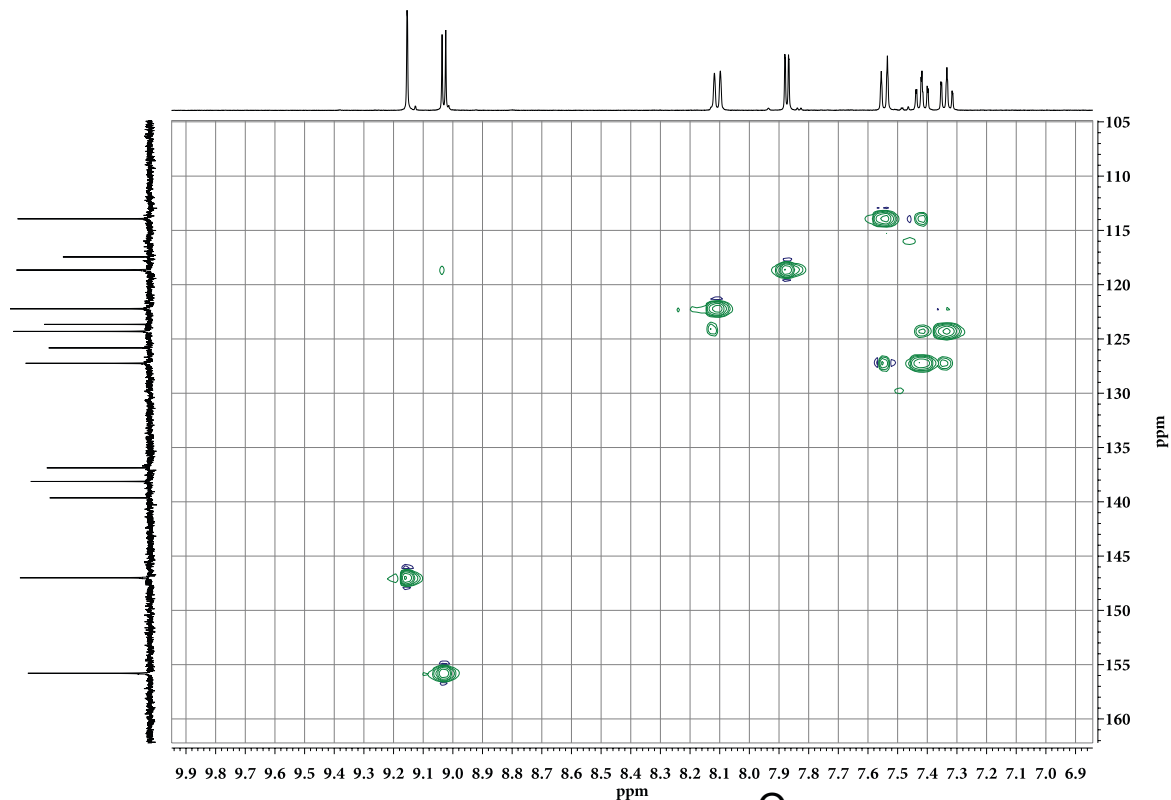
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (HMBC)
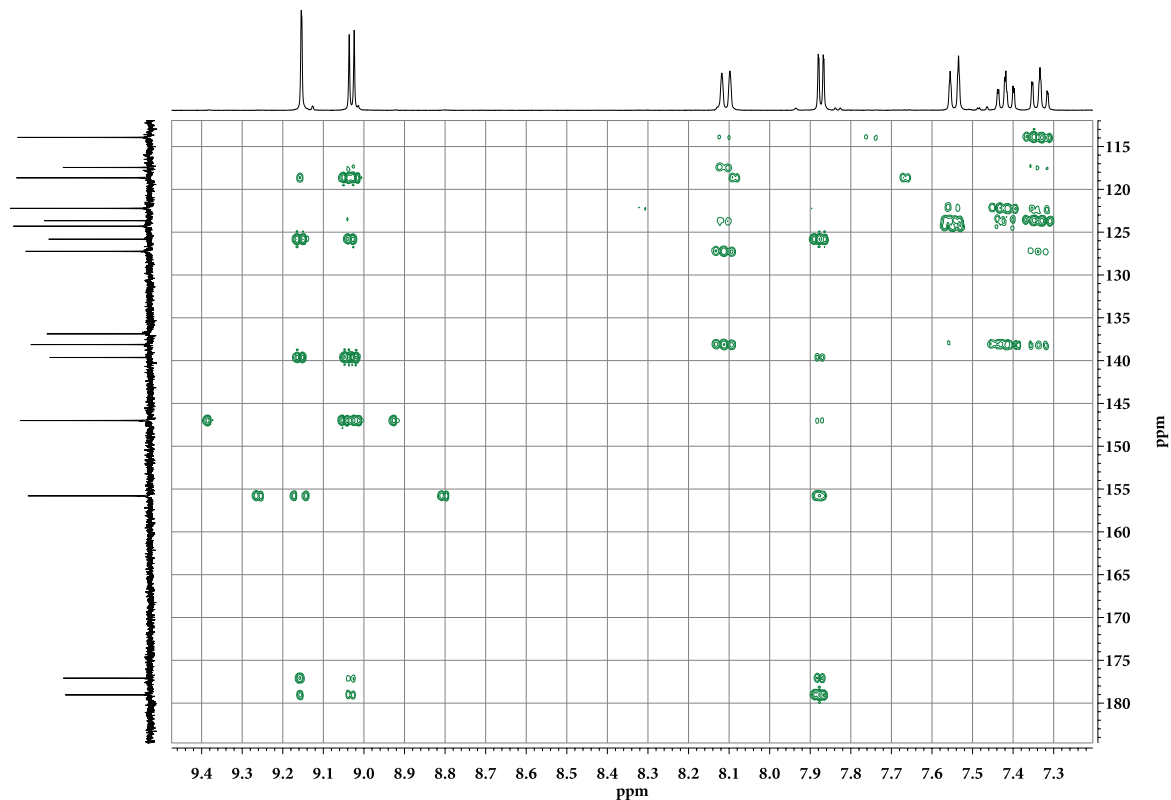
5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (15N HMBC)

5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (15N HSQC)

5H-Pyrido[3,4-b]carbazole-5,11(10H)-dione (COSY)

15
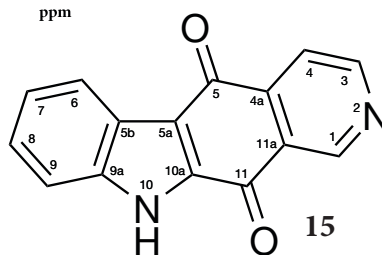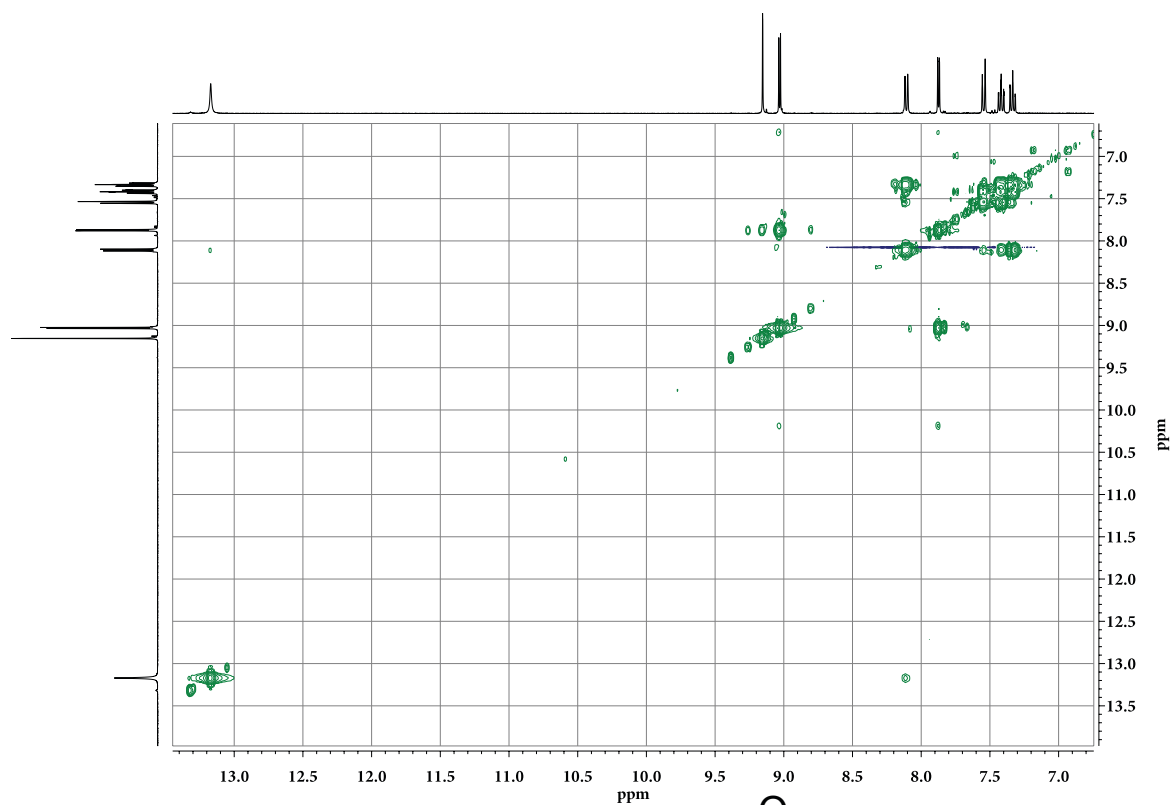
Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN087_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 11:15:12 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Intens.
x10^5

+MS, 0.0-2.6min #1-149

250.2138

271.0474

249.0655

264.2293

272.2942

251.2170 253.2633 255.2790 258.2786 268.2742

x10^4

$C_{15}H_9N_2O_2$, 249.0659

1+
249.0659

1+
250.0690

1+
251.0716

x10^4

$C_{15}H_8N_2NaO_2$, 271.0478

1+
271.0478

1+
272.0509

1+
273.0535

15

Bruker Compass DataAnalysis 4.2   printed: 11/10/2014 11:21:46 AM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 11/10/2014 11:15:12 AM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN087_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m		
Sample Name	approx. 0.01 mg/mL in MeOH	Operator	MZ
Comment		Instrument	maXis HD 1820881.21300

Acquisition Parameter
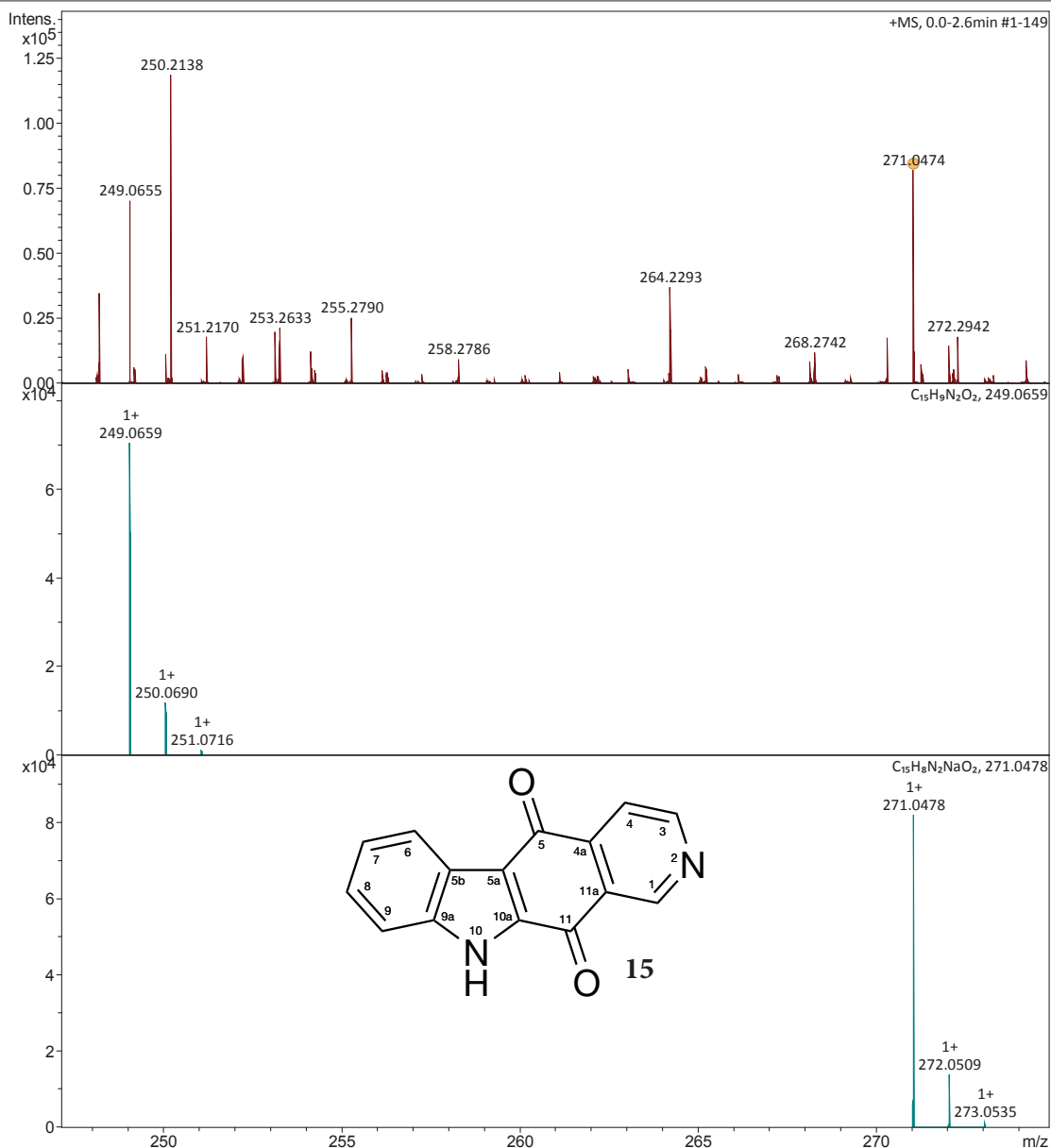
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C
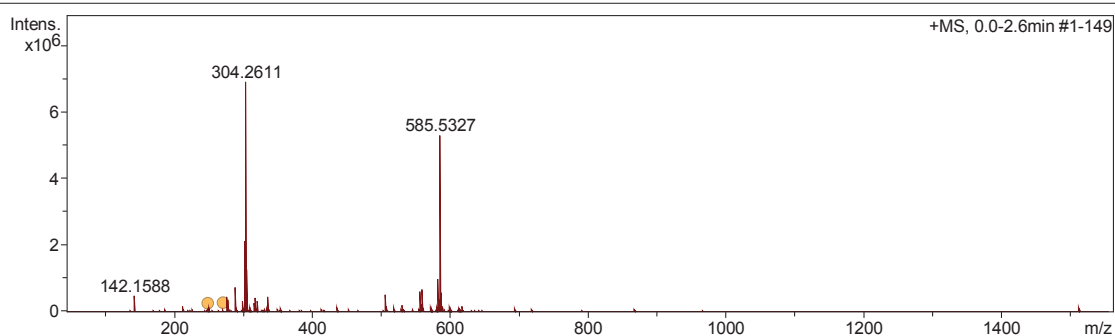
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
249.0655	1	C15H9N2O2	249.0659	1.5	12.1	1	100.00	12.5	even	ok
271.0474	1	C15H8N2NaO2	271.0478	1.6	10.9	1	100.00	12.5	even	ok

15

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 APT)

16

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (C13 APT)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (HSQC)

16

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (HMBC)

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (15N HMBC)

16

10-[2-(Dimethylamino)ethyl]-5H-pyrido[3,4-b]carbazole-5,11(10H)-dione (COSY)

16

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN060_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:43:18 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 11/10/2014 10:43:18 AM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN060_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 0.01 mg/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
320.1393	1	C19H18N3O2	320.1394	0.1	9.1	1	100.00	12.5	even	ok
342.1211	1	C19H17N3NaO2	342.1213	0.5	3.0	1	100.00	12.5	even	ok

16

N,N-Diethylisonicotinamide (PROTON)

17
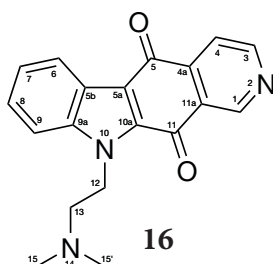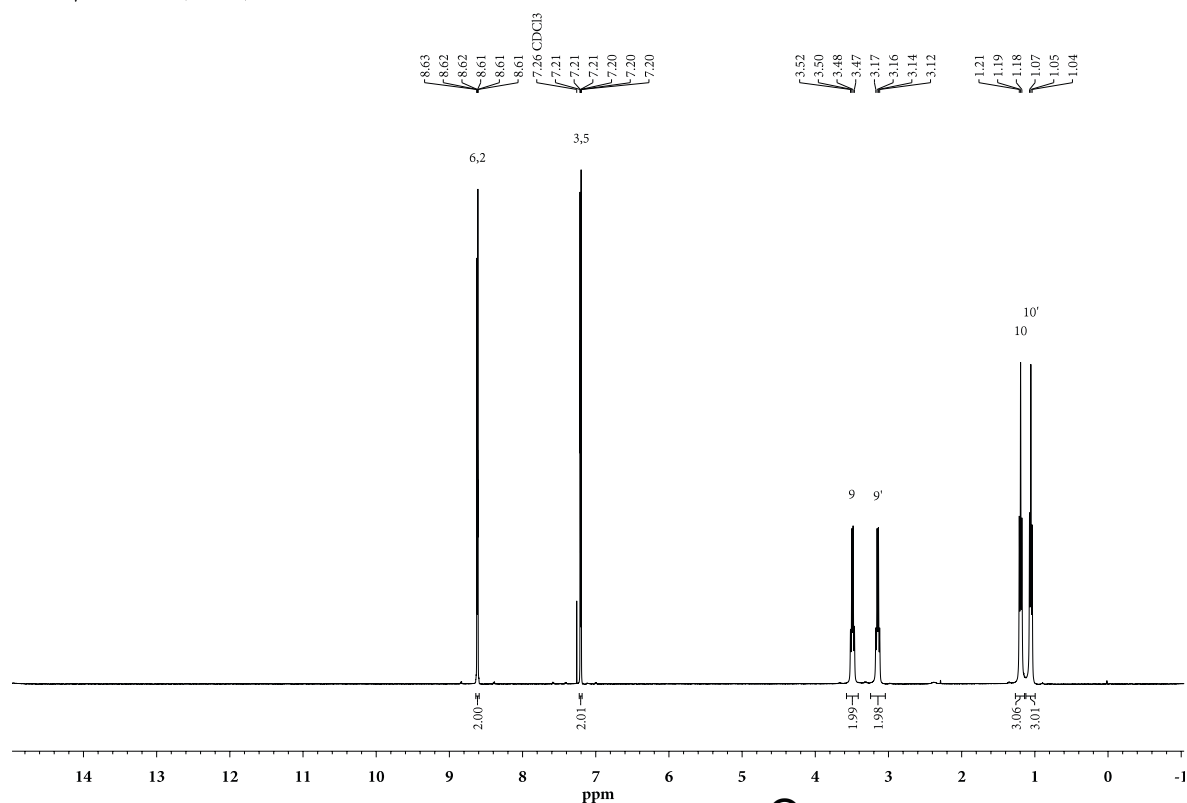
N,N-Diethylisonicotinamide (PROTON)

N,N-Diethylisonicotinamide (PROTON)
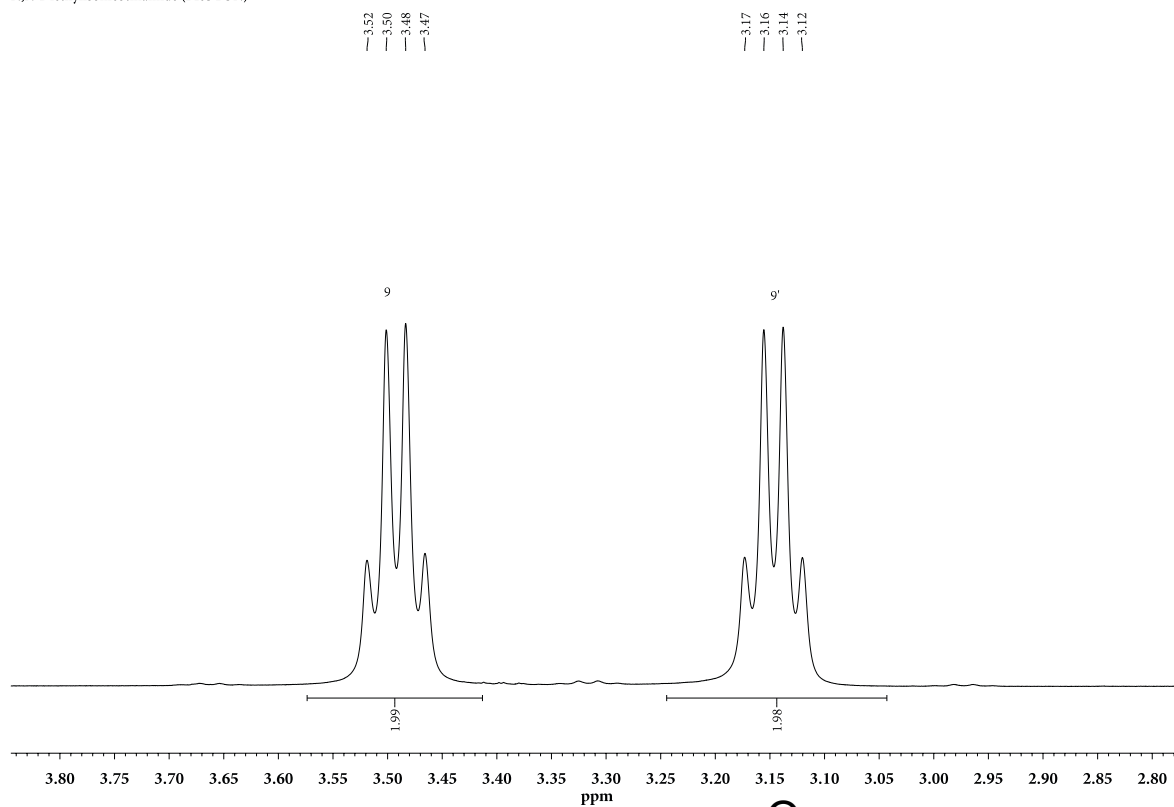
3.52
3.50
3.48
3.47

3.17
3.16
3.14
3.12

9

9'

1.99

1.98

3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 3.00 2.95 2.90 2.85 2.80
ppm

17

N,N-Diethylisonicotinamide (PROTON)

1.21
1.19
1.18

1.07
1.05
1.04

10

10'

3.06

3.01

.90 1.85 1.80 1.75 1.70 1.65 1.60 1.55 1.50 1.45 1.40 1.35 1.30 1.25 1.20 1.15 1.10 1.05 1.00 0.95 0.90 0.85 0.80 0.75 0.70 0.65 0.60 0.55
ppm
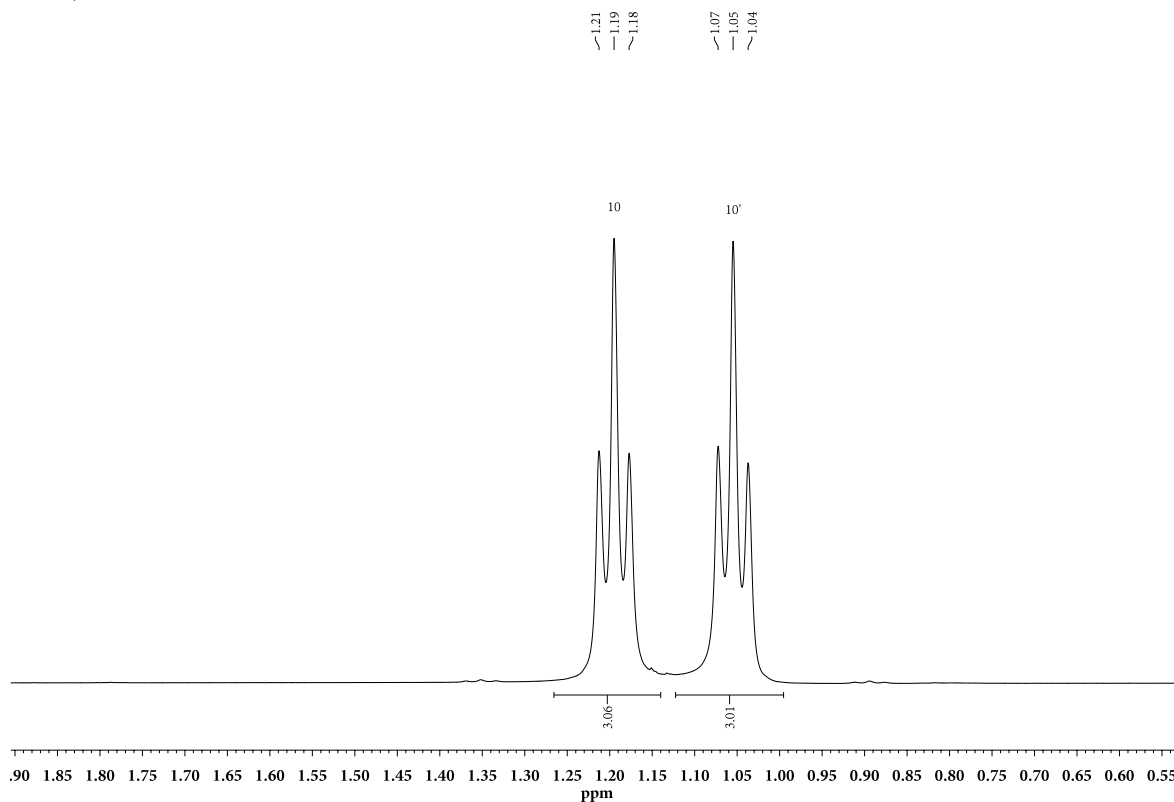
N,N-Diethylisonicotinamide (C13 CPD)
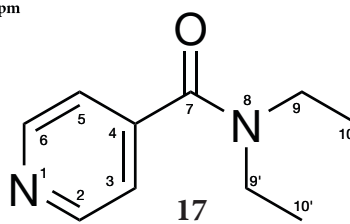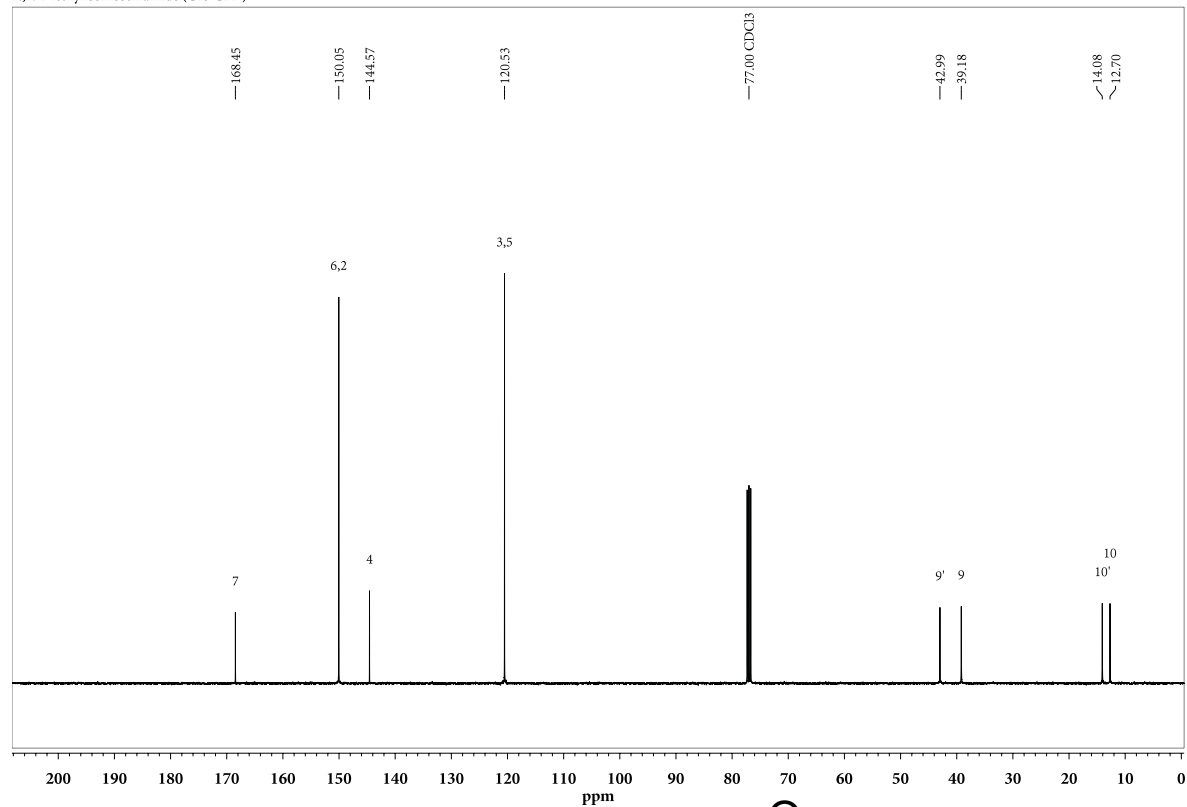
N,N-Diethylisonicotinamide (HSQC)
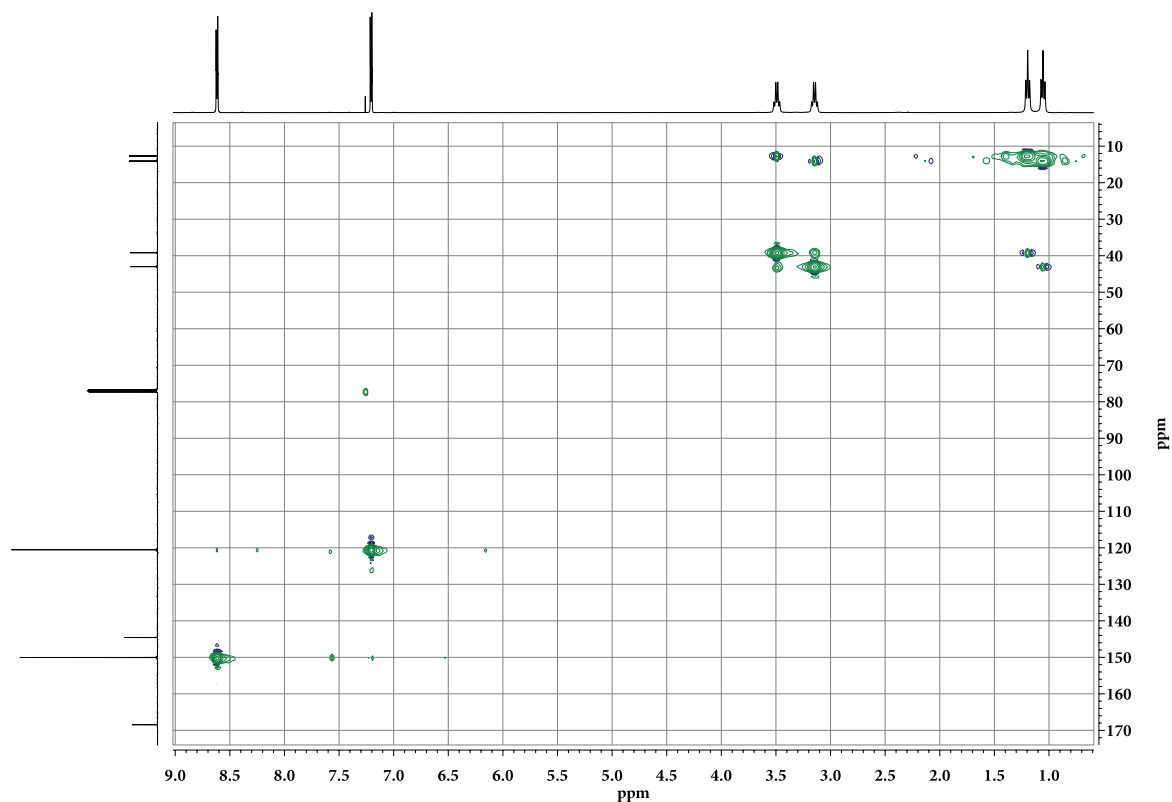
N,N-Diethylisonicotinamide (HMBC)
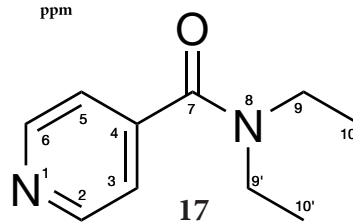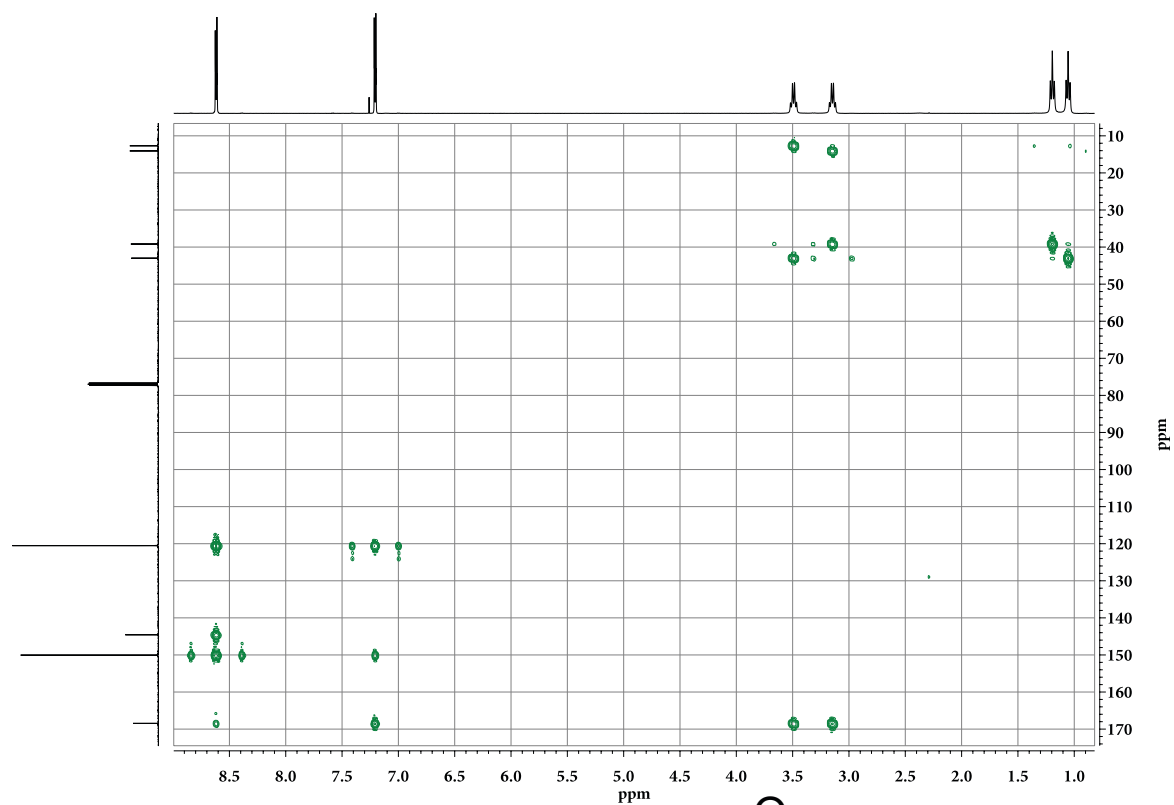
17

N,N-Diethylisonicotinamide (15N HMBC)
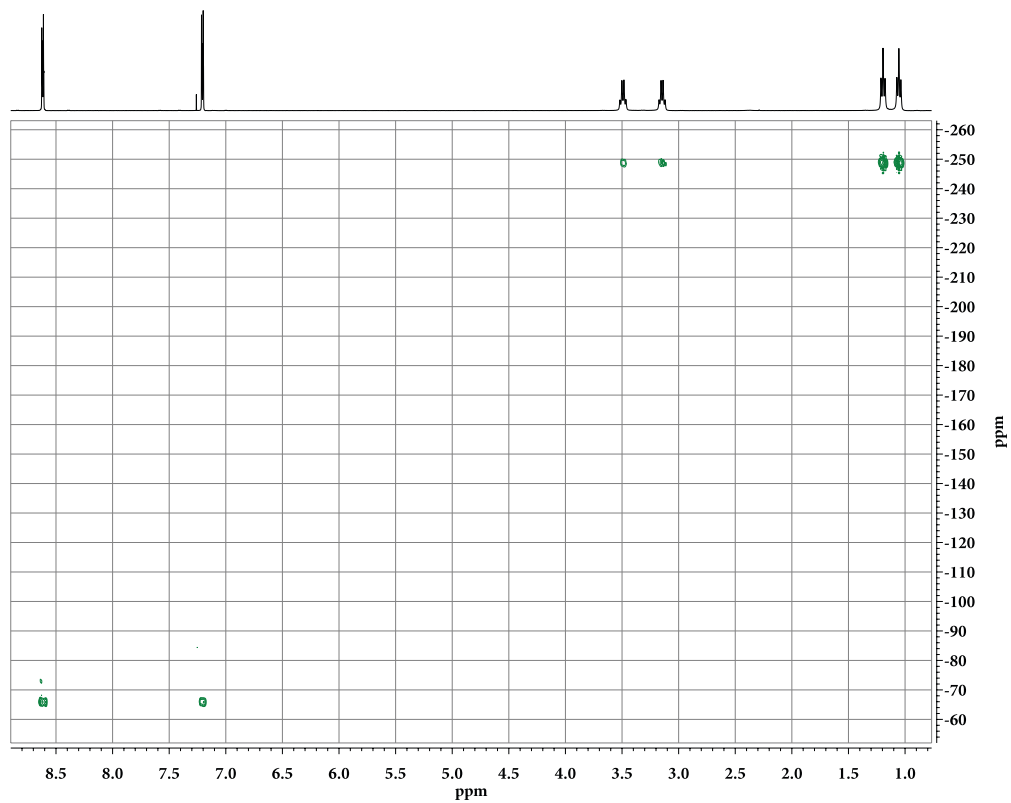
6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

7.92
7.92
7.91
7.91

7.62
7.62
7.61
7.60
7.59
7.59

7.52
7.51
7.50
7.50
7.49
7.48
7.47
7.43
7.41
7.41
7.39
7.39

7.26 CDCl3

4

7

8

9

1.03

1.04

1.06

1.06

7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20
ppm

18

6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

180.82
177.67

155.04

148.27
140.07
138.86
134.34
128.60
125.91
125.35
123.98
123.61
120.15
118.55
111.91

77.00 CDCl3
75.35

56.55

10a
10
8   9
4
7

13

3
1

6a
4a 5a
11a
11 5   10b

12

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

281
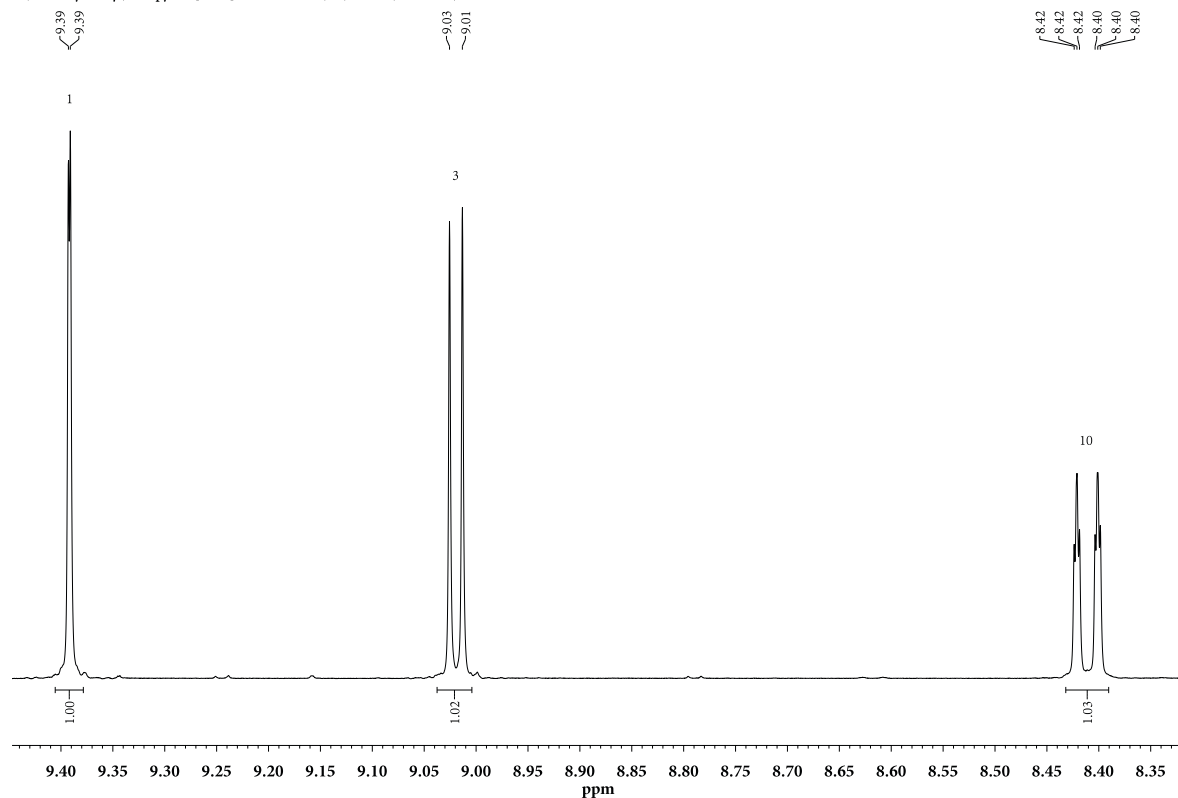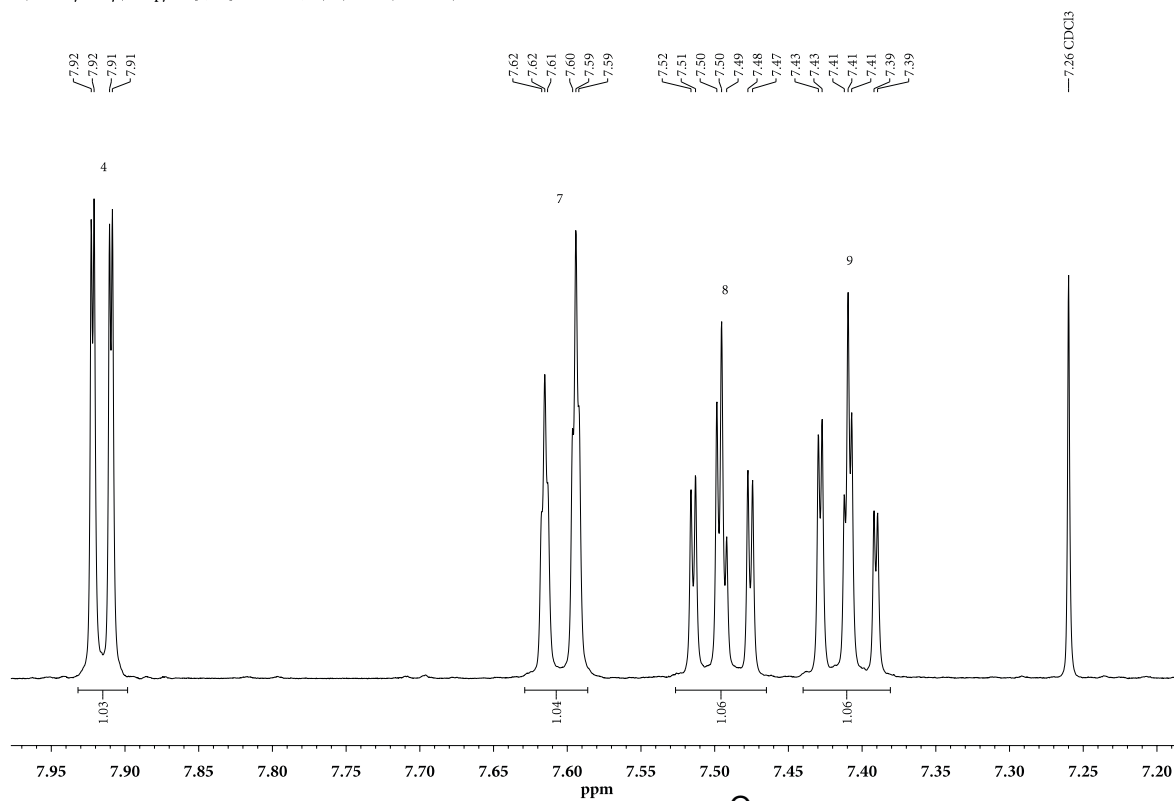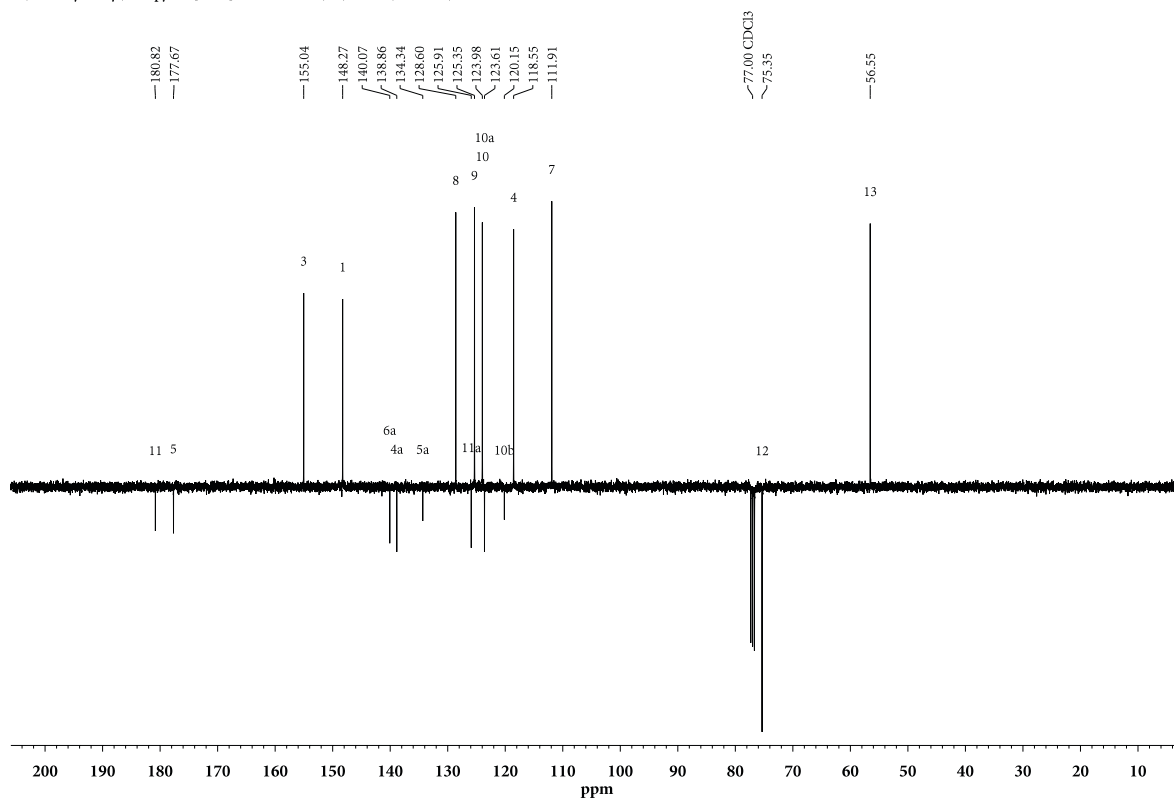
6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)
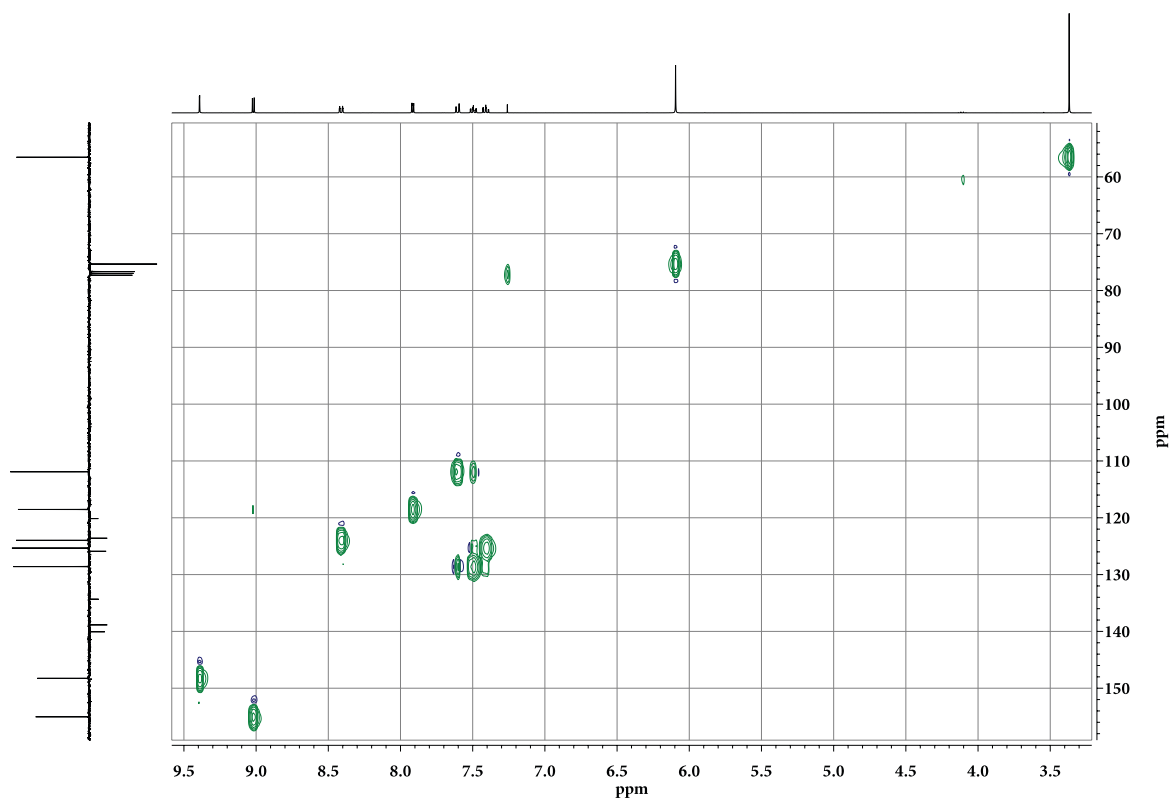
6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-(Methoxymethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 10:21:31 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN053_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Bruker Compass DataAnalysis 4.2   printed: 11/10/2014 1:37:56 PM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 10:21:31 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN053_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
315.0740	1	C17H12N2NaO3	315.0740	0.1	5.2	1	100.00	12.5	even	ok
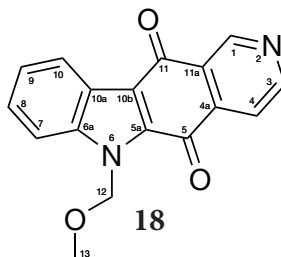
5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

19

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)

—180.19
—176.82
—155.06
—147.36
138.48
138.40
136.89
127.46
126.50
124.32
123.65
122.44
118.30
117.41
113.93
—39.50 DMSO

11a
5a   10a
6a   9   10b
4a   8   10   4   7
11 5   3   1

19

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)

—180.19
—176.82
—155.06
—147.36
138.48
138.40
136.89
127.46
126.50
124.32
123.65
122.44
118.30
117.41
113.93

11   5   3   1   6a   8   9   10   4   7
                    4a   11a   10a
                    5a   10b
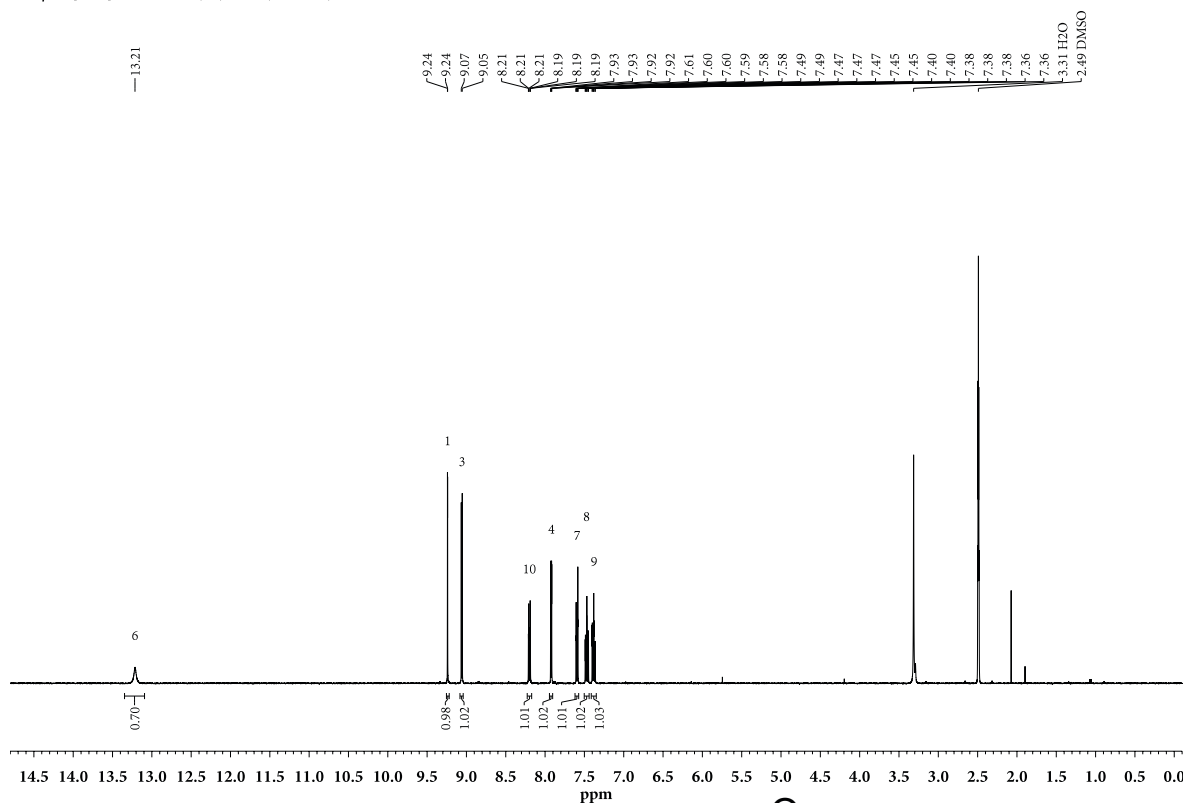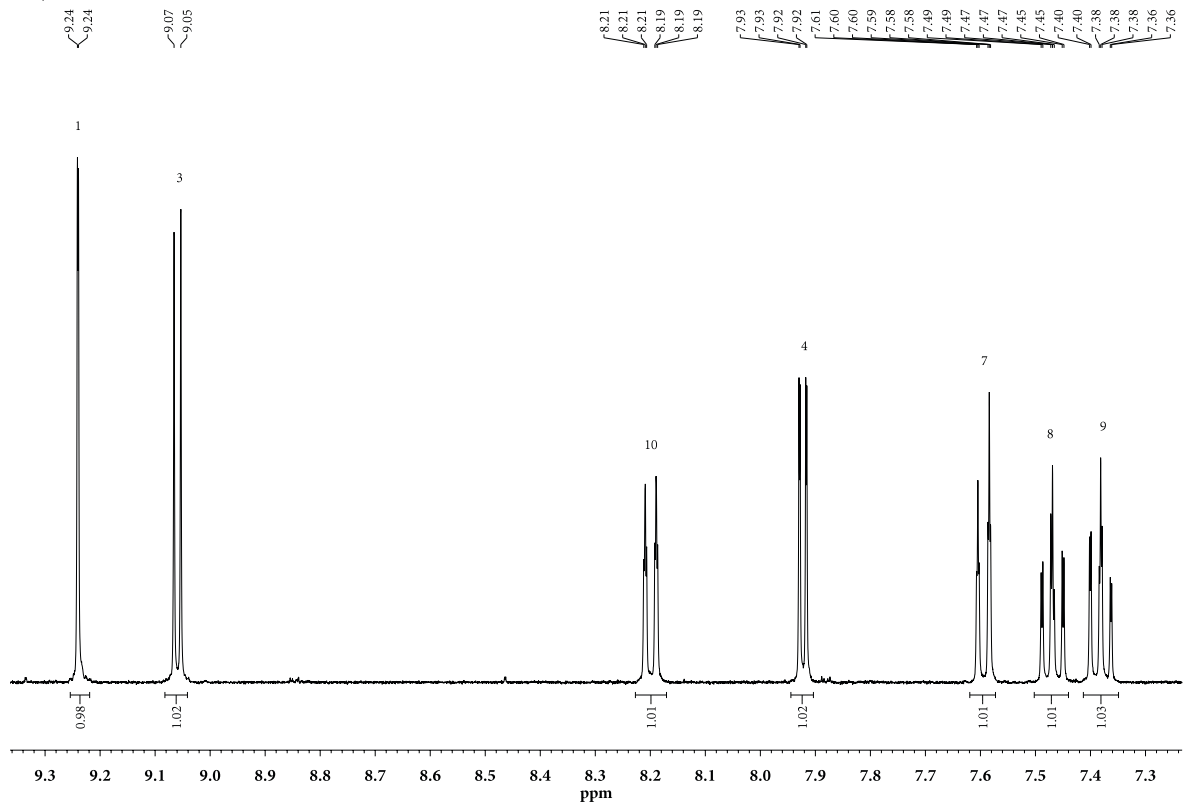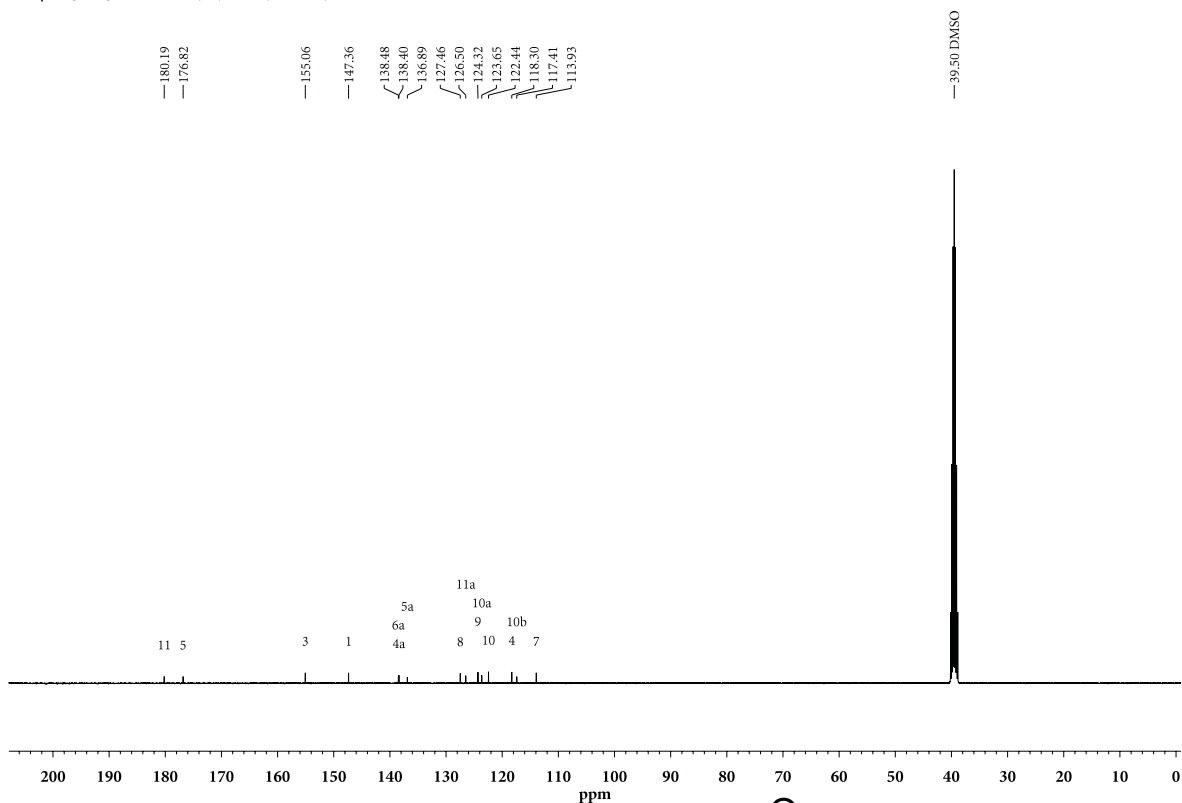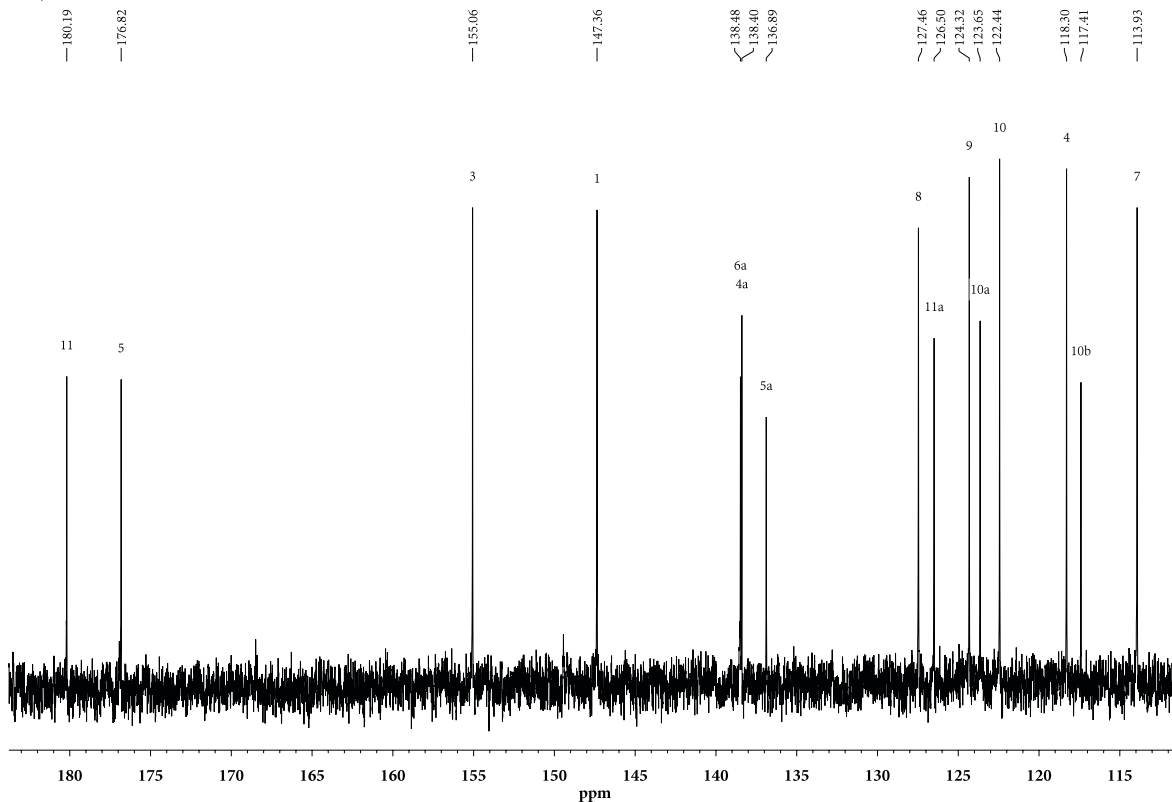
5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HSQC)

Generic Display Report

Analysis Info
Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN086_DI_ESI_HRMS.d
Method   DI_mz_50-1550.m
Sample Name   approx. 0.01 mg/mL in MeOH
Comment

Acquisition Date   11/10/2014 11:04:10 AM
Operator   MZ
Instrument   maXis HD

+MS, 0.0-1.8min #1-101

271.0477

249.0656 250.2139 251.2171 253.2636 254.2667 255.2792 256.2994 258.2789 261.1306 264.2295 267.2793 268.2745 269.2778 272.2945

$C_{15}H_9N_2O_2$, 249.0659

1+ 249.0659
1+ 250.0690
1+ 251.0716

$C_{15}H_8N_2NaO_2$, 271.0478

1+ 271.0478
1+ 272.0509
1+ 273.0535

19

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 11:04:10 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN086_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
249.0656	1	C15H9N2O2	249.0659	0.9	11.9	1	100.00	12.5	even	ok
271.0477	1	C15H8N2NaO2	271.0478	0.5	0.3	1	100.00	12.5	even	ok

19

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

20

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

7.93
7.93
7.92
7.92

7.49
7.49
7.49
7.48
7.48

4

7

8

9

1.13

1.06
1.07

1.13

7.98 7.96 7.94 7.92 7.90 7.88 7.86 7.84 7.82 7.80 7.78 7.76 7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32
ppm

20

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

4.82
4.80
4.79

2.75
2.73
2.72

2.37

15',15

12

13

5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2
ppm

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

20

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

N,N-Diethyl-2-pyrazinecarboxamide (NOESY)

Generic Display Report

Analysis Info

Analysis Name D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN061_DI_ESI_HRMS.d
Method DI_mz_50-1550.m
Sample Name approx. 10 ug/mL in MeOH
Comment

Acquisition Date 11/4/2014 10:18:15 AM
Operator MZ
Instrument maXis HD

Bruker Compass DataAnalysis 4.2   printed: 11/4/2014 11:40:26 AM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN061_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 10:18:15 AM

Method	DI_mz_50-1550.m	Operator	MZ	
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD	1820881.21300
Comment				

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
320.1394	1	C19H18N3O2	320.1394	-0.1	9.0	1	100.00	12.5	even	ok
342.1213	1	C19H17N3NaO2	342.1213	-0.0	9.7	1	100.00	12.5	even	ok
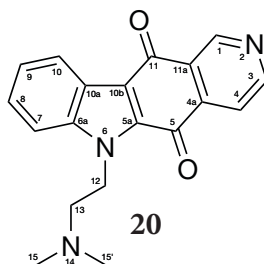
20

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (PROTON)
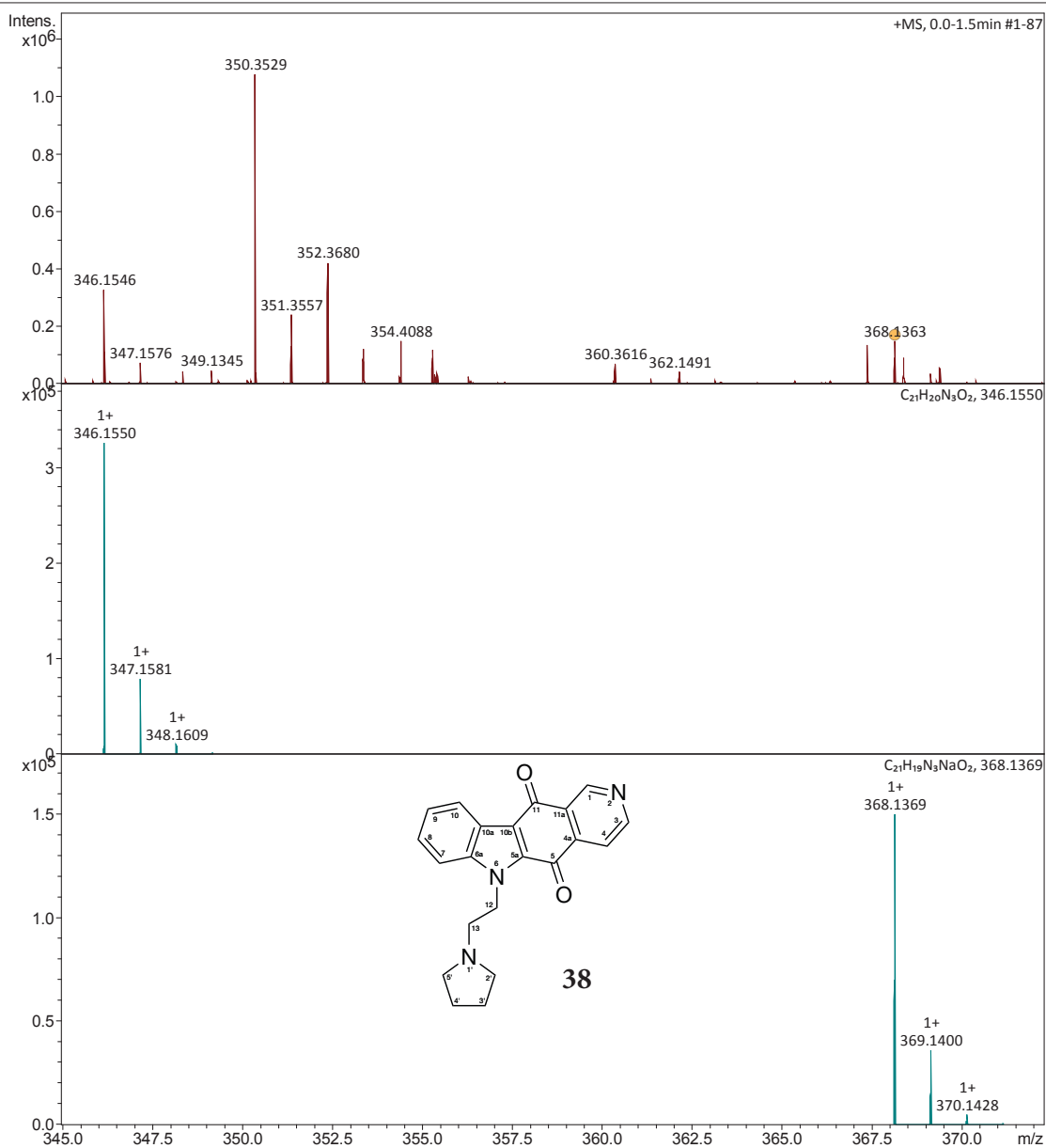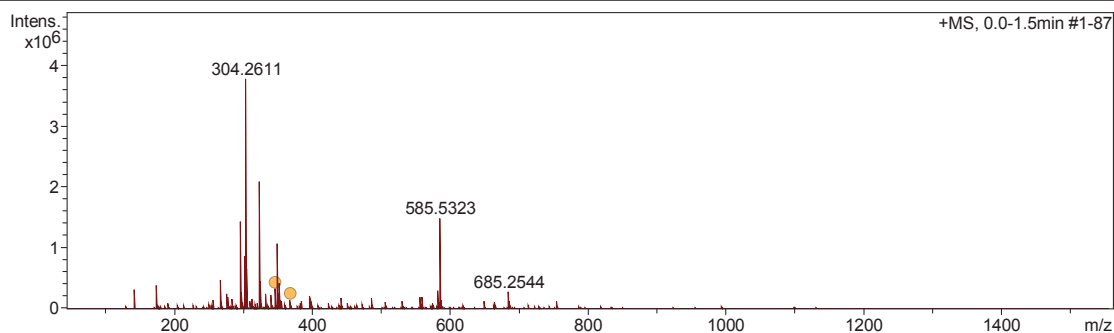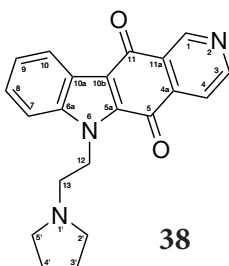

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (PROTON)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (C13 CPD)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (C13 CPD)

5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (HSQC)

21
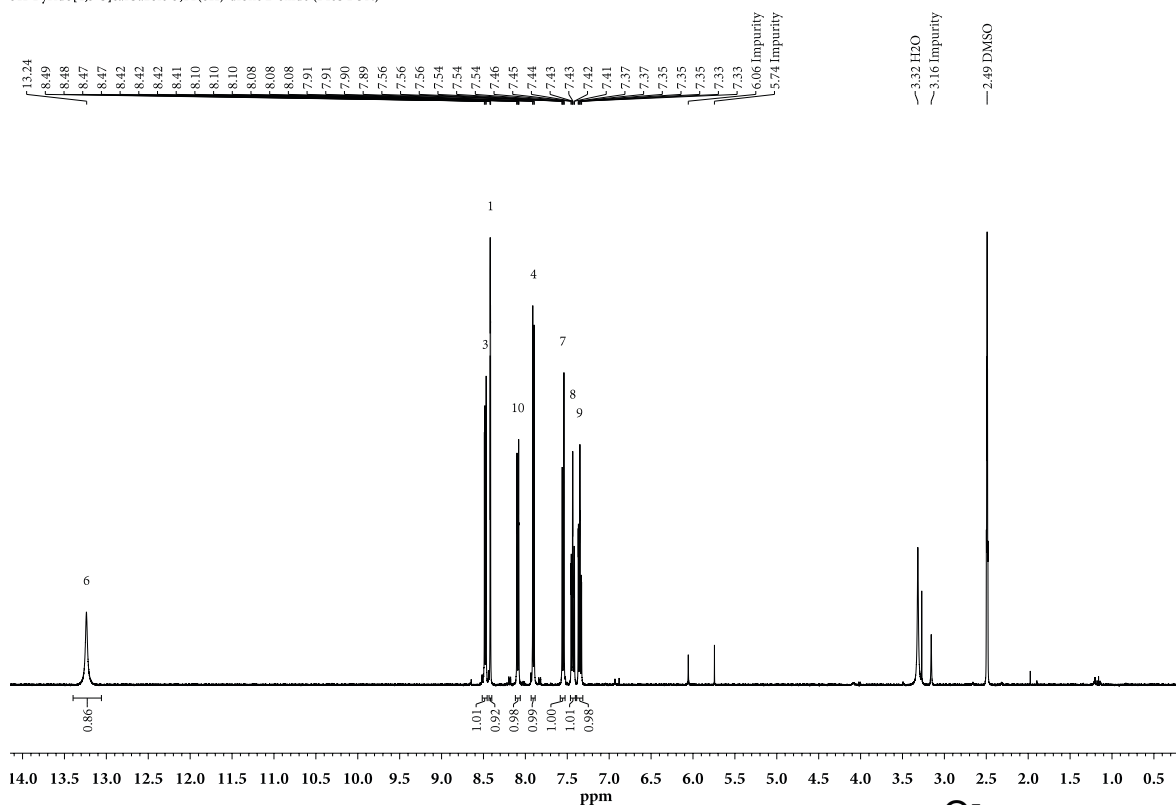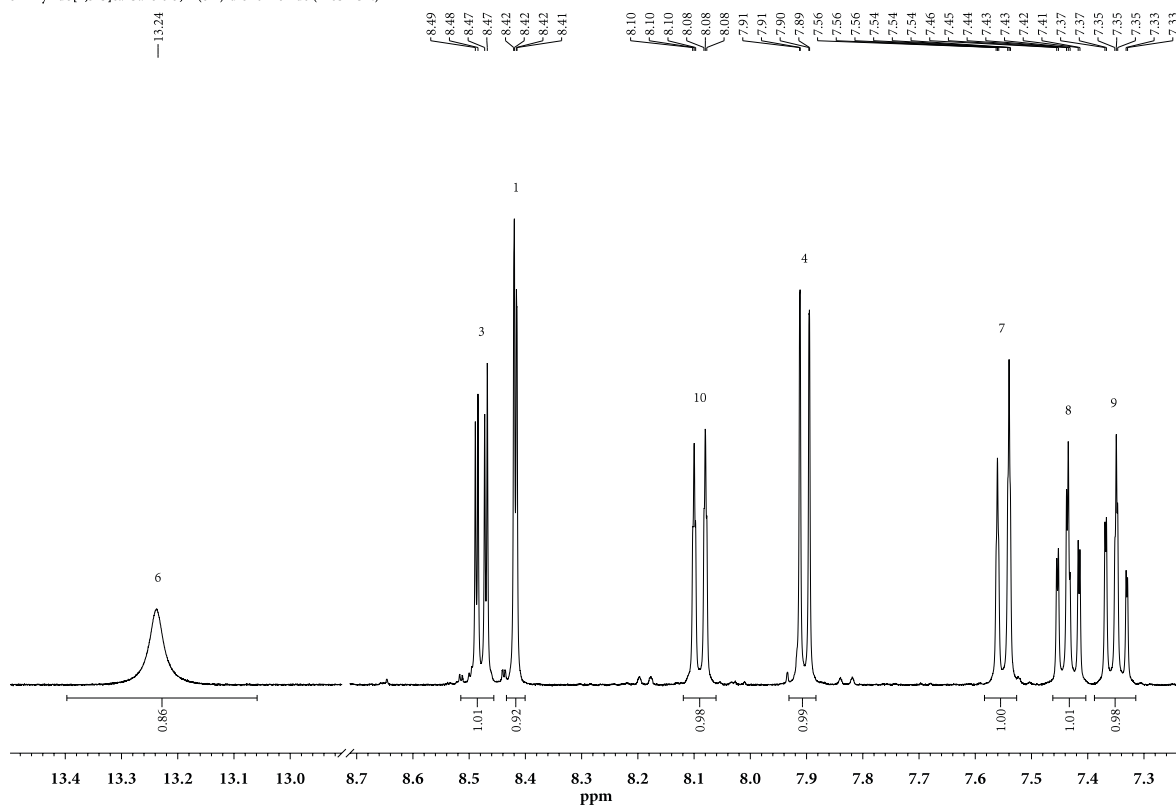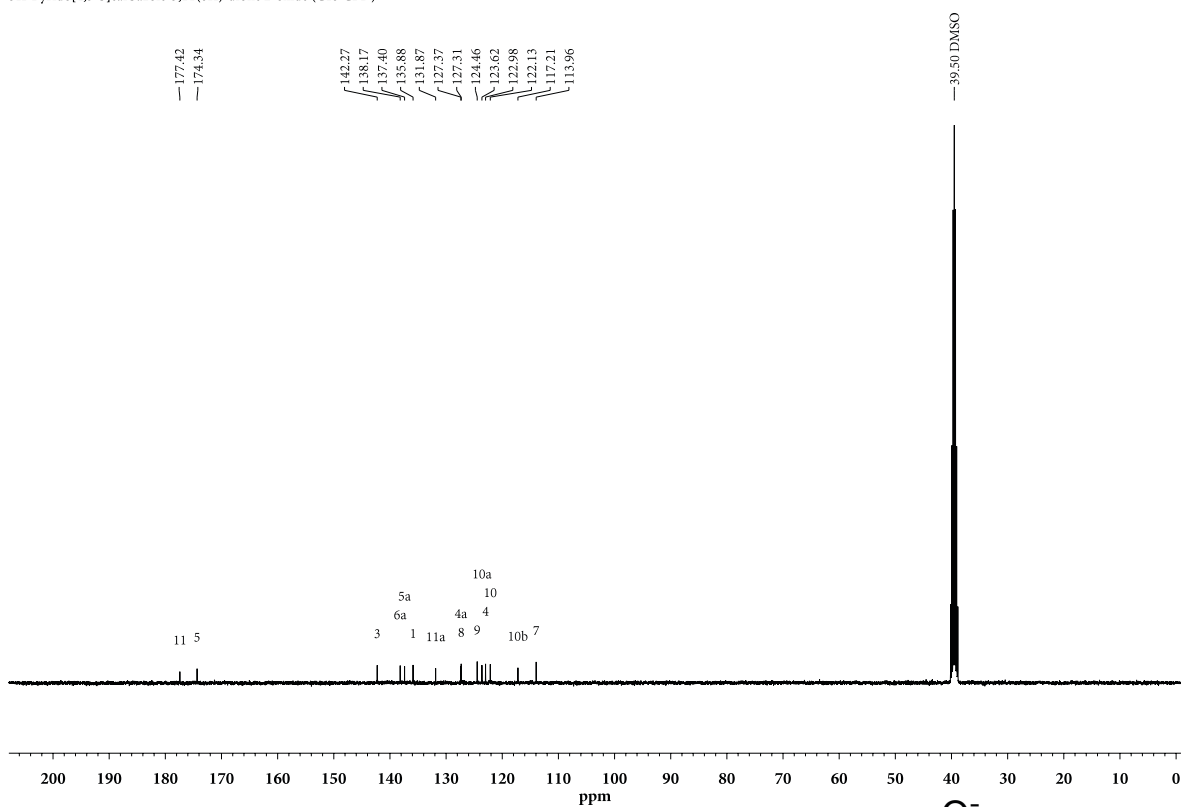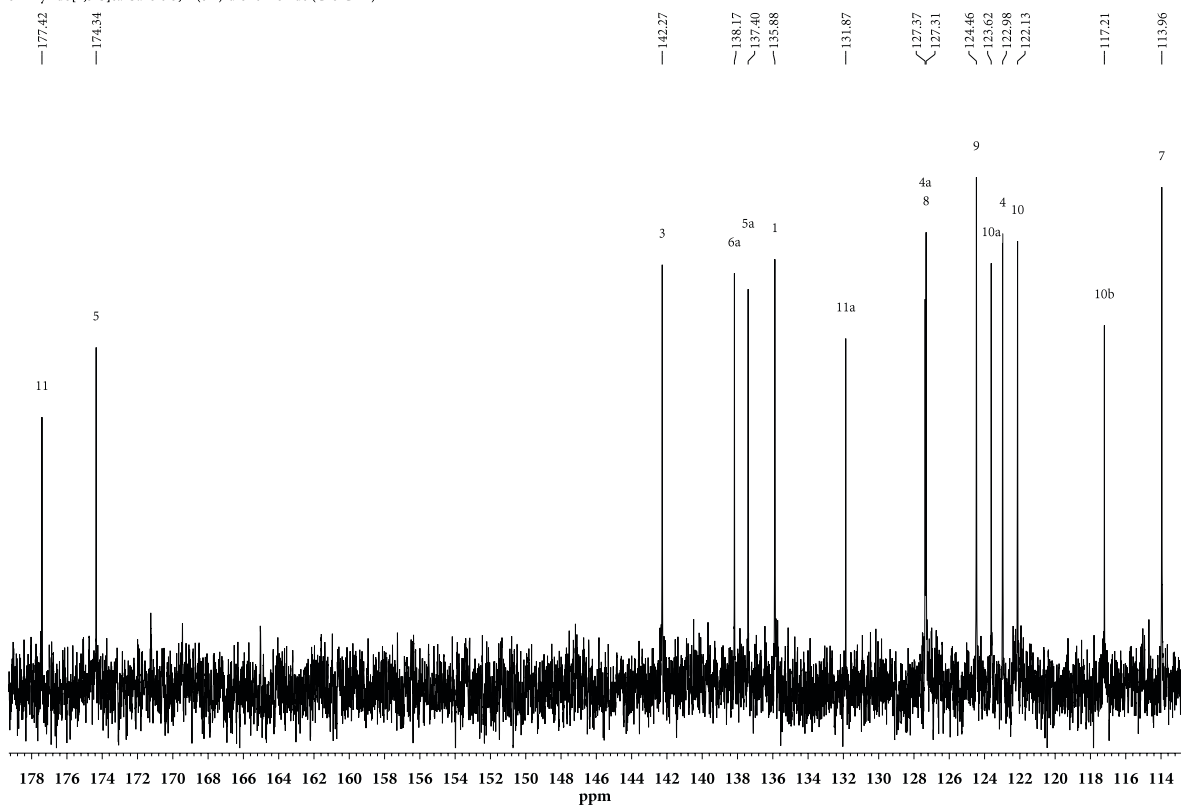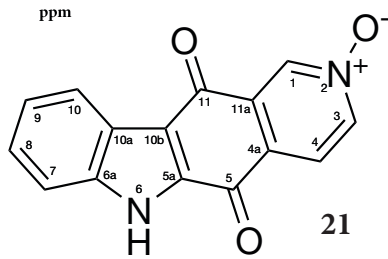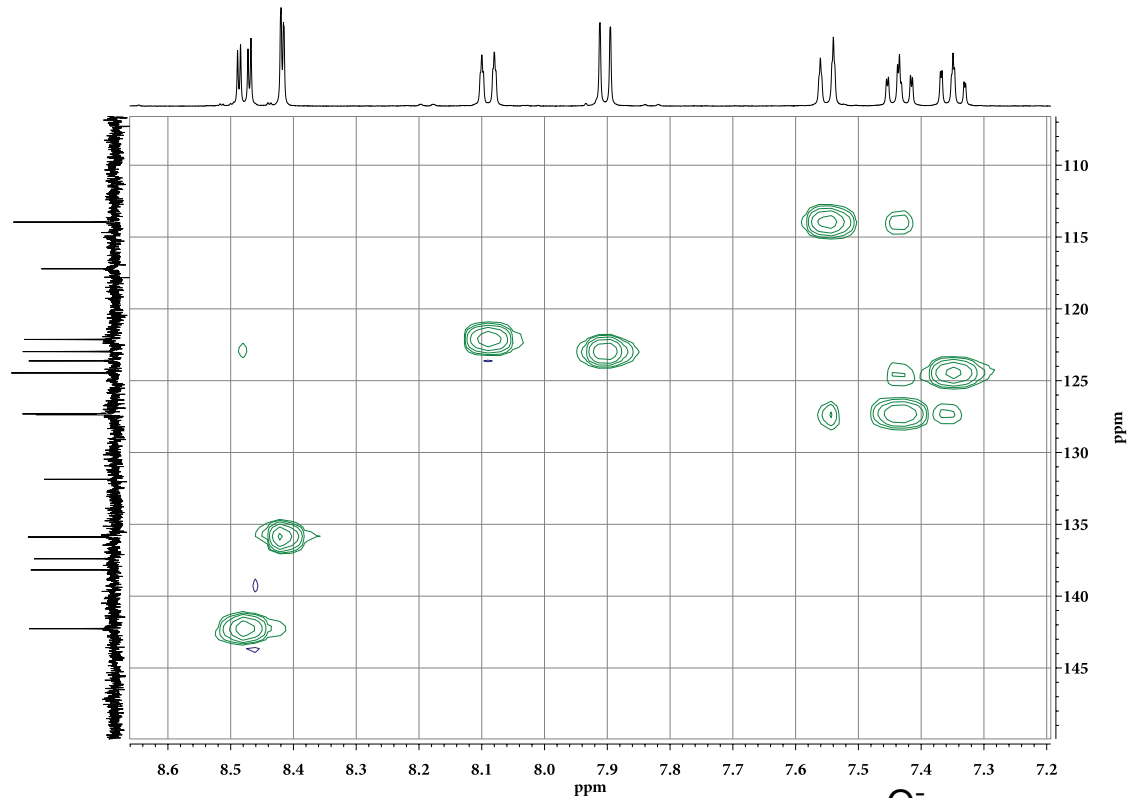
5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (HMBC)
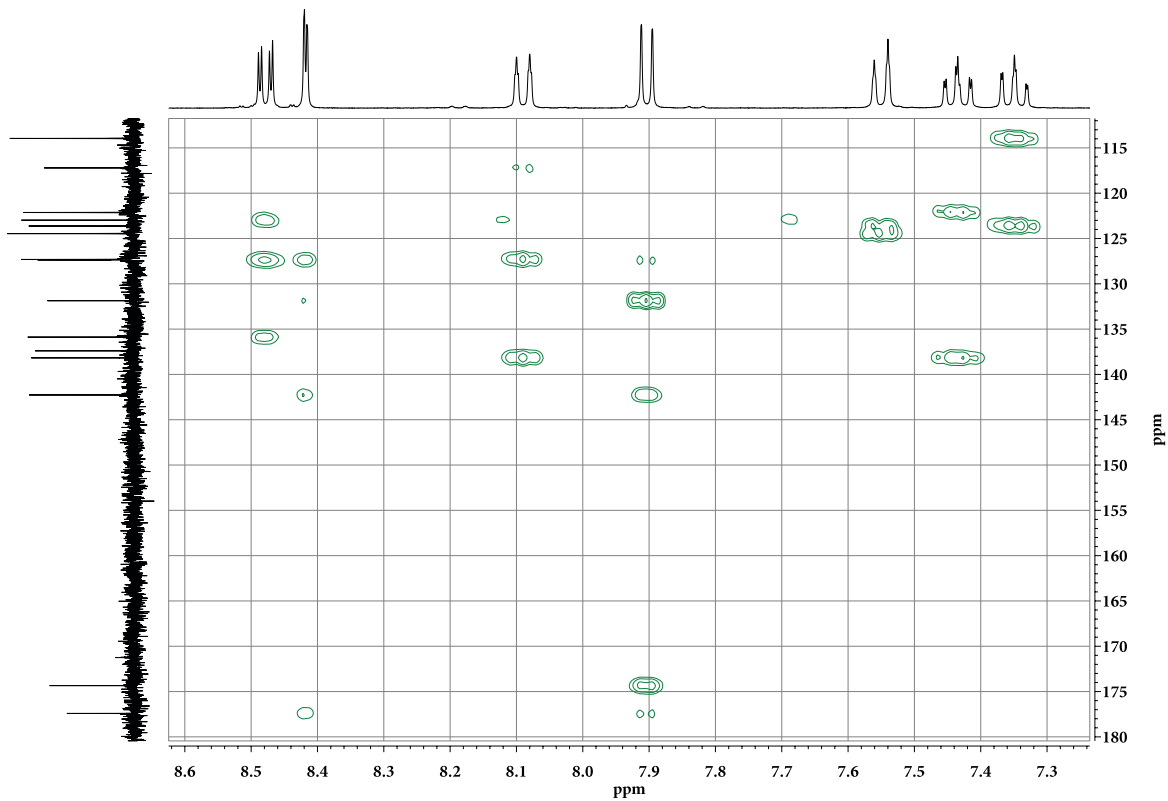
5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (15N HMBC)
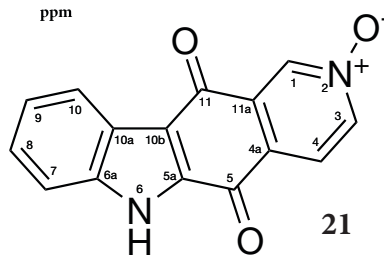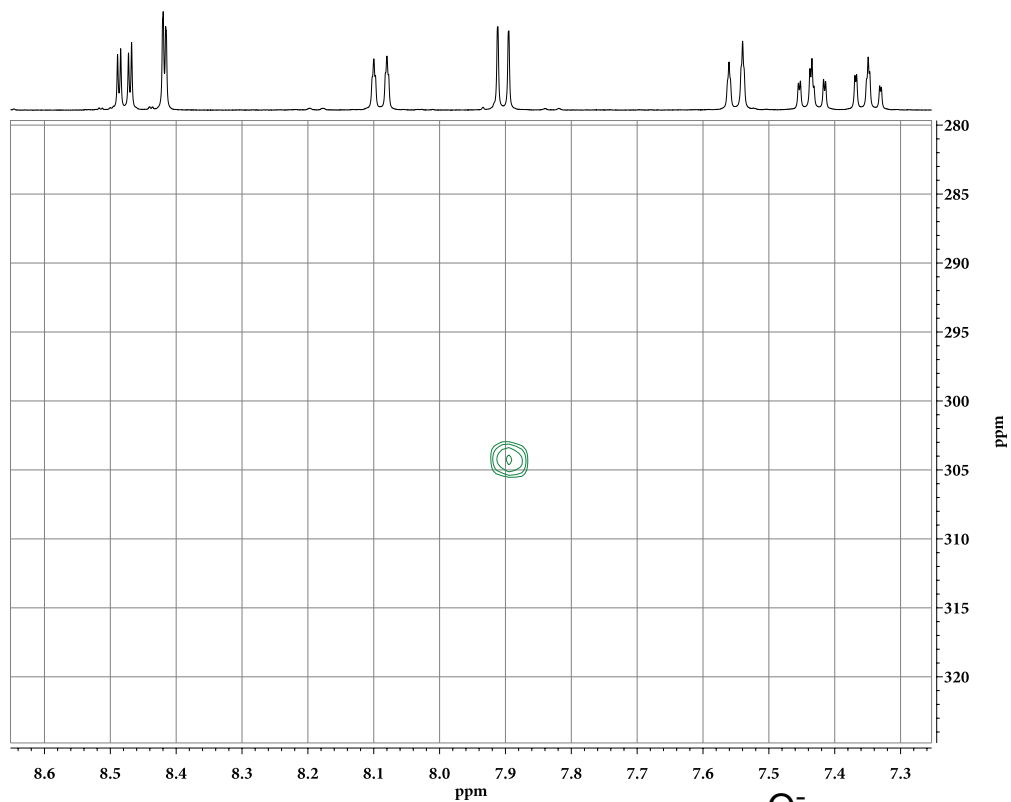
5H-Pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (15N HSQC)

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 5:16:08 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN157_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:10 in MeOH	Instrument	maXis HD
Comment			

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Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 1/12/2015 5:16:08 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN157_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:10 in MeOH
Comment	

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.7min #1-98

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
265.0607	1	C15H9N2O3	265.0608	0.3	12.0	1	100.00	12.5	even	ok
287.0427	1	C15H8N2NaO3	287.0427	0.0	12.2	1	100.00	12.5	even	ok
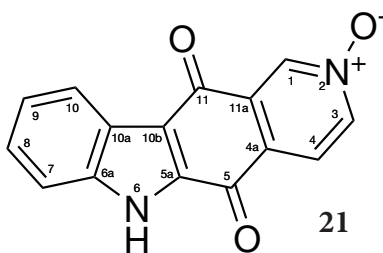
21

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (PROTON)

4.81
4.79
4.77

2.74
2.72
2.70

2.35

15,15'

12

13

1.98

1.99

5.99

5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2
ppm

22

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (C13 APT)

177.30
174.85

142.16
139.51
136.76
134.55
131.53
128.30
128.12
125.32
123.73
123.63
122.92
118.90
111.19

77.00 CDCl3

58.55

45.82
43.67

15,15'

10a
10
8
9
4a 4
7

3
1

5a

11 5
6a 11a 10b

13

12

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm
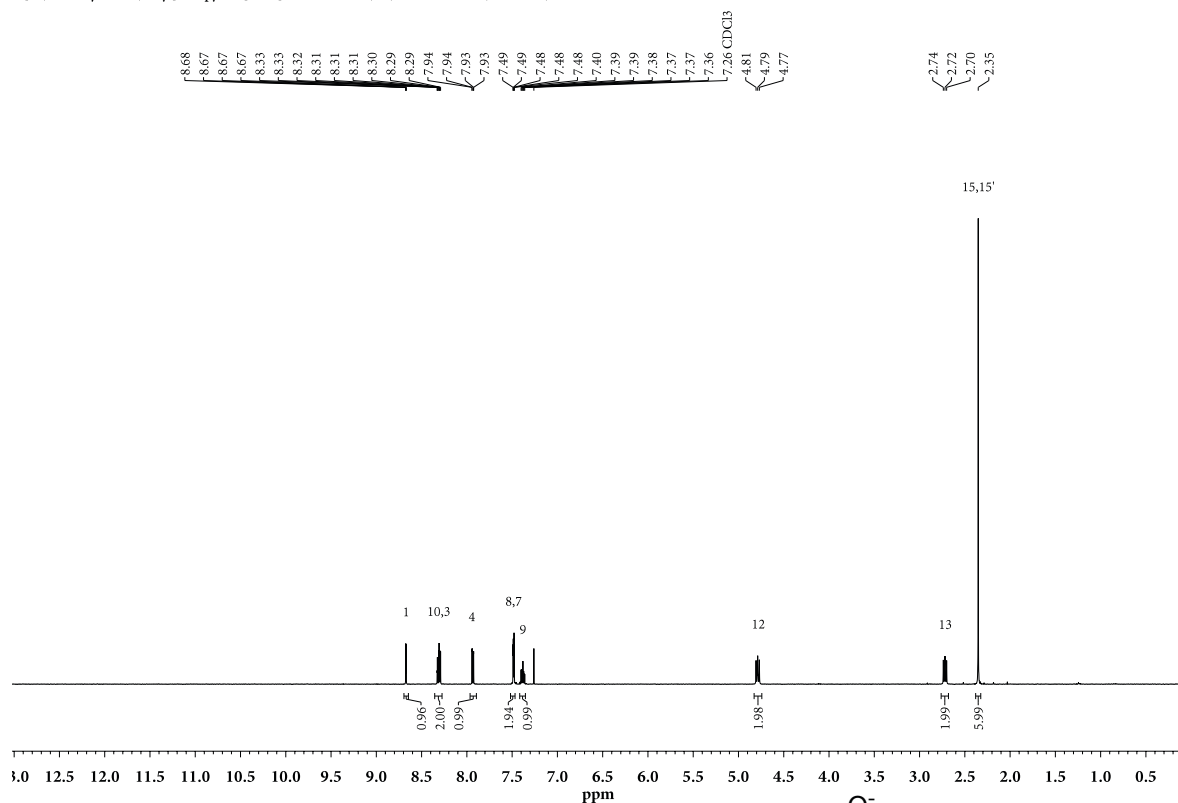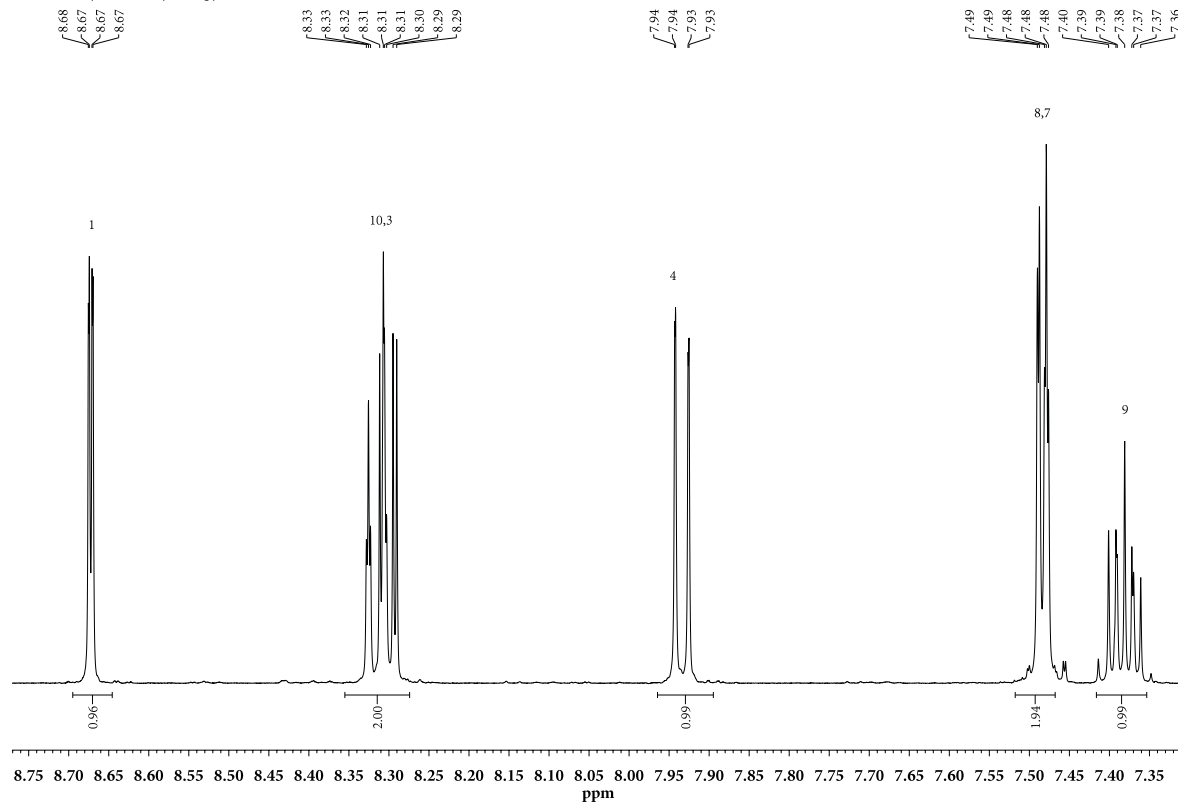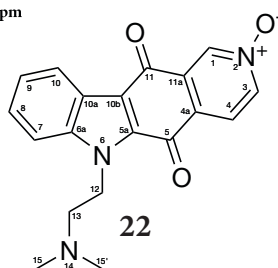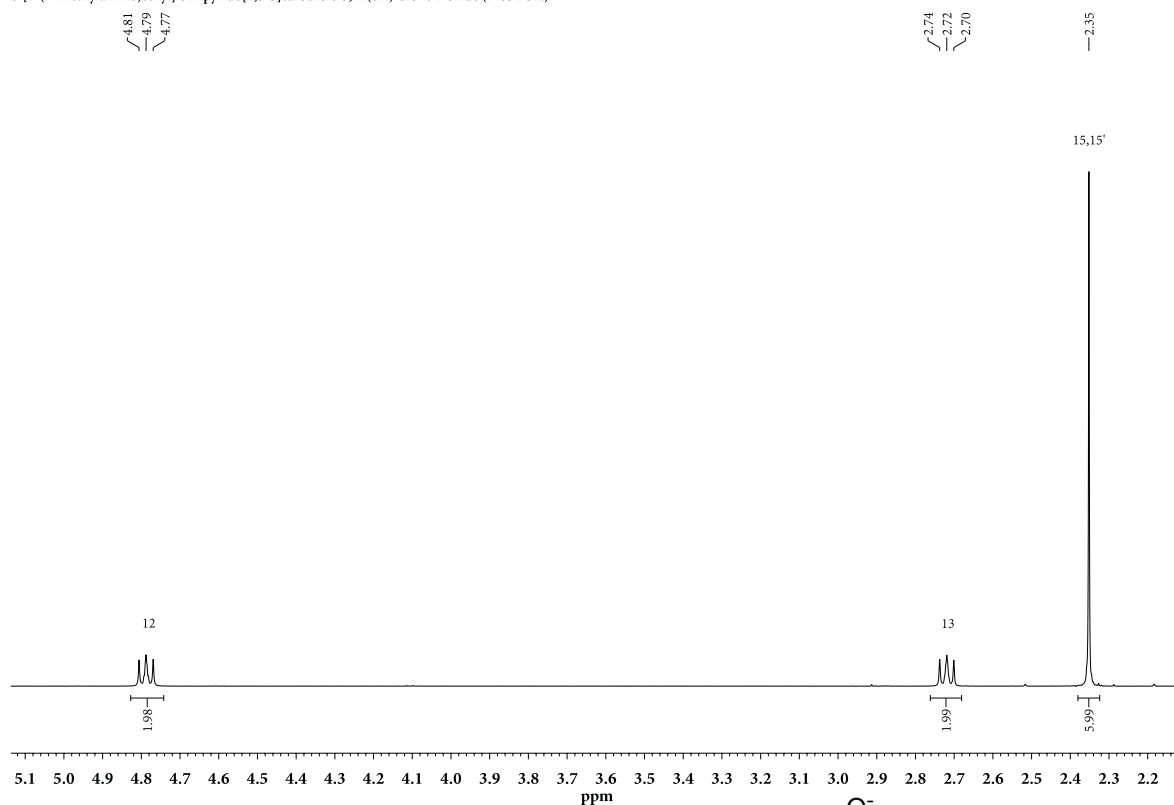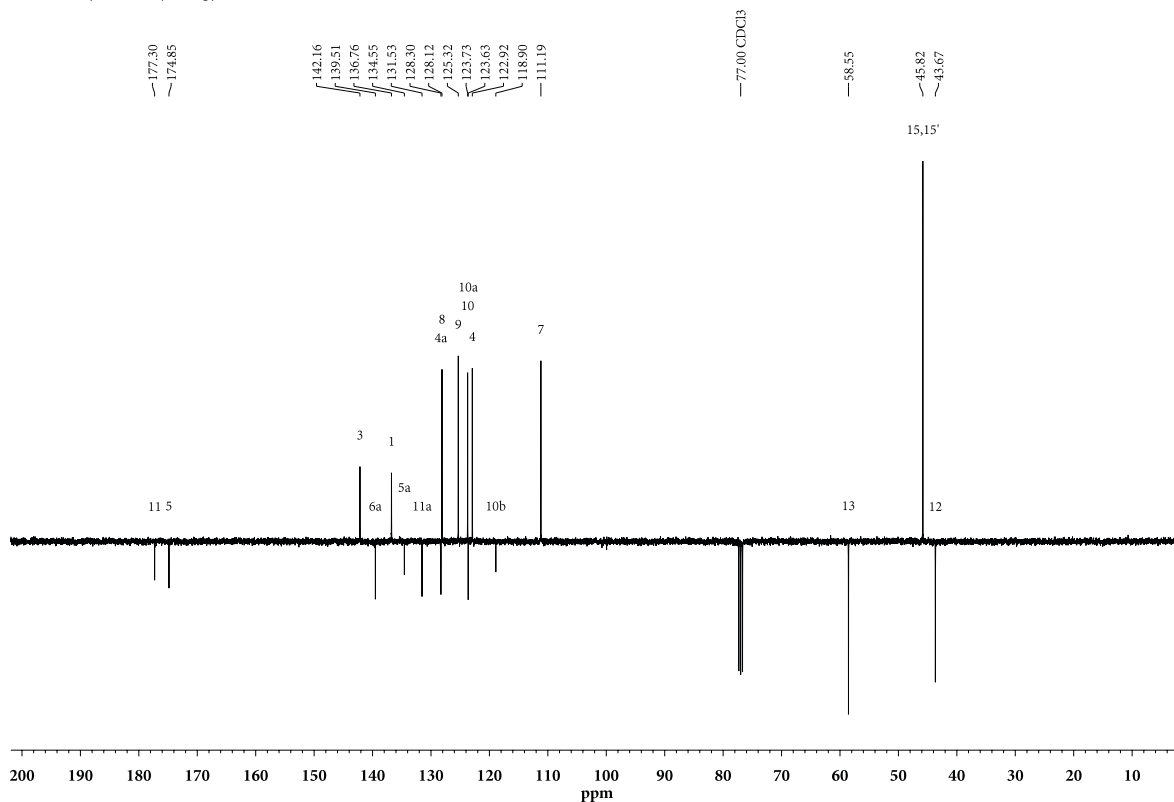
6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (C13 APT)
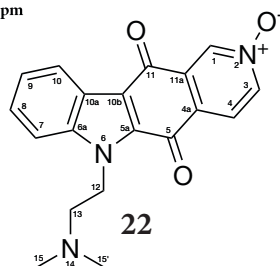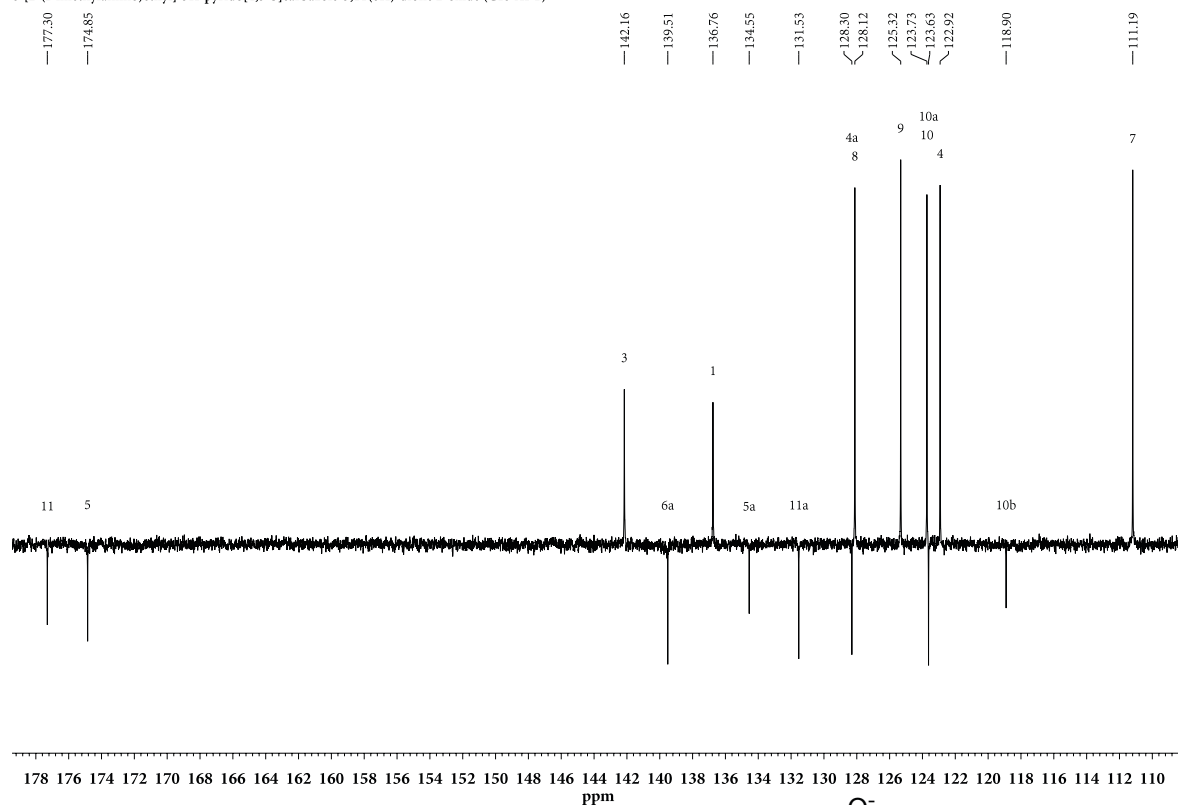
6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (HSQC)

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (HMBC)

22

6-[2-(Dimethylamino)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione 2-oxide (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 12:36:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN158_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.8min #1-101

Intens. x10⁵: 336.1345, 337.1376, 349.1353, 352.3684, 356.2779, 358.1166, 359.1195, 360.1222

C₁₉H₁₈N₃O₃, 336.1343

x10⁵: 1+ 336.1343, 1+ 337.1373, 1+ 338.1399

C₁₉H₁₇N₃NaO₃, 358.1162

x10⁵: 1+ 358.1162, 1+ 359.1193, 1+ 360.1219

22

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 12:36:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN158_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD	1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
336.1345	1	C19H18N3O3	336.1343	-0.8	6.8	1	100.00	12.5	even	ok
358.1166	1	C19H17N3NaO3	358.1162	-1.2	8.9	1	100.00	12.5	even	ok

22

N,N-Diethyl-2-pyrazinecarboxamide (PROTON)

23

N,N-Diethyl-2-pyrazinecarboxamide (PROTON)

N,N-Diethyl-2-pyrazinecarboxamide (C13 CPD)

N,N-Diethyl-2-pyrazinecarboxamide (HSQC)

N,N-Diethyl-2-pyrazinecarboxamide (HMBC)

23

N,N-Diethyl-2-pyrazinecarboxamide (15N HMBC)

N,N-Diethyl-2-pyrazinecarboxamide (NOESY)

23

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

24

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

9.01
9.01
8.98
8.98

2

3

10

1.01

0.99

1.04

9.08 9.06 9.04 9.02 9.00 8.98 8.96 8.94 8.92 8.90 8.88 8.86 8.84 8.82 8.80 8.78 8.76 8.74 8.72 8.70 8.68 8.66 8.64 8.62 8.60 8.58 8.56 8.54 8.52 8.50 8.48
ppm

24

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

7.69
7.69
7.69
7.68
7.67
7.67

7.58
7.57
7.56
7.56
7.56
7.54
7.54

7.49
7.49
7.48
7.48
7.48
7.46
7.46

7

8

9

1.06

1.06

1.06

7.73 7.72 7.71 7.70 7.69 7.68 7.67 7.66 7.65 7.64 7.63 7.62 7.61 7.60 7.59 7.58 7.57 7.56 7.55 7.54 7.53 7.52 7.51 7.50 7.49 7.48 7.47 7.46 7.45 7.44 7.4
ppm

317

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

6-(Methoxymethyl)-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

24

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 10:37:07 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN056_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

24

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 10:37:07 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN056_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
316.0691	1	C16H11N3NaO3	316.0693	0.5	7.7	1	100.00	12.5	even	ok

24

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

13.28

9.04 9.03 9.01 9.01 8.25 8.24 8.24 8.23 8.22 8.22 7.63 7.61 7.51 7.50 7.49 7.48 7.47 7.42 7.42 7.40 7.40 7.38

3.31 H2O

2.49 DMSO

3,2
3,2

10

7 8 9

6

1.00

1.04 1.01

1.00

1.05 1.05 1.06

14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

25

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

13.28

9.04 9.03 9.01 9.01

8.25 8.24 8.24 8.23 8.22 8.22

7.63 7.61 7.51 7.50 7.49 7.49 7.48 7.47 7.47 7.42 7.42 7.40 7.40 7.38

3,2
3,2

10

7

8 9

6

1.00

1.04 1.01

1.00

1.05

1.05 1.06

13.6 13.4 13.2 13.0 12.8 9.7 9.6 9.5 9.4 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9
ppm

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 CPD)

177.84
175.27

147.64
147.05
146.38
145.43
138.40
137.64

127.48
124.36
123.80
122.52
117.93
113.98

39.50 DMSO

4a,11a
4a,11a
3,2   10a
3,2   5a   9
11 5   6a   8 10 10b 7

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 CPD)

177.84
175.27

147.64
147.05
146.38
145.43

138.40
137.64

127.48

124.36
123.80
122.52

117.93

113.98

3,2
3,2

10

9

8

4a,11a
4a,11a

10a

6a

5a

10b

7

11 5

180 175 170 165 160 155 150 145 140 135 130 125 120 115 110
ppm

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

25

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)
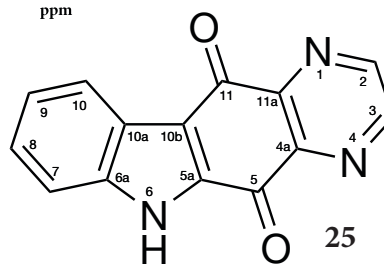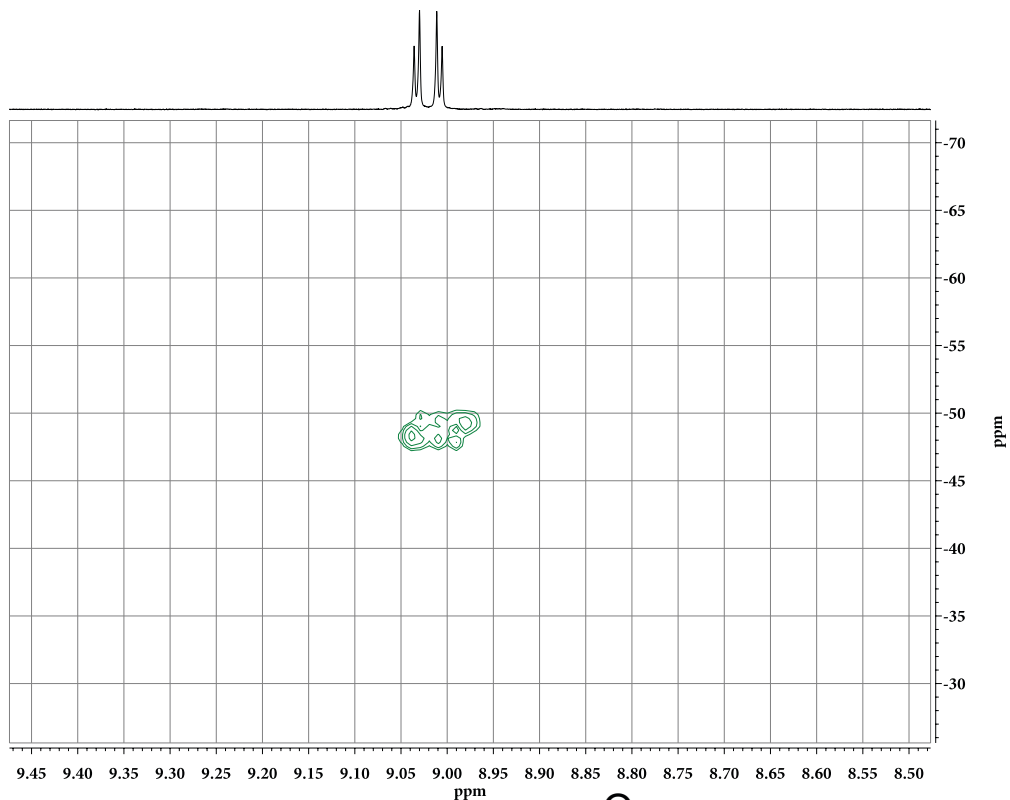
25

5H-Pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HSQC)
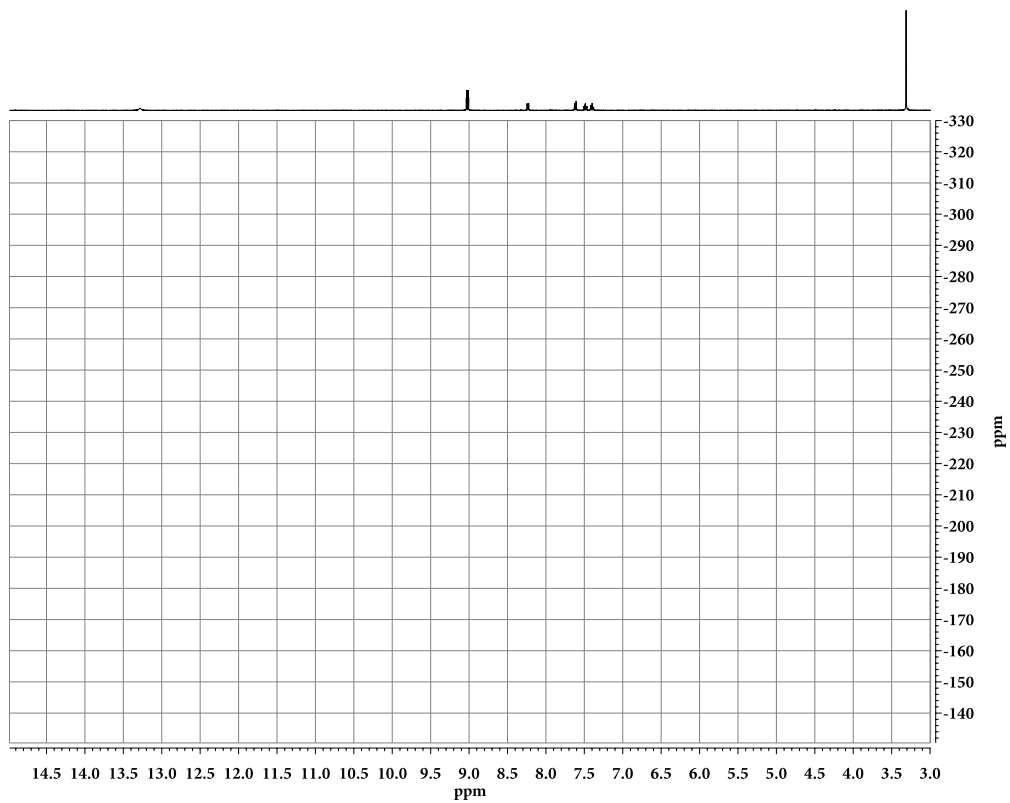
Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN072_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 10:50:32 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

$C_{14}H_7N_3NaO_2$, 272.0430

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 10:50:32 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN072_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
272.0431	1	C14H7N3NaO2	272.0430	-0.3	9.4	1	100.00	12.5	even	ok
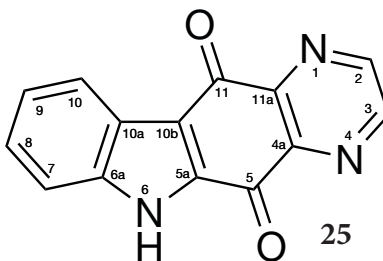
25

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

26

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

26

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 CPD)

26
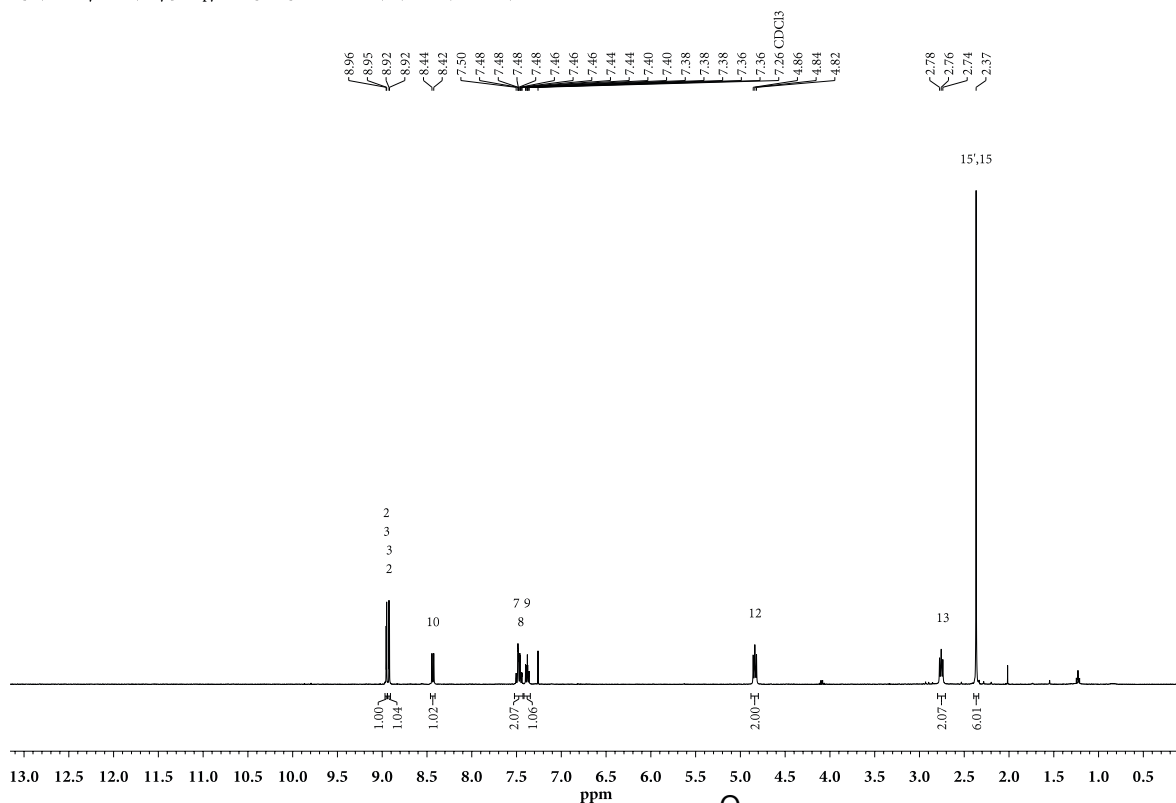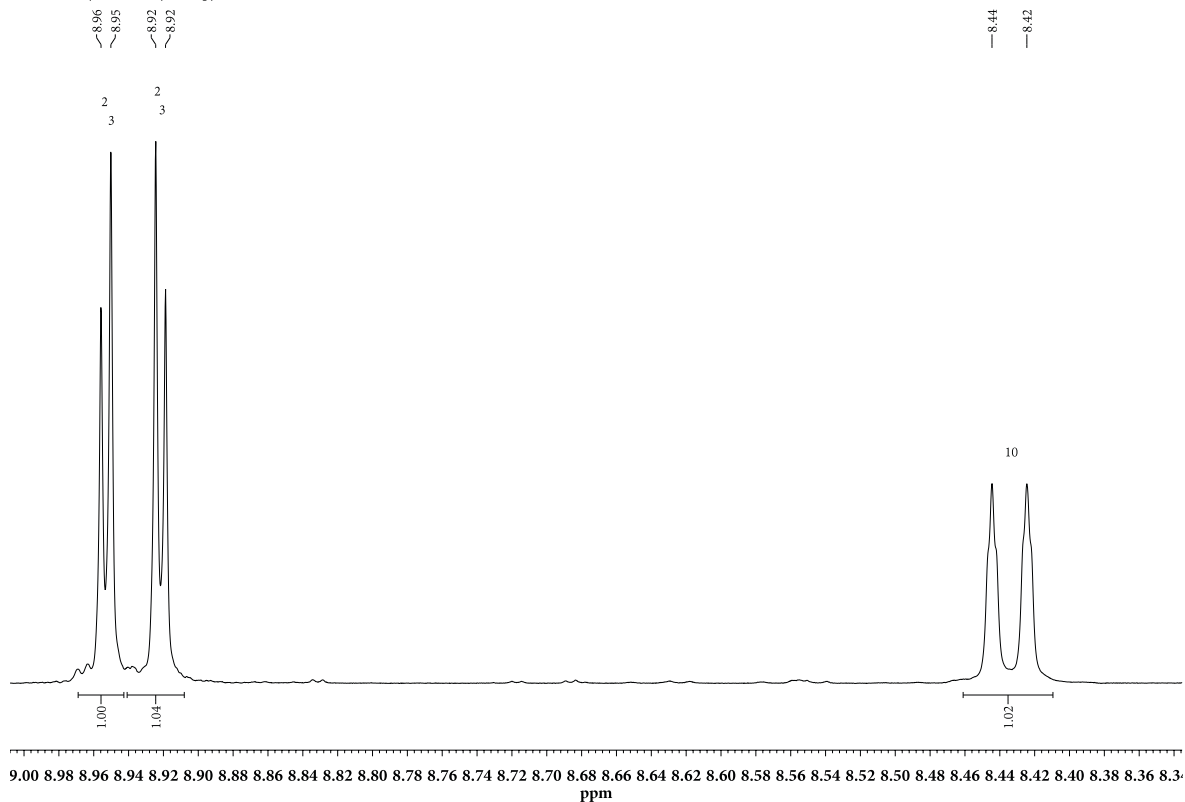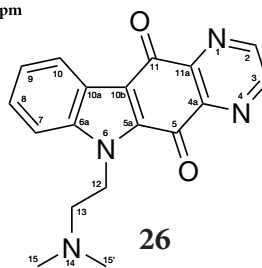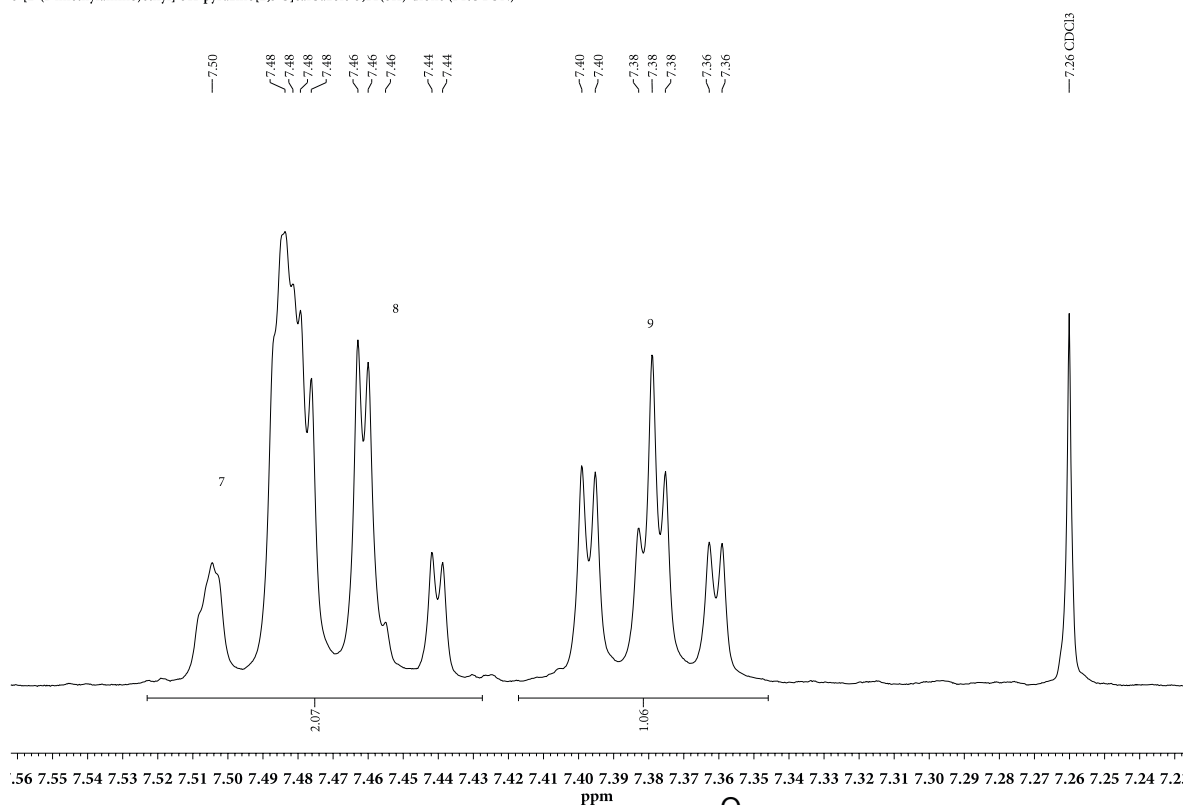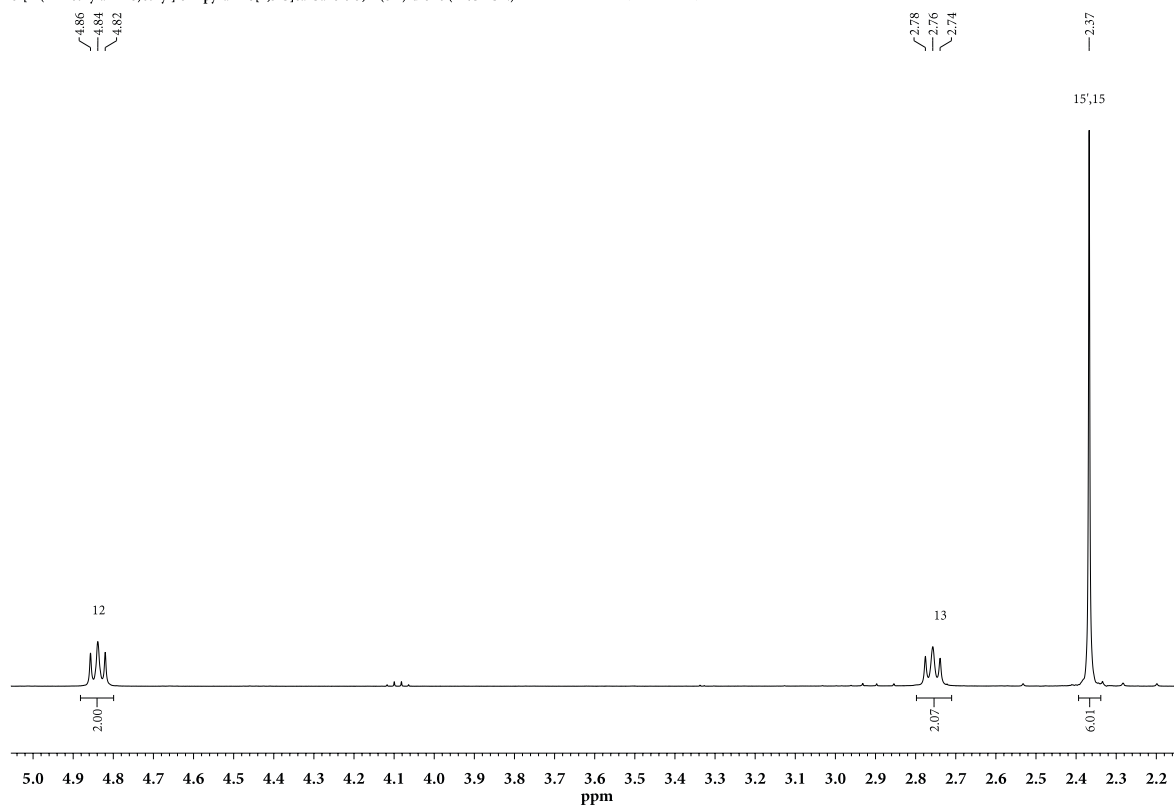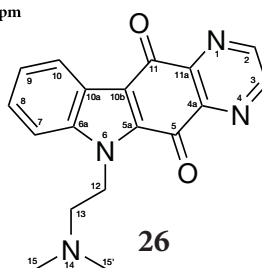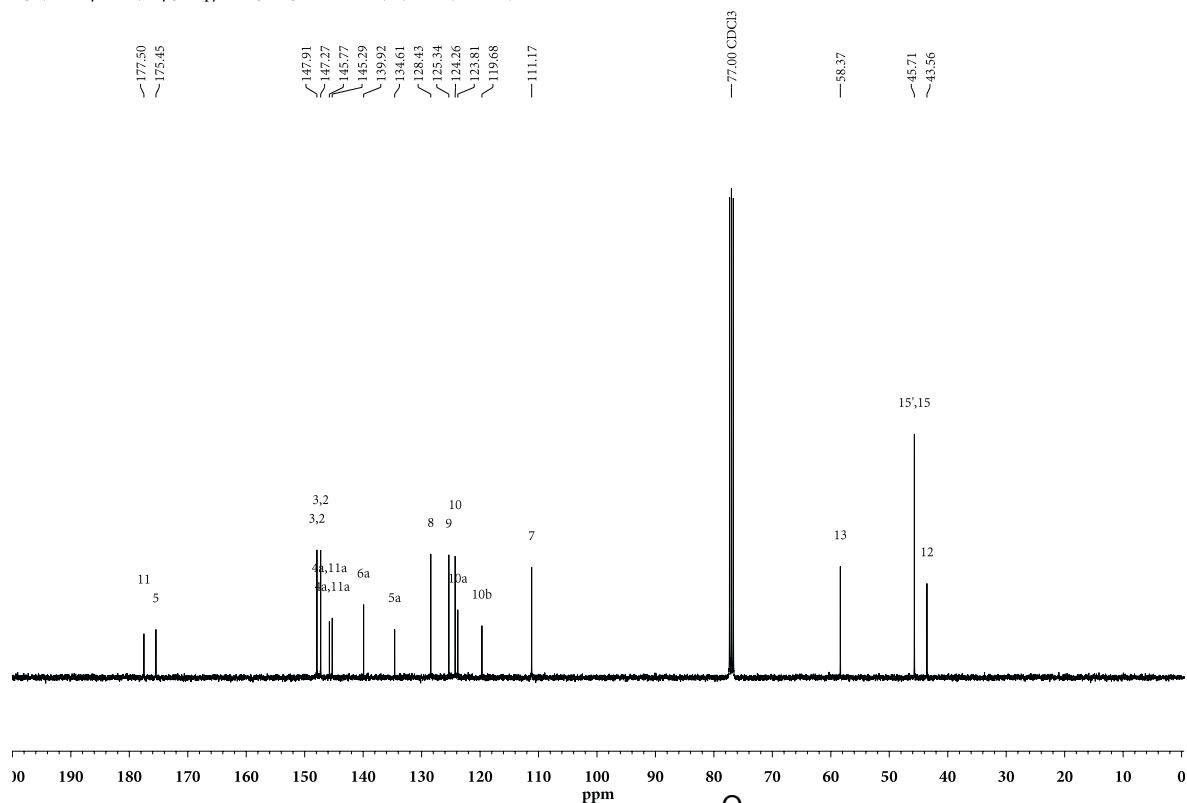
6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 CPD)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

26

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (COSY)

6-[2-(Dimethylamino)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (NOESY)

26

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 10:42:25 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN091_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/4/2014 10:42:25 AM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN091_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
321.1347	1	C18H17N4O2	321.1346	-0.2	6.4	1	100.00	12.5	even	ok
343.1168	1	C18H16N4NaO2	343.1165	-0.8	6.8	1	100.00	12.5	even	ok
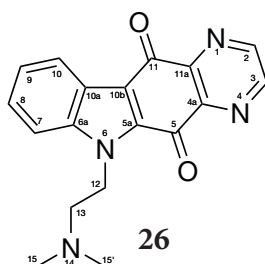
26

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (PROTON)

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (PROTON)

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (PROTON)

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (PROTON)

337
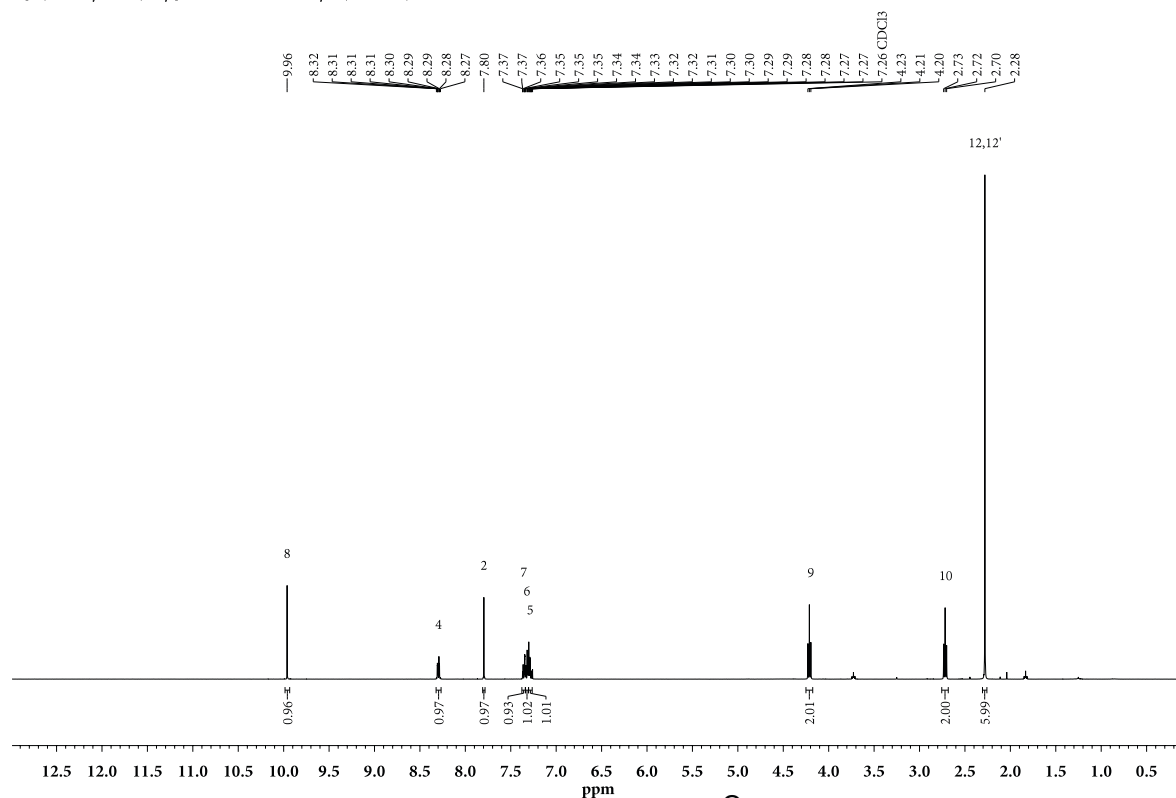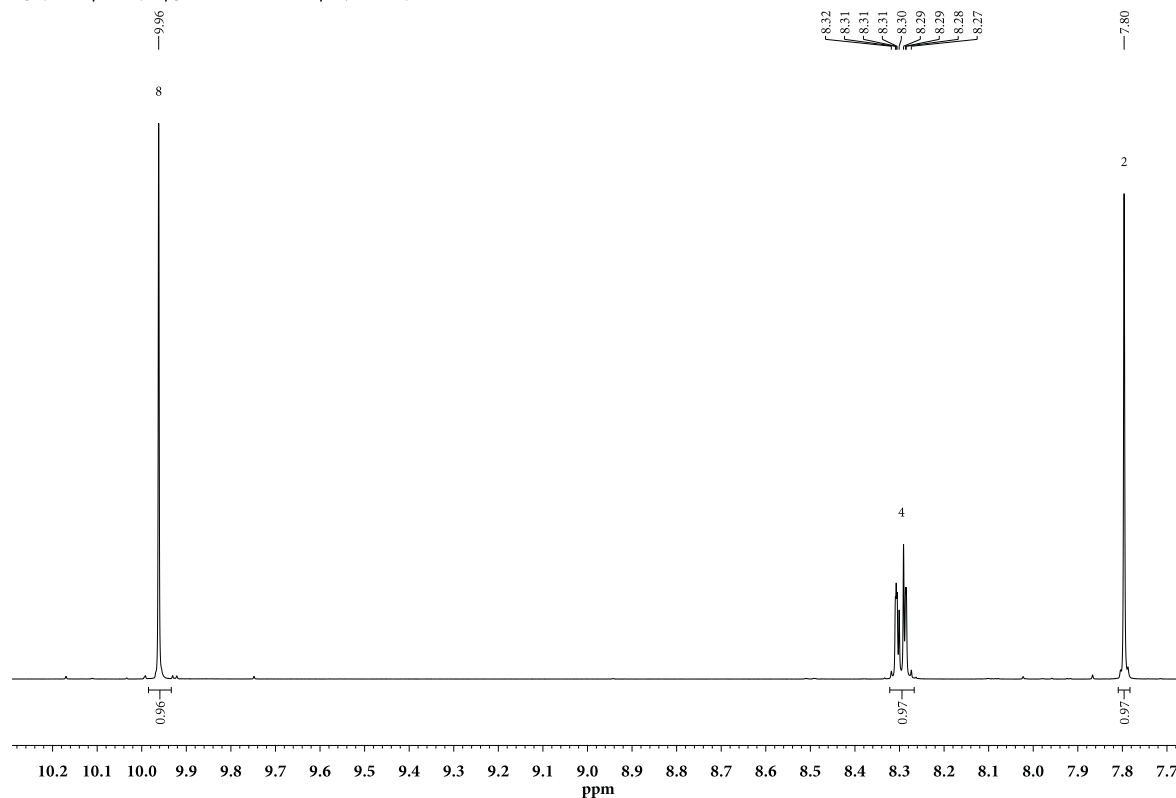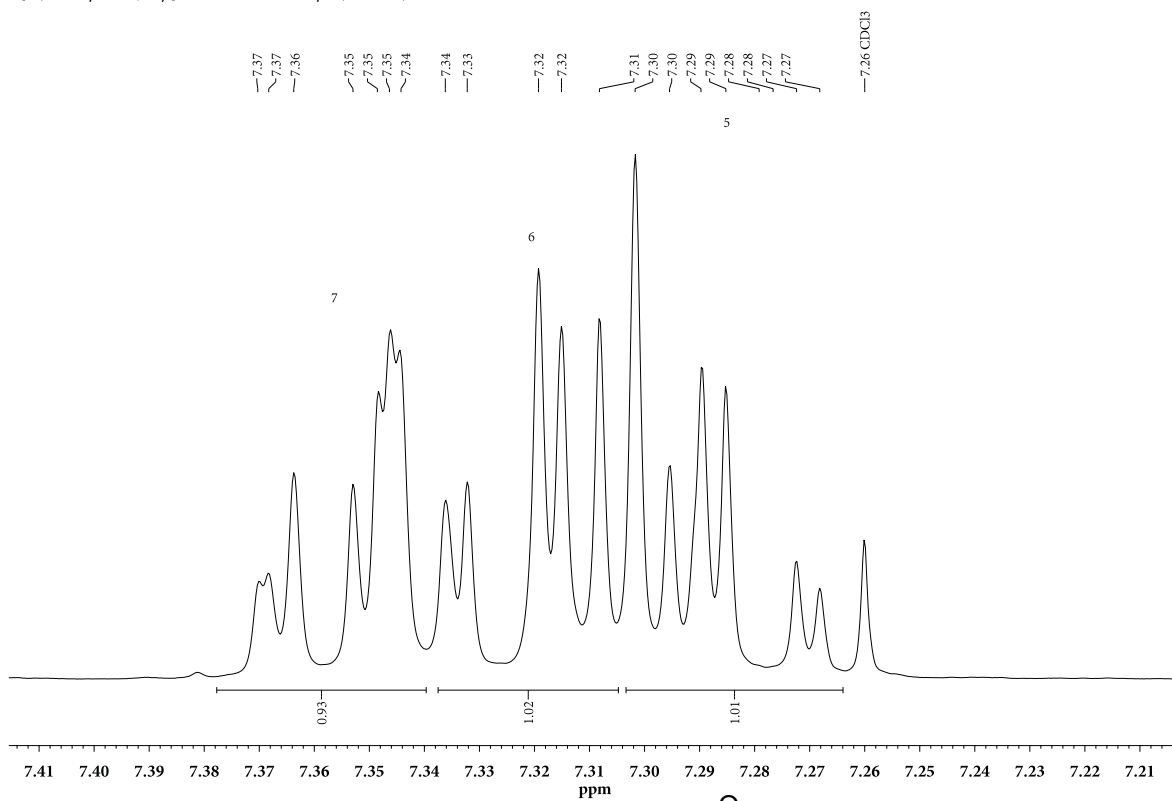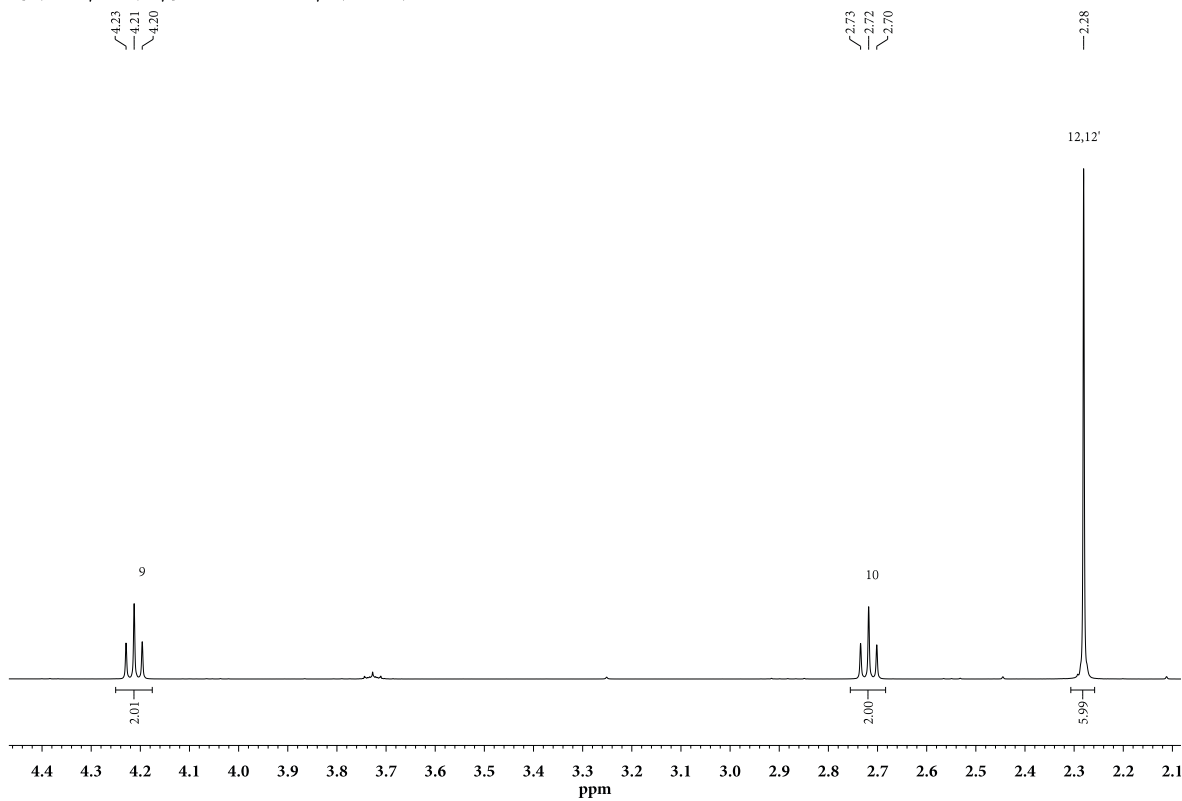
1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (C13 CPD)
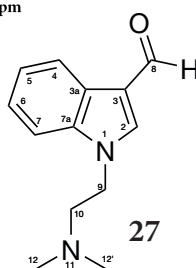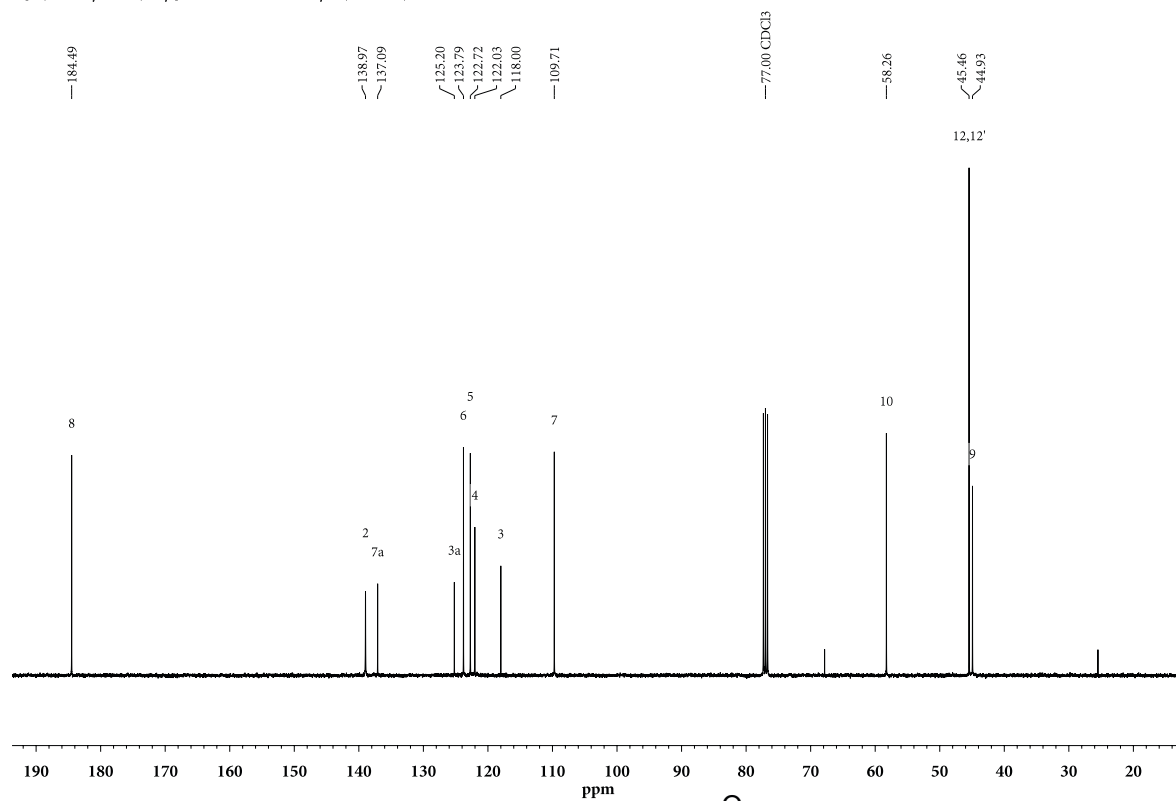
1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (HSQC)
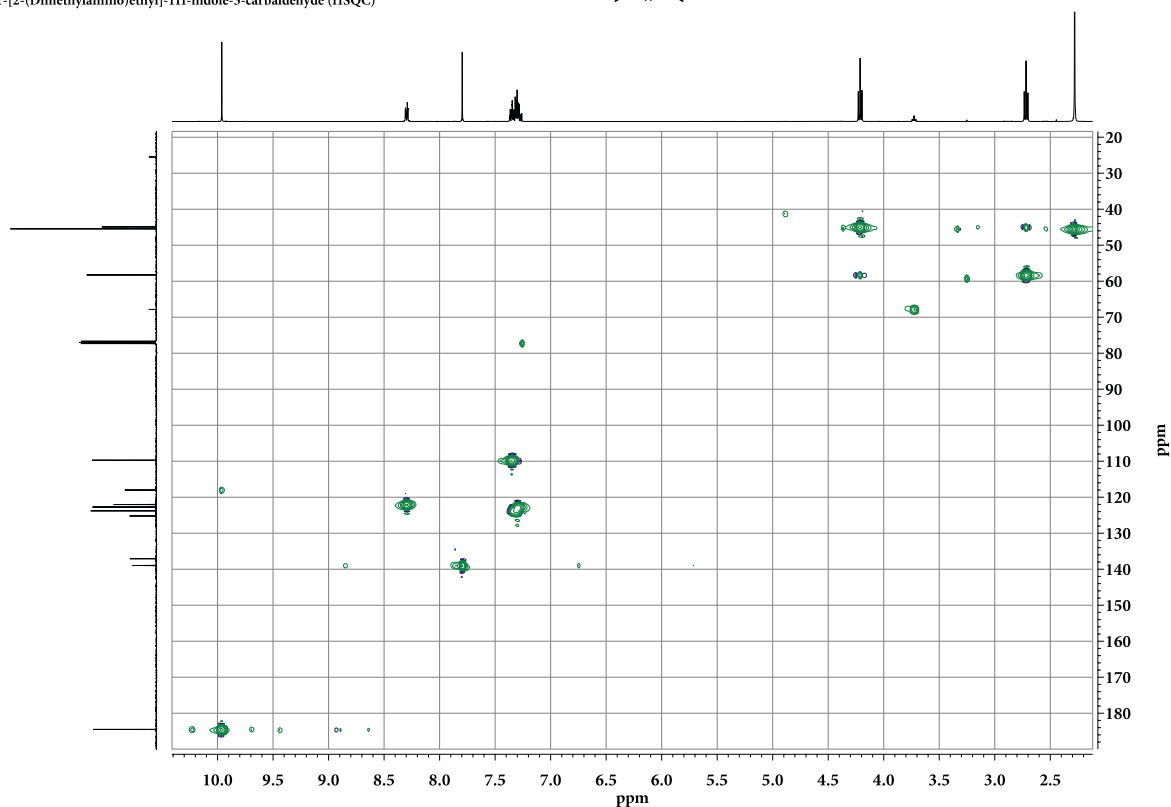
1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (HMBC)

1-[2-(Dimethylamino)ethyl]-1H-indole-3-carbaldehyde (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 2:19:59 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN097_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:100 in MeOH	Instrument	maXis HD
Comment			

Bruker Compass DataAnalysis 4.2   printed: 1/12/2015 2:24:30 PM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   1/12/2015 2:19:59 PM

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN097_DI_ESI_HRMS.d
Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   in MeOH --> 1:100 in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
217.1339	1	C13H17N2O	217.1335	-1.8	12.1	1	100.00	6.5	even	ok
239.1160	1	C13H16N2NaO	239.1155	-2.0	10.9	1	100.00	6.5	even	ok
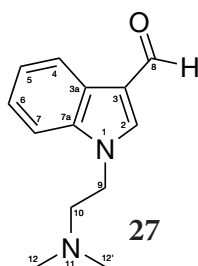
27

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (PROTON)

28

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (PROTON)

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (PROTON)

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (C13 APT)

28
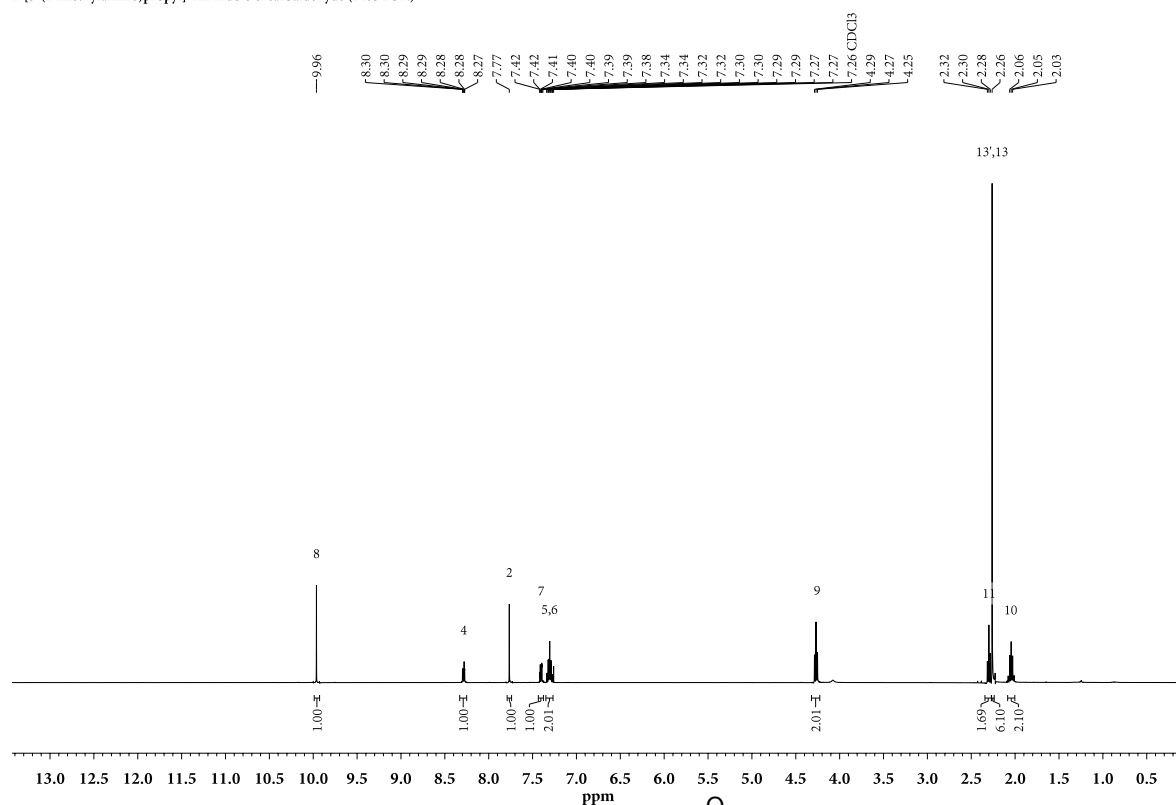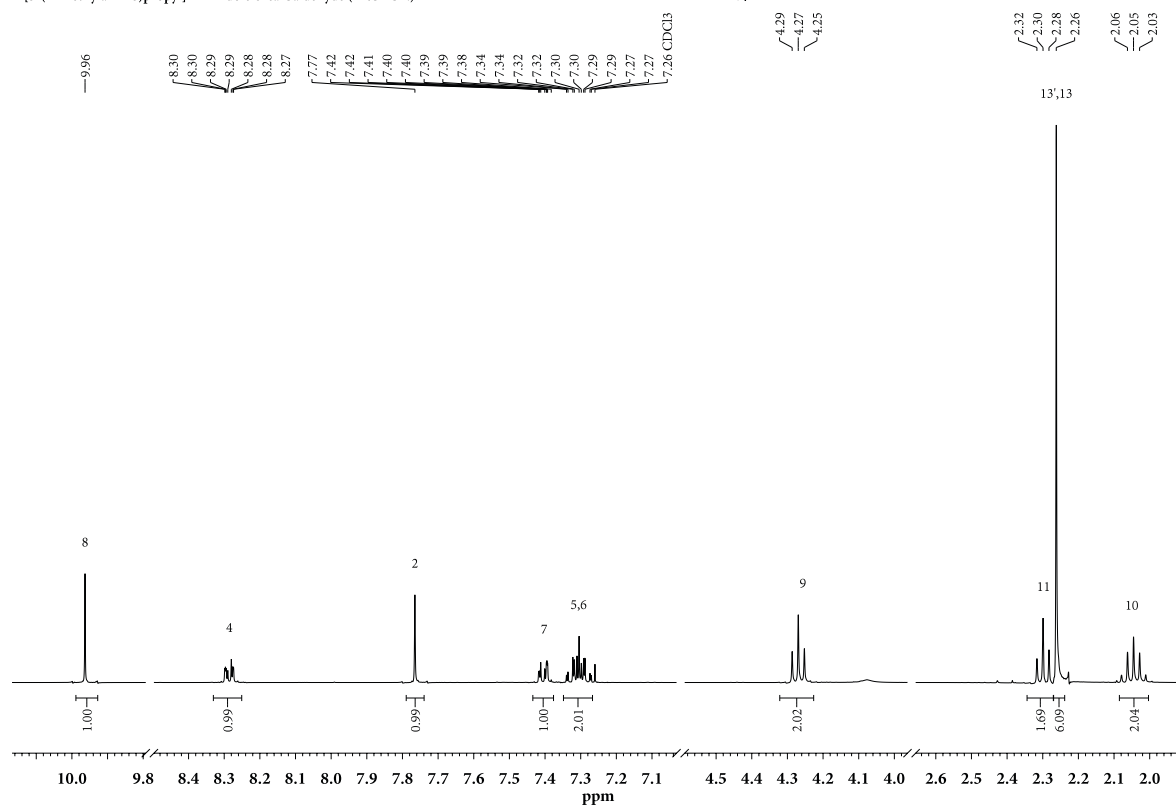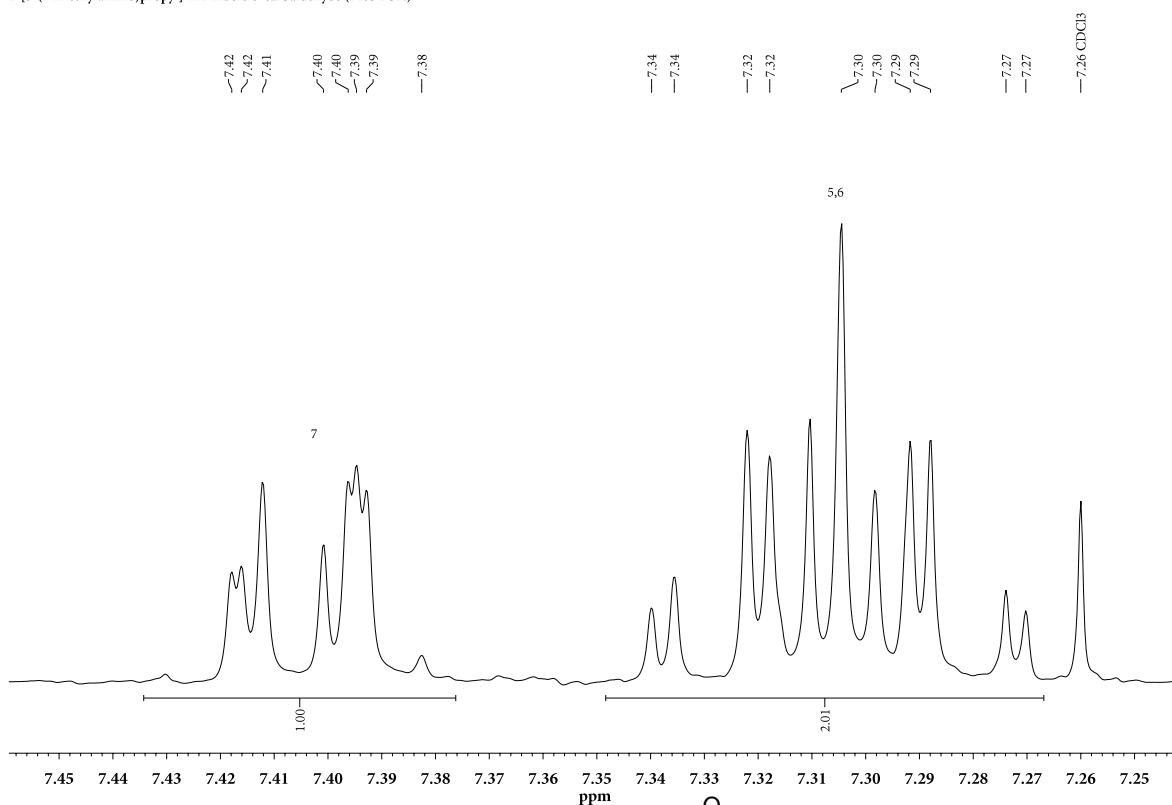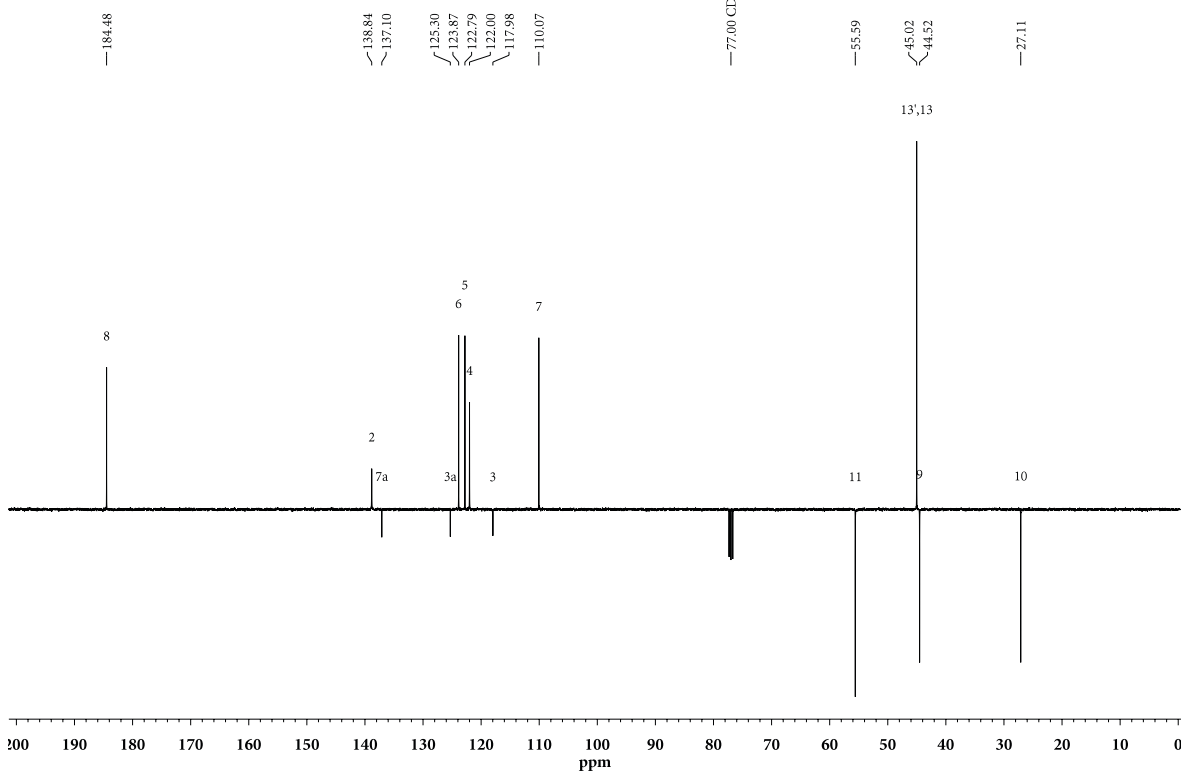
1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (C13 APT)

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (HSQC)

28

344
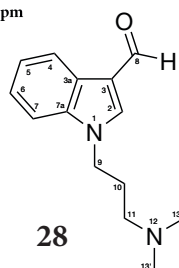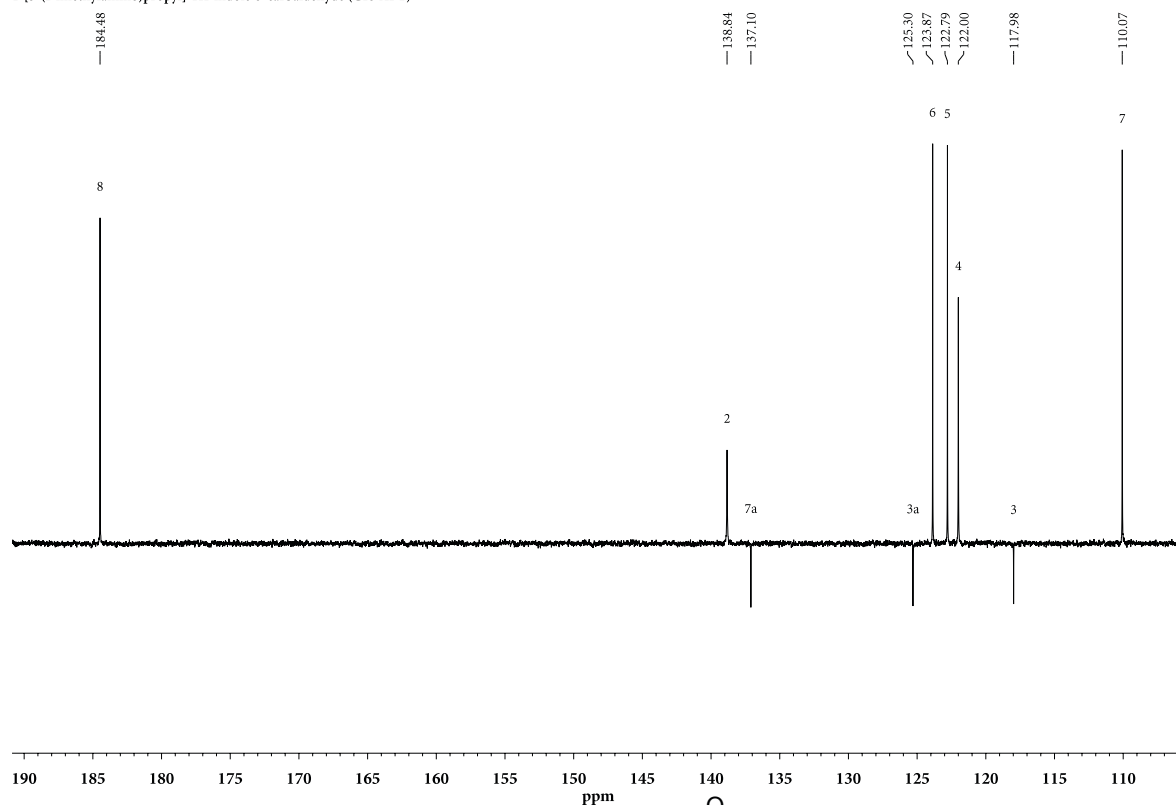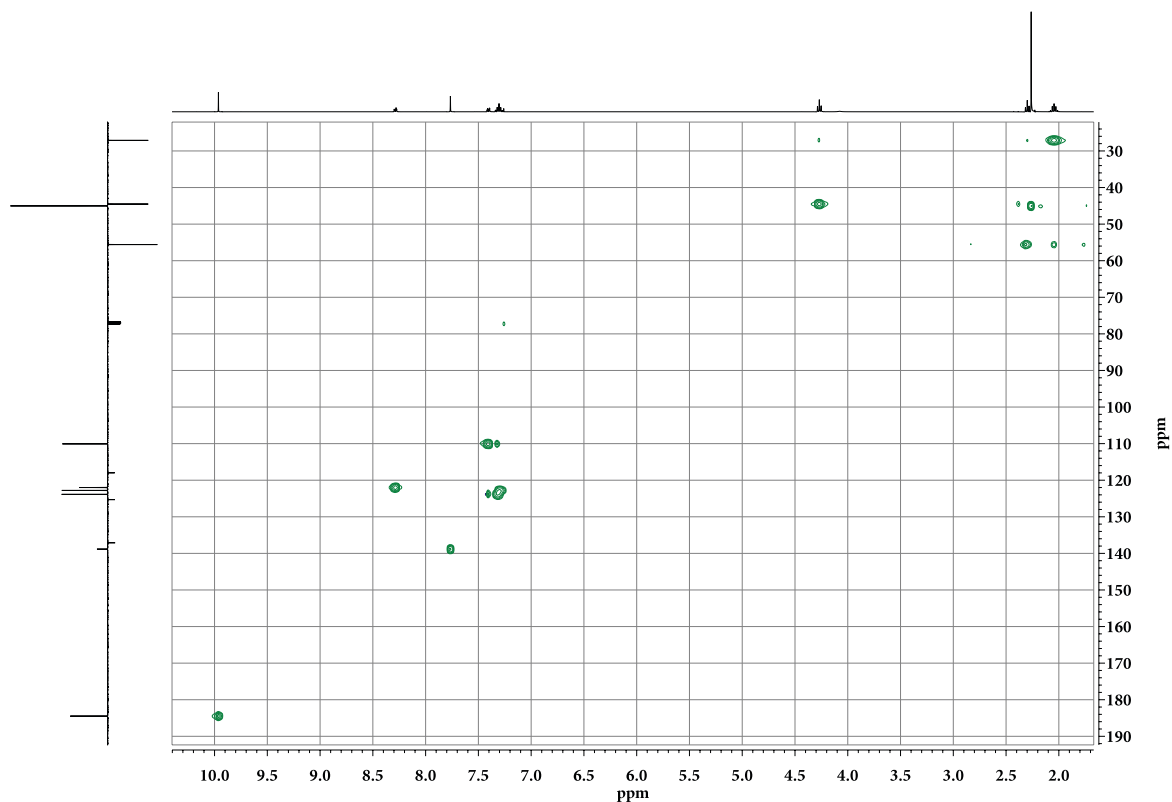
1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (HMBC)

1-[3-(Dimethylamino)propyl]-1H-indole-3-carbaldehyde (15N HMBC)
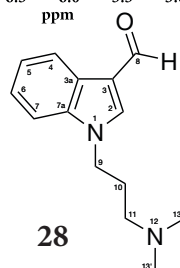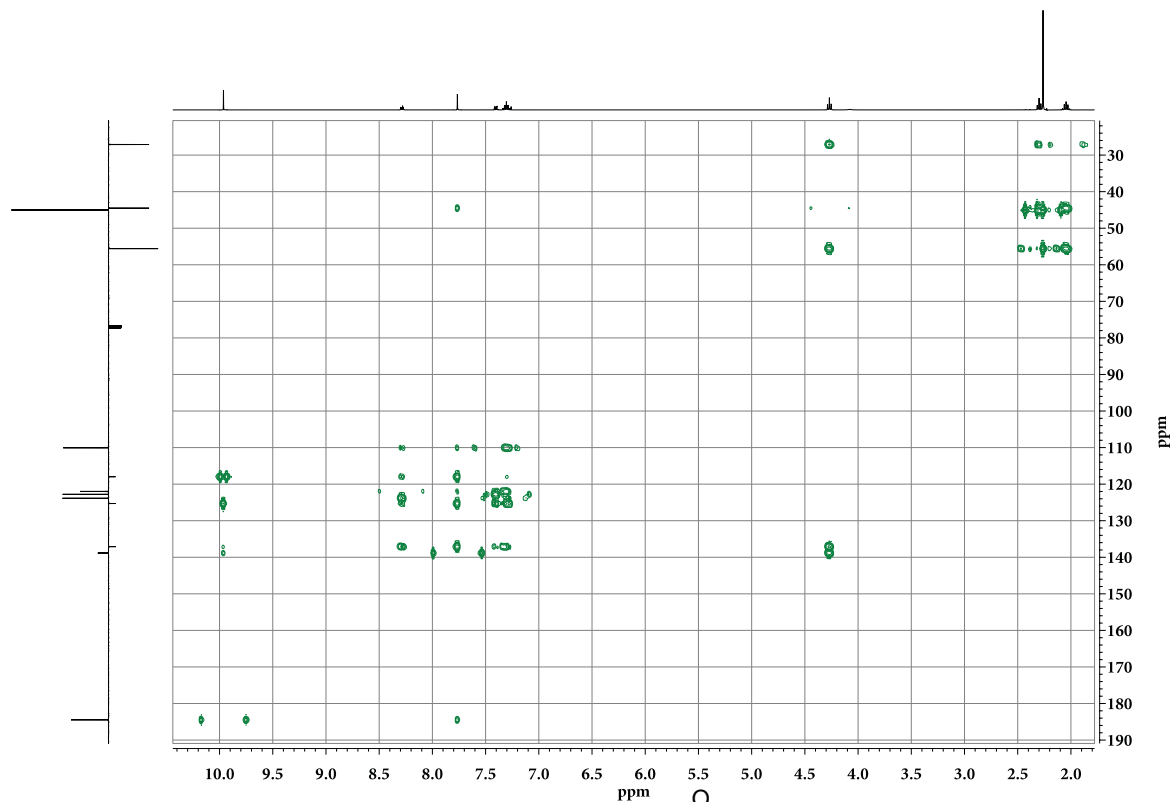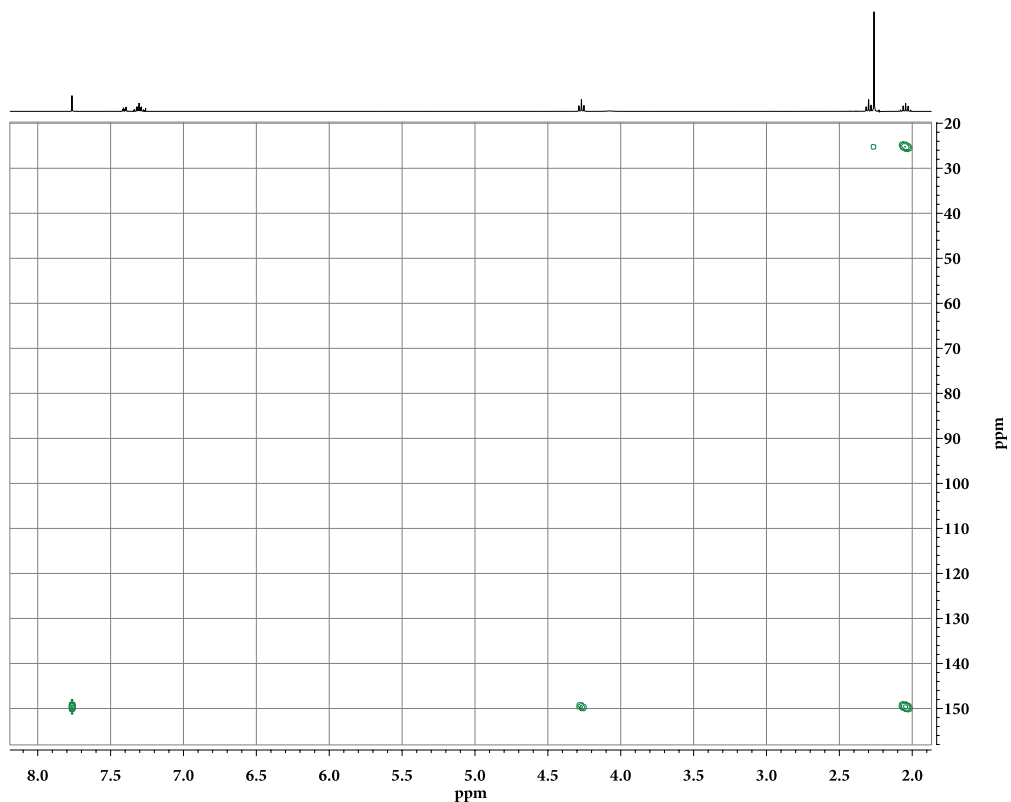
28

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

29

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

29

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

347

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

29
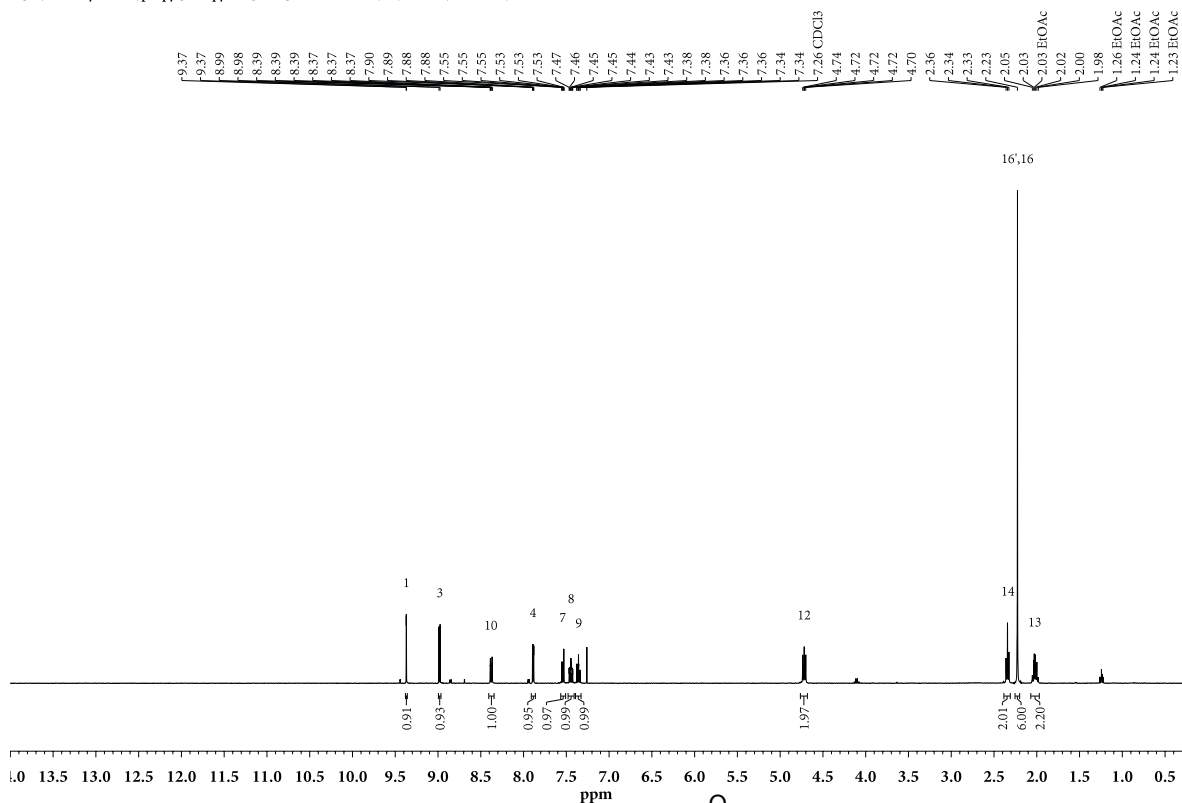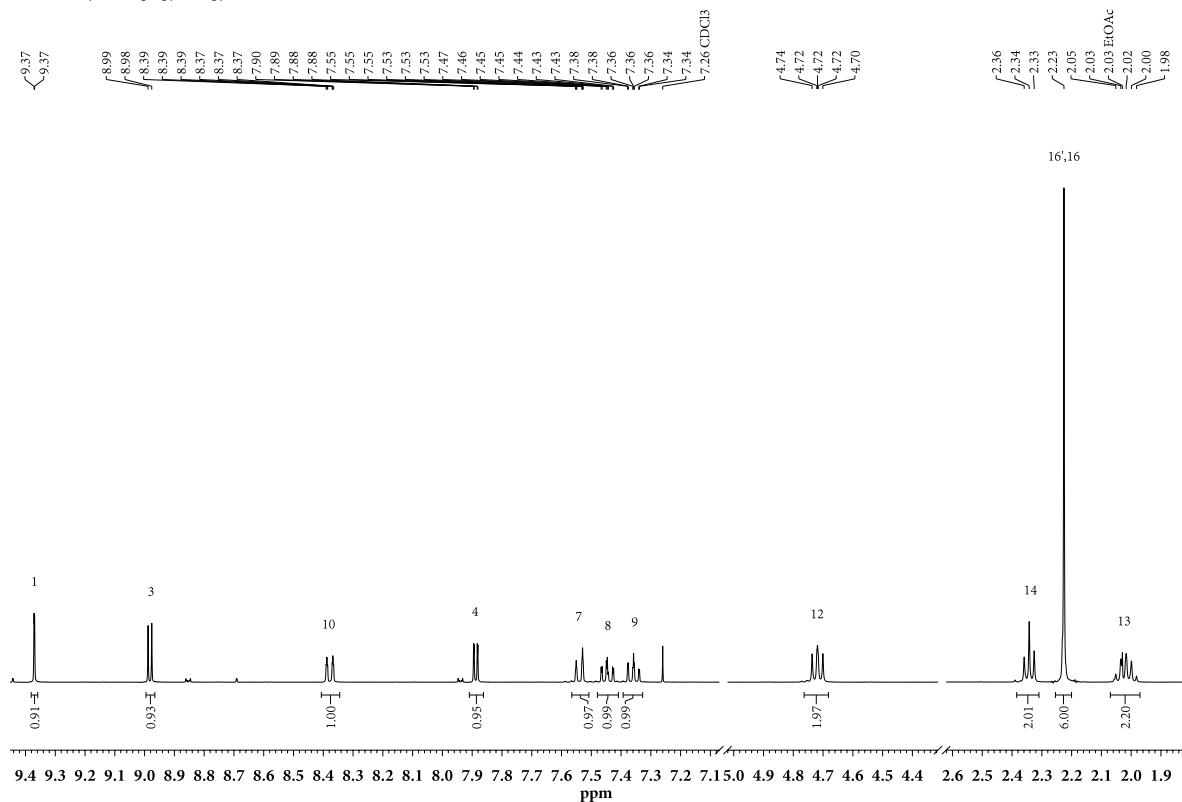
6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

29

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

29

6-[3-(Dimethylamino)propyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (NOESY)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN118_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 12:18:40 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

29

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/4/2014 12:18:40 PM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN118_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.6min #1-88

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
334.1549	1	C20H20N3O2	334.1550	0.3	21.6	1	100.00	12.5	even	ok

29

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

30

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

30

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

30
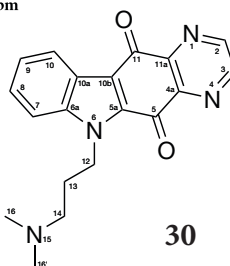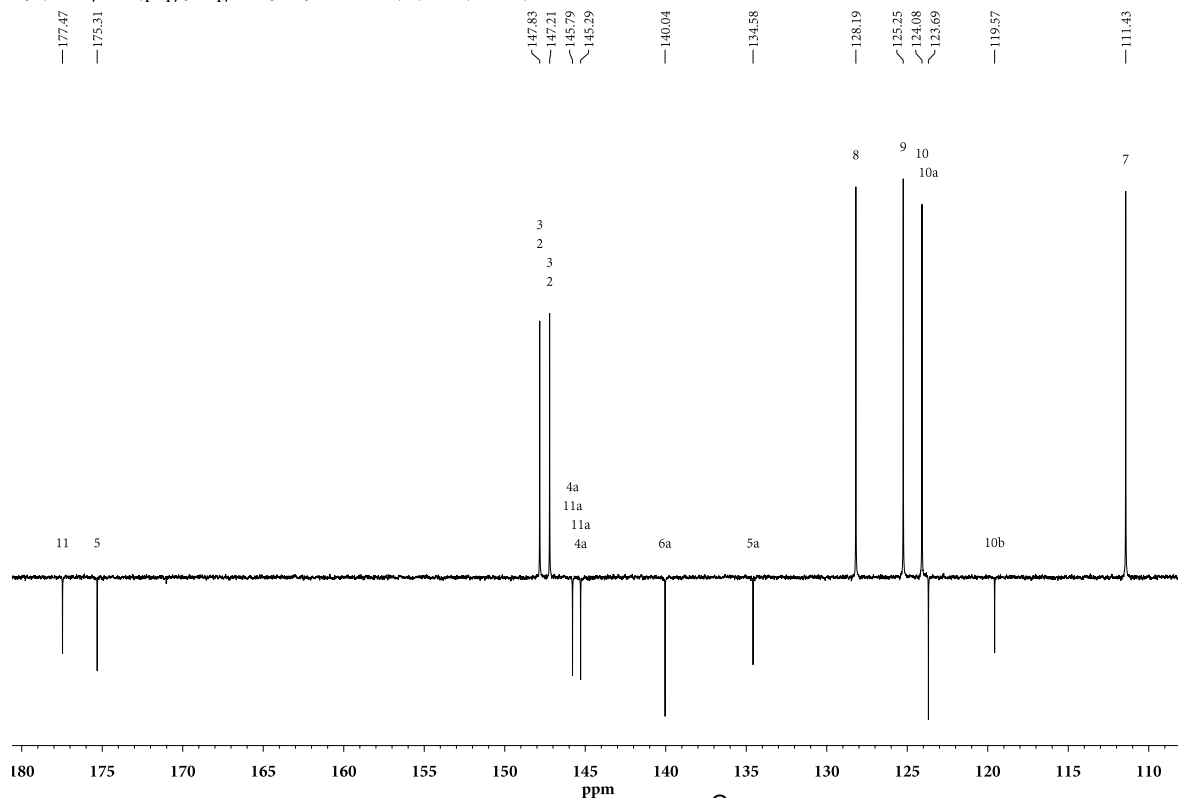
6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

30

6-[3-(Dimethylamino)propyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN116_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 12:08:26 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 12:08:26 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN116_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
335.1506	1	C19H19N4O2	335.1503	-1.1	9.5	1	100.00	12.5	even	ok
357.1326	1	C19H18N4NaO2	357.1322	-1.3	12.5	1	100.00	12.5	even	ok

30

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

31
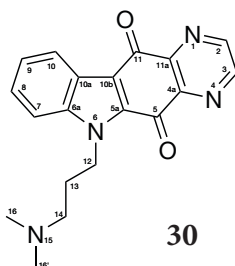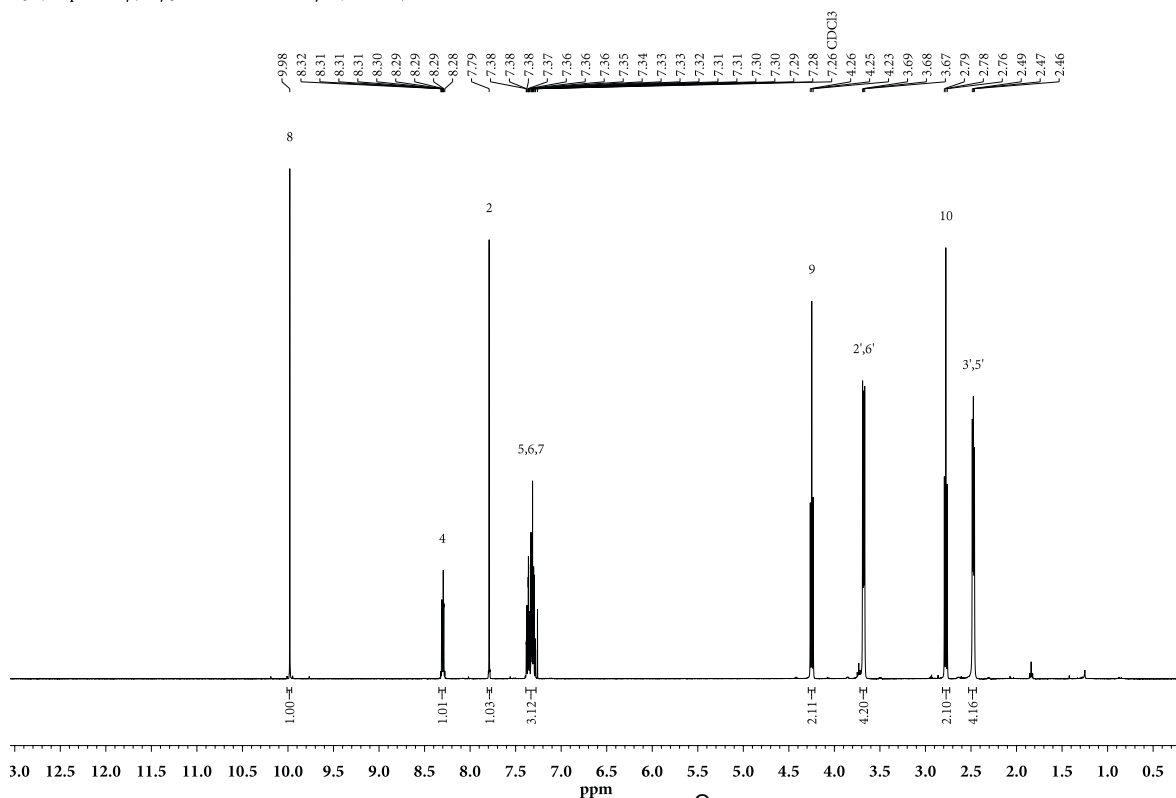
1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (C13 CPD)
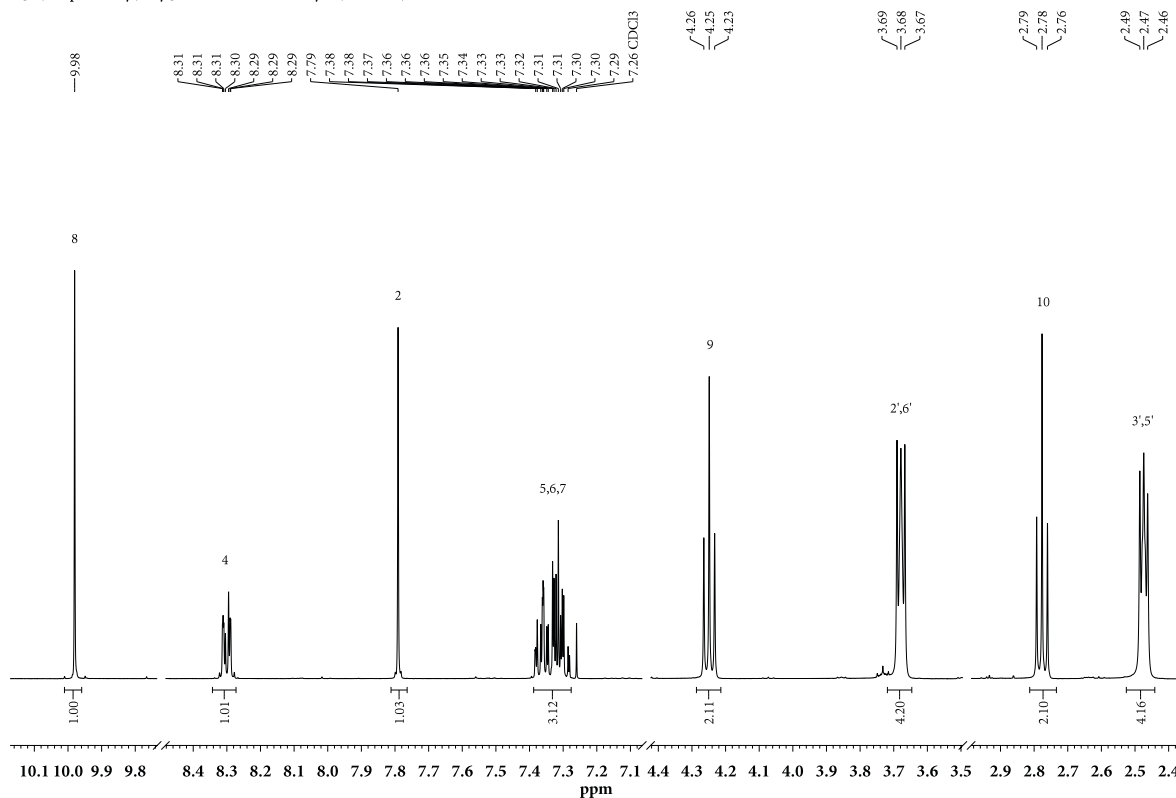
31

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (HSQC)

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (HMBC)

31

1-[2-(Morpholin-4-yl)ethyl]-1H-indole-3-carbaldehyde (15N HMBC)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

32

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

7.49
7.49
7.48
7.48
7.48
7.47

7.42
7.41
7.41
7.40
7.39
7.39
7.38

8,7

9

2.01

0.91

7.61 7.60 7.59 7.58 7.57 7.56 7.55 7.54 7.53 7.52 7.51 7.50 7.49 7.48 7.47 7.46 7.45 7.44 7.43 7.42 7.41 7.40 7.39 7.38 7.37 7.36 7.35 7.34 7.33
ppm

32

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

4.84
4.82
4.80

3.64
3.62
3.61

2.78
2.76
2.74

2.56
2.56
2.55
2.54

12

2',6'

3',5'

13

1.97

4.04

1.99

3.98

5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4
ppm

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

180.46
177.66
154.88
148.33
139.75
138.95
134.45
128.05
126.25
125.04
124.11
123.74
119.11
118.44
111.05
77.00 CDCl3
66.93
57.84
53.92
42.85

32

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

180.46
177.66
154.88
148.33
139.75
138.95
134.45
128.05
126.25
125.04
124.11
123.74
119.11
118.44
111.05

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

32
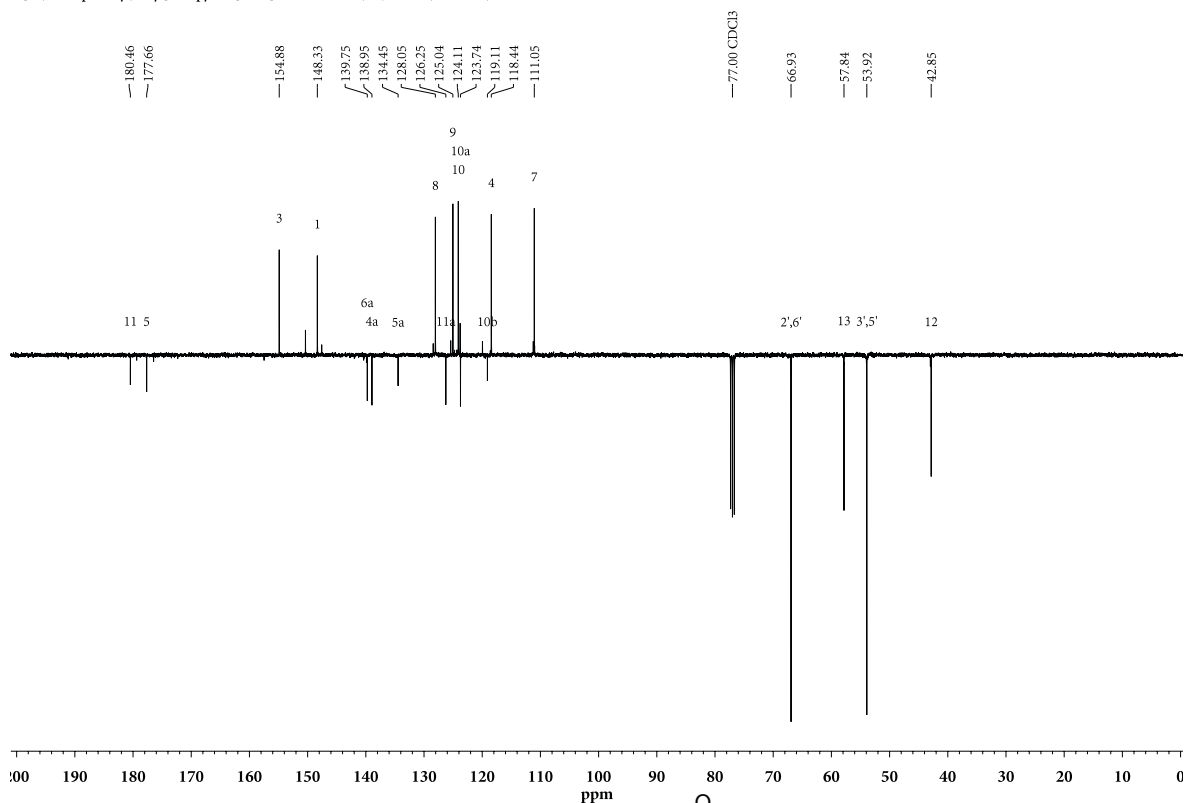
6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

32

6-[2-(4-Morpholinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (NOESY)

Generic Display Report
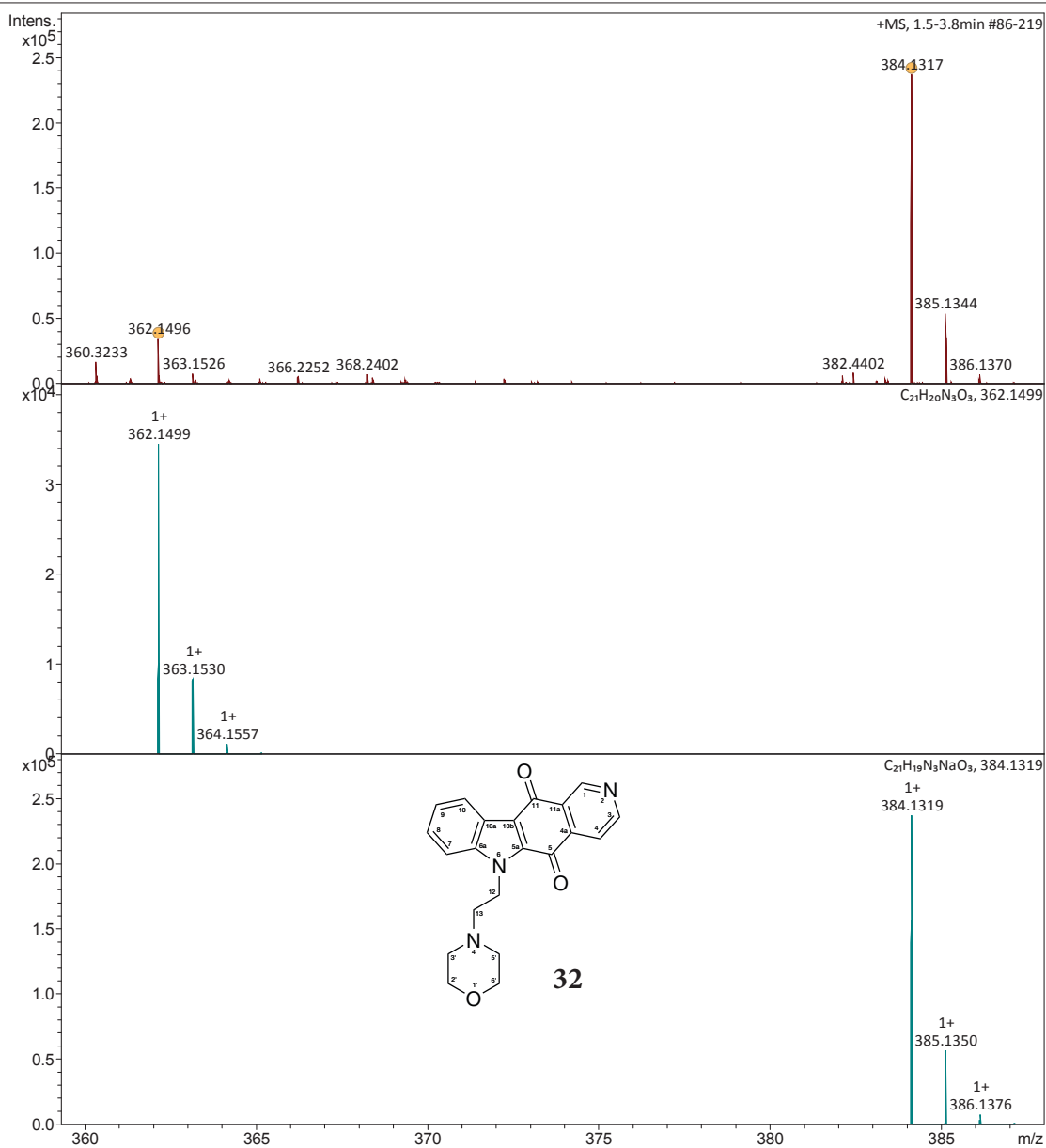
Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN108_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 10:56:41 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 1.5-3.8min #86-219

384.1317
385.1344
360.3233
362.1496
363.1526
366.2252
368.2402
382.4402
386.1370

$C_{21}H_{20}N_3O_3$, 362.1499

1+ 362.1499
1+ 363.1530
1+ 364.1557

$C_{21}H_{19}N_3NaO_3$, 384.1319

1+ 384.1319
1+ 385.1350
1+ 386.1376

32

Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date   11/4/2014 10:56:41 AM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN108_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 1.5-3.8min #86-219

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
362.1496	1	C21H20N3O3	362.1499	1.0	20.3	1	100.00	13.5	even	ok
384.1317	1	C21H19N3NaO3	384.1319	0.4	8.3	1	100.00	13.5	even	ok
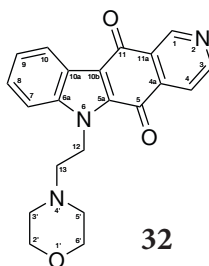
32

6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

33
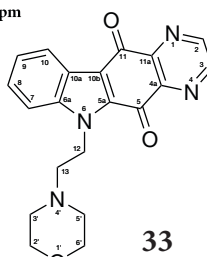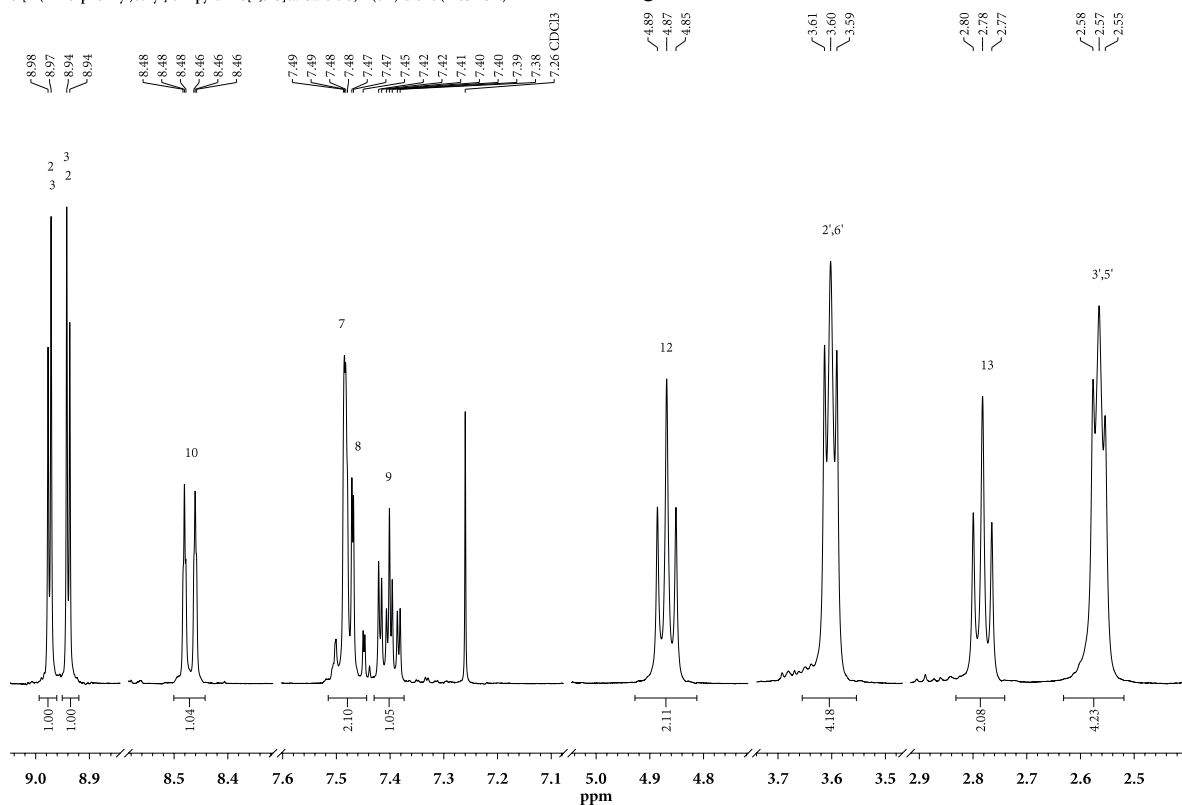
6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)
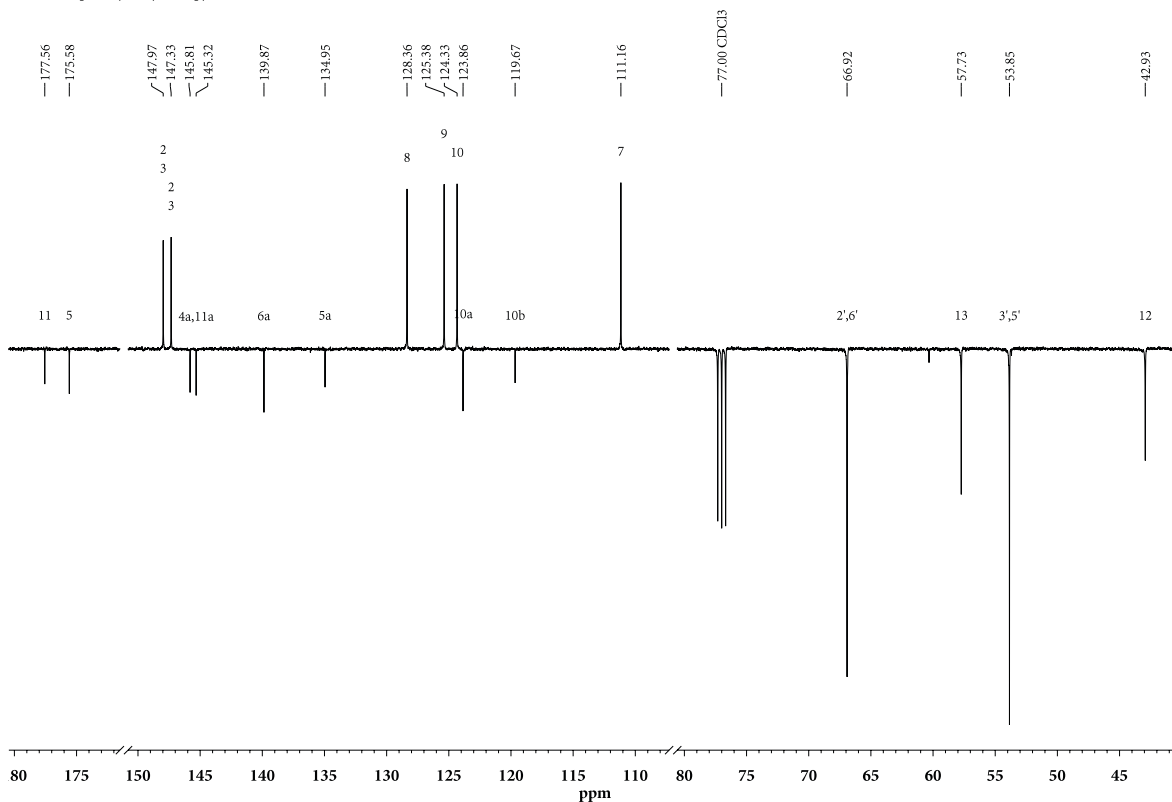
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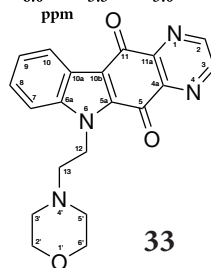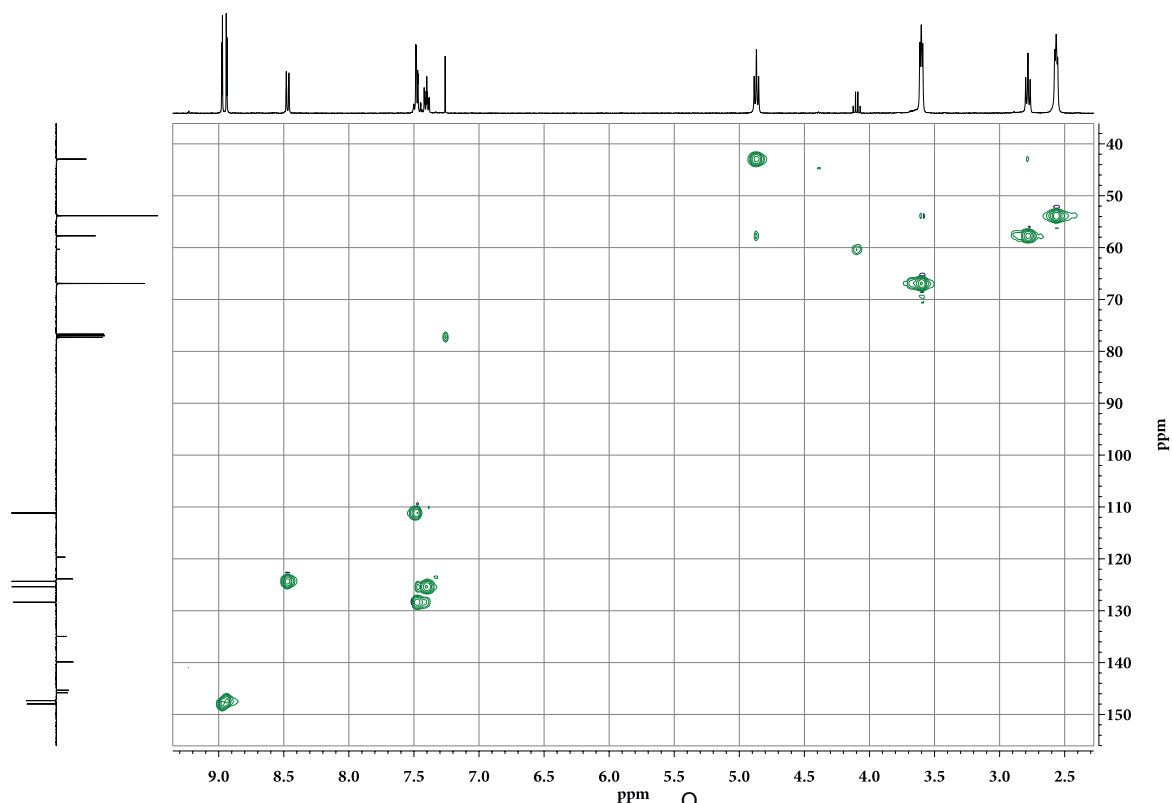
6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)
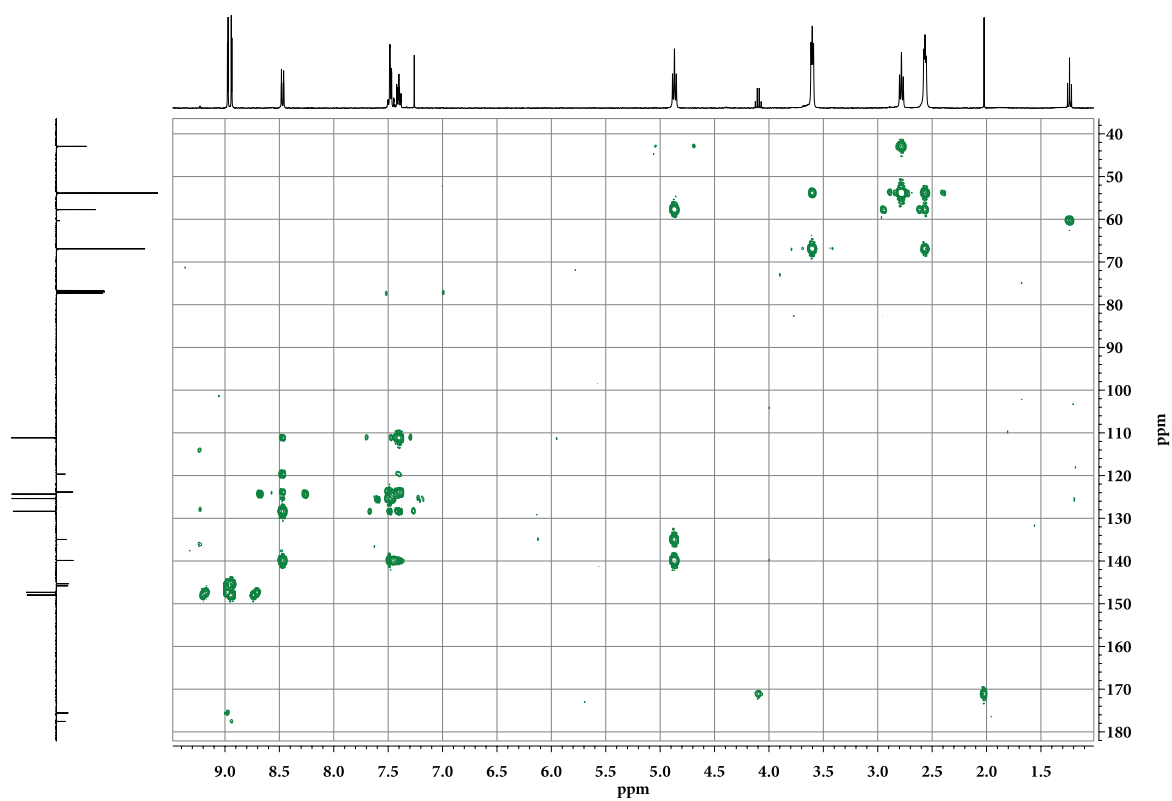
6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

33

6-[2-(4-Morpholinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (NOESY)

Mass Spectrum SmartFormula Report
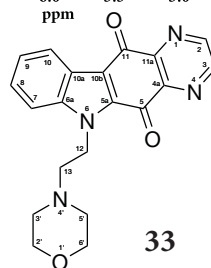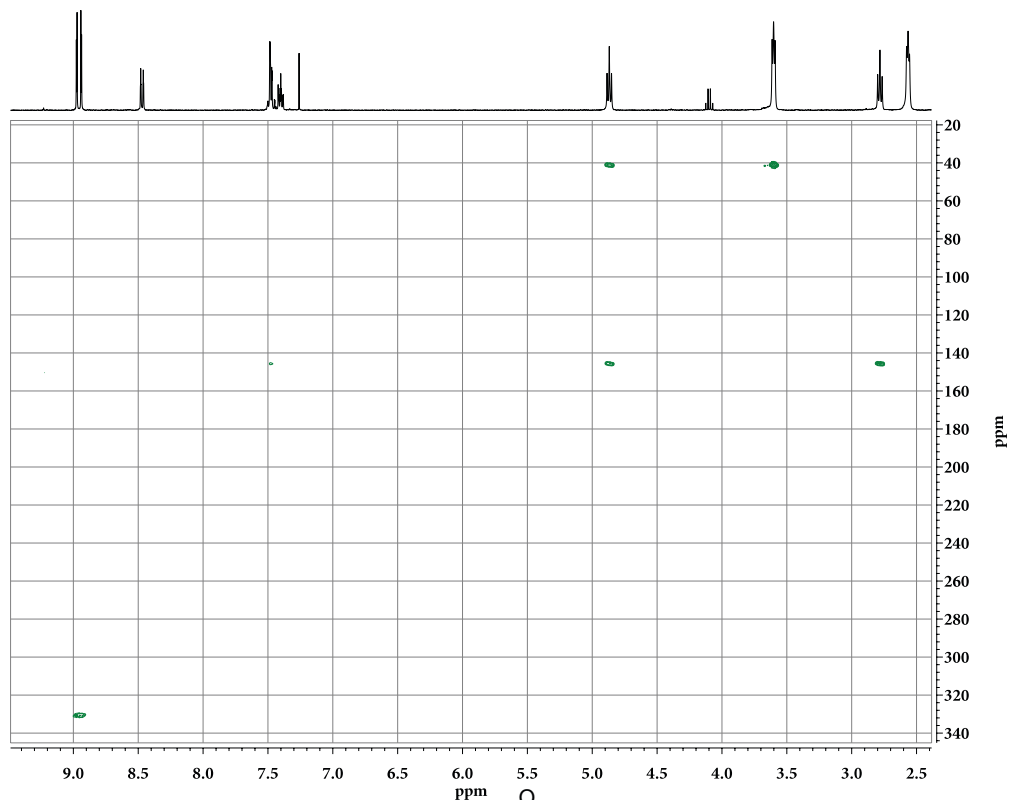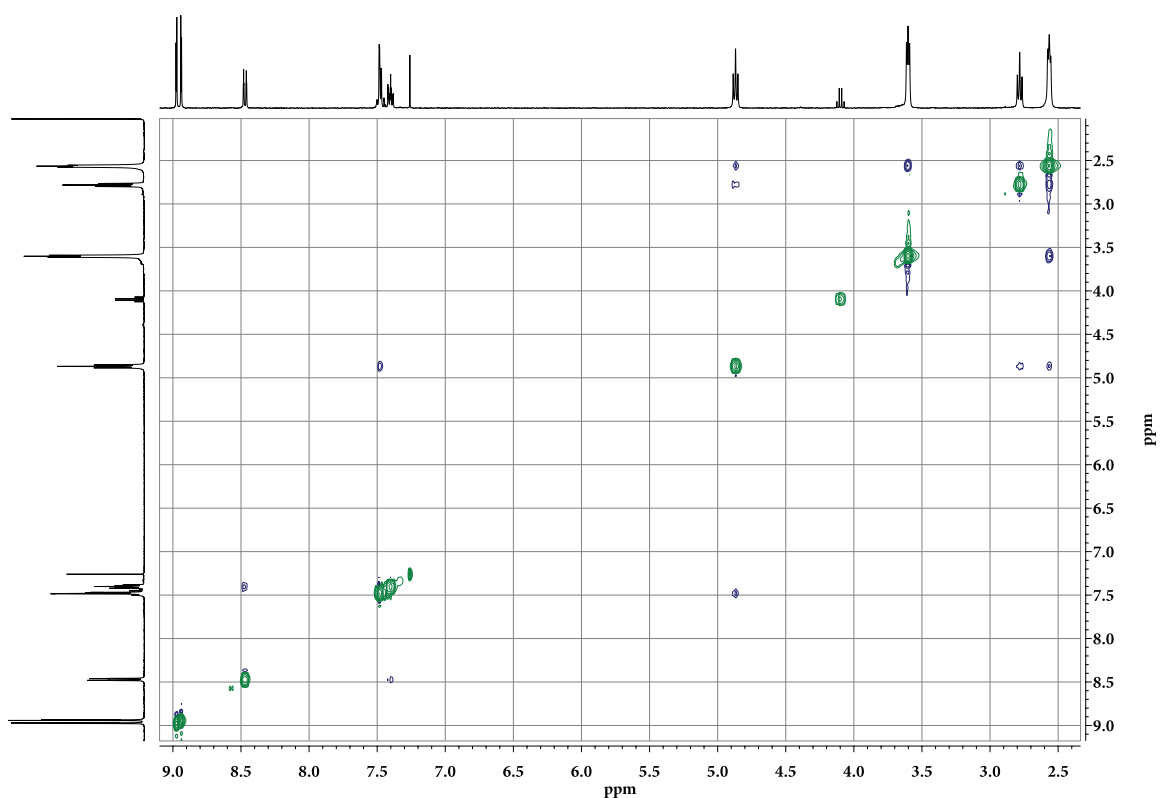
Analysis Info

		Acquisition Date	11/4/2014 10:50:10 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN107_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.7min #1-95

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
363.1450	1	C20H19N4O3	363.1452	0.5	19.7	1	100.00	13.5	even	ok
385.1272	1	C20H18N4NaO3	385.1271	-0.2	11.0	1	100.00	13.5	even	ok

33

1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

34
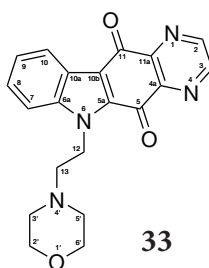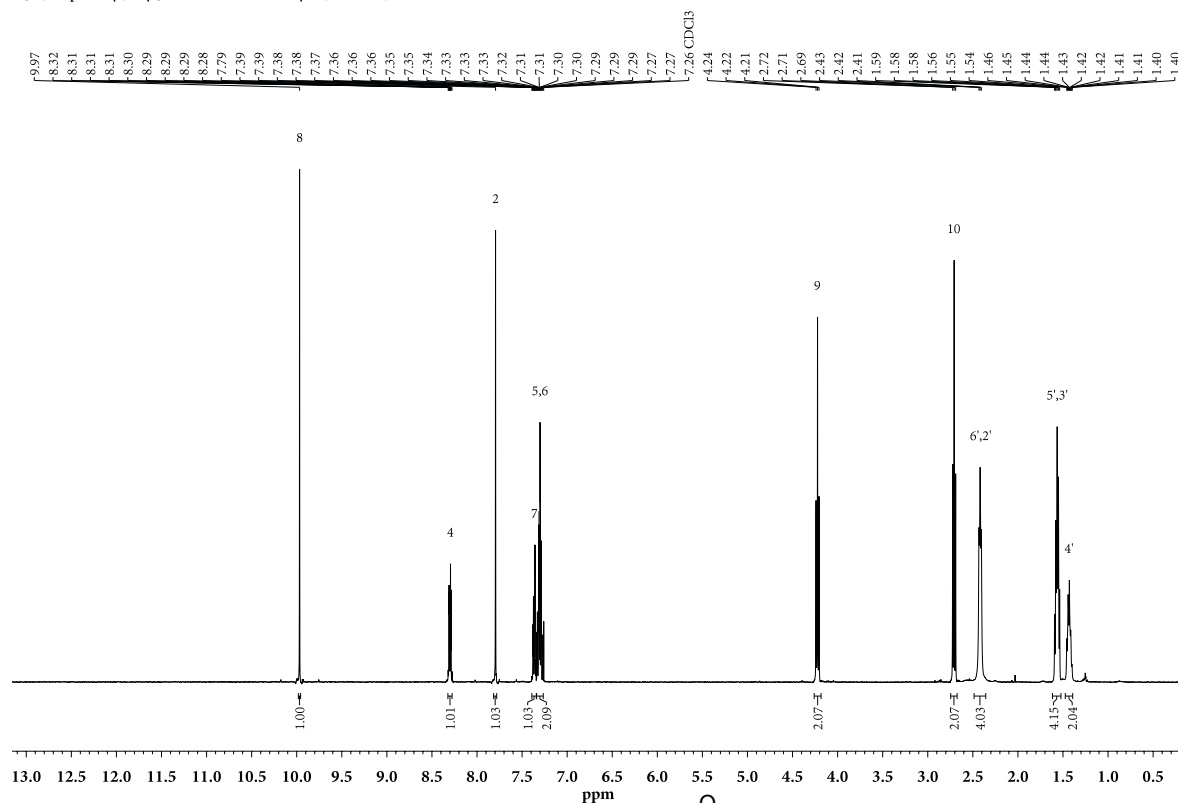
1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)
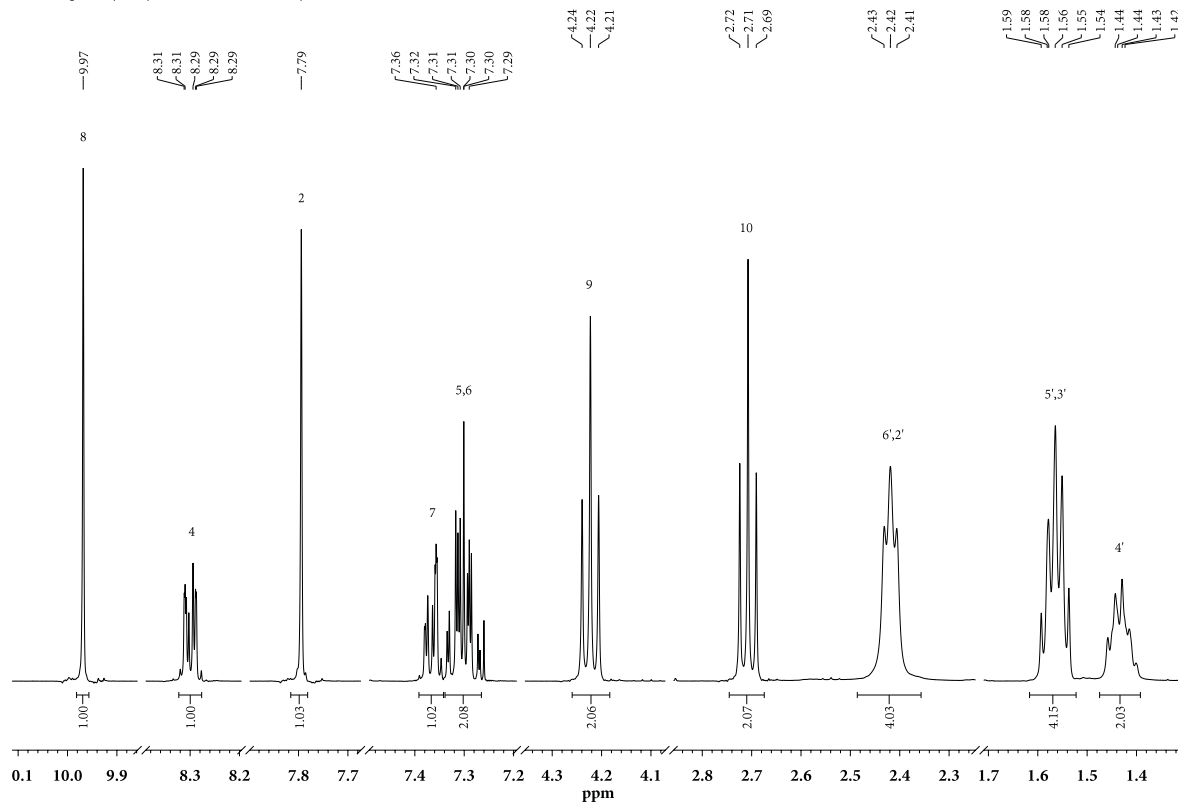
1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (C13 APT)
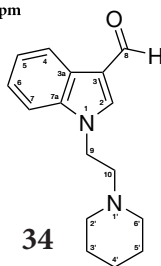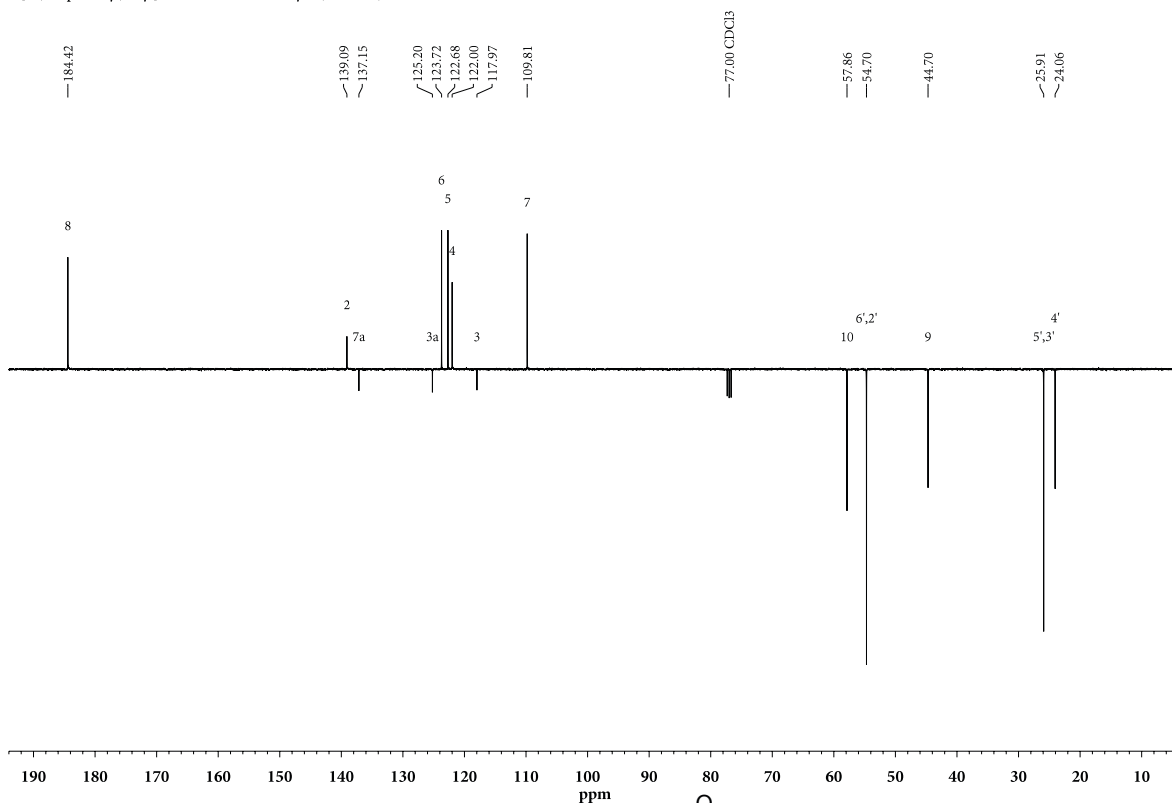
1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (C13 APT)
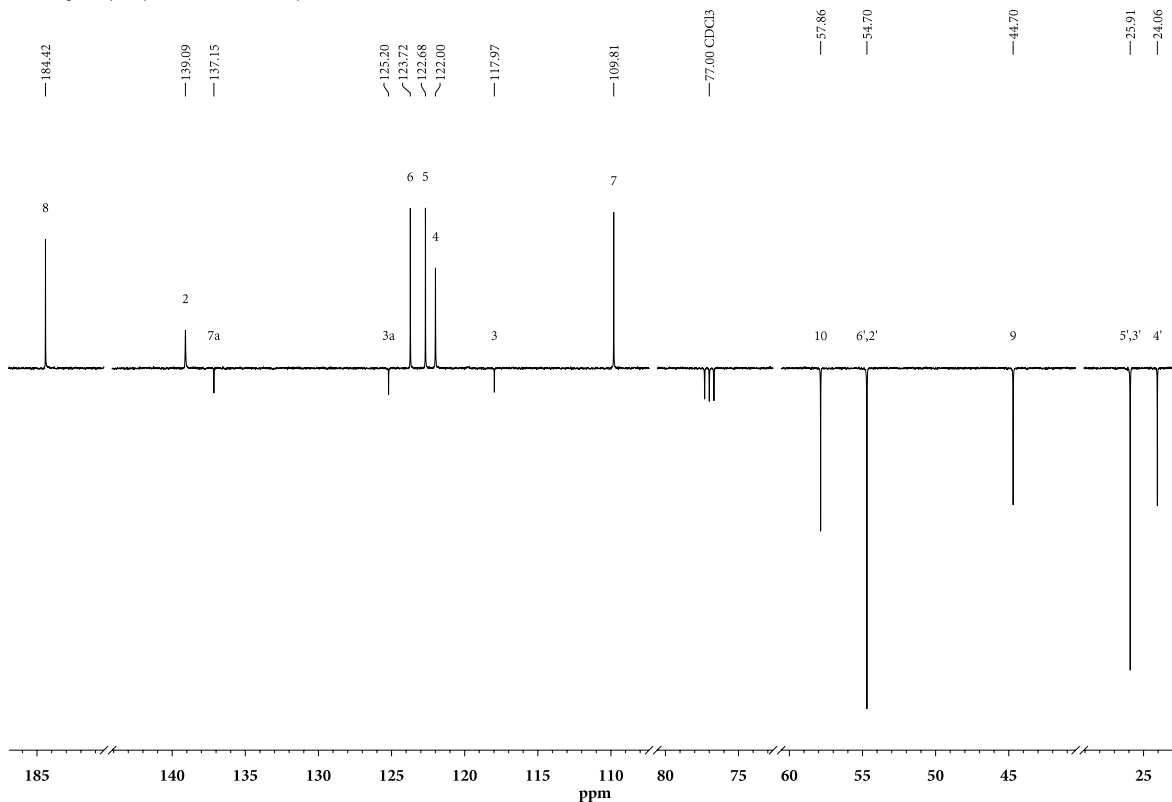
34

1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (HSQC)

34
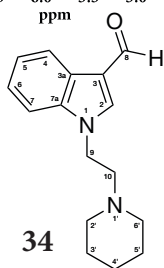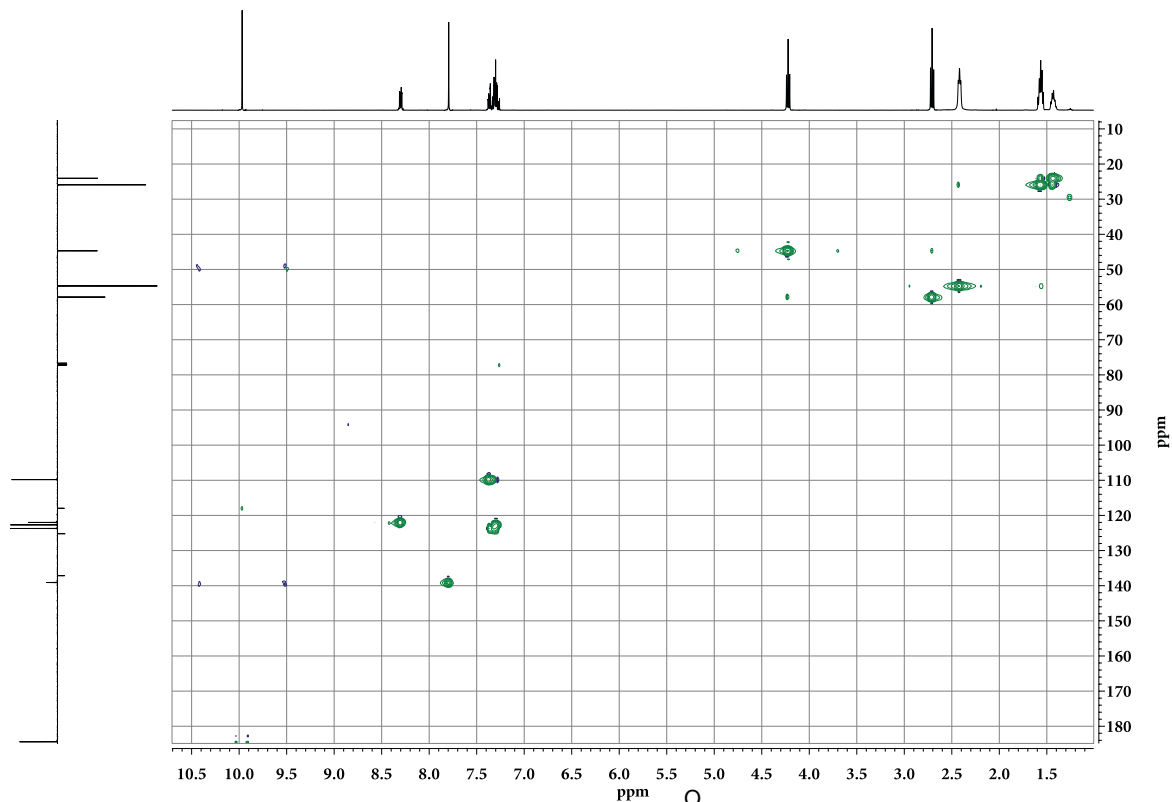
1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (HMBC)

N,N-Diethyl-2-pyrazinecarboxamide (15N HMBC)

34
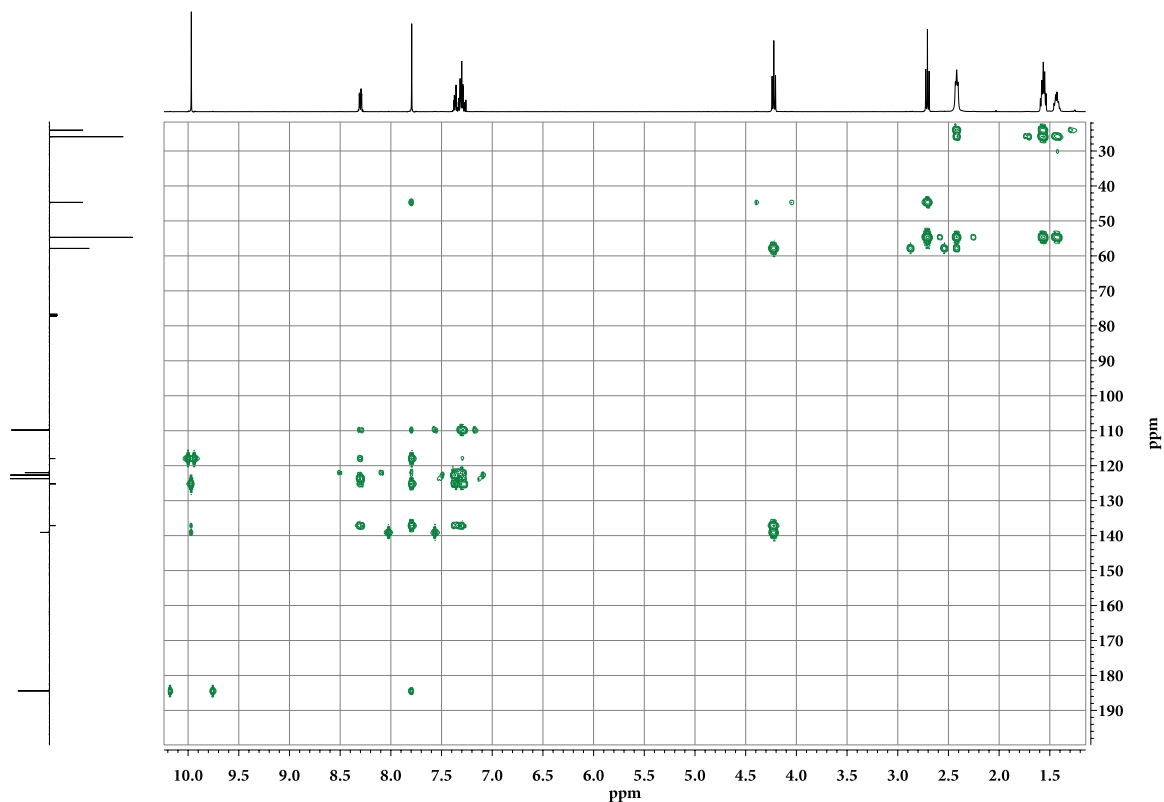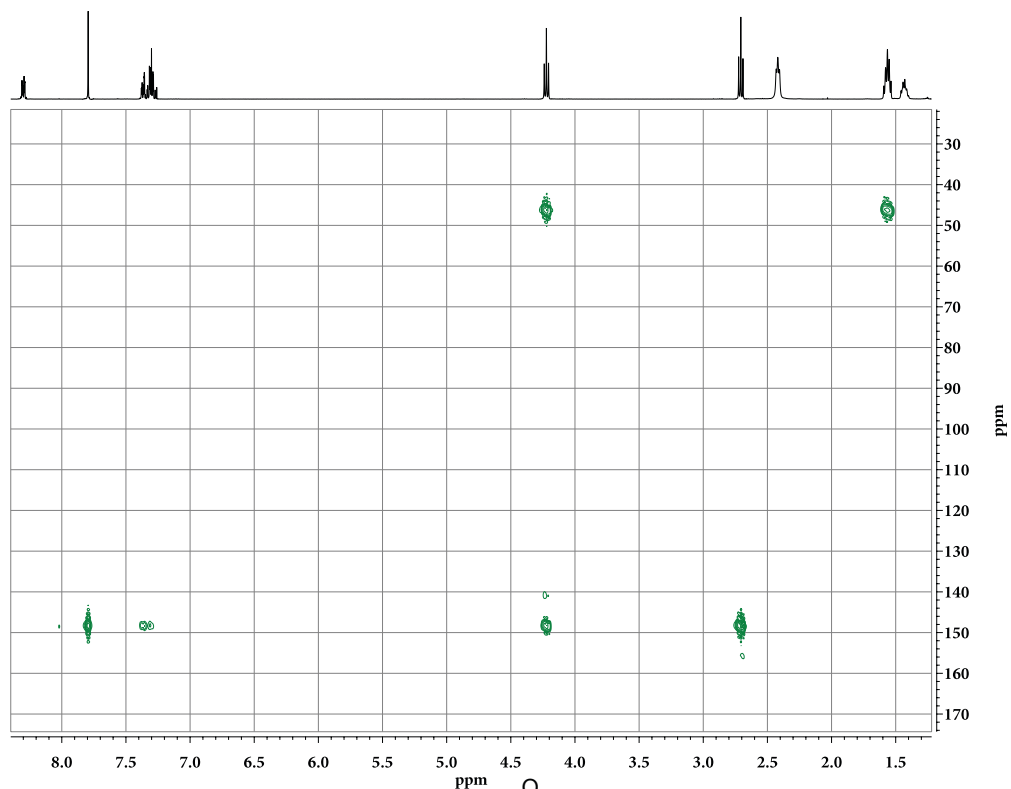
1-[2-(1-Piperidinyl)ethyl]-1H-indole-3-carbaldehyde (NOESY)

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 3:43:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN109_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.5min #1-86

257.1652

279.1469

$C_{16}H_{21}N_2O$, 257.1648

1+
257.1648

$C_{16}H_{20}N_2NaO$, 279.1468

1+
279.1468

34

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/12/2015 3:43:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN109_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
257.1652	1	C16H21N2O	257.1648	-1.3	10.0	1	100.00	7.5	even	ok
279.1469	1	C16H20N2NaO	279.1468	-0.5	9.2	1	100.00	7.5	even	ok
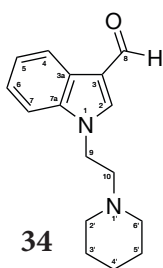
34

Bruker Compass DataAnalysis 4.2   printed: 1/12/2015 3:50:04 PM   by: MZ   Page 1 of 1

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

35
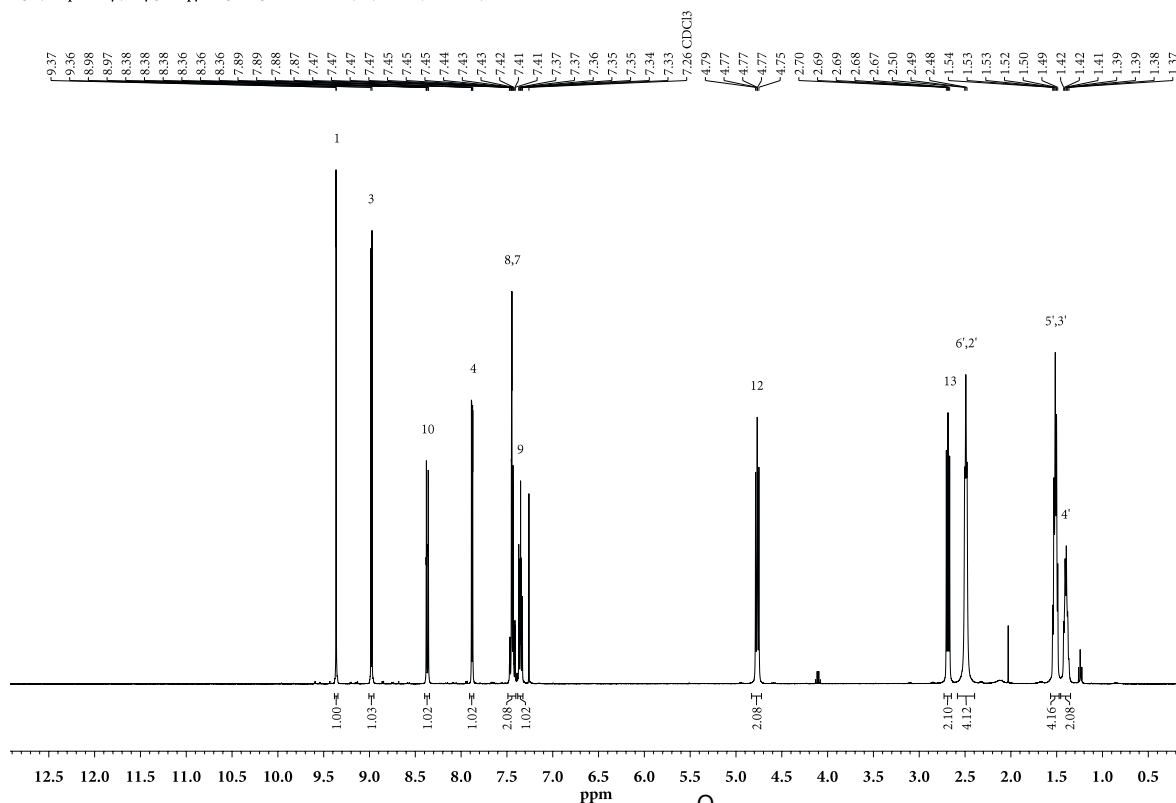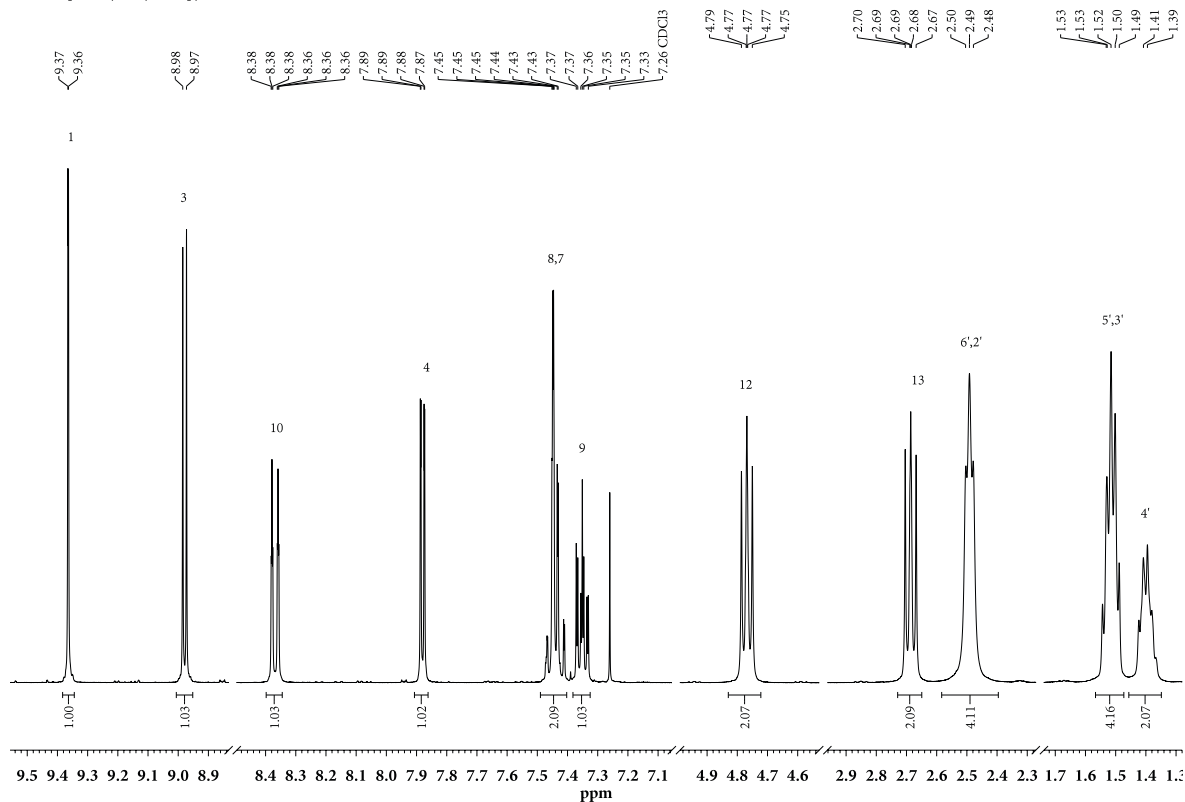
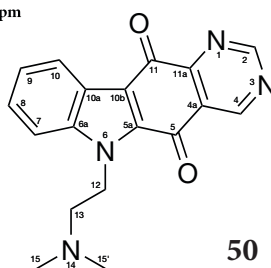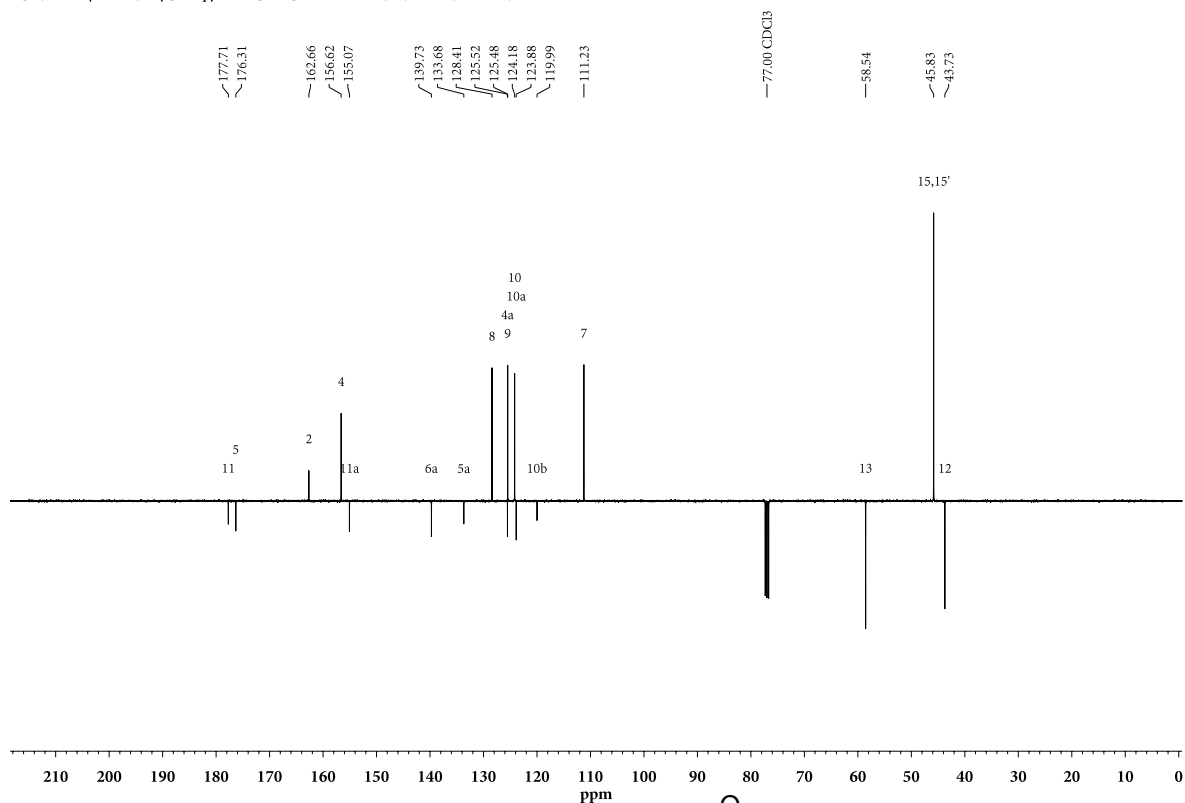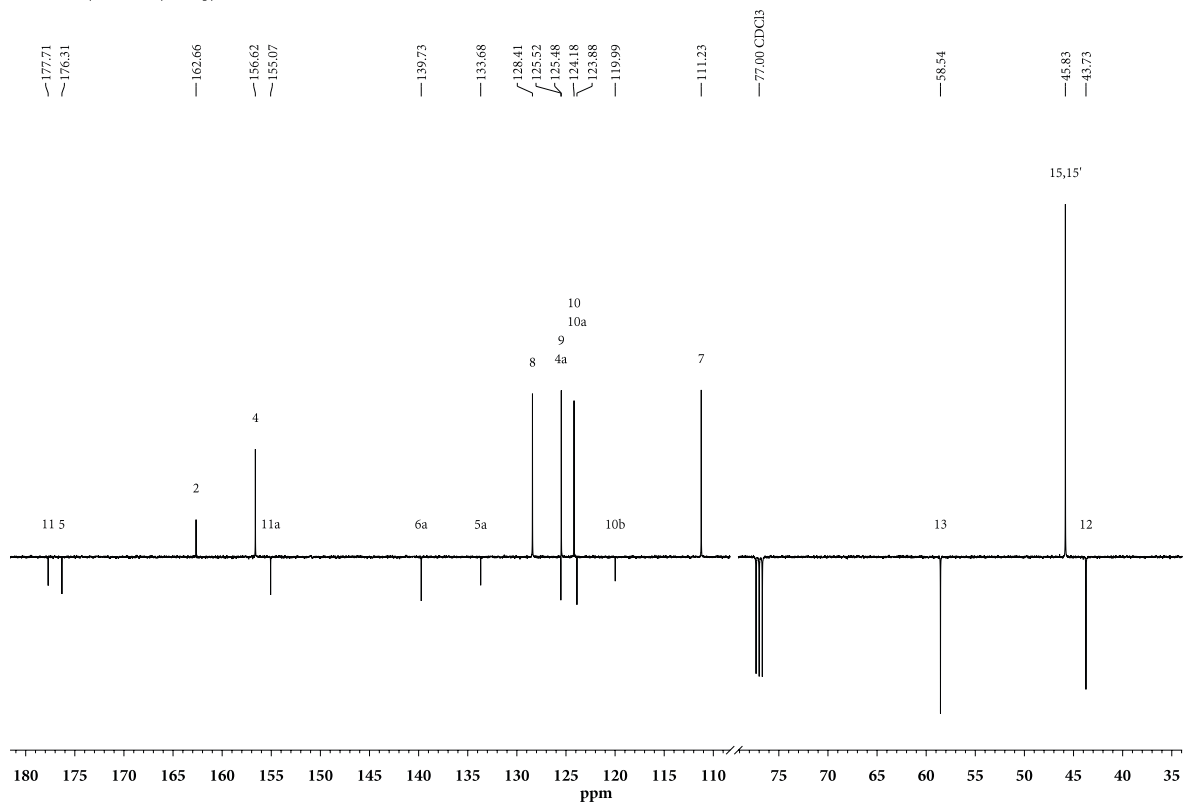6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

35

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

35

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)
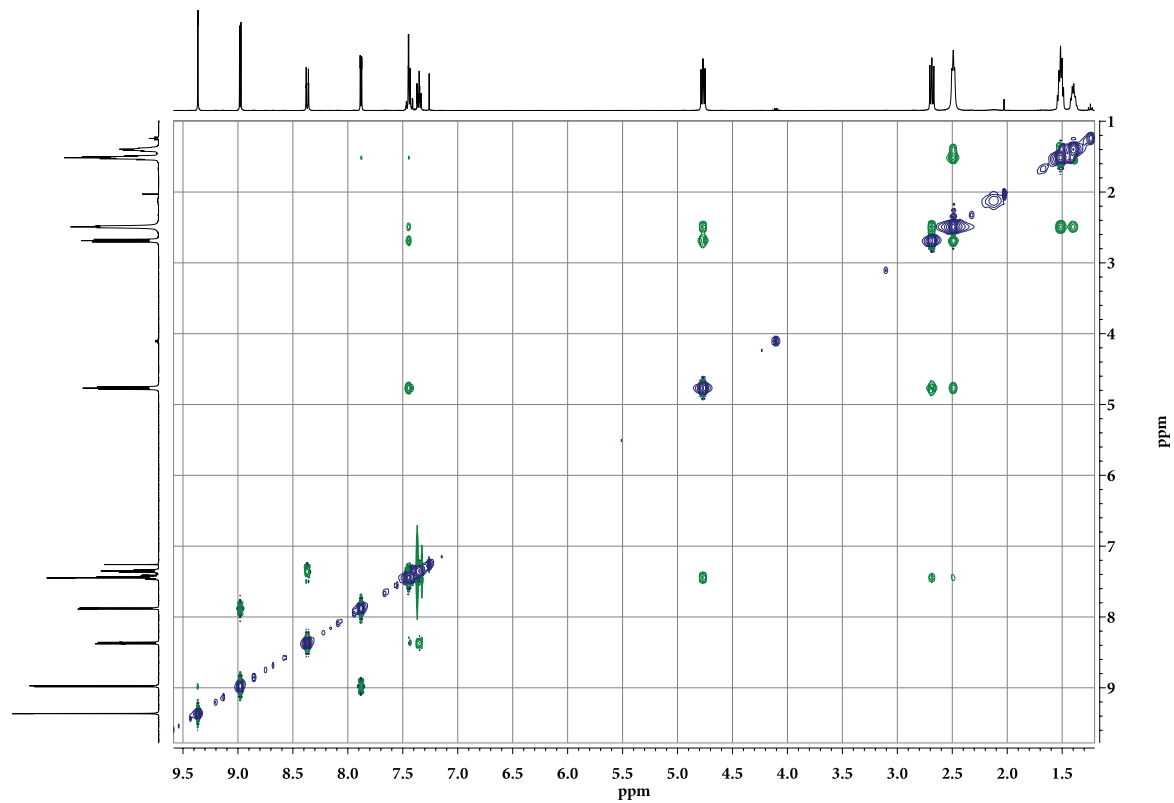
35

6-[2-(1-Piperidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (NOESY)

Generic Display Report

Analysis Info
Analysis Name   D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN111_DI_ESI_HRMS.d
Method   DI_mz_50-1550.m
Sample Name   approx. 10 ug/mL in MeOH
Comment

Acquisition Date   11/4/2014 11:15:02 AM
Operator   MZ
Instrument   maXis HD

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 11:15:02 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN111_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
360.1707	1	C22H22N3O2	360.1707	0.0	7.8	1	100.00	13.5	even	ok
382.1525	1	C22H21N3NaO2	382.1526	0.4	7.6	1	100.00	13.5	even	ok
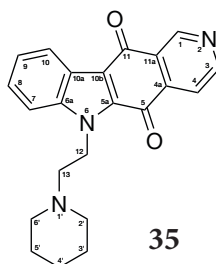
35

6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

36

6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

36

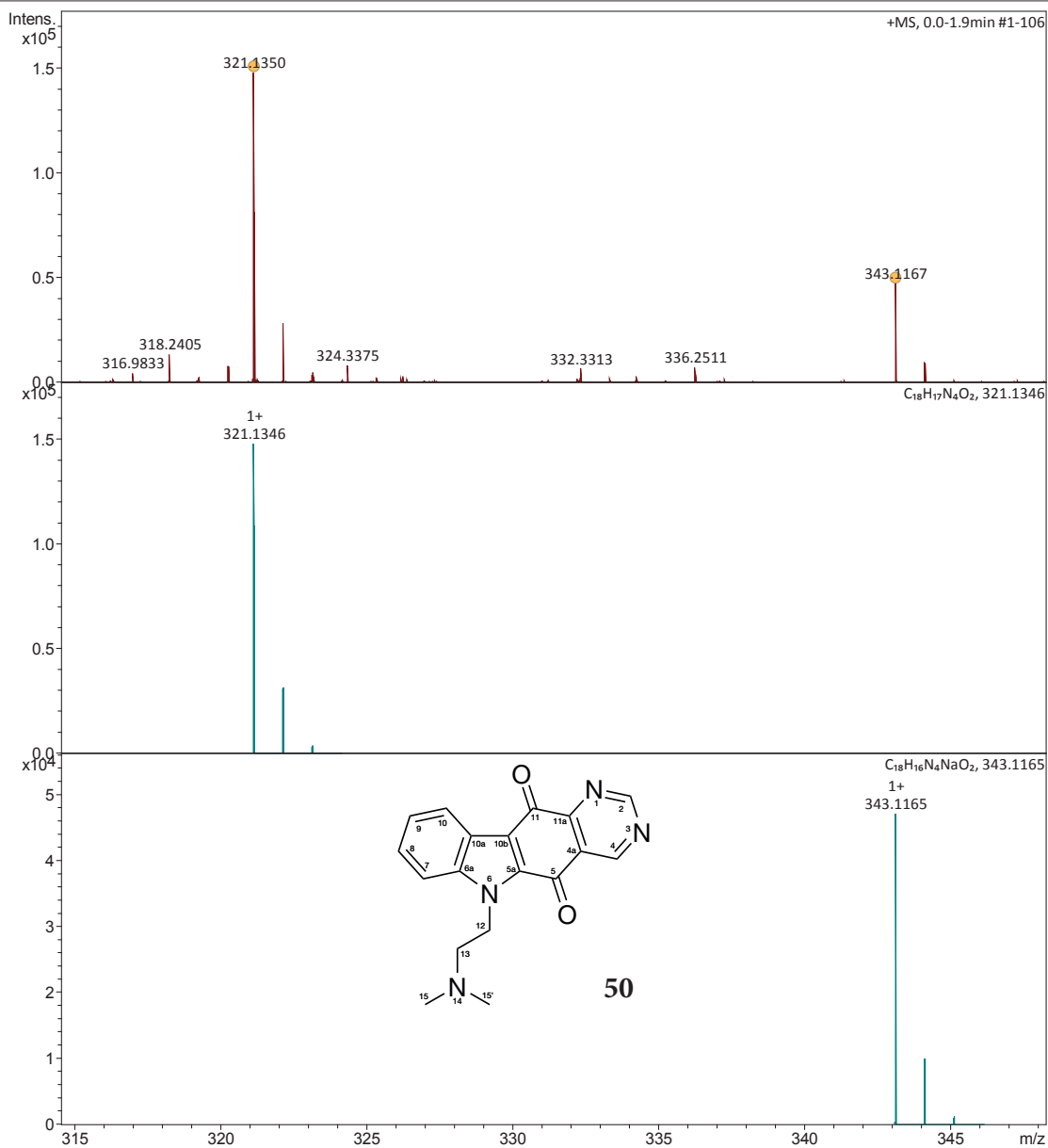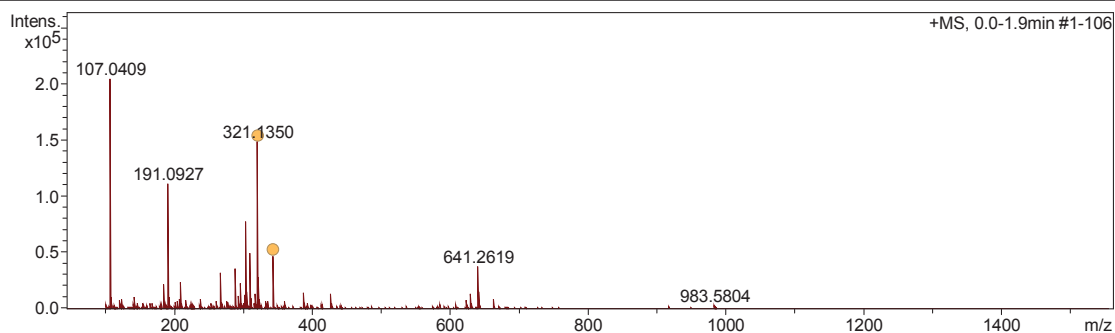6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

36

6-[2-(1-Piperidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 11:07:59 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN110_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/4/2014 11:07:59 AM

Analysis Name   D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN110_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   approx. 10 ug/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
361.1658	1	C21H21N4O2	361.1659	0.2	5.3	1	100.00	13.5	even	ok
383.1479	1	C21H20N4NaO2	383.1478	-0.1	11.5	1	100.00	13.5	even	ok

36

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

37
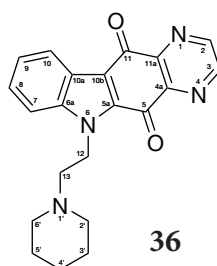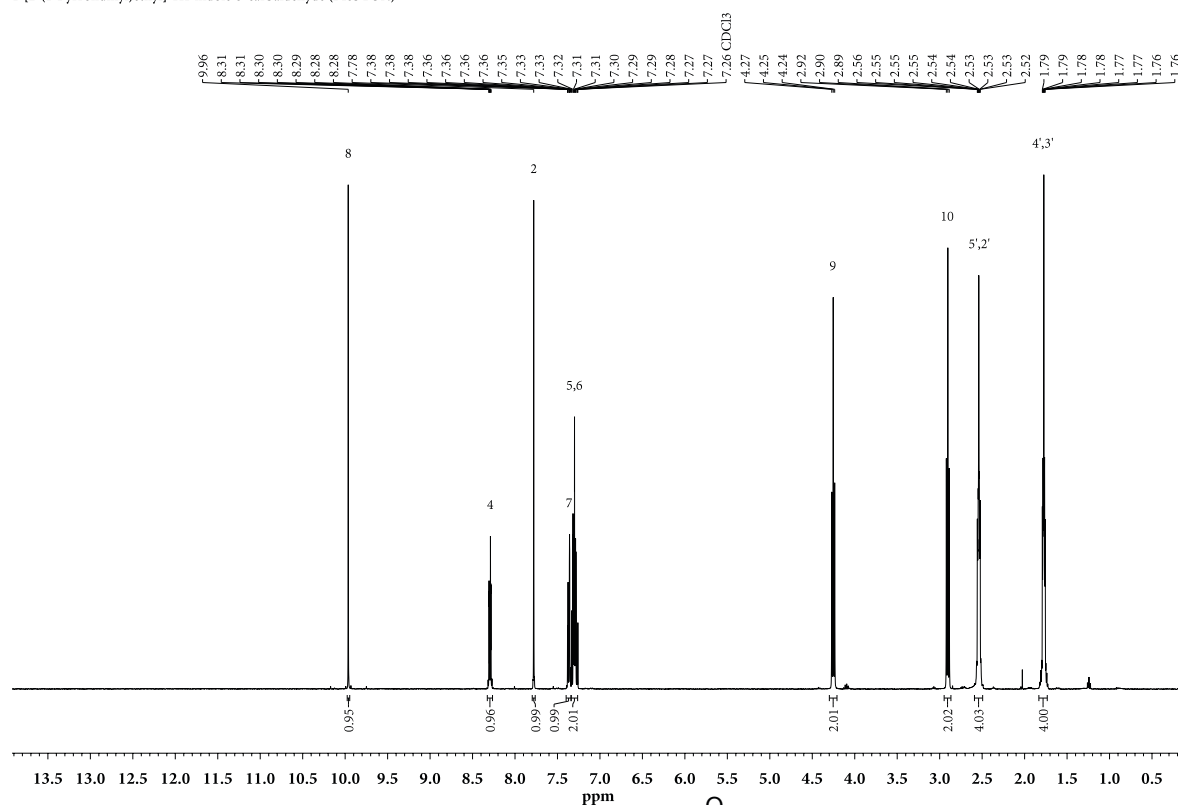
1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)
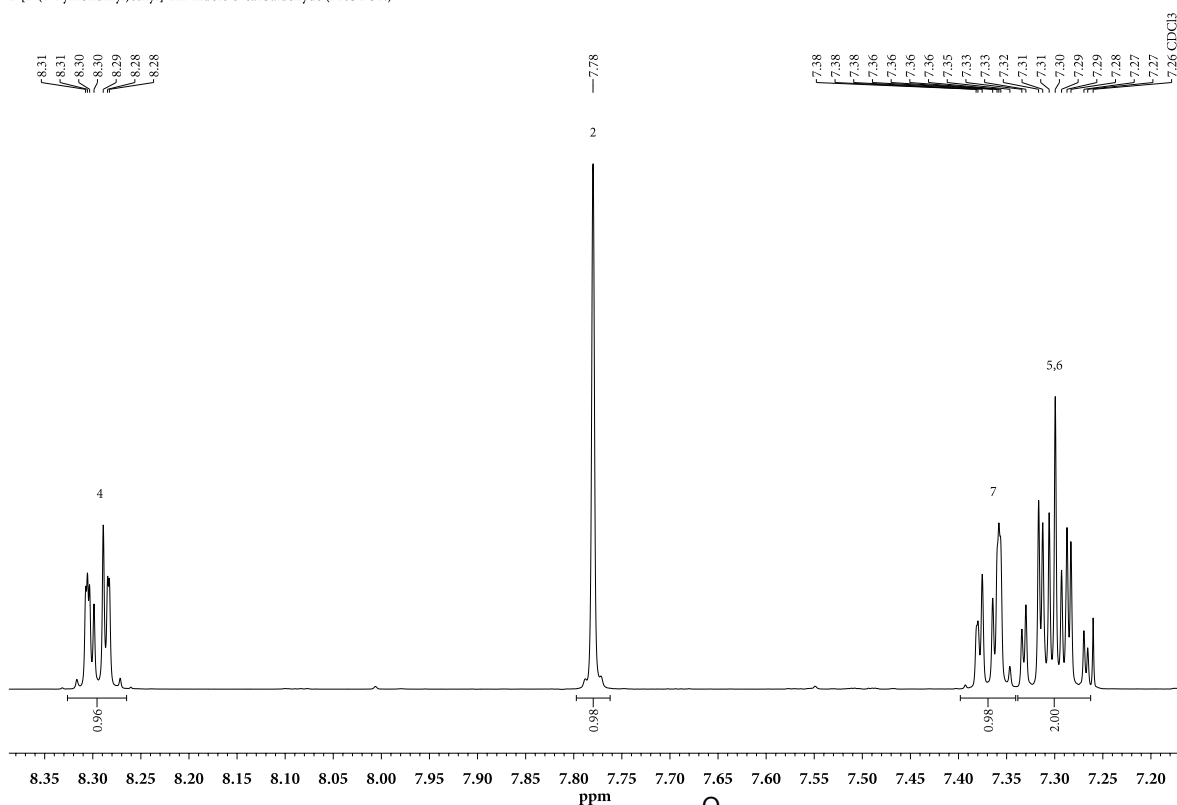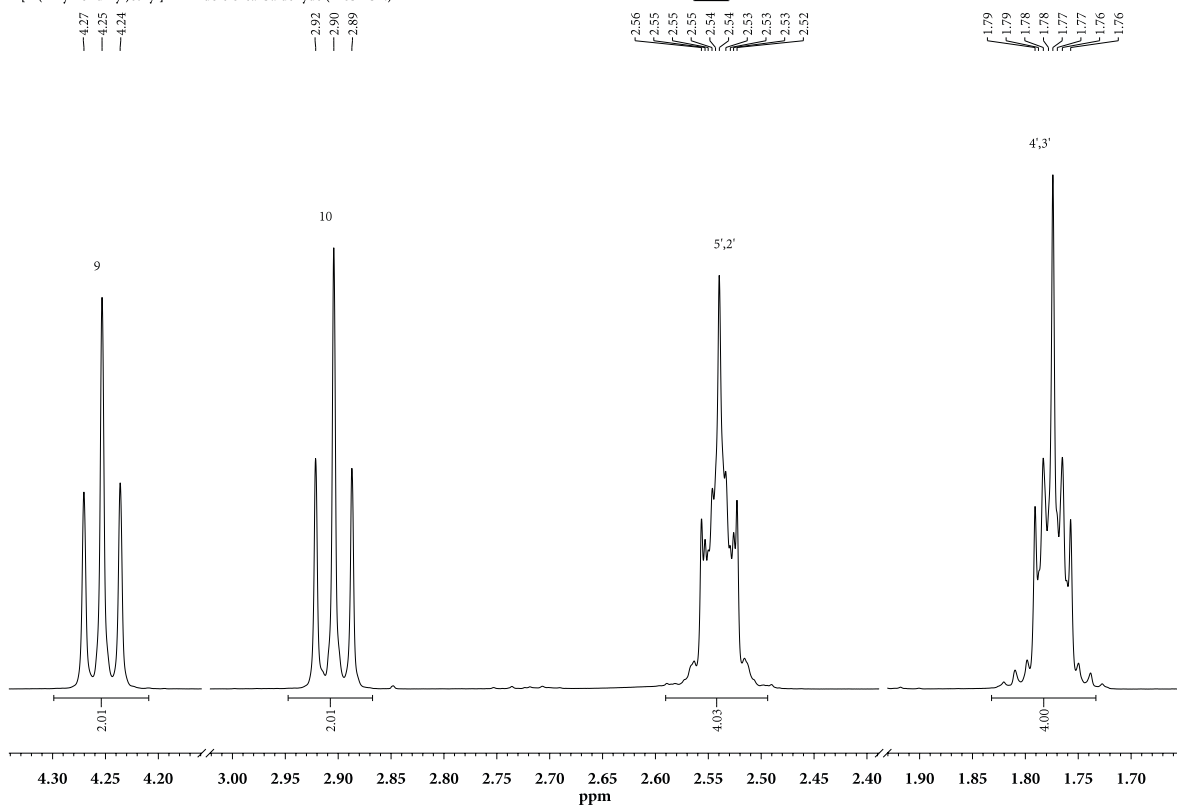
8.31
8.31
8.30
8.30
8.29
8.28
8.28

7.78

7.38
7.38
7.38
7.36
7.36
7.36
7.35
7.33
7.33
7.32
7.31
7.31
7.30
7.29
7.29
7.28
7.27
7.27
7.26 CDCl3

2

5,6

4

7

0.96

0.98

0.98

2.00

8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20
ppm

37

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (PROTON)

4.27
4.25
4.24

2.92
2.90
2.89

2.56
2.55
2.55
2.55
2.54
2.54
2.53
2.53
2.52

1.79
1.79
1.78
1.78
1.77
1.76
1.76

4',3'

10

9

5',2'

2.01

2.01

4.03

4.00

4.30 4.25 4.20 3.00 2.95 2.90 2.85 2.80 2.75 2.70 2.65 2.60 2.55 2.50 2.45 2.40 1.90 1.85 1.80 1.75 1.70
ppm

395

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (C13 CPD)

37
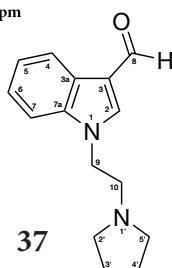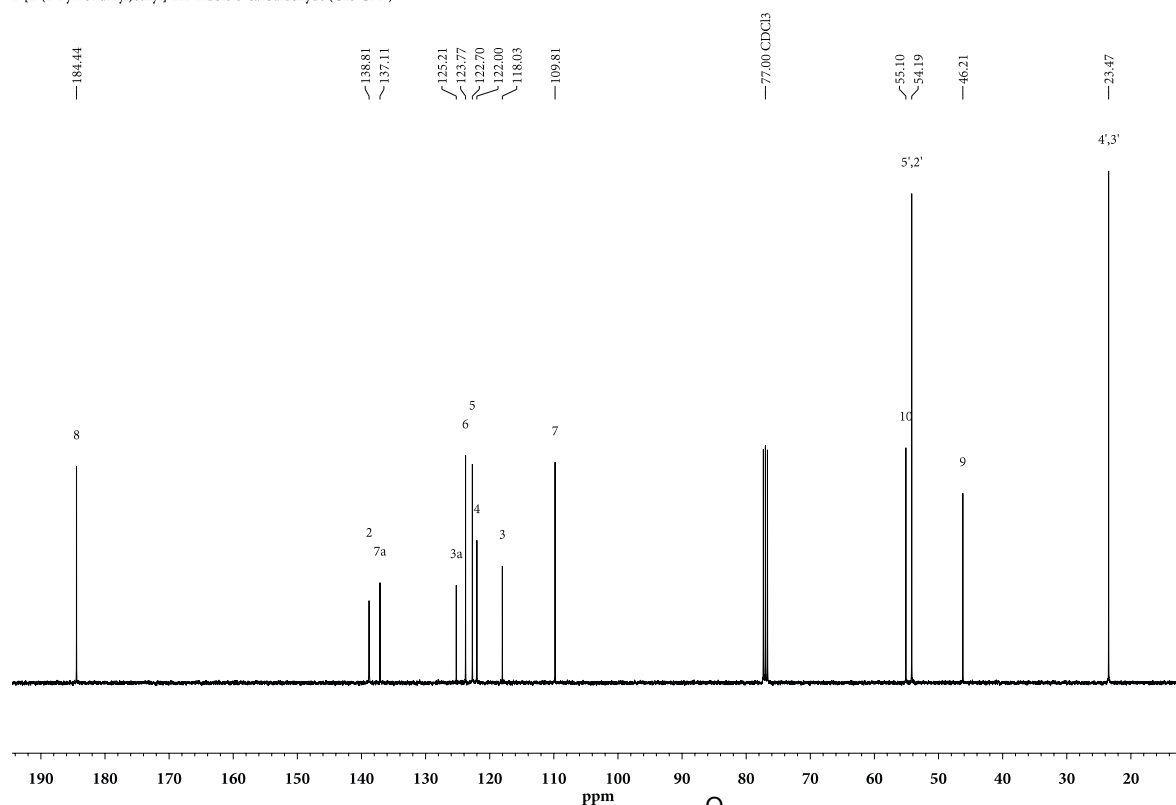
1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (HSQC)

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (HMBC)
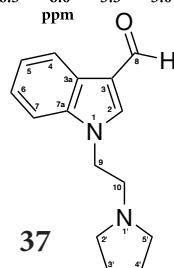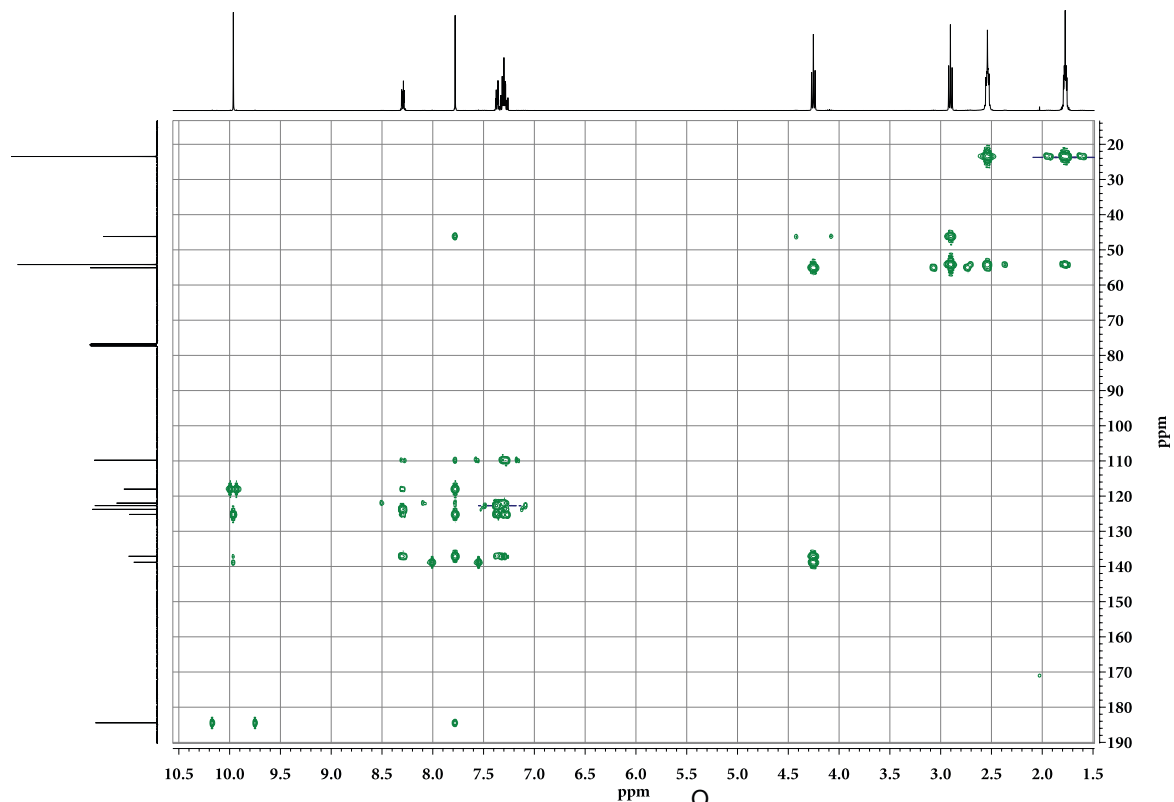
37

1-[2-(1-Pyrrolidinyl)ethyl]-1H-indole-3-carbaldehyde (15N HMBC)
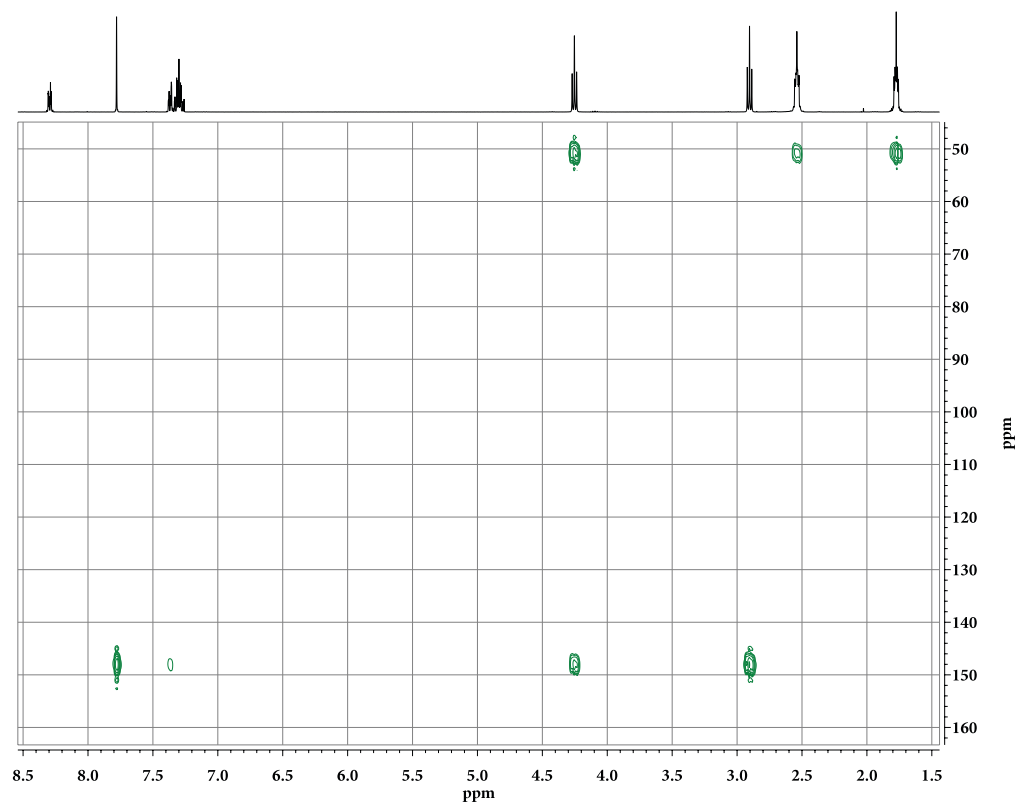
Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN112_DI_ESI_HRMS.d	Acquisition Date	11/17/2014 2:18:00 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Bruker Compass DataAnalysis 4.2   printed: 11/17/2014 2:23:30 PM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date 11/17/2014 2:18:00 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN112_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.5min #1-85

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
243.1495	1	C15H19N2O	243.1492	-1.2	6.1	1	100.00	7.5	even	ok
265.1313	1	C15H18N2NaO	265.1311	-0.8	5.8	1	100.00	7.5	even	ok
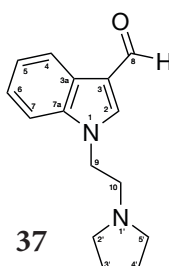
37

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

38
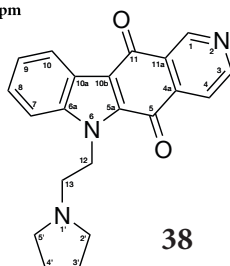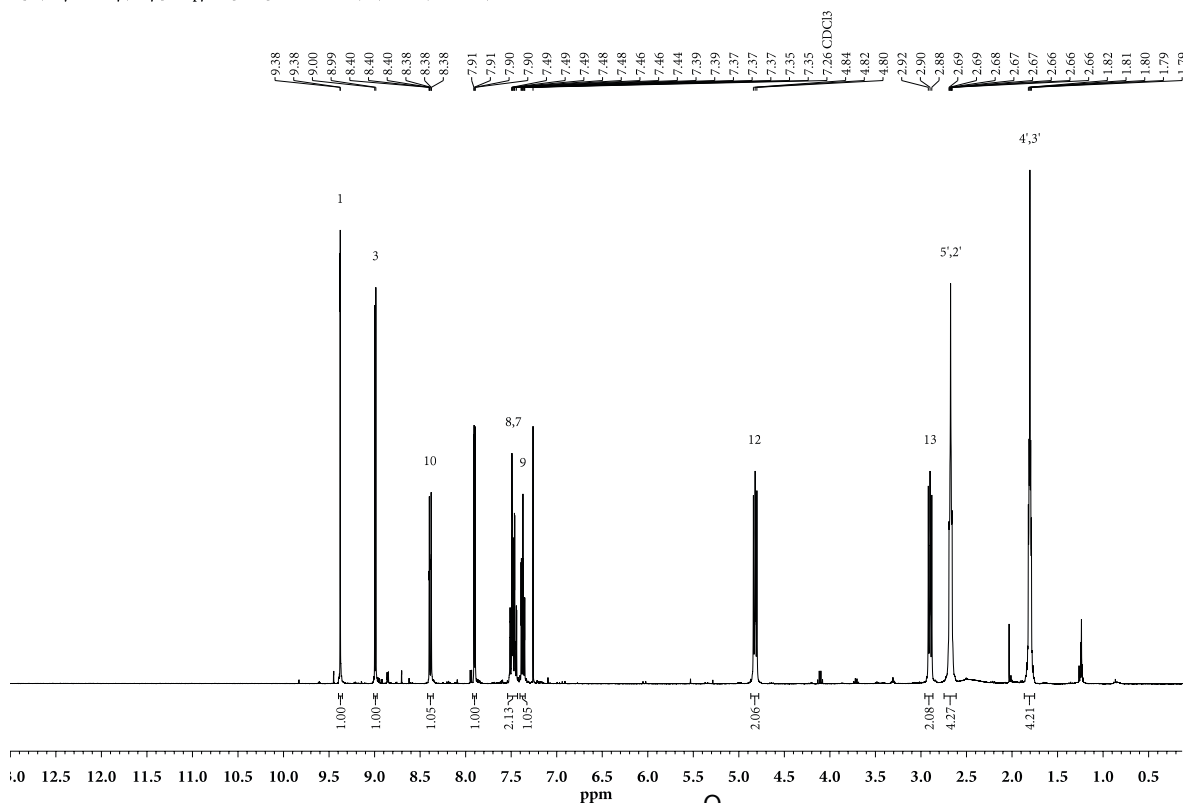
6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)
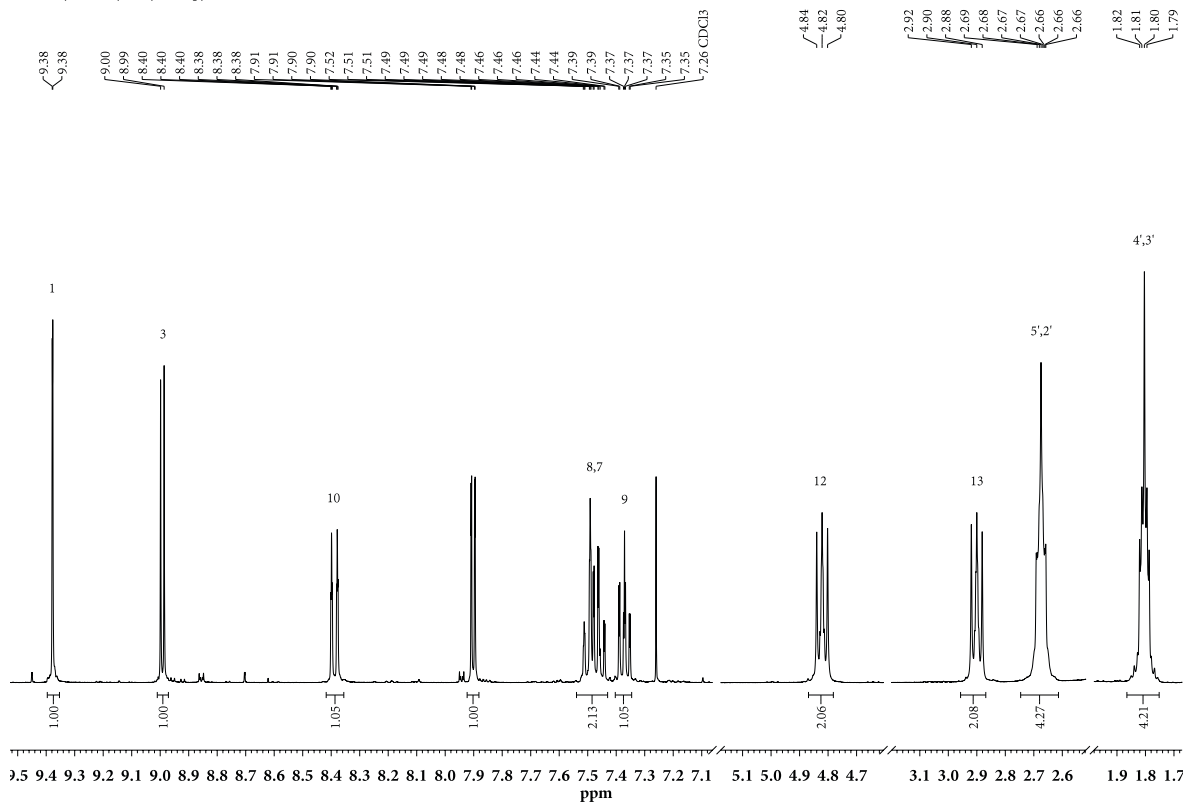
6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

38

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

38
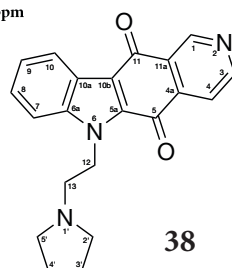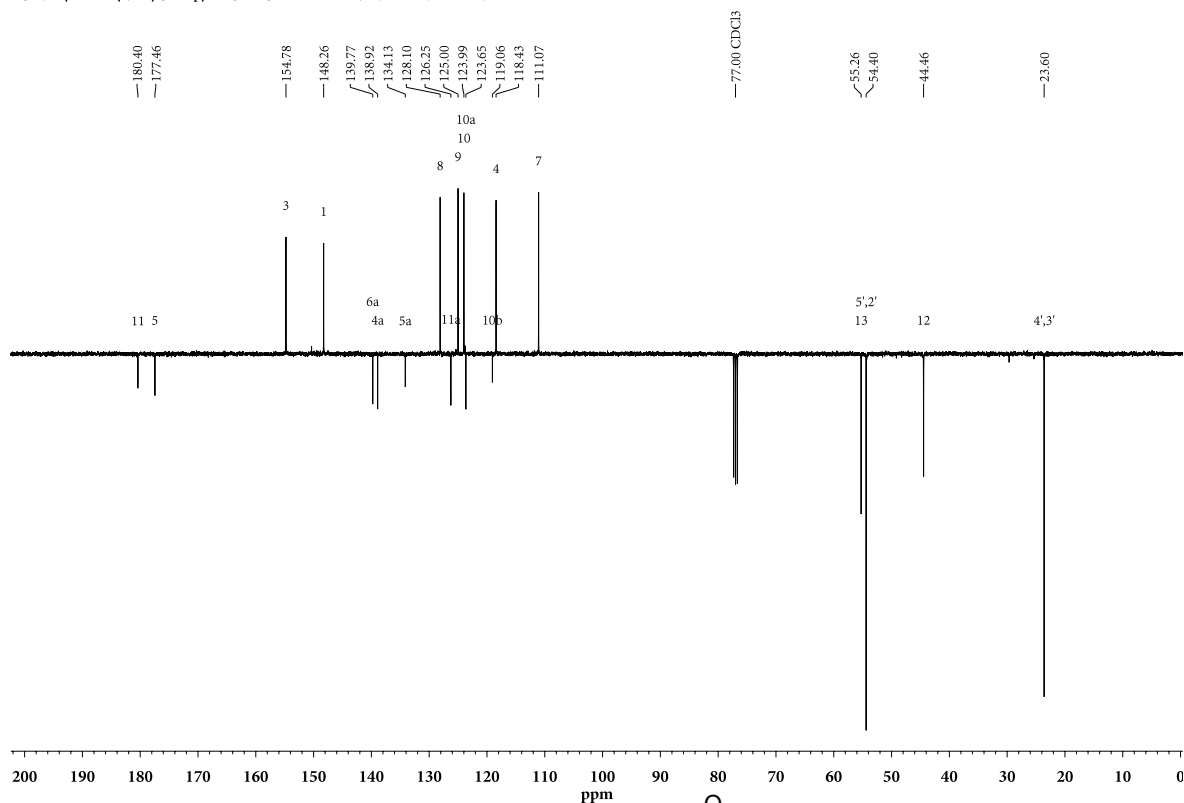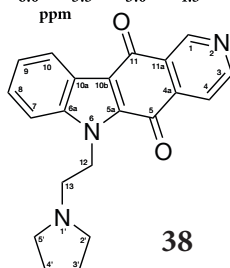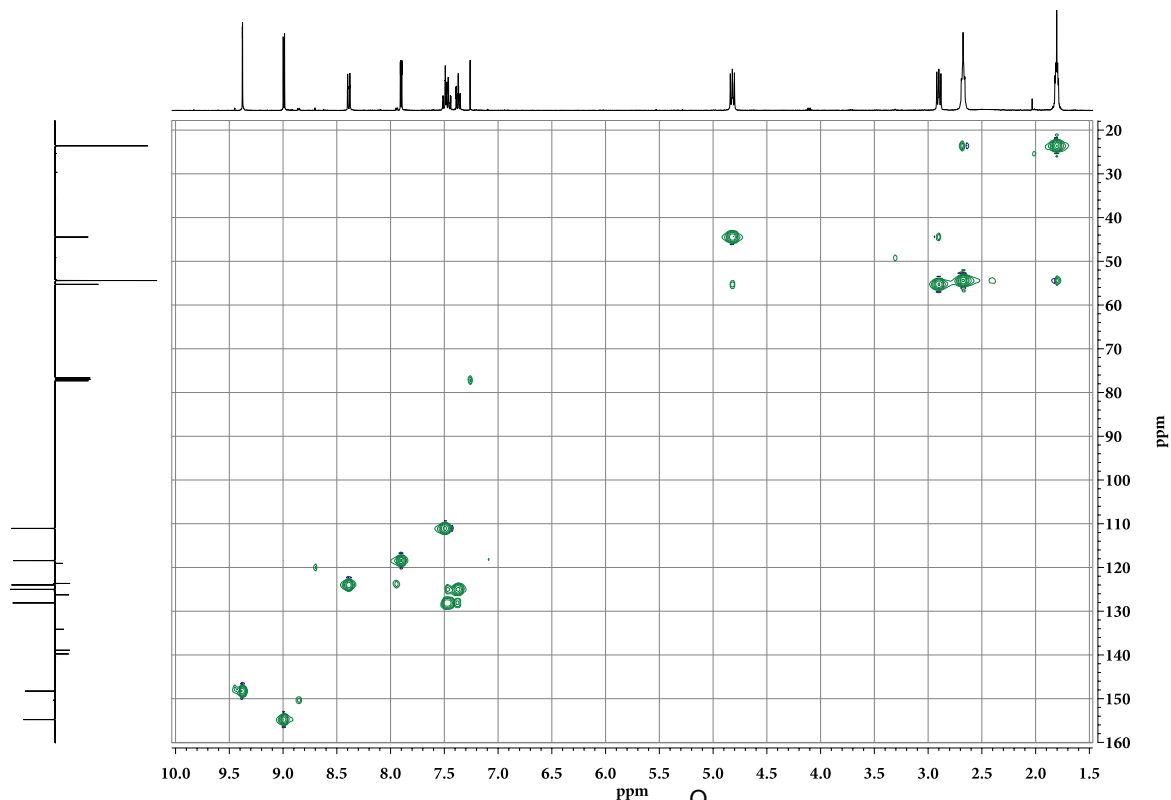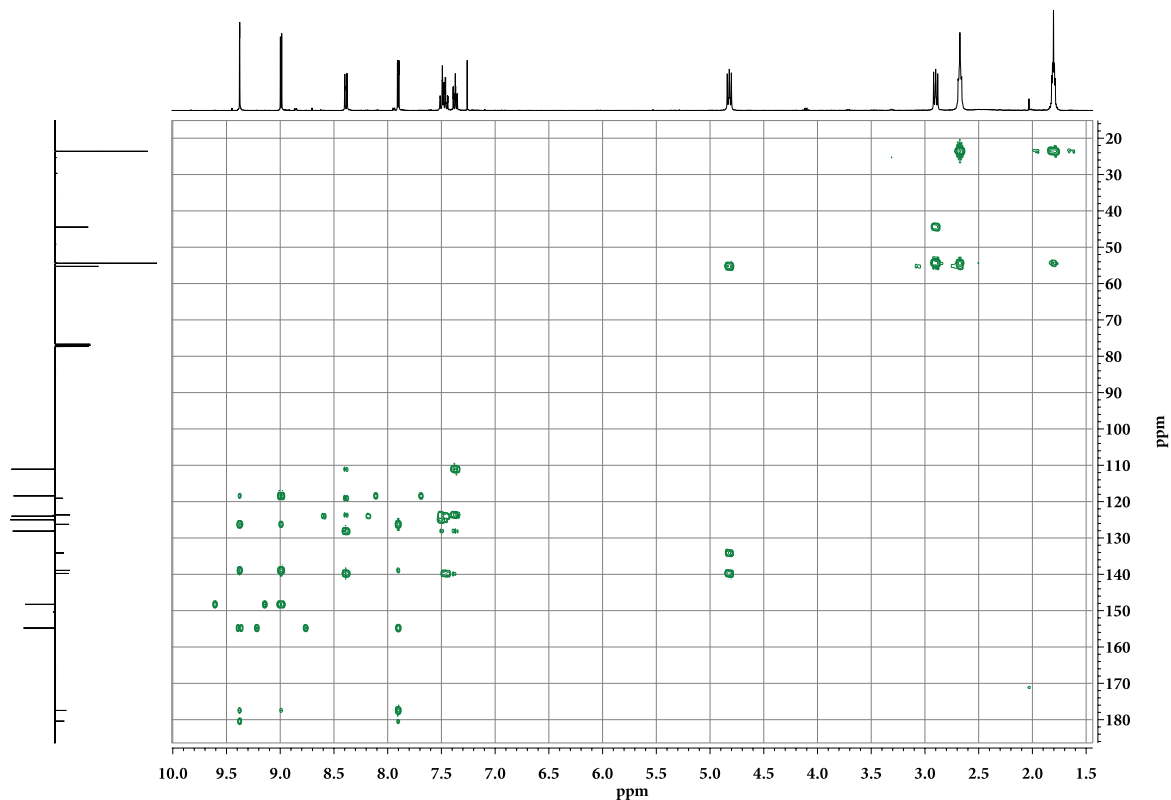
6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

38

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN113_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 11:22:01 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 11:22:01 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN113_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
346.1546	1	C21H20N3O2	346.1550	1.1	9.3	1	100.00	13.5	even	ok
368.1363	1	C21H19N3NaO2	368.1369	1.7	6.3	1	100.00	13.5	even	ok

38

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

39

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

8.95
8.94
8.92
8.91

8.43
8.41

7.51
7.51
7.51
7.49
7.49
7.47
7.46
7.45
7.44
7.43
7.39
7.38
7.37
7.37
7.36
7.35
7.26 CDCl3

3
2

2
3

8,7

10

9

1.00 1.00

1.03

2.16 1.13

9.05 9.00 8.95 8.90 8.85 8.80 8.75 8.70 8.65 8.60 8.55 8.50 8.45 8.40 8.35 8.30 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20
ppm

39

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

177.48
175.35

147.88
147.25
145.74
145.25
139.90
134.58
128.38
125.31
124.19
123.76
119.62

111.17

77.00 CDCl3

55.15
54.30

44.58

23.56

5
11

3,2
3
2

4a,11a
4a,11a 6a 5a

8
10
9

10a

10b

7

13
5',2'

12

4',3'

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

39

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

39

6-[2-(1-Pyrrolidinyl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

409

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN114_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 11:31:08 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-2.1min #1-122

Intens.
x10^5

347.1504

348.1534

350.3529

353.3527

354.3559

357.1321

360.3236

363.1451

367.1165

369.1325

370.1354

371.1381

$C_{20}H_{19}N_4O_2$, 347.1503

x10^5

1+
347.1503

1+
348.1532

1+
349.1559

$C_{20}H_{18}N_4NaO_2$, 369.1322

x10^5

1+
369.1322

1+
370.1352

1+
371.1379

39

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 11:31:08 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN114_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2300 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C
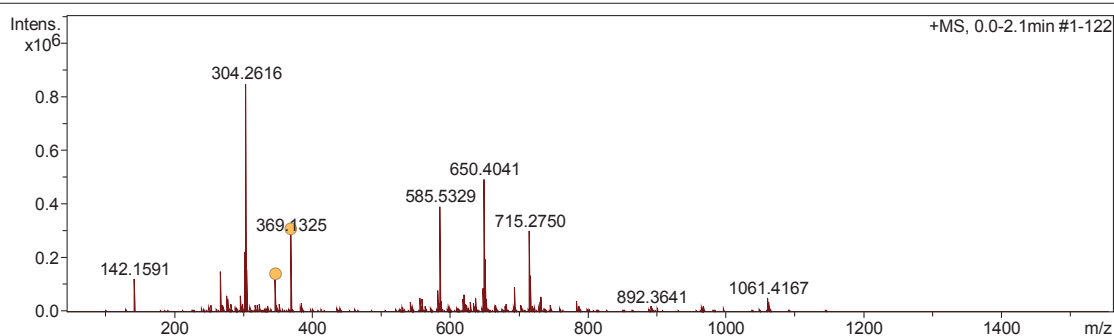
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
347.1504	1	C20H19N4O2	347.1503	-0.5	8.5	1	100.00	13.5	even	ok
369.1325	1	C20H18N4NaO2	369.1322	-0.8	8.4	1	100.00	13.5	even	ok
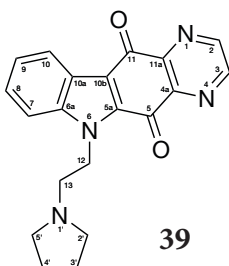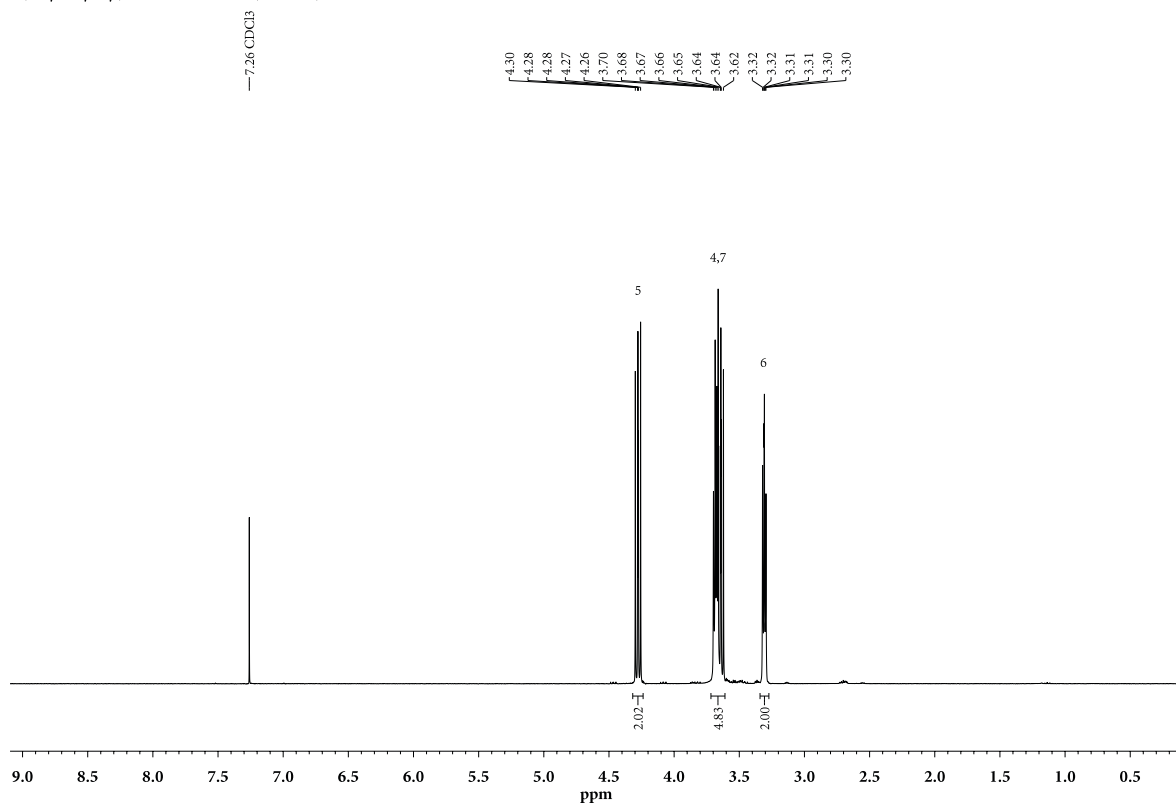
39

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (PROTON)

—7.26 CDCl3

4.30
4.28
4.28
4.27
4.26
3.70
3.68
3.67
3.66
3.65
3.64
3.64
3.62
3.32
3.32
3.31
3.31
3.30
3.30

5

4,7

6

2.02
4.83
2.00

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

40

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (PROTON)

4.30
4.28
4.28
4.27
4.26

3.70
3.68
3.67
3.66
3.65
3.64
3.64
3.62

3.32
3.32
3.31
3.31
3.30
3.30

5

4,7

6

2.02
4.83
2.00

4.30 4.25 4.20 4.15 4.10 4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 3.30 3.25
ppm
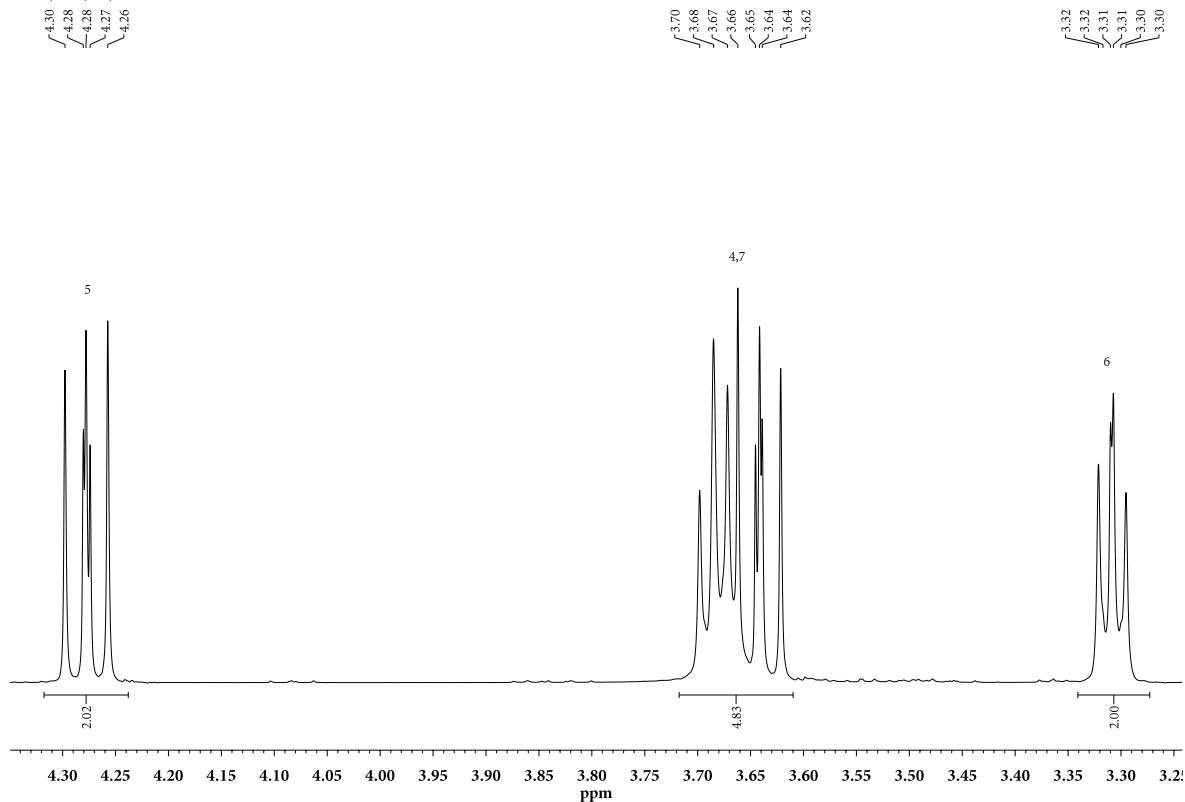
3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (C13 CPD)

40

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (HSQC)

413

3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (HMBC)

40
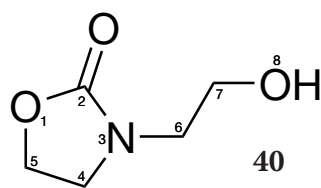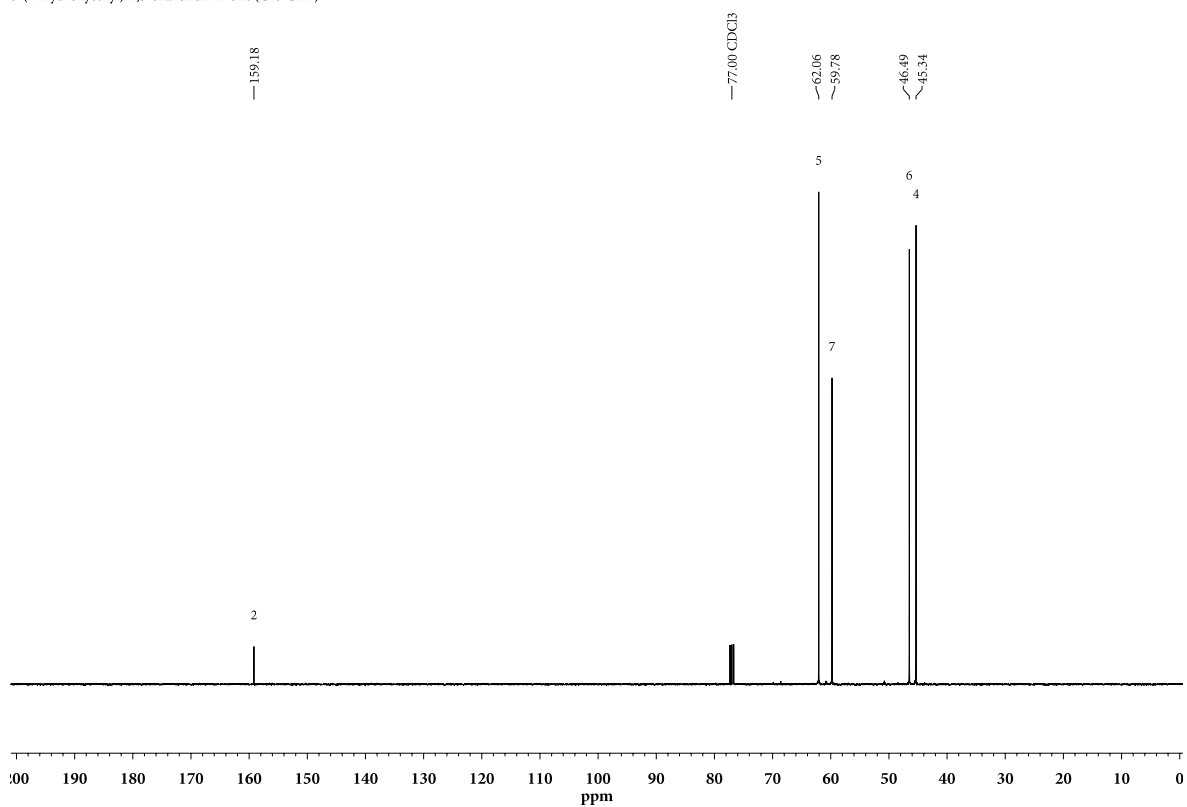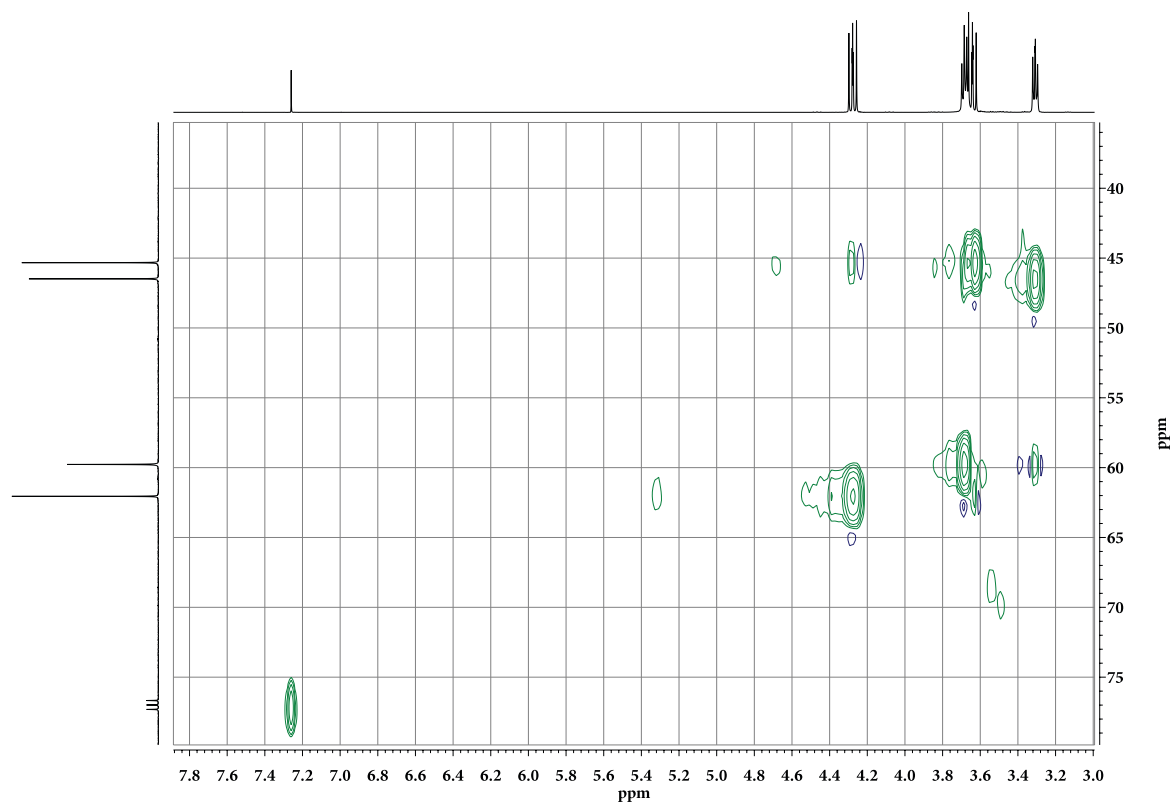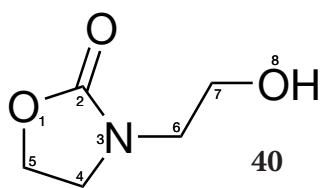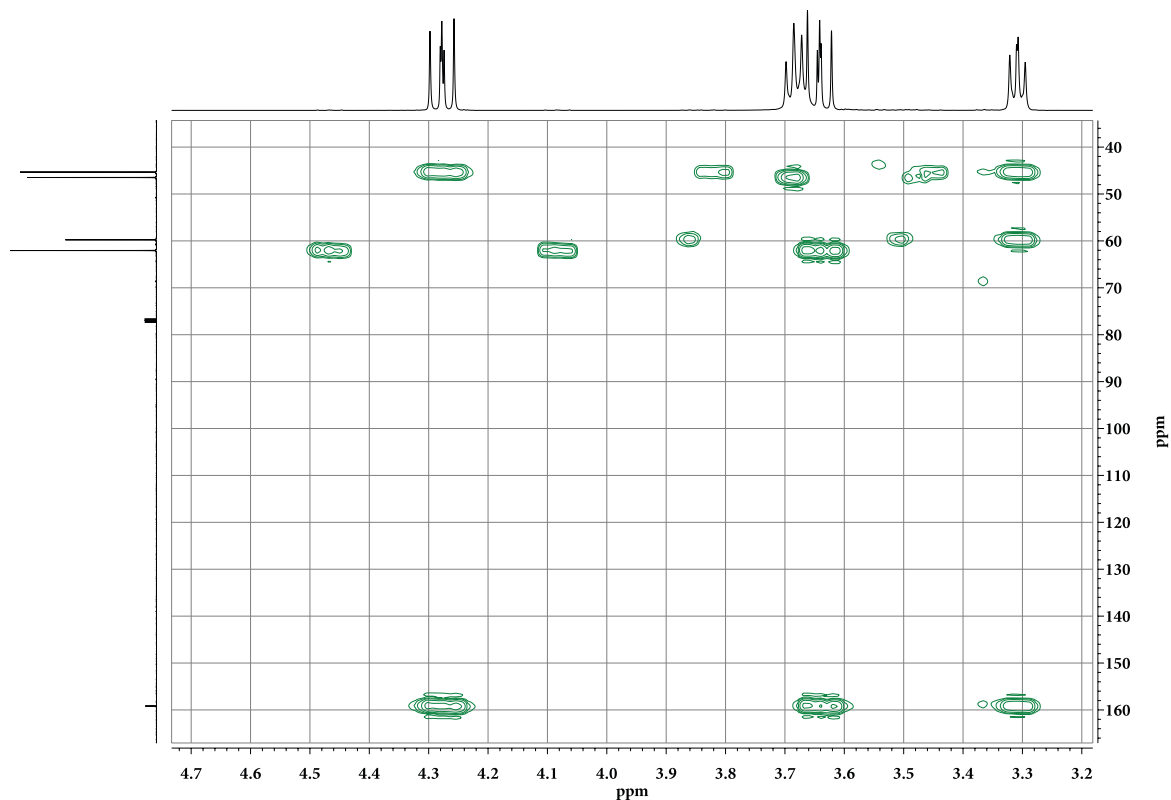
3-(2-Hydroxyethyl)-1,3-oxazolidin-2-one (15N HMBC)

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (PROTON)

—7.26 CDCl3

4.34
4.33
4.32
4.30 Impurity
4.28 Impurity
3.76 Impurity
3.75 Impurity
3.74 Impurity
3.71
3.70
3.69
3.67
3.66
3.66
3.65
3.65
3.64
3.63
3.63
3.59
3.58
3.58
3.57
3.56
3.55
3.55
3.37 Impurity
3.36 Impurity
3.34 Impurity

5

6
4,7

2.11
4.18
2.00

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

41

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (PROTON)

4.34
4.33
4.32
4.32
4.30 Impurity
4.28 Impurity
3.76 Impurity
3.75 Impurity
3.74 Impurity
3.71
3.70
3.69
3.69
3.67
3.67
3.66
3.66
3.65
3.65
3.64
3.63
3.63
3.59
3.58
3.58
3.57
3.57
3.56
3.56
3.55
3.55

5

4,7

6

2.11

4.18

2.00

4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50
ppm

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (C13 APT)

—158.31

—77.00 CDCl3

61.95
60.40 Impurity

46.74 Impurity
46.00
45.45
41.81

2

5

6
4 7

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

41

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (HSQC)

30
35
40
45
50
55
60
65
70
ppm

5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4
ppm

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (HMBC)

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (15N HMBC)

417

3-(2-Chloroethyl)-1,3-oxazolidin-2-one (15N HMBC)

41

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

42

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

42

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (COSY)

42

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 1:01:07 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN132_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 1:01:07 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN132_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.8min #1-103

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
362.1135	1	C20H16N3O4	362.1135	0.2	2.5	1	100.00	14.5	even	ok
384.0955	1	C20H15N3NaO4	384.0955	-0.2	6.7	1	100.00	14.5	even	ok
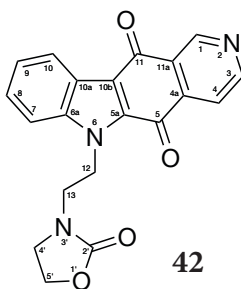
42

6-{2-[(2-Hroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

43
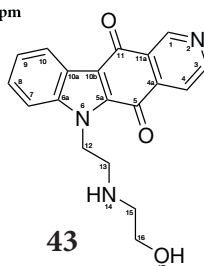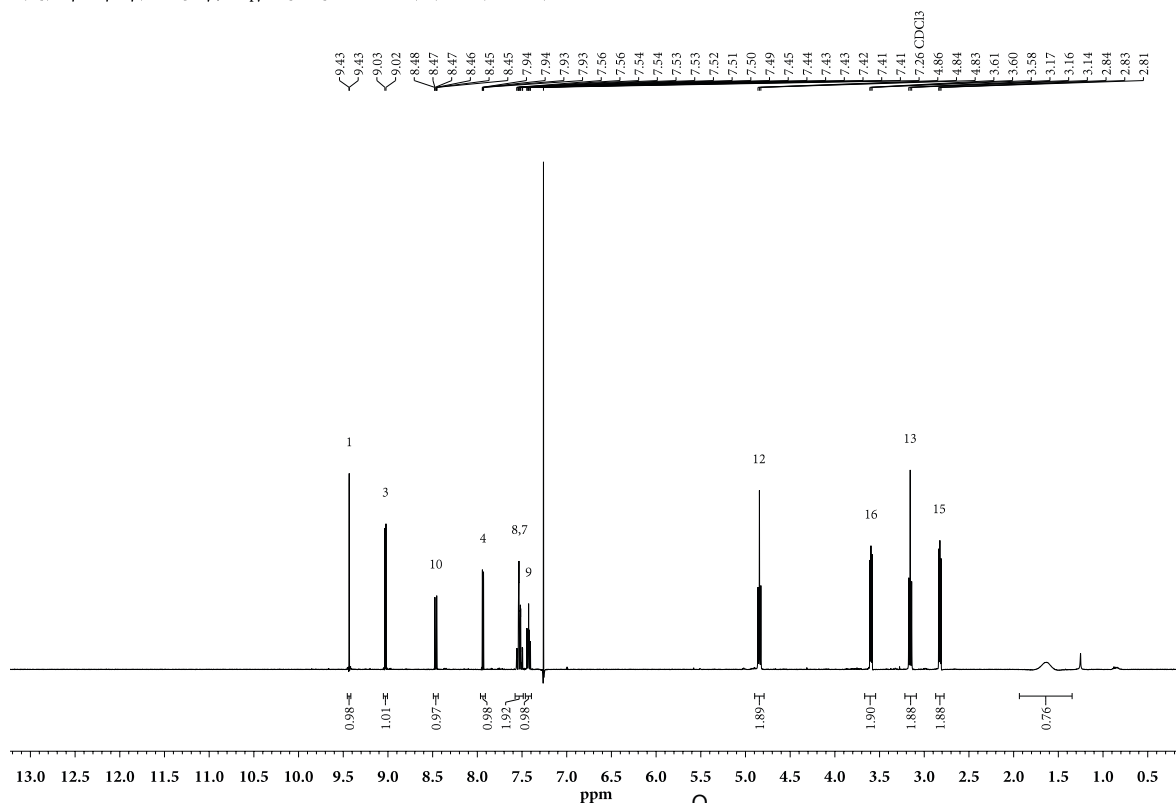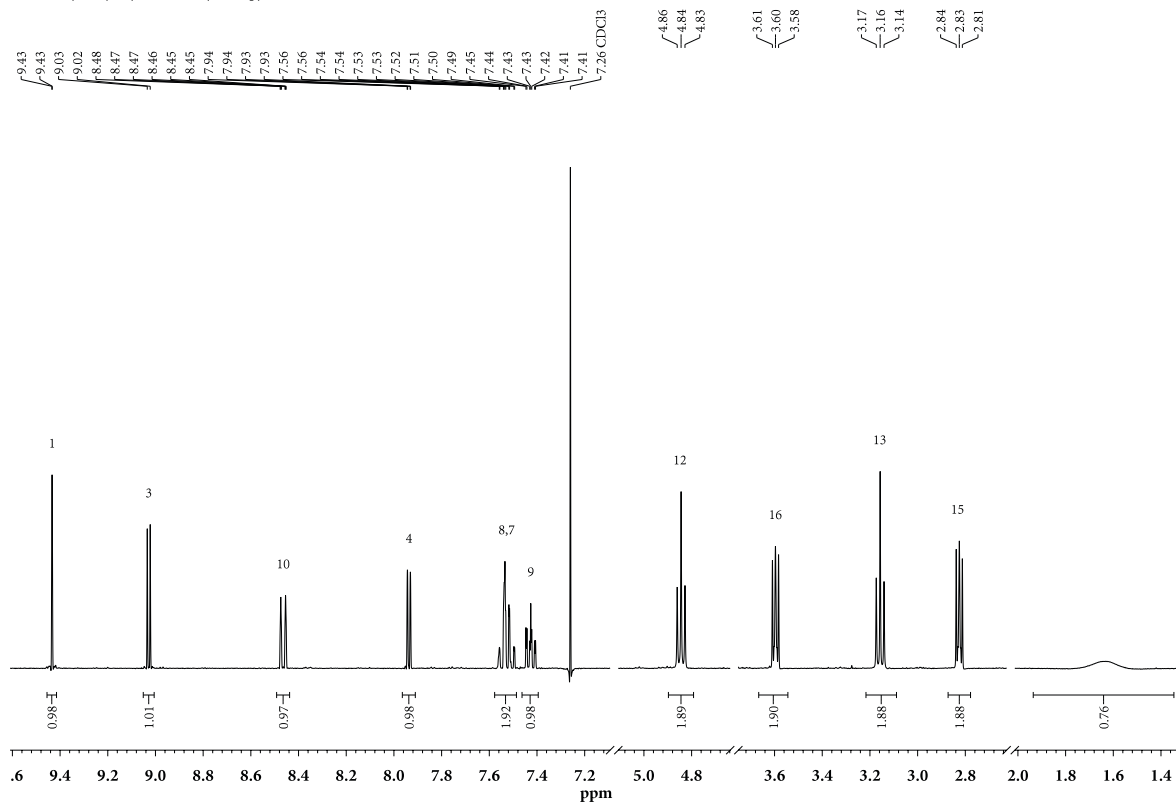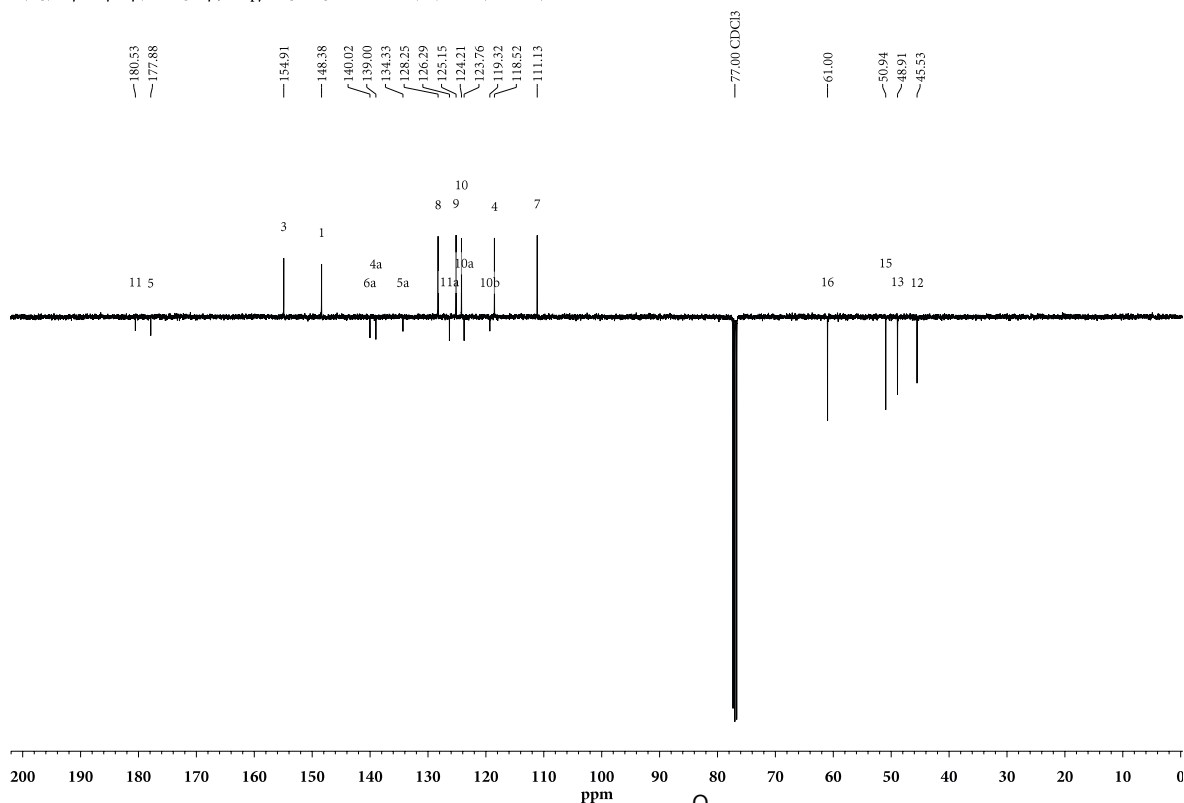
6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)
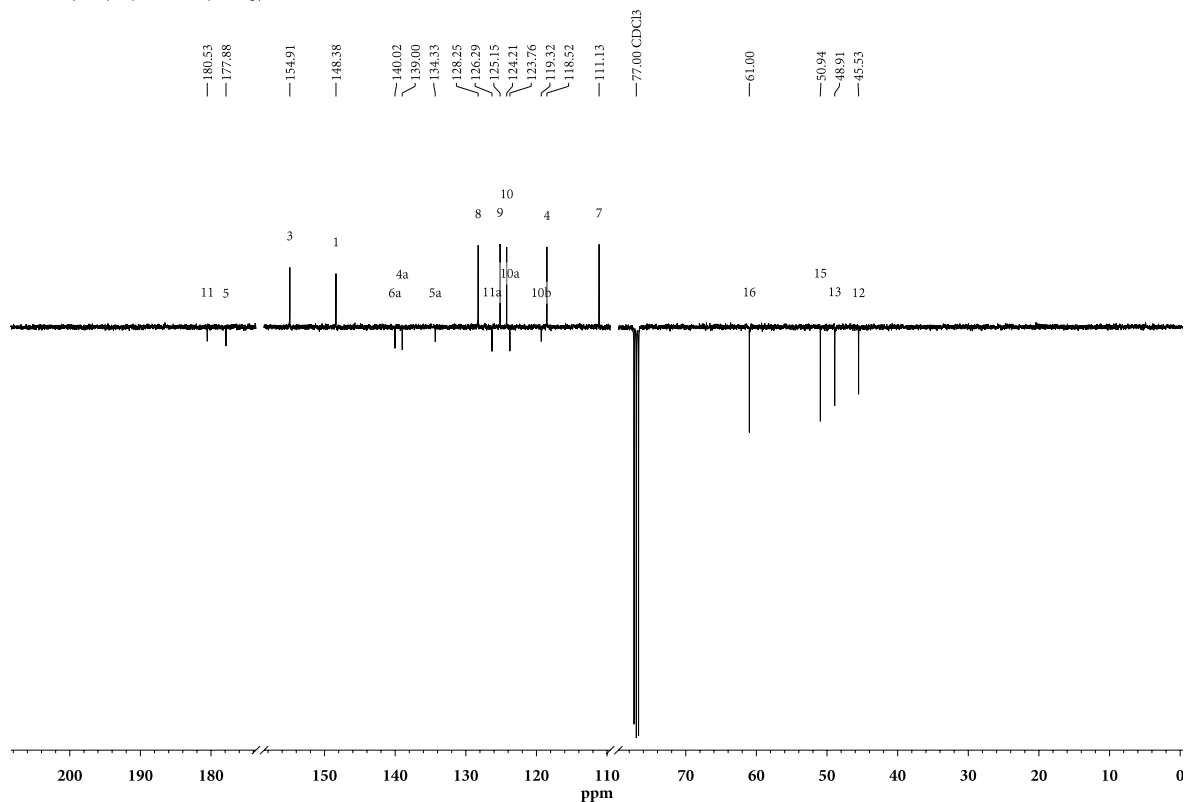
6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

43

6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

43
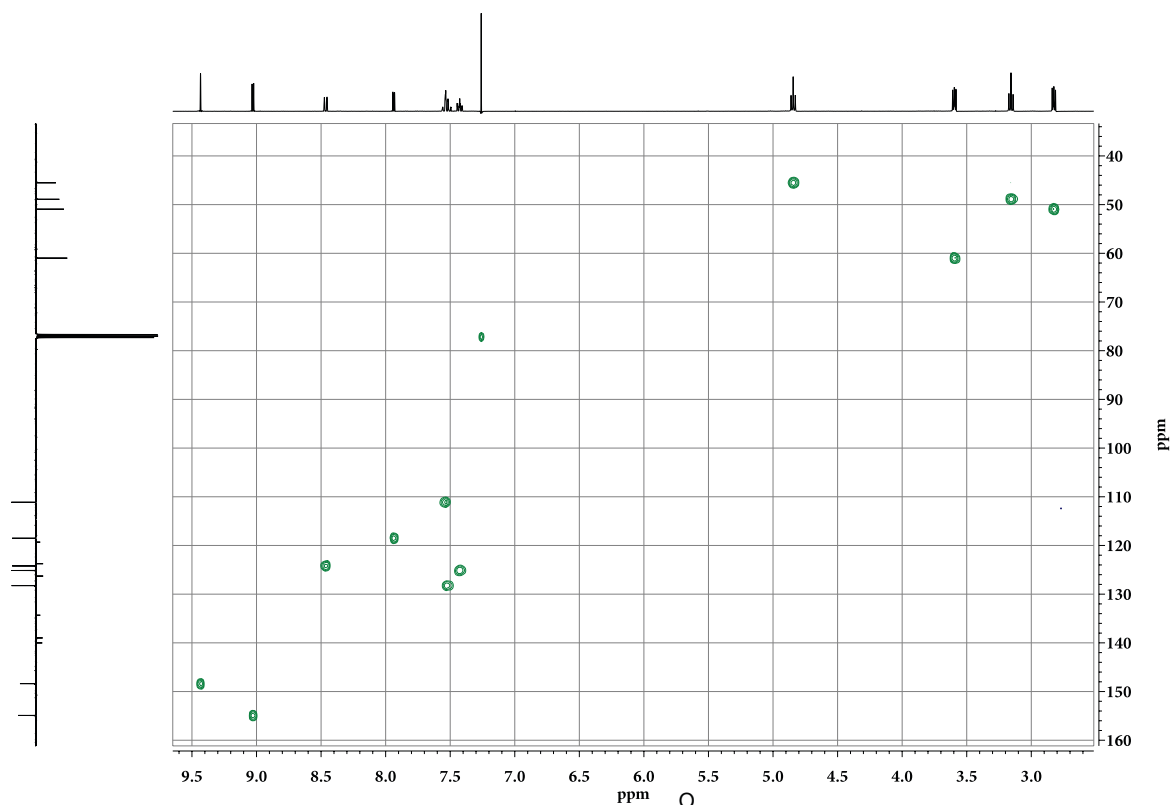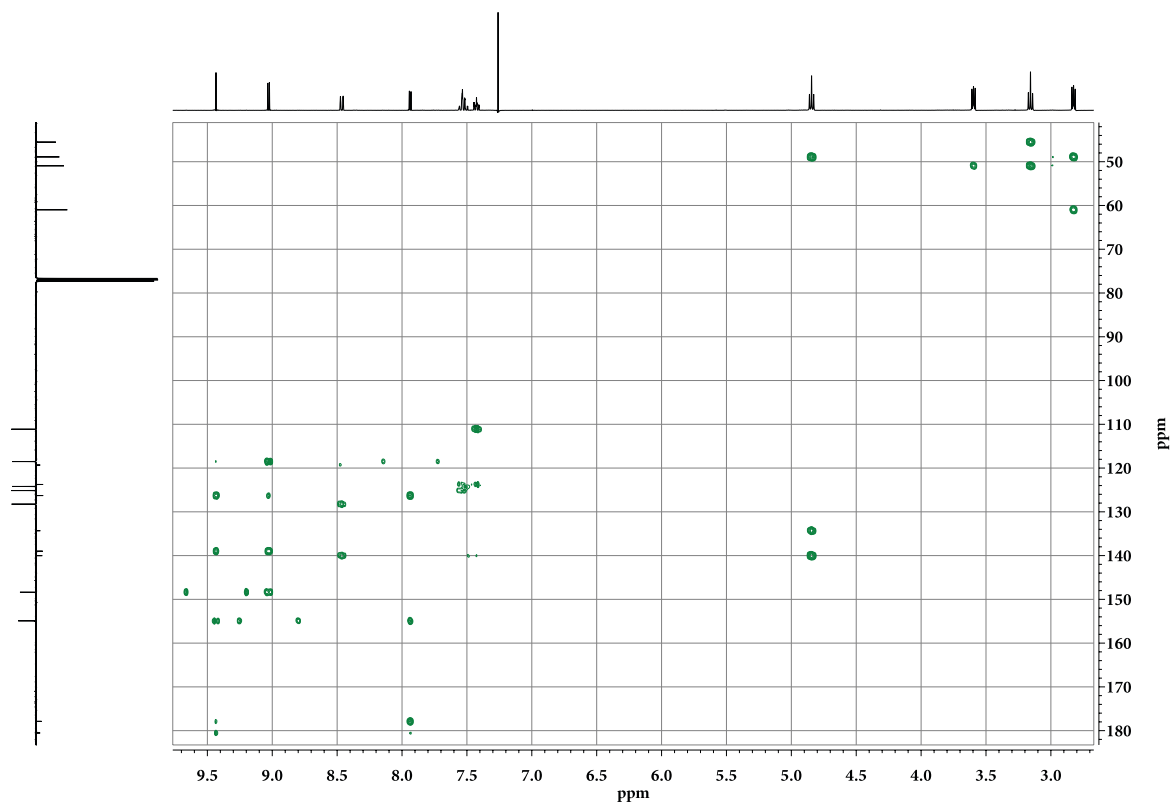
6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

6-{2-[(2-Hydroxyethyl)amino]ethyl}-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (COSY)

43

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN152_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 12:30:33 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 12:30:33 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN152_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.3min #1-134

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
336.1347	1	C19H18N3O3	336.1343	-1.2	11.2	1	100.00	12.5	even	ok
358.1165	1	C19H17N3NaO3	358.1162	-0.8	6.8	1	100.00	12.5	even	ok

43

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

44

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HSQC)

44

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (HMBC)

436

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

6-[2-(2-Oxo-1,3-oxazolidin-3-yl)ethyl]-5H-pyrazino[2,3-b]carbazole-5,11(6H)-dione (15N HMBC)

44

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 2:05:44 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN164_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Intens.
x10^5

+MS, 0.0-1.6min #1-90

385.0908

386.0938

363.1087
369.2976
382.4404
387.0962

C$_{19}$H$_{15}$N$_4$O$_4$, 363.1088

1+
363.1088

1+
364.1118

1+
365.1142

C$_{19}$H$_{14}$N$_4$NaO$_4$, 385.0907

1+
385.0907

1+
386.0937

1+
387.0962

x10^5

44

365   370   375   380   385   m/z

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 2:05:44 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN164_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar		
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C		
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min		
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste		
		Set Corona	0 nA	Set APCI Heater	0 °C		

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
363.1087	1	C19H15N4O4	363.1088	0.3	19.8	1	100.00	14.5	even	ok
385.0908	1	C19H14N4NaO4	385.0907	-0.3	11.7	1	100.00	14.5	even	ok

44

N,N-Diethyl-1H-indole-3-carboxamide (PROTON)

45

N,N-Diethyl-1H-indole-3-carboxamide (PROTON)

N,N-Diethyl-1H-indole-3-carboxamide (PROTON)

7.70
7.70
7.69
7.69
7.68
7.68

7.58
7.58

7.42
7.42
7.41
7.40
7.40

7.15
7.15
7.13
7.13
7.11
7.11
7.08
7.07
7.06
7.06
7.05
7.04
7.04

4

2

7

6

5

1.00

1.01

1.00

1.01

1.01

'.80   7.75   7.70   7.65   7.60   7.55   7.50   7.45   7.40   7.35   7.30   7.25   7.20   7.15   7.10   7.05   7.00   6.95

ppm

45

N,N-Diethyl-1H-indole-3-carboxamide (C13 CPD)

165.63

135.51
126.48
125.79
121.73
120.31
119.84
111.70
110.43

40.77
39.50 DMSO

13.79

8

7a

3a
2

4
6 5

3
7

10',10

11,11'

200   190   180   170   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0

ppm

N,N-Diethyl-1H-indole-3-carboxamide (C13 CPD)

45

N,N-Diethyl-1H-indole-3-carboxamide (HSQC)

N,N-Diethyl-1H-indole-3-carboxamide (HMBC)

45

N,N-Diethyl-1H-indole-3-carboxamide (15N HSQC)

N,N-Diethyl-1H-indole-3-carboxamide (15N HMBC)

45

N,N-Diethyl-1H-indole-3-carboxamide (NOESY)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (PROTON)

46

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (PROTON)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (PROTON)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (PROTON)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (C13 CPD)

46

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (C13 CPD)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (HSQC)

46

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (HMBC)

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (15N HMBC)

46

N,N-Diethyl-1-(methoxymethyl)-1H-indole-3-carboxamide (NOESY)

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (PROTON)

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (PROTON)
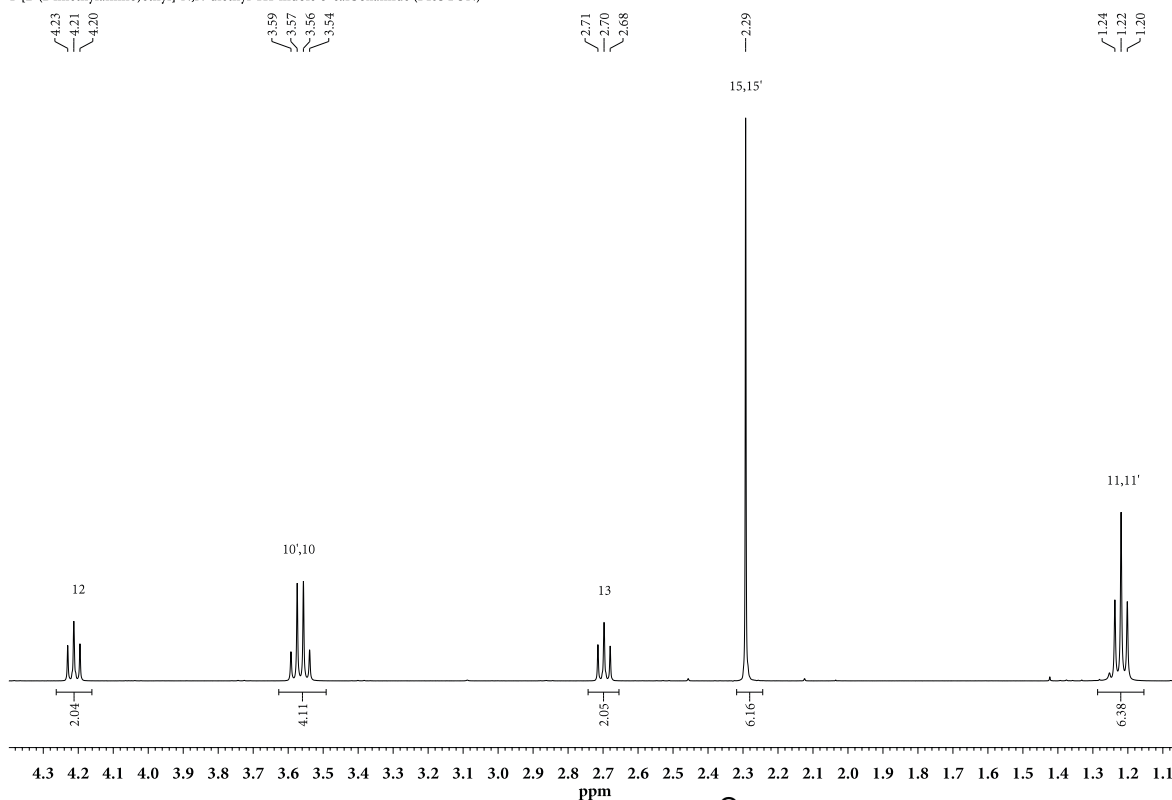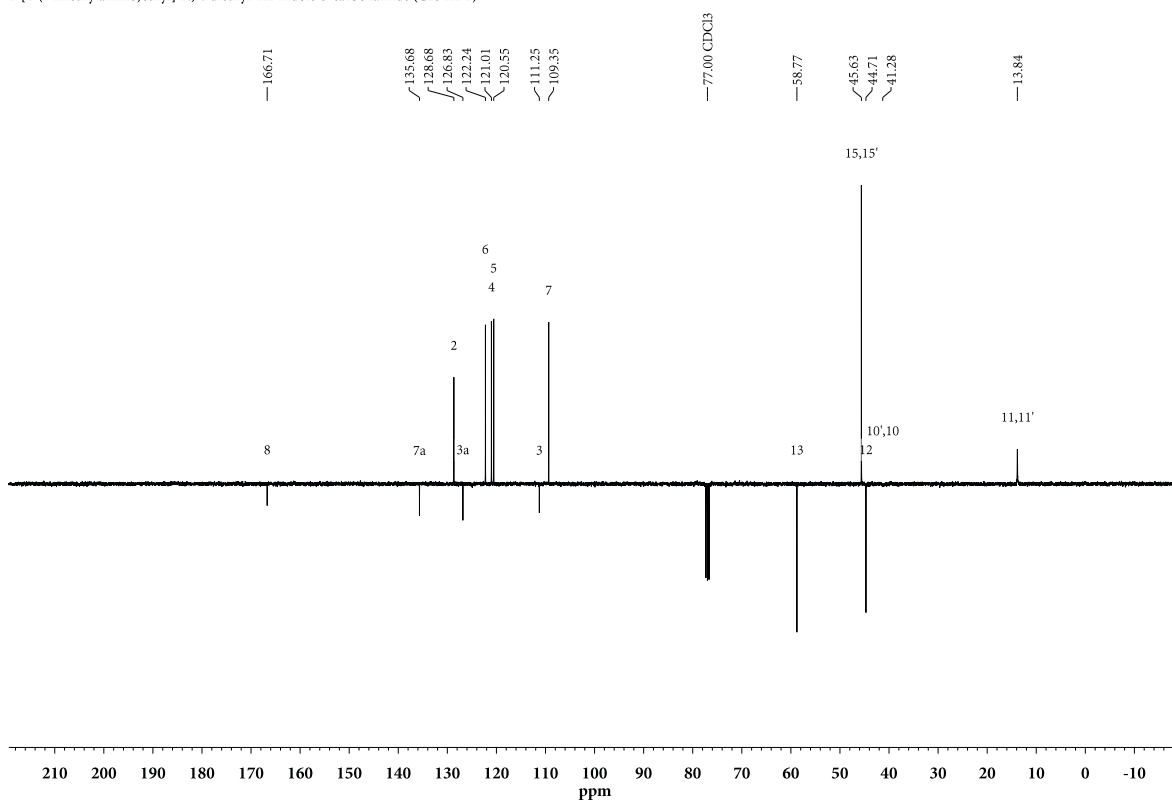
450

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (PROTON)

4.23
4.21
4.20

3.59
3.57
3.56
3.54

2.71
2.70
2.68

2.29

1.24
1.22
1.20

15,15'

11,11'

10',10

12

13

2.04

4.11

2.05

6.16

6.38

4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1
ppm

47

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (C13 APT)

166.71

135.68
128.68
126.83
122.24
121.01
120.55

111.25
109.35

77.00 CDCl3

58.77

45.63
44.71
41.28

13.84

15,15'

6
5
4

7

2

8

7a

3a

3

13

10',10
12

11,11'

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

451

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (C13 APT)

47
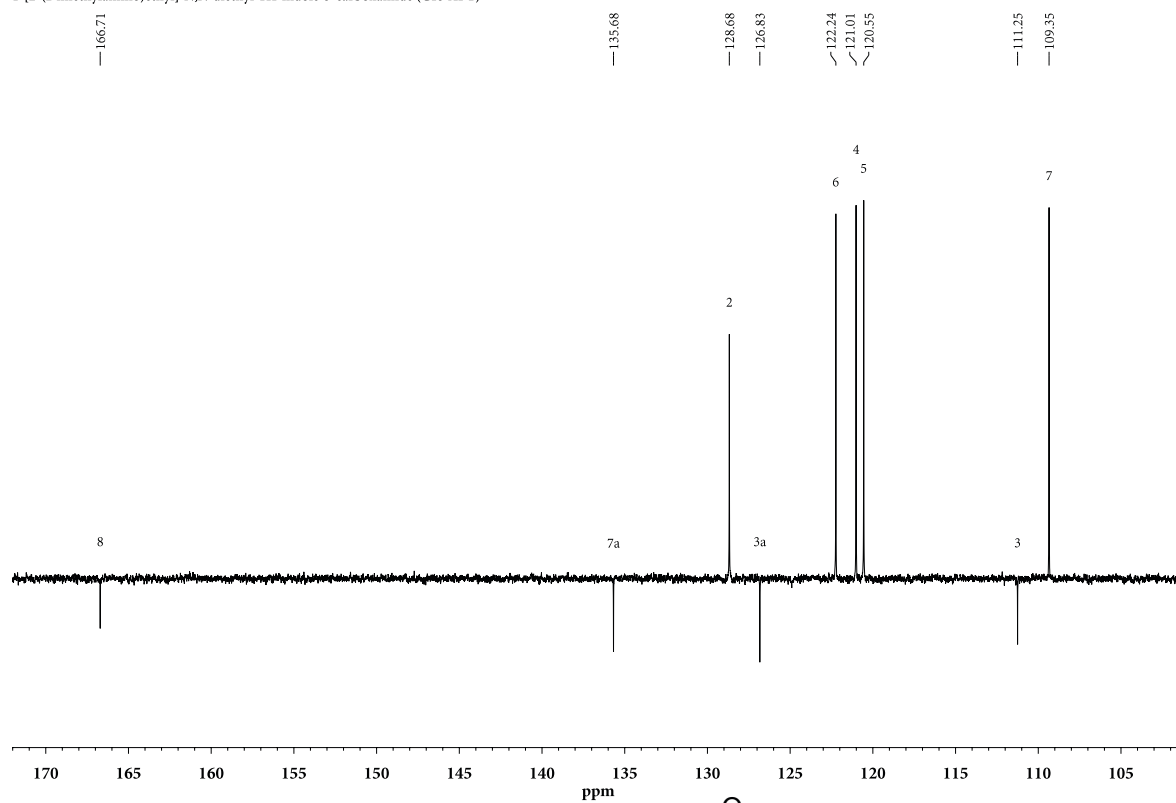
1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (HSQC)

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (HMBC)
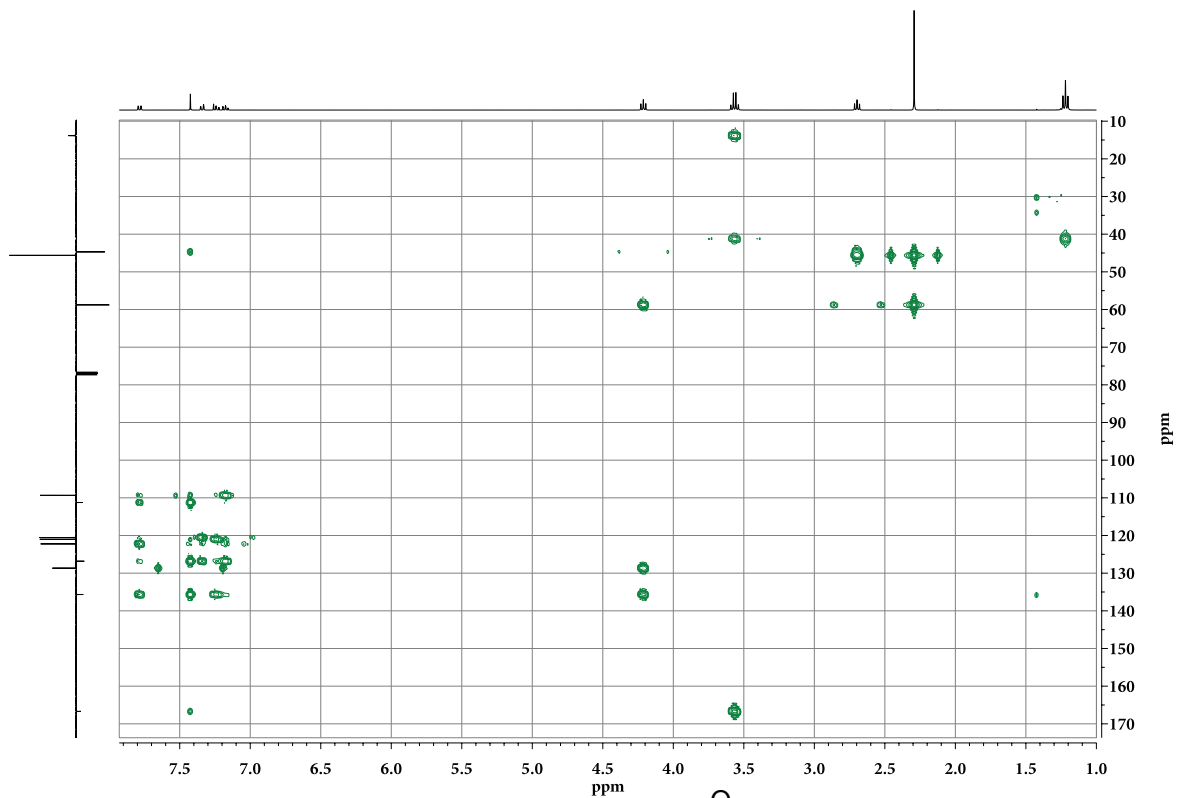
47

1-[2-(Dimethylamino)ethyl]-N,N-diethyl-1H-indole-3-carboxamide (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 2:03:54 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN078_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:200 in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.5min #1-86

310.1892

288.2072

302.2226 304.2616 307.1808

$C_{17}H_{26}N_3O$, 288.2070

1+
288.2070

$C_{17}H_{25}N_3NaO$, 310.1890

1+
310.1890

47

Mass Spectrum SmartFormula Report
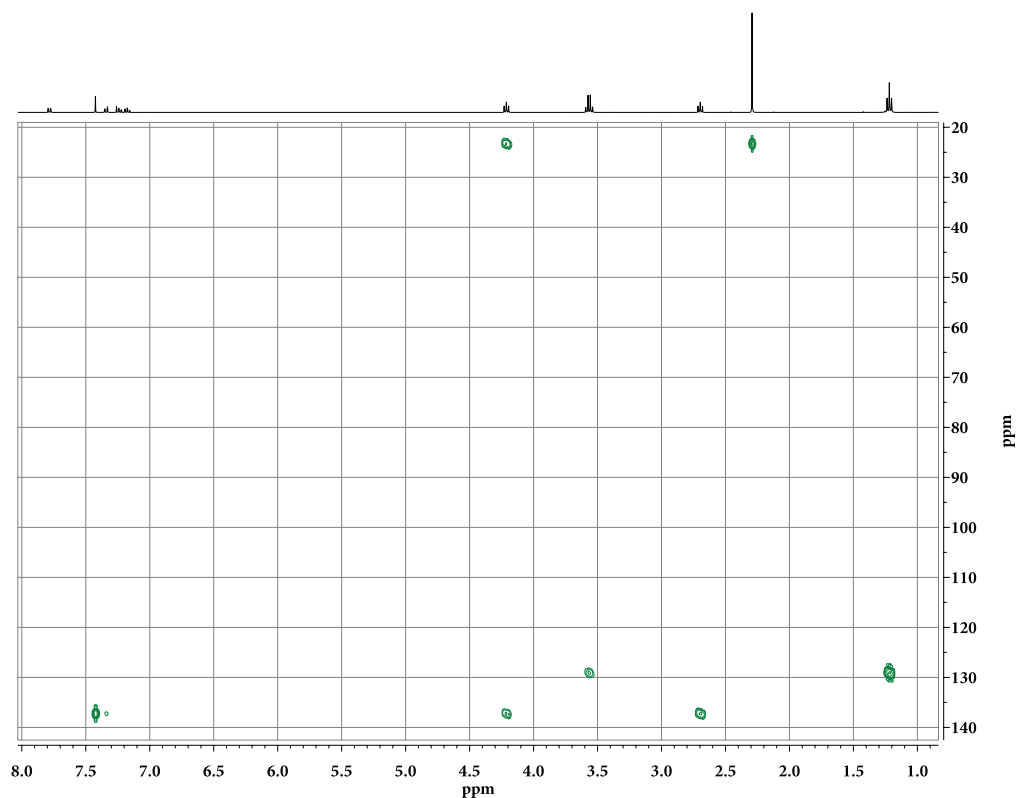
Analysis Info
Acquisition Date 1/12/2015 2:03:54 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN078_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:200 in MeOH
Comment	

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1400 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C
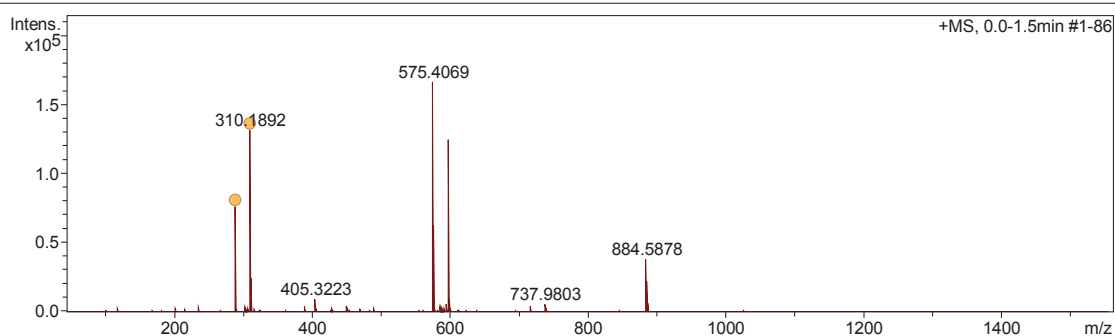
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
288.2072	1	C17H26N3O	288.2070	-0.4	8.0	1	100.00	6.5	even	ok
310.1892	1	C17H25N3NaO	310.1890	-0.8	8.4	1	100.00	6.5	even	ok

47

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

48

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 APT)

48

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 APT)

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (HSQC)

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (HMBC)

48

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (15N HMBC)

6-(Methoxymethyl)-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (15N HMBC)

48

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 6:41:17 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN176_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info　　　　　　　　　　　　　　　　　　　　Acquisition Date　11/17/2014 6:41:17 PM

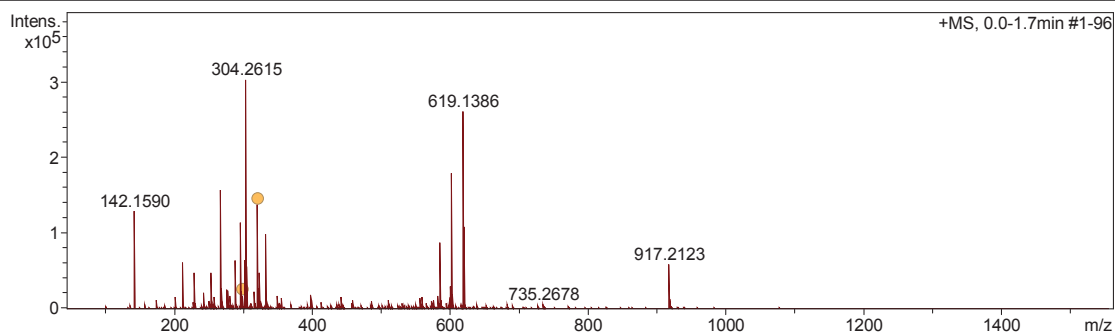|	
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN176_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator　　MZ
Instrument　maXis HD　　1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.5min #1-86

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
294.0873	1	C16H12N3O3	294.0873	0.2	13.5	1	100.00	12.5	even	ok
316.0696	1	C16H11N3NaO3	316.0693	-1.0	9.2	1	100.00	12.5	even	ok
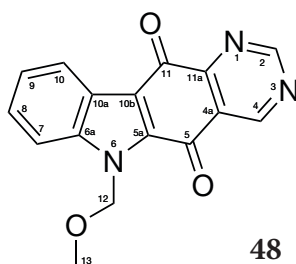
48

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)
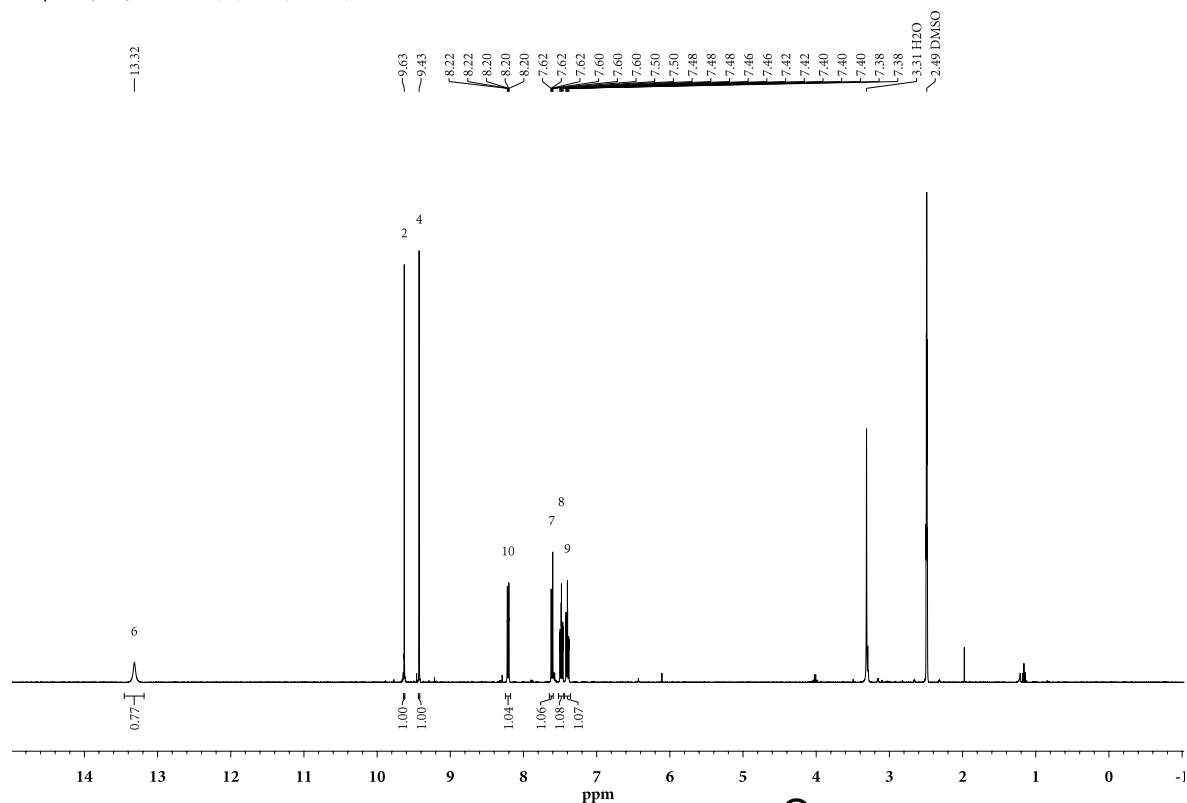
5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)
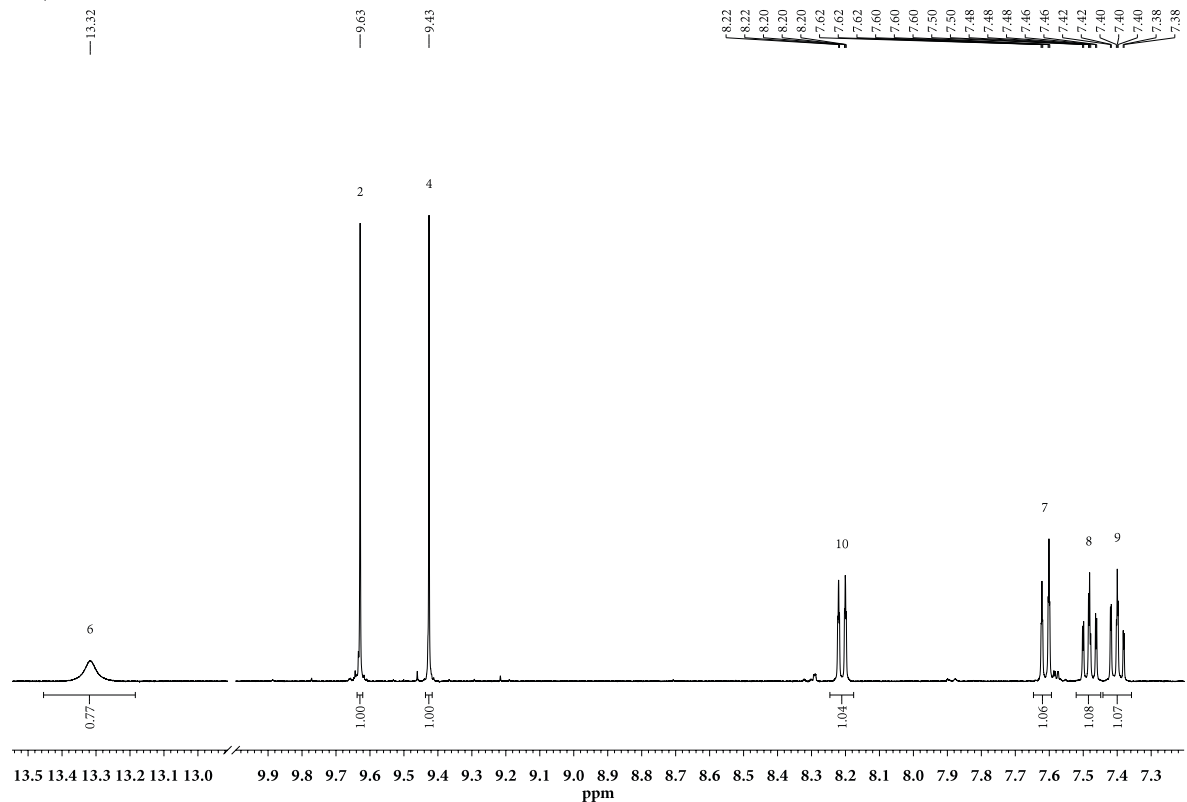
5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 CPD)

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 CPD)

49

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (HSQC)

49

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (HMBC)

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (15N HMBC)

5H-Pyrimido[5,4-b]carbazole-5,11(6H)-dione (15N HSQC)

49

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 1:27:30 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN181_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.8min #1-100

C₁₄H₈N₃O₂, 250.0611

C₁₄H₇N₃NaO₂, 272.0430

49

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/12/2015 1:27:30 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN181_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD	1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
250.0610	1	C14H8N3O2	250.0611	0.4	n.a.	1	100.00	12.5	even	ok
272.0433	1	C14H7N3NaO2	272.0430	-0.8	11.7	1	100.00	12.5	even	ok

49

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

50

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

50

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 APT)

50

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (HSQC)

50

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(Dimethylamino)ethyl]-5H-pyrimido[5,4-b]carbazole-5,11(6H)-dione (15N HMBC)

50

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 5:23:11 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN177_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.9min #1-106

321.1350

343.1167

316.9833 318.2405 324.3375 332.3313 336.2511

$C_{18}H_{17}N_4O_2$, 321.1346

1+
321.1346

$C_{18}H_{16}N_4NaO_2$, 343.1165

1+
343.1165

50

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 1/12/2015 5:23:11 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN177_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:500 in MeOH
Comment	

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Intens.
x10⁵

+MS, 0.0-1.9min #1-106

107.0409
321.1350
191.0927
641.2619
983.5804

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
321.1350	1	C18H17N4O2	321.1346	-1.2	10.7	1	100.00	12.5	even	ok
343.1167	1	C18H16N4NaO2	343.1165	-0.6	5.6	1	100.00	12.5	even	ok

50

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (C13 APT)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (C13 APT)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (C13 APT)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (HSQC)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (HMBC)

Ethyl 3-[hydroxy(5-pyrimidinyl)methyl]-1H-indole-2-carboxylate (15N HMBC)

51

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN160_DI_ESI_HRMS.d	Acquisition Date	11/17/2014 5:27:54 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Intens. x10⁵ — +MS, 0.0-1.7min #1-96

296.3062, 298.1186, 300.2296, 302.2455, 303.2488, 304.2613, 305.2644, 306.2729, 310.3219, 312.3376, 316.3210, 317.3243, 320.1007, 321.1037, 322.1062

x10⁴ — C₁₆H₁₆N₃O₃, 298.1186

1+ 298.1186
1+ 299.1216
1+ 300.1241

x10⁵ — C₁₆H₁₅N₃NaO₃, 320.1006

1+ 320.1006
1+ 321.1036
1+ 322.1060

51

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN160_DI_ESI_HRMS.d	
Method	DI_mz_50-1550.m	
Sample Name	0.1 mg/mL in MeOH	
Comment		

Acquisition Date 11/17/2014 5:27:54 PM

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
298.1186	1	C16H16N3O3	298.1186	0.1	7.4	1	100.00	10.5	even	ok
320.1007	1	C16H15N3NaO3	320.1006	-0.5	6.1	1	100.00	10.5	even	ok
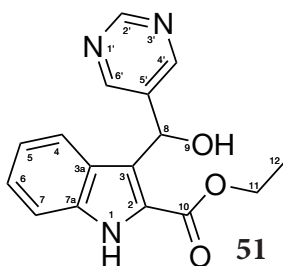
51

Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)
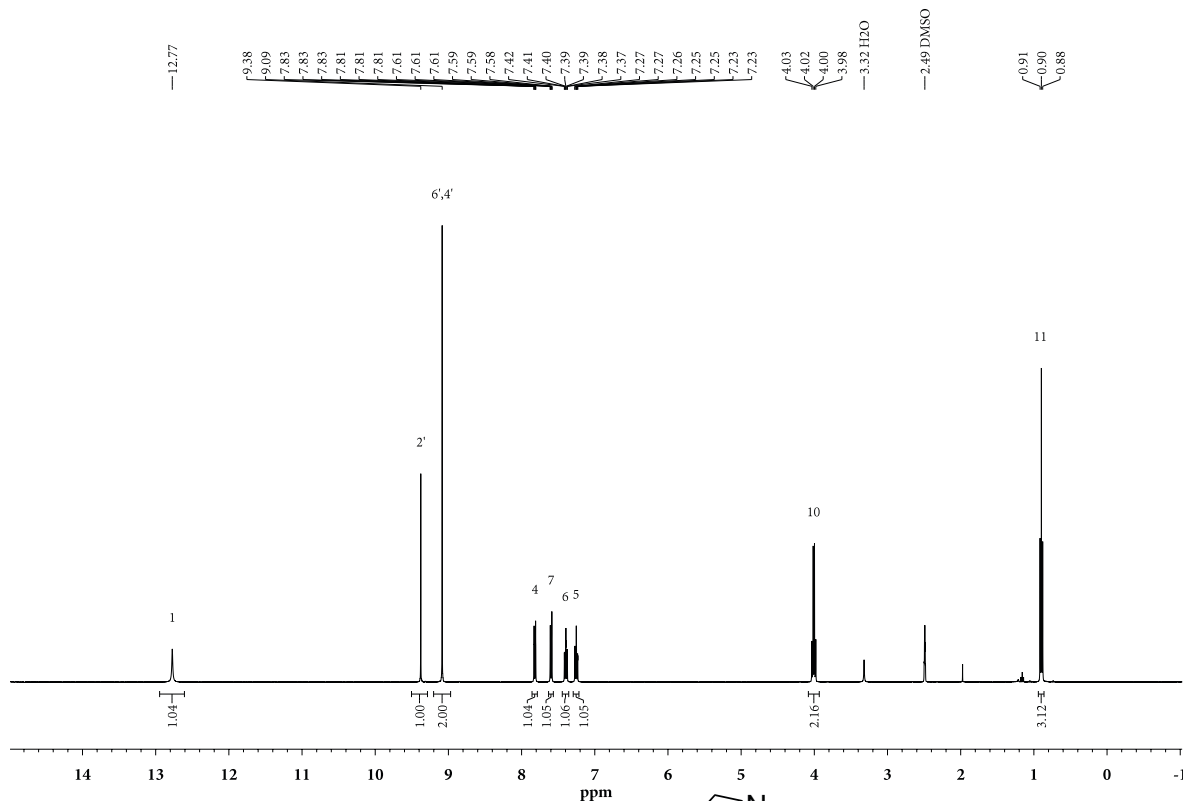
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)
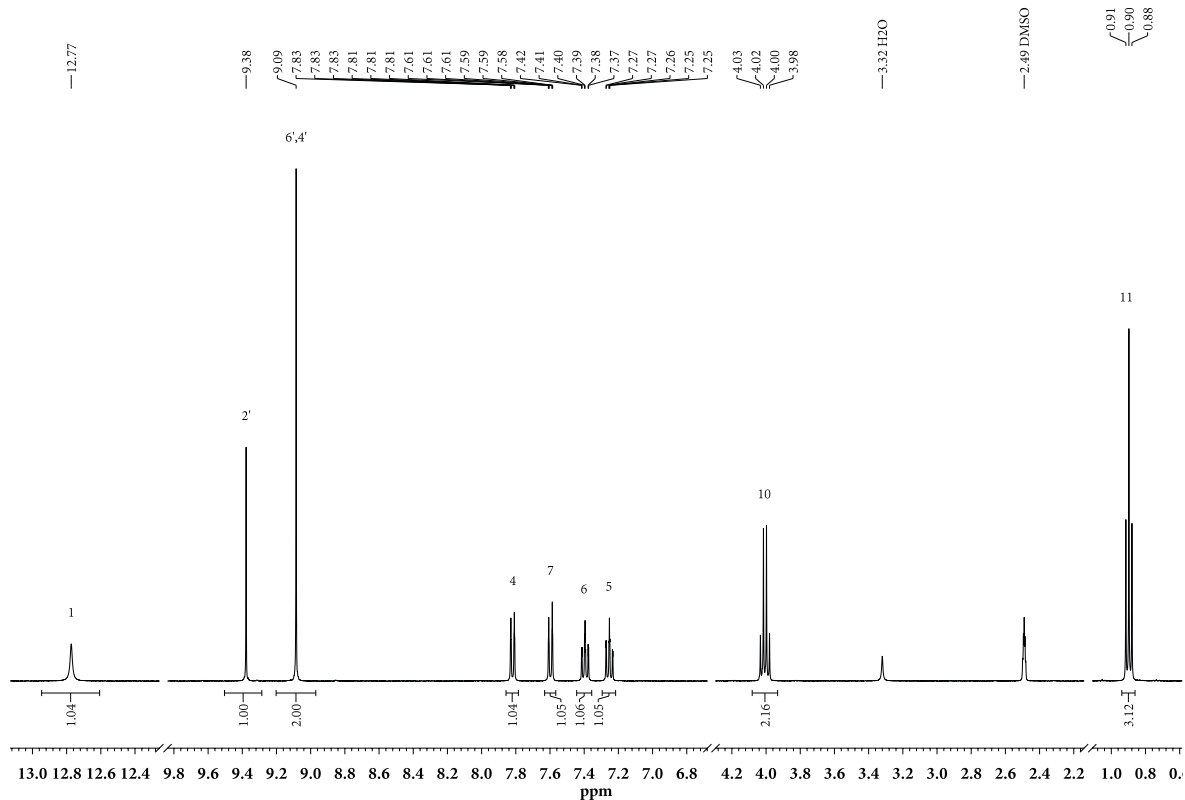
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (C13 APT)
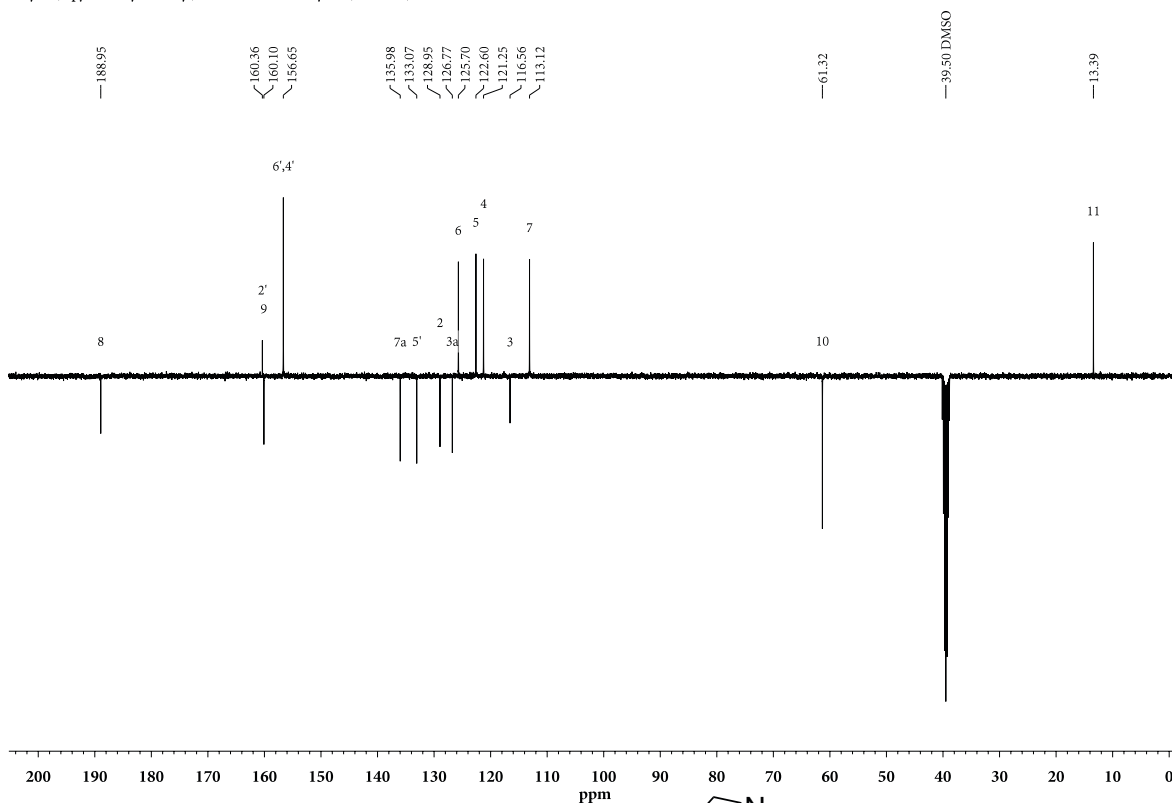
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (C13 APT)
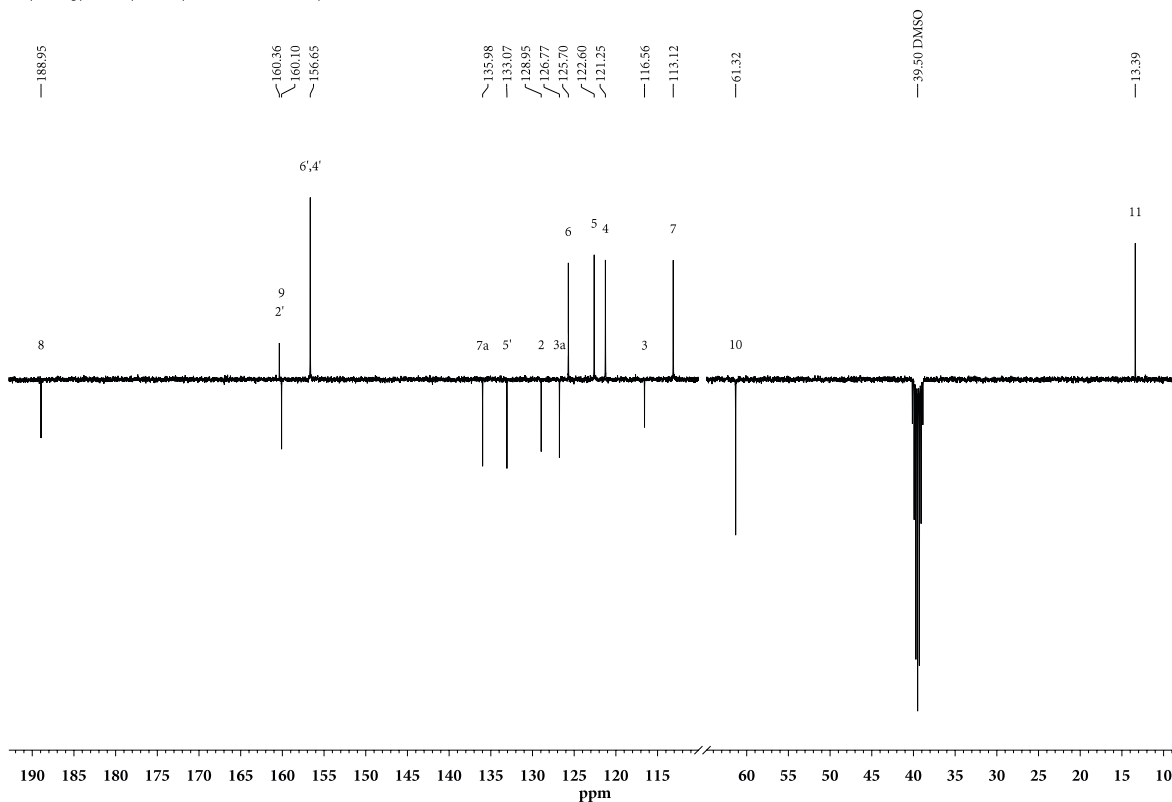
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (HSQC)
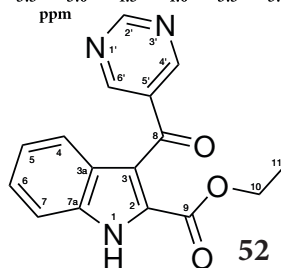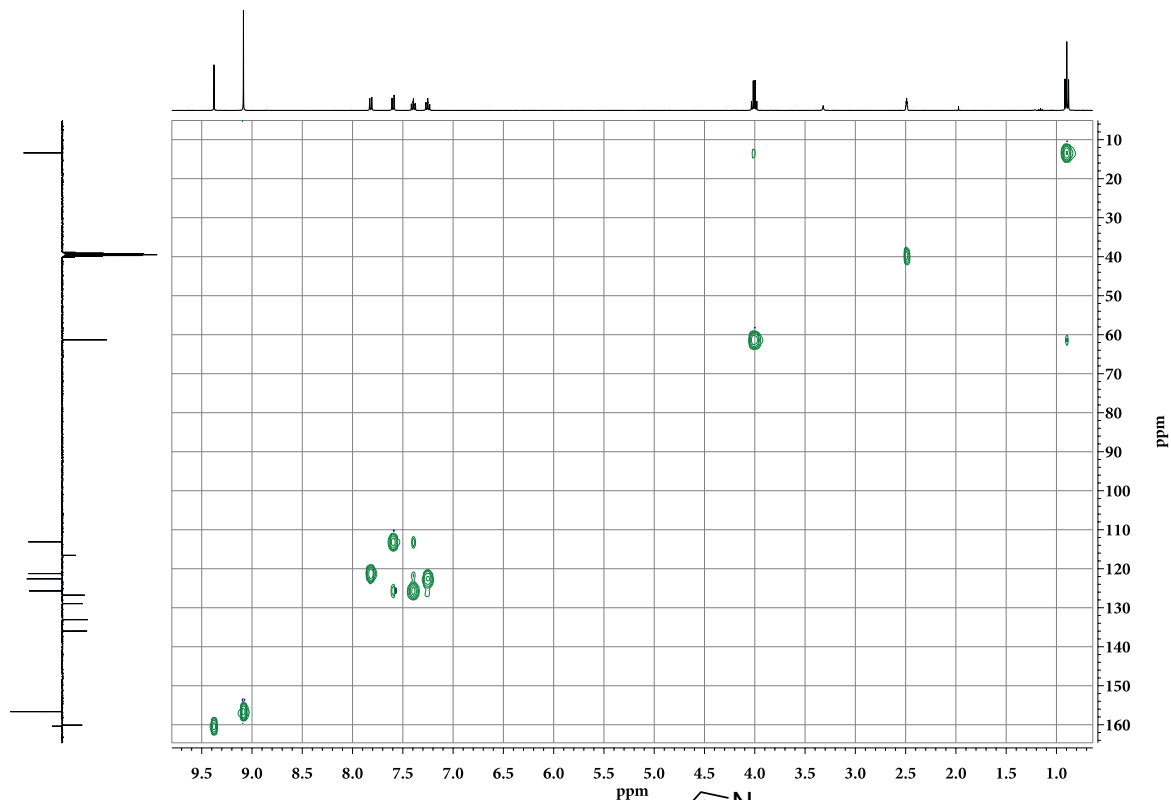
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (HMBC)
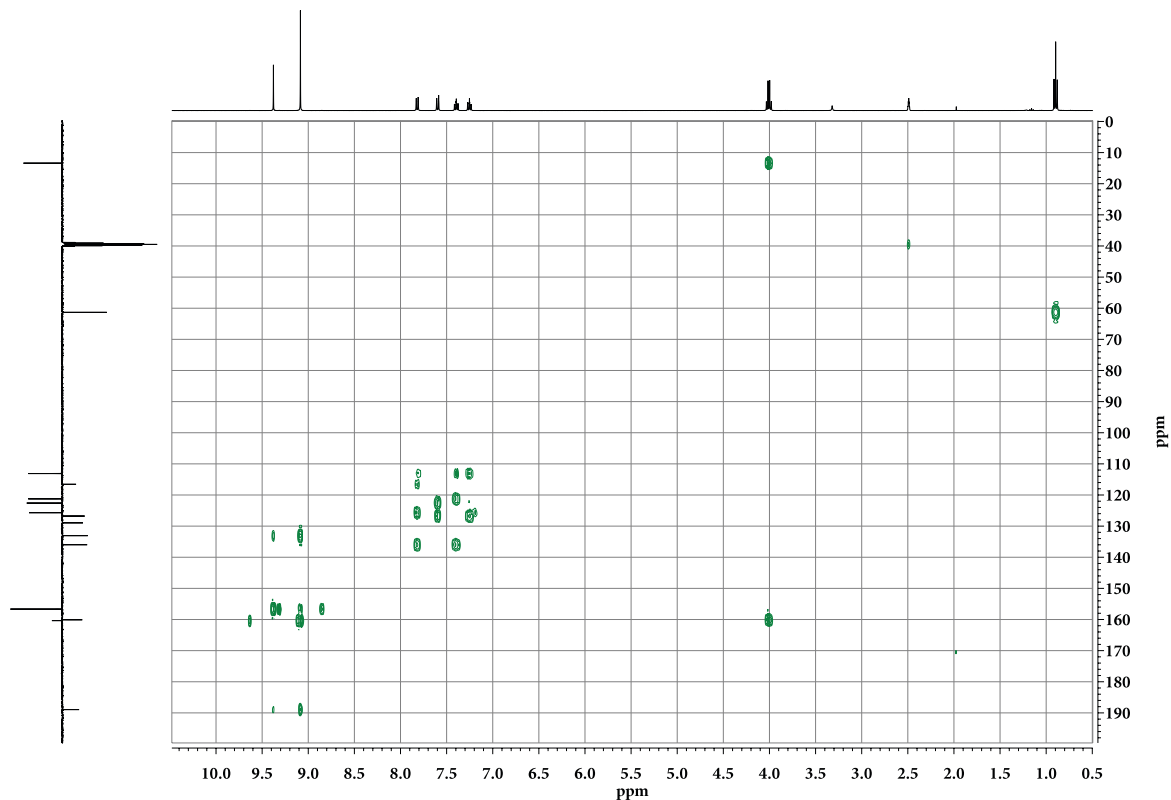
Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (15N HMBC)

Ethyl 3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (15N HSQC)

52

487

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN159_DI_ESI_HRMS.d	Acquisition Date	11/17/2014 5:19:59 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-3.3min #1-185

304.2613

318.0851

302.2455

296.3061

305.2644

297.3093 300.2297 303.2487 306.2727 308.1648 311.2557 313.3319 316.3209 319.0880 320.2556

$C_{16}H_{14}N_3O_3$, 296.1030

1+
296.1030

1+
297.1060

1+
298.1084

$C_{16}H_{13}N_3NaO_3$, 318.0849

1+
318.0849

1+
319.0879

1+
320.0904

52

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/17/2014 5:19:59 PM

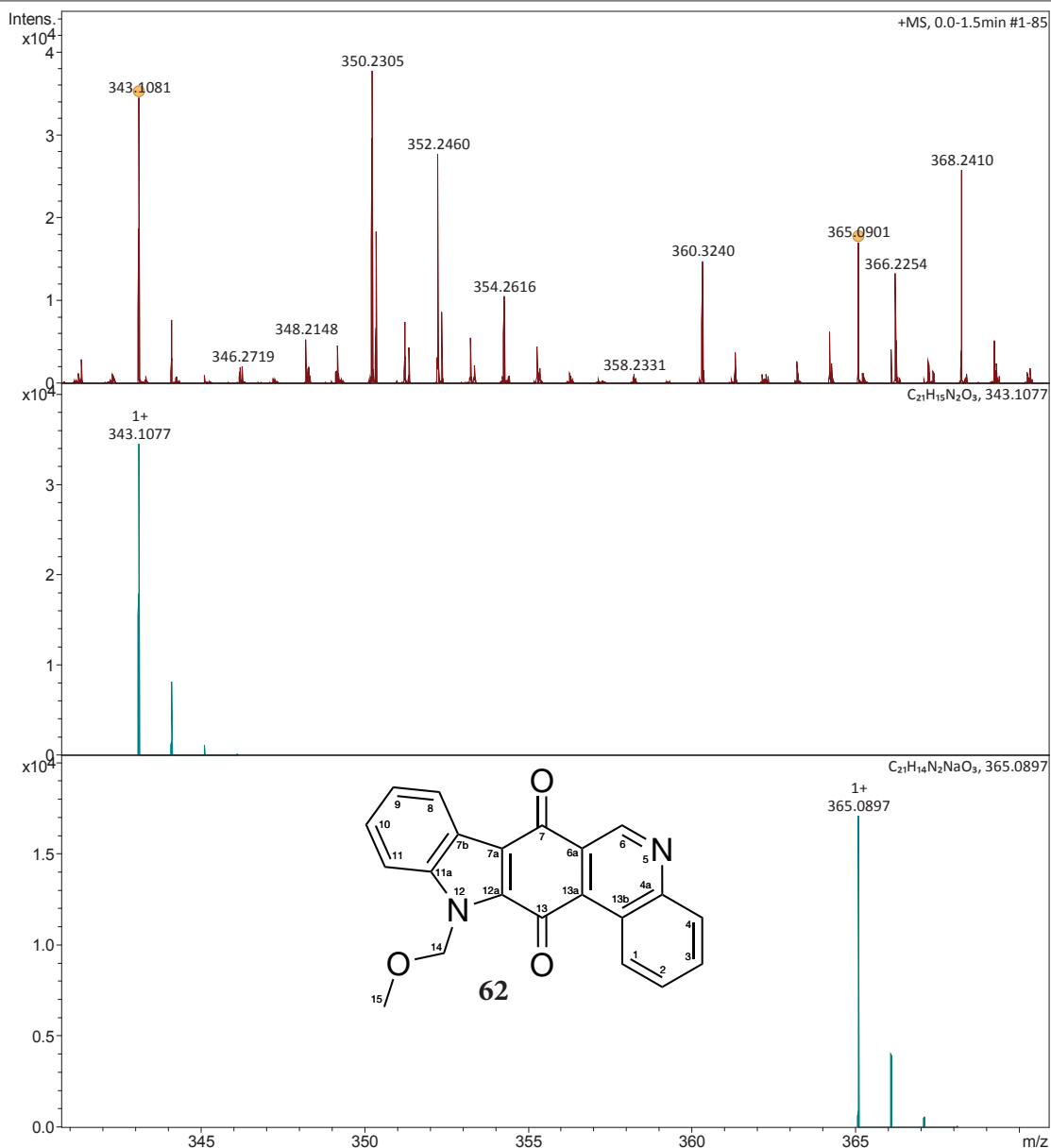|	
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN159_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
296.1030	1	C16H14N3O3	296.1030	-0.1	4.9	1	100.00	11.5	even	ok
318.0851	1	C16H13N3NaO3	318.0849	-0.5	11.8	1	100.00	11.5	even	ok

52

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)
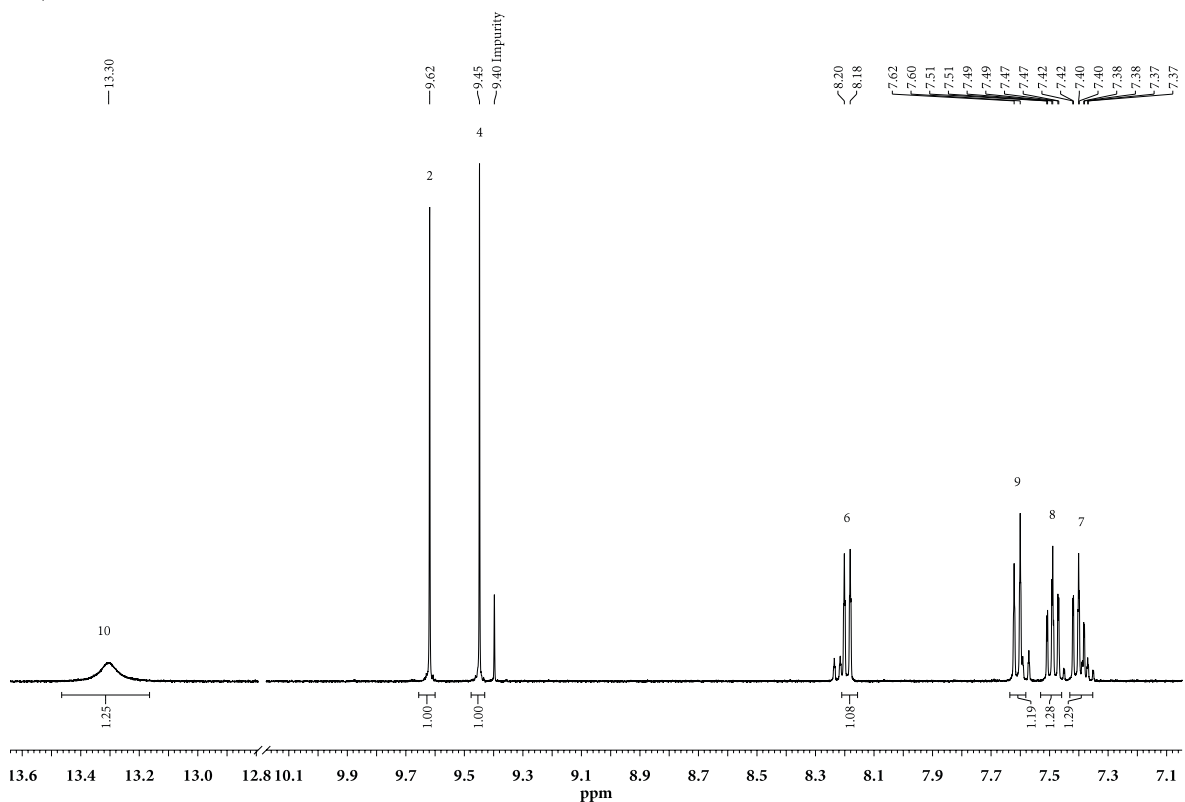
5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (C13 CPD)

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (C13 CPD)

53

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (HSQC)

53

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (HMBC)

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (15N HMBC)

5H-Pyrimido[4,5-b]carbazole-5,11(10H)-dione (15N HSQC)

53

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 5:41:25 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN162_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/17/2014 5:41:25 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN162_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e‾ Conf	N-Rule
250.0612	1	C14H8N3O2	250.0611	-0.4	11.0	1	100.00	12.5	even	ok
272.0432	1	C14H7N3NaO2	272.0430	-0.7	10.8	1	100.00	12.5	even	ok

53

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)

54

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)

9.34
9.08
7.82 7.81 7.81 7.80 7.79 7.79
7.51 7.49 7.45 7.45 7.44 7.43 7.43 7.41 7.41 7.30 7.29 7.28 7.28 7.27 7.26 7.26 CDCl3

6',4'
2'
4
7
6
5

1.00
2.00
1.02
1.05
1.04
1.02

9.40 9.35 9.30 9.25 9.20 9.15 9.10 9.05 9.00 8.95 8.90 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.1
ppm

54

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (PROTON)

4.64 4.63 4.63 4.62 4.61
3.99 3.97 3.95 3.93
2.69 2.67 2.67 2.65
2.28
0.98 0.96 0.94

15,15'
12
10
13
11

2.03
2.05
2.06
6.05
3.05

.70 4.65 4.60 4.55 4.50 4.05 4.00 3.95 3.90 3.85 2.75 2.70 2.65 2.60 2.55 2.50 2.45 2.40 2.35 2.30 2.25 2.20 2.15 1.15 1.10 1.05 1.00 0.95 0.9
ppm

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (C13 APT)

188.67

160.80
160.60
156.96

137.34
132.96
130.50
125.99
125.79
123.22
121.78
117.99

110.67

77.00 CDCl3

61.76
58.74

45.77
43.46
15,15'

13.51

6',4'

3a
6
4
5

7

9
2'

8

7a
5' 2

3

10 13

12

11

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

54

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (C13 APT)

188.67

160.80
160.60
156.96

137.34
132.96
130.50
125.99
125.79
123.22
121.78
117.99

110.67

77.00 CDCl3

61.76
58.74

45.77
43.46

13.51

6',4'

6
3a
5 4

7

9
2'

8

7a
5' 2

3

10 13

12

11

190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110   75 70 65 60 55 50 45 40 35 30 25 20 15
ppm

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (C13 APT)

—188.67

160.80
160.60
—156.96

—137.34
—132.96
—130.50
—125.99
—125.79
—123.22
—121.78
—117.99
—110.67

54

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (HSQC)

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (HMBC)

54

Ethyl 1-[2-(dimethylamino)ethyl]-3-(5-pyrimidinylcarbonyl)-1H-indole-2-carboxylate (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 6:21:37 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN175_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 1.6-3.2min #89-180

367.1770
368.1800
369.1828
372.1922
375.1432
381.1927
382.1957
383.1986
389.1589
390.1620

$C_{20}H_{23}N_4O_3$, 367.1765

1+ 367.1765
1+ 368.1795
1+ 369.1821

$C_{20}H_{22}N_4NaO_3$, 389.1584

1+ 389.1584
1+ 390.1614
1+ 391.1640

54

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/17/2014 6:21:37 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN175_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
367.1770	1	C20H23N4O3	367.1765	-1.5	8.3	1	100.00	11.5	even	ok
389.1589	1	C20H22N4NaO3	389.1584	-1.4	7.5	1	100.00	11.5	even	ok
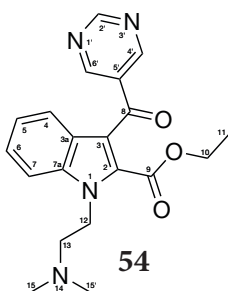
54

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (PROTON)

55

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (PROTON)

55

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (PROTON)

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (C13 APT)

55
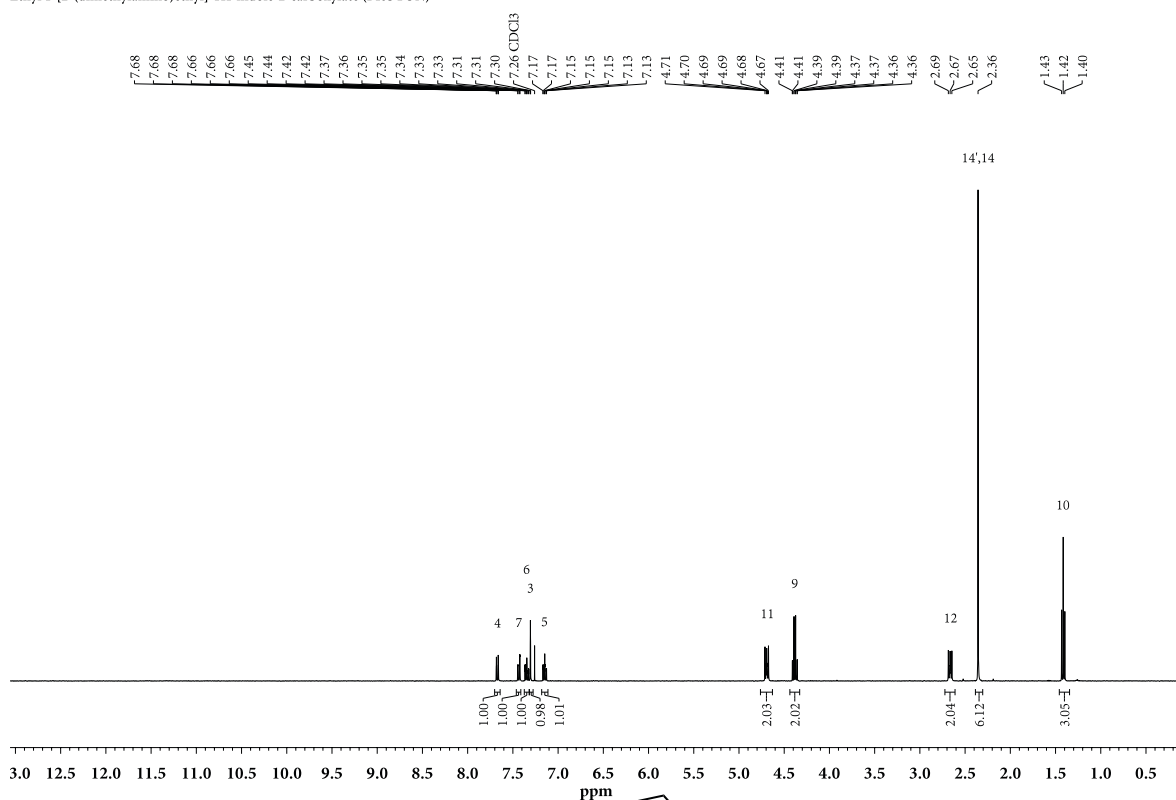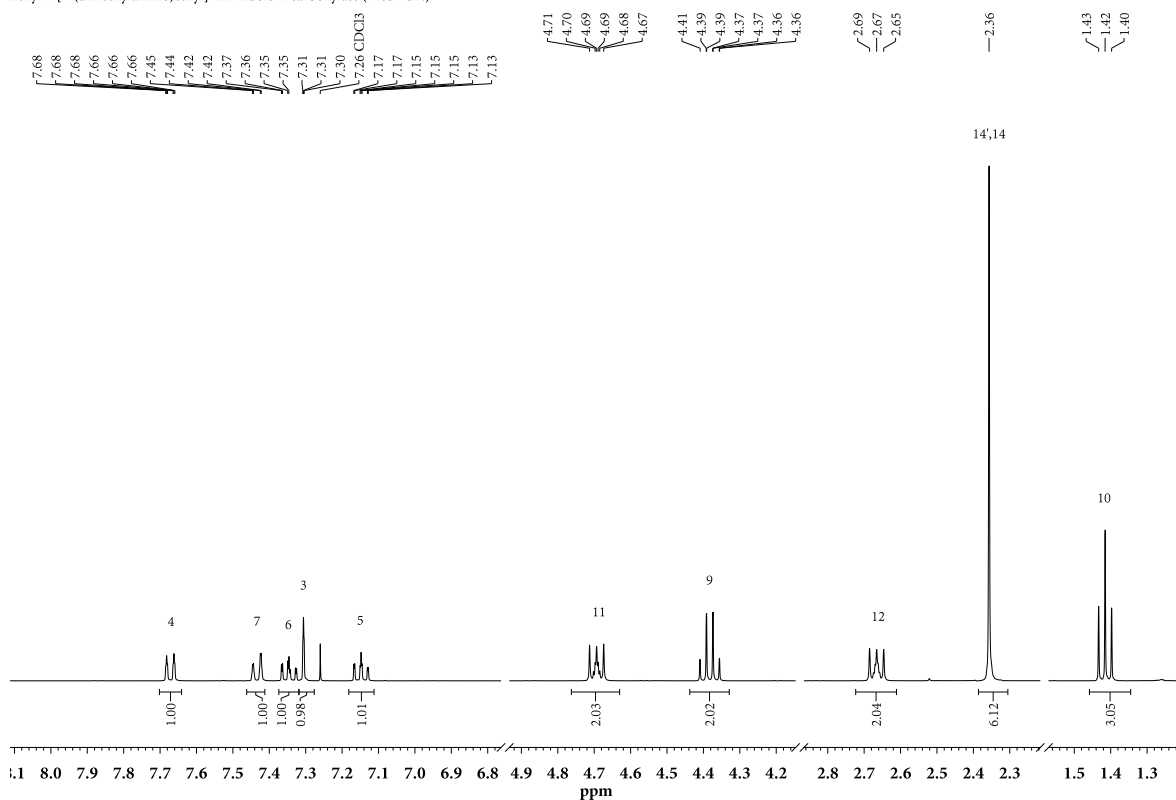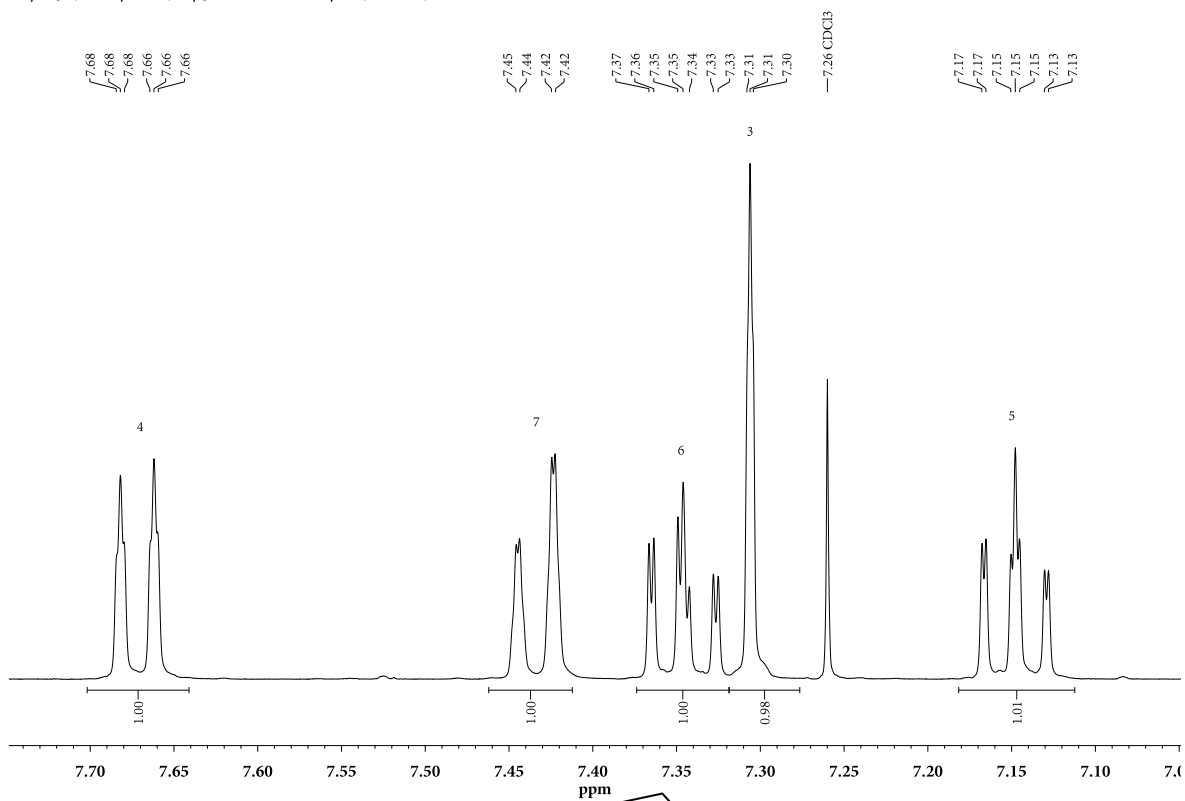
Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (C13 APT)

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (C13 APT)

— 60.51
— 58.88
— 45.82
— 42.92
— 14.37

14',14

10

9 12

11

66 64 62 60 58 56 54 52 50 48 46 44 42 40 38 36 34 32 30 28 26 24 22 20 18 16 14 12 1(

ppm

55

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (HSQC)

ppm

8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5

ppm

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (HMBC)

55

Ethyl 1-[2-(dimethylamino)ethyl]-1H-indole-2-carboxylate (15N HMBC)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)

56

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)

56

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (PROTON)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (C13 APT)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (C13 APT)

56

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (C13 APT)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (HSQC)

56

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (HMBC)

10-[2-(Dimethylamino)ethyl]-5H-pyrimido[4,5-b]carbazole-5,11(10H)-dione (15N HMBC)

56

513

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN163_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 12:45:21 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-2.2min #1-127

343.1170
321.1349
322.1379
324.3374
327.3361
332.3313
338.3031
344.1199
345.1225

$C_{18}H_{17}N_4O_2$, 321.1346

1+ 321.1346
1+ 322.1375
1+ 323.1402

$C_{18}H_{16}N_4NaO_2$, 343.1165

1+ 343.1165
1+ 344.1195
1+ 345.1221

56

514

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/4/2014 12:45:21 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN163_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
321.1349	1	C18H17N4O2	321.1346	-0.8	3.3	1	100.00	12.5	even	ok
343.1170	1	C18H16N4NaO2	343.1165	-1.5	6.4	1	100.00	12.5	even	ok
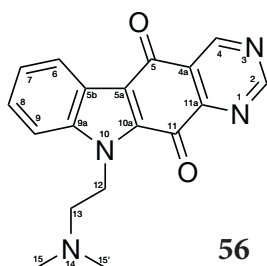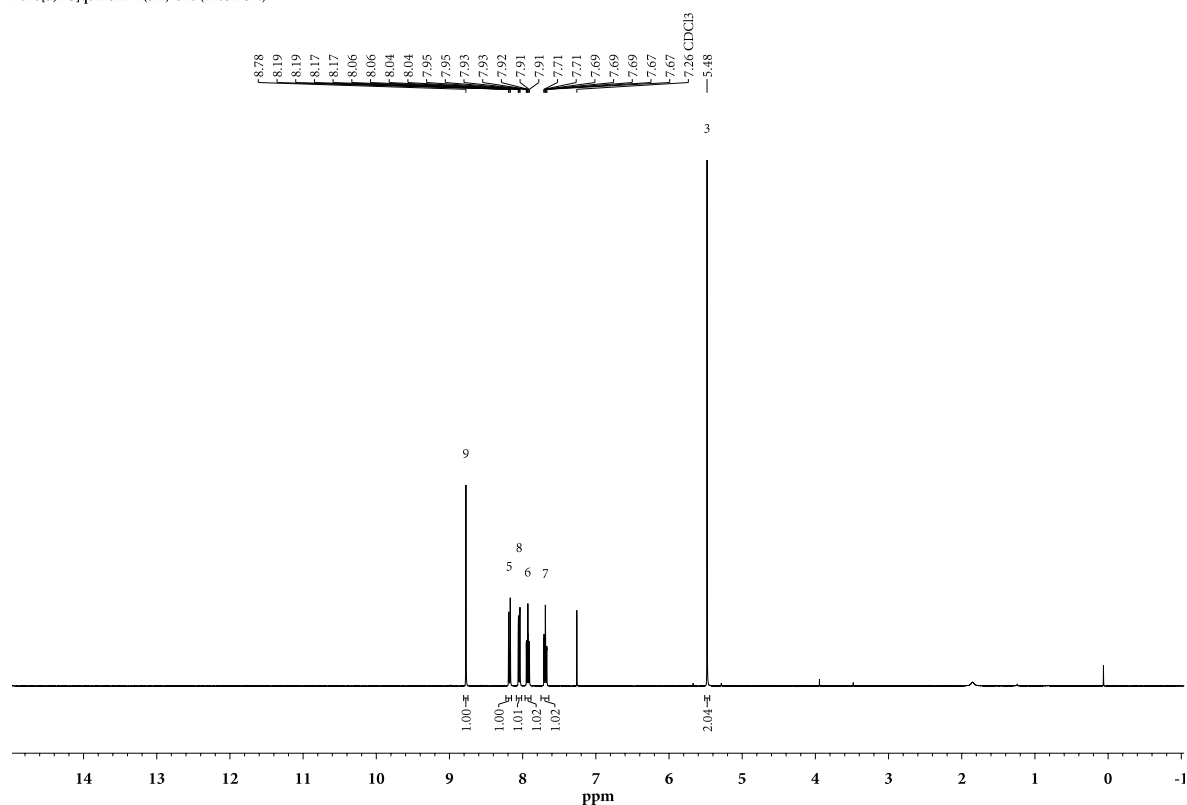
56

Furo[3,4-b]quinolin-1(3H)-one (PROTON)
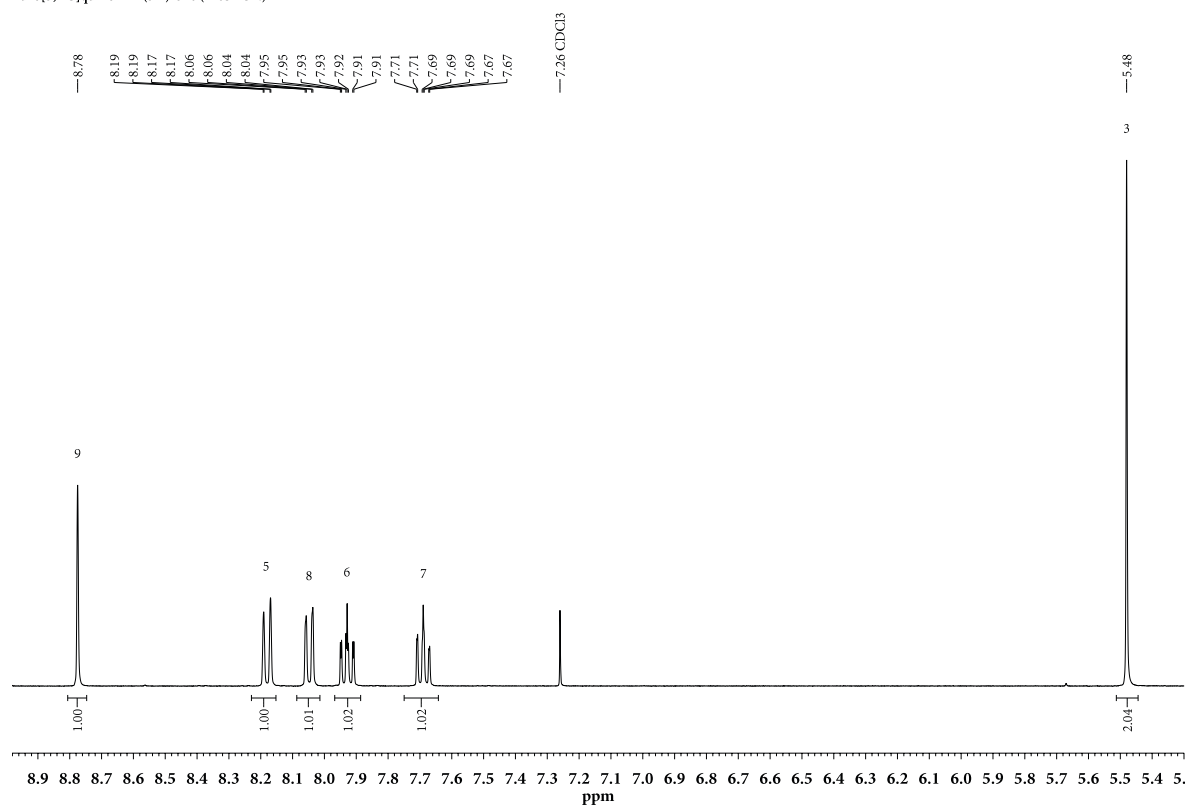
Furo[3,4-b]quinolin-1(3H)-one (PROTON)
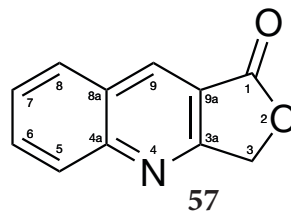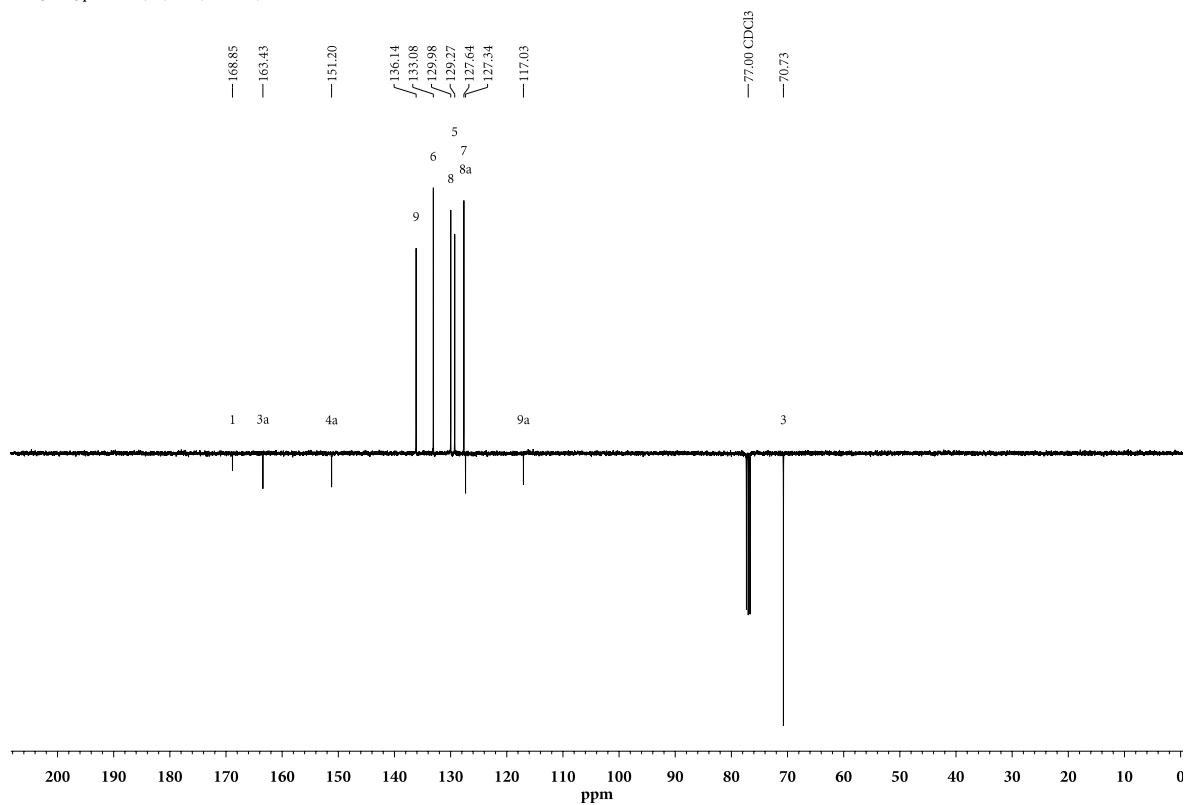
Furo[3,4-b]quinolin-1(3H)-one (C13 APT)
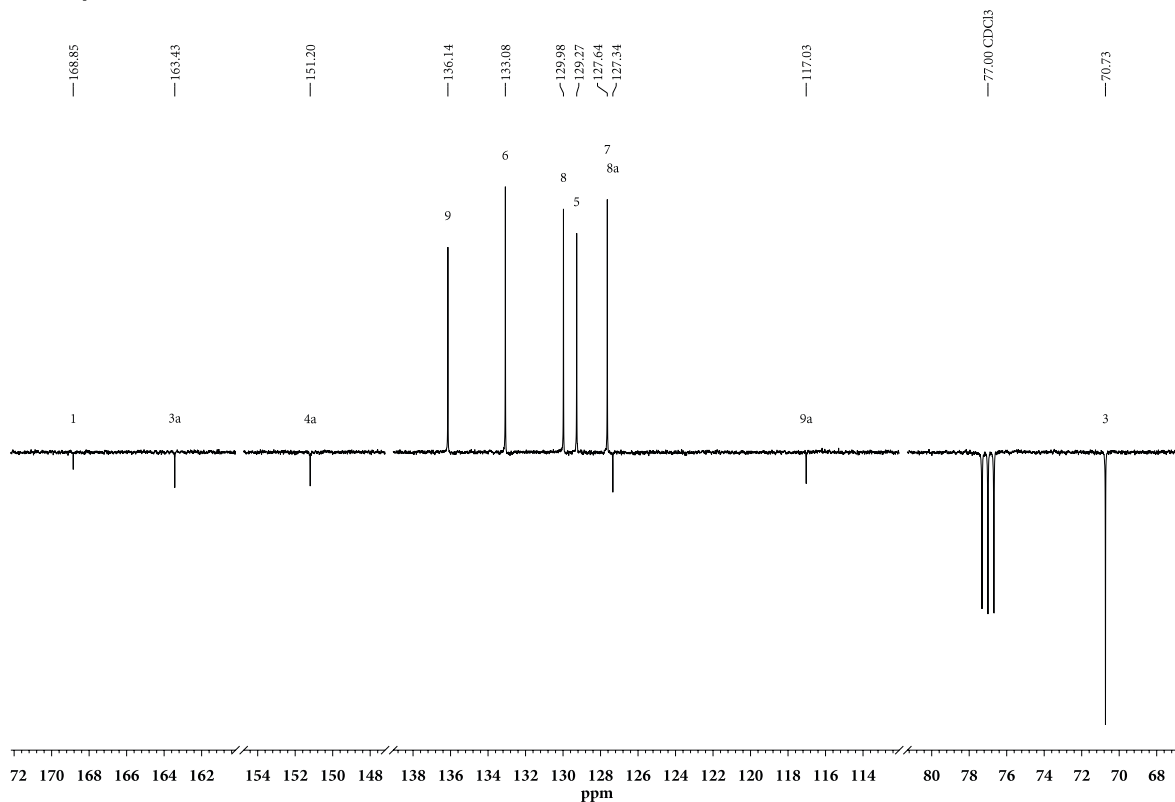
Furo[3,4-b]quinolin-1(3H)-one (C13 APT)

Furo[3,4-b]quinolin-1(3H)-one (HSQC)

57
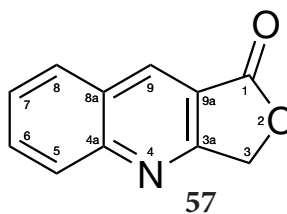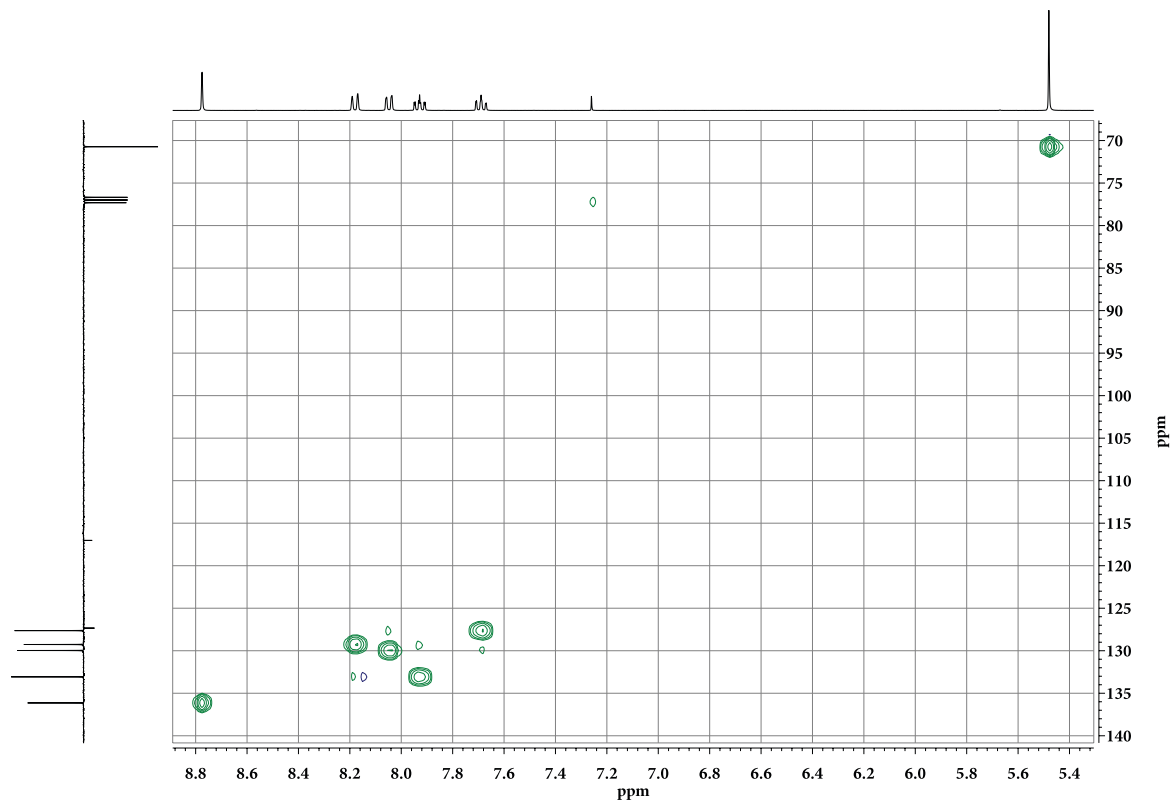
Furo[3,4-b]quinolin-1(3H)-one (HMBC)
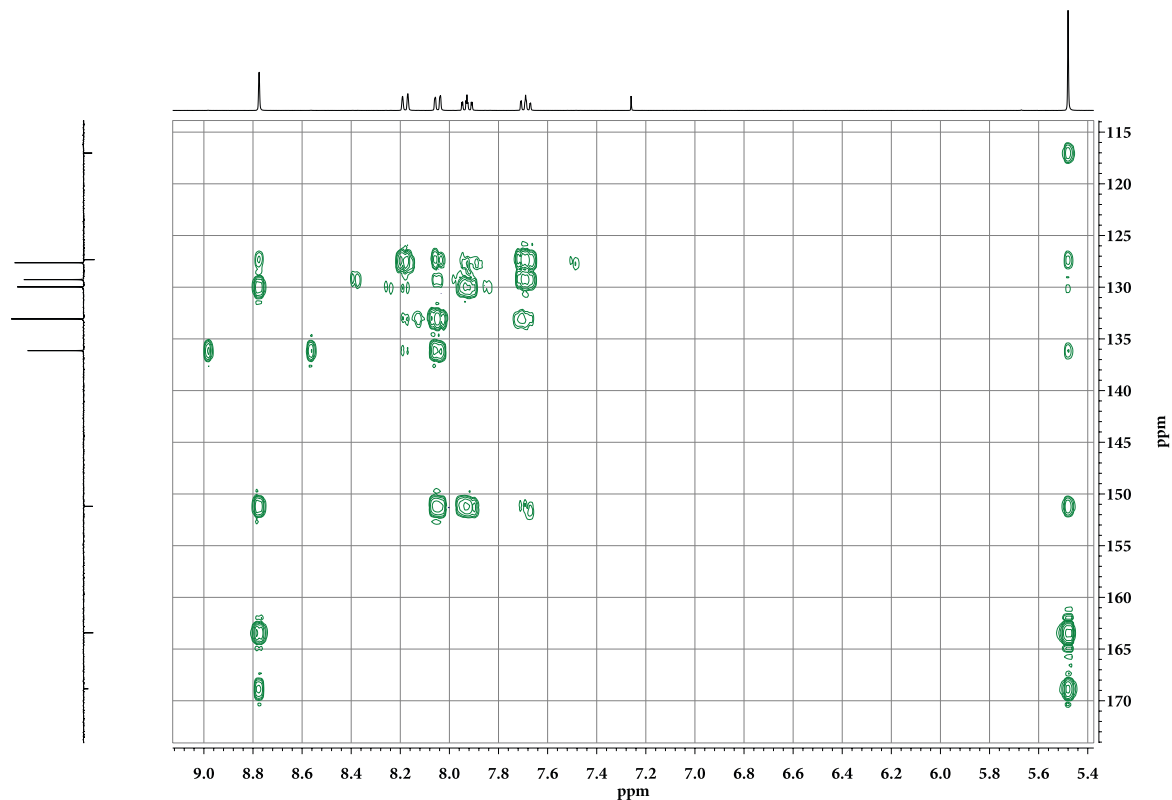
Furo[3,4-b]quinolin-1(3H)-one (15N HMBC)
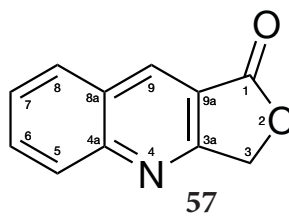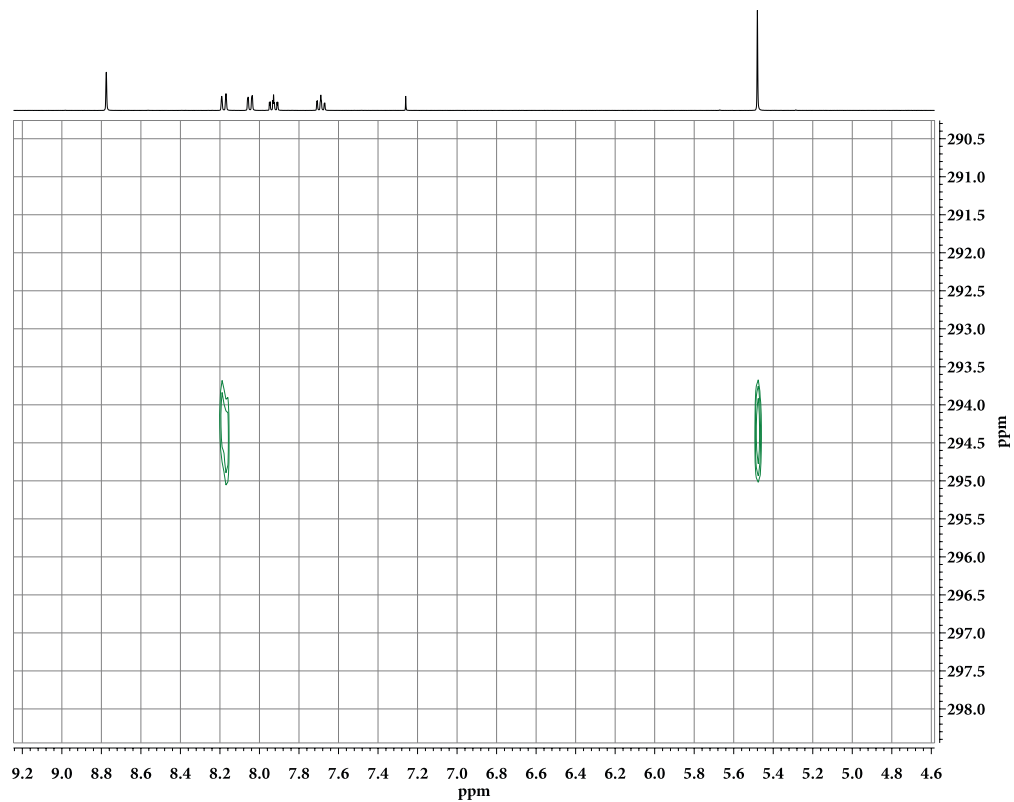
57

Furo[3,4-b]quinolin-1(3H)-one (NOESY)
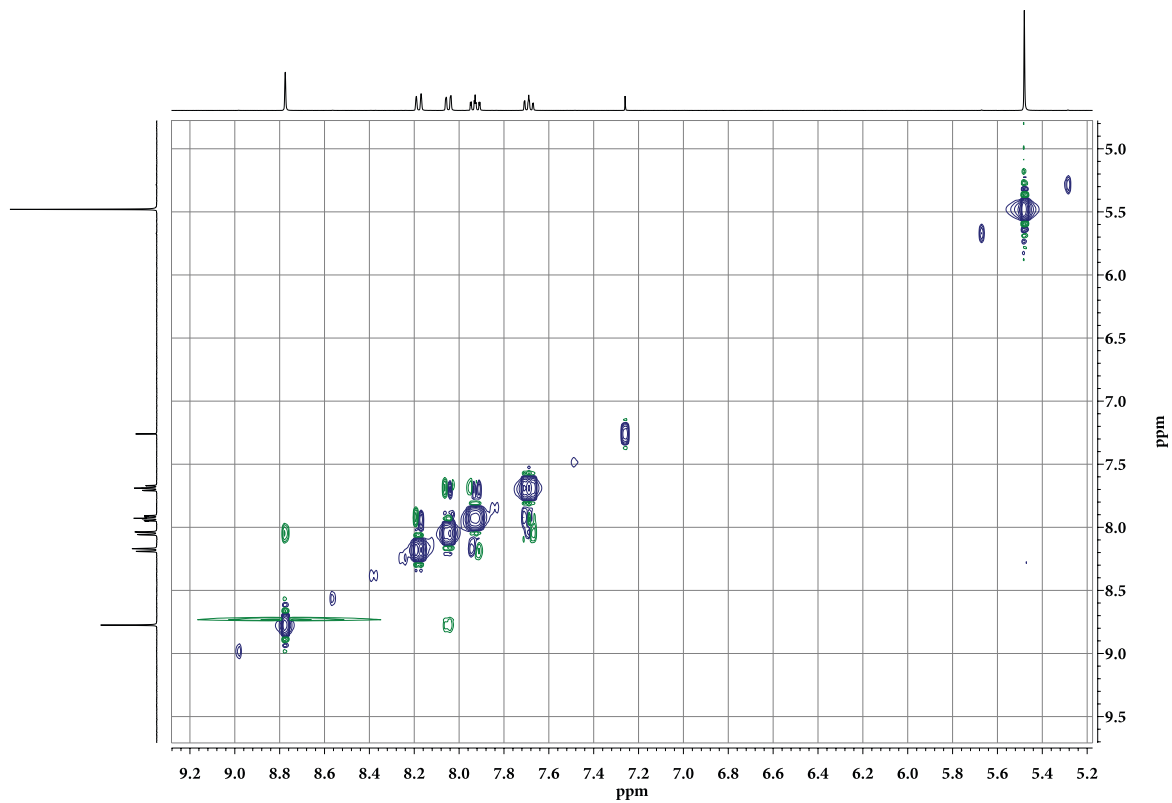
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (PROTON)
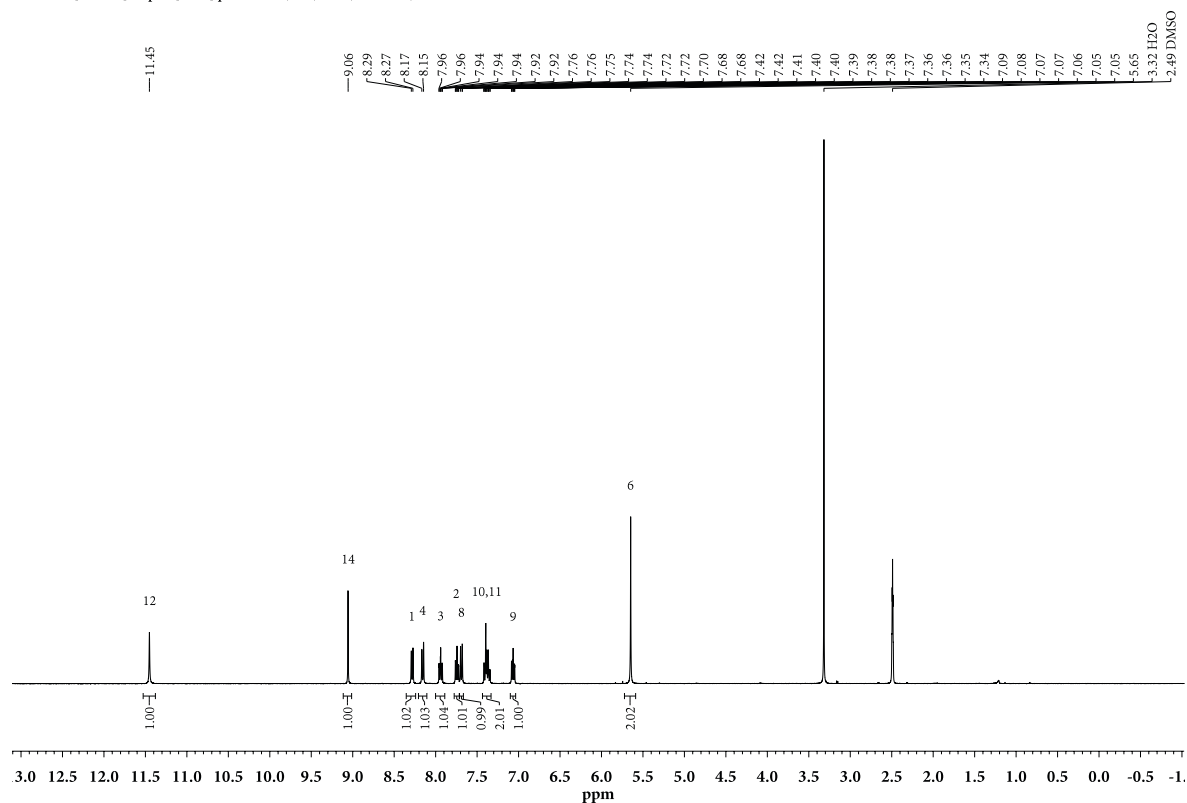
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (PROTON)
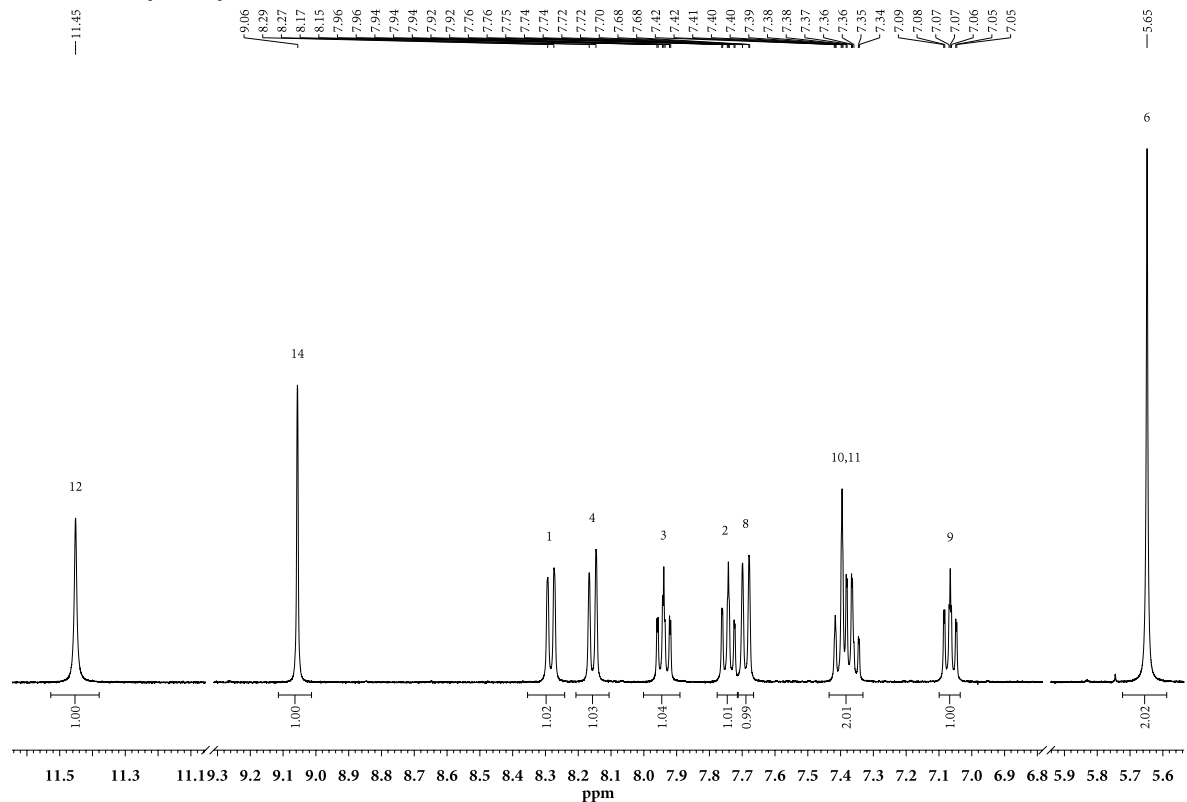
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (C13 APT)
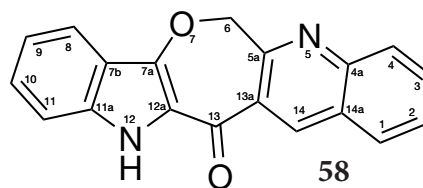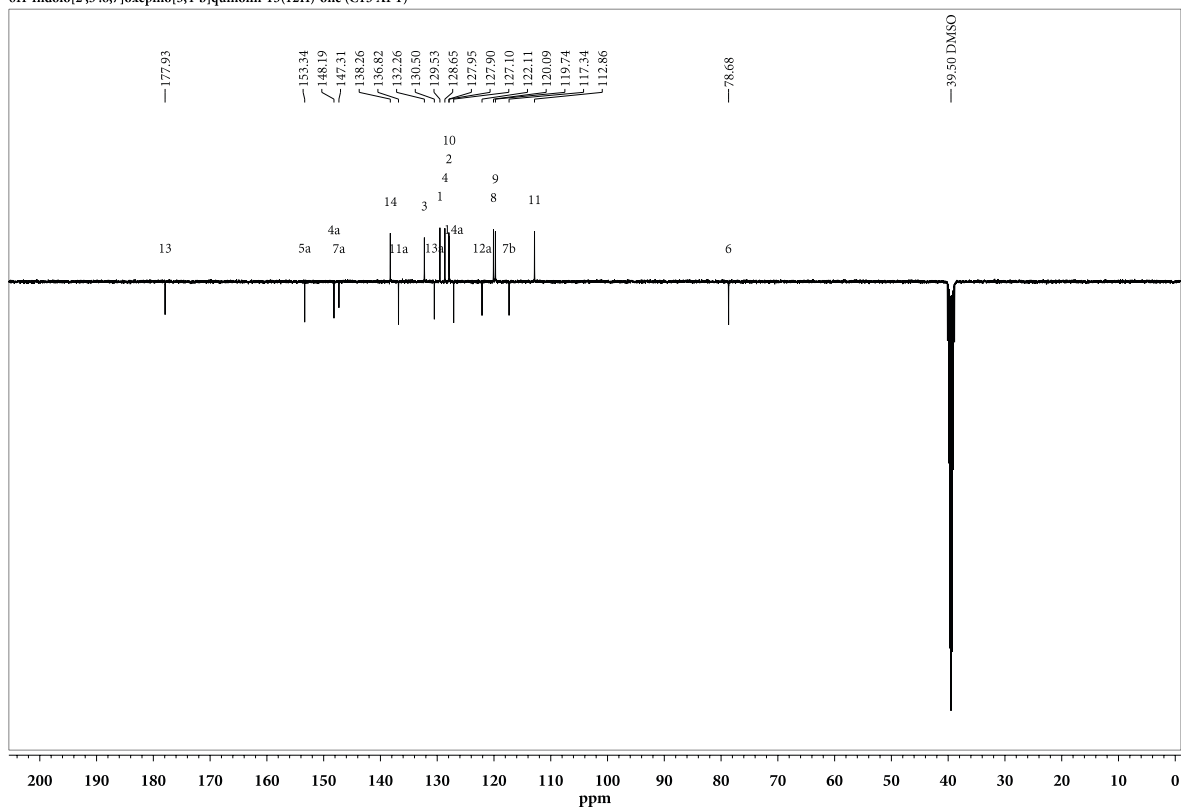
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (C13 APT)
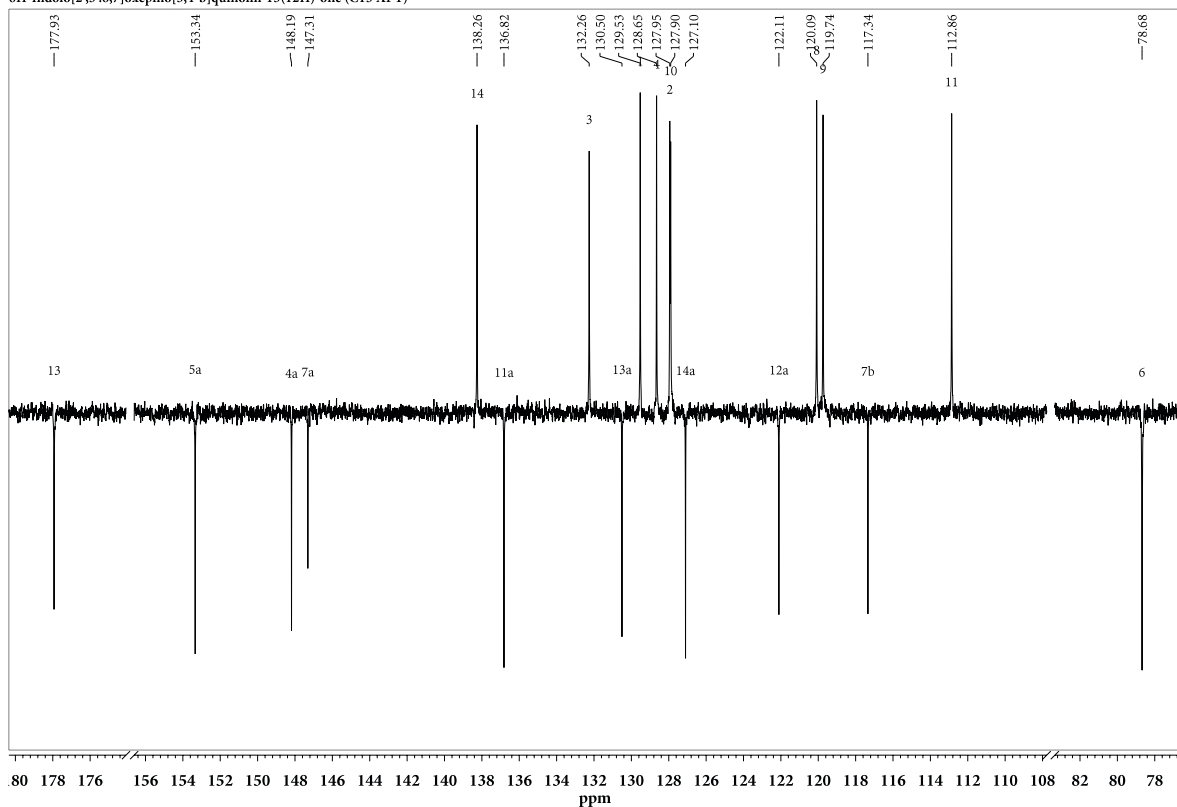
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (C13 APT)
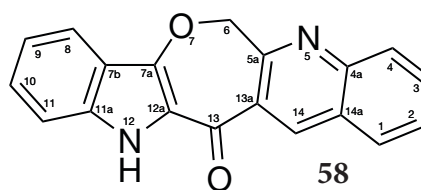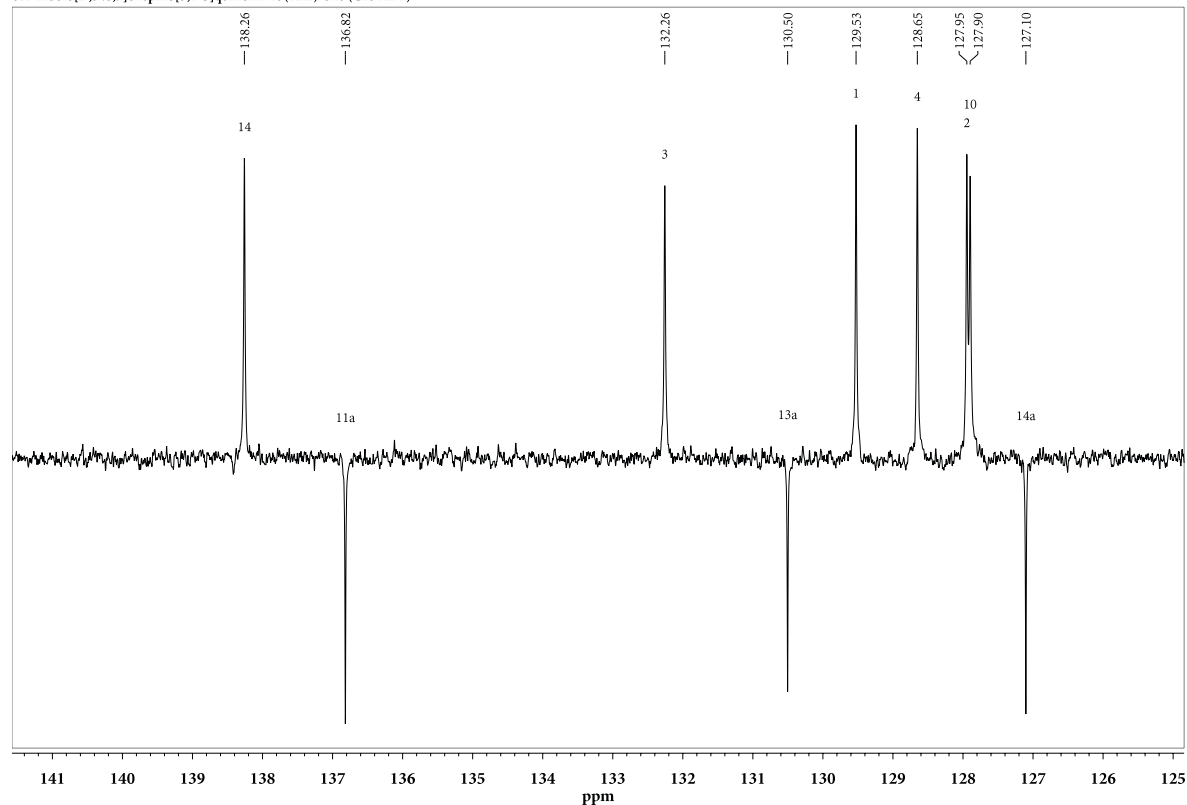
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (HSQC)
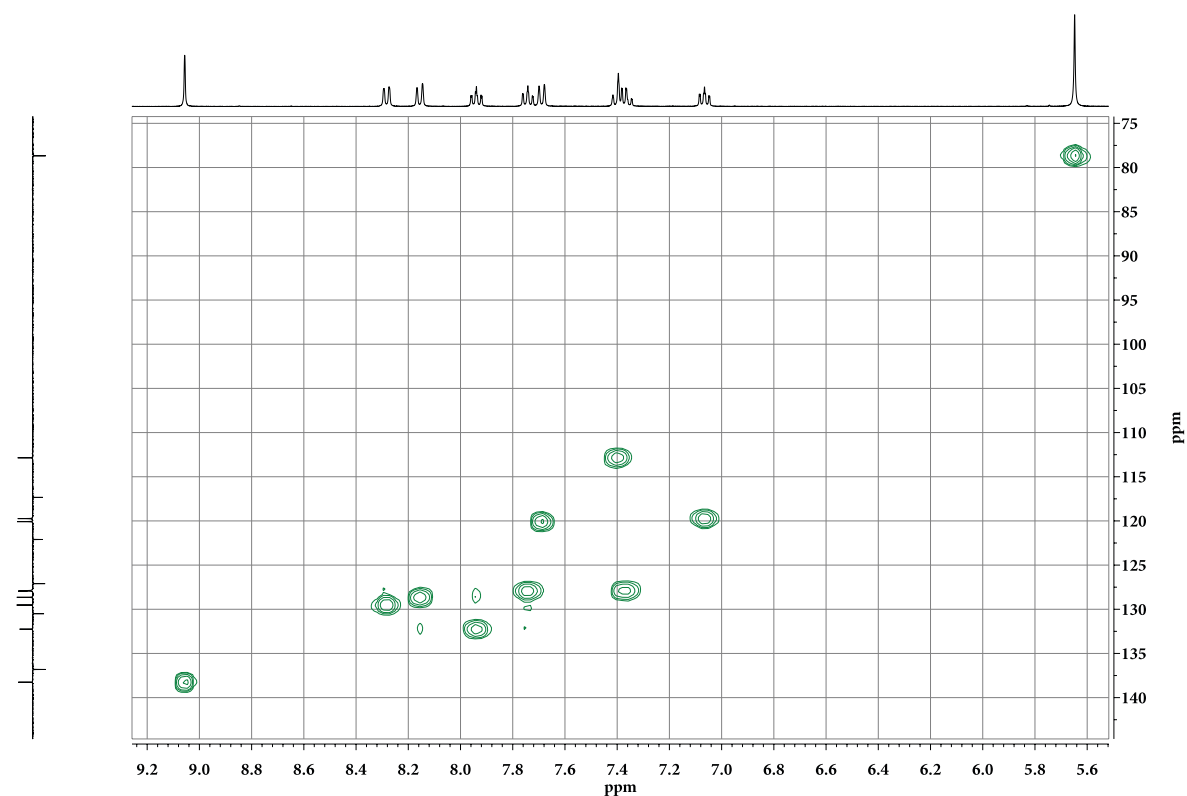
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (HMBC)
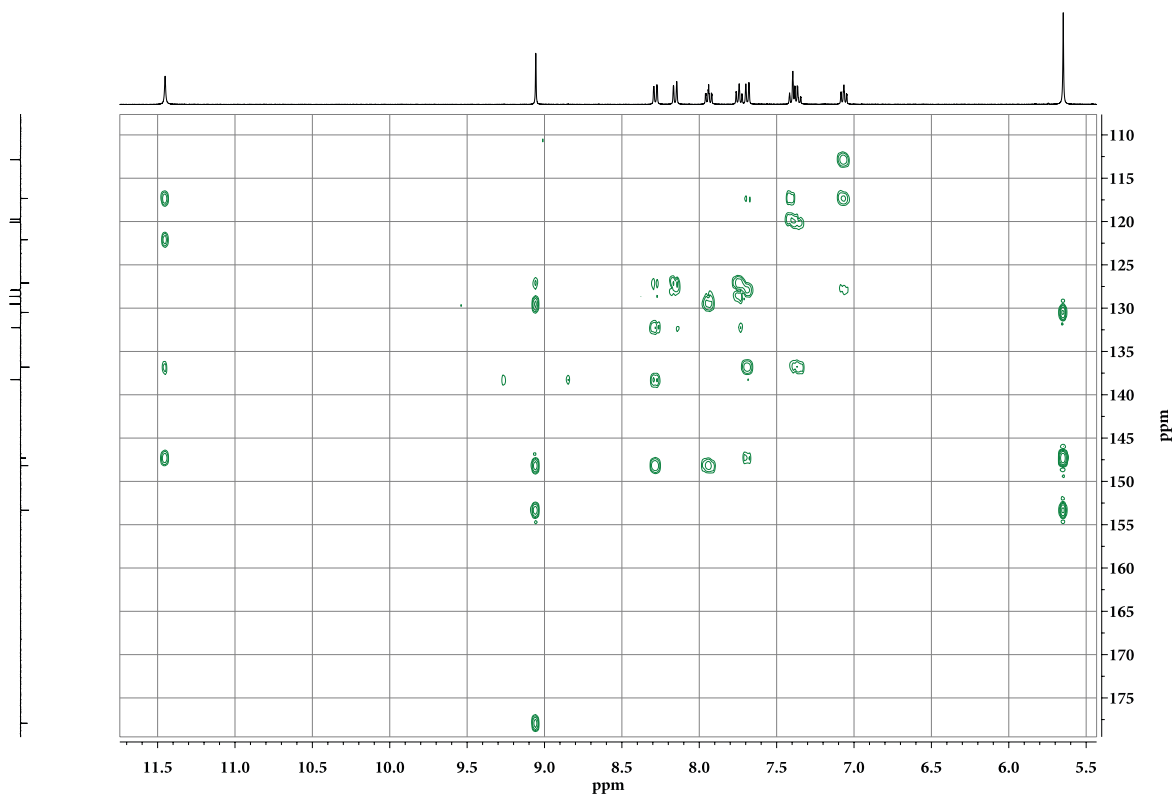
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (15N HMBC)

523
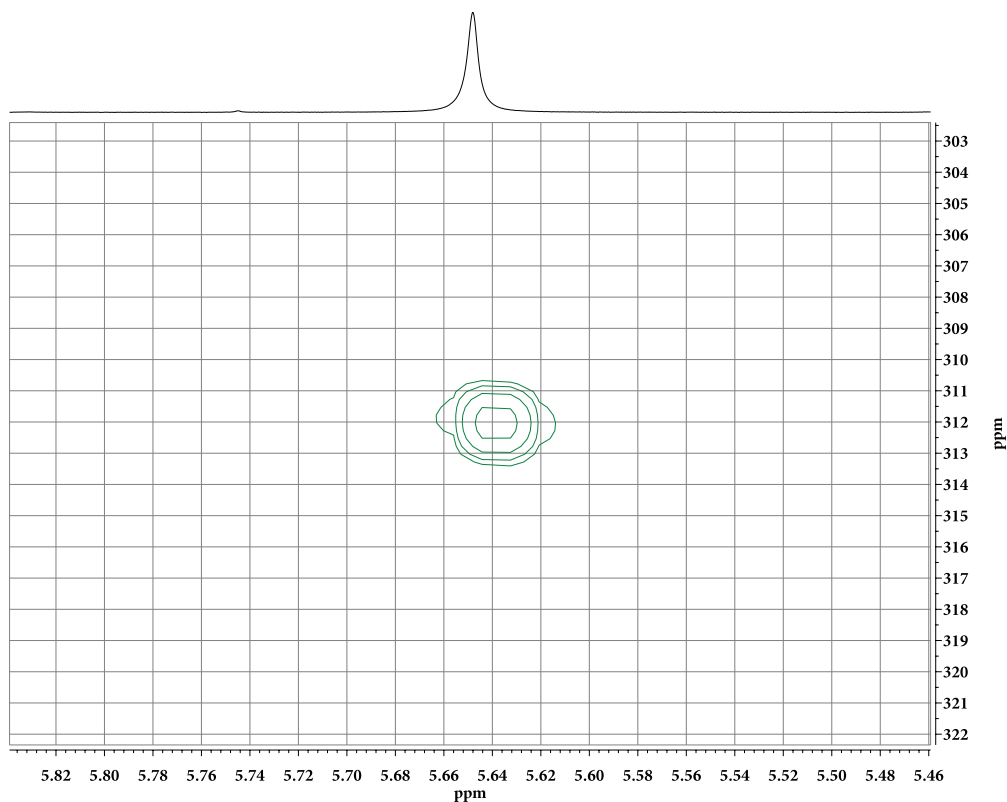
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (15N HSQC)
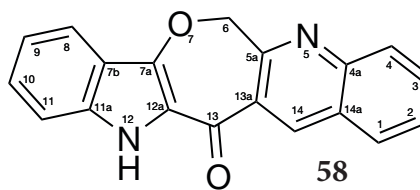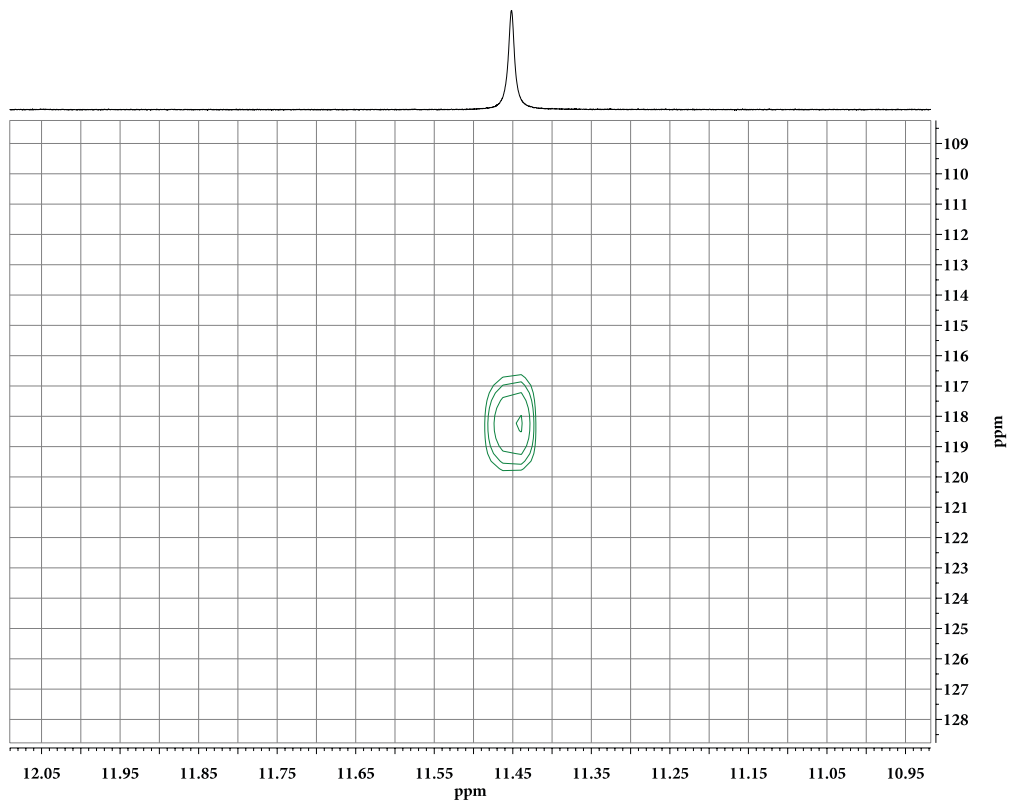
6H-Indolo[2',3':6,7]oxepino[3,4-b]quinolin-13(12H)-one (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 6:10:10 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN171_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-2.0min #1-116

300.2299
306.2735
308.2198
309.2230
311.2559
312.3392
313.3325
314.3182
316.2227
317.3246
319.2439
320.2356
321.2593
323.0794
324.0825
325.3409
326.3782

$C_{19}H_{13}N_2O_2$, 301.0972

1+
301.0972

1+
302.1003

1+
303.1031

$C_{19}H_{12}N_2NaO_2$, 323.0791

1+
323.0791

1+
324.0823

1+
325.0850

58

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/17/2014 6:10:10 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN171_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar		
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C		
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min		
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste		
		Set Corona	0 nA	Set APCI Heater	0 °C		

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
301.0974	1	C19H13N2O2	301.0972	-0.7	15.1	1	100.00	14.5	even	ok
323.0794	1	C19H12N2NaO2	323.0791	-0.9	27.6	1	100.00	14.5	even	ok
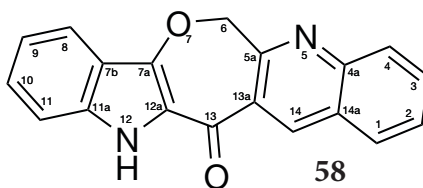
58

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

59

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (HSQC)

59

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (HMBC)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (15N HMBC)

59
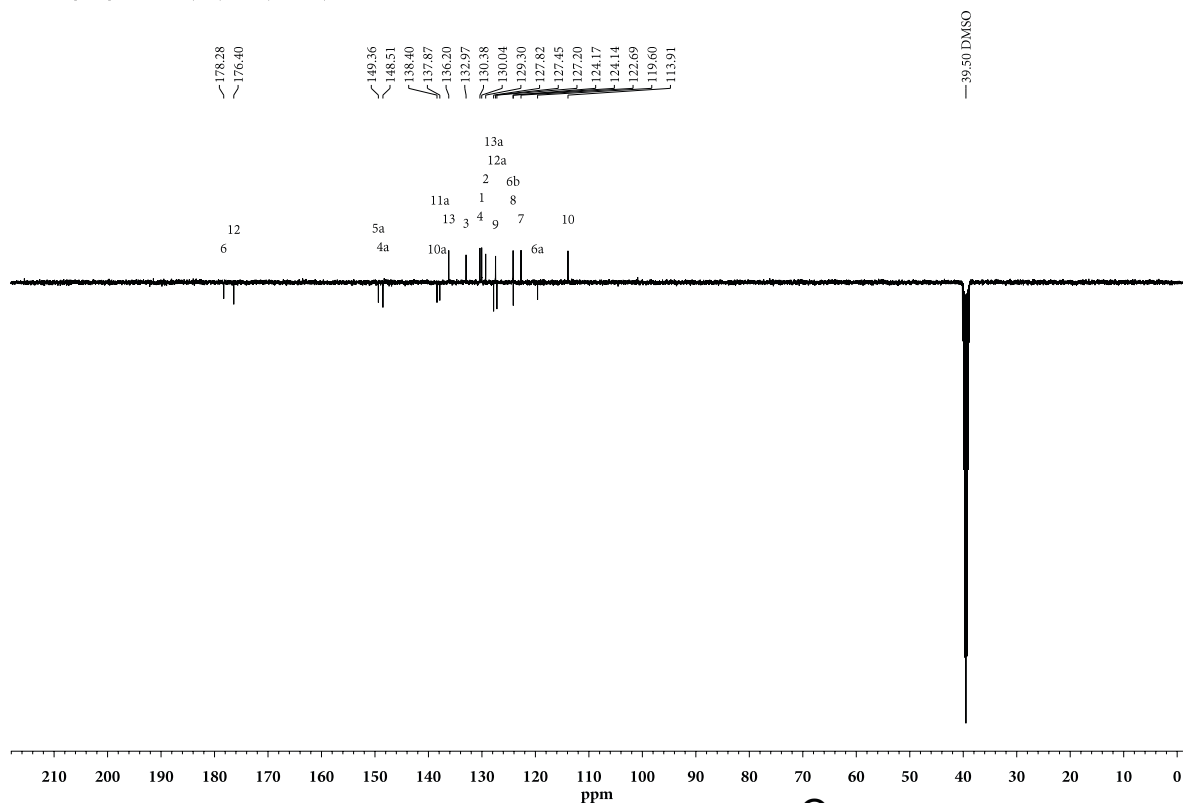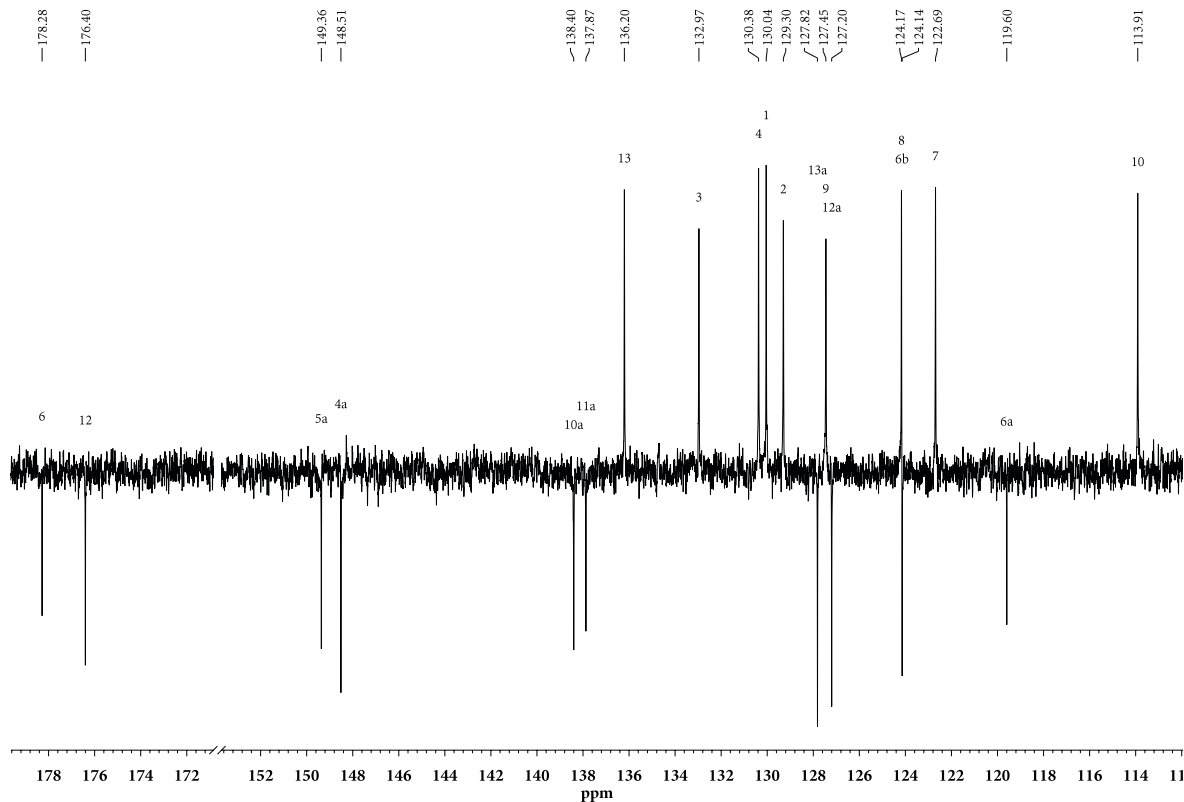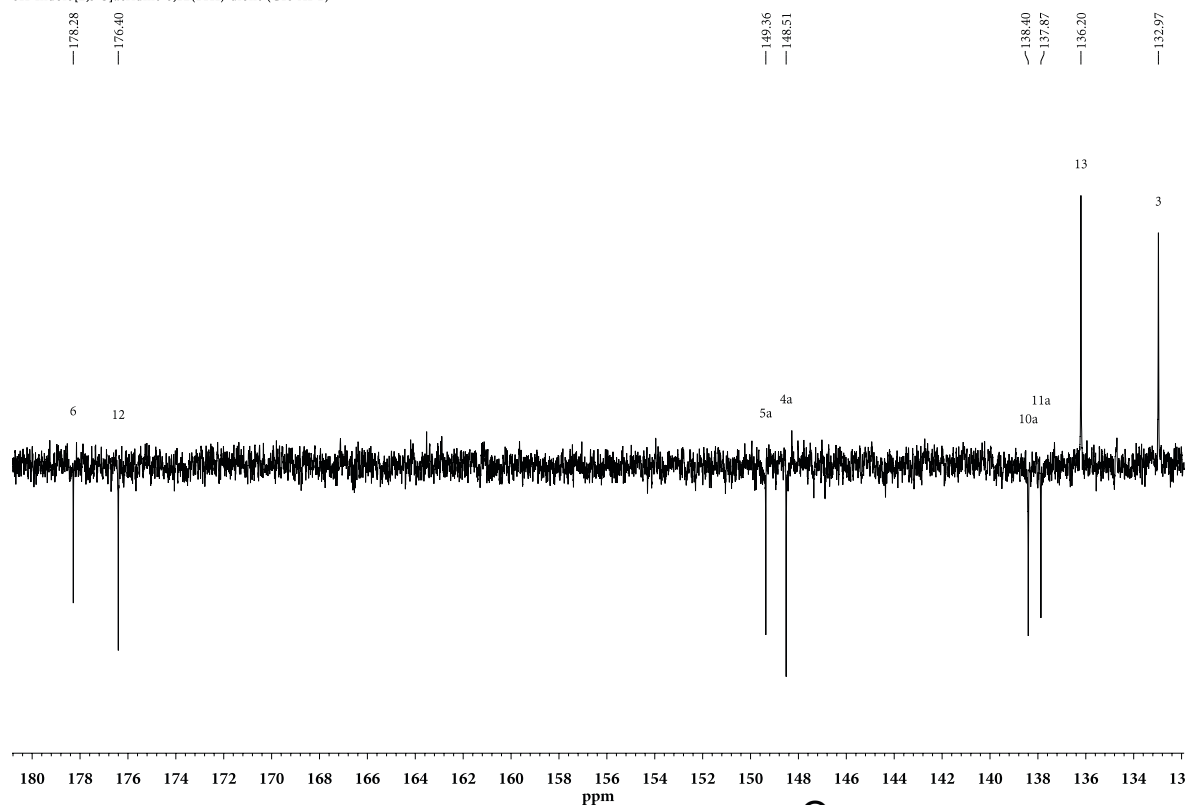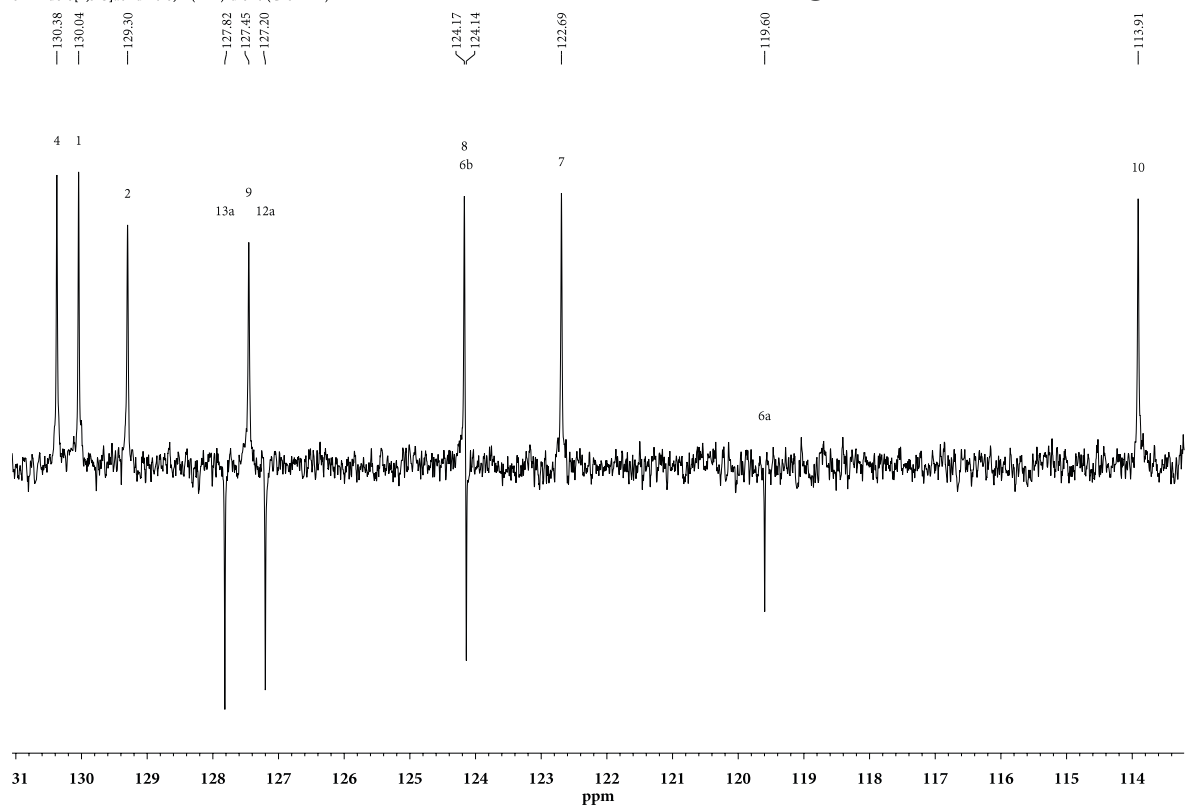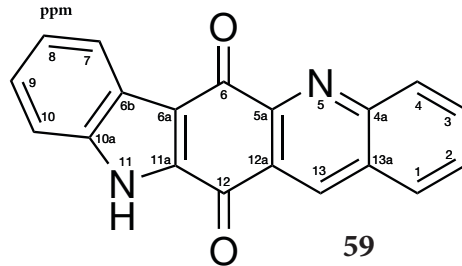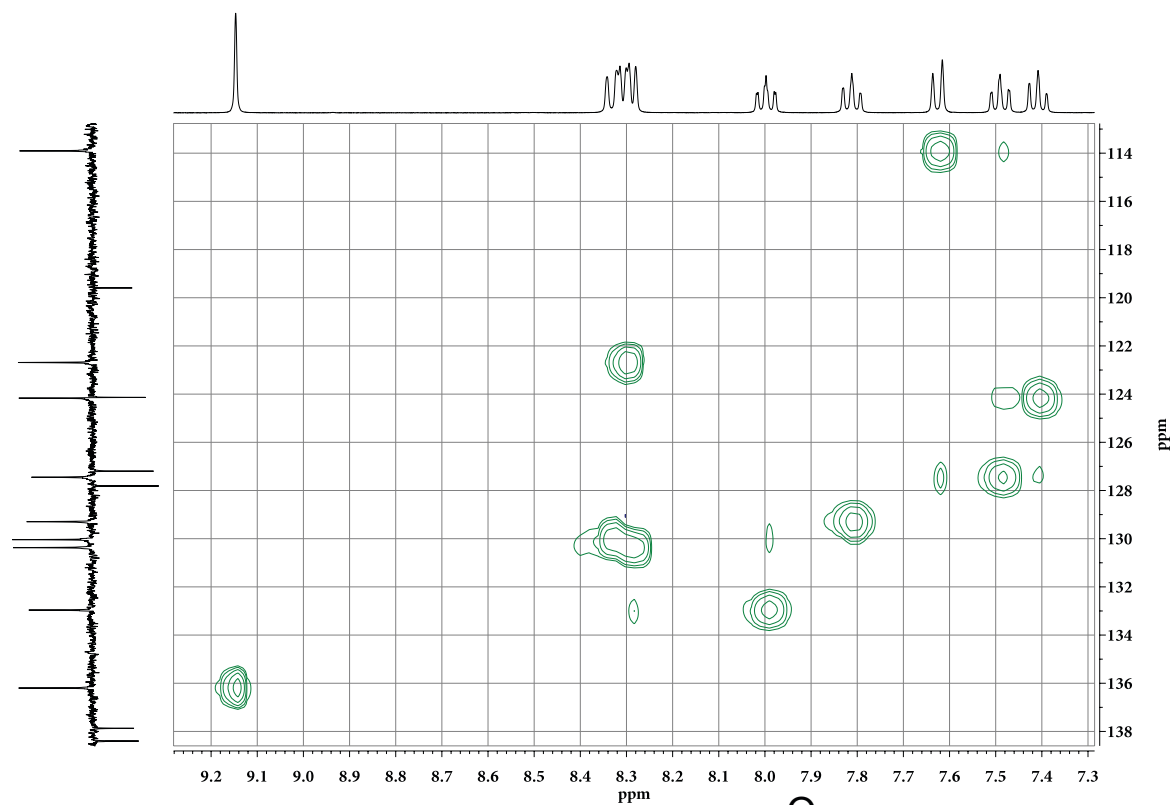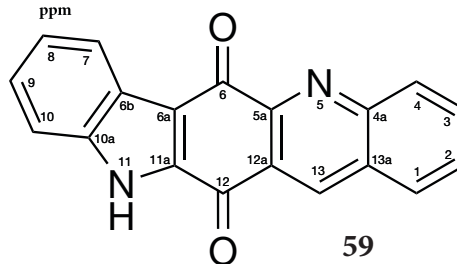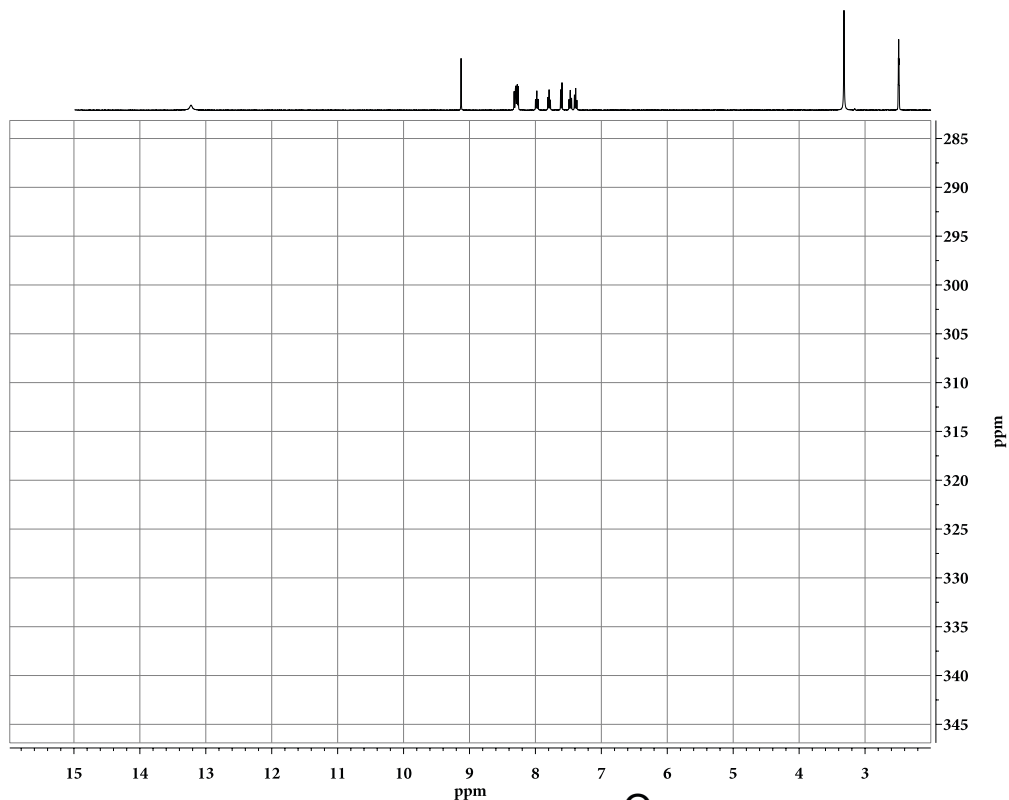
6H-Indolo[2,3-b]acridine-6,12(11H)-dione (15N HSQC)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (COSY)

6H-Indolo[2,3-b]acridine-6,12(11H)-dione (NOESY)

59

Generic Display Report

Analysis Info   Acquisition Date 11/17/2014 6:15:51 PM
Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN172_DI_ESI_HRMS.d
Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   0.1 mg/mL in MeOH   Instrument   maXis HD
Comment

$C_{19}H_{11}N_2O_2$, 299.0815

$C_{19}H_{10}N_2NaO_2$, 321.0634

59

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/17/2014 6:15:51 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN172_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
299.0816	1	C19H11N2O2	299.0815	-0.3	3.3	1	100.00	15.5	even	ok
321.0638	1	C19H10N2NaO2	321.0634	-1.0	9.1	1	100.00	15.5	even	ok
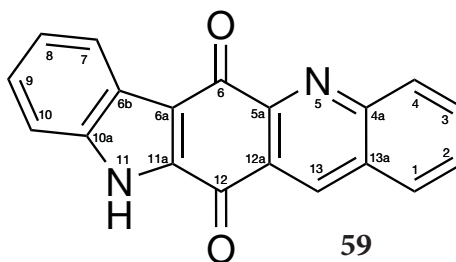
59

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

60

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

60

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (PROTON)

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

60
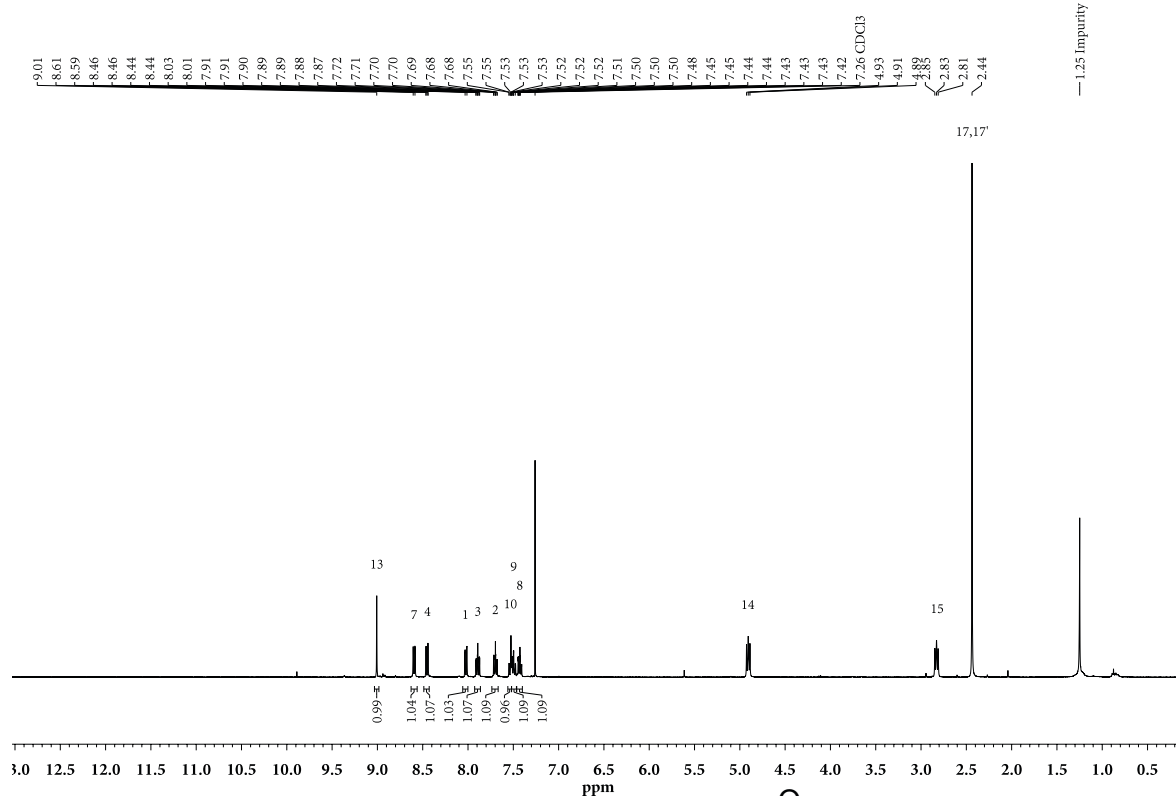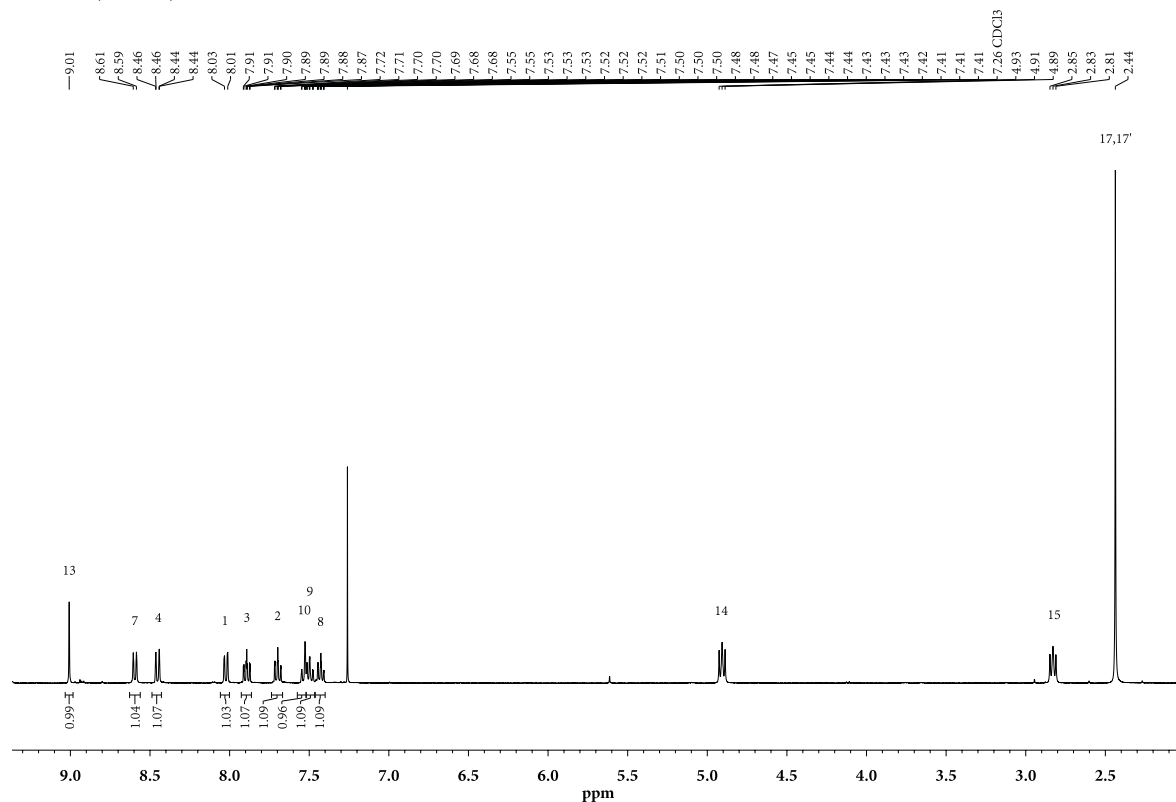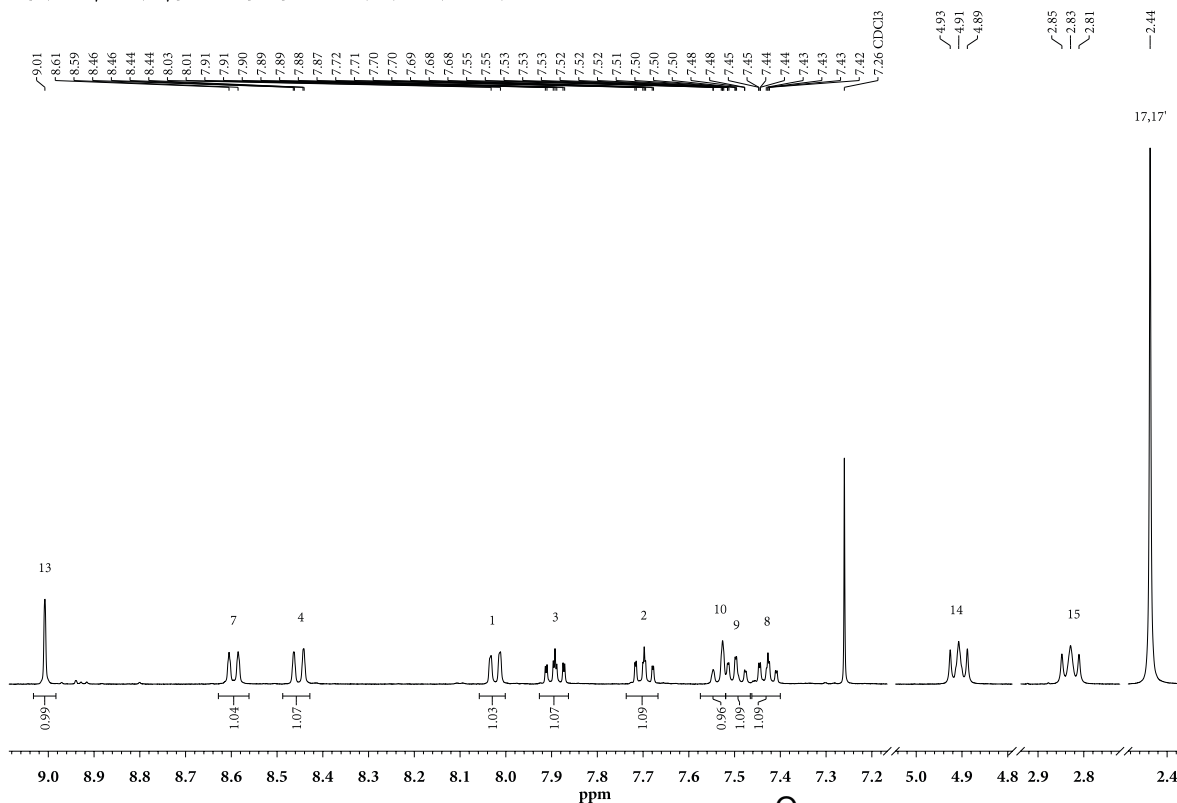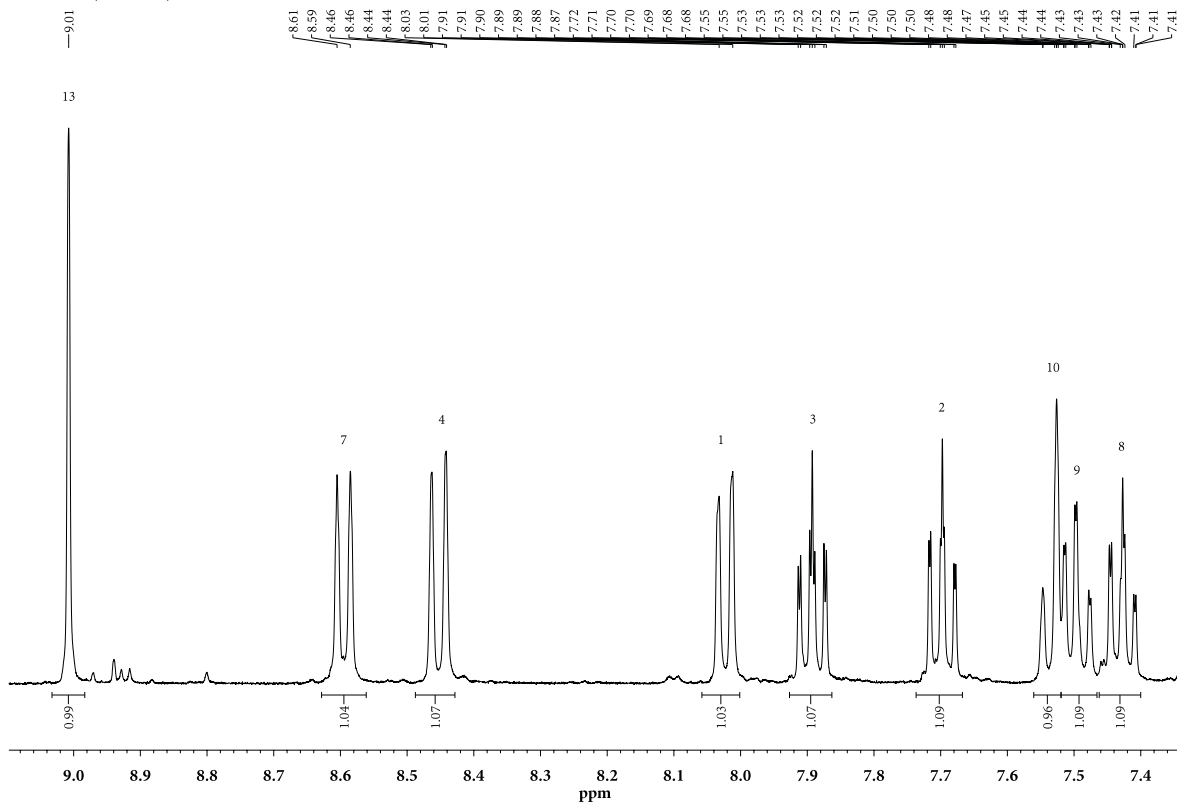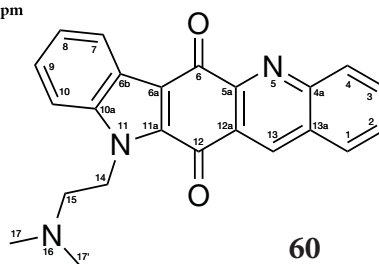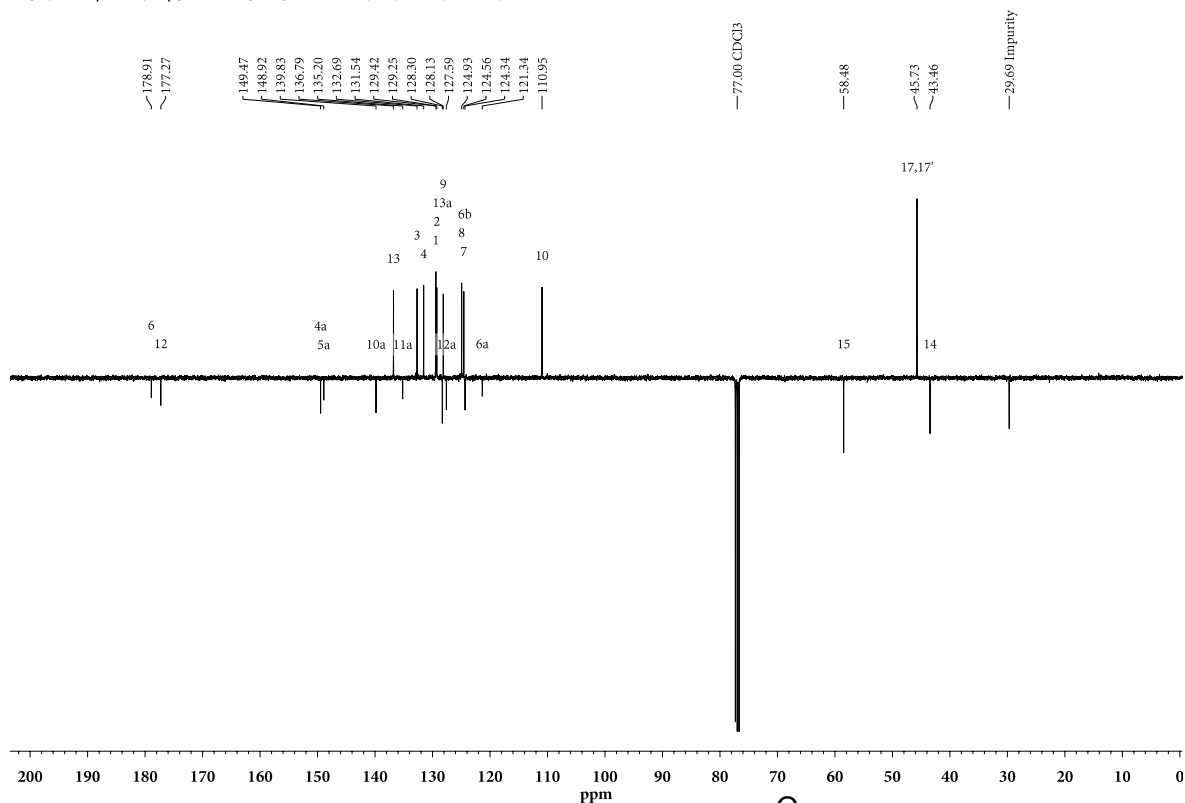
11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)
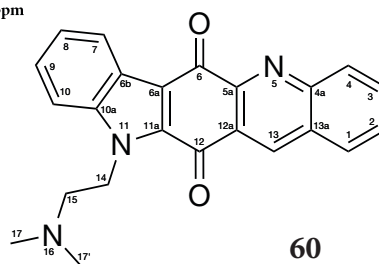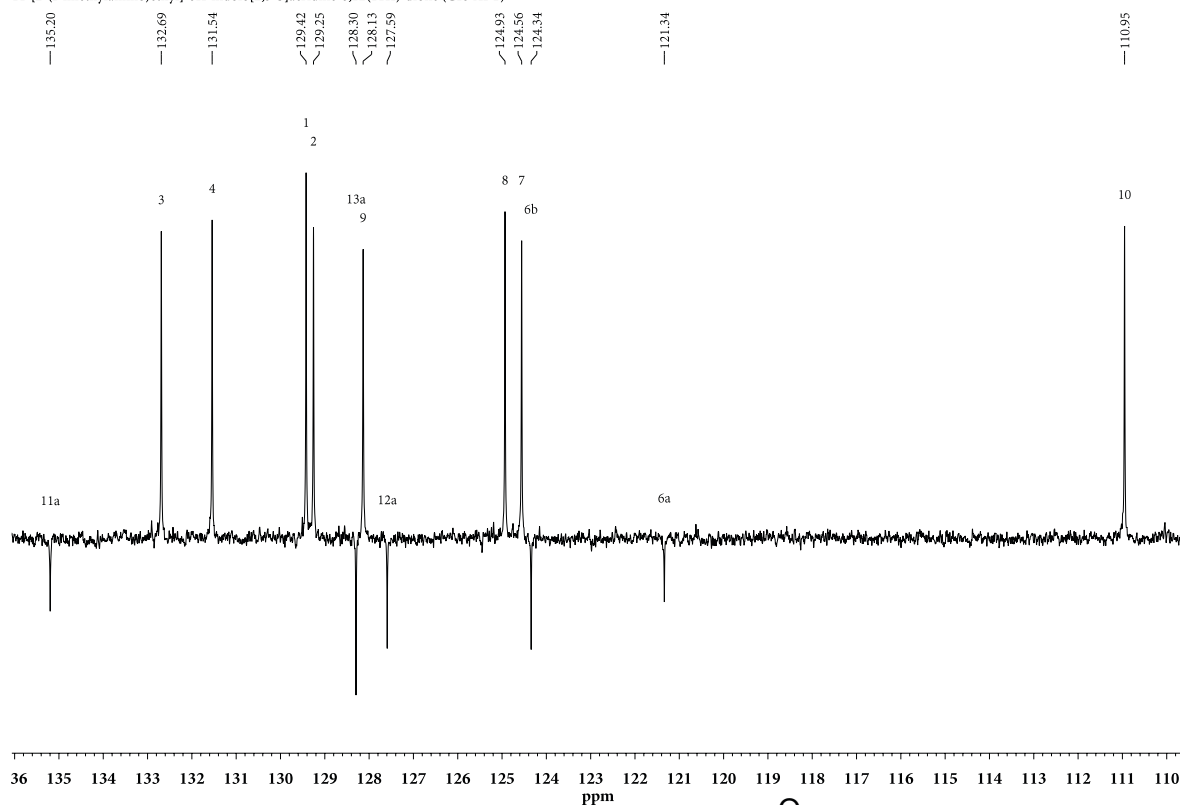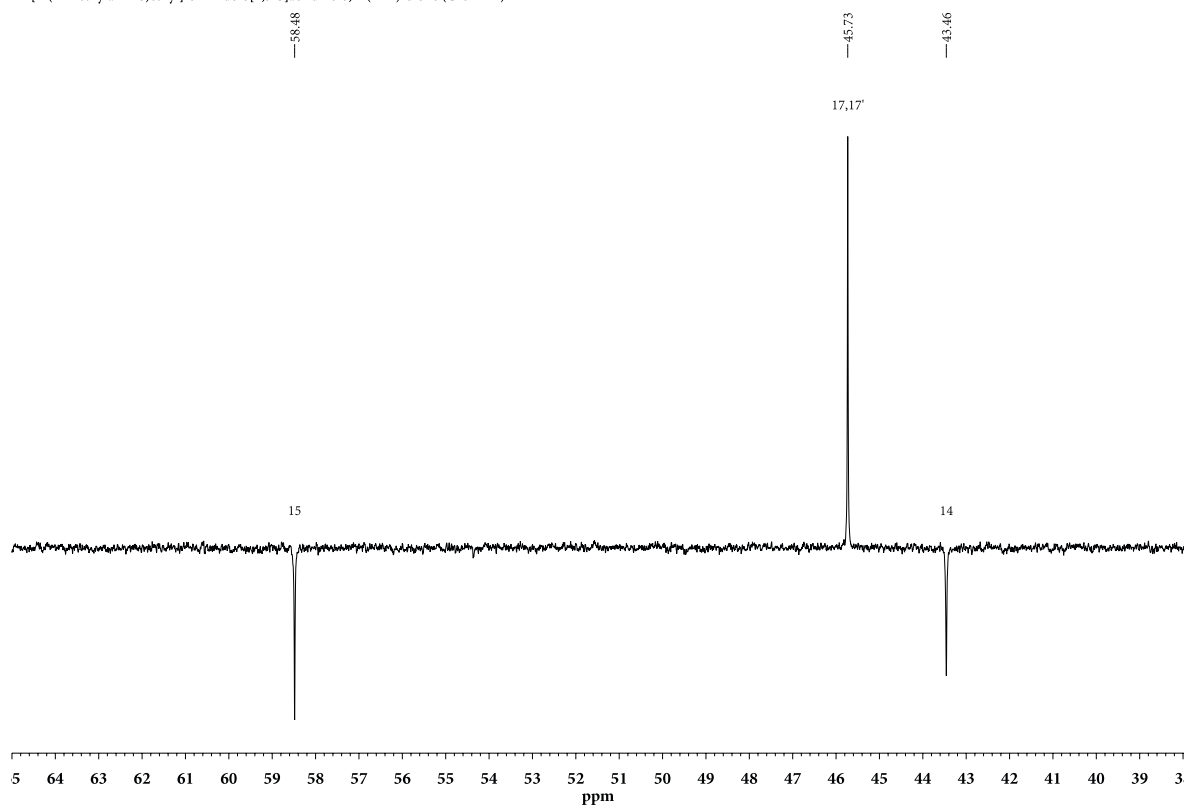
135.20
132.69
131.54
129.42
129.25
128.30
128.13
127.59
124.93
124.56
124.34
121.34
110.95

1
2
3
4
13a
9
8 7
6b
10
11a
12a
6a

36 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 115 114 113 112 111 110
ppm

60

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (C13 APT)

58.48
45.73
43.46

17,17'

15
14

5 64 63 62 61 60 59 58 57 56 55 54 53 52 51 50 49 48 47 46 45 44 43 42 41 40 39 3
ppm

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (HSQC)

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (HMBC)

60
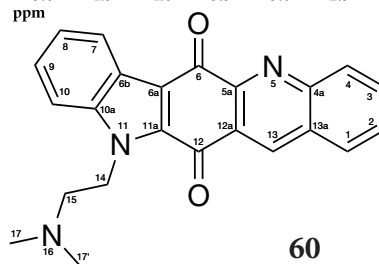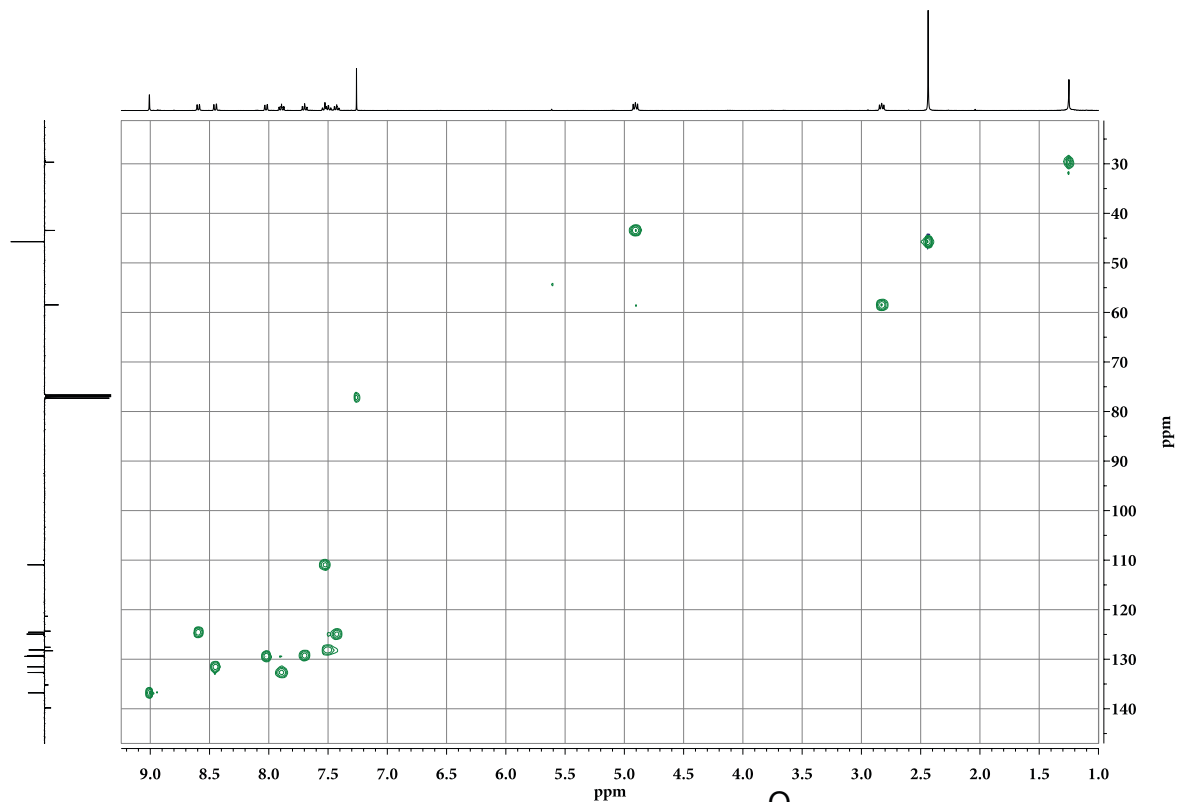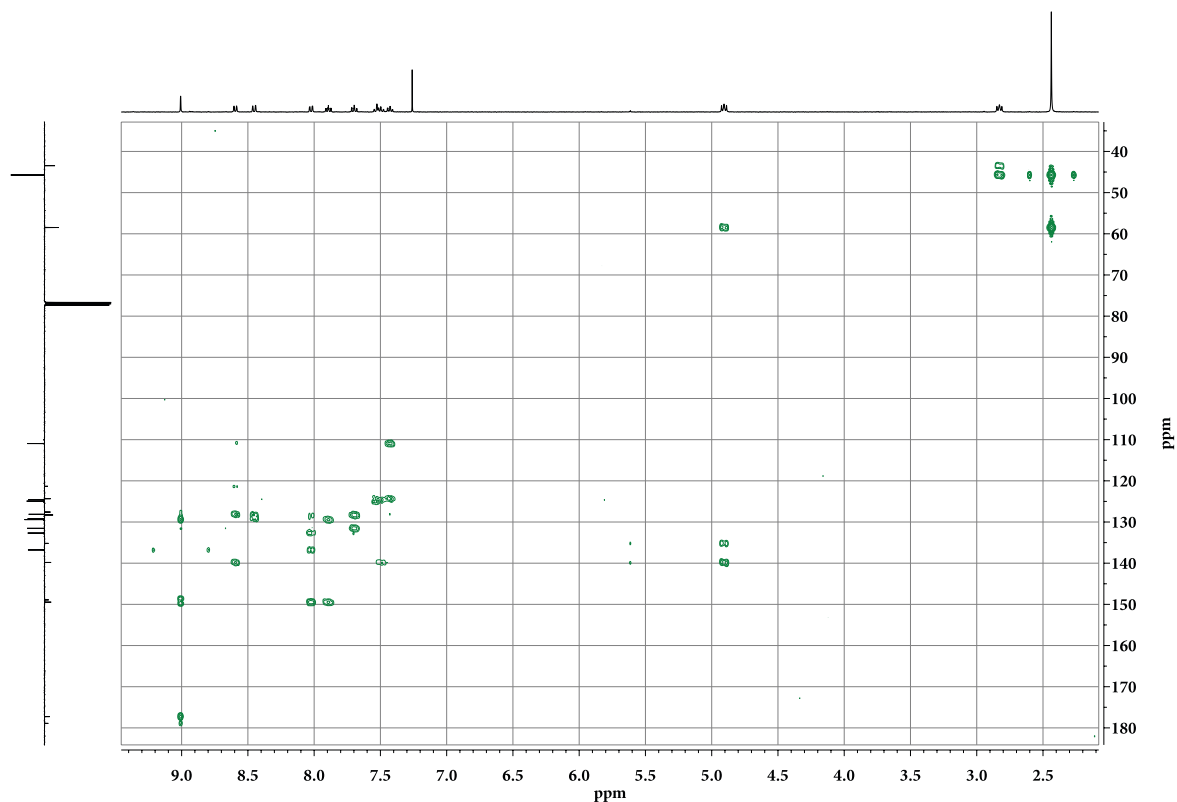
11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (15N HMBC)

11-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]acridine-6,12(11H)-dione (COSY)

60
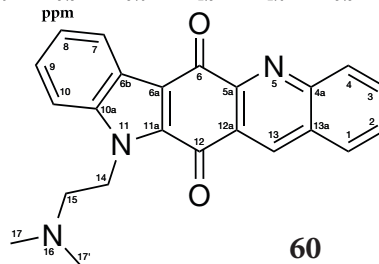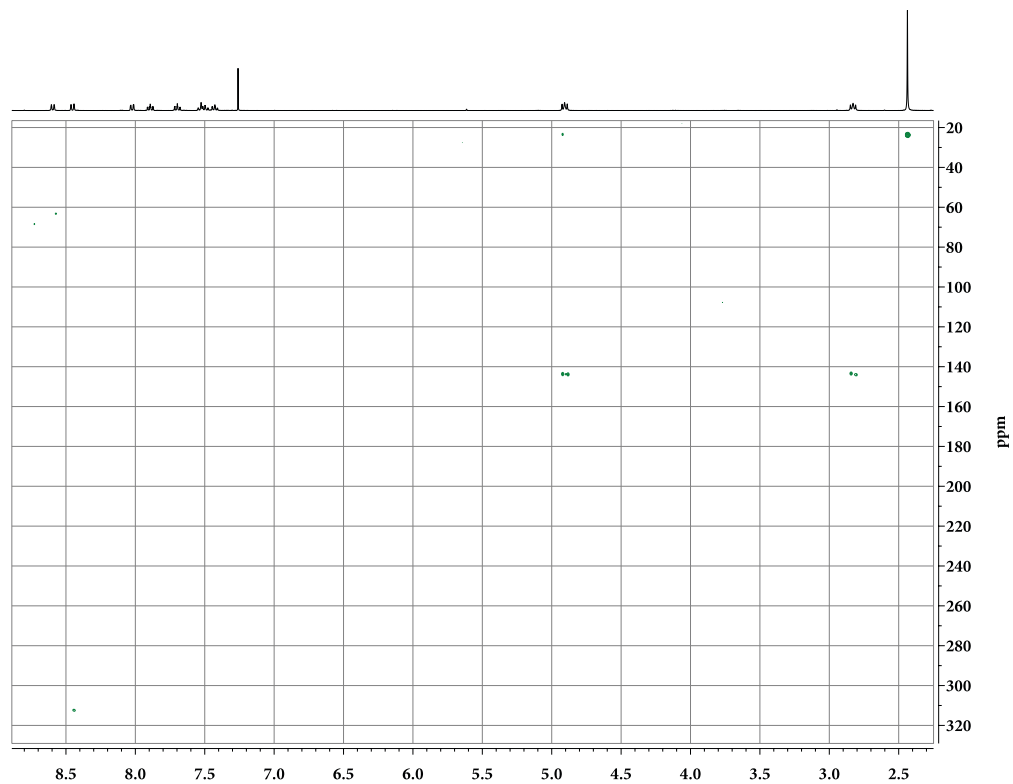
Generic Display Report

Analysis Info
Analysis Name   D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN173_DI_ESI_HRMS.d
Method   DI_mz_50-1550.m
Sample Name   approx. 10 ug/mL in MeOH
Comment

Acquisition Date   11/4/2014 1:25:35 PM
Operator   MZ
Instrument   maXis HD

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/4/2014 1:25:35 PM

Analysis Name   D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN173_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   approx. 10 ug/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
370.1545	1	C23H20N3O2	370.1550	1.4	21.6	1	100.00	15.5	even	ok
392.1368	1	C23H19N3NaO2	392.1369	0.4	10.4	1	100.00	15.5	even	ok
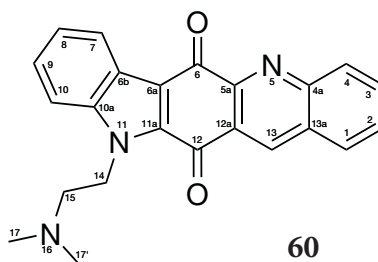
60

N,N-Diethyl-4-quinolinecarboxamide (PROTON)

N,N-Diethyl-4-quinolinecarboxamide (PROTON)

N,N-Diethyl-4-quinolinecarboxamide (PROTON)

8.90
8.89

8.10
8.08
8.08
7.76
7.75
7.75
7.75
7.73
7.73
7.73
7.72
7.72
7.70
7.70
7.70
7.68
7.68
7.55
7.54
7.53
7.53
7.52
7.51
7.26 CDCl3
7.26
7.25

2

8

5 7

6

3

1.00

1.00

2.03

1.02

1.08

8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2
ppm

61

N,N-Diethyl-4-quinolinecarboxamide (C13 APT)

167.58

149.85
148.22
143.05

129.89
129.82
127.40
124.55
124.16
117.26

77.00 CDCl3

42.92
42.13 Impurity
38.99
38.13 Impurity

14.13
13.84 Impurity
12.90
12.43 Impurity

7   4a
8   5
6

3

2

9

8a   4

11' 11

12
12'

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

N,N-Diethyl-4-quinolinecarboxamide (C13 APT)

61
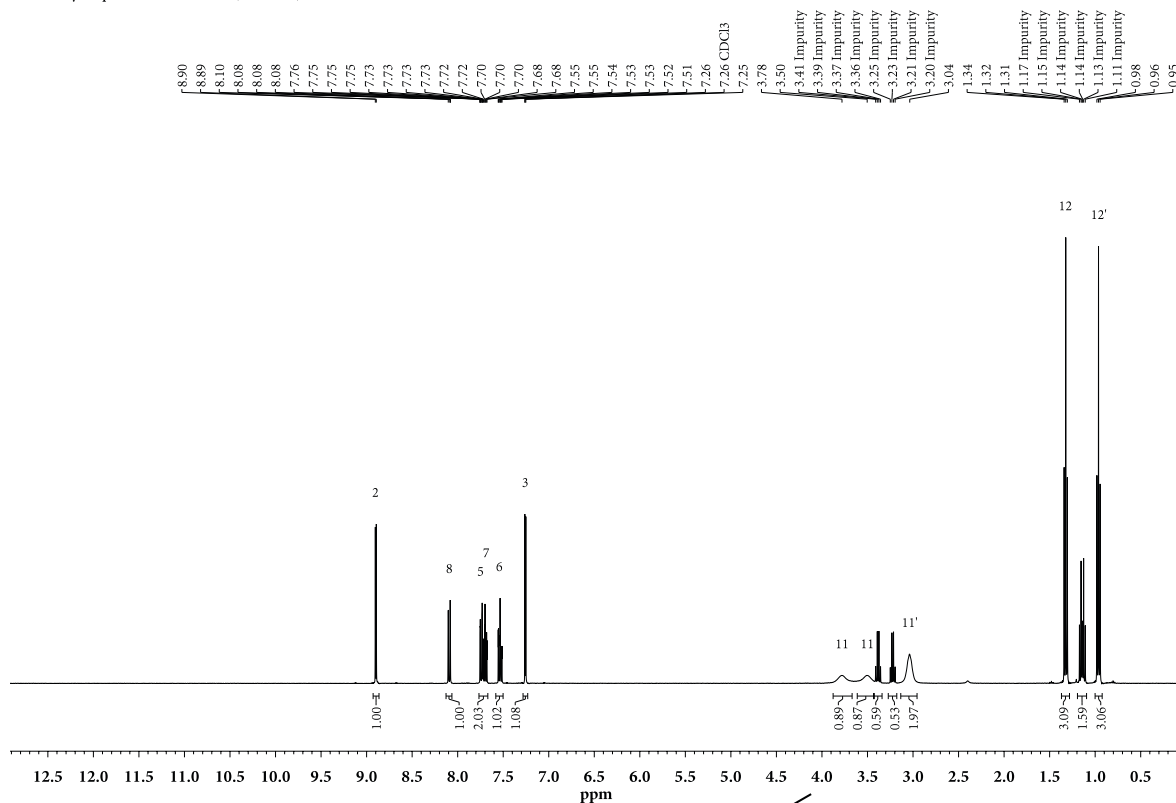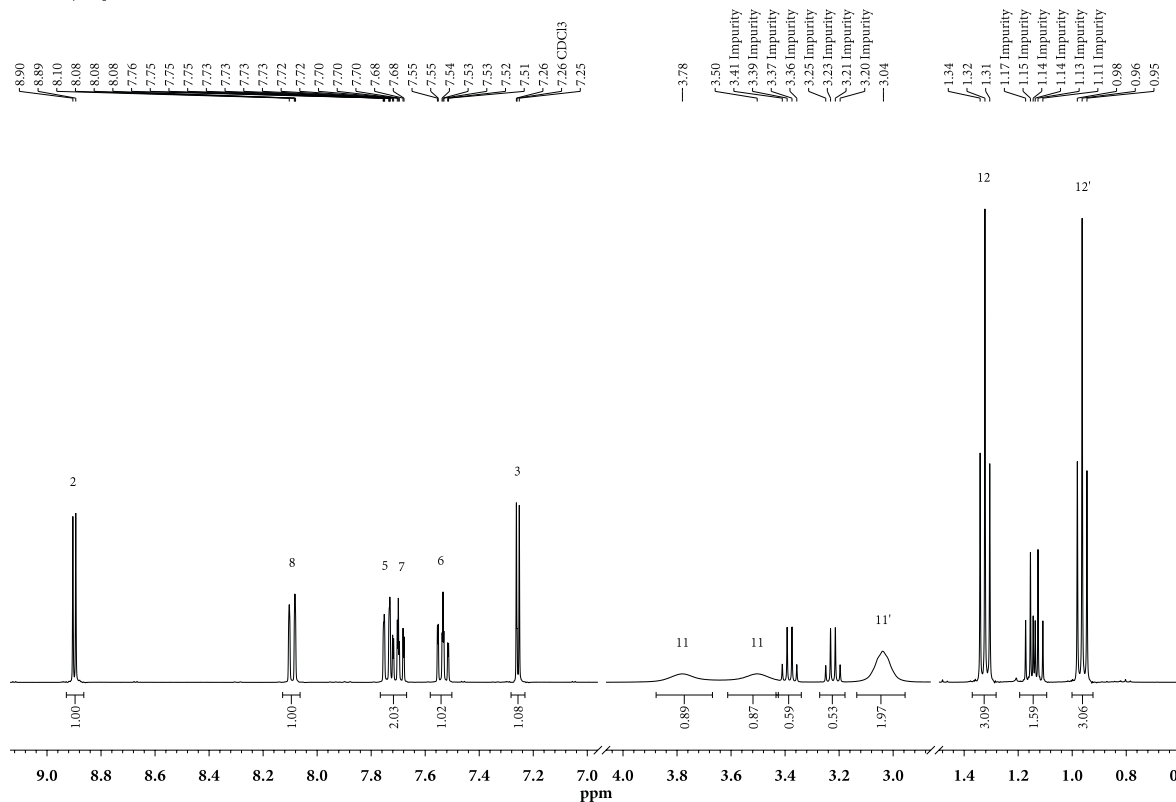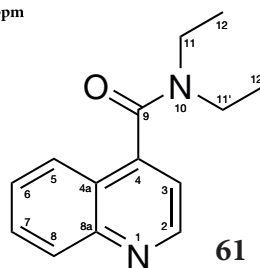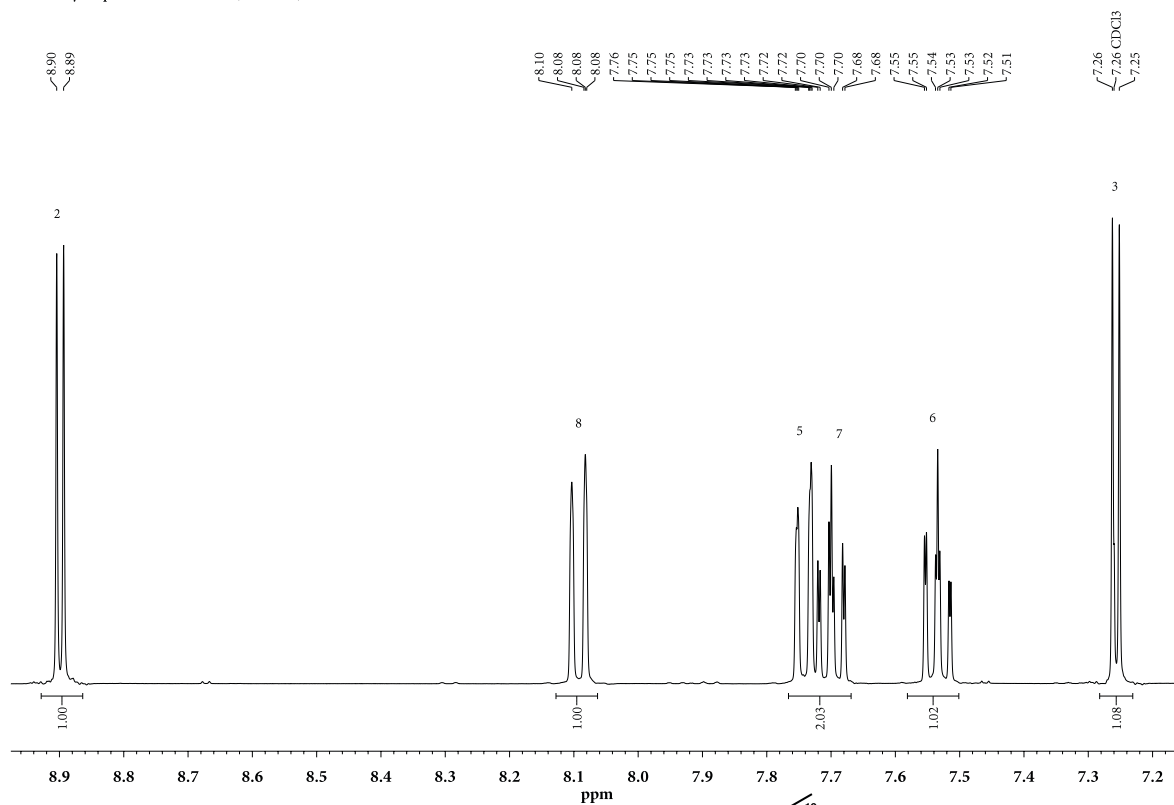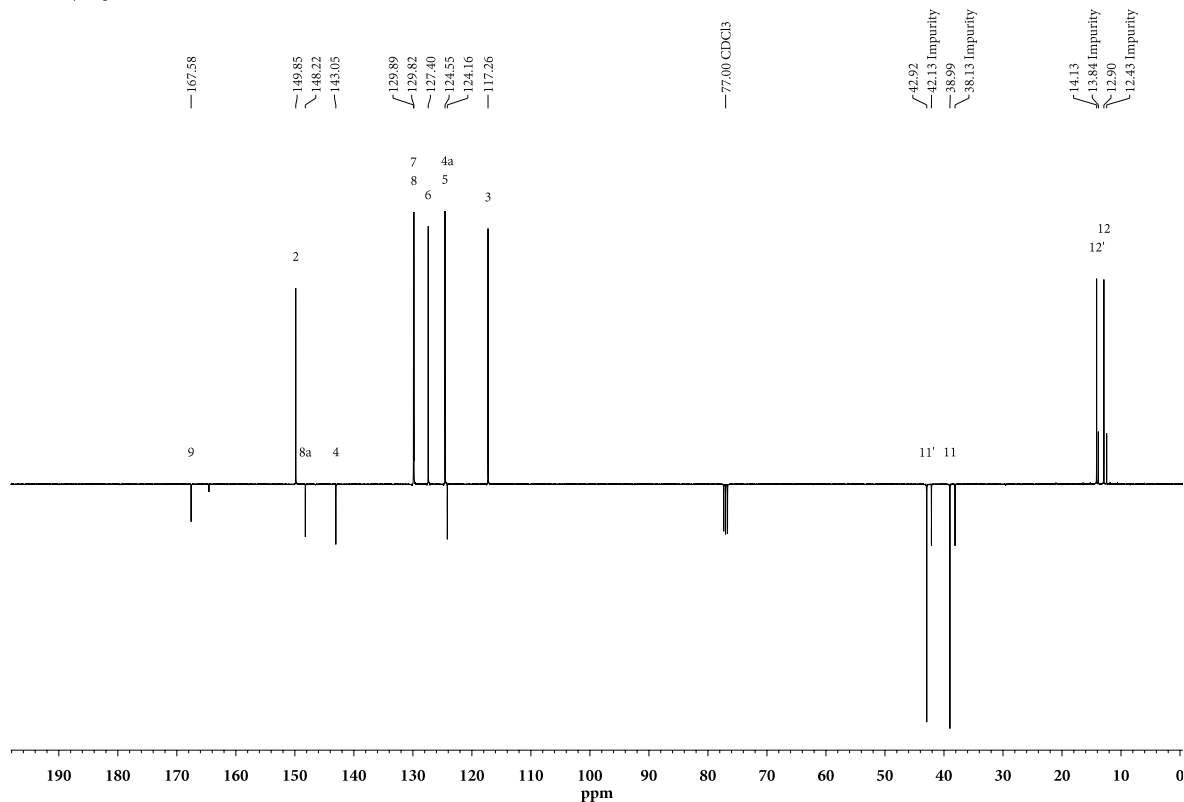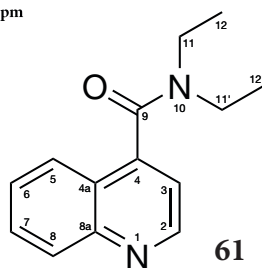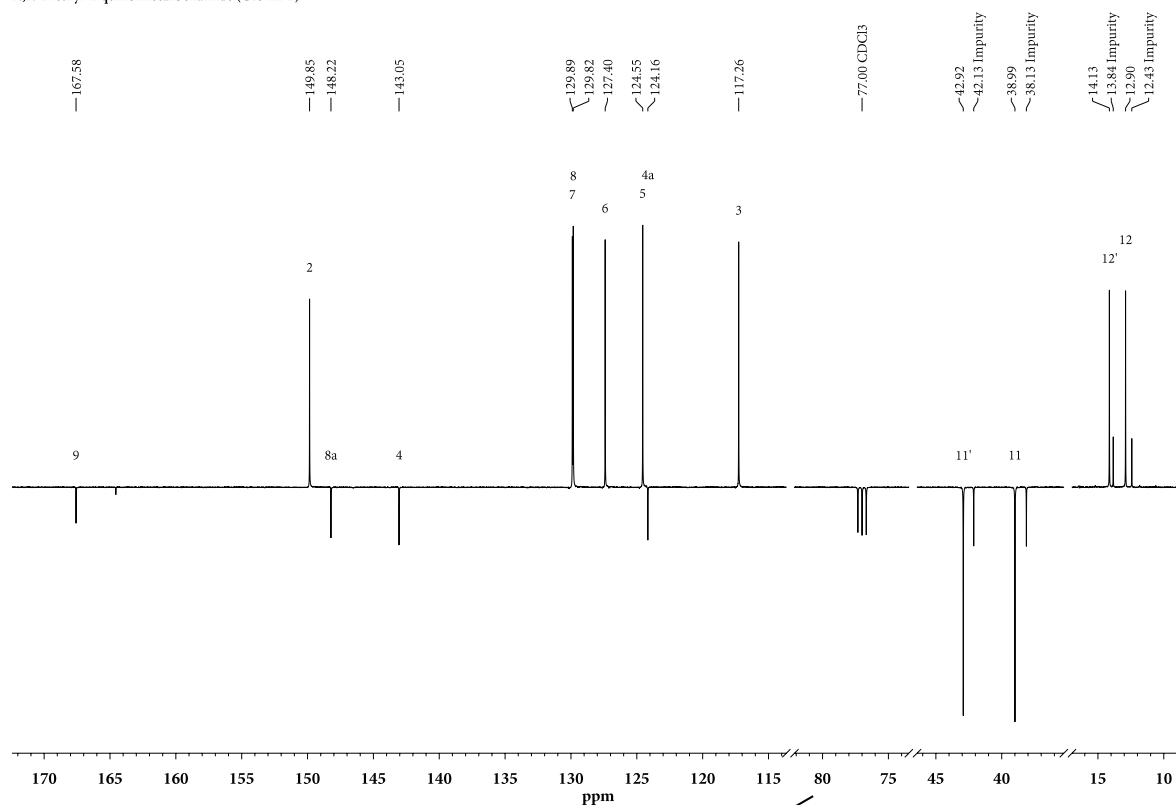
N,N-Diethyl-4-quinolinecarboxamide (C13 APT)

N,N-Diethyl-4-quinolinecarboxamide (HSQC)
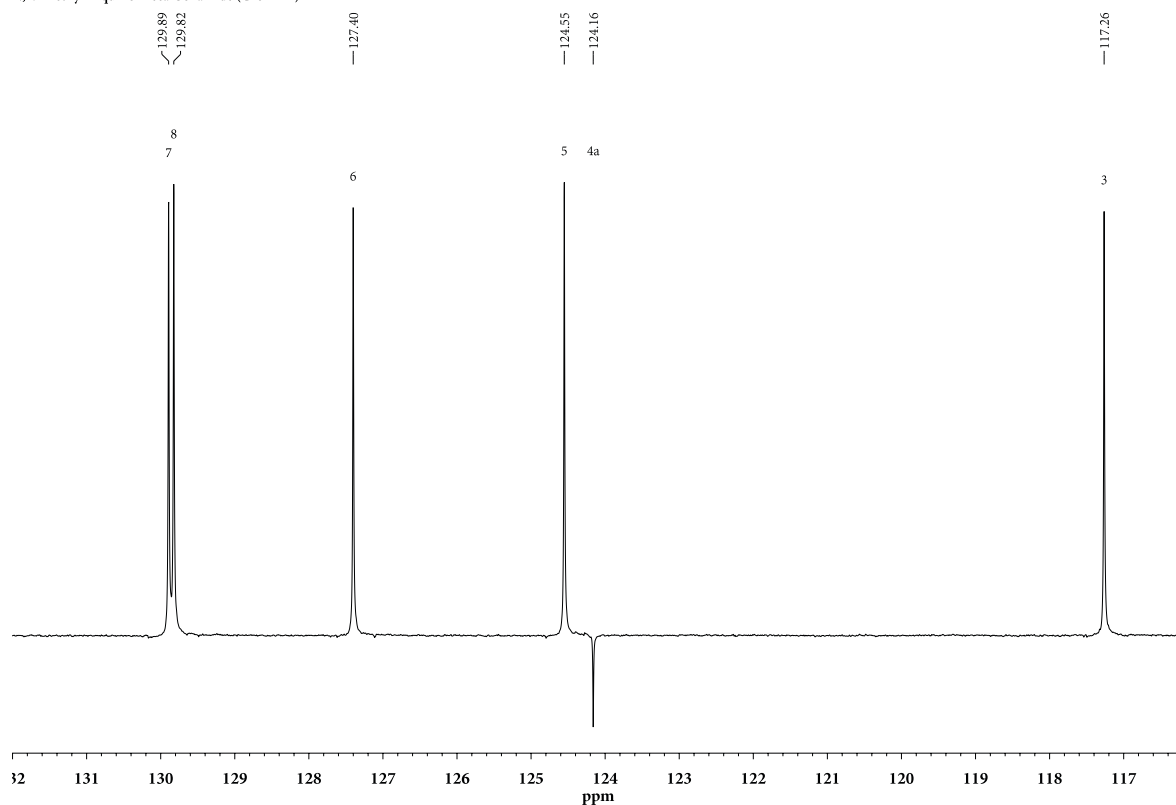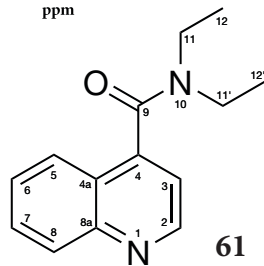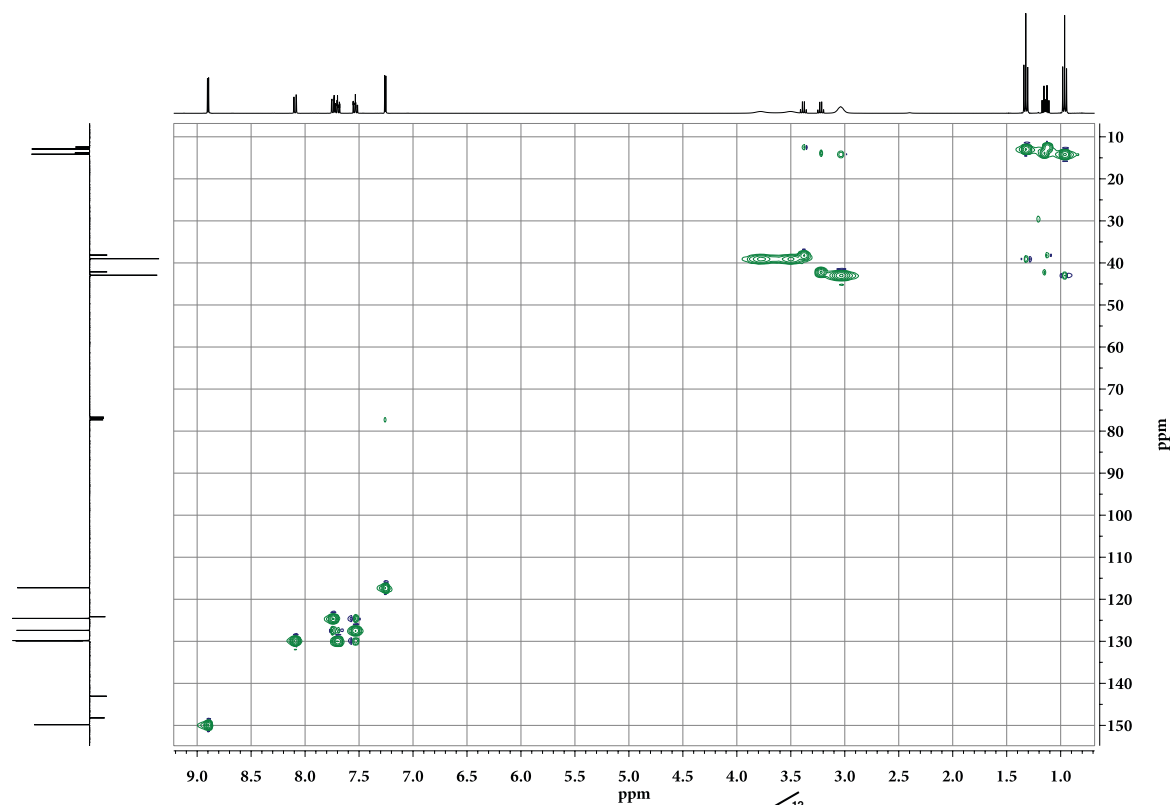
N,N-Diethyl-4-quinolinecarboxamide (HMBC)

61
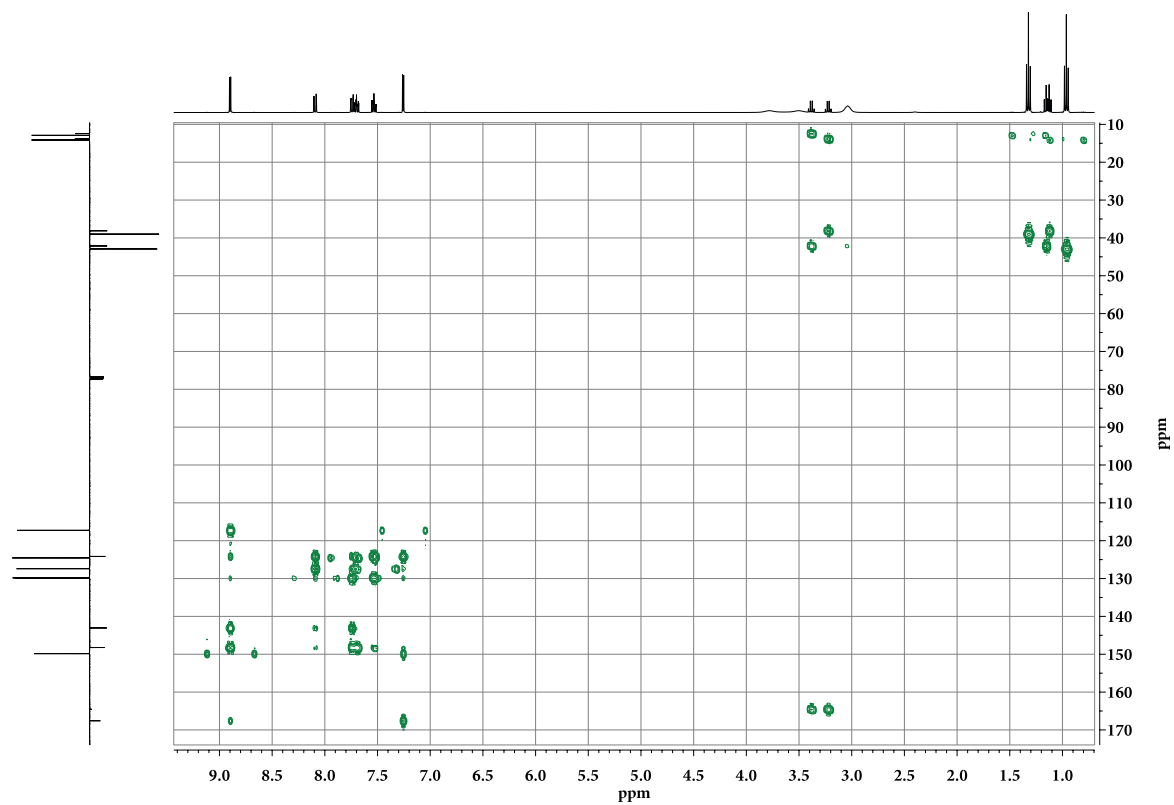
N,N-Diethyl-4-quinolinecarboxamide (15N HMBC)

N,N-Diethyl-4-quinolinecarboxamide (COSY)

61

12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

62
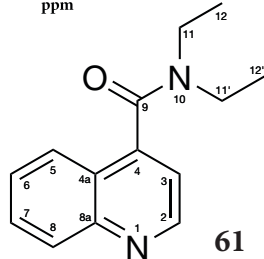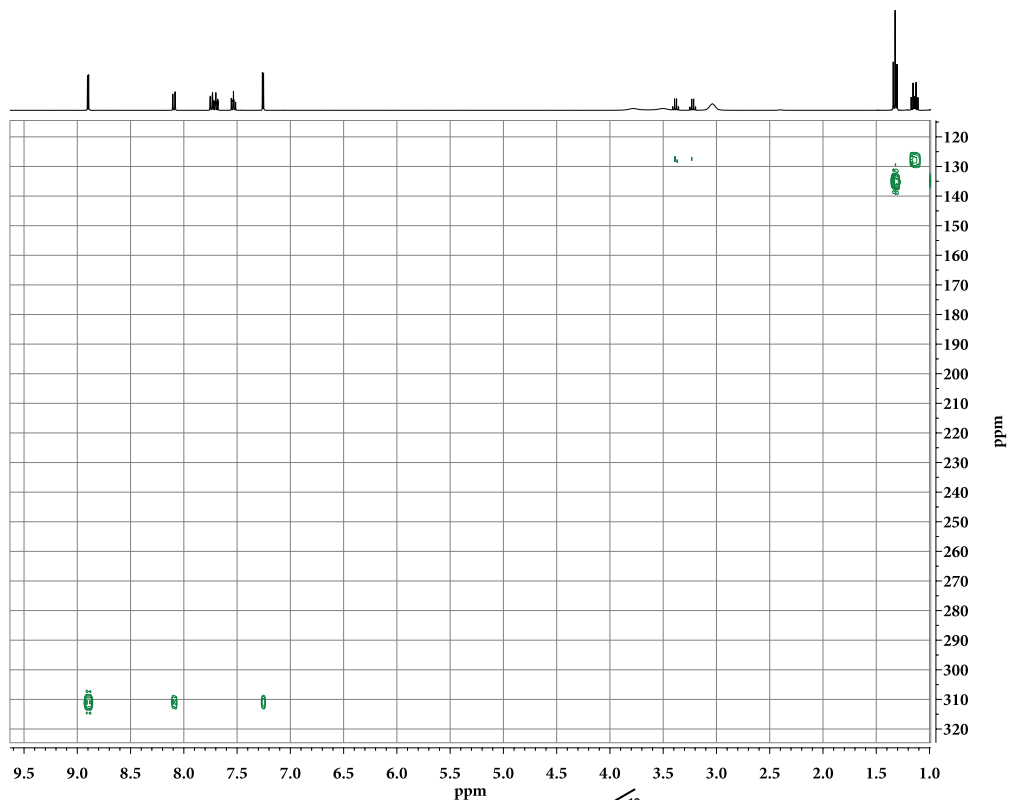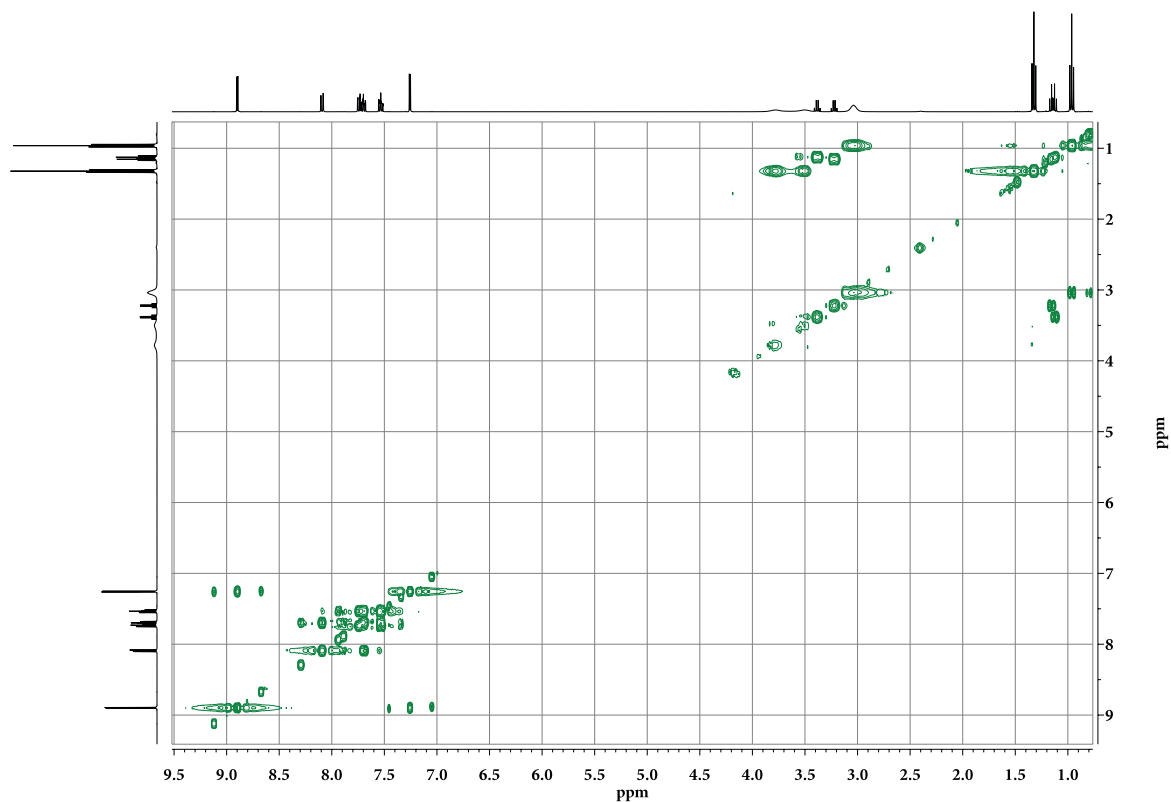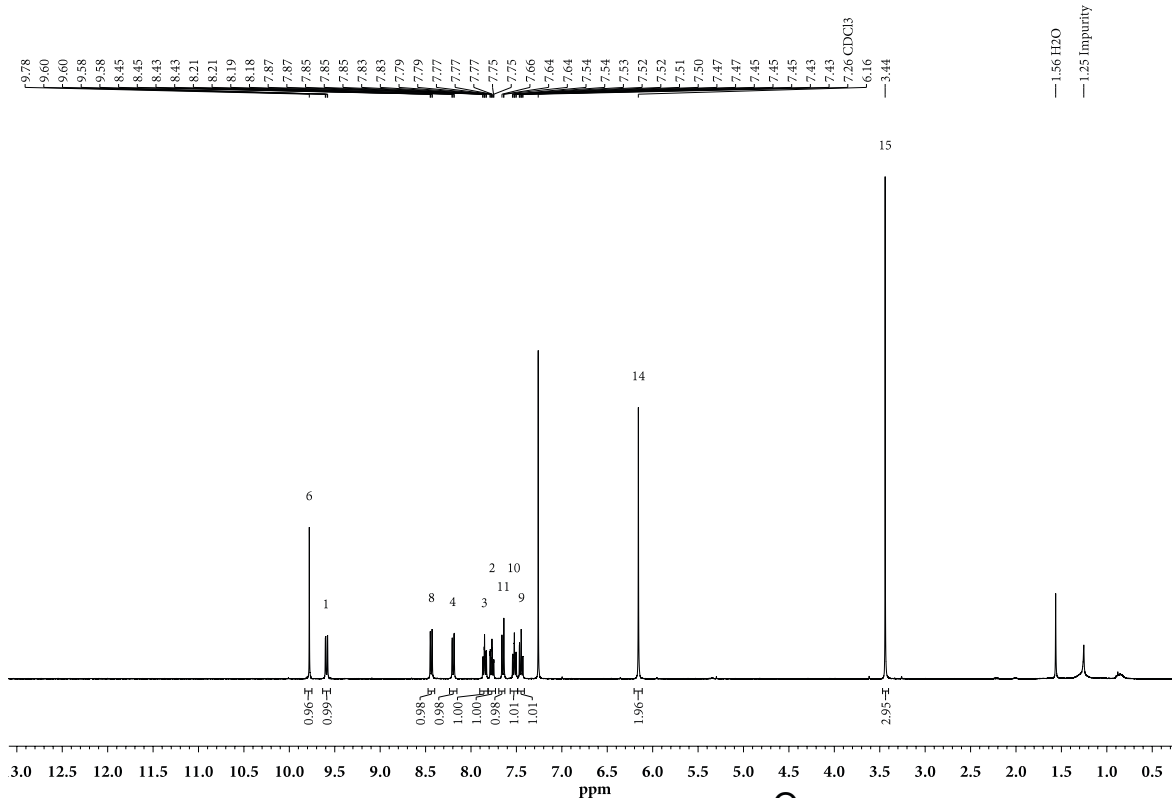
12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 APT)
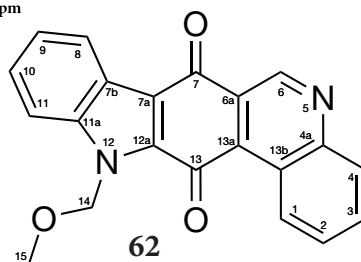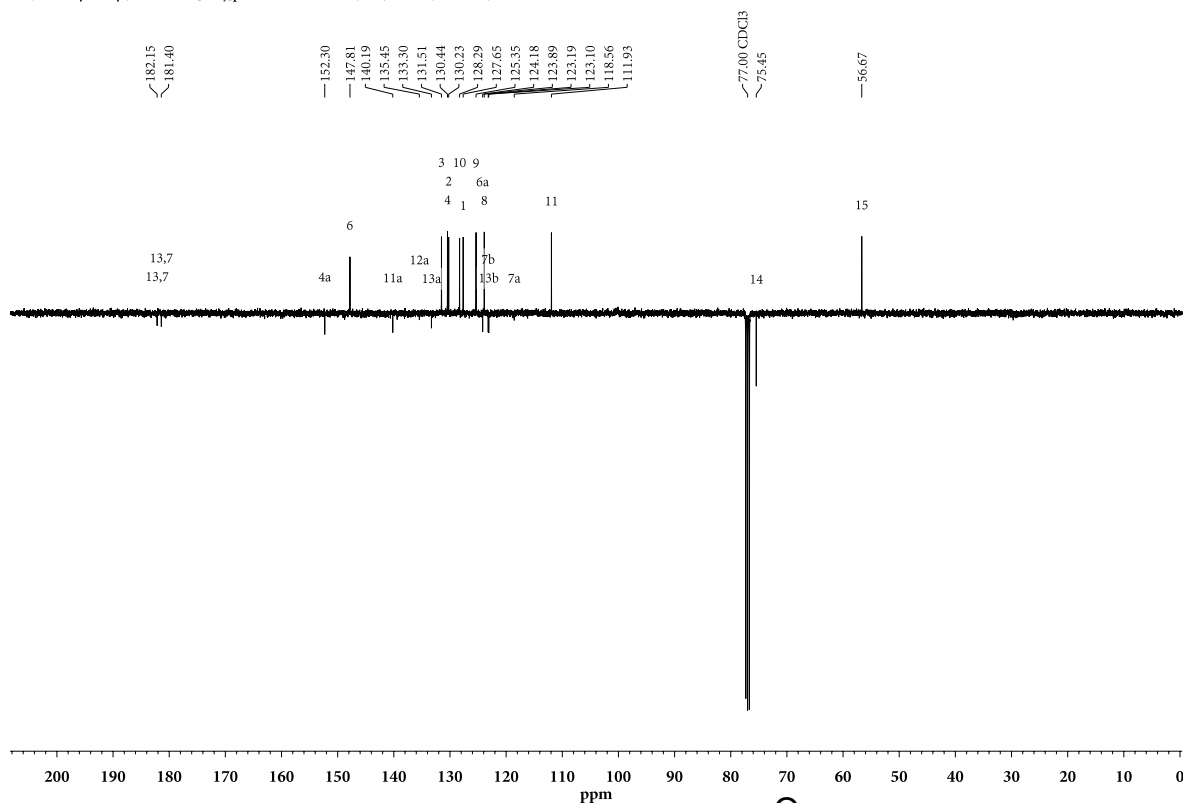
12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 APT)
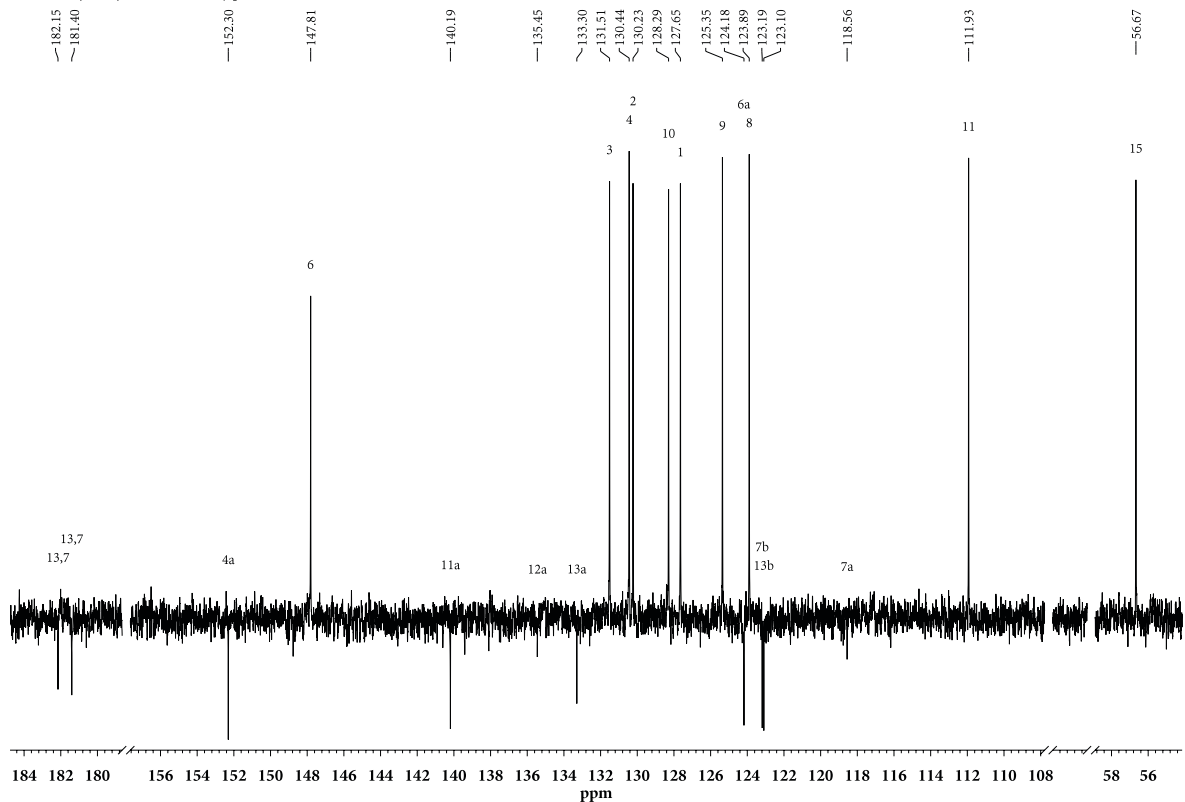
12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (HSQC)

12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (HMBC)

62
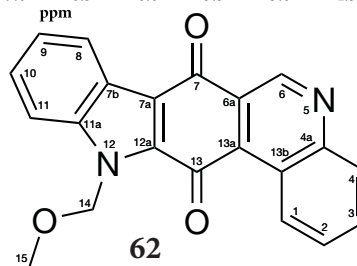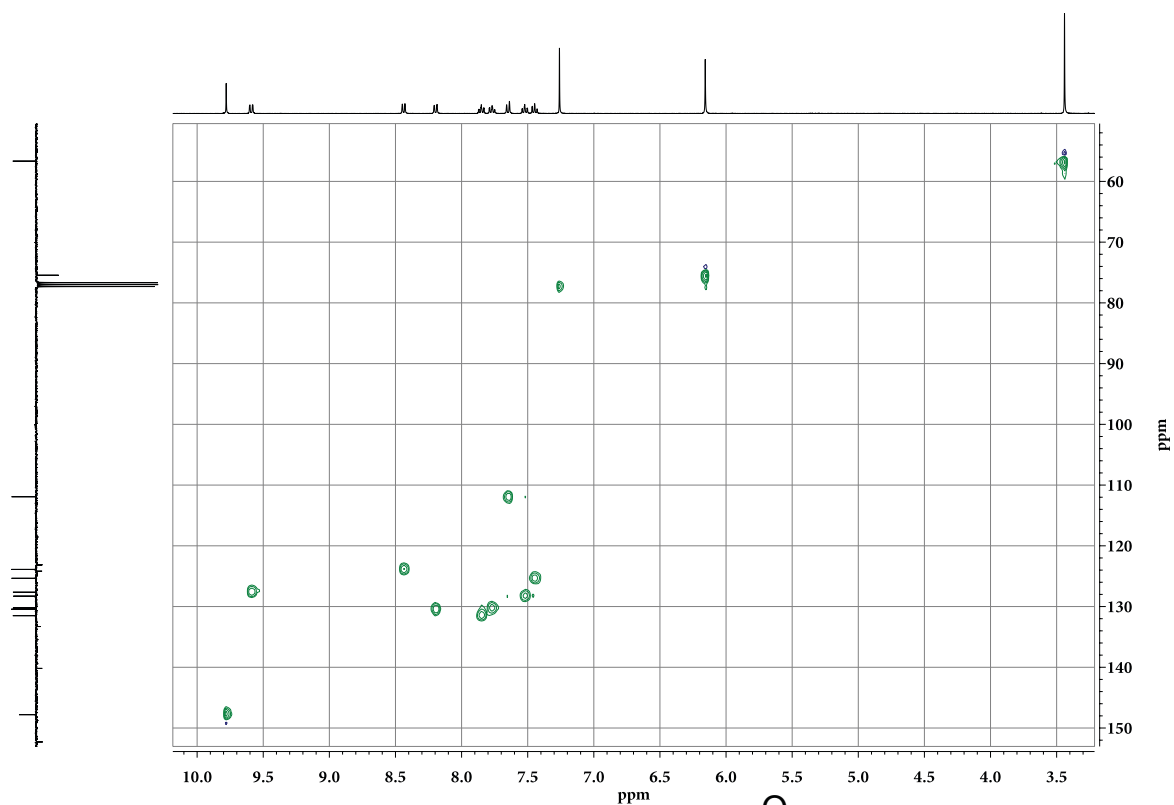
12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (15N HMBC)

12-(Methoxymethyl)-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (COSY)
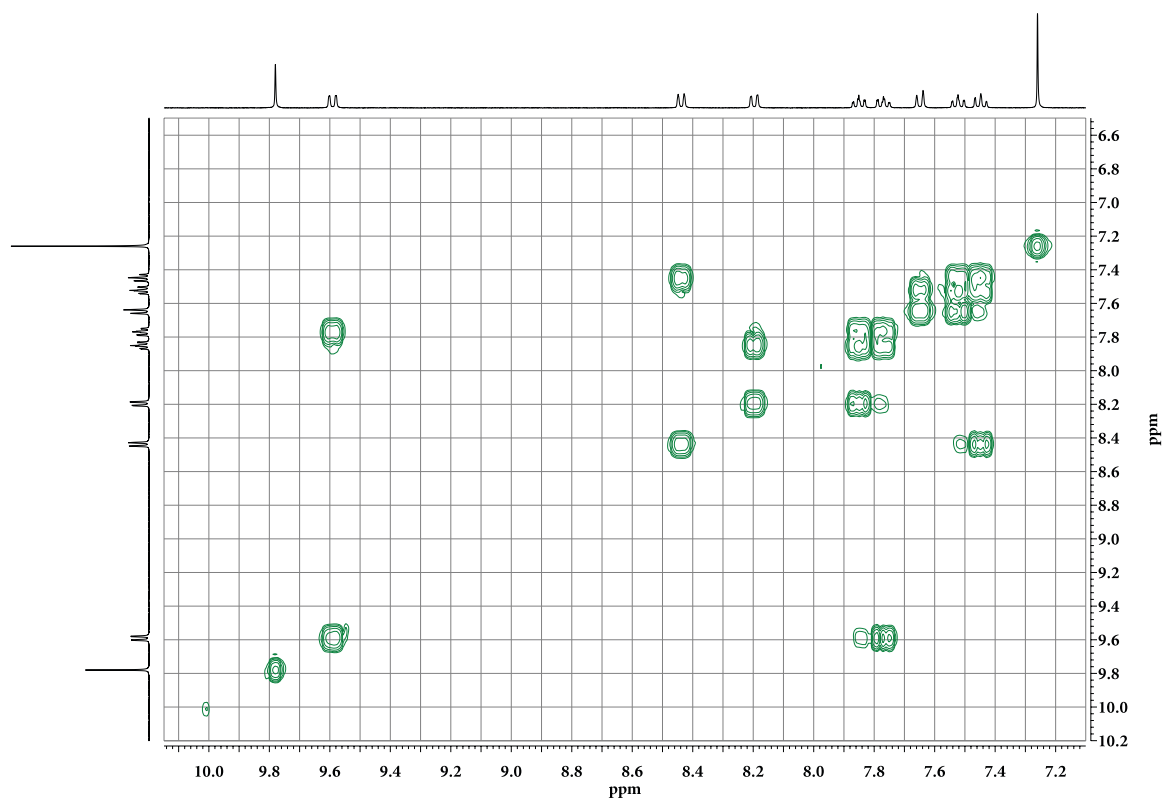
62

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 5:33:29 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN161_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

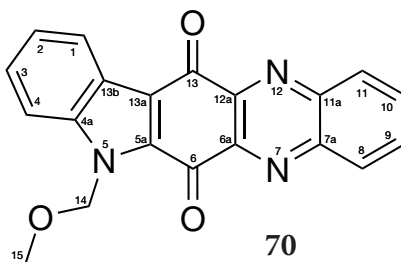|		
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN161_DI_ESI_HRMS.d	
Method	DI_mz_50-1550.m	
Sample Name	0.1 mg/mL in MeOH	
Comment		

Acquisition Date 11/17/2014 5:33:29 PM

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.5min #1-85
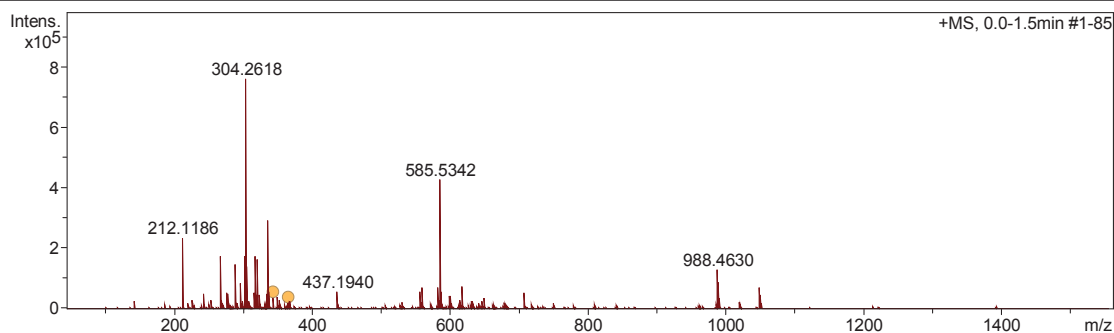
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
343.1081	1	C21H15N2O3	343.1077	-1.0	9.0	1	100.00	15.5	even	ok
365.0901	1	C21H14N2NaO3	365.0897	-1.1	19.1	1	100.00	15.5	even	ok

62

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

63

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

8.13
8.11
8.10

7.92
7.92
7.91
7.90
7.89
7.88
7.86
7.85
7.84
7.84
7.83
7.82
7.81

7.58
7.57
7.57
7.56
7.55
7.55

7.44
7.43
7.42
7.42
7.41
7.40
7.39

7.36
7.35
7.34
7.34
7.33

8,4

3

2

11

10

9

2.03

1.08

1.10

1.02

1.07

1.05

.25   8.20   8.15   8.10   8.05   8.00   7.95   7.90   7.85   7.80   7.75   7.70   7.65   7.60   7.55   7.50   7.45   7.40   7.35   7.30

ppm

63

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 CPD)

180.76
180.23

151.15
147.43
138.38
137.96
132.49
131.51
130.17
129.78
127.13
127.07
124.78
124.25
123.29
122.51
122.22
115.46
113.90

39.50 DMSO

6a
13a   7b
3   9
13,7   12a   2 10 13b   7a
13,7   4a 6 11a   4 1   8   11

200   190   180   170   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0

ppm

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 CPD)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 CPD)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (HSQC)

63

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (HMBC)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (15N HMBC)

63

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione (COSY)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN165_DI_ESI_HRMS.d	Acquisition Date	11/17/2014 5:49:40 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/17/2014 5:49:40 PM
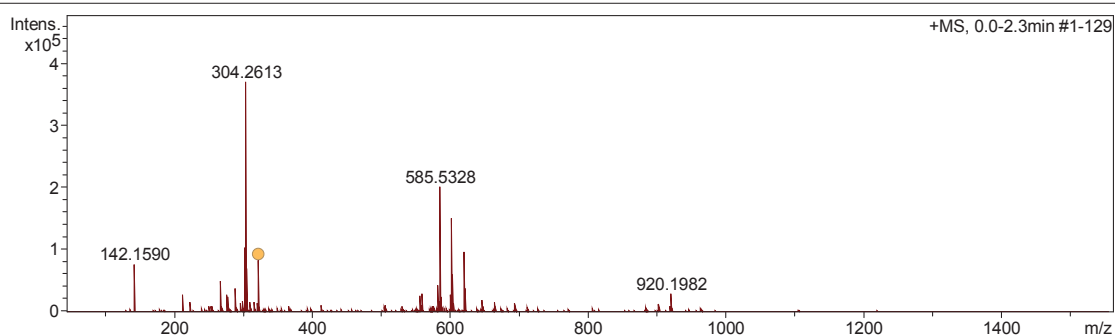
Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN165_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
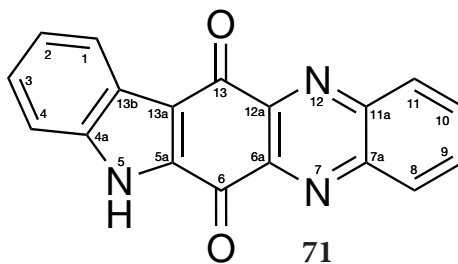Sample Name   0.1 mg/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
299.0814	1	C19H11N2O2	299.0815	0.5	n.a.	1	100.00	15.5	even	ok
321.0634	1	C19H10N2NaO2	321.0634	0.0	17.3	1	100.00	15.5	even	ok

63

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

64
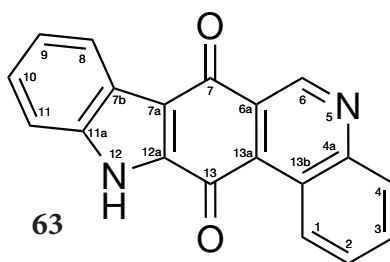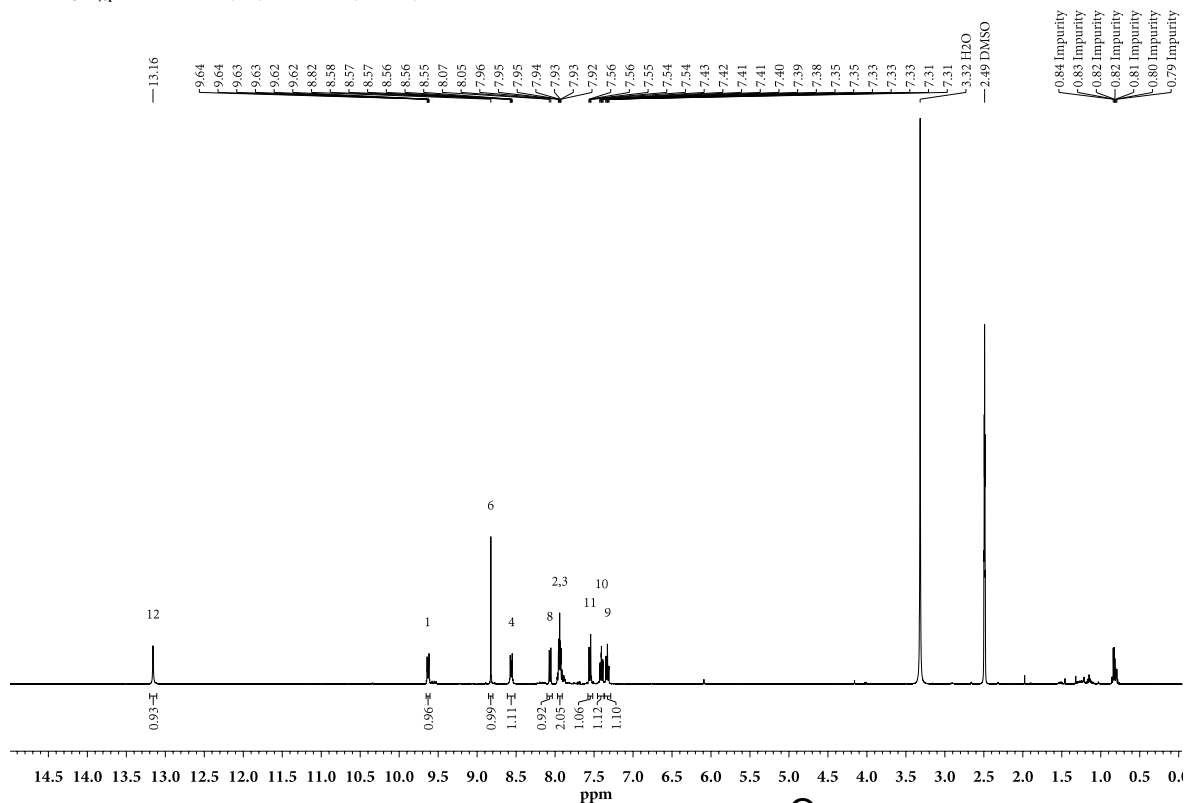
7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)
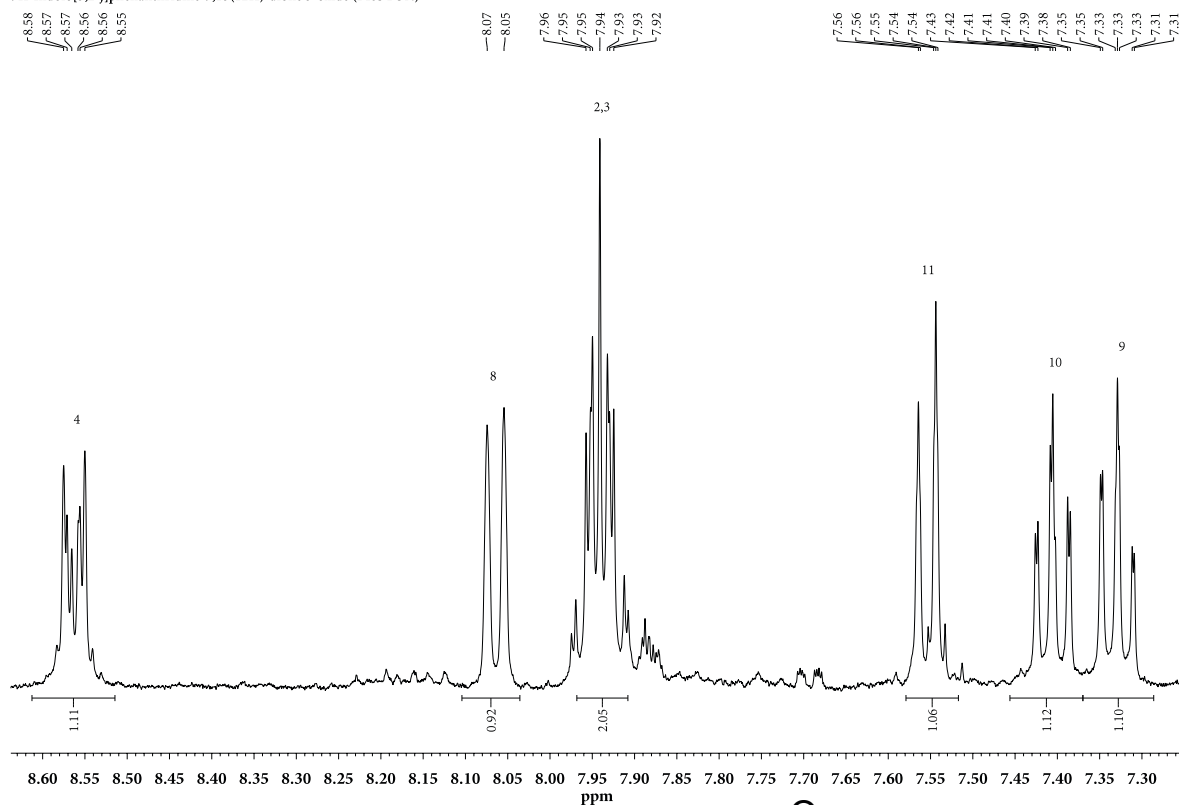
8.58
8.57
8.57
8.56
8.56
8.55

8.07
8.05
7.96
7.95
7.95
7.94
7.93
7.93
7.92

7.56
7.56
7.55
7.54
7.54
7.43
7.42
7.41
7.41
7.40
7.39
7.38
7.35
7.35
7.33
7.33
7.31
7.31

2,3

4

8

11

10

9

1.11

0.92

2.05

1.06

1.12

1.10

8.60 8.55 8.50 8.45 8.40 8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30
ppm

64

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

178.14
177.69

143.03
138.58
138.03
131.92
131.81
131.69
129.79
128.08
126.97
126.77
124.41
123.35
121.96
121.76
119.07
115.03
113.94

39.50 DMSO

6a
10 7b
6 13b
2 9 8 4 7a
13 12a 3 1 13a 11
7 4a 11a

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)
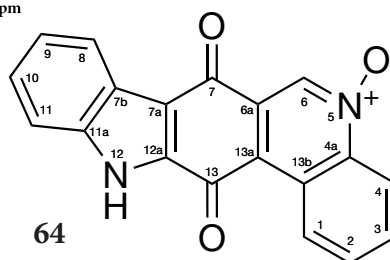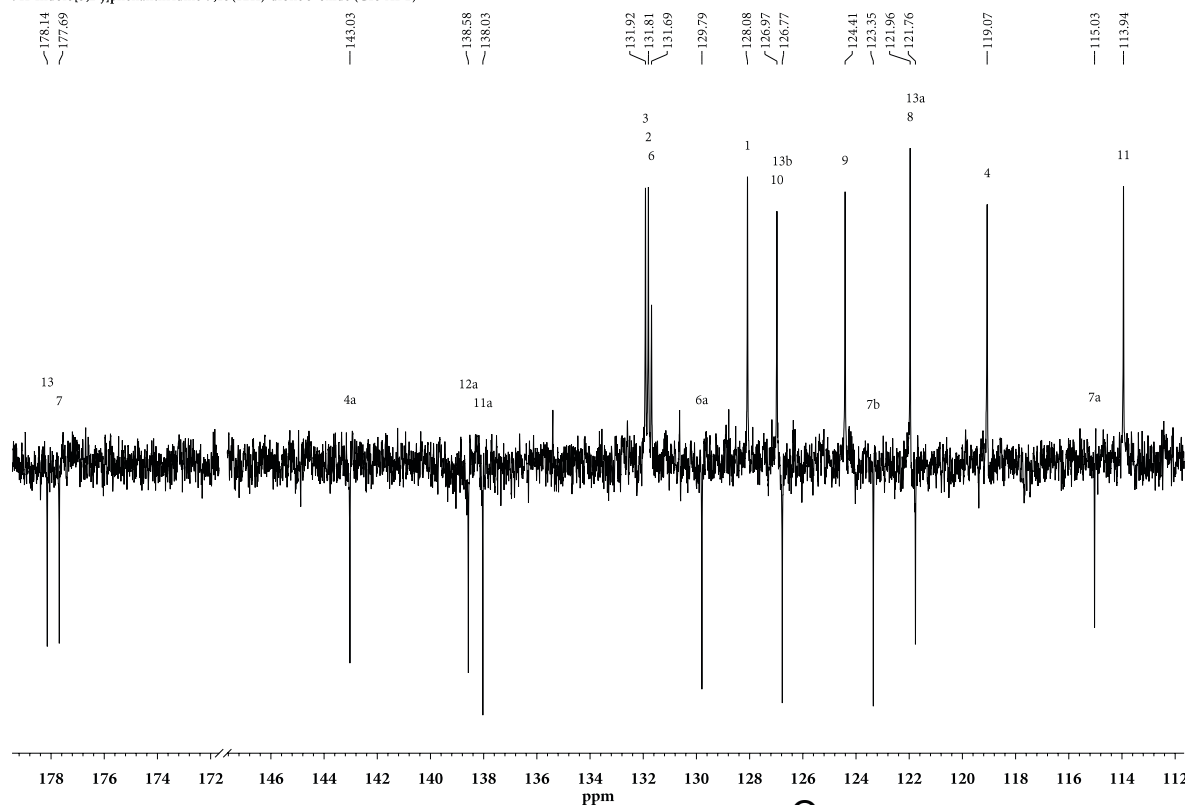
7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (HSQC)
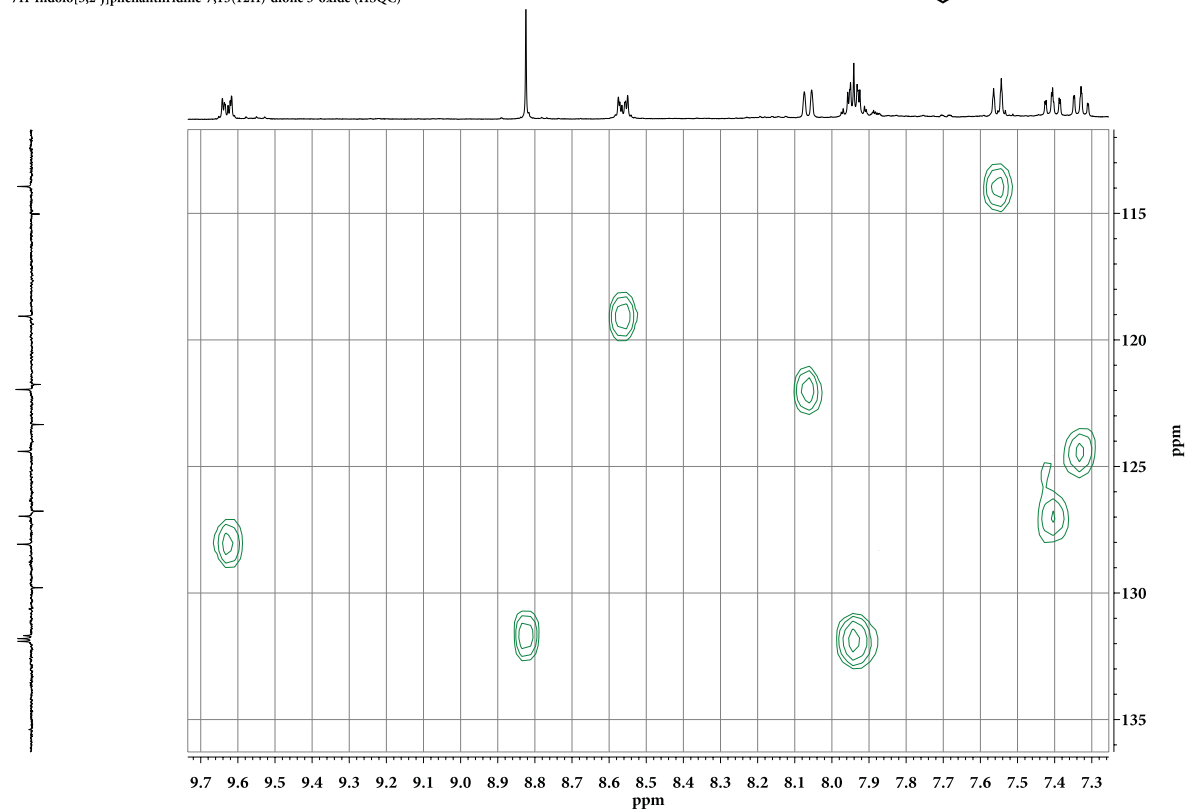
7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (HMBC)
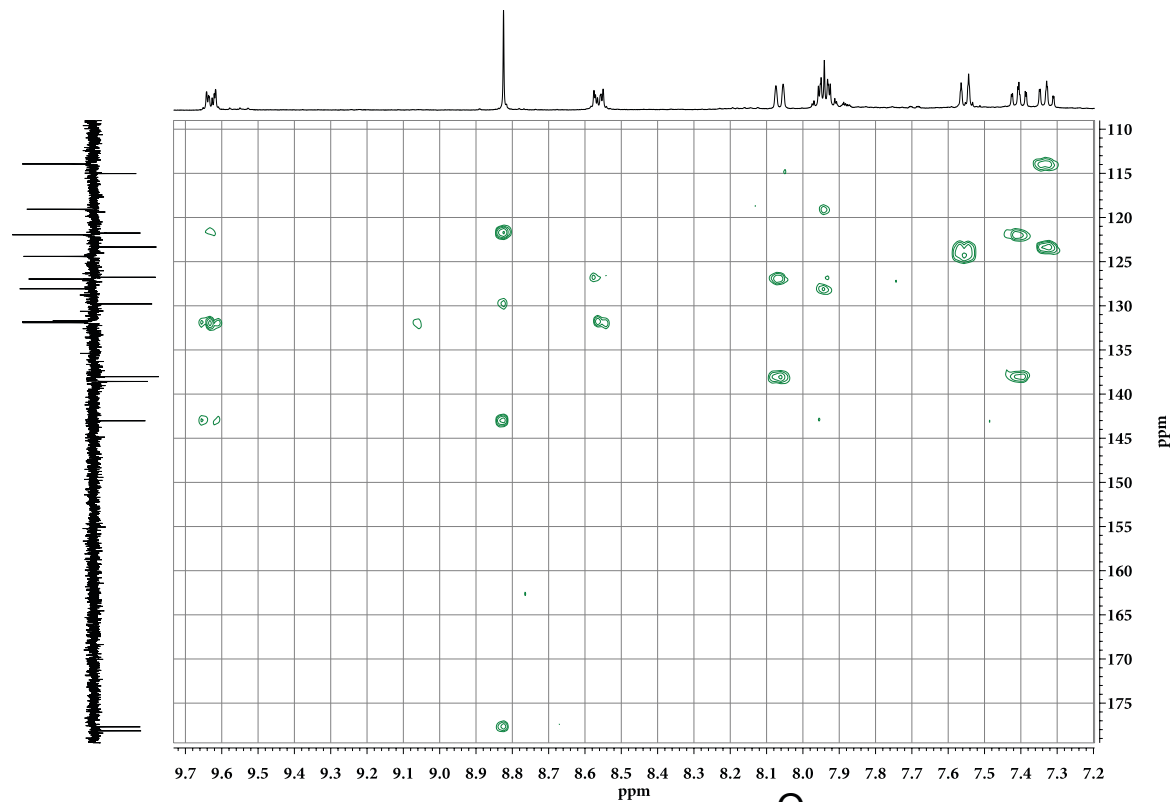
7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (15N HMBC)

64

567
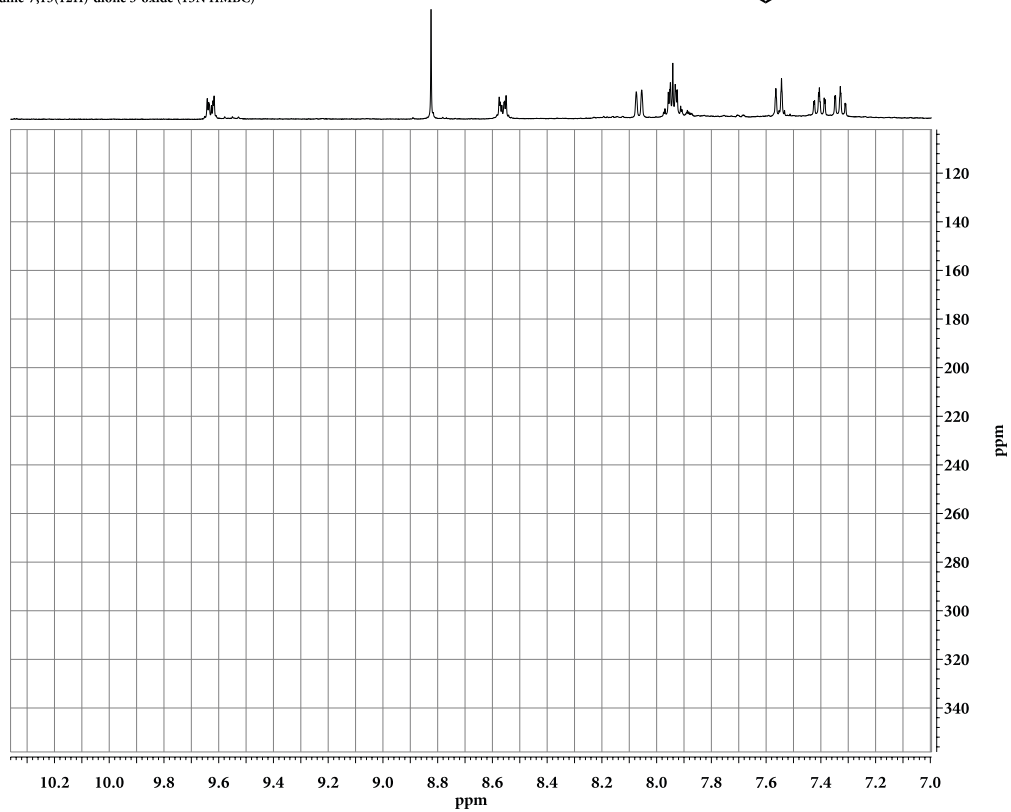
7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (15N HSQC)

7H-Indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 6:00:27 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN166_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Bruker Compass DataAnalysis 4.2   printed: 11/17/2014 6:06:32 PM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/17/2014 6:00:27 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN166_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.8min #1-160

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
315.0767	1	C4H7N14O4	315.0769	0.7	n.a.	1	100.00	8.5	even	ok
	2	C19H11N2O3	315.0764	-0.8	400.7	2	0.00	15.5	even	ok
337.0585	1	C19H10N2NaO3	337.0584	-0.3	5.5	1	100.00	15.5	even	ok

64

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)
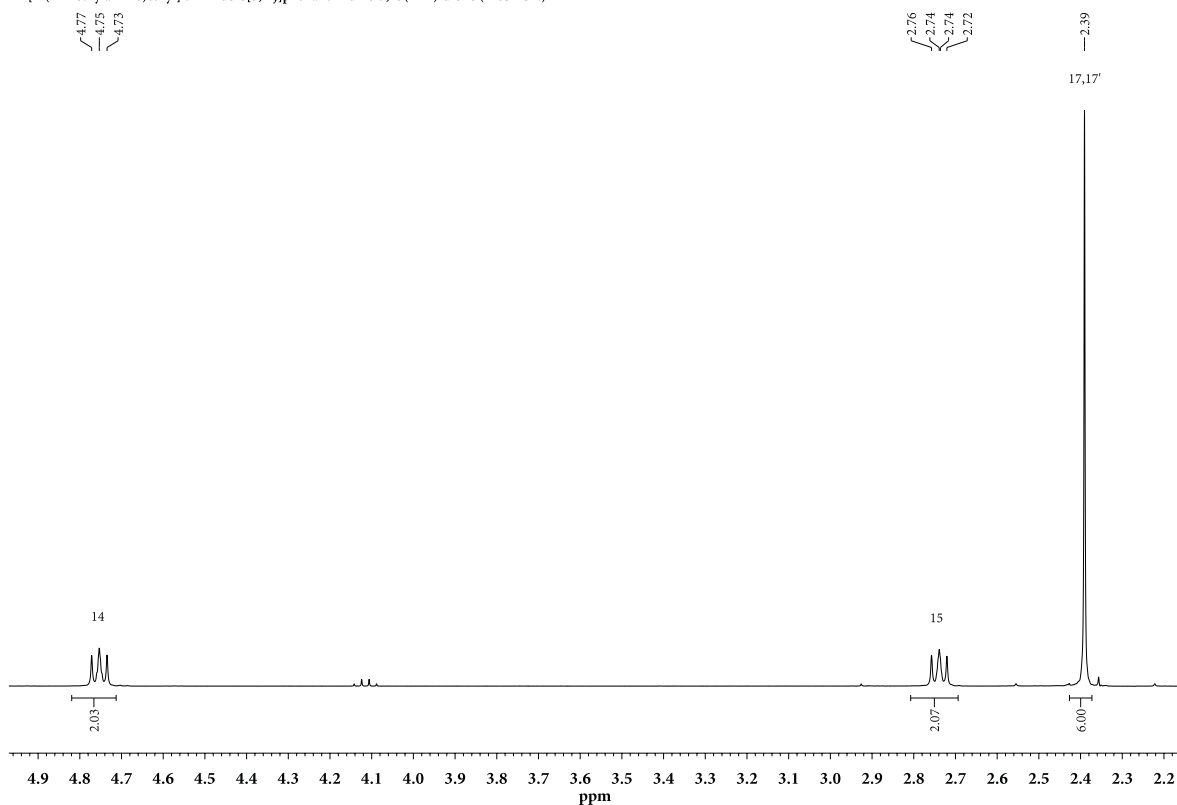
65

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

7.80
7.80
7.79
7.78
7.77
7.76
7.73
7.72
7.71
7.71
7.70
7.69
7.69

7.43
7.43
7.42
7.41
7.41
7.40
7.40
7.38
7.38
7.34
7.34
7.33
7.32
7.32
7.31
7.30

10,11

3

2

9

1.04   1.03   2.00   1.00

7.84 7.82 7.80 7.78 7.76 7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.2
ppm

65

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (PROTON)

4.77
4.75
4.73

2.76
2.74
2.74
2.72

2.39

17,17'

14   15

2.03   2.07   6.00

4.9   4.8   4.7   4.6   4.5   4.4   4.3   4.2   4.1   4.0   3.9   3.8   3.7   3.6   3.5   3.4   3.3   3.2   3.1   3.0   2.9   2.8   2.7   2.6   2.5   2.4   2.3   2.2
ppm

573

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 APT)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 APT)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (C13 APT)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (HSQC)

65
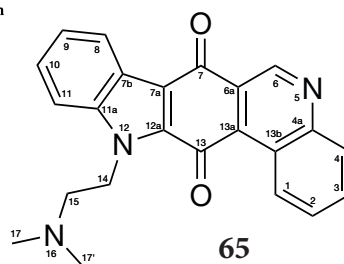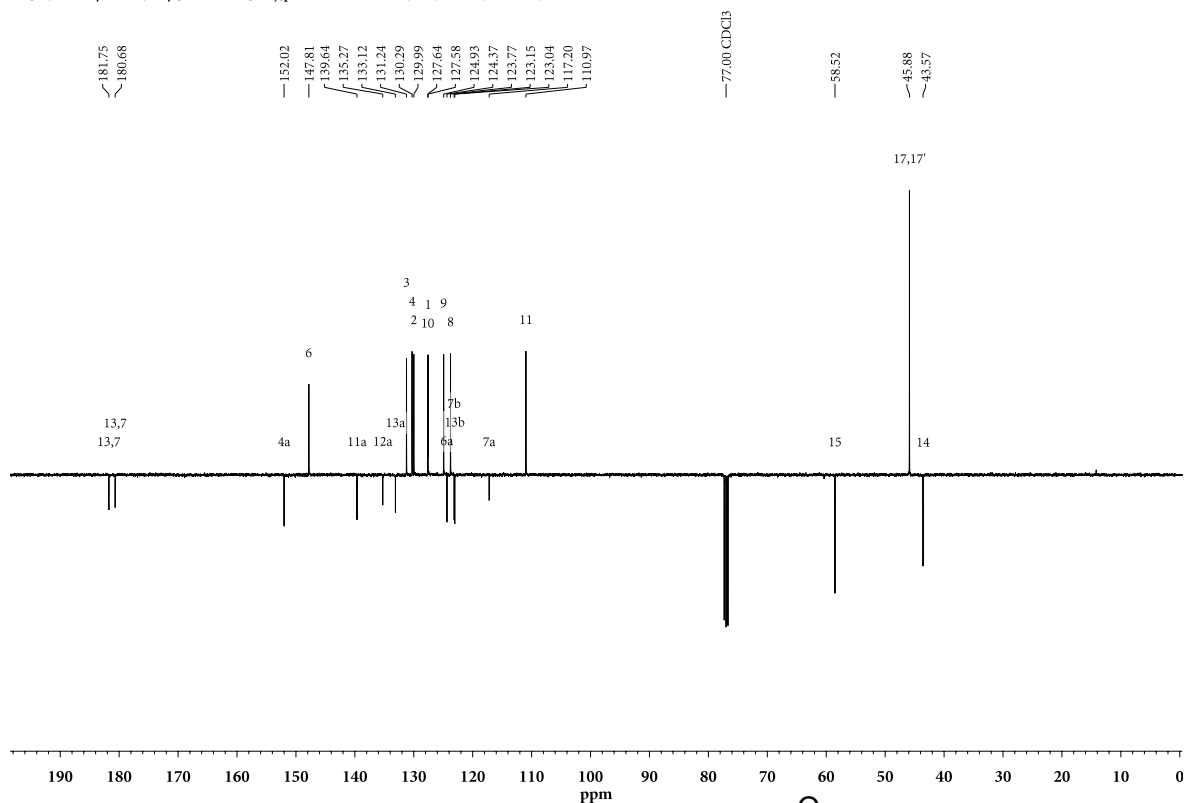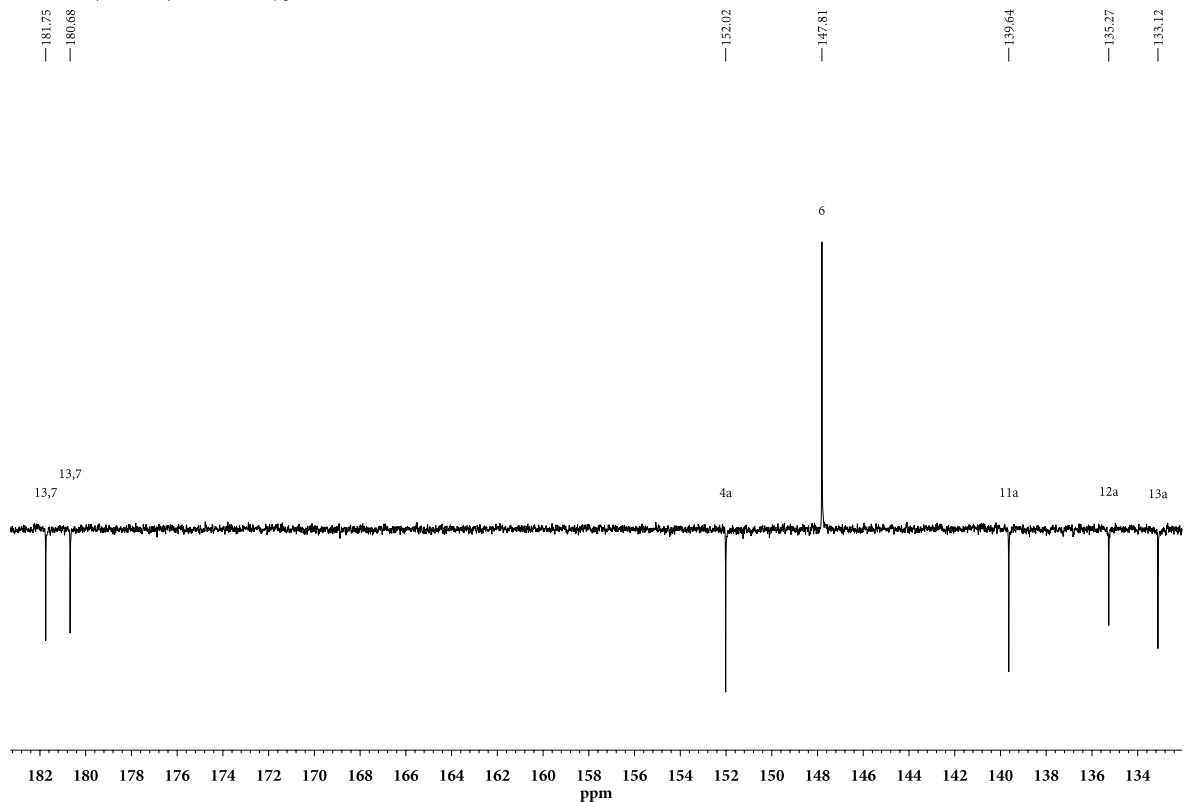
12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (HMBC)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (15N HMBC)

65

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (15N HMBC)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione (COSY)

65

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 12:54:07 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN167_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/4/2014 12:54:07 PM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN167_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
370.1551	1	C23H20N3O2	370.1550	-0.4	8.6	1	100.00	15.5	even	ok
392.1371	1	C23H19N3NaO2	392.1369	-0.4	7.4	1	100.00	15.5	even	ok

65

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

66

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (PROTON)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (C13 APT)

66

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (HSQC)

66

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (HMBC)

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (15N HMBC)

66

12-[2-(Dimethylamino)ethyl]-7H-indolo[3,2-j]phenanthridine-7,13(12H)-dione 5-oxide (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 1:21:03 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN168_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/4/2014 1:21:03 PM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN168_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.2min #1-124

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
386.1491	1	C23H20N3O3	386.1499	2.0	1.3	1	100.00	15.5	even	ok
408.1311	1	C23H19N3NaO3	408.1319	1.9	8.3	1	100.00	15.5	even	ok
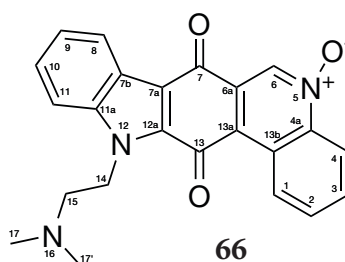
66

1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (PROTON)

1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (PROTON)

1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (C13 CPD)

67
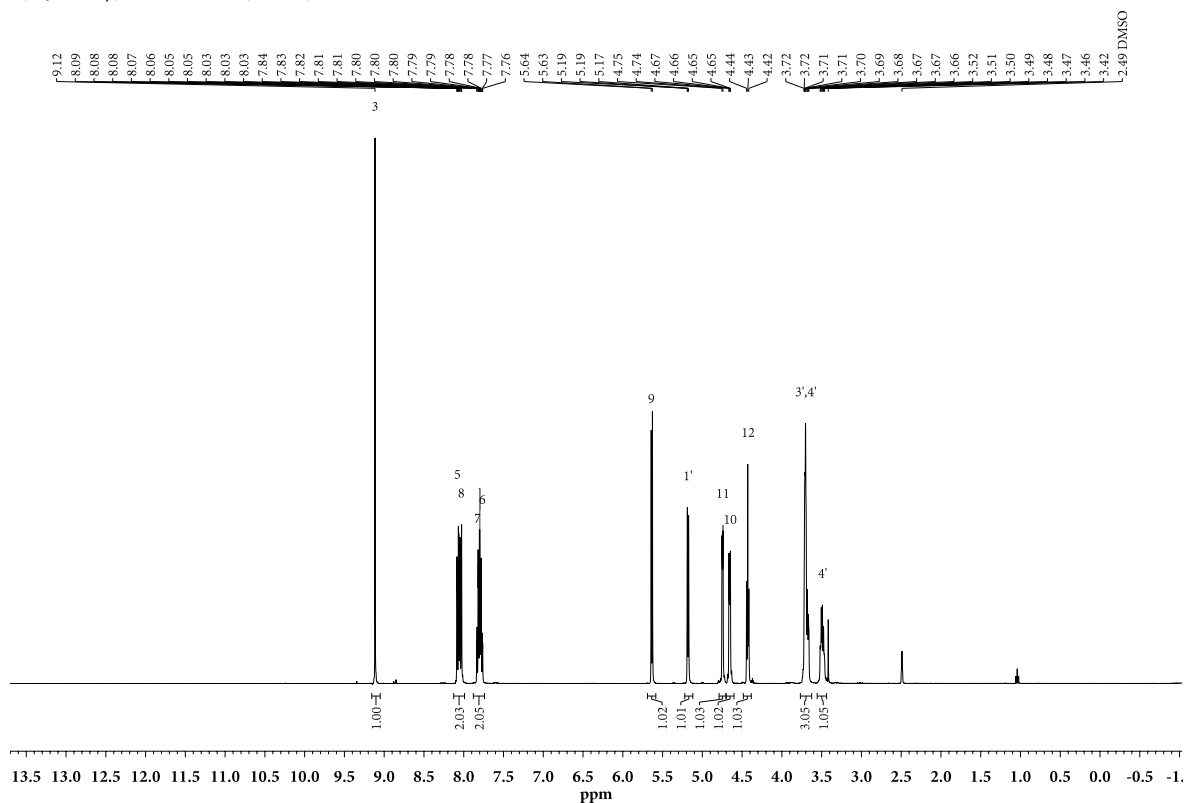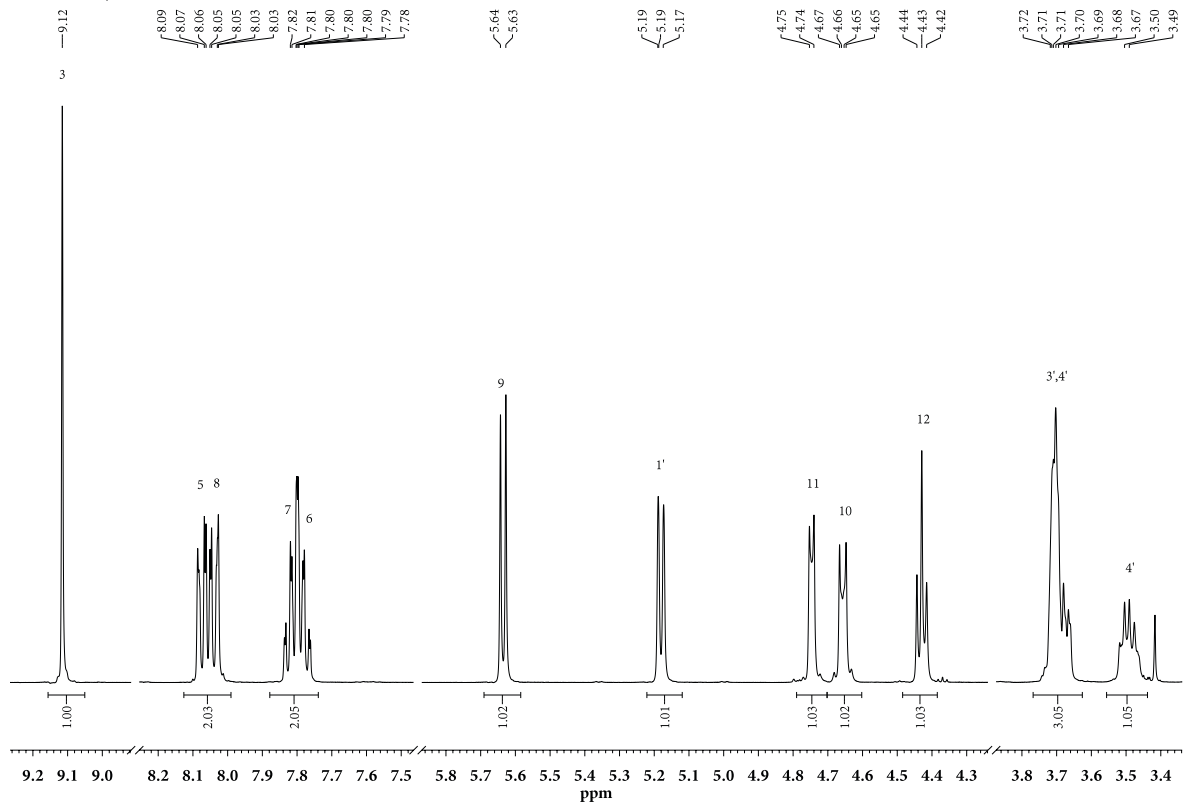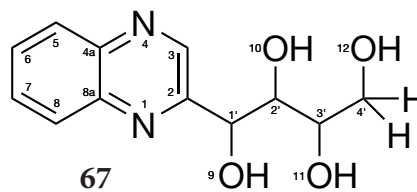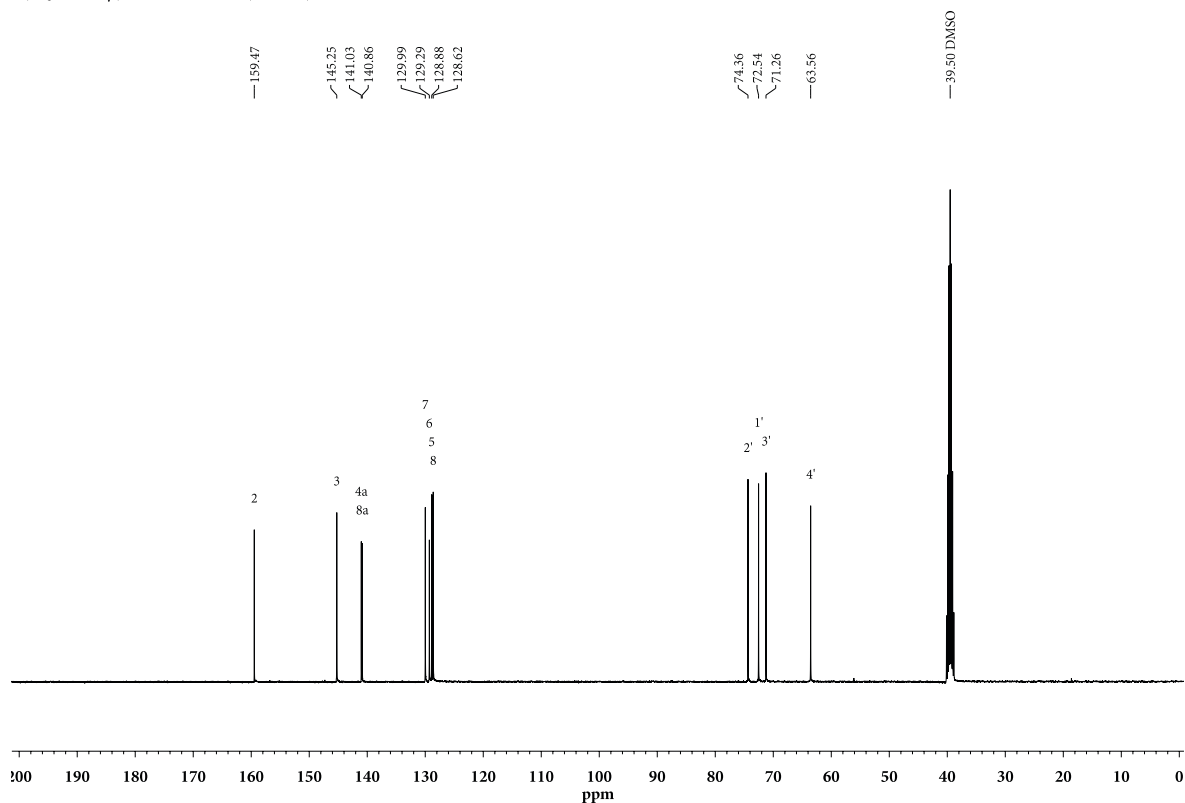
1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (C13 CPD)

1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (C13 CPD)

67
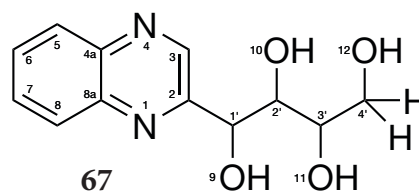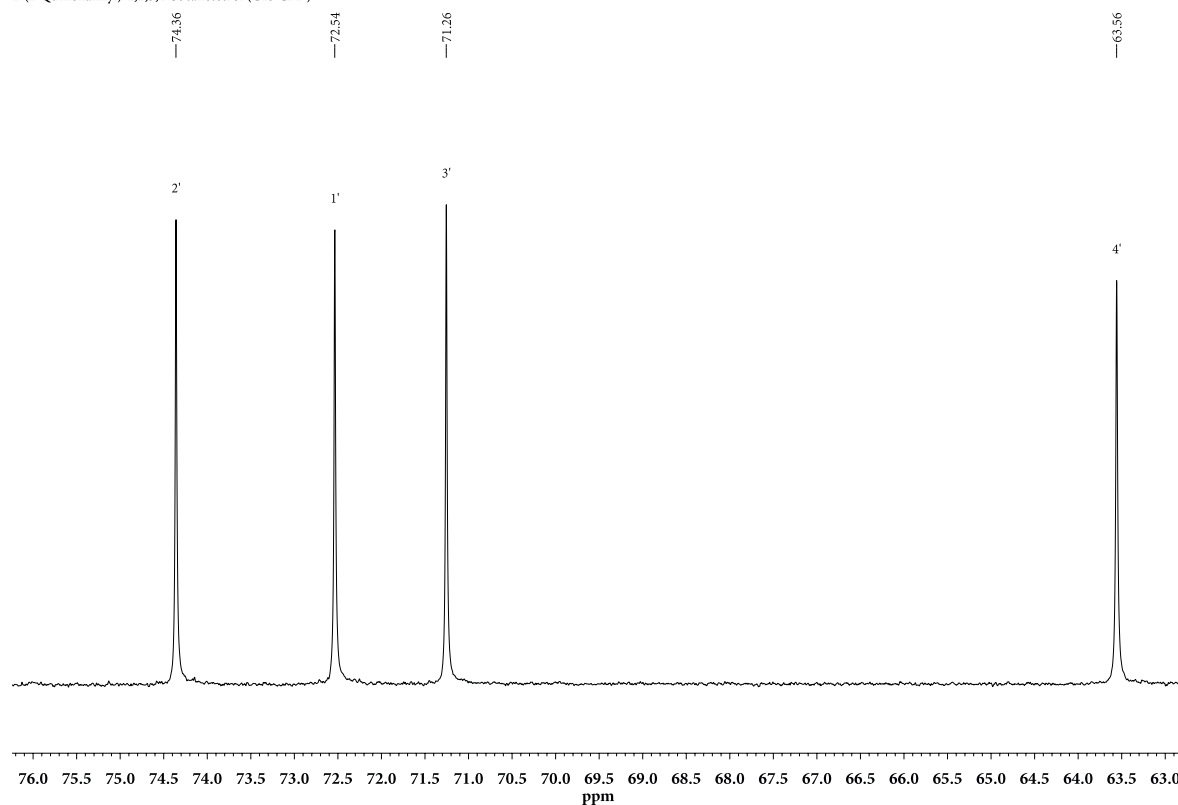
1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (HSQC)
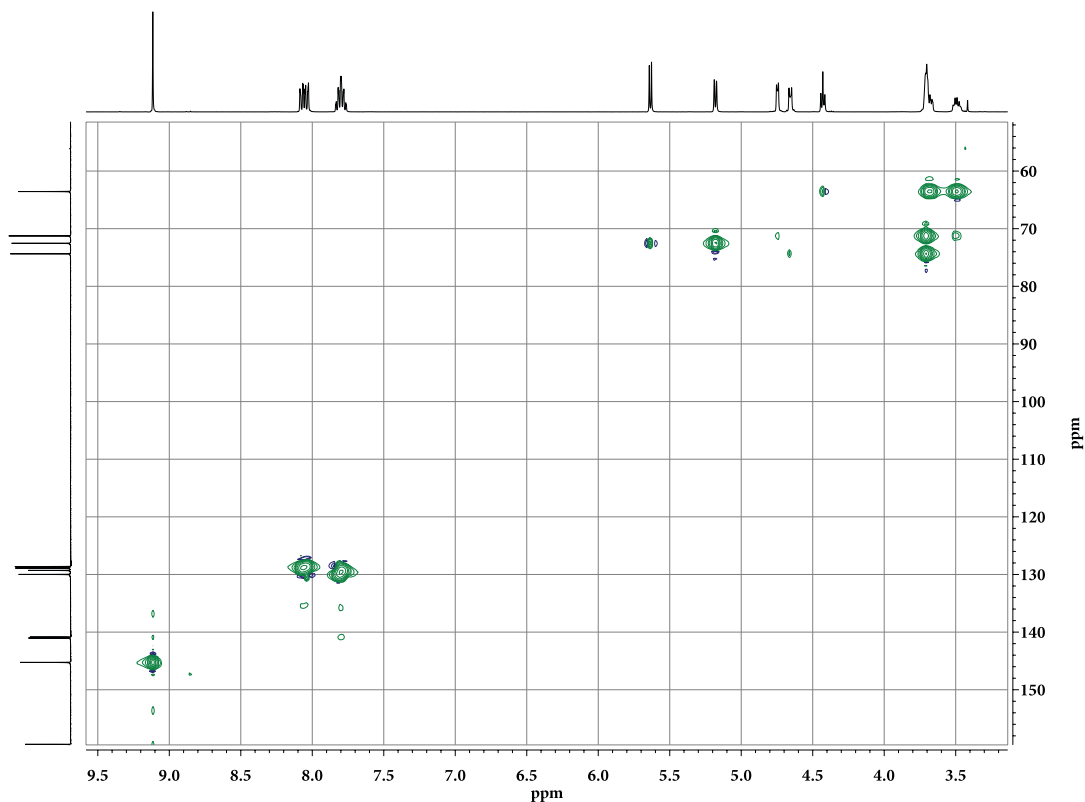
1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (HMBC)
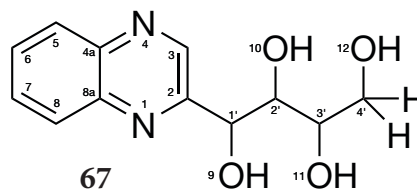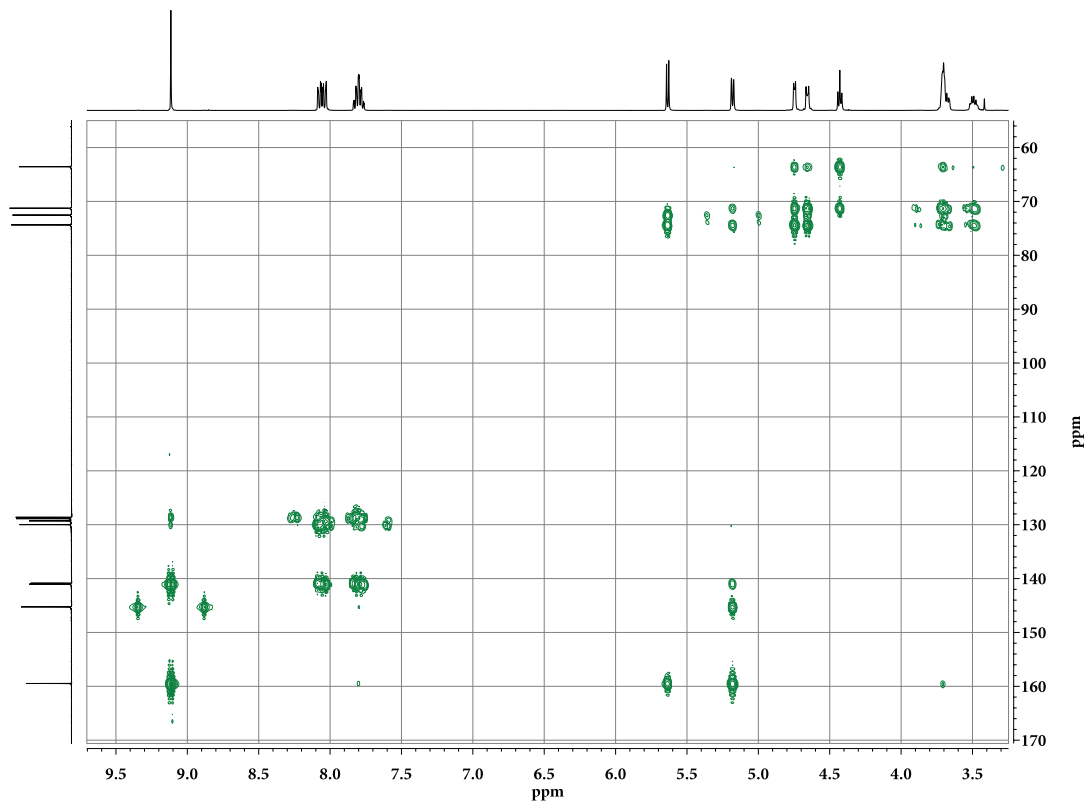
1-(2-Quinoxalinyl)-1,2,3,4-butanetetrol (15N HMBC)

67

2-Quinoxalinecarboxylic acid (PROTON)

68
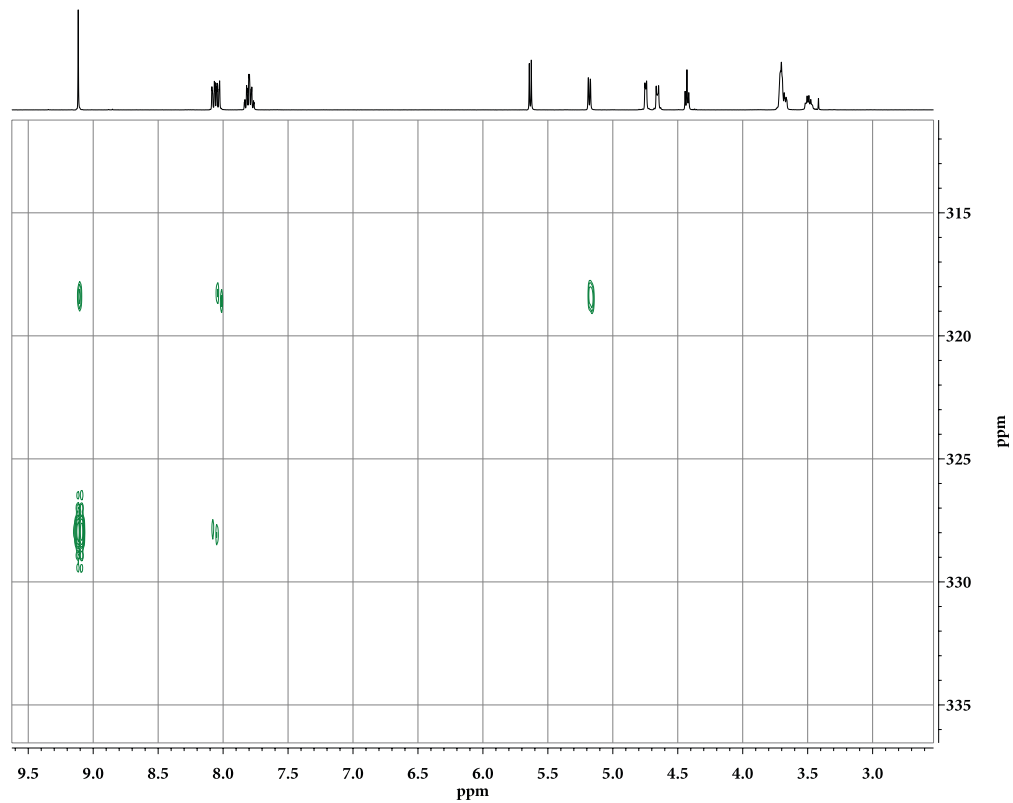
2-Quinoxalinecarboxylic acid (PROTON)

2-Quinoxalinecarboxylic acid (C13 CPD)
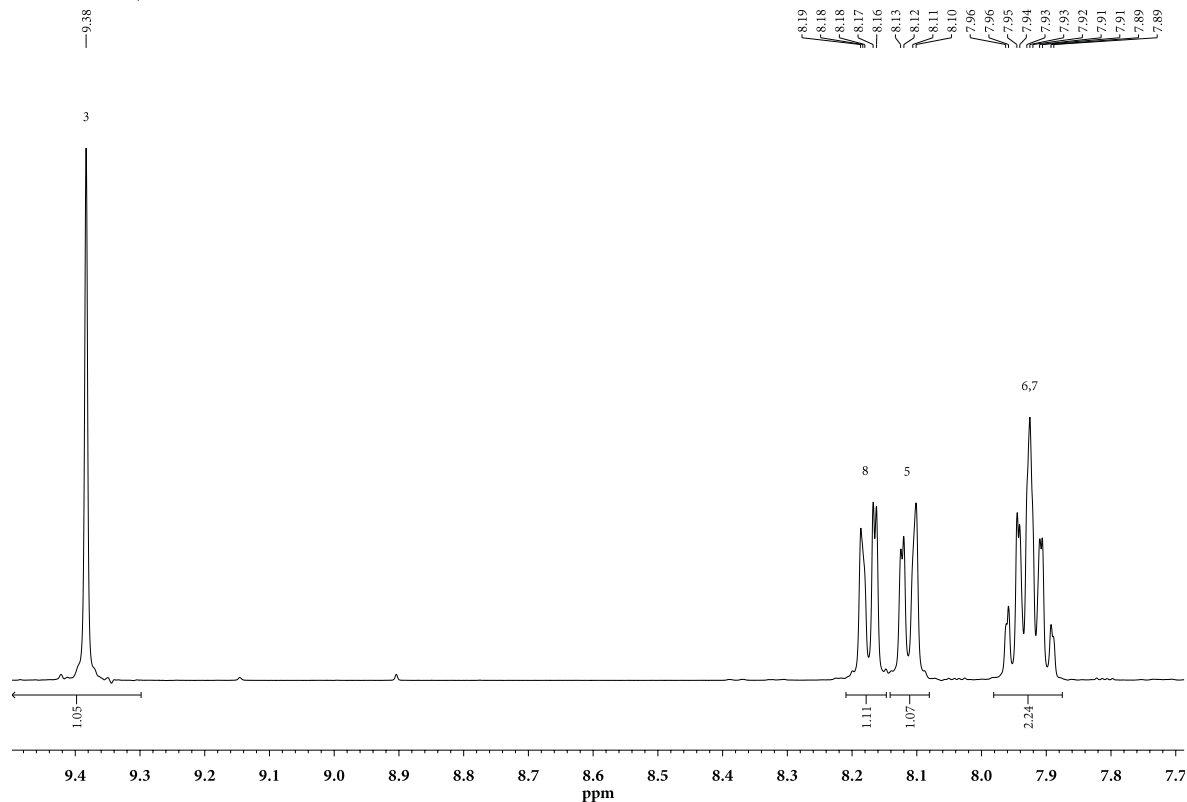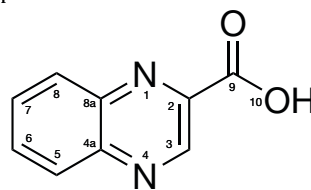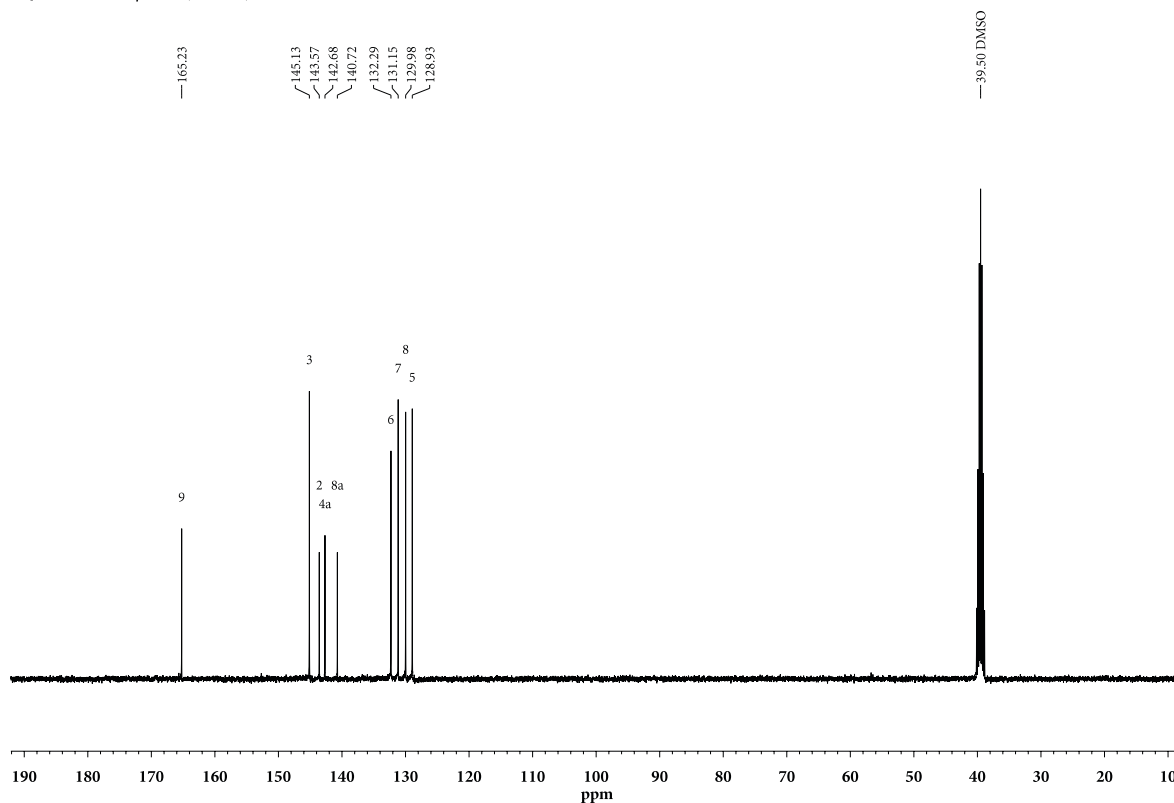
165.23

145.13
143.57
142.68
140.72
132.29
131.15
129.98
128.93

39.50 DMSO

3

8
7
5

6

9

2 8a
4a

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

68

2-Quinoxalinecarboxylic acid (HSQC)

9.9 9.8 9.7 9.6 9.5 9.4 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5
ppm
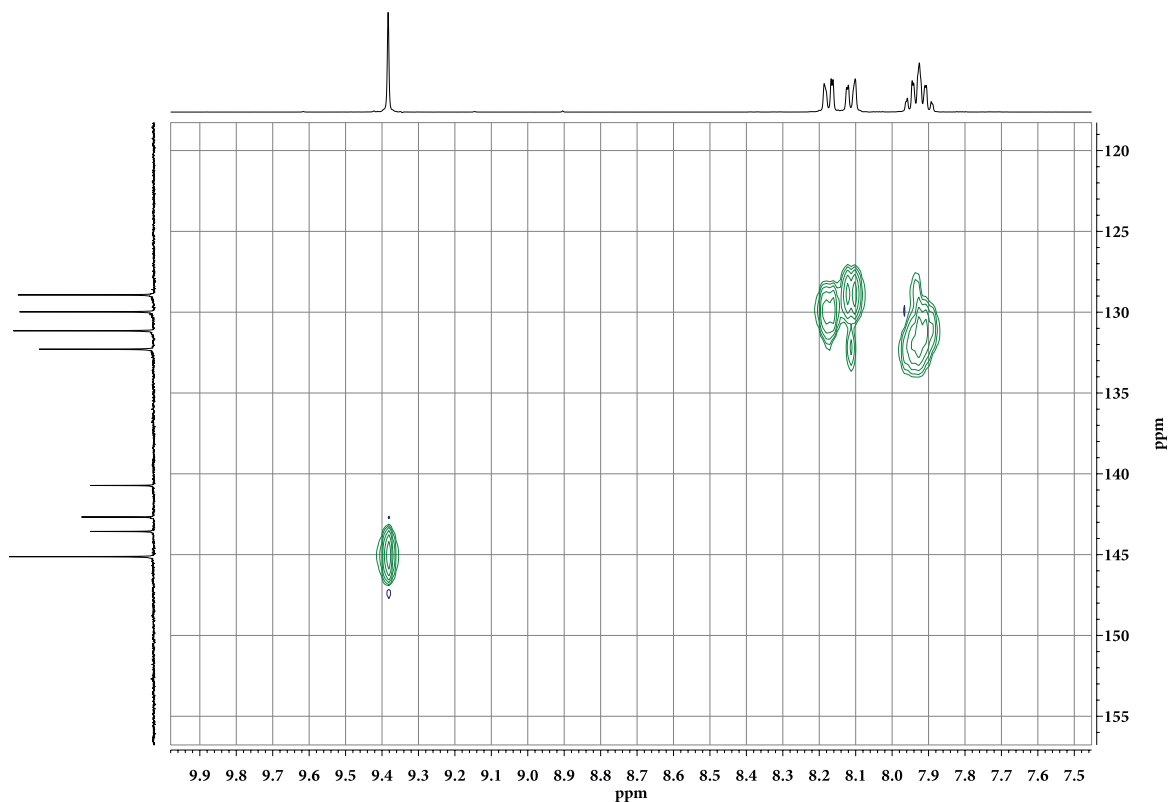
2-Quinoxalinecarboxylic acid (HMBC)

68
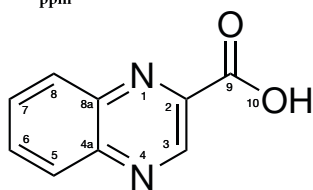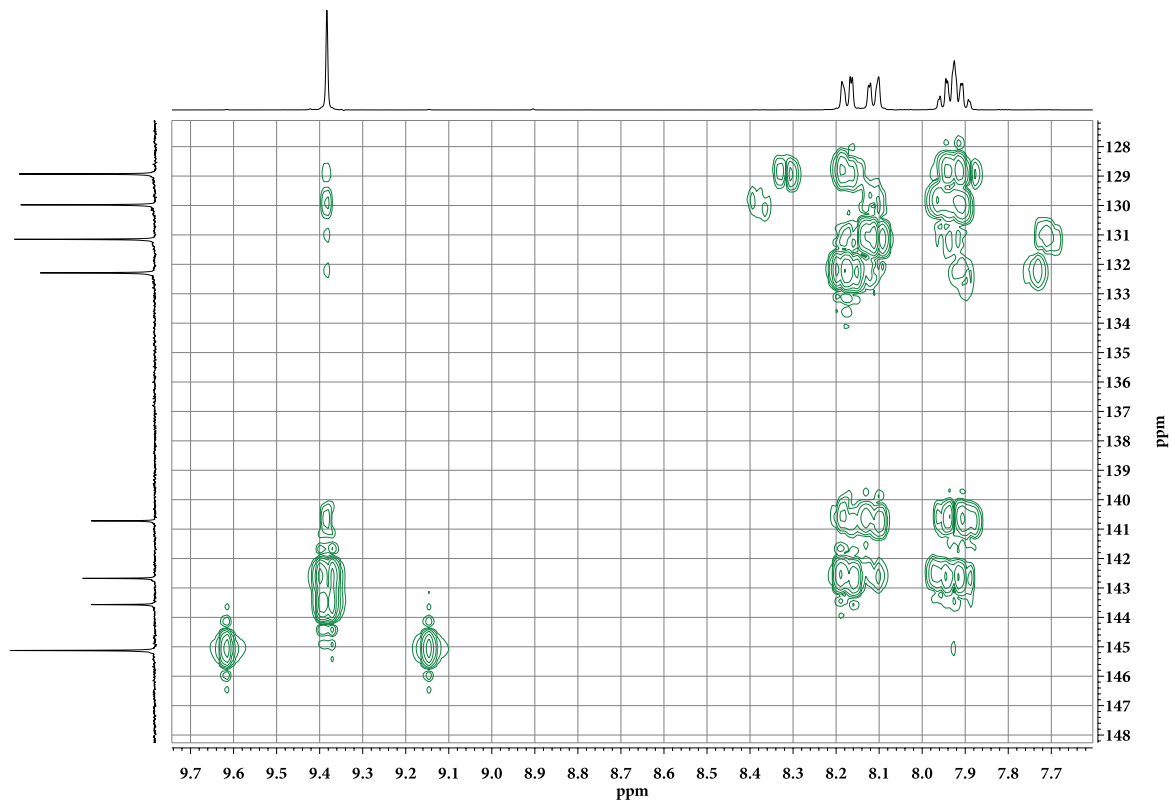
2-Quinoxalinecarboxylic acid (15N HMBC)

N,N-Diethyl-2-quinoxalinecarboxamide (PROTON)

N,N-Diethyl-2-quinoxalinecarboxamide (PROTON)

N,N-Diethyl-2-quinoxalinecarboxamide (PROTON)

3.65 3.63 3.62 3.60
3.53 3.51 3.50 3.48
1.33 1.31 1.30 1.29 1.27 1.26

12',12

11'

11

2.07

2.06

6.28

3.68 3.66 3.64 3.62 3.60 3.58 3.56 3.54 3.52 3.50 3.48 3.46 3.44 3.42 3.40 3.38 3.36 3.34 3.32   1.48 1.46 1.44 1.42 1.40 1.38 1.36 1.34 1.32 1.30 1.28 1.26 1.24
ppm

69

N,N-Diethyl-2-quinoxalinecarboxamide (C13 APT)

166.34

148.89 144.83 142.12 140.29

130.83 130.51 129.67 129.20

77.00 CDCl3

43.47 40.69

14.48 12.81

5
8
7
6

3

12'
12

9

2

4a
8a

11'
11

190   180   170   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0
ppm

N,N-Diethyl-2-quinoxalinecarboxamide (C13 APT)

N,N-Diethyl-2-quinoxalinecarboxamide (HSQC)

69

N,N-Diethyl-2-quinoxalinecarboxamide (HMBC)

69
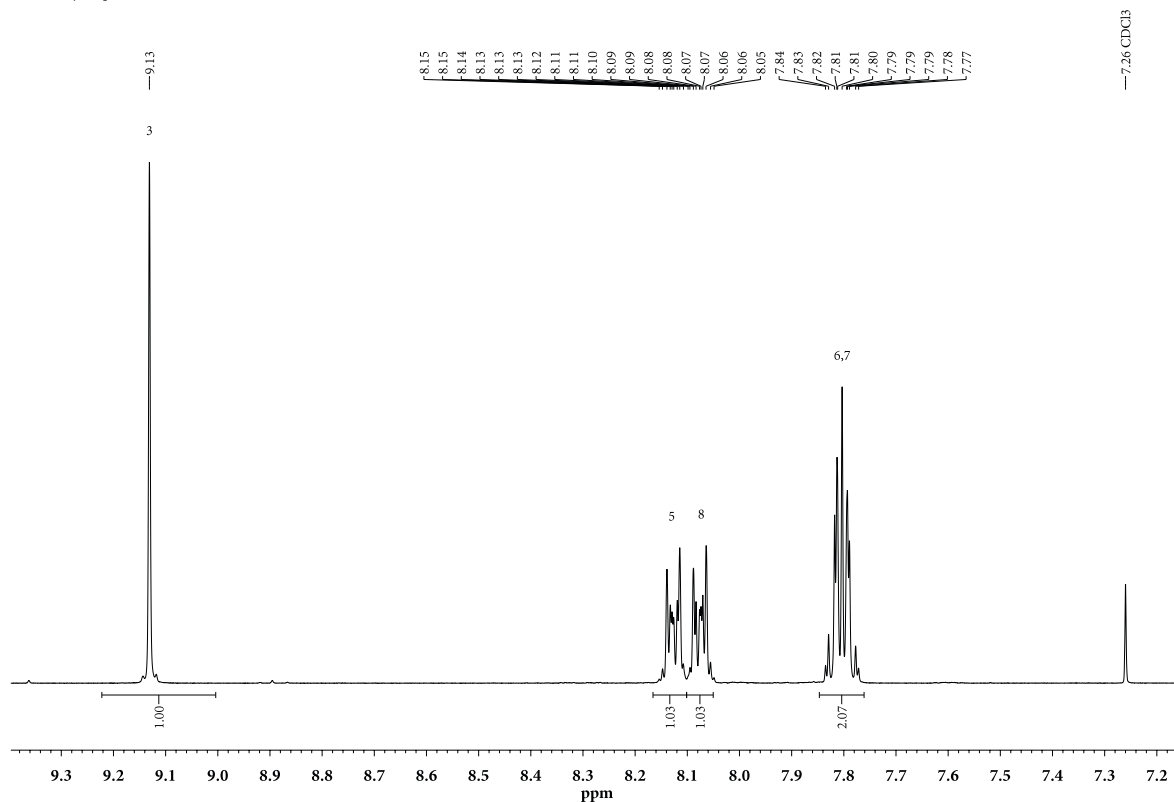
N,N-Diethyl-2-quinoxalinecarboxamide (15N HMBC)

5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)

70
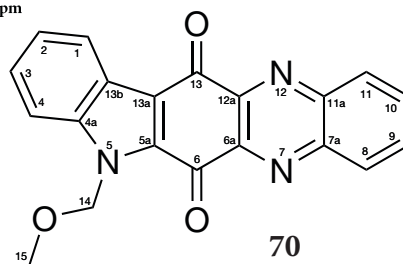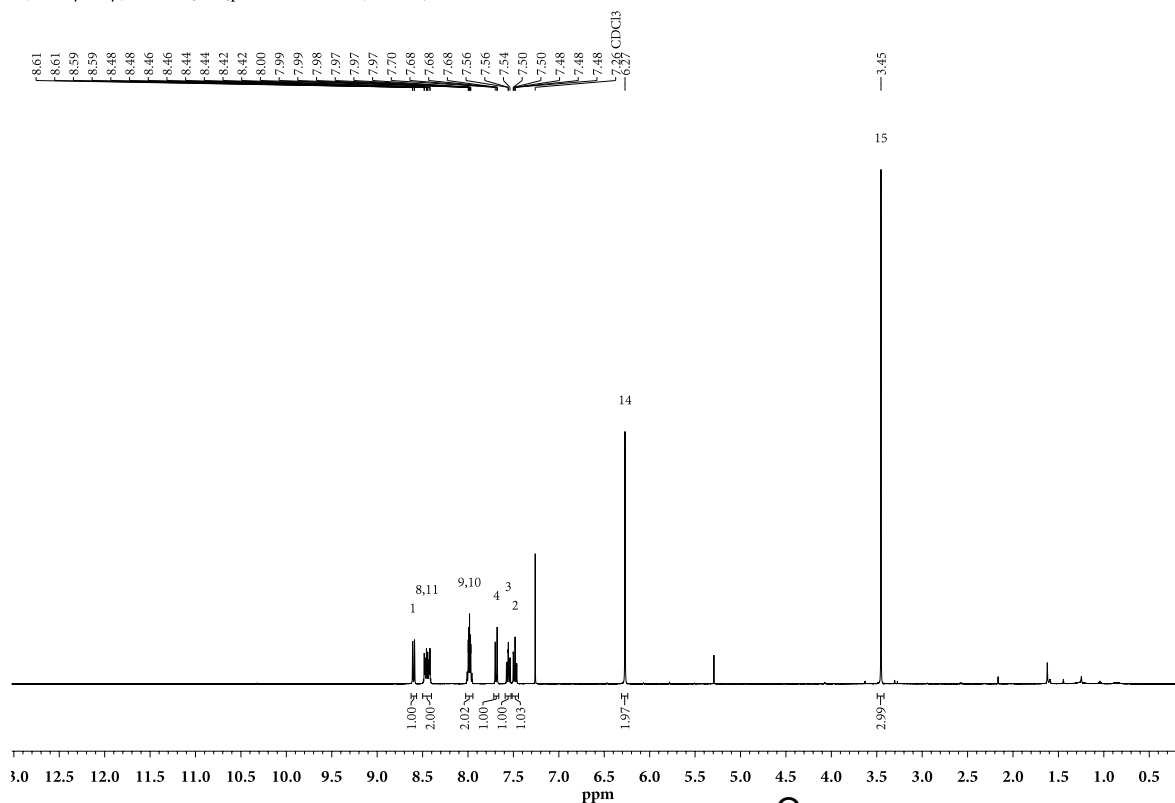
5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)
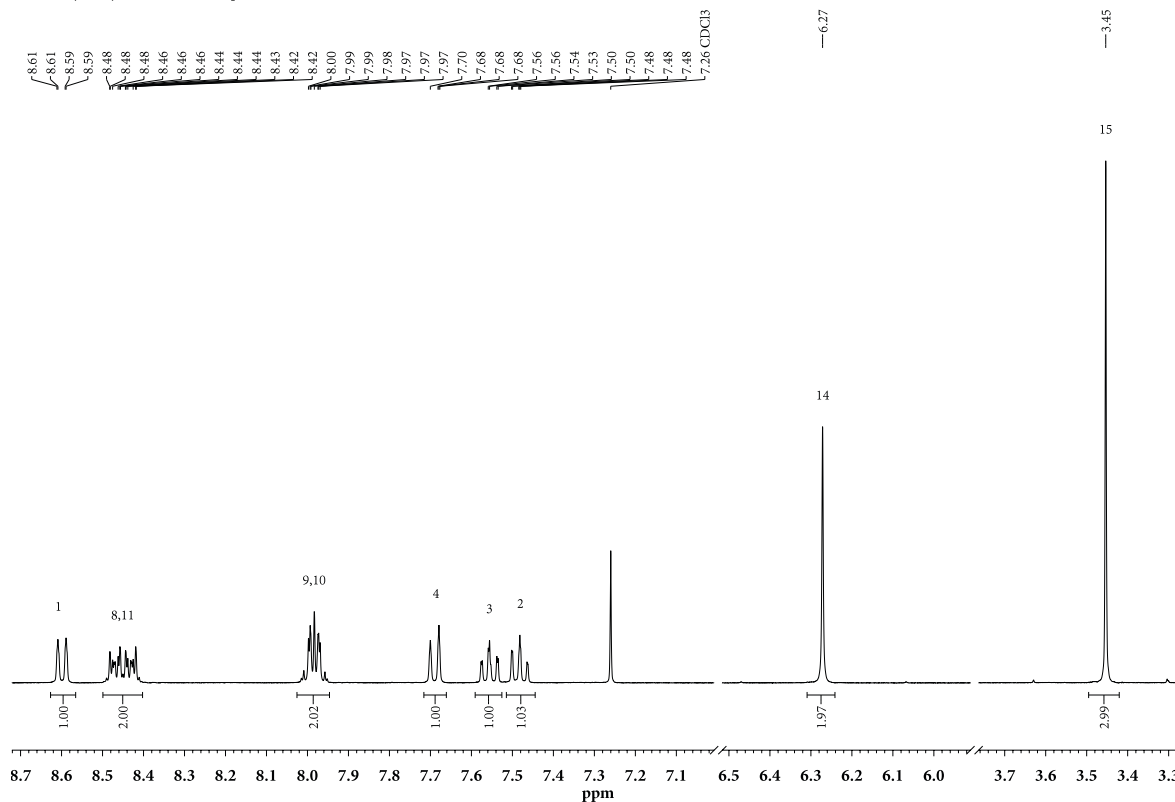
SPECTROSCOPIC INFORMATION

5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (C13 APT)
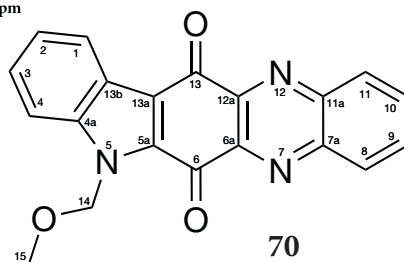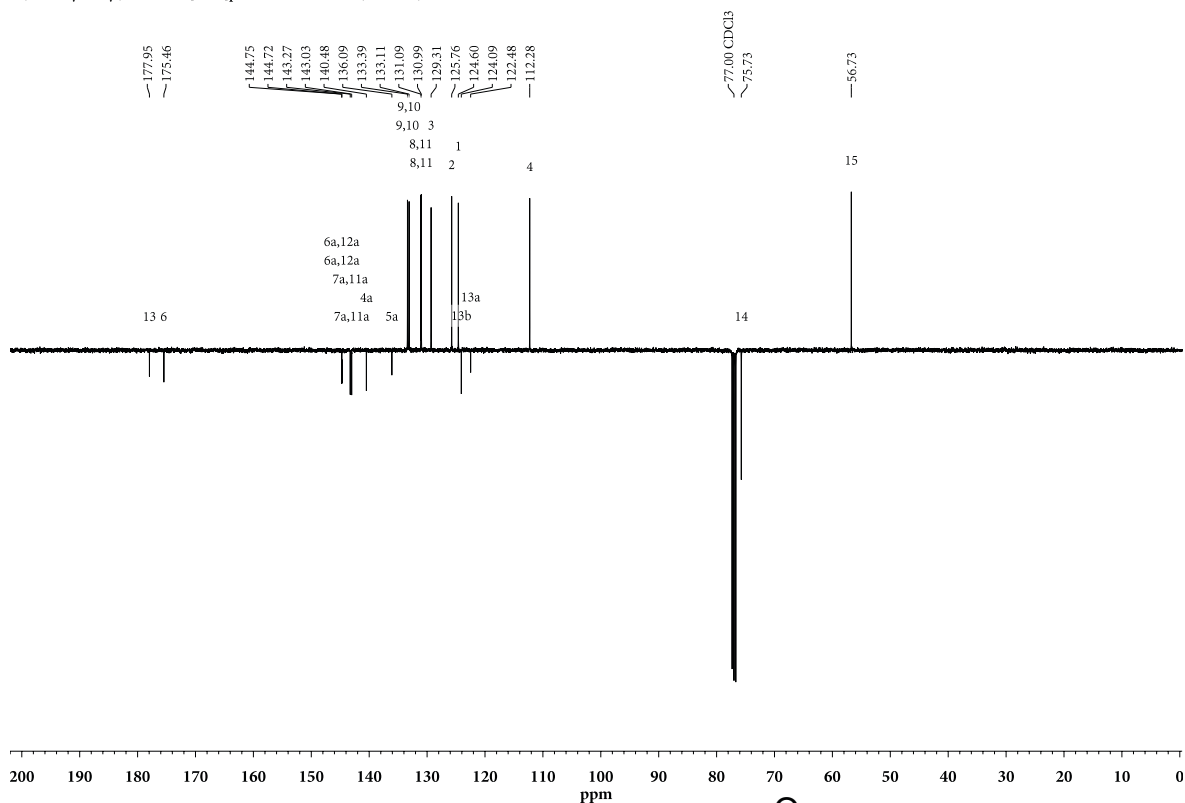
5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (C13 APT)
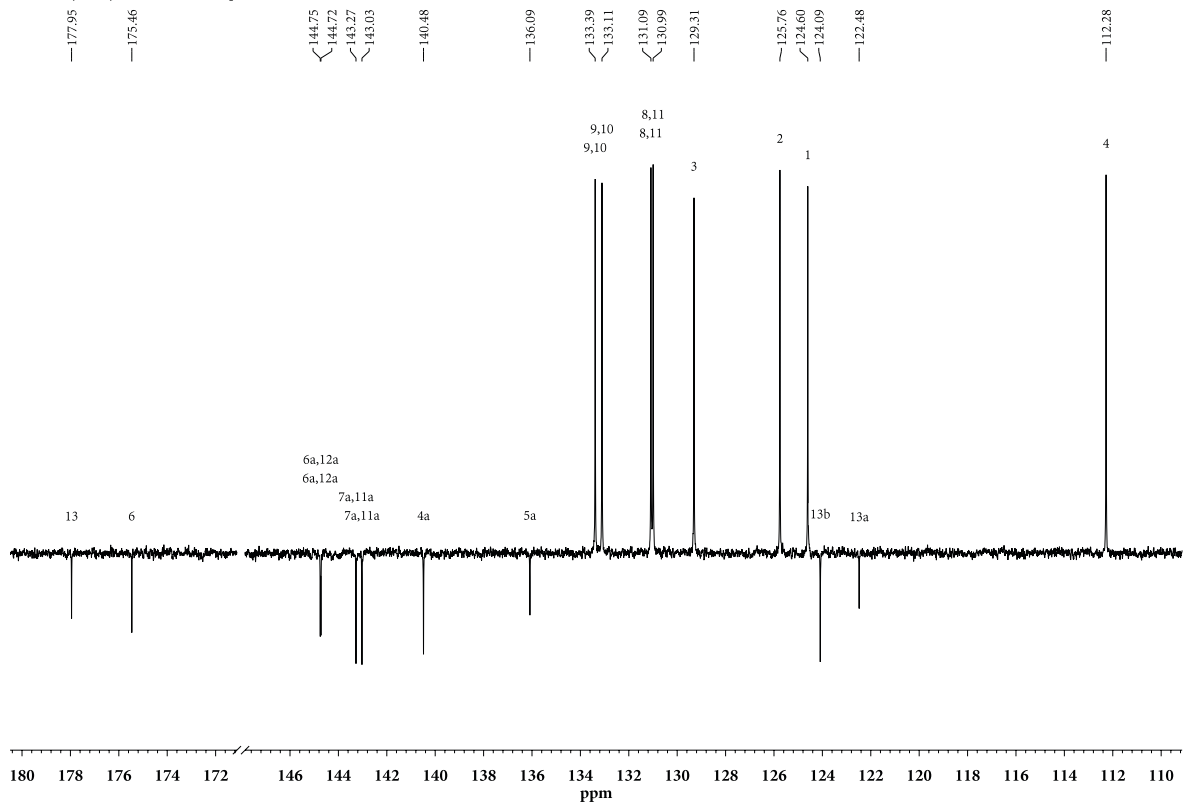
5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (HSQC)

5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (HMBC)

70

5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (15N HMBC)

5-(Methoxymethyl)-5H-indolo[2,3-b]phenazine-6,13-dione (COSY)

70

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 1:19:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN137_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

70

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 1:19:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN137_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3300 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
366.0849	1	C20H13N3NaO3	366.0849	-0.1	9.8	1	100.00	15.5	even	ok

70

5H-Indolo[2,3-b]phenazine-6,13-dione (PROTON)

71
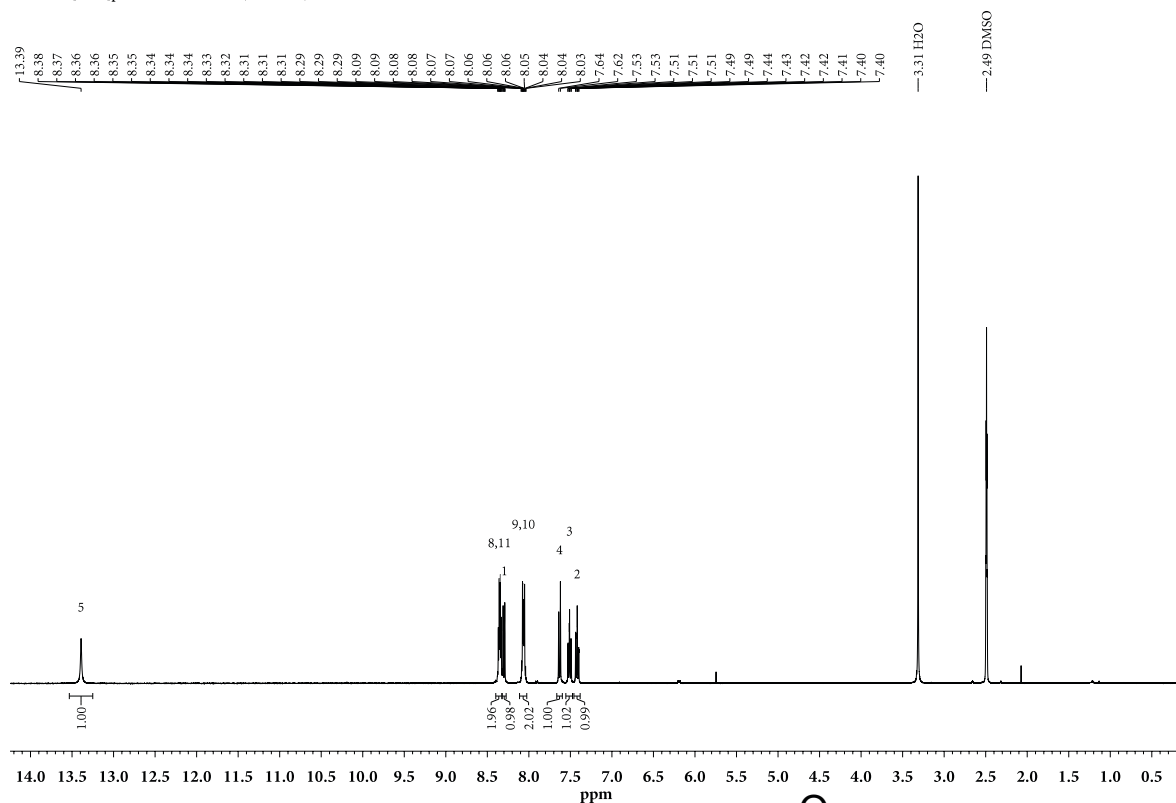
5H-Indolo[2,3-b]phenazine-6,13-dione (PROTON)

5H-Indolo[2,3-b]phenazine-6,13-dione (C13 APT)
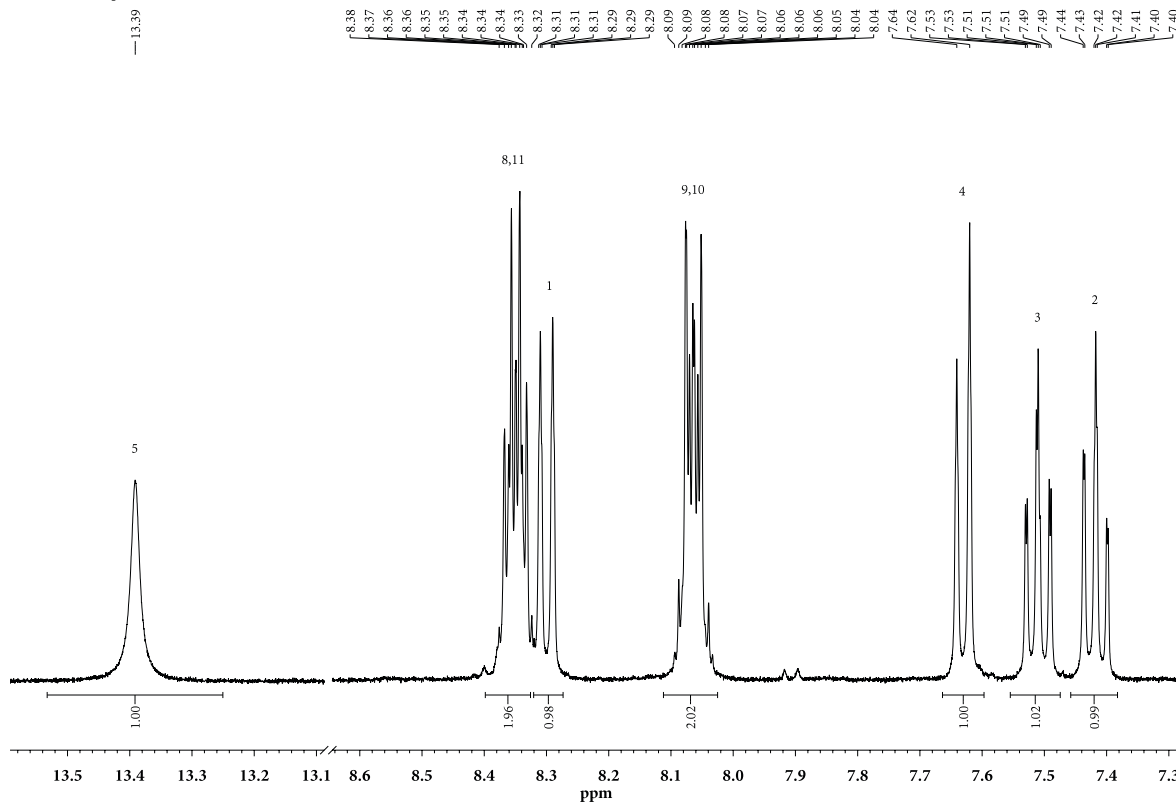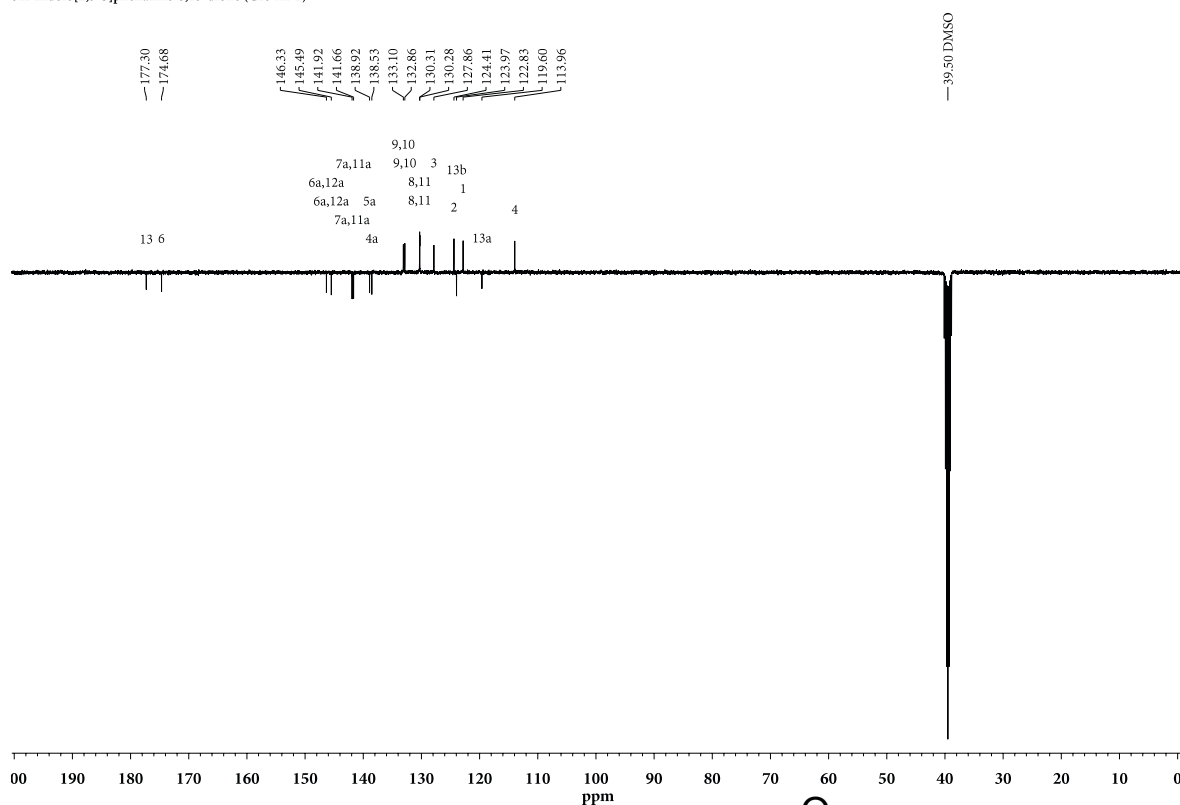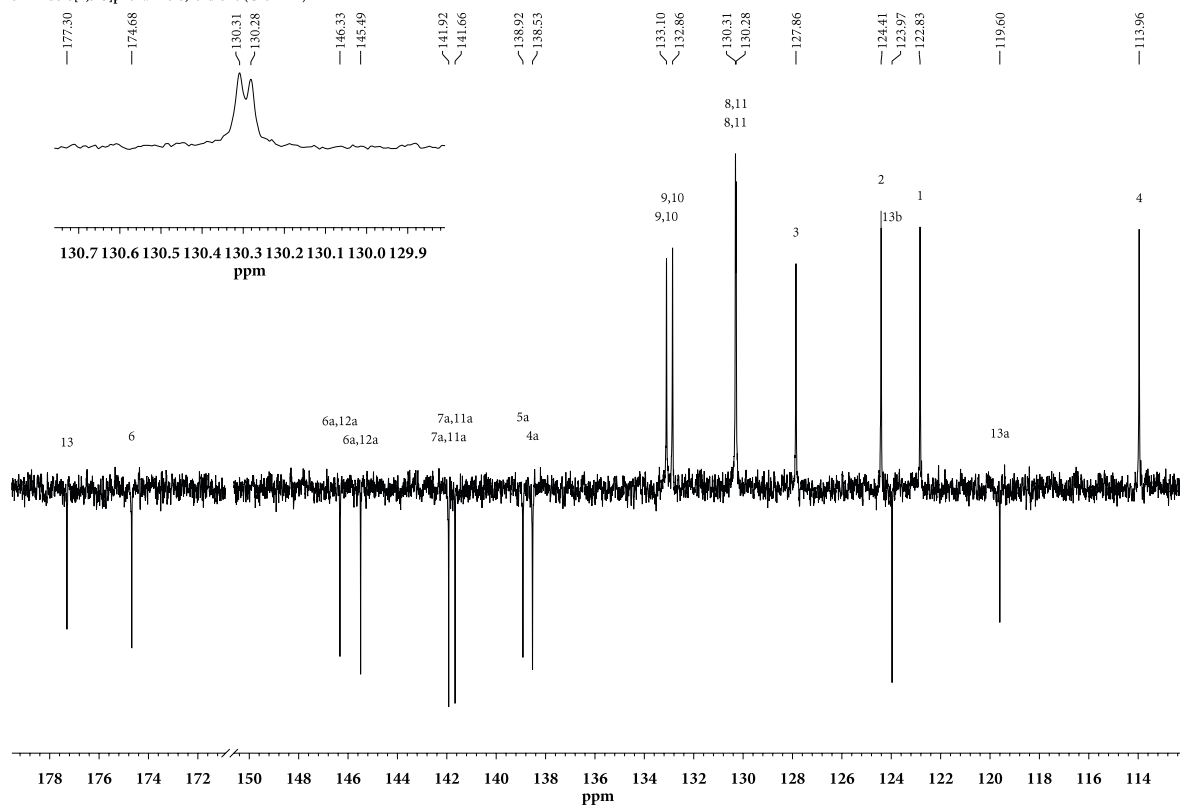
5H-Indolo[2,3-b]phenazine-6,13-dione (C13 APT)

5H-Indolo[2,3-b]phenazine-6,13-dione (HSQC)
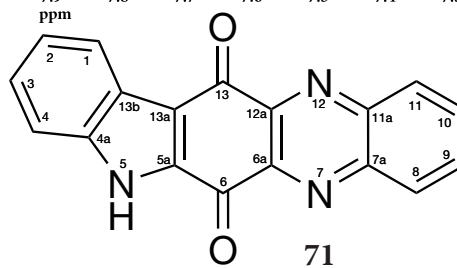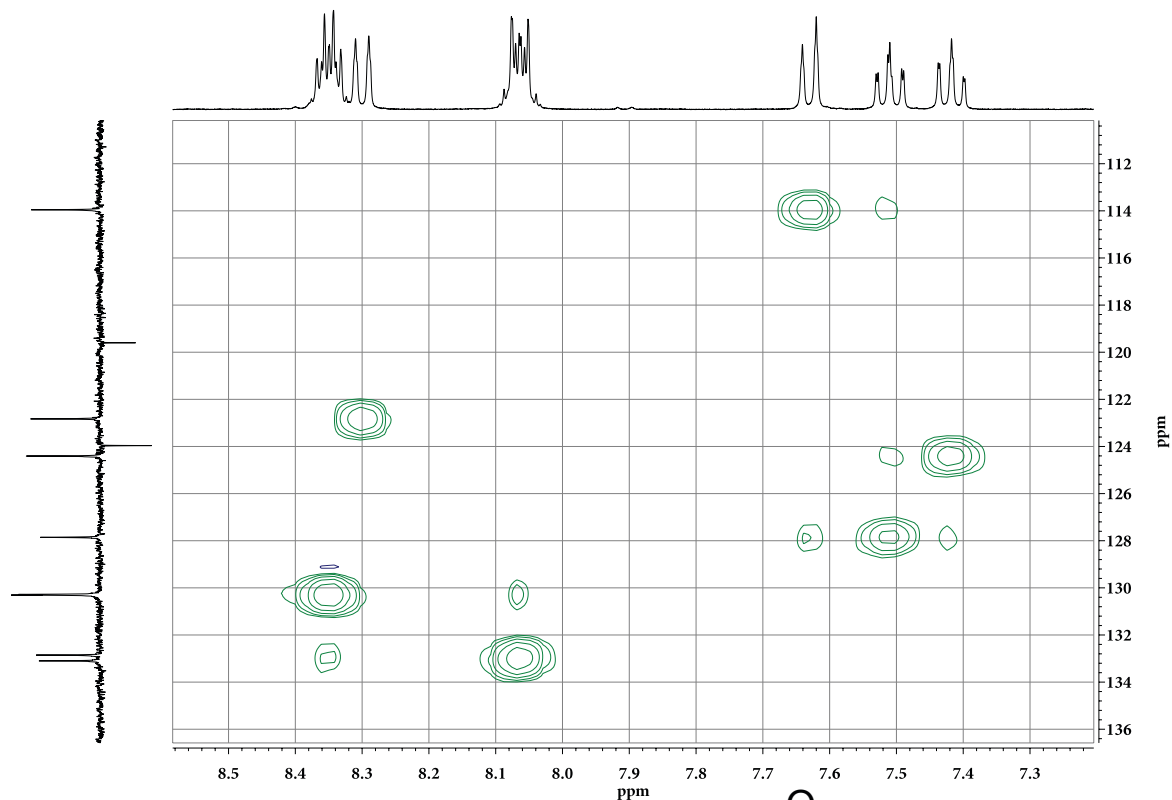
5H-Indolo[2,3-b]phenazine-6,13-dione (HMBC)
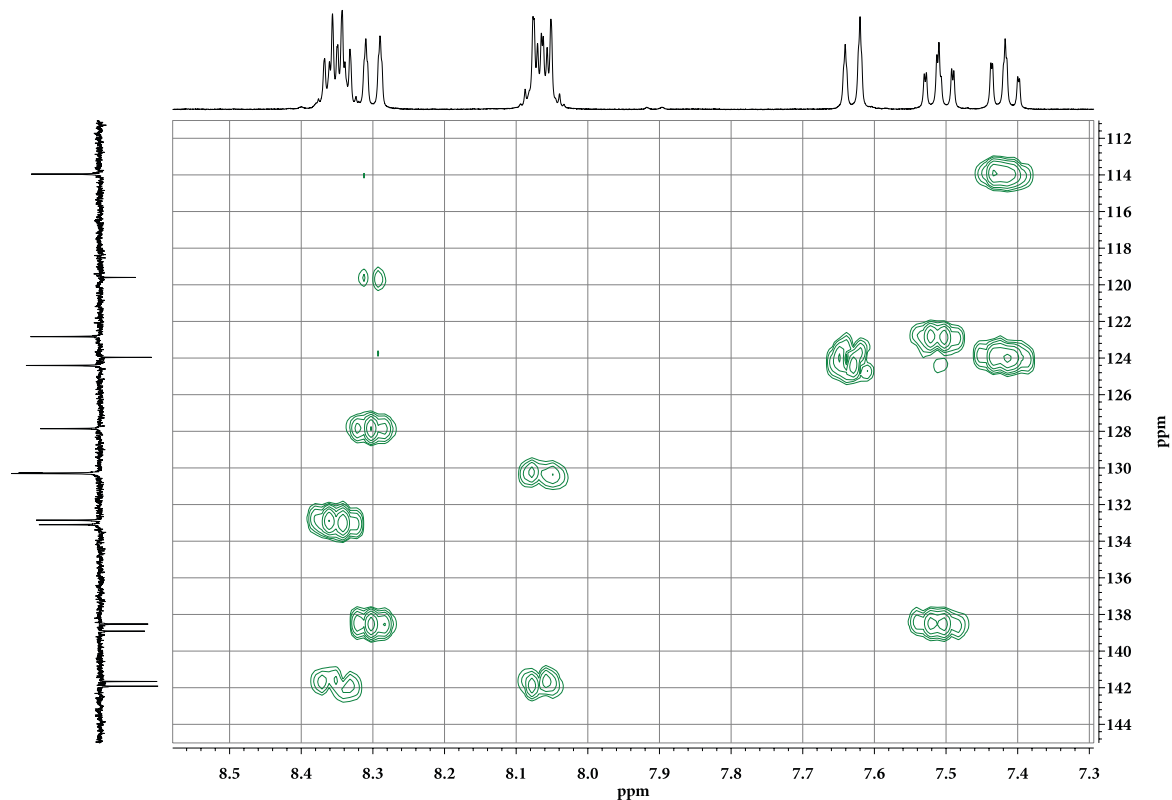
5H-Indolo[2,3-b]phenazine-6,13-dione (15N HMBC)
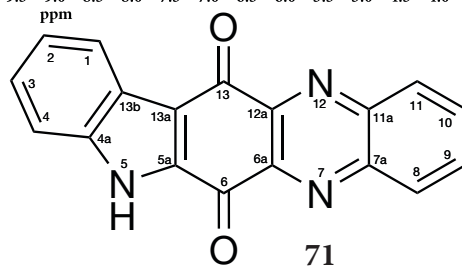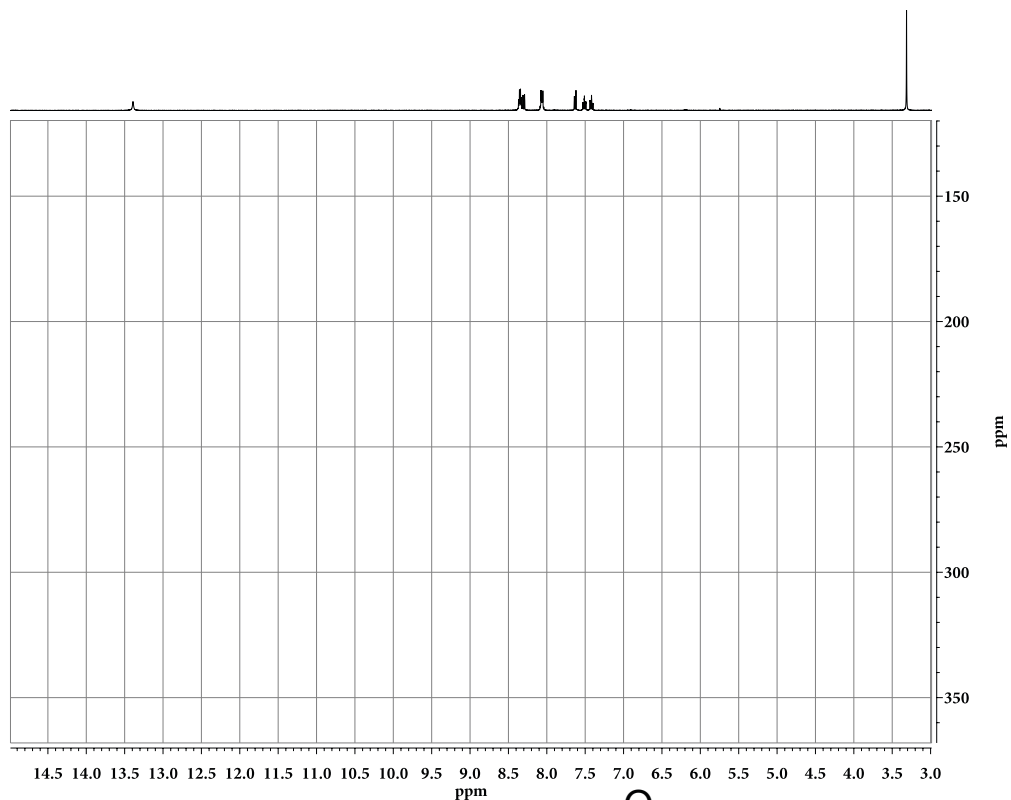
5H-Indolo[2,3-b]phenazine-6,13-dione (15N HSQC)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN138_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 1:27:10 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

71

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/10/2014 1:27:10 PM

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN138_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
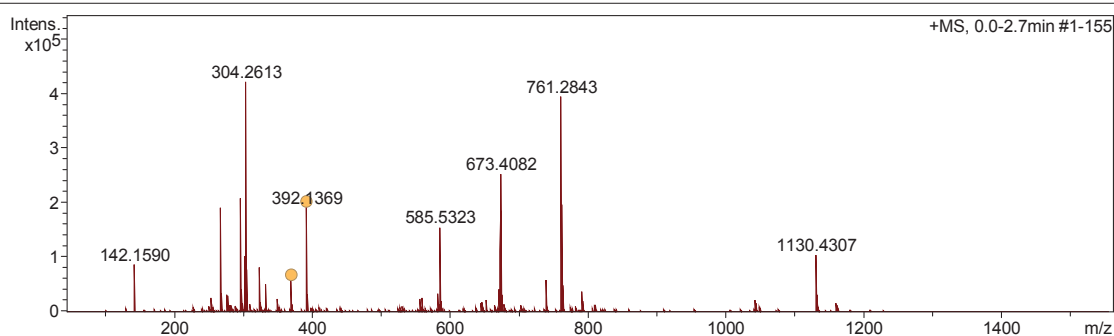Sample Name   approx. 0.01 mg/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3300 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
322.0589	1	C18H9N3NaO2	322.0587	-0.6	6.8	1	100.00	15.5	even	ok

71
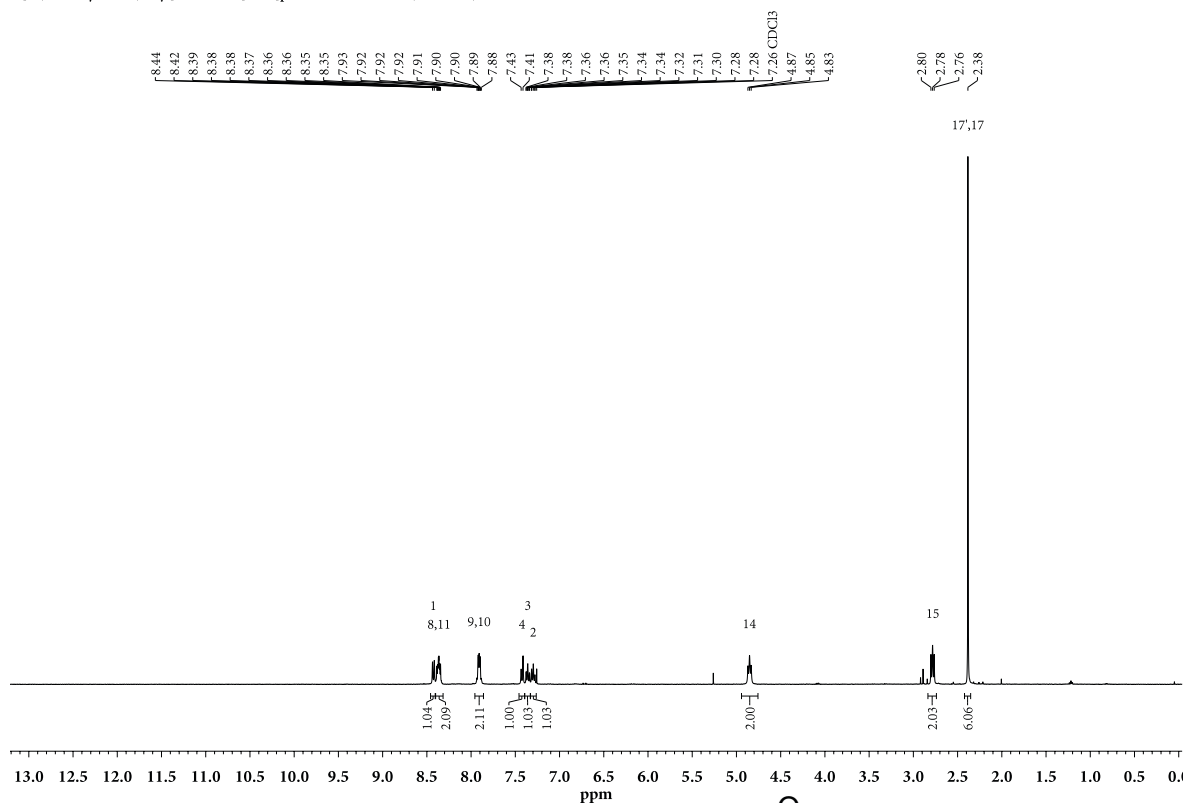
5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)

5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)
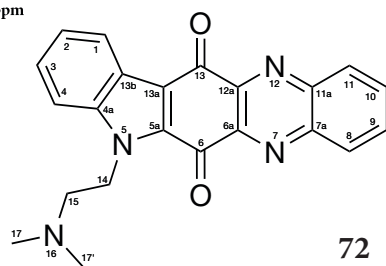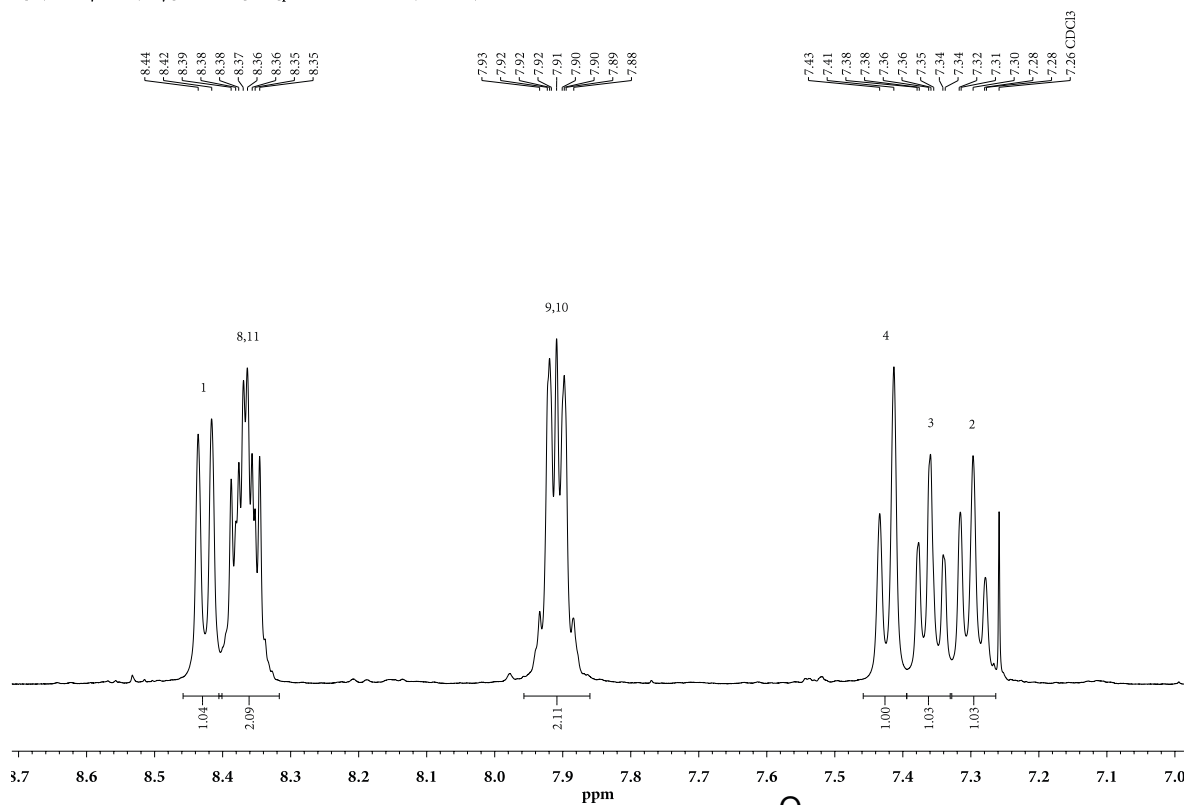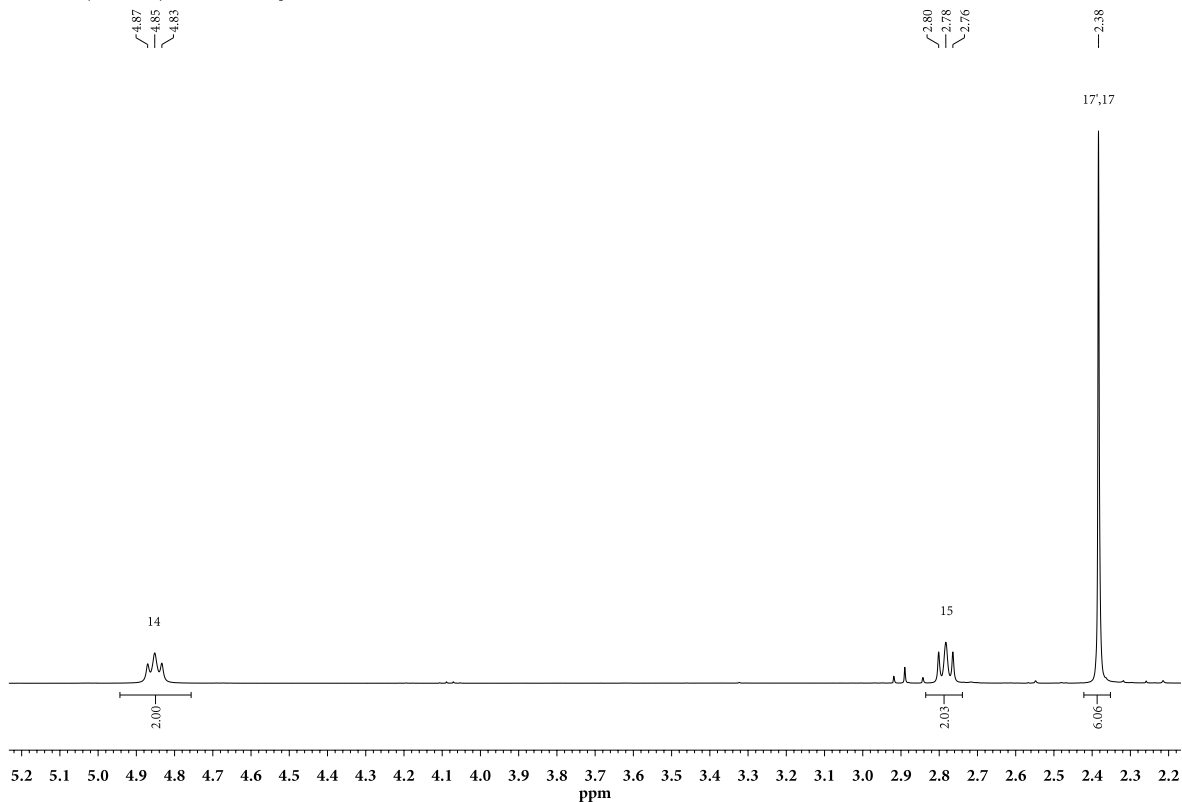
72

5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)

8.44
8.42
8.39
8.38
8.37
8.36
8.35
8.35

7.93
7.92
7.92
7.92
7.91
7.90
7.90
7.89
7.88

7.43
7.41
7.38
7.38
7.36
7.36
7.35
7.34
7.34
7.32
7.31
7.30
7.28
7.26 CDCl3

9,10

8,11

1

4

3

2

1.04
2.09

2.11

1.00
1.03
1.03

ppm

5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (PROTON)

72

4.87
4.85
4.83

2.80
2.78
2.76

2.38

17',17

14

15

2.00

2.03

6.06

ppm

N,N-Diethyl-4-quinolinecarboxamide (C13 APT)

72

N,N-Diethyl-4-quinolinecarboxamide (C13 APT)

614
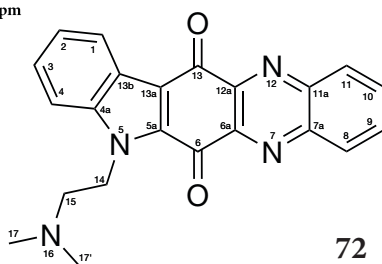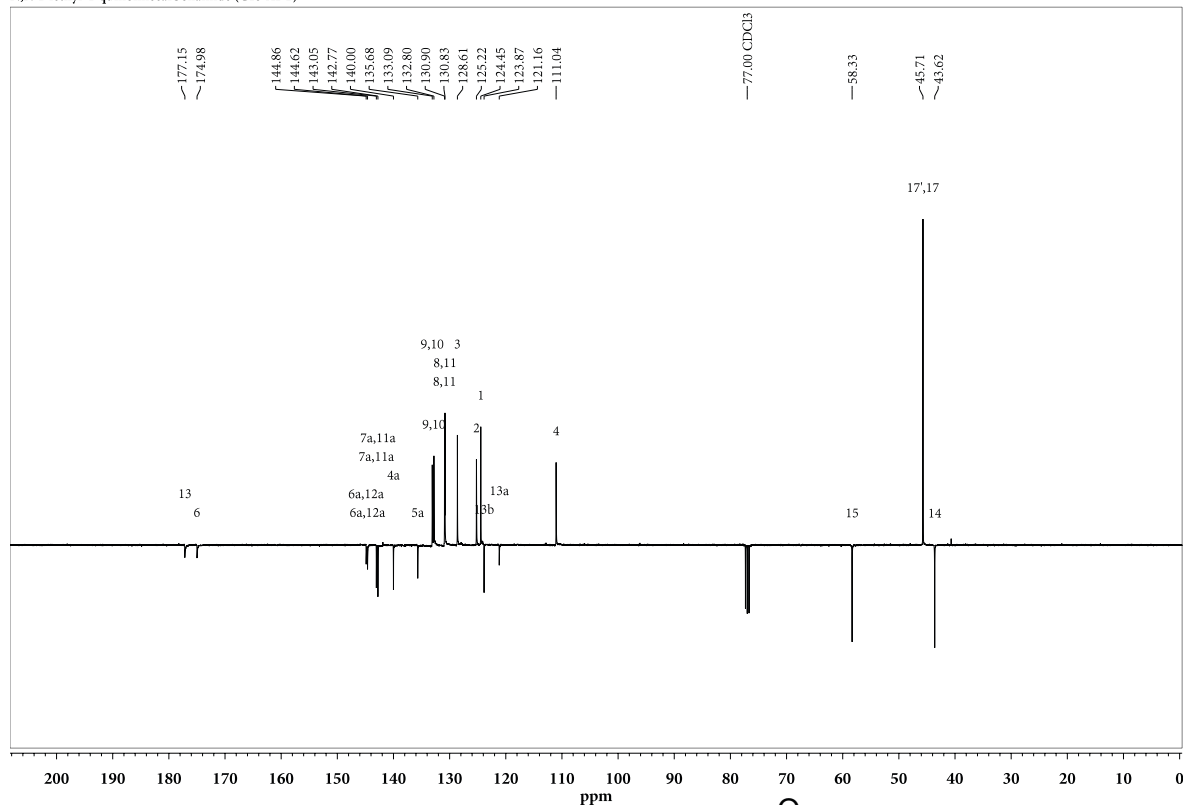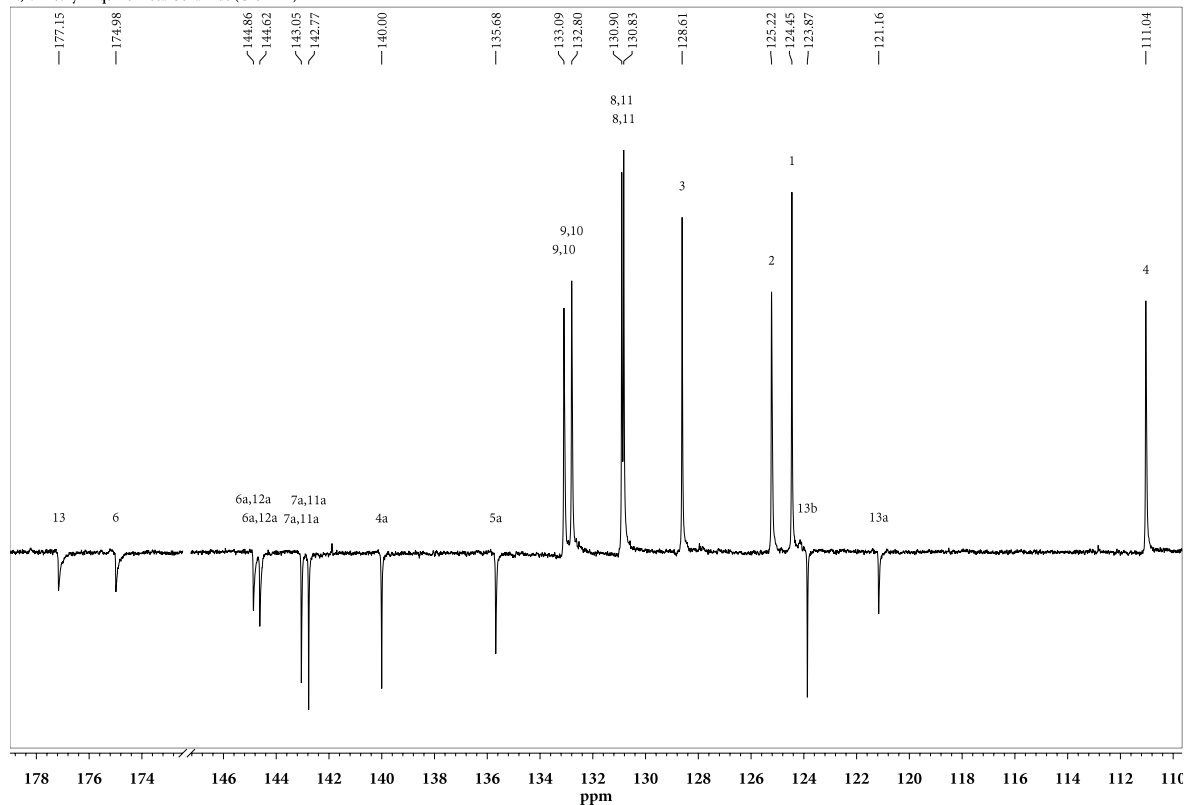
5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (HSQC)

5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (HMBC)

72

5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (15N HMBC)

72
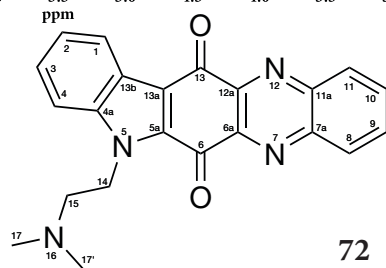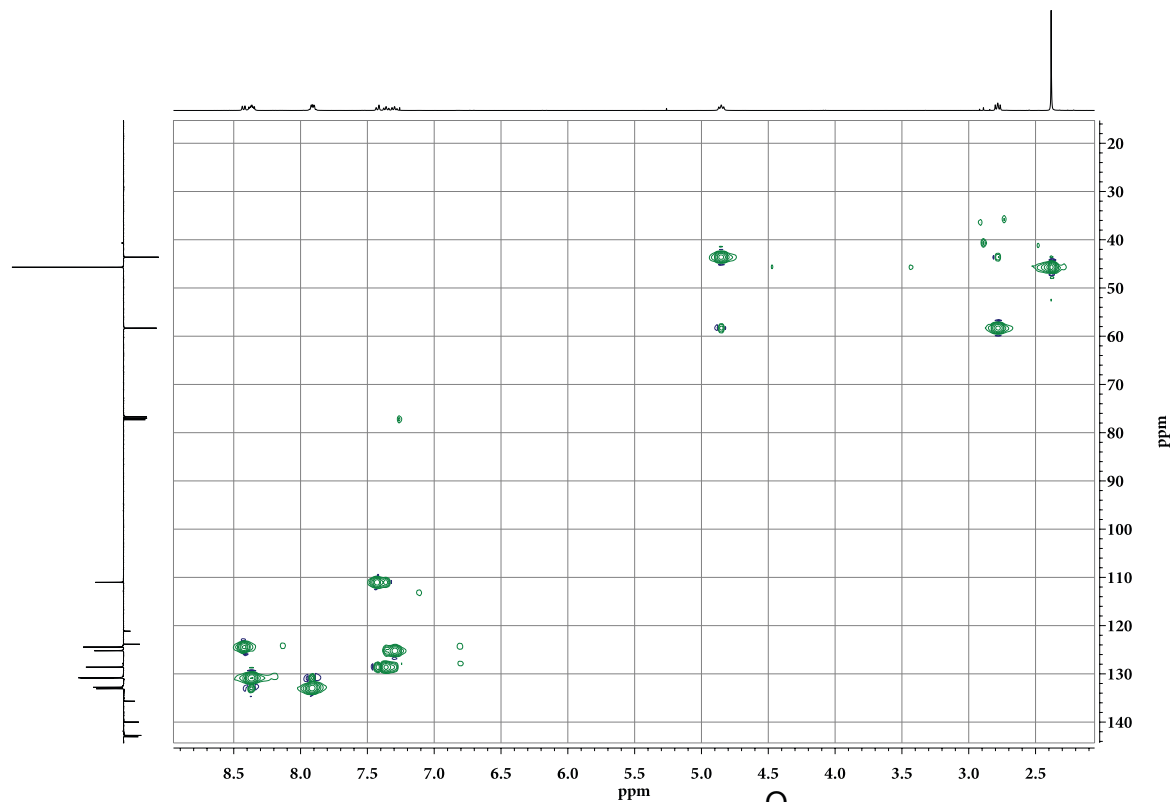
5-[2-(Dimethylamino)ethyl]-5H-indolo[2,3-b]phenazine-6,13-dione (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 12:25:53 PM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN139_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Intens. x10^5 — +MS, 0.0-1.5min #1-87

393.1323
394.1352
371.1502
372.1533
383.3849 385.2924
391.1164
395.1381

x10^4 — $C_{22}H_{19}N_4O_2$, 371.1503

1+ 371.1503
1+ 372.1533
1+ 373.1560

x10^5 — $C_{22}H_{18}N_4NaO_2$, 393.1322

1+ 393.1322
1+ 394.1352
1+ 395.1380

72

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\TN139_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Acquisition Date   11/4/2014 12:25:53 PM

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
371.1502	1	C22H19N4O2	371.1503	0.2	14.0	1	100.00	15.5	even	ok
393.1323	1	C22H18N4NaO2	393.1322	-0.4	14.9	1	100.00	15.5	even	ok
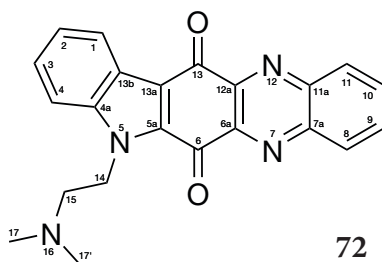
72

N,N-Diethyl-2-quinolinecarboxamide (PROTON)

N,N-Diethyl-2-quinolinecarboxamide (PROTON)

N,N-Diethyl-2-quinolinecarboxamide (C13 APT)

73

N,N-Diethyl-2-quinolinecarboxamide (C13 APT)

N,N-Diethyl-2-quinolinecarboxamide (HSQC)

73
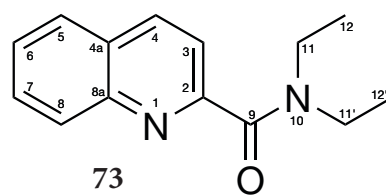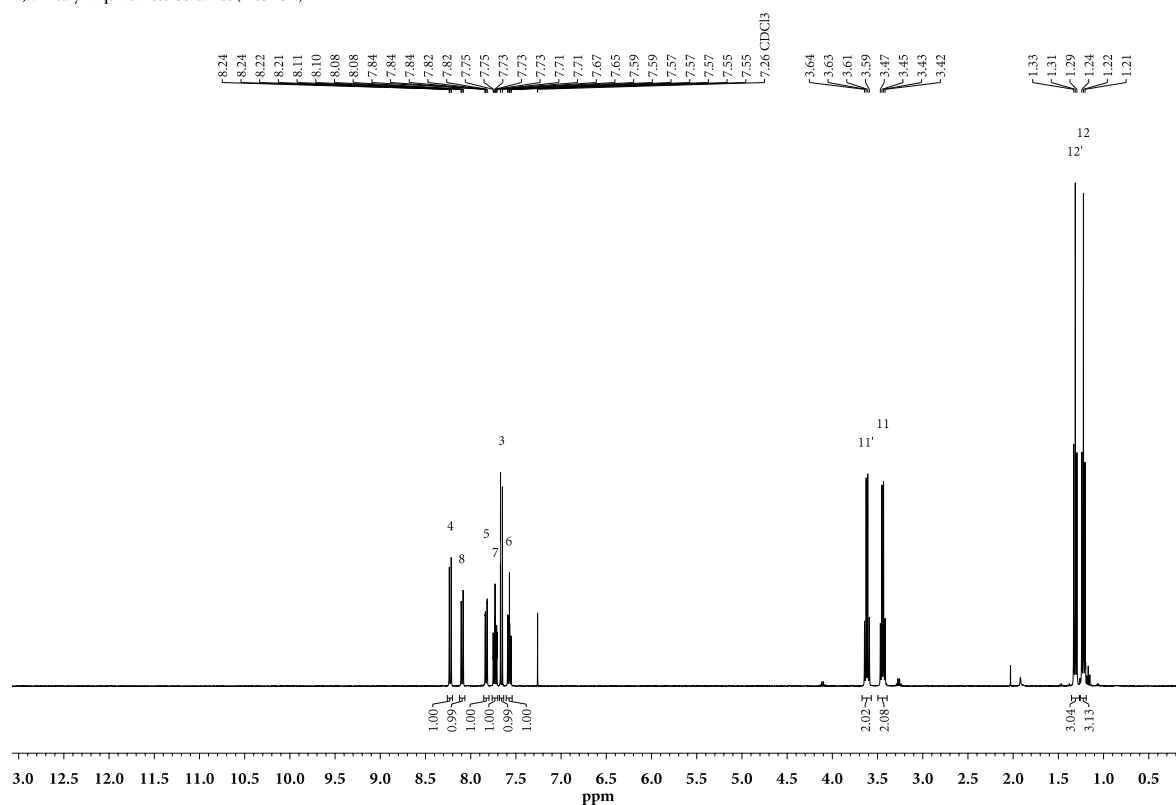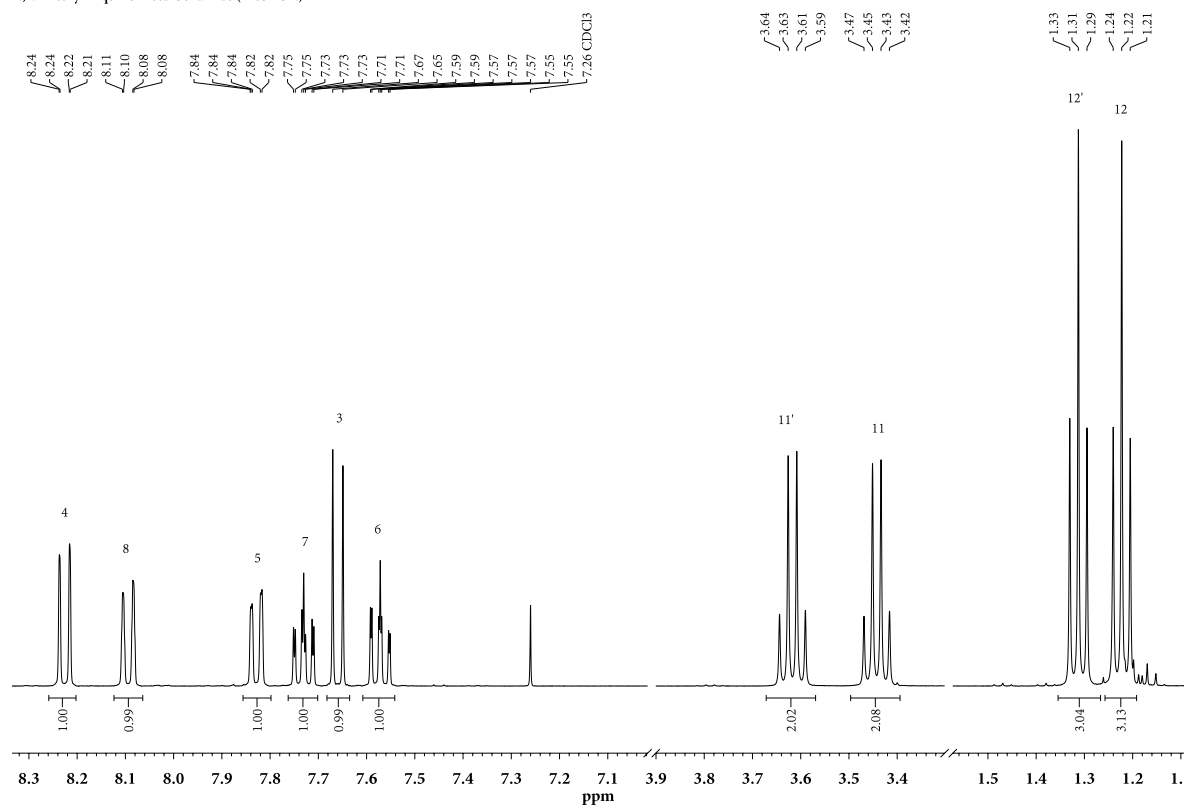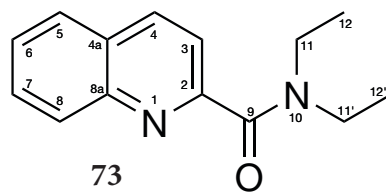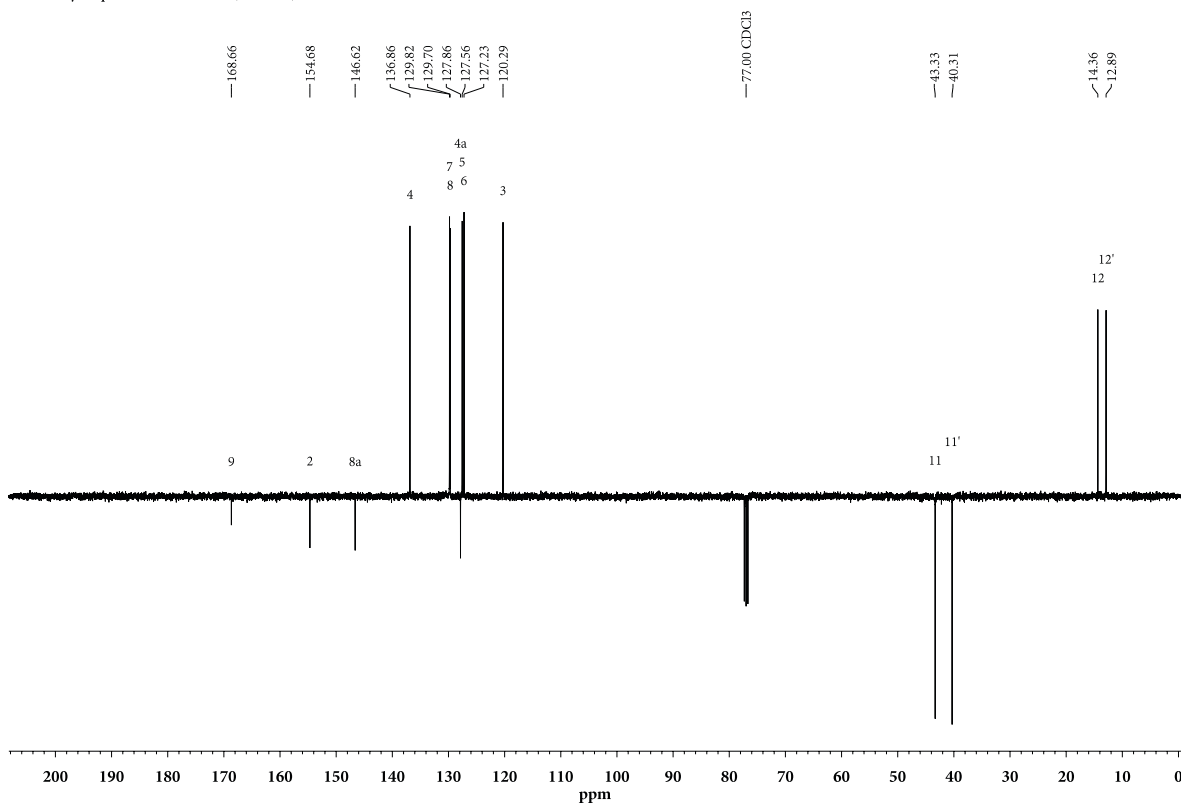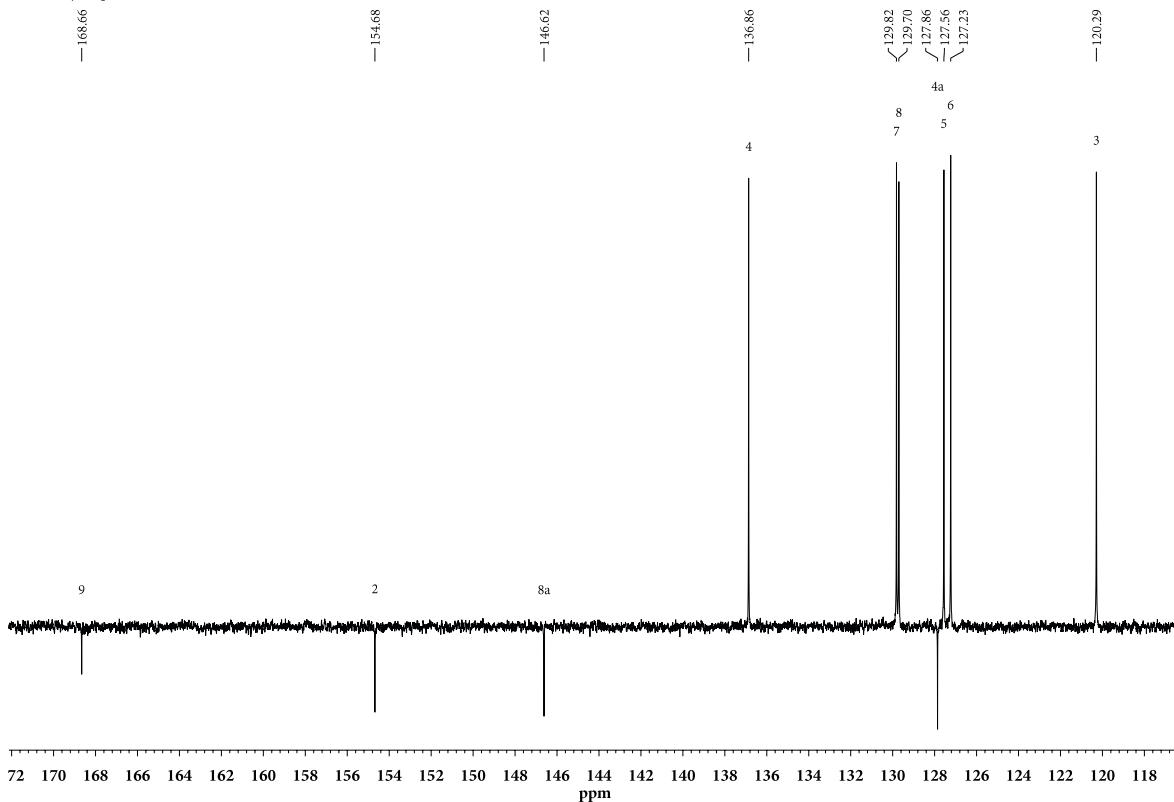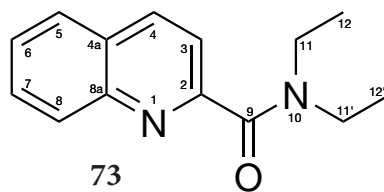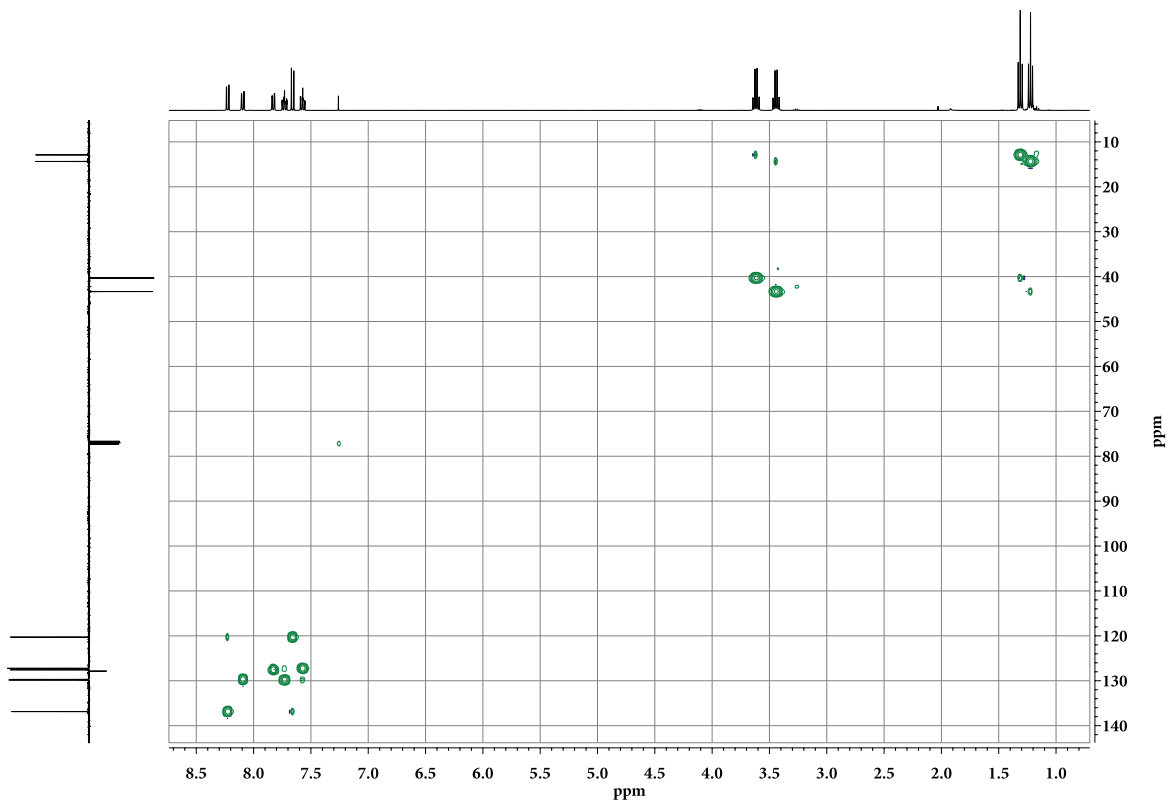
N,N-Diethyl-2-quinolinecarboxamide (HMBC)

N,N-Diethyl-2-quinolinecarboxamide (15N HMBC)

73

N,N-Diethyl-2-quinolinecarboxamide (COSY)

623

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

74
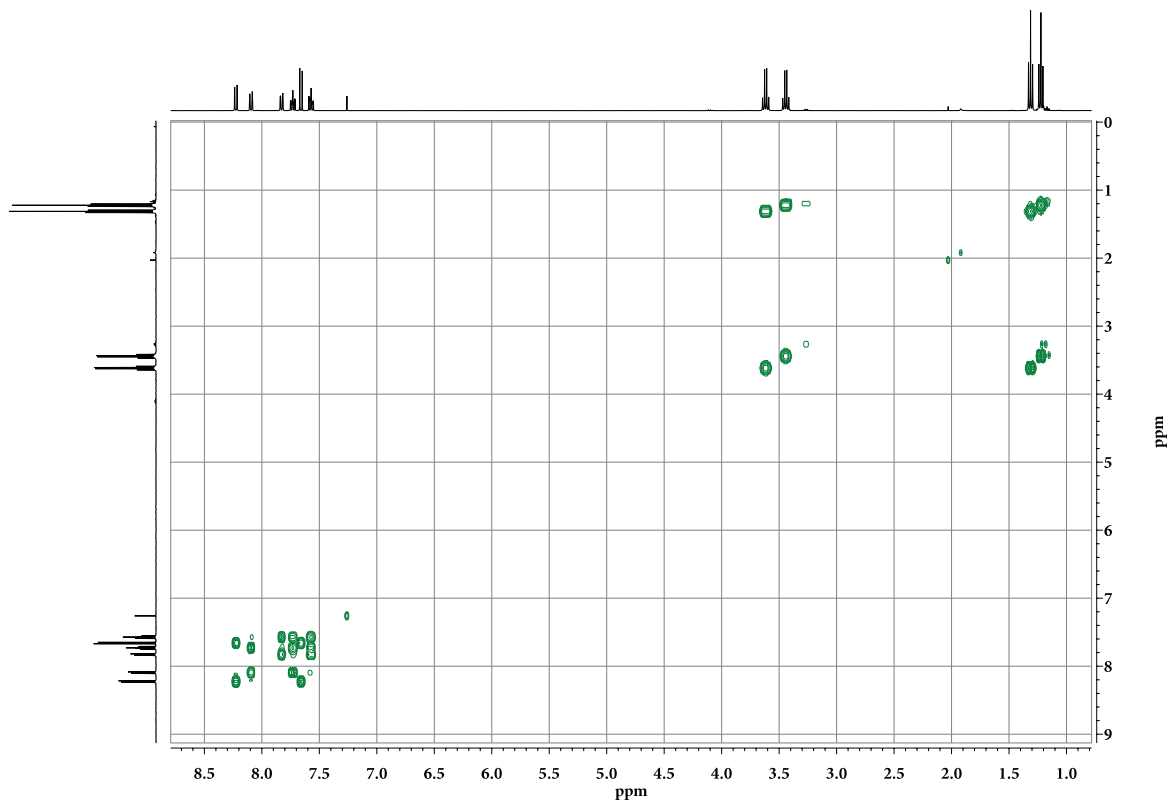
7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (C13 APT)
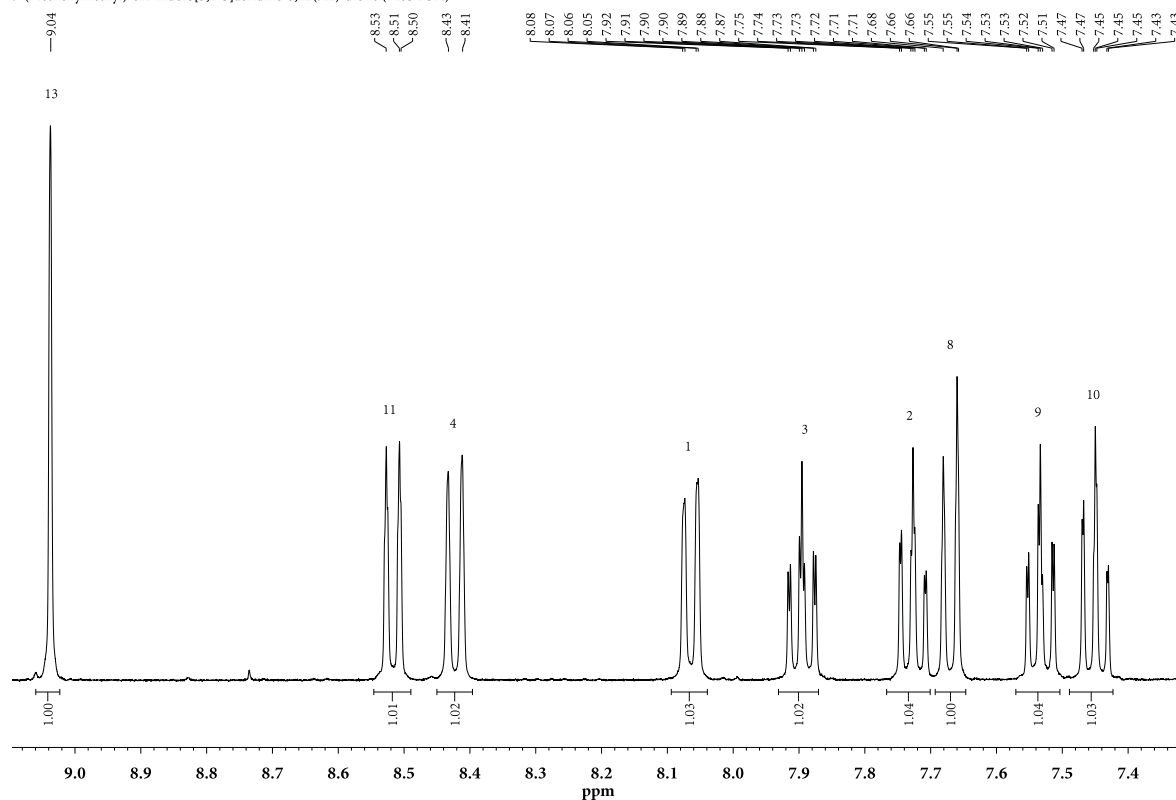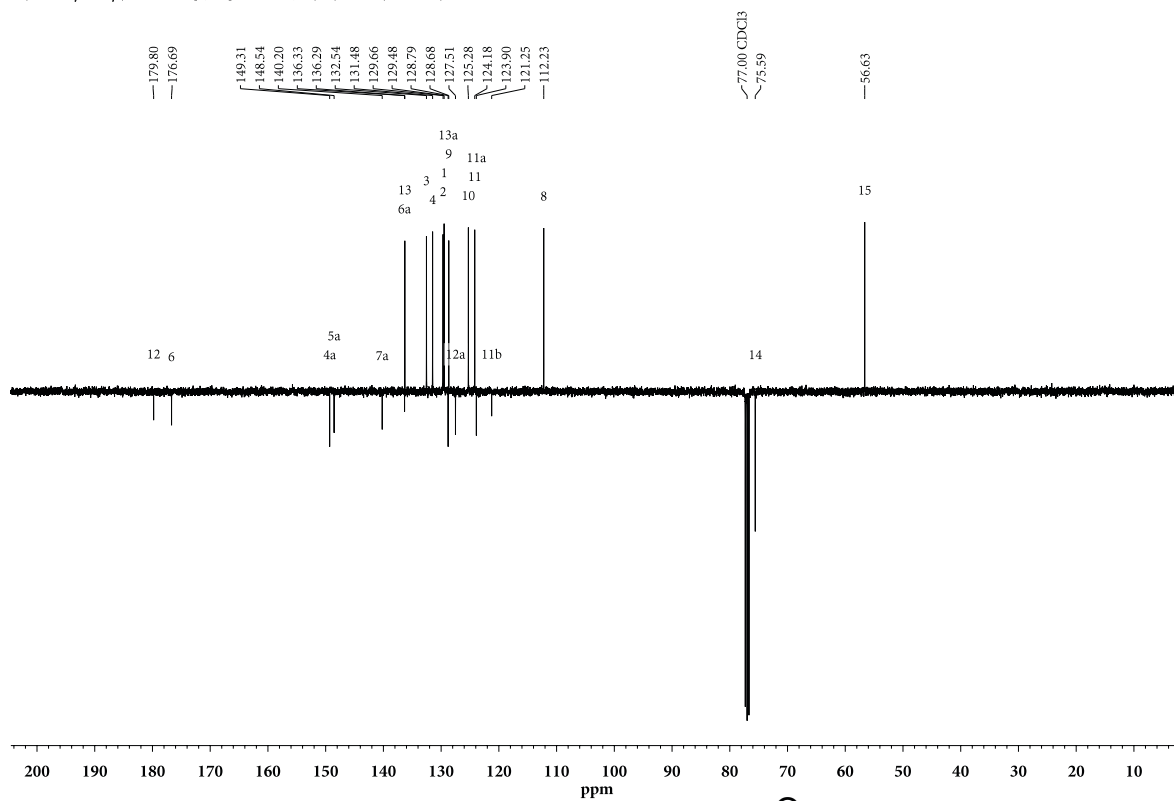
74

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (C13 APT)

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (C13 APT)

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (HSQC)

74
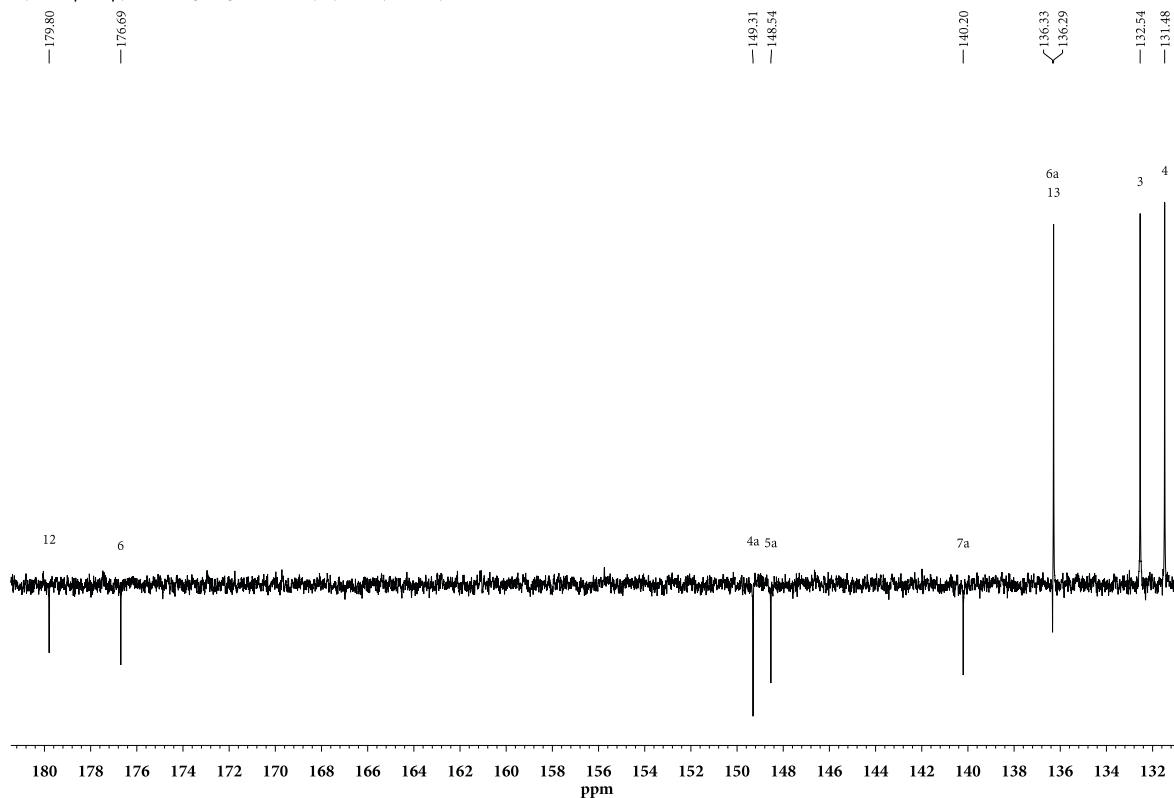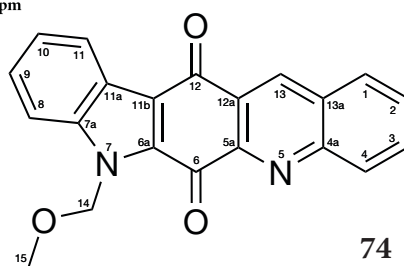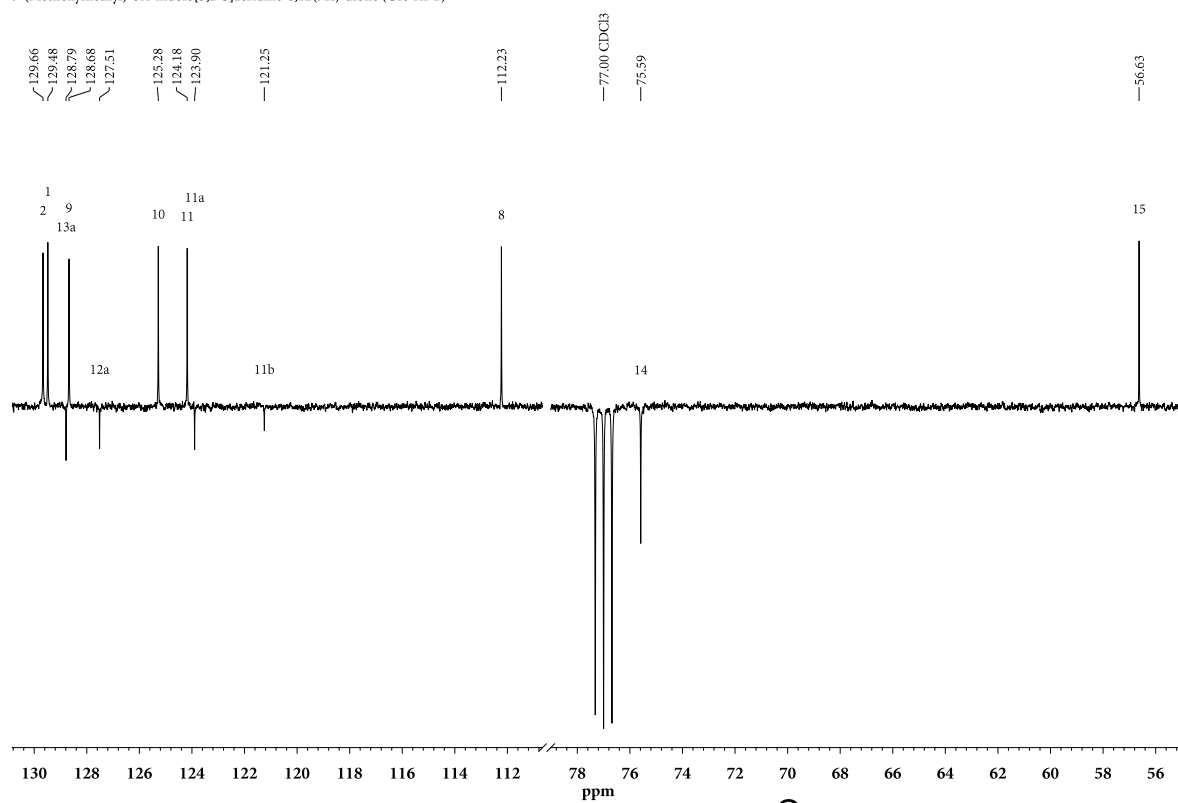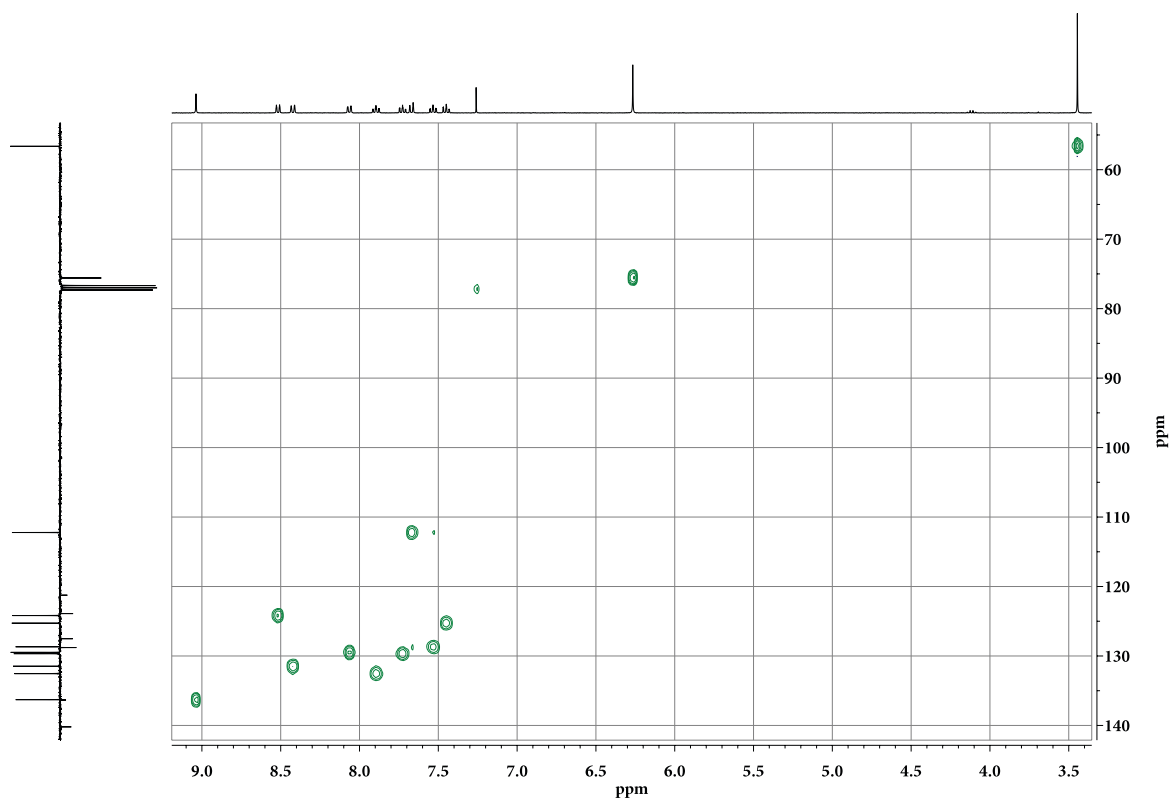
7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (HMBC)

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (15N HMBC)

74
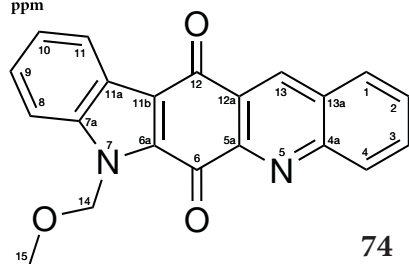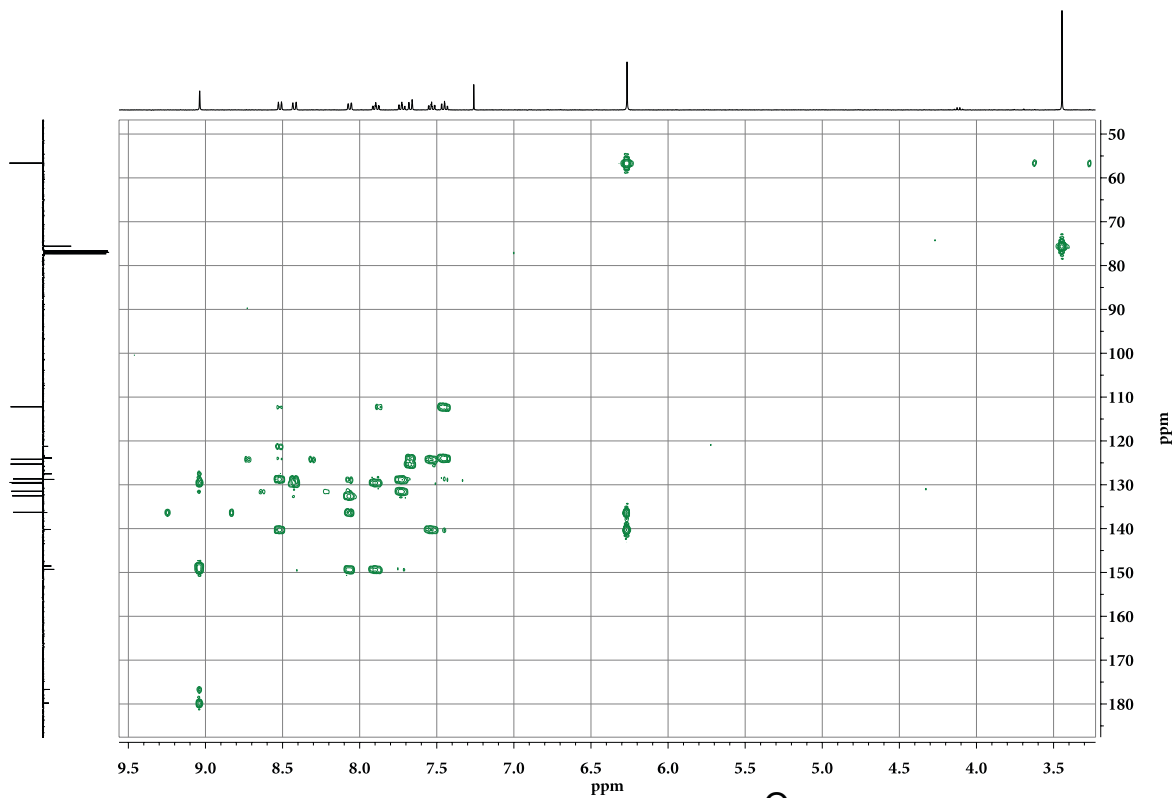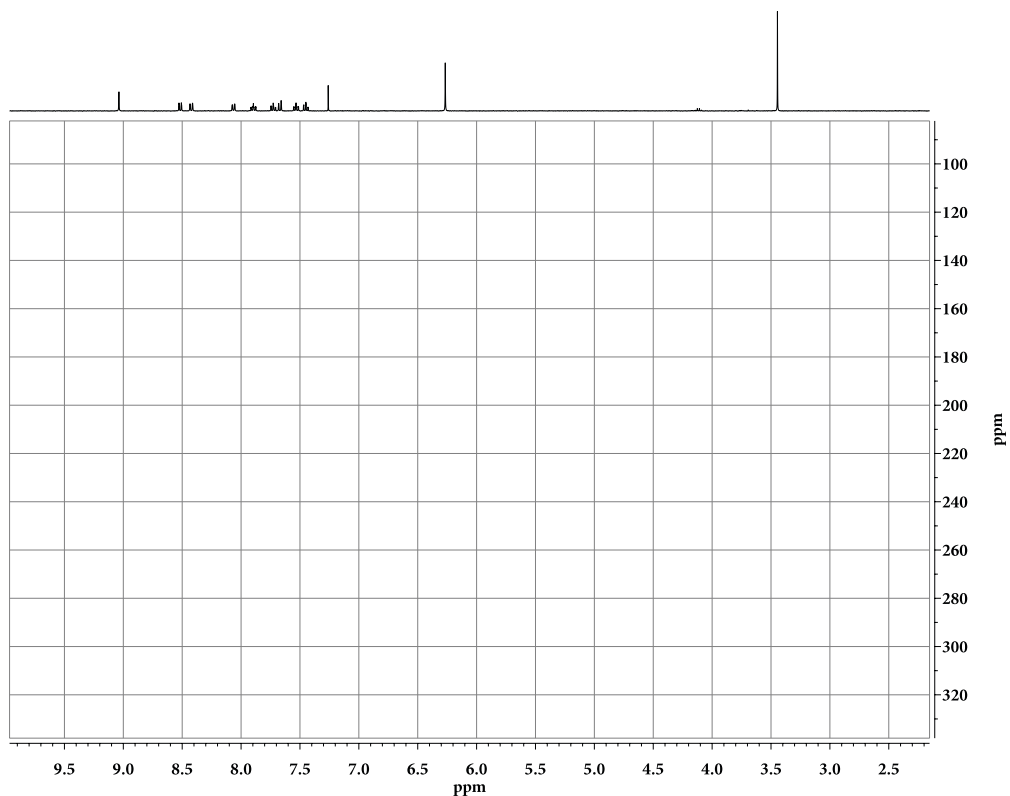
7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (15N HMBC)

7-(Methoxymethyl)-6H-indolo[3,2-b]acridine-6,12(7H)-dione (COSY)

74

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN145_DI_ESI_HRMS.d	Acquisition Date	11/10/2014 1:46:51 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	11/10/2014 1:46:51 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN145_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD	1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
343.1080	1	C21H15N2O3	343.1077	-0.7	4.3	1	100.00	15.5	even	ok
365.0900	1	C21H14N2NaO3	365.0897	-1.0	3.6	1	100.00	15.5	even	ok
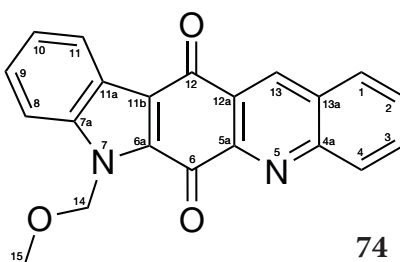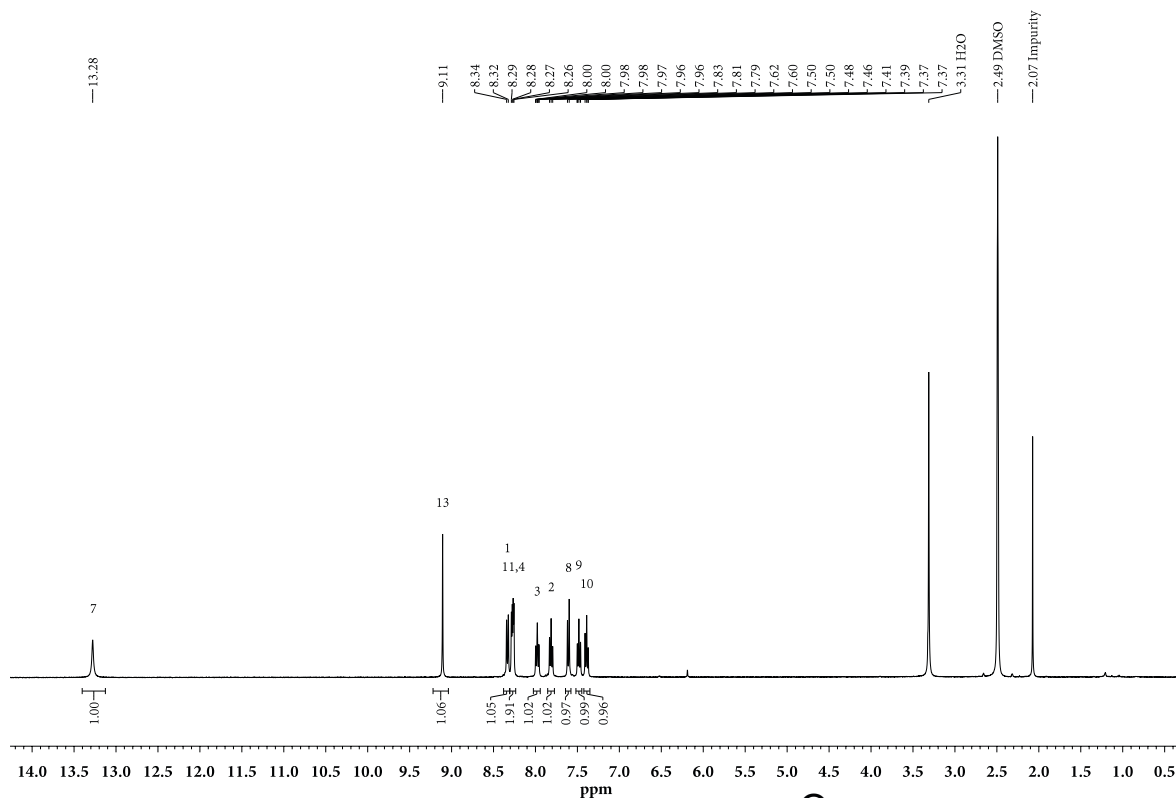
74

TN145_DI_ESI_HRMS.d

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

13.28

9.11
8.34
8.32
8.29
8.28
8.27
8.26
8.00
8.00
7.98
7.97
7.96
7.83
7.81
7.79
7.62
7.60
7.50
7.50
7.48
7.46
7.41
7.39
7.37
7.37
3.31 H2O
2.49 DMSO
2.07 Impurity

13

7

1
11,4

3
2
8 9
10

1.00
1.06
1.05
1.91
1.02
0.97
0.99
0.96

14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

75

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

13.28

9.11

8.34
8.32
8.29
8.27
8.00
8.00
7.98
7.97
7.96
7.83
7.81
7.79
7.62
7.60
7.50
7.50
7.48
7.46
7.41
7.39

13

11,4

1

3

2

8

9

10

7

1.00
1.06
1.05
1.91
1.02
1.02
0.97
0.99
0.96

13.5 13.4 13.3 13.2 13.1 13.0 12.9 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
ppm

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (C13 CPD)

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (C13 CPD)

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (HSQC)

75
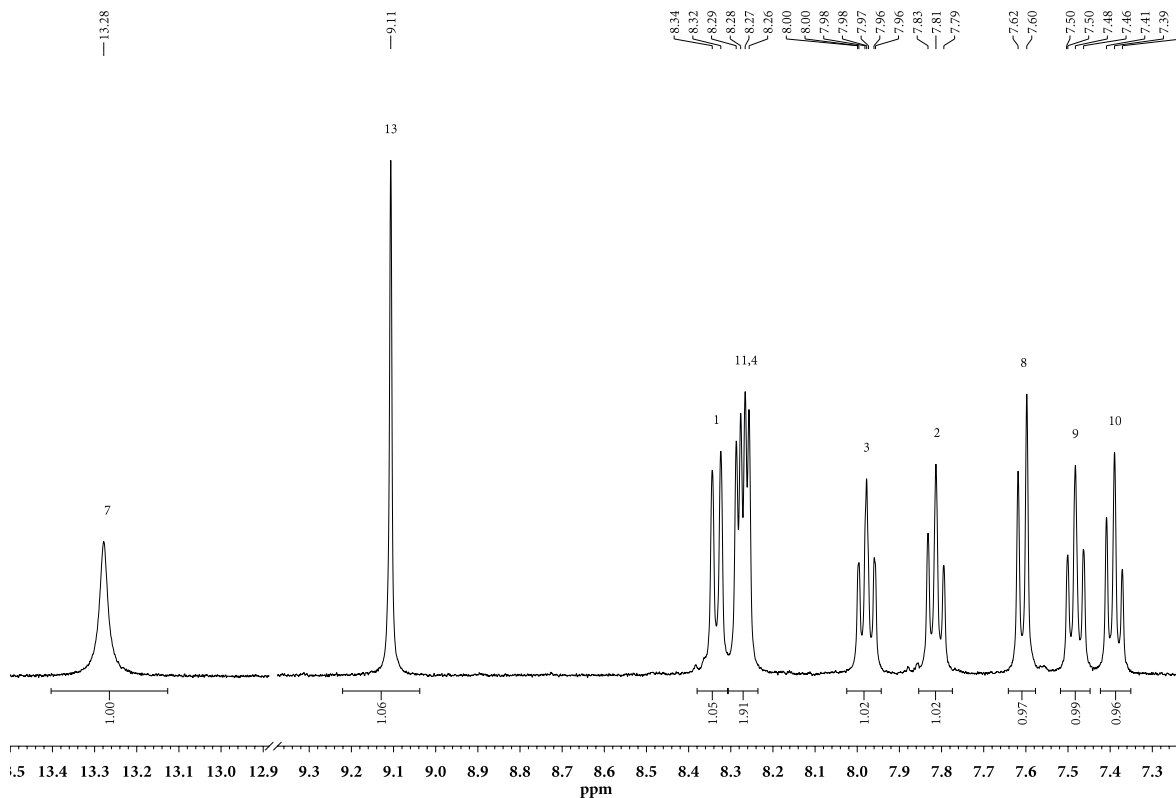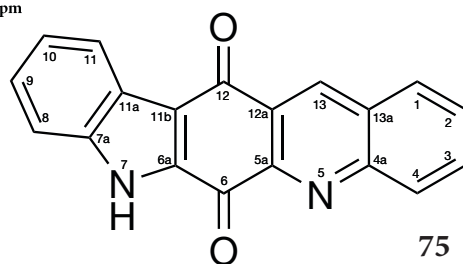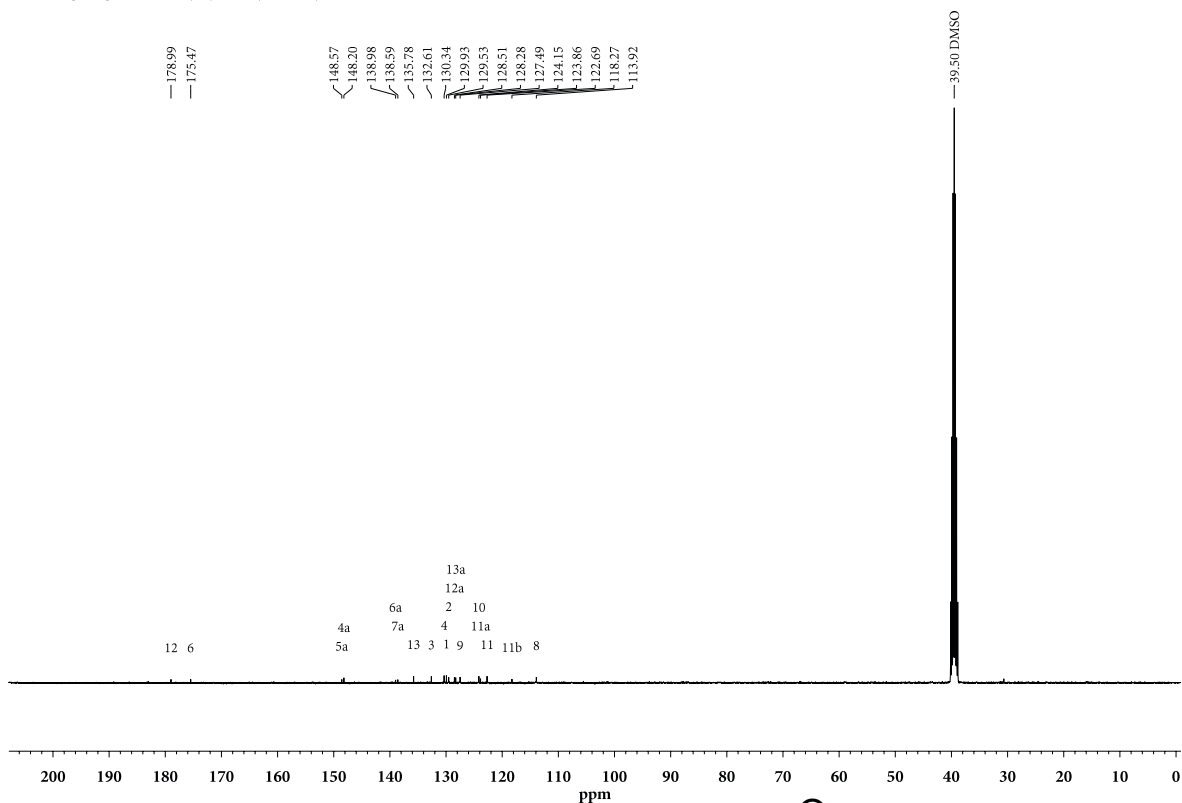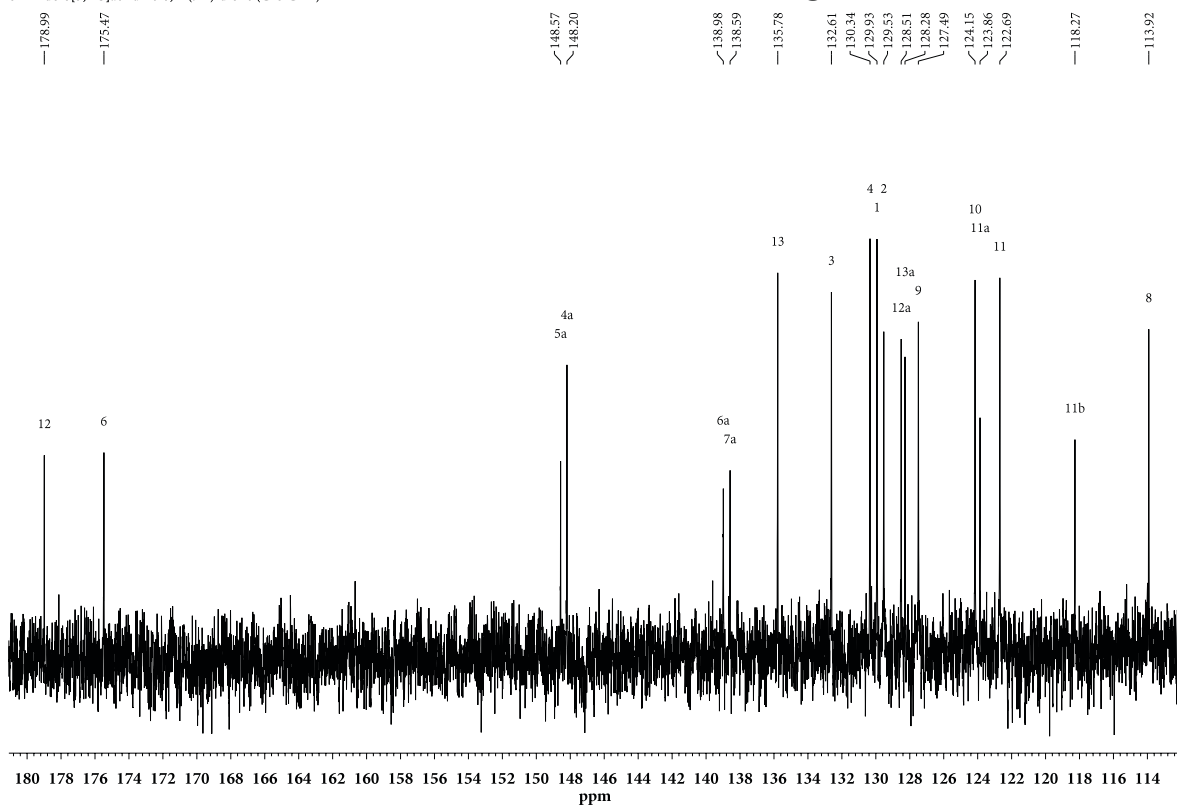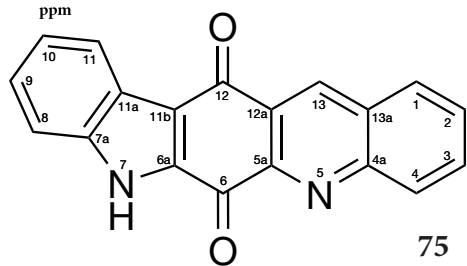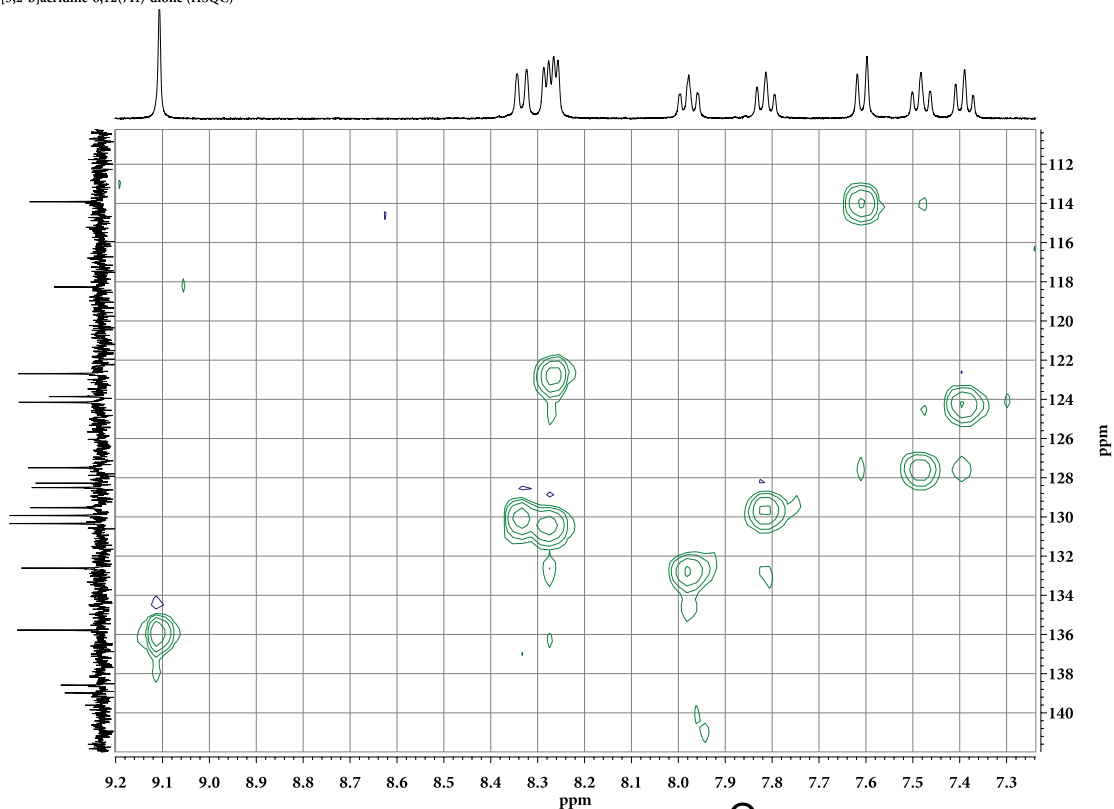
6H-Indolo[3,2-b]acridine-6,12(7H)-dione (HMBC)

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (15N HMBC)

75

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (15N HSQC)

6H-Indolo[3,2-b]acridine-6,12(7H)-dione (COSY)

75

Generic Display Report

Analysis Info

		Acquisition Date	11/10/2014 1:11:29 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN136_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 0.01 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/10/2014 1:11:29 PM

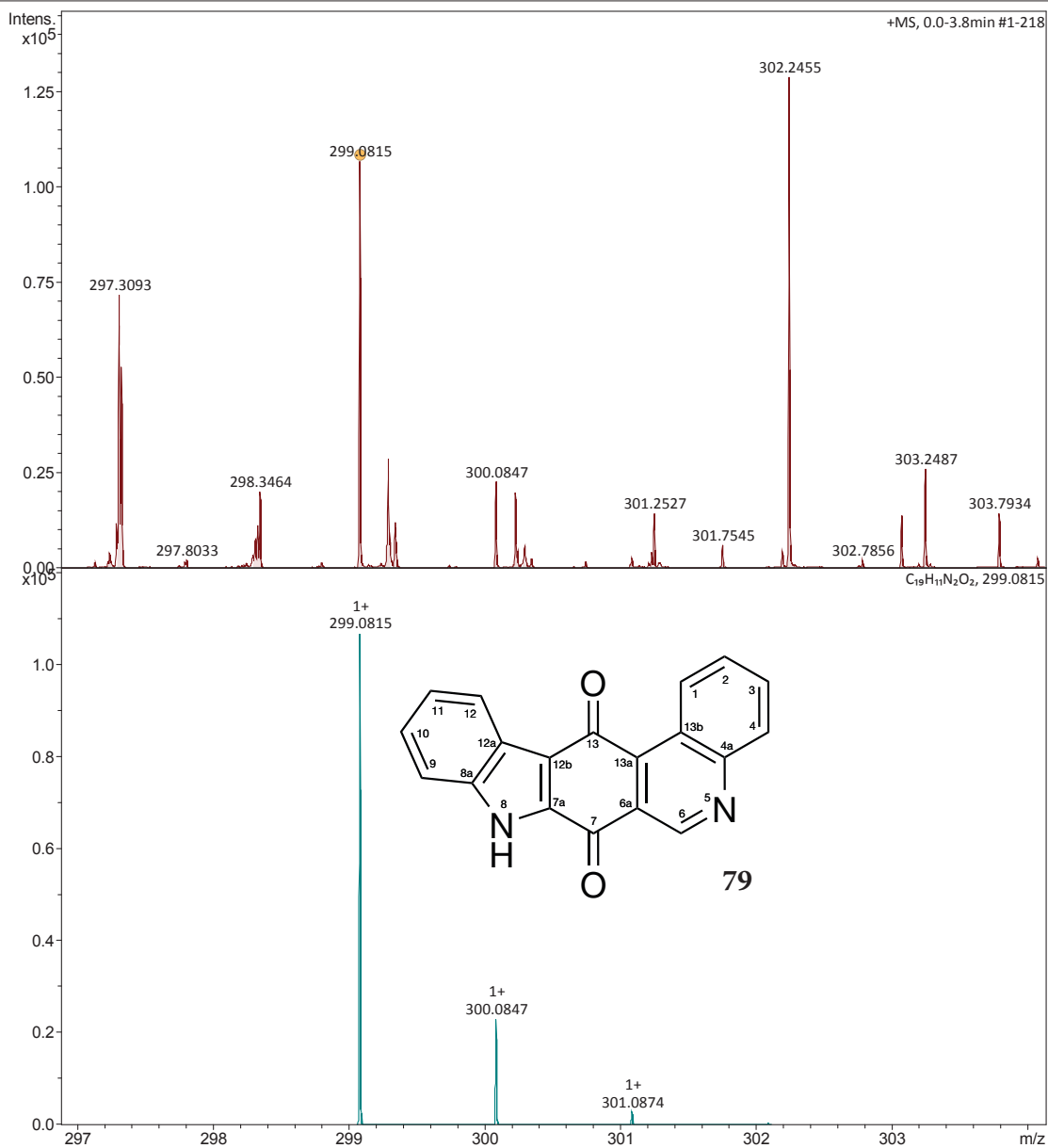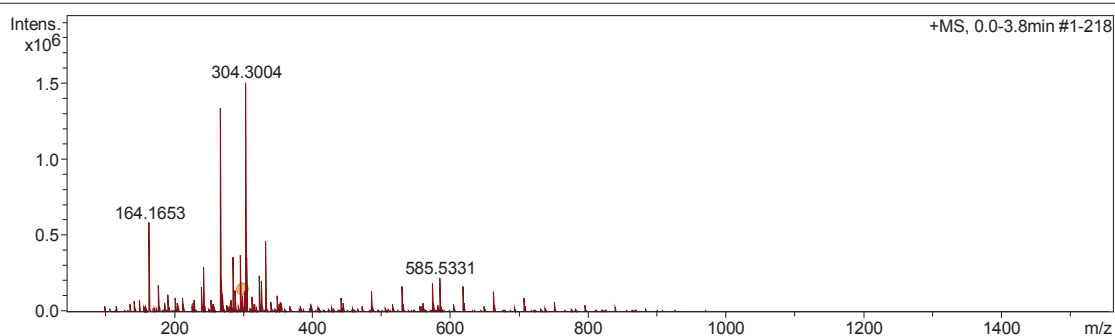Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141110\TN136_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
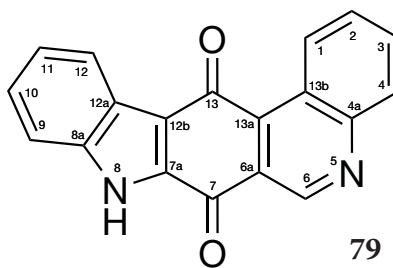
Sample Name   approx. 0.01 mg/mL in MeOH   Instrument   maXis HD   1820881.21300

Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
321.0635	1	C19H10N2NaO2	321.0634	-0.3	12.0	1	100.00	15.5	even	ok
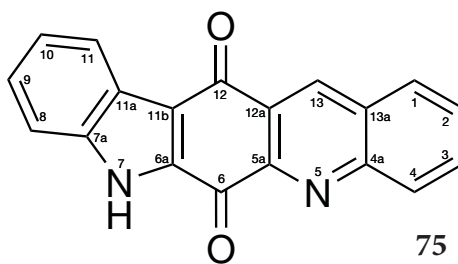
75

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (PROTON)

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (C13 APT)

76
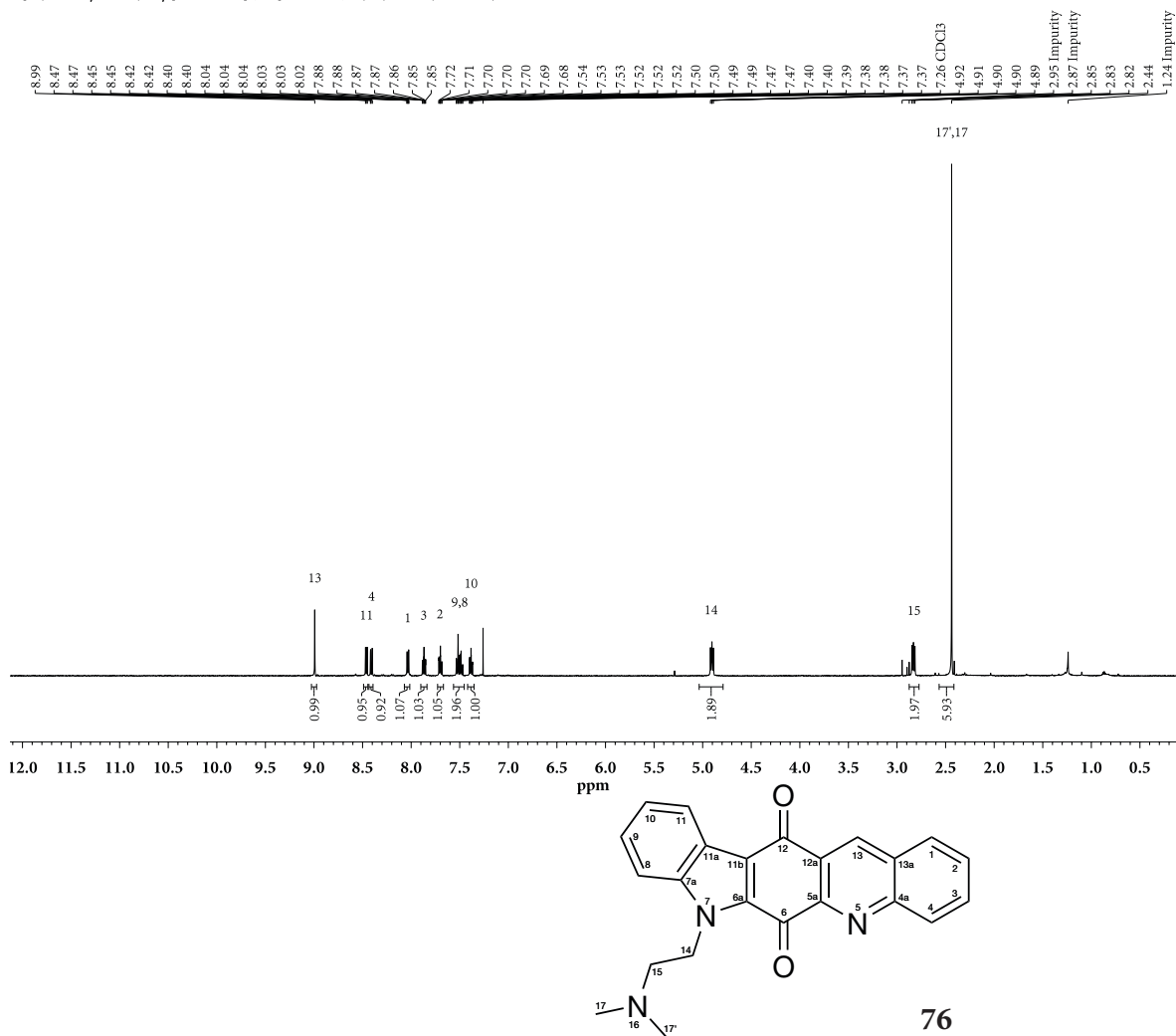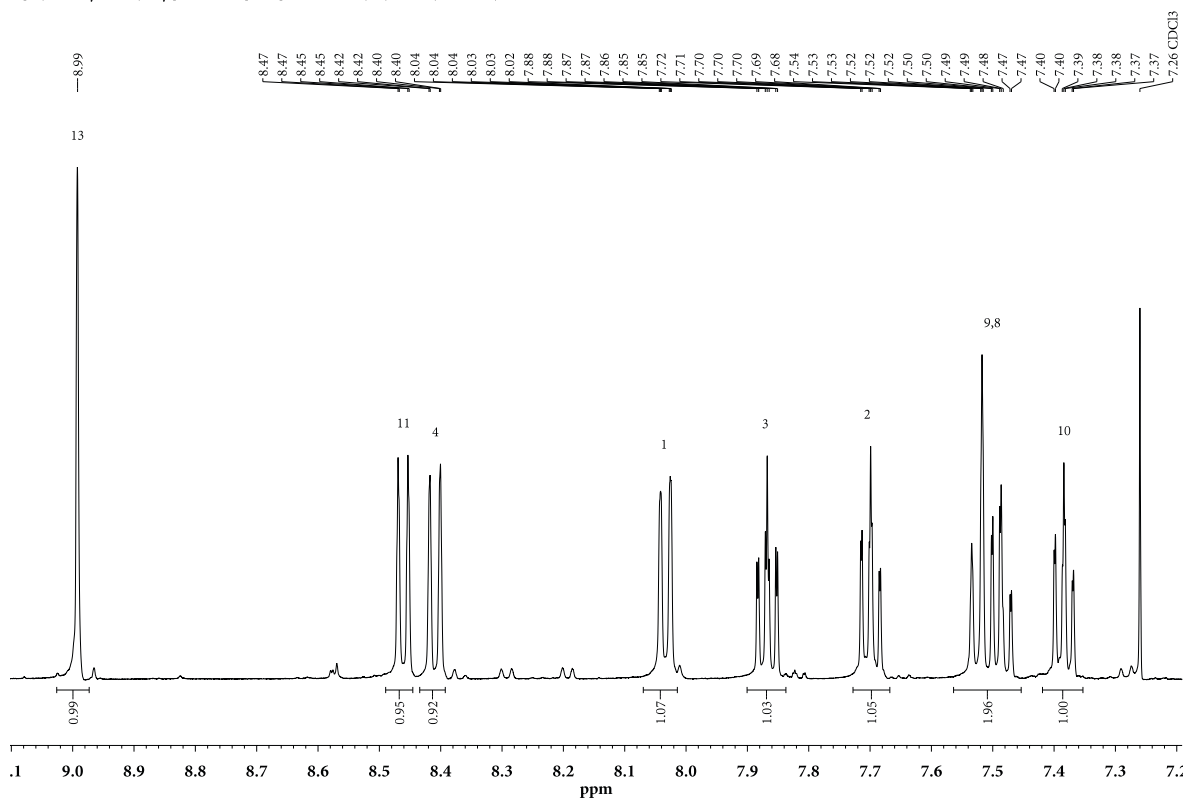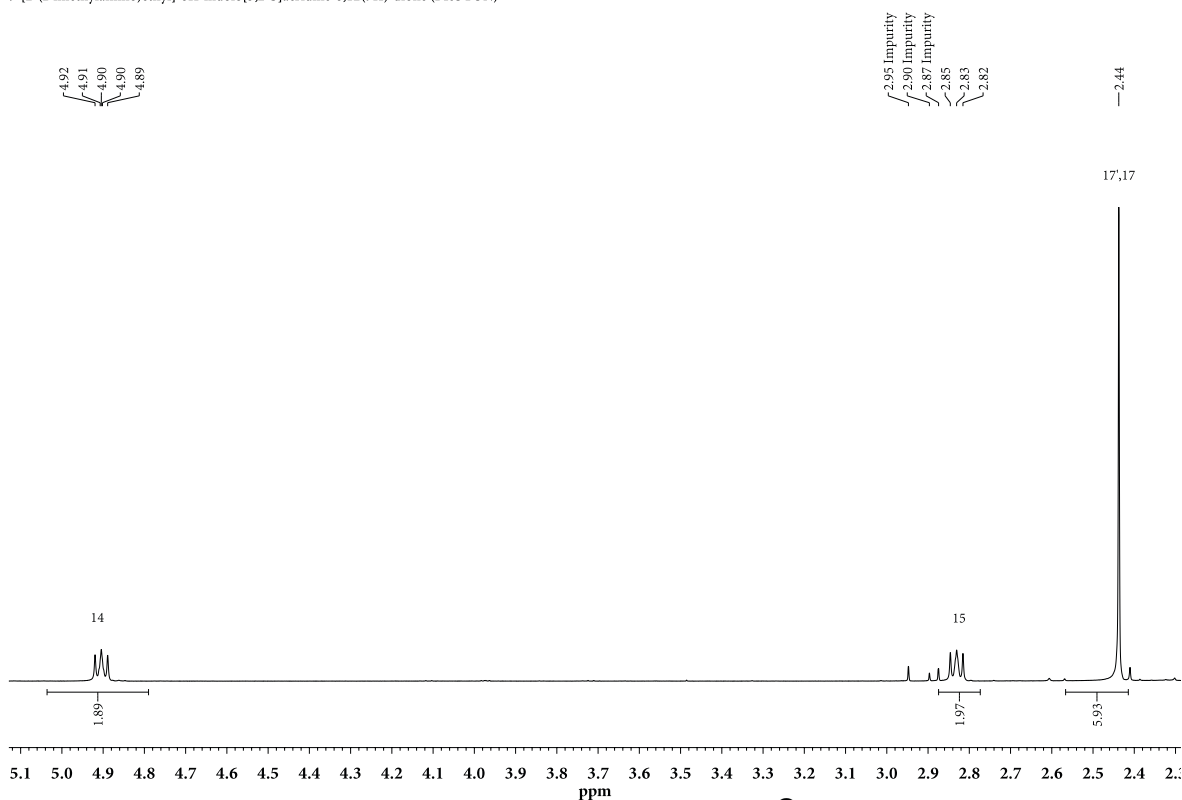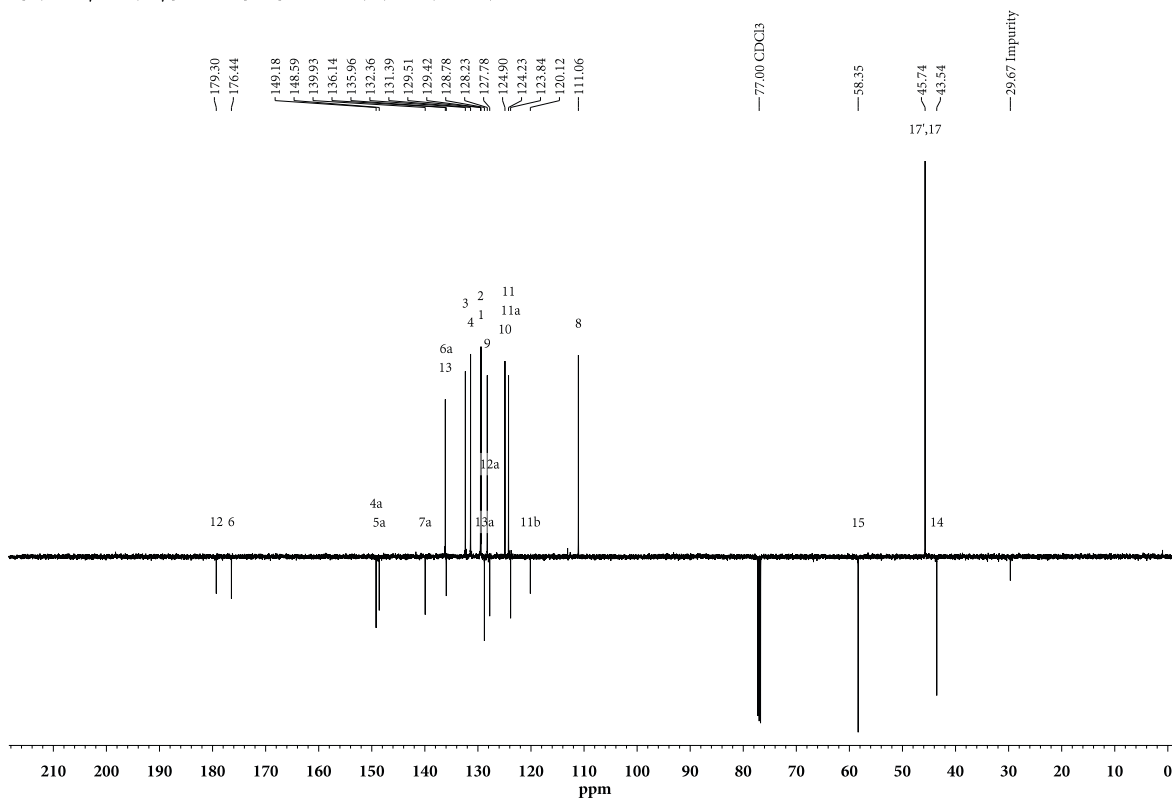
7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (C13 APT)

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (HSQC)

76
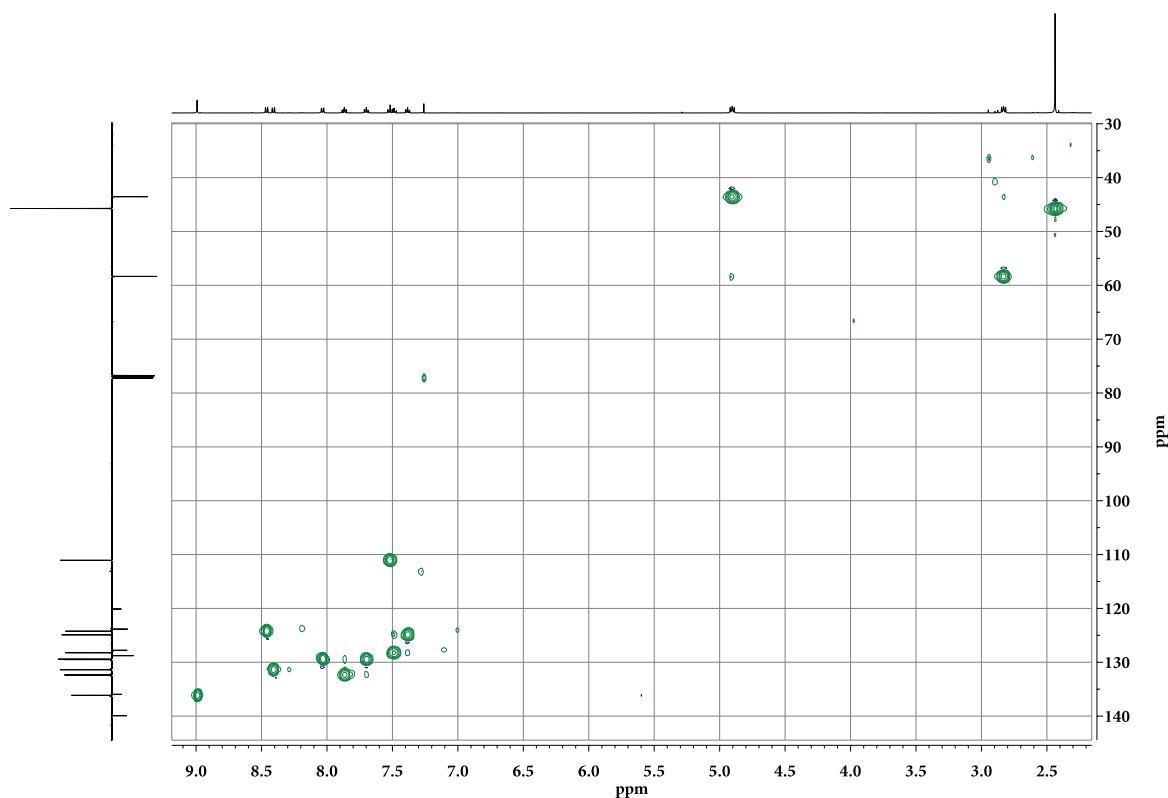
7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (HMBC)

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (15N HMBC)
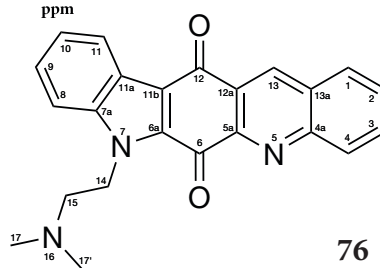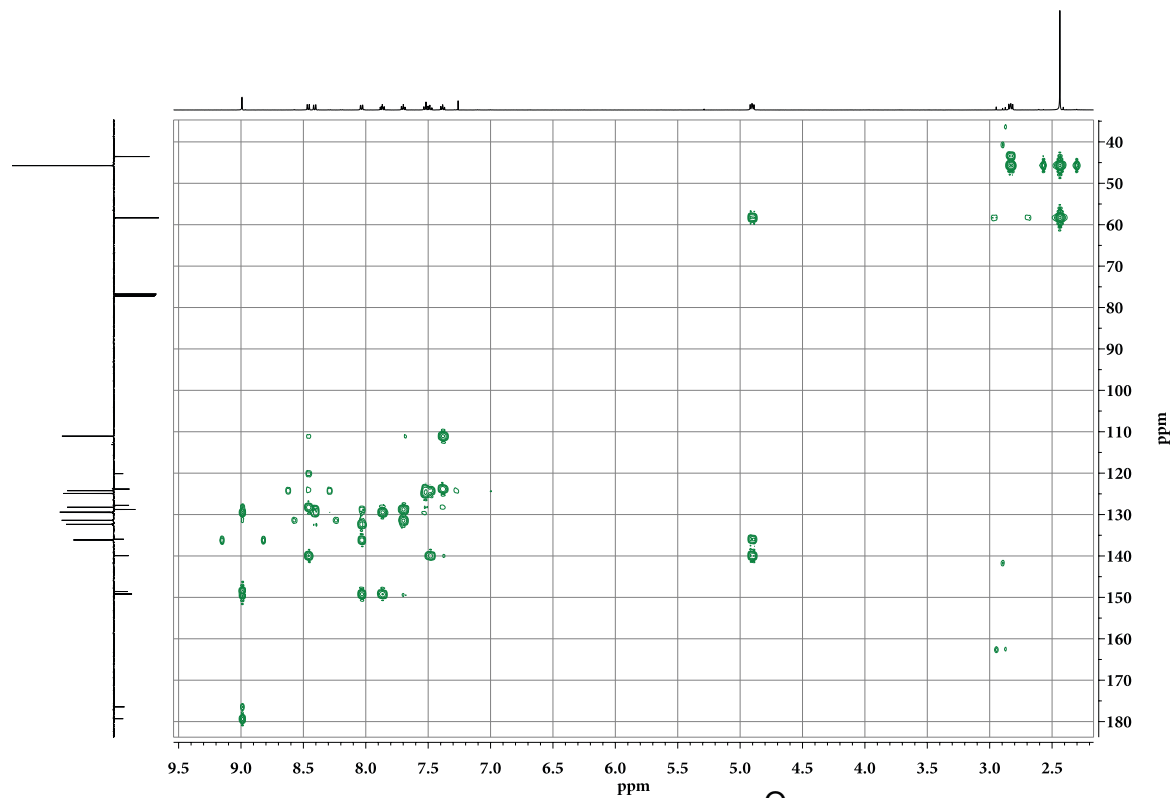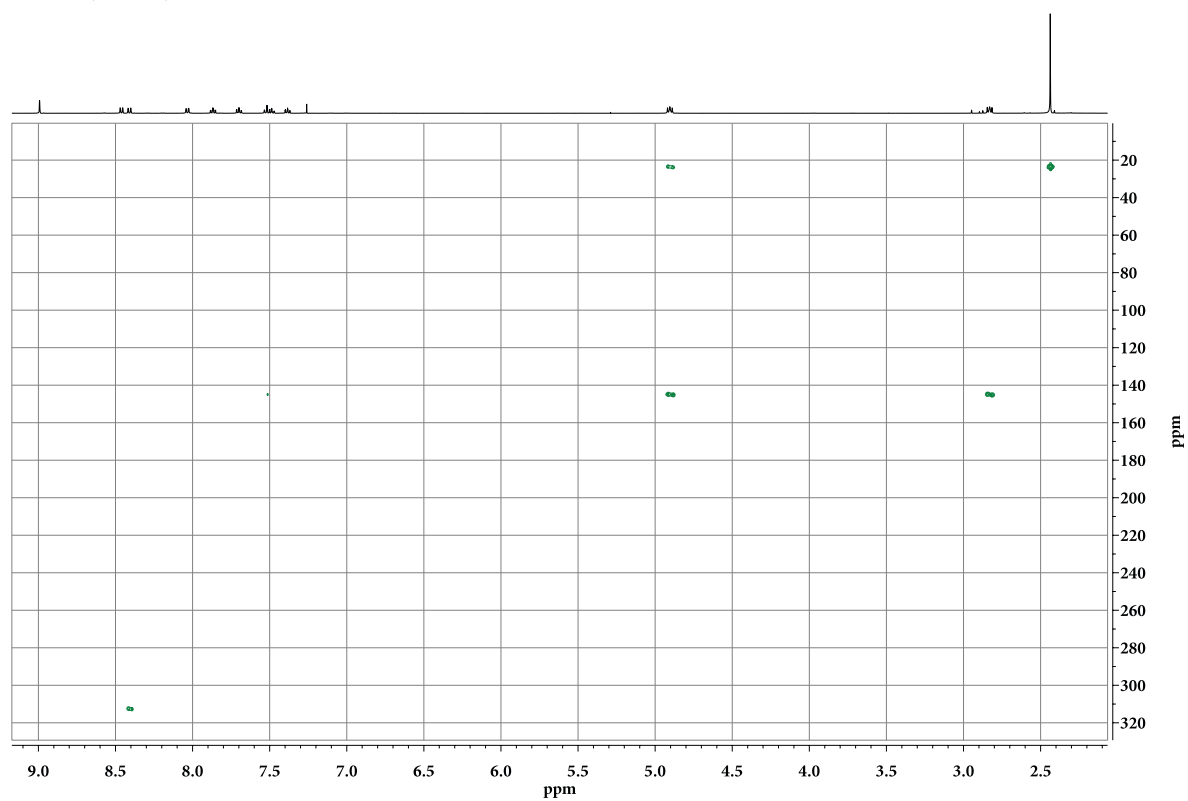
76

7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (COSY)

76
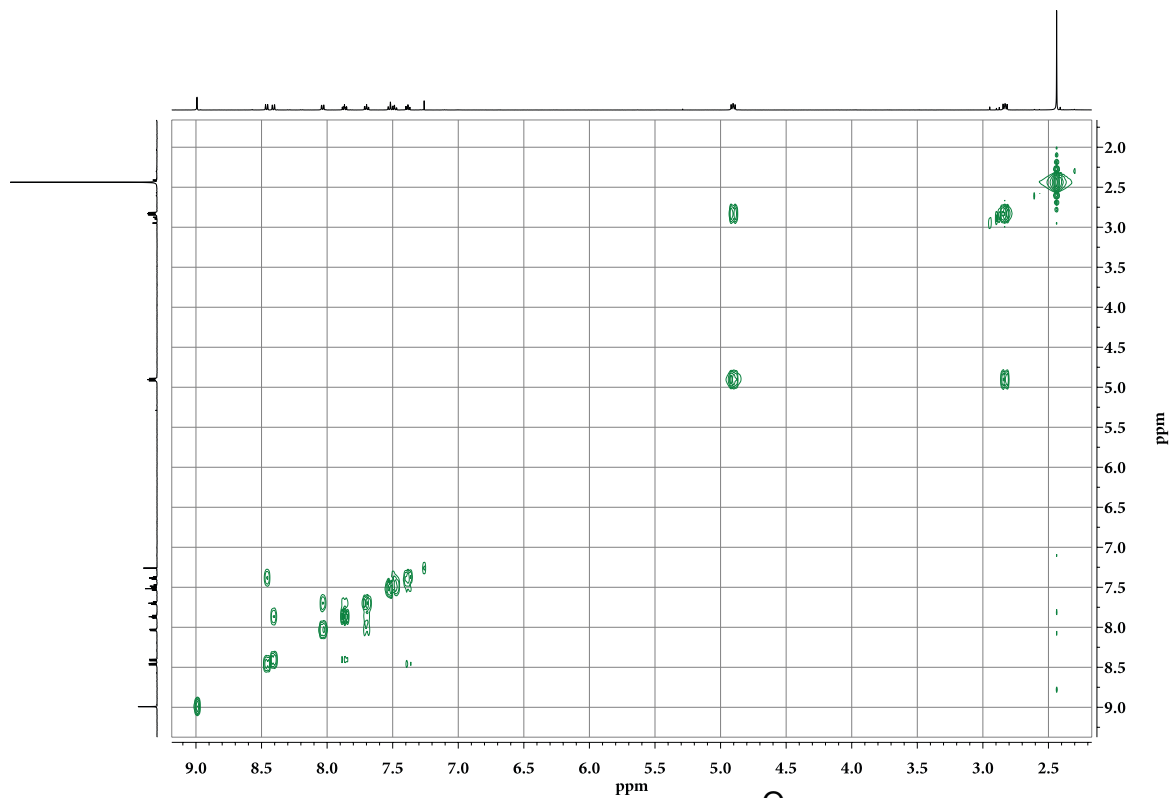
7-[2-(Dimethylamino)ethyl]-6H-indolo[3,2-b]acridine-6,12(7H)-dione (NOESY)

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 10:34:54 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\XXQ3_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/4/2014 10:34:54 AM

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\XXQ3_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	approx. 10 ug/mL in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.7min #1-155

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
370.1548	1	C23H20N3O2	370.1550	0.4	6.4	1	100.00	15.5	even	ok
392.1369	1	C23H19N3NaO2	392.1369	0.2	7.8	1	100.00	15.5	even	ok

76
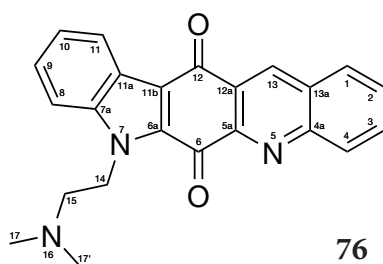
N,N-Diethyl-3-quinolinecarboxamide (PROTON)

77

N,N-Diethyl-3-quinolinecarboxamide (PROTON)

N,N-Diethyl-3-quinolinecarboxamide (C13 APT)

168.55

147.79

134.18
130.48
130.10
129.18
128.13
127.39
127.07

77.00 CDCl3

43.53
39.65

14.35
12.89

4a
5

6

8a
7

4

8

2

9

11' 11

12
12'

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

77

N,N-Diethyl-3-quinolinecarboxamide (C13 APT)

43.53

39.65

14.35

12.89

11'

11

12'

12

50 49 48 47 46 45 44 43 42 41 40 39 38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9
ppm
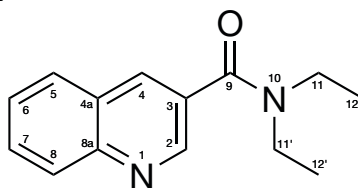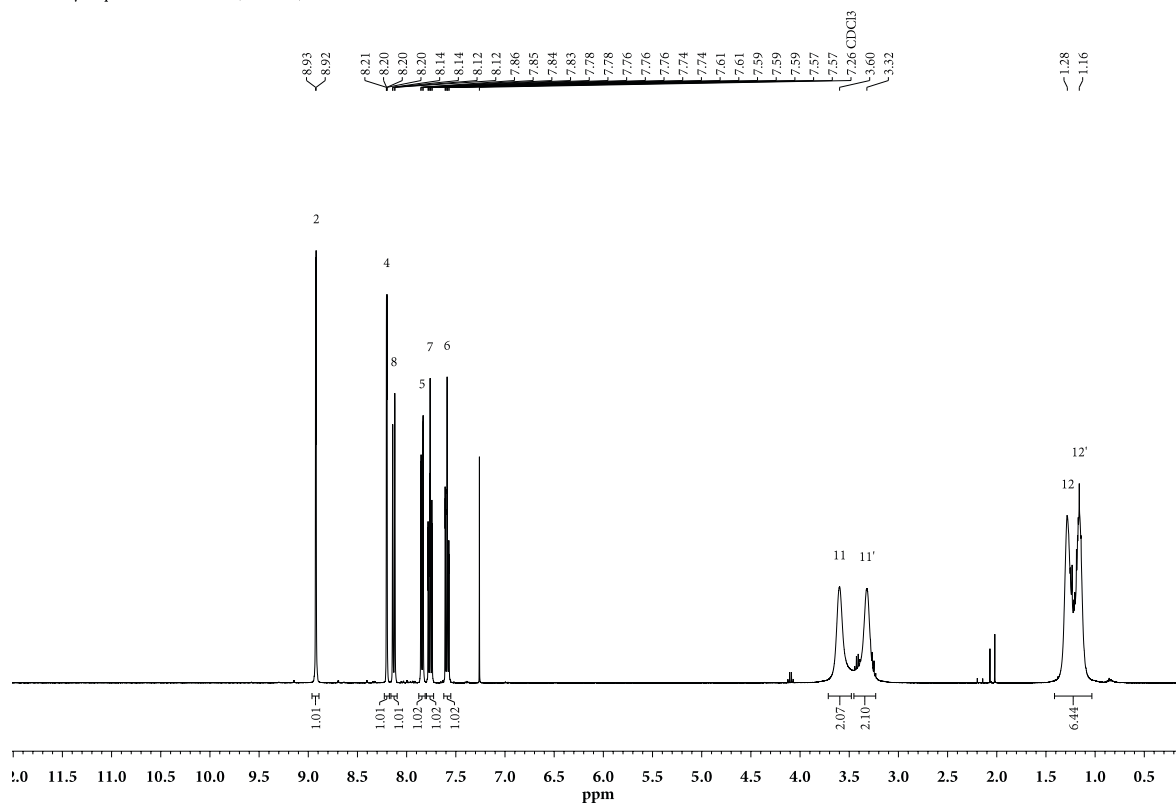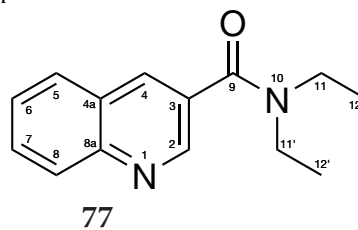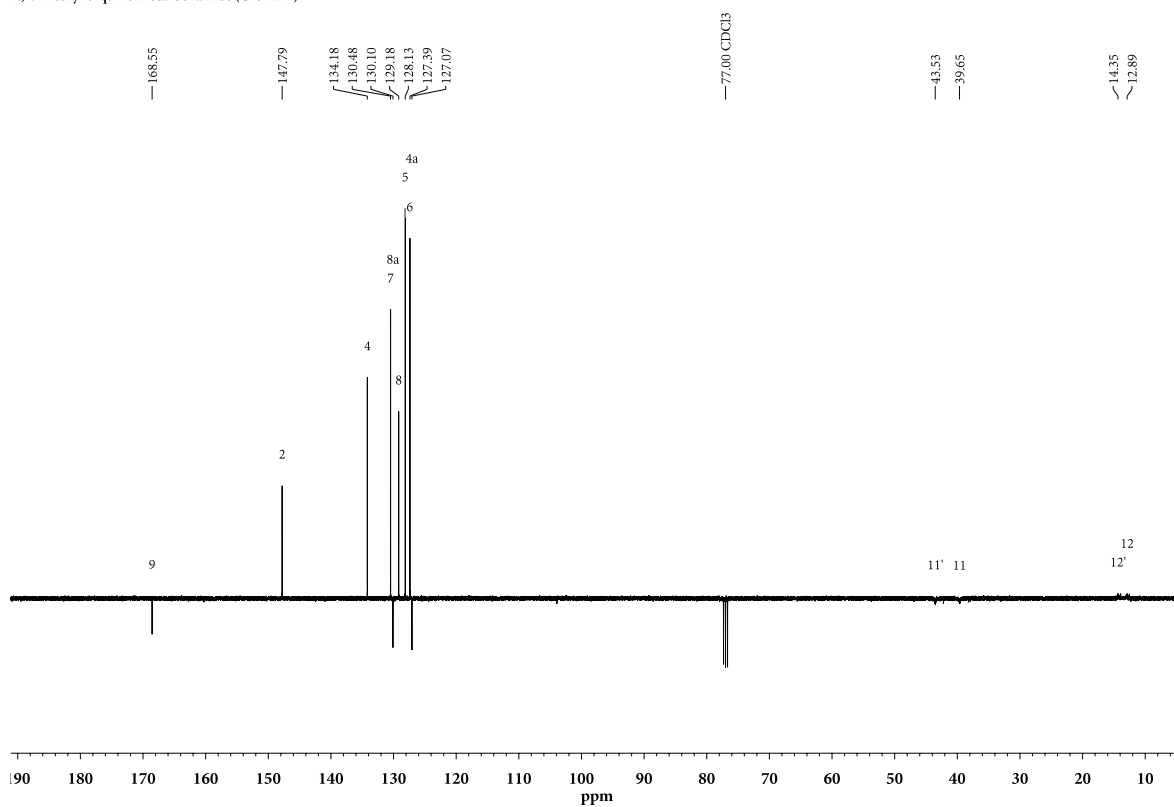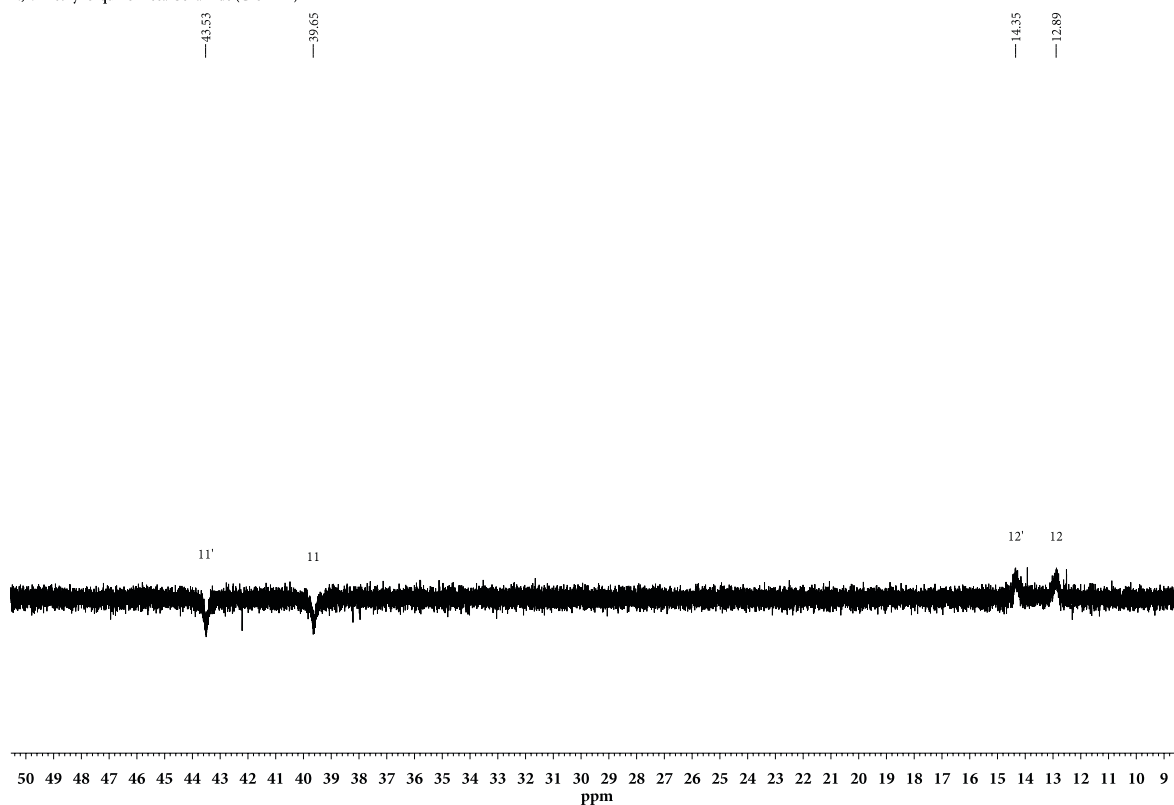
N,N-Diethyl-3-quinolinecarboxamide (HSQC)

N,N-Diethyl-3-quinolinecarboxamide (HMBC)

77
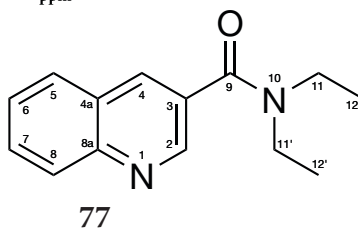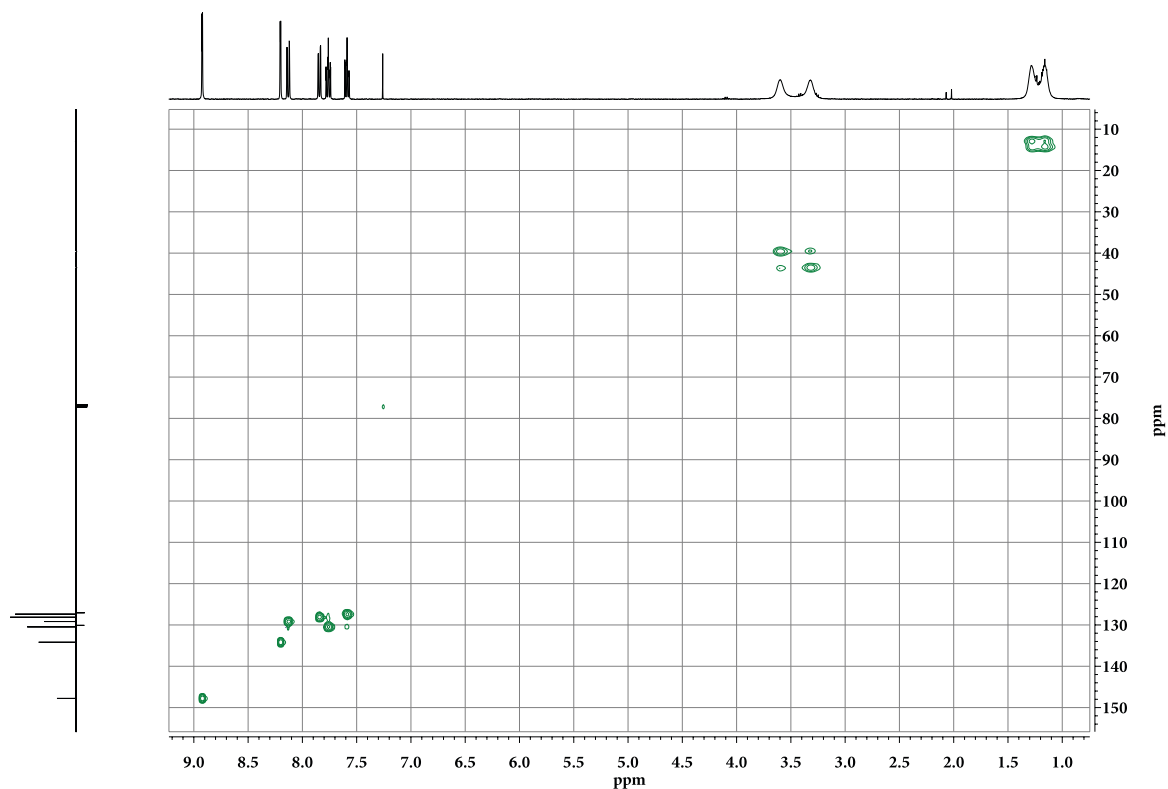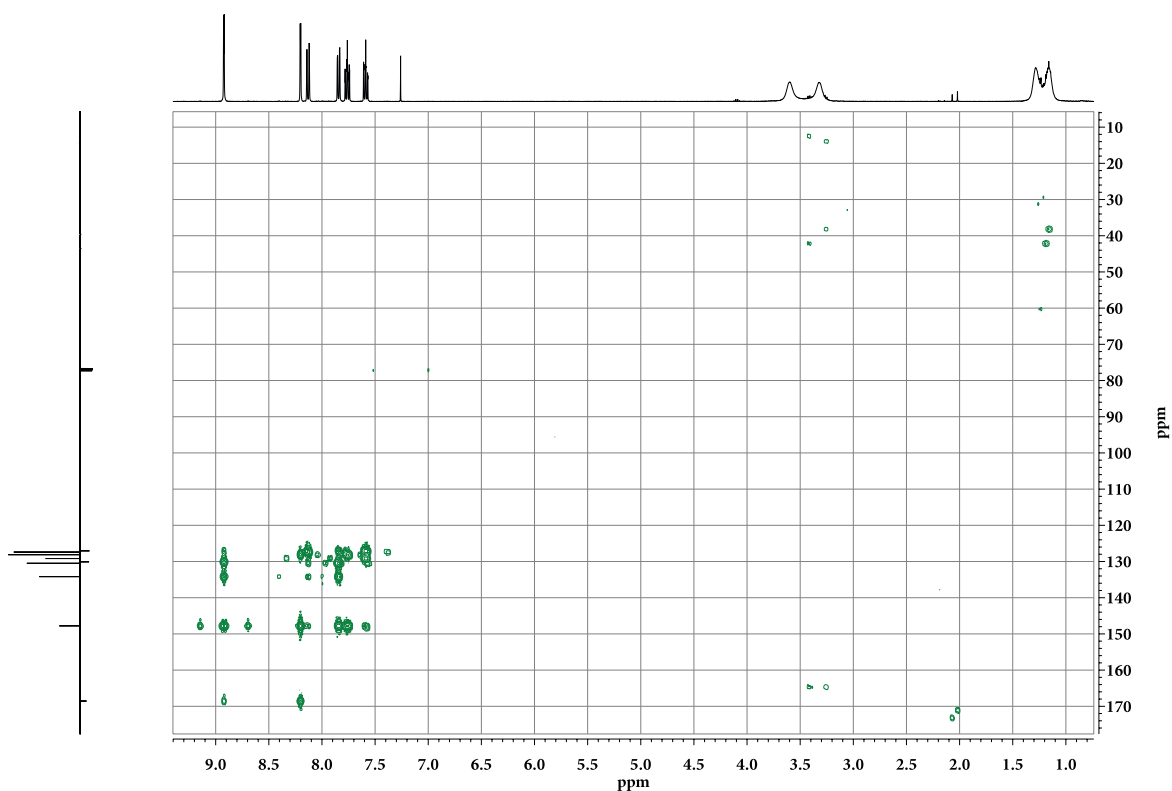
N,N-Diethyl-3-quinolinecarboxamide (15N HMBC)

77

N,N-Diethyl-3-quinolinecarboxamide (NOESY)
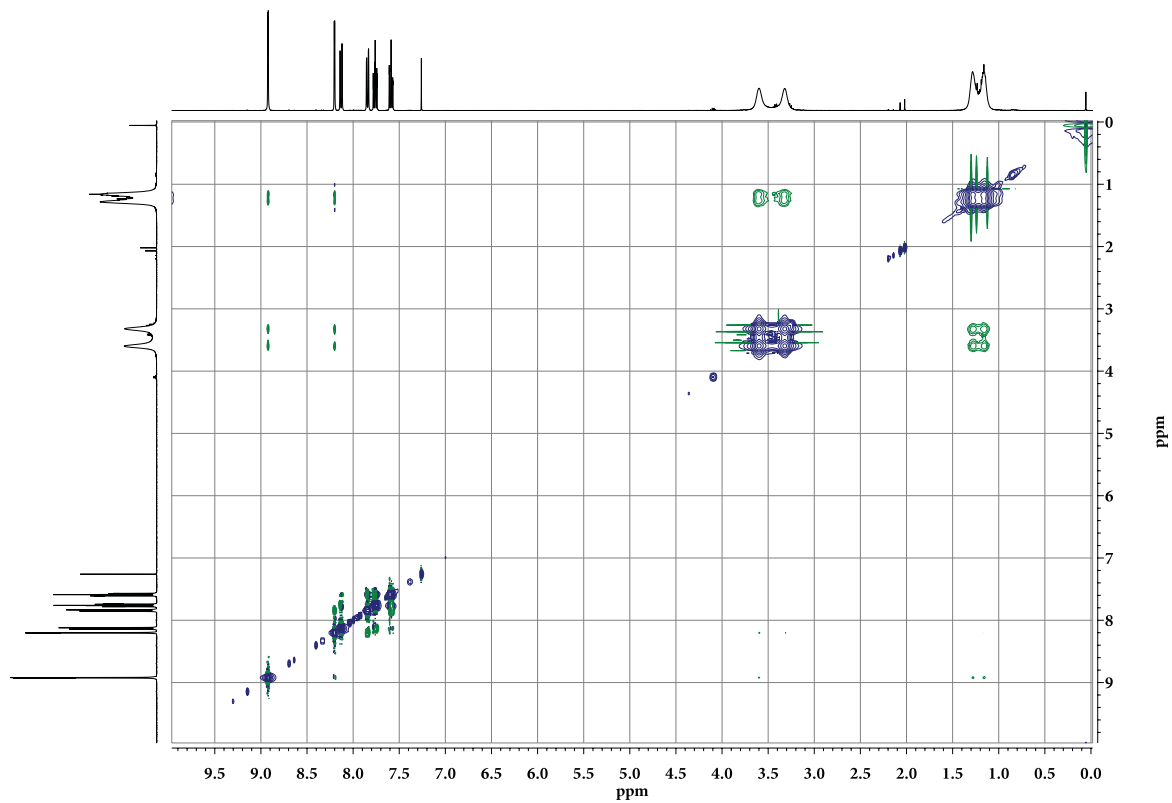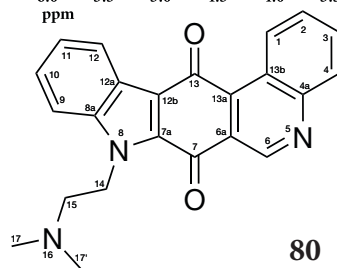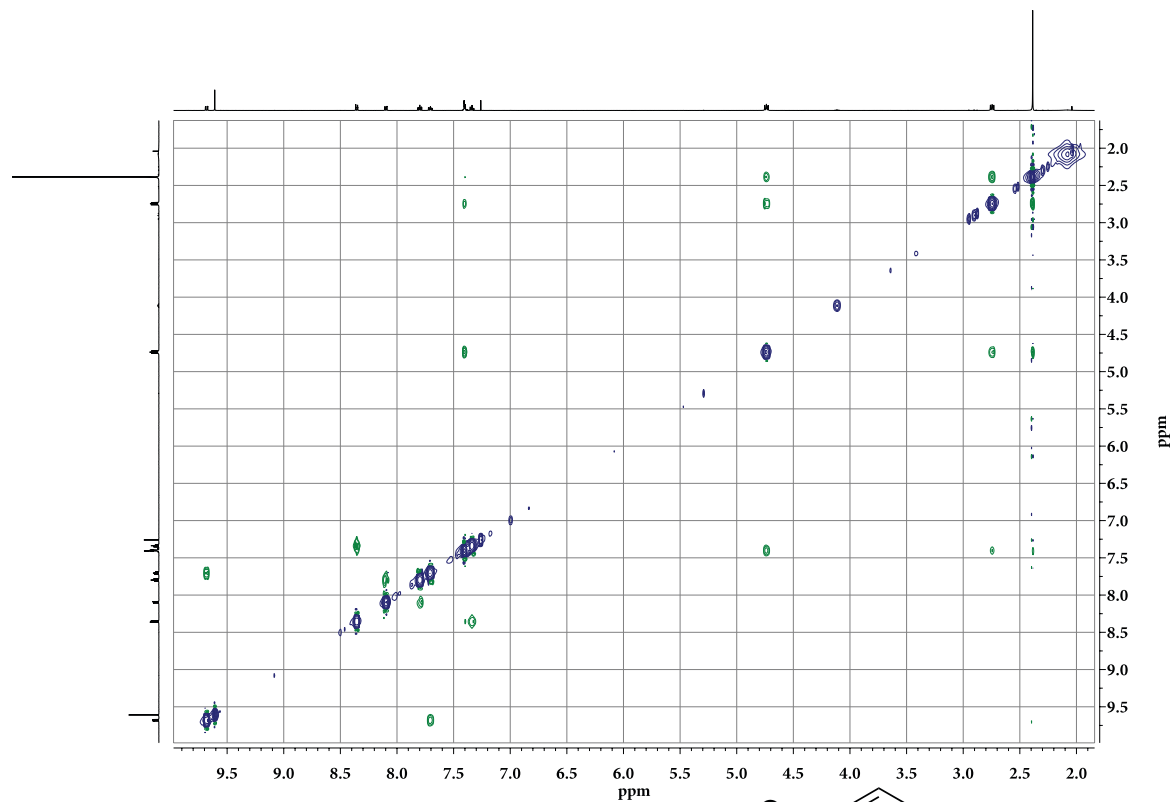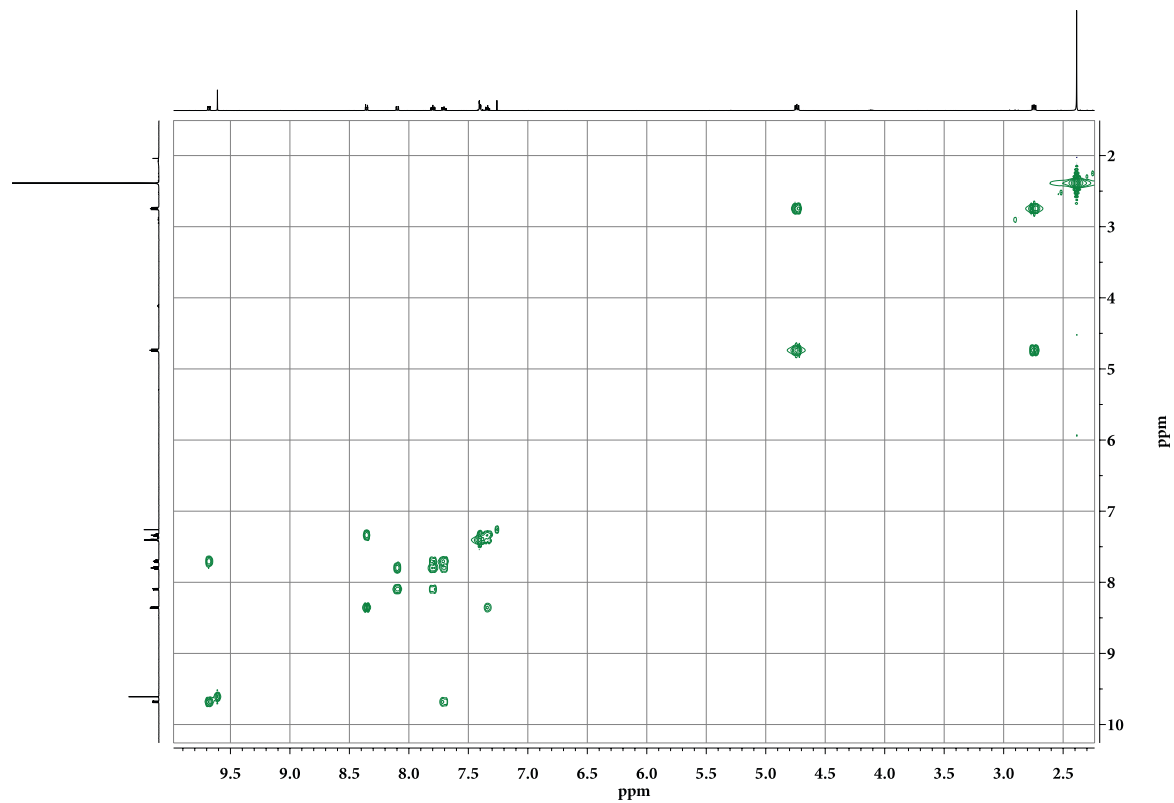
8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

78

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

78
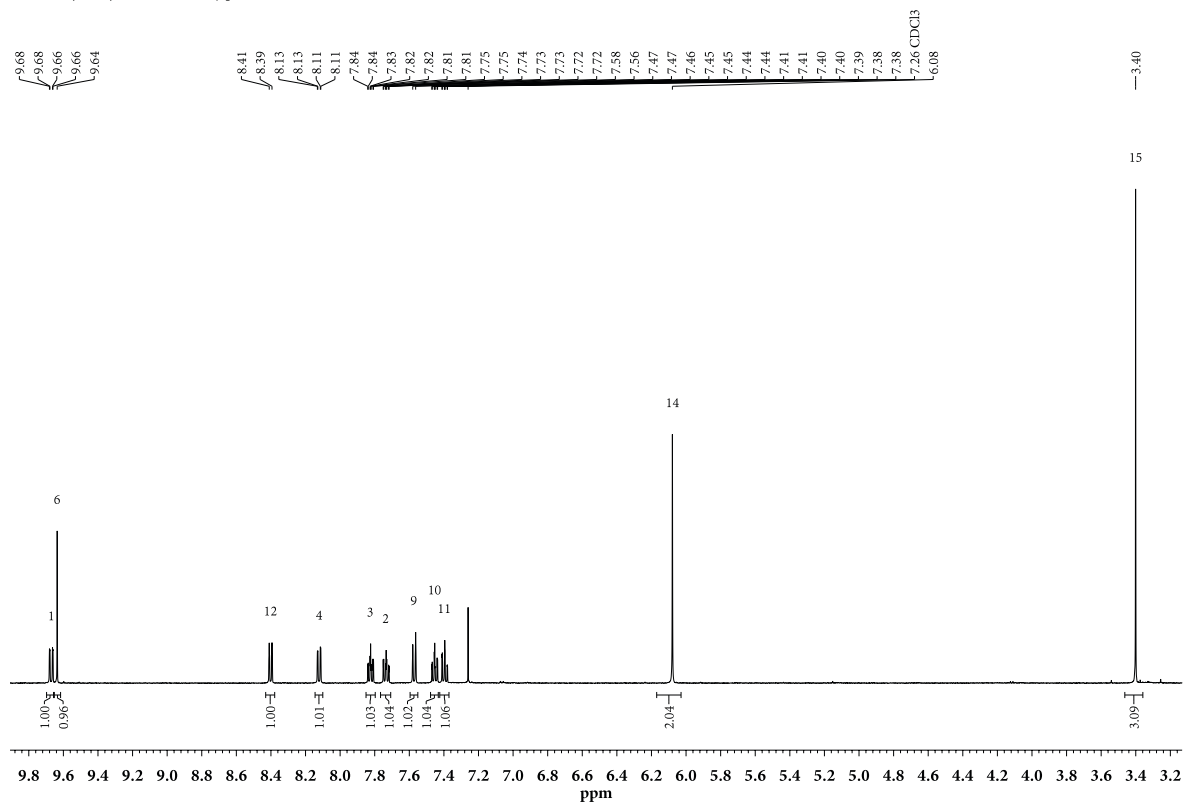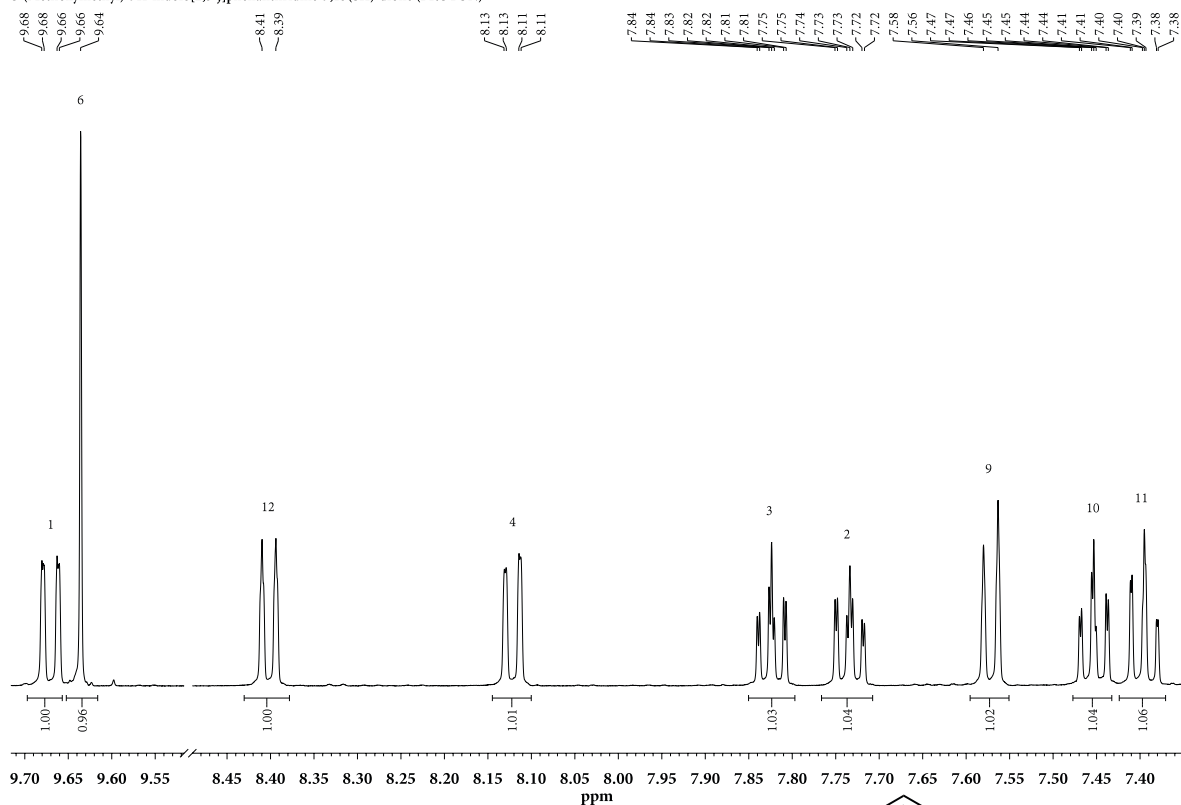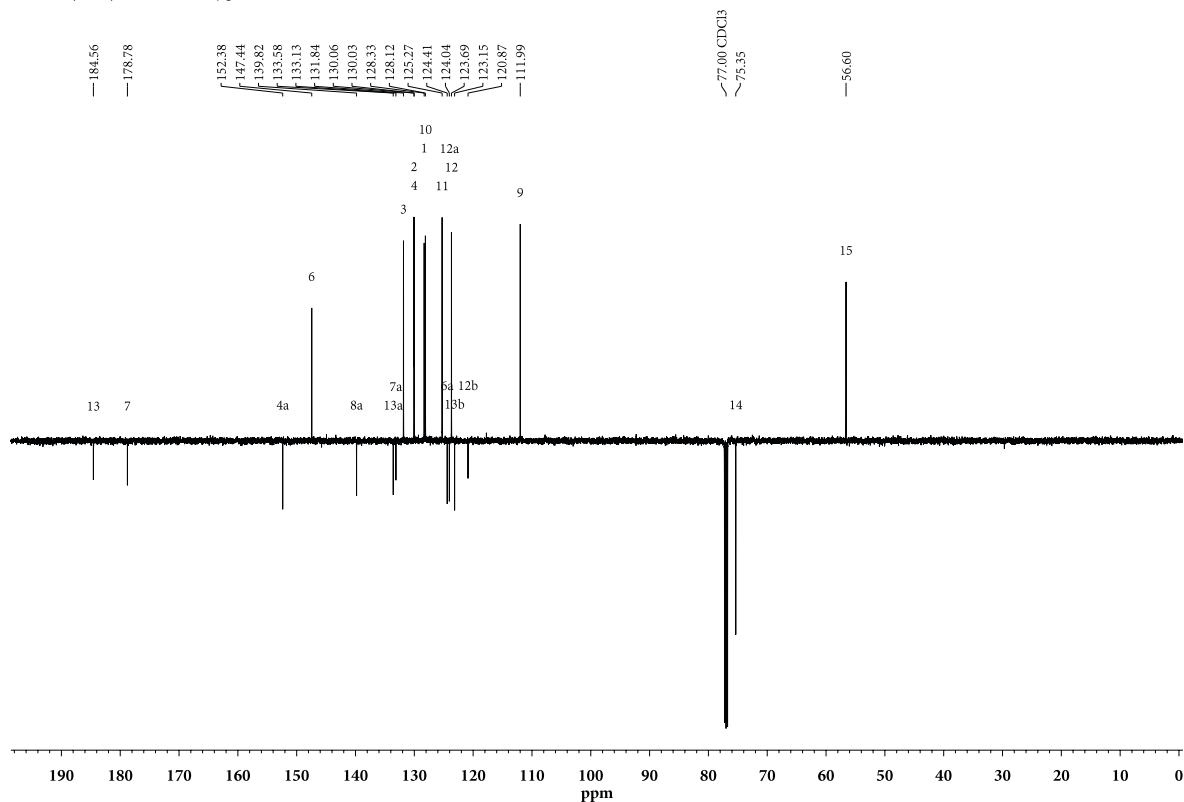
8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)
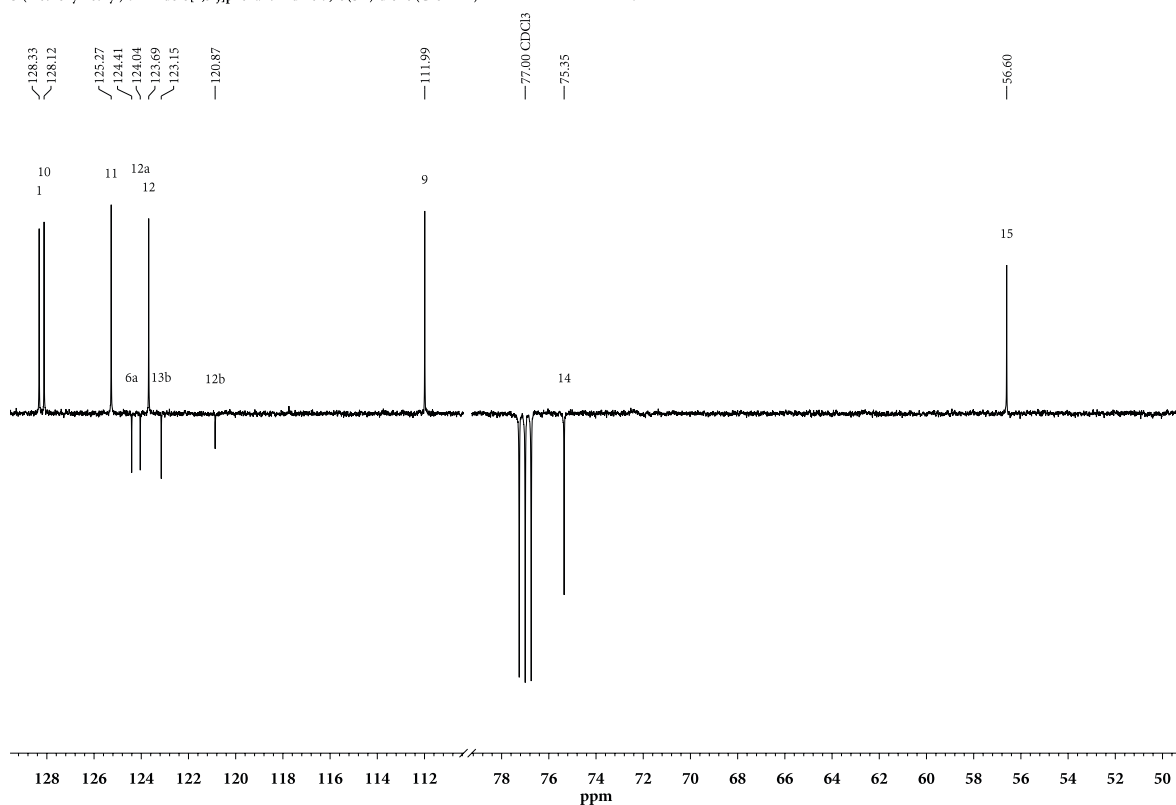
8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (HSQC)

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (HMBC)

78
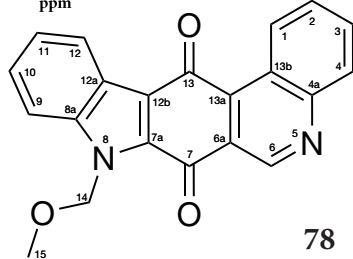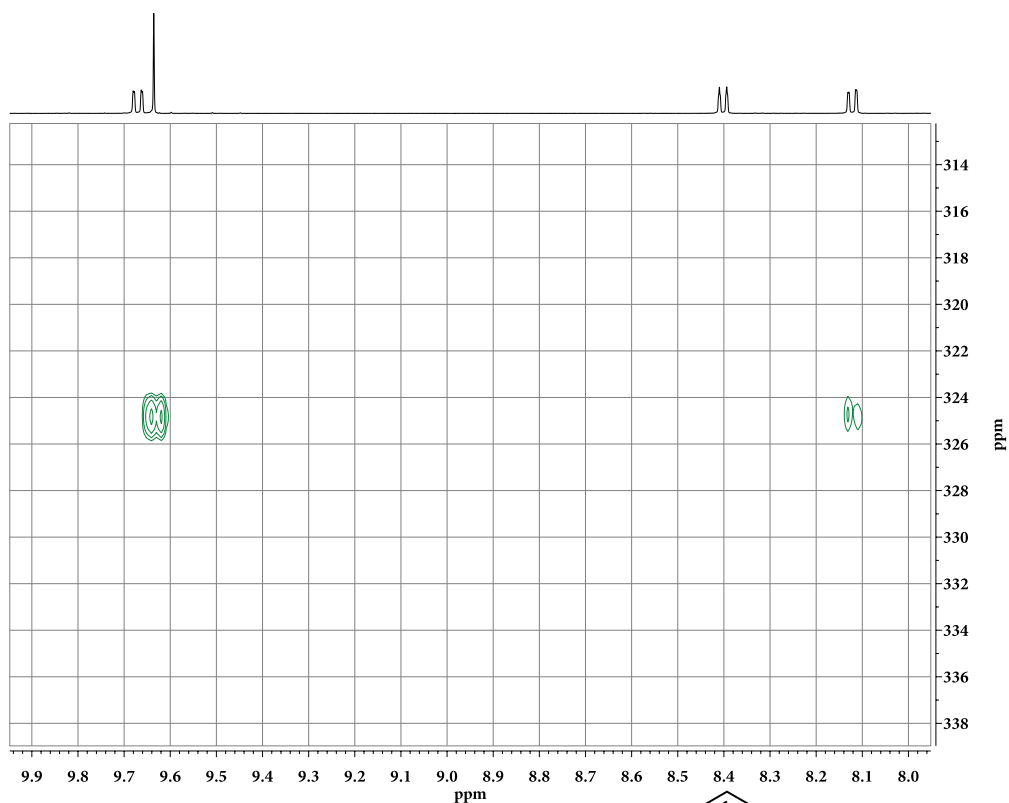
8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (15N HMBC)

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (15N HMBC)

78

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (NOESY)

78

8-(Methoxymethyl)-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (COSY)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 6:45:01 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\AK018_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-2.7min #1-152

343.1078
347.3058
349.1350
350.3527
352.2459
355.2817
358.1260
360.3234
365.0896
367.3316

$C_{21}H_{15}N_2O_3$, 343.1077

1+
343.1077

$C_{21}H_{14}N_2NaO_3$, 365.0897

1+
365.0897

78

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   11/17/2014 6:45:01 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\AK018_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.7min #1-152

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
343.1078	1	C21H15N2O3	343.1077	-0.3	291.4	1	100.00	15.5	even	ok
	2	C6H11N14O4	343.1082	1.2	360.5	2	0.01	8.5	even	ok
365.0896	1	C21H14N2NaO3	365.0897	0.1	2.0	1	100.00	15.5	even	ok

78
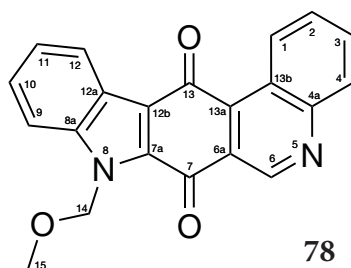
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)
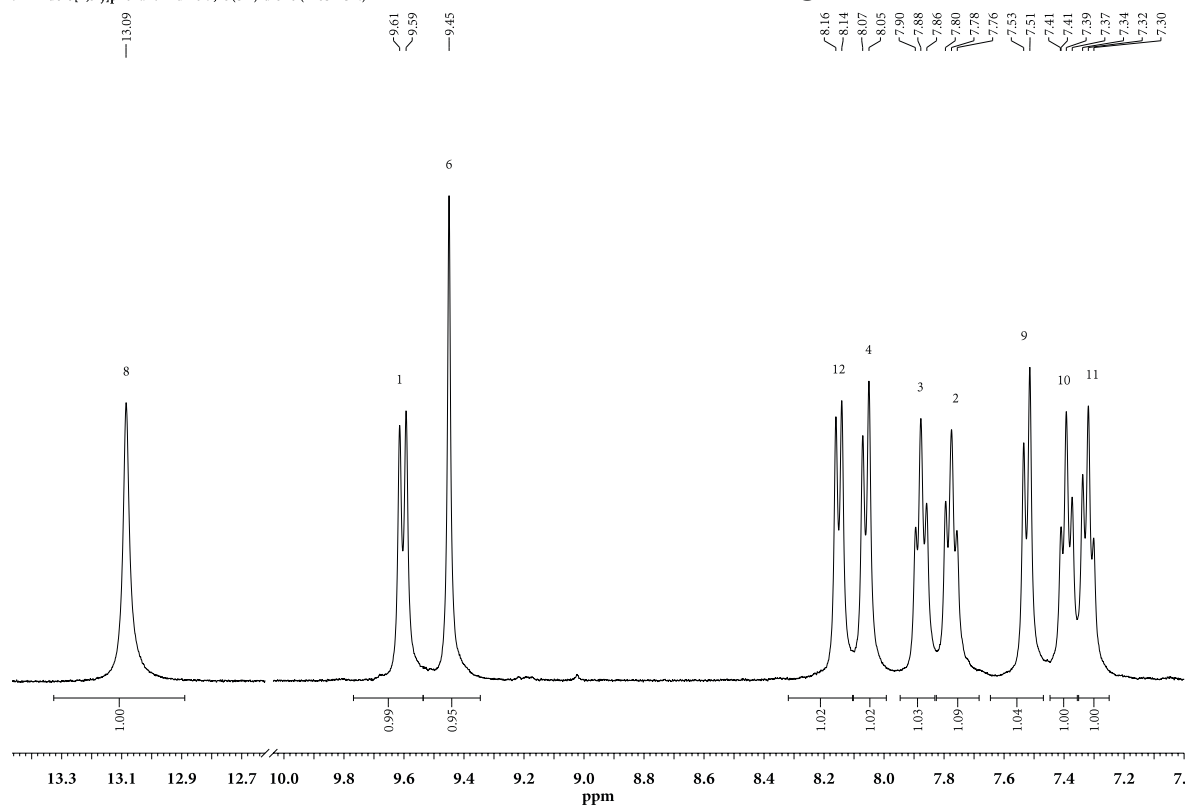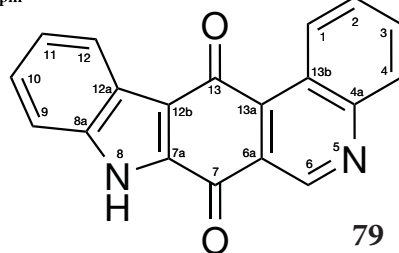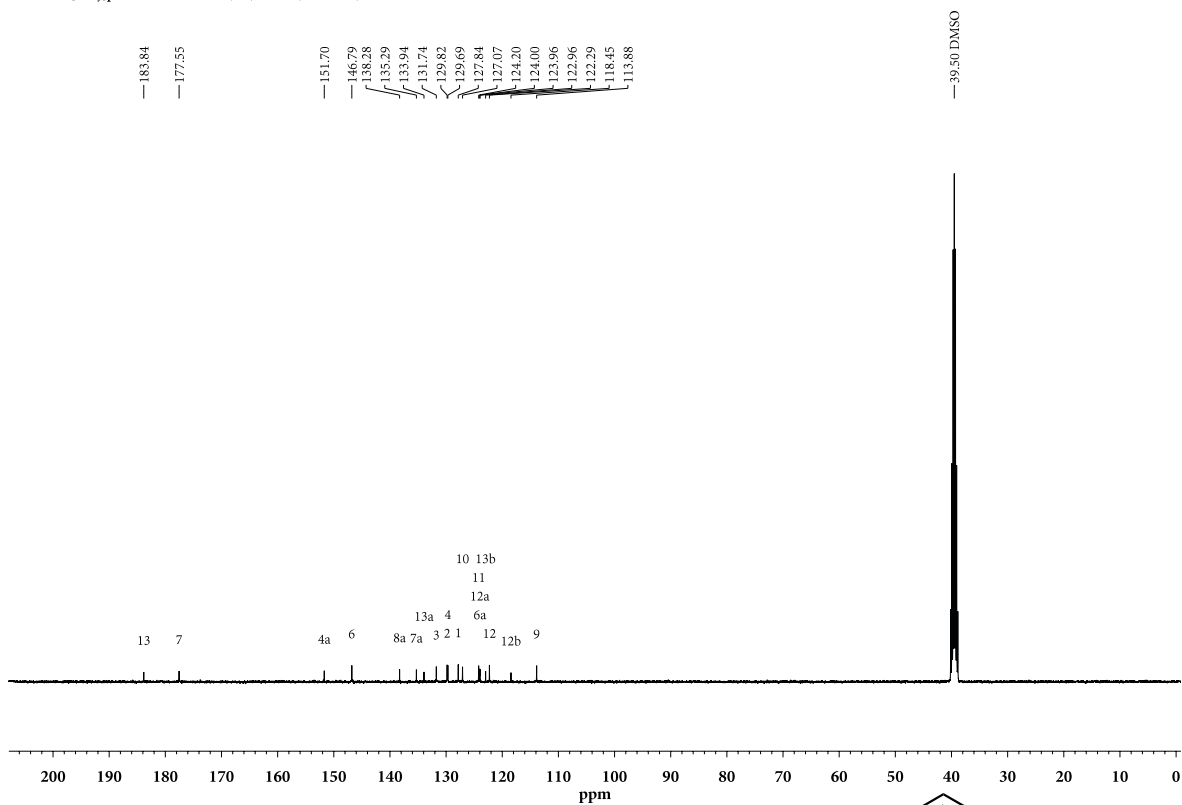
79

7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 CPD)

79

7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 CPD)
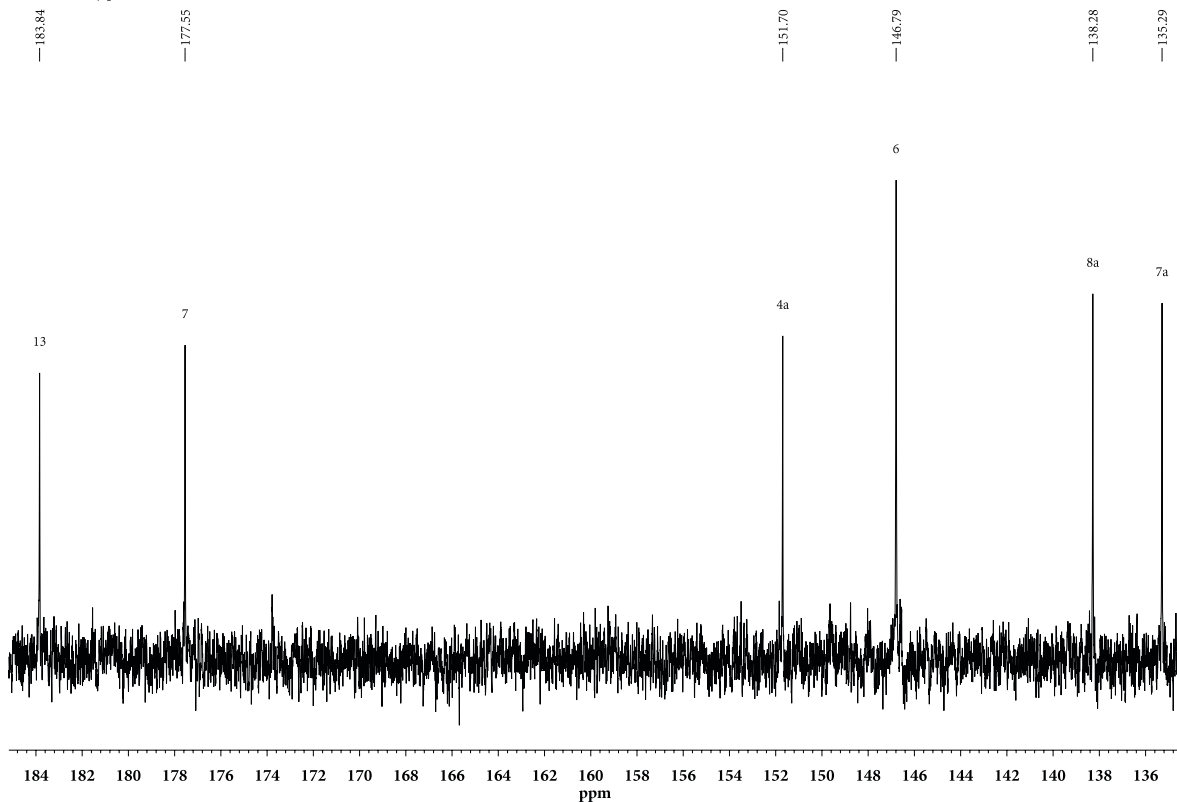
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 CPD)
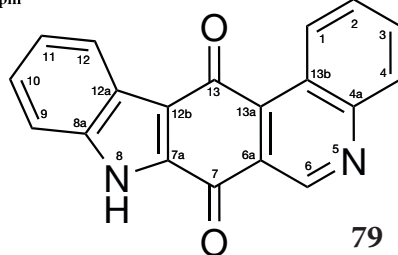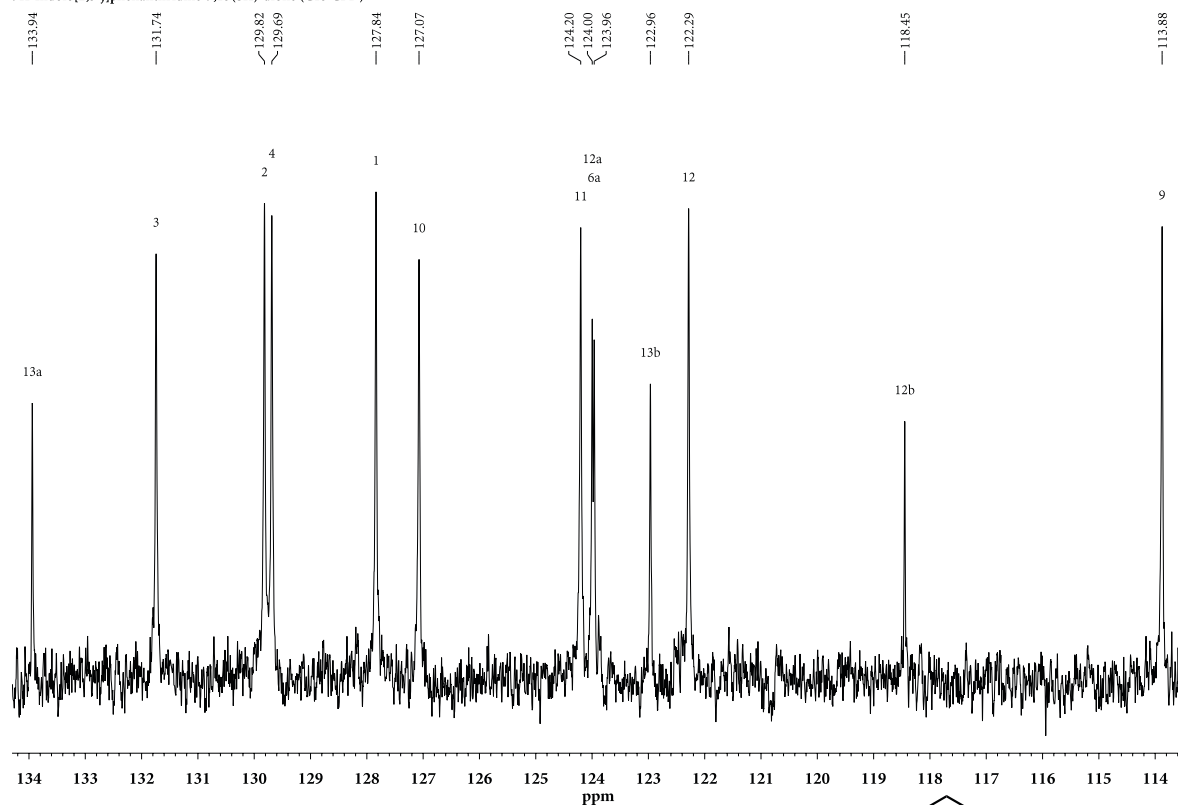
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (HSQC)

7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (HMBC)

79
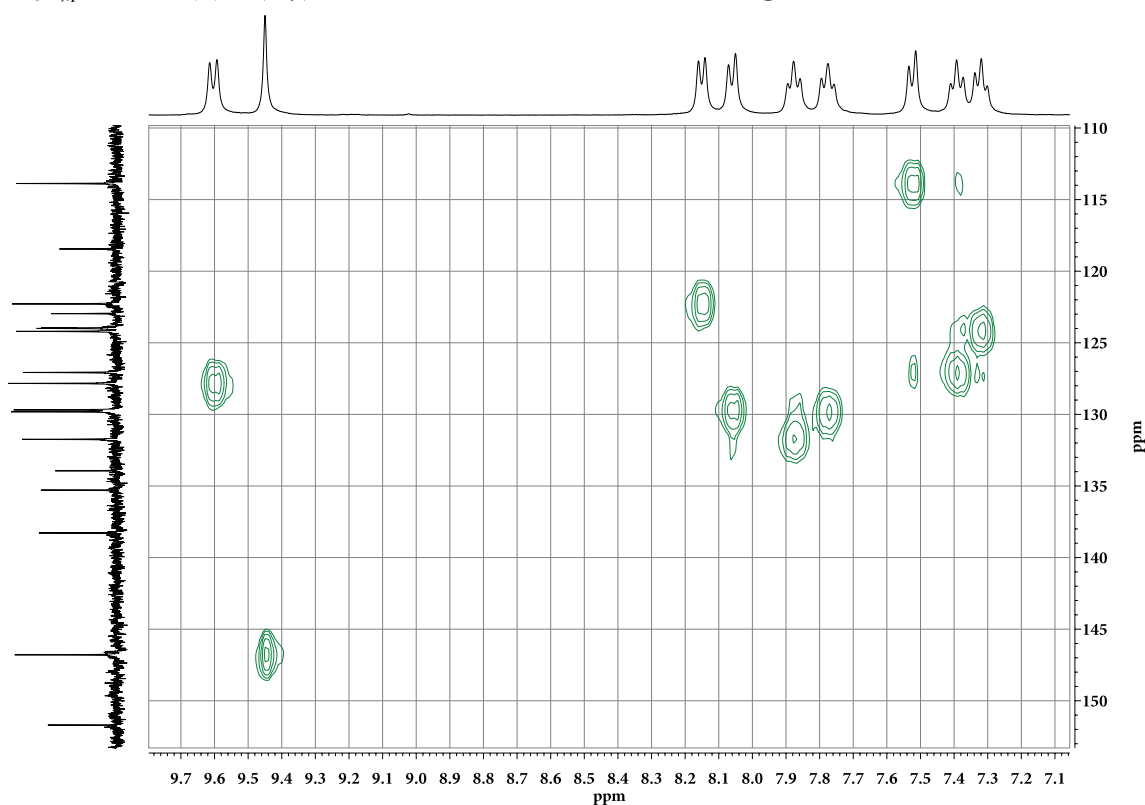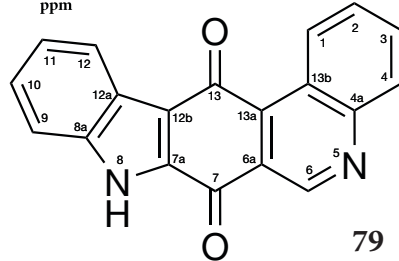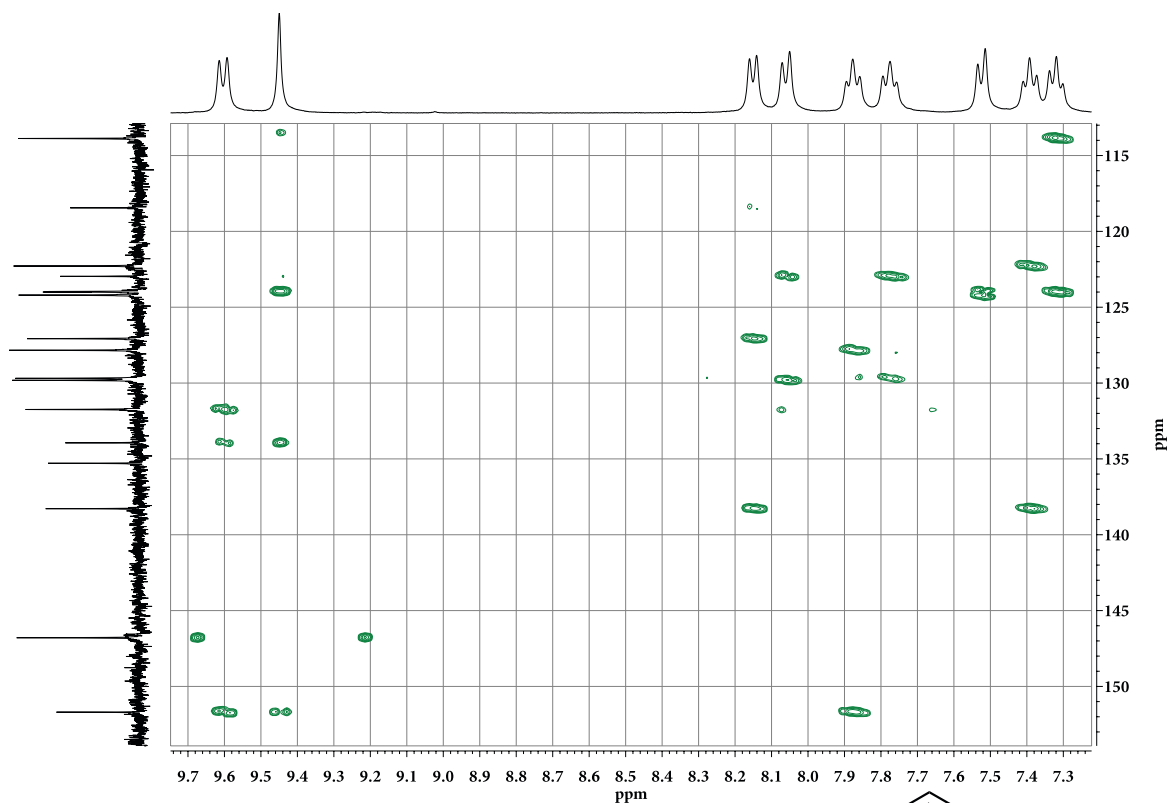
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (15N HMBC)
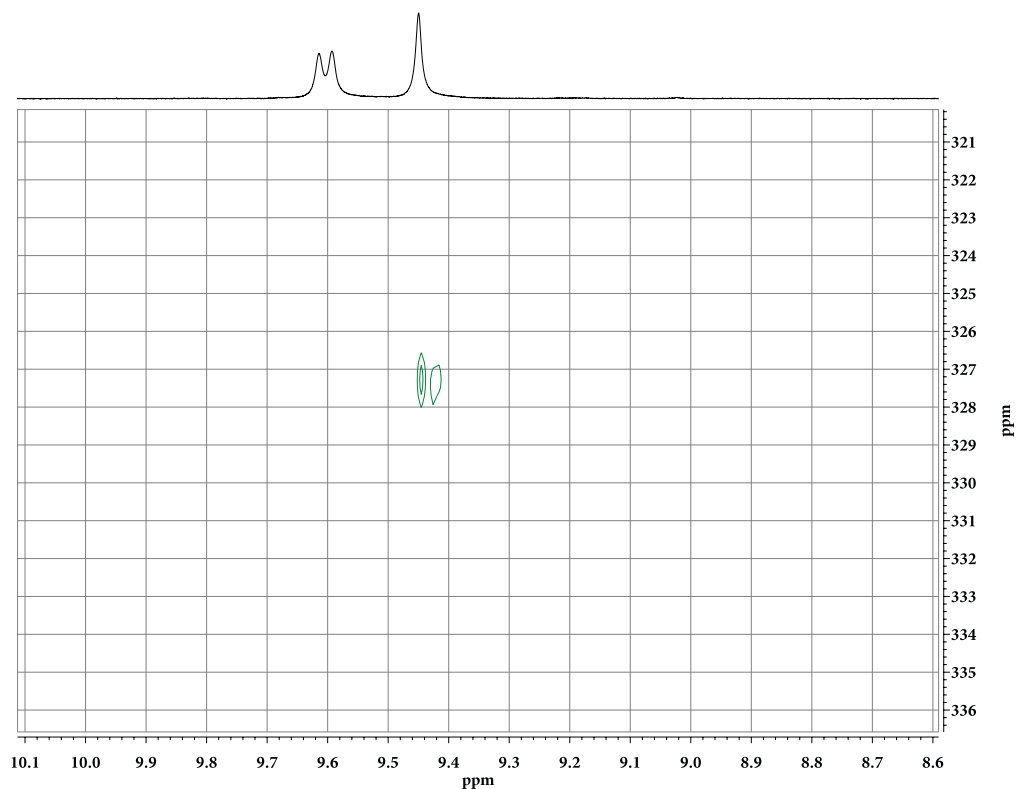
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (15N HSQC)

79
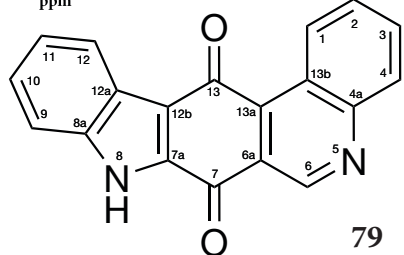
7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (COSY)

7H-Indolo[2,3-j]phenanthridine-7,13(8H)-dione (NOESY)

79

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 5:05:05 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN149_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Bruker Compass DataAnalysis 4.2   printed: 11/17/2014 5:10:28 PM   by: MZ   Page 1 of 1

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Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/17/2014 5:05:05 PM

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN149_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
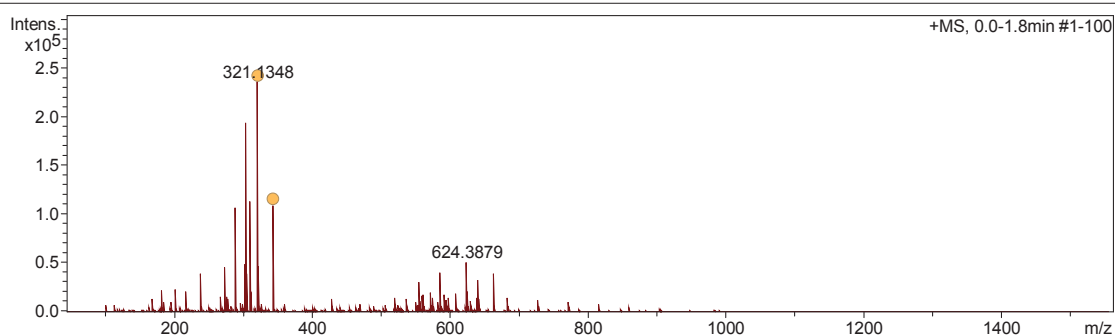Sample Name   0.1 mg/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
299.0815	1	C19H11N2O2	299.0815	-0.1	1.8	1	100.00	15.5	even	ok

79

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (PROTON)

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

80

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

184.06
178.53
152.29
147.43
139.50
133.90
132.91
131.69

3

6

13   7   4a   8a   13a 7a

184 182 180 178 176 174 172 170 168 166 164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132
ppm

80

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

129.97
129.81
128.43
127.60
124.92
124.44
124.09
123.72
123.28
119.83

4 2   1   10   11

12a   12

6a   13b   12b

130.0 129.5 129.0 128.5 128.0 127.5 127.0 126.5 126.0 125.5 125.0 124.5 124.0 123.5 123.0 122.5 122.0 121.5 121.0 120.5 120.0 119.
ppm

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (C13 APT)

—58.45

—45.79

—43.38

17,17'

15

14

ppm

67 66 65 64 63 62 61 60 59 58 57 56 55 54 53 52 51 50 49 48 47 46 45 44 43 42 41 40 39 38 37

80

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (HSQC)

ppm

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5

ppm

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (HMBC)

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (15N HMBC)

80
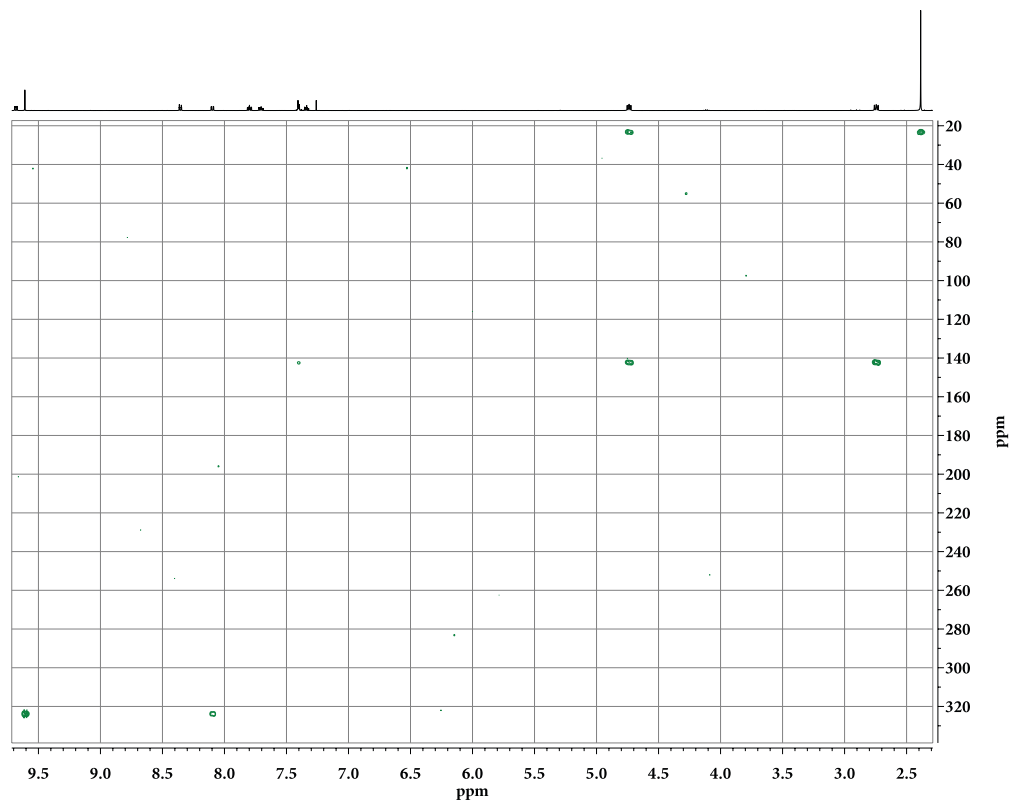
8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (NOESY)

8-[2-(Dimethylamino)ethyl]-7H-indolo[2,3-j]phenanthridine-7,13(8H)-dione (COSY)

80

Generic Display Report

Analysis Info

		Acquisition Date	11/4/2014 9:58:40 AM
Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\AK024_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer\Thomas_N\141104\AK024_DI_ESI_HRMS.d	Acquisition Date	11/4/2014 9:58:40 AM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	approx. 10 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive		Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V		Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V		Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V		Set Divert Valve	Waste
		Set Corona	0 nA		Set APCI Heater	0 °C

+MS, 0.0-1.7min #1-96

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
370.1555	1	C23H20N3O2	370.1550	-1.3	2.2	1	100.00	15.5	even	ok
392.1374	1	C23H19N3NaO2	392.1369	-1.0	8.4	1	100.00	15.5	even	ok

80

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

81

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

81

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 APT)

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (HSQC)

81

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (HMBC)

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (15N HMBC)

81

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (15N HMBC)

81

6-[2-(Dimethylamino)ethyl]-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (HOESY)

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN178_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 2:44:59 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:100 in MeOH	Instrument	maXis HD
Comment			

81

Mass Spectrum SmartFormula Report
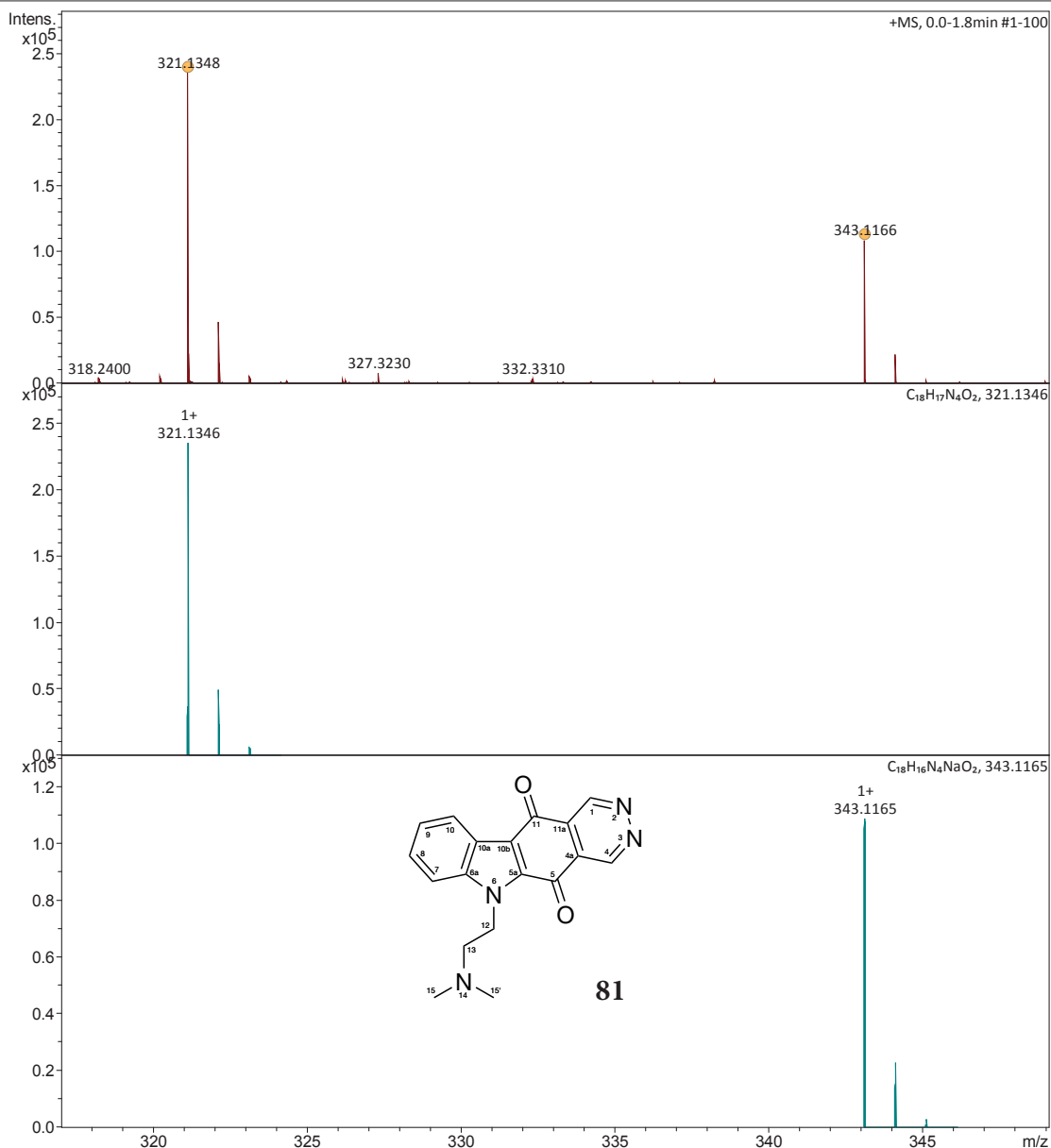
Analysis Info

Acquisition Date   1/12/2015 2:44:59 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN178_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:100 in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.8min #1-100

321.1348

624.3879

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
321.1348	1	C18H17N4O2	321.1346	-0.6	8.4	1	100.00	12.5	even	ok
343.1166	1	C18H16N4NaO2	343.1165	-0.1	4.1	1	100.00	12.5	even	ok

81

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

82

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

9.91
9.91
9.86
9.86

8.43
8.43
8.43
8.41
8.41
8.41

1

4

10

1.00

1.01

1.06

).96 9.94 9.92 9.90 9.88 9.86 9.84 9.82 9.80 9.78 9.76 9.74 9.72 9.70 9.68   8.64 8.62 8.60 8.58 8.56 8.54 8.52 8.50 8.48 8.46 8.44 8.42 8.40 8.38 8.36 8.34 8.3
ppm

82

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

7.69
7.69
7.67
7.67
7.66

7.59
7.57
7.57
7.55

7.50
7.50
7.48
7.48

7.26 CDCl3

7

8

9

1.07

1.08

1.10

7.76 7.74 7.72 7.70 7.68 7.66 7.64 7.62 7.60 7.58 7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.2
ppm

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 APT)

82

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 APT)

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (HSQC)

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (HMBC)

82

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (15N HMBC)

6-(Methoxymethyl)-5H-pyridazino[4,5-b]carbazole-5,11(6H)-dione (15N HMBC)

82

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN179_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 2:13:01 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:10 in MeOH	Instrument	maXis HD
Comment			

82

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN179_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:10 in MeOH
Comment	

Acquisition Date 1/12/2015 2:13:01 PM

Operator	MZ	
Instrument	maXis HD	1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
294.0874	1	C16H12N3O3	294.0873	-0.2	13.9	1	100.00	12.5	even	ok
316.0695	1	C16H11N3NaO3	316.0693	-0.9	6.1	1	100.00	12.5	even	ok

82

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

—9.80
—9.80

—9.77
—9.77

1

4

0.96

0.87

9.840   9.830   9.820   9.810   9.800   9.790   9.780   9.770   9.760   9.750   9.740   9.730   9.720   9.710
ppm

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (PROTON)

—8.21
—8.19

—7.64
—7.62

7.53
7.52
7.51
7.51
7.50
7.50
7.49
7.49
7.45
7.45
7.43
7.43
7.41
7.41

83

7

8

9

10

1.00

1.20

1.27

1.15

8.35   8.30   8.25   8.20   8.15   8.10   8.05   8.00   7.95   7.90   7.85   7.80   7.75   7.70   7.65   7.60   7.55   7.50   7.45   7.40   7.35   7.30   7.25
ppm

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 CPD)

179.45
176.92

146.49
145.69
138.29
136.55
127.76
126.42
126.11
124.82
123.40
122.30
117.28
114.12

39.50 DMSO

10a
4a,11a
4a,11a
4   5a
1   6a   8 9 10   10b 7
11 5

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

83

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 CPD)

179.45
176.92

146.49
145.69

138.29
136.55

127.76
126.42
126.11
124.82
123.40
122.30

117.28

114.12

11   5

1
4

6a

5a

8
4a,11a
4a,11a

9

10
10a

10b

7

180   175   170   165   160   155   150   145   140   135   130   125   120   115
ppm

694

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (C13 CPD)

146.49
145.69
138.29
136.55
127.76
126.42
126.11
124.82
123.40
122.30
117.28
114.12

1
4
6a
5a
8
4a,11a
4a,11a
9
10
10a
7
10b

148 147 146 145 144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127 126 125 124 123 122 121 120 119 118 117 116 115 114 113
ppm

83

5H-Pyridazino[4,5-b]carbazole-5,11(6H)-dione (HSQC)

110
115
120
125
130
135
140
145
150
ppm

9.9 9.7 9.5 9.3 9.1 8.9 8.7 8.5 8.3 8.1 7.9 7.7 7.5 7.3
ppm

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 1:43:34 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN180_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD
Comment			

Intens.
x10^5

+MS, 0.0-2.1min #1-118

272.0434

276.2300

278.2456

267.1106

271.1055

273.0462

277.2331

279.2488

268.1137

x10^5

$C_{14}H_7N_3NaO_2$, 272.0430

1+
272.0430

1+
273.0460

1+
274.0485

83

268   270   272   274   276   278   280   m/z

Bruker Compass DataAnalysis 4.2   printed: 1/12/2015 1:57:24 PM   by: MZ   Page 1 of 1

Mass Spectrum SmartFormula Report

Analysis Info
Acquisition Date   1/12/2015 1:43:34 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN180_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar		
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C		
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min		
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste		
		Set Corona	0 nA	Set APCI Heater	0 °C		

+MS, 0.0-2.1min #1-118
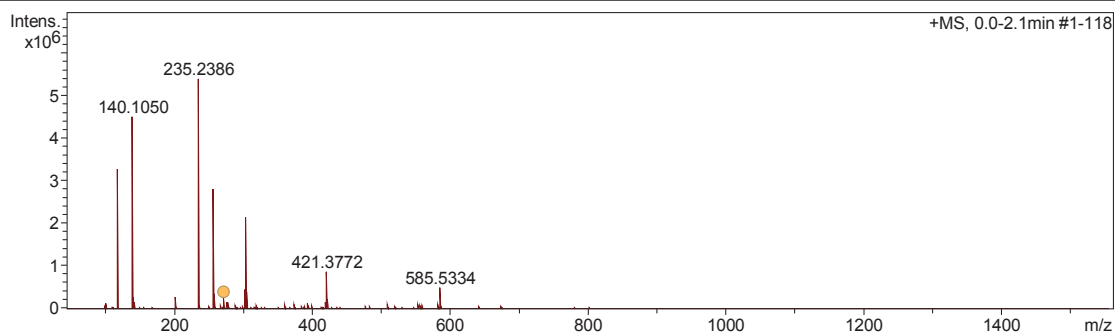
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
272.0434	1	C14H7N3NaO2	272.0430	-1.2	10.7	1	100.00	12.5	even	ok
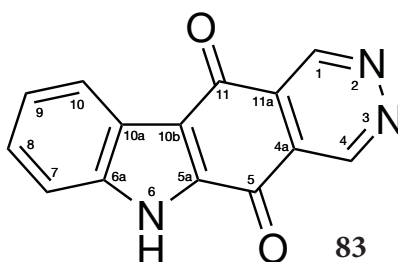
83
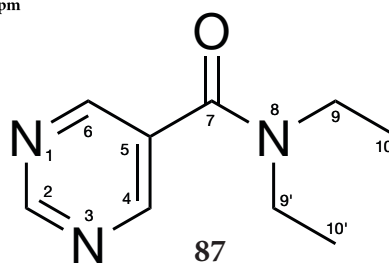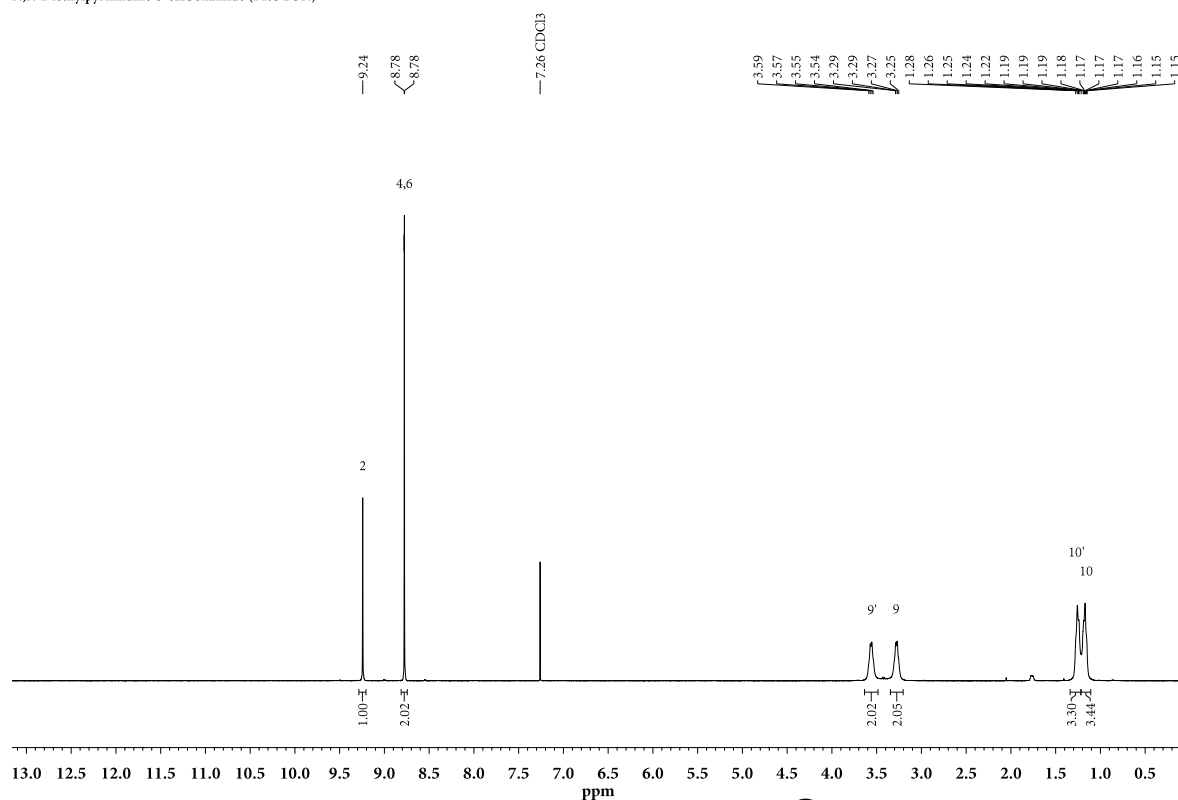
N,N-Diethylpyrimidine-5-carboxamide (PROTON)
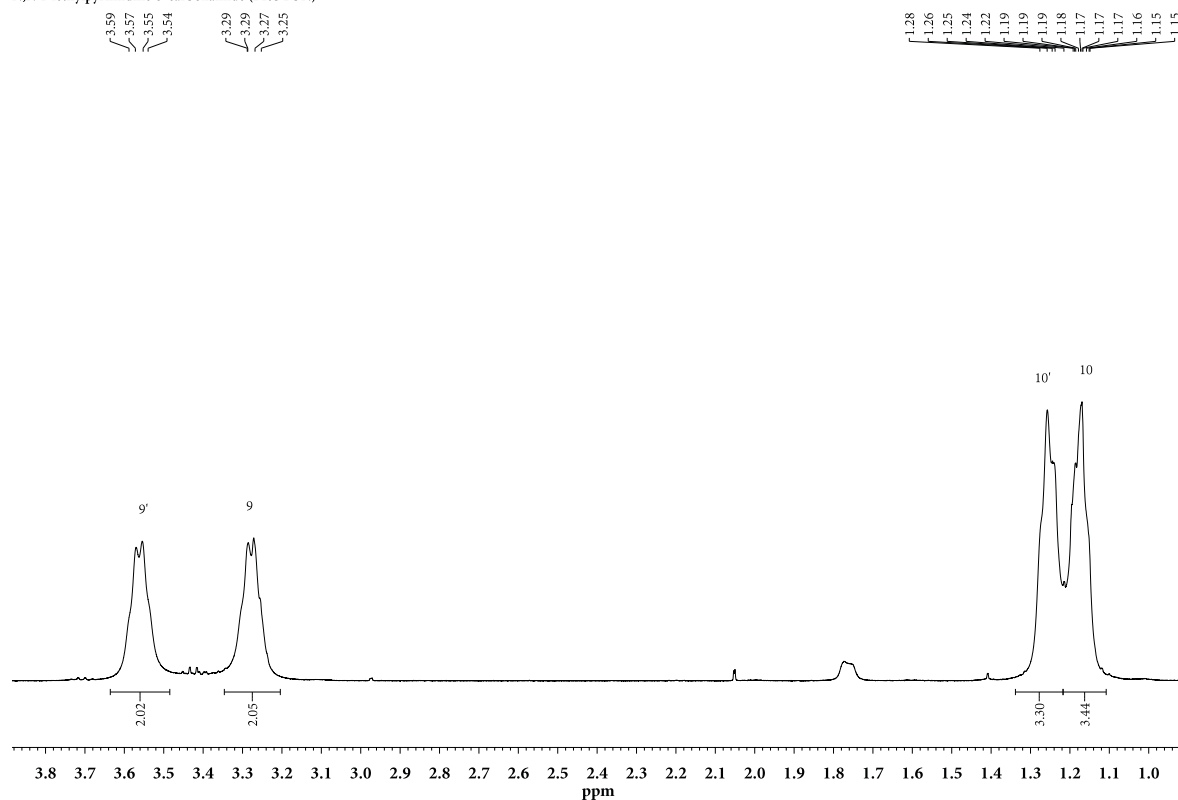
N,N-Diethylpyrimidine-5-carboxamide (PROTON)

N,N-Diethylpyrimidine-5-carboxamide (C13 CPD)

N,N-Diethylpyrimidine-5-carboxamide (HSQC)

87
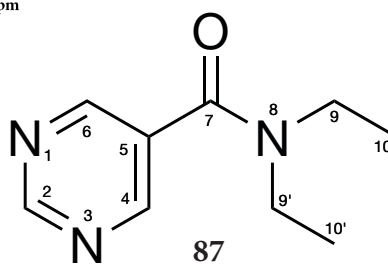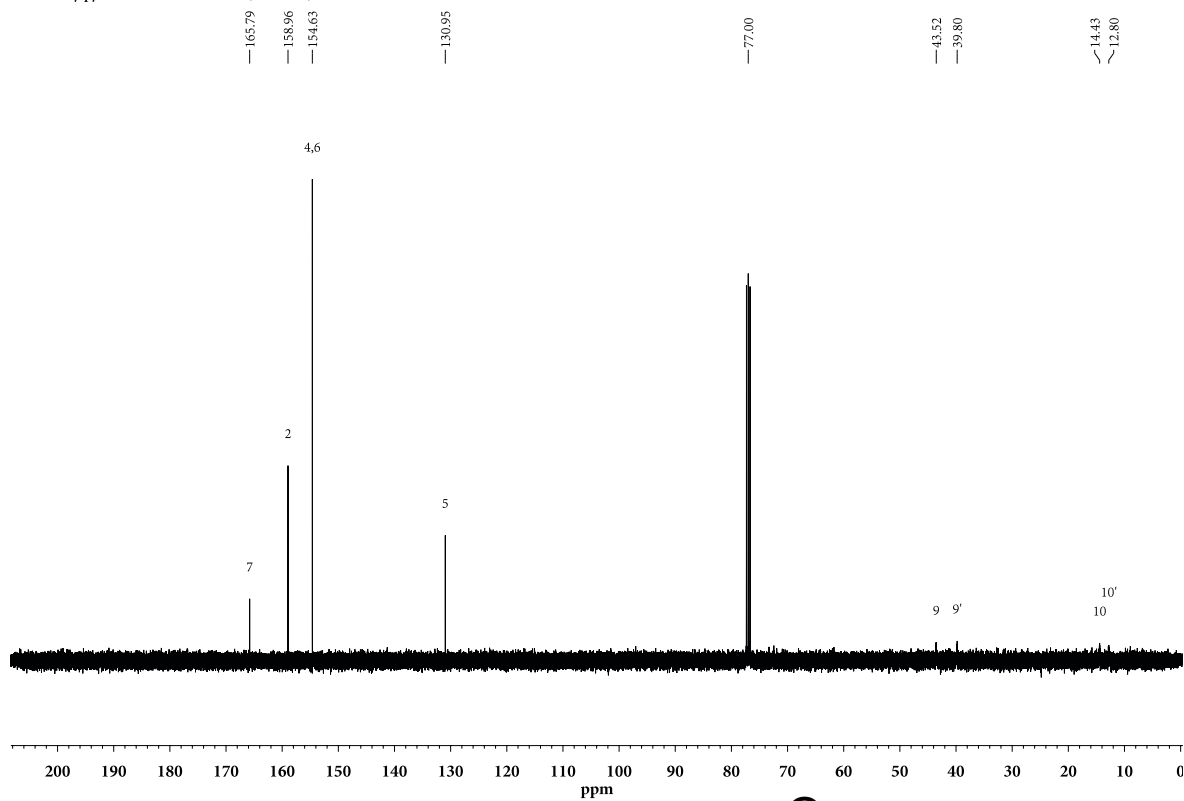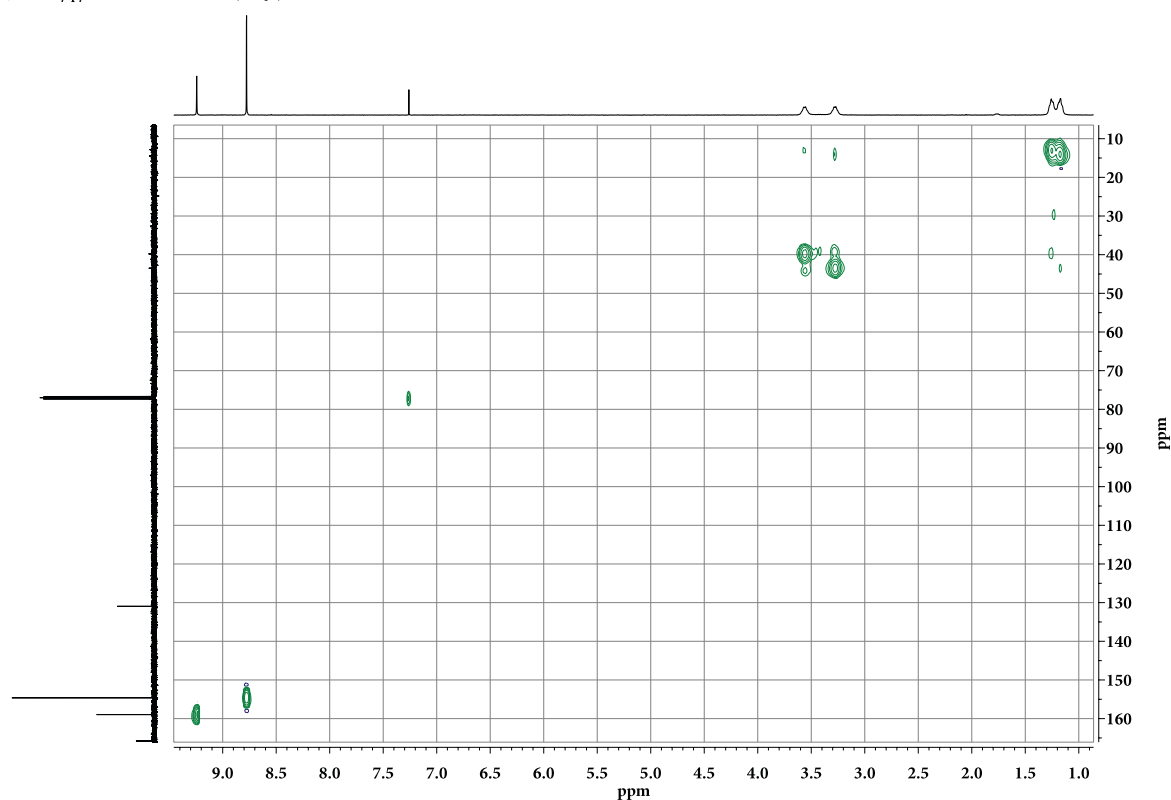
N,N-Diethylpyrimidine-5-carboxamide (HMBC)
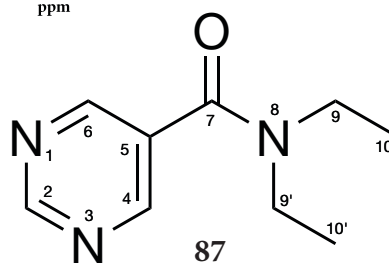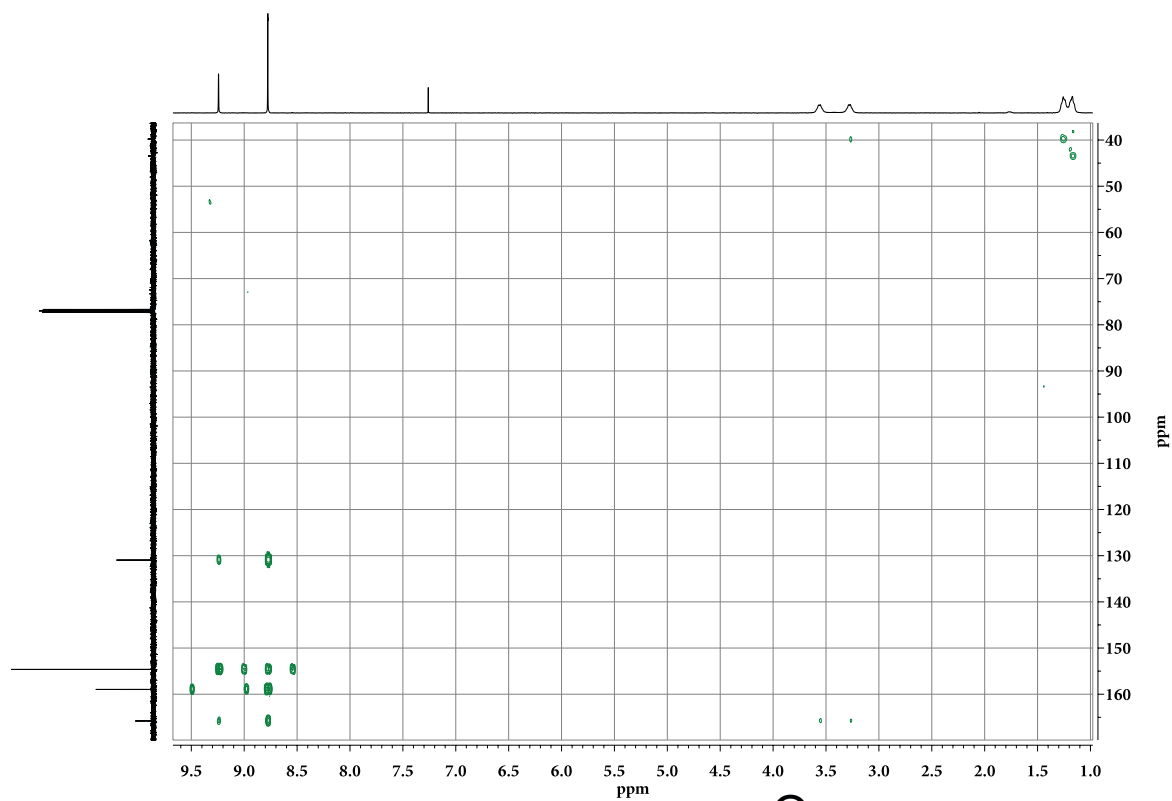
N,N-Diethylpyrimidine-5-carboxamide (15N HMBC)
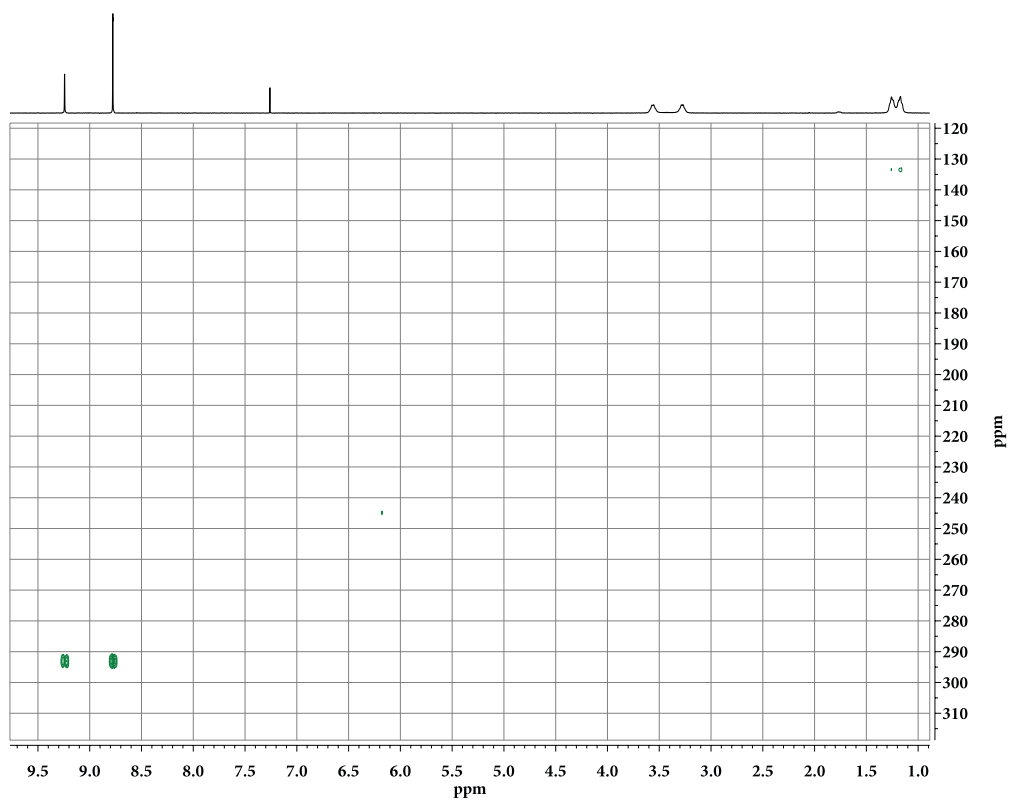
N,N-Diethylpyrimidine-5-carboxamide (NOESY)

87
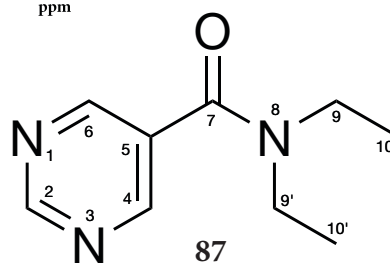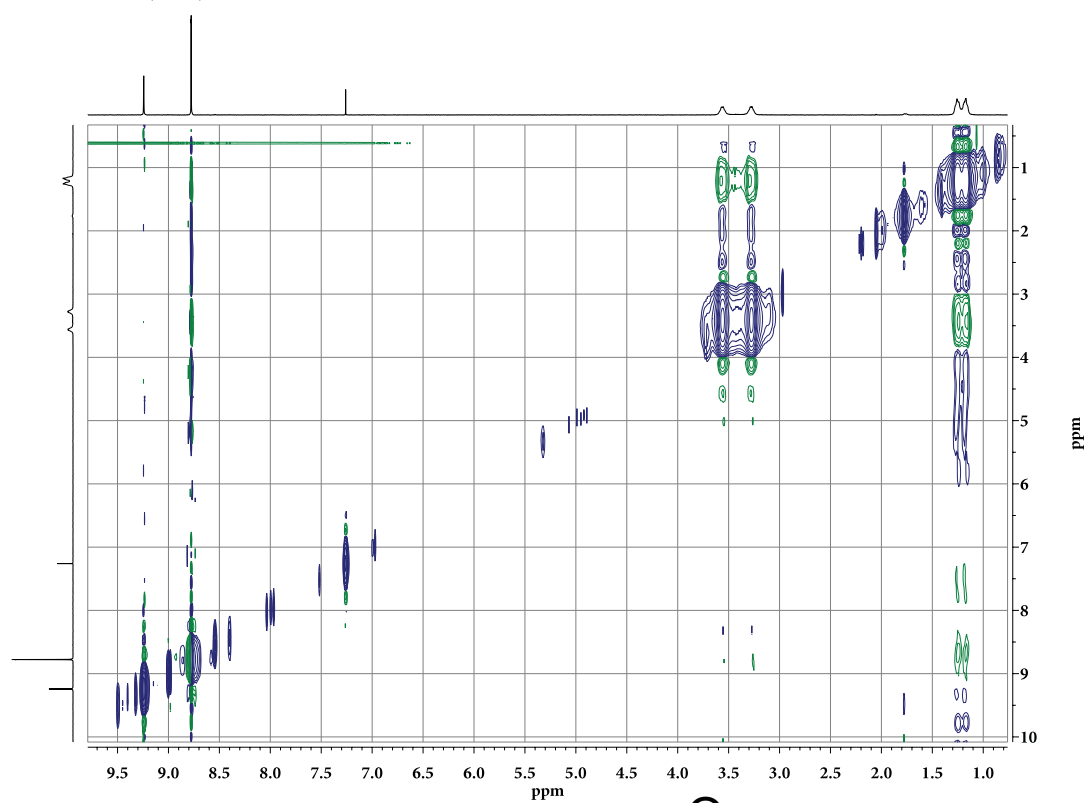
3,6-Dimethoxy-2-nitrobenzaldehyde (PROTON)

88
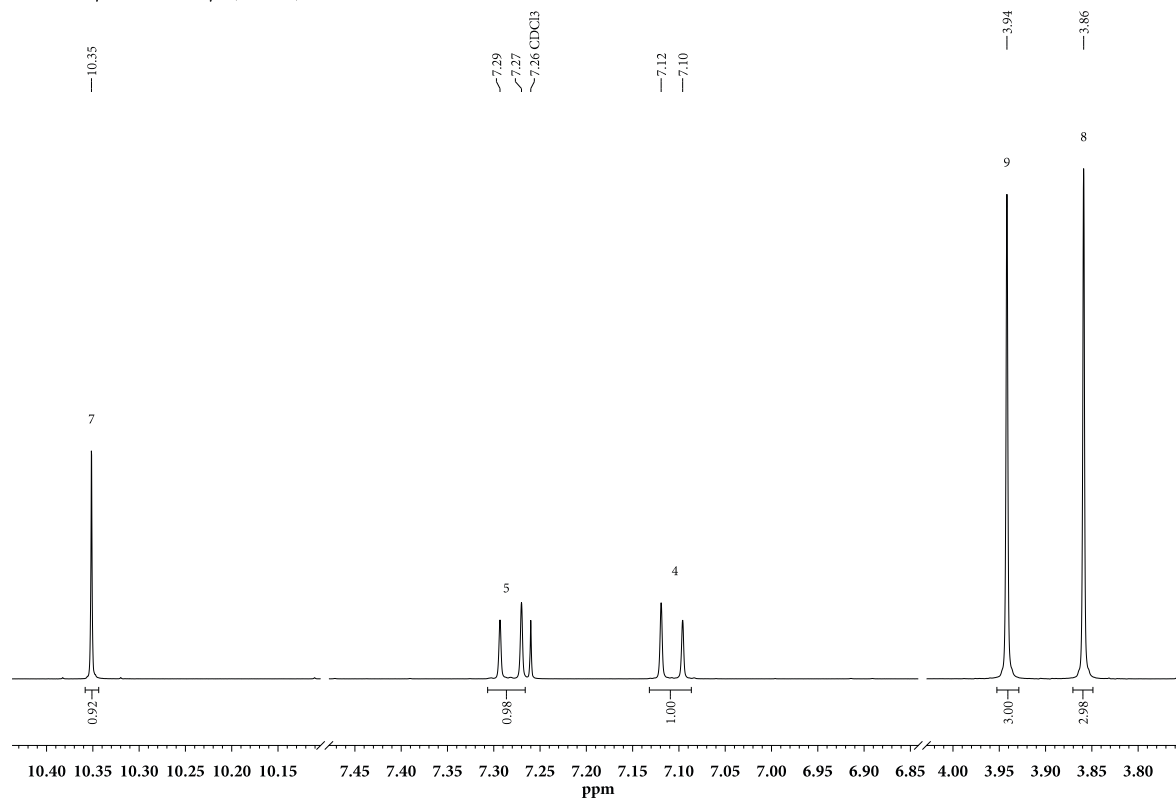
3,6-Dimethoxy-2-nitrobenzaldehyde (C13 CPD)
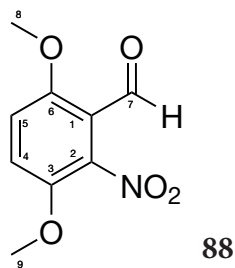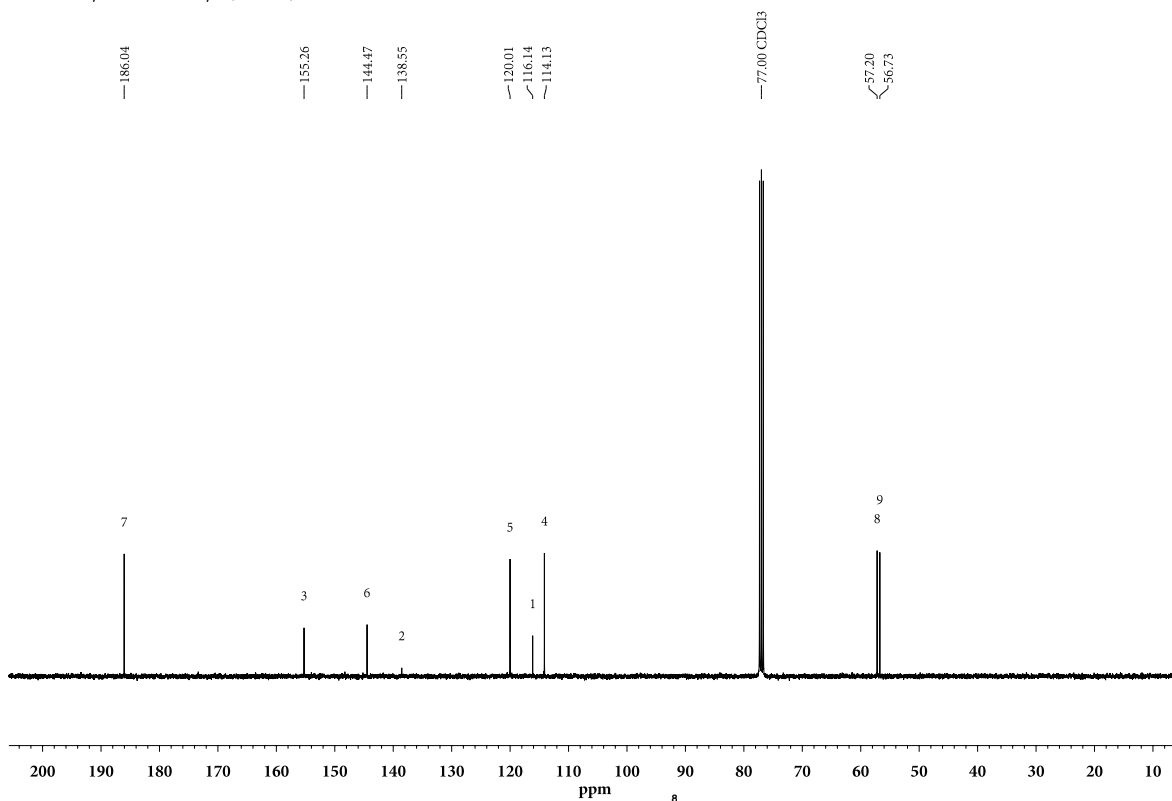
88

3,6-Dimethoxy-2-nitrobenzaldehyde (HSQC)
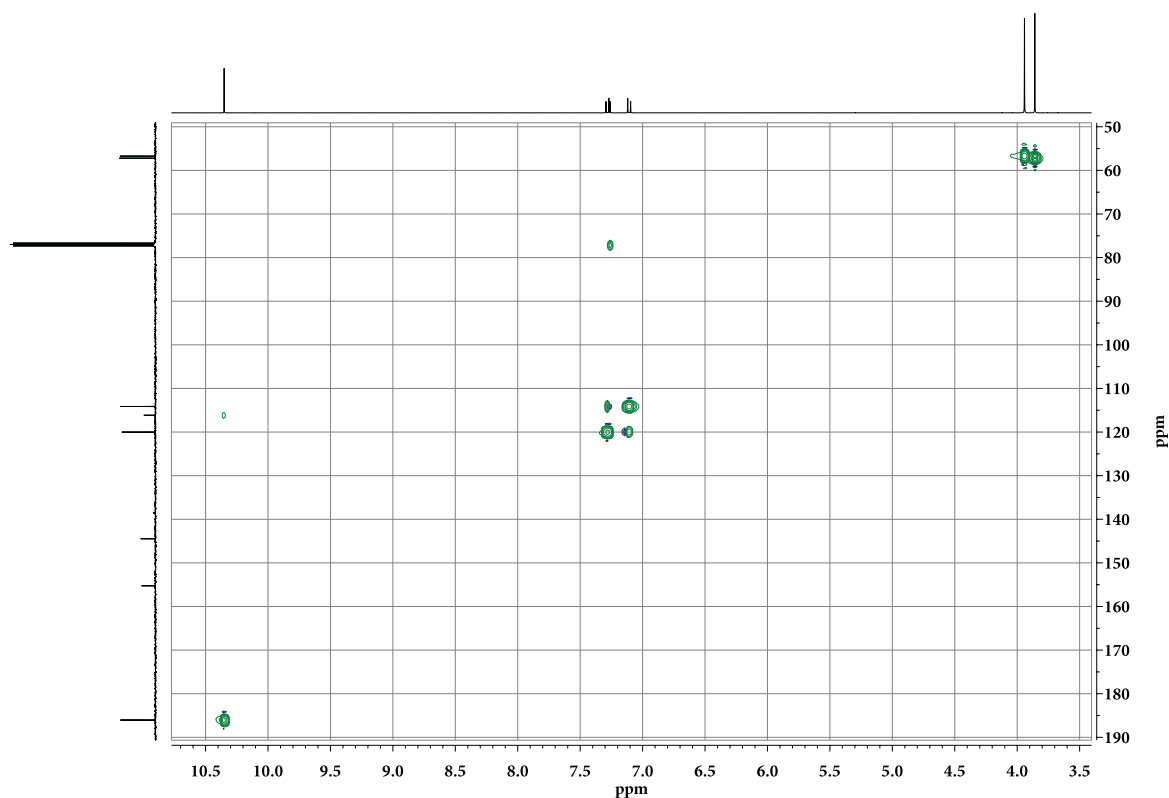
3,6-Dimethoxy-2-nitrobenzaldehyde (HMBC)

88
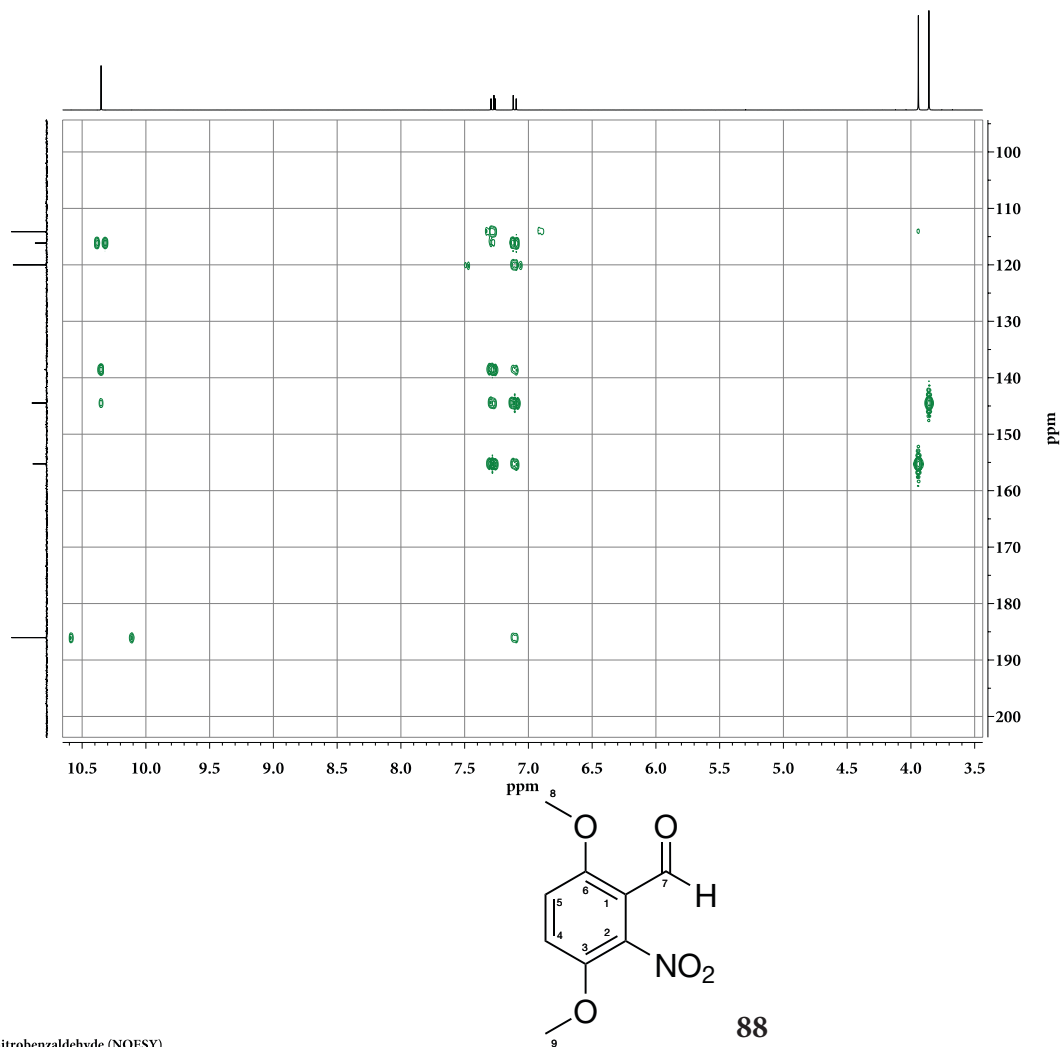
3,6-Dimethoxy-2-nitrobenzaldehyde (NOESY)
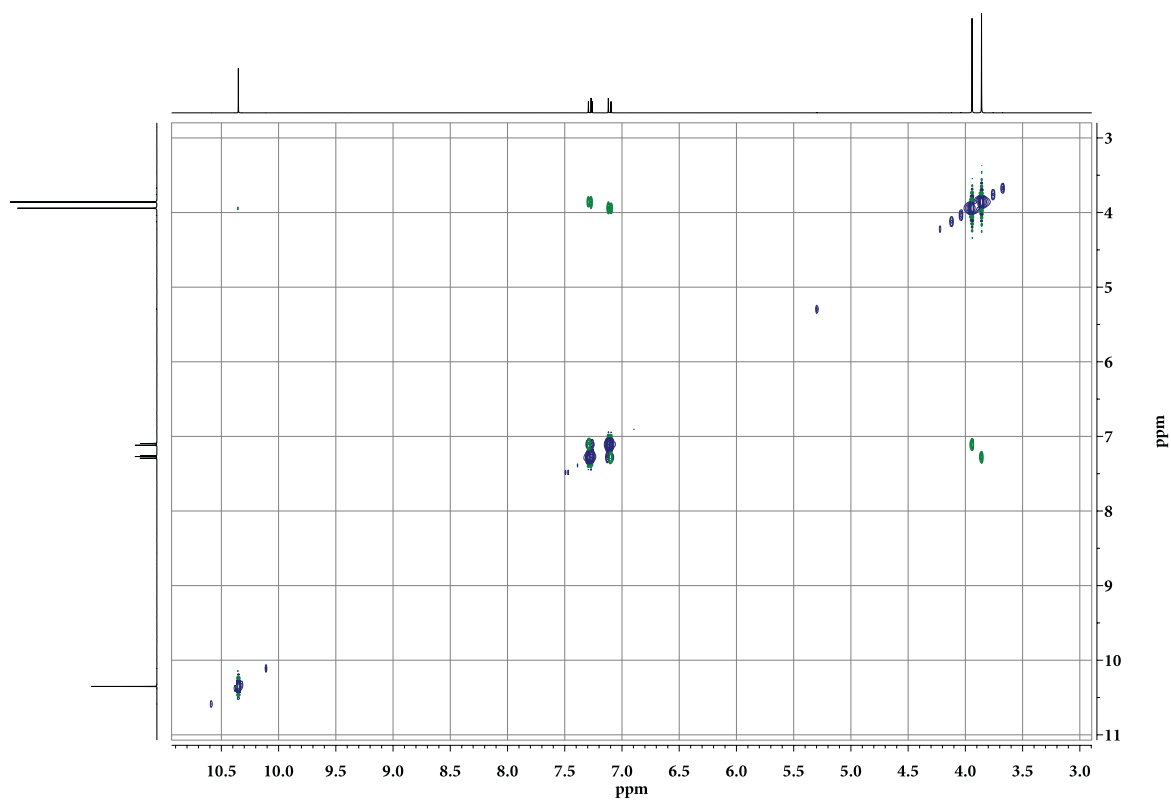
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (PROTON)
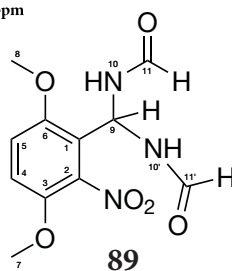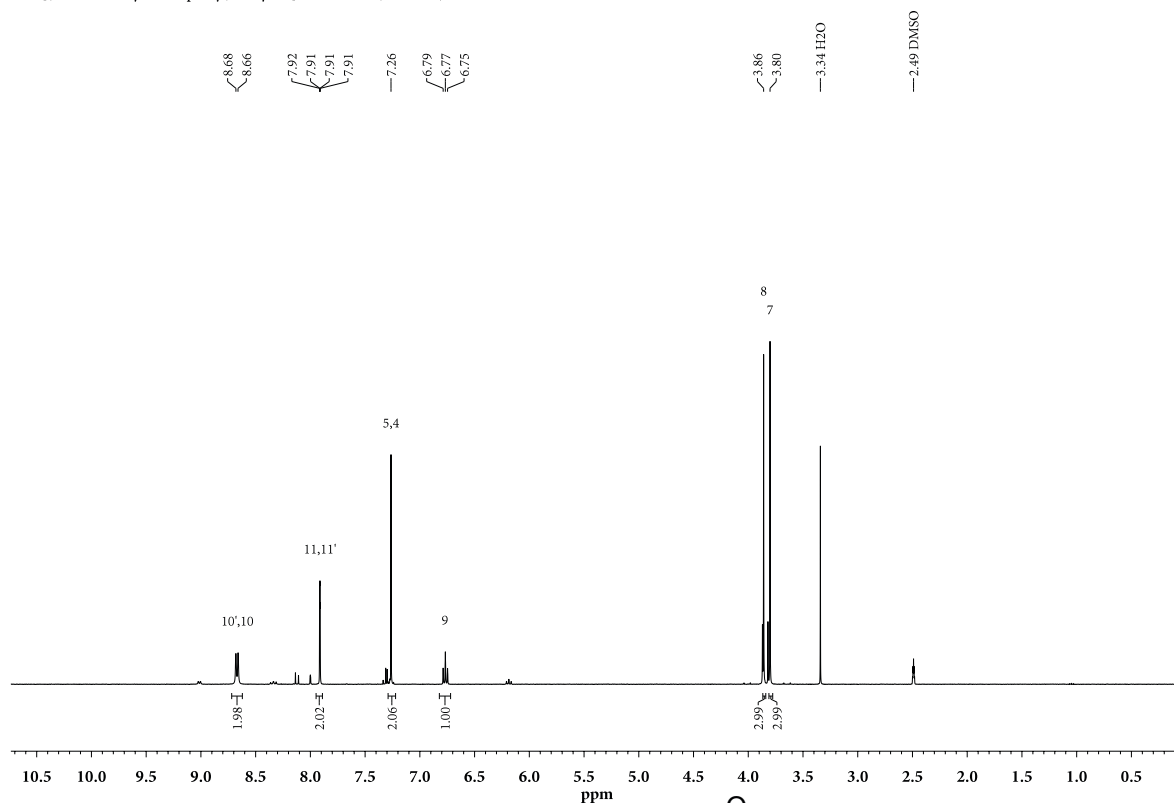
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (PROTON)
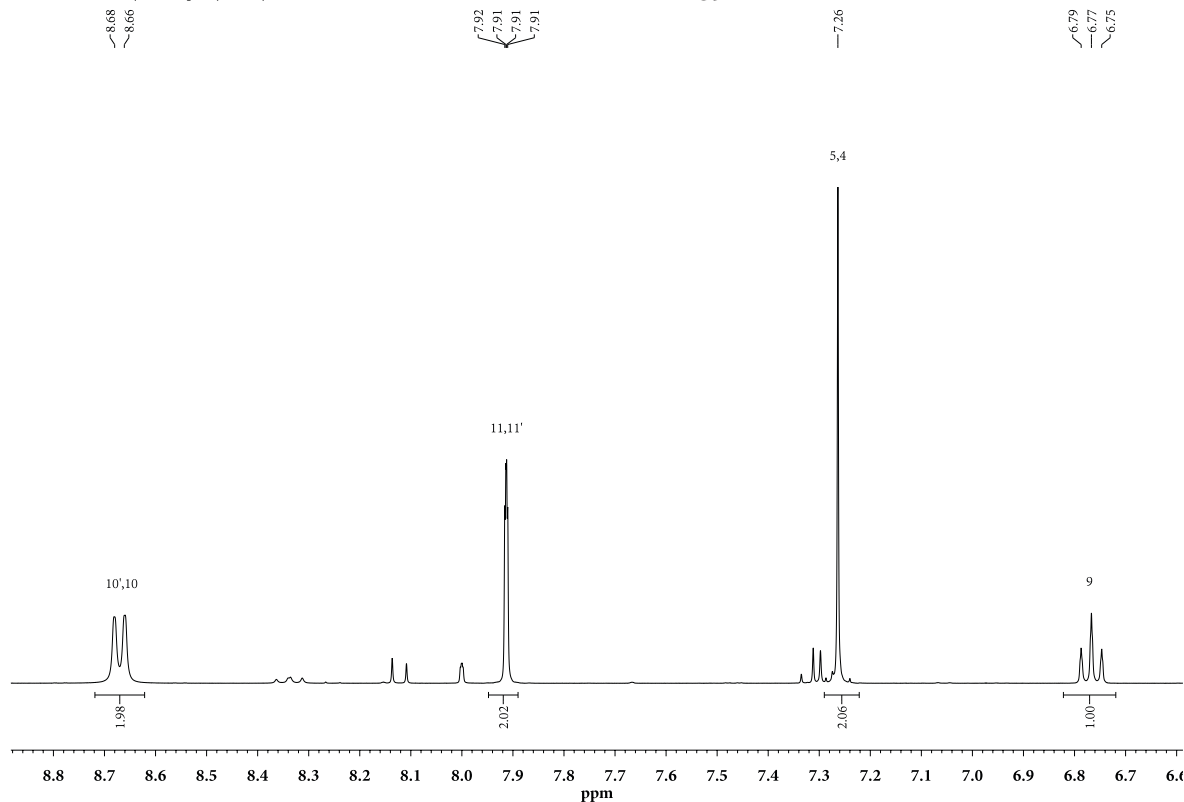
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (C13 CPD)
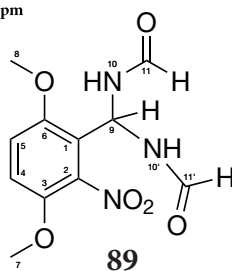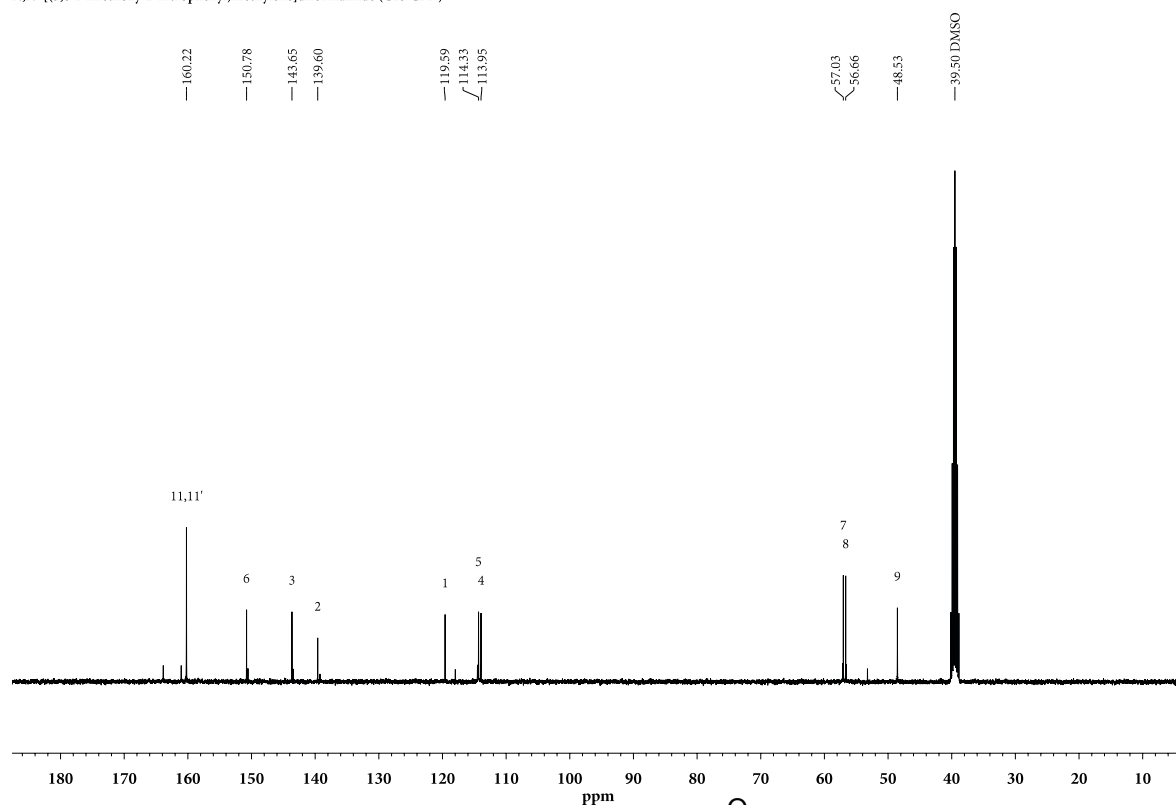
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (HSQC)

89
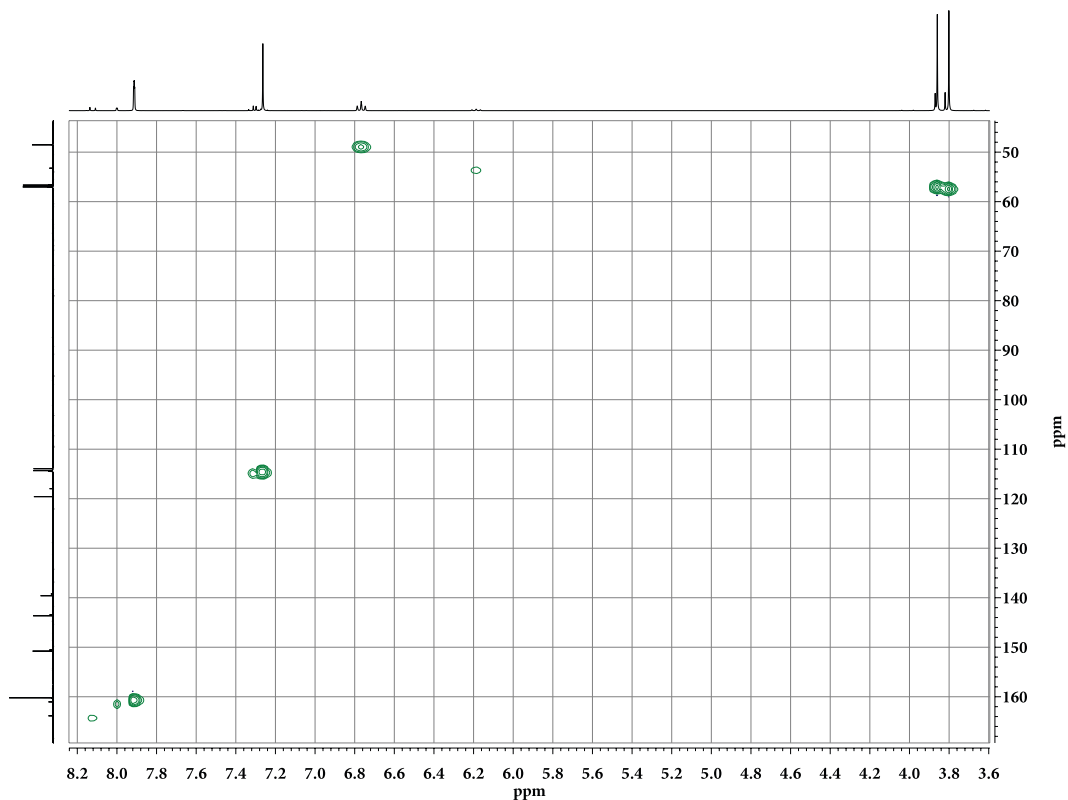
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (HMBC)
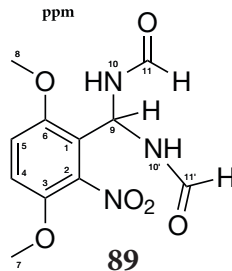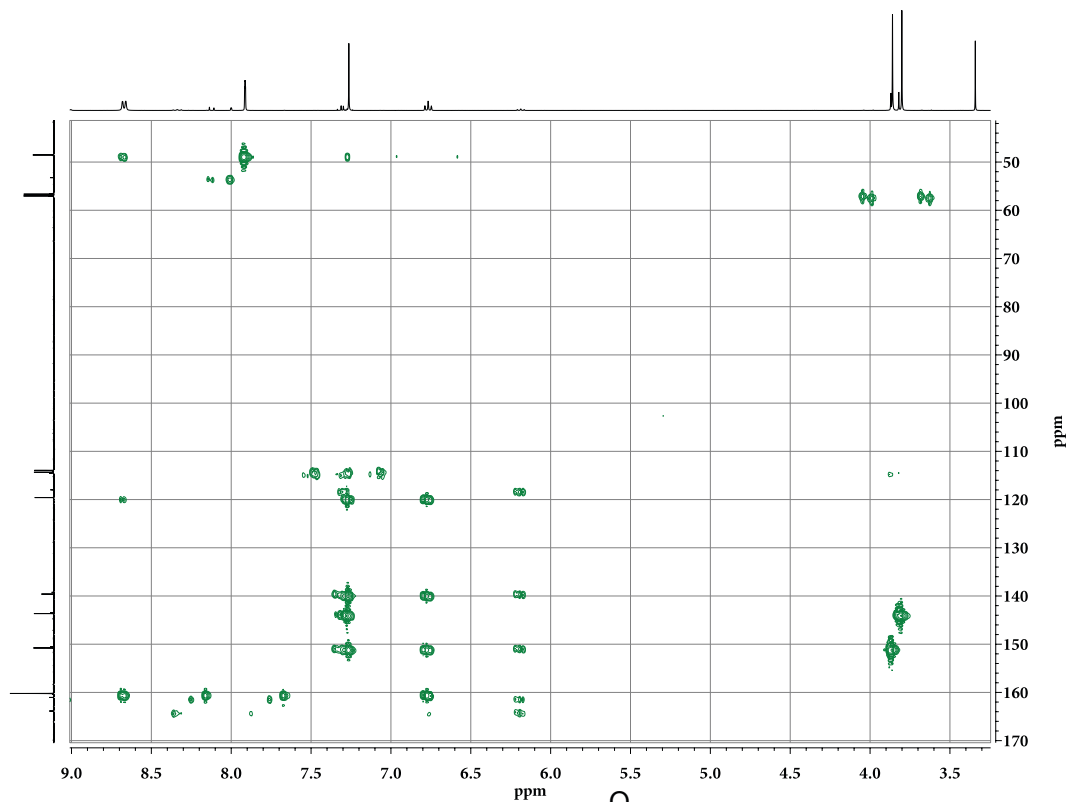
N,N'-[(3,6-Dimethoxy-2-nitrophenyl)methylene]diformamide (NOESY)

89
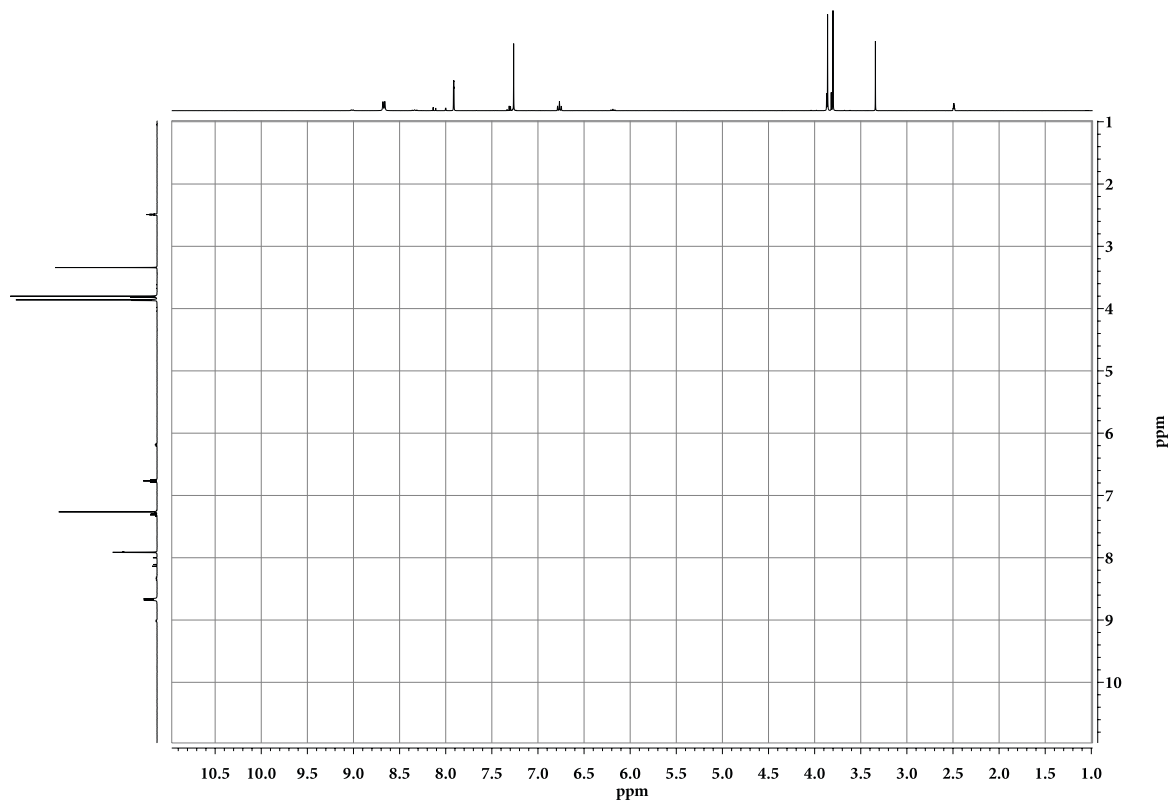
5,8-Dimethoxyquinazoline (PROTON)
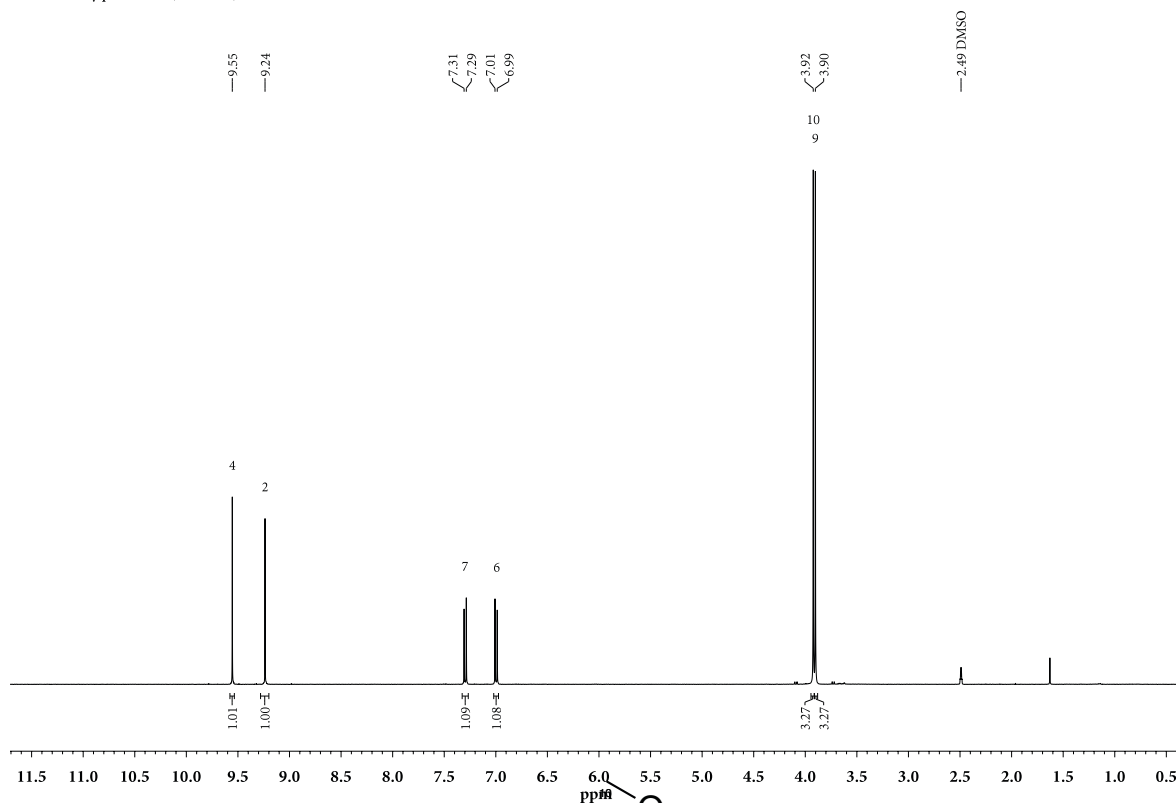
5,8-Dimethoxyquinazoline (C13 CPD)
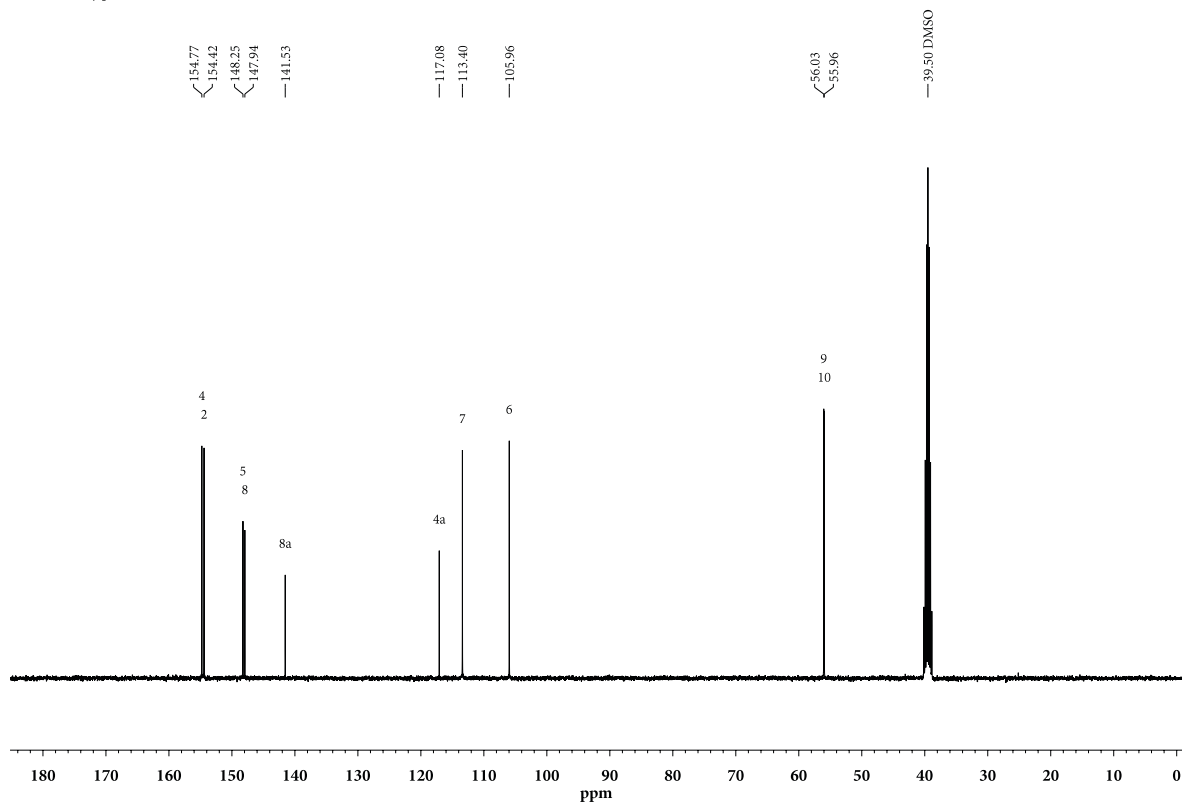
5,8-Dimethoxyquinazoline (HSQC)
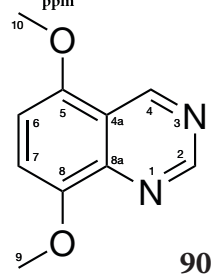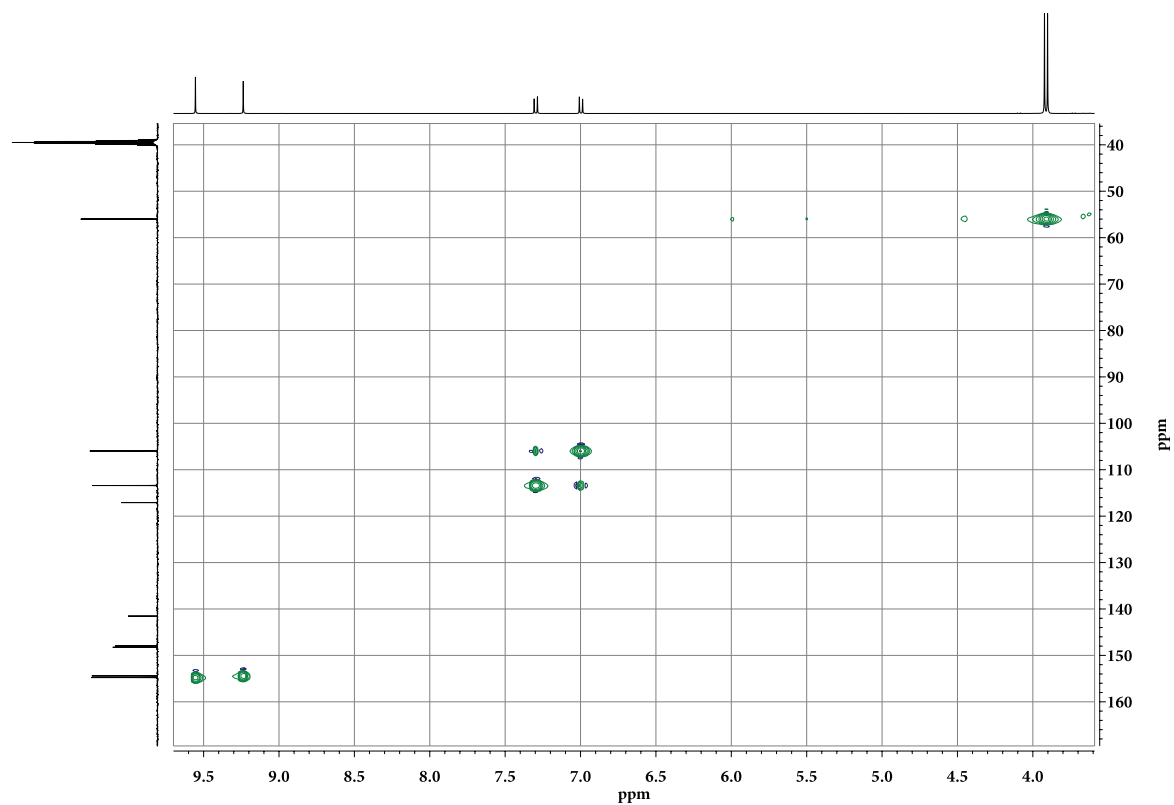
5,8-Dimethoxyquinazoline (HMBC)

90
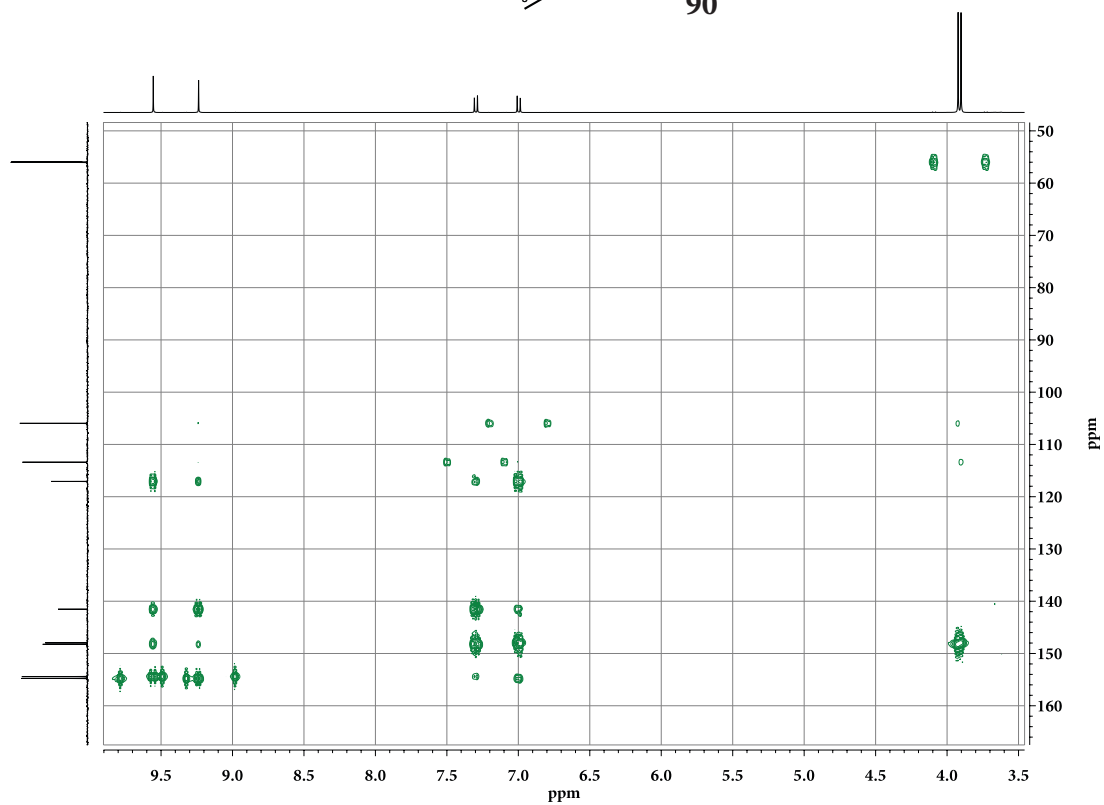
5,8-Dimethoxyquinazoline (15N HMBC)

5,8-Dimethoxyquinazoline (NOESY)

90
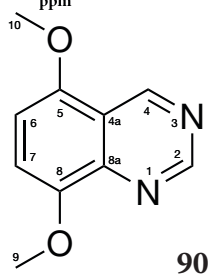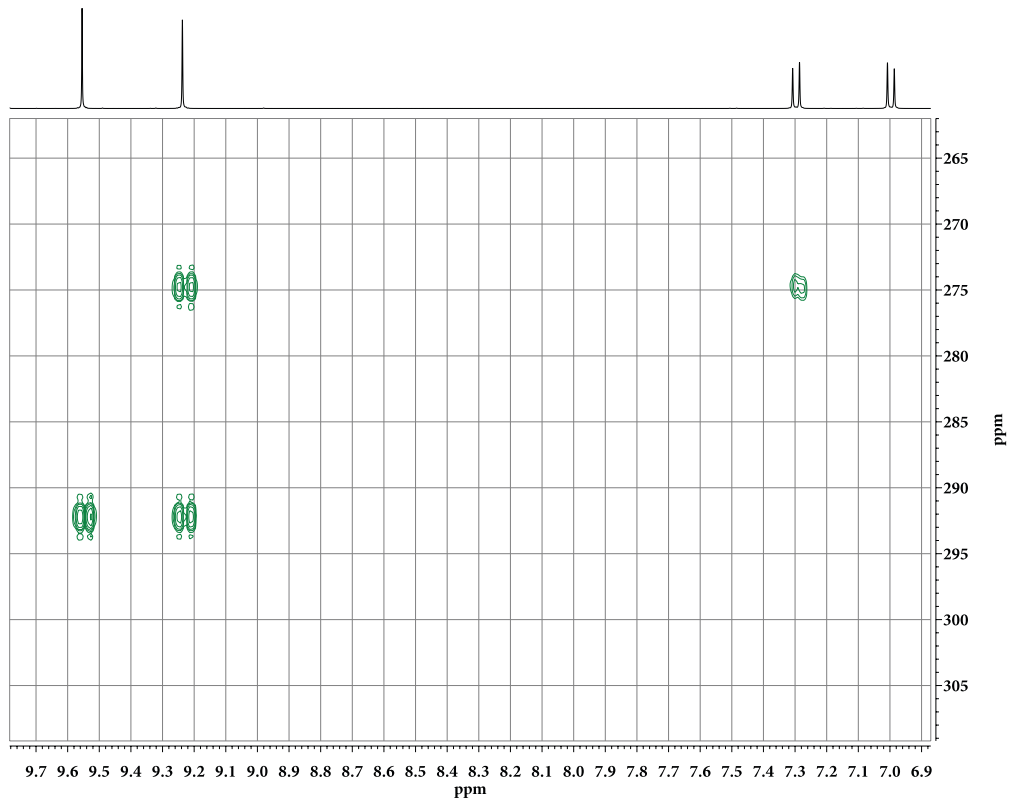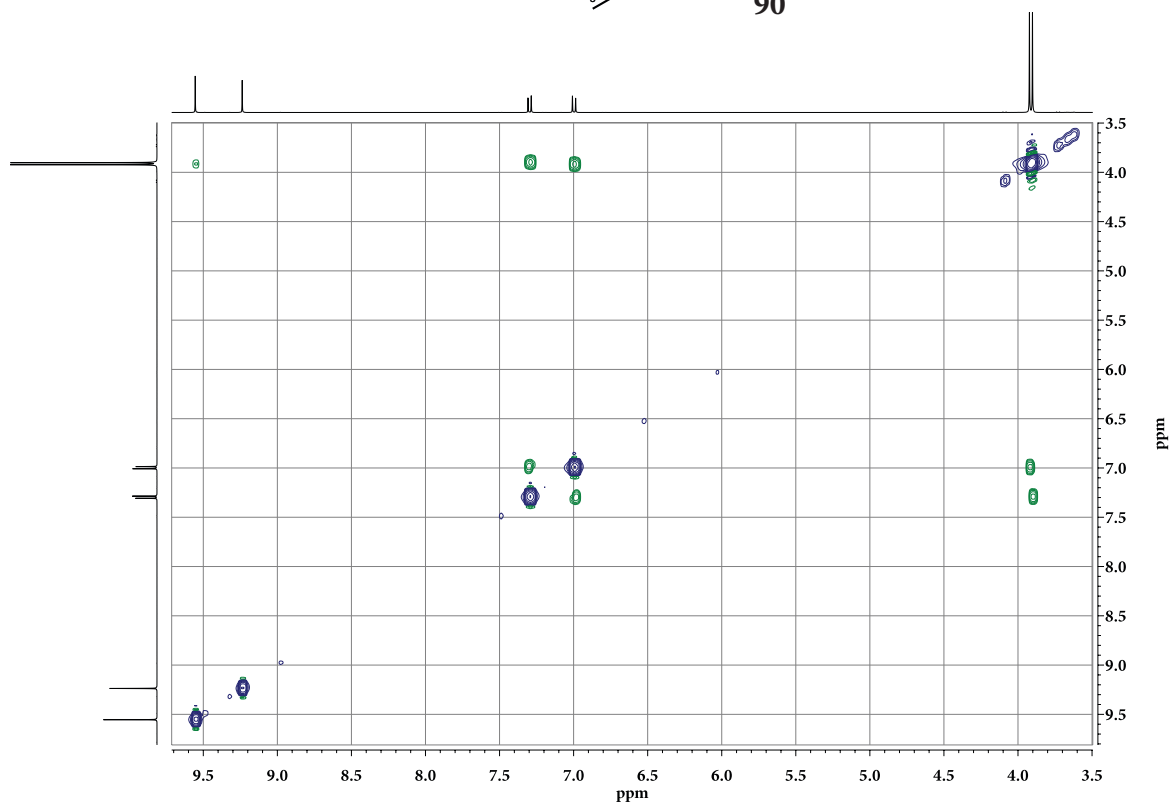
5,8-Quinazolinedione (PROTON)

5,8-Quinazolinedione (PROTON)

91
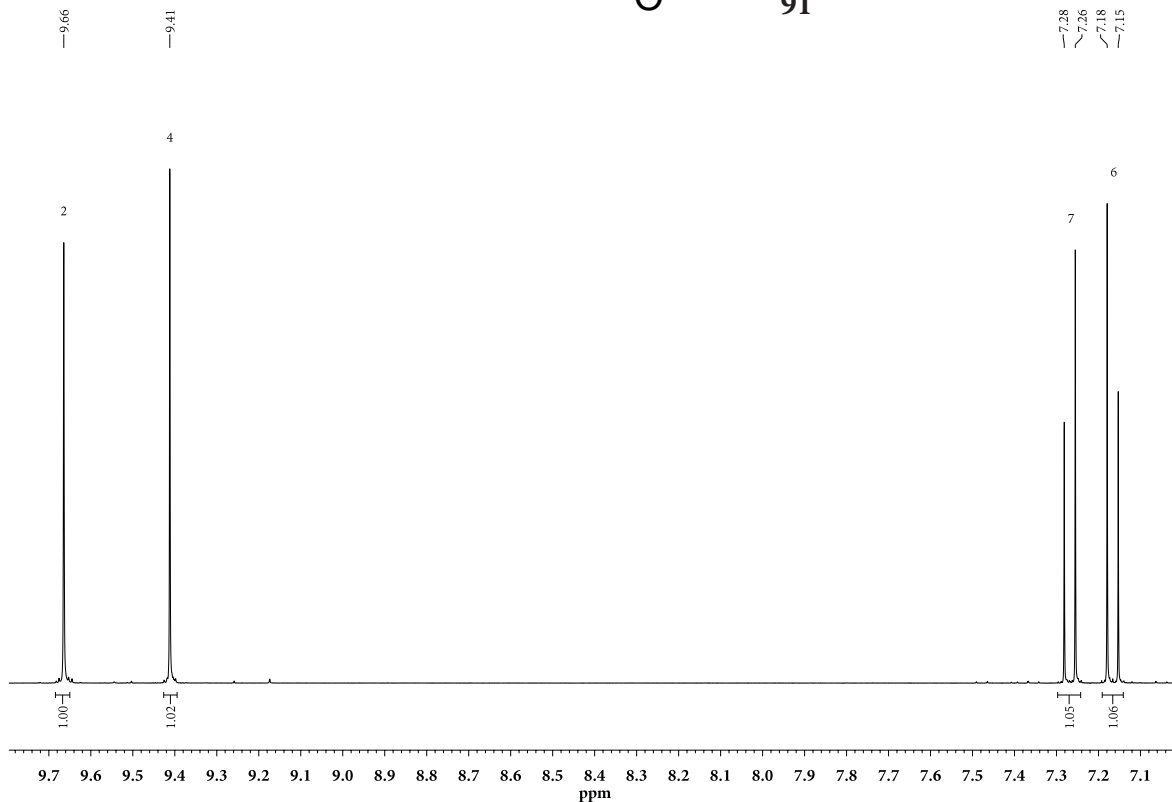
5,8-Quinazolinedione (C13 CPD)

5,8-Quinazolinedione (HSQC)

91

5,8-Quinazolinedione (HMBC)

91

5,8-Quinazolinedione (15N HMBC)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 2:03:35 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN082_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

+MS, 0.0-1.6min #1-89

Intens. x10^4

163.0503

183.0166

186.2217

185.0322

161.0345

164.0535

168.1495

173.0785 175.0504

184.0197

$C_8H_4N_2NaO_2$, 183.0165

x10^4

1+ 183.0165

1+ 184.0199

$C_8H_5N_2O_2$, 161.0346

1+ 161.0346

1+ 162.0379

91

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date 11/17/2014 2:03:35 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN082_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator MZ
Instrument maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
161.0345	1	C8H5N2O2	161.0346	0.2	n.a.	1	100.00	7.5	even	ok
183.0166	1	C8H4N2NaO2	183.0165	-0.6	5.1	1	100.00	7.5	even	ok
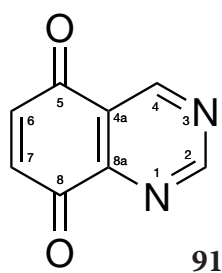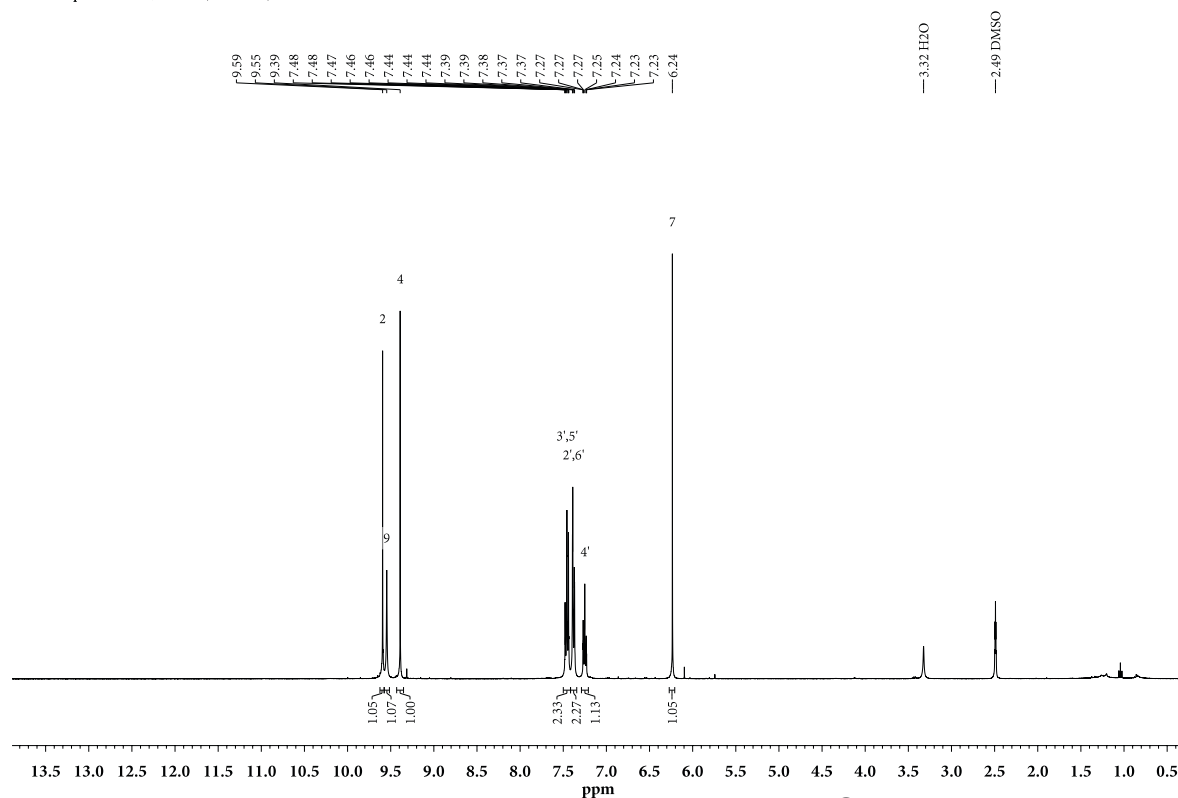
91

6-Anilinoquinazoline-5,8-dione (PROTON)

6-Anilinoquinazoline-5,8-dione (PROTON)

6-Anilinoquinazoline-5,8-dione (PROTON)

7.48
7.48
7.47
7.46
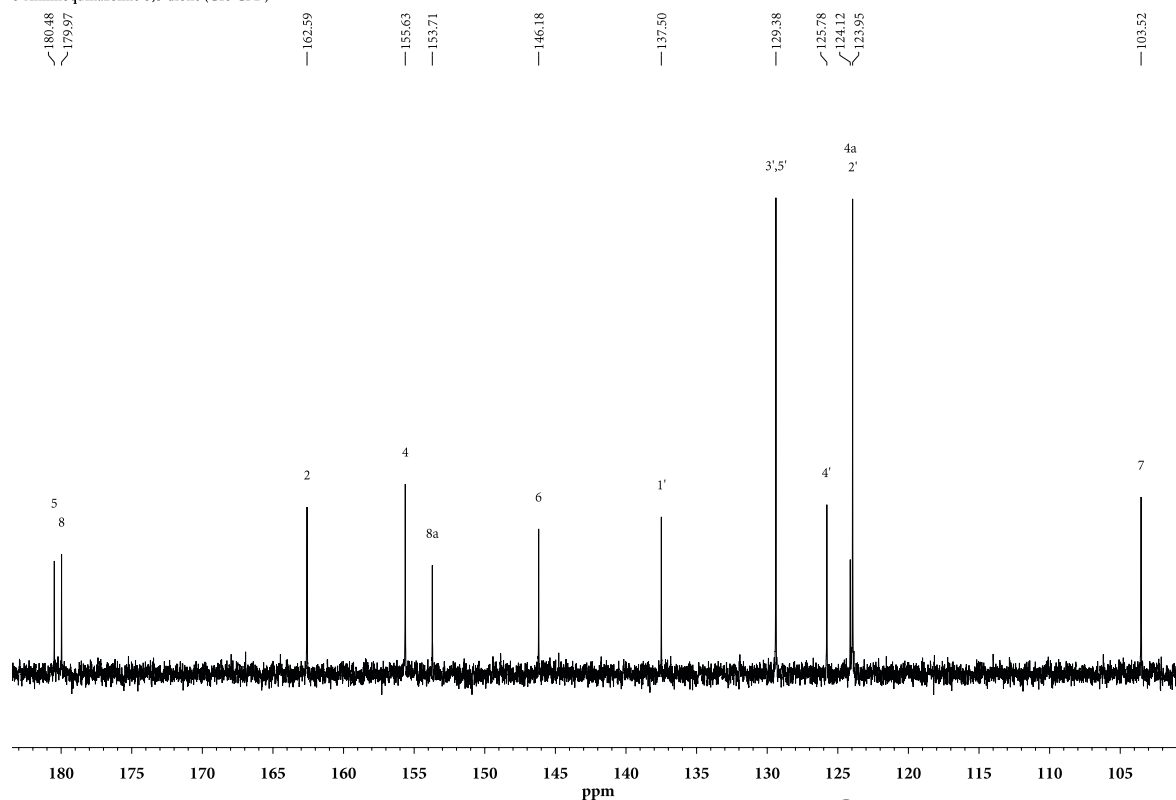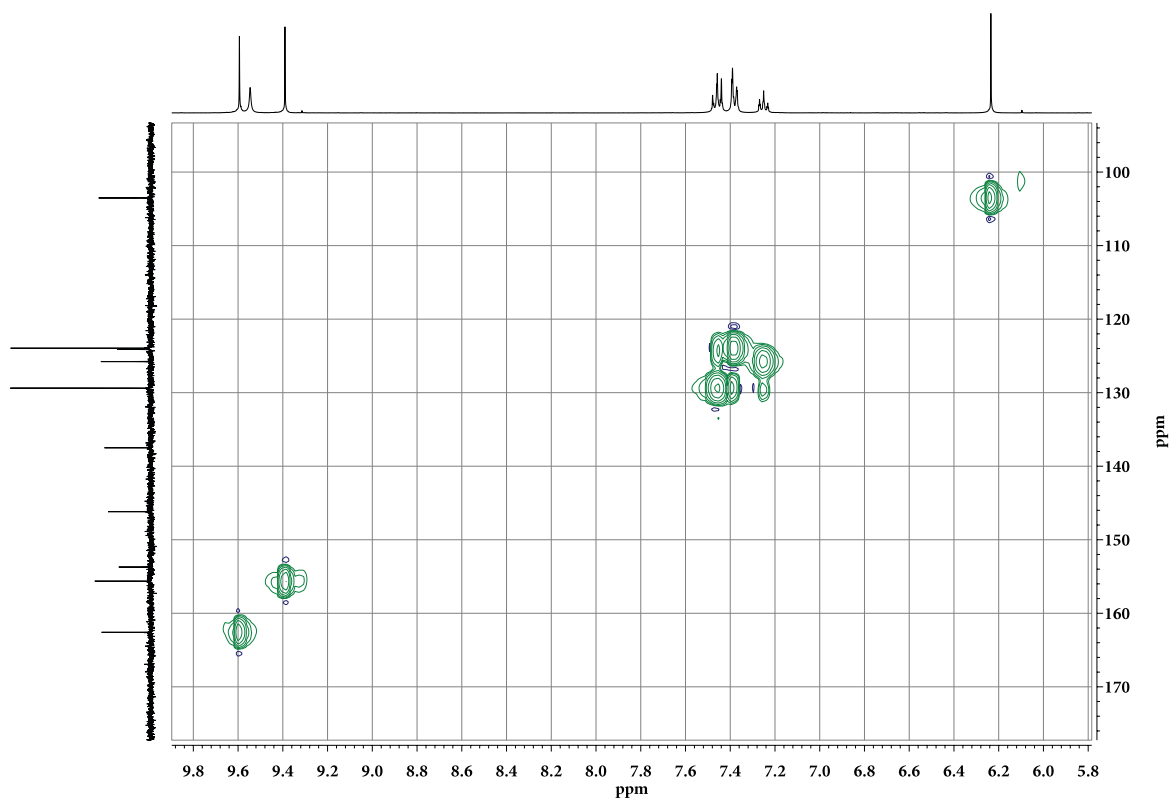7.46
7.44
7.44
7.44
7.39
7.39
7.38
7.37
7.37
7.27
7.27
7.27
7.25
7.24
7.23
7.23

3',5'

2',6'

4'

2.33

2.27

1.13

7.56 7.54 7.52 7.50 7.48 7.46 7.44 7.42 7.40 7.38 7.36 7.34 7.32 7.30 7.28 7.26 7.24 7.22 7.20 7.18 7.16
ppm

92

6-Anilinoquinazoline-5,8-dione (C13 CPD)

180.48
179.97
162.59
155.63
153.71
146.18
137.50
129.38
125.78
124.12
123.95
103.52
39.50 DMSO

3',5'

4a
2'

5
8

2

4
8a

6

1'

4'

7

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm
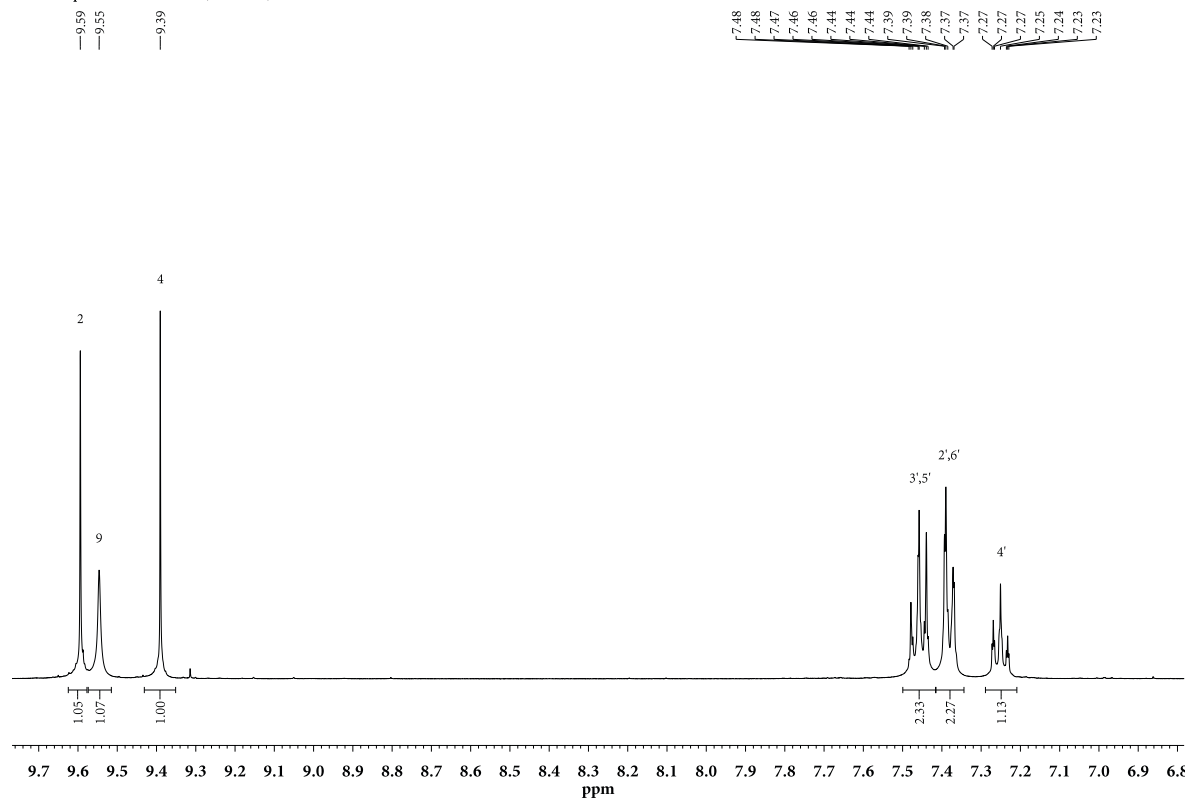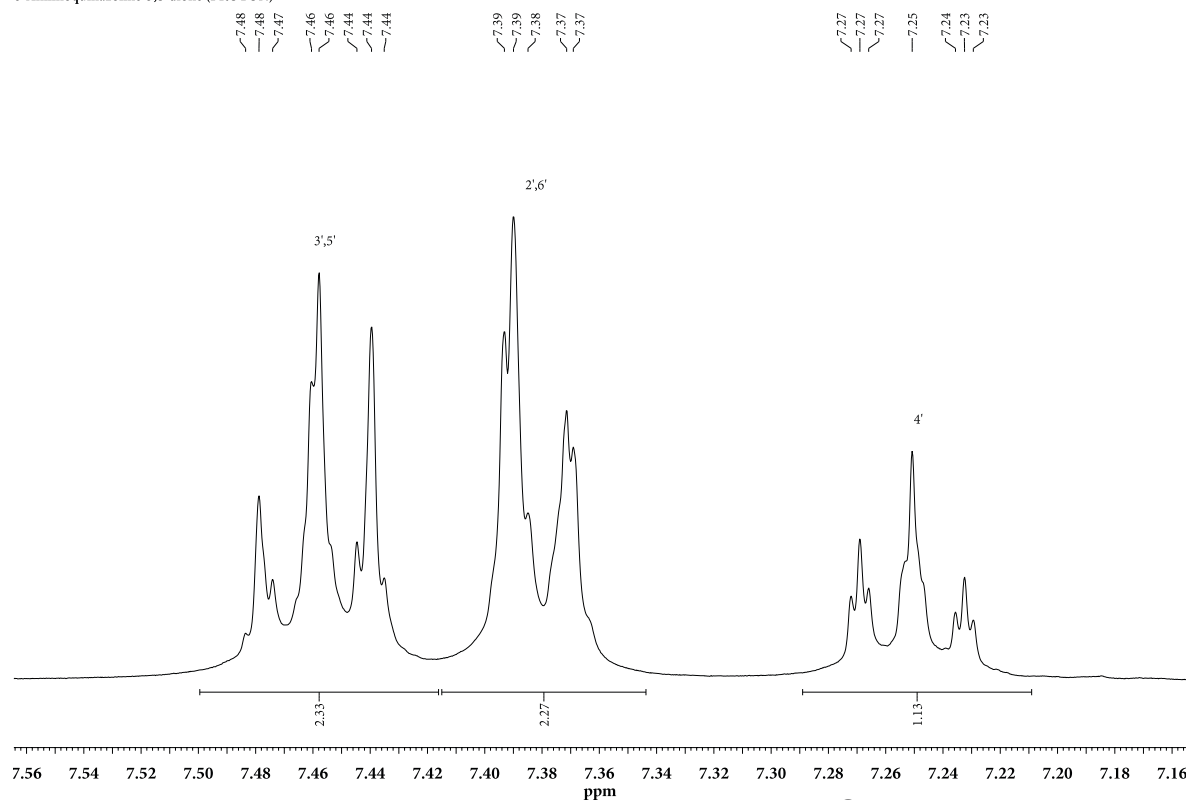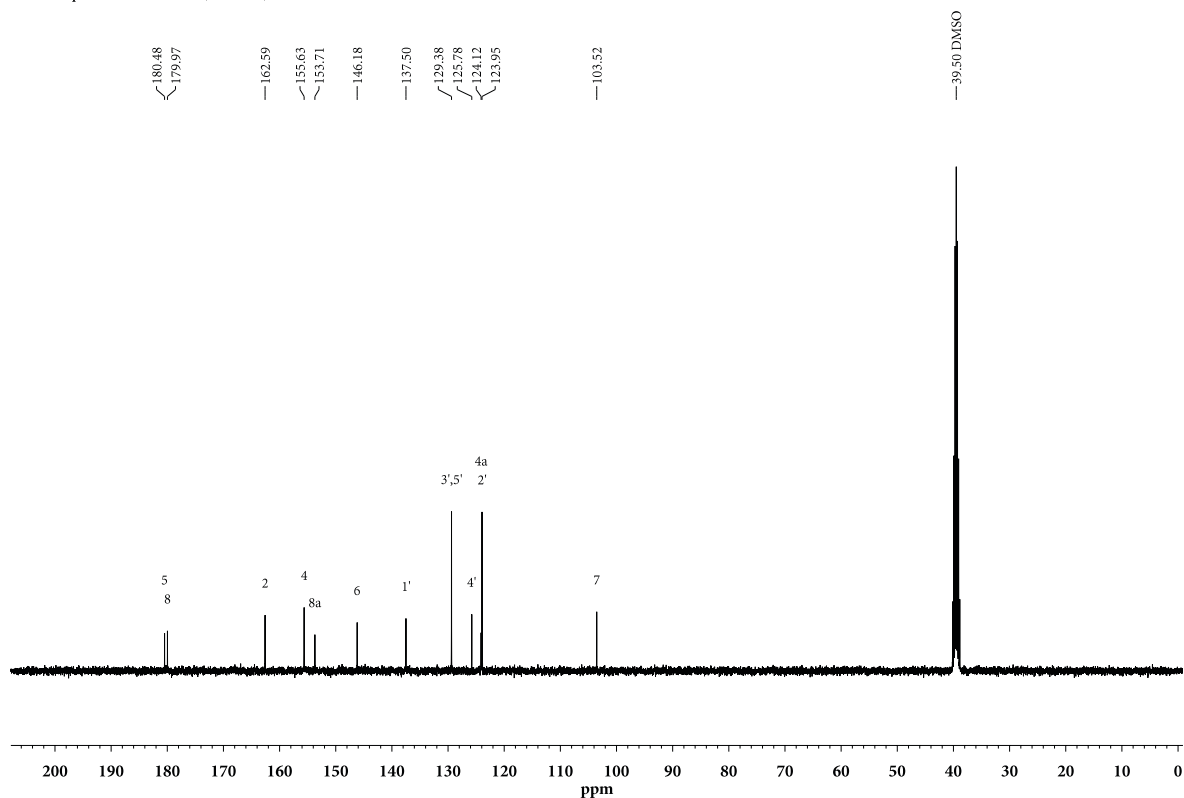
6-Anilinoquinazoline-5,8-dione (C13 CPD)
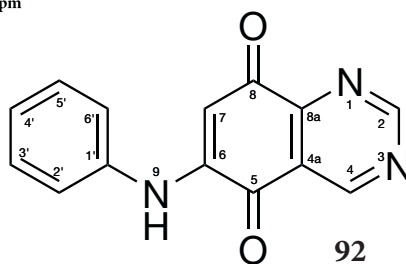
6-Anilinoquinazoline-5,8-dione (HSQC)

6-Anilinoquinazoline-5,8-dione (HMBC)
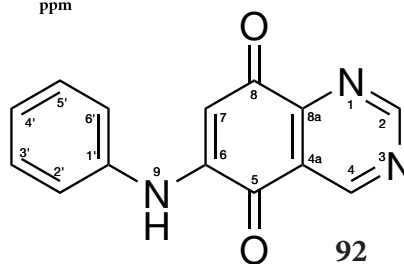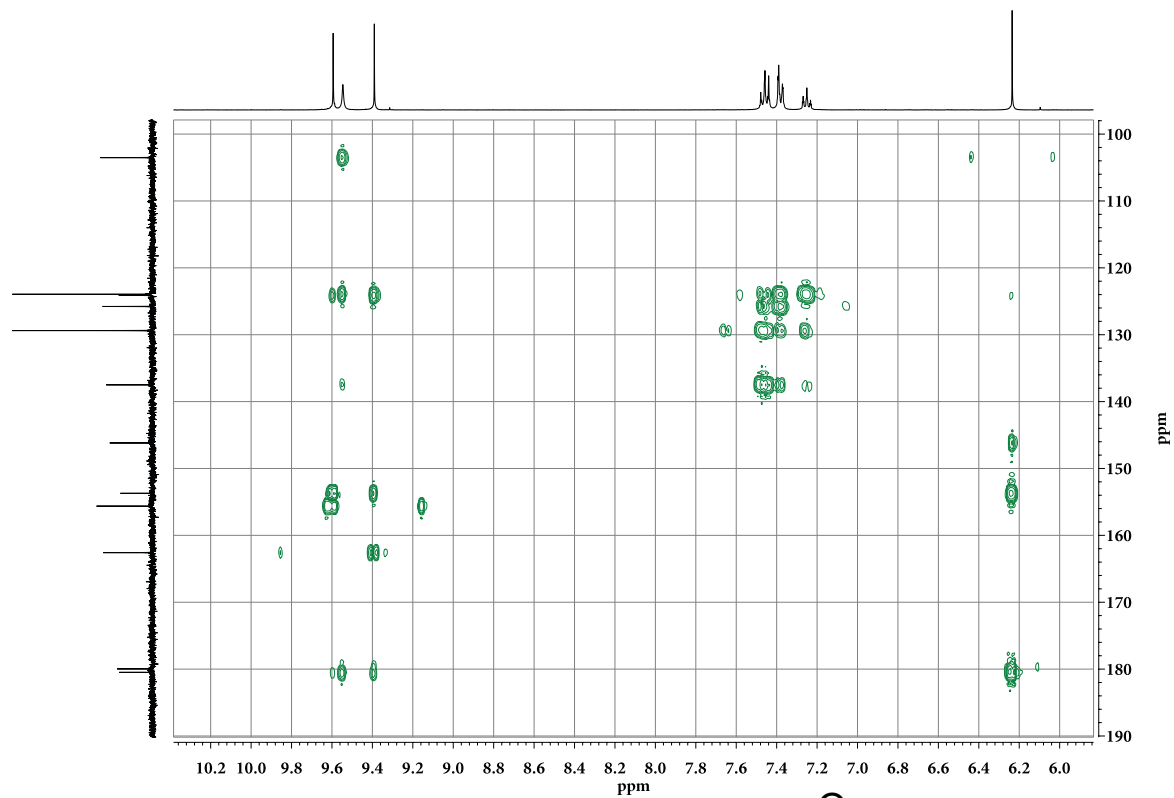
6-Anilinoquinazoline-5,8-dione (15N HMBC)

92
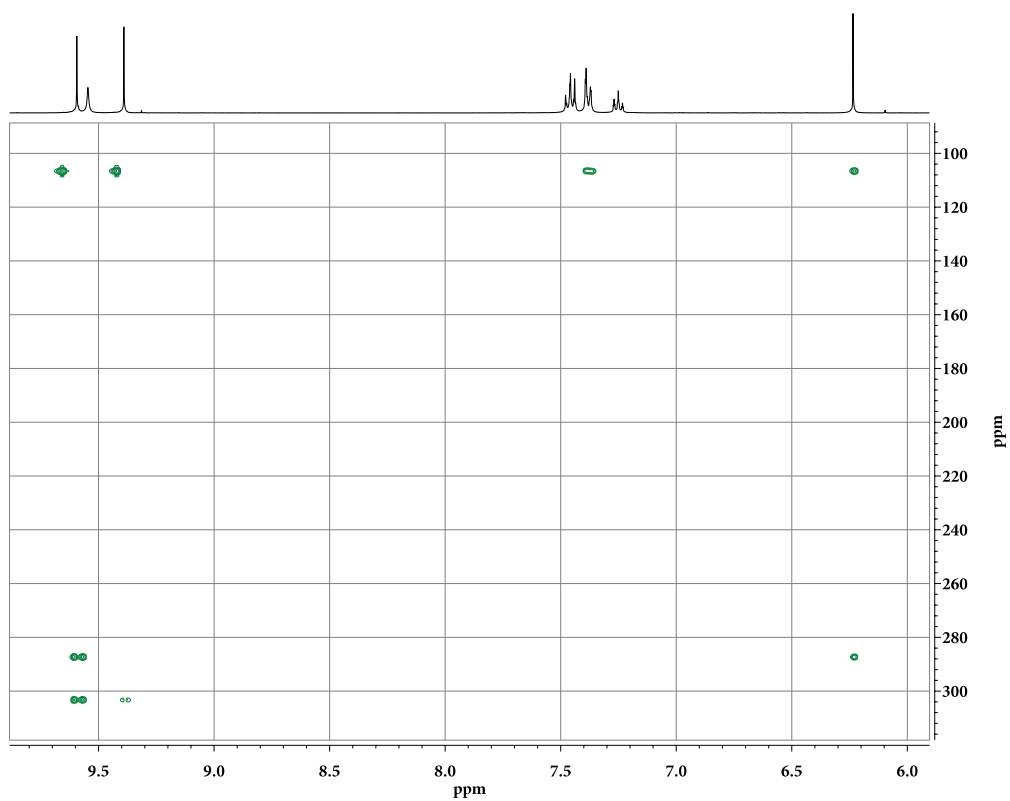
Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN104_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 2:30:26 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

+MS, 0.5-1.6min #29-88

274.0587

252.0765
250.2139
257.1257
261.1306
268.2744
271.1876
276.2295

$C_{14}H_{10}N_3O_2$, 252.0768

1+
252.0768

$C_{14}H_9N_3NaO_2$, 274.0587

1+
274.0587

92

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   1/12/2015 2:30:26 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN104_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:500 in MeOH
Comment	

Operator	MZ
Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.5-1.6min #29-88

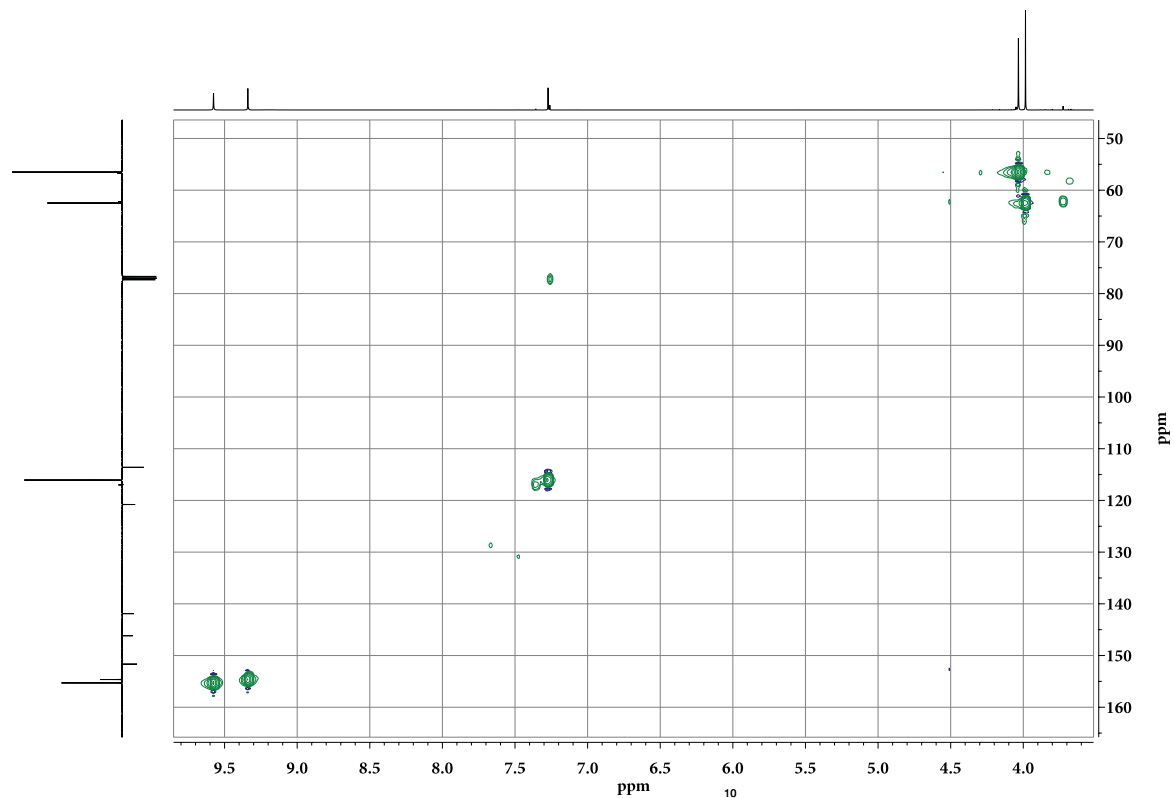| Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e‾ Conf	N-Rule
252.0765	1	C14H10N3O2	252.0768	1.0	7.4	1	100.00	11.5	even	ok
274.0587	1	C14H9N3NaO2	274.0587	-0.2	10.6	1	100.00	11.5	even	ok

92

TN104_DI_ESI_HRMS.d
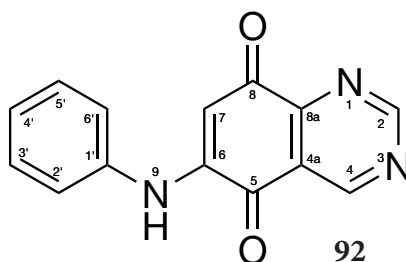
6-Bromo-5,8-dimethoxyquinazoline (PROTON)
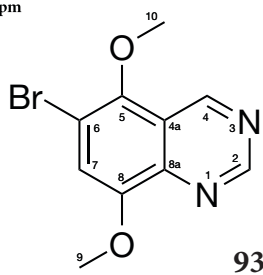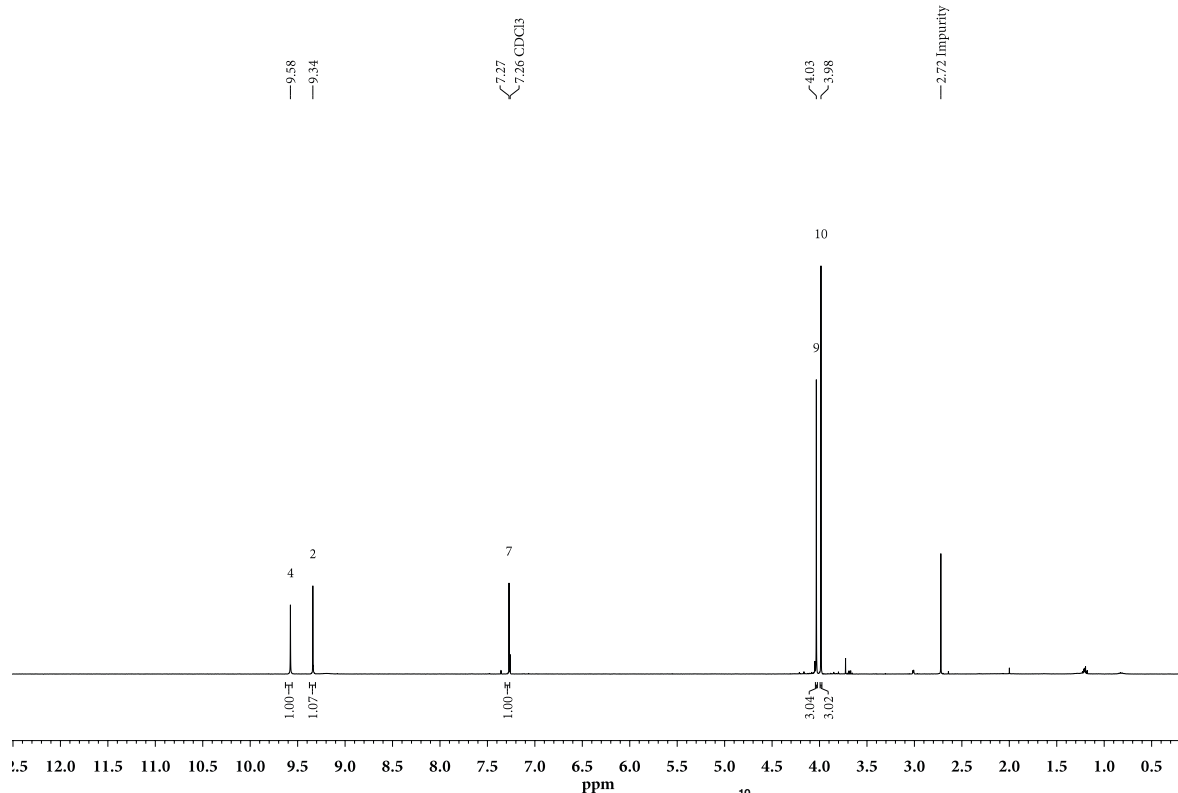
6-Bromo-5,8-dimethoxyquinazoline (C13 APT)
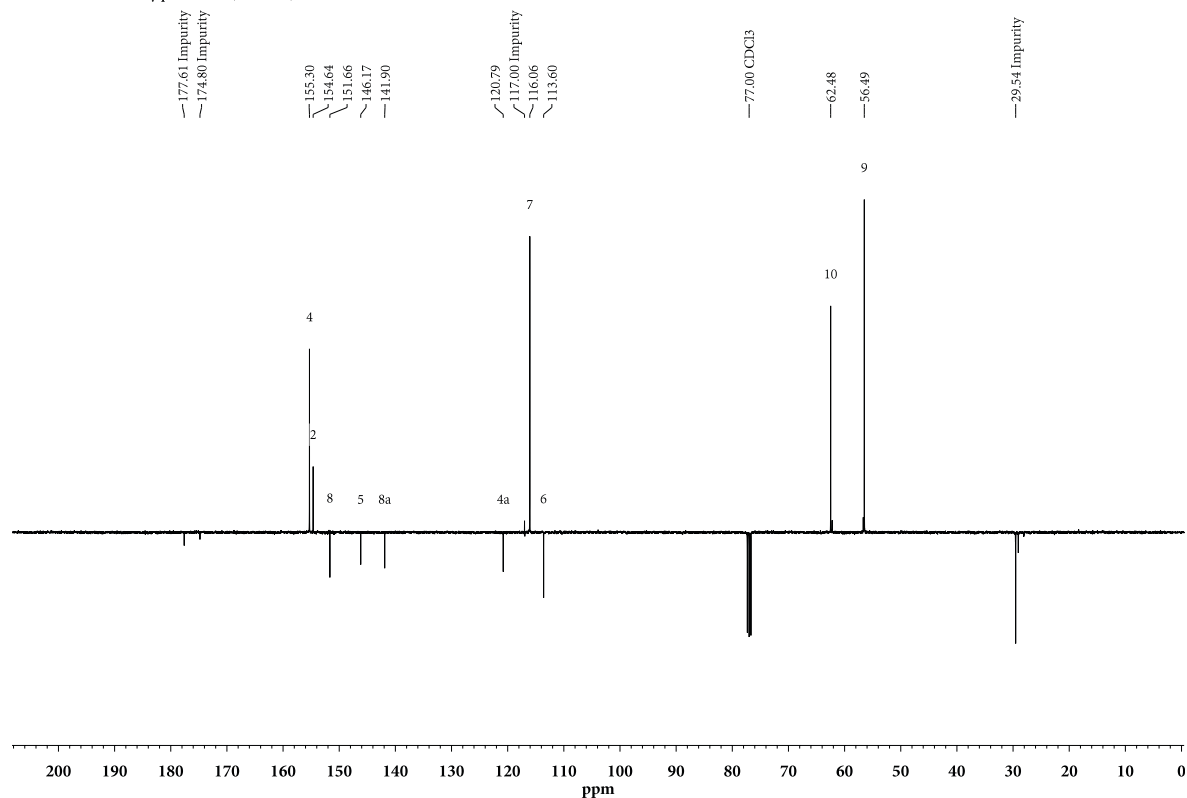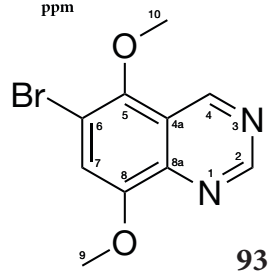
93
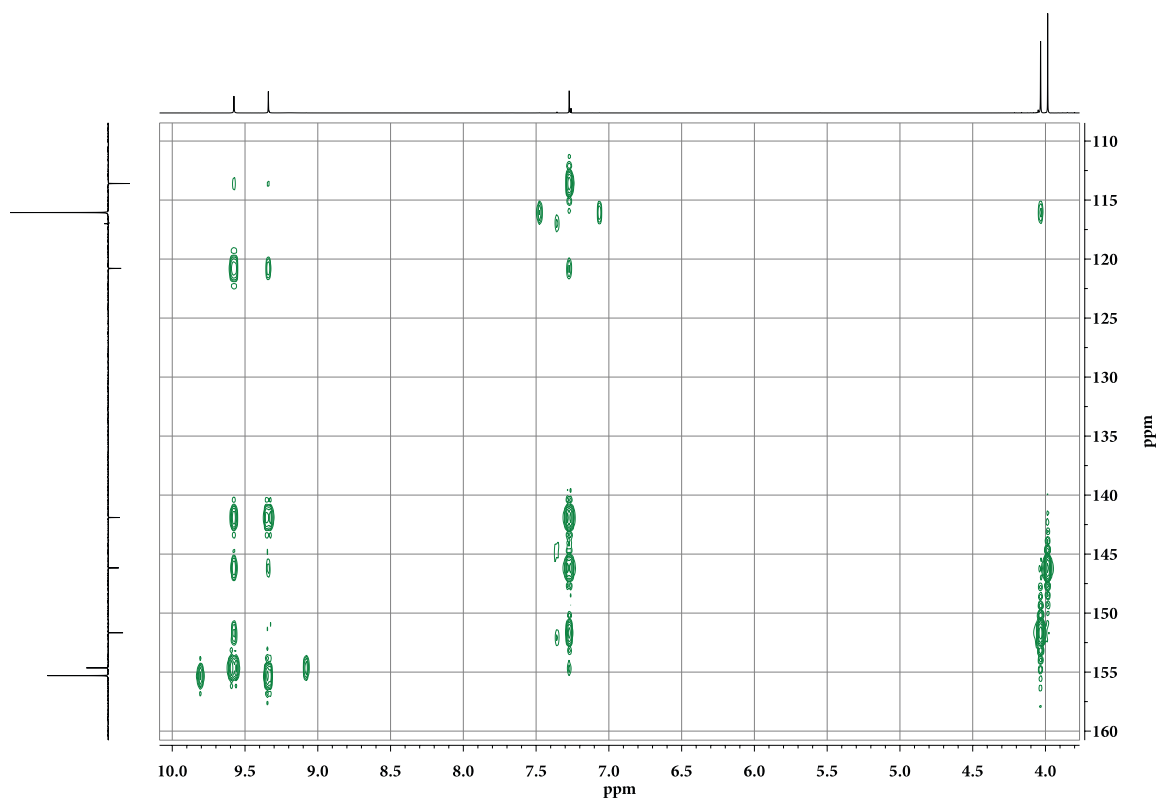
6-Bromo-5,8-dimethoxyquinazoline (HSQC)

93
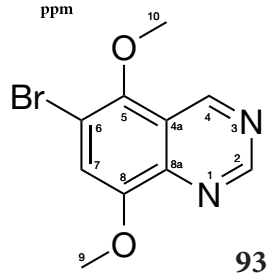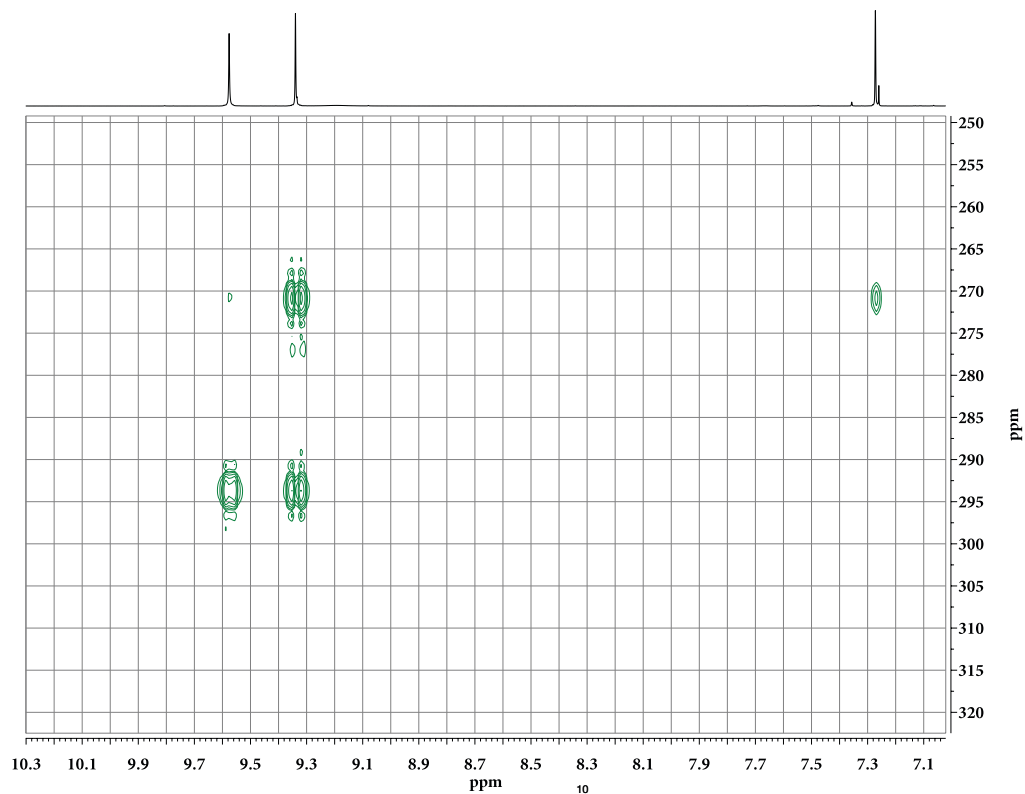
6-Bromo-5,8-dimethoxyquinazoline (HMBC)
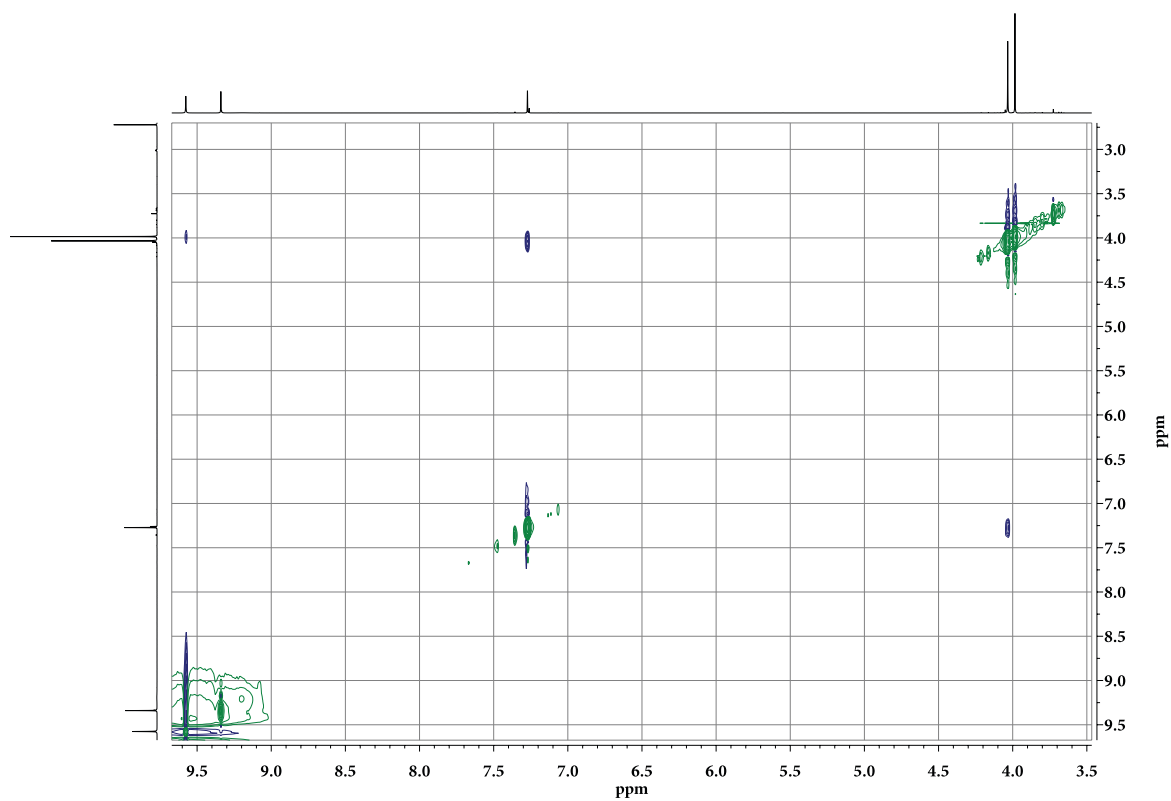
6-Bromo-5,8-dimethoxyquinazoline (15N HMBC)

93

6-Bromo-5,8-dimethoxyquinazoline (NOESY)

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 2:23:02 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN117_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

93

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/17/2014 2:23:02 PM

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN117_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   0.1 mg/mL in MeOH   Instrument   maXis HD   1820881.21300
Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
268.9922	1	C10H10BrN2O2	268.9920	-0.6	9.2	1	100.00	6.5	even	ok
290.9743	1	C10H9BrN2NaO2	290.9740	-1.2	10.3	1	100.00	6.5	even	ok
	2	C10H4NaO9	290.9748	1.5	544.9	2	0.00	8.5	even	ok

TN117_DI_ESI_HRMS.d

Bruker Compass DataAnalysis 4.2   printed: 11/17/2014 2:32:08 PM   by: MZ   Page 1 of 1

2-Anilino-6-methyl-1,4-benzoquinone (PROTON)

7.40
7.38
7.37
7.36
7.26 CDCl3
7.21
7.20
7.20
7.19
7.19
7.18
7.17
7.17
7.15
6.52
6.51
6.51
6.50
6.13
6.12

2.07
2.07

2.11
0.94
3.10

1.00

1.00

3.07

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

94a

2-Anilino-6-methyl-1,4-benzoquinone (PROTON)

7.40
7.40
7.39
7.38
7.37
7.36
7.35
7.31
7.26 CDCl3
7.21
7.20
7.20
7.19
7.19
7.18
7.18
7.17
7.17
7.16
7.15
7.15
6.52
6.52
6.51
6.51
6.50
6.13
6.12

2.11
0.94
3.10

1.00

1.00

.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10 7.05 7.00 6.95 6.90 6.85 6.80 6.75 6.70 6.65 6.60 6.55 6.50 6.45 6.40 6.35 6.30 6.25 6.20 6.15 6.10 6.05
ppm

2-Anilino-6-methyl-1,4-benzoquinone (PROTON)

6.52
6.52
6.51
6.51
6.50

6.13
6.12

2.07
2.07

1.00

1.00

3.07

ppm

6.55 6.50 6.45 6.40 6.35 6.30 6.25 6.20 6.15 6.10 6.05 6.00   2.15 2.10 2.05 2.00

94a

2-Anilino-6-methyl-1,4-benzoquinone (C13 APT)

186.76
184.40

142.99
141.22
137.55
135.92
129.56
125.30
122.14

100.73

77.00 CDCl3

15.45

5',3'

6',2'

5

4'

3

7

4 1

2
6 1'

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

ppm

2-Anilino-6-methyl-1,4-benzoquinone (HSQC)

2-Anilino-6-methyl-1,4-benzoquinone (HMBC)

94a
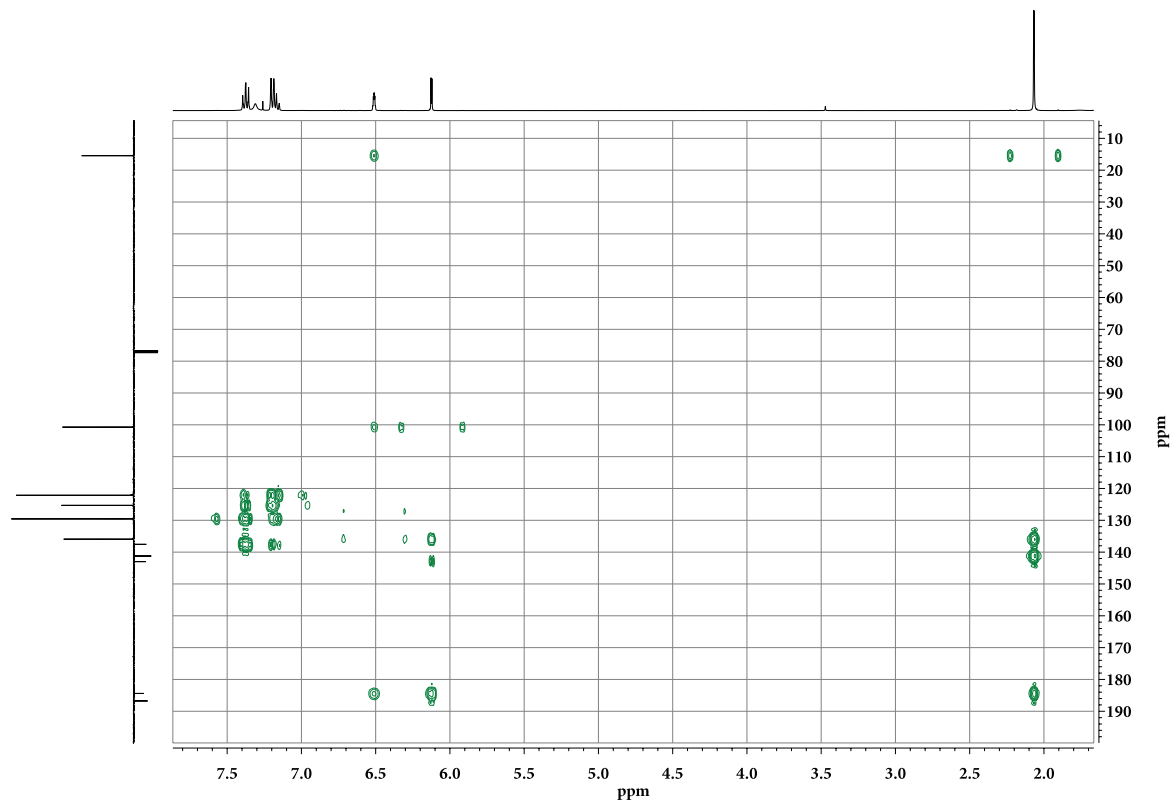
2-Anilino-6-methyl-1,4-benzoquinone (15N HMBC)

2-Anilino-6-methyl-1,4-benzoquinone (NOESY)

94a

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 3:49:48 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN123_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date   1/12/2015 3:49:48 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN123_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	in MeOH --> 1:500 in MeOH
Comment	

Operator	MZ		
Instrument	maXis HD	1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

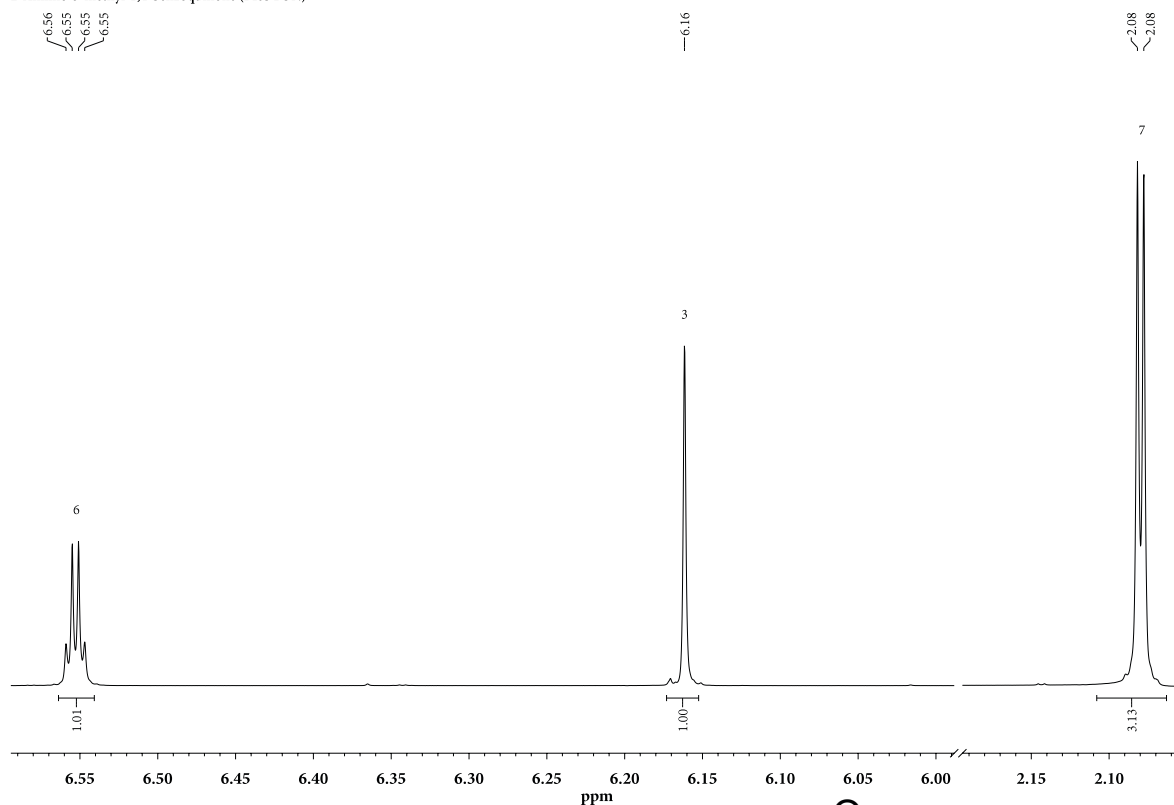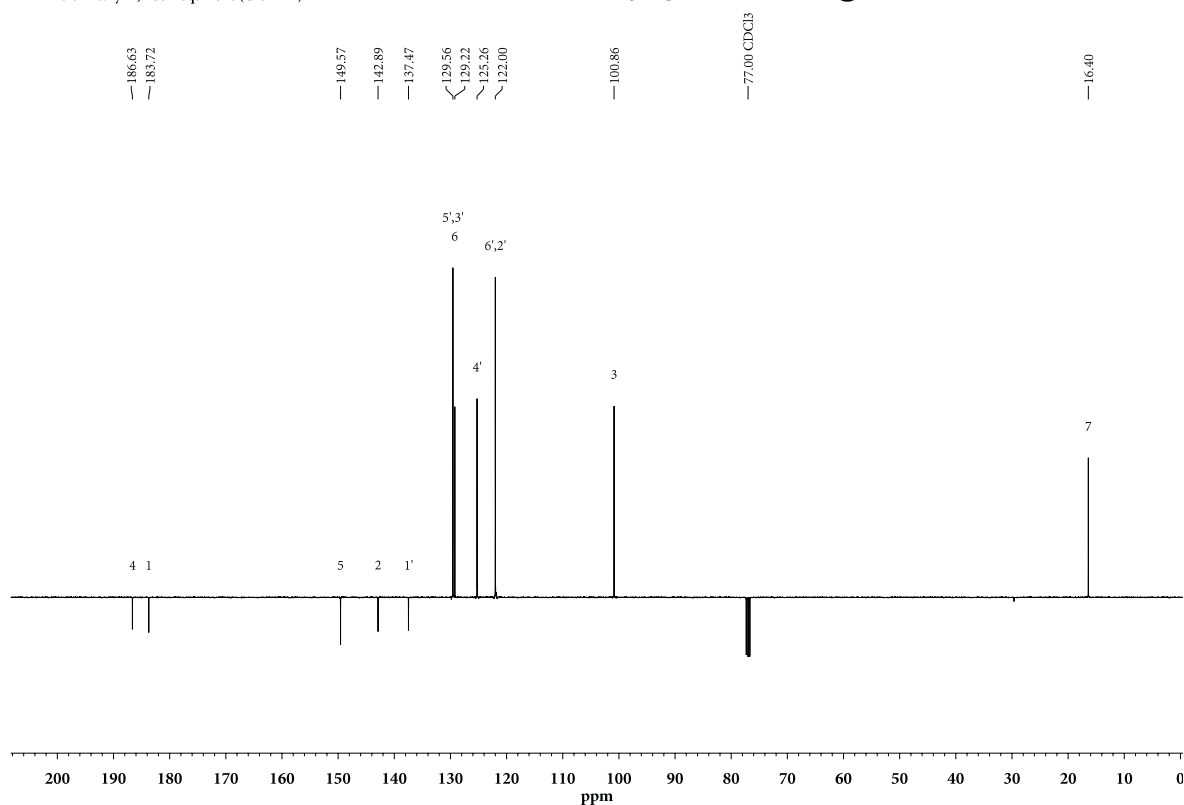| Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
214.0862	1	C13H12NO2	214.0863	0.1	7.6	1	100.00	8.5	even	ok
236.0684	1	C13H11NNaO2	236.0682	-0.9	0.8	1	100.00	8.5	even	ok

94a
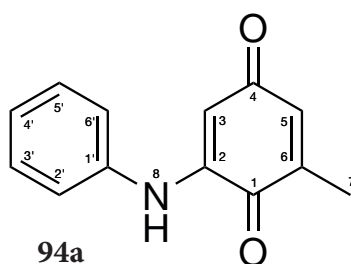
2-Anilino-5-methyl-1,4-benzoquinone (PROTON)

2-Anilino-5-methyl-1,4-benzoquinone (PROTON)
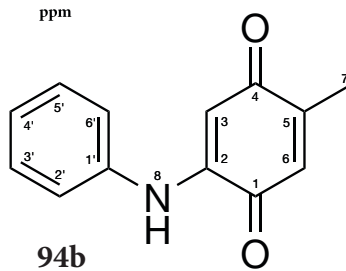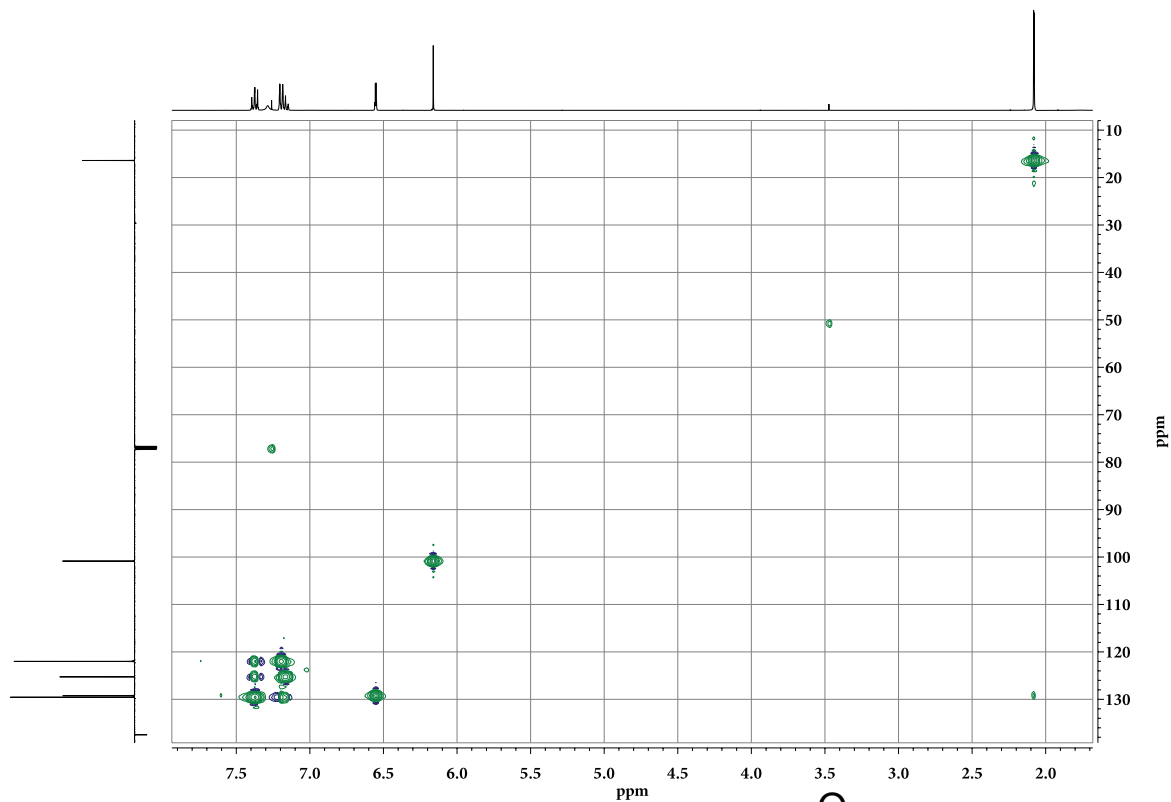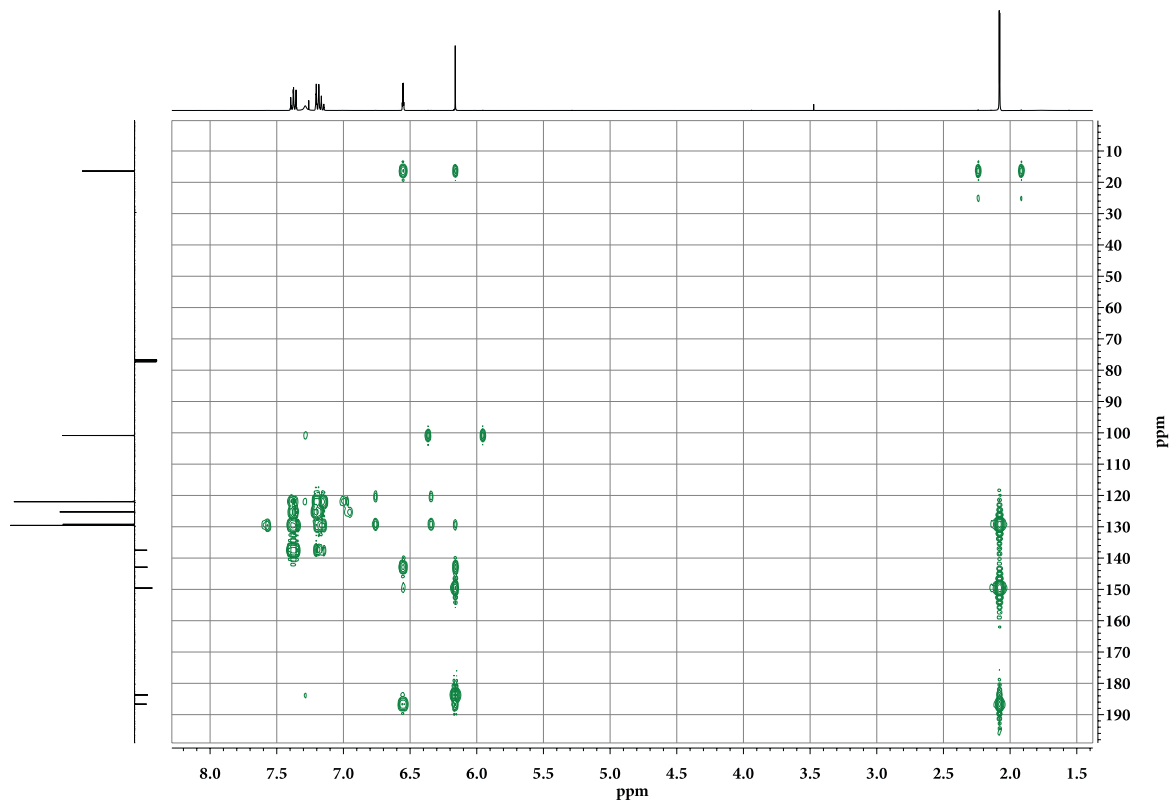
94b

2-Anilino-5-methyl-1,4-benzoquinone (PROTON)

6.56
6.55
6.55
6.55

6.16

2.08
2.08

7

3

6

1.01

1.00

3.13

6.55 6.50 6.45 6.40 6.35 6.30 6.25 6.20 6.15 6.10 6.05 6.00 2.15 2.10

ppm

94b

2-Anilino-5-methyl-1,4-benzoquinone (C13 APT)

186.63
183.72

149.57
142.89
137.47

129.56
129.22
125.26
122.00

100.86

77.00 CDCl3

16.40

5',3'
6

6',2'

4'

3

7

4 1

5 2 1'

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

ppm

735

2-Anilino-5-methyl-1,4-benzoquinone (HSQC)

94b

2-Anilino-5-methyl-1,4-benzoquinone (HMBC)

2-Anilino-5-methyl-1,4-benzoquinone (15N HMBC)

94b
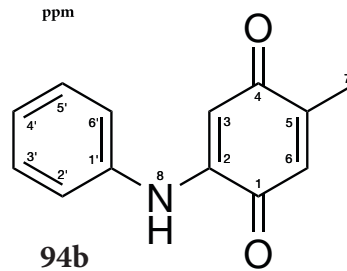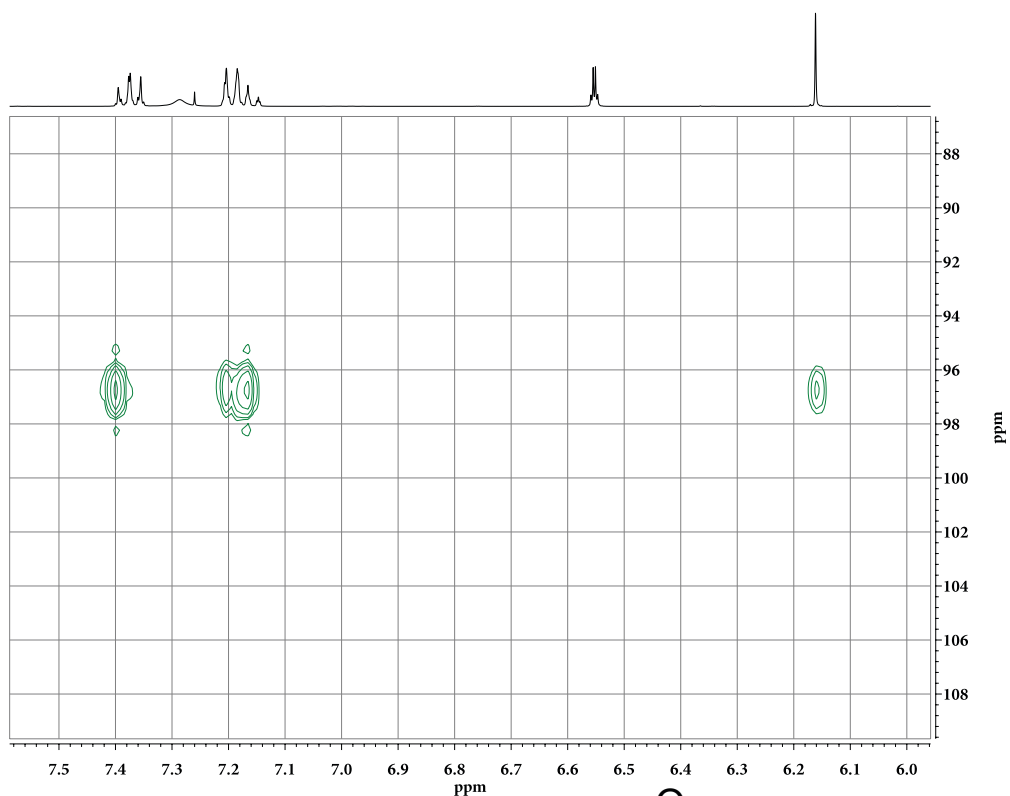
2-Anilino-5-methyl-1,4-benzoquinone (NOESY)

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 4:05:56 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN124_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

94b

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/12/2015 4:05:56 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN124_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-2.2min #1-124

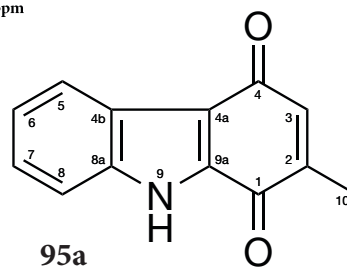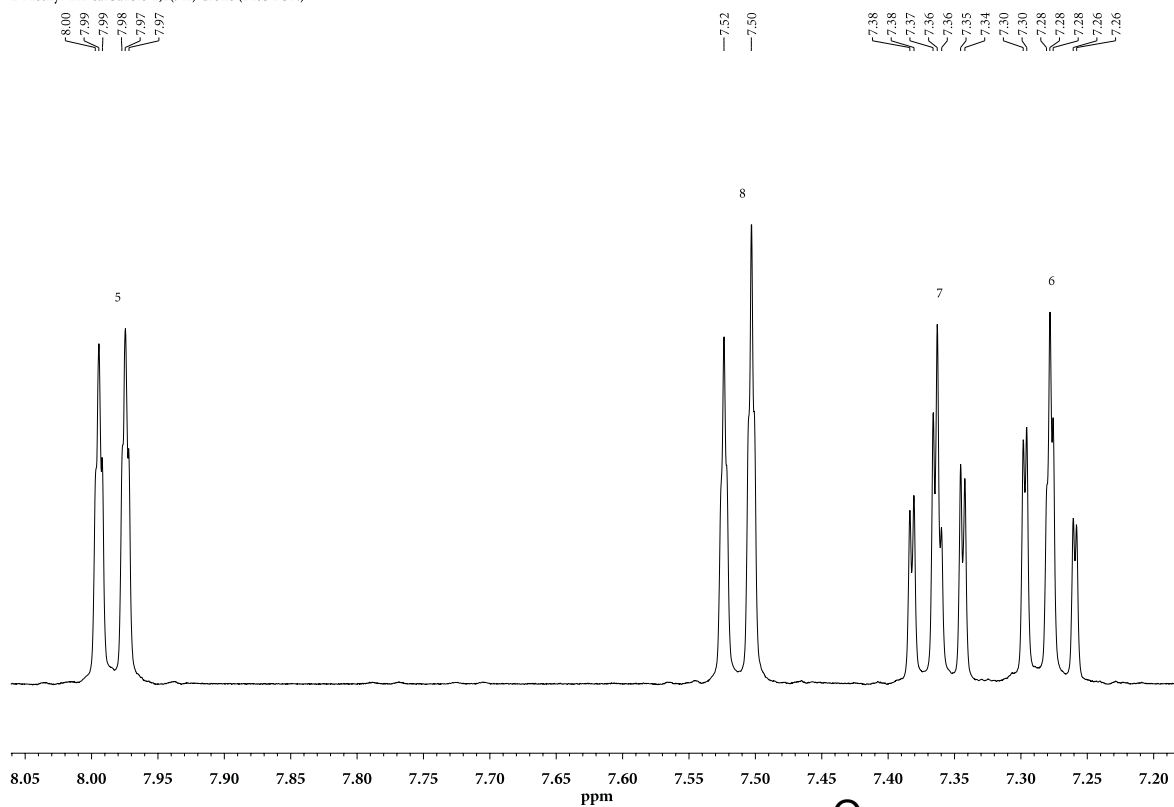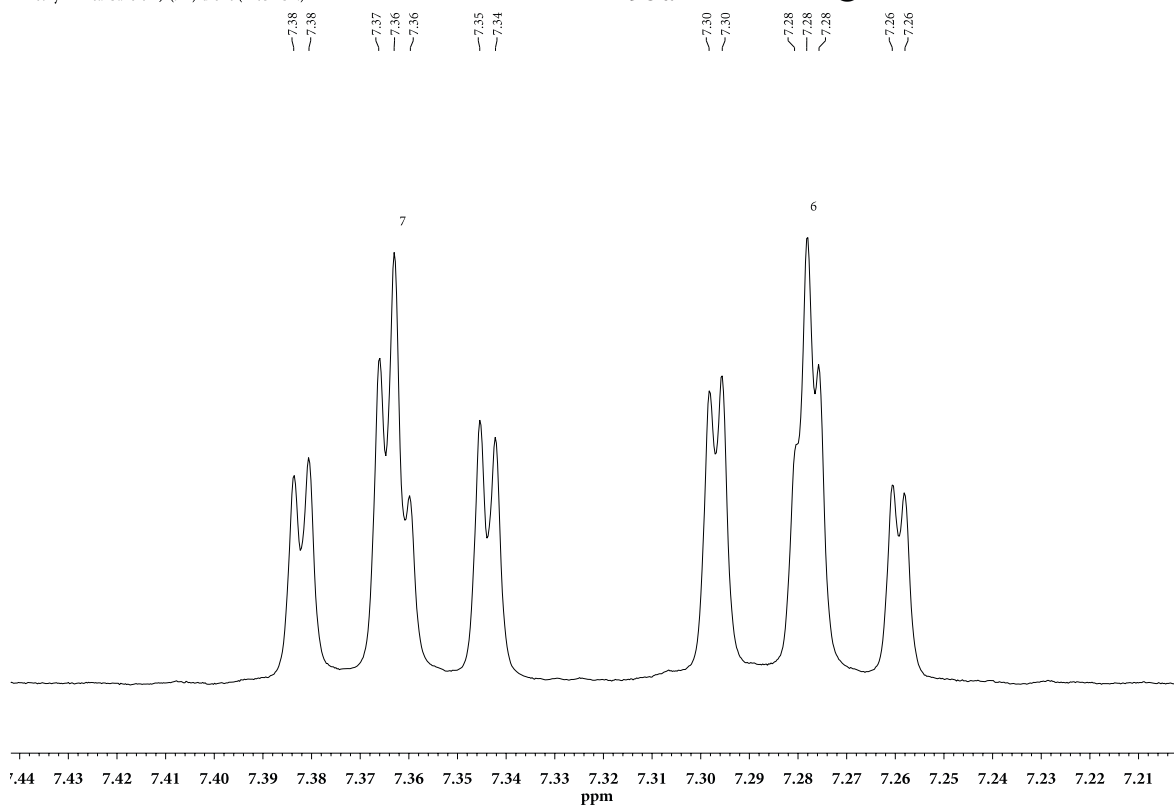| Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
214.0863	1	C13H12NO2	214.0863	-0.1	19.4	1	100.00	8.5	even	ok
236.0684	1	C13H11NNaO2	236.0682	-1.0	2.0	1	100.00	8.5	even	ok
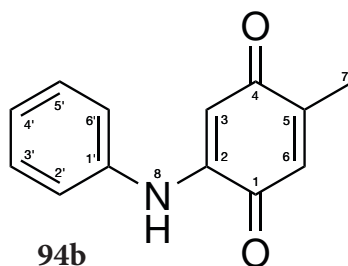
94b

2-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

95a
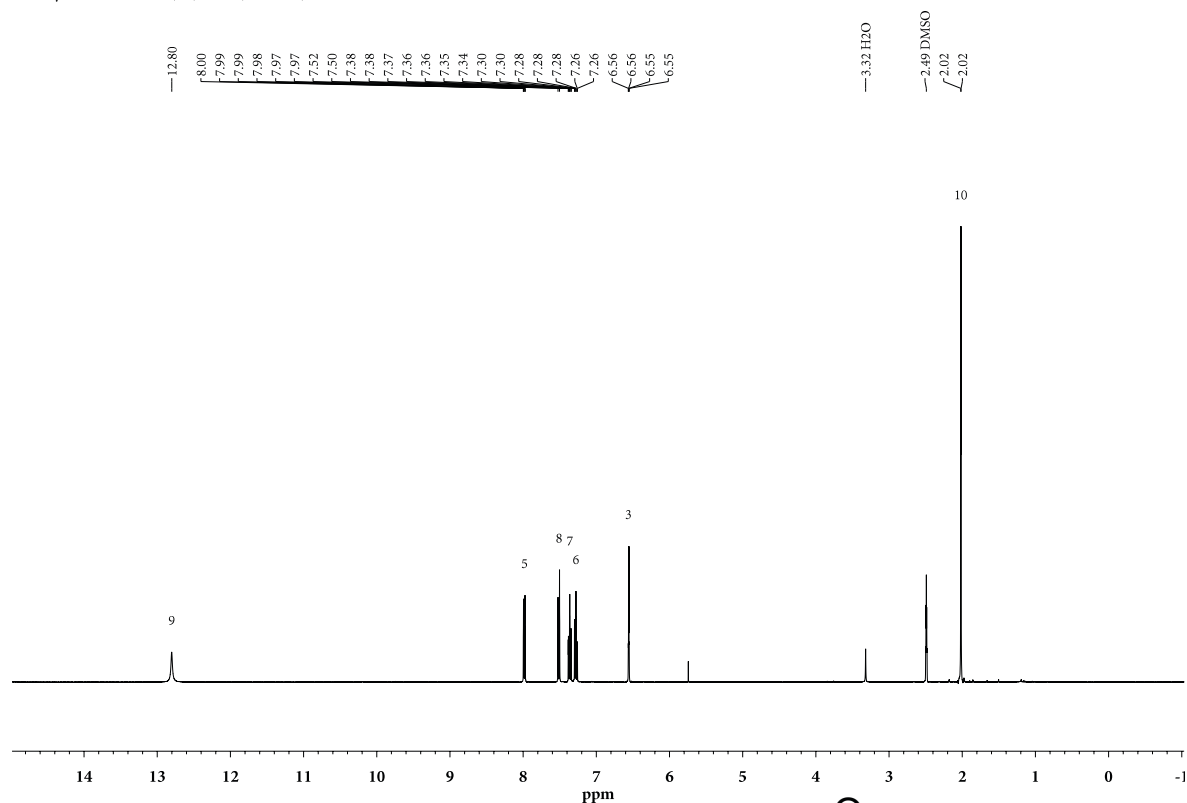
2-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

2-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)
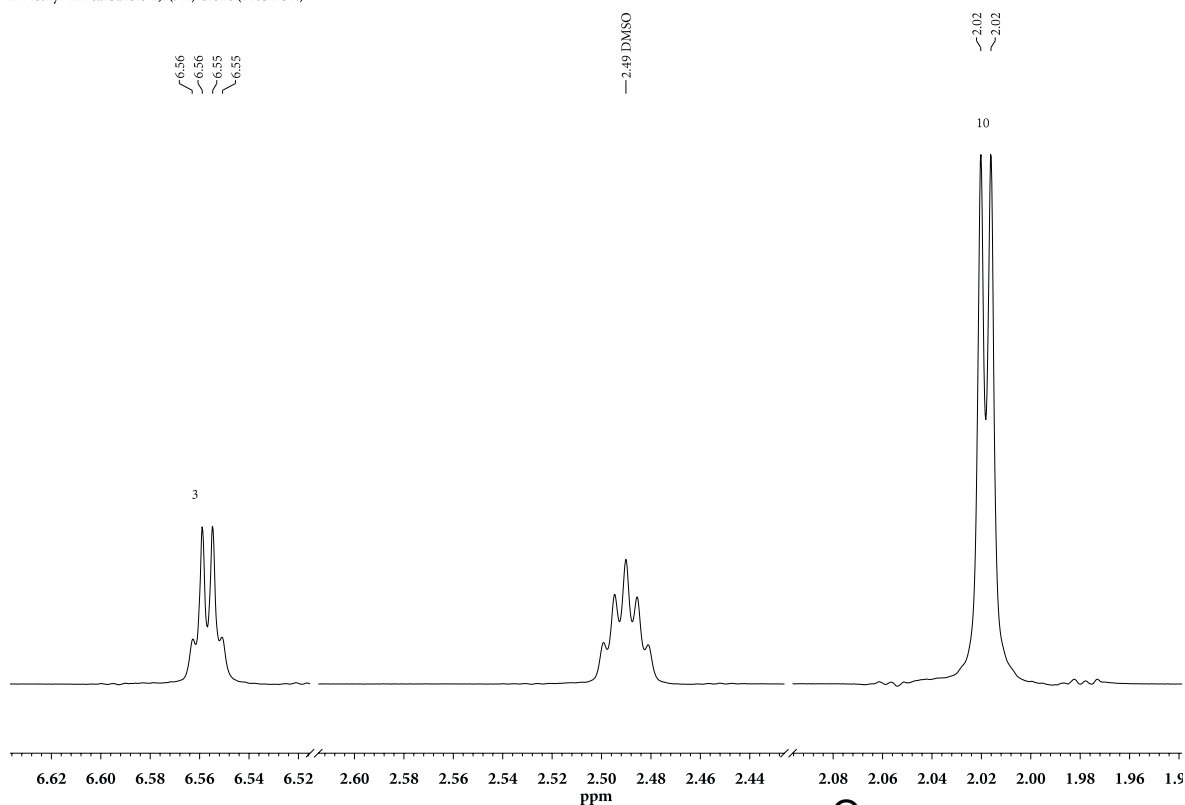
8.00
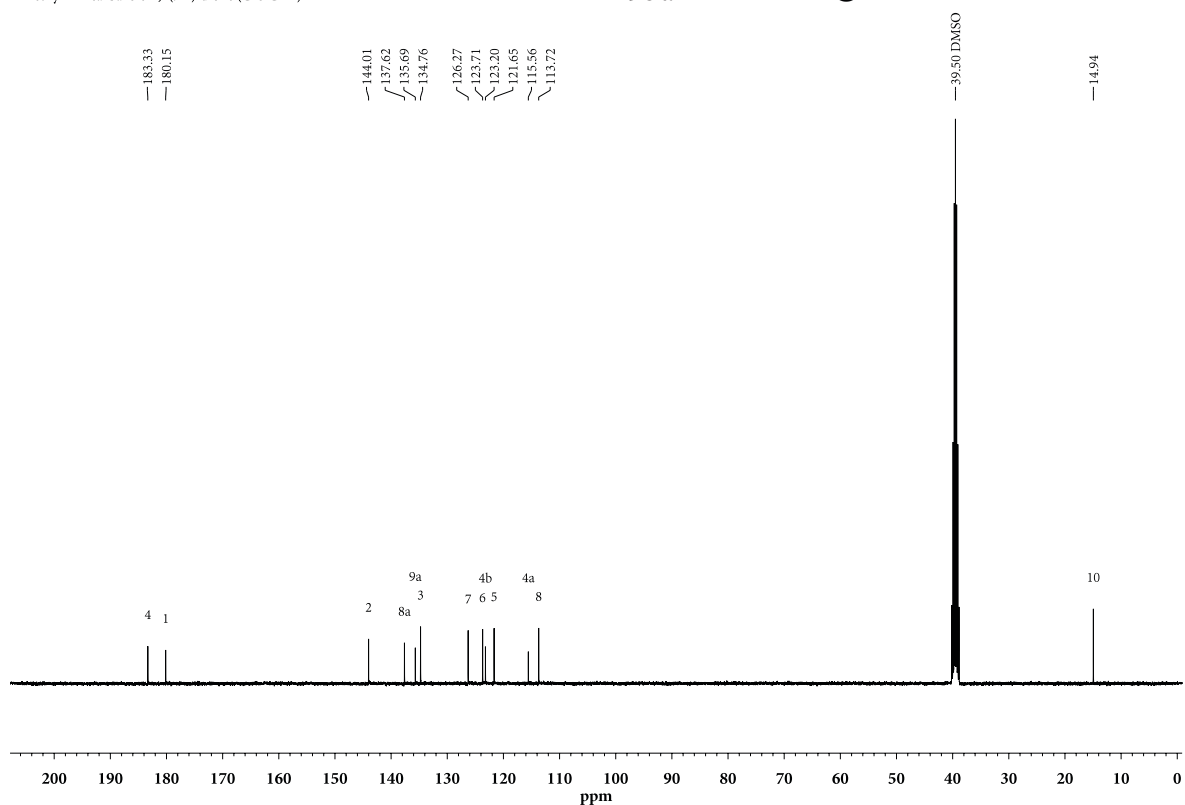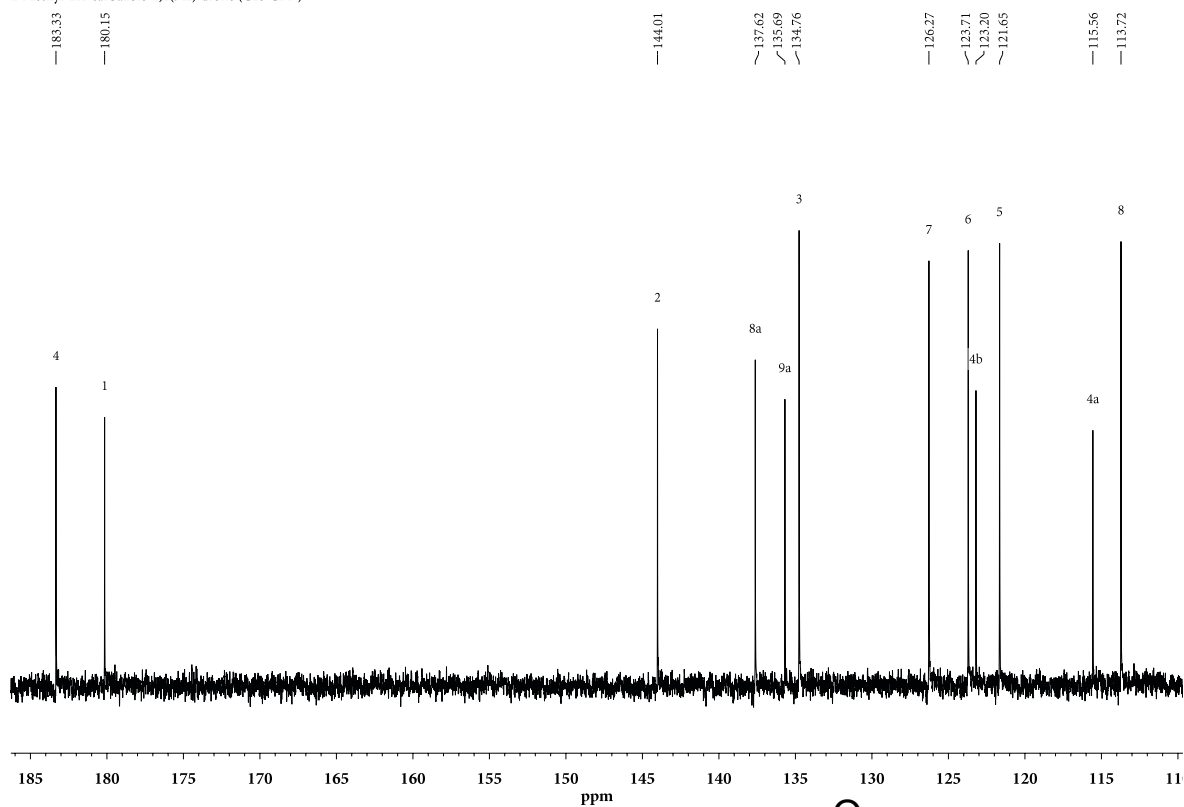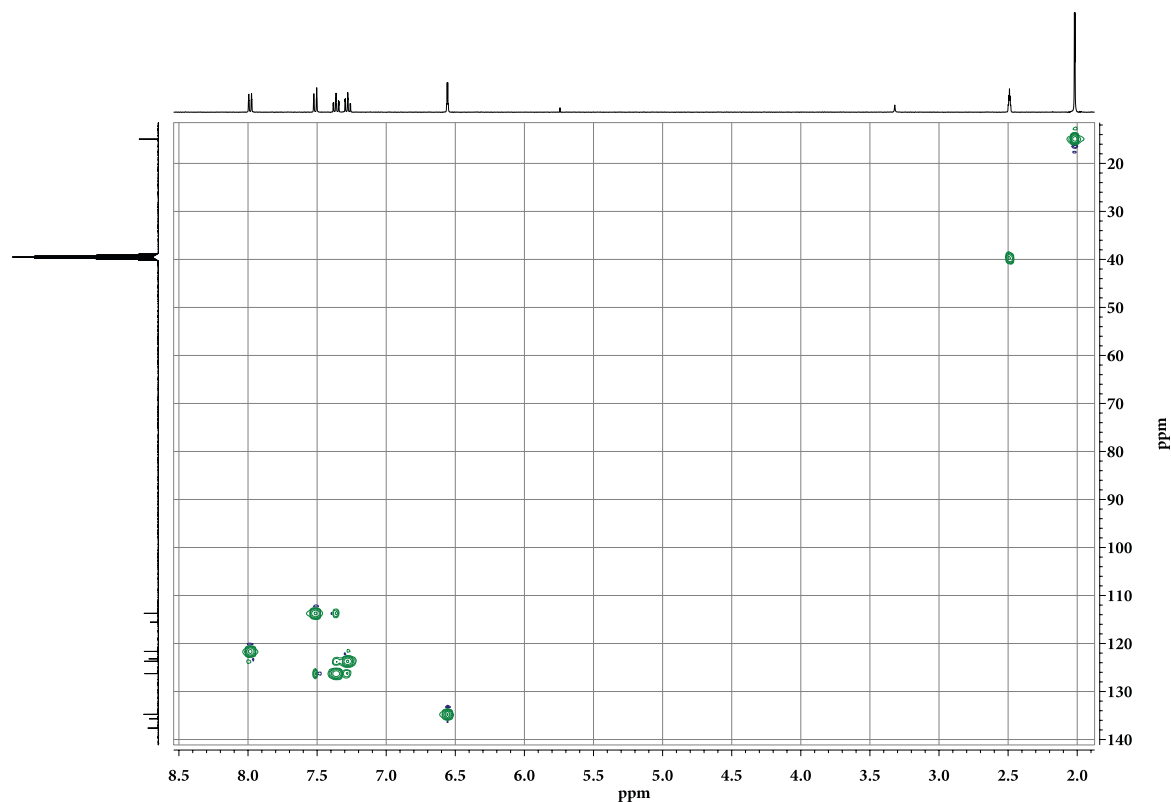7.99
7.99
7.98
7.97
7.97

7.52
7.50

7.38
7.38
7.37
7.36
7.35
7.34
7.30
7.30
7.28
7.28
7.26
7.26

2-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

7.38
7.38

7.37
7.36
7.36

7.35
7.34

7.30
7.30

7.28
7.28
7.28

7.26
7.26

95a

2-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

6.56
6.56
6.55
6.55

—2.49 DMSO

2.02
2.02

3

10

6.62 6.60 6.58 6.56 6.54 6.52   2.60 2.58 2.56 2.54 2.52 2.50 2.48 2.46 2.44   2.08 2.06 2.04 2.02 2.00 1.98 1.96 1.9
ppm

95a

2-Methyl-1H-carbazole-1,4(9H)-dione (C13 CPD)

—183.33
—180.15

144.01
137.62
135.69
134.76

126.27
123.71
123.20
121.65
115.56
113.72

—39.50 DMSO

—14.94

4   1

2
8a
9a
3
7 6 5
4b
4a
8

10

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

2-Methyl-1H-carbazole-1,4(9H)-dione (C13 CPD)

—183.33
—180.15
—144.01
—137.62
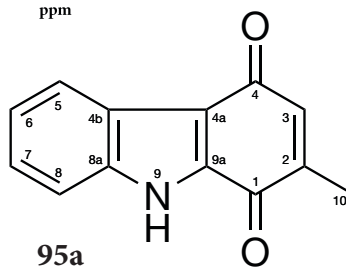—135.69
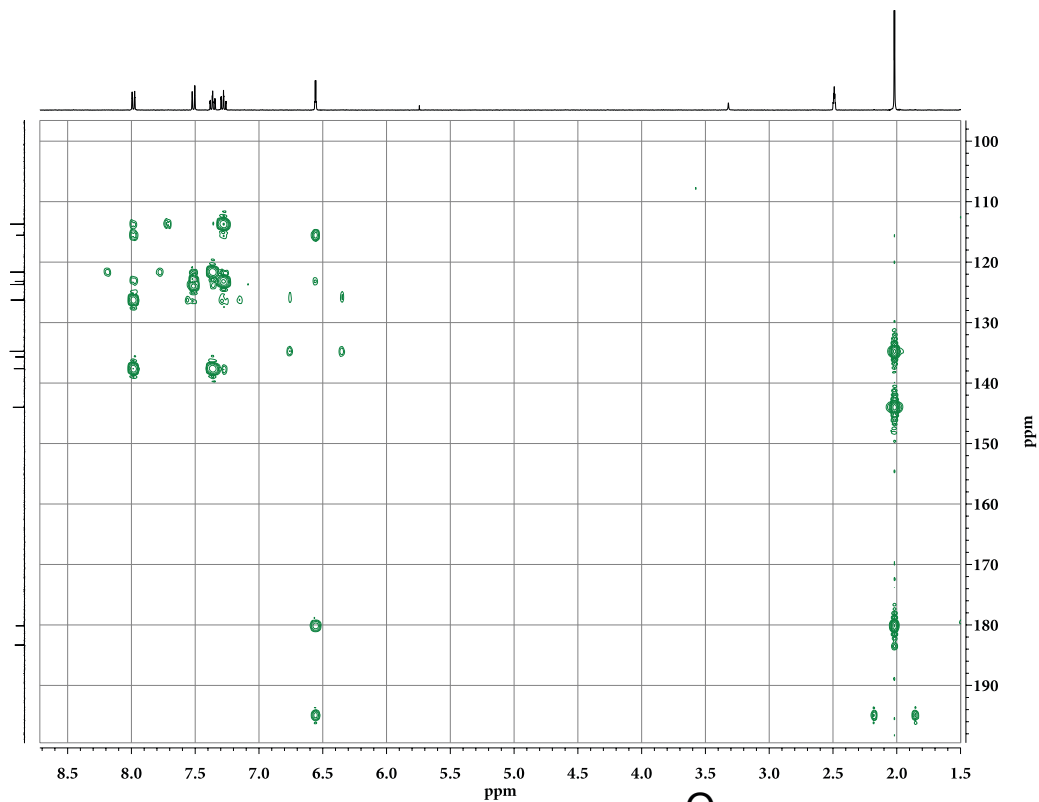—134.76
—126.27
—123.71
—123.20
—121.65
—115.56
—113.72

95a

2-Methyl-1H-carbazole-1,4(9H)-dione (HSQC)
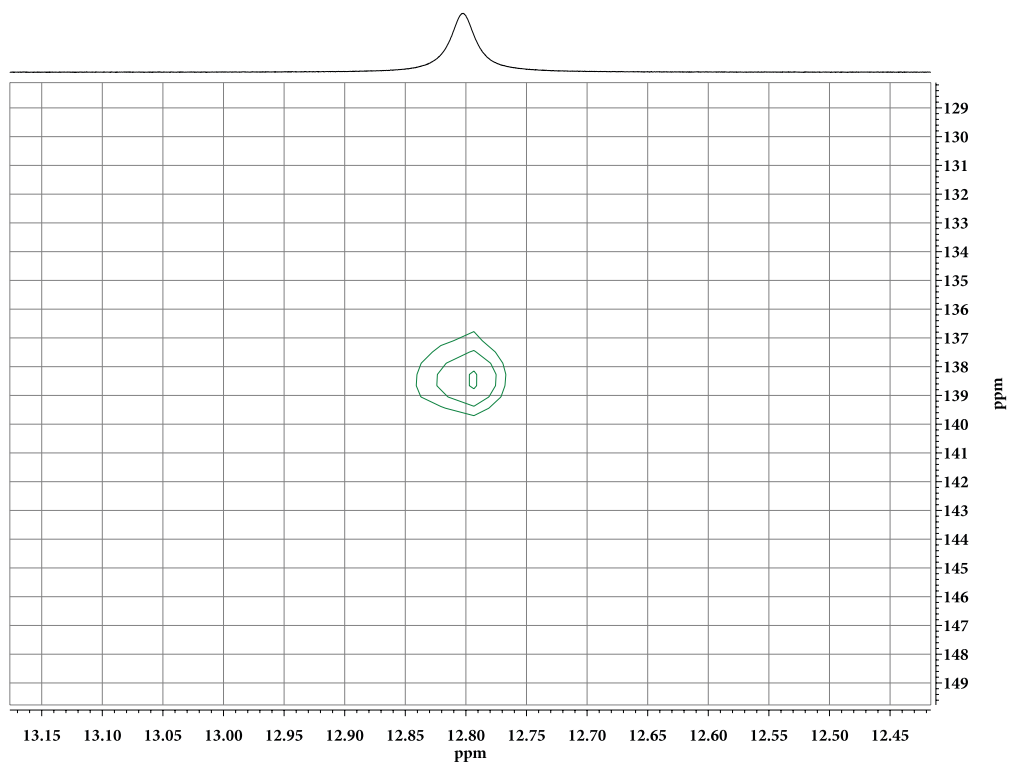
2-Methyl-1H-carbazole-1,4(9H)-dione (HMBC)

95a

2-Methyl-1H-carbazole-1,4(9H)-dione (15N HSQC)

Generic Display Report

Analysis Info   Acquisition Date   1/14/2015 10:42:38 AM
Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\150114\TN126_DI_APCI_HRMS.d
Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   100 ug/mL in MeOH   Instrument   maXis HD
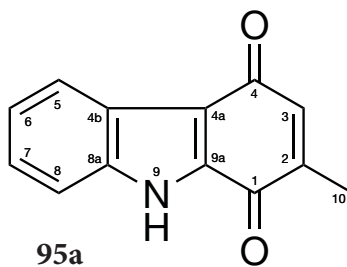Comment

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/14/2015 10:42:38 AM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150114\TN126_DI_APCI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	100 ug/mL in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Active	Set Capillary	1600 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	3.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	3000 nA	Set APCI Heater	470 °C

+MS, 0.0-1.5min #1-86
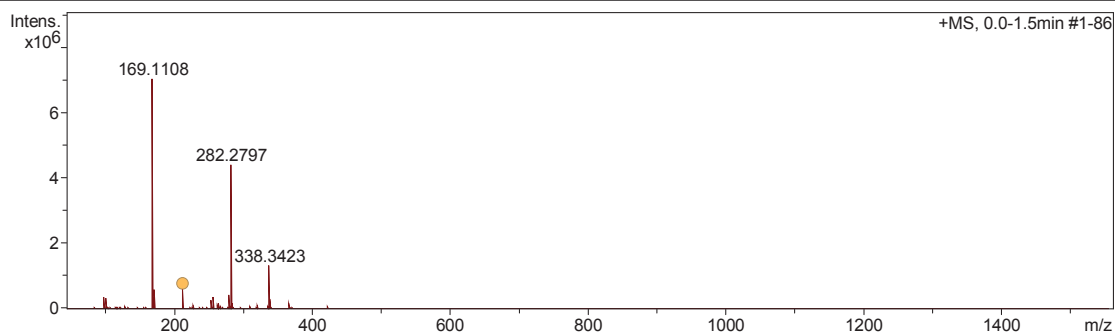
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
212.0709	1	C13H10NO2	212.0706	-1.5	2.7	1	100.00	9.5	even	ok

95a
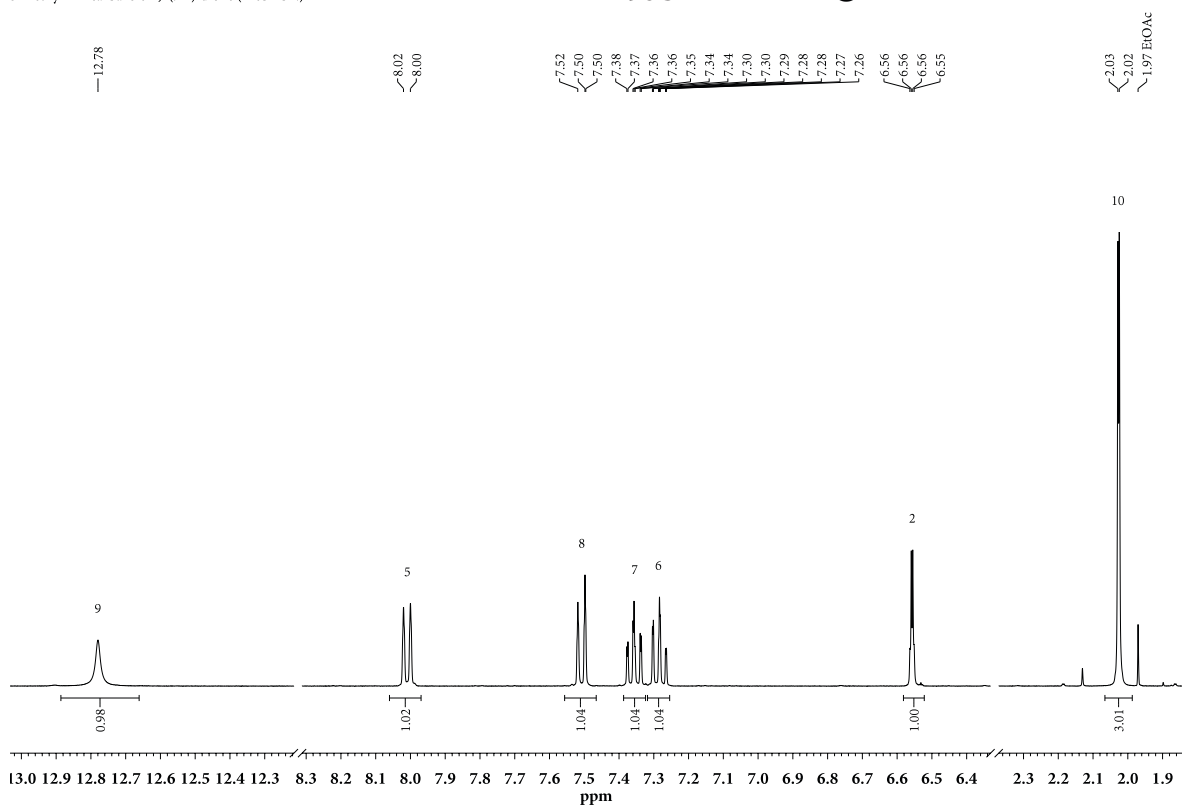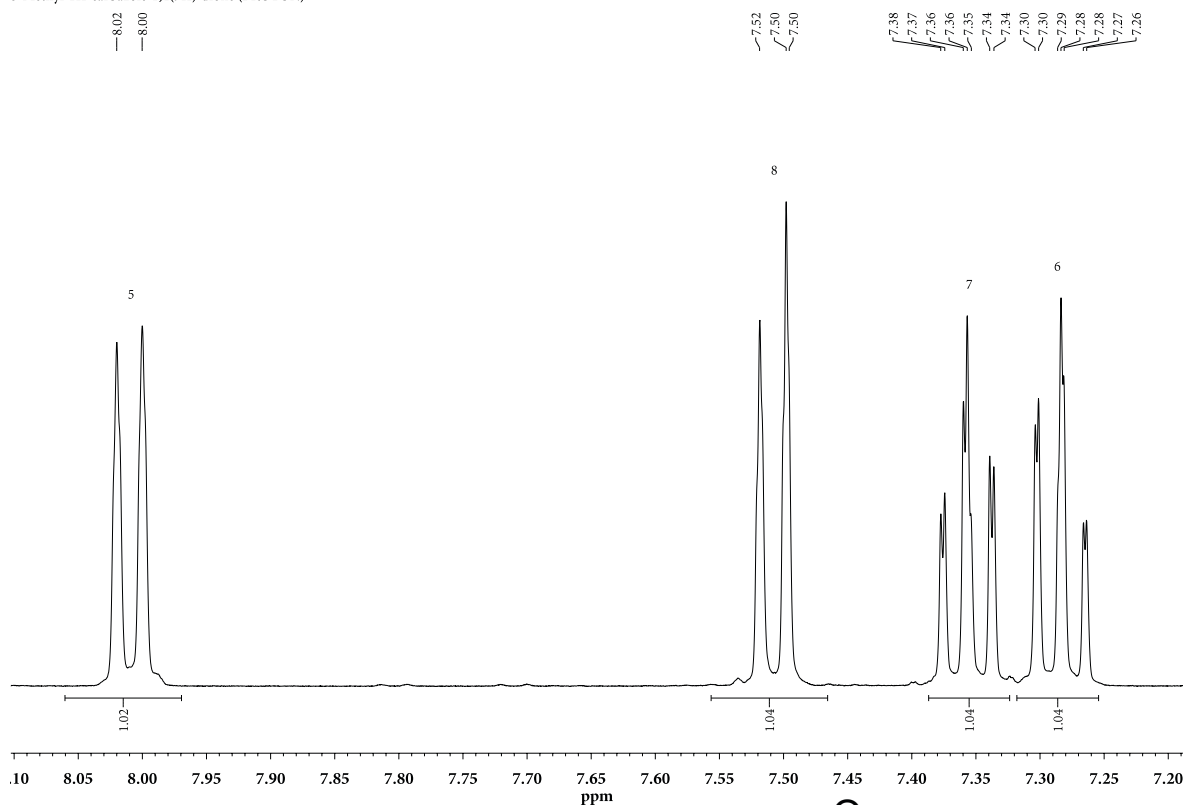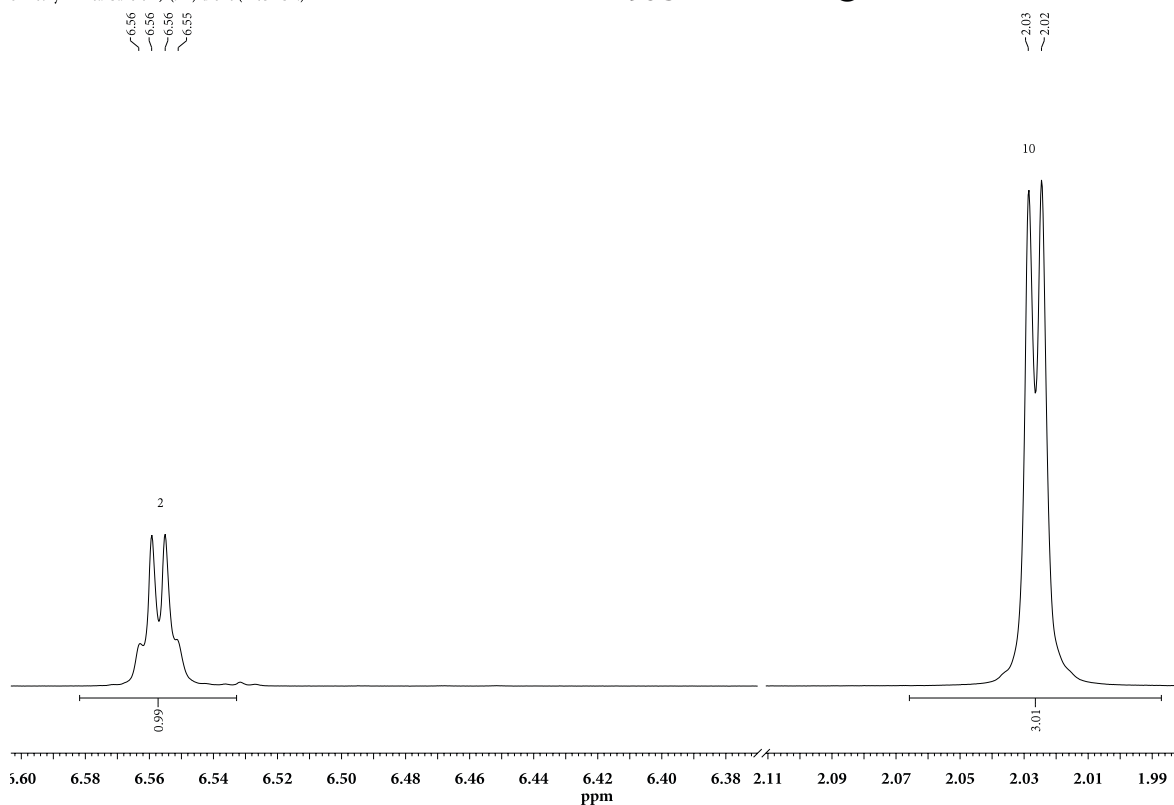
3-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

3-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

95b

3-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

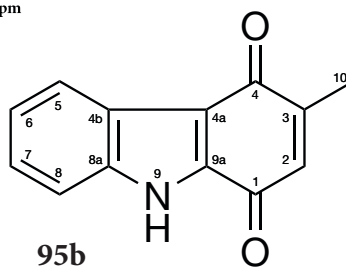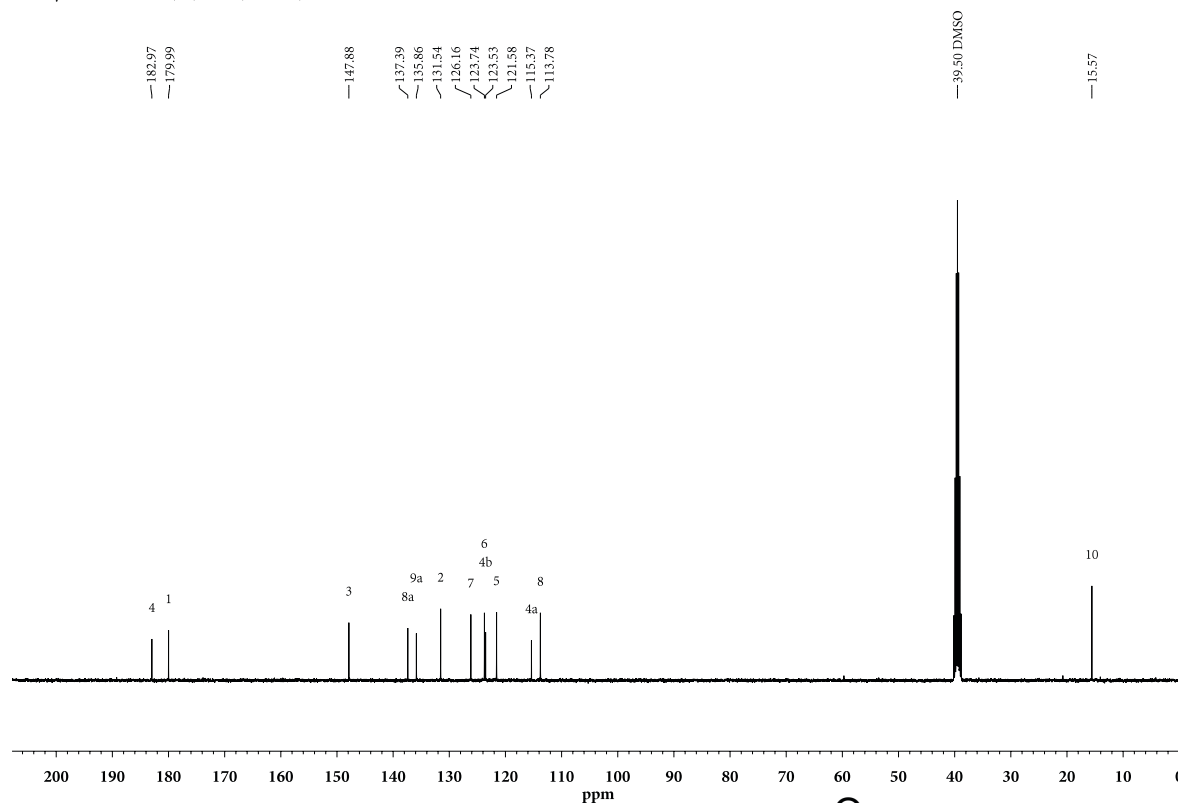8.02
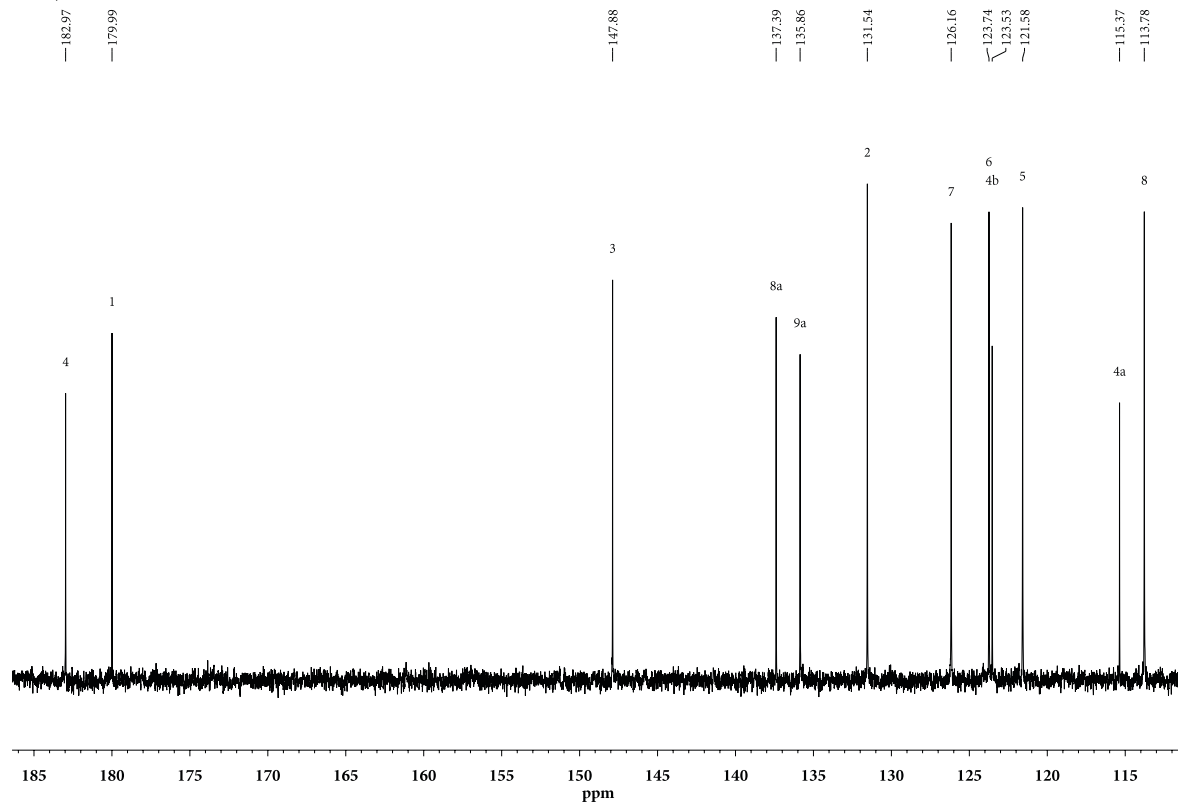8.00

7.52
7.50
7.50

7.38
7.37
7.36
7.36
7.35
7.34
7.30
7.30
7.29
7.28
7.27
7.26

5

8

7

6

1.02

1.04

1.04

1.04

| 8.05 | 8.00 | 7.95 | 7.90 | 7.85 | 7.80 | 7.75 | 7.70 | 7.65 | 7.60 | 7.55 | 7.50 | 7.45 | 7.40 | 7.35 | 7.30 | 7.25 | 7.20 |

ppm

95b

3-Methyl-1H-carbazole-1,4(9H)-dione (PROTON)

6.56
6.56
6.56
6.55

2.03
2.02

10

2

0.99

3.01

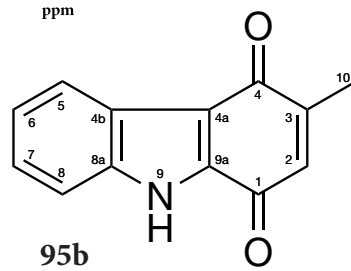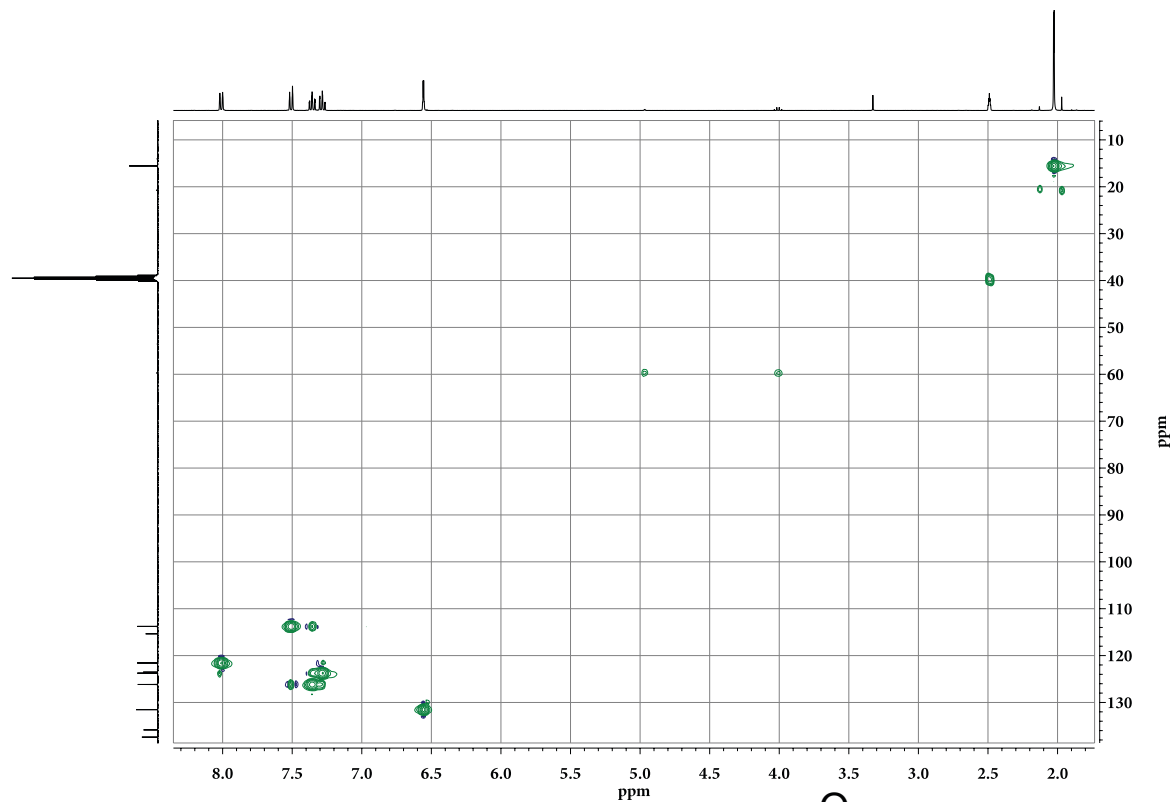| 6.60 | 6.58 | 6.56 | 6.54 | 6.52 | 6.50 | 6.48 | 6.46 | 6.44 | 6.42 | 6.40 | 6.38 | 2.11 | 2.09 | 2.07 | 2.05 | 2.03 | 2.01 | 1.99 |

ppm

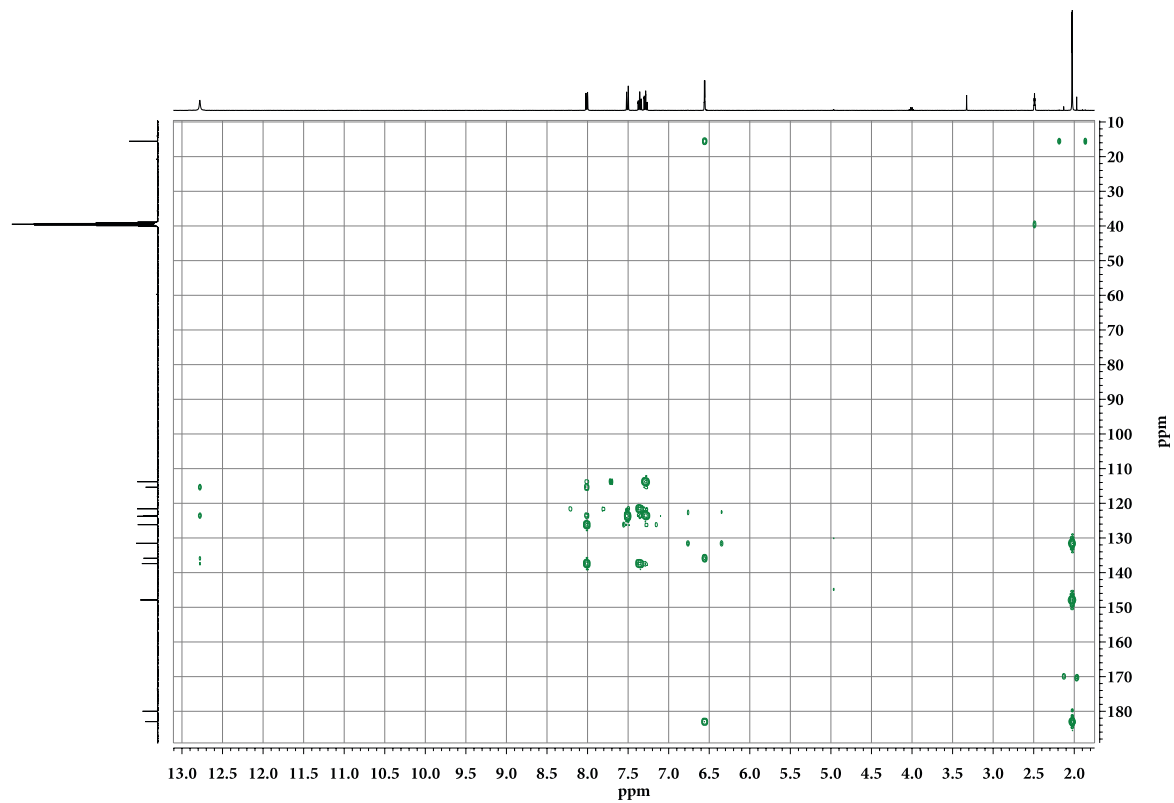3-Methyl-1H-carbazole-1,4(9H)-dione (C13 CPD)

95b

3-Methyl-1H-carbazole-1,4(9H)-dione (C13 CPD)
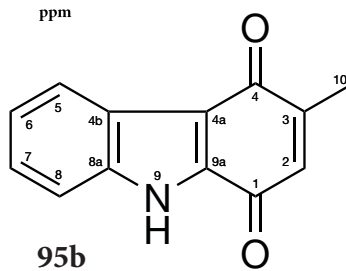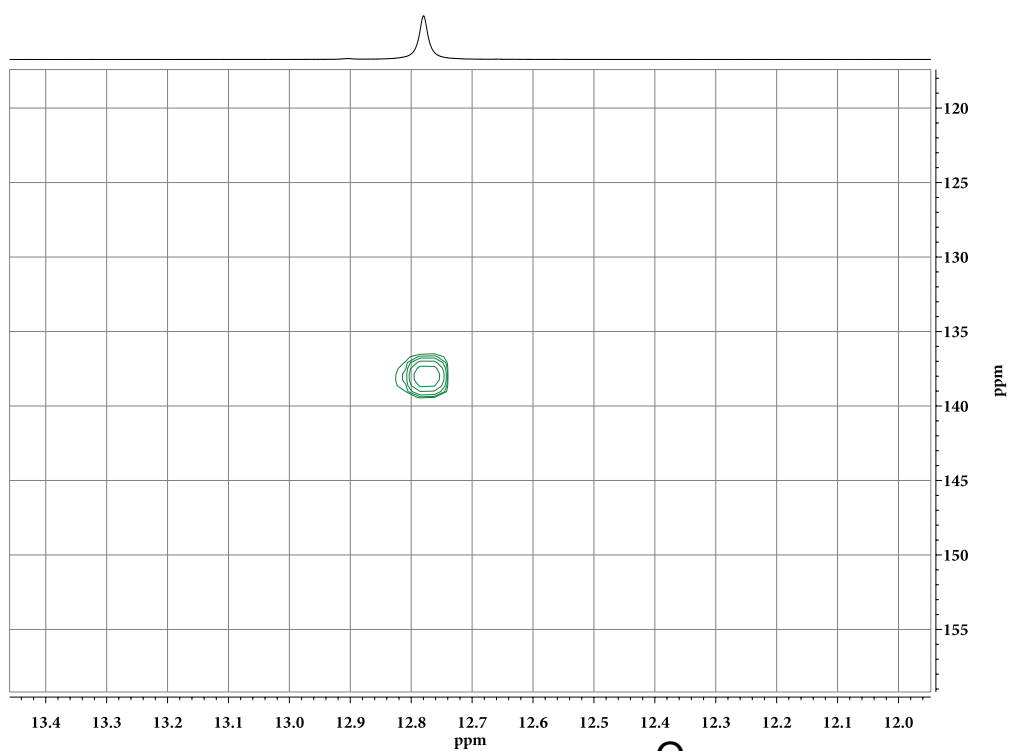
3-Methyl-1H-carbazole-1,4(9H)-dione (HSQC)

95b

3-Methyl-1H-carbazole-1,4(9H)-dione (HMBC)

3-Methyl-1H-carbazole-1,4(9H)-dione (15N HSQC)

95b

Generic Display Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN125_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 4:28:32 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD
Comment			

95b

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/12/2015 4:28:32 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN125_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar		
Focus	Active	Set Capillary	3000 V	Set Dry Heater	200 °C		
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min		
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste		
		Set Corona	0 nA	Set APCI Heater	0 °C		

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
234.0522	1	C13H9NNaO2	234.0525	1.5	8.3	1	100.00	9.5	even	ok

95b

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

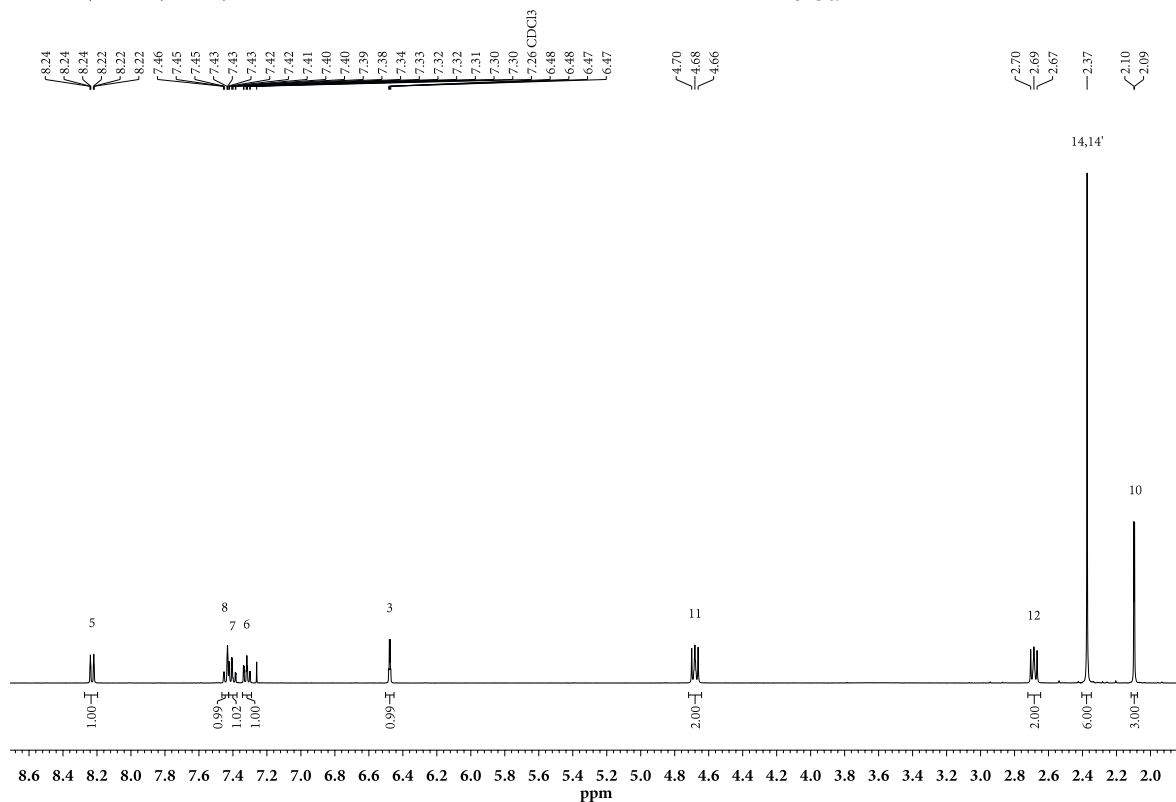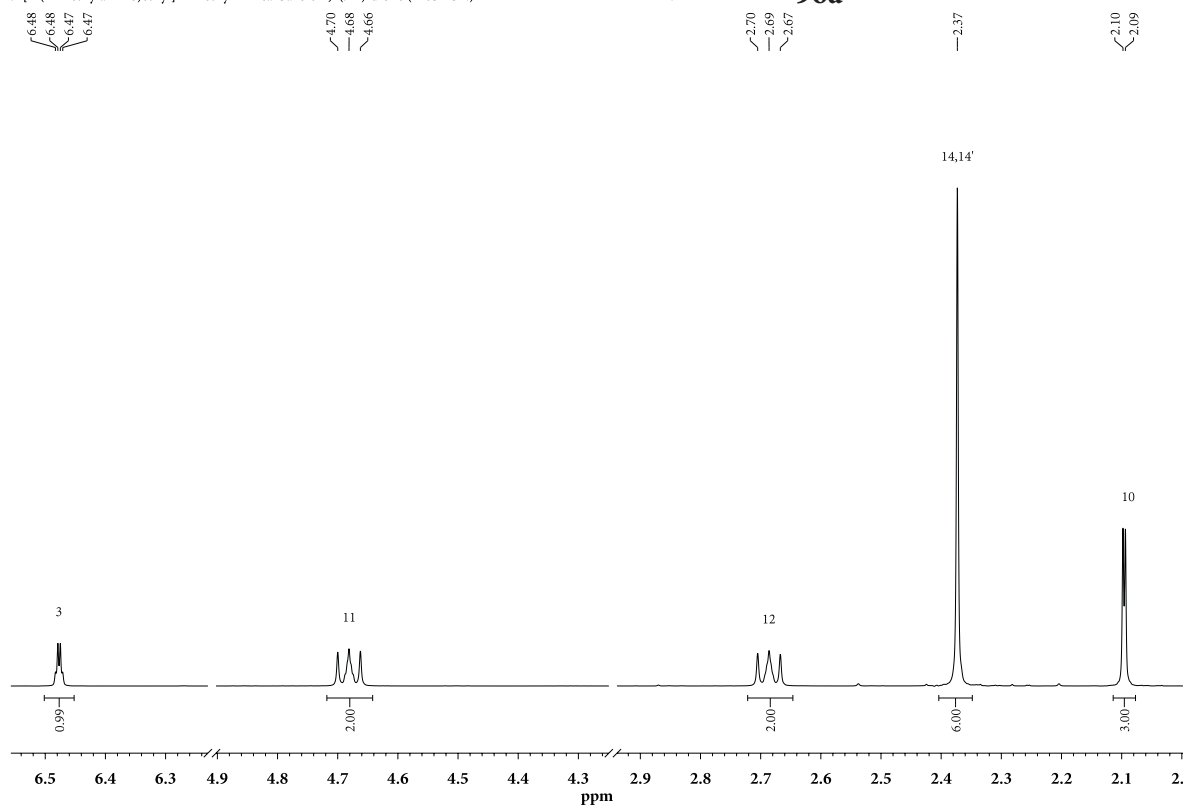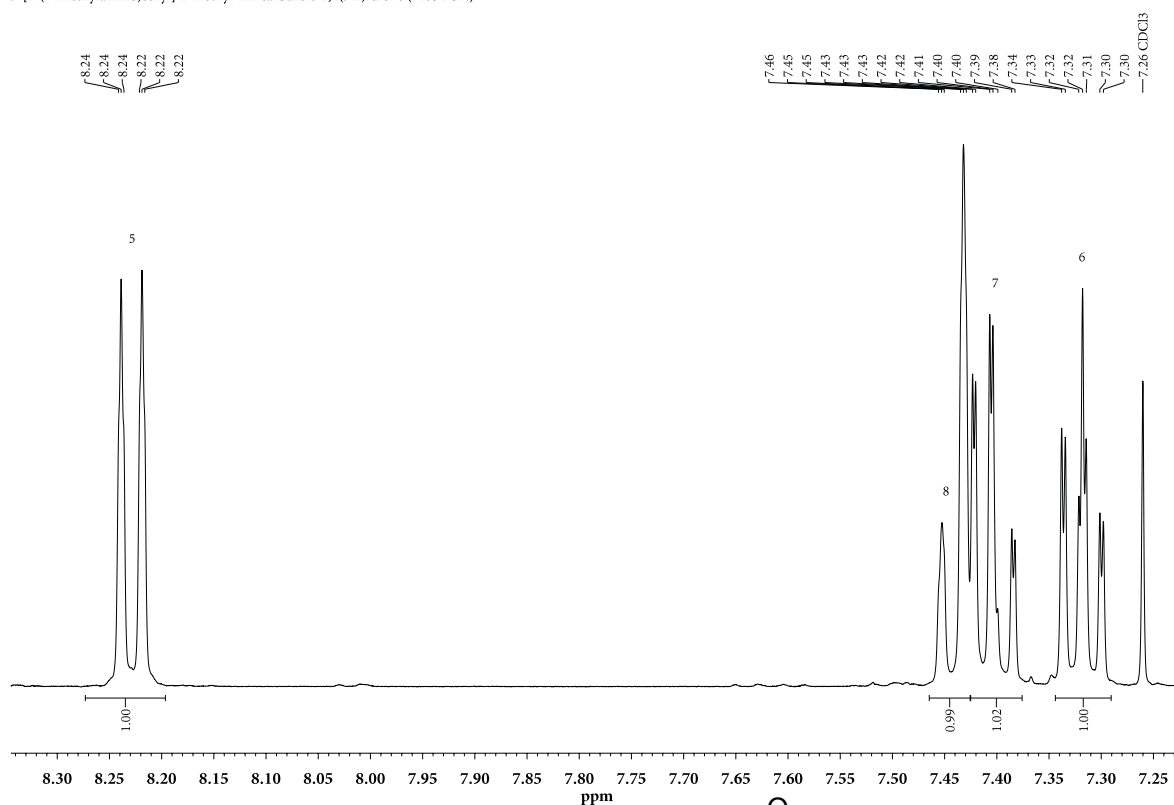**96a**

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)
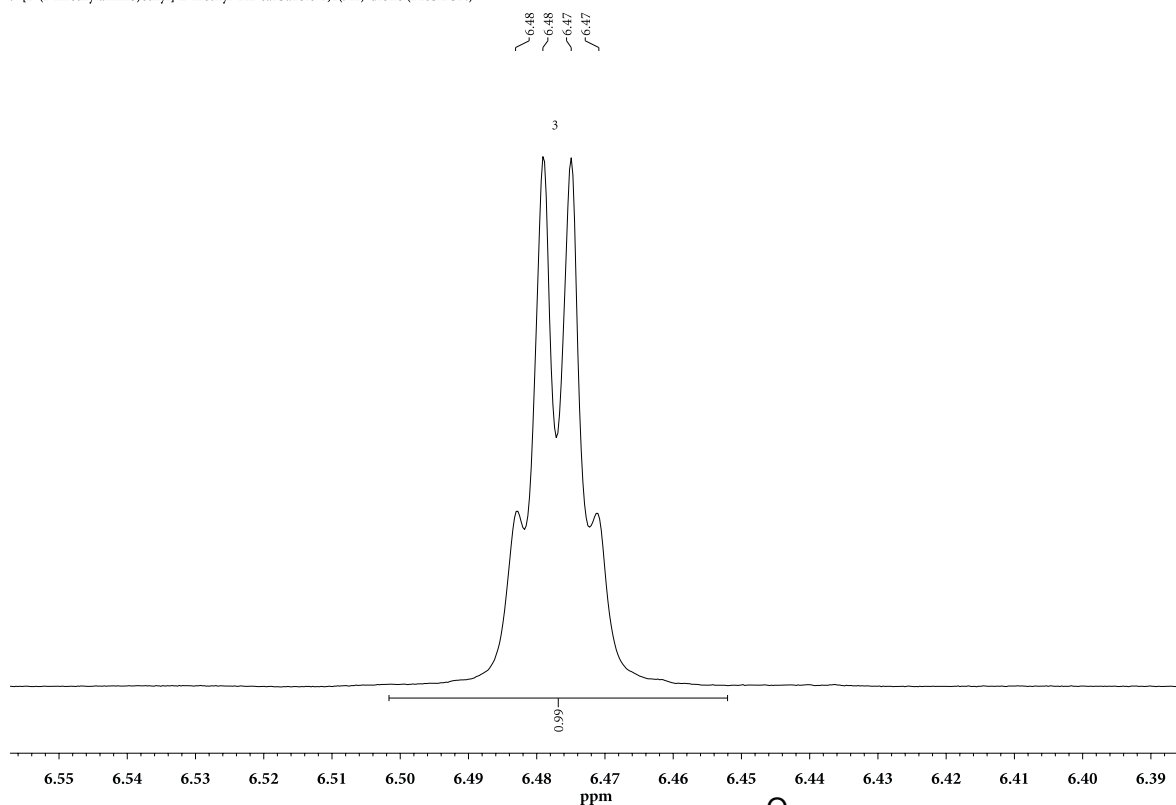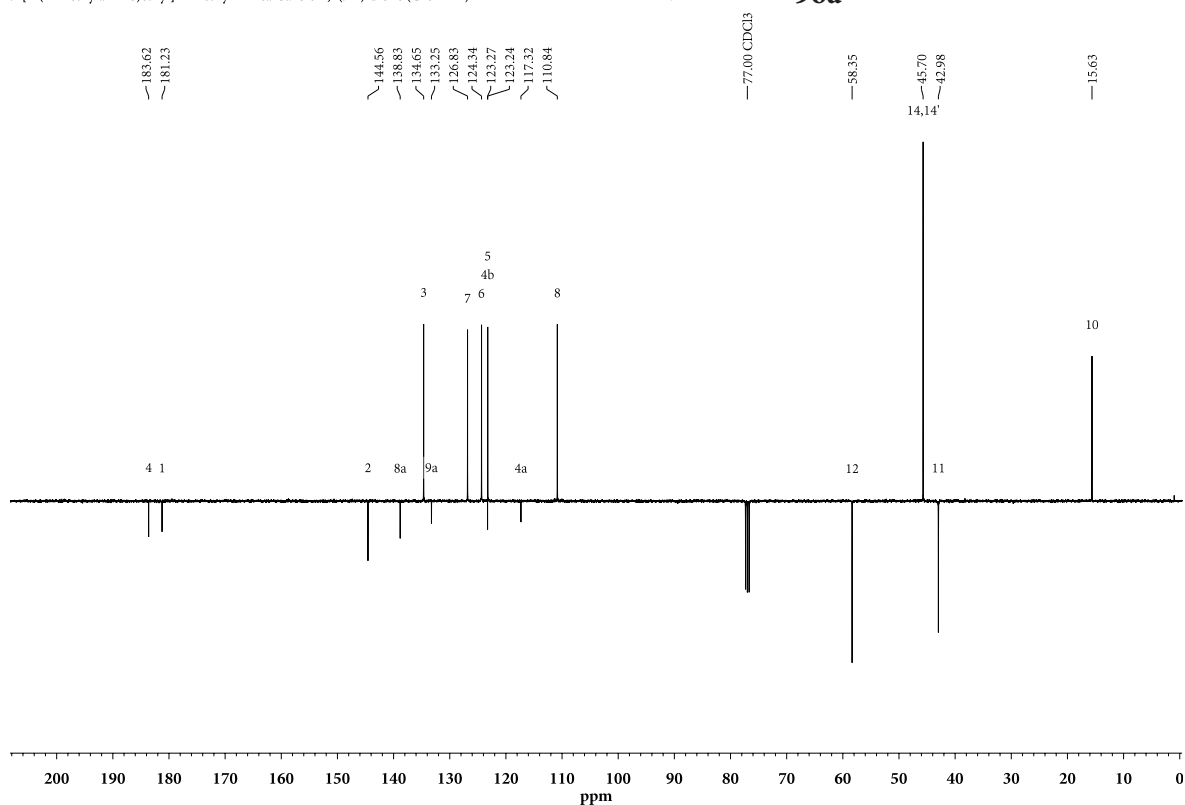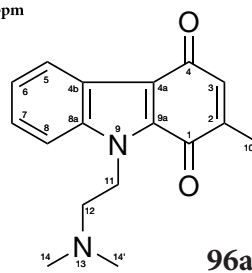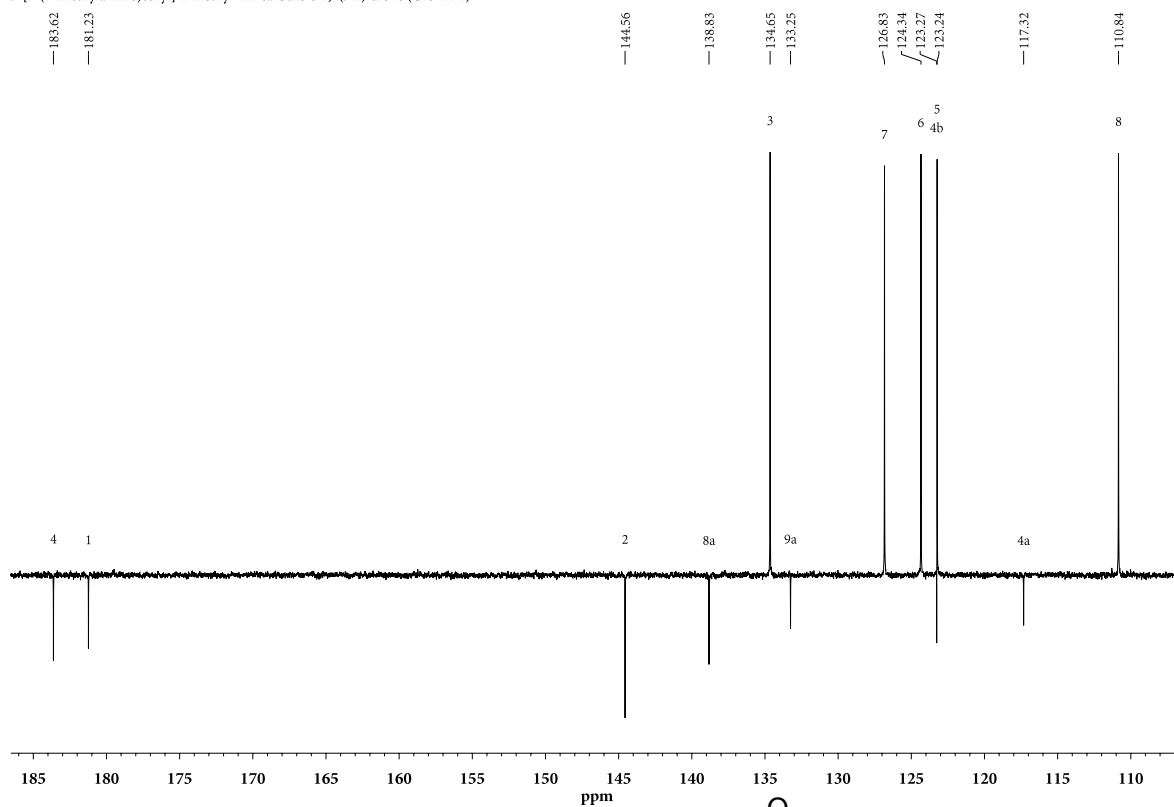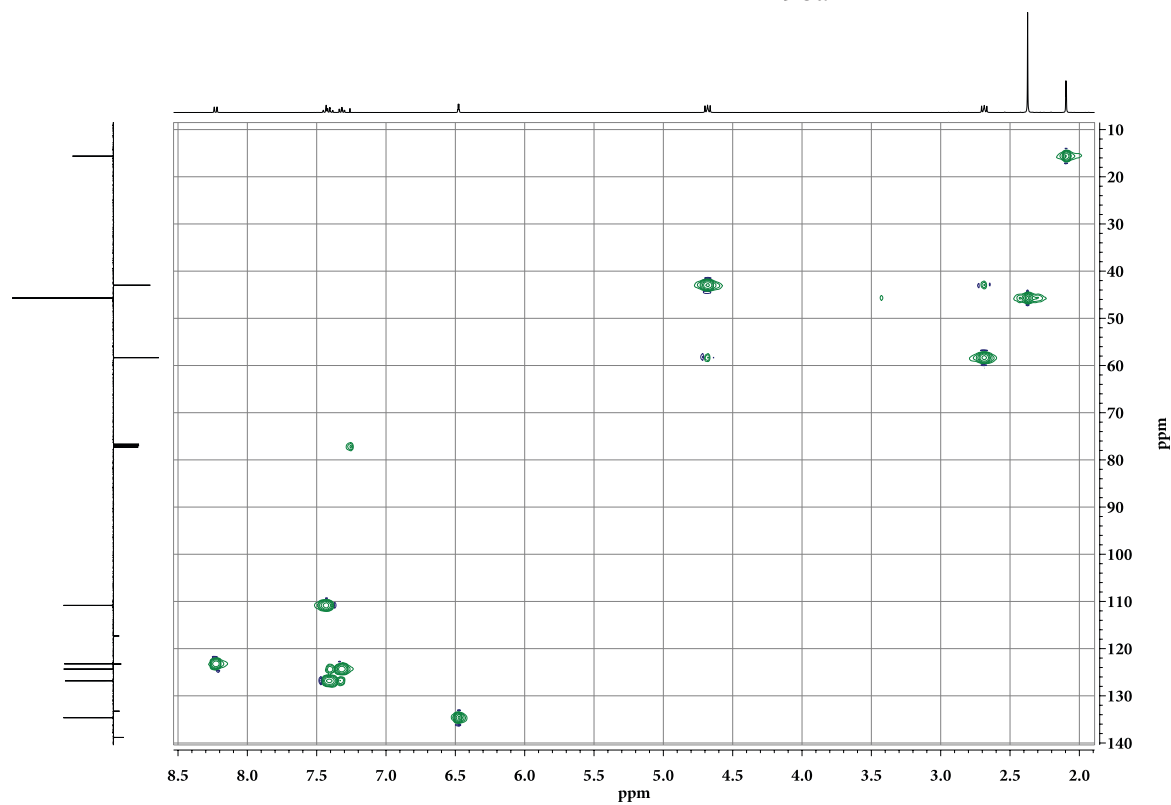
96a

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

6.48
6.48
6.47
6.47

3

0.99

6.55 6.54 6.53 6.52 6.51 6.50 6.49 6.48 6.47 6.46 6.45 6.44 6.43 6.42 6.41 6.40 6.39
ppm

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

96a

183.62
181.23

144.56
138.83
134.65
133.25
126.83
124.34
123.27
123.24
117.32
110.84

77.00 CDCl3

58.35

45.70
42.98
14,14'

15.63

5
4b
3   7 6   8
4 1   2 8a 9a   4a   12   11   10

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

—183.62
—181.23

—144.56

—138.83

—134.65
—133.25

—126.83
—124.34
—123.27
—123.24

—117.32

—110.84

96a

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HSQC)
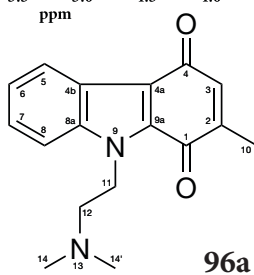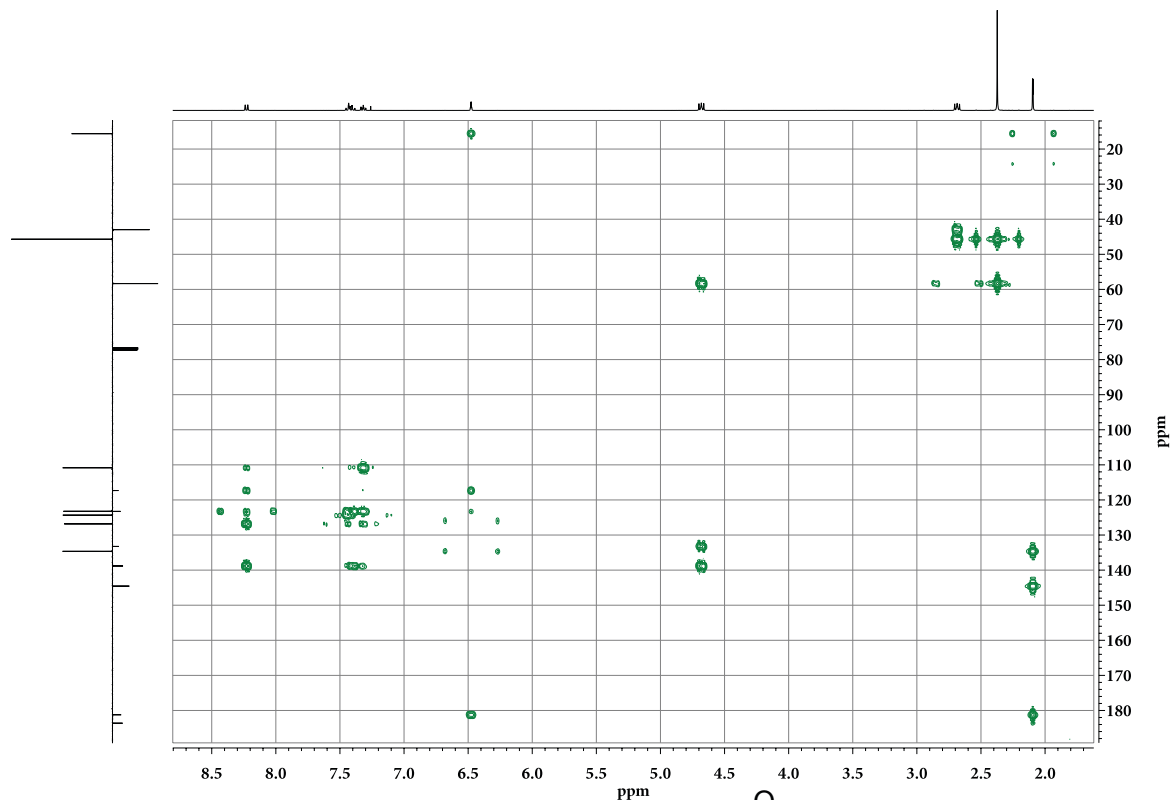
9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HMBC)

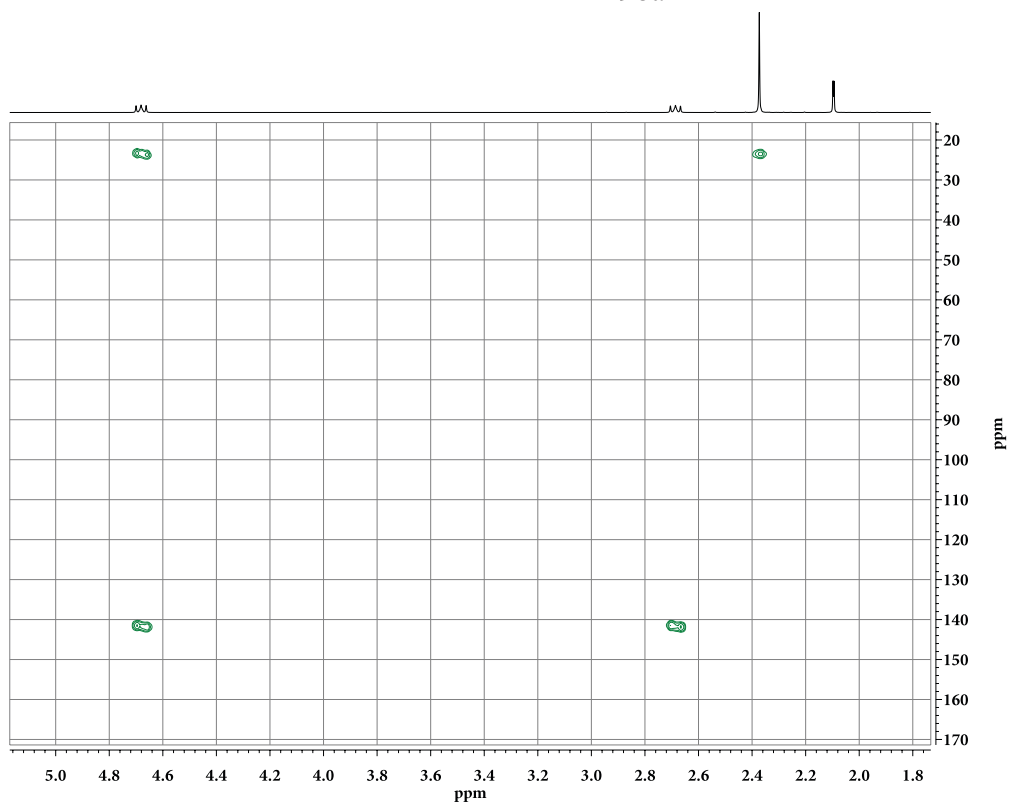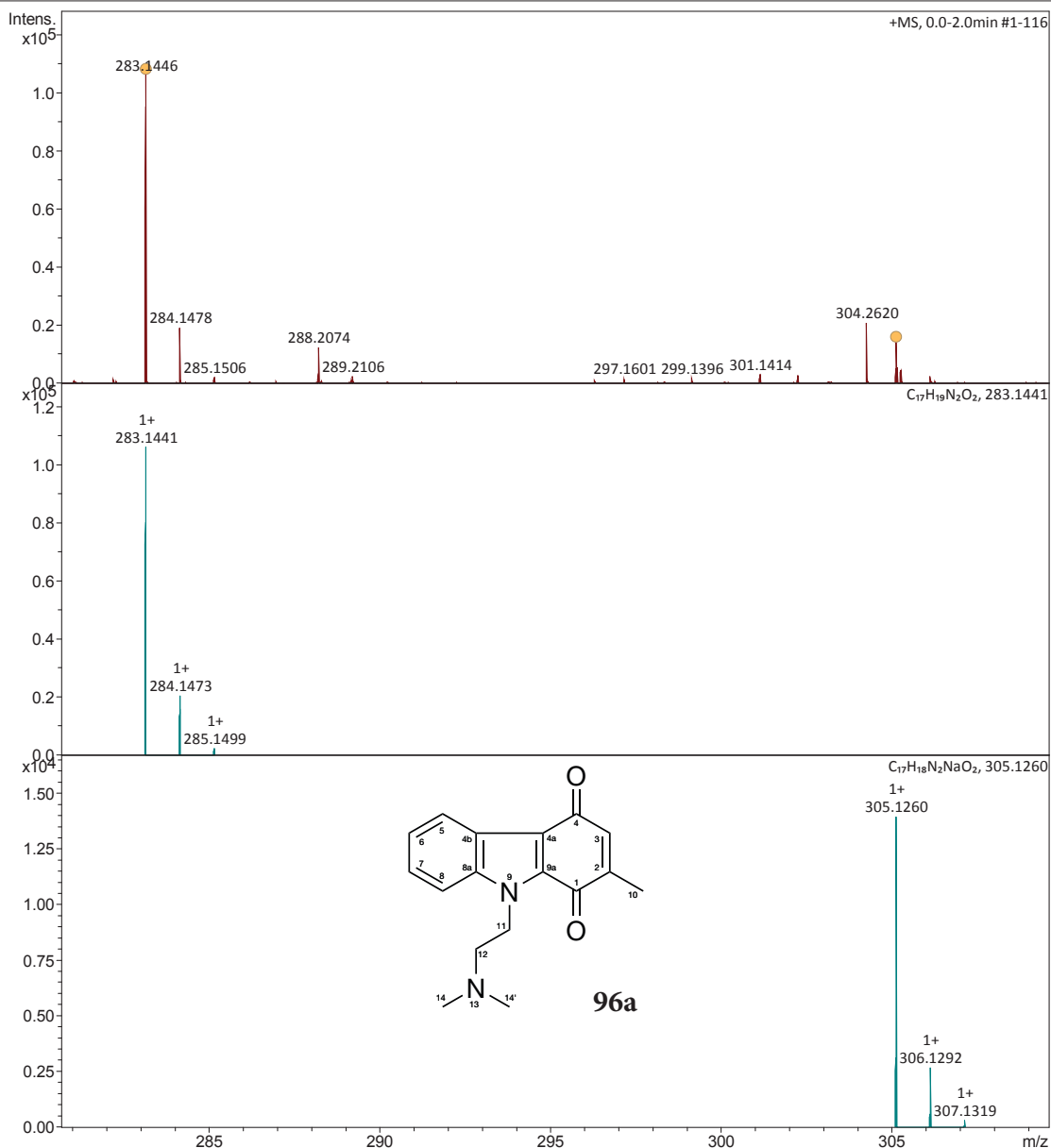9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (15N HMBC)

96a

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 4:14:56 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN129_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

$C_{17}H_{19}N_2O_2$, 283.1441

$C_{17}H_{18}N_2NaO_2$, 305.1260

96a

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   1/12/2015 4:14:56 PM

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN129_DI_ESI_HRMS.d

Method   DI_mz_50-1550.m   Operator   MZ
Sample Name   in MeOH --> 1:500 in MeOH   Instrument   maXis HD   1820881.21300
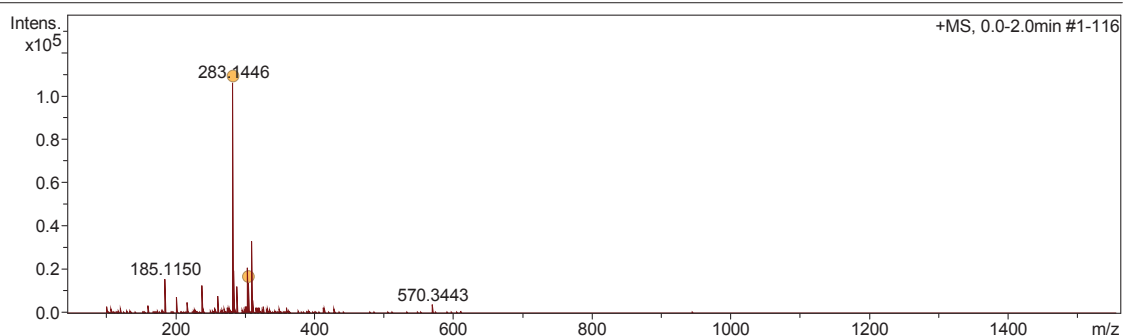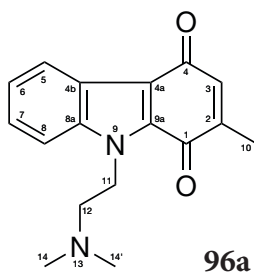Comment

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1300 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C
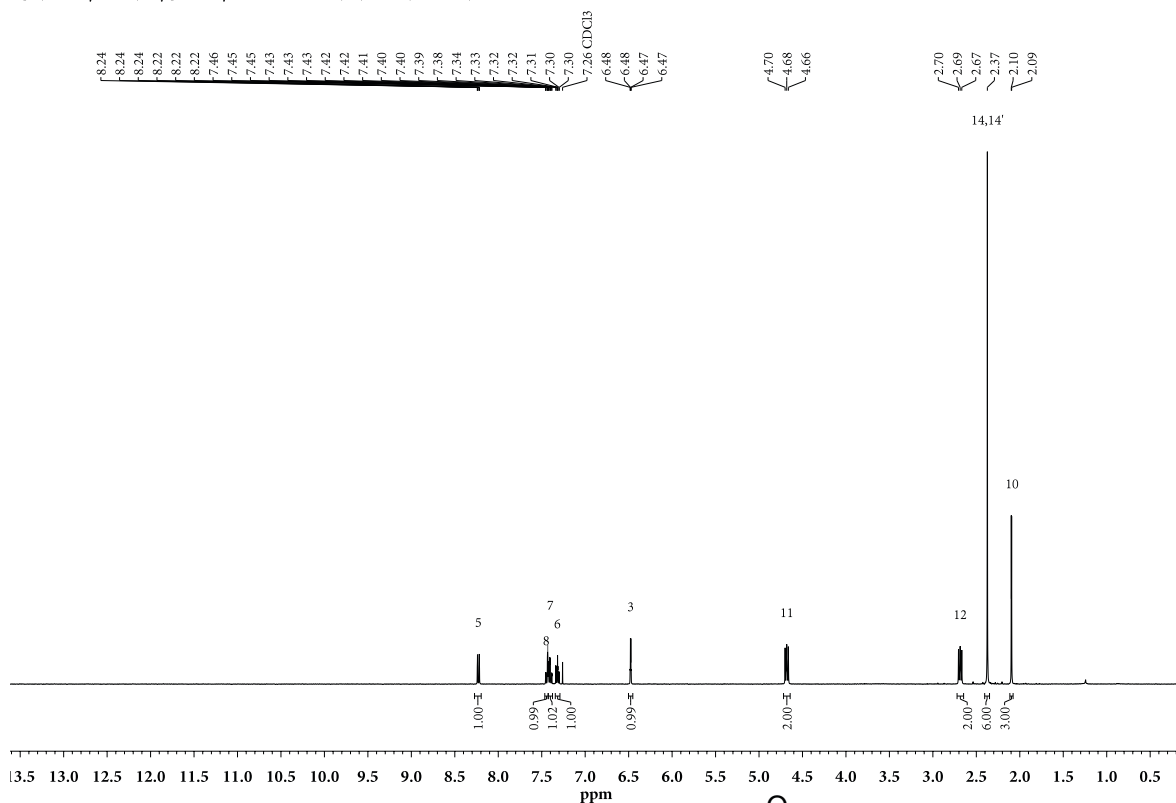
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
283.1446	1	C17H19N2O2	283.1441	-1.9	7.0	1	100.00	9.5	even	ok
305.1264	1	C17H18N2NaO2	305.1260	-1.3	4.7	1	100.00	9.5	even	ok

96a

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)
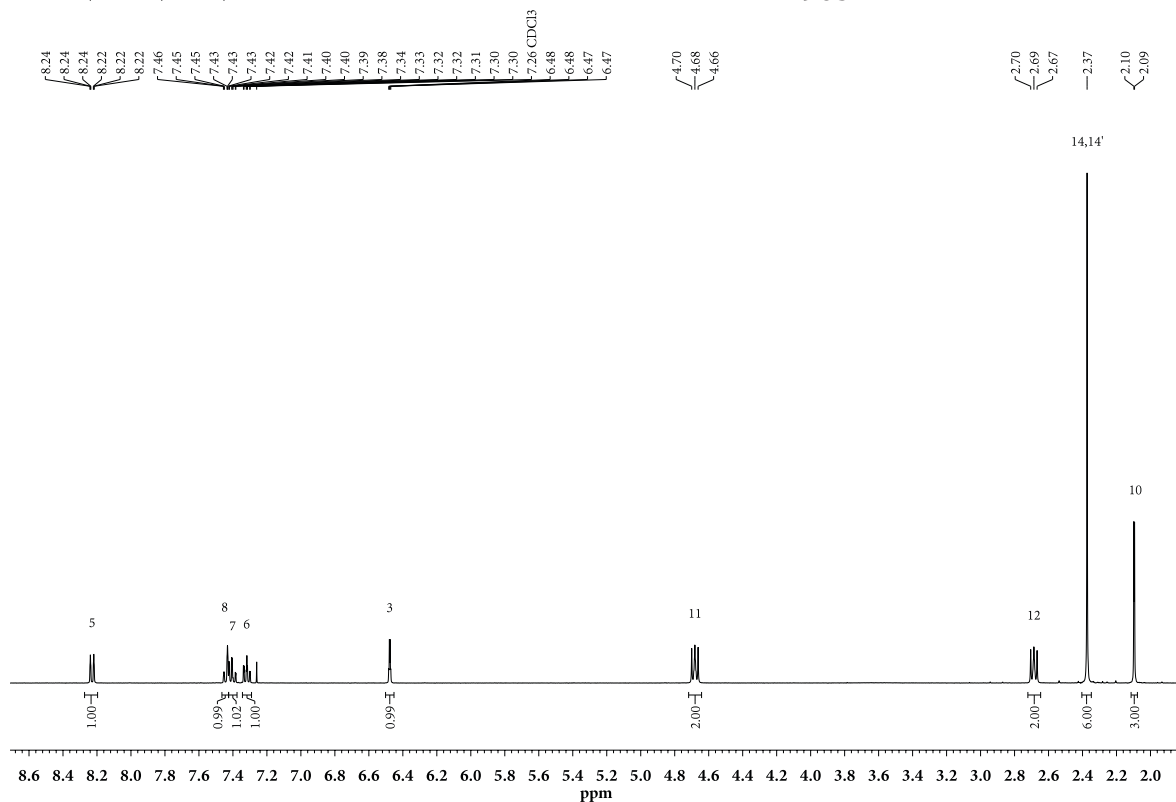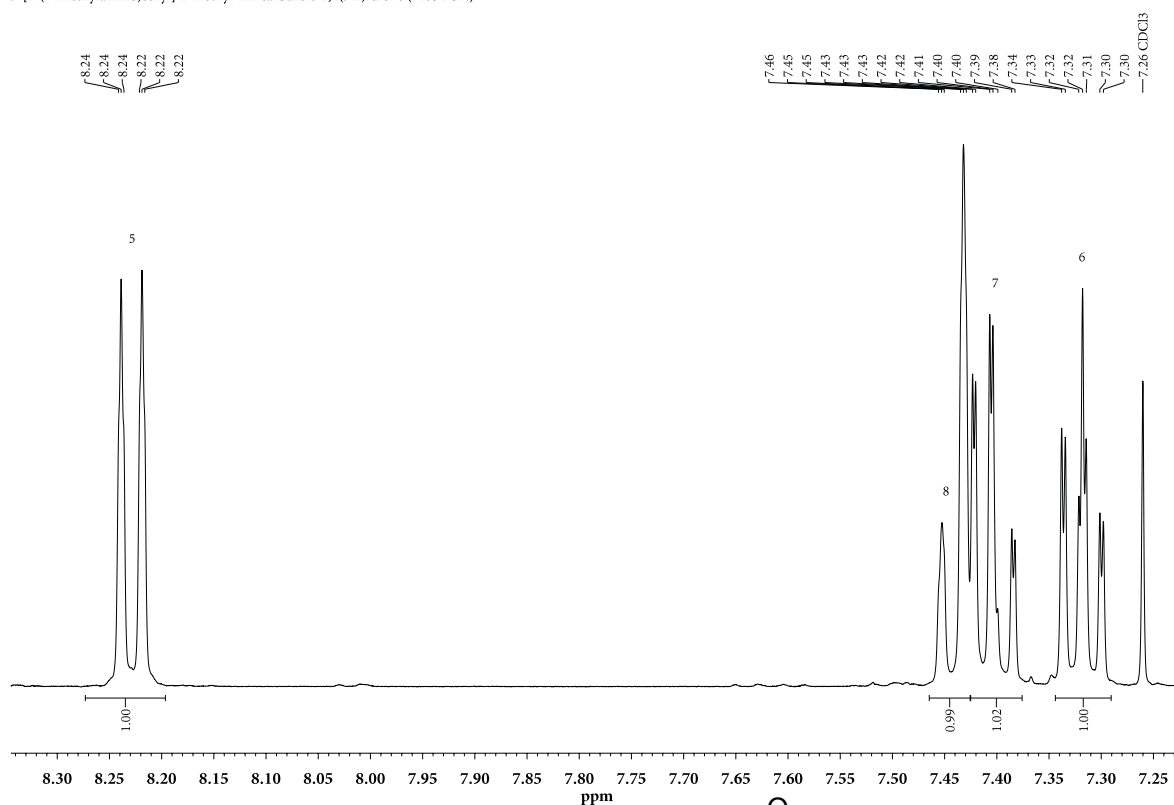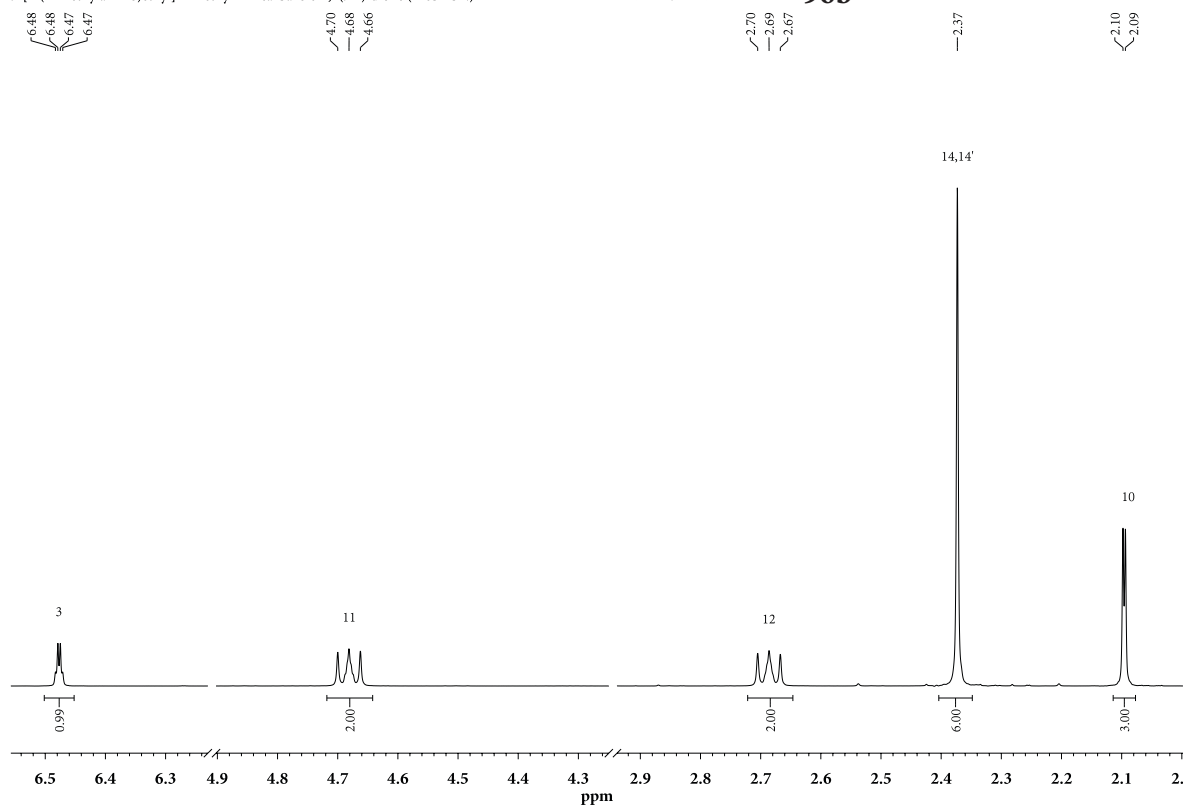

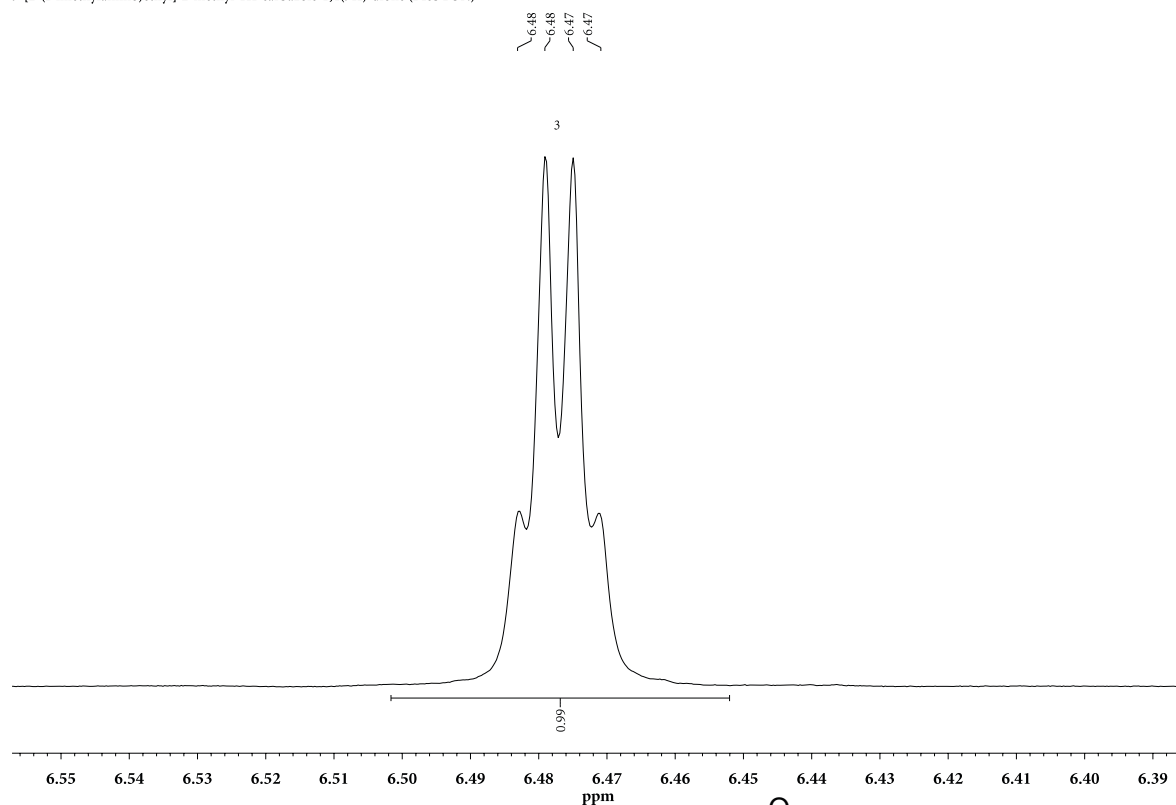9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

96b

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

8.24
8.24
8.24
8.22
8.22
8.22

7.46
7.45
7.45
7.43
7.43
7.43
7.42
7.42
7.41
7.40
7.40
7.39
7.38
7.34
7.33
7.32
7.32
7.31
7.30
7.30
7.26 CDCl3

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

6.48
6.48
6.47
6.47

4.70
4.68
4.66

2.70
2.69
2.67

2.37

2.10
2.09

96b

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

6.48
6.48
6.47
6.47

3

0.99

6.55　6.54　6.53　6.52　6.51　6.50　6.49　6.48　6.47　6.46　6.45　6.44　6.43　6.42　6.41　6.40　6.39
ppm

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

96b

183.62
181.23

144.56
138.83
134.65
133.25
126.83
124.34
123.27
123.24
117.32
110.84

77.00 CDCl3

58.35

45.70
42.98
14,14'

15.63

5
4b
3   7 6   8

4 1

2 8a 9a   4a   12   11   10

200　190　180　170　160　150　140　130　120　110　100　90　80　70　60　50　40　30　20　10　0
ppm

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

183.62
181.23
144.56
138.83
134.65
133.25
126.83
124.34
123.27
123.24
117.32
110.84

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HSQC)

96b

9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HMBC)

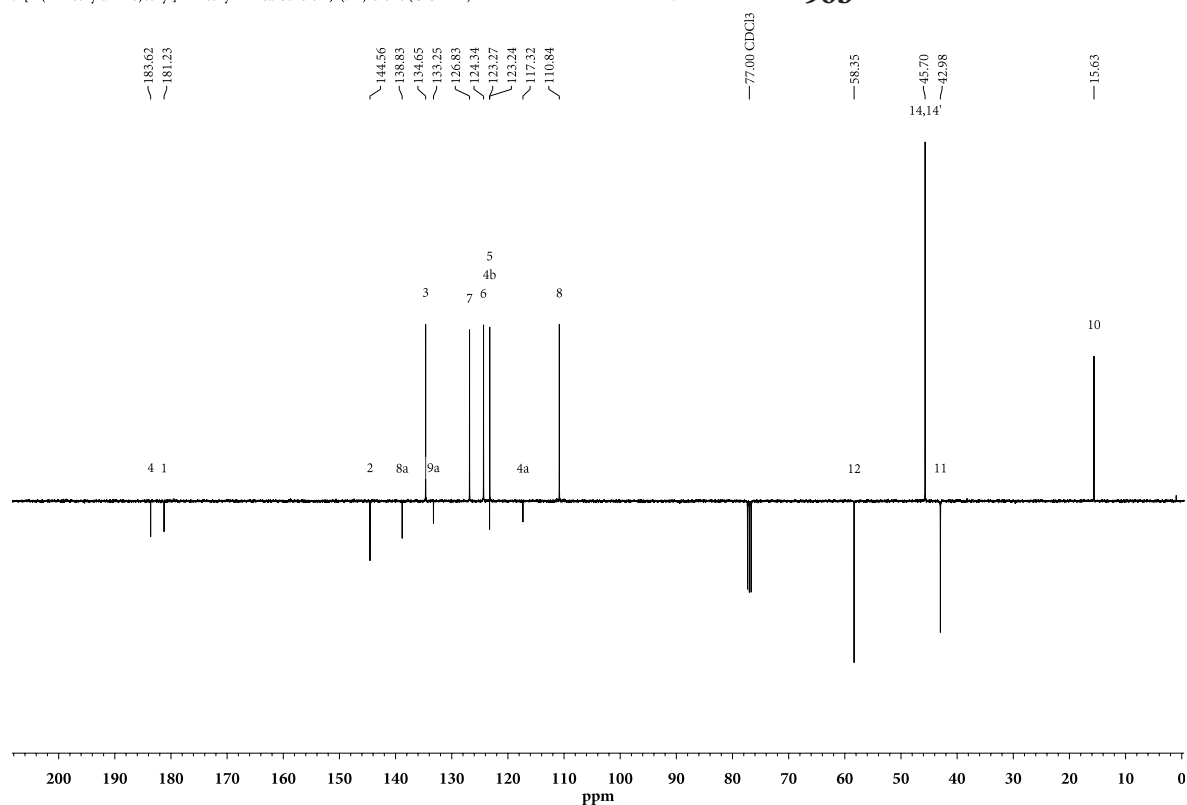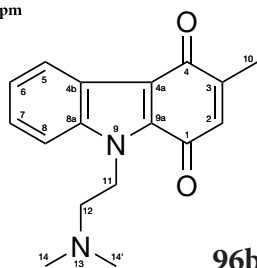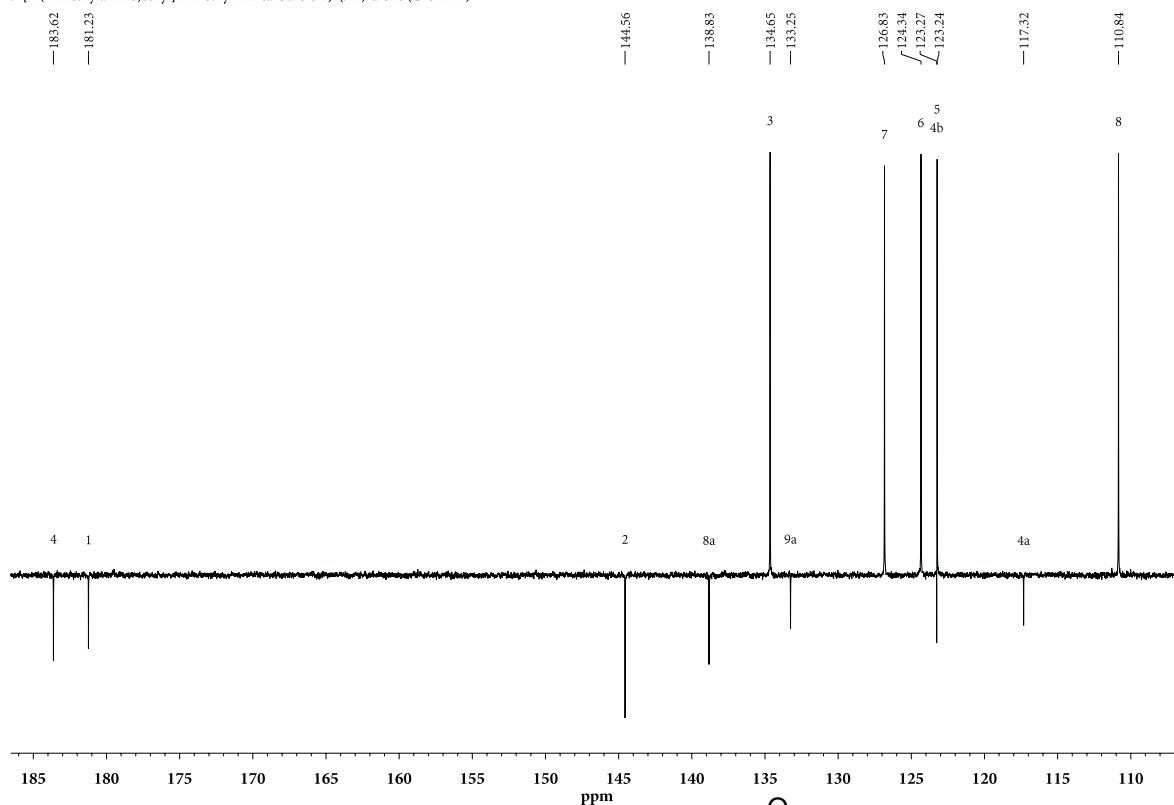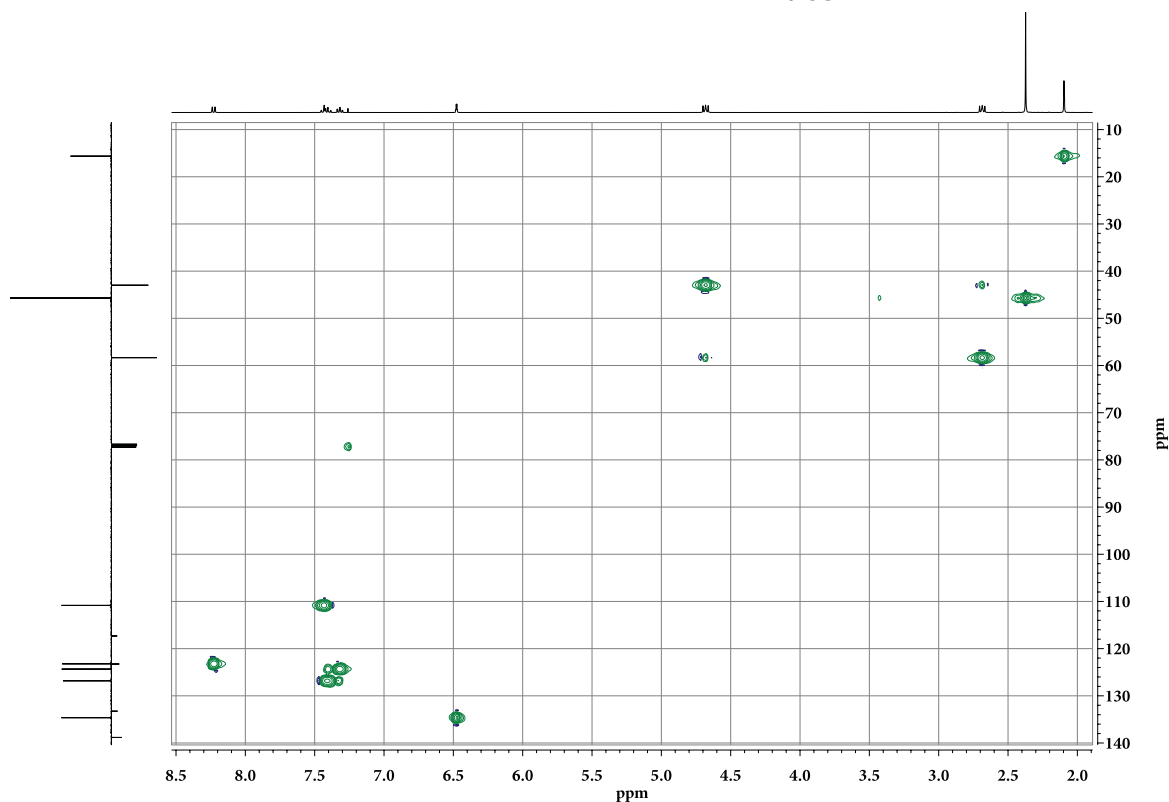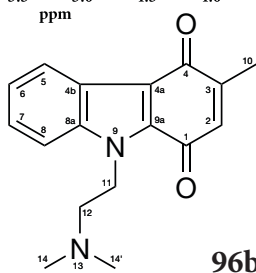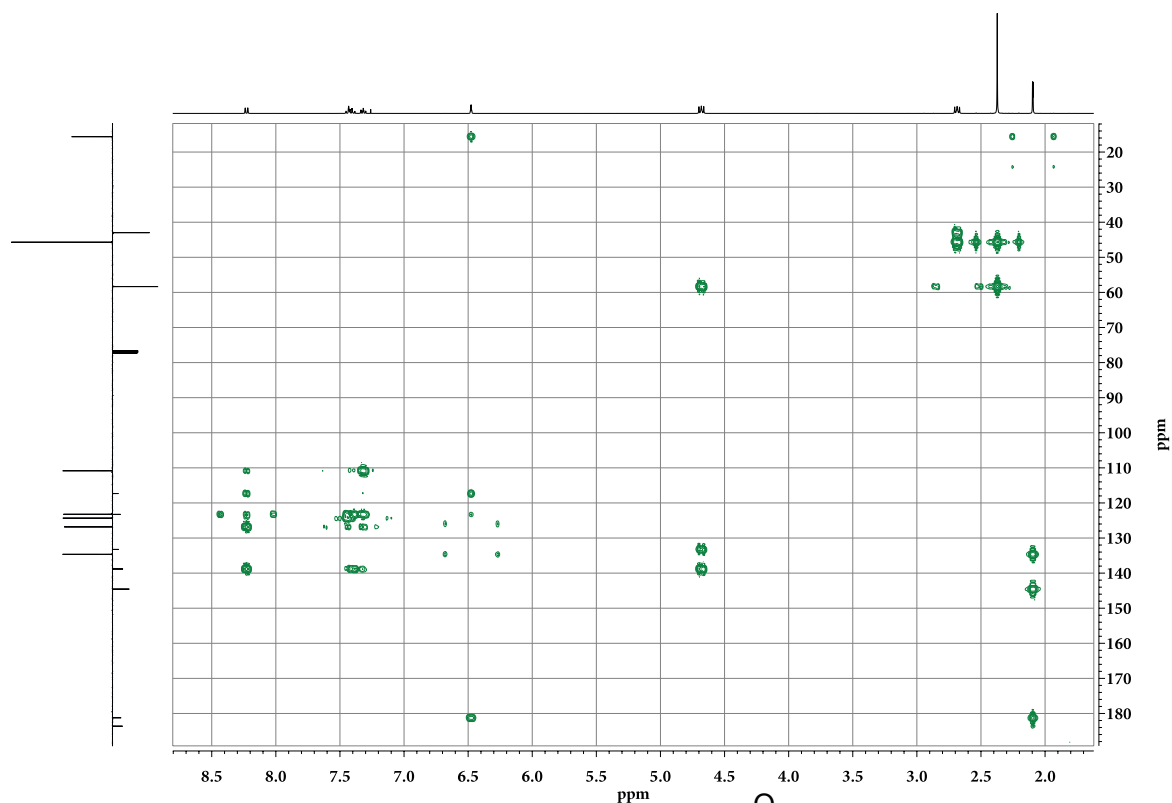9-[2-(Dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (15N HMBC)

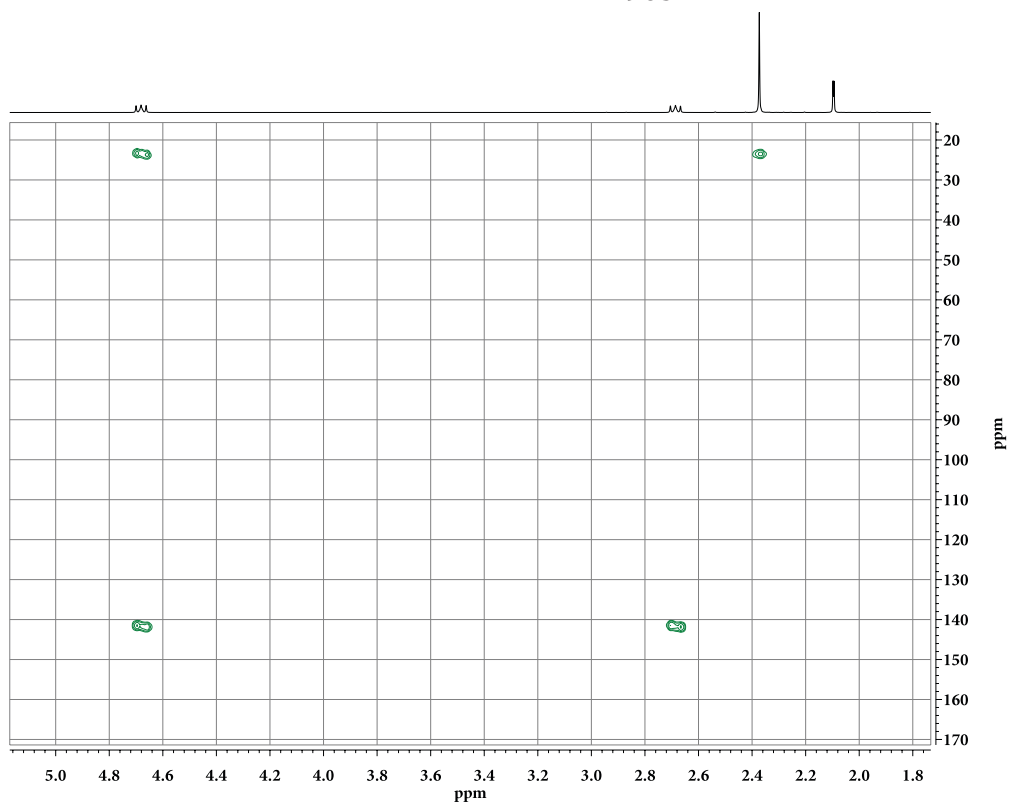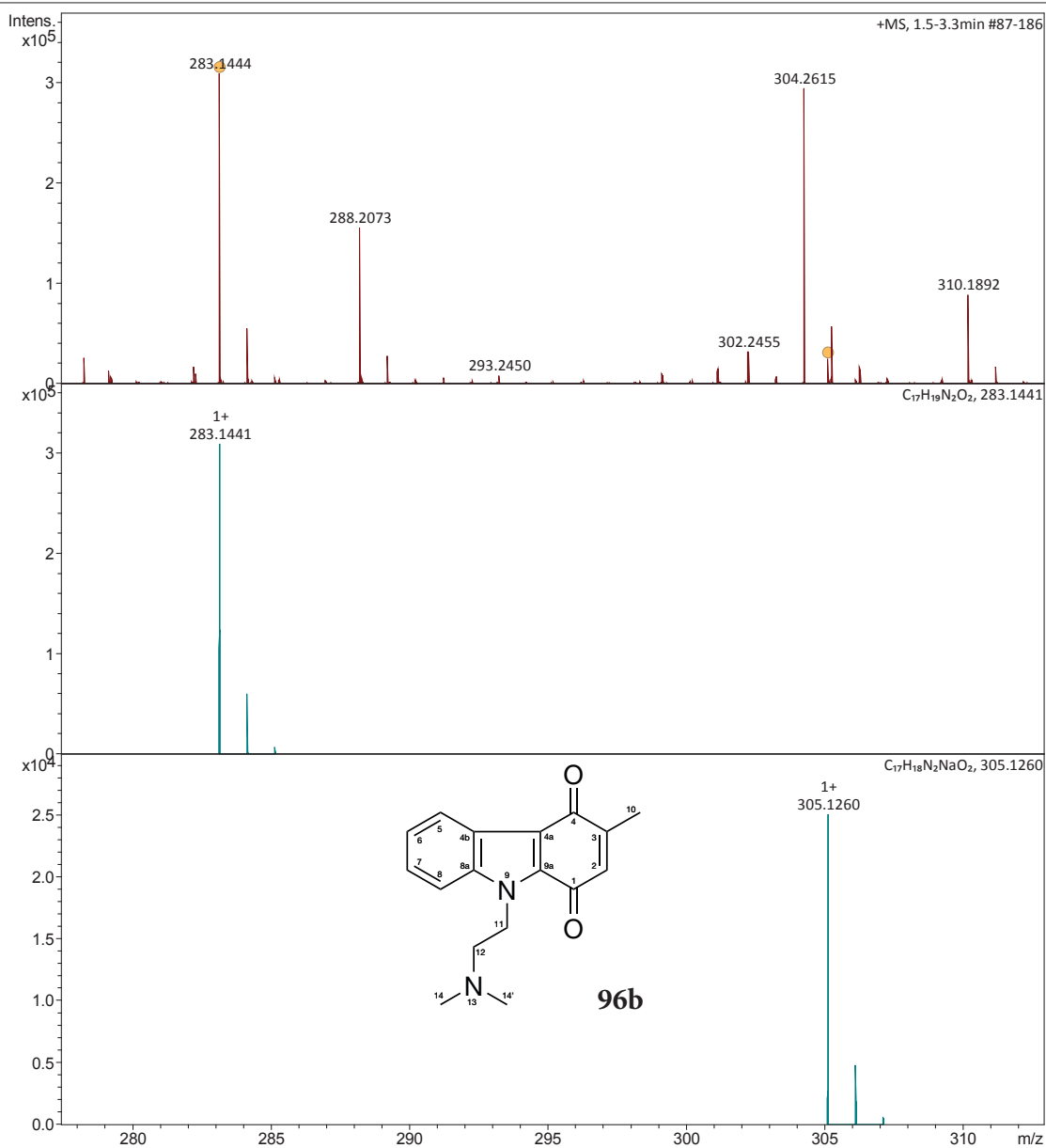**96b**

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 3:55:11 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN128_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			

+MS, 1.5-3.3min #87-186

283.1444

304.2615

288.2073

310.1892

302.2455

293.2450

$C_{17}H_{19}N_2O_2$, 283.1441

1+
283.1441

$C_{17}H_{18}N_2NaO_2$, 305.1260

1+
305.1260

96b

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN128_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 3:55:11 PM
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	2800 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 1.5-3.3min #87-186

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
283.1444	1	C17H19N2O2	283.1441	-1.2	8.1	1	100.00	9.5	even	ok
305.1260	1	C17H18N2NaO2	305.1260	-0.0	13.6	1	100.00	9.5	even	ok
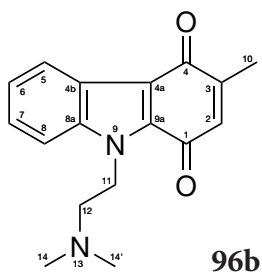
96b
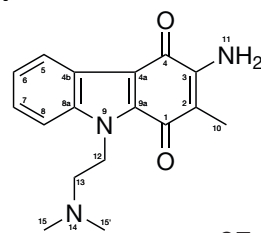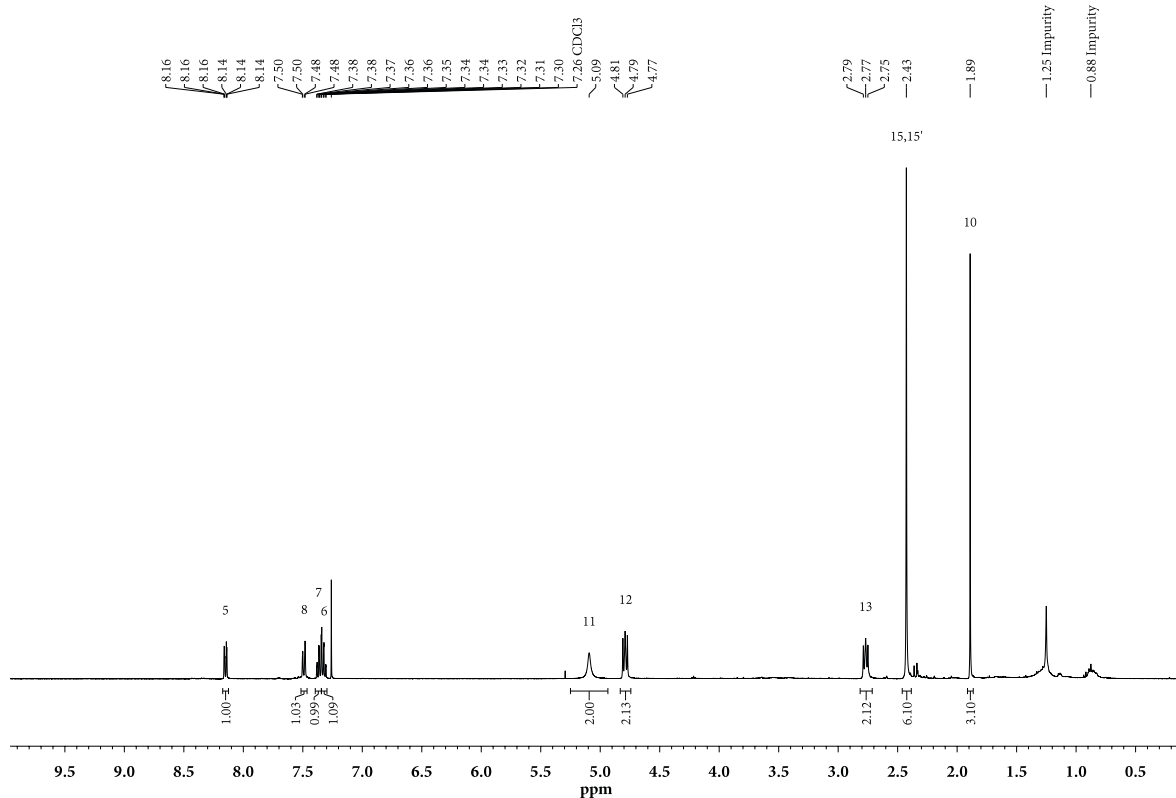
3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

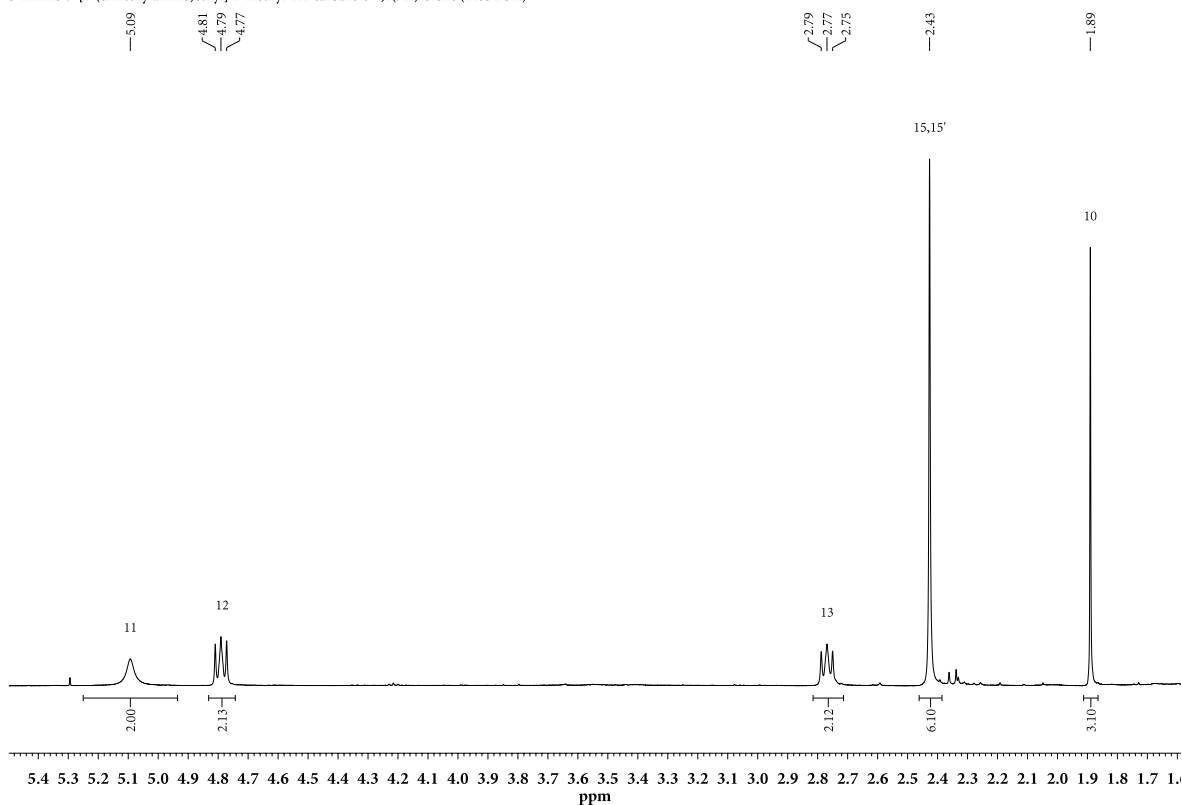**3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)**

97a

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (PROTON)

5.09
4.81
4.79
4.77
2.79
2.77
2.75
2.43
1.89

15,15'

10

11

12

13

2.00
2.13
2.12
6.10
3.10

5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.
ppm

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

97a

179.73
177.77
146.07
138.29
136.13
125.55
124.48
123.69
122.31
113.33
111.13
107.77
77.00 CDCl3
58.04
45.51
42.89
29.68 Impurity
8.33

15,15'

7
6 5

8

10

8a

1 4

3

9a

4b

4a 2

13

12

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (C13 APT)

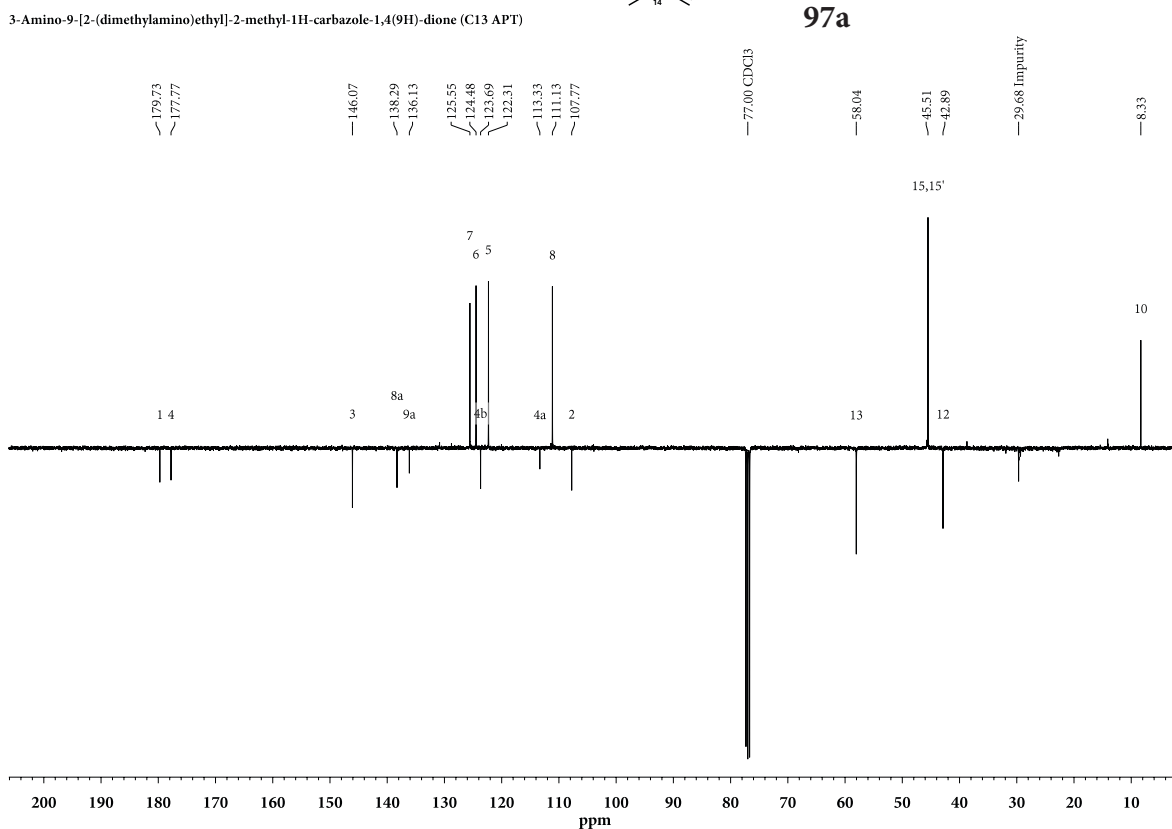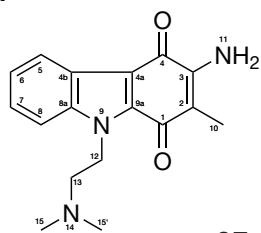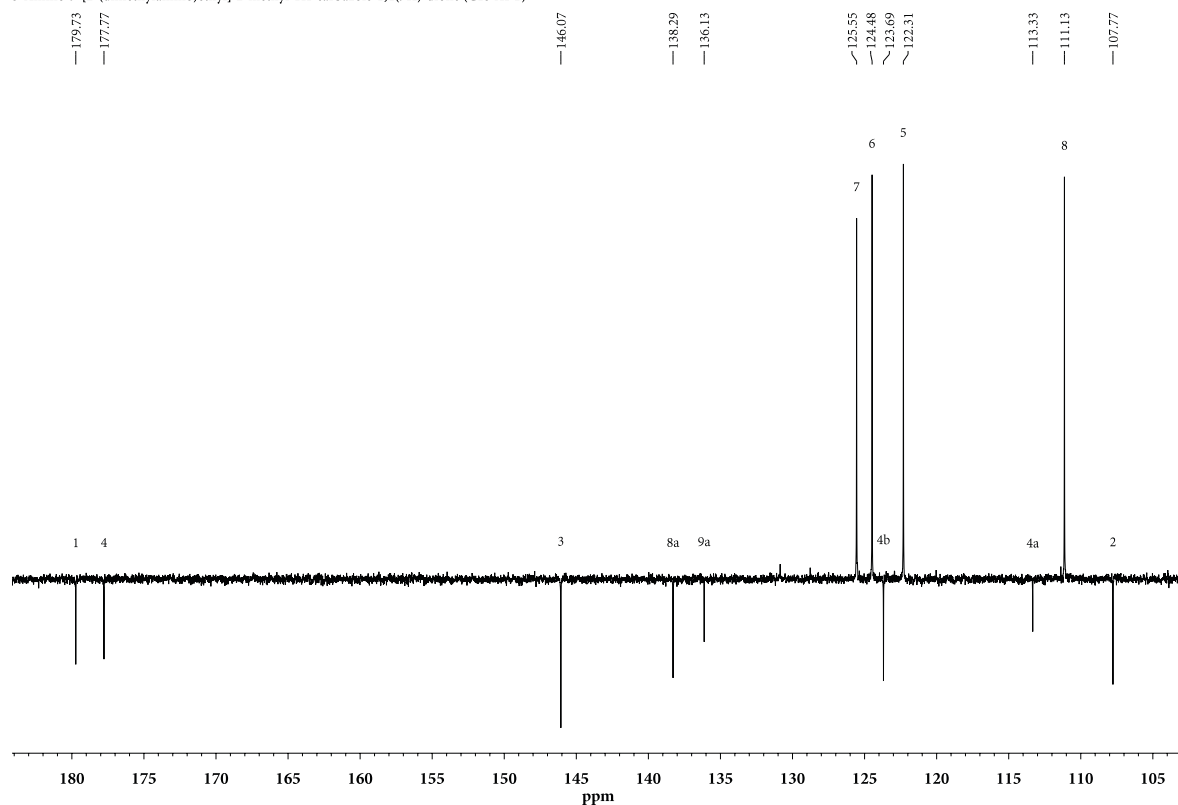3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HSQC)

97a

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (HMBC)

3-Amino-9-[2-(dimethylamino)ethyl]-2-methyl-1H-carbazole-1,4(9H)-dione (15N HMBC)
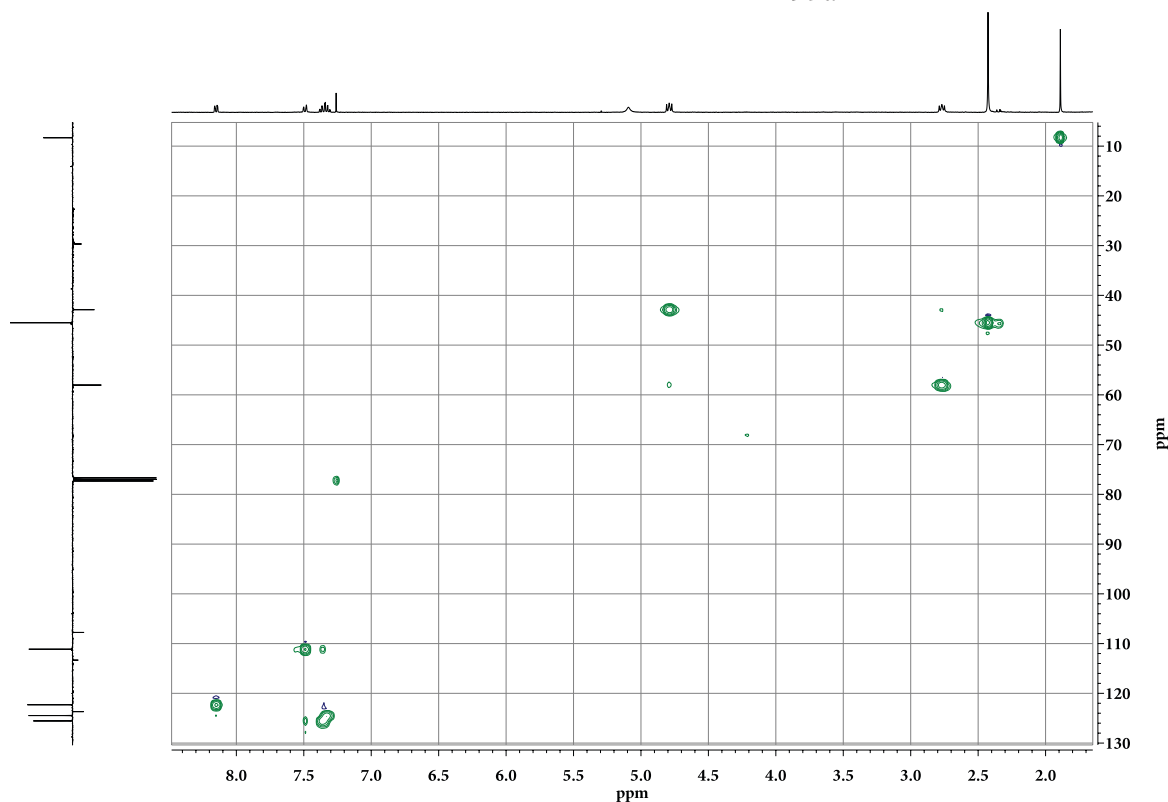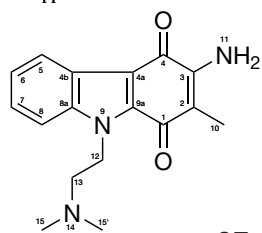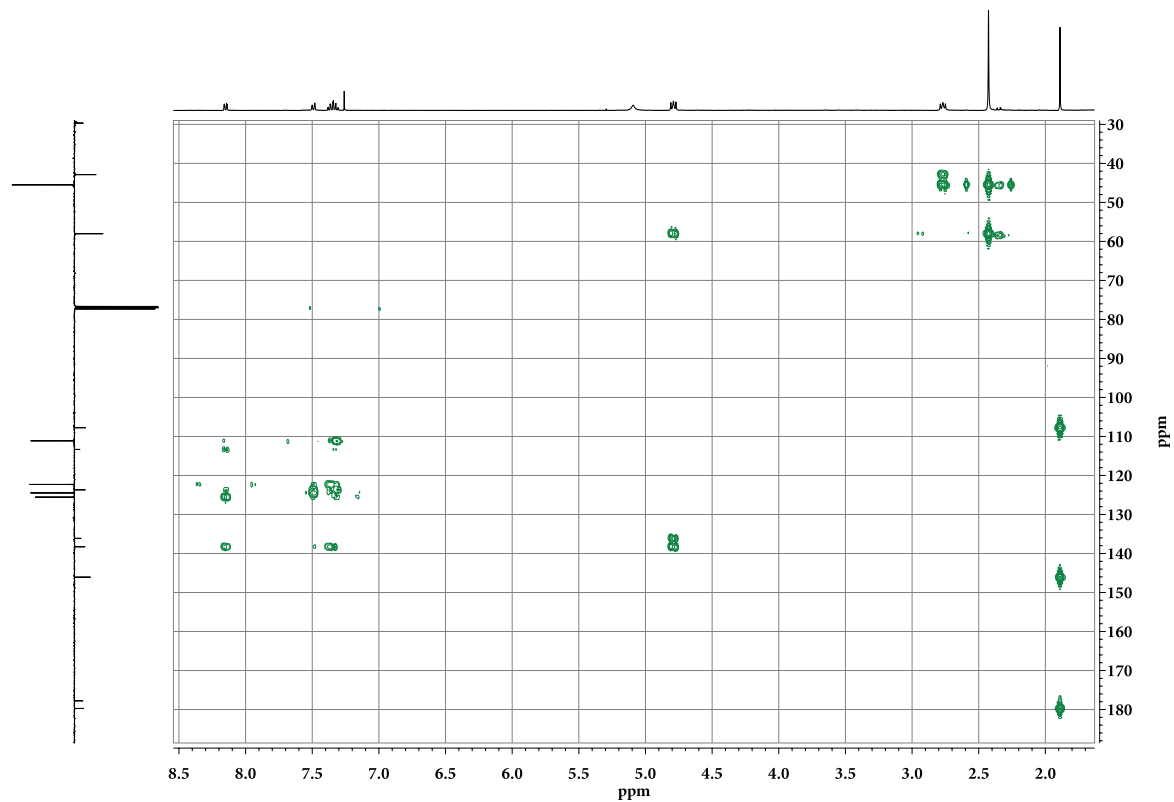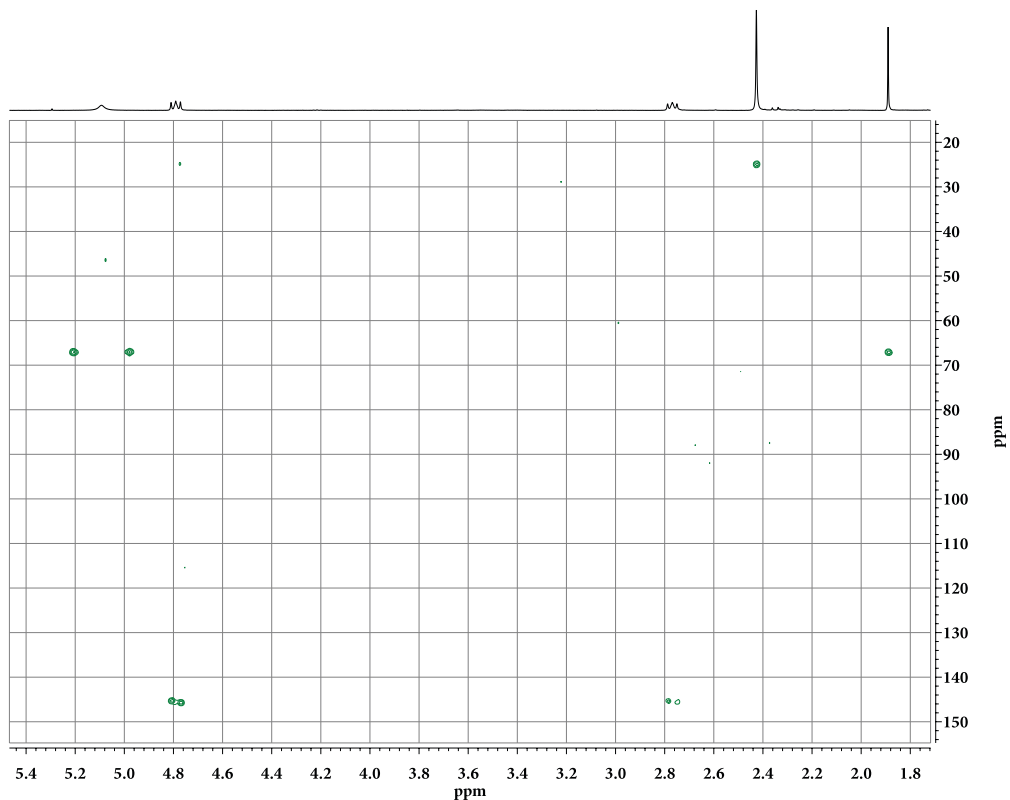
97a

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 4:43:36 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN131_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH --> 1:500 in MeOH	Instrument	maXis HD
Comment			
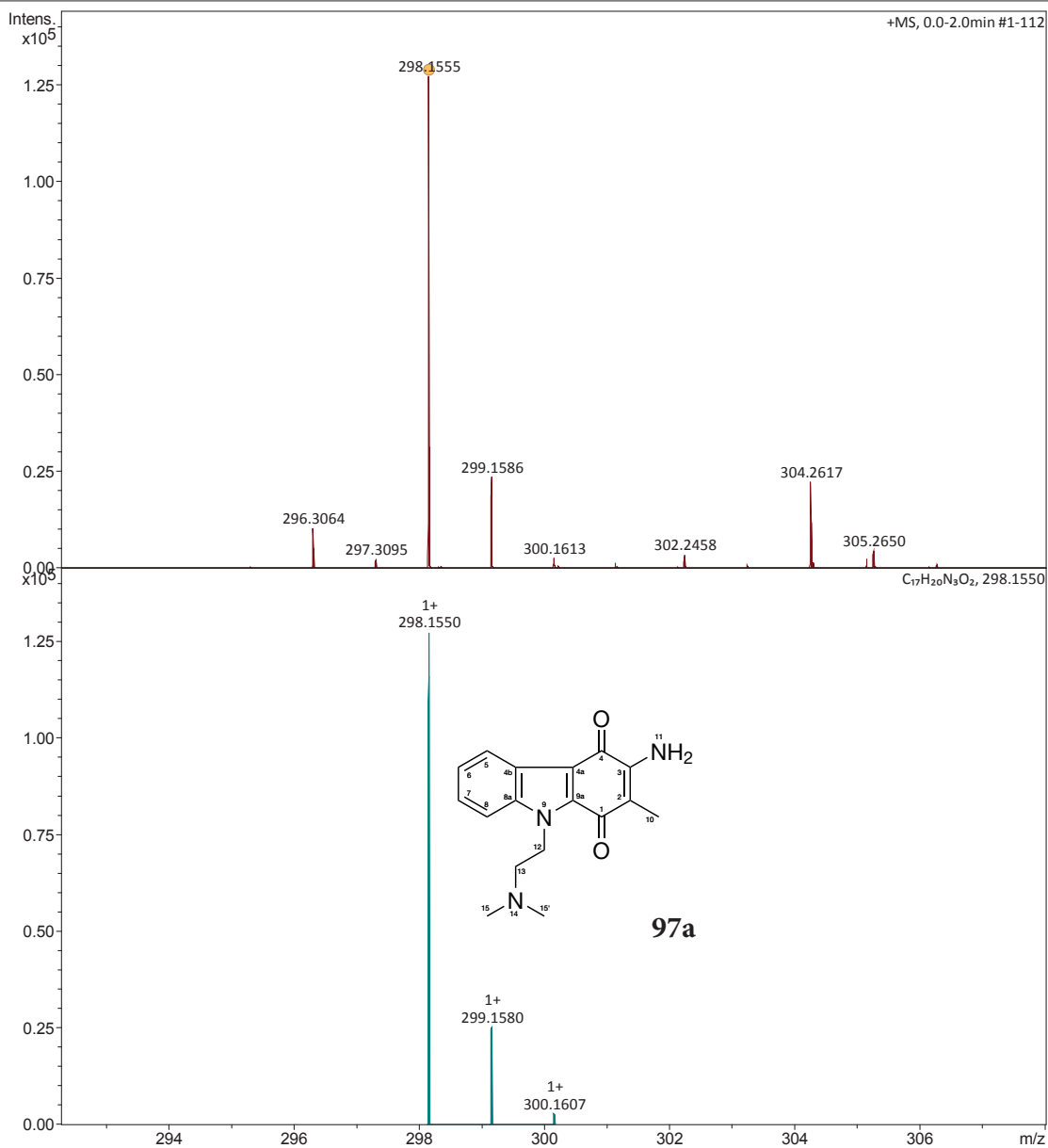
Mass Spectrum SmartFormula Report

Analysis Info

|			
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN131_DI_ESI_HRMS.d	Acquisition Date	1/12/2015 4:43:36 PM
Method	DI_mz_50-1550.m		
Sample Name	in MeOH --> 1:500 in MeOH	Operator	MZ
Comment		Instrument	maXis HD 1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1200 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C
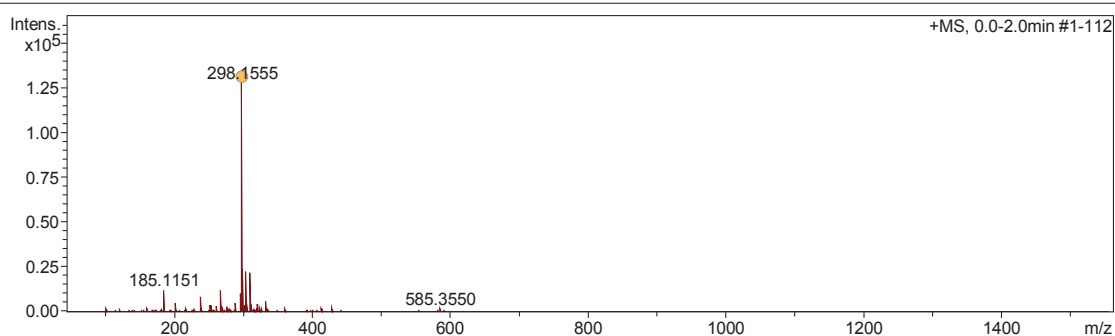
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
298.1555	1	C17H20N3O2	298.1550	-1.8	7.4	1	100.00	9.5	even	ok
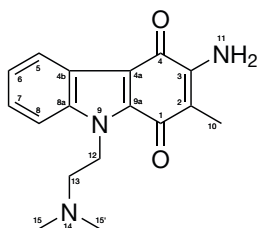
97a
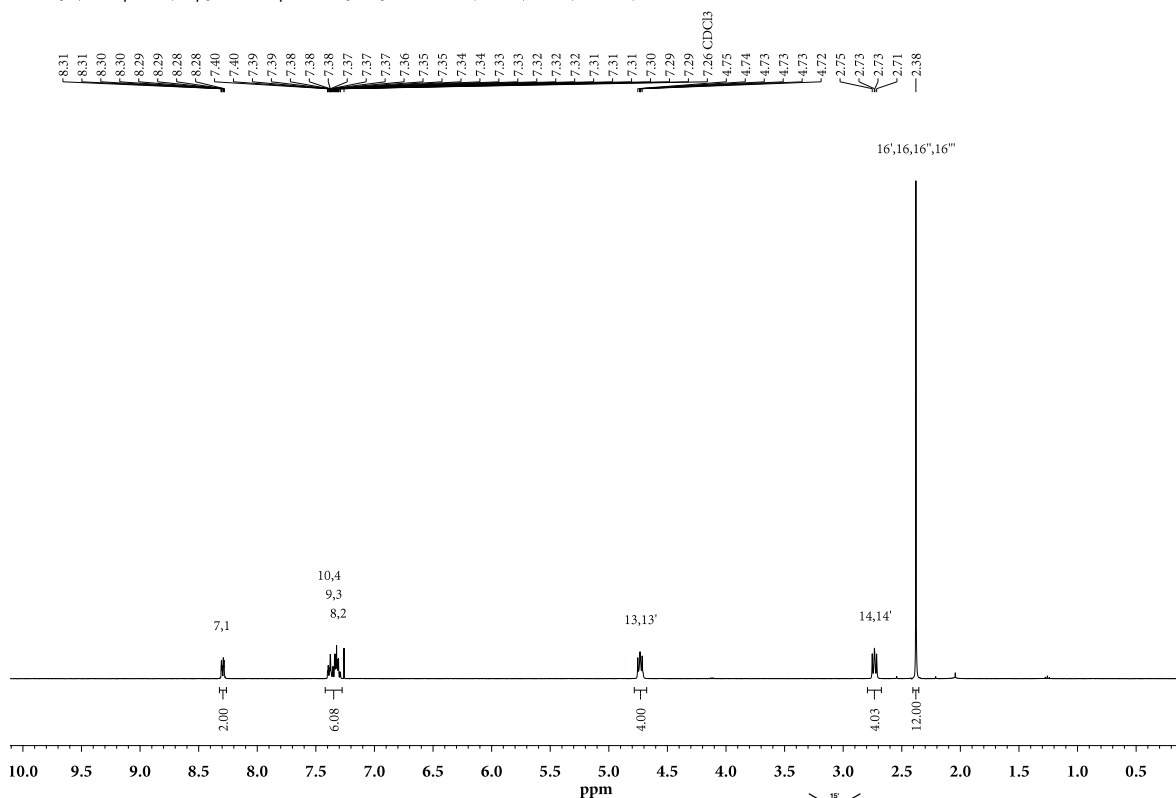
5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (PROTON)

5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (PROTON)
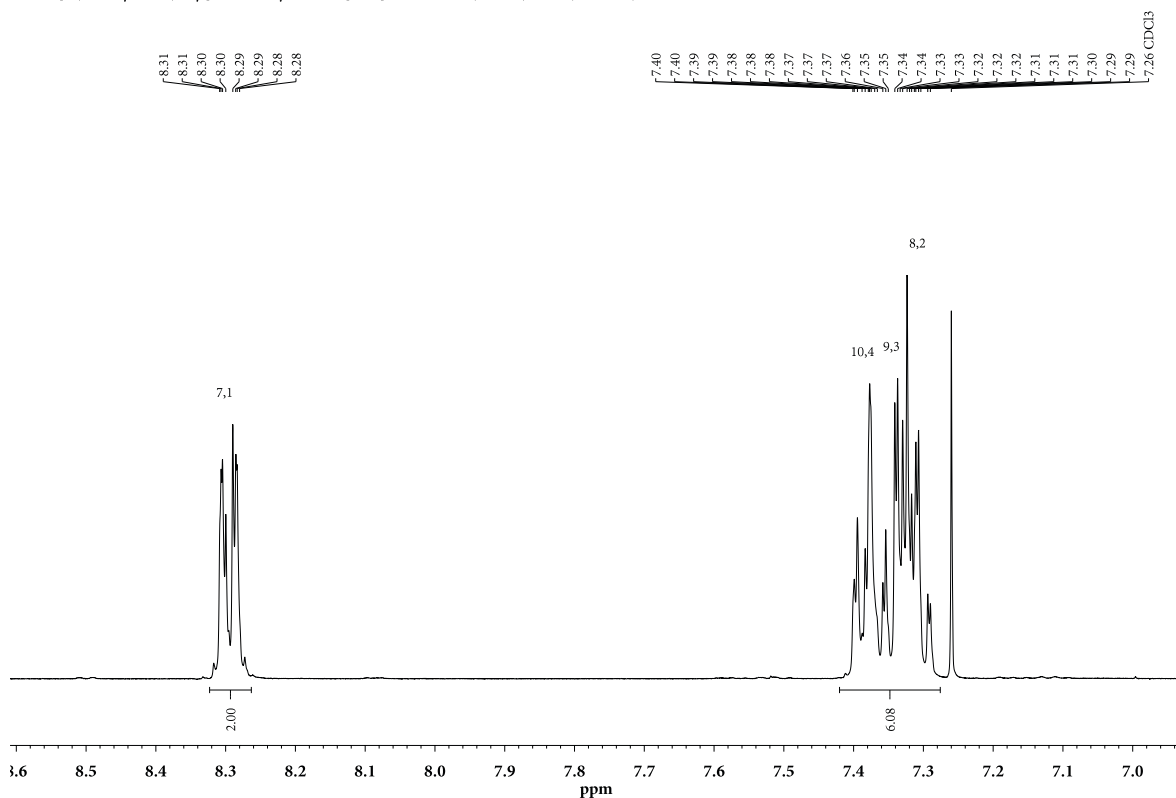
98

5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (PROTON)

5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (C13 APT)

98
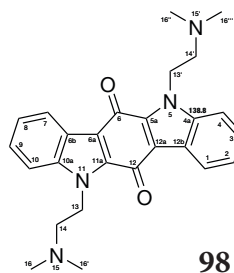
5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (C13 APT)
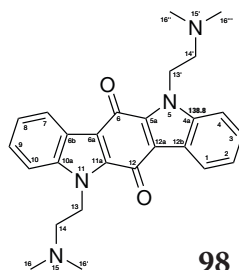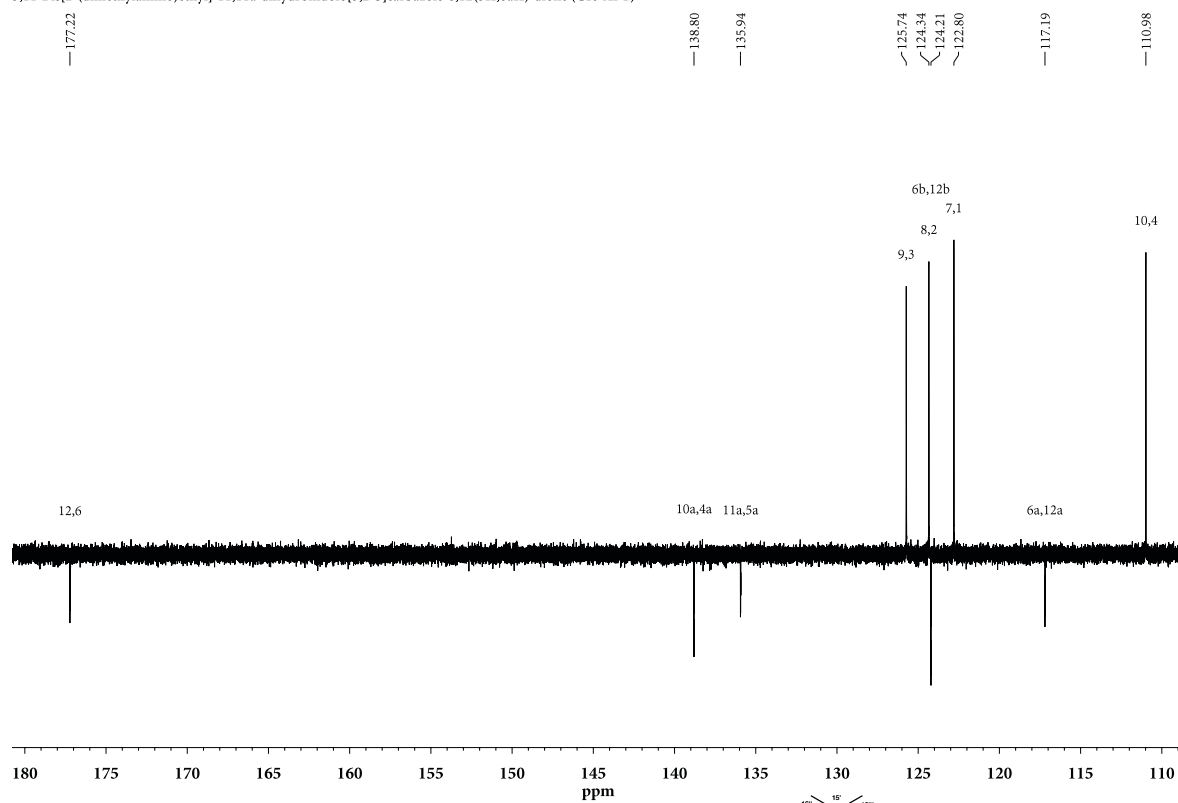
5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (HSQC)

98
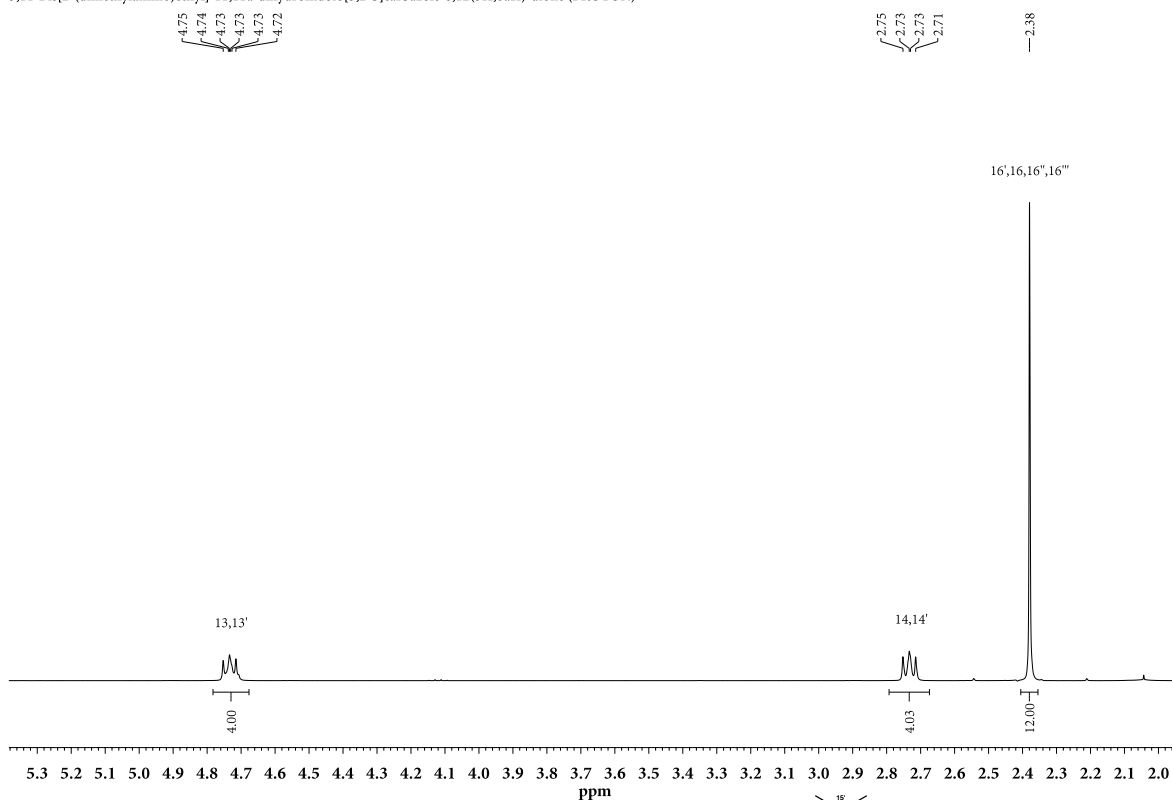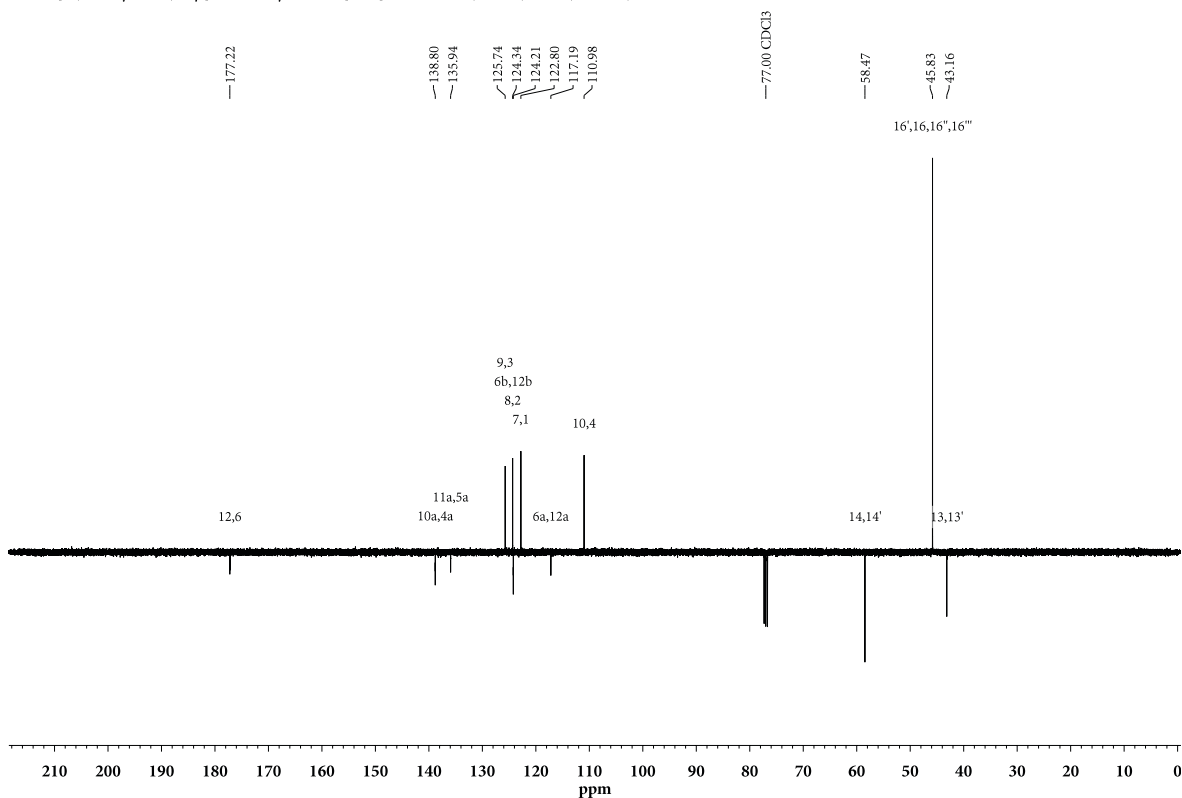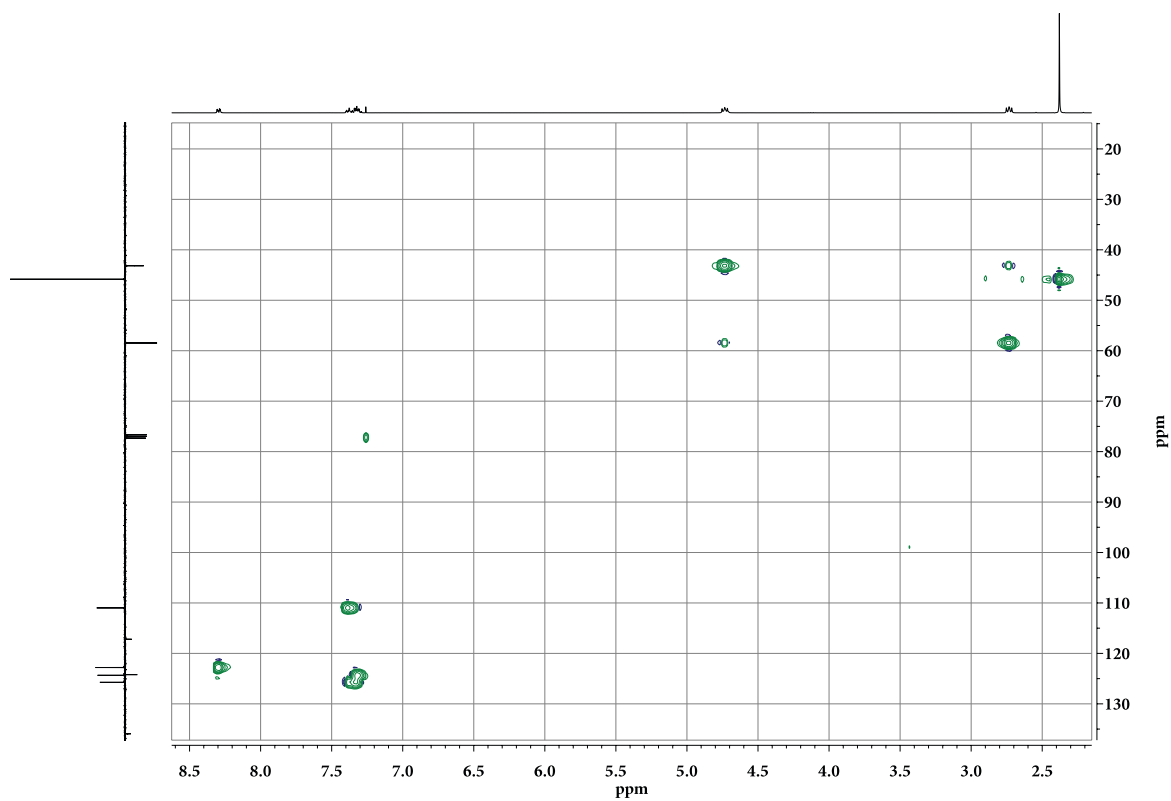
5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (HMBC)
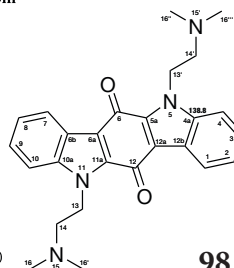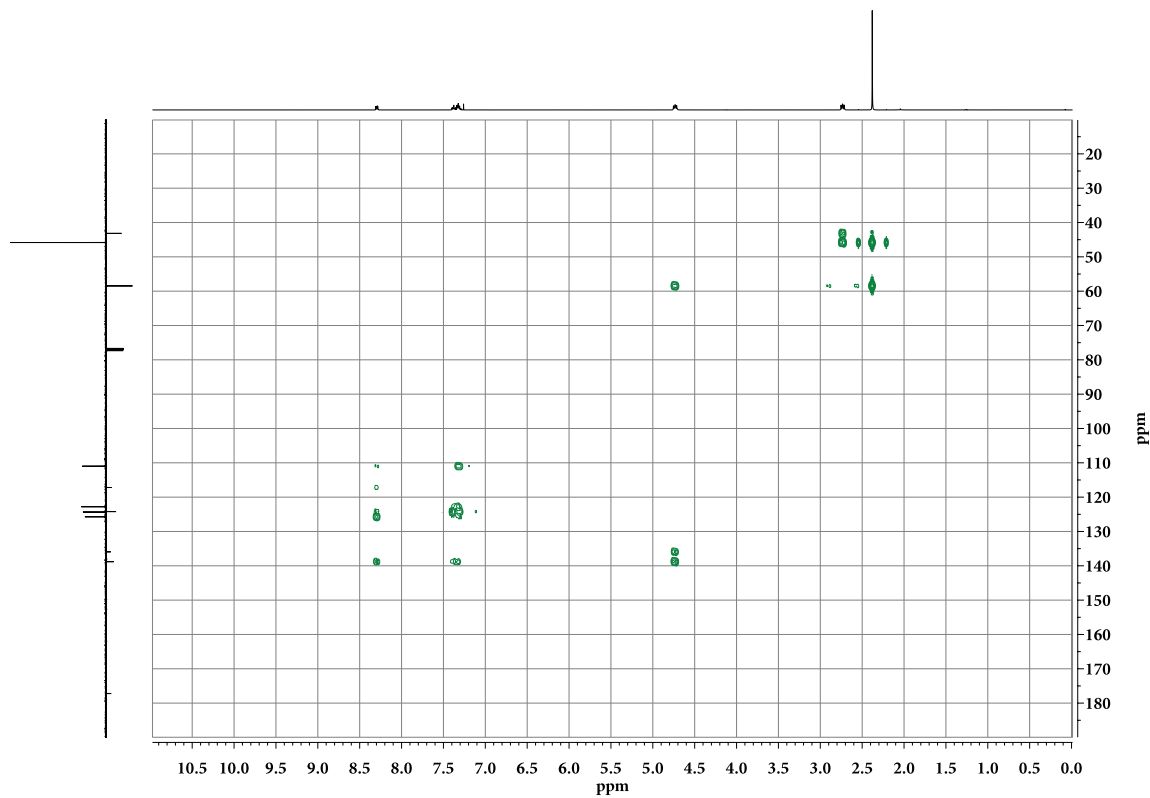
5,11-Bis[2-(dimethylamino)ethyl]-11,11a-dihydroindolo[3,2-b]carbazole-6,12(5H,6aH)-dione (15N HMBC)

98

Generic Display Report

Analysis Info

		Acquisition Date	1/12/2015 1:17:34 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN182_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

		Acquisition Date	1/12/2015 1:17:34 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\150112\TN182_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	in MeOH	Instrument	maXis HD 1820881.21300
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	1500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 0.0-1.5min #1-85

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
429.2284	1	C26H29N4O2	429.2285	0.2	11.1	1	100.00	14.5	even	ok
451.2103	1	C26H28N4NaO2	451.2104	0.3	15.6	1	100.00	14.5	even	ok
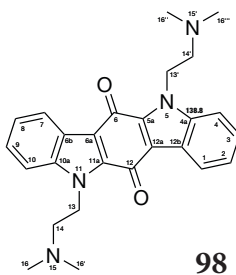
98
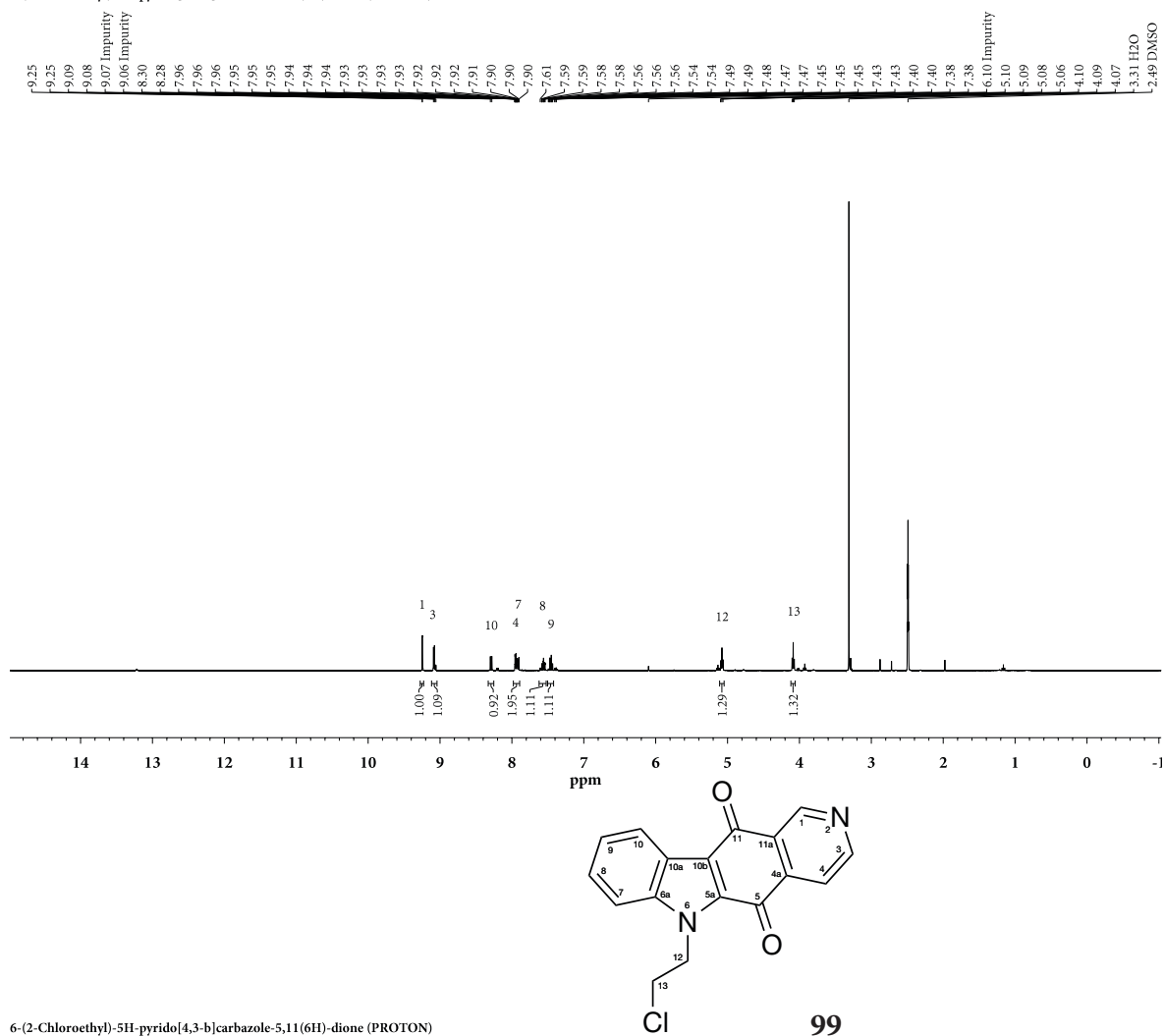
6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)
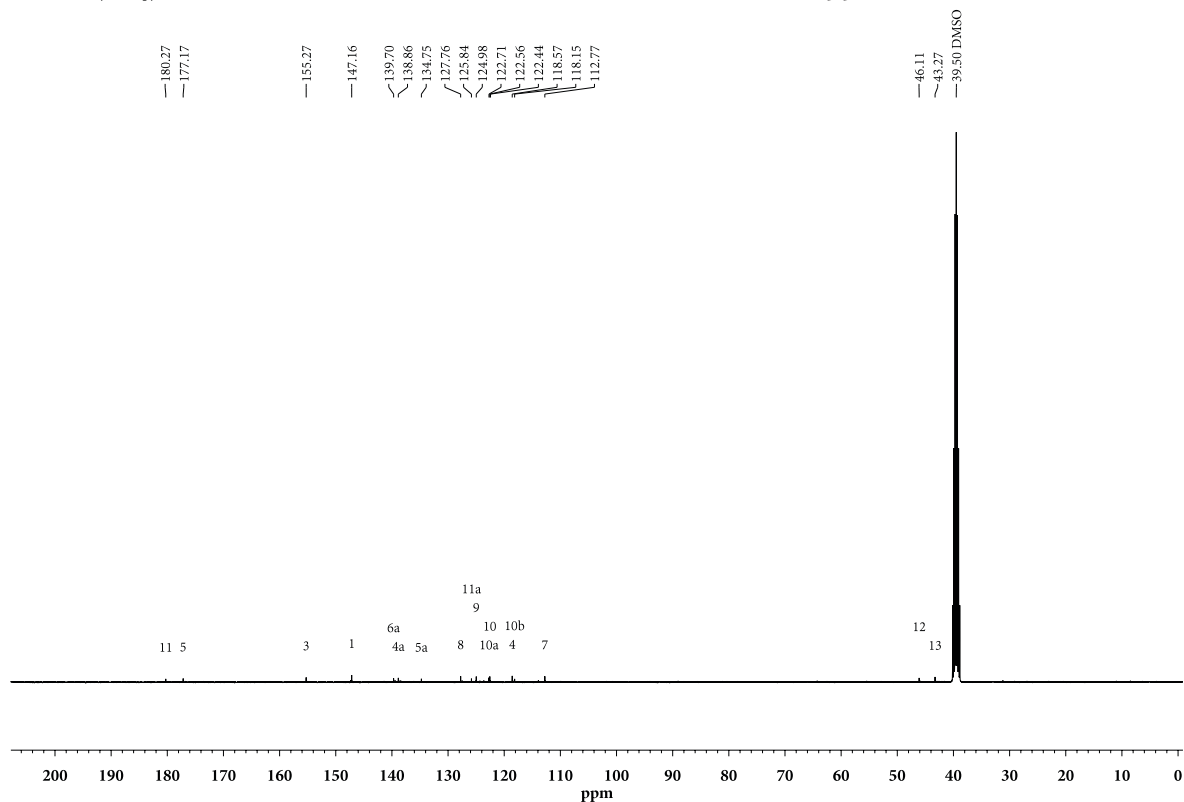
99

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

5.10
5.09
5.08
5.06

4.10
4.09
4.07

12

13

1.29

1.32

5.10 5.05 5.00 4.95 4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05 4.0
ppm

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)

99

180.27
177.17
155.27
147.16
139.70
138.86
134.75
127.76
125.84
124.98
122.71
122.56
122.44
118.57
118.15
112.77

46.11
43.27
39.50 DMSO

11a
9
6a
10 10b
11 5
3
1
4a 5a
8
10a 4
7
12
13

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 CPD)
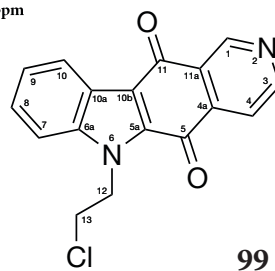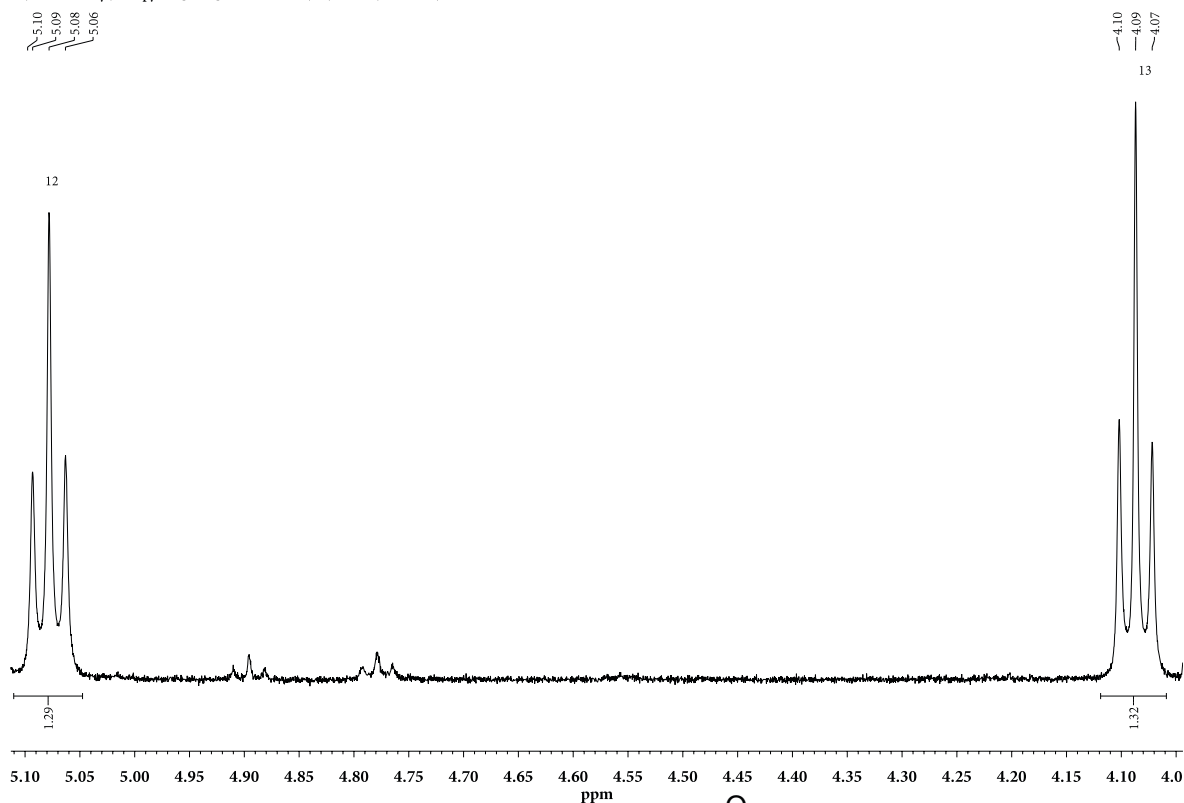

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

99

787
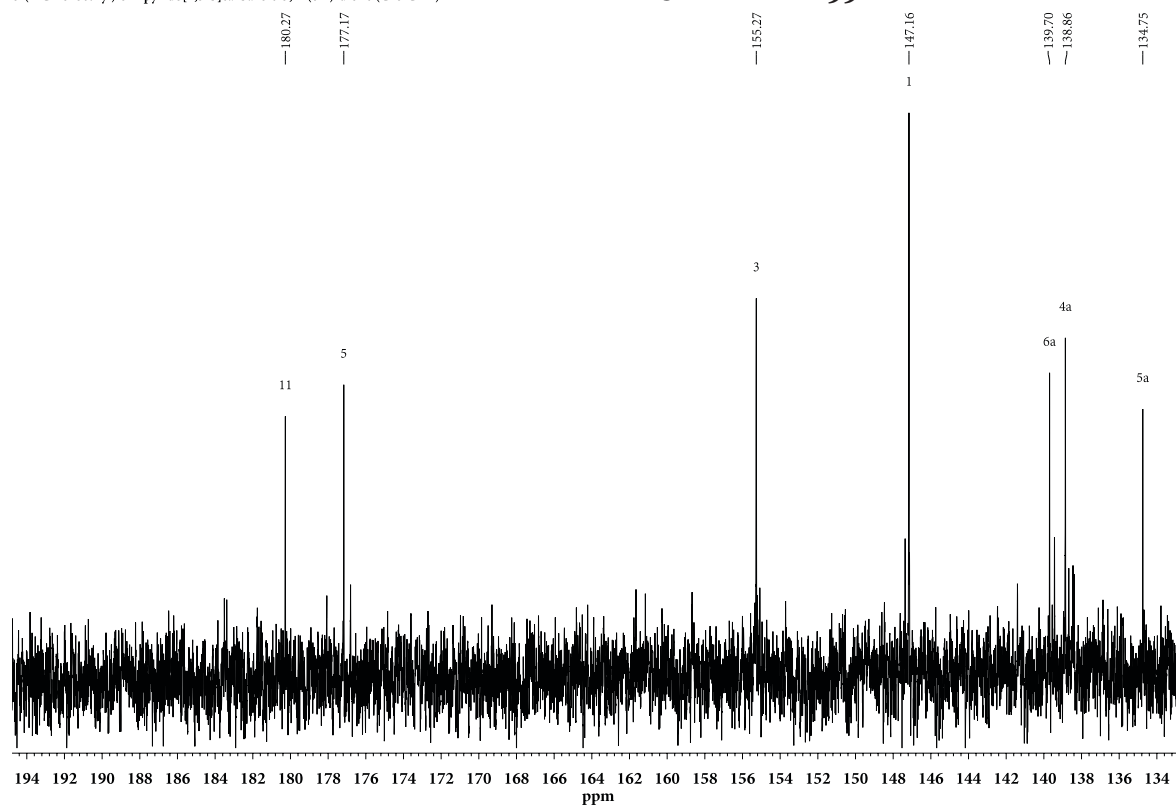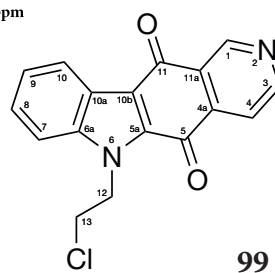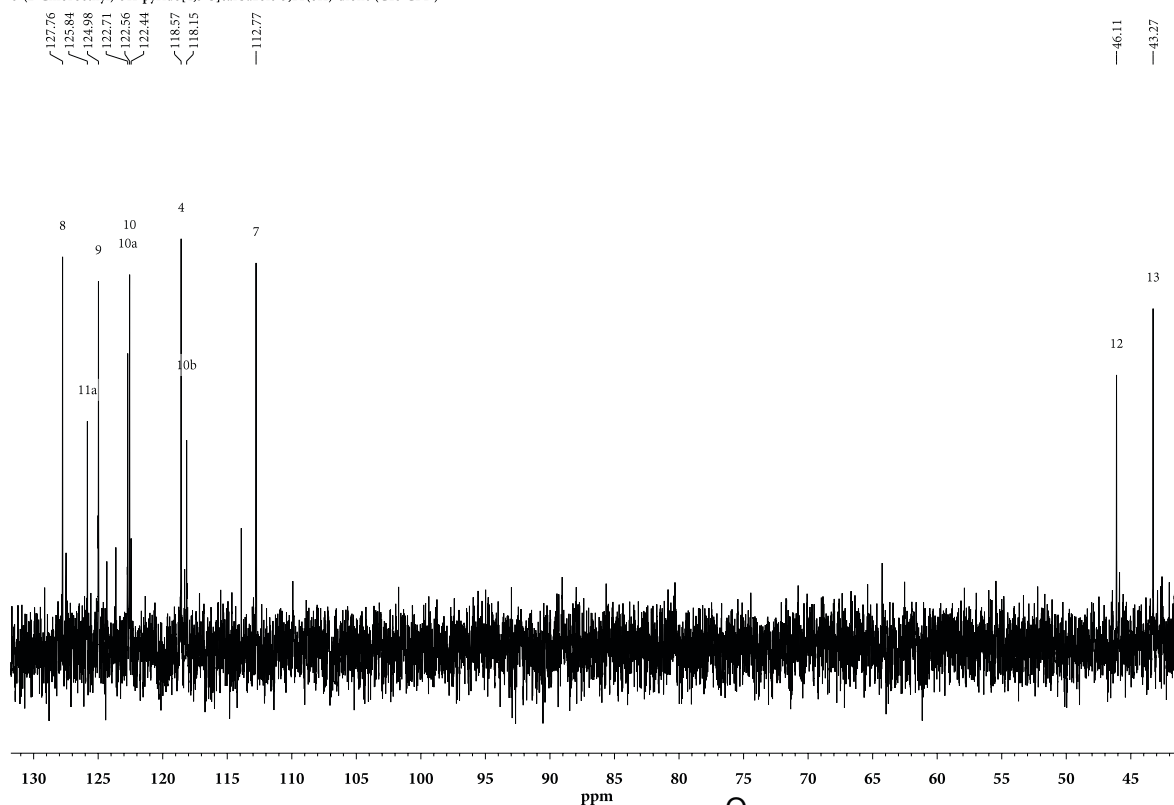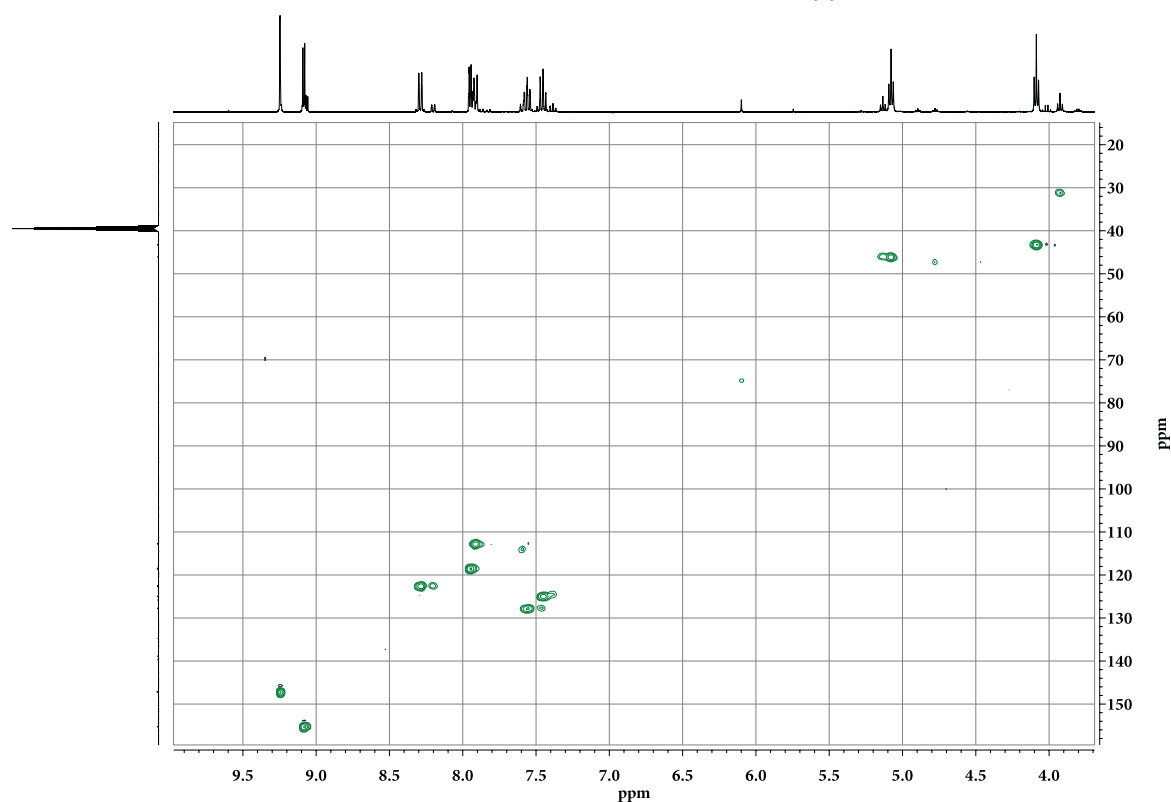
6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-(2-Chloroethyl)-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)

99

Generic Display Report

Analysis Info

		Acquisition Date	11/17/2014 2:42:19 PM
Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN122_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info   Acquisition Date   11/17/2014 2:42:19 PM

Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN122_DI_ESI_HRMS.d
Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
311.0580	1	C17H12ClN2O2	311.0582	0.5	3.9	1	100.00	12.5	even	ok
333.0399	1	C17H11ClN2NaO2	333.0401	0.7	32.3	1	100.00	12.5	even	ok
	2	C12H12Cl2N6Na	333.0393	-1.9	144.8	2	0.91	8.5	even	ok
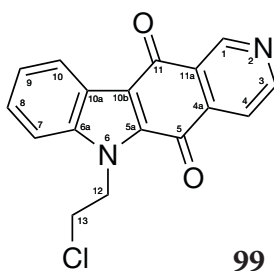
6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)
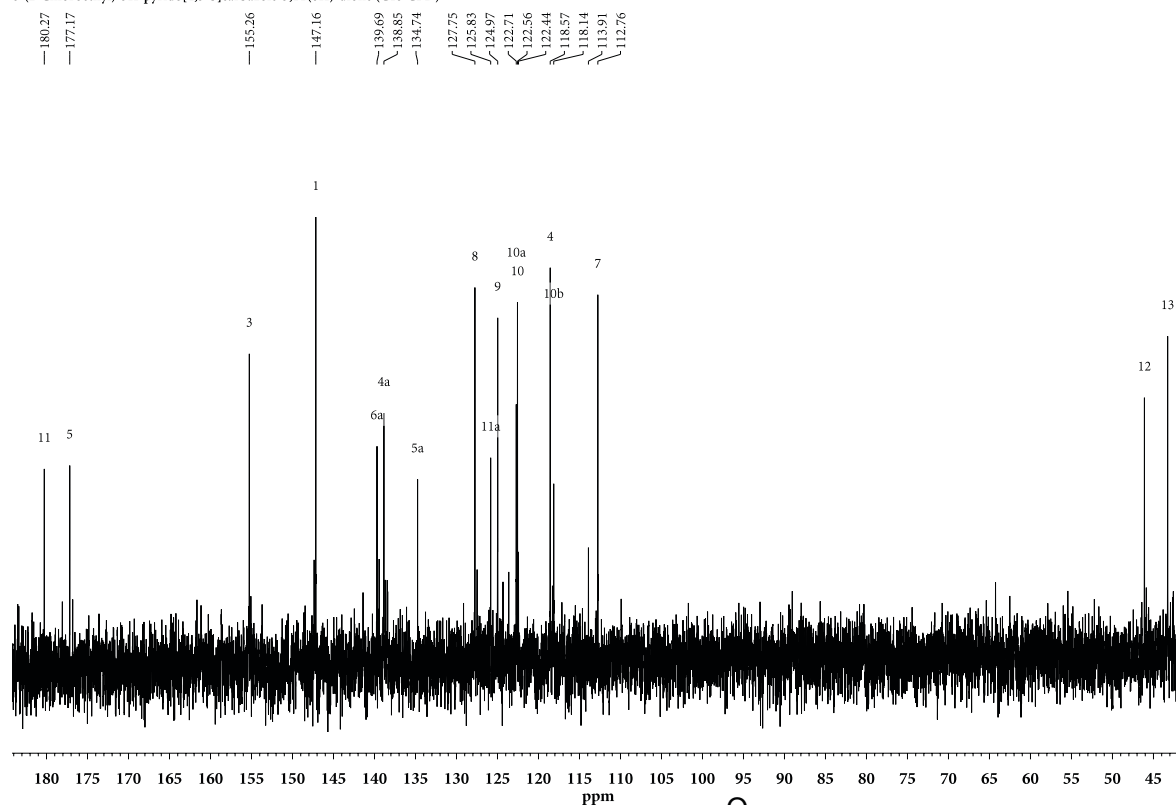
6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (PROTON)

5.72
5.71
5.68
5.67
5.60
5.60
5.58
5.58

13

13

1.00

0.99

5.82 5.81 5.80 5.79 5.78 5.77 5.76 5.75 5.74 5.73 5.72 5.71 5.70 5.69 5.68 5.67 5.66 5.65 5.64 5.63 5.62 5.61 5.60 5.59 5.58 5.57 5.56 5.55 5.54 5.53 5.52 5.51 5.50 5.49
ppm

100

6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

180.71
177.23
155.00
148.29
138.65
138.47
133.70
130.99
128.64
126.00
125.44
124.28
124.10
119.45
118.53
112.94
111.58
77.00 CDCl3

10
10a
8   9
4   7

3
1

12

6a
4a   5a
11a   10b
5   13

11 5

200   190   180   170   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0
ppm
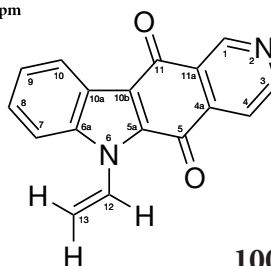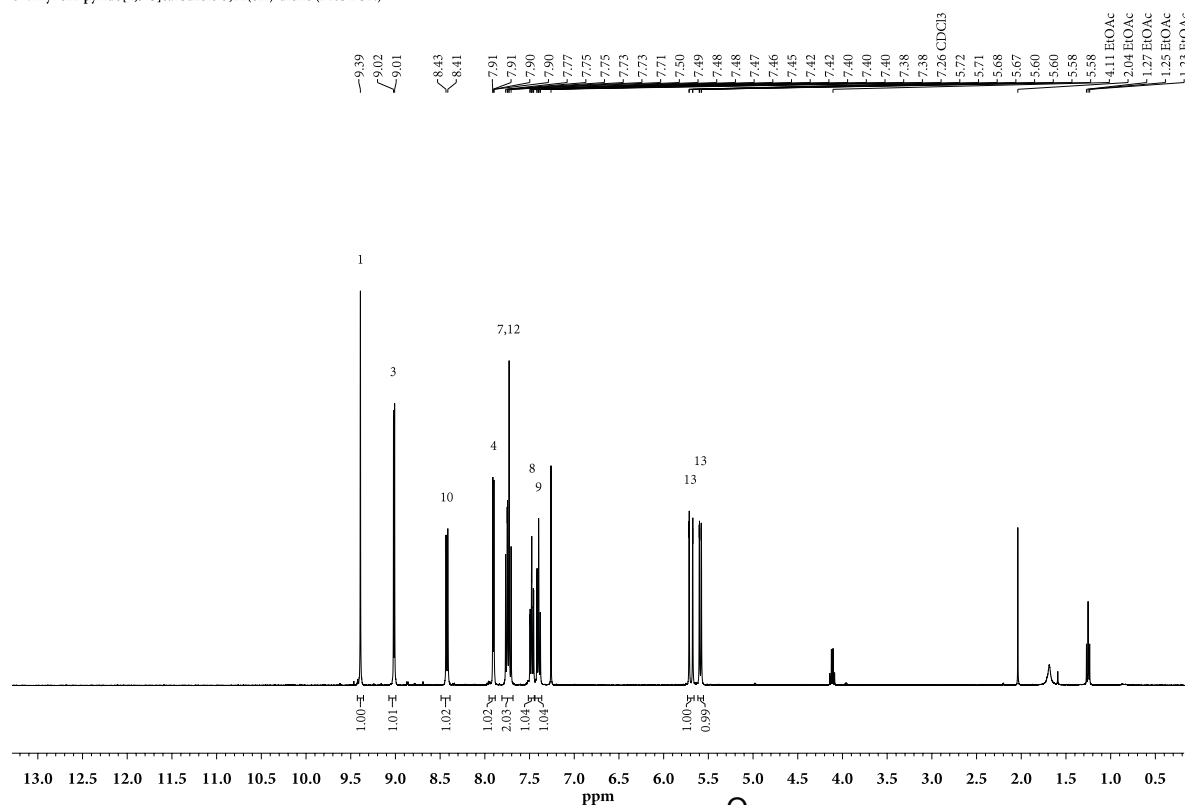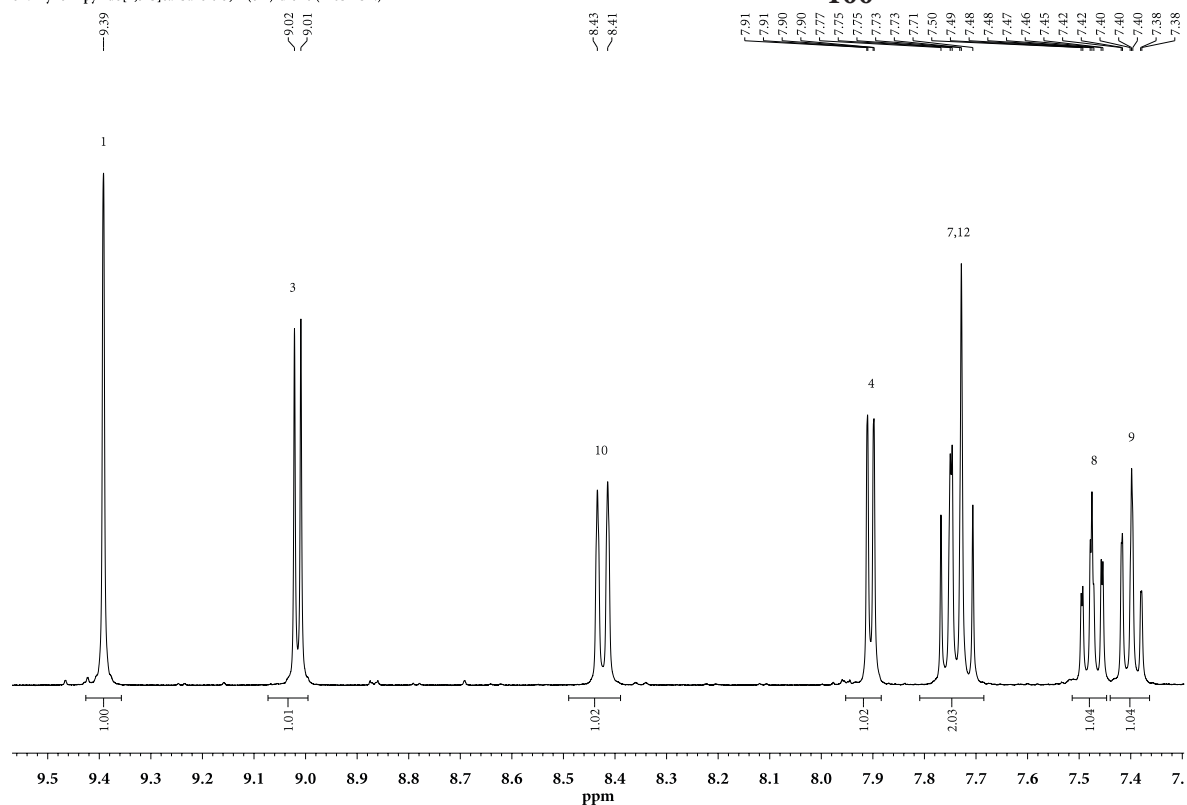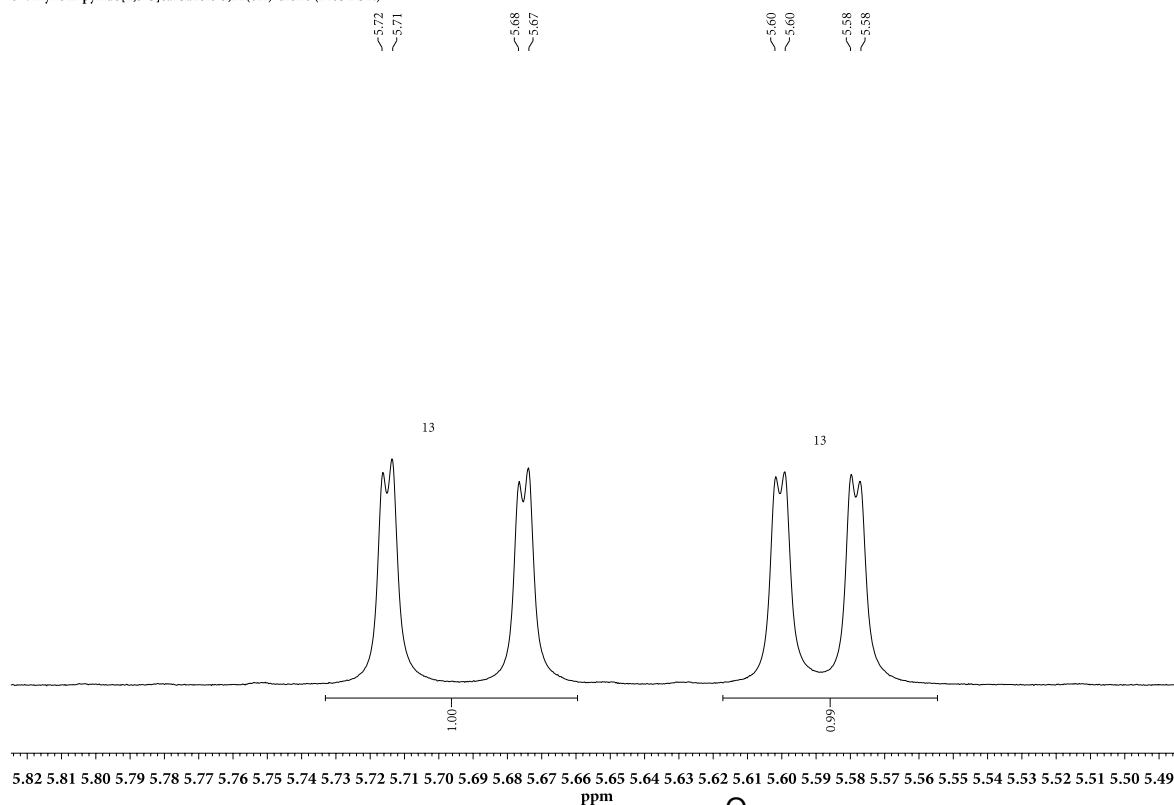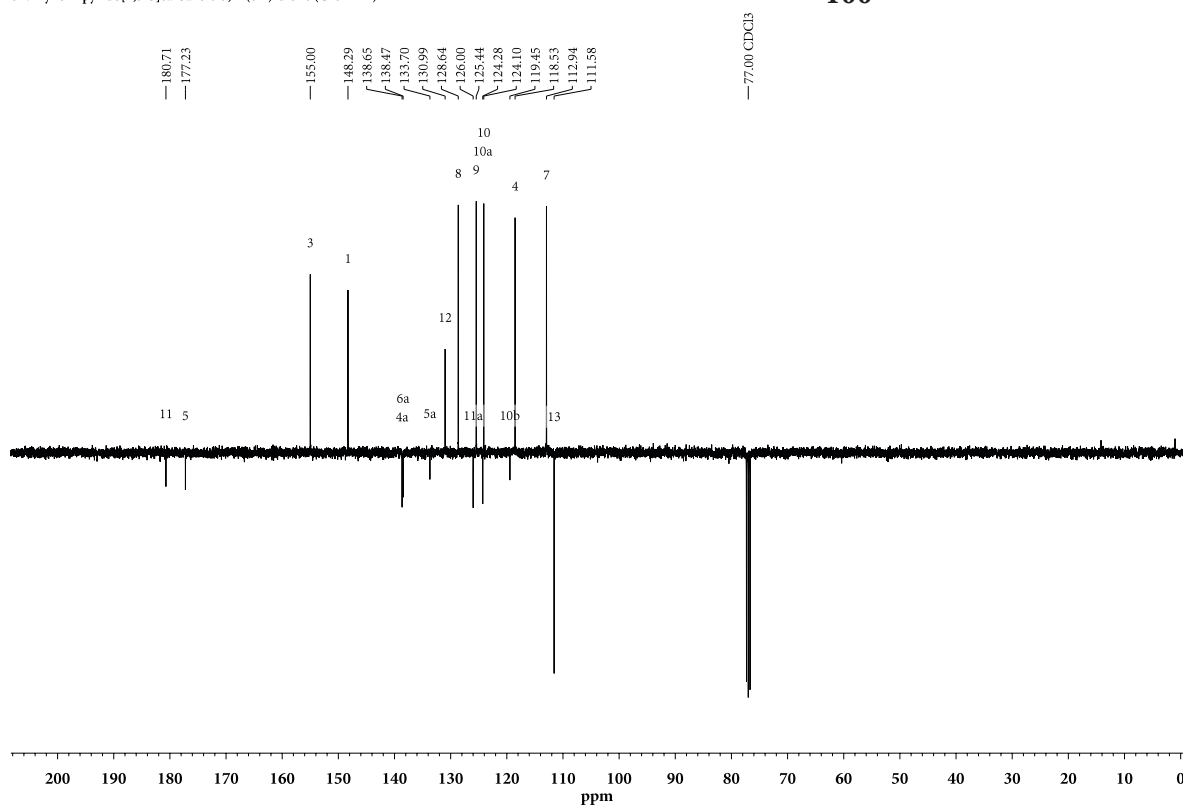
6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (C13 APT)

6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HSQC)

100
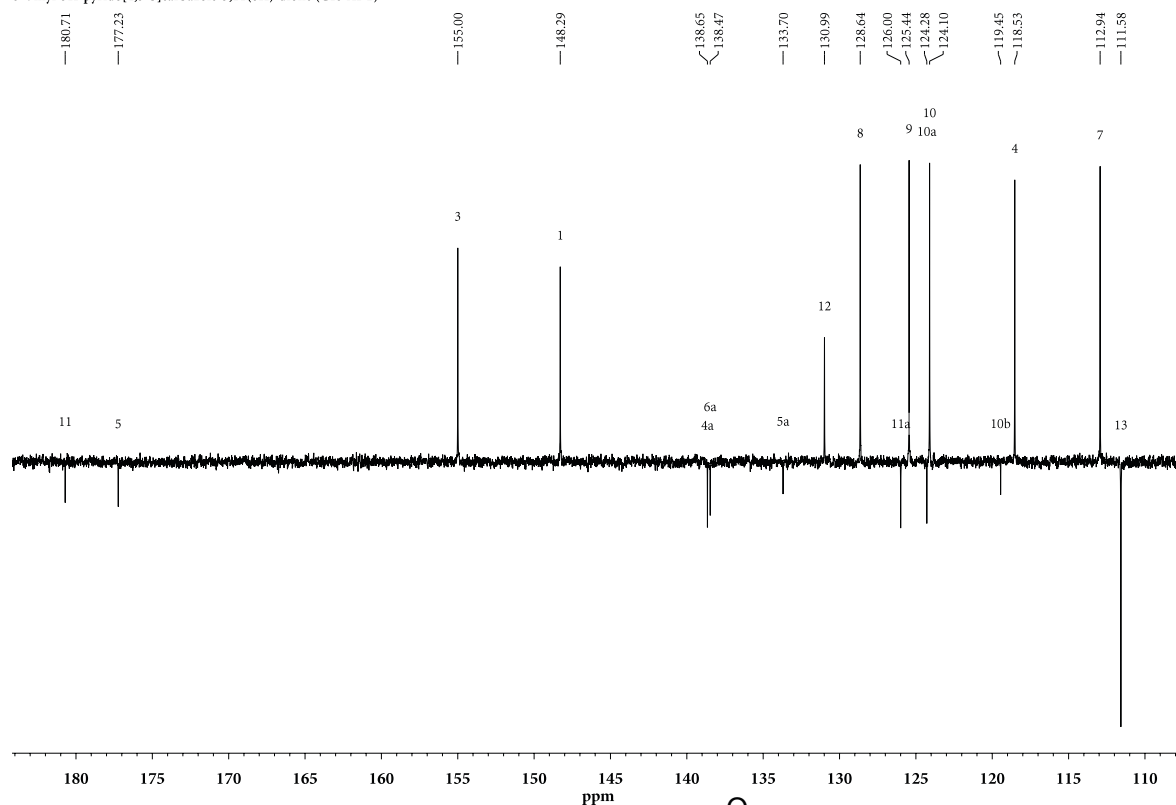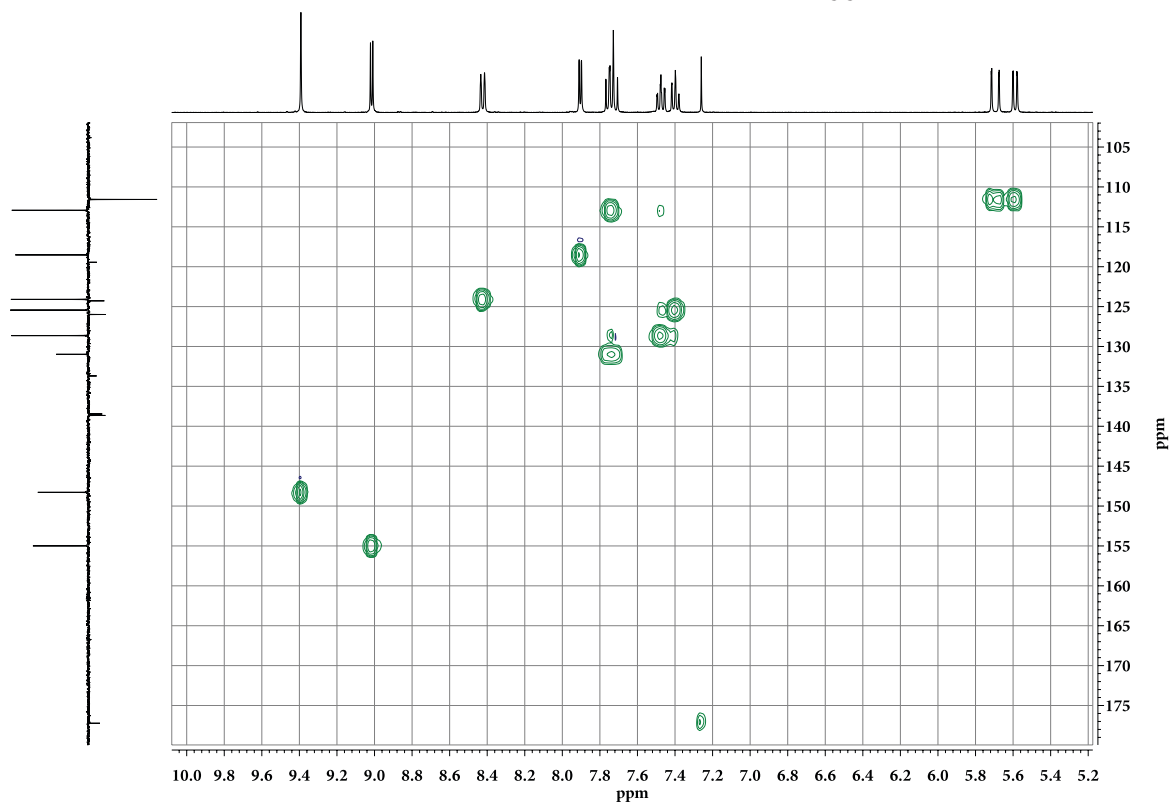
6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (HMBC)

6-Vinyl-5H-pyrido[4,3-b]carbazole-5,11(6H)-dione (15N HMBC)
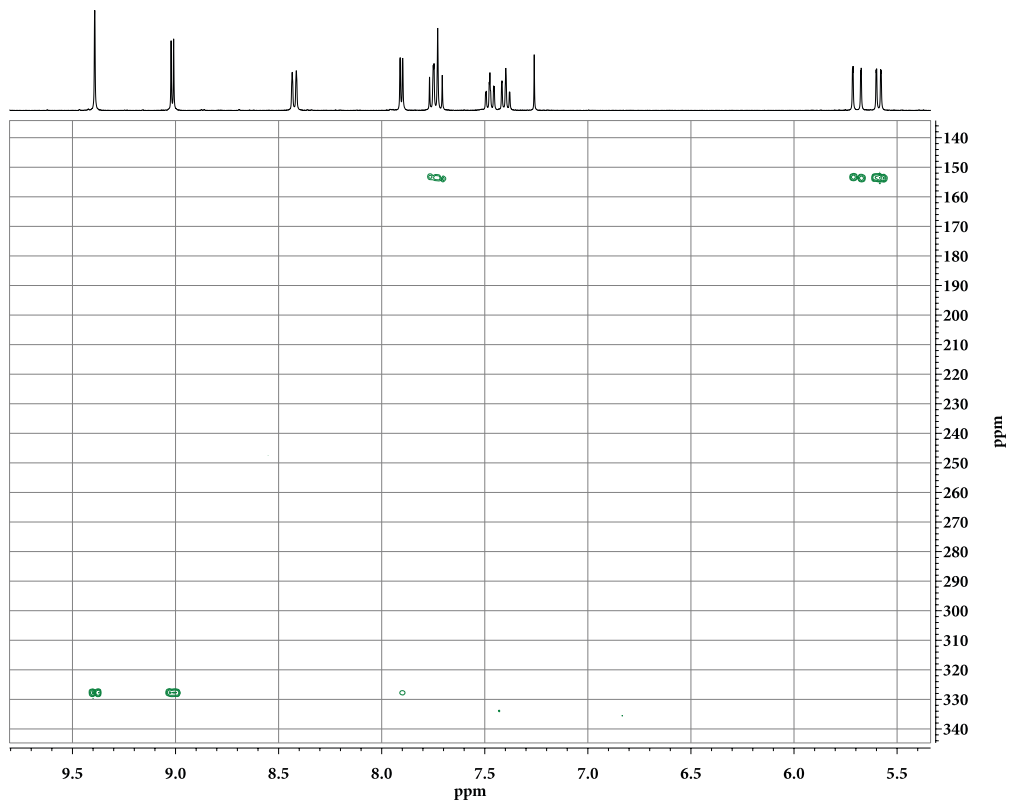
100

795

Generic Display Report

Analysis Info

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Analysis Name	D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN120_DI_ESI_HRMS.d		
Method	DI_mz_50-1550.m	Operator	MZ
Sample Name	0.1 mg/mL in MeOH	Instrument	maXis HD
Comment			

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name   D:\Data_MZ_data\Spreitzer_H\Thomas_N\141117\TN120_DI_ESI_HRMS.d

Method	DI_mz_50-1550.m
Sample Name	0.1 mg/mL in MeOH
Comment	

Acquisition Date   11/17/2014 2:31:53 PM

Operator   MZ
Instrument   maXis HD   1820881.21300

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1550 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
275.0820	1	C17H11N2O2	275.0815	-1.8	13.5	1	100.00	13.5	even	ok
297.0637	1	C17H10N2NaO2	297.0634	-0.8	12.7	1	100.00	13.5	even	ok

100
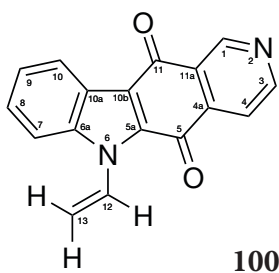
10   ZUSAMMENFASSUNG

Mit über 14 Millionen neuen Krebsfällen weltweit (2012) und über 8.2 Millionen Krebs-assoziierten Todesfällenbesteht ein starkes Interesse an der Entwicklung von neuen Medikamenten. Trotz vieler Fortschritte in der Entwicklung von Krebstherapeutika lassen die bislang eingesetzten hinsichtlich Anwendungsbreite, Selektivität und Nebenwirkungen nach wie vor viele Wünsche offen.
In der vorliegenden Dissertation wurden neue zytotoxische Strukturen mit einem tetrazyklischen Carbazol-Grundgerüst vom Ellipticinchinon-Typ (**1**) synthetisiert. Es wurden die Synthesen einer Reihe tetrazyklischer Ringsysteme mit einer basischen *N,N*-dimethylethanamin-Seitenkette am Carbazol-Stickstoff unternommen. Um die Aktivität zu erhöhen, wurde anschließend eine Erweiterung des Ringsystems um eine Benzoanellierung vorgenommen (mit pentazyklischem Calothrixin-Grundgerüst und dessen Isomeren **2a**, **2b**, **2c**) und derselben Seitenkette ausgestattet. In den anschließenden Antiproliferations-Assays stellten sich Derivate des tetrazyklischen Ellipticingrundkörpers als die vielversprechendsten antiproliferativen Substanzen heraus. Daraufhin wurden weitere Seitenketten-Modifikationen unternommen und erneut biologische Tests durchgeführt.

Als Ergebnis der vorliegenden Arbeit wurden drei zentrale Fragen beantwortet:

1.   Welchen Einfluss auf die zytotoxische Aktivität hat die Variation des Pyridin-Systems durch andere π-Elektronenmangelstrukturen (Pyridin-, Pyrimidin-, Pyrazin- und Pyridazin-Isomere).
2.   Kann durch Erweiterung dieses π-Elektronenmangels um eine weitere Benzoanellierung, die zu Calothrixin-Isomeren führt, die Wirkung zusätzlich erhöht werden? Hintergrund dieses Vorhabens war die bekannte hohe wachstumshemmende Aktivität der Calothrixine, die bekannte Naturstoffe aus *Calothrix cyanobacteria* darstellen.
3.   Durch welche Arten von synergistisch wirksamen Seitenketten mit basischen funktionellen Gruppen kann eine weitere Optimierung erzielt werden?

Mit der vorliegenden Arbeit ist es berechtigt, zu erwarten, dass mit all den bisher hergestellten Verbindungen nun eine Substanzbibliothek in einer Größe und Diversität vorliegt, die in der Folge auch ein Rational Drug Design für die weitere Entwicklung sinnvoll erscheinen lassen.

Pyridine
Pyrimidine
Pyrazine
Pyridazine
R = basic amino functions
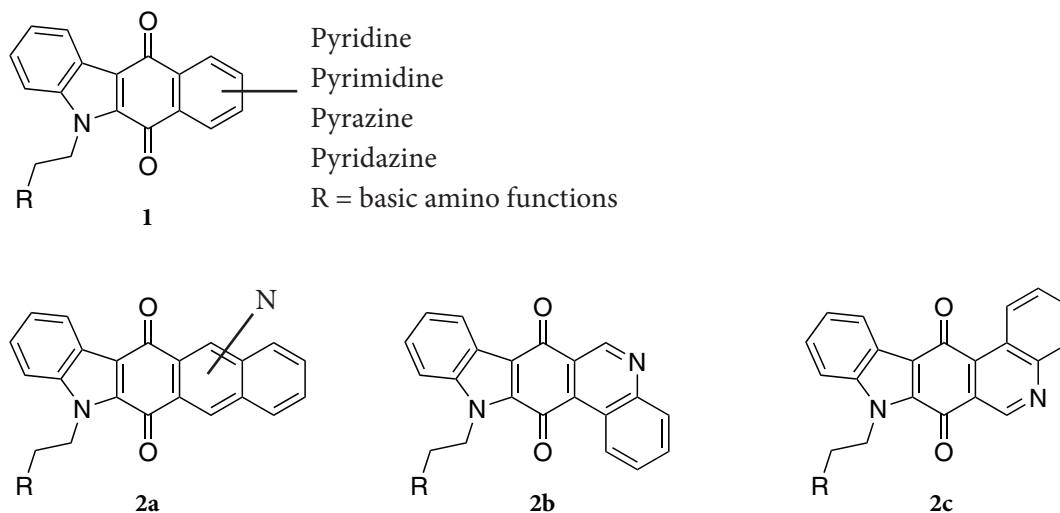
11 ABSTRACT

With over 14 million new cases of cancers worldwide (2012) and over 8.2 million cancer related deaths, there is a strong interest in the development of new drugs fighting this disease. Despite recent advances in chemotherapy, the goal to develop agents with a broad range of application, high selectivity and low toxicity is still unachieved.

In this thesis new cytotoxic compounds were developed based on previous structures containing a tetracyclic carbazole skeleton of the ellipticine quinone type (**1**) with cytotoxic properties. Tetracyclic analogues of this compound endowed with a basic *N,N*-dimethylethanamine side chain were synthesised. Subsequently, the tetracyclic ring system was expanded by a benzene ring leading to the pentacyclic calothrixin skeleton, which is known to possess cytotoxic properties. Subsequently, calothrixin isomers (**2a**, **2b**, **2c**) with the same side chain were synthesised. In the following, the compounds were subjected to viability assays in which derivatives of the tetracyclic series demonstrated the highest cytotoxic potential. Subsequent side chain modifications and further biological tests were performed.

As a result, three main questions were answered:

1. Does the substitution of the pyridine system by other π-electron deficient heterocycles (pyrazine, pyrimidine, pyridazine) enhance the antiproliferative activity of the compounds?
2. Does the expansion of the π-electron deficient system by a further benzo-annulation, which leads to calothrixin isomers, enhance the cytotoxic properties? The rationale for this modification is the documented cytotoxic properties of the calothrixins, which are natural compounds of *Calothrix cyanobacteria*.
3. Can the antiproliferative activity be enhanced by linking different basic substituents to the skeleton?

With the compounds synthesised, our library was expanded sufficiently to attempt further developments via a rational drug design approach.

Pyridine
Pyrimidine
Pyrazine
Pyridazine
R = basic amino functions

1

2a   **2b**   **2c**
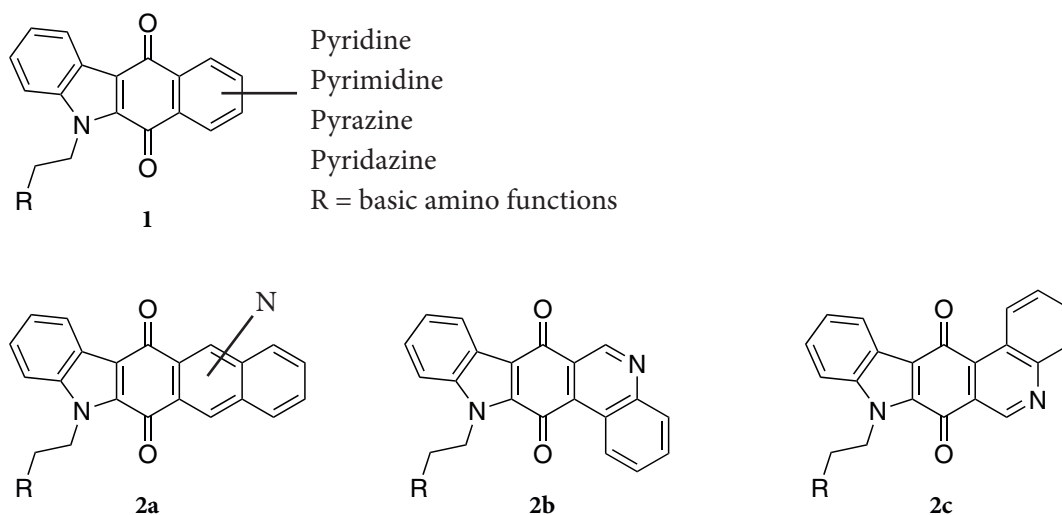
12 CURRICULUM VITAE

Personal Information

Name:	Thomas Nagel
Date of Birth:	1977 - 03 -01
Nationality	Austrian
E-Mail:	pharmazeut@gmx.at

Education

1983-1987	Primary school
	Volksschule Neulandschule, 1100 Vienna
1987-1995	Secondary school
	Bundesgymnasium, 2320 Schwechat
2002-2003	Civilian Service
	BPD Schwechat
2005-2010	Undergraduate Study of Pharmacy, University of Vienna
2008-2010	Tutor in practical courses for drug synthesis at the
	Dept. of Drug and Natural Product Synthesis, University of Vienna
2009-2010	Diploma Thesis at the Dept. for Drug and Natural
	Product Synthesis, University of Vienna.
2010-2015	Doctoral Thesis at the Dept. of Pharmaceutical Chemistry,
	Division of Drug Synthesis
2012-2015	University course "Pharmaceutical Quality Management"

Conferences

2013	Oral Presentation at the 3rd Meeting of the Paul Ehrlich Medchem
	Euro-PhD Network, Cagliari
2014	Poster Presentation at the 4th Meeting of the Paul Ehrlich
	MedChem Euro-PhD Network, Hradec Králové