universität wien

Diplomarbeit

Development of Pyrimidine- and Purine Bioisosters

angestrebter akademischer Grad

Magistra der Pharmazie (Mag. pharm.)

Verfasserin: Irene Maria Lagoja
Matrikel-Nummer: 8917045
Studienrichtung: A449 Diplomstudium Pharmazie
Betreuer: Prof. Helmut Spreitzer

Wien, 23. 06. 2009

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides Statt, dass ich die vorliegende Arbeit selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Der praktische Teil der in dieser Diplomarbeit vorgestellten Synthesen wurde am Rega Instituut, Universiteit Leuven zwischen 2005-2006 durchgeführt. Die Resulate dieser Arbeit wurden in meiner Habilitationsschrift mit dem Titel „Nitrogen Heterocyles, Synthesis, Isotopic Labeling and Biological Activity“ zusammengefasst, sind bis jetzt aber weder in Form von Patenten noch als wissenschaftliche Artikel in peer-reviewed Journals veröffentlicht.

Wien, am 23. 06. 2009

Unterschrift

Mein spezieller Dank gilt Prof. Spreitzer für die Annahme des Themenvorschlages als Diplomarbeit sowie all meinen Kollegen und Freunden.

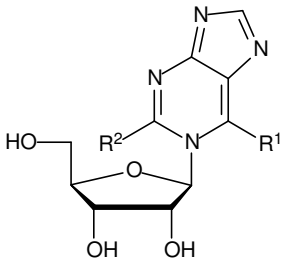
Meiner Familie

Content:

SUMMARY

This thesis describes the development of a series of novel pyrimidine- and purine bioisosters.

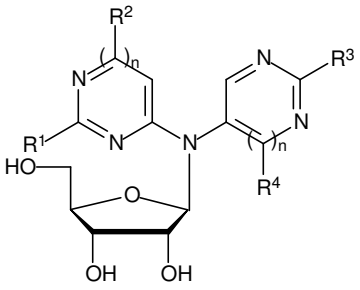
Systematically purines are called imidazo[4,5-d]pyrimidine. In nucleoside chemistry the synthesis and incorporation of N^9-purinylnucleoside - glycosylated at the imidazole nitrogen atom is well established. In these cases the classical Watson-Crick base pairing A-U (T), G-C is observed. This study deals with the chemical synthesis of N^1-glycosylated purinylnucleoside building blocks. In this substance-class, bearing the sugar moiety at the pyrimidine nitrogen, beside the classical Watson-Crick-base pairing additional hydrogen bridges to the unsubstituted imidazole moiety are conceivable.



to simplify this general structure N^1-inosine and N^1-xanthosine are given in the form of the hydroxy tautomer

R^1	R^2
NH_2	H
OH	H
OH	H

The second part of this thesis deals with the development and synthesis of a novel, flexible purine bioisoster, which also can be described as a glycosylated dihetaryl amine derivative. In this novel class the typical purine and pyrimidine recognition elements are retained.

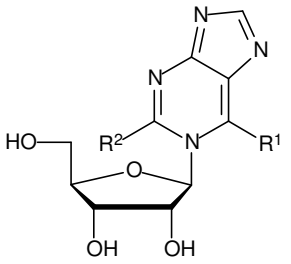


The proposed dihetaryl amines may either be obtained by palladium catalysed amination reaction or *via* reductive desulfurination of the 3-cyclic azaphenothiazin precursor.

ZUSAMMENFASSUNG

In dieser Arbeit wird die Darstellung einer Gruppe von neuen Purin- und Pyrimidin Bioisosteren diskutiert.

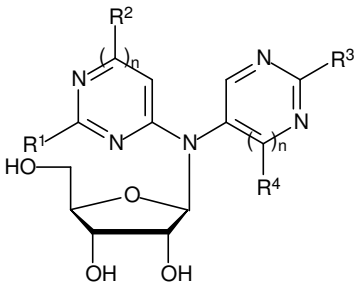
Der systematische Name von Purin lautet Imidazo[4,5-d]pyrimidine. Als Nucleosidbausteine finden vor allem die am Imidazolring glycosylierten N^9-Purinylnucleoside Anwendung. Beim Einbau in Oligonucleotide wird die klassische Watson-Crick Basenpaarung A-U(T), G-C beobachtet. In dieser Studie wurden am N^1 glycosylierte Purinylnucleosidbausteine hergestellt. In dieser am Pyrimidinstickstoff glycosylierten Verbindungsklasse sind neben der klassischen Watson-Crick-Basenpaarung auch weitere Wasserstoffbrückenbindungen zum unsubstituierten Imidazolteil des Nucleosides vorstellbar.



um die allgemeine Struktur
zu vereinfachen werden
N^1- Inosin bzw. N^1- Xanthosin
in der Form des Hydroxytautomers
wiedergegeben

R^1	R^2
NH_2	H
OH	H
OH	H

Im zweiten Teil wird die Synthese eines neuartigen, flexiblen Purinbioisosters diskutiert, das sich als glycosyliertes Dihetarylamin beschreiben lässt. Dabei werden die charakteristischen Erkennungselemente für sowohl Purine alsauch für Pyrimidine beibehalten.



Die Synthese des zugrunde liegenden Dihetarylamins erfolgt entweder durch eine palladiumkatalysierte Aminierungsreaktion oder durch reduktive Entschwefelung des 3-cyclischen Azaphenothiazin Vorläufers

One never notices what has been done; one can only see what remains to be done.
Marie Curie

INTRODUCTION

Nitrogen containing heterocyclic compounds featured prominently in early studies of chemistry and they were closely associated with the development of "organic" chemistry, which was concerned with the study of materials isolated from living sources, whilst "inorganic" chemistry dealt with the study of inanimate materials.

Over the years, the pyrimidine[1] and purine[2] systems turned out to be important pharmacophors, interacting with the synthesis and function of nucleic acids.

When one inspects the relevant literature, one recognizes that, during recent years, purine chemistry has enjoyed a renaissance. Purine receptors -particularly adenosine receptors[3] - are found in all organs of the human body, and the aim of modern purine research is to find new agonists and antagonists for purine receptors.[4]

Four adenosine receptor sub-types, A1, A2A, A2B, and A3, which have been all cloned, are currently known. Purine derivatives, needed as tools to study these receptors, also have the potential to be pharmacologically active. Thus, adenosine receptors play an important role in blood pressure regulation (besides adrenaline and angiotensine receptors).

Moreover: i) These receptors are important for the regulation of pain signals, e.g., when A1-receptors are activated, pain signal transmission is inhibited, leading to local analgesia. ii) In certain tumour tissues, A3 receptors are over expressed, and application of A3 agonists inhibits the growth of cancerous cells. iii) In the ongoing battle against asthma, new A2A agonists are looked upon as alternatives for glucocorticoides.

In this chapter the synthesis of a series of new pyrimidine- and purine bioisosters is described. A series of novel N^1-purinyl nucleosides has been investigated as possible pyrimidine bioisosters, whereas split-nucleosides, likewise bearing imidazole- and pyrimidine rings, have been developed as novel purine bioisosters.

According to the definition of Hansch, structures are called bioisoster when showing the same biological activity. [5] The investigation wheter these new compounds fulfil the biological requests of being a bioisoster is currently under process.

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1) Lagoja, I., *Chemistry & Biodiversity*, **2005**, *2*, 1-50.
2) Rosemeyer, H., *Chemistry & Biodiversity,* **2004**, *1*, 361-401.
3) Van der Wenden E.M., von Frejtag, D., Künzel, J.K., Mathot, R.A.A., Danhof, M.,. Iizerman, A.P, Soudijn, W., *J. Med. Chem*., **1995**, *38*, 4000.
4) Camaioni, E., Costanzi, S., Vitori, S., Volpini, R., Klotz, K.-N., Cristalli, G., *Bioorg. Med. Chem. Lett*. **1998**, 6, 523; http://www.nottingham.ac.uk/~mqzwww/adenosine.html.
5) Hansch, C., *Intra-Science Chem. Rep.,* **1974**, *8*, 17.

1. N^1-PURINYLNUCLEOSIDES

Nucleosides were among the first organic compounds formed at beginning of evolution in the early history of our planet earth. It is still believed that these nucleosides have been formed by reaction of a heterocycle with sugars. To support this hypothesis, guanine and adenine were heated with D-ribose in sea water, containing the Lewis acid magnesium chloride as catalyst[6] obtaining guanosine and adenosine, whereas the *in vitro* synthesis of pyrimidine nucleosides remains as yet an enigma.

The introduction of Friedel-Crafts catalysts such as $SnCl_4$[7] in 1970-1976 for the sugar-base condensation reaction as a more effective version of the Silyl-Hilbert-Johnson synthesis of nucleosides, was followed in 1975 by the use of the Lewis acid trimethylsilyl triflate TMSOTf in the condensation reaction.[8] The whole range of the different methods for pyrimidine and purine nucleoside syntheses as well as the syntheses of nucleosides of various heterocycles has been excessively reviewed previously.[9]

It turned out that TMSOTf, a much weaker Lewis acid than $SnCl_4$, is a superior, if not the optimal, catalyst for nucleoside synthesis. – Not only for mechanistic reasons, but also for simplifying the work-up procedure. By addition of an ice-cold solution of aqueous sodium bicarbonate to the reaction mixtures no emulsions are obtained as nearly always encountered on aqueous workup of reactions employing $SnCl_4$. Ethylene chloride (which favors σ-complex formation of the purine moieties with TMSOTf and which also favors the rearrangement of the initially formed kinetically controlled intermediates to give eventually the thermodynamically most stable products) is thus the preferred solvent for the synthesis of purine nucleosides.[222, 223] Understanding the mechanisms involved in these reactions is essential for their effective application. One of the aims of this project was the isolation and characterization of the kinetically controlled products and to incorporate the obtained purinyl-

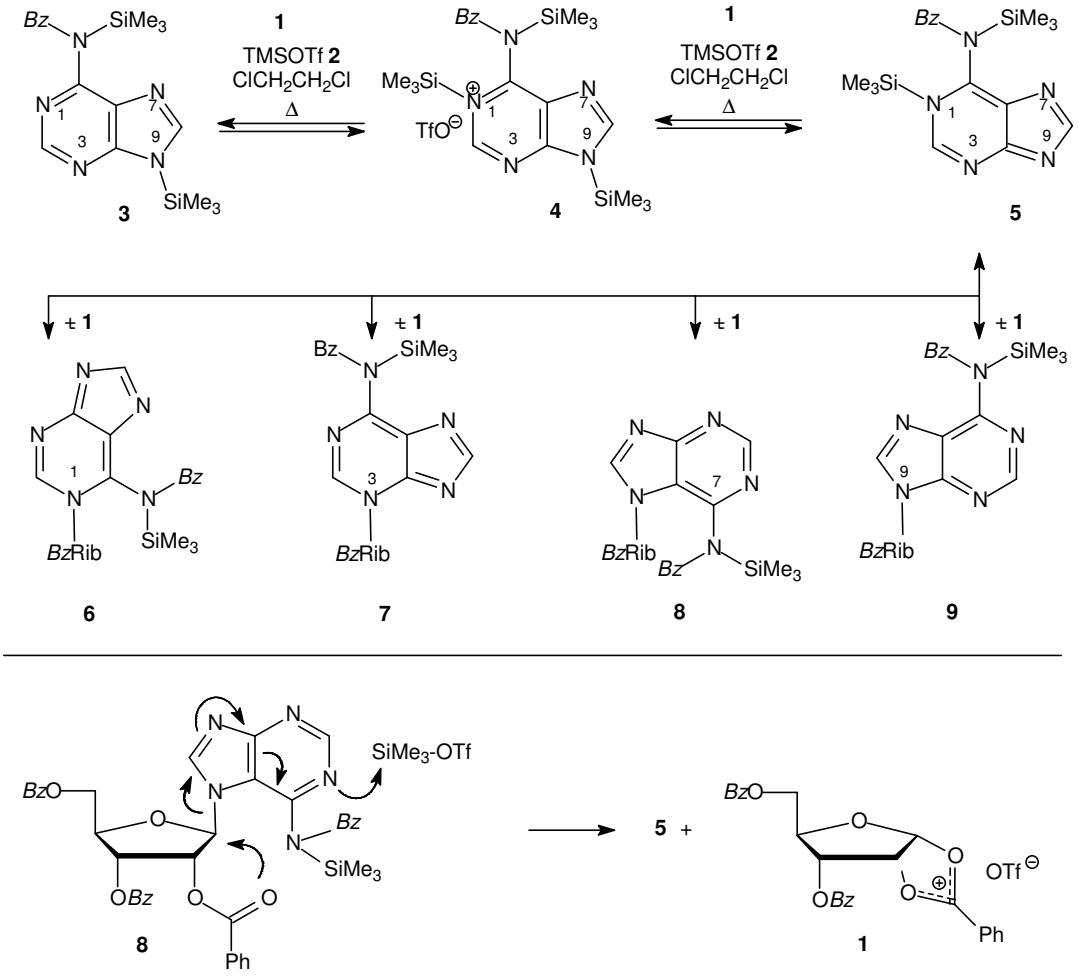
6) a) Fuller, W.D., Sanchez, R.A., Orgel, L.E., *J. Mol. Evol*., **1972**, *1*(3), 249-57; b) Fuller, W.D., Sanchez, R.A., Orgel, L.E., *J. Mol. Biol*., **1972**, *67*(1), 25-33.

7) a) Niedballa, U., Vorbruggen H., *Angew.Chem.* (Int. ed.), **1970**, *9*(6), 461-2. b) Niedballa, U., Vorbrueggen, H., *J. Org. Chem*., **1974**, *39*(25), 3654-9; c) Niedballa, U., Vorbrueggen, H., *J. Org. Chem.* **1974**, *39*(25), 3660-63; c) Niedballa, U., Vorbrueggen, H., *J. Org. Chem*., **1974**, *39*(25), 3664-7; d) Niedballa, U., Vorbrueggen, H., *J. Org. Chem.,* **1974**, *39*(25), 3668-71; e) Niedballa, U., Vorbrueggen, H., *J. Org. Chem*., **1974**, *39*(25), 3672-3; f) Niedballa, U., Vorbrueggen, H., *J. Org. Chem*., **1976**, *41*(12), 2084-6; g) Vorbrueggen, H.; Hoefle, G. *Chem. Ber*. **1981**, *114*(4), 1256-68.

8) a) Vorbrueggen, H., Krolikiewicz, K., *Angew. Chem*., **1975**, *87*(11), 417; b) Vorbrueggen, H., Krolikiewicz, K., Bennua, B., *Chem. Ber*., **1981**, *114*(4), 1234-55.

9) a) Vorbruggen, H., Ruh-Pohlenz, C. "Synthesis of nucleosides**"** *Organic Reactions* (New York) **2000**, *55*, 1-630. b) Vorbrueggen, H., Ruh-Pohlenz, C. "Handbook of nucleoside synthesis" Wiley Interscience, New York, **2001**;

nucleosides into oligonucleotides as a novel group of pyrimidine bio-isosters, offering an additional possibility of Hoogsteen pairing, due to the present imidazole moiety. The reaction mechaninsm of the Vorbrüggen condensation was investigated with adenine and hypoxanthine to obtain pyrimidine bioisosters bearing a hydrogen-bridge donor (adenine) as well as an acceptor (hypoxanthine). Furthermore, the obtained nucleosides should be evaluated for their activity against DNA viruses, RNA viruses and against HIV-1 and HIV-2.

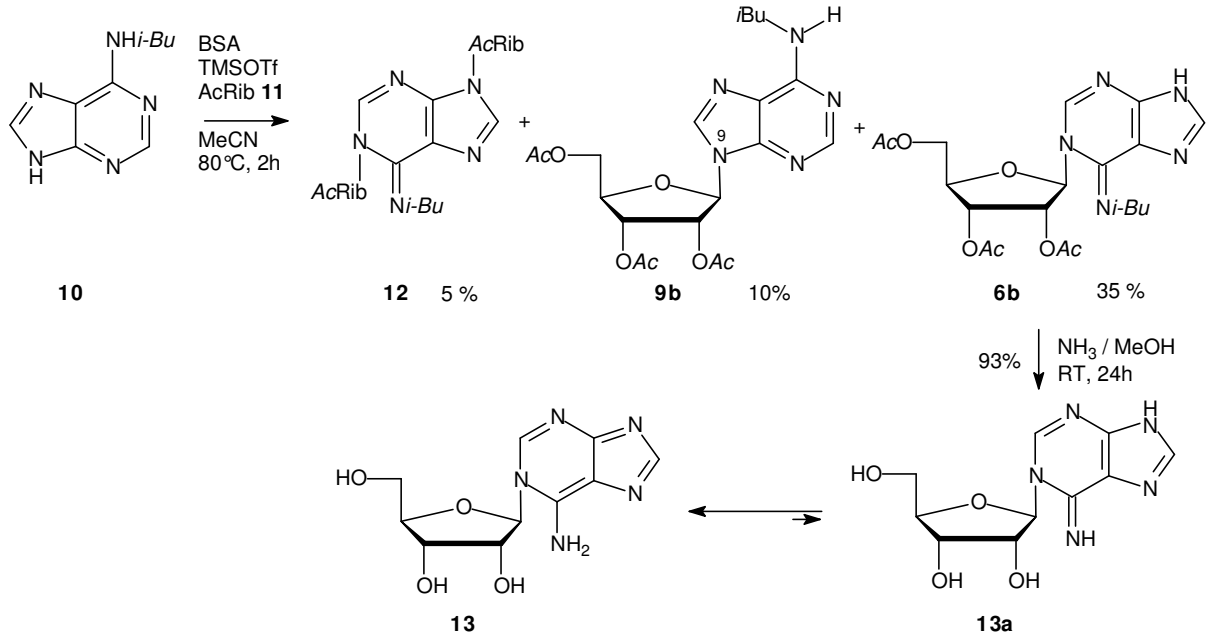


Scheme 1. Lewis acid catalysed glycosylation of N^6-Bz-adenine

On the basis of ^{13}C-NMR-studies[7g] (following the downfield shift of C8) it is assumed that the most basic N^1-nitrogen in persilylated N^6-benzoyl-adenine **3** is trimethylsilylated by TMSOTf **2** to form the σ-complex **4.** This complex eliminates **2** to form the activated putative isomeric silylated N^6-benzoyl-adenine **5**. Vorbrüggen and Ruh-Pohlenz[9] have suggested that compounds **3, 4** or **5** react reversibly with the sugar salts **1** in an initially kinetically controlled reaction to give the protected N^1-nucleoside **6** and the N^3-adenosine **7.** The latter can be

isolated at the start of the reaction but **6** and **7** occur in equilibrium with **5** and **1** (Scheme C1). Subsequent reaction of **5** with the sugar salt **1** furnishes primarily a mixture of the undesired protected N^7-nucleoside **8** and the desired N^9-nucleoside **9**. On boiling the reaction mixture in the rather unpolar solvent ethylene chloride (compared to acetonitrile) σ-complex formation of the undesired products **6, 7** and **8** with TMSOTf **2** is favored and thus rearrangement and formation of equilibria between **6, 7** and **8** with **5** and the sugar salts **1** occurs (cf. the depicted TMSOTf **2** catalyzed cleavage of **8** to **5** and **1**). This eventually results in the nearly exclusive formation of the desired and thermodynamically favored protected natural N^9-nucleoside adenosine **9.** On saponification of **9** with methanolic ammonia, crystalline adenosine is obtained in 81% overall yield starting from **2** and **3**.[8b]

However, it can be demonstrated, that on reacting N^6-isobuturyl-adenine **10** in the presence of TMSOTf **2** with 1,2,3,5-tetra-*O*-acetyl-ß-D-ribofuranose **11** (whose bridged cation corresponding to **1** is less stable than that of the benzoylated intermediate **1** and whose nucleosides consequently rearrange more slowly) the initially formed N^1-nucleoside **6** can be isolated, together with the corresponding N^9-nucleoside **9** as well as the N^1,N^9-bisriboside **12**, as the major product (Scheme C2).



Scheme 2. Synthesis of N^1-ribofuranosyladenine **13**

These results also were confirmed from Boryski et. al.[10] To stabilize the initially formed kinetic products the reaction is carried out in acetonitrile under exclusion of oxygen. Saponification with methanol /ammonia results in crystalline N^1-ribofuranosyladenine **13**.

Table C1. Comparison of ^{1}H and ^{13}C NMR shifts of N^9- and N^1-adenosine

	N^9-adenosine		N^1-adenosine	
^{1}H-NMR	H1´	5.91 d, J = 6.2 Hz	H1´	5.95 d, J=6.0 Hz
sugar	H2´	4.64 m	H2´	4.12 t, J=5.4 Hz
	H3´	4.17 m	H3´	4.12 d
	H4´	3.99 m	H4´	4.07 d
	H5´	3.68 m, 2H	H5´	3.72 m, 2H
	2-, 3-, 5-OH	5.20, 5.49 m, br, ex 3H	2-, 3-, 5-OH	3.38 br, s, ex, 3H
^{1}H-NMR	H-2	8.37 s	H-2	8.39 s
base	H-8	8.16 s	H-8	7.83 s
	NH_2	7.38 br, s, ex	NH_2	6.80 br, s, ex
^{13}C-NMR	C1´	88.4	C1´	92.23
sugar	C2´	73.9	C2´	72.74
	C3´	71.2	C3´	69.90
	C4´	86.4	C4´	86.27
	C5´	62.2	C5´	60.55
^{13}C-NMR	CH-2	152.9	CH-2	140.54
base	C-4	149.5	C-4	154.70
	C-5	119.8	C-5	118.99
	C-6	156.6	C-6	148.68
	CH-8	140.5	CH-8	150.73

In order to obtain building blocks suitable for oligonucleotide synthesis protection of N^9 and N^6 and 5´-OH were investigated. However, all attempts of benzoyl protection of the amino functions as well as the introduction of a mono-methoxytrityl protection of the 5´-OH failed. In the X-ray structure[11] of N^1-adenosine it can be seen that the tautomer of 1-ribofuranosyl-1*H*-purine-6-amine **13** is found in place of 1-

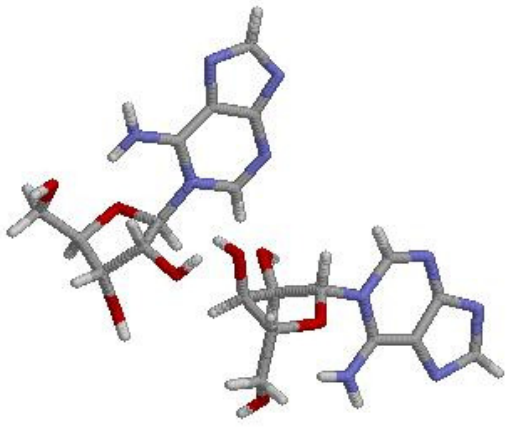
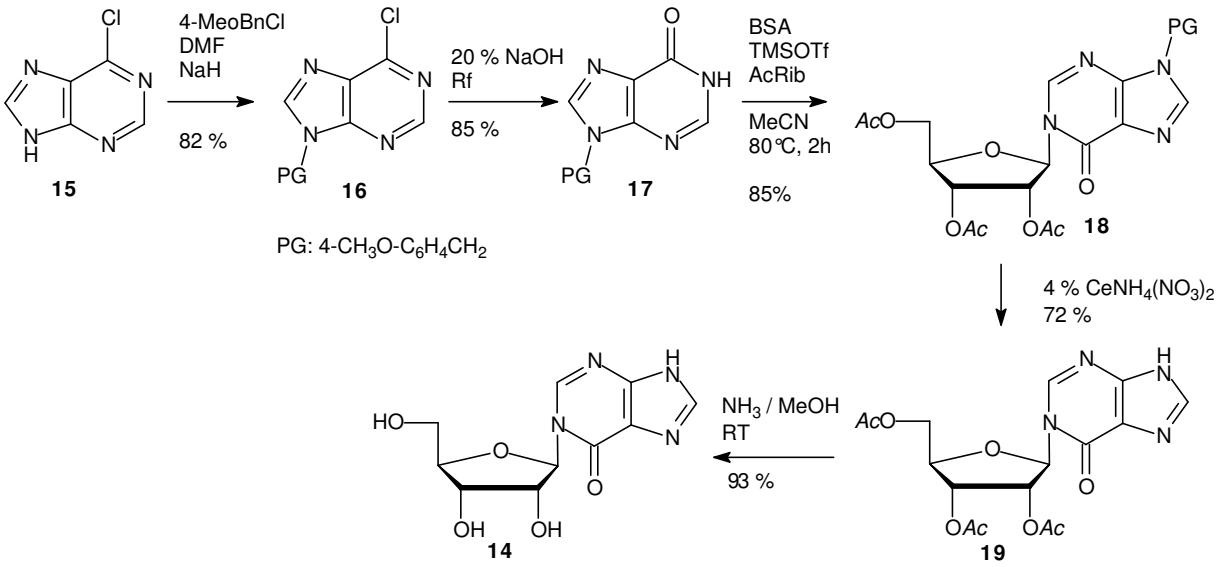


Figure 1. X-ray structure of N^1 adenosine **13**

10) Boryski, J., Framski, G. *Coll. Sympos. Series* **2005**, *7* (Chemistry of Nucleic Acid Components), 177-181.
11) X-ray structure was kindly provided from Prof. Jerzy Boryski, Poznan University, Poland.

ribofuranosyl-1,9-dihydro-6*H*-purin-6-imine **13a.** This may explain the difficulties to protect the position *N*9, whereas strong hydrogen-bridge between 5´OH and the 6-NH_2 causes the low reactivtity of these two positions. Compound **13** turned out to be stable in alkaline or neutral reaction conditions; however, in presence of Lewis acids a migration of the sugar moiety to obtain the thermodynamically more stable *N*9 nucleoside has been observed.

N^1-inosine **14** also could be isolated as the kinetic product (24 % yield) obtained by Vorbrüggen-Condensation of hypoxanthine and 1,2,3,5-tetra-*O*-acetyl-ß-D-ribofuranose **11.** However, a more favorable approach starts from 6-chloropurine **15**. After protection of 9-position to obtain **16**, the 6-chloro can be converted to a hydroxy function by refluxing in 1N NaOH yielding **17**. Glycosylation to **18** followed by selective deprotection to **19** finally afforded N^1-inosine **14** (Scheme 3).



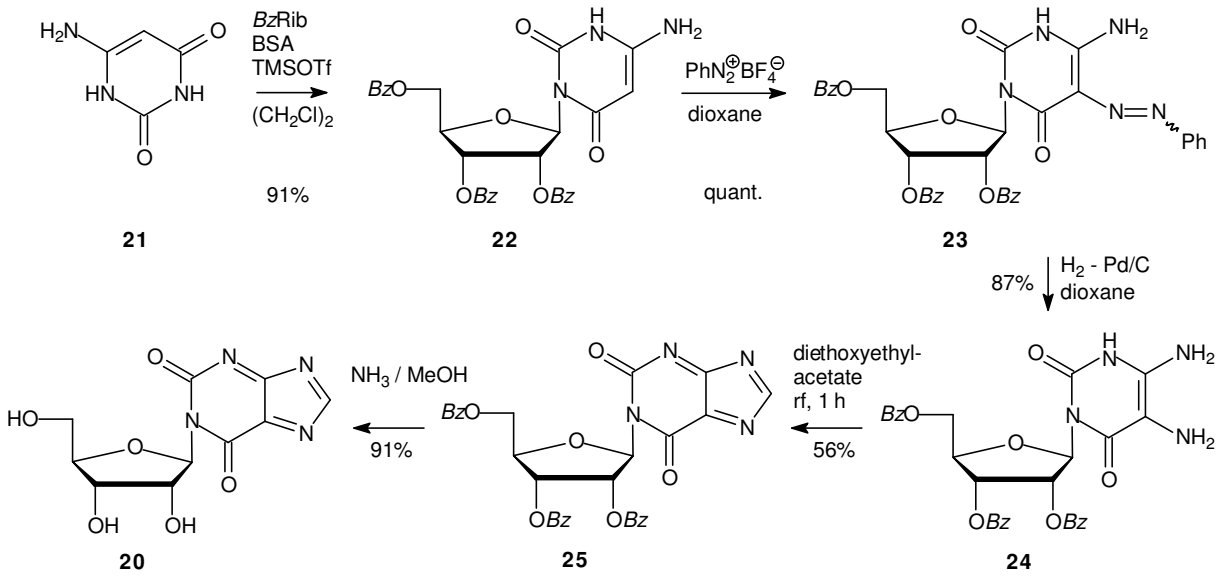
Scheme 3. Synthesis of N^1-inosine **14**

In first instance a benzyl protecting group has been applied, however, reductive deprotection was difficult at best. Also allyl-protection was not favourable since the conversion to the 9- protected hypoxanthine derivative **17** suffered from bad yield. These difficulties could be overcome using a 4-methoxybenzyl protecting group, which could be smoothly removed under mild oxidative conditions $(NH_4)_2Ce(NO_3)_3$. Saponification with MeOH / NH_3 finally derives N^1-inosine **14** in good yield.

Table 2. Comparison of ^{1}H NMR shifts of N^9- and N^1-inosine

	N^9-inosine		N^1-inosine	
^{1}H-NMR	H1´	5.88 d, J = 5.73 Hz)	H1´	6.15 d, J = 4.12 Hz
sugar	H2´	4.49 m	H2´	4.00 m. 3H
	H3´	4.12 m	H3´	4.00 m. 3H
	H4´	4.00 m	H4´	4.00 m. 3H
	H5´	3.55-3.60 m, 2H	H5´	3.68 m, 2H
	2-, 3-, 5-OH	5.06, 5.18, 5.48 m, br, ex	2-, 3-, 5-OH	5.50 m, br, ex
^{1}H-NMR	H-2	8.07 s	H-2	8.55 s
base	H-8	8.34 s	H-8	8.09 s
	NH / OH	12.38 s, br, ex	NH / OH	5.50 br, m, ex

As a last product of the series of N^1-nucleosides, N^1-xanthosine **20** has been prepared by total synthesis starting from 6-aminouracil **21**. Vorbrüggen Condensation exclusively derives the N^3 glycosylated derivative **22**. The formation of the *N3* isomer **22** was verified by NOE-Difference Spectroscopy (i.e. irradiation of the (1)NH causes a 12 % NOE at the exocyclic amino group). A nitrosation / reduction sequence proved not applicable due to decomposition problems of the nitroso intermediate.



Scheme 4. Synthesis of N^1-xanthosine **20**

However, the problem could be circumvented by preparing the diazo-intermediate **23** followed by reductive cleavage (H_2 / Pd-C) to obtain the 5,6-diaminopyrimidyl derivative **24**. Ring closure with diethoxyethyl acetate affords the protected xanthosine **25** in moderate yield.

Finally, upon saponification with MeOH / NH_3 the desired N^1-xanthosine **20** is obtained (Scheme 4).

Table 3. Comparison of ^{1}H NMR shifts of N^9- and N^1-xanthosine

	N^9-xanthosine		N^1-xanthosine	
^{1}H-NMR	H1´	5.73 d, *J* = 6.84 Hz	H1´	6.04 (d, J = 1.8 Hz),
sugar	H2´	4.22 m	H2´	4.42 m
	H3´	4.06 m	H3´	3.54 m
	H4´	4.01 m	H4´	3.54 m
	H5´	3.66 m, 2H	H5´	3.54 m
	2-, 3-, 5-OH	5.28, 5.48 m, br, ex	2-, 3-, 5-OH	5.00 m, br, ex
^{1}H-NMR	H-2	7.87 s	H-2	8.04 s
base	NH / OH	10.87	NH / OH	6.37, 8.60, br, ex

The nucleosides **13**, **14** and **20** have been evaluated for their activity against DNA viruses, RNA viruses and against HIV-1 and HIV-2. However, no significant activity or toxicity has been found.

2. SPLIT-NUCLEOSIDES

Purines are the most widely distributed heterocycles in nature. They appear in nucleic acids, are part of the energy supply mechanism (ATP) of the cell, they may function as cofactor (NAD) and ligands for receptors. In all these cases, the purine base is involved in molecular recognition and a perfect fit between ligand and protein is important for biological activity. All purinergic targets (ATP, NAD, receptors, nucleic acids) have been exploited for antagonist design (i.e. for the development of new potential drugs).

Therefore it might be important to develop a new general concept for modifying purine bases that can be used in the drug discovery process. The aim of this project is the search for a boycotter of purine bases and the use of this concept in several fields of inhibitor design.

One way to realize this concept is to develop “split” purine nucleosides in order to retain the typical purine recognition elements (i.e. the pyrimidine and the imidazole moiety) and to increase flexibility of the ring system to increase the adaptability to biological targets.

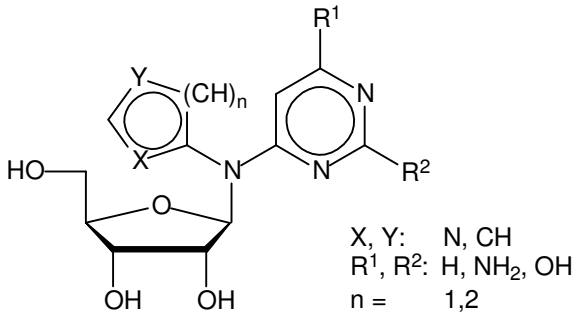


Figure 2. General structure of split-nucleosides

This concept has been developed before mostly for the sugar moiety (i.e. acyclic nucleosides) of nucleosides but it is unexplored for the base moiety.

The flexibility of a base is of utmost importance for the biological function of a nucleoside. In naturally occurring nucleotides the anti conformation (as shown in Figure C2) predominates, however, examples of the syn conformation are known. For example, the conformation of the base at the glycosidic bond shows a high impact on the enzymatic reactions catalyzed by 5'-nucleotidase and adenosine deaminase.[12] The importance of purine recognition in biomedical research can be demonstrated with several examples.

As an example at the level of receptors, adenosine has an important role in modulating the function of the cardiovascular, endocrine, and nervous system.[13] Till now, four receptor sub-types (A1, A2A, A2B, and A3)[14] are known and have been all cloned and pharmacologically characterized. Purines are needed as tools for the study of these receptors, and purine analogues have the potential to function as new pharmacologically active compounds.[15] Untill now, nucleoside ligands at the agonist-antagonist boundary have been either modified at the sugar,[16] or the intact purine moiety has been manipulated by substitution.[17] Due to their flexible character and specific recognition abilities the here proposed split-nucleosides represent a new generation of potential purine receptor agonists and antagonists. With the

12) Stolarski, R., Dudycz, L., Shugar, D., *Eur. J. Biochem.*, **1980**, *108*, 111-121.
13) Daly, J.W., *J. Med.Chem.*, **1982**, *25*, 197.
14) Olah, M.E., Stiles, G.L., *Pharmacol.Ther.*, **2000**, *85*, 55-75.
15) a) Jacobson, K.A., Knutsen, L.S., in "*Purinergic and Pyrimidinergic Signaling I*", Handbook of Experimental Pharmacology, Vol. 151/I, Abbracchio, M.P, Williams, M, Eds., Springer-Verlag: Berlin, Germany, **2001**, 129 – 175; b) Müller, C.E., *Curr. Med. Chem.*, **2000**, *7*, 1269 – 1288.
16) Gao, Z.G., Kim, S.K., Biadatti, T., Chen, W., Lee, K., Barak, D., Kim, S.G., Johnson, C.R., Jacobson, K.A. *J. Med. Chem.*, **2002**, *45*, 4471 – 4484.
17) a) Beauglehole, A.R., Baker, S.P., Scammells, P.J., *Biorganic & Med.Chem. Let.*, **2002**, *12*, 3179-3182; b) Rieger, J.M., Brown, M.L., Sullivan, G.W., Linden, J., Macdonald, T.L., *J. Med. Chem.*, **2001**, *44*, 531-539.

increasing availability of crystal structures for various enzyme-substrate complexes, it has become apparent that many binding sites for purine nucleosides are more flexible than previously assumed and can, therefore, adjust to fit to a wide range of substrates.[18] The split-nucleosides still have the molecular elements needed for recognition and they provide a unique perspective to investigate enzyme binding-site parameters. This information might serve to overcome the problems typically associated with using crystallographic data in modelling when dealing with flexible enzyme binding sites. The obtained data may be used to design a second-generation of more effective inhibitors. An example of this approach is the search for inhibitors of S-adenosyl-L-homocysteine hydrolase[19] (an antiviral target) which may evaluate the concept at the level of enzyme recognition.

At the level of DNA recognition, the flexibility and the possible variation of the substitution pattern of both heterocyclic rings of the split-nucleosides allows specific purine and / or pyrimidine recognition by the same molecule. This makes them unique candidates for investigation of hybridization properties within the double- and triple-helix motif and for nucleic acid-protein interactions. The degeneracy of the genetic code leads to a multitude of possible DNA sequences coding for a particular protein. Oligonucleotides taking this redundancy into account must be synthesized and used for the screening of the potential DNA or RNA candidates. This has led to the concept of mixed primers or probes. The fundamental problem of this approach is reflected by the fact that the replacement of natural nucleosides by a chemically modified congener within a certain sequence may have an impact on the helix-form (A, B, Z).[20] The use of less discriminatory base analogs (universal or ambiguous bases[21]) has not fulfilled the expectations yet, despite of twenty years of research. A true universal base should hybridize with all four natural bases without discrimination and significant destabilization of the DNA duplex. The split-nucleosides, as proposed here, increases the potentiality for discovery of new universal base moieties. Besides the antisense gene therapy, which aims to turn off a mutated gene in a cell by targeting the mRNA transcripts copied from the gene, triple-helix-forming oligonucleotide gene therapy targets the DNA sequence of a

18) a) Hu, Y., Yang, X., Yin, D., Mahadevvan, J., Kuczera, K., Schowen, R.L., Borchardt, R.T., *Biochem*., **2001**, *40*, 15143; b) Yin, D., Yang, X., Hu, Y., Kuczera, K., Schowen, R.L., Borchardt, R.T. Squier, T.C., *Biochem*., **2000**, *39*, 8323.

19) Seley, K.L., Quirk, S., Salim, S., Zhang, L., Hagos, A., *Biorg. & Med. Chem. Let*., **2003**, *13*, 1985 - 1988

20) Luyten, I., Herdewijn, P., *Eur. J. Med. Chem*., **1998**, *33*, 515-576.

21) a) Loakes, D., Brown, D.M., Linde, S., Hill, F., *Nucleic Acids Res.*, **1995**, *23*, 2361–2366; b) Loakes, D., Brown, D.M., *Nucleic Acids Res.*, **1994**, *22*, 4039–4043; c) Nichols, R., Andrews, P.C., Zhang, P., Bergstrom, D.E., *Nature*, **1994,** *369*, 492–493; d) Moran, S., Ren, R.X.-F., Kool, E.T., *Proc. Natl Acad. Sci. USA*, **1997,** *94*, 10506–10511; e) Takeshita, M., Chang, C.-N., Johnson, F., Will, S., Grollman, A.P. *J. Biol. Chem.*, **1987,** *262*, 10171–10179.

mutated gene to prevent its transcription. This technique involves the delivery of short, single-stranded oligonucleotides (TFO), binding specifically in the groove between the double strands of the mutated gene's DNA.[22] This produces a triple-helix structure preventing that segment of DNA from being transcribed into mRNA.[23] Reverse Transcription of retroviral RNA into double-stranded DNA is catalyzed by the reverse transcriptase (RT). A highly conserved polypurine tract, PPT, which is found twice, in HIV-1 viral RNA and in the nef gene adjacent to U3 and in the pol gene, serves as primer for plus strand DNA synthesis. The PPT and seven additional purine nucleotides[24] constitute a suitable target for triple-helix formation inhibiting the RT and RNaseH activities. Therefore, the incorporation of the flexible split-nucleosides into oligonucleotides allows the investigation of a new generation of TFOs.

2.1. Synthesis of Di-Hetaryl Amines by Hartwig-Buchwald Condensation (Method A):

Di-hetaryl amines can be prepared by direct condensation of a hetarylamine with a hetaryl-halogen compound. Condensation can be achieved in acidic[25] – or alkaline[26] medium, however, yields are not satisfying. In heterocyclic chemistry, the formation of a new C-N bond using palladium catalyzed amination reaction has received growing attention.[27] A possible catalytic cycle is given in Scheme 5.

At first instance, a Pd-BINAP (2,2´-bis(dipenylphosphino)-1,1´-binaphtyl) complex is formed which can undergo oxidative addition of the hetarylhalogen compound. Further coordination of the amine affords an amido complex, which in turn can undergo reductive elimination to form the desired product and regenerating the Pd(0) catalyst.

22) Szewczak, A.A., Ortoleva-Donnelly, L., Ryder, S.P., Moncoeur, E., Strobel, S.A. *Nature Struct. Biol*., **1998**, *5* (12), 1037 – 1042.

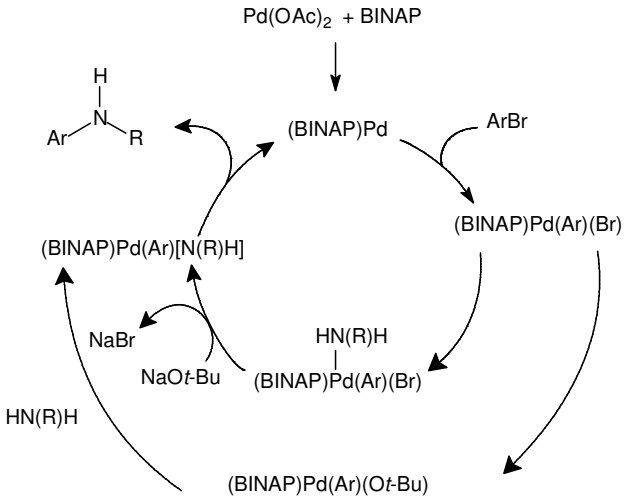
23) Vuyisich,M, Beal, P.A., *Nucl. Acids Res*. **2000**, *28* (12), 2369 – 2374.

24) a) Volkmann, S., Jendis J., Frauendorf, A., Moelling, K., *Nucleic Acid Res.,* **1995,** *23*, 1204-1212; b) Jendis, J., Strack, B., Volkmann, S., Moelling, K., *AIDS Res Hum Retroviruses,* **1996**, *12*, 1161-1168.

25) Banks, K., *J. Am. Chem. Soc*., **1944**, 1131.

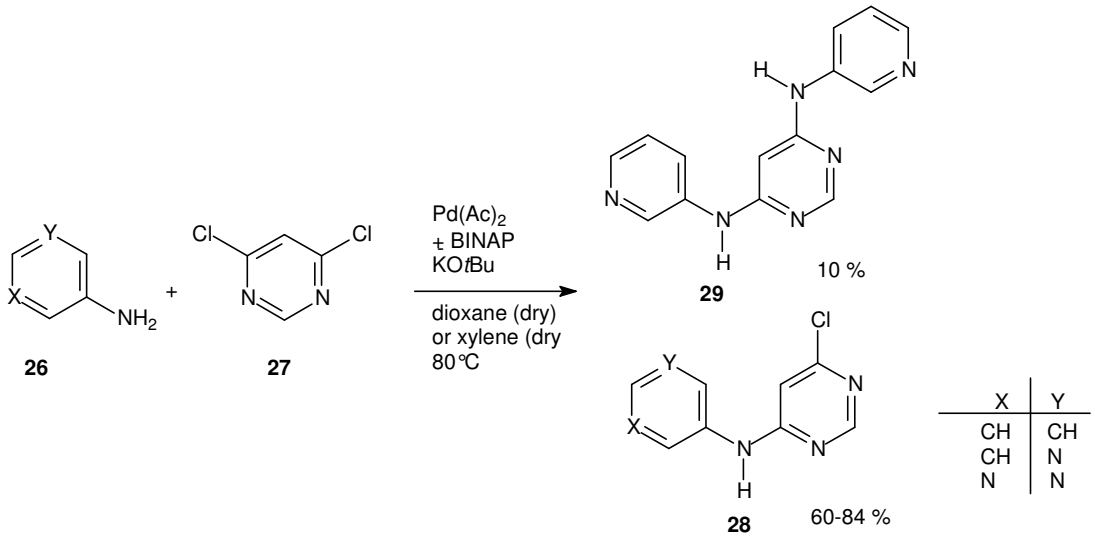
26) Banks, K., *J. Am. Chem. Soc*., **1944**, 1127-1130.

27) for review see: a) Wolfe, J.P., Wagaw, S., Marcoux, J.F., Buchwald, S.L., *Acc. Chem. Res*., **1998**, *31*, 805– 818; b) Hartwig, J.F., *Angew. Chem. Int. Ed*. **1998**, *37*, 2046– 2067; further references: c) Driver, M.S., Hartwig, J.F. *J., Am. Chem. Soc*., **1996**, 118, 7217 – 7218; d) Stauffer, S.R., Lee S., Stambuli, J.P., Hauck, S.I., Hartwig, J.F., *Org. Lett*., **2000**, 2, 1423; e) Wolfe, J.P., Buchwald, S.L., *Angew. Chem. Int. Ed*., **1999**, *38*, 2413- 2416; f) Wolfe, J.P., Buchwald, S.L., *J. Org. Chem*. **2000**, *65*, 1144-1157; g) Ali, M.H., Buchwald, S.L., *J. Org. Chem*. **2001**, *66*, 2560 - 2565.



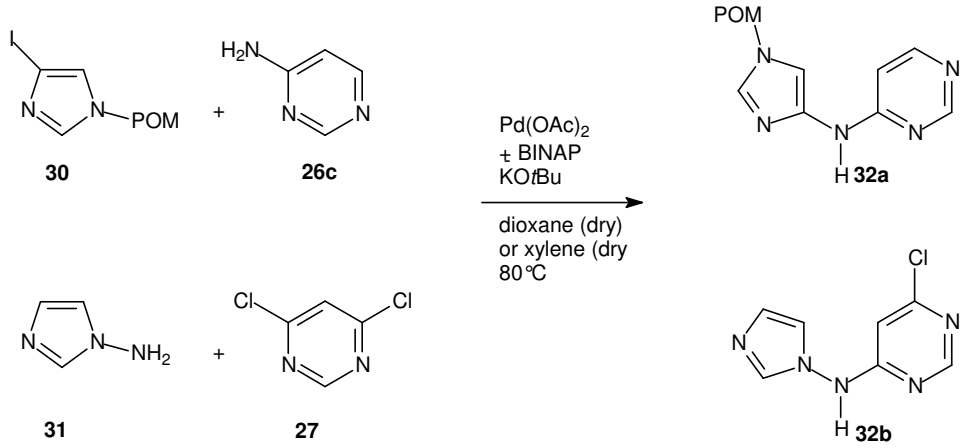
Scheme 5. Catalytic cycle of Pd-catalysed amination reaction

The solubility of amines HN(R)H **26** in xylene or dioxane limits the use of this methology. However, using the above method allows the condensation of anilines **26a**, aminopyridines **26b** and aminopyrimidines **26c** with i.e. 4-6-dichloropyrimidine **27** (Scheme 6).



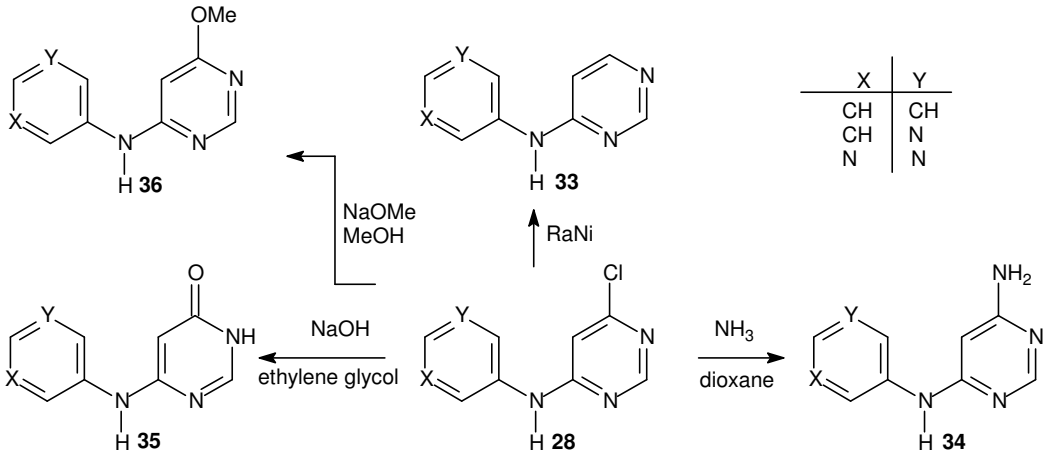
Scheme 6. Synthesis of di-hetaryl amines **28** (bearing two 6-membered rings) by Pd-catalyzed coupling

Besides the desired dihetarylamine derivatives **28** in some case (i.e. using **26b** as starting material) the bisamination product **29** could also be isolated as a minor product. Upon using imidazole derivatives **30** and **31**, respectively, a di-hetaryl amine **32** bearing as well a 5- as a 6-membered ring is obtained, respectivley showing even closer relationship to the naturally occurring purines (Scheme 7).



Scheme 7. Synthesis of di-hetaryl amines **32** (bearing a 5- and 6-membered ring) by Pd-catalyzed coupling

Further modifications could be achieved by nucleophilic substitution of the chlorine to a hydroxyl function or methoxyfunction or by reductive removal of the chlorine using Raney nickel (Scheme 8).

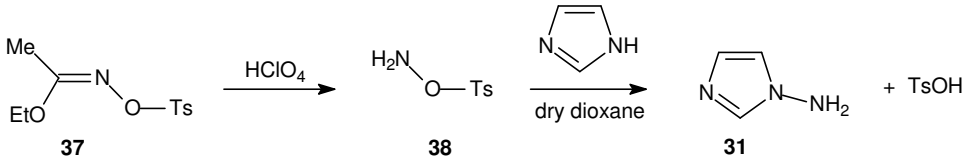


Scheme 8. Further modification of the di-hetaryl amines **28**

However, should a second amino-function in position 2 of the pyrimidine ring be desired, the here described method[28] did not work satisfyingly, and an alternative approach (compare Chapter 2.2) was chosen. Aniline **26a** and 3-aminopyridine **26b** are commercially available, whereas amino-imidazole derivatives **30**, **31** and amino-pyrimidine derivative **26c** have to be synthesized. 1-*N*-Aminoimidazole **31** can be prepared by electrophilic amination of imidazole

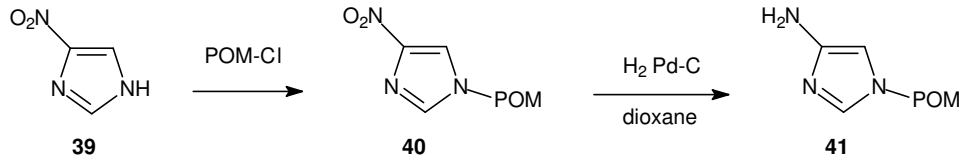
28) Prim, D., Campagne, J.-M., Joseph, D., Andrioletti, B., *Tetrahedron*, **2002**, *58*, 2041-2975.

using TSH **38**[29] (*O*-tosyl hydroxylamine, *in situ* prepared from ethyl-*O*-tosyl hydroxyimidoacetate **37**) as amination reagent (Scheme 9). 1-*N*-Aminoimidazole can be isolated after column chromatography in 64 % yield.



Scheme 9. Amination of imidazole with TSH

Choice of the protecting group is critical, since the literature indicated that deprotection of the imidazole nitrogen proved to be difficult at best. Starting from 4(5)-nitroimidazole **39** reaction with allyl chloride allows the introduction of a protecting group at N1, which later on can be easily removed with $KMnO_4$ / acetone.[30] However, reduction of the obtained 1-allyl-4-nitro-imidazole using 10 % Pd-C (1 mol%) in dry dioxane leads to 4-amino-1-propylimidazole. To overcome this difficulty, POM (pivaloyloxymethyl) turned out to be the protecting group of choice. Product **41** is not stable in oxygen atmosphere and has to be stored under argon atmosphere (Scheme 10).



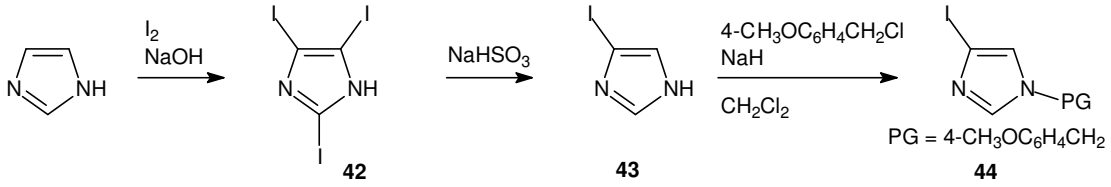
Scheme 10. Synthesis of 1-POM-4-amino-imidazole **41**

4(5)-Iodoimidazole **43** was prepared from imidazole following a literature protocol.[31] Treatment of **42** with $NaHSO_3$ allows the selective removal of two iodine functions. The obtained 4(5)-iodoimidazole **43** can be protected to **44** using a 4-methoxybenzyl protecting group (Scheme C11), which can be cleaved easily under mild oxidative circumstances.

29) a) Glover, E.E., Rowbottom, K.T., *J., Chem. Soc. Perkin Trans. I*, **1976**, 376; b) Messmer, A. Hajós, G., *J. Org. Chem.,* **1981**, 46, 843-846.

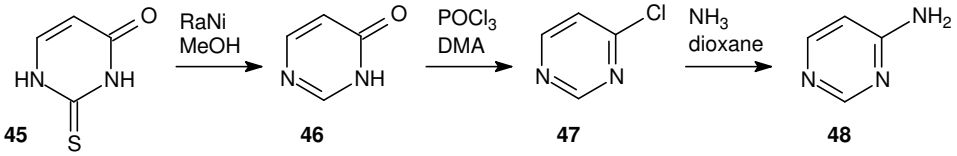
30) a) Lemaire-Audoire, S., Savignac, M., Genêt, J.P., Bernard, J.-M., *Tetrahedron Lett*., **1995**, *36*, 1267-1270, b) Kimbonguila, A.M., Boucida, S., Guibe, F., Loffet, A., *Tetrahedron,* **1997**, *53*, 12525-12538; c) Seley, K.L., Januszczyk, P., Hagos, A., Zhang, L., *J. Med. Chem*., **2000**, *43*, 4877-4883.

31) Panosyan, F.B., Still I.W.J., *Can. J. Chem*., **2001**, 1110-1114.



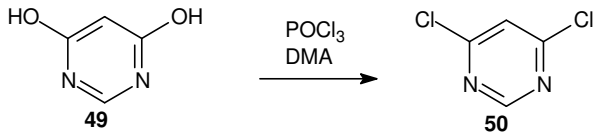
Scheme 11. Synthesis of 4(5)-iodo imidazole **43** and 1-(4-methoxybenzyl)-5-iodo imidazole **44**

Due to the high cost of 4-aminopyrimidine **48**[32] it was prepared starting from 2-mercapto uracil **45**. Removal of the thione function with Raney nickel affords 4-(3*H*)pyrimidone **46**, which in turn can be converted into 4-chloropyrimidine **47** by reaction with $POCl_3$-DMA (dimethylaniline). Nucleophilic exchange of the chlorine to an amino function is achieved treating 4-chloropyrimidine **47** with NH_3 in dioxane (NH_3 / water or NH_3 / MeOH should be avoided, since a mixture of 4-amino- and 4-hydroxy- or 4-methoxy pyrimidine, respectively, is obtained) (Scheme 12).



Scheme 12. Synthesis of 4-aminopyrimidine **48**

4,6-Dichloropyrimidine **50** can be obtained by treating 4,6-dihydroxypyrimidine **49** with $POCl_3$ and DMA (dimethylaniline).[33] For all chlorination reactions the relationship between pyrimidine (1 eq.), $POCl_3$ (11 eq.) and DMA (0.3 eq.) as well as reaction time and temperature during the work-up procedure play critical roles. If less DMA is used, the reaction time increases, a larger excess leads to problems during the work-up-procedure (Scheme 13).



Scheme 13. Synthesis of 4,6-dichloropyrimidine **50**

32) a) Buschauer, A., *Pharmazeutische Zeitung*, **1985**, *130* (33) 2067-70; b) von Angerer, S., Product class 12: pyrimidines**,** *Science of Synthesis*, **2004**, *16*, 379-572.
33) Hull, R., *J. Chem. Soc*., **1951**, 2214.

2.2. Synthesis of Di-Hetaryl Amines by "splitting" Azaphenothiazine Derivatives (Method B)

Till present, only a few aza-derivatives of phenothiazines have been described in literature.[34] Applying the known chemistry of phenothiazines to the aniline, pyridine, pyrimidine and imidazole precursors, a series of novel di-, tri- and tetraazaphenothiazines **55** and **56**, respectively, are generated. Due to their structural relationship with known pharmacophors - e.g. the *lin*-benzadenine system[35] and the phenothiazine skeleton[36] - these new tricyclic ringsystems are tested for their fluorescent properties[37] and biological activity.

Especially their abilities as minor groove intercalators will be investigated since phenothiazine derivatives are known to be redox active (low reduction potential). They could be of potential use as probes for electrochemically-based hybridization assays and for DNA-mediated charge-transfer studies.[38] While having intercalating properties, these tetraazaphenothiazine derivatives have the advantage of engaging in additional hydrogen-bonds between the intercalator and the cavity. In addition, analogues bearing a peptide side chain at the nitrogen of the middle ring therefore will be investigated for their ability of targeting conserved regions and structures in the viral genome (interaction of Tat protein with the transactivation responsive sequence (TAR RNA)[39]).

The acid catalyzed cyclization to azaphenothiazines **55** and **56**, is considered to proceed *via* initial protonation of a pyrimidine ring nitrogen of the halogen substituted ring of **53**. This activates nucleophilic attack by the non-protonated amino-pyrimidine function of **51**. The resulting dihetarylamine **54**, eliminates HX and forms the cyclization product (Scheme 14). If only a short reaction time is chosen, instead of the desired tri-cyclic compound the di-hetarylamine intermediate **54** could be isolated (no fluorescence at 364 nm, characteristic chemical shift of the bridging NH at δ = 6.89-7.54, comparable to the chemical shift of the NH of diphenylamine).

34) a) Phillips, A.P., Metha, N.B., Strelitz, J. Z., *J. Org. Chem*., **1962**, *28*, 1488 – 1490; b) Okafor, C.O., *J. Org. Chem*. **1967**, *32*, 2006 – 2007; c) Okafor, C.O., *J. Org. Chem*., **1973**, *38*, 4386 – 4390; d) Okoro, U.C., *Indian J. Chem. Sec*. **1990**, *29B*, 117 – 120.

35) a) Leonard, N.J., Morrice, A.G., Specker, M.A., *J. Org. Chem*., **1975**, *40*, 356-366; b) Leonard, N.J., Kazmierczak, F., Rykowski, A., *J. Org. Chem*., **1987**, *52*, 2933 – 2935.

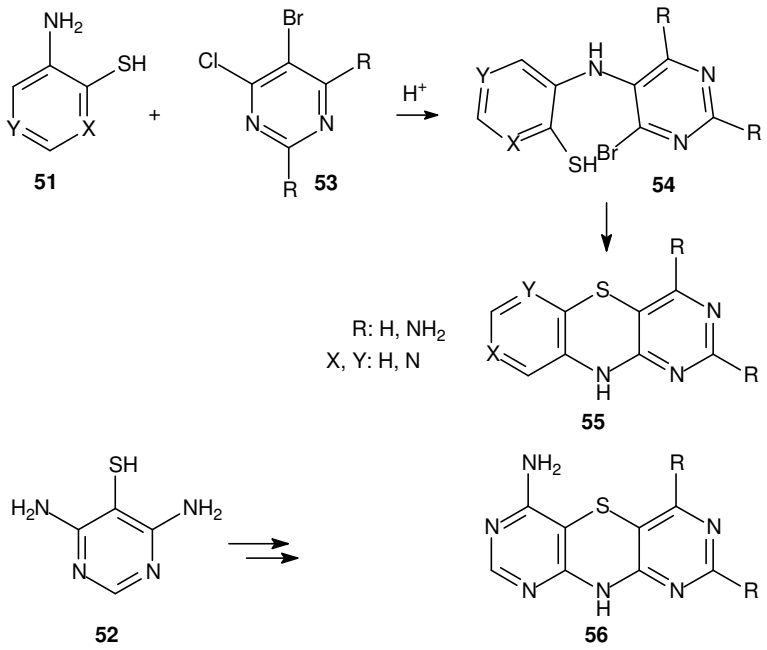
36) a) Massie, S.P., *Chem. Rev*., **1954**, *54*, 797; b) Bodea, C., Silberg, I. in „Advances in Heterocyclic Chemistry" ed. Katritzky, A.R., Boulton A.J., Academic Press, New York, **1968**, 321 – 460.

37) Rosendahl, M.S., Omann, G.M., Leonard, N.J., *Proc. Natl. Acad. Sci. USA*, **1982**, *79*, 3480 – 3484.

38) Hashmi, S.A., Hu, X., Immoos, C.E., Lee, S.J., Grinstaff, M.W., *Org. Lett*., **2002**, *4* (26), 4571 – 4574.

39) Gait, M., Karn, J., *Trends Biochem. Sci*., **1993**, *18*, 255 – 259.

A search for optimal conditions indicated that maximum yields could be obtained using 0.2-1 N mineral acids (HCl, H_2SO_4), whereas weak acids such as acetic acid, are ineffective. To improve the solubility of the ortho-dihalo-compound the condensation was carried out in mixtures of dioxane-water (1 : 3; 1 : 4).



Scheme 14. Ring-Closure to azaphenothiazine derivatives in acidic medium

The structure of the tricyclic compounds has been determined by means of HMBC and HMQC spectroscopy. The result is shown in Figure 3.

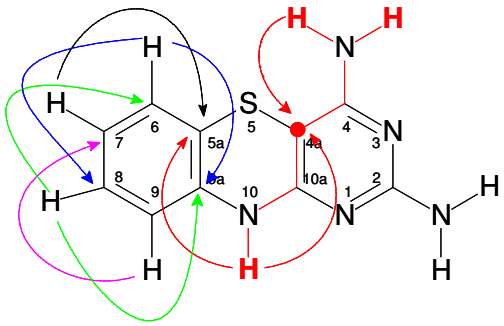
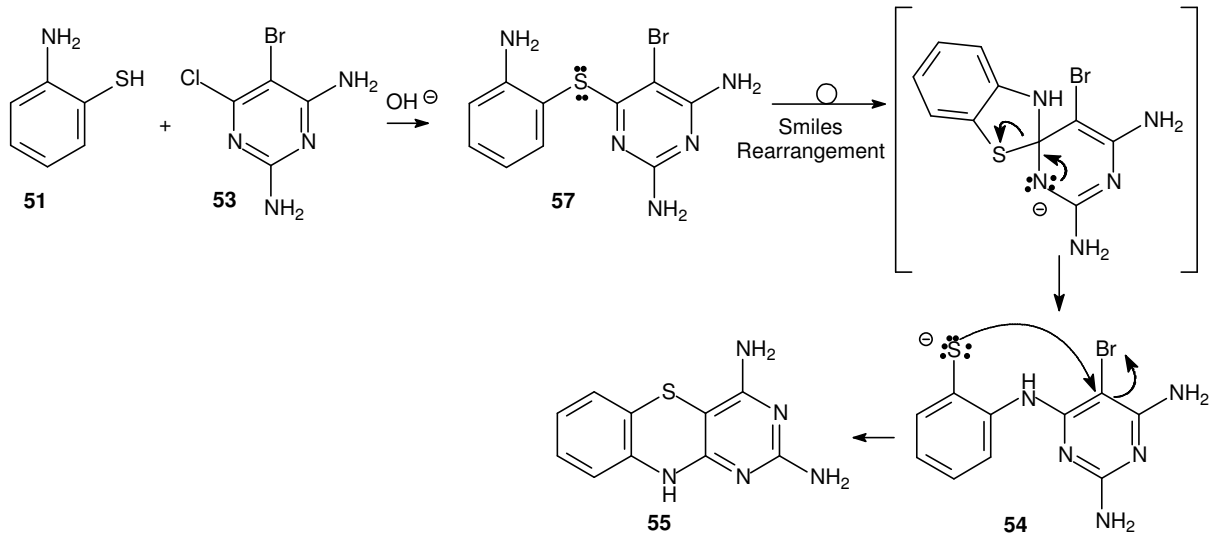


Figure 3. Results ghmbc spectra

Alternatively, the ring-closure reaction to obtain **55** and **56** can be carried out in alkaline medium (Scheme 15).[40] Under these circumstances the mercapto-group turns out to be the stronger nucleophile, giving rise to the formation of a di-hetarylsulfide **57**. Under the applied reaction conditions a "Smiles rearrangement"[41] is taking place, again leading to a di-hetararyl amine **54**, which can undergo ring-closure to an azaphenothiazine derivative **55**.



Scheme 15. Ring-closure to azaphenothiazine derivatives in alkaline medium

The products obtained under acidic or alkaline conditions have been proven identical by spectroscopic investigations.

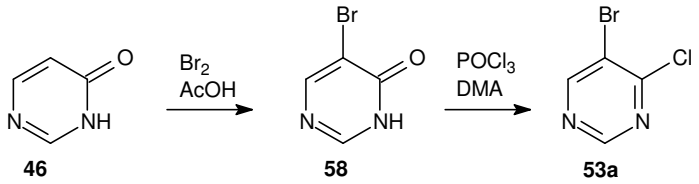
Characteristic for azaphenothiazine derivatives **55** and **56** is a strong absorption band in the ultraviolet spectrum at λ ~ 258 nm and a chemical shift of the thiazine NH at δ ~ 9.6 ppm. All derivatives are brightly colored (yellow-orange) and show melting points above 300°C. Figure 4 shows a solution of an azaphenothiazine derivative in a küvette at 364 nm.

Figure 4: Fluorescence of azaphenothiazines at 364 nm

For the here described condensation reaction two sorts of starting compounds, both not commercially available, are required. Direct bromination reaction introduces bromine into position 5 of the pyrimidine moiety. Therefore,

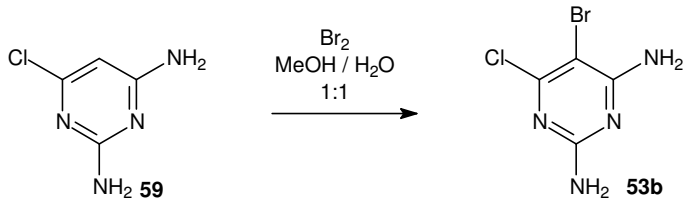
40) Okafor, C.O, Uche, I.O., Akpanisi, L.E.S., *J. Het. Chem.*, **1981**, *18*, 1589-1593.
41) Evans, W.J., Smiles, S., *J. Chem. Soc.*, **1935**, 181.

ortho dihalo substituted pyrimidine derivatives **53** either can be obtained by treating the corresponding halo-pyrimidone derivative **58** with $POCl_3$ as exemplified by the synthesis of 5-bromo-4-chloropyrimidine **53a** (Scheme 16). In this case 4(3*H*)-pyrimidone **46** first is brominated to obtain 5-bromo-4(3*H*)pyrimidone **58** [42] followed by chlorination with $POCl_3$ / DMA.



Scheme 16. Synthesis of 5-bromo-4-chloropyrimidine **53a**

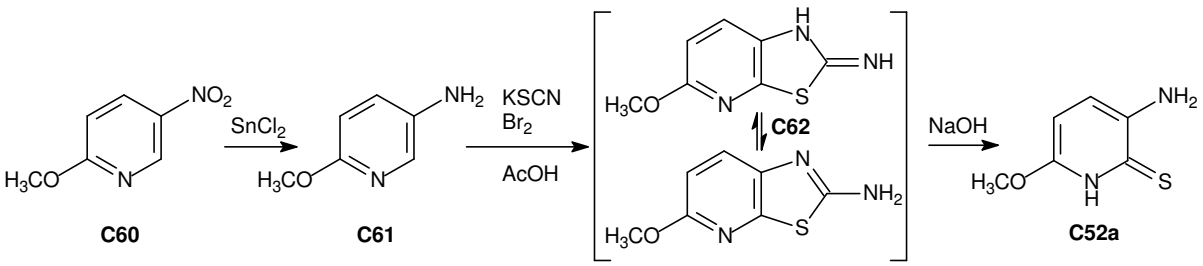
At the other hand, starting from commercially available 6-chloro-2,4-diaminopyrimidine **59** brominating in MeOH / water in presence of $NaHCO_3$ yields 5-bromo-6-chloro-2,4-diaminopyrimidine **53b** (Scheme 17).[43]



Scheme 17. Synthesis of 5-bromo-6-chloro-2,4-diaminopyrimidine **53b**

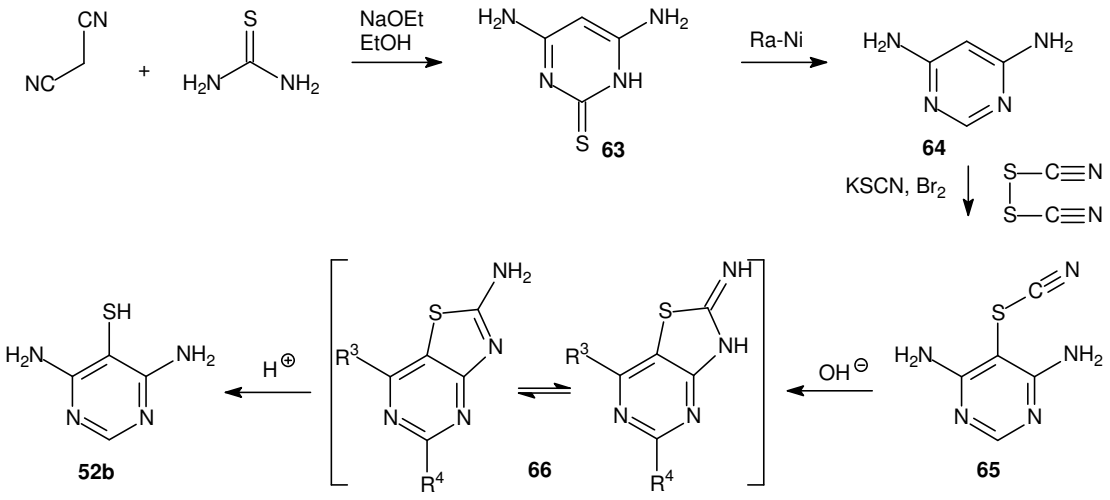
The second required starting material, *ortho* amino mercapto substituted heterocycles **52**, also are not commercially available. However, they can be smoothly prepared by a "Kaufmann reaction[44] - i.e. by cleavage of an intermediately formed 2-aminothiazole ring of **62** (Scheme 18).

42) Chesterfield, J., Mc Omie, J.F.W., Sayer, E.R., *J. Chem. Soc*. **1955**, 3478 – 3481
43) Okafor, C., *J. Org. Chem*. **1973**, *38*, 4386 – 4390
44) Kaufmann, H.P., Schulz, P., *Arch. Pharm*. **1935**, *273*, 31; b) Kaufmann, H.P., *Ber*. **1929**, *62B*, 390; c) Kaufmann, H.P., Schubert M., *German Patent* 493,025, **1927**, (*Chem. Abstr*. **1930**, 24, 2754).



Scheme 18. Synthesis of 3-amino-6-methoxypyridine 2-(1H)-thione **52a**

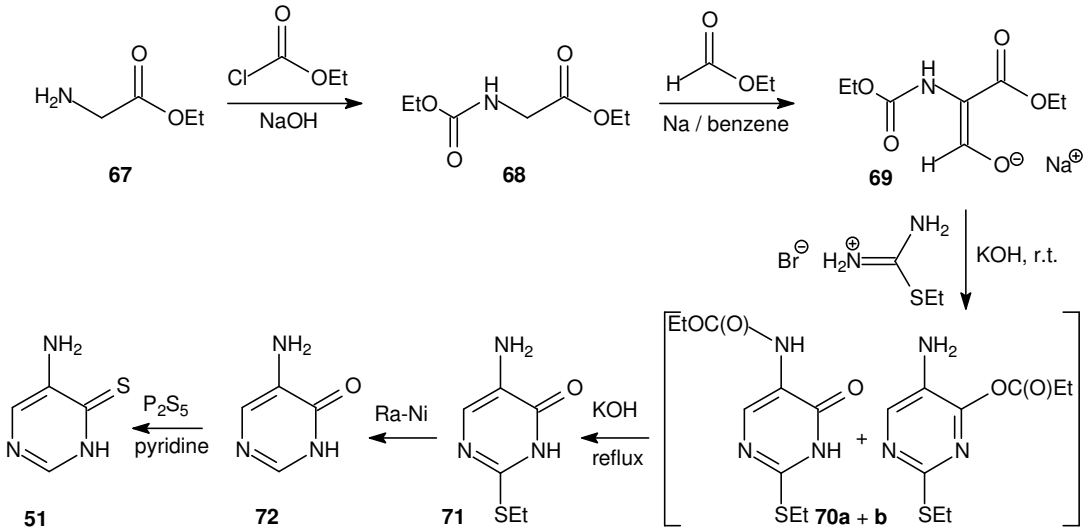
This method is also applicable for the synthesis of 4-amino-5-mercapto substituted pyrimidine derivatives **52**. Condensation of malononitrile with thiourea yields 4,6-diamino-1*H*-pyrimidine-2-thione **63**[45]. After desulfurization with Ra-Ni, the corresponding 4,6-diaminopyrimidine **64**[46] is obtained in good yield. Introduction of a 5-thiocyanato moiety[47] by using *in situ* generated di-cyan yields 4,6-diamino-5-thiocyanatopyrimidine **65**. This compound can undergo a Kaufmann reaction transforming the thiocyanato function into a mercapto function by an intramolecular ring-closure reaction to 7-amino-2-imino-2,3-dihydro[1,3]thiazolo[4,5-*d*]-pyrimidine **66**, which can be hydrolyzed to obtain the desired mercapto derivative **52b**. The reaction therefore allows the selective introduction of the mercapto function in position 5 of the pyrimidine ring (Scheme 19).



Scheme 19. Synthesis of 4,6-diamino-5-mercaptopyrimidine **52b**

45) Bendich, A., Tinker, J.F., Brown, G.B., *J. Am. Chem. Soc*., **1948**, *70*, 3109 – 3113.
46) Cavalieri, L.F., Bendich, A., *J. Am. Chem. Soc*, **1950**, *72*, 2587 – 2594.
47) Okafor, C.O., *J. Heterocycl. Chem.*, **1980**, **17**, 1587 – 1592.

Besides a Kaufmann reaction, a mercapto function can be introduced into the pyrimidine system by treatment of the corresponding pyrimidone with P_2S_5 in pyridine, in order to obtain 4-mercapto-5-amino substituted pyrimidine derivatives **51** (Scheme 20).[48]



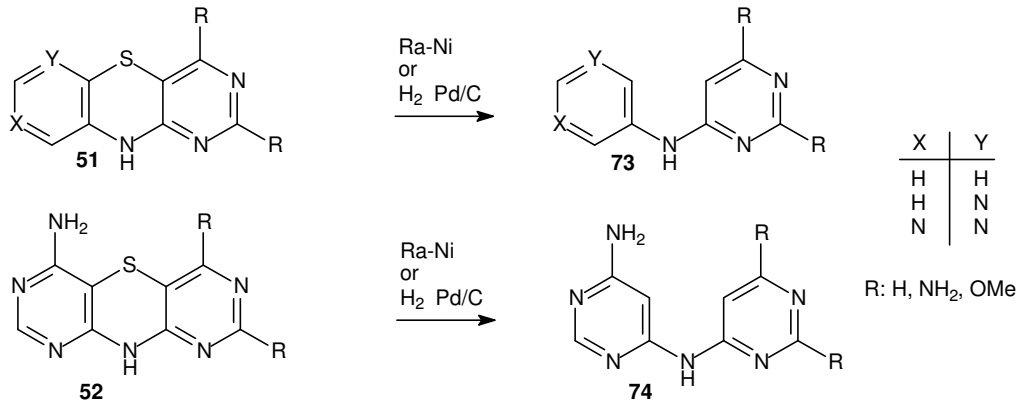
Scheme 20. Synthesis of 5-amino-(3*H*)-pyrimidine-4-thione **51**

Hereto, 5-amino-4-(3*H*)pyrimidone **72** needs to be synthesized. Starting from glycine ethyl ester hydrochloride **67**, reaction with ethyl chloroformate yields ethyl [(ethoxycarbonyl)-amino]acetate **68**[49] which after addition of ethylformate affords sodium (1*Z*)-3-ethoxy-2-[(ethoxycarbonyl)amino]-3-oxo-1-propen-1-olate **69.**[50] The obtained yellow, hygroscopic salt is condensed with ethylthioisouronium bromide[51] to give a mixture of two pyrimidine isomers **70a+b**, which are treated (without prior separation) with KOH to obtain 5-amino-2-(ethylsulfanyl)-4(3*H*)-pyrimidinone **71**. After removal of the 2-ethylsulfanyl moiety with Ra-Ni, the obtained 5-amino-4-(3*H*)pyrimidone **72** is reacted with P_2S_5 to finally obtain 5-amino- (3*H*)-pyrimidine-4-thione **51**.

The azophenothiazine derivatives **51** and **52** can be converted into the corresponding di-hetaryl amines **73** and**C74**, respectively, by reductive removal of the sulfur atom[52] partially

48) Duro, F., Santagati, N.A., Scapini, G., *Il Farmaco*, **1978**, *33*(12), 954-962.
49) Kende, A.S., Lurzio, M.J., Mendoza, J.S., *J. Org. Chem*., **1990**, *55* (31), 918-924.
50) Boarland M.P.V., McOmie J.F.W., *J. Chem. Soc*. **1952**, 4942 – 4945.
51) Spivak, D. Shea, K.J. *J. Org. Chem*. **1999**, *64*(13), 4627-4637.
52) Seley, K.L., Zhang, L., Hagos, A., Quirk, S., *J. Org. Chem*. **2002**, *67*, 3365 -3373;

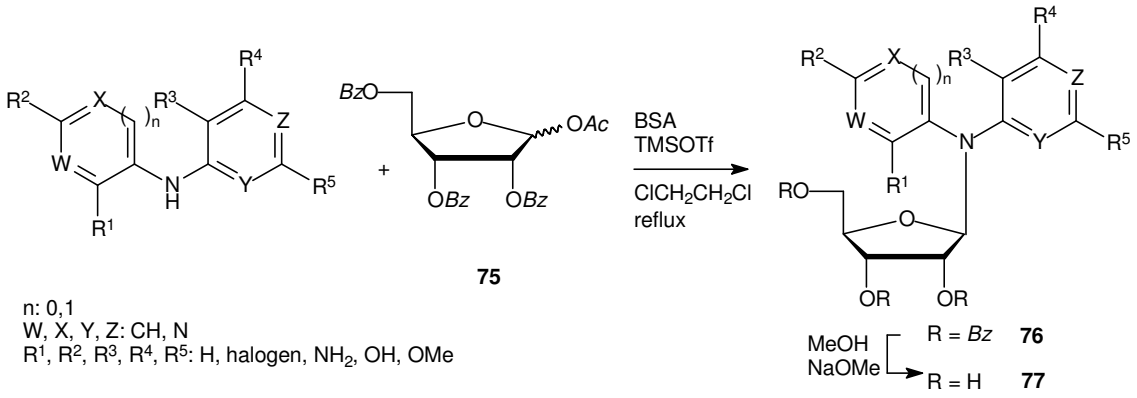
splitting the middle ring. This reaction can be seen as an inversion of the "Bernthsen" synthesis[53] of phenothiazines (Scheme 21).



Scheme 21. Splitting reaction to obtain dihetarylamines **73** and **74**

2.3. Synthesis of "Split-Nucleosides"

Di-hetaryl amines are seen as purine isosters. Glycosylation under Vorbrüggen conditions[54] allows the introduction of the sugar moiety at the bridging nitrogen atom (Scheme 22). To avoid extreme alkaline conditions the products were deprotected with NaOMe / MeOH.



Scheme 22. Synthesis of split-nucleosides **C77** by Vorbrüggen condensation

The reaction conditions were optimized using **76b** (obtained from 6-chloro-*N*-phenyl-4-pyrimidinamine **28a.** Attempts to deprotect **76b** with simultaneous conversion of the

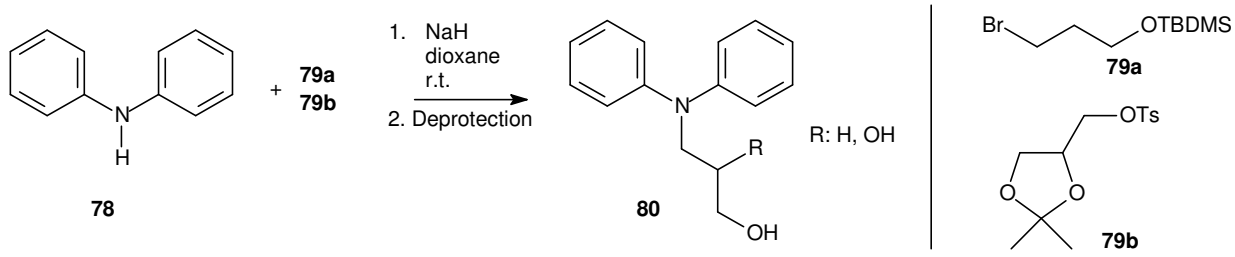
53) Eicher, T., Hauptmann, S., in "Chemie der Heterocyclen, Thieme Verlag, Stuttgart, New York, **1994**, 378.

54) a) Niedballa, U., Vorbrüggen, H., *J. Org. Chem.,* **1974**, *39*, 3654; b) Vorbrüggen, H., Krolikiewicz, K., Bennua, B., *Chem. Ber.*, **1981**, *114*, 1234; c) Vorbrüggen, H., Höfle, G., *Chem. Ber.*, **1981**, *114*, 1256; d) Vorbrüggen, H., Bennua, B., *Chem. Ber.*, **1981**, *114*, 1279.

6-chloro into a 6-amino-function failed. Treatment of **76b** with NH_3/MeOH at room temperature quantitatively forms the corresponding nucleoside **77b** but no nucleophilic substitution reaction is taking place. Carring out the reaction at elevated temperature (80°C, 1h) leads to decomposition of the nucleoside. Therefore, it is favourable to introduce first the desired functionalities followed by Vorbrüggen condensation.

Since the main concept of the split-nucleosides is flexibility with retainment of the recognition elements of purine bases, it seems rational to introduce also a "flexible" sugar moiety. First attempts were carried out using diphenylamine **78** as the test-aglycon (Scheme 23).

Attempts to condense 3-bromopropanol with the sodium salt of diphenylamine in dioxane failed, due to polymerisation of the bromoalcohol. Therefore, bromopropanol first was protected using TBDMS to obtain **79a**. Furthermore condensation reaction with 1-*O*-methylphenylsulfonyl-2,3-*O*-isopropylidene-glycerol **79b**[55] was attempted. However, so far, no satisfying yields of **80** were obtained; the reaction conditions have to be further optimized.



Scheme 23. Introduction of "open-sugars" to the bridging nitrogen

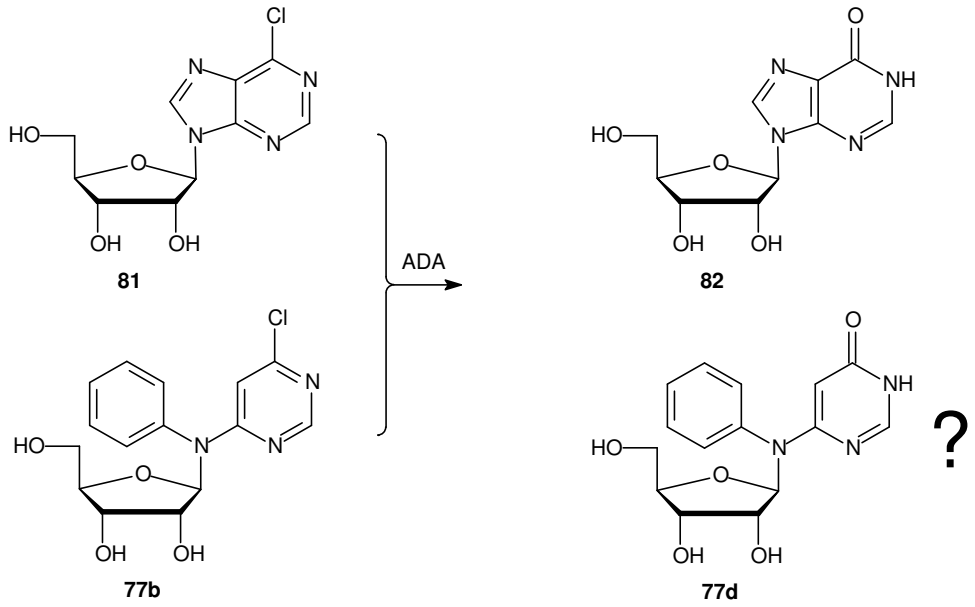
2.4. Activity of Adenosine Deaminase towards 6-Chloropurine Riboside and its "Split "Analogues

Adenosine deaminase (ADA, EC 3.5.4.4.) from calf intestinal mucosa catalyses the hydrolytic deamination of adenosine to inosine *via* a tetrahedral intermediate.[56] Furthermore, it is known,

55) Zhou, D., Lagoja, I. M., Rozenski, J., Busson, R., Van Aerschot, A., Herdewijn, P., *Chem. & Biochem.*, **2005**, *6* (12), 2298-2304.
56) Ciuffreda, P., Buzzi, B., Alessandrini, L., Santaniello, E*., Eur. J. Org. Chem.*, **2004**, 4405-4409.

that ADA is able to catalyse the deamination of structurally modified purine nucleoside analogues[57], provided that a hydroxy group is present at the 5´-position.[58]

In order to gain more information on the biocatalytic capability of ADA the conversion of 6-chloropurine riboside **81,** a widely used intermediate in the synthesis of adenosine derivatives, into inosine **82** has been compared with an example from the split-series (Scheme 24).



Scheme 24. ADA catalyzed transformation of **77** and **81**

Split-nucleosides were designed as a new group of purine bioisosteres, therefore it was an interesting question, if the enzyme can recognize the pyrimidine ring of **77b** as a part of a "flexible purine analogue" or not. The enzymatic dechlorination of compounds **81** and **77b** was carried out in a 3% DMSO aqueous solution, conditions that do not influence the deaminating activity of ADA towards chloropurine nucleosides.[59] The reaction has been monitored by HPLC. For 6-chloropurine riboside **81** the conversion to inosine **82** was completed after 2 hours, whereas in case of the split-analogue **77b** only minor minor amounts were converted after 10 hours. This first investigation of the enzymatic reaction has been studied with the simplest split-analogue (**77b**) of **81**, bearing a phenyl- and a chloropyrimidine

57) a) Ferrero, M., Gotor, V., *Chem.Rev*., **2000**, *100*, 4319-4347; b) Margolin, A.L., Borcherding, D.R., Wolf-Kugel, D., Margolin, N.*, J. Org. Chem*., **1994**, *59*, 7214-7218.
58) Bloch, A., Robins, M.J., Mc Carthy, Jr. J.R.*, J. Med. Chem*., **1967**, *10*, 908-912.
59) Ciuffreda, P., Loseto, A., Santaniello, E., *Tetrahedron,* **2002**, *58*, 5767-5771.

moiety. It is plausible, that better results can be obtained using split-derivatives with a more pronounced structural analogy to the parent 6-chloropurine riboside **81.**

2.5. Modeling

One of the concepts of developing split-nucleosides was to design a flexible molecule able to overcome problems typically associated with using crystallographic data in modelling when dealing with flexible enzyme binding sites. Since split-nucleosides still offer all required recognition elements, the overall energy of the enzyme-inhibitor complex should be lowered if both, enzyme and inhibitor are allowed to achieve their lowest energy conformation. This conformation should then be the most highly populated, increasing the chance of being the conformation to crystallize.[60]

An enzyme fulfilling the requirement of a flexible binding site[61] is SAHase (S-adenosyl-L-homocysteine hydrolase). SAHase cleaves SAH into its two cellular components, adenosine (Ado) and homocysteine (Hcy), and requires the assistance of an enzyme-bound cofactor, NADH.[62] Inhibition of SAHase by nucleoside inhibitors involves depletion of the NADH cofactor, which causes an intracellular accumulation of SAH, thereby elevating the SAH/SAM ratio. Many modified nucleosides have proven to be exceptional inhibitors of SAHase, and as a consequence, methyltransferases *via* this biofeedback mechanism, exhibit activity against numerous viruses including the pox viruses, arenaviruses, rhabdoviruses, renoviruses, vaccinia virus, parainfluenza virus, measles, ebola virus, sarcoma virus and the herpes viruses as well as against colon carcinoma, leukemia cells, among other cancer types.[63]

Amber9 software[64] was used to create the model. Atomic electrostatic charges and atom types for the split-nucleoside molecule to be used in the amber software package were calculated using Antechamber.[65] The other atomic charges (enzyme and NADH) and parameters (bond, angle, dihedral, van der Waals, ...) used in the amber simulations are those from the parm99 dataset.[66] The model was created starting from the x-ray structure of

60) Seley, K.L. Quirk, S., Salim, S., Zhang, L., Hagos, A., *Bioorganic & Medical Chemistry Letters*, **2003**, *13*, 1985-1988.
61) a) Yin, D., Yang, X., Hu, Y., Kuczera, K., Schowen, R. L., Borchardt, R. T., Squier, T.C. *Biochemistry* **2000**, 39, 9811 - 9818; b) Turner, M. A., Yang, X., Yin, D., Kuczera, K., Borchardt, R.T., Howell, P.L. *Cell Biochem. Biophys*., **2000**, 33, 101 - 125.
62) Palmer, J.L., Abeles, R.H., *J. Biol. Chem*. **1979**, *254*, 1217 - 1226.
63) a) Chiang, P.K., *Pharmacol. Ther*., **1998**, 77, 115-134; b) De Clercq, E., *Nucleosides Nucleotides,* **1994**, *13*, 1271-1295.
64) Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R. Merz, K.M. Onufriev, A. Simmerling, C. Wang, B. Woods, R., *J. Comput. Chem.* **2005***, 26*, 1668-1688.
65) Wang, J.1., Wang ,W.1, Kollman, P.A.1, Case, D.A.2, *J Mol Graph Model,* **2006**, *25*, 247-260.
66) Wang, J., Cieplak, P.,; Kollman, P.A. *J., Comput. Chem*., **2000**, *21*, 1049-1074.

S-Adenosyl-L-Homocysteine Hydrolase (pdb code 1li4).[67] The split-nucleoside was constructed in a C2' endo conformation using Macromodel.[68] Quatfit[69] was then used to fit the split-nucleoside onto the Neplanocin A molecule, present in the pdb file (the base ring having the 2 amino groups was fit onto the base of Neoplanocin A). Neoplanocin A was then removed. The NADH molecule present in the x-ray structure was kept while the crystal water molecules were removed.

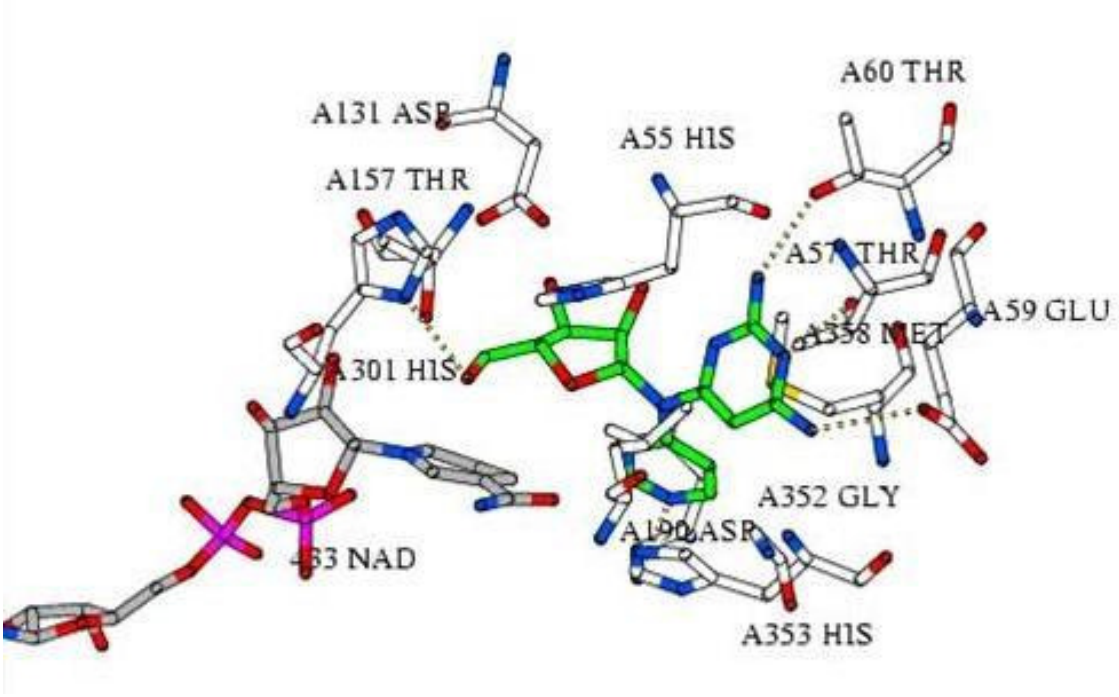


Figure 5. Close-up of the inhibitor in its binding pocket. The H-bonds are shown as well as all residues making hydrophobic contact with the inhibitor as determined by the Ligplot software

Molecular energy minimization was applied to the complex using Sander, while restraining the positions of the enzyme atoms that are at a distance of at least 5 Ångstrom from the inhibitor. A ligplot & hbplus analysis resulting in the H-bonds and hydrophobic contacts has also been performed.[70]

67) Yang, X., Hu, Y., Yin, D.H., Turner, M.A., Wang M., Borchardt., R.T., Howell P.L., Kuczera K., Schowen, R.L., *Biochemistry,* **2003**; *42*, 1900 – 1909.

68) Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M.; Caufield, C., Chang, G., Hendrickson, T., Still, W.C., *J. Comput. Chem.,* **1990**, *11*, 440-467.

69) Heisterberg, D.J. Quatfit program in CCL software archives. The Ohio Supercomputer Center Columbus, Ohio 43212.

70) a) McDonald, I.K., Thornton, J.M., *J. Mol. Biol.,* **1994**, *238*, 777-793; b) Wallace, A.C., Laskowski, R.A., Thornton, J.M., *Protein Engineering,* **1995**, *8*, 127-134.

Figure 5 shows a close-up of the inhibitor interacting with the enzyme while Figure 6 shows final complex structure. They were created by bobscript, molscript and Raster3D. [71]

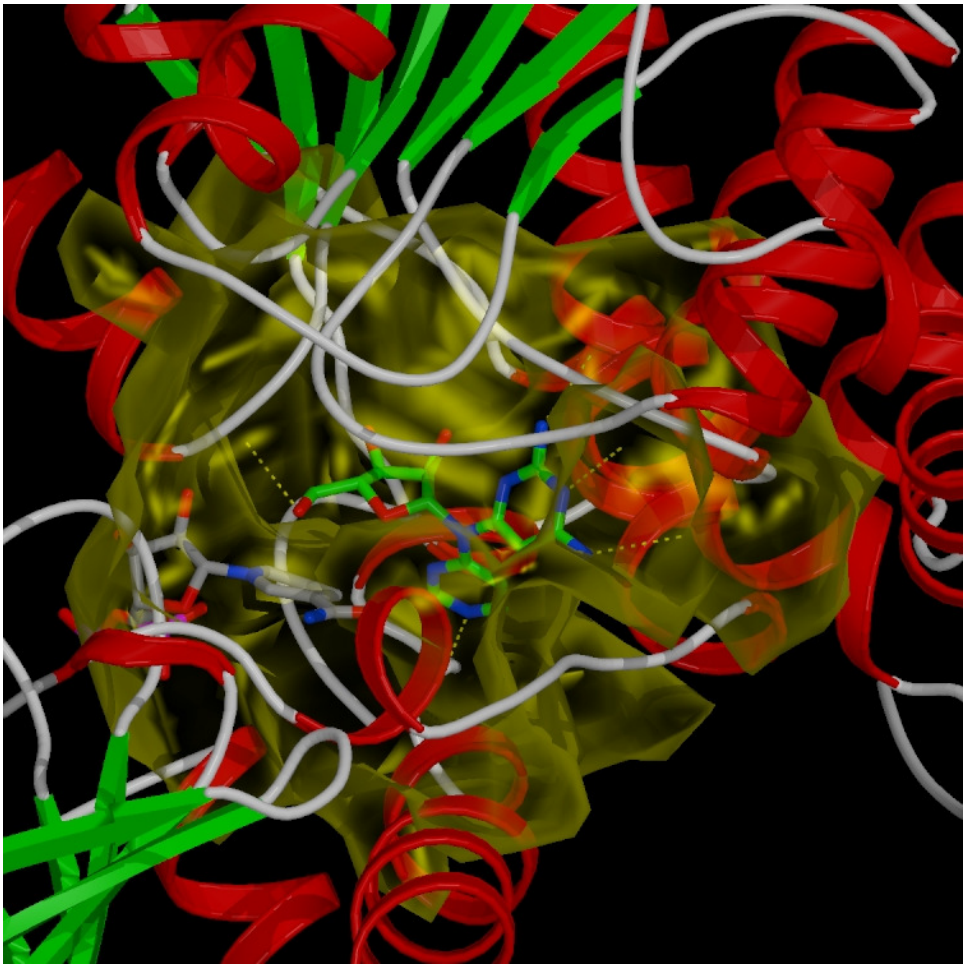


Figure 6. Overall picture of the inhibitor bound to the enzyme close to the NADH molecule. The binding site is coloured yellow. Secondary structure elements (alpha-helices, beta-sheets and coils are shown.

The modeling structures indicate a nice binding between split-nucleosides and enzyme. However, further studies using dynamic models to calculate quantitative binding energies are postponed until experimental results about the SAHase activity of split-nucleosides are available. Since binding to an enzyme always is entropically disfavoured,[72] split-nucleosides are designed to overcome this entropic cost by the ability to adopt more favourable conformations, therefore allowing more favourable binding interactions than the more rigid nucleoside analogues. The fact, that the split-nucleosides are found to bind in a "twisted" form seem to proof this hypothesis.

71) a) Esnouf, R.M., *J. Mol. Graphics Modell.,* **1997**, *15*, 132-134; b) Kraulis, P.J., *J. Appl. Cryst.* **1991**, *24*, 946-950; c) Merritt, E.A., Bacon, D.J. Raster3D: *Methods in Enzymology* **1997**, *277*, 505-524.

72) Seley K.L., Salim, S., Zhang. L., O´Daniel, P.I., *J. Org. Chem.*, **2005**, *70*, 1612-1619.

CONCLUSION

The first part of this chapter describes the syntheses of N^1-purinylnucleosides as a new class of pyrimidine bioisosteres. N^1-Adenosine (page 7) could be isolated as the kinetic product during studies of the mechanism of the Vorbrüggen condensation. N^1-Xanthosine (page 10) could be build up in a total synthesis, whereas N^1-inosine (page 9) was obtained from suitably protected 6-chloropurine. However, the compounds turned out to be biologically inactive. Incorporation into oligonucleotides failed due to strong hydrogen-bridges between 5´-OH and 6-XH (X= O, NH). To overcome these difficulties the interest now gets focused on the synthesis and incorporation of N^3-purinylnucleosides,[73] also fulfilling the criteria of pyrimidine-bioisosters.

Split-nucleosides offer a new field in medicinal chemistry. This work gives a first impression about synthetic routes and possible applications of this novel class of substances.

In this chapter the interest is focussed mainly on three different substance classes: split-nucleosides and their synthetic precursours: dihetaryl amines and azaphenothiazines.

With help of palladium catalyzed amination reaction (Hartwig-Buchwald condensation) (page 15) dihetarylamines can be synthesized in only one step starting from an amino- and a halogeno compound. The reaction could be verified by several examples; however, a synthetic challenge lies in further optimization of the reaction conditions.[74] Yields can be improved by choice of the base[75] and use of various catalysts.[76] The application of protection groups and modification of the solvent should allow a general application of this method. Investigation of the biological activities of these compounds is in progress.

The reversal of the Bernthsen synthesis, the reductive removal of the sulphur of an azaphenothiazine derivative is an alternative pathway (page 26) to obtain the desired dihetarylamines. Since ring-closure reaction to azaphenothiazines is possible in acidic (page 21) as well as in alkaline (page 22) circumstances high attention was paid to structure elucidation of the obtained reaction products. Azaphenothiazines are strongly fluorescent compounds showing strong structure analogy to known major groove intercalators and

73) Schmidt, C.L., Townsend, L.B., *J. Heterocyclic Chem.*, **1973**, 10(4), 687-8.
74) Buchwald, S.L., Mauger, C., Mignani, G., Scholz, U., *Adv. Synth. Catal.*, **2006**, *348*, 23 – 39.
75) Lakshman, M.K., *J. Organomet. Chem.,* **2002**, *653*, 234- 251.
76) a) Prim, D., Campagne, J.-M., Joseph, D., Andrioletti, B., *Tetrahedron*, **2002**, *58*, 2041-75; b) Charles, M.D., Schultz, P., Buchwald, S.L., *Organic Letters*, **2005,** *7* (18), 3965-68; c) Cristau, H.-J., Cellier, P.P., Spindler, J.-F., Taillefer, M., *Chem. Eur. J.*, **2004**, *10*, 5607 – 5622.

fluorescence markers. Therefore, in future more attention will be paid to these features of azaphenothiazines, their use as precursors of dihetarylamines will diminish. Initial tests to evaluate the quantum yield, fluorescence measurements, and the evaluation of the biological features of this substance class are currently under investigation.

Starting from the corresponding dihetarylamines, split-nucleosides can be obtained by Vorbrüggen condensation (page 26) in good yields. Currently the investigation of the biological properties of this novel class is in progress. Having in hand the ribo-derivatives, attention now is focused on the synthesis of further glycosides, using deoxy-sugars or open-chain sugars as starting material. Since an efficient synthetic pathway to obtain ribo-split-nucleosides is now available, the next step will be the incorporation of these compounds into oligonucleotides and the study of their hybridisation properties.

The best way to have a good idea is to have a lot of ideas.
Linus Pauling

SCHLUSSFOLGERUNG

Im ersten Teil dieses Kapitels wird die Synthese von N^1-Purinylnukleosiden als neue Pyrimidin Bioisostere beschrieben. N^1-Adenosin (Seite 7) wurde als kinetisches Produkt bei Studien zur Vorbrüggenkondensation erhalten, wohingegen N^1-Xanthosin (Seite 10) durch eine Totalsynthese aufgebaut und N^1-Inosin (Seite 9) durch die Anwendung geeigneter Schutzgruppen aus 6-Cl-Purin synthetisiert wurde. Die Verbindungen erwiesen sich als biologisch inaktiv, der Einbau dieser Verbindungen in Oligonukleotide ist aufgrund einer starken Wasserstoffbrückenbindung zwischen dem 5´-OH und dem 6-XH (X= O, NH) nicht gelungen. N^3-Purinylnukleoside[73] erfüllen ebenfalls die erforderlichen Kriterien eines Pyrimidin Bioisosters, der Einbau dieser Verbindungen in Oligonukleotide soll die Untersuchung der Hybridisierungseigenschaften erlauben.

Split-Nukleoside bieten ein neues, weitläufiges Betätigungsfeld auf dem Gebiet der medizinischen Chemie. Diese Arbeit gibt einen ersten Eindruck über mögliche Anwendungsgebiete sowie über mögliche Syntheserouten.

In diesem Kapitel werden vor allem drei Substanzklassen beschrieben: Split-Nukleoside, sowie ihre synthetischen Vorstufen: Dihetarylamine und Azaphenothiazine.

Mit Hilfe einer Palladium katalystierten Aminierungsreaktion (Hartwig-Buchwald Kondensation) (Seite 15) lassen sich Dihetarylamine in nur einem Reaktionssschritt ausgehend von einer Amino- und einer Halogenoverbindung herstellen. Die Reaktion konnte anhand von einigen Beispielen vorgestellt werden, eine Herausforderung liegt aber sicher noch in der weiteren Optimierung der Reaktionsbedingungen.[74] Vor allem durch die Wahl der Base,[76] aber auch durch den Einsatz verschiedener Katalysatoren[76] kann die Ausbeute noch verbessert werden. Durch die Anwendung von Schutzgruppen sowie Variation der Lösungsmittel soll eine generelle Anwendbarkeit dieser Methode erreicht werden. Untersuchungen zu den biologischen Eigenschaften der Dihetarylamine sind im Gange.

Die Umkehrung der Bernthsen Synthese – die reduktive Entfernung des Schwefels aus einem Azaphenothiazin Derivat (Seite 26) ist eine weitere Möglichkeit um die gewünschten Dihetarylamine herzustellen. Da eine Ringschluss Reaktion sowohl in saurem - (Seite 21) als auch in basischem Milieu (Seite 22) möglich ist wurde der Strukturaufklärung des entstandenen Reaktionsproduktes besondere Aufmerksamkeit geschenkt. Azaphenothiazine sind stark fluoreszierende Verbindungen, die eine starke Strukturanalogie zu bekannten Major-groove-Interkalatoren bzw. zu Fluoreszenzmarkern aufweisen. Aus diesem Grunde soll

in Zukunft auch mehr Andacht an die Verbindungsklasse selbst geschenkt werden. Erste Untersuchungen zur biologischen Aktivität, sowie Fluorenszenzmessungen und Messungen der Quantenausbeute wurden bereits durchgeführt.

Split-Nukleoside lassen sich in guten Ausbeuten durch Vorbrüggen Kondensation (Seite 26) aus den entsprechenden Dihetarylaminen herstellen. Die Verbindungen werden auf ihre biologischen Eigenschaften untersucht. Die Kondensation mit weiteren Zuckerbausteinen (Deoxyribose, offenkettige Zucker) wird untersucht. Da nun eine effiziente Synthesemöglichkeit für Split-Nukleoside besteht, ist der folgende Schritt der Einbau dieser neuartigen Purin-Bioisostere in Oligonukleotide und die Untersuchung der Hybridisierungseigenschaften.

The best way to have a good idea is to have a lot of ideas.
Linus Pauling

3. EXPERIMENTAL PART

A theory is something nobody believes, except the person who made it.
An experiment is something everybody believes, except the person who made it.
Albert Einstein

GENERAL REMARKS:

NMR spectra were recorded on a Varian, Gemini 200 spectrometer (^{1}H 200 MHz, ^{13}C 50 MHz) and a Bruker AM-300 spectrometer (^{1}H 300 MHz, ^{13}C 75 MHz). All NH protons were assigned by exchange with D_2O. In case of AA'BB' systems determination of *J* is based on the assumption of an AB quartet.[77] gHMQC and gHMBC NMR spectra were recorded on a Varian 500 MHz Unity spectrometer, operating at 499.505 MHz. Quadrature detection was achieved by States-Haberkorn hypercomplex mode.[78] The gHMQC and gHMBC spectra in DMSO consisted of 1024 data points in t_2 and 512 increments in t_1. The data were apodized with a shifted sine-bell square function in both dimensions and processed toa 2K × 2K matrix.

Mass spectrometrie was carried out at a Finnigan LC Duo spectrometer (FAB^+, FAB^-). Exact mass measurements were performed on a quadrupole - time of flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a propan-2-ol : water (1 : 1) mixture at 3 µL/min.

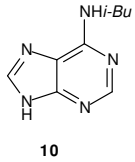
The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and / or spraying with a solution of phosphomolybdic acid, followed by charring at 150°C. Preparative TLC was carried out with precoated TLC-Plates (0.25 mm, Merck, Silica gel 60 F_{254}). Column chromatography was performed on Silica Gel 60 M (0.040-0.063 mm, E. Merck).

HPLC purifications were performed on a HS Prep100 BDS C18 8µ (250 x 10 mm) column, using H_2O/MeCN gradients as eluens. For UV spectroscopy UV-grade MeOH was used. Melting points were determined with a Kofler-block.

Nomenclature of the obtained compounds follows the rules of IUPAC and was checked with Autonome.[79]

77) Becker, E.D., "*High Resolution NMR, Theory and Chemical Application*", Academic Press, New York, **1969**, 169.
78) States, D.J., Haberkorn, R.A., Ruben, D. J., *J. Magn. Res.*, **1982**, *48*, 286.
79) ACD-Chem Sketch; http://www.acdlabs.com.

N^6-Isobutyryladenine (**10**):



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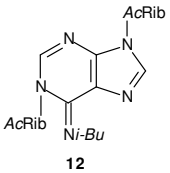
Adenine (6.70 g, 49.58 mmol) (vacuum dried) was stirred in isobutyric anhydride (160 mL) at 70°C (oil bath temperature) for 4 h. The TLC analysis showed the presence of two products, mono- and disubstituted ones. The mixture was cooled down to room temperature, absolute methanol (170 mL) was added, and the mixture was refluxed until the disubstituted product was completely decomposed (2 h). The solvent was evaporated, and the resulting syrup was crystallized from boiling ethanol. The product was crystallized from boiling ethanol to give 9.15 g of white crystals (44.6 mmol, 90%). Rf (MeCN : CH_3Cl = 1 : 1) 0.14; ^{1}H NMR (300 MHz, dmso-d_6): δ = 1.15, 1.18 (6H, 2 x s, 2 x CH_3), 2.92 (1H, q x q, *J* = 6.8 Hz, CH), 8.40 (1H, s, 8-H), 8.63 (1H, s, 2-H), 11.13 (1H, s, br, ex, NH), 12.28 (1H, s, br, ex, NHCO); ^{13}C NMR (75 MHz, dmso-d_6): δ = 19.0, 19.3 (2 x CH_3), 34.2 (CH), 113.3 (6-C), 144.4 (4-C), 146.0 (8-CH), 151.5 (2CH), 162.3 (5C), 177.5 (C=O); FAB$^+$ $[M+H]^+$: 206.1.

N^6-Isobutyryl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)adenine

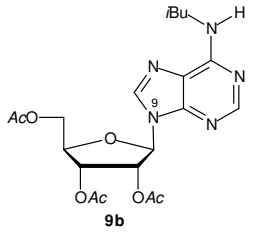


12

An anhydrous suspension of N^6-isobutyryladenine (0.90 g, 4.38 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.79 g, 5.63 mmol) in dry acetonitrile (20 mL) was washed with argon for 30 min, then N,O-bis(trimetylsilyl)acetamide (BSA; 1.64 g, 8.09 mmol) was added. The mixture was stirred at 75°C for 30 min until a clear solution was obtained. Trimethylsilyl triflate (TMSOTf; 0.48 g, 2.11 mmol) was then added and the mixture was stirred at 75°C for 3 h. After cooling down to room temperature the obtained solution CH_2Cl_2 was diluted with dichloromethane (180 mL) and extracted with cold saturated solution of $NaHCO_3$ (150 mL). The organic layer was dried over Na_2SO_4, evaporated to a yellow solid foam and then chromatographed on a silica gel column (12 cm x 4.5 cm) in a chloroform-acetonitrile gradient (from 2:1 to 1:1) to give (in order of elution):

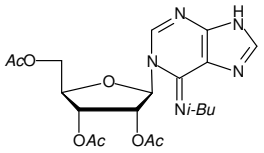
N^6-Isobutyryl-1,9-bis-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (**12**):

Yield: 0.16 g, 0.20 mmol, 5% Rf (MeCN : CH_3Cl = 1 : 1) 0.67; Exact Mass Calcd: $C_{31}H_{40}N_5O_{15}$ 722.2521$[M+H]^+$; Found: 722.2524.



9b

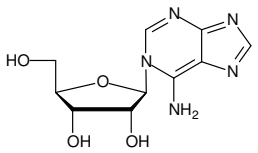
N^6-Isobutyryl-9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (**9b**): Yield: 0.21 g, 0.46 mmol, 10%), Rf (MeCN : CH_3Cl = 1 : 1) 0.49; FAB^+ $[M+H]^+$: 464.1.



6b

N^6-Isobutyryl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)adenine (**6b**): Yield: 0.66 g, 1.42 mmol, 33%). Rf (MeCN : CH_3Cl = 1 : 1) 0.25; **6b** was crystallized from boiling 40% aqueous ethanol to give white crystals (0.59 g). mp 159-161°C; UV (MeOH): λ_{max} 313.2 nm, λ_{min} 255.2 nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18 (6H,dd, $CH(C\underline{H}_3)_2$), 2.06, 2.18, 2.24 (9H, 3s, 3 x 3, $OCOCH_3$), 2.65 (1H, septet, $C\underline{H}(CH_3)_2$), 4.41 (1H,d, 5'b-H), 4.51-4.55 (2H, m, 5'a-H, 4'-H), 5.34 (1H, t, 3'-H), 5.63 (1H, d, *J* = 4.8 Hz, 1, 2'-H), 6.65 (1H, s, 1'-H), 8.13 (1H, s, 8-H), 8.82 (1H, s, 2-H), 12.49 (s, 1, N7-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.55, 19.84 (2 x $CH(\underline{C}H_3)_2$), 20.2, 20.3, 20.7 (3 x $OCO\underline{C}H_3$), 39.5 ($\underline{C}H(CH_3)_2$), 61.0 (C-5'), 67.7 (C-3'), 74.5 (C-2'), 78.9 (C-4'), 90.3 (C-1'), 114.3 (C-5), 141.5 (C-8), 142.2 (C-2), 148.0 (C-6), 157.0 (C-4), 168.9, 169.2, 170.2 (3 x $O\underline{C}OCH_3$), 188.9 ($N\underline{C}OCH$), Exact Mass Calcd: $C_{20}H_{26}N_5O_8$: 464.1781$[M+H]^+$, Found: 464.1784.

1-Ribofuranosyladenine (**13**):

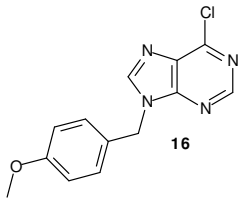


13

A solution of N^6-isobutyryl-1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-adenine (**6b**) (0.22 g, 0.48 mmol) in methanol saturated with ammonia (10 mL) was stirred at room temperature for 24 h. The reaction mixture was evaporated to give a white crystalline material, which was stirred in chloroform-methanol (1:1) at room temperature for 2 h. The precipitate was filtered off and the crystals were washed with chloroform-methanol (1:1). The product was crystallized from boiling water to give 1-ribofuranosyladenine (0.1 g, 0.45 mmol; 93%). mp decomp. >169°C; UV (MeOH): λ_{max} 274.8 nm, λ_{min} 247.6 nm, λ_{max} 228.4 nm, Rf (MeOH : CH_2Cl_2 = 1 : 4) 0.35; ^{1}H NMR (300 MHz, dmso-d_6): δ = 3.38 (3H, bs, ex, 2'-OH, 3'-OH, 5'-OH), 3.72 (2H, q, 5'a,b-H), 4.07 (1H, d, 4'-H), 4.12 (1H, d, 3'-H), 4.12 (1H, t, *J* = 5.4 Hz, 2'-H), 5.95 (1H, d, *J* = 6.0 Hz, 1'-H), 6.80 (2H, br, ex, NH_2), 7.83 (1H, s, 8-H), 8.39 (1H, s, 2-H); ^{1}H NMR (300 MHz, D_2O): δ = 4.05 (2H, q, 5'a,b-H), 4.46 (1H, d, 4'-H), 4.49 (1H, d, 3'-H), 4.79 (1H, t, *J* = 6.0 Hz, 2'-H), 6.15 (1H, d, *J* = 6.0 Hz, 1'-H), 8.21 (1H, s, 8-H), 8.60 (1H, s, 2-H). ^{13}C NMR (75 MHz, dmso-d_6): 60.5 (C-5'), 69.9 (C-3'), 72.7 (C-2'), 86.3 (C-4'), 92.2 (C-1'), 119.0 (C-

5), 140.5 (C-2), 148.7 (C-6), 150.7 (C-8), 154.7 (C-4); Exact Mass $C_{10}H_{14}N_5O_4$ Calcd: 268.1046 $[M+H]^+$, Found: 268.1049.

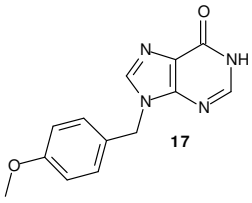
6-Chloro-9-(4-methoxybenzyl)-9*H*-purine **(16):**



16

A mixture of 6-chloropurine (1.00g, 6.47 mmol), 4-methoxybenzylchloride (1.11 g, 1.1 eq) and K_2CO_3 (1.00g) is kept in dry DMF (10 mL) overnight. Following dilution of the reaction mixture with water (400 mL) and extraction with CH_2Cl_2 (4 x 20 mL) the combined organic layers are washed with brine and dried over Na_2SO_4. *N*7 and *N*9 isomer (ratio 1 : 7) can be separated by column chromatography (CH_2Cl_2 : MeOH = 95 : 5). Yield: 82 %; Rf (CH_2Cl_2 : MeOH = 95 : 5) 0.46; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 3.79 (3H, s, CH_3), 5.38 (2H, s, CH_2), 6.85, 6.89 - 7.27, 7.29 (4H, AA´BB´), 8.09 (1H, s, 8-H), 8.77 (1H, s, 2-H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ = 47.5 (CH_2), 55.3 (CH_3), 114.6 (3,5H Bn), 128.9 (2,6H Bn), 128.9 (1C, Bn), 160.0 (4C, Bn), 126.5 (5-C), 145.0 (2-CH), 149.0 (6-C), 152.0 (8-CH), 152.5 (4-C); FAB$^+$ $[M+H]^+$: 274.1.

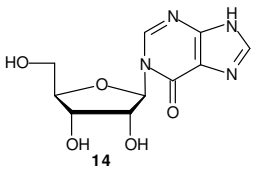
9-(4-Methoxybenzyl)-hypoxanthine (**17**):



17

A mixture of **16** (1.00 g, 3.2 mmol) in NaOH (1N, 25 mL) is heated under reflux for 1 hour. After cooling to room temperature the mixture is carefully neutralized with AcOH (pH = 7). Oil is formed. The emulsion is extracted with CH_2Cl_2 (2 x 40 mL). After drying the organic layer over Na_2SO_4 the volatiles are removed. The obtained oil crystallized in the refrigerator. Yield: 85 %; Rf (CH_2Cl_2 : MeOH = 95 : 5) 0.24; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 3.76 (3H, s, CH_3), 5.26 (2H, s, CH_2), 6.82, 6.85 – 7.21, 7.24 (4H, AA´BB´) 7.65 (1H, s, 8-H), 8.35 (1H, s, 2-H), 12.20 (1H, s, br, ex, NH); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ = 46.5 (CH_2), 55.3 (CH_3), 120.0 (5-C), 138.0 (8-CH), 146.6 (4-C), 152.5 (2-CH), 154.9 (6-C), 114.3 (3,5CH Bn), 128.6 (2,6CH Bn), 133.5 (1C Bn), 159.5 (4C Bn); FAB$^+$ $[M+H]^+$: 257.3.

N^1-Inosine **14:**



14

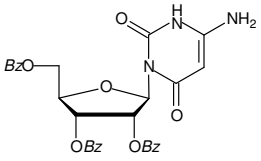
Under nitrogen protection the base **17** (0.65 g, 2.50 mmol) is dissolved in dichloroethane (20 mL). BSA (0.62 g, 3.00 mmol) is added and the mixture is stirred at room temperature for 20 min. After cooling to 0°C 1,2,3,5-tetra-*O*-acetyl-ribofuranose (0.80 g, 2.50 mmol) and TMSOTf (0.56 g, 2.5 mmol) is added. The mixture is allowed to come to room temperature and further

heated under reflux for 3 hours. After cooling to room temperature, the mixture was neutralized with saturated $NaHCO_3$–solution (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure and the residual oil was purified by column chromatography (silica, CH_2Cl_2 : MeOH = 9 : 1) to obtain 2,3,5-tri-*O*-acetyl-N^1-(4-methoxybenzyl)inosine **18** in 85 % yield. Rf (CH_2Cl_2 : MeOH = 9 : 1) 0.67; Exact Mass Calcd: $C_{39}H_{33}N_4O_9$: 701.2247 $[M+H]^+$, Found: 701.2247.

A mixture of **18** (0.55g, 1.07 mmol) and $(NH_4)_2Ce(NO_3)_2$ in CH_3CN / water (4 : 1, 20 mL) is kept at room temperature overnight. TLC monitoring showed complete conversion of **18** (Rf 0.67, CH_2Cl_2 : MeOH = 9 : 1) to the 2,3,5-tri-*O*-acetyl-N^1-inosine **19** (Rf 0.47, CH_2Cl_2 : MeOH = 9 : 1). After concentration of the reaction mixture, compound **19** was further purified by gel-filtration (removal of the inorganic salts). The product-containing fractions were combined and following removal of the volatiles, compound **19** (Yield 73 %) was deprotected by treatment with MeOH / NH_3 at room temperature. After TLC montoring indicated complete deprotection, the volatiles were removed under reduced pressure. The obtained oily product was dissolved in MeOH (2 mL) and precipitated with Et_2O to give white crystals, which further were recrystallized from water (93 %). The obtained spectroscopic data are identical with those described in literature.[80]

2,3,5-Tri-*O*-benzoyl-6-amino-N^3-uridine (**22**):

6-Aminouracil **21** (1.50 g, 2.50 mmol) is suspended in HMDS (10 mL), a catalytic amount of NH_4Cl is added and the mixture is heated under reflux until a clear solution is obtained (1h). The excess HMDS is removed under reduced pressure. The obtained precipitate is dissolved in dichloromethane (20 mL). Tribenzoylriboseacetate (3.97 g, 2.50 mmol) and TMSOTf (0.56 g, 2.5 mmol) are added and the mixture was kept stirring under N_2-atmosphere at room temperature overnight. After quenching with $NaHCO_3$–solution (30 mL) the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure and the resulted oil was purified by column chromatography (silica, CH_2Cl_2 : MeOH = 9 :1). Yield: 91 %; Rf (CH_2Cl_2 : MeOH = 9 : 1) 0.68; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ = 4.46 (3H, m,

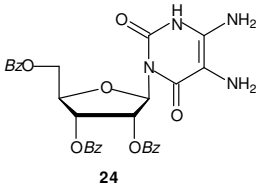


22

80) Montgomery, J.A., Thomas, H. J., in "*Nucleic Acid Chemistry*", 553-557.

H4´, 2 x H5´), 4.92 (1H, s, 5-H), 5.75 (2H, s, br, ex, NH_2), 6.68 (2H, m, H2´, H3´), 6.68 (1H, s, H1´), 7.24-7.99 (15H, m, 3 x Bz), 10.61 (1H, s, br, ex, NH); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ = 63.3 (5-CH_2), 70.7 (2H´), 74.3 (3H´), 75.9 (4H´), 85.2 (1CH´), 77.9 (5-CH), 151.8 (2-C), 153.8 (5-C), 163. 5 (4-C), 127.4-133.4 (C, CH Ar Bz), 165.8, 166.5 (C=O); Exact Mass $C_{30}H_{26}N_3O_9$ Calcd: 572.1669 $[M+H]^+$, Found: 572.1702.

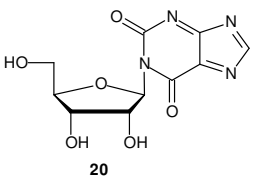
2,3,5-Tri-*O*-benzoyl-5,6-diamino-N^3-uridine (**24**):



24

A mixture of 2,3,5-tri-*O*-benzoyl-6-amino-N^3-uridine (**22**) (3.20 g, 5.6 mmol) and PhN_2BF_4 (1.57g, 1 eq., 5.6 mmol) is dissolved in dry dioxane (100 mL). Immediately a bright yellow colour is observed. Stirring under exclusion of moisture is continued, till TLC monitoring indicates, that all starting material has been converted (2 h) (CH_2Cl_2 : MeOH = 9 : 1: **22**: Rf 0.68, Diazo intermediate **23**: 0.88, bright yellow). The reaction mixture is flushed with argon; Pd-C (0.6g, 10 %) is added. The mixture is set under vacuum and flushed with hydrogen (ballon). The procedure is repeated 4 times, and then the mixture was left under H_2-atmosphere vigorously stirring overnight. Following filtration over a bed of Celite the volatiles were removed and the resulting-brown oil was purified by column chromatography (500 mL silica; CH_2Cl_2 : MeOH = 95 : 5 1L, CH_2Cl_2 : MeOH = 9 : 1 600 mL); After removal of the solvent from the product-containing fractions **24** is obtained as a yellow foam. Yield: 85 % both steps. %; Rf (CH_2Cl_2 : MeOH = 9 : 1) 0.57; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ = 4.74 (3H, m, H4´, 2 x H5´), 5.75 (4H, s, br, ex, 2 x NH_2), 6.27 (2H, m, H2´, H3´), 6.75 (1H, s, H1´), 7.29 – 8.04 (15H, m, 3 x Bz); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ = 62.6 (5-CH_2), 67.0 (2H´), 70.3 (3H´), 74.3 (4H´), 85.7 (1CH´), 93.9 (5-CH), 148.3 (2-C), 151.2 (5-C), 161.2 (4-C), 127.4-133.5 (C, CH Ar Bz), 165.6, 166.0, 166.4 (C=O); Exact Mass $C_{30}H_{27}N_4O_9$: 587.1778 $[M+H]^+$; Found: 587.1782.

N^1-Xanthosine (**20**):

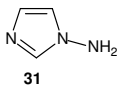


20

2,3,5-Tri-*O*-benzoyl-5,6-diamino-N^3-uridine (**24**) (1.8 g, 3.1 mmol) is dissolved in diethoxymethylacetate (15 mL) and triethylorthoformiate (10 mL). A catalytic amount of TsOH is added, the mixture is heated under reflux for 1 hour and left at room temperature overnight. After concentratin the resulting oil is dissolved in CH_2Cl_2 and purified by column chromatography (silica, CH_2Cl_2 : MeOH = 95 : 5). After removal of the solvent from the product-containing fractions, 2,3,5-tri-*O*-benzoylxanthosine (**25**) was obtained as a slight tan coloured foam

(56 %). After verification by MS spectroscopy (Exact Mass $C_{31}H_{24}N_4O_9$: Calcd: 596.1543 $[M+H]^+$, Found: 596.1551) compound **25** was deprotected by treatment with MeOH / NH_3 at room temperature overnight. The reaction was monitored by TLC. Following the removal of the volatiles, the obtained oily product was dissolved in MeOH (2 mL) and precipitated with Et_2O to give white crystals (91%). After recrystallization from water, the obtained spectroscopic data are identical with those described earlier in literature.[81]

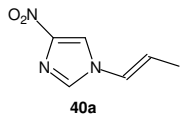
1-Aminoimidazole (**31**):



31

Ethyl*O*-tosylhydroxyimidoacetate (9.10 g, 35.3 mmol) is suspended in 60% $HClO_4$ (69 mL) and vigorously stirred for 30 min. The reaction mixture is quenched on crushed ice (300 mL) to free the reactive intermediate *O*-tosylhydroxylamine (TSH). The mixture is extracted with CH_2Cl_2 (3 x 30 mL), the extracts dried over Na_2SO_4, filtered and kept below 0°C. Imidazole (2.00 g) is dissolved in a minimum of dry dioxane (5 mL) and cooled to 0-5°C. The cooled TSH solution is added dropwise *via* a dropping funnel. Stirring at 0°C is continued for another 40 min, followed by stirring at room temperature for another 2 hours. After removing the volatiles the obtained crude product is purified by column chromatography (silica, CH_2Cl_2 : EtOH 2 : 1). Yield: 1.53 g (61 %) light yellow crystals; Rf (CH_2Cl_2 : EtOH 2 : 1) 0.23; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.85 (2H, s, br, ex, NH_2), 7.14 (1H, d, J = 7.2 Hz, 4-H), 7.47 (1H, dd, J^1 = 7.2 Hz, J^2 = 1.4 Hz, 5-H), 7.58 (1H, (t), J ~ 1.4 Hz, 2-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 122.6 (5-C), 128.3 (4-C), 135.0 (2-C); $FAB^+[M+H]^+$: 84.1.

1-Allyl-4-nitroimidazole (**40a**):

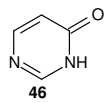


40a

4(5)-Nitroimidazole (2.00 g, 17.7 mmol), allyl bromide (2.31 mL, 26.5 mmol) and NaH (0.64 g, 26.5 mmol) in DMF (50 mL) were stirred at room temperature overnight. The solvent was removed under reduced pressure and the residual oil was purified by column chromatography (silica, CH_2Cl_2). Yield: 2.50 g (92 %); Rf (CH_2Cl_2 : MeOH 95 : 5) 0.77; ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 4.67 (2H, d, J = 5.8 Hz, CH_2=), 5.25-5.40 (2H, m, CH_2N), 5.97-6.04 (1H, m, CH=), 7.46 (1H, d, J = 1.4 Hz, 2-H), 7.79 (1H, d, J = 1.4 Hz, 5-H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 50.4 (CH_2N), 119.4 (CH_2=), 120.7 (5-C), 130.7 (2-C), 135.9 (CH=), 141.1 (4-C); FAB^+ $[M+H]^+$: 154.1.

81) Winkley, M.W. *J. Chem. Soc. [Section] C*: **1970**, *13*, 1869-74.

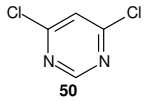
4-(3*H*)-Pyrimidone (**46**):



46

A mixture of 2-thiouracil (**45**) (3.00 g, 23.4 mmol) and Raney nickel (4.50 g, 50% emulsion in water) are suspended in water (60 mL) and heated under reflux for 3h. After filtering hot over a bed of Celite the residue is washed with boiling MeOH (4 x 10 mL). The combined filtrates are concentrated to a volume of ~ 5 mL and kept in the refrigerator overnight. The colorless crystals formed are sucked off and dried over P_2O_5. Yield: 1.14 g (51 %); Rf (CH_2Cl_2 : MeOH 99 :1) 0.31; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.33 (1H, d, *J* = 7.2 Hz, 5-H), 7.91 (1H, d, *J* = 7.2 Hz, 6-H), 8.16 (1H, s, 2-H), 12.52 (1H, s, br, ex, NH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 116.1 (5-CH), 150.7 (2-CH), 154.3 (6-CH), 161.4 (4-C); FAB$^+$[M+H]$^+$: 97.1.

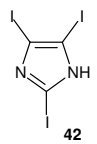
4,6-Dichloropyrimidine (**50**):



50

A mixture of 4,6-dihydroxypyrimidine (**49**) (5.00 g, 0.045 mol) and DMA (2.0 mL) in $POCl_3$ (20 mL) is heated under reflux for 3 hours. The obtained dark brown solution is cooled to room temperature, followed by removal of the excess $POCl_3$. The resulting oily residue is treated with ice-water (200 mL) and neutralized ($NaHCO_3$ – attention – foaming!!); After extraction with CH_2Cl_2 (5 x 20 mL), the combined organic layers are dried over Na_2SO_4, and filtered through SiO_2 (50 g). The column was washed with additional CH_2Cl_2 (500 mL); after removal of the volatiles the product crystallized in the refrigerator. Yield: 4.65 g (64 %); Rf (CH_2Cl_2); 0.44; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 8.10 (1H, s, 5-H), 8.96 (1H, s, 2-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 122.5 (5-CH), 159.3 (2-CH), 161.1 (4,6-C); FAB$^+$[M+H]$^+$: 150.0.

Triiodoimidazole (**42**):



42

A solution of iodine (22.50 g, 0.09 mol) in 20 % aqueous KI solution (30.00 g KI in 150 mL) was added dropwise to a stirred solution of imidazole (3.53 g, 0.05 mol) in 2M NaOH (24.00 g NaOH in300 mL) at room temperature. The resulting mixture is stirred at room temperature overnight. After neutralization with glacial acetic acid the white precipitate formed is filtered off, washed with water and dried over P_2O_5. Yield: 20.67g (89%); m.p. 186-190°C.[82]

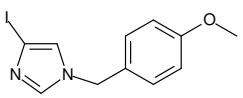
82) Panosyan, F.B., Still, I.J.W., *Can. J. Chem.,* **2001**, 1110-1114.

4(5)-Iodoimidazole (**43**):

43

Triodoimidazole (3.00 g, 6.72 mmol) and sodium sulfite (12.60 g, 99.9 mmol) were heated at reflux in 30% ethanol water solution (150 mL) for 24 hours. Ethanol was distilled off under reduced pressure. The remaining solution was filtered and the filtrate was extracted with diethyl ether. (4 x 30 mL). After drying the organic layer over Na_2SO_4, the volatiles were removed to obtain a colorless product. Yield: 1.29 g (quant); mp: 136-137°C (Lit[297]: 137-138°C) ^{1}H-NMR (200 MHz, dmso-d_6): δ = 7.31 (1H, s, 4(5)-H), 7.65 (1H, s, 2-H); FAB$^+$[M+H]$^+$: 195.0.

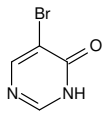
1-(4-Methoxybenzyl)-4-iodoimidazole (**44**):



44

A mixture of 4(5)-iodoimidazole (**43**) (1.20 g, 6.18 mmol), 4-methoxybenzyl chloride (1.07g, 6.80 mmol) and potassium carbonate (1.00g) in dry DMF (10 mL) is kept at room temperature overnight. Following dilution with water (400 mL) the suspension is extracted with CH_2Cl_2 84 x 20 mL). The combined organic layers are washed with brine (100 mL), dried over Na_2SO_4 and further purified by column chromatography (silica, CH_2Cl_2 : MeOH = 95 : 5). Yield: 85 %; Rf (CH_2Cl_2 : MeOH 95 : 5); 0.64; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 3.72 (s, 3H, CH_3), 4.97 (2H, s, CH_2), 6.82, 6.85 – 7.21, 7.24 (4H, AA´BB´), 6.89 (1H, s, 4CH), 7.35 (1H, s, 2CH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 50.6 (CH_2), 55.3 (CH_3), 114.4, 128.5, 128.8, 159.7 (3,5CH, 1C, 2,6-CH, 4C Bn), 81.7, 124.6, 138.7 (4-C, 5-CH, 2CH imidazole); FAB$^+$[M+H]$^+$: 315.0.

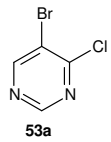
5-Bromo-4-(3*H*)pyrimidone (**58**):



58

Bromine (3.3 mL, 62 mL) is added dropwise at room temperature to a solution of 4-hydroxy(3*H*)pyrimidone (**46**) (6.00 g, 39 mmol) in AcOH (50 mL). The mixture was left at room temperature overnight. The formed precipitate (pyrimidine x HBr) is filtered off dried, and crystallized from water to afford the free base. Yield: 8.85 g, (81 %); Rf (CH_2Cl_2 : MeOH 99 : 1); 0.44; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 8.42 (1H, s, 2-H), 8.56 (1H, s, 6-H), 12.79 (1H, s, br, ex, NH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 113.5 (5-C), 150.4 (2-CH), 151.1 (6-CH), 157.5 (4-C); FAB$^+$[M+H]$^+$: 176.0.

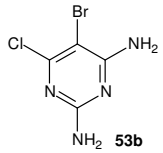
5-Bromo-4-chloropyrimidine (**53a**):



53a

A mixture of 5-bromo-4-(3*H*)pyrimidone (**58**) (6.00 g, 34.2 mmol), dimethylaniline (DMA) (1.4 mL) and $POCl_3$ (40.0 mL) is heated under reflux for 4 hours. The volatiles are distilled off, the resulted oil is treated with ice-water (200 mL), carefully neutralized with $NaHCO_3$ (foaming!) and extracted with ether (5 x 30 mL). The combined organic layers are dried over Na_2SO_4. After removal of the volatiles, the residue is purified by a small flash column. (Yield: 4.23 g (64 %); Rf (CH_2Cl_2); 0.36; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 8.80 (1H, d, *J* = 2.2 Hz, 2-H), 8.87 (1H, d, *J* = 2.2 Hz, 6-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 120.5 (5-C), 156.6 (156.6 C-CH), 159.8 (2-CH), 160.2 (4-C); $FAB^+[M+H]^+$: 194.2.

5-Bromo-6-chloro-2,6-diaminopyrimidine (**53b**):



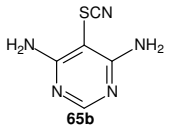
53b

To a rapidly stirred solution of the pyrimidine **59** (6.00g 41.5 mmol) and $NaHCO_3$ (4.00 g, 46.6 mmol) in MeOH / H_2O (120 mL, 1 : 1 H_2O dest.; MeOH HPLC grade) a solution of bromine (2 mL) in MeOH (20 mL) was added dropwise. After addition of half of the amount of bromine more $NaHCO_3$ (2.00 g, 23.3 mmol) was added and the addition was completed. Following stirring for another hour the flocculent precipitate formed is filtered off and washed with cold water. Yield: 5.19 g (56 %); Rf (CH_2Cl_2 : MeOH 4 : 1); 0.43; ^{13}C-NMR (50 MHz, dmso-d_6): δ = 97.8 (5-C), 158.3 (2-C), 161.2 (6-C), 167.6 (4-C); $ES^+[M]^+$: 223.4.

Kaufmann Reaction: Synthesis of the Thiocycanato- Intermediates, General Procedure:

A mechanical stirred mixture of pyrimidine (i.e. **64**) or pyridine (**61**), respectively (97.1 mmol) in acetic acid (134 mL) is cooled to -10°C. After stirring for 15 min potassium thiocyanate dihydrate (15.72 g, 118.0 mmol) is added keeping the temperature below 0°C. After dropwise addition of bromine (5.40 mL, 16.9 g, 106.0 mmol) the reaction mixture turns from bright yellow to deep orange after 2 hours. After additional stirring at 0°C for 5 hours the mixture is allowed to come to room temperature overnight. Following addition of water (34 mL) the mixture is heated to 80°C and filtered hot. The deep orange precipitate is washed with hot acetic acid. Under ice-bath cooling, the combined filtrates are neutralized to pH 6.5 with concentrated ammonia. The temperature has to be maintained below 30°C. The obtained yellow precipitate is filtered off and dried over P_2O_5.

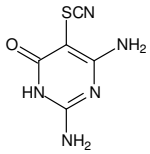
4,6-Diamino-5-thiocyanatopyrimidine (**65b**):



65b

Yield: 14.21 g (88 %); Rf (CH_2Cl_2); 0.44; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 7.08 (4H, s, br, ex, 2 x NH_2), 7.85 (1H, s, 2-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 73.7 (5-C), 111.3 (SCN), 159.6 (2-CH), 164.4 (4,6-C); $ES^+[M]^+$: 167.7.

2,6-Diamino-5-thiocyanato4-(3*H*)-pyrimidinone (**65c**):



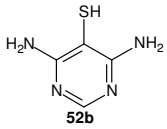
65c

Yield: 96 %; m.p. 182-185°C (H_2O); Rf (CH_2Cl_2 : MeOH 2 : 1) 0.80; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.73, 7.05 (2H, s, br, ex, 2 x NH_2), 10.46 (1H, s, br, ex, NH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 66.7 (5-C), 113.0 (SCN), 156.0 (2-C), 161.6 (6-C), 166.2 (4-C); $ES^+[M]^+$: 183.3.

Kaufmann Reaction: Synthesis of the Mercapto- Compounds, General Procedure:

A mixture of thiocycanato compounds **52** (30.6 mmol), KOH (24.0 g 42.8 mol) and water (61 mL) is heated under reflux overnight. At the end of the refluxing period, charcoal (0.2 g) is added and heating is continued for another 15 min. After filtering hot over a bed of Celite the filtrate is cooled to 0°C and carefully neutralized with acetic acid (temperature has to be maintained below 10°C). After filtration (paper filter) the filtrate is concentrated to a volume of 4 mL, water (3 mL) is added and pH is adjusted to 4. The yellow precipitate formed is sucked off and dried over P_2O_5.

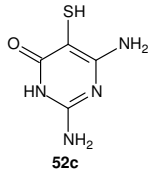
4,6-Diamino-5-mercaptopyrimidine (**52b**):



52b

Yield: 4.35 g (quant.); Rf (CH_2Cl_2 : MeOH 5 : 1); 0.21; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.39 (4 H, s, br, ex, 2 x NH_2), 7.67 (1H, s, 2-H, 11.5 (1H, br, ex, SH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 95.9 (5-C), 155.5 (4,6-C), 163.4 (2-CH); $FAB^+[M+H]^+$: 143.0.

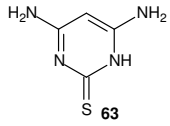
2,6-Diamino-5-mercapto-4-(3*H*)-pyrimidinone (**52c**):



52c

Yield: 97 % (CH_2Cl_2 : MeOH 95 : 5) 0.21; ^{13}C-NMR (50 MHz, dmso-d_6): δ = 80.5 (5-C), 156.5 (2-C), 164.1 (4-C), 167.6 (6-C); ES^+: 158.2 $[M]^+$.

4,6-Diamino-1*H*-pyrimidine-2-thione (**63**):

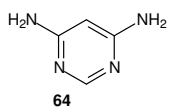


63

Sodium (9.40 g, 40.8 mmol) is dissolved in dry EtOH (300 mL). After a homogeneous solution is obtained, thiourea (30.00 g, 39.4 mmol) is added in a single portion. Stirring is continued for 10 minutes followed by addition of

freshly distilled malonitrile (25.00 g, 39.4 mmol). The mixture is heated under reflux for 2 hours. After cooling to room temperature water (50 mL) is added and pH is adjusted to pH 7 by dropwise addition of glacial acetic acid. The precipitate formed is sucked off and dried over P_2O_5. Yield: 31.20 g (70 %); Rf (CH_2Cl_2 : MeOH 2 : 1); 0.14; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 5.04 (1H, s, 5-H), 6.71 (5H, s, br, ex, NH, 2 x NH_2); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 74.3 (5-CH), 159.3 (br, 4,6-C), 177.5 (2-C); FAB$^+$[M+H]$^+$: 143.1, [M+Na]$^+$: 165.0.

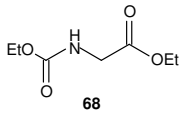
4,6-Diaminopyrimidine (**64**):



64

A mixture of 4,6-diamino-1*H*-pyrimidine-2-thione (**63**) (13.00 g, 91 mmol) and Raney nickel (13.00 g, 50 % suspension in H_2O) in water (160 mL) is heated under reflux for 2 hours. After filtering hot over a bed of Celite the residue is washed with boiling MeOH (4 x 10 mL). The combined filtrates are concentrated to a volume of ~ 5 mL. The compound is kept in the refrigerator overnight. The formed precipitate is sucked off and dried over P_2O_5. Yield: 9.61 g (95 %); Rf (CH_2Cl_2: MeOH 2 : 1); 0.10; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 5.38 (1H, s, 5-H), 6.02 (4H, s, br, ex, 2 x NH_2), 7.78 (1H, s, 2-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 82.9 (5-CH), 158.3 (2-CH), 163.9 (4,6-C); ES$^+$[M]$^+$: 110.7.

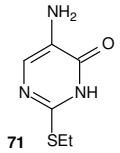
Ethyl[(ethoxycarbonyl)amino]acetate (**68**):



68

A solution of glycine ethyl ester hydrochloride (35.00 g, 0.25 mol) in distilled water (50 mL) cooled to -5°C was carefully neutralized with a 40% aqueous sodium hydroxide solution (15 mL), keeping the internal temperature below 5°C. Ethyl chloroformate (30.00 g, 0.26 mol) was then added dropwise with vigorous stirring at a rate to maintain the internal temperature below 5°C. After all the ethyl chloroformate was added, the reaction mixture was stirred at 0°C for 30 min, at which time a second portion of a 40% aqueous sodium hydroxide solution (10 mL) was added. After extraction the reaction mixture with ether (5 x 100 mL) the organic layers were combined, dried (Na_2SO_4), and concentrated *in vacuo* to give a crude liquid. This liquid was then distilled to afford 32 g (87%) of **68** as a colorless liquid: bp 135-139 °C/15 mmHg; ^{1}H NMR (200 MHz, dmso-d_6): δ = 1.15-1.22 (6H, 2 x t, *J* = 6.9 Hz, 2 x CH_3), 3.84 (2H, d, *J* = 5.6 Hz, CH_2N), 4.02-4.13 (4H, 2 x q, *J* = 6.9 Hz, 2 x CH2), 7.43 (1H, t, *J* = 5.6 Hz, NH); ^{13}C NMR (50 MHz, dmso-d_6): δ = 13.8, 14.2 (2 x CH_3), 42.4 (CH_2N), 60.9, 61.0 (2 x CH_2O), 156.4 (NCO_2), 170.0 (CO_2).

5-Amino-2-ethylsulfanyl-4-(3*H*)-pyrimidone (**71**):

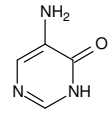


71

Ethylisothiouronium bromide (strong smell!!) is prepared according to literature.[83] Finely powdered sodium (4.87 g, 0.21 mol) is suspended in dry benzene (260 mL). Ethylformate (22.38 g, 0.3 mol, 24.4 mL) and ethyl[(ethoxycarbonyl)amino]acetate (**68**) (32.00 g, 0.2 mol) are added. Under exclusion of moisture the mixture is kept stirring at room temperature for two days, after which the metallic sodium has disappeared and the yellow Na-salt (**69**) is formed. Dry ether ($CaCl_2$, 200 mL) is added, the salt **69** is quickly sucked off, stored over P_2O_5 and used as soon as possible without further purification.

Ethylisothiouronium bromide (24.00 g, 0.131 mol) is dissolved in aq. KOH (9.50 g KOH, 55 mL H_2O). A solution of salt **69** (40.31 g, 0.179 mol) in water (55 mL) is added. The mixture is kept at room temperature for 2 days. After acidifying with AcOH a yellowish-white precipitate (**70**) is obtained and filtered off. The precipitate is dissolved in NaOH (10%, 150 mL) and heated under reflux for 25 min. After cooling to room temperature the solution was acidified with AcOH, the formed precipitate was filtered off and dried over P_2O_5. Yield: 8.08 g, 36 % Rf (CH_2Cl_2 : MeOH 5 : 1) 0.88; m.p. 158-160 °C (H_2O); ^{1}H-NMR (200 MHz, dmso-d_6): δ = 1.21 (3H, t, *J* = 7.4 Hz, CH_3), 2.98 (2H, q, *J* = 7.4 Hz, CH_2), 5.47 (3H, s, br, ex, NH, NH_2), 7.15 (1H, s, 6-H); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 15.0 (CH_3), 24.3 (CH_2), 128.6 (6-CH), 131.8 (5-C), 147.9 (2-C), 161.0 (4-C); $ES^+[M]^+$: 171.6 (28 %), $[M+Na]^+$: 193.9 (100%).

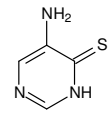
5-Amino-4-(3*H*)-pyrimidone (**72**):



72

Pyrimidine **71** (8.00 g, 0.047 mol) is suspended in water (100 mL). NH_4OH (25%) is added, until a clear solution is obtained. Raney nickel (15.00 g, 50 % slurry in water) is added and the mixture is heated under reflux for 3 hours. The mixture is filtered hot over a bed of Celite. After removal of the volatiles, the obtained product is crystallized from EtOH. Yield: 84 %; Rf (CH_2Cl_2: MeOH = 2 : 1) 0.65; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 4.88 (s, br, ex NH), 7.23 (1H, s, 6-CH), 7.59 (1H, s, 2-CH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 127.8 (2-CH), 135.9 (5-C), 139.9 (6-CH), 160.0 (4-C); $ES^+[M]^+$: 111.8 (100%), $[M+Na]^+$: 133.8 (37%).

5-Amino(3*H*)-pyrimidin-4-thione (**73**):



51

A mixture of pyrimidine **72** (5.00 g, 0.05 mol) and P_2S_5 (11.00 g, 0.05 mol) in dry

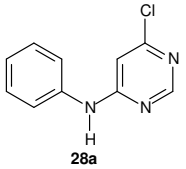
83) Spivak, D., Shea, K.J., *J. Org. Chem.*, **1999**, *64*(13), 4627-4637.

pyridine (100 mL) is kept at 120°C under exlusion of moisture for 6 hours. After removal of the volatiles, the residue is dissolved in EtOAc and purified by gel-filtration (60 g silica, CH_2Cl_2 : MeOH = 2: 1). The black tar sticks on the column, the product containing fractions are combined and further extracted with water (6 x 50 mL). The water layer contains the product, whereas pyridine residues are found in the organic layer. After treatment of the water layer with charcoal and hot filtration, the volatiles are removed under reduced pressure to obtain yellow crystals. Yield: 56 %, Rf (CH_2Cl_2: MeOH = 2 : 1) 0.82; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 4.50 (s, br, ex NH), 7.49 (1H, s, 2-CH), 7.80 (1H, s, 6-CH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 126.7 (6-CH), 137.5 (2-CH), 144.2 (5-C), 170.5 (4-C); FAB$^+$[M+H]$^+$: 128.0.

Hartwig-Buchwald Reaction: General Procedure

An oven-dried flask was purged with argon, charged with (±) BINAP (46 mg, 0.0375 mmol) and capped with a rubber septum. The flask was purged with argon and toluene (5 mL) was added. The mixture was heated to 80°C with stirring until the BINAP was dissolved (~ 1 min). The solution was cooled to room temperature, $Pd(OAc)_2$ (11 mg, 0.05 mmol, 1 mol%) was added, purged with argon and toluene was used to rinse the Pd from the sides of the reaction vessel. The mixture was stirred at room temperature for 5 min, the solution turned bright orange. 4,6-dichloropyrimidine (745 mg, 5.0 mmol), arylamine (6.0 mmol) and KO*t*Bu (785 mg, 7.0 mmol) were added. The mixture was purged with argon; toluene (3 mL) was added to rinse all compounds from the wall. The mixture was kept at 80°C till all starting material had been consumed (TLC; CH_2Cl_2 : MeOH = 10 : 1). After cooling to room temperature the mixture was diluted with ether (20 mL), filtered and purified by flash chromatography.

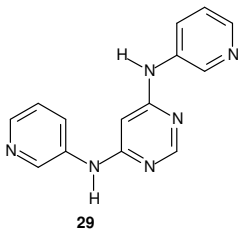
6-Chloro-*N*-phenyl-4-aminopyrimidine (**28a**):



28a

Yield: 92 %; colorless; Rf 0.86 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.70 (1H, s, 5-H pyrim), 7.22 - 7.34 (3H, m, 2,4,6H ph), 7.43 (2 H, (t), *J* = 7.6 Hz, 3,5-H ph), 7.54 (1H, s, br, ex, NH), 8.45 (1H, s, 2-H pyr); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 102.6 (5-CH pyr), 123.2 (2,6-CH ph), 126.0 (4-CH ph), 129.8 (3,5-CH ph), 137.1 (1-C ph), 158.7 (2-CH pyr), 160.7 (6-C pyr), 166.2 (1-C pyr); FAB$^+$[M+H]$^+$: 206.1.

*N*⁴,*N*⁶-Di(3-pyridinyl)-4,6-pyrimidinediamine (**29**):



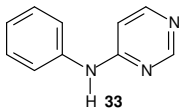
29

Yield: 10 % Rf (CH_3Cl : n-hexane 1 : 1) 0.82; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.70 (1H, s, 5-H pyrim), 7.20-7.38 (8H, m, 2 x 2,4,5,6H pryrid), 7.58 (1H, s, br, ex, NH), 8.46 (1H, s, 2-H pyr); Exact Mass: $C_{14}H_{13}N_6$ Calcd.: 265.1201 $[M+H]^+$, Found: 265.1204.

Dechlorination reaction: Typical Procedure:

To a solution of halo-dihetarylamine (500 mg) in ethanol (200 mL) Raney nickel (50 % suspension in water; 2.5 g) was added. The mixture was heated under reflux for 1 hour. TLC (CH_2Cl_2 : MeOH = 99 :1) indicated, that all starting material had been consumed. The mixture was filtered hot; the precipitate was washed with ethanol. The solvent of the combined filtrates was removed. The obtained residue was crystallized from ether/hexane.

N-Phenyl-4-aminopyrimidine (**33a**):



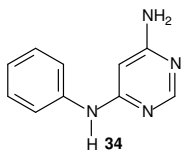
33

Yield: 86 %; colorless; Rf 0.81 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR (dmso-d_6, 300 MHz): δ = 6.73 (1H, d, 5-H pyrim), 7.20 - 7.36 (3H, m, 2,4,6H Ph), 7.48 (2 H, (t), *J* = 7.6 Hz, 3,5H Ph), 7.52 (1H, d, 6-H Pyrim.), 7.54 (1 H, s, br, ex, NH), 8.45 (1H, s, 2-H pyr); ^{13}C-NMR (dmso-d6, 75 MHz): δ = 107.7, 154.7, 158.0, 160.4 (5-CH, 2-CH, 6-CH, 4-C pyrim), 120.3, 123.1, 129.2, 140.9 (2,6-CH, 4-CH, 3,5-CH, 1-C ph); $FAB^+[M+H]^+$: 172.1.

Exchange of Cl to NH_2: Typical Procedure:

A pressure vessel is charged with halo-dihetarylamine (500 mg) and MeOH / NH_3 (200 mL). After sealing, the mixture is kept at 80°C overnight. After removal of the volatiles the obtained product is purified by crystallization from ether.

*N*⁴-Phenyl-4,6-pyrimidinediamine (**34**):



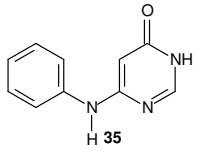
34

Yield: 78 %; colorless; Rf 0.74 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR (dmso-d_6, 300 MHz): δ = 6.25 (1H, d, 5-H pyrim), 6.91 (1H, t, *J* = 7.08 H, 4-H, Ph), 7.26 (2H, (t), J^1 = 7.8 Hz, J^2 = 7.6 Hz, 3,5-H Ph), 7.55 (2H, d, *J* = 7.6 Hz, 2,6-H, Ph), 8.24 (1H, s, 2-H pyr), 9.26 (1H, s, br, ex, NH); ^{13}C-NMR (dmso-d_6, 75 MHz): δ = 86.9, 149.6, 158.0, 160.9 (5-CH, 2-CH, 6-C, 4-C pyrim), 120.2, 122.1, 129.1, 141.0 (2,6-CH, 4-CH, 3,5-CH, 1-C ph); $FAB^+[M+H]^+$: 187.1.

Exchange of Cl to OH: Typical Procedure:

A solution of halo-dihetarylamine (0.5 g) in ethyleneglycol was treated with NaOH (0.6 g) at 180°C for 30 minutes. Complete solution was achieved when the internal temperature reached 110°C. After cooling to room temperature the mixture was diluted with water (25 mL), treated with charcoal and filtered. The clear yellow filtrate was acidified with glacial acetic acid. The precipitate formed was filtered off and recrystallized from EtOH / water.

6-Anilino-4(3*H*)-pyrimidinone (**35**):

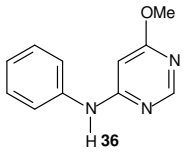


Yield: 72 %; colorless; Rf 0.66 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.70 (1H, s, 5-H pyrim), 7.24-7.54 (6H, m, 2, 3, 4, 5,6H ph, 2-H pyr), 7.58 (1 H, s, br, ex, NH), 9.85 (1H, s, br, ex NHCO); FAB$^+$[M+H]$^+$: 188.1.

Exchange of Cl to OMe: Typical Procedure:

A pressure vessel is charged with halo-dihetarylamine (500 mg) and sodium methoxide (1.2 equ.) in MeOH (200 mL). After sealing, the mixture is kept at 80°C overnight. After careful neutralization and removal of the volatiles, the obtained product is purified by crystallization from ether.

6-Methoxy-*N*-phenyl-4-aminopyrimidine (**36**):



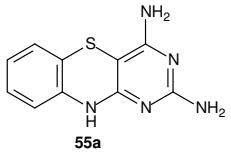
Yield: 81 %; Rf (CH_2Cl_2 : MeOH 9 : 1) 0.72; ^{1}H-NMR (300 MHz, dmso-d_6): δ = 3.32 (3H, s, CH_3), 6.10 (1H, s, 5-H pyrim), 6.96 (1H, m, 4H ph), 7.28 (2H, (m, 3,5H ph), 7.54 (2H, m, 2,6H ph), 8.34 (1H, s, 2-H pyrim), 9.12 (1H, s, br, ex, NH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 53.8 (CH_3), 88.0, 158.2, 161.0, 163.2 (5-CH, 2-CH, 4-C, 6-C pyrim), 120.3, 122.3, 129.1, 140.9 (2,6-CH, 4-CH, 3,5-CH, 1C ph); FAB$^+$[M+H]$^+$ 202.1.

Ring Closure in acidic medium: Typical Procedure:

A mixture of ortho mercapto-hetarylamine (4.0 mmol) and ortho-bromo-hetarylchloride (4.0 mmol) is dissolved in a mixture of dioxane (5 mL) and water (10 mL). $NaHSO_3$ (104 mg, 1 mmol) and conc. H_2SO_4 (4 drops) are added and the reaction mixture is heated under reflux for 4 hours. Almost immediately a light yellow precipitate is formed, which turns to a fluffy bright yellow- orange precipitate while heating was continued. During the whole heating process the pH has to remain slightly acidic. After cooling to room temperature the mixture is

carefully neutralized to pH ~ 7; the solvents are reduced to a volume of ~ 4 mL and after cooling the precipitate formed is filtered off, dried over P_2O_5 and crystallized from acetone.

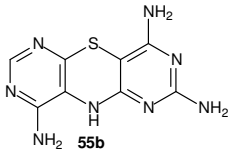
2,4-Diamino-10*H*-pyrimido[5,4-*b*][1,4]benzothiazine (**55**):



55a

Yield: 94 %; yellow; Rf: (CH_2Cl_2: MeOH = 9 . 1) 0.42; green fluorescence at 365 nm; mp: 255-256 °C; UV UV (λ_{max}[nm], log ε) (pH = 1): 268 (4.78), 295 (3.82), 291 (3.81), ^{1}H-NMR (dmso-d6, 500 MHz): δ = 6.58 (2H, s, br, ex, 2-NH_2), 6.67 (2H, s, br, ex, 4-NH_2), 6.85 (1H, t, *J* = 7.8 Hz 7-H), 6.88 (1H, d, *J* = 7.5 Hz, 9-H), 6.96 (1H, d, *J* = 7.8 Hz, 6-H), 7.01 (1H, t, *J* = 7.6 Hz, 8-H), 9.36 (1H, s, br, ex, 10-H); ^{13}C-NMR (dmso-d6, 125 MHz): δ = 73.4 (4a-C), 116.2 (9-CH), 116.7 (5aC), 123.6 (7-CH), 126.2 (6-CH), 127.5 (8-CH), 139.3 (9a-C), 154.3 (2-C), 157.0 (4-C), 159.2 (10a), FAB$^+$[M+H]$^+$: 232.1.

10*H*-Dipyrimido[4,5-*b*:4,5-*e*][1,4]thiazine-2,4,9-triamine (**55b**):



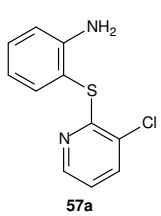
55b

Yield: 92 %; orange; Rf CH_2Cl_2: MeOH = 9 . 1) 0.31; green fluorescence at 365 nm; mp: > 300 °C; UV (λ_{max}[nm], log ε): (pH = 1): 258 (2.72), 430 (2.92); FAB$^+$[M+H]$^+$: 249.2.

Ring Closure in alkaline medium: Typical Procedure:

A mixture of ortho mercapto-hetarylamine (4.0 mmol) and *ortho*-bromo-hetarylchloride (4.0 mmol) is dissolved in 1N NaOH (20 mL). Almost immediately a bright red solution is obtained. Stirring is continued for 4 more hours. After neutralization with glacial acetic acid a fluffy bright orange-red precipitate is obtained. The volatiles are concentrated to a volume of 4 mL. After cooling the precipitate formed is filtered off, dried over P_2O_5 and crystallized from acetone / charcoal.

2-[(3-Chloro-2-pyridinyl)sulfanyl]aniline (**57a**):

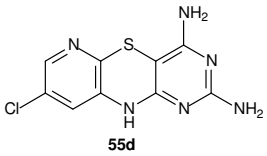


57a

Yield: 76 %; Rf (CH_2Cl_2 : MeOH = 9 : 1) 0.54 colorless, no fluorescence at 365 nm; ^{1}H-NMR (200 MHz, dmso-d_6): δ = 6.73-6.83 (2H, m, 5,6 CH an), 6.92-6.98 (1H, d xd, *J* = 4.8 Hz, *J* = 3.0 Hz, 4CH an), 7.25 (1H, (t), *J* = 7.6 Hz, *J* = 1.2 Hz, 5-H py), 7.40 (1H, d x d, *J* = 7.6 Hz, *J* = 1.3 Hz, 3-H an), 7.55 (1H, d x d, *J* = 1.2 Hz, *J* = 8.0 Hz, 4-H py), 8.00 (2H, s, br, ex, NH_2), 8.22 (1H, d x d, *J* = 1.3 Hz, *J* = 8.0 Hz, 6-H py); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 112.1, 115.5, 118.6, 131.4, 137.7, 149.7 (2-C, 6-

CH, 3-CH, 4-CH, 5-CH, 1-C an), 120.5, 128.9, 136.2, 147.6, 162.5 (5-CH, 3-C, 4-CH, 6-CH py); $FAB^+[M+H]^+$: 237.0.

8-Chloro-10*H*-pyrido[2,3-*b*]pyrimido[4,5-*e*][1,4]thiazine-2,4-diamine (**55d**):



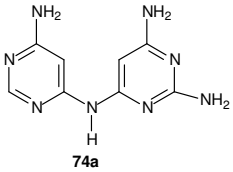
55d

Yield: 89 %; yellow; Rf: (CH_2Cl_2: MeOH = 9 : 1) 0.40; green fluorescence at 365 nm; mp: > 300 °C; UV (λ_{max}[nm], log ε): (pH = 1): 232 (4.10), 251 (4.15), 283 (3.79), 306 (3.96), 333 (3.67); $FAB^+[M+H]^+$: 267.1.

"Splitting Reaction:" Typical procedure:

To a solution of the azaphenothiazine derivative (500 mg) in ethanol (60 mL) Raney nickel (50 % suspension in water; 2.5 g) was added. The mixture was heated under reflux for 4 hour. TLC (CH_2Cl_2 : MeOH = 99 :1) indicated, that all starting material had been consumed. The mixture was filtered hot, the precipitate was washed with ethanol. The solvent of the combined filtrates was removed. The obtained residue was recrystallized from ether/hexane.

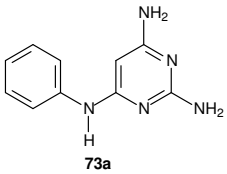
N^4-(6-Amino-4-pyrimidinyl)-2,4,6-pyrimidinetriamine (**74a**):



74a

Yield: 83 %; colorless; Rf: 0.32 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR (dmso-d_6, 200 MHz): δ = 4.49 (s, 1H, 5-H-triNH_2-pyr), 6.51 (s, 4H, 2 xNH_2), 6.89 (s, br, 3H, NH_2, 5-H, NH_2pyr), 7.68 (s, 1H, NH), 8.02 (s, 1H, 2-H, NH_2pyr); ^{13}C-NMR (dmso-d6, 50 MHz): δ = 85.9 (2 x 5-CH), 156.8 (2-CH NH_2pyr), 160.7 (3 x C-NH_2), 161.7 (2 x 1-C pyrim); $FAB^+[M+H]^+$: 219.2.

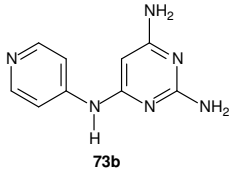
N^4-Phenyl-2,4,6-pyrimidinetriamine (**73a**):



73a

Yield: 78 %; Rf (CH_2Cl_2 : MeOH = 9 : 1) 0.41 ; ^{1}H-NMR (300 MHz, dmso-d_6): δ = 5.25 (1H, s, 5-H pyrim), 6.23, 6.30 (4H, 2 x s, br, ex, 2 NH_2), 6.92(1H, t, J = 7.2 Hz, 4-H Ph), 7.24 (2H, (t), J^1 = 7.2 Hz, J^2 = 8.0 Hz), 7.55 (2H, d, J = 8.0 Hz), 8.85 (1H, s, br, ex, NH); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 76.1, 160.5, 161.7, 162.5 (5-CH, 2-C, 6-C, 4-C pyrim), 120.5, 121.9, 129.0, 141.2 (2,6-CH, 4-CH, 3,5-CH, 1C ph); $FAB^+[M+H]^+$: 202.1.

N^4-(4-pyridinyl)-2,4,6-pyrimidinetriamine (**73b**):



73b

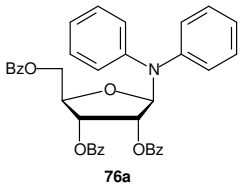
Yield: 85 %; colorless; Rf: 0.22 (CH_2Cl_2: MeOH = 9 : 1); ^{1}H-NMR (dmso-d6, 200 MHz): δ = 5.96 (1H, s, 5-H pyrim), 6.52 (2H, s, br, ex, 2-

NH_2), 6.75 (1H, s, br, ex, NH), 6.93 (2H, s, br, ex, 4-NH_2), 6.95 (2H, AA´BB´, *J* ~ 7.6 Hz, 2,6-H py), 8.64 (2H, AA´BB´, *J* ~ 7.6 Hz, 3,5-H py), ^{13}C-NMR (dmso-d6, 50 MHz): δ = 83.1 (5-CH pyr), 109.7 (3,5CH py), 139.2 (2,6CH py), 146.0 (1C py), 158.3 (1-C pyr), 160.9 (6-C pyr), 166.8 (4-C pyr); FAB$^+$[M+H]$^+$: 203.1.

Vorbrüggen Condensation to **76**: Typical Procedure:

Under nitrogen protection the corresponding base (4.0 mmol) is dissolved in dichloroethane (20 mL). BSA (0.94 g, 4.5 mmol) is added and the mixture is stirred at room temperature for 20 min. After cooling to 0°C tribenzoylriboseacetate (1.55 g, 3.0 mmol) or tetraacetylribose (0.95g, 3.0 mmol), respectively, and TMSOTf (1.02 g, 4.5 mmol) is added. The mixture is allowed to come to room temperature and further heated under reflux for 3 hours. After cooling to room temperature the mixture is then neutralized with saturated $NaHCO_3$–solution (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure and the resulting oil was purified by column chromatography (silica, CH_2Cl_2 : MeOH = 99 :1).

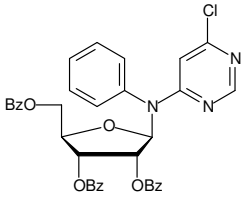
2,3,5-Tri-*O*-benzoyl-*N*,*N*-diphenylribofuranosylamine (**76a**):



76a

Yield: 34 %; Rf 0.38 (9: 1 CH_2Cl_2 : MeOH); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 1.83, 2.10 (9H, 3 x CH_3CO), 4.09-4.21 (3H, 2xH5´, H4´), 4.89 (1H, m, H3´), 5.07 (1H, m, H2´), 5.87 (1H, d, *J* = 8.2 Hz, H1´), 7.05-7.34 (10H, m, 2 x ph); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 20.1, 20.3 (3 x CH_3), 63.5 ($CH_2$5´), 70.5 (CH3´, CH4´), 77.5 (CH2´), 89.3 (CH1´), 123.9 (2 x 4-CH ph), 124.2 (2 x 2,6-CH ph), 129.0 (2 x 3,5-CH ph), 145.2 (2 x C-1 ph), 169.3, 169.6, 170.3 (3 x C=O); FAB$^+$[M+H]$^+$: 450.0.

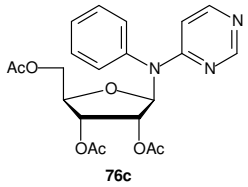
2,3,5-Tri-*O*-benzoyl-*N*-(6-chloro-4-pyrimidinyl)-*N*-phenylribofuranosylamine (**76b**):



76b

Yield: 45 %; Rf 0.38 (CH_2Cl_2 : MeOH 9: 1); ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.72 (1H, s, 5-CH pyr), 4.40-4.73 (3H, m, 2 x H5´, H4´), 5.40 (1H, m, H3´), 5.53 (1H, m, H2´), 5.99 (1H, d, *J* = 2.7 Hz, H1´), 7.17 – 7.98 (20H, m, 3 x Bz, ph), 8.60 (1H, s, 2-H pyr); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 63.5 ($CH_2$5´), 71.1 (CH3´, CH4´), 78.8, (CH2´), 86.1 (CH1´), 105.1 (5-CH pyr), 128.5-133.5 (C, CH Ar), 136.4 (1-C ph), 157.9 (2-CH pyr), 160.4 (6-C pyr), 164.3 (1-C pyr), 165.1, 166.0 (3 x C=O); FAB$^+$[M+H]$^+$: 650.1.

2,3,5-Tri-*O*-acetyl-*N*-(4-pyrimidinyl)-*N*-phenylribofuranosylamine (**76c**):



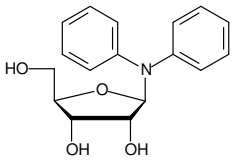
76c

Yield: 47%; Rf 0.41 (CH_2Cl_2 : MeOH 9: 1); ^{1}H-NMR (300 MHz, dmso-d_6): δ = 4.29 (2H, m, 2 x H5´), 5.31 (3H, m, H2´, H3´, H4´), 6.03 (1H, d, *J* = 3.7 Hz, H2´), 7.26 (1H, t, *J* = 7.5 Hz, 4-CH Ph), 7.37 (2H, (t), J^1 = 7.50 Hz, J^2 = 7.70 Hz), 7.48 (1H, d, *J* = 7.65 Hz, 5-H pyrim), 7.73 (2H, d, *J* = 7.70 Hz, 2,6-H Ph), 8.25 (1H, d, *J* = 7.65 Hz, 6-H pyrim), 8.77 (1H, s, 2-H pyrim); ^{13}C-NMR (75 MHz, dmso-d_6): δ = 20.3, 20.4, 20. 7 (3 x CH_3 Ac), 62.3 ($CH_2$5´), 69.4 (CH2´), 74.8 (CH3´), 81.5 (CH4´), 93.7 (CH1´), 109.4, 140.5, 150.1, 160.5 (5-CH, 6-CH, 2-CH, 4-C pyrim), 122.7, 127.0, 129.1, 135.8 (2,6-CH, 4-CH, 3,5-CH, 1-C ph), 169.7, 170.0, 170.1 (3 x C=O Ac); FAB$^+$[M+H]$^+$: 430.1.

Deprotection to **77**: Typical Procedure:

The protected nucleoside (2.0 mmol) is dissolved in NH_3/ MeOH (20 mL). The mixture is kept at room temperature till no more starting material could be detected on TLC. After removal of the volatiles under reduced pressure the resulting oil was purified by flash column chromatography.

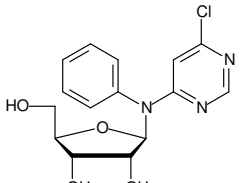
N,*N*-diphenylribofuranosylamine (**77a**):



77a

Yield: 85 %; Rf 0.38 (CH_2Cl_2 : MeOH 4: 1); ^{1}H-NMR (dmso-d6, 200 MHz): δ = 3.54-3.78 (3H, 2 x H5´, H4´), 4.06 (1H, m, H3´), 4.50 (1H, m, H2´), 4.91 (1H, t, *J* = 6 Hz, 5´-OH), 5.13 (1H, d, *J* = 6 Hz, OH), 5.25 (1H, d, *J* = 6 Hz, OH), 6.05 (1H, d, *J* = 8.2 Hz, H1´), 7.05-7.34 (10H, m, 2 x ph); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 61.0 ($CH_2$5´), 69.0 (CH3´), 71.4 (CH4´), 75.8 (CH2´), 89.4 (CH1´), 124.1 (2 x 4-CH ph), 125.0 (2 x 2,6-CH Ph), 129.4 (2 x 3,5-CH Ph), 145.0 (2 x C-1 ph); Exact Mass Calcd: $C_{17}H_{20}NO_4$: 302.1392 [M+H]$^+$, Found: 302.1395.

N-(6-chloro-4-pyrimidinyl)-*N*-phenylribofuranosylamine (**77b**):

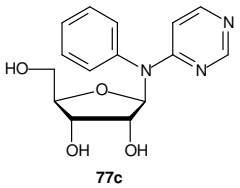


77b

Yield: 91 %; Rf (CH_2Cl_2 : MeOH = 4 : 1) 0.42; ^{1}H-NMR (300 MHz, dmso-d_6): 3.60 (2H, m, 2 x H5´), 4.09 (2H, m, H3´, H4´), 4.31 (1H, m, H2´), 5.10 (1H, t, *J* = 6 Hz, 5´-OH), 5.32 (1H, d, *J* = 6 Hz, OH), 5.56 (1H, d, *J* = 6 Hz, OH), 5.85 (1H, d, *J* = 6.8 Hz, H1´), 6.71 (1H, s, 5-H pyrim), 7.23-7.36 (3H, m, 2,4,6H ph), 7.48 (2H, (t), *J* = 7.6 Hz, 3,5H ph), 8.67 (1H, s, 2-H pyr); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 61.1 ($CH_2$5´), 70.3 (CH2´), 76.2 (CH3´), 87.5 (CH4´), 96.0 (CH1´), ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 102.8 (5-CH), 123.2, 126.0, 129.8, 137.1 (2,6-CH, 4-CH, 3,5-CH, 1-C

ph), 156.8 (2-CH pyr), 160.3 (6-C pyr), 164.2 (1-C pyr); Exact Mass $C_{15}H_{17}ClN_3O_4$ Calcd: 338.0908 $[M+H]^+$, Found: 338.0910.

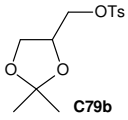
<u>*N*-(4-pyrimidinyl)-*N*-phenylribofuranosylamine (**77c**):</u>



77c

Yield: 83 %; Rf (CH_2Cl_2 : MeOH = 4 :1) 0.40 ; ^{1}H-NMR (300 MHz, dmso-d_6): δ = 3.68 (2H, m, 2 x H5´), 4.10 (2H, m, H3´, H4´), 4.21 (1H, m, H2´), 5.30 (1H, t, *J* = 6 Hz, 5´-OH), 5.42 (1H, d, *J* = 6 Hz, OH), 5.70 (1H, d, *J* = 5.8 Hz, H1´), 5.76 (1H, d, *J* = 6 Hz, OH), 7.15 (1H, d, *J* = 7.5 Hz, 5-H pyrim), 7.28 (1H, t, *J* = 7.4 Hz, 4-H ph), 7.47 (2H, (t), J^1 = 7.4 Hz, J^2 = 7.7 Hz, 3,5-H ph), 7.66 (2H, d, *J* = 7.7 Hz, 2,6-H ph), 8.59 (1H, d, *J* = 7.5 Hz, 6-H pyrim), 9.15 (1H, s, 2-H pyrim); ^{13}C-NMR (50 MHz, dmso-d_6): δ = 61.1 ($CH_2$5´), 70.7 (CH2´), 76.7 (CH3´), 87.9 (CH4´), 96.3 (CH1´), 108.9, 142.2, 152.5, 152.5 (5-CH, 6-CH, 2-CH, 4-C pyrim), 122.8, 126.5, 129.6, 136.9 (2,6-CH, 4-CH, 3,5-CH, 1-C ph); Exact Mass $C_{15}H_{18}N_3O_4$ Calcd: 304.1297 $[M+H]^+$; Found: 304.1300.

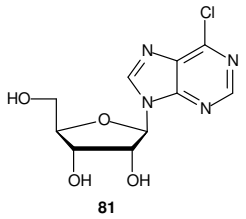
<u>1-*O*-Methylphenylsulfonyl-2,3-*O*-isopropylidene-glycerole (**79b**):</u>



C79b

Under ice-cooling *(rac)*-1,2-*O*-isopropylidene-1,2,3-propanetriole (2.00 g, 0.015 mol) was added in two portions to a solution of p-toluenesulfonyl chloride (3.80 g, 0.020 mol) in pyridine (20 mL). The mixture was stirred at 0°C for 4 hours and left overnight at room temperature. Methanol (3 mL) was added and the mixture was stirred for another 10 minutes. After evaporation of the volatiles, the residual oil was dissolved in CH_2Cl_2 (30 mL) and washed with water (2 x 20 mL). After being dried over Na_2SO_4, the organic layer was concentrated and the residue was purified by column chromatography (silica, CH_2Cl_2 ; CH_2Cl_2 : MeOH 99:1). Yield: 4.08 g (94%); Rf= 0.85 (CH_2Cl_2: MeOH = 99:1); ^{1}H-NMR ($CDCl_3$): δ =1.30 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 2.44 (s, 3H, CH_3-Ar), 3.71-3.78 (dd, 1H, H-3'A, $J_{(3'A,, 2')}$ =5.2 Hz, $J_{(gem)}$ =8.8 Hz), 3.98-4.06 (m, 3H, H-3'B + 2H-1'), 4.21-4.33 (t x t, 1H, H-2', $J_{(3',2')}$ = 5.2 Hz, $J_{(1',2')}$ = 6.0 Hz), 7.33-7.37 (d, 2H, 3,5-H-Ar, *J* =8.6 Hz), 7.76-7.80 (d, 2H, 2,6-H-Ar, J =8.6 Hz); ^{13}C-NMR ($CDCl_3$): δ = 21.3 (CH_3-Ar), 24.9, 26.3 (2 x CH_3), 65.9 (C-1'), 69.4 (C-3'), 72.7 (C-2'), 109.8 (C-4'), 127.8 (C-3,5-Ar), 129.8 (C-2,6-Ar), 132.6 (C-4-Ar), 145.0 (C-1-Ar). Exact Mass Calcd. $C_{13}H_{18}O_5S$ 287.0953$[M+H]^+$; Found: 287.0952.

6-Chloropurine Riboside (**81**):



81

Under nitrogen protection the 6-Cl-purine (0.46g, 2.5 mmol) is dissolved in dichloroethane (20 mL). BSA (0.62g, 3.0 mmol) is added and the mixture is stirred at room temperature for 20 min. After cooling to 0°C tetraacetylribose (0.79 g 2.5 mmol) and TMSOTf (0.56 g, 2.5 mmol) is added. The mixture is allowed to come to room temperature and further heated under reflux for 3 hours. After cooling to room temperature the mixture was neutralized with saturated $NaHCO_3$–solution (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure and the resulted oil was purified by column chromatography (silica, CH_2Cl_2 : MeOH = 95 : 5). Yield: 76 % protected nucleoside **C81a**; Rf (CH_2Cl_2 : MeOH 95 : 5) 0.80; ^{1}H-NMR (300 MHz, dmso-d_6): δ = 2.08, 2.11, 2.15 (9H, 3 x s, 3 x CH_3 Ac), 4.39-4.49 (3H, m, H4´2 x H5´), 5.64 (1H, (t), *J* = 5 Hz, H3´), 5.95 (1H, (t), *J* = 5 Hz, H2´), 6.24 (1H, d, *J* = 5.0 Hz, H1´), 8.31 (1H, s, 2-H), 8.77 (1H, s ,8-H); ^{13}C-NMR (75 MHz, dmso-d_6): δ = 20.3, 20.5, 20.7 (3 x CH_3 Ac), 62.8 (CH5´), 70.5 (CH3´), 73.1 (CH2´), 80.5 (CH4´, 86.9 (CH1´), 132.3 (5-C), 143.6 (8-CH), 151.2 (6-C), 151.6 (4-C), 152.3 (2-CH), 169.3, 169.5, 170.2 (3 x C=O Ac); FAB$^+$[M+H]$^+$: 413.1.

The protected nucleoside **81a** (2.0 mmol) is dissolved in NH_3/ MeOH (20 mL). The mixture is kept at room temperature till no more starting material could be detected on TLC. To remove traces of adenosine formed, the compound was further purified by flash chromatography (silica, CH_2Cl_2 : MeOH = 4 : 1); Yield: 81 % Rf (CH_2Cl_2 : MeOH = 4 = 1) 0.56; ^{1}H-NMR (300 MHz, dmso-d_6): δ = 3.57- 3.72 (2H, m, 2 x H5´), 3.99 (1H, m, H4´), 4.20 (1H, m, H3´), 4.59 (1H, m, H2´), 5.11, 5.26, 5.58 (3H, 3 x s, br, ex, 3 x OH), 6.05 (1H, d, *J* = 4.9 Hz, H1´), 8.81 (1H, s, 2-H), 8.95 (1H, s ,8-H); ^{13}C-NMR (75 MHz, dmso-d_6): δ = 61.4 (CH5´), 70.5 (CH3´), 74.4 (CH2´), 86.2 (CH4´, 88.6 (CH1´), 131.8 (5-C), 146.2 (8-CH), 149.7 (6-C), 152.1 (4-C), 152.2 (2-CH); FAB$^+$[M+H]$^+$: 286.0.

Enzymatic Deamination of Nucleosides **81** and **77b**:

Nucleosides **81** and **77b** (0.02 g) in phosphate buffer (50mol, 6 mL, pH 7.4) containing 3 % DMSO were treated with ADA (2 mg). The reaction progress was monitored by HPLC using phosphate buffer (pH 6.0) / acetonitrile as eluent (9:1). When the reaction was complete, the solution was lyophilized to afford inosine **82** (from **81**).

ABBREVIATIONS

Ac	Acetyl
AcOEt	Ethylacetate
AcOH	Acetic acid
AcRib	1,2,3,5-Tetra-*O*-acetyl-ribofuranose
ADA	Adenosine deaminase
AICA	5-Amino-4-imidazolecarboxamide
ATP	Adenosine Triphosphate
BINAP	2,2´-bis(dipenylphosphino)-1,1´-binaphtyl
Bn	Benzyl
br	broad (i.e. OH, or NH signals)
BSA	*N,O*-bis(trimethylsilyl)acetamide
Bz	Benzoyl
BzRib	1-*O*-Acetyl-2,3,5-tri-*O*-benzoylribofuranose
DEPT	Distortionless Enhancement by Polarization Transfer
DMA	Dimethylaniline
DMF	Dimethylformamide
DNA	Desoxyribonucleic Acid
ES	= EI; Electron Impact Ionisation, detection of positive charged particels
Et	Ethyl
Et_2O	Diethylether
Et_3N	Triethylamine
EtOH	Ethanol
ex	exchangeable
FAB	Fast Atom Bombardment, either positive (FAB^+) or negative (FAB^-) charged particels can be detected
FMO	Frontier Molecular Orbital
HMBC	Heteronuclear Multible Bond Correlation
HMDS	Hexamethyldisilazane
HMQC	Heteronuclear Multible Quantum Coherence
HOMO	Highest Occupied Molecular Orbital
HPLC	High Pressure Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence
LUMO	Lowest unoccupied Molecular Orbital
m.p.	Melting point
Me	Methyl
MeCN	Acetonitile
MeOH	Methanol

MMTr	Monomethoxytrityl
NaOEt	Sodium ethoxide
NOE	Nuclear Overhauser Effect
3NT	3-Nitrotriazole
Ph	Phenyl
POMCl	Pivaloyloxymethyl chloride
Py	Pyridine
Rf	Retention factor
RNA	Ribonucleic Acid
rt	Room temperature
s, d, t	singlet, dublet, triplet
SAHase	S-adenosyl-L-homocysteine hydrolase
t-Bu	tert. Butyl
TFA	Trifluoroacetic acid
TMSOTf	Trimethylsilyltrifluoromethanesulfonate
TsOH	toluene sulfonic acid

CURRICULUM VITAE

Personal:

Name:	Dr. Irene Maria Lagoja, PhD Sci.
Nationality:	Austrian
Date of Birth:	March 21, 1971
Place of Birth:	Salzburg, Austria

Education:

1977-1989	Elementary School, High School, Salzburg; final exam with highest distinction
1989-1994	Leopold-Franzens-Universität; Innsbruck Major in chemistry.
Nov. 1993 - Sept. 1994	Diploma thesis with Prof. J. Schantl ("Direkter synthetischer Zugang zu funktionalisierten Imidazolen"; "Direct Synthetic Approach to Functionalized Imidazoles")
October 1994	Mag. rer. nat. (Master's degree) with highest distinction
December1994-1997	PhD-Thesis with Prof. J. Schantl ("*N*-Aminoimidazole zur Generierung nucleophiler Carbene: Synthesen, Reaktionen und Strukturuntersuchungen"; "*N*-Amino-imidazoles as Precursors for Nucleophilic Carbenes: Syntheses, Reactions and Structure Elucidations")
08.01.1998	Graduation to PhD. Sci. in the subjects Organic- and Pharmaceutical Chemistry
18. 12. 2006	„Habilitation", University of Frankfurt; *venia legendi* in the subject Organic Chemistry

There's only one corner of the universe you can be certain of improving,
and that's your own self.
Aldous Huxley