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„Mefloquine-based chiral selectors and stationary phases (MFQ-CSPs)“

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Denise Wolrab

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Table of Contents

List of Abbreviations

ACN	acetonitrile
AcOH	acetic acid
AIBN	2,2´-Azobis(2-methylpropionnitril)
AX	anion exchanger
CDA	chiral derivatizing agent
CIS	complexation-induced chemical shift
CSA	chiral solvating agent
CSP	chiral stationary phase
CX	cation exchanger
DATAAN	(*O*,*O*)-diacetyl-(R,R)-tartaric acid anhydride
DBTAAN	(*O*,*O*)-dibenzoyl-(R,R)-tartaric acid anhydride
DBTAM	(*O*,*O*)-dibenzoyl-(R,R)-tartaric acid monoester
DCC	dicyclohexylcarbodiimide
DCM	dichlormethane
DCU	dicyclohexylurea
DEA	diethylamine
DMAP	4-dimethylaminopyridin
DMF	dimethylformamide
DNB-DL-Leu	*N*-3,5-dinitrobenzoyl-DL-leucine
EA	ethyl acetate
FA	formic acid
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
Linker-ACHSA	*trans*-2-(*N*-4-(allyloxy)-3,5-dichlorobenzoyl)-aminocyclohexanesulfonate
LSR	lanthanide shift reagent
MeOH	methanol
MeOD	methanol-d_4
MFQ	mefloquine
Mol.Wt.	molecular weight
MS	mass spectrometry

N-allyl MFQ	*N*-allyl mefloquine
N-allyl-F-CMFQ	3,5-Bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine
NHS	*N*-hydroxy succinimidyl
N-Me-MFQ	*N*-methyl mefloquine
NMR	nuclear magnetic resonance
N-undec-MFQ	*N*-undecenoyl mefloquine
PE	petrolether
PFB-DL-Leu	*N*-2,3,4,5,6-pentafluorobenzoyl-DL-leucine
QN/QD	quinine/quinidine
RP	reversed phase
SA	selectand
SCX	strong cation exchanger
SO	selector
Syringic-ACHSA	*trans*-2-(*N*-)4-allyloxy-3,5-dimethoxybenzoyl)-aminocyclohexanesulfonate
*t*BuCMFQ	*tert*-butylcarbamoyl mefloquine
*t*BuCQN/*t*BuCQD	*tert*-butylcarbamoyl quinine/quinidine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMB-DL-Ala	3,4,5-trimethoxybenzoyl-DL-alanine
TMD-DL-Leu	3,4,5-trimethoxybenzoyl-DL-leucine
ZWIX	zwitterion exchanger

Mefloquine-based chiral selectors and stationary phases (MFQ-CSPs)

1. Introduction

1.1 Mefloquine

The most lethal human parasitic infection is still malaria. Millions of people die due to this disease every year worldwide, especially in Africa region. Therefore an effective treatment and malaria prevention is indispensable. The human malaria is caused by a parasite of the genus *Plasmodium* and it is mostly transferred by mosquitoes[1]. Mefloquine (MFQ) is one of the best known and most widely used antimalarial discovered during 1960s. Despite its side-effects such as dizziness, headache, insomnia, nightmares, severe depression etc.[2,3], mefloquine is still used as an antimalarial drug because it is effective against resistant malaria species. Mefloquine is used as a *suppressive prophylactic* against malaria. That means that the drug is effective as long as the parasites are invading erythrocytes (red blood cells). However, in some regions, especially in Thai border areas, a mefloquine resistance has been observed. The mefloquine resistance is closely related to, the quinine resistance[4].

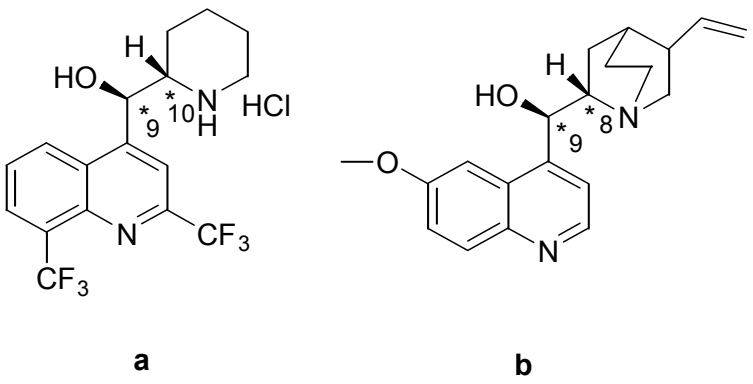


Figure 1: General structures of (9R,10S)-mefloquine hydrochloride (a) and (8S,9R)-quinine (b)

Since it is structurally an aminoethanol, mefloquine has been extensively tested also for different pharmacological activites, e.g. as cholinesterases inhibitor[5], antituberculosis[6], or antibacterial agent[7].

Synthetic mefloquine is a racemate[8,9], the *erythro* isomers of α-(2-piperidinyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol; however, an asymmetric total synthesis of (9R,10S)-mefloquine has been published recently[10]. The structure of mefloquine is derived from cinchona alkaloids. The quinoline ring of mefloquine is substituted by two electron withdrawing groups instead of the electron donating methoxy group and the quinuclidine moiety of cinchonas is replaced by a less bulky piperidine ring. The absolute configuration of MFQ (+) and (-) enantiomers was assigned by comparing the circular dichroism spectra of (+)-MFQ and (-)-MFQ with those of (+)-QN and (-)-QD[11], the absolute configuration of those was confirmed by x-ray analysis[12]. Furthermore, it was found that the crystallographic structure of MFQ hydrochloride is similar to the crystal structures of QN and QD with respect to the quinoline moiety and the hydroxyl group. Also the distance between the aliphatic nitrogen and the hydroxyl group was found to be the same as in cinchona alkaloids[13]. Based on these similarities we decided to design and synthesize a new type of selector for chiral HPLC separation (see section 1.5 **the aim of work**).

1.2 Chiral recognition

Most of the natural products are chiral, *e.g*. sugars and amino acids. Chirality depends on the structural arrangement of the different substituents. A chiral compound cannot be converted to its mirror image by rotation. The mirror image of a chiral compound is called an enantiomer. Many of the biochemical processes depend on chirality and therefore a lot of drugs are chiral. It is known that one enantiomer of a chiral compound achieves the desired effect of the drug (eutomer) and the other one can cause unwanted adverse effects (distomer). As a result it is necessary to have a separation technology, e.g. chromatography, to resolve the enantiomers of a racemic compound.

The fact that enantiomers have the same physicochemical properties, except the optical rotation, makes them indistinguishable under achiral conditions. However,

there are certain analytical methods (chiral chromatography, NMR, etc.) that distinguish enantiomers directly.
Therefore it is necessary to transform the enantiomers into diastereomers, which have distinguishable physicochemical properties. In chiral chromatography a distinction is drawn between indirect and direct enantiomer separation, according to the transformation mechanism of the enantiomer to the diastereomer[14].

The “indirect” approach means that the mixture of the to be separated enantiomers reacts with an enantiomerically pure chiral derivatizing agent (CDA) to form a pair of covalent bonded stereoisomers which are diastereomeric to each other and therefore separable with achiral chromatographic techniques[15]. For this approach it is necessary that the enantiomers contain a functional group, which allows a reaction with the chiral derivatizing agent. Furthermore it is indispensable that the chiral dervatizing agent is highly enantiomerically pure or at least the enantiomerical impurity is known. In case of enantiomerical impurities four stereoisomers will be formed whereby two pairs of them are enantiomeric to each other and therefore indistinguishable with achiral chromatographic techniques[15]. During derivatization racemisation of the enantiomer and the chiral derivatizing agent must be avoided, otherwise a mixture of stereoisomers will be obtained[15] and quantification of the content of enantiomers in the sample will be impossible.
The “direct” approach means that the enantiomers are reversibly converted to non-covalent diastereomeric associates, which can be separated using chiral chromatographic techniques. The diastereomeric associates are formed by complexation of the enantiomers with an enantiomerically pure chiral auxiliary, which is often covalently linked to the surface of the stationary phase or added as additive to the mobile phase. In this approach a derivatization to create diastereomeres is not needed. The chiral auxiliary is often referred to as chiral selector[15].
Adding the selector to the mobile phase as additive and using an achiral stationary phase is termed as chiral mobile phase mode. The enantioseparation, and therefore a difference in elution times of the associates is caused by different adsorption strength of the diastereomeric associates on the stationary phase or by adsorption of the selector on the surface of the achiral stationary phase and binding the enantiomers with different energies[15]. Nowadays the chiral mobile phase mode has

limited practical importance in HPLC, because of the necessity to dissolve the chiral selector in the mobile phase, the influence of the selector concentration on the separation factor and so forth[15].

The use of an achiral mobile phase and an enantiomerically pure compound, the selector, covalently linked to the surface of a carrier material is termed the chiral stationary phase mode. The enantioseparation occurs due to the formation of a diasteromeric complex on the surface of the stationary phase between selectands and the selector. The selectands (enantiomers) have different affinity towards the selector and thus the complexes formed have diverse stabilities[15]. The separation of the enantiomers can be improved by varying the mobile phase conditions, like changing solvents, pH and additives or temperature. The classification of the chiral stationary phases depends on the used selectors. The following selector-types are used in chiral stationary phases: Polysaccharides, proteins, synthetic polymers, macrocyclic compounds, crown ethers, and selectors whose interactions are based on donor-acceptor principles or on ion-exchange principles.

The basic concept of chiral recognition is the same for interactions between analytes and selectors, and interactions between chiral drugs and biological receptors. These association forces are of non-covalent nature, *e.g*. hydrogen-bonding, ionic interactions, π-π and dipole-dipole interactions as well as hydrophobic interactions [14,15]. The chiral recognition can be then explained by a three-point-rule[16], which defines that at least three simultaneous interactions have to take place between the selector and the analyte for efficient separation of its enantiomers. The selectand exposes its binding sites to the surface of the stationary phase and if the interaction sites of the selector are suitably oriented in space, they can bind to the selector and an ideal fit is achieved. If less than three interactions are possible, then such a binding is called a non-ideal fit. The enantiomer, which achieves the ideal fit, is the stronger bound one, and thus elutes as the second peak the column[15].

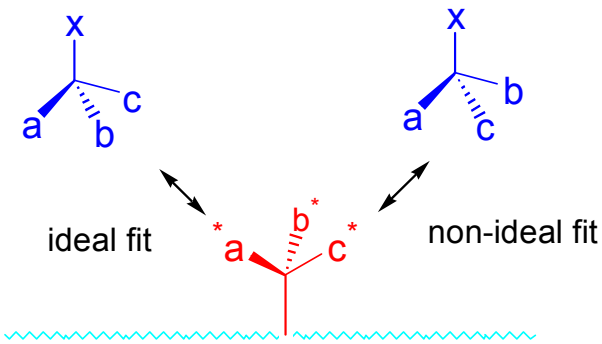


Figure 2: Schematical illustration of the 3-point rule

Another fast and effective chiral recognition method is NMR. NMR itself is an achiral method and so it is necessary to transform the enantiomers in diastereomers with a chiral auxiliary. There are two ways in which the chiral auxiliary can be used for the formation of diastereomers respectively diastereomer associates, similar to the direct and indirect approach of chromatography. The chiral auxiliary can react with the enamtiomers prior to the NMR experiment in order to produce diastereomers or it is used as additive or solvent during the NMR experiments in order to form diastereomer associates which differ in chemical shifts according to the geometry and stability of the formed complexes[17]. In both cases the chiral auxiliary has to be enantiomerically pure and there should be no racemization during formation of distereomers. By the direct approach either a chiral solvating agent (CSA) or a paramagnetic chiral lanthanide shift reagent (LSR) is added to the solution of the sample as chiral auxiliary for the formation of diastereomer associates. The enantiomeric ratio and therefore the enantiomeric excess (ee) can be determined directly by comparing the integrals of the same functionality of the diastereomeric associates with different chemical shifts[17]. The chemical shift differences are usually small using chiral solvating agents for the formation of diastereomeric associates compared to the use of a paramagnetic chiral lanthanide shift reagent[17]. The chemical shift differences for the enantiomers are large when the functionality of the enantiomer which reacts with the LSR is close to the stereogenic centre of the analyte. The chemical shift differences are low for compounds which contain halogens or π -systems like aromatics[17] when using of LSRs.

1.3 Chiral stationary phases (CSPs) for HPLC

As previously mentioned a common method for enantiomer separation is the use of chiral stationary phases, where the selector is immobilized on a silica surface. Phases containing a selector with a low molecular weight are generally called brush type phases. A short overview of Pirkle phases (Donor-Acceptor-type CSPs) and ionic exchanger phases, which belong to the brush type phases, is presented in the following paragraphs.

Donor-Acceptor-type CSPs (Pirkle phases):

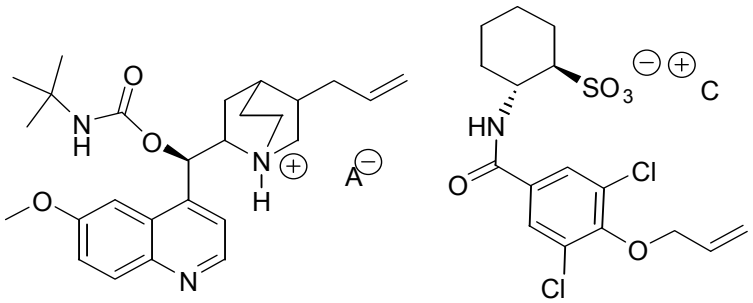
In Pirkle phases the chiral recognition is based on non-ionic attractive interactions, like hydrogen bonding, π-π -stacking, dipole-dipole- and sterical interactions[14]. Pirkle and coworkers developed the first commercially available CSP (DNBPG, Regis Technology) based on these interactions. This CSP was based on 3,5-dinitrobenzoyl-phenylglycine, which was immobilized on modified silica. This selector and in general some types of donor-acceptor type-CSPs are based on low-molecular weight molecules and that allow immobilization with a high surface coverage. Additional advantages are good solubility and the possibility of structural modifications of this selectors[14]. Amines, amino acids, carboxylic acids, esters and ethers represent analytes whose enantiomers can be separated with a Pirkle phase[18]. These CSPs are usually operated in normal phase mode because in reversed-phase or polar organic phase mode the polar interactions (mostly hydrogen bonding) are diminished and the enantioselectivity of a Pirkle phase drops significantly[14].

Ion-exchange-type CSPs:

The difference between Pirkle phases and ion-exchange type CSPs is that for the ion-exchange type CSP the main interaction is the long-range enantioselective ion-pairing interaction between the complementary charged selector and the analyte species[19,20]. The ion-pairing interaction brings the analyte close to the selector and permits non-covalent interactions like π-π stacking, hydrogen bonding and steric

interactions. All these interactions cause the separation of enantiomers i.e., the discrimination of one enantiomer and its reduced retention[19,20]. These CSPs are usually operated in polar organic or reversed-phase mode[15]. A distinction is made between chiral anion exchanger and chiral cation exchanger corresponding to the charge of the analyte. Anion exchanger CSPs adopt a protonated form and are applicable for the enantio-separation of chiral acids, such as *N*-derivatized amino acids, peptides, aminosulfonic acids etc. On the other hand, cation exchangers CSPs represent a separation tool for chiral bases, e.g. chiral amines[22,23,36].

A combination of both properties is realized within zwitterionic CSPs, where the selector consists of a protonated and an anionic part, and thus it is possible to separate zwitterions such as free amino acids.



a **b**

Figure 3: a: Anion exchanger CSP based on *t*BuCQN for the separation of chiral acids; b: Cation exchanger CSP based on linker acid derivatized with *trans*-2-aminocyclohexanesulfonic acid for the separation of chiral bases

1.4 CSPs based on chinchona alkaloids

In the workgroup of Professor Lindner many new selectors based on cinchona alkaloids have been developed and some of them have been commercialized[21,22,23,24].

The structure of a cinchona selector is based on π-basic quinoline ring which is connected via a hydroxymethylene spacer to the quinuclidine moiety. The strongly basic quinuclidine moiety is responsible for interactions with acidic groups, while the quinoline unit permits a π–π stacking with an aromatic part of the analytes.

Quinine has been used as antimalarial drug and it has antipyretic properties. It was also used as uterotonics, because of the muscle relaxant effects. Due to its bitter

taste, quinine found the biggest application as an additive in tonic water. By UV irradiation of an acidic solution of quinine fluorescence can be observed. Quinidine is a pseudoenantiomer of quinine and has a reversed configuration on the C8 and C9 position compared to quinine. It was frequently used as an antiarrythmic agent. Both quinine and quinidine and their derivatives are often employed as catalysts in stereoselective organic synthesis[25.26] and as chiral solvating agents in NMR spectroscopy[27,28].

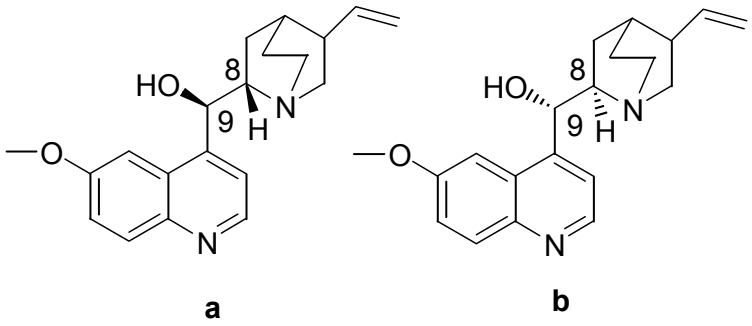


Figure 4: Cinchona alkaloids (8S,9R)-quinine (a) and (8R,9S)-quinidine (b)

Quinine and quinidine can work as selectors immobilized through their double bond on mercatopropyl modified silica gel. Such CSPs can be employed in high-performance liquid chromatography as weak chiral anion exchangers for enantioseparation of chiral acids. In this anion exchanger mode a buffered hydro-organic mobile phase is used to obtain a positively charged nitrogen in the quinuclidine ring, which can interact with the negatively charged selectands[29].

Derivatization of the hydroxyl group located at C_9 (see **Figure 5**) in the chinchona alkaloids quinine and quinidine can improve the chiral recognition for chiral acids, especially by introducing of a carbamoyl functionality[29]. Retention and enantioselectivity can be improved by optimization of the mobile phase conditions, like modifying the buffer concentration, the solvent composition, the pH and the temperature.

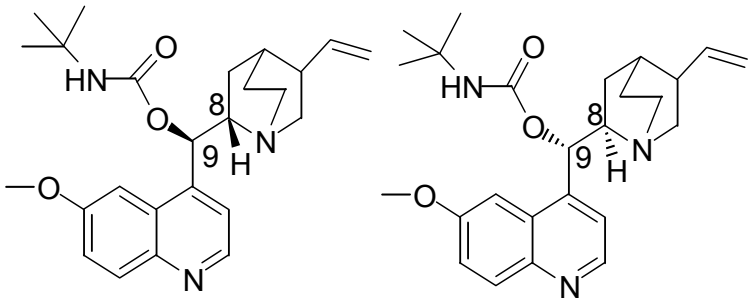


Figure 5: (8S,9R)-*t*BuCQN and (8R,9S)-*t*BuCQD

Another type of chiral stationary phase developed in Professor Lindner`s research group is a zwitterionic CSP (ZWIX CSP). This chiral stationary phase combines ion-exchange principles of QN/QD-based anion exchanger and *trans*-2-aminocyclohexanesulfonic acid-based cation exchanger. This type of selector was designed for the separation of a wide spectrum of chiral compounds. It can be used for separation of chiral acids, chiral bases and chiral amphoteric compounds like amino acids. The zwitterionic CSP is again applicable mainly for polar organic mobile phases[15,24].

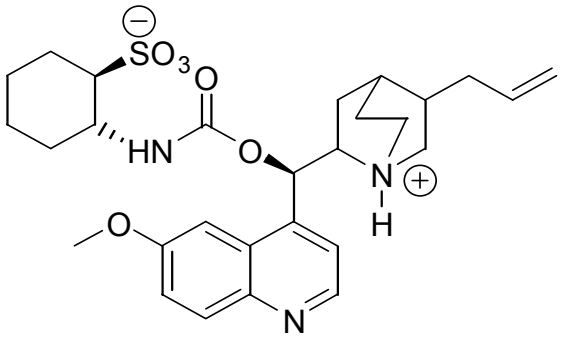


Figure 6: Zwitterionic exchanger-type CSP based on quinine derivatized with *trans*-2-aminocyclohexanesulfonic acid for the separation of chiral acids, chiral bases and zwitterionic compounds

The elution order of chiral acidic analytes obtained with the ZWIX CSPs (**Figure 6**) can be reversed by changing to the CSP of opposite configuration. This corresponds to the behaviour of the parent anion exchanger CSP based on quinine and quinidine. It was identified that the chiral recognition mechanism of chiral acidic analytes measured with this ZWIX CSPs is similar to the one of the parent anion exchanger based on quinine or quinidine. As a result it was observed that the attached *trans*-2-aminocyclohexanesulfonic acid causes reduced run times compared to the parent anion exchangers. This means that the chiral recognition for acidic analytes depends

on the AX site and the CX site represented by the sulfonic acid acts as intermolecular counter ion[23].

For basic chiral analytes similar behaviour was observed. Enantioselectivity corresponds to the selectivity of the parent SCX based on *trans*-2-aminocyclohexanesulfonic acid. The retention is reduced because of the intermolecular counter ion activity of the quinine/quinidine scaffold. The elution order of the basic analytes observed with the ZWIX CSP (**Figure 6)** cannot be reversed by changing the configuration of the *trans*-2 aminocyclohexanesulfonic acid. This indicates that the chiral recognition for basic analytes depends on the chiral environment of the quinine/quinidine scaffold and the *trans*-2-aminocyclohexanesulfonic acid has only secondary influence[23].

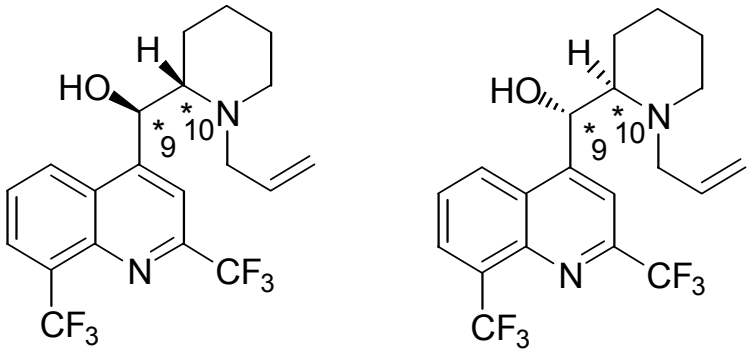
Also amphoteric analytes, like amino acids, can be effectively separated using a ZWIX column (see **Figure 6**). The elution order is not reversed by using the opposite configuration of the *trans*-2-aminocyclohexanesulfonic acid part of the selector. This indicates that the elution order depends on the absolute configuration of the used chinchona-moiety. Therefore by changing the AX unit from quinine to quinidine, the elution order of amphoteric, acidic as well as basic analytes can be reversed[23].

The structure of mefloquine (see **Figure 1**) is closely related to the structure of cinchona derivatives and therefore it is very promising for the development of novel chiral stationary phases. We assumed that the introduction of a π-acidic quinoline unit (caused by electron drawing trifluoromethyl groups) would provide a pronounced $\pi-\pi$ stacking with π-basic analytes. As many pharmaceutical substances are based on π-basic aromatic systems it is of interest having a selector with a π-acidic aromatic counterpart because such a property may lead to a better separation of enantiomers. Using mefloquine as a starting building block, we decided to mimic the structure of 9-O-*tert*-butylcarbamoyl quinine (*t*BuCQN)[29], which is commercially available from Chiral Technologies Europe as Chiralpak QN-AX. Such a selector should work as π-acidic anion exchanger. Moreover, mefloquine can be used for the development of zwitterionic CSP.

1.5 The aim of work

A, Synthesis of enantiomerically pure *N*-allyl mefloquine

Starting from racemic mefloquine we needed to introduce a functionality which allows us to immobilize the selector on the modified mercaptopropyl silica gel. This can be achieved by introduction of a double bond at either the hydroxyl or the secondary amino group. We decided to allylate the nitrogen in the piperidine ring. For effective chiral recognition it is necessary to immobilize the enantiomerically pure selector, and thus the racemic starting compound has to be separated into its enantiomers (**2a**, **2b**). Such compounds can be subsequently immobilized or further functionalized.



2a **2b**

Figure 7: Enantiomers of the (9R,10S)-*N*-allyl mefloquine (2a) and (9S,10R)-*N*-allyl mefloquine (2b)

B, Synthesis of *N*-allyl mefloquine carbamate and evaluation of its properties

For the synthesis of mefloquine-based anion exchangers (**4a**, **4b**) we decided to use a well-established procedure that had been used for the preparation of 9*O*-*tert*-butylcarbomyl quinine (QN-AX)[29].

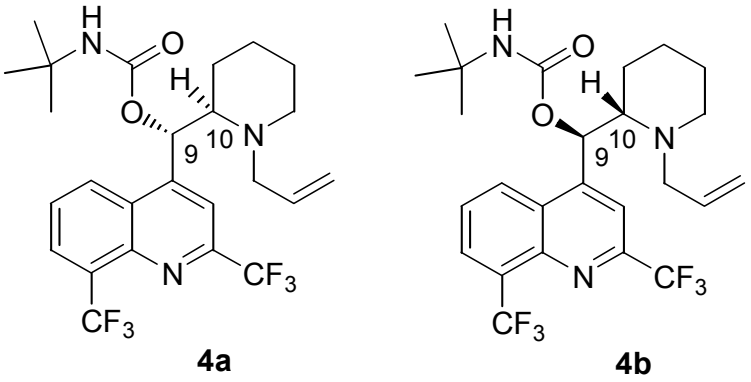


Figure 8: Novel chiral selectors based on mefloquine carbamates

For testing efficiency of our mefloquine-based selector, we decided to carry out an NMR study, which should give us the first insight into molecular recognition properties of the new SO candidate. Furthermore, all immobilized selectors were tested on a wide spectrum of analytes using HPLC in polar organic mode. In order to test the contribution of π-acidic quinoline part of the new selector, specially designed selectands were prepared.

C, Synthesis of novel cation exchanger CSP

In addition to the anion exchanger we decided to develop a novel cation exchanger based on *O*-allyl syringic acid derivatized with *trans*-2-aminocyclohexane-sulfonic acid (**9a**, **9b**), which shall allow an efficient separation of mefloquine derivatives. The derivatized *O*-allyl syringic acid is a π-base and therefore an effective π-π stacking should be possible. Furthermore it represents a strong acid, which protonates tertiary amines and consequently also the nitrogen of the selector.

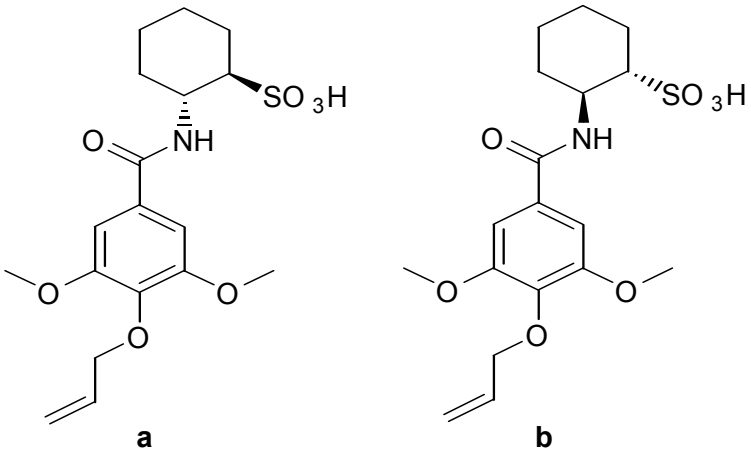


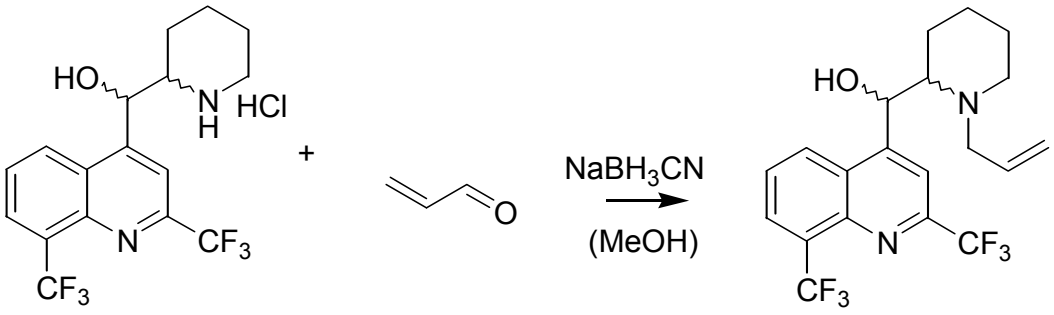
Figure 9: Novel cSCX- CSPs

2. Synthesis

As it was mentioned in the introduction, the main aim of the thesis was to prepare a mefloquine-based analogue of commercially available QN-AX and QD-AX columns. In order to mimic the structure of respective CSPs we decided to synthesize *N*-allyl mefloquine, which should be immobilizable through its double bond on mercaptopropyl-modified silica gel.

2.1 Synthesis of the starting compound: *N*-allyl mefloquine

The easiest way to synthesize tertiary amines out of secondary amines is to employ the Eschweiler Clarke reaction[30]. We decided to use a modified method[31], where sodium cyanoborohydride is used as a source of hydride ion (**Scheme 1**).



1

$C_{17}H_{17}ClF_6N_2O$
Mol. Wt.: 414.77

C_3H_4O
Mol. Wt.: 56.06

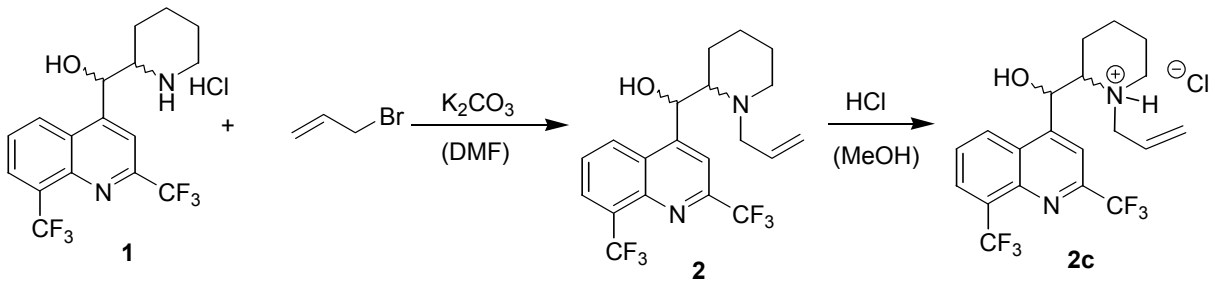
2

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

Scheme 1: Synthesis of *N*-allyl mefloquine using acrolein

Previous results with formaldehyde proved the applicability of this method for the *N*-alkylation of mefloquine. However, acrolein turned out not to be suitable for allylation of compound **1**. The performed LC-MS analysis showed a complex mixture of compounds among which the desired product was not identified. It seems that the reactivity of acrolein is too high under given conditions and rather a kind of oligomerization is preferred instead of *N*-allylation.

Consequently, we decided to use a procedure for *N*-alkylation of mefloquine, which was recently described in the literature[6].



$C_{17}H_{17}ClF_6N_2O$
Mol. Wt.: 414.77

C_3H_5Br
Mol. Wt.: 120.98

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_{20}H_{21}ClF_6N_2O$
Mol. Wt.: 454.84

Scheme 2: *N*-allylation of the mefloquine hydrochloride with allyl bromide and the synthesis of the *N*-allyl mefloquine hydrochloride

At first, the commercially available mefloquine hydrochloride (**1**) was transformed into a free base with K_2CO_3, otherwise the nucleophilic substitution would not work. The nucleophilic weaker oxygen is not allylated in this way and selectively *N*-allyl mefloquine was formed. This reaction was very straightforward and also the work-up of the reaction mixture was elegant because the mixture was only diluted with water and the precipitated product was simply filtered.

The molecular mass of the target compound (**2**) was checked by LC-MS; however, we were not successful in interpretation of NMR spectra. This was caused by high complexity of the proton spectrum and badly distinguished signals in its aliphatic part. Moreover, signals of the piperidine moiety in ^{13}C NMR were completely missing. We assumed that such originally not expected behaviour could be caused by the high flexibility of the piperidine ring. As a result we decided to prepare the *N*-allyl mefloquine hydrochloride with a methanolic solution of hydrogen chloride (see **Scheme 2**). We anticipated that the salt should not be that flexible, because of the intramolecular hydrogen bonding between the hydrogen of the nitrogen with the chlorine atom, and the hydrogen of the hydroxyl group with chlorine as well. The ^1H NMR spectra of compound **2c** were actually more explicit and a full set of signals was obtained for ^{13}C NMR. With the characterization of compound **2c** we proved that the site-selective reaction led to the desired product.

To further characterize compound **2** we separated the enantiomers using chiral HPLC.

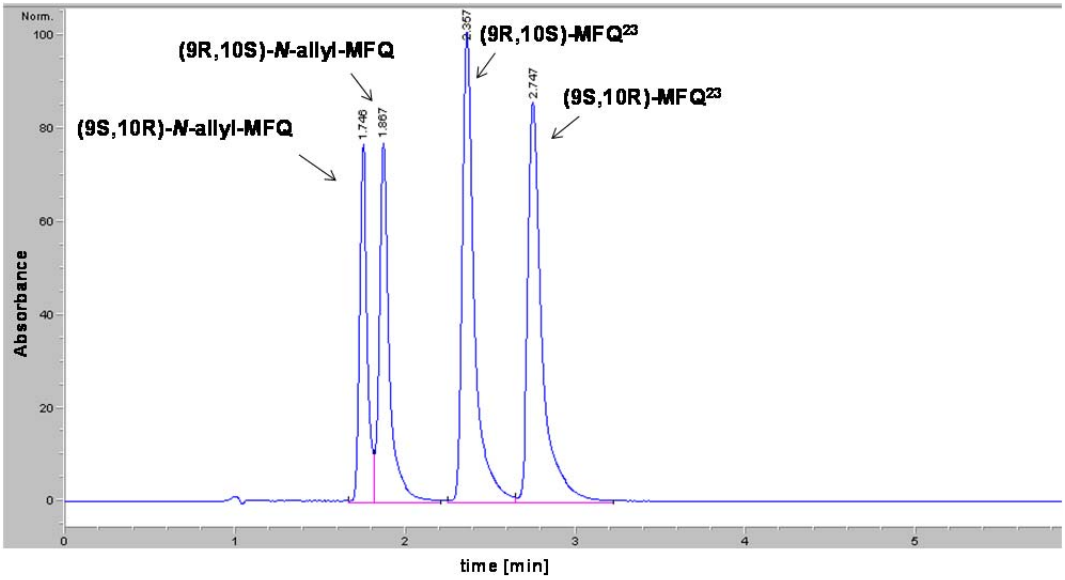
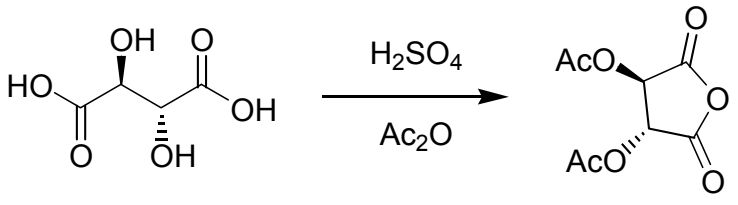


Figure 10: A mixture of mefloquine hydrochloride and *N*-allyl mefloquine (Stationary phase: Tau-QN (SW A11) 15 x 0.3 cm, 3 µm; mobile phase: MeOH, 50 mM AcOH, 25 mM NH_3; wavelength= 254 nm)

Figure 10 shows an HPLC chromatogram of the racemic mixture of compound **1** the mefloquine hydrochloride (t_3= 2.357 and t_4=2.747 min) and **2** the *N*-allyl mefloquine (t_1= 1.746 and t_2=1.867 min), which was obtained when the reaction was terminated prematurely. Even if the reaction was complete according to TLC, it was necessary to crystallize the crude product from methanol in order to remove completely the educt. **Figure 10** further shows that the elution order changes when the secondary amino group of the piperidine moiety of mefloquine hydrochloride is transformed into tertiary amino group. This behaviour is most probably caused by the change of the lipophilicity of the compound.

For the CSPs based on mefloquine it was necessary to receive the enantiomerically pure compounds. Although we tried to modify our conditions in order to obtain better separation of the *N*-allyl mefloquine enantiomers using different columns and mobile phases, we were not successful to achieve α-values feasible for preparative separation. Therefore we decided to derivatize the *N*-allyl mefloquine with (*O*,*O*)-diacetyl-(R,R)-tartaric acid anhydride (DATAAN), in the hope that the diastereomers would be separable via crystallization.

2.1.1 Synthesis of (R,R)-DATAAN



5

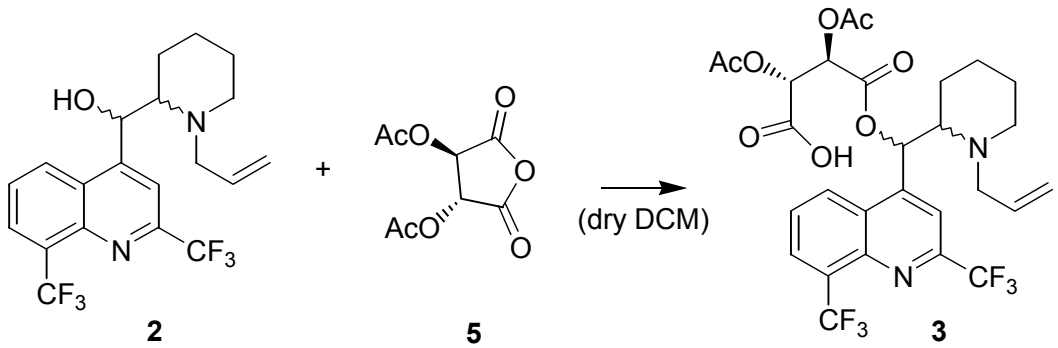
$C_4H_6O_6$
Mol. Wt.: 150.09

$C_8H_8O_7$
Mol. Wt.: 216.14

Scheme 3: Synthesis of (*O*,*O*)-diacetyl-(R,R)-tartaric acid anhydride (DATAAN) starting from the (R,R)--tartaric acid

In the previous work, Professor Lindner had shown the applicability of enantioseparation of alkanolamines by derivatization with (R,R)-DATAAN and following separation of the diastereomers via crystallization[32]. As has already been mentioned, mefloquine is structurally an ethanolamine and thus we decided to use the same procedure.

The synthesis of DATAAN (**Scheme 3**) is described in the literature[33]. (R,R)-tartaric acid, sulfuric acid and acetic anhydride were heated up for a few minutes. The reaction mixture was cooled with an ice bath, the precipitate was filtered and washed with dry benzene and stirred in absolute diethyl ether[33]. In this way, anhydride **5** was obtained as a white crystalline compound in a good yield.



2 **5** **3**

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_8H_8O_7$
Mol. Wt.: 216.14

$C_{28}H_{28}F_6N_2O_8$
Mol. Wt.: 634.52

Scheme 4: Esterification of the *N*-allyl mefloquine with (*O*,*O*)-diacetyl-(R,R)-tartaric acid anhydride

Consequently mefloquine monotartrate **3** was prepared (**Scheme 4**). The reaction was carried out under an inert atmosphere of nitrogen, because DATAAN is very sensitive to air moisture and in the presence of water quickly hydrolyzes to the acid. The reactivity of DATAAN can be checked by simply dissolving it in dichloromethane. The reactive anhydride is soluble while the acid is insoluble.

The separation of the diastereomeres with crystallization turned out to be not as easy as expected. We tried several solvents, like aceton, acetonitrile, ethanol, without and with additives like benzene, ethyl methyl keton and *tert*-butyl methyl ether. In the end we found out that it is possible to separate the diastereomeres with hot toluene, because one of them is soluble while the other is almost insoluble. However, it was necessary to wash the racemic precipitate several times with hot toluene and decant the solvent.

Therefore we employed soxhlet extraction, which was more efficient. The racemic mixture was placed in a soxhlet extractor and continuously washed with hot toluene. After 6 h the crystals were removed from the soxhlet extractor, dissolved in a solution of *tert*-butyl methyl ether and ACN (2:3) and the solvent was evaporated. The solid was again placed in the extractor and the washing with hot toluene was repeated. In this way we were able to obtain the “insoluble” diastereoisomer as crystalline powder in >99% diastereomeric excess. The diastereomer soluble in toluene was obtained by evaporating the solvent. The course of the separation was observed using HPLC (**Figure 11**).

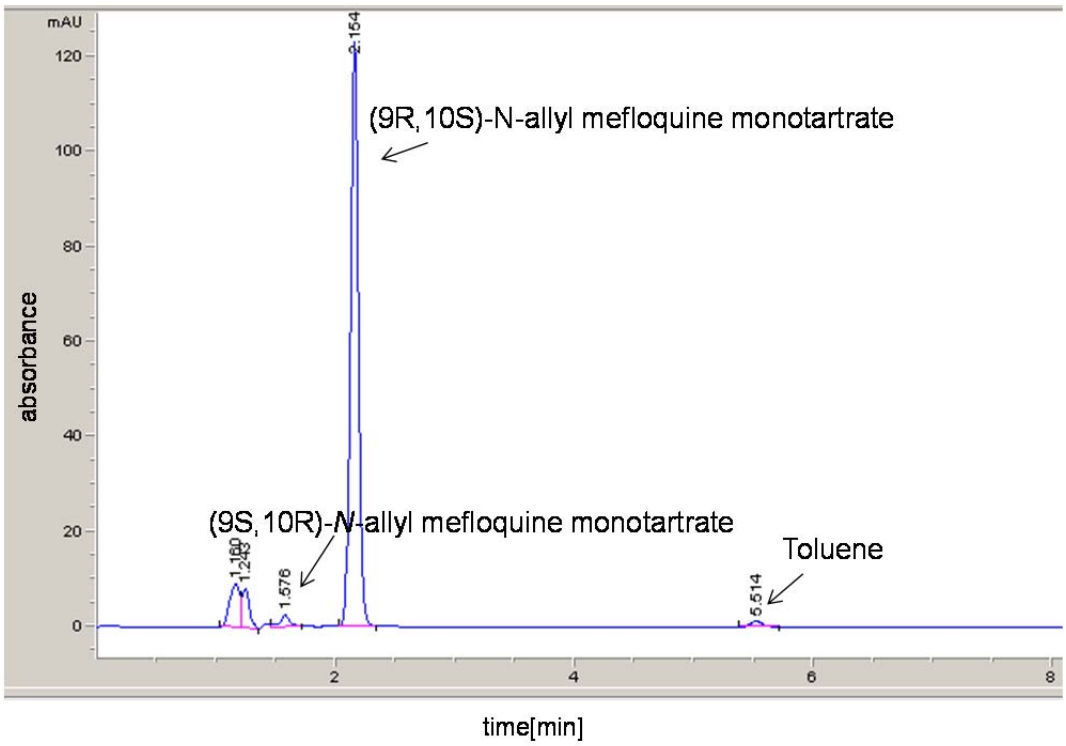


Figure 11: RP-HPLC chromatogram of *N*-allyl mefloquine (*O*,*O*)-diacetyl-(R,R)-tartaric esters after 6 h of soxhlet extraction (ratio 1:32; t_1=1.576 and t_2=2.154) (Mobile phase: 50% H_2O, 0.1% FA; 50% ACN, 0.1% FA; Stationary phase: eclipse XDB-C18 5 µm 4.6 x 150 mm; wavelength =254 nm) (The signal at t = 5.514 corresponds to toluene.)

Figure 11 shows the tartaric ester (t_1=2.154. min) which is insoluble in toluene. This HPLC sample was taken from the residue in the soxhlet extractor after 6 h of extraction. To be certain that the other diastereomer was dissolved in the extraction solution an HPLC measurement was carried out as well.

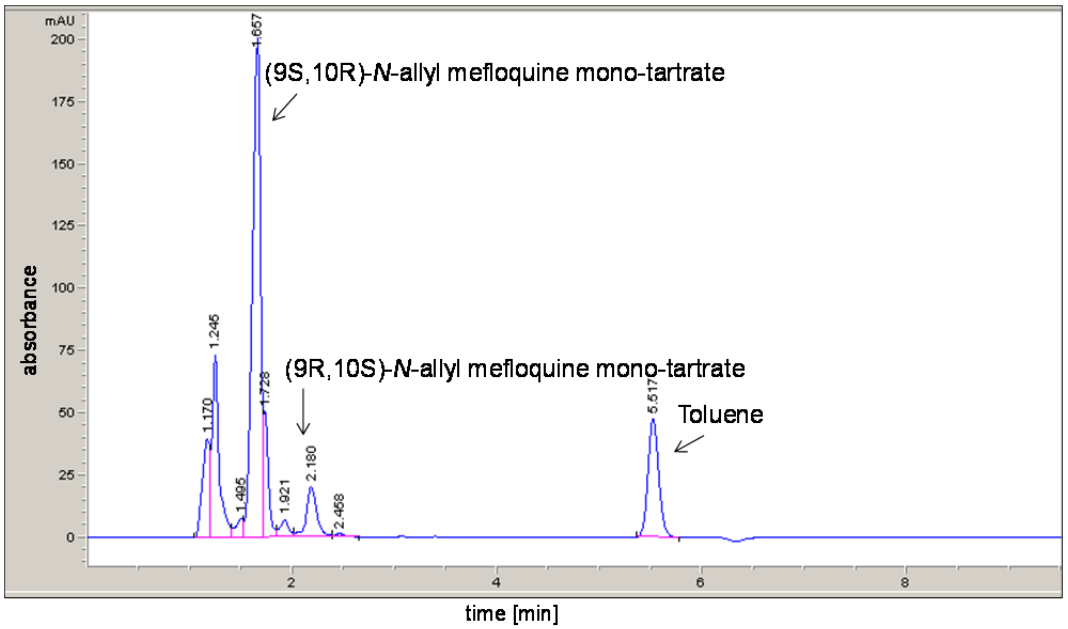


Figure 12: RP-HPLC chromatogram of the soxhlet solution (Mobile phase: 50% H_2O, 0.1% FA, 50% ACN, 0.1% FA Stationary phase: eclipse XDB-C18 5 µm, 4.6 x 150 mm; wavelength = 254 nm)

Figure 12 shows that besides impurities the concentration of the first ester (t_1=1.657 min) is much higher than that of the second one (t_2=2.180 min). In this way of separation of diasteroemers it is not unusual that one diastereomer is obtained totally pure and the second contains impurities of the first one. One has to find then a convenient method for purification of the second diasteroisomer. In our case we were able to find an optimal system for crystallization, and after a single crystallization from methanol we obtained the desired second diastereoisomer in a high purity (>99% de).

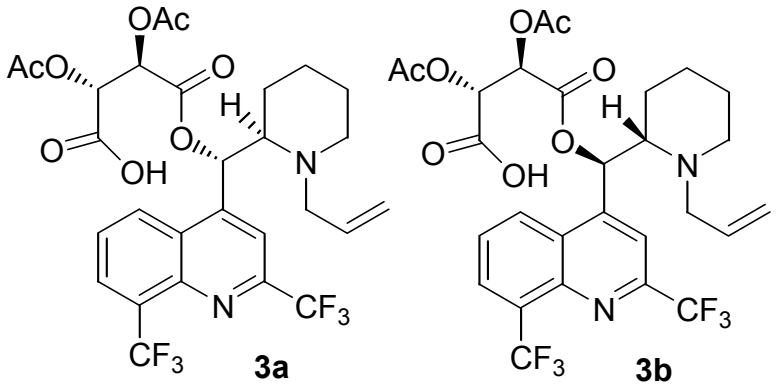
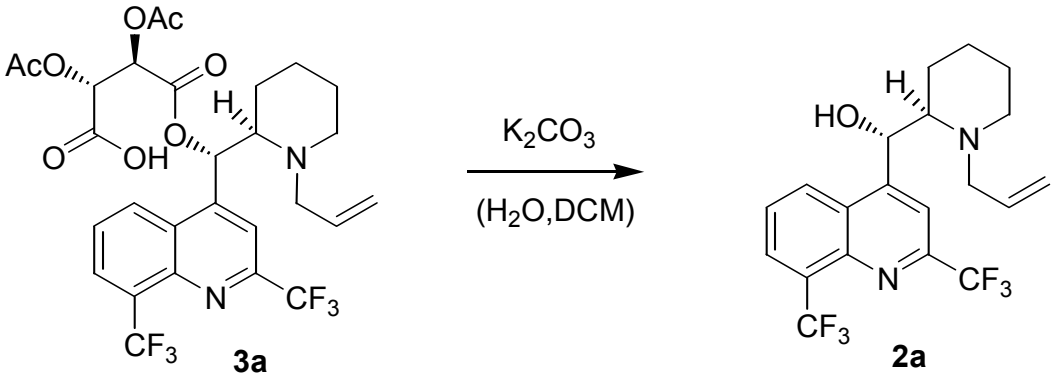


Figure 13: Synthesized diastereomeres by esterification with (R,R)-DATAAN

It is necessary to hydrolyze each of the separated diastereomers to obtain entiomerically pure *N*-allyl mefloquine. The hydrolysis must not affect the absolute configuration of mefloquine, which may happen under strong basic conditions. Therefore we decided to use K_2CO_3 as a mild base[32] (**Scheme 5**). The reaction control was done with TLC by comparing the reaction solution with corresponding standards (the tartaric ester and the *N*-allyl mefloquine). After several hours reaction time only the *N*-allyl mefloquine could be observed. The purification of the product was achieved with column chromatography (CH_2Cl_2/MeOH, 20:1).



$C_{28}H_{28}F_6N_2O_8$
Mol. Wt.: 634.52

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

Scheme 5: Hydrolysis of the (9S,10R)-*O,O*-diacetyl-(R,R)-tartaric mefloquine monoester

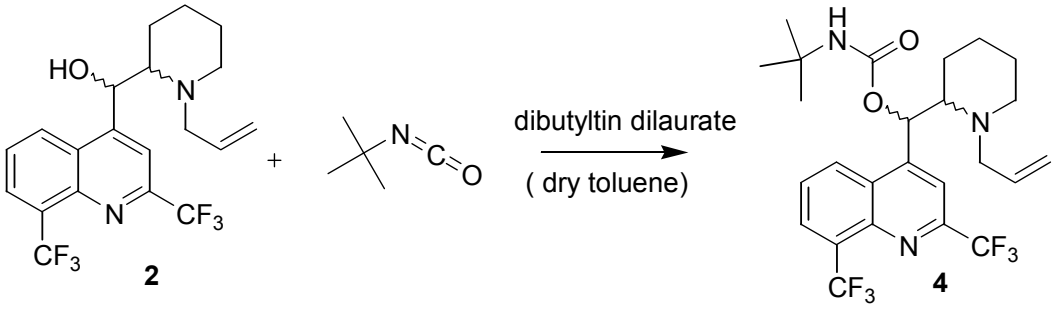
The obtained pure enatiomers of (9S,10R)-*N*-allyl mefloquine (**2a**) and (9R,10S)-*N*-allyl mefloquine (**2b**) were the primary objective of the thesis. With these key compounds it was possible to proceed with immobilization for having a novel CSP

(see **section 2.3**) and these enantiomers were the starting compounds for the design of other selectors and CSPs.

2.2 Preparation of carbamates based on (9S,10R)- and (9R,10S)-*N*-allyl mefloquine

2.2.1 *tert*-butylcarbamoyl-*N*-allyl mefloquine (*t*BuCMFQ)

As it has been mentioned above, the commercially available Chiralpak QN/QD-AX columns based on *t*BuCQN and *t*BuCQD selectors were the main motivation for the synthesis of the *N*-allyl mefloquine analogues.



$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_{25}H_{29}F_6N_3O_2$
Mol. Wt.: 517.51

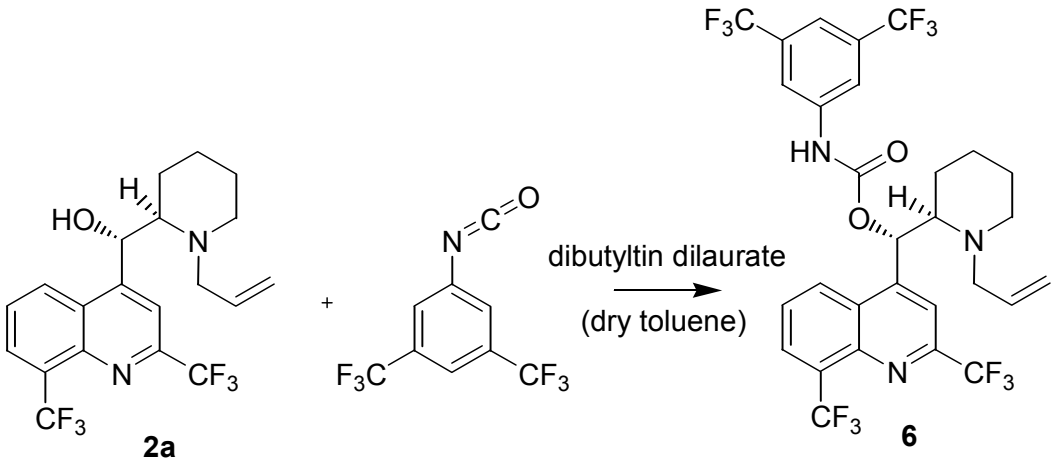
Scheme 6: Synthesis of the *t*BuCMFQ (the chiral bonding is undefined, but the carbamoylation was performed with the pure erythro enantiomers)

Enantiomerically pure **2a** or **2b** reacted with *tert*-butyl isocyanate in a presence of dibutyltin dilaurate as a catalyst (**Scheme 6**). According to a standard operating procedure developed in our working group, the *t*BuCQN/*t*BuCQD started to crystallize immediately after pouring the reaction mixture into petrol ether. Therefore we decided to proceed with purification of the crude *N*-allyl mefloquine carbamate **4** in the same way. Although we tried to cool down the resulting solution of the reaction mixture in petrol ether, no crystals appeared. Subsequently we employed also hexane as a less polar solvent but again no precipitate was formed. Therefore we used column chromatography for final purification of the product. To our surprise it

turned out that both the carbamates are highly viscous liquids and we were able to crystallize the (9S,10R) *t*BuCMFQ only after several weeks.

2.2.2 3,5-Bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine (*N*-allyl-F-CMFQ)

We decided to synthesize the 3,5-Bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine because this compound should have more potent fluorophilic and π-π interaction sites than the *tert*-butylcarbamoyl derivatives **4**. At first, we decided to use the same procedure as for the synthesis of *t*BuCMFQ in hot toluene with a tin catalyst (**Scheme 7**).



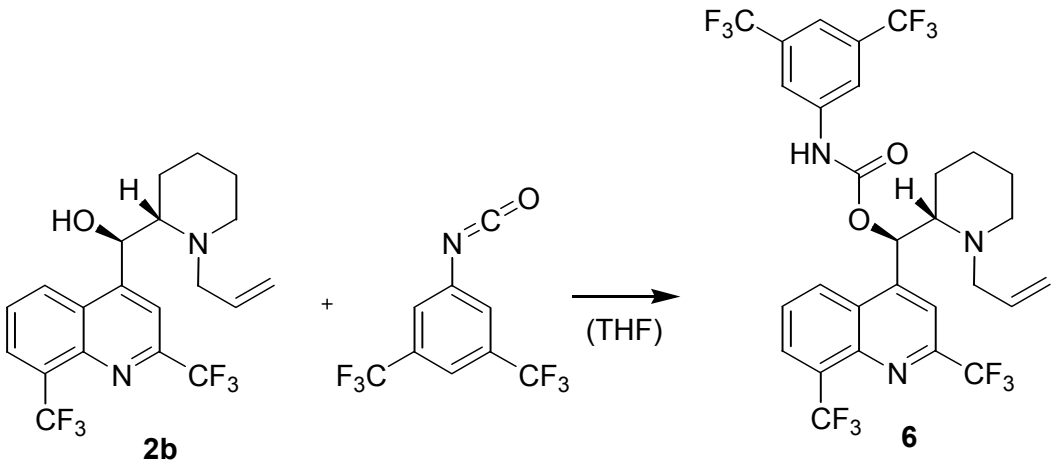
$C_{20}H_{20}F_6N_2O$ Mol. Wt.: 418.38 | $C_9H_3F_6NO$ Mol. Wt.: 255.12 | $C_{29}H_{23}F_{12}N_3O_2$ Mol. Wt.: 673.49

Scheme 7: Synthesis of the 3,5 bis{trifluoromethy}lphenylcarbamoyl-*N*-allyl mefloquine with (9S,10R)-*N*-allyl mefloquine in toluene

In spite of purification with column chromatography the NMR showed a diversity of impurities and it was hard to identify the product. The failure of the reaction could be caused either by reaction conditions, *i.e.* decomposition of isocyanate because of high temperature (boiling toluene), or the isocyanate was already decomposed due to long storage. In order to avoid these problems, we decided to use a new batch of isocyanate and work at reduced temperature.

Before we started this new experiment, we found a different reaction pathway in the literature, which was employed for the synthesis of a chinchona-based organic

catalyst[34]. This approach is based on the use of etheric solvent in which the catalyst is not needed.



2b

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_9H_3F_6NO$
Mol. Wt.: 255.12

6

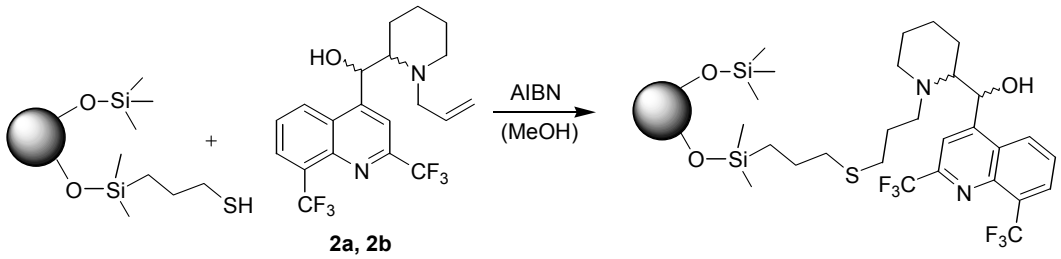
$C_{29}H_{23}F_{12}N_3O_2$
Mol. Wt.: 673.49

Scheme 8: Synthesis of 3,5-bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine with the (9R,10S)-*N*-allyl mefloquine in THF

The reaction was carried out in freshly distilled and dried THF and therefore under nitrogen atmosphere (**Scheme 8**). The course of the reaction was monitored with TLC, and the pure product was obtained by double column chromatography.

2.3 Immobilization of the mefloquine-based selectors

The immobilization should have been with the pure enantiomers of *N*-allyl mefloquine. The standard operating procedure of the immobilization (**Scheme 9**) was developed in our working group[35]. However, it turned out that the coverage of the selector on silica gel was low using the standard conditions.



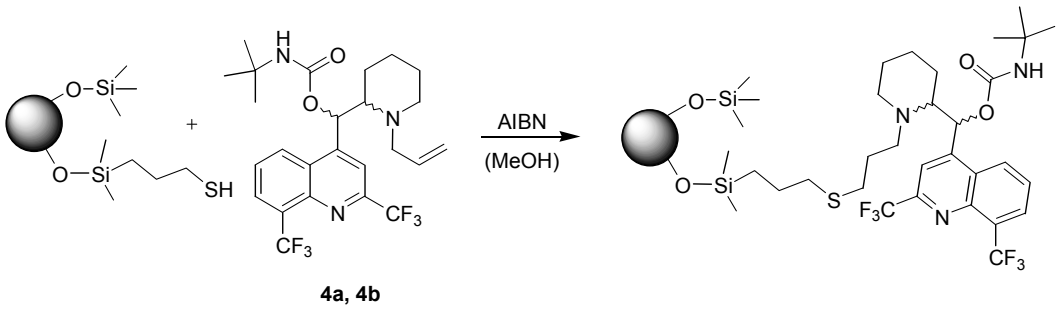
Scheme 9: Immobilization of *N*-allyl mefloquine (the stereocenter is not specified, but the immobilization was carried out with the pure erythro enantiomers)

Therefore we decided to perform a set of reactions with racemic *N*-allyl mefloquine using different reaction conditions and to save the enantiomerically pure compound for optimized immobilization. To improve the coverage, the amount of the radical starter was increased, the solvent amount was decreased and the reaction times were modified. It is known from previous experiments in our working group that the coverage of a selector on silica gel depends on the used amount of solvent, the less the better becomes the coverage. Moreover, the selector immobilized on silica gel is sensitive towards scratching. Therefore a certain carefulness is required by all mechanical manipulations like washing of the CSP, otherwise it can happen that the selector is removed. Although all these influences were considered and the handling with CSP was careful, the coverage was not improved. The coverage was determined by elemental analysis and the first estimation was done by comparing the selector weight in the washing solution with the amount used for immobilization. When the tare of the selector obtained by evaporation of the washing solutions was similar to the amount used for immobilization no elemental analysis was performed.

Table 1: Optimization trials for the immobilization of mefloquine based selectors with 3 g of mercaptopropyl modified silica gel.

	selector [mg]	MeOH [mL]	AIBN [mol%]	immobilization time [h]
1	500	40	20	14
2	500	40	25	14
3	500	30	40	16
4	500	25	40	8
5	500	25	20	8

For immobilization of *N*-allyl *t*BuCMFQ 500 mg of the selector per 3 g of mercaptopropyl modified silica gel, in 30 mL of MeOH with 20 mol% of AIBN was used. After 6 h immobilization time further 10 mg of AIBN were added to the immobilization suspension under increased nitrogen flow. The solution was stirred further for another 12 h.

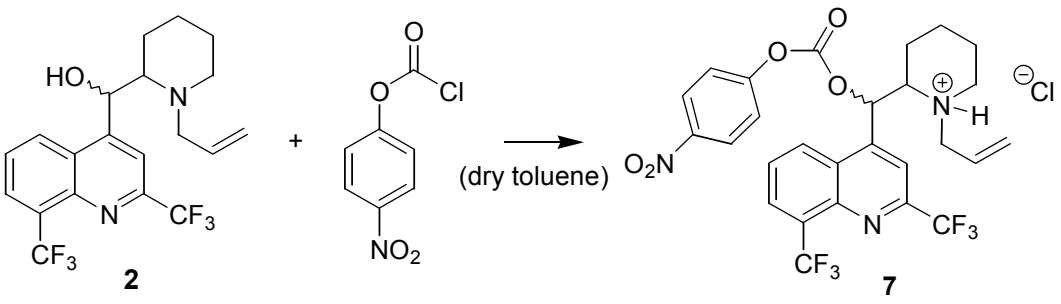


4a, 4b

Scheme 10: Immobilization of *N*-allyl mefloquine carbamate (the stereocenter is not specified, but the immobilization was done with the pure erythro enantiomer)

2.4 Synthesis of the ZWIX CSP based on mefloquine

One of the aims of the thesis was the synthesis of a zwitterionic chiral stationary phase based on mefloquine derivatized with *trans*-2-aminocyclohexanesulfonic acid. Such a CSP would be a π-acidic but also fluorophilic analogue to the known ZWIX phase based on quinine[23]. For the synthesis of the zwitterionic chiral stationary phase of quinine it is necessary to activate the starting compound with *p*-nitrophenyl chloroformate. Consequently, we decided to use this described procedure for mefloquine and employ *p*-nitrophenyl chloroformate as an activating group[23].



$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

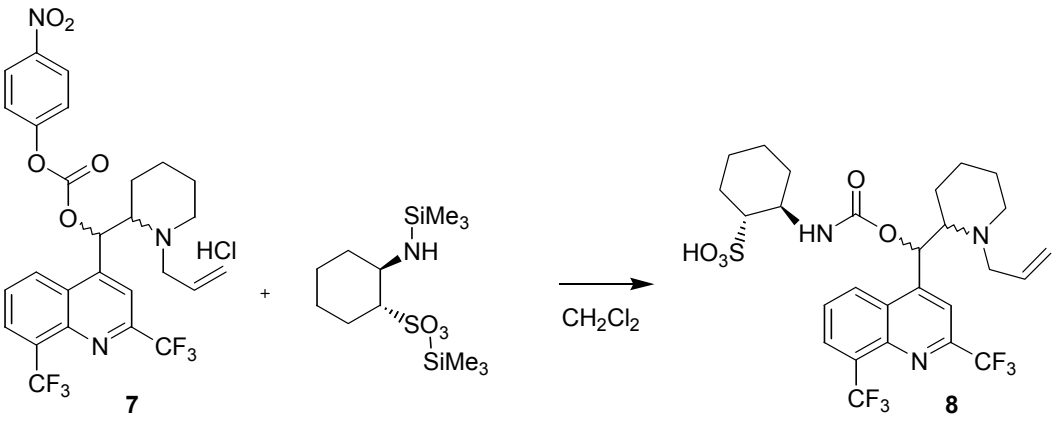
$C_7H_4ClNO_4$
Mol. Wt.: 201.56

$C_{27}H_{24}ClF_6N_3O_5$
Mol. Wt.: 619.94

Scheme 11: Synthesis of the active ester of the racemic *N*-allyl mefloquine

In case of QN or QD a quantitative yield of the active ester is usually obtained. Although a white precipitate was formed, NMR measurements revealed a complex mixture of products. The purification of the crude product with column chromatography afforded only *p*-nitophenol and mefloquine.

Nevertheless, the synthesis of the carbamate with *trans*-aminocyclohexylsulfonic acid was tried. Therefore we decided to carry out the derivatization of protected *trans*-2-aminosulfonic acid with the crude active ester.



$C_{27}H_{23}F_6N_3O_5$
Mol. Wt.: 583.48

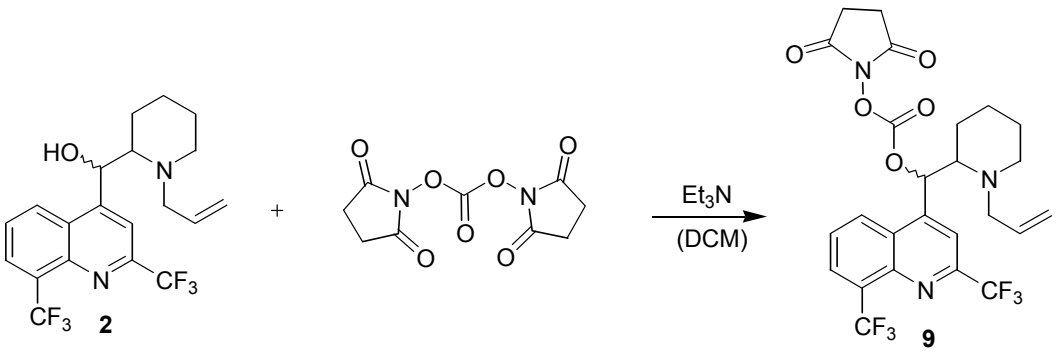
$C_{12}H_{29}NO_3SSi_2$
Mol. Wt.: 323.60

$C_{27}H_{31}F_6N_3O_5S$
Mol. Wt.: 623.61

Scheme 12: Synthesis of a zwitterionic selector based on mefloquine

The reactants were stirred for 3 days in dry DCM under nitrogen atmosphere. The course of the reaction was monitored with HPLC using a weak zwitterionic exchanger CSP. No change, i.e. formation of the product, during the reaction was found.

Because the synthesis of compound **7** through the activation with *p*-nitophenyl chloroformate did not work satisfactory, we decided to try an esterification with disuccinimidyl carbonate. We assumed that the formed activated ester **9** would react with the *trans*-2-aminocyclohexanesulfonic acid to, compound **7**.



$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_9H_8N_2O_7$
Mol. Wt.: 256.17

$C_{25}H_{23}F_6N_3O_5$
Mol. Wt.: 559.46

Scheme 13: Synthesis of the NHS- ester with racemic *N*-allyl mefloquine

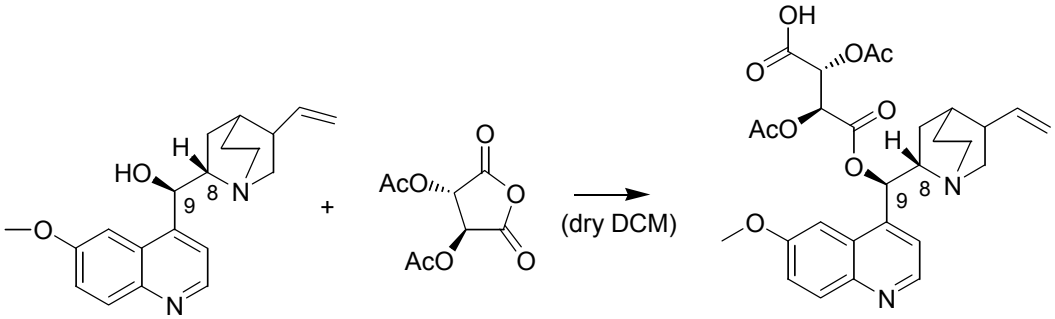
However, even this way did not work. A possible solution could be the use of another catalyst or the reaction of mefloquine with the corresponding isocyanate of the *trans*-aminocyclohexanesulfonic acid without the intermediate stage of the succinimide ester.

2.5 Analysis of the elution order of *N*-allyl mefloquine enantiomers with HPLC

The absolute configuration[11] and the elution order of free mefloquine are well known[23]. However, modification of the basic site of the molecule can cause a subtle change in the nature of a compound and can lead to the reversal of the elution order[23,29]. Therefore we decided to derivatize quinine and quinidine, with diacetyl-tartaric esters and compare the elution order of them with the one of the *N*-allyl mefloquine analogues.

Surprisingly, the cinchona derivatives were in this case less reactive than mefloquine. A huge excess of the (*O*,*O*)-diacetyl-(R,R)-tartaric anhydride was needed for the

reaction (**Scheme 14**). However, the chromatograms showed that just a little amount reacted to the desired monoesters **11**. There was also a possibility that the product partially hydrolyzed under the conditions of the HPLC measurement and therefore only a small amount of expected compound was detected.



10 **5** **11**

$C_{20}H_{24}N_2O_2$ Mol. Wt.: 324.42

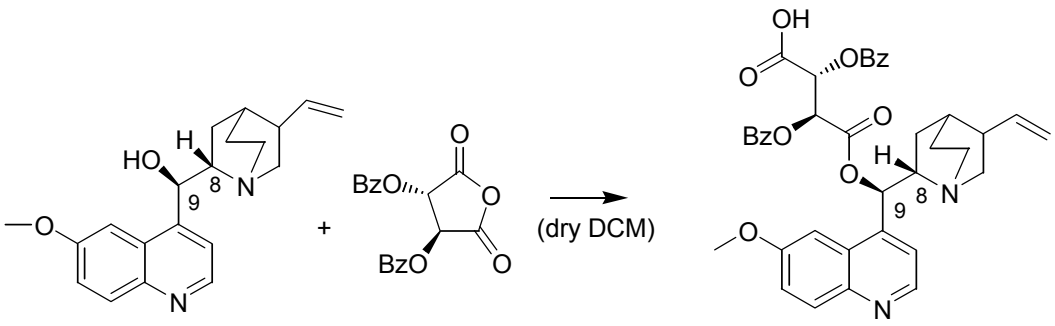
$C_8H_8O_7$ Mol. Wt.: 216.14

$C_{28}H_{32}N_2O_9$ Mol. Wt.: 540.56

Scheme 14: Synthesis of the (8S,9R) quinine (*O*,*O*)-diacetyl-(R,R)-tartaric monoester, the same procedure was used for the derivatization of (8R,9S) quinidine

Because the (R,R)-DATAAN approach was not successful, we decided to try the derivatization of the chinchona derivatives (**Scheme 15**) and *N*-allyl mefloquine (**Scheme 16**) with (*O*,*O*)-dibenzoyl-(R,R)-tartaric acid anhydride. We assumed that the so called DBTAAN would react slower but the stability of the resulting derivatives would be higher in comparison to (R,R)-DATAAN derivatives.

The synthesis of the chinchona derivatives with DBTAAN showed better results (**Scheme 15**) than the esterification with (R,R)-DATAAN. Nevertheless, DBTAAN had to be added in excess[15]. Although a total conversion of the chinchona derivatives to the corresponding esters was not achieved, the amount of the esters was sufficient for the safe determination of the elution order. The reaction control was done with TLC; however, the product was not isolated and the reaction mixture was submitted directly to the HPLC measurement.



10

$C_{20}H_{24}N_2O_2$
Mol. Wt.: 324.42

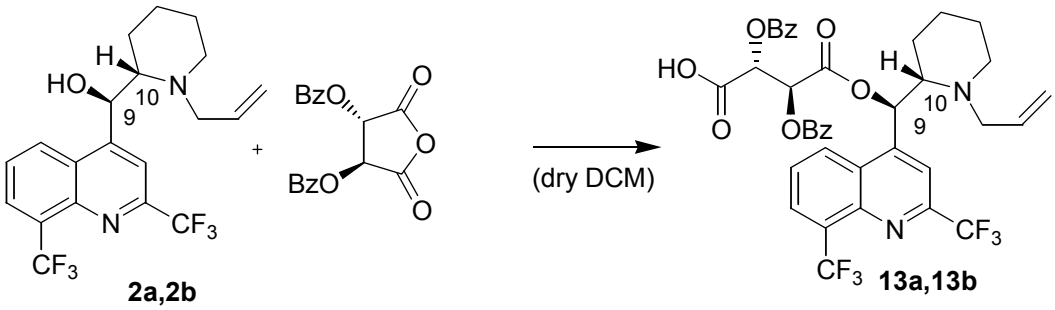
$C_{18}H_{12}O_7$
Mol. Wt.: 340.28

12

$C_{38}H_{36}N_2O_9$
Mol. Wt.: 664.70

Scheme 15: Synthesis of the (8S,9R) quinine (*O*,*O*)-dibenzoyl-(R,R)-tartaric monoester, the same procedure was used for the derivatization of (8R,9S) quinidine

N-Allyl mefloquine seems to react much better with both (R,R)-DATAAN and (R,R)-DBTAAN in comparison to the chinchona derivatives (see analytical part, section assignment of elution order of mefloquine). The product was not isolated and it was directly submitted to HPLC measurements.



2a,2b

$C_{20}H_{20}F_6N_2O$
Mol. Wt.: 418.38

$C_{18}H_{12}O_7$
Mol. Wt.: 340.28

13a,13b

$C_{38}H_{32}F_6N_2O_8$
Mol. Wt.: 758.66

Scheme 16: Reaction of the (*O*,*O*)-dibenzoyl-(R,R)-tartaric acid anhydride with (9R,10S)-*N*-allyl mefloquine, the same procedure was used for the derivatization of the (9S,10R)-*N*-allyl mefloquine

2.6 Synthesis of analytical probes used for the evaluation of the mefloquine-based CSPs

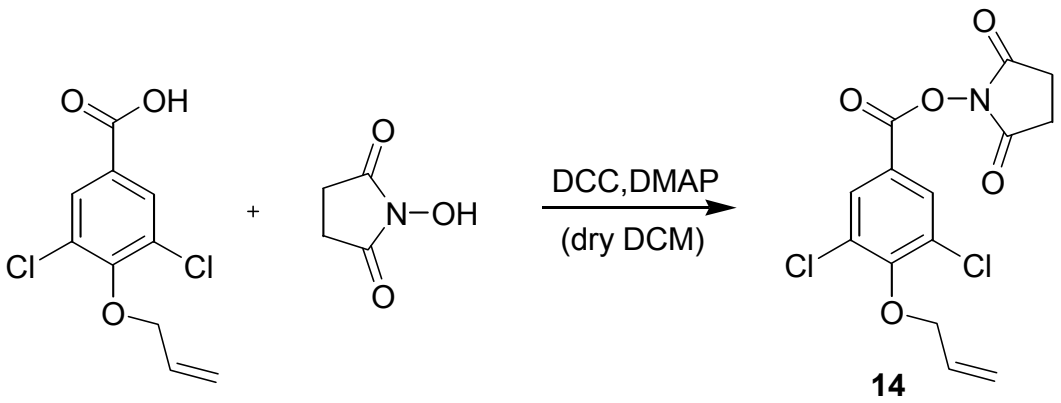
The novel mefloquine-based chiral stationary phases have been tested for their applicability toward enantioselective separation. For that purpose chiral analytes were necessary. Some of them were commercially available and some were

synthesized by us. Especially analytes with a π-basic aromatic system were of interest because of the assumed enhanced π–π interactions with π-acidic but also fluorophilic quinoline ring of mefloquine.

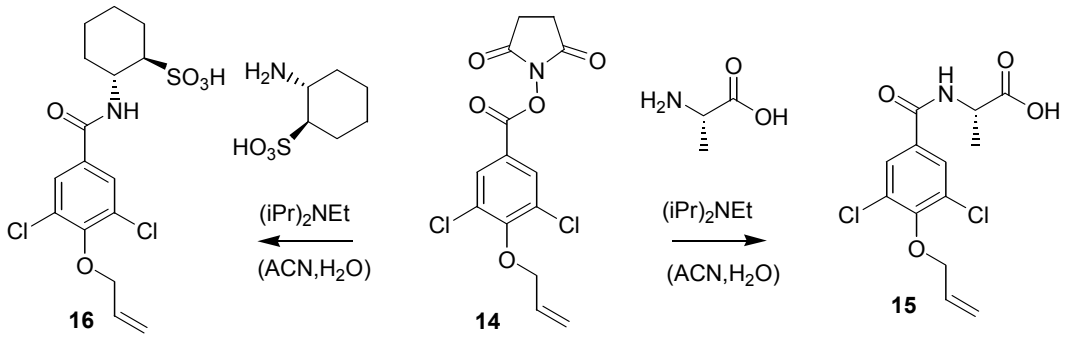
2.6.1 Derivatives of the 4-allyloxy-3,5-dichlorobenzoic acid

SCX (**16**) CSP previously developed in our working group is based on 4-(allyloxy)-3,5-dichlorobenzoic acid. It was found that mefloquine can be efficiently separated on this CSP[36], and thus we supposed that the 4-(allyloxy)-3,5-dichlorobenzoic acid is a useful starting compound for the design of analytes for the evaluation of mefloquine-based CSPs as well.

Except for the described strong cation exchanger, we decided to prepare also a derivative of a proteinogenic amino acid. Hence we employed Steglich esterification of the 4-(allyloxy)-3,5-dichlorobenzoic acid with hydroxysuccinimide (**Scheme 17**) in order to prepare an active ester which can be easily derivatized with amino acids (**Scheme 18**) according to a procedure described in the literature[37].



Scheme 17: Synthesis of the NHS-ester of the linker acid



$C_{16}H_{19}Cl_2NO_5S$
Mol. Wt.: 408.30

$C_{14}H_{11}Cl_2NO_5$
Mol. Wt.: 344.15

$C_{13}H_{13}Cl_2NO_4$
Mol. Wt.: 318.15

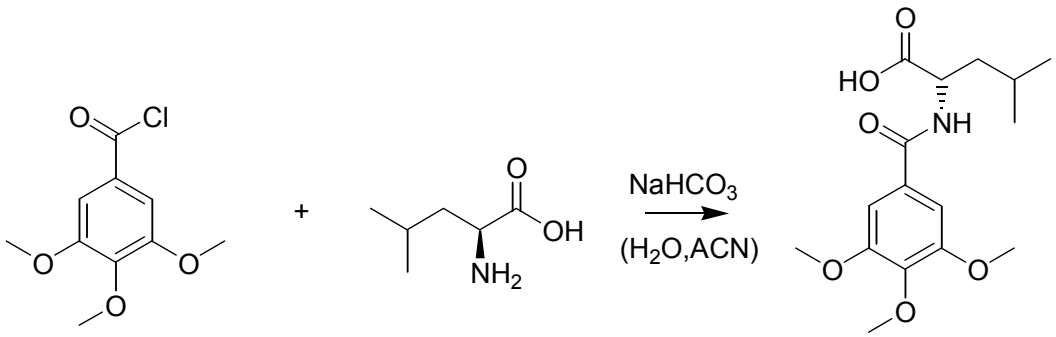
Scheme 18: Derivatization of the NHS ester of the 4-(allyloxy)-3,5-dichlorobenzoic acid with *trans*-2-aminocyclohexanesulfonic acid and L-alanine

The activated 4-(allyloxy)-3,5-dichlorobenzoic acid and L-alanine or the *trans*-2-aminocyclohexanesulfonic acid reacted in aqueous acetonitrile in the presence of Hünig base to the corresponding amide. It has to be noted that the isolation of the products via extraction is not an easy task. Especially the aminosulfonic acid derivative was highly soluble in water and therefore we used continuous extraction for its isolation. This synthetic pathway was quite efficient and allowed us to prepare the strong cation exchanger type selector. The yield of the target compound using our synthetic procedure turned out to be higher than that of the published synthesis[36].

2.6.2 Derivatives of the 3,4,5-trimethoxybenzoic acid

The derivatives of the 3,4,5-trimethoxybenzoic acid represent analytes for the evaluation of the mefloquine-based chiral stationary phases. The electron rich aromatic system of the acid should allow an effective $\pi-\pi$ stacking with electron deficient quinoline core of mefloquine.

An easy way for the synthesis of an amide is the Schotten-Baumann reaction between an acid chloride and an amino acid. This reaction is usually performed in a buffered mixture of polar organic solvent and water. We used commercially available 3,4,5-trimethoxybenzoic acid chloride which reacted with leucine in 50% aqueous acetonitrile with sodium hydrogen carbonate as a base[30] (**Scheme 19**).



17

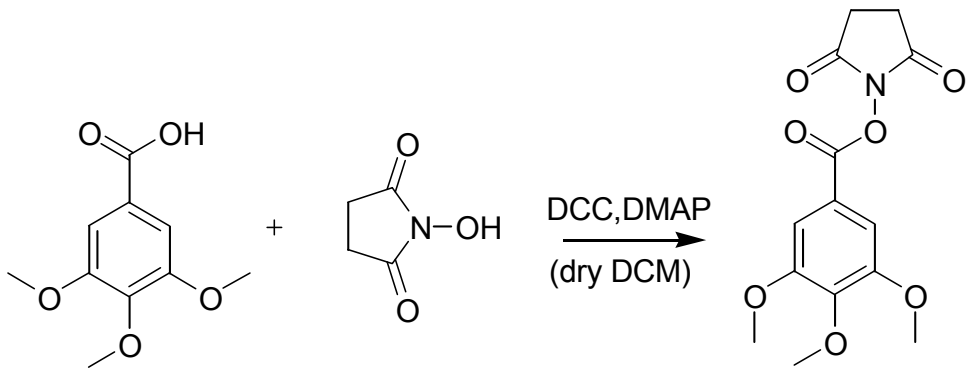
$C_{10}H_{11}ClO_4$
Mol. Wt.: 230.64

$C_6H_{13}NO_2$
Mol. Wt.: 131.17

$C_{16}H_{23}NO_6$
Mol. Wt.: 325.36

Scheme 19: Amidation of 3,4,5-trimethoxybenzoic acid with L-leucine

However, the Schotten-Baumann reaction did not turn out to be the right option for the synthesis of **17**. One possible explanation is a bad quality of used chloride, which was stored in the laboratory for some time. Therefore we decided to completely hydrolyze this old material and transform the obtained acid into an active ester. For this purpose we again employed the Steglich esterification which proved to be efficient for the synthesis of the "linker acid" active ester. In this case 3,4,5-trimethoxybenzoic acid reacted with *N*-hydroxysuccinimide in a DCC mediated, DMAP catalyzed esterification (**Scheme 20**). The corresponding active ester **18** was obtained in good yield.



18

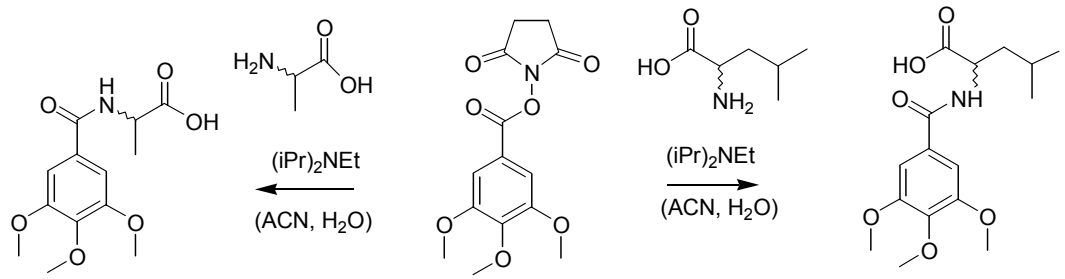
$C_{10}H_{12}O_5$
Mol. Wt.: 212.20

$C_4H_5NO_3$
Mol. Wt.: 115.09

$C_{14}H_{15}NO_7$
Mol. Wt.: 309.27

Scheme 20: Esterification of the 3,4,5-trimethoxybenzoic acid with *N*-hydroxysuccinimide

The active ester **18** was further derivatized with L-alanine, L/D-alanine, L-leucine and D-leucine (**Scheme 21**).



19

$C_{13}H_{17}NO_6$
Mol. Wt.: 283.28

18

$C_{14}H_{15}NO_7$
Mol. Wt.: 309.27

20a,20b

$C_{16}H_{23}NO_6$
Mol. Wt.: 325.36

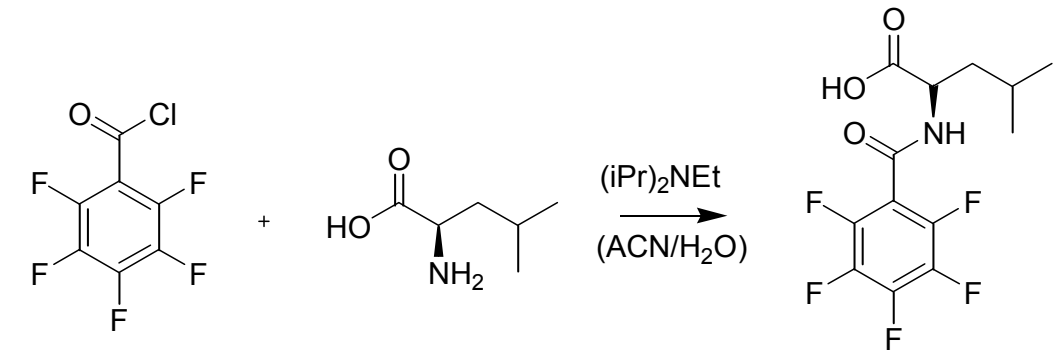
Scheme 21: Syntheses of analytes based on 3,4,5-trimethoxybenzoic acid for the evaluation of the mefloquine based CSPs

All analytes were prepared as enantiomerically pure compounds. 3,4,5-trimethoxybenzoyl L- and D-leucine represent π-basic analytes which we designed as the first choice selectands for π-acidic mefloquine-based CSPs. Moreover, these SAs were further used for NMR study with *t*BuCMFQ, where we evaluated the chiral recognition properties of the novel selector.

2.6.3 Derivatives of the pentafluorobenzoic acid

The two trifluoromethyl groups of mefloquine open another option in a possible application of mefloquine-based selectors. We decided to design and synthesize analytes with a high mass percentage rate of fluorine to determine a possible effect of fluorophilic interactions between an analyte and the potential selector. We chose *N*-pentafluorobenzoyl (L)- and (D)-leucine as appropriate selectands.

The Schotten-Baumann reaction with pentafluorobenzoic acid chloride and D-leucine turned out not to be the best choice again (**Scheme 22**). Simple monitoring of the reaction by TLC showed a slow progress but checking the product with HPLC revealed a mixture of compounds. It seems that under given conditions the chloride hydrolyzed faster than it reacted with the amino group of leucine.



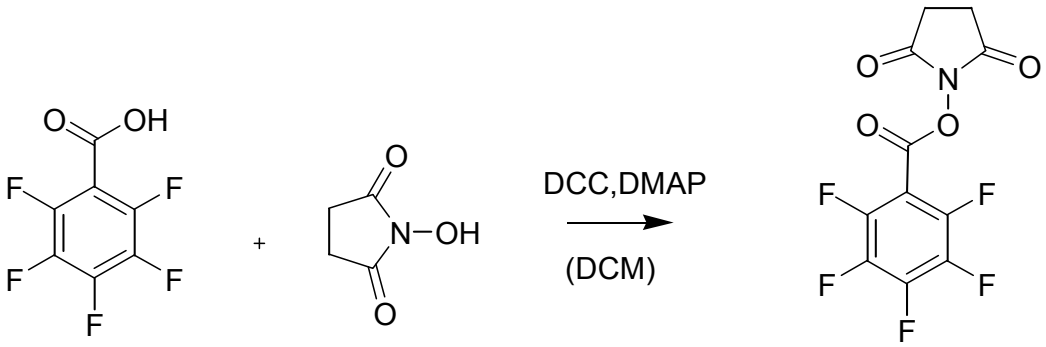
C_7ClF_5O
Mol. Wt.: 230.52

$C_6H_{13}NO_2$
Mol. Wt.: 131.17

$C_{13}H_{12}F_5NO_3$
Mol. Wt.: 325.23

Scheme 22: Schotten-Baumann reaction of the pentafluorobenzoic acid chloride with L-leucine

The *N*-succinimidyl pentafluorobenzoic acid ester was synthesized in the same way as the other succinimide active esters (**Scheme 23**).



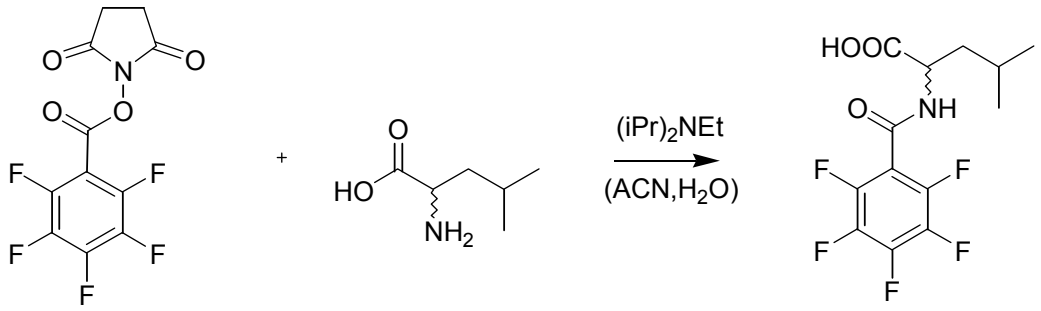
$C_7HF_5O_2$
Mol. Wt.: 212.07

$C_4H_5NO_3$
Mol. Wt.: 115.09

$C_{11}H_4F_5NO_4$
Mol. Wt.: 309.15

Scheme 23: Synthesis of the NHS- ester based on pentafluorobenzoic acid

The synthesis of target pentafluorobenzoyl L-and D-leucine proceeded in the same way as for the other analytes (**Scheme 25**).



22

$C_{11}H_4F_5NO_4$
Mol. Wt.: 309.15

$C_6H_{13}NO_2$
Mol. Wt.: 131.17

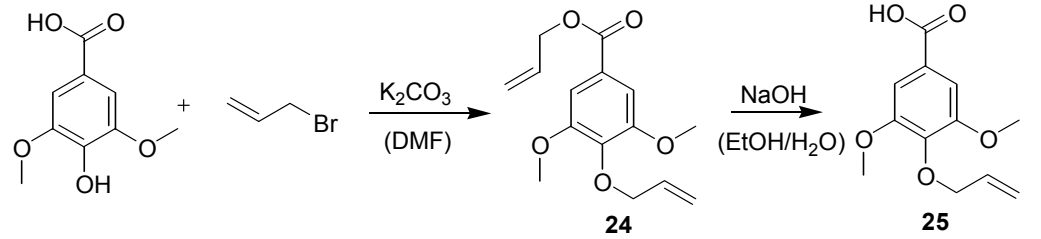
23a, 23b

$C_{13}H_{12}F_5NO_3$
Mol. Wt.: 325.23

Scheme 24: Amidation of the active ester of pentafluorobenzoic acid with D- or L-leucine (the stereocenter is not defined, but the synthesis was carried out with the enantiomerically pure amino acids)

2.7 Synthesis of a strong cation exchanger based on *O*-allyl syringic acid

The *O*-allyl syringic acid was chosen for the synthesis of a strong cation exchanger, a π–basic counterpart to the SCX CSP (**16**) based on the π–acidic linker acid developed in our working group[37].



24

25

$C_9H_{10}O_5$
Mol. Wt.: 198.17

C_3H_5Br
Mol. Wt.: 120.98

$C_{15}H_{18}O_5$
Mol. Wt.: 278.30

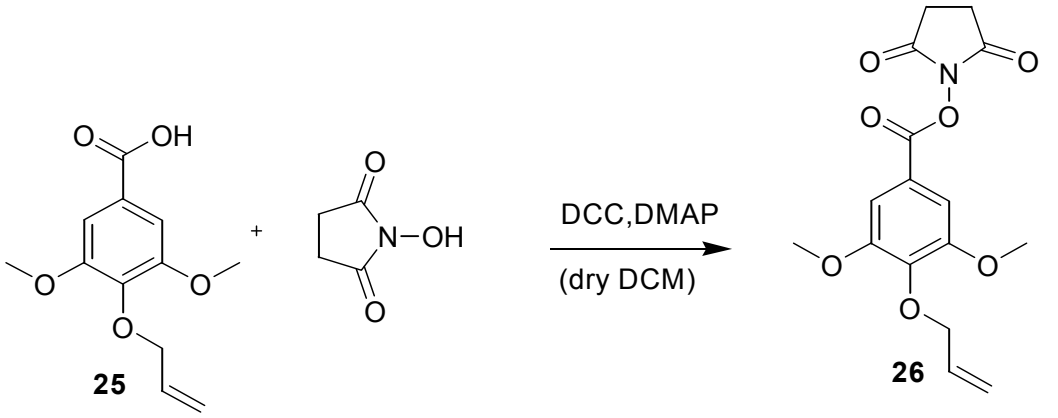
$C_{12}H_{14}O_5$
Mol. Wt.: 238.24

Scheme 25: Synthesis of the *O*-allyl syringic acid

At first, we used the standard allylation reaction with allyl bromide and potassium carbonate as a base in boiling acetone. This reaction was, however, too slow and thus after 2 days we added DMF in order to evaluate the influence of the better dipolar aprotic solvent. The reaction was finished in several hours and therefore the second reaction was carried out in DMF as a solvent (**Scheme 25**). The reaction mixture was heated up 60 °C in an oil bath and the reaction was accomplished in several hours.

Allyl 4-allyloxy-3,5-dimethoxybenzoate (**24**) could be easily transformed to the carboxylic acid **25** by hydrolysis. The basic reaction mixture was heated up, afterwards ice cooled and acidified. During this reaction only the ester function was affected not the alkoxy groups, which are stable under basic conditions.

The active ester of *O*-allyl syringic acid was then the starting compound for the synthesis of the SCX. For its synthesis we decided to use the well-proven Steglich esterification with the *O*-allyl syringic acid, *N*-hydroxysuccinimide, the coupling reagent DCC and DMAP as a base (**Scheme 26**).



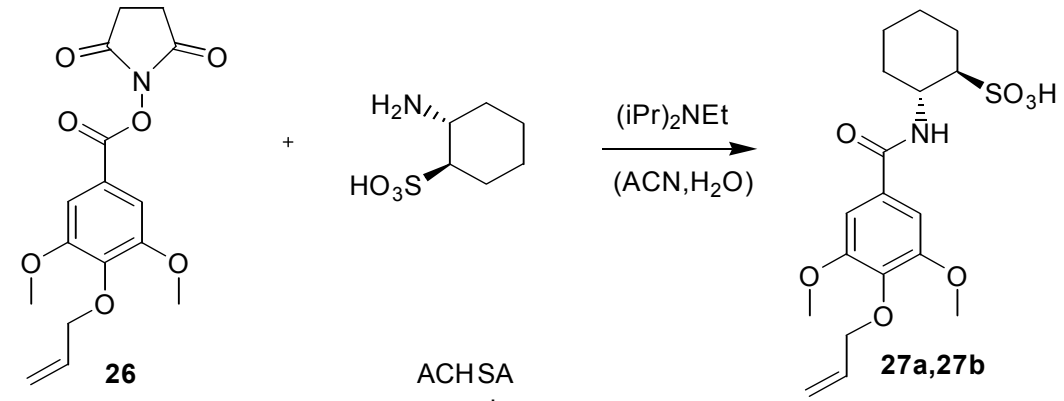
$C_{11}H_{11}O_5^{\bullet}$
Mol. Wt.: 223.20

$C_4H_5NO_3$
Mol. Wt.: 115.09

$C_{17}H_{19}NO_6$
Mol. Wt.: 333.34

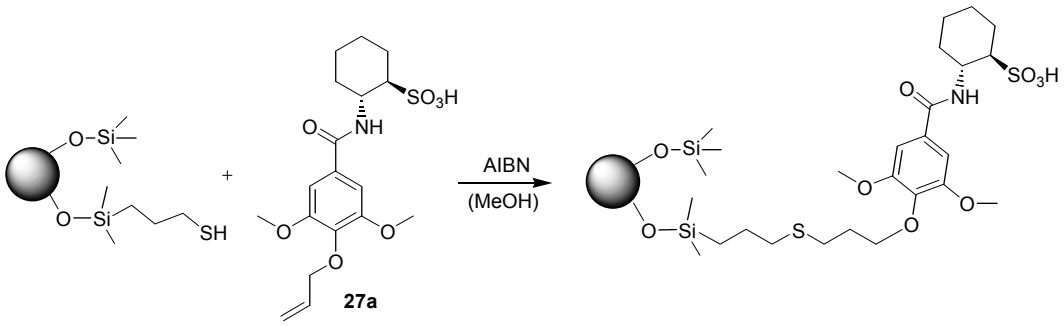
Scheme 26: Steglich esterification of the *O*-allyl syringic acid

We decided to perform the synthesis with racemic aminosulfonic acid (ACHSA) because the product can be easily enantioseparated. Therefore the activated carboxylic acid was derivatized with *trans*-2-aminocyclohexanesulfonic acid and the racemic strong cation exchanger was obtained (**Scheme 27**).



Scheme 27: Synthesis of a strong cation exchanger based on *O*-allyl syringic acid

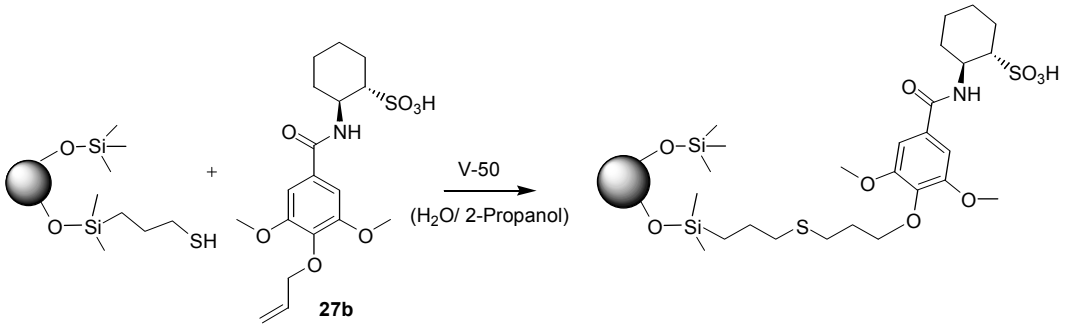
After the separation of enantiomers, which was performed on semipreparative column, the enantiomerically pure selector was immobilized on a 5 μm mercaptopropyl modified silica gel. The immobilization was done in methanol, with AIBN and under nitrogen atmosphere. In comparison to previously prepared SCX, the coverage was slightly lower calculated by the nitrogen content (see **experimental part**).



Scheme 28: Immobilization of the SCX CSP based on *O*-allyl syringic acid with AIBN

However, the column shows great performances for the separation of basic amines and the mefloquine derivatives (see **analytical part**).

In order to achieve a better coverage we changed the conditions for the immobilization. We employed a different radical starter which can be used in aqueous isopropanol. We supposed that the higher solubility of the selector in this solvent mixture would lead to a better reactivity and thus higher coverage.



Scheme 29: Immobilization of the SCX CSP based on *O*-allyl syringic acid with V-50

Unfortunately a better coverage was not obtained.

3. Analytical Part

3.1 Determination of the elution order of *N*-allyl mefloquine

For the separation of racemic *N*-allyl mefloquine diacetyl mono-tartrates were synthesized. Therefore we decided to functionalize quinine and quinidine, which are structurally related to mefloquine, with (R,R)-DATAAN as well. Although, QN/QD are usually called pseudoenantiomers they are in fact diastereoisomers. This can clearly be seen in **Figure 14** where both QN and QD are separated on non-chiral stationary phase.

The elution order of the quinine and quinidine derivatives could be compared with the elution order of the diacetyl monotartrates of MFQ. However, the reaction of QN and QD with (R,R)-DATAAN did not yield the expected product in a sufficient amount. This might have been caused either by lower activity of (R,R)-DATAAN, which is not very stable under long storage, or by hydrolysis of the product. Finally, we accomplished the derivatization of QN/QD as well as mefloquine with (+)-(*O*,*O)*-dibenzoyl-(R,R)-tartaric anhydride, which yielded more stable monoesters of respective compounds[32].

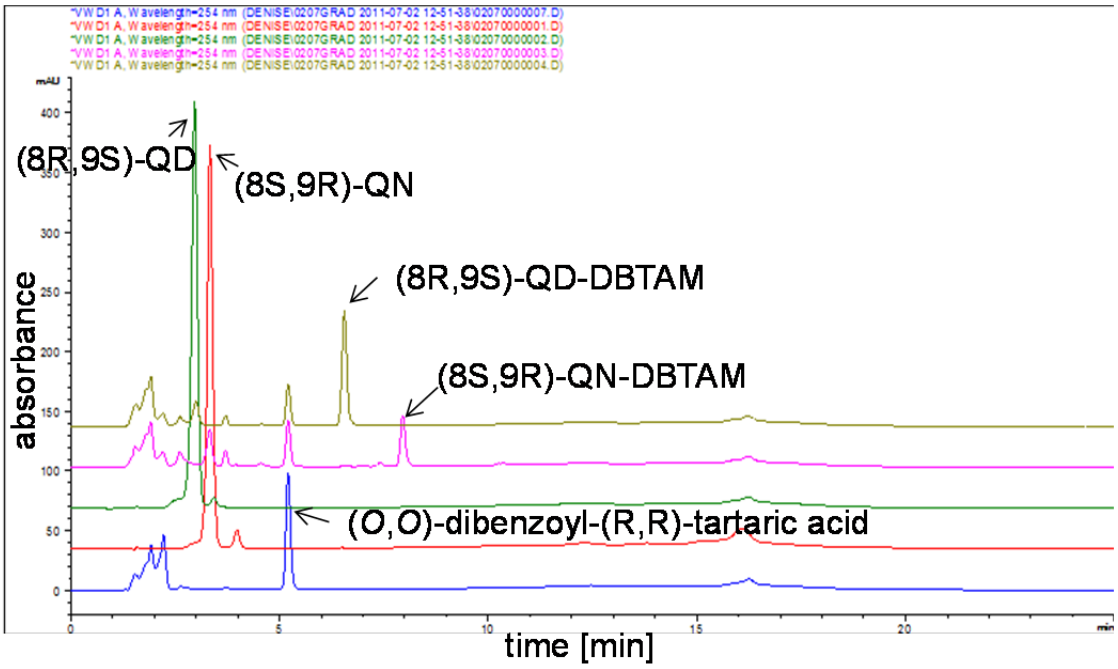


Figure 14: Elution order of the (red) (8S,9R)-QN standard, the (green) (8R,9S)-QD standard, the (pink) (8S,9R)-QN-(*O*,*O*)-dibenzoyl-(R,R)-tartaric acid monoester, the (military green) (8R,9S)-QD-(*O*,*O*)-dibenzoyl-(R,R)-tartaric acid monoester and the (blue) (*O*,*O*)-dibenzoyl-(R,R)-tartaric acid in RP mode.

All measurements for the assignment of the elution order of *N*-allyl mefloquine were done on Eclipse C_{18} RP column (150 x 4,6mm; 5 µm) as stationary phase and as a mobile phase water with 2% AcOH/MeOH mixture was used. The following gradient was applied:

Table 2: Gradient which was used for the assignment of the elution order of mefloquine[32]

t [min]	H_2O+AcOH[%]	MeOH[%]
0	50	50
5	35	65
10	20	80
15	20	80
20	35	65
25	50	50

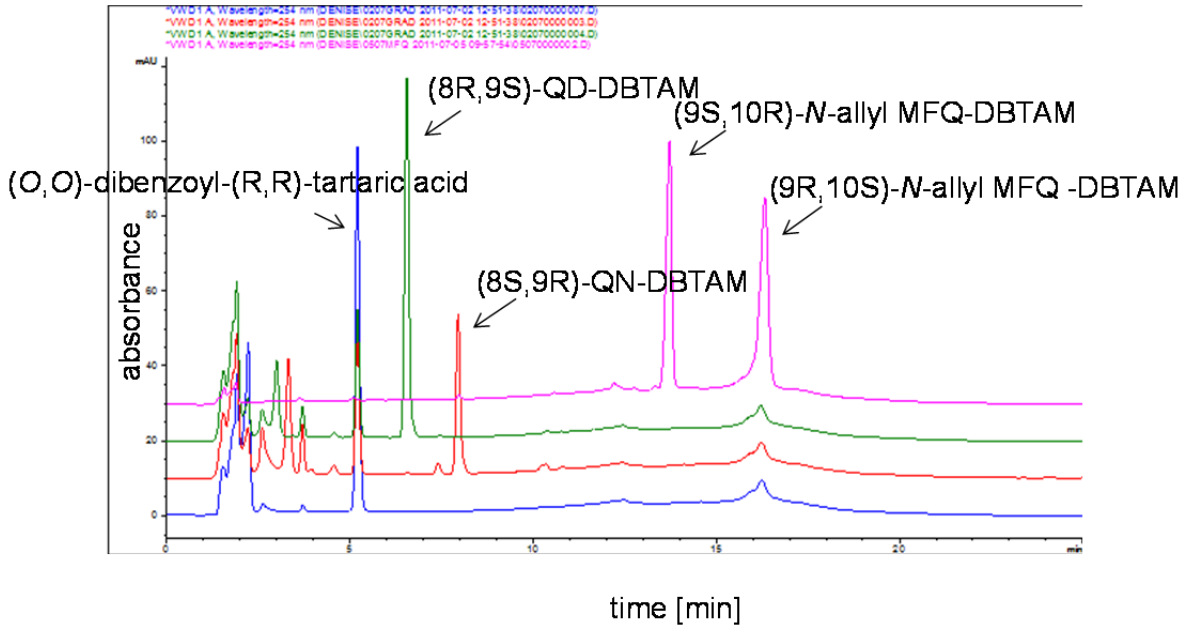


Figure 15: Elution order of *N*-allyl mefloquine. Colour code: (blue) (*O*,*O*)-dibenzoyl-(R,R)-tartaric acid, (red) (8S,9R)-QN-DBTAM, (green) (8R,9S)-QD-DBTAM and (pink) *N*-allyl MFQ-DBTAM

It was observed that the (*O*,*O*)-dibenzoyl-(R,R)-monotartrate of quinidine elutes before the quinine derivative and thus the elution order of the chinchona derivatives is (8R,9S) before (8S,9R). Because of the similarity of the chinchona derivatives to mefloquine we decided to assign the absolute configuration of the *N*-allyl mefloquine enantiomers in the same order[32].

For being absolutely sure of the absolute configuration we decided to carry out x-ray structure analysis of the assumed (9R,10S)-*N*-allyl mefloquine. The results of the x-ray analysis confirm our assignment.

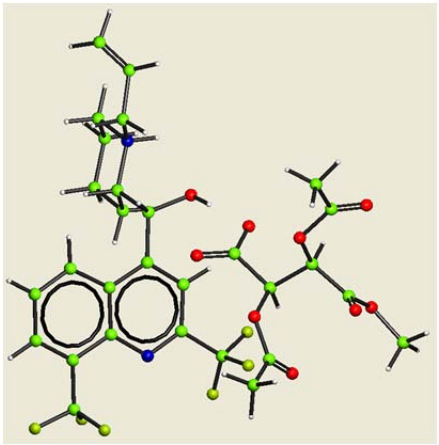


Figure 16: X-ray structure of a co-crystal of (9R,10S)-*N*-allyl mefloquine and methyl-(*O*,*O*)-diacetyl-(R,R)-tartrate

3.2 Evaluation of the novel CSPs

In this chapter the evaluation of different novel CSPs will be discussed. For describing the chromatographic behaviour we chose the following parameters, the retention factors k_i, the selectivity factor α_{ij}, the theoretical plate number N_i and the chromatographic resolution R.

The retention factor k_i is defined as the quotient of the difference of the retention time of the compound t_i and the void time t_0.

$$k_i = \frac{t_i - t_0}{t_0}$$

The retention factor indicates the interaction strength between a selector immobilized on the stationary phase and the analytes (selectand). The selector forms a kind of diastereomeric complex with each of the enantiomers and therefore the retention times differ. The retention time, and consequently the retention factor increases the stronger the interaction between the selector and the selectands gets. The retention factor is strongly dependent on the coverage of the selector on the stationary phase. Values between 1 and 5 are preferred for the retention factor, otherwise the retention time becomes too long or the interaction is too weak and no separation can be observed[19,20].

In following k_1 and k_2 are used for the retention factors of the first and the second eluting enantiomer.

The selectivity factor α_{ij} is defined as the quotient of the retention factors.

$$\alpha_{ij} = \frac{k_2}{k_1}$$

It represents the potential of a stationary phase to separate enantiomers. When the selectivity factor has a value of 1 no enantioseparation can be observed. That means that the selector of the chiral stationary phase does not prefer an interaction with one of the enantiomers.

The number of theoretical plates N_i is defined as follows:

$$N_i = 16\left(\frac{t_i}{b}\right)^2 = 5{,}54\left(\frac{t_i}{b_{FWHM}}\right)^2$$

where *b* comprises the width of a peak at its base and b_{FWHM} stands for the width of the peak at the half.

The plate number *N* indicates the chromatographic performance or the efficiency of the column. The higher its value the sharper becomes the peak, and thus the efficiency of the column. One has to bear in mind that there are many parameters which influence the number of theoretical plates *N*, e.g. injection volume, composition of the mobile phase, coverage of the selector on the stationary phase, flow rate and so forth. Consequently, it is difficult to compare the number of plates with values reported in the literature.

The chromatographic resolution *R* can be described with the Purnell equation:

$$R = \frac{1}{4} \cdot \sqrt{N} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{k_2 + 1}$$

The resolution describes the distance between two peaks. According to the equation, the resolution depends on the interaction strength of analytes with the selector (the retention factor), on the enantioseparation capability of the chromatographic system (selectivity factor *α*) and on the peak shape (the plate number *N*).

Another option for calculating the resolution is:

$$R = 2 \cdot \frac{t_2 - t_1}{b_1 + b_2}$$

where b is the width of the peak on the base and t the retention time.

The presented *R* values as well as the *N* values in the following chapter (see below) were calculated automatically by the HPLC software, if not noted otherwise.

3.2.1 Evaluation of the mefloquine based CSPs

The novel columns based on mefloquine selector, discussed in this section represent an anion exchanger type of CSP. Therefore, all the tested chiral analytes were of acidic nature.

3.2.1.1 *N*-allyl mefloquine CSP and *t*-butylcarbamoyl-*N*-allyl mefloquine CSP

The coverage of the selector immobilized on mercaptopropyl silica gel was in both cases low. In spite of that, the columns were laboratory-packed. The low coverage of the selector should only affect the retention times, not the capability for enantioseparation of the columns. In contrary to quinine, mefloquine is π-acidic and fluorophilic, therefore mainly π-basic analytes have been chosen for the evaluation of the columns.

For the evaluation of the columns methanol with acetic acid and ammonium acetate in different concentrations was used as mobile phase.

The chromatographic measurements were carried out on a 1100 series HPLC system from Agilent Technologies. The data analysis was done using the Agilent Technologies Chemstation software. Because all the analytes contained an aromatic moiety, 254 nm was chosen as the detection wavelength. The flow rate was set to 1 mL/min and the void volume was determined by injection of acetone. The sample concentrations were 1 mg/mL methanol and the injection volume was 5 or 10 µL, depending on the UV response.

The following analytes were tested:

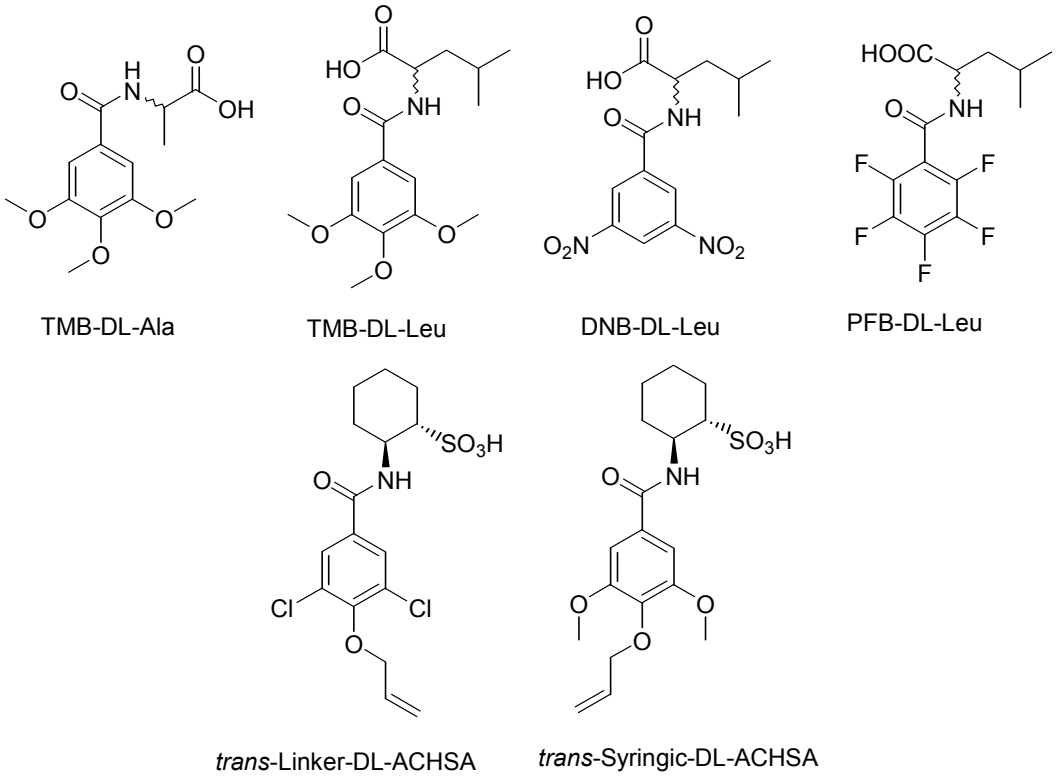


Figure 17: Analytes for evaluation of the mefloquine based columns

All analytes (except for DNB-Leu) were synthesized within the frame of this thesis (see **Chapter 2**). DNB-Leu analytes were previously prepared in our laboratory. For the derivatization of the 4-(allyloxy)-3,5-dichlorobenzoic acid (also called π-acidic linker acid) and the 4-*O*-allyl-syringic acid (also called π-basic syringic acid) *trans*-2-aminocyclohexanesulfonic acid was used.

In general the retention factors were quite low and enantioseparation was only observed for the strongly acidic analytes based on *trans*-2-aminocyclohexanesulfonic acid. The retention factor could be slightly increased by decreasing the amount of acetic acid, actually the amount of ammonium acetate in the mobile phase, as it is shown in **Figure 19**. It seems that a carboxylic acid compared to the sulfonic acid, is too weak for a discrete ionic pair interaction with the tertiary amino group of the piperidine ring.

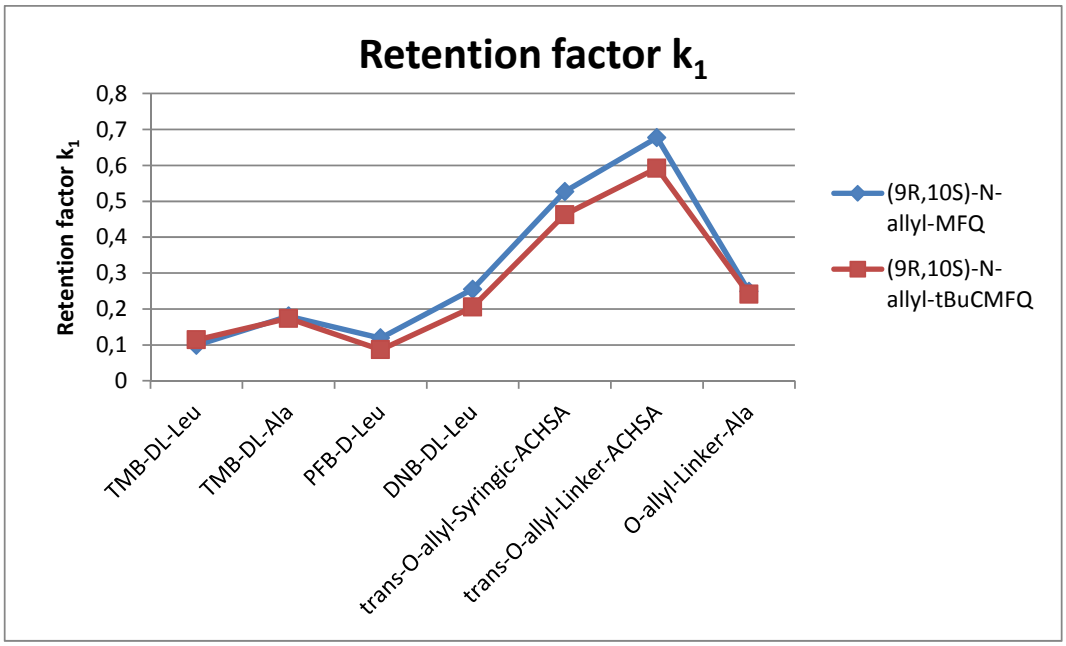


Figure 18: k_1 values, mobile phase: MeOH, 0.5% AcOH, 0.125% NH_4OAc

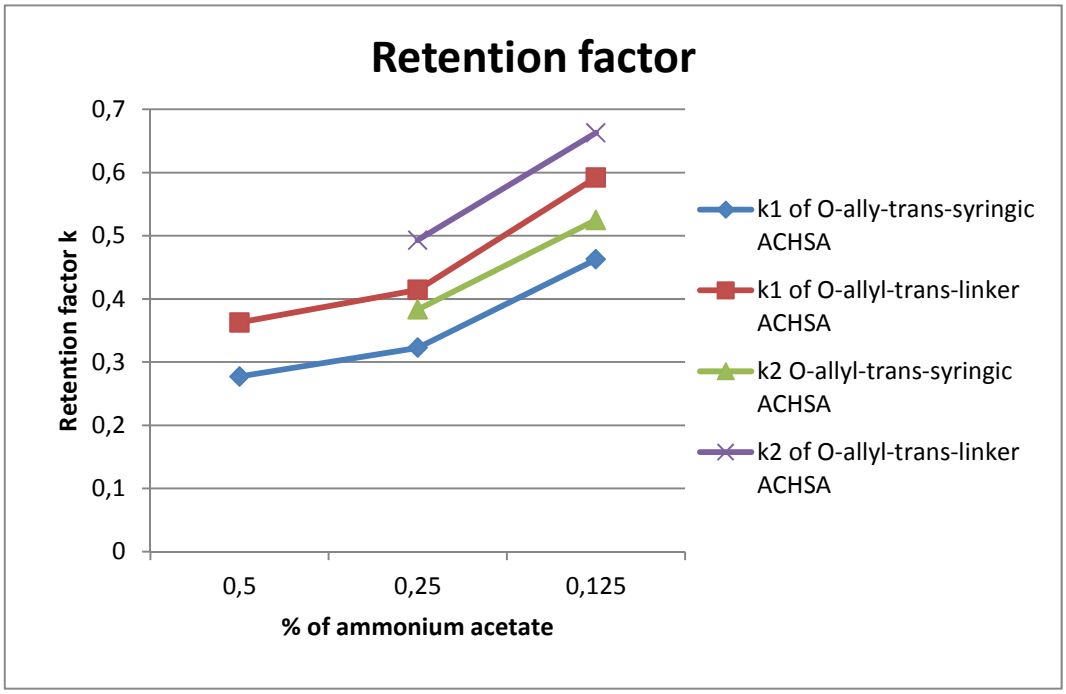


Figure 19: Increase of the retention factor depending on the amount of ammonium acetate in the mobile phase (conditions see table 3).

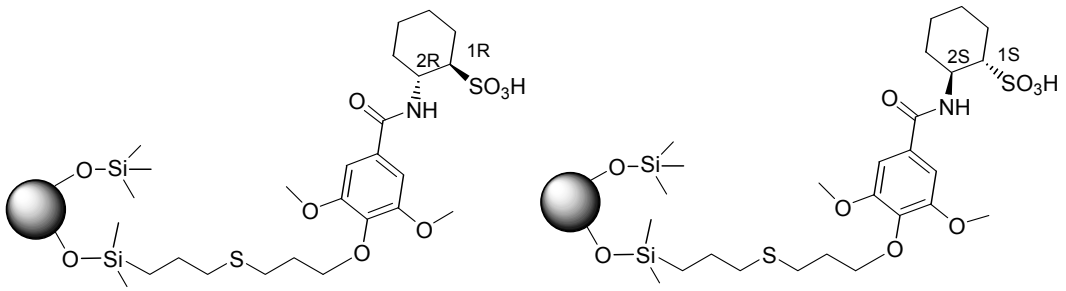
Table 3: The α values of *trans*-2-aminocyclohexanesulfonic acid type analytes; mobile phase conditions: 1st row: MeOH, 1% AcOH, 0.25% NH_4OAc; 2nd row: MeOH, 0.5% AcOH, 0.125% NH_4OAc

	(9R,10S)-*N*-allyl-tBuCMFQ-CSP		(9R,10S)-*N*-allyl-MFQ-CSP	
	trans-O-allyl-Syringic-ACHSA	*trans*-O-allyl-Linker-ACHSA	*trans*-O-allyl-Syringic-ACHSA	*trans*-O-allyl-Linker-ACHSA
NH_4OAc[%]	α	α	α	α
0,25	1,19	1,19	-	-
0,125	1,14	1,12	1,11	1,10

As a consequence of the low coverage of the selectors by immobilization and the obvious problems in enantioseparation we decided to carry out NMR studies. We assumed that such a study would clarify whether there is a complexation between the mefloquine-based selector and the selectands (see below).

3.2.2 Evaluation of the SCX CSPs based on syringic acid

As shown above, in spite of the low coverage of the mefloquine selectors, a separation of the enantiomers with the motif of aminosulfonic acid was observed. (see section 3.2.1.1). Therefore we decided to discover how the novel SCX CSPs based on syringic acid work for the enantioseparation of mefloquine based analytes.



CSP3 **CSP4**

Figure 20: Novel SCX CSPs based on ACHSA and syringic acid. They represent enantiomeric CSPs to each other

The coverage was calculated assuming that the novel SCXs is the ammonium salt of syringic acid derivative. However, it is possible that a mixture of the ammonium salt and the free selector is present. Therefore the coverage of carbons was determined in order to compare it with the calculated values for the nitrogen. The values are similar and therefore we can assume that the coverage corresponds to the presented values in **Table 4** (**(1R,2R)-CSP 3** and **(1S,2S)-CSP 4**). In general the coverage is

slightly lower for the novel SCXs than for the previously developed ones (**CSP 3** vs. **CSP 5** and **CSP 4** vs. **CSP 6**).

Table 4: Coverage of the novel SCX CSPs

	µmol N/g	µmol C/g
CSP3	153	164
CSP4	202	206
CSP5	184	-
CSP6	221	-

Figure 21 shows various analytes based on mefloquine, which were tested with and **CSP 4**.

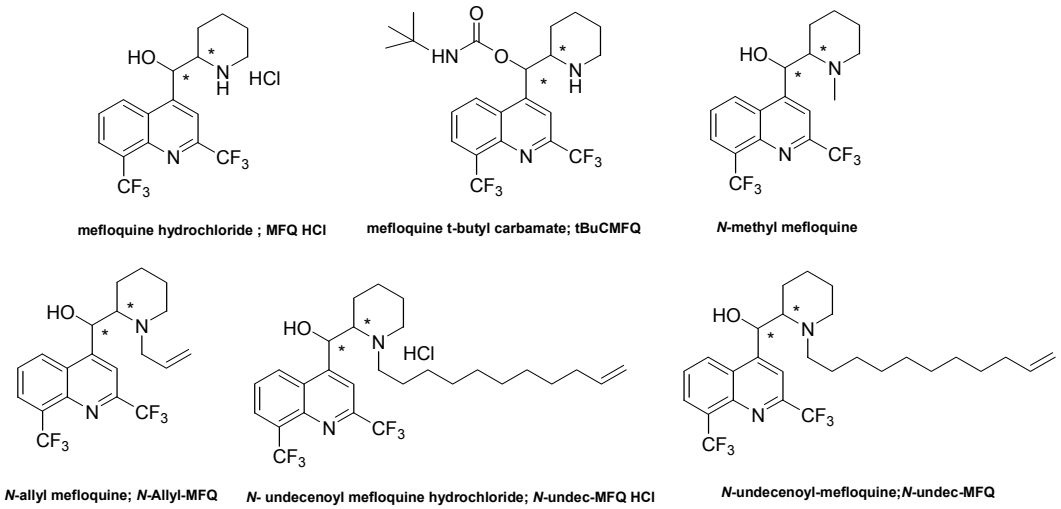
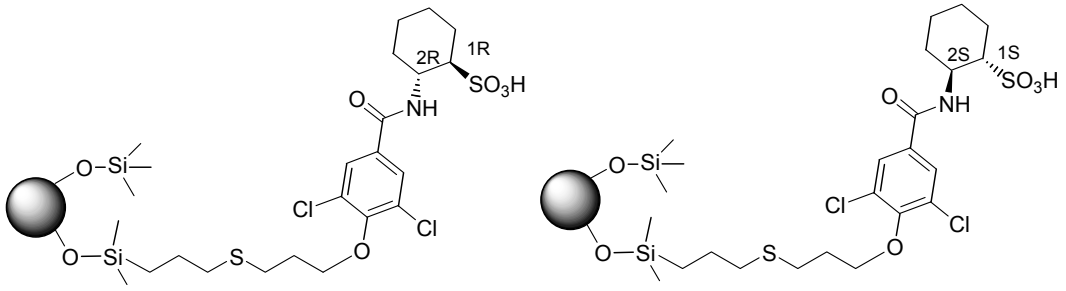


Figure 21: Analytes based on mefloquine for the evaluation of the novel SCX CSP.

For estimation of the quality of the CSPs regarding selectivity and resolution, a comparison was done with a SCX CSP based on π-acidic linker acid (**(1R,2R)-CSP5**, see **Figure 22**). This SCX CSP was developed by a previous coworker and shows great applicability for the enantioseparation of basic analytes.



CSP5 **CSP6**

Figure 22: SCX CSPs which were previous developed in our working group

As mobile phase a mixture of 90% ACN, 10% methanol with acidic and basic additives (50 mM formic acid and 25 mM DEA) as co- and counterion was used. The optimization of the mobile phase composition is an important parameter for optimising the elution strength. However, we used this composition of mobile phase, as it had already been optimized in our working group for the separation of chiral amines on **CSP 5** and **6**. The chromatographic measurements were carried out on a 1100 Series HPLC or on a 1260 Infinity HPLC from Agilent Technologies. Chromatographic data analysis was done using the Agilent Chemstation software. For the presented chromatographic parameters in the following chapter 254 nm wavelength was chosen as detector signal. The flow rate was 1 mL/min and the injection volume was 5 or 10 µL. The concentration of the analytes was 1 mg/mL methanol. The void volume was determined by injection of 5 µL of methanolic acetone (70 µL/mL) solution.

Table 5: Results for the evaluation of the novel SCX CSP 3 and 4 and for comparison CSP 5.

	(1R,2R)-CSP3						(1S,2S)-CSP4						(1R,2R)-CSP5					
Analytes	k_1	k_2	α	**Res.**	$N_1[m^{-1}]$	$N_2[m^{-1}]$	k_1	k_2	α	**Res.**	$N_1[m^{-1}]$	$N_2[m^{-1}]$	k_1	k_2	α	**Res.**	$N_1[m^{-1}]$	$N_2[m^{-1}]$
MFQ*HCl	7.34	11.44	**1.56**	**9.99**	68287	68947	7.72	11.52	**1.49**	**6.65**	38487	37353	9.19	12.11	**1.32**	**5.54**	52193	52727
MFQ*_N_-allyl	1.90	2.29	**1.20**	**3.05**	64140	63833	2.04	2.39	**1.17**	**1.92**	33227	33453	2.10	2.33	**1.11**	**1.53**	47473	48220
tBuCMFQ	5.19	7.46	**1.44**	**7.19**	57907	57860	5.25	7.35	**1.40**	**4.99**	29880	34327	5.23	7.51	**1.44**	**6.73**	51233	50260
_N_-undec-MFQ*HCl	3.38	4.69	**1.39**	**6.59**	70887	66633	2.60	3.65	**1.41**	**4.04**	24073	29093	2.77	3.44	**1.24**	**3.67**	54953	52787
_N_-methyl-MFQ	1.97	2.3	**1.17**	**2.89**	78860	77900	2.41	2.82	**1.17**	**2.15**	38233	37787	2.48	2.81	**1.13**	**2.22**	62507	63113

Table 5 shows the chromatographic data of the separation of mefloquine based analytes. It can be seen that the novel SCXs based on *O*-allyl syringic acid are performing similarly to the established **CSP 5**. A comparison shows that the new columns provide better enantioseparation for all tested analytes. The **CSP 5** is comparative to **CSP 3** with regard to the configurations of the chiral centres. The elution order of *N*-allyl mefloquine was determined with the novel **CSP 3** and **4**. On

the **CSP 3** the (9R,10S)-*N*-allyl mefloquine elutes before the (9S,10R)-*N*-allyl mefloquine and therefore on **CSP 4** conversely.

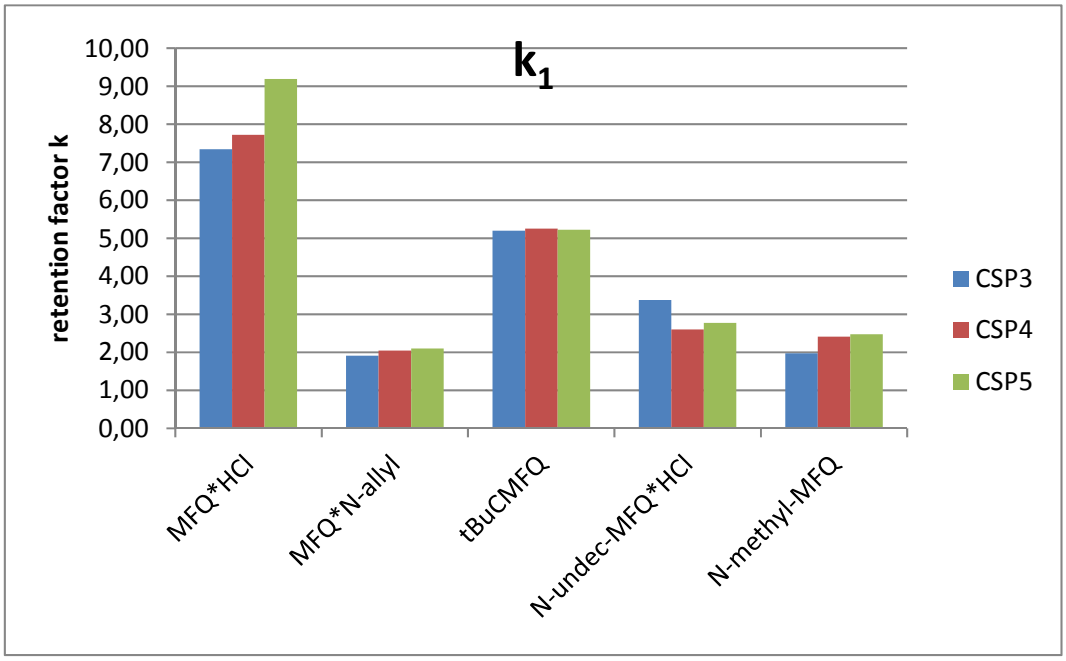


Figure 23: The retention factor k_1 values for the SCX CSPs

Figure 23 shows the retention factor k_1 values for the SCX CSPs. **CSP 5** shows a higher k_1 value for mefloquine itself. The other values for the mefloquine-based analytes are similar for all CSPs. Furthermore, it has to be noted that the k_1 values are higher, when the nitrogen of the piperidine ring in mefloquine is not derivatized. This fact indicates that the interaction strength of the selector with the selectand depends on the accessibility of the nitrogen in the piperidine ring. Moreover, additional H-bonding is possible in case of the free N-H (secondary amine) group.

As the k_1 values depend on the selector coverage on the silica surface, the direct comparison of the performance of columns is difficult.

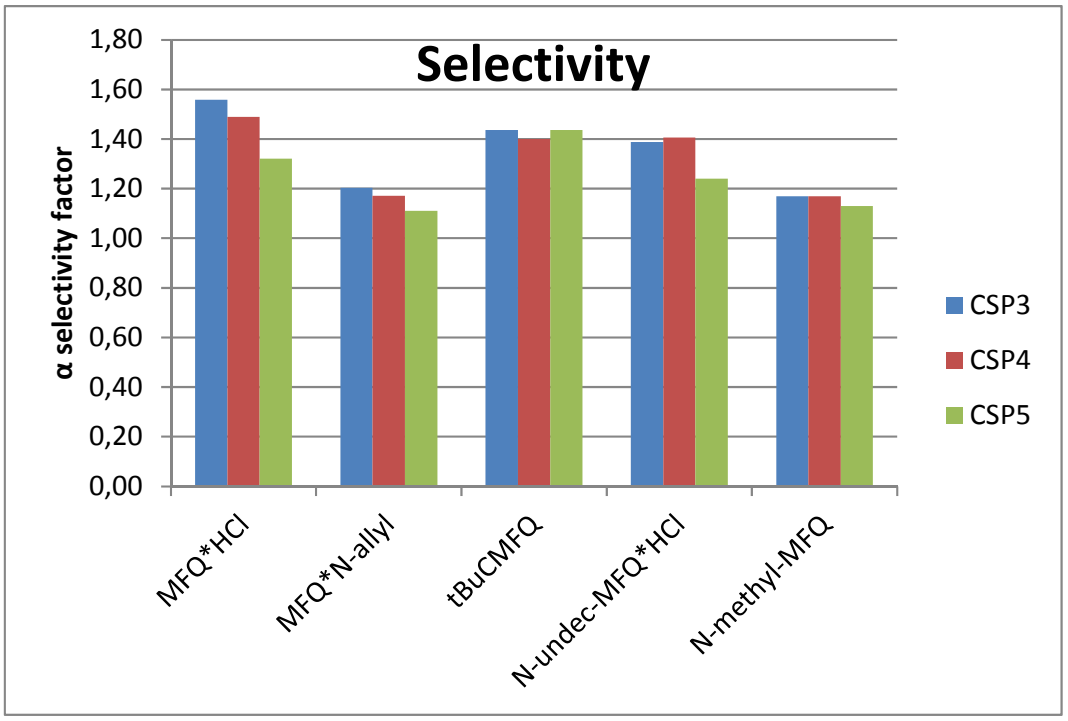


Figure 24: α values for the SCX CSPs

Figure 24 shows that the selectivity factor values for **CSP 3** and **4** are slightly better or at least equal to **CSP 5**. **CSP 3** and **CSP 4** are enantiomerically to each other and this leads to a reversal of the elution order. The *N*-allyl *t*BuCMFQ was also tested on all columns; however, no enantioseparation was observed using the given mobile phase.

By changing the mobile phase condition to methanol instead of acetonitrile the π-π interactions will be enhanced and the hydrogen bonding will be depressed. Thus, we changed the mobile phase and used MeOH with 50 mM formic acid and 25 mM diethyl amine for separation of *N*-allyl-*t*BuCMFQ on **CSP 3** and **5**. Enantioseparation was observed on **CSP 3**: α=1.06 or **CSP 5**: α=1.05 (same elution order).

Figure 25 shows that the resolution values are higher for **CSP 3** than **CSP 4** and **CSP 5**. Regarding **CSPs 4** and **5** resolution is more or less equal for most of the analytes based on mefloquine. In general it can be seen that the resolution values for analytes where the nitrogen in the piperidine ring is substituted are lower.

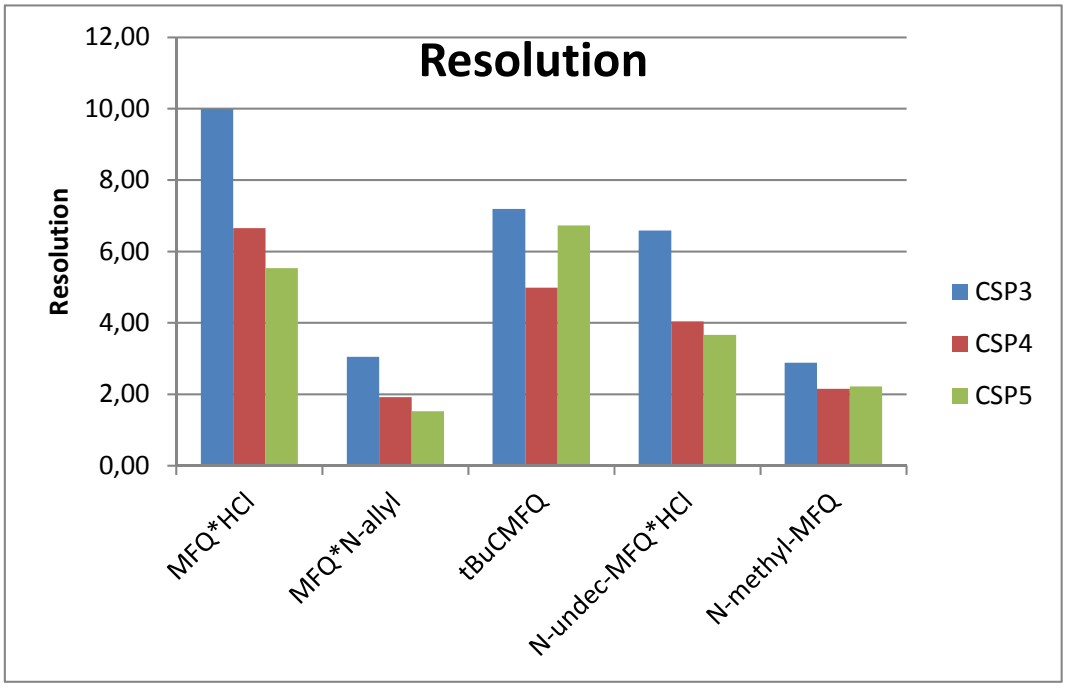
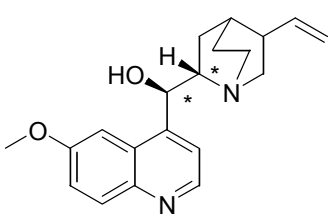
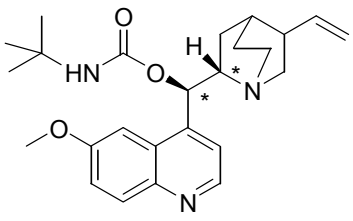


Figure 25: Resolution values for the SCX CSPs

The structural resemblance of mefloquine with the chinchona derivatives was the motivation for analysing quinine, quinidine and the corresponding *tert*-butyl carbamates on **CSP 3** and **CSP 4**.



(8s,9R) quinine QN (quinidine was also tested)



(8S,9R)-*tert*- butylcarbamoyl quinine (the pseudoenantiomer tBuCQD was also tested)

Figure 26: Analytes based on quinine and quinidine

Furthermore, for the evaluation of **CSP 3** and **CSP 4** several basic analytes were tested. Most of the tested analytes were secondary amines. The selected analytes are shown in **Figure 27**.

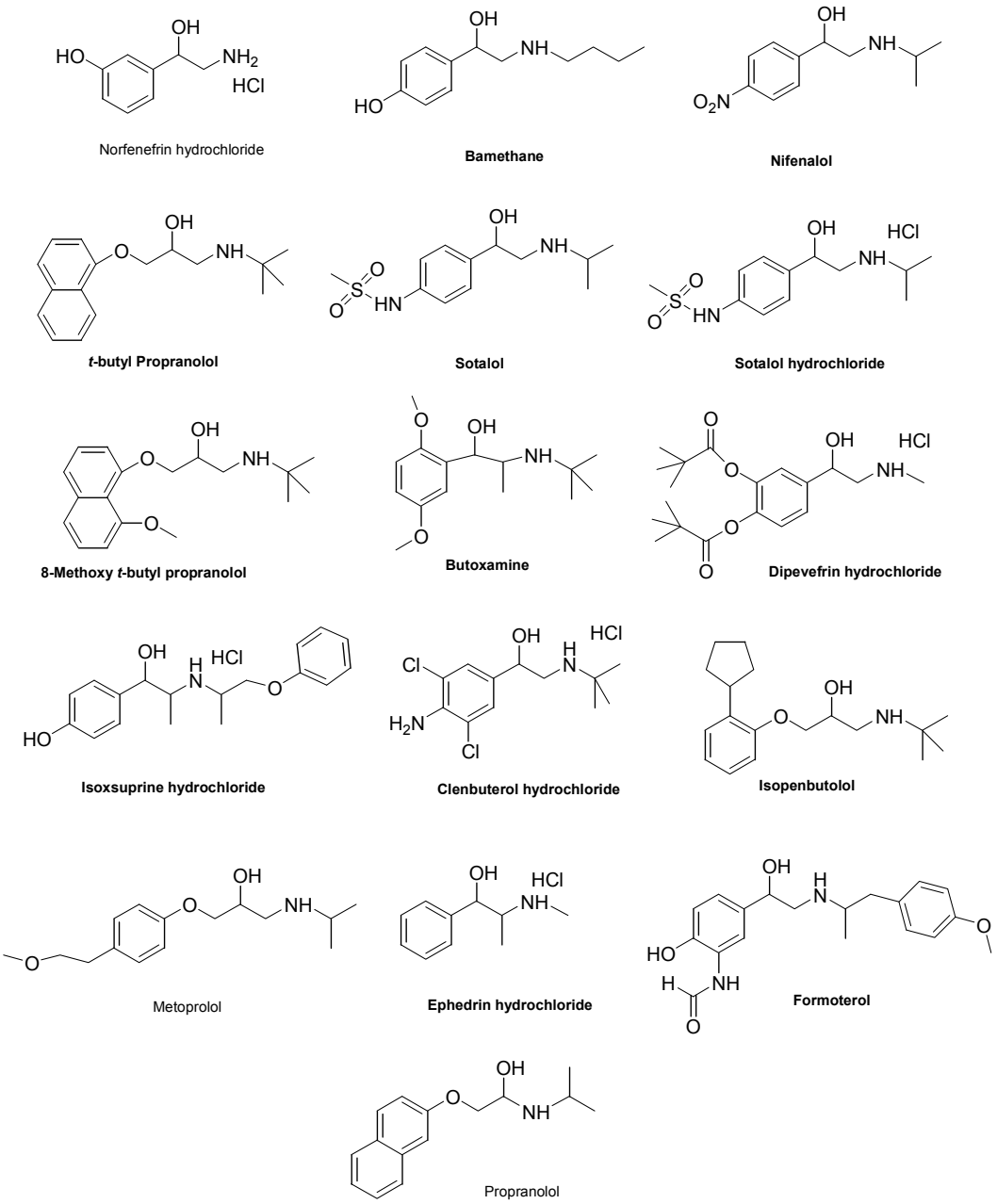


Figure 27: Basic analytes for the evaluation of the novel SCX CSP

Table 6: Chromatographic data obtained by the evaluation of basic analytes with the novel SCX CSPs based on syringic acid.

	(1S,2S) CSP4						(1R,2R) CSP3					
Analyt	***k1***	***k2***	**alpha**	**Res.**	**$N_1[m^{-1}]$**	**$N_2[m^{-1}]$**	***k1***	***k2***	**alpha**	**Res.**	**$N_1[m^{-1}]$**	**$N_2[m^{-1}]$**
QN/QD	7,47	8,49	**1,14**	**1,99**	33087	32667	5,91	7,81	**1,32**	**5,93**	65240	62787
*t*BuCQN/*t*BuCQD	2,02	5,71	**2,83**	**13,34**	32007	33387	2,13	4,45	**2,09**	**12,7**	58920	59480
Propranolol	7,08	7,45	**1,05**	**0,7**	26420	27620	6,90	7,22	**1,05**	**1,03**	71907	72427
Norfenefrin HCl	3,97	5,27	**1,33**	**1,17**	2333	3153	-	-	**-**	**-**	-	-
Isopenbutolol	5,51	6,12	**1,11**	**1,64**	34420	36107	4,99	5,45	**1,09**	**1,93**	70147	70373
Ephedrine HCl	6,86	7,78	**1,13**	**2,17**	38113	43873	5,09	5,73	**1,13**	**2,74**	78727	80740
Bamethan base	4,55	5,99	**1,32**	**1,03**	1713	2580	-	-	**-**	**-**	-	-
Nifenalol	6,34	8,25	**1,30**	**4,27**	36187	37360	5,47	6,98	**1,28**	**5,56**	77373	75113
*t*Bu-Propranolol	6,28	6,85	**1,09**	**1,36**	34887	35013	5,48	5,89	**1,08**	**1,59**	70507	70907
Sotalol*HCl	7,98	8,80	**1,10**	**1,57**	35227	35080	6,08	6,63	**1,09**	**1,89**	68627	69173
Metoprolol	4,95	5,11	**1,03**	**0,49**	25207	49573	4,35	4,49	**1,03**	**0,61**	51960	68527
Sotalol	7,94	8,72	**1,10**	**1,22**	18827	27647	6,08	6,62	**1,09**	**1,88**	68560	68713
8-Methoxy-*t*Bu-propranolol	6,34	6,85	**1,08**	**1,17**	32047	32707	5,53	5,90	**1,07**	**1,39**	65973	67200
Dipivefrin*HCl	4,94	5,37	**1,09**	**1,05**	23707	26687	4,36	4,70	**1,08**	**1,69**	67653	72320
Formoterol	15,00	16,33	**1,09**	**1,42**	34547	34060	13,29	14,34	**1,08**	**1,83**	72327	70767
Butoxamine	4,28	5,21	**1,22**	**2,96**	36013	35387	3,76	4,63	**1,23**	**4,17**	67713	66587
Isoxsuprine HCl	6,63	8,08	**1,22**	**3,09**	31687	36233	5,03	6,08	**1,21**	**3,99**	67440	65307
Clenbuterol *HCl	7,32	9,53	**1,30**	**4,34**	36073	36760	6,23	7,94	**1,27**	**5,5**	71187	72907
Piperoxilidide	9,04	10,25	**1,13**	**2,15**	37873	38727	5,51	6,28	**1,14**	**1,63**	23873	22060

The elution order for some of the analytes like propranolol was determined, by injecting only one pure enantiomer of the analyte and comparing the retention time with the retention times of the racemic mixture. On **CSP 4** the D enantiomers elute before the L enantiomers and therefore on **CSP 3** conversely.

Figure 28 shows the retention factor for the first eluting enantiomer. **CSP 4** shows higher k_1 values for all analytes.

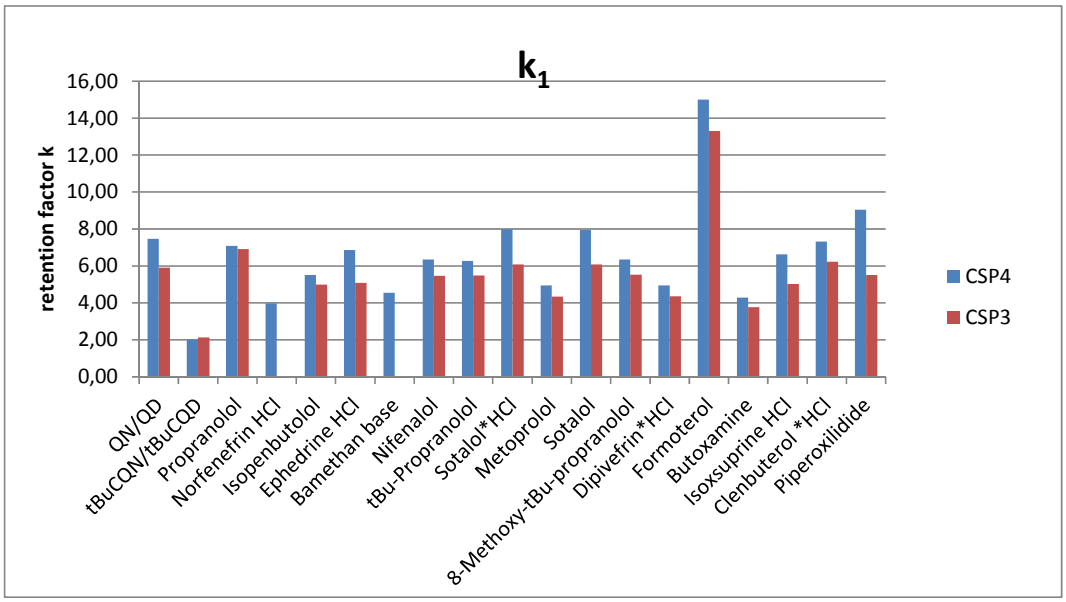


Figure 28: k_1 values for basic analytes measured on syringic acid based SCX CSPs.

Figure 29 shows slightly higher α values for analytes measured on **CSP 4**.

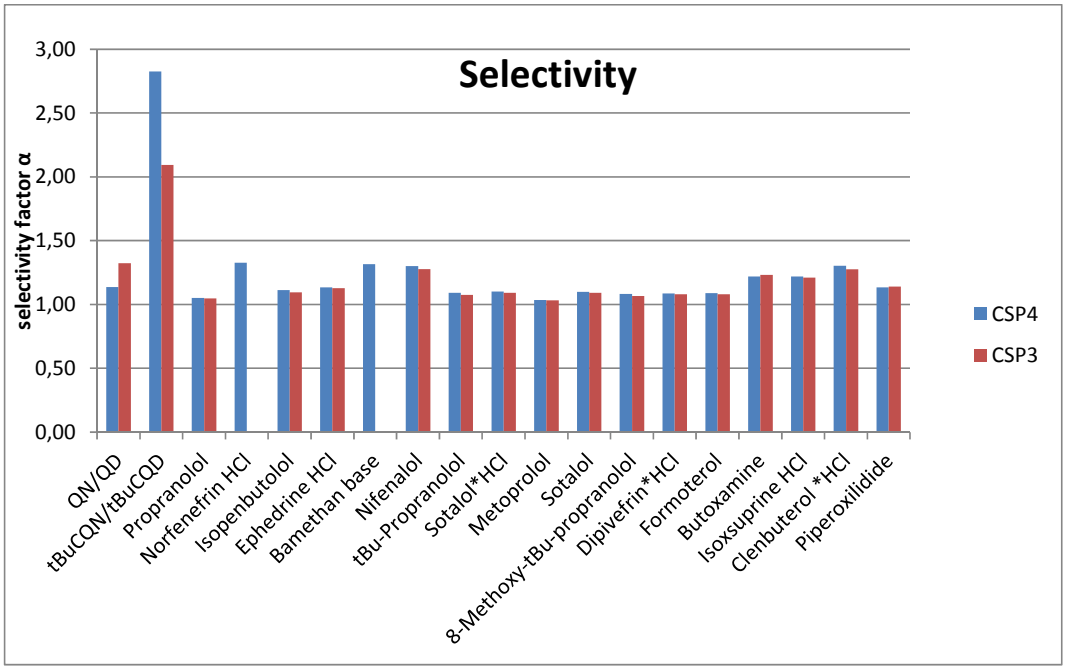


Figure 29: α values for basic analytes measured on syringic acid based SCX CSPs.

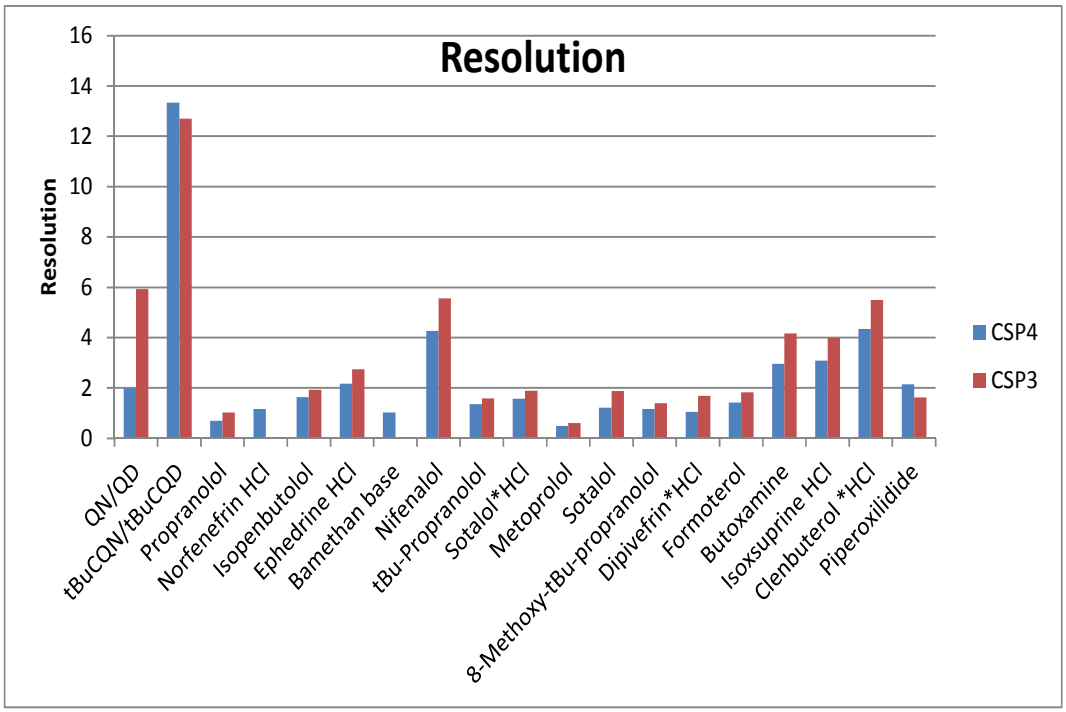


Figure 30: Resolution values for basic analytes measured on syringic acid based SCX CSPs.

Contrary to the *k* and the *α* values **CSP 3** shows higher resolution values for the analytes than **CSP 4**. This is caused by the different number of plates in **CSP 3** and **4**. As mentioned before the resolution values are, besides the *α* and the *k* values, influenced by the plates number. This indicates that the interaction strength of the (1S,2S)-**CSP 4** with the selectands is higher than that of (1R,2R)-**CSP 3**.

The number of plates also depends on the packing quality. The columns were packed in house and therefore a difference in the number of plates can be possible.

Table 7: Shows selected analytes for comparison of CSP 4 (4-allyloxy-3,5-dimethoxybenzoyl derivative) and 6 (4-allyloxy-3,5-dichlorobenzoyl derivative)

	(1S,2S)-CSP6						(1S,2S)-CSP4					
Analyt	***k1***	***k2***	**α**	**Res.**	**$N_1[m^{-1}]$**	**$N_2[m^{-1}]$**	***k1***	***k2***	**alpha**	**Res.**	**$N_1[m^{-1}]$**	**$N_2[m^{-1}]$**
QN/QD	9.45	10.91	**1.15**	**2.96**	55867	53867	7.47	8.49	**1.14**	**1.99**	33087	32667
*t*BuCQD/*t*BuCQN	2.82	9.34	**3.31**	**19.3**	49600	46067	2.02	5.71	**2.83**	**13.34**	32007	33387
Bamethan base	12.69	13.59	**1.07**	**1.61**	69000	68733	4.55	5.99	**1.32**	**1.03**	1713	2580
Nifenalol	8.15	11.05	**1.36**	**6.69**	63467	63733	6.34	8.25	**1.30**	**4.27**	36187	37360
Sotalol	8.40	9.49	**1.13**	**2.66**	62800	62867	7.94	8.72	**1.10**	**1.22**	18827	27647
Dipivefrin*HCl	6.15	6.85	**1.11**	**2.23**	59933	61867	4.94	5.37	**1.09**	**1.05**	23707	26687
Formoterol	18.19	20.54	**1.13**	**2.71**	59133	59333	15.00	16.33	**1.09**	**1.42**	34547	34060
*t*Bu-Propranolol	7.66	8.49	**1.11**	**2.22**	63600	62733	6.28	6.85	**1.09**	**1.36**	34887	35013
Butoxamine	5.10	6.28	**1.23**	**4.21**	62200	61000	4.28	5.21	**1.22**	**2.96**	36013	35387
Isopenbutolol	7.02	7.75	**1.10**	**2.09**	61400	62000	5.51	6.12	**1.11**	**1.64**	34420	36107
Isoxsuprine	7.14	8.68	**1.22**	**4.18**	63267	61400	6.63	8.08	**1.22**	**3.09**	31687	36233
Clenbuterol *HCl	9.64	13.85	**1.44**	**8.02**	63533	62933	7.32	9.53	**1.30**	**4.34**	36073	36760

The enantioseparation of **CSP 3** is comparable to the established SCX **CSP 6**.

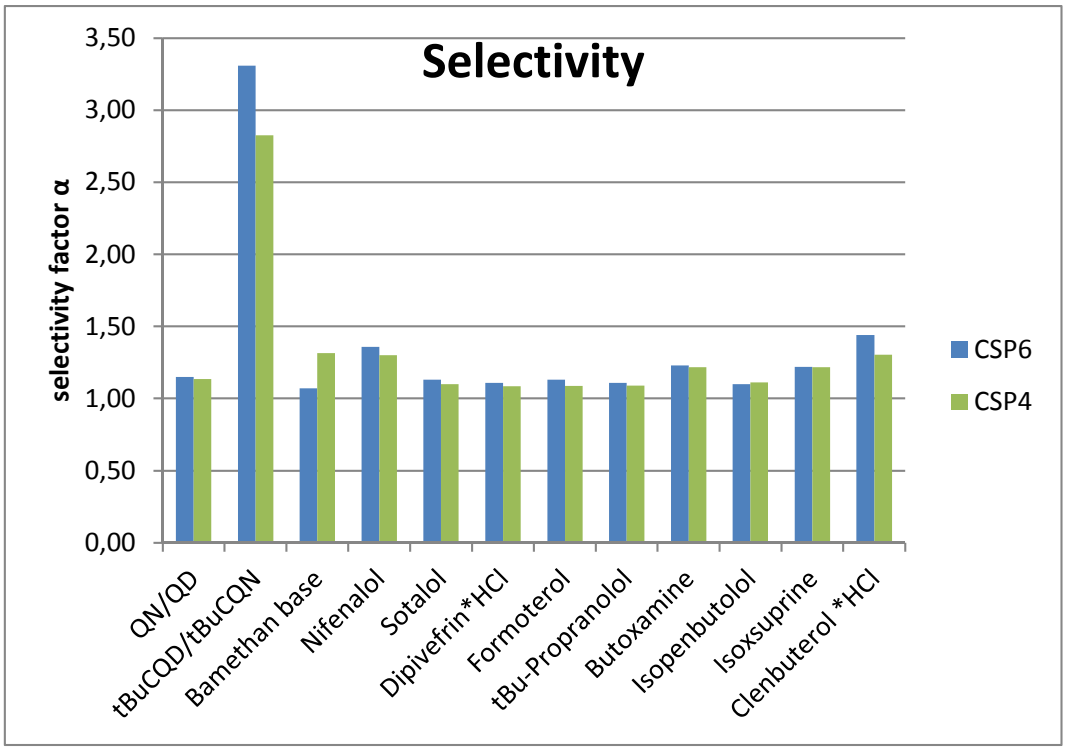


Figure 31: Comparison of the α values for selected analytes on SCX CSPs

The resolution is generally higher for the **CSP 6** than for the **CSP 4**.

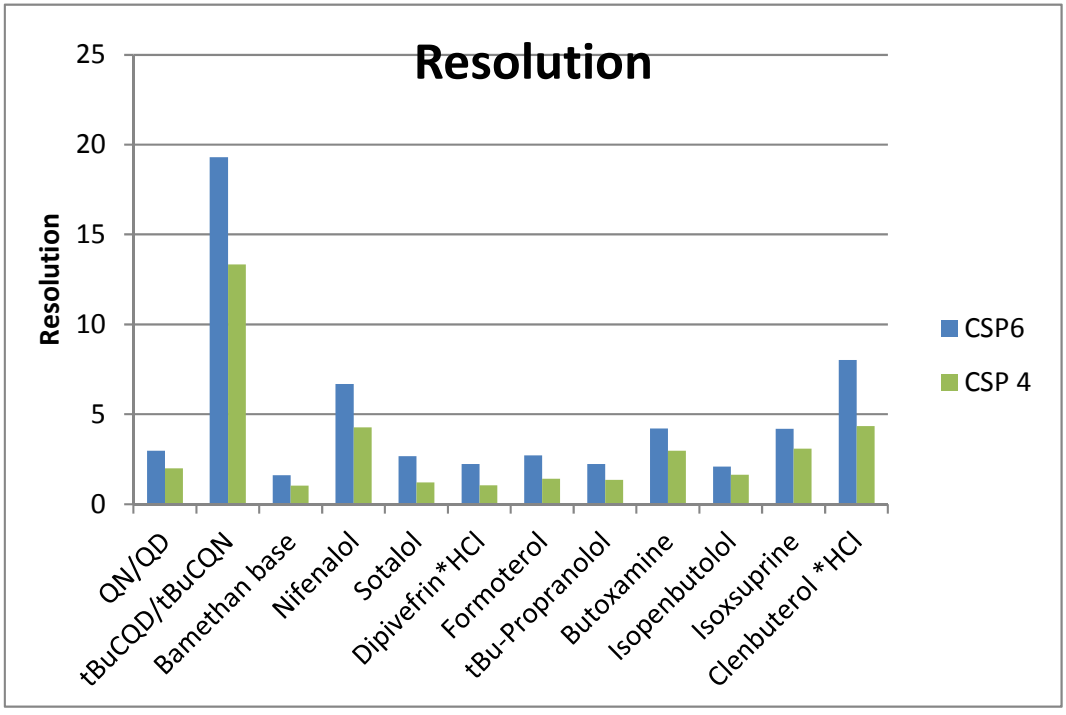


Figure 32: Comparison of the resolution values of analytes measured on SCX CSPs

3.3 NMR studies of interactions between mefloquine and analytes

The low selector coverage on silica used in the novel columns and the fact that enantioseparation can only be observed for sulfonic acid derivatives makes it difficult to decide, whether mefloquine fulfill the necessary interactions according to the three point model recomended for effective separation. Therefore we decided to carry out NMR studies as well.

Recently, several detailed NMR studies concerning the specific interactions of cinchona derived chiral selector (SO) and negatively charged chiral selectands (SAs) have been published[38,39]. Following the chemical and functional reciprocity principle we envisioned that 9-O-*tert*-butylcarbamoyl-*N*-allyl mefloquine should follow similar intermolecular recognition pathways, as the 9-O-*tert*-butylcarbamoyl quinine type chiral SO but being driven by a π-acidic quinoline moiety and a π-basic counterpart of the selectand.

We chose derivatives of 3,4,5-trimethoxybenzoic acid as appropriate selectands with sufficiently electron rich aromatic cores. Previously, the interactions of quinine-based selector with 3,5-dinitrobenzoylleucine were studied in our work group[38]. Therefore we decided to use leucine as a suitable amino acid part and synthesized *N*-3,4,5-trimethoxybenzoyl leucine, which was used as a selectand during the initiate NMR study. Later on we decided to extend our study to ^{19}F core measurements as well. We assumed that the trifluoromethyl groups of mefloquine may exhibit a kind of fluorophilic interactions with a fluoro substituted selectand. Such attractive forces may again enhance the chiral recognition and the selector itself might be helpful in trace analysis of herbicides and pesticides because many of them are nowadays fluorinated or perfluorinated. In order to discover whether such type of interactions is present, we prepared also *N*-2,3,4,5,6-pentafluorobenzoyl leucine.

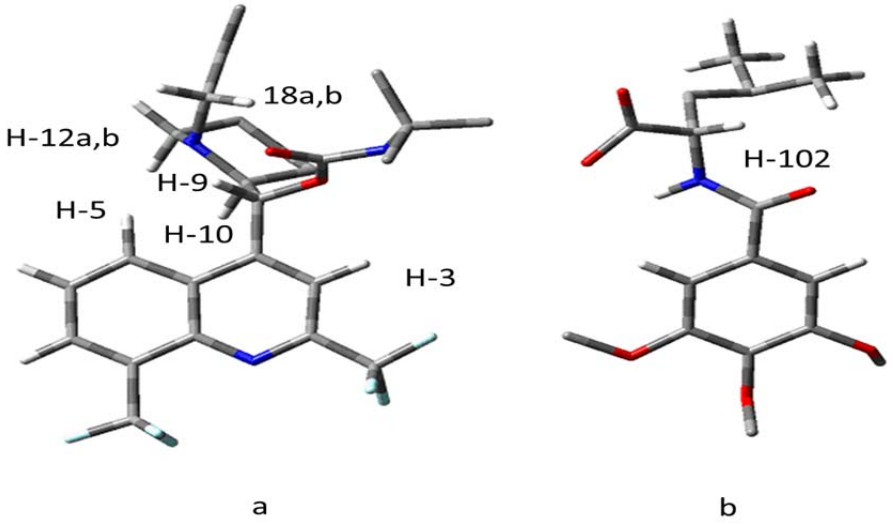


Figure 33: a: Assignation of diagnostic protons of *N*-allyl-*t*BuCMFQ (other protons omitted for clarify); b: Assignation of diagnostic protons of L-Leu-TMB (other protons omitted for clarify)

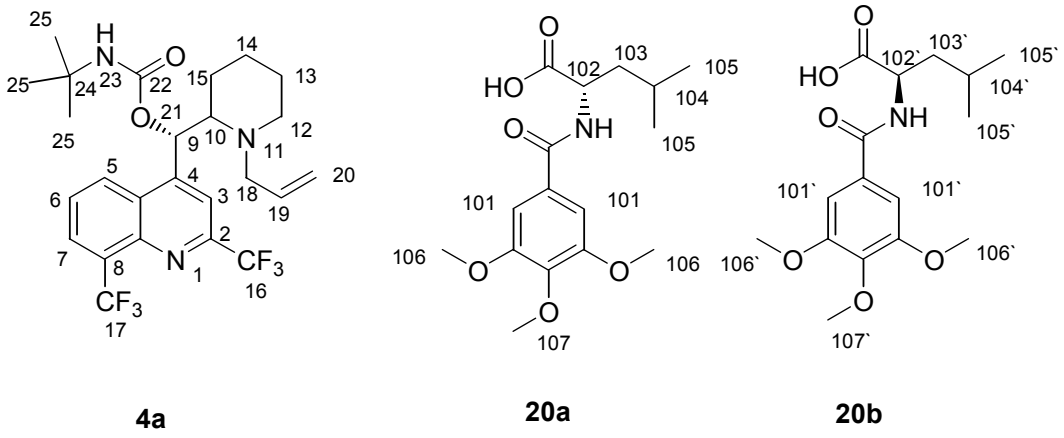


Figure 34: Atom numbering scheme of the *N*-allyl-*t*BuCMFQ and TMB-Leu

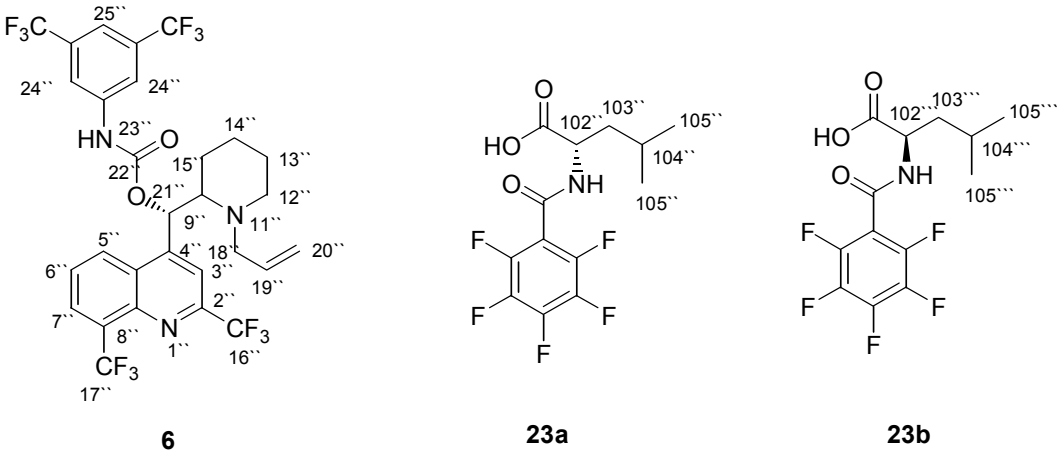


Figure 35: Atom numbering scheme of the *N*-allyl-F-CMFQ and PFB--Leu

The NMR measurements were carried out in methanol-d_4. This solvent was chosen for having comparable properties to an HPLC mobile phase. For the assignment of the interactions between the selector (mefloquine) and the selectand (TMB-DL-Leu, PBF-DL-Leu) a serial dilution was prepared[40,41]. A stock solution (20 µL/mL) was used for each of the compounds. Samples were prepared. A stock solution (20 µL/mL) was used for each of the compounds. Samples were prepared, as shown in **Table 8**, by pipetting a given volume directly into an NMR tube.

Table 8: Serial dilution of mefloquine based carbamates and the leucine derivatives

(9S,10R)-tBuCMFQ	DL-Leu derivatives	CMFQ	DL-Leu derivatives
[µmol]	[µmol]	[µL]	[µL]
18	2	738	82
16	4	656	164
14	6	574	246
12	8	492	328
10	10	410	410
8	12	328	492
6	14	246	574
4	16	164	656
2	18	82	738

The first NMR study was carried out with (9S,10R)-*N*-allyl-*t*BuCMFQ and TMB-L-Leu, TMB-D-Leucine, respectively. The corresponding job plot of ^{1}H NMR is shown in **Figure 36** for the H-9 (a diagnostic proton located at C-9 chiral centre).

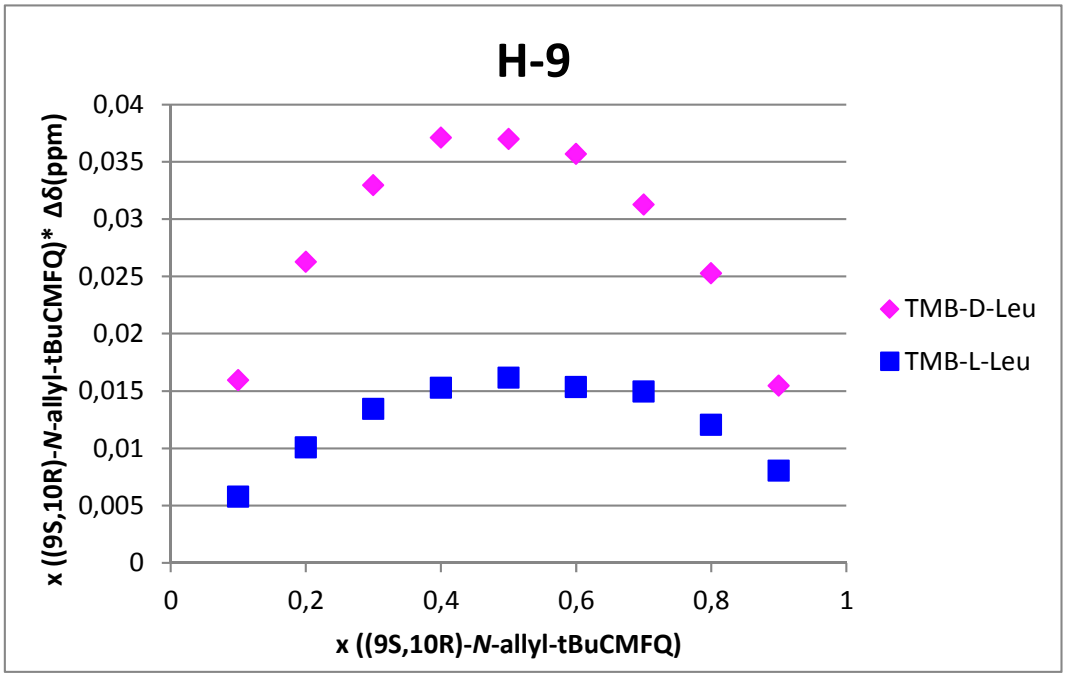


Figure 36: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and TMB-D-Leu, TMB-L-Leu respectively. The chiral H-9 of mefloquine was used for interpretation. The total concentration was 20 µM.

Figure 36 shows that a stoichiometric 1:1 complex of the mefloquine based selector and the leucine derivatives is formed. It has to be noted that the complexation of TMB-L-Leu is weaker than that of TMB-D-Leu.

The presence of two trifluoromethyl groups in the mefloquine molecule makes it interesting also for ^{19}F NMR studies with the advantage that ^{19}F NMR spectra are usually much easier to interpret because of a reduced number of fluorinated functionalities signals. In **Figure 37** and **Figure 38** the corresponding ^{19}F NMR job plot is shown for the trifluoromethyl groups of mefloquine.

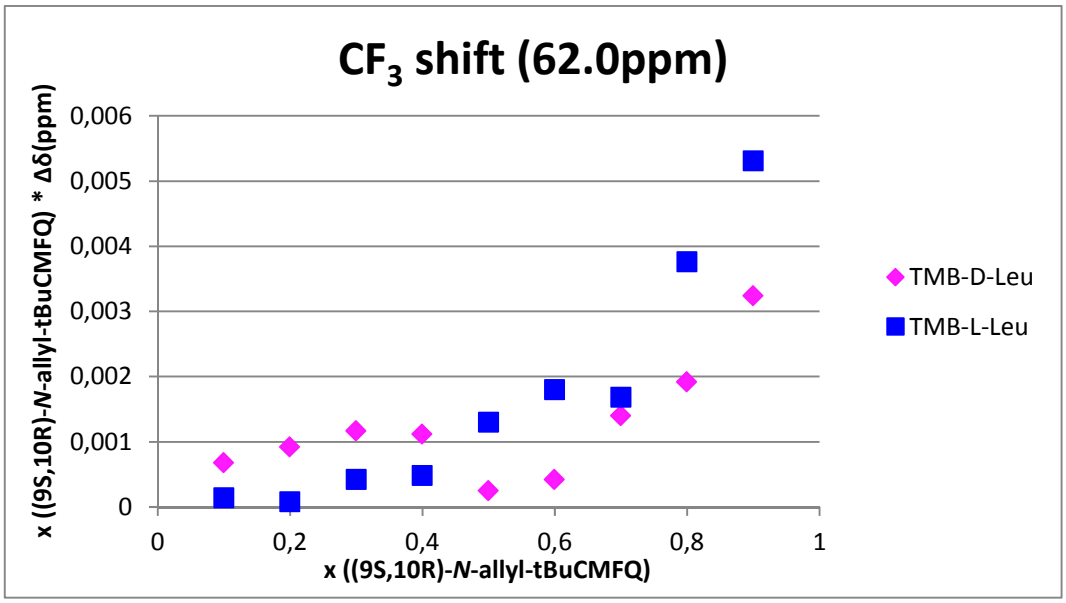


Figure 37: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and TMB-D-Leu, TMB-L-Leu respectively. The CF_3 (C-17) group was used for interpretation (62.0 ppm). The total concentration was 20 µM.

It can be observed that the complexation of TMB-L-Leu and mefloquine carbamate increase with the amount of the selector constantly. Contrary to this the interaction strength between TMB-D-Leu and mefloquine carbamate increases slightly until a ratio of 1:1 SO:SA, where it collapsed and afterwards it increase again.

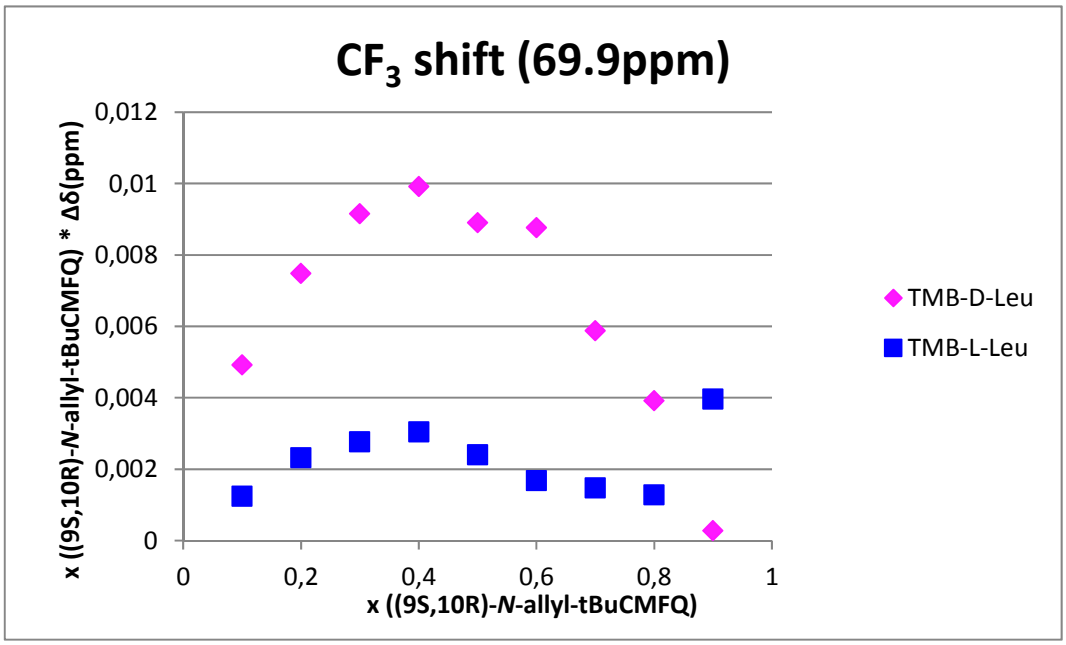


Figure 38: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and TMB-D-Leu TMB-L-Leu respectively. The CF_3 (C-16) group was used for interpretation (69.9 ppm). The total concentration was 20 µM.

Figure 38 shows that the interaction strength of TMB-D-Leu with (9S,10R)-*N*-allyl-*t*BuCMFQ is higher than the one of TMB-L-Leu. In general the same parabolic trend

of complexation can be observed for the selectands and the mefloquine carbamate with the highest point for 0.4 molar fraction of the selector.

These results indicate that the complexation of mefloquine with TMB-Leu is probably not based on strong $\pi-\pi$ interactions and thus the CF_3 (C-17) group is almost not affected. Therefore we decided to carry out a job plot for (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. We expected that we could observe stronger interactions between SO and SA based on fluorophilic interactions between the perflourinated core of protecting group and triflouromethyl groups of mefloquine. Because of stronger complex SO-D-Leu (see above) we decided to carry out the job plot measurement only with PFB-D-Leu.

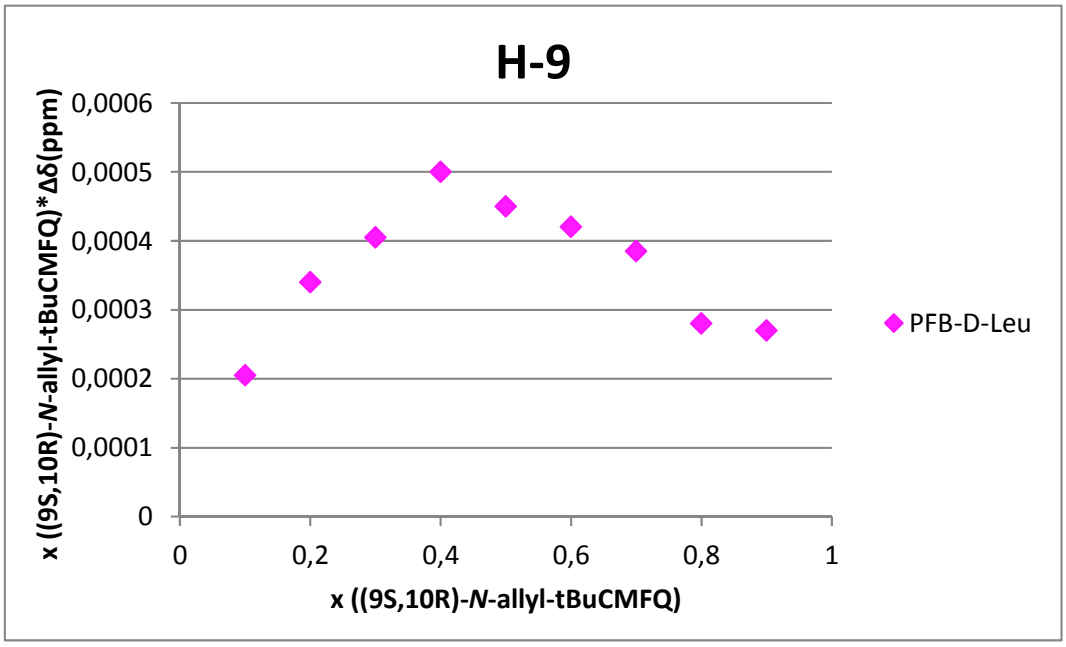


Figure 39: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. The chiral H-9 of mefloquine was used for interpretation. The total concentration was 20 µM.

Figure 39 shows a similar parabolic behaviour for the chiral proton located at the stereogenic centre of mefloquine like the complexation of the mefloquine carbamate and the TMB-D-Leu. However, in this case the highest interaction strength is observed by a molar ratio 0.4 of (9S,10R)-*N*-allyl-*t*BuCMFQ and 0.6 of PFB-D-Leu. Also it has to be noted that the SO-SA interaction is in this case almost 10 times weaker than for non-fluorinated SA (TMB-D-Leu).

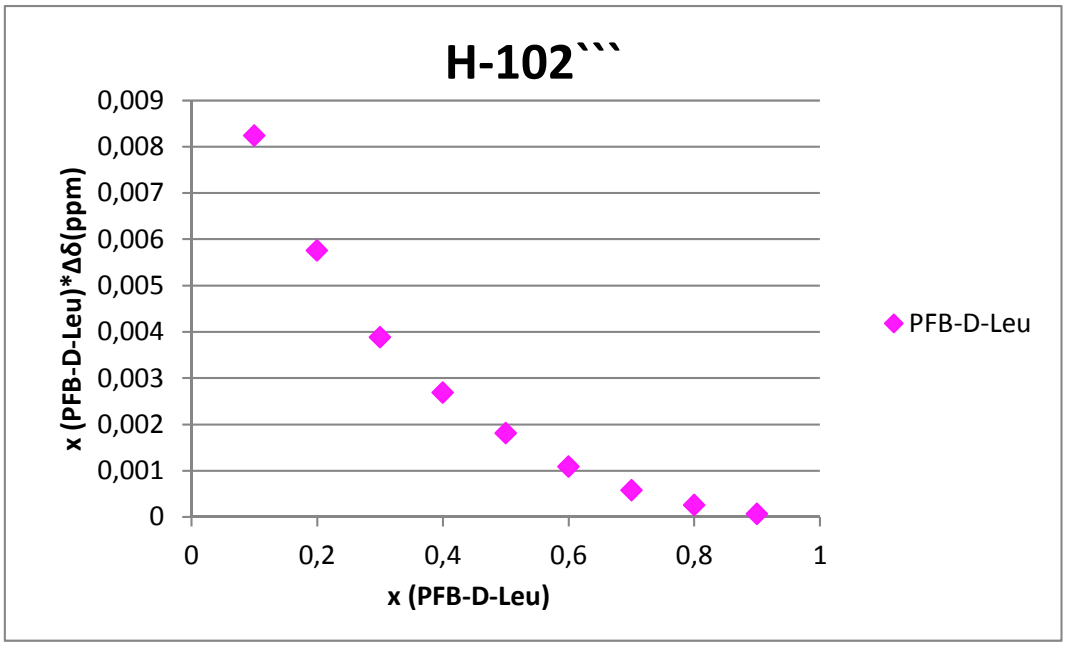


Figure 40: Job plot for the complexation of (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. The chiral H-102 of the fluorinated D-leucine derivative was used for interpretation. The total concentration was 20 µM.

For the chiral proton located on the stereogenic centre of the PFB-D-Leu it can be seen (**Figure 40**) that the interaction strength decreases with the amount of selectand. This could be caused by increasing dimerization of the selectand with its increasing concentration[42].That would mean that the fluorophilic interactions take place rather between two molecules of the SA than between one molecule of SO and one molecule of SA. Such behaviour would be further favoured by strong tendency of carboxylic acids to dimerize[43].

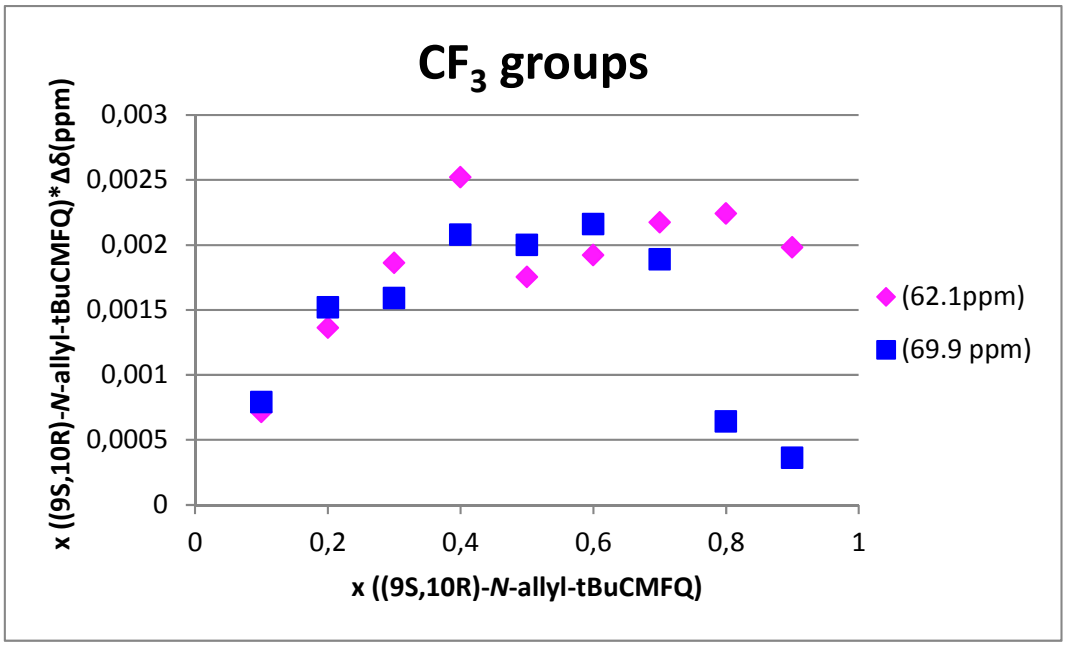


Figure 41: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. The CF_3 groups of mefloquine were used for interpretation. The total concentration was 20 µM.

For the trifluoromethyl groups of mefloquine no defined interactions can be observed, as it is seen in **Figure 41**.

In comparison to *N*-allyl-*t*BuCMFQ-TMB-Leu couple the CISs of trifluoromethyl groups are again almost 10 times weaker. In such a case it is hard to say whether the observed shifts are caused by direct SO-SA interaction or by some kind of diffusion processes in the diluted solution.

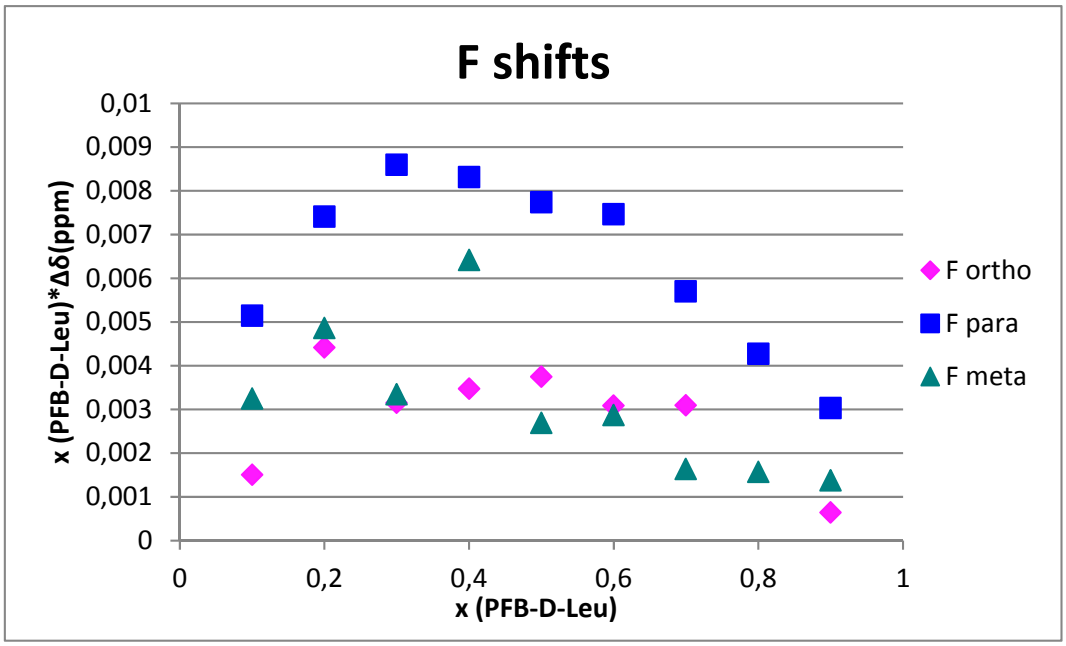


Figure 42: Job plot for the diastereomeric complexes of (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. The F signals of PFB-D-Leu were used for interpretation. The total concentration was 20 µM.

For the fluorine signals of the benzoyl group of the selectand also no defined interaction can be observed, with the exception of the fluorine atom in the para position. It exhibits a parabolic interaction profile (see **Figure 42**) with the highest interaction strength at 30% of the PFB-D-Leu. This behaviour might be caused by concentration dependent dimerization of the acid. That would mean that the interaction with the selector increased until a molar ration of 0.3 and 0.7 of SA and SO, respectively. After reaching this limit concentration the dimerization prevailed and the hydrogen bonding between piperidine nitrogen of the SO and the carboxylic group of the SA diminished.

Such behaviour would also explain the shift of *para*-fluorine of the SA, which is according to Hammett equation the most influenced functional group by hydrogen bonding of the carboxylic group[44].

Because we were not able to induce fluorophilic interaction by changing the structure of the protective group of the SA, we decided to modify also the structure of the mefloquine SO. We synthesized 3,5-bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine and carried out an NMR study with the pentafluorobenzoyl L-leucine (PFB-L-Leu) and pentafluorobenzoyl D-leucine (PFB-D-Leu). This selector and selectand should have stronger π-π- and fluorophilic interactions than the non-fluorinated ones.

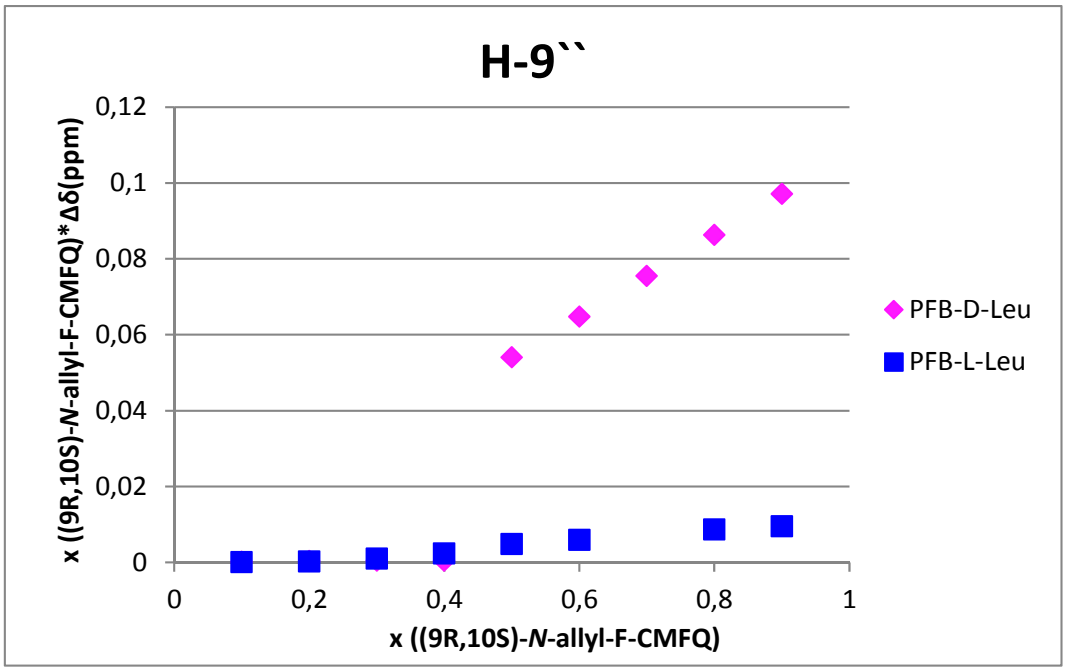


Figure 43: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The diagnostic H-9 of mefloquine was used for interpretation. The total concentration was 20 µM.

Figure 43 shows that there is no interaction between the selector and the selectand, the pentafluorobenzoyl L-Leu anymore. For pentafluorobenzoyl D-Leu it seems that interactions between the selector and the selectand occure only at higher concentrations of the selector and at lower concentrations, than a stoichiometric 1:1 ratio, no interactions can be observed.

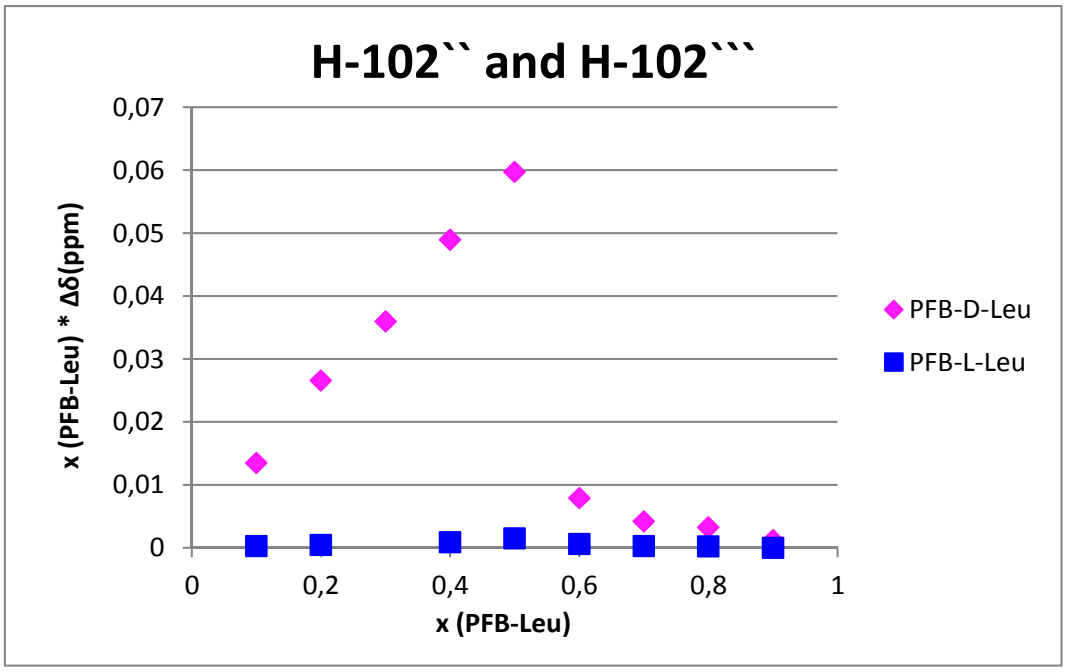


Figure 44: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The chiral H-102 of the fluorinated leucine derivatives was used for interpretation. The total concentration was 20 µM (the point at 0.3 molar fraction is a discordant value).

Figure 44 shows that no interaction can be observed for PFB-L-Leu with the fluorinated mefloquine selector. TMB-D-Leu exhibited the increasing interactions depending on the amount of selectand and they drop significantly once a complexation ratio of 1:1 was achieved.

Here we can again speculate about some kind of concentration dependent dimerization, which is supported by the linearity of the interactions depicted in **Figure 43** and **Figure 44**. However, in this case the dimer formation would be higher for lower concentrations of the acid, which is in contradiction to the theory and observed behaviour[39,42]. In order to clarify our observations additional experiments with a higher concentration of SO and SA are needed.

We also performed ^{19}F NMR measurements in the hope that we will obtain some additional information from potential fluorophilic interactions.

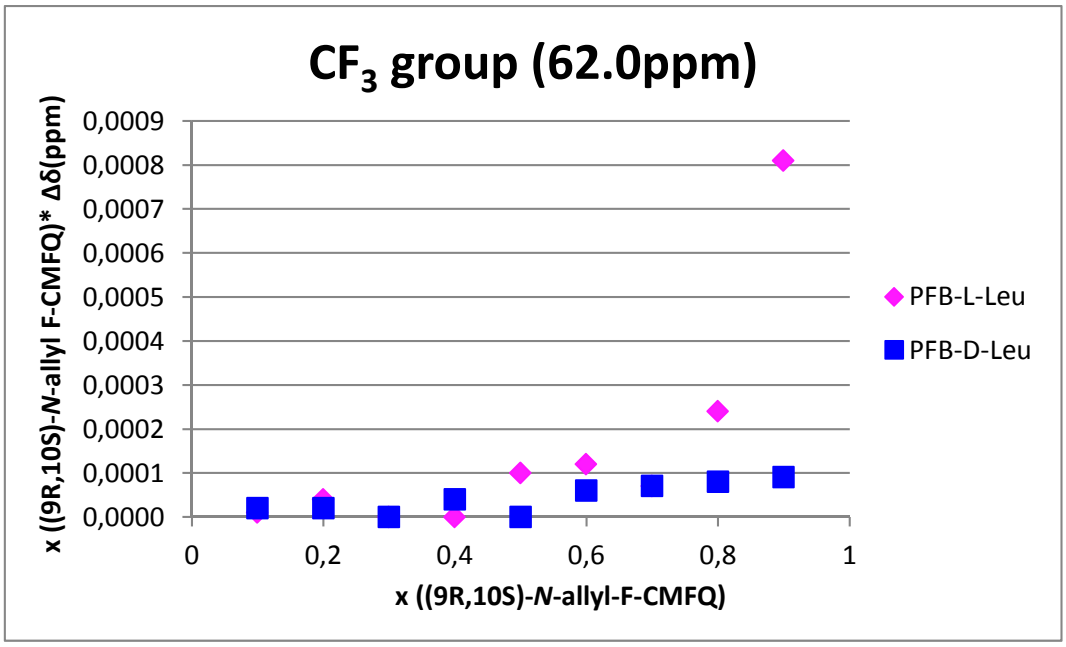


Figure 45: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The CF_3 (C-17) group of fluorinated mefloquine carbamate was used for interpretation (62.0 ppm). The total concentration was 20 µM.

Figure 45 and 46 show that almost no interaction can be observed among the trifluoromethyl groups of the quinoline ring (62.0 ppm) and the one of the carbamate functionality (65.1 ppm) and the pentafluorobenzoyl moiety of PFB-L-Leu. The CIS of the CF_3 groups increased only slightly with increasing concentration of the selector.

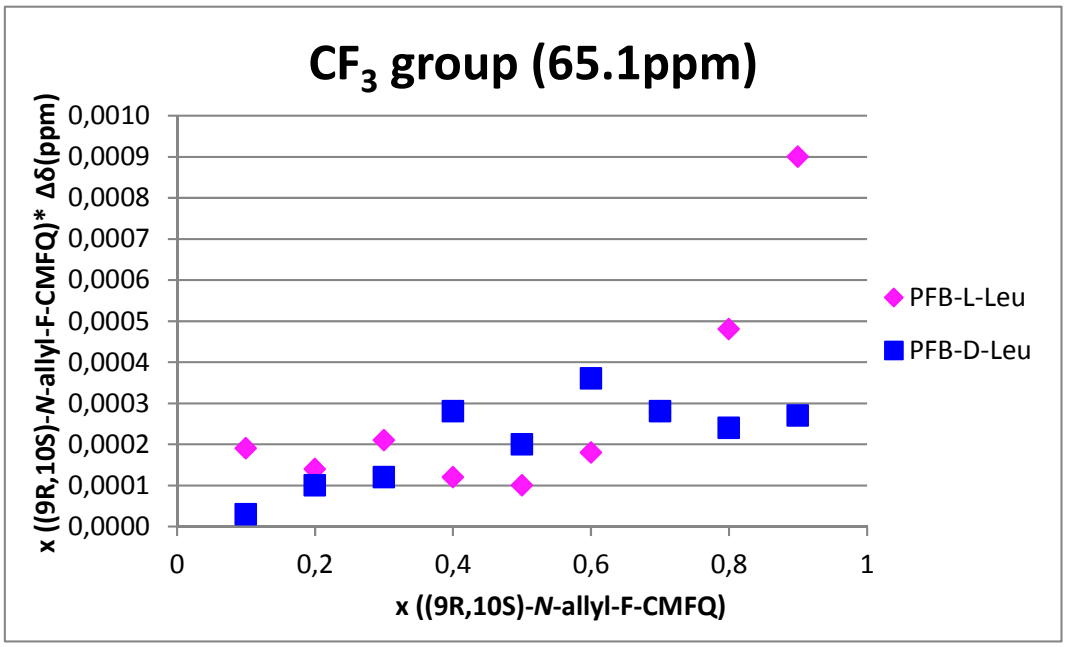


Figure 46: Job plot for the diastereomeric complexes of the (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, OFB-L-Leu respectively. The CF_3 group of the fluorinated carbamate functionality of mefloquine was used for interpretation (65.1 ppm). The total concentration was 20 µM.

Figure 47 shows slightly increasing interactions of PFB-D-Leu with the amount of fluorinated carbamate interpreted for the trifluoromethyl group next to the nitrogen in the quinoline of mefloquine (69.9ppm). For PFB-L-Leu the interactions increase with the amount of the selector.

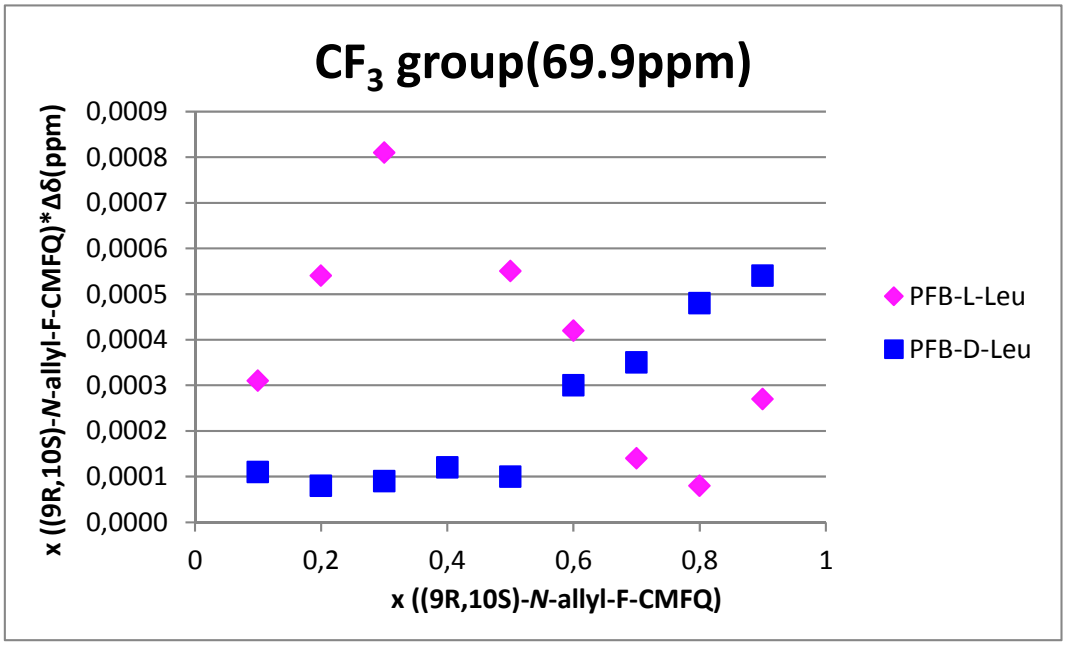


Figure 47: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The CF_3 group (C-16) of fluorinated mefloquine carbamate was used for interpretation (69.9 ppm). The total concentration was 20 µM.

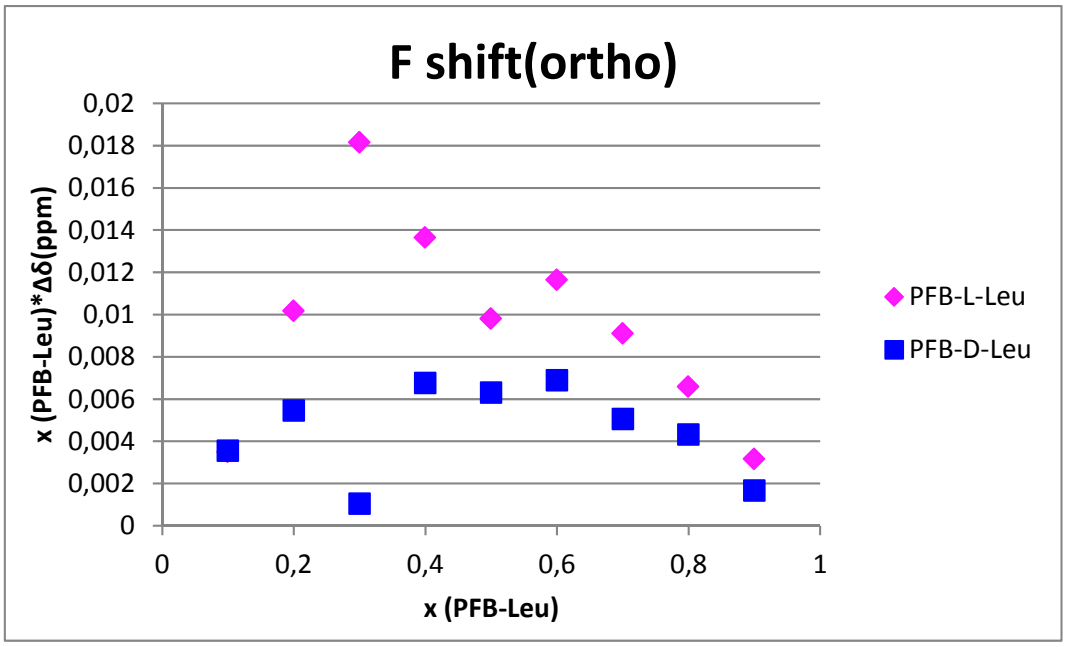


Figure 48: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The ortho F of fluorinated leucine deivative was used for interpretation. The total concentration was 20 µM.

For the F- signals of the selectand (pentafluorobenzoyl-leucine) the same trend can be observed (seen in **Figure 48, 49, 50**). For PFB-L-Leu the interactions increase until 0.3 molar fraction of selectand and then an increase of interactions can be observed. For PFB-D-Leu almost no interactions of the selectand and the selector can be observed.

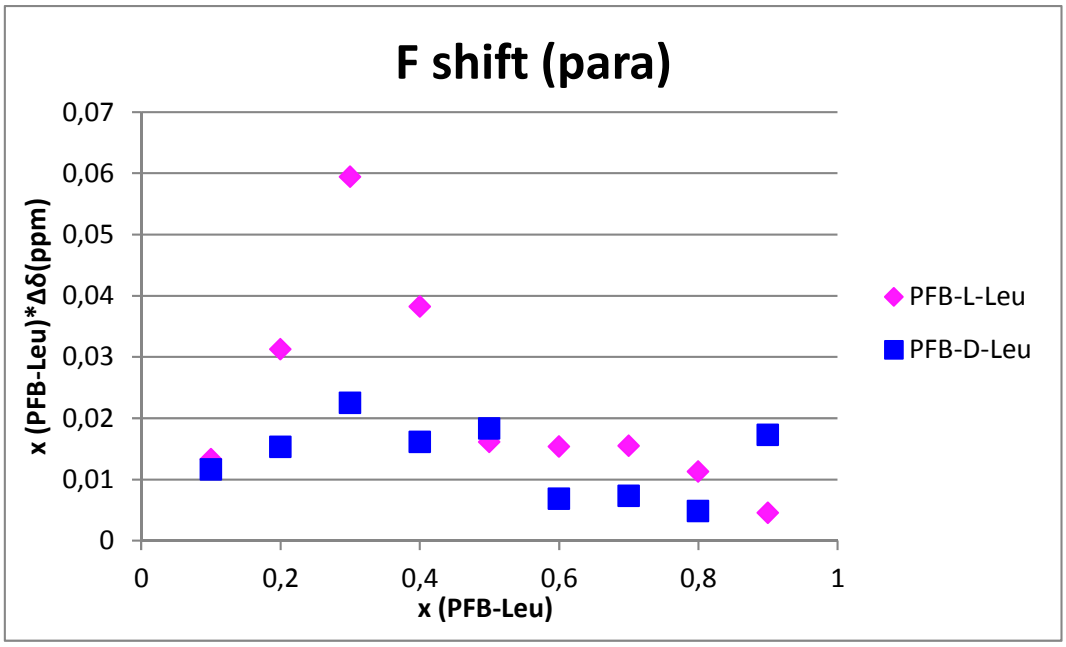


Figure 49: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The para F of fluorinated leucine deivative was used for interpretation. The total concentration was 20 µM.

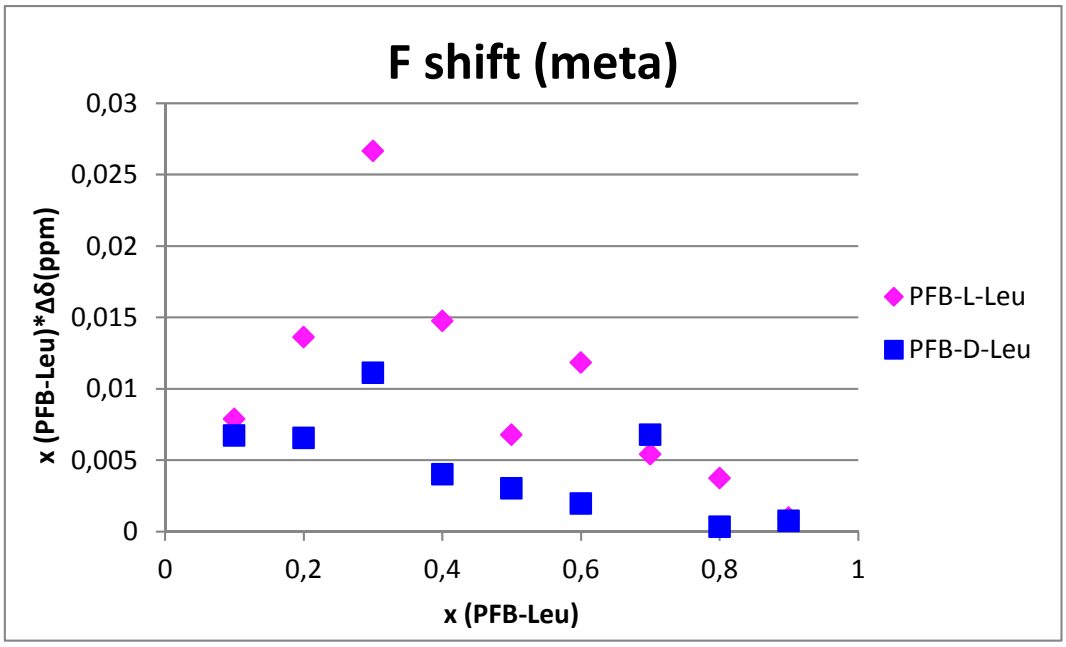


Figure 50: Job plot for the diastereomeric complexes of (9R,10S)-*N*-allyl-3,5-bis{trifluoromethyl}phenylcarbamoyl mefloquine and PFB-D-Leu, PFB-L-Leu respectively. The meta F of fluorinated leucine deivative was used for interpretation. The total concentration was 20 µM.

The expected increase of fluorophilic interactions by using the polyfluorinated mefloquine selector with the polyfluorinated selectand was not observed. Despite the high amount of fluorine in the selector and the selectand it seems that the

electrostatic interactions (like hydrogen bonding, formation of ion pairs) and the steric interactions are the main interactions for the enantiodiscrimination.

In general, interactions of the mefloquine carbamates and the selectands seemed to be much weaker compared to previous job plots which were carried out for chinchona derivatives and DNB-Leu[38]. Therefore we decided to carry out a job plot with (9S,8R)-*t*BuCQD and PFB-D-Leu. We chose quinidine carbamate because of the similarity to the mefloquine carbamate in terms of absolute configuration at C-9 and C-10 which allow us a direct comparison with the mefloquine selector.

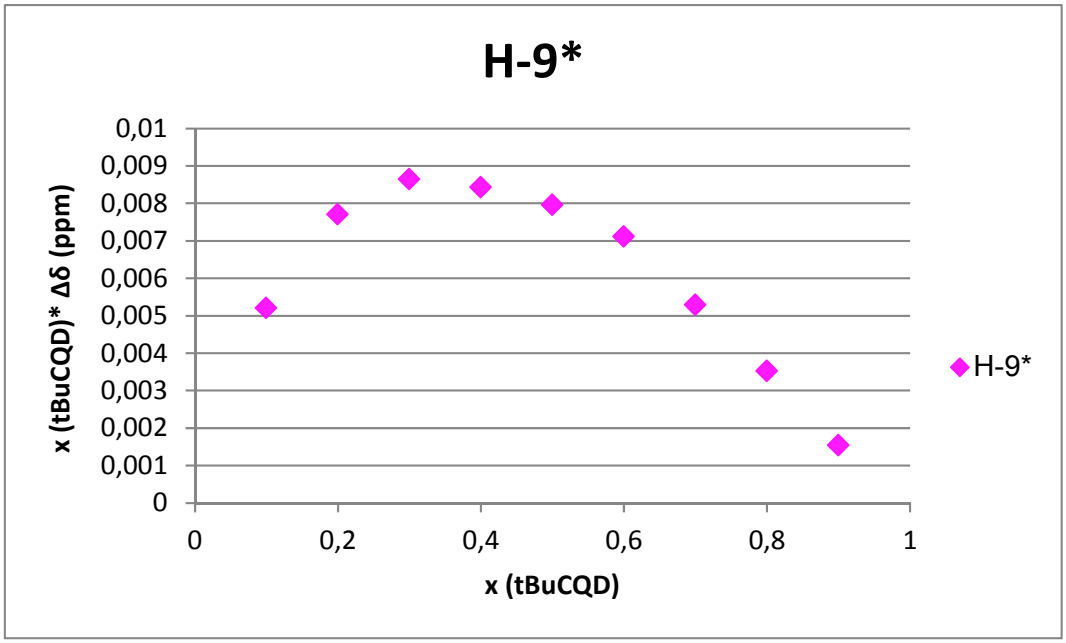


Figure 51: Job plot for the complexes of (9S,8R)-*t*BuCQD and PFB-D-Leu. The chiral H-9 of quinidine was used for interpretation. The total concentration was 20 µM.

Figure 51 shows a parabolic interaction profile of the quinidine carbamate and the PFB-D-Leu, similar to the one of the (9S,10R)-*N*-allyl-*t*BuCMFQ and the PFB-D-Leu with the exception that the highest interaction strength was observed for 0.3 mol fraction of quinidine carbamate. However, the interaction strength is about 10 times higher in case of *t*BuCQD than that of *t*BuCMFQ.

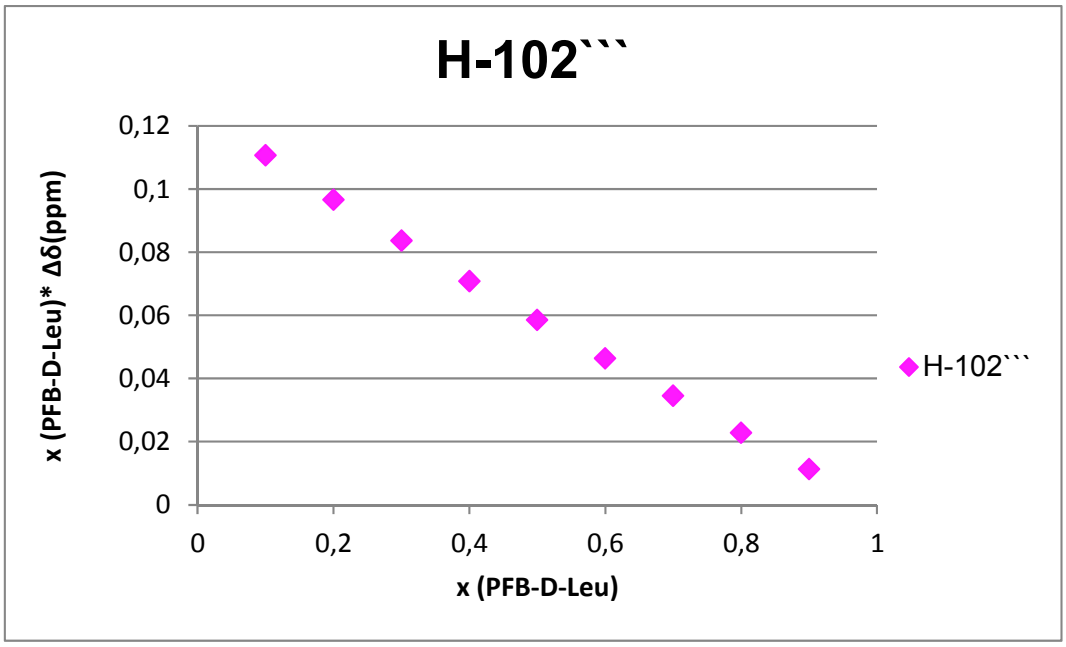


Figure 52: Job plot for the complexes of (9S,8R)-*t*BuCQD and PFB-D-Leu. The chiral H-102 of the fluorinated D-leucine derivative was used for interpretation. The total concentration was 20 µM.

The interaction strength of the PFB-D-Leu and the (9S,8R)-*t*BuCQD decreased with increasing concentration of PFB-D-Leu interpreted for the chiral proton located at the stereogenic centre of the selectand (see **Figure 52**). This might again be caused by dimerization of PFB-D-Leu as a similar behaviour was observed for the mefloquine carabamate and the same selectand.

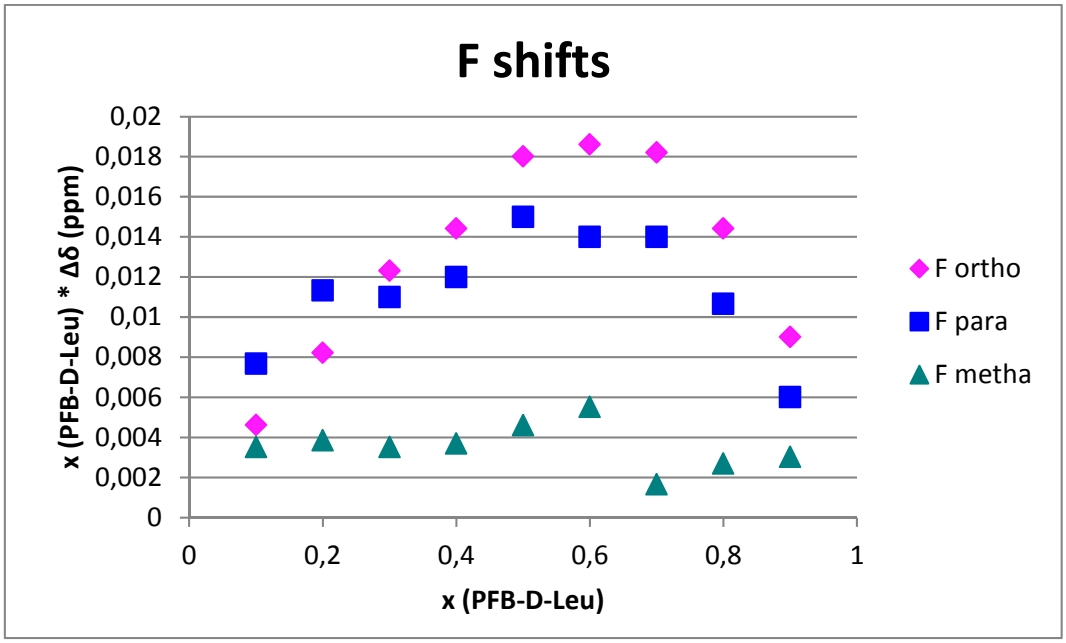


Figure 53: Job plot for the diastereomeric complexes of (9S,8R)-*t*BuCQD and PFB-D-Leu. The F signals of PFB-D-Leu were used for interpretation. The total concentration was 20 µM.

The interactions of (9S,8R)-*t*BuCQD and PFB-D-Leu according to the fluorine signals of the selectand seem more directed than for (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu. On the basis that quinidine has no fluorines the interactions cannot be driven by fluorophilic interactions. The electrostatic and steric interactions are most probably responsible for the enantiodiscrimination. The highest interaction strength is observed for *ortho*-fluorine and the lowest for *meta*-fluorine. As shown in **Figure 53** the interaction strength profile is again parabolic, with the maximum at 1:1 ratio of selector and selectand (*meta*-fluorine) or at 0.6 molar fraction of the selectand for *para*-fluorine. This corresponds with the shift of electron density in the aromatic core caused by hydrogen bonding[44]. As the CISs were generally more pronounced in this case, we assume that also an effective although weak π-π stacking was involved.

4. Discussion and Conclusion

4.1 Novel chiral stationary phases for HPLC

Chiral stationary phases based on *N*-allyl mefloquine

The evaluation of the *N*-allyl mefloquine based CSPs showed that enantioseparation could only be observed for analytes based on sulfonic acids. In general retention factors were quite low because of the low coverage of the selector on CSPs which was a consequence of immobilization problems. Despite optimization trials, no coverage improvement was observed; however, there are still options which can be tried to achieve better coverage in the future. Furthermore, the evaluation of the mefloquine based CSPs was done with a relatively small analyte set which should be expanded for a full characterization of the applicabilities of the novel CSPs. Therefore at least mobile phase composition should be varied.

Nevertheless, once the coverage of the selector is improved, mefloquine based CSPs should become an effective tool for separation of at least derivatives of sulfonic acids.

Novel SCX CSPs based on syringic acid

The novel SCX CSPs were developed for the analysis of mefloquine-based analytes. The evaluation showed that the new columns provide enantioseparation for all these compounds, except for *N*-allyl-*t*BuCMFQ. By enhancing π-π interactions with varying the composition of the mobile phase, we were able to achieve enantioseparation for *N*-allyl-*t*BuCMFQ. In general, the applicability of the new CSPs has to be further tested under different mobile phase conditions.

Moreover, we observed that the enantioseparation was improved for mefloquine derivatives where the nitrogen in the piperidine moiety remains non-derivatized and remains a secondary amino group. As a consequence of this fact, we designed a novel selector based on mefloquine which has a free nitrogen in the piperidine ring. This compound will be synthesized in frame of a subsequent diploma thesis.

The novel SCX CSPs showed also enantioseparation for several basic analytes. The (1S,2S) **CSP 4** shows slightly higher retention factors and selectivity values. On the other hand, (1R,2R) **CSP 3** shows higher resolution values than **CSP 4**. This difference was caused by higher number of plates in case of **CSP 3**, which can be explained by an inhomogeneous packing of the column. Because **CSP 3** and **4** are enantiomeric to each other, they also allowed the determination of elution orders of the analytes.

4.2 NMR studies of interactions between mefloquine and analytes

^{1}H-NMR and ^{19}F-NMR studies were carried out for the determination of complexation strength between mefloquine-based selectors and leucine derivatives.

NMR study for complexation of (9S,10R)-*N*-allyl-*t*BuCMFQ and TMB-DL-Leu

The interpretation of the ^{1}H-NMR job plot of the diagnostic proton located at the stereogenic centre C-9 shows (**Figure 36**) the strongest interaction strength at the equivalent amount of the selector and the selectand, which indicates that a 1:1 complex is formed. Based on the CISs obtained by NMR we assigned the complex with TMB-D-Leu as the stonger one. That means that in case of an efficient HPLC

column the TMB-D-Leu would be the more retained enantiomer. This result is completely in accordance with the well-established Chiralpak QD-AX column where (R)-enantiomers usually elutes before (S)-enantiomers.

The ^{19}F-NMR job plot of the trifluoromethyl groups shows different interaction behaviour (see **Figure 37** and **38**) compared to the ^{1}H-NMR job plot of the proton located at C-9. The complexation behaviour of the trifluoromethyl group located next to the nitrogen in the quinoline (69.9ppm) is parabolic with the highest shift by a ratio of 3:2 SA:SO. This indicates directed interactions of the selector and the selectand, at least in terms of protonation of nitrogen of the piperidine ring.

The second trifluoromethyl group shows an exponential behaviour for the interaction of TMB-L-Leu with increasing concentration of mefloquine carbamate, whereas TMB-D-Leu shows an exponential behaviour until an equivalent amount of selector and selectand is achieved.

In general, the chemical shift in NMR depends on the concentration of analytes. As a result, by constantly varying the concentration of the analyte the chemical shifts will change directly as well. If there are no specific interactions between the selector and the selectand, the difference in chemical shifts will be only concentration dependent. It is hard to say, if the exponential behaviour interpreted for trifluoromethyl group (62.0ppm) occurred only because of variation of the concentration or by other reasons, like unspecific interactions. Because the interaction strength is generally quite low, we suppose that this behaviour can be explained by insufficient SO-SA π-π stacking. As it is shown in **Figure 33** the aromatic part of TMB-Leu is quite spatially demanding, which most probably led to disruption of the π-π stacking. In that case the CISs, we observed, were only concentration dependent.

NMR study for complexation of (9S,10R)-*N*-allyl-tBuMFQ and PFB-D-Leu

In the hope to induce additional directed fluorophilic interactions between the selector and the selectand, we decided to carry out a job plot for (9S,10R)-*N*-allyl-*t*BuCMFQ and PFB-D-Leu.

For the diagnostic proton located at the sterogenic centre C-9 a parabolic interaction behaviour calculated for shift differences was observed. The maximum was observed

at a ratio of 7:3 SA:SO. Nevertheless a higher interaction strength caused by additional flourophilic interactions was not observed compared to the job plot where TMB-D-Leu was used as the selectand. The parabolic complexation behaviour indicates that directed interaction took place between the selector and the selectand.
The diagnostic proton located at the stereogenic centre of the PFB-D-Leu exhibited an exponential decrease with increasing concentration of the selectand. This indicates that interactions between selector and selectand are located in the region of the stereogenic centre of mefloquine carbamate and do not influence the stereogenic centre of the selectand. Such behaviour indicates that PFB-D-Leu favours dimerization at higher concentration. This can be ascribed to the ability of carboxylic acids to dimerize which in this case is supported by fluorophilic interactions between the pentafluorobenzoyl moieties.
Interpretation of the CISs of the trifluoromethyl groups of (9S,10R)-*N*-allyl-*t*BuCMFQ and the fluorine atoms of PFB-D-Leu measured with ^{19}F-NMR indicates only a specific interaction like ion pairing which influenced only *para*-fluorine in PFB-D-Leu.
In general no fluorophilic interactions were observed, because such interactions would influence the complexation behaviour of the ^{19}F signals in a similar way and there is no trend which can be observed. Consequently we decided to enhance the amount of fluorine with an expectation, if fluorophilic interactions are achievable they have to be observable with a high amount of fluorine.

NMR study for complexation of (9R,10S)-*N*-allyl-F-MFQ and PFB-DL-Leu

As mentioned before we decided to carry out ^{1}H-NMR and ^{19}F-NMR studies with (9R,10S)-*N*-allyl-3,5-bis-{trifluoromethyl}phenylcarbamoyl mefloquine, and PFB-L-Leu and PFB-D-Leu, in order to improve fluorophilic interactions. We also supposed that the additional aromatic functionality of the mefloquine carbamate could enhance π-π interactions abilities of the selector.
As a consequence of the reversal of absolute configuration of the (9R,10S)-*N*-allyl-F-CMFQ compared to the previous NMR studies (9S,10R)-*N*-allyl-*t*BuCMFQ, we suggested that the CISs are higher for PFB-L-Leu than for PFB-D-Leu. However, no defined complexation behaviour between the selector and the selectands can be

observed for the stereogenic protons of selector and selectand measured with ^{1}H-NMR.
Generally it seems that the high amount of fluorine (in total 17 fluorine) in the selector and the selectand disturb selective interactions. The CISs of trifluoromethyl groups and fluorine atoms of the selectand indicate that formation of dimers is probably a preferred form of interaction. This theory has to be confirmed by NMR experiments at higher concentrations.

NMR study for complexation of (9S,8R)-tBuCQD and PFB-D-Leu

For a direct comparison of the behaviour of our new selector we decided to carry out a ^{1}H-NMR and a ^{19}F-NMR study with *t*BuCQD and PFB-D-Leu.
Interpretation of the complexation induced shifts for the proton located at the stereogenic centre C-9 of quinidine showed a parabolic behaviour with the maximum at 0.3 molar fraction of *t*BuCQD, which is comparable to the mefloquine selector.
For the proton located at the stereogenic centre of PFB-D-Leu an almost linear decrease can be observed, perhaps caused by the variation of the concentration or by formation of dimers caused by favoured PFB-Leu:PFB-Leu interactions.
The interpretation of the ^{19}F-NMR study shows that the interaction of the quinidine carbamate and the PFB-D-Leu generated a parabolic profile for the fluorine signals as well. These interactions are most probably of electrostatic nature. Compared to the NMR study carried out with mefloquine carbamate, where only the para substituted fluorine shows a similar behaviour, it seems that in case of quinidine also π - π stacking took place.

Conclusion:

NMR studies are a convenient method to determine the applicability of a novel selector for enantioseparation. Therefore only 150 mg of the novel selector is needed and so it is not necessary to synthesize the novel selector in a big scale, which is often quite time-consuming and expensive.

Based on the results of the NMR study, we can say that the *N*-allyl-*t*BuCMFQ selector is not as efficient for enantioseparation as the *t*BuCQD selector.

5. Materials and Instruments

Mefloquine hydrochloride was purchased from Kreamer & Martin Pharma Handels-GmbH (Germany). Synthesis reagents were predominantly purchased by Sigma Aldrich (Vienna, Austria) and were of reagent grade or higher purity. The solvents used for syntheses were of technical grade and purchased from VWR (Darmstadt, Germany). The HPLC grade solvents were acquired from VWR (Darmstadt, Germany), from Sigma-Aldrich (Vienna,Austria) or from Carl Roth GesmBH (Karlsruhe, Germany).

Mobile phase additives (diethylamine, formic acid, *etc*.) were either from Sigma-Aldrich or Fluka (via Sigma Aldrich, Germany).

The NMR solvents were purchased from Deutero GesmBH (Kastellaun, Germany).

Analytes for evaluation of novel SCX and AX columns were synthesized on our own, by coworkers or were gifts from other research groups.

Reactions were often controlled with TLC plates on Silica gel 60 F_{254} (Merck, Germany) with UV detection at 254 nm in a cabinet from CAMAG. The silica gel 60 which was used for column chromatography in order to purify syntheses products was purchased from Merck KGAA (Darmstadt, Germany)

MS measurements were performed on 4000 Q TRAP LC/MS/MS System (AB Applied MDS SCIEX) with a ESI source for ionisation and the coupled HPLC system was from Agilent Technologies (Series 1200).

NMR measurements were carried out on a Bruker DRX 600 MHz or DRX 400 MHz NMR spectrometer. NMR spectra were evaluated with the NMR software SpinWorks.

Elemental analysis from the immobilized selectors on mercaptopropyl was performed on EA 3000 (eurovector).

Melting points were performed on a Kofler from Gallen^{TM} III Reichert Jung.

HPLC measurements were carried out either on a 1100 Series HPLC or on a 1260 Infinity HPLC from Agilent Technologies.

6. Experimental

6.1 Synthesis of the starting material *N*-allyl mefloquine

N-allyl mefloquine (2):

In a 250 mL round bottom flask the MFQ salt (**1**) (10.0 g; 24 mmol) and K_2CO_3 (3.33 g; 24 mmol) were placed with a stirrer. The flask was flushed with nitrogen. Dry DMF (60 mL) was added and the suspension was stirred for 15 min. Subsequently, allyl bromide (2.09 mL; 24 mmol) was added, the nitrogen source was removed and the reaction flask was fitted with a drying tube ($CaCl_2$). The reaction mixture was stirred for 17 h at room temperature, diluted with distilled water (150 mL) and stirred further for 30 min. The precipitate was filtered, washed with methanol (2x20 mL) and dried in vacuum. The crude product was purified by crystallization from methanol[6]. It was obtained 6.94 g (69%) of compound **2;** m.p 124.8-125.9 °C; m.p. lit.[6] 121-123 °C. **^{1}H NMR** (400 MHz,$CDCl_3$): 0.74(m); 0.94(d); 1.08(m); 1.33(m); 1.576(m); 1.72(m); 1.92(m); 2.05(m); 2.22(m); 2.80(s); 2.88(s); 2.93(m); 3.24(m); 3.41(d); 3.63(d); 3.74(m); 3.98(d); 4.07(dd); 4.17(m); 4.69(m); 4.87(d); 5.13(d); 5.26(dd); 5.35(d); 5.42(s); 5.47(s); 5.74(d) ;5.91(m); 5.96(s); 6.08(m); 6.22(m); 6.28(s); 6.45(s); 7.15(t); 7.62(t); 7.73(m); 7.93(m); 8.01(s); 8.05(t); 8.15(d); 8.94(d).
The complexity of the spectrum did not allow a precise assignment of all signals.

N-allyl-mefloquine HCl salt (2c):

To a solution of **2** (0.20 g; 0.48 mmol) in methanol (10 mL), a 3 M solution of HCl in methanol (239 µL; 0.71 mmol) was added. The reaction mixture was stirred without presence of air moisture ($CaCl_2$ drying tube) at room temperature over night. Evaporation of the solvent afforded 0.19 g (87%) of **2c**. **^{1}H NMR** (400 MHz, MeOD): 1.0 (d, 1 H, CH_2); 1.3 (dt, 2 H, CH_2); 1.75 (m, 2 H, CH_2); 1.85 (m, 2 H, CH_2); 3.2 (dt, 2 H, CH_2); 3.6 (d, 1 H, CH); 3.8 (d,1 H, CH); 4.2 (dt, 1 H, CH); 4.4 (dd, 1 H, CH); 5.85 (m, 2 H, CH_2); 6.3 (m ,2 H, CH_2); 8.0 (t, 1 H, *J* = 8.0 Hz, CH); 8.2 (s,1 H, CH); 8.3 (d, 1 H, *J* = 7.3 Hz, CH); 8.4 (d, 1 H, *J* = 8.5 Hz, CH). **^{13}C NMR** (100 MHz, MeOD): 23.0(CH_2); 24.5(CH_2); 25.0(CH_2); 54.7(CH_2); 57.0(CH_2); 66.4(CH); 66.7(CH); 117.4(CH); 124.0(C_{ar}); 126.8(CF_3); 127.3($\underline{CH_2}$=CH); 127.8(CF_3); 128.4(CH_{ar});

129.2(CH_{ar}); 129.9(CH_{ar}); 131.0(CH=CH_2); 145.3(C_{ar}); 149.5(C_{ar}); 149.9 (C_{ar}); 151.6(C_{ar}).

(9S,10R)-*N*-allyl mefloquine (*O*,*O*)-diacetyl-(R,R) mono-tartrate (3a):

In a 250 mL nitrogen flushed round bottom flask *N*-allyl mefloquine (**2)** (6 g; 14.34 mmol) and (R,R)-DATAAN (7.75 g; 35.85 mmol) were placed with a stirrer. Dry DCM (60 mL) was added and the solution was stirred over night. To avoid decomposition of the anhydride because of humidity the flask was fitted with a drying tube ($CaCl_2$). The reaction mixture was extracted with 5% aqueous $NaHCO_3$ (150 mL; pH~ 8 of the water layer), with 10% aqueous HCOOH (100 mL) and with saturated solution of NaCl (20 mL). The organic layer was dried with $MgSO_4$ and the solvent was evaporated. The crude racemic product (8.5 g; 93%) was placed in a soxhlet extractor and continuously washed with toluene for 6 h. The crystals (97% de) were removed from the extraction tube, dissolved in a solution of *tert*-butyl methyl ether and ACN, and the solvent was evaporated. The resulting solid was placed into the soxhlet extractor and washed with hot toluene further for 6 h. The system was left to cool down to room temperature and the monotartrate **3a** 3.6 g (93 %) was obtained as a crystalline material from the soxhlet extractor tube. m.p.186.2-187.8 °C.

^{1}H NMR (400 MHz, $CDCl_3$): 1.21 (m, 1 H, CH_2); 1.35 (m, 1 H, CH_2); 1.91 (m, 2 H, CH_2); 2.05 (s, 3 H, CH_3C=O); 2.07 (s, 3 H, CH_3C=O); 2.25 (m, 1 H, CH_2); 2.48 (m, 1 H, CH_2); 3.07 (m, 1 H, CH_2); 2.42 (m, 1 H, CH_2); 3.75 (m, 1 H, CH_2); 4.21 (m, 1 H, CH_2); 4.49 (m, 1 H, CH); 5.71 (s, 1 H, CH); 5.83 (d, 1 H, CH_2=); 5.88 (d, 1 H, CH_2=); 6.30 (m, 1 H, CH=); 6.57 (bs, 2 H, 2xCH); 7.80 (t, 1 H, *J* = 7.6 Hz, CH); 7.98 (s, 1 H, CH); 8.03 (d, 1 H, J = 8.3 Hz, CH); 8.16 (d, 1 H, *J* = 7.4 Hz, CH). **^{13}C NMR** (100 MHz, $CDCl_3$): 20.0(CH_3); 20.7(CH_3); 22.8(CH_2); 24.1(CH_2); 24.3(CH_2); 56.2(CH_2); 58.5(CH_2); 66.7(CH); 70.1(CH); 74.4(CH); 74.8(CH); 117.3(CH_{ar}); 123.6(C_{ar}); 126.8(C_{ar}); 128.1(CH_2=); 128.8(CF_3); 129.0(CH_{ar}); 129.7(CH=); 129.9(CF_3); 130.8(CH_{ar}); 130.9(CH_{ar}); 145.0(C_{ar}); 145.2(C_{ar}); 148.7(C_{ar}); 168.0 (C=O); 171.9(C=O); 172.0(C=O); 172.8 (C=O).

(9R,10S)-*N*-allyl mefloquine (*O*,*O*)-diacetyl-(R,R)-mono-tartrate (3b):

The extraction solvent was evaporated and the crude product was crystallized from MeOH. It was obtained 3.0 g (77%) of the diastereomer **3b**, m.p.: (9R) 195.4-196.9 °C. The reaction control was performed with HPLC (mobile phase: 50% H_2O and 0.1% FA; 50% ACN and 0.1 % FA; stationary phase: eclipse XDB-C18 5 µm, 4.6 x 150 mm).

^{1}H NMR (400 MHz, $CDCl_3$): 1.18 (m, 1 H, CH_2); 1.28 (m, 1 H, CH_2); 1.85 (m, 2 H, CH_2); 2.05 (s, 3 H, CH_3C=O); 2.07 (s, 3 H, CH_3C=O); 2.22 (m, 1 H, CH_2); 2.40 (m, 1 H, CH_2); 3.03 (m, 1 H, CH_2); 2.36 (m, 1 H, CH_2); 3.70 (m, 1 H, CH_2); 4.18 (m, 1 H, CH_2); 4.44 (m, 1 H, CH); 5.70 (d, 1 H, *J* = 1.4 Hz, CH); 5.86 (d, 1 H, CH_2=); 5.93 (d, 1 H, CH_2=); 6.26 (m, 1 H, CH=); 6.55 (bs, 1 H, CH); 6.58 (d, 1 H, J = 1.4 Hz, CH); 7.79 (t, 1 H, *J* = 7.8 Hz, CH); 8.00 (s, 1 H, CH); 8.03 (d, 1 H, J = 8.4 Hz, CH); 8.17 (d, 1 H, *J* = 7.2 Hz, CH); 11.1 (bs, 1 H, COOH). **^{13}C NMR** (100 MHz, $CDCl_3$): 20.4(CH_3); 21.1(CH_3); 22.6(CH_2); 22.9(CH_2); 24.4(CH_2); 55.6(CH_2); 56.2(CH_2); 63.4(CH); 69.3(CH); 70.6(CH); 71.7(CH); 117.1(CH_{ar}); 124.9(C_{ar}); 125.4(C_{ar}); 125.7(CH_{ar}); 126.1(CH_{ar}); 128.1(CH_2=); 128.1(CH_{ar}); 128.8(CH=); 128.8(CF_3); 129.7(CF_3); 142.4(C_{ar}); 144.4(C_{ar}); 165.9 (C=O); 166.6(C=O); 170.8(C=O); 171.4 (C=O). **MS:** [M+H^+]=635.3 calculated [M+H^+]=634.17+1.01.

(9S,10R)-*N*-allyl mefloquine (2a):

The compound **3a** (3.5 g, 5.81 mmol) was dissolved in a mixture of distilled H_2O (60 mL) and DCM (60 mL). The pH was set to 10 with K_2CO_3 (10% in distilled H_2O) and the solution was stirred for 16 h. The separated aqueous phase was extracted with dichloromethane (2x40 mL). The combined organic solution was dried with $MgSO_4$, filtered and evaporated. After purification with column chromatography (CH_2Cl_2/ MeOH; 20:1) (9S, 10R)-*N*-allyl mefloquine (**2a**) 2.0 g (82%) was obtained.

(9R,10S)-*N*-allyl mefloquine (2b):

In the similar way, (9R,10S)-*N*-allyl mefloquine (**2b**), 1.5 g, (75%) was prepared.

6.1.1 Synthesis of (R,R)-DATAAN (5)

The (R,R)-tartaric acid (20 g; 133 mmol) was placed in a round bottom flask, which was fitted with two reflux condensers and a mechanical stirrer. A solution of concentrated H_2SO_4 (0.6 mL) in acetic anhydride (63 mL) was added and the resulting solution was stirred and slowly heated. After the boiling point was reached the solution was stirred for further 10 min. The reaction mixture was cooled down to room temperature, transferred into an ice bath cooled Erlenmeyer flask and kept in ice for 90 min. The precipitate was filtered and in the inert atmosphere of nitrogen washed with benzene (2x20 mL). The product was placed in a round bottom flask suspended in dry diethylether (87 mL) and mechanically stirred at 4 °C for 30 min. The product was filtered under nitrogen atmosphere and 19.38 g (67%) of compound **14** were isolated. The product was stored in an evacuated dessicator above P_2O_5 and parafine.

6.2 Preparation of carbamates of (9S,10R)- and (9R,10S)-*N*-allyl mefloquine

(9S,10R)-*tert*-butylcarbamoyl-*N*-allyl mefloquine (4a):

N-Allyl mefloquine (**2a**) (0.5 g; 1.20 mmol) was dissolved in toluene (30 mL). A part of the solvent (5 mL) was distilled off and *tert*-butylisocyanate (0.341 µL; 2.99 mmol) and dibutyltin dilaurate as a catalyst (20 µL) were added and the reaction mixture was heated to 90 °C for 24 h. The solvent was evaporated and the procedure was repeated. The crude product was dissolved in dry toluene (30 mL), from which 5 mL was distilled off, and *tert*-butylisocyanate (136 µL; 1.19 mmol) and the catalyst (20 µL) were added and the solution was heated up for 2 days. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (CH_2Cl_2/MeOH 20:1). It was obtained 0.52 g (84%) of compound **4**, m.p. 49.5-50.5 °C**.** **^{1}H NMR** (600 MHz, $CDCl_3$): 1.13 (m, 2 H, $H_{14a,b}$); 1.32 (s, 9 H, $(CH_3)_3$); 1.61 (m, 2 H, $H_{13a,b}$); 1.72 (m, 2 H, $H_{15a,b}$); 2,52 (dt, 1 H, H_{12b}, ^2J = 11.8 Hz, ^3J = 3.3 Hz); 2.91 (d, 1 H, H_{10}, ^3J = 9.6 Hz); 3.12 (d, 1 H, H_{12a}, ^2J = 11.8 Hz); 3.46 (dd, 1 H, H_{18b}, ^2J = 14.2 Hz, ^3J = 8.2 Hz); 3.71 (dd, 1 H, H_{18a}, ^2J = 14.2 Hz, ^3J = 4.1 Hz); 5.4 (d, 1 H, H_{20b}, ^3J = 10.3 Hz); 5.46 (d, 1 H, H_{20a}, ^3J = 17.3 Hz); 6.11 (m, 1 H, H_{19}); 6.88

(d, 1 H, H_9, ^3J = 3.6 Hz); 7.93 (t, 1 H, H_6, ^3J = 8.1 Hz); 7.97 (s, 1 H, H_3); 8.30 (d, 1 H, H_7, ^3J = 7.2 Hz); 8.54 (d, 1 H, H_5, ^3J = 8.5 Hz). **^{13}C NMR** (150 MHz, $CDCl_3$): 154.6(C=O); 150.3(C_4); 148.0(C_2); 143.9(C_{8a}); 134.5(C_{19}); 129.6(C_7); 128.5(C_5); 128.0(C_6); 128.4(C_{17}); 127.9(C_{16}); 126.8(C_{4a}); 123.1(C_8); 118.7(C_{20}); 115.4(C_3); 69.6(C_9); 62.6(C_{10}); 56.9(C_{18}); 53.0(C_{12}); 50.3(C_{25}); 28.0(C_{26}); 24.6(C_{15}); 24.4(C_{13}); 23.4(C_{14}). **^{19}F NMR** (565 MHz, MeOD): -62.10(CF_3); -69.97(CF_3). **MS**: $[M+H]^+$ = 518.0, calculated $[M+H]^+$ = 517.22+1.01.

Using the same procedure, **(9R, 10S)-*tert*-butylcarbamoyl-*N*-allyl mefloquine** (**4b**) was prepared. It was obtained 0.44 g (71%) of highly viscous oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (m, 2 H, CH_2); 1.21 (bs, 9 H, $(CH_3)_3$); 1.49 (m, 2 H, CH_2); 1,60 (m, 2 H, CH_2); 2.38 (m, 1 H, CH_2); 2.77 (m, 1 H, CH_2); 3.00 (m, 1 H, CH_2,); 3.33 (m, 1 H, CH); 3.56 (m, 1 H, CH_2,); 5.26 (d, 1 H, $\underline{CH_2}$=CH); 5.32 (d, 1 H, $\underline{CH_2}$=CH); 5.97 (d, 1 H, CH, $\underline{CH}$=CH_2); 6.75 (d, 1 H, CH, H_9); 7.81 (d, 1 H, t, *J* = 8.3 Hz, H6); 7.90 (s, 1 H, H_3); 8.19 (d, 1 H, *J* = 7.4 Hz, H_7); 8.43 (d, 1 H, *J* = 7.8 Hz, H_5). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 151.9 (C=O); 149.5 (C_{ar}); 148.0 (C_{ar}); 143.9 (C_{ar}); 136.2 ($\underline{CH}$=CH_2); 130.9 (CH_{ar}); 129.9 (CH_{ar}); 129.3 (CH_{ar}); 128.3 (CF_3); 126.8 (CF_3); 124.1 (C_{ar}); 119.8 ($\underline{CH_2}$=CH); 118.3 (C_{ar}); 116.8 (CH_{ar}); 71.2 (CH); 64.0 (CH); 58.3 (CH_2); 54.3 (CH_2); 51.7 (C_q); 29.4 (CH_3); 25.8 (CH_2); 24.9 (CH_2); 24.0 (CH_2). **^{19}F NMR** (375 MHz, MeOD): -62.10(CF_3); -69.97(CF_3). **MS**: $[M+H]^+$ = 518.0, calculated $[M+H]^+$ = 517.22+1.01.

3,5-bis{trifluoromethyl}phenylcarbamoyl-*N*-allyl mefloquine (6):

N-Allyl mefloquine (**2b**) (0.100 g; .0.24 mmol) was dissolved in dry THF (2 mL) and a solution of 3,5-bis{trifluoromethyl}phenyl isocyanate (65.8 mg; 0.26 mmol) in THF (1 mL) was added drop wise at 0 °C and the reaction mixture was stirred in the nitrogen atmosphere over night. After purification with double column chromatography and drying the product for several hours in high vacuum, the compound **31** was obtained 0.126 mg (78 %), m.p. 47.2-49.3 °C. **^{1}H NMR** (400 MHz, MeOD): 1.20 (m, 2 H, CH_2); 1.50 (m, 2 H, CH_2); 1.68 (m, 2 H, CH_2); 2.35 (dt, 1 H, CH_2); 2.89(dt, 1 H, CH); 3.01(dt, 1 H, CH_2); 3.33 (dd, 1 H, CH_2); 3.66 (dd, 1 H, CH_2);5.17 (d, 1 H, CH_2); 5.26 (d, 1 H, CH_2); 5.82(m, 1 H, CH); 7.06 (d, 1 H, CH); 7.50 (s, 1 H, CH); 7.85 (t, 1 H, CH); 7.94

(s, 1 H, CH); 7.99(s, 1 H, CH); 8.22(d, 1 H, CH); 8.52(d, 1 H, CH). **^{13}C NMR** (100 MHz, MeOD): 24.46(CH_2); 25.46(CH_2); 53.80(CH_2-N); 58.51(CH_2-N); 64.03(CH-N); 72.14(CH-O); 114.87(CH_{ar}); 116.99(CH=C); 117.43(CH_{ar}); 119.74(CH_{ar}); 119.28(C_{ar}); 120.19(CH_{ar}); 128.32(C_{ar}); 129.48(CH_{ar}); 129.86(CH_{ar}); 131.07(CH_{ar}); 133.66(C_{ar}); 134.00 (C_{ar}); 136.67 (CH_{ar}); 142.55 (C_{ar}); 150.84 (C=C); 154.56 (C=O). **^{19}F NMR** (375 MHz, MeOD): -62.10(CF_3); -65.30(CF_3); -70.02(CF_3). **MS**: $[M+H]^+$ = 674.4 calculated $[M+H]^+$= 673.16+1.01

6.3 Immobilization of pure enantiomers of *N*-allyl mefloquine and its *t*Bu-carbamates

Immobilization of *N*-allyl mefloquine (2)

The selector (0.5 g; 1.19 mmol) and the mercaptopropyl modified silica gel (3 g) were placed in a 500 mL three necked round bottom flask. The flask was fitted with a mechanical stirrer, a reflux condenser and it was flushed with nitrogen. Methanol (30 mL) and AIBN (39 mg; 0.24 mmol) were added and the suspension was heated under reflux for 18 h. The reaction mixture was cooled down to room temperature and filtered. The product was washed with methanol. The chiral stationary phase was transferred into a round bottom flask and boiled in methanol. The filtered silica gel was dried for 3 h in a drying cabinet (60 °C) and for 12 h in a vacuum drying cabinet (60 °C).

Table 9: The elemental analysis of the *N*-allyl mefloquine selector (2a: (9S,10R)-*N*-allyl mefloquine; 2b: (9R,10S)-*N*-allyl mefloquine)

	N/g silica gel	C/ g silica gel
	[µmol]	[µmol]
2b	436	244
2b*	72	67
2a	83	76

The ratio of the coverage calculated for nitrogen and carbon should be in the same range. Consequently, the prepared CSP was washed with acidified methanol (10 mL

CH_3COOH, 40 mL H_2O and 50 mL MeOH) and it was dried for 14 h in the vacuum drying cabinet at 60 °C, which afforded **CSP 2b***. Despite the coverage of **2a** and **2b** was low, columns were packed. Therefore the sieved silica gel (2 g) was transferred in an Erlenmeyer flask. A suspension of acetic acid (1 mL) and isopropanol (20 mL) with the silica gel was prepared and sonicated for 10 min. The column was packed in house.

Immobilization of *tert*-butylcarbamoyl-*N*-allyl mefloquine (4):

The same procedure as for the immobilization of *N*-allyl mefloquine (**2**), described above, was employed. The following compounds were used: *tert*-butylcarbamoyl-*N*-allyl mefloquine (**4a**; **4b**) (0.5 g; 0.97 mmol), mercaptopropyl-silica (3 g) and AIBN (32 mg; 0.19 mmol).

Table 10: The elemental analysis of 4a: (9S,10R)- t-butylcarbamoyl-*N*-allyl mefloquine and 4b: (9R,10S)-t-butylcarbamoyl-*N*-allyl mefloquine)

	N/g silica gel	C/ g silica gel
	[µmol]	[µmol]
4a	109	113
4b	133	133

For packing a column of the immobilized selectors **4a,b** a suspension of the sieved silica (2 g) in acetic acid (1 mL) and isopropanol (20 mL) was prepared. The suspension was degassed in an ultrasonic bath and the columns were packed in house.

6.4 Analysis of the elution order of *N*-allyl mefloquine

(*O*,*O*)-dibenzoyl-(R,R)-tartaric-*N*-allyl mefloquine monoester (13)

A solution of *N*-allyl mefloquine (**2**) (0.15 g; 0.36 mmol) and DBTAAN (0.18 g; 0.54 mmol) in dry dichloromethane (5 mL) was stirred for 18 h under nitrogen atmosphere. The solution was extracted with a 5% aqueous $NaHCO_3$ solution (5 mL), 10%

aqueous HCOOH (5 mL) and saturated solution of NaCl (2 mL). The organic layer was dried with $MgSO_4$ and evaporated. The product was directly used for the HPLC measurements.

6.5 Synthesis of analytical probes used for the evaluation of the mefloquine-based CSPs

6.5.1 Derivatives of the 4-allyloxy-3,5-dichlorobenzoic acid

Succinimidyl 4-allyloxy-3,5-dichlorobenzoate (14):

The 4-(allyloxy)-3,5-dichlorobenzoic acid (0.247 g; 1 mmol), hydroxysuccinimide (0.126 g; 1.1 mmol), dicyclohexylcarbodiimid (0.237 g; 1.15 mmol) and dimethylaminopyridin (50 mg; 0.41 mmol) were dissolved in dry DCM (40 mL) and the flask was fitted with a drying tube ($CaCl_2$). The solution was stirred for 4 h at room temperature. The precipitated DCU was filtered and the solution was evaporated. After column chromatography (PE/EA 1:1) 0.17 g (49%) of the pure compound **14** was obtained, m.p. 119.2-119.9 °C

^{1}H NMR (400 MHz, $CDCl_3$): 2.80 (s, 4 H, 2xCH_2); 4.60 (d, 2 H, CH_2); 5.25 (d, 1 H, $\underline{CH_2}$=CH); 5.35 (d, 1 H, $\underline{CH_2}$=CH); 6.05 (m, 1 H, $\underline{CH}$=CH_2); 8.05 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, $CDCl_3$): 26.1(CH_2); 75.2(CH_2O); 120.2($\underline{CH_2}$=CH); 122.4(C_{ar}); 131.0(C_{ar}); 131.6(CH_{ar}); 132.6($\underline{CH}$=CH_2); 157.1(C_{ar}); 160.1(C=O); 169.2(C=O).

***N*-4-allyloxy-3,5-dichlorobenzoyl L-alanine (15)**

L-Alanine (25.8 mg; 0.29 mmol) and $(iPr)_2NEt$ (49.7 µL; 0.29 mmol) were dissolved in a diluted aqueous solution of ACN (1:1, 10 mL). The succinimide ester (100 mg; 0.29 mmol) (**22**) was added portion wise. The flask was fitted with a reflux condenser and the solution was heated to 40 °C over night. The reaction mixture was acidified with formic acid (10 µL) and extracted with ethyl acetate (10x20 mL). For transferring the product in the organic phase a saturated solution of NaCl (100 mL) was added. The

combined organic phases were dried with $MgSO_4$ and evaporated. 0.17 g (49%) of the compound **15** was obtained, m.p. 113.6-115.6 °C.

^{1}H NMR (400 MHz, MeOD): 1.40 (d, 3 H, CH_3); 2.55 (s, 1 H, NH); 4.45 (q, 1 H, CH); 4.55 (d, 2 H, CH_2); 5.15 (d, 1 H, $\underline{CH_2}$=CH); 5.30 (d, 1 H, $\underline{CH_2}$=CH); 6.05 (m, 1 H, CH); 7.80 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, $CDCl_3$): 17.6(CH_3); 50.6(CH-N); 76.0(CH_2O); 119.7($\underline{CH_2}$=CH); 129.9(2xCH_{ar}); 131.3(C_{ar}); 133.0 (C_{ar}); 134.6($\underline{CH}$=CH_2); 155.43(C_{ar}); 167.2(C=O); 176.3(C=O).

Ammonium *trans*-2-(*N*-4-allyloxy-3,5-dichlorobenzoyl)-aminocyclohexanesulfonate (16)[36]

trans-2-Aminocyclohexanesulfonic acid (21 mg; 0.12 mmol) was dissolved in a diluted aqueous solution of ACN (1:1, 10 mL). Hünig base (18.8 µL; 0.12 mmol) was added and the solution was heated to 40 °C. The ester **14** (40 mg; 0.12 mmol) was added portion wise and the reaction was stirred over night. The reaction mixture was acidified with formic acid (10 µL) and extracted for several times with ethyl acetate (10x20 mL). For better solubility of the product in the organic phase a saturated solution of NaCl was added. The combined organic solution was dried with $MgSO_4$, filtered and the solvent was evaporated. 0.04 g (85%) of the compound **16** was obtained, m.p. 55-62 °C.

^{1}H NMR (400 MHz, MeOD): 1.20 (m, 3 H, CH_2); 1.46 (m, 1 H, CH_2); 1.69 (m, 2 H, CH_2); 2.12 (m, 1 H, CH_2); 2.28 (m, 1 H, CH_2); 2.66 (dt, 1 H, CH); 3.95 (dt, 1 H, CH); 4.49 (d, 2 H, CH_2); 5.16 (m, 1 H, $\underline{CH_2}$=CH); 5.31 (m, 1 H, $\underline{CH_2}$=CH); 6.02 (m, 1 H, $\underline{CH}$=CH_2); 7.78 (s, 2 H, 2xCH_{ar}).

6.5.2 Derivatives of the 3,4,5-trimethoxybenzoic acid

Succinimidyl 3,4,5-trimethoxybenzoate (18)

Trimethoxybenzoic acid (2 g; 9.4 mmol), hydroxysuccinimide (1.19 g; 10.3 mmol), dicyclohexylcarbodiimide (2.2 g; 10.8 mmol) and dimethylaminopyridine (70 mg) were dissolved in dry DCM (50 mL) and stirred for 5 h without presence of air moisture ($CaCl_2$ drying tube). The precipitated dicyclohexylurea was filtered and the

filtrate was evaporated. The crude product was purified by column chromatography. It was obtained 2.5 g (87%) of active ester **18**, m.p. 130.4-131.8 °C.
^{1}H NMR (400 MHz, $CDCl_3$): 2.84 (s, 4 H, 2xCH_2); 3.82 (s, 6 H, 2xCH_3); 3.85 (s, 3 H, CH_3); 7.29 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, $CDCl_3$): 25.2(CH_2); 56.8 (CH_3); 61.6 (CH_3); 108.34 (CH_{ar}); 120.0(C_{ar}); 144.5(C_{ar}); 153.6 (C_{ar}); 161.9 (C=O); 169.5 (C=O).

***N*-3,4,5-trimethoxybenzoyl L-alanine (19a)**

The same procedure as for the derivatization of the 4-(allyloxy)-3,5-dichlorobenzoyl succinimide ester (**22**) with L-alanine, described in section 5.4.1, was employed. The following compounds were used: succinimidyl trimethoxybenzoate **18** (50 mg; 0.16 mmol), L- alanine (14.4 mg; 0.16 mmol) and $(iPr)_2NEt$ (27.7 µL ; 0.16 mmol). It was obtained 42 mg (81%) of the derivative **19a**, m.p. 107.6-109.8 °C.
^{1}H NMR (400 MHz, $CDCl_3$): 1.49 (d, 3 H, CH_3); 3.80 (s, 3 H, OCH_3); 3.82 (s, 6 H, 2xOCH_3); 4.69 (m, 1 H, CH); 6.98 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, $CDCl_3$): 18.8(CH_3); 49.4(CH); 56.7(OCH_3); 61.6(OCH_3); 105.1(CH_{ar}); 129.03(C_{ar}); 141.8(C_{ar}); 153.4(C_{ar}); 172.7(C=O); 176.3(C=O).

***N*-3,4,5-trimethoxybenzoyl D/L-alanine (19b)**

The racemic compound **19b** was obtained in the same way as the enantiomerically pure selectand **19a**.

***N*-3,4,5-trimethoxybenzoyl L-leucine (20a)**

A solution of L-leucine (0.127 g; 0.97 mmol) and $(iPr)_2NEt$ (166 µL, 0.97 mmol) in aqueous ACN (1:1, 10 mL) was warmed up to 40 °C, and the flask was fitted with a reflux condenser. The active ester **18** (0.3 g; 0.97 mmol) was added portion wise, and the reaction mixture was stirred and heated over night. The solution was acidified with formic acid (10 µL), diluted with a saturated solution of NaCl (100 mL) and extracted with ethyl acetate (15x30 mL). The combined organic solution was dried with $MgSO_4$, filtered and evaporated. The crude product was purified by column

chromatography (PE/EA, 1:2). It was obtained 0.19 g (60%) of compound **20a**, m.p. 80.6-81.7 °C.

^{1}H NMR (400 MHz, MeOD): 1.0 (dd, 6 H, 2xCH_3); 1.79 (m, 3 H, CH, CH_2); 2.7 (s, 1 H, NH); 3.82 (s, 3 H, OCH_3); 3.9 (s, 6 H, 2xOCH_3); 4.64 (m, 1 H, CH); 7.22 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, MeOD): 22.2(CH_3); 26.7(CH); 41.8(CH_2); 53.2(CH); 57.2(CH_3); 61.5(CH_3); 106.7(CH_{ar}); 131.0(C_{ar}); 142.6(C_{ar}); 154.8(C_{ar}); 170.2(C=O); 176.7(C=O).

N-3,4,5-trimethoxybenzoyl D-leucine (20b)

The analogic procedure was utilized for the derivatization of the succinimidyl 3,4,5-trimethoxybenzoate (**18**) with D-leucine. The following compounds were used: D-leucine (72.1 mg; 0.54 mmol), trimethoxybenzoic acid succinimide ester (0.170 g; 0.54 mmol) and $(iPr)_2NEt$ (94 µL, 0.54 mmol). It was obtained 50 mg (66%) of the compound **20b**.

^{1}H NMR (400 MHz, MeOD): 0.89 (dd, 6 H, 2xCH_3); 1.65 (m, 1 H, CH); 1.73 (m, 2 H, CH_2); 3.72 (s, 3 H, OCH_3); 3.79 (s, 6 H, 2xOCH_3); 4.6 (m, 1 H, CH); 7.1 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, MeOD): 22.2(CH_3); 26.7(CH); 35.2(CH_2); 53.2(CH); 57.1(CH_3); 61.6(CH_3); 106.7(CH_{ar}); 130.9(C_{ar}); 142.7(C_{ar}); 154.8(C_{ar}); 170.0(C=O); 176.9(C=O).

6.5.3 Derivatives of the pentafluorobenzoic acid

Succinimidyl 2,3,4,5,6-pentafluorobenzoate (22)

Pentafluorobenzoic acid (1.50 g; 7.07 mmol), hydroxysuccinimide (0.90 g; 7.78 mmol), dicyclohexylcarbodiimide (1.68 g; 8.14 mmol) and dimethylaminopyridine (40 mg; 0.40 mmol) were placed in round bottom flask, dissolved in dry DCM (30 mL) and stirred for 6 h. The precipitated DCU was filtered and the filtrate was partially removed under reduced pressure. The crude product was purified by column chromatography (PE/ EA, 1:2). It was obtained 1.25 g (57%) of ester **22**, m.p. 101.8-102.3 °C.

^{1}H NMR (400 MHz, $CDCl_3$): 2.82 (s, 4 H, 2xCH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): 26.0 (CH_2); 168.5 (C=O). **^{19}F NMR** (375 MHz, $CDCl_3$): -159.19 (*meta*-F); -143.48(*para*-F); -133.17(*ortho*-F).

***N*-2,3,4,5,6-pentafluorobenzoyl D-leucine (23a)**

D-leucine (0.13 g; 0.97 mmol) was dissolved in a diluted aqueous solution of ACN (1:1, 20 mL). $(iPr)_2NEt$ (0.17 mL; 0.97 mmol) and the active ester **22** (0.30 g; 0.97 mmol) were added. The reaction mixture was stirred and heated to 40 °C over night. The solution was acidified with formic acid (10 µL), diluted with a saturated solution of NaCl (100 mL) and extracted with ethyl acetate (10x20 mL). The combined organic layers were dried with $MgSO_4$, filtered and evaporated. The crude product (**23a**) was purified by double column chromatography (1st: PE/ EA , 1:2; 2nd: CH_2Cl_2/MeOH, 5:1). It was obtained 0.21 g (66%) of amide **23a**, m.p. 169.7-172.4 °C.

^{1}H NMR (400 MHz, MeOD): 1.00 (m, 6 H, 2xCH_3); 1.70 (m, 1 H, CH); 1.75 (m, 2 H, CH_2); 2.65 (s, 1 H, NH); 4.60 (m,1 H, CH). **^{13}C NMR** (100 MHz, MeOD): 22.06(CH_3); 23.81(CH_3); 26.48(CH); 26.69(CH_2); 53.52(CH); 171.8(C=O); 177.6(C=O). **^{19}F NMR** (375 MHz, MeOD): -143.9 (*ortho*-F); -156.0 (*para*-F); -164.6 (*meta*-F).

***N*-2,3,4,5,6-pentafluorobenzoyl L-leucine (23b)**

The same procedure (see above) was employed for the synthesis of *N*-2,3,4,5,6-pentafluorobenzoyl L-leucine (**23b**). Following amounts were used: L-leucine (0.09 mg, 0.68 mmol), pentafluorobenzoic acid succinimide ester (**22**) (0.21 g; 0.68 mmol) and $(iPr)_2NEt$ (116 µL; 0.68 mmol). It was obtained 0.16 g (70%).

^{1}H NMR (400 MHz, MeOD): 0.89 (d, 6 H, 2xCH_3); 1.27 (m, 2 H, CH_2); 1.62 (m, 1 H, CH); 4.50 (m, 1 H, CH). **^{13}C NMR** (100 MHz, MeOD): 22.13(CH_3); 23.86(CH_3); 26.50(CH); 26.68(CH_2); 53.96(CH); 170.9(C=O); 176.4(C=O). **^{19}F NMR** (375 MHz, MeOD): -143.91(*ortho*-F); -156.19(*para*-F); -164.62(*meta*-F).

6.6 Synthesis of a strong cation exchanger based on 4-allyloxy-3,5-dimethoxybenzoic acid

Allyl-4-allyloxy-3,5-dimethoxybenzoate (24):

To a suspension of syringic acid (5.0 g; 25.2 mmol) and K_2CO_3 (8.7 g; 63.0 mmol) in dry DMF (60 mL), allyl bromide (5.5 mL; 63.0 mmol) was added. The reaction mixture was stirred and heated to 60 °C over night and then decomposed with distilled water (200 mL). The brown solution was extracted with ethyl acetate (5x100 mL). The combined organic layers were washed with a saturated solution of NaCl, dried with $MgSO_4$, filtered and evaporated. In order to remove the rest of DMF toluene was added for several times to the brown liquid (**24**) and re-evaporated. It was obtained 6.54 g (93%) of compound **24**, which was directly used in the subsequent hydrolysis. In this case we used only TLC characterization of the product.

4-allyloxy-3,5-dimethoxybenzoic acid (Allyloxy syringic acid) (25):

The compound **24** (6.54 g; 23.5 mmol) was dissolved in a mixture of 1.4 M NaOH solution (2.8 g; 70 mmol, 50 mL) and ethanol (50 mL). The reddish solution was refluxed for 90 min. Then the flask was cooled with an ice bath and the solution was acidified with a diluted HCl solution (1:1); the pH was set to 3. The precipitate was filtered and washed with distilled water. 4.08 g (73%) of the allyloxy syringic acid **25** were obtained, m.p.111.2-113.4 °C.

^{1}H NMR (400 MHz, MeOD): 3.90 (s, 6 H, 2xOCH_3); 4.50 (d, 2 H, CH_2); 5.15 (d, 1 H, $\underline{CH_2}$=CH); 5.3 (d, 1 H, $\underline{CH_2}$=CH); 6.05 (m, 1 H, $\underline{CH}$=CH_2); 7.35 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, MeOD): 57.0(OCH_3); 75.5(CH_2); 108.6(CH_{ar}); 118.5(CH_2); 127.6(C_{ar}); 136.0(CH); 142.4(C_{ar}); 155.0(C_{ar}); 169.8(C=O).

Succinimidyl 4-allyloxy-3,5-dimethoxy benzoate (Allyloxy syringic succinimide ester) (26):

The same procedure as for the synthesis of the succinimide ester of the 3,4,5-trimethoxybenzoic acid, described in section 5.4.2, was employed. The following compounds were used: allyloxy syringic acid (**25**) (1 g; 4.20 mmol),

hydroxysuccinimide (0.53 g; 4.61 mmol), dicyclohexylcarbodiimide (1 g; 4.82 mmol) and dimethylaminopyridine (60 mg). It was obtained 0.70 g (50%) of allyloxy syringic succinimide ester **26**, m.p. 131.7-134.5 °C.

^{1}H NMR (400 MHz, $CDCl_3$): 2.82 (s, 4 H, CH_2); 3.82 (s, 6 H, 2xOCH_3); 4.55 (d, 2 H, CH_2); 5.11 (d, 1 H, $\underline{CH_2}$=CH); 5.21 (d, 1 H, $\underline{CH_2}$=CH); 5.99 (m, 1 H; $\underline{CH}$=CH2); 7.3 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, $CDCl_3$): 25.2(CH_2); 56.7(CH_3); 74.6(CH_2); 108.2(CH_{ar}); 118.9(CH_2); 120.0(C_{ar}); 134.1(CH); 153.9(C_{ar}); 162.0(C=O); 169.7(C=O).

Ammonium *trans*-2-(*N*-(4-allyloxy-3,5-dimethoxybenzoyl)-aminocyclohexane-sulfonate (27):

The same procedure as in case of the derivatization of the 4-(allyloxy)-3,5-dichlorobenzoic succinimide ester **14** with *trans*-2-aminocyclohexansulfonic acid, described in section 5.4.1, was utilized. The following compounds were used: allyloxy syringic succinimide ester (0.82 g; 2.44 mmol), *trans*-2-aminocyclohexanesulfonic acid (0.44 g; 2.44 mmol) and $(iPr)_2NEt$ (0.42 mL; 2.44 mmol).

It was not possible to extract the product from the aqueous solution. Therefore a continuous extraction was performed and the aqueous solution was washed with chloroform for 2 days. The layers were separated and the organic solution was dried with $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the pure enantiomers were obtained by preparative separation on HPLC (stationary phase: QD-AX (250x20 mm, 10 µm), mobile phase: MeOH, 0.5% HCOOH and 0.5% NH_4OOCH). After evaporation of the mobile phase and sublimation of the formic acid, the pure compounds **27a** (0.44 g; 45%) and **9b** (0.44 g; 45%) were obtained.
(R,R): m.p. 146.3-148.6 °C.
^{1}H NMR (400 MHz, MeOD): 1.26(m, 2 H, CH_2); 1.50 (m, 2 H, CH_2); 1.70 (m, 2 H, CH_2) 2.28 (m, 2 H, CH_2); 2.79 (dt, 1 H ,CH); 3.75 (s, 6 H, 2xOCH_3); 3.86 (dt, 1 H, CH); 4.38 (d, 2 H, CH_2); 5.05 (m, 1 H, $\underline{CH_2}$=CH); 5.16 (m, 1 H, $\underline{CH_2}$=CH); 5.93 (m, 1 H, $\underline{CH}$=CH_2); 7.09 (s, 2 H, 2xCH_{ar}). **^{13}C NMR** (100 MHz, MeOD): 26.2(CH_2); 26.8(CH_2); 29.8(CH_2); 34.2(CH_2); 53.5(CH); 57.1(CH_3); 63.1(CH); 75.4(CH_2); 106.4(CH_{ar}); 118.5(CH_2); 132.4(C_{ar}); 136.0(CH); 140.7(C_{ar}); 155.0(C_{ar}); 169.4(C=O).

Immobilization of the ammonium *trans*-2-(*N*-allyloxy-syringic acid)-aminocyclohexanesulfonate (27)

The mercaptopropyl modified silica gel (2.5 g) was placed in a three necked round bottom flask, which was fitted with a mechanical stirrer and a reflux condenser and the apparatus was flushed with nitrogen. The compound **27** (0.45 g; 1.13 mmol) was dissolved in methanol and poured in the three necked flask. A solution of AIBN (50 mg; 0.30 mmol) in methanol (3 mL) was added and the reaction mixture was heated under reflux for 8 h in the inert atmosphere of nitrogen. The reaction mixture was cooled down to room temperature. The CSP was filtered and washed with methanol (3x10 mL) and CH_2Cl_2 (2x15 mL). The product was dried for 3 h in a drying cabinet (60 °C) and for 12 h in a vacuum drying cabinet (60 °C). The coverage was 182 µmol/ g S.

Table 11: Coverage of the novel SCXs immobilized on mercaptoporpyl silica gel

	S/g silica
	[µmol]
27a	182
27b	202

7. References

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8. Attachments

Abstract

In the last decades chromatographic enantioseparation with chiral stationary phases (CSPs) has become a widely used analytical and industrial tool, mainly because of the importance of enantioseparation in the drug development.

The aim of the presented thesis was to synthesize novel selectors based on mefloquine and to evaluate their potential as a new anion exchange type of chiral stationary phases. In the design of the novel selector we were inspired by a commercially available QN-AX and QD-AX CSP respectively, which were developed in our working group. We assumed that a π-acidic quinoline unit of mefloquine (in contrast to a π-basic core of quinine) would lead to an altered separation of π-basic analytes.

We synthesized several different enantiomerically pure selectors based on *N*-allyl mefloquine and immobilized some of them on mercaptopropyl modified silica gel. The structural resemblance of mefloquine to chinchona alkaloids (quinine, quinidine) allowed us to determine the elution order of *N*-allyl mefloquine enantiomers. The absolute configuration of (9R,10S)-*N*-allyl mefloquine was verified by the x-ray analysis.

In order to evaluate the prepared CSPs based on mefloquine, several new π-basic and fluorophilic leucine derivatives were prepared. These selectands (SAs) were further used for NMR studies with selected selectors. Corresponding to the NMR

studies, we found out that the mefloquine selectors interact with selected leucine analytes in a similar way as the chinchona based selectors. We have not found any additional fluorophilic interactions for *N*-allyl mefloquine with polyfluorinated analytes, which could be considered as a drawback of the new selectors.

We found that *N*-allyl mefloquine derivatives and mefloquine itself can be effectively separated on strong cation exchanger columns. Therefore we developed a novel strong cation exchanger based on *O*-allyl syringic acid with *trans*-2-aminocyclohexanesulfonic acid. We proved that the separation of several types of mefloquine-based compounds was better with our new SCX CSPs.

Furthermore, we discovered during the evaluation of the novel SCX CSPs that a selector based on mefloquine with non-derivatized nitrogen in the piperidine moiety will be more efficiently enantioseperated, which was also non expected.

To conclude, we designed and synthesized several novel chiral selectors based on mefloquine and evaluated the applicability in enantioseparation using NMR and HPLC methods. In addition we developed novel SCX chiral stationary phases, which can be used for enantioseparation of mefloquine derivatives and chiral amines in general.

Zusammenfassung

Die Trennung von Racematen in ihre Enantiomere mittels chiralen stationären Phasen nahm sowohl in der Analytischen Chemie als auch in der Industrie in den letzten Jahrzehnten an Bedeutung zu. Nicht zuletzt wegen der Notwendigkeit enantiomerenreine Medikamente, die die gewünschte Wirkung erzielen, zu produzieren.

Die Synthese von neuen Selektoren basierend auf Mefloquin als auch die Evaluierung von chiralen stationären Phasen immobilisiert mit Mefloquinderivaten, waren Ziele der vorliegenden Diplomarbeit. Die Anionenaustauscher stationären Phasen basierend auf Chinchona Alkoloiden (Chinin und Chinidin), entwickelt in unserer Arbeitsgruppe, waren Motivation für die Entwicklung von auf Mefloquin beruhenden stationären Phasen. Mefloquin sollte aufgrund der vermuteten π-sauren Natur der Chinolin-Einheit für die Enantiomerentrennung von π-basischen Analyten geeignet sein.

Wir synthetisierten verschiedene enantiomerenreine *N*-allyl Mefloquinderivate. Ausgewählte Derivate wurden als Selektoren auf modifiziertem Kieselgel immobilisiert, um neue chirale stationäre Phasen zu erhalten.

Die strukturelle Ähnlichkeit von Mefloquin und den Chinchona Alkaloiden ermöglichte, durch Vergleich der Elutionsreihenfolge der entsprechenden Weinsäureester, die Bestimmung der Elutionsreihenfolge von den erythro Enantiomeren des *N*-allyl Mefloquins. Zusätzlich konnte die Absolutkonfiguration des (9S,10R)-*N*-allyl Mefloquins mittels Röntgenstrukturanalyse bestimmt werden, womit unser chromatographischer Ansatz bestätigt wurde.

Für die Evaluierung der neuen stationären Phasen wurden π-basische als auch polyfluorierte Analyte synthetisiert. Des Weiteren wurden diese Selektanden auch für NMR Studien mit ausgewählten Selektoren verwendet. Die NMR Studien ergaben, dass Mefloquinderivate und Chinidin in vergleichbarer Weise mit Selektanden interagieren. Es konnten allerdings keine zusätzlichen Fluor-Wechselwirkungen zwischen Mefloquin und polyfluorierten Analyten beobachtet werden.

Eine effiziente Trennung der Mefloquinderivate wurde mittels eines Kationenaustauschers als chirale stationäre Phase erreicht. Aufgrund dessen, entwickelten wir neue Kationenaustauscher-Säulen basierend auf Syringasäure. Die Evaluierung der neuen Kationenaustauscher-Säulen ergab eine bessere Enantiomerentrennung für Mefloquinderivate im Vergleich zu einer in unserer Arbeitsgruppe etablierten Kationenaustauscher-Säule. Des Weiteren ergab die Evaluierung der neuen Kationenaustauscher-Säulen, dass ein Selektor basierend auf Mefloquin effizienter in seine Enantiomere getrennt werden kann, wenn der Stickstoff des Piperidins als sekundäres Amin, wie eben im Mefloquin, vorliegt.

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Curriculum Vitae

Denise WOLRAB

September 15, 1986 in Vienna, Austria

Address: Weißgerberlände 40/ 7

A-1030 Vienna

Contact: denise.wolrab@gmail.com

Education:

September 1993- June 1997	Primary School Volksschule Löwengasse 7, A-1030 Vienna
September 1997- June 2005	Secundary School BRG3 Radetzkystrasse 2a , A-1030 Vienna
October 2005- November 2011	Student of Chemistry Facultiy of Chemistry University of Vienna Währingerstrasse 38, A-1090 Vienna
January 2011-November 2011	Diploma Student Reasearch Group for Molecular Recognition Dept. Analytical Chemistry University of Vienna Währingerstrasse 38, A-1090 Vienna

Work experience with relevance for chemistry:

01.07.2008-31.08.2008	-
06.07.2009-31.08.2009	-
05.07.2010-31.07.2010	Baxter AG Lange Allee 24, A-1220 Vienna

01.10.2009-28.02.2010	-
01.03.2010-31.07.2010	-
01.10.2010-28.02.2011	Teaching Assistant Department of Organic Chemistry University of Vienna Währingerstrasse 38, A-1090 Viena
01.03.2011-31.07.2011	Teaching Assistant Department of Analytical Chemistry University of Vienna Währingerstrasse 38, A-1090 Viena