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DIPLOMARBEIT

Development and Evaluation of Zwitterionic Chiral Stationary Phases

Entwicklung und Erprobung von Zwitterionischen Chiralen Stationären Phasen

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1 Chirality – Biological and Pharmaceutical Relevance

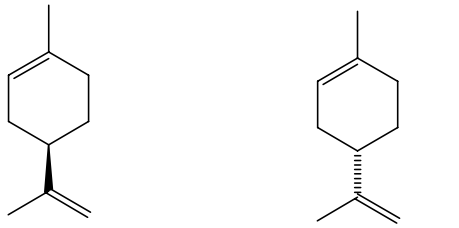
The goal of the presented diploma work was to design, synthesize and evaluate a novel type of zwitterionic chiral stationary phases (zwitterionic CSPs). The development of these CSPs, merging the fields of organic and analytical chemistry, is targeted on chromatographic separation of enantiomers. When talking about chromatographic stereoselective separation one is inevitably faced with the concept of chirality.

Chirality is a unique property of molecules that are non-superimposable with their mirror images. Thus, structures which are not identical but are mirror images of each other are called enantiomers. They have identical chemical and physical properties with one single exception - the antipodal rotation of plane polarized light - which is called optical activity. Enantiomers are usually denoted with a prefix of R (for right handed molecules) or S (for left handed molecules) and are called chiral. Chiral molecules are defined as ones that are not identical with their mirror images. A racemic mixture is an equal mixture of two enantiomers.[1] Deviation from an equimolar ratio is called enantiomeric excess and the mixture is denoted non racemic.

Chiral molecules are of utmost importance in living systems. Almost all of the chiral substances in living organisms are present in only one enantiomeric form. All but one of the proteinogenic amino acids are left handed (S-form) whereas the vast majority of sugars is right handed (R-form). (In addition to (R)- or (S)-nomenclature an old – but still widely spread – (L)- and (D)- nomenclature can be used to differentiate left and right handed molecules). As chiral amino acids are building blocks for peptides and proteins and chiral sugars are for polysaccharides, a chiral nature is created in all living systems.[2] Chiral monosaccharides also build up the backbone of the DNA, which is – due to its helicale structure – chiral itself.

Enzymes, which are almost all proteines, are catalyzing countless biochemical reactions in living systems. Such enzymatic reactions are amongst others based on stereospecific recognition between the converted molecule (substrate) and the enzyme. The stereospecifity of enzymes, which means that enzyme and substrate possess specific complementary geometric shapes, was already stated by Emil Fischer in 1894 and was later referred to as "Lock and Key" model.[3]

Regarding these examples makes it obvious that the absolute configuration of chiral molecules is of central importance. Although enantiomers have identical chemical and physical properties in an achiral environment, it makes a big difference which enantiomer (R or S) is present in a living system, since it is a chiral environment.
One typical example is the limonene molecule found in citrus fruits (**Figure 1.1**). While (R)-limonene smells of orange (S)-limonene smells of lemon.

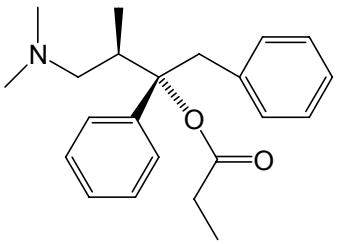


(R) - limonene (S) - limonene

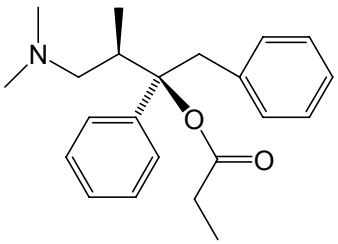
Figure 1.1

A tragic consequence of the different impact of chiral substances on humans took place in the late 1950s and early 1960s. A drug called Contergan® comprising the pharmaceutically active substance thalidomide was widely used for treating morning sickness and insomnia in pregnant women. The years after, thousands of babys were born having deformities because their mothers were taking thalidomide. Later investigations showed that the (R)-form was the beneficial enantiomer while (S)-form was teratogenic (it has to be added that even if enantiomerically pure (R)-form had been used, it would have caused malformities because of in vivo racemisation in the special case of thalidomide.[4])

Another examples of chiral drugs whose enantiomers have different therapeutic properties are dextro- and levopropoxyphene (**Figure 1.2**).[5] Dextropropoxyphene (known under the trade name Darvon®) is a painkiller while its enantiomer levopropoxyphene (Novrad®) shows antitussive effects.



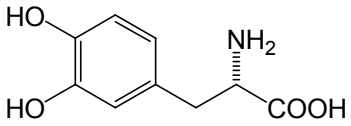
Dextropropoxyphene



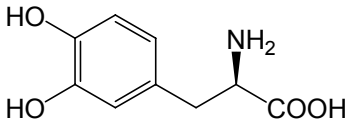
Levopropoxyphene

Figure 1.2

L-DOPA (**Figure 1.3**) is a chiral non-proteinogenic amino acid and is in use for treatment of Parkinson`s disease whereas the other enantiomer, D-DOPA, is toxic. Consequently, only the L-form can be commercially used.[6]



L-DOPA



D-DOPA

Figure 1.3

Hundreds of other drugs could be named whose enantiomers have different effects on humans to back up the importance of using enantiomerically pure compounds in the pharmaceutical industry.

Especially the Contergan tragedy made scientists – and the pharmaceutical industry - aware of the prime importance of the chiral character of drugs. Nowadays health and regulatory authorities make strict obligations to companies which are marketing drugs in racemic form. Detailed reports of the pharmacological and pharmacokinetic effects of the single enantiomers and the racemic mixtures must be given.[2]

Not only in pharmaceutical industry but also in agrochemical and pheromone chemistry the use of enantiomerically pure substances is desired. For example, a herbicide is used as a racemate where only one enantiomer is active, the other inactive represents an unnecessary contamination and burden for the environment.

Although the importance of stereochemistry and its consequences is well known for more than 30 years producing single enantiomers is still a challenging task. Main advances in the preparation of enantiomerically pure substances – especially driven by the pharmaceutical industry – have been made by the fields of asymmetric synthesis and analytical and preparative separation techniques in the last two decades.[2] Today asymmetric synthesis is seen as the most important area in organic chemistry.

The term "atom economy" gained major importance, which means that producing an enantiopure product is bringing down costs because of avoiding the opposite, probably useless enantiomer. However, designing and carrying out asymmetric synthetic ways is not always easy – especially when it comes to industrial scale productions. And even if asymmetric synthesis is feasible enantioselective analytical methods are still needed in order to analyze and quantitate impurities.

Therefore, enantioseparation techniques can be considered as essential (at least in analytical scale) in the field of chiral organic chemistry and are widely used in both analytical and preparative scale.

2 Enantioseparation

Enantioseparation techniques can be classified into two categories – direct and indirect enantiomer separation approaches. They are distinguished in different binding interactions between the single enantiomer (selectand, SA) and the chiral auxiliary (selector, SO).[7]

2.1 Indirect Approach

Indirect separation techniques are based on the formation of covalent diastereomers from the single enantiomer and a chiral derivatization agent (CDA). The CDA has to be of high enantiomeric purity and undergoes a chemical reaction with the racemic mixture. Thus, two diastereomers are formed by the CDA and the respective enantiomer having different chemical and physical properties. The diastereomers can be separated by common achiral separation techniques like crystallization or liquid chromatography. After separation the diastereomers are split into the enantiomers and the CDAs offering the chiral compound in its desired enantiomeric form.

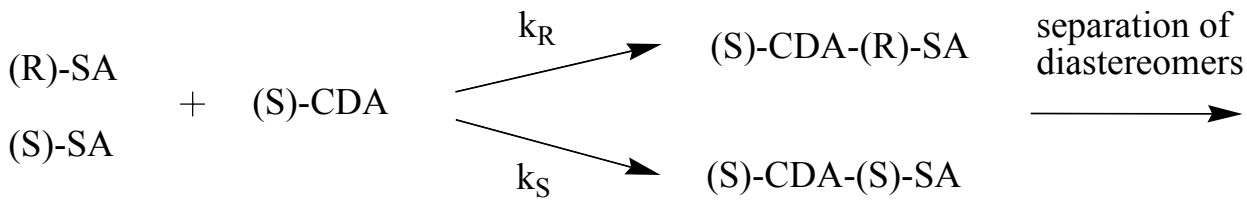


Figure 2.1: Scheme for indirect separation

The main advantage of the indirect approach can be seen in the use of conventional, well established achiral separation techniques. Especially crystallization is a frequently applicated method when carrying out large scale separations and is still widely spread in synthetic chemical industry.

On the other hand, the indirect separation approach is limited in its field of application. For example, only enantiomers with at least one functional group available for chemical derivatization (for the formation of the diastereomer) can be used. Moreover, the transformation into diastereomers has to be carried out in conditions that avoid inversions of the stereogenic centers. The kinetic resolution phenomena must also be taken into consideration, by assuming that the reaction is driven to completion avoiding any incomplete reactions.[7] The chiral derivatization agents (which are often isolated natural compounds or of synthetic origins) must be of very high enantiomeric purity.

Indirect approaches were typically used in the beginnings of enantioseparation and are still widely used in industrial scale separations.[8] However, the disadvantages listed above and the labor-intensive derivatization steps are decreasing the popularity of this method, and direct separation techniques for high throughput analysis are preferred nowadays.

2.2 Direct Approach

Direct enantiomer separation methodologies differ from indirect ones in formation of transient, noncovalent diastereomeric complexes. The single enantiomers which are getting in contact with the chiral selectors form a reversible SO – SA associate. These diastereomeric associates have different equilibrium constants resulting in different retention.

Direct approach techniques performed with liquid chromatography (LC) are carried out either by use of chiral mobile phase additives (CMPA) or chiral stationary phases (CSP).

The CMPA technique combines an achiral stationary phase and a chiral mobile phase additive. The racemate is injected onto a chromatographic column together with the chiral selector. Diastereomeric complexes are reversibly formed between the SAs and the CMPAs, which exhibit different physicochemical properties (e.g. association/dissociation rates, thermodynamic properties) and thus, make them separable on an achiral stationary phase. The CMPAs have to be separated from the enantiomers and recycled after separation which can be seen as a major disadvantage for high throughput applications.

An essential prerequisite is solubility of the CMPA in the mobile phase, which can be seen as another limiting factor as well as difficulties in reproducibility. Although application of CMPAs is rather inexpensive because of cheap achiral stationary phases and uncomplicated preparation of the chiral phase this type of direct separation approach is of only minor importance in present enantiomer separation.[9]

The most important direct approach technique is immobilizing the chiral selector onto a chromatographic support material (most often silica is used) and are referred to as chiral stationary phases (CSP). Immobilization can be carried out in different ways like covalent attachment of the SO onto functionalized support materials or application of coating techniques.

CSPs are operated with achiral mobile phases applying common solvent compositions as they are used in normal phase or reversed phase chromatography. When injecting a racemic mixture (under the prerequisite of a successful separation) the eluted analytes are obtained directly in their enantiomerically pure form. Thus, the detector signals (of the single enantiomers showing identical spectroscopic properties towards achiral detection systems) are a direct measure for enantiomeric impurities.

Additionally, a chiral stationary phase with its permanently bonded chiral selector is more robust than a CMPA separation system and can typically be reused oftentimes without any conditioning/preparation steps. The use of achiral mobile phases produces much lower background signals than CMPAs containing chiral selectors and, thus, results in an increased sensitivity. This can be of special interest when combining a CSP with mass sensitive detection systems.

Having properties like robustness in application, a broad variety of LC operation modes and the use of achiral bulk solvents (which can be easily evaporated off after separation) made CSPs an important tool for economic chromatographic separation in analytical and even preparative scale.[7]

2.3 Principles of Chiral Recognition

Several attempts have been made in the last two decades to enlighten the processes of chiral recognition on a molecular level. Among numerous scientific essays one concept gained major importance and is currently seen as the state of art: it is known as the **three-point rule** last modified by Pirkle and states that "*for chiral recognition to occur a minimum of three simultaneous interactions between SO and at least one of the enantiomers is required, with at least one of these interactions being stereochemically dependent."*[10]

This means that when the chiral selectand molecules approach the chiral selector both can interact with the selector on basis of two interactions, but only one of the enantiomeric forms is able to establish a simultaneous third interaction because of its unique spatial arrangement. This stereochemically dependent interaction is a prerequisite for the chiral recognition between the selector and selectand.

The concept of the three-point model is depicted in **Figure 2.2**

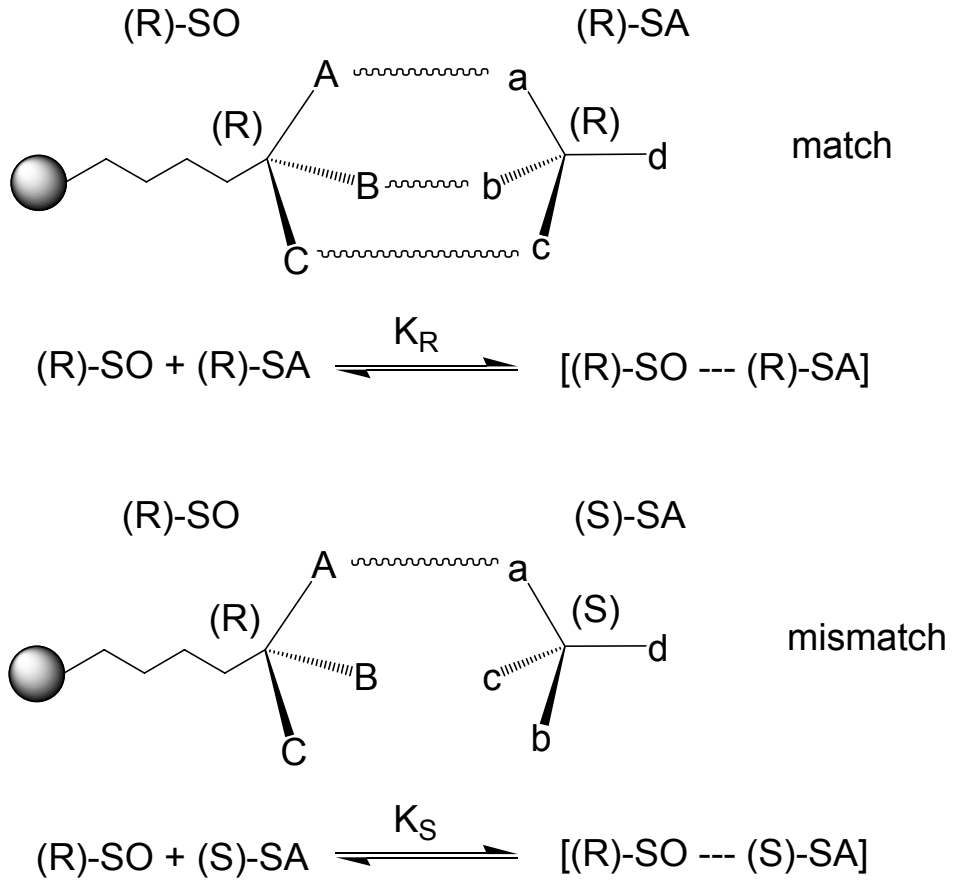


Figure 2.2: Principle of chiral recognition

There are single-point and multiple-point interactions. Ionic forces or hydrogen bondings, which are targeted on specific single atoms of the SOs and SAs, are referred to as single-point interactions. Dipole-dipole stacking and aromatic interactions (face-to-face and edge-to-face) are considered as multi-point contacts.

Because of differences between these two types of interactions one can enlarge the definition of the three-point model. Therefore, it can be sufficient for chiral recognition if two simultaneous SO-SA interactions take place, as long one of them will be of multi-point quality.

These spatial aspects of chiral recognition are closely connected with thermodynamic considerations. According to **equation 2.1** enantioseparation is achieved when the free energy changes ΔG of the process of single enantiomers binding to SOs are differing. $\Delta G_{R,S}$ represents the affinity of the selectand enantiomers to the chiral selectors and are related to the equilibrium constants K_R and K_S by **equation 2.2**.

$$\Delta G_R \neq \Delta G_S \qquad [2.1]$$

$$\Delta G_R = - RT \ln K_R \qquad \Delta G_S = - RT \ln K_S \qquad [2.2]$$

T = temperature in K

R = general gas constant

The retention factors $k_{R,S}$ are related to the equilibria constants $K_{R,S}$ by **equation 2.3** where Φ is the phase ratio:

$$k_R = K_R \cdot \Phi \qquad k_S = K_S \cdot \Phi \qquad [2.3]$$

Selectivity coefficient α is defined as the ratio of the retention factors of the single enantiomers. By convention $\alpha_{R,S}$ is calculated by dividing the retention factor of the more retained enantiomer by that of the less retained ($k_R > k_S$)

$$\alpha_{R,S} = k_R / k_S = K_R \cdot \Phi / K_S \cdot \Phi \qquad [2.4]$$

Combining **equations 2.2** and **2.4** gives **equation 2.5**. It can be seen that α is related to the difference of the free energy changes between the (R) - and (S)-enantiomers ($\Delta\Delta G_{R,S}$). The energy differences $\Delta\Delta G_{R,S}$ required for separation depend on the peak resolution capabilities (the efficiency) of the applied separation technique.

$$\Delta\Delta G_{R,S} = - RT \ln \alpha_{R,S} \qquad [2.5]$$

To have a closer look at thermodynamic parameters **equation 2.5** can be rewritten in the form of the Gibbs-Helmholtz **equation 2.6**:

$$\Delta\Delta G_{R,S} = \Delta\Delta H_{R,S} - T\,\Delta\Delta S_{R,S} \qquad [2.6]$$

$$\ln \alpha_{R,S} = - \Delta\Delta H_{R,S} / RT + \Delta\Delta S_{R,S} / R \qquad [2.7]$$

Equation 2.7 shows the relation of enantioselectivity α to enthalpic ($\Delta\Delta H_{R,S}$) and entropic ($\Delta\Delta S_{R,S}$) contributions as well as its dependency on the temperature. With the help of van`t Hoff plots (plots of ln α vs. 1/T where ΔΔH and ΔΔS can be extracted by the slope and intercept, respectively) the enthalpic and entropic magnitudes can be experimentally obtained.

Generally, the enthalpic values are mostly negative favoring chiral recognition while the entropic values tend to be positive counteracting chiral recognition. The opposite absolute values of $\Delta\Delta H_{R,S}$ and $\Delta\Delta S_{R,S}$, which are observed most of the times, assume that chiral recognition is usually enthalpically controlled.
The forms of enthalpic contribution like number and strenght of ionic interactions are better investigated in contrary to entropic contributions (solvation effects, re-ordering of SO and SA), which are difficult to study.[11]

2.4 Different Types of Chiral Stationary Phases

2.4.1 Polymeric CSPs

2.4.1.1 Polysaccharide Type CSPs

Chiral stationary phases based on natural polysaccharides can be considered as pioneers in chromatographic enantiomer separation. They date back to the early 1950s when amino acids were resolved on cellulose papers but showing only low enantioselectivity values.[12] An improvement in enantioselectivity was achieved by the development of microcrystalline cellulose triacetate (MCTA). Fusing high enantioselectivity and loading capacities those materials were suitable for commercial preparative use and became well known under the tradenames CTA-I (Merck) and CHIRALCELL CA-1 (Daicel Chemical Industries, Ltd.)

New coating techniques on silica support materials and development of further polysaccharide derivatives led to an improvement in mechanical stability and enlarged the spectra of chiral analytes to be resolved.[13] These CSPs can be operated in normal mode, polar organic and in reversed phase mode.

The introduction of aromatic substituents on cellulose and amylose materials created CSPs which were able to resolve a broad range of chemically diverse chiral analytes.[14] Especially cellulose and amylose tris(3,5-dimethylphenylcarbamates), which are sold under the trade names CHIRALCEL® OD and CHIRALPAK® AD, are currently among the most often applicated CSPs.

Despite of the positive aspects stated before these CSPs have still the disadvantage of instability in non standard mobile phases like dichloromethane, chloroform, tetrahydrofurane, toluene, dioxane, ethylacetate etc. as the polysaccharide is only adsorbed and not covalently attached to the silica support material. Operation with that kind of mobile phases causes changes in the tertiary structure and even dissolution of the stationary phase.

Therefore, dedicated research was made to invent new immobilization techniques. Hence, a new strategy containing a photochemical and thermal cross-linking in presence of a free radical initator was established obtaining new CSPs with stability towards all kinds of mobile phases.[15]

Widely spread polysaccharide phases like CHIRALCEL and CHIRALPAK were improved by using the novel immobilization technique enlarging their application modes. Thus, these types of polysaccharide-based CSPs are market leaders and can be considered as the currently most important tools in chromatographic enantiomer separation.

However, one negative aspect of polysaccharide-based CSPs is the limited understanding of the molecular chiral recognition process. Studies pointed out the importance of the helical supramolecular structure of cellulose- and amylose-tris(phenylcarbamate) derivatives for chiral recognition.[16] But detailed studies regarding chiral recognition on a molecular level are hardly realizable as polysaccharides show low solubility in solvents which favor chiral recognition or give badly resolved NMR spectra because of shift averaging. Another disadvantage is the great number of binding interactions between the single glucose monomers and the analytes which aggravates spectroscopic studies.

For this reasons systematic selector design is almost impossible and developments of new polysaccharide stationary phases are rather based on a "trial and error" principle.

2.4.1.2 Protein Type CSPs

Proteins immobilized onto support materials (most often silica) also play a role in chromatographic enantiomer separation. Protein selectors of commercial relevance are α1-acid glycoprotein (AGB), human serum albumin (HSA), bovine serum albumin (BSA), chicken ovomucoid (OMCHI) and cellobiohydrolase I (CHB I).[7] Especially the AGP-based CSPs are of biggest importance because they were found to resolve the broadest range of chiral compounds.[17]

Protein-type CSPs have relevance in bioanalytical chemistry exploiting their broad enantiomer separation abilities towards chiral drugs and drug-like molecules.[18] They are also used for studies on protein-drug binding interactions like determination of equilibrium binding constants or binding kinetics.

However, operation of protein based CSPs is strongly limited in several aspects. They are only applicable in aqueous mobile phases (reversed phase conditions) and within a certain pH-range to avoid denaturation. The possibility of biodegradation, low analytical loading capacity because of rather poor selector coverage and complex method development are other negative aspects which are limiting the application of protein-type CSPs for commercial use.

2.4.1.3 CSPs based on synthetic polymers

Among numerous synthetic polymeric chiral stationary phases of more or less promising enantioselectivities and commercial importance one special type is selected and introduced in this chapter, the concept of molecularly imprinted polymers (MIPs). In contrast to the CSPs described in the previous chapters the MIP-CSP is designed target-specifically. The preparation of MIPs comprises the equilibration of the SA (the template) with an excess of monomer, a cross-linking agent and a porogenic solvent. The monomers accumulate around the SA and form noncovalent complexes. After equilibration polymerization (thermal or photochemical) is started. When the polymerization process is finished the templates will be eluted obtaining a polymeric CSP with defined cavities in form of negatives of the templates. These molecular imprinted polymers are simple in preparation, show chemical stability and are applicable for a broad range of chiral analytes.[19]

However, a main disadvantage of MIPs is their low binding site density. The bulk polymerization process leads to big particles and low mass transfer properties (which is chromatographically recognized as peak tailing). This problem was adressed by replacement of the bulk polymerization process with polymerization on preformed particles of unique size and the use of monolithic stationary phases, respectively. In spite of these improvements MIPs are still not competitive to meanwhile well established CSPs.

2.4.2 Macrocyclic CSPs

Macrocyclic CSPs represent the field of intermediate molecular weight selectors and comprise cyclodextrin-type, antibiotic glycopeptide-type and crown ether-type CSPs. Cyclodextrines are basket-shaped D-(+)-glucopyranose oligomers consisting of either six, seven or eight monosaccharides. They have been used for chromatographic enantiomer separation for a long time.[20] Cyclodextrine-type CSPs which are prepared by covalent attachment of the cyclodextrine SO onto modified silica can be operated in normal phase mode, polar organic mode or reversed phase mode.

Macrocyclic CSPs based on glycopeptide antiobiotics are of particular commercial interest. Substances like vancomycin (**Figure 2.3**), teicoplanine, the aglycon of teicoplanine (TAG) and ristocetin A are primarily investigated and used as chiral selectors.

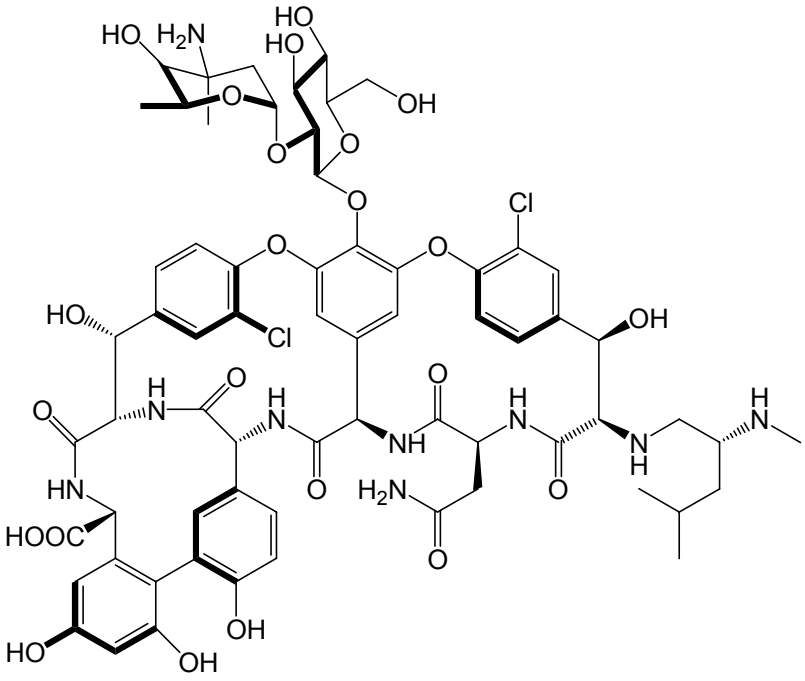


Figure 2.3: Structure of vancomycin

Vancomycin (and also the antibiotics named before which are similar in structure) possesses a basket-shaped framework and shows a variety of functional groups and aromatic moieties providing potential interaction possibilities for chiral analytes. On the other hand, the complexity of these molecules aggravates the molecular recognition understanding. Glycopeptide antibiotic CSPs operated in reversed phase and polar organic phase mode exhibit excellent enantiomer separation performance. A large variety of organic compounds – among other things underivatized amino acids – can be resolved.[21]
Another advantage of these phases is their so-called overlapping substrate specificitiy which means that a poorly resolved analyte on one antibiotic-type CSP may be well separated using another, similar antibiotic CSP.[22]

Another important species of macromolecular chiral stationary phases is that of crown ether-type CSPs. One crown ether-type selector – a bis-(1,1'-binaphthyl)crown ether – is depicted in **Figure 2.4**.

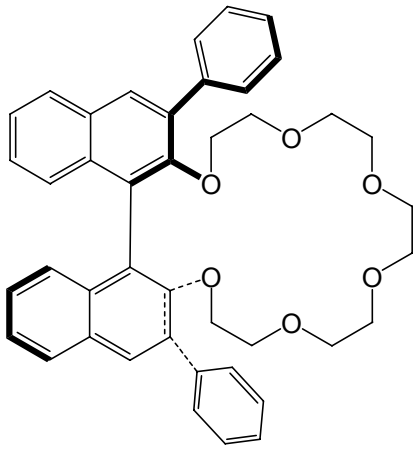


Figure 2.4: (3,3'-diphenyl-1,1'-binaphtyl)-20-crown-6

Most important selector-selectand interaction is hydrogen bonding, for instance, between the ether oxygens of the crown ether and the hydrogens of an amino group. Thus, this class of CSP is suitable for enantiomer separation of the narrow range of amines, amino acids and amino acid derivates, which has been successfully proven.[23]

2.4.3 Low Molecular Weight CSPs

Synthetic or semisynthetic chiral selectors of a molecular weight smaller than of macrocycles and which are attached onto a support material are referred to as low molecular weight CSPs. They have distinct advantages compared to high- and intermediate molecular weight CSPs. Low molecular weight SOs show definite and clearly arranged binding interactions with the selectands and, thus, are the selectors of choice for investigations of molecular recognition mechanisms. The simplicity of this SO type allows accurate and target-orientated selector design enabling resolution of specific analytes. The selectors require little space when being attached onto a support material which enables high selector coverage and, consequently, high loading capacity. Moreover, low molecular weight SOs are usually available in both enantiomeric forms facilitating a switch in elution orders of the resolved analytes.

CSPs with selectors being covalently attached to the surface of a modified silica gel are referred to as **brush type phases**. They are highly resistant to chemical and physical stress because of stable covalent bindings to the silica support material.

2.4.3.1 Donor – Acceptor Type CSPs

This type of low molecular weight selector interacts with analytes via nonionic interactions such as hydrogen bonding, π-π stacking, dipole-dipole stacking and steric interactions. A donor-acceptor type SO became the first commercialized CSP designed by the Pirkle group. It is known under the trade name DNBPG® and is depicted in **Figure 2.5**.

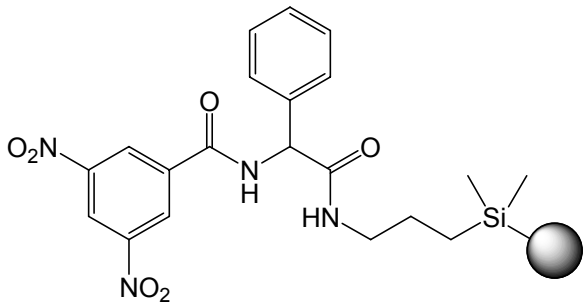


Figure 2.5

According to their limited functional groups these selectors are only feasible for a few classes of analytes, in particular those bearing an aromatic moiety. Furthermore, operation of these phases is limited to normal phase conditions because highly polar and protic solvents disturb the chiral recognition process.

2.4.3.2 Ion Exchanger CSPs

The principle of ion exchange selectors is based on ion pairing interactions between the SO and the chiral analyte as main interaction force. A prerequisite for an enantioselective ion exchange process are ionizable groups within the chiral selector (and within the analytes, respectively). Within this chapter ion exchange SOs based on synthetic or semisynthetic low molecular weight scaffolds are presented because they are considered as commercially most relevant. Apart from electrostatic interactions as dominating long range attractive force there exist additional attractive and / or repulsive interactions like hydrogen bonding, π-π stacking, dipole-dipole stacking or steric interactions, which may finally enable enantiodiscrimination.
Intensive and thourough research has been made by our group designing and preparing brush type chiral ion exchanger stationary phases. **Quinine and Quinidine O9 carbamate selectors** covalently attached onto silica showed high resolution values for numerous chiral acids.[24] The most promising one, O9-tert-butyl carbamoylated quinine, is illustrated in **Figure 2.6**.

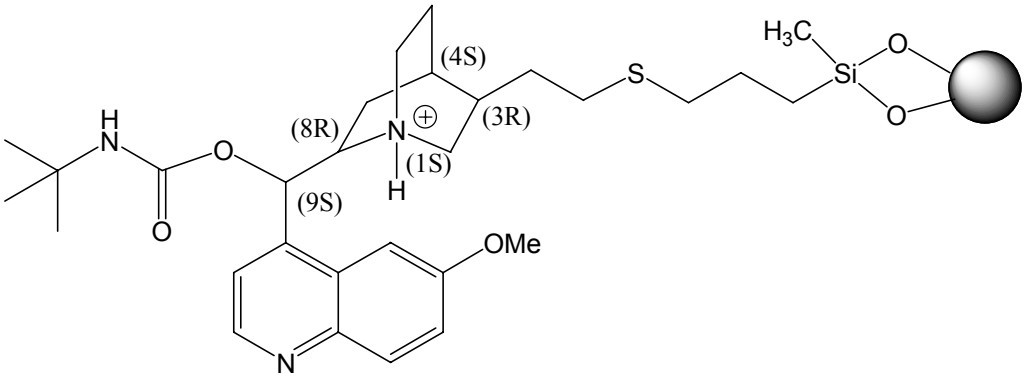


Figure 2.6: O9-tert-butyl carbamoylated quinine

The cinchona alkaloids quinine (QN) (8R, 9S) and the diastereomer quinidine (QD) (8S, 9R), which are serving as scaffolds for the ion exchanger SO, are advantageous in several aspects. First of all, quinine and quinidine show pseudoenantiomeric behaviour which results in reversal of elution orders when switching from a QN-CSP to a QD-CSP.[25] This can be of practical relevance when having the choice of eluting the desired analyte enantiomer first or second – especially when it comes to preparative separations, detection and quantification of minor enantiomeric impurities. Furthermore, the QN / QD scaffold provides well accessible binding interactions. The protonated tertiary amine in the quinuclidine moiety serves as a weak anion exchanger as primary interaction force, whereas the quinoline moiety provides possibilities for π-π stacking and the carbamate modification at O9 offers possibilities for hydrogen bonding as secondary, supportive interaction modes.[7] Additionally, the bulky tert-butyl substituent can favour enantiomer separation.[26]

The quinine and quinidine based anion exchange CSPs (which are from now on referred to as QN-AX CSP and QD-AX CSP, respectively) show high chemical and mechanical stability under liquid chromatographic conditions.[26] Both CSPs are used in analytical and preparative scale and have been comercialized by Chiral Technology Europe (Strasbourg, France) (CHIRALPAK® QN-AX and CHIRALPAK® QD-AX).

For chromatographic evaluation buffered reversed phase and buffered polar organic mobile phases are applied.[27] By varying concentration and type of the buffer salts as well as the type of organic solvent retention and enantioselectivity can be tuned.

Complementary to QN-AX CSPs cation exchange (CX)-type CSPs based on synthetic low molecular weight scaffolds have been recently prepared.[28] The CX-SO is depicted in **Figure 2.7**

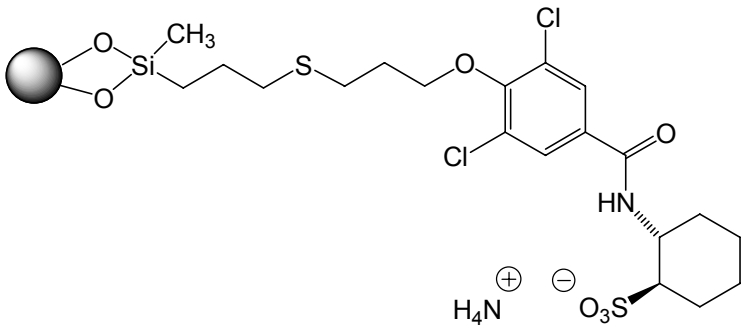


Figure 2.7: Cation exchange-type CSP

The sulfonic acid moiety serves as a strong cation exchanger and, thus, forms ion pairing interactions with basic compounds like chiral amines or aminoalcohols. The electrostatic interactions are supported by SO-SA π-π interactions or hydrogen bonding. In analogon to QN-AX phases inversion of stereoconfiguration at the chiral SO leads to reversal of elution order of separated analytes.[28] This CSP achieved baseline separation for a diverse set of basic chiral analytes in combination with high peak efficiencies.

Like QN-AX CSPs cation exchange phases are preferably operated in buffered polar organic mobile phases. Acidic additives create a slightly acidic mobile phase to protonate the basic analytes which is required for SO-SA electrostatic interactions.

To conclude, the concept of brush type ion exchange phases partially shows excellent enantioselectivity. In combination with high physicochemical stability and loading capacity they are predestinated for preparative enantiomer separation. However, the respective CSP (either CX or AX) can only be applied for analytes of opposite charge which is limiting the field of application.

2.5 Enantiomer Separation of Amino Acids

An extra chapter is dedicated to enantiomer separation of amino acids as a very important class of organic compounds. Also synthesis of the chiral selectors for this diploma work concentrated on enantioseparation of amphoteric analytes like underivatized amino acids. Nowadays direct amino acid separation is feasible on macrocyclic glycopeptide-, crown ether type- and protein-type CS Ps, which have been mentioned in the previous chapters. However, now the attention is turned to **ligand exchange-type CSPs**, the historically oldest CSPs ever. The ligand exchange technique was developed by Davankov et al. being the first who has ever achieved baseline separation of chiral analytes and being the first completely resolving racemic amino acids.[29] Moreover, ligand exchange phases became the first comercially available CSPs and were the method of choice for separation of amino acids in the beginning era of enantioseparation.

The principle of ligand exchange chromatography is based on formation of transient diastereomeric metal complexes whose different thermodynamical stabilities and formation rates are leading to different retention times. The ternary metal complexes consist of a bidentate chiral ligand which is attached to a stationary phase, a transition-metal ion and another chiral (racemic) ligand being the analyte. Rigid cyclic amino acids like proline or hydroxyproline are most often used as ligand attached to the solid phase support.

As divalent, chelating metal ions Cu (II), Ni (II), Zn (II), Cd (II) and Hg (II) can be used with Cu (II) being preferentially employed.[30] The Cu (II) ions are generally added to the aqueous mobile phase (most often water-methanol mixtures are applied) together with the racemic analytes. After isocratic elution Cu (II) is removed from the separated enantiomer. Fullfilling the prerequisite of bivalent chiral selectors most often amino acids are used, for example L-hydroxyproline in the commercial Nucleosil Chiral-1 column.

The chelate complex is then formed by coordination of the chiral selector ligand and one enantiomer of the injected racemate to the copper ion. Additionally, the complex is stabilized by coordination of solvent molecules to the metal ion in axial positions. Stereoconfiguration of the chelated analyte enantiomer can either enforce or weaken solvent coordination which results in different kinetic and thermodynamic stability between the diastereomeric complexes.

Figure 2.8 shows a model for such complexes.

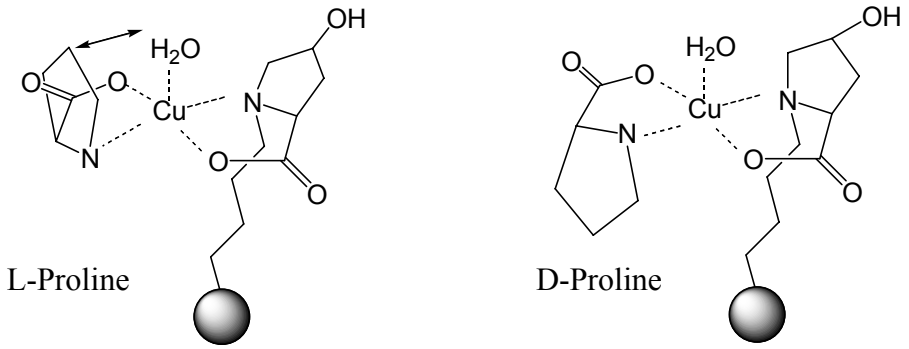


Figure 2.8: Model for complex of proline enantiomers on L-hydroxyproline selector

Regarding **Figure 2.8**, one can see that the complex formed with the L-Proline enantiomer is of less stability because of steric interactions with the coordinated water molecule (used as solvent). Therefore, retention of L-proline will be lower than that of D-proline.

Generally, additional intermolecular interactions like π- π interactions, hydrophobic effects or hydrogen bonding may also occur and contribute to differences in free energies of the diastereomeric complexes.

For obtaining high resolution it is necessary to guarantee fast kinetics (fast formation and dissociation of the diastereomeric complexes). This can be achieved by selection of appropriate metal-ions and positioning of the electron donor groups of the chiral selector. Additionally, the system can be optimized by increasing column temperature and modifying solvent composition and mobile phase pH.[31]

Nevertheless, poor efficiency still remains a main problem due to low rate of ligand exchange reactions. Generally, one can say that ligand exchange chromatography is characterized by excellent enantioselectivity but rather poor column efficiency.[30]

The limited application for just bidentate (tridentate) analytes and labor intensive method development for obtaining adequate separations are another reasons why ligand exchange chromatography has lost commercial relevance compared to e.g. polysaccharide based CSPs.

3 Design and Synthesis of Novel Chiral Zwitterionic Ion Exchange Stationary Phases

The main synthetic focus of the diploma work was on the development of novel zwitterionic chiral stationary phases. The fundamental idea was to fuse chiral anion and cation exchange units into one single zwitterionic selector.

Therefore, well established chiral weak anion exchangers (WAX) based on cinchona alkaloid derivatives (like Chiralpak® QD-AX and Chiralpak QN-AX Lindner phase columns) were merged with recently investigated strong cation exchange (SCX) chiral stationary phases (CSPs)[28].

Figure 3.1 visualizes the concept of the zwitterionic CSPs.

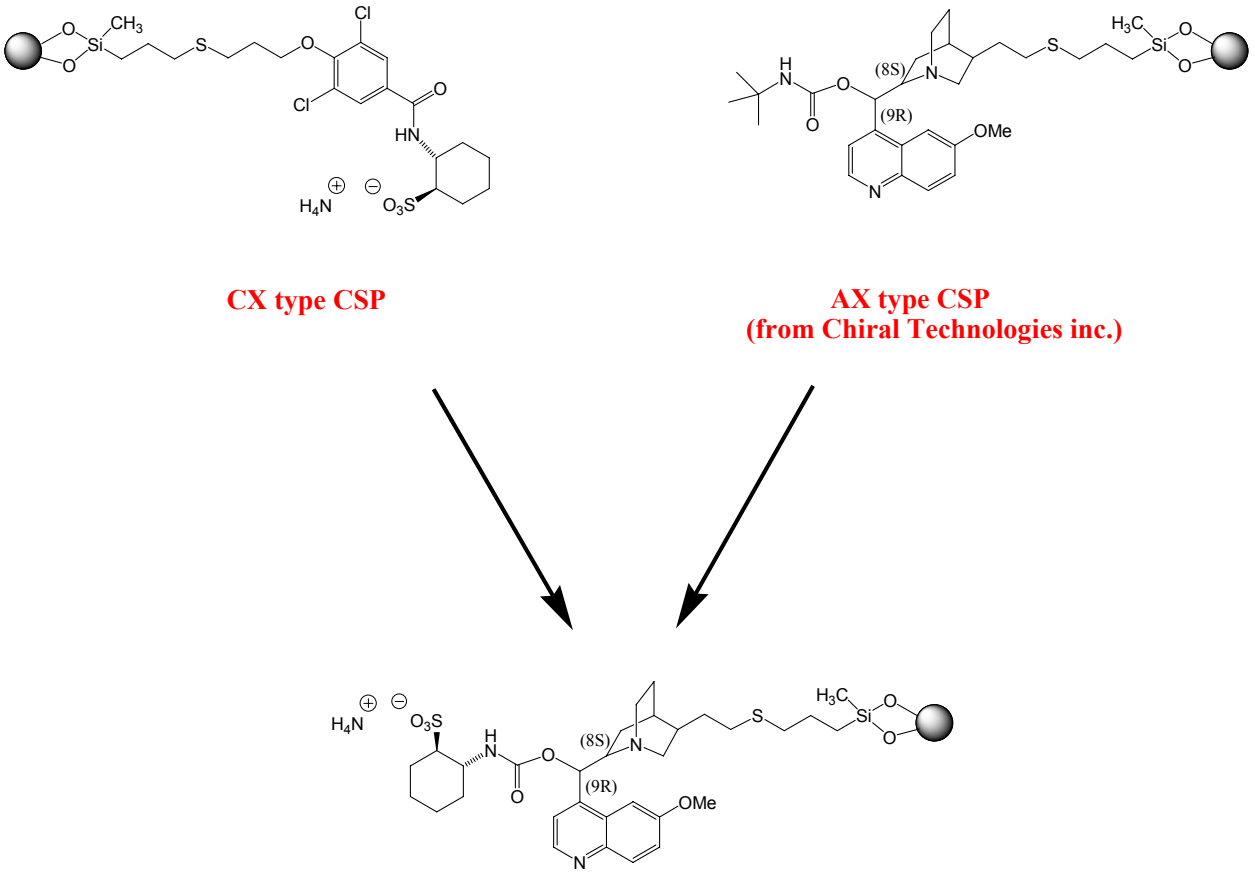


Figure 3.1

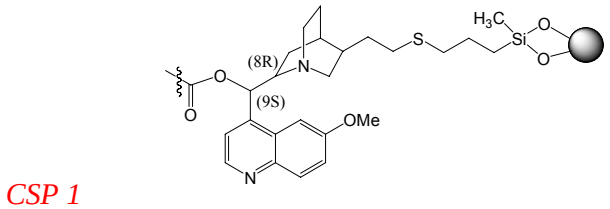
The sulfonic acid based strong cation exchange-type (SCX) CSP shown in **Figure 3.1** has proven to be a powerful tool in enantioselective separation of chiral amines. On the other hand, quinine/quinidine based carbamoylated weak anion exchange CSPs are capable to separate a great many of chiral acids. As a logical consequence, the idea came up to fuse these acidic and basic SO units into novel zwitterionic chiral SOs.

Previous investigations showed that the C9-position of quinine and of its pseudoenantiomer quinidine is of vital importance for chiral molecular recognition and, therefore, chiral separation ability.[25, 32] As a matter of this it was suggested to leave the C9 configuration unaltered and new acidic functionalities were introduced via chemically stable carbamate linkages.

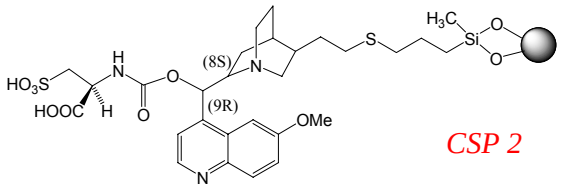
Several quinine based selectors have been prepared by our group varying acidic cation exchange units to make thorough chromatographic investigations possible.[33]

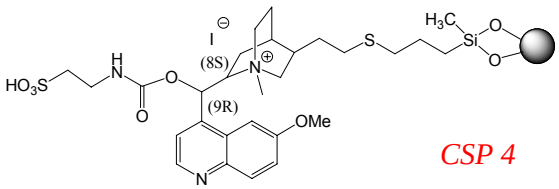
Hence, the synthetic work was not only limited in introducing new acidic moieties into cinchona scaffolds but also varying other crucial units of these novel zwitterionic CSPs.

Figure 3.2 gives an overview of all synthesized CSPs.

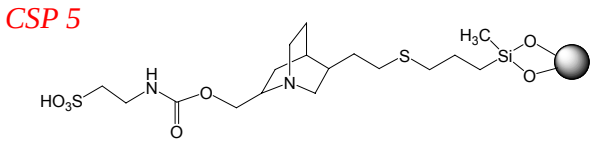


Quinidine-type (8R, 9S)

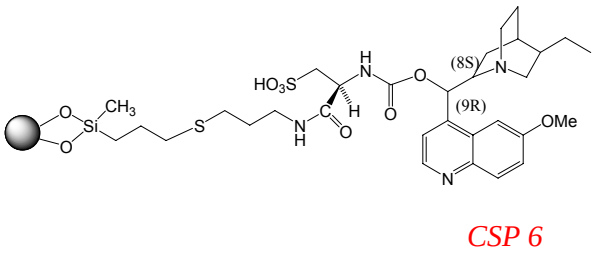




Quinine-type (8S, 9R)



Quincorine-type



Dihydro-Quinine-type

Table 3.1 shows the short names, full names and the selector coverages of the synthesized CSPs. The short names are referred to throughout the rest of the chapters. All colums were packed in house.

CSP Number	Short Name	Full Name	Coverage μmol [SO] g^{-1} [CSP]
CSP 1	Tau-QD	taurine-quinidine	210
CSP 2	L-CSA-QN	L-cysteic acid-quinine	240
CSP 3	Quinu-QD	quinuclidine-quinidine	218
CSP 4	Tau-N-Methyl-QN	taurine-1-N-methyl-quinine	105
CSP 5	Tau-Quinc	taurine-quincorine	290
CSP 6	L-CSAD-DH-QN	amido-L-cysteic acid-dihydroquinine	260

The aim was to prepare CSPs with selector loadings at about 200 μmol g^{-1}. In fact, the actual coverages vary between 105 and 255 μmol g^{-1}. Possible reasons for these fluctuations will be discussed on the following pages.

However, previous investigations in our group showed that selector density affects retention (higher selector loadings means having more possibilities for selector – analyte interactions and, therefore, increases retention time) but not so much enantioselectivity value α and enantiomeric separation can be achieved with SO loadings down to 100 μmol g^{-1}.

As it was stated before, the main focus was on varying the AX and CX moieties within the zwitterionic SO to create a new basis for posing more questions on the molecular recognition mechanism.

CSP 1 (Tau-QD) was primarily prepared for comparison with previously synthesized Tau-QN The comparison of SOs which only differ in their cinchona alkaloid scaffold (quinidine versus its pseudoenantiomeric form quinine) should confirm previous investigations in obtaining reversed enantiomer elution orders when switching from a quinine to a quinidine based SOs. It should be tested if this behaviour is maintained in the concept of these novel zwitterionic CSPs.

Chromatographic investigations on zwitterionic CSPs bearing a second acidic functionality led to the design of L-CSA-QN (**CSP 2**). In this case an additional carboxylic functionality was introduced whose effects on enantiomer separation should be investigated. Moreover, the L-cysteic acid site created another stereogenic center apart from the C8 and C9 centers of quinine. It was interesting to see how far this case would affect separation of enantiomers.

CSP 3 differs from the zwitterionic CSP scheme, so it is a "double anion exchange CSP". It was mainly synthesized to compare it with standard QN/QD-AX CSP (Chiralpak® QD-AX and Chiralpak® QN-AX) phases to evaluate how a second anion exchanger site affects enantioselectivity and retention, of course. Another aspect was if this bisbasic CSP is able to coordinate bisacidic analytes via double ion pairing in analogon to zwitterionic CSPs which are coordinating amphoteric analytes.

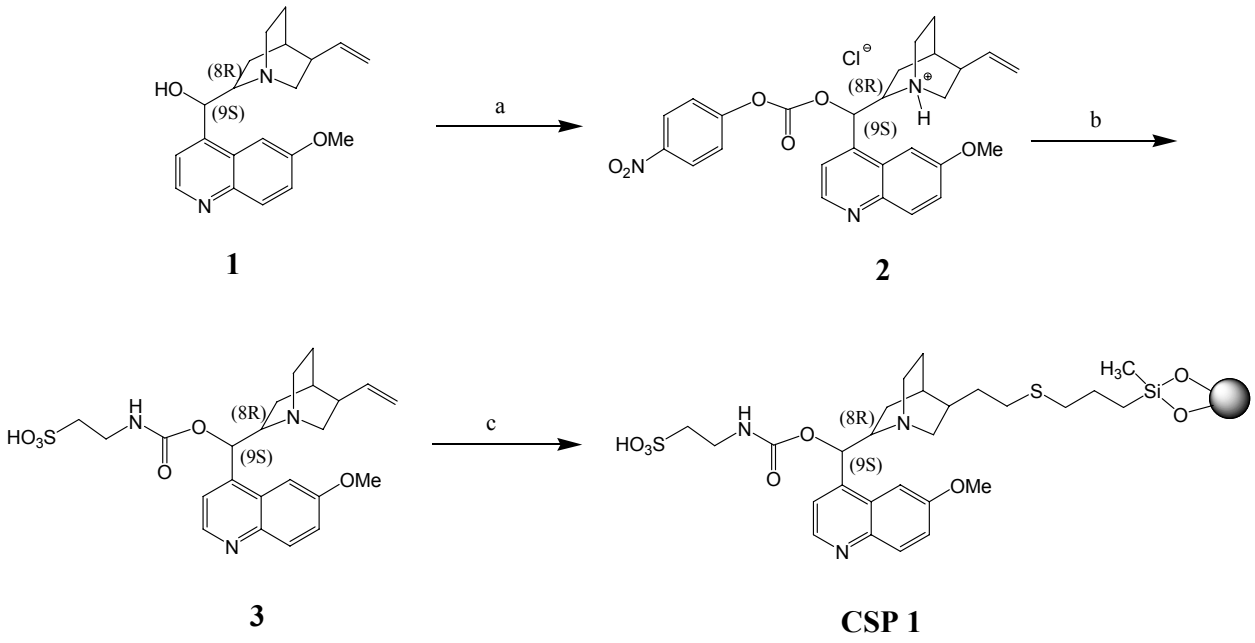
CSP 4 could be seen as the strong anion exchange (SAX) analog to Tau-QN. It was methylated at the quinuclidine nitrogen position in order to obtain a quaternary ammonium ion and hence, a strong anion exchange binding site. So far, strong cation exchange (SCX) sites like sulfonic acids turned out to be the more powerful selector than for example weak cation exchangers like carboxylic acids. As a consequence, it has to be investigated if a strong anion exchanger is able to improve enantiomeric separation as well.

Furthermore, the effect of π-π interactions for enantiomeric separation was a topic of detailed analysis. For this reason Tau-Quinc (**CSP 5**), which is characterized by a missing quinoline moiety, was synthesized. As π-π interactions are seen as an important factor for enantiomeric separation of aromatic analytes it was interesting to see wether enantiomeric separation is degraded or not. Another important aspect was how the missing quinoline moiety would affect separation of non aromatic analytes.

At last steric aspects were taken into consideration in preparing the L-CSAD-DH-QN selector (**CSP 6**). It is a modification of the carboxylic group of the L-cysteic acid moiety and could have also been made with L-CSA-QN (**CSP 2**). Because of the dihydroquinine scaffold a double bond for SO immobilization had to be introduced via allylamine coupling. With the preparation of **CSP 6** it was hoped to get new ideas how a more volatile SO (because of the completely different binding site between SO and silica) would influence enantioselectivity values.

On the whole, this overview should only give an idea on the background of the sythesized CSPs. More specific details according to the SO`s structure and its impact on enantiomeric separation will be discussed in the chromatography chapter.

3.1 Synthesis of Tau-QD (CSP 1)



*Scheme 3.1: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) taurine, BSA, CH_2Cl_2, reflux, 24 h, 2) **2**, CH_2Cl_2, r.t., 24 h (c) AIBN, MeOH, reflux, 5 h.*

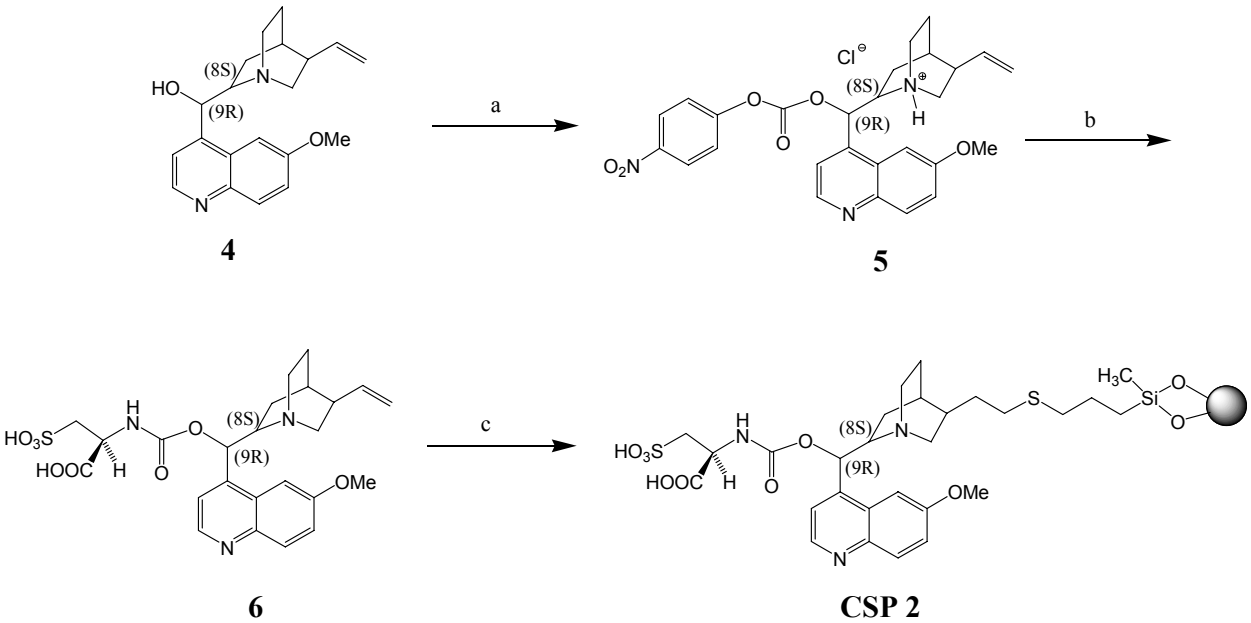
Scheme 3.1 shows the synthetic route to novel **CSP 1**. Commercially available quinidine **1** was transformed into its activated ester hydrochloride **2** by reaction with of 4-nitrophenyl chloroformate. The starting material had to be used as free base to achieve quantitative yields of **2** because of precipitation of the product (purification by chromatography would not have been possible due to instability). The activated ester is slightly sensitive to hydrolysis and should be stored under inert gas. The zwitterionic taurine-quinidine selector **3** was achieved by reaction of the silylated taurine with the activated ester hydrochloride **2**. First it was necessary to silylate taurine with O,N-bis(trimethylsilyl)acetamide (BSA) to avoid solubility problems due to inner salt formation. After that, nucleophilic addition of the silylated taurine to the activated ester hydrochloride led to novel taurine-quinidine selector **3**. It has turned out that only complete silylation (clear solution indicates complete silylation) of the corresponding amino acid led to high yields of the zwitterionic selector.

Compound **3** was purified by flash chromatography especially for removing acetamide, a byproduct that is formed from the silylating reagent.

Reaction step c was the most crucial one in synthesis of the CSP. Covalent selector immobilization was carried out via radical addition of the thiol-modified silica with the double bond of the taurine-quinidine selector. Experimental investigations showed that high selector loadings can only be achieved by using as little solvent as possible. What is more, the structure of the selector (zwitterionic, bisbasic, bisacidic) could have some influence on immobilization, too.

Therefore, it was generally not possible to create CSPs with equal selector loadings and they varied between 105 and 255 μmol g^{-1} whereas the low number was actually the exception.

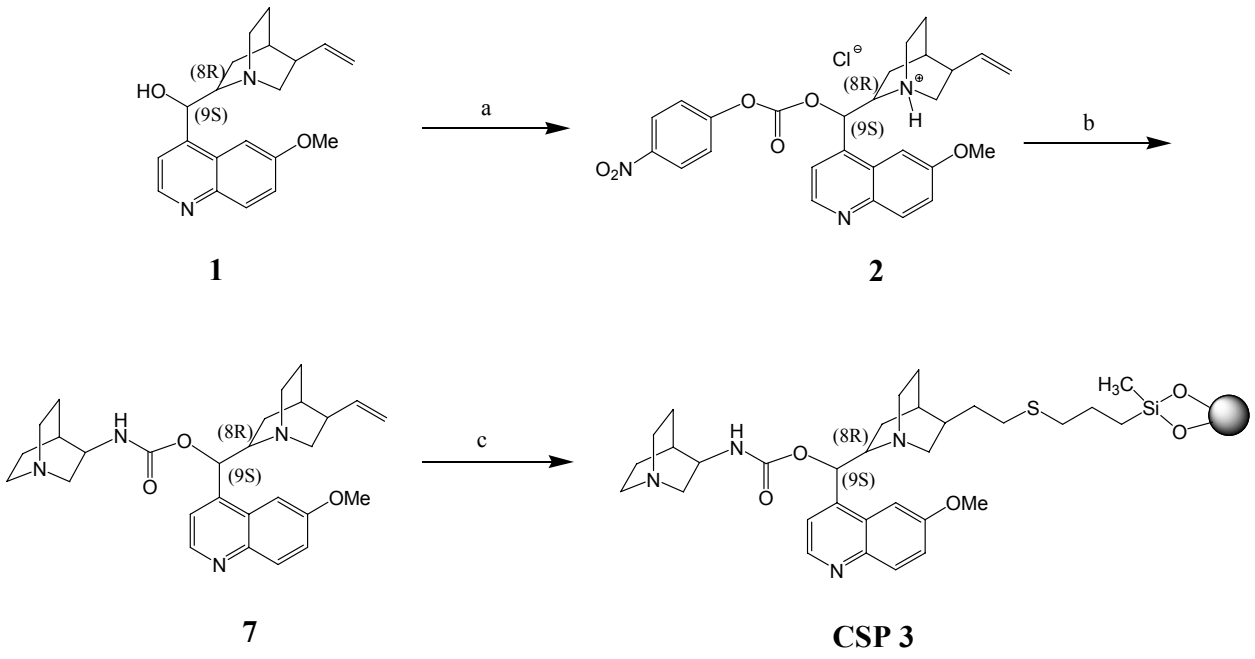
3.2 Synthesis of L-CSA-QN (CSP 2)



Scheme 3.2: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) L-cysteic acid, BSA, CH_2Cl_2, reflux, 48 h, 2) ***5****, CH_2Cl_2, r.t., 24 h (c) AIBN, MeOH, reflux, 5 h.*

Synthesis of L-CSA-QN (**CSP 2**) was similar to the preparation of **CSP 1**. This time the base free of quinine **4** was used as a starting material instead of quinidine. Addition of 4-nitrophenyl chloroformate led to quinine activated ester hydrochloride **5**. For getting the desired L-cysteic-QN selector **6** it was also necessary to convert the enantiomerically pure L-cysteic acid in its silylated form by addition of BSA. Nucleophilic additon of the silylated L-cysteic acid to the activated ester hydrochloride **5** led to L-cysteic acid quinine selector **6**. When quenching this reaction with MeOH (as it was done when synthesizing **CSP 1**) a viscous, polymer-like substance was usually formed. This residue could only be redissolved in MeOH and spectroscopic investigations showed that it was pure L-CSA-QN selector **6**. As a consequence, when carrying out these reaction again it was done without purification by flash chromatography to avoid unnecessary loss of product. When preparing **CSP 2** selector immobilization was carried out in completely the same way as it was done in Scheme 3.1.

3.3 Synthesis of Quinu-QD (CSP 3)



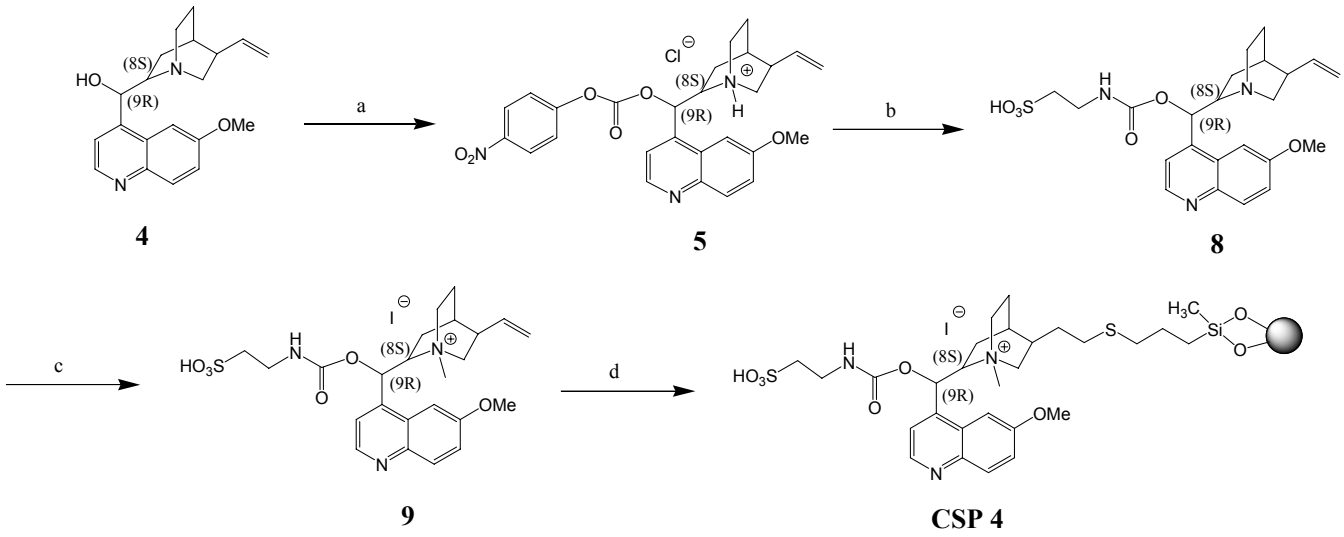
1 **2** **7** **CSP 3**

Scheme 3.3: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 3-aminoquinuchlidine (racemic mixture), CH_2Cl_2, r.t., over night, (c) AIBN, MeOH, reflux,5 h.

Scheme 3.3 shows the synthetic route to Quinu-QD (**CSP 3**). This CSP consists of two anion exchanger moieties instead of one AX and one CX moiety and is not zwitterionic anymore. It was designed to investigate enantiomeric separation behaviour for acidic analytes (especially analytes with two acidic functionalities) which will be discussed more detailed in the chromatography chapter.

Step a and b of the synthetic scheme followed standard procedures as described for **CSP 1** and **2**, respectively. The quinuclidine-quinidine SO **7** was obtained by nucleophilic addition of a racemic mixture of the free base of 3-aminoquinuchlidine to activated quinidine ester hydrochloride **2**. Because a racemic reagent was used two diastereomeric selectors were produced and immobilized. For the investigation of basic properties of this bisbasic CSP the use of a racemic selector was adequate.

3.4 Synthesis of Tau-N-Methyl-QN (CSP 4)



Scheme 3.4: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) taurine, BSA, CH_2Cl_2, reflux, 24 h, 2) ***5****, CH_2Cl_2, r.t., 48 h, (c) 1) KOH, MeOH 2) MeI, DMF, r.t., 60 h, (d) AIBN, MeOH, reflux, 5 h.*

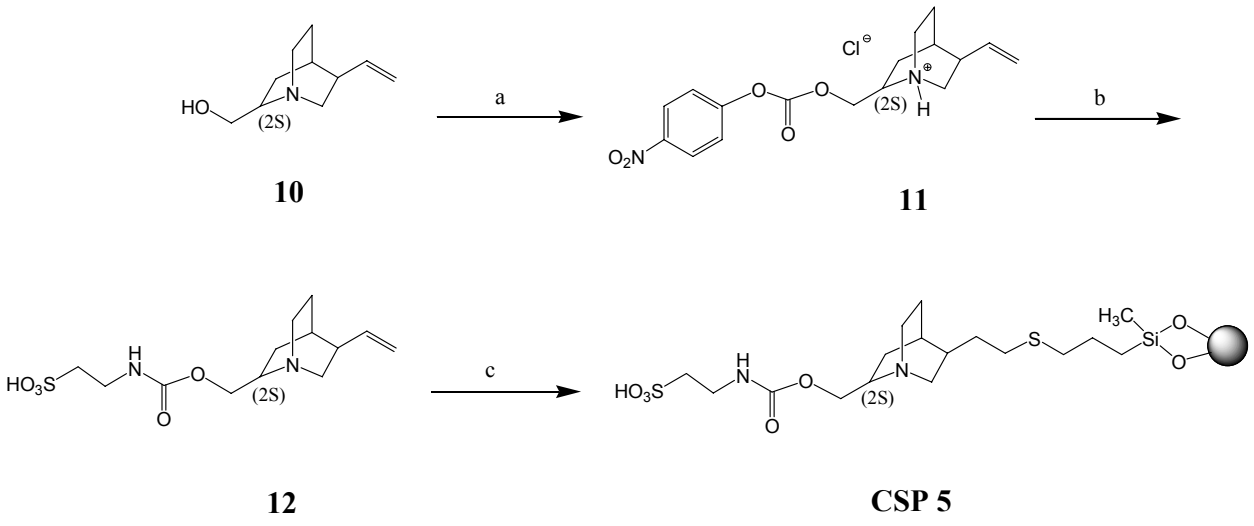
During the preparation of Tau-N-Methyl-QN (**CSP 4**) steps a and b followed standard procedures. Although this time Tau-QN **8** was synthesized instead of Tau-QD **2** no differences were noticed in practical synthetic work which is probably another aspect of the pseudoenantiomeric behaviour of quinine and quinidine.

However, quaternization of the quinuclidine nitrogen by methylation (step c) turned out to be not as straightforward as expected. Several attempts were tried varying solvents (switching from the protic solvent MeOH to aprotic solvents like CH_2Cl_2 and DMF), temperature and reaction time without any satisfying results. It was concluded that compound **8** in its zwitterionic form (meaning that sulfonic acid is deprotonated and the quinuclidine nitrogen is therefore protonated) is not accessible for alkylation. A protonated quinuclidine nitrogen transforms the tertiary amine into a quaternary one which is badly accessible for methylation, of course. Therefore, compound **8** had been transformed into its potassium salt before it was subjected to methylation.

Despite of varying reaction parameters like temperature, reaction time and equivalents of iodomethane no satisfying results could be obtained. The desired product **9** was always accompagnied by species with additional methylation at the carbamate nitrogen on one hand and non methylated, unreacted educt on the other hand (it must be pointed out that under such reaction conditions no methylation at the quinoline nitrogen occurs).[34] Therefore, the product mixture was purified by preparative RP-HPLC (reversed phase conditions had to be used because the very polar products could not be eluted on normal phase silica any more) obtaining pure compound **9**.

Unfortunately, immobilization of the Tau-N-Methyl-QN selector wasn`t working as expected where a selector loading of only 105 μmol $g^{-1}$ could be achieved. One possible explanation could be that the quaternary nitrogen affects radical addition negatively. This case has to be subjected to further investigations which couldn`t have been carried out any more.

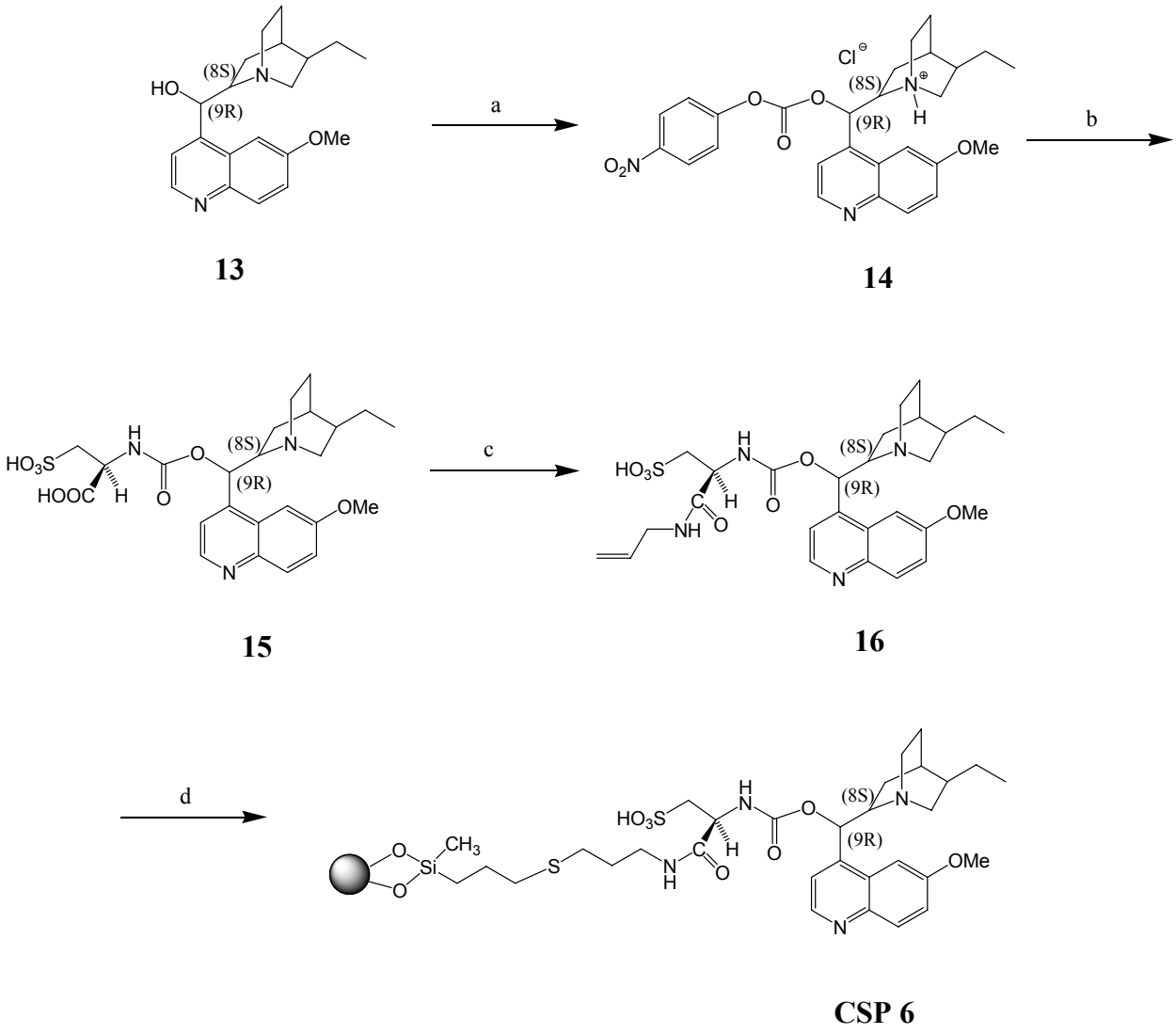
3.5 Synthesis of Tau-Quinc (CSP 5)



Scheme 3.5: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) taurine, BSA, CH_2Cl_2, reflux, over night, 2) ***11****, CH_2Cl_2, r.t., 40 h, (c) AIBN, MeOH, reflux, 5 h.*

Scheme 3.5 gives an overview of the synthetic route to **CSP 5**. At first sight one can see that with the quinoline moiety a significant part of the alkaloid motif is missing. So this CSP was designed to make investigations on how a missing position for π-π interactions affects enantiomer separation ability of zwitterionic CSPs. For the synthesis quincorine **10** was used as starting material (its pseudoenantiomeric form quincoridine is principally available, too). Addition of 4-nitrophenyl chloroformate led to activated quincorine ester hydrochloride **11** (a white solid - similar to previous activated quinine and quinidine esters - was formed which could easily be filtrated off). Preparation of the Tau-Quinc selector **12** followed the same procedure as it was carried out for the quinine/quinidine selectors. However, purification by flash chromatography was hampered because of missing UV-light absorption of the product and no colouring reagents could be found to make the product spots visible. Therefore, the use of flash chromatography was limited to the removal of the 4-nitrophenol byproduct. Acetamide as another byproduct was removed by iterative trituration using acetone. This purification process turned out to be sufficient regarding the following immobilization leading to **CSP 5**, which was obtained in a high selector loading of 290 μmol g^{-1}.

3.6 Synthesis of L-CSAD-DH-QN (CSP 6)



*Scheme 3.6: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) L-cysteic acid monohydrate, BSA, CH_2Cl_2, reflux, 24 h, 2) **14**, CH_2Cl_2, r.t., 60 h, (c) allylamine, DCC, DMF, r.t., 70 h, (d) AIBN, MeOH, reflux, 5 h.*

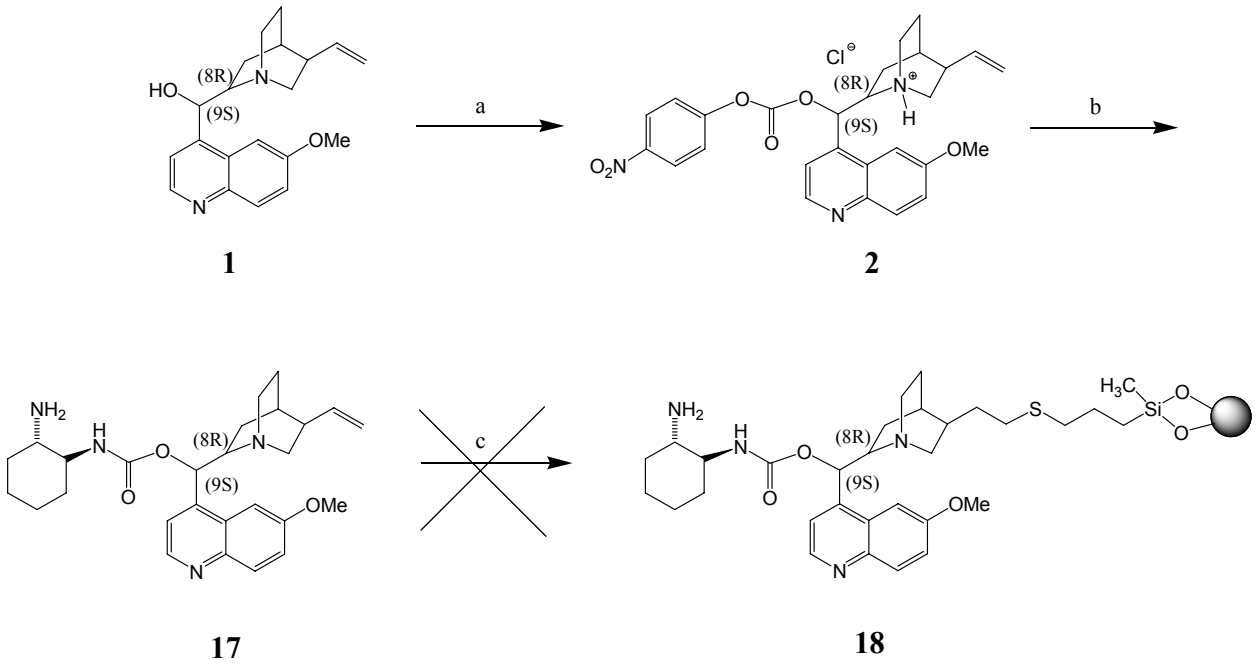
Scheme 3.6 shows the synthetic route of **CSP 6**. The goal was to create a selector with a completely different binding position to the silica compared to the other CSPs shown in Table 3.1. Since an olefinic double bond is required for radical addition it had to be introduced elsewhere within the selector molecule while the olefine group on the alkaloid substructure had to be eliminated to avoid double immobilization and inhomogeneous selector structures on the surface of the stationary phase. Therefore, dihydroquinine **13** with a saturated ethyl group in 3-position was used as convenient starting material (in its base free form). Steps a and b were carried out in meanwhile well known synthetic procedures as they were applied for preparation of CSP 2. However, it has to be noted that L-cysteic acid was not commercially available any more, so L-cysteic acid monohydrate had to be used instead in connection with a high excess of BSA to absorb undesired amounts of water.

For preparing the L-CSAD-DH-QN selector **16** several attempts for the allylamine coupling were carried out. Generally, no quantitative yields for the amide formation could be obtained. One explanation may be found in a sterically difficult access to the carboxylic function. The use of DCC as coupling reagent in combination with DMF as solvent was the best compromise regarding practical synthetic work and yield. Compound **16** was not purified by flash chromatography because of very close elution times of educt and product in a variety of eluent systems. Therefore, impurities due to unreacted starting material were accepted when doing the final immobilization step d because only the desired product was expected to be immobilized leading to **CSP 6**.

3.7 Synthesis of Selectors of non-realized CSPs

3.7.1 Synthesis of DACH-QD

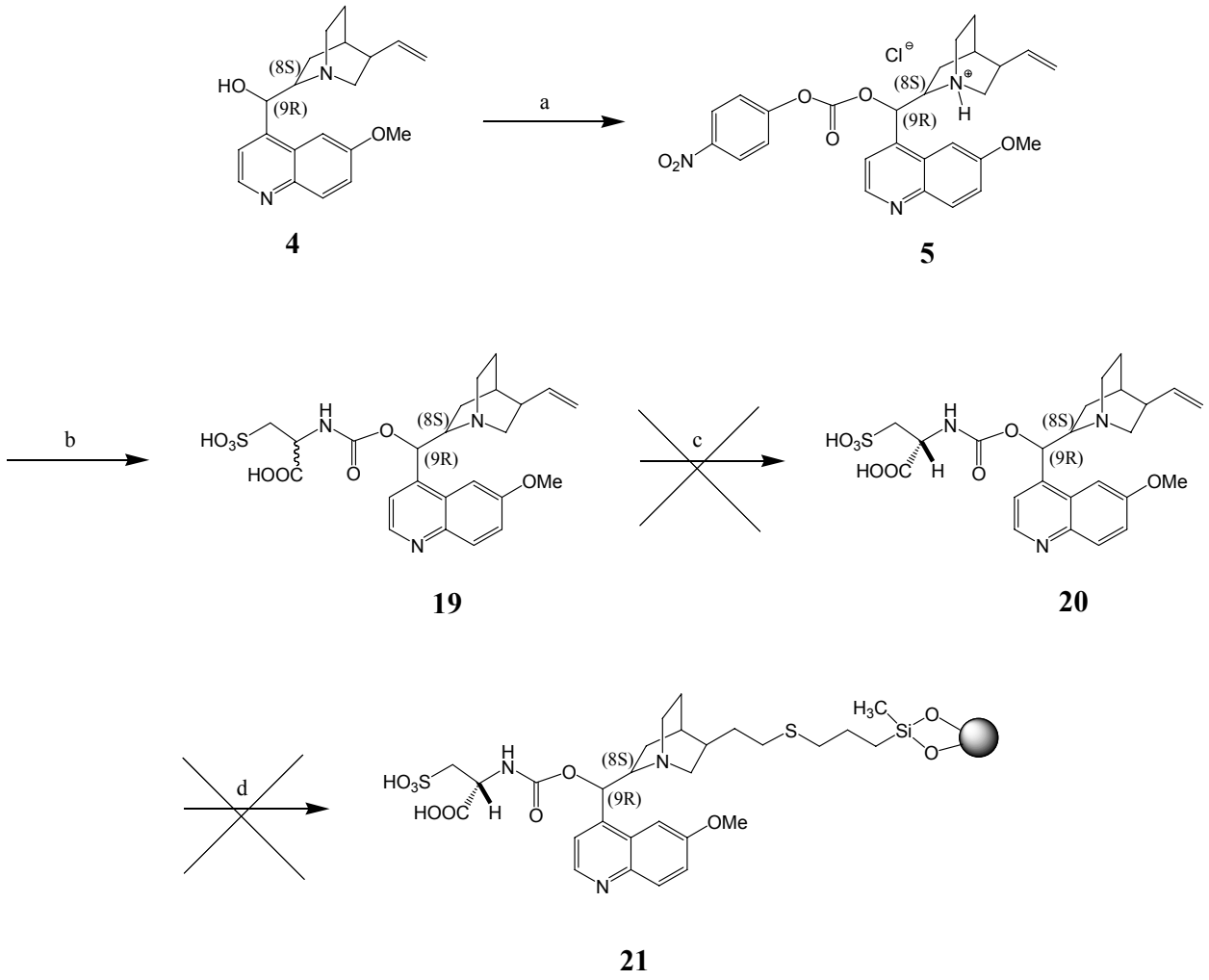
At the beginning of my diploma work a selector with purely AX character similar to **CSP 3** was synthesized, as outlined in **Scheme 3.7**. It could either be seen as an AX-analog to a previously prepared SCX-type CSP (see **Figure 3.1**), which is bears a sulfonic acid group instead of the amino group, or as supplementary derivative of the Quinu-QD type, now having a primary amine as the second AX site. The DACH-QD (diaminocyclohexane-quinidine) selector **17** was also designed as a promising building block employing the cyclohexane motif for synthetic concepts based on the primary amino group as functional group for further modifications.



Scheme 3.7: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1R,2R-diaminocyclohexane, , CH_2Cl_2, r.t., over night (c) AIBN, MeOH, reflux, 5 h.

The DACH-QD selector **17** was prepared by nucleophilic addition of enantiomerically pure diaminocyclohexane to activated quinidine ester hydrochloride **2**. After the standard immobilization step, however, no significant increase of nitrogen content was measured in the elemental analysis of the resulting silica material, indicating that no covalent selector attachment had taken place. LC-MS analysis of the reaction filtrate revealed that bisamine **17** had been converted completely to starting material quinidine **1**. In contrast to all other C9-carbamate-type CSPs in this study, the carbamate of **17** is obviously not stable under the immobilization conditions of MeOH at reflux. Due to proximity, an attack of the primary amino group at the carbamate carbonyl group could be a possible explanation although the resulting cyclic urea species has not been detected in MS. For the purpose of a better understanding of the observations on selector **17** stability tests were carried out where variations of solvents and reaction times were accomplished. The studies clearly showed that in an aprotic solvent like CH_2Cl_2 the selector remained unaltered during the immobilization process indicating that protic MeOH could be involved in the carbamate cleavage. Although successful alternative reaction conditions were hence established, the immobilization was not repeated on a larger scale in an aprotic solvent that would yield enough CSP for packing a column because of lack of selector **17** while syntheses of other CSPs were preferred.

3.7.2 Synthesis of D-CSA-QN



Scheme 3.8: Conditions: (a) 4-nitrophenyl chloroformate, toluene, r.t., over night, (b) 1) L-cysteic acid, BSA, CH_2Cl_2, reflux, 24 h, 2) 5, CH_2Cl_2, r.t., 24 h, (c) diasteromer separation on Tau-QD type CSP, (d) AIBN, MeOH, reflux, 5 h.

Scheme 3.8. gives an overview of another unrealized CSP. The D-CSA-QN selector **20** would have been synthesized to obtain the diastereomeric form of CSP 2. Differences in enantiomer separation ability between L- and D-CSA-QN would have been investigated. Though, after preparing the mix of diastereomers of selector **19** according to procedures as they were applied for CSP 2 the diastereomer separation turned out not to be feasible: No satisfying separation was achieved on a QN-AX CSP which was available in dimensions for preparative scale.

A satisfying separation of the two diastereomers was established only on Tau-QD (**CSP 1**) where parameters could be optimized.

However, this CSP was available only in analytical dimensions, and in combination with the poor solubility of the mixture of **19** a productive chromatographic separation was not practical.

4 Evaluation of Novel Chiral Zwitterionic Ion Exchange Stationary Phases

In this chapter the results of the chromatographic evaluations of **CSP 1** – **CSP 6** are presented. Chromatographic parameters like retention factor k, selectivity coefficient α_{ji}, plate number N and resolution R_S are discussed. First of all, a principal evaluation will be made of all CSPs regarding the chromatographic parameters and in later sections a more detailed comparison between single columns will be shown.

4.1 Experimental

Chromatographic measurements were carried out on a 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with an autosampler, binary pump, degasser for the mobile phase, multiple wavelength detector (MWD) and a 6-column switching valve. For analytes with weak UV-absorbance properties a corona charged aerosol detector (CAD) from ESA Biosciences, Inc. (Chelmford, USA) was used instead. An Agilent 1200 series 2/10-valve for switching between waste and recycle mode was used as well for the purpose of solvent economy: The waste position was chosen when switching from one column to another and for column conditioning, while recycle mode was used for analysis in order to save solvents. After use the columns were washed and stored in MeOH.
Data analysis was carried out with Chemstation chromatographic data software from Agilent Technologies. Elution was performed in isocratic mode at a mobile phase flow rate of 1.0 ml/min. The void volumes of the columns were determined by injecting acetone with detection at 230 nm. For chromatographic measurements no thermostat was used and therefore, temperature may have varied between 25° and 30°C. UV-detection was accomplished at selected wavelengths between 230 and 280 nm. All analytes were applied as methanolic solutions in concentrations between 1.0 – 3.0 mg/ml. To determine elution orders of selected solute enantiomers either single enantiomers or enantioenriched samples of known absolute configuration were injected.

4.2 Mobile Phases

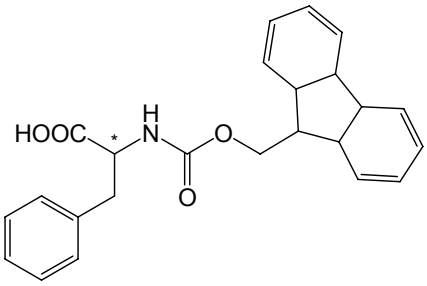
Generally, mobile phase composition is one of the most important tuning parameters in HPLC. In conventional ion exchange chromatography the elution strength is strongly influenced by varying concentrations of co- and counterions or by changing the pH-value and, therefore, the charge of ionic solutes in aqueous systems.

In the case of ion exchange chromatography using zwitterionic CSPs, polar organic mobile phases were used. They consist of the bulk solvent methanol with acidic and basic additives that act as co- and counterions. This kind of mobile phase composition has been thouroughly studied by our group when investigating CSPs of purely anionic or cationic character.[28, 32]

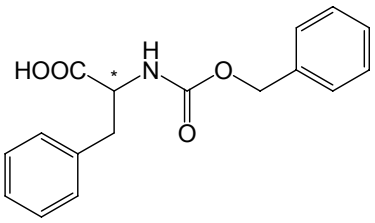
For better comparison of the chromatographic data being displayed in this chapter one common mobile phase for all columns was applied and no mobile phase optimizations were carried out. The analytes were measured using a mobile phase composition of methanol as bulk solvent and 25 mM acetic acid (HOAc) and 25 mM ammonium acetate (NH_4Ac) as additives.

4.3 Analytes

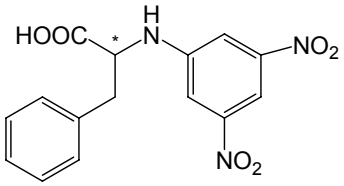
The main goal of designing and synthesizing novel brush type zwitterionic chiral stationary phases was to enable enantiomer separation for amphoteric analytes (see **Fig. 4.1**). Therefore, a broad range of unprotected amino acids was set up comprising aminoacids with aromatic, cyclic and aliphatic side chains, hydroxyl-, thiol-, carboxyl- and sulfonic acid functionalities. Not only zwitterionic analytes were chosen for a representative set of analytes but also chiral acids and chiral amines. When evaluations with acidic analytes were carried out the goal was to evaluate if the cinchona alkaloid based anion exchange properties could be conserved in the zwitterionic CSPs**. CSP 3** with its "double AX" character was only tested for acidic analytes. Hence, a set of eight acidic analytes (see **Fig. 4.2**) comprising N-protected amino acids with commonly used N-derivatizing groups was used. After testing the stereoselectivity between the AX-site and acidic analytes a set of chiral amines (see **Fig. 4.2**) was applied to investigate the CX-part. Five chiral pharmacologically relevant bases like β-blockers, β-sympathomimetics and others were selected to see if the cation exchange motif is still working in a zwitterionic CSP.



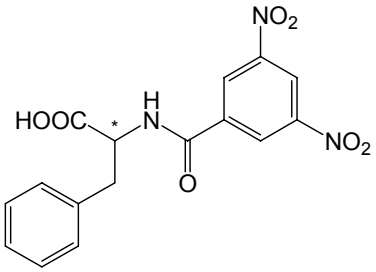
Fmoc-Phenylalanine



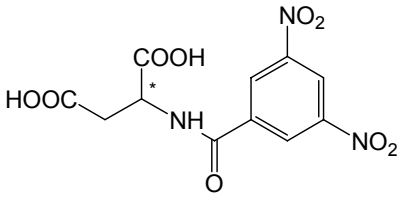
Z-Phenylalanine



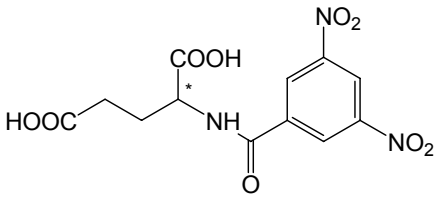
DNP-Phenylalanine



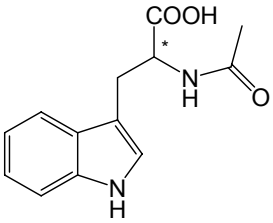
DNB-Phenylalanine



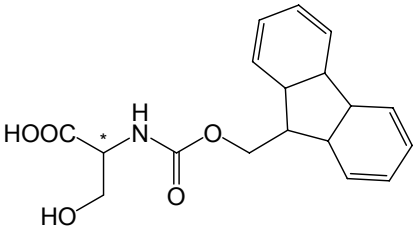
DNB-Aspartic Acid



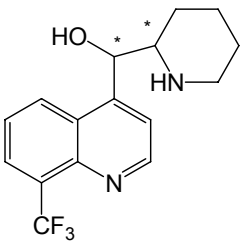
DNB-Glutamic Acid



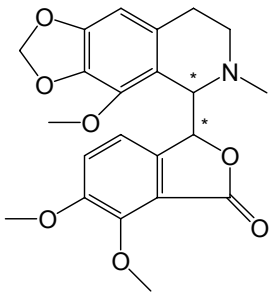
Ac-Tryptophane



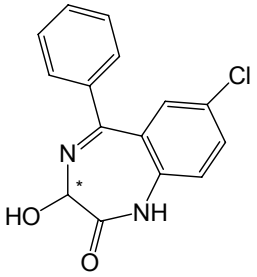
Fmoc-Serine



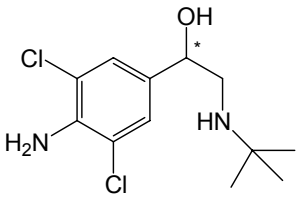
Mefloquine



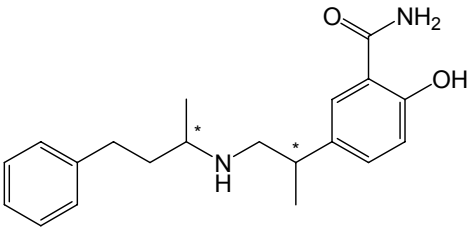
Noscapine



Oxazepam



Clenbuterol



Labetalol

Figure 4.1: Acidic and basic analytes used in the presented chromatography studies

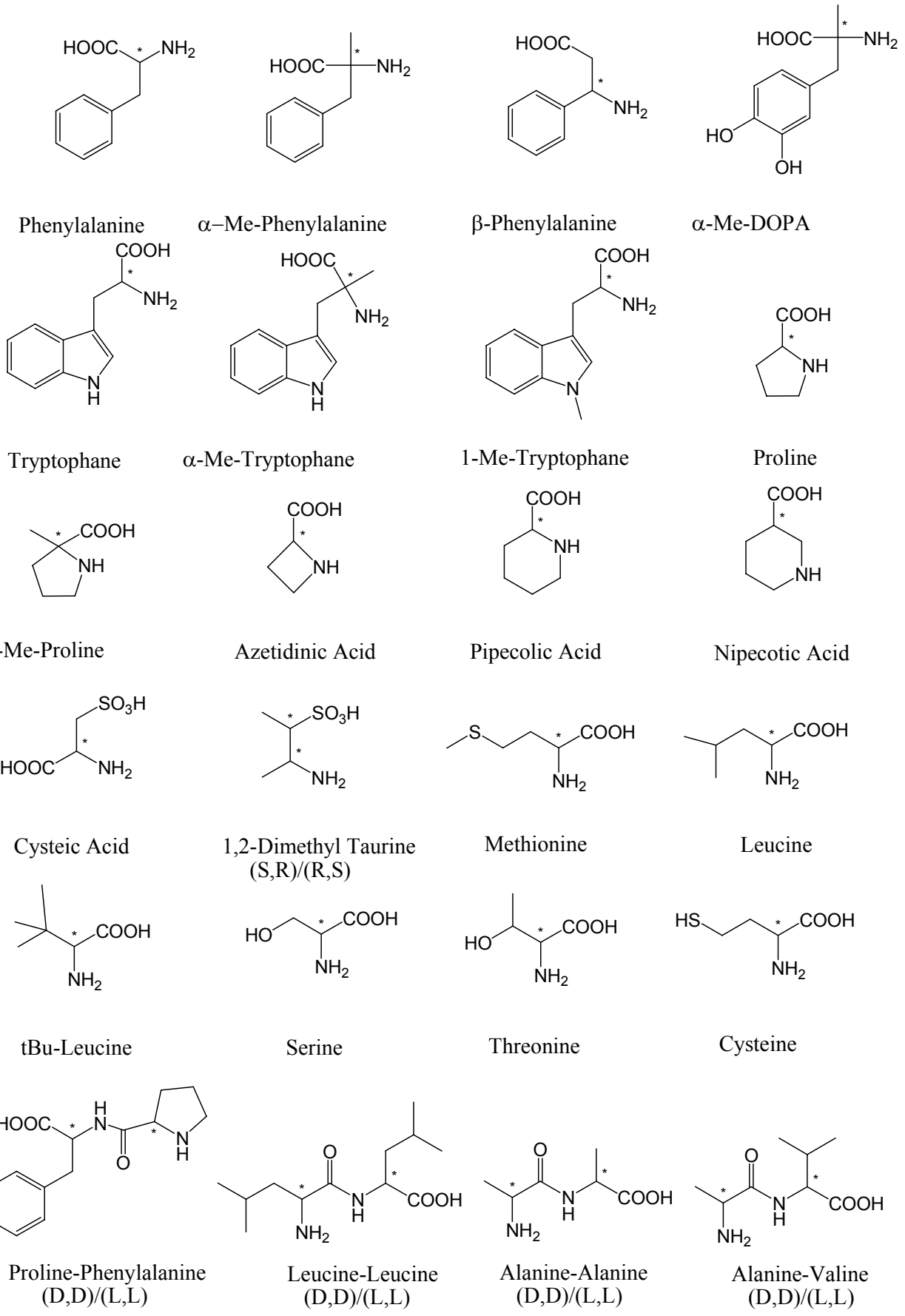


Figure 4.2: Zwitterionic analytes

4.4 General CSP Comparison

In the general comparison part principal differences of the synthesized CSPs are subject to investigation. A brief look will be taken at common chromatographic parameters, namely capacity factor k, selectivity coefficent α, plate number N and resolution R_S, of course. As written above, mobile phase composition throughout all compared data consisted of methanol with 25 mM HOAc and 25 mM NH_4Ac resulting in a acid to base ratio of 2:1.

CSP 3 is of purely anion exchange character and will be discussed in an extra chapter. In contrary, **CSP 4** (Tau-N-Methyl-QN) is of zwitterionic origin but of very low selector loading which doesn`t allow to draw conclusions regarding retention and plate numbers. Therefore, chromatographic parameters of **CSP 4** like enantioselectivity $\alpha$, which isn`t strongly affected by significantly lower selector loadings, will be discussed in an extra chapter. Consequently, **CSP 3** (Quinu-QD) and **CSP 4** (Tau-N-Methyl-QN) weren`t undergone these common chromatographic evaluations.

4.4.1 Retention Factor k

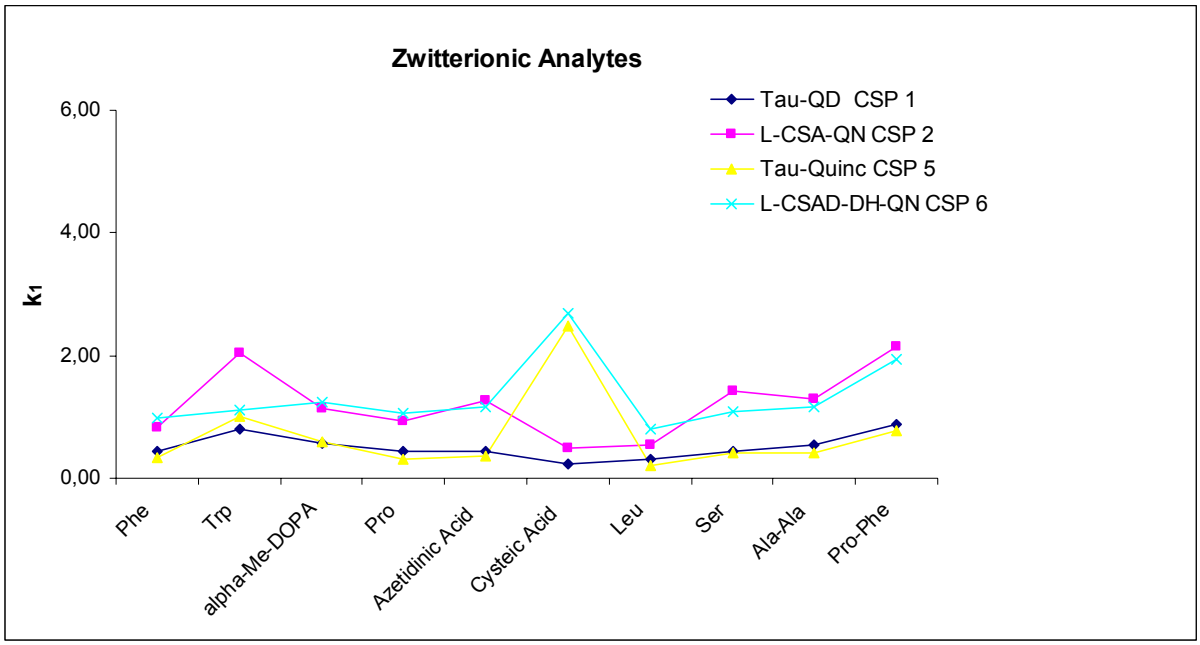
In the case of ion exchange-type chiral stationary phases under organic mobile phase conditions retention factor k reflects the binding strength between the chiral selector and the selectand. There are always k_1 values shown for separated analytes and for non separated analytes the k value of the single peak is denoted.

As stated before, those four zwitterionic CSPs possessing similar selector loadings are compared to each other. One has to take into consideration that selector loading – in other words the amount of interaction possibilities between analytes and selector – is mainly responsible for retention under the given, nonaqueous polar organic elution conditions. The synthesized zwitterionic CSPs differ in 80 µmol g^{-1} of selector loading which can be considered as a quite big difference. Nevertheless, comparison of a Tau-QD CSP with 150 µmol g^{-1} of selector loading and that of Tau-QD (**CSP 1**) with a coverage of 210 µmol g^{-1} did not show significant differences in retention time, which is an indication that the accessible selector density may be differing from the amount of immobilized selectors.

Table 4.1 shows retention factors k_1 for all investigated acidic, basic and zwitterionic analytes. In **Figure 4.3** results of elected analytes are graphically reflected.

k_1 Values				
	Tau-QD CSP 1	**L-CSA-QN CSP 2**	**Tau-Quinc CSP 5**	**L-CSAD-DH-QN CSP 6**
Selector Loadings [µmol g^{-1}]	**210**	**240**	**290**	**260**
Fmoc-Phe	0,37	0,26	1,63	2,84
Z-Phe	0,17	0,12	1,13	2,14
DNP-Phe	0,38	0,36	**2,02**	**3,15**
DNB-Phe	0,25	0,15	1,40	2,25
Ac-Trp	0,25	0,28	1,68	2,69
DNB-Glu	0,59	0,49	**3,94**	**9,86**
DNB-Asp	2,85	1,26	**5,69**	**16,50**
Fmoc-Ser	0,35	0,33	1,86	**3,05**
Phe	0,43	0,82	0,34	0,98
beta-Phe	0,87	1,15	0,30	1,09
alpha-Me-Phe	0,35	0,63	0,25	0,88
Trp	0,79	2,03	1,00	1,12
alpha-Me-Trp	0,75	1,41	0,88	1,40
1-Me-Trp	0,81	1,72	0,77	1,46
alpha-Me-DOPA	0,56	1,15	0,60	1,25
Pro	0,44	0,93	0,30	1,06
alpha-Me-Pro	0,40	0,58	0,17	0,86
Azetidinic Acid	0,44	1,27	0,37	1,16
Nipecotic Acid	0,90	1,93	0,29	1,38
Pipecolic Acid	0,42	0,73	0,30	0,94
Cysteic Acid	0,24	0,50	2,48	2,69
Dimethyltaurine	0,23	1,21	0,43	0,93
Met	0,46	0,90	0,37	1,04
Leu	0,31	0,54	0,21	0,80
tBu-Leu	0,33	0,53	0,19	0,79
Ser	0,45	1,42	0,42	1,08
Thr	0,41	0,92	0,32	0,94
Cys	0,50	///	0,58	1,10
Ala-Ala	0,55	1,30	0,42	1,17
Ala-Val	0,51	1,23	0,41	1,31
Leu-Leu	0,43	0,93	0,32	1,05
Pro-Phe	0,87	2,14	0,77	1,94
Mefloquine	1,40	**3,94**	0,19	1,25
Oxazepam	0,40	**0,60**	0,36	0,02
Clenbuterol	1,45	**3,35**	0,11	0,89
Noscapine	0,32	**0,38**	0,21	0,70
Labetalol	2,72	**11,48**	0,48	1,73

Table 4.1



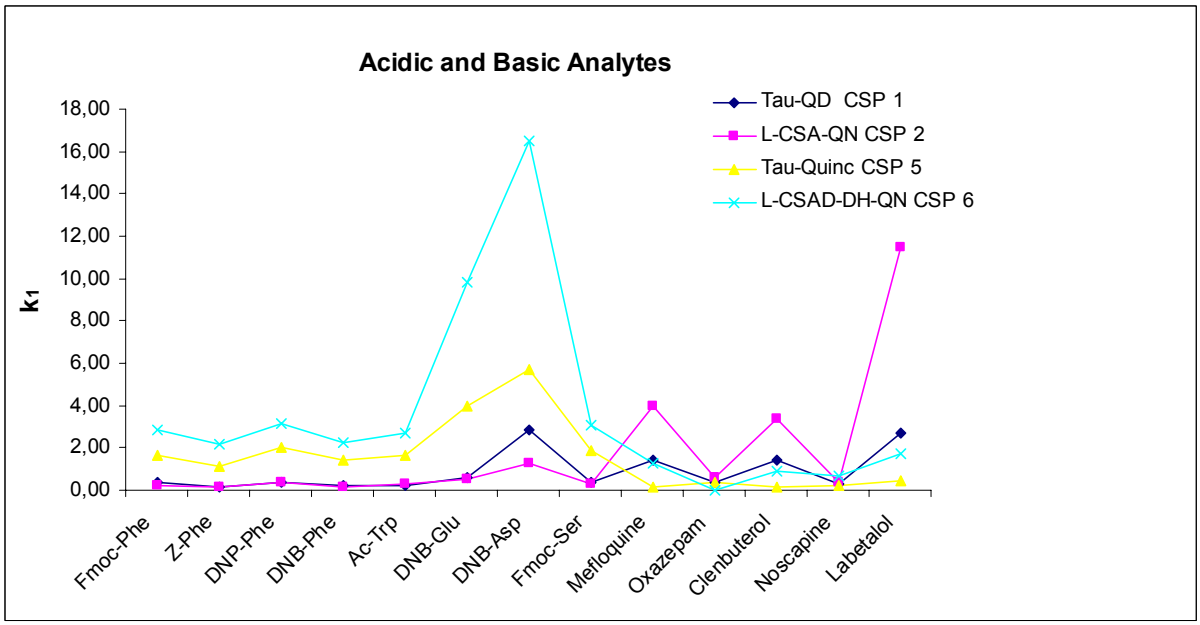


Figure 4.3: k_1 diagrams

When interpreting the obtained k_1 values the influence of different selector loadings has to be taken into consideration. Nevertheless, some genereal trends can be derived from **Table 4.1**. Previous investigations on molecular recognition showed that ionic interactions contribute at most to retention.[35] Therefore, a closer look to acid and basic functionalities of the selectors has to be taken. All CSPs presented in the study principally contain a sulfonic acid (strong cation exchanger) and a tertiary amine (weak anion exchanger) binding site within the selector. **CSP 2** provides with its extra carboxylic functionality an additional ionizable group. Consequently, it shows stronger retention for the basic amines (at least 3 fold higher than the other CSPs) which can be attributed to stronger ionic interactions (because of the additional carboxylic group) with the basic amines.

This conclusion is also confirmed by higher retention factors for amphoteric analytes compared to e.g. Tau-QD with only one acidic functionality. As a consequence, **CSP 2** shows lower retention for acidic N-protected amino acids, wherein the additional acid functionality of the SO contributes to increased elution strength. A stronger acid is a more dominant counter ion and, consequently, leads to shorter retention times.

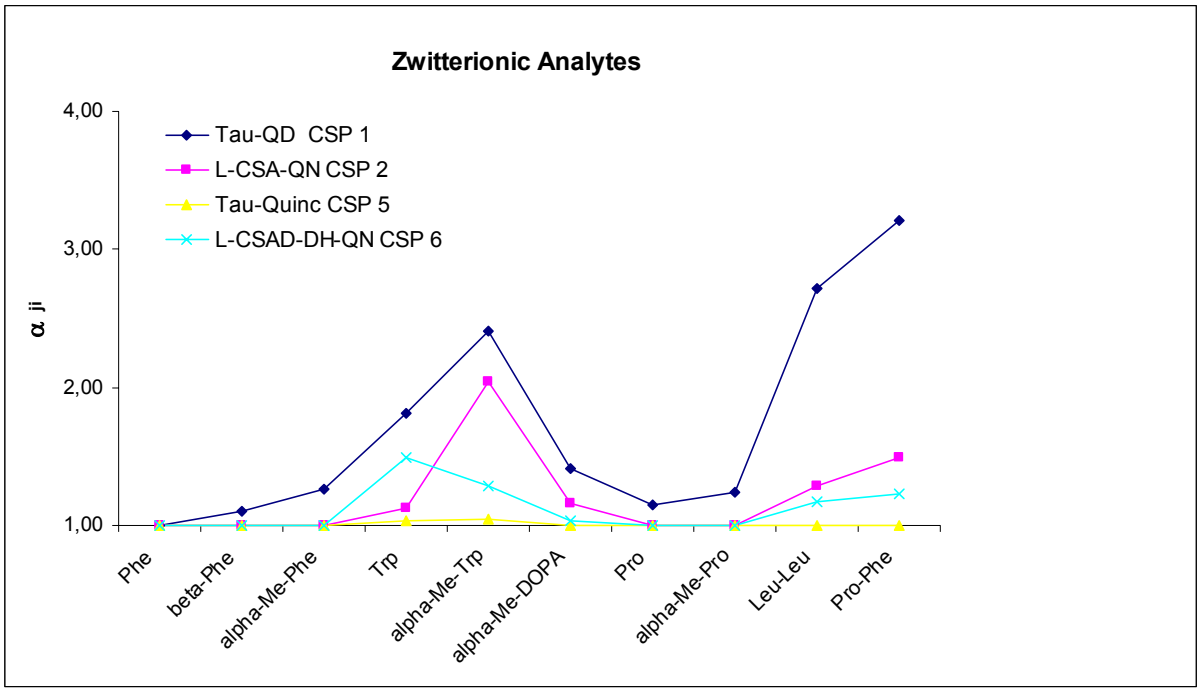
When comparing the three remaining CSPs – namely Tau-QD (**CSP 1**), Tau-Quinc (**CSP 5**) and L-CSAD-DH-QN (**CSP 6**) – one could expect similar retention between these phases if only ionic interactions were taken into account. However, the observed retention factors are differing strongly. Especially L-CSAD-DH-QN (**CSP 6**) showed stronger retention towards all classes of analytes. A possible reason may lie in different steric conditions because of an opposite binding site to the silica. Besides, there exist a stereogenic center on the cation exchanger site in contrary to **CSP 1** and **CSP 5** with achiral acidic side chains. However, it is not yet clear how far retention time is affected by these factors. Another remarkable fact is that the acidic N-derivatized amino acids are stronger retained on Tau-Quinc (**CSP 5**) than on Tau-QD (**CSP 1**). Altough **CSP 5** does not possess any possibility for aromatic interactions – which is seen as another important SO–SA interaction mode – it shows much higher retention than **CSP 1** whith its aromatic quinoline moiety. Besides, k_1 values for zwitterionic analytes are roughly equal for both **CSP 1** and **CSP 5**. The chiral amines (all containing aromatic functionalities) are even stronger retained on **CSP 1** which goes along according to prognosed interaction mechanisms.

4.4.2 Selectivity Coefficient α_{ji}

Generally, α values are an indicator for separation performance of a chromatographic system. Regarding chiral stationary phases, α gives information about the stereoselective discriminating character of a selector and, thus, its enantioselectivity. High α values are desirable because they can allow baseline separation even if unfavorable peak shapes are encountered. Especially in preparative chromatography sample loading capacities and the grade of flexibility towards mobile phase compositions profit from a higher enantioselectivity. In the presented chromatographic evaluations two different retention times were noticed whenever a clear valley between two peak maxima was observable and, therefore, α was > 1.

α Values				
	Tau-QD CSP 1	**L-CSA-QN CSP 2**	**Tau-Quinc CSP 5**	**L-CSAD-DH-QN CSP 6**
Fmoc-Phe	1,55	1,33	1,00	1,13
Z-Phe	1,46	1,00	1,00	1,07
DNP-Phe	1,78	1,00	1,00	1,06
DNB-Phe	**9,89**	**6,55**	1,00	2,06
Ac-Trp	**3,41**	**2,52**	1,00	1,21
DNB-Glu	**3,82**	**2,76**	1,00	1,74
DNB-Asp	1,90	1,19	1,00	1,23
Fmoc-Ser	1,75	1,50	1,00	1,15
Phe	1,00	1,00	1,00	1,00
beta-Phe	1,11	1,00	1,00	1,00
alpha-Me-Phe	1,26	1,00	1,00	1,00
Trp	1,82	1,13	1,03	1,49
alpha-Me-Trp	**2,41**	2,04	1,05	1,28
1-Me-Trp	1,20	1,00	1,00	1,00
alpha-Me-DOPA	1,41	1,16	1,00	1,03
Pro	1,15	1,00	1,00	1,00
alpha-Me-Pro	1,25	1,00	1,00	1,00
Azetidinic Acid	1,16	1,00	1,00	1,16
Nipecotic Acid	1,24	1,05	1,00	1,06
Pipecolic Acid	1,00	1,00	1,00	1,00
Cysteic Acid	1,00	1,00	1,00	1,04
Dimethyltaurine	1,00	1,00	1,00	1,00
Met	1,00	1,00	1,00	1,00
Leu	1,00	1,00	1,00	1,00
tBu-Leu	1,00	1,00	1,00	1,00
Ser	1,00	1,00	1,00	1,00
Thr	1,00	1,00	1,00	1,00
Cys	1,00	///	1,00	1,09
Ala-Ala	1,13	1,17	1,00	1,00
Ala-Val	1,69	1,29	1,00	1,00
Leu-Leu	**2,72**	1,29	1,00	1,17
Pro-Phe	**3,21**	1,50	1,00	1,23
Mefloquine	**1,88**	**2,45**	1,00	1,05
Oxazepam	1,00	1,00	1,00	1,00
Clenbuterol	1,03	1,11	1,00	1,00
Noscapine	1,00	1,00	1,00	1,00
Labetalol	1,07	1,14	1,00	1,00

Table 4.2: Selectivity coefficients for all investigated analytes



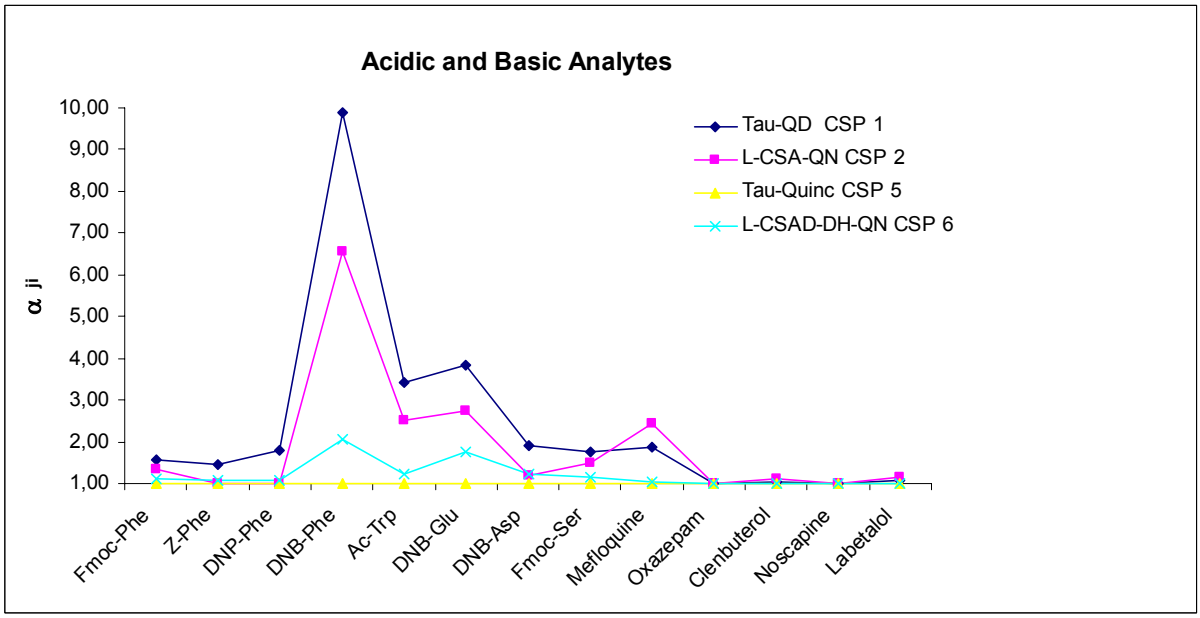


Figure 4.4: α diagrams

When looking at the results of **Table 4.2** and **Figure 4.4**, respectively, one can see evident differences in α values and, therefore, in separation abilities of the single SOs. **CSP 1** showed best overall performance of all tested CSPs. This concept of a weak anion exchange and an achiral cation exchanger site was investigated in previous studies in our group when tests on a Tau-QN CSP showed promising results.[33]

A more specific discussion comparing pseudoenantiomeric CSPs Tau-QD and Tau-QN will be made up on the following pages. **CSP 1** can also be seen in direct competition to **CSP 6** in terms of comparing the achiral and chiral sulfonic acid side chain, respectively. Zwitterionic selectors with chiral sulfonic acid side chains have been recently tested and showed so far the best enantioselectivity values. [33] Consequently, there were high expectations in improving selectivitiy and, hence, resolution when designing and synthesizing **CSP 6** – but without satisfying results. Selectivity factors are lower than for Tau-QD throughout all groups of analytes – acidic, zwitterionic and basic ones. This can be due to the fact that with L-CSAD-DH-QN an unfavorable SO configuration was chosen (when fusing chiral cysteic acid and a quinine/quinidine motif four diastereomeric SOs can be synthesized). An interesting point is that retention factors were generally higher for **CSP 6** than for **CSP 1** – another argument that high retention factors do not at all imply high stereoselectivity coefficients.

CSP 2 also shows lower α values than **CSP 1**. It seems that an additional acidic functionality hampers molecular recognition and, therefore, decreases enantioselectivity.

When testing the analyte set on Tau-Quinc (**CSP 5**) almost no enantioseparations were observed. Only partial separation for two analytes was obtained. The reason for this obviously lies in the absence of π – π interactions between selector and selectand due to the missing quinoline moiety in **CSP 5**. Furthermore, separation for non aromatic compounds could not be obtained either. Probably, this example confirms the theory that molecular recognition (and therefore enantiomer separation) is not only a consequence of ionic interactions, hydrogen bonding and π – π interactions, but also of steric interactions. In the presence of a quinoline moiety a binding pocket can be made up by quinuclidine and quinidine which is closed by the carbamate modification. It seems that this kind of binding pocket is another essential part for successful enantioseparation.

This theory is confirmed by exceptional selectivity (and retention as well) of some CSPs towards specific analytes. When looking at **Table 4.2** α values of DNB-protected amino acids (especially DNB-Phe), Tryptophane, dipeptides and Mefloquine are striking. The stronger binding enantiomers of these analytes are obviously fitting better into the binding pocket provided by the zwitterionic SO.

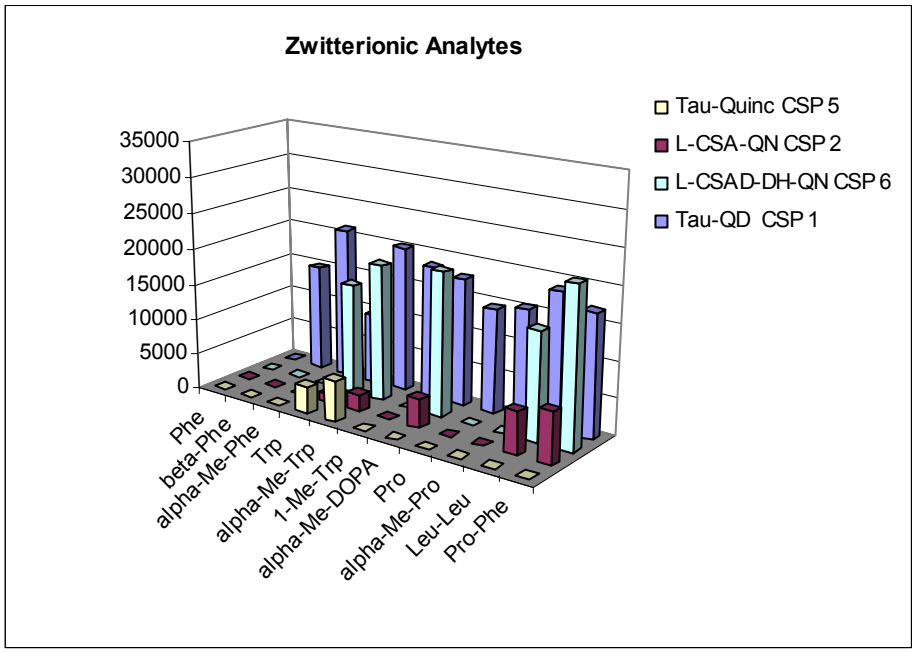
4.4.3 Plate number N

Generally, N values provide information about column performance. In context with chiral stationary phases N is correlated to selector coverage and especially to packing quality. All of the columns have been packed in house which may result in small individual differences.
In **Table 4.3** N values for all separated analytes are shown.

N Values / m				
	Tau-QD CSP 1	**L-CSA-QN CSP 2**	**Tau-Quinc CSP 5**	**L-CSAD-DH-QN CSP 6**
Selector Loadings [µmol g^{-1}]	**210**	**240**	**290**	**260**
Fmoc-Phe	13020	7260	0	16313
Z-Phe	16413	0	0	34847
DNP-Phe	16607	0	0	33687
DNB-Phe	19193	13333	0	32853
Ac-Trp	24133	10120	0	15807
DNB-Glu	12193	5867	0	6100
DNB-Asp	8160	3020	0	9640
Fmoc-Ser	14693	9880	0	27053
Phe	0	0	0	0
beta-Phe	14967	0	0	0
alpha-Me-Phe	21253	0	0	0
Trp	9773	1187	3717	15400
alpha-Me-Trp	20367	2307	5766	19127
1-Me-Trp	18567	0	0	0
alpha-Me-DOPA	17873	4067	0	20020
Pro	14593	0	0	0
alpha-Me-Pro	15533	0	0	0
Azetidinic Acid	9653	0	0	23813
Nipecotic Acid	8400	9327	0	15533
Pipecolic Acid	0	0	0	0
Cysteic Acid	0	0	0	15147
Dimethyltaurine	0	0	0	0
Met	0	0	0	0
Leu	0	0	0	0
tBu-Leu	0	0	0	0
Ser	0	0	0	0
Thr	0	0	0	0
Cys	0	0	0	24187
Ala-Ala	8620	2947	0	0
Ala-Val	16133	6647	0	0
Leu-Leu	18940	5893	0	15133
Pro-Phe	17147	7160	0	22087

Mefloquine	4053	4307	0	5067
Oxazepam	0	0	0	0
Clenbuterol	15027	15127	0	0
Noscapine	0	0	0	0
Labetalol	6493	2033	0	0

Table 4.3: N values for all investigated analytes



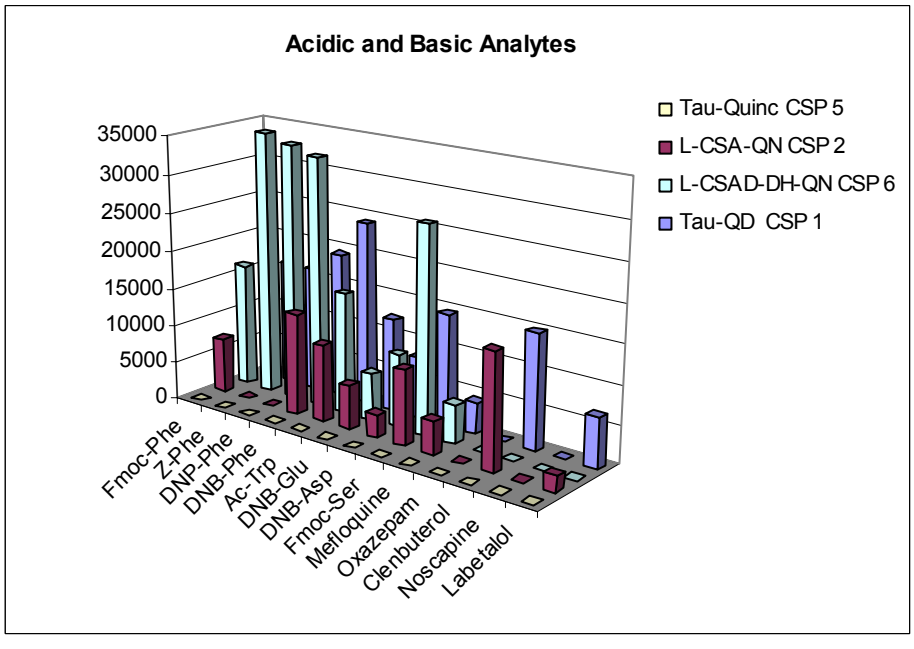


Figure 4.5: N diagrams

First of all, one has to consider that N values were calculated by the original software program. If only partial separation was achieved, integration provided a vertical line between the two peaks. Therefore, the integrated peaks are smaller and calculated peak numbers are higher than they actually would be.

Although N values can just roughly be compared with each other, some characteristics have become clear. Highest N values were obtained for L-CSAD-DH-QN (**CSP 6**). This means if separation took place the best peak shape could be achieved. Performance of Tau-QD (**CSP1**) was almost as good as **CSP 6** obtaining slightly lower N values. Column performance of **CSP 2** is roughly half as good as performances of **CSP 1** and **CSP 6**, respectively.

No meaningful statement can be issued for **CSP 5**, because partial separation was achieved only for two analytes.

4.4.4 Resolution R_S

The R_S value is doubtlessly the most significant criteria when looking at chromatographic peak separation. In contrast to selectivity α resolution R_S is a more practical tool for optimizing an appropriate stationary and mobile phase.

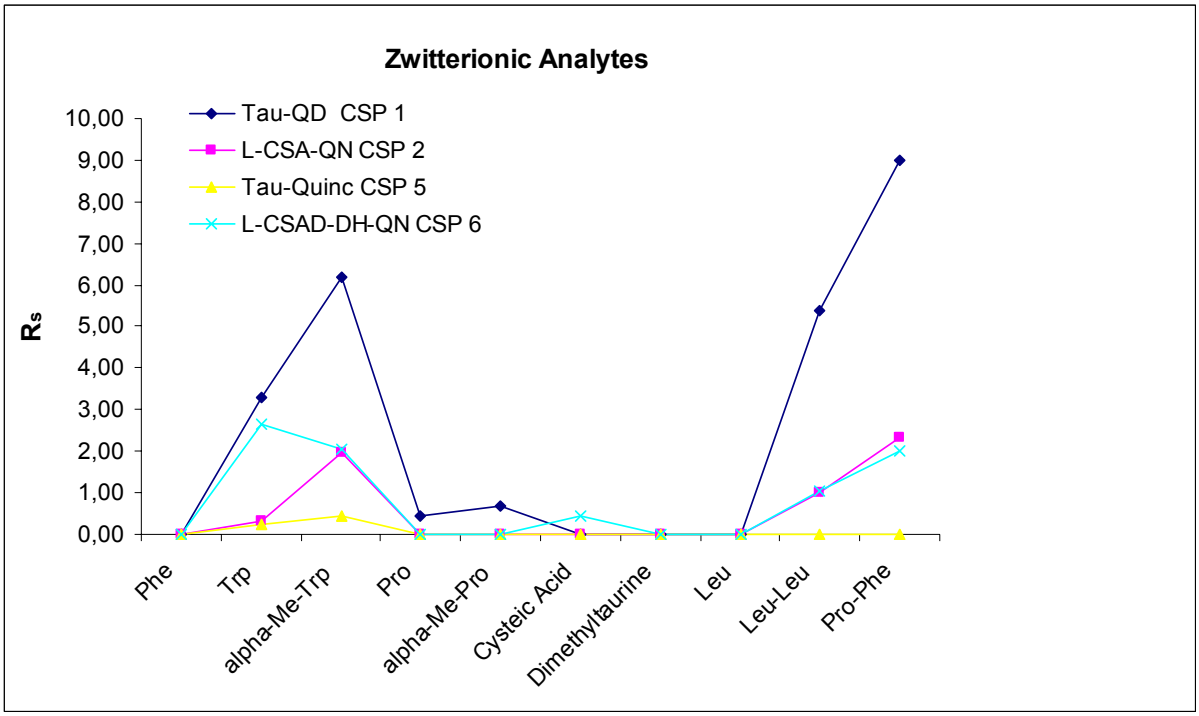
Resolution combines selectivity α, terms of peak shape (plate number N) and SO-SA binding strength (retention factor k).

Baseline separation typically requires R_S values higher than 1,5. Values above 2,0 suggest changing the chromatographic system to avoid unnecessarily long retention times (regarding analytical questions). On the contrary, in preparative chromatography high resolution values are desired because they allow higher sample loadings.

Table 4.4 depicts R_S values for the chosen set of analytes.

Rs Values				
	Tau-QD CSP 1	**L-CSA-QN CSP 2**	**Tau-Quinc CSP 5**	**L-CSAD-DH-QN CSP 6**
Fmoc-Phe	1,31	0,64	0	1,24
Z-Phe	0,49	0	0	0,79
DNP-Phe	2,66	0	0	0,80
DNB-Phe	**12,25**	**5,43**	0	**9,57**
Ac-Trp	**5,03**	**2,98**	0	2,04
DNB-Glu	**6,81**	**3,34**	0	**3,92**
DNB-Asp	**4,48**	0,63	0	1,89
Fmoc-Ser	2,02	1,16	0	1,81
Phe	0	0	0	0
beta-Phe	0,62	0	0	0
alpha-Me-Phe	0,95	0	0	0
Trp	**3,29**	0,31	0,25	**2,64**
alpha-Me-Trp	**6,18**	1,96	0,45	**2,05**
1-Me-Trp	1,18	0	0	0
alpha-Me-DOPA	1,78	0,56	0	0,27
Pro	0,45	0	0	0
alpha-Me-Pro	0,70	0	0	0
Azetidinic Acid	0,58	0	0	0,93
Nipecotic Acid	0,94	0,38	0	0,49
Pipecolic Acid	0	0	0	0
Cysteic Acid	0	0	0	0,43
Dimethyltaurine	0	0	0	0
Met	0	0	0	0
Leu	0	0	0	0
tBu-Leu	0	0	0	0
Ser	0	0	0	0
Thr	0	0	0	0
Cys	0	///	0	0,77
Ala-Ala	0,52	0,65	0	0
Ala-Val	**2,34**	1,24	0	0
Leu-Leu	**5,38**	1,01	0	1,03
Pro-Phe	**8,98**	**2,32**	0	**2,02**
Mefloquine	**2,76**	**3,73**	0	0,33
Oxazepam	0	0	0	0
Clenbuterol	0,23	1,02	0	0
Noscapine	0	0	0	0
Labetalol	0,44	0,57	0	0

Table 4.4



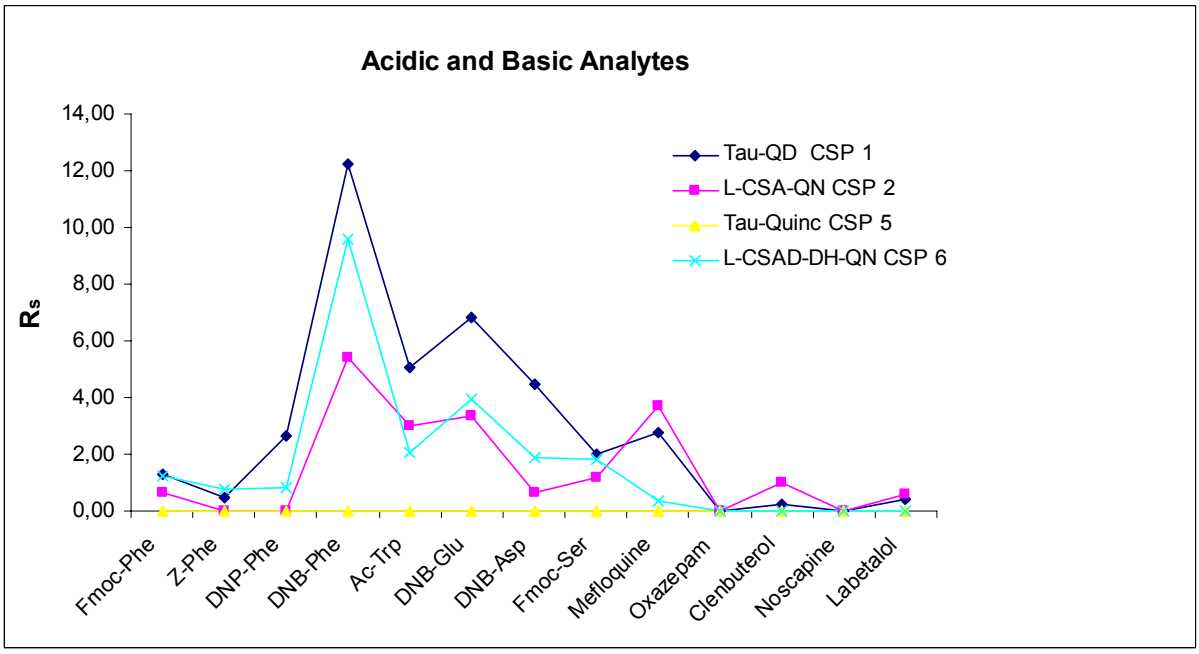


Figure 4.6: R_s Values

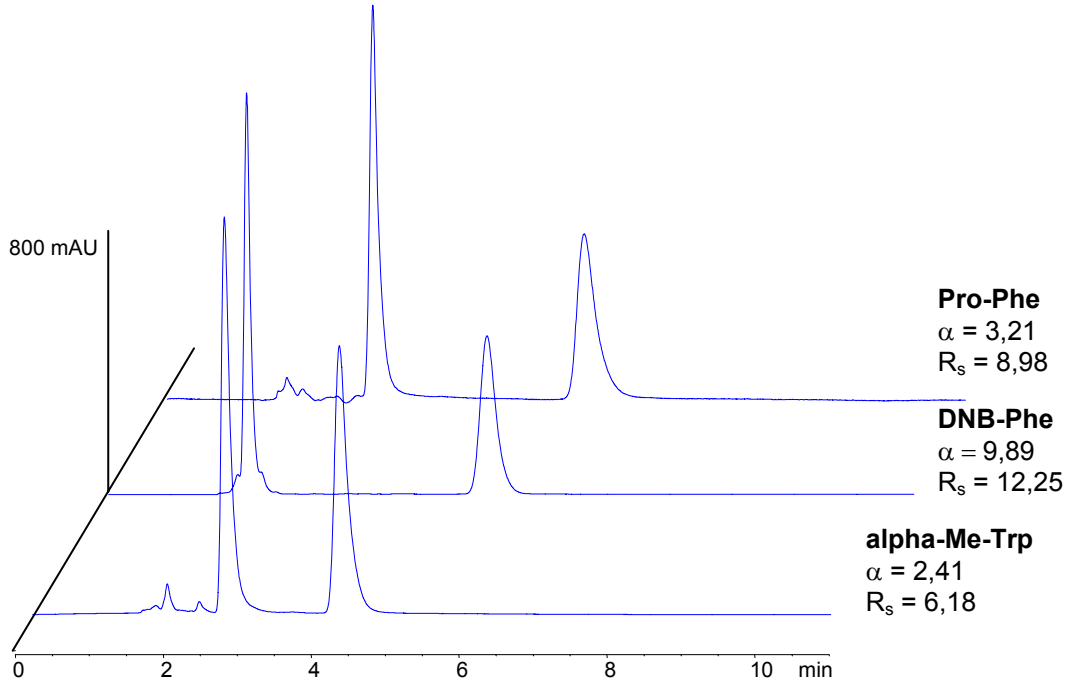


Figure 4.7: Separation of zwitterionic and acidic analytes on Tau-QD (CSP1) (λ=254 nm, t_0=1,51 min)

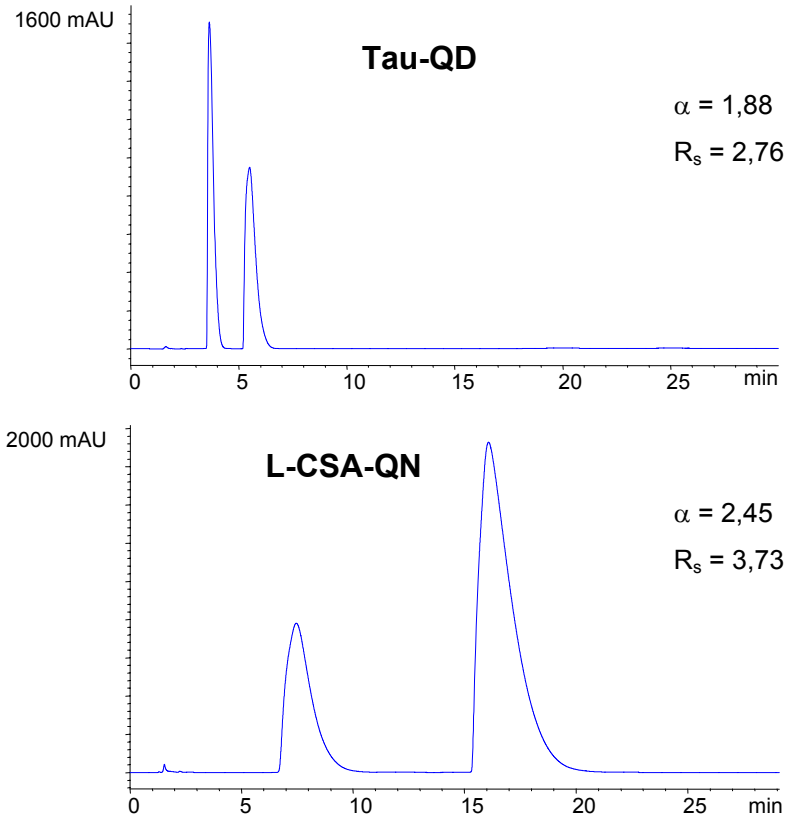


Figure 4.8: Separation of mefloquine on Tau-QD and L-CSA-QN (CSP2) (λ=254 nm, t_0=1,51 min)

Tau-QD **(CSP 1)** shows best overall performance which is a logical consequence of high selectivity values and plate numbers. L-CSAD-DH-QN (**CSP 6**) comes in second followed by L-CSA-QN **(CSP 2)**. However, interesting aspects can be found when looking more detailed at single analytes.

First of all it has to be pointed at the extraordinary R_S values for separation of DNB-Phe. The combination of an electron-rich and an electron poor aromatic moiety together with a sulfonic acid group results in strong retention and high R_S values as well. DNB-Phe seems to ideally fit into the selector's binding pocket. The importance of this steric interactions is also fortified by comparison of DNB-Glu and DNB-Asp. N-protected glutamic acid differs from DNB-Asp in only one additional methylen group but achieves by far better resolution values. Some of the free amino acids could also be well separated – predominantly tryptophane and its α–methylated derivative. On the other hand, for amino acids containing also aromatic groups like phenylalanine, tyrosine (it was tested although not listed here) and α-Me-DOPA no separation at all or partial separation only was achieved.

Nonaromatic, cyclic amino acids were also subject to investigation. Partial separation could be obtained (without recognizing any clear trends between the cyclic homologues), in contrast to their acyclic aliphatic counterparts where no separation took place at all. Astonishingly, no separation was achieved for dimethyltaurine (a derivative of taurine which is an essential moiety of CSP 1, 5, and 6) while cysteic acid was at least partially separated on **CSP 6**.

All four dipeptides were separated on **CSP 1** and **2** whereas the single amino acid e.g. leucine was not resolved in its enantiomers.

Anti malarial drug mefloquine achieved extraordinary high R_S values on **CSP 1** and **CSP 2**. Similar to DNB-Phe this analyte enables π-π stacking between electron rich and electron poor aromatic moieties. Overall, **CSP 2** performed best in separating chiral bases which can be reduced to its additional carboxylic functionality increasing ionic interaction between SO and the amine analytes.

4.5 Tau-QN versus Tau-QD

After the general comparison part in the previous chapter the focus is now set on a direct comparison between single CSPs to underline specific characteristics of the synthesized CSPs.

The Taurine-Quinine selector (Tau-QN) was previously prepared and investigated by our group and is the pseudoenantiomeric analog to Tau-QD **(CSP 1).**[33] The chromatographic comparison between Tau-QN and Tau-QD can be considered as a fundamental part of the presented thesis as it offers valuable clues to the pseudoenantiomeric behaviour of both selectors. So far experiments have shown that elution order is switching from one enantiomer to the other when changing from a quinine-based- to the pseudoenantiomeric quinidine-based CSP (and vice versa).

The coherences between elution orders and enantioselective interactions in the field of QN/QD anion exchanger CSPs have been thouroghly studied by our group and are well understood.[36] Now it has to be evaluated if the pseudoenantiomeric behaviour is preserved in the zwitterionic CSP motif. In **Table 4.5** the chromatographic results are shown – capital letters L and D are indicating which enantiomer was eluting first.

Comparison of Tau-QN versus Tau-QD

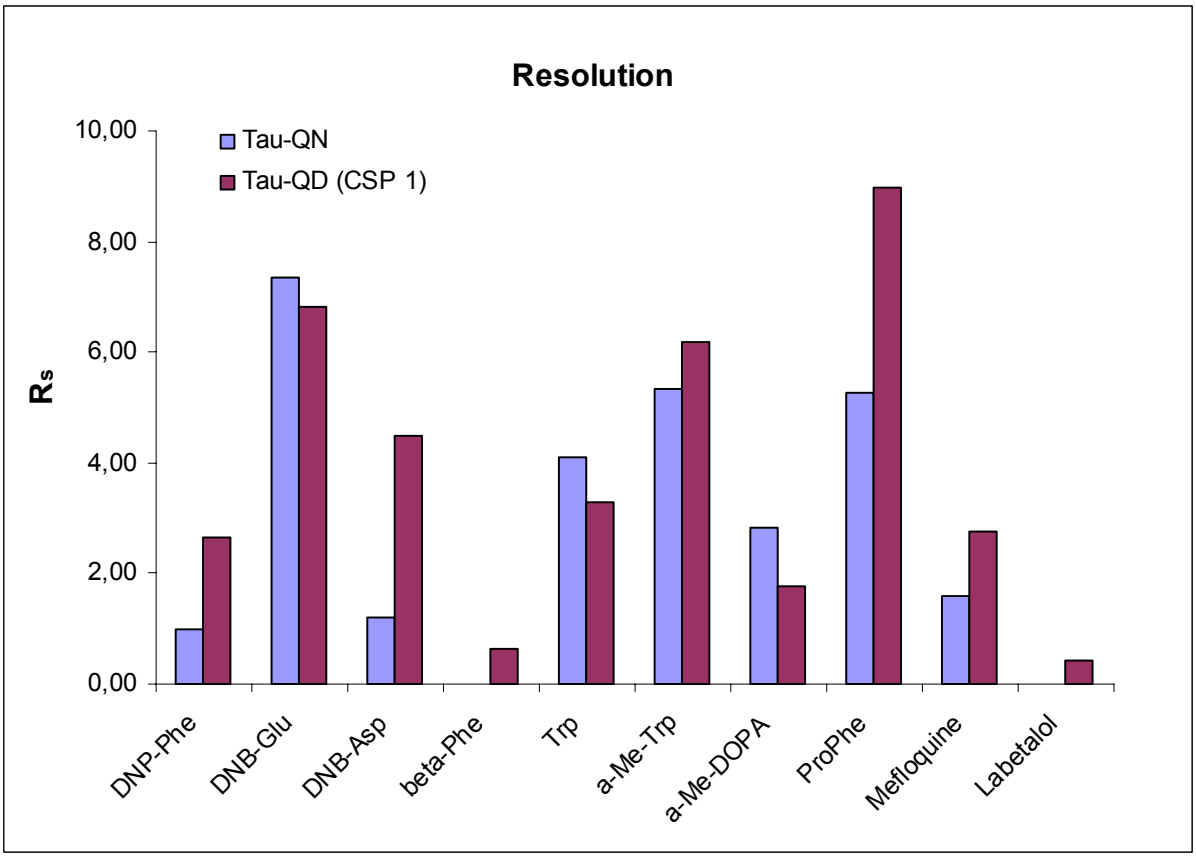
	Tau-QN				**Tau-QD (CSP 1)**			
	EO	**k_2**	**α**	**R_s**	**EO**	**k_2**	**α**	**R_s**
Fmoc-Phe	D-L	0,75	1,47	1,71	L-D	0,58	1,55	1,31
Z-Phe	D-L	0,36	1,25	0,78	L-D	0,25	1,46	0,49
DNP-Phe	L-D	0,62	1,18	**0,99**	D-L	0,68	1,78	**2,66**
DNB-Phe	D-L	3,88	10,10	11,81	L-D	2,42	9,89	12,25
Ac-Trp	D-L	1,15	2,40	4,28	L-D	0,83	3,41	5,03
DNB-Glu	n.d.	3,67	3,46	7,36	n.d.	2,25	3,82	6,81
DNB-Asp	n.d.	4,64	1,17	**1,21**	n.d.	5,40	1,90	**4,48**
Fmoc -Ser	D-L	0,83	1,52	1,80	L-D	0,62	1,75	2,02
Phe		0,79	1,00	0		0,43	1,00	0
beta-Phe		1,23	1,00	**0**	L-D	0,97	1,11	**0,62**
alpha-Me-Phe	n.d.	0,58	1,26	1,61	n.d.	0,44	1,26	0,95
Trp	D-L	1,72	1,60	**4,11**	L-D	1,44	1,82	**3,29**
alpha-Me-Trp	n.d.	3,08	2,46	**5,34**	n.d.	1,80	2,41	**6,18**
1-Me Trp	n.d.	1,36	1,33	1,88	n.d.	0,98	1,20	1,18
alpha-Me-DOPA	D-L	1,18	1,44	2,82	L-D	0,79	1,41	1,78
ProPhe	n.d.	3,69	2,06	**5,28**	n.d.	2,81	3,21	**8,98**

Mefloquine	n.d.	3,83	1,26	**1,60**	n.d.	2,64	1,88	**2,76**
Oxazepam		0,54	1,00	0		0,40	1,00	0
Clenbuterol		2,40	1,00	0	n.d.	1,50	1,03	0,23
Noscapine		0,41	1,00	0		0,32	1,00	0
Labetalol		4,72	1,00	**0**	n.d.	2,90	1,07	**0,44**

Table 4.5

A switch of elution orders is obtained for every tested analyte, which confirms that the pseudoenantiomeric properties are maintained in the concept of zwitterionic CSPs. This experiment also certifies that the quinine/quinidine motif is the driving force for enantioselective recognition in the zwitterionic selector and the steric influence by the acidic cation exchange moieties is limited.

Figure 4.9 depicts resolution- and retention values for both Tau-QN and Tau-QD CSP.



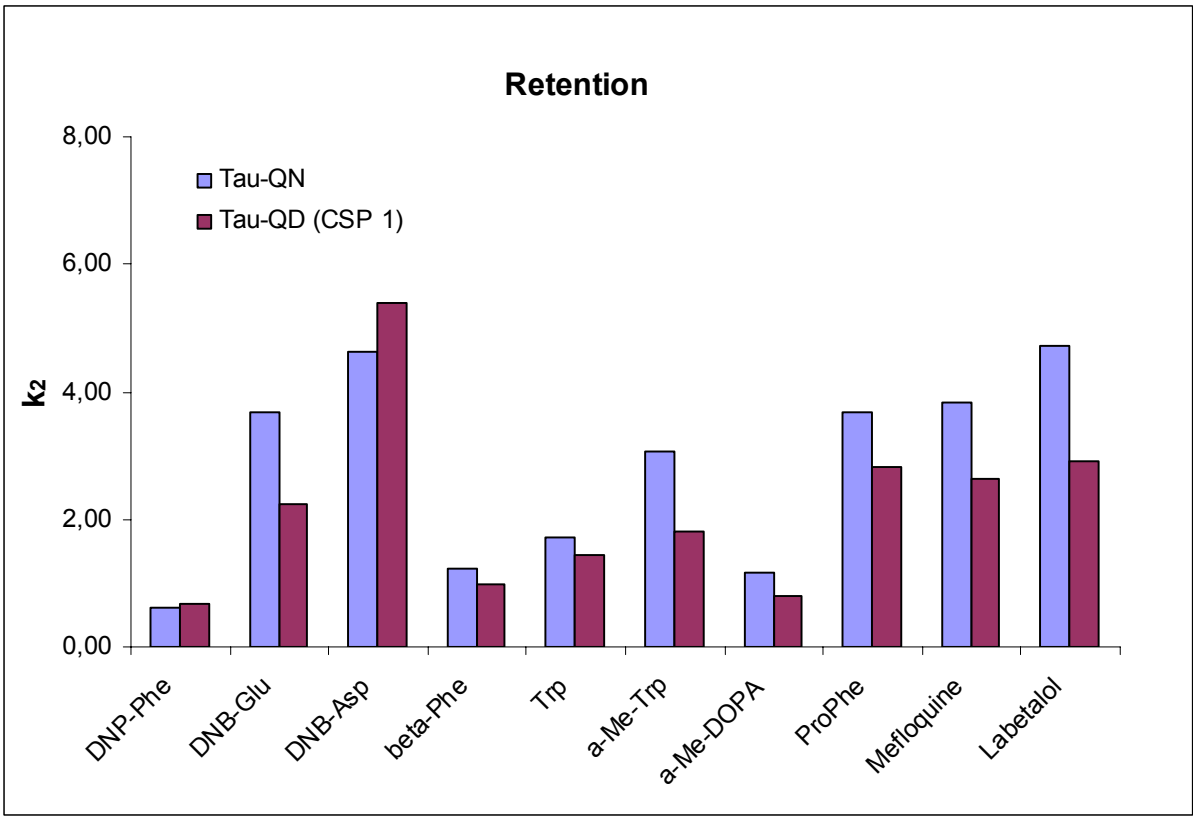


Figure 4.9

Although Tau-QN and Tau-QD CSPs possess almost the same SO coverage (205 µmol g^{-1} and 210 µmol g^{-1}, respectively) and have identical ionic interaction sites (as main attractive force), their retention factors are differing. Also by interpretation of resolution values one can see significant differences in stereoselectivity, which can only be reduced to the fact that configuration of the alkaloid scaffold plays a very important role in stereodiscrimination.

The arguments of obtaining different chromatographic values in combination with a change in elution orders between Tau-QN and Tau-QD clearly describe pseudoenantiomeric behaviour and impressively confirm that the pseudoenantiomeric properties are preserved in zwitterionic CSPs.

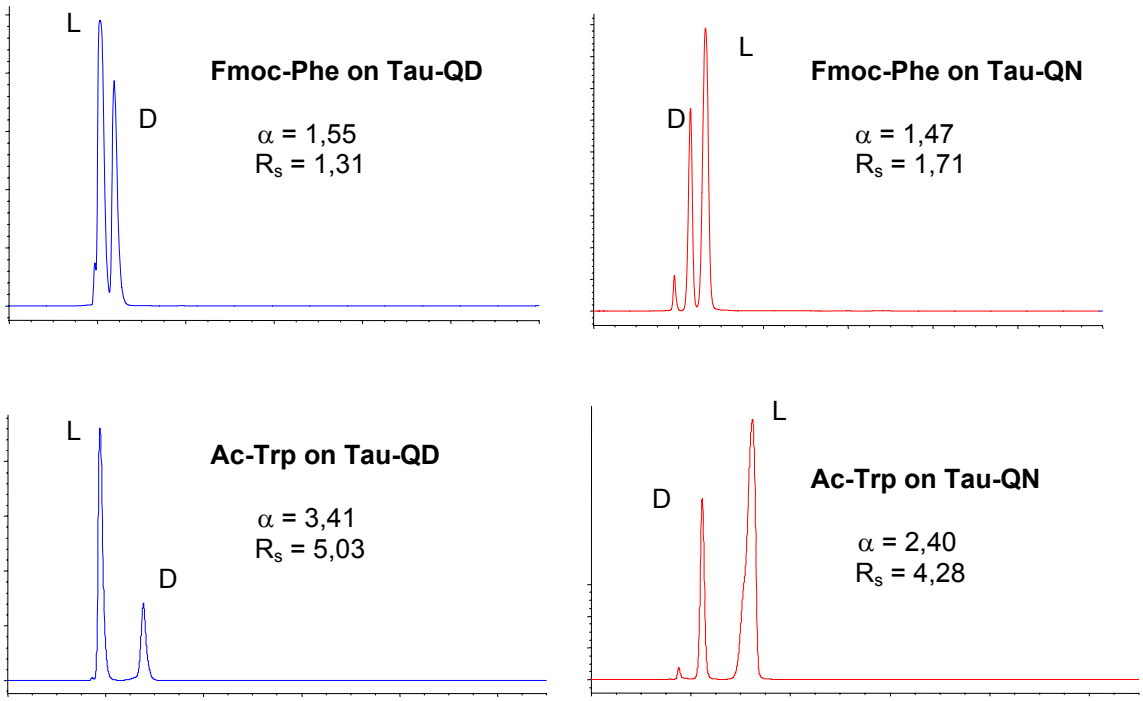


Figure 4.10: Switch of elution orders between Tau-QD and Tau-QN (λ=254 nm, t_0=1,51 min, enantioenriched samples)

In **Table 4.6** elution orders of the remaining zwitterionic CSPs are shown. **CSP 2** and **CSP 6** employ the quinine motif and provide the same elution orders, which is in contrast to quinidine based **CSP 1** where elution order is inverted.

Elution Order				
	Tau-QD CSP 1	**L-CSA-QN CSP 2**	**Tau-Quinc CSP 5**	**L-CSAD-DH-QN CSP 6**
Fmoc-Phe	L	D	///	D
Z-Phe	L	///	///	D
DNP-Phe	D	///	///	L
DNB-Phe	L	D	///	D
Ac-Trp	L	D	///	D
Fmoc-Ser	L	D	///	D
beta-Phe	L	///	///	///
Trp	L	D	///	D
alpha-Me-DOPA	L	D	///	D

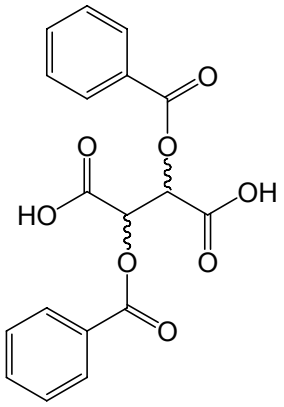
Table 4.6

4.6 Tau-QN versus Tau-N-Methyl-QN

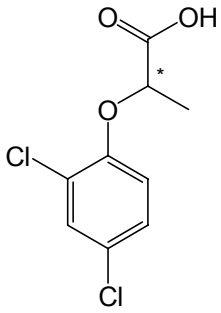
Tau-N-Methyl-QN **(CSP 4)** was mainly designed and synthesized for comparison to the Tau-QN CSP. By comparison **CSP 4** to Tau-QN a strong anion exchange (SAX) motif is opposed to the weak one of Tau-QN. The motivation for designing this selector was to generate a SAX-complement to the meanwhile well established strong cation exchange motif.
CSPs bearing a strong cation exchange binding site (e.g. Tau-QD) generally provide better enantioseparations than selectors comprising weak cation exchangers (e.g. CSPs with a carboxylic functionality). This fact has been shown by our group when carrying out chromatographic studies on a Tau-QN (a sulfonic acid carbamoyl-QN) and a carboxylic acid carbamoyl-QN.

Therefore, the concept of a strong exchanger was introduced on the alkaloid motif via methylation of the quinuclidine nitrogen. In conventional QN/QD-AX CSPs the tertiary amine moiety in the quinuclidine system is positively charged due to protonation. When introducing a methyl group the tertiary amine is transformed into a permanent quaternary ammonium ion becoming a strong anion exchanger. However, hydrogen bond arranged ionic interactions – as they occur in the weak anion exchange motif – are ceased to exist in the quaternary ammonium ion.

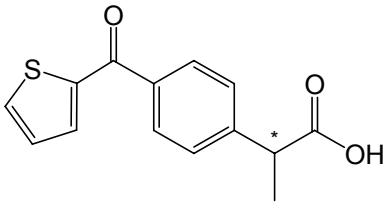
As mentioned in the synthetic chapter, **CSP 4** is of only low selector coverage of 105 μmol g^{-1} in contrast to the Tau-QN CSP with a SO loading of 205 μmol g^{-1}. This matter didn`t allow comparisons of retention times and, thus, terms of selectivity and resolution were discussed only as they are not that much influenced by SO coverage.
For chromatographic investigations the set of analytes was slightly modified including some new analytes which are depicted in **Figure 4.11.**



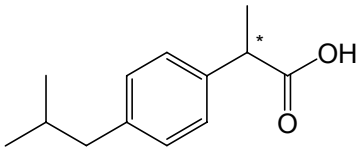
Dibenzoyl tartaric acid



Dichlorprop



Suprofen



Ibuprofen

Figure 4.11

In **Table 4.7** selectivity and resolution values for Tau-N-Methyl-QN and Tau-QN are depicted.

Comparison of Tau-QN versus Tau-N-Methyl-QN				
	Tau-QN		**Tau-N-Methyl-QN**	
	α	**R_s**	**α**	**R_s**
Fmoc-Phe	1,47	1,71	1,22	1,21
Z-Phe	1,25	0,78	1,22	1,31
DNP-Phe	1,18	0,99	1,26	2,31
DNB-Phe	10,10	11,81	1,93	5,25
Ac-Trp	2,40	4,28	1,48	2,45
DNB-Glu	3,46	7,36	1,39	1,22
DNB-Asp	1,17	1,21	1,06	0,36
Fmoc -Ser	1,52	1,80	1,34	2,03
Dibenzoyl tartaric acid	1,36	1,68	1,16	0,63
Dichlorprop	1,73	0,71	1,30	0,98
Ibuprofen	1,00	0	1,00	0
Suprofen	1,19	0,61	1,04	0,31

Phe	1,00	0	1,00	0
beta-Phe	1,00	0	1,00	0
alpha-Me-Phe	1,26	1,61	1,00	0
Trp	1,60	4,11	1,09	0,39
alpha-Me-Trp	2,46	5,34	1,64	1,87
1-Me Trp	1,33	1,88	1,00	0
alpha-Me-DOPA	1,44	2,82	1,33	0,71
ProPhe	2,06	5,28	1,51	2,39
Mefloquine	1,26	1,60	1,00	0
Oxazepam	1,00	0	1,00	0
Clenbuterol	1,00	0	1,00	0
Noscapine	1,00	0	1,00	0
Labetalol	1,00	0	1,00	0

Table 4.7

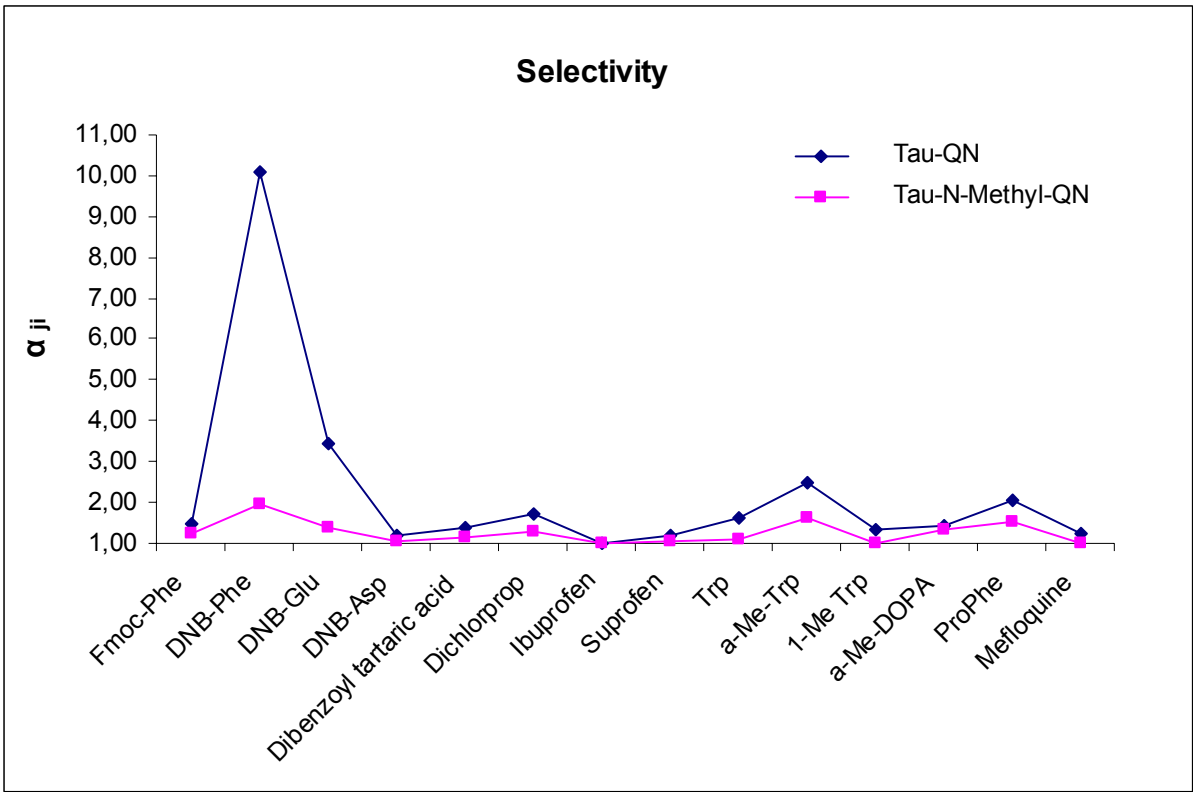


Figure 4.12: Selectivity values for Tau-QN and Tau-N-Methyl-QN

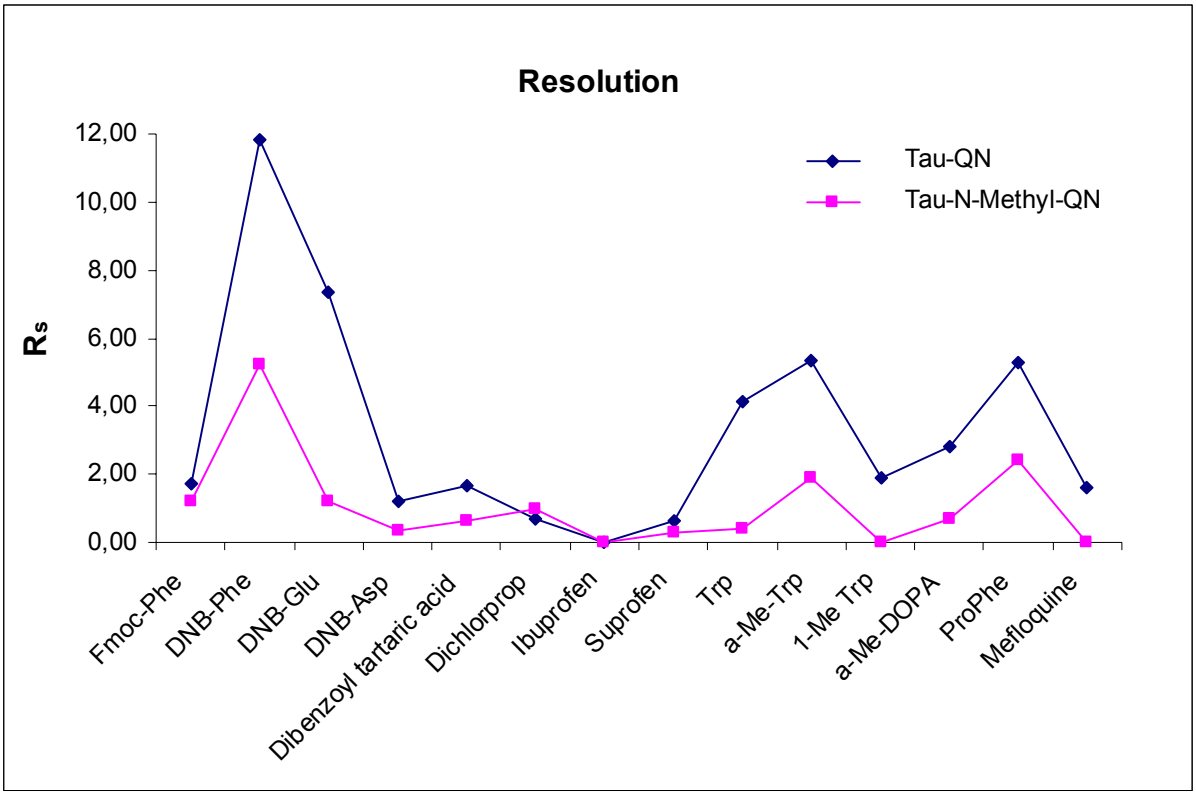


Figure 4.13: Resolution values for Tau-QN and Tau-N-Methyl-QN

The obtained results clearly show the negative influence of a strong anion exchange binding site on enantioseparation of a zwitterionic CSP. On **CSP 4** both selectivity and resolution values for all classes of analytes (acidic, basic and zwitterionic) are lower compared to Tau-QN bearing the weak anion exchange motif. Another striking fact is that no enantioseparation for chiral amines was obtained on Tau-N-Methyl-QN. Even Mefloquine was not separated which normally led to extraordinary high resolution values on Tau-QD, Tau-QN or L-CSA-QN. This can be attributed to the fact that the stronger repulsive interactions between the amine analyte and the SAX moiety badly affect the enantiomer recognition process.

On the other hand, a strong anion exchange binding site should result in higher retention for acidic analytes. However, this theory could not be empirically confirmed due to the strongly differing SO coverage.

4.7 Tau-QN versus L-CSAD-DH-QN

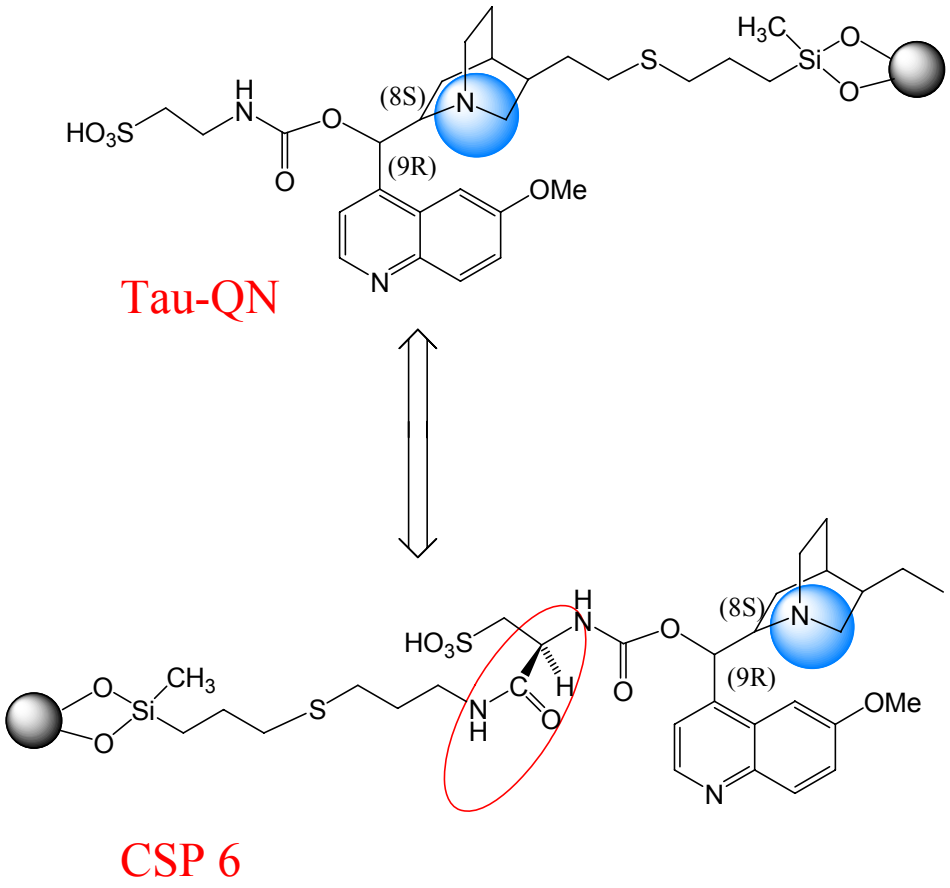


Figure 4.14: Comparison of Tau-QN and L-CSAD-DH-QN (CSP 6)

Within this chapter two selectors are related to each other which are mainly differing in their connectivity to their support material. L-CSAD-DH-QN (**CSP 6**) is bound to the silica on the carbamate site of the selector and, therefore, seen as a more volatile SO compared to the other herein presented CSPs. This selector is supposed to have better accessibility for ionic interactions due to a greater distance to the support material. The additional stereogenic center on the cation exchange site was hoped to positively affect enantioseparation, too.

In contrary to the general comparison chapter, CSP 6 is now set in contrast to its Tau-QN equivalent in stead of the pseudoenantiomeric Tau-QD CSP.

Comparison of Tau-QN versus L-CSAD-DH-QN						
	Tau-QN			L-CSAD-DH-QN (CSP 6)		
	k_2	α	R_s	k_2	α	R_s
Fmoc-Phe	0,75	1,47	1,71	3,20	1,13	1,24
Z-Phe	0,36	1,25	0,78	2,28	1,07	0,79
DNP-Phe	0,62	1,18	0,99	3,33	1,06	0,80
DNB-Phe	3,88	10,10	**11,81**	4,63	2,06	**9,57**
Ac-Trp	1,15	2,40	4,28	3,26	1,21	2,04
DNB-Glu	3,67	3,46	7,36	17,15	1,74	3,92
DNB-Asp	4,64	1,17	1,21	20,26	1,23	1,89
Fmoc -Ser	0,83	1,52	1,80	3,51	1,15	1,81
Phe	0,79	1,00	0	0,98	1,00	0
beta-Phe	1,23	1,00	0	1,09	1,00	0
alpha-Me-Phe	0,58	1,26	1,61	0,88	1,00	0
Trp	1,72	1,60	**4,11**	1,67	1,49	**2,64**
a-Me-Trp	3,08	2,46	**5,34**	1,79	1,28	**2,05**
1-Me Trp	1,36	1,33	1,88	1,46	1,00	0
a-Me-DOPA	1,18	1,44	2,82	1,28	1,03	0,27
ProPhe	3,69	2,06	**5,28**	2,39	1,23	**2,02**
Mefloquine	3,83	1,26	1,60	1,32	1,05	0,33
Oxazepam	0,54	1,00	0	0,02	1,00	0
Clenbuterol	2,40	1,00	0	0,89	1,00	0
Noscapine	0,41	1,00	0	0,70	1,00	0
Labetalol	4,72	1,00	0	1,73	1,00	0

Table 4.8

Results in **Table 4.8** are very similar to those in the general comparison part which means that capacity factors of **CSP 6** are extraordinary high for acidic analytes and lower for basic analytes in comparison to Tau-QN CSP (outlined in **Figure 4.15**). This is an unexpected result because ion exchange binding sites (as primary interaction force) and the alkaloid scaffold are identical for both selectors. Consequently, the significant differences in retention times must rely on a spatially different molecular recognition process. It is not yet cleared in what way the newly generated steric bulk (on the cation exchange site) affects stereoselectivity. Due to lack of time other diastereomeric forms of this SO (e.g. D-CSAD-DH-QN or L-CSAD-DH-QD) could not have been synthesized whose configurations are probably better appropriated to stereodiscrimination.

What needs also to be underlined is that high retention times do not automatically imply high enantioseparation values as for **CSP 6** lower selectivity and resolution values are obtained for all classes of analytes than for Tau-QN.

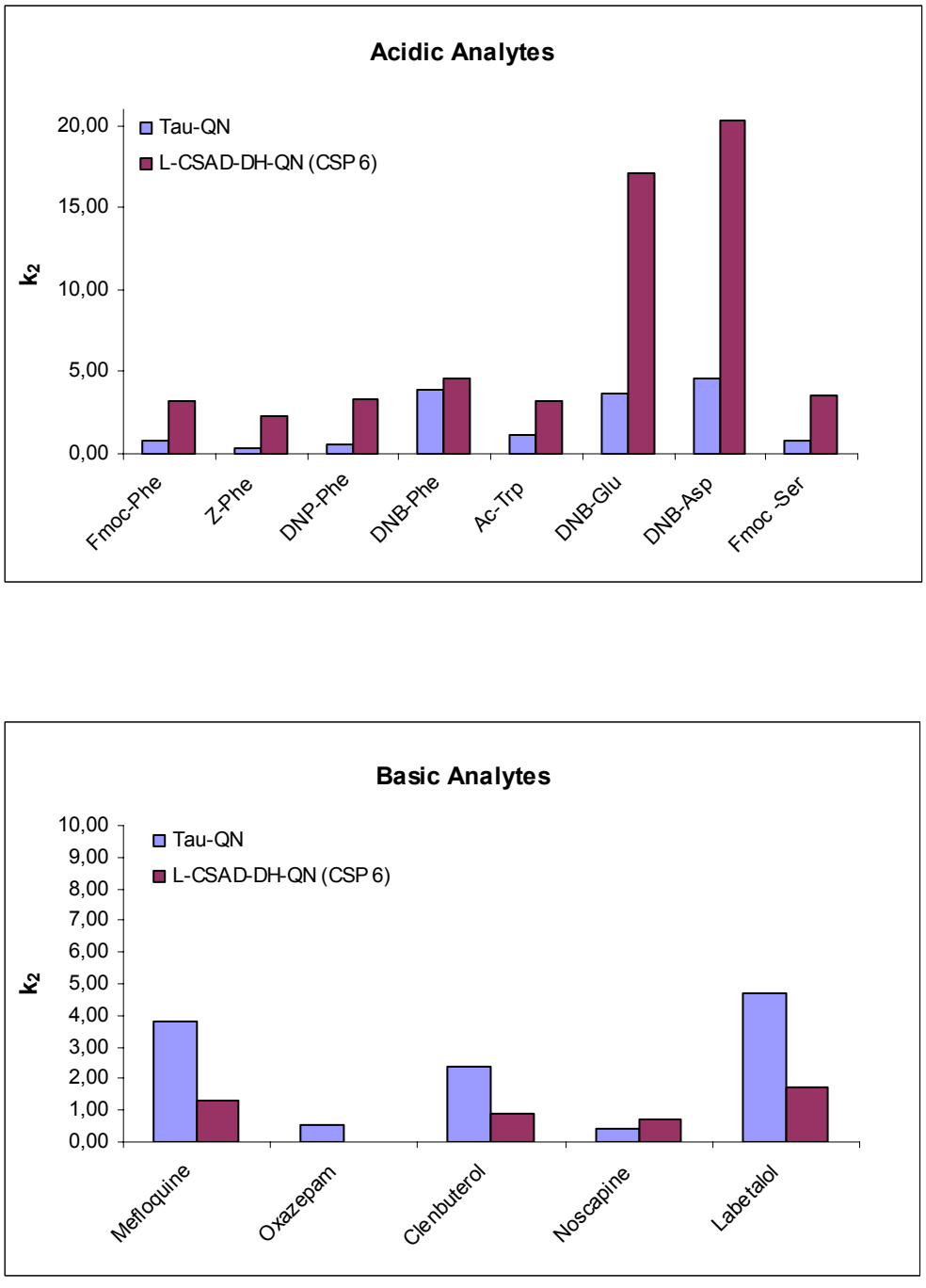


Figure 4.15: k_2 values

4.8 Quinu-QD versus QD-AX

This comparison could be interpreted as a challenge between single and double anion exchange CSPs. Quinu-QD (a quinuclidine carbamoyl QD CSP) comprises two basic anion exchange moieties. Like the QD-AX CSP it was mainly designed for separation of chiral acidic analytes like N-protected amino acids and profenes. Additionally, bisacidic analytes were subject to investigation to study molecular recognition processes of bisbasic – bisacidic selector – selectand interactions. **Figure 4.16** depicts both slectors Quinu-QD (**CSP 3**) and the well established standard QD-AX selector.

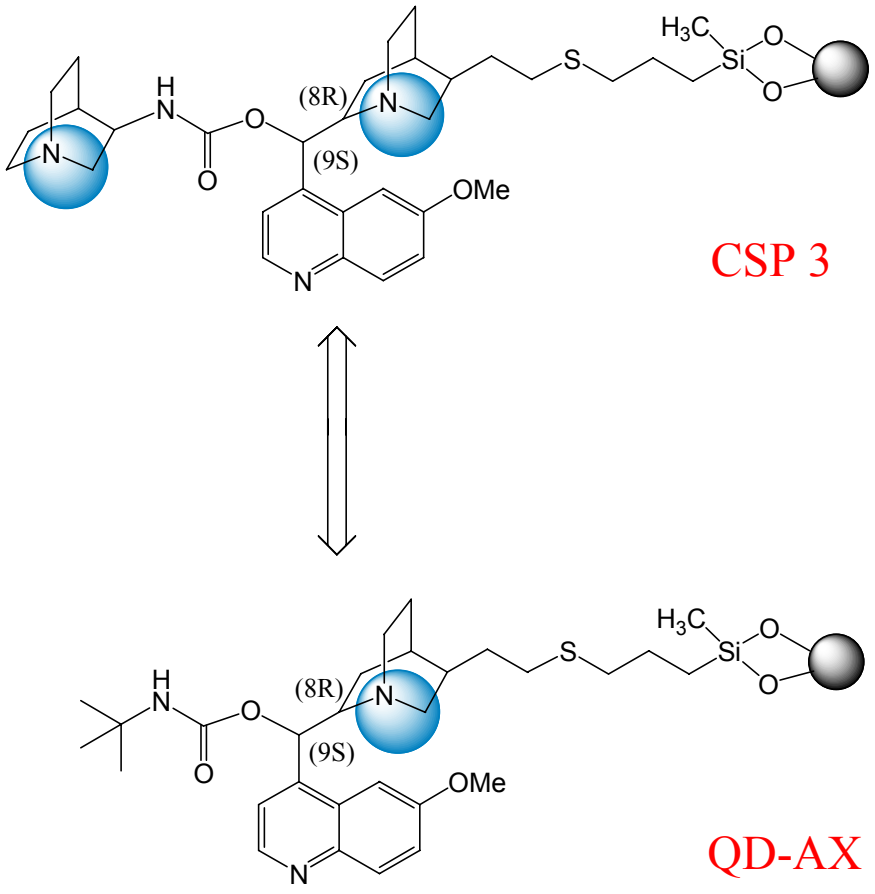


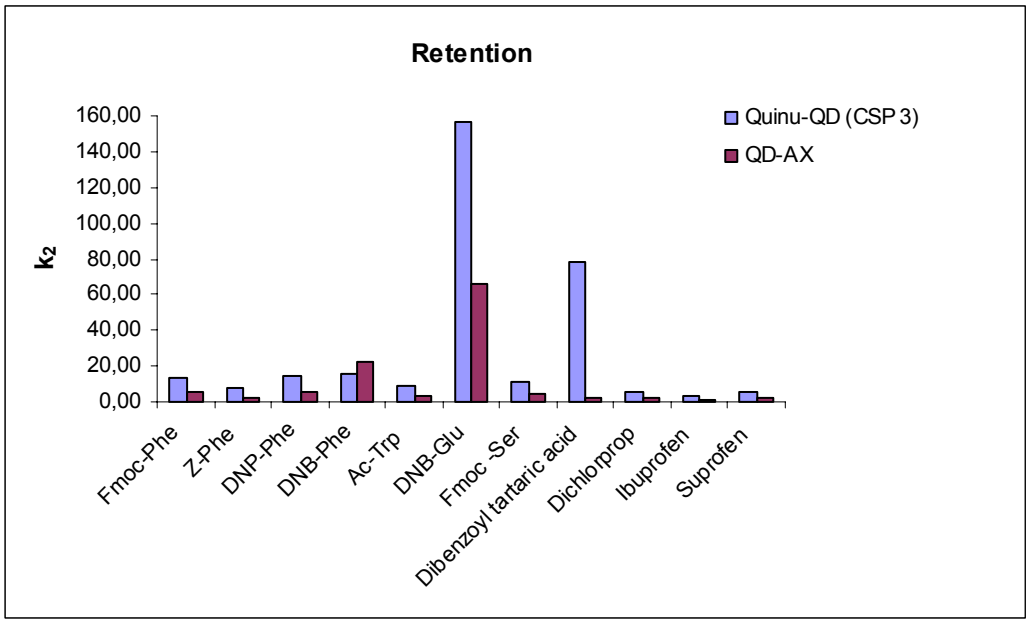
Figure 4.16

Results of chromatographic investigations are shown in **Table 4.9** and **Figure 4.17**. Before some additional information has to be given: Both **CSP 4** and QD-AX CSP were tested on non target molecules as there are chiral bases and zwitterionic analytes. Most basic analytes injected on both CSPs eluted around the void volume or even before the void volume. This behaviour can be attributed to slight repulsion instead of attractive molecular interaction. Furthermore, no enantioseparation was obtained for zwitterionic analytes.

	Quinu-QD (CSP 3)				
	EO	**k_1**	**k_2**	**α**	**R_s**
Fmoc-Phe	L-D	12,39	13,36	1,08	1,44
Z-Phe	L-D	7,89	8,28	1,05	0,85
DNP-Phe		14,39	14,39	1,00	0
DNB-Phe	L-D	7,90	15,30	1,94	13,15
Ac-Trp	L-D	7,74	8,52	1,10	1,64
DNB-Glu	n.d.	**110,89**	**156,76**	**1,41**	**5,09**
DNB-Asp		///	///	///	///
Fmoc -Ser	L-D	10,30	10,97	1,06	1,20
Dibenzoyl tartaric acid		**78,36**	**78,36**	**1,00**	**0**
Dichlorprop	n.d.	4,97	5,52	1,11	2,00
Ibuprofen		3,05	3,05	1,00	0
Suprofen		5,48	5,48	1,00	0

	QD-AX CSP				
	EO	**k_1**	**k_2**	**α**	**R_s**
Fmoc-Phe	L-D	3,06	5,11	1,67	6,18
Z-Phe	L-D	2,01	2,67	1,33	3,29
DNP-Phe	D-L	4,47	5,41	1,21	2,93
DNB-Phe	L-D	2,19	22,27	10,19	22,22
Ac-Trp	L-D	2,02	3,29	1,63	4,28
DNB-Glu	n.d.	**14,49**	**65,81**	**4,54**	**12,32**
DNB-Asp	n.d.	**60,03**	**109,92**	**1,83**	**4,51**
Fmoc -Ser	L-D	2,67	4,29	1,60	5,56
Dibenzoyl tartaric acid	n.d.	**1,89**	**2,03**	**1,07**	**0,76**
Dichlorprop	n.d.	1,77	2,49	1,40	2,69
Ibuprofen	n.d.	0,87	1,01	1,17	0,86
Suprofen	n.d.	2,44	2,75	1,13	1,45

Table 4.9



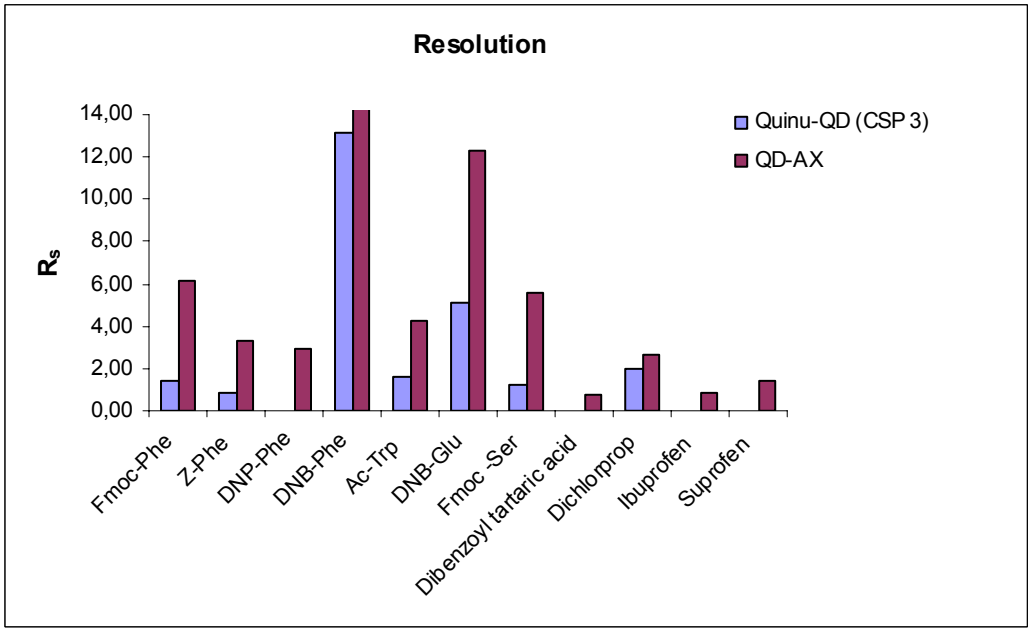


Figure 4.17

Quinu-QD (**CSP 3**) obtained extraordinary high capacity factors, in particular for the bisacidic analytes DNB-Glu, DNB-Asp and dibenzoyl tartaric acid. For example, the second enantiomer of DNB-Glu eluted after 238 min (!). The non separated peak of dibenzoyl tartaric acid came at 119 min similar to DNB-Asp, which showed very strong peak tailing and, therefore, chromatographic parameters could not be determined. Chromatograms of these analytes are shown in **Figure 4.18** and **4.19.**

Generally, Quinu-QD obtained signifcantly higher capacity factors also for monoacidic analytes like N-protected amino acids. This behaviour can be attributed to the bisbasic properties of Quinu-QD which means that this CSP has more ionic interaction sites per surface unit and, thus, builds up stronger ionic interactions with acidic analytes in contrast to the monobasic standard QD-AX CSP.

Detailed comparison of resolution values is not meaningful because the Quinu-QD selector was immobilized in its diastereomeric form (see synthetic chapter 3.3) which probably hampers stereodiscrimination of analytes.

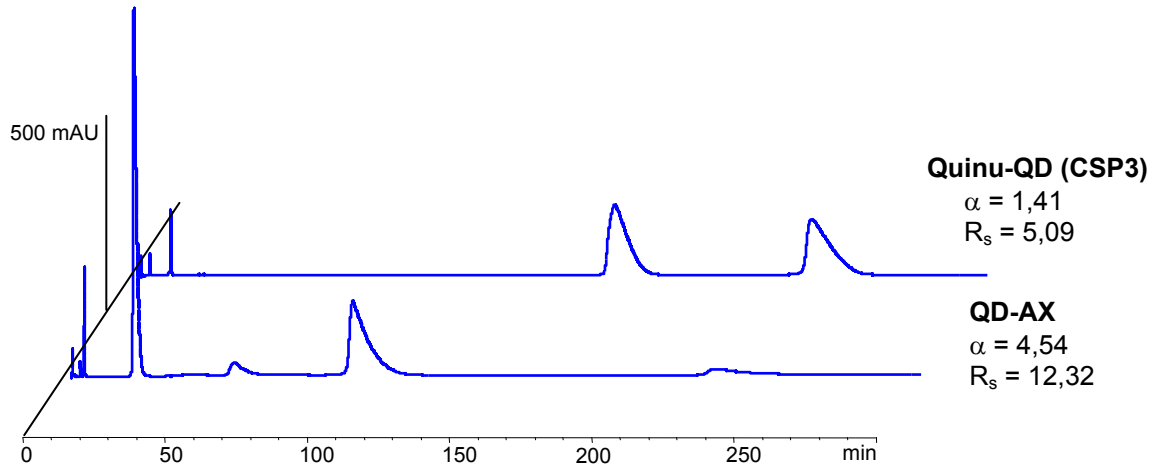


Figure 4.18: Separation of DNB-Glu
(λ=254 nm, t_0=1,51 min)

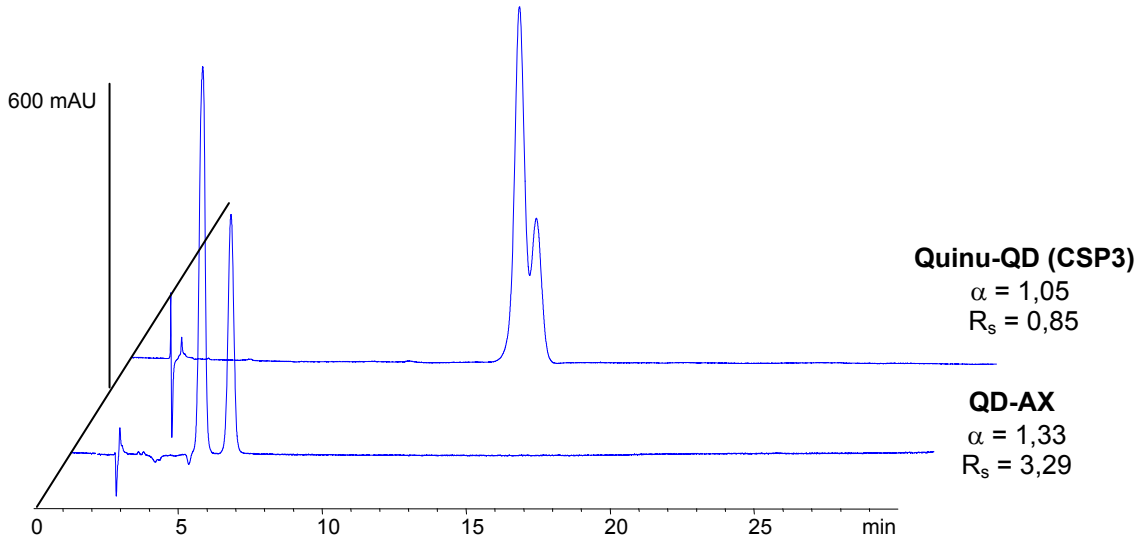


Figure 4.19: Separation of Z-Phe
(λ=230 nm, t_0=1,51 min, enantioenriched samples)

4.9 Conclusion and Outlook

Novel ion exchange chiral stationary phases have been designed, synthesized and chromatographically evaluated. Based on the concept of recently – in house - prepared zwitterionic CSPs the main focus was on development and preparation of carbamate- and cinchona-modified zwitterionic chiral stationary phases.

In addition to the zwitterionic CSPs a bisbasic quinidine selector was prepared for comparison to the standard quinidine anion exchange CSP. Six zwitterionic CSPs and two anion exchanger type chiral stationary phases were chromatographically evaluated whereas chiral acids, chiral bases and chiral amino acids had been used as analytes. Successful enantioseparations for all classes of analytes again confirmed that the ZWIX motif is promising and working.

Chromatographic key parameters of retention, selectivity and resolution were compared to each other for all synthesized CSPs to elucidate general trends and to enlighten the complex selector – selectand molecular recognition process.

Remarkable differences in the obtained chromatographic data amongst the evaluated CSPs are giving a good reason to further structural modifications of zwitterionic chiral stationary phases. Firstly, to learn more about stereochemically driven molecular interactions and, secondly, to support enhancement of the selectors` separation abilities. For example efforts on the non realized DACH-QD selector could be made to use it as a building block for a more complex zwitterionic selector.

What could not be realized in this work were experiments to optimize the mobile phase in terms of retention and enantioselectivity parameters. No doubt, tuning of mobile phase parameters (especially choice of the bulk solvent and concentration of co- and counterions) will certainly ameliorate enantioseparation in some special cases.

5 Experimental

Materials & Methods:

^{1}H-NMR and ^{13}C-NMR were recorded at room temperature with a Bruker DRX400 spectrometer. The spectra were recorded in $CDCl_3$, $CDOD_3$ and DMSO-d_6 and the solvent signals were used as reference. The raw data were processed with SpinWorks Version 2.5.5. software.

Analytical thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} plates from Merck (Darmstadt, Germany). Column chromatography was performed on silica gel 60 (0.040-0.063mm) (Merck). ESI-mass spectra were recorded on a PE Sciex API 365 spectrometer.

MeOH (gradient grade quality, Merck), n-hexane (gradient grade quality, Roth, Karlsruhe, Germany), n-heptane (gradient grade quality, Roth), CH_2Cl_2 (p.a. puriss quality, Fluka, Vienna, Austria), diethylether (Et_2O, puriss. quality), DMF (purum >99%, anhydrous) and toluene (>99%) were used as solvents. For column chromatography methanol and dichloromethane were used in technical grade quality. For using dried dichloromethane it was distilled over CaH_2. All chemical reactions were carried out under nitrogen atmosphere.

Generally, all the CSPs were prepared by immobilization of the chiral selectors onto 3-mercaptopropyl-modified, endcapped silica gel via radical addition with AIBN (about 40 mg AIBN per mmol selector were used). Thiol-activated silica was initially prepared from Daisogel 120-5 (pore size 120 Å, particle size 5 μm) from Daiso (Osaka, Japan) and was available from stock. The selector loadings were calculated based on the nitrogen content (w-% N). Analysis of the filtrate by ESI-MS confirmed selector integrity during immobilization conditions while unreacted selector could be recovered from the filtrate by simple flash chromatography if appropriate. Each CSP was slurry packed in house into stainless steel columns (each 150x4.0 mm I.D.).

9-O-(4-nitrophenyloxycarbonyl)quinidine hydrochloride (2, activated quinidine ester hydrochloride)

8.00 g of quinidine as a free base (24.7 mmol) were dissolved in 300 ml of toluene followed by azeotropic distillation (removal of 1/3 of the solvent) using a Dean-Stark trap. After cooling to room temperature (r.t.) 5.04 g (25.0 mmol) of 4-nitrophenyl chloroformate were added as a solid. The mixture was stirred at r.t. over night. Hydrochloride **2** was formed as a yellowish precipitate. The solid was filtrated off, washed in n-hexane (3x50 ml) and stored in a nitrogen atmosphere to avoid hydrolysis (almost quantitative yield).

MS (ESI, positive): 490.2 $[M+H]^+$, 383.3 [methoxycarbonate of **2**]$^+$.

N-[[[(8R,9S)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-taurine (3)

Finely grounded taurine (0.50 g, 4.0 mmol) was suspended in dried CH_2Cl_2. While stirring the suspension BSA (3.0 ml, 12.0 mmol) was added with a Hamilton syringe via septum followed by refluxing for 24 h until the solution became clear. After cooling to r.t. 2.10 g (4.0 mmol) of quinidine activated ester hydrochloride **2** were added and stirring continued for 24 h. The reaction was quenched with MeOH. The turbid mixture was stirred for another 30 min and was then transferred directly onto a silica flash column. Purification by gradient chromatography (CH_2Cl_2 : MeOH 15:1 – 3:1) yielded 1.24 g (65 %) of **3** as yellowish crystals.

^{1}H-NMR [CD_3OD]: δ = 1.47 (m,1H), 1.83-2.02 (m, 2H), 2.06 (m,1H), 2.42 (m, 1H), 2.77 (m, 1H), 3.02 (m, 2H), 3.37 (m, 1H), 3.51-3.65 (m, 5H), 3.87 (m, 1H), 4.01 (s, 3H), 5.24-5.33 (m, 2H), 6.14 (m, 1H), 7.11 (s, 1H), 7.50-7.56 (m, 2H), 7.78 (d, 1H), 7.96 (d, 1H), 8.79 (d, 1H).

MS (ESI, positive): 476.2 $[M+H]^+$.

CSP 1 (Immobilization of the chiral selector 3)

2.62 g of silica were suspended/elutriated in 20 ml MeOH. The selector **3** (0.50 g, 1.1 mmol) was dissolved in 15 ml MeOH and added to the slurry. After flushing for 15 minutes with nitrogen AIBN (70 mg, 0.43 mmol) was added and the suspension was refluxed for 5 h. After immobilization the modified silica gel was collected by filtration, washed with MeOH (3x30 ml) and CH_2Cl_2 (2x30 ml), dried under reduced pressure at 60°C over night and subjected to elemental analysis: 0.603 % N results in SO loading of 210 μmol g^{-1}.

9-O-(4-nitrophenyloxycarbonyl)quinine hydrochloride (5, activated quinine ester hydrochloride)

16.00 g of quinine as a free base (49.3 mmol) were dissolved in 600 ml of toluene. Azeotropic distillation (ca. 1/3 of the solvent was removed) using a Dean-Stark trap. After cooling to r.t. 10.08 g (50.0mmol) of 4-nitrophenyl chloroformate were added as a solid. The mixture was stirred at r.t. over night. A yellowish precipitate was formed. The solid was filtrated off, washed in n-hexane and stored in a nitrogen atmosphere to avoid hydrolisation (quantitative yield).

MS (ESI, positive): 383.3 [methoxycarbonate of **5**]$^+$.

N-[[[(8S,9R)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-L-cysteic acid (6)

Finely grounded L-cysteic acid (0.75 g, 4.4 mmol) was suspended in dried CH_2Cl_2. While stirring the suspension BSA (4.0 ml, 16.3 mmol) was added with a Hamilton syringe via septum. Refluxing for 48 h until solution became clear. After cooling to r.t. 2.10 g (4.0 mmol) quinine activated ester hydrochloride were added and again stirred for additional 24 h at r.t. The reaction was quenched with MeOH. The turbid mixture was stirred for 30 more minutes and transferred directly onto a flash column. Purification by chromatography (dichloromethane : methanol 10:1 – 1:1) yielding 1.50 g (72 %) of yellowish crystals.

^{1}H-NMR [CD_3OD]: δ = 1.72 (m, 1H), 1.93 (m, 1H), 2.09 (m, 1H), 2.24 (m, 2H), 2.78 (m, 1H), 3.20 (m, 1H), 3.33-3.48 (m, 3H), 3.65 (m, 1H), 3.84 (t, 2H), 4.03 (s, 3H), 4.59 (t, 1H), 5.06 (m, 2H), 5.75 (m, 1H), 6.96 (s, 1H), 7.42-7.59 (m, 2H), 7.84 (d, 1H), 8.03 (d, 1H), 8.03 (d, 1H), 8.78 (d, 1H).

MS (ESI, positive): 520.3 [M+H]$^+$.

CSP 2 (Immobilization of the chiral selector 6)

2.89 g of 3-mercaptopropylmethyl-silica were elutriated in 20 ml of MeOH. 0.60 g (1.2 mmol) of selector **6** were dissolved in 15 ml of MeOH. 60 mg (0.37 mmol) of AIBN were taken. Elemental analysis: 0.998 % N results in SO loading of 240 μmol g^{-1}.

N-[[[(8R,9S)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-3-quinuclidine (7)

First, the free base of 3-aminoquinuclidine dihydrochloride (racemic mixture) was prepared by reacting with equimolar amounts of KOH (methanolic solution). 0.58 g (4.6 mmol) of 3-aminoquinuclidine were dissolved in CH_2Cl_2 and 2.00 g (3.8 mmol) of activated quinidine hydrochloride **2** were added portionwise to the turbid solution. Stirring at r.t. was maintained until TLC showed complete conversion of **2** (18 h) and purification by chromatography (CH_2Cl_2 : MeOH 10:1 – 1:1) yielded 0.65 g (36 %) of **7** as a yellowish solid.

MS (ESI, positive): 477.4 $[M+H]^+$.

CSP 3 (Immobilization of the chiral selector 7)

3.00 g of 3-mercaptopropylmethyl-silica were elutriated in 25 ml of MeOH. 0.58 g (1.2 mmol) of selector **7** were dissolved in 20 ml of MeOH. 60 mg (0.37 mmol) of AIBN were used. Elemental analysis: 1.22 % N results in SO loading of 218 μmol g^{-1}.

N-[[[(8S,9R)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-taurine (8)

Finely grounded taurine (0.50 g, 4.0 mmol) was suspended in dried CH_2Cl_2. While stirring the suspension BSA (3.0 ml, 12.0 mmol) was added with a Hamilton syringe. Refluxing over night until solution became clear. After cooling to r.t. 2.10 g (4.0 mmol) activated quinine ester hydrochloride **5** were added and stirred for 48 h. The reaction was quenched with MeOH and the turbid mixture stirred for 1 more h. It was directly transferred onto a flash column and purified by chromatography (dichloromethane : methanol 15:1 – 1:1) yielding 0.88 g (47 %) of a pale yellow solid.

^{1}H-NMR [CD_3OD]: δ = 1.79 (m, 1H), 2.00 (m, 1H), 2.15 (m, 1H), 2.28 (m, 2H), 2.84 (m, 1H), 3.00 (m, 2H), 3.34 (m, 2H), 3.54 (t, 2H), 3.68 (t, 1H), 3.84 (m, 2H), 4.13 (s, 3H), 5.10 (m, 2H), 5.78 (m, 1H), 7.07 (s, 1H), 7.78 (m, 2H), 8.07 (d, 1H), 8.15 (d, 1H), 8.95 (d, 1H).

MS (ESI, positive): 476.2 $[M+H]^+$.

N-[[[(8S,9R)-1-N-methyl-6'-methoxycinchonan-9-yl]oxy]carbonyl]-taurine (9)

First taurine quinine **8** was converted into its potassium salt by addition of equimolar amounts of KOH (methanolic solution). 0.89 g (1.7 mmol) of the potassium salt were dissolved in DMF. While stirring 0.54 ml (8.7 mmol) of iodomethane were added dropwise with a Hamilton syringe via septum. Stirring at r.t. for 60 h. The solvent and excess iodomethane were removed using a rotary evaporator and the residue was resolved in 15 ml MeOH. By addition of Et_2O a yellow precipitate was formed which was filtrated off and washed in diethylether yielding a yellow powder.

The raw product was purified by preparative RP-HPLC (Phenomenex Gemini C18 column, 100x21.20 mm, 10µm, MP: H_2O/MeOH 50/50 50mM NH_4Ac, flow rate 4-6 ml/min, samples dissolved in DMSO) yielding **9** as an auburn oil (0.80 g, 70%).

MS (ESI, positive): 476.2 $[educt+H]^+$, 490.2 $[M]^+$

CSP 4 (Immobilization of the chiral selector 9)

2.30 g of 3-mercaptopropylmethyl-silica were elutriated in 20 ml of MeOH. 0.51 g (1.0 mmol) of selector were dissolved in 15 ml of MeOH. 45 mg (0.27 mmol) of AIBN were used. Elemental analysis: 0.442 % N results in SO loading of 105 µmol g^{-1}

4-nitrophenyl (8-vinylquinuclidin-2-yl)methyl carbonate (11, activated quincorine ester hydrochloride)

Azeotropic distillation of toluene using a Dean-Stark trap, then 1.00 g (6.0mmol) of quincorine was added dropwise with a Hamilton Syringe. While stirring the solution 1.26 g (6.3 mmol) of 4-nitrophenyl chloroformate was added as a solid. The mixture was stirred at r.t. over night. The precipitate was filtrated off, washed in n-heptane and stored in a nitrogen atmosphere to avoid hydrolisation (quantitative yield).

MS (ESI, positive): 226.3 [methoxycarbonate of **11**]$^+$.

2-(((8-vinylquinuclidin-2-yl)methoxy)carbonylamino)ethanesulfonic acid (12)

Finely grounded taurine (0.31 g, 2.5 mmol) was suspended in dried dichloromethane. While stirring the suspension BSA (1.9 ml, 7.5 mmol) was added with a Hamilton syringe. Refluxing over night until solution became clear. After cooling to r.t. 0.83 g (2.5 mmol) of activated quincorine ester hydrochloride were added and stirred for 40 h. The reaction was quenched with MeOH. The turbid mixture was stirred for 1 h. To remove excess 4-nitrophenol the raw product was purified by flash column chromatography (dichloromethane : methanol 25:1 – 10:1). The product and byproduct acetamid coeluted at high MeOH-content of the eluent. Acetamide was removed from the product by trituration in acetone to afford solid **12** (almost quantitative yield).

^{1}H-NMR [CD_3OD]: δ = 1.36 (m, 1H), 1.97 (m, 2H), 2.07 (m, 1H), 2.18 (m,1H), 2.89 (m,2H), 2.97 (m, 1H), 3.24 (m, 2H), 3.60 (m, 4H), 3.77 (m, 1H), 4.20 m, 1H), 4.32 (m, 1H), 5.23 (m, 2H), 5.96 (m, 1H).

MS (ESI, positive): 319.4 $[M+H]^+$.

CSP 5 (Immobilization of the chiral selector 12)

2.20 g of 3-mercaptopropylmethyl-silica were elutriated in 25 ml of MeOH. 0.30 g (0.9 mmol) of selector **12** were dissolved in 15 ml of MeOH. 40 mg (0.24 mmol) of AIBN were used. Elemental analysis: 0.817 % N results in SO loading of 290 μmol g^{-1}

9-O-(4-nitrophenyloxycarbonyl)dihydroquinine hydrochloride (14, activated dihydroquinine ester hydrochloride)

8.00 g of dihydroquinine as a free base (24.5 mmol) were dissolved in 300 ml of toluene. Azeotropic distillation (removal of 1/3 of the solvent) using a Dean-Stark trap. After cooling to r.t. 5.04 g (25.0 mmol) of 4-nitrophenyl chloroformate were added as a solid. The mixture was stirred at r.t. over night. A yellowish precipitate was formed. The solid was filtrated off, washed in n-hexane and stored in a nitrogen atmosphere to avoid hydrolisation (quantitative yield).

MS (ESI, positive): 385.3 [methyoxycarbonate of **14**]$^+$.

N-[[[(8S,9R)-6'-methoxy-(10,11-dihydro-cinchonan)-9-yl]oxy]carbonyl]-L-cysteic acid (15)

Finely grounded L-cysteic acid monohydrate (0.83 g, 4.4 mmol) was suspended in dried CH_2Cl_2. While stirring the suspension BSA (5.4 ml, 22.2 mmol) was added with a Hamilton syringe. Refluxing for 24 h until solution became clear. After cooling to r.t. 2.34 g (4.4 mmol) of activated dihydroquinine ester hydrochloride **14** were added and the solution was stirred for 60 h. The reaction was quenched with MeOH. After a few minutes a viscous gum was formed. The supernatant was decanted off and the residue was resuspended in MeOH. The solvent was evaporated yielding 1.75 g (76 %) of pure product.

^{1}H-NMR [CD_3OD]: δ = 0.83 (t, 3H), 1.32 (m, 2H), 1.74 (m, 1H), 1.85 (m, 1H), 2.09 (m, 1H), 2.26 (m, 2H), 3.00 (m, 1H), 3.22(m, 1H), 3.40 (m, 2H), 3.63 (t, 1H), 3.81 (m, 2H), 4.09 (s, 3H), 4.32 (m, 1H), 4.68 (m, 1H), 7.03 (s, 1H), 7.65 (m, 2H), 8.04 (d, 1H), 8.10 (d, 1H), 8.85 (d, 1H).

MS (ESI, positive): 520.3 $[M+H]^+$.

N-[[[(8S,9R)-6'-methoxy-(10,11-dihydro-cinchonan)-9-yl]oxy]carbonyl]-allylamido-L-cysteic acid (16)

1.37 g (2.6 mmol) of diacid **15** were dissolved in anhydrous DMF. DCC (0.54 g, 2.6 mmol) were added and stirring was maintained for 10 min when 0.2 ml (2.6 mmol) allylamine were added dropwise with a Hamilton syringe. The solution was stirred at r.t. for 70 h while monitoring the reaction progress via TLC. The reaction was quenched with 0.1 ml acetic acid and was stirried at r.t. for 1 h. before being cooled to 0°C and stirred for 3 h. Dicyclohexylurea precipitated and was filtrated off. The filtrate was concentrated yielding 1.20 g (82 %) of raw product. No further purification was applied.

^{1}H-NMR [CD_3OD]: because of educt and solvent impurities no exact peak identifications could be carried out. Nevertheless, the crucial peaks reflecting the allylamide moiety could be ascertained: δ = 5.39 (m, 2H), 5.90 (m, 1H).

MS (ESI, negative): 520.3 $[educt-H]^-$, 559.4 $[M-H]^-$.

CSP 6 (Immobilization of the chiral selector 16)

Because of the unknown amount of pure product all the raw product was used for selector immobilization. 2.20 g of 3-mercaptopropylmethyl-silica were elutriated in 20 ml of MeOH. 1.20 g of selector were dissolved in 15 ml of MeOH using 80 mg (0.49 mmol) of AIBN.
Elemental analysis: 1,47 % N results in SO loading of 260 μmol g^{-1}

N-[[[(8R,9S)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-1R,2R-diaminocyclohexane *(17)*

3.45 g (6.6 mmol) of activated quinidine ester hydrochloride **2** were suspended in CH_2Cl_2. To the stirred solution 0.90 g (7.9 mmol) of (1*R*,2*R*)-1,2-diaminocyclohexane were added in portions. The solution was stirred at r.t. over night. Purification by chromatography (CH_2Cl_2 : MeOH 20:1 – 3:1) yielded diamine **17** (0.76 g, 25%) as yellow crystals.
^{1}H-NMR [CD_3OD]: δ = 1.22-1.54 (m, 5H), 1.73-1.92 (m, 5H), 1.95 (m, 1H), 2.06 (m, 1H), 2.31 (m, 1H), 2.63 (m, 1H), 3.00 (m, 1H), 3.14 (m, 1H), 3.32-3.44 (m, 3H), 3.54 (m, 1H), 3.66 (m, 1H), 4.04 (s, 3H), 5.50 (m, 2H), 6.22 (m, 1H), 6.94 (s, 1H), 7.42-7.58 (m, 3H), 7.97 (d, 1H), 8.67 (d, 1H).
MS (ESI, positive): 465.4 $[M+H]^+$.

Immobilization of the chiral selector 17

5.00 g of 3-mercaptopropylmethyl-silica were elutriated in 50 ml of MeOH. 0.70 g (1.5 mmol) of selector were dissolved in 20 ml of MeOH. 60 mg (0.37 mmol) of AIBN were used. According to MS-analysis selector breakdown took place after immobilization.

N-[[[(8S,9R)-6'-methoxycinchonan-9-yl]oxy]carbonyl]-D,L-cysteic acid (19)

1.15 (6.8 mmol) of finely grounded cysteic acid (racemic mixture) were suspended in dried CH_2Cl_2. While stirring the suspension 6.1 ml (24.9 mmol) of BSA were added with a Hamilton syringe. Refluxing for 24 h until solution became clear. After cooling to r.t. 3.22 g (6.1mmol) activated quinine ester hydrochloride **5** were added and stirred for additional 24 h. The reaction was quenched with MeOH. After a few minutes a viscous gum was formed which was removed, redissolved in MeOH and combined with the reaction solution. Purification by chromatography (dichloromethane : methanol 10:1 – 1:1) yielded 2.10 g (92 %) of yellowish crystals.

^{1}H-NMR [CD_3OD]: δ = 1.7 (m, 1H), 1.79-1.98 (m, 1H), 2.08 (m, 1H), 2.22 (m, 2H), 2.78 (m, 1H), 3.18-3.28 (m, 1H), 3.35-3.51 (m, 2H), 3.66 (m, 1H), 3.83 (t, 1H), 4.01 (s, 3H), 4.58-4.72 (m, 1H), 4.99-5.14 (m, 2H), 5.74 (m, 1H), 7.04 (s, 1H), 7.45-7.57 (m, 2H), 7.83 (m, 1H), 8.00(m, 1H), 8.78 (m, 1H).

MS (ESI, positive): 520.3 $[M+H]^+$.

6 List of Abbreviations

///	no value
AIBN	azo-bisisobutyronitril
AX	anion exchange
BSA	N,O-bistrimethylsilylacetamide
CSA	cysteic acid
CSAD	cysteic acid derivative
CSP	chiral stationary phase
CX	cation exchange
DACH	diaminocyclohexane
DCC	N,N'-dicyclohexylcarbodiimide
DH-QD	dihydroquinidine
DH-QN	dihydroquinine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
e.g.	exempli gratia, for example
ESI-MS	electrospray ionization mass spectrometer
et al..	et alii, and others
etc.	et cetera, and so on
h	hours
HOAc	acetic acid
HPLC	high performance liquid chromatography
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
MeI	methyliodide
MeOH	methanol
MP	mobile phase
n.d.	not determined
NMR	nuclear magnetic resonance
QD	quinidine
QN	quinine
QD-AX	quinidine anion exchanger
QN-AX	quinine anion exchanger

RP-HPLC	reversed phase - high performance liquid chromatography
r.t.	room temperature
SA	selectand
SAX	strong anion exchange
SO	selector
TLC	thin layer chromatography
WAX	weak anion exchanger

Summary

Recent studies in our group established the concept of zwitterionic cinchona-alkaloid based chiral stationary phases (ZWIX-CSPs) by fusing anion- and cation exchanger selectors into single ZWIX-selector molecules. In the course of further exploration of such ZWIX-type CSPs the main focus of the presented thesis was therefore the synthesis and the chromatographic evaluation of newly designed zwitterionic ion exchange-type CSPs.
The diploma work was based on the Taurine-Quinine (Tau-QN) selector motif and starting from there, the synthetic efforts focused on one hand on O9-carbamate modifications to introduce further ion exchange functionalities, and on the other hand on variations of the cinchona scaffold.

In particular, with Tau-QD (**CSP 1**) the pseudoenantiomeric CSP to Tau-QN was prepared. Furthermore, the introduction of a cysteic acid functionality to the well established quinine based anion exchanger (QN-AX) led to L-CSA-QN (**CSP 2**), which provided both a weak and a strong cation exchanger (WCX, SCX) site. By quaternizing the initially weak anion exchanger site (WAX) of the cinchona scaffold a strong anion exchanger (SAX) resulted in Tau-N-Methyl-QN (**CSP 4**). With Tau-Quinc (**CSP 5**), which employed quincorine instead of quinine as alkaloid scaffold, the importance of the aromatic quinoline group in the SO could be assessed. With preparation of L-CSAD-DH-QN (**CSP 6**) an alternative site for covalent immobilization to the silica support – via the cation exchanger part instead of the anion exchanger alkaloid – was tested. Apart from these zwitterionic CSPs, a bisbasic selector was synthesized via introduction of a quinuclidine moiety into the QN-AX motif (Quinu-QD, **CSP 3**).

The prepared chiral stationary phases were subject to investigation by HPLC. Enantiomer separation performance as well as general chromatographic key parameters like retention, resolution and selectivity, were monitored for a diverse set of chiral acids, bases and amino acids while no mobile phase optimizations were carried out due to time limitations.

Comparison of pseudoenantiomeric CSPs Tau-QN and –QD essentially showed that switch of elution order of amino acid analytes could be effected - thereby indicating the guiding influence of the alkaloid scaffold.

The quaternized anion exchanger group in **CSP 4** is also functional in zwitterion exchange, whereas the site of SO immobilization has not shown major impact on CSP performance. The quinoline moiety in the SO was found to be fundamental for enantiomer separation as with Tau-Quinc **(CSP 5**) only two analytes could be partially resolved.
Generally, structural modifications on the selectors` zwitterionic scaffolds and interpretation of the obtained chromatographic data helped to enlarge the understanding of the complex molecular recognition process.

To summarize, synthesis of novel ZWIX-selectors was carried out and optimized, which led to the preparation of six novel CSPs followed by their chromatographic evaluation. The CSPs show high chemical and mechanical stability, the degree of flexibility in terms of synthetic pathways following a combinatorial concept in combination with the potential of mobile phase optimization will open up an area to establish ZWIX-CSPs as a powerful addition to the pool of already established CSPs on the market.

Zusammenfassung

Die vorliegende Arbeit handelt von der Herstellung und chromatographischen Evaluierung zwitterionischer chiraler stationärer Phasen (CSPs). Basierend auf vorangegangenen Arbeiten unserer Gruppe wurden Säurefunktionalitäten in einen (bereits etablierten) Chinin-basierten Anionenaustauscher (QN-AX) eingeführt und somit das Konzept eines zwitterionischen Selektors (SO) realisiert. Durch Immobilisierung des Selektors auf modifiziertem Kieselgel wurden neuartige chirale stationäre Zwitterionenaustauscher-Phasen (ZWIX-CSPs) geschaffen, welche Enantiomerentrennung von chiralen Säuren, Basen und amphoteren Verbindungen ermöglichen.

Insgesamt konnten fünf verschiedene zwitterionische chirale Austauscherphasen hergestellt werden. Es wurden Sulfon- und Carbonsäuregruppen an der O9-Carbamat Position von Cinchona-Alkaloiden (vorrangig Chinin und sein Pseudoenantiomer Chinidin) eingeführt und dadurch Selektoren mit starken und schwachen Ionenaustausch-Mechanismen geschaffen. Durch strukturelle Modifikationen an den Alkaloidgerüsten, wie z.B. Methylierung der 1-N Position an Chinin oder die Einführung einer neuen Selektor-Bindungsstelle an das Kieselgel, wurde das Spektrum der Interaktionsmöglichkeiten zwischen Selektoren und Analyten erweitert. Es wurde auch ein Selektor auf Basis des Chincorins synthetisiert, welcher durch einen fehlenden Chinolin-Rest gekennzeichnet ist. Zusätzlich zu den zwitterionischen Phasen wurde durch Fusion eines Chinuclidin-Motivs mit einem Chinidin-basierten Anionenaustauscher eine bisbasische CSP hergestellt.

Als Ergänzung zur synthetischen Arbeit erfolgte eine Evaluierung der CSPs auf einem HPLC-System. An Hand eines breit angelegten Sets an chiralen Säuren, Basen und freien Aminosäuren wurden die Phasen sowohl auf ihre chirale Trennleistung als auch auf allgemeine chromatographische Parameter hin untersucht.
Mit den ZWIX-CSPs konnte auch unter nicht-optimierten Elutionsbedingungen Basislinientrennung für alle drei Analytklassen erzielt werden. Außerdem konnte gezeigt werden, dass das pseudoenantiomere Verhalten von Chinin und Chinidin in den ZWIX-Selektoren erhalten bleibt, was sich durch eine Umkehr der Elutionsreihenfolge der getrennten Enantiomere widerspiegelt. Jedoch führten weder die Veränderung der Selektorbindungsstelle an das Kieselgel noch die Einführung einer bisaciden Seitenkette zu einer Verbesserung der Trennleistung.

Weiters können aromatische Reste in den ZWIX-Selektoren als essentielle Bestandteile für Enantioselektivität angesehen werden, da auf dem Chincorin-basierten SO keine Basislinientrennung erzielt werden konnte.

Zusammenfassend kann angemerkt werden, dass neue Synthesekonzepte erstellt und Synthesen für zwitterionische Selektoren durchgeführt und optimiert wurden. Die durch Immobilisierung auf modifiziertem Kieselgel realisierten CSPs lieferten großteils vielversprechende Trennleistungen. Das breite Anwendungsspektrum auf saure, basische und amphotere Verbindungen, die hohe mechanische und chemische Stabilität, sowie die milden Elutionsbedingungen zeigen das große Potential dieser zwitterionischen chiralen stationären Phasen.

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