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Journal of Chromatography A, 741 (1996) 33–48

JOURNAL OF CHROMATOGRAPHY A

Quinine and quinidine derivatives as chiral selectors I. Brush type chiral stationary phases for high-performance liquid chromatography based on cinchonan carbamates and their application as chiral anion exchangers

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Received 12 December 1995; revised 8 February 1996; accepted 8 February 1996

Abstract

Quinine and quinidine carbamates, respectively, have been immobilized onto porous silica utilizing two different types of spacer and/or grafting modes, obtaining four brush type chiral stationary phases (CSPs). These CSPs have preferentially been operated with buffered aqueous mobile phases to resolve the enantiomers of acidic analytes involving ion pair mechanisms as the dominating binding and/or retention principle beside additional adsorption towards the modified silica surface. The spectrum of successfully resolved racemates contains chiral aryl-, aryloxy- and arylthiocarboxylic acids, as well as N-derivatized amino acids (DNB-, B-, Ac-, F-, DNP-, DBD-, Fmoc-, Boc-, Z-, DNS-amino acids, etc.) and many other chiral acids including also sulfonic, phosphonic and phosphoric acids. The influence of mobile phase parameters, as well as of structure variations of analytes on retention and enantioselectivity was evaluated. Thus, a chromatographically derived tentative chiral recognition model is proposed for DNB-amino acids which are resolved into enantiomers with exceptionally high α-values (e.g., DNB-Leu with an α-value of about 7).

Keywords: Chiral stationary phases, LC; Chiral selectors; Enantiomer separation; Chiral recognition model; Cinchonan carbamates; Quinine carbamate; Quinidine carbamate; Amino acids, DNB derivatives

1. Introduction

Enantioseparation by liquid chromatography (LC) became in many instances the method of choice to recognize and/or separate stereoisomers. Several review articles and books deal with the wide variety of chiral stationary phases (CSPs), many of them commercially available, and with the attempts to interpret how these CSPs may operate with respect to molecular recognition [1,2].

For some time, cinchona alkaloids, especially quinine (QN) and quinidine (QD) (see Fig. 1), have received much attention as a cheap natural source for stereodiscriminating auxiliaries (selectors, SOs). Thus, cinchona alkaloids are widely used as resolving agents for chiral acids via their diastereomeric salts, as highly effective catalysts in asymmetric syntheses [3,4], as phase transfer catalysts in

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PII S0021-9673(96)00137-9

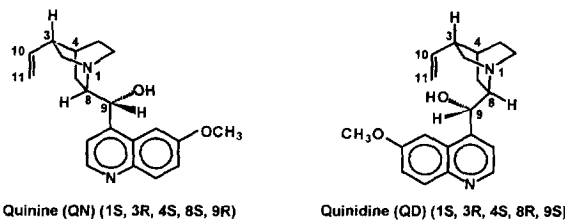


Fig. 1. Structure and stereochemistry of the "pseudo-enantiomeric" quinine and quinidine.

stereoselective syntheses [5,6] and finally as chiral selectors for direct chromatographic enantioseparations. For this purpose, quinine and quinidine are either added to the mobile phase as counterions in chiral ion-pair chromatography [7–9] or covalently immobilized onto silica thus obtaining chiral stationary phases (CSPs) [10–23].

Structurally, quinine (QN) and quinidine (QD) consist of a planar quinoline and a rigid quinuclidine ring, which are connected by a secondary methyl alcohol bridge. Conformational flexibility is available only for the torsions of the C_8-C_9 and C_9-$C_{4'}$ bonds (see Fig. 1). In whole, these molecules can be viewed as a semirigid framework and thus as promising SO molecules. More detailed information about the preferential conformations of free quinine and quinidine as well as of some derivatives thereof can be excerpted from [24].

The arrangement of the five stereogenic centers within the rigid molecules QN and QD (at position 1, 3, 4, 8 and 9) may provide an excellent basis for effective chiral recognition. N1, C3 and C4 have identical (1*S*, 3*R*, 4*S*) and C_8, C_9 have opposite configurations for QN and QD (see Fig. 1). Although they are diastereomers, QN and QD often display quasi-enantiomeric behavior. Therefore they are sometimes called "pseudo-enantiomers". This term can be addressed to the fact that in most cases stereoselectivity is under C_8/C_9 control and also to the opposite configurations at these chiral centers. This "pseudo-enantiomeric" behavior may also be observed concerning elution order of enantiomeric solutes on quinine and quinidine based CSPs. Opposite elution order on quinine and quinidine based CSPs may be advantageous especially for the application in trace analysis and is often not achievable for other type CSPs which are based on SOs from the naturally occurring chiral pool such as (cyclo)-dextrins, polysaccharides, macrocyclic antibiotics, proteins, etc.

Whereas the previously reported cinchonan derived CSPs were based on O-underivatized or O-esterified quin(id)ine derivatives as chiral selectors we decided to immobilize quin(id)ine via a carbamate group and a linking spacer onto silica. The obtained CSPs provide several sites for intermolecular interaction with the analytes distributed around the stereogenic center C_9 of the cinchonan skeleton, i.e., the basic aliphatic nitrogen group of the quinuclidine ring for electrostatic interaction (ion-pairing) preceded by protonation, the hydrogen donor-acceptor site of the carbamate group for hydrogen bonding and/or dipole–dipole interaction, the π-basic quinoline ring for intermolecular $\pi-\pi$ interaction, the bulky quinuclidine group and also the large planar quinoline ring for steric interaction (attraction and/or repulsion).

These new quinine and quinidine carbamate based CSPs (see CSP I–IV on Fig. 2) exhibit considerable stereoselectivity not only in the normal-phase, but especially in the reversed-phase mode. Normal-phase mode chiral separations according to the Pirkle concept include mainly solutes which are already resolved by the cinchonan based CSPs described earlier [10–23] (binaphthols, benzodiazepines, arylalkylamides, etc.) but which are not subject of the present investigation.

Working with aqueous mobile phases these new CSPs are, however, highly effective in resolving enantiomers of acidic analytes, selectands (SAs), with complementary intermolecular interaction sites, whereby ionic interactions seem to be the prime driving force for molecular association. Since a tertiary amine (quinuclidine) with a pK_a of about 9 is involved in ion-pair formation with acidic counterions, these CSPs can be classified as weak chiral anion-exchangers (WAX). Although there are a few examples reported in literature [25–27], the class of low molecular synthetic chiral ion-exchangers is in general underestimated among the wide variety of published but also commercially available CSPs.

In this paper, which is the first out of a series

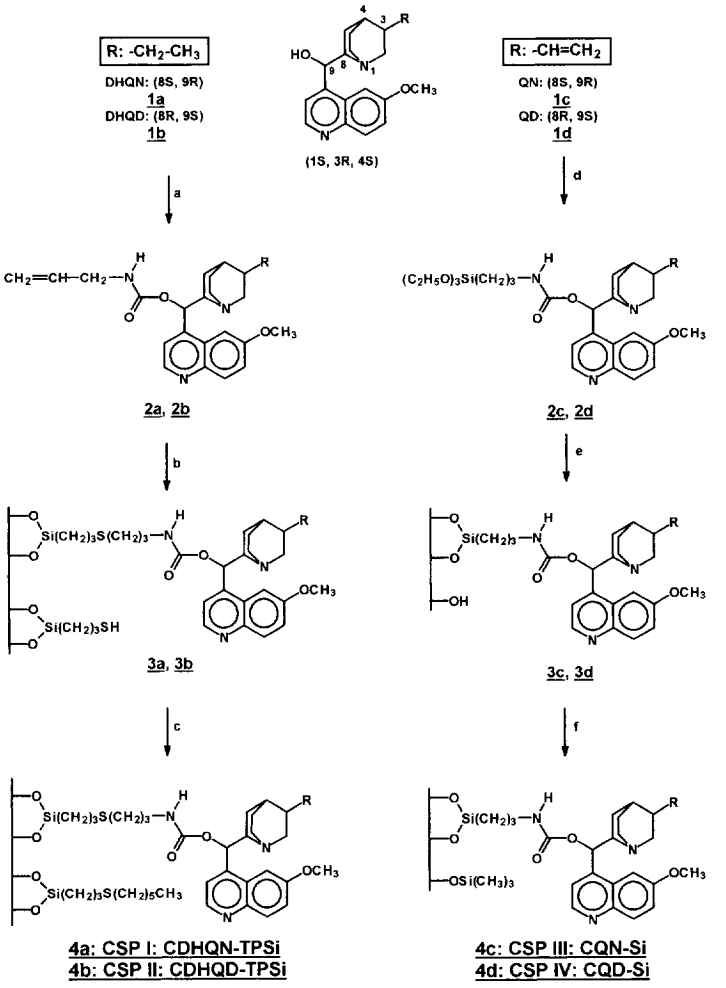


Fig. 2. Reaction scheme for the syntheses of the four chiral stationary phases (CSPs): (a) allyl isocyanate, dibutyl tin dilaurate, toluene, reflux; (b) 3-mercaptopropyl silanized silica, AIBN, chloroform, reflux, N_2; (c) 1-hexene, AIBN, chloroform, reflux, N_2; (d) 3-isocyanatopropyl triethoxysilane, dibutyl tin dilaurate, toluene, reflux; (e) silica, toluene, reflux; (f) N,O-bis-(trimethylsilyl)acetamide, toluene, reflux.

dealing with chiral anion-exchangers, the syntheses and the stereodiscriminating potential of the new CSPs for acidic selectands using aqueous buffered mobile phases are reported. Two different types of spacer and/or grafting modes of the selectors (SO) to silica are compared concerning retention and selectivity. The influence of mobile phase parameters and of structure variations of the selectands on enantioselectivity will be evaluated.

2. Experimental

2.1. Materials

10,11-Dihydroquinine (DHQN) and 10,11-dihydroquinidine hydrochloride (DHQD–HCl) were obtained as a gift from Boehringer Mannheim, (Mannheim, Germany). Quinine (QN) and quinidine hydrochloride (QD–HCl) were from Apoka AG and

supplied by Herba (Graz, Austria). Kromasil 100Å–5μm from EKA Nobel (Bohus, Sweden) was used as the porous silica material for all four CSPs, I–IV. 3-Mercaptopropyl silanized silica was prepared as described elsewhere [27] and afforded 4.83% C, 1.19% H. This corresponds to a calculated coverage of about 0.9 mmol thiol groups per gram silica. Allyl isocyanate α,α'-azo-bis-isobutyronitrile (AIBN), dibutyl tin dilaurate and 3-mercaptopropyl trimethoxysilane were purchased from Aldrich Chemie (Steinheim, Germany). Further, 1-hexene and N,O-bis-trimethylsilyl acetamide were obtained from EGA Chemie (Steinheim, Germany) and 3-isocyanatopropyl triethoxysilane from ABCR (Karlsruhe, Germany). The solvents used for the syntheses, toluene and chloroform, were of p.a. quality.

Mobile phases for chromatography were prepared from ammonium acetate p.a. from Loba Feinchemie, (Fischamend, Austria) and HPLC grade water (purified by a Milli-Q-Plus filtration unit from Millipore). The organic modifier, methanol (MeOH), was of HPLC grade and supported by Baker.

The chiral test compounds (analytes) were provided by different sources, mainly Aldrich, Sigma, Bachem and Degussa. N-derivatized amino acids were, if not deliverable by the prior mentioned companies, synthesized according to standard derivatization procedures.

2.2. Instrumentation

Each modified sorbent, CSP I–IV, was slurry packed into a stainless-steel column of the dimension 150×4.6 mm I.D. by Forschungszentrum Seibersdorf, Austria. The chromatographic system consisted of L-6200 intelligent pump, L-4250 UV–Vis detector, D-6000 interface, AS-2000A autosampler, D-6000 Chromatography Data Station software, HPLC Manager Vers. 2.09 from Merck (Darmstadt, Germany) and a column thermostat of W.O. Electronics (Langenzersdorf, Austria). The pH of the mobile phases (always apparent pH, pH_a) was measured with an Orion pH-meter, Model 520A.

IR spectra were recorded on a Perkin Elmer (Beaconsfield, UK) 881 infra-red spectrometer and NMR spectra were recorded on a 360 MHz machine, Bruker (Karlsruhe, Germany) AM 360 spectrometer. Optical rotation was measured with a Perkin Elmer 241 MC polarimeter. Melting points are uncorrected and were determined using a Tottoli apparatus of Büchi (Switzerland).

2.3. Standard chromatographic conditions

Unless otherwise stated, a mixture of 80% methanol–20% 0.1 *M* ammonium acetate was used as standard mobile phase. The pH of the mixture (apparent pH, pH_a) was adjusted to 6.0 by adding glacial acetic acid of p.a. quality. Mobile phases were filtered through a Nalgene nylon membrane filter (0.2 μm; Nalge Company, New York, USA) and degassed before use. Flow-rate was 1 ml min^{-1} and temperature was held constantly at 25°C with a column thermostat. UV detection at 254 nm and 230 nm, respectively, was the standard detection method.

2.4. Synthesis of CSP I and II (see Fig. 2)

2.4.1. 10,11-Dihydroquinine (DHQN) and 10,11-dihydroquinidine (DHQD) allylcarbamate (**2a** and **2b**)

A 3.0-g amount of **1a** and **1b** (as free bases), respectively, were dissolved in dry toluene; 0.9 ml of allyl isocyanate and 1 drop of dibutyl tin dilaurate as catalyst were added. The mixture was refluxed for 4 h, the solvent evaporated and the remaining raw material washed with *n*-hexane. The white solid was crystallized with chloroform/cyclohexane resulting in **2a** and **2b**, respectively, with 80% yield.

Physical properties of **2a**: m.p.: 162–164°C; $[\alpha]^{23}_{Na589}=+62.7°$, $[\alpha]^{23}_{Hg546}=+74.5°$ ($c=1.00$; MeOH); IR (KBr): 3200, 1700, 1620, 1580, 1500 cm^{-1}. ^1H NMR (TMS, dMeOD): 8.67 (d, 1H), 7.98 (d, 1H), 7.53 (m, 2H), 7.45 (dd, 1H), 6.50 (d, 1H), 5.82 (m, 1H), 5.20 (d, 1H), 5.07 (d, 1H), 4.00 (s, 3H), 3.70 (d, 2H), 3.27 (m, 1H), 3.08 (m, 2H), 2.73 (m, 1H), 2.37 (m, 1H), 2.00–1.40 (m, 6H), 1.28 (m, 2H), 0.85 (t, 3H) ppm.

Physical properties of **2b**: m.p.: 134–135°C; $[\alpha]^{23}_{Na589}=-0.3°$, $[\alpha]^{23}_{Hg546}=-0.8°$ ($c=1.00$; MeOH); IR (KBr): 3400, 1720, 1620, 1510, 1560 cm^{-1}. ^1H NMR (TMS, dMeOD): 8.64 (d, 1H), 7.94 (d, 1H),

7.52 (d, 1H), 7.50 (d, 1H), 7.42 (dd, 1H), 6.53 (d, 1H), 5.79 (m, 1H), 5.14 (d, 1H), 5.04 (d, 1H), 3.98 (s, 3H), 3.69 (m, 2H), 3.24 (q, 1H), 2.90 (q, 1H), 2.80 (m, 2H), 2.70 (m, 1H), 2.01 (t, 1H), 1.57 (s, 1H), 1.50 (m, 5H), 1.40 (m, 1H), 0.93 (t, 3H) ppm.

*2.4.2. Grafting of **2a** and **2b** to 3-mercaptopropylsilanized silica yielding **3a** and **3b***

A 4.0-g amount of 3-mercaptopropylsilanized silica (4.83% C, 1.19% H, about 0.9 mmol SH g^{-1} silica) were suspended in chloroform. After addition of 2 g of **2a** and **2b**, respectively, and 200 mg of the radical initiator AIBN the suspension was refluxed for 15 h flushing with a soft stream of nitrogen. The modified silica was sedimented, the solvent removed and the chiral sorbent washed with chloroform, methanol and petrol ether. The dried modified silica (**3a** and **3b**, respectively) was subjected to elemental analysis.

CHN analysis of **3a**: 14.53% C, 2.28% H, 1.55% N; CHN analysis of **3b**: 13.8% C, 2.13% H, 1.47% N.

This corresponds to a calculated selector density of about 0.35 mmol g^{-1} silica for **3a** and 0.32 mmol g^{-1} silica for **3b**, respectively.

*2.4.3. End-capping of **3a** and **3b***

According to previous studies, which indicated a positive influence of end-capping on enantioselectivity and column efficiency (data not shown), end capping was performed to reduce non-stereoselective hydrogen bonding of unmodified thiol groups of the silica backbone with selectands that are capable for hydrogen bonding. Thus, 4.0 g of **3a** and **3b**, respectively, 2.0 ml 1-hexene and 200 mg of AIBN were refluxed in chloroform for 15 h under nitrogen. The modified silica was washed as described above, dried and sieved.

CHN analysis of **4a**: 15.52% C, 2.46% H, 1.55% N; CHN analysis of **4b**: 14.72% C, 2.30% H, 1.46% N.

Thus, about 140 μmol thiol groups per gram silica of **3a** and about 130 μmol thiol groups per gram silica of **3b** were modified with lipophilic hexyl groups.

Finally, 4 g of **4a** (CSP I: carbamoylated dihydroquinine thiopropylsilanized silica, CDHQN-TPSi) and **4b** (CSP II: carbamoylated dihydroquinidine thiopropylsilanized silica, CDHQD-TPSi) were obtained and packed into stainless-steel columns by a conventional slurry packing method.

2.5. Synthesis of CSP III and IV (see Fig. 2)

*2.5.1. Quinine (QN) and quinidine (QD) 3-triethoxysilylpropylcarbamate (**2c** and **2d**)*

A 2.0-g amount of **1c** and **1d** (as free bases), respectively, and 1.5 ml of 3-triethoxysilyl isocyanate were refluxed for 4 h in dry toluene using 1 drop of dibutyl tin dilaurate as catalyst. Evaporation of the solvent and stirring with dry diethylether gave **2c** as white solid and **2d** as yellowish oil, respectively, in 99% yield.

Physical properties of **2c**: m.p.: 86°C; $[\alpha]^{23}_{Na589}=+3.5°$, $[\alpha]^{23}_{Hg546}=-2.2°$ ($c=1.00$; MeOH); IR (KBr): 3185, 2974, 2932, 2890, 1718, 1621, 1590, 1541, 1509, 1472, 1258, 1124, 1079 cm^{-1}. ^1H NMR (TMS, dMeOD): 8.67 (d, 1H), 7.97 (d, 1H), 7.55 (m, 2H), 7.45 (dd, 1H), 6.50 (d, 1H), 5.82 (m, 1H), 4.97 (m, 2H), 4.01 (s, 3H), 3.78 (q, 6H), 3.30 (m, 1H), 3.09 (m, 3H), 2.70 (m, 2H), 2.38 (m, 1H), 2.00–1.45 (m, 8H), 1.20 (t, 9H), 0.58 (m, 2H) ppm.

Physical properties of **2d**: m.p.: –; $[\alpha]^{23}_{Na589}=+37.5°$, $[\alpha]^{23}_{Hg546}=+41.4°$ ($c=2.04$; MeOH); IR (NaCl-window): 3340, 2973, 2934, 2880, 1715, 1622, 1593, 1511, 1475, 1435, 1244, 1103, 1079 cm^{-1}. ^1H NMR (TMS, dMeOD): 8.68 (d, 1H), 7.97 (d, 1H), 7.54 (m, 2H), 7.43 (dd, 1H), 6.58 (d, 1H), 6.20 (m, 1H), 5.16 (d, 1H), 5.09 (s, 1H), 4.00 (s, 3H), 3.77 (q, 6H), 3.40–2.60 (m, 5H), 2.32 (m, 1H), 2.10 (m, 1H), 1.80–0.80 (m, 17H), 0.58 (m, 2H) ppm.

*2.5.2. Covalent binding of **2c** and **2d** onto silica resulting in **3c** and **3d***

A 5.0-g amount of silica and 3.0 g of **2c** and **2d**, respectively, were refluxed in toluene for 20 h. The toluene was removed and the modified silica washed several times with toluene, methanol, acetone and petrolether. Elemental analysis gave 9.77% C, 1.32% H and 1.23% N for **3c**, corresponding to a selector coverage of 0.30 mmol g^{-1} silica and 10.52% C, 1.46% H and 1.41% N for **3d**, corresponding to a selector coverage of 0.32 mmol g^{-1} silica.

*2.5.3. End-capping of **3c** and **3d***

A 5.0-g amount of the modified silica **3c** and **3d**, respectively, and 3.0 ml of N,O-bis(trimethylsilyl) acetamide were refluxed for 20 h in toluene. Washing, drying and sieving of the modified silica yielded 5.0 g of the chiral sorbent **4c** (CSP III: CQN-Si) and **4d** (CSP IV: CQD-Si), respectively. Elemental analysis gave 11.71% C, 1.67% H and 1.24% N for **4c**, corresponding to 0.49 mmol trimethylsilyl groups per gram silica and 12.33% C, 1.82% H and 1.41% N for **4d**, corresponding to 0.50 mmol trimethylsilyl groups per gram silica.

3. Results and discussion

3.1. Considerations concerning retention and chiral recognition mechanisms

The new, easily accessible CSPs I–IV can be classified as weak chiral anion-exchangers (WAX). At the working pH (pH 4–7) of the buffered aqueous mobile phases the tertiary amine of the quinuclidine group is protonated to a high degree and therefore available for strong, non-directed long ranged Coulomb attraction with deprotonated acidic SAs. Consequently, retention of acidic analytes is predominantly controlled by an ion pairing mechanism, which is the driving force for specific molecular association of the SAs with the SO molecules, paired or combined with other adsorption processes to the modified silica surface. The molecular electrostatic field that surrounds the binding sites guides the correct orientation of the SAs towards the SO and is responsible for the first but reversible SO-SA contact to form transient diastereomeric SO-SA complexes or ion-pairs. Whereas ionic interaction and pure hydrophobic interaction with the SO and the modified silica surface, which itself has also hydrophobic character, are not stereoselective processes, additionally, specific intermolecular interactions between SO and SA may occur. These can be in particular hydrogen bonding and/or dipole–dipole forces, $\pi-\pi$ interactions and steric attraction/repulsion. All such SO-SA binding increments, which may markedly differ in strength towards the two SA enantiomers, may be responsible for molecular recognition and discrimination between enantiomers.

Table 1
Chromatographic retention and resolution data of diverse acidic compounds on CSP II[a]

Compound	k_1'	α	R_s[b]	Elution order[c]
1	19.13	5.17	17.68	L
2	9.47	1.00	0.00	
3	5.37	1.15	1.93	*S*
4	30.08	1.31	4.06	*S*
5	1.40	1.00	0.00	
6	3.96	1.15	1.82	*R*
7	8.99	1.12	1.46	
8	2.17	1.11	1.02	
9	5.28	1.20	1.93	*S*
10	2.14	1.04	<1	*S*
11	6.57	1.00	0.00	
12	12.99	1.10	1.27	

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 3.
[b] Calculated by the formula: $R_s = 1.18(t_{R_2} - t_{R_1})/(w_{0.5,1} + w_{0.5,2})$.
[c] Elution order: configuration of the first eluted enantiomer.

3.2. Influence of various (acidic) structural features on resolvability upon WAX type CSPs

Table 1 summarizes the separation results of various acidic chiral compounds on CSP II (for structures see Fig. 3). This selection of analytes (SAs) demonstrates the great potential of cinchonan carbamate based CSPs to resolve a very broad spectrum of SA stereoisomers exposing heterogeneous structural features and diversified acidities. As can be seen, an exceptionally high α-value is obtained for the N-3,5-dinitrobenzoyl-tryptophan (DNB-Trp) (Table 1, entity 1). The D-enantiomer matches the SO's binding prerequisite (complementarity with respect of functionalities, shape and space as well as favorable conformational arrangement) exceptionally well whereas the L-enantiomer is more or less excluded from the "binding groove" that is built by the quinoline, quinuclidine and the carbamate residue. However, blocking the carboxylic function by esterification brings about a complete loss of enantioselectivity (Table 1, compare entity 1 and 2). This finding emphasizes the importance of the acidic character of the analyte and demonstrates the role of ion-pairing for chiral recognition on these CSPs. They permit the resolution not only of the enantiomers of acidic compounds with a carbon as stereogenic center, but also acids with axial chirality (Table 1, compounds 3 and 4). Strong acids such as

carboxylic, sulfonic, phosphoric and phosphonic acids at the working pH (usually around pH 5–6) are deprotonated to a high degree and thus accessible for ionic interaction with the positively charged nitrogen of the quinuclidine moiety. Significantly weaker acids such as phenols, imides, etc., are hardly capable of ionic interaction at this pH, where the WAX type CSPs have their optimum exchanging capacity. This is illustrated by means of compound 4 and 5 listed in Table 1. The strong acidic 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (4) is resolved into enantiomers with an outstanding α-value. In contrast, the weak acidic, but structurally closely related, atropisomeric 1,1′-binaphthyl-2,2′-diol is lacking dissociation. Therefore, it is hardly retained and cannot be resolved. But, binaphthols, however, can be well resolved on these CSPs under normal-phase conditions involving a chiral discrimination mechanism according to the Pirkle concept (data not shown). Camphor-10-sulfonic acid (6) is shown as an example, for the discrimination force of quin(i-d)ine carbamate type CSPs for chiral sulfonic acids. The separation of the vitamin K antagonist acenocoumarol (7), which is due to stabilizing mesomeric effects acidified, should round off the broad spectrum of chiral selectands that are successfully resolved into enantiomers.

As expected, the distance of the acidic function,

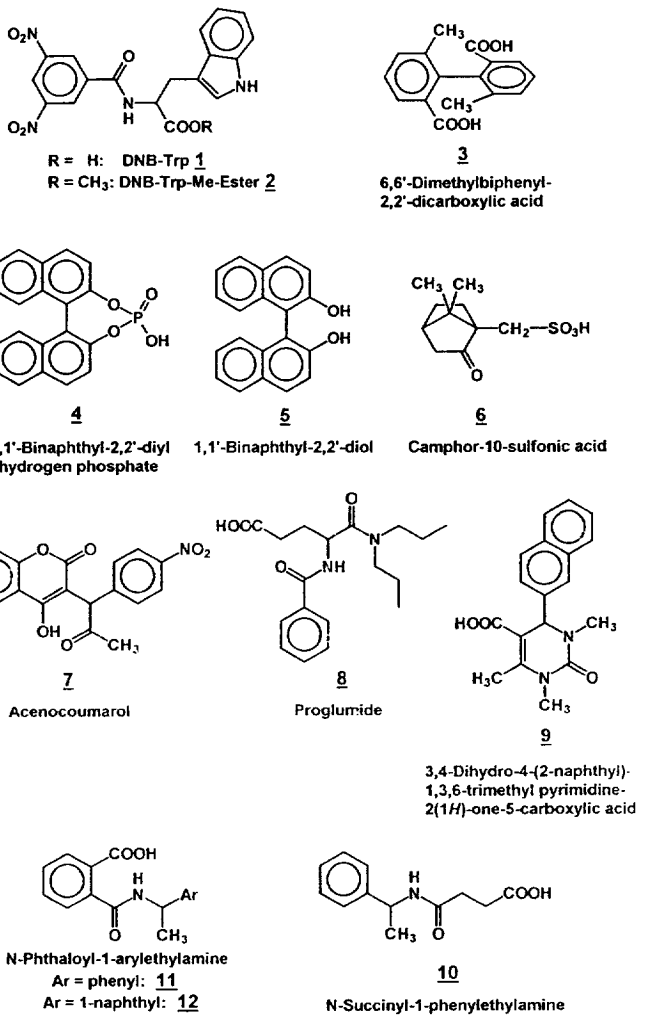


Fig. 3. Structure of analytes (continued on next page).

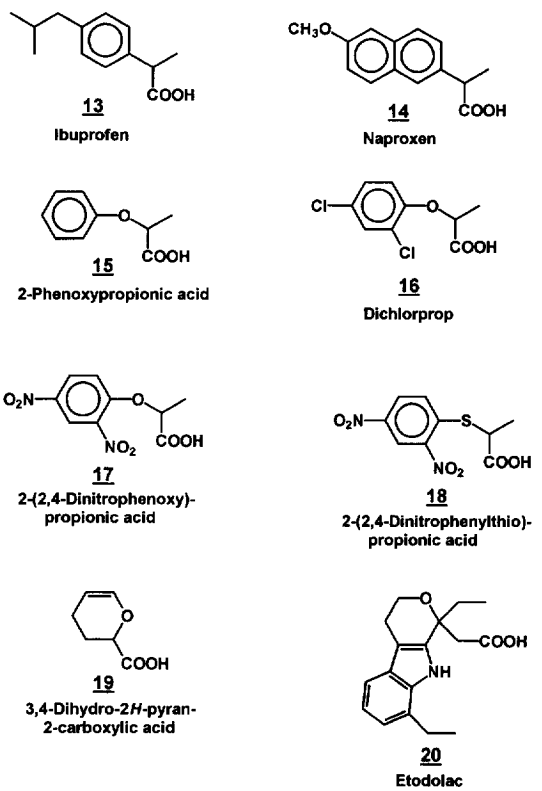


Fig. 3. (*continued*)

the dominant SO-SA binding site, from the chiral center within the SA molecule may influence the degree of chiral resolution. Nevertheless, as demonstrated by the examples of proglumide (8) and 3,4-dihydro-4-(2-naphthyl)-1,3,6-trimethyl pyrimidine-2(1*H*)-one-5-carboxylic acid (9), the stereodiscriminating potential is not restricted to compounds having the acidic function directly attached to the stereogenic center. This observation is a favorable feature of the presented separation systems: if the SAs to be resolved do lack an appropriate acidic functionality, such an interaction site may be conveniently introduced by achiral derivatization. Thus, the separation of chiral amines and alcohols becomes possible after derivatization, for instance with succinic acid anhydride or phthalic acid anhydride, as is demonstrated for alkylarylamines (Table 1, entities 10, 11 and 12).

The presence of two or more functionalities of similar nature (e.g., two $\pi-\pi$ interaction sites) in the SA molecule may lead to perturbation of the preferred unidirectional orientation of the SA into the cleft of the SO due to competitive effects of the analogues interaction sites for binding with the corresponding (complementary) SO binding site (e.g., quinoline π-base). Unfortunately, several distinct diastereomeric SO-SA complexes of different stability may be formed which are not unidirectional with respect to chiral discrimination. Usually, they tend to cancel one another partially or even totally, causing a significant decrease in overall stereoselectivity. Indeed, phenylethylamine is partially resolved as succinamide (Table 1, entry 10), whereas it could not be fractionated into enantiomers as phthalamide (Table 1, entry 11) which provides an additional $\pi-\pi$ interaction site stemming from the derivatizing reagent. However, since it constitutes a π-acid it may bind to the π-basic quinoline ring even stronger than the phenyl group originating from the arylethylamine SA. Undoubtedly, this competition of homologous functional groups for interaction with a certain binding group of the SO and the resulting non-uniform binding mode with respect to the total SO molecule is also the reason why dicarboxylic acids are resolved with a much lower α-value than their corresponding monocarboxylic acids although retention is dramatically increased. Consequently and according to the concept of reciprocity, more than one functional group of the same kind, particularly remote from the prime binding domain of the chiral host molecule may reduce the level of chiral recognition of the SO. This guideline, in return, should especially be encountered for the strongest, the ionic interaction.

3.3. Influence of mobile phase parameters on retention and enantioselectivity

Numerous mobile phase variables (type and concentration of buffer, eluent pH, type and content of organic modifier, temperature, charged and uncharged additives, etc.) can be used to control solute retention and enantioselectivity. The broad choice and combination of these variables indicate the great flexibility of this method and allow the separation of a wide variety of acidic analytes. Furthermore, gradient elution concerning ionic strength and/or organic modifier can be adopted to control overall

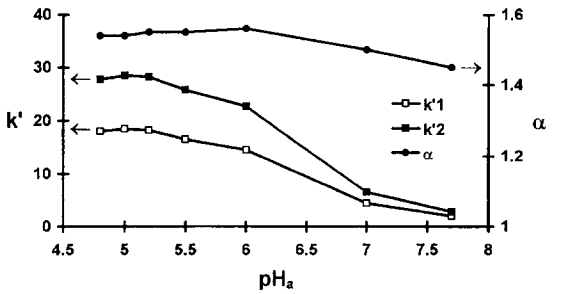


Fig. 4. Influence of pH on retention and enantioselectivity of N-2,4-dinitrophenyl-threonine (DNP-Thr) on CSP I. Chromatographic conditions: mobile phase: 80% methanol–20% 0.1 *M* ammonium acetate; pH adjusted to the respective value by adding glacial acetic acid; T: 25°C; flow-rate: 1 ml min^{-1}; det.: UV 254 nm.

retention and selectivity and to reduce the total run time.

Fig. 4 depicts a typical pH-curve of the chromatographic behaviour of carboxylic acids on the WAX type CSPs I to IV. As expected, a strong dependence of retention and selectivity on mobile phase pH was observed. Retention of (the weak acidic analyte) N-2,4-dinitrophenyl-threonine (DNP-Thr) on the WAX type stationary phase CSP II follows a typical ion-exchange process. As is common knowledge, in ion-exchange chromatography several equilibrium processes must be considered which are controlled by the respective mass-action equilibrium constants, by the pH-value of the mobile phase, and by p*K*-values of the ion exchanger (in our case of the cinchonan derivative), analytes and counterions. It is essential that both partners, ion exchanger as well as analyte (in our case selector, SO, as well as selectand, SA), are to a certain degree ionized. The degree of ionization of SO and SA depends on the respective pK_a-values and the pH of the mobile phase which can be varied to adjust the pH where the product of the degree of SO and SA ionization reaches maximum values. This relationship is also demonstrated in Fig. 4. Accordingly, maximum retention is reached on this type of weak chiral anion exchangers (CSP I to IV) at a pH close to the pK_a of the analyte. Generally, they should be used in this pH range (e.g., for carboxylic acids between pH 5–6) to exploit the full ion-exchange capacity and, hence, to develop optimal enantioselectivity.

In contrast, the lipophilic retention increment, which contributes to a great extent to non-stereoselective retention, is increasingly forced by lowering the pH (reflecting the conditions of reversed-phase chromatography of protonized acidic compounds). Nevertheless, it is negligible at the usual working pH of the mobile phase with respect to ionic interaction.

An important factor to influence retention in ion-exchange chromatography is the type and concentration of buffer or counterions of the mobile phase. As demonstrated by Fig. 5, retention decreases with increasing buffer concentrations. Higher concentrations of, for instance, acetate ions displace the SA anions more easily from the bound charge of the ion exchanger. With respect to different types of buffer ions which have been experimentally investigated, the elution strength of formate, acetate, phosphate and citrate increased in this order (data not shown). Concomitantly, enantioselectivity was more or less unaffected by the type of buffer salt over a wide range of buffer concentrations. Thus, one can accommodate the optimum retention time window by adjusting these variables without any significant loss of enantioselectivity.

3.4. Separation of N-derivatized amino acids

The outstanding high α-value of DNB-Trp (Table 1, compound 1; for structure see Fig. 3) led us to a more detailed study of the reason for the specific molecular recognition capability of these CSPs for

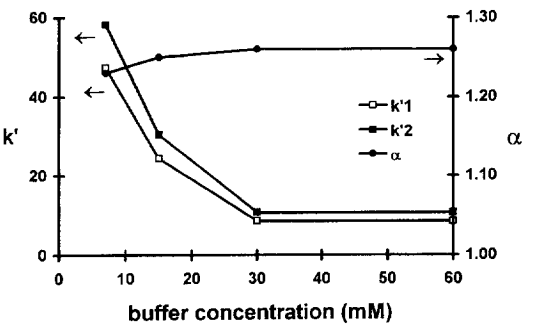


Fig. 5. Influence of buffer concentration on retention and enantioselectivity of N-2,4-dinitrophenyl-norleucine (DNP-NLeu) on CSP II. Chromatographic conditions: mobile phase: 80% methanol–20% ammonium acetate; pH$_a$=6.0; T: 25°C; flow-rate: 1 ml min^{-1}; det.: UV 254 nm; indicated values of buffer concentration refer to the actual buffer concentration of the mixture.

amino acid (AA) derivatives. Free amino acids could not be resolved into the enantiomers since appropriate functionalities to contact the SO are obviously lacking. However, additional sites for intermolecular interaction with the SO, beside the carboxylic function, can be introduced by the derivatization of the amino group. Fortunately, a broad spectrum of appropriate derivatizing agents as well as many AA derivatives thereof as reference substances are commercially available and the respective chemistry to generate them is well documented. Table 2 lists the chromatographic data for N-protected phenylalanine (Phe) derivatives reflecting a gross range of the most utilized reagents (for structures of protecting groups see Fig. 6). As can be seen from these data, beside the carboxylic group, the N-protecting group must also be a dominant SO-SA binding site. It controls by its functionality, shape and conformational arrangement the relative orientation of the SAs at the SO binding domain. Hence, the protecting group determines the elution order of the two enantiomers which is of significance with respect to the underlying molecular recognition mechanism and which may be different for structurally very similar derivatives. This is most pronounced for 3,5-dinitrobenzoyl (DNB) and 2,4-dinitrophenyl (DNP) amino acids and should be illustrated by the example of the respective leucine (Leu) derivatives. Both, DNB- and

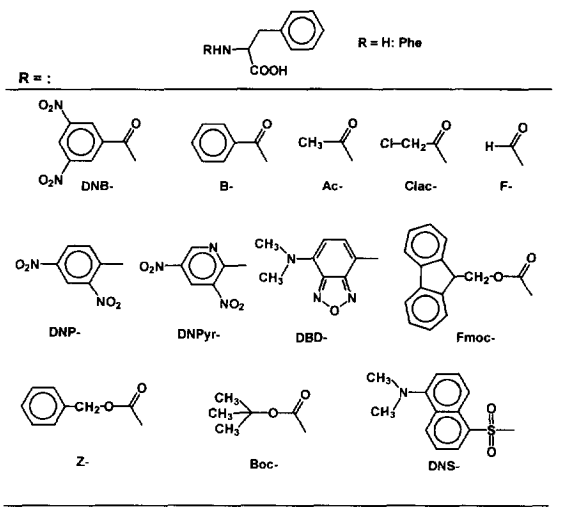


Fig. 6. Structure of various phenylalanine (Phe) derivatives.

DNP-Leu bear the strong π-acidic dinitrophenyl molecule structure increment available for $\pi-\pi$ interaction with the π-basic quinoline ring. The interstranded carbonyl function between the dinitrophenyl group and the Leu backbone in the DNB-AA molecule constitutes a rigid amide group that may build strong hydrogen bonds to the carbamate group of the SO. Obviously, the DNB-Leu enantiomers are completely differently arranged toward the SO compared to the DNP-Leu enantiomers, having

Table 2
Chromatographic retention and resolution data of phenylalanine (Phe) derivatives[a]

Compound	CSP I			CSP II			CSP III			CSP IV		
	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]
DNB-Phe	17.34	6.96	D	11.43	8.05	L	5.39	4.96	D	14.12	5.22	L
B-Phe	9.27	1.57	D	6.74	1.70	L	4.12	1.50	D	7.93	1.58	L
Ac-Phe	4.89	1.26	D	3.52	1.38	L	2.49	1.25	D	3.22	1.31	L
Clac-Phe	7.17	1.26		5.88	1.38		3.41	1.22		4.94	1.32	
F-Phe	5.81	1.17		4.02	1.26		2.70	1.15		3.50	1.20	
DNP-Phe	39.24	1.30	L	27.45	1.23	D	7.32	1.39	L	20.26	1.43	D
DNPyr-Phe	33.31	1.07		28.32	1.28		11.24	1.05		31.11	1.19	
DBD-Phe	25.65	1.23		18.14	1.26		8.71	1.16		21.34	1.15	
Fmoc-Phe	26.47	1.42	D	19.37	1.46	L	5.71	1.37	D	9.61	1.38	L
Z-Phe	12.37	1.18	D	9.04	1.20	L	5.16	1.19	D	8.68	1.22	L
Boc-Phe	6.45	1.16	D	5.28	1.13	L	3.36	1.18	D	5.65	1.11	L
DNS-Phe	33.93	1.28	D	25.23	1.30	L	8.89	1.27	D	15.60	1.30	L

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 6; SO density: CSP I: 0.35 mmol g^{-1}; CSP II: 0.32 mmol g^{-1}; CSP III: 0.30 mmol g^{-1}; CSP IV: 0.32 mmol g^{-1}.

[b] Elution order: configuration of the first eluted enantiomer.

an aromatic amine tether. However, the hydrogen donor acceptor site seems to be of higher priority than the $\pi-\pi$ contact point, corresponding to the relative strength of the given interaction forces, and is therefore responsible for the different binding mechanism and the reversal of elution order with respect to the both derivatives (compare Fig. 7, A and D).

The simultaneous occurrence of hydrogen bonding and effective $\pi-\pi$ interaction substantially pushes up the enantioselectivity of DNB-Leu ($\alpha=6.83$ on CSP I, see Fig. 7A). However, the presented chiral discrimination systems seem to be very sensitive to steric modifications at or nearby the binding and/or interacting groups of the SA and the SO. Accordingly, the underlying chiral recognition mechanism for DNB-AAs is severely disturbed, if the small α-positioned proton of the Leu residue is replaced by a more space filling substituent like the methyl group. It should be pointed out that the steric constraints do

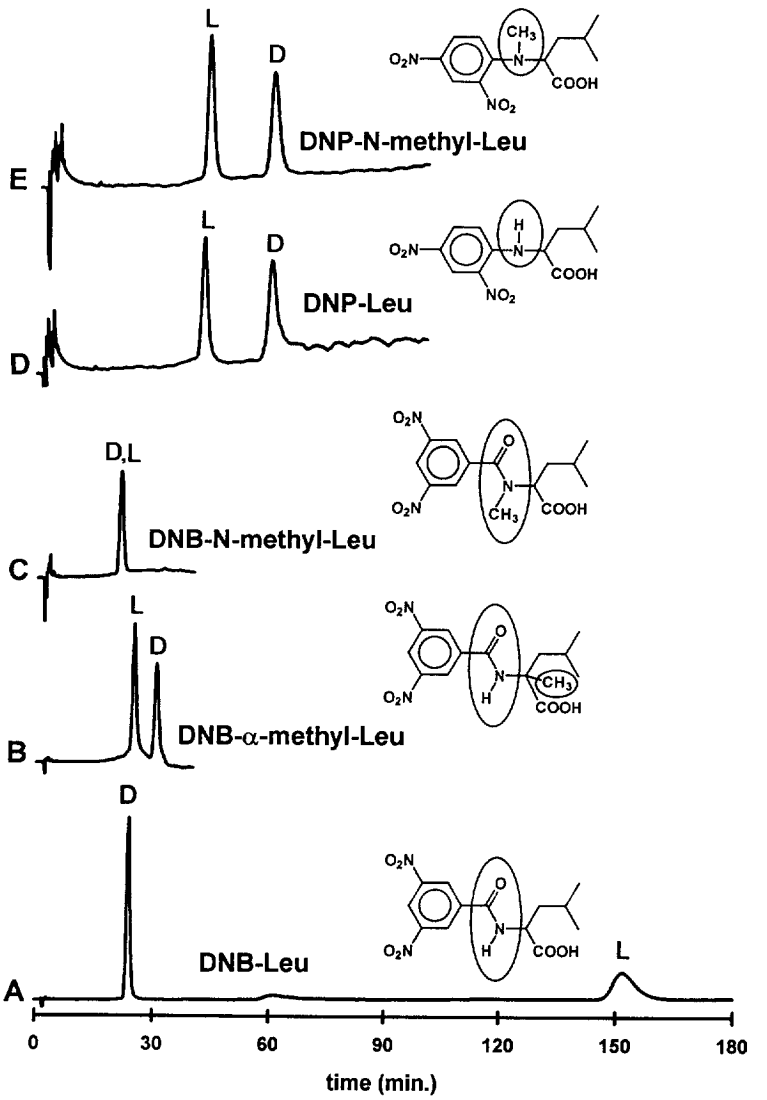


Fig. 7. Influence of N- and α-methylation on enantioselectivity of N-3,5-dinitrobenzoyl- (DNB-) and N-2,4-dinitrophenyl-leucine (DNP-Leu), respectively. Stationary phase: CSP I; chromatographic conditions: see Section 2; all chromatograms have the same time scale.

not only reduce the α-value dramatically (from 6.83 for DNB-Leu to 1.25 for DNB-α-methyl-Leu) but also leads to an inversion of the elution order (compare Fig. 7, A and B). On the other side, replacement of the amido hydrogen of DNB-Leu by a methyl group provokes a complete loss of stereoselectivity (see Fig. 7C). This indicates the importance of hydrogen bonding for chiral recognition and suggests that the hydrogen bonding from the N-H of the SA amido group to the carbonyl of the SO carbamate arises stereoselectively. Thus, a chromatographically derived tentative chiral SO-SA recognition model may be drawn for DNB-AAs (see Fig. 8) which could also be applied for structural homologues. That the $\pi-\pi$ interaction of the DNB-group with the quinoline π-base of the SO must be of stereoselective nature may be deduced from the dramatic diminution of enantioselectivity by exchanging the strong π-acidic DNB group against the much weaker π-acidic benzoyl (B) group (compare α-values of DNB-Phe and B-Phe from Table 2, e.g., on CSP II 8.05 for DNB-Phe versus 1.70 for B-Phe). Nevertheless, it should be emphasized that a $\pi-\pi$ binding site within the SA is not an essential prerequisite. If a well-disposed hydrogen donor–acceptor group (amide, carbamate, sulfonamide group, etc.) is provided by the analyte, these CSPs possess effective stereodiscriminating potential. Thus, on these CSPs aliphatic amino acids are also resolvable, as their acetyl (Ac), chloroacetyl (Clac), formyl (F) and *tert.*-butoxycarbonyl (Boc) derivatives. The rigid hydrogen donor–acceptor group (amido and carbamoyl group, respectively) already supplies two contact points between SA and SO of which one may arise stereoselectively and by which the model of multiple interaction points for efficient stereodiscrimination may be fulfilled. As an example, Fig. 9, A depicts the baseline separation of N-acetyl-valine (Ac-Val).

On the other side, the N-H hydrogen of N-aryl amino acids, i.e., 2,4-dinitrophenyl (DNP), 3,5-dinitro-2-pyridyl (DNPyr), 4-dimethylaminobenzo-2,1,3-oxadiazol-7-yl (DBD) amino acids (for structures see Fig. 6), does not participate on the chiral discriminating mechanism and therefore also secondary amino acids, e.g., N-methyl-leucine (N-methyl-Leu), proline (Pro), etc. can be fractionated into enantiomers as N-aryl derivatives. Consequent-

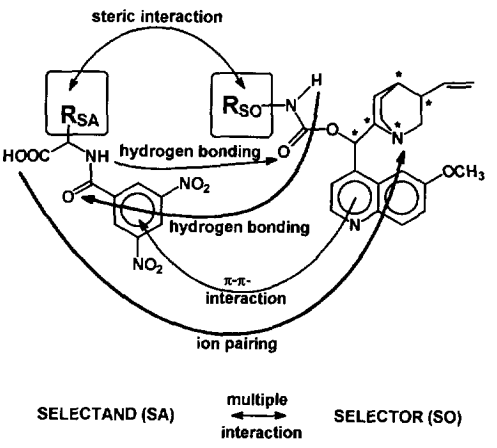
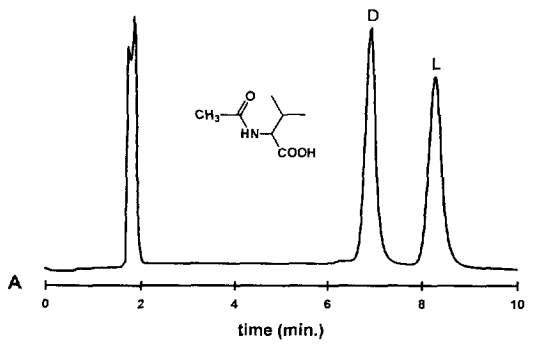


Fig. 8. Tentative chiral selector-selectand recognition model for N-3,5-dinitrobenzoyl amino acid derivatives.



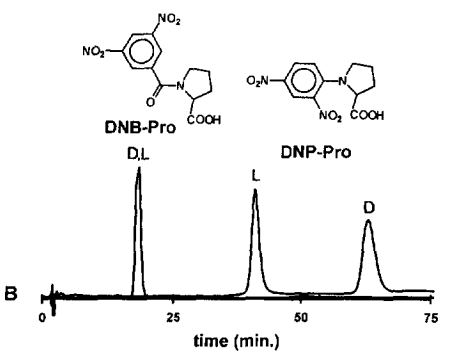


Fig. 9. Chromatograms of the enantioseparation of diverse N-derivatized amino acids on CSP I. Chromatographic conditions: see Section 2. (A) N-acetyl-valine (Ac-Val), (B) proline (Pro) as 3,5-dinitrobenzoyl (DNB) and 2,4-dinitrophenyl (DNP) derivatives, respectively.

ly, N-methyl-Leu and Leu are well resolved as DNP-derivatives nearly with the same α-value (see Fig. 7, D and E). Fig. 9, B clearly outlines the advantage of the DNP-protecting group over the DNB-derivative for secondary amino acids like Pro: DNB-Pro could not be resolved, whereas baseline separation could easily be achieved for the DNP-Pro racemate.

Aryl and alkyloxycarbonyl amino acid derivatives, as fluorenylmethoxycarbonyl (Fmoc), benzyloxycarbonyl (Z, Cbz), *tert.*-butoxycarbonyl (Boc), and arylsulfonyl amino acids, as dansyl (DNS), supplement the broad spectrum of resolvable amino acid derivatives. They possess also a hydrogen donor-acceptor binding site for hydrogen bonding and/or dipole stacking with the SO carbamate functionality and thus they behave quite similarly as their amido congeners, indicated by the same elution order. This suggests an analogous chiral recognition mechanism.

Briefly summarizing the above discussed findings, quinine and quinidine carbamate CSPs possess stereodiscriminating potential for α- as well as for β-amino acid derivatives. For primary amino acids the DNB, B, Ac, Clac, F, Fmoc, Z, Boc, DNS, DNP, DNPyr, DBD derivatives are well suited whereas secondary amino acids are only resolvable as DNP, DNPyr, DBD and similar N-aryl derivatives.

3.5. Influence of side chain of N-derivatized amino acids on retention and enantioselectivity

Involving ionic interaction and additional contact points of the protecting group (hydrogen bonding, dipole stacking) the SA is locked into the binding cleft of the SO. The type of side chain of the AA has a modifying effect on overall retention and enantioselectivity owing to their different lipophilicity and bulkiness but also functionality. The possibility for additional (competing, non-competing or supporting) interactions of functional groups of the side chain, which may also bind non-selectively to the SO, usually results in a poorer enantiomeric separation. Commonly, the elution order of the two enantiomers remains the same within a series of homologous derivatives. However, this general rule is occasionally disordered. If the side chain possesses a functional group of higher priority (i.e., stronger interaction) than the protecting group, it may force a reorientation of the SA enantiomers at the SO binding domain and/or a competing binding mechanism. This could be the case, for instance, for aspartic acid derivatives where we noticed a reversal of the elution order.

The influence of the amino acid residue should be illustrated for a series of DNB-congeners (see Fig. 10). A plot of the log k' values against the respective α-value illustrates some pattern or trends of chiral recognition. The log k' values of the less strongly bound enantiomers (first eluted antipodes) are more or less clustered around a constant retention increment of the DNB-amino acid's fundamental backbone. This is mostly caused by ionic interaction, and contains also the non-stereoselective contribution of hydrophobic interaction, of hydrogen bonding of the amide linkage together with eventually occurring π-π-interaction of the DNB group. Only the two aromatic AAs of the chosen set, phenylalanine (Phe) and phenylglycine (Phegly), show higher retention (see Fig. 10, especially indicated by arrows) due to an additional $\pi-\pi$ interaction increment. For the Phe-derivative, this additional retention increment is approximately as large for the first as well as for the second eluting enantiomer, whereas for the Phegly-analogue the additional $\pi-\pi$ interaction increment for the first eluting enantiomer is about twice as large as for the second peak, leading to an overall decrease in enantioselectivity. If only aliphatic amino acids, featuring basically the same retention mechanism, are considered (excluding the aromatic AAs as Phe and Phegly), it is no surprise that the log α values significantly correlate with the log k'_2 values ($r^2=$ 0.9889). As a general trend: the higher the lipophilicity and steric bulk of the side chain the higher the retention of the second eluted antipode and consequently the obtained α-values will be. It seems that the more strongly retained enantiomers of DNB-AAs match the SO's geometric requirements (complementary functionalities including shape and conformation) exceptionally well. Hence, α-values up to 10 could be obtained. The unlike accessibility of the binding site for the individual SA enantiomers is, however, forced by rigid and bulky elements in the SA and SO. Interestingly, SAs with a short non-bulky side chain residue, which should have better steric accessibility towards the binding site of the sterically bulky SO, have smaller k'_2 and α-values. However, increasing the size and bulkiness of the

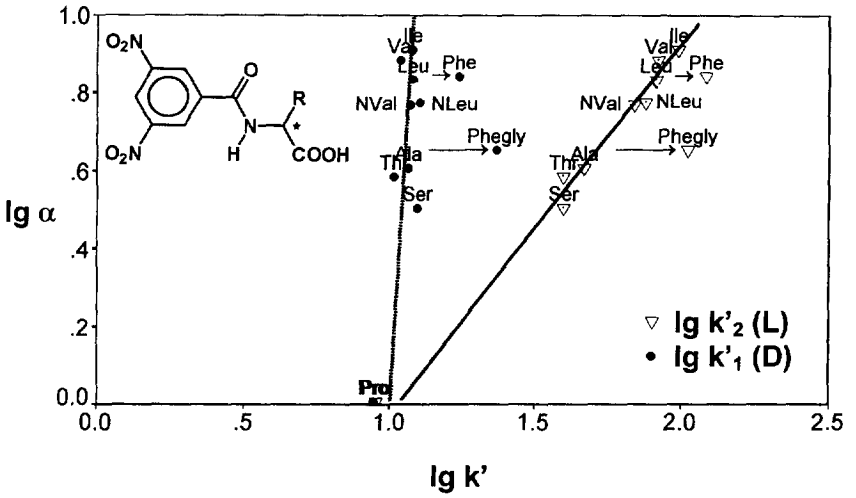


Fig. 10. Influence of N-3,5-dinitrobenzoyl-amino acids side chains on retention and enantioselectivity: Plot of log α values versus the respective log k' values of a series of DNB-amino acids. Coefficient of linear correlation of log α with log k'_2 not considering Phe and Phegly: $r^2=0.9889$. Stationary phase: CSP I, chromatographic conditions: see Section 2.

side chain leads to an increase of the retention of the second eluted antipodes. Therefore, α-values increase in the order: hydroxmethyl (serine, Ser: $\alpha=3.19$), 1-hydroxyethyl (threonine, Thr: $\alpha=3.85$) and methyl (alanine, Ala: $\alpha=4.06$), *n*-propyl (norvaline, NVal: $\alpha=5.89$), isopropyl (valine, Val: $\alpha=7.67$) and *n*-butyl (norleucine, NLeu: $\alpha=5.96$), isobutyl (leucine, Leu: $\alpha=6.83$), *sec.*-butyl (isoleucine, Ile: $\alpha=8.16$). Possibly, the rate of dissociation of the more stable SO-SA-complex is reduced by voluminous residues or the spatially pretentious requirements of the SA evoke an "induced fit" of the SO.

3.6. Comparison of the four CSPs with respect to the different binding modes

Generally, all four CSPs (with comparable selector coverage) provide reasonable enantioselectivity for aryl-, aryloxy-, arylthiocarboxylic acids, derivatized amino acids and several other chiral acids (see Table 2 and Table 3). CSP III and IV are less lipophilic than CSP I and II due to different spacer type and length, and the distinct lipophilized surface (different modes of endcapping) of the silica matrix. Apparently, non-enantioselective lipophilic interaction is sup-

Table 3
Chromatographic retention and resolution data of diverse acidic analytes on the four CSPs I–IV[a]

Compound	CSP I			CSP II			CSP III			CSP IV		
	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]	k'_1	α	Elution order[b]
13	4.83	1.05	*S*	3.42	1.06	*R*	3.64	1.08	*S*	4.13	1.09	*R*
14	8.42	1.06	*S*	5.62	1.06	*R*	4.14	1.07	*S*	6.06	1.08	*R*
15	7.41	1.04		4.58	1.10		3.19	1.13		5.80	1.16	
16	11.80	1.26	*S*	7.85	1.41	*R*	4.73	1.60	*S*	9.77	1.72	*R*
17	13.03	1.85		8.18	2.19		4.51	2.53		11.25	2.66	
18	25.02	1.64		6.59	2.22		7.78	2.04		18.41	2.23	
19	6.65	1.12		5.69	1.08		3.40	1.00		3.98	1.05	
20	8.58	1.12		7.55	1.22		3.73	1.11		4.27	1.13	

[a] For chromatographic conditions see Section 2, for structures of analytes see Fig. 3; SO density: CSP I 0.35 mmol g^{-1}; CSP II: 0.32 mmol g^{-1}; CSP III: 0.30 mmol g^{-1}; CSP IV: 0.32 mmol g^{-1}.

[b] Elution order: configuration of the first eluted enantiomer.

pressed on CSP III and IV resulting in somewhat higher α-values for lipophilic analytes as aryl-, aryloxy- and arylthiocarboxylic acids (see Table 3) and also for the relatively lipophilic DNP-amino acids (see Table 2) on the quinine based CSP III, compared to the corresponding quinine based CSP I, and also on the quinidine based CSP IV, compared to the corresponding quinidine based CSP II, respectively. On the other side, N-derivatized amino acids, which possess a hydrogen donor-acceptor group or other relative polar groups, may also develop non-enantioselective interactions to the silica matrix preferentially to the more polar CSPs, III and IV. Therefore, somewhat lower α-values could be observed on CSP III and IV compared to the corresponding, more lipophilic and/or better endcapped CSP I and II.

The α-aryl propionic acids, profens (13, 14), are relatively poorly resolved, since they possess only a α-basic aromatic group but no additional polar interstrand between the stereogenic center and the aromatic group for an additional hydrogen-bonding and/or dipole interaction as it is the case for the structurally related aryloxy- and arylthiocarboxylic acids (compare Table 3, entity 13 and 15). If such interaction sites are accessible, especially in combination with a π-acidic aromatic group (16, 17, 18), pronounced enantioselectivity arises. If the geometric arrangement of the binding sites of the SA fits reasonably well the shape of the SO binding domain, already weak interactions are sufficient to get baseline separation of the enantiomers as demonstrated for 3,4-dihydro-2*H*-pyran-2-carboxylic acid (19) (see Fig. 11). On the other side, etodolac (20) combines favorable functionalities for SO-SA interaction as an aromatic group for $\pi-\pi$ interaction and

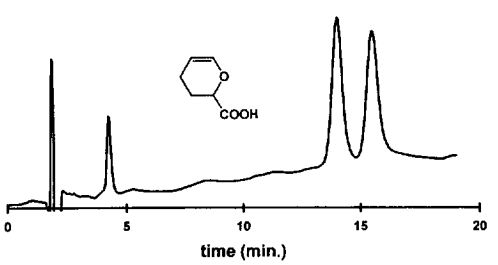


Fig. 11. Separation of 3,4-dihydro-2*H*-pyran-2-carboxylic acid enantiomers on CSP I. Chromatographic conditions: see Section 2.

an α-positioned oxygen (relative to the stereogenic center) for hydrogen bonding or dipole-dipole interaction. The arrangement of these groups as well as steric constraints (bulky ethyl substituent on stereogenic center, as earlier stressed in case of DNB-α-methyl-Leu) does not seem optimal. Nevertheless, baseline separation could still be achieved.

Without any exception, the corresponding quinine and quinidine CSPs exhibit opposite elution order. The former mentioned term "pseudo-enantiomers" still seems to be appropriate for both alkaloids, as well as for the corresponding carbamate derivatives and the resultant CSPs.

4. Conclusion

Quinine and quinidine carbamate based CSPs successfully proved their resolving capability in the anion exchanging mode for a broad spectrum of acidic compounds using buffered aqueous mobile phases. These CSPs can be readily prepared from inexpensive natural products and are stable over month when operated with commonly used mobile phase pH (4–8) and buffer concentrations. The spectrum of resolvable racemates is, however, restricted to acidic compounds. The lack of acidity in analytes may be overcome by the introduction of an acidic group through derivatization (or by changing to the normal-phase mode, where these CSPs operate with different retention mechanisms according to the Pirkle concept). For the retention and separation processes, ionic interaction is the driving force for prime molecular association but additional molecular increments accessible for intermolecular SO-SA binding are important to obtain reasonable enantioselectivity.

Retention can be easily adjusted by the ionic strength of the running buffer of the aqueous mobile phase without any remarkable influence on enantioselectivity. Usually, the respective quinidine carbamate type CSPs develop somewhat higher stereoselectivity than the corresponding quinine CSPs, and without any exception, elute the investigated SA enantiomers in opposite order, according to the "pseudo-enantiomeric" behavior of the two alkaloids.

The possibility of a specific and dedicated de-

rivatization of the diverse functional groups of the cinchona alkaloids' selector backbone opens a wide field of selector modification to optimize overall stereoselectivity, to enlarge the spectrum of resolvable racemates and to deduce SO-SA recognition relationships. Along this line, further investigations are currently being undertaken.

Acknowledgments

The authors thank Johannes Lämmerhofer and Paolo di Eugenio for their highly appreciated and skilful accomplishment of the experimental work. Also we would like to acknowledge the financial support of the Austrian "Fonds zur Förderung der wissenschaftlichen Forschung" FWF project number P8898.

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