HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF AMINO COMPOUNDS ON CHIRAL STATIONARY PHASES

Anita Sztojkov-Ivanov

Ph.D. Thesis

Szeged
2008

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF AMINO COMPOUNDS ON CHIRAL STATIONARY PHASES

Anita Sztojkov-Ivanov

Ph.D. Thesis

Supervisors:
Prof. Ferenc Fülöp, Ph.D., D.Sc.
Prof. Antal Péter, Ph.D., D.Sc.

Institute of Pharmaceutical Chemistry, University of Szeged
2008

CONTENTS

ABBREVIATIONS AND SYMBOLS

(v/v)	Volume to volume ratio
AcOH	Glacial acetic acid
CD	Cyclodextrin
CDA	Chiral derivatizing agent
CE	Capillary electrophoresis
CMA	Chiral mobile phase additive
CSP	Chiral stationary phase
DANI	1,3-Diacetoxy-1-(4-nitrophenyl)-2-propylisothiocyanate
DEA	Diethylamine
DNB	3,5-Dinitrobenzoyl
Et	Ethyl-
EtOH	Ethanol
FDA	US Food and Drug Administration
GC	Gas chromatography
GITC	2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate
HPLC	High-performance liquid chromatography
IPA	2-Propanol
*i*Pr	Isopropyl-
m	*meta*-
Me	Methyl-
MeCN	Acetonitrile
MeO	Methoxy-
MeOH	Methanol
NIFE	*N*-(4-Nitrophenoxycarbonyl)phenyl-alanine methoxyethyl ester
NP	Normal-phase
o	*ortho*-
OPA	*o*-Phthalaldehyde
p	*para*-
Ph	Phenyl-
PI	Polar-ionic
PO	Polar-organic
Pr	Propyl-
RP	Reversed-phase
*t*Bu	*Tert*-butyl-
TEA	Triethylamine
TEAA	Triethylammonium acetate
TFA	Trifluoracetic acid
TLC	Thin-layer chromatography

k'	Retention factor; defined as $(t_R-t_0)/t_0$; t_R, retention time; t_0, column dead-time
α	Separation factor; defined as k'_2/k'_1; 1: first- and 2: second-eluting peak
R_S	Resolution; defined as $2(t_{R2}-t_{R1})/(w_1+w_2)$; w, peak width measured on the baseline

1. INTRODUCTION

1.1. The importance of enantiomer separation

The separation of enantiomers of chiral compounds by chromatographic methods and related techniques is one of the most important tasks in modern analytical chemistry, especially in the analysis of compounds of biological and pharmaceutical interest.

On a molecular level of living organisms, chirality is an important property of the building blocks of life, such as amino acids and sugars, and therefore of peptides, proteins and polysaccharides. In biological systems, these biomolecules exist in only one of the possible enantiomeric forms and display different responses to one of a pair of enantiomers of drugs, agrochemicals, food additives, fragrances, *etc*. Stereoselectivity is often a characteristic feature of enzymatic reactions, messenger-receptor interactions and metabolic processes.

Accordingly, chirality is also a major concern in the pharmaceutical industry. When the enantiomers of a drug are administered into a chirally selective living system, these enantiomers often exhibit differences in bioavailability, distribution, metabolic and excretion behavior, and action. One of the enantiomers is often the more active stereoisomer for a given action (eutomer), while the other, less active one (distomer) may either contribute side-effects, display toxicity or act as an antagonist [1,2]. The differences in biological properties of enantiomers arise from the differences in protein transport and binding, the kinetics of their metabolism and their stability in the environment [3-5]. A tragic case in point is the racemic drug *n*-phthalylglutamic acid imide, which was marketed in the 1960s as the sedative Thalidomide (Contergan). Exclusively the *R*-(+) enantiomer possesses therapeutic activity; the *S*-(+) enantiomer is teratogenic, as proved in rats [6].

As a result, the pharmaceutical industry has raised its emphasis on the generation of enantiomerically pure compounds in the search for safer and more effective drugs. The US Food and Drug Administration (FDA) has required evaluation of each enantiomer in the development of stereoisomeric drugs [7]. According to the survey of Caner *et al*. [8], the distributions of wordwide-approved drugs from 1983-2002 and FDA-approved drugs from 1991-2002 indicate that single enantiomers surpassed achirals, and racemic drugs accounted for only the minor part.

Single enantiomers can be obtained via (*a*) the selective synthesis of one enantiomer or (*b*) the separation of racemic mixtures. Stereoselective syntheses are rarely selected for large-scale separations, particularly at the early stages of development of new drugs in the pharmaceutical industry because they are both expensive and time-consuming. The

enantiomers of a racemic mixture can be separated *indirectly* when diastereomer pairs are formed covalently; their separation can be achieved by taking advantage of their different chemical or physical properties on crystallization, nonstereoselective chromatography or distillation. Such methods are frequently used, especially for preparative-scale separations. Alternatively, *direct* processes are based on the formation of noncovalent diastereomeric pairs of molecules. These methods yield the enantiomerically pure substances and are generally the most advantageous. The direct resolution of enantiomers can be achieved by interaction of a racemic mixture with a chiral selector, either a part of a chiral stationary phase (CSP) or as a chiral mobile phase additive (CMA). On the preparative scale, enantioseparation can also be achieved by kinetic resolution procedures, with enantioselective membranes or simulated moving bed techniques. Biotransformations involving the use of enzymes are of recognized importance in industrial technologies and will multiply in the future.

To control the enantiomeric purity of starting materials and products, reliable and accurate analytical methods are necessary. At an analytical level, sensitivity and selectivity are important requirements in many fields of academic, industrial and pharmaceutical research. Accordingly, several techniques should be mentioned as being of particular value for analytical purposes: gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical liquid chromatography, capillary electrophoresis (CE), capillary electro chromatography and thin-layer chromatography (TLC). HPLC is the most widespread chiral separation technique in analytical and preparative resolutions and drug discovery.

Chromatographic chiral separation dates back to 1939 when Henderson and Rule demonstrated the separation of *d,l-p*-phenylenediiminocamphor on *d*-lactose [9]. Kotake *et al*. in 1951 [10] and Dalgliesh in 1952 [11] applied paper chromatography, using a cellulose support to separate chiral amino acids. Dalgliesh first correctly attributed enantioselectivity to adsorption and suggested the three-point rule for asymmetric recognition. This rule was later restated by Pirkle and Pochapski [12] as “Chiral recognition requires a minimum of three simultaneous interactions, with at least one of these interactions to be stereochemically dependent.” In 1960, Klemm and Reed [13] first reported the use of a silica gel support for chiral HPLC separation. Racemic naphthyl ether and phenanthrene were partially separated on silica gel. The first successful GC direct enantiomeric separation was reported by Gil-Av *et al*. in 1966 [14]. Enantiomers of a drug (the above-mentioned Thalidomide) were first separated by liquid chromatography on chiral stationary phases by Blaschke *et al*. [6].

1.2. Aims

The primary aim of this work was to develop simple, convenient, readily available chiral HPLC methods that are suitable not only for the separation of the enantiomers of racemic aminonaphthols and *β*-amino acids, but also for monitoring their synthesis and for determining their absolute configuration.

The first objective was to develop systematic methods for the optimized resolution of different *α*-aminobenzyl- and *α*-aminoalkyl 1- and 2-naphthol analogs on 3,5-dimethylphenyl carbamoylated cellulose- and *β*-cyclodextrin (CD)-based CSPs. In order to investigate the thermodynamic functions of enantioselective adsorption, the effects of temperature on the chromatographic parameters were investigated on a cellulose *tris–3,5–διμετηψλπηενψλ χαρβαμοψλατεδ ΧΣΠ.*

[illegible] *β*-substituted-*β*-amino acids (*β*-homoamino acids) on CSPs containing macrocyclic antibiotics and chiral crown ether.

The influence of different parameters, such as the nature and composition of the mobile phases and mobile phase modifiers, the buffer concentration, pH, *etc*. on chromatographic separations were extensively investigated for both substituted aminonaphthol analogs and *β*-amino acids. The effects of the structural features of the investigated analytes on the discrimination between the enantiomers were examined through the chromatographic parameters (retention, selectivity and resolution).

2. A LITERATURE REVIEW OF CHROMATOGRAPHIC METHODS FOR RESOLVING ENANTIOMERIC AMINO COMPOUNDS

The physicochemical properties of the enantiomers of racemates are identical in an achiral environment. The discrimination of enantiomers is required to generate a chiral environment. In this respect, the procedures used for chromatographic enantioseparation can be classified as either indirect or direct methods. *Indirect separation* is based on the use of chiral derivatization reagents to form diastereomeric derivatives which differ in their chemical and physical behavior and which can therefore be separated on achiral stationary phases. *Direct methods* involve two techniques; the enantiomers are separated either on achiral stationary phases by means of CMAs or on CSPs.

2.1. Indirect chromatographic methods

The application of chiral derivatizing agents (CDAs) was the first method widely used for the enantioseparation of optically active molecules in liquid chromatography. They have been applied from the early 1970s up to the present time. After the introduction of newer chromatographic techniques, the importance of separations based on covalent chiral derivatization has naturally decreased to some extent. However, this general method is still frequently used in HPLC. The reasons for this are the large number of commercially available homochiral derivatizing reagents and well-established reactions leading to diastereomer pairs with appropriate separation and detection possibilities and the use of less expensive achiral columns.

Labeling with CDAs is carried out by reaction with a functional group in the analyte, *e.g*. amine [15], carboxy [16,17], carbonyl [18], hydroxy [19] or thiol [20]. The characteristics and applications of the individual reagents for various functional groups have been widely described in numerous scientific contributions, but in this thesis the indirect approach will be overviewed in terms of the amino compounds with regard to all of the analytes discussed in the following sections that possess an $–NH_2$ group.

The important requirements, and the main advantages and disadvantages of chiral derivatization reactions and reagents are summarized in Table 1.

Table 1.
Comparison of advantages and disadvantages of indirect chromatographic method

Advantages	Disadvantages
1. Good chromatographic properties of derivatives	1. The purity of the CDA is critical
2. Elution sequence predictable	2. The molar absorptivities of the diastereomers may differ
3. Good chromophoric or fluorophoric properties of the reagent (enhanced sensitivity can be achieved)	3. Possibility of racemization
4. Low cost of achiral columns	4. Possibility of kinetic resolution
5. Method development is simple	5. The excess of reagent and side-products may interfere with separation
6. Selectivity can be increased (better separation is often achieved than with a direct method)	6. Preparative application is restricted
7. Possibility of appropriate selection of the elution sequence	7. Derivatization may be time-consuming

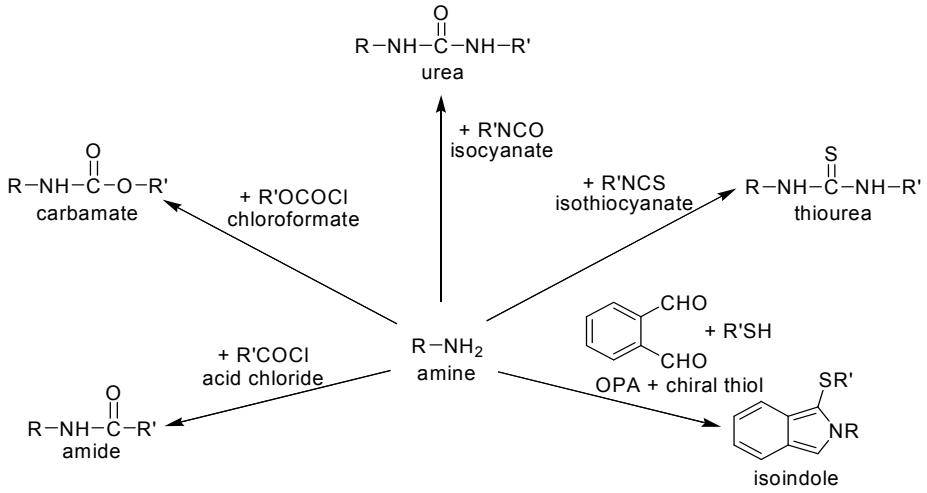
There is no denying the fact that the direct chromatograpic enantioseparations based on CSPs have greatly overshadowed the importance of indirect separations based on covalent chiral derivatization. In some areas (*e.g*. the determination of small concentrations of D-amino acids in various biological samples), the well-established classical indirect methods are still in use.

2.1.1. Derivatization of the amino group

Many biochemically important compounds, such as amines, amino alcohols, amino acids and drugs, have at least one amino functional group in their structure. Among various functional groups, the tagging reactions of primary and secondary amines are unquestionably of major significance. The derivatization reactions for chiral amines are mainly based on the formation of amides, carbamates, ureas and thioureas. Only the most important members of the CDA types are shown in Scheme 1.

The reactions with carboxylic chlorides proceed rapidly to produce the corresponding carboxamides, which are separable on HPLC columns. The classical reagent, Mosher's acid chloride ((*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride), is still in use for GC separations. Some 'dual-purpose' variation of carboxylic chlorides with strong UV adsorption and fluorescence allows a reduction of the detection limit: Spahn and colleagues first reported

the application of (*S*)-(+)-2-(*p*-chlorophenyl)-α-methylbenzoxazole-5-acetyl chloride (benzoxaprofen chloride) [21,22], (*S*)-(+)-2-(*p*-fluorophenyl)-α-methylbenzoxazole-5-acetyl chloride (flunoxaprofen chloride) [23,24] and (*S*)-(+)-2-(6-methoxy-2-naphthyl)propionyl chloride (naproxen chloride) [25,26] in the indirect enantiomeric separation of amino compounds. Toyo'oka and Liu developed optically active fluorescent reagents with the 2,3,1-benzoxadiazole structure of acid chloride and isothiocyanate types [27], which were applied by Jin *et al*. to separate racemates of D,L-amino acids in foodstuffs [28]. Another amide formation is the reaction with *N*-succinimidyl ester, which is a fairly stable reagent and can be used in aqueous media [29].



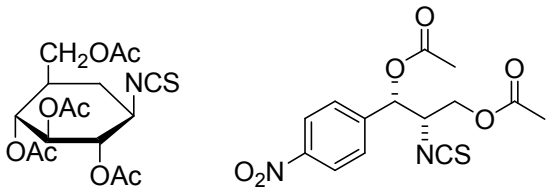
Scheme 1.
Possible chiral derivatization reactions for amino groups (both R and R' contain a chiral center)

A widely used reagent of the chloroformate type, (–)-menthyl chloroformate, is a classical CDA for the transformation of amines into carbamates [30,31]. (+)-1-(9-Fluorenyl)ethyl chloroformate is a fluorescence labeling reagent developed by Einarsson *et al*. [32]. The reaction conditions at room temperature in basic solution are mild and the resulting diastereomers are stable. Other highly fluorescent derivatization reagents are 9-fluorenylmethyl chloroformate-L-phenylalanine and 9-fluorenylmethyl chloroformate-L-proline [33].

Isocyanates form the corresponding urea derivatives with both primary and secondary amines. (*R*)-(–)- and (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate [34,35], (*R*)-α-methylbenzyl isocyanate [36] and (*R*)-(+)- and (*S*)-(–)-phenylethyl isocyanate [37,38] are among the most frequently used CDAs of this type. The derivatization reactions generally require more time and a higher temperature than reactions with other CDAs.

Isothiocyanates react with primary and secondary amines to form thiourea derivatives. The reactions are rapid and clean, and also selective for derivatization of the amino function.

The thiourea moiety imparts significant UV light-absorbing properties to the derivatives. Nimura *et al*. first applied 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) (Fig. 1) to the HPLC analysis of amino acids 20 years ago, initiating the breakthrough of isothiocyanate CDAs in HPLC [39]. Similar reagents, such as the GITC isomer 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl isothiocyanate [40], 2,3,4-tri-*O*-acetyl-*α*-D-arabinopyranosyl isothiocyanate [41] and 2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl isothiocyanate [42] have also been reported in drug analysis. Kleidernigg *et al*. [43] synthetized three isothiocyanate-based chiral *trans*-1,2-diaminocyclohexanes to separate a number of *β*-blockers. Other labels having the aromatic structure as a fluorophore are 1-phenylethyl- and 1-(1-naphthyl)ethyl isothiocyanate [44]. (1*S*,2*S*)- and (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate [(*S*,*S*)- and (*R*,*R*)-DANI] (Fig. 1) were developed by Péter *et al*. [45].



GITC (*S*,*S*)-DANI

Figure 1.
Structures of isothiocyanate derivatizing reagents

From the group of *N*-haloarylamino derivatives, Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alaninamide) (Fig. 2) is one of the most generally used reagents in the analysis of amino acids and peptides [46]. This reagent belongs in the family of Sanger reagents. Because of the strong electron-withdrawing activity of the dinitro groups on the aromatic ring, the derivatization generally proceeds under mild conditions, but the hydrolysis product of the CDA appears as an interfering peak in the chromatogram. Brückner and Gah developed a number of new reagents analogous to Marfey's reagent [47].

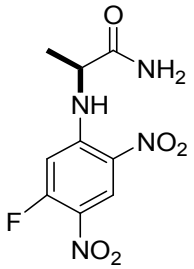


Figure 2.
Structure of Marfey's reagent

The dual derivatization reaction of *o*-phthalaldehyde (OPA) and chiral thiols leading to fluorimetrically highly active isoindole derivatives is another generally used method in the chiral analysis of amino acids, biogenic amines, amino alcohols and drugs. It can be routinely used in automated reversed-phase (RP)-HPLC analyses, in spite of the fact that the chiral amines are unstable. Examples of thiol reagents are *N*-acetyl-L-cysteine [48], *N*-acetyl-D-penicillamine [49,50], *N*-*t*Boc-L-cysteine [49,51], 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-glucopyranosyl isothiocyanate [52] and *N*-isobutyryl-L-cysteine [53].

A new "active uretane type" CDA, (*S*)-*N*-(4-nitrophenoxycarbonyl)phenyl-alanine methoxyethyl ester ((*S*)-NIFE, Fig. 3) was developed by Péter *et al*. for the enantioseparation of sterically constrained amino acids [54,55]. Derivatization was carried out rapidly under mild conditions and the urea derivatives formed were stable for several weeks.

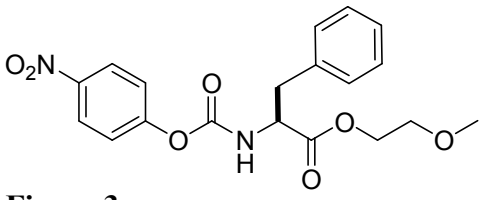


Figure 3.
Structure of (*S*)-NIFE

2.2. Direct chromatographic methods

2.2.1. Chiral mobile phase additives

Enantiomers can be resolved on conventional achiral stationary phases by adding an appropriate chiral selector to the mobile phase. Since the advent of chiral eluents introduced by Hare and Gil-Av [56] and LePage *et al*. [57], several approaches involving the application of different CMAs have been accomplished. These additives can be CDs, chiral crown ethers, chiral counter-ions or chiral ligands which are capable of forming ternary complexes with the solute enantiomers in the presence of a transition metal ion [58].

2.2.2. Chiral stationary phases

The 1980s proved to be a major turning-point in the field of enantiomer separation in HPLC. A tremendous number of new and improved CSPs were introduced and accompanied by a corresponding increase in the number of publications in this area. CSPs can be grouped in several ways. Depending on their separation principles, the main classes are as follows: Pirkle CSPs, helical polymers, mainly cellulose and amylose, cavity phases, macrocyclic antibiotic phases and protein- and ligand-exchange phases.

2.2.2.1. Pirkle phases

Pirkle and Sikkenga [59] immobilized (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (previously used in magnetic resonance studies) on a silica support and separated the enantiomers of several π-acidic racemates, *e.g*. 3,5-dinitrobenzoyl (DNB) derivatives of amines, amino acids, amino alcohols, *etc*. [60]. Such stationary phases became the first commercially available CSPs in the early 1980s. *π*-Basic phases such as 1-aryl-1-

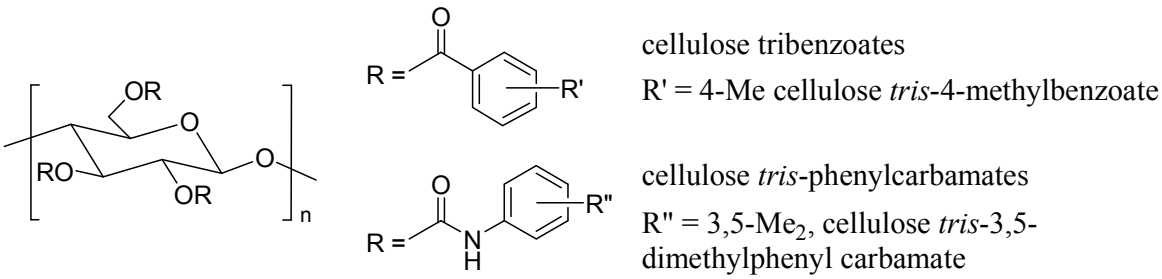
aminoalkanes, *N*-arylamino esters, phthalides, phospine oxides, *etc*. contain a π-electron-donor group and are expected to interact and resolve compounds bearing a π-electron-acceptor group. Typically, DNB and 3,5-dinitrophenylurea derivatives of amino acids and amines [61] were resolved on these CSPs. Among the DNB derivatives, phenylglycine exhibited large separation factors. This observation led to the investigation of DNB-phenylglycine as a π-acid-based CSP [62].

Finally, Hyun and Pirkle designed CSPs containing both π-acidic and π-basic sites [63]. These phases were expected to allow the efficient resolution of compounds containing appropriately located π-acidic and π-basic moieties.

2.2.2.2. Polysaccharide phases

Polysaccharides (especially cellulose and amylose) are naturally-occurring polymers; their derivatives were found to exhibit the ability of chiral recognition as CSPs.

Cellulose is a crystalline polymer composed of linear poly-β-D-1,4-glucoside residues which form a helical structure. Although crystalline cellulose displays chiral recognition, it does not yield practical CSPs. The reason for this is the poor resolution, and broad peaks are obtained due to slow mass transfer and slow diffusion through the polymer network. The highly polar hydroxy groups of cellulose often lead to nonstereoselective binding with the enantiomers of the analyte. Additionally, cellulose is unable to withstand normal HPLC pressures.



cellulose tribenzoates
R' = 4-Me cellulose *tris*-4-methylbenzoate

cellulose *tris*-phenylcarbamates
R" = 3,5-Me_2, cellulose *tris*-3,5-dimethylphenyl carbamate

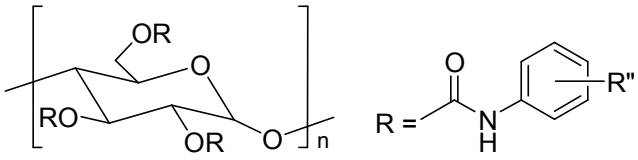
Figure 4.
Structures of derivatized cellulose CSPs

Derivatization of cellulose brings about practically useful CSPs which can separate a wide range of racemic compounds with high selectivities. Yashima and Okamoto [64,65] developed new types of CSPs by converting natural cellulose to a variety of derivatives, such as triesters and tricarbamates, by reaction at one or more of the hydroxy groups, and by coating the polysaccharide on a solid support, *e.g.* silica gel. Among the derivatives, cellulose *tris*-4-methylbenzoate and *tris*-3,5-dimethylphenyl carbamate (Fig. 4) exhibit excellent

resolving ability for a variety of racemic compounds and appear to be practically useful CSPs [66]. Recently, a series of phenylcarbamate derivatives containing both an electron-donating and an electron-withdrawing group on the phenyl moiety were prepared to perfect their chiral recognition abilities [67].

According to thermodynamic [68], nuclear magnetic resonance and mass spectroscopic studies and molecular mechanistic calculations [69], the general mechanism for chiral recognition on derivatized cellulose CSPs probably involves inclusion of the analytes into the chiral cavities created between sheets formed by the polymer chains and the attractive interactions which can occur with the solute through H-bonding, dipole-dipole or π-π interactions.

Amylose is a natural polymer possessing the same constituents as cellulose, but differing in the glycoside linkage, in this case a helical α-D-1,4-glucoside, and therefore amylose and cellulose have different higher-order structures. Derivatized amyloses exhibit similar behavior in discriminating racemic analytes as derivatized celluloses.



amylose *tris*-phenylcarbamates
R"= 3,5-Me_2, amylose *tris*-3,5-dimethylphenyl carbamate

Figure 5.
Structure of derivatized amylose CSP

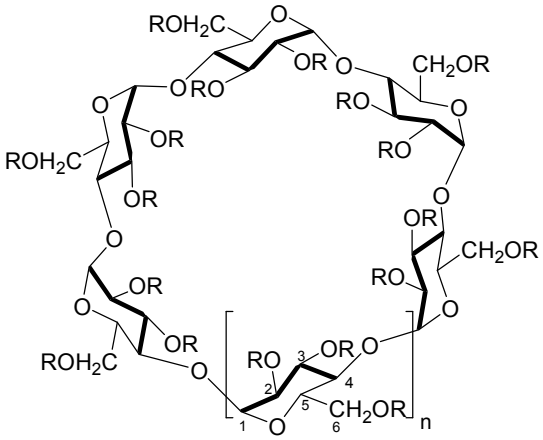
If the derivative group of an amylose and cellulose CSP is the same, the expected enantioseparating abilities of the two CSPs should be complementary [70]. Among the prepared derivatives of amylose, *tris*-3,5-dimethylphenyl carbamate (Fig. 5) shows excellent enantioselective properties toward a wide range of chiral compounds.

Polysaccharide CSPs can be used in both normal-phase (NP) and RP modes. Some solvents are prohibited as HPLC solvents, such as ethyl acetate, tetrahydrofuran, methyl *tert*-butyl ether, dichloromethane and chloroform, in which the polysaccharide derivatives themselves are dissolved or swollen; these solvents cannot be used as eluents [71].

2.2.2.3. Cavity phases

Chiral separations based on inclusion are achieved through a mechanism by which the guest molecule is accepted into the cavity in a host molecule. The exterior of the host molecule generally possesses functional groups that act as barriers or interact with the guest molecule in a fashion that includes enantioselectivity.

The most often used CSP of this type is CD bound to a silica support. CDs are cyclic oligomers with 6 (α-CD), 7 (β-CD) or 8 (γ-CD) D-glucopyranose units through an α-1,4-linkage that adopt a tapered cylindrical or toroidal shape (Fig. 6). The toroid has a maximum diameter ranging from 5.7 Å (α-CD) to 9.5 Å (γ-CD) with a depth of about 7 Å. The exterior of the toroid is relatively hydrophilic because of the presence of the primary C-6 hydroxy groups at the smaller rim of the toroid and the secondary C-2 and C-3 hydroxy groups at the opposite end. The internal cavity is hydroxy-free and hydrophobic, which favors the enantioseparation of partially nonpolar compounds via selective inclusion. Chiral discrimination by unmodified CDs has been intensively studied and extensively exploited, most notably through the work of Armstrong *et al*. in the development of CD-based CSPs [72].



α-CD: n=6; β-CD: n=7; γ-CD: n=8;

Figure 6.
Structures of α-, β- and γ-CDs

The modification of CDs and their complexation behavior involves substitution of one or more of the C-2, C-3 and C-6 hydroxy groups. The most commonly used derivatized CDs are sulfated, acetylated, permethylated, perphenylated, 2-hydroxypropylated, 3,5-dimethylcarbamoylated, and naphthylethyl-carbamoylated [73] CDs. Most of the studies involving CDs as CSPs in HPLC were accomplished in the RP mode, in the NP mode or in the polar-organic (PO) mode. Thus, CD-bonded CSPs are regarded as multi-modal phases.

Crown ethers are heteroatomic macrocycles with repeating (-X-C_2H_4-) units, where X, the heteroatom, is commonly oxygen, but may also be a sulfur or nitrogen atom. Unlike CDs, the host-guest interaction within the chiral cavity is hydrophilic in nature. Crown ethers, and especially 18-crown-6 ethers, can complex inorganic cations and alkylammonium compounds. This inclusion interaction is based mainly on H-bonding between the hydrogens of the ammonium group and the heteroatom of the crown ether. Additional electrostatic interaction occurs between the nitrogen and the crown ether's oxygen lone pair electrons. Crown ethers were first introduced as CSPs by Pedersen [74], Sousa *et al*. [75] and Shinbo *et al*. [76,77]. Recently, Machida *et al*. [78] and Hyun [79] designed covalently bonded CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

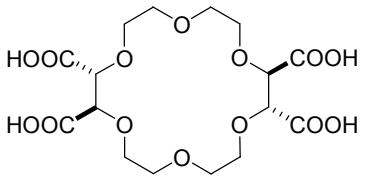
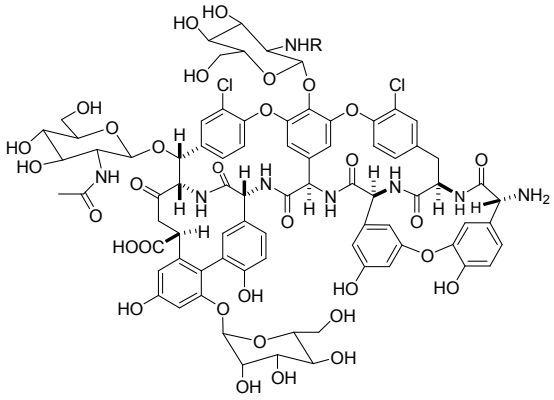


Figure 7.
Structure of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

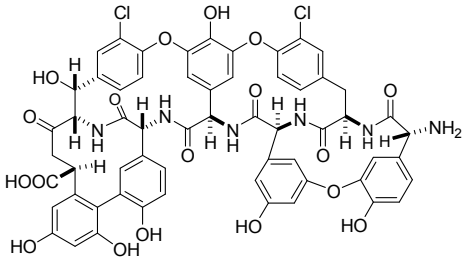
(Fig. 7). These CSPs were very successful in the resolution of various racemic primary amino compounds, including α- and β-amino acids [80,81], α-amino acid derivatives [82], racemic amines, amino alcohols [83] and secondary amines, including β-blockers [84]. The chiral recognition mechanism is not completely clear at present, but diastereoselective complexation of the primary ammonium group ($R\text{-}NH_3^+$) of the analyte enantiomers within the chiral crown ether cavity of the CSP is expected to be important [85].

2.2.2.4. Macrocyclic antibiotic phases

In the past decade, macrocyclic antibiotics have proved to be an exceptionally useful class of chiral selectors for the separation of enantiomers of biological and pharmaceutical importance by means of HPLC, TLC and CE. In 1994, Armstrong proposed their use in the separation of amino acid enantiomers [102]. The macrocyclic antibiotics are covalently bonded to silica gel via chains of different linkages, such as carboxylic acid, amine, epoxy or isocyanate-terminated organosilanes. This kind of attachment ensures the stability of the chiral selectors, while their chiral recognition properties are retained. All macrocyclic antibiotic stationary phases are multimodal CSPs, *i.e.* they can be used in NP, RP, PO or polar-ionic (PI) mode. The antibiotics used for chiral separations in HPLC include the ansamycins (rifamycins), the glycopeptides (avoparcin, teicoplanin, ristocetin A, vancomycin and their analogs) and the polypeptide antibiotic thiostrepton.



Teicoplanin



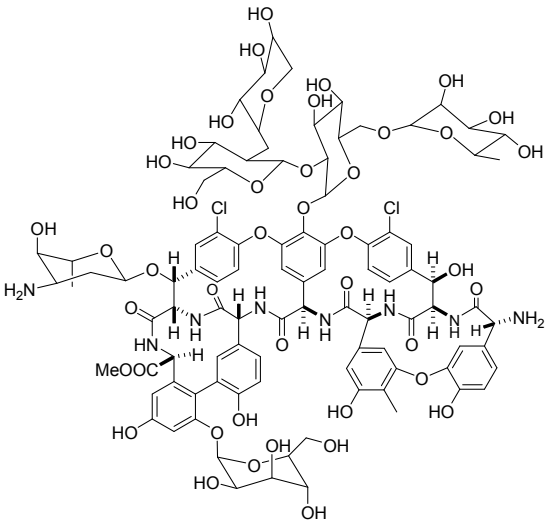
Teicoplanin aglycone

Figure 8.
Structures of the macrocyclic glycopeptide teicoplanin and teicoplanin aglycone

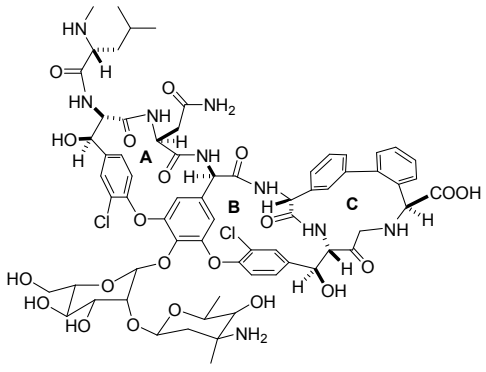
One of the most frequently used teicoplanin (Fig. 8) consists of four fused macrocyclic rings, which contain seven aromatic rings, two of them bearing a chlorine substituent, and the others having a phenolic character. The molecule possesses a carboxy and a primary amine group, which are responsible for the formation of a zwitterionic form in the pH range 3.5-8.1.

A D-mannose and two D-glucosamines are attached to the aromatic rings via ether bonds; one of the glucosamines contains an *N*-acyl hydrocarbon chain. Teicoplanin has 23 stereogenic centers. In recent years, a new teicoplanin-based CSP was introduced by Berthod *et al*. [103]: teicoplanin without sugar units, teicoplanin aglycone (Fig. 8). Berthod [103] obtained better resolutions for α-amino acids on teicoplanin aglycone than on teicoplanin. Péter *et al.* [104,105] reported similar observations in the separation of unusual α- and β-amino acids. It was concluded that sugar units hindered the chiral recognition by occupying space inside the "basket", which limits the access of other molecules to the binding sites. Additionally, sugar moieties block the possible interaction sites on the aglycone and also offer competing interaction sites (the latter may be advantageous in some cases).

The basic frame of ristocetin A (Fig. 9) is similar to that frame of teicoplanin: it consists of four macrocyclic rings with seven aromatic rings, to which two monosaccharides and a tetrasaccharide are covalently bonded. Thus, the number of stereogenic centers is increased to 38. An important difference between ristocetin and teicoplanin is that ristocetin A contains a methyl ester group instead of a carboxy group. In consequence of the similar structures, the interactions between the selectors and analytes are likewise similar, except that the separation of analytes with a positive charge is not favorable on the ristocetin A CSP because of the lack of a carboxy group [106].



Ristocetin A



Vancomycin

Figure 9.
Structures of the macrocyclic glycopeptide ristocetin A and vancomycin

Vancomycin (Fig. 9) exists as a mixture of similarly structured compounds; the parent component is vancomycin B. There are three macrocyclic portions in the molecule, which include five aromatic rings, two side-chains (a carbohydrate dimer and an *N*-methylamino acid) and two chlorine moieties besides the hydroxy and amino groups. There are 18

stereogenic centers in the macromolecule. The macrocyclic glycopeptides are to some extent complementary to one another: where partial resolution is obtained with one CSP, there is a high probability that better or baseline separation can be obtained with another [107].

Due to the number and variability of the functional groups and stereogenic centers, the above-mentioned chiral selectors can interact by electrostatic, hydrophobic, dipole-dipole, π-π interactions, H-bonding and steric repulsion, depending on the nature of the analyte and the mode of chromatography [108-110]. One of the most important interactions is ionic or charge-to-charge interaction [111].

2.2.2.5. Ligand-exchange phases

Introduced by Davankov and Rogozhin [86], chiral ligand-exchange chromatography is a widely used and sensitive method for separating enantiomers of amino acids, hydroxy acids, peptides, amino alcohols, alkaloids and β-blockers capable of chelating a divalent transition metal-ion through a pair of coordinating electronegative atoms. Separation is based on the formation of reversible diastereomeric complexes comprised of an immobilized bidentate chiral selector, a divalent transition metal cation (Zn(II), Ni(II) or Cu(II)) and either of the enantiomers to be resolved in the mobile phase. Usually, the commercially available ligand-exchange phases include immobilized derivatives of L-hydroxyproline [87,88], L-proline [89] or L-histidine [90].

2.2.2.6. Protein phases

Proteins are high-molecular mass polymers composed of chiral subunits (L-amino acids) and known to bind small molecules. A protein can be immobilized on a solid support by a variety of bonding chemistries; the choice of bonding chemistry affects the selectivity of the CSP obtained [91]. A milestone in this field was the introduction by Hermansson of α_1-acid glycoprotein chemically bonded to a silica particle support, which is a commercially available column [92]. While α_1-acid glycoprotein and crude ovomucoid [93,94] probably have the broadest fields of application, numerous reports in the literature have described chromatographic separations and binding studies using bovine serum albumin [95], human serum albumin [96], cellobiohydrolase I [97], avidin [98] and pepsin [99] as immobilized chiral selectors. Riboflavin-binding protein [100] and amyloglucosidase [101] are promising candidates for the chiral separation of basic drugs.

2.3. Effect of temperature on retention of chiral separations

The variation of column temperature has effects on the retention and enantioselectivity of chiral analytes on a CSP. Enantioselective retention mechanisms are sometimes influenced by temperature to a greater extent than are ordinary RP separations. There are at least two completely different effects of temperature, and both can affect resolution. One effect changes the separation factor (α), *i.e*. the peak-to-peak separation distance. The separation factor may either increase or decrease, depending on the type of interaction mechanism. This occurs because the partition coefficients and therefore the free energy change (ΔG^{o}) of transfer of the analyte between the stationary phase and the mobile phase vary with temperature. This is the *thermodynamic effect*. In the case of multicomponent or ionizable mobile phases or ionizable solutes, both the distribution of the solvent components and the pK_a of the ionizable compound can also vary with temperature. Another completely different effect of temperature is the influence on viscosity and on diffusion coefficients. This is largely a *kinetic effect*, which improves efficiency (*i.e*. peak width). There are two different mass transfer effects here. One is mobile phase mass transfer. An increase of temperature reduces the viscosity of the mobile phase. However, an increase of temperature also increases the diffusion coefficient of the solute in both the mobile phase and the stationary phase, and it decreases the viscosity of the stationary phase (enhancing stationary phase mass transfer).

The relationship between retention and temperature can be derived from the van't Hoff equation. Retention data obtained at different temperatures allow an evaluation of the variations in enthalpy (ΔH^{o}) and entropy (ΔS^{o}), which are related to the process of transfer of the analyte from the mobile phase to the stationary phase [112,113] and also allow a comparison with the data obtained by conventional RP chromatography.

The separation of the (*R*) and (*S*) enantiomers on a CSP involves the reversible formation of a pair of reversible diastereomeric analyte-CSP complexes. The difference in stability between these diastereomeric complexes leads to a difference in retention time. The enantiomer that forms the less stable complex will be eluted first. These diastereomeric complexes must therefore differ adequately in free energy for enantiomer separation to be observed. In chromatography, the magnitude of solute retention is expressed by the retention factor, *k'*, which is a measure of the stochiometric mass distribution of the analyte between the stationary and mobile phases:

k'= analyte mass in stationary phase/analyte mass in mobile phase

k' is related to the thermodynamic equilibrium constant (*K*) according to the following equation:

$$k' = K\phi \quad (1)$$

where ϕ is the phase ratio of the column (the volume of the stationary phase divided by the volume of the mobile phase). The free energy charge for the process is expressed by:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K = -RT \ln\left(\frac{k'}{\phi}\right) \quad (2)$$

Therefore:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi \quad (3)$$

where ΔG^o is the standard free energy of transfer of the analyte from the mobile phase to the CSP, ΔH^o is the enthalpy of transfer of the analyte from the mobile phase to the CSP, ΔS^o is the entropy of transfer of the analyte from the mobile phase to the CSP, R is the universal gas constant and T is the absolute temperature. Equation 3 shows that a plot of ln k' *vs* $1/T$ has a slope of $-\Delta H^o/R$ and an intercept of ($\Delta S^o/R + \ln\phi$) if ΔH^o is invariant with temperature (*i.e.* a linear van't Hoff plot is obtained). This provides a convenient way of calculating the thermodynamic constants ΔH^o and ΔS^o for a chromatographic system if the phase ratio is known or can be calculated. Determination of ϕ is relatively easy in pure liquid-liquid chromatography, but the situation is much more complex in RP chromatography with chemically bonded materials.

The corresponding $\Delta(\Delta H^o)$ and $\Delta(\Delta S^o)$ values can be obtained as the differences $\Delta H^o_2 - \Delta H^o_1$ and $\Delta S^o_2 - \Delta S^o_1$ or can be obtained from the selectivity factor ($\alpha = k_2'/k_1'$), which is related to the difference in Gibbs free energy of assiciation for an enantiomeric pair, $\Delta(\Delta G^o)$:

$$\Delta(\Delta G^\circ) = -RT \ln\alpha \quad (4)$$

and

$$R \ln\alpha = \frac{-\Delta(\Delta H^\circ)}{T} + \Delta(\Delta S^\circ) \quad (5)$$

If $\Delta(\Delta H^o)$ is constant within the temperature range, a straight line should be obtained when R ln α is plotted *vs* $1/T$. This means that the retention mechanism in the temperature range under study is invariant. The slope is $-\Delta(\Delta H^o)$ and the intercept is $\Delta(\Delta S^o)$. The difference $\Delta(\Delta G^o)$ is solely responsible for enantioselectivity. The larger the value of $\Delta(\Delta G^o)$, the better the enantiomeric separation. The designer of a CSP should maximize $\Delta(\Delta G^o)$ while minimizing the adsorption energies, ΔG^o_1 and ΔG^o_2. Large ΔG^o_1 and ΔG^o_2 lead to long retention times and broad chromatographic peaks.

The separation of enantiomers on a CSP is said to be enthalpy-driven when the enthalpy term, $\Delta(\Delta H^o)$, dominates over the entropy term, $\Delta(\Delta S^o)$. In this case, lowering the temperature

improves the enantioselectivity and hence the enantioseparation. As regards the van't Hoff plots of the (*R*) and (*S*) enantiomers, this is manifested by a large difference in slopes that corresponds to $\Delta(\Delta H^{o})$. That is, the two lines diverge from each other. In an entropy-controlled enantioseparation, $\Delta(\Delta S^{o})$ dominates over $\Delta(\Delta H^{o})$. This means that a decrease in temperature decreases the enantioselectivity. The van't Hoff plots show a large difference in the intercepts of the two lines, which corresponds to $\Delta(\Delta S^{o})$.

One of the first studies in the field of enantioselective retention mechanisms of chiral separation was accomplished by Pirkle in 1991 [114]. There are a number of other, generally acknowledged methods for the investigation of chiral retention mechanisms, such as the bi-Langmuir-isothermal model developed by Guiochon *et al*. [115]. The selective and nonselective interactions responsible for retention can be distinguished with the help of this method, and thus the chiral separation can be optimized. The intermolecular interactions between the chiral selector and the analyte can also be studied by nuclear magnetic resonance [116,117] or mass spectrometry [118,119]. In summary, independently neither of the above-mentioned methods provide exact results, but they complementing each other to provide an overall view of the retention mechanism developed on a certain CSP.

3. EXPERIMENTAL

3.1. Apparatus

Our measurements were carried out with three HPLC systems.

System I: An M-600 low-pressure gradient pump, equipped with an M-996 photodiode-array detector and a Millenium^{32} 2.1 Chromatography Manager data system (all Waters Chromatography, Milford, MA, USA).

System II: A 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (all Waters Chromatography, Milford, MA, USA).

System III: An MD-2089 PLUS quaternary low-pressure gradient pump, an MD-2010 PLUS photodiode-array detector and a ChromPass 1.8 Chromatography Data System (all JASCO International Co., Tokyo, Japan).

All of the HPLC systems were equipped with a Rheodyne Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA).

A Radelkis OP/20811 pH-meter (Budapest, Hungary) was employed for pH measurements.

The columns were thermostated in a water bath, a cooling-heating thermostat (MK70, Mechanik Prüfgeräte, Medlingen, Germany) being applied. The accuracy of temperature adjustment was ±0.1 °C.

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal and reproducible retention factors were obtained for the subsequent injections. This procedure was always followed when a new mobile phase or temperature was chosen.

3.2. Applied columns

The columns used for chiral separations:

Polysaccharide-based CSPs: cellulose *tris*-3,5-dimethylphenyl carbamate-containing Chiralcel OD-H, 250 × 4.6 mm I.D., 5 μm particle size, and Chiralcel OD-RH, 150 × 4.6 mm I.D., 5 μm particle size (Diacel Chemical Industries Ltd., Tokyo, Japan).

Cavity-type CSPs: 3,5-dimethylphenyl carbamoylated *β*-CD-containing Cyclobond DMP, 250 × 4.6 mm I.D., 5 μm particle size (Astec, Whippany, NJ, USA), and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP, 150 × 4.6 mm I.D., 5 μm particle size.

Macrocyclic antibiotic-based CSPs: teicoplanin-containing Chirobiotic T and T2, teicoplanin aglycone-containing Chirobiotic TAG, vancomycin-containing Chirobiotic V and V2 and ristocetin A-containing Chirobiotic R, 250 × 4.6 mm I.D., 5 μm particle size for each column (Astec, Whippany, NJ, USA).

3.3. Chemicals and reagents

Acetonitrile (MeCN), methanol (MeOH), ethanol (EtOH), 2-propanol (IPA), *n*-hexane and chloroform of HPLC grade were from Merck (Darmstadt, Germany). Milli-Q water was further purified by filtering it on a 0.45 μm filter, type HV, Millipore (Molsheim, France). Diethylamine (DEA), triethylamine (TEA), glacial acetic acid (AcOH), trifluoracetic acid (TFA), H_3PO_4, $HClO_4$, H_2SO_4, KOH and other reagents of analytical reagent grade were from Aldrich (Steinheim, Germany). Phosphate buffer was made from appropriate volumes of H_3PO_4 dissolved in Milli-Q water and adjusted to the appropriate pH with 5.0 M KOH solution, and diluted to a final volume of 1000 ml in a volumetric flask. 1% Triethylammonium acetate (TEAA) buffer was prepared by dissolving 10 ml of TEA in ca. 900 ml of water, and adjusting the pH with AcOH to 4.1 or 6.5 and diluting to a final volume of 1000 ml.

Mobile phases were prepared by mixing the indicated volumes of solvents and were further purified by filtration through a 0.45-μm Millipore filter, type HV. The eluents were degassed in an ultrasonic bath and helium gas was purged through them during the analysis. Stock solutions of analytes (1 mg ml^{-1}) were prepared by dissolution in the starting mobile phase. The analytes were chromatographed without pre- or postcolumn derivatization.

The void volumes of the columns were determined by injecting 20 μl of a 0.01 M methanolic solution of KBr. In the case of cellulose-based columns, eluents with different compositions were injected in the flow of the mobile phase for the measurement of void volumes.

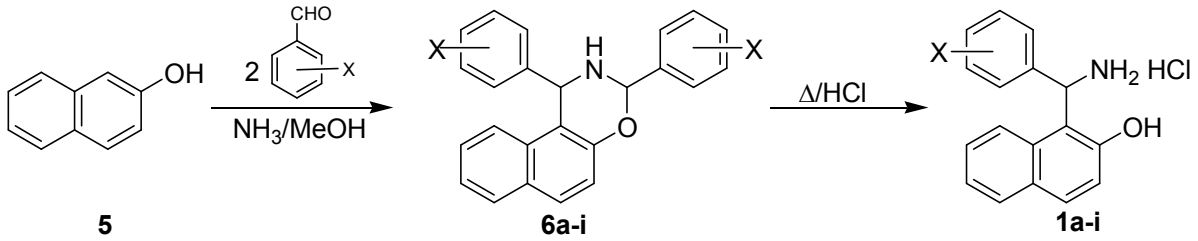
3.4. Ab initio calculations

The minimum-structure search protocol involved preliminary molecular mechanics (MMFF94) minimization, which was followed by geometry optimization at the *ab initio* level by using the Gaussian 03 program package. The B3LYP DFT hybrid method and the 6-31G* double zeta split valence basis set were used. The NBO population analyses were calculated at the same level of theory.

3.5. Investigated compounds

3.5.1. α-Aminobenzyl- and α-aminoalkyl 1- and 2-naphthol analogs

The group of 1-(aminobenzyl)-2-naphthol analogs (Scheme 2) includes 1-(*α*-aminobenzyl)-2-naphthol (Betti base, **1a**), 1-[amino(4-methylphenyl)methyl]-2-naphthol (**1b**), 1-[amino(4-methoxyphenyl)methyl]-2-naphthol (**1c**), 1-[amino(4-fluorophenyl)methyl]-2-naphthol (**1d**), 1-[amino(4-chlorophenyl)methyl]-2-naphthol (**1e**), 1-[amino(4-bromophenyl)methyl]-2-naphthol (**1f**), 1-[amino(4-nitrophenyl)methyl]-2-naphthol (**1g**), 1-[amino(3-bromophenyl)methyl]-2-naphthol (**1h**) and 1-[amino(3-nitrophenyl)methyl]-2-naphthol (**1i**).



5 **6a-i** **1a-i**

X = **a**: H, **b**: *p*-Me, **c**: *p*-OMe, **d**: *p*-F, **e**: *p*-Cl, **f**: *p*-Br, **g**: *p*-NO_2, **h**: *m*-Br, **i**: *m*-NO_2

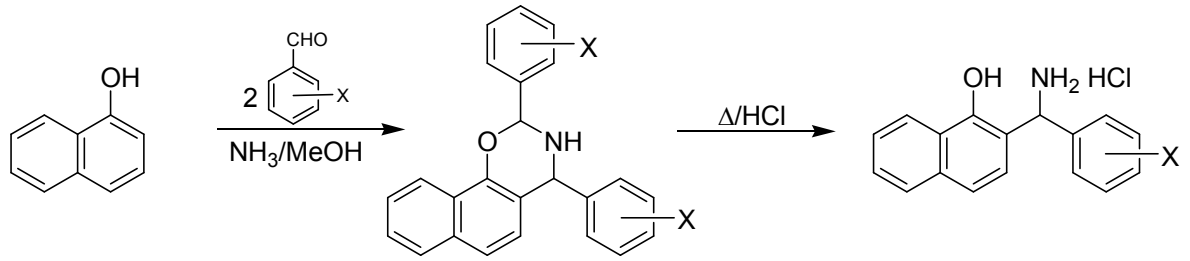
Scheme 2.
Synthesis of 1-(aminobenzyl)-2-naphthol analogs

The 2-(aminobenzyl)-1-naphthol derivatives (Scheme 3) are 2-(*a*-aminobenzyl)-1-naphthol (**2a**), 2-[amino(4-methylphenyl)methyl]-1-naphthol (**2b**), 2-[amino(4-methoxyphenyl)methyl]-1-naphthol (**2c**), 2-[amino(4-fluorophenyl)methyl]-1-naphthol (**2d**), 2-[amino(4-chlorophenyl)methyl]-1-naphthol (**2e**), 2-[amino(4-bromophenyl)methyl]-1-naphthol (**2f**), 2-[amino(3-bromophenyl)methyl]-1-naphthol (**2h**) and 2-[amino(3-nitrophenyl)methyl]-1-naphthol (**2i**).

The compounds 1-(1-aminoethyl)-2-naphthol (**3a**), 1-(1-aminopropyl)-2-naphthol (**3b**), 1-(1-aminobutyl)-2-naphthol (**3c**), 1-(1-amino-2-methylpropyl)-2-naphthol (**3d**) and 1-(1-

amino-2,2-dimethylpropyl)-2-naphthol (**3e**) belong in the group of 1-(aminoalkyl)-2-naphthols (Scheme 4).

The syntheses of all the substituted naphthol derivatives investigated in this thesis were based on the Betti reaction. In 1941, Betti [120] reported the Mannich aminoalkylation of 2-naphthol for the preparation of 1-(*α*-aminobenzyl)-2-naphthol (Scheme 2, **1a**), which has subsequently been utilized by Szatmári *et al*. [121,122].

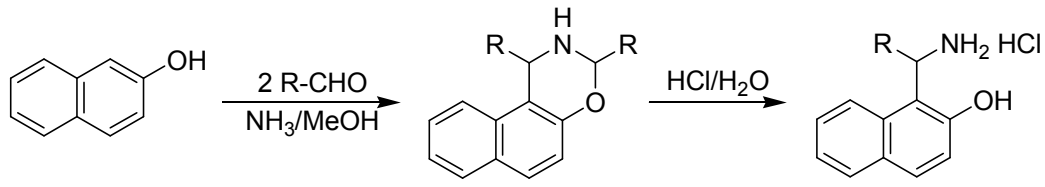


7 **8a-f,h,i** **2a-f,h,i**

X = **a**: H, **b**: *p*-Me, **c**: *p*-OMe, **d**: *p*-F, **e**: *p*-Cl, **f**: *p*-Br, **h**: *m*-Br, **i**: *m*-NO_2

Scheme 3.
Synthesis of 2-(aminobenzyl)-1-naphthol derivatives

The preparation of substituted Betti base derivatives by the modified Mannich reaction was performed with 2-naphthol (**5**), substituted aromatic aldehydes and ammonia (in proportions of 1:2:1) to obtain 1,3-diaryl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3]oxazines (**6a-i**). Their subsequent acidic hydrolysis gave the desired 1-(aminobenzyl)-2-naphthol analogs (**1a-i**) (Scheme 2). By the reaction of aliphatic aldehydes (in place of aromatic aldehydes), 1,3-dialkyl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3]oxazines (**9a-e**) have been prepared by the same method. The acidic hydrolysis of **9a-e** led to the 1-(aminoalkyl)-2-naphthol derivatives (**3a-e**) (Scheme 4) [123].



5 **9a-e** **3a-e**

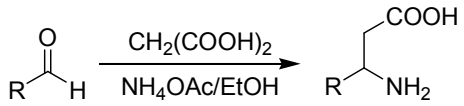
R = **a**: Me, **b**: Et, **c**: Pr, **d**: *i*Pr, **e**: *t*Bu

Scheme 4.
Synthesis of 1-(aminoalkyl)-2-naphthols

2-(Aminobenzyl)-1-naphthols (**2a-f**,**h**,**i**) were obtained in a manner similar to that for regioisomeric 1-(*α*-aminobenzyl)-2-naphthol derivatives: the classical Betti aminoalkylation of 1-naphthol (**7**) with benzaldehyde or substituted benzaldehydes in the presence of ammonia yielded 2,4-diaryl-3,4-dihydro-2*H*-naphth[2,1-*e*][1,3]oxazines (**8a-f**,**h**,**i**), and their acidic hydrolysis furnished **2a-f**,**h**,**i** (Scheme 3) [124].

3.5.2. Unnatural β-amino acids

The racemic 3-aryl- or 3-heteroaryl-substituted 3-aminopropanoic acids (Scheme 5), 3-amino-3-phenylpropanoic acid (**4a**), 3-amino-3-(4-methylphenyl)propanoic acid (**4b**), 3-amino-3-(4-trifluoromethylphenyl)propanoic acid (**4c**), 3-amino-3-(4-methoxyphenyl)-propanoic acid (**4d**), 3-amino-3-(3-methoxyphenyl)propanoic acid (**4e**), 3-amino-3-(2-methoxyphenyl)propanoic acid (**4f**), 3-amino-3-(3,4-dimethoxyphenyl)propanoic acid (**4g**), 3-amino-3-(4-fluorophenyl)propanoic acid (**4h**), 3-amino-3-(4-chlorophenyl)propanoic acid (**4i**), 3-amino-3-(3-chlorophenyl)propanoic acid (**4j**), 3-amino-3-(2-chlorophenyl)propanoic acid (**4k**), 3-amino-3-(3,4-dichlorophenyl)propanoic acid (**4l**), 3-amino-3-(4-bromophenyl)propanoic acid (**4m**), 3-amino-3-(3-bromophenyl)propanoic acid (**4n**), 3-amino-3-(2-furyl)propanoic acid (**4o**), 3-amino-3-(2-thienyl)propanoic acid (**4p**), 3-amino-3-(3-pyridyl)propanoic acid (**4q**) and 3-amino-3-(1-naphthyl)propanoic acid (**4r**) were synthetized from the corresponding aromatic aldehydes by a modified Rodionov procedure: the aldehydes were condensed with an equimolar amount of malonic acid in refluxing 96% ethanol in the presence of two equivalents of ammonium acetate (Scheme 5) [125,126].



14a-r **4a-r**

R = **a**: Ph; **b**: *p*-Me-C_6H_4; **c**: *p*-CF_3-C_6H_4; **d**: *p*-OMe-C_6H_4; **e**: *m*-OMe-C_6H_4; **f**: *o*-OMe-C_6H_4; **g**: *m,p*-diOMe-C_6H_3; **h**: *p*-F-C_6H_4; **i**: *p*-Cl-C_6H_4; **j**: *m*-Cl-C_6H_4; **k**: *o*-Cl-C_6H_4; **l**: *m,p*-diCl-C_6H_3; **m**: *p*-Br-C_6H_4; **n**: *m*-Br-C_6H_4; **o**: 2-furyl; **p**: 2-thienyl; **q**: 3-pyridyl; **r**: 1-naphthyl

Scheme 5.

Synthesis of 3-aryl- and 3-heteroaryl-substituted 3-aminopropanoic acids

The 3-aryl-substituted *β*-amino acid enantiomers were prepared by Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin)-catalyzed enantioselective ring cleavage of the corresponding racemic 4-aryl-substituted *β*-lactams with water in diisopropyl ether [127]. The enantiomerically pure heteroaryl-substituted *β*-amino acids were prepared by *Candida antarctica* lipase A-catalyzed enzymatic kinetic resolution of the corresponding esters in ethyl butyrate [128].

4. RESULTS AND DISCUSSION

4.1. Separation of α-aminobenzylnaphthol analogs

The chemistry of the Betti bases started in 1941, when Betti reported the synthesis of 1-(α-aminobenzyl)-2-naphthol (Scheme 2, **1a**) [120]. The first resolution by crystallization of naphthol derivatives was reported by Betti and Pratesi and the enantiomers of **1a** were used for the resolution of aldehydes [129]. However, in spite of the ready availability and low cost of the Betti base enantiomers, the early publications were followed by a long silence over this material. In the past decade, interest in these types of compounds has intensified, while enantiomers of the Betti base and its *N*-substituted derivatives are of significance since they can serve as chiral catalysts [130,131], while the nonracemic **1a** was applied successfully as a new chiral auxiliary [132]. On the other hand, Betti base derivatives also provide convenient access to many useful synthetic building blocks [133]. The biological effects of naphthol derivatives have been investigated in *in vivo* systems. Desai *et al*. [134] found that the presence of the thiazolidine nucleus, produced from the racemic Betti base, was necessary for good antituberculotic activity, and the presence of halogen atoms enhances the antibacterial activity. The chemical and biological applications of the enantiomers of Betti base derivatives required a precise separation and detection method.

4.1.1. Separation on cellulose tris-3,5-dimethylphenyl carbamate-based CSP

Extensive searches in different databases indicated that no chromatographic data have been published in the literature on the separation and identification of Betti base derivatives. On the evidence of the functional groups present in the investigated analytes (**1a**-**1i**, **2a**-**f**,**h**,**i**), a cellulose *tris*-3,5-dimethylphenyl carbamate-based CSP (Chiralcel OD-H, Fig. 4) was expected to be a very successful tool in the separation of the enantiomers of the α-aminobenzyl 1- and 2-naphthol analogs, in consequence of the presence of aromatic rings capable of π-π interactions, involving both the analytes and the selector of the CSP. The dimethylphenyl carbamate groups of the Chiralcel OD-H CSP also allow additional H-bonding sites and the potential for dipole stacking interactions [69]. The results are discussed below, in terms of different parameters, such as the effects of the mobile phase composition and the structural features of the investigated analytes on the chiral recognition and separation.

The application of *n*-hexane and IPA as alcoholic modifier resulted in chromatograms with broad and asymmetric peaks (data not shown). Acidic or basic mobile phase additives are widely used on polysaccharide-based CSPs with the intent of minimizing interactions with residual silanols, giving better peak shape and increased resolution and they also affect the retention and selectivity. Addition of a small percentage of DEA to the mobile phase significantly improved the peak shapes and resolutions (Tables 2 and 3).

Table 2.
Chromatographic data on the enantioseparation of 2-naphthol derivatives

Analyte	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S
1a	60/40/0.1	0.92	3.00	3.26	10.0
	50/50/0.1	0.73	2.35	3.21	8.50
	40/60/0.1	0.67	2.07	3.09	7.36
1b	60/40/0.1	0.82	2.54	3.10	9.11
	50/50/0.1	0.69	2.17	3.16	7.66
	40/60/0.1	0.55	1.81	3.29	7.41
1c	60/40/0.1	1.24	3.25	2.62	8.73
	50/50/0.1	1.01	2.90	2.87	8.85
	40/60/0.1	0.79	2.13	2.70	7.36
1d	60/40/0.1	0.87	2.79	3.21	9.20
	50/50/0.1	0.67	2.45	3.64	9.60
	40/60/0.1	0.53	1.85	3.49	6.92
1e	60/40/0.1	0.95	3.02	3.18	9.80
	50/50/0.1	0.74	2.57	3.47	9.09
	40/60/0.1	0.58	1.94	3.34	7.52
1f	60/40/0.1	1.02	3.15	3.08	9.37
	50/50/0.1	0.78	2.46	3.15	9.09
	40/60/0.1	0.67	2.19	3.27	9.17
1g	60/40/0.1	1.70	4.19	2.46	8.17
	50/50/0.1	1.26	3.37	2.67	9.00
	40/60/0.1	0.98	2.57	2.62	7.42
1h	60/40/0.1	1.04	2.83	2.72	8.40
	50/50/0.1	0.81	2.32	2.86	7.57
	40/60/0.1	0.72	2.29	3.18	8.23
1i	60/40/0.1	1.69	3.55	2.10	5.83
	50/50/0.1	1.19	2.67	2.24	6.43
	40/60/0.1	0.92	1.99	2.16	4.93

Column, Chiralcel OD-H; mobile phase, *n*-hexane/IPA/DEA; detection, 232 nm; flow rate, 0.5 ml min^{-1}; temperature, 298 K

It is generally recognized that, at the supramolecular level, the lamellar arrangement of the polysaccharide chains provides a multitude of chiral cavities, which result in multiple interaction sites [68]. In the case of the cellulose *tris*-3,5-dimethylphenyl carbamate-based CSP, the polar carbamate residues are located inside, while the hydrophobic aromatic groups

are outside the polymer chain. Enantiomers can therefore interact with the carbamate groups via H-bonding with the –NH– and –CO= groups and dipole-dipole interactions using the –CO= moiety. Besides these polar interactions, π-π interactions between the phenyl groups of the CSP and an aromatic group of the solute may play some role in chiral recognition.

Table 3.
Chromatographic data on the enantioseparation of 1-naphthol derivatives

Analyte	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S
2a	60/40/0.1	1.66	4.84	2.92	12.5
	40/60/0.1	1.22	3.41	2.80	10.6
2b	60/40/0.1	1.62	3.75	2.31	10.0
	40/60/0.1	1.21	2.70	2.23	7.08
2c	60/40/0.1	2.56	4.07	1.59	5.60
	40/60/0.1	1.66	2.65	1.60	4.43
2d	60/40/0.1	1.32	2.80	2.11	7.81
	40/60/0.1	0.90	1.89	2.10	5.25
2e	60/40/0.1	1.60	3.18	1.99	7.40
	40/60/0.1	1.20	2.27	1.89	5.38
2f	60/40/0.1	1.80	3.64	2.02	7.70
	40/60/0.1	1.31	2.48	1.89	5.43
2h	60/40/0.1	1.70	4.46	2.62	11.6
	40/60/0.1	1.25	3.44	2.75	9.23
2i	60/40/0.1	2.42	3.87	1.60	4.23
	40/60/0.1	1.48	2.37	1.60	4.00

Column, Chiralcel OD-H; mobile phase, *n*-hexane/IPA/DEA; detection, 232 nm; flow rate, 0.5 ml min^{-1}; temperature, 298 K

As shown in Tables 2 and 3, the alcohol content in the mobile phase is responsible for retention as well as chiral discrimination. At ambient temperature, increase of the IPA content of the mobile phase from 40 to 60% decreased the retention factors of both the 2- and 1-naphthol derivatives. It is hypothesized that, with increase of the mobile phase polarity, the strength of the H-bonds between the analytes and the CSP decreases and the solubility of the analytes in the mobile phase increases. Furthermore, it is possible that some alcohol molecules are associated with the CSP and cause swelling of the column, which leads to opening of the chiral cavities. Thus, the inclusion interactions of the enantiomers are diminished and *k'* is decreased. The separation factor (α) improved on increase of the concentration of IPA from 40 to 60% in the case of 2-naphthol analogs (with the exception of **1a**). Opposite behavior was observed for the 1-naphthol derivatives (with the exception of **2h**), as shown in Table 3. For both groups of the investigated aryl-substituted naphthol analogs, the resolution (R_S) decreased as the concentration of IPA in the mobile phase was increased (Tables 2 and 3). At a constant mobile phase composition, the 1-naphthol

derivatives exhibited larger retention factors and smaller separation factors than the 2-naphthol analogs (Tables 2 and 3). This indicates that the steric arrangements of the 1-naphthol derivatives are more favorable for chiral recognition. At the same time, the interactions of the two stereoisomers with the selector did not differ appreciably, which resulted in smaller α values for the 1-naphthol analogs.

In an effort to describe the effects of the aromatic substituents of the analytes, the chromatographic parameters were studied at constant mobile phase composition. Methyl substitution on the aromatic ring resulted in lower retention, while methoxy subtitution led to a higher retention as compared with the nonsubstituted compounds (**1b** and **1c** *vs* **1a**; **2b** and **2c** *vs* **2a**). In the cases of **1c** and **2c**, additional H-bonding interactions may be formed between the methoxy group of the analytes and the CSP. This achiral interaction results in an increase in retention and decreases in selectivity and resolution. Halogen substitution on the aromatic ring increased the π-acidic character of the analytes and therefore a stronger interaction with the π-basic 3,5-dimethylphenyl carbamate group of the selector would be expected. Nevertheless, this expectation was not fulfilled for the 2-naphthol derivatives, with the exception of **1d**. The retentions of halogen-substituted 1-naphthol analogs were somewhat smaller than those for nonsubstituted ones (with the exceptions of k_1' for **2f** and **2h**). A slight increase was observed in the sequence fluorine-chlorine-bromine for both groups of α-aminobenzylnaphthols. For the 2-naphthol derivatives, the comparison of analytes possessing the same substituent in different positions revealed that the retentions of the first-eluting enantiomers were similar, but the k_2', α and R_S values of the 4-substituted analogs were higher (**1f** and **1h**; **1g** and **1i**). For the 1-naphthol analogs, a slightly higher selectivity and significantly higher resolution were observed for the 3-substituted analog (**2h**) than for the 4-substituted **2f**. The more π-acidic nitro substituents on the aromatic ring led to larger retention times for **g** and **i** than those for the nonsubstituted or less π-acidic halogen-substituted analytes, especially for the first-eluted stereoisomers. These increased retention factors were generally not associated with increased selectivity. A possible explanation appears to be that, besides the nature of the aromatic substituents, other steric effects also influenced the retention and chiral discrimination of the naphthol derivatives.

The absolute configuration and elution sequence were determined for analyte **1a**: the elution sequence (*S*)<(*R*) was detected. A similar elution sequence can probably be presumed for 2-naphthol analogs. As seen from the chromatograms in the Appendix, the enantiomers of the 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs are baseline-resolved and elute as symmetrical peaks.

4.1.2. Effects of temperature on chromatographic parameters of 1-(α-aminobenzyl)-2-naphthol and 1-(α-aminobenzyl)-2-naphthol analogs

The temperature dependence of the chromatographic parameters of α-aminobenzyl-substituted 1- and 2-naphthol analogs was studied on the Chiralcel OD-H CSP, at 10 ºC increments in the temperature range 5-35 ºC, using *n*-hexane/IPA/DEA=55/45/0.1 (v/v/v) as mobile phase. Table 4 lists the results obtained. Increasing temperature led to a diminished enantioselectivity (α) and reduced retention (k'). This tendency is illustrated in Fig. 10, which also shows that an increase of temperature improves the peak symmetry. The resolution of the enantiomers decreased when the temperature was increased for all of the investigated naphthol analogs. Similar tendencies were measured at other mobile phase compositions (data not shown). In order to access the thermodynamic functions of enantioselective adsorption, which may be interpreted in terms of mechanistic aspects of chiral recognition, van't Hoff plots were constructed (Eq. 5) and different thermodynamic parameters were calculated. For all analytes in this study, the plots of $R \ln \alpha$ *vs* $1/T$ could be fitted by straight lines over the investigated temperature range, as illustrated in Fig. 11 on the example of analyte **1b**. Correlation coefficients are reported in Table 5. The linear behavior of the plot suggests that the retention process remains unchanged over the investigated temperature range. The slope of the straight line is $-\Delta(\Delta H^o)$ and the intercept is $-\Delta(\Delta S^o)$. The free energy can be calculated from Eqs 4 and 5; the values obtained are listed in Table 5. The enthalpy term $\Delta(\Delta H^o)$ provides information on the relative ease with which the preferentially adsorbed enantiomer

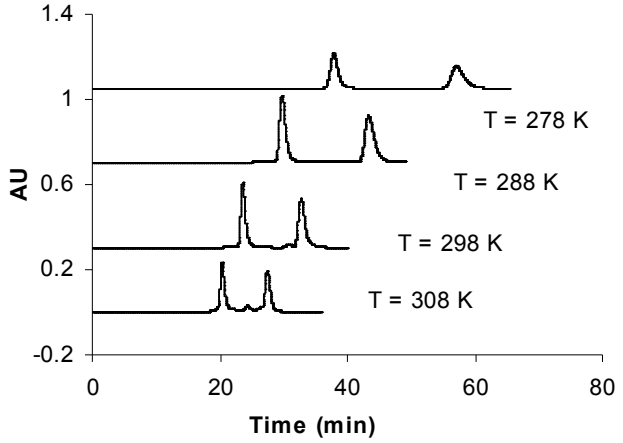


Figure 10.
Temperature dependence of the chiral separation of **2c** at an *n*-hexane/IPA/DEA mobile phase composition of 55/45/0.1

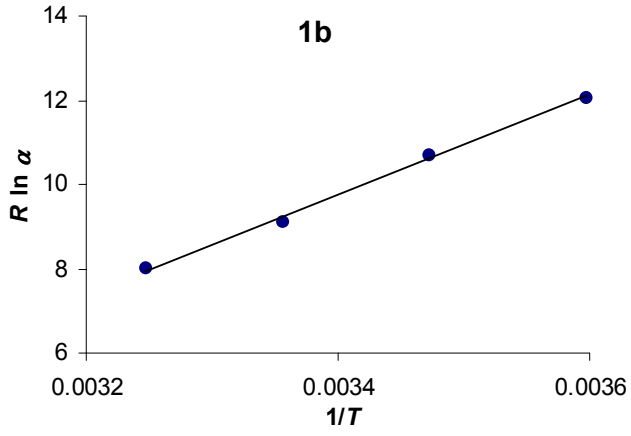


Figure 11.
Plot of $R \ln \alpha$ for enantiomer **1b** as a function of the inverse of temperature ($1/T$)

Table 4.
Temperature dependence of retention factors (k'), separation factors (α) and resolutions (R_S) of enantiomers of 1- and 2-naphthol analogs

Temp. (K)	Analyte	k_1'	k_2'	α	R_S	Analyte	k_1'	k_2'	α	R_S
278	**1a**	1.32	6.04	4.57	12.8	**2a**	2.78	9.47	3.41	13.2
288		1.12	4.25	3.80	12.2		2.26	7.00	3.10	13.1
298		0.93	2.89	3.11	10.4		1.73	4.68	2.71	12.9
308		0.82	2.22	2.71	7.81		1.45	3.61	2.49	8.20
278	**1b**	1.24	5.31	4.28	13.6	**2b**	2.81	6.99	2.49	10.7
288		1.08	3.90	3.62	11.6		2.49	5.35	2.15	10.3
298		0.88	2.63	3.00	9.47		1.67	3.54	2.12	8.20
308		0.74	1.94	2.62	7.37		1.39	2.90	2.09	7.11
278	**1c**	2.04	7.11	3.49	11.7	**2c**	4.32	7.02	1.63	6.09
288		1.64	4.84	2.95	10.2		3.25	5.18	1.59	5.48
298		1.24	3.15	2.54	8.42		2.32	3.61	1.56	5.33
308		1.07	2.43	2.27	7.74		1.94	2.97	1.53	4.67
278	**1d**	1.28	5.92	4.63	13.8	**2d**	2.04	4.60	2.25	7.66
288		1.08	4.40	4.07	12.4		1.67	3.51	2.10	7.11
298		0.88	2.81	3.19	10.1		1.33	2.69	2.03	6.66
308		0.79	2.22	2.80	7.90		1.18	2.21	1.87	5.87
278	**1e**	1.37	5.82	4.25	12.2	**2e**	2.46	4.95	2.01	7.20
288		1.15	4.37	3.79	11.6		2.08	3.91	1.88	6.66
298		0.96	2.95	3.06	9.26		1.69	3.16	1.87	6.40
308		0.85	2.31	2.72	8.40		1.41	2.50	1.77	5.75
278	**1f**	1.43	6.55	4.58	13.0	**2f**	2.84	5.72	2.02	7.74
288		1.27	4.56	3.59	12.7		2.27	4.32	1.90	7.20
298		1.02	3.07	3.01	10.4		1.83	3.45	1.88	7.00
308		0.90	2.35	2.61	7.11		1.53	2.78	1.82	4.89
278	**1g**	2.46	7.83	3.18	10.7	**2i**	3.47	5.75	1.66	5.34
288		1.95	5.75	2.94	10.5		2.88	4.66	1.62	5.21
298		1.62	4.01	2.47	8.40		2.40	3.78	1.58	4.56
308		1.38	3.08	2.23	7.20		1.92	2.92	1.52	4.16

Column, Chiralcel OD-H; eluent, *n*-hexane/IPA/DEA = 55/45/0.1 (v/v/v); detection, 232 nm; flow rate, 0.5 ml min^{-1}

is transferred from the mobile phase to the selector molecules of the CSP. The entropy term $\Delta(\Delta S^{o})$ is a measure of the changes in the state of order induced by enantioselective analyte enantiomer-selector binding. The structure of the analyte obviously has a strong influence on the thermodynamics of enantioselective adsorption to the selector of the Chiralcel OD-H column. The enantioselective binding of the 2-naphthol analogs was characterized by highly negative $\Delta(\Delta H^{o})$ values, ranging from -9.4 to -13.4 kJ mol^{-1}. Change of the α-aminobenzyl substituent to position 2 led to a conformationally more flexible structure and a 60-80% reduction in the interaction energy observed ($\Delta(\Delta H^{o})$ ranging from -1.5 to -7.6 kJ mol^{-1}).

Table 5.
Thermodynamic parameters $\Delta(\Delta H°)$, $\Delta(\Delta S°)$, correlation coefficients (r^2), $\Delta(\Delta G^{o})_{298K}$ and Q values of enantiomers of 2-naphthol and 1-naphthol derivatives

Analyte	$\Delta(\Delta H^{o})$ (kJ mol^{-1})	$\Delta(\Delta S^{o})$ (J mol^{-1} K^{-1})	r^2	$\Delta(\Delta G^{o})_{298K}$ (kJ mol^{-1})	Q
1a	-13.4	-34.0	0.997	-3.3	1.3
1b	-12.6	-31.8	0.997	-3.2	1.3
1c	-10.3	-26.9	0.995	-2.3	1.3
1d	-13.3	-33.3	0.998	-3.4	1.3
1e	-11.8	-28.8	0.994	-3.2	1.4
1f	-13.3	-35.5	0.997	-2.8	1.3
1g	-9.4	-23.0	0.998	-2.6	1.4
2a	-7.6	-17.3	0.994	-2.5	1.5
2b	-4.0	-6.9	0.986	-1.9	1.9
2c	-1.5	-1.3	0.992	-1.1	3.8
2d	-4.2	-8.0	0.990	-1.7	1.7
2e	-2.8	-4.3	0.996	-1.5	2.2
2f	-2.3	-2.5	0.999	-1.5	3.1
2i	-2.0	-3.4	0.993	-1.0	2.0

Column, Chiralcel OD-H; mobile phase, *n*-hexane/IPA/DEA=55/45/0.1 (v/v/v); detection, 232 nm; flow rate, 0.5 ml min^{-1}; temperature, 298-308 K; $Q=\Delta(\Delta H^{o})/(\Delta(\Delta S^{o})\times 298\ K)$

These observations suggest that the rigidity of the 2-naphthol analogs may promote not only the establishment of retention, but even the process of chiral discrimination. Negative $\Delta(\Delta H^{o})$ values indicate an exothermic transfer of the preferentially adsorbed enantiomer from the mobile phase to the stationary phase and therefore a favorable process. The negative entropy values, on the other hand, can be interpreted as an increase in order and/or a loss in the number of degrees of freedom due to adsorption and association to the CSP, respectively. Typically, the formation of enantiomer-selector complexes is accompanied by a significant loss in the number of degrees of freedom and therefore represents a thermodynamically unfavorable process. The more negative $\Delta(\Delta S^{o})$ values of the 2-naphthol derivatives can be explained by the fact that the difference in the number of degrees of freedom between the stereoisomers on the CSP was large. In the case of 2-naphthol derivatives, the enantioselective

association is highly favored by enthalpic contributions; this process is associated with more destabilizing entropic increments in the entire set of analytes **1a**-**g**. These analytes might allow multiple intermolecular interactions (dipole-dipole, H-bonding and π-π interactions) and hence highly efficient binding to the selector of the CSP, as reflected by the highly negative $\Delta(\Delta H^{o})$ values. However, the rigidity of the 2-naphthol analogs might also require extensive conformational rearrangements prior to an adsorption mechanism with an exceedingly unfavorable entropy ($\Delta(\Delta S^{o})$) balance. This behavior was not so expressed for the 1-naphthol derivatives.

As evident from the data listed in Table 5, enthalpically favored separations were observed for all the investigated naphthol analogs with the CSP; however, the relative contributions of the enthalpic and entropic terms to the free energy of adsorption depend on the nature of the analytes. As these effects are difficult to visualize directly from the thermodynamic data, the enthalpy/entropy ratios, $Q=[\Delta(\Delta H^{o})]/[\Delta(\Delta S^{o})\text{x}298\text{K}]$, were calculated and are listed in Table 5. This arbitrary factor represents the relative contributions of enthalpic *vs* entropic effects to $\Delta(\Delta G^{o})$ at 298 K. A comparison of the Q values for the individual classes of naphthol derivatives revealed that the enantioselective association of the 1-naphthol analogs (**2b**-**i**) is significantly more enthalpy-driven (Q ranges between 1.7 and 3.8). This again reflects the ideal steric preorganization, which facilitates highly stereoselective analyte-selector interactions. 2-Naphthol analogs (**1a**-**g**) and the nonsubstituted **2a** exhibited lower Q values, typically $Q<1.5$. For this class of analytes, a major proportion of the favorable $\Delta(\Delta H^{o})$ balance is counterbalanced by unfavorable entropic contributions.

As a practical aspect of these studies, temperature has proven to be a powerful tool to control and adjust the retention and the enantioselectivity of different analytes on the Chiralcel OD-H CSP. For optimization of enantiomer separations, retention and selectivity factors can be varied independently via the composition of the mobile phase and the column temperature, respectively. While the variation of the mobile phase composition allows tuning of the retention with negligible consequences for the enantioselectivity, temperature strongly affects both k' and α values. Subambient temperature chromatography with weak eluents may be employed to facilitate challenging separations and/or for preparative applications. If short run times are of concern, increased temperature in combination with mobile phases containing high buffer concentrations is advised.

4.1.3. Separation on 3,5-dimethylphenyl carbamoylated β-CD-based CSP

The enantiomers of 1-(α-aminobenzyl)-2-naphthol (**1a-i**) and 2-(α-aminobenzyl)-1-naphthol (**2a-f**,**h**,**i**) analogs were separated on a 3,5-dimethylphenyl carbamoylated β-CD-based CSP (Cyclobond DMP, Fig. 12). Variation of the mobile phase composition was investigated for optimization of the enantioseparation of racemic naphthol derivatives. The influence of specific structural features of the analytes (aryl substituents) on the retention and separation were also explored. Several kinds of mobile phase compositions were investigated, by changing the nature and percentage of the alcohol modifier and by applying acidic and basic modifiers. To simplify the presentation, only the chromatographic results relating to a partial or baseline separations are given in Table 6. For purposes of comparison, examples are presented where no separation occurred.

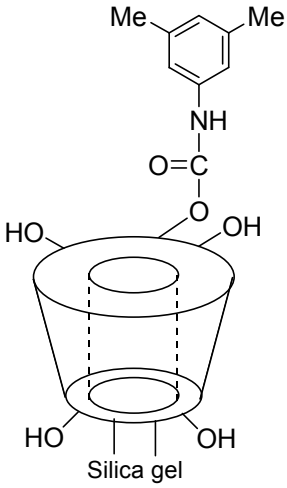


Figure 12.
The selector of Cyclobond DMP CSP

Enantioseparation was first tried with *n*-hexane/IPA as a mobile phase in different compositions; these conditions resulted in no separation (data not shown). Changing the alcoholic modifier from IPA to EtOH significantly improved the separations in most cases. At the constant mobile phase composition *n*-hexane/EtOH=80/20 (v/v), the retention factors of the 2- and 1-naphthol derivatives possessing the same substituents did not differ substantially. The exceptions were **2a** and **2i**, which exhibited significantly higher retention factors than those of the corresponding 1-naphthol analogs **1a** and **1i**, and the enantiomers of **1c** eluted later than **2c**. The separation factors were also similar, except for analogs **h** and **i**, where the 2-naphthol analogs (**1h** and **1i**) could be satisfactorily resolved, but the 1-naphthol analogs (**2h** and **2i**) could not.

Investigations of the enantioseparation characteristics of α-aminobenzyl-substituted 1- and 2-naphthol analogs at a mobile phase composition of *n*-hexane/EtOH=80/20 (v/v) revealed that both retention and enantioselectivity are slightly influenced by the nature of the substituents on the aromatic ring of the analytes. The methyl-substituted analogs **1b** and **2b** were less retained than the nonsubstituted **1a** and **2a**, respectively. Methoxy substitution entailed higher (**1c**) or lower (**2c**) retention factors than those of **1a** and **2a**, respectively. While the enantiomers of the nonsubstituted analytes were not separated, the increased bulkiness or hydrophobicity of the methyl-substituted analogs and the possibility of H-bonding interactions between the carbamate groups of the selector and the methoxy group of the analyte probably enhanced the enantioselectivity. Analytes **d-f** and **h**, with increased π-acidic character due to the halogen atoms, would undergo a stronger interaction with the π-

basic 3,5-dimethylphenyl carbamate group of the selector. Nevertheless, the k_1' values were somewhat smaller than those for the nonsubstituted analogs (with the exception of k_1' for **1f**), but the k_2' values of 2-naphthol derivatives containing halogen atoms were larger than those for **1a**. The increasing sequence of the retention was F<Cl<Br. In the cases of **2d**-**h**, the stronger interaction was more expressed by means of the k' values and the selectivities and resolutions improved (with the exception of **2h**) as compared with the nonsubstituted **2a**. The Appendix illustrates typical chromatograms attained with the methods described above.

Table 6.
Chromatographic data, retention factors (k'), separation factors (α) and resolutions (R_S) of the enantioseparation of 2-naphthol and 1-naphthol derivatives

Analyte	Mobile phase (v/v/v/v/v)	k_1'	k_2'	α	R_S	Analyte	Mobile phase (v/v/v/v)	k_1'	k_2'	α	R_S
1a	80/20, **a**	1.91	1.91	1.00	0.00	**2a**	80/20, **a**	3.46	3.46	1.00	0.00
	95/5, **g**	11.65	13.12	1.13	1.60		80/10/10, **c**	1.75	1.97	1.13	0.80
	80/10/10/0.1/0.1, **f**	1.83	1.83	1.00	0.00		80/10/10/0.1, **d**	1.79	2.03	1.13	1.15
							80/5/15/0.1, **e**	1.68	1.86	1.11	1.45
1b	80/20, **a**	1.64	1.73	1.06	1.10	**2b**	80/20, **a**	1.61	1.72	1.07	0.80
	80/10/10/0.1/0.1, **f**	1.52	1.63	1.07	0.95		80/10/10, **c**	1.44	1.66	1.15	1.00
							80/5/15, **c**	1.38	1.60	1.16	1.20
							80/10/10/0.1, **d**	1.84	2.26	1.23	1.25[h]
							80/5/15/0.1, **e**	1.39	1.58	1.14	1.64
1c	80/20, **a**	2.90	3.03	1.05	0.95	**2c**	80/20, **a**	2.11	2.45	1.16	0.90
	80/20/0.1, **b**	2.72	2.88	1.06	1.00		80/10/10, **c**	2.72	3.12	1.15	0.90
	80/10/10/0.1/0.1, **f**	2.56	2.69	1.05	0.80		80/5/15, **c**	2.49	2.89	1.16	1.20
							80/10/10/0.1, **d**	3.70	4.44	1.20	1.30[h]
							80/5/15/0.1, **e**	2.46	2.79	1.13	1.50
1d	80/20, **a**	1.87	2.00	1.07	0.70	**2d**	80/20, **a**	2.02	2.20	1.09	0.90
	80/10/10/0.1/0.1, **f**	1.77	1.92	1.09	1.05		80/10/10, **c**	1.86	2.80	1.50	1.40
							80/5/15/0.1, **e**	1.91	2.20	1.15	1.75
1e	80/20, **a**	1.89	2.30	1.22	1.80	**2e**	80/20, **a**	2.08	2.44	1.17	1.00
	80/20/0.1, **b**	1.89	2.36	1.25	2.80		80/10/10, **c**	1.77	2.21	1.25	1.70
	80/10/10/0.1/0.1, **f**	1.66	2.07	1.25	2.55		80/5/15/0.1, **e**	1.80	2.17	1.21	2.30
1f	80/20, **a**	1.93	2.46	1.28	2.10	**2f**	80/20, **a**	2.05	2.39	1.16	1.10
	80/20/0.1, **b**	1.91	2.47	1.29	2.40		80/10/10, **c**	1.84	2.30	1.25	1.80
	80/10/10, **c**	1.61	1.97	1.22	2.45		80/5/15/0.1, **e**	2.00	2.43	1.22	2.60
	80/10/10/0.1/0.1, **f**	1.76	2.30	1.31	3.30						
1h	80/20, **a**	1.80	1.97	1.09	1.10	**2h**	80/20, **a**	1.83	1.83	1.00	0.00
	80/20/0.1, **b**	1.86	2.07	1.11	1.25		80/10/10, **c**	1.62	1.80	1.11	0.90
	80/10/10/0.1/0.1, **f**	1.73	1.94	1.12	1.50		80/5/15/0.1, **e**	1.62	1.78	1.10	1.30
1i	80/20, **a**	3.84	4.47	1.16	1.60	**2i**	80/20, **a**	5.23	5.23	1.00	0.00
	80/10/10/0.1/0.1, **f**	3.36	3.92	1.17	2.60		80/10/10, **c**	4.16	4.66	1.12	0.90
							80/5/15/0.1, **e**	3.80	4.23	1.11	1.65
1g	80/20, **a**	4.25	5.98	1.41	3.10						
	80/10/10/0.1/0.1, **f**	3.85	4.71	1.32	4.75						

Column, Cyclobond DMP, mobile phase, **a,** *n*-hexane/EtOH, **b,** *n*-hexane/EtOH/DEA, **c**, *n*-hexane/EtOH/MeOH, **d,** *n*-hexane/EtOH/MeOH/DEA, **e**, *n*-hexane/EtOH/MeOH/TEA **f**, *n*-hexane/EtOH/MeOH/AcOH/TEA, **g**, *n*-hexane/chloroform; detection, 232 nm; flow rate, 0.8 ml min^{-1}; temperature, 298 K, [h]283 K

The comparison between analogs **f** and **h** showed the effects of the same substituent at the positions 3 and 4: **f** is always more retained with higher selectivity than **h** for both the 1- and 2-naphthol analogs. Similar patterns were observed for the retention and selectivity of **1g**

and **1i**. The retention factors of the more π-acidic nitro-substituted analogs (**g** and **i**) were the largest among the investigated analytes. The nitro moiety is an electron-withdrawing group and probably increases the capacity of the molecule to generate π-π interactions with the π-basic 3,5-dimethylphenyl carbamate residue of the CSP. Furthermore, a nitro group may also promote dipole-dipole interactions or H-bondings. In the cases of **1g** and **1i**, increased enantioselectivities were observed relative to the nonsubstituted analog **1a**. On the other hand, the enantiomers of **2i** remained unresolved: in spite of the stronger achiral interactions, the nitro group hindrance may be unfavorable for the chiral discrimination process.

Finally, to determine the reason for the similar chromatographic behavior of the 1- and 2-naphthol analogs on both the Chiralcel OD-H and Cyclobond DMP columns, *ab initio* calculations were performed. Analytes **1e** and **2e**, possessing an electron-withdrawing substituent (X=Cl), and as **1c** and **2c**, bearing an electon-donating substituent (X=OMe), were selected as model compounds. The calculations established that the torsional angle between the naphthyl and the phenyl planes was around 70º for the 2-naphthol analogs and around 66º for the 1-naphthol derivatives. The differences in the torsional angle produced by the X substituents were small: 1.5º for **1c**-**e** and 0.1º for **2c**-**e**. The calculations suggested that there is an intramolecular H-bond between the $-NH_2$ and the –OH group of the analytes, the distance between the N and the H atoms being 1.8353 Å for **1e** and 1.8099 Å for **1c**. The corresponding distances were 1.7462 Å for **2e** and 1.7402 Å for **2c**. The shorter distances suggested a stronger intramolecular H-bond for the 1-naphthol derivatives, which may explain the lower enantioselectivity for analytes **2** than for analytes **1** on either the Chiralcel OD-H or the Cyclobond DMP CSP. It may be considered that in series **1** the X substitution has a greater influence on the strength of the intramolecular H-bond, but overall these differences seemed to be of no significance in the chiral recognition process and the different substituents had only a slight influence on the separation.

4.2. Separation of α-aminoalkylnaphthol analogs

The enantiomers of various 1-(aminoalkyl)-2-naphthol analogs (**3a**-**e**, Scheme 4) were separated on cellulose *tris*-3,5-dimethylphenyl carbamate-based CSPs (Chiralcel OD-H and Chiralcel OD-RH), using *n*-hexane/IPA/DEA or phosphate buffer/organic modifier mobile phases. In the NP mode, the effects of the concentration of alcohol in the mobile phase were studied. In the RP mode, the effects of the mobile phase composition, the pH and the buffer concentration on the enantioseparation were investigated. The influence of the structural features of the solutes on the discrimination between the stereoisomers was examined through

the chromatographic parameters (retention factors, selectivity and resolution) in both NP and RP modes. Typical chromatograms of α-aminoalkylnaphthol enantiomers are presented in the Appendix.

4.2.1. Separation on Chiralcel OD-H column in NP mode

To the best of our knowledge, no report involving the use of HPLC methods for the analytical resolution of 1-(aminoalkyl)-2-naphthol enantiomers has been published. Chiralcel OD-H has proved to be an extremely useful stationary phase for chiral resolution of the structurally similar 1-(α-aminobenzyl)-2-naphthol (**1a**-**i**) and 1-(α-aminobenzyl)-2-naphthol (**2a**-**f**,**h**,**i**) analogs, as presented in Section 4.1. Therefore, it was obvious to adopt this CSP for the separation of 1-(aminoalkyl)-2-naphthol stereoisomers (**3a**-**e**). As expected, baseline separations (R_S>1.5) were easily obtained in all cases.

Table 7.
Volume of substituents (V^a) and chromatographic data, retention factors (k'), separation factors (α) and resolutions (R_S) of the enantioseparation of 1-(aminoalkyl)-2-naphthol analogs

Compound	V^a	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S
1a	-	70/30/0.1	1.32	3.82	2.89	8.85
3a	2.84	90/10/0.1	4.12	5.56	1.35	3.79
		70/30/0.1	1.26	1.80	1.43	2.62
		60/40/0.1	0.93	1.34	1.44	2.40
		50/50/0.1	0.68	0.98	1.44	2.38
		30/70/0.1	0.54	0.79	1.46	2.28
		10/90/0.1	0.45	0.70	1.56	2.44
3b	4.31	70/30/0.1	1.13	1.68	1.49	2.90
3c	4.78	70/30/0.1	1.02	1.62	1.59	3.19
3d	5.74	70/30/0.1	0.91	1.54	1.69	4.08
3e	7.16	70/30/0.1	0.82	1.43	1.74	4.52

Column, Chiralcel OD-H; mobile phase, *n*-hexane/IPA/DEA; detection, 232 nm; flow rate, 0.5 ml min^{-1}; temperature, 298 K

Table 7 reveals that the retention factor of **3a** is increased on decrease of the percentage of IPA in the mobile phase, in accordance with the decrease in the solvent polarity. With decreasing mobile phase polarity, the strength and the number of H-bonds between the solute and the polar carbamate residues of the CSP increase and the solubility of the solute in the mobile phase decreases. The separation factor (α) slightly decreased, while the resolution (R_S) changed in parallel with the k' values, *i.e.* R_S increased with decreasing IPA content. At higher alcohol content, the IPA molecules may not only compete for chiral binding sites with the solute, but can also cause swelling of the CSP by binding to achiral sites at or near the chiral

cavities. With loss of the chiral cavities, the inclusion interactions are lost, and *k'* and R_S therefore decrease. Inclusion and steric phenomena seem to be important factors in the resolution mechanism.

To determine the specific structural effects of the 1-(aminoalkyl)-2-naphthol analogs (**3a**-**e**) on the chromatographic parameters, the dependence of the experimental chromatograpic *k'* data on the volume of the anchor sphere of the alkyl substituents (V^a) was investigated (Fig. 13) at an *n*-hexane/IPA/DEA mobile phase composition of 70/30/0.1 (v/v/v). The steric effects of the substituents on the reaction rates were characterized by the size-descriptor, V^a (Meyer parameter) [28]. All the plots of V^a *vs k'* were fitted by straight lines with good correlation coefficients. Linear regression analyses revealed that the retention of the analytes depended strongly on the volumes of the alkyl substituents; larger or more bulky substituents inhibited the interaction with the selector, and at the same time the interactions of the two stereoisomers with the CSP differed appreciably. Interestingly, larger *k'* values were associated with smaller enantioselectivities and resolutions. The increased electron-donating ability of the larger alkyl chains increased the electron density on the amino and hydroxy groups of the analytes, which influenced the H-bonding interaction with the cellulose *tris*-3,5-dimethylphenyl carbamoylated selector. Moreover, the enantioselectivity was not mostly influenced by the H-bonds: the steric interactions caused by the substituent groups R of the analyte and the CSP had a more prominent effect in stabilizing the solute-CSP complex. Analyte **1a**, included in the study for purposes of comparison, showed the highest retention among the investigated analytes, due to the presence of the most bulky phenyl substituent. The especially large α and R_S values of this compound may be attributed to the additional π-π interactions between the aromatic moiety of the analyte and the phenyl groups of the CSP.

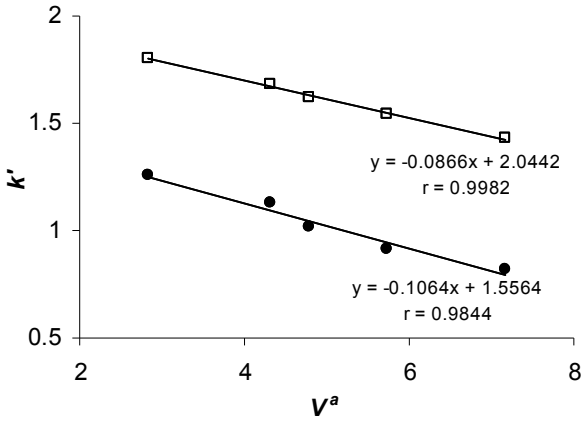


Figure 13.
Dependence of retention factors of 1-(aminoalkyl)-2-naphthol analogs on the Meyer substituent parameter (V^a); ●, k'_1; □, k'_2

The elution sequence was determined for analyte **3d** and was found to be (*S*)<(*R*). A similar elution sequence can probably be presumed for other 1-(aminoalkyl)-2-naphthol analogs, as observed for 1-(α-aminobenzyl)-2-naphthol and 1-(α-aminobenzyl)-2-naphthol analogs in Section 4.1.

4.2.2. Separation on Chiralcel OD-RH column in RP mode

The polysaccharide CSPs are mostly used with NP eluents and there have been significantly fewer reports of the separation of enantiomers with the use of these CSPs in aqueous mobile phase systems. The Chiralcel OD-RH column has the same cellulose *tris*-3,5-dimethylphenyl carbamate selector as Chiralcel OD-H, but it has a separation ability under RP conditions.

Table 8.
Chromatographic data, retention factors (k'), separation factors (α) and resolutions (R_S) of the enantioseparation of 1-(aminoalkyl)-2-naphthol analogs

Compound	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S
1a	**a**	16.89	35.09	2.08	9.00
	b	4.55	6.61	1.45	2.67
	d	27.87	44.60	1.60	2.00
3a	**a**	0.68	0.84	1.24	0.74
	c	4.78	6.38	1.30	1.23
3b	**a**	1.13	1.57	1.39	1.25
	c	5.09	7.81	1.53	1.71
3c	**a**	2.55	3.86	1.51	2.57
	c	13.10	20.63	1.57	1.70
3d	**a**	1.85	2.69	1.45	1.73
	c	11.20	16.66	1.49	1.47
3e	**a**	4.06	6.01	1.46	2.84
	c	27.25	38.15	1.40	2.50

Column, Chiralcel OD-RH; mobile phase, **a,** 0.2 M aqueous KH_2PO_4(pH 6.0)/MeCN=80/20, **b,** 0.2 M aqueous KH_2PO_4(pH 5.0)/MeCN=80/20, **c,** 0.2 M aqueous KH_2PO_4(pH 6.0)/MeOH=75/25, **d,** 0.2 M aqueous KH_2PO_4(pH 5.0)/MeOH=75/25; detection, 232 nm; flow rate, 0.5 ml min^{-1}; temperature, 298 K

Table 8 presents the retention factors (k'), separation factors (α) and resolutions (R_S) of the 1-(aminoalkyl)-2-naphthol enantiomers (**3a**-**e**), with several kinds of mobile phase, involving change of the nature and percentage of the organic component and the pH of the aqueous buffer. All compounds were evaluated with a minimum of three mobile phases in different compositions, where baseline separations were generally obtained.

The effect of pH in the aqueous part of the mobile phase can be seen for analyte **3c** as model compound in Fig. 14A. Increase of the pH from 2.0 to 6.0 in the inorganic part of the 0.2 M KH_2PO_4/MeOH=75/25 (v/v) eluent led to strong increases in the retention factors, while the selectivity and resolution exhibited only slight increases. All of the other analytes displayed similar tendencies with the same buffer between pH 2.0 and 6.0 in either MeCN or MeOH as organic modifier (data not shown).

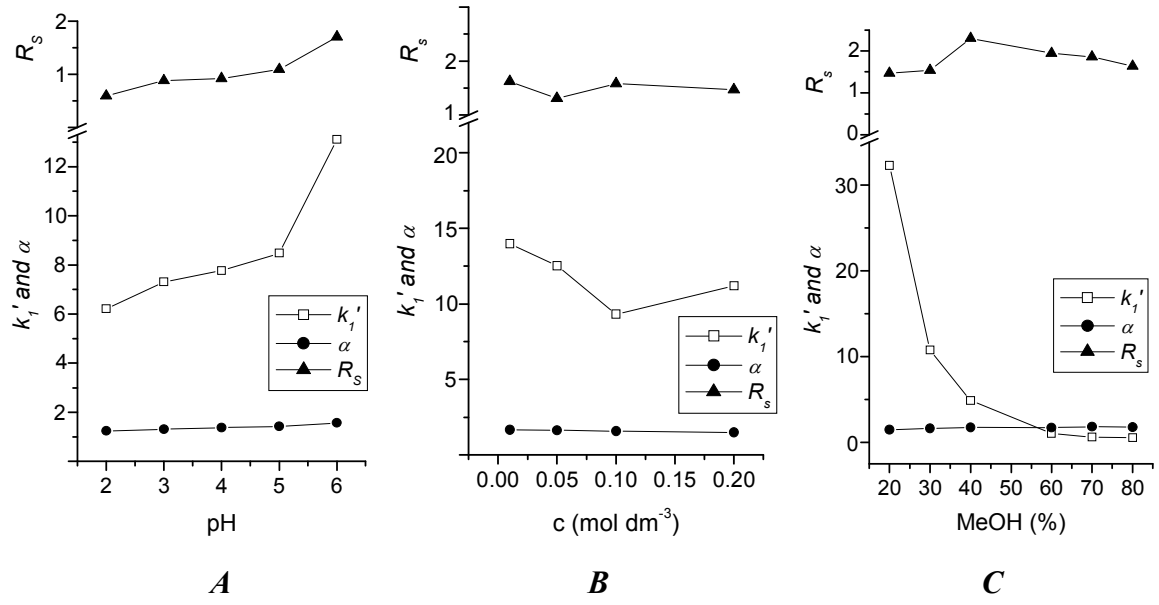


Figure 14.

Influence of pH (**A**), buffer concentration (**B**) and MeOH content of mobile phase (**C**) on retention, selectivity and resolution of analytes **3c** (**A** and **C**) and **3d** (**B**). Chromatographic conditions: column, Chiralcel OD-RH; mobile phase, (**A**) 0.2 M aqueous KH_2PO_4(pH 2.0-6.0)/MeOH = 75/25 (v/v), (**B**) 0.01-0.2 M aqueous KH_2PO_4(pH 6.0)/MeOH = 75/25 (v/v), (**C**) 0.05 M aqueous KH_2PO_4(pH 6.0)/MeOH = 80/20-20/80 (v/v); detection, 230 nm; flow rate, 0.5 ml min^{-1}; temperature, 298 K

Figure 14B depicts the effect of the phosphate buffer concentration (ionic strength) on the chromatographic data for analyte **3d**. Similar tendencies were observed for the other analytes (data not shown). At lower buffer concentration, the retention factors were always somewhat higher than at the highest concentration, while the selectivity and resolution remained unchanged.

The retention depended strongly on the organic modifier content of the mobile phase, as shown in Fig. 14C for analyte **3c**. Decreases in *k'* for all compounds were observed on increase of the percentage of organic modifier, as expected from the decrease in eluent polarity, but this was not accompanied by significant decreases in α and R_S. These observations suggest the importance of hydrophobic interactions in the retention mechanism. As shown in Table 8, both MeCN and MeOH provided good chromatographic results for the 1-(aminoalkyl)-2-naphthol analogs. At similar eluent strength (eluent **a** and **c**; or eluent **b** and **d**), the change of the mobile phase modifier from MeCN to MeOH resulted in increases in the retention factors, while no definite trend was found for the changes in selectivity and resolution.

Since the selector of the CSP lacks acid-base functionalities, the dependence of the chromatographic parameters on the pH and ionic strength is probably due to the acid-base

properties of the analytes. The pK_a values of analytes **1a** and **3a**-**e** are not known. For analytes with a similar structure, *e.g.* for 2-(aminomethyl)phenol [29] in an organic-water phase, values of pK_1=12.8 and pK_2=6.3; and 2-(4-hydroxyphenyl)ethyl-amine [30] in a water phase, pK_1=9.2 and pK_2=7.4 have been reported. From these data, we can assume that under acidic conditions both the amino and phenolic hydroxy groups of the 1-(aminoalkyl)-2-naphthol derivatives are protonated, so the molecules are positively charged. With increasing pH, the more acidic phenolic group loses its proton and the molecule assumes a zwitterionic form, becoming more hydrophobic and resulting in stronger hydrophobic interactions. The retention therefore increases drastically with increasing pH. The effect of the organic modifier on the retention also supported the importance of hydrophobic interactions. The hydrophobic interactions are probably similar for both enantiomers and therefore the selectivity did not change significantly with the change of pH or organic modifier content. The ionic strength generally affects separations involving coulombic interactions. Here, the concentration of phosphate buffer can influence only the state of ionization of the analyte and not that of the selector, and thus its effect was neghigible.

At constant mobile phase composition of eluent **a** or eluent **c**, larger or more bulky alkyl chain substituents resulted in larger retention factors (Table 8). At the same time, increases in selectivity and resolution were obtained. The higher retention factors in the RP mode may be explained by the additional hydrophobic interactions, which are absent in the NP mode, where the *k'* values changed in the opposite way with the length and bulkiness of the alkyl substituents.

The elution sequence was determined for analyte **3d** and, as in the NP mode, it was (*S*)<(*R*).

4.3. Separation of the enantiomers of β-substituted-β-amino acid analogs

β-Amino acids have increased in importance in the past few decades with regard to their unique biological activity and their meaningful presence in synthetic chemistry and drug research. In their free forms, they can exhibit neurological activity and are known to be receptor antagonist and enzyme inhibitors [135]. The structural moiety of *β*-amino acids is found in numerous biologically active natural products, *e.g.* taxane alkaloids, *β*-lactam antibiotics and compounds with antitumor properties. Besides their own pharmacological activity, *β*-amino acids can be used as building blocks for the preparation of modified (unnatural) analogs of biologically active peptides. By insertion of a *β*-amino acid instead of an *α*-amino acid into the naturally-occurring pharmacologically active peptides, the activity or

the effect can be modified and the stability of the natural peptides can be increased. These investigations are applied for determination of the fine structures of receptors [136]. On the other hand, *β*-amino acids can be used as the starting substances of different heterocycles with the aim of preparing potential pharmacons [137]. Their enantiomerically pure forms can serve as chiral auxiliaries or additives. Their use in combinatorial chemistry is also in progress.

In consequence of the wide-ranging utility of these compounds, considerable attention has been paid to the preparation of *β*-amino acids in enantiopure forms via enantioselective synthesis or resolution of the racemates. This requires enantioselective analytical methods to control the enantiomeric purity of the final products. For this purpose, HPLC is widely used.

The HPLC enantioseparation of *β*-amino acids includes both direct and indirect methods. Winnacker *et al*. [138], Yamada *et al*. [139], and Török *et al*. [140] applied different CDAs. Davankov *et al*. [141], Lindner and Hirschbock [142] and Yamakazi *et al*. [143] reported ligand-exchange chromatographic methods. Griffith *et al*. [144] used a *π*-complex column, while D'Acquarica *et al*. [145], Péter *et al*. [146,147] and Hyun *et al*. [148] separated different alicyclic, cyclic and aromatic *β*-amino acids on new types of CSPs, containing a macrocyclic glycopeptide antibiotic, quinine-derived chiral anion-exchanger and crown ether as chiral selectors.

Stereoisomers of analytes **4a**, and **4o-q** were examined previously on macrocyclic glycopeptide-based CSPs [149], and the present work therefore takes the results published in the literature as its starting point. In our study, the direct separations of the *β*-homoamino acid enantiomers (**4a-r**, Scheme 5) were performed on six CSPs: teicoplanin-based (Chirobiotic T and T2) columns, a teicoplanin aglycone-based (Chirobiotic TAG) column, vancomycin-based (Chirobiotic V and V2) columns and a ristocetin A-based (Chirobiotic R) column. All of the examined synthetic *β*-amino acids were evaluated by using all stationary phases with a minimum of five of the following PI or RP mobile phases: MeOH/AcOH/TEAA in different compositions, 100% MeOH and 0.1% TEAA (pH=4.1 or 6.5)/MeOH in different compositions. To simplify the presentation, only the chromatographic results for a partial or baseline separation are given in the Tables. However, for purposes of comparison, examples are included in some cases where no separations occurred. Chromatograms obtained are shown in the Appendix.

4.3.1. Enantioseparations on ristocetin A and vancomycin-based CSPs

On all the macrocyclic antibiotic CSPs, several factors can affect the retention and selectivity, such as the nature and concentration of the organic modifier, the presence of mobile phase additives or the pH of the buffered mobile phase. In some cases, variation of the flow rate had a beneficial effect on the resolution. The selectivity of these CSPs can be related to the amino groups. The most important enantioselective binding site of vancomycin is the secondary amine group of the *N*-methylleucine moiety [150]. In the case of ristocetin A, the primary amine attached to the macrocyclic ring is a necessary interaction site for chiral recognition. Under the working conditions applied, the actual pH of the aqueous component of the mobile phase was 4.1 or 6.5. Thus, the molecules of the chiral selector and the analytes exist in ionic form. The primary predominant step in chiral recognition in the RP mode is the strong charge-to-charge interaction between the carboxy group of the amino acid and the ammonium group of the CSP. The secondary and tertiary structures of vancomycin and ristocetin A molecules play an additional important role in chiral recognition by supplying appropriate H-bonding, hydrophobic and steric interaction sites.

Table 9.
Chromatographic data, retention factor of the first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) of the enantioseparation of β-amino acids on ristocetin A and vancomycin-based CSPs

Analyte	Column	Mobile phase (v/v/v)	k_1'	α	R_S	Elution sequence
4a	R	100/0.1/0.1	1.38	1.09	0.80	*R*<*S*
	V	100/0.1/0.1	0.70	1.13	0.80	*S*<*R*
4b	R	100/0/0	1.51	1.14	0.80	*R*<*S*
	R	100/0.1/0.1*	1.26	1.14	0.90	*R*<*S*
4d	R	100/0.04/0.01	1.80	1.10	0.80	n.d.
4e	R	100/0.1/0.1	1.43	1.15	1.00	*R*<*S*
	V	100/0.1/0.1	0.75	1.16	0.80	n.d.
	V	100/0/0	0.85	1.13	0.85	*S*<*R*
4g	R	100/0.1/0.1	1.75	1.15	1.00	n.d.
4j	R	100/0.01/0.01	1.20	1.10	0.80	*R*<*S*
	V	100/0.1/0.1	0.71	1.13	0.80	*S*<*R*
4k	R	100/0.1/0.1	1.23	1.11	0.70	*R*<*S*
4n	R	100/0.01/0.01	1.33	1.10	0.75	*R*<*S*
	V	100/0.1/0.1	0.77	1.13	0.75	n.d.
4r	V	100/0/0	1.02	1.07	0.70	n.d.

Column, R, Chirobiotic R; V, Chirobiotic V; mobile phase, MeOH/AcOH/TEA; flow rate, 0.5 ml min^{-1}, * 0.25 ml min^{-1}; detection, 205 nm; temperature, 298 K; n.d., elution sequence not determined.

In the first experiments, 0.1% TEAA (pH=4.1 or 6.5)/MeOH was applied as mobile phase in different compositions, but unfortunately most of the β-amino acid enantiomers were

poorly resolved or could not be separated at all (data not shown). The enantioselectivity and resolution underwent only a small improvement in the cases of analytes **4b**,**e**, and **r**, even when 100% MeOH was used as mobile phase.

In order to achieve improved separation, a PI mobile phase system was applied. In the PI mode, the key factor is the acid-to-base ratio. Altering the ratio can have a dramatic effect on the selectivity. The concentrations of the organic acid and base at a suitable ratio can affect the retention of the analytes. In the PI mode, the main interactions are H-bonding, π-π and steric interactions. To some extent, charge-charge interactions may occur, similarly as in the RP mode.

The PI mode exhibited some selectivity for these types of β-amino acids, but despite the large variations in the conditions, half of the stereoisomers were unresolved and the stereoisomers of **4a**,**b**,**d**,**e**,**g**,**j**,**n** exhibited only partial resolution, with R_S<1 (Table 9). The ristocetin A selector generally displayed a better separation performance than that of the vancomycin-based CSP, possibly due to the fact that the position and order of the amino group on the macrocyclic glycopeptide selector is a determining factor for the establishment of chiral recognition and enantioselectivity.

The elution sequence of enantiomers was in most cases determined by cochromatography of the racemate with a stereoisomer of known configuration. It was found that on the vancomycin-containing CSP the (*S*) enantiomers elute before the (*R*) enantiomers, while on the ristocetin A-containing column the reverse elution sequence was observed.

4.3.2. Enantioseparations on teicoplanin and teicoplanin aglycone-based CSPs

The three other stationary phases applied for direct enantioseparation that contained a chiral antibiotic as chiral selector were teicoplanin (Chirobiotic T and T2) and teicoplanin aglycone (Chirobiotic TAG). On these CSPs, the β-amino acids were analyzed by working in the RP or PI mode. The chiral selector molecules of T and T2 are both bound to 5 nm particle size silica gel, but they differ from each other in the pore size of the silica particles, which are 120 and 200 Å, respectively. Moreover, the linkage chain on Chirobiotic T2 is approximately twice the length of that on Chirobiotic T. Hence, the coverage and spacing are different for the two CSPs. Due to this fact, it was expected that the form of the steric interactions would differ between the two columns.

Table 10.

Chromatographic data on the enantioseparation of *β*-amino acids on teicoplanin and teicoplanin aglycone-based CSPs

Analyte	Column	Mobile phase (v/v/v)	k_1'	α	R_S	Elution sequence
4a	T	100/0.01/0.01,**a***	1.67	1.00	0.00	-
	T2	100/0.01/0.01,**a**	2.08	1.17	1.00	*S*<*R*
4b	T	100/0.01/0.01,**a***	1.62	1.00	0.00	-
	T2	100/0.01/0.01,**a**	2.13	1.13	0.95	*S*<*R*
4c	T	100/0.01/0.01,**a***	1.32	1.14	<0.40	n.d.
	T2	100/0.01/0.01,**a**	1.93	1.23	1.09	
4d	TAG	100/0.01/0.01,**a**	3.32	1.05	0.50	
	T	100/0.01/0.01,**a***	2.01	1.00	0.40	n.d.
	T2	100/0.01/0.01,**a**	2.66	1.13	0.90	
4e	T	100/0.01/0.01,**a***	1.70	1.00	0.00	n.d.
	T2	100/0.01/0.01,**a**	2.66	1.00	0.00	
4f	TAG	100/0.01/0.01,**a**	2.67	1.12	0.90	*S*<*R*
	T	100/0.01/0.01,**a***	1.67	1.00	<0.40	-
	T2	100/0.01/0.01,**a**	1.97	1.13	0.80	*S*<*R*
4g	TAG	100/0/0,**a**	5.46	1.06	0.75	
	T	100/0.01/0.01,**a***	2.34	1.00	0.00	n.d.
	T2	100/0.01/0.01,**a**	3.08	1.00	0.00	
4h	T	100/0.01/0.01,**a***	1.76	1.06	<0.40	-
	T2	100/0.01/0.01,**a**	2.45	1.20	1.15	*S*<*R*
4i	T	100/0.01/0.01,**a***	1.88	1.06	<0.40	-
	T2	100/0.01/0.01,**a**	2.72	1.18	1.10	*S*<*R*
4j	T	100/0.01/0.01,**a***	1.61	1.00	0.00	-
	T2	100/0.01/0.01,**a**	2.21	1.15	0.90	*S*<*R*
4k	T	100/0.01/0.01,**a***	1.46	1.08	<0.40	-
	T2	100/0.01/0.01,**a**	2.17	1.00	0.00	*S*<*R*
4l	T	100/0.01/0.01,**a***	1.83	1.08	<0.40	n.d.
	T2	100/0.01/0.01,**a**	2.85	1.11	0.85	
4m	T	100/0.01/0.01,**a***	1.94	1.07	<0.40	-
	T2	100/0.01/0.01,**a**	3.00	1.18	1.10	*S*<*R*
4n	T	100/0.01/0.01,**a***	1.77	1.00	0.00	n.d.
	T2	100/0.01/0.01,**a**	2.35	1.14	1.00	
4o	TAG	10/90,**b***	4.29	1.10	1.00	-
	T	100/0.1/0.1,a*	3.84	1.15	1.00	*S*<*R*
	T2	100/0.01/0.01,**a**	1.99	1.27	1.65	*S*<*R*
4p	TAG	10/90,**b***	4.53	1.07	0.80	*S*<*R*
	T	100/0.4/0.1,**a***	3.79	1.03	<0.40	*S*<*R*
	T2	100/0.01/0.01,**a**	2.28	1.00	0.00	-
4q	TAG	10/90,**b***	10.26	1.18	1.10	*S*<*R*
	T	100/0.1/0.1,**a**	6.29	1.09	1.00	*S*<*R*
	T2	100/0.01/0.01,a	3.93	1.00	0.00	-
4r	TAG	100/0.01/0.01,a	3.23	1.16	1.00	
	T	100/0.01/0.01,a*	1.81	1.09	<0.40	n.d.
	T2	100/0.01/0.01,a	2.33	1.15	0.95	

Column, T, Chirobiotic T; T2, Chirobiotic T2; TAG, Chirobiotic TAG; mobile phase, **a**, MeOH/AcOH/TEA, **b,** 0.1% aqueous TEAA (pH 4.1)/MeOH; flow rate, 0.5 ml min^{-1}, * 0.8 ml min^{-1}; detection, 205 nm; temperature, 298 K; n.d., elution sequence not determined

Berthod *et al*. [151] earlier found that teicoplanin aglycone afforded a higher separation capability for α-amino acids than did native teicoplanin in the PI mode. Opposite behavior was observed for β-3-homoamino acids by Árki *et al*. Péter *et al*. [149,152]. Table 10 summarizes the results concerning the best resolutin of the investigated β-3-homoamino acids (**4a**-**r**). With the same PI mobile phase composition, the retention factors of the first-eluted enantiomers were lower on the teicoplanin CSP than on the aglycone phase, because of the overall polarity differences between the T and TAG columns. From the chiral separation point of view, the sugar moieties of native teicoplanin may cause steric hindrance by occupying space inside the "basket", which limits the access of analyte molecules to the binding sites. In place of the three sugar units, there are two phenols and a hydroxy group on the TAG CSP, which further enhance the interaction with the amino acid enantiomers. At the same eluent composition, MeOH/AcOH/TEA=100/0.01/0.01 (v/v/v), for analytes **4d**,**f**,**r**, higher separation factors were obtained on the Chirobiotic TAG column than on the Chirobiotic T and T2 CSPs. The presence of methoxy and naphthyl groups in **4d**,**f**,**r** allows them to associate more strongly and easily with the active binding site of the aglycone than they can on the native teicoplanin selector. This closer approach produces a stronger enantioreaction and better enantioselectivity.

A comparison of the separation performances of the Chirobiotic T and T2 CSPs under the same chromatographic conditions revealed that the retention factors of the first-eluting enantiomers on Chirobiotic T2 were larger by 25-30% (exceptions were analytes **4o-q**, for which the larger k_1' values were obtained on Chirobiotic T). A possible explanation appears to be that Chirobiotic T2 is a more densely covered phase, and thus stronger interactions may form between the analytes and this recently developed CSP.

Resolution of a compound on the macrocyclic antibiotic CSPs is sensitive to the fine structure of the studied analyte. Due to this fact, it was expected that a substituent group, even situated far from the chiral center, can produce some impact on the chromatographic parameters of these compounds. For analogs **4d**-**g** or **4i-l**, having the same substituents but in different positions, the *para*-substituted compounds had larger retention factors and generally larger α values than the *meta*- and *ortho*-substituted ones on the T2 column. On comparison of the analytes containing halogen atoms in the same positions (**4h**,**i**,**m** or **4j**,**n**), larger retention factors were obtained for the bromo-substituted analogs than for the chloro- or fluoro-substituted ones, whereas the α and R_S values did not change significantly. While the primary "docking" interactions between the amine of the CSP and the carboxylate of these series of compounds should be similar, different secondary interactions (such as H-bonding, steric and hydrophobic) can cause the difference in the process of chiral recognition. If the

steric repulsive interactions are very different, they could affect the other interactions, *e.g.* they could prevent or minimize the primary interaction. As regards H-bonding, its ability decreases in the sequence F>Cl>Br, whereas the hydrophobicity and bulkiness of the analytes increase in the same sequence. In consequence of the above-mentioned effects, the bromo-substituted analogs exhibited the largest retention among the halogen-containing compounds. These results indicate the importance of steric and hydrophobic interactions in the separation of *β*-substituted-*β*-amino acids.

In most cases, the sequence of elution of the enantiomers was identified by cochromatography of racemic mixtures with an enantiomer with known configuration. The elution sequence for *β*-substituted-*β*-amino acids on the macrocyclic antibiotic CSPs seems to follow a general rule: the (*S*) enantiomers eluted before the (*R*) enantiomers, with the exception of the Chirobiotic R column, where the reverse elution sequence was observed. These results shed light on the importance of the selection of the most suitable chiral column. Trace levels of enantiomeric impurity are determined more easily and more exactly when the impurity peak appears before the main peak of a drug administered as the pure enantiomer.

4.3.3. Separation on chiral crown ether-based CSP

In the past decade, extensive research has been performed on the resolution of various primary amino compounds by recently developed crown-ether based CSPs with a chiral selector of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid [78,79]. With the aim of finding a more uniformly applicable CSP with enhanced chiral recognition ability in the separation of *β*-substituted-*β*-amino acid enantiomers, we developed direct RP methods applying a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP.

First of all, the effect of the organic modifier on the enantioselectivity exerted by the CSP was investigated at lower (20% v/v) and higher (80% v/v) MeOH contents, at a constant AcOH concentration of 10.0 mM. Chromatographic results are summarized in Table 11, together with data obtained at an optimized mobile phase composition with a MeOH content of 50% (v/v) and an AcOH concentration of 5.0 mM. As expected, all the investigated *β*-amino acids were resolved very well on this CSP, with the exception of **4f**. As the content of MeOH in the aqueous mobile phase was increased from 20 to 50% (v/v), the retention factors (*k'*) increased. As the concentration of MeOH was increased, the mobile phase became less polar and more hydrophobic. In this instance, the hydrophilic interactions between the mobile phase and the polar protonated analytes decreased, and consequently the retention increased.

Table 11.
Retention factors (*k'*), separation factors (α), resolutions (R_S) and elution sequences of stereoisomers of β-amino acids

Analyte	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S	Elution sequence
4a	80/20,**a**	2.27	2.94	1.30	1.44	
	20/80,**a**	8.66	9.89	1.14	1.13	*R*<*S*
	50/50,**b**	2.98	3.93	1.32	1.77	
4b	80/20,**a**	2.96	3.71	1.26	0.85	
	20/80,**a**	6.85	8.21	1.20	1.06	*R*<*S*
	50/50,**b**	2.86	3.63	1.27	1.46	
4c	80/20,**a**	4.16	5.68	1.36	0.90	
	20/80,**a**	17.87	23.55	1.32	2.38	n.d.
	50/50,**b**	3.52	5.33	1.51	1.97	
4d	80/20,**a**	2.66	3.43	1.29	1.31	
	20/80,**a**	7.09	8.56	1.21	1.05	n.d.
	50/50,**b**	2.92	3.83	1.31	1.73	
4e	80/20,**a**	3.60	4.51	1.25	1.45	
	20/80,**a**	10.50	12.28	1.17	1.00	n.d.
	50/50,**b**	3.83	5.02	1.31	1.71	
4f	80/20,**a**	1.31	1.31	1.00	0.00	
	20/80,**a**	2.71	2.71	1.00	0.00	n.d.
	50/50,**b**	2.18	2.18	1.00	0.00	
4g	80/20,**a**	3.06	3.94	1.29	1.43	
	20/80,**a**	17.44	21.30	1.22	1.84	n.d.
	50/50,**b**	4.43	5.68	1.28	1.88	
4h	80/20,**a**	2.84	4.02	1.42	1.97	
	20/80,**a**	10.74	13.72	1.28	1.58	*R*<*S*
	50/50,**b**	3.25	4.53	1.39	1.59	
4i	80/20,**a**	4.24	6.06	1.43	1.86	
	20/80,**a**	10.39	13.84	1.34	2.03	*R*<*S*
	50/50,**b**	6.82	9.89	1.45	2.40	
4j	80/20,**a**	5.40	8.09	1.50	1.79	
	20/80,**a**	12.27	18.64	1.52	2.40	*R*<*S*
	50/50,**b**	5.69	9.19	1.62	2.53	
4k	80/20,**a**	1.81	1.81	1.00	<0.40	
	20/80,**a**	3.04	3.47	1.14	1.06	*R*<*S*
	50/50,**b**	2.51	2.76	1.10	0.85	
4l	80/20,**a**	8.97	14.95	1.62	2.27	
	20/80,**a**	24.35	39.70	1.63	2.40	n.d.
	50/50,**b**	6.27	7.66	1.22	1.91	
4m	80/20,**a**	3.80	5.61	1.48	1.54	
	20/80,**a**	14.58	20.15	1.38	2.32	*R*<*S*
	50/50,**b**	3.56	5.26	1.48	1.91	
4n	80/20,**a**	5.78	8.83	1.53	1.87	
	20/80,**a**	22.30	34.52	1.55	1.80	n.d.
	50/50,**b**	6.66	11.32	1.70	3.07	

Column, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP; mobile phase, **a**, H_2O/MeOH (v/v), 10 mM AcOH; **b**, H_2O/ MeOH (v/v), 5 mM AcOH; flow rate, 0.5 ml min^{-1}; detection, 210 nm; temperature, 298 K; n.d., elution sequence not determined

These trends were observed for all the investigated *β*-amino acid analogs. The results in Table 11 revealed that the α values generally decreased or did not change significantly (analytes **4j**,**l**,**n**) with increasing MeOH content, while for analyte **4k** a slight increase was observed. At high MeOH content, the interactions between the first- and second-eluting enantiomers and the CSP were different from those at low MeOH content. When the MeOH content is increased, nonchiral hydrophobic interactions are favored for both enantiomers. As a consequence, the overall retention increased, while the role played by the chiral recognition decreased and so the selectivity also decreased. In the case of analyte **4k**, the very small *k'* values may indicate that the *ortho* position of the chlorine atom hindered the interaction with the CSP, but increased MeOH content promoted the chiral retention, probably due to a different interaction mechanism with the CSP. The resolution factors usually do not display significant trends with variation of the MeOH content: the R_S values slightly decreased with increasing organic modifier content for analytes **4a**,**d**,**e**,**h**,**n**, but the opposite behavior was observed for analyte **4b**,**c**,**g**,**i-m**.

In order to investigate the effects of acidic modifiers on the resolution behavior of the CSP, analytes **4f**,**i**,**k** were chosen for more detailed study. The concentration of MeOH was fixed at 50% (v/v). Even though the chiral recognition mechanism is still controversial, it has been generally accepted that, for the crown ether-based CSP, the most important interaction is the host-guest complexation of the primary ammonium ion ($R\text{-}NH_3^+$) and the oxygen atoms in the crown ether ring. The pK_a values of the investigated *β*-amino acids are not known, except for analyte **4a**, for which is about 9. Under all the applied mobile phase conditions, supposing similar pK_a values, the amino groups can be considered to be always completely protonated. The pK_a values of the four carboxy groups of the CSP are 2.13, 2.84, 4.29 and 4.88 [78]. Therefore, above pH=2 these carboxy groups dissociate and can contribute to the electrostatic interaction with the cationic analytes. As shown in Table 12, a comparison of the chromatographic results obtained by using AcOH, $HClO_4$, H_2SO_4, H_3PO_4 and TFA as acidic modifiers demonstrates that AcOH is much more suitable than the others in terms of retention, enantioselectivity and resolution. The retention factors (*k'*) were significantly diminished as the concentration of AcOH in the mobile phase was increased. The reason for this chromatographic behavior is not yet clear, but it is assumed to be a result of the greater ionic strength of the mobile phase at a higher concentration of AcOH. In this instance, the hydration or the dissolution of polar protonated analytes by the mobile phase increases and, consequently, the retention of polar protonated analytes decreases. The enantioselectivity (α), however, remained almost constant with variation of the content of AcOH in the aqueous mobile phase, as shown in Table 12. The resolution factors (R_S) were found to exhibit a

slightly decreasing trend on increase of the AcOH concentration in the case of analyte **4i**. The application of stronger acids in the same amount instead of AcOH diminished the retention times, selectivities and resolution factors considerably. However, for analyte **4f** a partial separation was observed in $HClO_4$, H_2SO_4 and H_3PO_4, while AcOH furnished unsuccessful results. The reason for this chromatographic resolution behavior is not yet clear.

Table 12.
Dependence of chromatographic parameters, retention factor of first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) on the concentration of the acidic modifier in the mobile phase

Compound	Nature of acid	Concentration of acid (mM)	k_1'	α	R_S
4f	AcOH	1.0	3.03	1.00	0.00
	AcOH	5.0	2.18	1.00	0.00
	AcOH	10.0	1.68	1.00	0.00
	AcOH	20.0	1.32	1.00	0.00
	$HClO_4$	5.0	0.78	1.00	0.00
	$HClO_4$	10.0	0.02	1.10	0.60
	H_2SO_4	5.0	0.18	1.50	0.60
	H_3PO_4	5.0	0.23	1.00	0.00
	H_3PO_4	10.0	0.20	1.25	>0.40
	TFA	5.0	0.0	1.00	0.00
4i	AcOH	1.0	8.84	1.45	2.41
	AcOH	5.0	6.82	1.45	2.40
	AcOH	10.0	5.70	1.45	2.38
	AcOH	20.0	4.58	1.45	2.20
	$HClO_4$	5.0	1.28	1.16	0.64
	H_2SO_4	5.0	2.65	1.32	1.56
	H_3PO_4	5.0	0.71	1.00	0.00
	TFA	5.0	0.97	1.23	0.75
4k	AcOH	1.0	3.27	1.11	0.70
	AcOH	5.0	2.51	1.10	0.85
	AcOH	10.0	2.01	1.10	0.70
	AcOH	20.0	1.64	1.11	0.70
	$HClO_4$	5.0	0.20	1.00	0.00
	H_2SO_4	5.0	0.35	1.00	0.00
	H_3PO_4	5.0	0.71	1.00	0.00
	TFA	5.0	0.10	1.00	0.00

Column, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP; mobile phase, H_2O/MeOH=50/50 (v/v), containing different amounts of acidic modifier; flow rate, 0.5 ml min^{-1}; detection, 210 nm, temperature, 298 K

The investigation of the influence of small structural changes in this series of analytes revealed that both the retention and the selectivity are unambiguously controlled by the nature and position of the substituents on the β-amino acids (Table 11). As a general trend, the *meta*- and *para*-substituted analogs exhibited larger retentions, while the *k'* values of analogs possessing *ortho*-substituents were smaller than that of the non-substituted **4a**, especially in

mobile phases containing a large amount of organic modifier. For the 3',4'-disubstituted analogs **4g**,**l**, especially large *k'* values were obtained. A comparison of the retention factors obtained for the analytes containing the same substituents in different positions on the aromatic ring (**4d-g**,**i**,**l**) revealed that the *meta*-substituted analogs interacted more strongly with the chiral selector molecules than did the *para*-substituted ones, and the compounds containing *ortho* substituents exhibited much weaker affinities. Comparison of the chromatographic data on analytes bearing different halogen substituents in the same position, **4h**,**i**,**m**,**j**,**n**, revealed that the retention factors were smaller for the fluoro- than the bromo-substituted analogs. Interestingly, the *k'* values of the chloro-substituted analyte **4i** were the largest among the three β-amino acids possessing halogen atoms on position 4' of the aromatic ring. The selectivity and the resolution increased in the sequence F<Cl<Br. All of the obtained data demonstrate that, besides complex formation in the crown ether cavity, intermolecular H-bonding interaction may occur between the halogen atoms of the analyte and the amino tethering group of the CSP [153].

The results of these investigations concerning the positions of the substituents reveal that electron-donating atoms (oxygen or halogen) in the *meta* or *para* position are favorable for H-bonding interactions, while those in the *ortho* position may hinder complex formation, since they are sterically situated very close to the primary amino function.

The elution sequences shown in Table 11 were determined by injecting configurationally known samples. However, the elution sequences in some cases were not determined, because configurationally known samples were not available. The elution sequences were consistent, the (*R*) enantiomers being retained longer than the (*S*) enantiomers. Selected chromatograms for the enantioseparation of analytes **4a**-**n** are depicted in the Appendix.

5. SUMMARY

The main purpose of this work was to develop chiral HPLC methods suitable for the resolution and identification of the enantiomers of differently substituted aminonaphthols and β-amino acids of both synthetic and pharmaceutical interest.

The enantiomers of the α-aminobenzyl- and α-aminoalkyl-substituted 1- and 2-naphthol analogs were separated by direct methods, using two cellulose *tris*-3,5-dimethylphenyl carbamate-based CSPs and a 3,5-dimethylphenyl carbamoylated β-CD-based CSP. To the best of our knowledge, to date there has not been any publication dealing with the HPLC separation of the enantiomers of these aminonaphthol analogs. The analytes could be separated with good resolutions in the applied RP or NP eluent systems on all three CSPs. Optimization of the separation was achieved by change of the pH and the buffer concentration in the RP mode and by variation of the mobile phase composition in both RP and NP modes. The retention factors, selectivities and resolutions depended strongly on the mobile phase composition in the NP mode, while in the RP mode the change of the organic modifier content, the concentration and the pH of the buffers involved the significant change only of the retention factors. It was established that the position of the α-aminobenzyl subtituent in the 1- and 2-naphthol analogs influenced the retention and selectivity. Methyl, methoxy, halogen or nitro substitution on the α-aminobenzyl group slightly influenced the chromatographic parameters. Nevertheless, the chain lengths of the substituents on the 1-(aminoalkyl)-2-naphthol analogs strongly affected the enantioseparations.

The temperature effect for the isocratic retention of the enantiomers of α-aminobenzyl-substituted 1- and 2-naphthol analogs was studied on the cellulose *tris*-3,5-dimethylphenyl carbamate-based Chiralcel OD-H CSP. Optimization of the separations was achieved by change of the column temperature rather than the mobile phase composition. The retention factors, selectivities and resolution for the enantiomers of all the investigated compounds decreased with increasing temperature. The natural logarithms of the selectivities ($\ln \alpha$) of the analytes depended linearly on the inverse of temperature ($1/T$). van't Hoff plots afforded thermodynamic parameters, such as the apparent change in enthalpy ($\Delta(\Delta H^{o})$), the apparent change in entropy ($\Delta(\Delta S^{o})$) and the apparent change in Gibbs free energy ($\Delta(\Delta G^{o})$) for the transfer of the analyte from the mobile phase to the stationary phase. The values of the thermodynamic parameters depended on the structures of the compounds. The thermodynamic data revealed that all the α-aminobenzyl-substituted 1- and 2-naphthol analogs separated via the same enthalpy-driven chiral recognition mechanism.

The direct separations of eighteen unnatural β-substituted-β-amino acids were performed on six macrocyclic antibiotic-based CSPs and a chiral crown ether-based CSP. In conclusion, the results obtained have demonstrated that macrocyclic antibiotic-based CSPs are quite successful for the direct separation of various β-substituted-β-amino acids. Each of the Chirobiotic CSPs showed some selectivity for the investigated analytes, but the newly developed Chirobiotic T2 proved to be the most suitable of the six applied CSPs. The effects of different parameters on the retention and selectivity, such as the nature of the organic modifier, the mobile phase composition, the pH and the structures of the analytes, were investigated and discussed. Among the applied eluent systems, the PI mode was the most successful for the resolution of the analytes. It was found that the substitution on the aromatic rings slightly influenced the chromatographic parameters. The results observed point to the fact that the macrocyclic glycopeptides are to some extent complementary to one another: where partial enantioresolution was obtained with one glycopeptide, there was a probability that better separation could be achieved with another. The reasons for this phenomena have to do with the subtle differences in diastereomeric binding sites between the different Chirobiotic phases.

Application of the recently developed (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP was noteworthy: it provided a very valuable means of enantioseparation of various β-amino acids. This chiral crown ether-containing CSP turned out to be superior to macrocyclic glycopeptide CSPs, especially in terms of selectivity (α) and resolution factors (R_S). The separations were optimized by application of acidic mobile phase modifiers, among which AcOH proved to be the most suitable. The chromatographic retention and resolution behavior were found to be dependent on the natures and positions of the substituents on the phenyl ring of the 3-amino-3-phenylpropanoic acid analogs.

The elution sequences in such cases were determined, when configurationally known samles were available.

The described procedures were applied for the separation and identification of the α-aminobenzyl- and α-aminoalkyl-substituted 1- and 2-naphthol and β-substituted-β-amino acid enantiomers. The application of direct methods ensures the availability of effective chromatographic procedures for chiral purity control. The results obtained by application of the different CSPs and methods show that there was no general method that met all the requirements. For analytical applications, enantioseparation will maintain and even gain in importance. In any case, the challenge of understanding chirality, a key to the major secrets of life, will continue to fascinate and stimulate future generations of scientists.

REFERENCES

[1] I.W. Wainer, *Drug Stereochemistry, Analytical Methods and Pharmacology*, 2nd Edition, Marcel Dekker, New York (1993).
[2] E. Ariens, *Med. Res. Rev*. 6 (1986) 451.
[3] M. Simonyi, I. Fitos, J. Visy, *Trends Pharmacol. Sci*. 7 (1986) 112.
[4] T. Walle, U.K. Walle, *Trends Pharmacol. Sci*. 7 (1986) 155.
[5] E.J. Äriens, *Eur. J. Clin. Pharmacol*. 26 (1984) 663.
[6] B. Blaschke, H.P. Kraft, K. Fickentscher, F. Kohler, *Arzneim.-Forsch*. 29 (1979) 1640.
[7] FDA's Policy Statement for the Development of New Stereoisomeric Drugs (1992) Last update made on July 6, 2005.
[8] H. Caner, E. Groner, L. Levy, I. Agranat, *Drug Discov. Today* 9 (2004) 105.
[9] G.M. Henderson, H.G. Rule, *J. Chem. Soc.* (1939) 1568.
[10] M. Kotake, T. Sakan, N. Nakamura, S. Senon, *J. Am. Chem. Soc*. 73 (1951) 2973.
[11] C.E. Dalgliesh, *J. Chem. Soc.* 47 (1952) 3940.
[12] W. Pirkle, T. Pochapski, *Chem. Rev*. 89 (1989) 347.
[13] L.H. Klemm, D. Reed, *J. Chromatogr*. 3 (1960) 364.
[14] E. Gil-Av, B. Feibush, R. Charles-Siegler, *Tetrahedron Lett.* (1966) 1009.
[15] S. Görög, *Encyclopedia of Separation Science*, Wilson, I.D., (T.R. Ablard, C.F. Poole, M. Cook, Editors), *Academic Press*, London (2000) pp. 2310-2321.
[16] Y. Yasaka, T. Matsumoto, M. Tanaka, *Anal. Sci*. 11 (1995) 295.
[17] T. Toyo'oka, M. Ishibashi, T. Terao, *Anal. Chim. Acta* 278 (1993) 71.
[18] R. Oshima, J. Kumanotani, C. Watanabe, *J. Chromatogr*. 259 (1983) 159.
[19] N. Harada, M. Watanabe, S. Kuwahara, A. Sugio, Y. Kasai, A. Ichikawa, *Tetrahedron: Asymmetry* 11 (2000) 1249.
[20] A. Sano, S. Takitani, H. Nakamura, *Anal. Sci*. 11 (1995) 299.
[21] H. Weber, H. Spahn, E. Mutschler, W. Möhrke, *J. Chromatogr*. 307 (1984) 145.
[22] H. Spahn, H. Weber, E. Mutschler, W. Möhrke, *J. Chromatogr*. 310 (1984) 167.
[23] P. Langguth, H. Spahn, H.P. Merkle, *J. Chromatogr*. 528 (1984) 55.
[24] H. Spahn-Langguth, B. Podkowik, E. Stahl, E. Martin, E. Mutschler, *J. Anal. Toxicol*. 15 (1991) 209.
[25] R. Büschges, R. Devant, E. Mutschler, H. Spahn-Langguth, *J. Pharm. Biomed. Anal*. 15 (1996) 201.
[26] H. Spahn, D. Krauß, E. Mutschler, *Pharm. Res*. 5 (1988) 107.
[27] T. Toyo'oka, Y.M. Liu, *Analyst* 120 (1995) 385.
[28] D. Jin, Y. Miyahara, T. Oe, T. Toyo'oka, *Anal. Biochem*. 269 (1999) 124.
[29] T. Toyo'oka, *Biomed. Chromatogr*. 10 (1996) 265.
[30] F. Li, S.F. Cooper, M. Cote, *J. Chromatogr. B* 668 (1995) 67.
[31] V.L. Lanchote, P.S. Bonato, P.M. Cerqueira, V.A. Pereira, E.J. Cesarino, *J. Chromatogr. B* 738 (2000) 27.
[32] S. Einarsson, B. Josefsson, P. Moller, D. Sanchez, *Anal. Chem*. 59 (1987) 1191.
[33] R. Herraez-Hernandez, P. Campins-Falco, L.A. Tortajada-Genaro, *Analyst* 123 (1998) 2131.
[34] F.J. Belas, M.A. Phillips, N.R. Srinivas, R.H. Barbhaiya, I.A. Blair, *Biomed. Chromatogr*. 9 (1995)140.
[35] E. Souri, H. Farsam, F. Jamali, *J. Chromatogr. B* 700 (1997) 215.
[36] J. Szymura-Oleksiak, M. Walczak, J. Bojarski, H.Y. Aboul-Einen, *Chirality* 11 (1999) 267.
[37] T.J. Novak, L. Berwick, *J. Liq. Chromatogr. Relat. Tecnol*. 21 (1998) 1883.
[38] I. Rondelli, R. Corsaletti, E. Redenti, D. Acerbi, M. Delcanale, G. Amari, *Chirality* 8 (1996) 381.
[39] N. Nimura, H. Ogura, T. Kinoshita, *J. Chromatogr*. 202 (1980) 375.
[40] L. He, J.T. Stewart, *Biomed. Chromatogr*. 6 (1992) 291.

[41] F.T. Noggle, C.R. Clark, *J. Forensic. Sci*. 31 (1986) 732.
[42] M. Lobell, M.P. Schneider, *J. Chromatogr. A* 633 (1993) 287.
[43] O.P. Kleidernigg, K. Posch, W. Lindner, *J. Chromatogr. A* 729 (1996) 33.
[44] J. Gal, M. Desai, S. Meyert-Lehnert, *Chirality* 2 (1990) 43.
[45] M. Péter, A. Péter, F. Fülöp, *J. Chromatogr. A* 871 (2000) 115.
[46] P. Marfey, *Carlsberg Res. Commun*. 49 (1984) 591.
[47] H. Brückner, C. Gah, *J. Chromatogr.* 555 (1991) 81.
[48] M. Catalá-Icardo, M.J. Medina-Hernandez, M.C. García Alvarez-Coque, *J. Liq. Chromatogr*. 18 (1995) 2827.
[49] M.R. Euerby, P.B. Nunn, L.Z. Partridge, *J. Chromatogr*. 466 (1989) 407.
[50] R.H. Buck, K. Krummen, *J. Chromatogr*. 387 (1987) 255.
[51] A. Hashimoto, T. Nishikawa, T. Oka, K. Takahashi, T. Hayashi, *J. Chromatogr*. 582 (1992) 41.
[52] D.M. Desai, J. Gal, *J. Chromatogr*. 629 (1993) 215.
[53] H. Brückner, T. Westhauser, H. Godel, *J. Chromatogr*. 711 (1995) 201.
[54] A. Péter, E. Vékes, G. Török, *Chromatographia* 52 (2000) 821.
[55] A. Péter, E. Vékes, G. Tóth, D. Tourwé, F. Borremans, *J. Chromatogr. A* 984 (2002) 283.
[56] P.E. Hare, E. Gil-Av, *Science* 204 (1979) 1226.
[57] J. LePage, W. Lindner, G. Davies, D. Seitz, B.L. Karger, *Anal. Chem*. 51 (1979) 433.
[58] S. Görög, M. Gazdag, *J. Chromatogr. B* 659 (1994) 109.
[59] W.H. Pirkle, D.L. Sikkenga, *J. Org. Chem*. 40 (1975) 3430.
[60] W.H. Pirkle, D.W. House, J.M. Finn, *J. Chromatogr*. 192 (1980) 143.
[61] W.H. Pirkle, T.C. Pochapsky, *J. Am. Chem. Soc*. 108 (1986) 352.
[62] W.H. Pirkle, J.M. Finn, J.L. Schreiner, B.C. Hamper, *J. Am. Chem. Soc*. 103 (1981) 3964.
[63] M.H. Hyun, W.H. Pirkle, *J. Chromatogr*. 393 (1987) 357.
[64] E. Yashima, Y. Okamoto, *Bull. Chem. Soc. Jpn*. 68 (1995) 3289.
[65] Y. Okamoto, E. Yashima, *Angew. Chem. Int. Ed*. 37 (1998) 1021.
[66] Application Guide For Chiral Columns Selection, 2nd Edition, Diacel Chemical Industries Ltd., Tokyo, Japan.
[67] B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, Y. Okamoto, *J. Chromatogr. A* 787 (1997) 67.
[68] T. O'Brien, L. Crocker, R. Thompson, K. Thompson, P.H. Toma, D.A. Conlon, B. Feibush, C. Moeder, G. Bicker, N. Grinberg, *Anal. Chem*. 69 (1997) 1999.
[69] E. Yashima, *J. Chromatogr. A* 906 (2001) 105.
[70] K. Tachibana, A. Ohnishi, *J. Chromatogr. A* 906 (2001) 127.
[71] P. Franco, A. Senso, L. Oliveros, C. Minguillón, *J. Chromatogr. A* 906 (2001) 155.
[72] D.W. Armstrong, T.J. Ward, R.D. Armstrong, T.E. Beesley, *Science* 232 (1986) 1132.
[73] Cyclobond Handbook, 7th Edition, Advanced Separation Technologies Inc., Astec, USA, (2005).
[74] C.J. Pedersen, *J. Am. Chem. Soc*. 89 (1967) 2495.
[75] L.R. Sousa, G.D.Y. Sogah, D.H. Hoffman, D.J. Cram, *J. Am. Chem. Soc*. 100 (1987) 4569.
[76] T. Shinbo, T. Yamaguchi, K. Nishimura, M. Sugiura, *J. Chromatogr.* 405 (1987) 145.
[77] T. Shinbo, T. Yamaguchi, H. Yanagishita, D. Kitamoto, K. Sakaki, M. Sugiura, *J. Chromatogr.* 625 (1992) 101.
[78] Y. Machida, H. Nishi, K. Nakamura, H. Nakai, T. Sato, *J. Chromatogr. A* 805 (1998) 85.
[79] M.H. Hyun, *Bull. Kor. Chem. Soc*. 26 (2005) 1153.
[80] M.H. Hyun, J.S. Jin, W. Lee, *J. Chromatogr. A* 822 (1998) 155.
[81] M.H. Hyun, Y.J. Cho, J.A. Kim, J.S. Jin, *J. Liq. Chromatogr. Relat. Technol*. 26 (2003) 1083.
[82] M.H. Hyun, H.J. Min, Y.J. Cho, *Bull. Kor. Chem. Soc*. 24 (2003) 911.

[83] M.H. Hyun, J.S. Jin, H.J. Koo, W. Lee, *J. Chromatogr. A* 837 (1999) 75.
[84] R.J. Steffeck, Y. Zelechonok, K.H. Gahm, *J. Chromatogr. A* 947 (2002) 301.
[85] M.H. Hyun, Y.J. Cho, Y. Song, H.J. Choi, B.S. Kang, *Chirality* 19 (2007) 74.
[86] V.A. Davankov, S.V. Rogozhin, *J. Chromatogr*. 60 (1971) 280.
[87] N. Sanaie, C.A. Haynes *J. Chromatogr. A* 1104 (2006) 164.
[88] M. Grobuschek, M.G. Schmid, C. Tuscher, M. Ivanova, G. Gübitz, *J. Pharm. Biomed. Anal*. 27 (2002) 599.
[89] A. Kurganov, *J. Chromatogr. A* 906 (2001) 51.
[90] M. Remelli, P. Fornasari, F. Pulidori, *J. Chromatogr. A* 761 (1997) 79.
[91] M.C. Millot, *J. Chromatogr. B* 797 (2003) 131.
[92] J. Hermansson, *J. Chromatogr*. 269 (1983) 71.
[93] T. Miwa, M. Ichikawa, M. Tsuno, T. Hattori, T. Miyakawa, M. Kayano, Y. Miyake, *Chem. Pharm. Bull*. 35 (1987) 682.
[94] J.S. Munro, T.A. Walker, *J. Chromatogr. A* 913 (2001) 275.
[95] J.H. Park, J.K. Ryu, J.K. Park, C.V. Mc Neff, P.W. Carr, *Chromatographia* 53 (2001) 405.
[96] V. Andrisano, R. Gotti, M. Recanatini, A. Cavalli, L .Varoli, C. Bertucci, *J. Chromatogr. B* 768 (2002) 137.
[97] H. Henriksson, I.G. Munoz, R. Isaksson, G. Pettersson, G. Johansson, *J. Chromatogr. A* 898 (2000) 63.
[98] T. Miwa, T. Miyakawa, Y. Miyake, *J. Chromatogr.* 457 (1988) 227.
[99] J. Haginaka, Y. Miyano, *Anal. Sci*. 12 (1996) 727.
[100] G. Massolini, E. De Lorenzi, E. Calleri, E. Tabolotti, G. Caccialanza, *J. Chromatogr. B* 738 (2000) 343.
[101] A. Nyström, A. Strandberg, A. Aspegren, S. Behr, A. Karlsson, *Chromatographia* 50 (1999) 209.
[102] D.W. Armstrong, *Pittsburg Conference Abstracts*, Pittcon, (1994) p.572.
[103] A. Berthod, X. Chen, J.P. Kullman, D.W. Armstrong, F. Gasparrini, D'Acquarica, C. Villani, A. Carotti, *Anal. Chem*. 72 (2000) 1767.
[104] A. Péter, A. Árki, E. Vékes, D. Tourwé, E. Forró, F. Fülöp, D.W. Armstrong, *J. Chromatogr. A* 1031 (2004) 159.
[105] A. Péter, R. Török, D.W. Armstrong, *J. Chromatogr. A* 1057 (2004) 229.
[106] T.J. Ward, A.B. Farris III, *J. Chromatogr. A* 906 (2001) 73.
[107] I. Ilisz, R. Berkecz, A. Péter, *J. Sep. Sci*. 29 (2006) 1305.
[108] A. Berthod, Y. Liu, C. Bagwill, D.W. Armstrong, *J. Chromatogr. A* 731 (1996) 123.
[109] A. Péter, G. Török, D.W. Armstrong, *J. Chromatogr. A* 793 (1996) 283.
[110] A. Berthod, B.L. He, T.E. Beesley, *J. Chromatogr. A* 1060 (2004) 205.
[111] T.J. Ward, C. Dann III, A. Blaylock, *J. Chromatogr. A* 715 (1995) 337.
[112] L.C. Sander, S.A. Wise, *Anal. Chem*. 61 (1989) 1749.
[113] L.A. Cole, J.G. Dorsey, *Anal. Chem*. 64 (1992) 1317.
[114] W. H. Pirkle, *J. Chromatogr.* 558 (1991) 1.
[115] G. Guiochon, S. G. Shirazi, A. M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, *Academic Press*, Boston (1994).
[116] C. Czerwenka, M. M. Zhang, H. Kählig, N. M. Maier, K. B. Lipkowitz, W. Lindner, *J. Org. Chem.* 68 (2003) 8315.
[117] C. Hellrieger, U. Skogsberg, K. Albert, M. Lämmerhofer, N. M. Maier, W. Lindner, *J. Am. Chem. Soc.* 126 (2004) 3809.
[118] C. Czerwenka, W. Lindner, *Anal. Bioanal. Chem.* 382 (2005) 599.
[119] K. A. Schug, W. Lindner, *Chem. Rev.* 105 (2005) 67.
[120] M. Betti, *Org. Synth. Coll. Vol*. 1 (1941) 381.
[121] I. Szatmári, F. Fülöp, *Curr. Org. Synth*. 1 (2004) 155.
[122] I. Szatmári, T.A. Martinek, L. Lázár, F. Fülöp, *Tetrahedron* 59 (2003) 2877.
[123] D. Tóth, I. Szatmári, F. Fülöp, *Eur. J. Org. Chem*. 20 (2006) 4664.

[124] I. Szatmári, T.A. Martinek, L. Lázár, F. Fülöp*, Eur. J. Org. Chem*. 10 (2004) 2231.
[125] W.M. Rodionov, E.F. Malivinskaya, *Chem. Ber*. 59 (1926) 2952.
[126] L. Lázár, T. Martinek, G. Bernáth, F. Fülöp, *Synth. Commun*. 28 (1998) 219.
[127] E. Forró, T. Paál, G. Tasnádi, F. Fülöp, *Adv. Synth. Catal*. 348 (2006) 917.
[128] M. Solymár, F. Fülöp, L.T. Kanerva, *Tetrahedron: Asymmetry* 13 (2002) 2383.
[129] M. Betti, P. Pratesi, *Biochem. Z.* 274 (1934) 1.
[130] C. Cimarelli, G. Palmieri, E. Volpini, *Tetrahedron: Asymmetry* 13 (2002) 2417.
[131] J. Lu, X. Xu, C. Wang, J. He, Y. Hu, H. Hu, *Tetrahedron Lett.* 43 (2002) 8367.
[132] X. Wang, Y. Dong, J. Sun, X. Xu, R. Li, Y.J. Hu, *J. Org. Chem.* 70 (2005) 1879.
[133] I. Szatmári, A. Hetényi, L. Lázár, F. Fülöp, *J. Heterocyclic Chem.* 41 (2004) 367.
[134] N.C. Desai, H.K. Shukla, N.A. Langalia, K.A. Thaker, *J. Indian Chem. Soc*. 61 (1984) 711.
[135] R.L. Wolin, A. Santillan, T. Barclay, L. Tang, H. Venkatesan, S. Wilson, D.H. Lee, T.W. Lovenberg, *Bioorg. Med. Chem*. 12 (2004) 4493.
[136] T. Yamakazi, A. Pröbstl, P.W. Schiller, M. Goodman, *Int. J. Peptide Protein Res*. 37 (1991) 6644.
[137] G. Bernáth, *Acta Chim. Hung., Models In Chemistry* 129 (1992) 107.
[138] E.L. Winnacker, M.M. Hebst, H.A. Barker, *Biochem. Biophys. Acta* 237 (1971) 280.
[139] T. Yamada, S. Nonomura, H. Fujiwara, T. Miyazawa, S. Kuwata, *J. Chromatogr*. 515 (1990) 475.
[140] G. Török, A. Péter, P. Csomós, L.T. Kanerva, F. Fülöp, *J. Chromatogr*. 797 (1998) 177.
[141] V.A. Davankov, Y.A. Zoltarev, A.A. Kurganov, *J. Liq. Chromatogr*. 2 (1979) 1191.
[142] W.L. Lindner, I. Hirschbock, *J. Liq. Chromatogr*. 9 (1986) 551.
[143] S. Yamakazi, T. Takeuchi, T. Tanimura, *J. Chromatogr*. 540 (1991) 169.
[144] O.W. Griffith, E.B. Campbell, W.H. Pirkle, A. Tsipouras, M.H. Hyun, *J. Chromatogr*. 362 (1986) 345.
[145] I. D'Acquarica, F. Gasparrini, D. Misiti, G. Zappia, C. Cimarelli, G. Palmieri, A. Carotti, S. Cellamare, C. Villani, *Tetrahedron: Asymmetry* 11 (2000) 2375.
[146] A. Péter, L. Lázár, F. Fülöp, D.W. Armstrong, *J. Chromatogr. A* 926 (2001) 229.
[147] A. Péter, *J. Chromatogr. A* 955 (2002) 141.
[148] M.H. Hyun, Y.J. Cho, J.S. Jin, *J. Sep. Sci*. 25 (2002) 648.
[149] A. Árki, D. Tourwé, M. Solymár, F. Fülöp, D.W. Armstrong, A. Péter, *Chromatographia* 60 (2004) S43.
[150] U.B. Nair, S.S.C. Chang, D.W. Armstrong, Y.Y. Rawjee, *Chirality* 8 (1996) 590.
[151] A. Berthod, X. Chen, J.P. Kullman, D.W. Armstrong, F. Gasparrini, I. D'Aquarica, C. Villani, A. Carotti, *Anal. Chem*. 72 (2000) 1767.
[152] A. Péter, A. Árki, E. Vékes, D. Tourwé, L. Lázár, F. Fülöp, D.W. Armstrong, *J. Chromatogr. A* 1031 (2004) 171.
[153] W. Lee, J.Y. Jin, C.S. Back, *Microchem. J.* 80 (2005) 213.

ACKNOWLEDGEMENTS

This work was performed at the Institute of Pharmaceutical Chemistry, University of Szeged, in the period 2003-2008.

I would like to express my sincere thanks to my supervisor, Professor Ferenc Fülöp, Head of the Institute of Pharmaceutical Chemistry, for his guidance of my work, his inspiring ideas and his constructive criticism during my work.

I also express my warmest thanks to my second supervisor, Professor Antal Péter, for his guidance of my work and for his professional opinions. I owe my endless thanks to him for his fruitful cooperation, his continuous interest in my work and his patience in helping me to understand the discipline of high-performance liquid chromatography.

I am grateful to Dr. Enikő Forró, Dr. Magdolna Solymár, Dr. László Lázár, Dr. István Szatmári and Diána Tóth for their pleasant cooperation and for providing me with the compounds investigated.

I would also like to thank my further co-author, Róbert Berkecz, for his helpful discussions and his participation in the common researches.

I am very grateful to Ilona Varga-Halasi for her valuable technical and practical assistance.

I would like to thank Dr. David Durham for revising the English language of all the English publications and the manuscripts.

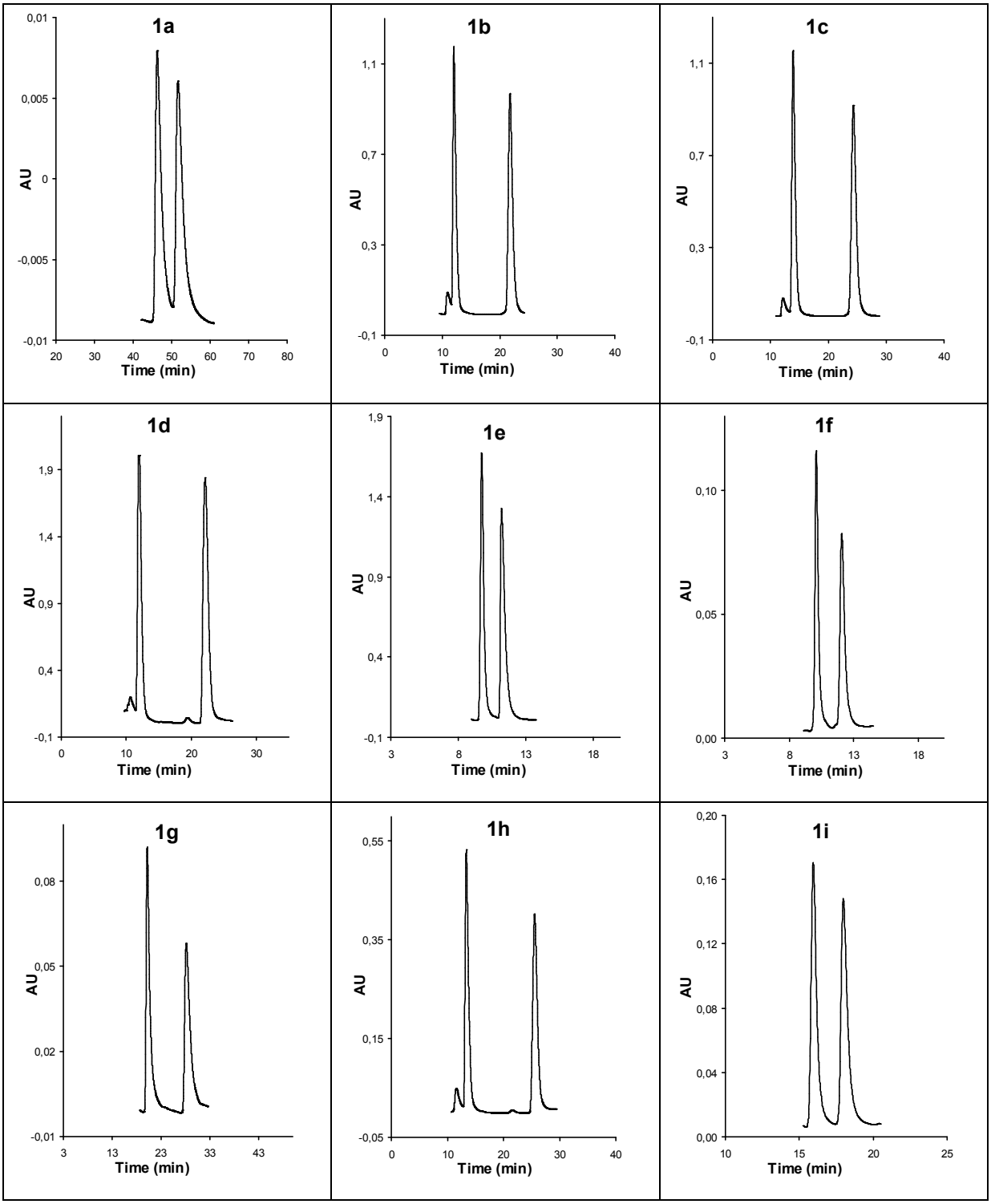
I thank Richter Gedeon Rt. Centenárium Alapítvány for supporting my Ph.D. study from September 1, 2006 to February 30, 2007.

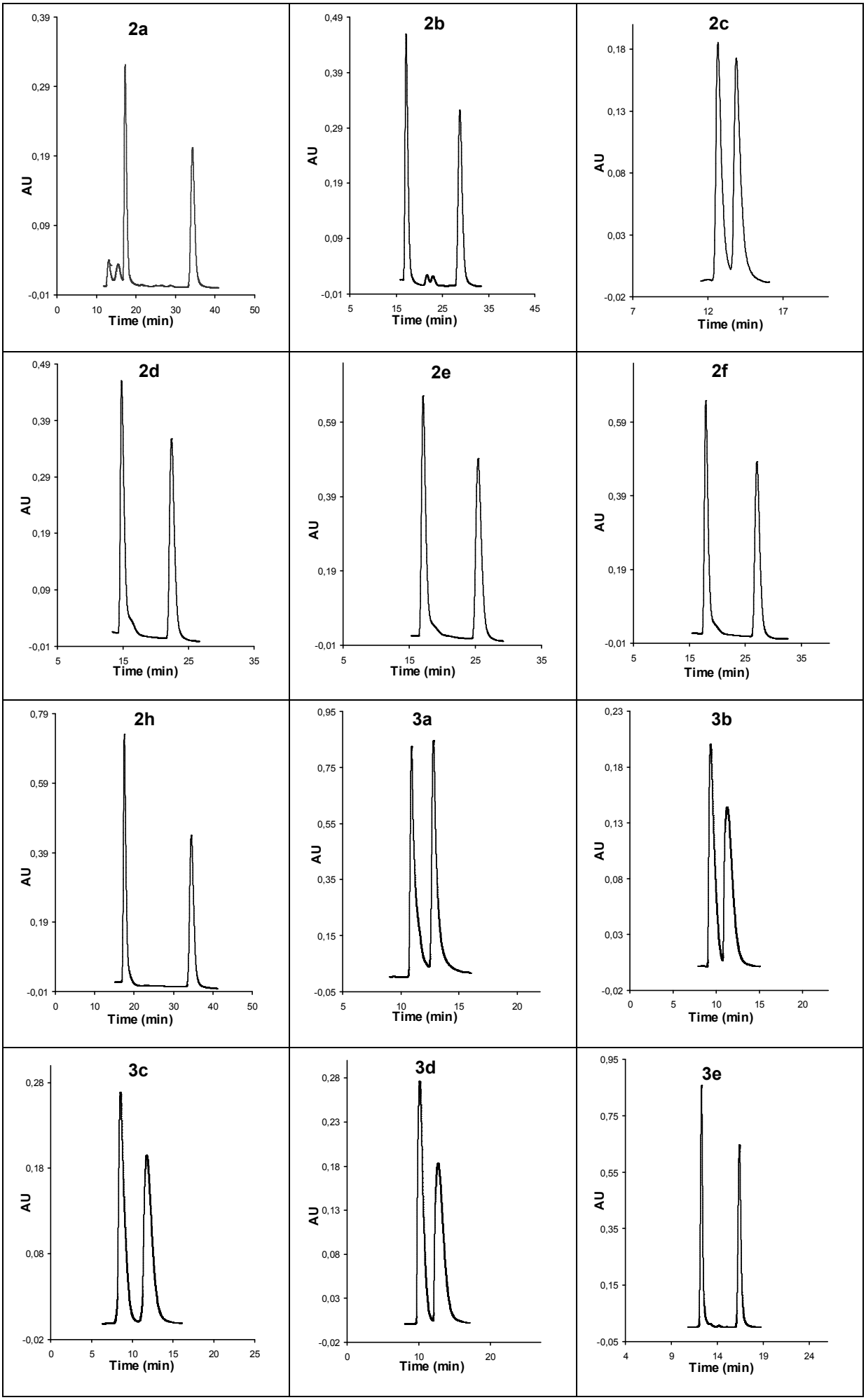
I wish to express my thanks to the whole staff of the Institute of Pharmaceutical Chemistry for their constant readiness to help, and especially to those with whom I have made a true friendship, for their encouragement, and inspiring discussions.

Last but not least, I would like to express my whole-hearted thanks to my family, my parents and my brother, who have always ensured such circumstances that I could carry out my work conscientiously. I also appreciate their warm affection, care, magnificient help and endless patience. Without their support, this work would not have been completed.

APPENDIX

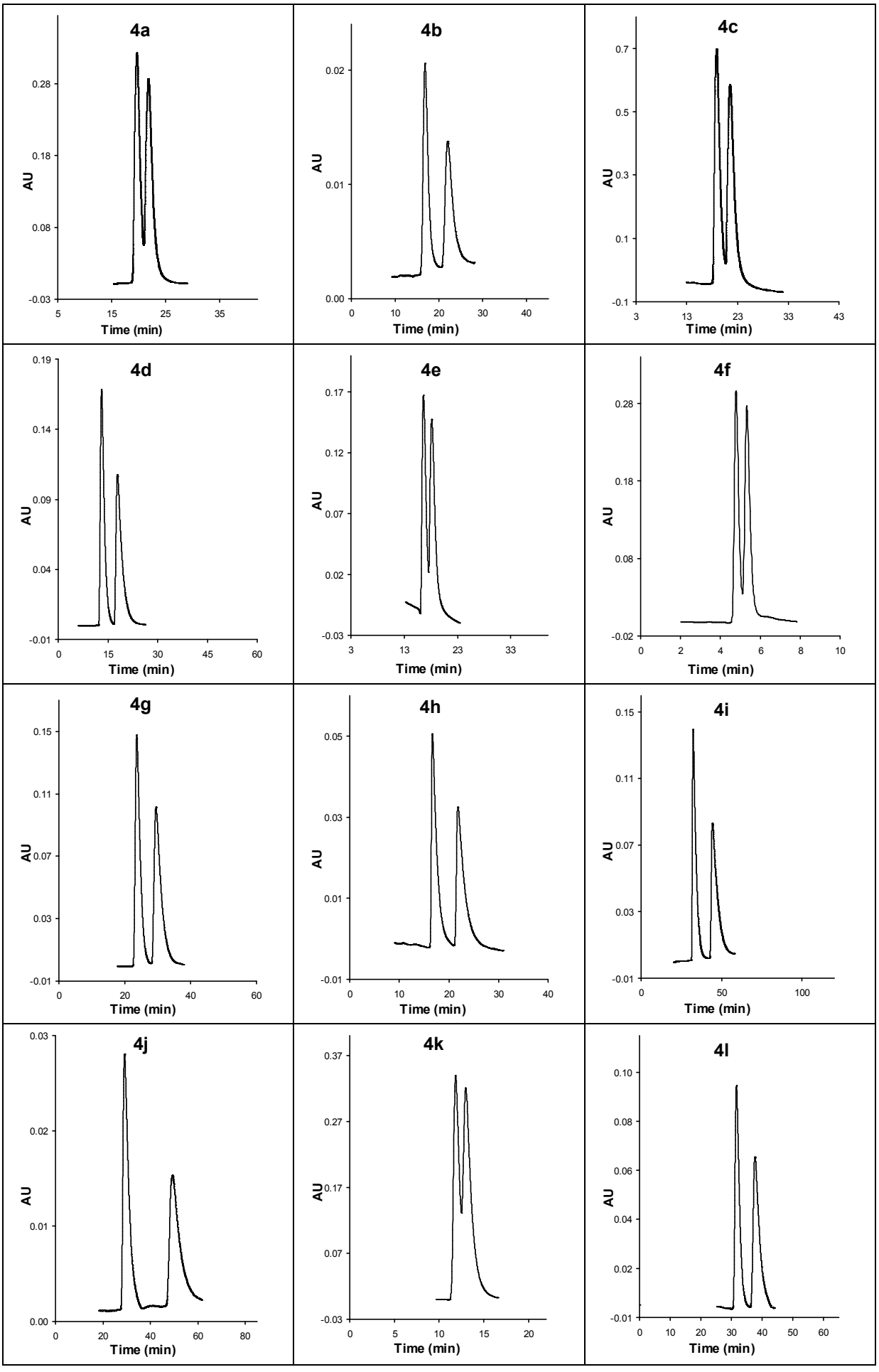
Selected chromatograms of the enantioseparation of 1- and 2-naphthol derivatives

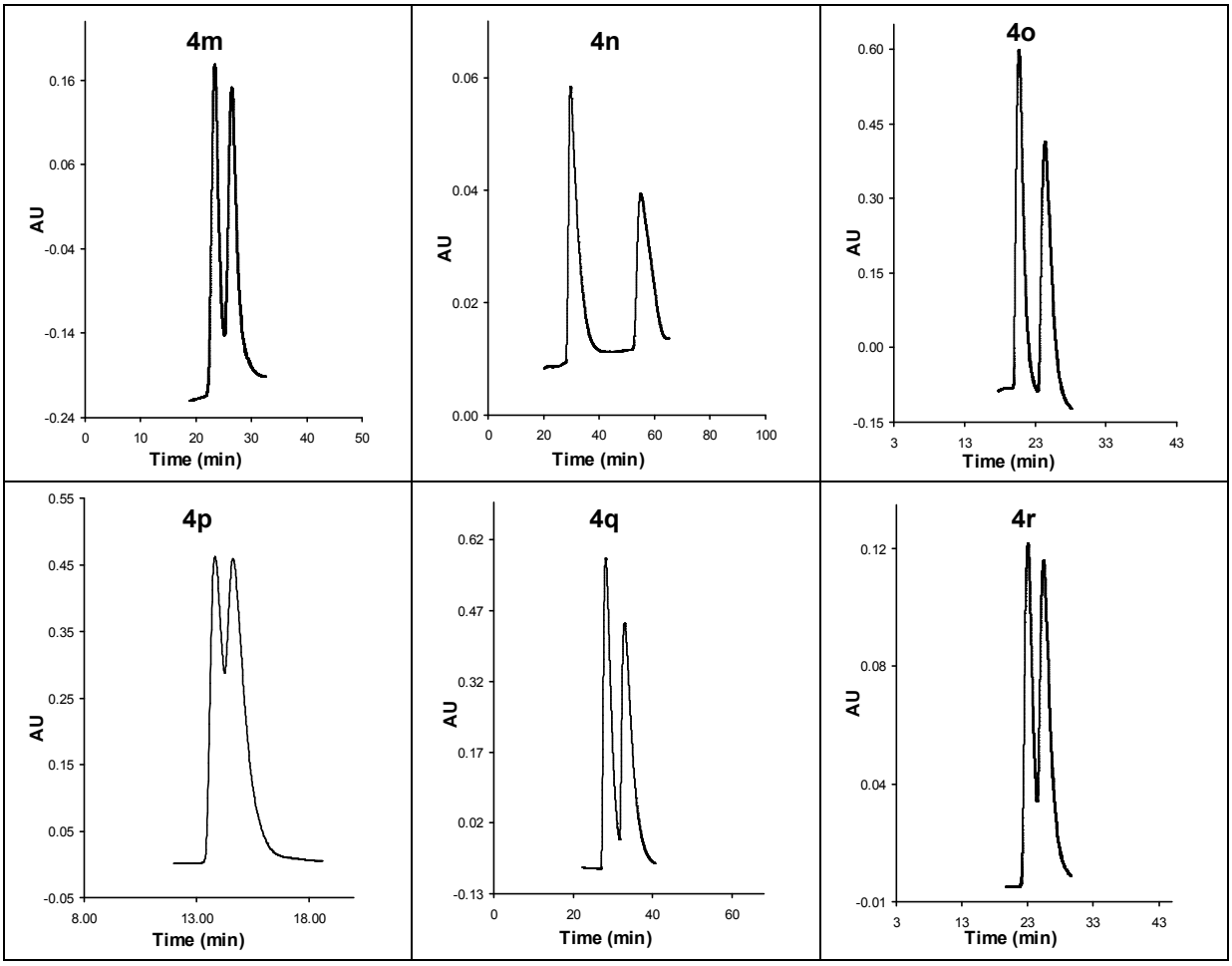



2a
2b
2c
2d
2e
2f
2h
3a
3b
3c
3d
3e
AU
Time (min)

Chromatographic conditions: column, Cyclobond DMP for **1a**,**e-g**, **i** and **2c**, Chiralcel OD-H for **1b-d**,**h**,**a**, **2b**,**d-f**,**h** and **3a**,**e**, Chiralcel OD-RH for **3b-d**; mobile phase, *n*-hexane/chloroform=95/5 (v/v) for **1a**, *n*-hexane/IPA/DEA=40:60:0.1 (v/v/v) for **1b-d**,**h** and **2a**,**b**,**d-f**,**h**, *n*-hexane/EtOH/MeOH/TEA=80/10/10/0.1 (v/v/v/v) for **1e**,**f**,**i**, *n*-hexane/EtOH/TEA=80/20/0.02 (v/v/v) for **1g**, *n*-hexane/EtOH/MeOH/TEA=80/5/15/0.1 (v/v/v/v) for **2c,** *n*-hexane/IPA/DEA=30/70/0.1 (v/v/v) for **3a**, 0.2 M aqueous KH_2PO_4 (pH=6.0)/MeCN=80/20 (v/v) for **3b**, 0.05 M aqueous KH_2PO_4 (pH=6.0)/MeOH=40/60 (v/v) for **3c**, 0.2 M aqueous KH_2PO_4 (pH=5.0)/MeCN=80/20 (v/v) for **3d**, *n*-hexane/IPA/DEA=70/30/0.1 (v/v/v) for **3e**; flow rate, 0.5 ml min^{-1} for Chiralcel OD-H and Chiralcel OD-RH columns and 0.8 ml min^{-1} for Cyclobond DMP column; detection, 230 nm; temperature, 298 K

Selected chromatograms of the enantioseparation of β-substituted β-amino acids





Chromatographic conditions: column, Chirobiotic T2 for **4a**,**c**,**m**,**o**,**r**, Chirobiotic TAG for **4p**,**q**, Chirobiotic R for **4e**, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP for **4b**,**d**,**f-l**,**n**; mobile phase, MeOH/AcOH/TEA=100/0.01/0.01 (v/v/v) for **4a**,**c**,**m**,**o**,**r**, MeOH/AcOH/TEA=100/0.1/0.1 (v/v/v) for **4e**, aqueous TEAA (pH=4.1)/MeOH=10/90 (v/v) for **4p**,**q**, H_2O/MeOH (5 mM AcOH)=50/50 (v/v) for **4b**,**d**,**g**,**j-l**, H_2O/MeOH (10 mM AcOH)=80/20 (v/v) for **4h**,**i**,**n**, H_2O/MeOH (20 mM H_2SO_4)=50/50 (v/v) for **4f**; flow rate, 0.4 ml min^{-1} for **4a**,**c**,**e**,**m**,**o**,**p**,**r**, 0.5 ml min^{-1} for **4b**,**d**,**f-l**,**n**, 0.8 ml min^{-1} for **4q**; detection, 205 nm for **4a**,**c**,**e**,**m**,**o-r**, 210 nm for **4b**,**d**,**f-l**,**n**; temperature, 298 K

PUBLICATIONS

I.

Anita Sztojkov-Ivanov[1]
István Szatmári[1]
Antal Péter[2]
Ferenc Fülöp[1]

[1]Institute of Pharmaceutical Chemistry, University of Szeged, Szeged, Hungary
[2]Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary

Structural and temperature effects in the high-performance liquid chromatographic enantioseparation of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs

The enantiomers of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs were separated isothermally on a cellulose-*tris*-3,5-dimethylphenyl carbamate-based chiral stationary phase (Chiralcel OD-H), at 10°C increments in the range of 5–35°C, using *n*-hexane/2-propanol/diethylamine as mobile phase. The mobile phase composition and temperature were varied to achieve baseline resolutions in a single chromatographic run. The dependence of the natural logarithms of selectivity factors, ln α, on the inverse of temperature, $1/T$, was used to determine the thermodynamic data of the enantiomers. The thermodynamic data revealed that all the compounds in this study separate *via* the same enthalpy-driven chiral recognition mechanism.

Key Words: 1-(α-Aminobenzyl)-2-naphthol analogs; 2-(α-Aminobenzyl)-1-naphthol analogs; Enantiomer separation; HPLC; Temperature effect

Received: March 24, 2005; revised: June 23, 2005; accepted: July 6, 2005

DOI 10.1002/jssc.200500138

1 Introduction

One hundred years ago, Betti reported the Mannich aminoalkylation of 2-naphthol for the preparation of 1-(α-aminobenzyl)-2-naphthol derivatives (Fig. 1, **1**, X = H, Betti base) [1]. The analogous transformation of 1-naphthol led to the regioisomeric Betti base derivative 2-(α-aminobenzyl)-1-naphthols (Fig. 1, **2**) [2]. The first resolution of naphthol derivatives was reported by Betti and Pratesi, and the enantiomers of **1** were used for the resolution of 2-(*p*-anisyl)-propanal [3] and glycerylaldehydes [4] and for discrimination between aldohexoses and ketohexoses [5]. However, in spite of the ready availability and low cost of the optically active Betti base, the early publications were followed by a long silence over this material. In the past decade, however, interest in the chemistry of the Betti base has intensified. Preparation of the enantiomers of the Betti base and its *N*-substituted derivatives is of significance since they can serve as chiral catalysts [6–12]. Betti base derivatives also provide convenient access to many useful synthetic building blocks [13].

The biological effects of naphthol derivatives have been investigated in *in vivo* systems. Desai *et al.* [14] found that the presence of the thiazolidine nucleus, produced from the racemic Betti base, was necessary for good antitubercular activity, and the presence of halogen atoms enhances the antibacterial activity.

The applications of Betti base enantiomers required the elaboration of a precise separation and detection method. For the separation of chiral compounds, chromatographic methods are widely used. HPLC on chiral stationary phases (CSPs) is an effective tool for the resolution of chiral compounds on both analytical and preparative scales.

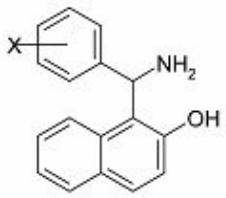
In all chromatographic modes, the selectivity and retention factors are mainly controlled by the concentration and nature of the mobile phase components, together with other variables, such as the flow rate and the pH of the mobile phase. Enantioselective retention mechanisms are sometimes influenced by temperature to a greater extent than are ordinary separations. Numerous studies have been devoted to the elucidation of the effects of temperature on the separation characteristics of chiral selector systems [15–21]. On alteration of the temperature, significant effects can often be seen.

In order to investigate the thermodynamic functions of enantioselective adsorption van't Hoff plots were constructed, which may be interpreted in terms of mechanistic aspects of chiral recognition:

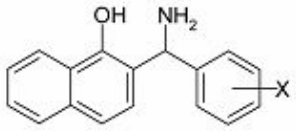
$$\Delta(\Delta G^\circ) = -RT \ln \alpha = \Delta(\Delta H^\circ) - T\Delta(\Delta S^\circ) \qquad (1)$$

If $\Delta(\Delta H^\circ)$ is invariant with temperature (*i.e.*, a linear van't Hoff plot is obtained), this expression shows that a plot of

Correspondence: Professor Antal Péter, Department of Inorganic and Analytical Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 7, Hungary. Fax: +36-62-420-505.
E-mail: apeter@chem.u-szeged.hu.



1



2

Position	Analyte (1 or 2)						
	a	**b**	**c**	**d**	**e**	**f**	**g**
4	H	CH_3	CH_3O	F	Cl	Br	NO_2
	h	**i**					
3	Br	NO_2					

Figure 1. Structures of analytes. **1a**, 1-(α-aminobenzyl)-2-naphthol; **1b**, 1-[amino(4-methylphenyl)methyl]-2-naphthol; **1c**, 1-[amino(4-methoxyphenyl)methyl]-2-naphthol; **1d**, 1-[amino(4-fluorophenyl)methyl]-2-naphthol; **1e**, 1-[amino(4-chlorophenyl)-methyl]-2-naphthol; **1f**, 1-[amino(4-bromophenyl)methyl]-2-naphthol; **1g**, 1-[amino(4-nitrophenyl)methyl]-2-naphthol; **1h**, 1-[amino(3-bromophenyl)methyl]-2-naphthol; and **1i**, 1-[amino(3-nitrophenyl)methyl]-2-naphthol. **2a**, 2-(α-aminobenzyl)-1-naphthol; **2b**, 2-[amino(4-methylphenyl)methyl]-1-naphthol; **2c**, 2-[amino(4-methoxyphenyl)methyl]-1-naphthol; **2d**, 2-[amino(4-fluorophenyl)methyl]-1-naphthol; **2e**, 2-[amino(4-chlorophenyl)methyl]-1-naphthol; **2f**, 2-[amino(4-bromophenyl)methyl]-1-naphthol; **2h**, 2-[amino(3-bromophenyl)methyl]-1-naphthol; and **2i**, 2-[amino(3-nitrophenyl)methyl]-1-naphthol.

$R \ln \alpha$ *versus* $1/T$ has a slope of $\Delta(\Delta H^\circ)$ and an intercept of $\Delta(\Delta S^\circ)$.

In this paper, normal-phase HPLC methods are described for the enantioseparation of racemic 1- and 2-naphthol analogs (for structures, see Fig. 1). The HPLC methods rely on the use of a cellulose-*tris*-3,5-dimethylphenyl carbamate-based CSP (Chiralcel OD-H). For comparison purposes, most of the separations were carried out at constant mobile phase compositions and at different temperatures under linear chromatographic conditions, *e.g.*, the van't Hoff plots exhibited linear behavior in the temperature range investigated. On the basis of the experimental data, the influence of mobile phase composition and of specific structural features of the analytes (aryl substituents) on the apparent thermodynamics of enantioselective adsorption will be discussed in the light of mechanistic aspects of chiral recognition.

2 Experimental

2.1 Chemicals and reagents

The Betti reaction was applied to prepare 1-(α-aminobenzyl)-2-naphthol derivatives (Fig. 1, **1a–1i**). The reaction was performed with 2-naphthol, benzaldehyde or substituted benzaldehydes and ammonia (in proportions of 1:2:1) to obtain 1,3-diaryl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3]oxazines. Their subsequent acidic hydrolysis and extraction with NH_4OH gave the desired aminonaphthols [1, 22, 23]. 2-(α-Aminobenzyl)-1-naphthols (Fig. 1, **2a–2f, 2h, 2i**) were prepared in a manner similar to that for their regioisomeric 1-(α-aminobenzyl)-2-naphthol derivatives, by Betti's classical aminoalkylation of 1-naphthol with benzaldehyde or substituted benzaldehydes in the presence of ammonia, which led to the formation of 2,4-diaryl-3,4-dihydro-2*H*-naphth[2,1-*e*][1,3]oxazines. The subsequent acidic hydrolysis of 2,4-diarylnaphthoxazines furnished aminonaphthol hydrochlorides [2].

n-Hexane and 2-propanol (IPA) of HPLC grade were from Merck (Darmstadt, Germany). Diethylamine (DEA) and other reagents of analytical reagent grade were from Aldrich (Steinheim, Germany). Mobile phases were prepared by mixing the indicated volumes of solvents and were further purified by filtration through a 0.45-μm Millipore filter, type HV. The eluents were degassed in an ultrasonic bath and He gas was purged through them during the analysis. Stock solutions of analytes (1 mg/mL) were prepared by dissolution in the starting mobile phase.

2.2 Apparatus

The HPLC system consisted of an M-600 low-pressure gradient pump, equipped with an M-486 tunable absorbance detector and an M-996 photodiode-array detector, and a Millenium 2.1 Chromatography Manager data system (all Waters Chromatography, Milford, MA, USA). The HPLC system was equipped with a Rheodyne Model 7125 injector with a 20-μL loop (Rheodyne, Cotati, CA, USA).

Analyses were carried out on a column of Chiralcel OD-H, 250 × 4.6 mm ID, 5-μm particle size (Daicel, Tokyo,

Japan). The column was thermostated in a water bath, applying a cooling–heating thermostat (MK 70, Mechanik Prüfgeräte, Medlingen, Germany). The accuracy of temperature adjustment was ±0.1°C.

3 Results and discussion

The application of *n*-hexane and IPA as alcoholic modifier resulted in chromatograms with broad and asymmetric peaks (data not shown). Addition of a small percentage of DEA significantly improved the peak shapes and resolutions. The retention factors of the first- and second-eluted stereoisomers depended strongly on the IPA content of the mobile phase. At ambient temperature, increase of the IPA content from 40 to 60% decreased the retention factors of both the 2- and the 1-naphthol derivatives (Tables 1, 2). The change in the separation factor, α, with increasing IPA content differed for the 2- and 1-naphthol derivatives. For the 2-naphthol derivatives a slight increase in α was registered with increasing IPA content (with the exception of **1a**), while for the 1-naphthol derivatives, α changed in parallel with the *k'* values, *i.e.*, α decreased with decreasing *k'* (with the exception of **2h**). For both the 1- and 2-naphthol analogs, the resolution, R_s, decreased with increasing IPA content.

At a constant mobile phase composition, comparison of the chromatographic behavior of the 2- and 1-naphthol analogs containing the same substituents revealed that the 1-naphthol derivatives exhibited larger retention factors and smaller separation factors (Tables 1, 2). It seemed that the steric arrangements of the aromatic rings in the 1-naphthol derivatives advanced the interaction with the selector, and therefore the retention factors were higher. At the same time, the interactions of the two stereoisomers with the selector did not differ appreciably, which resulted in smaller α values. For the 2-naphthol derivatives, despite the smaller *k'* values, larger α values were obtained than for the 1-naphthol derivatives.

Aromatic substitution exhibited a slight effect on both the retention and the selectivity. At a constant mobile phase composition Me substitution on the aromatic ring resulted in a lower retention, while MeO substitution led to a higher retention as compared with the nonsubstituted compounds; **1b** and **1c** *versus* **1a** and **2b**, **2c** *versus* **2a**. The higher retention factors of **1c** and **2c** can be attributed to the possible H-bonding interactions of the carbamate groups of the selector with the MeO group of the analytes. Despite the additional H-bonding interactions of **1c** and **2c**, which led to higher retention, the selectivity and resolution of the MeO-substituted analogs remained lower than those of the nonsubstituted analogs (especially in the case of the 1-naphthol analogs).

Halogen substitution on the aromatic ring increased the π-acidic character of the analytes and therefore a stronger interaction with the π-basic 3,5-dimethylphenyl carbamate group of the selector would be expected. The increased π-acidity of the aromatic ring of halogen-substituted naphthol analogs (Fig. 1, **d–f, h**) slightly influenced the retention; k_1' and k_2' were somewhat smaller than those for the nonsubstituted analogs (with the exception of k_1' for analog **f**) and a slight increase was observed in the sequence fluorine–chlorine–bromine. The position (3 or 4) of the substituent slightly influenced the retention and selectivity; for the 1-naphthol analogs, a slightly higher selectivity was observed for the 3-substituted analog (**2h**) than for the 4-substituted analog (**2f**). The more π-acidic

Table 1. Chromatographic data, retention factors (*k'*), separation factors (α), and resolutions (R_s) of the enantioseparation of 2-naphthol derivatives

Analyte	Eluent Hex/IPA/DEA v/v/v	k_1'	k_2'	α	R_s
1a	60/40/0.1	0.92	3.00	3.26	10.0
	50/50/0.1	0.73	2.35	3.21	8.50
	40/60/0.1	0.67	2.07	3.09	7.36
1b	60/40/0.1	0.82	2.54	3.10	9.11
	50/50/0.1	0.69	2.17	3.16	7.66
	40/60/0.1	0.55	1.81	3.29	7.41
1c	60/40/0.1	1.24	3.25	2.62	8.73
	50/50/0.1	1.01	2.90	2.87	8.85
	40/60/0.1	0.79	2.13	2.70	7.36
1d	60/40/0.1	0.87	2.79	3.21	9.20
	50/50/0.1	0.67	2.45	3.64	9.60
	40/60/0.1	0.53	1.85	3.49	6.92
1e	60/40/0.1	0.95	3.02	3.18	9.80
	50/50/0.1	0.74	2.57	3.47	9.09
	40/60/0.1	0.58	1.94	3.34	7.52
1f	60/40/0.1	1.02	3.15	3.08	9.37
	50/50/0.1	0.78	2.46	3.15	9.09
	40/60/0.1	0.67	2.19	3.27	8.17
1g	60/40/0.1	1.70	4.19	2.46	8.17
	50/50/0.1	1.26	3.37	2.67	9.00
	40/60/0.1	0.98	2.57	2.62	7.42
1h	60/40/0.1	1.04	2.83	2.72	8.40
	50/50/0.1	0.81	2.32	2.86	7.57
	40/60/0.1	0.72	2.29	3.18	8.23
1i	60/40/0.1	1.69	3.55	2.10	5.83
	50/50/0.1	1.19	2.67	2.24	6.43
	40/60/0.1	0.92	1.99	2.16	4.93

Column, Chiralcel OD-H; eluent, *n*-hexane/2-propanol/DEA; detection, 232 nm; flow rate, 0.5 mL/min; and temperature, 298 K.

Table 2. Chromatographic data, retention factors (k'), separation factors (α), and resolutions (R_s) of the enantioseparation of 1-naphthol derivatives

Analyte	Eluent Hex/IPA/DEA v/v/v	k'_1	k'_2	α	R_s
2a	60/40/0.1	1.66	4.84	2.92	12.5
	40/60/0.1	1.22	3.41	2.80	10.6
2b	60/40/0.1	1.62	3.75	2.31	10.0
	40/60/0.1	1.21	2.70	2.23	7.08
2c	60/40/0.1	2.56	4.07	1.59	5.60
	40/60/0.1	1.66	2.65	1.60	4.43
2d	60/40/0.1	1.32	2.80	2.11	7.81
	40/60/0.1	0.90	1.89	2.10	5.25
2e	60/40/0.1	1.60	3.18	1.99	7.40
	40/60/0.1	1.20	2.27	1.89	5.38
2f	60/40/0.1	1.80	3.64	2.02	7.70
	40/60/0.1	1.31	2.48	1.89	5.43
2h	60/40/0.1	1.70	4.46	2.62	11.6
	40/60/0.1	1.25	3.44	2.75	9.23
2i	60/40/0.1	2.42	3.87	1.60	4.23
	40/60/0.1	1.48	2.37	1.60	4.00

Column, Chiralcel OD-H; eluent, *n*-hexane/2-propanol/DEA; detection, 232 nm; flow rate, 0.5 m mL/min; and temperature, 298 K.

nitro-substituted analogs exhibited a more pronounced effect than that of the less π-acidic halogen-substituted analogs. The retention factors of analogs **g** and **i** were higher than those of the nonsubstituted or halogen-substituted analogs, especially for the first-eluted stereoisomers, but these increased retention factors were generally not associated with increased selectivity. It seems that, besides the nature of the aromatic substituents, other steric effects also influenced the retention and chiral discrimination of naphthol derivatives.

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out between 5 and 35°C. The chromatographic data were measured and calculated in 10°C increments over the temperature range studied, using ternary mixtures. Experimental data for the *n*-hexane/IPA/DEA = 55/45/0.1 v/v/v mobile phase are collected in Table 3. Comparison of the retention factors k'_1 and k'_2 in Table 3 shows that all of the recorded values decrease with increasing temperature (similar tendencies were measured at other mobile phase compositions; data not shown). This decrease is illustrated in Fig. 2 for the separation of 2-[amino(4-methoxyphenyl)methyl]-1-naphthol (**2c**). It is evident that an increase in separation temperature lowers the separation factor, α, for these enantiomers, but it also may improve the peak symmetry. The dependence of the resolution, R_s, on temperature, however, is more complex. The data listed in Table 3 show that the resolution factors for 2-naphthol and 1-naphthol analogs at higher temperatures decreased to a larger extent.

In order to calculate the thermodynamic parameters and to acquire information of value for an understanding of the enantiomeric retention, selectivity and mechanism on this CSP, van't Hoff plots were constructed (Eq. (1)). In the present study, all the plots of $R\ln\alpha$ *versus* $1/T$ could be fitted by straight lines with good correlation coefficients (Table 4).

The structure of the analyte obviously has an influence on the thermodynamics of *enantioselective adsorption* to the Chiralcel OD-H selector. The thermodynamic quantities ($\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, and $\Delta(\Delta G^\circ)$) originating from the $R\ln\alpha$

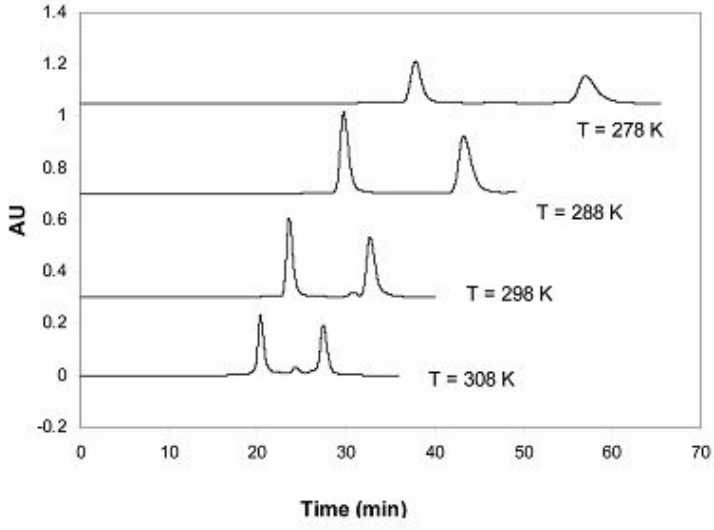


Figure 2. Temperature dependence of the chiral separation of 2-[amino(4-methoxyphenyl)methyl]-1-naphthol (**2c**). Column, Chiralcel OD-H; mobile phase, *n*-hexane/2-propanol/DEA = 55/45/0.1 v/v/v; detection, 232 nm; and flow rate, 0.5 mL/min.

Table 3. Temperature dependence of retention factor (k'), separation factor (α), and resolution (R_s) of enantiomers of 1- and 2-naphthol analogs

Temperature, K	Analyte	k_1'	k_2'	α	R_s	Analyte	k_1'	k_2'	α	R_s
278	**1a**	1.32	6.04	4.57	12.8	**2a**	2.78	9.47	3.41	13.2
288		1.12	4.25	3.80	12.2		2.26	7.00	3.10	13.1
298		0.93	2.89	3.11	10.4		1.73	4.68	2.71	12.9
308		0.82	2.22	2.71	7.81		1.45	3.61	2.49	8.20
278	**1b**	1.24	5.31	4.28	13.6	**2b**	2.81	6.99	2.49	10.7
288		1.08	3.90	3.62	11.6		2.49	5.35	2.15	10.3
298		0.88	2.63	3.00	9.47		1.67	3.54	2.12	8.20
308		0.74	1.94	2.62	7.37		1.39	2.90	2.09	7.11
278	**1c**	2.04	7.11	3.49	11.7	**2c**	4.32	7.02	1.63	6.09
288		1.64	4.84	2.95	10.2		3.25	5.18	1.59	5.48
298		1.24	3.15	2.54	8.42		2.32	3.61	1.56	5.33
308		1.07	2.43	2.27	7.74		1.94	2.97	1.53	4.67
278	**1d**	1.28	5.92	4.63	13.8	**2d**	2.04	4.60	2.25	7.66
288		1.08	4.40	4.07	12.4		1.67	3.51	2.10	7.11
298		0.88	2.81	3.19	10.1		1.33	2.69	2.03	6.66
308		0.79	2.22	2.80	7.90		1.18	2.21	1.87	5.87
278	**1e**	1.37	5.82	4.25	12.2	**2e**	2.46	4.95	2.01	7.20
288		1.15	4.37	3.79	11.6		2.08	3.91	1.88	6.66
298		0.96	2.95	3.06	9.26		1.69	3.16	1.87	6.40
308		0.85	2.31	2.72	8.40		1.41	2.50	1.77	5.75
278	**1f**	1.43	6.55	4.58	13.0	**2f**	2.84	5.72	2.02	7.74
288		1.27	4.56	3.59	12.7		2.27	4.32	1.90	7.20
298		1.02	3.07	3.01	10.4		1.83	3.45	1.88	7.00
308		0.90	2.35	2.61	7.11		1.53	2.78	1.82	4.89
278	**1g**	2.46	7.83	3.18	10.7	**2i**	3.47	5.75	1.66	5.34
288		1.95	5.75	2.94	10.5		2.88	4.66	1.62	5.21
298		1.62	4.01	2.47	8.40		2.40	3.78	1.58	4.56
308		1.38	3.08	2.23	7.20		1.92	2.92	1.52	4.16

Column, Chiralcel OD-H; eluent, *n*-hexane/2-propanol/DEA = 55/45/0.1 v/v/v; detection, 232 nm; and flow rate, 0.5 mL/min.

versus $1/T$ plots are listed in Table 4. The enantioselective binding of the 2-naphthol analogs was characterized by negative $\Delta(\Delta H^\circ)$ values, ranging from 9.4 to −13.4 kJ/mol. Shift of the α-aminobenzyl substitution to position 2 led to a 60–80% reduction in interaction energy, $-\Delta(\Delta H^\circ)$ ranging from 1.5 to 7.6 kJ/mol. The trends in the change in $\Delta(\Delta S^\circ)$ confirmed that the 2-naphthol analogs exhibited the more negative entropy values (Table 4). This can be explained by the fact that the difference in the number of degrees of freedom between the stereoisomers on the CSP was large. In summary, the actual complex formation *via* multiple intermolecular interactions was generally exothermic, but the corresponding entropic contribution was negative because of the restriction of the analytes on the CSP. The final balance between these contributions may lead to either an enthalpy- or an entropy-dominated process. As evident from the $\Delta(\Delta G^\circ)$ data listed in Table 4, the separations for all investigated analytes on this CSP were enthalpically favored.

However, the relative contributions of the enthalpy and entropy to the free energy of adsorption depend on the nature of the *analytes*. To visualize this term, the enthalpy/entropy ratios, $Q = [\Delta(\Delta H^\circ)]/[\Delta(\Delta S^\circ) \times 298^\circ\ \mathrm{K}]$, were calculated and are listed in Table 4. This factor indicates the relative contribution of the enthalpy *versus* the entropy term to $\Delta(\Delta G^\circ)$ at 298 K. A comparison of the Q values for the individual analytes revealed that the enan-

Table 4. Thermodynamic parameters $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $\Delta(\Delta G^\circ)_{298\,K}$ and correlation coefficients (r^2) of enantiomers of 2-naphthol and 1-naphthol derivatives at a mobile phase composition of *n*-hexane/IPA/DEA = 55/45/0.1 v/v/v

Analyte	$\Delta(\Delta H^\circ)$, kJ/mol	$\Delta(\Delta S^\circ)$, J/mol × K	r_2	$\Delta(\Delta G^\circ)_{298\,K}$, kJ/mol	Q	Analyte	$\Delta(\Delta H^\circ)$, kJ/mol	$\Delta(\Delta S^\circ)$, J/mol × K	r_2	$\Delta(\Delta G^\circ)_{298K}$, kJ/mol	Q
1a	−13.4	−34.0	0.997	−3.3	1.3	**2a**	−7.6	−17.3	0.994	−2.5	1.5
1b	−12.6	−31.8	0.997	−3.2	1.3	**2b**	−4.0	−6.9	0.986	−1.9	1.9
1c	−10.3	−26.9	0.995	−2.3	1.3	**2c**	−1.5	−1.3	0.992	−1.1	3.8
1d	−13.3	−33.3	0.998	−3.4	1.3	**2d**	−4.2	−8.0	0.990	−1.7	1.7
1e	−11.8	−28.8	0.994	−3.2	1.4	**2e**	−2.8	−4.3	0.996	−1.5	2.2
1f	−13.3	−35.5	0.997	−2.8	1.3	**2f**	−2.3	−2.5	0.999	−1.5	3.1
1g	−9.4	−23.0	0.998	−2.6	1.4	**2i**	−2.0	−3.4	0.993	−1.0	2.0

Column, Chiralcel OD-H; eluent, *n*-hexane/2-propanol/DEA; detection, 232 nm; flow rate, 0.5 mL/min; temperature, 298–308 K; and $Q = [\Delta(\Delta H^\circ)]/[(\Delta(\Delta S^\circ) \times 298\ K]$.

tioselective association for substituted 1-naphthol analogs (**2b–2i**) is significantly more enthalpy-driven (Table 4; *Q* ranges between 1.7 and 3.8). 2-Naphthol analogs (Table 4; **1a–1g**) and the unsubstituted 1-naphthol analog (**2a**) exhibit significantly lower *Q* values: typically, $Q < 1.5$. For this class of analytes, a major proportion of the favorable $\Delta(\Delta H^\circ)$ balance is counterbalanced by unfavorable entropic contributions.

Absolute configuration [6] and elution sequence were determined for analyte **1a**, and (*S*) < (*R*) elution sequence was detected. Probably a similar elution sequence can be presumed for 2-naphthol analogs.

4 Concluding remarks

Chiralcel OD-H can be used as CSP to separate the enantiomers of α-aminobenzyl-substituted 1- and 2-naphthol analogs. The separation can be accomplished in normal-phase mode by using different mobile phases and temperatures. The position of the α-aminobenzyl substituent of the 1- and 2-naphthol analogs has an influence on the retention and the selectivity. Halogen or nitro substitution on the α-aminobenzyl group has little effect on the separation.

Optimization of the separation can be achieved by the change of the column temperature rather than the mobile phase composition. Evaluation of van't Hoff plots was applied to determine thermodynamic data and can also be used for a mechanistic study of the chiral recognition process.

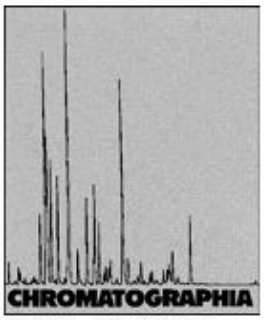
This work was supported by the Hungarian National Science Foundation grants, OTKA: T 042451, TS 40888, and F 034218.

5 References

[1] Betti, M., *Org. Synth. Coll. Vol.* 1941, *1*, 381–383.
[2] Szatmári, I., Martinek, T. A., Lázár, L., Fülöp, F., *Eur. J. Org. Chem.* 2004, 2231–2238.
[3] Betti, M., Pratesi, P., *Berichte der Deutschen Chemischen Gesellschaft* 1930, *63B*, 874–875.
[4] Betti, M., Pratesi, P., *Biochem. Z.* 1934, *274*, 1–3.
[5] Betti, M., *Gazz. Chim. Ital.* 1912, *42*, 288–294.
[6] Cardellicchio, C., Ciccarella, G., Naso, F., Schingaro, E., Scordari, F., *Tetrahedron: Asymm.* 1998, *9*, 3667–3675.
[7] Cardellicchio, C., Ciccarella, G., Naso, F., Perna, F., Tortorella, P., *Tetrahedron* 1999, *55*, 14685–14692.
[8] Palmieri, G., *Tetrahedron: Asymm.* 2000, *11*, 3361–3373.
[9] Liu, D.-X., Zhang, L.-C., Wang, Q., Da, C.-S., Xin, Z.-Q., Wang, R., Choi, M. C. K., Chan, A. S. C., *Org. Lett.* 2001, *3*, 2733–2735.
[10] Cimarelli, C., Palmieri, G., Volpini, E., *Tetrahedron: Asymm.* 2002, *13*, 2417–2426.
[11] Lu, J., Xu, X., Wang, S., Wang, C., Hu, Y., Hu, H., *J. Chem. Soc., Perkin Trans.* 2002, *1*, 2900–2903.
[12] Lu, J., Xu, X., Wang, C., He, J., Hu, Y., Hu, H., *Tetrahedron Lett.* 2002, *43*, 8367–8369.
[13] Szatmári, I., Hetényi, A., Lázár, L., Fülöp, F., *J. Heterocyclic Chem.* 2004, *41*, 367–373.
[14] Desai, N. C., Shukla, H. K., Langalia, N. A., Thaker, K. A., *J. Indian Chem. Soc.* 1984, *61*, 711–713.
[15] Péter, A., Tőrők, G., Fülöp, F., *J. Chromatogr. Sci.* 1998, *36*, 311–317.
[16] Yashima, E., Shavattanapong, P., Okamoto, Y., *Chirality* 1996, *8*, 446–451.
[17] Fornstedt, T., Sajonz, P., Guichon, G., *J. Am. Chem. Soc.* 1997, *119*, 1254–1264.
[18] Pirkle, W. H., Burke, J. A., *J. Chromatogr. A* 1991, *557*, 173–185.
[19] Péter, A., Tőrők, G., Armstrong, D. W., Tóth, G., Tourwé, D., *J. Chromatogr. A* 1998, *828*, 177–190.
[20] Péter, A., Vékes, E., Armstrong, D. W., *J. Chromatogr. A* 2002, *958*, 89–107.
[21] Dungelova, J., Lehotay, J., Krupcik, J., Cizmarik, J., Armstrong, D. W., *J. Sep. Sci.* 2004, *27*, 983–990.
[22] Szatmári, I., Fülöp, F., *Curr. Org. Synth.* 2004, *1*, 155–165.
[23] Szatmári, I., Martinek, T. A., Lázár, L., Fülöp, F., *Tetrahedron* 2003, *59*, 2877–2884.

II.

Comparison of Separation Efficiency of Macrocyclic Glycopeptide-Based Chiral Stationary Phases for the LC Enantioseparation of β-Amino Acids



2006, *64*, 89–94

A. Sztojkov-Ivanov[1], L. Lázár[1], F. Fülöp[1], D. W. Armstrong[2], A. Péter[3,✉]

[1] Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös utca 6, 6720, Szeged, Hungary
[2] Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington TX, 76019-0065, USA
[3] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, 6720, Szeged, Hungary; E-Mail: apeter@chem.u-szeged.hu

Received: 20 March 2006 / Revised: 26 April 2006 / Accepted: 1 May 2006
Online publication: 28 June 2006

Abstract

Direct reversed-phase high-performance liquid chromatographic methods were developed for the separation of enantiomers of eighteen unnatural β-amino acids, including several β-3-homo-amino acids. The direct separations of the underivatized analytes were performed on chiral stationary phases containing macrocyclic glycopeptide antibiotics such as teicoplanin (Chirobiotic T and T2), teicoplanin aglycone (Chirobiotic TAG), vancomycin (Chirobiotic V and V2), and ristocetin A (Chirobiotic R) as chiral selectors. The effects of the organic modifier, mobile phase composition and pH on the separations were investigated. A comparison of the separation performances of the macrocyclic glycopeptide stationary phases revealed that the Chirobiotic T2 column exhibited better selectivity than the Chirobiotic T column for the separation of β-3-homo-amino acid enantiomers; vancomycin or ristocetin A exhibited lower selectivity. The elution sequence was determined in some cases: the *S* enantiomers eluted before the *R* enantiomers, with the exception of the Chirobiotic R column, where the elution sequence *R* < *S* was observed.

Keywords

Column liquid chromatography
Macrocyclic glycopeptide-based chiral stationary phases
Chirobiotic columns
β-Amino acids

Introduction

In recent years, β-amino acids have received increased attention either because of their unique pharmacological properties or in consequence of their being key components of numerous bioactive molecules (*e.g.* taxane derivatives and bestatin), developmental pharmaceuticals and peptidomimetics [1–4]. Their oligomers, the β-peptides, display a high tendency to the formation of secondary structures including helices, sheets and reverse turns [5–7].

The wide-ranging utility of these compounds has led to an increased attention to their enantioselective syntheses [8–10], which require analytical methods for a check on the enantiopurity of the final products.

For this purpose, mainly direct HPLC separation methods are widely used, as are chiral stationary phases (CSPs) based on macrocyclic antibiotics. The latter allow the resolution of many classes of compounds, often with improved selectivity. In the early stages of the application of macrocyclic antibiotics, Beesley and Scott [11], and more recently Aboul-Enein et al. [12], Ward et al. [13] and Dungelova et al. [14], surveyed the application of macrocyclic antibiotics in different fields of chromatography, including the enantioseparation of amino acids.

The HPLC enantioseparation of β-amino acids has been performed by Winnacker et al. [15], Davankov et al. [16], Lindner and Hirshbock [17], Griffith et al. [18], Yamazaki et al. [19], and Yamada [20], who applied mainly indirect chromatographic methods. D'Acquarica et al. [21] separated different alicyclic and cyclic β-amino acids on a new type of CSP, containing a macrocyclic glycopeptide antibiotic as chiral selector. Péter et al. made use of different chiral derivatizing agents [22–24], while D'Acquarica et al. [21] and Péter et al. [25–30] separated different alicyclic, cyclic and aromatic β-amino acids on new types of CSPs, containing a macrocyclic glycopeptide antibiotic, a quinine-derived chiral anion-exchanger, or an (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol derivative as chiral selector.

In the present paper, direct HPLC methods are described for the enantioseparation of racemic β-substituted-β-amino acids (β-homo-amino acids),

DOI: 10.1365/s10337-006-0824-8
0009-5893/06/07

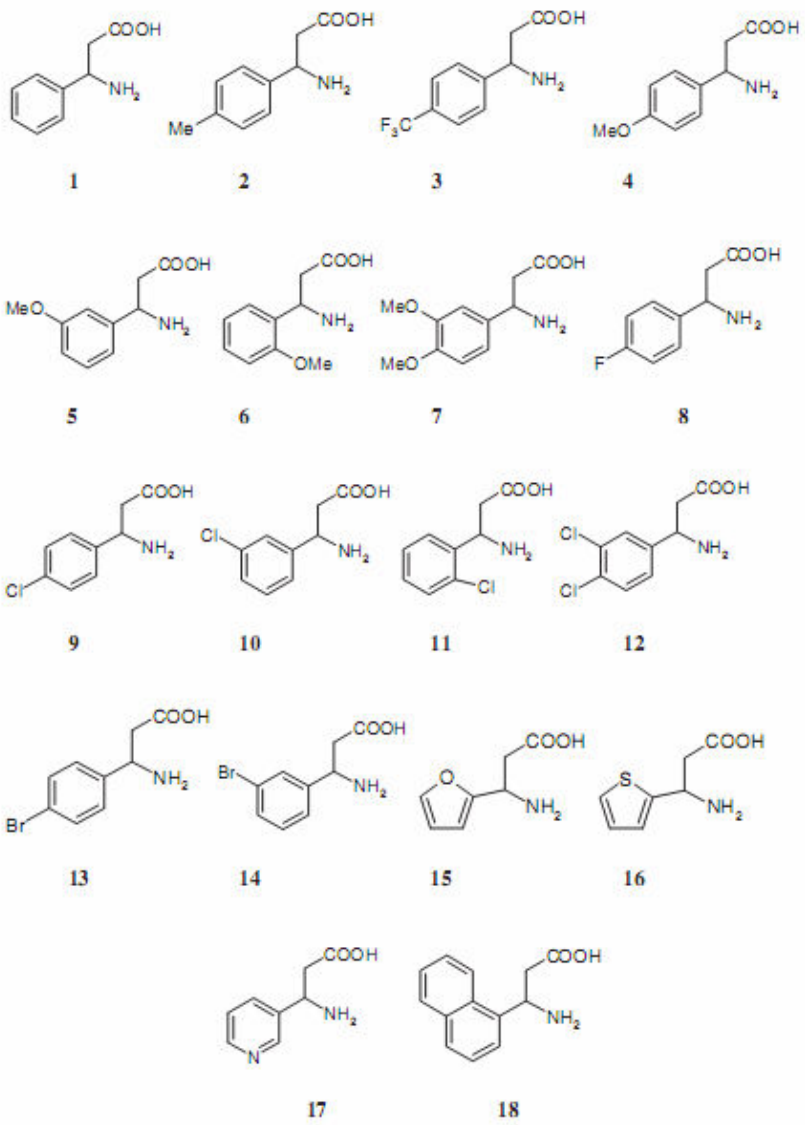


Fig. 1. Structures of investigated analytes Compounds: **1**, 3-amino-3-phenylpropanoic acid; **2**, 3-amino-3-(4-methylphenyl)propanoic acid; **3**, 3-amino-3-(4-trifluomethylphenyl)propanoic acid; **4**, 3-amino-3-(4-methoxyphenyl)propanoic acid; **5**, 3-amino-3-(3-methoxyphenyl)propanoic acid; **6**, 3-amino-3-(2-methoxyphenyl)propanoic acid; **7**, 3-amino-3-(3,4-dimethoxyphenyl)propanoic acid; **8**, 3-amino-3-(4-fluorophenyl)propanoic acid; **9**, 3-amino-3-(4-chlorophenyl)propanoic acid; **10**, 3-amino-3-(3-chlorophenyl)propanoic acid; **11**, 3-amino-3-(2-chlorophenyl)propanoic acid; **12**, 3-amino-3-(3,4-dichlorophenyl)propanoic acid; **13**, 3-amino-3-(4-bromophenyl)propanoic acid; **14**, 3-amino-3-(3-bromophenyl)propanoic acid; **15**, 3-amino-3-(2-furyl)propanoic acid; **16**, 3-amino-3-(2-thienyl)propanoic acid; **17**, 3-amino-3- (3-pyridyl)propanoic acid; **18**, 3-amino-3-(1-naphthyl)propanoic acid

with the application of different macrocyclic glycopeptide antibiotic-based CSPs. Columns were used in the reversed-phase (RP) or the polar-ionic (PI) mode. The effects of different parameters on the selectivity, such as the nature of the organic modifier, the mobile phase composition, the pH and the structure of the analytes (Fig. 1) are examined and discussed. The separation of the stereoisomers was optimized by variation of the chromatographic parameters. The elution sequence was in most cases determined by spiking the racemate with an enantiomer with a known absolute configuration.

Experimental

Chemicals and Reagents

Racemic 3-aryl- or 3-heteroaryl-substituted 3-aminopropanoic acids (**1**–**18**) were synthetized by a modified Rodionov synthesis, starting from the corresponding aromatic aldehydes [31–33]. The 3-aryl-substituted β-amino acid enantiomers were prepared by Lipolase-catalyzed (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) enantioselective ring cleavage of the corresponding racemic 4-aryl-substituted β -lactams with water in diisopropyl ether [34]. The enantiomerically pure heteroaryl-substituted β-amino acids were prepared by *Candida antarctica* lipase A-catalyzed enzymatic kinetic resolution of the corresponding esters in ethyl butyrate [33].

Acetonitrile (MeCN) and methanol (MeOH) of HPLC grade were purchased from Merck (Darmstadt, Germany). Triethylamine (TEA), glacial acetic acid (AcOH) and other reagents of analytical reagent grade were from Merck. The Milli-Q water was further purified by filtration on a 0.45-µm filter, type HV, Millipore (Molsheim, France).

Apparatus

The HPLC measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium^{32} Chromatography Manager data system; the alternative Waters Breeze system consisted of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (both systems from Waters Chromatography, Milford, MA, USA). Both chromatographic systems were equipped with Rheodyne Model 7125 injectors (Cotati, CA, USA) with 20-µL loops.

The macrocyclic glycopeptide-based stationary phases used for analytical separation were teicoplanin-containing Chirobiotic T and T2, teicoplanin aglycone-containing Chirobiotic TAG, vancomycin-containing Chirobiotic V and V2, and ristocetin A-containing Chirobiotic R columns, 250 mm × 4.6 mm I.D., 5-µm particle size (for each column) (Astec, Whippany, NJ, USA). The differences between the Chirobiotic T

and T2 are that they are both on 5-µm particle size silica gel, but the Chirobiotic T uses a 120 Ängstrom pore size material while Chirobiotic T2 uses a 200 Ängstrom pore material. Also the linkage chain on the Chirobiotic T2 is approximately twice the length of the Chirobiotic T. Hence the coverage and spacing will be different for the two. This will manifest itself mainly in the form of steric interaction differences between the two columns.

The columns were thermostated in a water bath, with a cooling-heating thermostat (MK 70, Mechanik Prüfgeräte, Medlingen, Germany). The precision of the temperature adjustment was ± 0.1 °C.

Results and Discussion

Six stationary phases were used for the direct enantioseparation of the β-amino acids in this study: teicoplanin-based (Chirobiotic T and Chirobiotic T2) columns, a teicoplanin aglycone-based (Chirobiotic TAG) column, vancomycin-based (Chirobiotic V and Chirobiotic V2) columns and a ristocetin A-based (Chirobiotic R) column. On all stationary phases, the examined synthetic β-amino acids were analyzed by working in the PI or RP mode. The analytes were chromatographed and detected without pre- or postcolumn derivatization. All compounds in Tables 1 and 2 were evaluated by using all stationary phases with a minimum of five of the following PI or RP mobile phases: MeOH/AcOH/TEA (100/0.1/0.1 *v/v/v*), (100/0.01/0.01 *v/v/v*), (100/0.4/0.1 *v/v/v*), (100/0.1/0.4 *v/v/v*), 100% MeOH, 0.1% aqueous triethylammonium acetate (TEAA, pH 4.1)/MeOH in different compositions and 0.1% aqueous triethylammonium acetate (TEAA, pH 6.5)/MeOH in different compositions. To simplify the presentation, only the chromatographic results for a partial or baseline enantiomeric separation are given in the tables. However, for purposes of comparison, examples are included in some cases where no separation occurred. The elution sequence was determined in some cases: on Chirobiotic V column the *S* enantiomers eluted before the *R* enantiomers, while on the Chirobiotic R column, $R < S$ elution sequence was observed.

Table 1. Chromatographic data, retention factor of the first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) of the enantioseparation of β-amino acids with the structure H_2N-CHX-CH_2-COOH on ristocetin A and vancomycin-based CSPs

Compound X	Column	Eluent (*v/v/v*)	k_1'	α	R_S	Elution sequence
1 	Chirobiotic R	100/0.1/0.1,a	1.38	1.09	0.80	*R<S*
	Chirobiotic V	100/0.1/0.1,a	0.70	1.13	0.80	*S<R*
2 	Chirobiotic R	100/0/0,d	1.51	1.14	0.80	*R<S*
	Chirobiotic R	100/0.1/0.1,a*	1.26	1.14	0.90	*R<S*
4 	Chirobiotic R	100/0.04/0.01,c	1.80	1.10	0.80	n.d.
5 	Chirobiotic R	100/0.1/0.1a	1.43	1.15	1.00	*R<S*
	Chirobiotic V	100/0.1/0.1,a	0.75	1.16	0.80	n.d.
	Chirobiotic V	100/0/0,d	0.85	1.13	0.85	*S<R*
7 	Chirobiotic R	100/0.1/0.1,a*	1.75	1.15	1.00	n.d.
10 	Chirobiotic R	100/0.01/0.01,a	1.20	1.10	0.80	*R<S*
	Chirobiotic V	100/0.1/0.1,a	0.71	1.13	0.80	*S<R*
11 	Chirobiotic R	100/0.1/0.1,a	1.23	1.11	0.70	*R<S*
14 	Chirobiotic R	100/0.01/0.01,b	1.33	1.10	0.75	*R<S*
	Chirobiotic V	100/0.1/0.1,a	0.77	1.13	0.75	n.d.
18 	Chirobiotic V	100/0/0,d	1.02	1.07	0.70	n.d.

Chromatographic conditions: column, Chirobiotic R, Chirobiotic V; mobile phase, MeOH/AcOH/TEA: **a**, (100/0.1/0.1 *v/v/v*), **b**, (100/0.01/0.01 *v/v/v*), **c**, (100/0.04/0.01 *v/v/v*), **d**, (100/0.0/0.0 *v/v/v*); flow rate, 0.5 mL min^{-1}, * 0.25 mL min^{-1}; detection, 205 nm; n.d., elution sequence not determined

Enantioseparation of β-Amino Acids on Ristocetin A and Vancomycin-Based CSPs

On all these CSPs, the retention and selectivity could be controlled by altering the nature and concentration of the organic modifier, but variation of the flow rate sometimes had a beneficial effect on the resolution (data not shown). To obtain higher resolution on the ristocetin A and vancomycin-containing CSPs, the separation was optimized by: (i) changing the MeOH/AcOH/TEA ratio in the mobile phase, (ii) application of 0.1% aqueous triethylammonium acetate (TEAA) pH 4.1 or 6.5 buffer in the eluent, or (iii) application of 100% MeOH as the organic modifier. Despite the large variations in the conditions, half of the stereoisomers were unresolved and the stereoisomers of 1, 2, 4, 5, 7, 10, 11, 14 and 18 exhibited only partial resolution, $R_S < 1.0$ (Table 1). The PI mode exhibited some selectivity for these types of β-amino acids, while the RP mode was ineffective in the separations of the β-amino acid enantiomers. The ristocetin A selector generally displayed a better separation performance than that of the vancomycin-based CSP.

Enantioseparation of β-Amino Acids on Teicoplanin and Teicoplanin Aglycone-Based CSPs and Comparison of Separation Performances of Chirobiotic T and T2 Columns

Enantioseparations were carried out on two teicoplanin-based CSPs (Chirobiotic T and T2) and on a teicoplanin aglycone-based CSP (Chiribiotic TAG) (Fig. 2); the results are listed in Table 2. For purposes of comparison Table 2 also includes results where separation was not achieved. Berthod et al. [35] earlier found that the teicoplanin aglycone afforded a higher separation capability for α-amino acids than the native teicoplanin. Opposite behavior was observed for β-3-homo-amino acids by Peter et al. [27,28]. For the α-amino acids, use of the PI mode with a MeOH/AcOH/TEA mobile phase system instead of the RP mode generally

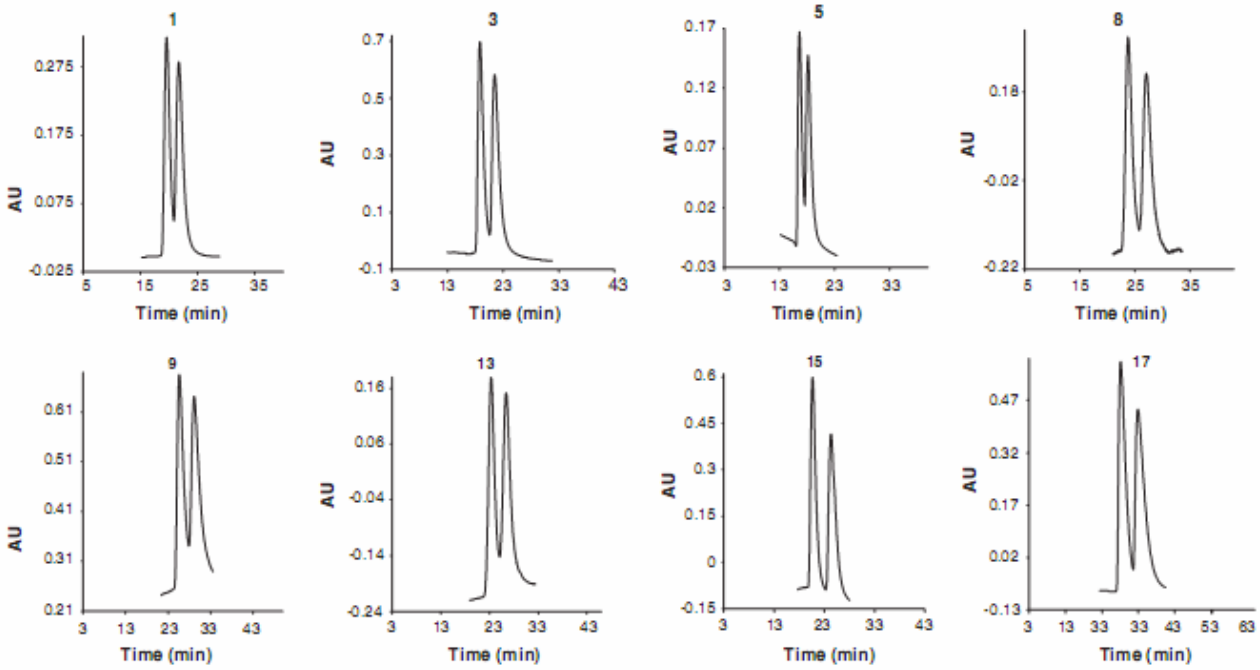


Fig. 2. Selected chromatograms of the enantioseparation for analytes **1, 3, 5, 8, 9, 13, 15** and **17**, evaluated by the direct method Chromatographic conditions: column, Chirobiotic T2 for **1, 3, 8, 9, 13** and **15**, Chirobiotic R for **5**, Chirobiotic TAG for **17**; mobile phase, MeOH/AcOH/TEA (100/0.01/0.01 *v*/*v*/*v*) for **1, 3, 8, 9, 13** and **15**; MeOH/AcOH/TEA (100/0.1/0.1 *v*/*v*/*v*) for 5; 0.1% aqueous TEAA (pH 4.1)/MeOH = 10/90 (*v*/*v*) for 17; temperature, ambient; detection, 205 nm; flow rate, 0.40 mL min^{-1} (for 17, 0.80 mL min^{-1}); for the elution sequences see, Tables 1 and 2

resulted in good enantioresolution on both the native teicoplanin and teicoplanin aglycone stationary phases [35]. On both teicoplanin and teicoplanin aglycone CSPs, use of the PI mode did not result in any improvement in enantioresolution for the β-3-homo-amino acids [27,28]. The data in Table 2 indicate that both Chirobiotic T and Chirobiotic TAG CSPs seemed to be less effective in the enantioseparation of the β-3-homo-amino acid enantiomers in either RP or PI separation mode.

A comparison of the separation performances of the Chirobiotic T and T2 CSPs revealed that the retention factors of the first-eluting enantiomers on Chirobiotic T2 were larger by 25–30% (exceptions were analytes 15–17, for which the larger k_1' values were obtained on Chirobiotic T). The newly developed Chirobiotic T2 CSP is a result of studies of the effects of the positions of the linkages, the lengths of the spacers and silica chemistry. Chirobiotic T2 is a more density covered phase and this may explain the stronger interactions between the enantiomers and this CSP.

Substitution on the aromatic rings of the β-amino acids (compounds 2–14) did not result in a significant change in the retention factors of the first-eluting enantiomers. At a constant mobile phase composition, MeOH/AcOH/TEA (100/0.01/0.01 *v*/*v*/*v*) on the Chirobiotic T, k_1's ranged from 1.32 to 2.34, while on Chirobiotic T2 they were from 1.93 to 3.08. The least and most retained analytes were 3 and 7, respectively. For analogs 4–7 or 9–12, having the same substituents but in different positions, the *para*-substituted compounds had larger retention factors, and generally larger a values on the T2 column. It appeared that the Chirobiotic T CSP produces less efficient separations for this class of compounds. On the T2 column, for analogs 8, 9, 13 or 10, 14 (having the halogen substituents in the same position), larger retention factors were obtained for the bromo-substituted analogs than for the chloro- or fluoro-substituted ones, while the α values did not change significantly. In the process of chiral recognition the primary "docking" interaction (between the amine of the CSP and the carboxylate of the analyte) should be similar. H-bonding, hydrophobic, steric etc. interactions should be taken into account. However different sterics (one of the three simultaneous interactions needed for chiral recognition) can be the lone difference or if the steric repulsive interactions are very different, they could effect the other interactions (for example, if the steric repulsion in one case is so great that it prevents or minimizes one of the primary interactions). As regards H-bonding, its ability decreases in the sequence fluoro > chloro > bromo. The bromo-substituted analogs exhibited the largest hydrophobicity and bulkiness of the analogs containing the three halogens. Our results shed light on the importance of steric and hydrophobic interactions in these separations.

The resolution (R_S values) of these compounds were larger on the Chirobiotic T2 column than found on the Chirobiotic T column, except for analytes 15–17. In these latter three cases, the reasons are unclear. This behavior may be attributed to the structures of the analytes: the aromatic ring positioned close to the chiral center have –O–, –S– or =N– groups containing unpaired electrons, while in all other cases (3–14) the atoms with unpaired electrons are not part of the aromatic ring. In summary, the newly developed teicoplanin CSP Chirobiotc T2 proved more suitable for the separation of the stereoisomers of β-3-homo-amino acids than Chirobiotic T.

The sequence of elution was determined in most cases. Since the Chirobiotic T, TAG, R and V columns proved to be less efficient than the Chirobiotic

T2 column, most of the data on the elution sequence in Table 2 relate to the latter column. A general rule could be established for the elution of the stereoisomers of β-3-homo-amino acids: the *S* stereoisomers eluted before the *R* stereoisomers.

Conclusions

In conclusion, this study has demonstrated that macrocyclic antibiotic-based CSPs are quite successful for the direct enantioseparation of various β-amino acids. The direct separations of the underivatized analytes were in most cases successfully achieved on the CSP containing the macrocyclic glycopeptide antibiotic teicoplanin (Chirobiotic T2). The teicoplanin (Chirobiotic T), teicoplanin aglycone (Chirobiotic TAG), vancomycin (Chirobiotic V2) and ristocetin A (Chirobiotic R) CSPs seemed to be less effective in the chiral separation of β-3-homo-amino acids. The effects of the organic modifier, mobile phase composition on the separation were investigated. In fact, the macrocyclic glycopeptides are to some extent complementary to one another: where partial enantioresolution was obtained with one glycopeptide, there was a probability that better separation could be obtained with another. The elution sequence was in some cases determined: the *S* enantiomers eluted before the *R* enantiomers, with the exception of the Chirobiotic R column where the elution sequence *R*<*S* was observed.

Acknowledgments

This work was supported by OTKA grants T 042451 and T 049407 and by the National Institutes of Healths grant, NIH RO1 GM53825–10.

Table 2. Chromatographic data, retention factor of the first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) of the enantioseparation of β-amino acids with the structure H_2N-CHX-CH_2-COOH (**1-18**) on teicoplanin and teicoplanin aglycone-based CSPs

Compound X	Column	Eluent (v/v/v)	k_1'	α	Rs	Elution sequence
1	T	100/0.01/0.01,a*	1.67	1.00	0.0	-
	T2	100/0.01/0.01,a	2.08	1.17	1.00	*S*<*R*
2	T	100/0.01/0.01,a*	1.62	1.00	0.0	-
	T2	100/0.01/0.01,a	2.13	1.13	0.95	*S*<*R*
3	T	100/0.01/0.01,a*	1.32	1.14	<0.40	n.d.
	T2	100/0.01/0.01,a	1.93	1.23	1.09	
4	T	100/0.01/0.01,a*	2.01	1.00	0.40	n.d.
	T2	100/0.01/0.01,a	2.66	1.13	0.90	
5	T	100/0.01/0.01,a*	1.70	1.00	0.0	n.d.
	T2	100/0.01/0.01,a	2.66	1.00	0.0	
6	T	100/0.01/0.01,a*	1.67	1.0	<0.40	-
	TAG	100/0.01/0.01,a	2.67	1.12	0.90	*S*<*R*
	T2	100/0.01/0.01,a	1.97	1.13	0.80	*S*<*R*
7	T	100/0.01/0.01,a*	2.34	1.00	0.0	n.d.
	TAG	100/0/0,c	5.46	1.06	0.75	
	T2	100/0.01/0.01,a	3.08	1.00	0.0	
8	T	100/0.01/0.01,a*	1.76	1.06	<0.40	-
	T2	100/0.01/0.01,a	2.45	1.20	1.15	*S*<*R*
9	T	100/0.01/0.01,a*	1.88	1.06	<0.40	-
	T2	100/0.01/0.01,a	2.72	1.18	1.10	*S*<*R*
10	T	100/0.01/0.01,a*	1.61	1.00	0.0	-
	T2	100/0.01/0.01,a	2.21	1.15	0.90	*S*<*R*
11	T	100/0.01/0.01,a*	1.46	1.08	<0.40	-
	T2	100/0.01/0.01,a	2.17	1.00	0.0	*S*<*R*
12	T	100/0.01/0.01,a*	1.83	1.08	<0.40	n.d.
	T2	100/0.01/0.01,a	2.85	1.11	0.85	
13	T	100/0.01/0.01,a*	1.94	1.07	<0.40	-
	T2	100/0.01/0.01,a	3.00	1.18	1.10	*S*<*R*
14	T	100/0.01/0.01,a*	1.77	1.00	0.0	n.d.
	T2	100/0.01/0.01,a	2.35	1.14	1.00	
15	T	100/0.1/0.1,b*	3.84	1.15	1.00	*S*<*R*
	T2	100/0.01/0.01,a	1.99	1.27	1.65	*S*<*R*
16	T	100/0.4/0.1,b*	3.79	1.03	<0.40	*S*<*R*
	TAG	10/90,d*	4.53	1.07	0.80	*S*<*R*
	T2	00/0.01/0.01,a	2.28	1.00	0.00	-
17	T	100/0.1/0.1,b	6.29	1.09	1.00	*S*<*R*
	TAG	10/90,d*	10.26	1.18	1.10	*S*<*R*
	T2	100/0.01/0.01,a	3.93	1.00	0.0	-
18	T	100/0.01/0.01,a*	1.81	1.09	<0.40	n.d.
	T2	100/0.01/0.01,a	2.33	1.15	0.95	

Chromatographic conditions: column, T and T2, Chirobiotic T and Chirobiotic T2; TAG, Chirobiotic TAG; mobile phase, a, MeOH/AcOH/TEA (100/0.01/0.01 v/v/v), b, MeOH/AcOH/TEA (100/0.1/0.1 v/v/v), c, (100/0.0/0.0 v/v/v); d, 0.1% aqueous triethylammonium acetate (TEAA, pH 4.1)/MeOH (10/90 v/v); flow rate, 0.5 mL min^{-1}, * 0.8 mL min^{-1}; detection, 205 nm; n.d., elution sequence not determined

References

1. Ojima I, Lin S, Wang T (1999) Curr Med Chem 6:927–954
2. Steer DL, Lew RA, Perlmutter P, Smith AI, Aguilar MI (2002) Curr Med Chem 9:811–822
3. Ma JS (2003) Chim. Oggi – Chem Today 21(6):61–68
4. Kuhl A, Hahn MG, Dumic M, Mittendorf J (2005) Amino Acids 29:89–100
5. Cheng RP, Gellman S, DeGrado WF (2001) Chem Rev 101:3219–3232
6. Fülöp F, Martinek T, Tóth GK (2006) Chem Soc Rev 35:323–334
7. Seebach D, Beck AK, Bierbaum DJ (2004) Chem Biodivers 1:1111–1238
8. Liu M, Sibi MP (2002) Tetrahedron 58:7991–8035
9. Ma JA (2003) Angew Chem Int Ed 42:4290–4299
10. Juaristi E, Soloshonok V (eds) (2005) Enantioselective Synthesis of β-Amino Acids, Wiley, New York

11. Beesley TE, Scott RPW (1998) Chiral Chromatography, John Wiley & Sons, Chichester
12. Aboul-Enein HY, Ali I (2000) Chromatographia 52:679–691
13. Ward TJ, Farris III AB (2001) J Chromatogr A 906:73–89
14. Dungelova J, Lehotay J, Rajkovicova T (2004) Chem Anal 49:1–17
15. Winnacker EL, Herbst MM, Barker HA (1971) Biochem Biophys Acta 237:280–283
16. Davankov VA, Zolotarev YA, Kurganov AA (1979) J Liq Chromatogr 2: 1191–1204
17. Lindner WL, Hirschbock I (1986) J Liq Chromatogr 9:551–571
18. Griffith OW, Campbell EB, Pirkle WH, Tsipouras A, Hyun MH (1986) J Chromatogr 362:345–352
19. Yamazaki S, Takeuchi T, Tanimura T (1991) J Chromatogr 540:169–175
20. Yamada T, Nonomura S, Fujiwara H, Miyazawa T, Kuwata S (1990) J Chromatogr 515:475–482
21. D'Acquarica I, Gasparrini F, Misiti D, Zappia G, Cimarelli C, Palmieri G, Carotti A, Cellamare S, Villani C (2000) Tetrahedron: Asymmetry 11:2375–2385
22. Péter A, Fülöp F (1995) J Chromatogr A 715:219–226
23. Péter A, Török G, Csomós P, Péter M, Bernáth G, Fülöp F (1997) J Chromatogr A 761:103–113
24. Török G, Péter A, Csomós P, Kanerva LT, Fülöp F (1998) J Chromatogr A 797:177–186
25. Péter A, Lázár L, Fülöp F, Armstrong DW (2001) J Chromatogr A 926:229–238
26. Péter A (2002) J Chromatogr A 955:141–150
27. Árki A, Tourwé D, Solymár M, Fülöp F, Armstrong DW, Péter A (2004) Chromatographia 60:S43
28. Péter A, Árki A, Vékes E, Tourwé D, Lázár L, Fülöp F, Armstrong DW (2004) J Chromatogr A 1031:171–178
29. Péter A, Török R, Wright K, Wakselman M, Mazaleyrat JP (2003) J Chromatogr A 1021:1–10
30. Berkecz R, Török R, Ilisz I, Forró E, Fülöp F, Armstrong DW, Péter A (2006) Chromatographia, in press
31. Rodionov WM, Malivinskaya EF (1926) Chem Ber 59:2952–2958
32. Tan CYK, Weaver DF (2002) Tetrahedron 58:7449–7461
33. Solymár M, Fülöp F, Kanerva LT (2002) Tetrahedron: Asymmetry 13:2383–2388
34. Forró E, Paál T, Tasnádi G, Fülöp F (2006) Adv Synt Catal, in press
35. Berthod A, Chen X, Kullman JP, Armstrong DW, Gasparrini F, D'Aquarica I, Villani C, Carotti A (2000) Anal Chem 72:1767–1780

III.

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Journal of Chromatography A, 1125 (2006) 138–143
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

High-performance liquid chromatographic enantioseparation of β-amino acid stereoisomers on a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase

Róbert Berkecz[a], Anita Sztojkov-Ivanov[b], István Ilisz[a], Enikő Forró[b], Ferenc Fülöp[b], Myung Ho Hyun[c], Antal Péter[a,*]

[a] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary
[b] Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös utca 6, H-6720 Szeged, Hungary
[c] Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735, South Korea

Received 7 March 2006; received in revised form 19 June 2006; accepted 20 June 2006
Available online 14 July 2006

Abstract

Direct reversed-phase high-performance liquid chromatographic methods were developed for the separation of enantiomers of 14 unnatural β-amino acids, including several β-3-homo-amino acids on a chiral stationary phase containing (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid bonded to 3-aminopropyl silica gel as chiral selector. The effects of the organic and the acidic modifiers and the mobile phase composition on the separation were investigated. The natures and positions of the substituents on the aromatic ring substantially influenced the retention and enantioseparation. The elution sequence in most cases was determined and the *R* enantiomers were eluteted before the *S* enantiomers.

Keywords: Column liquid chromatography; β-Amino acids; (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase

1. Introduction

β-Aryl-substituted β-amino acids have been intensively investigated in the past few years, with regard to their unique biological activity [1] and their utility in synthetic chemistry and drug research [2]. In the free form, they can exert neurological activity and are known to be receptor antagonist and enzyme inhibitors [3]. They are also components of naturally occurring compounds with antitumor properties [2]. The importance of β-aryl-substituted β-amino acids is reflected by the large number of syntheses published for their preparation in both racemic and enantiopure form [4–7]. Control of the enantiopurity of the final products requires efficient analytical methods.

For these purposes, mainly high-performance liquid chromatographic (HPLC) separation methods are widely used. Application of chiral crown ethers as chiral stationary phases (CSPs) was initiated by Cram and co-workers [8] and nowadays chiral crown ethers are known to be very effective in the resolution of racemic compounds containing a primary amino group [9]. Among others, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid covalently bonded to silica gel has been successfully applied as a CSP [10,11].

HPLC enantioseparations of β-amino acids have been performed by indirect and direct methods. In the past decade new types of chiral derivatizing agents and CSPs were applied for the enantioseparation of β-amino acids by D'Acquarica et al. and by Péter et al. [12–15].

In the present paper, direct HPLC methods are described for the enantioseparation of racemic β-substituted-β-amino acids (β-homo-amino acids). Direct methods were carried out in reversed-phase mode by applying the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP. The effects of different parameters on the selectivity, such as the nature of the organic modifier, the mobile phase composition, the acidic modifier and the structure of the analytes, were examined and are discussed. The elution sequence was in most cases determined.

* Corresponding author. Tel.: +36 62544000/3656; fax: +36 62420505.
E-mail address: apeter@chem.u-szeged.hu (A. Péter).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.06.064

2. Experimental

2.1. Chemicals and reagents

Racemic 3-aryl-substituted 3-aminopropanoic acids were synthetized by a modified Rodionov and Malivinskaya synthesis, starting from the corresponding aromatic aldehydes [16]. The racemic aryl-substituted β-lactams were prepared according to the literature procedure [17]. The enantiomers of (*R*)-**1**, (*R*)-**2**, (*R*)-**9**, (*R*)-**10**, (*R*)-**11**, (*S*)-**8** and (*R*)-**13** were prepared by Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin)-catalyzed enantioselective ring cleavage of the corresponding racemic 4-aryl-substituted β-lactams [17].

Acetonitrile (MeCN) and methanol (MeOH) of HPLC grade were purchased from Merck (Darmstadt, Germany). Triethylamine (TEA), glacial acetic acid (AcOH), trifluoroacetic acid (TFA) and other reagents of analytical reagent grade were also from Merck. The Milli-Q water was further purified by filtration it on a 0.45 μm filter, type HV, Millipore (Molsheim, France).

2.2. Apparatus

The HPLC measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium^{32} Chromatography Manager data system (Waters Chromatography, Milford, MA, USA). The chromatographic system was equipped with Rheodyne Model 7125 injector (Cotati, CA, USA) with 20 μl loop.

The (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP used for direct separations, 5 μm particle size, 150 mm × 4.0 mm I.D., was prepared via the method described in the previous paper except for the use of Kromasil aminopropylsilica gel (Kromasil® NH_2, 5 μm) instead of Rainin AMINO silica gel [11]. The column was thermostated in a water bath, with a cooling–heating thermostat (MK 70, Mechanik Prüfgeräte, Medlingen, Germany). The precision of temperature adjustment was ±0.1 °C.

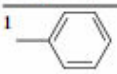
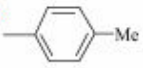
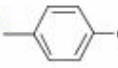
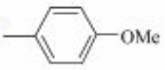
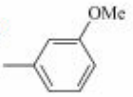
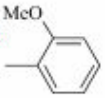
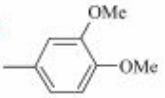
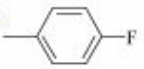
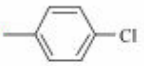
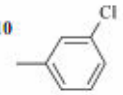
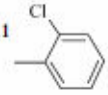
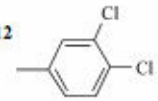
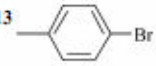
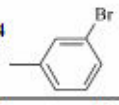
3. Results and discussion

Chromatographic results on the separation of 14 β-amino acid enantiomers are listed in Table 1; the data were obtained at lower (20%, v/v) and higher (80%, v/v) MeOH contents, at a constant AcOH concentration of 10.0 mM, and at an optimized mobile phase composition with an MeOH content of 50% (v/v) and an AcOH concentration of 5.0 mM. For the crown ether-based CSP, the most important interaction of analytes containing a primary amino group is complex formation between the protonated primary amino group and the oxygen atoms in the crown ether ring. The data in Table 1 reveals that an increase in the content of MeOH in the aqueous mobile phase increases the retention, as denoted by the retention factors (k'). As the content of MeOH in the aqueous mobile phase is increased, the polarity of the mobile phase is expected to decrease, and consequently the polar interactions between the mobile phase and polar β-amino acids should decrease. In this case, the retention factors will increase as the content of MeOH in the aqueous mobile phase increases. These trends were observed for all the investigated analytes.

A detailed investigation on the effect of the MeOH content and a deeper analysis of the data revealed that the separation factor (α) and resolution (R_S) changed in different ways as the MeOH content of the mobile phase was increased (Table 1 and Fig. 1). The α values generally decreased or did not change significantly (analytes **10**, **12** and **14**) with increasing MeOH content; for analyte **11** a slight increase was observed. At high MeOH content there was an increase in retention, while the interactions of the first- and second-eluting enantiomers with the CSP were different from those at low MeOH content. When MeOH content increases, non-chiral hydrophobic interactions are favoured for both enantiomers. As a consequence the overall retention increases while the role played by the chiral recognition decreases and so the selectivity. The exception of analyte **11** may be due to a different interaction mechanism of the 2′-chloro-substituted analog with the CSP. The *ortho* position of the chlorine atom hindered the interaction with the CSP (very small k' values), but in this case increase of the MeOH content promoted the chiral recognition. As concerns the R_S values, both a decrease and an increase were observed with increasing MeOH content (Fig. 1). The R_S values slightly decreased with increasing MeOH content for analytes **1**, **4**, **5**, **8** and **14**, but increased for analytes **2**, **3**, **7** and **9–13**. The enantiomers of analyte **6** were unresolved under these conditions.

The natures and positions of the substituents had substantial effects on the retention, but the selectivity (α) changed to a much lower extent than the k' value (Table 1). The k' values of *meta* and *para*-substituted analogs were larger than that of the non-substituted **1**, especially in mobile phases containing a large amount of the organic modifier. As concerns a comparison of the retention factors obtained for the analytes containing the same substituents in different positions on the aromatic ring, analytes **4–7** and **9–12** revealed that the *meta*-substituted analogs were more strongly retained than the *para*-substituted ones, and *ortho* position of the substituents was unfavorable regarding the enantioseparation. The enantiomers of analyte **6** were not separated, while those of analyte **11** were only partially resolved in a mobile phase containing AcOH as acidic modifier. For 3′,4′-disubstituted analogs **7** and **12**, especially large k' values were obtained. As regards the chromatographic data for the analytes bearing different halogen substituents in the same position, **8**, **9** and **13**; **10** and **14**, the retention factors were smaller for the fluoro- than for the bromo-substituted analogs. All of these data may be explained by a separation mechanism in which, besides complex formation inside the crown ether cavity, there may be an intermolecular H-bonding interaction between the halogen atoms of the aromatic ring of the analyte and the amino tethering group of the CSP (another intermolecular H-bonding interaction between the analyte and the CSP may occur between the two protonated carboxy groups [18]). It seems that the H-bonding effect is favorable when electron-donating atoms (oxygen or halogen) are in the *meta* or *para* position, and unfavorable when electron-

Table 1
Retention factors (k'), separation factors (α), resolutions (R_S) and elution sequences of stereoisomers of β-amino acids with the structure H_2N–CHR–CH_2–COOH (**1–14**)

Compound, –R	Eluent (v/v)	k_1'	k_2'	α	R_S	Elution sequence
1 	80/20, **a**	2.27	2.94	1.30	1.44	*R*<*S*
	20/80, **b**	8.66	9.89	1.14	1.13	
	50/50, **c**	2.98	3.93	1.32	1.77	
2 	80/20, **a**	2.96	3.71	1.26	0.85	*R*<*S*
	20/80, **b**	6.85	8.21	1.20	1.06	
	50/50, **c**	2.86	3.63	1.27	1.46	
3 	80/20, **a**	4.16	5.68	1.36	0.90	n.d.
	20/80, **b**	17.87	23.55	1.32	2.38	
	50/50, **c**	3.52	5.33	1.51	1.97	
4 	80/20, **a**	2.66	3.43	1.29	1.31	n.d.
	20/80, **b**	7.09	8.56	1.21	1.05	
	50/50, **c**	2.92	3.83	1.31	1.73	
5 	80/20, **a**	3.60	4.51	1.25	1.45	n.d.
	20/80, **b**	10.50	12.28	1.17	1.00	
	50/50, **c**	3.83	5.02	1.31	1.71	
6 	80/20, **a**	1.31	1.31	1.00	0.00	n.d.
	20/80, **b**	2.71	2.71	1.00	0.00	
	50/50, **c**	1.36	1.36	1.00	0.00	
7 	80/20, **a**	3.06	3.94	1.29	1.43	n.d.
	20/80, **b**	17.44	21.30	1.22	1.84	
	50/50, **c**	4.43	5.68	1.28	1.88	
8 	80/20, **a**	2.84	4.02	1.42	1.97	*R*<*S*
	20/80, **b**	10.74	13.72	1.28	1.58	
	50/50, **c**	3.25	4.53	1.39	1.59	
9 	80/20, **a**	4.24	6.06	1.43	1.86	*R*<*S*
	20/80, **b**	10.39	13.84	1.34	2.03	
	50/50, **c**	3.18	4.62	1.45	1.73	
10 	80/20, **a**	5.40	8.09	1.50	1.79	*R*<*S*
	20/80, **b**	12.27	18.64	1.52	2.40	
	50/50, **c**	5.69	9.19	1.62	2.53	
11 	80/20, **a**	1.81	1.81	1.00	<0.40	*R*<*S*
	20/80, **b**	3.04	3.47	1.14	1.06	
	50/50, **c**	1.73	1.94	1.12	0.69	
12 	80/20, **a**	8.97	14.95	1.62	2.27	n.d.
	20/80, **b**	24.35	39.70	1.63	2.40	
	50/50, **c**	6.27	7.66	1.22	1.91	
13 	80/20, **a**	3.80	5.61	1.48	1.54	*R*<*S*
	20/80, **b**	14.58	20.15	1.38	2.32	
	50/50, **c**	3.56	5.26	1.48	1.91	
14 	80/20, **a**	5.78	8.83	1.53	1.87	n.d.
	20/80, **b**	22.30	34.52	1.55	1.80	
	50/50, **c**	6.66	11.32	1.70	3.07	

Chromatographic conditions: column, crown ether-based CSP; mobile phase, **a**, H_2O/MeOH (80/20, v/v), 10 mM AcOH; **b**, H_2O/MeOH (20/80, v/v), 10 mM AcOH; **c**, H_2O/MeOH (50/50, v/v), 5 mM AcOH; flow rate, 0.5 ml min^{-1}; detection, 210 nm; n.d., not determined.

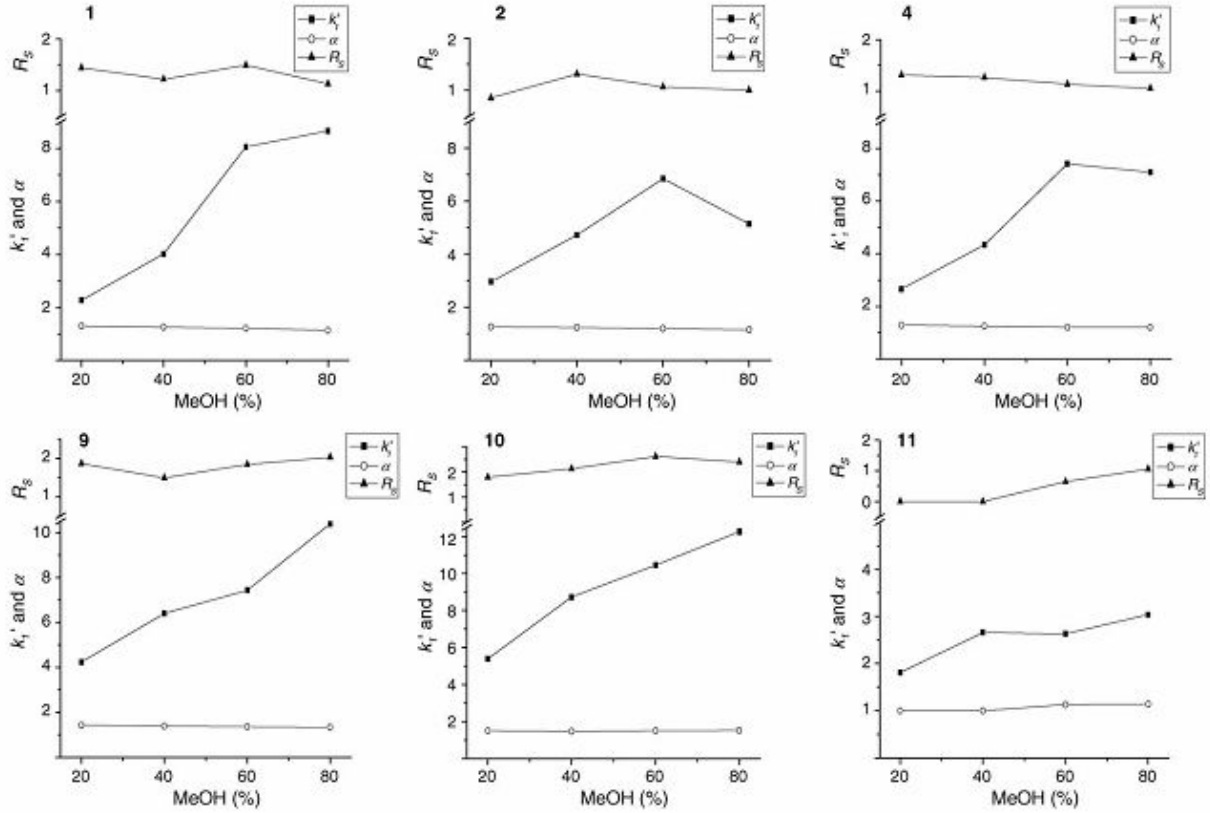


Fig. 1. Dependence of the retention factor (k'), separation factor (α) and resolution (R_S) on the MeOH content in the hydroorganic mobile phase; compounds: (**1**) 3-amino-3-phenylpropanoic acid, (**2**) 3-amino-3-(4-methylphenyl)propanoic acid, (**4**) 3-amino-3-(4-methoxyphenyl)propanoic acid, (**9**) 3-amino-3-(4-chlorophenyl)propanoic acid, (**10**) 3-amino-3-(3-chlorophenyl)propanoic acid, (**11**) 3-amino-3-(2-chlorophenyl)propanoic acid; chromatographic conditions: mobile phase, H_2O/MeOH (10 mM AcOH), 80/20, 60/40, 40/60 and 20/80 (v/v); flow rate, 0.5 ml min^{-1}; detection, 210 nm.

donating atoms are in the *ortho* position. The substituents in the *ortho* position may hinder the complex formation, since they are sterically situated very close to the primary amino function.

The effects of the nature and content of the acidic modifier on the separation were investigated by resolution in an aqueous mobile phase at a constant concentration of the organic modifier MeOH (50%, v/v). Host–guest complexation of the primary ammonium ion ($-NH_3^+$) inside the cavity of the chiral crown ether ring is essential for the chiral recognition. Actually the influence of pH on complexation is minimal, if pH is 2 or 3 units lower than log K value. The log K values of these analytes are not known, except for analyte **1** for which it is about 9. Under all the investigated experimental conditions supposing a similar log K values, the amino groups can be always considered completely protonated. Chromatographic data on analytes **6**, **9** and **11**, obtained by variation of the natures and concentrations of the acidic modifiers are depicted in Table 2. A comparison of the chromatographic data obtained by using AcOH, $HClO_4$, H_2SO_4, H_3PO_4 and TFA as acidic modifier in a hydroorganic mobile phase containing MeOH (50%, v/v) demonstrates that AcOH is much more suitable than the others in terms of retention, enantioselectivity and resolution. With the use of AcOH in the hydroorganic mobile phase, the retention factors continuously decreased with the amount of AcOH added to the mobile phase. At present, the reason for this chromatographic behavior with the variation of the acid content in the mobile phase is not clear. It may be explained by the higher ionic strength of the mobile phase at higher concentration of AcOH. The ionic strength can act as a "shield" with respect to the host–guest complex formation, reducing the retention, when increased.

According to Table 2, when stronger acid TFA (5 mM) is used instead of AcOH (5 mM) the retention time decreased quite much. This also can be rationalized by the improved ionic strength of the mobile phase. In mobile phases containing AcOH, the separation factor (α) did not change with variation of the acid content, while for analyte **9** the resolution (R_S) exhibited a slight decrease as the AcOH concentration in the mobile phase was increased. In the presence of other acidic modifiers, for analytes **9** and **11** both α and R_S were decreased as compared with data for AcOH. For analyte **6** a partial separation was observed in perchloric, sulfuric and phosphoric acids (Table 2). Selected chromatograms for the enantioseparation of analytes **1–14** are depicted in Fig. 2.

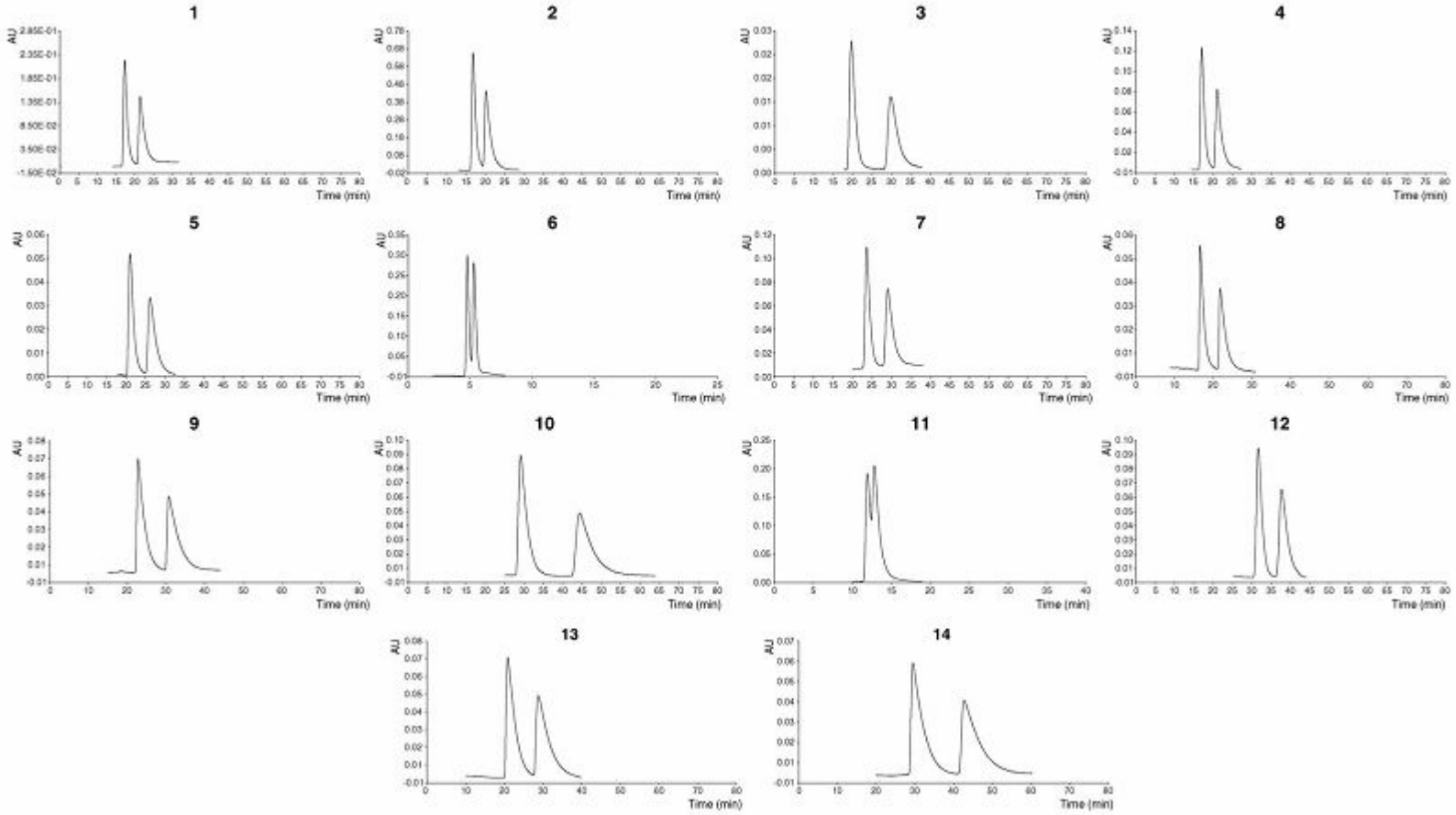
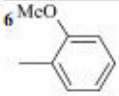
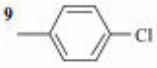
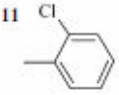


Fig. 2. Selected chromatograms of β-amino acids (**1–14**). Compounds: (**1**) 3-amino-3-phenylpropanoic acid, (**2**) 3-amino-3-(4-methylphenyl)propanoic acid, (**3**) 3-amino-3-(4-trifluomethylphenyl)propanoic acid, (**4**) 3-amino-3-(4-methoxyphenyl)propanoic acid, (**5**) 3-amino-3-(3-methoxyphenyl)propanoic acid, (**6**) 3-amino-3-(2-methoxyphenyl)propanoic acid, (**7**) 3-amino-3-(3,4-dimethoxyphenyl)propanoic acid, (**8**) 3-amino-3-(4-fluorophenyl)propanoic acid, (**9**) 3-amino-3-(4-chlorophenyl)propanoic acid, (**10**) 3-amino-3-(3-chlorophenyl)propanoic acid, (**11**) 3-amino-3-(2-chlorophenyl)propanoic acid, (**12**) 3-amino-3-(3,4-dichlorophenyl)propanoic acid, (**13**) 3-amino-3-(4-bromophenyl)propanoic acid, (**14**) 3-amino-3-(3-bromophenyl)propanoic acid; chromatographic conditions: column, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP; mobile phase, for compounds **1–5**, **7** and **10–12** H_2O/MeOH, 5 mM AcOH (50/50, v/v), for compounds **8**, **9**, **13** and **14** H_2O/MeOH, 10 mM AcOH (80/20, v/v), for analyte **6** H_2O/MeOH, 20 mM H_2SO_4 (50/50, v/v); flow rate, 0.5 ml min^{-1}; detection, 210 nm; for elution sequence, see Table 1.

Table 2
Dependence of chromatographic parameters, retention factor of first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) on the concentration of the acidic modifier in the mobile phase

Compound	Nature of acid	Concentration of acid (mM)	k_1'	α	R_S
6 	AcOH	1.0	3.03	1.00	0.0
	AcOH	5.0	2.18	1.00	0.0
	AcOH	10.0	1.68	1.00	0.0
	AcOH	20.0	1.32	1.00	0.0
	$HClO_4$	5.0	0.78	1.00	0.0
	$HClO_4$	10.0	0.02	1.10	0.60
	H_2SO_4	5.0	0.18	1.50	0.60
	H_3PO_4	5.0	0.23	1.00	0.0
	H_3PO_4	10.0	0.20	1.25	<0.40
	TFA	5.0	0.0	1.00	0.0
9 	AcOH	1.0	8.84	1.45	2.41
	AcOH	5.0	6.82	1.45	2.40
	AcOH	10.0	5.70	1.45	2.38
	AcOH	20.0	4.58	1.45	2.20
	$HClO_4$	5.0	1.28	1.16	0.64
	H_2SO_4	5.0	2.65	1.32	1.56
	H_3PO_4	5.0	0.71	1.00	0.0
	TFA	5.0	0.97	1.23	0.75
11 	AcOH	1.0	3.27	1.11	0.70
	AcOH	5.0	2.51	1.10	0.85
	AcOH	10.0	2.01	1.10	0.70
	AcOH	20.0	1.64	1.11	0.70
	$HClO_4$	5.0	0.20	1.00	0.0
	H_2SO_4	5.0	0.35	1.00	0.0
	H_3PO_4	5.0	0.71	1.00	0.0
	TFA	5.0	0.10	1.00	0.0

Chromatographic conditions: mobile phase, H_2O/MeOH (50/50, v/v), containing different amounts of acidic modifier; AcOH, acetic acid; TFA, trifluoroacetic acid; flow rate, 0.5 ml min^{-1}; detection, 210 nm.

4. Conclusions

In conclusion, this study has demonstrated that this CSP is quite successful for the direct enantioseparation of various β-amino acids. The optimum mobile phase composition was found to be a mixed solvent of 50% MeOH in water containing 5.0 mM AcOH, but for the *ortho*-substituted 3-amino-3-phenylpropionic acid analogs variation of the natures and contents of the organic and acidic modifiers in the aqueous mobile phase was needed. The chromatographic retention and resolution behavior was found to be dependent on the natures and positions of the substituents on the phenyl ring of the 3-amino-3-phenylpropionic acid analogs. The elution sequence was *R* < *S*.

Acknowledgments

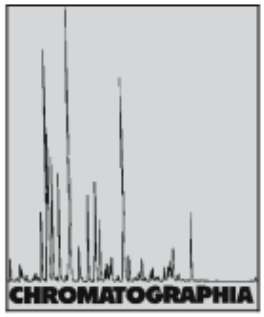
This work was supported by OTKA grants T 042451 and T 049407 and Korea Science and Engineering Foundation (NRL program: M1-0318-00-0005).

References

[1] H.H. Wasserman, H. Matsuyama, R.P. Robinson, Tetrahedron 58 (2002) 7177.
[2] E. Juaristi, V.A. Soloshonok, Enantioselective Synthesis of β-Amino Acids, second ed., Wiley-VCH, New York, 2005.
[3] R.L. Wolin, A. Santillan, T. Barclay, L. Tang, H. Venkatesan, S. Wilson, D.H. Lee, T.W. Lovenberg, Bioorg. Med. Chem. 12 (2004) 4493.
[4] D. Seebach, S. Abele, K. Gademann, B. Jaun, Angew. Chem. Int. Ed. 38 (1999) 1595.
[5] M. Liu, M.P. Sibi, Tetrahedron 58 (2002) 7991.
[6] J.A. Carr, T.F. Al-Azemi, T.E. Long, J. Shim, C.M. Coates, E. Turos, K.S. Bisht, Tetrahedron 59 (2003) 9147.
[7] H. Groeger, H. Werner, European Patent Applied, EP 1361279 (2003). (Chem. Abst. 139 (2003) 363709).
[8] E.P. Kyba, M.G. Siegel, L.R. Sousa, G.D.Y. Sogah, D.J. Cram, J. Am. Chem. Soc. 95 (1973) 2691.
[9] M.H. Hyun, Bull. Kor. Chem. Soc. 26 (2005) 1153.
[10] Y. Machida, H. Nishi, K. Nakamura, H. Nakai, T. Sato, J. Chromatogr. A 805 (1998) 85.
[11] M.H. Hyun, J.S. Jin, W. Lee, J. Chromatogr. A 822 (1998) 155.
[12] I. D'Acquarica, F. Gasparrini, D. Misiti, G. Zappia, C. Cimarelli, G. Palmieri, A. Carotti, S. Cellamare, C. Villani, Tetrahedron: Asymmetry 11 (2000) 2375.
[13] A. Péter, L. Lázár, F. Fülöp, D.W. Armstrong, J. Chromatogr. A 926 (2001) 229.
[14] A. Péter, J. Chromatogr. A 955 (2002) 141.
[15] A. Árki, D. Tourwé, M. Solymár, F. Fülöp, D.W. Armstrong, A. Péter, Chromatographia 60 (2004) S43.
[16] W.M. Rodionov, E.F. Malivinskaya, Chem. Ber. 59 (1926) 2952.
[17] E. Forró, T. Paál, G. Tasnádi, F. Fülöp, Adv. Synth. Catal. 348 (2006) 917.
[18] W. Lee, J.Y. Jin, C.S. Back, Microchem. J. 80 (2005) 213.

IV.

HPLC Enantioseparation of 1-(α-Aminobenzyl)-2-naphthol and 2-(α-Aminobenzyl)-1-naphthol Analogs on a β-Cyclodextrin-Based Chiral Stationary Phase



2007, *65*, 337–341

R. Berkecz[1], I. Ilisz[1], A. Ivanov-Sztojkov[2], I. Szatmári[2], F. Fülöp[2], D. W. Armstrong[3], A. Péter[1,✉]

[1] Department of Inorganic and Analytical Chemistry, University of Szeged, 6720, Szeged, Dóm tér 7, Hungary; E-Mail: apeter@chem.u-szeged.hu
[2] Institute of Pharmaceutical Chemistry, University of Szeged, 6720, Szeged, Eötvös u. 6, Hungary
[3] Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019-0065, USA

Received: 28 September 2006 / Revised: 5 December 2006 / Accepted: 7 December 2006
Online publication: 10 January 2007

Abstract

The enantiomers of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs were separated isothermally on a 3,5-dimethylphenylcarbamoylated β-cyclodextrin-based chiral stationary phase (Cyclobond DMP), with an *n*-hexane/alcohol modifier as mobile phase. Optimization of the separation was achieved by variation of combinations of the polar mobile phase additives ethanol and methanol. The nature and position of the α-aminobenzyl substituent of the 1- and 2-naphthol analogs influenced the retention and the selectivity.

Keywords

Column liquid chromatography
Enantiomer separation
1-(α-Aminobenzyl)-2-naphthol analogs
2-(α-Aminobenzyl)-1-naphthol analogs
Cyclobond column

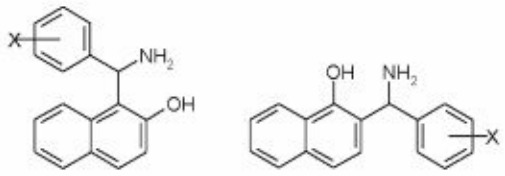
Introduction

The Mannich aminoalkylation of 2-naphthol for the preparation of 1-(α-aminobenzyl)-2-naphthol derivatives (Fig. 1, **1**, X = H, Betti base) was first reported by Betti [1]. Betti and Pratesi [2] described the first resolution of 2-naphthol derivatives, and the enantiomers of **1** were used for the resolution of aldehydes [2–4]. The subsequent long silence over this material was interrupted in the past decade, and interest in the chemistry of the Betti base has since intensified. The Betti base and its *N*-substituted derivatives serve as chiral catalysts [5–11]. Betti base derivatives also provide convenient access to many useful synthetic building blocks [12]. The analogous transformation of 1-naphthol recently led to the regioisomeric Betti base derivative 2-(α-aminobenzyl)-1-naphthols (Fig. 1) [13].

The biological effects of naphthol derivatives have been investigated in in vivo systems. Desai et al. [14] found that the presence of halogen atoms and of the thiazolidine nucleus produced from the racemic Betti base were necessary for good antituberculous activity.

The chemical and biological applications of Betti base enantiomers required a precise separation and detection method. Chromatographic methods are widely used for the separation of chiral compounds. High-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs) is an effective tool for the resolution of chiral compounds on both analytical and preparative scales [15–17]. Few chromatographic data have been published on the separation and identification of Betti bases. Sztojkov-Ivanov et al. [18] separated 1- and 2-naphthol analogs on a *tris*-dimethylphenylcarbamate cellulose CSP at different temperatures with various *n*-hexane/2-propanol (IPA)/diethylamine (DEA) mobile phases.

In this paper, normal-phase HPLC methods are described for the enantioseparation of racemic 1- and 2-naphthol analogs (for the structures, see Fig. 1). The HPLC methods rely on the use of a 3,5-dimethylphenylcarbamoylated β-cyclodextrin-based CSP (Cyclobond DMP). The experimental data are utilized to discuss the influence of the mobile phase composition and specific structural features of the analytes (aryl substituents) on the retention and separation.



1 2

Position	Analyte (1 or 2)						
	a	b	c	d	e	f	g
4	H	CH_3	CH_3O	F	Cl	Br	NO_2
	h	i					
3	Br	NO_2					

Fig. 1. Structures of analytes **1a**, 1-(α-aminobenzyl)-2-naphthol; **1b**, 1-[amino(4-methylphenyl) methyl]-2-naphthol; **1c**, 1-[amino(4-methoxyphenyl)methyl]-2-naphthol; **1d**, 1-[amino(4-fluorophenyl) methyl]-2-naphthol; **1e**, 1-[amino(4-chlorophenyl)methyl]-2-naphthol; **1f**, 1-[amino(4-bromophenyl)methyl]-2-naphthol; **1g**, 1-[amino(4-nitrophenyl)methyl]-2-naphthol; **1h**, 1-[amino (3-bromophenyl)methyl]-2-naphthol; **1i**, 1-[amino(3-nitrophenyl)methyl]-2-naphthol. **2a**, 2-(α-aminobenzyl)-1-naphthol; **2b**, 2-[amino(4-methylphenyl)methyl]-1-naphthol; **2c**, 2-[amino(4-methoxyphenyl)methyl]-1-naphthol; **2d**, 2-[amino(4-fluorophenyl)methyl]-1-naphthol; **2e**, 2-[amino (4-chlorophenyl)methyl]-1-naphthol; **2f**, 2-[amino(4-bromophenyl)methyl]-1-naphthol; **2h**, 2-[amino(3-bromophenyl)methyl]-1-naphthol; **2i**, 2-[amino(3-nitrophenyl)methyl]-1-naphthol

Table 1. Chromatographic data, retention factors (k), separation factors (α) and resolutions (R_S) of the enantioseparation of 2-naphthol derivatives

Compound	Mobile phase (v/v/v/v)	k_1	k_2	α	R_S
1a	80/20, **a**	1.91	1.91	1.00	0.0
	95/5, **g**	11.65	13.12	1.13	1.60
	80/10/10/0.1/0.1, **f**	1.83	1.83	1.00	0.00
1b	80/20, **a**	1.64	1.73	1.06	1.10
	80/10/10/0.1/0.1, **f**	1.52	1.63	1.07	0.95
1c	80/20, **a**	2.90	3.03	1.05	0.95
	80/20/0.1, **b**	2.72	2.88	1.06	1.00
	80/10/10/0.1/0.1, **f**	2.56	2.69	1.05	0.80
1d	80/20, **a**	1.87	2.00	1.07	0.70
	80/10/10/0.1/0.1, **f**	1.77	1.92	1.09	1.05
1e	80/20, **a**	1.89	2.30	1.22	1.80
	80/20/0.1, **b**	1.89	2.36	1.25	2.80
	80/10/10/0.1/0.1, **f**	1.66	2.07	1.25	2.55
1f	80/20, **a**	1.93	2.46	1.28	2.10
	80/20/0.1, **b**	1.91	2.47	1.29	2.40
	80/10/10, **c**	1.61	1.97	1.22	2.45
	80/10/10/0.1/0.1, **f**	1.76	2.30	1.31	3.30
1g	80/20, **a**	4.25	5.98	1.41	3.10
	80/10/10/0.1/0.1, **f**	3.58	4.71	1.32	4.75
1h	80/20, **a**	1.80	1.97	1.09	1.10
	80/20/0.1, b	1.86	2.07	1.11	1.25
	80/10/10/0.1/0.1, **f**	1.73	1.94	1.12	1.50
1i	80/20, **a**	3.84	4.47	1.16	1.60
	80/10/10/0.1/0.1, **f**	3.36	3.92	1.17	2.60

Column, Cyclobond DMP, dimethylphenylcarbamate-derivatized β-cyclodextrin; mobile phase, **a** *n*-hexane/ethanol (v/v), **b** *n*-hexane/ethanol/diethylamine (v/v/v), **c** *n*-hexane/ethanol/methanol (v/v/v), **f** *n*-hexane/ethanol/methanol/AcOH/TEA (v/v/v/v/v), **g** *n*-hexane/chloroform (v/v); detection 232 nm; flow rate 0.8 mL min^{-1}; temperature 25 °C

Experimental

Chemicals and Reagents

The Betti reaction was used to prepare 1-(α-aminobenzyl)-2-naphthol derivatives **1a–1i** (Fig. 1). The reactions were performed with 2-naphthol, benzaldehyde or substituted benzaldehydes and ammonia (in proportions of 1:2:1) to obtain 1,3-diaryl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3] oxazines. Their subsequent acidic hydrolysis and extraction with NH_4OH gave the desired aminonaphthols [1, 19, 20]. 2-(α-Aminobenzyl)-1-naphthols **2a–f**, **h**, **i** (Fig. 1) were prepared in a manner similar to that for their regioisomeric 1-(α-aminobenzyl)-2-naphthol derivatives, by Betti's classical aminoalkylation of 1-naphthol with benzaldehyde or substituted benzaldehydes in the presence of ammonia, which led to the formation of 2,4-diaryl-3,4-dihydro-2*H*-naphth[2,1-*e*][1,3]oxazines, acidic hydrolysis of which furnished aminonaphthol hydrochlorides [13].

n-Hexane, methanol (MeOH), ethanol (EtOH), 2-propanol (IPA), chloroform and glacial acetic acid (AcOH) of HPLC grade were from Merck (Darmstadt, Germany). DEA, triethylamine (TEA) and other reagents of analytical reagent grade were from Aldrich (Steinheim, Germany). Mobile phases were prepared by mixing the indicated volumes of solvents and were further purified by filtration through a 0.45-μm Millipore filter, type HV. The eluents were degassed in an ultrasonic bath and He gas was purged through them during the analysis. Stock solutions of analytes (1 mg mL^{-1}) were prepared by dissolution in the starting mobile phase.

Apparatus

The HPLC measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium^{32} Chromatography Manager data system; the alternative Waters Breeze system consisted of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (both systems from Waters Chromatography, Milford, MA, USA). Both chromatographic systems were equipped with Rheodyne Model 7125 injectors (Cotati, CA, USA) with 20-μL loops.

The β-cyclodextrin-based stationary phase used for analytical separations was the 3,5-dimethylphenylcarbamoylated β-cyclodextrin (Cyclobond DMP) column, 250 mm × 4.6 mm I.D., 5-μm particle size (Astec, Whippany, NJ, USA). The columns were thermostated in a water bath, with a cooling–heating thermostat (MK 70, Mechanik Prüfgeräte, Medlingen, Germany). The precision of the temperature adjustment was ±0.1 °C.

Ab Initio Calculations

To obtain information on the torsional angles between the naphthyl and the phenyl planes and on the influence of *X* substitution on the cyclic hydrogen bond in analytes **1** and **2**, ab initio calculations were performed for **1e** and **2e** (*X* = Cl; an electron-withdrawing substituent) and for

1c and **2c** (X = OMe; an electron donating substituent). The minimum-structure search protocol involved preliminary molecular mechanics (MMFF94) minimization, followed by geometry optimization at the ab initio level by using the Gaussian 03 program package. The B3LYP DFT hybrid method and the 6–31G* double zeta split valence basis set were used. The NBO population analyses were performed at the same level of theory.

Results and Discussion

The analytes examined in this study (Tables 1, 2) can be grouped into class **1** (2-naphthol analogs) and class **2** (1-naphthol analogs). These two classes of analytes differed in the steric arrangement of the aromatic rings around the chiral center. Relevant separation data on these two classes of compounds, including the retention factors, separation factors and resolutions for each analyte for several mobile phases, are given in Tables 1 and 2. All compounds were evaluated with a minimum of three of the following mobile phases: *n*-hexane/IPA, *n*-hexane/EtOH, *n*-hexane/EtOH/DEA, *n*-hexane/EtOH/MeOH, *n*-hexane/EtOH/MeOH/DEA, *n*-hexane/EtOH/MeOH/AcOH/DEA, *n*-hexane/EtOH/MeOH/AcOH/TEA, *n*-hexane/IPA/MeOH, *n*-hexane/IPA/MeOH/DEA. To simplify the presentation, only the chromatographic results relating to a partial or baseline enantiomeric separation are given in Tables 1 and 2. However, in a few cases, for purposes of comparison, examples are included where no separation occurred.

The use of *n*-hexane with IPA as the alcoholic modifier in different compositions resulted in unseparated chromatographic peaks (data not shown). The use of EtOH instead of IPA significantly improved the separations in most cases. At a constant mobile phase composition of *n*-hexane/EtOH = 80/20 (*v*/*v*), most of the enantiomers of the 1- and 2-naphthol analogs were partially or baseline-resolved (with the exceptions of **1a** and **2a**, **2h**, **2i**). At the same mobile phase composition of *n*-hexane/EtOH = 80/20 (*v*/*v*), comparison of the chromatographic behavior of the 2- and 1-naphthol analogs otherwise containing the same substituents revealed that the retention factors did not differ substantially (**2a** and **2i** exhibited higher k_1 values those than for **1a** and **1i**, while the k of **1c** was higher than that of **2c**). The separation factors were also similar, with the exceptions of the **h** and **i** analogs, where the 2-naphtol analogs were separable, but the 1-naphthol analogs were not (Tables 1, 2).

Table 2. Chromatographic data, retention factors (k), separation factors (α) and resolutions (R_S) of the enantioseparation of 1-naphthol derivatives

Compound	Mobile phase (*v*/*v*/*v*/*v*)	k_1	k_2	α	R_S
2a	80/20, **a**	3.46	3.46	1.00	0.0
	80/10/10, **c**	1.75	1.97	1.13	0.80
	80/10/10/0.1, **d**	1.79	2.03	1.13	1.15
	80/5/15/0.1, **e**	1.68	1.86	1.11	1.45
2b	80/20, **a**	1.61	1.72	1.07	0.80
	80/10/10, **c**	1.44	1.66	1.15	1.00
	80/5/15, **c**	1.38	1.60	1.16	1.20
	80/10/10/0.1, **d**	1.84	2.26	1.23	1.25[a]
	80/5/15/0.1, **e**	1.39	1.58	1.14	1.64
2c	80/20, **a**	2.11	2.45	1.16	0.90
	80/10/10, **c**	2.72	3.12	1.15	0.90
	80/5/15, **c**	2.49	2.89	1.16	1.20
	80/10/10/0.1, **d**	3.70	4.44	1.20	1.30[a]
	80/5/15/0.1, **e**	2.46	2.79	1.13	1.50
2d	80/20, **a**	2.02	2.20	1.09	0.90
	80/10/10, **c**	1.86	2.80	1.50	1.40
	80/5/15/0.1, **e**	1.91	2.20	1.15	1.75
2e	80/20, **a**	2.08	2.44	1.17	1.00
	80/10/10, **c**	1.77	2.21	1.25	1.70
	80/5/15/0.1, **e**	1.80	2.17	1.21	2.30
2f	80/20, **a**	2.05	2.39	1.16	1.10
	80/10/10, **c**	1.84	2.30	1.25	1.80
	80/5/15/0.1, **e**	2.00	2.43	1.22	2.60
2h	80/20, **a**	1.83	1.83	1.00	0.0
	80/10/10, **c**	1.62	1.80	1.11	0.90
	80/5/15/0.1, **e**	1.62	1.78	1.10	1.30
2i	80/20, **a**	5.23	5.23	1.00	0.0
	80/10/10, **c**	4.16	4.66	1.12	0.90
	80/5/15/0.1, **e**	3.80	4.23	1.11	1.65

Column, Cyclobond DMP, dimethylphenylcarbamate-derivatized β-cyclodextrin; mobile phase, **a**, *n*-hexane/ethanol (*v*/*v*), **c**, *n*-hexane/ethanol/methanol (*v*/*v*/*v*), **d**, *n*-hexane/ethanol/methanol/diethylamine (*v*/*v*/*v*/*v*), **e**, *n*-hexane/ethanol/methanol/triethylamine (*v*/*v*/*v*/*v*); detection, 232 nm; flow rate, 0.8 mL min^{-1}; temperature, 25 °C
[a] 10 °C

The aromatic substitution exerted slight effects on both the retention and the selectivity. At a constant mobile phase composition of *n*-hexane/EtOH = 80/20 (*v*/*v*), methyl substitution on the aromatic ring resulted in lower retention, while methoxy substitution led to a higher (**1c**) or a lower (**2c**) retention factor as compared with the non substituted compounds. In both cases, methyl and methoxy substitution led to the better separation of analogs **b** and **c** than **a**. This may be attributed to the increased bulkiness (steric effect) or hydrophobicity (**b** analogs) or to the possible H-bonding interactions of the carbamate groups of the selector with the methoxy group of the analyte (**c** analogs).

Halogen substitution on the aromatic ring increased the π-acidic character of the analyte, and stronger interactions with the π-basic 3,5-dimethylphenylcarbamate group of the selector was therefore to be expected. Halogen substitution in naphthol analogs **d–f** and **h** (Fig. 1), slightly influenced the retention; the k_1 values were somewhat smaller than those for the non-substituted analogs (with the exception of k_1 for analogs **1f**), and a slight increase in k was observed in the sequence fluorine–chlorine–bromine.

To explain the similar chromatographic behavior of the 1- and 2-naphthol analogs and the slight influence of X substitution on the enantioseparation, ab initio calculations were carried out. These demonstrated that the torsional angle between the naphthyl and the phenyl planes was around 70° for analytes **1** and around 66° for analytes **2**, and the differences in the torsional angle in consequence of the substitution (X) were also small: 1.5° for **1c–1e** and 0.1° for **2c–2e**. These calculations further revealed that the strength of the intramolecular hydrogen bond between the $-NH_2$ and the –OH groups differs

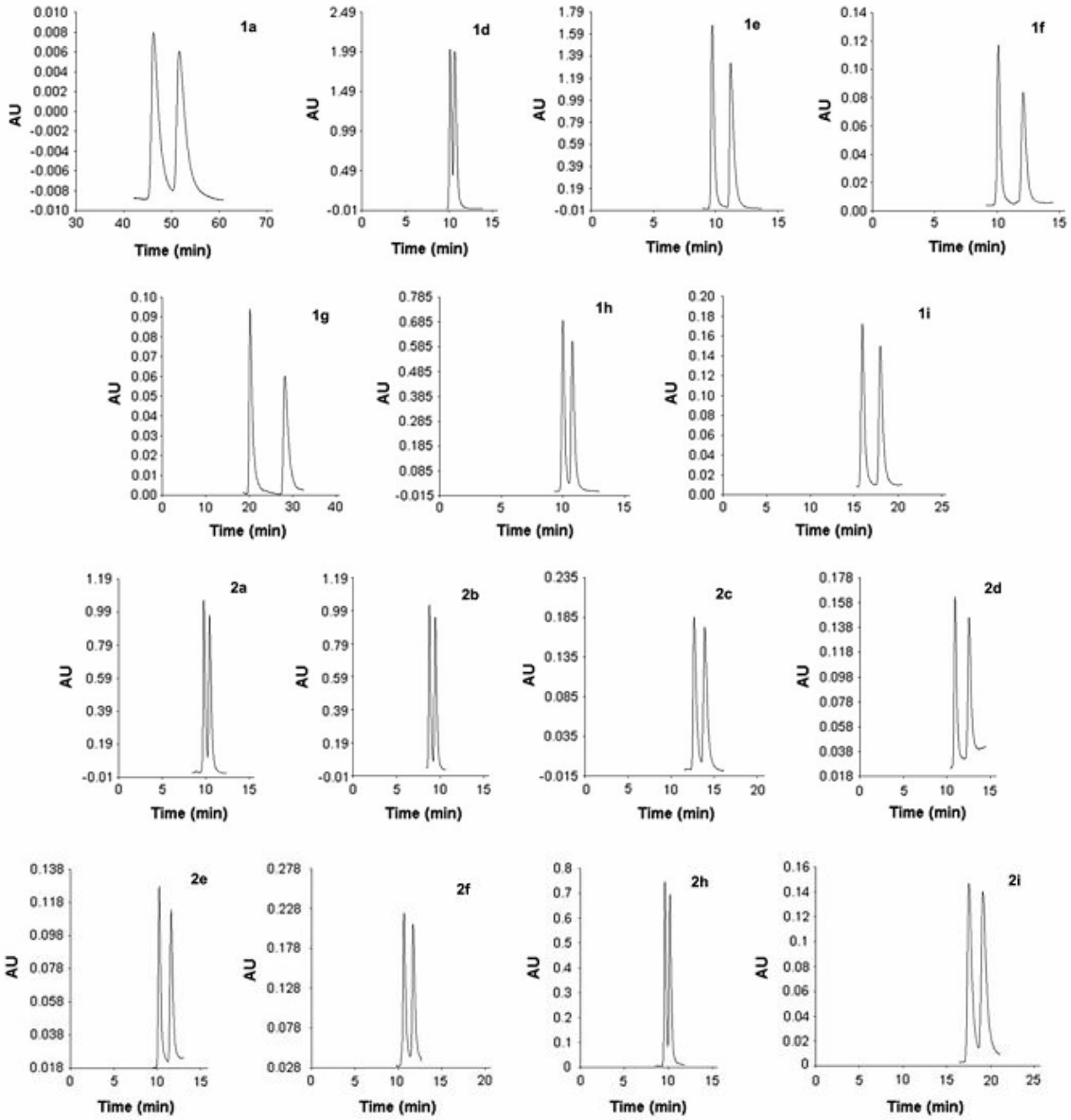


Fig. 2. Selected chromatograms for the separation of 1- and 2-naphthol derivatives. Chromatographic conditions: column, Cyclobond DMP; mobile phase, *n*-hexane/chloroform = 95/5 (*v*/*v*) for compound **1a**, *n*-hexane/EtOH/MeOH/TEA = 80/10/10/0.1 (*v*/*v*/*v*/*v*) for compounds **1d–1f**, **1h**, **1i**, *n*-hexane/EtOH/TEA = 80/20/0.02 (*v*/*v*/*v*) for **1g**, *n*-hexane/EtOH/MeOH/TEA = 80/5/15/0.1 (*v*/*v*/*v*/*v*) for compounds **2a–2f**, **2h**, **2i**; flow rate 0.8 mL min^{-1}; detection 230 nm; temperature 25 °C

somewhat in series **1** and **2**. The distances between the nitrogen of the $-NH_2$ and the hydrogen of the –OH group were 1.8353 Å for **1e** and 1.8099 Å for **1c**, respectively. Somewhat shorter distances (stronger hydrogen bonds) were observed for **2e**, 1.7462 Å, and for **2c**, 1.7402 Å, but it can be considered that in series **1** the substitution (*X*) has a greater influence on the strength of the hydrogen bond. These differences seemed to be of no significance. It can be concluded that substituent *X* has only a small influence on the torsional angle between the naphthyl and the phenyl rings and on the strength of the intramolecular hydrogen bond. Since the intramolecular hydrogen bond was slightly stronger for analytes **2** than for analytes **1**, this may explain the lower enantioselectivity for analytes **2**.

The position (3 or 4) of the substituent influenced the retention and selectivity; for both the 1- and 2-naphthol analogs, higher selectivity was observed for the 4-substituted analogs (**f**) than for the 3-substituted ones (**h**).

The more π-acidic nitro-substituted analogs exhibited more pronounced chromatographic effects than the less π-acidic halogen-substituted analogs. The retention factors of analogs **g** and **i** were higher than those of the nonsubstituted or halogen-substituted analogs, and these increased retention factors were generally associated with increased selectivity (with the exception of **2i**).

Combinations of the EtOH and MeOH modifiers in the mobile phase led, in most cases, to good separations of the enantiomers. For 2-naphthol analogs the best separation conditions were achieved by application of *n*-hexane/EtOH or *n*-hexane/EtOH/MeOH mobile phases which ensured baseline or almost baseline separation for the stereoisomers (with the exception of **1a**). Addition of 0.1% DEA to the *n*-hexane/EtOH or 0.1% AcOH/TEA to the *n*-hexane/EtOH/MeOH mobile phases in some cases increased the resolution (Table 1). Compound **1a** was baseline separated in the *n*-hexane/chloroform mobile phase (Table 1).

For 1-naphthol analogs the best separation conditions were achieved by application of *n*-hexane/EtOH/MeOH mobile phases with addition of 0.1% DEA or TEA. It was observed that the increase of MeOH/EtOH ratio from 1/1 to 3/1 (*v*/*v*) significantly improved the resolution.

Selected chromatograms for the enantioseparation of 1- and 2-naphthol analogs are depicted in Fig. 2.

Conclusions

Cyclobond DMP can be used as CSP to separate the enantiomers of α-aminobenzyl-substituted 1- and 2-naphthol analogs. The separations can be accomplished in the normal phase mode by using different mobile phases. The nature and position of the α-aminobenzyl substituent of the 1- and 2-naphthol analogs influences the retention and the selectivity. Methyl, methoxy and halogen substituents on the α-aminobenzyl group slightly influenced the retention and selectivity, while nitro substitution promoted chiral recognition. Optimization of the separation was achieved by variation of the mobile phase additives and their composition. Addition of 0.1% DEA, TEA or AcOH/TEA to *n*-hexane/EtOH or *n*-hexane/EtOH/MeOH mobile phases improved the enantioseparation of most of these analogs.

Acknowledgments

This work was supported by OTKA grants T 042451 and T 049407, and by National Institutes of Healths grant NIH RO1 GM53825-10. The computational resources of HPC U-Szeged (ALAP4-00092/2005) is acknowledged. I. Ilisz wishes to express his thanks to the Bolyai János Postdoctoral Scholarship for supporting his research work.

References

1. Betti M (1941) Org Synth Coll 1:381–383
2. Betti M, Pratesi P (1930) Berichte 63B:874–875
3. Betti M, Pratesi P (1934) Biochem Z 274:1–3
4. Betti M (1912) Gazz Chim Ital 42:288–294
5. Cardellicchio C, Ciccarella G, Naso F, Schingaro E, Scordari F (1998) Tetrahedron Asymmetry 9:3667–3675
6. Cardellicchio C, Ciccarella G, Naso F, Perna F, Tortorella P (1999) Tetrahedron 55:14685–14692
7. Palmieri G (2000) Tetrahedron Asymmetry 11:3361–3373
8. Liu D-X, Zhang L-C, Wang Q, Da C-S, Xin Z-Q, Wang R, Choi MCK, Chan ASC (2001) Org Lett 3:2733–2735
9. Cimarelli C, Palmieri G, Volpini E (2002) Tetrahedron Asymmetry 13:2417–2426
10. Lu J, Xu X, Wang S, Wang C, Hu Y, Hu H (2002) J Chem Soc [Perkin 1] 1:2900–2903
11. Lu J, Xu X, Wang S, Wang C, He J, Hu Y, Hu H (2002) Tetrahedron Lett 43:8367–8369
12. Szatmári I, Hetényi A, Lázár L, Fülöp F (2004) J Heterocyclic Chem 41:367–373
13. Szatmári I, Martinek TA, Lázár L, Fülöp F (2004) Eur J Org Chem 10:2231–2238
14. Desai NC, Shukla HK, Langalia NA, Thaker KA (1984) J Indian Chem Soc 61:711–713
15. Subramanian G, (1994) A practical approach to chiral separations by liquid chromatography, VCH, Weinheim
16. Jinno K, (1997) Chromatographic separations based on molecular recognition. Wiley, New York
17. Beesley TE, Scott RPW, (1998) Chiral chromatography. Wiley, Chichester
18. Sztojkov-Ivanov A, Szatmári I, Péter A, Fülöp F (2005) J Sep Sci 28:2505–2510
19. Szatmári I, Fülöp F (2004) Curr Org Synth 1:155–165
20. Szatmári I, Martinek TA, Lázár L, Fülöp F (2003) Tetrahedron 59:2877–2884

V.

WILEY InterScience® DISCOVER SOMETHING GREAT CHIRALITY 19:374–379 (2007)

High-Performance Liquid Chromatographic Enantioseparation of 1-(Aminoalkyl)-2-naphthol Analogs on Polysaccharide-Based Chiral Stationary Phases

ANITA SZTOJKOV-IVANOV,[1] DIANA TÓTH,[1] ISTVÁN SZATMÁRI,[1] FERENC FÜLÖP,[1] AND ANTAL PÉTER[2]*

[1]*Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary*

[2]*Department of Inorganic and Analytical Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 7, Hungary*

ABSTRACT The enantiomers of various 1-(α-aminobenzyl)-2-naphthol and 1-(aminoalkyl)-2-naphthol analogs were separated on cellulose-*tris*-3,5-dimethylphenyl carbamate-based chiral stationary phases (Chiralcel OD-H and Chiralcel OD-RH), using *n*-hexane/2-propanol/diethylamine or phosphate buffer/organic modifier mobile phases. The 3,5-dimethylphenyl carbamoylated cellulose columns were effective in both normal and rev ersed-phase modes. The effects of the mobile phase composition, the pH, the buffer concentration, and the structures of the substituents on the 2-naphthol on the enantioseparations were studied. The absolute configuration and elution sequence were determined for 1-(1-amino-2-methylpropyl)-2-naphthol: the elution sequence was *S* < *R*. *Chirality 19:374–379, 2007.* © 2007 Wiley-Liss, Inc.

KEY WORDS: enantioselective HPLC; 1-(α-aminobenzyl)-2-naphthol; 1-(aminoalkyl)-2-naphthol analogs; polysaccharide-based chiral stationary phases; Chiralcel OD-H; Chiralcel OD-RH

INTRODUCTION

Study of the chemistry of the Betti bases started at the beginning of the 20th century, when Betti reported the synthesis of 1-(α-aminobenzyl)-2-naphthol.[1] The preparation of substituted Betti base derivatives by the modified Mannich reaction has subsequently become of considerable importance because a C—C bond is formed under mild experimental conditions.[2]

In the past decade, interest in the chemistry of the Betti bases has intensified. Preparation of the enantiomers of the Betti bases and their N-substituted derivatives is of significance since they can serve as chiral catalysts[3–12] or the nonracemic Betti base can be applied successfully as a new chiral auxiliary.[13] Betti base derivatives also provide convenient access to many useful synthetic building blocks because the amino and phenolic hydroxy groups can be converted into a wide variety of compounds.[14,15]

Only moderate attention has been paid to the compounds discussed as far as their biological activity is concerned. Desai et al. have found activity against the $H_{37}D_V$ strain of *Mycobacterium tuberculosis* in vitro.[16]

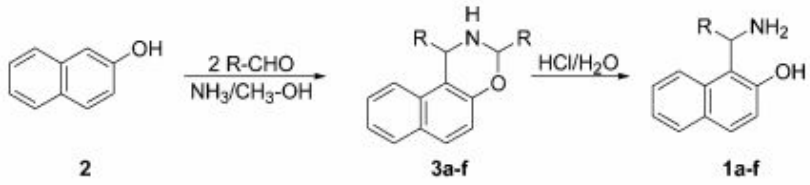
The applications of Betti base enantiomers required the elaboration of a precise separation and detection method. For the separation of chiral compounds, chromatographic methods are widely used. High-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs) is an effective tool for the resolution of chiral compounds.[17–19] A number of CSPs for HPLC have been prepared and more than 100 CSPs have been commercialized. Among them, polysaccharide-based CSPs are among the most useful columns for both analytical and preparative scales.[20–22] Few chromatographic data have been published on the separation and identification of Betti bases. Sztojkov-Ivanov et al.[23] separated 1- and 2-naphthol analogs on a *tris*-dimethylphenyl carbamate cellulose CSP at different temperatures with various *n*-hexane/2-propanol (IPA)/diethylamine (DEA) mobile phases. The enantiomers of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs were separated isothermally by Berkecz et al.[24] on a 3,5-dimethylphenyl carbamoylated β-cyclodextrin-based chiral stationary phase (Cyclobond DMP), with *n*-hexane/plus an alcohol modifier as mobile phase.

In this study, normal and reversed-phase HPLC methods are described for the enantioseparation of racemic 1-(aminoalkyl)-2-naphthol analogs (for the structures, see Scheme 1). These HPLC methods rely on the use of cellulose-*tris*-3,5-dimethylphenyl carbamate-based CSPs (Chiralcel OD-H and Chiralcel OD-RH), which were effective in both normal and reversed-phase modes. The effects of mobile phase composition, pH, and buffer concentration on the enantioseparations were studied, and the effects of the

Contract grant sponsor: Hungarian National Science Foundation grants; Contract grant numbers: T 67563, TS 40888.

*Correspondence to: Prof. Antal Péter, Department of Inorganic and Analytical Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 7, Hungary. E-mail: apeter@chem.u-szeged.hu

Received for publication 6 December 2006; Accepted 29 January 2007
DOI: 10.1002/chir.20392
Published online 22 March 2007 in Wiley InterScience (www.interscience.wiley.com).



2 3a-f 1a-f

R : **a**, phenyl; **b**, CH_3-; **c**, CH_3CH_2-; **d**, $CH_3CH_2CH_2$-; **e**, $(CH_3)_2CH$-; **f**, $(CH_3)_3C$-

Scheme 1. **1a**, 1-(α-Aminobenzyl)-2-naphthol; **1b**, 1-(1-aminoethyl)-2-naphthol; **1c**, 1-(1-aminopropyl)-2-naphthol; **1d**, 1-(1-aminobutyl)-2-naphthol; **1e**, 1-(1-amino-2-methylpropyl)-2-naphthol; **1f**, 1-(1-amino-2,2-dimethylpropyl)-2-naphthol.

structures of the substituents on the 2-naphthol were also examined.

EXPERIMENTAL

Materials

The synthesis of 1-(α-aminobenzyl)-2-naphthol from 2-naphthol, benzaldehyde, and ammonia is straightforward.[1] By the reaction of aliphatic aldehydes (in place of aromatic aldehydes) with 2-naphthol in the presence of methanolic ammonia solution in methanol at 60°C, 1,3-dialkyl-2,3-dihydro-1*H*-naphth[1,2-*e*][1,3]oxazines have been prepared (Scheme 1). The acidic hydrolysis of naphthoxazines led to the desired aminonaphthol hydrochlorides. The overall yield was improved considerably when the solvent was evaporated off after the formation of the intermediate naphthoxazines and the residue then directly hydrolyzed with hydrochloric acid.[25]

n-Hexane, acetonitrile (MeCN), methanol (MeOH), and IPA of HPLC grade were from Merck (Darmstadt, Germany). DEA and other reagents of analytical reagent grade were from Aldrich (Steinheim, Germany). Mobile phases were prepared by mixing the indicated volumes of solvents and were further purified by filtration through a 0.45-μm Millipore filter, type HV. The eluents were degassed in an ultrasonic bath and purged with He gas during analysis. Stock solutions of analytes (1 mg/ml) were prepared by dissolution in the starting mobile phase.

Apparatus and Chromatography

The HPLC system consisted of an M-600 low-pressure gradient pump, equipped with an M-486 tunable absorbance detector, and a Millenium 2.1 Chromatography Manager data system (all Waters Chromatography, Milford, MA). The HPLC system was equipped with a Rheodyne Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA).

Analyses were carried out on columns of Chiralcel OD-H, 250 × 4.6 mm I.D, 5-μm particle size and Chiralcel OD-RH, 150 × 4.6 mm I.D, 5-μm particle size (Daicel, Tokyo, Japan).

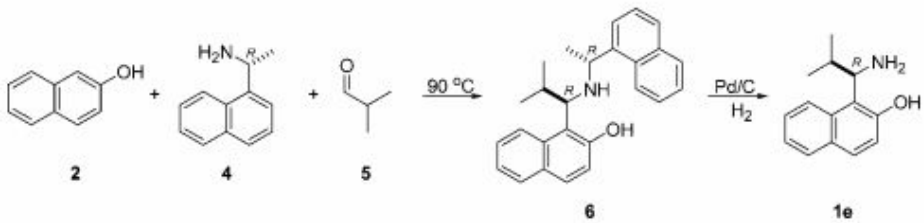
Absolute Configuration and Elution Sequence

The elution sequence of the two enantiomers of (1-(1-amino-2-methylpropyl)-2-naphthol) was determined as given in Scheme 2. 1-[(1*R*)-2-Methyl-1-{[(1′*R*)-1′-(1-naphthyl)ethyl]amino}propyl]-2-naphthol (**6**) was prepared from 2-naphthol,(**2**) (*R*)-(+)-1-(1-naphthyl)ethylamine (**4**) and isobutyraldehyde (**5**).[7] Compound **6** was hydrogenated in methanol in the presence of palladium on charcoal for 15 h.[26] The residue was purified by column chromatography (elution with ethyl acetate) and was isolated as the *R* enantiomer of analyte **1e**. The elution sequence was detected on Chiralcel OD-H and Chiralcel OD-RH columns.

RESULTS AND DISCUSSION

Separation on Chiralcel OD-H Column in Normal-Phase Mode

The application of *n*-hexane and IPA as alcoholic modifier resulted in chromatograms with broad and asymmetric peaks (data not shown). Addition of a small percentage of DEA significantly improved the peak shapes and resolutions. The retention factors of the first and second-eluted stereoisomers depended strongly on the IPA content of the mobile phase. For analyte **1b** at ambient temperature, increasing IPA content from 10 to 90% decreased the retention factors (Table 1). With increasing IPA content, the changes in the separation factor, α, and resolution, R_S differed. For the 1-(aminoalkyl)-2-naphthol analogs, slight increases in α were registered with increasing IPA content,



2 4 5 6 1e

Scheme 2. Stereoselective synthesis of (*R*)-1-(1-amino-2-methylpropyl)-2-naphthol analog.

TABLE 1. Volume of substituents (V^a) and chromatographic data, retention factors (k'), separation factors (α), and resolutions (R_s) of the enantioseparation of 1-(aminoalkyl)-2-naphthol analogs

Compound	V^a	Mobile phase (v/v/v)	k_1'	k_2'	α	R_S
1a	–	70/30/0.1	1.32	3.82	2.89	8.85
1b	284	90/10/0.1	4.12	5.56	1.35	3.79
		70/30/0.1	1.26	1.80	1.43	2.62
		60/40/0.1	0.93	1.34	1.44	2.40
		50/50/0.1	0.68	0.98	1.44	2.38
		30/70/0.1	0.54	0.79	1.46	2.28
		10/90/0.1	0.45	0.70	1.56	2.44
1c	431	70/30/0.1	1.13	1.68	1.49	2.90
1d	478	70/30/0.1	1.02	1.62	1.59	3.19
1e	574	70/30/0.1	0.91	1.54	1.69	4.08
1f	716	70/30/0.1	0.82	1.43	1.74	4.52

Column, Chiralcel OD-H, cellulose-*tris*-3,5-dimethylphenyl carbamate; mobile phase, *n*-hexane/2-propanol/diethylamine (v/v/v); detection, 232 nm; flow rate, 0.5 ml/min; dead time, t_M = 6.8 min; temperature, 25°C.

while R_S changed in parallel with the k' values, i.e. R_S decreased with decreasing k'. The changes in selectivity might be explained on the change of the specific structure of the selector and analyte. Wang et al. have found that the structural change of the CSP caused by the change of alcohol concentration may affect the chiral selectivity of the CSP, depending on the size and structure of the analytes.[27]

At a constant mobile phase composition of *n*-hexane/IPA/DEA = 70/30/0.1 (v/v/v), comparison of the chromatographic behavior of the 2-naphthol analogs containing different alkyl substituents revealed that analytes with a larger or more bulky alkyl chain exhibited smaller retention factors and larger α and R_S values (Table 1). To determine the specific structural effects of the analytes (alkyl substituents) on the chromatographic parameters, the dependence of the experimental chromatographic data k' on the volume in the anchor sphere of the substituents (V^a) was investigated (see Fig. 1). According to Meyer, the steric effects of substituents on reaction rates was characterized by the size-descriptor V^a (Meyer parameter).[28] All the plots of V^a versus k' were fitted by straight lines with good correlation coefficients. Linear regression analysis revealed that the retentions of analytes depended strongly on the volumes of the substituents; bulkier substituents inhibited the interaction with the selector; and at the same time, the interactions of the two stereoisomers with the selector differed appreciably. Despite the smaller k' values, larger α and R_S values were obtained.

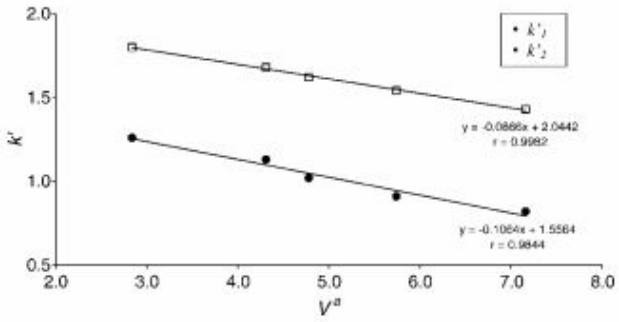


Fig. 1. Dependence of retention factors of 1-(aminoalkyl)-2-naphthol analogs on the Meyer substituent parameter (V^a). Chromatographic conditions: column, Chiralcel OD-H; mobile phase, *n*-hexane/IPA/DEA = 70/30/0.1 (v/v/v); detection, 230 nm; flow rate, 0.5 ml/min.

For the analyte 1-(α-aminobenzyl)-2-naphthol (**1a**), especially large α and R_S values were obtained, which may be attributed to the additional aromatic π-π-interactions between the analyte and the *tris*-3,5-dimethylphenyl carbamate group of the selector.

The absolute configuration[7,26] and elution sequence were determined for analyte **1e**: the elution sequence was (*S*) < (*R*). A similar elution sequence can probably be presumed for other alkyl-substituted 2-naphthol analogs, as observed for 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs.[23]

Separation on Chiralcel OD-RH Column in Reversed-Phase Mode

Relevant separation data on the Chiralcel OD-RH column, including the retention factors, separation factors, and resolutions for several mobile phases, are given in Table 2. All compounds were evaluated with a minimum of three of the following mobile phases: 0.2 M aqueous KH_2PO_4 (pH 2, 3, 4, 5, and 6)/MeCN = 80/20 (v/v), 0.2 M aqueous KH_2PO_4 (pH 2 and 6)/MeCN = 90/10 (v/v), 0.2 M aqueous KH_2PO_4 (pH 2 and 6)/MeOH = 75/25 (v/v) in different compositions. To simplify the presentation, only the chromatographic results relating to a partial or baseline enantiomeric separation are given in Table 2.

TABLE 2. Chromatographic data, retention factors (k'), separation factors (α), and resolutions (R_s) of the enantioseparation of 1-(aminoalkyl)-2-naphthol analogs

Compound	Mobile phase	k_1'	k_2'	α	R_S
1a	a	16.89	35.09	2.08	9.00
	b	4.55	6.61	1.45	2.67
	d	27.87	44.60	1.60	2.00
1b	a	0.68	0.84	1.24	0.74
	c	4.78	6.38	1.30	1.23
1c	a	1.13	1.57	1.39	1.25
	c	5.09	7.81	1.53	1.71
1d	a	2.55	3.86	1.51	2.57
	c	13.10	20.63	1.57	1.70
1e	a	1.85	2.69	1.45	1.73
	c	11.20	16.66	1.49	1.47
1f	a	4.06	6.01	1.46	2.84
	c	27.25	38.15	1.40	2.50

Column, Chiralcel OD-RH, cellulose-*tris*-3,5-dimethylphenyl carbamate; mobile phase, **a**, 0.2M aqueous KH_2PO_4(pH 6.0)/MeCN = 80/20 (v/v), **b**, 0.2 M aqueous KH_2PO_4(pH 5.0)/MeCN = 80/20 (v/v), **c**, 0.2 M aqueous KH_2PO_4(pH 6.0)/MeOH = 75/25 (v/v), **d**, 0.2 M aqueous KH_2PO_4(pH 5.0)/MeOH = 75/25 (v/v); detection, 232 nm; flow rate, 0.5 ml/min, dead time, t_M = 4.9 min; temperature, 25°C.

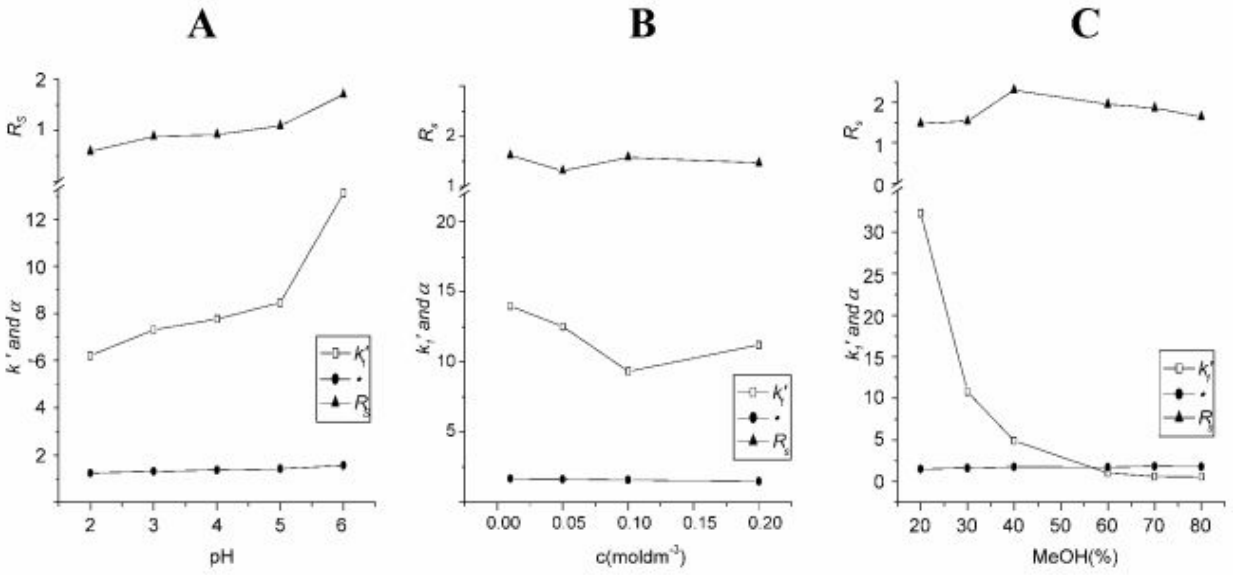


Fig. 2. Influence of pH (A), buffer concentration (B), and methanol content of mobile phase (C) on retention, selectivity and resolution of 1-(1-aminobutyl)-2-naphthol (**1d**). Chromatographic conditions: column, Chiralcel OD-RH; mobile phase, (A) 0.2 M aqueous KH_2PO_4 (pH 2.0–6.0)/MeOH = 75/25 (v/v), (B) 0.01–0.2 M aqueous KH_2PO_4 (pH 6.0)/MeOH = 75/25 (v/v), (C) 0.05 M aqueous KH_2PO_4 (pH 6.0)/MeOH = 80/20–20/80 (v/v); detection, 230 nm; flow rate, 0.5 ml/min.

However, in a few cases, for purposes of comparison, examples are included where no separation occurred.

First, experimental conditions such as the pH of the mobile phase, the buffer type and concentration, and the organic modifier type and concentration were optimized by using analytes **1a–1f** as model compounds. The main tendencies of these results are depicted in Figure 2A. With increase of the pH from 2.0 to 6.0 in the inorganic part of the mobile phase of 0.2 M aqueous KH_2PO_4/MeOH = 75/25 (v/v), the retention factor increased, while the selectivity and the resolution exhibited slight increases, as can be seen for analyte **1d** in Figure 2A. Similar tendencies were observed for all of the analytes using the same buffer with either MeOH or MeCN as organic modifier (data not shown).

Figure 2B depicts the effects of the phosphate buffer concentration (ionic strength) on the retention, selectivity, and resolution for analyte **1e.** The variation of the buffer concentration exhibited a slight effect. Similar tendencies were observed for the other analytes; it should be noted that the retention factor at the lowest buffer concentration was always somewhat higher than at the highest concentration, while the selectivity and resolution were very similar (data not shown).

In terms of the type of organic modifier, both MeCN and MeOH exhibited good enantioselectivity for the 1-(aminoalkyl)-2-naphthol analogs (Table 2). At similar eluent strength (eluent **a** and **c;** or **b** and **d**; Table 2), higher retention, and selectivity factors were generally obtained with MeOH as organic modifier, while for the resolution both higher and lower values were obtained. The retention depended strongly on the MeOH (or MeCN) content of the mobile phase. With increasing organic modifier content, the k' values decreased, but this was not accompanied by significant decreases in α and R_S (Fig. 2C). Similar curves were obtained for all the analytes investigated (data not shown).

On the cellulose-*tris*-3,5-dimethylphenyl carbamate-based CSP, the hydrogen-bonding, hydrophobic, steric, and dipole-dipole interactions principally govern retention and selectivity. The dependence of the separation on the pH and ionic strength was probably due to the acid-base properties of the analytes (the selector lacks acid-base functionalities). The pK values of analytes **1a–1f** are not known. For analytes with a similar structure, e.g. 2-(aminomethyl)phenol[29] in an organic-water phase, values of pK_1 = 12.8 and pK_2 = 6.3, and 2-(4-hydroxyphenyl)ethylamine[30] in a water phase, pK_1 = 9.2 and pK_2 = 7.4 have been reported. In light of these data, we can assume that, under acidic conditions, both the amino and the phenolic hydroxy groups of the 1-(aminoalkyl)-2-naphthol analogs are protonated, and the molecule is positively charged. With increasing pH, the more acidic phenolic hydroxy group loses its proton, and the molecule exhibits a "zwitterionic" form, becoming more hydrophobic and resulting in stronger hydrophobic interactions. The retention therefore increases drastically with increasing pH. The influence of the organic modifier content also supported the importance of hydrophobic interactions, i.e. at high water content the retention increased significantly. It seems that the hydrophobic interactions are similar for both enantiomers, and therefore the selectivity did not change significantly with the change of pH or organic modifier content. The ionic strength generally affects separations involving coulombic interactions. Here, the ionic strength can influence the state of ionization of the analytes only, and not of the selector, and therefore its effect was negligible.

At a constant mobile phase composition of 0.2 M aqueous KH_2PO_4 (pH 6.0)/MeCN = 80/20 (v/v) or 0.2 M aque-

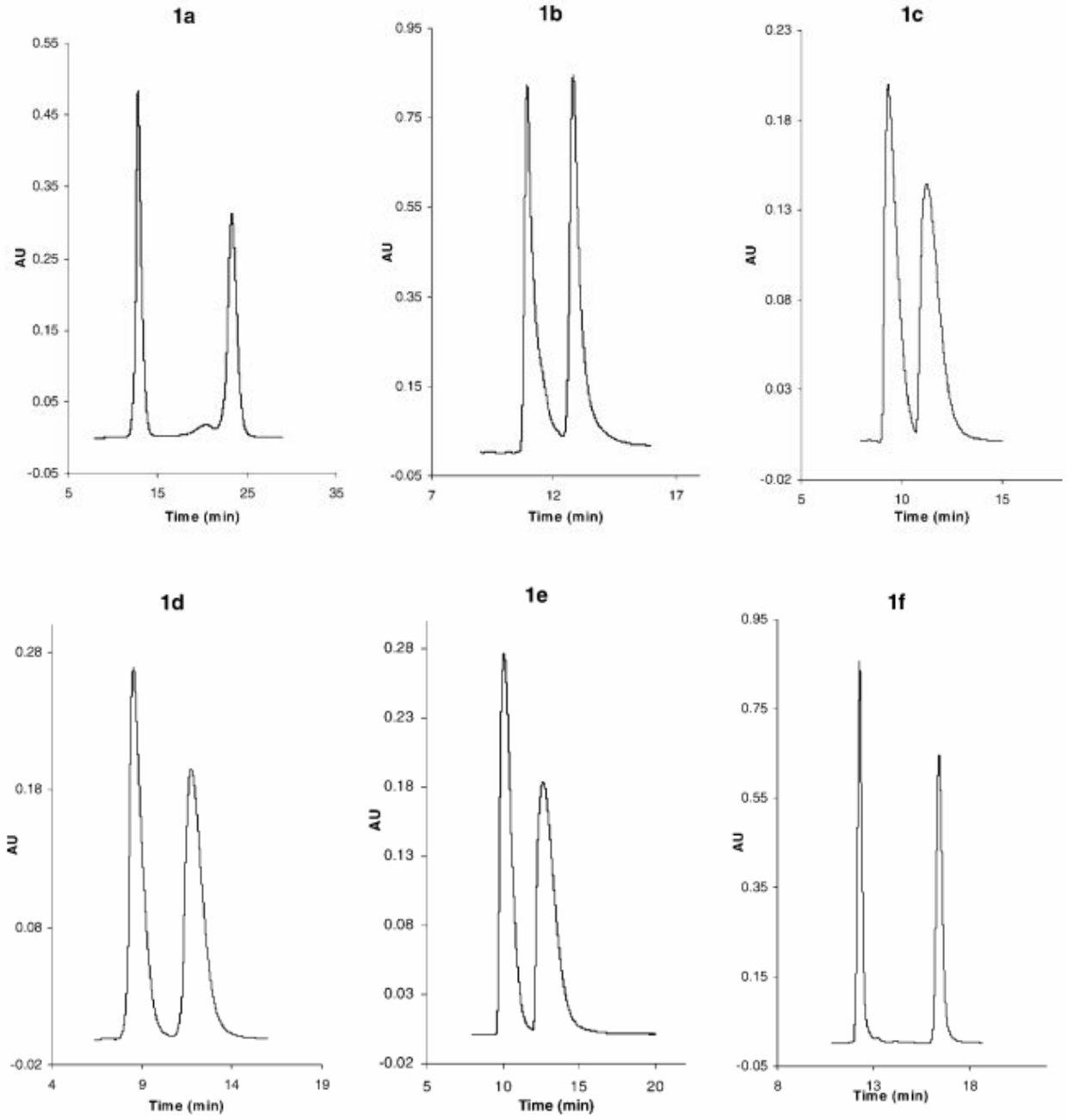


Fig. 3. Selected chromatograms of 1-(aminoalkyl)-2-naphthol analogs. Chromatographic conditions: column, Chiralcel OD-H for analytes **1b** and **1f** and Chiralcel OD-RH for analytes **1a**, **1c**, **1d**, and **1e**; mobile phase, **1a**, 0.05 M aqueous KH_2PO_4 (pH 6.0)/MeOH = 20/80 (v/v); **1b**, *n*-hexane/IPA/DEA = 30/70/0.1 (v/v/v); **1c**, 0.2 M aqueous KH_2PO_4 (pH 6.0)/MeCN = 80/20 (v/v); **1d**, 0.05 M aqueous KH_2PO_4 (pH 6.0)/MeOH = 40/60 (v/v); **1e**, 0.2 M aqueous KH_2PO_4 (pH 5.0)/MeCN = 80/20 (v/v); **1f**, *n*-hexane/IPA/DEA = 70/30/0.1 (v/v/v); detection, 230 nm; flow rate, 0.5 ml/min.

ous KH_2PO_4(pH 6.0)/MeOH = 75/25 (v/v), comparison of the chromatographic behavior of the 2-naphthol analogs containing different alkyl substituents revealed that analytes with larger or more bulky alkyl chain substituents exhibited larger retention factors and generally larger α and R_S values (Table 2). In the normal-phase mode on the Chiralcel OD-H column containing the same selector, the retention factor decreased, while the α and R_S values also increased with increasing length and bulkiness of the alkyl chain. The increased electron-donating ability of larger alkyl chains increased the electron density on the amino and hydroxy groups of the analytes, which influenced the hydrogen-bonding interaction with the *tris*-3,5-dimethylphenyl carbamoylated cellulose selector. The increased hydrogen-bonding ability and steric effect resulted in the improved separation. The higher retention factors in the

reversed-phase mode may be explained by the hydrophobic interactions, which absent in normal-phase mode.

The elution sequence was determined for analyte **1e**. As in normal-phase mode, the elution sequence was (*S*) < (*R*).

Selected chromatograms for analytes **1a–1f** are depicted in Figure 3.

CONCLUSIONS

Chiralcel OD-H and Chiralcel OD-RH can be used as CSPs to separate the enantiomers of 1-(aminoalkyl)-2-naphthol analogs in normal-phase and reversed-phase modes. Optimization of the separation can be achieved by change of pH and buffer concentration in the reversed-phase mode, and by change of the mobile phase composition in both the normal and the reversed-phase modes. The chain lengths of the substituents on 2-naphthol affected the enantioseparations. The absolute configuration and elution sequence were determined for 1-(1-amino-2-methylpropyl)-2-naphthol; the elution sequence was *S* < *R* in both the normal and reversed-phase mode.

LITERATURE CITED

1. Betti M. α-Naphthol phenylaminomethane. Org Synth Colloid 1941; 1:381–383.
2. Szatmári I, Fülöp F. Synthesis and transformations of 1-(α-aminobenzyl)-2-naphthol derivatives. Curr Org Synth 2004;1:155–165.
3. Cardellichio C, Ciccarella G, Naso F, Schingaro E, Scordari F. The Betti base: absolute configuration and routes to a family of related chiral nonracemic bases. Tetrahedron: Asymmetry 1998;9:3667–3675.
4. Cardellicchio C, Ciccarella G, Naso F, Perna F, Tortorella P. Use of readily available chiral compounds related to the Betti base in the enantioselective addition of diethylzinc to aryl aldehydes. Tetrahedron 1999;55:14685–14692.
5. Cimarelli C, Mazzanti A, Palmieri G, Volpini E. Solvent-free asymmetric aminoalkylation of electron-rich aromatic compounds: stereoselective synthesis of aminoalkylnaphthols by crystallization-induced asymmetric transformation. J Org Chem 2001;66:4759–4765.
6. Liu DX, Zhang LC, Wang Q, Da CS, Zin ZQ, Wang R, Choi MKC, Chan ASC. The application of chiral aminonaphthols in the enantioselective addition of diethylzinc to aryl aldehydes. Org Lett 2001;3:2733–2735.
7. Cimarelli C, Palmieri G, Volpini E. A practical stereoselective synthesis of secondary and tertiary aminonaphthols: chiral ligands for enantioselective catalysts in the addition of diethylzinc to benzaldehyde. Tetrahedron: Asymmetry 2002;13:2417–2426.
8. Lu J, Xu X, Wang S, Wang C, Hu Y, Hu H. Novel preparation of nonracemic 1-[α-(1-azacycloalkyl)benzyl]-2-naphthols from Betti base and their application as chiral ligands in the asymmetric addition of diethylzinc to aryl aldehydes. J Chem Soc Perkin Trans 1 2002;2900–2903.
9. Lu J, Xu X, Wang S, Wang C, He J, Hu Y, Hu H. Synthesis of chiral ligands derived from the Betti base and their use in the enantioselective addition of diethylzinc to aromatic aldehydes. Tetrahedron Lett 2002;43:8367–8369.
10. Ji JX, Qui LQ, Yip CW, Chan ASC. A convenient, one-step synthesis of optically active tertiary aminonaphthol and its applications in the highly enantioselective alkenylations of aldehydes. J Org Chem 2003;68:1589–1590.
11. Ji JX, Wu J, Au-Yeung TTL, Yip CW, Haynes RK, Chan ASC. Highly enantioselective phenyl transfer to aryl aldehydes catalyzed by easily accessible chiral tertiary aminonaphthol. J Org Chem 2005;70:1093–1095.
12. Dong Y, Li Rui, Lu J, Xu X, Wang X, Hu Y. An Efficient kinetic resolution of racemic betti base based on an enantioselective N, O-deketalization. J Org Chem 2005;70:8617–8620.
13. Wang X, Dong Y, Sun J, Xu X, Li R, Hu Y. Nonracemic betti base as a new chiral auxiliary: application to total syntheses of enantiopure (2*S*,6*R*)-dihydropinidine and (2*S*,6*R*)-isosolenopsins. J Org Chem 2005;70:1897–1900.
14. Szatmári I, Hetényi A, Lázár L, Fülöp F. Transformation reactions of the Betti base analog aminonaphthols. J Heterocyclic Chem 2004; 41:367–373.
15. Heydenreich M, Koch A, Klod S, Szatmári I, Fülöp F, Kleinpeter E. Synthesis and conformational analysis of naphth[1′,2′:5,6][1,3] oxazino[3,2-*c*][1,3]benzoxazine and naphth[1′,2′:5,6][1,3]oxazino[3,4-*c*] [1,3]benzoxazine derivatives. Tetrahedron 2006;62:11081–11089.
16. Desai NC, Shukla HK, Langalia NA, Thaker KA. Preparation and antibacterial activites of 2-aryl-3-[α-(2-hydroxy-1-naphthyl)benzyl]-4-thiazolidinones. J Indian Chem Soc 1984;61:711–713.
17. Subramanian G. A practical approach to chiral separations by liquid chromatography. Weinheim: Wiley-VCH; 1994.
18. Jinno K. Chromatographic separations based on molecular recognition. New York: Wiley-VCH; 1997.
19. Beesley TE, Scott RPW. Chiral chromatography. New York: Wiley; 1998.
20. Oguni K, Oda H, Ichida A. Development of chiral stationary phases consisting of polysaccharide derivatives. J Chromatogr A 1995;694:91–100.
21. Yashima E. Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. J Chromatogr A 2001;906:105–125.
22. Franco P, Senso A, Oliveros L, Minguillón C. Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. J Chromatogr A 2001;906:155–170.
23. Sztojkov-Ivanov A, Szatmári I, Péter A, Fülöp F. Structural and temperature effects in the high-performance liquid chromatographic enantioseparation of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs. J Sep Sci 2005;28:2505–2510.
24. Berkecz R, Ilisz I, Sztojkov-Ivanov A, Szatmári I, Fülöp F, Armstrong DW, Péter A. High-performance liquid chromatographic enantioseparation of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs on a β-cyclodextrin-based chiral stationary phase. Chromatographia (in press).
25. Tóth D, Szatmári I, Fülöp F. Substituent effects in the ring-chain tautomerism of 1-alkyl-3-arylnaphth[1,2-*e*][1,3]oxazines. Eur J Org Chem 2006;20:4664–4669.
26. Gong Y, Kato K. Diastereoselective Friedel–Crafts reaction of α-trifluoromethyl imines derived from chiral amines. Tetrahedron: Asymmetry 2001;12:2121–2127.
27. Wang T, Wenslow RM Jr. Effect of alcohol mobile-phase modifiers on the structure and chiral selectivity of amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. J Chromatogr A 2003;1015:99–110.
28. Meyer AZ. Molecular mechanics and molecular shape. IV. Shape and steric parameters. J Chem Soc Perkin Trans 2 1986;1567–1986.
29. Jabarpurwala K, Venkatachalam K, Kabadi M. Proton–ligand stability constants of some ortho-substituted phenols. J Inorg Nucl Chem 1964;26:1011–1026.
30. Sugimori T, Shibakawa K, Masuda H, Odani A, Yamauchi O. Ternary metal(II) complexes with tyrosine-containing dipeptides. Structures of copper(II) and palladium(II) complexes involving L-tyrosylglycine and stabilization of copper(II) complexes due to intramolecular aromatic ring stacking. Inorg Chem 1993;32:4951–4959.