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High-performance liquid chromatographic enantioseparation of bicyclic 1,3-amino alcohols

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Abstract

Different high-performance liquid chromatographic methods were developed for the separation and identification of enantiomers of *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol derivatives. Direct separation was carried out on a naphthylethyl carbamate-derivatized β-cyclodextrin (Cyclobond I 2000 SN) stationary phase, which was used in the polar-organic mode. This allowed the simultaneous separation of stereoisomers of alcohol and ester analogs of the bicyclic 1,3-amino alcohols. Alternatively, the derivatization of amino alcohols on the amino group with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide produced diastereomers which were separable with high resolution (R_s>5–10) on a LiChrospher RP-18 stationary phase. The order of elution of the enantiomers was determined by both direct and indirect methods.

Keywords: Enantiomer separation; Bicyclic 1,3-amino alcohols; 3-Aminobicyclo[2.2.1]heptane-2-methanol; 3-Aminobicyclo[2.2.1]hept-5-ene-2-methanol

1. Introduction

The importance of 1,2- and 1,3-amino alcohols is increasing: these compounds and their derivatives play important roles in the syntheses of various pharmaceutically important compounds [1–3]. The naturally occurring amino sugars, for example, are important segments of several antibiotics [4]. Carbocyclic nucleosides containing an aminocyclanol moiety exhibit potent antiviral activity [5]. Enantiopure *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanols and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanols (Fig. 1, **1**–**4**) can be used as chiral building blocks of pharmacologically active, fused saturated 1,3-heterocycles [6]. In these cases, the enantiopurity is crucial. It is therefore very important to have available enantiopure and defined substances and analytical methods for the separation and identification of the different stereoisomers.

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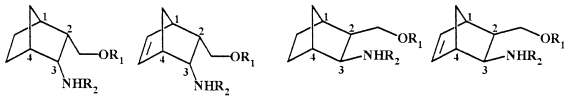


1(a-c) (1*S*,2*S*,3*R*,4*R*; 1*R*,2*R*,3*S*,4*S*)

2(a-c) (1*R*,2*S*,3*R*,4*S*; 1*S*,2*R*,3*S*,4*R*)

3(a-f) (1*S*,2*R*,3*S*,4*R*; 1*R*,2*S*,3*R*,4*S*)

4(a-c) (1*R*,2*R*,3*S*,4*S*; 1*S*,2*S*,3*R*,4*R*)

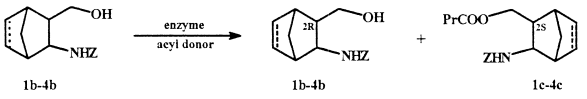
Substituent	a	b	c	d	e	f
R_1	H	H	COPr	COMe	H	COPr
R_2	H	Z	Z	Z	Boc	Boc

Fig. 1. Structures of *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol derivatives. **1a–c**, (1*S*,2*S*,3*R*,4*R*)- and (1*R*, 2*R*, 3*S*, 4*S*)-*diendo*-3-aminobicyclo[2.2.1]heptane-2-methanol derivatives; **2a–c**, (1*R*,2*S*,3*R*,4*S*)- and (1*S*,2*R*,3*S*,4*R*)-*diendo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol derivatives; **3a–f**, (1*S*,2*R*,3*S*,4*R*)- and (1*R*,2*S*,3*R*,4*S*)-*diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol derivatives; **4a–c**, (1*R*,2*R*,3*S*,4*S*)- and (1*S*,2*S*,3*R*,4*R*)-*diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol derivatives.

A valuable method for the preparation of enantiopure compounds is the enzymatic resolution of racemic mixtures. *N*-Benzyloxycarbonyl (*Z*)-protected 1,3-amino alcohols are excellent substrates for lipase-catalyzed *O*-acylation to produce (2*R*)-alcohols (Fig. 1) and (2*S*)-esters (Fig. 2). Analytical methods are required to estimate the enantioselectivity of the enzymatic acylation.

For these purposes, chromatographic separations are widely used. Successful high-performance liquid chromatographic (HPLC) methods for enantioresolution include both indirect and direct methods. Indirect methods involve pre-column derivatization reaction with a chiral reagent, with subsequent separation of the diastereoisomers on an achiral column [7,8]. Direct methods are performed by ligand-exchange chromatography [9,10], or with the application of chiral stationary phases [11,12].

In this work, HPLC methods are described for the enantioseparation of derivatives of racemic *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanols and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanols. A naphthylethylcarbamate-derivatized β-cyclodextrin (Cyclobond I 2000 SN) chiral stationary phase was used in the polar-organic mode [13,14]. The effects of mobile phase composition, temperature and flow-rate on the separation were investigated, and the conditions affording the best resolution were determined. The indirect chiral separations were performed on a C_{18} column in the reversed-phase mode after pre-column derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide. The order of elution of the enantiomers was determined by spiking racemic samples with enantiomers with known absolute configuration.



1b-4b 1b-4b 1c-4c

Fig. 2. Reaction scheme and enantioselectivity of lipase-catalyzed *O*-acyl transfer of *N*-*Z*-protected bicyclic 1,3-amino alcohols.

2. Experimental

2.1. Chemicals and reagents

The racemic 1,3-amino alcohols **1a**–**4a** (Fig. 1) were obtained from the corresponding β-amino acids by $LiAlH_4$ reduction [15–18]. Enantiopure or enantiomerically enriched compounds were prepared by lipase-catalyzed *O*-acylation of *N*-protected racemic 1,3-amino alcohols [19]. Derivatives of amino alcohols were synthesized by standard methods.

The reagents for *N*-protection, di-*tert*.-butyl dicarbonate, benzyl chloroformate and 3,5-dinitrobenzoyl chloride (3,5-DNB-Cl), and for chiral derivatization, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent, FDAA), were purchased from Aldrich (Steinheim, Germany). Hexane (Hex), *i*-propanol (IPA), methanol (MeOH) and acetonitrile (MeCN) were from Merck (Darmstadt, Germany); all were of HPLC grade. Triethylamine (TEA), glacial acetic acid (HOAc) and other reagents of analytical-reagent grade were also from Merck. The Milli-Q water was further purified by filtering it on a 0.45-μm filter, type HV, Millipore (Molsheim, France).

2.2. Apparatus

Two HPLC systems were used. The Waters chromatographic system consisted of an M-600 low-pressure gradient pump, equipped with an M-996 photodiode-array detector and a Millennium 32 Chromatography Manager data system (Waters

Chromatography, Milford, MA, USA). The system working under isocratic conditions included an L-6000 Merck–Hitachi pump (Tokyo, Japan) with a Shimadzu SPD-6AV variable-wavelength UV–Vis detector (Shimadzu, Tokyo, Japan). For data processing, a Hewlett-Packard HP 3395 integrator (Waldbronn, Germany) was applied. The Model 7125 injectors with a 20-μl loop were from Rheodyne (Cotati, CA, USA).

The columns used were (I) Cyclobond I 2000 SN [(*S*)-naphthylethyl carbamate-derivatized β-cyclodextrin], 250×4.6 mm I.D., 5 μm particle size (Astec, Whippany, NJ, USA), (II) Chiralcel OD-RH [cellulose tris(3,5-dimethylphenyl carbamate)], 150×4.0 mm I.D., 5 μm particle size (Daicel, Tokyo, Japan), (III) Crownpak CR(+), 150×4.0 mm I.D., 5 μm particle size (Daicel) and (IV) LiChrospher 100 RP-18, 125×4.0 mm I.D., 5 μm particle size (Merck). The columns were thermostated with a water bath. The temperature was regulated and controlled by a heating–cooling circulator system, type MK-70 (Mechanik Prüfgeräte, Medlingen, Germany).

The normal- and reversed-phase mobile phases were prepared by mixing Hex with IPA or Milli-Q water with MeCN or MeOH (by volume), respectively. The polar-organic mobile phases were prepared in a similar way by mixing MeCN, HOAc and TEA (by volume). The eluents were degassed in an ultrasonic bath, and during the analysis helium gas was bubbled through the solution.

3. Results and discussion

3.1. Direct separation of derivatives

Direct stereoselective analyses were performed on an (*S*)-naphthylethylcarbamate-derivatized β-cyclodextrin chiral stationary phase. On this column, the separation of stereoisomers of compounds **1b**–**c**, **2b**–**c**, **3b**–**f** and **4b**–**c** in reversed-phase mode was unsatisfactory. Table 1 reports chromatographic data on *Z*-protected alcohols and esters, obtained in polar-organic mode. Under the same conditions, alcohols displayed higher retention and lower resolution than the corresponding esters. Also, the separation of saturated compounds was better than that of related unsaturated compounds. The difference were fairly small and could have resulted for example from the higher selectivity (α) of saturated compounds (Table 1). The separation had to be optimized, especially for unsaturated analogs; accordingly, the composition of the mobile phase, the flow-rate and the column temperature were varied. In the polar-organic mode a decrease in the buffer concentration (HOAc/TEA) increased the retention factors and resolutions (**2b, c** and **4b, c**). A decrease in the flow-rate from 0.8 ml min^{-1} to 0.25 or 0.15 ml min^{-1}, and of the temperature from 25 to 5°C, also improved the resolution. By optimizing these three factors, an optimum resolution of $R_s \approx 1.5$ was achieved in most cases (except for compound **2b**).

Regarding the mechanism of chiral discrimination, the most important docking sites of these type of columns are considered to be the polar naphthylethyl carbamate groups, which can interact with enantiomers via H-bonding, π–π interaction and/or dipole–dipole interaction (Fig. 3) [20,21]. For alcohols, the predominating H-bond can be formed between the OH proton of the analyte and the carbonyl oxygen of the naphthylethyl carbamate, while for esters the carbonyl oxygen of the ester function may interact with the NH proton of the carbamate residue through H-bonding. In Cyclobond I 2000 SN there is only an average of five naphthylethyl carbamate groups on each cyclodextrin. This means that there are several cyclodextrin-OH groups still available for ester carbonyl oxygen for hydrogen bonding. However, in consequence of the electron-donating character of the methyl group in naphthylethyl carbamate, the acidity of the NH proton decreases, while the electron density on the carbonyl oxygen of the carbamate is expected to increase. The H-bond can therefore be stronger in its interactions with alcohols than in those with esters, and the former can be strongly retained. Our results support these expectations: the discussed effects contributed to the observed differences in retention, i.e., the alcohols were more strongly retained than the esters. The differences in retention were so high, that the α and R_s factors between the second-eluting ester peak and the third-eluting alcohol peak in most cases exceeded 2.0 (Fig. 4). The role of the *Z* group in the analyte is also crucial. When the substituent in the R_2 position was Boc (**3e** and **3f**), no separation of the stereoisomers was

Table 1
Chromatographic data, retention factors (k), separation factors (α) and resolutions (R_s) for derivatives of racemic *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanols and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanols

Compound	R_1	R_2	Eluent, MeOH–HOAc–TEA (%, v/v)	Flow-rate (ml min^{-1})	Temperature (°C)	k_1	k_2	α	R_s	Elution order
1b	H	Z	100:0.8:0.2	0.25	25	0.67	0.75	1.12	1.25	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)
1c	COPr	Z	100:0.8:0.2	0.25	25	0.25	0.42	1.68	1.85	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)
2b	H	Z	100:0.8:0.2	0.25	25	0.65	0.74	1.14	0.82	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.04:0.01	0.15	5	1.08	1.17	1.08	1.05	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
2c	COPr	Z	100:0.8:0.2	0.25	25	0.24	0.28	1.16	0.76	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.04:0.01	0.15	5	0.28	0.46	1.43	1.48	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
3b	H	Z	100:0.8:0.2	0.80	25	0.83	0.95	1.14	1.05	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.8:0.2	0.40	25	0.84	0.96	1.14	1.23	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.8:0.2	0.25	25	0.87	0.99	1.14	1.43	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
3c	COPr	Z	100:0.8:0.2	0.80	25	0.34	0.37	1.08	0.97	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.8:0.2	0.40	25	0.35	0.41	1.17	1.28	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
			100:0.8:0.2	0.25	25	0.29	0.38	1.31	1.60	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
3d	COMe	Z	100:0.8:0.2	0.25	25	0.32	0.43	1.34	1.66	(1*R*,2*S*,3*R*,4*S*)<(1*S*,2*R*,3*S*,4*R*)
4b	H	Z	100:0.8:0.2	0.15	5	0.79	0.89	1.13	0.83	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)
			100:0.04:0.01	0.15	5	0.89	1.03	1.17	1.43	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)
4c	COPr	Z	100:0.8:0.2	0.15	5	0.29	0.35	1.20	1.08	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)
			100:0.04:0.01	0.15	5	0.31	0.48	1.54	1.42	(1*S*,2*S*,3*R*,4*R*)<(1*R*,2*R*,3*S*,4*S*)

Column, Cyclobond I 2000 SN; detection, 205 nm; k_1: retention factor of first-eluting stereoisomer; k_2: retention factor of second-eluting stereoisomer; t_M=3.6 min (0.8 ml min^{-1}).

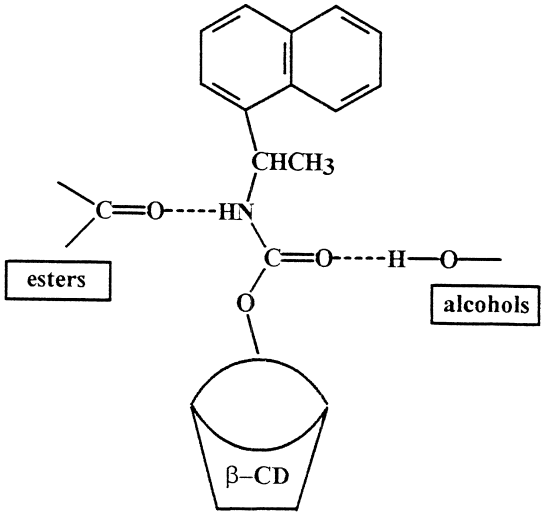


Fig. 3. Presumed interactions on (*S*)-naphthylethyl carbamate-derivatized β-cyclodextrin (Cyclobond I 2000 SN) stationary phase.

observed. This indicates the possible role in the retention mechanism of π–π interactions between the naphthyl group of the stationary phase and the aromatic group of the solute. It looks like the alcohol group contributes to retention, but not to chiral recognition. When the ester is made, this serves to do one of two things. Either it disrupts a hydrogen bond that decreases retention, or it provides steric bulk, which contributes to chiral recognition, via a steric repulsion interaction. Or both things could be happening.

Racemic **1b**–**4b** were found to be appropriate starting substrates for enzymatic resolution [19]. Lipase-catalyzed *O*-acylation of these *Z* derivatives produces four possible analogs in the reaction mixture: two alcohol enantiomers **1b**–**4b** and two ester enantiomers **1c**–**4c**. Fig. 4 reveals that all of these stereoisomers were separable on the Cyclobond I 2000 SN column in a single chromatographic run, thereby offering a very convenient method for the

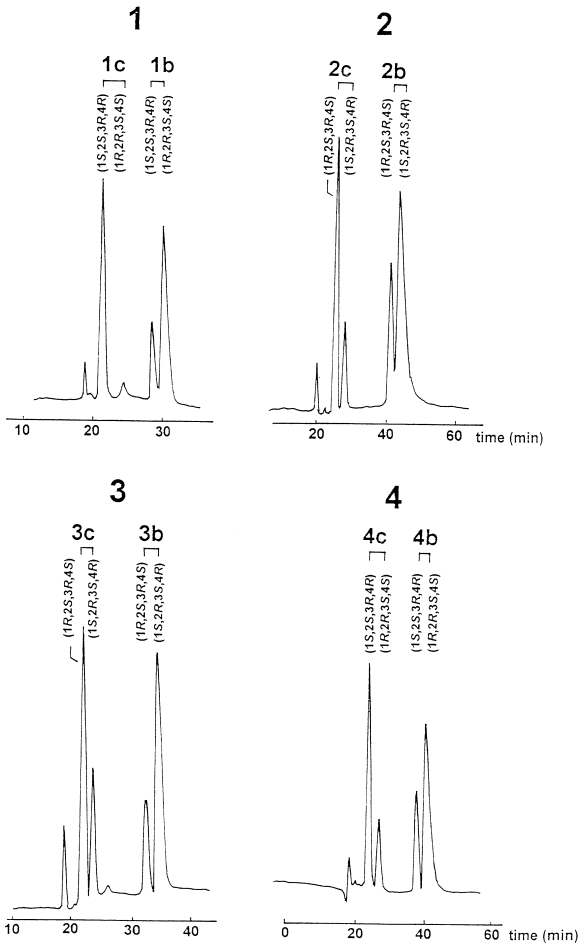


Fig. 4. Selected chromatograms of lipase-catalyzed *O*-acylation of *N-Z* derivatives of *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol; *N-Z*-alcohols and their butyrate analogs. Column, Cyclobond I 2000 SN; flow-rate, **1, 3**, 0.25 ml min^{-1}, **2, 4**, 0.15 ml min^{-1}; temperature, **1, 3**, 25°C, **2, 4**, 5°C; detection, 205 nm; mobile phase, MeOH–HOAc–TEA, **1, 3**, (100:0.8:0.2, v/v), **2, 4**, (100:0.04:0.01, v/v); **1b**, (1*S*,2*S*,3*R*,4*R*)- and (1*R*,2*R*,3*S*,4*S*)-*diendo*-3-aminobicyclo[2.2.1]heptane-2-methanol, **1c**, (1*S*,2*S*,3*R*,4*R*)- and (1*R*,2*R*,3*S*,4*S*)-*diendo*-3-aminobicyclo[2.2.1]heptane-2-methanol butyrate; **2b**, (1*R*,2*S*,3*R*,4*S*)- and (1*S*,2*R*,3*S*,4*R*)-*diendo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol, **2c**, (1*R*,2*S*,3*R*,4*S*)- and (1*S*,2*R*,3*S*,4*R*)-*diendo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol butyrate; **3b**, (1*R*,2*S*,3*R*,4*S*)- and (1*S*,2*R*,3*S*,4*R*)-*diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol; **3c**, (1*R*,2*S*,3*R*,4*S*)- and (1*S*,2*R*,3*S*,4*R*)-*diexo*-3-aminobicyclo[2.2.1]heptane-2-methanol butyrate; **4b**, (1*S*,2*S*,3*R*,4*R*)- and (1*R*,2*R*,3*S*,4*S*)-*diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol; **4c**, (1*S*,2*S*,3*R*,4*R*)- and (1*R*,2*R*,3*S*,4*S*)-*diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanol butyrate.

simultaneous monitoring of the conversion and enantioselectivity of an enzymatic resolution.

The order of elution of the enantiomers is indicated on the chromatograms in Fig. 4 and Table 1; it was determined by addition of standards [19]. The order of elution follows a rule. In both cases (*N-Z*-protected alcohols and *N-Z*-protected esters), the first-eluting enantiomers always have the 2*S*,3*R*-configuration, while the second-eluting enantiomers have the 2*R*,3*S*-configuration. The general rule for elution order on Cyclobond I 2000 SN for racemates with one stereogenic center is $R<S$ [22]. Since the more strongly retained stereoisomer has the *S* configuration at the C(3) position, it seems that the configuration around C(3) plays an important role in chiral discrimination.

Cellulose tris(3,5-dimethylphenyl carbamate) as stationary phase (Chiralcel OD) in the normal-phase mode has been previously used in the separation of monocyclic 1,3-amino alcohol derivatives [23]. In this study Chiralcel OD-RH in reversed-phase mode was effective in the separation of *N*-Boc-protected ester derivatives (**3f**), whereas *N*-Boc-protected alcohols (**3e**) and *N-Z*-protected esters (**1c**–**4c**) and alcohols (**1b**–**4b**) underwent only a partial separation on this stationary phase in the reversed-phase mode.

3.2. Separation of bicyclic 1,3-amino alcohols

In other work, attempts were made to separate enantiomers of racemic bicyclic 1,3-amino alcohols **1a**–**4a** without any derivatization on a chiral crown-ether-containing stationary phase, Crownpak CR(+). This column has been successfully applied earlier for the enantioseparation of amino acids and amines containing a primary amino group [24]. Unfortunately, no separation was achieved in the present work, despite variation of the eluent composition (pH of perchloric acid), flow-rate and temperature.

The enantiomers of the racemic bicyclic 1,3-amino alcohols **1a**–**4a** were separated after pre-column derivatization with both achiral and chiral reagent. Achiral derivatization was performed with 3,5-DNB-Cl, and the 3,5-DNB derivatives were separated on a Cyclobond I 2000 SN column in the normal-phase mode. Table 2 depicts the best results achieved, in the Hex–IPA (70:30, v/v) eluent system. At ambient temperature, the enantiomers of *diexo* compounds

Table 2
Chromatographic data, retention factors (k), separation factors (α) and resolutions (R_s) for FDAA and 3,5-DNB derivatives of racemic *diendo*- and *diexo*-3-aminobicyclo[2.2.1]heptane-2-methanols and *diendo*- and *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-methanols

Compound	Eluent (%, v/v)	k_1	k_2	α	R_s	Column	Flow-rate (ml min^{-1})	Elution order
FDAA derivatives	Water–MeCN							
1a	65:35	3.90	6.56	1.68	8.15	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	60:40	2.26	3.96	1.75	5.82	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
2a	65:35	2.49	4.26	1.71	6.36	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
	60:40	1.95	3.24	1.66	5.02	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
3a	65:35	4.38	9.65	1.99	11.73	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
	60:40	2.33	3.98	1.71	6.23	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
4a	65:35	3.77	6.86	1.82	8.14	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	60:40	2.14	3.53	1.65	4.21	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	Water–MeOH							
1a	45:55	3.99	11.85	2.97	14.37	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	35:65	1.31	3.24	2.47	4.89	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
2a	45:55	3.47	9.65	2.78	10.81	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
	35:65	1.18	2.84	2.40	5.25	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
3a	45:55	2.38	9.10	3.82	11.30	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
	35:65	1.21	3.41	2.82	7.14	IV	0.80	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
4a	45:55	3.30	9.94	3.01	10.52	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	35:65	1.13	2.83	2.50	5.03	IV	0.80	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
3,5-DNB derivatives	Hex–IPA							
1a	70:30	6.27	7.07	1.13	1.09	I	1.00	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
	70:30	6.17	6.99	1.13	1.38	I	0.50	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)
2a	70:30	7.32	8.04	1.10	1.02	I	1.00	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
	70:30	7.28	8.00	1.10	1.05	I	0.50	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
3a	70:30	4.98	6.23	1.25	1.45	I	1.00	(1*S*,2*R*,3*S*,4*R*)<(1*R*,2*S*,3*R*,4*S*)
4a	70:30	5.37	6.45	1.20	1.52	I	1.00	(1*R*,2*R*,3*S*,4*S*)<(1*S*,2*S*,3*R*,4*R*)

Column, I, Cyclobond I 2000 SN, IV, LiChrospher RP-C_{18}; eluent, Hex: hexane, IPA: 2-propanol; detection, 205 nm; k_1: retention factor of first-eluting stereoisomer; k_2: retention factor of second-eluting stereoisomer; t_M=3.1 min (1.0 ml min^{-1}) I; t_M=1.47 min (0.8 ml min^{-1}) IV.

underwent baseline separation, while the R_s values for the stereoisomers of *diendo* compounds were somewhat lower, especially for the unsaturated analyte **2a**. In all of these cases the selectivity (α) was higher and retention was less. Thus *diendo* compounds were retained longer and this additional retention was due to a nonenantioselective retention mechanism. Probably, the *diexo* compounds, due to their configuration, are able to better maximize their enantioselective interactions and, in particular, minimize their nonenantioselective retention relative to the *diendo* compounds. The elution order was the reverse of that observed for *N*-*Z*-protected derivatives in polar-organic mode. The reverse elution order points to the different mechanism of separation. The DNB group is π-acidic (i.e., π-electron acceptor) and forms a strong π–π interaction with the π-donor moiety (i.e., the naphthylethyl carba-

mate group) on the CSP. This interaction is particularly strong in the normal-phase mode where non-polar solvents are used.

The chiral derivatization was performed with FDAA [25], and analyses were carried out on a LiChrospher RP-18 column at ambient temperature. The FDAA derivatives separated well when MeCN or MeOH was used as the organic component of the mobile phase. The α and especially R_s factors were very high. Of the two organic modifiers, MeOH seemed to be more effective. A probable explanation of this effect may be, that in reversed-phase mode MeOH can participate in hydrogen-bonding interactions while MeCN not. At similar eluent strength, with MeOH as organic modifier (a 40% MeCN content corresponds to 55% MeOH content in respect of eluent strength), the retention factors for the second-eluting components displayed a twofold increase, which resulted in a twofold increase in the R_s values, too: $R_s>10$ was obtained. Mobile phases containing 65% MeOH produced high resolution and relatively short retention times. The order of elution of the diastereomers was determined by standard addition, and was found to be 2*R*,3*S*<2*S*,3*R* for both *diendo* and *diexo* derivatives. The amino group attached to stereogenic center C(3) takes part in the derivatization reaction, and the configuration of C(3) in the first-eluting diastereomer was 3(*S*) in every case. This follows the general rule relating to the order of elution of FDAA derivatives [26].

4. Conclusions

HPLC methods were developed for the separation of stereoisomers of racemic bicyclic 1,3-amino alcohol derivatives. Direct separations were performed on a (*S*)-naphthylethyl carbamate-derivatized β-cyclodextrin (Cyclobond I 2000 SN) stationary phase. This column is suitable for the simultaneous separation of bicyclic 1,3-amino alcohols and their ester analogs. Indirect separation of the free amino alcohols, either involving pre-column derivatization with 3,5-DNB-Cl or 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide permitted the differentiation of bicyclic 1,3-amino alcohol enantiomers with high resolution on a Cyclobond I 2000 SN or a LiChrospher RP-18 column, respectively. In combination with the addition of standards, both direct and indirect methods can be used to identify absolute configurations and hence to determine enzyme selectivity in lipase-catalyzed *O*-acylation processes.

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