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Resolution studies on two regioisomeric chiral stationary phases: Effects from reversed orientation of an amide group

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Abstract

Two new polymeric chiral stationary phases, incorporating the selectors *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid bis-allylamide, **1** (DEABA) and *trans*-11,12-diamino-9,10-dihydro-9,10-ethanoanthracene bis-butenoylamide, **2** (DDEBB), respectively, have been evaluated by chromatographic resolution of a series of structurally different racemates. For some groups of compounds, where large separation factors were obtained, more detailed studies were performed by the use of different retention modifiers. As an effect from the reversed orientation of the amide group in the two selectors, the enantiomers of the racemates investigated are separated in opposite order of elution on the two columns.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Amide group orientation; Retention modifiers

1. Introduction

The increasing demand, particularly in pharmaceutical research, for racemate resolution into pure enantiomers, has led to the development of a variety of chiral stationary phases used in liquid chromatography. The Kromasil CHI-DMB and CHI-TBB columns, developed some years ago and today commercially available, have been shown to possess physical stability and high capacity, due to covalently bound network polymerized selectors [1,2]. The selectors of the Kromasil CHI-TBB (TBB) and CHI-DMB (DMB) phases are based on derivatives of *N*,*N*′-diallyl-L-tartar-diamide (DATD), containing *tert*-butylbenzoyl and 3,5-dimethylbenzoyl substituents, respectively (Fig. 1). The selectors are polymerized and immobilized to vinyl-silica by multifunctional hydrosilylation reactions.

This work is focused on two selectors, *trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid bis-allylamide, **1** (DEABA) and *trans*-11,12-diamino-9,10-dihydro-9,10-ethanoanthracene bis-butenoylamide, **2** (DDEBB), which have their stereogenic centres incorporated into a rigid structure and a mutually reversed orientation of the amide groups. The synthesis of the selectors is shown in Fig. 2. Both selectors are derivatives of a C_2-symmetric dicarboxylic acid (*trans*-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid, **3**) [3] which is conformationally more restrained than the chiral synthon of the selectors of the TBB and DMB phases, DATD.

The dicarboxylic acid, **3**, is obtained by a Diels–Alder reaction between anthracene and fumaric acid [4] and is easily resolved by recrystallization of its brucine salts; the less soluble salt giving the (−)-(*S*,*S*)-enantiomer [5]. Both enantiopure forms of **3** can also be obtained by asymmetric Diels–Alder reactions [6,7], to avoid use of the expensive and toxic brucine on a large scale. After synthesis of the selectors, they have been crosslinked and immobilized to vinyl-silica by methods similar to those used to obtain the DMB and TBB phases.

On the two chiral stationary phases, DEABA and DDEBB, the effects from the reduced mobility at the central part and from the reversed orientation of the amide groups have been studied by chromatography of a series of racemates (Fig. 3). The DEABA phase has previously been shown to give high selectivity factors for a series of benzodiazepinones [8]. Now the study has been expanded to include the influence of the retention modifier on the resolution of seven carbamoylated amino acids on

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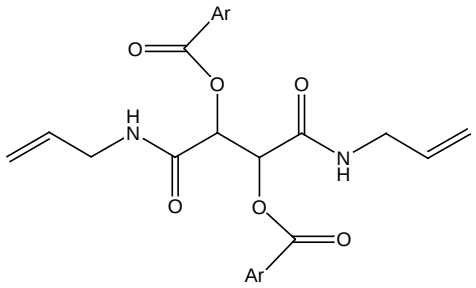

TBB: Ar = 4-*tert*-butylbenzoyl
DMB: Ar = 3,5-dimethylbenzoyl

Fig. 1. The chiral selectors used in the Kromasil CHI-TBB and CHI-DMB sorbents.

the DEABA and the DDEBB phases. A series of halogenated benzoins has also been studied on both phases. By CD spectroscopy, the order of elution on the two phases has been determined for a majority of the resolved racemates.

2. Experimental

2.1. Instruments

Analytical liquid chromatography was performed on a system composed of a Varian 9012Q solvent delivery pump and a Varian 9050 variable wavelength UV detector. Samples were injected via a Rheodyne injector. Optical rotations were determined with a Perkin-Elmer (Norwalk, CT) 341 LC instrument using a quartz microcell of 1 dm pathlength at 20 °C. CD spectra were recorded with a Jasco J-715 instrument in a 0.1 cm quartz cell at 20 °C. ^{1}H NMR spectra were obtained with a 400 MHz Varian VXR-400 spectrometer with $CDCl_3$ or acetone-d_6 as solvent.

2.2. Chemicals

The vinyl-silica was a gift from Eka Chemicals (Bohus, Sweden) and the pharmaceutical compounds were a gift from Astra Zeneca (Mölndal, Sweden).

2.3. Chromatography

The Kromasil CHI-DMB and CHI-TBB columns were of size 250 mm × 4.6 mm i.d. The flow of the mobile phase was 1.5 ml/min and the samples injected were of volume 20 μl and concentration 1 mg/ml dissolved in 2-propanol. The columns with the DEABA and the DDEBB phases, packed by HiChrom Ltd. (Berkshire, UK), were of size 250 mm × 3.2 mm i.d. The flow of the mobile phase was 0.75 ml/min and the samples injected were of volume 5 μl and concentration 1 mg/ml dissolved in 2-propanol. Detection was made at λ = 225 nm.

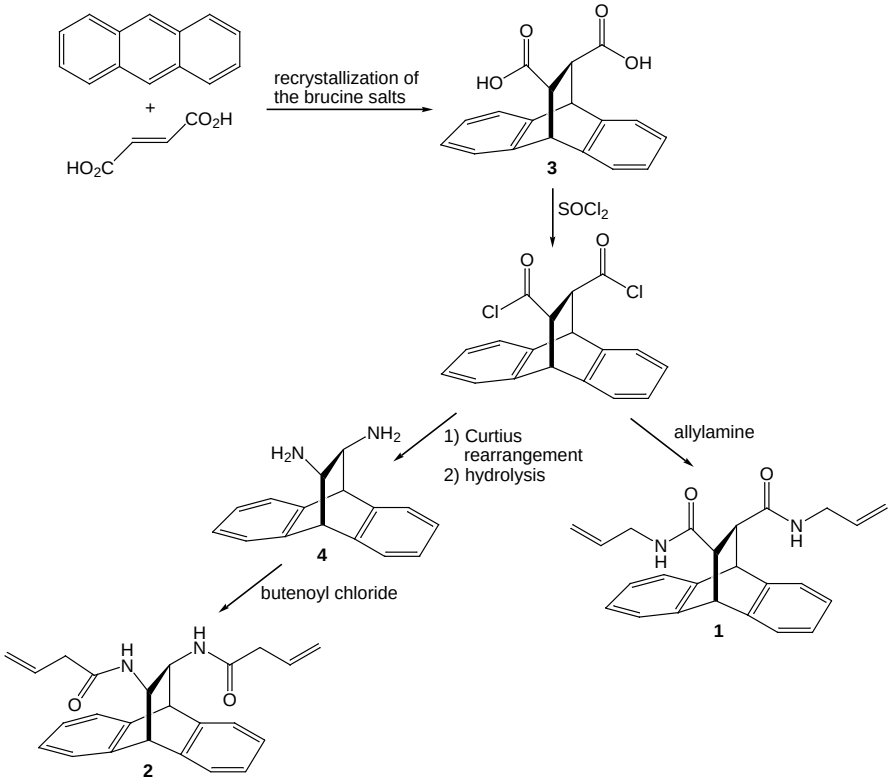

Fig. 2. Synthesis of the chiral selectors used in the DEABA phase (**1**) and in the DDEBB phase (**2**).

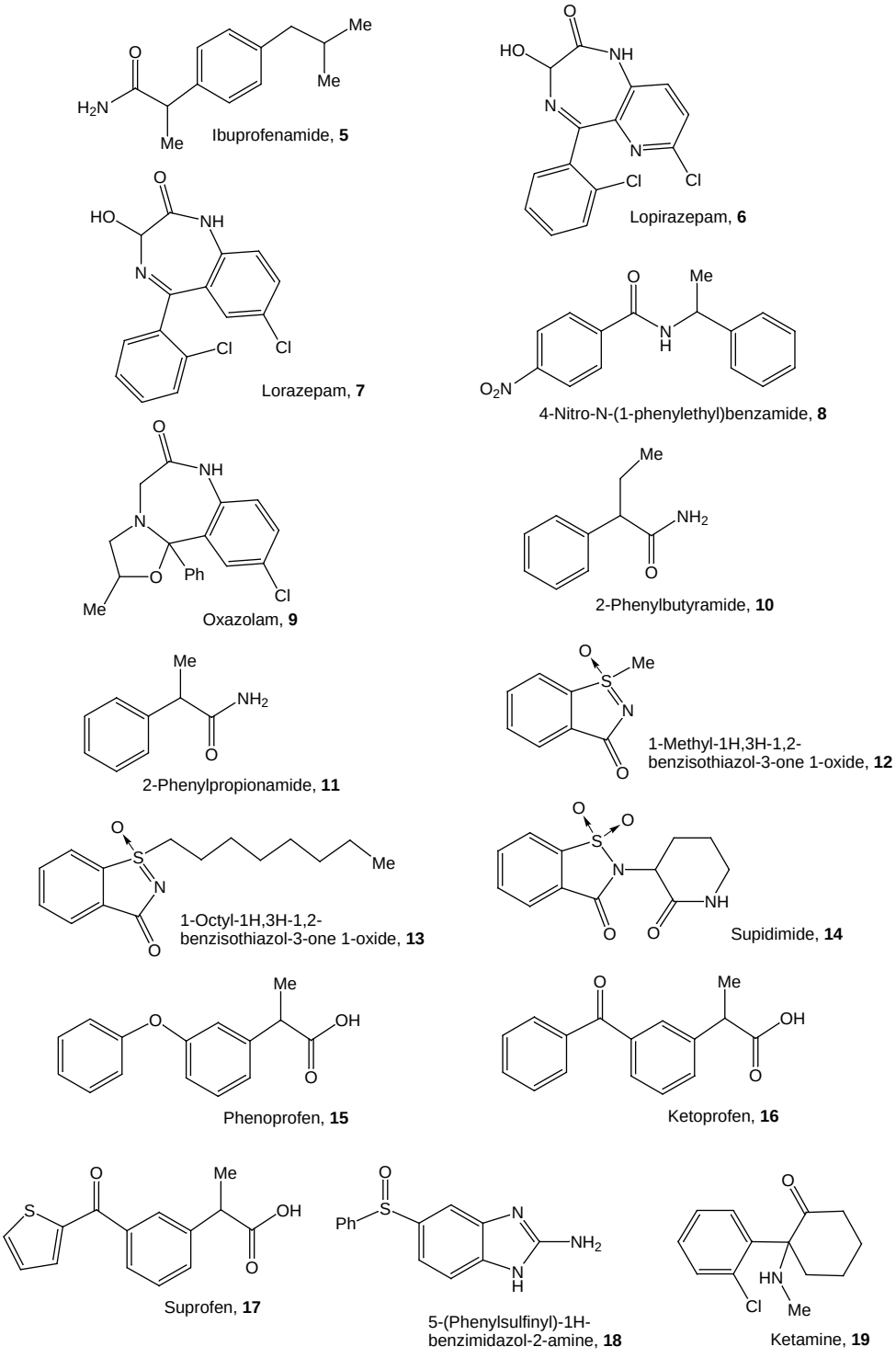

Fig. 3. Racemates chromatographed on the DEABA and DDEBB phases.

2.4. Synthesis

2.4.1. (±)-trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid (3) [4]

A solution of anthracene (96.11 g, 0.512 mol) and fumaric acid (20.19 g, 0.172 mol) in 1,4-dioxane (770 ml) was refluxed for 70 h. The solvent was removed under reduced pressure and the residue was stirred with 2.5% sodium carbonate (1.0 l) for 24 h. The mixture was filtered and conc. hydrochloric acid was added to the filtrate until pH 1. By filtration of the hot solution the product was isolated from the remaining fumaric acid in

a yield of 86.5% (43.8 g); mp 253.1–253.7 °C ([5] mp 252.5 °C).

*2.4.2. Resolution of (±)-**3** [5]*

A mixture of (±)-**3** (10.09 g, 34.27 mmol) and brucine (32.08 g, 78.89 mmol) was crystallized from 37% ethanol. After two recrystallizations of the brucine salt, it was added to 6 M hydrochloric acid and the aqueous phase was extracted with diethyl ether. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure, yielding 97.6% of (−)-(*S*,*S*)-**3** in ee > 99%; mp 219.8–220.2 °C ([5] mp 220.5 °C). $[\alpha]^{20}_{546} = -15.5$ (*c* 2.03, 1,4-dioxane) ([5] $[\alpha]^{25}_{578} = -15.3$ (*c* 2, 1,4-dioxane). CD (acetonitrile): λ_{ext} (nm), $\Delta\varepsilon_{ext}$ ($cm^2\,mmol^{-1}$) 218, −4.74; 206, +3.39; 197, −8.60. HPLC (Kromasil CHI-TBB, 2% 2-propanol and 0.2% acetic acid in hexane, flow: 2 ml/min): $k_1 = 7.48$, $\alpha = 1.28$. ^{1}H NMR (400 MHz, acetone-d_6) $\delta = 7.42$ (m, 2H), 7.32 ppm (m, 2H), 7.11 ppm (m, 4H), 4.83 ppm (s, 2H), 3.38 ppm (s, 2H).

*2.4.3. (+)-trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dicarboxylic acid bis-allylamide (**1**) [8]*

To a slurry of (−)-(*S*,*S*)-**3** (3.71 g, 12.6 mmol) in anhydrous benzene (35 ml) one drop of anhydrous DMF and thionyl chloride (3.65 ml, 50.16 mmol) was added. After reflux for 4 h under nitrogen, the solvent with the excess of thionyl chloride was evaporated under reduced pressure. Anhydrous benzene (30 ml) was added in two portions and removed under reduced pressure.

The acid chloride and allylamine (12.0 ml, 0.157 mol) was dissolved in benzene (175 ml) and (45 ml), respectively. The solutions were cooled on ice and the acid chloride was added dropwise to the stirred allylamine causing formation of a white precipitate. After stirring for 45 min while attaining room temperature, the mixture was filtered. The crude product was dissolved in chloroform (700 ml) and the organic layer was washed with 2 M hydrochloric acid (3 × 150 ml), 1 M sodium hydroxide (3 × 150 ml) and saturated aqueous sodium chloride. The solvent was dried over $MgSO_4$, filtered and evaporated under reduced pressure, yielding 68.2% (3.20 g) of (+)-(*S*,*S*)-**1** in ee 98.7%; mp 183.4–183.7 °C. $[\alpha]^{20}_{D} = +39.4$ (*c* 0.17, ethyl acetate). CD (acetonitrile): λ_{ext} (nm), $\Delta\varepsilon_{ext}$ ($cm^2\,mmol^{-1}$) 233, +4.06; 207, +22.0. HPLC (Kromasil CHI-TBB, 5% 2-propanol in hexane, flow: 2.0 ml/min): $k_1 = 0.58$, $\alpha = 2.3$. ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.42$ (m, 2H), 7.31 ppm (m, 2H), 7.15 ppm (m, 4H), 6.88 ppm (s, 2H), 5.76 ppm (m, 2H), 5.06 ppm (m, 2H), 4.67 ppm (s, 2H), 3.08 ppm (m, 4H), 2.88 ppm (s, 2H).

*2.4.4. (+)-(S,S)-11,12-Diamino-9,10-dihydro-9,10-ethanoanthracene (**4**) [9]*

(−)-(*S*,*S*)-**3** (7.80 g, 26.5 mmol) was converted to the corresponding acid chloride by the same method as above (Section 2.4.3). The acid chloride was dissolved in anhydrous DMF (70 ml). The solution was stirred for 1h at 0 °C and sodium azide (5.14 g, 79.1 mmol) was added. After 3.5 h of stirring, while attaining room temperature, the reaction mixture was poured into cold water (300 ml) and extracted with cold toluene (6 × 150 ml). The organic layer was dried over $MgSO_4$, filtered and the solvent was reduced to 500 ml by evaporation. After 14 h of stirring, the solution was refluxed for 3 h. The solvent was evaporated under reduced pressure. The obtained diisocyanate was dissolved in dichloromethane (150 ml) and 8 M hydrochloric acid (150 ml) was added. The two-phase system was stirred at room temperature for 20 h and then at 40–50 °C for 2 h. The organic layer was washed with 8 M hydrochloric acid (50 ml). To the total aqueous phase, sodium hydroxide was added until pH 14. The aqueous phase was extracted with diethyl ether (4 × 200 ml). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure, yielding 52.6% (3.29 g) of (+)-(*S*,*S*)-**4**; mp 152.5–153.9 °C ([9] mp 153–156 °C). $[\alpha]^{20}_{D} + 20.1$ (*c* 1.0, methanol) ([9] $[\alpha]_D = +20.5$ (*c* 2.275, methanol)). CD (acetonitrile): λ_{ext} (nm), $\Delta\varepsilon_{ext}$ ($cm^2\,mmol^{-1}$) 242, −1.01; 210, +18.4. ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.32$ (m, 4H), 7.16 ppm (m, 4H), 4.05 ppm (s, 2H), 2.67 ppm (s, 2H), 1.33 ppm (s, 4H).

2.4.5. Butenoyl chloride

A mixture of vinyl acetic acid (6.0 ml, 68.5 mmol) and oxalyl chloride (12.0 ml, 135 mmol) was stirred at 40 °C for 18 h. The butenoyl chloride was isolated by distillation (bp 98–99 °C), yielding 67.7% (4.85 g). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 5.91$ (m, 1H), 5.31 ppm (m, 2H), 3.63 ppm (d, 2H).

*2.4.6. (+)-(S,S)-11,12-Diamino-9,10-dihydro-9,10-ethanoanthracene bis-butenoyl-amide (**2**)*

(+)-(*S*,*S*)-**4** (2.60 g, 11.0 mmol) in diethyl ether (300 ml) was added to 5% sodium carbonate (100 ml). While cooling with ice, a solution of butenoyl chloride (4.82 g, 46.1 mmol) and 4-*tert*-butylcatechol (100 mg, 0.59 mmol) in diethyl ether (50 ml) was added. The two-phase system was stirred at 0 °C for 1.5 h and then at room temperature for 2.5 h. The phases were separated and the organic layer was washed with 2 M hydrochloric acid (5 × 100 ml) and 2 M sodium hydroxide (8 × 100 ml), until the aqueous phase no longer turned red. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The product was purified by flash chromatography on silica with ethyl acetate/dichloromethane 60:40 as eluting solvent, yielding 79.2% (3.25 g) of (+)-(*S*,*S*)-**2** in ee 98.2%; mp 198.2–198.9 °C. $[\alpha]^{20}_{D} = +123.0$ (*c* 1.0, methanol). CD (acetonitrile): λ_{ext} (nm), $\Delta\varepsilon_{ext}$ ($cm^2\,mmol^{-1}$) 234, −3.57; 209, +47.9. HPLC (Kromasil CHI-TBB, 2% 2-propanol in hexane, flow: 2.0 ml/min): $k_1 = 1.96$, $\alpha = 2.32$. ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.38$ (m, 2H), 7.28 ppm (m, 2H), 7.19 ppm (m, 4H), 5.80 ppm (m, 2H), 5.28 ppm (d, 2H), 5.13 ppm (m, 4H), 4.35 ppm (d, 2H), 3.91 ppm (d, 2H), 2.91 ppm (m, 4H).

2.4.7. Preparation of the chiral stationary phases

The chiral selector (+)-(*S*,*S*)-**1** or (+)-(*S*,*S*)-**2** (8.0 mmol) was dissolved in anhydrous 1,4-dioxane (140 ml). *Tetrakis*-(dimethylsiloxy)silane (8.0 mmol) and a solution of hydrogen hexachloroplatinate in 2-propanol (10 μl, 55 mg/ml) was added and the solution was stirred for 24 h at 95 °C. To vinyl-silica (4.0 g) in anhydrous 1,4-dioxane (60 ml), hydrogen hexachloroplatinate in 2-propanol (10 μl, 55 mg/ml) was added. After 15 min the solution of polymerized selector was added to the slurry of vinyl-silica. The slurry was carefully stirred for 60 h at 95 °C. DMF (50 ml) was added and careful stirring continued at 110 °C for 3 h. The resulting sorbent was filtered while warm and washed with warm 1,4-dioxane, THF, dichloromethane and diethyl ether. The sorbents were dried (4.53 g DEABA of and 4.15 g of DDEBB) and columns (250 mm × 3.2 mm i.d.) were packed by HiChrom Ltd. Elementary analysis: DEABA: C, 13.3%; N, 0.45%; DDEBB: C, 11.8%; N, 0.41%.

3. Results and discussion

3.1. Synthesis of the chiral stationary phases

The synthesis of (+)-(*S*,*S*)-**1** and (+)-(*S*,*S*)-**2** is shown in Fig. 2. The two selectors are prepared from the same enantiopure dicarboxylic acid, (−)-(*S*,*S*)-**3**, which is obtained by a Diels–Alder reaction between anthracene and fumaric acid and easily resolved by two recrystallizations of its brucine salts. The bis-allyl amide, (+)-(*S*,*S*)-**1**, is obtained in a yield of 68.2% via the acid chloride, which is reacted with allyl amine. The acid chloride can also be converted to the corresponding diamine in a yield of 52.6% by a Curtius rearrangement [9], followed by hydrolysis. The rearrangement reaction takes place with complete retention of configuration, giving (+)-(*S*,*S*)-**4** in an ee > 99%. Reaction between the diamine and butenoyl chloride, obtained from vinylacetic acid and oxalyl chloride, gives (+)-(*S*,*S*)-**2** in a yield of 79.2%. The chiral selectors were polymerized and immobilized to vinyl-silica by multifunctional hydrosilylation reactions [1,2]. From elementary analysis of carbon and nitrogen, the amount of selector covering the vinyl-silica was calculated to 0.16 mmol/g sorbent for the DEABA phase and 0.15 mmol/g sorbent for the DDEBB phase. Fig. 4 shows the CD spectra of the two selectors. The strong positive Cotton effect at 207 nm for (+)-(*S*,*S*)-**1** and 209 nm for (+)-(*S*,*S*)-**2** corresponds to both a ^1L_a transition in the aromatic parts and also to an $n \rightarrow \pi^*$ transition in the carbonyl groups of the selectors [10,11]. The Cotton effect at 233 nm for (+)-(*S*,*S*)-**1** and 234 nm for (+)-(*S*,*S*)-**2** have different signs. These bands also correspond to an overlap of two transitions, an aromatic ^1L_b transition and a $\pi \rightarrow \pi^*$ transition in the carbonyl groups. Due to the overlapping bands, analysis and comparison of the two spectra is difficult.

3.2. Chromatographic evaluation

The two chiral stationary phases, DEABA and DDEBB, where evaluated by chromatography of a series of racemates (Fig. 3). Under comparable conditions, chromatography of the same compounds was performed also on the Kromasil CHI-DMB and CHI-TBB columns. The retention factors (k) and the selectivity factors (α) from resolution of the racemates **5**–**19** are shown in Table 1. The two new phases do not retain the compounds as much as the TBB and DMB phases, but the DDEBB phase mostly gives larger retention factors than the DEABA phase. For almost all the racemates, except for supidimide, suprofen and the two amines **18** and **19**, larger selectivity factors were obtained on the DEABA phase than on the DDEBB phase, the only phase resolving ketamine, **19**. As compared to the results obtained from chromatography of the racemates **5**–**19** on the TBB

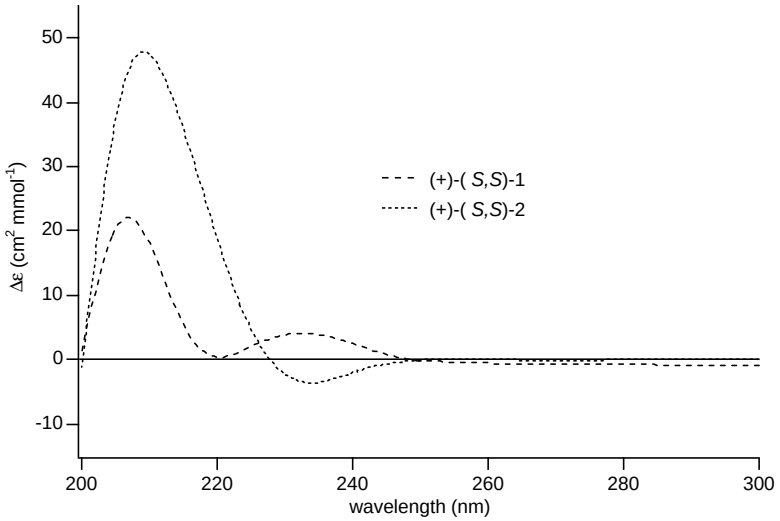

Fig. 4. CD spectra of (+)-(*S*,*S*)-**1** and (+)-(*S*,*S*)-**2**.

Table 1
Chromatographic resolution of racemates **5**–**19**

Compound	TBB		DMB		DEABA		DDEBB	
	k_1	α	k_1	α	k_1	α	k_1	α
5	1.97	1.26	2.22	1.14	1.13	1.34	1.58	1.00
6[a]	23.6	1.59	29.3	1.75	7.01	2.84	10.4	1.19
7	11.8	1.61	13.8	1.61	4.47	2.11	6.07	1.14
8	11.7	1.00	15.0	1.06	3.79	1.24	5.05	1.00
9[a]	2.55	1.12	0.97	1.27	0.95	1.70	0.96	1.55
10	2.94	1.16	3.03	1.00	1.45	1.28	2.26	1.00
11	3.26	1.18	3.50	1.06	1.69	1.21	1.96	1.00
12	19.5	1.24	32.1	1.11	18.1	2.48	11.7	1.46
13[a]	8.21	1.18	11.9	1.07	6.54	2.09	4.25	1.25
14[a]	36.6	1.70	26.8	1.06	17.4	1.82	7.73	1.99
15[b]	1.06	1.21	2.85	1.00	0.72	1.08	1.06	1.07
16[b]	2.03	1.14	2.87	1.09	1.35	1.16	2.04	1.07
17[b]	3.15	1.43	4.73	1.08	1.97	1.06	2.71	1.09
18[c]	30.2	1.00	43.2	1.50	15.5	1.00	15.6	1.22
19[c]	0.71	1.00	0.92	1.00	0.52	1.00	0.61	1.08

Mobile phase: 5% 2-propanol in hexane. See Section 2.3 for chromatographic conditions.
[a] The eluted enantiomers were collected and analysed by CD spectroscopy.
[b] Mobile phase: 5% 2-propanol and 0.1% acetic acid in hexane.
[c] Mobile phase: 5% 2-propanol and 0.1% triethylamine in hexane.

and DMB phases, the α-values were larger for quite half of the racemates on the DDEBB phase and almost all of the racemates, except for the amines, were better resolved on the DEABA phase. The sulfoximides, **12** and **13**, were well separated on the two new phases, particularly **12** which gave an α-value of 2.48 on the DEABA phase. Fig. 5 shows the chromatograms of **12** resolved on the DMB, DEABA and DDEBB phases.

It has previously been shown that for most of the benzodiazepinones the DEABA phase gives larger selectivity factors than the TBB and DMB phases [8]. The only benzodiazepinone that is better resolved on the DDEBB phase is oxazolam, having no hydroxyl group. The reverse orientation of the amide group in the selectors probably effects the direction by which the analyte interacts with the selector. This determines the direction of the hydroxyl group of an interacting benzodiazepinone in relation to the selector structure and will probably cause the less selective interaction observed with the selector of the DDEBB phase as compared to that of the DEABA phase. Moreover, with respect to oxazolam the difference in enantioselectivity of the two selectors is not as remarkable as with the benzodiazepinones containing a hydroxyl group.

The effect from the difference in the structure of the two selectors on the enantiomeric elution order has been studied for eight of the racemates, assuming the same behaviour for structural analogues. The chromatographic peaks were collected from the analytical runs and fractions were analyzed by CD spectroscopy. Fig. 6 shows the CD spectra of the two collected enantiomers of oxazolam. On the DEABA phase, b was eluted prior to a, whereas the DDEBB phase gave the opposite order of elution. For all the racemates investigated, marked with a star in Tables 1–3, corresponding results were obtained. The enantiomers first eluted from the two columns showed Cotton effects of different signs at the same wavelength in the CD spectra. Consequently, as an effect from the reversed orientation of the amide group in the two selectors, the enantiomers of the racemates are separated in opposite order of elution on the two columns, despite the same absolute configuration of the selectors. This shows that the main

Table 2
Chromatographic resolution of the benzoins **20**–**25**

Compound	TBB		DMB		DEABA		DDEBB	
	k_1	α	k_1	α	k_1	α	k_1	α
20[a]	1.63	1.18	2.13	1.06	0.70	1.33	1.21	1.33
21	1.05	1.32	1.92	1.22	0.66	1.00	0.99	1.14
22[a]	3.87	1.24	5.10	1.16	1.38	1.32	2.21	1.19
23	3.86	1.19	4.70	1.09	1.20	1.36	1.90	1.31
24	1.87	1.00	2.28	1.00	0.87	1.00	1.34	1.12
25[a]	4.72	1.16	5.53	1.10	1.27	1.30	2.10	1.27

Mobile phase: 1% 2-propanol in hexane. See Section 2.3 for chromatographic conditions.
[a] The eluted enantiomers were collected and analysed by CD spectroscopy.

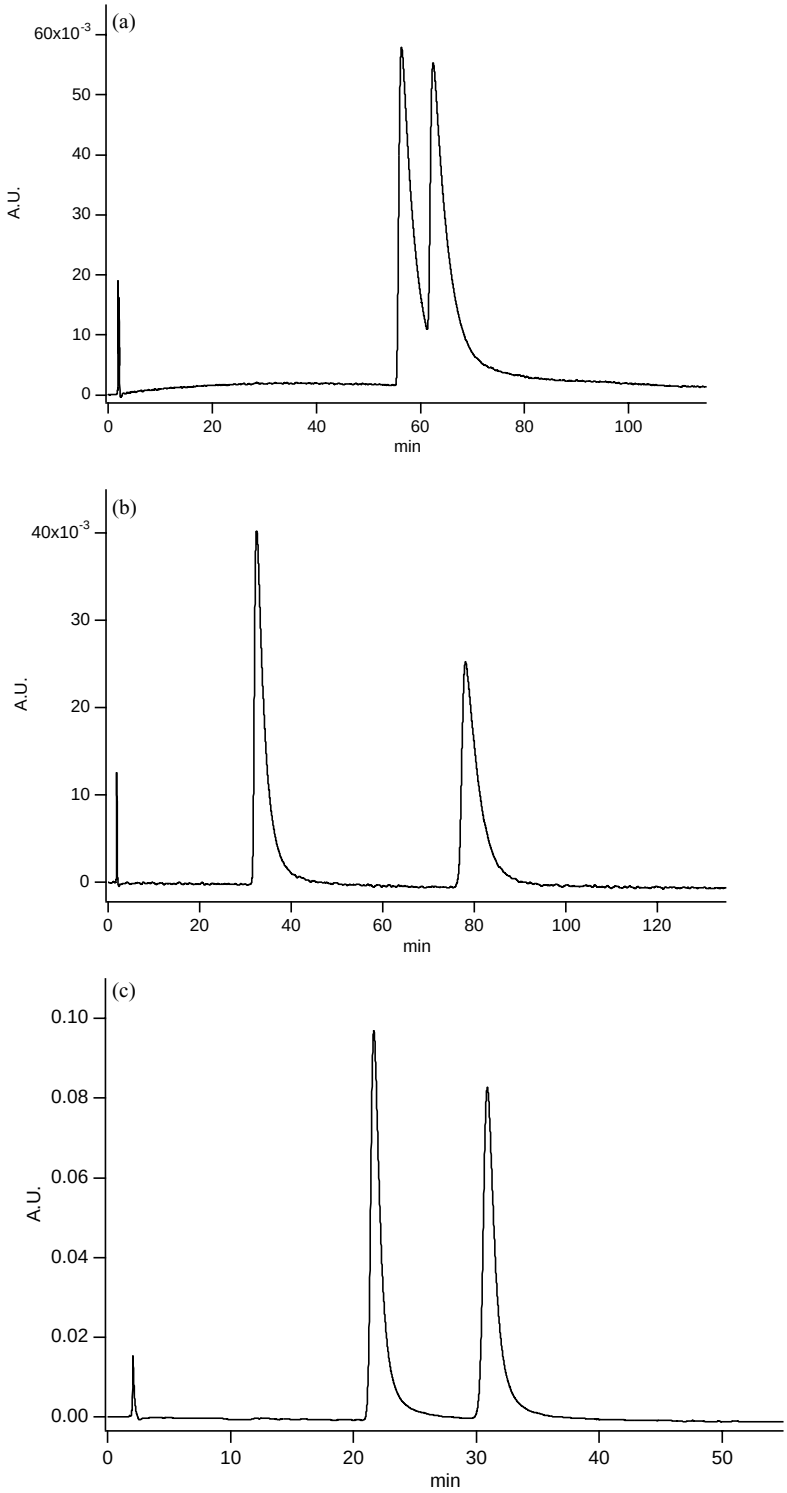

Fig. 5. Chromatographic resolution of **12** on (a) the DMB phase; (b) the DEABA phase; and (c) the DDEBB phase. Mobile phase: 5% 2-propanol in hexane. See Section 2.3 for chromatographic conditions.

contribution to enantiodiscrimintion of the analytes comes from interaction with the amide group of the selectors, confirming previous results obtained for the DEABA phase [8].

By chromatography of a series of halogenated benzoins (Fig. 7) on the columns with the TBB, DMB, DEABA and DDEBB phases (Table 2) both electronic and steric effects from the analyte can be studied. First, the reduced selectivity for the *ortho*-substituted compounds **21** and **24** is evident. This most likely reflects the increased steric bulk near the hydrogen bonding groups in the analytes. Moreover,

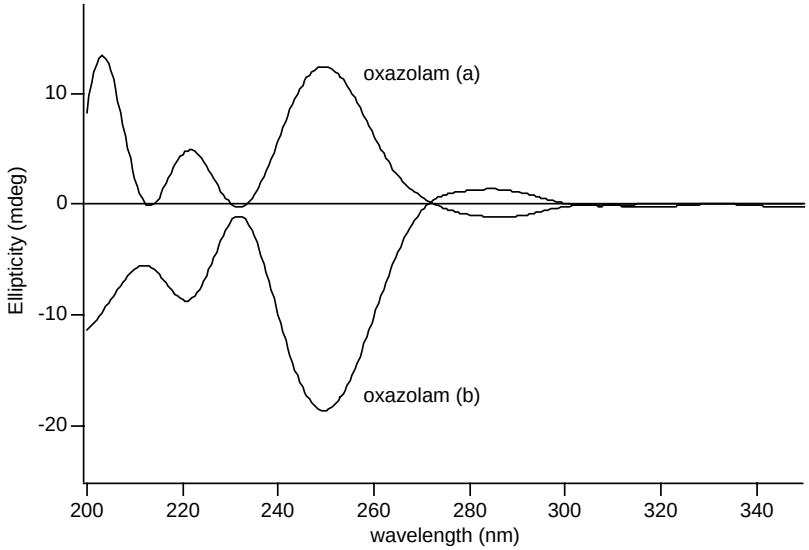

Fig. 6. CD spectra of eluted oxazolam enantiomers.

the inductive effect caused by the fluorine substituents in the *meta*-substituted benzoin **22** is reflected in the generally higher affinity shown as compared to the parent benzoin **20**. Similar results have been obtained from chromatography of the same series of benzoins on two different BSA fragment columns [12]. For all the benzoins studied, smaller retention factors were obtained on the DEABA and DDEBB phases than on the TBB and DMB phases. In contrast to the other halogenated benzoins on both new phases, analyte **23** is better resolved on the DEABA phase than the unsubstituted

Table 3
Resolution of the carbamoylated amino acids **26**–**31** with the use of different retention modifiers in hexane

Mobile phase/column	**26**		**27**		**28**[a]		**29**		**30**		**31**	
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
A/DEABA												
85/15/0.1	1.57	1.55	0.94 (0.94)	1.59 (1.89)	1.04	1.47	2.01	1.36	1.68	1.31	2.57	1.11
90/10/0.1	2.54	1.62	1.46 (1.46)	1.67 (1.99)	1.58	1.53	3.32	1.42	2.91	1.36	5.12	1.13
95/5/0.1	6.25	1.81	2.99 (3.35)	2.03 (2.22)	3.59	1.62	8.39	1.48	8.12	1.42	17.7	1.15
A/DDEBB												
85/15/0.1	1.83	1.27	1.23	1.36	1.18	1.32	2.55	1.17	2.48	1.14	3.56	1.20
90/10/0.1	2.96	1.31	1.95	1.42	1.80	1.38	4.19	1.20	4.31	1.17	6.87	1.25
95/5/0.1	7.35	1.45	4.24 (4.67)	1.74 (1.58)	4.18	1.52	11.3	1.28	12.3	1.24	25.0	1.36
B/DEABA												
30/70/0.1	3.03	1.25	1.62 (1.79)	1.22 (1.37)	2.09	1.11	3.12	1.07	4.45	1.16	2.96	1.00
40/60/0.1	4.41	1.27	2.30 (2.52)	1.24 (1.40)	2.98	1.14	4.65	1.08	7.11	1.18	4.68	1.00
50/50/0.1	6.94	1.30	3.55 (3.83)	1.26 (1.44)	4.74	1.16	7.74	1.11	12.8	1.18	8.18	1.00
B/DDEBB												
30/70/0.1	4.94	1.45	2.85 (3.15)	1.86 (1.68)	3.21	1.67	6.03	1.28	8.93	1.28	5.53	1.20
40/60/0.1	7.53	1.48	4.75	1.74	4.92	1.74	9.48	1.30	15.7	1.28	9.41	1.24
50/50/0.1	11.8	1.54	7.30	1.79	7.50	1.78	15.1	1.33	29.3	1.22	16.3	1.27
C/DEABA												
50/50/0.1	2.01	1.35	1.17 (1.17)	1.45 (1.70)	1.38	1.32	2.07	1.18	3.34	1.22	1.70	1.00
60/40/0.1	3.08	1.38	1.68 (1.76)	1.47 (1.66)	2.02	1.34	3.15	1.20	5.50	1.24	2.93	1.00
70/30/0.1	5.98	1.43	3.13 (3.35)	1.57 (1.76)	3.91	1.38	6.32	1.24	12.6	1.24	6.64	1.00
C/DDEBB												
50/50/0.1	4.89	1.35	3.50	1.59	3.35	1.49	5.29	1.21	7.59	1.22	4.40	1.13
60/40/0.1	8.06	1.42	5.35 (5.68)	1.65 (1.55)	5.66	1.56	8.84	1.22	13.5	1.24	7.71	1.17
70/30/0.1	13.0	1.42	8.72 (9.40)	1.73 (1.60)	9.22	1.60	16.1	1.25	27.6	1.28	16.1	1.21

A: hexane/2-propanol/acetic acid, B: hexane/methyl *tert*-butyl ether/acetic acid, C: hexane/ethyl acetate/acetic acid.
[a] The eluted enantiomers were collected and analysed by CD spectroscopy.

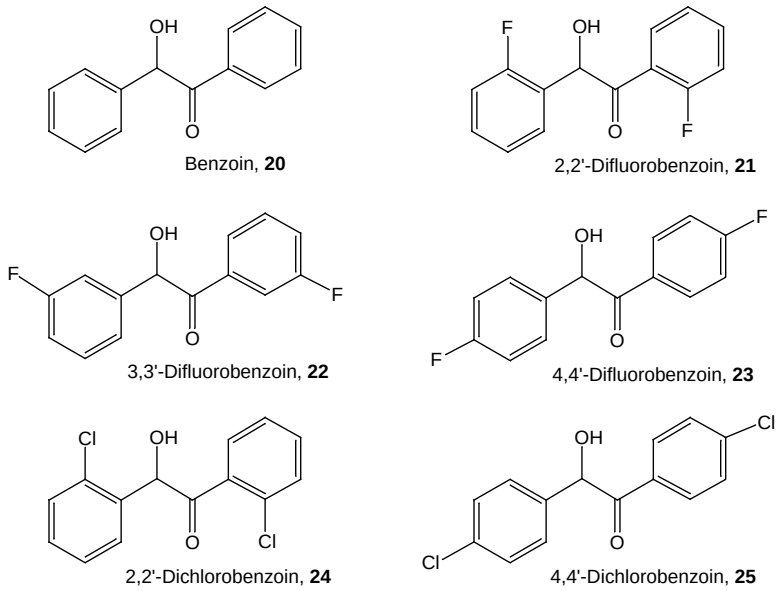

Fig. 7. Halogenated benzoins chromatographed on the DEABA and DDEBB phases.

benzoin **20**. Analyte **20** and the *para*-halogenated benzoins **23** and **25** were resolved with higher α-values on the two new phases than on the TBB and DMB phases.

For some carbamoyl derivatives of amino acids (Fig. 8) the influence of the retention modifier has been studied on the DEABA and DDEBB phases (Table 3). The retention modifiers in hexane were 2-propanol, methyl *tert*-butyl ether and ethyl acetate. All the carbamoylated amino acids, **26**–**31**, showed higher enantioselectivity on the DEABA and the DDEBB phases with the mobile phase 5% 2-propanol in hexane than on the DMB and TBB phases under corresponding conditions. On the DEABA phase higher selectivity factors were obtained with 2-propanol as retention modifier than with methyl *tert*-butyl ether or ethyl acetate. This is probably due to less contribution from achiral interactions on the stationary phase using a protic retention modifier. The selectivity factor is dependent on both chiral and achiral interaction on the stationary phase [13–15]. A protic retention modifier can form strong hydrogen bonds with, for example, remaining silanol groups on the sorbent, preventing some achiral interaction with the analytes [16]. Larger contribution from chiral interactions will give higher selectivity factors. The effects from different retention modifiers on the DEABA phase are shown by Fig. 9a. Extrapolation of the *k*-values

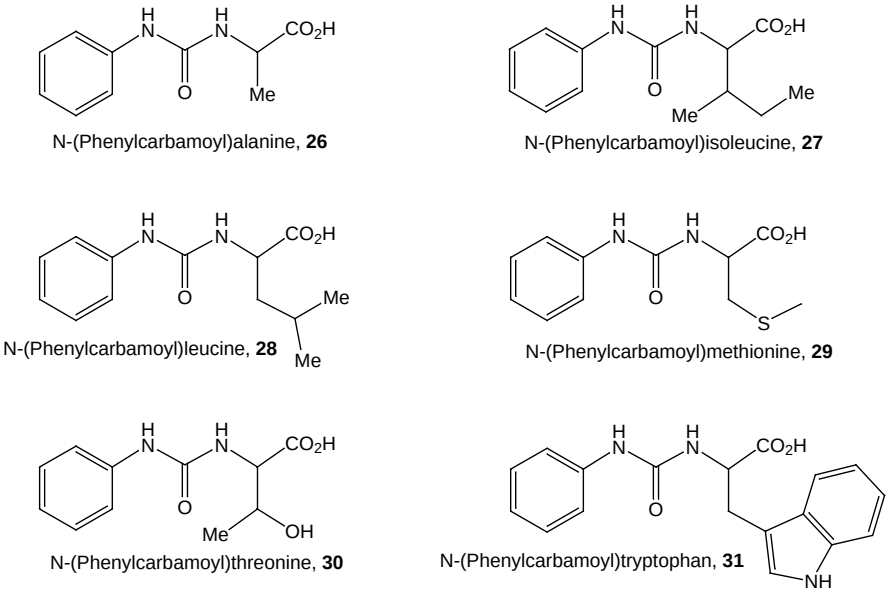

Fig. 8. Carbamoylated amino acids chromatographed on the DEABA and DDEBB phases.

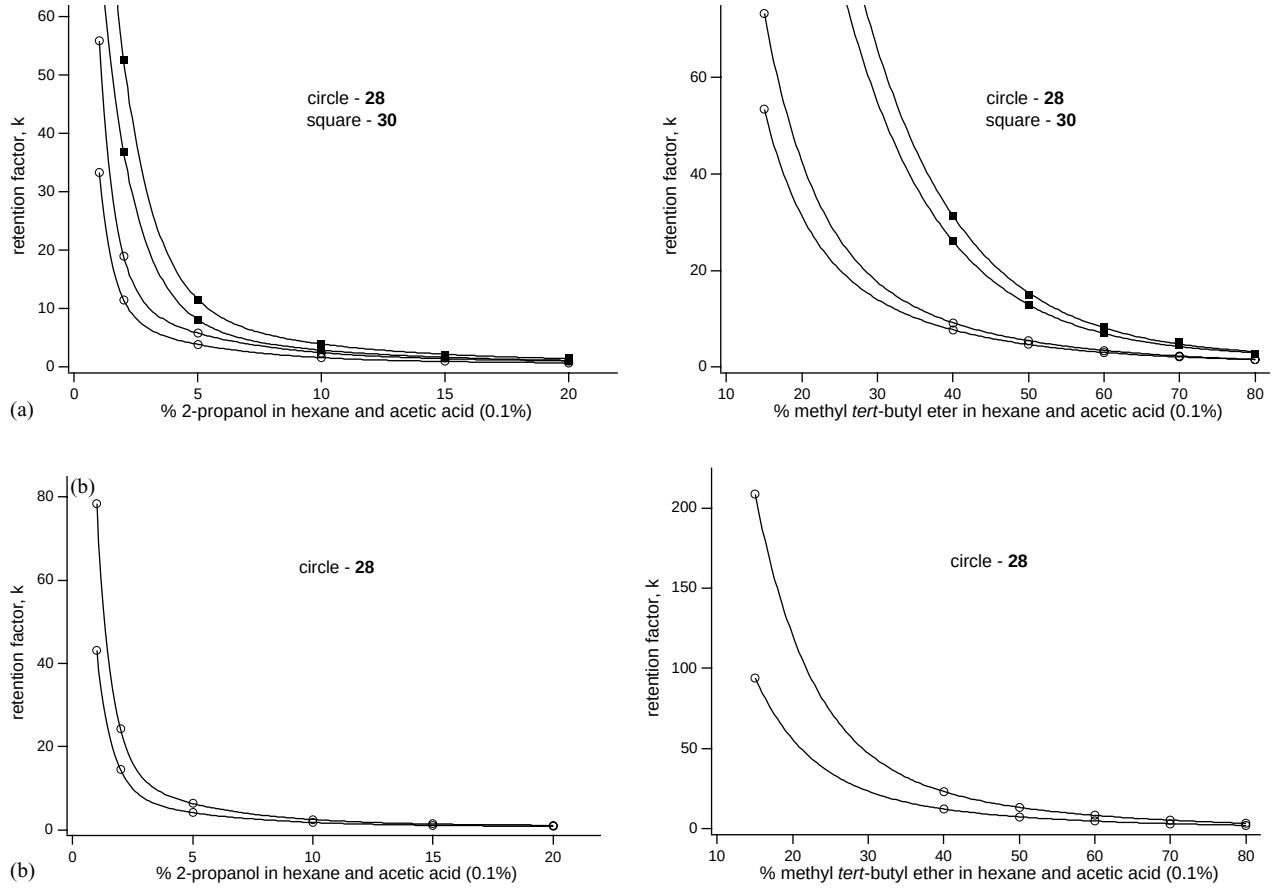

Fig. 9. Variation of k and α with the content of retention modifier 2-propanol or methyl *tert*-butyl ether on (a) the DEABA phase; and (b) the DDEBB phase.

for **28** to zero concentration of the retention modifier shows a larger k_2/k_1 ratio in the system with the protic solvent than in the system with methyl *tert*-butyl ether. This indicates that the two systems differ in the contribution of chiral interaction, most likely due to a more efficient covering of achiral sites by the protic modifier. In the system with 2-propanol as retention modifier, the difference in *k*-values between the two carbamoylated amino acids **28** and **30** is smaller than in the system with methyl *tert*-butyl ether. In the latter system, free silanol groups in the sorbent can strongly interact with the hydroxyl group of **30**, resulting in a larger difference in *k*-values between the two analytes.

One noticeable example of the effects obtained from changing the retention modifier is the separation of **31** on the DEABA phase, where resolution was lost when going from 2-propanol to either of the aprotic retention modifiers, probably due to a larger contribution from achiral interactions in the latter systems. Remarkably, this is not the case on the DDEBB phase, where the enantioselectivity seems to be less dependent on the retention modifier. For many of the carbamoylated amino acids, the largest selectivity factors were obtained using methyl *tert*-butyl ether as retention modifier on the DDEBB phase. Since the amount of selector covering the silica was almost the same for both selectors, this might be an effect from the structure of the polymer bound to the silica. Fig. 9b illustrates some of the effects from changing retention modifier on the DDEBB phase. Extrapolation of the *k*-values for **28** to zero concentration of retention modifier shows, as expected, that the *k*-values are higher in the system with methyl *tert*-butyl ether than in the system with 2-propanol. However, like for many of the carbamoylated amino acids (Table 3), the k_2/k_1 ratio for **28** is larger when methyl *tert*-butyl ether rather than the protic solvent is used as retention modifier on the DDEBB phase. Methyl *tert*-butyl ether might, by interaction with the sorbent, modify the polymer structure to become enantioselectively more favourable than in the system with 2-propanol. Many factors [17], for example, the reversed orientation of the amide group, could influence the two selectors to form slightly different polymer structures which show different solvation behaviour in the mobile phase.

On the DEABA phase all four diastereomers of the isoleucine **27** were separated (Fig. 10a). On the DDEBB phase, the two pairs of diastereomers, I and II, were partly resolved, giving three peaks in the chromatogram (Fig. 10b), whereas the DMB and TBB phases failed to resolve the diastereomers.

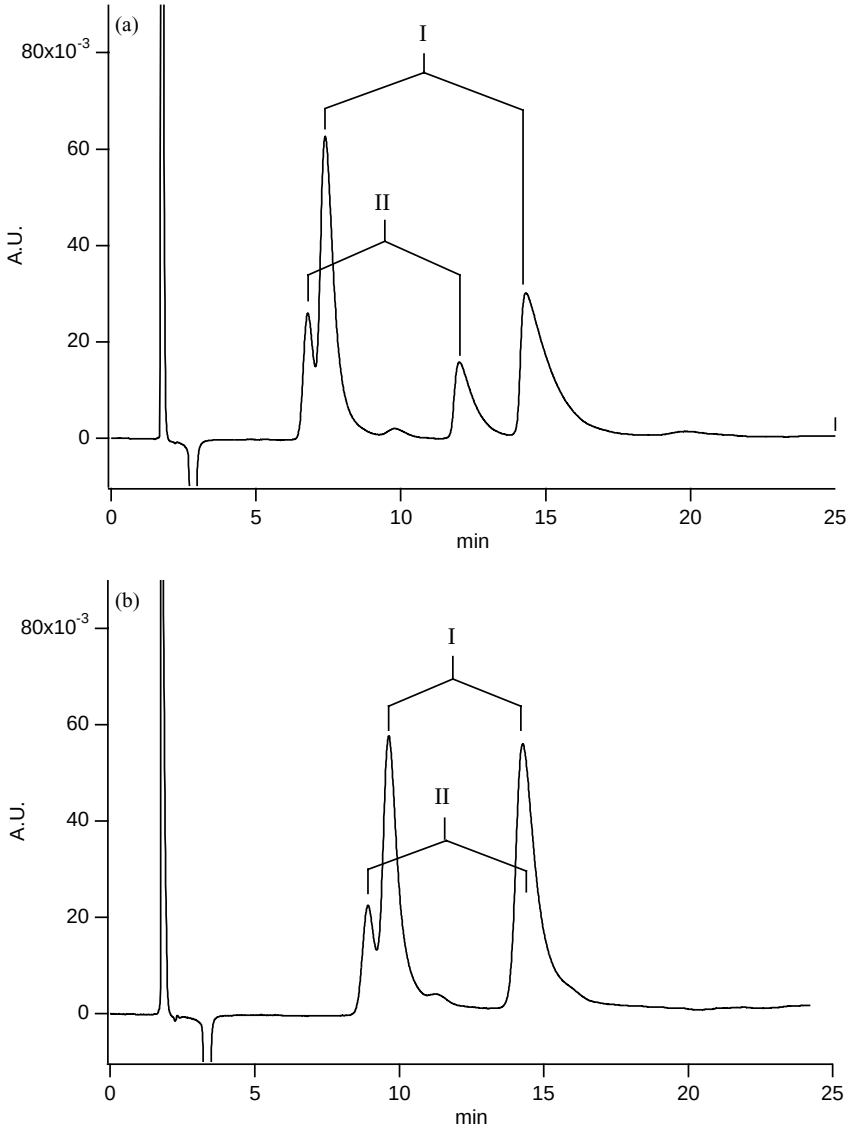

Fig. 10. Chromatographic resolution of the diastereomers of **27** on (a) the DEABA phase; and (b) the DDEBB phase. Mobile phase: 5% 2-propanol in hexane. See Section 2.3 for chromatographic conditions.

4. Conclusions

For many of the racemates investigated, the two new chiral stationary phases gave improved enantioselectivity as compared to the commercially available Kromasil CHI-TBB and CHI-DMB phases. The DEABA phase resolved more racemates than the DDEBB phase, but the two phases complement each other. As an effect from the reversed orientation of the amide group in the two selectors, the enantiomers of the racemates investigated were separated in opposite order of elution on the two columns. This confirms previous studies of the importance of hydrogen bonds between the selectors and the analyte in the enantiodiscriminating interaction. The study of the influence of different retention modifiers on the resolution of the carbamoylated amino acids suggests a difference in the polymer structure and solvation properties of the two phases, as an effect from the mutually reversed orientation of the amide group.

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