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Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol

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Abstract

Direct separation of enantiomeric amines using mainly N-benzyloxycarbonylglycyl-L-proline (L-ZGP) but also N-benzyloxycarbonylglyclglycl-L-proline (L-ZGGP) as the chiral counter ion in methanol is described. The solid phase was Hypercarb porous graphitic carbon. Several amines of pharmacological interest (e.g., alprenolol, sotalol, terbutaline, promethazine and trimipramine) were separated with high enantioselectivity ($\alpha = 1.16–1.98$) using L-ZGP and L-ZGGP as chiral selectors. In accordance with ion-pair chromatography, the retention of the enantiomeric amines was found to increase with increasing concentration of the anionic form of L-ZGP. Addition of a base (sodium hydroxide or an alkylamine) in excess of L-ZGP gave rise to a decrease in retention and enantioselectivity. The enantioselective retention was also affected by adding 2-propanol or acetonitrile to the mobile phase.

1. Introduction

Chiral ion-pair chromatography was first reported in the late 1970s. Yoneda [1] used chiral counter ions in aqueous mobile phases for the resolution of optically active metal complexes. A chiral zwitterion (L-leucyl-L-leucyl-L-leucine) in a phosphate buffer was used as eluent by Knox and Jurand [2] for the separation of racemic tryptophan and glycylphenylalanine. A chiral counter ion present in an organic mobile phase promotes a high degree of ion-pair formation and has been used successfully for the chiral separation of amines and acids [3]. (+)-10-Camphorsulphonic acid and N-benzyloxycarbonylglycyl-L-proline (L-ZGP) as counter ions in dichloromethane permitted chiral separations of, e.g., amino alcohols [4–6]. Enantioselective retention of carboxylic acids and "N-blocked amino acids" has been obtained by addition of quinine and analogues as chiral counter ions to dichloromethane [7]. Modified silica supports were used as the achiral solid phase. Introduction of porous graphitic carbon as the solid phase made it possible to apply the chiral counter ions L-ZGP [8,9] and (−)-2, 3:4,6-di-O-isopropylidene-2-keto-L-gulonic acid [10] in polar mobile phases (e.g., methanol) for the separation of enantiomeric amines. The possibility of excluding hazardous organic solvents has great

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importance in routine analyses. Further, exchanging dichloromethane for methanol or methanol–water mixtures allows the direct injection of biological fluids and facilitates integration of the chiral separation system in coupled-column chromatography for bioanalysis.

The aim of this study was to extend the earlier investigations on L-ZGP in methanol for chiral separations of amines. The influence of the mobile phase composition (total concentration of L-ZGP, concentration of anionic form of L-ZGP, addition of alkylamines and organic modifiers) on the retention and enantioselectivity was studied in detail. A model for the retention of the enantiomers based on the distribution of diastereomeric ion pairs to the solid phase is proposed.

The chiral separation of several amines of pharmacological interest, e.g., β-blocking agents and α-adrenoreceptor stimulating agents, is presented in order to illustrate the potential of L-ZGP as a chiral selector in methanol.

2. Experimental

2.1. Apparatus

The pumps used were a Model 114 M (Beckman Instruments, Fullerton, CA, USA) and a ConstaMetric III (LDC, Riviera Beach, FL, USA). A SpectroMonitor 3100 detector (Milton Roy, Riviera Beach, FL, USA) was set at 270 nm. A Rheodyne (Berkeley, CA USA) Model 7120 injector with a 20-μl loop was used. The column and the solvent reservoir were kept at 17.00 ± 0.1°C using a type 02 PT 923 TC waterbath thermostat (HETO, Birkerød, Denmark).

2.2. Chemicals

All chemicals were of analytical-reagent grade unless indicated otherwise, and were used without further purification. Methanol, 2-propanol and acetonitrile were obtained from Merck (Darmstadt, Germany), sodium hydroxide pellets from Eka Nobel (Surta, Sweden), triethylamine (TEA) and dimethyloctylamine (DMOA) (95% purity) from Janssen (Beerse, Belgium), tributylamine (TBA) from Fluka (Buchs, Switzerland), tripentylamine (TPA) and trihexylamine (THA) (golden label) from Eastman Kodak (New York, USA), N-benzyloxycarbonylglycyl-L-proline from Nova Biochem (Leufärlfingen, Switzerland) and N-benzyloxycarbonylglycylglycyl-L-proline from Sigma (St. Louis, MO, USA). The solutes used are listed in Table 1.

The Hypercarb column (100×4.7 mm I.D., 7 μm) was purchased form Shandon (Astmoor, UK).

2.3. Chromatographic technique

The mobile phase was prepared by dissolving the counter ion, L-ZGP or L-ZGGP (Fig. 1), in methanol. The base, sodium hydroxide or an alkylamine, was added to the mobile phase using a 50 m*M* stock standard solution in methanol.

The column was washed with 250 ml of pure methanol before introducing a new mobile phase. After the breakthrough of the counter ion, as recorded by the UV trace, the mobile phase was recirculated. Less than $2 \cdot 10^{-5}$ mol of L-ZGP was adsorbed on ca. 1 g of the solid phase, Hypercarb.

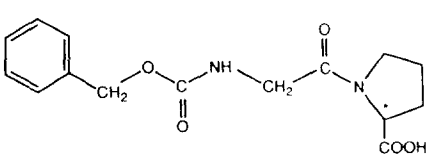
Stock standard solutions were prepared by dissolving the solutes in methanol. Before injection, these solutions were diluted at least tenfold with the mobile phase, giving a solute concentration of 0.2 mmol/l.

3. Results and discussion

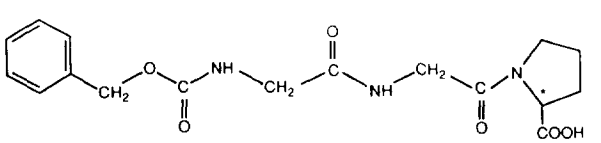
Previous studies of the separation of enantiomeric amines and acids by the ion-pair technique have been based on the addition of a chiral acid or an amine to an organic mobile phase of low polarity [3]. The acid–base interaction (Eq. 1) in inert solvents of low polarity gives rise to the formation of ion pairs or "hydrogen bonded ion pairs" [11]. The chromatographic resolution of the enantiomers is due to differences in ion-pair formation in the organic phase or distribution of the diastereomeric ion pairs between the organic

Table 1
Solutes studied

Entry No.	Compound	Source
1	(*R*,*S*)-Alprenolol · HCl	Astra Hässle
2	(*R*,*S*)-1-(2-Allyl-4-hydroxyphenoxy)-3-isopropyl-amino-2-propanol(*p*-hydroxy alprenolol)	Astra Hässle
3	(*R*,*S*)-Oxprenolol	Astra Hässle
4	(*R*,*S*)-1-(4-Allyloxyphenoxy)-3-isopropylamino-2-propanol (*p*-Oxprenolol)	Astra Hässle
5	(*R*,*S*)-Metoprolol	Astra Hässle
6	(*R*,*S*)-Atenolol	Astra Hässle
7	Pafenolol	Astra Hässle
8	(*R*,*S*)-Sotalol	Bristol Meyers Squibb
9	(*R*,*S*)-Isoprenaline	Apotek Bolaget
10	(*R*,*S*)-Terbutaline	Astra Draco
11	(*R*,*S*)-Salbutamol	Glaxo
12	(*R*,*S*)-Mepivacaine	Astra Pain Control
13	(*R*,*S*)-Ropivacaine	Astra Pain Control
14	(*R*,*S*)-Bupivacaine	Astra Pain Control
15	(*R*,*S*)-Promethazine	Kabi Pharmacia
16	(*R*,*S*)-N-Hydroxyethylpromethazine	Kabi Pharmacia
17	(*R*,*S*)-Trimipramine	Rhône-Poulenc Rorer
18	(*R*)-Alprenolol · HCl	Astra Hässle
19	(*S*)-Alprenolol · HCl	Astra Hässle
20	(*R*)-Sotalol	Bristol Meyers Squibb
21	(*S*)-Sotalol	Bristol Meyers Squibb
22	(*R*)-Terbutaline	Astra Draco
23	(*S*)-Terbutaline	Astra Draco



N-Benzyloxycarbonyl-glycyl-*L*-proline (*L*-ZGP)



N-Benzyloxycarbonyl-glycyl-glycyl-*L*-proline (*L*-ZGGP)

Fig. 1. Structures of counter ions.

mobile phase (org) and the adsorbing stationary phase (stat):

$$(R)\text{-HA}_{org} + (S,R)\text{-B}_{org} \Leftrightarrow (R)\text{-A}^-\text{:}(S)\text{–HB}^+_{org},$$
$$(R)\text{-A}^-\text{:}(R)\text{–HB}^+_{org} \Leftrightarrow (R)\text{-A}^-\text{:}(S)\text{–HB}^+_{stat},$$
$$(R)\text{-A}^-\text{:}(R)\text{–HB}^+_{stat} \quad (1)$$

where (*R*)-HA is a chiral acid, (*S*,*R*)-B is the enantiomeric pair of an amine and (*R*)-A$^-$:(*S*)-HB$^+$ and (*R*)-A$^-$:(*R*)-HB$^+$ are the corresponding diastereomeric ion pairs between the chiral acid and the (*S*)- and (*R*)-enantiomers of the amine, respectively.

Organic mobile phases of low polarity promote a high degree of ion-pair formation, but may give rise to disturbing side-reactions (association of the ion pairs). Thus, achiral and also several competing enantioselective equilibria

may decrease or even ruin the enantioselective separation [3]. Chiral ion-pair chromatography in polar mobile phases with medium or high dielectric constants, e.g., methanol, has a great advantage as unfavourable association processes of the diastereomeric ion pairs and their components are less pronounced in these solvent. Mayer discussed, in a review [12], the solvent effects on ion-pair equilibria. Obviously, the ion association behaviour of ionic species in many solvent systems cannot be adequately explained by the electrostatic model (electrostatic interaction, solvent's dielectric constant). Methanol, which has strong electrophilic properties, can solvate the nucleophilic ions by hydrogen bonding. The free energy of solvation depends also on the ion size and influences the ion-pair association constant.

In methanol, acids and bases are partly dissociated and the amount of charged protolytes depends on the degree of protolysis. Coulombic attraction between the charged enantiomer of the amine and the anionic counter ion give rise to the formation of a neutral complex (ion pair) that can be selectively distributed to the solid phase:

$$(R)\text{-}A^-_{mob} + (S,R)\text{-}HB^+_{mob} \Leftrightarrow (R)\text{-}A^-\text{:}(S)\text{–}HB^+_{stat},\ (R)\text{-}A^-\text{:}(R)\text{–}HB^+_{stat} \quad (2)$$

3.1. Retention model

The ion pair and its components can be adsorbed on the surface of the solid support. The adsorption is reversible and the assumption is made that the surface has a limited capacity (K^0) of one type of adsorption sites (A_S) [13]:

$$K^0 = [A_S] + [\text{L-ZGPA}_S] + [(R)\text{-BA}_S] + [(S) - \text{BA}_S] + [\text{L-ZGP:}(R)\text{-HBA}_S] + [\text{L-ZGP:}(S)\text{-HBA}_S] \quad (3)$$

where $[A_S]$ are the available free adsorption sites, $[\text{L-ZGPA}_S]$ are sites occupied by free L-ZGP, $[(R)\text{-BA}_S]$ and $[(S)\text{-BA}_S]$ are sites occupied by the enantiomers of the solute and $[\text{L-ZGP:}(R)\text{-BA}_S]$ and $[\text{L-ZGP:}(S)\text{-BA}_S]$ are those occupied by their ion pairs with L-ZGP. The ion pair of L-ZGP$^-$ and sodium ion is not included in the K^0 expression in order to simplify the retention model. Since an increase in sodium hydroxide concentration gave an increase retention time, it was reasonable to believe that the ion-pair formation between sodium ion and L-ZGP$^-$ was small and did not affect the retention of the enantiomeric amines to a great extent.

The adsorption equilibria for the retention of the ion pairs and the uncharged form of the acid and amine is given by

$$\text{L-ZGP}_{mob} + A_S \overset{K_{\text{L-ZGP}}}{\Longleftrightarrow} \text{L-ZGPA}_S \quad (4)$$

$$(R)\text{-B}_{mob} + A_S \overset{K_{(R)\text{-B}}}{\Longleftrightarrow} (R)\text{-BA}_S \quad (5)$$

$$\text{L-ZGP}^-_{mob} + (R)\text{-HB}^+_{mob} \overset{K_{\text{L-ZGP:}(R)\text{-HB}}}{\Longleftrightarrow} \text{L-ZGP}^-\text{:}(R)\text{-HB}^+A_S \quad (6)$$

The chiral selector, L-ZGP, is added in uncharged form. The pK_a value of L-ZGP in methanol was estimated to be 7–9 [14], which should result in negligible protolysis of L-ZGP. Ionization of L-ZGP was obtained by neutralization with sodium hydroxide. Thus, the concentration of the charged form of the selector, i.e., the counter ion (L-ZGP$^-$), is equal to the concentration of sodium hydroxide added to the mobile phase. The free uncharged L-ZGP is consequently the total concentration of L-ZGP in the mobile phase minus the added concentration of sodium hydroxide.

When the solute concentration is so low that $[(R)\text{-BA}_S] + [(S)\text{-BA}_S] + [\text{L-ZGP:}(R)\text{-HBA}_S] + [\text{L-ZGP:}(S)\text{-HBA}_S] \ll [A_S] + [\text{L-ZGPA}_S]$, the adsorption isotherm is linear and the capacity factor for the enantiomers is given by

$$k' = \frac{qK_{\text{L-ZGP:}(R)\text{-HB}}K^0[\text{L–ZGP}^-]_{mob}}{1 + K_{\text{L-ZGP}}[\text{L-ZGP}]_{mob}} \quad (7)$$

The retention can be controlled by the counter ion (L-ZGP$^-$) concentration and the concentration of uncharged acid (L-ZGP).

The enantiomers of alprenolol showed low retention times ($k' = 0.5$) and no enantioselectivity when using an acidic mobile phase pre-

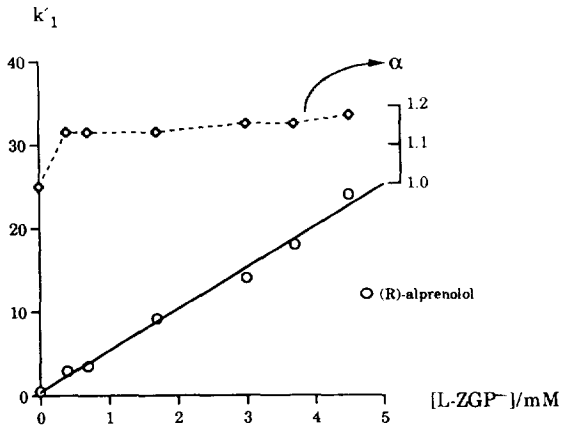


Fig. 2. Enantioretention of (*R*)- and (*S*)-alprenolol related to the anionic form of counter-ion concentration. Solid phase: Hypercarb. Mobile phase: 5 m*M* L-ZGP and *X* m*M* sodium hydroxide in methanol ($0.4 \leq X \leq 4.5$).

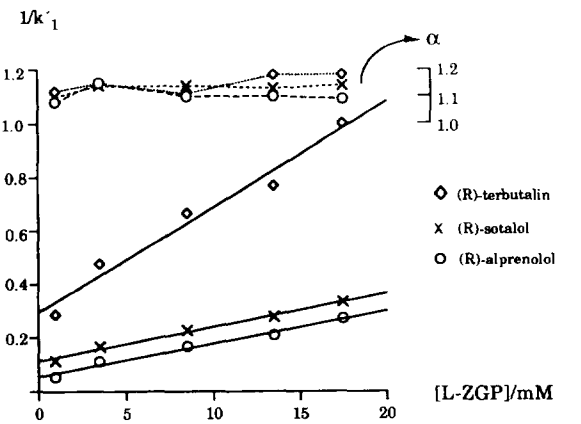


Fig. 3. Influences of the uncharged concentration of counter ion on the enantioretention of amines. Solid phase: Hypercarb. Mobile phase: *X* m*M* L-ZGP and 1.5 m*M* sodium hydroxide in methanol ($2.5 \leq X \leq 19$).

pared by dissolving L-ZGP in methanol (Fig. 2). Clearly, the counter ion concentration generated by the protolysis of L-ZGP was too low to promote enantioselective ion-pair retention. The linear increase in capacity factors (Fig. 2) with increase in the amount of sodium hydroxide in the mobile phase, i.e., increasing concentration of the charged form of the selector, L-ZGP$^-$, is in accordance with ion-pair chromatographic principle (Eq. 7).

A competition for the limited adsorption capacity of the stationary phase by the diastereomeric ion pairs and the uncharged selector, L-ZGP, would give rise to reduced retention for the enantiomeric amines. Inversion of Eq. 7 gave a linear relationship on plotting the inverse of the capacity factor ($1/k'$) versus the concentration of the uncharged form of L-ZGP (Fig. 3). Values for the adsorption constant of the uncharged L-ZGP can be obtained, since the ratio between the slope and intercept is equal to $K_{\text{L-ZGP}}$. The adsorption constants for L-ZGP obtained from the plots for terbutaline and sotalol were in a good agreement, 7.5 and 8.7, respectively. The lower value of $K_{\text{L-ZGP}}$, 4.5, estimated from the plot for alprenolol is probably due to a large uncertainty in the low intercept.

Adjustment of the retention times for the enantiomers by the concentration of either the counter ion (L-ZGP$^-$) or the uncharged acid (L-ZGP) is uncomplicated as it does not affect the enantioselectivity to a significant extent (Figs. 2 and 3).

3.2. Mobile phase amine

An amine is often added to the mobile phase in order to reduce retention times and improve the peak shapes when chromatographing amines on silica-based solid phases [15]. It is generally believed that the improved peak symmetry is due to deactivation of unfavourable adsorption sites on the solid phase by the mobile phase amine [15]. A beneficial effect on the peak symmetry in the presence of a mobile phase amine has also been observed when using porous graphitic carbon, Hypercarb, as the adsorbing phase [8]. Hence, it was of interest to exchange sodium hydroxide for an alkylamine in the mobile phase in order to improve the peak shape of enantiomeric amines. The acid–base reaction between L-ZGP and the alkylamine will promote the necessary counter ion, L-ZGP$^-$. However, the presence of the alkylamine will shorten the retention time and the denominator of the k' expression will be expanded to $1 + K_{\text{L-ZGP}^-}[\text{L-ZGP}^-] + K_{\text{Am}^+\text{L-ZGP}^-}[\text{Am}^+][\text{L-ZGP}^-]$.

Table 2
Influence of alkylamines on enantioselective retention and peak symmetry

Solute	NaOH			Alkylamine													
				TEA			TBA			TPA			THA			DMOA		
	k'_1	a	*asf*	k'_1	a	*asf*	k'_1	a	*asf*	k'_1	a	*asf*	k'_1	a	*asf*	k'_1	a	*asf*
Alprenolol	17	1.10	6.5	14	1.11	5.0	13	1.12	5.5	11	1.14	3.6	7.2	1.14	3.1	9.7	1.16	2.7
Sotalol	6.8	1.10	5.0	7.5	1.11	3.2	7.0	1.12	3.8	6.6	1.14	3.5	4.5	1.14	3.2	5.6	1.11	2.9
Terbutaline	2.4	1.15	2.8	2.7	1.14	1.6	2.7	1.14	3.3	2.5	1.14	2.5	1.6	1.16	1.7	2.0	1.15	1.8

Solid phase: Hypercarb. Mobile phase: 2.5 m*M* L-ZGP and 1.5 m*M* sodium hydroxide or alkylamine in methanol. TEA = triethylamine; TBA = tributylamine; TPA = tripentylamine; THA = trihexylamine; DMOA = dimethyloctylamine; asf = asymmetry factor of the first-eluted enantiomer measured on the baseline.

$K_{Am^+L\text{-}ZGP^-}$ represents the formation constant of the ion pair between the alkylammonium ion (Am^+) and L-ZGP$^-$. The lowest retention of the enantiomeric amines was obtained when trihexylamine (THA) or dimethyloctylamine (DMOA) was added to the mobile phase (Table 2). It is reasonable to assume that the more hydrophobic amines have a higher affinity for the porous graphitic carbon phase and are more effective as competing agents for the limited adsorption capacity of the solid phase. Unfortunately, the improvement in peak shape was modest even for the hydrophobic mobile phase amines THA and DMOA.

The effect of the concentration of the mobile phase amine on the chiral separation was investigated using triethylamine in a solution of 2.5 m*M* L-ZGP in methanol [Table 3(a)]. The retention of the enantiomeric amines was highest when using a triethylamine concentration that was about the same as that of the chiral selector. As discussed above, a low concentration of the mobile phase amine will not neutralize the selector to a sufficient extent, and accordingly the counter ion concentration will be too low. However, a large excess of the mobile phase amine should be avoided as it will result in an alkaline solution where the enantiomeric amines are mainly present as uncharged amines. A decrease in capacity factor was observed at 6.0 m*M* or higher of triethylamine in the mobile phase, indicating that the free amine is less retained (Eq. 5) than the diastereomeric ion pairs (Eq. 6). The enantioselectivity decreased with increasing amounts of mobile phase amines as a result of the retention of the free enantiomeric amines being achiral [Table 3(b)].

3.3. Organic solvent

The elution power of the mobile phase, 5.0 m*M* L-ZGP and 3.0 m*M* sodium hydroxide in methanol, was modified by addition of a second organic solvent to the mobile phase. 2-Propanol and acetonitrile had a profound effect on the retention and enantioselectivity (Tables 4 and 5).

Table 3
Influence of triethylamine on enantioselective retention

Conditions	Solute	[TEA] (m*M*)									
		0.5		1.0		1.5		2.0		2.3	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
(a) [TEA] < [L-ZGP]	Alprenolol	5.5	1.13	10	1.10	14	1.11	17	1.12	23	1.13
	Sotalol	3.4	1.12	5.5	1.12	7.5	1.11	9.2	1.11	10	1.10
	Trimipramine	11	1.18	18	1.19	24	1.20	29	1.20	33	1.19
	Terbutaline	1.3	1.11	2.0	1.13	2.7	1.14	3.3	1.16	3.8	1.15
	Ropivacaine	2.0	1.03	3.0	1.02	3.17	1.12	–	–	5.1	1.03
		[TEA] (m*M*)									
		3.0		6.0		9.0		18			
		k'_1	α	k'_1	α	k'_1	α	k'_1	α		
(b) [TEA] > [L-ZGP]	Alprenolol	23	1.09	14	1.08	12	1.07	8.8	1.05		
	Sotalol	8.3	1.10	5.6	1.06	4.5	1.07	3.4	1.05		
	Trimipramine	15	1.09	8.6	1.03	7.7	1.0	7.0	1.0		
	Terbutaline	3.7	1.15	2.6	1.16	2.1	1.11	1.6	1.09		
	Ropivacaine	4.9	1.0	4.8	1.0	4.7	1.0	4.5	1.0		

Solid phase: Hypercarb. Mobile phase: 2.5 m*M* L-ZGP and *X* m*M* TEA in methanol.

Table 4
Influence of 2-propanol on enantioselective retention

2-Propanol (%, v/v)	Solute							
	Alprenolol		Terbutaline		Trimipramine		Promethazine	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
0	14	1.15	3.3	1.19	22	1.21	70	1.28
20	–	–	2.4	1.13	17	1.29	–	–
40	11	1.13	2.6	1.18	14	1.38	45	1.44
60	12	1.11	3.8	1.18	11	1.58	37	1.58
80	33	1.02	12	1.13	9.4	1.76	33	1.78
94	–	–	–	–	6.0	1.98	21	2.02

Solid phase: Hypercarb. Mobile phase: 5.0 m*M* L-ZGP and 3.0 m*M* sodium hydroxide and *X*% (v/v) 2-propanol in methanol.

Table 5
Influence of acetonitrile on enantioselective retention

MeOH:AcN (v/v)	Solute							
	Alprenolol		Sotalol		Trimipramine		Terbutaline	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α
100:0	17	1.10	6.8	1.10	28	1.16	2.4	1.15
50:50	13	1.07	5.1	1.14	9.9	1.21	3.4	1.21
20:80	33	1.0	16	1.09	15	1.29	16	1.21
6:94	19	1.14	17	1.10	4.1	1.27	64	1.03

Solid phase: Hypercarb. Mobile phase: 2.5 m*M* L-ZGP and 1.5 m*M* sodium hydroxide in methanol–acetonitrile.

The capacity factors for the hydrophobic tricyclic amines (trimipramine and promethazine) decreased whereas the enantioselectivity improved with increasing concentration of 2-propanol in the mobile phase. A different effect with the addition of 2-propanol was observed for the enantiomers of alprenolol and terbutaline. The capacity factor decreased with 20–40% 2-propanol in the mobile phase but increased at higher concentrations of the alcohol. The enantioselectivity for alprenolol and terbutaline deteriorated at 80% 2-propanol in the mobile phase.

There was no clear trend in the changes in retention and enantioselectivity on addition acetonitrile to the mobile phase (Table 5). Clearly, a high concentration of acetonitrile in the mobile phase is favourable for the enantioselectivity of alprenolol and trimipramine but not for the separation of (*R*,*S*)-terbutaline. The best separation of trimipramine and promethazine was observed at 94% acetonitrile in the mobile phase (Fig. 4), whereas the separation factor for sotalol seems less affected by the content of acetonitrile.

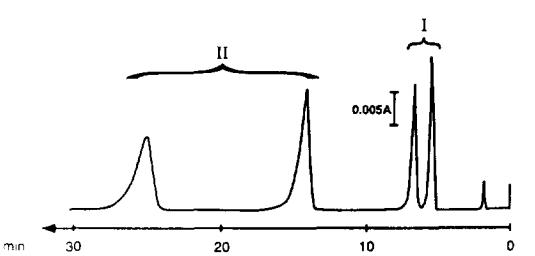


Fig. 4. Separation of racemic trimipramine (I) and promethazine (II). Solid phase: Hypercarb. Mobile phase: 2.5 m*M* L-ZGP and 1.5 m*M* sodium hydroxide in methanol–acetonitrile (6:94, v/v).

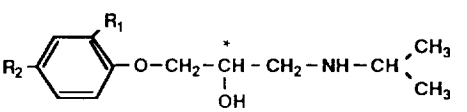
Further studies on this kind of separation system might improve the understanding of the resolution of enantiomeric ions by a chiral counter ion in mixed organic solvents as the mobile phase.

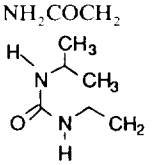
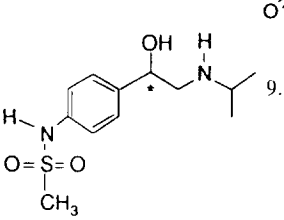
3.4. Structure and stereoselectivity

Previous studies using L-ZGP as chiral counter ion in dichloromethane have shown that intermolecular interactions (e.g., hydrogen bonding) between the counter ion and the enantiomeric solutes are of great importance for the enantioselectivity [8,16]. This study showed that the enantioselectivity for amino alcohols was significantly lower when using methanol (α = 1.08–1.17) as mobile phase solvent than with dichloromethane (α = 1.20–1.90] [8,16]. A detailed discussion of the effect of the organic solvent and the solute structure on the enantioselectivity is not possible at present. It would require a knowledge of the ion-pair formation constants and also the adsorption constants of the ion pair and the free amine of all solutes in both solvent systems. However, several enantiomeric amines with different hydrogen bonding properties were studied in order to establish the influence of molecular structure on the enantioselectivity when using L-ZGP as chiral counter ion in methanol (Tables 6–9).

Alprenolol and *p*-hydroxyalprenolol (**1** and **2**), lacking an O or an N atom in the *ortho* or *para* substituent in the aromatic ring, gave high enantioselectivies (α = 1.15 and 1.17) in methanol as compared with the other β-blockers (Table 6). The retention of *p*-hydroxyalprenolol (**2**) as an ion pair with L-ZGP was significantly higher than that for alprenolol (**1**). The same retention order between alprenolol and *p*-hydroxyalprenolol was observed using alkaline methanol without L-ZGP as the mobile phase (k'_{alp} = 8.2, $k'_{p\text{-OH-alp}}$ = 11.0), i.e. retention of the β-blockers as free amines. However, almost the same enantioselectivity was observed for alprenolol and *p*-hydroxyalprenolol

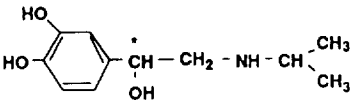
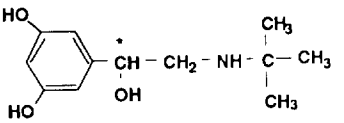
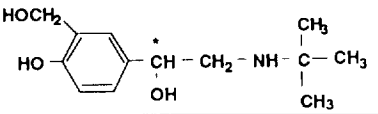
Table 6
Solute structures and enantioselective retention for β-receptor blocking agents



Entry no.	Solute	R_1	R_2	k'_1	α
1	Alprenolol	CH_2=$CHCH_2$	H	22	1.17
2	*p*-Hydroxyalprenolol	CH_2=$CHCH_2$	OH	38	1.15
3	Oxprenolol	CH_2=$CHCH_2O$	H	34	1.09
4	H 74/16	H	CH_2=$CHCH_2O$	50	1.10
5	Metoprolol	H	$CH_2OCH_2CH_2$	18	1.09
6	Atenolol	H	NH_2COCH_2	13	1.09
7	Pafenolol[a]	H		21	1.09
8	Sotalol		9.8	9.8	1.13

Solid phase: Hypercarb. Mobile phase: 5 m*M* L-ZGP and 4.5 m*M* NaOH in methanol.
[a] Mobile phase: 5 m*M* L-ZGP and 3 m*M* NaOH in methanol.

Table 7
Solute structures and enantioselective retention for bronchodilators

Entry No.	Solute	Structure	k'_1	α
9	Isoprenaline		3.9	1.0
10	Terbutaline		3.6	1.17
11	Salbutamol		3.2	1.09

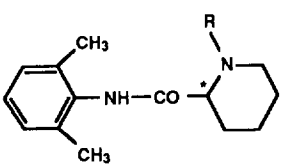
Solid phase: Hypercarb. Mobile phase: 5 m*M* L-ZGP and 4.5 m*M* NaOH in methanol.

(Table 6). Hence it is reasonable to assume that the *p*-hydroxyl group only affects the distribution properties of the diastereomeric ion pairs and is not involved in the interaction with the counter ion. This is also supported by the fact that the same enantioselectivity was obtained for compounds **4–7** with different hydrogen-bonding substituents in the *para* position to the alkanol amine chain. However, the structure of the substituent in the *ortho* position, i.e., closer to the chiral centre, had a significant effect on the enantioselectivity (compounds **1** and **3**).

Despite the low enantioselectivity obtained using L-ZGP in methanol as the mobile phase, it permitted the stereoselective determination of hydrophilic amino alcohols, e.g., sotalol (Table 6), terbutaline (Fig. 5) and salbutamol (Table 7). No enantioselective retention was found for the structurally related α-adrenoreceptor stimulating agent isoprenaline (Table 7).

The retention for enantiomeric local anaesthetics (**9–11**) (Table 8) could be correlated with the hydrophobicity (number of methylene groups) of the alkyl chain attached to the amine function in accordance with ion-pair retention theory [17], whereas the length of the alkyl chain did not affect the enantioselectivity. The slight decrease in separation factor for ropivacaine and

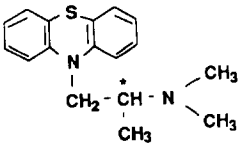
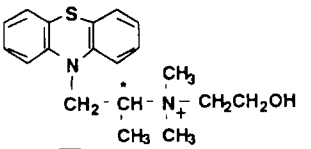
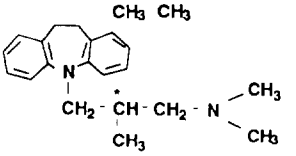
Table 8
Solute structures and enantioselective retention for local anaesthetics



Entry No.	Solute	R	k'_1	α
12	Mepivacaine	CH_3	2.32	1.11
13	Ropivacaine	$CH_2CH_2CH_3$	2.78	1.08
14	Bupivacaine	$CH_2CH_2CH_2CH_3$	3.09	1.08

Solid phase: Hypercarb. Mobile phase: 15 m*M* L-ZGP and 9 m*M* NaOH in methanol–2-propranol (90:10, v/v).

Table 9
Solute structures and enantioselective retention for other amines

Entry No.	Solute	Structure	k'_1	α
15	Promethazine		58	1.23
16	N-Hydroxyethyl promethazine		47	1.0
17	Trimipramine		21	1.19

Solid phase: Hypercarb. Mobile phase: 5 m*M* L-ZGP and 4.5 m*M* NaOH in methanol.

bupivacaine compared with mepivacaine is probably due to steric effects.

Polyaromatic tertiary amines were enantioselectively retained by L-ZGP (Table 9). The chiral separation of trimipramine was as good as that of promethazine; the former has one more methylene group between the asymmetrical carbon and the nitrogen. However, the retention time of promethazine, containing a sulphur atom with free electron pairs, was higher. Previous studies have shown that the retention on porous graphitic carbon with hexane in the mobile phase is sensitive to changes in the solute electron density caused by the electron-donating or -withdrawing ability of substituents and the number and position of the electrondense bonds in the solute [18].

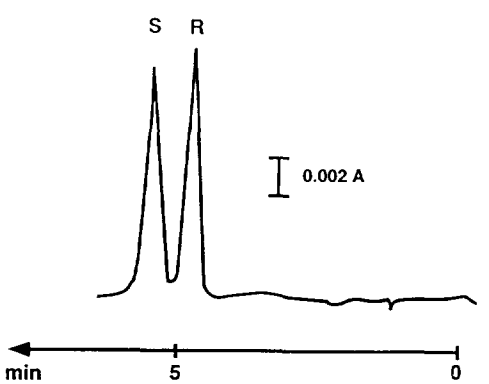


Fig. 5. Separation of racemic terbutaline. Solid phase: Hypercarb. Mobile phase: 5.0 m*M* L-ZGP and 3.0 m*M* sodium hydroxide in methanol.

The influence of the counter-ion structure on the stereoselective retention of β-receptor blocking agents and an α-adrenergic stimulation agent, terbutaline, is presented in Table 10. Exchange of L-ZGP for L-ZGGP resulted in additional hydrogen bonding functions in the counter ion (Fig. 1). Lower retention was observed for the enantiomeric amines when L-ZGGP was the chiral counter ion, probably owing to the lower distribution of the ion pair caused by stronger competition of the uncharged form of L-ZGGP. The effect on enantioselectivity of introducing a glycyl moiety into the counter ion is highly dependent on the solute structure. The use of L-ZGGP instead of L-ZGP gave improved enantioselectivity for metoprolol and pafenolol, whereas no change was found for alprenolol, atenolol and oxprenolol. Several β-blocking agents could be separated in one chromatographic system using L-ZGGP as counter ion, as shown in Fig. 6. The same counter ion was applied to the determination of enantiomeric impurity in (*S*)-alprenolol (Fig. 7). For sotalol

Table 10
Counter-ion structures and enantioselective retention

Entry No.	Solute	Counter ion			
		L-ZGP		L-ZGGP	
		k'_1	α	k'_1	α
5	Metoprolol	13	1.08	9.2	1.16
1	Alprenolol	14	1.15	8.8	1.16
6	Atenolol	9.1	1.08	5.8	1.10
2	Oxprenolol	23	1.08	14	1.09
7	Pafenolol	21	1.09	12	1.13
8	Sotalol	9.9	1.13	9.4	1.06
10	Terbutaline	2.8	1.21	2.6	1.07

Solid phase: Hypercarb. Mobile phase: 5 m*M* of the counter ion and 4.5 m*M* NaOH in methanol.

and terbutaline, with the aromatic ring bound directly to the asymmetric carbon atom, L-ZGP is the more suitable counter ion (Table 10).

4. Conclusion

N-Benzyloxycarbonylglycyl-L-proline and N-benzyloxycarbonylglycylglycyl-L-proline can be used as chiral counter ions in the reversed-phase mode with methanol-containing bases as mobile phase. A retention model based on ion-pair adsorption and competition for adsorption sites according to Langmuir isotherms is shown to be valid. The retention and enantioselectivity can mainly be controlled by the ratio of charged and uncharged chiral selector and the presence of a second organic solvent such as acetonitrile of 2-propanol. Many different chiral amine drugs belonging to several pharmacological groups, i.e., β-blocking agents, local anaesthetics, bronchodilators, antihistamines and antidepressants,

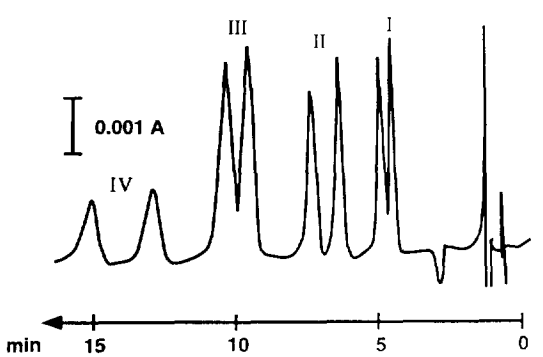


Fig. 6. Separation of β-blocking agents: I = (*R*)- and (*S*)-atenolol; II = (*R*)- and (*S*)-alprenolol; III = (*R*)- and (*S*)-oxprenolol; IV = (*R*)- and (*S*)-*p*-hydroxyalprenolol. Solid phase: Hypercarb. Mobile phase: 5.0 m*M* L-ZGGP and 3.0 m*M* sodium hydroxide in methanol.

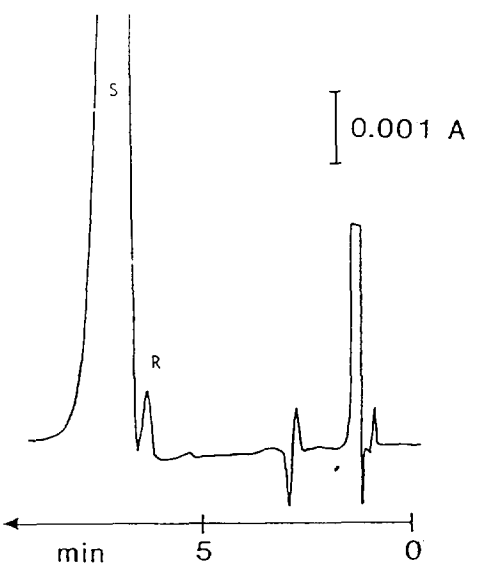


Fig. 7. Determination of enantiomeric impurity in (*S*)-alprenolol using L-ZGGP in methanol as mobile phase. Solid phase: Hypercarb. Mobile phase: 5.0 m*M* L-ZGGP and 3.0 m*M* sodium hydroxide in methanol. Solute: (*S*) and (*R*)-alprenolol (99:1).

can be separated. The relationship between chemical structure and enantioselectivity is complex and cannot easily be predicted.

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