Gas chromatographic enantiomer separation on a chiral self-associating selector

F. Betschinger, J. Libman, A. Shanzer*

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

Abstract

A novel chiral stationary phase bearing a tripodal selector is described. In gas chromatography this phase exhibits high enantioselectivity towards derivatized amino acids and moderate capability for the enantiomer separation of derivatized hydroxy acids. Due to a C_3 symmetric arrangement of three N-acyl-valine-*tert.*-butyl amide groups the tripodal selector adopts a hydrogen bonding network involving inter- and intrastrand hydrogen bonds. Thus a conformationally constrained structure is formed which is stable up to 150°C.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Amino acids; Hydroxy acids

1. Introduction

Gas chromatography based on chiral stationary phases represents a powerful tool for enantiomer analysis. However, the mechanism by which a chiral stationary phase (CSP) retains one enantiomer in preference to its antipode is still a matter of controversy [1]. Some of the difficulties derive from the often rudimentary knowledge of the conformation of the selector, especially under working conditions. This is particularly true for diamide phases, such as the most successful and most thoroughly investigated Chirasil-Val [2,3] and modifications thereof [4–9]. For these phases different association states between selector molecules [10,11], various structures and conformations of selector moieties bound to siloxane [12,13] or even an induced fit model [14] have been suggested.

In an attempt to shed light on the molecular basis

*Corresponding author.

of enantiomer separation by chiral diamide phases, we aimed at a selector molecule whose conformation would be constrained. Towards this end we assembled three N-acyl-L-valine-*tert.*-butyl amide residues (which are the most frequently used chiral moieties in diamide phases), on a tripodal anchor in order to form a selector of defined conformation by virtue of intramolecular hydrogen bond networks.

Tripodal, C_3 symmetric structures were selected as basic skeletons, because such systems have previously been shown to form intramolecular hydrogen bonding networks [15,16]. In this context, several homologous C_3 symmetric tripeptides Et-C-$\{CH_2O(CH_2)_n$–CONH–CHR–CONH–C$(CH_3)_3\}_3$, $n=1$, 2, R=iBu, sBu, iPr, Me have already been synthesized in our group. Both molecules have been found to form fully hydrogen bound networks, involving inter- and intrastrand hydrogen bonds. Due to the presence of asymmetric centers in the strands, these molecules adopted propeller-like arrangements of the preferred chiral sense. The subtle structural

difference between these two homologous tripeptides was expressed in an unexpected solvent dependence of their hydrogen bond formation. In acetonitrile, the tripeptide with a short methylene bridge (type 1, $n=1$) showed anisotropy of the C_{quart}–CH_2O protons in the ^1H NMR spectra, indicating the formation of interstrand H-bonds, but no anisotropy in chloroform solution. The tripeptide with the long methylene bridge (type 2, $n=2$) showed the opposite behavior [15].
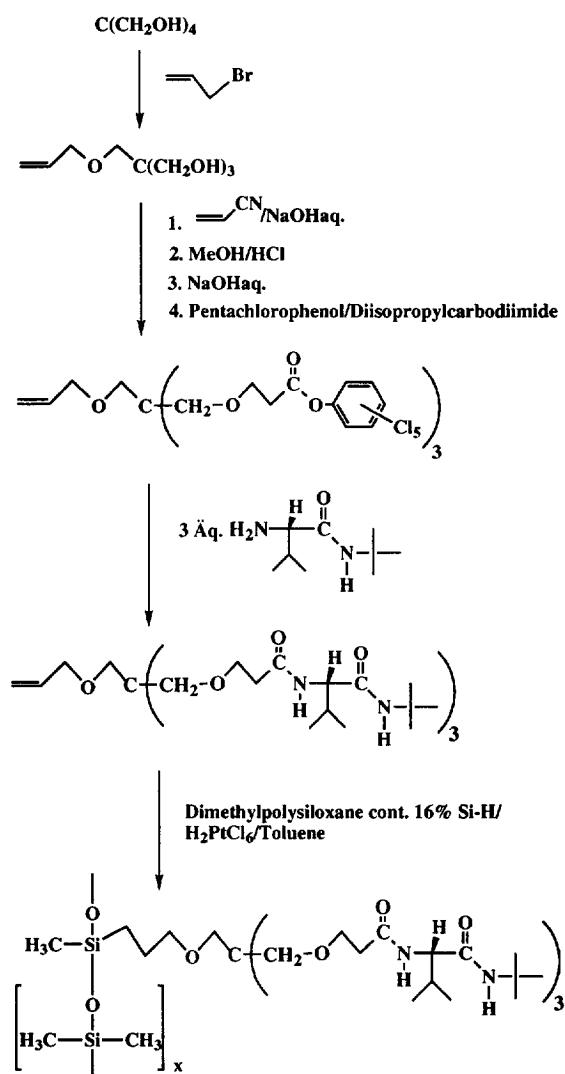

Fig. 1. Synthetic route for the preparation of the novel CSP consisting of a tripodal, C_3 symmetric tripeptide bound to a polysiloxane backbone.

We considered tripeptides of type 2 to be better suited for the preparation of our target selector than tripeptides of type 1. Type 2 tripeptides were anticipated to have sufficient conformational freedom to accommodate bulky isopropyl substituents (valine residues) within a fully H-bonded network, while sterically demanding isopropyl substituents were thought to hinder the formation of such a hydrogen bond network in type 1 tripeptides.

The preparation of the desired tripodal selector molecule bearing three N-acyl-L-valine-*tert.*-butyl amide moieties was accomplished according to the synthetic route outlined in Fig. 1. Subsequently the corresponding CSP was obtained by Pt-catalyzed hydrosilylation [17] of the selector's double bond with a dimethylpolysiloxane containing 16% Si–H groups [18] affording a viscous, colorless and clear oil (Fig. 1).

2. Experimental

2.1. Synthesis

L-Valine-*tert.*-butyl amide was prepared according to a previously published procedure for the synthesis of L-leucine-isopropyl amide from Cbz-L-leucine [19] with the modification that anhydrous CH_2Cl_2 was used instead of tetrahydrofuran as solvent in the amide formation step. Overall yield: 90%.

2.2. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OH)_3$

To a suspension of pentaerythritol (204 g, 1.5 mol) in water (215 ml), 4 M NaOH (90 ml), tetra-*n*-butyl ammonium iodide (100 mg) and 2-propanol (2 ml) were added. Subsequently allylbromide (45 ml, 0.26 mol) was added dropwise and the reaction mixture was heated overnight to 65–70°C. After filtration, the filtrate was evaporated to dryness and the crude product extracted in a Soxhlet with chloroform. The product was purified by chromatography on silica gel with $CHCl_3$–MeOH (90:10) as eluent. Yield: 34.3 g (13%).^1H NMR (C^2HCl_3): 2.78 (s, 3H, OH), 3.48 (s, 2H, C_{quart}–CH_2–O–CH_2), 3.72 (s, 6H, CH_2OH), 3.97 (m, 2H, CH_2 in allylic position), 5.22 (m, 2H, $CH_2=CH$), 5.86 (m, 1H, $CH=CH_2$).

2.3. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OCH_2CH_2CN)_3$

Pentaerythritol monoallyl ether (21.7 g, 123.1 mmol) was placed in a 1 l flask cooled by a water bath. A solution of NaOH in water (1.2 ml, 40%) was added and subsequently acrylonitrile (30 ml, 456 mmol, purified by chromatography over neutral Al_2O_3) was added dropwise such that the temperature did not exceed 30°C. The reaction mixture was stirred overnight at room temperature, neutralized by addition of 1 M HCl and extracted with ethyl acetate. The extracts were washed with water and dried over Na_2SO_4. The crude product was purified by column chromatography over silica gel with $CHCl_3$ as eluent. Yield: 35.9 g (87%).^{1}H NMR (C^2HCl_3): 2.57 (t, 6H, CH_2CN), 3.40 (s, 2H, $CH_2-O-CH_2-CH=CH_2$), 3.47 (s, 6H, $C_{quart}-CH_2O$), 3.63 (t, 6H, CH_2-CH_2-CN), 3.94 (m, 2H, CH_2 in allylic position), 5.16 (m, 2H, $CH_2=CH$), 5.83 (m, 1H, $CH=CH_2$).

2.4. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OCH_2CH_2COOCH_3)_3$

A solution of trinitrile (25.7 g, 76.6 mmol) in MeOH (200 ml) was saturated with gaseous HCl. Slowly inserting HCl was continued for 1 h after the appearance of an exothermic reaction, then the reaction mixture was refluxed for 2 h. The main part of the solvent was evaporated, the residue taken up in CH_2Cl_2, washed twice with water and dried over $MgSO_4$. The product was obtained by evaporation of the solvent. Yield: 15.9 (48%).^{1}H NMR (C^2HCl_3): 2.56 (t, 6H, CH_2COO), 3.34 (s, 2H, $CH_2-O-CH_2-CH=CH_2$), 3.37 (s, 6H, $C_{quart}-CH_2O$), 3.68 (t, 6H, CH_2-CH_2-COO), 3.92 (m, 2H, CH_2 in allylic position), 5.16 (m, 2H, $CH_2=CH$), 5.87 (m, 1H, $CH=CH_2$).

2.5. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OCH_2CH_2COOH)_3$

Trimethyl ester (15.9 g, 36.6 mmol) was dissolved in MeOH (250 ml), then 4 M KOH solution (70 ml) was added dropwise. The mixture was stirred at 50°C overnight, neutralized with 4 M HCl and subsequently the main part of the methanol evaporated. After acidification to pH 1 with 4 M HCl under ice cooling the product was extracted with diethyl ether. The extracts were washed with water, dried over $MgSO_4$ and evaporated. Yield: 14.07 g (98%) of a yellow oil.^{1}H NMR (C^2HCl_3): 2.58 (t, 6H, CH_2COOH), 3.35 (s, 2H, $CH_2-O-CH_2-CH=CH_2$), 3.41 (s, 6H, $C_{quart}-CH_2O$), 3.64 (t, 6H, CH_2-CH_2-COOH), 3.94 (m, 2H, CH_2 in allylic position), 5.21 (m, 2H, $CH_2=CH$), 5.85 (m, 1H, $CH=CH_2$), 8.95 (s(b), 3H, COOH).

2.6. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OCH_2CH_2COOC_6Cl_5)_3$

Triacid (6.0 g, 15.3 mmol), pentachlorophenol (16.3 g, 61.2 mmol) and 4-dimethyl-amino pyridine (700 mg, 5.7 mmol) were dissolved in the minimum amount of anhydrous CH_2Cl_2. At 0°C a solution of diisopropylcarbodiimide (7.72 g, 61.2 mmol) in anhydrous CH_2Cl_2 (50 ml) was added dropwise. The solution was stirred for 3 days at room temperature, the solvent was evaporated and the residue chromatographed over silica gel with n-hexane, n-hexane–CH_2Cl_2 (1:1) and CH_2Cl_2. Yield: 5.23 g (30%).^{1}H NMR (C^2HCl_3): 2.93 (t, 6H, CH_2COO), 3.42 (s, 2H, $CH_2-O-CH_2-CH=CH_2$), 3.49 (s, 6H, $C_{quart}-CH_2O$), 3.82 (t, 6H, CH_2-CH_2-COO), 3.95 (m, 2H, CH_2 in allylic position), 5.18 (m, 2H, $CH_2=CH$), 5.87 (m, 1H, $CH=CH_2$).

2.7. Preparation of $CH_2=CH-CH_2-O-CH_2-C(CH_2OCH_2CH_2CONH-CH(iPr)CONHC(CH_3)_3)_3$

Triactive ester (2.48 g, 2.18 mmol), L-valine-*tert.*-butyl amide (1.50 g, 8.71 mmol), imidazole (130 mg) and N-hydroxysuccinimide (100 mg) were dissolved in anhydrous CH_2Cl_2 (100 ml). The mixture was stirred at room temperature for 3 days. After the first and second day, triethylamine (0.44 g, 4.36 mmol) was added. The solvent was evaporated and the crude product was purified by chromatography on silica gel with $CHCl_3$, $CHCl_3$–MeOH (95:5) and $CHCl_3$–MeOH (90:10) as eluent and subsequently recrystallized from acetonitrile–$CHCl_3$ (5:1). Yield: 1.07 g (64%). ^{1}H NMR (C^2HCl_3): 0.94 (dd, 18H, CH_3 of isopropyl), 1.32 (s, 27H, *tert.*-butyl), 2.01 (m, 3H, $CH(CH_3)_2$), 2.45+2.56 (AM, 6H, CH_2COO), 3.34 (AB, 8H, $C_{quart}-CH_2-O-CH_2-CH=CH_2+C_{quart}-CH_2-O-CH_2-CH_2-COO$), 3.63

(AB, 6H, CH_2–CH_2–COO), 3.91 (m, 2H, CH_2 in allylic position), 4.23 (m, 3H, CH-iPr), 5.08 (m, 2H, CH_2=CH), 5.82 (m, 1H, CH=CH_2), 6.00 (s, 3H, NH-*tert.*-butyl), 7.48 (d, 3H, NH-CH). ^{13}C NMR (C^2HCl_3): 19.25, 19.87, 29.45, 31.98, 37.47, 45.86, 51.92, 59.74, 68.03, 69.85, 70.12, 72.90, 116.76, 135.83, 171.68, 172.28.

2.8. Preparation of the CSP by hydrosilylation of CH_2=CH–CH_2–O–$CH_2C(CH_2OCH_2$–CH_2CONH–$CH(iPr)$–$CONH$–$C(CH_3)_3)_3$

In an argon atmosphere, olefin (105 mg, 0.137 mmol), dimethylpolysiloxane containing 16% of Si–H groups (215 mg) [18], diethylaminomethyl–polystyrene (200 mg, Fluka, Buchs, Switzerland) and anhydrous toluene (50 ml) were placed into a 0.1 l, three-necked flask equipped with an argon inlet and a reflux condenser fitted with a valve. To the refluxing reaction mixture were added 1 ml volumes of a solution of the catalyst H_2PtCl_6 (50 mg) in anhydrous tetrahydrofuran (10 ml) and anhydrous 2-propanol (0.1 ml) at intervals of 4 h. After 24 h the reaction mixture was filtered and the filtrate evaporated. The oily residue was taken up in anhydrous methanol (10 ml), filtered, the filtrate evaporated. The brownish (Pt) oily residue was dissolved in the minimum volume necessary of anhydrous methanol and the solution was stored in NMR tubes (I.D. 5 mm) overnight. The Pt precipitates and the overstanding solution was evaporated, yielding a clear, viscous oil (114 mg).^{1}H NMR (C^2HCl_3): 0.07 (s, 173H, Si(CH$_3$)$_2$, 0.48 (m, 2H, Si–CH_2), 0.94 (dd, 18H, CH$_3$ of valine), 1.32 (s, 27H, *tert.*-butyl), 1.55 (m, 2H, CH_2–CH_2–Si), 2.01 (m, 3H, CH(CH$_3$)$_2$), 2.45+2.56 (AM, 6H, CH_2COO), 3.34 (AB, 8H, CH_2–O–CH_2–CH=CH_2+C_{quart}–CH_2O), 3.48 (m, 2H, O–CH_2–CH_2–CH_2–Si), 3.63 (AB, 6H, CH_2–CH_2–COO), 4.23 (m, 3H, C–H), 6.42 (s, 3H, NH-*tert.*-butyl), 7.55 (s, 3H, NH–CH).

The ratio of selector groups in the polysiloxane to dimethylsilyloxy groups was determined by ^{1}H NMR and was found to be 1:35.

3. Instrumentation

A Carlo-Erba gas chromatograph (Hofheim, Taunus, Germany), Fractovap 2150, equipped with flame ionization detection was used. The carrier gas was helium and the split ratio was set at 1:100.

3.1. Preparation of open-tubular columns

Fused-silica columns (Israel Scientific Instruments, Ramat Gan, Israel) were prepared according to the following procedures.

3.1.1. Acid leaching and rinsing

The capillary column was dynamically coated with 35% HNO_3, sealed and heated to 200°C for 10 h. The ends were opened and the column was rinsed with 2 volumes of 0.1% HNO_3. Finally, the column was dried for 4 h at 250°C under a low flow of helium.

3.1.2. Deactivation with DPTMDS (diphenyltetramethyldisilazane)

The column was dynamically coated with DPTMDS, connected to vacuum for 30 min, subsequently sealed under vacuum and heated to 200°C for 3 h. The temperature was increased to 280°C and held for another 3 h. The column ends were opened under toluene, and the column was rinsed with 2 volumes of toluene, methanol and diethyl ether, respectively.

3.1.3. Coating

Column coating was performed by the static method using a 0.4% solution of the CSP in *n*-pentane–dichloromethane (2:1).

4. Results and discussion

The structure of the tripodal selector, and particularly the occurrence of intramolecular hydrogen bonding, was tested by IR, circular dichroism (CD) and especially NMR spectroscopy in solvents of different polarity (Table 1).

The IR spectra of the tripeptide show low frequency carbonyl and NH-absorptions in chloroform, and undergo moderate blue shifts when chloroform is replaced by acetonitrile, indicating the presence of hydrogen bonds in chloroform which are weakened in acetonitrile.

NMR enabled us to establish the conformational consequences of hydrogen bonding in the tripodal

Table 1
Spectroscopic data of the tripodal selector.

	IR (cm^{-1})	NMR (ppm)	UV (nm)	CD, λ_{ex} (nm), ($\Delta\epsilon$)
Chloroform	3330 (NH)	2.45 + 2.56 (AM, CH$_2$CO)		229,(−4.50)
	1650 (amide I)	3.36 (AB, $\Delta\delta$ = 0.0196,		
	1518 (amide II)	C$_{quart}$–(CH$_2$O)$_3$)		
Acetonitrile	3381 (NH)	2.36 + 2.45 (AM, CH$_2$CO)	λ_{max} 217,	232, (−1.09)
	1654 (amide I)	3.30 (AB, $\Delta\delta$ = 0.0084,	ϵ_{max} 2130	217, (0)
	1540 (amide II)	C$_{quart}$–(CH$_2$O)$_3$)		209, (1.67)
Methanol	not determined	2.49 (t,CH$_2$CO)	λ_{max} 216,	218, (−2.58)
		3.38 (AB, $\Delta\delta$ = 0.0054,	ϵ_{max} 2250	
		C$_{quart}$–(CH$_2$O)$_3$)		

selector molecule. The spectra in C^{2}HCl$_3$, C^{2}H$_3$CN and even in C^{2}H$_3$O^{2}H show nonequivalence of the diastereotopic –C$_{quart}$–(CH$_2$O)$_3$– protons, indicating restricted conformational freedom. The extent of anisotropy decreases with increasing solvent polarity, but remains constant over a 100-fold concentration range (between 0.2 and 20 mM, measured in C^{2}HCl$_3$), indicating that it derives from intramolecular interactions.

The strong anisotropy, expressed in an AM system for the CH$_2$CO-methylene protons in C^{2}HCl$_3$ and C^{2}H$_3$CN (triplet-splitting of the four lines regularly observed for an AM system), can be explained by the presence of intrastrand hydrogen bonds from the NH of the external tert.-butyl amide group to the C=O of the internal amide. This conclusion is in line with the hydrogen bond pattern suggested for the tripeptides Et-C-{CH$_2$O(CH$_2$)$_n$–CONH–CHR–CONH–C(CH$_3$)$_3$}$_3$, n=1, 2. The interstrand hydrogen bonds, probably formed between the NH of the internal amide group and the C=O of the external tert.-butyl amide group, together with the intrastrand hydrogen bonds between the internal C=O group and external NH, strongly decrease the conformational freedom of the molecule and particularly of the –CH$_2$CO protons, resulting in the observed AM system. In C^{2}H$_3$O^{2}H solution the hydrogen bonds seem to be weakened or broken as the AM system collapses to a regular triplet.

The parallel solvent dependence of the IR and NMR spectra of the tripodal selector molecule suggests that hydrogen bonding and restricted conformational freedom (as expressed by the nonequivalence of the diastereotopic protons) are interrelated phenomena, and confirm the presence of interstrand and intrastrand hydrogen bonds in apolar and even medium polar solvents.

CD spectroscopy was used to further support this conclusion and to establish the presence of a preferred chiral sense (Table 1). In acetonitrile solution two Cotton effects were observed, a positive one at longer wavelength and a negative one at shorter wavelength. These Cotton effects derive from exciton coupling between adjacent chromophores and demonstrate chiral alignment of the amide groups. In chloroform only the negative Cotton effect could be detected due to the end-absorption of the solvent. Yet, the size of the observed Cotton effect is significantly larger than that recorded in acetonitrile, in compliance with stronger H-bonding in chloroform solution. In methanol a single Cotton effect is observed close to the absorption maximum, indicating lack of exciton coupling and lack of alignment, which is attributed to cleavage of the hydrogen bond network.

These data taken together led us to conclude that the tripodal selector molecule adopts in apolar and medium polar solvents a conformationally constrained propeller-like arrangement due to the presence of both interstrand and intrastrand hydrogen bonds. A structure compatible with the spectroscopic results is schematically illustrated in Fig. 2.

Since the polysiloxane bound selector can be regarded as solute "dissolved" in apolar siloxane, it is most likely that the bound selector adopts the same structure as the free selector. This assumption is supported by the NMR spectrum of the chiral stationary phase in C^{2}HCl$_3$, which is close to that of the free selector, except for line broadening.

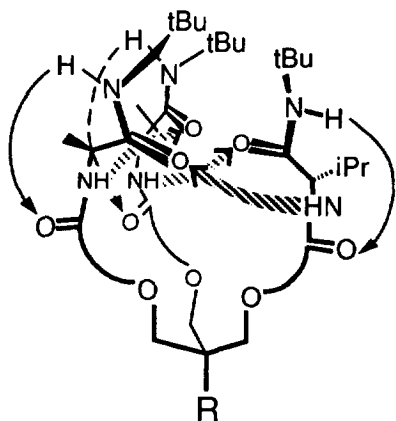In order to establish the relationship between

Fig. 2. Schematic representation of the intramolecular hydrogen bonding network of the tripodal selector (interstrand hydrogen bonds, broken arrows; intrastrand hydrogen bonds, full arrows).

structure and enantioselectivity in gas chromatography, information on the structural changes of the selector on temperature increase is of great relevance. We therefore recorded the ^{1}H NMR spectra of the selector in C^2HCl_3 at elevated temperatures, and determined the temperature dependence of the separation factors of derivatized amino acids (vide infra).

The ^{1}H NMR spectra measured in intervals of 10 degrees from 20°C to 50°C show a slight decrease of the $\Delta\delta$ values for the AB and the AM systems of the methylene protons (Fig. 2) with increasing temperature. This indicates increased conformational freedom, probably due to partial cleavage of the hydrogen bonds. Although the overall structure is still retained at 50°C, a careful examination reveals that the $\Delta\delta$ for the CH_2CO protons decreases more with increasing temperature than the $\Delta\delta$ for the $-C_{quart}-$
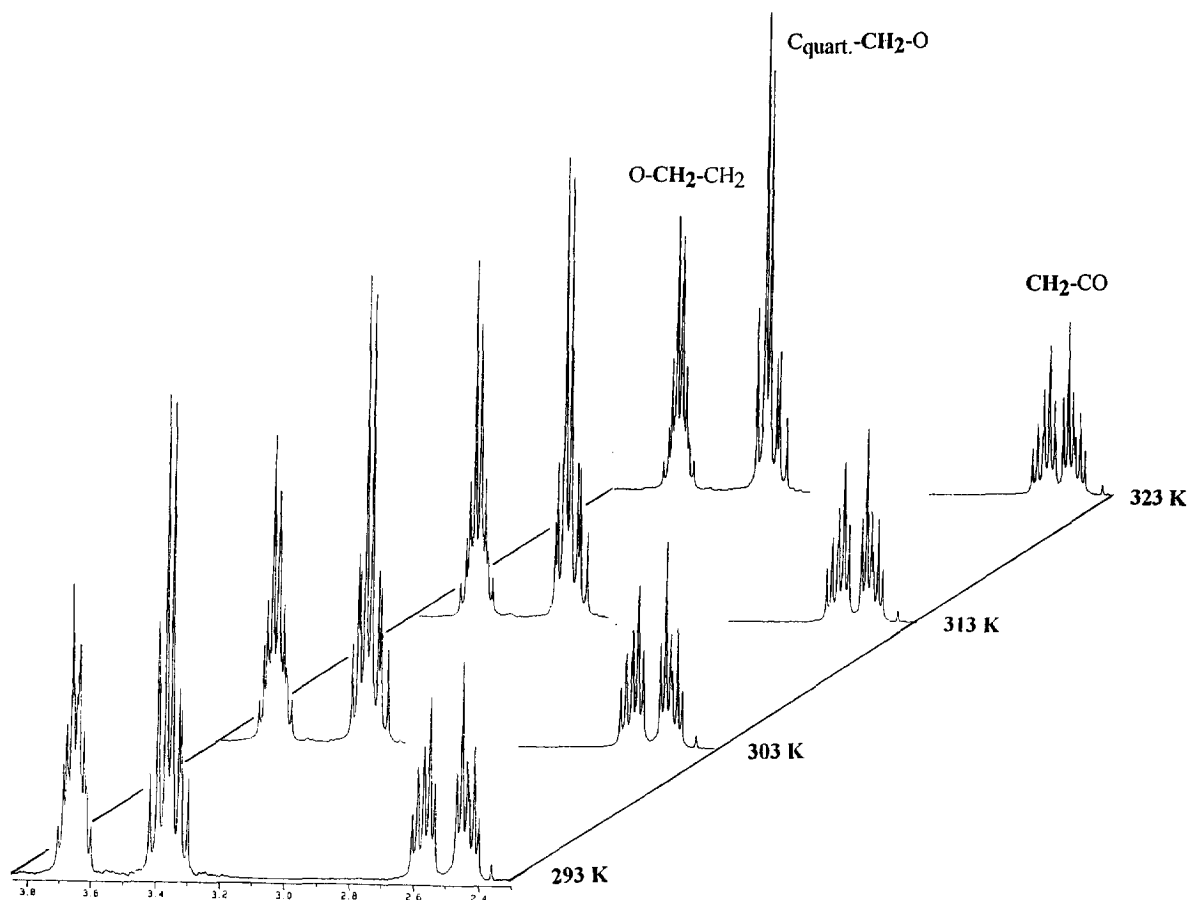

Fig. 3. Temperature dependence of the ^{1}H NMR spectra of the selector in C^2HCl_3. The range of the methylene proton signals is shown.

$(CH_2O)_3$ protons, implying that the interstrand hydrogen bonds are more stable than the intrastrand hydrogen bonds (Fig. 3).

5. Chromatography

The ability of chiral recognition of the novel CSP was investigated using derivatives of racemic amino acids and hydroxy acids. Table 2 summarizes capacity factors and separation factors for the resolution of racemic amino acids. Under the conditions given in Table 1 baseline separation was achieved for all amino acids employed except for proline, which is almost baseline resolved. The order of elution for all amino acids investigated is D before L as regularly found on phases employing L-valine-alkyl amide selectors. The separation factors observed on the CSP bearing the tripodal selector are close to the values previously reported for the single strand analog, Chirasil-Val [2], suggesting that the molecular interactions between the solutes and the selector are analogous.

Fig. 4 demonstrates the temperature dependence of enantioselectivity for the alanine, leucine and tyrosine, N-trifluoroacetyl-isopropyl esters. These plots of ln α versus $1/T$ show linear dependence for temperatures below 150°C. The Gibbs-Helmholtz parameters $\Delta_{(R,S)}\Delta H°$ and $\Delta_{(R,S)}\Delta S°$ for chiral recognition on the novel phase were calculated from these lines according to Eq. (1) [20,21].

$$\ln\alpha = \Delta_{(R,S)}\Delta H°/RT + \Delta_{(R,S)}\Delta S°/R \qquad (1)$$

The slope of the line represents the difference between the enthalpy of association of the enantiomers with the chiral stationary phase, while the intercept represents the difference of the entropy of association.

The constant $\Delta_{(R,S)}\Delta H°$ value below 150°C implies that the enantiomer separation mechanism on the novel phase, and therefore the structure of the tripodal selector is unchanged between 50°C and 150°C.

At higher temperatures a deviation from linearity to relatively higher separation factors is found. These changes of the values of $\Delta_{(R,S)}\Delta H°$ correspond to a change in the mechanism of enantiomer separation, and suggest that the hydrogen bond network of the selector is stable up to 150°C under gas chromatog-

Table 2
Enantiomer separation data of amino acids (derivatized as N,O,S-trifluoroacetylisopropyl esters, histidine as N^{im}-ethoxycarbonyl. In each case the D-enantiomer is eluted prior to the L-enantiomer, k' is given for the last eluted enantiomer).

| Racemate	Temperature (°C)	Separation factor, α	Capacity factor, k'
Ala	100	1.14(3)	2.23
Val	100	1.10(4)	3.83
Thr	100	1.09(1)	4.09
allo-	100	1.12(4)	6.07
Ile	100	1.11(1)	6.52
Ile	100	1.22(8)	8.87
Leu	100	1.02(5)	9.52
Pro	120	1.06(3)	2.85
Ser	120	1.06(0)	8.17
Cys	120	1.02(8)	9.45
Asp	140	1.09(2)	5.92
Met	140	1.07(5)	8.62
Glu	140	1.17(3)	8.74
Phe	170	1.05(1)	5.04
Tyr	170	1.07(6)	8.75
Orn	170	1.06(4)	12.47
Lys	170	1.03(2)	14.00
His	170	1.08(5)	18.34
Arg	170	1.05(4)	21.24
Trp			
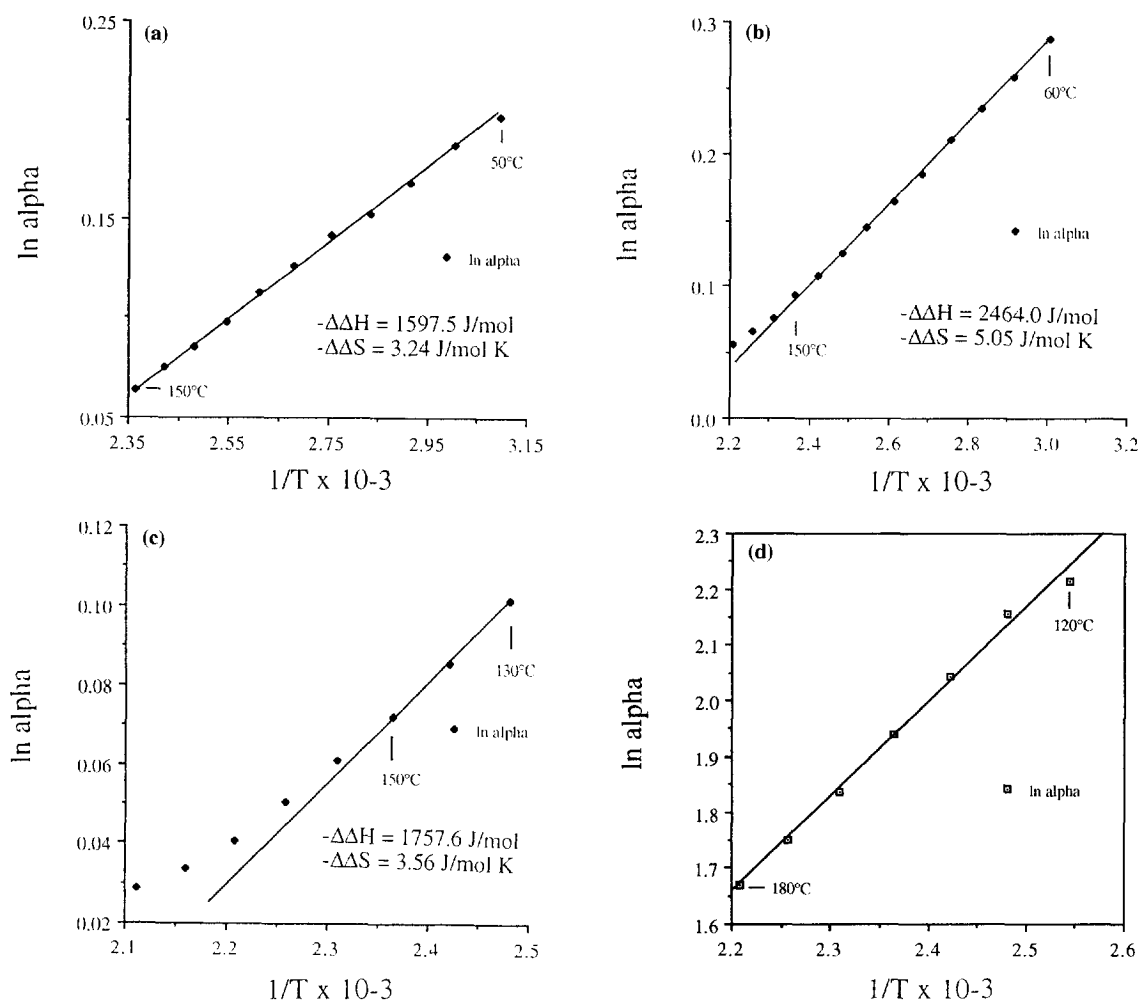
Fig. 4. Van't Hoff plots for the enantioseparation of (a) alanine, (b) leucine and (c) tyrosine N-TFA-isopropyl esters and (d) ln α of the separation of C_{14} and C_{18}.

raphy conditions. At higher temperatures the hydrogen bonds of the selector seem to be cleaved, thus providing easy access to all hydrogen bonding sites, and resulting in a change of separation mechanism and in an increase of enantioselectivity.

In order to examine the validity of the above interpretation, the separation of two simple alkanes was measured in a control experiment in the temperature range from 120°C to 180°C (cf. Fig. 4). The resulting Van't Hoff plot shows linearity. This unambiguously confirms that the deviation from linearity observed for the enantioseparation of amino acids arises from structural changes of the selector.

The separation of amino acids is summarized in Fig. 5 showing the enantioseparation of eighteen racemic N-TFA-amino acid isopropyl esters on the novel phase.

For the separation of hydroxy acids, ethyl esters were selected as derivatives, because of their increased retention compared to methyl esters, and because of their commercial availability as pure enantiomers. The resolution of these derivatives ranges from partial resolution to baseline separation (Table 3). The observed elution orders of the enantiomers are identical to those found for Chirasil-Val, and the dependence of the separation factors on
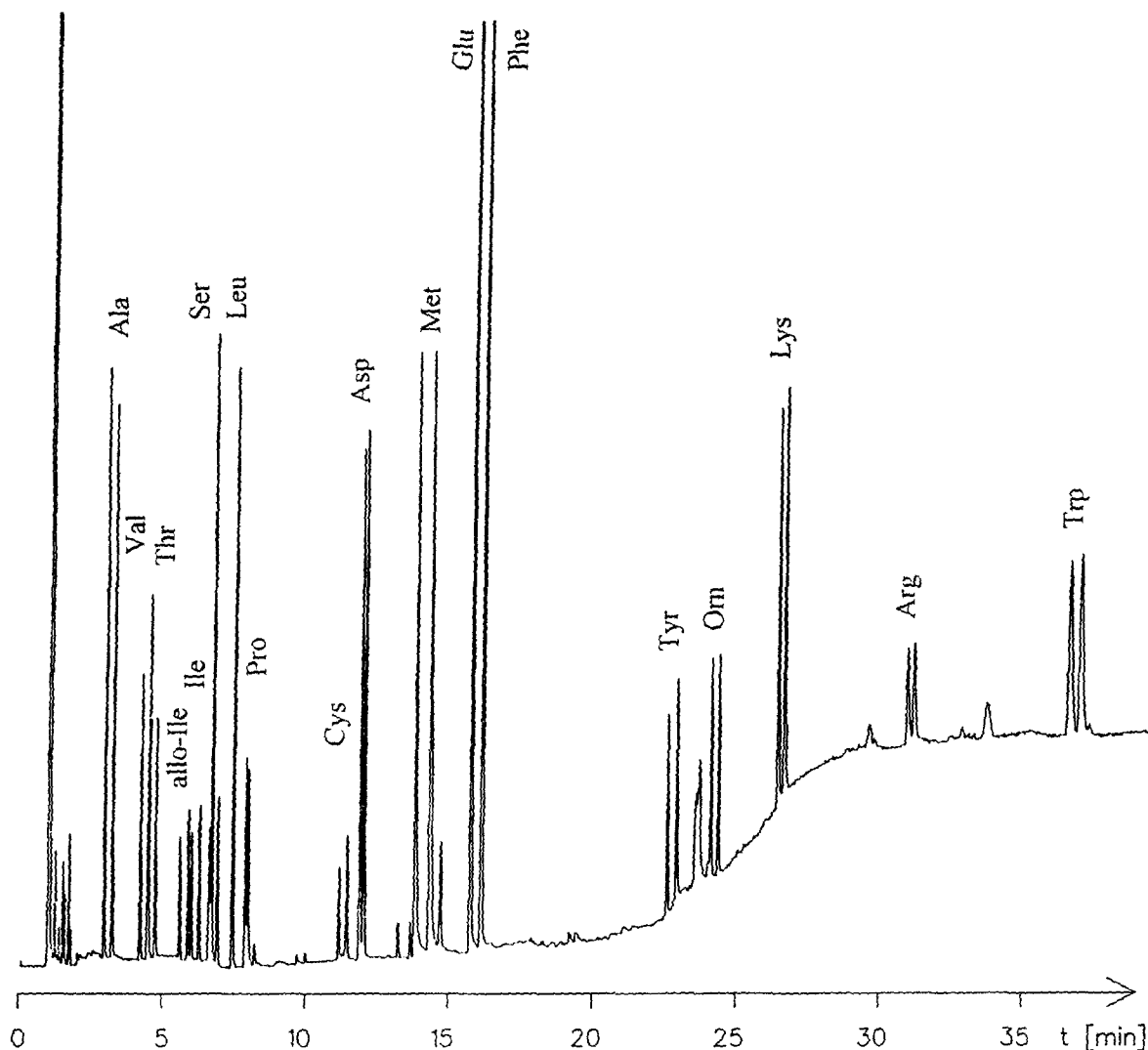
Fig. 5. Enantiomer separation of eighteen racemic amino acids (N-TFA-isopropyl esters) on the tripodal CSP under the applied conditions. The D and L enantiomers of Glu and Phe show perfect peak overlap. GC conditions: 20 m×0.25 mm undeactivated fused-silica column (heated at 250°C for 4 h under a low stream of helium) (d_i = 0.25 μm); carrier gas, He, 0.5 bar; temperature program, 100°C (3 min), 5 C°/min to 220°C, 220°C.

the structure of the solutes similar to those observed for Chirasil-Val [22,23].

6. Conclusion

The above described results show that the novel tripodal selector exhibits high enantioselectivity towards derivatized amino acids, and moderate capa-

bility for the enantiomer separation of derivatized hydroxy acids.

Due to the C_3 symmetric arrangement of three amino acid residues, the tripodal selector forms a intramolecular hydrogen bonding network involving both inter- and intrastrand hydrogen bonds. Thus a conformationally constrained selector is formed which retains this conformation up to temperatures of 150°C.

Table 3
Enantiomer separation data of hydroxy acids (derivatized as ethyl esters).

Racemate	T (°C)	α	k'	R_s	Elution order
Lactic acid	50	1.04(5)	2.74	1.18	S before R
2-Hydroxybutyric acid	50	1.05(0)	5.55	1.08	Not determined
3-Hydroxybutyric acid	50	1.00	9.17	0	–
2-Hydroxyisovaleric acid	50	1.05(6)	9.33	1.67	S before R
2-Hydroxycaproic acid	50	1.04(0)	30.62	1.48	Not determined
2-Hydroxyisocaproic acid	50	1.04(2)	22.95	0.67	S before R
Malic acid	90	1.02(7)	17.67	0.99	S before R
Mandelic acid	90	1.04(4)	29.10	1.86	S before R
3-Phenyllactic acid	90	1.01(6)	49.27	0.79	S before R

The conformationally constrainted structure of the present selector might enable us to elucidate the nature of the selector–solute interactions. Efforts in this direction applying a combination of experimental and theoretical tools are in progress.

Acknowledgments

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