Published in final edited form as:

J Sep Sci. 2008 December ; 31(21): 3688–3697. doi:10.1002/jssc.200800388.

Comparison of performance of Chirobiotic T, T2 and TAG columns in the separation of β^2 - and β^3 -homoamino acids

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Abstract

The enantiomers of eight unusual β^2 - and β^3 -homoamino acids were separated on chiral stationary phases containing the macrocyclic glycopeptide antibiotic teicoplanin (Chirobiotic T or T2) or teicoplanin aglycone (Chirobiotic TAG) as chiral selectors. The effects of the organic modifier, the mobile phase composition and temperature on the separations were investigated. Linear van't Hoff plots were observed in the studied temperature range, 280–318 K, and the changes in enthalpy, (H°), entropy, (S°), and free energy, (G°), were calculated. The values of the thermodynamic parameters depended on the nature of the selectors, the structures of the analytes, and especially the positions of the substituents on the analytes. A comparison of the separation performances of the macrocyclic glycopeptide stationary phases revealed that the Chirobiotic TAG column exhibited much better selectivity for β^2 -homoamino acids, while the separation of β^3 -homoamino acid enantiomers was better on Chirobiotic T or T2. The elution sequence was determined in some cases and was observed to be $R < S$.

Keywords

Column liquid chromatography; β^2 -homoamino acids; β^3 -homoamino acids; Macrocyclic glycopeptide-based chiral stationary phases; Chirobiotic columns

1. Introduction

β -Amino acids are key components of numerous bioactive molecules, developmental pharmaceuticals, β -peptides and peptidomimetics [1–3]. The wide-ranging utility of these compounds has led to increased attention being paid to their enantioselective syntheses

[4,5], which require analytical methods for a check on the enantiopurity of the final products.

In the past decade, new types of chiral derivatizing agents and chiral stationary phases (CSPs) have been applied by D'Acquarica *et al.*, Hyun *et al.* and Péter *et al.* for the high-performance liquid chromatographic (HPLC) enantioseparation of β -amino acids. [6–23].

In all chromatographic modes, the selectivity and retention factors are controlled mainly by the concentrations and nature of the mobile phase components, together with other variables, such as the flow rate, and the pH of the mobile phase. Enantioselective retention mechanisms are sometimes influenced by temperature to a greater extent than are ordinary separations. This was noted first in chiral gas chromatography [24]. Additionally, it is known that there are achiral and chiral contributions to retention that can vary with a wide variety of experimental parameters [25–28]. Accordingly, the column temperature has often been optimized in chiral HPLC separations [29–35]. The dependence of the retention of an analyte on the temperature can be expressed by the van't Hoff equation, which may be interpreted in terms of mechanistic aspects of chiral recognition:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

where ΔH° is the enthalpy of transfer of the solute from the mobile phase to the CSP, ΔS° is the entropy of transfer of the solute from the mobile phase to the CSP, R is the gas constant, T is the temperature and ϕ is the phase ratio of the column (the volume of the stationary phase divided by the volume of the mobile phase). This equation reveals that a plot of $\ln k'$ vs $1/T$ is linear, with a slope of $-\Delta H^\circ/R$ and an intercept of $\Delta S^\circ/R + \ln \phi$, if ΔH° is invariant with temperature. Since the value of ϕ is often not known, the $\Delta S^{*\circ}$ values [$\Delta S^{*\circ} = \Delta S^\circ + R \ln \phi$] are generally used. Any uncertainty in the phase ratio affects the $\Delta S^{*\circ}$ values virtually equally.

The corresponding (ΔH°) and (ΔS°) values for the separated enantiomers can be determined from a modification of Eq. 1:

$$\ln \alpha = -\frac{\Delta(\Delta H^\circ)}{RT} + \frac{\Delta(\Delta S^\circ)}{R} \quad (2)$$

where α is the selectivity factor ($\alpha = k_2'/k_1'$).

The aim of the present work was to investigate the effectiveness of different macrocyclic glycopeptide-based CSPs for the separation of β^2 - and β^3 -homoamino acids. The direct HPLC enantioresolution of some β^3 -homoamino acid analogs on Chirobiotic T and TAG CSPs was reported earlier by Péter *et al.* [9,11,12]. For comparison purposes, most of the separations were carried out at constant mobile phase compositions at different temperatures. The influence of specific structural features of the analytes and selectors and the effects of temperature on the retention will be discussed on the basis of the experimental data. The elution sequence was in some cases determined by spiking the racemate with an enantiomer with known absolute configuration.

2. Experimental

2.1. Chemicals and reagents

Racemic 3-amino-2-methylpropanoic acid (**B²-1**), 2-aminomethyl-butanoic acid (**B²-2**), 2-aminomethyl-3-methyl-butanoic acid (**B²-3**) and 3-amino-2-benzylpropanoic acid (**B²-4**) (Fig. 1) were prepared in two-step syntheses from methyl cyanoacetate, which in the first step was either alkylated [36] or condensed with aromatic aldehydes [37,38] and in the next step reduced. (*S*)-**B²-1** and (*S*)-**B²-3** were generous gifts from Prof. D. Tourwé (Vrije Universiteit Brussels, Belgium). The addition of benzylamine to ethyl ethacrylate furnished the corresponding *N*-benzylamino ester, which was transformed to the racemic ethyl ester of **B²-2** by catalytic hydrogenolysis. ($\pm$)-**B²-2** was resolved via *Candida antarctica* lipase-A-catalyzed *N*-acylation in *tert*-amyl alcohol with ethyl butanoate, yielding (*R*)-**B²-2** [39]. Racemic 3-aminobutanoic acid (**B³-1**) and 3-aminopentanoic acid (**B³-2**) (Fig. 1) were prepared from the corresponding α , β -unsaturated acids by benzylamine addition and subsequent debenzylation of the products with 20% metallic palladium on charcoal in a hydrogen atmosphere [40,41]. (*R*)-**B³-1** was prepared by the same method, but (*R*)-(+)- α -methylbenzylamine was used in the addition step instead of benzylamine [42]. 3-Amino-4-methylpentanoic acid (**B³-3**) and 3-amino-3-phenylpropanoic acid (**B³-4**) were synthesized from the corresponding aldehydes by a modification of the procedure of Rodionov and Malivinskaia [43]: the aldehydes were condensed with an equimolar amount of malonic acid in refluxing 96% ethanol in the presence of two equivalents of ammonium acetate [44,45].

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

2.2. Apparatus and Chromatography

The HPLC measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millennium³² Chromatography Manager data system; the alternative Waters Breeze system consisted of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (both systems from Waters Chromatography, Milford, MA, USA). Both chromatographic systems were equipped with Rheodyne Model 7125 injectors (Cotati, CA, USA) with 20- μ l loops.

The macrocyclic glycopeptide-based stationary phases [46] used for analytical separation were teicoplanin-containing Chirobiotic T and T2 and teicoplanin aglycone-containing Chirobiotic TAG columns, 250 mm $\times$ 4.6 mm I.D., 5- μ m particle size (for each column) (Astec, Whippany, NJ, USA). The differences between the Chirobiotic T and T2 columns are that they are both on 5- μ m particle size silica gel, but the Chirobiotic T uses a 120 Ångstrom pore size material and Chirobiotic T2 uses a 200 Ångstrom pore material. Also, the linkage chain on Chirobiotic T2 is approximately twice as long as that on Chirobiotic T.

Hence, the coverage and spacing are different for the two phases. This manifests itself mainly in the form of steric interaction differences between the two columns.

The columns were thermostated in a Spark Mistral column thermostat (Spark Holland, Emmen, The Netherlands). The precision of the temperature adjustment was ± 0.1 °C.

3. Results and discussion

3.1. Separation of enantiomers on Chirobiotic T, T2 and TAG columns

The analytes in this study (Fig. 1) can be arranged into two classes. The β^2 - and β^3 -homoamino acids differ in the positions of their substituents. Compounds **1** (β^2 -**1** and β^3 -**1**) can be considered as analogs of Ala, while compounds **3** (β^2 -**3** and β^3 -**3**) as analogs of Val. In both classes, analogs **1**, **2** and **3** bear alkyl groups, which may have different steric effects. This influences the hydrophobicity, bulkiness and rigidity of the molecules. Compounds **4** (β^2 -**4** and β^3 -**4**; analogs of Phe) bear aromatic rings, potentially able to make steric/rigid or other interactions with the numerous aromatic rings of the CSPs. The relevant chromatographic data on these two classes of compounds are given in Table 1. All compounds in Table 1 were evaluated with a minimum of three mobile phases, the eluent composition varying in the interval 0.1% triethylammonium acetate (TEAA, pH 4.1)/MeOH = 10/90 – 90/10 (v/v). For comparison purposes, Table 1 lists the chromatographic results obtained when enantiomeric separation was achieved at the 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v) mobile phase composition on the three different Chirobiotic columns. With the same mobile phase composition, the retention factors of the first-eluted enantiomers are lower on Chirobiotic T and T2 than on Chirobiotic TAG. Similar trends of higher k'_I values on a Chirobiotic TAG than on a Chirobiotic T column were observed by Berthod *et al.* [47], D'Acquarica *et al.* [8] and Péter *et al.* [11,12,14,48,49]. Comparison of the Chirobiotic T and T2 CSPs revealed that the retention factors of the first-eluting enantiomers on Chirobiotic T were somewhat larger (Table 1). Slightly higher k'_I values on Chirobiotic T2 than on Chirobiotic T were observed by Péter *et al.* [48] for β^3 -homoamino acids possessing aryl or heteroaryl side-chains. β^3 -Homoamino acids always exhibited lower k'_I values than β^2 -homoamino acids (the only exception was β^3 -**2** on Chirobiotic T).

The effect of the MeOH content on the enantiomeric separation was investigated for β^2 -**1**, β^3 -**1**, β^2 -**3**, β^3 -**3**, β^2 -**4** and β^3 -**4** on the three stationary phases. For analogs with an alkyl side-chain (β^2 -**1** and β^3 -**1**), when the MeOH content of the mobile phase was increased, the retention factor progressively increased. This was due to the reduced solubility of the polar amino acids in the MeOH-rich, more apolar mobile phase (Fig. 2). Similar results were obtained for analytes with a branched alkyl side-chain: β^2 -**3** and β^3 -**3** (data not shown). This behavior was observed on all three stationary phases. For analogs with an aromatic side-chain (β^2 -**4** and β^3 -**4**), in most cases a U-shaped retention curve was observed. At higher water content, the retention factor increased again with increasing water content; this was due to enhanced hydrophobic interactions between the analyte and the CSP in the water-rich mobile phases (Fig. 3). In this study, as in an earlier one [48], the position of minimum of k' differed somewhat for each compound. In the cases, when separations were occurred the α values increased very slightly, while the R_S values progressively increased with increasing MeOH content on all three CSPs (Figs 2 and 3).

A deeper analysis of the results reported in Table 1 shows that at a mobile phase composition of 0.1% TEAA(pH 4.1)/MeOH = 30/70 (v/v) on a given column, the k_I' values for the same type of analyte (β^2 - or β^3 -homoamino acid) differed slightly. Overall, the k_I' values were smallest on the Chirobiotic T2 column and largest on the Chirobiotic TAG column. For the appropriate proteinogenic α -amino acids, Ala, Val, Phe, also a similarity in k_I' values were observed [49,50]. A possible explanation for this an enantioselective recognition mechanism in which the first-eluted enantiomers do not experience selective interactions and accordingly are eluted at similar retention times (the slight variances in k_I' may originate from structural differences between the analytes).

The corresponding α and R_S values on the three CSPs for the β^2 -homoamino acids were larger than those for the β^3 -homoamino acids. However, the α and R_S values were lower than that of for the proteinogenic α -amino acids [49,50]. For the β^2 -homoamino acids, the Chirobiotic TAG exhibited higher selectivities (the only exception was **β^2 -1**), while for the β^3 -homoamino acids the Chirobiotic T or T2 column did so (the only exception was **β^3 -1**; Table 1). The enantiomers of **β^2 -1**, **β^3 -1**, **β^2 -2** and **β^3 -2** with short alkyl side-chains were retained on the CSPs, but they exhibited small α and R_S values. In reversed-phase mode one of the most important interaction between analyte and CSP is the hydrophobic interaction inside the “basket” of glycopeptide. The interactions of the short alkyl side-chains with the hydrophobic wall of the cavity of glycopeptides are probably too weak for effective chiral recognition, small α and R_S values were obtained. From the k_I' values for **β^2 -3** and **β^3 -3** in Table 1, it is evident that the 2-propyl group has a negative role in the stabilization of the molecule–CSP complex, causing a slight decrease in k_I' , but α (and R_S) in most cases slightly improved relative to **β^2 -1** and **β^3 -1**. A comparison of α and R_S values for Ala and Val also showed the negative role of the presence of 2-propyl group in Val; both α and R_S values were lower for Val comparing to Ala [49].

β^2 -4 and **β^3 -4** possess aromatic rings capable of steric/rigid or other interactions with the CSP. The data in Table 1 for the β^2 - and β^3 -homoamino acids with aromatic side-chains indicate that (i) on a given column the k_I' values did not differ considerably from those for amino acids with alkyl side-chains, and (ii) the α and R_S values were in some cases higher (**β^3 -4** was separable only on the Chirobiotic T2 column). Similarly one of the highest k' , α and R_S values were obtained for Phe in the case of proteinogenic α -amino acids [49,50].

A comparison of the results obtained on the Chirobiotic TAG column relative to Chirobiotic T may contribute to an understanding of the role of the pendant sugar moieties. To quantify the effects of the sugar units, the differences in enantioselective free energies between the two CSPs, $(G^\circ)_{\text{TAG}} - (G^\circ)_{\text{T}}$, were used. (G°) values were taken from Tables 4 and 5. The $(G^\circ)_{\text{TAG}} - (G^\circ)_{\text{T}}$ values were plotted as shown in Fig. 4. A negative number means that the stereoisomers are better separated on the aglycone CSP, and a positive number means that the stereoisomers are better separated on the native teicoplanin CSP, which contains the carbohydrate units. As can be seen in Fig. 4, the β^2 -homoamino acid enantiomers are much better separated by the aglycone CSP (an exception was **β^2 -1**), while for the β^3 -homoamino acids the native teicoplanin CSP was favorable. It also indicates that the aglycone basket of the teicoplanin molecule is solely responsible for the enantioselective recognition of most of the β^2 -homoamino acid enantiomers. From the aspect of

enantiomeric separations, the sugar moieties of the native teicoplanin may intervene in the chiral recognition process in at least three ways [47]: (i) the sugar units occupy space inside the basket; (ii) they block the possible interaction sites on the aglycone (phenolic hydroxy groups and an alcohol moiety); and (iii) they offer competing interaction sites, since the three sugars are themselves chiral and have hydroxy, ether and amido functional groups.

In general, it seems that the steric hindrance effect of the sugar moieties predominates for the β^2 -homoamino acids, while for the β^3 -homoamino acids sugar units support the enantio-recognition. Better enantio-separation of the enantiomers was observed for α -amino acids and cyclic- β -amino acids on the teicoplanin aglycone phase [14,47,48], whereas the β^3 -homoamino acids were better separated on the native teicoplanin CSP [12,51].

The elution sequence was determined for β^2 -1, β^2 -2, β^2 -3 and β^3 -1, and in all cases was observed to be $R < S$.

3.2. Effects of temperature and thermodynamic parameters

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out between 280 and 318 K. For β^2 -1, β^2 -3, β^2 -4, β^3 -1, β^3 -3 and β^3 -4, the chromatographic data were measured and calculated in 10 K increments over the temperature range studied (Tables 2 and 3). A comparison of the retention factors in Tables 2 and 3 shows that all of the recorded values decrease with increasing temperature. It is evident that an increase in separation temperature lowers the separation factor, α , but it may also improve the peak symmetry. The dependence of the resolution, R_S , on temperature, however, is more complex. The data listed in Tables 2 and 3 show that R_S for β^2 -3, β^3 -1 and β^3 -4 progressively decreased with increasing temperature on all three columns, while β^2 -1, β^2 -4 and β^3 -3 exhibited a maximum R_S with increasing temperature. At higher temperatures, R_S decreased due to the smaller α , while the decrease in R_S at lower temperatures may be explained by the dominating effect of the decreased column efficiency. Since the effect of temperature on the separation was more complex, an extensive study dealing with the thermodynamics of enantiomer separation was carried out.

In order to calculate the thermodynamic parameters and to acquire information of value for an understanding of the enantiomeric retention on these CSPs, van't Hoff plots were constructed [Eq. (1)]. The H° and S° values calculated from the slopes and intercepts of the plots of Eq. (1) for the enantiomers on all three columns were negative (Tables 4 and 5; since the thermodynamic parameters on Chirobiotic T and T2 for β^2 -1, β^3 -1, β^2 -3 and β^3 -3 were very similar, only the results obtained for β^2 -4 and β^3 -4 on Chirobiotic T2 are depicted in Table 4). Further, the H° , S° and G° values for the first-eluting enantiomer were always less negative than those for the second-eluting enantiomer. It was also observed that the G° values for the Chirobiotic T and T2 columns were less negative than those for the Chirobiotic TAG column.

As concerns the six analytes, β^2 -3 exhibited the smallest and β^2 -4 the largest $-H^\circ$ and $-S^\circ$ values. The branched side-chain in β^2 -3 is possibly responsible for steric repulsive effects when in the cavity. The largest $-H^\circ$ and $-S^\circ$ values for β^2 -4 show that the aromatic ring may promote the steric/rigid or polar interactions with the CSP.

The data on the changes in (H°) , (S°) and (G°) are depicted in Tables 4 and 5. For the β^2 -homoamino acid analogs the $-(H^\circ)$ values were more negative than those for the β^3 -homoamino acid analogs on the three different columns, (the exception was **β^3 -3** on Chirobiotic T2; data not shown). The largest values were obtained for **β^2 -4** on the Chirobiotic T and TAG columns, indicating the importance of steric/rigid effects or polar interactions. The $-(H^\circ)$ value on Chirobiotic T2 was less for **β^2 -4** than for **β^2 -1** (data not shown). The linkage chain on Chirobiotic T2 is approximately twice as long as that on Chirobiotic T, and the coverage and spacing will be different for the two columns. The increased hydrophobicity of Chirobiotic T2 may improve the interaction between an amino acid with an alkyl side-chain and the CSP. The small negative $-(H^\circ)$ values [$-(H^\circ) < 1.5 \text{ kJ mol}^{-1}$] for the β^3 -homoamino acids on all three columns indicate that the enantiorecognition is relatively unfavorable enthalpically.

The trends in the change in $-(S^\circ)$ demonstrated that in most cases β^2 -homoamino acids exhibited more negative $-(S^\circ)$ values than did the β^3 -homoamino acids; exceptions were **β^3 -3** and **β^3 -4** on the Chirobiotic T2 column. It was also observed that the $-(S^\circ)$ values were more negative for the amino acids possessing an aromatic side chain than for those with an alkyl side-chain (Tables 4 and 5).

The data on the changes in $-(G^\circ)$ indicate that enantioseparation on macrocyclic glycopeptide CSPs is more favorable for β^2 -homoamino acids (Tables 4 and 5). The largest negative $-(G^\circ)$ values for **β^2 -4** suggest that the steric/rigid or polar interactions induce highly efficient binding to the selector. The big difference in $-(G^\circ)$ values on Chirobiotic TAG column for **β^2 -1** and **β^2 -3** may be explained by the difference in steric and hydrophobic effect of the alkyl side-chains. On the basis of the $-(G^\circ)$ values, it can be stated that, of the three columns, Chirobiotic TAG (without sugar units) may promote the interactions of the enantiomers of β^2 -homoamino acids, while for β^3 -homoamino acids Chirobiotic T with sugar moieties seems to be more favorable. However, the $-(G^\circ)$ values also indicate that the enantioseparation of amino acids with alkyl side-chains is more favorable on native teicoplanin, while for amino acids possessing an aromatic side-chain the teicoplanin aglycone is favored.

In summary, complex formation that involved multiple intermolecular interactions was generally exothermic, and the corresponding entropic contribution was also negative. As is evident from the $-(G^\circ)$ data listed in Tables 4 and 5, the separations for all the investigated analytes on this CSP were enthalpically favored.

4. Concluding remarks

The enantioseparation of β^2 - and β^3 -homoamino acid analogs was investigated by using macrocyclic glycopeptide-based CSPs, *i.e.* Chirobiotic T, T2 and TAG columns, at either subambient or elevated temperatures. The separations could be accomplished in reversed-phase mode with 0.1% TEAA(pH 4.1)/MeOH mobile phases of various compositions and at different temperatures. Linear van't Hoff plots were observed in the studied temperature range, 280–318 K, and the apparent changes in enthalpy, H° , entropy, S° , and free energy, G° , were calculated. The values of the thermodynamic parameters depended on the

structures of the compounds and the CSPs applied. The elution sequence was determined in some cases. The macrocyclic glycopeptide-based CSPs for α - and β^2 -homoamino acids complement one another: if enantiomers exhibit partial resolution on one CSP, they would probably be baseline-resolved on the other macrocyclic glycopeptide-based CSP. However, this statement is not always valid for β^3 -homoamino acids.

Acknowledgments

This work was supported by Hungarian National Science Foundation grant OTKA K 67563 and UE grant LSHC-CT-2006-037733. I. Ilisz wishes to express his thanks for a Bolyai János Postdoctoral Fellowship supporting his research work. The authors gratefully acknowledge the support of this work by the National Institutes of Health (NIH R01 GM53825-11i).

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Figure 1.

3-Amino-2-methylpropanoic acid (β^2-1), 2-aminomethylbutanoic acid (β^2-2), 2-aminomethyl-3-methylbutanoic acid (β^2-3), 3-amino-2-benzylpropanoic acid (β^2-4), 3-aminobutanoic acid (β^3-1), 3-aminopentanoic acid (β^3-2), 3-amino-4-methylpentanoic acid (β^3-3) and 3-amino-3-phenylpropanoic acid (β^3-4)

Chirobiotic T

Chirobiotic T2

Chirobiotic TAG

Figure 2.

Effects of the MeOH content on the retention factor of the first-eluting enantiomer (k'_1), the separation factor (α) and the resolution (R_S) for β^2-1 and β^3-1 on Chirobiotic T, T2 and TAG CSPs.

Chromatographic conditions: mobile phase, 0.1% TEAA(pH 4.1)/MeOH = 30/70 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm, (■) k'_1 ; (●) α ; (▲) R_S

Chirobiotic T

Chirobiotic T2

Chirobiotic TAG

Figure 3.

Effects of the MeOH content on the retention factor of the first-eluting enantiomer (k'_1), the separation factor (α) and the resolution (R_S) for β^2-4 and β^3-4 on Chirobiotic T, T2 and TAG CSPs.

Chromatographic conditions: mobile phase, 0.1% TEAA(pH 4.1)/MeOH = 30/70 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm; (■) k'_1 ; (●) α ; (▲) R_S

Figure 4.

Enantioselectivity free energy differences, $(\Delta G^\circ)_{TAG} - (\Delta G^\circ)_T$, between aglycone and native teicoplanin CSPs.

Chromatographic conditions: mobile phase, 0.1% TEAA(pH 4.1)/MeOH = 30/70 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm

Table 1

Chromatographic data, retention factor of first-eluting enantiomer (k'_f), separation factor (α) and resolution (R_S) for the direct separation of the stereoisomers of β^2 - and β^3 -homoamino acids on macrocyclic glycopeptide CSPs at a mobile phase composition of 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v)

Column	Analyte	k'_f	α	R_S
T	β^2 - 1	2.58	1.14	0.90
	β^3 - 1	2.00	1.00	0.00
T2	β^2 - 1	2.26	1.00	0.00
	β^3 - 1	1.52	1.00	0.00
TAG	β^2 - 1	4.66	1.07	0.30
	β^3 - 1	3.30	1.00	0.00
T	β^2 - 2	2.34	1.15	1.20
	β^3 - 2	2.50	1.04	0.50
T2	β^2 - 2	2.00	1.08	0.70
	β^3 - 2	1.44	1.06	0.60
TAG	β^2 - 2	3.71	1.16	1.65
	β^3 - 2	3.32	1.00	0.00
T	β^2 - 3	2.10	1.14	1.13
	β^3 - 3	1.97	1.07	0.90
T2	β^2 - 3	1.63	1.14	0.95
	β^3 - 3	1.29	1.06	0.55
TAG	β^2 - 3	3.36	1.20	1.30
	β^3 - 3	3.23	1.00	0.00
T	β^2 - 4	2.35	1.32	3.50
	β^3 - 4	2.10	1.00	0.00
T2	β^2 - 4	1.95	1.19	1.70
	β^3 - 4	1.66	1.07	0.45
TAG	β^2 - 4	4.64	1.40	2.60
	β^3 - 4	3.83	1.00	0.00

Columns, **T**, Chirobiotic T, **T2**, Chirobiotic T2, **TAG**, Chirobiotic TAG; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm; temperature, ambient

Retention factor of first-eluting enantiomer (k_1'), separation factor (α) and resolution (R_S) of enantiomers of β^2 -homoamino acids as a function of temperature

Column	k_I', α, R_S	280	288	298	308	318
β^2-1						
T	k_I'	3.26	2.95	2.58	2.36	2.15
	α	1.22	1.19	1.15	1.12	1.09
	R_S	1.15	1.80	1.40	1.30	1.05
β^2-1						
T2	k_I'	2.90	2.66	2.26	1.90	1.67
	α	1.21	1.16	1.11	1.07	1.03
	R_S	0.40	0.60	0.65	0.40	<0.40
β^2-1						
TAG	k_I'	6.30	5.36	4.66	3.96	3.46
	α	1.10	1.09	1.07	1.05	1.05
	R_S	0.80	0.85	0.70	0.60	0.55
β^2-3						
T	k_I'	2.43	2.23	2.10	1.95	1.80
	α	1.20	1.17	1.14	1.12	1.10
	R_S	1.70	1.60	1.45	1.25	0.75
β^2-3						
T2	k_I'	2.05	1.79	1.63	1.43	1.31
	α	1.17	1.17	1.15	1.15	1.14
	R_S	1.70	1.65	1.60	1.55	1.45
β^2-3						
TAG	k_I'	4.11	3.72	3.36	2.98	2.78
	α	1.28	1.26	1.22	1.19	1.17
	R_S	3.20	3.10	3.00	1.75	1.50
β^2-4						
T	k_I'	2.94	2.64	2.35	2.05	1.85

Column	k_I' , α , R_S	Temperature (K)				
		280	288	298	308	318
	α	1.47	1.39	1.31	1.23	1.18
	R_S	3.00	3.10	2.50	2.05	1.70
T2	k_I'	2.75	2.35	1.95	1.64	1.43
	α	1.27	1.23	1.21	1.19	1.17
	R_S	1.55	1.65	1.65	1.70	1.55
TAG	k_I'	6.61	5.37	4.64	3.83	3.37
	α	1.57	1.47	1.38	1.31	1.24
	R_S	2.50	2.80	3.10	3.05	2.95

Columns, **T**, Chirobiotic T, **T2**, Chirobiotic T2, **TAG**, Chirobiotic TAG; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm

Table 3

Retention factor of first-eluting enantiomer (k_I'), separation factor (α) and resolution (R_S) of enantiomers of β^3 -homoamino acids as a function of temperature

Column	k_I', α, R_S	Temperature (K)				
		280	288	298	308	318
$\beta^3\text{-1}$						
T	k_I'	5.10	4.53	4.02	3.59	3.26
	α	1.07	1.06	1.04	1.03	1.02
	R_S	0.70	0.65	0.45	0.30	0.15
T2	k_I'	4.32	3.72	3.11	2.70	2.38
	α	1.09	1.08	1.07	1.06	1.05
	R_S	0.75	0.70	0.65	0.60	0.55
TAG	k_I'	9.05	7.78	6.64	5.97	5.08
	α	1.07	1.06	1.00	1.00	1.00
	R_S	0.55	0.45	0.00	0.00	0.00
$\beta^3\text{-3}$						
T	k_I'	4.07	3.72	3.28	2.92	2.64
	α	1.14	1.11	1.09	1.07	1.06
	R_S	1.16	1.32	1.10	0.90	0.70
T2	k_I'	2.79	2.39	2.05	1.82	1.63
	α	1.12	1.11	1.10	1.08	1.07
	R_S	1.05	1.15	1.00	0.85	0.75
TAG	k_I'	6.36	5.74	4.82	4.26	3.64
	α	1.00	1.00	1.00	1.00	1.00
	R_S	0.00	0.00	0.00	0.00	0.00
$\beta^3\text{-4}$						
T	k_I'	3.93	3.47	3.10	2.68	2.30

Column	k_I', α, R_S	Temperature (K)				
		280	288	298	308	318
T2	α	1.04	1.03	1.00	1.00	1.00
	R_S	0.30	0.20	0.00	0.00	0.00
	k_I'	3.33	2.94	2.49	2.18	1.92
	α	1.14	1.12	1.10	1.08	1.06
TAG	R_S	1.15	1.10	1.05	0.90	0.70
	k_I'	6.40	5.80	4.87	4.28	3.87
	α	1.00	1.00	1.00	1.00	1.00
	R_S	0.00	0.00	0.00	0.00	0.00

Columns, **T**, Chirobiotic T, **T2**, Chirobiotic T2, **TAG**, Chirobiotic TAG; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v); flow rate, 0.5 ml min⁻¹; detection, 205 nm

Table 4

Thermodynamic parameters, H° , $S^{\circ*}$, G° , (H°) , (S°) , (G°) and correlation coefficient (R^2) of analytes measured on Chirobiotic T and T2 columns

Analyte Stereoisomer	$-H$ (kJ mol ⁻¹)	$-S^{\circ*}$ (J mol ⁻¹ K ⁻¹)	Correlation coefficient, R^2	$-G^{\circ}_{298K}$ (kJ mol ⁻¹)	$-(H^\circ)$ (kJ mol ⁻¹)	$-(S^\circ)$ (J mol ⁻¹ K ⁻¹)	$-(G^\circ)$ (kJ mol ⁻¹)
Chirobiotic T							
β^2 -1	1	8.2	0.9977	2.4	2.2	6.3	0.35
	2	10.4	0.9974	2.7			
β^3 -1	1	8.7	0.9988	3.5	0.9	2.6	0.11
	2	9.6	0.9988	3.6			
β^2 -3	1	5.7	0.9963	1.8	1.6	4.3	0.32
	2	7.3	0.9971	2.2			
β^3 -3	1	8.5	0.9992	2.9	1.5	4.3	0.22
	2	10.0	0.9993	3.2			
β^2 -4	1	9.0	0.9988	2.1	4.2	12.0	0.67
	2	13.2	0.9993	2.8			
β^3 -4	1	10.3	0.9943	2.8	0.9	2.8	0.05
	2	11.2	0.9989	2.8			
Chirobiotic T2							
β^2 -4	1	12.9	0.9989	1.7	1.4	3.2	0.47
	2	14.4	0.9991	2.2			
β^3 -4	1	10.8	0.9994	2.3	1.4	3.8	0.24
	2	12.1	0.9995	2.5			

Column, Chirobiotic T; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v); $S^{\circ*} = S^\circ + \ln \phi$, where ϕ is the phase ratio; R^2 , correlation coefficient of van't Hoff plot, $\ln k - 1/T$ curves

Table 5

Thermodynamic parameters, H° , $S^{\circ*}$, G° , (H°), (S°), (G°) and correlation coefficient (R^2) of analytes measured on Chirobiotic TAG column

Analyte	$-H$ (kJ mol ⁻¹)	$-S^{\circ*}$ (J mol ⁻¹ K ⁻¹)	Correlation coefficient, R^2	$-G^{\circ*}_{298K}$ (kJ mol ⁻¹)	$-(H^\circ)$ (kJ mol ⁻¹)	$-(S^\circ)$ (J mol ⁻¹ K ⁻¹)	$-(G)$ (kJ mol ⁻¹)
Stereoisomer							
β^2-1	1	11.5	25.7	0.9980	3.8	1.1	3.3
	2	12.6	29.0	0.9982	4.0		0.17
β^3-1	1	10.9	20.8	0.9953	4.7	0.7	2.0
	2	12.4	25.6	0.9919	4.8		0.12
β^2-3	1	7.7	15.9	0.9969	3.0	1.9	4.6
	2	9.6	20.5	0.9979	3.5		0.49
β^3-3	1	10.9	23.5	0.9966	3.9	0.0	0.0
	2	10.9	23.5	0.9966	3.9		0
β^2-4	1	13.0	30.9	0.9933	3.8	4.5	12.5
	2	17.5	43.3	0.9949	4.6		0.8
β^3-4	1	10.1	20.7	0.9954	4.0	0.0	0.0
	2	10.1	20.7	0.9954	4.0		0

Column, Chirobiotic TAG; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 30/70 (v/v); $S^{\circ*} = S^\circ + \ln \phi$, where ϕ is the phase ratio; R^2 , correlation coefficient of van't Hoff plot, $\ln k - 1/T$ curves