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High-performance liquid chromatographic enantioseparation of monoterpene-based 2-amino carboxylic acids on macrocyclic glycopeptide-based phases

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ABSTRACT

The enantiomers of five monoterpene-based 2-amino carboxylic acids were directly separated on chiral stationary phases containing macrocyclic glycopeptide antibiotics such as teicoplanin (Astec Chirobiotic T and T2) and teicoplanin aglycone (Chirobiotic TAG) as chiral selectors. The effects of pH, the mobile phase composition, the structure of the analyte and temperature on the separations were investigated. Experiments were performed at constant mobile phase compositions in the temperature range 10–40 °C to study the effects of temperature and thermodynamic parameters on separations. Apparent thermodynamic parameters and T_{iso} values were calculated from plots of $\ln k$ or $\ln \alpha$ versus $1/T$. Some mechanistic aspects of the chiral recognition process are discussed with respect to the structures of the analytes. It was found that the enantioseparations were in most cases enthalpy driven. The sequence of elution of the enantiomers was determined in all cases.

1. Introduction

β-Amino acids are key building blocks of numerous bioactive molecules [1–4]. Icofungipen (PLD-118; (1*R*,2*S*)-2-amino-4-methylenecyclopentanecarboxylic acid), a β-amino acid, upsets the biosynthesis of protein in *Candida albicans* [5]. β-Amino acids and their foldameric oligomers are currently at the focus of research interest [6,7].

Enantiomerically pure α-pinene, δ-pinene and 3-carene can be transformed into β-amino acid derivatives [8,9,10,11], which are excellent building blocks for the syntheses of monoterpene-fused saturated 1,3-heterocycles [9,12]. Also, they were used successfully as chiral auxiliaries in the enantioselective reactions of Et_2Zn with aromatic aldehydes [10,12,8,9]. Apopinane-based β-amino acids were used as building blocks in the construction of stable H12 foldameric helices and in Ugi four-centre three-component reactions [11,13,14]. The latter new family of monoterpene-based chiral β-lactams and β-amino acid derivatives derived from (−)- and (+)-apopinene recently were reported to eliminate the disadvantageous steric effect of the methyl substituent on the pinane ring system [11]. The regioisomeric *trans* apopinane-based β-amino acids could be prepared by conjugate addition of lithium amides to (−)- and (+)-*tert*-butyl myrtenate, derived from natural (−)-myrtenal and (+)-α-pinene [3].

The wide-ranging utility of these compounds requires analytical methods to check on the stereochemistry of the final product. One of the most frequently applied techniques is chiral high-performance liquid chromatography (HPLC). HPLC enantioseparations of β-amino acids have been performed by both indirect and direct methods. In the past decade, chiral derivatizing agents (CDAs) such as Marfey' reagent (FDAA), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), *N*-(4-nitrophenoxycarbonyl)-phenylalanine methoxyethyl ester [(*S*)-NIFE] [15], chiral stationary phases (CSPs) such as macrocyclic glycopeptides [16,17], quinine derived [18], crown ether based [19,20], rapid double derivatization technique with gas chromatography [21] and (18-crown-6)-2,3,11,12-tetracarboxylic acid as a chiral NMR solvating agent have been used for the enantioseparation of β-amino acids [22].

In all chromatographic modes, the selectivity and retention factors are mainly controlled by the concentration and nature of the mobile phase components, together with other variables, such as

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the pH of the mobile phase. Enantioselective retention mechanisms are often influenced by temperature. This has been noted for some time in chiral gas chromatography [23,24]. It additionally known that there are both achiral and chiral contributions to retention that can vary with a wide variety of experimental parameters [24–28]. Several papers have been published that discuss the effects of temperature on enantiomers HPLC separation [29–33].

The dependence of the retention of an analyte on temperature can be expressed by the van't Hoff equation, which may be interpreted in terms certain of mechanistic aspects of chiral recognition:

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

in which k is retention factor, ΔH° is the enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the entropy of transfer of the solute from the mobile phase to the stationary phase, R is the gas constant, T is the temperature and ϕ is the phase ratio of the column. This equation reveals that a plot of $\ln k$ versus $1/T$ is linear, with slope $-\Delta H^\circ/R$ and intercept $\Delta S^\circ/R + \ln \phi$, if ΔH° is invariant with temperature. Since the value of ϕ is often not known, the $\Delta S^{\circ *}$ values [$\Delta S^{\circ *} = \Delta S^\circ + R \ln \phi$] calculated from the intercepts of the plots via Eq. (1) are generally used. Any uncertainty in the phase ratio affects all $\Delta S^{\circ *}$ values in the same manner. In chiral chromatography, however, the van't Hoff plots often deviate from linearity, possibly as a result of the inhomogeneity of the CSP surface, leading to a mixed retention mechanism [31,32].

The corresponding $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ 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 ($\alpha = k_2/k_1$).

In the present paper, direct HPLC methods are described for the enantioseparation of monoterpene-based 2-amino carboxylic acids, with the application of different macrocyclic glycopeptide-based CSPs. For comparison purposes, most of the separations were carried out at constant mobile phase compositions at different temperatures. The effects of pH, the mobile phase composition, the specific structural features of the analytes and selectors and temperature on the retention are discussed on the basis of the experimental data. The elution sequence was determined in all cases.

2. Experimental

2.1. Materials and methods

The enantiomers of monoterpene-based *cis*-β-amino acids were prepared in two steps by literature methods [10,11,8]. Chlorosulfonyl isocyanate addition to the corresponding chiral monoterpene ((−)-apopinene for **1A** and **2A**, (+)-apopinene for **1B** and **2B**, (−)-α-pinene for **4A**, (+)-α-pinene for **4B**, (−)-δ-pinene for **5A**, and (+)-δ-pinene for **5B**) afforded β-lactams in highly regio- and stereospecific reactions, subsequent acidic hydrolysis resulted in the corresponding *cis*-β-amino acids. The base-catalysed isomerization of the esters of the *cis*-amino acids derived from apopinene (**1A** and **1B**), followed by hydrolysis, afforded the corresponding *trans* enantiomers **2A** and **2B** in excellent yields [11]. The regioisomeric *trans* apopinane-based β-amino acids **3A** and **3B** could be prepared by conjugate addition of lithium amides to (−)- and (+)-*tert*-butyl myrtenate, derived respectively from natural (−)-myrtenal and (+)-α-pinene, followed by catalytic debenzylation and hydrolysis [3].

Methanol (MeOH) of HPLC grade was purchased from Scharlau (Sentmenat, Spain). Triethylamine (TEA), glacial acetic acid (AcOH), triethylammonium-acetate (TEAA), ethanol (EtOH), *n*-propanol (PrOH), 2-propanol (IPA) 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-μm filter, type HV, Millipore (Molsheim, France).

All the compounds mentioned in Fig. 1 were evaluated with different mobile phases. Reversed-phase mobile phases consisted of 0.1% TEAA (pH 4.1)/MeOH = 98/2, 90/10, 80/20, 60/40, 40/60, 20/80 and 10/90 (v/v), 100% MeOH while polar ionic mobile phase was MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v).

2.2. Apparatus

The HPLC separations were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode-array detector and a Millenium^{32} Chromatography Manager data system (Waters Chromatography, Milford, MA, USA) equipped with a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20-μL loop.

The macrocyclic glycopeptide-based stationary phases used for analytical separation were teicoplanin-containing Chirobiotic T and T2 and teicoplanin aglycone-containing Chirobiotic TAG columns, 250 mm × 4.6 mm I.D., 5-μm particle size (for each column) (Astec, Whippany, NJ, USA). Chirobiotic T and T2 are both based on silica gel with a 5-μm particle size, but the Chirobiotic T material has a 120 Å pore size and the Chirobiotic T2 material a 200 Å pore size. Moreover, the linkage chain in Chirobiotic T2 is approximately twice as long as that in Chirobiotic T. Hence, the coverage and spacing will be different for the two. This is manifested in the form of steric and non-enantioselective hydrophobic interaction differences between the two columns.

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

3. Results and discussion

The experimental conditions, including the pH of the mobile phase, the buffer type, the concentration of the organic modifier and the temperature, were investigated. The analytes in this study (Fig. 1) possess a monoterpene-based skeleton. Besides carboxy and primary amino groups (in *exo* or *endo* position), analogues **4** and **5** bear one methyl group, in position 2 or 4. These differences result in different steric effects and influence the hydrophobicity, bulkiness and rigidity of the molecules, depending on how their atoms are linked and how capable they are of different interactions with the selector.

The effects of pH on the separation were investigated in the acidic pH range. A decrease in the pH of the 0.1% aqueous TEAA/MeOH = 60/40 (v/v) eluent system from 6.50 to 4.00 considerably increased the retention factors of analyte **4** on both the Chirobiotic T and TAG columns; the selectivity decreased slightly, while the resolution did so considerably. Similar results were obtained by Armstrong et al. [34] on a teicoplanin CSP for analytes with free carboxylic acid groups. The pH 6–7 that produced the highest α also yielded the best resolution. According to the Armstrong's work [34] the protonation of teicoplanin either directly affects the coulombic or dipolar interactions between the analyte and the CSP, or indirectly influences the separation by changing the conformation of the selector.

All data relating to the separation of the compounds, including the retention factors, separation factors and resolutions for each analyte on the three different Chirobiotic columns, are given in Table 1 . For purposes of comparison and to simplify the presentation, Table 1 lists only the chromatographic results obtained when the enantiomeric separation was achieved with mobile phase com-

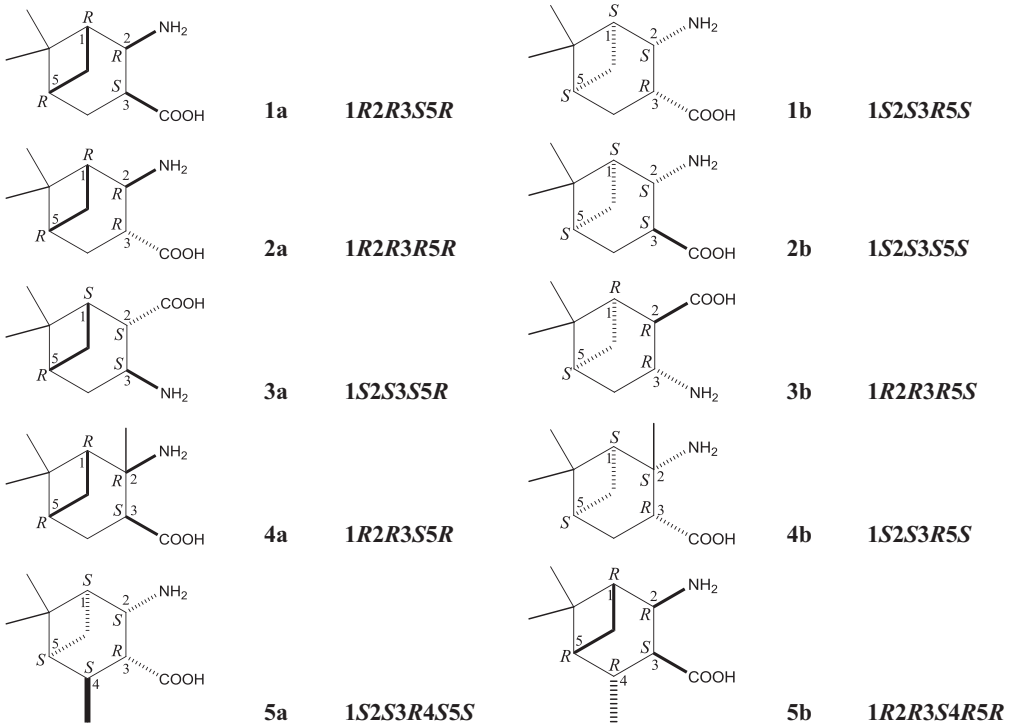

Fig. 1. Chemical structures of analytes. **1a** (1*R*,2*R*,3*S*,5*R*)-2-amino-6,6-dimethylbicyclo[3.1.1]heptane-3-carboxylic acid; **1b** (1*S*,2*S*,3*R*,5*S*)-2-amino-6, 6-dimethylbicyclo[3.1.1]heptane-3-carboxylic acid; **2a** (1*R*,2*R*,3*R*,5*R*)-2-amino-6,6-dimethylbicyclo[3.1.1]heptane-3-carboxylic acid; **2b** (1*S*,2*S*,3*S*,5*S*)-2-amino-6,6-dimethylbicyclo[3.1.1]heptane-3-carboxylic acid; **3a** (1*S*,2*S*,3*S*,5*R*)-3-amino-6,6-dimethylbicyclo[3.1.1]heptane-2-carboxylic acid; **3b** (1*R*,2*R*,3*R*,5*S*)-3-amino-6,6-dimethylbicyclo[3.1.1]heptane-2-carboxylic acid; **4a** (1*R*,2*R*,3*S*,5*R*)-2-aminopinane-3-carboxylic acid; **4b** (1*S*,2*S*,3*R*,5*S*)-2-aminopinane-3-carboxylic acid; **5a** (1*S*,2*S*,3*R*,4*S*,5*S*)-2-amino-4,6,6-trimethylbicyclo[3.1.1]heptane-3-carboxylic acid; **5b** (1*R*,2*R*,3*S*,4*R*,5*R*)-2-amino-4,6,6-trimethylbicyclo[3.1.1]heptane-3-carboxylic acid.

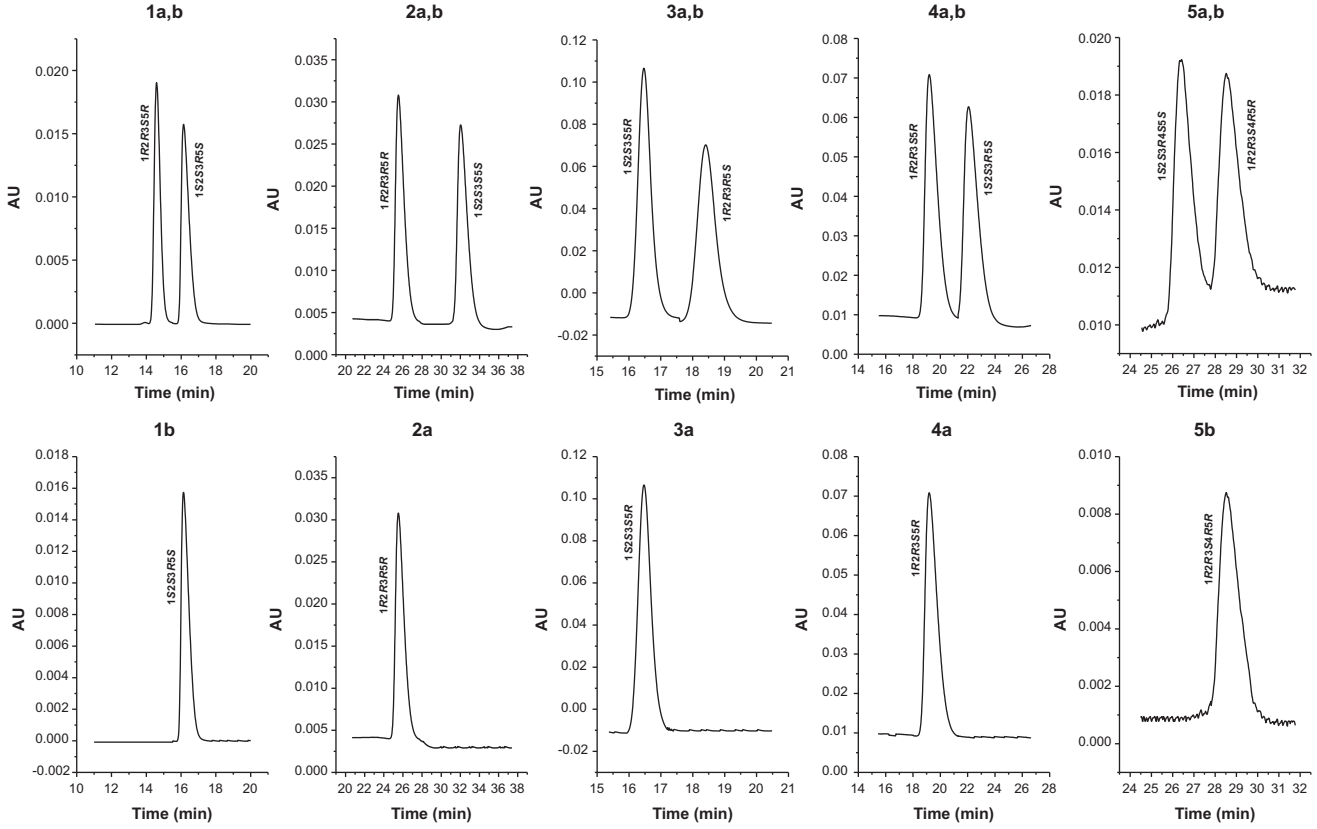

Fig. 2. Selected chromatograms for analytes **1a**,**b**–**5a**,**b** and **1b**, **2a**–**4a** and **5b** chromatographic conditions: column, Chirobiotic T for analytes **1** and **3**, Chirobiotic T2 for analytes **2** and Chirobiotic TAG for analytes **4** and **5**; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v) for analytes **1**, **3** and **5**, 0.1% TEAA (pH 4.1)/MeOH = 40/60 (v/v) for **4** and 0.1% TEAA (pH 4.1)/MeOH = 98/2 (v/v) for **2**; temperature, ambient for analytes **1**–**4**, 20 °C for **5**; flow rate, 0.5 mL min^{-1}; detection, 210 nm.

Table 1
Chromatographic data, retention factor of first eluted enantiomer (k_1'), separation factor (α), resolution (R_S) and configuration of the first eluted enantiomer of monoterpene-based 2-amino acids on different monocyclic glycopeptide-based columns.

Analyte	Column	Mobile phase TEAA/MeOH (v/v), **a** MeOH/AcOH/TEA (v/v/v), **b**	k_1'	α	R_S	Configuration of the first eluted enantiomer
	T	90/10, **a**	3.48	1.14	1.27	1*R*2*R*3*S*5*R*
		40/60, **a**	1.98	1.08	0.72	
		10/90, **a**	2.65	1.15	1.74	
		0/100, **a**	4.18	1.19	1.85	
		100/0.1/0.1, **b**	3.05	1.24	1.57	
	T2	90/10, **a**	3.21	1.00	0.00	
		40/60, **a**	1.59	1.12	1.24	
1		10/90, **a**	2.00	1.16	1.40	
		0/100, **a**	2.67	1.19	1.17	
		100/0.1/0.1, **b**	2.01	1.00	0.00	
	TAG	90/10, **a**	6.92	1.00	0.00	
		40/60, **a**	3.74	1.15	1.79	
		10/90, **a**	4.89	1.22	1.63	
		0/100, **a**	6.61	1.23	1.13	
		100/0.1/0.1, **b**	3.88	1.00	0.00	
	T	90/10, **a**	3.28	1.00	0.00	1*R*2*R*3*R*5*R*
		40/60, **a**	2.30	1.04	0.39	
		10/90, **a**	3.65	1.06	0.80	
		0/100, **a**	4.73	1.08	0.90	
		100/0.1/0.1, **b**	3.39	1.08	0.56	1*S*2*S*3*S*5*S*
	T2	90/10, **a**	3.13	1.18	1.35	1*R*2*R*3*R*5*R*
		40/60, **a**	2.23	1.03	0.27	
2		10/90, **a**	3.35	1.05	0.51	
		0/100, **a**	5.98	1.06	0.60	
		100/0.1/0.1, **b**	2.32	1.09	0.42	1*S*2*S*3*S*5*S*
	TAG	90/10, **a**	7.63	1.19	1.06	1*R*2*R*3*R*5*R*
		40/60, **a**	4.88	1.00	0.00	
		10/90, **a**	7.05	1.00	0.00	
		0/100, **a**	8.96	1.02	1.53	
		100/0.1/0.1, **b**	4.09	1.00	0.00	1*S*2*S*3*S*5*S*
	T	90/10, **a**	11.95	1.00	0.00	1*R*2*R*3*R*5*S*
		40/60, **a**	7.49	1.00	0.00	
		10/90, **a**	3.68	1.15	1.34	
		0/100, **a**	1.30	1.48	3.49	
		100/0.1/0.1, **b**	3.17	1.32	2.67	
	T2	90/10, **a**	10.11	1.00	0.00	
		40/60, **a**	2.43	1.12	0.62	
3		10/90, **a**	2.00	1.22	1.22	
		0/100, **a**	1.80	1.29	1.97	
		100/0.1/0.1, **b**	2.84	1.09	0.95	
	TAG	90/10, a	11.90	1.07	0.81	
		40/60, **a**	5.96	1.08	0.35	
		10/90, **a**	4.75	1.29	1.83	
		0/100, **a**	4.84	1.32	2.76	
		100/0.1/0.1, **b**	3.13	1.35	1.97	
	T	90/10, **a**	3.28	1.34	2.95	1*S*2*S*3*R*5*S*
		40/60, **a**	1.79	1.08	0.66	
		10/90, **a**	2.17	1.15	1.32	
		0/100, **a**	3.29	1.22	1.38	
		100/0.1/0.1, **b**	2.07	1.30	2.31	
	T2	90/10, **a**	3.22	1.20	1.78	
		40/60, **a**	1.40	1.12	1.07	
4		10/90, **a**	1.52	1.27	1.78	
		0/100, **a**	1.90	1.45	1.92	
		100/0.1/0.1, **b**	1.25	1.65	2.11	
	TAG	90/10, **a**	9.68	1.06	0.48	
		40/60, **a**	3.86	1.17	1.68	
		10/90, **a**	3.25	1.25	1.80	
		0/100, **a**	4.63	1.30	1.83	
		100/0.1/0.1, **b**	2.69	1.49	3.27	

Table 1 (*Continued*)

Analyte	Column	Mobile phase TEAA/MeOH (v/v), **a** MeOH/AcOH/TEA (v/v/v), **b**	k_1'	α	R_S	Configuration of the first eluted enantiomer
	T	90/10, **a**	4.40	1.00	0.00	1*R*2*R*3*S*4*R*5*R*
		40/60, **a**	1.91	1.00	0.00	
		10/90, **a**	2.44	1.05	0.67	
		0/100, **a**	3.69	1.09	0.81	
		100/0.1/0.1, **b**	2.51	1.12	1.78	
	T2	90/10, **a**	4.38	1.12	0.89	
		40/60, **a**	1.59	1.08	0.74	
5		10/90, **a**	1.90	1.10	0.86	
		0/100, **a**	2.24	1.14	0.90	
		100/0.1/0.1, **b**	1.54	1.17	0.75	
	TAG	90/10, **a**	10.93	1.07	0.66	
		40/60, **a**	4.49	1.09	0.81	
		10/90, **a**	3.86	1.11	1.09	
		0/100, **a**	5.34	1.16	1.11	
		100/0.1/0.1, **b**	2.88	1.25	1.36	

Chromatographic conditions: columns, **T**, Chirobiotic T, **T2**, Chirobiotic T2, **TAG**, Chirobiotic TAG; mobile phase, **a**, 0.1% TEAA (pH 4.1)/MeOH (v/v), **b**, MeOH/AcOH/TEA (v/v/v); flow rate, 0.5 mL min^{-1}; detection, 210 nm.

positions of 0.1% TEAA (pH 4.1)/MeOH = 90/10, 40/60 and 10/90 (v/v), 100% MeOH and MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v). At a given mobile phase composition, the retention factors were lower on the teicoplanin CSPs (T and T2) than on the aglycone CSP (TAG) (except for analyte **3** in some cases). The native teicoplanin phase (Chirobiotic T) exhibited intermediate k' values. Similar trends, with higher k' values on Chirobiotic TAG than on a Chirobiotic T column, were observed by Berthod et al. [35], D'Acquarica et al. [16] and Péter et al. [36–38] for unusual α-amino acids and cyclic β-amino acids. Comparison of the data for the Chirobiotic T and T2 columns revealed that the retention factors on Chirobiotic T were somewhat larger (except for analytes **2** and **3** at 100% MeOH) (Table 1). Slightly higher k' values on Chirobiotic T2 than on Chirobiotic T were observed by Péter et al. [38] for β^3-homoamino acids.

The effects of the MeOH content of the mobile phase were investigated on all three CSPs. In most cases, a U-shaped retention curve was observed for all analogues (the only exception was analyte **3**). At higher water contents, the retention factor increased with increasing water content; this was probably due to enhanced hydrophobic interactions between the analyte and the CSP in the water-rich mobile phases (Table 1). In the reversed-phase mode, one of the most important interactions between the analyte and the CSP is the hydrophobic interaction inside the "basket" of the glycopeptide. Here, an increase in k' at high water content was observed for all analytes (Table 1). When the MeOH content of the mobile phase exceeded ~50%, the retention factor increased (an exception was analyte **3**). This suggests that the separation may rather be controlled by the hydrophilic interaction chromatography (HILIC) than by the reversed-phase mechanism at high MeOH contents. In this study, as earlier [34], the inflection point and the slope of the U-shaped curve at higher and lower MeOH concentrations differed somewhat for each compound. Different extents of solvation of the stationary phase during HILIC and under the reversed-phase conditions may explain the observed retention behaviour. For analyte **3**, an increase in k' was observed with increasing water content. The different behaviour of analyte **3** may be due to the different positions of the carboxy and amino groups, resulting in differences in the steric interaction with the selector. In regard to the variations in the separation factors (α) and resolutions (R_S) with change of the MeOH content, no general trends were observed.

Use of the MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v) mobile phase system generally resulted in lower retention than with 100% MeOH (except for analyte **3**). However, for analytes **4** and **5**, in most cases despite the lower k' values, higher α and R_S values were obtained, indicating that the level of chiral discrimination improved in the polar ionic mode probably due to the steric effect of methyl group (Table 1).

The structures of the analytes influenced the chiral recognition. In the reversed-phase mode in water-rich mobile phases, analyte **3** exhibited large k_1' values, not accompanied by high resolution. At high water contents, the non-specific hydrophobic interaction resulted in high retention without chiral recognition. The value of α reached its maximum when using mobile phases of high MeOH content. Analytes **4** and **5**, which have an additional methyl group, are sterically constrained. In most cases this led to higher α (and R_S) values relative to those for analytes **1** and **2**. Interestingly, the position of the methyl group on the cycloalkane skeleton (position 2 or 4) influenced the values of α and R_S considerably; in that the more constrained analyte **4** displayed higher α and R_S values than those of analyte **5**.

Elution sequences were determined in all cases. For analytes **1**–**5** on the Chirobiotic T, T2 and TAG columns, no consistent elution sequence was observed. Neither the configuration of the carbon atom attached to the carboxyl group nor that of the carbon atom attached to the amino group determined the elution sequence, and in the case of analyte **2** the elution sequence differed when the mobile phase was changed from reversed-phase to polar ionic mode. Selected chromatograms for analytes **1**–**5** are depicted in Fig. 2.

3.1. 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 on Chirobiotic T and TAG columns over the temperature range 10–40 °C (in 5 or 10 °C increments). Experimental data for the mobile phase 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v) are listed in Table 2. A comparison of the retention factors in Table 2 and Fig. 3 reveals that all of the recorded values decreased with increasing temperature (similar tendencies were measured at other mobile phase compositions). It is evident that an increase in the separation temperature lowers the separation factor, α. However, for analyte **3** on the Chirobiotic T column, α (and R_S) increased with increasing temperature (Fig. 3). Increasing temperature may improve the peak symmetry and efficiency, and therefore the resolution may also improve.

Since the effect of temperature on the separation was complex, an extensive study relating to the thermodynamics of this system was carried out. The initial step of this process is to accumulate accurate chromatographic data from which van't Hoff plots were constructed [Eq. (1)]. The ΔH° and $\Delta S^{\circ *}$ values calculated from the slopes and intercepts of these plots for the enantiomers on all three

Table 2
Retention factor of first-eluting enantiomer (k'), separation factor (α) and resolution (R_S) of enantiomers of monoterpene-based 2-amino carboxylic acids **1**–**5** as a function of temperature.

Analyte	Columns	k_1', α, R_S	Temperature (°C)				
			10	15	20	30	40
1	**T**	k_1'	4.49	4.18	4.05	3.58	3.21
		α	1.13	1.12	1.12	1.11	1.10
		R_S	1.66	1.64	1.51	1.59	1.62
	TAG	k_1'	6.79	6.29	5.81	5.09	4.63
		α	1.17	1.16	1.15	1.14	1.12
		R_S	1.46	1.48	1.66	1.71	1.88
2	**T**	k_1'	5.84	5.54	5.31	4.97	4.63
		α	1.04	1.03	1.03	1.02	1.01
		R_S	0.53	0.50	0.39	0.38	0.46
	TAG	k_1'	10.66	9.96	9.65	8.68	8.12
		α	1.00	1.00	1.00	1.00	1.00
		R_S	0.00	0.00	0.00	0.00	0.00
3	**T**	k_1'	7.30	6.26	5.66	4.76	4.05
		α	1.09	1.11	1.12	1.14	1.17
		R_S	0.70	0.81	0.85	1.07	1.09
	TAG	k_1'	13.37	10.21	8.09	6.18	4.08
		α	1.20	1.17	1.13	1.09	1.04
		R_S	1.97	1.33	1.04	0.75	0.33
4	**T**	k_1'	3.74	3.51	3.35	3.06	2.73
		α	1.13	1.12	1.11	1.10	1.09
		R_S	1.27	1.28	1.30	1.32	1.35
	TAG	k_1'	5.18	4.86	4.48	4.13	3.75
		α	1.20	1.19	1.18	1.17	1.16
		R_S	1.47	1.74	1.84	1.95	2.09
5	**T**	k_1'	4.29	4.02	3.77	3.43	3.08
		α	1.05	1.05	1.04	1.04	1.03
		R_S	0.72	0.67	0.61	0.48	0.38
	TAG	k_1'	6.23	5.82	5.64	4.89	4.37
		α	1.12	1.11	1.10	1.09	1.07
		R_S	1.24	1.25	1.30	1.29	1.22

Chromatographic conditions: column, **T**, Chirobiotic T, **TAG**, Chirobiotic TAG; mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v); flow rate, 0.5 ml min^{-1}; detection, 210 nm.

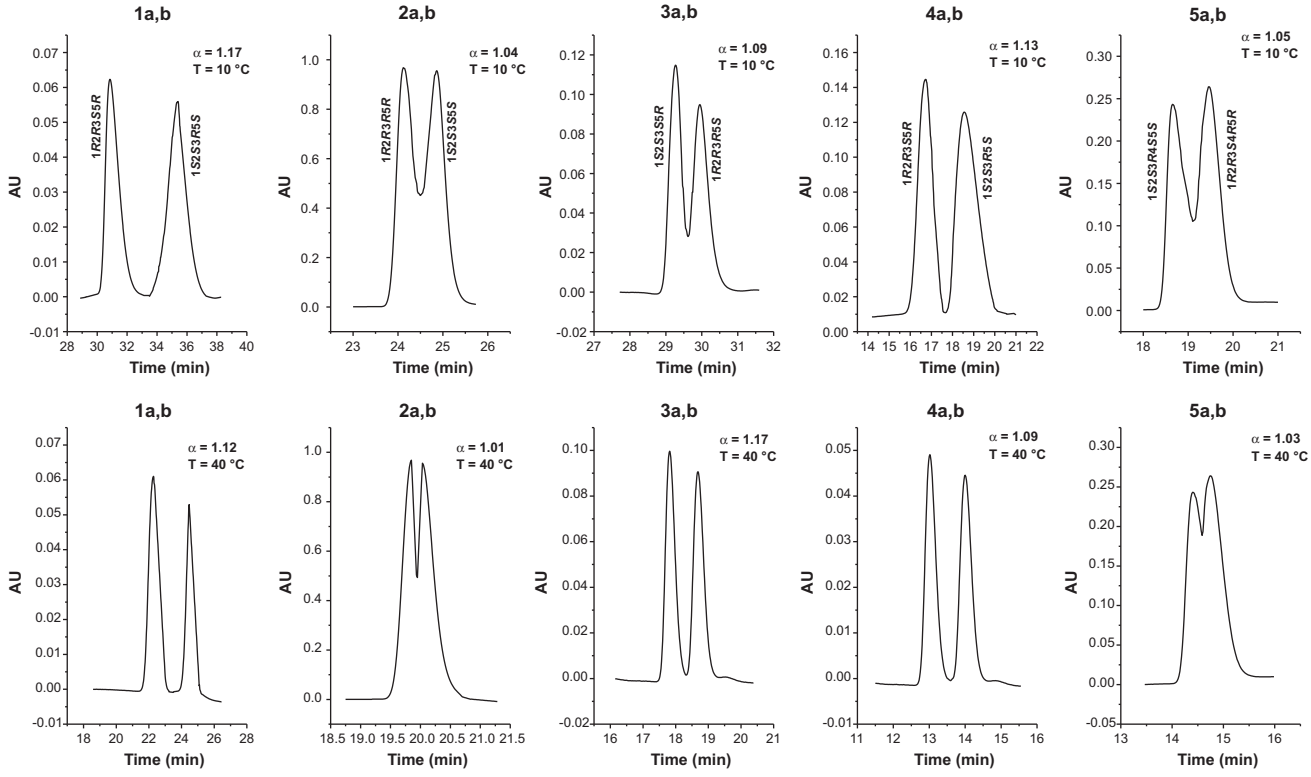

Fig. 3. Temperature and enantioselectivity correlation of chiral separation of analytes **1a**,**b**–**5a**,**b** chromatographic conditions: column, Chirobiotic T for analytes **2**, **3**, **4** and **5**, Chirobiotic TAG for analyte **1**; mobile phase 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v); temperature, 10 °C (upper) and 40 °C (lower); detection, 210 nm; flow rate 0.5 ml min^{-1}.

Table 3
Thermodynamic parameters, ΔH°, $\Delta S^{\circ *}$, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $\Delta(\Delta G^\circ)$, correlation coefficients (R^2) and T_{iso} temperature of analytes **1–5** on Chirobiotic T and TAG columns.

Analyte	Column	Stereoisomer	$-\Delta H^\circ$ (kJ mol^{-1})	$-\Delta S^{\circ *}$ (J mol^{-1} K^{-1})	Correlation coefficient (R^2)	$-\Delta(\Delta H^\circ)$ (kJ mol^{-1})	$-\Delta(\Delta S^\circ)$ (J mol^{-1} K^{-1})	$-\Delta(\Delta G^\circ)_{298\,K}$ (kJ mol^{-1})	T_{iso} (°C)
1	**T**	1	8.1 ± 0.3	16.1 ± 1.2	0.9946	0.61 ± 0.02	1.17 ± 0.07	0.30	248
		2	8.7 ± 0.4	17.3 ± 1.3	0.9950				
	TAG	1	9.5 ± 0.4	17.7 ± 1.3	0.9949	0.98 ± 0.05	2.16 ± 0.16	0.35	179
		2	10.5 ± 0.4	19.8 ± 1.3	0.9960				
2	**T**	1	5.6 ± 0.2	5.1 ± 0.6	0.9966	0.61 ± 0.03	1.86 ± 0.12	0.06	57
		2	6.2 ± 0.2	7.0 ± 0.6	0.9977				
	TAG	1	6.7 ± 0.3	3.9 ± 1.2	0.9921	0.00	0.00	0.00	–
		2	6.7 ± 0.3	3.9 ± 1.2	0.9921				
3	**T**	1	14.1 ± 0.8	33.5 ± 2.6	0.9911	−1.50 ± 0.08	−6.05 ± 0.27	0.30	−26
		2	12.6 ± 0.7	27.4 ± 2.4	0.9903				
	TAG	1	27.9 ± 1.6	77.3 ± 5.4	0.9901	3.31 ± 0.17	10.22 ± 0.56	0.25	50
		2	31.2 ± 1.7	87.6 ± 5.8	0.9910				
4	**T**	1	7.6 ± 0.3	15.7 ± 0.9	0.9960	0.91 ± 0.05	2.21 ± 0.18	0.25	139
		2	8.5 ± 0.3	17.9 ± 1.0	0.9961				
	TAG	1	7.8 ± 0.4	14.1 ± 1.5	0.9906	0.79 ± 0.03	1.30 ± 0.08	0.40	338
		2	8.6 ± 0.4	15.4 ± 1.5	0.9922				
5	**T**	1	8.0 ± 0.2	16.1 ± 0.6	0.9985	0.54 ± 0.02	1.47 ± 0.08	0.10	93
		2	8.5 ± 0.2	17.6 ± 0.6	0.9985				
	TAG	1	8.8 ± 0.4	15.7 ± 1.5	0.9924	1.02 ± 0.05	2.67 ± 0.18	0.20	108
		2	9.8 ± 0.4	18.4 ± 1.4	0.9944				

Mobile phase, 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v); $\Delta S^{\circ *} = \Delta S^\circ + R \ln \Phi$, where Φ is the phase ratio; R^2, correlation coefficient of van't Hoff plot, $\ln k - 1/T$ curves; T_{iso}, temperature of $\ln k - 1/T$ curves where enantioselectivity cancels.

columns were negative (Table 3). The second-eluting enantiomers have more negative $\Delta S^{\circ *}$ values, it is likely that they have fewer degrees of freedom on the CSP, i.e., they are held at more points or are less able to move or rotate. It is widely accepted that both enantiomers undergo the same non-specific interactions, whereas the more strongly retained one is subject to additional stereospecific interactions. Moreover, it was observed that $\Delta H^\circ{}_1$ and $\Delta H^\circ{}_2$, and in parallel $\Delta S^{\circ *}_1$ and $\Delta S^{\circ *}_2$, for the Chirobiotic T column were in most cases less negative than those for the Chirobiotic TAG column. This may be due to the fact that the teicoplanin aglycone (TAG) has been the sugar units removed and this may promote the interaction between the analyte and the CSP.

Of the five analytes, analyte **2** exhibited the smallest, and analyte **3** the largest $-\Delta H^\circ$ and $-\Delta S^{\circ *}$ values. The positions (2 and 3) of the amino and carboxy groups and their *trans* configuration in **2** probably inhibit fitting and orientation in the cavity (the sugar moieties on Chirobiotic T improved the chiral recognition, while on teicoplanin aglycone no separation was observed), and the separation was thermodynamically less favorable. The largest $-\Delta H^\circ$ and $-\Delta S^{\circ *}$ values for **3** indicate that, despite the *trans* configuration, the separation sterically was favorable.

The differences in the changes in enthalpy and entropy, $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$, are also presented in Table 3. The $-\Delta(\Delta H^\circ)$ values ranged from −1.5 to 3.3 kJ mol^{-1}. The interactions of **3** with the Chirobiotic TAG stationary phase were characterized by the highest negative $\Delta(\Delta H^\circ)$ value, while analyte **3** on Chirobiotic T exhibited a positive $\Delta(\Delta H^\circ)$. The trends in the change in $-\Delta(\Delta S^\circ)$ showed that analyte **3** on Chirobiotic TAG displayed the largest negative entropies, $-\Delta(\Delta S^\circ)$ ranging from −6.1 to 10.2 J mol^{-1} K^{-1} (Table 3). For analyte **3**, similarly to $\Delta(\Delta H^\circ)$ on Chirobiotic T column $\Delta(\Delta S^\circ)$ was also positive. The $\Delta(\Delta S^\circ)$ values are controlled by the difference in the degrees of freedom between the stereoisomers on the CSP, and mainly by the number of solvent molecules released from both the chiral selector and the analyte when the analyte is associated with the CSP.

The thermodynamic parameter $-\Delta(\Delta G^\circ)$ suggests that teicoplanin without sugar units induces highly efficient binding to the selector, as reflected by the large negative $\Delta(\Delta G^\circ)$ values for **1**, **3**, **4** and **5**. For analyte **2** on Chirobiotic T $\Delta(\Delta G^\circ)$ exhibits a small negative value while on Chirobiotic TAG, the enantiomers of analyte **2** were not separable at this eluent composition. For analytes **1**–**5** on both columns (with exception of analyte **3** on Chirobiotic T) the selector–selectand complex formation proceeds via multiple intermolecular interactions and was generally exothermic, with a corresponding negative entropic contribution.

For analyte **3**, on Chirobiotic T, the positive $\Delta(\Delta S^\circ)$ compensated for the positive $\Delta(\Delta H^\circ)$ and resulted in a relatively high $-\Delta(\Delta G^\circ)$ value. The teicoplanin, which contains several sugar moieties having more chiral centres, ensures more interaction sites for the analytes, leading to more negative $-\Delta(\Delta G^\circ)$ values. In this temperature range, enantioresolution is entropically driven, and the selectivity increases with increasing temperature (Fig. 3).

From the data, the temperature, T_{iso}, was calculated at which the enantioselectivity balances out and the elution sequence changes (Table 3). In most cases, T_{iso} was above 50 °C, but for analyte **3** on Chirobiotic T it was −26 °C. These temperatures indicate that lower temperatures are preferable for the best separation of most of the analytes, with the exception of analyte **3** on the Chirobiotic T, where positive $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ were observed.

4. Conclusions

HPLC methods were developed for the separation of the enantiomers of monoterpene-based 2-amino carboxylic acids, using macrocyclic glycopeptide-based CSPs: (i.e., Chirobiotic T, T2 and TAG). Baseline resolution was achieved in all cases.

The values of thermodynamic parameters such as the changes in enthalpy, $\Delta(\Delta H^{\circ})$, entropy, $\Delta(\Delta S^{\circ})$, and Gibbs free energy, $\Delta(\Delta G^{\circ})$, depended on the structures of the analytes and on the chiral selectors used. The elution sequence was determined in all cases, but no general predictive rule could be found to describe the elution behaviour of these compounds.

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