Enantiomeric Separation of Bicyclo[2.2.2]octane-Based 2-Amino-3-Carboxylic Acids on Macrocyclic Glycopeptide Chiral Stationary Phases

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ABSTRACT Direct high-performance liquid chromatographic (HPLC) separation of four bicyclo[2.2.2]octane based 2-amino-3-carboxylic acid enantiomers were developed on chiral stationary phases (CSPs) containing different macrocyclic glycopeptide antibiotic selectors. The analyses were performed under reversed-phase, polar organic and polar ionic mode on macrocyclic-glycopeptide-based Chirobiotic T, T2, TAG, and R columns. The effects of the mobile phase composition including the acid and base modifier, the structure of the analytes, and the temperature on the separations were investigated. Experiments were achieved at constant mobile phase compositions on different stationary phases in the temperature range 5–40°C. Thermodynamic parameters were calculated from plots of ln k or ln α versus 1/T. It was recognized that the enantioseparations in reversed-phase and polar organic mode were enthalpically driven, but under polar-ionic conditions entropically driven enantioseparation was observed as well. Baseline separation and determination of elution sequence were achieved in all cases. *Chirality 26:200–208, 2014.*

KEY WORDS: column liquid chromatography; enantiomer separation; β-amino acid analogs; macrocyclic glycopeptide-based columns

INTRODUCTION

The role of nonnatural amino acids is significant and increasing due to their interesting chemical and biological activities. Among them, bicyclic amino acids are important components in biological systems. One of the most significant representatives of this family is the α-amino acid, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH). This compound has several potentially useful effects in biological systems: it is able to increase insulin secretion,[1,2] it plays a role in cell membrane transport,[3] and cancer cell apoptosis.[4,5]

β-Amino acids with similar structures could be effective analogs of α-amino acids; in addition, their biological activities also have been noted.[6,7] In addition, they have been used as intermediates for the synthesis of pharmaceutical compounds and other organic molecules such as alkaloids, peptides, and β-lactam antibiotics.[8–13] Moreover, these compounds are building blocks of novel β-peptides with unique properties, which has been attracting attention because of the feasibility of derivatization with various side chains at α- and β-positions.[14–17] Several β-amino acid derivatives were shown to be effective inhibitors of glycine transport.[18]

The antigen-4 (VLA-4) is a heterodimeric cell surface glycoprotein transmembrane receptor,[19] which is implicated in several inflammatory and autoimmune disease states.[20] The inhibition of VLA-4 may produce a reduction in the migration and/or activation of cell types important to sustaining a prolonged inflammatory response.[21] The cyclic β-amino acids were found to be effective as VLA-4 antagonists.[22]

Conformationally constrained bicyclo[2.2.2]octane β-amino acids are also of great interest, owing to their role in both synthetic and medicinal chemistry.[23–28] These compounds simultaneously combine the particular structural properties of constrained cyclic amino acids and those of β-amino acids that are more resistant than α-amino acids to enzymatic degradations. The interest in bicyclic amino acids is highlighted by publications on several investigations in recent years.[29–31]

The wide-ranging utility of these compounds has led to increased interest in their enantioselective syntheses, which demand analytical methods for checking the enantiopurity of these chemical products. The direct and indirect chiral high-performance liquid chromatography (HPLC) are well-known, frequently used, and reliable applied techniques in the enantioseparation of different analytes.

Due to their biological importance, many papers deal with the enantioseparation of chiral β-amino acids on different types of chiral stationary phases (CSPs). Successful separations were achieved on macrocyclic glicopeptide,[32–36] crown-ether,[37–39] cyclodextrin,[40,41] cinchona alkaloid,[42] and cyclofructan-based[43] chiral columns.

The macrocyclic glycopeptide antibiotics (especially teicoplanin, ristocetin A and vancomycin) and their analogs, appear to be among the most successful chiral selectors in HPLC and capillary electrophoresis (CE).[44] The variety of chiral interactive sites, which are able to create hydrogen bonding, steric hindrance, π–π interaction, inclusion complexation, electrostatic, van der Waals forces, and hydrophobic interactions with the stereoisomers makes them so broadly successful.[45] Their excellent chiral discrimination capability

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can be achieved in normal phase (NPM), reversed phase (RPM), polar-organic (POM), and polar-ionic (PIM) modes.[46] Moreover, to some extent these macrocyclic glycopeptides are complementary to each other.

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 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.[47,48] Additionally, it is known that there are both achiral and chiral contributions to retention that can vary with changes in experimental parameters.[48–52] Several papers have been published discussing the effects of temperature on enantiomeric HPLC separations.[53–57]

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 of mechanistic aspects of chiral recognition:

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

in which k is the 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 absolute temperature, and ϕ is the inverse of the phase ratio of the column: $\phi = V_S/V_M$.

This equation reveals that a plot of ln k vs. 1/T is linear, with slope of -ΔH°/R and intercept ΔS°/R + ln ϕ, if ΔH° is invariant with temperature. Since the value of ϕ is often not known, the ΔS°* values [ΔS°* = ΔS° + R ln ϕ] calculated from the intercepts of the plots via Eq. 1 are generally used. Any uncertainty in the phase ratio affects the ΔS°* values virtually equally. In chiral chromatography, however, the van't Hoff plot often deviates from linearity, possibly as a result of the inhomogeneity of the CSP surface, leading to a mixed retention mechanism.

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

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

where α is the selectivity factor ($\alpha = k_2/k_1$). If α is 1.0 (ln α is 0), there must exist a temperature at which enthalpic and entropic contributions to chiral recognition exactly compensate each other and the enantiomers coelute.[46,58]

This paper describes direct HPLC methods for the separations of the enantiomeric mixtures of four bicyclo[2.2.2]octane-based 2-amino-3-carboxylic acids on the macrocyclic glycopeptide-based CSPs Chirobiotic T, Chirobiotic T2, Chirobiotic TAG, and Chirobiotic R under RPM, POM, and PIM. Additionally, in order to better understand the chromatographic behavior of the β-amino acids, the effects of the different conditions — the mobile phase composition, the amount and ratio of acid and base modifier, the structure of the analytes, and the column temperature — were investigated. The separation of the stereoisomers was optimized by variation of the chromatographic parameters. The elution sequence was determined in all cases.

MATERIALS AND METHODS

Chemicals

The Diels Alder reaction of 1,3-cyclohexadiene with maleic anhydride resulted in diendo-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic acid anhydride diastereoselectively. The starting diendo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid (**1**) was prepared selectively by hypochlorite-mediated Hoffman degradation of the carboxamide obtained by ammonolysis of diendo-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic acid anhydride. Compound **1** was transformed into cis-amino acid **3** with H_2 in the presence of Pd/C.[59]

Racemic ethyl diendo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylate was prepared by a literature method.[59] The C-2 isomerization of ethyl diendo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylate with NaOEt in EtOH at 70°C resulted in ethyl 2-exo-3-endo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylate. Diendo- and endo-exo-amino ester derivatives were transformed into cis- and trans-amino ester derivatives of **3** and **4** with H_2 in the presence of Pd/C. Continuous flow hydrogenations were carried out in the H-Cube system. When subjected to microwave irradiation in H_2O at 150°C for 1 h, cis- and trans-esters gave amino acids **2** and **4**. Ethyl diendo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylate was resolved with O, O'-dibenzoyltartaric acid via diastereomeric salt formation. The synthesis of the enantiomers of diendo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid (**1a** and **1b**), 2-exo-3-endo-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid (**2a** and **2b**), cis- and trans-3-aminobicyclo[2.2.2]octane-2-carboxylic acid (**3a**, **3b**, **4a**. and **4b**) was achieved via isomerization, hydrogenation. and hydrolysis of the corresponding ester enantiomers.[60] The stereochemistry and absolute configurations of the synthesized compounds were determined by nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography.[59,60]

The difference between analytes **a** and **b** is the difference in the steric orientation in positions 1, 2, 3, and 4 (Fig. 1); they are enantiomers. Hence, the configurations of the enantiomers are 1S2R3S4R (**a**) and 1R2S3R4S (**b**) in case of sample **1** and 1S2S3S4R (**a**), 1R2R3R4S (**b**) in case of sample **2**, the analytes **1** and **2** are diastereomers. The same relationship exists between sample **3** and **4**.

Methanol (MeOH) and acetonitrile (MeCN) 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). The Milli-Q water was further purified by filtration on a 0.45-μm type HV Millipore filter (Molsheim, France).

Instruments and Chromatography

The HPLC measurements were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode-array detector, and an Empower 2 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 Empower 2 data manager software (both systems from Waters Chromatography, Milford, MA). Both chromatographic systems were equipped with Rheodyne Model 7125 injectors (Cotati, CA) with 20-μL loops.

The macrocyclic glycopeptide-based stationary phases used for analytical separation were teicoplanin-containing Chirobiotic T and T2, teicoplanin aglycone-containing Chirobiotic TAG, and ristocetin A-containing Chirobiotic R columns, 250 x 4.6 mm I.D., 5 μm particle size (for each column) (Sigma-Aldrich). Teicoplanin has 23 chiral centers surrounding four cavities. Hydrogen donor and acceptor sites are readily available, close to seven aromatic rings. Chirobiotic T and T2 are both based on silica gel with a 5-μm diameter, but the Chirobiotic T material has a 120 Å pore size and the Chirobiotic T2 material has 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 CSPs leading to steric interaction differences between the two columns. Teicoplanin aglycone is made by chemical synthesis with the removal of the three sugar moieties of teicoplanin. After the modification it contains eight chiral centers and four inclusion cavities. Ristocetin is the largest and most complex selector. It has 38 chiral centers surrounding four cavities. Six sugar moieties, a peptide chain,

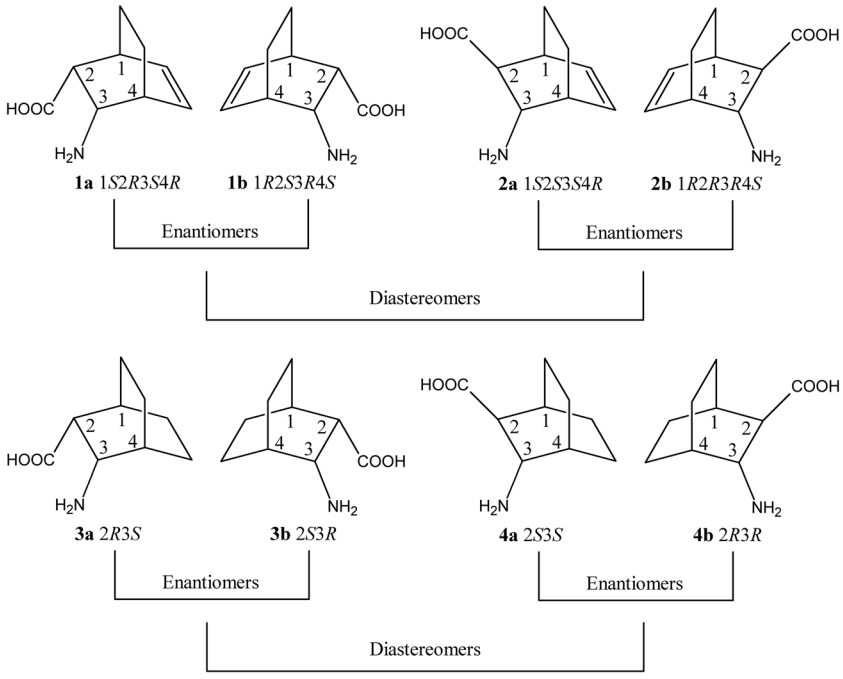

Fig. 1. Structures of analytes (1S,2R,3S,4R)-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid **(1a)**, (1R,2S,3R,4S)-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid **(1b)**, (1S,2S,3S,4R)-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid **(2a)**, (1R,2R,3R,4S)-3-aminobicyclo[2.2.2]oct-5-ene-2-carboxylic acid **(2b)**, (2R,3S)-3-aminobicyclo[2.2.2]octane-2-carboxylic acid **(3a)**, (2S,3R)-3-aminobicyclo[2.2.2]octane-2-carboxylic acid **(3b)**, (2S,3S)-3-aminobicyclo[2.2.2]octane-2-carboxylic acid **(4a)**, (2R,3R)-3-aminobicyclo[2.2.2]octane-2-carboxylic acid **(4b)**.

and additional ionizable groups give this structure the complexity and diversity to separate a wide variety of analytes.

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.

RESULTS AND DISCUSSION

The experimental conditions, including the nature and concentration of the mobile phase components and the temperature were investigated. The analytes in this study (Fig. 1) possess unsaturated and saturated bicyclic skeletons with carboxy and primary amino groups (in exo or endo position). 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 simultaneous interactions with the selector.

Enantioseparation in RPM

In RP chromatographic mode all the compounds in Figure 1 were evaluated with a minimum of six mobile phases, the eluent composition being varied between 0.1% TEAA (pH 4.1)/MeOH = 2/98 – 90/10 (v/v). For comparison purposes and to simplify the presentation, Figure 2 exhibits only the chromatographic results obtained when the enantiomeric separation was achieved; enantiomers of analyte **1** were separable on Chirobiotic T and TAG, enantiomers of **2** and **3** on Chirobiotic R, while enantiomers of analyte **4** exhibited a partial separation on Chirobiotic T at 0.1% TEAA (pH 4.1)/MeOH = 10/90 (v/v) (R_S = 0.55) and on Chirobiotic R at 0.1% TEAA (pH 4.1)/MeOH = 2/98 (v/v) eluent composition (R_S = 0.85) in the RPM.

At a given mobile phase composition, the retention factors were lowest on the ristocetin A CSP (Chirobiotic R) and highest on the aglycone CSP (Chirobiotic TAG). 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.[61] D'Acquarica et al.[62] and Péter et al.[63,64] for unusual α-amino acids and cyclic β-amino acids. Teicoplanin and teicoplanin aglycone differ in the lack of sugar units in the latter. The possible interactions strictly depend on how the enantiomers fit into the aglycone cavity which is determined, among others,by the presence of the sugar moieties and the chemical structure of the selector. The absence of the sugar groups in case of teicoplanin aglycone manifested in the largest retention, while the structure of the ristocetin A was unfavorable for the investigated analytes to the fitting of the cavity.

The effects of the MeOH content of the mobile phase were investigated on all three CSPs (Fig. 2). In most cases, a U-shaped retention curve was observed for all analogs (the exceptions were analyte **2** and analyte **4** on Chirobiotic T and TAG columns; data not shown). 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. In the RPM, one of the most important interactions between the analyte and the CSP is the hydrophobic interaction inside the "basket" of the glycopeptide. When the MeOH content of the mobile phase exceeded ~50%, the retention factor increased. This suggests that the separation may rather be controlled by the hydrophilic interaction liquid chromatography (HILIC) than by the RPM mechanism at high MeOH contents. In this study, as earlier,[44,57] 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 RPM conditions may explain the observed retention behavior. On Chirobiotic T and TAG columns a continuous increase in k was observed for analytes **2** and **4** with increasing MeOH content. The different behavior

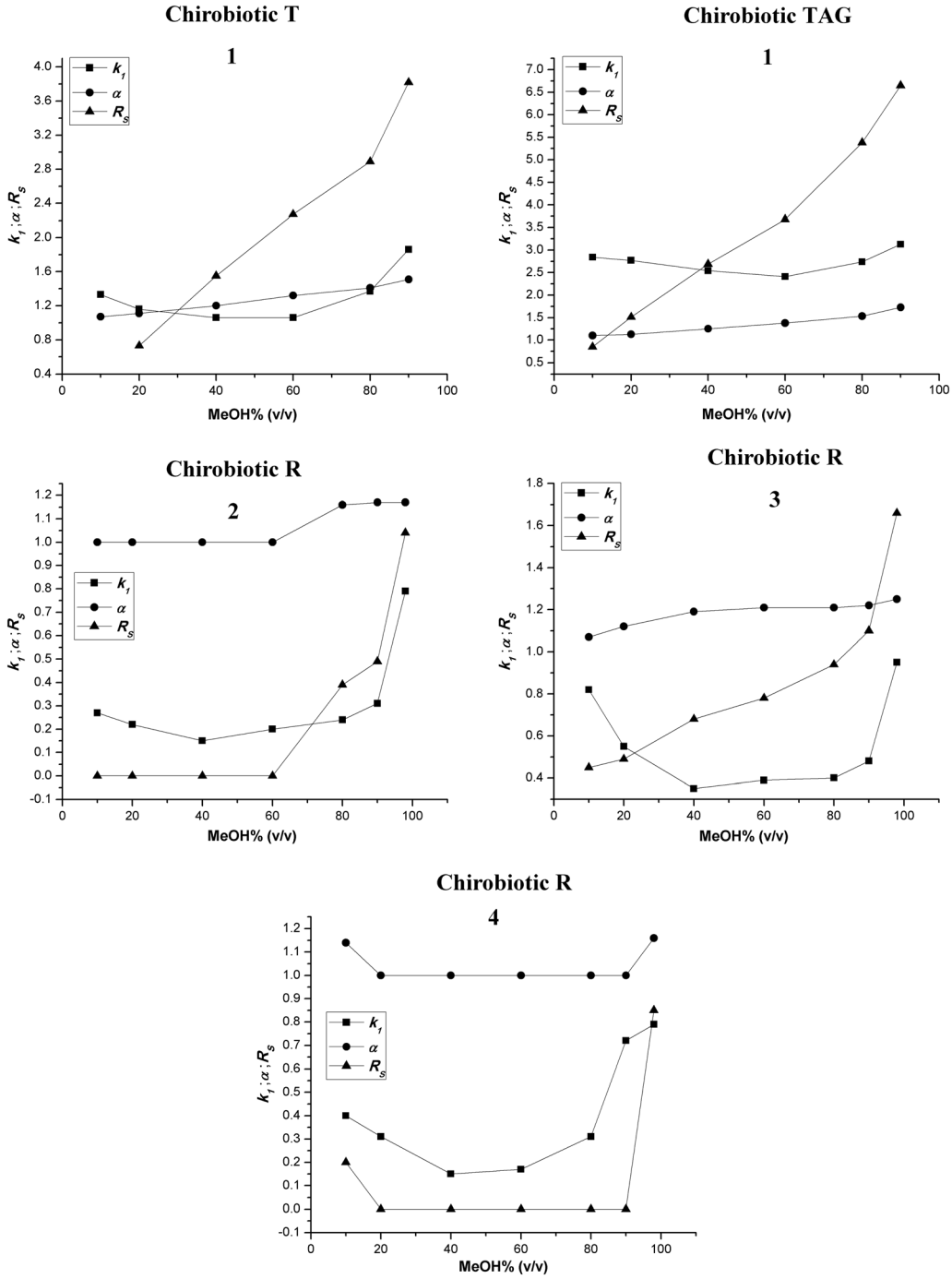

Fig. 2. Effects of MeOH content on retention factor of first-eluting enantiomer (k_1), separation factor (α) and resolution (R_S) for analog **1** on Chirobiotic T and TAG and for analogs **2**, **3** and **4** on Chirobiotic R CSP. Chromatographic conditions: mobile phase, TEAA(pH 4.1)/MeOH = 90/10 – 2/98 (v/v); temperature, 25°C; flow rate, 1.0 mL min-1; detection, 215 nm; *k*, ■; *a*, ● ; *Rs*, ▲.

of analytes **2** and **4** may be due to the different positions of the carboxy and amino groups, resulting in differences in their steric interactions with the selector.

With regard to the variations in the separation factors (α) and resolutions (R_S) with change of the MeOH content, no general trends were observed.

In the RPM in MeOH-rich mobile phases, on Chirobiotic R analytes **1** and **3** exhibited larger k_1 values than analytes **2** and **4**, respectively, indicating how the structures of the analytes influences the chiral recognition. At high MeOH contents, for the more polar analytes **1** and **3** probably the HILIC interaction resulted in higher retention and in better chiral recognition. The value of α reached its maximum when using mobile phases of high MeOH content.

Elution sequences were determined in all cases. In RPM when separation occurred on Chirobiotic T and TAG column for analyte **1** b < a while on Chirobiotic R CSP for analytes **1** and **3** a < b and for analytes **2** and **4** b < a elution sequence was obtained.

Enantioseparation in POM and PIM

Use of the MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v) mobile phase system on four columns at constant temperature (35ºC) generally resulted in lower retention and higher resolution than with 100% MeOH (Table 1). In the case of PIM and POM the k

values of the first (and the second) eluted enantiomers were lower on the teicoplanin CSPs (T and T2) than on the aglycone CSP. The different separation ability of the Chirobiotic TAG column compared to Chirobiotic T for amino acids indicates a possible difference in the separation mechanism on the teicoplanin versus the teicoplanin aglycone CSPs. 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[61]: (i) sugar units occupy the space inside of 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.

To quantify the effects of the sugar units, the differences in enantioselective free energies between the two CSPs, $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, were used. $\Delta(\Delta G^\circ)$ values were taken partly from Table 1 [$-\Delta(\Delta G^\circ) = RT \ln \alpha$]. The $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, values were plotted as shown in Figure 3. A negative number means that the stereoisomers were better separated on the aglycone CSP. A positive number means that the stereoisomers are better separated on the native teicoplanin CSP. The negative energy difference means that the absence of sugar units increases the amino acid enantiorecognition. It also indicates that the aglycone basket of the teicoplanin molecule is solely responsible for the enantiorecognition. As can be seen in Figure 3, no general trends could be observed. The differences in $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, depends on the nature of analyte and mobile phase composition, too.

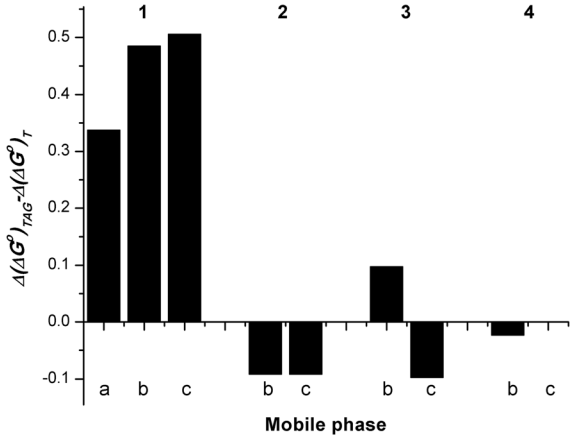

Fig. 3. Enantioselectivity free energy differences, $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, between aglycone and native teicoplanin CSPs. Chromatographic conditions: column, Chirobiotic T and Chirobiotic TAG; mobile phase, **a**, 0.1% TEAA(pH 4.1)/MeOH = 10/90 (v/v), **b**, MeOH = 100%, **c**, MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v); temperature, 25 °C; flow rate, 1.0 mL min-1; detection, 215 nm.

Comparison of the Chirobiotic T and T2 columns revealed that the retention factors on Chirobiotic T were somewhat larger (Table 1). Similar behavior was observed by Sipos et al. for the enantioseparaton of β^3-amino acids,[34] but slightly higher k values on Chirobiotic T2 than on Chirobiotic T were observed by Péter et al.[64]. These small differences may come from the different physical properties of the silica gel and the linkage of the selector. The teicoplanin-based CSPs were very

TABLE 1. Chromatographic data, retention factor (k), separation factor (α), and resolution (R_S) for direct separation of the stereoisomers of bicyclic β-amino acids on macrocyclic glycopeptide CSPs

Analyte	Column	Mobile phase MeOH/MeCN/AcOH/TEA (v/v/v/v)	k_1	α	R_S	Elution sequence
1	**T**	100/0/0/0/0	2.36	1.62	3.79	b < a
	T	100/0/0.1/0.1	2.62	1.59	3.23	b < a
2	**T**	100/0/0.1/0.1	1.51	1.10	0.76	b < a
3	**T**	100/0/0.1/0.1	1.88	1.04	<0.20	—
4	**T**	100/0/0.1/0.1	1.72	1.05	0.29	b < a
1	**T2**	100/0/0/0/0	1.90	1.49	3.14	b < a
	T2	100/0/0.1/0.1	1.82	1.48	2.63	b < a
2	**T2**	100/0/0.1/0.1	1.39	1.00	0.00	—
3	**T2**	100/0/0.1/0.1	1.42	1.00	0.00	—
4	**T2**	100/0/0.1/0.1	1.59	1.00	0.00	—
1	**TAG**	100/0/0/0/0	3.70	1.97	9.20	b < a
	TAG	100/0/0.1/0.1	3.49	1.95	6.95	b < a
2	**TAG**	100/0/0.1/0.1	2.11	1.06	0.63	b < a
3	**TAG**	100/0/0/0/0	3.33	1.04	<0.20	—
	TAG	100/0/0.1/0.1	3.14	1.00	0.00	—
4	**TAG**	100/0/0/0/0	3.80	1.05	0.30	b < a
	TAG	100/0/0.1/0.1	2.37	1.05	0.29	b < a
1	**R**	100/0/0/0/0	1.14	1.09	0.46	a < b
	R	100/0/0.1/0.1	1.12	1.07	0.50	a < b
	R	50/50/0.1/0.1	3.53	1.09	0.93	a < b
2	**R**	100/0/0/0/0	0.57	1.22	1.13	b < a
	R	100/0/0.1/0.1	0.54	1.22	1.51	b < a
	R	50/50/0.1/0.1	4.47	1.10	1.08	b < a
3	**R**	100/0/0/0/0	0.72	1.25	1.36	a < b
	R	100/0/0.1/0.1	0.72	1.24	1.93	a < b
	R	50/50/0.1/0.1	2.81	1.15	1.81	a < b
4	**R**	100/0/0/0/0	1.37	1.11	0.80	b < a
	R	100/0/0.1/0.1	0.58	1.17	1.33	b < a
	R	50/50/0.1/0.1	5.17	1.17	1.88	b < a

Chromatographic conditions: columns, **T**, Chirobiotic T, **T2**, Chirobiotic T2, **TAG**, Chirobiotic TAG, **R**, Chirobiotic R; mobile phase, MeOH/MeCN/AcOH/TEA (v/v/v/v); flow rate, 1.0 mL min^{-1}; detection, 215 nm; temperature, 35°C.

effective (Chirobiotic TAG was the most effective) for the enantioseparation of analyte **1**, but less effective in case of analytes **2–4**. The selectivity and the resolution observed in the case of **1** and **2** are similar or somewhat higher compared with samples **3** and **4** on T, T2, and TAG. The presence of the double bond in **1** and **2** ensures π–π interactions which is one of the most important interactions in case of macrocyclic glycopeptides in PIM and POM conditions.

Another obvious trend indicated by the data in Table 1 was that on the Chirobiotic R CSP, 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 PIM and POM (an exception was analyte **1**) (Table 1). The smallest k values on Ristocetin A was probably due to the lack of interaction between the carboxymethyl ester group on the selector and protonated ammonium group of analytes. On Chirobiotic R, baseline separations were achieved in cases **2**, **3**, and **4**, while for analyte **1** this CSP was less effective.

The effects of the MeOH/MeCN ratio of the mobile phase in PIM were investigated on Chirobiotic R. With increasing MeCN content, the k value and R_S also increased (except for analyte 2). This was probably due to the solvation effect both of the enantiomers and the CSP. When the MeOH content decreased, solvation of analytes in mobile phase also decreased, resulting in higher retention.

The effect of the concentration of the acid and base components was examined in the following mobile phase systems: MeOH/AcOH/TEA, **a**, 100/0.1/0.1, **b**, 100/0.05/0.05, **c**, 100/0.01/0.01, **d**, 100/0.05/0.01 and **e**, 100/0.01/0.05 (v/v/v). Decreasing the AcOH/TEA content from 0.1/0.1 (v/v) to 0.01/0.01 (v/v) k increases, indicating the importance of coulombic interactions in the separation process, while α and R_S remained similar or slightly decreased. With the variation of the ratio of acid and base additives, AcOH/TEA between 0.05/0.01 – 0.01/0.05 (v/v) a small increase in k, α, and R_S in TEA-rich mobile phase was observed.

The sequence of elution of the enantiomers was determined by spiking the racemic samples with enantiopure analytes. For analytes **1–4** on the Chirobiotic T, T2, TAG, and R columns, no consistent elution sequence was observed in PIM and POM. On the Chirobiotic T, T2, and TAG CSPs, if the separation occurred b < a an elution sequence was observed, while on Chirobiotic R analytes **1** and **3** exhibited a < b and analytes **2** and **4** b < a elution sequence. The different steric position of $–NH_2$ and –COOH groups in analytes **1**, **3** and **2**, **4** may explain the different elution sequence on Chirobiotic R.

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, TAG, and R columns over the temperature range 5–40°C (with 5 or 10°C increments). Experimental data for analyte **1** on Chirobiotic T and TAG in the mobile phase 0.1% TEAA (pH 4.1)/MeOH = 50/50 (v/v) and for analytes **2–4** on Chirobiotic R in the mobile phase MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v) are listed in Table 2. A comparison of the retention factors in Table 2 reveals that all of the recorded values decreased with increasing temperature. It is evident that an increase in the separation temperature lowers the separation factor, α. However, for analyte **3** on the Chirobiotic R column a slight increase in α (and R_S) were registered with increasing temperature

TABLE 2. Retention factor of first-eluting enantiomer (k_1), separation factor (α), and resolution (R_S) of enantiomers of bicyclic β-amino acids 1, 2, 3, and 4 on different Chirobiotic columns as a function of temperature

Column	Eluent	k_1, α, R_S	Temperature (°C)				
			5*	15	20	30	40
1							
T	**a**	k_1	1.28	1.16	1.06	0.88	0.77
		α	1.29	1.28	1.26	1.25	1.23
		R_S	2.46	2.39	2.23	1.97	1.72
TAG	**a**	k_1	3.10	2.65	2.52	2.22	1.99
		α	1.41	1.37	1.34	1.30	1.26
		R_S	3.25	3.43	3.46	3.54	3.55
2							
R	**b**	k_1	0.75	0.68	0.65	0.59	0.55
		α	1.22	1.22	1.21	1.20	1.19
		R_S	1.23	1.36	1.42	1.47	1.50
3							
R	**b**	k_1	1.05	0.91	0.84	0.76	0.68
		α	1.24	1.25	1.26	1.26	1.27
		R_S	1.71	1.87	1.91	1.96	2.05
4							
R	**b**	k_1	0.74	0.67	0.66	0.61	0.57
		α	1.23	1.22	1.21	1.20	1.18
		R_S	1.54	1.49	1.45	1.37	1.35

Columns, **T**, Chirobiotic T, **TAG**, Chirobiotic TAG, **R**, Chirobiotic R; mobile phase, **a**, 0.1% TEAA (pH 4.1)/MeOH = 50/50 (v/v), **b**, MeOH/AcOH/TEA = 100/0.1/0.1 (v/v); flow rate, 1.0 mL min^{-1}; detection, 215 nm;
*exact temperature on **T** was 8.7°C, on **TAG** 6.3°C and on **R** 5°C.

(Table 2). As concerns resolution, R_S generally decreases with an increase of temperature but for analyte **1** on Chirobiotic **TAG** and for analytes **2** and **3** on Chirobiotic **R** it increases with temperature. Increasing temperature may improve the peak symmetry (improves the kinetics of separation) 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 $\Delta H°$ and $\Delta S°^*$ values calculated from the slopes and intercepts of these plots for the enantiomers on three columns were negative (Table 3). Since the second-eluting enantiomers have more negative $\Delta S°^*$ values (except for analyte **3** on Chirobiotic R), 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 nonspecific interactions, whereas the more strongly retained one is subject to additional stereospecific interactions. Moreover, applying 0.1% TEAA(pH 4.1)/MeOH = 50/50 (v/v) mobile phase system, it was observed in the case of analyte **1** that $\Delta H°_1$ and $\Delta H°_2$, and in parallel $\Delta S°^*_1$ and $\Delta S°^*_2$, for the Chirobiotic T column were more negative than those for the Chirobiotic TAG column. This may be due to the fact that the teicoplanin aglycone (TAG) had the sugar units removed and this may hinder the interaction between the analyte and the CSP (but the difference in the interaction energies of the two enantiomers in the basket of the aglycone was higher).

In the case of the Chirobiotic R column, using the MeOH/MeCN/TEA = 100/0.1/0.1 (v/v/v) eluent system for analytes

TABLE 3. Thermodynamic parameters, ΔH°, ΔS°*, Δ(ΔH°), Δ(ΔS°), Δ(ΔG°), and T_{iso} of analytes 1, 2, 3, and 4 on Chirobiotic T, TAG and R columns

Analyte	Column	Mobile phase	Stereo isomer	-ΔH° (kJ mol^{-1})	-ΔS°* (J mol^{-1} K^{-1})	-Δ(ΔH°) (kJ mol^{-1})	-Δ(ΔS°) (J mol^{-1} K^{-1})	-TΔ(ΔS°)$_{298K}$ (kJ mol^{-1})	-Δ(ΔG°)$_{298K}$ (kJ mol^{-1})	T_{iso} (°C)
1	**T**	**a**	1	12.0	40.4	1.1	1.8	0.5	0.6	340
			2	13.1	42.2					
	TAG	**a**	1	9.4	24.4	2.4	5.6	1.7	0.7	150
			2	11.8	30.0					
2	**R**	**b**	1	6.2	24.8	0.5	0.2	<0.1	0.4	—
			2	6.7	25.0					
3	**R**	**b**	1	8.8	31.2	−0.1	−2.3	−0.7	0.6	−220
			2	8.7	28.9					
4	**R**	**b**	1	5.2	21.5	0.9	1.4	0.4	0.5	350
			2	6.1	22.9					

Chromatographic conditions: column, **T**, Chirobiotic T, **TAG**, Chirobiotic TAG, **R**, Chirobiotic R; mobile phase, **a**, 0.1% TEAA (pH 4.1)/MeOH = 50/50 (v/v), **b**, MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v); ΔS°* = ΔS° + lnΦ, where Φ is reversal of the phase ratio

2–4, the ΔH°_1 and ΔH°_2 were less negative than those for analyte **1** on Chirobiotic T and TAG. It seems that the energy of transfer from the mobile phase to the CSP in this case is less favorable. The relatively more negative ΔH° and ΔS°* values for analyte **3** indicate that the different position of the functional groups, compared with analytes **2** and **4**, may promote steric effects or different simultaneous interactions with the CSP. The -ΔH° and -ΔS°* values of **2** and **4** were similar, but slightly higher values were obtained for analyte **2** possessing an unsaturated skeleton, indicating the importance of π–π interactions in the PIM.

The differences in the changes in enthalpy and entropy, Δ(ΔH°) and Δ(ΔS°), are also presented in Table 3. The -Δ(ΔH°) values ranged from −2.4 to +0.1 kJ mol^{-1}. The interactions of **1** with the Chirobiotic TAG stationary phase were characterized by the highest negative Δ(ΔH°) value, while analyte **3** on Chirobiotic R exhibited a small positive Δ(ΔH°). The trends in the change in −Δ(ΔS°) showed that analyte **1** on Chirobiotic TAG displayed the largest negative entropies; −Δ(ΔS°) ranged from −5,6. to +2.3 J mol^{-1} K^{-1} (Table 3). For analyte **3**, similar to the Δ(ΔH°) on the Chirobiotic R column, Δ(ΔS°) also was positive. The Δ(ΔS°) 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 -Δ(ΔG°) suggests that the teicoplanin without sugar units (i.e., the TAG) induces highly efficient binding to the selector, as reflected by the large negative Δ(ΔG°) values for **1**. Analytes **2–4** exhibit a negative Δ(ΔG°) value on the Chirobiotic R column. For analytes **1**, **2**, and **4** the selector-selectand complex formation proceeds via multiple intermolecular interactions and was generally exothermic, with corresponding negative entropic contributions.

From the data of the -TΔ(ΔS°) values for analyte **3**, on Chirobiotic R, the positive Δ(ΔS°) compensated for the positive Δ(ΔH°) and resulted in a relatively high -Δ(ΔG°) value (Table 3). The ristocetin A, which contains several sugar moieties having more chiral centers, ensures more interaction sites for the analytes, leading to more negative −Δ(ΔG°) values. In this temperature range, enantioresolution is entropically driven, and the selectivity increases with increasing temperature (Fig. 4).

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 150°C, but for analyte **3** on Chirobiotic R it was −220°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 R, where positive Δ(ΔH°) and Δ(ΔS°) were observed.

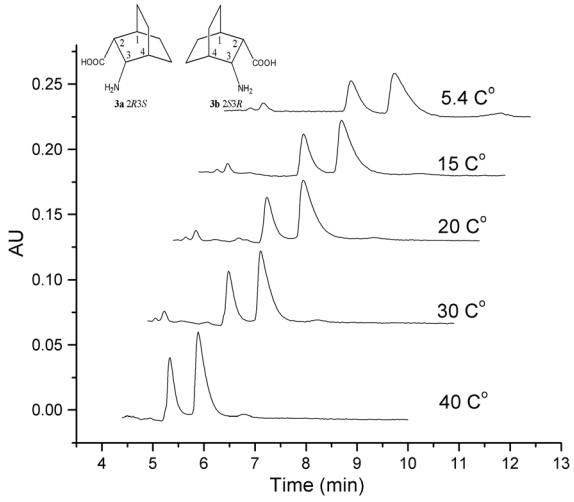

Fig. 4. Temperature dependence of separation of analyte **3**. Chromatographic conditions: column, Chirobiotic R; mobile phase, 0.1% MeOH/AcOH/TEA = 100/0.1/0.1 (v/v/v); flow rate, 1.0 mL min-1; detection, 215 nm.

CONCLUSION

HPLC methods were developed for the separation of the enantiomers of bicyclo[2.2.2]octane-based 2-amino-3-carboxylic acids, using macrocyclic glycopeptide-based CSPs: (i.e., Chirobiotic T, T2, and TAG). Baseline resolution was achieved applying different chromatographic methods. The values of thermodynamic parameters such as the changes in enthalpy, Δ(ΔH°), entropy, Δ(ΔS°), and Gibbs free energy, Δ(ΔG°), 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 behavior of these compounds.

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LITERATURE CITED

1. Abdel-Ghany M, Sharp GWC, Straub SG. Glucose stimulation of protein acylation in the pancreatic β-cell. Life Sci 2010;87:667–671.
2. Carobbio S, Ishihara H, Fernandez-Pascual S, Bartley C, Martin-Del-Rio R, Maechler P. Insulin secretion profiles are modified by overexpression of glutamate dehydrogenase in pancreatic islets. Diabetologia 2004;47:266–276.
3. Tager HS, Christensen HN. 2-Aminonorbornane-2-carboxylic acid. Preparation, properties and identification of the four isomers. J Amer Chem Soc 1972;94:968–972.
4. Kim CS, Cho SH, Chun HS, Lee SY, Endou H, Kanai Y, Kim DK. BCH, an inhibitor system L amino acid transporters, induces apoptosis in cancer cells. Biol Pharm Bull 2008;31:1096–1100.
5. Sebastianelli L, Ledonne A, Marrone MC, Bernadi G, Mercuri NB. The L-amino acid carrier inhibitor 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) reduces L-dopa-elicited responses in dopaminergic neurons of the substantia nigra pars compata. Exp Neurol 2008;212:230–233.
6. Csende F, Fülöp F, Stájer G. Chemistry of norbornane/ene and heteronorbornane/ene β-amino acids. Curr Org Synth 2008;5:173–185.
7. Kiss L, Fülöp F. Synthesis of carbocyclic and heterocyclic β-aminocarboxylic acids. Chem Rev 2014;114:1116–1169.
8. Forró E, Fülöp F. Lipase-catalyzed enantioselective ring opening of unactivated alicyclic-fused β-lactams in an organic solvent. Org Lett 2003;5:1209–1212.
9. Cheng RP, Gellman SH, DeGrado WF. β-Peptides: from structure to function. Chem Rev 2001;101:3219–3232.
10. Forró E, Fülöp F. The first direct enzymatic hydrolysis of alicyclic β-amino esters, -a route to enantiopure cis and trans β-amino acids. Chem Eur J 2007;22:6397–6401.
11. Hyyryläinen AR, Pakarinen JM, Forró E, Fülöp F, Vainiotalo P. Chiral differentiation of some cyclic β-amino acids by kinetic and fixed ligand methods. J Mass Spectrom 2010;45:198–204.
12. Steer DL, Lew RA, Perlmutter P, Smith AI, Aguilar MI. β-Amino acids: versatile peptidomimetics. Curr Med Chem 2002;9:811–822.
13. Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH. Non-haemolytic β-amino-acid oligomers. Nature 2000;404:565–565.
14. Cheng RP, Gellman SH, DeGrado WF. β-Peptides: from structure to function. Chem Rev 2001;101:3219–3232.
15. Seebach D, Beck AK, Bierbaum D. The world of β- and γ-peptides comprised of homologated proteinogenic amino acids and other components. J Chem Biodivers 2004;1:1111–1239.
16. Horne WS, Gellman SH. Foldamers with heterogeneous backbones. Acc Chem Res 2008;41:1399–1408.
17. Seebach D, Gardiner J. β-Peptidic peptidomimetics. Acc Chem Res 2008;41:1366–1375.
18. Wolin RL, Santillán A Jr, Barclay T, Tang L, Venkatesan H, Wilson S, Lee DH, Lovenberg TW. Novel glycine transporter type-2 reuptake inhibitors. Part 2: β- and γ-amino acid derivatives. Bioorgan Med Chem 2004;12:4493–4509.
19. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994;76:301–314.
20. Elices M. Review of pharmacology and therapeutic potential. Curr Opin Anti-Inflam Immunomod Invest Drugs 2000;2:228–235.
21. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 1993;11:767–804.
22. Chang LL, Troung Q, Doss GA, MacCoss M, Lyons K, McCauley E, Mumford R, Forrest G, Vincent S, Schmidt JA, Hagmann KW. Highly constrained bicyclic VLA-4 antagonists. Bioorg Med Chem Lett 2007;17:597–601.
23. Bunue E, Cativiela C, Diaz-de-Villages MD, Galvez JA. A new conformationally restricted aspartic acid analogue with a bicyclo[2.2.2]-octanes skeleton. Acta Cryst C 1996; 52:2292–2294.
24. Ruebsam F, Murphy DE, Tran CV, Li LS, Zhao J, Dragovich PS, McGuire HM, Xiang AX, Sun Z, Ayida BK, Blazel JK, Kim SH, Zhou Y, Han Q, Kissinger CR, Webber SE, Showalter RE, Shah AM, Tsan M, Patel RA, Thompson PA, LeBrun LA, Hou HJ, Kamran R, Sergeeva, MV, Bartkowski DM, Nolan TG, Norris DA, Khandurina J, Brooks J, Okamoto E, Kirkovsky L. Discovery of tricyclic 5,6-dihydro-1H-pyridin-2-ones as novel, potent, and orally bioavailable inhibitors of HCV NS5B polymerase. Bioorg Med Chem Lett 2009;19:6404–6412.
25. Songis O, Didierjean C, Laurent C, Martinez J, Calmes M. Asymmetric Diels–Alder cycloaddition of 1-aminocyclohexadiene to chiral acrylate: synthesis of enantiopure bridgehead-aminobicyclo[2.2.2]octane-2-carboxylic acid derivatives. Eur J Org Chem 2007;19:3166–3172.
26. Todorov PT, Wesselinova DW, Pavlov ND, Martinez J, Calmes M, Naydenova ED. Cytotoxic activity of new racemic and optically active N-phosphonoalkyl bicyclic β-amino acids against human malignant cell lines. Amino Acids 2012;43:1445–1450.
27. Charifson PS, Clark MP, Bandarage UK, Davies I, Duffy JP, Gao H, Feng J, Liang J, Kennedy JM, Ledeboer MW. Inhibitors of influenza viruses replication. PCT Int Appl 2012; WO 2012083117 A1 20120621.
28. Freddo JL. Preparation of [1,2,4]thiadiazine 1,1-dioxide compounds for lowering serum uric acid. PCT Int Appl 2012; WO 2012170536 A1 20121213.
29. Calmes M, Escale F, Didierjean C, Martinez J. Synthesis of enantiopure trans N-Boc-3-aminobicyclo[2.2.2]octane-2-carboxylic acids and their bicyclic 1,3-amino alcohol derivatives via the [4 + 2] cycloaddition of 1,3-cyclohexadiene to a chiral *β*-nitroacrylate. Chirality 2011;23:245–249.
30. Todorov PT, Pavlov ND, Shivachev BL, Petrova RN, Martinez J, Naydenova ED, Calmes M. Synthesis of new racemic and optically active N-phosphonoalkyl bicyclic β-amino acids via the Kabachnik-Fields reaction as potential biologically active compounds. Heteroat Chem 2012;23:123–130.
31. Andre CL, Baptiste ML, Wenger E, Didierjean C, Aubert E, Averlant-Petit MC, Martinez J, Amblard M, Calmes M. Mixed oligoureas based on constrained bicyclic and acyclic β-amino acids derivatives: on the significance of the subunit configuration for folding. Chemistry 2013;19:16963–16971.
32. Pataj Z, Berkecz R, Ilisz I, Misicka A, Tymecka D, Fülöp F, Armstrong DW, Péter A. High-performance liquid chromatographic chiral separation of β^2-homoamino acids. Chirality 2009;21:787–798.
33. Pataj Z, Ilisz I, Berkecz R, Misicka A, Tymecka D, Fülöp F, Armstrong DW, Péter A. Comparison of performance of Chirobiotic T, T2 and TAG columns in the separation of β^2- and β^3-homoamino acids. J Sep Sci 2008;31:3688–3697.
34. Sipos L, Ilisz I, Nonn M, Fülöp F, Pataj Z, Armstrong DW, Péter A. High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on macrocyclic glycopeptide-based chiral stationary phases. J Chromatogr A 2012;1232:142–151.
35. Sipos L, Ilisz I, Pataj Z, Szakonyi Z, Fülöp F, Armstrong DW, Péter A. High-performance liquid chromatographic enantioseparation of monoterpene-based 2-amino carboxylic acids on macrocyclic glycopeptide-based phases. J Chromatogr A 2010;1217:6956–6963.
36. Winkler M, Klempier N. Enantioseparation of nonproteinogenic amino acids. Anal Bioanal Chem 2009;393:1789–1796.
37. Sipos L, Ilisz I, Aranyi A, Gecse Z, Nonn M, Fülöp F, Hyun MH, Péter A. High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phases. Chirality 2012;10:817–824.
38. Berkecz R, Ilisz I, Fülöp F, Pataj Z, Hyun MH, Péter A. High-performance liquid chromatographic enantioseparation of β-3-homo-amino acid stereoisomers on a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase. J Chromatogr A 2008;1189:285–291.
39. Choi HJ, Ha HJ, Han SC, Hyun MH. Liquid chromatographic resolution of β-amino acids on CSPs based on optically active (3,3-diphenyl-1,1-binaphthyl)-20-crown-6. Anal Chim Acta 2008;619:122–128.
40. Ilisz I, Berkecz R, Forró E, Fülöp F, Armstrong DW, Péter A. The role of π-acidic and π-basic chiral stationary phases in the high-performance liquid chromatographic enantioseparation of unusual β-amino acids. Chirality 2009;21:339–348.
41. Berkecz R, Török R, Ilisz I, Forró E, Fülöp F, Armstrong DW, Péter A. LC enantioseparation of β-lactam and β-amino acid stereoisomers and a comparison of macrocyclic glycopeptide- and β-cyclodextrin-based columns. Chromatographia 2006;63:37–43.
42. Hoffmann CV, Pell R, Lämmerhofer M, Lindner W. Synergistic effects on enantioselectivity of zwitterionic chiral stationary phases for separations

of chiral acids, bases, and amino acids by HPLC. Anal Chem 2008;80:8780–8789.

43. Sun P, Wang C, Padivitage NLT, Nanayakkara YS, Perera S, Qiu H, Zhang Y, Armstrong DW. Evaluation of aromatic-derivatized cyclofructans 6 and 7 as HPLC chiral selectors. Analyst 2011;136:787–800.
44. Ward TJ, Farris AB. Chiral separations using the macrocyclic antibiotics: a review. J Chromatogr A 2001;906:73–89.
45. Berthod A. Chiral recognition mechanisms with macrocyclic glycopeptide selectors. Chirality 2009;21:167–175.
46. He X, Lin R, He H, Sun M, Xiao D. Chiral separation of ketoprofen on a Chirobiotic T column and its chiral recognition mechanisms. Chromatographia 2012;75:1355–1363.
47. Schurig V, Ossig J, Link R. Evidence for a temperature dependent reversal of enantioselectivity in complexation gas chromatography on chiral phases. Angew Chem 1989;101:197–198.
48. Allenmark S, Schurig V. Chromatography on chiral stationary phases. J Mater Sci 1977;7:1955–1963.
49. Spánik I, Krupcik J, Schurig V. Comparison of two methods for the gas-chromatographic determination of thermodynamic parameters of enantioselectivity. J Chromatogr A 1999;843:123–128.
50. Götmar G, Fornstedt T, Guiochon G. Retention mechanism of β-blockers on an immobilized cellulase. Relative importance of the hydrophobic and ionic contributions to their enantioselective and nonselective interactions. Anal Chem 2000;72:3908–3915.
51. Cavazzini A, Nadalini G, Dondi F, Gasparrini F, Ciogli A, Villani C. Study of mechanisms of chiral discrimination of amino acids and their derivatives on a teicoplanin-based chiral stationary phase. J Chromatogr A 2004;1031:143–158.
52. Oxelbark J, Allenmark S. Enantiomerization kinetics studied by dynamic enantioselective liquid chromatography: Solvent, temperature and stationary phase effects on the rate of N-benzyl-1,3,2-benzodithiazole 1-oxide enantiomer interconversion. J Chem Soc Perkin Trans 1999;2:1587–1590.
53. Thunberg L, Allenmark S, Friberg A, Ek F, Frejd T. Evaluation of two pairs of chiral stationary phases: effects from the length of the achiral spacers. Chirality 2004;16:614–624.
54. Fornstedt T, Sajonz P, Guichon G. Thermodynamic study of an unusual chiral separation. Propranolol enantiomers on an immobilized cellulase. J Am Chem Soc 1997;119:1254–1264.
55. Fornstedt T, Sajonz P, Guichon G. A closer study of chiral retention mechanisms. Chirality 1998;10:375–381.
56. Peyrin E, Guillaume YC, Guinchard C. Interactions between dansyl amino acids and human serum albumin using high-performance liquid chromatography: mobile-phase pH and temperature considerations. Anal Chem 1997;69:4979–4984.
57. Armstrong DW, Liu Y, Ekborg-Ott KH. A covalently bonded teicoplanin chiral stationary phase for HPLC enantioseparations. Chirality 1995;7:474–497.
58. Castells CB, Carr RW. A study of the thermodynamics and influence of temperature on chiral high-performance liquid chromatographic separations using cellulose tris(3,5-dimethylphenylcarbamate) coated zirconia stationary phases. Chromatographia 2000;52:535–542.
59. Palkó M, Sohár P, Fülöp F. Synthesis and transformations of di-endo-3-aminobicyclo-[2.2.2]oct-5-ene-2-carboxylic acid derivatives. Molecules 2011;16:7691–7705.
60. Palkó M, Hänninen M, Sillanpää R, Fülöp F. Syntheses of four enantiomers of 2,3-diendo- and 3-endo-aminobicyclo[2.2.2]oct-5-ene-2-exo-carboxylic acid and their saturated analogues. Molecules 2013;18:15080–15093.
61. Berthod A, Chen X, Kullman JP, Armstrong DW, Gasparini F, D'Acquarica I, Villani C, Carotti A. Role of the carbohydrate moieties in chiral recognition on teicoplanin-based LC stationary phases. Anal Chem 2000;72:1767–1780.
62. D'Acquarica I, Gasparrini F, Misiti D, Zappia G, Cimarelli C, Palmieri G, Carotti A, Cellamare S, Villani C Application of a new chiral stationary phase containing the glycopeptide antibiotic A-40,926 in the direct chromatographic resolution of β-amino acids. Tetrahedron Asymmetr 2000;11:2375–2385.
63. Péter A, Árki A, Tourwé D, Forró E, Fülöp F, Armstrong DW. A comparison of the separation efficiencies of Chirobiotic T and TAG columns in the separation of unusual amino acids. J Chromatogr A 2004;1031:159–170.
64. Sztojkov-Ivanov A, Lázár L, Fülöp F, Armstrong DW, Péter A. Comparison of separation efficiency of macrocyclic glycopeptide-based chiral stationary phases for the LC enantioseparation of β-amino acids. Chromatographia 2006;64:89–94.