ELSEVIER

Journal of Chromatography A, 838 (1999) 121–129

JOURNAL OF CHROMATOGRAPHY A

Comparison of enantioselective separation of *N-tert.*-butyloxycarbonyl amino acids and their non-blocked analogues on teicoplanin-based chiral stationary phase

Eva Tesařová[a,*], Zuzana Bosáková[b], Věra Pacáková[b]

[a]*Department of Physical and Macromolecular Chemistry, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic*
[b]*Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic*

Abstract

A teicoplanin-based chiral stationary phase (CSP) was tested for enantioseparation of underivatized amino acids and their *N-tert.*-butyloxycarbonyl (*t*-Boc) derivatives, important precursors in peptide synthesis. Mobile phase composition was optimized for organic modifier and triethylamine acetate buffer (TEAA) contents, and retention and enantioresolution of *t*-Boc-amino acids and their non-blocked analogues were compared. The importance of the amino group of amino acids in the interaction mechanism was evaluated. Native amino acids have better possibility for interaction with teicoplanin; they are more retained on the CSP and better enantioresolved than the blocked amino acids. Presence of triethylamine in the aqueous portion of the mobile phase was shown to be important for separation of enantiomers of *t*-Boc-amino acids; while native amino acids were almost not affected by the addition of TEAA. Results obtained for blocked amino acid on teicoplanin CSP with mobile phase composed of 1% triethylamine acetate, pH 4.1 and organic modifier were compared to those got on hydroxypropyl-β-cyclodextrin CSP. Good enantioseparation in a reasonable analysis time was obtained on the teicoplanin-based chiral stationary phase.

Keywords: Enantiomer separation; Teicoplanin stationary phases; Chiral stationary phases, LC; Mobile phase composition; Amino acids; Amino acids, butyloxycarbonyl derivatives

1. Introduction

Diverse properties and vital functions of enantiomers of amino acids (AAs) make their separation important in various fields of science and technology.

Blocked amino acids, among them mostly *N-tert.*-butyloxycarbonyl (*t*-Boc) derivatives, are important precursors in peptide synthesis [1]. They are resistant to racemization during synthesis of the peptide chain but their basic enantiomeric purity is essential for the quality of final products. Therefore, a fast and a powerful method to determine the enantiomeric purity of the precursors, as well as a semipreparative or preparative procedures to obtain highly purified substances are required.

Various chiral stationary phases (CSPs) were successfully used for enantioseparation of non-blocked amino acids but also for amino acids with differently blocked functional groups. Underivatized amino acids were enantioresolved in HPLC by ligand-exchange chromatography [2,3], on cyclodextrin (CD)-based CSP [4], on CSP with crown ethers

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00089-8

as chiral selectors (CSs) [5] or on teicoplanin-based chiral stationary phase [6–8]. Other CSs (bonded to silica support) with less general applications (e.g. chymotrypsin, naproxen, ergot alkaloids) were also proved to be suitable for enantioseparation of native or derivatized amino acids [9].

Electromigration methods with chiral selectors added to the background electrolyte as well as micellar chiral systems or electrochromatography with CS bonded to the capillary wall are other powerful methods which can be employed in analysis of enantiomers of amino acids [9]. Similar CSs which have been successfully employed in HPLC, e.g. crown ethers [10], amino acid–Cu^{2+} complexes [11], CDs [12–16] or macrocyclic antibiotics [17,18] are also utilized for enantioresolution of amino acids in capillary electrophoresis (HPCE). Moreover, this technique enables to combine two different CSs which can be advantageous in some cases [9,19].

Amino acids are often derivatized prior to analysis in order to improve their separation and/or to enhance the limits of detection. Derivatizing of the amino group of amino acids can change the enantioselectivity of separation significantly. Aromatic substituents can improve the enantioseparation in some cases while non-aromatic blocking groups yield worse results or even make the enantioresolution impossible. Therefore, a separation system for chiral resolution of *t*-Boc-amino acids is quite difficult to find. Amide and urea derivatives bonded on silica gel were used for enantioseparation of some *t*-Boc-amino acids in HPLC [20]. CSP with an anion-binding CS-based on metalloporphyrin gave another possibility to separate some of these enantiomers [21]. *t*-Boc-phenylalanine was also enantioresolved with CSs as mobile phase additives [22]. Some derivatives were enantioseparated on teicoplanin-based CSP [23]. Hydroxypropyl-β-cyclodextrin CSP was reported to be probably the only suitable stationary phase for enantiomeric separation of the majority of *t*-Boc-amino acid derivatives in HPLC [24]. If hydroxypropyl-β-CD was used as chiral selector for enantioseparation of these blocked amino acids in HPCE worse resolution was obtained [25].

In this work a comparison of retention behavior and enantioseparation of some *t*-Boc-amino acids and their non-blocked analogues on teicoplanin-based CSP in HPLC is presented. Enantioselective interaction mechanism is discussed with a special emphasis on the influence of the blocking group on the teicoplanin–analyte interaction.

Enantiomeric resolution of *t*-Boc-amino acids utilizing the teicoplanin-based CSP was compared with results obtained with hydroxypropyl-β-CD bonded CSP [24].

2. Experimental

2.1. Chemicals

Enantiomers of amino acids and *N*-*t*-Boc-amino acids were obtained from Sigma (St. Louis, MO, USA). The following set of compounds, both L- and D-enantiomers, was used: *N*-*t*-Boc-tyrosine (*N*-*t*-Boc-Tyr), *N*-*t*-Boc-phenylalanine (*N*-*t*-Boc-Phe), *N*-*t*-Boc-tryptophan (*N*-*t*-Boc-Trp), *N*-*t*-Boc-arginine-fosyl (*N*-*t*-Boc-Arg-Fos), *N*-*t*-Boc-leucine (*N*-*t*-Boc-Leu), *N*-*t*-Boc-alanine (*N*-*t*-Boc-Ala), and tyrosine (Tyr), phenylalanine (Phe), tryptophan (Trp), leucine (Leu) and alanine (Ala).

Chemicals used for preparation of mobile phases: Acetonitrile, gradient grade purity, and methanol, purity for chromatography, were purchased from Merck (Darmstadt, Germany). Triethylamine, purity ≥ 99%, was from Sigma and analytical-reagent grade glacial acetic acid from Lachema (Brno, Czech Republic). Deionized water was used with these experiments.

2.2. Method

HPLC equipment (Pye Unicam, Cambridge, UK) consisted of an LC–XPD pump, LC–UV detector and an injection valve Model 7125 Rheodyne (Cotati, CA, USA) with a 10-μl sample loop. Signal acquisition and data handling were performed with PC 486 software.

Chirobiotic T (Astec, Whippany, NY, USA), a commercially available steel column 250×4.6 mm I.D. packed with teicoplanin bonded to silica gel, particle size 5 μm, was utilized.

Detection wavelength was 255 nm for amino acids possessing an aromatic group and 214 nm for aliphatic derivatives. Measurements were carried out at a temperature of 24°C.

Mobile phases were composed of either water or 1.0 or 0.1% triethylamine acetate (TEAA) buffer, pH 4.1, with different portions of methanol (MeOH) or acetonitrile (ACN) as organic modifiers. The buffer pH was adjusted with acetic acid to the appropriate value before the addition of an organic modifier. Buffers were filtered through a 0.45 μm filter and sonificated before use. Flow-rate of mobile phases was 0.6 ml/min.

Void volume of the column was determined with an aqueous solution of KI and was 2.2 ml.

Samples were dissolved in methanol in concentrations 1–3 mg/ml.

3. Results and discussion

3.1. HPLC on teicoplanin-based chiral stationary phase

All measurements were performed on teicoplanin-based chiral stationary phase (see the structure in Fig. 1) in reversed-phase separation mode. This CSP proved to be suitable for chiral separation of native amino acids [6]. The influence of TEAA buffer concentration and type and content of organic modifier – methanol (MeOH) or acetonitrile (ACN) – was tested in order to find conditions for enantioresolution of *t*-Boc-amino acids. Retention data of some *t*-Boc-amino acids were compared with those obtained for their non-blocked analogues in order to reveal the influence of the blocking group on interaction with teicoplanin. It was reported that pH has almost no effect on enantioresolution of some amino acids on the teicoplanin-based CSP in the recommended pH range (pH=4.0–6.5) but lower pH seemed to be a little bit more advantageous [6,26]. Therefore, the buffer pH was adjusted to 4.1.

Fig. 2 illustrates the difference of retention behavior of *t*-Boc-phenylalanine and phenylalanine in pure aqueous–methanolic mobile phase. While enantiomers of native amino acids are well separated in this mobile phase – mainly at higher methanol content – enantiomers of the *t*-Boc-amino acids are not resolved. The sterically hindered NH_2 group has reduced possibility for electrostatic interaction with teicoplanin, thus yielding lower *t*-Boc-amino acids retention and worse enantioresolution. This result shows the importance of the amino group in the binding mechanism. Only at lower methanol content

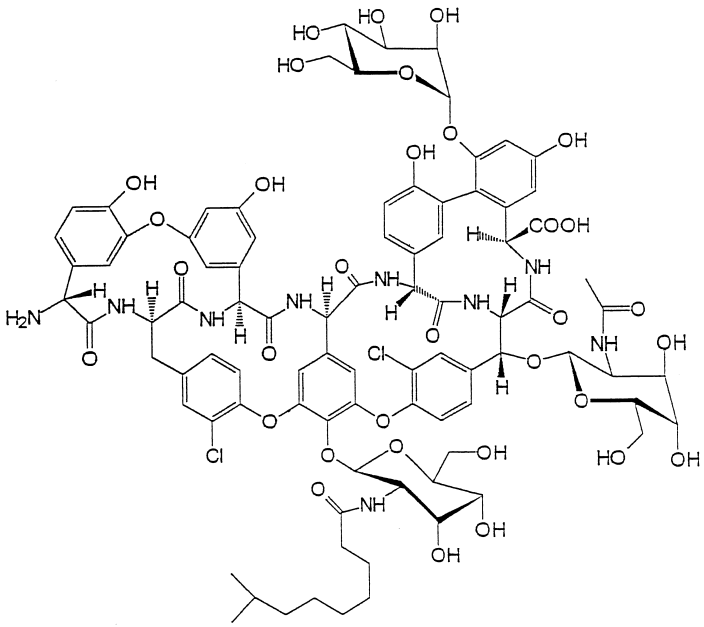

Fig. 1. Structure of teicoplanin chiral selector.

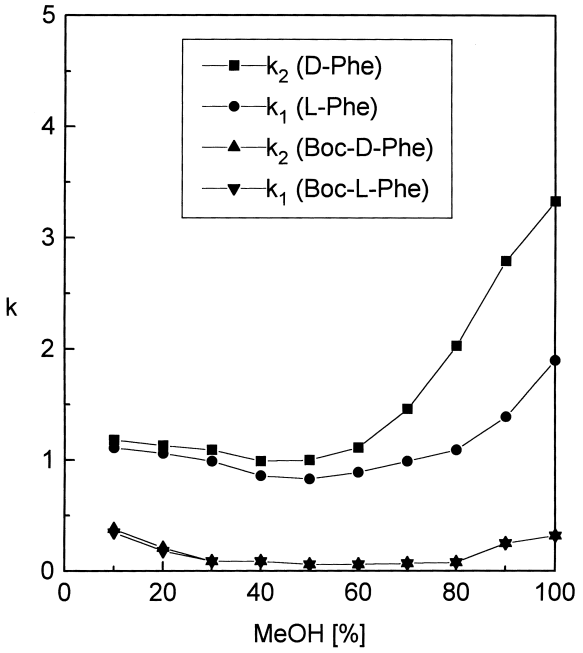

Fig. 2. Comparison of retention behavior of phenylalanine (Phe) and *N-tert*.-butyloxycarbonyl-phenylalanine (Boc-Phe) in aqueous–methanolic mobile phases with different portions of methanol. Stationary phase, Chirobiotic T; mobile phase, pure water with methanol. Note: k_1 and k_2 are the capacity factors of the L- and D-enantiomers, respectively.

in the mobile phase the retention of *t*-Boc-Phe slightly increases which is accompanied by partial enantioresolution. Enhanced hydrophobic interactions are responsible for this behavior in the water-rich mobile phase.

Table 1 summarizes the data of *t*-Boc-amino acids (retention factors of the first eluted enantiomer, k_1, and enantioresolution, R) obtained in mobile phases composed of 1% aqueous TEAA, pH 4.1, and various amounts of methanol (Table 1a) and acetonitrile (Table 1b), respectively. The retention decreases with increasing methanol content in the measured range. A similar trend has the enantioresolution up to 60% of methanol. At 80% methanol, resolution of some enantiomers again increases. An increase of retention of *t*-Boc-amino acids in the mobile phase with acetonitrile was observed at 80% of this organic modifier. It indicates a change of the interaction mechanism. Nevertheless, these stronger interactions seem to be non-enantioselective because they do not enhance the enantioresolution. Methanol, as the organic modifier, was proved to be more suitable for enantioseparation of *t*-Boc-amino acids. The best separation of enantiomers of *t*-Boc-amino acids was obtained in 20% methanol in 1% aqueous triethylamine acetate buffer; with an exception for *t*-Boc-tyrosine that was better enantioresoved in 20% acetonitrile in this buffer.

Results in Table 2a, b show the effect of buffer to organic modifier ratios on chromatographic behavior of some non-blocked amino acids. On the contrary to *t*-Boc-amino acids enantioresolution of the non-blocked amino acids increases with increasing amount of organic modifier added to the buffer. The best resolution of enantiomers of native amino acids in mobile phases containing TEAA was obtained, in a reasonable analysis time, if the ratio of methanol to 1% TEAA was 80:20. This result also indicates that

Table 1
Effect of methanol or acetonitrile contents in the mobile phase (1% aqueous TEAA, pH=4.1) on the chromatographic data of Boc-amino acids[a]

Boc-amino acid	(a) Methanol (%)								(b) Acetonitrile (%)							
	80		60		40		20		80		60		40		20	
	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R
Boc-Tyr	0.22	0.96	0.30	0.51	0.62	0.57	1.33	0.86	n		0.03	0.00	0.33	0.00	0.66	1.16
Boc-Phe	0.21	0.91	0.28	0.82	0.75	0.98	1.71	1.02	0.30	0.00	0.21	0.00	0.42	0.00	1.00	0.65
Boc-Trp	0.56	2.27	0.71	2.34	1.93	2.47	6.18	2.61	0.59	0.00	0.23	0.57	0.25	0.88	1.87	1.78
Boc-Arg	0.57	1.46	0.73	1.42	2.57	1.76	6.85	1.79	0.62	0.00	0.25	0.31	0.67	0.45	1.64	2.00
Boc-Leu	0.21	0.90	0.27	0.00	0.51	1.38	1.06	1.96	n		0.24	0.00	0.19	0.00	0.79	1.32
Boc-Ala	0.26	0.00	0.30	0.00	0.51	2.45	0.81	2.97	n		0.32	0.00	0.41	0.00	0.62	1.38

[a] Note: k_1=Retention factor of L-enantiomer. n=Not measured, solubility problems.

Table 2
Effect of methanol or acetonitrile contents in the mobile phase (1% aqueous TEAA, pH=4.1) on the chromatographic data of non-blocked amino acids[a]

Amino acid	(a) Methanol (%)										(b) Acetonitrile (%)							
	100		80		60		40		20		80		60		40		20	
	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R
Tyr	2.44	2.95	1.20	4.31	0.79	2.06	0.67	1.25	0.89	0.98	3.43	2.79	0.87	1.10	0.66	0.60	0.53	0.00
Phe	3.61	5.94	1.29	4.16	0.93	2.59	0.85	1.77	1.51	1.04	2.88	2.44	0.84	1.54	0.71	0.71	0.35	0.00
Trp	3.14	3.50	1.50	3.36	1.04	2.29	1.04	2.25	1.32	1.30	2.89	3.00	0.89	1.52	0.70	1.11	0.80	0.94
Leu	1.54	2.20	0.72	1.96	0.31	1.42	0.62	0.00	0.92	0.00	n		0.88	2.09	0.52	0.94	0.27	0.00
Ala	3.23	3.28	1.47	2.91	0.56	2.02	0.55	1.16	1.05	0.97	n		0.87	1.73	0.60	0.82	0.39	0.00

[a] Note: k_1=Retention factor of L-enantiomer; n=Not measured, solubility problems.

the presence of TEAA acid is not essential in the case of non-derivatized amino acids.

The effect of organic modifier content in the buffer containing mobile phase on enantioseparation of *t*-Boc-L-/D-Trp and L/D-Trp can be seen in Fig. 3a and b. Similar retention of both the blocked and non-blocked L-/D-tryptophan, and worse enantioresolution of the former were observed in the mobile phase composed of 1% TEAA and acetonitrile in the ratio 40:60. If this ratio is shifted to 80:20 (1% TEAA–acetonitrile) the retention of enantiomers of Boc-L-/D-Trp increases and their resolution is enhanced. Comparison of retention and enantioresolution of *t*-Boc-amino acids and their non-blocked analogues in these mobile phases shows the importance of TEAA on resolution of enantiomers of *t*-Boc-amino acids. As a consequence, lower amount of organic modifier in the mobile phase is required for satisfactory enantioresolution of *t*-Boc derivatives.

The influence of the concentration of triethylamine acetate on retention and enantioseparation of amino acids was studied in mobile phases in which the organic modifier to buffer (or water) ratio was kept constant (Table 3). Blocked amino acids exhibited much higher retention in mobile phases with TEAA, while the retention of non-blocked amino acids was only slightly affected by this factor (their highest affinity to the CSP was observed in water–methanolic mobile phase). The higher the buffer concentration the higher was the retention of *t*-Boc-amino acids. Only a weak interaction of *t*-Boc-amino acids with the teicoplanin stationary phase was observed in pure aqueous–methanolic mobile phases. Complete enantioseparation of *t*-Boc-amino acids was reached only in the presence of the TEAA buffer. This result shows on stereoselective character of interactions which enhance retention in the buffered mobile phase. The effect of triethylamine as an agent limiting interactions of analytes with free silanol groups on a C_{18} stationary phase, known from RP–HPLC, was not observed in this case; it would lower the retention. The spatial arrangement of the bulky chiral selector with saccharide moieties, which are free to rotate, does not allow the analyte to reach the silica surface. In addition teicoplanin is bonded to the carrier by three linkages. Probably triethylamine forms ion pairs with the amino acids (or teicoplanin). Such behavior can then support the hydrophobic interactions which are important in the retention mechanism of the more hydrophobic blocked amino acids.

Primary interaction of amino acids with teicoplanin (no matter if amino acids are *N*-blocked or not) is between the carboxyl group of an amino acid and amino group of the CS [6,8,27]. The carboxylic group of teicoplanin is partly sterically hindered between two sugar moieties. It is thus less suitable for interaction with the NH_2 group of the amino acid. Simultaneous interaction of NH_2 group of the amino acid with the carboxyl of teicoplanin and the carboxyl group of the amino acid with amino group of this CS is not possible, as we confirmed by molecular modelling. As is obvious from the dependencies in Fig. 2 an interaction of the NH_2 group of the amino acids with teicoplanin is necessary in

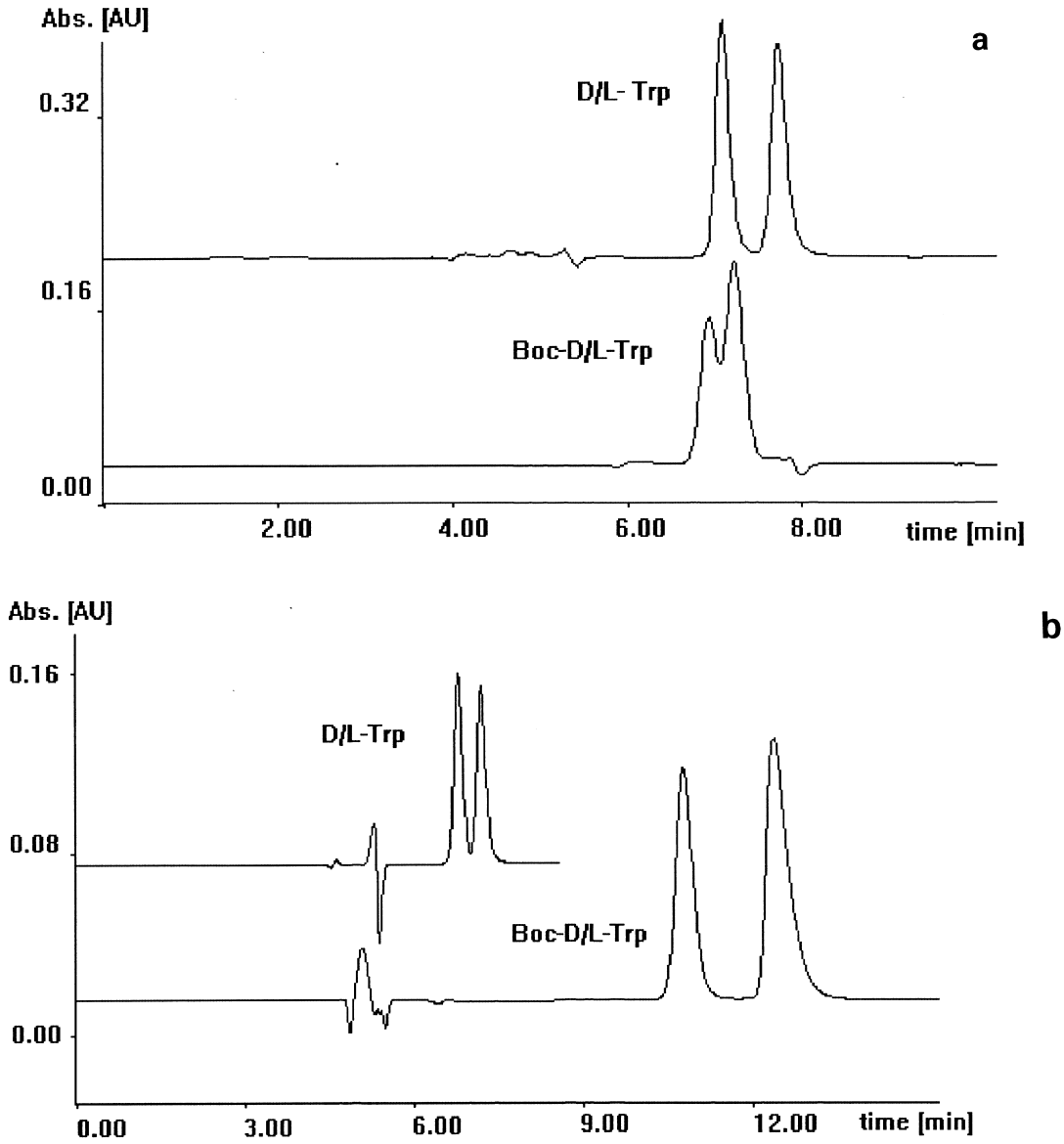

Fig. 3. Examples of enantioseparation of tryptophan (L/D-Trp) and *N-tert*.-butyloxycarbonyl-tryptophan (L/D-Boc-Trp) under two different mobile phase compositions. Stationary phase, Chirobiotic T; mobile phase (a) acetonitrile–1% triethylamine acetate, pH 4.1, (60:40, v/v), (b) acetonitrile–1% triethylamine acetate, pH 4.1 (20:80, v/v).

order to get the enantioseparation. Electrostatic interactions are most likely responsible for the enantiorecognition mechanism. If the amino group of an amino acid is blocked, the required bonding must be substituted by another type of interaction, e.g. hydrophobic and/or sterical. In addition, $\pi-\pi$ interactions contribute to the chiral recognition of amino acids in less polar mobile phases (with higher methanol content).

L-Enantiomer was always eluted first, no matter if

Table 3
Effect of concentration of TEAA in the mobile phase on the chromatographic data of some Boc-amino acids and amino acids[a]

Amino acid	40% Methanol						20% Acetonitrile					
	1.0% TEAA		0.1% TEAA		0.0% TEAA		1.0% TEAA		0.1% TEAA		0.0% TEAA	
	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R	k_1	R
Boc-Phe	0.75	0.98	0.45	0.53	0.09	0.00	1.00	0.65	0.34	0.00	0.10	0.00
Phe	0.85	1.77	0.95	1.63	0.99	1.57	0.35	0.00	0.45	0.00	0.50	0.00
Boc-Trp	1.93	2.47	1.20	0.28	0.08	0.00	1.87	1.78	1.23	1.68	0.05	0.00
Trp	1.04	2.25	1.04	1.50	1.12	1.10	0.80	0.94	1.10	1.01	1.15	0.00
Boc-Arg	2.57	1.76	2.38	1.13	0.11	0.00	1.64	2.00	1.33	1.26	0.38	0.00

[a] Note: k_1 = Retention factor of L-enantiomer.

blocked or free amino acids were enantioseparated. Neither the mobile phase composition had any influence on the retention order of these enantiomers.

3.2. Comparison of enantioseparation of N-t-Boc-amino acids using teicoplanin and hydroxypropyl-β-cyclodextrin bonded chiral stationary phases

Table 4 summarizes the enantioseparation data obtained for blocked amino acids in our measurements on teicoplanin-based CSP and those on hydroxypropyl-β-cyclodextrin stationary phase – Cyclobond I RSP, published by other authors [24]. Our experiments gave good enantioseparations at shorter separation times. Mobile phases yielding the best separation of enantiomers of *N-t*-Boc-amino acids required lower organic modifier content in both separation systems. On Cyclobond I RSP the possibility for inclusion of an analyte into the CD-cavity is reduced due to the competition with acetonitrile present in the mobile phase. The inclusion plays an important role in the interaction mechanism if CD-CS is used in a reversed-phase separation mode. With teicoplanin-based CSP the role of organic modifier and TEAA, respectively, is different. Lower methanol and/or acetonitrile contents, and thus higher amount of TEAA buffer in the mobile phase enhanced the electrostatic but also hydrophobic interactions which are responsible for enantiorecognition of *N-t*-Boc-amino acids.

4. Conclusion

Teicoplanin-based CSP proved to be suitable for enantioresolution of *N-t*-Boc-amino acids as well as non-blocked amino acids but both under different mobile phase composition. Preliminary results have shown that teicoplanin used as chiral selector in capillary electrophoresis is much more efficient for resolution of enantiomers of non-derivatized amino acids. These results support the idea that the amino group of amino acids plays an important role in stereoselective CS–analyte interaction. The work is proceeding in HPCE in order to elucidate the complexity of the interaction mechanism in different buffer solutions.

Acknowledgements

The authors would like to thank Professor Dr. D.W. Armstrong from the University of Missouri-Rolla who kindly supplied the Chirobiotic T column, and some of the derivatives of amino acids. Support of this work by the Grant Agency of the Charles University, Czech Republic (grant GA UK 12-980009) is gratefully acknowledged.

Table 4
Comparison of enantioseparation of some Boc-amino acids on teicoplanin-based and hydroxypropyl-β-cyclodextrin-based chiral stationary phases[a]

Boc-amino acid	Chirobiotic T			Cyclobond I RSP [24]		
	k_1	R	Mobile phase	k_1	R	Mobile phase[d]
Boc-Phe	1.7	1.0	20:80[b]	3.0	2.7	7:93
Boc-Tyr	0.7	1.2	20:80[c]	2.1	3.0	7:93
Boc-Trp	1.9	2.5	40:60[b]	4.6	1.9	7:93
Boc-Arg	2.6	1.8	40:60[b]	–	–	–
Boc-Ala	0.8	3.0	20:80[b]	1.3	1.8	5:95
Boc-Leu	1.1	2.0	20:80[b]	1.5	4.6	5:95

[a] Note: Chirobiotic T=teicoplanin bonded CSP; Cyclobond I RSP=hydroxypropyl-β-cyclodextrin-based CSP; k_1=Retention factor of L-enantiomer.
[b] Mobile phase: methanol–1% aqueous TEAA, pH 4.1.
[c] Mobile phase: acetonitrile–1% aqueous TEAA, pH 4.1.
[d] Mobile phase: acetonitrile–1% aqueous TEAA, pH 7.0.

References

[1] Catalog and Peptide Synthesis Handbook, Bovabiochem, Calbiochem-Novabiochem AB, Laufelfingen, Switzerland, 1997.

[2] V.A. Davankov, A.S. Bochkov, A.A. Kurganov, P. Roumeliotis, K.K. Unger, Chromatographia 13 (1980) 677.

[3] G. Gübitz, F. Juffman, W. Jellenz, Chromatographia 14 (1982) 103.

[4] D.W. Armstrong, X. Yang, S.M. Han, R.A. Menges, Anal. Chem. 59 (1987) 2594.

[5] M. Hilton, D.W. Armstrong, J. Liq. Chromatogr. 14 (1991) 9 and 3673.

[6] A. Berthod, Y. Liu, C. Bagwill, D.W. Armstrong, J. Chromatogr. A 731 (1996) 123.

[7] T.E. Beesley, New Methodology for the Purification of Amino acids and Peptides, Advanced Separation Technologies, Whippany, NJ, 1998.

[8] A. Péter, G. Török, D.W. Armstrong, J. Chromatogr. A 793 (1998) 283.

[9] E. Tesařová, D.W. Armstrong, in: Z. Deyl, I. Mikšík, F. Tagliaro, E. Tesařová (Eds.), Advanced Chromatographic and Electromigration Methods in Biosciences, Elsevier, Amsterdam, 1998, p. 197, Chapter 5.

[10] K. Kuhn, F. Erni, T. Bereuter, J. Hauser, Anal. Chem. 64 (1992) 2815.

[11] P. Gozel, E. Gassman, H. Michelsen, R.N. Zare, Anal. Chem. 59 (1987) 44.

[12] J. Snopek, E. Smolková-Keulemansová, in: D. Duchéne (Ed.), New Trends in Cyclodextrins and Derivatives, Edition de Santé, Paris, 1991, p. 483, Chapter 14.

[13] J. Snopek, E. Smolková-Keulemansová, T. Cserháti, K.H. Gahm, A. Stalcup, in: J. Szejtli, T. Osa (Eds.), Comprehensive Supramolecular Chemistry, Cyclodextrins, Pergamon, Elsevier, Oxford, 1996, p. 515.

[14] S. Pálmarsdóttir, L.-E. Edholm, J. Chromatogr. A 666 (1994) 337.

[15] A. Werner, T. Nassauer, P. Kiechle, F. Erni, J. Chromatogr. A 666 (1994) 375.

[16] T. Ueda, F. Kitamura, R. Mitchell, T. Metcalf, T. Kuwana, A. Nakamoto, Anal. Chem. 63 (1991) 2979.

[17] D.W. Armstrong, K.L. Rundlett, J. Chen, Chirality 6 (1994) 496.

[18] K.L. Rundlett, M.P. Gasper, E.Y. Zhou, D.W. Armstrong, Chirality 8 (1996) 88.

[19] D.W. Armstrong, L.W. Chang, S.S.C. Chang, J. Chromatogr. A 793 (1998) 115.

[20] N. Oi, H. Kitahara, F. Aoki, N. Kiso, J. Chromatogr. 689 (1995) 195.

[21] K. Konishi, K. Yahara, H. Toshishige, T. Aida, S. Inoue, J. Am. Chem. Soc. 116 (1994) 1337.

[22] C. Petterson, J. Chromatogr. 316 (1984) 553.

[23] D.W. Armstrong, Y. Liu, K.H. Ekborg-Ott, Chirality 7 (1995) 474.

[24] S.C. Chang, L.R. Wang, D.W. Armstrong, J. Liq. Chromatogr. 15 (1992) 1411.

[25] G.G. Yowell, S.D. Fazio, R.V. Vivilecchia, J. Chromatogr. A 745 (1996) 73.

[26] Chirobiotic Handbook, Advanced Separation Technology, Whippany, NY, 1996.

[27] M.P. Gasper, A. Berthod, U.B. Nair, D.W. Armstrong, Anal. Chem. 68 (1996) 2501.