Anal Bioanal Chem (2009) 393:1789–1796
DOI 10.1007/s00216-008-2564-0

ORIGINAL PAPER

Enantioseparation of nonproteinogenic amino acids

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Received: 23 October 2008 / Revised: 28 November 2008 / Accepted: 3 December 2008 / Published online: 11 January 2009

Abstract The enantioseparation of structurally related *N*-protected β-/γ-amino acids, β-/γ-amino amides, and β-/γ-amino nitriles by using six different commercially available chiral stationary phases (CSPs) is reported. The synthetic key step to introduce stereochemical asymmetry into all compounds is an enzymatic kinetic resolution of the racemic nitriles to the respective amino amides and/or amino acids, depending on the class of enzyme (nitrile hydratase or nitrilase) applied. The separation efficiencies of all CSPs with regard to functional groups as well as structural variations of the amino acid derivatives depicted in Fig. 1 are discussed.

Keywords Enantioseparation · HPLC · Biotransformation · β-Amino acids · γ-Amino acids · Macrocyclic glycopeptide-based CSPs · Chiral crown ether · Polysaccharide-based CSPs · Protein-based CSPs

Abbreviations

(HP)LC	(high pressure) liquid chromatography
RP	reversed phase
CSP	chiral stationary phase
3-/2-ACPA	3-aminocyclopentanecarboxylic acid
3-/2-ACHA	3-aminocyclohexanecarboxylic acid
Ts	tolylsulfonyl
ACN	acetonitrile
MeOH	methanol
AcOH	acetic acid
EtOH	ethanol
i-PrOH	isopropanol
TEA	triethylamine
TEAA	triethylammonium acetate
PCA	perchloric acid

Presented at the 9th International Symposium on Instrumental Analysis, Pécs, Hungary, July 29 to August 2, 2008

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Introduction

Nonproteinogenic amino acids such as β- and γ-amino acids play a significant role in many areas of chemistry. They occur as part of natural products many of which are of important therapeutical use. This has led to an increased demand in providing these compounds in enantiopure manner, resulting in a number of novel synthetic methods published recently [1–6].

β-Amino acids, for example, are known to possess antibiotic [7, 8], cytotoxic [9], antifungal [10], and other useful pharmacological properties [11, 12]. They serve as chiral building blocks for asymmetric catalysts [13] and for nonnatural modified peptides and also frequently occur incorporated in peptides, depsipeptides, and peptidomimetics [14].

The importance of (carbocyclic) γ-amino acids is mainly related to their potential to mimic the structure of γ-amino butyric acid (GABA) on specific receptor sites as well as to their occurrence in biologically active natural products, e.g., pepstatin [15]. GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) [16]. In recent times, the application of conformationally restricted GABA mimics—typically cyclic compounds containing a

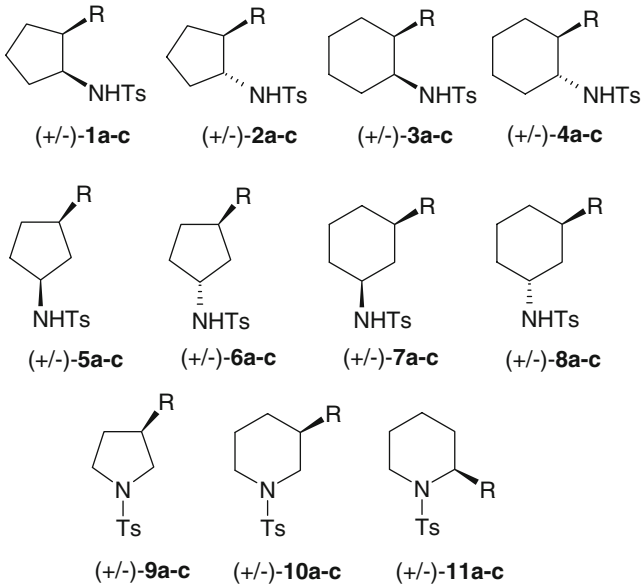


Fig. 1 Structures of β- and γ-amino acid derivatives; **a**: R=CN (all nitriles are racemic); **b**: R=$CONH_2$; **c**: R=COOH; (only one enantiomer is depicted)

rigid carbon backbone [17]—has contributed to a better understanding in GABA neuroreceptor research [18]. For example, 3-aminocyclopentanecarboxylic acid isomers were recognized to be particularly efficient stereomeric probes for GABA binding-site topography. 3-Aminocyclohexanecarboxylic acids, however, were found to act selectively as GABA uptake inhibitors [19]. Besides, analogues therefrom were investigated as therapeutic agents for a range of CNS system disorders [20].

Recently, highly enantioenriched β- and γ-amino acids and amides, respectively, have been made accessible in an enzymatic kinetic resolution step by biohydrolysis of the corresponding racemic β- and γ-amino nitriles, applying novel biocatalysts, such as nitrile hydratase [21, 22] or nitrilase [23, 24] (Scheme 1).

The determination of the enantiomeric purity of the products of an enzymatic/microbial nitrile biotransformations—amides and acids—is a challenging task. First, the strongly basic and polar amino group gives rise to serious troubles during all kinds of chromatographic analyses. Second, the isolation of this type of products from an aqueous reaction (so far the only medium for this type of biotransformation) has to take into account the high water solubility of the products—acids and amides—thus rendering an extraction into the organic phase always accompanied with high losses. Therefore and not at last by our long experience with these compounds and their pitfalls, we felt prompted to carry out our work with tolylsulfonyl protected amino groups (*N*-Ts) in all substrates [25].

In this paper, we wish to report on the enantioseparation of a range of *N*-protected β- and γ-amino acids, amides, and nitriles, respectively. The enantioseparation of β-amino acids by using chiral gas chromatography [26], chiral liquid chromatography (LC) [27–33], as well as by chiral derivatization LC [34–37] has been addressed recently. However, separations of γ-amino acids were reported less frequently [38], and a separation of *N*-Ts-protected acids presented in this work has not yet been reported. The same is true for the enantioseparation of the present amino amides and amino nitriles.

Experimental

Chemicals

n-Heptane (VWR), isopropanol (*i*-PrOH; Merck), ethanol (EtOH; Merck), methanol (MeOH; VWR), and acetonitrile (ACN; Merck) were high-performance liquid chromatography (HPLC) grade. *n*-Butanol (Roth), *i*-butanol (Roth), trifluoroacetic acid (Aldrich), triethylamine (TEA; Fluka), acetic acid (AcOH; Sigma-Aldrich), perchloric acid (PCA; 85%; Roth) and H_3PO_4 (85%; Roth) were all reagent grade. All aqueous mobile phases were filtered through 0.45 μm cellulose nitrate filter (Sartorius) prior to use.

Apparatus

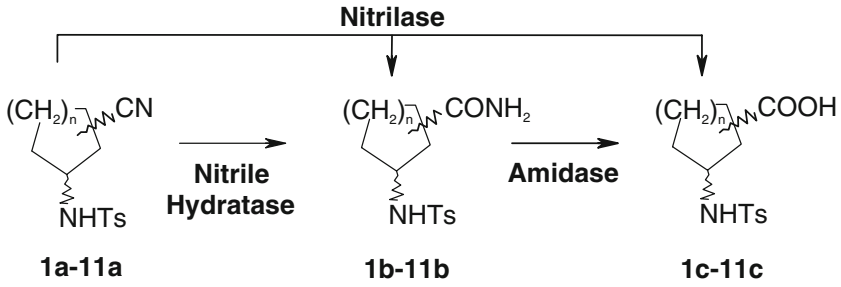
The HPLC chromatographic system used for water compatible chiral columns was a Hewlett Packard Series 1100 HPLC equipped with a G1315A diode array detector. For nonwater-compatible chiral columns, an Agilent Series 1100 HPLC equipped with a multiwavelength UV-detector was used. All analytes bearing an aromatic ring were analyzed at 254 nm, all other analytes at 205 nm.

Chiral stationary phases

The columns used were a Chirobiotic R (250×4.6 mm) 5 μm particle size (Astec); a Chiral AGP 100.4 (100×4 mm) 5 μm particle size (ChromTech); a Crownpak CR(+) (150×4 mm) 5 μm particle size (Daicel); a Chiralpak AD-H (250×4.6 mm) 5 μm particle size (Daicel); a Chiralcel OD-H column (250×4.6 mm) 5 μm particle size (Daicel); and Chiralcel OJ column (250×4.6 mm) 5 μm particle size (Daicel). The hold-up time t_0 was determined by injection of air.

From time to time, each HPLC-chiral stationary phase (CSP) was checked for unchanged and best performance using test analytes. Thus, α_1 acid glycoprotein (AGP) was found to be the only CSP which had to be regenerated several times by flushing with EtOH over night.

Scheme 1 Pathways of biotransformation of amino nitriles (n=1,2)



Substances

The syntheses as well as the associated physical and spectroscopic data for all compounds are given in [21–24] and in the literature cited therein.

Separation conditions

Chiralcel OD-H: **1a**: heptane/*i*-PrOH 50/50 (0.38 mL min^{-1}; 15 °C); **1b**: heptane/*i*-PrOH 50/50 (0.38 mL min^{-1}; 15 °C); **3a**: heptane/*i*-PrOH 50/50 (0.38 mL min^{-1}; 15 °C); **3b**: heptane/*i*-PrOH 50/50 (0.38 mL min^{-1}; 15 °C); **4a**: heptane/*i*-PrOH 85/15 (0.45 mL min^{-1}; 15 °C); **11a**: heptane/*i*-PrOH 50/50 (0.38 mL min^{-1}; 20 °C).

Chiralpak AD-H: **1a**: heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 15 °C); **1b**: heptane/*i*-PrOH 70/30 (0.5 mL min^{-1}; 15 °C); heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 15 °C); **2a**: heptane/*i*-PrOH 70/30 (0.5 mL min^{-1}; 15 °C); **2b**: heptane/*i*-PrOH 70/30 (0.5 mL min^{-1}; 15 °C); **2c**: heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 15 °C); **3a**: heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 15 °C); **3c**: heptane/n-butanol 70/30 (0.5 mL min^{-1}; 15 °C); **4a**: heptane/*i*-PrOH 70/30 (0.8 mL min^{-1}; 15 °C); **4b**: heptane/EtOH 70/30 (0.8 mL min^{-1}; 15 °C); **6a**: heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 20 °C);**7a**: heptane/EtOH 50/50 (0.5 mL min^{-1}; 20 °C); **8a**: heptane/EtOH 50/50 (0.5 mL min^{-1}; 20 °C); **9a**: heptane/EtOH 50/50 (0.8 mL min^{-1}; 20 °C); **10a**: heptane/*i*-PrOH 50/50 (0.5 mL min^{-1}; 20 °C).

Chirobiotic R: **1c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 23 °C); **2c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 23 °C); **5a**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C); **5c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C); **6a**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 23 °C); **6c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C);

9c: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C); **10c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C); **11c**: MeOH/TEA/AcOH 1000/4/1 (0.8 mL min^{-1}; 30 °C).

Chiral AGP: **2a**: phosphate buffer 10 mM (pH 7.02)/ ACN 90/10 (0.8 mL min^{-1}; 15 °C); **2c**: phosphate buffer 10 mM (pH 7.02)/ACN 90/10 (0.8 mL min^{-1}; 15 °C); **4b**: phosphate buffer 10 mM (pH 4.05)/ACN 95/5 (0.8 mL min^{-1}; 15 °C);**4c**: phosphate buffer 10 mM (pH 7.02)/ ACN 95/5 (0.8 mL min^{-1}; 15 °C); **7c**: phosphate buffer 10 mM (pH 7.02) (0.8 mL min^{-1}; 15 °C); Crownpak CR (+): **1a-c-4a-c**: PCA (pH 1; 0.5 mL min^{-1}; 15 °C).

Results and discussion

Generally, most of the existing work on enantioseparation of β- and γ-amino acids [27–37] was carried out using either pure amino acid standards or free racemic acids prepared by chemical methodology, thus reducing the problem to the chiral separation of a pair of pure enantiomers without other interfering compounds. Different from that, the analytes in our investigation are isolates from a biotransformation reaction, therefore a mixture of several enantiomeric compounds.

The amino nitriles (**1a–11a**)—substrates for the enzymatic transformation reaction shown in Scheme 1—as well as the expected products—amino acids (**1c–11c**) and amino amides (**1b–11b**)—are depicted in Fig. 1.

Initially, our ambitious goal was to separate all possible products of the biotransformation and determine the enantiomeric excess within a single chromatographic run. This goal, however, turned out to be impracticable. Besides, we also expected at the least the respective structurally related analytes (bearing identical functionalities, ring size as well as identical relative 1,2- and 1,3-substituent configuration) to share some chromatographic features to a certain extent, e.g., to be resolvable by the same CSP. In the end, however, it turned out that predictable similarities in separation behavior of even structurally closely related compounds could only be noticed for the chiral crown ether-based CSP .

Chiral crown ether-based CSP

Chiral crown ethers (*Crownpak CR*(+)) are known to be selectors for compounds bearing primary amine functionalities near a chiral center, capable of complex formation with the crown ether oxygen atoms. Hence, the separation of amides (e.g., sulfonamides or benzamides) is not possible on these CSPs. Unprotected β-amino acids (Fig. 2) have been

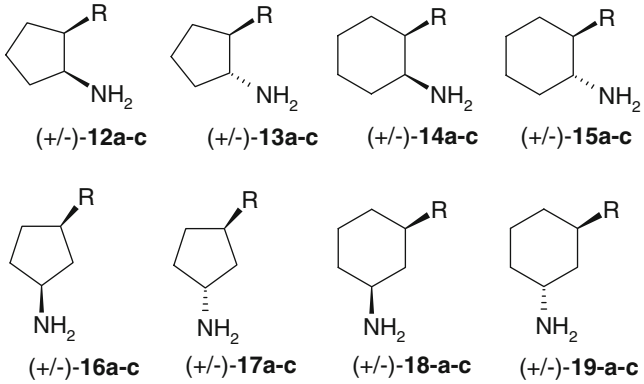


Fig. 2 *N*-unprotected β- and γ-amino acid derivatives; **a**: R=CN (all nitriles are racemic); **b**: R=$CONH_2$; **c**: R=COOH; (only one enantiomer is depicted)

successfully resolved on Crownpak CR(+) [39] (Table 1). The complex stability can be modulated by variation of the pH of the mobile phase.

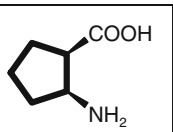
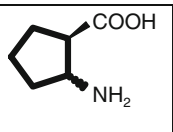
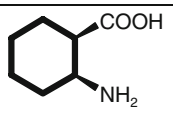
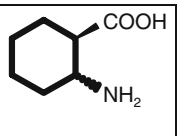
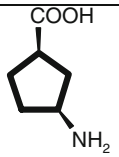
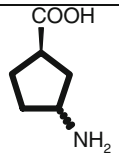
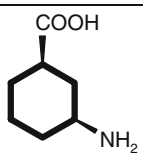
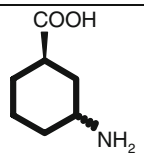
The ring size of the alicyclic analytes **12a**–**c**–**15a**–**c** was found not to affect the separation of the enantiomers substantially; however, due to different solubility of nitrile, amide and acid, the content of organic modifier had to be adapted in order to achieve suitable retention times ($HClO_4$aq/ACN 90:10; pH=1). Generally, we achieved better resolution for *trans*-isomers (**13a**–**c** and **15a**–**c**) than for the *cis*-isomers (**12a**–**c** and **14a**–**c**), which could only be partially separated, in accordance to previously reported results [35]. Increasing resolution efficiency was noticed in the order from β-amino amides and acids, respectively, to amino nitriles. As far as the free γ-amino acid derivatives **16a**–**c**–**19a**–**c** were concerned, none of them could be separated using Crownpak CR(+). Instead, we had to resort to a traditionally successful method, the indirect separation via chiral derivatization by *o*-phthalic dialdehyde/*N*-acetyl cysteine using a RP-18 column.

Polysaccharide-based CSPs

The enantioseparation of amino nitriles **1a** through **11a**—the remaining enantiomers of the kinetic resolution—is best accomplished by Chiralpak AD-H, an amylose tris-(3,5-dimethylphenylcarbamate) CSP (Tables 2, 3, and 7). Interestingly, Chiralcel OD-H, a cellulose tris-(3,5-dimethylphenylcarbamate) CSP, completely failed in separating all γ-amino nitriles **5a**–**8a** (Table 3) as well as heterocyclic nitriles **9a**–**11a** (Table 7). Generally, the separation of *trans*-carbocyclic nitriles **2a**, **4a**, **6a**, and **8a** was superior to the *cis*-isomers, and Chiralpak AD-H was also found to be superior to Chiralcel OD-H in terms of resolution power. The results from the separation of β-amino amides are more diverse. With the exception of carbocyclic six-ring amides **b** and **b**, which could be separated complementary by Chiralcel OD-H and Chiralpak AD-H, all amides **1b**–**4b** were resolved by these CSPs (Table 4). This was rather unexpected since we anticipated the high polarity and low solubility of the amides in the eluents (hexane/*i*-PrOH) to be an obstacle. The separation results of the respective carboxylic acids **1c** through **11c** are somewhat more diverse. Chiralpak-AD-H resolved all β-amino acids **1c**–**4c**, but not

Table 1 Separation of free β-amino acids **12c**-**15c** and γ-amino acids **16c**-**19c**

Separation of free β-amino acids **12c** - **15c** and γ-amino acids **16c** - **19c**

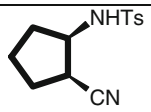
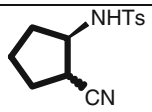
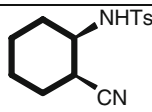
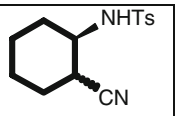
				
Crownpak CR (+)	+	+	+	+
OPA/NAC	+	+	+	+
				
Crownpak CR (+)	-	-	-	-
OPA/NAC	+	+	+	+

+ baseline separation - no separation

+ baseline separation, − no separation

Table 2 Separation of β-amino nitriles **1a**–**4a**

Separationof β-amino nitriles**1a**–**4a**

				
Chiralcel OD-H	+	-	+	+
Chiralpak AD-H	+	+	+	+
Chirobiotic R	-	-	-	-
Chiral AGP	-	+	-	-

+ baseline separation - no separation

+ baseline separation, − no separation

so did Chiralcel OD-H (Table 5). Likewise, neither of the acids **5c**–**11c** was separable on Chiralpak-AD-H or Chiralcel OD-H (Table 6). For synthetic reasons, none of the separations of γ-amino acid **8c** could be worked out, since all attempts in preparing the racemic reference acid failed. This was due to extensive epimerization of the *trans*-amino nitrile **8a** at carbon-1 to the respective all-equatorial 1,3-*cis*-amino acid **7c** when subjected to strong alkaline hydrolysis to yield *trans*-acid **8c** [23].

The obscure separation behavior of Chiralcel OJ only enabled the separation of **3c**, **4c**, and **4b** (Table 4), independent of their *cis*/*trans*-relative stereochemistry.

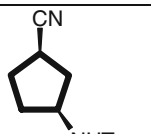
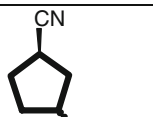
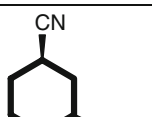
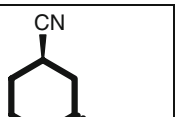
As mentioned in "Experimental," the portion of alcohol in the eluent composition is generally high (15–50% *i*-PrOH in *n*-heptane when operating with Chiralcel OD-H and up to 30% EtOH in *n*-heptane, in certain cases 100% EtOH, when operating with Chiralpak AD-H). As a consequence of this unusually high alcohol portion in the mobile phase, the flow rates had to be adjusted according to maximum pressure requirements of the columns. In addition, to lower eluent viscosity, the temperature was raised to 40 °C when 100% EtOH was used. Irrespective of this, **3c** could be only sufficiently separated when using *n*-BuOH in the eluent composition with *n*-heptane; all other alcohols failed.

Macrocyclic glycopeptide-based CSP

Ristocetin A derived CSPs (Chirobiotic R) are capable to offer multiple interaction sites, such as electrostatic, hydrogen bonding, dipole–dipole, π–π complexation as well as hydrophobic effects to diverse analytes. Armstrong and coworkers [40] have separated a plethora of free and *N*-protected α-amino acid derivatives and small peptides on Chirobiotic R. These authors knowingly commented on the benefits for detection and quantification of protected amino acids in "real world samples," such as from a biotransformation reaction. However, a separation of *N*-protected β- and γ-amino acid

Table 3 Separation of γ-amino nitriles **5a**–**8a**

Separation of γ-amino nitriles **5a** – **8a**

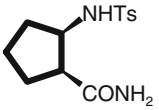
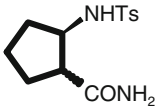
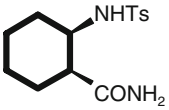
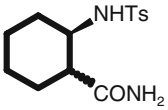
				
Chiralcel OD-H	-	-	-	-
Chiralpak AD-H	-	+	+	+
Chirobiotic R[a]	+	-	-	-
Chiral AGP[a]	-	-	-	-

+ baseline separation - no separation

+ baseline separation, − no separation

Table 4 Separation of β-amino amides **1b**–**4b**

Separation of β-amino amides **1b** – **4b**

				
Chiralcel OD-H	+	+	+	-
Chiralpak AD-H	+	+	-	+
Chiralcel OJ	-	-	+	-
Chirobiotic R	-	-	-	-
Chiral AGP	-	-	-	+

+ baseline separation - no separation

+ baseline separation, − no separation

derivatives of the present kind has not been reported with macrocyclic glycopeptidic CSPs up to now.

We investigated the separation capability of this CSP using reversed-phase eluents (MeOH/H_2O or ACN/H_2O mixtures) and the reportedly more successful polar organic mode elution conditions, utilizing MeOH/TEAA (MeOH/TEA/AcOH 100/0.4/0.1; *v*/*v*/*v*). Although Chirobiotic R represents the CSP of choice for (free and *N*-derivatized) amino acids, to our surprise, only cyclopentane carbocyclic acids **1c**, **2c**, **5c**, and **6c** could be sufficiently resolved but none of the respective six-membered acids, irrespective of any *cis*/*trans* stereoisomery as well as position of the two ring substituents. All heterocyclic amino acids (**9c**–**11c**, Table 7) were not nearly baseline separated.

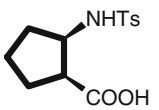
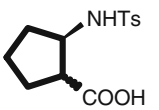
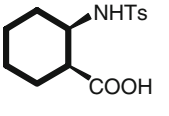
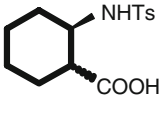
Moreover, the complete failure of Chirobiotic R to separate a single amide among the amides **1b**–**11b** came notably unexpected. The failure of resolution of amino nitriles **1a**–**11a** was likely due to the Ristocetin macrocycle lacking the strong ionic interaction between its free amino group and the carboxylic functionality of the acids. However, in this case, the carbocyclic ring size also played an additional influential role on the separation behavior.

Protein-based CSP

Proteolytic analytes such as carboxylic acids, amines, etc. are the classical analytes for protein-based CSP. During our work, Chiral AGP, an α_1 acid glycoprotein CSP, was found

Table 5 Separation of β-amino acids **1c**–**4c**

Separation of β-amino acids **1c** – **4c**

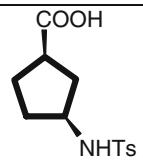
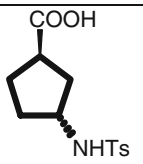
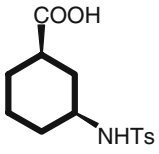
				
Chiralcel OD-H	-	-	-	-
Chiralpak AD-H	+	+	+	+
Chiralcel OJ	-	-	+	+
Chirobiotic R	+	+	-	-
Chiral AGP	-	+	-	+

+ baseline separation -no separation

+ baseline separation, − no separation

Table 6 Separation of γ-amino acids **5c**–**8c**

Separation of γ-amino acids **5c** – **8c**

			
Chiralcel OD-H	-	-	-
Chiralpak AD-H	-	-	-
Chirobiotic R[a]	+	+	-
Chiral AGP[a]	-	-	+

+ baseline separation -no separation [a] the separation of amino acid **8c** could not be accomplished since all attempts to prepare the racemic reference acid failed

+ baseline separation, − no separation
[a] The separation of amino acid **8c** could not be accomplished since all attempts to prepare the racemic reference acid failed

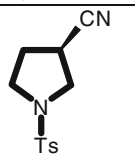
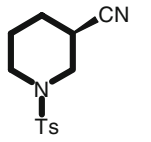
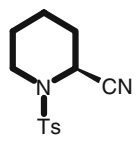
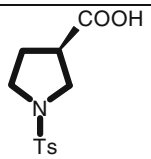
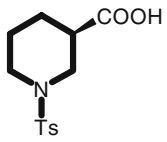
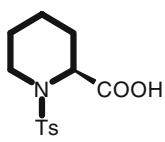
to be best applicable in resolving *trans*-β-amino carboxylic acids **2c** and **4c** (Table 5) as well as γ-amino acid **7c** (Table 6). The separation of acid **8c** (Table 6) could not be worked out due to the reasons mentioned before. The separation of amide **4b** (Table 4) as well as nitrile **2a** (Table 2) was achieved by slightly varying the organic modifier between 0% and 15% for optimal capacity ratio of the more lipophilic nitriles when compared to the acids. This is consistent with a certain reversed-phase behavior reported for this kind of CSP [41]. The complete loss of resolving power for five-membered amide *trans*-**2b** and six membered amide *cis*-**3b** is pointing out once more the unpredictable response of the CSP with respect to ring size, position of ring substituents, and their relative stereochemistry. In the instance of the amino acids not separable on this CSP, attempts to add ACN up to 5% resulted in a drop of retention and complete loss of separation. Therefore, the organic modifier was omitted giving rise to higher capacity ratios for the acids. Regardless of that, the resolution remained insufficient for a quantitative determination of the enantiomeric excess.

Conclusions

In view of the inconsistency in separation behavior of the present CSPs towards structurally closely related analytes (Fig. 1), the benchmark to separate a mixture of nitrile-, amide-, and acid-enantiomers in a single chromatographic run was initially set too high. However, this was not only

Table 7 Separation of heterocyclic amino nitriles **9a**–**11a** and amino acids **9c**–**11c**

Separation of heterocyclic amino nitriles **9a** – **11a** and amino acids **9c** – **11c**

			
Chiralpak AD-H	+	+	+
			
Chirobiotic R	+/-	+/-	+/-

+ baseline separation - no separation +/- no adequate separation

+ baseline separation, − no separation, +/− no adequate separation

due to the failure of the CSPs in separating them but also due to overlapping peaks even in the rare incident of concomitant enantioseparation of each of the acid derivatives.

Moreover, most of the resolutions required tedious optimization efforts.

In summary, the investigation of the separation properties of four different types of CSPs (six CSPs) towards *N*-protected β-amino acid and γ-amino acid derivatives has nevertheless revealed some trends: α_1 acid glycoprotein CSP (Chiral AGP) and Chiralpak AD-H were found to be the most versatile CSPs for the compounds presented in this investigation, covering the majority of enantioseparations as depicted in Tables 2, 3, 4, 5, 6, and 7. Chiralcel OJ turned out to be the least suitable of all the CSP tested.

The overall performance of Chirobiotic R was somewhat disappointing, and good resolution could be achieved only for the cyclic five ring acids **1c**, **2c**, **5c**, and **6c**, regardless of the relative *cis*/*trans*-configuration of the ring substituents. At least, in this case, there is a certain consistency to notice in the separation behavior of the CSP.

The crown ether-based Crownpak CR(+) CSP efficiently separated all free β-amino acid derivatives and—evidently—any *N*-protection prevented from separation.

Acknowledgments We acknowledge the financial support of his work by the Austrian Science Fund (FWF Project No. P15810).

References

1. Juaristi E (ed) (1997) Enantioselective synthesis of β-amino acids. Wiley-VCH, New York
2. Cardillio G, Tomasini C (1996) Chem Soc Rev 25:117–128
3. Liljeblad A, Kanerva LT (2006) Tetrahedron 62:5831–5854
4. Mazzini C, Lebreton J, Alphand V, Furstoss R (1997) J Org Chem 62:5215–5218
5. Ordonez M, Cativiela C (2007) Tetrahedron Asymmetry 18:3–99
6. Chenevert R, Martin R (1992) Tetrahedron Asymmetry 3:199–200
7. Grieco PA, Hon YS, Perez-Medrano A (1988) J Am Chem Soc 110:1630–1631
8. Hamada M, Takeuchi T, Kondo S, Ikeda Y, Naganawa H, Maeda K, Umezawa H (1970) J Antibiotics 23:170–171
9. Georg GI, Chen TT, Ojima I, Vyas DM (eds) (1995) Taxane anticancer agents: basic science and current status. ACS, Washington DC
10. Kuhl A, Hahn MG, Dumic M, Mittendorf J (2005) Amino Acids 29:89–100
11. Abdel Magid AF, Cohen JA, Maryanoff CA (1999) Curr Med Chem 6:955–970
12. Ojima I, Lin S, Wang T (1999) Curr Med Chem 6:927–954
13. Davies SG, Sheppard RL, Smith AD, Thomson JE (2005) Chem Commun 30:3802–3804
14. Steer DL, Lew RA, Perlmutter P, Smith AI, Aguilar MI (2002) Curr Med Chem 9:811–822
15. Umezawa H, Aoyagi T, Morishima H, Matsuzaki M, Hamada M (1970) J Antibiot 23:259–262
16. Andersen KE, Sorensen JL, Lau J, Lundt BF, Petersen H, Huusfeldt PO, Suzdak PD, Swedberg MD (2001) J Med Chem 44:2152–2163, (and references therein)
17. Simonyi M (1996) Enantiomer 1:403–414
18. Chebib M, Johnston GAR (1999) Clin Experim Pharmacol Physiol 26:937–940
19. Krogsgaard-Larsen P, Froelund B, Frydenvang K (2000) Curr Pharm Des 6:1193–1209
20. Chebib M, Duke RK Allan RD, Johnston GAR (2001) Eur J Pharmacol 430:185–192
21. Preiml M, Hillmayer K, Klempier N (2003) Tetrahedron Lett 44:5057–5059
22. Winkler M, Martínková L, Knall AC, Krahulec S, Klempier N (2005) Tetrahedron 61:4249–4260
23. Winkler M, Knall AC, Kulterer MR, Klempier N (2007) J Org Chem 72:7423–7426
24. Winkler M, Meischler D, Klempier N (2007) Adv Synth Catal 349:1475–1480
25. Preiml M, Hönig H, Klempier N (2004) J Mol Catal B Enzym 29:115–121
26. Forró E, Fülöp F (2007) Chem Eur J 13:6397–6401
27. Miyazawa T, Minowa H, Imagawa K, Yamada T (2004) Chromatographia 60:45–50
28. Péter A (2002) J Chromatogr A 955:141–150
29. Péter A, Török G, Armstrong DW (1998) J Chromatogr A 793:283–296
30. Péter A, Lázár L, Fülöp F, Armstrong DW (2001) J Chromatogr A 926:229–238
31. D'Acquarica I, Gasparrini F, Misiti D, Zappia G, Cimarelli C, Palmieri G, Carotti A, Cellamare S, Villani C (2000) Tetrahedron Asymmetry 11:2375–2385
32. Péter A, Árki A, Vékes E, Tourwé D, Lázár L, Fülöp F, Armstrong DW (2004) J Chromatogr A 1031:171–178
33. Péter A, Árki A, Tourwé D, Forró E, Fülöp F, Armstrong DW (2004) J Chromatogr A 1031:159–170
34. Pétér A, Fülöp F (1995) J Chromatogr A 715:219–226
35. Pétér A, Török G, Csomós P, Péter M, Bernáth G, Fülöp F (1997) J Chromatogr A 761:103–113
36. Péter M, Péter A, Fülöp F (2000) J Chromatog A 871:115–126
37. Ilisz I, Berkecz R, Peter A (2008) J Pham Biomed Anal 47:1–15
38. Lee SJ, Cho HS, Choi HJ, Hyun MH (2008) J Chromatogr A 1188:318–321
39. Hyun MH, Cho YJ, Jin JS (2002) J Sep Sci 25:648–652
40. Ekborg-Ott KH, Liu Y, Armstrong DW (1998) Chirality 10:434–483
41. Kern J, Kirkland K (1997) Chiral separations. In: Snyder LR, Kirkland JJ, Glajch JL (eds) Practical HPLC method development. Wiley, New York