Lehrstuhl für Pharmazeutische Chemie, Universität Freiburg, Germany

Asymmetric synthesis and chromatographic resolution of all four stereomers of the α,β-propanoleucine

P. Bisel, E. Breitling, M. Schlauch, F.-J. Volk and A. W. Frahm

Dedicated to Prof. Dr. Dr. h.c. mult. H. Oelschläger on the occasion of his 80th birthday

The paper describes the first synthesis of the enantiomerically pure *cis*-α,β-propanoleucines **6c** and **6d** by means of asymmetric Strecker synthesis. Furthermore, an improved procedure for the preparation of the stereomeric *trans* compounds **6a** and **6b** is proposed. Finally, the four feasible stereomeric α,α-quaternary-α-amino acids are resolved on a penicillamine based chiral stationary phase allowing the determination of *ee* values ranging from 92.9% to > 98%.

1. Introduction

Carbocyclic α-amino acids have gained much interest in the last decade. Indeed, the α,α-disubstituted α-amino acids are widely used in the isosteric replacement of proteogenic amino acids, resulting in interesting specific backbone conformations and increased stability towards enzymatic and chemical degradations [1–3]. Furthermore, this type of non proteogenic amino acids have been described as agonists and/or antagonists of both the ionotropic and metabotropic glutamate receptors [4, 5]. Some representatives served as a tool for the family and subtype classification of the latter.

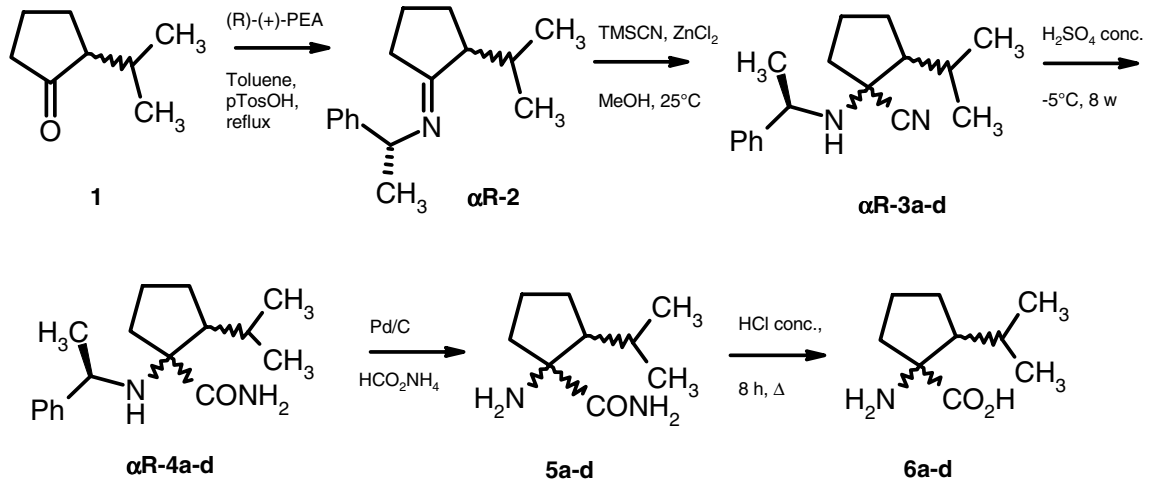
2. Investigations and results

2.1. Chemistry

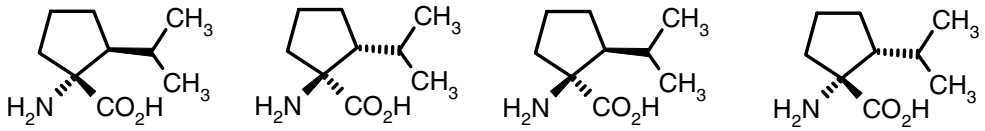
In a series of earlier works, we reported on the successful synthesis of all four feasible stereomers of various β-substituted cyclic analogues of naturally occuring amino acids by means of the asymmetric Strecker sequence starting from racemic 2-substituted cyclic ketones and (*R*)-(+)- or (*S*)-(−)-phenylethylamine (PEA) as chiral auxilliaries [6]. The major issues of this synthetic pathway were the stereochemical outcome of the cyanide addition to the imine **2** mixture and the stepwise hydrolysis of the resulting nitriles **3** to the corresponding carboxylic acids **6** (Scheme). It is noteworthy that the hydrolysis of the nitrile group of these sterically hindered compounds could not be achieved under mild acidic conditions (formic acid/HCl or HCl-gas/EtOH) and that the conversion to the corresponding amides **4** was exclusively possible in conc. H_2SO_4. Very recently, we have described the synthesis of twelve 2-alkylated-1-aminocyclopentane carboxylic acids together with a theoretical model for the cyanide addition to the nitriles [7]. Even if all four stereomers were formed during the cyanide addition step, we were not able to isolate the *cis* configured amino acids arising from 2-methylethylcyclohexanone. Indeed, unexpected side reactions occuring during the key hydrolysis step denied the access to the enantiomerically pure *cis*-2-methylethyl-1-aminocyclopentanecarboxylic acids. Herein, we wish to report on a modified procedure giving access to enantiomerically pure *cis*- and *trans*-α,β-propanoleucines **6a–d**. Furthermore, we describe the chromatographic resolution of both the enantiomers and the diastereomers of **6** on a chiral column and on the resulting enantiomeric excesses of the final amino acids.

The racemic 2-methylethylcyclopentanone **1** was prepared according to literature procedures [8] and subsequently treated with one equivalent of (*R*)-(+)-PEA to the corresponding imine mixture **αR-2** the respective ^{13}C NMR of which shows four sets of signals accounting for the four

Scheme



Asymmetric Strecker sequence exemplified with (R)-(+)-PEA. **a**: *trans* compound with "like-induction" at C-1; **b**: *trans* compound with "unlike-induction" at C-1; **c**: cis compound with "like-induction" at C-1; **d**: cis compound with "unlike-induction" at C-1



6a 6b 6c 6d

theoretically feasible diastereomeric imines. The following cyanide addition was performed in methanol under thermodynamic control giving rise to a 32/7/43/18 mixture of the four diastereomeric nitriles **αR-3a–d**. The hydrolysis of these nitriles revealed troublesome since a concuring hydrogenolysis reaction was observed under the classical conditions. Indeed, when treated with conc. sulfuric acid at room temperature, the nitrile mixture afforded four compounds, two of which with M_R 274 and the other two with M_R 169. Thus, a concomitant hydrogenolysis must have occured during the hydrolysis and it was shown that the *cis* amino nitriles suffered complete hydrogenolysis (loss of the chiral auxiliary) while the corresponding *trans* amino nitriles were only partly cleaved [7]. In order to isolate the enantiomerically pure *cis* derivatives it became essential to develop a milder hydrolysis protocol leaving the secondary amine intact. Thus, the treatment of the amino nitriles with conc. sulfuric acid at −5 °C led to smooth hydrolysis to the corresponding primary amides **αR-4a–d** within 8 weeks without any cleavage of the chiral auxiliary. The separation of the diastereomeric secondary amines **αR-4a–d** asked for successive gravity and preparative HPLC chromatography on silica gel. The gravity chromatography allowed the isolation of the minor *trans* and the major *cis* compound in 5 and 15% yield, respectively. In analogy to our previous results and assuming "like-induction", their absolute stereochemistry was assigned *trans*-**αR-1S,2R-4b** and *cis*-**αR-1R,2R-4c**, respectively. Alongside with the pure compounds we have gained several mixed fractions from which additional portions of pure *trans*-**αR-1S,2R-4b** (2%) and *cis*-**αR-1R,2R-4c** (10%), respectively, could be isolated besides the major *trans*-**αR-1R,2S-4a** (25%) diastereomer upon preparative HPLC. However, while the three pure diastereomers *trans*-**αR-1R,2S-4a**, *cis*-**αR-1R,2R-4c** and *trans*-**αR-1S,2R-4b** could be gained with 25,25 and 7% yield, respectively, the fourth diastereomer was not to be obtained from this reaction sequence. Nevertheless, from the reaction with the enantiomeric chiral auxiliary (*S*)-(−)-PEA the respective enantiomeric secondary amines *trans*-**αS-1S,2R-4a′**, *cis*-**αS-1S,2S-4c′** and *trans*-**αS-1R,2S-4b′** could be obtained.

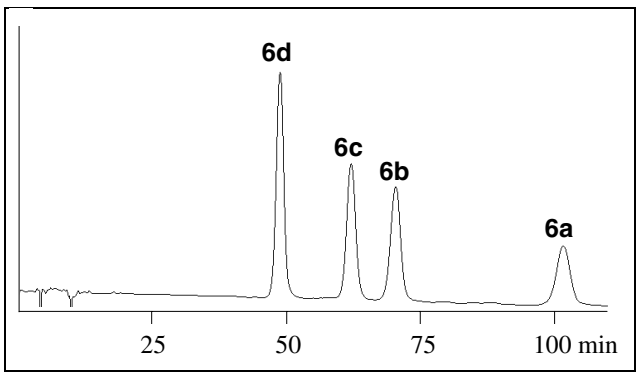


Fig.: Chromatogram of the four stereoisomers of **6** on a copper(II)-D-penicillamine CSP with a 3 mM $CuSO_4$ in methanol/water (25/75, v/v) mobile phase at 20 °C, 0.75 ml/min, UV detection 254 nm

Thus the cleavage of the chiral auxiliary by means of transfer hydrogenolysis opened the access to all four stereomers of the primary amino amides **5**. Indeed, the N-benzyl cleavage of **4a** or **4b′**, **4b** or **4a′**, **4c** and **4c′** afforded **5a**, **5b**, **5c** and **5d**, respectively, in quantitative yields. Since the free bases of the primary amino amides **5a–d** are spontaneously converted to their instable carbonates, they have been converted to the stable hydrochlorides **5a–d** · **HCl** for analysis purpose. Final hydrolysis of the crude free bases with conc. HCl afforded all four desired amino acids **6a–d** upon elution from a strong acidic ion exchange resin with ammonia.

2.2. Chromatographic resolution of the stereoisomers

Since the biological activity of amino acids, like most of the chiral compounds, is usually strongly related to their absolute stereochemistry, one of our main concerns remains the determination of both the diastereomeric and the enantiomeric excesses of the final amino acids. The classical analyses via derivatisation with chiral reagents suffer from major drawbacks including lack of enantiopurity of the reagents and the need for completion of the derivatisation reactions. Thus, we have developed HPLC methods for the direct determination of *ee* values of amino acids on chiral stationary phases. The Chirobiotic T column based on the glycopeptide antibiotic teicoplanin was found to be an effective tool for the separation of several α-quaternary-β-substituted-α-amino acids [9]. However, the resolution of **6a–d** could not be achieved on this column probably due to dramatic steric effects. Indeed, since the corresponding 2-ethyl-substituted amino acids are well separated, it seems obvious that the bulk of the methyl-ethyl moiety hinders the chiral discrimination process. In contrast, the Chirex (D) penicillamine column introduced by Ôi et al. [10] for the chiral analysis of amino acids proved successful for the separation of various carbocyclic α,α-disubstituted-β-substituted amino acids [11]. Chiral discrimination on this type of columns is based on the diastereomeric interaction of the enantiomeric analytes and the chiral selector with Cu^{2+} as metal ion [12]. Here, the amine and the carboxyl functionalities of the solute interact with the copper (II)-penicillamine complex of the coated reversed phase silica gel chiral stationary phase. The optimised chromatographic conditions with 3 mM $CuSO_4$ in MeOH/H_2O (1/3, v/v) mobile phase eluting at a rate of 0.75 ml/min and 20 °C allowed the baseline separation of all four stereomers of **6** in one single run (Fig.).
The observed elution order with the 1*S*-enantiomer eluting in front of the 1*R*-enantiomer in both the *cis* and *trans* series is in agreement with the rule established by Davankov et al [12]. Furthermore, it is also in accordance with the elution order observed for the corresponding 2-ethyl substituted carbocyclic amino acids. However, the 2-methyl substituted analogues cannot be baseline separated under similar chromatography conditions [11] confirming that the hydrophobic interactions between the analyte and the apolar surface of the packing material play an

essential role in the separation mechanism [13]. The analysis of the pure samples under the optimised conditions gave *ee* values ranging from 92.9% to >98% and *de* values >98%.

3. Experimental

3.1. Chemistry

Gravity column chromatography was performed on Merck silica gel (70–230 mesh ASTM). The uncorrected open capillary melting points were determined with a Mel-Temp II apparatus (Devices Laboratory USA). Optical rotations were measured on a Perkin-Elmer 241 spectrometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD with tetramethylsilane as internal standard on a Varian Unity 300 spectrometer. The chemical shifts are given in δ ppm. Microanalyses were carried out at the Chemische Laboratorium der Universität Freiburg. All the results were in an acceptable range. Preparative HPLC separation was carried out on a silica gel column (LiChrosorb Si 60, 5 µm, L = 250 mm, Ø 10 mm) with detection at 254 nm. The elution rate was 0.7 ml/min with ethylacetate/hexane 3.5/6.5 as the mobile phase.

3.1.1. E/Z-2-(RS)-N-[(R)-1-Phenylethyl]-2-(methylethyl)cyclopentylidenamine (**2**)*, αR-2-(methylethyl)-1-(phenylethylamino)cyclopentanecarbonitriles* ***3a–d*** *and αS-2-(methylethyl)-1-(phenylethylamino)cyclopentanecarbonitriles* ***3a′–d′***

The nitrile mixtures have been obtained from the addition of TMSCN to **2** in methanol at RT according to ref. [7] where the compounds **2**, **3a–d** and **3a′–d′** are described.

3.1.2. αR-2-(Methylethyl)-1-(phenylethylamino)cyclopentanecarboxamides ***4a–c***

The nitrile mixtures **3a–d** (3.3 g, 13 mmol) dissolved in 1.5 ml CH_2Cl_2, were added dropwise to 25 ml H_2SO_4 conc. at −5 °C. Stirring was maintained at the same temperature for 8 weeks. The reaction mixture was poured on ice, adjusted to pH 8 with a 25% ammonia solution and extracted 3 times with 1 volume diethylether. The combined organics were dried, filtered and evaporated in vacuo. The residue (2.7 g, 76%) was submitted to flash chromatography on silica gel (440 g) eluted with ethylacetate/hexane : 3/7 and **4b** (135 mg, 5%), **4c** (405 mg, 15%) and a mixed fraction (1.08 g, 40%) were isolated. The mixed fraction was submitted to preparative HPLC on a silica gel column eluted at 0.7 ml/min with ethylacetate/hexane 3.5/6.5 as the mobile phase. In each run 10 mg of the mixture were injected in 500 µl solvent. Several runs finally yielded **4b** (21 mg), **4c** (108 mg) and **4a** (270 mg, 25%).
trans-**αR-1R,2S (4a)** and *trans*-**αR-1S,2R (4b)**: for analytical data see ref. [7].
cis-**αR-1R,2R (4c)**: colorless oil; $[\alpha]_D^{20}$ −6.5 (c = 0.4, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (1 H, s br, CONH_2), 7.35-7.05 (5 H, m, *H-arom.*), 6.65 (1 H, s br, CONH_2), 3.88 (1 H, q, J = 6.6 Hz, *H*-α), 2.20–1.80 (4 H, m, H-2, H-3, 2 × H-5), 1.75–1.40 (3 H, m, H-3, H-4, C*H*(CH$_3$)$_2$), 1.36 (3 H, d, J = 6.6 Hz, CHC*H*$_3$), 1.40–1.20 (2 H, m, H-3, NH), 1.16–0.98 (1 H, m, H-4), 0.96 (3 H, d, J = 6.5 Hz, CH(C*H*$_3$)$_2$), 0.88 (3 H, d, J = 6.5 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 181.67 (CONH$_2$), 147.41 (C-1′), 128.52 (C-3′/5′), 126.83 (C-4′), 125.87 (C-2′/6′), 70.58 (C-1), 57.26 (C-2), 54.02 (C-α), 33.47 (C-5), 28.81 (C-3), 28.08 (CH(CH$_3$)$_2$), 26.15 (C-β), 22.15 (CH(*C*H$_3$)$_2$), 22.01 (CH(CH$_3$)$_2$), 21.38 (C-4).

3.1.3. αS-2-(Methylethyl)-1-(phenylethylamino)cyclopentanecarboxamides **4a′–c′**

Same procedure as *3.1.2.* but with (S)-PEA.
trans-**αS-1S,2R (4a′)** and *trans*-**αS-1R,2S (4b′)**: for analytical data see ref. [7].
cis-**αS-1S,2S (4c′)**: colorless oil; $[\alpha]_D^{20}$ +5.3 (c = 0.5, MeOH); NMR data are identical to those of **4c**.

3.1.4. 1-Amino-2-(methylethyl)cyclopentanecarboxamide hydrochlorides **5a–d · HCl**

The pure secondary amino amides **4a–d** (150 mg, 0.55 mmol each) were taken up in 25 ml MeOH abs., 150 mg Pd/C (10%) and 280 mg ammonium formate were added. The mixtures were stirred and refluxed for 2 h. The catalyst was filtered off and the solvent evaporated in vacuo to yield the respective primary amino amides **5a-d** (76–81 mg, 0.45 mmol, 81–83%). For analysis the residues of **5c** and **5d**, respectively, were taken up in Et_2O. Et_2O/HCl was then added. The solvent was evaporated and the residues washed with acetone and dried in high vacuo to yield the respective **5c · HCl** and **5d · HCl** in quantitative yields.
trans-**1R,2S (5a)** and *trans*-**1S,2R (5b)**: for analytical data see ref. [7].
cis-**1R,2R · HCl (5c · HCl)**: m.p. > 240 °C (decomp.) ; $[\alpha]_D^{20}$ −6.5 (c = 0.5, MeOH); ^{1}H NMR (300 MHz, MeOD) δ 2.44–2.12 (3 H, m, H-2, H-3, H-5), 2.12–1.74 (3 H, m, 2 × H-4, H-5), 1.72–1.48 (2 H, m, C*H*(CH$_3$)$_2$, H-3) 0.99 (3 H, d, J = 6.6 Hz, CH(C*H*$_3$)$_2$), 0.95 (3 H, d, J = 6.6 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (75.4 MHz, MeOD) δ 175.44 (CONH$_2$), 69.84 (C-1), 56.1 (C-2), 39.57 (C-5), 30.09 (C-3), 30.07 (*C*H(CH$_3$)$_2$), 22.96 (CH(*C*H$_3$)$_2$), 21.99 (C-4), 21.60 (CH(*C*H$_3$)$_2$).
$C_9H_{18}N_2O \cdot HCl$ (206.7)
cis-**1S,2S · HCl (5d · HCl)**: m.p. > 240 °C (decomp.); $[\alpha]_D^{20}$ +5.3 (c = 0.5, MeOH); NMR data are identical to those of **5c · HCl**

Table: ee and de-values of compound 6

	Stereochemistry	ee	de
6a	*trans*-**1R,2S**	95.4%	>98%
6b	*trans*-**1S,2R**	92.9%	>98%
6c	*cis*-**1S,2S**	94.0%	>98%
6d	*cis*-**1R,2R**	>98%	>98%

3.1.5. 1-Amino-2-(methylethyl)cyclopentanecarboxylic acids **6a–d**

The crude amino amides **5a–d** (76 mg, 0.45 mmol each) were taken up in 6 ml HCl conc., stirred for 8 h at 80 °C and evaporated to dryness. The residues were taken up in 1.5 ml H_2O and eluted from a strong acidic ion exchange resin (Merck Eurolab GmbH Art. 4765) with 1M ammonia. The combined fractions were evaporated yielding the respective free amino acids **6a–d** (52–54 mg, 68–70%)
trans-**1R,2S (6a)** and *trans*-**1S,2R (6b)**: for analytical data see ref. [7].
cis-**1R,2R (6c)**: m.p. > 250 °C (decomp.); $[\alpha]_D^{20}$ −22.4 (c = 0.6, MeOH); ^{1}H MNR (300 MHz, MeOD) δ 2.40–2.20 (2 H, m, H-2, H-5), 2.18–2.00 (3 H, m, 2 × H-4, H-5), 1.60–1.36 (2 H, m, H-3, C*H*(CH$_3$)$_2$), 0.98 (3 H, d, J = 6.6 Hz, CH(C*H*$_3$)$_2$), 0.93 (3 H, d, J = 6.6 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (75.4 MHz, MeOD) δ 177.90 (COOH), 70.43 (C-1), 55.36 (C-2), 39.16 (C-5), 30.39 (C-3), 30.00 (*C*H(CH$_3$)$_2$), 23.10 (CH(*C*H$_3$)$_2$), 22.61 (C-4), 21.84 (CH(*C*H$_3$)$_2$).
$C_9H_{17}NO_2$ (171.2)
cis-**1S,2S (6d)**: m.p. > 250 °C (decomp.); $[\alpha]_D^{20}$ +21.2 (c = 0.5, MeOH); NMR data are identical to those of **6c**

3.2. HPLC analysis

The HPLC system consisted of a Waters 515 HPLC Pump, a Waters 717 plus Autosampler, and a Waters 2487 Dual λ Absorbance Detector. The column temperature was controlled by a Jetstream 2 plus Peltier-Column-Thermostat (Water, Milford, MA, USA). The chiral analysis was carried out on a Chirex (D) Penicillamine column, 250 × 4.6 mm I.D. (Phenomenex Ltd., Aschaffenburg, Germany), at 20 °C. The mobile phase was 3mM $CuSO_4$ in MeOH/H_2O (1/3, v/v) at a flow rate of 0.75 ml/min; *ee* values were determined from the peak areas under the curve at 254 nm. The samples were dissolved in water to a concentration of 1 mg/ml; the injection volume was 5 µl.

Acknowledgements: The Fonds der Chemischen Industrie, Frankfurt am Main, Germany, is gratefully acknowledged for financial support. The authors also thank Volker Brecht for careful and skillful NMR measurements.

References

1 Kaul, R.; Balaram, B.: Bioorg. Med. Chem. Let. **7**, 105 (1999)
2 Ohfune, Y.; Nanba, K.; Takada, I.; Kann, T.: Chirality **9**, 459 (1997)
3 Horikawa, M.; Shigeni, Y.; Yumato, N.; Yoshikawa, S.; Nakajima, T.; Ohfune, Y.: Bioorg. Med. Chem. Lett. **8**, 2027 (1998)
4 Trist, D. G. S.: Pharm. Act. Helv. **74**, 221 (2000)
5 Karcz-Kubicka, M.; Wedzony, K.; Zajaczkowski, W.; Danysz, W. J.: Neural. Transm. **105**, 1189 (1999)
6 Volk, F. J.; Frahm, A. W.: Liebigs Ann. 1893 (1996)
7 Wede, J.; Volk, F. J.; Frahm, A. W.: Tetrahedron: Asymmetry **11**, 3231 (2000)
8 Case, F. H.; Reid, E. E.: J. Am. Chem. Soc., **50**, 3062 (1928)
9 Schlauch, M.; Frahm, A. W.: J. Chromatogr. A, **868**, 197 (2000)
10 Ôi, N.; Kitahara, R.; Kira, R.: J. Chromatogr. **592**, 291 (1992)
11 Schlauch, M.; Volk, F. J.; Pai Fondekar, K.; Wede, J.; Frahm, A. W.: J. Chromatogr. A **897**, 145 (2000)
12 Davankov, V. A.; Bochkov, A. S.; Kurganov, A. A.; Rouneliotis, P.; Unger, K. K.: Chromatographia **13**, 677 (1980)
13 Davankov, V. A.; Kurganov, A. A.: Chromatographia **17**, 686 (1983)

Received March 2, 2001
Accepted June 19, 2001

Prof. Dr. A. W. Frahm
Lehrstuhl für Pharmazeutische Chemie
Albertstr. 25
D-79104 Freiburg
awfrahm@sun2.ruf.uni-freiburg.de