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Synthesis of Optically Pure α-Hydroxyglycine Peptides

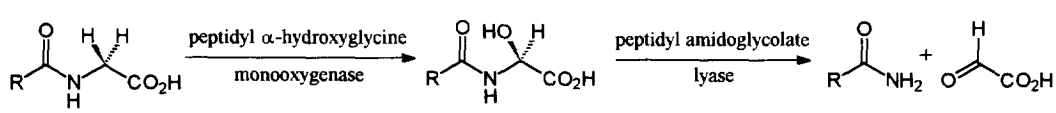
Michael Bogenstätter and Wolfgang Steglich*

Institut für Organische Chemie der Ludwig-Maximilians-Universität, Karlstraße 23, D-80333 München, Germany

Abstract: Optically pure α-hydroxyglycine peptides can be prepared by enzymatic resolution of DL-α-(benzyloxy)glycine peptide esters with subtilisin Carlsberg followed by hydrogenolysis of the benzyl protecting group. The DL-α-(benzyloxy)glycine peptide esters were obtained from the corresponding serine derivatives *via* lead tetraacetate oxidation and reaction of the resulting electrophilic glycine equivalents with benzyl alcohol. © 1997 Elsevier Science Ltd.

INTRODUCTION

α-Hydroxyglycine peptides play a crucial role in the biosynthesis of peptide hormones having a *C*-terminal amide group.[1] In this process a *C*-terminal glycine peptide **1** is transformed into a peptide amide **3** by stereoselective enzymatic α-hydroxylation to **2** followed by enzymatic cleavage into **3** and glyoxylic acid (Scheme 1).[2] α-Hydroxyglycine peptides could therefore be useful for the design of inhibitors for the biosynthesis of *C*-terminally amidated hormones[3] and may serve as starting materials for the synthesis of glycopeptides with sugar residues directly attached to the peptide backbone.[4]



1 **2** **3**

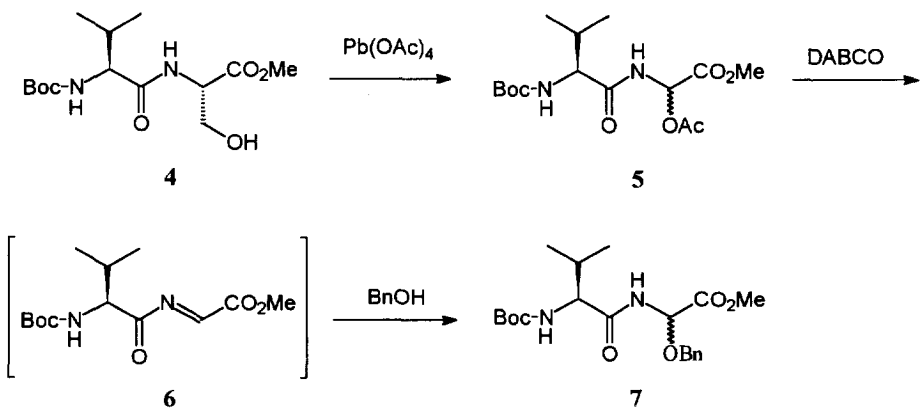
Scheme 1

Due to the inherent instability of α-hydroxyglycine the incorporation of this amino acid in peptides cannot be accomplished by the usual methods of peptide coupling. As an alternate approach the addition of glyoxalates to amino acid amides has been used,[5] however, this technique gives mixtures of diastereomers and sometimes

experiences low yields.[6] The same problems apply to a recently published solid phase synthesis of α-hydroxyglycine peptides *via* their *O*-methyl derivatives.[3] Hermann[7] was able to resolve *N*-acyl-(α-ethylthio)glycine esters and the corresponding peptide esters by enzymatic hydrolysis with thermitase and used his technique for the first synthesis of optically active α-hydroxyglycine peptides.[8] In this publication we describe an efficient synthesis of optically pure α-hydroxyglycine peptides based on the oxidative transformation of serine peptides to electrophilic glycine equivalents[4] and enzymatic resolution.

RESULTS AND DISCUSSION

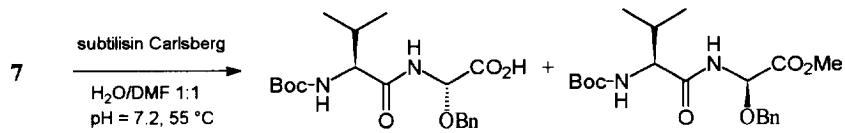
The synthesis of diastereomeric α-(benzyloxy)glycine peptides is illustrated by the preparation of Boc-L-Val-DL-Gly(OBn)-OMe (7) (Scheme 2). Peptide ester **7** can be easily derived from the corresponding L-serine derivative **4** by oxidative cleavage with lead tetraacetate.[4] The resulting diastereomers of α-acetoxyglycine peptide **5** are transformed to the reactive acylimino intermediate **6** on treatment with 1,4-diazabicyclo[2.2.2]-octane (DABCO) in tetrahydrofuran at -78 °C. Subsequent addition of benzyl alcohol affords Boc-L-Val-DL-Gly(OBn)-OMe (7) as a 1:1 mixture of diasteromers in 90% overall yield from serine derivative **4**.



Scheme 2

The diastereomers of **7** can be efficiently resolved by enzyme catalysed ester hydrolysis with subtilisin Carlsberg. This enzyme is one of the best known alkaline serine proteases[9] and is used for the stereoselective hydrolysis of *N*-acylpeptide esters with L-amino acid residues.[10]

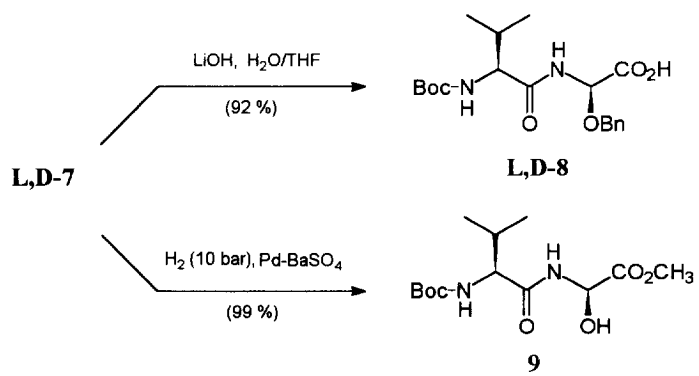
In the case of peptide esters **7** the hydrolysis is carried out in a mixture of water and dimethylformamide (1:1) at 55 °C and pH 7.2 with an enzyme-substrate ratio of 1:10[4]. The reaction is monitored by the consumption of NaOH added to keep the pH constant. It stops after complete hydrolysis of the L,L-diastereomer to Boc-L-Val-L-(BnO)Gly-OH (**L,L-8**), whereas the diastereomeric ester **L,D-7** remains unaffected.[11] After separation by extraction with aqueous $KHCO_3$ both compounds are obtained in nearly quantitative yield with a diastereomeric excess of >99% in both cases (Scheme 3).



L,L-8 **L,D-7**

Scheme 3

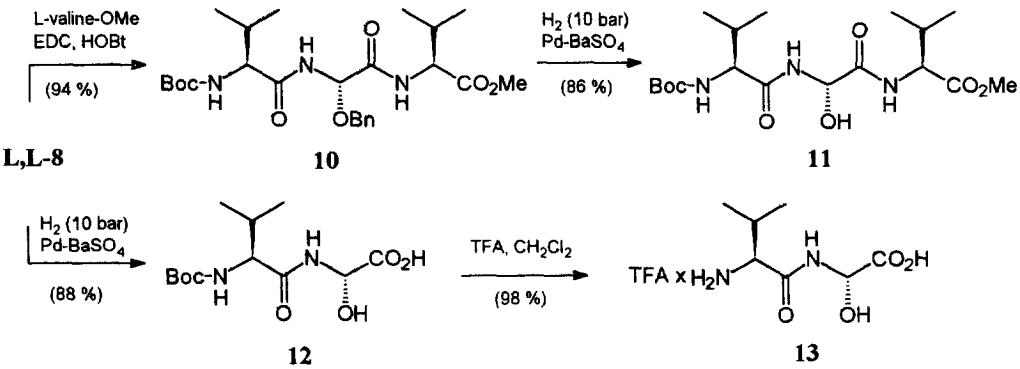
The optically pure peptide derivatives of α-(benzyloxy)glycine are stable compounds which can be used for the usual peptide transformations. Thus, treatment of Boc-L-Val-D-Gly(OBn)-OMe (**L,D-7**) with lithium hydroxide in a mixture of water and tetrahydrofuran[12] yields the free acid **L,D-8** without any epimerisation. **L,D-7** is easily converted to the enantiomerically pure hydroxyglycine peptide **9** by hydrogenolysis in ethanol with palladium on barium sulphate (Scheme 4). The hydrolysis of α-hydroxyglycine peptide ester **9** was unsuccessful even under mild conditions.



Scheme 4

Coupling of **L,L-8** with methyl L-valinate according to König and Geiger[13] affords the tripeptide Boc-L-Val-L-Gly(OBn)-L-Val-OMe (**10**) in 94% yield. Hydrogenolysis of the α-(benzyloxy)glycyl derivatives

10 and **L,L-8** gives the enantiomerically pure α-hydroxyglycyl peptides **11** and **12**, respectively, without epimerisation. Removal of the Boc residue from Boc-L-Val-L-Gly(OH)-OH (**12**) with trifluoroacetic acid in dichloromethane[14] yields optically pure L-valyl-L-α-hydroxyglycine (**13**) as its trifluoroacetate (Scheme 5).



Scheme 5

The optical purity of the peptides was determined by comparison of their ^{1}H and ^{13}C NMR spectra with those of the corresponding diastereomeric mixtures, prepared by the same methods from unresolved starting materials.

The use of Z and benzyl ester protecting groups and solid phase techniques for the synthesis of free α-hydroxyglycine peptides is under active investigation.

EXPERIMENTAL

General Methods. Melting points were determined with a Büchi melting-point apparatus and are uncorrected. Infrared spectra were obtained using Perkin Elmer 100 FT-IR and Bruker IFS 45 spectrometers. NMR spectra were recorded with Bruker ARZ 300 and Varian VRX 400S instruments (Me_4Si as internal reference). Mass spectra were obtained using Finnigan MAT 90 and Finnigan MAT 90Q instruments equipped with a data system. Optical rotations were measured with a Perkin Elmer 241 polarimeter. The enzyme reaction was carried out with a Metrohm 718 STAT Titrino pH controller. Flash chromatography was performed according to ref.[15]. Organic solutions were dried over anhydrous $MgSO_4$ and solvent evaporation was carried out at reduced pressure using a rotatory evaporator. Elemental analyses were performed at the Institut für Organische Chemie, Universität München. Subtilisin Carlsberg was purchased from Sigma.

Methyl *N-tert*-Butyloxycarbonyl-L-valyl-DL-α-(benzyloxy)glycinate (7). To a stirred solution of methyl *N-tert*-butyloxycarbonyl-L-valyl-L-serinate (**4**)[16] (1.59 g, 5 mmol) in dry EtOAc (75 mL) under argon were added molecular sieve 4 A (1 g) and $Pb(OAc)_4$ (6.65 g, 15 mmol). The mixture was heated at reflux for 2 h and cooled to rt. After filtration through Celite, the organic layer was stirred with 10% aqu. citric acid (150 mL) until the solution became nearly colourless. The organic layer was separated and washed with 10% aqu. citric acid, water, and brine. Concentration of the dried solution yielded a 1:1 mixture of the diastereomeric α-acetoxyglycine derivatives **5** as a colourless solid. This material was immediately dissolved in dry THF (100 mL), and a solution of 1,4-diazabicyclo[2.2.2]octane (DABCO) (1.25 g, 12 mmol) in dry THF (15 mL) was added at -78°C. After the solution was stirred for 5 min at -78 °C, benzyl alcohol (0.62 mL, 6 mmol) was added. After being stirred for additional 6 h at -78°C, the mixture was allowed to warm to rt and then stirred again for 24 h. Then, 10% aqu. citric acid was added and the volatile compounds were removed *in vacuo*. The residue was extracted with EtOAc and the combined organic layers were washed with 10% aqu. citric acid, saturated aqu. $NaHCO_3$, water and brine. The solution was dried, concentrated, and purified by flash silica gel column chromatography (petroleum ether/acetone, 3:1) to give **7** as a mixture of diastereomers (1.78 g, 90%) as a colourless oil: IR (KBr): 3422 (sh), 3391 (s), 3322 (s), 3033 (w), 2966 (m), 2935 (w), 2874 (w), 1752 (s), 1703 (sh), 1692 (sh), 1666 (s), 1523 (s), 1456 (w), 1440 (w), 1391 (w), 1367 (m), 1294 (w), 1246 (m), 1218 (m), 1168 (m), 1099 (m), 1069 (w), 1022 (w), 734 (w), 698 (w) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.10-1.15 (m, 6 H), 1.45/1.48 (2s, 9 H), 2.37-2.51 (m, 1 H), 3.51/3.63 (2s, 3 H), 4.65-4.75 (m, 1 H), 4.83-4.03 (m, 2 H), 6.25/6.30 (2d, J = 12.0 Hz, J = 12.4 Hz, 1 H), 7.24-7.40 (m, 3 H), 7.47-7.62 (m, 2 H), 8.02/8.09 (2d, J = 8.4 Hz, J = 8.7 Hz, 1 H), 10.23/10.33 (2d, J = 9.0 Hz, J = 9.3 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.32/18.69, 19.70/19.75, 28.43/28.47, 31.56/31.67, 52.20/52.33, 60.99/61.14, 70.44/70.67, 77.70/77.85, 78.76, 128.09, 128.14, 128.48, 128.64, 128.68, 128.72, 138.13/138.18, 156.86/157.00, 168.87/169.17, 173.66/173.84; FAB MS: *m/z* 789 (3%) [$2MH^+$], 417 (1) [MNa^+], 395 (22) [MH^+], 339 (11), 231 (22), 200 (31), 144 (14), 116 (22), 91 (53), 88 (100). Anal. Calcd for $C_{20}H_{30}N_2O_6$ (394.47): C, 60.90; H, 7.67; N, 7.10. Found: C, 61.15; H, 7.88; N, 6.99.

Enzyme Catalysed Saponification of 7 (Mixture of Diastereomers). To a stirred solution of **7** (1.73 g, 4.39 mmol) in water (40 mL) and DMF (40 mL) was added at 55 °C subtilisin Carlsberg (37 mg). The pH of the reaction mixture was kept constant at 7.2 by adding 1 N NaOH under pH-control. After the consumption of 2.48 ml 1 M NaOH the reaction stopped and the solvent was removed *in vacuo*. The residue was dissolved in 5% aqu. $KHCO_3$, extracted with EtOAc, and the combined organic layers were washed with 5% aqu. $KHCO_3$, 10% aqu. citric acid, water and brine. Evaporation of the dried solution yielded **L,D-7** as a colourless solid. The aqueous layer was adjusted to pH 3 with 30% aqu. citric acid and extracted with EtOAc. The combined extracts were washed with 10% citric acid, water and brine and yielded after the usual workup **L,L-8** as a colourless foam.

Methyl *N-tert*-Butyloxycarbonyl-L-valyl-D-α-(benzyloxy)glycinate (L,D-7). Yield 0.70 g (93%); mp 88.5-90°C; $[\alpha]_D^{20}$ = -14.8 (*c* 0.58, $CHCl_3$); IR (KBr): 3325 (s), 3031 (w), 2966 (m), 2936 (w), 2870 (w), 1749 (s), 1689 (s), 1666 (s), 1527 (s), 1470 (w), 1455 (w), 1442 (m), 1391 (m), 1366 (m), 1355 (m), 1337 (m), 1295 (m), 1248 (m), 1232 (m), 1213 (m), 1170 (m), 1155 (m), 1096 (m), 1061 (m), 1029 (w), 1020 (w), 700 (w) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.13 (d, J = 6.9 Hz, 3 H), 1.44 (d, J = 6.9 Hz, 3 H), 1.45 (s, 9 H), 2.40-2.51 (m, 1 H), 3.51 (s, 3 H), 4.73 (dd, J = 6.9 Hz, J = 8.1 Hz, 1 H), 4.88 (d, J = 11.4 Hz, 1 H), 5.01 (d, J = 11.4 Hz, 1 H), 6.26 (d, J = 9.0 Hz, 1 H), 7.26-7.35 (m, 3 H), 7.50-7.56 (m, 2 H), 8.02 (d, J = 8.4 Hz, 1 H), 10.23 (d, J = 8.7 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.32, 19.75, 28.43, 31.67, 52.20, 60.98, 70.66, 77.85, 78.76, 128.14, 128.48, 128.71, 138.18, 156.86, 168.87, 173.65; FAB MS: *m/z* 417 (39%) [MNa^+], 395 (13) [MH^+], 339 (12), 231 (27), 200 (31), 144 (15), 116 (26), 91 (61), 88 (100), 72 (32), 57 (37). Anal. Calcd for $C_{20}H_{30}N_2O_6$ (394.47): C, 60..90; H, 7.67; N, 7.10. Found: C, 60.79; H, 7.47; N, 7.17.

***N-tert*-Butyloxycarbonyl-L-valyl-L-α-(benzyloxy)glycine (L,L-8).** Yield 0.91 g (96%); $[\alpha]_D^{20}$ = +10.6 (*c* 0.70, $CHCl_3$); IR (KBr): 3326 (m), 3065 (w), 3035 (w), 2975 (m), 2934 (m), 2878 (w), 1734 (s), 1668 (s), 1521 (s), 1456 (m), 1393 (m), 1368 (m), 1293 (m), 1247 (m), 1211 (m), 1164 (m), 1096 (m), 1072 (m), 1028 (w), 737 (w), 698 (m) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.14 (d, *J* = 6.9 Hz, 3 H), 1.17 (d, *J* = 6.9 Hz, 3 H), 1.49 (s, 9 H), 2.44-2.55 (m, 1 H), 4.78 (dd, *J* = 6.9 Hz, *J* = 8.7 Hz, 1 H), 4.95 (d, *J* = 11.4 Hz, 1 H), 5.06 (d, *J* = 11.7 Hz, 1 H), 6.45 (d, *J* = 9.3 Hz, 1 H), 7.23-7.32 (m, 3 H), 7.52-7.60 (m, 2 H), 8.07 (d, *J* = 8.7 Hz, 1 H), 10.13 (d, *J* = 9.0 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.60, 19.78, 28.46, 31.67, 61.04, 70.42, 78.43, 78.69, 127.87, 128.55, 128.63, 138.59, 156.97, 171.18, 173.76; FAB MS: *m/z* 403 (100%) [MNa^+], 381 (5) [MH^+], 325 (16), 295 (6), 239 (9), 217 (27), 116 (31), 91 (82), 57 (71). Anal. Calcd for $C_{19}H_{28}N_2O_6$ (380.44): C, 59.99; H, 7.42; N, 7.36. Found: C, 59.54; H, 7.31; N, 7.43. HR FAB Calcd: [MH^+] 381.2025. Found: 381.2046.

***N-tert*-Butyloxycarbonyl-L-valyl-D-α-(benzyloxy)glycine (L,D-8).** To a stirred solution of **L,D-7** (70 mg, 0.2 mmol) in THF (1 mL) at 0 °C was added a solution of LiOH (14 mg, 0.6 mmol) in water (0.5 mL). After being stirred for 1 h at 0°C, the mixture was allowed to warm to rt and then stirred again for 2 h. The pH was adjusted to pH 3 by the addition of 10% aqu. citric acid and the solution extracted with EtOAc. The combined organic layers were washed with 10% aqu. citric acid, water and brine. The solution was dried and concentrated to give **L,D-8** as a colourless foam. Yield 70 mg (92%); $[\alpha]_D^{20}$ = -9.1 (*c* 1.21, $CHCl_3$); IR (KBr): 3410 (sh), 3319 (m), 3078 (w), 3034 (w), 2972 (m), 2935 (m), 2876 (w), 1740 (s), 1675 (s), 1522 (s), 1456 (m), 1393 (m), 1368 (m), 1248 (m), 1210 (m), 1164 (m), 1092 (m), 1069 (m), 1029 (w), 736 (w), 697 (m) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.15 (d, *J* = 6.6 Hz, 3 H), 1.17 (d, *J* = 6.9 Hz, 3 H), 1.45 (s, 9 H), 2.44-2.56 (m, 1 H), 4.81 (dd, *J* = 6.9 Hz, *J* = 7.8 Hz, 1 H), 5.00 (d, *J* = 11.7 Hz, 1 H), 5.11 (d, *J* = 11.7 Hz, 1 H), 6.42 (d, *J* = 8.7 Hz, 1 H), 7.25-7.35 (m, 3 H), 7.56-7.61 (m, 2 H), 8.00 (d, *J* = 8.4 Hz, 1 H), 9.98 (d, *J* = 9.0 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.28, 19.81, 28.42, 31.76, 60.94, 70.65, 78.60, 78.69, 127.96, 128.40, 128.65, 138.65, 156.87, 170.97, 173.62; FAB MS: *m/z* 783 (4%) [$2MNa^+$], 761 (5) [$2MH^+$], 403 (43) [MNa^+], 381 (35) [MH^+], 325 (41), 217 (47), 116 (41), 91 (100). Anal. Calcd for $C_{19}H_{28}N_2O_6$ (380.44): C, 59.99; H, 7.42; N, 7.36. Found: C, 59.04; H, 7.43; N, 7.27. HR FAB Calcd: [MH^+] 381.2025. Found: 381.2023.

Methyl *N-tert*-Butyloxycarbonyl-L-valyl-L-α-(benzyloxy)glycyl-L-valinate (10). To a stirred solution of **L,L-8** (0.76 g, 2 mmol), methyl L-valinate hydrochloride (0.34 g, 2 mmol) and HOBt (0.41 g, 3 mmol) in THF (60 mL) were added at 0 °C *N*-ethylmorpholine (0.51 mL, 4 mmol) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.4 g, 2.1 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed up to rt and stirred for further 3 h. After the addition of 10% aqu. citric acid, the solvent was evaporated, the residue dissolved in EtOAc and the solution washed with 10% aqu. citric acid, saturated aqu. $NaHCO_3$, and brine. Usual work-up yielded the crude peptide **10** that was purified by flash chromatography on silica gel (petroleum ether/acetone, 3:1). Yield 0.93 g (94%); colourless fine needles; mp 137-137.5 °C; $[\alpha]_D^{20}$ = -19.7 (*c* 1, $CHCl_3$); IR (KBr): 3425 (s), 3313 (s), 3066 (w), 3032 (w), 2967 (m), 2935 (m), 2876 (w), 1744 (m), 1712 (sh), 1694 (s), 1655 (s), 1520 (s), 1469 (w), 1456 (w), 1438 (w), 1392 (m), 1368 (m), 1245 (m), 1213 (m), 1168 (m), 1112 (w), 1084 (w), 1065 (w), 1018 (w), 743 (w), 698 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.81 (d, *J* = 6.8 Hz, 3 H), 0.84 (d, *J* = 6.8 Hz, 3 H), 0.92 (d, *J* = 6.8 Hz, 3 H), 0.97 (d, *J* = 6.8 Hz, 3 H), 1.42 (s, 9 H), 2.07-2.15 (m, 2 H), 3.71 (s, 3 H), 3.98-4.03 (m, 1 H), 4.44 (dd, *J* = 4.8 Hz, *J* = 8.8 Hz, 1 H), 4.63-4.74 (AB-System, 2 H), 5.15 (d, *J* = 8.4 Hz, 1 H), 5.68 (d, *J* = 8.8 Hz, 1 H), 6.98-7.03 (m, 2 H), 7.28-7.39 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.63, 17.76, 18.83, 19.22, 28.30, 31.07, 31.13, 52.22, 57.27, 60.06, 70.59, 76.93, 79.90, 128.14, 128.24, 128.60, 137.00, 155.78, 167.62, 171.75, 173.16; FAB MS: *m/z* 987 (1%) [$2MH^+$], 494 (19) [MH^+], 438 (5), 286 (7), 187 (100), 91 (49), 72 (28). Anal. Calcd for $C_{25}H_{39}N_3O_7$ (493.60): C, 60.83; H, 7.96; N, 8.51. Found: C, 60.39; H, 7.61; N, 8.56. HR FAB Calcd: [MH^+] 494.2866. Found 494.2854.

General Procedure for the Hydrogenolysis of the (α-Benzyloxy)glycine Peptides. To a solution of the peptide (0.3 mmol) in ethanol (10 mL) was added 5% palladium on $BaSO_4$ (0.1 g). The reaction mixture was stirred at rt under a hydrogen pressure of 10 bar for 24 h. Filtration and evaporation of the solvent yielded the crude products. If necessary, they can be purified by flash chromatography on silica gel oder by recrystallisation.

***N-tert*-Butyloxycarbonyl-L-valyl-D-α-hydroxyglycinate (9).** From **L,D-7** (118 mg); colourless fine needles; mp 124-126; yield 80 mg (88%); $[\alpha]_D^{20}$ = +8.6 (*c* 0.97, $CHCl_3$); IR (KBr): 3330 (s), 3300 (s), 2972 (m), 2961 (m), 2940 (m), 2876 (w), 1761 (s), 1689 (s), 1667 (s), 1524 (s), 1472 (w), 1448 (m), 1393 (m), 1370 (m), 1321 (m), 1299 (m), 1247 (m), 1231 (m), 1213 (m), 1171 (m), 1093 (m), 1046 (m), 1020 (m), 905 (w), 781 (w) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.06 (d, *J* = 6.3 Hz, 3 H), 1.08 (d, *J* = 6.6 Hz, 3 H), 1.44 (s, 9 H), 2.36-2.47 (m, 1 H), 3.54 (s, 3 H), 4.71 (dd, *J* = 6.9 Hz, *J* = 8.7 Hz, 1 H), 6.45 (d, *J* = 8.4 Hz, 1 H), 7.89 (d, *J* = 9.0 Hz, 1 H), 9.18 (br, 1 H), 10.05 (d, *J* = 7.8 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.22, 19.67, 28.42, 31.94, 51.98, 60.46, 72.63, 78.60, 156.76, 171.32, 172.90; FAB MS: *m/z* 913 (1%) [$3MH^+$], 631 (4) [$2MNa^+$], 609 (22) [$2MH^+$], 343 (1) [MK^+], 327 (9) [MNa^+], 305 (80) [MH^+], 249 (54), 231 (52), 88 (100). Anal. Calcd for $C_{13}H_{24}N_2O_6$ (304.34): C, 51.31; H, 7.95; N, 9.20. Found: C, 51.24; H, 7.82; N, 9.25.

Methyl *N-tert*-Butyloxycarbonyl-L-valyl-L-α-hydroxyglycyl-L-valinate (11). From **10** (148 mg); colourless foam; yield 104 mg (86%); $[\alpha]_D^{20}$ = -2.0 (*c* 2.19, $CHCl_3$); IR (KBr): 3326 (s), 2966 (s), 2935 (m), 2877 (w), 1745 (s), 1690 (s), 1663 (s), 1521 (s), 1470 (w), 1437 (w), 1393 (m), 1367 (m), 1312 (w), 1248 (m), 1213 (m), 1166 (m), 1115 (w), 1094 (w), 1046 (w), 1018 (w), 874 (w), 770 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.87-0.93 (m, 12 H), 1.38 (s, 9 H), 2.03-2.19 (m, 2 H), 3.70 (s, 3 H), 4.04-4.08 (m, 1 H), 4.46 (dd, *J* = 5.0 Hz, *J* = 9.0 Hz, 1 H), 5.37-5.47 (m, 2 H), 5.67 (dd, *J* = 5.6 Hz, *J* = 6.4 Hz, 1 H), 7.47 (d, *J* = 9.2 Hz, 1 H), 7.71-7.76 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.74, 18.90, 19.24, 28.28, 31.08, 31.15, 52.23, 57.50, 59.61, 72.77, 79.90, 155.90, 169.27, 171.71, 173.62; FAB MS: *m/z* 829 (2%) [$2MNa^+$], 807 (1) [$2MH^+$], 426 (60) [MNa^+], 404 (6) [MH^+], 348 (8), 330 (6), 286 (13), 210 (15), 187 (100), 127 (22), 72 (53), 57 (39). Anal. Calcd for $C_{18}H_{33}N_3O_7$ (403.47): C, 53.58; H, 8.24; N, 10.41. Found: C 53.48; H 8.45; N 10.08.

***N-tert*-Butyloxycarbonyl-L-valyl-L-α-hydroxyglycine (12).** From **L,L-8** (114 mg); yield 86 mg (99%), colourless foam; $[\alpha]_D^{20}$ = -13.3 (*c* 1.16, $CHCl_3$); IR (KBr): 3327 (s), 3069 (w), 2974 (m), 2935 (m), 2885 (w), 1727 (s), 1670 (s), 1528 (s), 1456 (w), 1394 (m), 1369 (m), 1292 (w), 1248 (m), 1165 (m), 1102 (m), 1019 (w), 869 (w), 779 (w) cm^{-1}; ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.09-1.15 (m, 6 H), 1.45 (s, 9 H), 2.43-2.52 (m, 1 H), 4.78 (dd, *J* = 6.8 Hz, *J* = 8.6 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 7.90 (br, 1 H), 10.00 (d, *J* = 8.4 Hz, 1 H); ^{13}C NMR (75 MHz, [D_5]pyridine): δ = 18.31, 19.70, 28.42, 32.04, 60.60, 73.01, 78.53, 156.74, 172.76, 173.62; FAB MS: *m/z* 291 (17%) [MH^+], 235 (10), 217 (9). Anal. Calcd. for $C_{12}H_{22}N_2O_6$ (290.32): C, 49.65; H, 7.64; N, 9.65. Found: C, 49.12; H, 7.70; N, 9.44.

L-Valyl-L-α-hydroxyglycine Trifluoroacetate (13). To a stirred solution of **12** (14 mg, 0.05 mmol) in CH_2Cl_2 (2 ml) at 20 °C was added trifluoroacetic acid (0.67 mL). After stirring for 90 min at rt, the solution was concentrated *in vacuo* and yielded the crude trifluoroacetate **13** as a colourless syrup. Yield 14 mg (96%); ^1H NMR (300 MHz, [D_5]pyridine): δ = 1.28-1.36 (m, 6 H), 2.64-2.76 (m, 1 H), 4.67 (d, *J* = 5.7 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 10.68 (d, *J* = 8.4 Hz, 1 H); FAB MS: *m/z* 213 (11%) [MNa^+], 191 (11) [MH^+], 139 (23), 119 (38), 72 (100); $C_7H_{11}N_2O_4$*TFA (304.23).

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