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A convenient chemoenzymatic synthesis of (*R*)-(−) and (*S*)-(+)-homo-β-proline

Fulvia Felluga,* Valentina Gombac, Giuliana Pitacco and Ennio Valentin

Dipartimento di Scienze Chimiche, Università di Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy

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Abstract—Both enantiomers of the heterocyclic GABA analogue homo-β-proline (3-pyrrolidineacetic acid) were synthesized by a chemoenzymatic method involving the use of two enantiocomplementary enzymes in the disymmetric hydrolyses of 3-nitromethylglutaric acid diethyl ester.

1. Introduction

GABA (γ-aminobutyric acid)[1] is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS), as approximately 40% of synapses in the CNS are GABA'ergic. Several neurological and psychiatric disorders, such as anxiety, Parkinson's disease, epilepsy and some forms of schizofrenia, are correlated with a disfunctioning of the GABA system.[1c]

Many unnatural γ-amino acids have been designed for their promising therapeutic use in the treatment of these diseases, acting either as specific agonists at postsynaptic $GABA_A$ receptors[2] or as inhibitors of the GABA-uptake mechanism.[3]

(*R*) and (*S*)-Homo-β-proline [(*R*)- and (*S*)-3-pyrrolidineacetic acid] are conformationally restrained γ-amino acids, which bind to the GABA transport system in the brain. They are known to act as potent inhibitors of the neuronal and glial uptake mechanism of GABA.[3,4] They bind the GABA receptor sites[2] with an opposite stereochemistry, as the (*R*)-enantiomer has a major affinity for the $GABA_A$ receptor, while the (*S*)-enantiomer is more potent as an inhibitor of the $GABA_B$ receptor. For this reason, the obtainment of both antipodes of this cyclic GABA analogue is an interesting synthetic target.

A few asymmetric syntheses of (*R*)- and (*S*)-homo-β-proline have already been described, based either on the use of (*S*)-(−)-1-phenylethylamine as a chiral auxiliary,[4,5] or on the use of aspartic acid as the chiral starting material,[6] through procedures involving several steps. Herein we report the chemoenzymatic synthesis of both enantiomers of homo-β-proline in high enantiomeric excesses.

2. Results and discussion

The synthetic approach to the target compounds started from diethyl 3-(nitromethyl)pentanedioate **2**, which was the product of a Michael addition of nitromethane to diethyl glutaconate **1**, catalyzed by 1,1,3,3-tetramethylguanidine (Scheme 1).[7]

The racemic γ-lactam **4**, precursor of (±)-homo-β-proline, was obtained in good yield from the intermediate **2** by a reduction/cyclization sequence carried out over Raney nickel, through the intermediacy of the amino diester **3**, not isolated.[8]

The prochiral nitrodiester **2** is an ideal substrate for enzymatic transformations. Desymmetrization of prochiral compounds is known to constitute an efficient route to single enantiomers in good yields (>50%) and, therefore, this method is well recognized in biocatalysis as a valuable tool for inducing asymmetry.[9] In particular, many 3-substituted glutaric acid diesters have been transformed by hydrolytic enzymes to the corresponding

* Corresponding author. Tel.: +39 040 5583924; fax: +39 040 5583903; e-mail: felluga@dsch.univ.trieste.it

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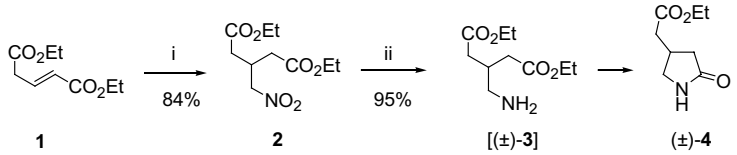
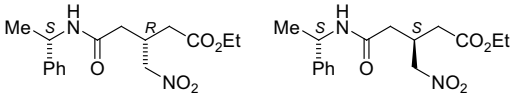


Scheme 1. Reagents and conditions: (i): CH_3NO_2 (5 equiv), 1,1,3,3-tetramethylguanidine (0.2 equiv), 12 h, rt; (ii) H_2, RaNi, 1 atm, 3 h, rt.

optically active half esters with enantiotopic specificity.[10]

During the screening of the possible enzymes able to discriminate between the enantiotopic ester groups of compound **2**, we have found that crude pig liver esterase (PLAP, pig liver acetone powder) hydrolyzed the pro-(*S*) ester group of **2** (Scheme 2), to give monoester (*S*)-(−)-**5** with 99% ee and 82% yield in only 2 h. When pure pig liver esterase (PLE) was used, the same (*S*)-enantiomer was isolated, although with a slightly lower enantiomeric excess (92%). These results are in accordance with the already observed pro-(*S*) enantiopreference of pig liver esterase in the hydrolysis of a series of 3-monosubstituted glutaric acid diesters.[10h] On the other hand, when Porcine pancreatic lipase (PPL) was used in the desymmetrization of diester **2**, monoester (*R*)-(+)-**5** with 98% ee was isolated, after 72 h, in 79% yield. In neither case, contrary to reported examples,[10d] was the 3-substituted pentanedioic acid detected as the product of hydrolysis of both ester groups in **2**.

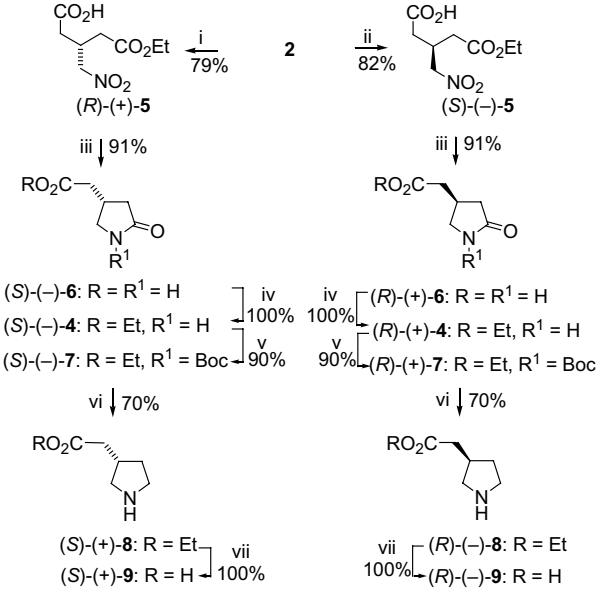
The enantiomeric excesses of (*R*)-(+)-**5** and (*S*)-(−)-**5** were determined by ^{1}H NMR (C_6D_6, 400 MHz) analysis of their diastereoisomeric amides (−)-**10** and (−)-**11**,[10f] obtained by coupling their carboxylic functions with (*S*)-(−)-1-phenylethylamine, followed by integration of the corresponding nitromethylenic proton signals, which resonated at 4.25, 4.35 (each dd) and 4.17, 4.37 (each dd) ppm, respectively.



Other enzymes were also checked, such as α-chymotrypsin, which hydrolyzed the substrate with no enantioselectivity, or lipases from *Candida rugosa*, *Aspergillus niger* and *Pseudomonas* species, which proved completely inactive.

The conversion of the half esters (*R*)-(+)-**5** and (*S*)-(−)-**5** into the corresponding enantiomerically pure (*S*)-(−)- and (*R*)-(+)-homo-β-proline, respectively,[†] **9** was performed within a few steps, as described in Scheme 2. Reduction of the nitro group under an atmospheric pressure of hydrogen, in the presence of Raney nickel as catalyst, at room temperature for 3 h, afforded the corresponding amino substituted diesters, which were not isolated. Heating the crude reaction mixture in an ethanol/toluene solution (1:1) for 2 h ensured the complete lactamization of the reduction intermediates into the desired lactams (*R*)-(+)-**6** and (*S*)-(−)-**6**. Interestingly, the cyclization reaction occurred exclusively at the ester function, as verified by the analysis of the ^{1}H NMR spectra of the crude reaction mixtures.

Removal of the lactamic carbonyl function was performed on the *N*-Boc protected γ-lactams (*R*)-(+)- and (*S*)-(−)-**7**, obtained from the corresponding acidic lactams (*R*)-(+)-**6** and (*S*)-(−)-**6** by esterification[11] of the carboxylic function and treatment with di-*tert*-butyl dicarbonate.[12] Thus, treatment of the *N*-protected γ-lactams (*R*)-(+)-**7** and (*S*)-(−)-**7** with BH_3–DMS[13] in THF afforded the corresponding ethyl (*R*)-(−)- and (*S*)-(+)-3-pyrrolidineacetate **8**, after removal of the *tert*-butoxycarbonyl group. These latter compounds were eventually hydrolyzed to the target molecules (*R*)-(−)- and (*S*)-(+)-homo-β-proline **9**, obtained in approximately 50% overall yield starting from **2**.



Scheme 2. Reagents and conditions: (i) PPL (1:1 w/w), pH 7.4, rt, 3 d; (ii) PLAP (1:1 w/w), pH 7, rt, 2 h; (iii) Raney nickel, EtOH, 1 atm, rt, 3 h, then refluxing in 1:1 ethanol/toluene, 2 h; (iv) EtOH, TMSCl, rt, 12 h; (v) $(BOC)_2O$ (2 equiv), DMAP, Et_3N (1 equiv), CH_2Cl_2 1 h; (vi) BH_3–DMS (2 equiv), dry THF, 0 °C; (vii) Amberlite 400 (OH^-), then AcOH.

[†] The change in the configurational descriptors in going from the linear compounds (*R*)-(+)- and (*S*)-(−)-**5** to the cyclic ones (*S*)-(−)- and (*R*)-(+)-**6** is due to a change in the order of priority of the groups attached to the stereocentre.

3. Conclusion

In conclusion, the synthetic pathway proposed offers an easy and efficient route to both enantiomers of homo-β-proline with high enantiomeric excess and satisfactory yields.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT-IR 200 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 (400 MHz for proton, 100.1 MHz for carbon), using deuterochloroform as a solvent and tetramethylsilane as the internal standard, unless otherwise stated. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter, at 25 °C. ESI-MS spectra were obtained on a PE-API spectrometer at 5600 V by infusion of methanolic solutions. Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer (Copenhagen). TLC's were performed on Polygram® Sil G/UV$_{254}$ silica gel pre-coated plastic sheets (eluent: light petroleum–ethyl acetate). Flash chromatography was run on silica gel, 230–400 mesh ASTM (Kieselgel 60, Merck), using mixtures of light petroleum 40–70 °C and ethyl acetate as the eluent. CHN analyses were run on a 1106 Carlo Erba Elemental Analyzer.

Raney® 2800 nickel, slurry in water, active catalyst, was purchased from Aldrich. Porcine pancreatic lipase type II (PPL), and Porcine liver acetone powder (PLAP) were supplied by Sigma, while α-chymotrypsin (α-CT; 53.1 U/mg) and esterase from pig liver (PLE, 220 U/mg) were purchased from Fluka.

4.1.1. Synthesis of diethyl 3-(nitromethyl)pentanedioate 2. A mixture of nitromethane (8.2 g, 135 mmol), diethyl 2-pentenedioate **1** (5.0 g, 27 mmol) and 1,1,3,3-tetramethylguanidine[7] (0.625 g, 5.3 mmol) was stirred at room temperature for 24 h. Excess nitromethane was removed in vacuo, the residue dissolved in diethyl ether and washed with 5% HCl and the organic phase dried over Na_2SO_4 and chromatographed on column (eluent: light petroleum–ethyl acetate, 9:1) to give pure **2** as a pale yellow oil, 5.5 g, 84% yield. IR, cm^{-1} (neat): 1728 (CO_2Et), 1545 and 1382 (NO_2); ^{1}H NMR, δ, ppm: 4.62 (d, J = 6.3 Hz, 2H, CH_2NO_2), 4.14 (q, 4H, 2 CH_2O), 3.05 (quint, 1H, H-3), 2.51 (d, 4H, J = 6.8 Hz, 2 H-2 and 2 H-4), 1.25 (t, 6H, 2 CH_3); ^{13}C NMR, δ, ppm: 170.4 (s), 77.2 (t), 60.4 (t), 34.8 (t), 30.1 (d), 13.6 (q); ESI-MS (*m*/*z*): 248.1 $[M+H]^+$, 270.0 $[M+Na]^+$, 286.0 $[M+K]^+$. Anal. Calcd for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 48.7; H, 6.9; N, 5.6.

4.1.2. Ethyl (±)-2-oxo-4-pyrrolidineacetate 4. To a solution of compound **2** (1.0 g, 4.0 mmol) in ethanol (15 mL), Raney nickel was added and the mixture hydrogenated at atmospheric pressure until disappearance of the starting material (TLC, eluent: light petroleum–ethyl acetate, 4:1). Toluene (10 mL) was then added to the reaction mixture and the solution heated to a complete cyclization. After filtering on a pad of Celite, the solvents were evaporated in vacuo and the residue chromatographed on a column (eluent: light petroleum–ethyl acetate, from 4:1 to 1:1) to give γ-lactam **4** (0.65 g, 95% yield). IR, cm^{-1} (neat): 3055 (NH), 1735 (CO_2Et), 1693 (NHCO); ^{1}H NMR, δ, ppm: 6.14 (br s, 1H, NH), 4.13 (q, 2H, CH_2O), 3.59 (dd, J = 8.0 and 9.7 Hz 1H, H-5), 3.08 (dd, J = 6.2 and 9.7 Hz, 1H, H-5), 2.87 (sept, 1H, H-4), 2.51 (dd, J = 8.8 and 16.8 Hz, 1H, H-3), 2.47 (dd, J = 1.5 and 8.8 Hz, 2H, CH_2CO_2Et), 2.04 (dd, J = 7.3 and 16.8 Hz, H-3), 1.24 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 177.4 (s), 171.6 (s), 60.7 (t), 47.5 (t), 38.7 (t), 36.2 (t), 31.1 (d), 14.2 (q); ESI-MS (*m*/*z*): 172.0 $[M+H]^+$, 194.1 $[M+Na]^+$, 209.9 $[M+K]^+$. Anal. Calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.1; H, 7.6; N, 8.2.

4.2. Enzymatic hydrolyses of compound 2

4.2.1. (*R*)-(+)- and (*S*)-(−)-3-(Nitromethyl)pentanedioic acid monoethyl ester 5. A suspension of the diester **2** (1.0 g, 4.0 mmol) in phosphate buffer (pH 7) (20 mL) was hydrolyzed with PPL (0.5 g) at room temperature under vigorous stirring. The pH was kept at its initial value by automatic continuous addition of 1 M NaOH. After consumption of 1 equiv of NaOH, the mixture was centrifuged and extracted with diethyl ether (2 × 10 mL). The aqueous layer was acidified to pH 2 with 2 M HCl and extracted with chloroform (5 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to give (*R*)-(+)-**5**, oil, 0.70 g, 79% yield, 98% ee; $[\alpha]_D^{25} = +2.2$ (*c* 1, MeOH); IR cm^{-1}(neat): 3600–2800 (br, OH), 1785, 1731 (CO_2H, CO_2Et), 1553, 1378 (NO_2); ^{1}H NMR, δ, ppm: 9.42 (br s, 1H, OH), 4.86 (apparent d, J = 5.9 Hz, 2H, CH_2NO_2), 4.13 (q, 4H, CH_2O), 3.04 (quint, 1H, H-3), 2.61 (apparent d, J = 6.6 Hz, 2H, 2 H-2 or 2 H-4), 2.54 (apparent d, J = 6.6 Hz, 2H, 2 H-2 or 2 H-4), 1.25 (t, 3H, CH_3); ^{13}C NMR (100.4 MHz): 176.6 (s), 170.9 (s), 77.3 (t), 61.1 (t), 35.2 (t), 35.0 (t), 30.3 (d), 14.0 (q); ESI-MS (*m*/*z*): 218.0 $[M-H]^+$. Anal. Calcd for $C_8H_{13}NO_6$: C, 43.84; H, 5.98; N, 6.39. Found: C, 43.9; H, 6.0; N, 6.35.

Under the same conditions as above, diester **2** (1.0 g, 4.0 mmol) was hydrolyzed with PLAP (0.5 g) in phosphate buffer (pH 7) (20 mL) to give (*S*)-(−)-**5**, oil, 0.72 g, 82% yield, 99% ee; $[\alpha]_D^{25} = -2.2$ (*c* 1, MeOH).

The same compound (*S*)-(−)-**5** with a 92% ee was obtained by running the hydrolysis of the diester **2** (1.0 g, 4.0 mmol) with PLE (10 mg, 400 U/mmol) in 20 mL phosphate buffer (pH 7), under the conditions described above; $[\alpha]_D^{25} = -2.0$ (*c* 1, MeOH).

4.3. Determination of the enantiomeric excess of (*R*)-(+)-5 and (*S*)-(−)-5

4.3.1. Ethyl (1′*S*,3*R*)-(−)- and (1′*S*,3*S*)-(−)-3-(nitromethyl)-4-(*N*-1-phenylethylcarbamoyl)butanoate 10 and 11. To a solution of the half ester (*R*)-(+)-**5** (0.05 g,

0.2 mmol) in 5 mL of CH_2Cl_2 with 1 drop of DMF added, oxalyl chloride (20 μL, 0.2 mmol) was added. The solution was stirred at room temperature for 2 h and then the solvents removed in vacuo, after which (*S*)-(+)-1-phenylethylamine (0.05 g, 0.4 mmol) was added at once. After 10 min of stirring, ether was added and the solution washed with 1 M HCl. After drying the organic phase, the solvent was evaporated to give the corresponding amide (−)-**10** in quantitative yield. Mp 112–114 °C. $[\alpha]_D^{25} = -24.9$ (*c* 0.22, $CHCl_3$); IR, cm^{-1} (Nujol): 3310 (NH), 3066 (Ph), 1720 (CO_2Et), 1640 (CONH), 1546 (NO_2), 1496 (Ph), 1378 (NO_2); ^1H NMR (C_6D_6), δ, ppm: 7.24 (m, 5H, ArH), 5.27 (quint, 1H, C*H*Ph), 5.13 (br s, 1H, NH), 4.35 dd, *J* = 5.9 and 12.4 Hz, 1H, C*H*NO_2), 4.25 dd, *J* = 6.2 and 12.4 Hz, 1H, C*H*NO_2), 4.00 (q, 2H, CH_2O), 3.06 (quint, 1H, H-3), 2.38 (part AB of an ABX system, *J* = 6.2, 7.0 and 16.8 Hz, 2H, 2 H-2), 1.94 (part AB of an ABX system, *J* = 6.6, 7.0 and 15.4 Hz, 2H, 2 H-4), 1.30 (d, 3H, *J* = 7.0 Hz, C*H*$_3$CHPh), 1.05 (t, 3H, CH_3); ^{13}C NMR (C_6D_6), δ, ppm: 171.3 (s), 168.9 (s), 144.1 (s), 128.3 (2d), 128.0 (d), 127.6 (2d), 77.9 (t), 60.6 (t), 48.9 (d), 36.8 (t), 35.1 (t), 31.4 (d), 21.8 (q), 14.0 (q); ESI-MS (*m*/*z*): 339.1 $[M+H]^+$, 362.2 $[M+Na]^+$, 378.6 $[M+K]^+$. Anal. Calcd for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.55; N, 8.28. Found: C, 56.9; H, 6.45; N, 8.3.

The same reaction was carried out on the opposite enantiomer (*S*)-(−)-**5** to give the corresponding amide (−)-**11**.

Mp 103–105 °C. $[\alpha]_D^{25} = -54$ (*c* 1, $CHCl_3$); IR, cm^{-1} (Nujol): 3308 (NH), 3066 (Ph), 1725 (CO_2Et), 1639 (CONH), 1546 (NO_2), 1498 (Ph), 1378 (NO_2); ^1H NMR (C_6D_6), δ, ppm: 7.24 (m, 5H, ArH), 5.43 (br s, 1H, NH), 5.25 (quint, 1H, C*H*Ph), 4.37 (dd, *J* = 5.8 and 12.4 Hz, 1H, C*H*NO_2), 4.17 (dd, *J* = 5.5 and 12.4 Hz, 1H, C*H*NO_2), 3.98 (q, 2H, CH_2O), 3.05 (quint, *J* = 6.6 Hz, 1H, H-3), 2.39 (apparent d, *J* = 6.6 Hz, 2H, CH_2CO_2Et), 1.98 (2 *pseudo*q, part AB of an ABX system, J_{AB} = 15.4 Hz, 2H, C*H*$_2$CONH), 1.27 (d, 3H, *J* = 7.0 Hz, C*H*$_3$CHPh), 1.03 (t, 3H, CH_3); ^{13}C NMR (C_6D_6), δ, ppm: 171.3 (s), 168.9 (s), 144.1 (s), 128.3 (2d), 128.0 (d), 127.6 (2d), 77.9 (t), 60.6 (t), 48.9 (d), 36.8 (t), 35.1 (t), 31.4 (d), 21.8 (q), 14.0 (q). ESI-MS (*m*/*z*): 339.1 $[M+H]^+$, 362.0 $[M+Na]^+$, 378.5 $[M+K]^+$. Anal. Calcd for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.55; N, 8.28. Found: C, 56.8; H, 6.4; N, 8.3.

4.4. Transformation of (*S*)-(−)-5 into (*R*)-(−)-3-pyrrolidineacetic acid 9

4.4.1. (*R*)-(+)-2-Oxo-4-pyrrolidineacetic acid 6. To a solution of (*S*)-(−)-**5** (1.0 g, 4.6 mmol) in ethanol (15 mL), Raney nickel was added and the mixture hydrogenated at atmospheric pressure until disappearance of the starting material (TLC, eluent: ethyl acetate). The mixture was then added with toluene (10 mL) and heated to obtain a complete cyclization. After removal of the solvents in vacuo, the residue was dissolved in 5% $NaHCO_3$ and extracted with diethyl ether (3×). The aqueous layer was acidified to pH 3, evaporated to dryness to give a residue which, on trituration with methanol, afforded the acidic lactam (*R*)-(+)-**6**, a semisolid material, 0.60 g, 91% yield. $[\alpha]_D^{25} = +2.9$ (*c* 0.5, MeOH); IR, cm^{-1} (Nujol): 3600–2600 (broad band, OH), 1726 (CO_2H), 1674 (NHCO); ^1H NMR (CD_3OD), δ, ppm: 3.59 (dd, *J* = 8.0 and 9.7 Hz, 1H, H-5), 3.08 (dd, *J* = 6.2 and 9.7 Hz, 1H, H-5), 2.87 (sept, 1H, H-4), 2.51 (dd, *J* = 8.8 and 16.8 Hz, 1H, H-3), 2.47 (dd, *J* = 1.5 and 8.8 Hz, 2H, C*H*$_2$CO$_2$H), 2.04 (dd, *J* = 7.3 and 16.8 Hz, H-3); ^{13}C NMR (CD_3OD), δ, ppm: 175.9 (s), 173.5 (s), 43.5 (t), 36.5 (t), 36.4 (t), 31.0 (d); ESI-MS (*m*/*z*): 144.0 $[M+H]^+$, 166.1 $[M+Na].^+$ Anal. Calcd for $C_6H_9NO_3$: C, 50.35; H, 6.34; N, 9.79. Found: C, 50.35; H, 6.4; N, 9.8.

The opposite enantiomer (*S*)-(−)-**6** was obtained under the same conditions from (*R*)-(+)-**5**. $[\alpha]_D^{25} = -2.8$ (*c* 1, MeOH).

4.4.2. Ethyl (*R*)-(+)-2-oxo-4-pyrrolidineacetate 4. The acidic lactam (*R*)-(+)-**6** (0.61 g, 4.1 mmol) was dissolved in EtOH, and trimethylchlorosilane[11] (1.0 g, 8.2 mmol) was added. The mixture was left at room temperature overnight, after which evaporation of the solvent to dryness gave ethyl (*R*)-(+)-2-oxo-4-pyrrolidineacetate **4** in a quantitative yield, $[\alpha]_D^{25} = +2.8$ (*c* 1, MeOH).

The opposite enantiomer (*S*)-(−)-**4** was obtained from (*S*)-(−)-**6**, $[\alpha]_D^{25} = -2.8$ (*c* 1, MeOH).

4.4.3. Ethyl (*R*)-(+)-2-oxo-1-*tert*-butoxycarbonyl-4-pyrrolidineacetate 7. To a solution of (*R*)-(+)-**5** (0.400 g, 2.3 mmol), having 99% ee, in 5 mL of dichloromethane, di-*tert*-butyldicarbonate (0.64 g, 4.8 mmol), DMAP (0.16 g, 2.4 mmol) and triethylamine (0.20 mL, 2.4 mmol) were added[12] and the resulting solution stirred at rt until disappearance of the substrate (TLC, ethyl acetate). The reaction mixture was washed with 5% citric acid, 5% $NaHCO_3$ and brine and the solvent removed in vacuo to give 0.58 g (90% yield) of pure ethyl (+)-2-oxo-1-*tert*-butoxycarbonyl-4-pyrrolidineacetate (*R*)-(+)-**7**. $[\alpha]_D^{25} = +2.7$ (*c* 1.1, MeOH); IR (film): 1788, 1742 (NCO_2Bu^t, CO_2Et), 1716 (NCO); ^1H NMR, δ, ppm: 4.06 (q, 2H, CH_2O), 3.88 (dd, *J* = 7.9 and 10.9 Hz, 1H, H-5), 3.32 (dd, *J* = 6.6 and 10.9 Hz, 1H, H-5), 2.64 (m, 1H), 2.58 (m, 1H), 2.39 (d, *J* = 6.6 Hz, CH_2CO_2Et), 2.23 (m, 1H), 1.17 (t, 3H, CH_3); ^{13}C NMR, δ, ppm: 172.6 (s), 170.9 (s), 149.6 (s), 82.6 (s), 60.5 (t), 51.1 (t), 38.6 (t), 37.8 (t), 27.7 (3q), 26.9 (d), 13.9 (q); ESI-MS (*m*/*z*): 272.1 $[M+H]^+$, 294.0 $[M+Na]^+$, 310.0 $[M+K]^+$. Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.5; H, 7.8; N, 5.2.

The opposite enantiomer (*S*)-(−)-**7** was obtained from (*S*)-(−)-**5**, $[\alpha]_D^{25} = -2.6$ (*c* 0.8, MeOH).

4.4.4. Ethyl (*R*)-(−)-3-pyrrolidineacetate 8. To a solution of (*R*)-(+)-**7** (0.4 g, 1.5 mmol), having 99% ee, in dry THF (20 mL), 1.5 mL of a 2 M THF solution of BH_3–DMS (3 mmol)[13] were slowly added at −10 °C, while stirring under Ar. After the addition was complete, the temperature was left to rise to room temperature and the mixture stirred until disappearance of the starting material (TLC, eluent: light petroleum/ethyl acetate, 1:1). Ethanol was then added (10 mL) with a few drops of 1 M HCl after which the solution was

refluxed for 1 h. The solvents were removed in vacuo, the residue dissolved in 5% $NaHCO_3$ and the solution extracted with ethyl acetate to give (*R*)-(−)-**8** (0.16 g, 70% yield); $[\alpha]_D^{25} = -14$ (*c* 0.25, MeOH). ^1H NMR (D_2O), δ, ppm: 4.23 (q, 2H, CH_2O), 3.64 (dd, $J = 7.7$ and 11.7 Hz, 1H, H-2), 3.48 (m, 1H, H-5), 3.32 (m, 1H, H-5), 3.03 (dd, $J = 8.8$ and 11.7 Hz, 1H, H-2), 2.75 (m, 1H, H-3), 2.65 (part AB of an ABX system, J = 6.6, 8.4 and 16.5 Hz, 2H, CH_2CO_2Et), 2.30 (m, 1H, H-4), 1.72 (m, 1H, H-4), 1.31 (t, 3H, CH_3); ^{13}C NMR (D_2O), δ, ppm: 172.9 (s), 61.4 (t), 50.5 (t), 45.8 (t), 37.2 (t), 35.1 (d), 30.7 (t), 14.3 (q). ESI-MS (*m*/*z*): 157.9 $[M]^+$, 159.1 $[M+H]^+$. Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.1; H, 9.5; N, 8.9.

The opposite enantiomer (*S*)-(+)-**8** was obtained from (*S*)-(−)-**5**, $[\alpha]_D^{25} = +13.2$ (*c* 1, MeOH).

4.4.5. (*R*)-(−)-3-Pyrrolidineacetic acid 9. Ester (*R*)-(−)-**8** was treated as described in the literature,[5a] on Amberlite IRA 400 (OH^- form) and eluted with an equivalent amount of aqueous acetic acid (6%), to quantitatively give (*R*)-(−)-3-pyrrolidineacetic acid **9** $[\alpha]_D^{25} = -9.1$ (*c* 2, H_2O) [lit.[4] −9.3 (*c* 1, H_2O); lit.[5a] −9.1 (*c* 1, H_2O), lit.[6] −10.1 (*c* 1, H_2O)]. ^1H NMR (D_2O), δ, ppm: 3.62 (dd, $J = 7.7$ and 11.7 Hz, 1H, H-2), 3.48 (m, 1H, H-5), 3.34 (m, 1H, H-5), 3.00 (dd, $J = 8.8$ and 11.7 Hz, 1H, H-2), 2.76 (m, 1H, H-3), 2.64 (m, 2H, CH_2CO_2Et), 2.30 (m, 1H, H-4), 1.74 (m, 1H, H-4). ^{13}C NMR (D_2O), δ, ppm: 178.6 (s), 50.5 (t), 46.0 (t), 38.9 (t), 35.1 (d), 30.4 (t). ESI-MS: 131.0 $[M^+]$, 144.1 $[M+H^+]$, 162.0 $[M+K^+]$.

Enantiomer (*S*)-(+)-3-pyrrolidineacetic acid **9** had $[\alpha]_D^{25} = +9.3$ (*c* 2, H_2O) [lit.[4] +9.6 (*c* 1, H_2O), lit.[5a] +9.2 (*c* 1, H_2O); lit.[6] +8.6 (*c* 1, H_2O)].

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References

1. (a) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*; Hardman, J. G., Limbird, L. E., Goodman Gilman, A., Eds., 10th ed.; Mc Graw-Hill: New York, 2001; Section III; (b) McGeer, P. L.; McGeer, E. G. In *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*; 4th ed.; Siegel, G. J., Agranoff, B., Albens, R. W., Molinoff, P., Eds.; Raven: New York, 1989; (c) *GABA-Neurotransmitters. Pharmacochemical, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Krueger, J., Kofod, H., Eds.; Munksgaard: Copenhagen, 1979.
2. (a) Krogsgaard-Larsen, P.; Frolund, B.; Ebert, B. *The GABA Receptors*, 2nd ed.; Humana: Totowa, New Jersey, 1997, pp 37–81; (b) Bowery, N. G.; Hudson, A. L.; Price, G. W. *Neuroscience* **1987**, *20*, 365–383; (c) *The GABA Receptors*; Enna, S. J., Ed.; Humana: Clifton, NJ, 1983; (d) Hill, D. R.; Bowery, N. G. *Nature* **1981**, *290*, 149–152.
3. (a) Krogsgaard-Larsen, P.; Hjeds, H.; Falch, E.; Jorgensen, F. S.; Nielsen, L. *Adv. Drug Res.* **1988**, *17*, 381–456; (b) Krogsgaard-Larsen, P.; Falch, E.; Larsson, O. M.; Schousboe, A. *Epilepsy Res.* **1987**, *1*, 77–93; (c) Krogsgaard-Larsen, P.; Falch, E.; Hjeds, H. *Prog. Med. Chem.* **1985**, *22*, 67–120.
4. Nielsen, L.; Brehm, L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1990**, *33*, 71–77.
5. (a) Galeazzi, R.; Geremia, S.; Mobbili, G.; Orena, M. *Tetrahedron: Asymmetry* **1996**, *7*, 79–88; (b) Galeazzi, R.; Mobbili, G.; Orena, M. *Tetrahedron* **1996**, *52*, 1069–1084; (c) Cardillo, B.; Galeazzi, R.; Mobbili, G.; Orena, M. *Synlett* **1995**, *11*, 1159–1160.
6. Thomas, C.; Orecher, F.; Gmeiner, P. *Synthesis* **1998**, 1491–1496.
7. Pollini, G. P.; Barco, A.; De Giuli, G. *Synthesis* **1972**, *9*, 44–45.
8. Seebach, D.; Brenner, M. *Helv. Chim. Acta* **1999**, *82*, 2365–2379.
9. (a) Davies, B. G.; Bojer, V. *Nat. Prod. Rep.* **2001**, *18*, 618–640; (b) Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer-Verlag: Berlin, 2000; (c) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769–3826; (d) Haraldsson, G. G. The Application of Lipases in Organic Synthesis. In *The Chemistry of Functional Groups, Supplement B: The Chemistry of Acid Derivatives*; Patai, S., Ed.; John Wiley and Sons: Chichester, 1992; Vol. 2.
10. (a) Moen, A. R.; Hoff, B. H.; Hansen, L. K.; Aanthonsen, T.; Jacobsen, E. E. *Tetrahedron: Asymmetry* **2004**, *15*, 1551–1554; (b) Chenevert, R.; Tchedam, N.; Yannick, S. R.; Goupil, D. *Tetrahedron: Asymmetry* **1998**, *9*, 4325–4329; (c) Loubinoux, B.; Sinnes, J.-L.; O'Sullivan, A. C.; Winkler, T. *Tetrahedron* **1995**, *51*, 3549–3558; (d) Huges, D. L.; Bergan, J. J.; Amato, J. S.; Bhupathy, M.; Leazer, J. L.; Mc Namara, J. M.; Sidler, D. R.; Reider, P. J.; Grabowski, E. J. *Org. Chem.* **1990**, *55*, 6252–6259; (e) Lam, L. K.-P.; Jones, B. *Can. J. Chem.* **1988**, *66*, 1422–1424; (f) Santaniello, E.; Chiari, M.; Ferraboschi, P.; Trave, S. *J. Org. Chem.* **1988**, *53*, 1567–1569; (g) Lam, L. K.-P.; Hui, A. H. F.; Jones, B. *J. Org. Chem.* **1986**, *51*, 2047–2050; (h) Francis, C. J.; Jones, B. *J. Chem. Soc., Chem. Commun.* **1984**, *9*, 579–580.
11. Brook, M. A.; Chan, T. H. *Synthesis* **1983**, *9*, 201–203.
12. Flynn, D. L.; Zelle, R.; Grieco, P. *J. Org. Chem.* **1983**, *48*, 2424–2426.
13. Felluga, F.; Pitacco, G.; Prodan, M.; Pricl, S.; Visintin, M.; Valentin, E. *Tetrahedron: Asymmetry* **2001**, *12*, 3241–3249.