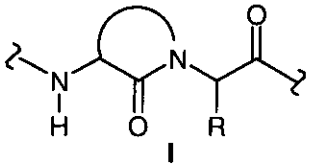
CHEMO- AND REGIOSELECTIVE SYNTHESES OF ENANTIOPURE AMINOPYRROLIDINONES AS BUILDING BLOCKS FOR CONSTRAINED PEPTIDOMIMETICS

Thomas Lehmann, Dorothee Michel, Markus Glänzel, Reiner Waibel, and Peter Gmeiner*

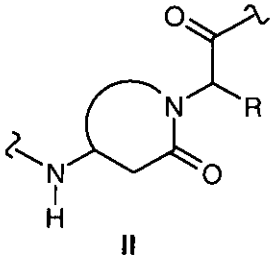
Institut für Pharmazie und Lebensmittelchemie der Universität Erlangen-Nürnberg, Schuhstraße 19, D-91052 Erlangen, Germany

Abstract - Starting from natural asparagine the synthesis of the *N*-protected enantiomerically pure 3- and 4-aminopyrrolidinones (**1**) and (**3**) was accomplished. The incorporation of these building blocks into conformationally constrained peptidomimetics was demonstrated by the synthesis of the potential dopamine receptor modulator (**14b**) (ß-PAOPA). Furthermore, *Freidinger* γ-lactams including the protected dipeptide mimetics (**8a-c**) and (**9**) were prepared. The optical integrity of the synthesis was established by NMR analysis of the ureas (**10a,b**).

Conformationally constrained peptide analogs represent an important family of compounds employed for the development of enzyme inhibitors and receptor ligands.[1] Additionally, interesting insights into the biologically active conformation of natural peptides are provided.[2] Because of their ability to serve as conformationally rigidized surrogates of peptide secondary structures, lactam-bridged peptidomimetics including *Freidinger* lactams (**I**) have proven particularly useful.[3] As an extension to these studies, the first investigations on the incorporation of ß-amino acid derived lactams (*Homo-Freidinger* lactams, **II**) were reported, very recently.[4-6]



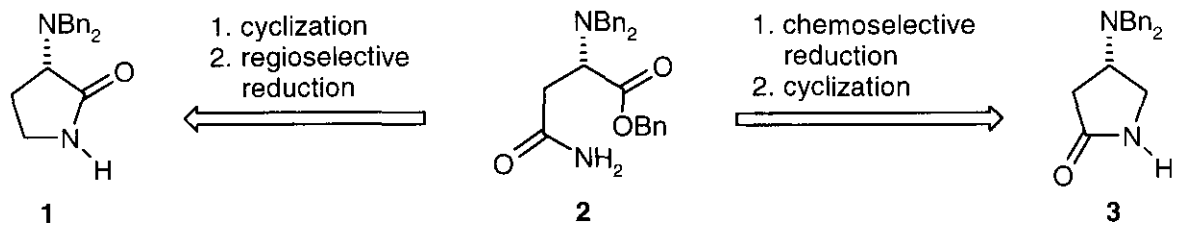
I



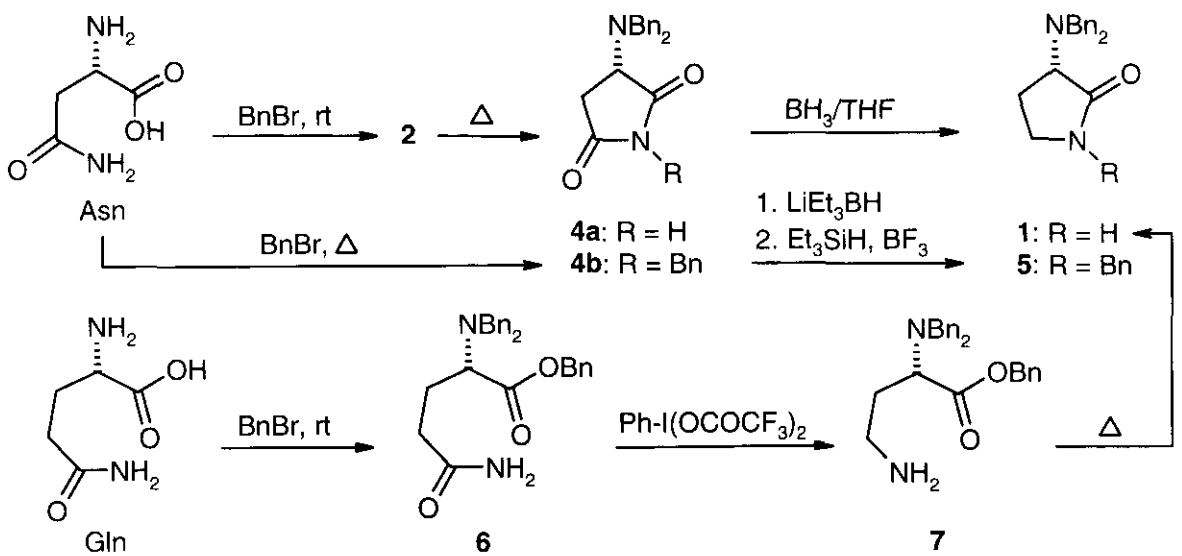
II

So far, only few synthetic methods for the construction of these molecular scaffolds in enantiomerically pure form are descibed in the literature.[7,8]

In this paper, we present a practical ex-chiral pool approach to 3- and 4-dibenzylaminopyrrolidinones which can serve as useful and flexible synthetic intermediates for the preparation of γ-lactam bridged peptidomimetics of type I and II, respectively. Our strategy was based on selective transformations of asparagine as a versatile bifunctional amino acid. Thus, cyclic imide formation of the benzyl protected asparagine derivative (**2**) [6,9,10] followed by regioselective reduction of the sterically less hindered carbonyl function should lead to the α-amino lactam (**1**). On the other hand, the ß-amino regioisomer should be approached by chemoselective reduction of the ester group of **2**, followed by activation of the thus formed primary alcohol and cyclization.



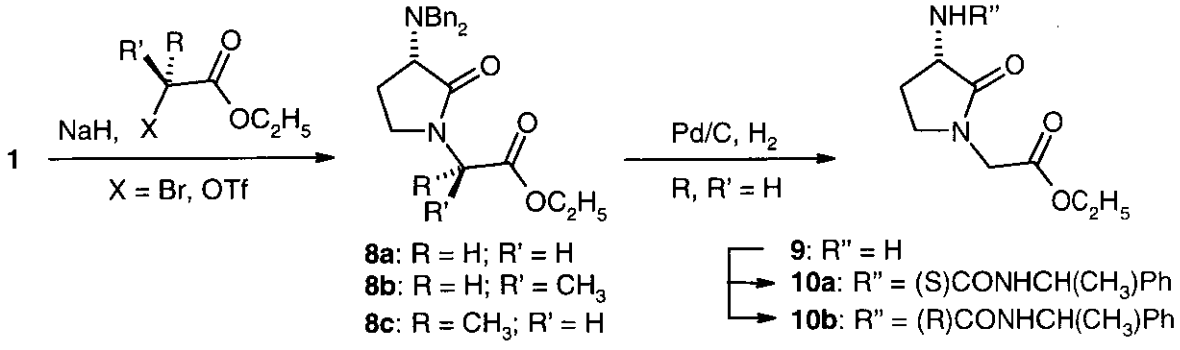
In practice, refluxing of the central intermediate (**2**) in toluene afforded the projected imide (**4a**) in high 91% yield (Scheme 1). Regioselective reduction of **4a** was attempted by a two-step procedure involving reduction with $LiEt_3BH$ and treatment of the thus formed hydroxylactam with a mixture of Et_3SiH and BF_3/Et_2O.



Scheme 1

Actually, product formation was observed, however, **1** could be isolated in only 8% yield. Employing BH_3/Et_2O as the reducing agent and CH_2Cl_2 as a solvent gave substantial improvement resulting in the formation of **1** in 48% yield. The synthesis of the regioisomer (**3**) as a side product could not be detected. Complete regioselectivity of the reduction was also observed for the *N*-alkylated imide (**4b**),[10] readily available by benzylation of asparagine under more drastic conditions. In this case, the two-step reduction procedure turned out to be advantageous affording the respective lactam (**5**) in 74% yield compared to 48% for the borane-reduction. As an alternative, a synthesis of **1** from natural glutamine involving a modified Hofmann degradation was elaborated.[11] Using our benzylation protocol described for asparagine, the glutamine derivative (**6**) as well as the enantiomer (**ent-6**) could be readily prepared from *(S)*- and *(R)*-glutamine, respectively. Subsequent treatment of **6** with bis-trifluoroacetoxyiodobenzene[12] resulted in form-ation of the primary amine (**7**) in 37% yield, which was converted into the lactam (**1**) upon refluxing in toluene.

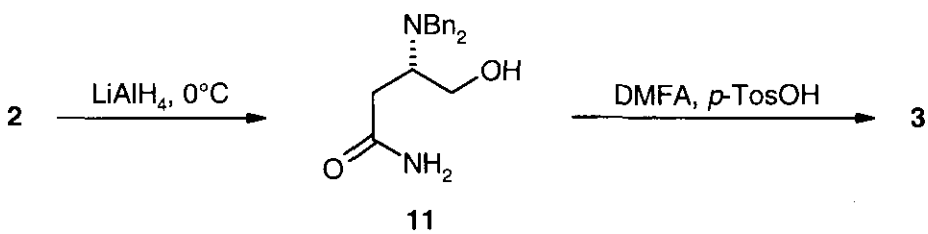
To verify whether the building block (**1**) is suitable as a central intermediate for lactam-bridged peptido-mimetics, further reactions including lactam *N*-alkylation and deprotection aw well as the optical integrity were investigated (Scheme2). Thus, preparation of the protected lactam-bridged dipeptide analog (**8a**) containing a glycine moiety was done by NaH induced deprotonation of **1** followed by lactam *N*-alkylation with ethyl bromoacetate. Subsequent hydrogenolytic deprotection afforded the primary amine (**9**). Starting from the commercially available amino acids *(R)*-asparagine and *(R)*-glutamine the enantiomeric building block (***ent*-9**) can be approached which is known as a synthetic intermediate of PAOPA,[13] a highly bioactive surrogate of the dopamine D2 receptor modulating peptide Pro-Leu-Gly-NH_2. The amino function of **9** was also used to determine the optical integrity of the synthesis. Thus, coupling of the dipeptide surrogate (**9**) with *(R)*- and *(S)*-1-phenylethyl isocyanate afforded the isomerically pure ureas (**10a**) and (**10b**), respectively. No diastereomeric impurity could be detected by careful ^1H NMR spectroscopic analysis of the crude products.



Scheme 2

For the incorporation of an alanine substructure the α-amino lactam (**1**) was deprotonated and alkylated with the commercially avaiable *(S)*- and *(R)*-configured ethyl 2-trifluormethylsulfonyloxypropionates to afford the dipeptide surrogates (**8b**) and (**8c**), respectively. In accordance to previous alkylation studies on Boc-protected α-amino-ε-caprolactams,[8] a partial epimerization at the acidic ester α-position was observed. Thus, the reaction of **1** with the *(S)*-configured malic acid equivalent resulted in formation of a 3.7:1 mixture of the diastereomers (**8b**) and (**8c**), which could be separated and purified by MPLC. On the other hand, employment of the *(R)*- configured electrophile resulted in a prefered formation of **8c** (**8b**:**8c** = 1:3)

The protected asparagine ester (**2**) was also chosen as the precursor of the 4-aminopyrrolidinone (**3**) representing a building block for *Homo-Freidinger* lactams (Scheme 3). Following our previously described protocol, chemoselective reduction of **2** afforded the *N,N*-dibenzylasparaginol (**11**)[10] which we attempted to cyclize under *Mitsunobu* conditions or by *O*-mesylation and subsequent ring closure. However, due to side reactions including lactone formation, migration of the amino fuctionality and ß-elimination, these reactions failed. Synthesis of the γ-lactam (**3**) was achieved by activation of the primary alcohol with dimethylformamide dimethyl acetal. Using *p*-TosOH as a catalyst and toluene as a solvent, pure product was formed in moderate yield (24%). However, due to the short reaction pathway and the inexpensive starting materials the synthesis is still very practical.



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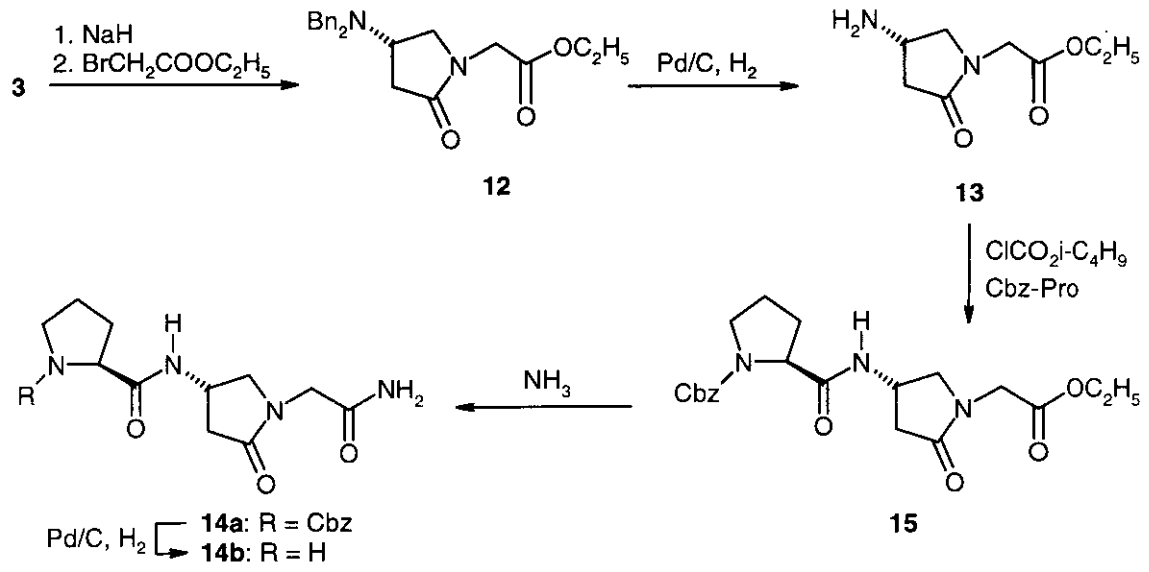
Scheme 3

The incorporation of the ß-amino-γ-lactams (**3**) into conformationally constrained peptide surrogates was representatively demonstrated by the synthesis of the tripeptide mimetic (**14b**) which can be regarded as a ß-amino isomer of the dopamine receptor modulator PAOPA (3*R*-[(2*S*-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide).[14] *N*-deprotonation of **3** with NaH, followed by reaction with ethyl bromoacetate resulted in the formation of **12**, which was hydrogenolytically debenzylated to give the amine (**13**) (Scheme 4). Proceeding through a mixed anhydrid, **13** was reacted with Cbz-Pro to afford the coupling product (**15**), which was transformed into **14a** by aminolysis of the glycine ester functionality. Subsequent hydrogenolytic *N*-deprotection gave the final product (**14b**) in 33% overall yield.



Scheme 4

EXPERIMENTAL

General: Solvents were purified and dried by standard procedures. Optical rotation was measured on a Perkin-Elmer Polarimeter 241 at 23°C. IR spectra were recorded on a Perkin-Elmer 1420. If not otherwise stated MS were run by EI ionization (70 eV) with solid inlet. ^{1}H-NMR spectra were obtained on Bruker AC 200 (200 MHz), AM 360 (360 MHz) and AM 400 (400 MHz) spectrometers, if not otherwise stated in $CDCl_3$ relative to TMS; ^{13}C-NMR spectra were run on a Bruker AC 250 (63 MHz) in DMSO-d_6 relative to the solvent resonance (δ = 39.5). Chromatographic purification was performed using Silica gel 60 (Merck).

(*S*)-(-)-*N*,*N*-Dibenzylasparagine benzyl ester (2)

L-Asparagine (22.5 g, 0.15 mol) was benzylated as earlier described[6] to give **2** (39.2 g, 65%) as a colorless solid; $[\alpha]_D$ -93° (c = 0.5, $CHCl_3$), ref. 6: $[\alpha]_D$ -93° (c = 0.5, $CHCl_3$).

(*S*)-(-)-3-*N*,*N*-Dibenzylaminopyrrolidine-2,5-dione (4a)

A solution of **2** (5 g, 12 mmol) in toluene (100 mL) was refluxed for 6 h. Evaporation of the solvent followed by flash chromatographic purification (petroleum ether-EtOAc 4:1) afforded **4a** (3.34 g, 91%) as colorless crystals; mp 93-95°C (petroleum ether); $[\alpha]_D$ -12.6° (c = 0.5, $CHCl_3$); IR (KBr) ν 3030, 2930, 1780, 1705, 1600 cm^{-1}; ^{1}H-NMR (200 MHz) δ 2.59 (dd, J = 18.6, 6.1 Hz, 1H, H-4a), 2.73 (dd, J = 18.6, 8.7 Hz, 1H, H-4b), 3.58 (d, J = 13.6 Hz, 2H, NCH_2Ph), 3.78 (d, J = 13.4 Hz, 2H, NCH_2Ph), 3.91 (dd, J = 8.7, 6.1 Hz, 1H, H-3), 7.14-7.35 (m, 10H, ar). *Anal.* Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.35; H, 6.16; N,

9.58.

(*S*)-(-)-1-Benzyl-3-*N*,*N*-dibenzylaminopyrrolidine-2,5-dione (4b)

To a solution of L-asparagine (7.5 g, 0.05 mmol) and K_2CO_3 (25 g) in H_2O (100 mL) benzyl bromide (35.63 mL, 0.3 mol) was added. The mixture was stirred under reflux for 2 h, then extracted 4 times with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated and the residue was separated by flash chromatography (petroleum ether-EtOAc 9:1) to give **4b** (5.1 g, 26%) as colorless crystals; mp 66-67°C (petroleum ether); $[\alpha]_D$ -18° (c = 0.5, $CHCl_3$), ref. 10: mp 65-66°C; $[\alpha]_D$ -19° (c = 0.5, $CHCl_3$). As a further product *N*,*N*-dibenzylasparaginic acid dibenzyl ester (6.8 g, 27%) was isolated.

(*S*)-(-)-3-*N*,*N*-Dibenzylaminopyrrolidin-2-one (1)

a) To a solution of **4a** (1.0 g, 3.4 mmol) in CH_2Cl_2 (30 mL) a solution (1 M) of BH_3 (20.4 mL, 20.4 mmol) in THF was added dropwise at 0°C under N_2. After 1 h the temperature was raised to rt and finally the reaction mixture was refluxed for 4 h. The reaction was quenched by acidifying to pH 1 with 6N HCl, the mixture was alkalized to pH 12 with 2N NaOH and extracted 4 times with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether-EtOAc 3:2) to give **1** (462 mg, 48%) as a colorless oil; $[\alpha]_D$ -24.7° (c = 0.5, $CHCl_3$); CI-MS (isobutane) *m/z* 281 (MH^+); IR (film) ν 3246, 3028, 2927, 2870, 1695 cm^{-1}; ^{1}H-NMR (360 MHz) δ 2.04-2.23 (m, 2H, H-4) 3.15-3.24 (m, 1H, H-5), 3.27-3.35 (m, 1H, H-5), 3.58 (dd, J = 9.3, 9.3 Hz, 1H, H-3), 3.66 (d, J = 13.9 Hz, 2H, NC$\underline{H}_2$Ph), 3.95 (d, J = 13.9 Hz, 2H, NC$\underline{H}_2$Ph), 6.50 (s, 1H, NH), 7.19-7.24 (m, 2H, Ar), 7.27-7.33 (m, 4H, Ar), 7.41-7.46 (m, 4H, Ar). *Anal.* Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 76.78; H, 7.29; N, 9.91.

b) To **4a** (150 mg, 0.51 mmol) dissolved in CH_2Cl_2 (20 mL) a solution (1 M) of $LiEt_3BH$ (0.61 mL, 0.61 mmol) in THF was added at -78°C under N_2. After 1 h a second portion of $LiEt_3BH$ (0.61 mL, 0.61 mmol) was added and the mixture was stirred for further 30 min before the reaction was quenched with a saturated solution of $NaHCO_3$ (5 mL). At 0°C H_2O_2 (0.15 mL) was added and after stirring for 30 min the mixture was brought to rt. Extraction with CH_2Cl_2, drying of the combined organic layers ($MgSO_4$) and evaporation of the solvent afforded a crude product (176 mg) which was used without purification for the following reaction.

To a solution of the crude product (176 mg) and Et_3SiH (0.32 mL, 2.24 mmol) in CH_2Cl_2 (20 mL) BF_3/Et_2O (8 M, 0.28 mL, 2.24 mmol) was added at -78°C under N_2. After 1 h a second portion of Et_3SiH (0.16 mL, 1.02 mmol) and BF_3/Et_2O (8 M, 0.14 mL, 1.12 mmol) was added. The mixture was stirred for 2 h brought to rt and after further 2 h stirring the reaction was quenched by adding a saturated solution of $NaHCO_3$ (10 mL). Extraction with CH_2Cl_2, drying of the combined extracts ($MgSO_4$), evaporation and subsequent separation by flash chromatography afforded **1** (11.5 mg, 8%) as a colorless oil; $[\alpha]_D$ -25° (c = 0.5, $CHCl_3$); identical with the product described above.

c) Crude **7** (1.8 g, 4.64 mmol) was refluxed in toluene (40 mL) for 17 h. To the reaction mixture a saturated solution of $NaHCO_3$ was added and the water layer was extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), evaporated and purified by flash chromatography (petroleum ether-EtOAc 1:1) to give **1** (0.5 g, 38.5%) as a colorless oil; $[\alpha]_D$ -25.4° (c = 1.0, $CHCl_3$); identical with the product described above.

(*S*)-(-)-1-Benzyl-3-*N*,*N*-dibenzylaminopyrrolidin-2-one (5)

Starting from **4b** (80 mg, 0.21 mmol) the above described reaction sequence a) afforded **5** (37.4 mg, 48%) as a colorless oil; reaction sequence b), starting from **4b** (1g, 2.59 mmol) gave **5** (671 mg, 74%) as a colorless oil, too; $[\alpha]_D$ -13.5° (c = 0.5, $CHCl_3$); IR (film) ν 3020, 2920, 1680, 1490, 1450 cm^{-1}; ^{1}H-NMR (400 MHz) δ 1.88 (m, 1H, H-4), 2.02 (m, 1H, H-4), 2.97 (ddd, J = 9.6, 8.1, 8.1 Hz, 1H, H-5), 3.08 (ddd, J = 9.6, 9.6, 2.4 Hz, 1H, H-5), 3.61 (m, 1H, H-3), 3.61 (d, J = 13.8 Hz, 2H, NCH_2Ph), 3.92 (d, J = 13.8 Hz, 2H, NCH_2Ph), 4.31 (d, J = 14.5 Hz, 1H, NCH_2Ph), 4.43 (d, J = 14.5 Hz, 1H, NCH_2Ph), 7.14-7.39 (m, 15H, Ar). *Anal.* Calcd for $C_{25}H_{26}N_2O$: C, 81.05; H, 7.07; N, 7.56. Found: C, 80.25; H, 7.12; N, 7.29.

***(S)*-(-)-*N*,*N*-Dibenzylglutamine benzyl ester (6)**

To a solution of L-glutamine (12.5 g, 85 mmol) in H_2O (575 mL), K_2CO_3 (57.5 g, 0.42 mol) and benzyl bromide (58.5 g, 0.34 mol) were added. After stirring for 10 d at rt the mixture was extracted with Et_2O, the combined organic layers were dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (petroleum ether-EtOAc 3:2 to 2:3) to yield **6** (15.9 g, 46%) as a colorless oil; $[\alpha]_D$ -100.3° (c = 1.0, $CHCl_3$); IR (film) ν 3459, 3343, 3191, 3029, 2939, 2846, 1727, 1672, 1605 cm^{-1}; ^{1}H-NMR (250 MHz) δ 1.99-2.35 (m, 4H, H-3, H-4), 3.36 (dd, J = 7.5, 6.5 Hz, 1H, H-2), 3.52 (d, J = 13.5 Hz, 2H, NCH_2Ph), 3.87 (d, J = 13.5 Hz, 2H, NCH_2Ph), 5.16 (d, J = 12.0 Hz, 1H, OCH_2Ph), 5.27 (d, J = 12.0 Hz, 1H, OCH_2Ph), 7.17-7.46 (m, 15H, Ar). *Anal.* Calcd for $C_{26}H_{28}N_2O_3$: C, 74.98; H, 6.78; N, 6.73. Found: C, 74.89; H, 6.72; N, 6.73.

Starting from D-glutamine the same procedure afforded **ent-6** as a colorless oil; $[\alpha]_D$ +100° (c = 1.0, $CHCl_3$).

(*S*)-(-)-4-Amino-2-*N*,*N*-dibenzylaminobutanoic acid benzyl ester (7)

To a solution of bis-trifluoroacetoxyiodobenzene (7 g, 16.3 mmol) in DMF (30 mL) and H_2O (45 mL), compound (**6**) (4.5 g, 10.8 mmol) in DMF (15 mL) was added slowly. After stirring at rt for 15 min pyridine (1.71 g, 21.6 mmol) was added, the reaction mixture was stirred for further 4 h at rt and then evaporated. The residue was mixed with a saturated solution of $NaHCO_3$ and extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated to give the crude product (3.6 g, 86%) as a colorless oil, which was used for the following reaction.

To characterize the compound a sample was purified by flash chromatography (CH_2Cl_2-MeOH 4:1) to yield pure **7** as a colorless oil; $[\alpha]_D$ -29.7° (c = 0.6, $CHCl_3$); CI-MS (methane) *m/z* 389 (MH^+); IR (film) ν 3500-3100, 3027, 2929, 2844, 1730 cm^{-1}; ^{1}H-NMR (360 MHz) δ 1.94-2.06 (m, 1H, H-3), 2.12-2.24 (m, 1H, H-3), 2.73-2.83 (m, 1H, H-4), 2.88-2.99 (m, 1H, H-4), 3.43 (dd, J = 10.6, 5.1 Hz, 1H, H-2), 3.47 (d, J = 13.4 Hz, 2H, NCH_2Ph), 3.85 (d, J = 13.4 Hz, 2H, NCH_2Ph), 5.12 (d, J = 12.0 Hz, 1H, OCH_2Ph), 5.25 (d, J = 12.0 Hz, 1H, OCH_2Ph), 7.18-7.45 (m, 15H, Ar). *Anal.* Calcd for $C_{25}H_{28}N_2O_2 \cdot ½H_2O$: C, 75.54; H, 7.35; N, 7.05. Found: C, 75.23; H, 7.07; N, 6.94.

(*S*)-(-)-3-*N*,*N*-Dibenzylaminopyrrolidin-2-on-1-ylacetic acid ethyl ester (8a)

A solution of **1** (150 mg, 0.535 mmol) in THF (5 mL) was treated at 0°C with 50% NaH in paraffin (21 mg, 0.535 mmol). After stirring at 0°C for 30 min bromoacetic acid ethyl ester (222 mg, 1.338 mmol) was added. The mixture was stirred for 4 h at 0°C, then 14 h at rt. After that EtOAc (15 mL) and a saturated $NaHCO_3$

solution (30 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petrol-EtOAc 7:3 to 3:7) to yield the product (140 mg, 72%) as a colorless oil; $[\alpha]_D$ -24.3° (c = 1.0, $CHCl_3$); CI-MS (methane) *m/z* 367 (MH^+); IR (film) ν 3026, 2979, 2925, 2852, 1747, 1695 cm^{-1}; ^{1}H-NMR (360 MHz) δ 1.25 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2O$), 2.00-2.23 (m, 2H, H-4), 3.31 (ddd, J = 9.1, 8.8, 8.8 Hz, 1H, H-5), 3.37 (ddd, J = 9.1, 9.1, 2.8 Hz, 1H, H-5), 3.68 (d, J = 13.9 Hz, 2H, NCH_2Ph), 3.69 (dd, J = 9.3, 9.3 Hz, 1H, H-3), 3.95 (d, J = 13.9 Hz, 2H, NCH_2Ph), 3.98 (d, J = 17.5 Hz, 1H, NCH_2CO), 4.09 (d, J = 17.5 Hz, 1H, NCH_2CO), 4.17 (q, J = 7.2 Hz, 2H, $CH_3C\underline{H}_2O$), 7.19-7.25 (m, 2H, Ar), 7.27-7.33 (m, 4H, Ar), 7.41-7.47 (m, 4H, Ar). *Anal.* Calcd for $C_{22}H_{26}N_2O_3 \cdot ¼ H_2O$: C, 71.23; H, 7.20; N, 7.55. Found: C, 71.25; H, 7.26; N, 7.43.

(-)-2*R*-(3*S*-*N*,*N*-Dibenzylaminopyrrolidin-2-on-1-yl)propionic acid ethyl ester (8b) and (-)-2*S*-(3*S*-*N*,*N*-dibenzylaminopyrrolidin-2-on-1-yl)propionic acid ethyl ester (8c)

A solution of **1** (36 mg, 0.13 mmol) in THF (5 mL) was treated at 0°C with 60% NaH in paraffin (36 mg, 0.13 mmol). After stirring for 30 min (*S*)-2-trifluoromethanesulfonyloxypropionic acid ethyl ester (32 mg, 0.128 mmol) was added. The mixture was stirred for 4 h at 0°C and then for 14 h at rt. EtOAc (5 mL) and a saturated solution of $NaHCO_3$ (10 mL) were added, the layers were separated and the water layer was extracted with EtOAc. After drying ($MgSO_4$) and evaporation of the solvent the mixture was separated by MPLC (petroleum ether-EtOAc 7:3 to 3:7) to yield **8b** (18 mg, 37 %) and **8c** (5 mg, 10 %) as colorless oils together with **1** (17 mg). The same reaction starting from **1** (106 mg, 0.378 mmol) and (*R*)-2-trifluoromethanesulfonyloxypropionic acid ethyl ester (95 mg, 0.378 mmol) afforded **8b** (20 mg, 14 %), **8c** (61 mg, 42 %), and **1** (33 mg).

8b: $[\alpha]_D$ -2.8° (c = 1.0, $CHCl_3$); IR (film) ν 3326, 2981, 2938, 1738, 1692 cm^{-1}; ^{1}H-NMR (C_6D_6, 360 MHz) δ = 0.83 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$), 1.10 (d, J = 7.4 Hz, 3H, $C\underline{H}_3CH$), 1.45 - 1.73 (m, 2H, H-4), 2.70 (ddd, J = 9.0, 9.0, 2.6 Hz, 1H, H-5), 2.90 (ddd, J = 9.0, 8.4, 8.4 Hz, 1H, H-5), 3.67 (d, J = 13.9 Hz, 2H, NCH_2Ph), 3.68 (dd, J = 9.2, 9.2 Hz, 1H, H-3), 3.82 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O$), 4.13 (d, J = 13.9 Hz, 2H, NCH_2Ph), 4.92 (q, J = 7.4 Hz, 2H, $CH_3C\underline{H}$), 7.06 - 7.12 (m, 2H, ar), 7.14 - 7.21 (m, 4H, ar), 7.46 - 7.51 (m, 4H, ar). *Anal.* Calcd for $C_{23}H_{28}N_2O_3$: C, 72.61; H,7.42; N, 7.36. Found: C, 72.53; H, 7.51; N, 7.27.

8c: $[\alpha]_D$ -22.5° (c = 1.0, $CHCl_3$); IR (film) ν 3326, 2981, 2927, 1737, 1691 cm^{-1}; ^{1}H-NMR (C_6D_6, 360 MHz) δ = 0.82 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$), 1.04 (d, J = 7.5 Hz, 3H, $C\underline{H}_3CH$), 1.49 - 1.81 (m, 2H, H-4), 2.43 (ddd, J = 9.0, 8.4, 8.4 Hz, 1H, H-5), 2.97 (ddd, J = 9.0, 9.0, 2.6 Hz, 1H, H-5), 3.59 (dd, J = 9.0, 9.0 Hz, 1H, H-3), 3.72 (d, J = 13.9 Hz, 2H, NCH_2Ph), 3.81 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O$), 4.19 (d, J = 13.9 Hz, 2H, NCH_2Ph), 4.98 (q, J = 7.5 Hz, 2H, $CH_3C\underline{H}$), 7.06 - 7.13 (m, 2H, ar), 7.15 - 7.23 (m, 4H, ar), 7.50 - 7.55 (m, 4H, ar). *Anal.* Calcd for $C_{23}H_{28}N_2O_3$: C, 72.61; H,7.42; N, 7.36. Found: C, 72.45; H, 7.26; N, 7.23.

(*S*)-(-)-3-Aminopyrrolidin-2-on-1-ylacetic acid ethyl ester (9)

A mixture of **8a** (138 mg, 0.377 mmol) and 10% Pd/C (30 mg) in EtOH (30 mL) was stirred under H_2 (1 bar) at rt for 4 h. The mixture was filtered, the filtrate evaporated and the residue purified by flash chromatography (CH_2Cl_2-MeOH 9:1) to yield the product (60 mg, 86%) as a colorless oil; $[\alpha]_D$ -45.3° (c = 1.0, $CHCl_3$); MS *m/z* 186 (M^+); IR (film) ν 3600 - 3000, 3370, 2982, 2931, 1741, 1681 cm^{-1}; ^{1}H-NMR (360 MHz) δ 1.28 (t,

J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$), 1.80 (dddd, J = 12.6, 9.7, 9.3, 9.3 Hz, 1H, H-4), 2.47 (dddd, J = 12.6, 8.4, 6.8, 1.9 Hz, 1H, H-4), 3.36 (ddd, J = 9.3, 9.3, 1.9 Hz, 1H, H-5), 3.45 (ddd, J = 9.3, 9.3, 6.8 Hz, 1H, H-5), 3.57 (dd, J = 9.7, 8.4 Hz, 1H, H-3), 3.97 (d, *J* = 17.5 Hz, 1H, NCH_2CO), 4.15 (d, *J* = 17.5 Hz, 1H, NCH_2CO), 4.20 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O$). *Anal.* Calcd for $C_8H_{14}N_2O_3 \cdot H_2O$: C, 47.05; H, 7.90; N, 13.72. Found: C, 46.42; H, 7.46; N, 14.25.

(-)-3*S*-[3-(1*S*-Phenylethyl)ureido]pyrrolidin-2-on-1-ylacetic acid ethyl ester (10a)

To a solution of **9** (10 mg, 0.054 mmol) in THF (1 mL) (*S*)-1-phenylethyl isocyanate (8 mg, 0.054 mmol) was added at 0°C. After 3 h the solvent was evaporated and the residue purified by flash chromatography (CH_2Cl_2-MeOH 19:1) to give **10a** as a colorless oil; $[\alpha]_D$ -26.4° (c = 0.4, $CHCl_3$); MS *m/z* 333 (M^+); IR (film) ν 3342, 2963, 2928, 1747, 1695, 1643 cm^{-1}; ^{1}H-NMR (360 MHz) δ 1.26 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$), 1.44 (d, J = 7 Hz, 1H, $CHC\underline{H}_3$) 1.89 (dddd, J = 12.5, 9.9, 9.5, 9.5 Hz, 1H, H-4), 2.65 (dddd, J = 12.5, 8.0, 6.6, 1.2 Hz, 1H, H-4), 3.35 (ddd, J = 9.5, 9.5, 1.2 Hz, 1H, H-5), 3.46 (ddd, J = 9.5, 9.5, 6.6 Hz, 1H, H-5), 3.94 (d, J = 17.8 Hz, 1H, NCH_2CO), 4.10 (d, J = 17.8 Hz, 1H, NCH_2CO), 4.17 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O$), 4.38 (ddd, J = 9.9, 8.0, 4.8 Hz, 1H, H-3), 4.88 (dq, J = 7.0, 7.0 Hz, 1H, $C\underline{H}CH_3$), 5.41 (br d, J = 4.8 Hz, 1H, $N\underline{H}CH$), 5.58 (br d, *J* = 7.0 Hz, 1H, $N\underline{H}CH$), 7.19 - 7.35 (m, 5H, ar). *Anal.* Calcd for $C_{17}H_{23}N_3O_4$: C, 61.25; H, 6.95; N, 12.60. Found: C, 60.97; H, 7.11; N, 12.56.

(-)-3*S*-[3-(1*R*-Phenylethyl)ureido]pyrrolidin-2-on-1-ylacetic acid ethyl ester (10b)

The same reaction as described above using (*R*)-1-phenylethyl isocyanate (8 mg, 0.054 mmol) afforded **10b** (17 mg, 94%) as a colorless oil; $[\alpha]_D$ -2.1° (c = 0.5, $CHCl_3$); MS *m/z* 333 (M^+); IR (film) ν 3345, 2978, 2932, 1744, 1695, 1642 cm^{-1}; ^{1}H-NMR (360 MHz) δ 1.26 (t, J = 7.1 Hz, 3H, $C\underline{H}_3CH_2O$), 1.43 (d, J = 7.0 Hz, 1H, $CHC\underline{H}_3$) 1.90 (dddd, J = 12.7, 10.0, 9.6, 9.6 Hz, 1H, H-4), 2.65 (dddd, J = 12.7, 8.0, 6.8, 1.2 Hz, 1H, H-4), 3.35 (ddd, J = 9.6, 9.6, 1.2 Hz, 1H, H-5), 3.45 (ddd, J = 9.6, 9.6, 6.8 Hz, 1H, H-5), 3.94 (d, J = 17.5 Hz, 1H, NCH_2CO), 4.12 (d, J = 17.5 Hz, 1H, NCH_2CO), 4.17 (q, J = 7.1 Hz, 2H, $CH_3C\underline{H}_2O$), 4.26 (ddd, J = 10.0, 8.0, 4.7 Hz, 1H, H-3), 4.87 (dq, J = 7.0, 7.0 Hz, 1H, $C\underline{H}CH_3$), 5.48 (br d, J = 4.7 Hz, 1H, $N\underline{H}CH$), 5.66 (br d, J = 7.0 Hz, 1H, $N\underline{H}CH$), 7.19 - 7.35 (m, 5H, ar). *Anal.* Calcd for $C_{17}H_{23}N_3O_4$: C, 61.25; H, 6.95; N, 12.60. Found: C, 60.97; H, 7.11; N, 12.56.

(*S*)-(+)-*N*,*N*-Dibenzylamino-4-hydroxybutanecarboxamide (11)

Compound **2** (5.2 g, 12.9 mmol) was reduced with $LiAlH_4$ as earlier described[10] to give **11** (2.3 g, 60%) as a colorless oil; $[\alpha]_D$ +8° (c = 1, $CHCl_3$), ref. 10: $[\alpha]_D$ +8° (c = 1, $CHCl_3$).

(*S*)-(+)-4-*N*,*N*-Dibenzylaminopyrrolidin-2-one (3)

A solution of **11** (298.4 mg, 1 mmol) in toluene (20 mL) was refluxed with *N*,*N*-dimethylformamide dimethyl acetal (0.67 mL, 5 mmol) and *p*-toluenesulfonic acid (19 mg, 0.1 mmol) for 3.5 h. After evaporation of the solvent the residue was purified by column chromatography (CH_2Cl_2-MeOH 19:1) to yield **3** (67 mg, 24%) as a colorless solid; mp 139°C (MeOH); $[\alpha]_D$ +26.2° (c = 0.5, $CHCl_3$); IR (KBr) ν 3600-3100, 3460, 3400, 1670 cm^{-1}; ^{1}H-NMR (400 MHz) δ 2.31 (dd, J = 17.3, 8.6 Hz, 1H, H-3), 2.38 (dd, J = 17.3, 6.6 Hz, 1H, H-3), 3.30

(dd, J = 10.3, 5.3 Hz, 1H, H-5), 3.39 (dd, J = 10.3, 7.9 Hz, 1H, H-5), 3.47 (d, J = 13.8 Hz, 2H, NCH_2Ph), 3.58 (d, J = 13.8 Hz, 2H, NCH_2Ph), 3.62-3.70 (m, 1H, H-4), 6.16 (s, 1H, NH), 7.15-7.28 (m, 10H, Ph). *Anal.* Calcd for $C_{21}H_{27}N_3O_2$: C, 77.10; H, 7.19; N, 10.00. Found: C, 77.00; H, 7.25; N, 9.89.

(*S*)-4-*N*,*N*-Dimethylaminopyrrolidin-2-on-1-ylacetic acid ethyl ester (12)

A solution of **3** (65.8 mg, 0.24 mmol) in THF (10 mL) was treated at 0°C with a suspension of 50% NaH (23.2 mg, 0.591 mmol) in paraffin. After stirring 1 h at 0°C bromoacetic acid ethyl ester (79.7 mg, 0.48 mmol) was added and the mixture was stirred at 0°C for further 3 h. Adding EtOAc (5 mL) and a saturated solution of NaCl quenched the reaction. The layers were separated and the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated, and the crude product was purified by column chromatography (petroleum ether-EtOAc 3:2) to yield **12** (47.3 mg, 54%) as a colorless oil; MS *m/z* 366 (M^+); IR (film) ν 3500-3100, 1730, 1680 cm^{-1}; ^{1}H-NMR (400 MHz) δ 1.23 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.50 (dd, J = 17.7, 9.0 Hz, 1H, H-3), 2.58 (dd, J = 17.7, 5.7 Hz, 1H, H-3), 3.53-3.57 (m, 2H, H-5), 3.55 (d, J = 13.8 Hz, 2H, NCH_2Ph), 3.70 (d, J = 13.8 Hz, 2H, NCH_2Ph), 3.70-3.74 (m, 1H, H-4), 3.95 (d, J = 17.5 Hz, 1H, H-6), 4.11 (d, J = 17.5 Hz, 1H, H-6), 4.16 (q, J = 7.1 Hz, 3H, $C\underline{H}_2CH_3$), 7.23-7.36 (m, 10H, Ph). *Anal.* Calcd for $C_{22}H_{26}N_2O_3$: C, 72.11; H, 7.15; N, 7.64. Found: C, 72.01; H, 7.23; N, 7.62.

(*S*)-4-Aminopyrrolidin-2-on-1-ylacetic acid ethyl ester (13)

A mixture of **12** (381 mg, 1.04 mmol) and 10% Pd/C (50 mg) in MeOH (15 mL) was stirred under H_2 (1 bar) at rt for 24 h. The mixture was filtered and the filtrate evaporated to give **13** (192 mg, 99%) as an unstable colorless oil. ^{1}H-NMR (400 MHz) δ 1.25 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.66 (br s, 2H, NH_2), 2.19 (dd, J = 16.7, 3.5 Hz, 1H, H-3), 2.71 (dd, J = 16.7, 6.4 Hz, 1H, H-3), 3.13-3.18 (m, 1H, H-4), 3.60-3.74 (m, 2H, H-5), 3.80-4.06 (m, 2H, H-6), 4.17 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$). HR-MS *m/z* 186.0999 (M^+) calcd for $C_8H_{14}N_2O_3$: 186.1004.

(*S*)-(-)-4-(*N*-Benzyloxycarbonylprolyl)aminopyrrolidin-2-on-1-ylacetic acid ethyl ester (15)

To a solution of L-Cbz-proline (267.0 mg, 1.08 mmol) in THF (15 mL) *N*-methylmorpholine (0.12 mL, 1.08 mmol) was added. After cooling the solution to -15°C i-butyl chloroformiate (0.134 mL, 1.03 mmol) and a solution of **13** (192 mg, 1.03 mmol) in THF (15 mL) were added dropwise. The reaction mixture was kept at -15°C for 5 min and after stirring at rt for 30 min it was filtered and evaporated. The residue was dissolved in a mixture of $CHCl_3$ (5 mL), H_2O (5 mL) and EtOAc (25 mL). The layers were separated and the organic layer was successively washed with a saturated solution of Na_2CO_3 and H_2O. Drying ($MgSO_4$), evaporation of the solvent and subsequent purification of the residue by column chromatography (CH_2Cl_2-MeOH 19:1) afforded **15** (312 mg, 73%) as a colorless oil; $[\alpha]_D$ -198° (c = 0.5, $CHCl_3$); CI-MS (methane) *m/z* 418 (MH^+); IR (film) ν 3320, 2980, 2940, 2880, 1750, 1700 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 360 MHz, 100°C) δ 1.21 (t, J = 7.0 Hz, 3H, $OCH_2C\underline{H}_3$), 1.73-1.93 (m, 3H, 2 x H-8 Pro, H-β Pro), 2.12 (m, 1H, H-β Pro), 2.20 (dd, J = 17.0, 5.5 Hz, 1H, H-3), 2.53 (dd, J = 17.0, 8.5 Hz, 1H, H-3), 3.18 (dd, J = 10.0, 5.0 Hz, 1H, H-5), 3.37-3.50 (m, 2H, 2 x H-δ Pro), 3.63 (dd, J = 10.0, 7.5 Hz, 1H, H-5), 3.93 (d, J = 17.0 Hz, 1H, H-α Gly), 3.99 (d, J = 17.0 Hz, 1H, H-α Gly), 4.13 (q, J = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.17 (dd, J = 8.5, 4.0 Hz, 1H, H-α Pro), 4.32 (m, 1H, H-4), 5.01 (d,

J = 13.0 Hz, 1H, OCH_2Ph), 5.07 (d, J = 13.0 Hz, 1H, OCH_2Ph), 7.24-7.37 (m, 5H, Ph), 7.96 (d, J = 5.5 Hz, 1H, CONH). At temperatures below 60°C a double set of signals was observed. *Anal.* Calcd for $C_{21}H_{27}N_3O_6 \cdot H_2O$: C, 57.92; H, 6.71; N, 9.65. Found: C, 58.05; H, 6.90; N, 9.45.

(*S*)-(-)-4-(*N*-Benzyloxycarbonylprolyl)aminopyrrolidin-2-on-1-ylacetic acid amide (14a)

A solution of **15** (295 mg, 0.71 mmol) in MeOH (10 mL) was treated at rt for 16 h with NH_3. The solvent was removed and the residue purified by flash chromatography (CH_2Cl_2-MeOH 9:1) to give **14a** (259 mg, 94%) as a colorless oil; $[\alpha]_D$ -58.1° (c = 0.3, MeOH); CI-MS (methane) *m/z* 389 MH^+); IR (KBr) ν 3387, 2957, 2884, 1680, 1551 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 360 MHz, 100°C) δ 1.75-1.90 (m, 3H, 2 x H-8 Pro, H-β Pro), 2.11 (m, 1H, H-β Pro), 2.17 (dd, J = 17.0, 5.5 Hz, 1H, H-3), 2.54 (dd, J = 17.0, 8.5 Hz, 1H, H-3), 3.14 (dd, J = 10.0, 4.5 Hz, 1H, H-5), 3.37-3.51 (m, 2H, 2 x H-δ Pro), 3.61 (dd, J = 10.0, 7.5 Hz, 1H, H-5), 3.73 (d, J = 17.0 Hz, 1H, H-α Gly), 3.77 (d, J = 17.0 Hz, 1H, H-α Gly), 4.15 (dd, J = 8.5, 3.5 Hz, 1H, H-α Pro), 4.30 (m, 1H, H-4), 5.01 (d, J = 13.0 Hz, 1H, OCH_2Ph), 5.07 (d, J = 13.0 Hz, 1H, OCH_2Ph), 6.85 (br s, 2H, $CONH_2$), 7.24-7.37 (m, 5H, Ph), 7.95 (br d, J = 5.5 Hz, 1H, CONH). At temperatures below 60°C a double set of signals was observed.

(*S*)-(-)-4-Prolylaminopyrrolidin-2-on-1-ylacetic acid amide (14b)

A mixture of **14a** (194.3 mg, 0.5 mmol) and 10% Pd/C (19 mg) in MeOH (15 mL) was stirred under H_2 (1 bar) at rt for 12 h. The mixture was filtered, the filtrate evaporated and the residue purified by column chromatography (CH_2Cl_2-MeOH-Me_2NH 9:1:0.1) to yield the product (90 mg, 71%) as a colorless oil; $[\alpha]_D$ -58° (c = 0.1, MeOH); IR (film) ν 3329, 2971, 2878, 1675, 1551 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 360 MHz) δ 1.53 - 1.69 (m, 3H, 2 x H-γ Pro, H-β Pro), 1.92 (m, 1H, H-β Pro), 2.22 (dd, 1H, J = 16.5, 5.0 Hz, H-3), 2.57 (dd, 1H, J = 16.5, 8.5 Hz, H-3), 2.80 (m, 2H, H-δ Pro), 3.13 (dd, 1H, J = 10.0, 4.5 Hz, H-5), 3.27 (s, 1H, NH), 3.48 (dd, 1H, J = 9.0, 5.5 Hz, H-α Pro), 3.64 (dd, 1H, J = 10.0, 7.5 Hz, H-5), 3.72 (1H, d, J = 17 Hz, H-α Gly), 3.78 (1H, d, J = 17 Hz, H-α Gly), 4.35 (m, 1H, H-4), 7.11 (s, 1H, $CONH_2$), 7.39 (s, 1H, $CONH_2$), 8.28 (d, 1H, J = 7.5 Hz, CONHCH); ^{13}C-NMR δ = 25.7 (C-β Pro), 30.4 (C-γ Pro), 36.8 (C-3), 41.8 (C-4), 44.7 (C-α Gly), 46.7 (C-δ Pro), 53.7 (C-5), 60.1 (C-α Pro), 169.6 (C=O Gly), 172.3 (C-2), 174.6 (C=O Pro). *Anal.* Calcd for $C_{11}H_{18}N_4O_3 \cdot H_2O$: C, 48.52; H,7.40; N, 20.53. Found: C, 48.31; H, 7.76; N, 20.85.

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