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Syntheses and ^{1}H NMR Spectroscopic Investigations of Some Pyrrolidine Carboxylic Acids Designed as Potential Glial GABA Uptake Inhibitors

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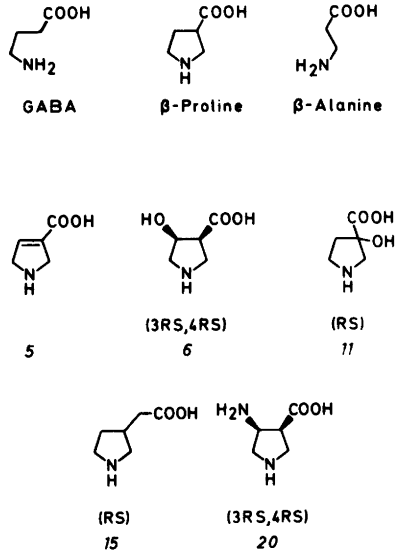
The syntheses of 3-pyrroline-3-carboxylic acid (*5*), *cis*-4-hydroxypyrrolidine-3-carboxylic acid (*6*), 3-hydroxypyrrolidine-3-carboxylic acid (*11*), pyrrolidine-3-acetic acid (homo-β-proline) (*15*) and *cis*-4-aminopyrrolidine-3-carboxylic acid (*20*) are described. Catalytic hydrogenation of appropriate cyclic β-oxoesters are keysteps in the preparation of *5* and *6*. Compound *11* was synthesized from the ketone *8* *via* the corresponding protected cyanohydrin *9*, and homo-β-proline (*15*) was prepared *via* a Knoevenagel reaction. The β-amino acid *20* was prepared by stepwise hydrogenation of the enamine *16* followed by acid treatment of the protected product *19*. 270 MHz ^{1}H NMR spectroscopic analyses of *4a*, *7* and *19* were carried out in order to establish the relative stereochemistry of *6* and *20*.

The glial uptake system probably contributes to the termination of GABA mediated synaptic transmission in the central nervous system.[1-3] β-Alanine[1,4] and in particular β-proline[3,5,6] are inhibitors of the glial GABA uptake system. In an attempt to develop more potent and more selective glial GABA uptake inhibitors we have synthesized a number of cyclic amino acids structurally related to β-proline, the structure of which combines the structural elements of GABA as well as of β-alanine.

Most of these compounds, *i.e.* *5*, *6*, *11* and *20* have little effect on glial as well as on neuronal GABA transport *in vitro*.[6-8] Pyrrolidine-3-acetic acid (homo-β-proline) (*15*), on the other hand, turned out to be a very potent competitive inhibitor of both GABA transport systems. The compound, however, is not a specific GABA uptake inhibitor but has also a high affinity for the GABA receptors.[7]

The compounds were prepared as described below. Dieckmann condensation of *2a* with methyl acrylate gave the β-oxoester *3a*, which according to the ^{1}H NMR spectrum exists as the enol-form (Scheme 1). High pressure hydrogenation of *3a*, *b* proceeded stereospecifically to give racemic *4a*, *b*. Treatment of *4a*, *b* with hydrochloric acid gave the hydroxy amino acid *6*, whereas prolonged treatment of *4a*, *b* with hydrobromic acid gave the α,β-unsaturated amino acid *5*. The ethyl ester *7* was



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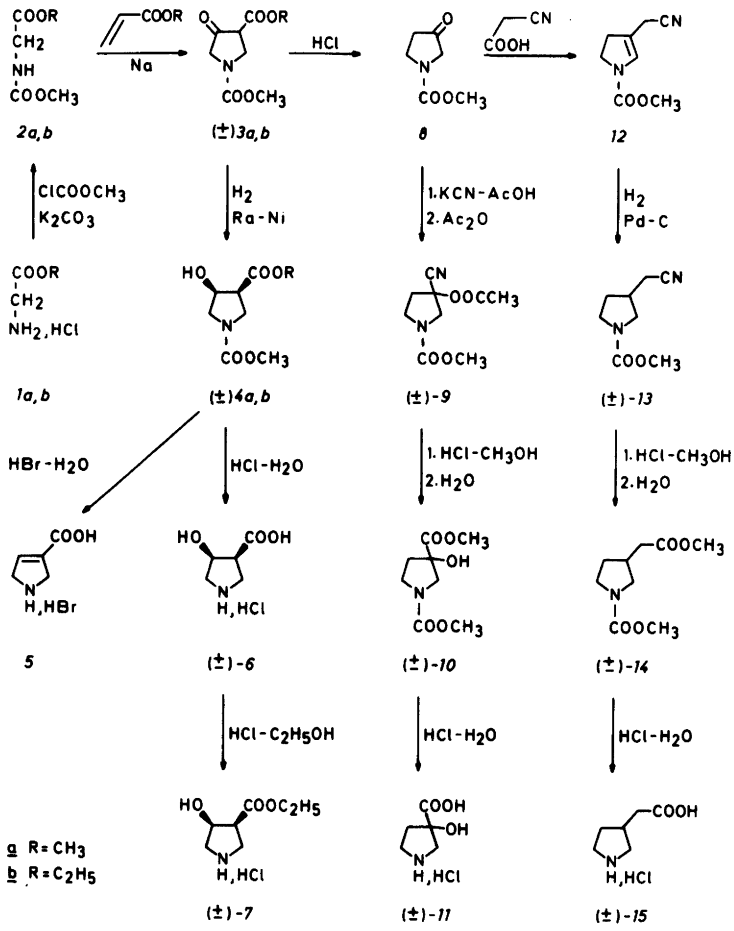


Scheme 1.

synthesized to facilitate the 270 MHz ^{1}H NMR spectroscopic analysis.

Reaction of the ketone *8* with potassium cyanide in glacial acetic acid under acylating conditions gave *9*.

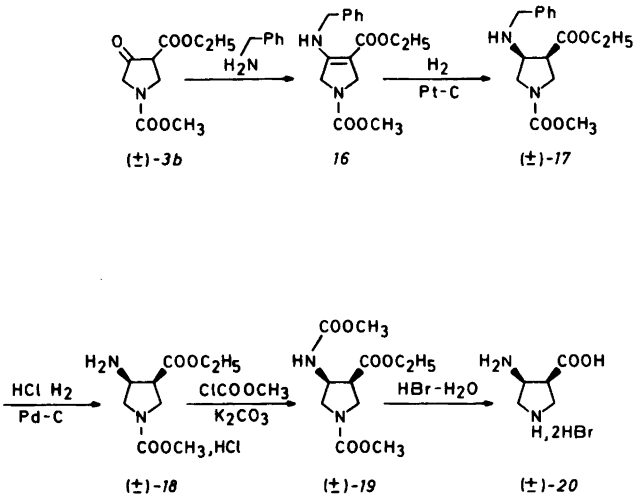
In order to prepare compound *13*, cyanoacetic acid was used as an active methylene compound in a Knoevenagel condensation with *8*. As the reaction was performed in pyridine using piperidine as a catalyst[9] the obtained product was the decarboxylated β,γ-unsaturated nitrile derivative *12*, which by low pressure hydrogenation was converted into the saturated compound *13*.

The nitriles *9* and *13* were transformed into the amino acids *11* and *15*, respectively, *via* the corresponding methyl esters *10* and *14*.

The β-amino acid *20* was prepared from *3b via* the enamine *16* (Scheme 2). High pressure hydrogenation of *16* using Pt–C as a catalyst resulted in reduction of the enamine double bond and yielded *17*. Hydrogenolysis of *17* as a hydrochloride gave a compound considered to be *18*. Crude *18* was transformed into the protected compound *19*, which was used in the 270 MHz ^{1}H NMR spectroscopic analysis. Treatment of *19* with hydrobromic acid gave compound *20*.

The structure elucidation of the new compounds *2a–4a*, *4b*, *5–7*, *9–17*, *19* and *20* was based on elemental analyses, IR and ^{1}H NMR spectroscopy, in the cases of *12* and *16* supported by UV spectroscopy.

The relative configurations of *4a*, *7* and *19* were



Scheme 2.

deduced by analysis of the 270 MHz ^{1}H NMR spectra.

In the literature comparatively little is reported concerning chemical shifts and coupling constants of pyrrolidines.[10-14] The scarcity of data reflects the lack of success NMR data have had in characterizing conformations of this ring system. The pyrrolidine ring is an almost planar pentagon with low barrier to pseudorotation. The NMR data represent accordingly the average over a number of probable conformations. The vicinal coupling constants are claimed to be represented by the Karplus equation[15] but the extensive averaging results in typical values in the range of 4 to 8 Hz for both *cis* and *trans* isomers. The key problem in connection with the 3,4-disubstituted compounds is the determination of the relative configuration of the substituents. The observed values of $J_{3,4}$ of the three compounds *4a*, *7* and *19* are 4.59, 5.0 and 6.0 Hz, respectively, permitting no final conclusion as to the *cis-trans* isomerism. The same consideration is reached based on the values of $J_{2x,3}$ and $J_{2y,3}$. The sum $J_{2x,3}+J_{2y,3}$ is expected to be less influenced by the pseudorotation and is quoted to be 13.3 Hz in pyrrolidine.[16] In all of the three compounds the sum $J_{2x,3}+J_{2y,3}+J_{4,5x}+J_{4,5y}$ is close to twice this value, but in *4a* and *7* $J_{2x,3}+J_{2y,3}$ has increased to 18 Hz, while the contribution from $J_{4,5x}+J_{4,5y}$ has fallen to 6 Hz. This indicates that *4a* as well as *7* are present in solution with strongly biased conformations.

Since no reliable conclusion regarding the *cis–trans* isomerism can be drawn from the NMR data above, we have attempted to gain this from proton–proton nuclear Overhauser effect (NOE).[17] On the qualitative level the experiment may be used to distinguish protons on basis of their spatial separation. The NOE depends on r^{-6}, r being the inter-proton distance, and this ensures that only protons with a distance of $r \leq 6$ Å will normally be capable of showing measurable effects.[17] For the NOE experiment H_4 is chosen for saturation, since it is well separated from the remaining signals of interest. The NOE difference spectrum and the reference spectrum of *19* are reproduced in Fig. 1. It is seen that a NOE of 12 % is found for H_3 in *19*. In a similar experiment concerning compound *4a* a NOE of 12 % is found for H_3. These effects are sufficiently large[17] to ensure that the protons H_3 and H_4 of *4a* and *19* are located in a *cis* configuration.

The chemical processes, which transform these compounds to the final products *6* and *20*, will retain the configuration at C_3 and C_4, leading to the conclusion, that *6* and *20* are also characterized by *cis* arrangement of the substituents at C_3 and C_4.

EXPERIMENTAL

Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished by using silica gel GF_{254} plates (Merck) and silica gel

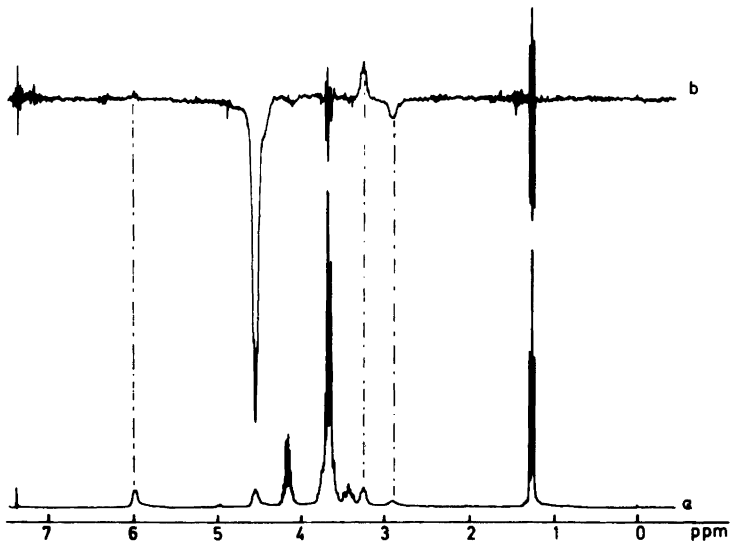


Fig. 1. In trace a the ^1H NMR spectrum of *19* is reproduced, obtained in FT mode at 270 MHz. Trace b shows the proton – proton difference nuclear Overhauser effect obtained by irradiating H_4. The appearance of a positive NOE of 12% is determined for H_3.

(Woelm 0.063 – 0.100 mm), respectively. Columns were developed by stepwise gradient elution. Melting points, determined in capillary tubes, are corrected. Elemental analyses were made by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen. The pK_A-values were determined as described in a previous paper.[18] A Perkin-Elmer grating infrared spectrophotometer model 247, a Perkin-Elmer ultraviolet-visible spectrophotometer model 402 and a JEOL JMN-C-60HL (60 MHz) ^1H NMR instrument were used. The 270 MHz ^1H NMR spectra were obtained on a Bruker HX 270 S instrument operating at 293 and 353 K.

The samples were contained in 5 mm o.d. sample tubes. The concentrations of the substances were *ca.* 4 w/v %. The analysis of the spectra was supplemented with selective decoupling experiments wherever appropriate. ^1H NMR spectra were recorded using TMS as an internal standard, except for the compounds dissolved in D_2O, where DSS was used.

The spectra of *4a*, *7* and *19* have been simulated using the programme MIMER[19] and the simulations were found to coincide with the experimental spectra.

The proton – proton nuclear Overhauser experiments were performed by difference technique. Two spectra are alternatingly accumulated and swopped from disc. In the active spectrum $C_4 - H$ was saturated using a 4 s pulse, after which the FID was sampled with the decoupler off. The passive spectrum is a reference spectrum where the irradiation is displaced outside the spectral region. The NOE spectrum corresponds to the difference between the two spectra accumulated. The magnitude of the NOE was determined by computer subtraction of a suitable fraction of the reference spectrum whereby nulling was obtained for the signal for which the NOE was to be determined. In the NOE difference spectrum a broad signal due to traces of water was observed at 2.91 ppm. The delay of 4 s between the experiments did not permit full relaxation of water. As a consequence the outbalancing was not complete.

Methyl N-*Methoxycarbonylglycinate (*2a*).* To a stirred ice-cooled solution of *1a* (50.2 g; 0.4 mol) in water (150 ml) was added an iced solution of potassium carbonate (138.2 g; 1.0 mol) in water (150 ml) followed by methyl chloroformate (45.4 g; 0.48 mol). The mixture was stirred at 0 °C for 1 h and at 24 °C for 1 h followed by extraction with ether (4 x 300 ml). The combined and dried (K_2CO_3) ether phases were evaporated *in vacuo*. Distillation of the residue gave *2a* (27.3 g; 46 %), collected at 82 – 84 °C/50 Pa. Anal. $C_5H_9NO_4$: C, H, N. IR (film): 3370 (m), 2930 (w), 1750 (s), 1720 (s), 1530 (m), 1280 (m), 1210 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 5.66 (1 H, broad signal), 3.93 (2 H, m), 3.77 (3 H, s), 3.70 (3 H, s).

*Methyl 1-methoxycarbonyl-4-oxopyrrolidine-3-carboxylate (*3a*).* To a suspension of sodium (3.3 g; 0.145 g-atom) in CaH_2-dried toluene – xylene [250

ml; (10:1)] was added *2a* (21.4 g; 0.145 mol). The mixture was stirred at 80 °C for 1/4 h and at room temperature overnight. After dropwise addition of methyl acrylate (13.1 g; 0.15 mmol) the suspension was refluxed for 3½ h. The reaction mixture was treated with 3 M hydrochloric acid (50 ml). The aqueous phase was extracted with chloroform (5 × 50 ml). Evaporation of the combined and dried ($MgSO_4$) organic phases followed by distillation of the residue gave *3a* (17.2 g; 59 %), collected at 132–142 °C/65 Pa. Anal. $C_8H_{11}NO_5$: C, H, N. IR (KBr): 3420 (w), 2950–2870 (several bands, w), 1700 (s), 1670 (s), 1450 (m), 1400 (m), 1250 (m), 1200 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.3–3.9 (4 H, m), 3.82 (3 H, s), 3.77 (3 H, s).

cis-*Methyl 1-methoxycarbonyl-4-hydroxypyrrolidine-3-carboxylate (4a)*. A solution of *3a* (7.2 g; 36 mmol) in methanol (400 ml) was hydrogenated (*ca.* 5 MPa) for 24 h using *ca.* 3 g Ra–Ni W–2 catalyst. The filtered and evaporated reaction mixture gave *4a* (7.2 g; 90 %) as a crude product. An analytical sample was purified by CC [silica gel; eluents: toluene containing ethyl acetate (78–84 %)] followed by ball-tube distillation at 50 Pa (oven temperature 200 °C). Found: C 46.75; H 6.93; N 6.77. Calc. for $C_8H_{13}NO_5$: C 47,29; H 6.45; N 6.89. IR (film): 3420 (m), 2960–2840 (several bands, w), 1720 (s), 1680 (s), 1460 (m), 1400 (m), 1210 (m) cm^{-1}. ^1H NMR (270 MHz, DMSO-d_6, 353 K): δ_{2x} 3.50, δ_{2y} 3.58, δ_3 3.16, δ_4 4.41, δ_{5x} 3.31, δ_{5y} 3.42, δ_{OH} 5.2, $\delta_{N-COOCH_3}$ 3.63, $\delta_{C-COOCH_3}$ 3.59. $J_{2x,2y}$ −10.92 Hz, $J_{2x,3}$ 8.43 Hz, $J_{2y,3}$ 9.63 Hz, $J_{3,4}$ 4.59 Hz, $J_{4,5x}$ 1.74 Hz, $J_{4,5y}$ 4.43 Hz, $J_{5x,5y}$ −11.55 Hz.

cis-*Ethyl 1-methoxycarbonyl-4-hydroxypyrrolidine-3-carboxylate (4b)*. A solution of *3b*[20] (2.0 g; 9.3 mmol) in ethanol (200 ml) was hydrogenated (*ca.* 3.5 MPa) for 24 h using *ca.* 1 g Ra–Ni W–2 catalyst. Ball-tube distillation of the evaporated reaction mixture at 50 Pa (oven temperature 210 °C) gave *4b* (1.8 g; 89 %). Found: C 48.35; H 6.79; N 6.49. Calc. for $C_9H_{15}NO_5$: C 49.76; H 6.96; N 6.45. IR (film): 3430 (m), 2980–2870 (several bands, w), 1730 (s), 1690 (s), 1460 (m), 1395 (m), 1200 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 5.00 (1 H, broad signal), 4.49 (1 H, m), 4.13 (2 H, q), 3.8–3.4 (m) and 3.65 (s) (a total of 7 H), 3.23 (1 H, m), 1.27 (3 H, t).

3-Carboxy-3-pyrrolinium bromide (5). A mixture of *4a* (2.0 g; 10 mmol) or *4b* (2.2 g; 10 mmol) and 48 % aqueous hydrobromic acid (10 ml) was refluxed for 24 h. Filtration of the hot reaction mixture, followed by cooling gave pure *5* (605 mg; 31 %), m.p. 255–257 °C (decomp.). Anal. $C_5H_8BrNO_2$: C, H, Br, N. IR (KBr): 3450 (m), 3070 (broad band, s), 1735 (s), 1720 (s), 1660 (m), 1200 (s) cm^{-1}. ^1H NMR [60 MHz, DMSO-d_6–D_2O (9:1)]: δ 6.75 (1 H, m), 4.4–4.1 (4 H, m). pK_A values (H_2O, 25 °C): 2.93 ± 0.01; 9.77 ± 0.03.

cis-*3-Carboxy-4-hydroxypyrrolidinium chloride (6)*. A solution of *4a* (900 mg; 4.5 mmol) or *4b* (1.0 g; 4.6 mmol) in 5 M hydrochloric acid (10 ml) was refluxed for 1½ h. Evaporation of the reaction mixture to dryness *in vacuo* and recrystallization (acetic acid) of the residue gave *6* (200 mg; 26 %), m.p. *ca.* 159 °C (decomp.). Anal. $C_5H_{10}ClNO_3$: C, H, Cl, N. IR (KBr): 3480–3200 (several bands, s–m), 3050–2450 (several bands, s–m), 1710 (s), 1590 (w), 1445 (m), 1240 (s) cm^{-1}. ^1H NMR [60 MHz, DMSO-d_6–D_2O (9:1)]: δ 4.58 (1 H, m), 4.12 (4 H, s), 3.8–3.1 (1 H, m). pK_A values (H_2O, 25 °C): 3.39 ± 0.01; 10.06 ± 0.02.

cis-*3-Ethoxycarbonyl-4-hydroxypyrrolidinium chloride (7)*. A solution of *6* (100 mg; 0.60 mmol) in 9 w/v % ethanolic hydrochloric acid (2 ml) was refluxed for 2 h. Evaporation *in vacuo* and recrystallization (ethanol–ether) gave *7* (40 mg; 34 %), m.p. 190–191 °C. Anal. $C_7H_{14}ClNO_3$: C, H, Cl, N. IR (KBr): 3450–3200 (several bands, m), 3020–2550 (several bands, s–w), 1730 (s), 1580 (w), 1460 (w), 1390 (m), 1200 (s) cm^{-1}. ^1H NMR (270 MHz, DMSO-d_6, 353 K): δ_{2x} 3.25, δ_{2y} 3.39, δ_3 3.16, δ_4 4.50, δ_{5x} 3.04, δ_{5y} 3.19, δ_{CH_2} 4.11 and 4.13, δ_{CH_3} 1.22. $J_{2x,2y}$ −11.2 Hz, $J_{2x,3}$ 8.3 Hz, $J_{2y,3}$ 9.9 Hz, $J_{3,4}$ 5.0 Hz, $J_{4,5x}$ 1.8 Hz, $J_{4,5y}$ 4.0 Hz, $J_{5x,5y}$ −12.0 Hz, $^3J_{CH_2CH_3}$ 7.1 Hz, $^2J_{CH_2}$ −10.78 Hz.

1-Methoxycarbonyl-3-acetoxy-3-cyanopyrrolidine (9). A solution of *8*[20] (3.0 g; 21 mmol) and potassium cyanide (2.1 g: 32 mmol) in glacial acetic acid (12 ml) was stirred at room temperature for ½ h. After addition of acetic anhydride (2.7 g; 26 mmol) the mixture was heated at 50–60 °C for 70 h. The evaporated reaction mixture was dissolved in water (50 ml) and extracted with ether (4 × 25 ml). The combined, dried and evaporated ether phases were subjected to CC [silica gel: 200 g; eluent: toluene–ethyl acetate–methanol (80:20:2)] to give *9* (3.4 g; 76 %), m.p. 74–75 °C. Anal. $C_9H_{12}N_2O_4$: C, H, N. IR (KBr): 2960–2880 (several bands, w), 2250 (w), 1750 (s), 1700 (s), 1450 (m), 1390 (s), 1200 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 3.9–3.4 (m) and 3.67 (s) (a total of 7 H), 2.50 (2 H, t), 2.12 (3 H, s).

Methyl 1-methoxycarbonyl-3-hydroxypyrrolidine-3-carboxylate (10). A solution of *9* (2.0 g; 9.4 mmol) in 10 w/v % methanolic hydrochloric acid (40 ml) was stirred at 24 °C overnight. The reaction mixture was concentrated *in vacuo* to *ca.* 15 ml. Upon addition of water (30 ml) and stirring at room temperature for 1/4 h the solvents were removed *in vacuo* and the residue was extracted with ethyl acetate–toluene (4:1) (3 × 15 ml). The combined and dried ($MgSO_4$) organic extract was evaporated to give *10* (1.5 g; 79 %). An analytical sample was purified by CC [silica gel; eluents: toluene containing ethyl acetate (80–84 %)] followed by ball-tube distillation at 25 Pa (oven temperature 170 °C). Anal. $C_8H_{13}NO_5$. Found: C 45.70, H 7.00, N 6.47. Calc.:

C 47.29, H 6.45, N 6.89. IR (film): 3400 (m), 2960 (m), 2900 (w), 1730 (s), 1690 (s), 1460 (s), 1400 (m), 1230 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 5.06 (1 H, broad signal), 4.0–3.5 (m), 3.88 (s), and 3.77 (s) (a total of 10 H), 2.5–2.0 (2 H, m).

3-Carboxy-3-hydroxypyrrolidinium chloride (11). A solution of *10* (300 mg; 1.5 mmol) in 5 M hydrochloric acid (6 ml) was refluxed for 1 ½ h. The reaction mixture was evaporated to dryness *in vacuo*. The crystalline residue was recrystallized (water–acetic acid–ether) to give *11* (100 mg; 40 %), m.p. 224–225 °C (decomp.). Anal. $C_5H_{10}ClNO_3$: C, H, Cl, N. IR (KBr): 3460–2730 (several bands, s–m), 1730 (s), 1575 (w), 1400 (m), 1210 (m) cm^{-1}. ^1H NMR [60 MHz, DMSO-d_6–D_2O (9:1)]: δ 3.5–3.0 (4 H, m), 2.4–1.9 (2 H, m). pK_A values (H_2O, 25 °C): 2.82±0.03; 10.11±0.03.

1-Methoxycarbonyl-3-cyanomethyl-2-pyrroline (12). To a solution of *8*[20] (10.0 g; 70 mmol) and cyanoacetic acid (12.0 g; 140 mmol) in pyridine (100 ml) was added piperidine (2 ml). The mixture was refluxed for 12 h. The solvent was removed *in vacuo* and CC [silica gel: 500 g; eluents: toluene containing ethyl acetate (20–30 %) and formic acid (1 %)] of the residue gave *12* (7.2 g; 62 %). An analytical sample was purified by ball-tube distillation at 65 Pa (oven temperature 150 °C). Found: C 57.00; H 6.21; N 16.62. Calc. for $C_8H_{10}N_2O_2$: C 57.82; H 6.07; N 16.86. IR (film): 3120 (w), 2970–2850 (several bands, m–w), 2250 (w), 1710 (s), 1660 (m), 1460 (s), 1410 (s), 1210 (m) cm^{-1}. UV [methanol (log ε)]: 234 (4.07) nm. ^1H NMR (60 MHz, $CDCl_3$): δ 6.63 (1 H, m), 3.87 (2 H, m), 3.77 (3 H, s), 3.17 (2 H, m), 2.65 (2 H, m).

1-Methoxycarbonyl-3-cyanomethyl-pyrrolidine (13). A solution of *12* (3.0 g; 18 mmol) in ethanol (125 ml) was hydrogenated (*ca.* 300 kPa) for 21 h in a PARR low pressure hydrogenation apparatus using a 10 % Pd–C catalyst (0.6 g). Ball-tube distillation of the evaporated solution at 130 Pa (oven temperature 170 °C) gave *13* (2.7 g; 89 %). Anal. $C_8H_{12}N_2O_2$: C, H, N. IR (film): 2970 (m), 2880 (m), 2250 (w), 1700 (s), 1460 (s), 1390 (s), 1200 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 3.8–2.9 (m) and 3.72 (s) (a total of 7 H), 2.6–2.3 (3 H, m), 2.3–1.6 (2 H, m).

Methyl 1-methoxycarbonylpyrrolidine-3-acetate (14). A solution of *13* (2.0 g; 12 mmol) in 20 w/v % methanolic hydrochloric acid (50 ml) was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* at 30 °C to *ca.* 10 ml. Upon addition of water (35 ml) the mixture was extracted continuously for 2 h in a Kutscher-Steudel apparatus with ether–methylene chloride (4:1) (200 ml). The dried (Na_2SO_4) and evaporated organic phase was submitted to CC [silica gel: 100 g; eluents: toluene containing ethyl acetate (50–54 %)] to give *14* (1.2 g; 50 %). An analytical sample was purified by ball-tube distillation at 50 Pa (oven temperature 175 °C. Found: C 53.10; H 7.68; N 6.78. Calc. for $C_9H_{15}NO_4$: C 53.72; H 7.51; N 6.96. IR (film): 2950 (m), 2870 (w), 1730 (s), 1695 (s), 1450 (s), 1390 (s), 1190 (m), 1170 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 3.8–2.7 (m) and 3.66 (s) (a total of 10 H), 2.6–2.3 (3 H, m), 2.3–1.5 (2 H, m).

Pyrrolidine-3-acetic acid (homo-β-proline) hydrochloride (15). A solution of *14* (500 mg; 2.5 mmol) in 5 M hydrochloric acid (10 ml) was refluxed for 1 ½ h. Evaporation to dryness *in vacuo* and recrystallization (water–acetic acid–ether) gave *15* (280 mg; 68 %), m.p. 90–91.5 °C. Anal. $C_6H_{12}ClNO_2$: C, H, Cl, N. IR (KBr): 3460–2700 (several bands, s–m), 1720 (s), 1590 (w), 1410 (m), 1210 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 3.8–3.0 (4 H, m), 3.0–2.4 (m) and 2.64 (d) (a total of 3 H), 2.4–1.5 (2 H, m). pK_A values (H_2O, 25 °C): 3.96±0.01; 11.03±0.05.

Ethyl 1-methoxycarbonyl-4-benzylamino-3-pyrroline-3-carboxylate (16). To a stirred solution of *3b*[20] (5.0 g; 23.2 mmol) in toluene (75 ml) was added benzylamine (2.7 g; 24.8 mmol) and 5 g of Molecular Sieve (Union Carbide 3 A). The mixture was refluxed for 20 h using a Dean-Stark water separator. Upon evaporation *in vacuo* the reaction product was purified by CC [silica gel: 100 g; eluents: methylene chloride containing ethyl acetate (50–65 %)] to give *16* (6.0 g; 85 %). IR (film): 3340 (m), 3100–2850 (several bands, m–w), 1700 (s), 1670 (s), 1620 (s), 1450 (s), 1390 (s), 1290 (s) cm^{-1}. UV [methanol (log ε)]: 288 (4.11) nm. ^1H NMR (60 MHz, $CDCl_3$): δ 7.25 (5 H, s), 4.4–3.8 (8 H, m), 3.63 (3 H, s), 1.22 (3 H, t).

cis-*Ethyl 1-methoxycarbonyl-4-benzylaminopyrroline-3-carboxylate (17).* A solution of *16* (4.8 g; 15.8 mmol) in ethanol (350 ml) was hydrogenated (*ca.* 9 MPa) using a 5 % Pt–C catalyst (2.5 g) for 72 h. Filtration and evaporation *in vacuo* followed by CC [silica gel: 150 g; eluents: methylene chloride containing ethyl acetate (45–70 %)] yielded starting material (1.8 g; 37 %) and *17* (1.1 g; 23 %). Anal. $C_{16}H_{22}N_2O_4$: Found: C 61.75, H 7.23, N 9.00. Calc.: C 62.72, H 7.24, N 9.14. IR (film): 3330 (w), 3100–2850 (several bands, m–w), 1730 (s), 1700 (s), 1450 (s), 1390 (s), 1190 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 7.17 (5 H, s), 4.05 (2 H, q), 3.8–3.4 (m), and 3.58 (s) (a total of 9 H), 3.4–2.7 (3 H, m), 1.18 (3 H, t).

cis-*Ethyl 1-methoxycarbonyl-4-methoxycarbonylaminopyrrolidine-3-carboxylate (19).* A solution of *17* (800 mg; 2.6 mmol) and 0.1 M aqueous hydrochloric acid (26 ml) in 50 % aqueous ethanol (120 ml) was hydrogenated (*ca.* 300 kPa) for 20 h in a PARR low pressure hydrogenation apparatus using a 5 % Pd–C catalyst (250 mg). The reaction mixture was concentrated *in vacuo* to *ca.* 30 ml and washed with methylene chloride (2 × 20 ml). The aqueous

phase was evaporated *in vacuo* to give an oil (0.7 g), characterized by TLC [R_F: 0.33; eluent: butanol–acetic acid–water (4:1:1)]. The compound was considered to be *18*. To a stirred ice-cooled solution of the crude product (0.7 g) and potassium carbonate (900 mg; 6.5 mmol) in water (9 ml) was added methyl chloroformate (300 mg; 3.1 mmol). After stirring for further 1 h at 0 °C and 1 h at room temperature the reaction mixture was extracted with ether (6 × 10 ml). Evaporation of the combined and dried (Na_2SO_4) ether phases gave *19* (50 mg; 7 % based on *17*), which was shown to be TLC-pure in a variety of eluent-systems. An analytical sample was purified by CC [silica gel; eluent: toluene containing ethyl acetate (78–82 %)] followed by ball-tube distillation at 65 Pa (oven temperature 250 °C). Anal. $C_{11}H_{18}N_2O_6$: C, H, N. IR (film): 3320 (m), 2990–2850 (several bands, m–w), 1730 (s), 1710 (s), 1690 (s), 1550 (m), 1460 (s), 1400 (s), 1200 (s) cm^{-1}. ^1H NMR (270 MHz, DMSO-d_6, 353 K): δ_{2x} 3.53, δ_{2y} 3.68, δ_3 3.25, δ_4 4.43, δ_{5x} 3.35, δ_{5y} 3.58, δ_{NH} 6.83, δ_{CH_2} 4.09 and 4.13, $\delta_{HN-NCOOCH_3}$ 3.63, $\delta_{1-COOCH_3}$ 3.59, δ_{CH_3} 1.22. $J_{2x,2y}$ −10.8 Hz, $J_{2x,3}$ 7.8 Hz, $J_{2y,3}$ 7.05 Hz, $J_{3,4}$ 6.0 Hz, J_{4NH} 8.1 Hz, $J_{4,5x}$ 5.15 Hz, $J_{4,5y}$ 6.45 Hz, $J_{5x,5y}$ −11.03 Hz, $^3J_{CH_2CH_3}$ 7.1 Hz, $^2J_{CH_2}$ −11.22 Hz.

cis-*3-Carboxy-4-aminopyrrolidinium dibromide (20)*. A mixture of *19* (500 g; 1.8 mmol) and 48 % aqueous hydrobromic acid (6 ml) was refluxed for 2 h. Evaporation to dryness *in vacuo* gave crude *20* (500 mg) as an oil, which slowly crystallized. Recrystallization from water–acetic acid–ether [45 ml; (2:5:2)] gave pure *20* (175 mg; 33 %), m.p. 178–181 °C. Anal. $C_5H_{12}Br_2N_2O_2$: C, H, Br, N. IR (KBr): 3440 (m), 3200–2600 (several bands, s–m), 1735 (s), 1590 (m), 1490 (m), 1390 (m) cm^{-1}. ^1H NMR [60 MHz, DMSO-d_6–D_2O (9:1)]: δ 4.15 (1 H, m), 3.8–3.3 (5 H, m).

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