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Syntheses of Some Aminopiperidinecarboxylic Acids Related to Nipecotic Acid

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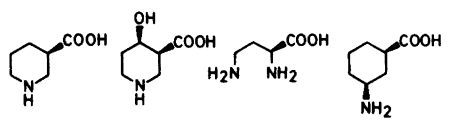
This paper describes the syntheses of (3*RS*,5*SR*)-5-hydroxypiperidine-3-carboxylic acid (*8*), (3*RS*,5*SR*)-5-aminoperidine-3-carboxylic acid (*9*), (*RS*)-3-hydroxypiperidine-3-carboxylic acid (*13*), (*RS*)-α-amino-3-pyridineacetic acid (*18*), and α-amino-3-piperidineacetic acid (*19*), compound *19* probably being a mixture of diastereomeric racemates. The compounds *8* and *9* were prepared from 5-aminonicotinic acid by catalytic hydrogenation. The relative stereochemistry of *9* was established by 270 MHz ^{1}H NMR spectroscopy. The α-hydroxy acid *13* was prepared *via* cyanhydrin reaction of the 3-piperidone derivative *11*. The α-amino acids *18* and *19* were prepared from 3-pyridineacetic acid by nitrosation and subsequent catalytic hydrogenation.

(*R*)-(−)-Nipecotic acid,[1–3] (*S*)-(+)-2,4-diaminobutyric acid (DABA),[4,5] (1*RS*,3*SR*)-3-aminocyclohexanecarboxylic acid (ACHC),[6] and probably also (3*RS*,4*SR*)-4-hydroxynipecotic acid[8] (Scheme 1), are substrate competitive inhibitors of the neuronal GABA uptake system. Of these inhibitors DABA and in particular ACHC have selective effects on the neuronal GABA uptake system, whereas nipecotic acid and *cis*-4-hydroxynipecotic acid, which are the most potent inhibitors, also interact with the transport process for GABA in glial cells.[7] As an attempt to develop compounds with high and specific affinity for the neuronal GABA uptake system a series of amino acids, analogous with the above-mentioned inhibitors, has been synthesized. These compounds, *i.e.* *8*, *9*, *13*, *18*, and *19*, however, have very little or no effect when tested as inhibitors of the neuronal uptake of [^{3}H]-GABA using the procedure described in a previous paper,[9] emphasizing the substrate specificity of the neuronal GABA transport system.[10]



Scheme 1.

Hydrogenation of 5-aminonicotinic acid (*2*) using PtO_2 as a catalyst gave a complex mixture of products. After treatment of the reaction mixture with methyl chloroformate the compounds *5* and *6* and an inseparable mixture of *3* and *4* could be isolated (Scheme 2), the desired protected diamino acid *6* being the major product. Subsequent acetylation of the mixture of *3* and *4* gave *4* and *7*, which were separated by column chromatography.

Stepwise hydrogenation of the pyridine ring of *2* could probably cause the formation of an intermediate ene-diamine (Scheme 2), which spontaneously would be hydrolyzed in the acidic aqueous medium to the corresponding 5-oxo derivative. Hydrogenation of this compounds followed by treatment with methyl chloroformate would give the 5-hydroxycarboxylic acid derivative *5*. In support of this proposal, low pressure hydrogenation of methyl 3-oxopiperidine-1-carboxylate[11] (*10*) using PtO_2 as a catalyst gave the corresponding 3-hydroxypiperidine *3* (Scheme 2), indicating the high reactivity of this α-aminoketone derivative.

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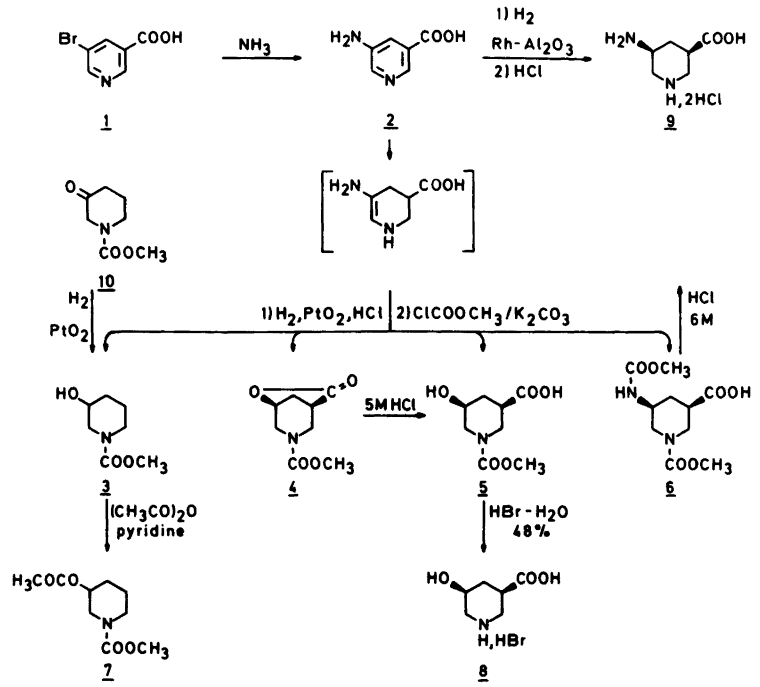


Scheme 2.

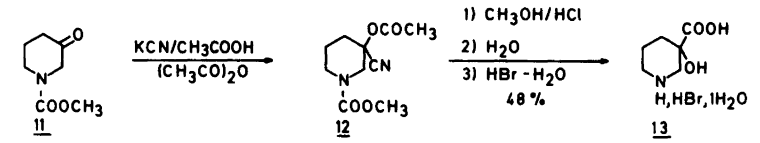
The 3,5-carbolactone *4* could be transformed into the 5-hydroxypiperidine-3-carboxylic acid derivative *5* using mild acidic conditions, and this conversion unequivocally indicates a 3,5-*cis* configuration of *5*. The mechanisms underlying the formation of *4* are unknown. A possible mechanism could be an intramolecular nucleophilic addition/elimination reaction of the proposed ene-diamine intermediate. Alternatively, the lactone *4* could be formed *via* a hydroxylated intermediate during the treatment with methyl chloroformate.

The mechanism for the formation of *3* is unknown, but hydrogenation of pyridine-3-carboxylic acids using PtO_2 as a catalyst is frequently accompanied by decarboxylation.[12,13]

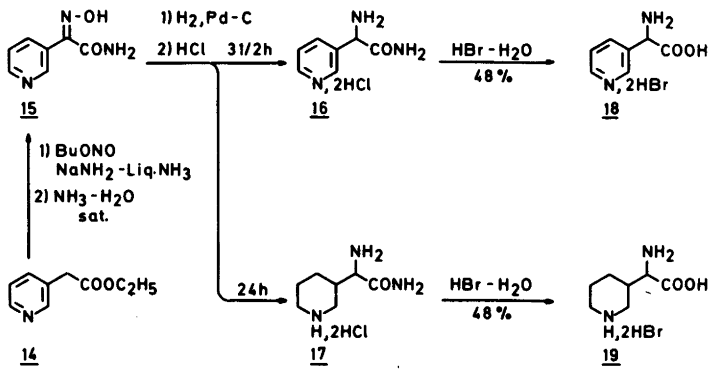
Hydrogenation of *2* using rhodium-Al_2O_3 as a catalyst by analogy with a method described for the hydrogenation of nicotinic acid[14] gave *9* as the only product. Acid treatment of the protected diamino acid *6* also gave *9* as the only product.

The α-hydroxy acid *13* was prepared *via* cyanhydrin reaction of *11* using acetylating conditions (Scheme 3). Methanolysis of *12* and subsequent acid hydrolysis of the intermediate α-hydroxy ester gave *13*.

Treatment of *14* with butyl nitrite in sodium amide-liquid ammonia (Scheme 4) gave a mixture of the corresponding α-hydroxyimino ethyl ester, butyl ester and amide (*15*), judging from the ^1H NMR spectra. Treatment of this mixture of compounds with saturated ammonia gave the amide *15* as the major product. The hydroxyimino group in *15* could be selectivity hydrogenated to give the pyridine derivative *16* (Scheme 4). Extended hydro-



Scheme 3.



Scheme 4.

genation with an increased amount of catalyst gave the piperidine derivative *17* as the only product. Acid treatment of *16* and *17* gave the α-amino acid dihydrobromides *18* and *19*.

The structures of the new compounds *3*–*9*, *12*, *13* and *15*–*19* were established by elemental analysis, IR and ^{1}H NMR spectroscopy. The relative configuration of *6*, and consequently that of *9* according to a previous paper,[18] was established by analysis of the 270 MHz ^{1}H NMR spectrum of *6*.

In simple piperidine derivatives the equatorial protons on C(2) and C(6) are found downfield from their axial counterparts.[15] In the 270 MHz ^{1}H NMR spectrum of *6* the signals for H_{2e} and H_{2a} are observed at δ 4.13 and 2.55, respectively. The vicinal coupling constants between the C(3) and C(2) protons, determined by decoupling experiments, are typical for axial-axial and axial-equatorial configurations of these protons. This is consistent with a predominantly equatorial orientation of the C(3) carboxylic acid group. An analysis producing the coupling constant between the C(3) and C(4) protons supports this assignment. The coupling patterns of the C(4), C(5), and C(6) protons unequivocally indicate an axial orientation of the C(5) proton, and therefore a 3,5-*cis* configuration of *6*.

The observed chemical shift values and coupling constants parallel data previously found in other piperidines.[16–18] The interpretation of the vicinal coupling constants in terms of ring conformation follows the general treatment of 6-membered rings.[19]

EXPERIMENTAL

Melting points, determined in capillary tubes, are corrected. Elemental analyses were performed by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen. TLC and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel (Woelm 0.063–0.100 mm), respectively. Columns were developed by stepwise gradient elution. The pK_A values were determined as earlier described.[20] A Perkin-Elmer grating infrared spectrophotometer model 247 and a JEOL JMN-C-60HL (60 MHz) ^{1}H NMR instrument were used. The 270 MHz ^{1}H NMR spectra were obtained on a Bruker HX 270 S instrument. Fourier transform method was used to obtain the spectrum with a spectral width of 1500 Hz. Quadrature detection and homodecoupling were used. A frequency of 19,506,582 Hz was used for nitrogen decoupling. ^{1}H NMR spectra were recorded using TMS as an internal standard, except for the compounds dissolved in D_2O, where DSS was used. The computations in connection with the analyses of the 270 MHz ^{1}H NMR spectra were performed on a Varian 620/I computer using the SIMEQ program[21] and on a Nicolet 1180 computer using the ITRCAL program.[22]

5-Aminonicotinic acid (2). The compound *2* was prepared from *1*[23] (15.0 g; 74.3 mmol) as described elsewhere.[23] The yield of recrystallized (water) *2* was 6.5 g (63 %), m.p. 293.0–295.0 °C (293–294 °C[23]). Anal. $C_6H_6N_2O_2$: C, H, N. IR (KBr): 3350 (s), 3200 (s), 2800–2100 (w, several bands), 1660 (s), 1595 (s), 1470 (m), 1400 (s), 1330 (s) cm^{-1}. ^{1}H NMR (60 MHz, D_2O+DMSO-d_6): δ 8.67 (1 H, m), 8.40 (2 H, m).

Reduction of 5-aminonicotinic acid (2) using PtO_2 as a catalyst. A solution of *2* (3.0 g; 24.6 mmol) in 4 M aqueous hydrochloric acid (12.3 ml; 49.2

mmol) and water (100 ml) was hydrogenated (*ca.* 300 kPa) for 24 h in a PARR low pressure hydrogenation apparatus using PtO_2 (0.5 g) as a catalyst. To an ice-cooled solution of the filtered and evaporated reaction mixture in water (30 ml) an iced solution of potassium carbonate (13 g; 94 mmol) in water (15 ml) was added with stirring followed by addition of methyl chloroformate (7.0 g; 74.4 mmol). Stirring was continued at 0 °C for 1 h and then at 24 °C for 1 h.

The basic reaction mixture was continuously extracted with ether–dichloromethane (3:1) for 3 h. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*, and to a solution of the crude evaporated product in pyridine (27 ml) was added excess of acetic anhydride (3 ml). The mixture was heated at 80 °C for 24 h. CC [silica gel: 50 g; eluents: toluene containing ethyl acetate (80–85 %) and formic acid (2 %)] of the evaporated reaction mixture gave the following products:

a. (*RS*)-Methyl-3-acetoxypiperidine-1-carboxylate (*7*) (1.0 g; 20 %). Anal. $C_9H_{15}NO_4$: C, H, N. IR (film): 3500 (w), 2950 (s), 2850 (m), 1730 (s), 1700 (s), 1440 (s), 1400 (s), 1360 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.8 (1 H, m), 3.70 (3 H, s), 3.5 (4 H, m), 2.03 (3 H, s), 1.7 (4 H, m).

b. (3*RS*,5*SR*)-1-Methoxycarbonylpiperidine-3,5-carbolactone (*4*) (0.45 g; 10 %). IR (film): 3500 (w), 3000–2800 (w, several bands), 1780 (s), 1700 (s), 2450 (s), 1400 (m), 1360 (m), 1230 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.9 (1 H, m), 4.5–4.0 (2 H, m), 3.72 (3 H, s), 3.4 (1 H, m), 3.2 (1 H, m), 2.8 (2 H, m), 2.0 (1 H, m).

To the basic aqueous extraction phase, from which the mixture of *3* and *4* was extracted, was added at 0 °C aqueous hydrochloric acid (2 M) to pH = 2. The solution was continuously extracted with ether–dichloromethane (3:1) for 24 h. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. CC [silica gel: 100 g; eluents: toluene containing ethyl acetate (80–90 %) and formic acid (2 %)] gave the following products:

c. (3*RS*,5*SR*)-Methyl 5-hydroxy-3-carboxypiperidine-1-carboxylate (*5*) (0.47 g; 10 %), recrystallized from ethyl acetate–cyclohexane, m.p. 127.5–129.0 °C. Anal $C_8H_{13}NO_5$: C, H, N. IR (KBr): 3400 (s), 3100–2600 (m, several bands), 1720 (s), 1660 (s), 1480 (s), 1440 (s), 1410 (m) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$ + DMSO-d_6): δ 7.4 (2 H, m), 4.1 (2 H, m), 3.65 (3 H, s), 3.6–3.4 (2 H, m), 3.0–2.6 (2 H, m), 2.0 (2 H, m).

d. (3*RS*,5*SR*)-Methyl 5-methoxycarbonylamino-3-carboxypiperidine-1-carboxylate (*6*) (1.91 g; 30 %), recrystallized from ethyl acetate–cyclohexane, m.p. 196.0–197.0 °C. Anal. $C_{10}H_{16}N_2O_6$: C, H, N. IR (KBr): 3600–2800 (m–s, several bands), 1720 (s), 1690 (s), 1660 (s), 1560 (m), 1530 (m), 1500 (m), 1450 (m), 1260 (s) cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ_{2a} 2.77, δ_{2e} 4.22, δ_{3a} 2.45, δ_{4a} 1.52, δ_{4e} 2.25, δ_{5a} 3.45, δ_{6a} 2.55, δ_{6e} 4.13, δ_{NH} 6.47, δ_{COOCH3} 3.66, δ_{COOCH3} 3.62, δ_{COOH} 9.0. J_{2a2e} −13.24 Hz, J_{2a3a} 11.03 Hz, J_{2e3a} 4.23 Hz, J_{3a4a} 11.4 Hz, J_{3a4e} 4.22 Hz, J_{4a4e} −12.68 Hz, J_{4a5a} 12.13 Hz, J_{4e5a} 5.2 Hz, J_{5a6a} 10.52 Hz, J_{5a6e} 4.41 Hz, J_{6a6e} −12.68 Hz.

Conversion of (3RS,5SR)-1-methoxycarbonylpiperidine-3,5-carbolactone (4) into (3RS,5SR)-methyl 5-hydroxy-3-carboxypiperidine-1-carboxylate (5). A mixture of *4* (100 mg; 0.54 mmol) and aqueous hydrochloric acid (5 ml; 5 M) was stirred at 24 °C for 5 days. The solution was continuously extracted with ether–dichloromethane (3:1) for 24 h. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. Recrystallization (ethyl acetate-cyclohexane) gave *5* (65 mg; 59 %), the IR spectrum of which was identical with that of *5* prepared as described above.

(3RS,5SR)-5-Hydroxy-3-carboxypiperidinium bromide hydrate (8). A solution of *5* (70 mg; 0.34 mmol) in aqueous hydrobromic acid (5 ml; 48 %) was refluxed for 45 min. Evaporation *in vacuo* and recrystallization (water–ethanol) gave *8* (40 mg; 52 %), m.p. *ca.* 170 °C (decomp.). Anal. $C_6H_{12}NO_3Br$, 1 H_2O: C, H, N, Br. IR (KBr): 3350 (s), 3000–2400 (m, several bands), 1700 (s), 1550 (m), 1460 (w), 1400 (w), 1350 (w), 1260 (s) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 4.1 (1 H, m), 3.6–3.0 (5 H, m), 2.1 (2 H, m).

(3RS,5SR)-5-Aminopiperidine-3-carboxylic acid dihydrochloride (9). Method a. A solution of *6* (300 mg; 1.2 mmol) in aqueous hydrochloric acid (10 ml; 6 M) was refluxed for 5 h. Evaporation *in vacuo* and recrystallization (water–acetic acid) gave *9* (170 mg; 50 %), m.p. 245 °C (decomp.). Anal. $C_6H_{14}N_2O_2Cl_2$: C, H, N, Cl. IR (KBr): 3450 (w), 3200–2400 (w–s, several bands), 1720 (s), 1600 (w), 1520 (w), 1380 (m), 1220 (s) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 3.8 (3 H, m), 3.1 (3 H, m), 2.0 (2 H, m). pK_A values (H_2O, 24 °C): 2.55 ± 0.05, 6.76 ± 0.01, 9.99 ± 0.01.

Method b. A solution of *2* (0.7 g; 5.1 mmol) in water (90 ml) and aqueous ammonia (2 ml; 25 %) was hydrogenated (*ca.* 300 kPa) for 72 h in a PARR low pressure hydrogenation apparatus by using a 5 % rhodium-Al_2O_3 (280 mg) catalyst. To the evaporated reaction product was added aqueous hydrochloric acid (5 ml; 4 M). Evaporation *in vacuo* and recrystallization (water–acetic acid) gave *9* (0.55 g; 55 %), the IR spectrum of which was identical with that of *9* prepared from *6* as described above.

(RS)-Methyl-3-hydroxypiperidine-1-carboxylate (3). A solution of *10*[11] (0.2 g; 1.3 mmol) in ethanol (100 ml) was hydrogenated (*ca.* 300 kPa) for 48 h in a PARR low pressure hydrogenation apparatus by using PtO_2 (60 mg) as a catalyst. CC [silica gel: 20 g; eluents: toluene containing ethyl acetate (80–90 %) and formic acid (2 %)] of the filtered and evaporated reaction mixture gave *3* (0.13 g; 60 %). Anal. $C_7H_{13}NO_3$: C, H, N. IR (film): 3400 (s, broad band), 2950 (s), 2850 (m), 1690 (s), 1480 (s), 1440 (s), 1410 (s),

1260 (s), 1240 (s) cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 4.0–3.5 (2 H, m), 3.76 (3 H, s), 3.4–3.0 (3 H, m), 3.05 (1 H, s), 1.9–1.5 (4 H, m).

(RS)-Methyl 3-cyano-3-acetoxypiperidine-1-carboxylate (12). A solution of *11*[11] (3.6 g; 22.9 mmol) and potassium cyanide (3.0 g; 46 mmol) in glacial acetic acid (15 ml) was left at 25 °C for 30 min. Acetic anhydride (4.0 ml; 42 mmol) was added and the solution was heated at 60 °C for 72 h. The evaporated reaction mixture was treated with water (100 ml), and the solution was extracted with ethyl acetate (3 × 75 ml). The combined organic phases were dried (Na_2SO_4) and evaporated *in vacuo*. CC [silica gel: 200 g; eluents: toluene containing ethyl acetate (50–75 %)] gave *12* (2.3 g; 45 %). An analytical sample was purified by ball tube distillation at 100 Pa (oven temperature 200 °C). Anal. $C_{10}H_{14}N_2O_4$: C, H, N. IR (film): 3500 (w), 2950 (s), 2850 (m), 1760 (s), 1700 (s), 1450 (s), 1410 (s), 1370 (m), cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 3.95 (2 H, s), 3.80 (3 H, s), 3.5 (2 H, m), 2.2 (2 H, m), 2.13 (3 H, s), 1.8 (2 H, m).

(RS)-3-Hydroxy-3-carboxypiperidinium bromide hydrate (13). A solution of *12* (2.0 g; 8.8 mmol) in methanolic hydrogen chloride (40 ml; 10 %) was left at 24 °C for 24 h. The solution was concentrated to 15 ml *in vacuo*. Water (30 ml) was added, and the solution was left at 24 °C for 15 min. The avaporated reaction product was extracted with dichloromethane (2 × 25 ml). The combined organic phases were dried (Na_2SO_4) and evaporated *in vacuo*. A solution of the crude reaction product in aqueous hydrobromic acid (30 ml; 48 %) was refluxed for 45 min. Evaporation *in vacuo* and recrystallization (water–acetic acid) gave *12* (1.3 g; 65 %), m.p. *ca.* 102 °C (decomp.). Anal. $C_6H_{12}NO_3Br$, 1 H_2O: C, H, N, Br. IR (KBr): 3400 (s), 3200–2600 (m–s, several bands), 1730 (s), 1630 (w), 1580 (s), 1170 (s) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 3.4 (4 H, m), 2.0 (4 H, m). pK_A values (H_2O, 24 °C): 2.86 ± 0.08, 10.29 ± 0.01.

α-Hydroxyimino-3-pyridineacetamide (15). Sodium (*ca.* 2 g) was added in small pieces at −70 °C to anhydrous liquid ammonia (150 ml) containing $Fe(NO_3)_3$, 9 H_2O (30 mg). When the blue colour had vanished, and the mixture had assumed a gray colour (about 15 min), *14* (3.0 g; 14.9 mmol) was added with stirring at −33 °C during 10 min. The mixture was left at −33 °C for 20 min, and then a solution of butyl nitrite (5.0 g; 44.3 mmol) in ether (10 ml) was added during 15 min. The mixture was left at −33 °C for 45 min, whereupon a solution of ammonium sulfate (10 g; 75 mmol) in water (40 ml) was added. After stirring for 1 h, the reaction mixture was saturated with ammonia at 0 °C, and then left with stirring for 24 h. A solution of the evaporated reaction product in warer (100 ml) was washed with ether (2 × 50 ml), and then continuously extracted with ethyl acetate for 24 h. The ethyl acetate phase was evaporated *in vacuo*. Recrystallization (water) gave *15* (1.4 g; 55 %), m.p. 198.0–200.0 °C. Anal. $C_7H_7N_3O_2$: C, H, N. IR (KBr): 3450 (m), 3300–2500 (m, several bands, 1700 (s), 1640 (w), 1580 (m), 1400 (m), 1050 (s) cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 12.2 (1 H, s), 8.6 (1 H, m), 7.8 (1 H, m), 7.5 (2 H, m).

(RS)-α-Amino-3-pyridineacetamide dihydrochloride (16). A solution of *15* (0.5 g; 3.1 mmol) in methanol (150 ml) was hydrogenated at *ca.* 300 kPa in a PARR low pressure hydrogenation apparatus for 3 h using a Pd-C catalyst (125 mg; 5 %). To the evaporated reaction product was added aqueous hydrochloric acid (5 ml; 4 M). Evaporation *in vacuo* and recrystallization (methanol–ether) gave *16* (390 mg; 57 %), m.p. 235 °C (decomp.). Anal. $C_7H_{11}N_3OCl_2$: C, H, N, Cl. IR (KBr): 3400–2500 (m, several bands), 1720 (s), 1640 (m), 1620 (w), 1560 (m), 1500 (m), 1480 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 9.0 (3 H, m), 8.3 (1 H, m), 5.53 (1 H, s).

α-Amino-3-piperidineacetamide dihydrochloride (17). A solution of *15* (0.5 g; 3.1 mmol) in methanol (150 ml) was hydrogenated at *ca.* 300 kPa in a PARR low pressure hydrogenation apparatus for 24 h using a Pd-C catalyst (1.2 g; 5 %). To the evaporated reaction mixture was added aqueous hydrochloric acid (5 ml; 5 M). Evaporation *in vacuo* and recrystallization (methanol–ether) gave *17* (0.4 g; 58 %), m.p. 250 °C (decomp.). Anal. $C_7H_{17}N_3OCl_2$, C, H, N, Cl. IR (KBr): 3350 (m), 3200–2400 (m–s, several bands), 1710 (s), 1700 (s), 1600 (m), 1500 (s), 1420 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 3.97 (1 H, d), 3.5 (2 H, m), 3.0 (3 H, m), 1.9 (4 H, m).

(RS)-α-Amino-3-pyridineacetic acid dihydrobromide (18). A solution of *16* (0.3 g; 1.3 mmol) in aqueous hydrobromic acid (10 ml; 48 %) was refluxed for 1 h. Evaporation *in vacuo* and recrystallization (water–acetic acid) gave *18* (0.2 g; 48 %), *m.p.* 190 °C (decomp.). Anal. $C_7H_{10}N_2O_2Br_2$: C, H, N, Br. IR (KBr): 3450 (w), 3100–2500 (m, several bands), 1750 (s), 1610 (m), 1560 (s), 1500 (s), 1470 (s), 1400 (m) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 8.8 (3 H, m), 8.3 (1 H, m), 5.51 (1 H, s).

α-Amino-3-piperidineacetic acid dihydrobromide (19). A solution of *17* (0.3 g; 1.3 mmol) in aqueous hydrobromic acid (10 ml; 48 %) was refluxed for 1 h. Evaporation *in vacuo* and recrystallization (water–acetic acid) gave *19* (0.2 g; 50 %), m.p. 240 °C (decomp.). Anal. $C_7H_{16}N_2O_2Br_2$: C, H, N, Br. IR (KBr): 3450 (w), 3100–2500 (m, several bands), 1740 (s), 1580 (m), 1490 (s), 1220 (s), 1210 (s) cm^{-1}. ^1H NMR (60 MHz, D_2O): δ 4.03 (1 H, d), 3.4 (2 H, m), 2.9 (3 H, m), 1.9 (4 H, m). pK_A values (H_2O, 24 °C): 2.20 ± 0.10, 8.30 ± 0.07, 10.46 ± 0.03.

Inhibition of neuronal GABA uptake. The procedures used for the isolation of the crude synaptosomal fraction from rat brains, and for the measurement of the inhibition of the uptake of [^3H]-GABA

are described elsewhere in detail.[9,24] None of the compounds concerned, *i.e. 8, 9, 13, 18* and *19*, inhibited GABA uptake by more than 50 % at concentrations of 2×10^{-4} M.

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