# Synthesis of *N*-(*N*-Acetyl-L-aspartyl)-4-aminobutyric Acid (Ac-Asp-γAbu)

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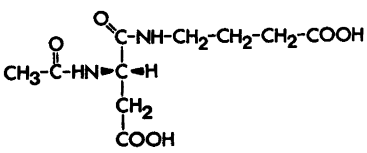
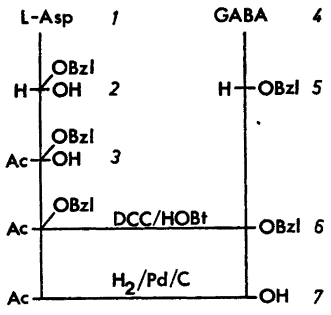
*N*-Acetyl-L-aspartic acid (Ac-Asp) has been found in mammalian brain tissue at a concentration of 5 – 6 μmol/g fresh tissue.[1,2] Reichelt and co-workers [3,4] have observed that an amine- and ATP-dependent synthesis of Ac-Asp containing oligopeptides takes place in homogenates of mouse cortex. This synthesis is apparently independent of protein synthesis. The function of these oligopeptides is as yet unknown. Reichelt [5] has postulated that *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid (Ac-Asp-γAbu) is one of these oligopeptides and the present communication describes its synthesis. The compound was tested for anticonvulsive and antimicrobial activity.

The title compound was prepared as outlined in Scheme 1. Asp(OBzl) [6] (*2*) was acetylated [7]



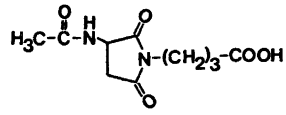
*7*

with acetic anhydride in an aqueous solution whose pH was kept at 7.5 by addition of sodium hydroxide. Ac-Asp(OBzl) (*3*) was subsequently coupled to γAbu-OBzl (*5*) using dicyclohexyl-




*Scheme 1.*

carbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) as coupling reagents. The protecting benzyl groups were removed by hydrogenolysis in the presence of acetic acid to prevent cyclization to the corresponding succinimide- or γ-lactam derivatives (*8* and *9*). Elemental analysis of the crystalline product confirmed that these side reactions had been avoided.



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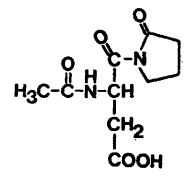

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In view of the fact that several succinimides are valuable antiepileptic drugs, *e.g.* ethosuximide (3-ethyl-3-methylpyrrolidine-2,5-dione), and the possibility of *in vivo* formation of the succinimide- and γ-lactam derivatives (*8* and *9*), Ac-Asp-γAbu (*7*) was tested for anticonvulsive activity by peritoneal administration (2.0 mg in 10.2 ml $H_2O$) to five mice 15 min prior to subcutaneous injection of pentylenetetrazole (2.0 mg in 0.2 ml $H_2O$). No activity, however, could be detected as judged from the number of deaths (4 of 5 animals) compared with the number of deaths (4 of 5) recorded for the animals receiving the convulsant only. All mice had convulsions in both groups.

Ac-Asp-γAbu (*7*) was also tested for antimicrobial activity using the diffusion technique described by Clausen [8,9] with *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* as test organisms. No inhibition of their growth could be seen when using a solution of 1 mg of *7* in 1 ml $H_2O$ as the highest concentration.

*Experimental.* Melting points (uncorrected), rotations, IR and mass spectra were recorded on Reichert, Perkin-Elmer 141, Jasco IRA-1, and Hitachi – Perkin-Elmer RMU 6L instruments, respectively. $^1H$ and $^{13}C$ NMR spectra were obtained with Varian A-60A, Varian HA-100 15 D and Jeol JMN-FX60 instruments, respectively. Analytical TLC and column chromatography was performed on silica gel: $F_{254}$ plates and Merck Kieselgel 60 (0.040 – 0.063 mm), respectively.

*Ac-Asp(OBzl) (3).* To a stirred, cooled (0 °C) solution of Asp(OBzl) [6] (*2*, 4.4 g, 19.7 mmol; [α]: see Table 1; m.p. 214 – 216 °C, lit.[6] m.p. 218 – 220 °C; $^{13}C$ NMR ($H_2O$/HCl): δ 137.46 (s), 131.29, 130.77, 70.19 (t) and signals given in Table 2) in 2 M NaOH (10.5 ml) and $H_2O$ (8.4 ml) was added dropwise acetic anhydride (2.35 ml, 25 mmol). pH of the reaction mixture was kept at 7.5 (pH-meter) by dropwise addition of 2 M NaOH. After 1.5 h the mixture was filtered, cooled (0 °C) and acidified with cold 6 M HCl to pH 2. The sirup obtained crystallized slowly at 4 °C yielding 2.96 g (57 %). M.p. 112 – 115 °C; $R_F$ 0.8 (BuOH – AcOH – pyridine – $H_2O$ = 15:3:10:12); [α]: see Table 1; $^1H$ NMR (60 MHz, $CD_3OD$): δ 1.90

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*Table 1.* Optical activities (°).

| Compound | λ/nm at 20 °C 589 | 578 | 546 | 436 | 365 | Solvent | c |
|---|---|---|---|---|---|---|---|
| 2 [a] | +27.7 | +29.1 | +33.0 | +57.7 | +93.5 | 1 M HCl | 1.9 |
| 3 [b] | +14.5 | +15.1 | +17.2 | +24.9 | +36.1 | MeOH/EtOH = 5/95 | 1.6 |
| 6 | −14.7 | −13.1 | −16.9 | −34.4 | −69.0 | $CHCl_3$ | 1.0 |
| 7 | −38.7 | −38.3 | −43.0 | −79.0 | −140.3 | $CH_3OH$ | 0.6 |

[a] Lit.[6]: $[\alpha]_D^{25}$ +28.1° (*c* 1; 1 M HCl). [b] Lit.[7]: $[\alpha]_D$ +14.7° (*c* 1; MeOH−EtOH = 5:95).

(3H, s), 2.85 (2H, m), 5.09 (2H, s), 7.30 (5H, s); $^{13}C$ NMR ($CD_3OD$): δ 173.74 (s), 173.15 (s), 171.92 (s), 137.36 (s), 129.56, 129.23, 67.64 (t), 37.23 (t), 22.42 (q), one signal was hidden under the solvent peak; ($CDCl_3$): δ 135.19 (s), 128.56, 128.17, 66.94 (t) and signals given in Table 2; *m/e* (%): 265 ($M^+$, 0.6), 91 (100), 43 (44), 108 (26), 88 (20), 158 (19), 79 (17), 86 (15), 107 (15).

*Tosylate of benzyl γ-aminobutyrate (γAbu-OBzl tosylate, 5)* γAbu (10.3 g, 0.1 mol, *4*) was esterified with benzyl alcohol (27 g, 0.25 mol) in refluxing benzene (100 ml) in the presence of *p*-toluenesulfonic acid (19 g, 0.1 mol); water was removed by azeotropic distillation (20 h). Removal of the solvent furnished an oil which crystallized (24.1 g, 66 %) from ethanol−ether. M.p. 105−107 °C; $R_F$ 0.3 ($CHCl_3$); $^1H$ NMR peaks at (100 MHz, $D_2O$) δ *ca.* 1.9 (2H, m), 2.3 (3H, s), 2.46 (2H, t, *J ca.* 7 Hz), 2.95 (2H, t, *J ca.* 7.5 Hz), 5.02 (2H, s), 7.18 (2H, d, *J ca.* 8 Hz), 7.27 (5H, s), 7.54 (2H, d, *J ca.* 8 Hz); $^{13}C$ NMR ($H_2O$): δ 144.28 (s), 142.46 (s), 137.98 (s), 131.74, 131.10, 130.91, 130.64, 127.91, 69.28 (t), 23.05 (q) and signals given in Table 2; *m/e* (%): 193 ($M^+$ for γAbu-OBzl, 0.7), 172 ($M^+$ for *p*-toluenesulfonic acid, 20).

*Ac-Asp(OBzl)-γAbu-OBzl (6).* A mixture of Ac-Asp(OBzl) (530 mg, 2.0 mmol, *3*), γAbu-OBzl tosylate (804 mg, 2.2 mmol, *5*), dicyclohexylcarbodiimide (1030 mg, 5 mmol), 1-hydroxybenzotriazole (810 mg, 6 mmol) and *N*-ethylmorpholine (257 mg, 2.2 mmol) in $CH_2Cl_2$ (10 ml) was stirred for 5 h at −15 °C. Acetic acid (0.5 ml) was added and the mixture filtered after 5 min. The solid material was washed with $CH_2Cl_2$ (30 ml) and the combined filtrates were washed twice with 1 M HCl and water respectively, dried over $Na_2SO_4$ and evaporated. The residue was chromatographed twice on silica gel columns and eluted with $CH_2Cl_2$ and 2 % $CH_3OH$ in $CH_2Cl_2$ yielding pure Ac-Asp(OBzl)-γAbu-OBzl (509 mg, 58 %, *6*). M.p. 92 °C; $R_F$ 0.3 (3 % $CH_3OH$ in $CHCl_3$); [α]: see Table 1; $^1H$ NMR peaks at (100 MHz, $CDCl_3$) δ 1.78 (2H, quintet, *J ca.* 7 Hz), 1.96 (3H, s), 2.34 (2H, t, *J ca.* 7 Hz), 2.63 (1H, dd, *J ca.* 7 and 17 Hz), 2.93 (1H, dd, *J ca.* 4.5 and

*Table 2.* $^{13}C$ NMR chemical shifts [a] (in ppm relative to external TMS) of Ac-Asp-γAbu and intermediates. Shifts of protecting groups are given in the experimental part.

| L-Aspartic acid C-4 | C-3 | C-2 | C-1 | $HNCOCH_3$ | | γ-Aminobutyric acid (γAbu) C-4 | C-3 | C-2 | C-1 |
|---|---|---|---|---|---|---|---|---|---|
| Asp (*1*) in $H_2O$/HCl | | | | | | γAbu (*4*) in $H_2O$ | | | |
| 173.50 [b] | 36.43 | 52.01 | 175.64 [b] | | | 42.08 | 26.23 | 36.88 | 183.81 |
| Asp(OBzl) (*2*) in $H_2O$ | | | | | | γAbu-OBzl tosylate (*5*) in $H_2O$ | | | |
| 173.30 [b] | 36.49 | 51.75 | 173.04 [b] | | | 41.43 | 24.61 | 33.11 | 176.54 |
| Ac-Asp(OBzl) (*3*) in $CDCl_3$ | | | | | | | | | |
| 173.30 [b] | 36.04 | 48.63 | 171.28 [b] | 171.09 [b] | 22.73 | | | | |
| Ac-Asp(OBzl)-γAbu-OBzl (*6*) in $CDCl_3$ | | | | | | | | | |
| 172.98 [b] | 35.97 | 49.35 | 171.54 [b] | 170.24 [b] | 23.05 | 38.96 | 24.41 | 31.49 | 170.24 [b] |
| Ac-Asp-γAbu (*7*) in $H_2O$ | | | | | | | | | |
| 176.61 [b] | 38.31 | 52.92 | 180.44 [b] | 174.92 [b] | 24.41 | 41.23 | 26.30 | 33.50 | 176.61 [b] |

[a] The assignments were confirmed by the splitting patterns observed in off-resonance decoupled spectra. [b] Assignments may be reversed.

17 Hz), 3.2 (2H, q, *J ca.* 6.5 Hz), 4.68 (1H, octet, *J ca.* 4.5, 7 and 8 ($J_{CHNH}$) Hz), 5.02 (4H, s), 6.66 (2H, m, NH), 7.20 (10H, s); $^{13}C$ NMR ($CDCl_3$): $\delta$ 135.77 (s), 135.32 (s), 128.50, 128.17, 66.75 (t), 66.36 (t) and signals given in Table 2; *m/e* (%): 439 ($M^+-1$, 2) 91 (100), 108 (93), 79 (80), 43 (75), 107 (69), 77 (44), 198 (36), 225 (28).

*Ac-Asp-γAbu (7).* Ac-Asp(OBzl)-γAbu-OBzl (196 mg, 0.44 mmol, *6*) dissolved in $CH_3OH$ (4 ml) and acetic acid (0.3 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10 % Pd/C (78 mg). The gas uptake was complete in 100 min and the solution was filtered through celite and evaporated to yield a crystalline product (104 mg, 90 %) which was recrystallized from $CH_3OH$–ether. M.p. 156–158 °C; $R_F$ 0.8 (ethanol–$H_2O$= 14:1); [α]: see Table 1; $^1H$ NMR peaks at ($CD_3OD$, 100 MHz) $\delta$ 1.78 (2H, quintet, *J ca.* 7 Hz), 1.98 (3H, s), 2.32 (2H, t, *J ca.* 7 Hz), *ca.* 2.7 (1H, dd (? the small peaks of AB-part of the ABX-system were barely visible), *J ca.* 6.5 and 16 (?) Hz), *ca.* 2.8 (1H, dd (?), *J ca.* 6.5 and 16 (?) Hz), 3.19 (2 (?) H, "t" (partly coinciding with solvent peak), *J ca.* 7 Hz), 4.61 (2H, t, *J ca.* 6.5 Hz); ($D_2O$, 100 MHz) $\delta$ 1.80 (2H, quintet, *J ca.* 7 Hz), 2.04 (3H, s), 2.39 (2H, t, *J ca.* 7 Hz), 2.82 (2H, d, *J ca.* 7 Hz), 3.23 (2H, t, *J ca.* 7 Hz), a signal at $\delta$ *ca.* 4.6 was blurred by water-peak; $^{13}C$ NMR: see Table 2. $\nu_{max}$ (KBr): 3375 (m), 3305 (m), 1735 (s), 1728 (s), 1658 (s), 1625 (s), 1575 (s), 1545 (s), 1268 (s), 1241 (s), 1202 (m), 1103 (m); *m/e* (%): 260 ($M^+$, 1), 43 (100), 85 (38), 88 (20), 183 (19), 131 (11), 196 (10), 86 (10). Found: C 46.32; H 6.08; N 10.58. Calc. for $C_{10}H_{16}N_2O_6$: C 46.15; H 6.20; N 10.77.

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