^{13}C NMR Study and X-Ray Analysis of N-(N-Acetyl-L-aspartyl)-4-aminobutyric Acid (Ac−Asp−γAbu), $C_{10}H_{16}N_2O_6$. A Comparison of the NMR Results with Semi-empirical Calculations

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The complete assignment of the ^{13}C NMR resonances at 22.5 MHz has been performed for N-(N-acetyl-L-aspartyl)-4-aminobutyric acid (Ac−Asp−γAbu) in aqueous solution. In the crystalline state the conformation of Ac−Asp−γAbu has been determined by X-ray crystallography. Semi-empirical CNDO/2 calculations based on a model from the solid state have been used to calculate the net charges on the C atoms and these charges show correlation with the ^{13}C NMR chemical shifts found in solution. The synthesis of a deuterium labelled analog N-(N-acetyl-L-aspartyl)-4-amino-2,2,3,3,4,4-hexadeuteriobutyric acid (Ac−Asp−γAbu-d_6) is also described.

Many ω-amino acids have been isolated from various sources of animals and plants and numerous investigations have been performed to accumulate data concerning their metabolic pathways and/or physiological functions. One of the most important results was that γ-aminobutyric acid (γAbu) was found to have a potent inhibitory action against epileptic seizure.[1] γAbu occurs especially in the mammalian brain where it is enzymatically produced. N-Acetyl-L-aspartic acid (Ac−Asp) has also been found in mammalian brain tissue.[2] Recently we reported[3] the synthesis of N-(N-acetyl-L-aspartyl)-4-aminobutyric acid (Ac−Asp−γAbu, 1) which has been postulated by Reichelt[4] to be one of the Ac−Asp-containing oligopeptides produced in an amine- and ATP-dependent synthesis in homogenates of mouse cortex.[5,6]

The search for possible structure−activity relationships in such compounds is of considerable interest and we thought it therefore worthwhile to make a combined ^{13}C NMR study and X-ray analysis of Ac−Asp−γAbu and try to correlate the results with semi-empirical calculations.

RESULTS AND DISCUSSION

Ac−Asp−γAbu-d_6 (2) was prepared by coupling Ac−Asp(OBzl)[3,7] (5) with benzyl 4-amino-2,2,3,3,4,4-hexadeuteriobutyrate (γAbu-d_6−OBzl, 3) using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents. The protecting benzyl groups were subsequently removed by hydrogenolysis in the presence of acetic acid to prevent cyclization to the corresponding succinimide- or γ-lactam derivatives.

In the paper describing the synthesis of Ac−Asp−γAbu (1),[3] we assigned all the ^{13}C resonances except those of the four carbonyl groups which were observed at 174.9, 176.6 (two coinciding resonances) and 180.4 ppm in its noise-decoupled spectrum; cf. Fig. 1a. The noise-decoupled spectrum of Ac−Asp−γAbu-d_6 (2) exhibited three singlets at 175.0, 176.6 and 176.8 ppm in addition to a barely visible multiplet at 180.4 ppm which could be ascribed to the carboxylic carbonyl resonance of the γAbu-d_6 moiety because of its complexity due to ^{2}H−^{13}C spin couplings and low intensity caused by increased relaxation time. Deuteration of a nearby protonated carbon is known to increase the T_1 of a nonprotonated carbon;[8] cf. Fig. 1c.
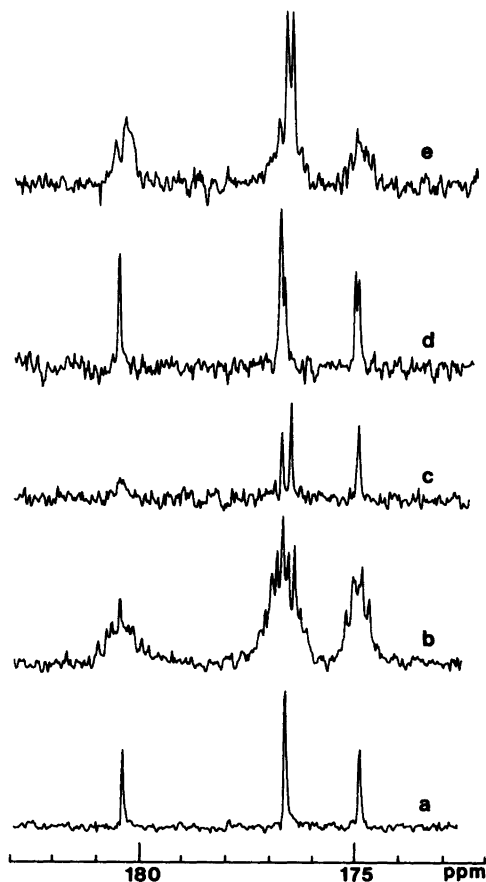
Fig. 1. ^{13}C spectra of carbonyl carbons. a, Ac — Asp — γAbu: ^{1}H-decoupled; b, Ac — Asp — γAbu: single-resonance; c, Ac — Asp — γAbu-d_6: ^{1}H-decoupled; d, Ac — Asp — γAbu in 50:50 (v/v) H$_2$O — D$_2$O: ^{1}H-decoupled; e, Ac — Asp — γAbu: selective low-power irradiation at the frequency of the CH$_3$CO-protons.

When recorded in a 50:50 mixture of H$_2$O and D$_2$O as solvent, amide NH protons will, as pointed out by Feeney *et al.*,[9] exchange slowly and amide carbonyl resonances will appear as doublets corresponding to CONH and COND ^{13}C signals which have slightly different δ values due to the deuterium isotope effect. Since no splitting is observed on the carboxylate signals because protons of the carboxylate are in rapid exchange with the solvent, amide and carboxylic carbonyl signals can be distinguished by recording the noise-decoupled spectrum in a mixture of H$_2$O and D$_2$O. Thus, the

noise-decoupled spectrum of Ac — Asp — γAbu (*1*) in H$_2$O/D$_2$O exhibited a singlet at *ca.* 180.4 ppm confirming it being a carboxylic carbonyl resonance. Furthermore, the spectrum revealed a doublet at *ca.* 176.6 ppm partly coinciding with a singlet which consequently could be ascribed to the carboxylic carbon of the Ac — Asp moiety, and a doublet at *ca.* 174.9 ppm; *cf.* Fig. 1d. The isotope shift, 0.08 ppm, observed in the latter doublet is comparable to that observed by Feeney *et al.*[9] The spectrum also displayed doublets at *ca.* 41.2 and 52.9 ppm (isotope shifts 0.14 and 0.08 ppm, respectively) ascribed to the C — 4 signal of the γAbu moiety and the C — 2 signal of the Ac — Asp moiety, respectively.

Assignments of the two amide carbonyl resonances were readily achieved employing selective low-power irradiation at the resonance frequency of the CH$_3$CO-protons; *cf.* Fig. 1e. The doublet which appeared at *ca.* 176.6 ppm revealed a residual ^{1}H — ^{13}C spin coupling constant of 3.1 Hz which is in excellent agreement with the $^3J_{^{13}C-C\alpha H}$ spin coupling constant of 3.1 ± 0.1 Hz reported by Feeney *et al.*[9] for a similar system (Ac — Asp). Hence, the final ^{13}C-signal at 174.9 ppm could be assigned to the amide carbonyl carbon linking the Ac — Asp and γAbu moieties (*cf.* Fig. 2).

The conformation of Ac — Asp — γAbu as found in the crystalline state and the numbering of the atoms are shown in Fig. 3. Bond lengths and angles for non-hydrogen atoms as calculated from the atomic coordinates in Table 1, are listed in Tables 2 and 3. Some torsion angles are given in Table 4. The molecule has an open structure with no intra-molecular contacts. The different parts of the molecule can be described by four planes P1 — P4 comprising the following atoms: P1: O(101), O(102), C(9), C(8) and C(7); P2: C(7), N(2), C(6), O(6) and C(3); P3: C(6), C(3), C(4), C(5), O(51) and O(52); and P4: C(3), N(1), C(2), O(2) and C(1). P1 and P4
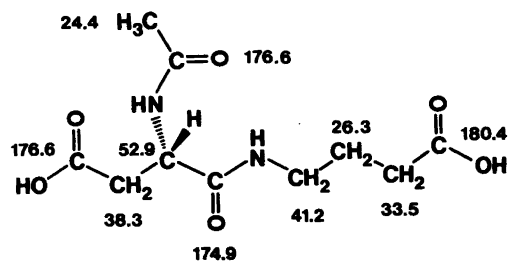

Fig. 2. ^{13}C NMR shift assignments of Ac — Asp — γAbu in H$_2$O. δ-Values are relative to external TMS.

Table 1. Atomic coordinates and temperature parameters $U_{ij}(\text{Å}^2 \times 10^4)$ for the oxygen, nitrogen and carbon atoms and $U(\text{Å}^2 \times 10^4)$ for the hydrogen atoms. The temperature factors are $\exp\{2\pi^2(h^2 a^{*2} U_{11} + \cdots 2hka^*b^* U_{12} + \cdots)\}$ and $\exp\{-8\pi^2 U(\sin^2\theta/\lambda^2)\}$, respectively.

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
C(1)	.2046(14)	.1447(0)	1.2216(6)	405(37)	188(25)	90(21)	0(0)	34(23)	0(0)
C(2)	.1326(11)	.1741(3)	1.0536(5)	315(29)	96(20)	136(22)	66(20)	66(21)	-14(16)
O(2)	-.0650(8)	.1392(2)	.9715(4)	337(23)	132(16)	170(16)	-52(15)	-11(15)	45(13)
N(1)	.2862(9)	.2376(3)	.9946(5)	283(24)	94(16)	114(18)	20(17)	-12(17)	-18(14)
C(3)	.2120(12)	.2734(3)	.8357(5)	315(32)	103(21)	111(21)	-29(21)	14(21)	17(17)
C(4)	.3448(12)	.3636(3)	.8185(6)	367(33)	83(19)	150(22)	-46(22)	88(21)	14(18)
C(5)	.1898(12)	.4350(3)	.9076(6)	557(33)	155(23)	125(22)	-92(22)	-31(22)	37(18)
O(51)	-.0128(11)	.4218(3)	.9787(5)	516(27)	163(18)	256(21)	-28(20)	100(20)	-40(16)
O(52)	.3205(9)	.5115(2)	.8930(4)	457(26)	80(15)	277(21)	-53(16)	72(18)	-22(14)
C(6)	.2964(11)	.2150(5)	.6988(5)	304(29)	87(20)	92(19)	-39(19)	24(19)	-16(16)
O(6)	.1848(9)	.2281(2)	.5626(4)	519(26)	142(17)	95(15)	88(17)	-69(16)	-12(13)
N(2)	.4925(10)	.1547(3)	.7296(5)	289(24)	108(18)	123(18)	-21(17)	14(18)	-29(15)
C(7)	.6085(12)	.0997(3)	.6071(6)	314(30)	97(21)	149(23)	-48(20)	53(21)	11(17)
C(8)	.4500(13)	.0182(3)	.5652(6)	411(35)	131(22)	113(23)	-12(21)	18(23)	-23(18)
C(9)	.4216(14)	-.0463(3)	.7035(6)	439(38)	165(23)	131(23)	-100(24)	-32(24)	30(20)
C(10)	.2787(12)	-.1321(4)	.6651(6)	398(34)	158(22)	134(22)	24(23)	-10(22)	-3(19)
O(101)	.3083(9)	-.1978(2)	.7470(4)	472(25)	127(17)	198(17)	-3(17)	-20(17)	39(13)
O(102)	.1044(10)	-.1299(3)	.5346(5)	582(31)	195(19)	239(21)	-170(21)	-186(20)	71(17)

ATOM	X	Y	Z	U	ATOM	X	Y	Z	U
H(11)	.336(15)	.169(5)	1.273(8)	413(199)	H(2)	.631(13)	.153(4)	.815(7)	280(162)
H(12)	-.079(12)	.157(4)	1.280(6)	187(158)	H(71)	.620(13)	.130(4)	.511(7)	342(169)
H(13)	.271(14)	.085(5)	1.210(8)	456(202)	H(72)	.803(10)	.077(3)	.644(5)	528(116)
H(1)	.448(10)	.265(3)	1.055(5)	281(127)	H(81)	.222(10)	-.034(3)	.529(6)	571(124)
H(3)	.036(12)	.282(3)	.820(5)	365(115)	H(82)	-.516(12)	-.012(3)	.484(6)	479(118)
H(41)	.571(11)	.368(4)	.856(6)	450(131)	H(91)	.623(12)	-.065(4)	.758(6)	512(131)
H(42)	.354(20)	.374(6)	.698(10)	537(324)	H(92)	.325(10)	-.017(3)	.787(6)	426(115)
H(52)	.199(10)	.559(3)	.925(5)	475(121)	H(102)	-.015(19)	-.171(5)	.532(9)	567(248)

Table 2. Bond lengths *l* in *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid. Standard deviations in parentheses.

Bond	*l*(Å)
C(1)−C(2)	1.499(7)
C(2)−O(2)	1.250(6)
C(2)−N(1)	1.322(7)
N(1)−C(3)	1.464(6)
C(3)−C(4)	1.521(7)
C(3)−C(6)	1.524(7)
C(4)−C(5)	1.529(7)
C(5)−O(51)	1.172(7)
C(5)−O(52)	1.329(6)
C(6)−O(6)	1.247(6)
C(6)−N(2)	1.319(7)
N(2)−C(7)	1.456(7)
C(7)−C(8)	1.530(7)
C(8)−C(9)	1.523(7)
C(9)−C(10)	1.499(8)
C(10)−O(101)	1.219(6)
C(10)−O(102)	1.332(7)

Table 4. Selected torsion angles (°) in Ac−Asp−γAbu.

Torsion angle	(°)
C(1)−C(2)−N(1)−C(3)	−175.3(4)
O(2)−C(2)−N(1)−C(3)	5.0(7)
C(2)−N(1)−C(3)−C(4)	159.9(5)
C(2)−N(1)−C(3)−C(6)	−76.8(6)
N(1)−C(3)−C(4)−C(5)	−75.6(5)
N(1)−C(3)−C(6)−O(6)	162.7(5)
C(4)−C(3)−C(6)−O(6)	−73.5(6)
C(4)−C(3)−C(6)−N(2)	104.2(5)
C(6)−C(3)−C(4)−C(5)	158.3(4)
C(3)−C(4)−C(5)−O(51)	−1.5(7)
C(3)−C(4)−C(5)−O(52)	177.9(4)
C(3)−C(6)−N(2)−C(7)	−174.8(5)
O(6)−C(6)−N(2)−C(7)	2.7(8)
C(6)−N(2)−C(7)−C(8)	−84.4(6)
N(2)−C(7)−C(8)−C(9)	−65.5(6)
C(7)−C(8)−C(9)−C(10)	−173.3(5)
C(8)−C(9)−C(10)−O(101)	162.3(6)
C(8)−C(9)−C(10)−O(102)	−20.2(7)

are almost parallel with an angle of 18.5° between them. P2 is almost perpendicular to both P3 and P4, and the values are 84.2 and 84.4°, respectively. The angle between P3 and P4 is 74.3°.

Table 3. Bond angles ∠(*ijk*) in *N*-(*N*-acetyl-L-aspartyl)-4-aminobutyric acid.

i − *j* − *k*	∠(*ijk*)°
C(1)−C(2)−O(2)	121.0(5)
C(1)−C(2)−N(1)	118.0(5)
O(2)−C(2)−N(1)	121.0(5)
C(2)−N(1)−C(3)	120.4(5)
N(1)−C(3)−C(4)	110.2(4)
N(1)−C(3)−C(6)	114.0(5)
C(4)−C(3)−C(6)	109.3(5)
C(3)−C(4)−C(5)	112.7(5)
C(3)−C(6)−O(6)	118.8(5)
C(3)−C(6)−N(2)	118.0(4)
C(4)−C(5)−O(51)	123.6(5)
C(4)−C(5)−O(52)	110.0(5)
O(51)−C(5)−O(52)	126.4(5)
O(6)−C(6)−N(2)	123.2(5)
C(6)−N(2)−C(7)	123.3(4)
N(2)−C(7)−C(8)	114.0(5)
C(7)−C(8)−C(9)	112.7(5)
C(8)−C(9)−C(10)	115.5(5)
C(9)−C(10)−O(101)	124.1(5)
C(9)−C(10)−O(102)	113.9(5)
O(101)−C(10)−O(102)	122.0(6)

The crystal structures of γ-aminobutyric acid and aspartic acid have been solved[10−15] and a comparison can therefore be made with the γAbu and Ac−Asp parts of Ac−Asp−γAbu. γAbu has been solved at low temperature[10] as well as at room temperature[11] and γAbu · HCl has also been solved.[12,13] Our results show that the γAbu part of Ac−Asp−γAbu is in a planar *trans* conformation with respect to the C_α−C_β bond C(9)−C(8). This is in more accordance with γAbu · HCl than with γAbu where a *gauche* conformation is found with respect to the C_α−C_β bond. For aspartic acid two previous investigations have been made,[14,15] one of L-aspartic acid[14] and the other of DL-aspartic acid.[15] In both structures the carbon chain is nearly planar with an average deviation from planarity of 0.01 and 0.03 Å, respectively. A least squares plane made up of the same four atoms C(6), C(3), C(4) and C(5) in the present molecule gives a mean deviation from the plane of about 0.1 Å. The atoms C(3), C(4), C(5), O(51) and O(52) lie, however, in the same plane to within 0.01 Å, with the atoms C(6) and N(1) −0.52 and 1.36 Å, respectively, out of the plane. A comparison of bond lengths in Ac−Asp−γAbu with relevant bonds in γAbu and Asp is given in Table 5. The packing of Ac−Asp−γAbu is illustrated in Fig. 4.[16] The crystal structure is stabilized by hydrogen bonds, one for each hydrogen atom covalently bonded to nitrogen
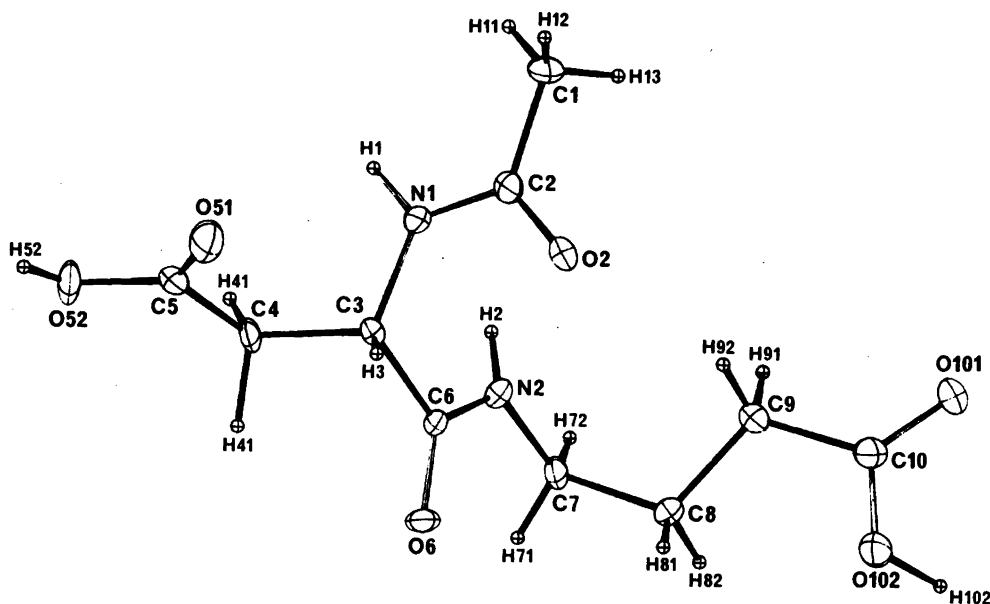
Fig. 3. ORTEP[16] drawing of the title compound with the numbering of the atoms in the molecule. Thermal ellipsoids for the non-hydrogen atoms are drawn at the 50 % probability level.

or oxygen. Hydrogen bond distances are given in Table 6.

CNDO/2 calculations − correlation with the ^{13}C *NMR results.* The results obtained from the X-ray study have been used to calculate net charges for the C atoms by semi-empirical CNDO/2 calculations.[17] The net charges are calculated from an analysis of the electron density-bond order matrix,

$$P_{\mu\nu} = 2 \sum_{i}^{occ} C_{\mu_i\nu_i},$$

where C_{μ_i} are LCAO coefficients and the summation being over all occupied molecular orbitals. The bond lengths and angles found in the X-ray crystallographic work were used as a model for the theoretical calculations, except for the C−H, O−H

Table 5. Comparison of bond lengths (Å) in Ac−Asp−γAbu with relevant bonds in related compounds. The numbering of the atoms refer to the present work.

| Bond	This work	Ref. 15	Ref. 14	Ref. 11	Ref. 10	Ref. 13
N(1)−C(3)	1.464(6)	1.491(3)	1.495(4)			
C(3)−C(4)	1.521(7)	1.519(3)	1.518(4)			
C(4)−C(5)	1.529(7)	1.505(3)	1.512(4)			
C(5)−O(51)	1.172(7)	1.219(3)	1.202(4)			
C(5)−O(52)	1.329(6)	1.305(3)	1.306(4)			
C(3)−C(6)	1.524(7)	1.538(3)	1.543(4)			
C(6)−O(6)	1.247(6)	1.250(3)	1.242(4)			
N(2)−C(7)	1.456(7)			1.469(5)	1.497(4)	1.49(2)
C(7)−C(8)	1.530(7)			1.502(6)	1.520(5)	1.56(2)
C(8)−C(9)	1.523(7)			1.519(6)	1.528(5)	1.52(2)
C(9)−C(10)	1.499(8)			1.520(6)	1.522(6)	1.53(2)
C(10)−O(101)	1.219(6)			1.226(6)	1.249(4)	1.22(2)
C(10)−O(102)	1.332(7)					1.32(2)
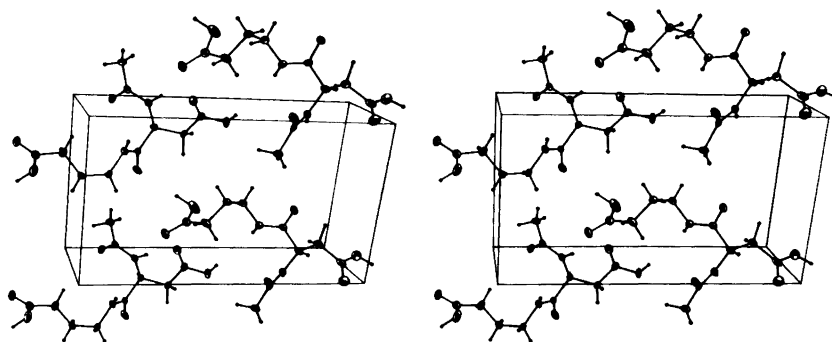
Fig. 4. A stereoscopic view of the arrangement of molecules in the unit cell.

and N−H bond lengths which were given the values 1.09, 0.96 and 1.01 Å, respectively.[18] The net charges have been plotted against the ^{13}C NMR chemical shift found on corresponding atoms, Table 7, and the plot is given on Fig. 5. The plot shows that there is some correlation between the calculated charges and the observed chemical shifts. The correlation coefficient from the least squares linear regression fit[19] is 0.93 if all ten carbon atoms are included.

A comparison of the results from the aspartic part of Ac−Asp−γAbu can be made with the theoretical work of Momany *et al.*[20] They have used CNDO/2 calculations to find the energetically most favoured conformations of polypeptides and proteins and our CNDO/2 results show a good agreement with their calculations. The largest difference in net charge is on nitrogen atom N(1), *cf.* Fig. 3; this work gives a value of −0.203 compared to the value of −0.356 calculated by Momany *et al.* This difference may be explained by the fact that in the present work an acetyl group is attached to N(1)

and this will lead to a decreased charge on N(1) relative to a nitrogen atom with only a hydrogen atom attached to it as in the work of Momany *et al.*[20]

EXPERIMENTAL

Melting points (uncorrected), rotations, IR and mass spectra were recorded on Mettler FP61, Perkin-Elmer 241, Beckmann and Micromass 7070H instruments, respectively, [1]H (89.55 MHz) and [13]C (22.5 MHz) NMR spectra were obtained on a Jeol FX90Q instrument operating in the pulsed-Fourier transform mode. Unless otherwise stated, ^{13}C NMR spectra were recorded using a pulse width of 5.5 μs (45° pulse), a spectral width of 5000 Hz (16K data points), an acquisition time of 0.998 s, and with TMS as external reference. Pulse delays are stated below. Analytical TLC and column chromatography were performed on silica gel: F_{254} plates and Merck Kieselgel 60 (0.040−0.063 mm), respectively.

Tosylate of benzyl 4-amino-2,2,3,3,4,4-hexadeuteriobutyrate (γ-Abu-d_6-OBzl tosylate, 3). γAbu-d_6

Table 6. Hydrogen bond distances (Å) and angles (°). Symmetry code:

(i) x, y, z
(ii) $1+x, y, z$
(iii) $-x, \frac{1}{2}+y, 1-z$

(iv) $1-x, \frac{1}{2}+y, 2-z$
(v) $-x, y-\frac{1}{2}, 2-z$

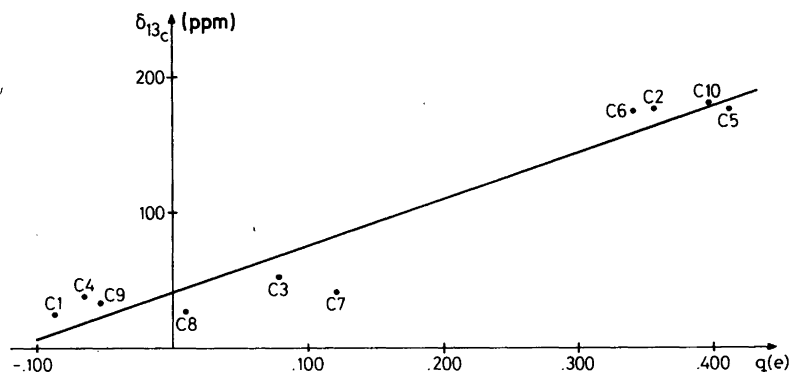| A−H···B	A−H	H···B	A···B	∠AHB
N(2)^i − H(2)···O(2)^ii	0.94	1.90	2.84	180
N(1)^i − H(1)···O(101)^iv	0.98	2.05	2.98	158
O(52)^i − H(52)···O(2)^v	0.97	1.65	2.58	159
O(102)^i − H(102)···O(6)^iii	0.84	1.89	2.66	152

Fig. 5. Correlation of net charges (*q*) calculated using CNDO/2 and ^{13}C NMR chemical shifts (δ_{13C}) in Ac—Asp—γAbu.

(242 mg, 2.22 mmol, *4*) was esterified with benzyl alcohol-d_1 (656 mg, 6.08 mmol) in refluxing benzene (7 ml) in the presence of *p*-toluenesulfonic acid-d_1 (554 mg, 2.92 mmol). The labile protons of the benzyl alcohol and the *p*-toluenesulfonic acid had in advance been exchanged with deuterium by shaking with D_2O and subsequent removal of the water by distillation *in vacuo*. Water was removed from the reaction mixture by azeotropic distillation (20 h). The solvent was removed and the residue recrystallized from ethanol—ether (763 mg, 93 %). M.p. 100—101 °C; R_F 0.3 (CHCl$_3$); ^{1}H NMR peaks at (D$_2$O) δ 2.38 (3H, s), 5.17 (2H, s), 7.38 (2H, d, *J ca.* 8.5 Hz), 7.44 (5H, s), 7.67 (2H, d, *J ca.* 8.5 Hz); ^{13}C NMR (D$_2$O, pulse delay: 2 s): δ 23.1 (q), 69.7 (t), 128.0, 130.1, 130.9, 131.3, 131.4, 131.5, 132.0, 138.3, 145.1; δ-values are relative to sodium 3-(trimethylsilyl)-propansulfonate.

Ac—Asp(OBzl)—γAbu-d$_6$—*OBzl (6).* A mixture of Ac—Asp(OBzl) (576 mg, 2.2 mmol, *5*), γAbu-

Table 7. Net charges (q) from CNDO/2 calculations and ^{13}C NMR chemical shifts (δ) in Ac—Asp—γAbu.

Atom	q(e)	δ (ppm)
C(1)	−0.087	24.4
C(2)	0.355	176.6
C(3)	0.078	52.9
C(4)	−0.065	38.3
C(5)	0.411	176.6
C(6)	0.340	174.9
C(7)	0.121	41.2
C(8)	0.010	26.3
C(9)	−0.054	33.5
C(10)	0.396	180.4

d_6—OBzl tosylate, 733 mg, 2.0 mmol, *3*), dicyclohexylcarbodiimide (1018 mg, 4.9 mmol), 1-hydroxybenzotriazole (800 mg, 5.9 mmol) and N-ethylmorpholine (227 mg, 2.0 mmol) in CH$_2$Cl$_2$ (10 ml) was stirred for 5 h at −15 °C and left in the refrigerator overnight. Acetic acid (0.5 ml) was added and the mixture filtered after 5 min. The solid material (dicyclohexylurea) was washed with CH$_2$Cl$_2$ (30 ml) and the combined filtrates were washed twice with 1 M HCl and water, respectively, dried over Na$_2$SO$_4$ and evaporated. The residue was chromatographed twice on silica gel columns and eluted with CH$_2$Cl$_2$, 1 % CH$_3$OH and 2 % CH$_3$OH in CH$_2$Cl$_2$ yielding pure Ac—Asp(OBzl)—γAbu-d_6—OBzl (540 mg, 61 %, *6*). M.p. 91—92 °C; R_F 0.3 (3 % CH$_3$OH in CHCl$_3$); $[\alpha]^{20}$ (*c* 1.23, CHCl$_3$) −15.5° (589 nm), −16.5° (578 nm), −19.3° (546 nm), −39.5° (436 nm), −78.2° (365 nm). ^{1}H NMR peaks at (CDCl$_3$) δ 1.99 (3H, s), *ca.* 2.7 (1H, dd, J *ca.* 6.6 and 16.8 Hz, B-part of an ABMX-system), *ca.* 3.0 (1H, dd, J *ca.* 4.4 and 16.8 Hz, A-part of an ABMX-system), *ca.* 4.8 (1H, m, M-part of an ABMX-system), 5.11 (4H, s), *ca.* 6.7 (2H, m), 7.33 (10H, s); ^{13}C NMR (CDCl$_3$, pulse delay: 1.5 s): δ 23.1 (q), 36.0 (t), 49.5 (d), 66.4 (t), 66.9 (t), 128.2, 128.3, 128.4, 128.6, 135.9, 136.0, 170.2 (s), 170.4 (s), 171.8 (s), 173.1 (barely visible multiplet); δ-values are relative to internal TMS; *m/e* (%): 446 (M$^+$, 0.4), 91 (100), 79 (49), 43 (47), 108 (40), 77 (31), 107 (30), 204 (29), 231 (24), 172 (21). The deuterium-content of *6*−d_6 was calculated on the basis of the intensities of the ions of *6*−d_6 around *m/e* 204 and 231 in relation to corresponding ions of *6*−h_6 around *m/e* 198 and 225 observed in the spectrum recorded for a mixture of the two compounds: d_6 *ca.* 92.4%, d_5 *ca.* 6.8%, d_4 *ca.* 0.8%.

Ac—Asp—γAbu-d$_6$ *(2).* Ac—Asp(OBzl)—γAbu-d_6—OBzl (470 mg, 1.1 mmol, *6*) dissolved in CH$_3$OH

(5 ml) and acetic acid (0.2 ml) was hydrogenated at room temperature and atmospheric pressure in the precence of 10 % Pd/C (40 mg) by bubbling hydrogen through the reaction mixture for 100 min. The solution was filtered through celite and evaporated to yield a crystalline product which was recrystallized from CH_3OH-ether (197.1 mg, 71 %). M.p. 157−158 °C; R_F 0.8 (ethanol−$H_2O = 14:1$); $[\alpha]^{20}$ (c 0.64, CH_3OH) −33.6° (589 nm), −35.1° (578 nm), −40.5° (546 nm), −76.1° (436 nm), −136.4° (365 nm); ^1H NMR peaks at (TMS, CD_3OD) δ 1.99 (3H, s), ca. 2.8 (1H, dd, J ca. 16.6 and 7.0 Hz, B-part of an ABX-system), ca. 2.7 (1H, dd, J ca. 16.6 and 6.3 Hz, A-part of an ABX-system), ca. 4.7 (1H, dd, J ca. 6.3 and 7.0 Hz, X-part of an ABX-system); ^{13}C NMR (D_2O, pulse delay: 12 s): δ 24.5 (q), 38.5 (t), 53.0 (d), 175.0 (s), 176.6 (s), 176.8 (s), ca. 180.4 (barely visible multiplet); m/e (%): M^+ not visible, 248 ($M-18$, 0.3), 43 (100), 85 (34), 70 (9), 46 (8), 187 (7), 186 (6), 92 (6), 91 (6), 201 (6), 112 (5), 229 (3).

Ac−Asp−γAbu (1).[1] ^{13}C NMR spectra (D_2O except in the case where a 1:1 mixture of H_2O-D_2O was employed) were recorded using the following conditions: ^1H-decoupled using a pulse delay of 6 s, single-resonance using a pulse delay of 3 s and acquisition time of 1.638 s, selective decoupling using a pulse delay of 1.5 s, and a pulse delay of 2 s in the case where H_2O-D_2O was used as solvent.

Crystal data. N-(N-Acetyl-ʟ-aspartyl)-4-aminobutyric acid (Ac−Asp−γAbu), $C_{10}H_{16}N_2O_6$, M = 260.25. Monoclinic space group $P2_1$. $a = 4.720(2)$, $b = 15.242(4)$, $c = 8.383(3)$ Å, $\beta = 93.19(3)°$, $Z = 2$, $D_m = 1.45$ g cm^{-3}, $D_x = 1.435$ g cm^{-3}, $\mu(MoK\alpha) = 1.29$ cm^{-1}. Cell dimensions were found from a least squares refinement of the 2θ values of 25 reflections.

Data collection. A crystal of size $0.5 \times 0.2 \times 0.2$ mm was used for all data collections. Unit cell dimensions and intensity data were measured on an automatic Enraf-Nonius CAD4 diffractometer using graphite monochromator and MoKα radiation ($\lambda = 0.71069$ Å). The data was collected at about −150 °C using liquid nitrogen equipment for cooling. Three-dimensional intensity data for 2103 independent reflections within $2\theta < 64°$ were collected by the $\omega - 2\theta$ scan technique and with $\Delta\omega = 0.60 + 0.35 \tan \theta$. After data reduction including Lp-correction but no absorption correction, 1512 reflections had net intensities $> 2.0\sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics. These were regarded as observed reflections and were used in the refinement procedure.

Structure solution and refinements. The structure was solved using the MULTAN system,[21] which revealed the 18 non-hydrogen atoms of the molecule. After a few full matrix least squares refinement cycles, the 16 hydrogen atoms were found from

difference maps. The atomic parameters were thereafter refined by full matrix least squares to an R of 0.066. At the end of the refinement the average shift/error ratio was 0.04.

The form factors used in the structure factor calculations were those of Stewart et al.[22] for hydrogen and Cromer and Mann[23] for the other atoms. Final atomic coordinates and temperature parameters are listed in Table 1.

All the calculations mentioned above were carried out on the Cyber 171MP computer at the University of Tromsø. The programs used were mainly those of the X-RAY 76 program system.[24] The data reduction programs used were adopted for the Cyber 171MP computer by L. K. Hansen and L. J. Sæthre, this University.

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