Aust. J. Chem., 1985, **38**, 1651–6

Synthesis of Analogues of GABA. XIV* Synthesis and Activity of Unsaturated Derivatives of 5-Aminopentanoic Acid (δ-Aminovaleric Acid)

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Abstract

The (*Z*) and (*E*) pairs of 5-aminopent-2-enoic acid and 5-aminopent-3-enoic acid, as well as the related 5-aminopent-3-ynoic acid, have been prepared for structure–activity studies on GABA receptors. Only the (*Z*) isomers were active as GABA agonists with (*Z*)-5-aminopent-2-enoic acid being two- to four-fold more active than 5-aminopentanoic acid.

γ-Aminobutyric acid (GABA) acts as an inhibitory transmitter at receptor sites in the mammalian central nervous system, and information regarding active conformations[1] has been derived from conformationally restricted analogues such as (*E*)- and (*Z*)-4-aminocrotonic acid.[2] 5-Aminopentanoic acid (δ-aminovaleric acid) interacts with $GABA_A$-receptor processes as a moderately active agonist,[1] and has been reported to antagonize the $GABA_B$-receptor activity of baclofen.[3,4]

Recent work in this laboratory has shown that the isothiouronium salt (1)[5] is a very potent $GABA_A$-receptor agonist. Comparison of the charge separation of (1) with that of GABA and of 5-aminopentanoic acid suggested that a conformationally restricted δ-aminovaleric acid analogue may adopt a similar shape and charge separation at the receptor.

The compound which most closely resembles the potent GABA agonist (1) is the conjugated (*Z*) unsaturated amino acid (2). The corresponding (*E*) isomer (3) can be considered as a homologue of (*E*)-4-aminocrotonic acid which is a potent $GABA_A$-receptor agonist and considerably more potent than (*Z*)-4-aminocrotonic acid.[2] Other compounds that are related to (2) and (3) and which are important for structure–activity studies on GABA are the β,γ-unsaturated derivatives (4)–(6). These compounds are particularly interesting because they contain an activated C–H bond

* Part XIII, *Aust. J. Chem.*, 1985, **38**, 1647.

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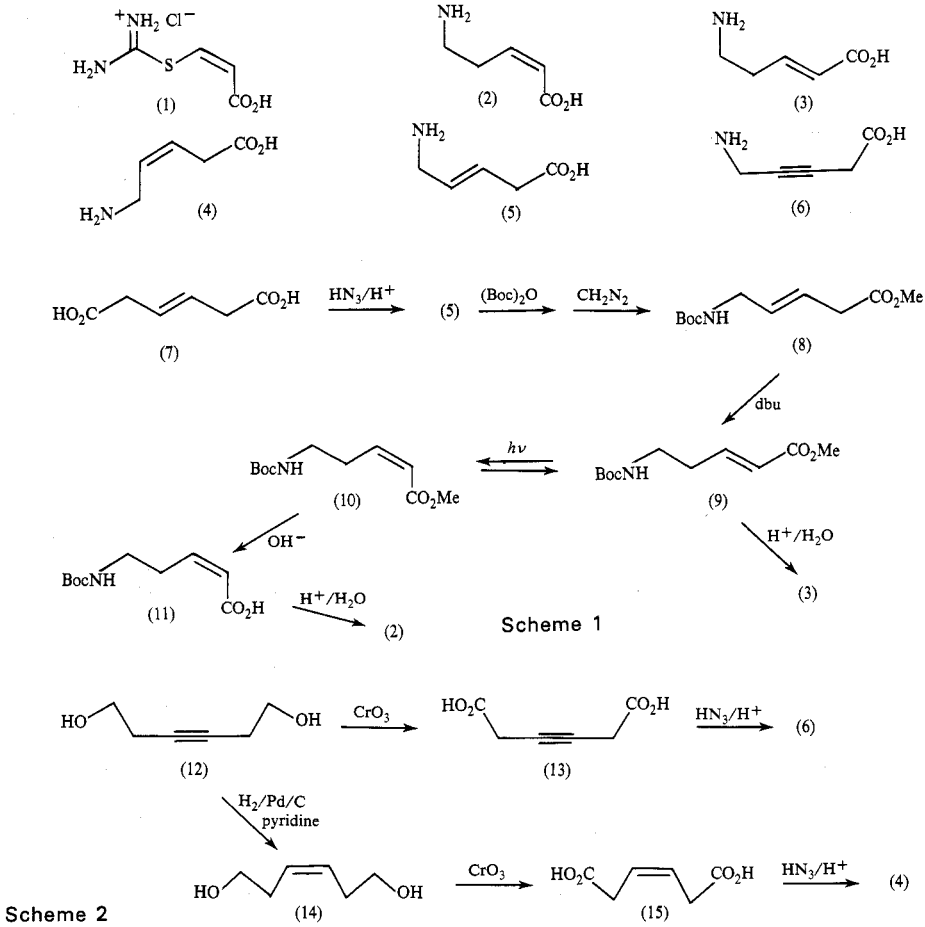

Table 1. Activity studies

ED_{50}, effective dose to produce 50% of the maximal response. IC_{50}, inhibitor concentration to reduce activity to 50% of control values. δApn, 5-aminopentanoic acid

Compound tested	ED_{50} (μM) Contraction of guinea-pig ileum	ED_{50} (μM) Enhancement of [^{3}H]diazepam binding	IC_{50} (μM) Uptake of [^{3}H]GABA	IC_{50} (μM) GABA-transaminase
GABA	2·2±0·1	0·46±0·06	—	—
(4)	74±6	[B]	84±3	295±46[E]
(5)	[A]	[B]	417±47	736±92[E]
(2)	26±3	13±1	[A]	[A]
(3)	[A]	[A]	>500[C]	[A]
(6)	[A]	[B]	>500	[B]
δApn	105±8	30±4	85±3[D]	295±46[E]
(1)	0·63±0·1	0·19±0·03	74±8	[A]

[A] Not significant (at 500 μM for guinea-pig ileum and uptake, at 100 μM for diazepam binding, at 1 mM for GABA-transaminase).

[B] Not tested.

[C] Cf. >4000 (Schousboe, A., Thorbek, P., Heutz, L., and Krogsgaard-Larsen, P., *J. Neurochem.*, 1979, **33**, 181).

[D] Cf. 181±9 (Krogsgaard-Larsen, P., and Johnston, G. A. R., *J. Neurochem.*, 1975, **25**, 797).

[E] Acts as substrate (Beart, P. M., Uhr, M. L., and Johnston, G. A. R., *J. Neurochem.*, 1972, **19**, 1849).

on the carbon attached to the nitrogen. Such compounds have potential to inhibit the GABA-degrading enzyme GABA-transaminase.[1]

Three amino acids were prepared from *trans*-β-hydromuconic acid [(*E*)-hex-3-enedioic acid (7)] as shown in Scheme 1. Schmidt reaction on this diacid with 1 equiv. of sodium azide gave a good yield of the (*E*) amino acid (5). Protection of the amino function was accomplished by treatment with di-t-butyl dicarbonate and subsequent methylation with diazomethane gave the *N*-t-butyloxycarbonyl ester (8). Conjugation of the double bond was readily effected by treatment with diazabicyclo[5.4.0]undec-7-ene (dbu) yielding the (*E*) conjugated protected amino acid (9). The (*E*) amino acid (3) was obtained as its hydrochloride salt on deprotection of (9) with hydrochloric acid.

The (*Z*) isomer (10) could be generated by photolysis of the (*E*) isomer (9) in a quartz vessel with ultraviolet light. This gave a (*Z*)/(*E*) mixture containing up to 35% of the required (*Z*) isomer (10) which could be readily purified by chromatography on silica gel. Deprotection of (10) was effected in two steps (Scheme 1) via the crystalline *N*-protected amino acid (11), yielding the (*Z*) amino acid (2) as its hydrochloride salt.

(*Z*)-5-Aminopent-3-enoic acid (4) and the acetylenic compound (6) were both made from the known diol (12)[6,7] (Scheme 2). The diacid (13) was formed by oxidation with Jones reagent.[6] Treatment under Schmidt conditions with sodium azide and concentrated sulfuric acid gave a very low yield of the desired acetylenic amino acid (6), and variations of conditions did not improve the yield which was low presumably because of competing additions to the triple bond. Partial reduction of the diol (12) in pyridine gave the (*Z*) olefinic diol (14) which was directly oxidized to the diacid (15) and converted into the amino acid (4) under the usual Schmidt conditions.

The activity of the analogues on various GABA assays is shown in Table 1. The transient contraction of the guinea-pig ileum,[8,9] and enhancement of [^{3}H]diazepam binding to rat brain membranes[8,10] were used to screen the compounds for $GABA_A$-receptor agonist activity. Other screening procedures used for GABA mimetic activity were inhibition of the uptake of [^{3}H]GABA into rat brain slices[8,11] and the inhibition of the transamination of [^{14}C]GABA by a rat brain mitochondrial preparation.[12] As $GABA_A$-receptor agonists, only the (*Z*) isomers (2) and (4) were active but even the more active compound (2) (ED_{50} 26 μM), although selective for GABA receptors, was tenfold weaker than GABA (ED_{50} 2·2 μM) at contracting the isolated guinea-pig ileum. This activity was also reflected in the ED_{50} values for the facilitation of [^{3}H]diazepam binding to rat brain membranes[8] (Table 1), and the higher potency of the (*Z*) isomer compared to the (*E*) is in contrast with that observed for (*Z*) and (*E*)-4-aminocrotonic acid.

The greater potency of the (*Z*) isomer (2) compared with the activities of the other amino acids in Table 1 fits the concept that interaction with GABA receptors may

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occur if the amino acid adopts a conformation analogous to that of the isothiouronium salt (1), but obviously there are other factors involved in receptor activation which are responsible for the very high potency of (1).

Experimental

^{1}H n.m.r. spectra were measured at 60 MHz on a Varian EM-360A spectrometer or, where indicated, at 89·6 MHz on a Jeol FX90Q instrument. ^{13}C n.m.r. spectra were obtained at 22·55 MHz on a Jeol FX90Q spectrometer. Values are given in ppm relative to internal tetramethylsilane for $CDCl_3$ solutions, and n.m.r. spectra run in D_2O solution are given relative to external $SiMe_4$. Melting points (uncorrected) were determined on a Reichert hot-stage apparatus. Infrared spectra were recorded on a Perkin–Elmer 177 spectrometer as Nujol mulls. Mass spectra were obtained on a Finigan 2300E instrument under chemical ionization conditions, methane being used as the reagent gas. Microanalyses were carried out by the Australian Microanalytical Service (AMDEL) in Melbourne. R_F values reported are for thin-layer chromatography on Merck Kieselgel precoated aluminium-backed plates in butan-1-ol/acetic acid/water (4 : 1 : 1)

(E)-*5-Aminopent-3-enoic Acid (5)*

trans-β-Hydromuconic acid (7) (1·4 g) was suspended in chloroform (40 ml), and concentrated sulfuric acid (4·0 ml) added. Sodium azide (0·65 g) was added in small amounts over 30 min while the mixture was stirred rapidly. After a further 4 h at 40° the chloroform layer was decanted from the viscous residue which was washed again with chloroform (30 ml). The acid layer was dissolved in water (150 ml), filtered and added to a column of Dowex 50W (H^+) ion-exchange resin (50 ml). The column was washed with water to neutral pH and then the amino acid eluted with aqueous pyridine (300 ml, 1 M). Evaporation of the solvent afforded an oil (600 mg) which slowly solidified. Recrystallization from water/ethanol gave *(E)-5-aminopent-3-enoic acid* (5) (437 mg, 42%), m.p. 165–167°, as white needles (Found: C, 52·0; H, 7·9; N, 12·4. $C_5H_9NO_2$ requires C, 52·2; H, 7·9; N, 12·2%). ν_{max} 3500–2400, 2180, 1625, 1580–1520, 1080, 1040, 980, 960, 720 cm^{-1}. ^{1}H n.m.r. δ (D_2O/DCl; 89·6 MHz) 6·28, 2H, m, $CH_2CH{=}CHCH_2$; 4·03, 2H, d, *J* 5·7 Hz, $H_2NCH_2CH{=}$; 3·64, 2H, d, *J* 6·1 Hz, $=CHCH_2CO_2H$. Mass spectrum *m/z* 116 (100%, M+H), 99 (86), 98 (11), 85 (10), 70 (13). R_F 0·44.

(Z)-*5-Aminopent-3-enoic Acid (4)*

Hex-3-yne-1,6-diol[6] (12) (1·1 g) was dissolved in pyridine (5 ml), and palladium/charcoal (100 mg) added. The mixture was hydrogenated for 14 h, filtered through Celite and the solvent evaporated to give (*Z*)-hex-3-ene-1,6-diol (14) (1·0 g) as an oil which was used without further purification. ^{1}H n.m.r. δ ($CDCl_3$) 5·56, 2H, d, *J* 6 Hz, $CH_2CH{=}$; 4·26, 2H, s, OH; 3·63, 4H, t, *J* 6 Hz, CH_2CH_2OH; 2·32, 4H, q, *J* 6 Hz, $=CHCH_2CH_2$.

This diol (14) (1·0 g) was dissolved in acetone (20 ml), and treated with excess Jones reagent at 0°. Ethanol was added to decompose excess reagent, the mixture was filtered and the filtrate evaporated to dryness below 50° in vacuum. The oily residue slowly solidified to give crude (*Z*)-hex-3-ene-1,6-dioic acid (15) (0·95 g). This material was dissolved in a mixture of carbon tetrachloride (50 ml) and concentrated sulfuric acid (3 ml). Sodium azide (0·5 g) was added, and the reaction mixture stirred vigorously at 40° for 4 h. The carbon tetrachloride was decanted, and the oily acid layer washed with chloroform (20 ml) which was also decanted and discarded. The residue was dissolved in water (30 ml), and added to a column of Dowex 50W (H^+) ion-exchange resin (30 ml). The resin was washed with water to neutral pH then the amino acid was eluted with aqueous pyridine (100 ml, 1 M). Evaporation of the solvent gave an oil which was dissolved in acetic acid (1 ml), and hydrogen bromide in acetic acid (3 drops, 40%) was added. This was evaporated to dryness producing a solid residue which was recrystallized from ethanol/ether giving (*Z*)-*5-aminopent-3-enoic acid* (4) (80 mg) as an amorphous buff-coloured hygroscopic solid (Found: C, 30·9; H, 4·9; N, 6·8. $C_5H_9NO_2$.HBr requires C, 30·6; H, 5·1; N, 7·1%). ν_{max} 3600–2500, 1720br, 1220, 1115, 735 cm^{-1}. ^{1}H n.m.r. δ (D_2O+DCl, 89·6 MHz) 6·28, 2H, m, $CH_2CH{=}CHCH_2$; 4·12, 2H, d, *J* 5·7 Hz, $=CHCH_2NH_3$; 3·72, 2H, d, *J* 7 Hz, $=CHCH_2CO_2H$. Mass spectrum *m/z* 116 (45%, M+H), 98 (89), 99 (100), 83 (26), 81 (60). R_F 0·44.

5-Aminopent-3-ynoic Acid (6)

To a stirred mixture of hex-3-yne-1,6-dioic acid (13) [232 mg, prepared from the diol (12)[6]] in concentrated sulfuric acid (1·0 ml) and chloroform (7 ml) at 10° was added sodium azide (110·9 mg) over a period of 10 min. The reaction mixture was heated to 50° and stirred for another hour. The chloroform was decanted, and ice (2 g) was added to the residue. The mixture was absorbed on Dowex 50W (H^+) ion-exchange resin (25 ml), the resin was washed with water until neutral, and then the amino acid eluted with aqueous pyridine (1 M, 100 ml). Evaporation of the solvent gave a residue (27 mg) which on recrystallization from water/ethanol (<50°) gave *5-aminopent-3-ynoic acid* (6) (5 mg, 2·7%), m.p. 170° (dec). The compound did not give a satisfactory microanalysis due to the presence of water of crystallization (between 1 and 2 molar proportions) which could not be removed without decomposing the sample. ^{1}H n.m.r. δ (D_2O, 89·6 MHz) 4·25, 2H, t, *J* 2·2 Hz, $NHCH_2C{\equiv}$; 3·63, 2H, t, *J* 2·2 Hz, $\equiv CCH_2CO_2$. ^{13}C n.m.r. 29·1, 30·6, 74·4, 84·4, 176·9 ppm. Mass spectrum *m/z* 114 (100%, M+H), 98 (4), 96 (11), 68 (7). R_F 0·37.

(Z)- *and* (E)-*5-Aminopent-2-enoic Acids (2) and (3)*

(*E*)-5-Aminopent-3-enoic acid (5) (7 g) was dissolved in water (15 ml) containing sodium hydroxide (2·5 g). t-Butyl alcohol (20 ml) was added followed by a solution of di-t-butyl dicarbonate (10·5 g) in t-butyl alcohol (20 ml). After stirring at room temperature for 5 min, a further amount of aqueous sodium hydroxide (2·5 g in 15 ml) was added giving a clear solution. After 4 h the mixture was evaporated to dryness below 50° in vacuum, dissolved in water (100 ml) and extracted with ether (30 ml). The aqueous phase was acidified with 6 M hydrochloric acid, and extracted with ether (3×20 ml). The ether layer was washed with brine, dried and evaporated to give an oil which was dissolved in methanol (15 ml), and excess ethereal diazomethane added. Evaporation of the solvents gave methyl (*E*)-5-[(t-butyloxycarbonyl)amino]pent-3-enoate (8) as a mobile oil (6·5 g). ^{1}H n.m.r. δ ($CDCl_3$) 5·60, 2H, m, $CH_2CH{=}CHCH_2$; 4·80, 1H, br m, NH; 3·73, 2H, m, $=CHCH_2NH$; 3·67, 3H, s, OCH_3; 3·13, 2H, m, $=CHCH_2CO_2$; 1·43, 9H, s, $C(CH_3)_3$.

The deconjugated ester (8) (6·5 g) was dissolved in dichloromethane (100 ml), and diazabicyclo[5·4·0]undec-7-ene (2 g) was added. After 3 days at room temperature the organic solvent was washed with 1 M hydrochloric acid (2×50 ml) and then water. Evaporation of the dichloromethane gave methyl (*E*)-5-[(t-butyloxycarbonyl)amino]pent-2-enoate (9) (6·4 g) as a colorless oil. ^{1}H n.m.r. δ ($CDCl_3$) 7·02, 1H, dt, *J* 16, 7 Hz, $CH_2CH{=}CHCO_2$; 5·90, 1H, dt, *J* 16, 2 Hz, $CH_2CH{=}CHCO_2$; 4·73, 1H, br s, NH; 3·77, 3H, s, OCH_3; 3·28, 2H, q, *J* 7 Hz, $NHCH_2CH_2$; 2·40, 2H, q, *J* 7 Hz, $CH_2CH_2CH{=}$; 1·45, 9H, s, $C(CH_3)_3$.

The ester (9) (6 g) in dichloromethane (200 ml) was photolysed in a quartz flask over a low-pressure 250-W mercury lamp at reflux temperature for 16 h [samples were taken at intervals and checked by n.m.r. to determine the (*Z*)/(*E*) ratio which at the end of photolysis was *c.* 1 : 2]. The dichloromethane was evaporated and the light brown residue was chromatographed on silica gel (t.l.c. grade). Elution with 15–20% ether/light petroleum gave pure methyl (*Z*)-5-[(t-butyloxycarbonyl)amino]pent-2-enoate (10) (2·2 g). ^{1}H n.m.r. δ ($CDCl_3$) 6·36, 1H, dt, *J* 11, 7 Hz, $CH{=}CHCH_2$; 5·93, 1H, d, *J* 11 Hz, $CH{=}CHCO_2$; 4·80, 1H, br m, NH; 3·78, 3H, s, OCH_3; 3·33, 2H, q, *J* 6 Hz, CH_2CH_2NH; 2·88, 2H, q, *J* 6 Hz, $=CHCH_2CH_2$; 1·48, 9H, s, $C(CH_3)_3$.

The ester (10) (2 g) was dissolved in t-butyl alcohol (15 ml), and a solution of potassium hydroxide (2 g) in water (15 ml) added. After stirring overnight at room temperature, the homogeneous solution was acidified with 1 M hydrochloric acid, and extracted with dichloromethane. Removal of the organic solvent gave (Z)-*5-[(t-butyloxycarbonyl)amino]pent-2-enoic acid* (11) (1·6 g) which was recrystallized from dichloromethane/light petroleum to give white needles, m.p. 115–117° (Found: C, 55·8; H, 7·9; N, 6·4. $C_{10}H_{17}NO_4$ requires C, 55·8; H, 8·0; N, 6·5%). ν_{max} 3360, 1670, 1640, 1520, 1245, 1170 cm^{-1}. ^{1}H n.m.r. δ ($CDCl_3$) 6·40, 1H, dt, *J* 11, 7 Hz, $CH{=}CHCH_2$; 5·93, 1H, d, *J* 11 Hz, $CH{=}CHCO_2$; 5·12, 1H, br m, NH; 3·30, 2H, q, *J* 7 Hz, $NHCH_2CH_2$; 2·82, 2H, q, *J* 7 Hz, $CH_2CH_2CH{=}$; 1·50, 9H, s, $C(CH_3)_3$. Mass spectrum *m/z* 160 (78%, M+H), 142 (10), 116 (100), 99 (11), 98 (68).

Methyl (*E*)-5-[(t-butyloxycarbonyl)amino]pent-2-enoate (9) (1 g) was suspended in 6 M hydrochloric acid (10 ml) under nitrogen, and heated to 80° for 10 min after which time no more gas evolution occurred. After standing overnight at room temperature, the solution was diluted with water (60 ml), and absorbed on a column of Dowex 50W (H^+) ion-exchange resin (50 ml). The resin was washed to neutral pH, and the amino acid eluted with aqueous pyridine (150 ml, 1 M). Removal of the solvent in vacuum gave an oil which was dissolved in 1 M hydrochloric acid (1 ml), and evaporated again to give a solid which, on recrystallization from ethanol/ethyl acetate, yielded (E)-*5-aminopent-2-enoic acid* (3) as the hydrochloride (300 mg, 45%), m.p. 204–206° (Found: C, 39·4; H, 6·6; N, 9·2. $C_5H_9NO_2.HCl$ requires C, 39·4; H, 6·7; N, 9·5%). ν_{max} 3500–2400, 1710, 1650, 1585, 1225, 1180, 975, 855, 740 cm^{-1}. ^1H n.m.r. δ (D_2O) 7·33, 1H, dt, *J* 16, 7 Hz, CH_2CH=CH; 6·35, 1H, dt, *J* 16, 2 Hz, CH_2CH=CH; 3·53, 2H, t, *J* 7 Hz, $NHCH_2CH_2$; 2·93, 2H, br q, *J* 7 Hz, CH_2CH_2CH=. Mass spectrum *m/z* 116 (100%, M+H), 99 (39), 98 (52), 77 (18). R_F 0·47.

(*Z*)-5-[(t-Butyloxycarbonyl)amino]pent-2-enoic acid (11) (300 mg) was heated in 6 M hydrochloric acid (2 ml) at 80° for 5 min. Evaporation of the solvent gave a solid which was recrystallized from ethanol/ethyl acetate to give (Z)-*5-aminopent-2-enoic acid* (2) as the hydrochloride (100 mg, 48%) as off-white crystals, m.p. 108–110° (Found: C, 39·5; H, 6·8; N, 9·3. $C_5H_9NO_2.HCl$ requires C, 39·6; H, 6·6; N, 9·2%). ν_{max} 3500–2300, 1750–1560, 1200, 1135, 1120, 1015, 820, 725 cm^{-1}. ^1H n.m.r. δ (D_2O) 6·78, 1H, dt, *J* 11·5, 7 Hz, CH_2CH=$CHCO_2$; 6·46, 1H, d, *J* 11·5 Hz, CH=$CHCO_2$; 3·56, 2H, m, CH_2CH_2CH=; 3·40, 2H, t, *J* 7 Hz, NCH_2CH_2. Mass spectrum *m/z* 116 (53%, M+H), 99 (87), 98 (100). R_F 0·48.

Acknowledgments

We are grateful to the National Health and Medical Research Council for funding this project, to the Department of Pharmacy, University of Sydney, for mass spectral measurements, and to the University of Sydney for an equipment grant for the purchase of the Jeol FX90Q n.m.r. spectrometer.

Manuscript received 3 June 1985