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THE SYNTHESES OF 4-AMINOBUTANOIC ACID-2,2-$^2H_2$ AND -4,4-$^2H_2$ AND PROGABIDE-2,2-$^2H_2$ AND -4,4-$^2H_2$

Bruce Davis
Psychiatric Research Division, Saskatchewan Health, Cancer and Medical Research Building, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.
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SUMMARY

4-Aminobutanoic acid-2,2-$^2H_2$ and -4,4-$^2H_2$ were synthesized in high yield with high deuterium incorporation, and then converted into the corresponding deuterium-labelled anti-convulsant drug, progabide, by means of a transimination reaction.

Key Words: Deuterium labelled, GABA, progabide

INTRODUCTION

Progabide, 4-([(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino)-butanamide, is an anticonvulsant drug, the lipophilic character of which permits it to cross the blood brain barrier (1,2). Metabolism of the drug in the brain produces 4-aminobutanoic acid (GABA) which, on its own, crosses the blood brain barrier very poorly. Alterations in GABA function have been implicated in epilepsy, spasticity and movement disorders. In the brain, GABA is metabolized chiefly by transamination, a reaction catalyzed by the enzyme transaminase in which a carbon-hydrogen bond alpha to the amino nitrogen atom is broken. This step, which is probably rate-determining, should therefore show a large deuterium isotope effect. Consequently, for a study of the metabolism of GABA and progabide it was necessary to synthesize GABA-4,4-$^2H_2$ and progabide-4,4-$^2H_2$, as well as GABA-2,2-$^2H_2$ and progabide-2,2-$^2H_2$ to serve as controls in which no isotope effect would be expected to occur. Previously reported syntheses of GABA-4,4-$^2H_3$ (3) and proteo progabide (1) were not successful in our hands; therefore, the new syntheses reported here were devised.

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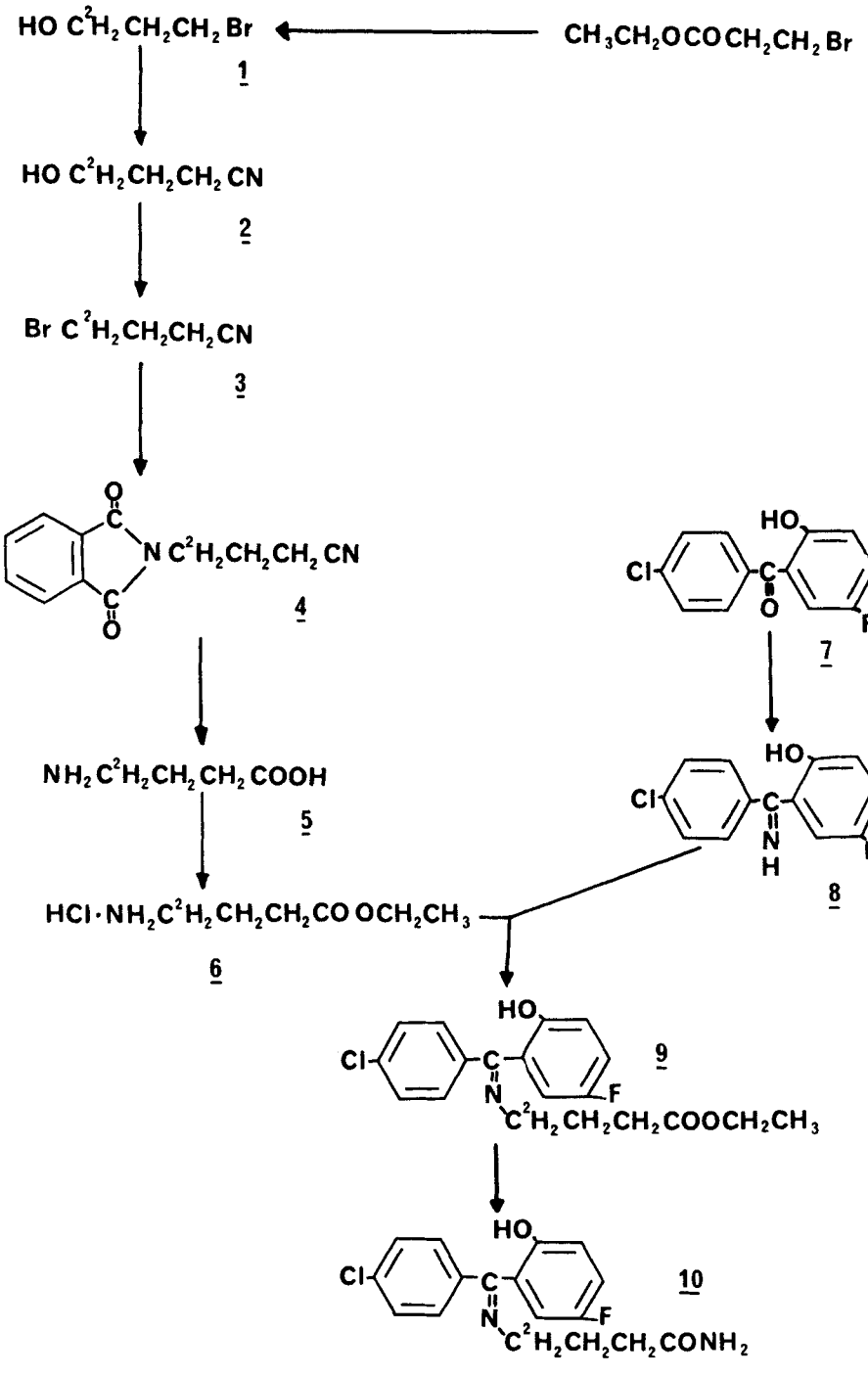
HO C²H₂ CH₂CH₂ Br
1
CH₃CH₂OCOCH₂CH₂ Br
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2
Br C²H₂CH₂CH₂CN
3
O
C
N C²H₂CH₂CH₂ CN
C
O
4
HO
Cl
C
O
F
7
NH₂ C²H₂CH₂ CH₂ COOH
5
HO
Cl
C
N
H
F
8
HCl·NH₂C²H₂CH₂CH₂CO OCH₂CH₃
6
HO
Cl
C
N
F
C²H₂ CH₂CH₂COOCH₂CH₃
9
HO
Cl
C
N
F
C²H₂CH₂CH₂CONH₂
10

RESULTS AND DISCUSSION

GABA-2,2-$^2H_2$ was prepared by a standard acid-catalyzed exchange of proteo-GABA. The synthesis of GABA-4,4-$^2H_2$ was achieved in five steps with a relatively high overall yield of 20% and in high chemical and isotopic purity (Figure). Only the bromination with N-bromosuccinimide and final hydrolysis steps gave poor yields (50%). Bromination with phosphorous tribromide, however, was even less successful (yield of 40%; not described). Losses of GABA-4,4-$^2H_2$ in the final hydrolysis step probably occurred during the filtration of the precipitated byproduct, phthalic acid. An earlier two-step synthesis of GABA-4,4-$^2H_2$ involved the partial reduction of succinimide with lithium aluminum deuteride to pyrrolidinone-5,5-$^2H_2$, followed by hydrolysis, but the yield was only about 5% (3).

The first synthesis of progabide (in the proteo form) was reported by Kaplan et al. (1) in which (4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methanone (7) was condensed with 4-aminobutanamide by evaporating an absolute ethanol solution of the two reactants. However, 4-aminobutanamide is very labile and readily cyclizes or hydrolyzes. After repeated failures in attempting Kaplan's method, the synthesis reported here was developed in which ethyl 4-aminobutanoate-4,4-$^2H_2$ hydrochloride (6) was condensed with the ketimine (8) by means of a transimination reaction (4). The resulting progabide ester was converted to the amide by the aluminum-mediated method of Levin et al. (5).

Experimental

Materials and Instruments

Lithium aluminum deuteride, deuterium chloride and deuterium oxide were purchased from Merck, Sharp and Dohme (Montréal, Canada) and all other reagents were purchased from Aldrich Chemical Co. (Milwaukee, USA; distributed by Terochem, Edmonton, Canada). The identities of the intermediate and final products were determined by means of their mass spectra which were obtained by direct probe injection into the ion source of an AEI MS902S mass spectrometer operated at 70 eV, ion source temperature of 200°C and resolution of 1000.

4-Aminobutanoic Acid-4,4-$^2H_2$ (5)

3-Bromopropanol-1,1-$^2H_2$ (1):

Lithium aluminum deuteride (8.4 g, 0.2 mol) suspended in dry ether (200 mL) was

treated with a solution of aluminum trichloride (26.6 g, 0.2 mol) in dry ether (200 mL) and cooled in a methanol-dry ice bath. To this solution was added slowly ethyl bromopropionate (33.4 g, 0.2 mol). One hour after the addition was completed, the cold mixture was treated cautiously with water (8.4 mL), 10% sodium hydroxide (8.4 mL) and water (25 mL). After stirring for another hour, the solids were filtered and washed well with ether, the combined filtrates were dried over anhydrous sodium sulfate, and the solvent was removed by rotary evaporation to give 26 g (100%) of 3-bromopropanol-1,1-$^2H_2$ (1), a colorless oil which was used without further purification.

3-Cyanopropanol-1,1-$^2H_2$ (2):

3-Bromopropanol-1,1-$^2H_2$ (1) (26 g, 0.185 mol) in acetonitrile (200 mL) and methanol (2 mL) was heated under reflux for 72 h with potassium cyanide (24 g, 0.370 mol) and 18-crown-6 (1.0 g, 3.7 mmol). After allowing to cool, the mixture was filtered with suction and the solvent was removed by rotary evaporation to give 15.8 g (100%) of a colorless oil which was not purified further. The mass spectrum exhibited major ions at m/z 87 (M^+), 55, 45 and 33, which is correct for 3-cyanopropanol-1,1-$^2H_2$.

2-Cyanopropyl bromide-1,1-$^2H_2$ (3):

To 3-cyanopropanol-1,1-$^2H_2$ (2) (15.8 g, 0.185 mol) was added triphenylphosphine (52 g, 0.198 mol) and then, in small portions through an efficient condenser, N-bromosuccinimide (29.5 g, 0.166 mol). The strongly exothermic reaction soon converted the solid reaction mixture to a dark liquid. Immediately after completion of the addition, the product was isolated as a colorless liquid (12.0 g, 50%) from the black product mixture by vacuum distillation, and its identity was confirmed by mass spectrometry.

3-Phthalimido-n-propyl cyanide-3,3-$^2H_2$ (4):

3-Cyanopropyl bromide-1,1-$^2H_2$ (3) (12 g, 0.080 mol) in acetonitrile (100 mL) and methanol (1 mL) was heated under reflux for 48 hours with potassium phthalimide (22.2 g, 0.12 mol) and 18-crown-6 (1.0 g). The product was isolated by filtration and rotary evaporation of the solvent to give 17.4 g (100%) of an off-white solid, mp. 64-66°C.

4-Aminobutanoic acid-4,4-$^2H_2$ (5):

3-Phthalimido-n-propyl cyanide-3,3-$^2H_2$ (4) (17.4 g, 0.080 mol) was hydrolyzed by boiling under reflux with concentrated hydrochloric acid (100 mL) for 4 hours. On cooling, phthalic acid precipitated and was filtered with suction and washed with a little water. The filtrate was concentrated by rotary evaporation to about 25 mL and

filtered again. The final filtrate was rotary evaporated to dryness, the residue was dissolved in 1N hydrochloric acid (30 mL), and loaded onto a column of DOWEX AG 50x2 (35 g) which had been prepared by washing successively with 1N hydrochloric acid, water, absolute ethanol, 4N ammonium hydroxide, water and 1N hydrochloric acid. Elution with water gave phthalic acid and ammonium chloride, then 4-aminobutanoic acid-4,4-$^2H_2$ was eluted with 2N ammonium hydroxide. The crude product (3.4 g) was recrystallized from water (5 mL) and absolute ethanol (200 mL) to give 2.9 g (34%) of white crystals, mp 219-220°C. The mass spectrum of the dansyl derivative (N-dansyl-2-pyrrolidone-5,5-$^2H_2$) showed 98% of the $^2H_2$ species (7) and 2% of the $^3H_1$ species.

4-Aminobutanoic acid-2,2-$^2H_2$

4-Aminobutanoic acid (6.0 g, 0.058 mol) was dissolved in 9% ^{2}HCl in ^2H_2O and divided equally among eight acylation tubes which were then sealed and heated at 120°C for 84 hours. The combined solutions were then rotary evaporated. Three more such exchanges were carried out to give 8.1 g (100%) of 4-aminobutanoic acid-2,2-$^2H_2$ hydrochloride, containing 93% of the $^2H_2$ species (7.0% of $^2H_1$) as shown by the mass spectrum of the dansyl derivative.

Ethyl 4-Aminobutanoate-4,4-$^2H_2$ Hydrochloride (6):

Ethanolic HCl was prepared by adding acetyl chloride (5 mL) to absolute ethanol (120 mL) (for the 2,2-$^2H_2$ ester, ethanol-O^{2}H was used). After 15 min, 4-aminobutanoic acid-4,4-$^2H^2$ was added and the mixture was boiled under reflux for 24 hours. The solvent was removed by rotary evaporation to give 9.67 g (99%) of ethyl 4-aminobutanoate-4,4-$^2H_2$ hydrochloride.

Progabide-2,2-$^2H_2$ and -4,4-$^2H_2$ (10):

(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methyleneimine (8):

(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methanone (7) (16 g) [prepared from 4-chlorobenzoyl chloride and 4-fluorophenol by the method of Kaplan et al. (1)] was dissolved in methanol (250 mL) and cooled in a dry-ice methanol bath to -70°C. Liquid ammonia (75 mL) was added and the stirred solution was allowed to warm to 20°C, then left lightly stoppered for 24 hours. The bright orange crystals were filtered, the filtrate was concentrated to 50 mL and filtered again to give 8 in a total yield of 15 g (94%), mp 163-164°C. Mass spectrum: m/z 249/251 (M^+), 248/250 (M^+-1), 214 (M^+-Cl), 138.

Ethyl 4-([(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene] amino) butanoate-4,4-$^2H_2$ (9):

The ketimine (8) (12.87 g, 0.052 moles) and ethyl 4-aminobutanoate hydrochloride-4,4-$^2H_2$ (or -2,2-$^2H_2$) (9.08 g, 0.053 moles) were dissolved in dichloromethane (250 mL) and stirred at 20°C for 24 hours. The precipitated ammonium chloride was filtered, the solvent was removed by rotary evaporation, and the residue was taken up in ether. The ethereal solution was washed once with water, dried and rotary evaporated to give a viscous orange-red oil (17.8 g, 94%) which very slowly crystallized, m.p. 49-51°C. Mass spectrum for the 4,4-$^2H_2$ ester: m/z 365/367 (M^+), 278 (M^+-$CH_2COOC_2H_5$), 264 (M^+-$CH_2CH_2COOC_2H_5$). For the 2,2-$^2H_2$ ester: m/z 365/367 (M^+), 276 (M^+-$C^2H_2COOC_2H_5$), 262 (M^+-$CH_2C^2H_2COOC_2H_5$).

4-([(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene] amino)butanamide-4,4-$^2H_2$ (10):

To a stirred suspension of ammonium chloride (9.86 g, 0.186 mol) in anhydrous benzene (180 mL), cooled in an ice-water bath with a slow stream of nitrogen passing through the reaction vessel, was added a 2N solution of trimethylaluminum in toluene (93 mL, 0.186 mol). After completion of the addition, the solution was allowed to warm to 20°C and was stirred for 2 hours or until all the ammonium chloride had dissolved. A benzene solution (180 mL) of the ester (9) (17.8 g, 0.146 mol) was added and the reaction solution was stirred at 45°C for 18 hours, at which time thin-layer chromatography showed no starting ester present. The reaction solution was cooled in ice-water and 2.5% hydrochloric acid was very slowly added with gentle stirring. Since prolonged contact of the product with acid results in hydrolysis of both the imine and amide bonds, the organic layer was immediately separated, the aqueous layer was extracted once with ethyl acetate and the combined organic solutions were dried, filtered and the solvent was removed at 20°C. Removal of the solvent at elevated temperatures results in nearly complete conversion of the amide to the nitrile. A yellow hard cake remained (14.85 g, 90%) and was recrystallized from ethyl acetate-petroleum ether (b.p. 60-110°C) to give 9.5 g of bright yellow crystals (m.p. 131-133°C). The mass spectra of the products exhibited major ions at m/z 336 (M^+, 100%), 278 (M-CH_2CONH_2, 80%) and 264 (M-$CH_2CH_2CONH_2$, 60%) for progabide-4,4-$^2H_2$ and at m/z 336, 276, and 262 for progabide-2,2-$^2H_2$. No undeuterated species was detected.

Conclusions

4-Aminobutanoic acid-4,4-$^2H_2$ and -2,2-$^2H_2$ have been prepared in good yields and exhibiting high chemical and isotopic purity. The esters of the acids were condensed with a lipophilic carrier in a transimination reaction and the resulting product was converted to deuterated progabide in a procedure which does not require the labile 4-aminobutanamide as intermediate.

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