PREPARATION OF DEUTERIUM LABELLED α-AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONIC ACID (AMPA)

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SUMMARY

The preparation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) with deuterium α to the carboxylic acid group (2) and in the exocyclic methyl group (5), respectively, are described. The deuterium labelled AMPA (2) was synthesized by hydrolysis and decarboxylation of the corresponding di-ester (1), whereas the deuterium labelled AMPA (5) was prepared *via* a catalytic deuteration of the bromoderivative (3).

Key Words: AMPA, Glutamic Acid Agonist, Deuterium

INTRODUCTION

L-Glutamic acid (GLU) is a putative excitatory transmitter in the mammalian central nervous system based on *in vivo* experiments.[1-4] The understanding of the mechanism involved in receptor mediated excitations produced by GLU, will be facilitated by the identification and characterization of the physiological GLU-receptor using ligand binding techniques. Several attempts have been made to identify the GLU-receptor using [^{3}H]GLU and [^{3}H]kainic acid (KAIN), a structural analogue of GLU (Fig. 1), as ligands.[5-18] However, the characteristics of the binding sites so

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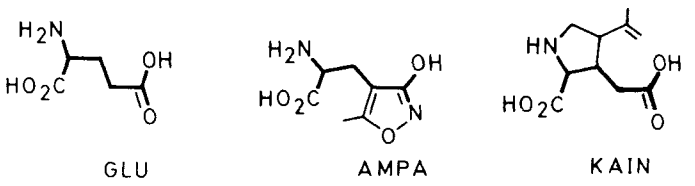


Figure 1. The structures of GLU, AMPA, and KAIN

far detected are inconsistent with the characteristics of a physiological GLU receptor.

The title compound α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, Fig. 1) has proved to be a very potent and selective GLU-agonist in *in vivo* experiments.[19] However, when tested as an inhibitor of [^{3}H]GLU and [^{3}H]KAIN binding AMPA was virtually inactive.[18,19] Consequently, tritium labelled AMPA is an important tool in the search for the physiological GLU-receptor. The present paper describes the synthesis of deuterium labelled AMPA, which can also be applied for the synthesis of tritiated AMPA.[20]

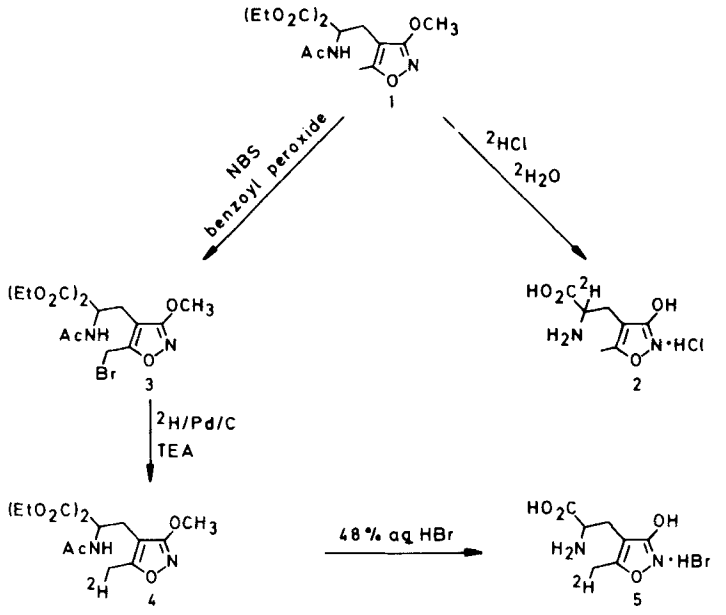
RESULTS AND DISCUSSION

AMPA was deuterium labelled i) in the position α to the carboxylic acid group and ii) in the exocyclic methyl group.

The syntheses are outlined in scheme 1.

Treatment of 1 with 6 M ^{2}HCl in ^2H_2O gave, after removal of labile deuterium with water, labelled AMPA hydrochloride (2) with deuterium in the α-position to the carboxylic acid group.

Treatment of 1 with N-bromosuccinimide (NBS) under free radical conditions was shown by ^{1}H NMR spectroscopy to give 3 as the major product in contrast to the findings that NBS bromination of 4,5-dimethyl-3-methoxyisoxazole resulted in bromination in the 4-me-



Scheme 1.

thyl group.[21] Catalytic low pressure deuteration of 3 using triethylamine (TEA) as a base afforded the deuterated compound 4. Deprotection of 4 using 48 % aqueous HBr gave labelled AMPA (5) with deuterium in the exocyclic methyl group.

In order to investigate the stability of deuterium in 2 the compound was dissolved in water and kept at ambient temperature for 36 h. The ^{1}H NMR spectrum of the evaporated solution showed a minor content of non-labelled AMPA, which was not quantified. The instability of 2 was confirmed by experiments with the tritium labelled compound.[20] In these experiments 27 per cent of tritium were labilized on storage of the tritium labelled compound in aqueous solution for 2 days at 3 ^{o}C.

The deuterium in compound 5 was considered to be permanent, as the deuterium in compound 4 was stable in refluxing 48 % aqueous HBr.

EXPERIMENTAL

Melting points, determined in capillary tubes, are corrected. Analyses indicated by elemental symbols were within ±0.4 % of the theoretical values and were performed by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen.

A JEOL JMN-C-60HL (60 MHz) ^{1}H NMR instrument was used. ^{1}H NMR spectra were recorded using TMS as an internal standard, except for the compounds dissolved in D_2O where DSS was used. TLC and column chromatography were accomplished by using silica gel F_{254} plates (Merck) and silica gel, 0.063-0.100 (Woelm), respectively.

[α-^{2}H]α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid, hydrochloride (2). A solution of 1 [21] (170 mg; 0.05 mmol) in 6 M ^{2}HCl in ^2H_2O (10 ml) was refluxed for 2 h. Evaporation followed by evaporation twice from H_2O and recrystallization (glacial acetic acid:water) gave 2 (80 mg; 72 %). TLC [butanol-glacial acetic acid-water (4:1:1)]: comparison with an authentic sample of AMPA [21] corresponded. ^{1}H NMR (D_2O): δ 4.75 (*ca.* 5 H, s), 3.00 (2 H, s), 2.30 (3 H, s).

Ethyl α-ethoxycarbonyl-α-acetylamino-3-methoxy-5-bromomethylisoxazole-4-propionate (3). To a solution of 1 [21] (2.05 g; 6 mmol) in CCl_4 (12 ml) was added NBS (1.07 g; 6 mmol) and benzoyl peroxide (10 mg). The reaction mixture was refluxed for 30 min. Filtration and evaporation followed by column chromatography [methylene chloride - 2-butanone (95:5)] and recrystallization (ethyl acetate-light petroleum) gave 3 (0.8 g; 32 %). M.p. 147.0-147.5 °C. Anal. $C_{15}H_{21}BrN_2O_7$: C, H, Br, N. IR (KBr): 3360 (m), 2980 (m), 1730 (s), 1660 (s), 1530 (s), 1500 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.7 (1 H, broad s), 4.5-4.0 (6 H, m containing a s), 3.95 (3 H, s), 3.45 (2 H, s), 2.05 (3 H, s), 1.30 (6 H, t, *J* 7 Hz).

Ethyl α-ethoxycarbonyl-α-acetylamino-3-methoxy-5-[^{2}H]methylisoxazole-4-propionate (4). To a solution of 3 (500 mg; 1.2 mmol)

in dioxane (10 ml) was added TEA (165 µl; 1.2 mmol). The solution was deuterated at low pressure using Pd/C (10 %, 50 mg) for 1½ h. After filtration and evaporation the crude product was dissolved in methylene chloride (5 ml) and washed with water (2 × 5 ml). The organic phase was dried (Na_2SO_4), evaporated and submitted to column chromatography [toluene-ethyl acetate (1:1)]. Yield of 4: 210 mg (52 %). TLC [toluene-ethyl acetate (1:1)]: comparison with 1 corresponded. ^{1}H NMR ($CDCl_3$): δ 6.7 (1 H, broad s), 4.5-4.0 (4 H, m), 3.90 (3 H, s), 3.35 (2 H, s), 2,2-2.1 (2 H, m), 2.00 (3 H, s), 1.25 (6 H, t, *J* 7.0 Hz).

α-Amino-3-hydroxy-[^{2}H]methylisoxazole-4-propionic acid (5).

A solution of 4 (130 mg; 0.4 mmol) in 48 % aqueous HBr (13 ml) was refluxed at 140 °C for 15 min. After evaporation and recrystallization (glacial acetic acid:ether) 5 (90 mg; 88 %) was obtained as s hydrobromide. TLC [butanol-glacial acetic acid-water (4:1:1)]: comparison with an authentic sample of AMPA [21] corresponded. ^{1}H NMR (D_2O): δ 4.75 (*ca.* 5 H, s), 4.30 (1 H, t, *J* 6.0 Hz), 3.00 (2 H, d, *J* 6.0 Hz), 2.4-2.2 (2 H, m).

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