THE SYNTHESIS OF [4-^{3}H]OXIRACETAM - A NOVEL NOOTROPIC AGENT

A M Crowe*, K W M Lawrie*, D Saunders*
and U Pfeiffer+

*SmithKline Beecham Pharmaceuticals
The Frythe, Welwyn, Hertfordshire AL6 9AR, England

+ISF, Via Leonardo Da Vinci, Trezzano, Milan, Italy

SUMMARY

The synthesis of [4-^{3}H]oxiracetam (6) from ethyl 2-(4-chloro-3-oxo-butyramido)acetate (1) is described. Ethyl 2-(4-chloro-3-oxo-butyramido)-acetate (1) was reduced with sodium borotritide at Amersham International plc (TR5 tritiation service) to give ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxy-butyramido)acetate (2). This was elaborated, in four steps, to [4-^{3}H] oxiracetam (6) in 4.8% overall radiochemical yield, furnishing 5735 μCi, 212 MBq, 41.8 μg, of specific activity 21.7 Ci/mmol, 802.9 GBq/mmol, and radiochemical purity of greater than 98%.

Key words: [4-^{3}H]Oxiracetam, nootropic agent, sodium borotritide, [^{3}H]pyrrolidin-2-one, h.p.l.c.

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INTRODUCTION

Cognitive deterioration in the elderly is a major medical and sociological problem. Nootropic agents, a class of psychotropic drugs with direct and selective activity on higher integrative mechanism of the brain, are of increasing interest for the treatment of impaired cognitive functions.[1] Oxiracetam, developed and introduced in therapy by ISF, Italy, a subsidiary of SmithKline Beecham Pharmaceuticals, is an effective example of this class of compounds.[2]

DISCUSSION

We required about 5 mCi, 185 MBq, of [4-^{3}H]oxiracetam (6) with a specific activity of approximately 20 Ci/mmol, 740 GBq/mmol, which represents about 40 µg of material. A number of syntheses of non-labelled oxiracetam have appeared in the literature.[3,4,5,6] We were required to introduce the tritium label efficiently, and into a stable position of the molecule. Accordingly we utilized a modification of the route described in reference 3 for the radiolabelled synthesis. The synthesis of [4-^{3}H]oxiracetam is illustrated in scheme 1. In initial experiments with non-labelled, and low specific activity material (26 mCi/mmol, 962 MBq/mmol) oxiracetam could be prepared reproducibly in 20% yield from ethyl 2-(4-chloro-3-hydroxybutyramido) acetate. Modifications to work up and purification procedures required for the microgram scale synthesis were developed during these experiments.

The radiolabel was introduced by reduction of ethyl 2-(4-chloro-3-oxo-butyramido)-acetate[7] (1) with sodium borotritide (5 Ci), in ethanol, at -30°C, by Amersham International plc (TR5 service). A portion of the crude ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxybutyramido)acetate (2) was purified by column chromatography before use. The hydroxyl function of ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxy butyramido)acetate (2) was readily protected as its THP ether, by treatment with

dihydropyran in dichloromethane in the presence of pyridinium p-toluene sulphonate[3,8] (PPTS). Although the crude THP ether (3) could be successfully purified by column chromatography (silica, CH_2Cl_2) on a milligram scale, however on a microgram scale loss of the THP protecting group was experienced presumably due to the acidic nature of the silica.

Consequently the PPTS was washed out of the reaction mixture with water, and the ethyl 2-[4-chloro-[3-^{3}H]-3-(tetrahydropyran-2-yloxy)butyramido]acetate (3) reacted without further purification.

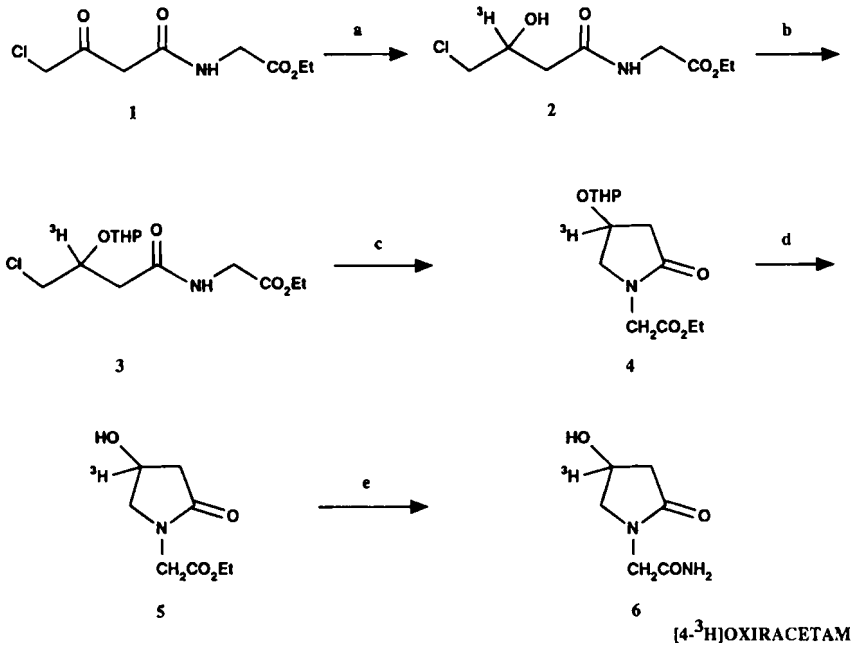
Cyclisation to the pyrrolidinone (4) was carried out by adding the THP protected material to sodium hydride in dry acetonitrile at 0°C. Subsequent warming to room temperature gave ethyl 2-oxo-[4-^{3}H]-4-(tetrahydropyran-2-yloxy)-1-pyrrolidineacetate (4). This material was found to be very unstable in basic conditions. On a microgram scale when dilute acid was added to destroy excess sodium hydride, complete decomposition of the product (4) was observed. This is believed to be due to the generation of localised highly basic conditions. However, by simply reversing the addition, i.e. adding the reaction mixture to the acid, this problem was readily overcome.

The remaining two steps in the synthesis were readily achieved. The THP protecting group was removed from the cyclised intermediate by treatment with PPTS in ethanol at 50°C, and the crude ethyl [4-^{3}H]-4-hydroxy-2-oxo-1-pyrrolidine acetate (5) purified by column chromatography. Ammonolysis of the ester functionality was carried out by treatment with saturated methanolic ammonia to give crude [^{3}H]oxiracetam.

The crude [^{3}H]oxiracetam (6) was purified by hplc on Spherisorb 10 μ amino stationary phase eluted with acetonitrile:water, 95:5 v/v, furnishing 5.735 mCi, 212.2 MBq, of radiochemical purity greater than 98% as assessed by tlc and hplc. The specific activity was determined by a u.v./hplc peak height assay

SCHEME 1

THE SYNTHESIS OF [4-^{3}H]OXIRACETAM



a) $NaBT_4$ b) dihydropyran c) NaH d) PPTS e) $MeOH/NH_3$

relative to non-labelled standard oxiracetam to be 21.7 Ci/mmol, 802.9 GBq/mmol. The [4-^{3}H]oxiracetam (6) was stored at 4°C in the hplc mobile phase, acetonitrile:water, 95:5 v/v.

EXPERIMENTAL

Ethyl 2-(4-chloro[3-^{3}H]-3-hydroxybutyramido)acetate (2) (4.7 Ci, 174 GBq) was obtained by reduction of ethyl 2-(4-chloro-3-oxo-butyramido) acetate (1) with sodium borotritide at Amersham International using their TR5 tritiation service. A portion of this material (500 mCi, 18.54 GBq, 40% radiochemically pure) was purified by column chromatography (silica, dichloromethane:methanol, 50:1 v/v followed by 20:1 v/v) to give ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxy butyramido)acetate (2) (150 mCi, 5550 MBq, 80% radiochemically pure). The low specific activity sodium borotritide reduction is detailed below.

Hplc purification of [4-^{3}H]oxiracetam (6) was carried out using a Gilson preparative system with a Spherisorb 10 μ amino column (4.5mm x 20cm) eluted with acetonitrile:water, 95:5 v/v at 1 ml/minute, UV detection at 220 nm. Radiochemical purities were determined by hplc and tlc. Tlc purities were determined using a Berthold LB2832 Automatic Linear Analyser in the following systems: Merck HPTLC silica 5642 developed in i) chloroform:methanol (12:8 v/v); ii) chloroform:methanol: glacial acetic acid (10:1:1 by volume); and iii) ethyl acetate:methanol: concentrated ammonium hydroxide (5:2:1 by volume). Analytical hplc was carried out using a Beckman 344 system, equipped with a Beckman 171 radioactivity detector and Nelson PC Integrator, software version 4.01. The hplc conditions were the same as above. The specific activity was calculated from a uv/hplc peak height assay relative to non-labelled oxiracetam as external standard, carried out using the analytical hplc system above. Liquid scintillation counting was performed using a Beckman LS6800 counter. The identity of all radiolabelled compounds was confirmed by tlc comparison to authentic samples.

Ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxybutyramido)acetate (2)

Sodium borotritide (6.7 mg, 17.97 mCi, 664.9 MBq, 0.176 mmoles) was cooled in dry ethanol (2 ml) to -30°C, and ethyl 2-(4-chloro-3-oxo-butyramido)acetate (1) (117 mg, 0.529 mmoles) added, to the stirred mixture. After an hour the reaction was acidified with acetic acid, added to water (5 ml), and extracted with ethyl acetate (3 x 10 ml). The organic layers were dried over magnesium sulphate, filtered and evaporated to give ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxy-butyramido)acetate (2) (11.4 mCi, 421.8 MBq, 63% radiochemical yield).

Ethyl 2-[4-chloro-[3-^{3}H]-3-(tetrahydropyran-2-yloxy)butyramido]acetate (3)

Ethyl 2-(4-chloro-[3-^{3}H]-3-hydroxybutyramido)acetate (2) (150 mCi, 5550 MBq, nominally 0.0055 mmol, 80% radiochemically pure) was dissolved in dichloro-methane (0.5 ml) containing dihydropyran (5 μl, 0.055 mmol) and a few crystals

of pyridinium p-toluenesulphonate. The resulting solution was stirred at room temperature. After 20 hours a further portion of dihydropyran (5 µl, 0.055 mmoles) and pyridinium p-toluenesulphonate (a few crystals) were added. After 24 hours the reaction mixture was diluted with diethyl ether, washed with brine, and water. The ether layer was dried over magnesium sulphate, filtered and evaporated to give the required ethyl 2-[4-chloro-[3-^{3}H]-3-(tetrahydropyran-2-yloxy)butyramido]acetate (3) (143 mCi, 5291 MBq, 95% radiochemical yield, 80% radiochemically pure as assessed by tlc (silica, ethyl acetate)).

Ethyl 2-oxo-[4-^{3}H]-4-(tetrahydropyran-2-yloxy)-1-pyrrolidineacetate (4)

Ethyl 2-[4-chloro-[3-^{3}H]-3-(tetrahydropyran-2-yloxy)butyramido]acetate (143 mCi 5291 MBq, nominally 0.0052 mmol, 80% radiochemically pure) was dissolved in dry acetonitrile (1 ml) and added to a suspension of NaH (1.7 mg, 50% in oil, 0.035 mmoles) in dry acetonitrile (0.5 ml) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for two hours. The reaction mixture was added to acetic acid (10 µl) in water (1 ml) and diethyl ether (2 ml) with cooling in an ice bath. The ether layer was separated, washed with water and dried over magnesium sulphate, filtered and evaporated to give the desired ethyl 2-oxo-[4-^{3}H]-4-(tetrahydropyran-2-yloxy)-1-pyrrolidineacetate (4) (106 mCi, 3922 MBq, 65% radiochemical yield, 70% radiochemically pure as assessed by tlc (silica, ethyl acetate)).

Ethyl [4-^{3}H]-4-hydroxy-2-oxo-1-pyrrolidineacetate (5)

Ethyl 2-oxo-[4-^{3}H]-4-(tetrahydropyran-2-yloxy)-1-pyrrolidineacetate (106 mCi, 3922 MBq, nominally 0.003 mmoles, 70% radiochemically pure) was dissolved in ethanol (2 ml), and a few crystals of pyridinium p-toluenesulphonate added. The reaction was stirred at 50°C, and further portions of pyridinium p-toluenesulphate were added after two hours, twenty hours and twenty four hours. After forty-four hours the reaction mixture was allowed to cool, evaporated and purified by column chromatography (silica, ethyl acetate), to

give the desired ethyl [4-^{3}H]-4-hydroxy-2-oxo-1-pyrrolidineacetate (5) (21 mCi, 777 MBq, 25% radiochemical yield; 88% radiochemically pure, as assessed by t.l.c (silica, ethyl acetate)).

[4-^{3}H]-4-Hydroxy-2-oxo-1-pyrrolidineacetamide, [4-^{3}H]oxiracetam (6)

Ethyl [4-^{3}H]-4-hydroxy-2-oxo-1-pyrrolidineacetate (5) (21 mCi, 777 MBq, nominally 0.0008 mmoles, 88% radiochemically pure) was dissolved in methanolic ammonia (1 ml) and stirred at room temperature. After four hours the reaction mixture was evaporated to give [4-^{3}H]-4-hydroxy-2-oxo-1-pyrrolidineacetamide (6) (14.5 mCi, 536.5 MBq, 67% radiochemical yield, 86% radiochemically pure, as assessed by t.l.c. (silica, chloroform:methanol 3:2 v/v)). This material was then purified by hplc (see above) to give [4-^{3}H]oxiracetam (6) (5735 µCi, 212.2 MBq, 21.7 Ci/mmol, 4.8% overall radiochemical yield) with a radiochemical purity by hplc of 98.0% and by tlc in system, i) 98.4%, system ii) 98.9% and system iii) >99%.

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