Synthesis of [^{14}C]ABT-418, a Cholinergic Channel Activator Labeled at Two Sites on the Isoxazole Ring

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Summary

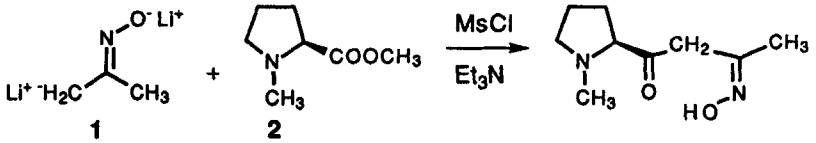
[^{14}C]ABT-418, (S)-3-[^{14}C]methyl-5-[N-methyl-2-pyrrolidinyl][4-^{14}C]isoxazole hydrochloride, was labeled in two positions at maximum specific activity. Starting with 100 mCi of sodium [2-^{14}C]acetate, 14.6 mCi at 105 mCi/mmol was obtained in 8 steps including the formation of [1,3-^{14}C]acetone in the pyrolysis of barium [2-^{14}C]acetate. The key step was the formation of the dianion of [1,3-^{14}C]acetone oxime and its condensation with L-proline methyl ester.

Key Words: ABT-418, cholinergic, Alzheimer's, pyrolysis, labeled, isoxazole

Introduction

The novel cholinergic channel activator, (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418), has been shown to be a very potent and selective ligand for the neuronal nicotinic acetylcholine receptor.[1] The easiest way to label this compound with high enough specific activity for ADME studies is *N*-[^{3}H]methylation of the pyrrolidine ring in one step. This has provided a wealth of information about the metabolism of ABT-418[2] but the issue of N-demethylation was resolved only with carbon-14 labeled material[3]. In order to achieve a suitably high specific activity for ADME studies using carbon-14, at least two carbon-14 atoms per molecule were required. A solution to this problem arose when an efficient synthesis of ABT-418 was developed by our Neuroscience Research Department.[4] In the key step of this synthesis (Scheme 1), a large excess of acetone oxime dianion (**1**)

Scheme 1



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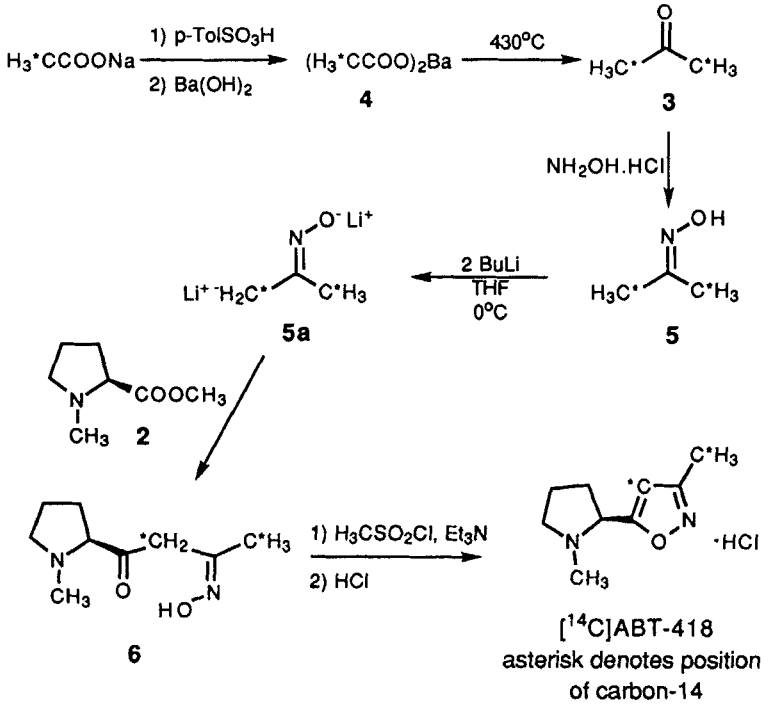
is condensed with *N*-methyl-L-proline methyl ester (**2**). This development pointed to [1,3-^{14}C]-acetone (**3**)[5] as the ideal precursor in a labeled synthesis provided its oxime dianion could be prepared on a semimicro scale and react on a more equivalent basis with **2**.

Results

The labeled acetone (**3**) was prepared pyrolytically in a classical labeled synthesis from barium [2-^{14}C]acetate (**4**), itself being efficiently obtained from the commercially available sodium salt. [1,3-^{14}C]Acetone (**3**) was converted to its oxime (**5**) by simply vacuum transferring the freshly prepared **3** into a solution of hydroxyl amine and allowing them to react at room temperature. Attempts to convert crude **5** to the dianion (**5a**) with butyl lithium in tracer reactions (not described) were unsuccessful and it was suspected that **5** had to be purified. Normally, one would consider recrystallization to accomplish this but for such a small amount of labeled material this approach was deemed too inefficient. We found that the labeled dianion (**5a**) formed nicely when **5** was sublimed prior to reaction. The dianion (**5a**) reacted with **2** reasonably well on a 1:1 mole ratio and the radiochemical yield did not change appreciably using a 2:1 ratio in tracer experiments (not described). This reaction provided the labeled ketooxime (**6**) which, without purification, was cyclized using mesyl chloride and triethylamine to yield [^{14}C]ABT-418. After purification of the final product by HPLC, the hydrochloride salt was made (Scheme 2).

Scheme 2



Experimental

Sodium [2-^{14}C]acetate was purchased from Amersham. N-Methyl-L-proline methyl ester (**2**) was supplied by our Neuroscience Research Department. Other chemicals were of reagent grade or better and purchased from common commercial suppliers. The pyrolysis was conducted using a Lindberg/Blue M tube furnace equipped with a programmable temperature controller (model 55035) and Aldrich Flash-Vacuum Thermolysis equipment. The product trap was modified to attach to a standard vacuum line equipped with a thermocouple pressure gauge used to monitor the reaction. Liquid scintillation counting was performed on a LKB-Wallac 1214 Rack Beta "Excel" counter. TLC plates were coated with Kieselgel 60 (0.25 mm, Merck) and were scanned for radioactivity with a Radiomatic RS chromatograph. For analytical HPLC, a Hitachi L-7000 Series HPLC system consisting of an auto-sampler, a pump, and a variable wavelength UV detector was used with a Flo-One/Beta Model A-500 liquid scintillation radioactivity flow detector (Packard Instruments, 0.5 mL flow cell, 3:1 ratio of Packard Ultima-Flo M scintillant to effluent). A Waters Delta-Prep 3000 system was used for preparative HPLC. Mass spectra were obtained by chemical ionization (NH_3) on a Finnigan MAT SSQ-700 mass spectrometer.

Barium [2-^{14}C]Acetate (4)

Sodium [2-^{14}C]acetate (100 mCi, 2 mmol) was dissolved in water (10 mL) with p-toluenesulfonic acid monohydrate (0.5 g, 3 mmol). The resulting aqueous [2-^{14}C]acetic acid was distilled and chased with two 4 mL portions of water. This solution was titrated to a phenolphthalein end-point with barium hydroxide (0.1 M) and evaporated to dryness, yielding **4** as a pinkish white solid (255 mg, 1.0 mmol, ~100 mCi, quantitative yield).

[1,3-^{14}C]Acetone (3)

Barium [2-^{14}C]acetate (**4**, 255 mg, 1.0 mmol, ~100 mCi) was placed in a platinum boat inside a quartz tube and heated slowly under vacuum to 430°C and held there for 10 min. The product (**3**) was collected in a liquid nitrogen-cooled trap and the entire apparatus was connected to a standard vacuum line. Progress of the reaction was monitored with the vacuum line pressure gauge which showed a peak in pressure during the reaction and a return to baseline afterwards. An assay of the radioactivity after the next reaction showed 64 mCi (64% yield).

[1,3-^{14}C]Acetone Oxime (5)

[1,3-^{14}C]Acetone (**3**, 64 mCi, 0.64 mmol) was vacuum transferred from the pyrolysis trap to a liquid nitrogen-cooled aqueous solution (2 mL) containing hydroxylamine hydrochloride (151 mg, 2.19 mmol). The stopcock to the vessel was shut and the mixture was allowed to warm to room temperature and was stirred for 20 h. The contents were again frozen into the bottom of the vessel, the vacuum was released, the mixture was warmed to room temperature, the pH was adjusted to 7-8 with mostly solid sodium bicarbonate (172 mg, 2 mmol) followed by a few drops of sat. sodium

bicarbonate, and the mixture was stirred for another 20 h. Ether extraction (4 times) removed all but 1 mCi from the aqueous phase. The organic phase was dried over magnesium sulfate, decanted, and concentrated using a 6 in. Vigreaux distillation column at 1 atm. The residue was vacuum sublimed using a dry ice-cooled condenser to afford **5** as colorless needles (55 mCi, 0.55 mmol, 86% yield).

[2,4-^{14}C]1-[(2S)-1-Methyl-2-pyrrolidinyl]-1,3-butanedione 3-Oxime (6)

[1,3-^{14}C]Acetone oxime (**3**, 55 mCi, 0.55 mmol) was dissolved in THF (2 mL) and cooled to 0°C under a nitrogen atmosphere. A solution of butyl lithium in hexane (2.6 M, 0.50 mL, 1.3 mmol) was added dropwise causing cloudiness at first but giving a clear solution as the addition progressed. This solution was stirred for 1.5 h at 0°C and then a solution of freshly vacuum distilled N-methyl-L-proline methyl ester (**2**, 79 mg, 0.55 mmol) in THF (0.2 mL) was added dropwise followed by a THF rinse (0.3 mL). After 6.5 h, the mixture was added with stirring to ice cold 10% hydrochloric acid (3 mL). Extraction with ether, basification with solid sodium bicarbonate and sodium carbonate to pH 8-9, and extraction with methylene chloride followed. The methylene chloride extract containing 35 mCi was dried over magnesium sulfate, decanted, and concentrated at 1 atm. The residue, 83% of which was **6** by radio-TLC (95:4.5:0.5, CH_2Cl_2 : MeOH : NH_4OH, silica gel) (54% yield), was dissolved in methylene chloride (2 mL), filtered, and used directly in the next step.

[^{14}C]ABT-418

The solution of crude ketooxime (**6**, 83% of 35 mCi, 0.29 mmol) in methylene chloride (2 mL) was cooled to 0°C under an argon atmosphere and stirred while triethylamine (63 μL, 0.45 mmol) and methanesulfonyl chloride (33 μL, 0.42 mmol) were added sequentially. The mixture was allowed to gradually warm to room temperature and, after stirring for 24 h, was extracted with 1 M hydrochloric acid (1 mL). The aqueous extract was washed with ether, basified to pH 8-9 with solid sodium bicarbonate and sodium carbonate, and extracted again with ether. This extract was dried over magnesium sulfate, filtered, and concentrated at 1 atm chasing the last of the ether with hexane. The residue (19 mCi) was dissolved in 9:1 hexane : 2-propanol (3.5 mL) for HPLC purification. Seven injections were made onto a Whatman Partisil 10 column (250 mm x 20 mm i.d.) and the analyte was eluted each time at a flow rate of 15 mL/min with a mobile phase consisting of 85:15 n-heptane : 2-propanol containing 0.6% ammonium hydroxide. Peaks were detected with a UV detector set at 215 nm. Fractions containing [^{14}C]ABT-418 were pooled and extracted with 6 M hydrochloric acid (4.5 mL) followed by two water washes. The pooled aqueous extract was pH adjusted with solid sodium bicarbonate and 4 N hydrochloric acid to pH 1 and concentrated in vacuo. The residue was dissolved in water (5 mL), basified to pH 8-9 with solid sodium bicarbonate, and extracted with ether. The ether was extracted with 1 M hydrochloric acid (1 mL) and this extract was concentrated in vacuo. The residue was dissolved in 10% aqueous ethanol to give 14.6 mCi of [^{14}C]ABT-418 (50% yield).

Determination of Purity

High Performance Liquid Chromatography (HPLC)

[^{14}C]ABT-418 was analyzed by reversed phase HPLC and compared to authentic ABT-418. Each was injected onto a Waters μ-Bondapak C-18 (300 x 3.9 mm i.d.) column and eluted at 1 mL/min with a mobile phase consisting of 50% ammonium acetate (0.1M) and 50% methanol. Peaks were detected with a UV detector at 215 nm and a liquid scintillation radioactivity flow detector. Greater than 99% of the radioactivity corresponded to the UV peak of ABT-418 at 8.3 min. An assay of the effluent showed that 101% of the radioactivity was recovered from the column. The specific activity was determined to be 105 mCi/mmol (517 μCi/mg) by measuring the mass and radioactivity concentrations of the material in a given solution. The concentration of radioactivity was determined by liquid scintillation counting of an accurately measured aliquot. The mass concentration was measured by comparing the HPLC UV peak area of an accurately measured aliquot to a standard curve fitting a straight line equation (correlation coefficients of 0.998) generated by injecting standard solutions of ABT-418 and measuring the resulting peak areas.

Mass Spectroscopy (MS)

The identity of [^{14}C]ABT-418 was confirmed by mass spectral analysis as the sample showed the same molecular ion (m/z 167, $[M+H]^+$) as authentic ABT-418. There was also a peak at m/z 169 and one at m/z 171 in the radioactive sample arising from the carbon-14 labeled molecules in the sample; the ratio of the areas of these peaks indicated that approximately 28% of the molecules were labeled with one carbon-14 and 68% were labeled with two. This translates to a specific activity of 102 mCi/mmol, in good agreement with the HPLC data.

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