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SYNTHESIS OF 1-ETHYL-1-(2-HYDROXYETHYL)AZIRIDINIUM-1,2-$^{14}C_2$ CHLORIDE (1) ([^{14}C]AF64A)

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SUMMARY

1-Ethyl-1-(2-hydroxyethyl)aziridinium-1,2-$^{14}C_2$ chloride (1) ([^{14}C]AF64A), the labelled analogue of a selective presynaptic cholinotoxin, was prepared from labelled ethylene oxide. The synthetic intermediate, 1,2-$^{14}C_2$-2[ethyl[2-[(tetrahydro-2H-pyran-2-yl)oxy]ethyl]amino]ethanol, obtained in good yield by the reaction of 2-[(tetrahydro-2H-pyran-2-yl)oxy]diethylamine with [^{14}C] ethylene oxide, was converted to the corresponding ethyl chloride derivative using CH_3SO_2Cl/n-BuLi. Subsequent removal of the THP group under mild acidic condition gave the mono-armed mustard as a stable hydrochloride salt in an overall chemical yield of 46% and radiochemical yield of 27%. Conversion of the mustard compound to the corresponding aziridinium species was followed by ^{1}H NMR.

Key Words: Synthesis, Cholinotoxin, AF64A, Aziridinium, Mono-armed Mustard

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INTRODUCTION

1-Ethyl-1-(2-hydroxyethyl)aziridinium chloride (1) (AF64A) is a selective presynaptic cholinotoxin capable of inducing long-term cholinergic hypoactivity without affecting other neurotransmitter systems such as the catecholaminergic, serotonergic, and GABAergic systems [1-4]. Because of its specificity, (1) has great potential to serve as a novel compound in developing animal models of human disorders including Alzheimer's disease, in which a central cholinergic hypofunction has been implicated [5,6].

In order to investigate the in vivo mechanism of action of the cholinotoxin (1) in experimental animals, the radiolabelled compound was synthesized, with ^{14}C in the aziridinium moiety.

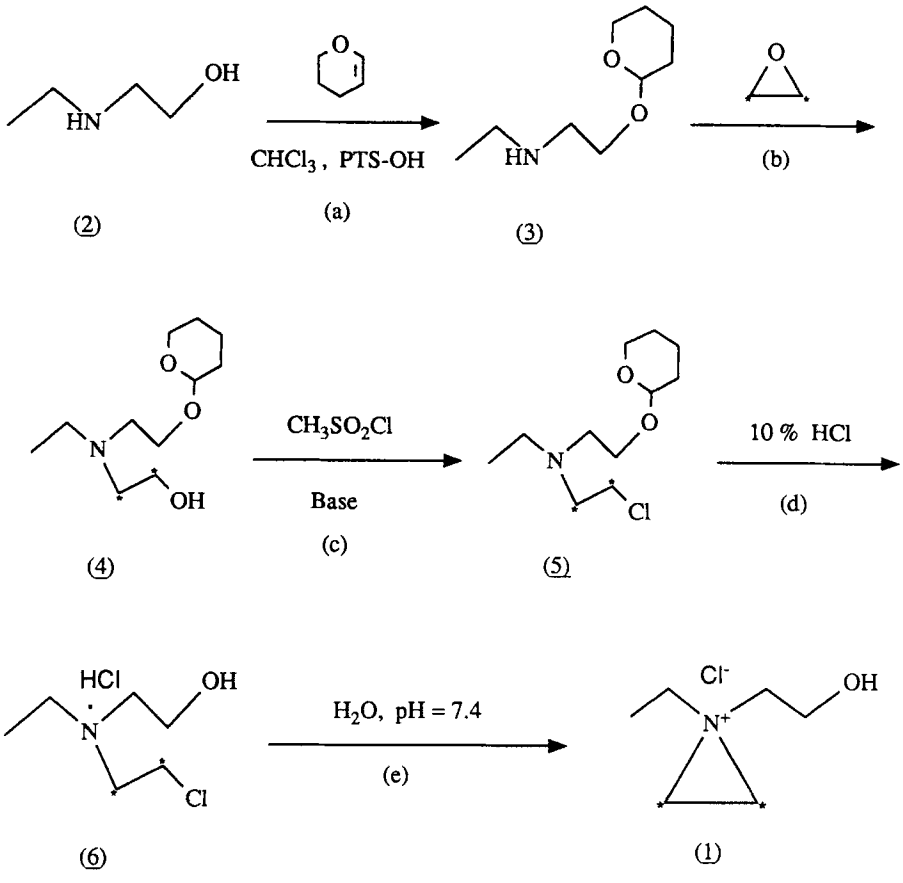
This paper describes the synthetic methodology developed to make the radiolabelled (1).

RESULTS AND DISCUSSION

The synthetic route to obtain ^{14}C-labelled (1) is shown in Scheme 1. N-ethylethanolamine (2) was allowed to react with dihydropyran, in the presence of a 1.1 equivalent of p-toluenesulfonic acid in $CHCl_3$ to obtain 2-[(tetrahydro-2H-pyran-2-yl)oxy]diethylamine (3) in good yield [step (a)]. Since ^{14}C-labelled ethylene oxide could be introduced through step (b), it was the most crucial step in the reaction scheme. Therefore, various conditions were tried to optimize the yield for this step. When THF was used as a solvent, the reaction did not take place and when phosphate buffer (pH=7.5) alone or a combination of phosphate buffer + 95% EtOH was used as the solvent system, it resulted in a longer reaction time and lower yields. The best results in terms of a shorter reaction time and higher yields (≈80%) of (4) were obtained when 95% EtOH was used as the solvent. The ^{14}C-labelled ethylene oxide was initially reacted with ≈ 10 fold molar excess of (3). Subsequently, unlabelled ethylene oxide was added to drive the reaction to completion. At the end of the reaction between (3) and ethylene oxide, the desired compound (4) could be selectively extracted from the aqueous layer using $CHCl_3$.

SCHEME 1

Synthesis of ^{14}C-labelled AF64A (1)



Attempts to convert (4) to (5) by using conventional reagents such as $SOCl_2/CHCl_3$ or $(C_6H_5)_3P/CCl_4$ failed. We, therefore, decided to make the mesylate of (4) and to subsequently displace the mesylate with Cl^-. However, we anticipated the possibility that the mesylate may not need to be isolated and the reaction with CH_3SO_2Cl might directly give the desired compound (5). When (4) was reacted with CH_3SO_2Cl in CH_2Cl_2, a small amount of (5) was indeed obtained. Various bases such as $(C_2H_5)_3N$, $C_2H_5N[CH(CH_3)_2]_2$ and n-BuLi were used to facilitate the reaction and to improve the yield of (5). The yield

was markedly improved when $C_2H_5N[CH(CH_3)_2]_2$ was used (≈50%) as compared to $(C_2H_5)_3N$ (≈20%). The poorer yield obtained by using $(C_2H_5)_3N$ is probably due to the formation of a quaternary ammonium structure of the type (I) (shown below) which was lost probably during aqueous work-up. On the other hand, $C_2H_5N[CH(CH_3)_2]_2$, being a more hindered base, has poorer nucleophilic properties and therefore, may avoid the formation of the quaternary ammonium structure (II) as shown:

$$R\text{-}CH_2\text{-}Cl \xrightarrow{(C_2H_5)_3N} R\text{-}CH_2\text{-}\overset{+}{N}(C_2H_5)_3\ Cl^- \quad (I)$$

(possible)

$$R\text{-}CH_2\text{-}Cl \xrightarrow{C_2H_5N[CH(CH_3)_2]_2} R\text{-}CH_2\text{-}\overset{+}{N}(C_2H_5)[CH(CH_3)_2]_2\ Cl^- \quad (II)$$

(less possible)

When n-BuLi was employed as the base in anhydrous ether, a considerably high yield (75-80%) of (5) was obtained. In the ^1H NMR of the unlabelled (5) prepared by the same procedure, a singlet at δ 2.8 (due to $-OSO_2C\underline{H}_3$) was absent, indicating that the mesylate was not present. Furthermore, ^{13}C NMR of unlabelled (5) indicated the absence of a peak at δ 59 ($\underline{C}H_2$-OH) and showed the presence of a new peak at δ 42 ($\underline{C}H_2$-Cl). This is consistent with the difference in the δ values between the two carbons [7]. An ethanolic-$AgNO_3$ test and mass spectral analysis of unlabelled (5) further confirmed the presence of 'Cl' in the compound. We postulate that mesylate of (4) may have first formed as an intermediate during the reaction, which in turn is converted to the aziridinium species via an intramolecular nucleophilic substitution (S_Ni). Subsequent attack by Cl^- on the aziridinium ring would result in the desired product (5).

Deprotection of the -OH group was easily achieved by stirring (5) with 10% HCl in H_2O [step (d)]. Removal of H_2O produced a pure, stable hydrochloride salt of (6) in ≈87% yield.

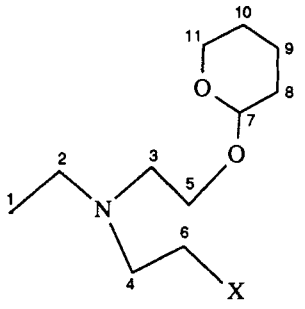
The conversion of (6) to the final aziridinium compound (1) and the stability of (1) were studied by ^1H NMR. Thus, to the hydrochloride salt (6) in D_2O was added 1.1 equivalent of $NaHCO_3$ and ^1H NMRs were recorded at various time intervals. A gradual disappearance of the triplet at δ 3.65 (-N-C$\underline{H}_2$-CH_2-Cl) was observed with a concurrent appearance of a singlet at δ 3.15 (corresponding to the 4H of the aziridinium ring). A simultaneous transformation of the multiplet at δ 4.15-3.83 (-N-CH_2-C$\underline{H}_2$-Cl and -N-CH_2-C$\underline{H}_2$-OH) into a first-order triplet at δ 4.0 (-N-CH_2-C$\underline{H}_2$-OH) indicated that the desired aziridinium compound (1) was being formed from the mustard compound (6). The conversion appeared to be complete within 30 min. Compound (1) was found to be stable for at least 3 hrs, under the experimental conditions used.

EXPERIMENTAL

Materials and Methods:

[^{14}C]Ethylene oxide was purchased from Amersham Corporation. Other chemicals and solvents were obtained from Aldrich Chemical Co., Fisher Scientific Co., and Eastman Kodak Co. All reactions were monitored with TLC (EM reagent aluminum oxide 60F, 0.20 mm thickness or cellulose #5577, Merck, Darmstadt) and visualized after incubation in an I_2 chamber. Alkylating agents were visualized as blue spots by spraying TLC plates with 1% solution of 4-(p-nitrobenzyl)pyridine (NBP) in acetone, heating the plates at 100°C for 2 min., followed by spraying with 2% solution of NaOH in EtOH. ^1H and ^{13}C NMR spectra were obtained with a JEOL FX90Q Fourier transform spectrometer and are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Electron ionization mass spectral analyses were conducted on a Finnigan 3200 mass spectrometer. High resolution FAB and SIMS mass spectral analyses were conducted on a V6 70-G double focusing mass spectrometer. Radioactivity was determined by using an LKB 1211 Rackbeta liquid scintillation counter.

Figure 1 shows the numbering system utilized for ^1H and ^{13}C NMR assignments for compounds (3)-(6).



X = OH or Cl

FIGURE 1: Numbering system used for ^{1}H and ^{13}C NMR assignments for compounds (3) - (6)

2-[(Tetrahydro-2H-pyran-2-yl)oxy]diethylamine (3)

To a solution of N-ethyl ethanolamine (500 mg, 5.62 mmol) in anhydrous $CHCl_3$(8 mL) and p-toluenesulfonic acid (1.080 g, 6.28 mmol) was added dihydropyran (500 mg, 5.95 mmol) in $CHCl_3$ (4 mL) over a period of 10 min. The solution was stirred for 16 hrs. at RT. $CHCl_3$ was then removed and the mixture treated with ammoniacol methanol (15 mL). After the methanol was removed, the oil was redissolved in $CHCl_3$, washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. Recovery of $CHCl_3$ afforded 0.747 g of the crude THP derivative (3) which was purified by chromatography on alumina, using $CHCl_3$ as the eluent. The desired product (3) was obtained as an oil; bp 80-84°C(1mm Hg); yield: 673 mg (3.9 mmol 69.2%). TLC ($CHCl_3$ + 10% MeOH), single spot (R_f=0.63).

^{1}H NMR ($CDCl_3$): δ 4.78-4.48(m, 1H, $C_7\underline{H}$), 4.10-3.70(m, 2H, $C_5\underline{H}_2$), 3.70-3.30(m, 2H, $C_{11}\underline{H}_2$), 3.05-2.90(m, 4H, $C_2\underline{H}_2$ and $C_3\underline{H}_2$), 2.20-1.32(m, 7H, $C_8\underline{H}_2$, $C_9\underline{H}_2$, $C_{10}\underline{H}_2$ and N$\underline{H}$), 1.13(t, 3H, $C_1\underline{H}_3$)

^{13}C NMR ($CDCl_3$): δ 98.7(C_7), 66.5(C_{11}), 61.9(C_5), 48.7(C_3), 43.5(C_2), 30.2(C_8), 25.0(C_{10}), 19.2(C_9), 14.7(C_1)

1,2-$^{14}C_2$-2-[Ethyl[2-[(tetrahydro-2H-pyran-2-yl)oxy]ethyl]amino]ethanol (4)

A "break-seal" ampule containing 1,2-$^{14}C_2$ ethylene oxide (1 mCi, 36.5mCi/mmol) was partially immersed in a dry-ice/acetone bath for 30 min.

The seal was broken, and prechilled EtOH (1 mL) was added to the tube which was quickly fitted on top of a 25 mL flask containing a solution of the tetrahydropyranyl ether (3) (50 mg, 0.29 mmol) in 95% EtOH (2 mL), at 4°C. The solution was stirred at 4°C for 30 min. and then at RT for 18 hrs. The reaction-flask was then immersed in an ice-bath and nonradioactive ethylene oxide (17.7 mg, 0.40 mmol) added. The reaction mixture was then stirred at 4°C for 30 min. and then at RT for a further period of 23 hrs. Ethanol and traces of ethylene oxide were removed on a rotary evaporator. Deionized H_2O (6 mL) was added and the solution extracted with $CHCl_3$ (3 x 20 mL). The $CHCl_3$ extract was passed through anhydrous Na_2SO_4 and the $CHCl_3$ removed. The desired ^{14}C-labelled (4) was obtained as an oil; bp 103-108°C (0.2 mm Hg); yield: 50 mg (0.23 mmol, 79.5%); specific activity: 1.752 mCi/mmol; total radioactivity: 0.404 mCi; TLC ($CHCl_3$ + 10% MeOH), single spot (R_f=0.71). Spectral analysis was conducted on a sample of the unlabelled compound prepared by the same synthetic route.

^1H NMR($CDCl_3$): δ 4.80-4.42(m, 1H, $C_7\underline{H}$), 4.10-3.75(m, 2H, $C_5\underline{H}_2$), 3.75-3.25(m, 4H, $C_{11}\underline{H}_2$ and $C_6\underline{H}_2$), 3.25-2.95(bs, 1H, OH), 2.90-2.27(m, 6H, $C_2\underline{H}_2$, $C_3\underline{H}_2$ and $C_4\underline{H}_2$), 2.10-1.27(m, 6H, $C_8\underline{H}_2$, $C_9\underline{H}_2$, and $C_{10}\underline{H}_2$), 1.13(t, 3H, $C_1\underline{H}_3$).

^{13}C NMR ($CDCl_3$): δ 98.8(C_7), 65.6(C_{11}), 62.2(C_5), 58.7(C_6), 55.6(C_3), 52.7(C_4, 48.5(C_2), 30.5(C_8), 25.3(C_{10}), 19.4(C_9) 11.7(C_1).

1,2-$^{14}C_2$-2-[Ethyl[2-[(tetrahydro-2H-pyran-2-yl)oxy]ethyl]amino]ethyl chloride (5)

To a magnetically stirred and ice-cooled solution of the ^{14}C-labelled (4) (48 mg, 0.22 mmol) was added n-BuLi (190 μl of 1.4 M solution, 0.26 mmol) in anhydrous ether (3mL) followed by CH_3SO_2Cl (30 mg, 0.31 mmol), under N_2 atmosphere. After the addition of CH_3SO_2Cl, the ice-bath was removed and the mixture stirred at RT for 15 hrs. under N_2 atmosphere. The reaction-mixture was then filtered under N_2 atmosphere, and the solid washed repeatedly with dry ether. The ether was removed from the combined filtrates. The oil obtained, was shaken with hexane (4 x 15 mL) and the hexane extract was

washed once with chilled brine solution and passed through anhydrous Na_2SO_4. Removal of hexane gave the desired ^{14}C-labelled (5) as an oil; bp 123-128°C(1mm Hg); yield: 36 mg (0.153 mmol, 69.6%); TLC ($CHCl_3$ + 10% MeOH), single NBP-positive spot (R_f=0.86). To determine the radiochemical purity of (5), the TLC plate was divided into 1 cm strips. Each strip was scraped off the plate, placed in a scintillation vial containing 1 mL of H_2O and 10 mL of scintillation liquid, and the radioactivity of each strip was measured using a scintillation counter. The results indicated that at least 98% of the radioactivity resided in the band corresponding to that with the R_f value of the unlabelled compound.

Unlabelled (5), prepared by the same synthetic procedure had the following spectral data:

^1H NMR ($CDCl_3$): δ 4.75-4.40(m, 1H, $C_7\underline{H}$), 4.10-3.60(m, 2H, $C_5\underline{H}_2$), 3.60-3.10(m, 4H, $C_{11}\underline{H}_2$ and $C_6\underline{H}_2$), 3.05-2.25(m, 6H, $C_2\underline{H}_2$, $C_3\underline{H}_2$ and $C_4\underline{H}_2$), 2.10-1.23(m, 6H, $C_8\underline{H}_2$, $C_9\underline{H}_2$ and $C_{10}\underline{H}_2$), 1.13(t, 3H, $C_1\underline{H}_3$)

^{13}C NMR ($CDCl_3$): δ 99.0 (C_7), 66.2(C_{11}), 62.2(C_5), 56.3(C_3), 53.5(C_4), 48.9(C_2), 42.0(C_6), 30.7(C_8), 25.5(C_{10}), 19.5(C_9), 12.3(C_1)

EIMS: m/e (relative intensity) 122(28.78, M^+-$C_7H_{15}O$), 120(100, M^+-$C_7H_{15}O$), 85(24.73, M^+-$C_7H_{15}ClO$)

2-[(2-chloroethyl-1,2-$^{14}C_2$)ethylamino]ethanol hydrochloride (6)

^{14}C-labelled (5) (36 mg, 0.15 mmol) was dissolved in 12% HCl (2 mL) and the solution stirred at RT for 30 hrs. Freeze-drying of the reaction mixture produced the desired (6) in the stable hydrochloride salt form; yield: 25 mg (0.133 mmol., 88.6%); specific activity: 2.045 mCi/mmol; total radioactivity: 0.272 mCi; TLC of free base ($CHCl_3$ + 2% MeOH) single, NBP-positive spot (R_f=0.67). The following spectral data obtained on the unlabelled (6), prepared by the same synthetic route, confirmed the chemical structure:

^1H NMR (D_2O): δ 4.15-3.83(m, 4H, $C_5\underline{H}_2$ and $C_6\underline{H}_2$), 3.65(t, 2H, $C_4\underline{H}_2$), 3.52-3.20(m, 4H, $C_2\underline{H}_2$ and $C_3\underline{H}_2$), 1.35(t, 3H, $C_1\underline{H}_3$)

^{13}C NMR (D_2O): δ 55.7(C_5), 54.6(C_3), 54.2(C_4), 49.7(C_2), 38.0(C_6), 8.42(C_1)

High Resolution FAB MS: m/z 152.0863 152.0863 ($C_6H_{15}NOCl$ requires 152.0843)

1-Ethyl-1-(2-hydroxyethyl)aziridinium-1,2-$^{14}C_2$ chloride (1)

A solution of the hydrochloride salt (6) (4 mg, 0.021 mmol) in D_2O (0.55 mL) was placed in a 5 mm NMR tube and its ^1H NMR was recorded. $NaHCO_3$ solution (1.98 mg in 50 μl D_2O, 0.024 mmol) was then added to the NMR tube and ^1H NMRs were recorded at RT, at 5, 10, 15, 30, 60, 120, 180 and 300 min., At the end of 30 min. the conversion appeared to be complete. The aziridinium compound was found to be stable for at least 180 min. At the end of 300 min., a slight decomposition of (1) was observed. TLC on cellulose plate: (n-BuOH:EtOH:CH_3CO_2H:H_2O, 8:2:2:1): R_f=0.5O

^1H NMR (D_2O): δ 4.0(t, 2H, $C_5\underline{H}_2$), 3.52-3.20(m, 4H, $C_2\underline{H}_2$ and $C_3\underline{H}_2$), 3.15(s, 4H, aziridinium protons), 1.35(t, 3H, $C_1\underline{H}_3$)

SIMS analysis conducted on lyophilized sample of unlabelled (1), further confirmed the identity of the product: m/z 116(M^+), 87 (M^+-C_2H_5), 72(M^+-CH_2CH_2OH), 56 ().

Quantitation of (1) formed from (6) was also studied by $Na_2S_2O_3/I_2$ titration method as follows:

The hydrochloride salt (6) (5 mg, 0.027 mmol) was dissolved in distilled H_2O (2 mL). A sufficient amount of 0.1N NaOH was then added to adjust the pH between 7.4 to 7.5. The required amount of H_2O (total volume 2.660 mL) was added to make up a final solution of 10 mM concentration. The solution was then stirred at RT for 30 min. at the end of which 1 mL solution was removed and transferred to a conical flask. Additional H_2O (5 mL) was added followed by ~2 drops of 2N HOAc. To this solution, $Na_2S_2O_3$-$5H_2O$ (2 mL of 11.513 mM solution) was added and the contents were stirred at RT for 20 min. Freshly prepared starch solution was then added and the excess $Na_2S_2O_3$ was back-titrated against 4.433 mM I_2 solution. From the amount of $Na_2S_2O_3$

consumed for the formation of the Bunte salt, the percent range of formation of the aziridinium compound was calculated to be 80-90.

The above TLC, spectral and titration data are identical to those of an authentic sample of (1).

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