Synthesis of Carbon-14 Labeled 1-(2-Hydroxyiminomethyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane Dichloride Monohydrate ([^{14}C]HI-6•H_2O)

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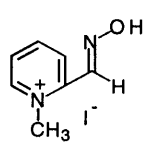
SUMMARY

The synthesis of the title compound ([^{14}C]-**2**) is described. Simultaneous quaternizations of 2-pyridine[^{14}C]aldoxime (**8**) and isonicotinamide (**10**) by bis(methylsulfonoxymethyl) ether gave 1-(2-hydroxyimino-[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane dimethanesulfonate (**11**) which was converted to [^{14}C]-**2** by ion-exchange chromatography. Purification of [^{14}C]-**2** was done by serial crystallization with isotopic dilution which gave product of 97% and 98% radiochemical and chemical purity, respectively with 3% overall radiochemical yield.

Key words: carbon-14, radiolabeled oximes, HI-6, organophosphates, reactivators, acetylcholinesterase.

INTRODUCTION

Some organophosphates, such as pinacoyl methylphosphonofluoridate (soman) and isopropyl methylphosphonofluoridate (sarin), cause central nervous respiratory depletion through inhibition of acetylcholinesterase (AChE). However, many quaternary pyridinium oximes have been developed (1-5) which can reactivate the inhibited AChE. It is believed that because of its good nucleophilicity, the oxime moiety of the pyridinium salt can easily displace the phosphoryl group which is covalently bound to the enzyme. Although pyridinium oximes like 2-PAM (**1**) can not reactivate[6] soman-inhibited AChE, HI-6 (**2**) has been found to be an effective reactivator of non-aged soman-inhibited human AChE.[7,8]



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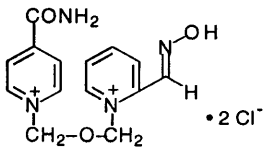

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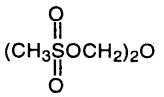

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Nicolas and co-workers reported[9] the synthesis of carbon-14 labeled HI-6 with the label in the oxime moiety. However, their synthesis involves the use of bis(chloromethyl) ether which is highly carcinogenic. More recently, Hsiao and Musallam[10] prepared HI-6 where bis(chloromethyl) ether is replaced by a less hazardous solid, bis(methylsulfonoxymethyl) ether (**3**).

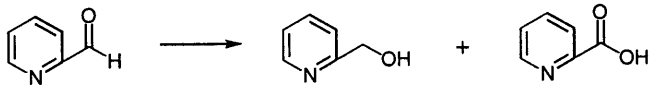
The present report summarizes our synthesis of [^{14}C]HI-6. The synthetic scheme essentially follows Nicolas[9] procedure, but uses the modification of Hsiao and Musallam.[10]

RESULTS AND DISCUSSION

The synthesis of [^{14}C]HI-6 ([^{14}C]-**2**) is summarized in Scheme 1. The scheme essentially consists of the preparation of 2-pyridine[^{14}C]aldoxime (**8**) followed by simultaneous quaternizations of **8** and isonicotinamide (**10**) by bis(methylsulfonoxymethyl) ether (**3**).

Preparation of oxime [^{14}C]-**8** was achieved through formylation of the lithium salt **5** with N-[^{14}C]methylformanilide ([^{14}C]-**6**). Nicolas and co-workers[9] report an 80% yield of aldehyde [^{14}C]-**7** by addition of [^{14}C]-**6** to **5**. Our attempts to repeat this reaction however, only afforded **7** in a maximum yield of 50%. A tracer synthesis of [^{14}C]-**7** revealed that the yield was reduced by the formation of two radioactive side products, one of which was identified as 2-pyridine[^{14}C]methanol (**12**) on the basis of its ^{1}H NMR spectrum. The formation of [^{14}C]-**12** suggests that aldehyde [^{14}C]-**7** is undergoing a base catalyzed Cannizzaro dismutation reaction as shown in Scheme 2. In an effort to

Scheme 2



7 **12** **13**

minimize this side reaction, the synthesis of **7** was repeated using an inverse addition procedure, i.e. a solution of lithium salt **5** was added slowly to a solution of N-methylformanilide (**6**). This increased the yield of **7** to 83%. Subsequently, it was found that oxime **8** could be prepared directly from the crude preparation of **7** in 89% yield.

The preparation of **11** is normally carried out by first allowing oxime **8** to react with ether **3** presumably to form intermediate **9**, followed by addition of amide **10** to give **11**.

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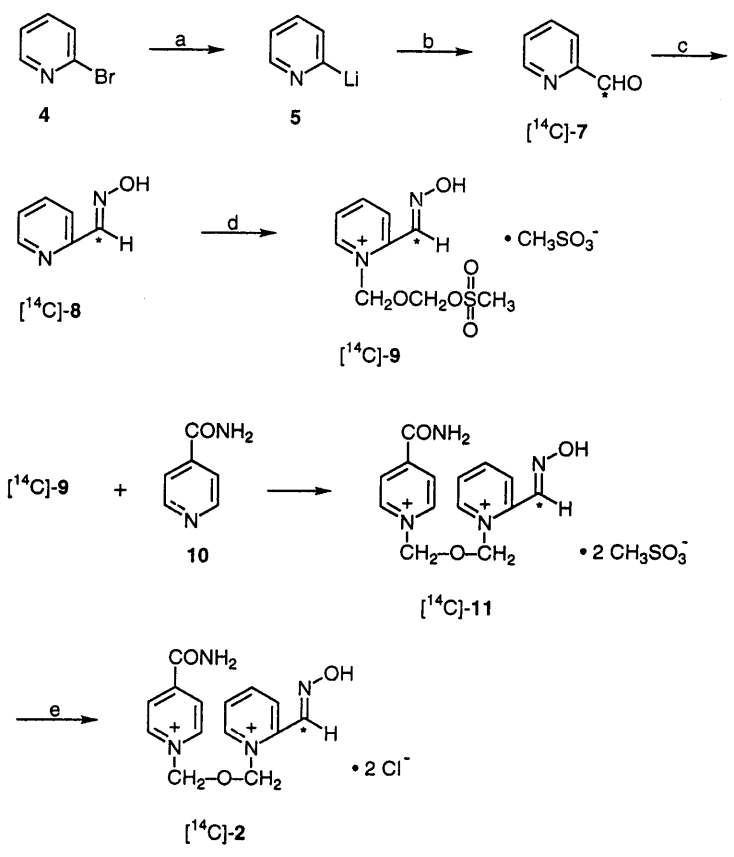
Scheme 1



a) n-BuLi
b) $C_6H_5N(Me)\overset{*}{C}HO$ ([^{14}C]-6)
c) NH_2OH•HCl
d) $(CH_3SOCH_2)_2O$ (3)
e) Bio-Rad AG 1-X8 ion exchange resin, Cl^-

The crude **11** contains the symmetrical salts **14a** and **15a**, as well as **16a** and **16b**, respectively as impurities. Repeated syntheses of **11** gave crude products which contained 19-26% yield of **11** by HPLC analysis. Crude **11** is difficult to purify mainly because of the presence of the symmetrical oxime salt **14a** which has solubility properties similar to **11**. Salts **15a**, **16a** and **16b** are relatively easy to separate because of solubility differences. Formation of **14a** consumes radioactive starting material in a

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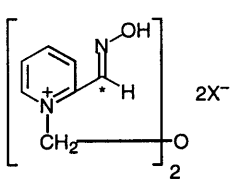
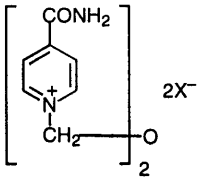
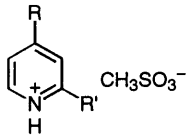
nonproductive manner, but more importantly its specific activity is twice that of **11** making it necessary to reduce its chemical contamination to <1% in order to prepare [^{14}C]-**2** of 98% radiochemical purity.



14a X = $CH_3SO_3^-$
14b X = Cl^-



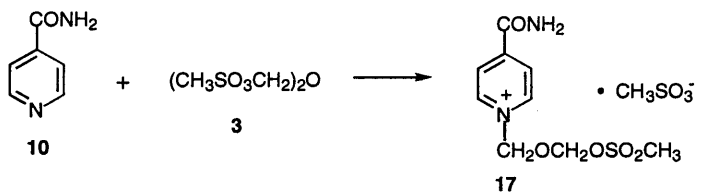
15a X = $CH_3SO_3^-$
15b X = Cl^-



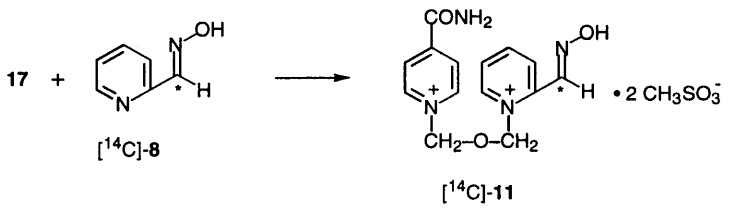
16a R = H, R' = $\overset{*}{C}$H=NOH
16b R' = H, R = $CONH_2$

The reactions shown in Scheme 3 were investigated with the hope of improving the yield of [^{14}C]-**11** with respect to the radioactive intermediate [^{14}C]-**8**. It was reasoned that if the intermediate **17** could be prepared and purified prior to reaction with [^{14}C]-**8**, that the formation of the symmetrical salts **14a** and **15a** could be avoided, thus increasing the utilization of [^{14}C]-**8** and affording crude [^{14}C]-**11** that would be easier to purify. However, when **10** was allowed to react with **3**, no **17** could be detected by ^{1}H NMR analysis. The only identifiable products were salts **15a** and **16b**.

Scheme 3



10 **3** **17**



[^{14}C]-**8** [^{14}C]-**11**

Purification of [^{14}C]-**11** was attempted by crystallization. Two successive crystallizations (ethanol/acetonitrile) of the crude product from a tracer run afforded [^{14}C]-**11** of 88% chemical and 82% radiochemical purity. In search of a better purification method

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ion-exchange chromatography, preparative thin layer chromatography, and preparative HPLC were investigated. Ion-exchange chromatography with a cation-exchange resin (AG 50W-X8) failed to separate the salts whereas preparative TLC on cellulose with butanol-water-acetic acid as eluant failed to yield material of acceptable purity with good recovery. The major problem with purification by HPLC was separation of **11** from the solvent modifier, sodium heptane sulfonate. In a control experiment, attempts to separate **11** from sodium heptane sulfonate by anion exchange chromatography failed because the sodium chloride contaminant which results from the removal of the solvent modifier could not be removed from HI-6 by crystallization. Efforts to develop an HPLC system to purify **11** without a solvent modifier were also unsuccessful.

The results from the crystallization experiments suggested that crystallization with isotopic dilution was likely to give [^{14}C]HI-6 of acceptable purity but in low yield. In order to test this hypothesis, a crude tracer sample containing an approximately 1:1 radiochemical mixture (2:1 molar mixture) of [^{14}C]-**11** and [^{14}C]-**14a** was converted to their chloride salts by ion-exchange chromatography and diluted with nonlabeled HI-6. Two successive crystallization of this mixture from water-ethanol gave [^{14}C]-**2** of 98% chemical and 89% radiochemical purity. The major radiochemical impurity was the high specific activity [^{14}C]-**14b** which was present as only a 2% chemical impurity. Repeated crystallizations would probably have afforded material of suitable radiochemical purity, but with considerable loss of product. Consequently, it was decided to attempt to improve the radiochemical purity of [^{14}C]HI-6, with possible sacrifice of some chemical purity by adding enough nonlabeled impurity **14b** to dilute the specific activity of [^{14}C]-**14b** to approximately that of the isotopically diluted [^{14}C]-**2** prior to the third crystallization. The third crystallization gave [^{14}C]-**2** of 94% and 98% chemical and radiochemical purity, respectively, with 40% radiochemical recovery (5% radiochemical yield overall).

The above technique of removing the last traces of the high specific activity radiochemical impurity by impurity-isotopic dilution was applied to the master synthesis of [^{14}C]HI-6 and gave material of 97% and 98% radiochemical and chemical purity, respectively. The specific activity was 15.8 μCi/mg, 5.97 mCi/mmol and the overall radiochemical yield was 3% not including a 20% yield of recovered oxime [^{14}C]-**16a**.

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EXPERIMENTAL

Melting points were recorded on a Fisher-Johns melting point apparatus. Ultraviolet spectra were recorded on a Varian Model 2290 spectrophotometer. Proton NMR spectra were recorded on a Bruker AM-250 spectrometer. Analytical TLC were performed using E. Merck silica-gel 60 F-254 plates. Column chromatographies were performed with E. Merck silica-gel 60 (230-400 mesh). Radioactive samples were counted on a Packard Tri-carb 4000 liquid scintillation counter using an internal standard in Ultima Gold cocktail. Developed TLC plates were scanned on a Berthold Model LB 285 Linear Analyzer system. HPLC was done using a Waters Associates Model 6000A dual pump system with a Model U6K septumless injector, and a IN/US System, Inc. Model 20725 β RAM Flow-Through Monitor.

2-Pyridine[^{14}C]carboxaldehyde (7)

A 50-mL two-neck round bottom flask containing a magnetic stirring bar was flushed with dry argon for 0.5 h. Anhydrous ether (2.6 mL) was added to the flask and the temperature was lowered to -50 °C. n-Butyllithium (3.98 mmol, 2.6 mL of 1.6 M solution in hexane) was added in one portion from a syringe, and the mixture was stirred for 0.5 h. 2-Bromopyridine (**4**, 3.98 mmol, 0.38 mL) in dry ether (4 mL) was then added slowly from a syringe over a period of 45 min with constant stirring. The reaction mixture was stirred for another 1 h while the solution slowly turned from light yellow to light red. TLC (SiO_2:hexane-acetone, 7:3) indicated the reaction was complete. The temperature was then lowered to -60 °C.

A 100-mL, two-neck round bottom flask containing a magnetic bar was flushed with dry argon for 0.5 h and N-[^{14}C]methylformanilide ([^{14}C]-**6**, 3.16 mmol, 427 mg, 180 mCi) in dry ether (25 mL) was added. The temperature was lowered to -60 °C with constant stirring. The solution from the initial 50-mL flask was slowly added to that in the second flask through a well insulated double-ended needle over a period of 0.5 h, the flow being controlled by argon pressure. The reaction mixture was stirred for another 3 h. TLC (SiO_2 : hexane-acetone, 7:3) indicated the reaction was complete.

The reaction flask was removed from the cooling bath and 1 N HCl (15 mL) solution was added slowly with constant stirring. The mixture was transferred to a separatory funnel and the ether layer was separated. The aqueous layer was washed once with ether (10 mL), neutralized with 1 N K_2CO_3 (8 mL) and extracted with CH_2Cl_2

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(6 x 25 mL). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and the radioactivity was measured (125.5 mCi). TLC (SiO_2:hexane-acetone, 7:3) indicated that the crude solution was 88% radiochemically pure. The solution was stripped in the fume hood (the aldehyde was found to be volatile under low pressure) with the bath temperature at 12-15 °C. The crude **7** was used in the next step without further purification.

2-Pyridine[^{14}C]aldoxime (8)

In a 25-mL round bottom flask were placed crude 2-pyridine[^{14}C]carboxaldehyde ([^{14}C]-**7**), ethanol-water (1:1, 8 mL), hydroxylamine hydrochloride (430 mg, 6.2 mmol) and K_2CO_3 (860 mg, 6.2 mmol). The mixture was stirred at room temperature overnight. TLC (SiO_2: hexane-acetone,7:3) indicated the reaction was complete. The mixture was concentrated by rotary evaporation and extracted with EtOAc (5 x 10 mL). The EtOAc extracts were combined, dried over Na_2SO_4 and stripped to give 437 mg of a brown solid. The solid was flash chromatographed on a 15 x 3.5 cm SiO_2 (230-400 mesh) column eluting with petroleum ether (30-60 °C)-EtOAc initially in 4:1 ratio until the N-methylaniline byproduct was removed and then in 1:1 ratio. The fractions were analyzed by TLC-RAM (SiO_2: hexane-acetone, 7:3) and the fractions containing pure product were combined, stripped and dried to a constant weight in vacuo to yield 263 mg (113 mCi, 429 mCi/mg, 52.4 mCi/mmol, 63% radiochemical and 66% chemical yields based on N-[^{14}C]methylformanilide) of oxime [^{14}C]-**8**.

Bis(1-(2-Hydroxyiminomethyl)-1-pyridino)-2-oxapropane Dimethanesulfonate (14a)

Bis(methanesulfonoxymethyl) ether (**3**, 641 mg, 2.74 mmol) was transferred to a 100-mL round bottom flask containing a magnetic stirring bar under nitrogen in a glove bag. Ether **3** was dissolved in anhydrous CH_3CN (5 mL) and the solution was cooled to 0 °C under nitrogen. Nonlabeled 2-pyridinealdoxime (**8**, 669 mg, 5.48 mmol) was dissolved in anhydrous CH_3CN (10 mL) and transferred to a syringe. The oxime solution was slowly added to the solution of **3** over a period of 20 min. The reaction flask was removed from the cooling bath and attached to a condenser fitted with a $CaCl_2$ drying tube. The reaction mixture was heated at 55 °C under nitrogen for 1 h with constant stirring. Anhydrous CH_3CN (10 mL) was added to the flask and heating continued for

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another 1 h. The resulting gummy brown material which precipitated was separated from the supernatant by decantation and washed further with CH_3CN (3 x 2 mL). The gum was triturated with EtOH (3 mL) and stirred for 1 h. The supernatant was separated by centrifugation and the residue was washed several times with EtOH (2 mL) until a clean white solid was obtained. HPLC[11] (R_t = 12 min) indicated the compound was more than 99% pure. The solid was dried to a constant weight in vacuo to give 272 mg (21% yield) of **14a**: m.p. 170-172 (d) °C; ^1H NMR (D_2O) δ 8.0-9.1 (m, 10, aromatic and CH), 6.5 (s, 4, CH_2), 2.8 (s, 6, CH_3).

The ethanol washes were combined, stripped and dried to a constant weight in vacuo to yield 250 mg of a gum which contained 56% and 44% of **14a** and **16a**, respectively by HPLC[11] analyses. An initial attempt to recover **14a** by crystallization failed and was not pursued further.

1-(2-Hydroxyimino[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane Dimethanesulfonate (11)

Bis(methanesulfonoxymethyl) ether (**3**, 457 mg, 1.95 mmol) was transferred to a 100-mL round bottom flask containing a magnetic stirring bar under nitrogen in a glove bag. Ether **3** was dissolved in anhydrous CH_3CN (3 mL) and the solution was cooled to 0 °C under nitrogen. 2-Pyridine[^{14}C]aldoxime ([^{14}C]-**8**), 238 mg, 1.95 mmol, 102 mCi) was dissolved in anhydrous CH_3CN (7 mL) and transferred to a syringe. The oxime solution was added slowly to the solution of **3** over a period of 20 min with constant stirring. Isonicotinamide (**10**, 238 mg, 1.95 mmol) was added portionwise over 15 min. The reaction flask was removed from the cooling bath and the reaction mixture was stirred at room temperature for 20 h. The resulting gummy brown material which precipitated was separated from the supernatant by decantation and washed with CH_3CN (3 x 1 mL). The gum was triturated with EtOH (10 mL) and stirred for 1 h. The supernatant was separated by centrifugation and the solid was washed with EtOH (5 x 1 mL). The solid was dried to a constant weight in vacuo to yield 174 mg of a mixture of **16b** and **15a**, with the former as the major component by ^1H NMR analyses. HPLC[11] analyses of the ethanol-solubles indicated a radiochemical mixture of [^{14}C]-**11**, [^{14}C]-**14** and [^{14}C]-**16a** in a ratio of 28:32:33, respectively. Removal of the solvent afforded a gum which was stirred overnight with anhydrous CH_3CN (20 mL), decanted and washed further with CH_3CN (3 x 2 mL). The residual gum was dried to a constant

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weight in vacuo to yield 440 mg (35 mCi) of a radiochemical mixture of [^{14}C]-**11**, [^{14}C]-**14a** and [^{14}C]-**16a** in a ratio of 39:39:16, respectively. This crude mixture was used in the next step without further purification.

1-(2-Hydroxyimino[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane Dichloride Monohydrate ([^{14}C]HI-6•H_2O) (2)

The crude mixture (440 mg, 35 mCi) containing 39% (radiochemically) of 1-(2-hydroxyimino[^{14}C]methyl)-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane dimethanesulfonate (**11**) was dissolved in water (1.5 mL) and passed through an ion exchange column (10 mL of Bio-Rad AG 1-X8 Cl^- form). The solution was held in the column for an hour and then eluted with water in 20 mL fractions. The fractions containing the product were combined and stripped followed by the addition of 530 mg of standard HI-6. The mixture (900 mg) was then crystallized from water (1 mL) and ethanol (5 mL) to yield 536 mg of solid. The solid contained 78:20:1.5 of [^{14}C]-**2**, [^{14}C]-**14b** and the chloride form of [^{14}C]-**16a**, respectively by HPLC[11] analyses. A second crystallization afforded 407 mg of solid and improved the radiochemical purity of [^{14}C]-**2** to 91% (8% [^{14}C]-**14b** and 1% [^{14}C]-**16a** as the chloride).

Bis(1-(2-hydroxyiminomethyl)-1-pyridino)-2-oxapropane dimethanesulfonate (**14a**, 40 mg, 0.083 mmol) was dissolved in water (0.5 mL) and passed through an ion-exchange column (1 mL of Bio-Rad AG 1-X8 Cl^- form). The fractions containing **14b** were collected and stripped. The residue was dissolved in water (0.8 mL) and added to the above 91% radiochemically pure [^{14}C]HI-6. Addition of ethanol (4 mL) gave 330 mg of crystalline solid. The solid contained (radiochemically) 96%, 3.6% and 0.5% of [^{14}C]-**2**, [^{14}C]-**14b** and [^{14}C]-**16a** (as the chloride), respectively. Final crystallization from water (0.6 mL) and ethanol (3 mL) afforded 200 mg (3.1 mCi) of [^{14}C]-**2**; 3% radiochemical yield overall; m.p.135-140 °C gradual decomposition (lit[10] m.p. 145-147 °C decomposition). The specific activity was determined to be 15.8 μCi/mg, 5.97 mCi/mmol. The radiochemical and chemical purity were found to be 97.4% and 98.3%, respectively by HPLC (t_R = 10 min 5 sec.) analyses.[11]

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11. Waters μBondapak C18, 10 μ, 3.9 x 300 mm, 20% CH_3CN-80% [0.01 M $CH_3(CH_2)_6 SO_3Na$ in aqueous 0.1% AcOH], 1.7 mL/min, 304 nm.

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