PREPARATION OF PYRIDOSTIGMINE BROMIDE LABELED WITH CARBON-14 AND TRITIUM[1]

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SUMMARY

[2-^{14}C]Pyridostigmine bromide was prepared in 17.6% radiochemical yield with specific activity of 18 mCi/mmol. The reaction sequence involved preparation of 2-furan[^{14}C]carboxylic acid by carbonation of 2-lithiofuran, followed by conversion to 2-amino[^{14}C]methylfuran by lithium aluminum hydride reduction of its carboxamide. Oxidative rearrangement of 2-amino[^{14}C]methylfuran gave 3-hydroxy[2-^{14}C]pyridine which was converted to [2-^{14}C]pyridostigmine bromide by reaction with dimethylcarbamyl chloride and quarternization with bromomethane. Pyridostigmine bromide labeled in the methyl group of the carbamate function was prepared in 73% yield with specific activity of 37.6 mCi/mmol by reaction of bis-3-pyridyl carbonate with [^{14}C]dimethylamine followed by quarternization with bromomethane. [6-^{3}H]-Pyridostigmine bromide with specific activity of 22.5 mCi/mmol was prepared by catalytic halogen-tritium replacement of 2,6-dibromo-3-dimethylcarbamyloxypyridine followed by quarternization with bromomethane and back-exchanging the labile 2-tritium.

Key Words: Pyridostigmine, carbon-14, tritium

INTRODUCTION

3-[[(Dimethylamino)carbonyl]oxyl]-1-methylpyridinium bromide (pyridostigmine bromide) is a synthetic anticholinesterase agent used in treatment of myasthenia gravis and as an antagonist to nondepolarizing muscle relaxants. The agent inhibits the destruction of acetylcholine by cholinesterase, thus permitting freer transmission of nerve impulses across the neuromuscular junction.[2] Pyridostigmine bromide has been investigated as a protective agent against organophosphate poisoning [3,4] and is currently being used by the military for this purpose.[5]

Carbon-14 and tritium labeled samples of pyridostigmine bromide were required for biological studies.

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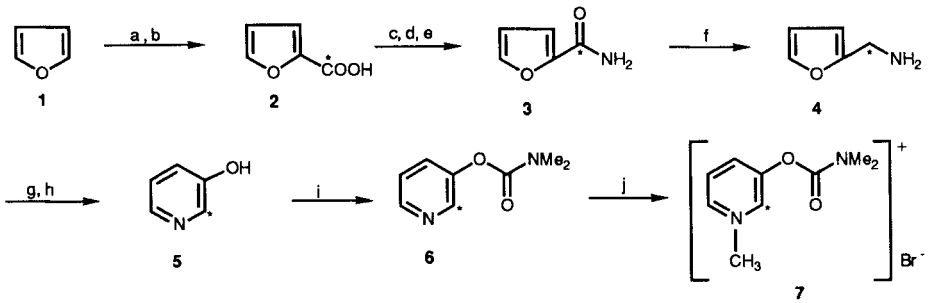
RESULTS AND DISCUSSION

The synthetic method used for preparing [2-^{14}C]pyridostigmine bromide (7) is outlined in Scheme 1. This method is based on a previous synthesis of 2-amino[^{14}C]methylfuran (4)[6] and the oxidative rearrangement of 2-aminomethylfurans to 3-hydroxypyridines by hydrogen peroxide under acidic conditions.[7] Carboxylation of 2-lithofuran under argon afforded furan-2-[^{14}C]carboxylic

Scheme 1

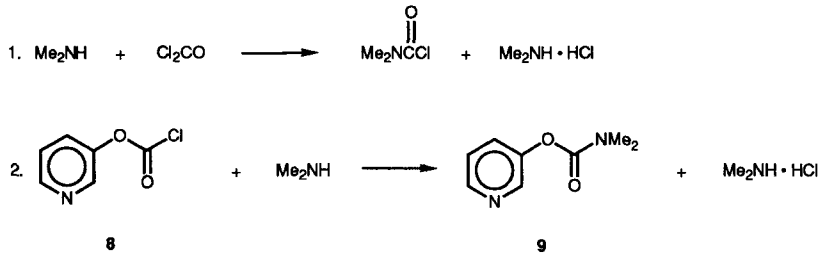


(a) BuLi; (b) $\overset{\bullet}{C}O_2$; (c) KOH; (d) $SOCl_2$; (e) NH_3; (f) $LiAlH_4$; (g) HCl, H_2O_2; (h) K_2CO_3; (i) $ClCONMe_2$; (j) CH_3Br

acid (2)[8] in 66% radiochemical yield. Treatment of the potassium salt of 2 with thionyl chloride and then ammonia gave a 73% radiochemical yield of amide 3. The amide was reduced with lithium aluminum hydride to amine 4 in 69% radiochemical yield. Oxidative rearrangement of 4 gave 3-hydroxy-[2-^{14}C]pyridine (5) in 68% radiochemical yield. Treatment of 5 with dimethylcarbamoyl chloride as described in the literature[9] gave carbamate 6 in 89% radiochemical yield. Subsequent treatment of 6 with methyl bromide in acetone gave [2-^{14}C]-pyridostigmine bromide 7 in 84% radiochemical yield with specific activity of 18 mCi/mmol. The overall radiochemical yield was 17.6% starting from barium [^{14}C]carbonate.

Besides having the pyridine ring of pyridostigmine labeled, it was also desirable to label the N-methyl groups of the carbamate residue. Two methods are described in the literature[8] for converting 3-hydroxypyridine (14, Scheme 3) to its dimethylcarbamate derivative (9). One involves reaction of 14 with dimethylcarbamoyl chloride and the other involves reaction of 14 with

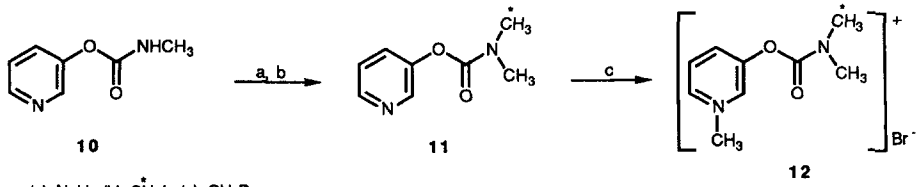
phosgene to form 3-hydroxypyridine chloroformate (8) followed by treatment with dimethylamine. Both of these methods are complicated by the formation of dimethylamine hydrochloride during the sequence; the former in the preparation of dimethylcarbamoyl chloride (Reaction 1) and the later in the reaction of dimethylamine with 8 (Reaction 2). Preliminary studies of these two reactions indicated that it would be difficult to make either one efficient with respect to dimethylamine.



8 9

The based catalyzed alkylation of carbobenzyloxy protected primary amines to give protected secondary amines has been reported.[9] Based on this report, we attempted to prepare [carbamate-N-methyl-^{14}C]pyridostigmine bromide (12) as outlined in Scheme 2, but all attempts to prepare 11 by alkylation of 10

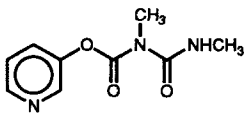
Scheme 2



10 11 12

(a) NaH; (b) $C\overset{*}{H}_3I$; (c) $C\overset{*}{H}_3Br$

failed to give appreciable quantities of desired product. The major side product of these attempts was identified as 13 on the basis of spectral data. The mass spectrum of 13 shows a molecular ion at m/z 209. Its IR spectrum has

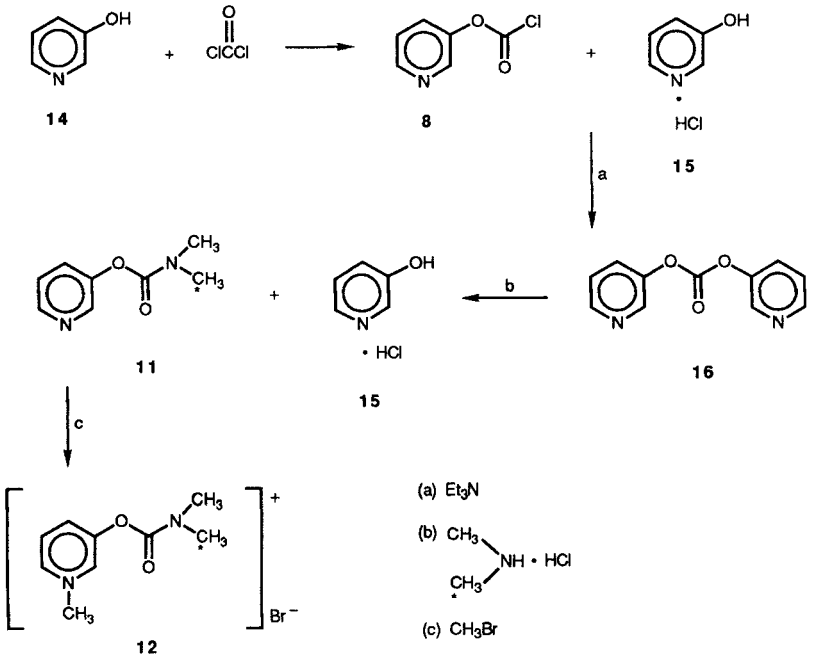


13

absorption bands at 3380 (N-H) and 1735 and 1690 (imide carbonyls) cm^{-1}. The ^1H NMR spectrum showed two singlets at δ 2.80 and 2.90 which integrated for

about 1.5 protons each and were assigned to the $CONHCH_3$ function, a 3-proton singlet at δ 3.46 assigned to the $CON(CH_3)CO$ group and resonances typical of a 3-substituted pyridine. It was presumed 13 was formed by reaction of 10 with itself under the basic reaction conditions. The isolation of 13 suggested that 3-hydroxypyridine carbonate (16) might react directly with dimethylamine to form 11 (Scheme 3). Using 16 as the acylating agent has the advantages of

Scheme 3

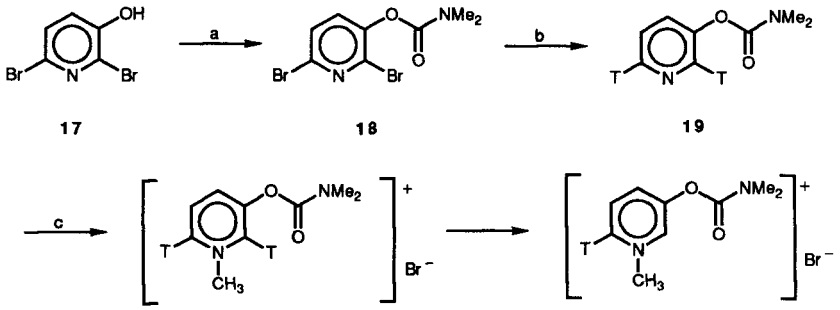


providing an intermediate that is more easily purified and characterized than chloroformate 8, and avoids the complication of dimethylamine hydrochloride formation. Carbonate 16 was prepared by modification of the method used to prepare chloroformate 8.[8] During the course of reaction of 14 with phosgene in acetonitrile, 2-hydroxypyridine hydrochloride (15) precipitates. When precipitation was completed the solvent was evaporated under vacuum to remove excess phosgene. The residue was resuspended in acetonitrile and triethylamine was added to neutralize 15 and catalyze its reaction with 8 to form 16. The yield of 16 ranged from 75 to 80%. Reaction of 16 with [^{14}C]dimethylamine hydrochloride prepared from 100 mCi of [^{14}C]methyl iodide by the method of

Dutton and Heath[10] afforded 11 which was contaminated with 14 and unreacted 16. This material was loaded on a silica gel column for chromatography, but was allowed to stand 18 h prior to carrying out the chromatography to ensure that all of the 16 had decomposed to 14. This was done because separation of 11 from 14 was much easier than its separation from 16. Completion of the chromatography gave a 75% yield of pure 11. The 11 was converted to [carbamate methyl-^{14}C]pyridostigmine bromide (12) by reaction with methyl bromide in acetone. The overall radiochemical yield of 12 based on [^{14}C]methyl iodide was 67%.

[6-^{3}H]Pyridostigmine bromide was prepared by the procedure outlined in Scheme 4. Reaction of 2,6-dibromo-3-hydroxy pyridine (17)[11] with dimethylcarbamoyl chloride gave carbamate 18. Reduction of 18 with carrier free tritium gas and 5% palladium on charcoal afforded 19 which was quarternized with methyl bromide to give [2,6,-$^{3}H_2$]pyridostigmine bromide (20). A methanol solution of 20 was found to contain a considerable amount of volatile radioactivity after storage overnight. Consequently, a series of methanol exchanges were carried out until there was less than 1% volatile radioactivity after storage in methanol for three days. The ^{3}H NMR spectrum of the product after the exchange showed that > 99% of the tritium was in the 6-position

Scheme 4



17 18 19 20 21

(a) Me_2NCOCl; b) T_2, Pd/C; c) CH_3Br

(δ 8.78) with no detectable tritium in the 2-position. Exchange studies in pH 11 and pH 7 buffers showed less than 1% exchange after 24 h. The specific

activity of the product was 22.5 Ci/mmol, and the radiochemical purity was 96%.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Varian Model 2290 spectrometer. ^3H NMR spectra were recorded on a JEOL FX90Q Fourier transform spectrometer. E. Merck silica gel 60 F-254 and aluminium oxide 150 F-254 analytical plates were used for analytical TLC. Radioactive samples were counted on a Packard Tri-Carb 4000 liquid scintillation spectrometer using an internal standard in Scintiverse E cocktail. Developed TLC plates were scanned on a Berthold Model LB 283 Linear Analyzer system. Carrier-free tritium gas was purchased from DuPont/New England Nuclear Corporation.

2-Furan[^{14}C]carboxylic Acid (2)[8]

To a 100 mL one-neck flask having a septum capped side arm was added tetrahydrofuran (28 mL) and furan (1.1 mL, 15 mmol). These two materials had been distilled from $LiAlH_4$ immediately prior to use. The flask was flushed with argon and attached to the vacuum manifold which was also flushed with argon. A positive pressure of argon was maintained in the manifold at all times it was not being held at high vacuum. The reaction flask was cooled to -25°C and n-BuLi (8.2 mL, 1.6 M, 13.1 mmol) was added via syringe through the septum. The resulting mixture was stirred for 4 h at -25° to -10°C. The mixture was isolated from the manifold under an argon atmosphere.

A CO_2 generator containing $BaCO_3$ (1791 mg, 9.07 mmol), [^{14}C]$BaCO_3$ (688 mg, 0.295 mCi/mg, 230 mCi) and H_2SO_4 (15 mL) was attached to the manifold, was evacuated (0.05 Torr) for 1.5 h, and then was isolated from the vacuum line while keeping full vacuum in the generator.

The reaction flask was cooled to -45°C and was opened to the CO_2 generator. The CO_2 was generated over 20 min by careful addition of H_2SO_4 to the [^{14}C]$BaCO_3$ mixture. The reaction mixture was cooled to -65°C while warming the CO_2 generator to 70°C with a warm water bath. The reaction mixture was stirred for 30 min and was warmed to -10°C. Water (1 mL) followed by H_2SO_4 (6 N, 5 mL) was added through the septum. The resulting mixture was poured into a separatory funnel, and the layers were separated. The aqueous

layer was extracted four times with Et_2O. The combined organic extracts were dried (Na_2SO_4) and stripped to give 820 mg (59%) of crude acid 2. The crude 2 was bulb-to-bulb distilled (105-120°C at 0.04-0.02 Torr) to give 737 mg (53% chemical yield) of white crystalline 2. The total radioactivity was 135 mCi (66.5%).

2-Furan[^{14}C]carboxamide (3)

A solution of potassium hydroxide (406 mg, 7.25 mmol) in H_2O (4 mL) was used to wash the 2-furan[^{14}C]carboxylic acid (737 mg, 6.58 mmol) from the distillation bulb into a 50 mL round bottom flask. This solution was stripped to dryness, benzene (5 mL) was added, and the slurry was again stripped to dryness. The white residue was dried in vacuum (0.1 Torr) for 1 h while being warmed with a water bath. The residue was slurried in benzene (8 mL), and thionyl chloride (15 mL) was added dropwise followed by dimethylformamide (2 drops). The mixture was stirred for 18 h at 40°C. The benzene and excess thionyl chloride were stripped, and the residue was dissolved in $CHCl_3$ (10 mL, dried by passing through a column of basic Al_2O_3). The $CHCl_3$ solution was cooled in an ice bath while ammonia gas was passed through it for 30 min. This mixture was stirred for 30 min and then was poured into a separatory funnel containing H_2O (10 mL). This solution was extracted seven times with $CHCl_3$. The combined $CHCl_3$ extracts were dried (Na_2SO_4) and stripped to give 605 mg (75%) of 3 as a tan solid of > 98% radiochemical purity. The total radioactivity was 102 mCi (73.3%).

2-Amino[^{14}C]methylfuran (4)

To a 50 mL round bottom flask containing $LiAlH_4$ (345 mg, 9.09 mmol) was added THF (4 mL, dried by passing through a column of basic Al_2O_3). A slurry of amide 3 (605 mg, 5.45 mmol) in THF (15 mL) was added dropwise to this mixture, and the reaction was heated at 40°C for 20 h. TLC (SiO_2:$CHCl_3$-acetone-NH_4OH, 17:8:1) at this time indicated ~ 85% conversion to the desired amine 4. Additional $LiAlH_4$ (~ 150 mg) was added and the mixture heated at 40°C for an additional 22 h. TLC (same system) indicated 92% conversion to 4. The reaction mixture was cooled, and 50% NaOH solution was added dropwise until no additional gas evolution was evident. Solid NaCl (~ 1 g) was added

with stirring. The organic layer was decanted and was dried over Na_2SO_4. The aqueous layer which stuck to the inside of the reaction flask was washed five times with Et_2O, and the Et_2O washes were combined with the first organic layer. Water (5 mL) was added to the reaction flask to obtain a mobile slurry. The slurry was poured into a separatory funnel and extracted three times with Et_2O. The ether extracts were combined with the other organic extracts and dried (Na_2SO_4). The solvents were stripped to leave an oily residue. The residue was transferred to a tared 50 mL centrifuge tube with Et_2O, and a solution of Et_2O saturated with HCl was added. The resulting white crystals were collected, washed three times with Et_2O and dried in vacuo to give 488 mg (67%) of the HCl salt of 4. The total radioactivity was 70.3 mCi (69% radiochemical yield).

3-Hydroxy[2-^{14}C]pyridine (5)

The hydrochloride salt of 4 (488 mg, 3.66 mmol) was dissolved in a solution of H_2O (0.8 mL) and HCl (3 N, 2.5 mL). Hydrogen peroxide (30%, 0.5 mL) was added dropwise, and the resulting mixture was heated at reflux for 1 h. TLC (SiO_2:CH_2Cl_2-CH_3OH, 9:1) indicated some amine 4 was still present. The reaction was heated at reflux for one additional hour and was stirred overnight at room temperature. TLC (same system) indicated the reaction was complete. The reaction mixture was placed in a continuous extractor and was extracted with Et_2O for 38 h. The Et_2O was stripped, the residue was dissolved in EtOAc and filtered through 5 cm of Na_2SO_4 in a Pasteur pipette. The EtOAc was stripped to give 253 mg (73%) of 5 as white crystals. The total radioactivity was 47.9 mCi (68% radiochemical yield).

3-(N,N-Dimethylcarbamoyloxy)[2-^{14}C]pyridine (6)

To a solution of 5 (253 mg, 2.66 mmol) dissolved in pyridine (5 mL) was quickly added a solution of dimethylcarbamyl chloride (1273 mg, 11.8 mmol) in pyridine (5 mL). (Note: This solution will set to a semi-solid gel if not quickly added after mixing.) The reaction mixture was heated at reflux for 2 h, was cooled, and the pyridine was stripped. The residue was dissolved in Et_2O and a small amount of H_2O. The layers were separated, and the aqueous layer was extracted five times with Et_2O. The combined ether washes were

washed with brine, dried (Na_2SO_4) and stripped. The resulting crude 6 (540 mg) was bulb-to-bulb distilled (70-100°C at 0.4 Torr) to give 380 mg (86%) of 6 as a colorless oil. The total radioactivity was 42.5 mCi (89% radiochemical yield).

[2-^{14}C]Pyridostigmine Bromide (7)

A solution of 6 (380 mg, 2.29 mmol) in Et_2O was placed in a tared 50 mL centrifuge tube and the Et_2O was removed with a stream of dry nitrogen. The oily residue was dissolved in acetone (1.5 mL). To this solution was added a 50% solution of CH_3Br in acetone (1.5 mL), and the mixture was allowed to stand at room temperature overnight whereupon crystallization occurred. The supernatant was pipetted from the white crystals. The crystals were rinsed twice with 1 mL of 50% Et_2O-acetone and three times with 5 mL of Et_2O. The residue was dried in vacuo for 1 h to give 519 mg (87%) of 7 as white crystals, mp 154-155°C (cap) (lit.[12]: 152-154°C). The specific activity was determined to be 18 mCi/mmol, 69 mCi/mg. The total radioactivity was 35.8 mCi (17.6% radiochemical yield from [^{14}C]-$BaCO_3$). The radiochemical purity was determined by radio-TLC as follows: 99%, [SiO_2, CH_3OH-HOAc (25:1), R_f 0.06]; 98%, [Al_2O_3, CH_3OH-CCl_4-HOAc (70:30:3), R_f 0.63].

Bis-3-pyridyl Carbonate (16)

A solution of 3-hydroxypyridine (14) (6.65 g, 70.0 mmol) in acetonitrile (500 mL) was saturated with phosgene over ~ 20 min. During this time a white precipitate formed. The resulting mixture was stripped to dryness in the hood. The residue was slurried with CH_3CN (50 mL) and again stripped to dryness to ensure removal of any excess phosgene. The residue was suspended in CH_3CN (200 mL) and triethylamine (7.08 g, 70.0 mmol, 9.75 mL) was added to give a homogenous solution. This solution was stripped to dryness and the residue was dissolved in CH_2Cl_2 and washed thrice with H_2O. The CH_2Cl_2 solution was dried (Na_2SO_4) and stripped to give a thick slurry. Hexane (100 mL) was added to the slurry and the resulting mixture was filtered. The residue was rinsed thrice with hexane and dried under a vacuum to give 5.55 g (73%) of 16 as a white solid: mp(cap) 85-90°C; IR (CH_2Cl_2) 1790 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.31 (ABq, 2, $J_{5,6}$ = 5 Hz, $J_{3,4}$ = 8 Hz, C_5 H), 7.63 (ddd, 2,

$J_{4,5}$ = 8 Hz, $J_{4,6}$ = 1.5 Hz, $J_{4,2}$ = 3 Hz, C_4 H), 8.37 (dd, 2, $J_{6,5}$ = 5 Hz, $J_{6,4}$ = 1.5 Hz, C_6 H), 8.57 (d, 2, $J_{2,4}$ = 3 Hz, C_2 H). The ^{1}H NMR assignments were confirmed by decoupling experiments.

3-(N-[^{14}C]Methyl-N-methylcarbamoyloxy)pyridine (11)

[^{14}C]Dimethylamine hydrochloride was prepared from 100 mCi of [^{14}C]methyl iodide (specific activity 36 mCi/mmol) by the method of Dutton and Heath.[10] The product from this preparation was placed in a 100 mL flask and 685 mg (3.17 mmol) of bis-3-pyridyl carbonate (16) and 4 mL of acetonitrile were added. The flask was tightly stoppered and was heated in an oil bath at 85° C or 2 h. After cooling, the mixture was stirred at room temperature for 64 h. The reaction mixture was concentrated to dryness with a stream of N_2 and the resulting residue was extracted thrice with hexane and twice with CH_2Cl_2-hexane (8:1) to remove 11 from 14. The progress of the extractions was followed by TLC (SiO_2:40% $CHCl_3$-Acetone). The extracts were stripped to leave a residue of 11 containing a small amount of 16. This material was applied to the top of a SiO_2 (22 g) column which had been packed with CH_2Cl_2. The mixture was washed into the top of column with CH_2Cl_2 and the column was allowed to stand 18 h before elution was started. This waiting period allowed the SiO_2 to decompose 16 to 14. The column was eluted with CH_2Cl_2 (400 mL), CH_2Cl_2-$CHCl_3$ (1:1, 200 mL) and then $CHCl_3$ while collecting 50 mL fractions. Pure 11 was obtained in fractions 18-33. These were combined and the solvent was removed on the rotary evaporator to give 78.6 mCi of an oily residue which was used in the next step without further purification.

[carbamate methyl-^{14}C]Pyridostigmine Bromide (12)

The oily residue of 11 prepared above was dissolved in Et_2O and filtered through a short column of Na_2SO_4. The filtrate was collected in a tared 15 mL screw cap centrifuge tube. The Et_2O was evaporated with a stream of N_2 and the residue was dissolved in acetone (1.5 mL). To this solution was added methyl bromide-acetone (1:1, 1.5 mL). The mixture was allowed to stand at room temperature overnight. The resulting crystals were stirred by means of the Vortex stirrer for 10 sec and then centrifuged. The supernatant was removed and saved. The crystals were rinsed (stirred as before and

centrifuged) twice with acetone-Et_2O (1:1, 1 mL) and thrice with Et_2O (3 mL). All of the rinses were added to the first supernatant. The combined solution was allowed to stand 4 h to allow a 2nd crop of crystals to form. These were collected and rinsed in the same manner as the first crop. The supernatant and rinses were evaporated with a stream of N_2. The residue was dissolved in acetone (0.5 mL) and CH_3Br-acetone (1:1, 0.5 mL) and a seed crystal of 11 was added. The mixture was allowed to stand at room temperature for 18 h. No additional crystals formed, so the solution was concentrated with a stream of nitrogen and the oily residue was dried under high vacuum. After 4 h the oily residue had crystallized to leave an off-white solid. Et_2O (5 mL) was added and the solid crushed with a glass stirring rod to obtain a white powder. The crystals were collected by centrifugation and were rinsed thrice with Et_2O. After drying under vacuum for 4 h, 71 mg of white powder was obtained.

The first and second crops each had mp 154-155°C (Lit.[12], 152-154°C) and were combined to give 73.2 mCi (508 mg) of 12 with specific activity of 37.6 mCi/mmol. The radiochemical purity was determined by radio-TLC as follows: > 99%, [SiO_2; CH_3OH-HOAc (25:1), R_f 0.02]; > 99% [Al_2O_3; CH_3OH-CCl_4-HOAc (70:30:3), R_f 0.66].

The third crop (71 mg) had mp 148-152°C, but was > 99% radiochemically pure when analyzed by the TLC systems described above.

2,6-Dibromo-3-dimethylcarbamyloxypyrdine (18)

A solution of dimethylcarbamoyl chloride (3.78 g, 35.2 mmol) in pyridine (15 mL) was prepared and quickly added to a solution of 2,6-dibromo-3-hydroxypyridine[11] (2.02 g, 8.00 mmol) in pyridine (20 mL) at 25°C. This mixture was heated at 120°C for 2 h and then cooled and stirred at 25°C for 18 h. Analysis by TLC [(Al_2O_3, $CHCl_3$), (SiO_2, 95% $CHCl_3$-CH_3OH)] indicated the reaction was complete. The pyridine was evaporated under reduced pressure and the residue was dissolved in Et_2O and H_2O. The ether layer was separated, and the aqueous layer was extracted thrice with Et_2O. The combined Et_2O extracts were washed once with saturated NaCl solution, and was dried over $MgSO_4$. After filtration, the Et_2O was evaporated to leave 2.78 g of a white solid. An

analytical sample was prepared by crystallization from CH_2Cl_2-hexane: mp 83-84°C; IR ($CHCl_3$) 1728 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.34 (s, 2, ring-H); 3.10 (s, 3, N-CH_3); 2.98 (br s, 3, N-CH_3).

Anal. Calcd. for $C_8H_8Br_2N_2O_2$: C, 29.66; H, 2.49; Br, 49.33; N, 8.65. Found: C, 29.81; H, 2.60; Br, 49.44; N, 8.72.

[6-^{3}H]Pyridostigmine Bromide (21)

A mixture of 2,6-dibromo-3-dimethylcarbamyloxypyridine (9.7 mg, 0.030 mmol), magnesium oxide (6 mg), 5% palladium on charcoal (3 mg) and ethanol (300 μL) was stirred under tritium (5 Ci) for 4 h. The reaction mixture was washed through a short column of Celite (3 mL) with ethanol. The ethanol was removed under vacuum and the residue taken up in ethanol (1 mL), and the solvent again removed under vacuum. This process was repeated twice to remove any easily exchangeable tritium. The residue was dissolved in $CHCl_3$, and the solution was washed through a short column of silica gel (3 mL). The $CHCl_3$ eluant (~ 25 mL) contained product, 19, that was ~ 98% radiochemically pure. The $CHCl_3$ was evaporated, and the residue was dissolved in CH_3Br-acetone (1:1, 20 mL) and stored in the refrigerator (5° C) for 18 h. TLC (SiO_2, 95% $CHCl_3$-CH_3OH) analysis indicated the reaction was 60% complete. The mixture was stored at ~ 25° C for 20 hours. TLC (same system) indicated the reaction was complete. The CH_3Br-acetone was evaporated and the residue was rinsed with Et_2O-acetone (1:1, 4 x 100 μL). The residue was dissolved in CH_3OH and transferred to a 500 mL volumetric flask. A determination of volatile radioactivity indicated the presence of a large amount of exchangeable tritium. The entire sample was evaporated and the residue taken up in CH_3OH (500 mL) and the solvent was removed on the rotary evaporator. This process was repeated (4 times) until there was less than 1% volatile radioactivity after storage for 3 days. Tritium NMR indicated the majority (> 99%) of the tritium was in the 6 position (δ 8.78). There was no detectable tritium in the 2 position. Exchange studies in pH 7 and pH 11 buffers showed 1% or less exchange after 24 hours. Based on a UV determined weight, the specific activity was found to be 22.5 Ci/mmol, 86 mCi/mg. A total of 342 mCi was prepared. The radiochemical purity was determined by radio-TLC to be: 96%, [SiO_2, CH_3OH-HOAc (25:1), R_f 0.12]; 96%, [Al_2O_3, CH_3OH-HOAc (25:1), R_f 0.59].

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