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Research Article

A convenient synthesis of [^{11}C]paraquat and other [*N*-methyl-^{11}C]bisquaternary ammonium compounds

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Summary

[^{11}C]Paraquat was synthesized by the reaction of [^{11}C]methyl triflate with the mono-triflate salt of 1-methyl-[4,4′]bipyridinyl. The product was selectively separated from the precursor by a microcolumn of Chelex 100 ion exchange resin. The method was applied to the synthesis of a variety of [*N*-methyl-^{11}C]bisquaternary ammonium compounds. This is the first reported use of a chelating cation exchange resin for the selective purification of organic dications. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: [^{11}C]paraquat; Chelex-100; [^{11}C]hexamethonium; [^{11}C]methyl triflate

Introduction

Bisquaternary ammonium compounds may be selectively concentrated in tissues by cation transport[1] and by affinity for anionic sites.[2] The availability of carbon-11 labelled bisquaternary ammonium compounds as PET tracers might help to explain the toxicity of such compounds as well as to suggest possible diagnostic uses based on dication transport

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systems or specific tissue affinities. Paraquat **1c** is a mitochondrial toxin that is avidly concentrated in the lungs, heart and other organs.[3] It has been implicated in some studies as a possible environmental cause of neurodegenerative disorders,[4,5] though the mechanisms by which it may cross the blood–brain barrier are not well explained.[6,7] Carbon-11 labelled paraquat may help to identify conditions resulting in enhanced CNS uptake in intact animals.

Hexamethonium **3c** is a ganglionic blocker, which also binds avidly to collagen.[2] Once used clinically, it has been implicated in a recent fatality[8] apparently as the result of unexpected lung damage from an aerosol preparation. Carbon-11 labelled hexamethonium may be useful as a diagnostic agent as well as to shed light on possible pulmonary toxicity. A synthesis of carbon-11 labelled hexamethonium has been reported,[9] however, the procedure required several steps, starting with *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine, and necessarily resulted in a product with low specific activity.

We observed the following considerations in developing a new, general synthesis of carbon-11 labelled bisquaternary ammonium compounds: (1) In order to obtain the highest possible specific activity the bisquaternary compounds should be obtained in one step by carbon-11 methylation of the monocationic precursor. (2) As hydrophilic dications such compounds should be capable of isolation by trapping on microcolumns of chelating ion exchange resin. (3) The higher reactivity of [^{11}C]methyl triflate compared to [^{11}C]methyl iodide should allow instantaneous methylation at room temperature. (4) Use of a triflate salt of a monocationic precursor should offer two advantages over halide salts. Triflate salts tend to be highly soluble in polar solvents such as DMSO, and the triflate anion cannot attack [^{11}C]methyl triflate as a competitive nucleophile as halide ions may. We describe here a simple synthesis of [^{11}C]paraquat which incorporates the above considerations. Other carbon-11 labelled bisquaternary ammonium compounds (see Figure 1) were prepared in the same way in high radiochemical purity and satisfactory yields. [^{11}C]Hexamethonium presented a special problem for purification in that it was not as strongly retained on the chelating resin relative to the monocationic precursor.

Results and discussion

The formation of [^{11}C]paraquat from [^{11}C]methyl triflate occurred within a few seconds in DMSO or acetonitrile at room temperature. The

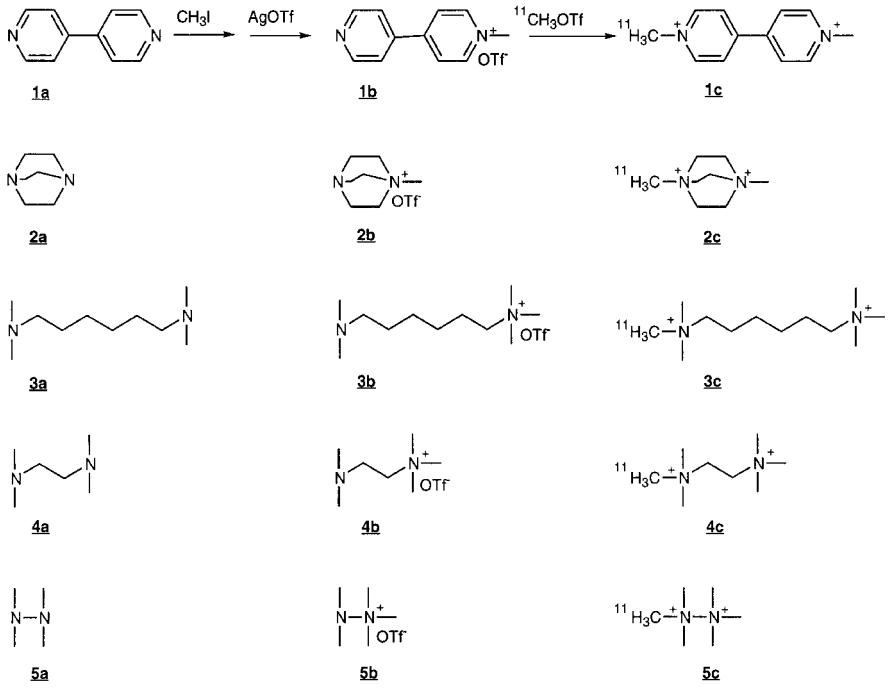

Figure 1. Synthesis of carbon-11 bisquaternary ammonium compounds and their precursors

subsequent steps – dilution with aqueous ammonia, trapping on the chelating cation exchange resin, selective elution of the precursor with aqueous *n*PrOH/NaCl/NH_4OH and recovery of the product in normal saline required about 4 min. In a representative experiment approximately 300 mCi [^{11}C]paraquat with a specific activity of 2200 Ci/mmole were obtained from 1400 mCi [^{11}C]CO_2. The radiochemical purity was $> 99\%$. The precursor was present at the level of 0.13 μg/ml, compared to 2.6 μg/ml for paraquat. Table 1 summarizes the results obtained for [^{11}C]paraquat and other carbon-11 labelled bisquaternary compounds obtained by the same method.

Figure. 2 shows elution profiles of paraquat **1c** and its precursor **1b** from microcolumns of two different forms of chelating ion exchange resin, Chelex-100 (Na^+ form, 200–400 mesh, Bio-Rad) and a chelating cation exchange fiber (TIN-600, Toray), simulating conditions for the radiochemical synthesis. Since it was necessary to use widely different UV detector settings to accommodate the large excess of precursor over product encountered in the radiochemical synthesis, the base line was adjusted after the elution of the precursor. Of the compounds labelled,

Table 1. Summary of results of radiosynthesis of carbon-11 bisquaternary ammonium compounds

Compound (*n*) experiments	Counter anion	Reaction solvent	Radiochem. yield[a]	Radiochem. purity (%)	Resin
1c (4)	OTf^-	MeCN	69	> 99	TIN-600
1c (2)	OTf^-	MeCN	63	> 99	Chelex-100
1c (1)	I^-	MeCN	74	> 99	TIN-600
1c (1)	OTf^-	Water	58	97	TIN-600
1c (1)	OTf^-	DMF	15	> 99	TIN-600
2c (1)	OTf^-	MeCN	63	> 99	Chelex-100
2c (1)	I^-	MeCN	26	> 98	TIN-600
3c (1)	OTf^-	MeCN	74	> 99	TIN-600
4c (1)	OTf^-	MeCN	52	> 99	Chelex-100
5c (1)	OTf^-	DMSO	1.6	88	TIN-600

[a] Approximate uncorrected radiochemical yield based on the amount of [^{11}C]methyl triflate distilled.

only paraquat had a sufficiently strong chromophore to permit obtaining an elution profile based on ultraviolet absorbance. However, compounds **2c** and **4c** behaved qualitatively the same as paraquat in their affinity for the chelating cation exchange resin, as judged by the well-defined trapping of radioactivity on the microcolumn and subsequent displacement by normal saline. In contrast [^{11}C]hexamethonium **3c** was not as strongly retained on the resin. Apparently, steric constraints interfered with specific binding to the dianionic site of the resin. For [^{11}C]hexamethonium, conditions have not been established for the selective removal of the precursor from the resin. While the results show that [^{11}C]hexamethonium can be obtained in high radiochemical purity, additional work must be done to find a weak ionic eluant to selectively remove the large excess of precursor for applications requiring a high specific activity.

The elution profiles indicate that both the fibrous and spherical forms of chelating cation exchanger are satisfactory for the separation of [^{11}C]paraquat from its monocationic precursor. The peak for paraquat is sharper for the Chelex-100 resin at the low flow rate used to obtain the UV elution profiles. In practice, the fibrous resin[10] was preferred because of the ease of packing and compatibility with high flow rates. This resin, however, is not commercially available at present. Apparently, this is the first reported use of a chelating resin to isolate organic dications such as paraquat.

The use of an ion-pairing HPLC method for monitoring the synthesis of hydrophilic organic dications[11] simplifies the interpretation of experiments, in that the retention times are substantially determined

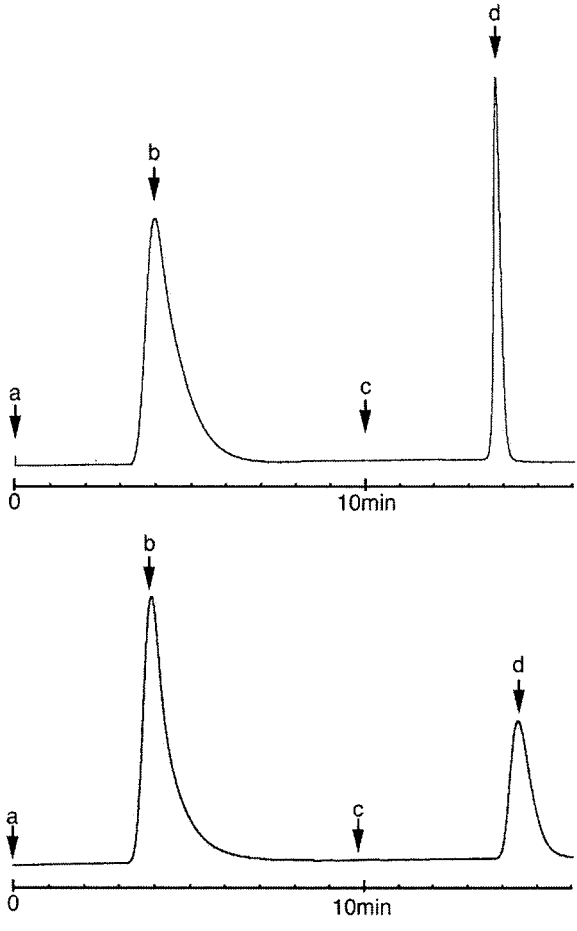

Figure 2. Elution profiles of paraquat (d) and its monocationic precursor (b) from chelating ion exchange resins. Upper trace: Chelex-100 resin. Lower trace: TIN-600 fibrous resin. (a) Inject simulated reaction mixture while eluting with a solution of 15% *n*-propanol, 0.5% NaCl and 0.3% ammonium hydroxide in water. (c) Begin elution with normal saline

by the charge alone. All of the carbon-11 labelled bisquaternary ammonium compounds had essentially the same retention times. Thus, we were able to obtain evidence for the stability of the previously unreported hexamethyl hydrazinium dication **5c**[12] by reacting compound **5b**[13] with [^{11}C]methyl triflate.

With the general method described above, high specific activities are possible provided that the monocationic precursors are not contaminated by small amounts of the unlabelled dication. In the case of

paraquat the presence of a strong UV chromophore allowed the precursor to be highly purified by recrystallization. While ^{1}H NMR analysis of the other precursors indicated them to be substantially pure, contamination by small but significant amounts of the dications could not be ruled out. For some applications of these compounds, as tracers, it would be necessary to develop HPLC methods able to detect them at trace levels, so that the precursors could be rigorously purified by recrystallization.

Experimental

General

Starting bisdiamines **1a**, **2a**, **3a** and **4a** were purchased from Aldrich Chemical Co. *N*,*N*,*N*′*N*′-tetramethylhydrazine **5a** was purchased from Fluka Chemical Co. Proton NMR spectra were obtained on a Bruker DRX500 instrument using a TMS internal standard. Melting points were obtained with a Mel-Temp apparatus (Laboratory Devices, Cambridge, MA). The HPLC system consisted of a Spectra Physics SP8800 ternary pump, Hitachi L4000 detector and HP3395 integrator. The radioactivity of the HPLC effluent was measured by a sodium iodide crystal coupled to a photomultiplier module and an Ortec 449 ratemeter.

General procedure for monocationic triflate precursors

(Compounds **1b**–**4b**). Methyl iodide was reacted at room temperature in acetonitrile with a ten-fold molar excess of the corresponding bisdiamine. In this way, the monocationic product was favored over the dication, albeit at the expense of a low chemical yield. The resulting monocationic iodide salt was washed with ether and recrystallized repeatedly from isopropanol, ethyl acetate, diisopropyl ether or mixtures thereof. This salt was then dissolved in water and reacted with a slight molar excess of aqueous silver triflate. Silver iodide was removed by filtration through diatomaceous earth. The aqueous filtrate was concentrated to dryness and the resulting solid was washed with ether. The precuresor for paraquat **1b** was recrystallized as the triflate salt from isopropanol. Due to the high solubility of the other triflate salts **2b**–**5b** in organic solvents, these were not recrystallized at the triflate salt stage.

Compound **1b**, triflate salt (from 4,4′-dipyridyl **1a**). ^{1}H-NMR (500 MHz, DMSO) δ 9.13 (2H, d, $J = 6.75$ Hz), 8.88 (2H, d, $J = 6.14$ Hz), 8.62 (2H, d, $J = 6.9$ Hz), 8.04 (2H, d, $J = 6.2$ Hz), 4.39 (3H, s, *N*-Me). HPLC UV 300 nm, >99%; m.p. 130–132°C (lit.[14] 135°C).

Compound **2b**, triflate salt[15] (from diazabicyclooctane **2a**). ^{1}H-NMR (500 MHz, DMSO) δ 3.25 (6H, t), 3.02 (6H, t), 2.96 (3H, s, *N*-Me).

Compound **3b**, triflate salt (from *N*,*N*,*N*′,*N*′-tetramethylhexanediamine **3a**). ^{1}H-NMR (500 MHz, DMSO) δ 3.24 (2H, m), 3.05 (9H, *N*-Me, s), 2.24 (2H, m), 2.15 (6H, *N*-Me, s), 1.68 (2H, m), 1.43 (2H, m), 1.33 (4H, CH_2, m).

Compound **4b**, triflate salt (from *N*,*N*,*N*′,*N*′-tetramethylethanediamine **4a**). ^{1}H-NMR (500 MHz, DMSO) δ 3.55 (2H, t), 3.31 (9H, *N*-Me, s), 2.76 (2H, m), 2.29 (6H, *N*-Me, s).

Compound **5b**. (from tetramethylhydrazine **5a**). Iodide salt m.p. 223°C, lit.[13] 223–224°C. ^{1}H-NMR (500 MHz, DMSO) δ 3.21 (9H, *N*-Me, s), 2.66 (6H, *N*-Me, s).

Elution profile for paraquat and monocationic precursor

A microcolumn containing 6 mg of Chelex-100 resin, or alternatively, Toray TIN-600 fibrous resin (sodium form, see below) was connected to an HPLC pump and injector via 30 m × 0.1 mm i.d. tubing. The latter provided a pressure drop necessary for proper operation of the pump and also provided a convenient delay between the time of injection and the beginning of the elution of the monocation. The outlet of the microcolumn was connected to a UV absorbance detector. Elution was begun with solution of 15% *n*-propanol, 0.05% NaCl and 0.3% ammonium hydroxide in water. A solution containing 1.0 mg precursor **1b** and 50 μg paraquat hydrochloride in 50 μL water was injected to simulate the amounts of product and precursor in a carbon-11 labelling reaction. Due to the large amount of monocation, the detector was initially set at 326 nm on the shoulder of the UV absorbance. After elution of the monocation was complete, the elution solvent was changed to 0.9% NaCl and the detector was set at 310 nm to monitor the elution of paraquat from the column.

Ion exchange column

The chelating ion-exchange column consisted of 6 mg iminodiacetate functionalized fibrous resin (Toray TIN-600, sodium form) slurry

packed in a 3 mm i.d. polyethylene column (bed length 5 mm). The resin was held in place by small plugs of polypropylene wool (Aldrich). The end fittings were miniature barbed polypropylene male luer adapters (Value Plastics, Fort Collins, CO 80525, USA).

Radiosynthesis

The precursor (triflate salt, 1 mg) was dissolved in 0.1 ml anhydrous acetonitrile. [^{11}C]Methyl triflate, prepared by the gas-phase reaction of [^{11}C]methyl iodide with silver triflate[16] at 180°C, was distilled into this solution in a stream of nitrogen at room temperature. When maximum radioactivity had accumulated the reaction mixture was diluted with 4 ml water containing 0.3% NH_4OH (pH 11) and passed through the ion exchange column. The column was washed with 10 ml of a solution of 15% *n*-propanol, 0.05% NaCl and 0.3% ammonium hydroxide in water, followed by 2 ml of water to remove the precursor. The radioactive product was eluted from the column with 4–10 ml 0.9% NaCl. In the case of [^{11}C]hexamethonium the column was washed with water but not with *n*PrOH/NH_4OH/NaCl, since this solution was sufficiently strong to elute the product. The radioactivity of the product and waste fractions were measured in a Capintec ionization counter. Approximate uncorrected radiochemical yields were obtained by dividing the radioactivity in the pure product by that of the total amount of radioactivity trapped as [^{11}C]methyl triflate.

Analysis by HPLC

The radioactive product was analyzed by reversed phase, ion-pair HPLC on a Keystone BetaBasic 8 column (4.6 × 150 mm). The solvent consisted of 7.5 mM sodium octanesulfonate and 20 mM $NH_4H_2PO_4$ in 20% aqueous acetonitrile, pH 4.0, 1.5 ml/min. Radioactivity was detected by a sodium iodide crystal flow detector. For [^{11}C]paraquat specific activity was determined by UV absorbance at 254 nm. Retention times were 6.1 min for paraquat and 2.7 min for the precursor.

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