I-125 and I-123 LABELLED IODOBENZYL BROMIDE, A USEFUL ALKYLATING AGENT FOR RADIOLABELLING BIOLOGICALLY IMPORTANT MOLECULES

Alan A. Wilson, Robert F. Dannals, Hayden T. Ravert, H. Donald Burns, and Henry N. Wagner, Jr.
Divisions of Nuclear Medicine and Radiation Health Sciences
The Johns Hopkins Medical Institutions, Baltimore, Maryland

SUMMARY

The radioiodination of 4-trimethylsilylbenzyl bromide in acetic acid with no carrier added I-125 and I-123 iodide ion and N-chlorosuccinimide (NCS) is a rapid, high yielding process which produces high specific activity 4-[I-125 or I-123]iodobenzyl bromide. This radiolabelled alkylating agent has been efficiently reacted with N,N-dimethylaniline and triphenylphosphine to generate radiolabelled quaternary ammonium and phosphonium salts, and should be useful for labelling other biologically important molecules which possess suitable nucleophilic functional groups.

Key Words: Radioiodination, 4-iodobenzyl bromide, 4-trimethylsilylbenzyl bromide, I-125, I-123, lipophilic cations

INTRODUCTION

The need for single photon emitting radiotracers of high specific activity has generated many new methods for the introduction of radionuclides of iodine and bromine into organic molecules.[1] In particular, demetallation of aryltrimethylsilanes[2] is promising as a general method for the fast, regiospecific introduction of radiohalogens into activated[3] and non-activated[4] aromatic rings. It is desirable to be able to introduce the radiolabel into compounds as the final synthetic step in a reaction sequence, in order to reduce the radiation exposure to the chemist and often results in higher specific activity and a better overall radiochemical, if not chemical, yield. Nevertheless, in some cases it may be necessary to introduce the radiolabel earlier in the reaction sequence for a variety of reasons, e.g., to ensure the regiospecificity of the label[5] or to protect a compound subsequent in the synthesis from harsh radiolabelling conditions.[6] Thus, there is a need

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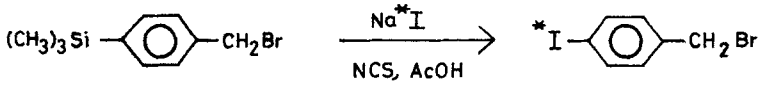
for small, reactive, radiolabelled agents which allow the introduction of a radiolabel into an otherwise difficult or inaccessible portion of some molecules. To this end we describe here the synthesis of high specific activity I-125 and I-123 labelled 4-iodobenzyl bromide 1, a facile alkylating agent, by the demetallation of 4-trimethylsilylbenzyl bromide 2. While I-125 labelled 1 has been prepared before,[7] it has never been synthesised without added carrier. The uses of this radiolabelled alkylating agent are also discussed.

RESULTS AND DISCUSSION

Preparation and isolation of radiolabelled 4-iodobenzyl bromide 1 .

4-Trimethylsilylbenzyl bromide 2 was prepared by slight modifications of published methods[8] from 4-bromotoluene via 4-trimethylsilyl toluene.[9] For radioiodination studies, 2 was purified by reverse-phase (C_{18}) column chromatography to remove trace amounts of impurities. When 2 was reacted with sodium iodide (I-125 Amersham IMS.30/300) in acetic acid containing N-chlorosuccinimide[2] for 12 min at 65°C near quantitative incorporation of iodide was achieved, yielding the desired product 1 (Table, Runs 1 and 2). The product was isolated from the crude reaction mixture by preparative HPLC to give 80-90% radiochemical yields of high specific activity 1 (600-1400 mCi/μmol) in a total synthesis time of 45 min.

The product was obtained in 3-4 mL of 55% aqueous acetonitrile (the HPLC solvent, see experimental section) and for many applications could be used directly in this solvent system (vide infra). If, however, a different solvent system is desired then the HPLC eluent is extracted with pentane and, after drying, the pentane cautiously removed by spin evaporation at 0°C. The solvent of choice may then be used to take up the radiolabelled 1 for further reactions. At the no carrier added level the product is exceedingly volatile, but with care ca. 80% of the activity collected from the HPLC may be recovered for use in a different solvent system.



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Effect of added base

Although the yields of labelled 1 were found to be comparable when small amounts (<1 mCi) of I-123 iodide (Crocker Nuclear Laboratories) were used instead of I-125 iodide (Amersham), yields were reduced to 30-40% when larger quantities (8-16 mCi) were used. The most striking difference between the commercial sources of radioactive iodide is the pH of the two solutions; the I-123 [iodide] is dissolved in 0.1N NaOH (pH 13) while the I-125 [iodide] is in pH 8-11 aqueous NaOH solution. We therefore investigated the effect of base on the production yields of 4-[I-125]iodobenzyl bromide (Table). As we suspected from the I-123 runs, the addition of hydroxide ions to the reaction mixture had a marked deleterious effect on the radiochemical yields (runs 3 and 4). However, the order of addition was also important. Addition of hydroxide before iodide did not reduce yields as severely as addition after iodide (runs 4 and 5). Also, the presence of large amounts of water reduced the rate of reaction, although the final yield was not markedly affected (run 7). Smaller amounts had little influence on the reaction (run 6). Since acetic acid was present in large excess over hydroxide in all cases, the effect of sodium acetate was investigated. It too reduced yields (runs 8 and 9) with a comparable effect to hydroxide addition prior to iodide (run 5) but the order of addition was not important in this case.

A plausible mechanistic explanation for these observations is not readily apparent to us although one might suspect that the presence or absence of acetyl hypoiodite is involved. In any case it is clearly necessary to use low pH solutions of I-123 for satisfactory radiochemical yields.

TABLE

The Effect of Base on the Production of 4-[I-125]Iodobenzyl Bromide

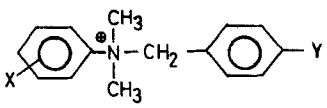
Run #	Conditions[a]	Time (min)	% Yield[b]
1	Standard	5	80
		10	98
		22	98
2	5 µL I-125 iodide	10	97
	(Amersham IMS.300, 2.5 mCi)	22	98
3	2.5 µL of 2M aq. NaOH	10	36
	added after iodide	22	41
		82	42
4	5 µL of 2M aq. NaOH	10	18
	added after iodide	22	13
		50	15
5	5 µL of 2M aq. NaOH	10	68
	added before iodide	22	63
6	93% aq. AcOH used	10	96
	instead of AcOH (glacial)	22	98
7	70% aq. AcOH used	10	78
	instead of AcOH (glacial)	22	92
8	2.0 mg of NaOAc. $3H_2O$	10	--
	added prior to NCS	22	67
		90	64
9	5 µL of 2M aq. NaOAc	10	63
	added after iodide	22	63

(a) Standard conditions were 5.0 mg of NCS dissolved in 80 µL of AcOH in a V-vial, followed by 4 µL of 2 then quickly 5 µL of Na I-125 iodide (Amersham IMS.30, 0.49 mCi). Other conditions were one variable changes of these conditions. (b) See Experimental Section for details.

Examples of uses

Radiolabelled lipophilic cations have been investigated by many groups as myocardial perfusion agents.[10] We were interested in preparing a series of radioiodinated quaternary ammonium cations (3, Y=I-125) in order to investigate the effect(s) of aniline ring substituents on the various parameters[11] which influence myocardial uptake of these radiotracers. One

synthetic approach involves the preparation and radioiodination of (3, Y=trimethylsilyl). However, electrophilic substitution of the parent compound (3,X=H, Y=trimethylsilyl) by radioiodide could not be effected under a variety of conditions. It would appear that the $-CH_2-\overset{+}{N}R_3$ substituent is sufficiently deactivating[12] to prevent this reaction from proceeding at any appreciable rate. However, radioiodinated (3, X=H, Y=I-125) could readily be prepared using 4-[I-125]iodobenzyl bromide. Coupling of the radiolabelled alkylating agent to N,N-dimethylaniline proceeds smoothly in aqueous acetonitrile to give, after HPLC purification, an 85% radiochemical yield of radiochemically pure (3, X=H, Y=I-125). In similar fashion 4-[I-125]iodobenzyltriphenylphosphonium acetate was prepared in 64% radiochemical yield using triphenylphosphine as the nucleophile.



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Thus, radioiodinated 4-iodobenzyl bromide can be prepared in excellent yield and with high specific activity from a readily available precursor, 4-trimethylsilylbenzyl bromide. It reacts with amines and phosphines to generate new labelled compounds not easily prepared by other methods.

We are currently evaluating its use with other nucleophiles, e.g., carboxylic acid anions, aliphatic amines and with carbanions. Such methodology should be useful for the synthesis of high specific activity radioligands for

biochemical investigations and receptor imaging. Since the introduction of radioiosotopes of bromine into non-activated aryltrimethylsilyl derivatives has been demonstrated,[2,4] it should be possible to prepare, e.g., 4-[Br-77]bromobenzyl bromide in a similar manner.[13]

EXPERIMENTAL

All analyses and purifications of radioactive mixtures were performed on a Perkin-Elmer Series 2 HPLC system. The column was a Waters C_{18} Novapak cartridge (10 cm x 8 mm) in a Waters Radial Compression Module. Detection of products was carried out by means of a variable wavelength UV detector and a flow radioactivity detector (CsF crystal) in series. Peak areas were measured using a Hewlett-Packard 3390A recording integrator. Isolated radiochemical yields were measured using a dose calibrator (Capintec CRC-7). No corrections were made for shielding of vessels but geometries were kept constant to minimize this problem. All radiolabelled products were found to co-elute with authentic unlabelled samples.

4-Trimethylsilylbenzyl bromide 2. A mixture of trimethyl-4-tolylsilane[9] (23.9 g, 145 mmol), N-bromosuccinimide (24.7 g, 137 mmol) and benzoyl peroxide (0.15 g, 0.6 mmol) in dry carbon tetrachloride (88 mL) was stirred and heated to reflux for 2.5 hrs then stirred at room temperature overnight. The mixture was filtered and the filtrate washed with ice-cold aqueous 5% NaOH solution (50 mL), ice-cold water (50 mL), dried (Na_2SO_4), and filtered. The solvent was removed to leave a yellow oil which was fractionally distilled through a Vigreux column. The fraction boiling at 105°-110°C/2.9 mm, (lit[8] 125°/10.2 mm) was collected as a colourless oil (21.45 g, 61%).

The product was contaminated by ca. 0.2% of starting material. A portion (1.3 g) was purified by flash chromatography using Waters prep Bondapak C_{18} (55-105 μm) with CH_3CN/H_2O (80/20 v/v) as eluent. A colourless oil (0.85 g), free of contaminants by analytical HPLC, was obtained.

4-Iodobenzyl bromide 1. A mixture of 4-iodotoluene (23.3 g, 107 mmol), N-bromosuccinimide (22.8 g, 127 mmol) and benzoyl peroxide (1.16 g, 4.8 mmol) in dry carbon tetrachloride (35 mL) was stirred and heated to reflux for 3.5 hrs then cooled and filtered. The red filtrate was washed with saturated sodium thiosulphate solution (20 mL), aqueous HCl (1 M, 20 mL), dried (Na_2SO_4), and filtered. The solvent was removed to leave a green solid (21.0 g) which was taken up in boiling hexane, filtered, and cooled to precipitate an off-white solid (15.8 g, 49.7%): mp 78-79.5°C (lit[14] 78.5-79.5°C).

4-[I-125]Iodobenzyl bromide 1. To a stirred solution of N-chlorosuccinimide (5.0 mg) in acetic acid (80 µL), in a 1 mL Reacti-vial, was added 4-trimethylsilylbenzyl bromide 2 (4 µL), followed by aqueous sodium [I-125]iodide (5 µL, 0.5 mCi, Amersham IMS.30). After heating to 65°C for 15 min the mixture was cooled and 100 µL of HPLC solvent added. The total mixture (~200 µL) was injected onto the HPLC column (55/45 CH_3CN/H_2O, 1 mL/min, R_T-4-iodobenzyl bromide, 25 min). Typically the product was collected in 3-4 mL of aqueous acetonitrile. Isolated yields of 80-85% were obtained with specific activities of 600-1200 mCi/µmol. In order to use the product in a solvent other than aqueous acetonitrile, water (4 mL) was added and the labelled material then extracted with pentane (2 x 3 mL). The combined pentane extracts were dried by passage through a small column of sodium sulphate (top half) and magnesium sulphate (bottom half). Careful spin evaporation of the pentane at 0°C left ca. 80% of the collected iodinated material which could then be taken up in the desired solvent.

4-[I-123]Iodobenzyl Bromide 1. To a vial containing aqueous sodium [I-123]iodide (3.0 µL, 0.5 mCi, Crocker Nuclear Laboratories) was added a solution of NCS (5.0 mg) in acetic acid (80 µL), followed quickly by 4-trimethylsilylbenzyl bromide (4 µL). The procedure described for I-125 labeling was then followed. A radiochemical yield of 78% was obtained with a

specific activity of 1100 mCi/μmol. When larger quantities of I-123 were used lower yields were obtained.

N,N,N-4-Iodobenzylphenyldimethylammonium Bromide (3, X=H, Y=I).
N,N-Dimethylaniline (0.456 g, 3.76 mmol) was added to a solution of 4-iodobenzyl bromide (0.77 g, 2.59 mmol) in ethanol (3 mL). The mixture was heated to reflux for 90 min, cooled, and added dropwise to vigorously stirred ether (50 mL). The off-white precipitate was collected by vacuum filtration, washed with ether, and dried in vacuo (0.92 g, 85%): mp 154-156°C, ^{1}HNMR (CD_3OD) = 3.67 (s, 6H), 5.01 (s, 2H), 6.83 (d, J = 7.5 Hz, 2H), 7.3-8.0 (m, 6H). ^{13}CNMR (CD_3OD) = 54.1, 74.1, 98.4, 122.6, 128.6, 131.6, 131.9, 135.4, 139.3, 146.2. Anal. $C_{15}H_{14}NIBr$ requires C, 43.09; H, 4.10; N, 3.35; I, 30.35. Found C, 43.2; H, 4.13; N, 3.33; I, 30.16.

N,N,N-4-[I-125]Iodobenzylphenyldimethylammonium Acetate (3, X=H, Y=I-125).
Ten microliters (10 μL) of freshly distilled N,N-dimethylaniline were added to a solution of 4-[I-125]iodobenzyl bromide (2.03 mCi) in 55% aqueous acetonitrile (4 mL). The solution was heated to 60°C for 10 min and the solvent removed by spin evaporation. The residue was taken up in 1.5 mL HPLC buffer and purified by preparative HPLC (45/55 CH_3CN/0.1N $NH_4CH_3CO_2$ (aq.), 2 mL/min, R_T of product 5.7 min). The product was obtained radiochemically pure in 85% yield with a specific activity of 1200 mCi/μmol.

N,N,N-4-Trimethylsilylbenzylphenyldimethylammonium Bromide (3, X=H, Y=trimethylsilyl). A solution of N,N-dimethylaniline (0.8 g, 6.6 mmol) and 2 (1.0 g, 4.1 mmol) in ethanol (3 mL) was heated to reflux for 2 hrs then cooled. Upon removal of the solvent by spin evaporation the residue was triturated with pentane to leave a white solid (1.51 g, 100%): mp 92.5-95.5°C, ^{1}HNMR (D_2O) = -0.02 (s, 9H), 3.67 (s, 6H), 5.08 (s, 2H), 6.85-7.8 (m, 9H). ^{13}CNMR (D_2O) = 1.2, 56.2, 75.6, 123.9, 131.0, 132.8, 133.0, 134.5, 136.0,

145.4, 146.2. Anal. $C_{18}H_{26}NSiBr.H_2O$ requires C, 56.53; H, 7.38. Found C, 56.68; H, 7.40.

4-Trimethylsilylbenzyltriphenylphosphonium Bromide. To a stirred solution of triphenylphosphine (1.0 g, 3.8 mmol) in THF (3 mL) was added 2 (0.5 g, 2.06 mmol). The resultant solution was heated to reflux for 30 min then cooled. The white precipitate was collected by vacuum filtration, washed thoroughly with ether, and dried in vacuo to give a white powder (0.90 g, 86%): mp 190-220°C (dependent upon the rate of heating); IR (KBr): V max = 2775, 1432, 1241, 1103, and 849 cm^{-1}; ^{1}HNMR ($CDCl_3$) = 6.85-7.72 (m, 19H), 5.11 (d, 2H, J=14.4 Hz), 0.008 (s, 9H). Anal. $C_{28}H_{30}PSiBr$ requires C, 66.01; H, 5.94; Br, 15.68. Found C, 66.29; H, 6.06; Br, 15.75.

4-[I-125]Iodobenzyltriphenylphosphonium Acetate. A solution of 4-[I-125]iodobenzyl bromide (1.25 mCi) in 4 mL of 55% aqueous acetonitrile was treated with 12 mg of triphenylphosphine and the solution stirred at 65°C for 1 hr. The solvent was then removed by spin evaporation and the residue suspended in aqueous ammonium acetate (0.1 N, 5 mL). This was filtered through a Millipore filter to remove triphenylphosphine and then concentrated to ca. 2 mL. The product was obtained by preparative HPLC in two injections (50/50 CH_3CN/ 0.1N $NH_4CH_3CO_2$ (aq.), 2 mL/min, R_T of product 16 min). A total of 795 µCi (64%) of radiochemically pure product was obtained with a specific activity of 370 mCi/µmol.

4-Iodobenzyltriphenylphosphonium Iodide. A solution of triphenylphosphine (2.6 g, 10mmol) and 4-iodobenzyl bromide (1.5 g, 5 mmol) in THF (5 mL) was heated to reflux for 1 hr then cooled. The white precipitate was collected by vacuum filtration, washed thoroughly with ether, and dried in vacuo to give a white powder (2.3 g, 81%). A sample which was recrystalised from aqueous 2-propanol (1:1) had mp 254-258°C; IR (KBr):V max = 2780, 1430, 1100, and 1010

cm^{-1}; ^1H NMR ($CDCl_3$): = 7.6-8.2 (m, 17H), 6.7-6.9 (m, 2H), and 5.23 (d, 2H, J = 15.8 Hz). Anal. $C_{25}H_{21}PIBr.H_2O$ requires C, 50.45; H, 4.23; Br, 13.42. Found C, 50.54; H, 4.23; Br, 13.49.

Radioiodinations with added base. After the addition of all of the reagents (see the Table) the reacti-vial was placed in an oil bath at 65°C and stirred. Aliquots were removed at the appropriate time and injected directly onto the HPLC for analysis (70/30 CH_3CN/H_2O, 2 mL/min, R_T of product 5.1 min). The yields quoted are the normalized ratios of product area to all other peak areas. These values represent real yields, as less than 20 µCi of non-eluting material was found on the column after injection of 2.5 mCi of activity.

Determination of specific activities[15]. An aliquot of the final solution was transferred to a thin plastic vial (to minimize shielding effects) by syringe and its activity measured using a dose calibrator. Another aliquot (of the same volume) was removed by the same syringe, injected onto the HPLC column and the peak area of the product measured. Using a standard curve, obtained by injecting the same volume of standard solutions of varying concentrations under the same conditions, the mass of the product, and hence its specific activity, could be calculated.

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