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Polymer-Supported Radiopharmaceuticals: [^{131}I]MIBG and [^{123}I]MIBG

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Summary

A new method has been developed that produces no-carrier-added [^{131}I]MIBG in ≥ 90 % radiochemical yield and high chemical purity. Isolation and purification are simple involving just filtration and absorption and desorption onto a C18 Sep-Pak™ cartridge. No-carrier-added material should avoid the potential pharmacological side effects accompanying the current method of production.

Key words: [^{131}I]MIBG, solid phase chemistry, 3-iodobenzylguanidine

Introduction

Radioiodinated MIBG (m-iodobenzylguanidine) has found use in nuclear medicine (1) as either an imaging agent for diagnosis or as a therapeutic agent for neural crest tumors such as neuroblastoma. With the shorter-lived iodine-123, $t_{½}$ = 13 h, [^{123}I]MIBG also provides diagnostic cardiac images as well as images of tumors. The longer-lived, $t_{½}$ = 8.02 d, [^{131}I]MIBG is used at much higher radiation and chemical doses for the treatment of tumors.

By far the most common method (1) of producing either [^{123}I]MIBG or [^{131}I]MIBG is by a Cu^{+} catalyzed exchange process which commences with 1-5 mg of MIBG and the desired amount of radioiodide. This method has the drawback of producing [^{131}I]MIBG

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of low specific activity resulting in administered doses of 1-5 mg when therapeutic samples are prepared. Doses of this magnitude carry concern for side effects (2). To this end, routes to no-carrier-added [^{131}I]MIBG have been developed which could reduce the administered dose of MIBG by up to a factor of 100. Precursors to no-carrier-added [^{131}I]MIBG include 3-tributylstannylbenzylamine (3) , 3-trimethylsilylbenzylguanidine (3), 3-trimethyl stannylguanidinium (4,5) and 3-bromobenzylguanidine (6) none of which have found widespread application.

As an attractive alternative route to no-carrier-added [^{131}I]MIBG or [^{123}I]MIBG which also carries the potential for ready isolation and purification, we have been investigating the usefulness of polymer-supported radiopharmaceutical precursors (7). The principle is to prepare an insoluble polymer in which the pharmaceutical to be radiohalogenated is bound to the polymer through a tin-aryl bond as illustrated below. Treatment with the radiohalide and an appropriate oxidant would result in release of the radiohalogenated pharmaceutical, but unreacted polymer and polymer side-products would remain insoluble and easily removed by filtration. This approach has the potential for formation of a no-carrier-added radiopharmaceutical which can be isolated by simple filtration and thus the added potential for 'kit' formulation.

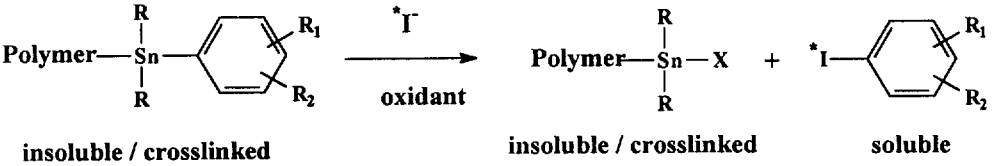

Figure 1. Route to radioiodinated pharmaceuticals using polymer-supported precursors

We wish to report on the conversion of a chlorostannane polymer, 1 (8), into a polymer-supported 3-benzylguanidinium reagent and its use in preparing no-carrier-added [^{131}I]MIBG or [^{123}I]MIBG.

Results and Discussion

Samples of the chlorostannane polymer 1 were prepared as previously reported (7,8) using unpurified commercial divinylbenzene and chlorodibutylstannane. Polymer 1 was characterized both chemically and spectroscopically. The MAS ^{119}Sn NMR showed

one peak at 148 ppm consistent with species of the general structure R_3Sn-Cl. Hydrolysis with NaOH in 50 % ethanol/water at room temperature gave chloride (9) at the level of 1.7 ± 0.1 mmol/g of polymer.

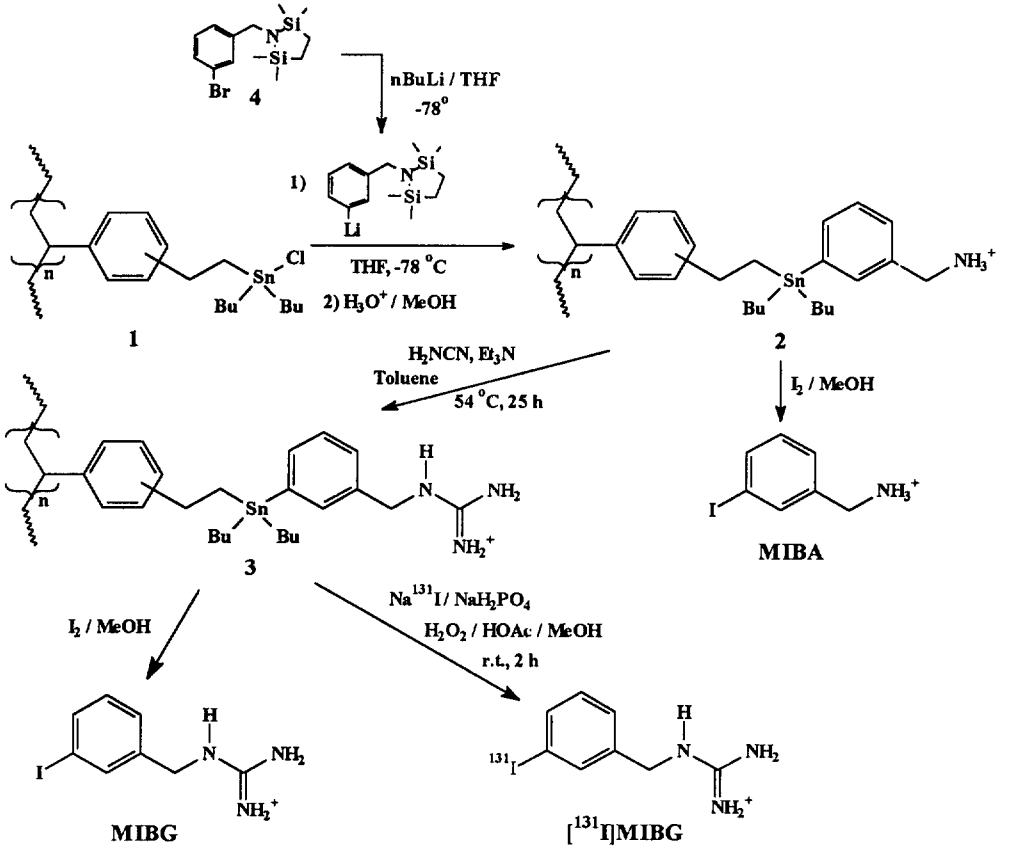

Figure 2. Synthetic sequence to [^{131}I]MIBG

As illustrated in Figure 2, polymer 1 was converted in two steps first into polymer 2 which bears the 3-benzylammonium moiety and then into polymer 3 functionalised with a 3-benzylguanidinium species. Polymer 2 was prepared by reaction of polymer 1 with an excess of the monolithium derivative of 3-bomobenzyl amine with the amino group protected as an azadisilolidine (10). After hydrolysis to remove the protecting group and washing to remove unreacted reagents and side-products, the MAS ^{119}Sn spectrum showed primarily one peak at -43.6 ppm with a small peak at 90 ppm consistent with SnOH and representing about 10 % of the tin signals. Thus all of the Sn-Cl bonds had reacted with the organolithium reagent with a small amount of concurrent hydrolysis.

The extent of reaction in forming polymer **2** and the availability of the 3-benzyl ammonium species in polymer **2** was probed by reaction with iodine as illustrated. The generated 3-iodobenzyl ammonium ion , MIBA, was identified and quantified by HPLC at 0.95 ± 0.05 mmol of MIBA per gram of polymer **2**. A comparison of the MAS ^{119}Sn NMR spectra for polymer **2** and for polymer **2** recovered after iodination, showed a complete loss of the signal at -43.6 ppm consistent with complete release of the 3-benzyl ammonium group. The major peak which appeared at 80 ppm is assigned to Sn-I by comparison to tributyliodostannane (80.6 ppm).

Treatment of polymer **2** with a large excess of cyanamide converted the ammonium group into a guanidinium group as illustrated in Figure 2. The progress of the reaction was followed by iodination. Unreacted polymer **2** results in the release of MIBA (Figure 2) while polymer **3** results in the release of MIBG. After polymer **2** was reacted for 25 h at 54 °C and after washing, polymer **3** was analyzed by iodination to yield MIBG at 1.1 ± 0.05 mmol/g of polymer **3** containing ≤ 0.5 % of MIBA.

With the successful iodination of polymer **3** completed, attention was turned to its radioiodination using sodium [^{131}I]iodide. The success of the radioiodination was assessed by HPLC using both an UV detector and a γ-ray detector. The two types of oxidants chosen, Iodobeads™ and H_2O_2/ acetic acid or their by-products, should be readily separable from the [^{131}I]MIBG produced. When ~ 0.5 mg of polymer **3** was treated with one or two Iodobeads™ in a mixture of methanol, 0.1 M KH_2PO_4 and Na ^{131}I , about 45 % of the radioactivity remained with the insoluble solids and so this oxidant was abandoned.

Instead, H_2O_2/HOAc was examined as a potential oxidizing system. Labelling reactions were run at room temperature on ≤ 1 mg of polymer **3** in a mixture of methanol and 0.1 M KH_2PO_4 using Na ^{131}I. After filtration, essentially all of the radioactivity was found in solution either as $^{131}I^-$ or as [^{131}I]MIBG by their HPLC retention times using a radioactivity detector. The radiochemical yield of [^{131}I]MIBG was increased to near quantitative by increasing the concentration of H_2O_2 and acetic acid at various reaction times. This led to an optimum reaction time of 2 hours using 60 mM H_2O_2 and 70 mM acetic acid.

Purification of the product from the radiolabelling reaction involved two steps. The first was simple filtration through a syringe tip filter. An HPLC analysis of this product using a UV detector showed a large peak at the solvent front and several minor peaks. The radioactivity detector showed two peaks corresponding to $^{131}I^-$ at about 2 % and the other to [^{131}I]MIBG at 98 %. The second step in the purification involved selective absorption and desorption of the [^{131}I]MIBG onto a C18 Sep-Pak™ cartridge. When the primarily aqueous solution from the filtration step was passed through the C18 Sep-Pak™ cartridge, essentially all of the radioactivity was retained on the cartridge with the small amount of iodide passing through. When the cartridge was washed with ethanol/water, 82 % of the radioactivity was released. An additional 10 % was released when methanol was used as eluant.

As shown in Figure 3, an HPLC analysis of these solutions, using an UV detector, showed two small peaks near the solvent front and a small peak at the retention time of MIBG. The area of this peak was too small to measure the specific activity of the [^{131}I]MIBG. The corresponding radioactivity trace showed but one peak at the retention time of MIBG. Thus the no-carrier-added [^{131}I]MIBG was produced in about 92 % radiochemical yield with a specific activity of $\geq$500 Ci/mmol.

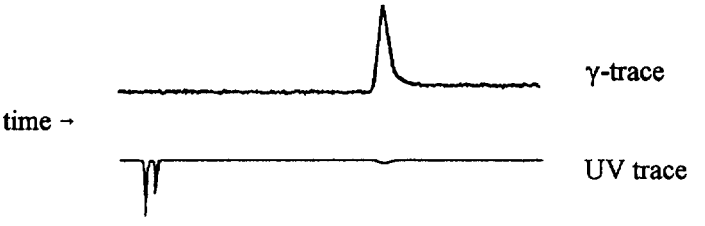

Figure 3. HPLC traces of the radioiodination product of polymer **3** after filtration and treatment with a C18 Sep-Pak™ cartridge.

An attempt was made to produce [^{123}I]MIBG using Iodobeads™ as the oxidant which resulted in a radiochemical yield of about 55 % with the remaining radioactivity absorbed on the polymer. Since experiments with Na ^{131}I showed that about 45 % of the radioactivity remained absorbed to the insoluble polymeric materials, this experiment suggests that polymer **3** may also be an effective precursor to [^{123}I]MIBG if H_2O_2 / acetic acid were used as oxidant. Further experiments with Na ^{123}I were not pursued given the cost of this material.

All three polymers have maintained their spectroscopic properties and chemical reactivities for as long as two years with no particular storage precautions. Solutions from both iodination and radioiodination have been analyzed for tin and none has been observed to the detection limits of the analyses (≤ 10 ppb).

Experimental

Materials and Equipment

NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer, mass spectra on a Finnigan-MAT 8230 mass spectrometer and IR spectra on a Perkin Elmer system 2000 FT-IR. All solid-state MAS NMR spectra of polymer samples were swollen with chloroform. Sodium [^{131}I]iodide solution was generously donated by the Radiopharmaceutical Division of Merck-Frosst Canada.

HPLC analyses of radioiodination reactions were performed on a Dionex Standard AGP equipped with an Eluent Degas Module, a Rheodyne Model 9126 Automatic Sample Injector, a Variable Wavelength Detector-II (VDM), a Harshaw sodium iodide flow scintillation detector and a 4400 integrator. Radioactivity was measured with a CAPINTEC Radioisotope Calibrator CRC-12.

The solutions from iodination of the polymers were anaylzed on a Milipore Waters 600E System controller with a Waters 486 Tunable Absorbance Detector at a wavelength of 231 nm, a U6K injector and a Waters 746 Data Module. The columns used were Millipore-Waters 3.9 x 300 mm columns containing 10 µm µBondapak C18. The HPLC was operated isocratically with acetonitrile/0.01 M sodium dihydrogen orthophosphate buffer (20:80) at a flow rate of 2 mL /min. Standard solutions of MIBA and MIBG were prepared in 0.01 M potassium dihydrogen orthophosphate.

Syringe filters were Gelman Sciences Nylon Acrodisc 4 (4 mm, 0.45 µm pore size). Sep-Pak cartridges were Waters Sep-Pak Vac 1 cc (100 mg) C18 cartridges. The Sep-Pak cartridges were pretreated with methanol overnight.

Preparation of 1-(3-Bromobenzyl)-2,2,5,5-tetramethyl-1,2,5-azadisilolidine , 4

Adapting the reported procedure (10), 3-bromobenzylamine hydrochloride (25.0 g, 112 mmol) was converted to a yellowish liquid which was distilled to yield 29.0 g (82% yield) of **4** as a light yellowish liquid (bp. 93-5°C/0.05 mm Hg).

^{1}H NMR spectrum (acetone-d_6): δ 0 (s, 12H, CH_3); 0.79 (s, 4H, Si-CH_2-CH_2-Si); 4.05(s, 2H, Ar-$\underline{C}H_2$-N); 7.25 (t, 1 H, 5-H), 7.30 (d, 1 H, 6-H), 7.38 (d, 1 H, 4-H), 7.47 (s, 1 H, 2-H). ^{13}C NMR spectrum (chloroform-d): δ 0 (CH_3); 8.2 (Si-CH_2-CH_2-Si); 45.8 (Ar-$\underline{C}H_2$-N); 122.4 (C-3), 126.3 (C-6), 129.5 (C-5), 129.7 (C-2),130.9 (C-4), 146.2 (C-1). MS: M/Z 329, 327, 314, 312, 169, 130, 116, 100, 90, 73, 59, 45. Anal. required for $C_{13}H_{22}BrNSi_2$, m/e 327.0474; found, m/e 327.0479.

Preparation of polymer-supported 3-benzylammonium chloride (Polymer 2)

To 25.0 g (76 mmol) of **4** in a three-neck flask equipped with an argon inlet, a serum cap and a powder addition sidearm containing polymer **1** (28.6 g) was added 250 mL of freshly distilled anhydrous tetrahydrofuran. To this flask cooled to -78 °C was added slowly 30.5 mL (76.1 mmol) of 2.5 M n-butyllithium. Polymer **1** was then tipped into the reaction solution and the mixture was stirred gently at -78 °C for 7 h. The temperature was allowed to rise to room temperature over 2 h and then at room temperature for another 1 h. Methanol (10 mL) was added followed by sufficient 1 M hydrogen chloride to give a pH of 4-5. The mixture was stirred overnight and allowed to stand unstirred for 15 min.

After the upper cloudy solution was decanted, 200 mL of methanol was added. Again the cloudy upper layer was decanted and this process was repeated 4 times. The polymer was filtered through a coarse sintered glass funnel and washed with 50 % methanol/water solution (100 mL), methanol (3 x 100 mL), and 95 % ethanol (50 mL). After vacuum drying at room temperature, 30.1 g (84 wt % yield) of a white grainy material was obtained.

Solid-state MAS ^{119}Sn NMR spectrum: δ -44 ppm

IR spectrum (KBr): cm^{-1} 3435 (broad, -NH_3^+ stretches); 3027(aromatic C-H stretch); 2927, 2857 (aliphatic C-H stretches); 1611, 1498 (aromatic C=C vibrations).

Iodination of Polymer 2 and Polymer 3

To either polymer (25-100 mg) suspended in 4 mL of methanol was added 300 μL of 0.2 M iodine in acetonitrile solution. After stirring for13.5 h at room temperature, 0.1 mL of 1 M sodium metabisulphite solution was added. The mixture was transferred into a 100 mL volumetric flask and diluted to the mark with 0.01 M KH_2PO_4 buffer. After filtering through a syringe filter, the filtrate was analysed by HPLC by comparison to

MIBA chloride solutions (0.4 mM) and MIBG chloride solutions (0.2 mM) which had a retention times of 8.7 min and 15.6 min respectively.

Preparation of polymer-supported 3-benzylguanidinium chloride (Polymer 3)

Under an argon atmosphere, 20.0 g of polymer 2, 15.1 g (360 mmol) of cyanamide, 100 μL (0.72 mmol) of triethylamine and 250 mL of toluene were added to a flask equipped with a reflux condenser. The mixture was heated for 25 h at 54° C and the hot reaction mixture was filtered through a coarse sintered glass funnel. The polymer was washed with acetonitrile (4 x 100 mL), methanol (4 x 100 mL) and acetonitrile (2 x 100 mL). After vacuum drying at room temperature overnight, 20.7 g of white grainy material was obtained.

Solid-state MAS ^{119}Sn NMR spectrum: δ -40 ppm

IR spectrum (KBr): cm^{-1} 3340, 3271, 3174 (N-H stretch); 3066, 3027 (aromatic C-H stretch); 2978, 2929, 2880, 2860 (aliphatic C-H stretch); 1677, 1658 (C=N stretch); 1521, 1496 (aromatic C=C vibrations); 719 (aromatic C-H bend).

Radioiodination of Polymer 3

Into a 2 mL vial was placed 0.5 mg of 3, 300 μL of methanol, 100μl of 0.1 M potassium dihydrogen orthophosphate, 50 μL (45.5 MBq) of a Na^{131}I solution (radiolabelling grade, reductant free), 450 μL of distilled water and 100 μL of a solution 700 mM in acetic acid and 600 mM in H_2O_2. The mixture was occasionally shaken for 2 h at room temperature and then 200 μL of 0.1 M sodium metabisulphite was added to the reaction mixture. After filtration through a syringe filter, the filtrate was analyzed by HPLC. The UV detector trace showed a large peak at the solvent front and several smaller peaks well before MIBG peak. The corresponding radioactivity detector trace showed two peaks at 1.9 and 15.6 min which were confirmed by coinjection to be $^{131}I^-$ (2.4 %) and [^{131}I]MIBG (97.6 %) respectively.

An aliquot of the reaction mixture , 25.4 MBq, was passed through a Sep-Pak cartridge and washed with 5 mL of water. Radioactivity, 1.0 MBq, was found in the washes and 24.4 MBq on the cartridge. Washing of the cartridge with 1.3 mL of 42 % ethanol, released 20.0 MBq of radioactivity into the washes. A wash with 1 mL of methanol released a further 3.1 MBq of [^{131}I]MIBG for a total of 23.1 MBq (95 %). The washes were analyzed by HPLC. The trace from the UV detector showed two small

peaks at the solvent front and a peak at 15.6 min which, by coinjection, was confirmed to be MIBG. The corresponding radioactivity trace showed a single peak at 15.6 min.

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