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Research Article

Synthesis of ^{123}I labeled Congo Red via solid phase organic chemistry

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Summary

A polymer supported synthesis of ^{123}I labeled Congo Red has been developed that provides a high yield of radiochemically pure product. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: Alzheimer's disease; trialkylchlorostannane; β-amyloid; Congo Red

Introduction

Radiopharmaceuticals used in nuclear medicine imaging techniques normally contain short-lived radioisotopes which complicate both their preparation and purification. Radiohalogens are often employed because of their widespread availability and the minimal effect they have on the *in vivo* properties of the substrate.[1] Due to their high reactivity, organometallic compounds based on mercury, thallium, boron, tin, silicon, germanium, and lead have all been used as precursors for radiohalogenated pharmaceuticals.[2] Among these organometallic reagents, organotin precursors are quite popular in radioiodinations because the radiolabeled product is generally produced in high radiochemical purity.[3]

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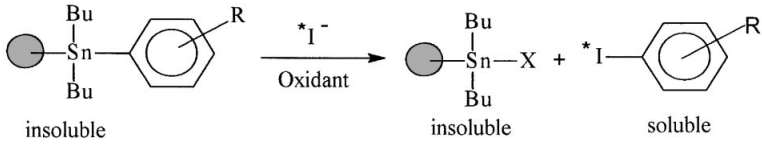
Unfortunately, organotin compounds are highly toxic and the toxicity of the tin waste is an ecological as well as economic concern. This limits the application of organotin reagents in the pharmaceutical industry.

The application of solid phase reagents in organic synthesis has become an area of intense interest because of the potential environmental advantages related to recoverability, handling, recycling, and expense. In addition, the use of sterically demanding polymers can lead to isomerically purer products which is especially important in the production of radiopharmaceuticals.[4–6] Greigk and his coworkers[7] developed an insoluble macroporous polystyrene-supported dialkylchlorostannane that can be filtered and recycled and thus provides an ecofriendly reagent in organic synthesis.[8,9]

The azo dye Congo Red has a high affinity for the β-pleated structure of all forms of amyloid.[10] Kabalka and his coworkers reported the synthesis of radioiodinated Congo Red which was found to localize in amyloid tissue.[11] Since this radiolabeled azo dye binds to β-amyloid fibrils, it might be possible to monitor *in vivo* levels of amyloid by single photon emission computerized tomographic imaging. The purpose of this study was to develop a readily isolable, tin-free, ^{123}I labeled Congo Red reagent via a solid phase organic synthesis using a polymeric trialkylarylstannane as a reactant (Scheme 1).

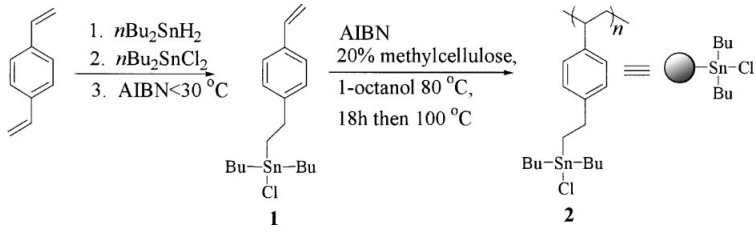


Scheme 1.

The advantage of this polymer-based approach is that the pure radiopharmaceutical can be easily isolated with minimal loss. The polymeric side product remains insoluble in the reaction medium and is easily removed by filtration.

Results and discussion

The monomer dibutyl[2-(4-ethenylphenyl)ethyl]tin chloride **1** was prepared by adding di-*n*-butylchlorostannane, generated *in situ*, to the double bond of divinylbenzene via free radical addition mechanism (Scheme 2) using α,α'-azo*bis*isobutyronitrile (AIBN).

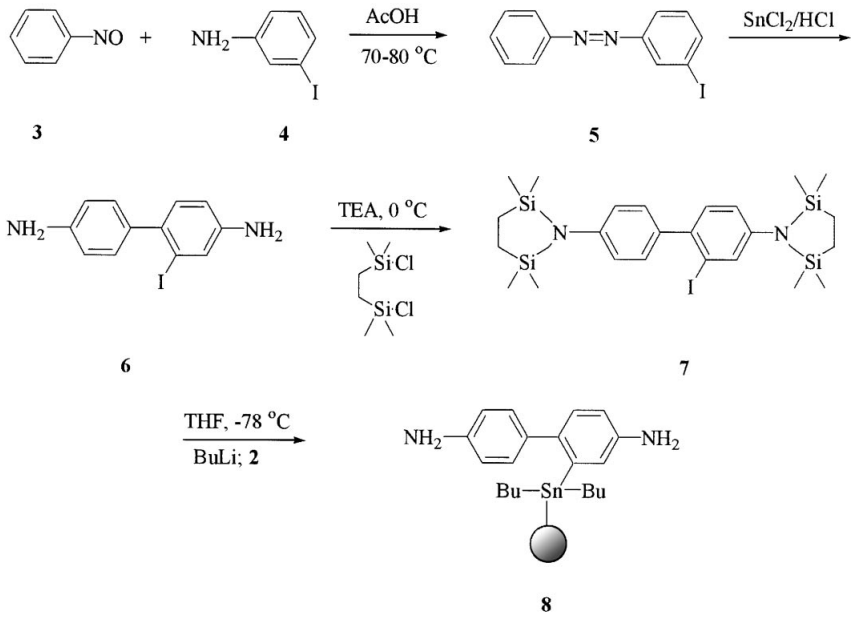


Scheme 2.

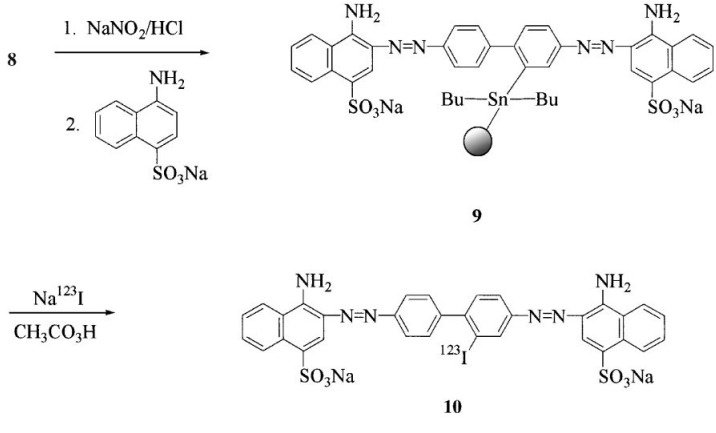
Poly{dibutyl[2-(4-ethenylphenyl)ethyl]tin chloride} **2** was prepared by copolymerizing the tin monomer and divinylbenzene.[8,9] Macroporous polymer beads were obtained after a free-radical suspension polymerization which utilized 1-octanol as diluent and methylcellulose as stabilizer.[7] The polymer beads were washed sequentially with water, methanol, THF, hexane and then a final washing was carried out using THF and a Soxhlet extractor. The yield of the polymer was 98%. Polymer **2** was characterized using ^{13}C and ^{119}Sn solid-state NMR spectroscopy. The tin solid-state NMR revealed a resonance centered at 148 ppm. In the ^{13}C NMR, resonances due to the butyl groups appeared at 14–28 ppm, the polymer chain carbons and aromatic carbons appeared at 40–50 and 120–150, respectively. The chlorine content in the sample was determined by refluxing the sample with sodium hydroxide in methanol.[12] The chlorine content was found to be 1.73 mmol/g of the polymer.

The preparation of benzidine polymer **8** was carried out in four steps from nitrosobenzene, **3**, and 3-iodoaniline, **4** (Scheme 3). 3-Iodoazobenzene, **5**, was prepared by the condensation of nitrosobenzene, **3**, with 3-iodoaniline, **4**, in acetic acid at 70–80°C.[13] Reduction of **5** with $SnCl_2$ in ethanol, accompanied by an acid promoted benzidine rearrangement, furnished 2-iodobenzidine, **6**. The amino groups of **6** were protected utilizing 1,2-*bis*(chlorodimethylsilyl)ethane prior to incorporation of the polymer. The protected iodobenzidine **7** was lithiated using *n*-butyllithium and then treated with **2** to obtain the polymer **8**.

Polymer **8** was converted to the Congo Red precursor **9** by diazotization using sodium nitrite and hydrochloric acid followed by treatment with the sodium salt of 4-aminonaphthalene-1-sulfonic acid to obtain a red polymer (Scheme 4). Diazotization was carried out at various concentrations of HCl to optimize the reaction conditions. It was found that 1.5 M HCl produced the maximum yield. Iodination of the polymer **9** was conducted using sodium iodide and peracetic acid.



Scheme 3. Preparation of I-123 Iodo Congo Red



Scheme 4. Preparation of ^{123}I iodo Congo Red

Iodinated Congo Red was isolated from the polymer by filtration and the purity was confirmed by TLC and HPLC. The yield of Congo Red was 250 mg/g of the polymer. Radioiodination was carried out using no-carrier-added $Na^{123}I$ (250 Ci/mmol) and peracetic acid to afford the title compound, **10**. The radiochemical purity of **10** was found to be $>99\%$ and the radiochemical yield was 56%.

Experimental

All solution NMR spectra were obtained using a Brucker 250 MHz spectrometer. The solid-state MAS-NMR spectra of the polymer and supported intermediates (after swelling with chloroform) were obtained using an INOVA 400 MHz solid-state NMR spectrophotometer. The IR spectra of the polymers (KBr pellet) were measured on a BioRad FT-IR spectrometer. HPLC analyses were performed using a Waters 501 unit with a 1 : 1 mixture of water and methanol as the solvent system. The flow rate was 1 ml/min. Radio thin layer chromatography was carried out using a radio-TLC scanner (Bioscan, Autochanger 300, system imaging scanner). $Na^{123}I$ was purchased from MDS Nordion Inc., Vancouver, Canada.

3-*Iodoazobenzene,* ***5***

3-Iodoaniline (11.0 g, 50.0 mmol) was added to a solution of nitrosobenzene (5.85 g, 50.0 mmol) in glacial acetic acid (10 ml). The reaction mixture was kept at 70–80°C for 7 h and then dissolved in benzene (200 ml). The solution was washed with several portions of 8 N sulfuric acid to remove the tarry material. The organic phase was further washed with 1% sodium hydroxide followed by brine and water. The solvent was dried, evaporated and the product purified by column chromatography (SiO_2) eluting with petroleum ether to yield 8.2 g (53%) of 3-iodoazobenzene as orange crystals. m.p. = 70–71°C (lit.[13]71–71.5°C).

2-*Iodobenzidine,* ***6***

3-Iodoazobenzene (3.10 g, 10.0 mmol) was dissolved in anhydrous ethanol (50 ml). Stannous chloride dihydrate (3.37 g, 15.0 mmol) was added followed by conc. HCl (1.2 ml). The resulting mixture was refluxed for 5 h, cooled to room temperature, and made alkaline using 6 N NaOH (to pH 11). The mixture was poured into ice cold water and thoroughly extracted with ether (3×100 ml). The combined ethereal extracts were washed with water, brine and dried over anhydrous $MgSO_4$. The organic solvent was removed under vacuum to obtain a gummy product which was purified by flash column chromatography (SiO_2), using ethyl acetate : petroleum ether (1 : 1) as eluent to yield 1.7 g (54%) of a colorless gum. m.p. = 97–98°C (lit.[14]98–99°C). ^{1}H NMR ($CDCl_3$) δ 7.26 (dd, 1H, $J = 7.5$, 1.3 Hz), 7.51 (d, 2H, $J = 7.5$ Hz), 7.79

(dd, 1H, $J = 7.5$, 1.3 Hz), 7.90 (d, 2H, $J = 7.5$ Hz) and 8.25 (d, 1H, $J = 1.3$ Hz). ^{13}C NMR ($CDCl_3$) δ 94.56, 123.03, 129.14, 130.62, 131.46, 131.56, 139.50, 152.33 and 153.37.

Protected benzidine, 7

2-Iodobenzidine (0.31 g, 1.0 mmol) was placed in a flame dried, argon flushed, 200 ml two-necked round bottomed flask. Dry dichloromethane (75 ml) and triethylamine (0.40 g, 4.0 mmol) were added and the solution cooled to 0°C. 1,2-*Bis*(chlorodimethylsilyl)ethane (0.43, 2.0 mmol) in dichloromethane (25 ml) was slowly added and the solution stirred for 1 h at 0°C and then at room temperature for 6 h. The solvent was then removed under reduced pressure using a rotary evaporator. Hexane (30 ml) was added to the flask to dissolve the protected amine and the solution was filtered under argon to remove unprotected amine and the triethylamine–HCl precipitate. Solvent was removed under reduced pressure to yield the protected amine as a light yellow liquid (0.52 g, 89%). ^{1}H NMR ($CDCl_3$) δ 0.26 (s, 12H, Si-CH_3), 0.27 (s, 12H, Si-CH_3), 0.862 (s, 4H, Si-$\underline{C}H_2\underline{C}H_2$-Si), 0.863 (s, 4H, $SiCH_2$-CH_2-Si), 7.00–8.00 (m, 7H, aromatic). ^{13}C NMR δ 0.00 (Si$\underline{C}H_3$), 8.44 (Si-CH_2-CH_2-Si), 96.97, 114.94, 115.42, 116.03, 125.28, 126.74, 129.74, 130.67, 130.77, 136.70, 145.99, 148.35 (aromatic).

Polymer-supported benzidine, ***8***

2-Iodobenzidine-2,2,5,5-tetramethyl-1,2,5-azadisilolidine (0.52 g, 0.89 mmol) was dissolved in anhydrous THF (20 ml), in a three-necked round-bottomed flask equipped with an argon inlet, septum, and a powder addition sidearm containing **2** (0.50 g, 0.89 mmol). The flask was cooled to −78°C, and *n*-butyllithium (0.89 mmol, 0.55 ml of a 1.6 M solution in hexanes) was slowly added. The reaction was allowed to proceed for 30 min. The polymer was then added and the mixture stirred overnight at −78°C. Ethanol (3 ml) was added and the precipitated polymer collected by filtration, washed sequentially with ethanol (3×5 ml), water (2×3 ml), dilute acetic acid (37% in ethanol, 1×3 ml), water (4×3 ml), and ethanol (4×3 ml). After drying in vacuum, white polymer beads (0.56 g) were obtained. Approximately, 77% of the theoretical quantity of benzidine was attached to the polymer. Solid-state MAS ^{13}C NMR spectrum: δ 10.4 (Sn-CH_2-); 14.0 (CH_3); 28.0 (-$\underline{C}H_2$-CH_3); 29.2 (Sn-CH_2-$\underline{C}H_2$-); 40.8 (polymer backbone-CH_2);

43.8 (polymer backbone, -CH-). 124.2,129.0 (backbone -CH aromatic); 136.0 (Sn-C aromatic), 142.6 (backbone quaternary C-aromatic). Solid-state MAS ^{119}Sn NMR spectrum: δ −60 ppm (phenyl-Sn). IR spectrum (KBR) cm^{-1} broad band near 3500; 3050 aromatic C–H stretch; 2930, 2857 (aliphatic C–H stretches); 1606, 1500 (aromatic C = C vibrations).

Polymer-supported Congo Red, ***9***

Polymer **8** (0.56 g, containing approximately 0.66 mmo1 of benzidine) was placed in a 100 ml beaker, toluene added (3 ml), and the mixture cooled to 0°C in an ice bath. Ice cold HCl (1.5 M, 1 ml) was added and the mixture stirred. Sodium nitrite (0.11 g, 1.3 mmol) was dissolved in water (1 ml), cooled to 0°C and then slowly added to the beaker at a rate such that temperature was maintained at 0°C. The reaction mixture was stirred for 5 min and a solution of 4-amino-l-naphthalenesulfonic acid sodium salt (0.32 g, 1.3 mmol) in water (2 ml) was added. The reaction was allowed to proceed for 15 min. The mixture was filtered and the polymer washed sequentially with water (3 × 5 ml), 5% aqueous sodium bicarbonate (2 × 5 ml), water (2 × 5 ml), methanol (3 × 5 ml), THF (3 × 5 ml) and methanol (2 × 5 ml). A small portion of Congo Red detached from the polymer during this sequence. The red colored polymer was dried under vacuum to yield 0.73 g (containing approximately 55% Congo Red by weight).

^{123}I labeled Congo Red, ***10***

Electrophilic radioiodination was carried out by placing no-carrier-added Na^{123}I (specific activity of 9.25×10^6 MBq/μmol, 85 MBq in 0.1% aqueous NaOH) into a 4 ml Wheaton vial containing a methanolic suspension of polymer **9** (10 mg in 200 μl). To this was added 100 μl of peracetic acid (0.3% methanolic solution). The reaction vial was sealed and the mixture stirred for 0.5 h. A drop of 10% aqueous sodium thiosulfate was added to destroy excess iodine. The solid matrix was removed by filtration. The filtrate contained the desired product. The radiochemical purity of the ^{123}I labeled Congo Red was measured utilizing an aluminum backed silica gel plate using *n*-butanol : acetone : water : ammonium hydroxide = 15 : 15 : 8 : 3 as solvent system, R_f = 0.53 (Figure 1). The chemical purity was verified by radio-HPLC (C_{18}, 3.9 mm × 390 mm) under gradient elution conditions (0 min 100%

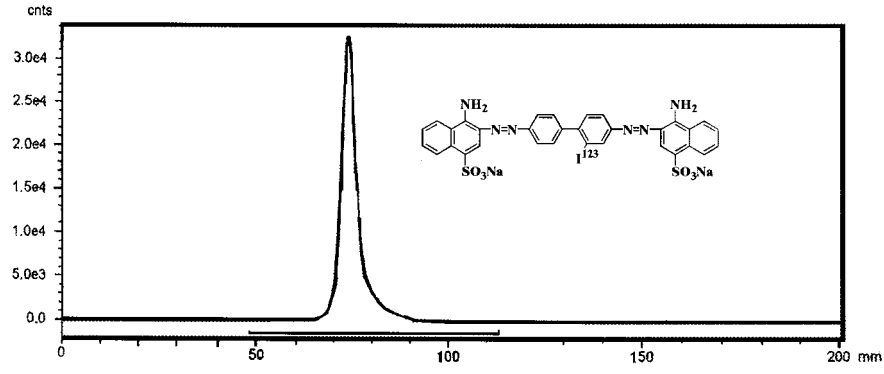


Figure 1. Radio-TLC of ^{123}I labeled Congo Red

MeOH, 20 min 100% H_2O, flow rate 1 ml/min). A single peak was obtained with a retention time of 15 min. The radiochemical purity was $>99\%$ and the decay corrected yield was 56%.

Conclusions

An ecofriendly method for preparing radiochemically pure ^{123}I labeled Congo Red has been developed. The polymer-supported tin reagent avoids possible toxic tin contamination during the synthesis. In addition, the polymer can be recycled which minimizes the release of metal wastes to the environment while making the reaction both ecofriendly and economically viable.

Acknowledgements

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