Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 23 December 2013, Revised 21 May 2014, Accepted 29 May 2014 Published online 28 July 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3214

A hybrid solid-fluorous phase radioiodination and purification platform†

James P. K. Dzandzi, Denis R. Beckford Vera, and John F. Valliant*

A new class of fluorous materials was developed to create a hybrid solid-solution phase strategy for the expedient preparation and HPLC-free purification of ^{125}I-labeled compounds. The system is referred to as a hybrid platform in that it combines solution phase labeling and fluorous solid-phase purification in one step as opposed to two separate individual processes. Treatment of fluorous arylstannanes coated on fluorous silica with [^{125}I]NaI and the appropriate oxidant made it possible to produce and selectively isolate the nonfluorous radiolabeled products in high purity (>98%) free from excess starting material and unreacted radioiodine. Examples included simple aryl and heterocyclic (click) derivatives, known radiopharmaceuticals including meta-iodobenzylguanidine (MIBG) and iododeoxyuridine (IUdR), and a new agent with high affinity for prostate-specific membrane antigen. The coated fluorous silica kits are simple to prepare, and reactions can be performed at room temperature using different oxidants generating products in minutes in biocompatible solutions.

Keywords: labeling methods; I-125; instant kits; fluorous; MIBG; IUdR

Introduction

Radiopharmaceuticals are typically produced using 'just-in-time' manufacturing methods where radioactive decay necessitates expedient and efficient synthesis and purification methods. For many targeted small molecule radiopharmaceuticals, unlike for more traditional perfusion-type agents, HPLC is necessary to separate the desired product from impurities and the large excess of unlabeled precursor that is often used in radiolabeling reactions. HPLC purification has a number of limitations particularly for routine production of radiopharmaceuticals in that it is time-consuming, requires extensive validation efforts for agents being translated to clinical use, and adds the risk of instrument failure during production runs.

To eliminate the need for HPLC, alternative labeling and purification methods employing solid-phase synthesis and solid-phase extraction (SPE) have been developed for a variety of medical isotopes. These include systems based on polymer-supported precursors[1–7] and fluorous-tagged molecules.[8–11] In solid-phase labeling, compounds are linked to insoluble supports via covalent bonds or ionic interactions in a way that the desired product is released upon labeling and isolated by simple filtration. For the fluorous labeling strategy (FLS),[8–12] fluorous-tagged precursors, which are prepared and purified using traditional synthetic methods,[13,14] are converted to nonfluorous analogues upon labeling. The fluorous precursor is separated from the desired radiolabeled compound by passing the reaction mixture through a fluorous SPE (FSPE) cartridge.[10,15–17]

The advantage of the solid-phase system is that labeling and purification is carried out in a single step. Unfortunately, the inability to purify cross-linked polymer derivatives limits this method to the small number of loading reactions that proceed in quantitative yield. Groups developing solid-phase labeling systems have in certain instances also reported reduced radiochemical yields compared with solution phase methods, which is associated with the heterogeneous nature of the reaction mixture and nonspecific binding of the product and isotope to the support. Solid-phase labeling can also require heating and/or use of organic solvents.[4]

One approach to addressing these limitations is to combine fluorous and solid-phase labeling methods. Fluorous precursors can be prepared, purified, and fully characterized using traditional solution-phase methods and then the material loaded onto a solid support by physisorption (taking advantage of the fluorine–fluorine interactions) to create a hybrid fluorous-solid-phase system. The product would be released from the support upon labeling because it is rendered 'nonfluorous'. The advantage of loading the material onto the fluorous support, as opposed to labeling and then passing the mixture through a FSPE cartridge, is that the combined system could be used to create a single step 'instant kit', which would minimize handling of the radioactive solutions and facilitate clinical translation. Such a system, including the preparation of the fluorous coated materials, was developed here for labeling and purifying a range of different molecules with iodine-125.

Results and discussion

Previous work on the FLS involved labeling fluorous tin derivatives in solution and then isolation of the products subsequently using a FSPE cartridge. Here, the goal was to develop a different paradigm where labeling takes place directly

Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON L8S 4M1, Canada

**Correspondence to: John F. Valliant, Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, L8S 4M1, Canada.*
E-mail: valliant@mcmaster.ca

†*Additional supporting information may be found in the online version of this article at the publisher's web site.*

on a hybrid fluorous-solid-phase material such that the product, free from residual starting materials and impurities, can be eluted directly and selectively.

The initial focus was to determine the range of loadings that can be achieved on fluorous silica (FS), which was carried out using a simple fluorous-tin benzoic acid (FBA) derivative **1a** (Figure 1), which can be prepared using a commercially available fluorous tin bromide[18] following a minor modification of a previously published method.[8] To prepare silica coated with tin precursor, varying quantities of **1a** as a 500 mg/mL solution in $CHCl_3$ were added to FS. The resulting chloroform-silica slurry was agitated periodically and the solvent allowed to evaporate to give loading ranges of 100–500 mg/g of coated FS in 50 mg/g increments. The hybrid fluorous-solid-phase labeling and purification cartridges were prepared by first loading normal (uncoated) FS (500 mg) that had been preconditioned with DMF, H_2O, and 80:20 (v/v) MeOH/H_2O into a polypropylene SPE tube (3 mL) followed by a sample (50 mg) of coated FS. Each cartridge was capped with a polyethylene frit and washed with H_2O followed by 80:20 MeOH/H_2O where fractions (1 mL) were collected and analyzed by HPLC to determine if any **1a** leached from the support.

Based on HPLC analysis, there was no elution of the tin precursor in the 100–300 mg/g samples of FS-**1a**.[19] However, the tin compound was detected in samples containing higher loadings (400–500 mg/g). Initial experiments were therefore performed with the 200 mg/g (FS(200)-**1a**) material. To identify the optimal elution conditions, a 50 mg sample of FS(200)-**1a** that also contained *para*-iodobenzoic acid (10 mg) was loaded onto a sample of preconditioned unmodified FS (500 mg). This combination of materials was washed with water followed by different alcohol-water mixtures. The aqueous fractions did not contain any *para*-iodobenzoic acid, whereas the desired product was eluted quantitatively using an 80:20 (v/v) MeOH-water mixture. None of the fractions contained any detectable amount of **1a**.

One of the concerns raised over using arylstannanes is their potential toxicity, where the recommended parenteral limit for tin is 300 ppm.[20] To further demonstrate that the FS is able to effectively retain the tin precursors, a fluorous-tin derivative of fluorescein was prepared and loaded onto FS. This provided a convenient tool to visualize the distribution of the fluorous precursor on the silica after loading and washing. Compound **3a** (Scheme 1) was prepared by coupling the fluorous-tin benzylamine **2a** to fluorescein 5(6)-isothiocyanate, which was achieved in 71% yield. FS(300)-**3a** was prepared as previously described for **1a,** where visually the precoated fluorous-tin dye was clearly retained on the top portion of the FS. There was little change after washing with water and 80:20 alcohol/H_2O (Figure 2), and the precursor remained bound to the FS and was not detected spectroscopically in any fraction where the detection limit for **3a** was 6 μg/mL (6 ppm), which is well below (50×) the USP recommended limit. Inductively coupled plasma mass spectrometry analysis was also performed on the product and showed that there was no tin present above the detection limit of the instrument (1 ppb).

Radiochemical experiments were performed subsequently using $Na^{125}I$ and a range of fluorous compounds to test the general utility of the approach and to allow for comparison to previously reported solution phase work. For the initial tests, a cartridge containing 50 mg of FS(200)-**1a** and preconditioned FS (500 mg) giving a ratio of loaded material to FS of 1:10 w/w was prepared. EtOH/H_2O (80:20, pH adjusted to 3–4 with AcOH) was added followed by iodogen and $Na^{125}I$ (3.7–7.4 MBq). The reaction was allowed to proceed for 20 min and then quenched with aqueous $Na_2S_2O_5$ followed by a water wash to remove any residual salts and unreacted $Na^{125}I$. The desired product was obtained by elution with 80:20 EtOH/H_2O (5 mL), which was used in place of MeOH/H_2O to ensure biocompatibility upon dilution of the product with isotonic saline or buffer.[21] HPLC analysis revealed no evidence of **1a** in the ultraviolet (UV)-trace and a single peak in the γ-trace. The identity of the peak was confirmed by comparing its retention time to that for an authentic sample of *para*-iodobenzoic acid (Figure 3). The product was obtained in 97% radiochemical purity and 49 ± 3% ($n = 3$) isolated radiochemical yield. There was some small amount of residual activity on the FSPE cartridge and the three-way-valve attached to the polypropylene SPE tube. The remainder of the activity was unreacted $Na^{125}I$, which was detected in the initial aqueous fractions.

Following the successful proof of concept experiment with FS (200)-**1a**, the general utility of the method was evaluated using other previously reported fluorous-precursors. The fluorous-tin

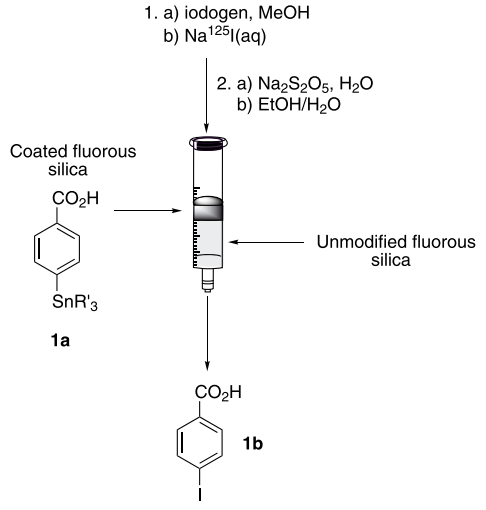
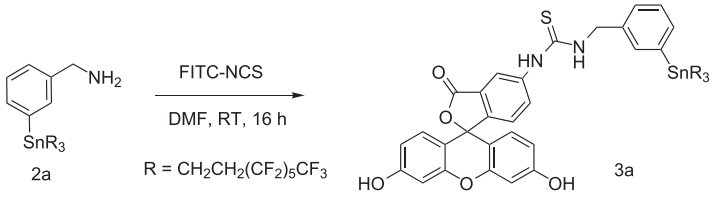


Figure 1. Schematic representation of the hybrid solid-fluorous phase radioiodination and purification system. R′ = $(CH_2)_2(CF_2)_5CF_3$. Additional examples of compounds tested are shown in Scheme 2.



Scheme 1. Synthesis of the fluorous-tin fluorescein derivative 3a.

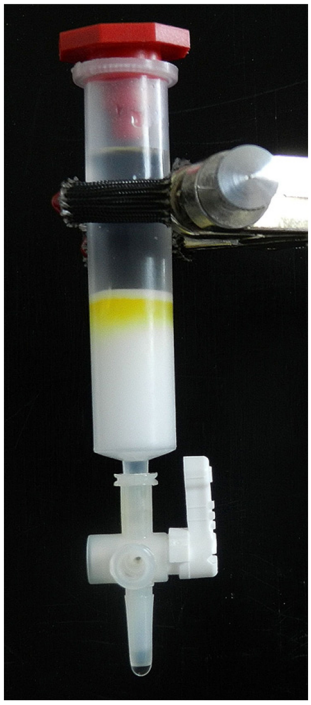


Figure 2. Fluorescein derivative **3a** (yellow color) loaded on unmodified fluorous silica gel. The picture shows the cartridge after loading and elution with water and 80:20 EtOH/H_2O.

compounds spanned simple aryl derivatives to known and emerging radiopharmaceuticals (Scheme 2). The FS-coated materials (200 mg/g) were prepared and labeled as previously described, and all reactions were performed in head-to-head comparisons with solution phase (two steps) FLS reactions.

In all cases, radiochemical purities were greater than 95% while isolated radiochemical yields ranged from 30% to 49%, except for the nitrobenzene derivative where the isolated yield was less than 5% (Table 1). For the anisole derivative **5b,** the yields were higher than for the phenyl (**4b**) and nitro (**6b**) derivatives, which is expected given the electrophilic nature of the starting material. The yields reported are lower than for the traditional FLS reactions but were comparable with yields reported for polymer supported aryltrifluoroborates.[4]

The coated FS system was also evaluated using a series of simple benzamides including an analogue of *N*-(2-(diethylamino) ethyl)-4-iodobenzamide (BZA), an agent that has been evaluated as a SPECT agent for imaging melanoma.[22] The fluorous precursors **7a–9a** were prepared via the tetrafluorophenol (TFP) active ester of **1a**.[8] The purified products were coated onto FS using the chloroform evaporation method to give FS(200)-**7a**, FS (200)-**8a**, and FS(200)-**9a**, which were loaded onto FS (1:10 w/w). Upon labeling, the yields for **7b**, **8b**, and **9b** were 42 ± 4%, 43 ± 6%, and 36 ± 2%, respectively, with radiochemical purities greater than 98% in all cases. The yields were again lower than the corresponding solution phase method, which were 78 ± 5%, 81 ± 2%, and 70 ± 3%, respectively. Elution of **7b** was not successful with 80:20 EtOH/H_2O as the majority of the product was retained on the FSPE cartridge. Because of the basicity of the tertiary amine, a small amount of acid (0.1% TFA) was needed to elute the product.

The system was also effective in producing *meta*-iodobenzylguanidine (MIBG, **10b**), a radiopharmaceutical which is used for imaging and treating neuroendocrine tumors.[23] The yield obtained with the one-step radioiodination was 40 ± 6%, which is a twofold and 1.5-fold reduction in yield compared with the solution phase FLS labeling and an existing polymer supported MIBG labeling method, respectively.[2] However, the radiochemical purity of the

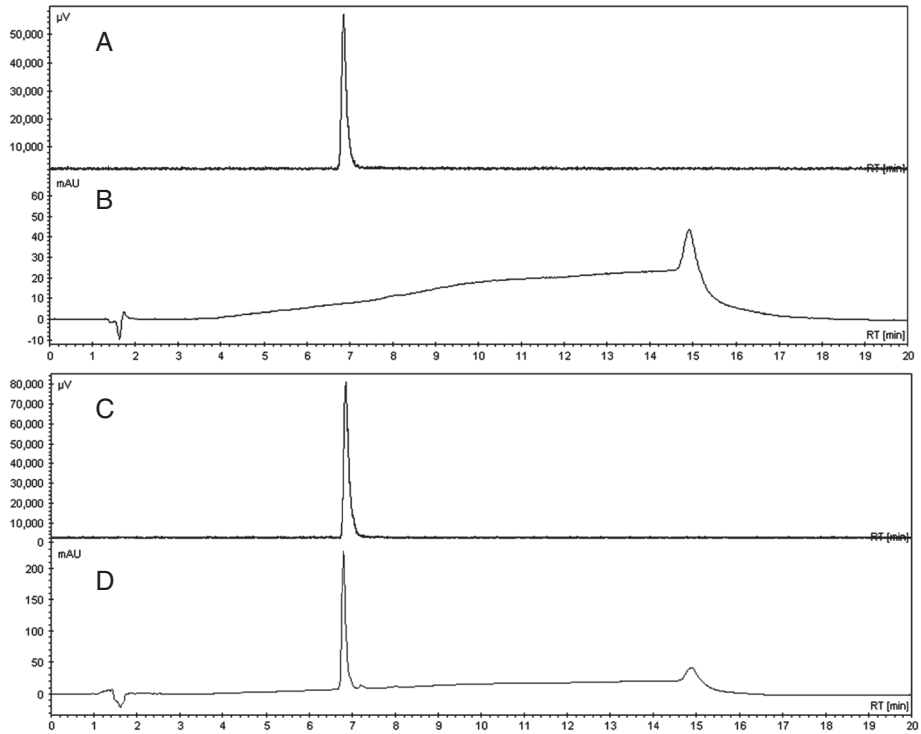
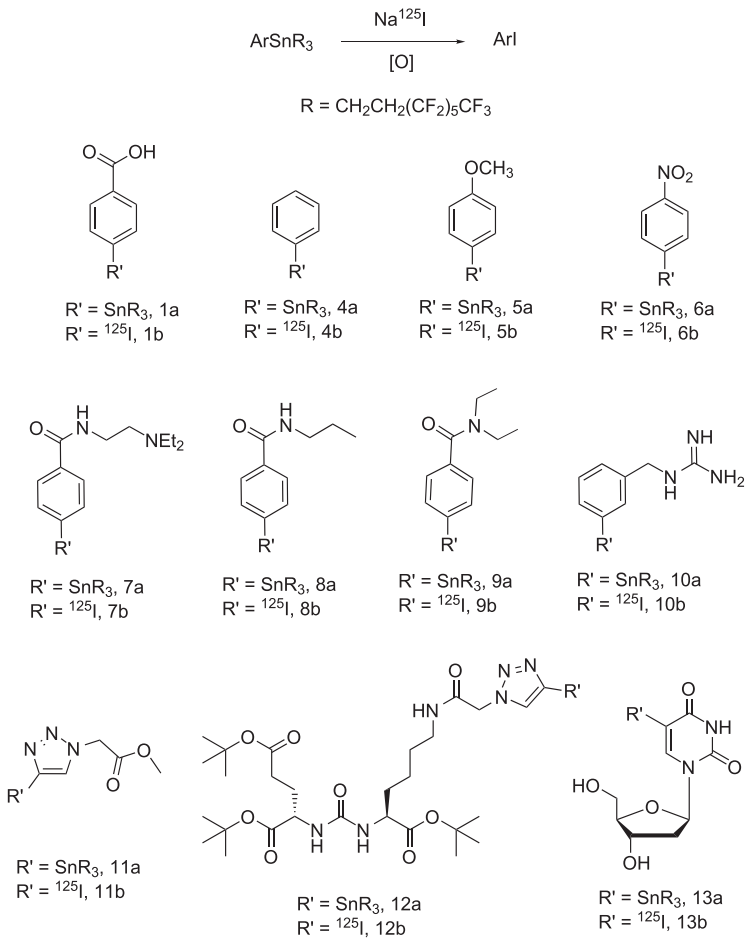


Figure 3. (A) γ-HPLC chromatogram of 1b; (B) UV-HPLC chromatogram of the same sample; (C) γ-HPLC chromatogram of 1b co-injected with a cold reference standard; (D) UV-HPLC chromatogram of the same sample. The small difference in the retention times between the UV and the corresponding γ-trace is due to the time lag between the detectors, which are connected in series (elution method A). The peak and change in the baseline between 14.5 and 15.5 min is a result of a rapid change in the gradient.



Scheme 2. Basic radioiodination reaction, fluorous precursors and radioiodinated products.

Table 1. Radiochemical yields for solution (fluorous) and hybrid fluorous-solid-phase radioiodination reactions with different oxidants

Precursor	Iodogen		Chloramine-T
	Solution	Coated	Coated
1a	83 ± 2	49 ± 3	75 ± 6
4a	80 ± 2	30 ± 4	47 ± 8
5a	86 ± 3	45 ± 5	73 ± 3
6a	13 ± 5	≤5	—
7a	78 ± 5	42 ± 4	72 ± 12
8a	81 ± 2	43 ± 6	—
9a	70 ± 3	36 ± 2	83 ± 6
10a	80 ± 3	40 ± 6	63 ± 10
11a	78 ± 5	45 ± 3	—
12a	81 ± 3	48 ± 6	67 ± 2
13a	—	—	57 ± 3

product was greater than 98%, and loading the material onto FS was simple and optimized in a couple of hours whereas developing a method to produce polymer supported MIBG quantitatively was nontrivial.[2]

One of the potential limitations of the fluorous system is in using FSPE to purify polar molecules which could co-elute with unreacted iodide. To test this, a new and polar heterocyclic iodine prosthetic group known as the triazole-appending agent (TAAG)[24] was investigated as a substrate. The fluorous TAAG precursor **11a** could be effectively loaded onto FS to give FS (200)-**11a** which when loaded onto FS (1:10 w/w) showed no evidence of breakthrough when using 80:20 EtOH/H_2O. Upon radioiodination however, some of the desired product, **11b**, eluted in the 100% aqueous wash along with free [^{125}I]NaI. To address this, a C-18 SPE cartridge was connected in series to the FSPE cartridge so that after elution with water the radioiodinated product was retained and subsequently selective eluted in 45 ± 3% radiochemical yield using 80:20 EtOH/H_2O.

The utility of the hybrid labeling system was tested further using a novel TAAG derivative that can bind to prostate-specific membrane antigen,[24] a protein that is over expressed on prostate cancers and associated metastases.[25,26] Compound **12a** was loaded onto F-silica to give FS(200)-**12a** (1:10 w/w) and labeled with Na^{125}I. The desired product **12b** was eluted with 80:20 EtOH/H_2O in 48 ± 6% radiochemical yield (n = 3) with a radiochemical purity that was greater than 98%, which is comparable with that for the solution phase method. To test whether or not the cartridges can be used multiple times, following the elution of the product, the labeling of FS(200)-**12a** was repeated using the same cartridge, which was washed with water followed by 80:20 EtOH/H_2O. The yield for the subsequent labeling was 30 ± 6% and the product isolated in 90% radiochemical purity. A third labeling was

performed, where the yield and radiochemical purity decreased to 12 ± 7 and 75%, respectively, indicating that the cartridges are best suited for single use applications.

Not all molecules can be iodinated effectively with iodogen; consequently, the general utility of the system with other oxidants was also explored. Preliminary experiments using peracetic acid were found to give inconsistent radiochemical yields. Chloramine-T in contrast showed that comparable or superior yields compared with iodogen could be obtained using less precursor. Isolated yields of 65 ± 5%, 71 ± 3%, and 70 ± 2% ($n = 3$) were obtained using 30 mg of FS(30)-**1a**, FS(50)-**1a**, and FS(100)-**1a**. With chloramine-T, it was advantageous to connect the FSPE cartridge to a C18 SPE cartridge and to acidify the 80:20 EtOH/H_2O eluent with dilute phosphoric acid. This eliminated the need to collect fractions and resulted in selective separation of the desired product from free iodide. For example, using this system isolated radiochemical yields of 75 ± 6% ($n = 5$) and radiochemical purity greater than 95% were obtained for **1b**. To demonstrate the elution conditions did not cause protodestannylation, compound **5a** was incubated in the acidified 80:20 EtOH/H_2O solution and samples analyzed by HPLC at 5 and 40 min, where there was no evidence of anisole at either time point. The system remains biocompatible as the eluted product when added to PBS was isotonic and within a pH and radioactivity concentration range suitable for injection.

As a result of the increased yield with chloramine-T at reduced ligand loading levels, several of the fluorous derivatives previously labeled using iodogen were radioiodinated using chloramine-T. Yields ranged from 47% to 83% (Table 1), where the radiochemical purity was greater than 95% for all products. In terms of specific activity, the limit of detection for **1b** was determined, and the value was greater than 240 Ci/mmol (average of three experiments). As an additional test of the labeling method, 5-iodo-2′-deoxyuridine (IUdR)-**13b**, an agent currently undergoing investigation as therapeutic radiopharmaceutical,[27] was also produced using the chloramine-T approach, where the isolated yield was 57% ± 3%. In general, the isolated yields obtained using chloramine-T are comparable or higher than those previously reported for other solid-phase radioiodination methods.[2,4,28–30] It should also be noted that the coated material was evaluated over 2 months after storage at 4 °C, where there was no significant change in radiochemical yield or purity.

Conclusion

The reported hybrid fluorous-solid phase labeling material is a convenient platform for producing radioiodinated compounds in high purity and effective specific activity (i.e., free from any residual precursor) without the need to use HPLC. An advantage of the hybrid system over covalent polymer modification is that the precursor compounds can be prepared and fully characterized prior to immobilization, which is convenient and can be carried out using a wide range of compounds. Reactions were performed at room temperature using either iodogen or chloramine-T and reached completion within 20 min, and the system did not require the use of organic solvents (other than ethanol). All products can be isolated in biocompatible solvents ready for injection by simple dilution with isotonic saline or PBS.

Experimental

Reagents and general procedures

Unless otherwise stated, all chemical reagents were purchased and used as received from Sigma-Aldrich without further purification. Toluene, dichloromethane, and tetrahydrofuran (THF) were distilled using a PURE SOLV distillation system. FC-72® was purchased from 3M; $(CF_3(CF_2)_5(CH_2)_2)_3SnPh$ and $(CF_3(CF_2)_5(CH_2)_2)_3SnH$ were purchased from Fluorous Technologies Inc. SiliaFlash® P60 Silica gel from SiliCycle was used for silica gel chromatography. Analytical thin-layer chromatograms (Merck F254 silicagel on aluminum plates) were visualized using ultraviolet light. Compounds **1a**, **2a**, and **4a–12a** were prepared according to literature methods.[8,9,12,24] 1,3,5,7-Tetramethyl-2,4,8-trioxa-(2,4-dimethoxyphenyl)-6-phosphaadamantane ligand for cross coupling reactions was generously provided by Prof. Alfredo Capretta (McMaster University, Hamilton, Ontario).[31] KF/silica stationary phase contained 10% w/w of KF and 90% w/w SiliaFlash P60 silica gel (finely ground together) loaded in a disposable glass pipette plugged with glass wool. Empty fritted polypropylene SPE tubes were purchased from Sigma-Aldrich. SepPak® Plus C18 cartridges were purchased from Waters Corporation. Sodium [^{125}I]iodide with a specific activity of ~17 Ci/mg was provided by the McMaster Nuclear Reactor (Hamilton, ON, Canada).

Caution: ^{125}I is radioactive and should only be handled in an appropriately equipped and licensed facility.

Instrumentation

NMR spectra were recorded using Bruker DRX-500 and 600 spectrometers with chemical shifts reported in parts per million relative to the residual proton signal of the deuterated solvent (^{1}H NMR) or the carbon signal of the solvent (^{13}C NMR). Reactions requiring microwave heating were performed using a Biotage Initiator 60 instrument. Infrared (IR) spectra were acquired using a BioRad FTS-40 FT-IR spectrometer. ESI mass spectrometry experiments were performed on a Waters/Micromass Quattro Ultima instrument, where samples were first dissolved in methanol. High resolution mass spectral data were obtained using a Waters-Micromass quadrupole/time-of-flight Ultima Global spectrometer. Elemental analyses were performed at the McMaster University Combustion Analysis and Optical Spectroscopy facility. HPLC was performed using a Varian ProStar Model 230 instrument, fitted with a Varian ProStar model 330 PDA detector, an IN/US γ-RAM gamma detector, a Star 800 analog interface module, and a Phenomenex Gemini-C18 column (4.6 × 100 mm, 11nm, 5 μm). The wavelength for UV detection was set at 254 nm, and the dwell time in the gamma detector was 5 s in a 10 μL loop. The mobile phase was composed of solvent A = H_2O (0.1% TFA) and solvent B = CH_3CN (0.1% TFA). Method A: 0–10 min, 40–100% B; 10–14 min, 100% B; 14–15 min, 100–40% B; 15–20 min, 40% B. Method B: 0–10 min, 2–100% B; 10–14 min, 100% B; 14–15 min 100–2% B; 15–18 min 2% B. Method C: 0–1 min, 40% B; 1–7 min, 40–100% B, 7–12 min, 100% B; 12–13 min, 100–40% B, 13–18 min 40% B. Method D: 0–1 min, 20% B; 1–7 min, 20–100% B, 7–12 min, 100% B; 12–13 min, 100–20% B, 13–18 min 20% B. Method E: 0–5 min, 5% B; 5–12 min, 5–100% B; 12–13 min 100–5% B, 13–18 min 5% B. For the anisole stability test, HPLC was performed using a Waters 1525 Binary HPLC system fitted with a 2998 photodiode array detector and a Bioscan γ detector. A Phenomenex Gemini column (5 μm, 4.6 Å 250 mm, C18) at a flow rate of 1.0 mL/min and monitoring at 215 and 254 nm was employed. The mobile phase was composed of solvent A = H_2O (0.1% TFA) and solvent B = CH_3CN (0.1% TFA). Method F: 0–15 min, 20–100% B; 15–25 min, 100% B. For calibration curves, each calibration solution was evaluated in triplicate and the data analyzed by the least-squares method. The limit of quantitation and the limit of detection were calculated using the standard deviation method.

31-(3′,6′-Dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthen]-6-yl)-3-(3-(tris(2-perfluorohexylethyl)stannyl)benzyl)thiourea (3a)

To a solution of compound **2a** (50 mg, 0.039 mmol) in DMF (5 mL) was added fluorescein 5(6)-isothiocyanate (41 mg, 0.11 mmol) and the reaction mixture stirred at room temperature overnight. The solution was subsequently concentrated to dryness, FC-72 was added (10 mL) and the solution extracted with DCM (3 × 5 mL). FC-72 was removed by rotary evaporation and the desired product isolated using column chromatography eluting with methanol/DCM (1:9, v/v) yielding **3a** as

an orange solid. Yield (46 mg, 71%). ^{1}H NMR (600 MHz, MeOD-d_4) δ 8.13 (s, 1H), 7.78–7.76 (m, 1H), 7.59 (s, 1H), 7.42–7.39 (s, 3H), 7.14–7.12 (m, 1H), 6.68–6.66 (m, 4H), 6.53–6.52 (m, 2H), 4.88 (s, 2H), 2.47–2.38 (m, 6H), 1.42–1.29 (m, 6H); HRMS (ESI$^+$) *m/z* calcd. for $C_{52}H_{32}F_{39}N_2O_5SnS$ $[M+H]^+$ 1657.0431, found: 1657.0454; FTIR (KBr, cm^{-1}): 2922, 1699, 1592, 1206; HPLC (method B) $t_R = 12.8$ min.

1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-(tris(2-perfluorohexyl-ethyl)stannyl)pyrimidine-2,4(1*H*,3*H*)-dione (13a)

A solution of diacetoxypalladium (4 mg, 0.02 mmol) in THF (0.5 mL) was added to 1,3,5,7-tetramethyl-2,4,8-trioxa-(2,4-dimethoxyphenyl)-6-phosphaadamantane (8 mg, 0.03 mmol) in THF (0.5 mL).[12] The resulting solution was stirred for 10 min, and tris-(2-perfluorohexylethyl)stannane (650 mg, 0.56 mmol) in THF (0.5 mL) was added. After 10 min, 1-(4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-iodopyrimidine-2,4(1*H*,3*H*)-dione (92 mg, 0.26 mmol) in THF (0.5 mL) was added and the resulting mixture was heated in a Biotage microwave reactor at 160 °C for 15 min. The reaction mixture was filtered through KF/silica and the solvent removed by rotary evaporation. The desired product was isolated using column chromatography eluting with methanol/DCM (1:9, v/v) yielding **13a** as a colorless oil. Yield (126 mg, 35%). ^{1}H NMR (600 MHz, MeOD-d_4) δ 7.92 (s, 1H), 6.31 (m, 1H), 4.41 (m, 1H), 3.95 (m, 1H), 3.78–3.72 (m, 2H), 2.47–2.41 (m, 6H), 2.32–2.21 (m, 2H), 1.35–1.23 (m, 6H); ^{13}C NMR (150 MHz, MeOD-d_4) δ 169.3, 152.7, 147.7, 110.8, 89.4, 87.0, 72.6, 62.9, 41.7, 28.7, 0.0; HRMS (ESI$^+$) *m/z* calcd. for $C_{33}H_{23}F_{39}N_2O_5Sn$ $[M+H]^-$ 1389.0061, found: 1389.0084; FTIR (KBr, cm^{-1}): 3410, 3043, 2944, 1691, 1238, 1144.

General procedure for preparing coated fluorous silica

Fluorous silica was added to the required concentration of the fluorous precursor in chloroform and the mixture agitated by hand until a slurry was formed. After sitting at room temperature overnight, the resulting powder was added to a polypropylene SPE tube that contained 500 mg of unmodified FS previously washed with DMF (1 mL), H_2O (5 mL), and 80:20 EtOH/H_2O (5 mL).

General procedure for solid-phase radioiodination with iodogen

80% (v/v) EtOH/H_2O (100 μL) (pH adjusted between 3 and 4 with conc. AcOH) was added to the SPE cartridge containing the coated FS. Iodogen (5 μL, 0.4 mg/mL in MeOH) was added followed by $Na^{125}I$ (10 μL, 3.7–7.4 MBq) in 0.1 M NaOH. After 20 min, the reaction was quenched with 0.1 M $Na_2S_2O_{5(aq)}$ (50 μL), and the cartridge was washed with water (5 mL) followed by 80% EtOH/H_2O (5 mL), where (0.5 mL) fractions were collected. HPLC retention times for all products were compared with that for authentic non radioactive standards.

General procedure for solution phase (FLS) labeling with iodogen

Iodogen (5 μL, 0.4 g/mL in MeOH) followed by $Na^{125}I$ (10 μL, 3.7–7.4 MBq in 0.1 M NaOH) was added to the fluorous-tin precursor (100 μL, 5 mg/mL in 5% AcOH/MeOH). After 5 min, $Na_2S_2O_{5(aq)}$ (50 μL, 0.1) was added and the mixture diluted to 1 mL with water. The solution was then transferred to a 2 g preconditioned FSPE cartridge. This was washed with water (3 mL) followed by 80:20 EtOH/H_2O (8 mL), and 1 mL fractions were collected.

General procedure for solid-phase radioiodination with chloramine-T

An SPE cartridge containing the coated FS was connected to a SepPak plus C18 cartridge. Ten percent acetic acid in EtOH (50 μL) was added, followed by chloramine-T (50 μL, 4 mg/mL in water) and $Na^{125}I$ (10 μL, 1.85 GBq/mL in 0.1 M NaOH). After 20 min, $Na_2S_2O_{5(aq)}$ (50 μL, 0.2 M) was added, and the system washed with water (5 mL) followed by 1.5:200:50 (v/v/v) H_3PO_4/EtOH/H_2O (5 mL) to elute the desired product. HPLC retention times were compared with those of nonradioactive authentic standards.

Stability study

Compound **5a** (20 μL of a 6.9 mg/mL solution in MeOH) was added to 1.5:200:50 (v/v/v) H_3PO_4/EtOH/H_2O (160 μL). Samples were taken at 5 and 40 min and analyzed by reverse-phase HPLC (method C).

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada and the Ontario Institute for Cancer Research (OICR) through funding provided by the Government of Ontario.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] P. A. Culbert, D. H. Hunter, *React. Polym.* **1993**, *19*, 247–253.
[2] D. H. Hunter, X. Zhu, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 653–661.
[3] R. Dunn-Dufault, A. Pollak, J. Fitzgerald, J. R. Thornback, J. R. Ballinger, *Nucl. Med. Biol.* **2000**, *27*, 803–807.
[4] L. Yong, M.-L. Yao, J. F. Green, H. Kelly, G. W. Kabalka, *Chem. Commun.* **2010**, *46*, 2623–2625.
[5] A. N. Gifford, S. Kuschel, C. Shea, J. S. Fowler, *Bioconjugate Chem.* **2011**, *22*, 406–412.
[6] S. Mundwiler, L. Candreia, P. Hafliger, K. Ortner, R. Alberto, *Bioconjugate Chem.* **2004**, *15*, 195–202.
[7] R. W. Riddoch, P. Schaffer, J. F. Valliant, *Bioconjugate Chem.* **2006**, *17*, 226–235.
[8] A. Donovan, F. Forbes, P. Dorff, P. Schaffer, J. Babich, J. F. Valliant, *J. Am. Chem. Soc.* **2006**, *128*, 3536–3537.
[9] A. C. Donovan, J. F. Valliant, *Nucl. Med. Biol.* **2008**, *35*, 741–746.
[10] R. Bejot, T. Fowler, L. Carroll, S. Boldon, J. E. Moore, J. Declerck, V. Gouverneur, *Angew. Chem. Int. Ed.* **2009**, *48*, 586–589.
[11] A. S. Zhang, C. S. Elmore, M. A. Egan, D. G. Melillo, D. C. Dean, *J. Label. Compd. Radiopharm.* **2005**, *48*, 203–208.
[12] J. W. McIntee, C. Sundararajan, A. C. Donovan, M. S. Kovacs, A. Capretta, J. F. Valliant, *J. Org. Chem.* **2008**, *73*, 8236–8243.
[13] W. Zhang, *Tetrahedron* **2003**, *59*, 4475–4489.
[14] W. Zhang, *Chem. Rev.* **2004**, *104*, 2531–2556.
[15] D. P. Curran. Handbook of Fluorous Chemistry, Wiley-VCH, Weinheim, **2004**, pp. 101–127.
[16] D. P. Curran, S. Hadida, M. He, *J. Org. Chem.* **1997**, *62*, 6714–6715.
[17] J.-M. Vincent, *Top. Curr. Chem.* **2012**, *308*, 153–174.
[18] M. Hoshino, P. Degenkolb, D. P. Curran, *J. Org. Chem.* **1997**, *62*, 8341–8349.
[19] C. C. Chan, H. Lam, Y. C. Lee, X.-M. Zhang. Analytical Method Validation and Instrument Performance Verification, 1st edn, John Wiley & Sons, Inc., Hoboken, New Jersey, **2004**, pp. 37–40.
[20] General chapter on inorganic impurities, United States pharmacopeia, USP ad hoc advisory panel on inorganic impurities. http://www.usp.org/sites/default/files/usp_pdf/EN/USPNF/2008-04-10InorganicImpuritiesStim.pdf. [24 May 2013].
[21] A. Vale, *Medicine* **2007**, *35*, 633–634.
[22] J. M. Michelot, M. F. C. Moreau, A. J. Veyre, F. J. Bonafous, F. J. Bacin, J. C. Madelmont, F. Bussiere, P. A. Souteyrand, L. P. Mauclaire, F. M. Chossat, J. M. Papon, P. G. Labarre, P. Kauffmann, R. J. Plagne, *J. Nucl. Med.* **1993**, *34*, 1260–1266.
[23] G. Prabhakar, A. Mathur, G. Shunmugam, Y. D. Teje, S. S. Sachdev, N. Sivaprasad, *Appl. Radiat. Isot.* **2011**, *69*, 63–67.
[24] A. Darwish, M. Blacker, N. Janzen, S. M. Rathmann, S. Czorny, S. M. Hillier, J. L. Joyal, J. W. Babich, J. F. Valliant, *Med. Chem. Lett.* **2012**, *3*, 313–316.

[25] K. P. Maresca, S. M. Hillier, F. J. Femia, D. Keith, C. Barone, J. L. Joyal, C. N. Zimmerman, A. P. Kozikowski, J. A. Barrett, W. C. Eckelman, J. W. Babich, *J. Med. Chem.* **2009**, *52*, 347–357.
[26] B. Youngjoo, M. Pullambhatla, H. Wang, R. C. Mease, M. G. Pomper, *Macromol. Res.* **2013**, *21*, 565–573.
[27] S. Lehnert, Y. Li, E. Bump, B. Riddoch, A. Chenite, M. Shive, *Open Nucl. Med. J.* **2011**, *3*, 19–24.
[28] T. J. Mangner, J. L. Wu, D. M. Wieland, *J. Org. Chem.* **1982**, *47*, 1484–1488.
[29] G. W. Kabalka, V. Namboodiri, M. R. Akula, *J. Label. Compd. Radiopharm.* **2001**, *44*, 921–929.
[30] L. Yong, M.-L. Yao, H. Kelly, F. F. Green, G. W. Kabalka, *J. Label. Compd. Radiopharm.* **2011**, *54*, 173–174.
[31] T. Brenstrum, D. A. Gerristma, G. M. Adjabeng, C. S. Frampton, J. Britten, A. J. Robertson, J. McNulty, A. Capretta, *J. Org. Chem.* **2004**, *69*, 7635–7639.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site.

