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Preparation and evaluation of reagents for tagging amino and thiol groups with fluorous stannanes. A convenient method for producing radioiodinated compounds in high effective specific activity†

Amanda C. Donovan, and John F. Valliant*

Building on the previously reported fluorous labeling strategy (FLS), a new approach for preparing molecular imaging and therapy agents derived from radioiodine in high purity without the need to employ HPLC was developed. A series of novel reagents containing a fluorous arylstannane, including fluorous benzaldehydes (1a/b) and an aryl-iodoacetamide (2), were prepared so that the FLS could be used to label and purify targeting vectors that contain free amines or thiol groups. The reagents were conjugated to model amines and thiols in generally high yields (79–95%) under mild conditions. The fully characterized products were radiolabeled with Na[^{125}I] in the presence of iodogen and the products were purified using fluorous solid-phase extraction to yield the desired iodinated products in high yield (>83%) and high effective specific activity (ESA). The work reported creates a convenient and flexible means of preparing targeted molecular imaging and therapy agents derived from radioisotopes of iodine in high ESA.

Keywords: fluorous chemistry; molecular imaging; high effective specific activity; radioiodination

Introduction

Existing methods used to prepare radiopharmaceuticals involve the use of a large excess of the compound to be labeled compared with the amount of radionuclide. This can be problematic in the case of molecular imaging probes[1,2] that are designed to seek out protein targets that have relatively low expression levels. The large excess of unlabeled ligand can compete with the labeled form for the limited number of target sites, consequently the reaction mixture in these cases must be purified prior to use.[2–4]

Purification is normally performed using HPLC, which is acceptable for research studies but is less desirable for routine clinical production. A number of alternative techniques have been developed to address the excess ligand issue including solid-phase labeling. In solid-phase labeling, a precursor is attached to a cross-linked polymer through an arylstannane.[5–7] On addition of radioiodine and an oxidant, which cleaves the polymer-tin-aryl bond, the desired product is released into solution and the precursor is removed by filtration. Although highly effective in removing excess ligand, solid-phase labeling has a number of challenges including difficulties in conjugating precursors to the polymer support quantitatively and the inability to remove any polymer-bound impurities prior to labeling.

A new solution phase technique for producing and purifying radioiodinated compounds in high effective specific activity (ESA), which addresses these limitations was developed through the use of fluorous supports.[8] In this work, fluorine-rich substituents are used in place of the polymer in solid-phase labeling. On reaction with radioiodine and an oxidant, the fluorous-tin aryl bond is cleaved and the product is separated from the precursor by passing the reaction mixture through a fluorine-rich solid-phase extraction (FSPE) cartridge. In FSPE, the precursor and all fluorous byproducts are retained while the desired compound is selectively eluted. This type of chemoselective filtration has been used to produce a library of iodobenzamides in high yield and high purity in mere minutes without the need to employ HPLC.[8] The fluorous labeling strategy (FLS) has also been used to produce clinically relevant radiopharmaceuticals including *meta*-iodobenzylguanidine and 5-iodo-2′-deoxyuridine in high ESA using different isotopes of iodine.[9,10]

The FLS has been used predominately for tagging small molecules. Given the rapidly expanding use of peptides and biomolecules as targeting vectors, it would be beneficial to have fluorous-tin synthons for tagging amino and thiol groups found on these vectors as a means to preparing and purifying the

Department of Chemistry, McMaster University, 1280 Main St. West, Hamilton, Ont., Canada L8S 4M1

**Correspondence to: John F. Valliant, Department of Chemistry, McMaster University, 1280 Main St. West, Hamilton, Ont., Canada L8S 4K1. E-mail: valliant@mcmaster.ca*

†*Supporting information may be found in the online version of this article.*

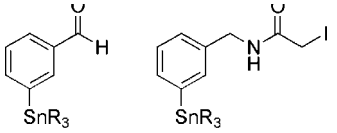


Figure 1. Targeted fluorous synthons ($R = CH_2CH_2(CF_2)_5CF_3$).

iodinated derivatives. To this end, the synthesis of a series of new fluorous 'tagging' groups and a study of their reactivities towards model compounds was undertaken along with developing the method for labeling and purifying the products.

Benzaldehyde derivatives are an attractive choice for modifying small molecules and biomolecules, as they are readily introduced into diverse scaffolds at free amine-sites under mild conditions. Furthermore, the resulting Schiff-base can be irreversibly reduced to a stable amine under mild conditions that are compatible with a number of sensitive functional groups.[11] Similarly, iodoacetamides react with a number of functional groups on proteins including the thioether in methionine, the sulfhydryl group of cysteine, the ε-amine of lysine and *N*-terminal α-amines.[12] Building on this general utility and versatility, methods for preparing the fluorous benzaldehyde (**1**) and aryl-iodoacetamide (**2**) derivatives were developed (Figure 1).

Experimental section

Reagents and general procedures

All chemicals were purchased from Sigma-Aldrich and Fluorous Technologies Inc. Compounds **3** and **5** were prepared as previously reported.[9,13] Sodium [^{125}I] iodide with a specific activity of $\sim$17 Ci/mg was obtained from the McMaster Nuclear Reactor (Hamilton, Ont.).

Caution

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

Instrumentation

NMR spectra were recorded using a Bruker DRX 500 spectrometer with chemical shifts reported in ppm relative to the residual proton (^{1}H NMR) or carbon (^{13}C NMR) signal of the deuterated solvent. Signals for C–F carbon atoms are enclosed in square brackets. Infrared (IR) spectra were acquired using a BioRad FTS-40 FT-IR spectrometer. All spectra were recorded at ambient temperature. Analytical thin-layer chromatograms (Merck F_{254} silica gel on aluminum plates) were visualized using UV light. Purification of products was carried out using Ultrapure Silica Gel from Silicycle (70–230 mesh). Electrospray ionization (ESI) mass spectrometry experiments were performed on a Waters/Micromass Quattro Ultima instrument, and samples were first dissolved in methanol. High-resolution mass spectrometry data were obtained using a Waters-Micromass Q-TOF Ultima Global spectrometer. High-performance liquid chromatography 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, and a Star 800 analog interface module. Reverse-phase chromatography was performed using an Agilent Zorbax SB-C18 column (4.6 $\times$ 250 mm, 300Å–5 μm). The wavelength for UV detection was set at 254 nm and the dwell time in the gamma detector was 1 s in a 10-μl loop. The elution conditions were as follows: **Method A**: Solvent A = CH_3CN containing 0.1% HOAc, Solvent B = H_2O containing 0.1% HOAc; Elution conditions: 60% A 0–2 min; 60–100% A 2–15 min; 100% A 15–25 min. **Method B**: Solvent A = CH_3CN (0.1 % H_3PO_4), Solvent B = H_2O (0.1% H_3PO_4); 0–10 min 23% A; 10–15 min 23–100% A; 15–30 min 100% A. For both the methods, the flow rate was maintained at 1 ml/min.

3-(*Tris*[2-perfluorohexylethyl]stannyl)benzaldehyde (1a)

Under an argon atmosphere, 3-bromobenzaldehyde diethyl acetal (311 mg, 1.16 mmol) was dissolved in diethyl ether and cooled to -100°C. A solution of *tert*-butyllithium (1.7 M, 1.7 mmol) in pentane was added dropwise via syringe. After 40 min, a solution of *tris*[2-perfluorohexylethyl]tin bromide (1.06 g, 856 μmol) in diethyl ether (5 ml) was added dropwise via cannula. The reaction mixture was stirred at -100°C for an additional 40 min, after which it was warmed slowly to room temperature. After stirring for a minimum of 3 h, the solvent was removed by rotary evaporation. A colorless oil was isolated by liquid–liquid extraction using FC-72® (3 $\times$ 5 ml) and dichloromethane (3 $\times$ 10 ml), which was subsequently stirred in 20 ml of 1 N HCl for 6 h. Following this, the fluorous components were extracted into FC-72® (3 $\times$ 10 ml) and was later purified by flash chromatography (5 % EtOAc in hexanes) to give a colorless oil. Yield: 440 mg, 35%. TLC(CH_2Cl_2): $R_f = 0.72$. ^{1}H NMR (500 MHz, $CDCl_3$): 10.03 (s, 1H, C*H*O), 7.89 (*m*, 2H, Ar-*H*), 7.65 (*m*, 1H, Ar-*H*), 7.59 (*m*, 1H, Ar-*H*), 2.33 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.37 (*t*, $J = 8.2$ Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125 MHz, $CDCl_3$): 193.0, 142.9, 138.5, 137.9, 137.2, 132.0, 130.3, 28.4, 0.1. FTIR (KBr, cm^{-1}): 2933, 2852, 1706, 1577.

4-(*Tris*[2-perfluorohexylethyl]stannyl) benzaldehyde (1b)

Prepared in the same fashion as described for **1a**. Yield: 377 mg, 30%. TLC(CH_2Cl_2): $R_f = 0.71$. ^{1}H NMR (500 MHz, $CDCl_3$): 10.02 (s, 1H, C*H*O), 7.88 (d, $J = 8.0$ Hz, 2H, Ar-*H*), 7.59 (*m*, 2H, Ar-*H*), 2.33 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.35 (*t*, $J = 8.1$ Hz, 6H, $SnCH_2CH_2C_6F_{13}$); ^{13}C NMR (125 MHz, $CDCl_3$): 192.2, 146.1, 137.1, 136.5, 129.3, [118.2, 116.2, 111.1], 27.6, -1.1; FTIR (KBr, cm^{-1}): 2933, 2831, 1707, 1592.

2-Chloro-*N*-(3-(*tris*[2-perfluorohexylethyl]stannyl)-benzyl) acetamide (6)

To a solution of 3-(*tris* [2-perfluorohexylethyl]stannyl)benzylamine (80 mg, 67 μmol) in chloroform was added chloroacetyl chloride (21 mg, 188 μmol) at 0°C, followed by slow addition of NEt_3 (500 μl, 3.6 mmol). The resulting mixture was stirred at ambient temperature overnight. Solvent and excess triethylamine were removed under reduced pressure; the resulting residue was dissolved in FC-72® (20 ml) and extracted with water (3 $\times$ 15 ml). The fluorous layer was dried over anhydrous sodium sulfate. Removal of the FC-72® gave dark yellow–brown oil, which was purified by silica gel chromatography where the product was isolated using 10% ethanol in chloroform. Yield: 62 mg, 78%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.41 (*m*, 1H, Ar-*H*), 7.31 (*m*, 3H, Ar-*H*), 6.89 (s, 1H, Ar-CH_2NH), 4.50 (d, $J = 5.9$ Hz, 2H, Ar-CH_2NH), 4.10 (s, 2H, CH_2Cl), 2.32 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.32 (t, $J = 8.2$ Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125 MHz, $CDCl_3$): 165.9, 138.0, 137.5, 135.3, 135.2, 129.3, 128.9, [118.3,] 43.8, 42.5, 27.7, -1.3. HRMS (QTOF+): $C_{33}H_{22}NOF_{39}SnCl$ $[M+H]^+ = 1343.9789$, found: 1343.9807. FTIR (KBr, cm^{-1}): 3298, 2943, 1666, 1550.

2-Iodo-*N*-(3-(*tris*[2-perfluorohexylethyl]stannyl)-benzyl) acetamide (2)

Compound **6** (45 mg, 33 μmol) was dissolved in acetonitrile (5 ml) and combined with sodium iodide (50 mg, 335 μmol). The resulting solution was heated at reflux (85°C) overnight and then allowed to cool to ambient temperature. The solvent was removed and the resulting residue was dissolved in FC-72® and extracted with water (3 × 10 ml). The fluorous layer was dried over anhydrous sodium sulfate. Removal of the FC-72® gave an off-white solid. Yield: 42 mg, 86 %. TLC (9:1, $CHCl_3$:EtOH) $R_f = 0.67$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.40 (*m*, 1H, Ar-*H*), 7.32 (*m*, 3H, Ar-*H*), 6.34 (s, 1H, Ar-CH_2NH), 4.47 (d, $J = 5.8$ Hz, 2H, Ar-CH_2), 3.73 (s, 2H, CH_2I), 2.32 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.30 (*t*, $J = 8.2$ Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125 MHz, $CDCl_3$): 166.7, 138.2, 137.5, 135.2, 129.0, 128.8, [120.3, 118.3, 116.2], 44.3, 27.7, −1.2 HRMS (QTOF+) calcd for $C_{33}H_{21}INOF_{39}Sn$ $[M+NH_4]^+$: 1452.9411, found: 1452.9417. FTIR (KBr, cm^{-1}): 3281, 3084, 2922, 2857, 1652, 1559.

Aldehyde conjugation via reductive amination: general procedure

To a solution of 3- or 4-*tris*[2-perfluorohexylethyl]stannyl-benzaldehyde (**1a** or **1b**) (75 mg, 61 μmol) in chloroform, the amine (234 μmol) of interest was added. The mixture was stirred for 2 h at which point sodium triacetoxyborohydride (50 mg, 234 μmol) was added. The reaction mixture was stirred at ambient temperature for 16 h prior to removal of the solvent under reduced pressure. The resulting white residue was suspended in FC-72® (10 ml) and extracted with 1 M Na_2CO_3 (20 ml) and twice with H_2O (2 × 20 ml). The fluorous layer was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford a colorless oil. The desired products were isolated using silica gel chromatography and 10% ethanol in chloroform.

N-Butyl-3(-*tris*[2-perfluorohexylethy]stannyl)benzylamine (7a)

Yield: 140 mg, 85%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.30$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.37 (*m*, 4H, Ar-*H*), 3.79 (s, 2H, Ar-CH_2NH), 2.64 (*t*, $J = 7.0$ Hz, 2H, $NHCH_2CH_2CH_2CH_3$), 2.31 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.51 (*m*, 2H, $NHCH_2CH_2CH_2CH_3$), 1.37–1.27 (*m*, 8H, $Sn(CH_2CH_2C_6F_{13})_3$, $NHCH_2CH_2CH_2CH_3$), 0.90 (*t*, $J = 7.3$ Hz, 3H, $NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 141.0, 136.4, 135.6, 134.5, 129.2, [120.3, 118.2, 116.2, 110.8, 108.6], 54.0, 49.2, 32.1, 27.7, 20.4, 13.8, −1.5. HRMS (QTOF+): mass calcd. for $C_{35}H_{28}NF_{39}Sn$: 1324.0707, found: 1324.0653. FTIR (KBr, cm^{-1}): 3395, 2922, 1646.

N-Butyl-4-*tris*[2-perfluorohexylethyl])stannyl-benzylamine (7b)

Yield: 132 mg, 80%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.30$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.42 (d, $J = 7.6$ Hz, 2H, Ar-*H*), 7.35 (d, $J = 7.8$ Hz, 2H, Ar-*H*), 3.83 (s, 2H, Ar-CH_2NH), 2.66 (*t*, $J = 7.3$ Hz, 2H, $NHCH_2CH_2CH_2CH_3$), 2.30 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$) 1.56 (*m*, 2H, $NHCH_2CH_2CH_2CH_3$), 1.33 (*m*, 8H, $Sn(CH_2CH_2C_6F_{13})_3$, $NHCH_2CH_2CH_2CH_3$), 0.91 (*t*, $J = 7.3$ Hz, 3H, $NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 136.1, 129.0, [118.3, 116.0,] 53.3, 48.7, 31.5, 27.7, 20.3, 13.8, −1.5. HRMS (QTOF+): mass calcd for $C_{35}H_{29}NF_{39}Sn$: 1324.0699, found: 1324.0653. FTIR (KBr, cm^{-1}): 3395, 2922, 1996, 1436, 1239.

N-3-(*Tris*[2-perfluorohexylethyl]stannyl)-1-(2-methoxyphenyl)-piperazine (8a)

Yield: 142 mg, 79%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.39 (*m*, 3H, Ar-*H*), 7.27 (*m*, 1H, Ar-*H*), 7.01–6.85 (*m*, 4H, Ar-*H*), 3.85 (s, 3H, OCH_3), 3.60 (s, 2H, Ar-CH_2-N), 3.10 (s, 4H, $N(CH_2CH_2)_2N$), 2.67 (s, 4H, $N(CH_2CH_2)_2N$), 2.32 (*m*, 6H, $Sn(CH_2CH_2C_6F_{13})_3$), 1.32 (*t*, $J = 8.3$ Hz, 6H, $Sn(CH_2CH_2C_6F_{13})_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 152.3, 141.4, 138.8, 136.6, 136.2, 134.6, 130.5, 128.8, 122.9, 121.0, 118.2, [116.1], 111.3, [111.1], 63.0, 55.3, 53.3, 50.6, 27.7, −1.5. HRMS (QTOF+): mass calcd for $C_{42}H_{33}F_{39}N_2OSn$: 1443.1070, found: 1443.1052. FTIR (KBr, cm^{-1}): 3422, 2940, 2817, 1501.

N-4-(*Tris*[2-perfluorohexylethyl])stannyl)-1-(2-methoxyphenyl)-piperazine (8b)

Yield: 150 mg, 83%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.68$. ^{1}H NMR (500 MHz, $CDCl_3$): 7.42 (d, $J = 7.3$ Hz, 2H, Ar-*H*), 7.34 (d, $J = 7.6$ Hz, 2H, Ar-*H*), 6.99–6.85 (*m*, 4H, Ar-*H*), 3.85 (s, 3H, OCH_3), 3.59 (s, 2H, Ar-CH_2-N), 3.10 (s, 4H, $N(CH_2CH_2)_2N$), 2.66 (s, 4H, $N(CH_2CH_2)_2N$), 2.31 (*m*, 6H, $Sn(CH_2CH_2C_6F_{13})_3$), 1.30 (*t*, $J = 8.3$ Hz, 6H, $Sn(CH_2CH_2C_6F_{13})_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 152.3, 141.5, 139.9, 135.9, 134.7, 129.8, 122.9, 121.0, 118.3, 111.2, 62.9, 55.3, 53.4, 50.7, 27.7, −1.5. HRMS (QTOF+): mass calcd for $C_{42}H_{33}F_{39}N_2OSn$ $[M+H]^+$: 1443.1070, found: 1443.1066. FTIR (KBr, cm^{-1}): 2943, 2819, 1596, 1502.

2-Amino-3-[(3-2-perfluorohexylethylstannyl-benzylcarbamoyl)-methylsulfanyl] propionic acid (9)

Fluorous iodoacetamide (**2**) (30 mg, 21 μmol) was dissolved in a 1:1 mixture of perfluorobutyl methyl ether (PFBME)/methanol (10 ml). A solution of L-cysteine (10 mg, 82 μmol) in 1 M methanolic KOH (5 ml) was then added and the resulting homogeneous solution was stirred for 2 h. The solvent was removed by rotary evaporation and the product (**9**) isolated by liquid–liquid extraction using FC-72® (3 × 5 ml) and water (15 ml). Yield: 28 mg, 95%. TLC (9:1, $CHCl_3$:EtOH): $R_f = 0.1$. ^{1}H NMR (500 MHz, CD_3OD): 7.44 (*m*, 4H, Ar-*H*), 4.41 (s, 2H, Ar-CH_2NH), 3.36 (*m*, 1H, $SCH_2CH(NH_2)$), 3.26 (d, 2H, $NHC(O)CH_2S$), 2.99 (*m*, 1H, CH_2SCH_2), (1H, CH_2SCH_2) 2.41 (*m*, 6H, $SnCH_2CH_2C_6F_{13}$), 1.42 (*t*, $J = 8.3$ Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125 MHz, CD_3OD): 180.3, 172.5, 140.2, 139.0, 136.4, 136.1, 129.8, 129.5, 56.6, 44.4, 39.8, 29.0, −0.5. HRMS (QTOF -): mass calcd for $C_{36}H_{26}N_2O_3F_{39}SSn$: 1427.0063 $[M-H]^-$, found: 1427.0048. FTIR (KBr, window, cm^{-1}): 3401, 1634.

General procedure for preparing iodo-arylamines (10, 11)

The desired amine (1.01 mmol) was dissolved in chloroform (10 ml). To this was added either 3- or 4-iodobenzylbromide (100 mg, 337 μmol) followed by triethylamine (363 mg, 36 mmol). The resulting mixture was heated to reflux and stirred overnight. The reaction solvent was removed under reduced pressure and the resulting yellow residue was subsequently purified by using silica gel chromatography (10 % ethanol in chloroform).

N-Butyl-3-iodobenzylamine (10a)

Yield: 76 mg, 78%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.5$. HPLC: $R_t = 6.0$ min (Method A). ^{1}H NMR (500 MHz, $CDCl_3$): 7.72 (s, 1H, Ar-*H*), 7.60 (d, $J = 7.8$ Hz, 1H, Ar-*H*), 7.30 (d, $J = 7.5$ Hz, 1H, Ar-*H*) 7.06 (*t*, $J = 7.7$ Hz, 1H, Ar-*H*), 3.76 (s, 2H, Ar-CH_2NH), 2.64 (*t*, $J = 7.2$ Hz, 2H, Ar-CH_2NH-CH_2), 1.61 (s, 1H, Ar-CH_2NH) 1.52 (*m*, 2H, NH-$CH_2CH_2CH_2$), 1.38 (*m*, 2H, NH-$CH_2CH_2CH_2CH_3$), 0.94 (*t*, $J = 7.3$ Hz, 3H, NH-$CH_2CH_2CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 142.6, 137.2, 136.0, 130.1, 127.4, 94.4, 53.2, 49.0, 32.0, 20.4, 13.9. HRMS (QTOF+): mass calcd for $C_{11}H_{17}NI$: 290.0406, found: 290.0413. FTIR (KBr, cm^1): 3448, 2962, 2935, 2865, 2796.

N-Butyl-4-iodobenzylamine (10b)

Yield: 76 mg, 78%. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.5$. ^1H NMR (500 MHz, $CDCl_3$): 7.64 (d, $J = 8.1$ Hz, 2H, Ar-*H*), 7.08 (d, $J = 8.1$ Hz, 2H, Ar-*H*), 3.74 (s, 2H, Ar-CH_2NH), 2.60 (*t*, $J = 7.3$ Hz, 2H, NH-CH_2), 1.48 (*m*, 2H, NH-CH_2CH_2), 1.35 (*m*, 2H, NH-$CH_2CH_2CH_2CH_3$), 0.91 (*t*, $J = 8.3$ Hz, 3H, NH-$CH_2CH_2CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 140.5, 137.5, 130.3, 92.2, 53.6, 49.3, 32.4, 20.6, 14.2. HRMS (QTOF+): mass calcd for $C_{11}H_{17}NI$: 290.0406, found: 290.0417. FTIR (KBr, cm^{-1}): 2956, 2927, 2797.

N-(3-Iodobenzyl)-1-(2-methoxyphenyl)-piperazine (11a)

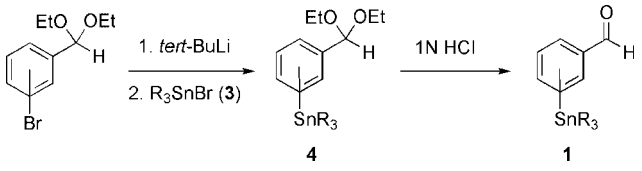
Yield: 131 mg, 95%. TLC ($CHCl_3$): $R_f = 0.38$. HPLC: $R_t = 14.5$ min (Method B). ^1H NMR (500 MHz, $CDCl_3$): 7.74 (s, 1H, Ar-*H*), 7.60 (d, $J = 7.9$ Hz, 1H, Ar-*H*), 7.33 (d, $J = 7.6$ Hz, 1H, Ar-*H*), 7.06 (*t*, $J = 7.7$ Hz,1H, Ar-*H*), 7.01–6.85 (*m*, 4H, Ar-*H*), 3.89 (s, 3H, OCH_3), 3.55 (s, 2H, Ar-CH_2-N), 3.13 (s, 4H, $N(CH_2CH_2)_2N$), 2.68 (s, 4H, $N(CH_2CH_2)_2N$). ^{13}C NMR (125 MHz, $CDCl_3$): 152.3, 141.4, 140.8, 138.1, 136.2, 130.1, 128.5, 122.9, 121.1, 118.3, 111.2, 94.5, 62.5, 55.4, 53.4, 50.7. HRMS (QTOF+): mass calcd for $C_{18}H_{22}N_2OI$: 409.0777, found: 409.0785. FTIR (KBr, cm^{-1}): 3063, 2935, 2816, 1500, 1241.

N-(4-Iodobenzyl)-1-(2-methoxyphenyl)-piperazine (11b)

Yield: 128 mg, 93%. TLC ($CHCl_3$): $R_f = 0.40$. ^1H NMR (500 MHz, $CDCl_3$): 7.66 (d, $J = 8.2$ Hz, 2H, Ar-*H*), 7.12 (d, $J = 8.2$ Hz, 2H, Ar-*H*), 7.00–6.85 (*m*, 4H, $C_6H_4OCH_3$), 3.86 (s, 3H, OCH_3), 3.52 (s, 2H, Ar-CH_2-N), 3.10 (s, 4H, $N(CH_2CH_2)_2N$), 2.64 (s, 4H, $N(CH_2CH_2)_2N$). ^{13}C NMR (125 MHz, $CDCl_3$): 152.4, 141.5, 138.1, 137.4, 131.3, 122.9, 121.1, 118.3, 111.4, 92.4, 62.6, 55.5, 53.4, 50.7. HRMS (QTOF+): mass calcd for $C_{18}H_{22}N_2OI$: 409.0777, found: 409.0795. FTIR (KBr, cm^{-1}): 2936, 2816, 1593.

2-Chloro-*N*-(3-iodobenzyl)acetamide (12)

3-Iodobenzylamine (501 mg, 1.85 mmol) was dissolved in chloroform (15 ml), then NEt_3 (500 µl, 3.6 mmol) was added. The solution was then cooled to 0°C prior to the addition of chloroacetyl chloride (313 mg, 2.78 mmol) and additional NEt_3 (3 ml, 21 mmol). The mixture was stirred at room temperature overnight prior to the removal of solvent and excess triethylamine under reduced pressure. The resulting residue was purified by silica gel chromatography where the desired product was eluted using a 10 % ethanol in chloroform. Yield: 348 mg, 61 %. TLC (9:1 $CHCl_3$:EtOH): $R_f = 0.70$. ^1H NMR (500 MHz, $CDCl_3$): 7.64 (*m*, 2H, Ar-*H*), 7.27 (*m*, 1H, Ar-*H*), 7.09 (*m*, 1H, Ar-*H*), 6.94 (s, 1H, Ar-CH_2N*H*), 4.44 (d, $J = 6.0$ Hz, 2H, Ar-CH_2), 4.11 (s, 2H, CH_2Cl). ^{13}C NMR (125 MHz, $CDCl_3$): 166.0, 139.9, 137.0, 136.8, 130.6, 127.1, 94.7, 43.2, 42.7. HRMS (QTOF+): mass calcd for $C_9H_{10}NOClI$: 309.9496, found: 309.9505. FTIR (KBr, cm^{-1}): 3272, 3068, 1653, 1555.



Scheme 1. Preparation of the fluorous benzaldehyde **1** ($R = CH_2CH_2(CF_2)_5CF_3$).

2-Iodo-*N*-(3-iodobenzyl)acetamide (13)

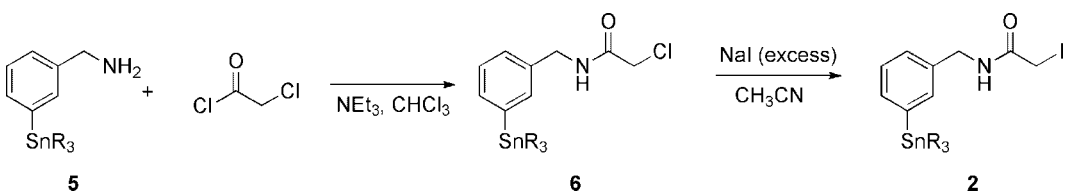
3-Iodobenzyl-chloroacetamide (**12**) (53 mg, 172 µmol) was dissolved in acetonitrile (5 ml) and combined with sodium iodide (258 mg, 1.72 mmol). The resulting solution was heated at reflux overnight and allowed to cool to room temperature. The solvent was removed and the resulting residue was purified by using silica gel chromatography where the product was eluted using 10 % ethanol in chloroform. Yield: 59 mg, 86%. TLC ($CHCl_3$) $R_f = 0.67$. ^1H NMR (500 MHz, $CDCl_3$): 7.64 (*m*, 2H, Ar-*H*), 7.27 (*m*, 1H, Ar-*H*), 7.09 (*m*, 1H, Ar-*H*), 6.38 (s, 1H, Ar-CH_2N*H*), 4.41 (d, $J = 5.9$ Hz, 2H, Ar-CH_2), 3.75 (s, 2H, CH_2Cl). ^{13}C NMR (125 MHz, $CDCl_3$): 167.0, 140.1, 137.0, 136.8, 130.7, 127.1, 94.7, 43.8. HRMS (QTOF+): mass calcd for $C_9H_{10}NOI_2$: 401.8825, found: 401.8860. FTIR (KBr, cm^{-1}): 3271, 1632, 1550.

2-Amino-3-[(3-iodo-benzylcarbamoyl)-methylsulfanyl] propionic acid (14)

L-Cysteine (30 mg; 247 mmol) was dissolved in methanolic KOH (5 ml, 1 M) and added to a solution of **13** (100 mg; 250 µmol) in methanol (2 ml). The resulting homogeneous mixture was stirred for 1 h whereupon the reaction solvent was removed by rotary evaporation. Yield: 95 mg, 97 %. TLC (9:1, $CHCl_3$:EtOH): $R_f = 0.1$. HPLC: $R_t = 11.1$ min (Method B). ^1H NMR (500 MHz, CD_3OD): 7.65 (s, 1H, Ar-*H*), 7.56, (d, $J = 7.7$ Hz, 1H, Ar-*H*), 7.28 (d, $J = 7.6$ Hz, 1H, Ar-*H*), 7.06 (*t*, $J = 7.8$ Hz, 1H, Ar-*H*), 4.32 (s, 2H, Ar-CH_2), 3.34 (*m*, 1H, $SCH_2CH(NH_2)$), 3.28 (*m*, 2H, CH_2SCH_2), 2.96 (*m*, 1H, CH_2SCH_2), 2.75 (*m*, 1H, CH_2SCH_2). ^{13}C NMR (125 MHz, CD_3OD): 180.3, 172.6, 142.6, 137.6, 137.4, 131.4, 127.9, 94.9, 56.6, 49.9, 43.6, 39.8. ESMS(+): 394.1 $[M+H]^+$. (KBr, cm^{-1}): 3417, 2505, 1625, 1592, 1418.

General radioiodination procedure

Compound **7**, **8**, or **9** (0.5 mg) was added to a sample vial previously coated with Iodogen® (prepared by evaporating 20 µl of a solution of Iodogen in CH_2Cl_2, 1 mg/ml). A solution of 5% acetic acid in methanol (100 µl) was added followed by sodium [^{125}I]iodide (5 µl, 20 mCi/ml, pH 10). After swirling the mixture for 3 min, the reaction was quenched by the addition of aqueous sodium metabisulfite ($Na_2S_2O_5$, 10 µl, 44 mg/ml). The reaction mixture was diluted with water (1 ml) and loaded onto a FluoroFlash® solid-phase extraction (F-SPE) cartridge which had been previously activated by washing first with 80:20 (v/v) solution of methanol–water (6 ml), followed by water (6 ml). The reaction vial was subsequently rinsed with an additional 3 ml of water that was also added to the F-SPE cartridge. The cartridge was eluted with water (6 ml), followed by 80:20 (v/v) solution of methanol–water (6 ml). Radioactive fractions eluted from the F-SPE cartridge were analyzed by using HPLC where in all cases,



Scheme 2. Preparation of iodoacetamide **2** ($R = CH_2CH_2(CF_2)_5CF_3$).

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one radioactive peak was observed where the retention times matched that for the authentic non-radioactive standards.

N-butyl-3- [^{125}I]iodobenzylamine ([^{125}I]**10a**): R_t: 6.4 min (Method A); RCY: 92 %; Radiochemical purity: > 98 %.

N-(3-[^{125}I]*iodobenzyl)-1-(2-methoxyphenyl)piperazine* ([^{125}I]**11a**): R_t: 14.8 min (Method B); RCY: 83 %; Radiochemical purity: > 98 %.

L-*Cysteine conjugate* ([^{125}I]**14**): R_t: 11.4 min (Method B); RCY: 86%; Radiochemical purity: > 98%.

Results and discussion

Preparation of fluorous conjugation synthons

To probe the differing *in vivo* stabilities of the positional isomers, the preparation of the first targets (meta and para-fluorous-tin benzaldehydes **1a/b**) was accomplished by combining the lithium salt of 3- or 4- bromobenzaldehyde diethyl acetal with *tris*(perfluorohexylethyl)stannyl bromide (Scheme 1). Note that to obtain good yields of the product it was essential to use *tert*-BuLi instead of *n*-BuLi. Compound **4a/b** was subsequently isolated from the reaction mixture following liquid–liquid extraction with perfluorinated hexanes (FC-72®) and dichloromethane. Immediately after isolating **4a/b**, the acetal was stirred in the presence of 1N HCl to effectively remove the protecting group. Purification via silica gel chromatography gave compounds **1a** or **1b** in 30 and 35 % yield, respectively.

The preparation of the iodoacetamide **2** involved conversion of the fluorous benzylamine **5,** whose synthesis has been previously reported,[9] to **6** *via* treatment with chloroacetyl chloride (Scheme 2).[14] A standard fluorous workup including liquid–liquid extraction between FC-72® and the reaction solvent (chloroform) was used to isolate the desired compound. Following purification by column chromatography, compound **6** was converted to the iodoacetamide **2** via a halogen exchange reaction. After purification by column chromatography, compound **2** was isolated in 86 % yield.

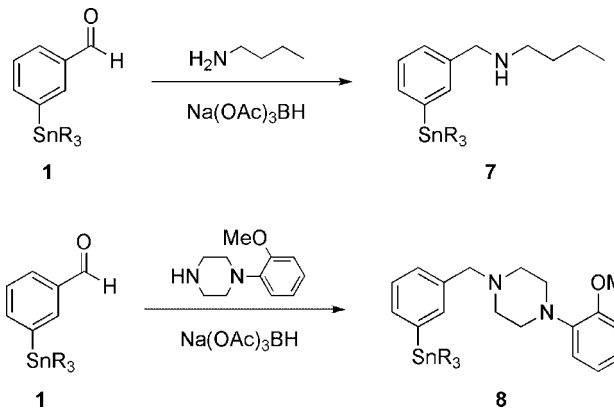
Conjugation chemistry

Reductive amination is often employed in bioconjugate chemistry to link aldehyde-containing molecules to a targeting vector including small molecules, proteins, and peptides that have free amine functionalities.[12] To establish the suitability of **1a/b** for reactions of this type and to probe the feasibility of the conjugation irrespective of the position of the functional group relative to the fluorous moiety, representative reductive amination reactions were conducted using *n*-butylamine (a primary amine) and 1-(2-methoxyphenyl)piperazine (a secondary amine). The latter compound is routinely used to prepare radiopharmaceuticals for targeting the $5HT_{1A}$ receptor.[15] Compound **1a/b** and the desired amine were combined in chloroform in the presence of an excess of sodium triacetoxyborohydride under an inert atmosphere (Scheme 3).[16] Once again, the reductive amination products were isolated *via* liquid–liquid extraction using FC-72® and water. The resulting oil was subsequently purified by silica gel chromatography, affording products **7a**/**b** in 80 and 85% yield, respectively, and **8a/b** in 79% and 83% yield, respectively.

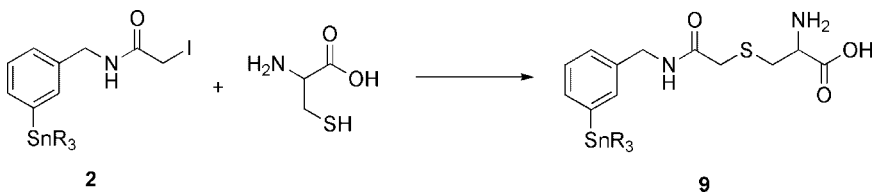
To probe the reactivity and selectivity of **2**, L-cysteine was chosen as a model ligand system. Ultimately, conjugation was achieved by utilizing a mixture of methanol and PFBME; a fluorous solvent which is miscible with most organic solvents (Scheme 4).[17] The two reagents were combined using an excess of cysteine in methanolic KOH for 1 h, according to a procedure adapted from Armstrong *et al.* [18] The desired product **9** was isolated in high yield (95%) by liquid–liquid extraction using perfluorinated hexanes (FC-72®) and water; no further purification was required.

Preparation of cold iodinated reference standards

Prior to radiolabeling the fluorous conjugates (**7a**, **8a** and **9**), the cold iodinated reference standards were synthesized and characterized. The non-radioactive iodinated aryl amines were



Scheme 3. Conjugation of **1** with model amines ($R = CH_2CH_2(CF_2)_5CF_3$).



Scheme 4. Reaction of **2** with a model thiol (cysteine) ($R = CH_2CH_2(CF_2)_5CF_3$).

prepared according to Scheme 5. The 3- and 4-iodobenzyl bromides were each treated with two mole equivalents of the respective amine; purification by silica gel chromatography gave compounds **10** and **11** in good yields (78–95%) and the characterization data for all products were consistent with that predicted for the desired products.

Compound **13** was prepared from 3-iodobenzylamine which was converted to the chloro precursor **12** using chloroacetyl chloride in the presence of base in 61% yield (Scheme 6). Using a similar procedure employed to make the fluorous analog (NaI in acetonitrile), compound **12** was converted to the iodoacetamide **13** in high yield (86%). This product was subsequently conjugated in nearly quantitative yield to L-cysteine in a 1 M methanolic KOH solution to give **14** (Scheme 7). Again, characterization data for each step were consistent with the formation of the desired products.

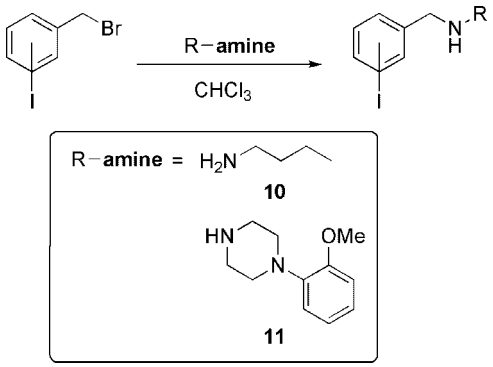
Radiochemistry

Radioiodination reactions using the fluorous conjugates were conducted using sodium [^{125}I]iodide and iodogen (Scheme 8). The radioiodination was performed to show that there were no interferences or complications due to the interactions between the substituents on the ring and to determine whether there were differences between the primary and secondary amine functionalities during the reaction. As these variables are unchanged in the positional isomers **7b** and **8b**, the radiolabeling reactions were not conducted. The average yield ($n = 2$) of the desired product **10a** was 92 % (Table 1).

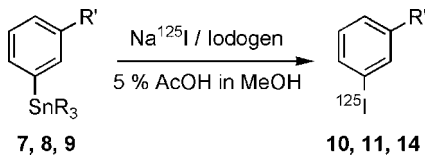
When the same approach was applied to the other fluorous conjugates (**8a** and **9**), the yields exceeded 83% (Table 1) and good correlation was observed in all cases between the reference standards (UV) and the product observed in the radio-HPLC traces (e.g. Figure 2). In all the experiments, there was no evidence of residual fluorous starting materials in the HPLC chromatogram, which reconfirms the effectiveness of F-SPE at removing unreacted starting material. Work in progress involves utilizing the synthons developed here and fluorous SPE for radioiodinating peptide and biomolecule derived vectors in which the weight percent of fluorine is greatly reduced.

Conclusions

Two new fluorous tagging reagents that can be used to link arylstannanes to amino and sulfhydryl groups were prepared and evaluated. The resulting conjugates were radiolabeled and isolated in high yield in mere minutes without the need for HPLC purification by employing fluorous solid-phase extraction (F-SPE). The conjugation and radiolabeling methodology reported in this work can be applied to the development of targeted molecular imaging probes using targeting vectors derived from small molecules, peptides, or possibly even higher molecular weight proteins.



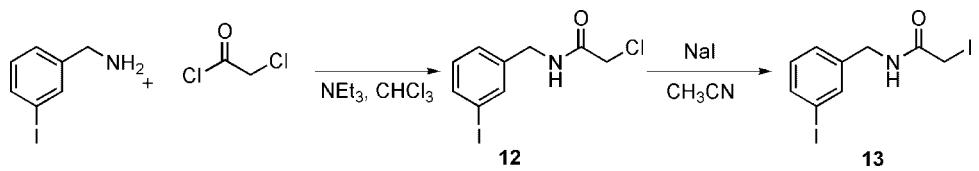
Scheme 5. Preparation of iodinated aryl amine standards.



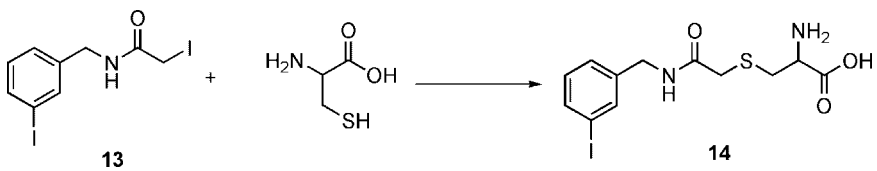
Scheme 8. Radioiodination of fluorous conjugates using Na [^{125}I]iodide.

Table 1. Summary of radiochemical yields

Compound number	Radiochemical yield ($n = 2$) (%)	Radiochemical purity (%)
10a	92 ± 2	> 98
11a	83 ± 5	> 98
14	86 ± 3	> 98



Scheme 6. Synthesis of compound **13**.



Scheme 7. Preparation of iodinated reference standard **14**.

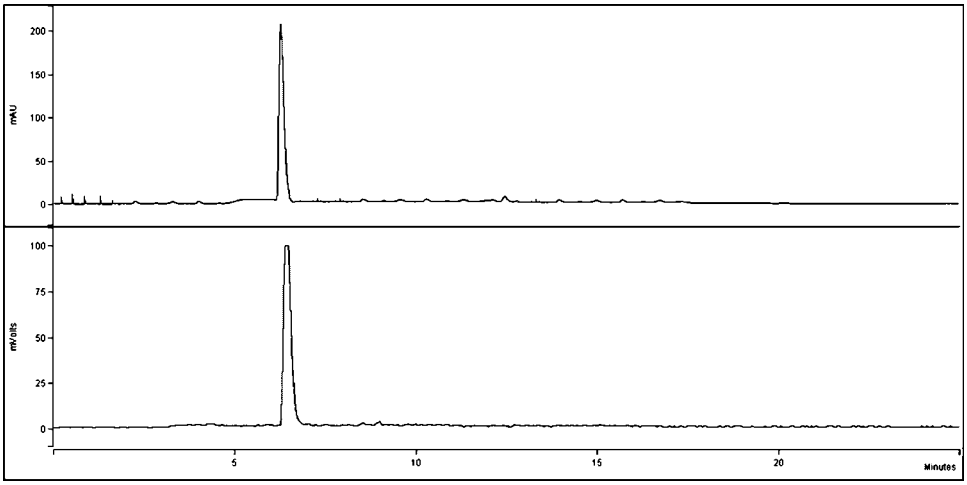


Figure 2. UV and γ-HPLC chromatograms (HPLC Method A) of non-radioactive **10a** (*top*) and F-SPE purified [^{125}I]**10a** (*bottom*). The slight difference in retention time is associated with the fact that the UV and γ-detectors are connected in series.

Acknowledgements

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