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Fluorous Isocyanates: Convenient Synthons for the Preparation of Radioiodinated Compounds in High Effective Specific Activity

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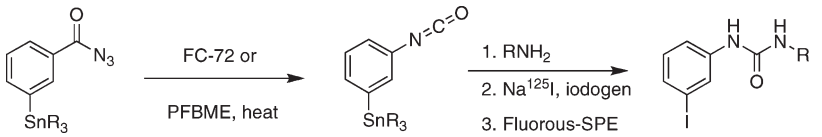

A convenient method for the preparation of fluorous−tin isocyanate derivatives was developed from the corresponding acyl azides as a novel route to targeted radiopharmaceuticals that can be produced in high effective specific activity without having to resort to using HPLC. The isocyanates, which were stable for greater than 20 days when dissolved in fluorous solvents, were conjugated to a series of amines including lysine derivatives by using a variety of miscible and biphasic solvent systems where yields were consistently greater than 95%. The resulting ureas were radiolabeled with ^{125}I by using iodogen as the oxidant and the desired products were isolated in high radiochemical yield (>87%) and purity by using a simple fluorous solid phase extraction procedure.

Introduction

With increasing interest in radionuclide-based molecular imaging probes and therapeutics that are capable of targeting specific proteins, there is a concomitant need for new compound discovery and production strategies.[1] For proteins whose expression levels are low and therefore saturable, it is generally desirable to produce radiolabeled compounds in high effective specific activity to minimize the amount of competition for the target.[2] While the removal of unlabeled ligand and reaction byproducts can be readily achieved by using HPLC, it is not a desirable strategy for routine clinical production of agents which requires handling large amounts of radioactivity.

Recently an alternative strategy for producing and purifying radioiodinated compounds in high effective specific activity without the use of HPLC has been reported.[3−5] Arylstannanes containing fluorous substituents were developed such that upon reaction with radioiodine the fluorous−tin aryl bond is cleaved. By passing the reaction mixture through a fluorine-rich solid-phase extraction cartridge, the precursor and all fluorous byproducts are retained while the desired nonfluorous compound is selectively eluted.[6−9] This type of chemoselective filtration has been used to produce a library of iodo-benzamides in high yield and high purity in mere minutes without the need to employ HPLC.[3] The fluorous labeling method (FLM) has also been used to produce clinically relevant radiopharmaceuticals including *m*-iodobenzylguanidine (MIBG) and iododeoxyuridine (IUdR) in high effective specific activity using different isotopes of iodine.[4,5]

There is sufficient evidence in the literature that the nature of the group carrying the isotope (the prosthetic group) and the linker between the prosthetic group and targeting vector can impact target uptake and overall distribution of a new agent.[10,11] Consequently there is a need to have a tool box of synthons so that different linker groups can be assessed for any given targeting vector. For the FLM, the focus of previous bioconjugate work involved the use of simple amide derivatives of a benzoic acid core.[3] To expand the utility of the methodology, a new generation of synthons is needed

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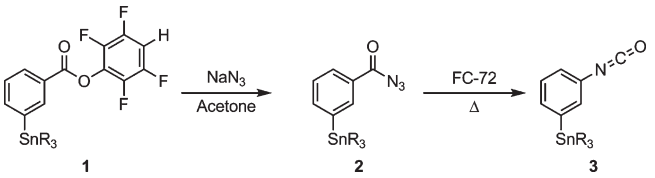
SCHEME 1. Preparation of the Acyl Azide 2 and the Isocyanate 3 (R = $CH_2CH_2(CF_2)_5CF_3$)



that can be used to derivatize both small molecule and lysine-containing targeting vectors in a complementary manner.

Isocyanates are attractive functional groups for tagging amine-containing targeting vectors because reactions, particularly with arylisocyanates, generally proceed in high yield to form robust ureas.[12] Despite the obvious benefits, this approach to conjugation chemistry has been largely overlooked in the preparation of radiohalogenated compounds. This is likely due in part to how easily isocyanates are hydrolyzed in aqueous media, which limits their general utility in bioconjugate chemistry. Because of the orthogonal solubilities of fluorous compounds and nonfluorous materials (i.e., water),[13] a fluorous isocyanate derivative could potentially be more resistant to hydrolysis than organic analogues. So long as suitable conjugation methods can be developed, fluorous arylisocyanates would offer a versatile platform for developing novel molecular imaging and therapy agents. To this end, research into the synthesis and reactivity of fluorous isocyanates and radiolabeling of their amine-derived conjugates was undertaken.

Results and Discussion

The preparation of the first fluorous arylisocyanate **3** was achieved via the acyl azide **2** (Scheme 1). The active ester **1**, which was reported by us previously,[3] was converted to **2** when combined with 4 mol equiv of sodium azide in a solution of acetone at ambient temperature.[14] Taking advantage of the fluorous nature of the product, the desired compound was isolated in quantitative yield by liquid−liquid extraction by using perfluorinated hexanes (FC-72) and water. The ^{1}H and ^{13}C NMR spectra were consistent with the formation of **2** and the IR spectrum displayed a prominent stretch at 2140 cm^{-1} that is characteristic of an acyl azide.[14]

Acyl azides can readily be converted to isocyanates via a simple Curtius rearrangement.[15] The first attempted preparation of **3** was based on the work reported by Zimmerman and co-workers that involved heating **2** at 90 °C for 4 h in toluene.[14] Following reaction workup, however, no product was isolated or detected with IR spectroscopy, which was attributed to the limited solubility of **2** in toluene. In order to circumvent this issue, the synthesis of **3** was repeated by dissolving compound **2** in FC-72 and heating at reflux (∼70 °C) for at least 4 h. Evaporation of the reaction solvent afforded a colorless oil. The IR spectrum of the product showed a peak at 2272 cm^{-1}, which is consistent with the formation of an isocyanate.[15] Furthermore, the ^{13}C NMR spectrum of the isolated product was significantly different from both the azide starting material and the possible hydrolysis product, fluorous benzoic acid. The carbonyl carbon atom was shifted from 172.4 ppm in the starting material **2** to 128.5 ppm in compound **3**.[16,17] The corresponding chemical shift for the carbonyl carbon in the acid is 170.6 ppm.

Stability Study

Once the synthesis and characterization of the isocyanate (**3**) were completed, the stability of the fluorous compound was evaluated since traditional organic isocyanates are generally susceptible to hydrolysis, particularly in the absence of good nucleophiles.[18] Initially, compound **3** was stored open to the atmosphere as a neat oil. After 4 h, the IR spectrum indicated that no isocyanate remained. Compound **3** was also dissolved in perfluorinated hexanes (10 mg/mL) and the solution, wrapped in aluminum foil, was stored as described above. IR spectroscopy demonstrated that the fluorous isocyanate did not degrade in solution even after 21 days (Figure 1). We attribute this stability to the poor miscibility of water in the fluorous solvent, thereby reducing the possibility of hydrolysis in solution.

An experiment was then performed to compare the stability of **3** dissolved in fluorous solvents with that of organic isocyanates stored in the comparable hydrocarbon solvent. Phenyl isocyanate was dissolved in hexanes (as an organic analogue of FC-72) and, when stored open to the atmosphere for 30 min, no isocyanate was visible by IR spectroscopy. Furthermore, an attempt was made to establish whether the organic isocyanates would also display an increased resistance to hydrolysis if stored in perfluorinated solvents; however, these compounds were completely insoluble and thus their stability in FC-72 could not be determined. Likewise, the stability of **3** in hexanes was not determined due to a similar lack of solubility.

Conjugation Chemistry. One of the main challenges to overcome with the fluorous isocyanates was their orthogonal solubilities with the solvents that dissolve most targeting vectors. Biovectors, such as peptides, were of particular concern as they are often used in buffered aqueous solvents while the isocyanate is prepared (and stored) in immiscible perfluorinated solvents. A series of preliminary biphasic reactions were conducted to probe the extent of this potential problem with use of a variety of solvent systems and *n*-butylamine as a model nucleophile (Scheme 2). Conditions included adding the amine as a solution in water, PBS buffer (pH 7.4) and carbonate buffer (pH 9.5), and neat, and in all cases the desired urea was formed as the major product. Yields were consistently greater than 95%.

The reactivity of the fluorous isocyanate toward secondary amines was also investigated by using (2-methoxyphenyl)-piperazine, which is a commercially available and widely used pharmacophore for developing serotonin receptor imaging probes.[19] Conjugation experiments were conducted by

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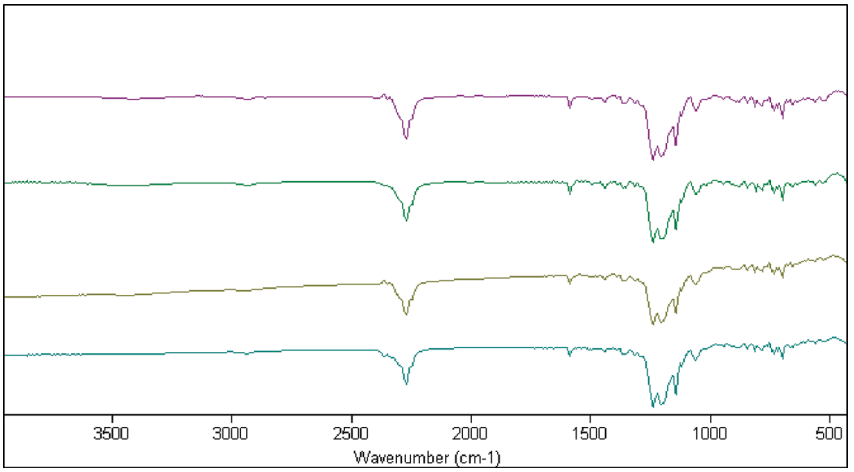
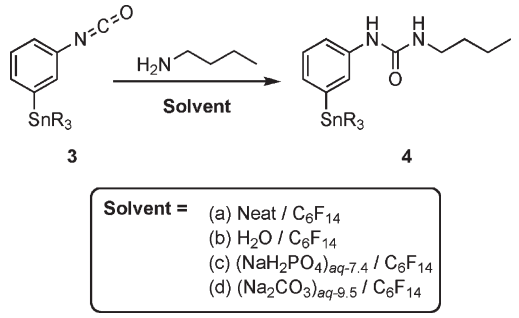


FIGURE 1. FTIR spectrum of **3** as a function of time in FC-72 at room temperature. From top to bottom: 7, 11, 14, and 21 days.

SCHEME 2. Treatment of 3 with a Model Amine (R = CH_2-$CH_2(CF_2)_5CF_3$)[a]

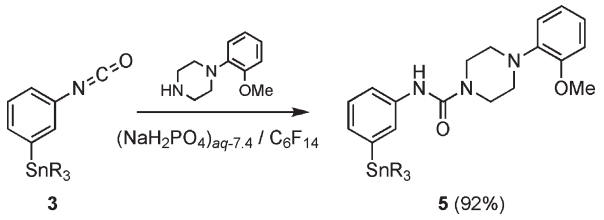


[a]pH denoted as subscripts.

combining a solution of the amine in phosphate buffered saline with the isocyanate in perfluorinated hexanes (FC-72). After 2 h of vigorous stirring and isolation via liquid−liquid extraction, MS and IR analyses of the product confirmed the presence of the desired compound, **5** (Scheme 3). The presence of the piperazine group was evident in the ^{1}H NMR spectrum with the signal for the methylene protons appearing as two sets of triplets at 3.78 (J = 5.0 Hz) and 3.14 ppm (J = 5.0 Hz), and the signal for the methyl protons on the methoxy group assigned to the singlet at 3.96 ppm. Furthermore, the spectrum confirmed the presence of the fluorous chains; in particular, the methylene protons appeared as a multiplet at 2.51 ppm and a triplet at 1.43 ppm (J = 8.4 Hz).

When similar conjugation reactions were performed with the water-soluble *N*α-acetyl-L-lysine methyl ester hydrochloride or the urea dimer Glu-U-Lys, in which the iodinated analogue is being pursued as a putative agent for diagnosing and staging prostate cancer,[20] the yields of the desired products were poor. This was most likely due to the biphasic nature of the reaction and the polar nature of the amine. By using the less expensive *N*α-acetyl-L-lysine-methyl ester as a model system, a conjugation strategy that is better suited for polar molecules was developed.

SCHEME 3. Synthesis of Compound 5[a] (R = $CH_2CH_2(CF_2)_5$-CF_3)



[a]pH denoted as subscript.

To facilitate better mixing of the orthogonal layers, perfluorobutyl methyl ether (PFBME) was investigated as a cosolvent as it, unlike FC-72, is miscible with most organic solvents including MeOH, DMF, toluene, hexane, Et_2O, THF, CH_3CN, and CH_2Cl_2.[21] Compound **3** could be prepared in PFBME and a stability test was performed as described above. PFBME was found to be equally good at preventing hydrolysis of the isocyanate as perfluorinated hexanes. To a solution of **3**, freshly prepared in PFBME, was added *N*α-acetyl lysine methyl ester (hydrochloride salt) dissolved in a solution of methanol containing 5% triethylamine (Scheme 4). The reaction mixture was homogeneous and TLC analysis indicated that the reaction was complete after 2 h at which point the solvent was removed and fluorous components were isolated by liquid−liquid extraction with use of FC-72 and water. Following silica gel chromatography, the desired product (**6**) was obtained as a colorless oil in 75% yield.

The same method was applied to the synthesis of the fluorous heterodimer **7** where the corresponding ^{123}I derivative is being investigated as a radiopharmaceutical for imaging prostate cancer.[20] Freshly prepared fluorous isocyanate synthesized in PFBME was added to a solution of Glu-U-Lys(^{t}Bu) dissolved in methanol containing 5% triethylamine (Scheme 5). After 2 h, the desired product was isolated by silica gel chromatography in 78% yield.

Radiochemistry. Prior to labeling the fluorous urea compounds, the cold iodinated standards were synthesized (Figure 2). Nonradioactive standards need to be prepared

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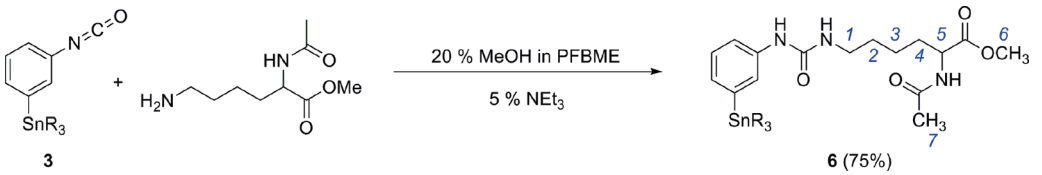
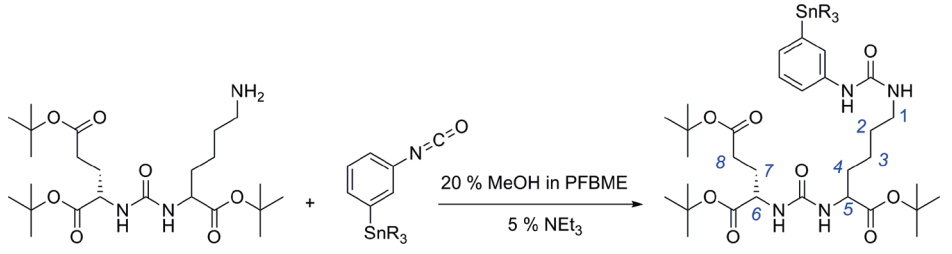
SCHEME 4. Conjugation of 3 with *N*α-Acetyl Lysine Methyl Ester (homogeneous conditions) (R = $CH_2CH_2(CF_2)_5CF_3$)[a]



3 6 (75%)

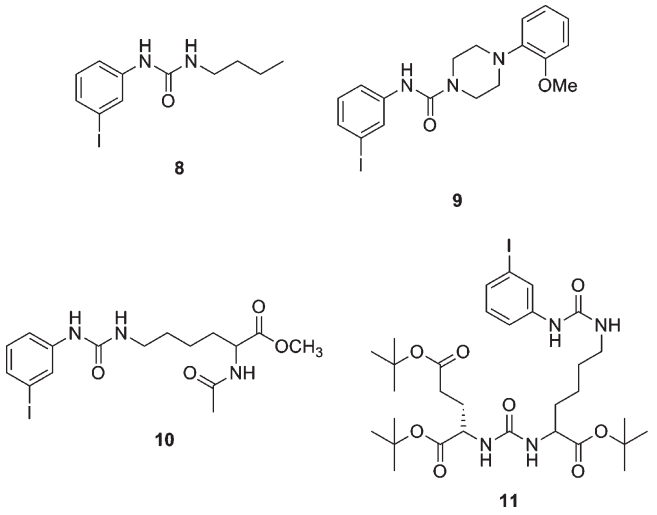
[a]The numbering scheme used for NMR assignments is shown.

SCHEME 5. Synthesis of Compound 7 (R = $CH_2CH_2(CF_2)_5CF_3$) and the Associated NMR Numbering Scheme



3 7

separately because the amounts of material produced at the tracer level are below the detection limits of standard characterization tools. In all cases, 3-iodophenyl isocyanate was added to a stoichiometric amount of the respective amine in acetone. The reaction was stirred under ambient conditions for 2 h at which point the solvent was removed. The preparation of the iodinated standards for **4** and **5** proceeded quantitatively to give the desired compounds in high yield. For compounds **10** and **11** it was necessary to utilize column chromatography to isolate the desired iodinated standards.



8 9 10 11

FIGURE 2. Nonradioactive urea standards **8**−**11**.

Radioiodination reactions with the fluorous ureas were conducted by using sodium [^{125}I]iodide and iodogen (Scheme 6). Using compound **4** as a typical example, approximately 0.5 mg of **4**, dissolved in methanol containing 5% acetic acid, was added to the reaction vial, which had been precoated with iodogen. Sodium iodide was added to the mixture, which was agitated manually. After 3 min the reaction was quenched with sodium metabisulfite and

SCHEME 6. General Scheme for the Radioiodination of Fluorous Ureas (R = $CH_2CH_2(CF_2)_5CF_3$)

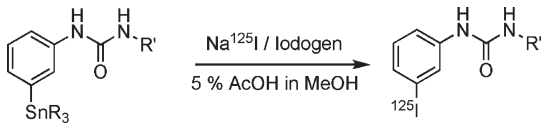


TABLE 1. Summary of Radiochemical Yields

compd no.	radiochemical yield (%) (n = 3)
4	88 ± 2
5	87 ± 4
6	91 ± 1
7	90 ± 2

diluted to 1 mL with water. The solution was passed through a commercially available fluorous solid-phase extraction cartridge, using water to remove all salts and then an alcohol−water solution to elute the desired compound. The average yield of the desired product was 88(±2)% (n = 3).

When the same approach was applied to the other fluorous ureas **5**−**7**, the yields routinely exceeded 87% (Table 1) and good correlation was observed in all cases between the reference standards (UV), which were prepared from the corresponding iodophenylisocyanate, and the product observed in the radio-HPLC traces.

Conclusions

Fluorous technology has helped facilitate the synthesis, product isolation, and stability of the compounds described herein. A fluorous isocyanate was prepared and its reactivity toward a variety of different model amines tested. The isocyanate reported here is stable under ambient conditions for weeks when stored in a fluorous solvent and can be used to prepare urea derivatives under both mono- and biphasic reaction conditions. The urea conjugates were radiolabeled and purified without the need for HPLC and the products

were isolated in high radiochemical yield and purity via fluorous solid-phase extraction. The conjugation and radiolabeling methodology reported here can be applied to the development of targeted molecular imaging probes by using any one of a number of promising amine-containing targeting vectors derived from small molecules or lysine-containing macromolecules. Future work will involve comparing the reactivity of the fluorous isocyanate with that of the previously reported 3-iodophenylisothiocyanate[22] when labeling proteins or other biomolecules in aqueous solution.

Experimental Section

3-(Tris[2-perfluorohexylethyl]stannyl)benzoyl Azide (2). Sodium azide (10 mg, 154 μmol) was dissolved in 3 mL of a 1% solution of water in acetone and combined with 3-tris[2-perfluorohexylethyl]stannyltetrafluorophenol benzoate (**1**)[3] (57 mg, 40 μmol) in acetone (5 mL). The resulting solution was stirred at room temperature overnight. The reaction solvent was then removed and the resulting residue was dissolved in FC-72 and extracted with water (3 × 5 mL each). The fluorous layers were combined and dried over anhydrous sodium sulfate. Subsequent removal of the FC-72 afforded a colorless oil. Yield 52 mg (>99%); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.64 (m, 1H), 7.51 (m, 1H), 2.33 (m, 6H), 1.36 (t, J = 8.3 Hz, 6H); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 172.4, 141.5, 137.9, 136.5, 131.0, 130.4, 129.0, 27.6, −1.1; FTIR (KBr, cm^{-1}) 2925, 2140, 1691.

3-(Tris[2-perfluorohexylethyl]stannyl)phenyl Isocyanate (3). Compound **2** (50 mg, 38 μmol) was dissolved in FC-72 (10 mL) and heated at reflux overnight. The reaction was then cooled and transferred to a vial pending use in further reactions. Yield 47 mg (>99%); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.37 (t, J = 7.6 Hz, 1H), 7.19 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 7.9 Hz, 1H), 7.09 (s, 1H), 2.32 (m, 6H), 1.32 (t, 6H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 138.7, 134.3, 132.9, 131.6, 129.9, 125.8, 27.6, −1.2; FTIR (KBr, cm^{-1}) 2272, 1585.

3-(Tris[2-perfluorohexylethyl]stannyl)phenyl Isocyanate: Stability Study. Compound **2** (100 mg; 77 μmol) was dissolved in FC-72 (15 mL) and the solution was heated at reflux for 16 h. After cooling to room temperature a small sample of the reaction mixture was collected for spectroscopic analysis and concentrated under reduced pressure. The remaining contents of the reaction vessel were transferred to a scintillation vial and stored under ambient conditions wrapped in aluminum foil and monitored weekly for decomposition with IR spectroscopy. No decomposition was observed for up to 3 weeks.

General Procedure for Biphasic Conjugation Reactions. To a solution of **3** (200 mg, 156 μmol) in FC-72 (3 mL) was added a 2 mL solution of the amine of interest (150 μmol) in either (A) sodium carbonate solution (500 mM; pH 9.5), (B) PBS (pH 7.4), (C) distilled deionized water, or (D) as a neat solution. The resulting biphasic mixture was stirred vigorously for 2 h then water and FC-72 were added (5 mL each). Following liquid−liquid extraction and concentration of the fluorous layer, the desired product was obtained as a white film. Identical products and yields were obtained irrespective of the solvent used to dissolve the amine.

1-Butyl-(3-tris[2-perfluorohexylethyl]stannylphenyl)urea (4): yield 184 mg (95%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.56; ^{1}H NMR (500.13 MHz, CD_3OD) δ 7.54 (s, 1H), 7.32 (m, 2H), 7.09 (d, J = 6.3 Hz, 1H), 3.21 (t, J = 7.0 Hz, 2H), 2.43 (m, 6H), 1.53 (m, 2H), 1.42 (m, 2H), 1.35 (t, J = 8.4 Hz, 6H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125.77 MHz, CD_3OD) δ 158.4, 141.3, 139.1, 131.0, 130.0, 127.5, 120.1, 40.6, 33.4, 28.8, 21.0, 14.1, −0.6; HRMS(+) mass calcd for $C_{35}H_{27}N_2OF_{39}Sn$ ([M + H]$^+$) 1353.0601, found 1353.0590; FTIR (KBr, cm^{-1}) 3331, 2945, 2876, 1641, 1551.

4-(2-Methoxyphenyl)-*N*-(3-tris[2-perfluorohexylethyl]stannylphenyl)piperazine-1-carboxamide (5): yield 211 mg (92%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.78; ^{1}H NMR (500.13 MHz, CD_3OD) δ 7.62 (s, 1H), 7.47 (m, 2H), 7.22 (d, J = 6.9 Hz, 1H), 7.05 (m, 4H), 3.96 (s, 3H), 3.78 (t, J = 5.0 Hz, 4H), 3.14 (t, J = 5.0 Hz, 4H), 2.51 (m, 6H), 1.43 (t, J = 8.4 Hz, 6H); ^{13}C NMR (125.76 MHz, CD_3OD) δ 158.0, 154.0, 142.3, 141.2, 138.9, 131.8, 129.8, 129.6, 124.9, 123.1, 122.2, 119.7, 113.0, 56.0, 52.1, 45.4, 28.8, −0.6; HRMS(+) mass calcd for $C_{42}H_{32}F_{39}N_3O_2Sn$ ([M + H]$^+$) 1472.0981, found 1472.0942; FTIR (KBr, cm^{-1}) 3331, 2950, 1640, 1583.

General Procedure for Single Phase Conjugation Reactions. To a solution of 3-tris[2-perfluorohexylethyl]stannylphenyl isocyanate (**3**) (200 mg, 156 μmol) in PFBME (3 mL) was added the amine (300 μmol), dissolved in methanol (1 mL) containing 5% triethylamine. The resulting mixture was stirred vigorously for 2 h, then the reaction solvent was removed by rotary evaporation. Fluorous products were isolated via liquid−liquid extraction, using equal volumes of FC-72 and water. Following concentration of the fluorous layer, the desired product was immediately purified by silica gel chromatography.

Conjugation to *N*α-Acetyl-Lys-OMe (6): yield 173 mg (75%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.5; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.50 (s, 1H, ArH), 7.33 (s,1H, NH), 7.32 (m, 1H, ArH), 7.21 (m, 1H, ArH), 6.98 (d, J = 7.0 Hz, 1H, ArH), 6.48 (d, J = 7.8 Hz, 1H, NH), 5.48 (m, 1H, NH), 4.54 (m, 1H, H-5), 3.72 (s, 3H, H-6), 3.27 (m, 1H, H-1), 3.20 (m, 1H, H-1), 2.27 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 2.02 (s, 3H, H-7), 1.82 (m, 1H, H-2), 1.71 (m, 1H, H-2), 1.56 (m, 2H, H-4), 1.41 (m, 2H, H-3), 1.25 (t, J = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 172.8, 170.6, 155.9, 139.9, 137.4, 129.8, 129.2, 126.1, 120.2, [118.6−108.5], 52.4, 51.8, 39.4, 32.3, 28.7, 27.6, 23.1, 22.3, −1.5; HRMS(+) mass calcd for $C_{40}H_{36}F_{39}N_3O_4Sn$ ([M + 2H]$^+$) 1483.1105, found 1483.1005; FTIR (KBr, cm^{-1}) 3314, 2952, 2943, 2868, 1747, 1667, 1554.

Conjugation to Glu-U-Lys (tri^{t}Bu ester) (7): yield 215 mg (78%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.6; ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.91 (s, 1H, ArH), 7.79 (s, 1H, NH), 7.26 (m, 1H, ArH), 7.18 (m, 1H, ArH), 6.92 (d, J = 7.1 Hz, 1H, ArH), 6.34 (br, 2H, NH), 5.71 (d, J = 6.6 Hz, 1H, NH), 4.32 (m, 1H, H-5), 4.00 (br, 1H, H-6), 3.50 (br, 1H, H-1), 3.01 (br, 1H, H-1), 2.34 (m, 2H, H-7), 2.29 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 2.08 (m, 1H, H-2), 1.86 (m, 1H, H-2), 1.74 (m, 2H, H-8), 1.52 (m, 2H, H-3), 1.41 (s, 9H, ^{t}Bu), 1.39 (s, 9H, ^{t}Bu), 1.36 (s, 9H, ^{t}Bu), 1.29 (t, J = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$), 1.24 (m, 2H, H-4); ^{13}C NMR (150.92 MHz, $CDCl_3$) δ 175.4, 172.3, 171.9, 158.6, 156.0, 141.1, 137.0, 129.1, 128.9, 125.4, 120.2, [119.5, 118.5, 116.7, 111.6−108.5], 83.7, 81.9, 81.1, 55.2, 53.9, 39.3, 32.2, 31.8, 29.8, 28.1 (9C), 27.9, 27.8, 24.6, −1.5; HRMS(+) mass calcd for $C_{55}H_{61}F_{39}N_4O_8Sn$ ([M + H]$^+$) 1767.2967, found 1767.2902; FTIR (KBr, cm^{-1}) 3339, 2980, 2935, 1733, 1644, 1558.

Iodophenyl−Isocyanate Conjugation Reaction: General Procedure (8−9). The desired amine (306 μmol) was dissolved in acetone (4 mL). To this was added 3-iodophenyl isocyanate (75 mg; 306 μmol). The reaction was stirred at room temperature for 6 h at which point the solvent was removed by rotary evaporation. A white crystalline solid was formed that required no further purification.

1-Butyl-3-(iodophenyl)urea (8): yield 92 mg (95%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.59; HPLC t_R = 10.3 min; ^{1}H NMR (500.13 MHz, CD_3OD) δ 7.87 (s, 1H), 7.28 (m, 2H), 6.98 (m, J = 8.0 Hz, 1H), 3.18 (t, J = 7.0 Hz, 2H), 1.51 (m, 2H), 1.39 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125.77 MHz, CD_3OD) δ 157.9, 142.6, 132.1, 131.4, 128.5, 119.0, 94.7, 40.5, 33.3, 21.0, 14.1; HRMS(+) mass calcd for $C_{11}H_{16}N_2OI$ ([M + H]$^+$) 319.0307, found 319.0321; FTIR (KBr, cm^{-1}) 3315, 2965, 2868, 1622, 1477.

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4−2-Methoxyphenyl)-*N*-(3-iodophenyl)piperazine-1-carboxamide (9): yield 127 mg (95%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.7; HPLC t_R = 14.1 min; ^{1}H NMR (600.13 MHz, CD_3OD) δ 7.87 (s, 1H), 7.38 (m, 2H), 6.98 (m, 5H), 3.86 (s, 3H), 3.69 (t, J = 5.0 Hz, 4H), 3.06 (t, J = 5.0 Hz, 4H); ^{13}C NMR (150.92 MHz, CD_3OD) δ 157.5, 154.0, 142.5, 142.1, 132.8, 131.2, 130.5, 124.9, 122.2, 120.9, 119.7, 112.9, 94.4, 56.0, 52.0, 45.4; HRMS(+) mass calcd for $C_{18}H_{21}N_3O_2I$ ([M + H]$^+$) 438.0679, found 438.0684; FTIR (KBr, cm^{-1}) 3302, 2904, 2822, 1639, 1581.

3-Iodophenyl−Isocyanate Conjugation with *N*α-Acetyl Lys-OMe (10). *N*α-Acetyl-lysine methyl ester (38 mg; 204 μmol) was dissolved in methanol containing 5% triethylamine (1 mL). To this was added 3-iodophenyl isocyanate (50 mg; 204 μmol). The reaction was stirred at room temperature for 6 h at which point the solvent was removed by rotary evaporation and **10** was purified by silica gel chromatography. The product was eluted with a 10% solution of ethanol in chloroform. Yield 59 mg (65%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.61; HPLC t_R = 6.5 min; ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ 7.80 (s, 1H), 7.76 (s, 1H), 7.27 (m, 2H), 6.92 (t, J = 8.0 Hz, 1H), 6.81 (d, J = 7.4 Hz, 1H), 5.80 (m, 1H), 4.44 (m, 1H), 3.70 (s, 3H), 3.18 (m, 2H), 2.0 (s, 3H), 1.76 (m, 2H), 1.48 (m, 2H), 1.36 (m, 2H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 172.9, 171.3, 156.4, 140.9, 131.5, 130.5, 127.9, 118.5, 94.4, 52.6, 52.5, 39.2, 31.6, 29.5, 23.2, 22.5; HRMS (+) mass calcd for $C_{16}H_{23}N_3O_4I$ ([M + H]$^+$) 448.0733, found 448.0735; FTIR (KBr, cm^{-1}) 3310, 3080, 2945, 2868, 1744, 1649, 1584, 1548.

3-Iodophenyl−Isocyanate Conjugation with Glu-U-Lys (11). 11 was prepared in an analogous fashion as **10**. Yield 84 mg (56%); TLC (9:1 $CHCl_3$:EtOH) R_f 0.7; HPLC t_R = 11.5 min; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 7.90 (s, 1H, NH), 7.87 (t, J = 2.1 Hz, 1H, Ar−H), 7.43 (ddd, J = 8.4, 2.1, 1.0 Hz, 1H, ArH), 7.24 (ddd, J = 8.4, 2.1, 1.0 Hz, 1H, ArH), 6.94 (t, J = 7.7 Hz, 1H, ArH), 6.41 (d, J = 7.7 Hz, 1H, NH), 6.30 (br m, 1H, NH), 5.78 (d, J = 7.0 Hz, 1H, NH), 4.28 (ddd, J = 9.8, 7.7, 4.9 Hz, 1H, H-5), 3.98 (ddd, J = 10.0, 6.7, 3.5 Hz, 1H, H-6), 3.52 (dddd, J = 13.8, 7.4, 7.0, 4.2 Hz, 1H, H-1), 3.04 (ddd, J = 17.3, 8.4, 3.5 Hz, 1H, H-1), 2.39 (m, 2H, H-7), 2.08 (dddd, J = 14.5, 9.5, 8.4, 4.9 Hz, 1H, H-2), 1.89 (m, 1H, H-2), 1.74 (tt, J = 6.3, 4.9 Hz, 1H, H-8), 1.52 (m, 3H, H-3, H-8), 1.42 (s, 9H, ^{t}Bu), 1.40 (s, 9H, ^{t}Bu), 1.36 (s, 9H, ^{t}Bu), 1.33 (m, 1H, H-4), 1.22 (m, 1H, H-4); ^{13}C NMR (176.09 MHz, $CDCl_3$) δ 175.3, 172.1, 158.6, 155.6, 141.8, 130.4, 130.3, 126.9, 117.4, 94.2, 83.8, 81.9, 81.0, 60.5, 55.1, 54.1, 39.2, 32.1, 29.8, 28.1, 28.0, 27.9, 27.2, 24.6, 21.1, 14.3; HRMS (+) mass calcd for $C_{31}H_{50}N_4O_8I$ ([M + H]$^+$) 733.2673, found 733.2690; FTIR (KBr, cm^{-1}) 3351, 2977, 2932, 1731, 1645, 1555.

General Radioiodination Procedure. An aliquot (20 μL) of iodogen dissolved in $CHCl_3$ (1 mg/mL) was added to an eppendorf tube and the solvent evaporated under reduced pressure (water aspirator). Compounds **4**, **5**, **6**, or **7** (0.5 mg) were added followed by a solution of 5% acetic acid in methanol (100 μL) and sodium [^{125}I]iodide (5 μL, 20 mCi/mL, pH 10). After the mixture was swirled 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 solid-phase extraction (F-SPE) cartridge that 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 an 80:20 (v/v) solution of methanol−water (6 mL). Radioactive fractions eluted from the F-SPE cartridge were analyzed by HPLC where in all cases one radioactive peak was observed. When coinjected with the nonradioactive standards, the peaks coeluted.

[^{125}I]**8:** t_R 10.6 min; RCY 88%; radiochemical purity >98%.
[^{125}I]**9:** t_R 14.4 min; RCY 87%; radiochemical purity >98%.
[^{125}I]**10:** t_R 6.9 min; RCY 91%; radiochemical purity >98%.
[^{125}I]**11:** t_R 11.8 min; RCY 90%; purity >98%.

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Supporting Information Available: Complete characterization data for novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.