The Fluorous Labelling Strategy: a New Paradigm in Preparing Radiolabelled Compounds in High Effective Specific Activity

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Abstract

A new radiolabelling strategy suitable for preparing radioiodinated compounds in high effective specific activity was developed. The core method employs fluorous chemistry in such a manner that unlabelled precursors can be separated from labelled compounds without the use of HPLC purification. The fluorous labelling strategy involved tin-aryl groups in which the substituents off the metal were fluorine rich. Upon reaction with radioiodide and an oxidant, the tin-aryl bond is cleaved. The non-fluorous product could then be separated from the fluorous starting material by fluorous solid-phase extraction (F-SPE).

The initial goal of this thesis was to first establish the viability of the fluorous labelling strategy (FLS). To this end, fluorous benzoic acids, 3- and 4-(*tris*[2-perfluorohexylethyl]stannyl) benzoic acid (*m*- and *p*-**2.1**) were prepared *via* organozinc derivatives and subsequent hydrolysis. Following direct iodinolysis of *m*-**2.1** using a sub-stoichiometric amount of iodine, the fluorous benzoic acids were treated with Na[^{125}I]I. After purification *via* fluorous solid-phase extraction, the resulting iodinated benzoic acids (*m*- and *p*-**2.5**) were prepared in 92 and 93 % yield and excellent purity (>98 % by HPLC).

Next, to demonstrate that the FLS could be used to create libraries of both precursors and labelled compounds, a small collection of benzamides were prepared from fluorous tetrafluorophenyl active esters (*m*- and *p*-**2.9**). An excess of a series of simple amines were added to reaction wells containing one of the two fluorous active esters and the products purified by taking advantage of the

non-fluorous nature of the reagents and reaction byproducts. All products, which were fully characterized and their purities verified by HPLC, were subsequently labeled with ^{125}I in high radiochemical yield (> 85 %), and the products were obtained in greater than 98% radiochemical purity.

To demonstrate the applicability of this method clinically relevant radiopharmaceuticals, a fluorous precursor of the radiopharmaceutical *meta*-iodobenzylguanidine (MIBG) was prepared *via* the fluorous benzylamine, 3-*tris*[2-perfluorohexylethyl]stannyl) benzylamine (**3.3**) upon reacting with *1*H-pyrazole carboxamidine. Following radioiodination, high purity [^{125}I]- and [^{123}I]MIBG were produced in 81±3% and 80% radiochemical yield respectively in less than 20 min without high-performance liquid chromatography (HPLC) purification. The purified product contained less than 1 ppm tin as determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Next, the FLS was expanded to include fluorous precursors suitable for reacting with groups common in peptides and proteins for use in bioconjugation reactions. Among these were a fluorous isocyanate (**4.1**) which was shown to be stable in fluorous solvents for up to 3 weeks, fluorous benzaldehydes (*m*- and *p*-**5.1**) and a fluorous iodoacetamide (**5.3**). Following their purification and characterization, these compounds were each reacted with model systems (*e.g.* amines, amino acids) and were subsequently radioiodinated using Na[^{125}I]I in good yields (> 80 %) and in very high purity (> 98 %) as determined by HPLC.

Publications

1. Donovan, A.; Forbes, J.; Dorff, P.; Schaffer, P.; Babich, J.; Valliant, J. F., A New Strategy for Preparing Molecular Imaging and Therapy Agents Using Fluorine-Rich (Fluorous) Soluble Supports *J. Am. Chem. Soc.*, **2006**, *128*, 3536-3537. Chapter 2.
   In this manuscript, the candidate was responsible for the synthesis, characterization and radioiodination of all compounds reported in the manuscript. In addition, the x-ray crystallography was performed by the candidate under the guidance of P. Schaffer

2. Donovan, A. C.; Valliant, J. F., A Convenient Solution-Phase Approach for the Preparation of Meta-Iodobenzylguandine (MIBG) in High Effective Specific Activity. *Nucl. Med. Biol.*, **2008**, *35*, 741-746. Chapter 3.

3. Donovan, A. C., Valliant, J. F., Fluorous Isocyanates: Convenient Synthons for the Preparation of Radioiodinated Compounds in High Effective Specific Activity. *J. Org. Chem*, **2009** *(Accepted 2009-09-22)*. Chapter 4.

4. Donovan, A. C., Valliant, J. F., The Next Generation of Fluorous Synthons for Producing Radioiodinated Compounds in High Effective Specific Activity. (*Manuscript in Preparation)* Chapter 5.

5. McIntee, J. W.; Sundararajan, C.; Donovan, A. C.; Kovacs, M. S.; Capretta, A.; Valliant, J. F., A Convenient Method for the Preparation of Fluorous Tin Derivatives for the Fluorous Labelling Strategy. *J. Org. Chem*, **2008**, *73*, 8236-8243.
   The work completed by the candidate for this paper is not described in this thesis.

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List of Abbreviations

A	
HOAc	Acetic acid
CH_3CN	Acetonitrile
α	Alpha particle
Å	Angstrom
aq	aqueous
Ar-Li	Aryllithium
At	Astatine
^{211}At	Astatine-211
B	
β^-	Beta particle
BP	British Pharmacopeia
Br	Bromine
Bu-X	Butylhalide
C	
calcd	Calculated
^{11}C	Carbon-11
^{13}C NMR	Carbon-13 NMR
cm	Centimeter(s)
CCD	Charged coupled device (x-ray)
δ	Chemical shift (NMR)
CAT	Chloramine-T
$CHCl_3$	Chloroform
CT	Computed tomography
$CuSO_4$	Copper sulphate
COSY	Correlated spectroscopy
J	Coupling constant (NMR)
Ci	Curie
D	
Da	Dalton(s)
d	Day(s)
°C	Degrees Celcius
DNA	Deoxyribonucleic acid
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
D_2O	Deuterium oxide
CH_2Cl_2	Dichloromethane

Et_2O	Diethyl ether
d	Doublet (NMR)
ddd	Doublet of doublet of doublets (NMR)
E	
ESA	Effective specific activity
ESI	Electrospray ionization
ES-MS	Electrospray mass spectrometry
ε	Epilson
EtOH	Ethanol
EtOAc	Ethyl acetate
F	
^{18}F	Fluorine-18
FLS	Fluorous labelling strategy
F-SPE	Fluorous solid-phase extraction
FTIR	Fourrier transform infrared spectroscopy
G	
γ	Gamma ray
Glu-U-Lys	Glutamic acid-lysine urea dimer
g	Gram(s)
H	
$t_{1/2}$	Half-life
Hz	Hertz
HMBC	Heternuclear multiple bond correlations
HSQC	Heteronuclear single quantum coherence
Hex	Hexanes
HPLC	High performance liquid chromatography
HTS	High throughput screening
HRMS	High-resolution mass spectrometry
h	Hour(s)
HCl	Hydrochloric acid
I	
ICP-MS	Inductively coupled plasma mass spectrometry
i.e.	id est
IR	Infrared spectroscopy
I	Iodine
^{122}I	Iodine-122
^{123}I	Iodine-123
^{124}I	Iodine-124
^{125}I	Iodine-125
^{131}I	Iodine-131

N=C=O	Isocyanate functional group
K	
K	Kelvin
keV	Kiloelectron volt(s)
kJ	Kilojoule(s)
L	
LET	Linear energy transfer
LDA	Lithium diisopropylamide
M	
MR	Magentic resonance
m/z	Mass-to-charge ratio
MHz	Megahertz
MIBG	*Meta*-iodobenzylguanidine
MeOH	Methanol
μCi	Microcurie(s)
μL	Microlitre(s)
μmol	Micromole(s)
μm	Micron(s)
mCi	Millicurie(s)
mg	Milligram(s)
mL	Millilitre(s)
mm	Millimeter(s)
mM	Millimolar (millimole(s) per litre)
mmol	Millimole(s)
min	Minute(s)
mol	Mole(s)
M	Molarity (mole(s) per litre)
m	Multiplet (NMR)
N	
[^{123}I]BZA	*N*-(2-diethylaminoethyl)-4-[^{123}I]iodobenzamide
EDC	*N,N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
DMF	*N,N*-dimethylformamide
Nα-Acetyl-Lys-OMe	N-alpha-acetyl-lysine methyl ester
nm	Nanometer(s)
^{14}N	Nitrogen-14
NMR	Nuclear magnetic resonance
O	
ORTEP	Oak Ridge thermal ellipsoid plot
HBTU	*O*-Benzotriazole-*N,N,N',N'*-tetramehyl-uronium-hexafluoro-phosphate
ON	Ontario
^{15}O	Oxygen-15

P	
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
C_6F_{14}	Perfluorinated hexanes
FC-72	Perfluorinated hexanes
PFBME	Perfluorobutyl methyl ether
PBS	Phosphate buffered saline
H_3PO_4	Phosphoric acid
PDA	Photodiode array
pg	Picogram(s)
PTFE	Polytetrafluoroethylene
β^+	Positron
PET	Positron emission tomography
KBr	Potassium bromide
KOH	Potassium hydroxide
pH	Potential of hydrogen
PSMA	Prostate specific membrane antigen
^{1}H NMR	Proton NMR
Q	
q	Quartet (NMR)
R	
RCY	Radiochemical yield
R_f	Retention factor
R_t	Retention time
^{186}Re	Rhenium-186
S	
s	Second(s)
Si	Silicon
SPECT	Single photon emission computed tomography
s	Singlet (NMR)
$NaHCO_3$	Sodium bicarbonate
Na_2CO_3	Sodium carbonate
NaH_2PO_4	Sodium dihydrogen phosphate
NaOH	Sodium hydroxide
NaI	Sodium Iodide
$Na_2S_2O_5$	Sodium metabisulfite
Na_2SO_4	Sodium sulfate
SB-C18	Stable bond octadecylsilyl packing (HPLC column)

^{89}Sr	Strontium-89
T	
^{99m}Tc	Technetium-99m
^{t}Bu	Tertiary butyl
THF	Tetrahydrofuran
θ	theta (x-ray)
TLC	Thin-layer chromatography
Sn	Tin
^{119}Sn	Tin-119
NEt_3	Triethylamine
t	Triplet (NMR)
TRIS	*tris*-(hydroxymethyl)aminomethane
U	
US	Ultrasound
UV	Ultraviolet
USP	US Pharmacopeia
V	
v/v	Volume by volume
W	
H_2O	Water
λ	Wavelength
cm^{-1}	Wavenumber(s)
Y	
^{90}Y	Yittrium-90

Chapter 1: Introduction

1.1. Nuclear medicine

Nuclear medicine is the medical specialty that is based on examining the regional chemistry of the living body through the use of radioactive isotopes which are injected into a subject and imaged non-invasively using a gamma-camera as they move through the body.[1] In 1971, nuclear medicine was first recognized as a separate discipline by the Atomic Energy Commission, and only 1200 medical institutions were licensed to handle radionuclides.[2] Since that time, the field has assumed an increasingly important role in the diagnosis, monitoring and treatment of disease, growing to such an extent that over 11 million procedures involving radionuclides are now conducted throughout the United States each year.[2] Similar growth is taking place in Canada, Great Britain and Japan where between on average, 44 procedures per 1000 people are carried out yearly, representing an average annual increase in nuclear medicine procedures of 7-14 % worldwide.[2, 3]

Nuclear medicine has developed into a multidisciplinary field combining fundamental aspects of chemistry, physics and clinical medicine. Driven by the constant need for new and better diagnostic tools, therapeutic agents and sensitive and specific methods for diagnosis and treatment, the field of nuclear medicine has three main active and interrelated areas of research and development. The first is the discovery of new radiopharmaceuticals that target specific biochemical aspects of disease while the second is the development of

new methods of producing radiotracers. The third area, which is beyond the scope of this thesis, is the development of new hardware and software (*i.e.* higher sensitivity detectors).[3]

1.2. Radiopharmaceuticals

Radiopharmaceuticals fall under two broad categories: perfusion agents and targeted radiopharmaceuticals. Perfusion agents currently represent the most widely used radiopharmaceuticals.[4] Generally, these agents are taken up into target organs or sites of disease *via* non-specific mechanisms and biological gradients. They allow for imaging of bone remodelling or turnover, hepatocyte clearance, blood circulation and perfusion in the brain.[5, 6] These agents are employed as clinical diagnostic imaging agents in the assessment of cardiac, renal, pulmonary functions, and the diagnosis of cancer.[7]

In the last decade, the development of radiopharmaceuticals has shifted from functional imaging to receptor and transporter imaging.[8] This change depends on the availability of radiolabelled compounds that bind with high affinity to a specific target molecule. Ideally the biological target should have a significant change in concentration at the site of disease versus healthy tissue and its upregulation should be directly associated with the disease state or progression to be as specific as possible for the disease. These conditions are needed to achieve good image quality (*i.e.* high target to non-target ratios), reduce the occurrence of false positives, and to reduce the radiation damage to healthy tissue which is particular of important in the case of therapeutic agents.[4]

Targeted radiopharmaceuticals, used for imaging or therapy, can be considered to have three components: (*i*) the targeting vector, (*ii*) the radionuclide and (*iii*) a means of linking the two together that may, or may not, include a pharmacokinetic modifier to optimize properties such as biological half-life and rate / mode of metabolism (Figure 1-1).[4] Targeted agents are typically prepared by tagging a vector that is known to have high affinity for the target of interest. The high affinity is needed to achieve high target to target ratios. Such compounds are designed as indicators of specific metabolic or biochemical dysfunction that are characteristic of a diseased state.[9] Since these molecular changes tend to occur before structural biological changes can be detected *in vivo*, validated molecular imaging probes can provide a sensitive way to identify and characterize the nature of the disease in its early stages of development.[10, 11]

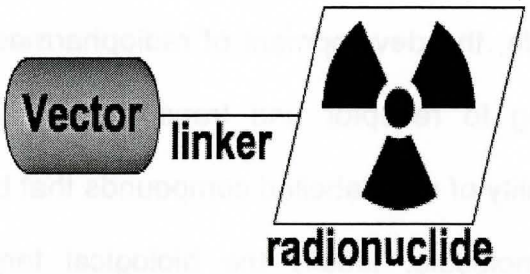


Figure 1-1. Components of a radiopharmaceutical

In addition to exhibiting strong binding affinity for the targeted receptor, the chemical stability of the putative radiopharmaceutical *in vivo* is critical. The radioisotope must remain associated with the targeting vector after introduction into the biological system so that the distribution of the radioactivity in the subject is an accurate reflection of the biological pathway of interest.[4] If the radionuclide

is placed on a drug candidate in such a manner that is rapidly metabolized or excreted, the radiotracer may not reach its target of interest before the radiolabel is lost. Extensive and rapid metabolism of the radiotracer creates radioactive metabolites that will likely have significantly different receptor affinities or specificities from the parent agent, which will detrimentally affect the therapeutic efficacy or image quality.[12]

1.2.1. Diagnostic molecular imaging

Anatomic imaging modalities such as X-ray computed tomography (CT), magnetic resonance (MR) and ultrasound imaging (US), provide very high resolution images suited to reveal structural abnormalities. Nuclear imaging however, stands apart as a technique because of its unparalleled sensitivity and ability to image biochemical processes, in addition to biological structures.[13, 14]

Radioimaging procedures require highly penetrating radiation such as γ-photons or X-rays (generally exceeding 100 keV in energy) that can pass through overlying tissues and organs and be detected by suitable instruments outside the body.[15] The radioimaging modalities which are currently used are positron emission tomography (PET) and single photon emission tomography (SPECT) and are classified by the nuclear decay properties of the radioisotopes detected.[4]

1.2.1.1. Positron emission tomography (PET)

Positron emission tomography (PET) is a diagnostic imaging technique which detects radiation emitted from radionuclides that emit a positron (β^+). When the positron emitted by the decaying isotope encounters an electron in the

surrounding tissue, an annihilation reaction occurs producing two 511 keV gamma rays at a 180° angle from each other (Figure 1-2). Thus, when two detectors positioned opposite each other detect a simultaneous event (coincidence), the disintegration must lie along a line connecting these two detectors. From this, the location of the decay event can be calculated.[12, 16]

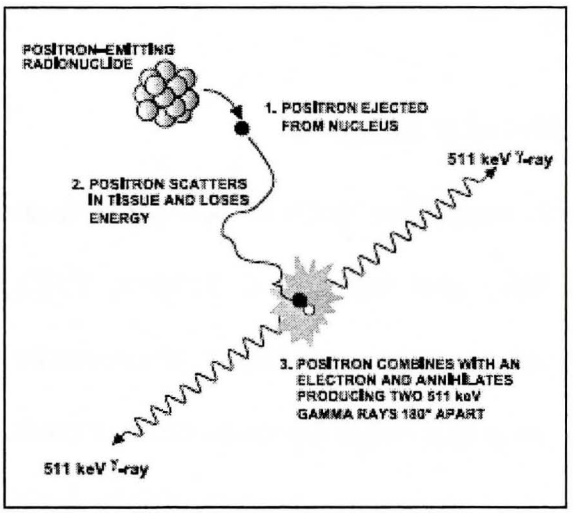


Figure 1-2. Schematic representation of radioactive (β^+) decay[17]

Some of the most common radionuclides used in PET are fluorine-18, carbon-11, and oxygen-15. Fluorine-18 is often considered the single most important radioisotope used in PET;[15] however, since very few drugs naturally contain this atom, carbon-11 is an alternative isotope which permits the synthesis of isotopically-labelled and isostructural versions of the drug of interest.[18] The widespread use of carbon-11 has been somewhat limited however due to the short half-life of this isotope ($t_{½}$ = 20.4 min) and the presence of carbon-12 impurities which results in lower specific activity formulations (*vide infra*). The use of other PET isotopes is currently being investigated and includes iodine-124, copper-64 and generator products like gallium-68.[17]

1.2.1.2. Single photon emission computed tomography (SPECT)

SPECT imaging differs from PET in that it monitors and records γ-ray emissions. The detectors employed in this technique are based on Anger cameras which move in a circle around the subject. These cameras are composed of an array of photomultiplier tubes coupled to a crystal which when exposed to γ-photons leads to a flash of light. Through collimation (which serves to focus the radiation) and the use of a back-projection algorithm similar to that used in X-ray computed tomography (CT), it is possible to determine the site of decay.[12, 16]

Presently over 50 different radiopharmaceuticals incorporating single photon emitters are in clinical use.[3] Their half-lives range from 6 hours to 8 days and their γ-photon energies are between 70-511 keV.[3] Some of the most commonly used radionuclides for SPECT include technetium-99m, iodine-123, gallium-67 and indium-111.[16]

1.2.2. Therapeutic agents

Therapeutic radiopharmaceuticals are molecules that are designed to deliver doses of ionizing radiation.[19] These compounds are designed to act against malignant and other diseased cells with high specificity, even when the location of diseased sites in the body is unknown, while causing minimal radiation damage to normal tissue.[20] Treatment regimes involving radionuclides do not, in general, cause acute chemical toxicity because considerably smaller

amounts of agent (*e.g.* 10^{-12} g or pg) are required to exert similar cytotoxicities as compared to other chemotherapeutic agents in which 100s of milligrams are used.[21] It should be noted that radionuclide-based methods of treatment also differs significantly from conventional external beam radiation in that the latter provides a localized therapeutic dose of radiation to a selected target area, whereas targeted radionuclides can seek out diseased sites throughout the body.[15] This is particularly important for cancer that has metastasized.

Developing therapeutic radiopharmaceuticals requires achieving a combination of specific *in vivo* targeting of the diseased site and clearance of radioactivity from non-target tissues[19] in order to maximize the radiation dose delivered to diseased cells versus healthy tissue. Considerations in this regard include the pharmacokinetics of the targeting vector, the physical decay properties of the radionuclide and the ratio of target to non-target binding.[22] With respect to the isotope, as a general guideline, the half-life of the radionuclide must be long enough for the agent to clear the blood and bind its intended receptor or antigen, and the energy of the particle must be high enough to induce apoptosis.[19] The most appropriate isotope will vary depending on the application, the size of disease tissue to be ablated and the accessibility of the diseased sites.[22] Some examples of therapeutic isotopes currently under investigation are provided in Table 1-1.

Radionuclide	Decay mode	Half-life	Clinical Use
^{89}Sr	β^-	50.5 d	bone metastases
^{90}Y	β^-	2.7 d	malignant tumours
^{131}I	β^-	8.0 d	Thyroid/neural crest/hepatic tumours
^{186}Re	β^-	3.8 d	bone metastases
^{125}I	Auger electrons	59.4 d	DNA targeting of tumours
^{211}At	α	7.2 h	leukaemia, vascularized tumours

Table 1-1. Examples of therapeutic radionuclides in pre-clinical or clinical trials[16]

Three types of radioactive decay result in the emission of particles whose properties are suitable for therapy: β-particles, α-particles and Auger electrons.[23] Beta-particle emitters (β^-) are the most common agents used in targeted radiotherapy and provide a highly homogeneous radiation dose even though their deposition may be heterogeneously distributed in target tissues.[19] Generally speaking, high energy β^- emitters are effective at treating bulky tumours whereas those with lower energy emissions are considered better at treating single cancer cells and metastases owing to their shorter path length.[23] Alpha-particle emitters show promise in situations where a tumour has "metastasized and tumour cells have disseminated throughout the body."[24] This is due to the fact that α-emitters provide a more cytotoxic type of radiation which can sterilize individual tumour cells by causing irreparable DNA damage.[24] Similarly, Auger electrons are able produce cytotoxic DNA damage by evoking double-strand breaks.[25] A challenge with these nuclides however, is that Auger-emitting radiopharmaceuticals must either be incorporated into the backbone of the genetic material or be placed in between the DNA strands of diseased cells in order to be effective.[2]

1.3 Preparation of radiopharmaceuticals

1.3.1. SPECT agents

Radiopharmaceuticals prepared from single-photon emitting isotopes are attractive because the required instrumentation is widely available and the radionuclides can be obtained at a relatively low cost since most of them have sufficiently long half-lives that transportation over long distances does not pose a problem.[12] Technetium-99m is by far the most commonly used radionuclide in nuclear medicine, representing over 80 % of the radiopharmaceuticals used in clinics.[26, 28] The reason for such a prominent position in clinical use is a combination of the nuclide's physical properties, widespread availability (commercial generators) of the nuclide at a low cost per dose ($1.00 / mCi)[12, 27] and the ease with which ^{99m}Tc pharmaceuticals can be prepared from lyophilized kits.[28]

Sterile kits for labelling with ^{99m}Tc generally contain the active ingredient (molecule to be labelled with the radionuclide), and reducing agent (usually Sn^{2+} ion in the form of stannous chloride). The reducing agent is required to reduce the technetium-99m, eluted from the generator in the form of pertechnetate ($^{99m}TcO_4^-$), to a lower (more reactive) oxidation state.[28, 29] The addition of pertechnetate to these "instant kits" produces the desired radiopharmaceutical in high yield in a single step, requiring no further purification.[5] Such commercial kits are ideal for producing perfusion agents and probes for high-capacity systems where the number of binding sites is not impacted by the presence of any other components (*e.g.* unreacted ligand). These kits are not well suited for

making radiopharmaceuticals for targets having a small number of docking sites (low B_{max}) since both the amount of the active ingredient is in excess of the desired radioactive compound in the radiopharmaceutical formulation and can therefore compete with the radiopharmaceutical for the site of interest.[5, 28]

1.3.2. PET agents

The aforementioned positron-emitting nuclides commonly incorporated in radiopharmaceuticals (^{11}C, ^{18}F, ^{15}O and ^{14}N) have very short half-lives. As a result, these isotopes are prepared using an on-site cyclotron. Still, the use of PET agents is growing and have the distinct advantage over compounds incorporating SPECT-nuclides in that image resolution is superior and the imaging data obtained can be calibrated in absolute units of radioactivity concentrated.[30] This allows parameters such as receptor occupancy to be quantified. An additional advantage to, the use of carbon-11 and fluorine-18 is that it is often possible to label the vector of interest *via* isotopic substitution thereby not changing the structure of the parent compound.[12]

Conventional methods of preparing PET radiopharmaceuticals often utilize a high level of radioactivity (> 1 Ci), multi-step procedures and manual manipulations, and are therefore likely to subject persons involved in the routine synthesis to high radiation exposure. In an attempt to minimize these risks, automated modules have been designed for the synthesis of PET agents.[31] The automated synthesis device, is designed to carry out the sequential physical and chemical steps needed to accomplish the entire synthesis of a radiolabelled product. With these synthesis modules, the various parameters pertinent to the

synthesis of the compound including time, pressure, volume, temperature etc., are all controlled remotely thereby minimizing the risk of undue radiation exposure during routine production.[31, 32]

1.4. Radiotracer purity: specific activity

In preparing targeted radiopharmaceuticals, the specific activity must be assessed in order to determine the total mass and isotopic purity of the compound that will be delivered or used. Specific activity is classically defined as the ratio of the number of radioactive atoms to the total number of atoms of a given radionuclide present in the same chemical form.[33] Simply put, the determination of specific activity describes the presence of isotopic impurities-compounds that have the same chemical form but bearing a different isotope. When describing final formulation of a radiopharmaceutical, one is often more specifically concerned with the apparent or *effective* specific activity (ESA) which takes into consideration the total mass of all radioactive and non-radioactive species present after purification.[33] ESA can be compromised by the presence of unreacted starting materials or other reagents used in the synthesis (Figure 1-3). If left-over starting material (Fig. 1-3: shaded circles) retain affinity for the target of interest (Fig. 1-3: black circles) the uptake of the labelled compound (Fig. 1-3: open circles) could be reduced versus a high ESA product.

In the case of targeted radiopharmaceuticals, the presence of unlabelled ligand can impact the ability of the agent to bind the target. The requirement for high effective activity is particularly important for agents targeting low-capacity

systems (*e.g.* receptors or antigens) that are uniquely over-expressed by cells and can be easily saturated by small quantities of competing ligands.

The requirement to produce agents in high effective specific activity will require a profound change on how agents are produced. First, radionuclides are used in nano- or pico-molar concentrations, which makes the stoichiometry of other reagents, and even minute impurities present in starting materials or reagents, extreme by comparison. It is important to note that standard radiolabelling methods, particularly for SPECT agents are designed for producing perfusion agents or targeted compounds for non-saturable systems. As such they are not geared for producing molecular imaging and therapeutic agents.

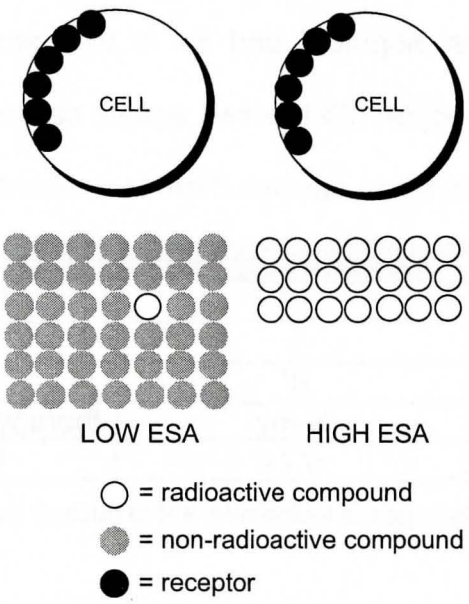


Figure 1-3. Pictoral comparison of low- and high-ESA formulations of a radiopharmaceutical

1.5. Radioisotopes of iodine

Radiohalogens have played and continue to play a major role in clinical nuclear medicine. Radioiodine, in particular, may be considered a cornerstone of

the field as it was the first nuclide used for both imaging and therapy.[34] Iodine has 30 radioisotopes, 5 of which have been found to have clinical applications (Table 1-2).[16] These include iodine-131 whose decay properties (γ-ray and β^- emitter) make it a suitable radionuclide for both imaging and therapeutic applications. Iodine-123 is an attractive isotope for SPECT imaging because of its relatively long half-life (13 h) and nearly ideal γ-ray energy. Iodine-124 is currently being investigated as a PET isotope for imaging slower biological processes because of its long half-life (4.15 days).[35, 36] Iodine-125 does not emit γ-rays of sufficient energy to be used for clinical diagnostic imaging however it is is an attractive isotope for Auger electron therapy and is often used for radiochemistry method development and for *in vitro* studies. Despite its low energy X-ray emissions, iodine-125 labelled agents can be used in small animal imaging studies because in these species there is reduced attenuation.[37]

Isotope	Half-life ($t_{1/2}$)	Primary decay mode	Applications
^{122}I	3.6 min	β^+	PET (blood flow)
^{123}I	13.3 h	γ	SPECT
^{124}I	4.15 d	β^+	PET
^{125}I	59.4 d	X-ray	therapy / small animal SPECT
^{131}I	8.0 d	β^- / γ	therapy / SPECT

Table 1-2. Isotopes of iodine relevant to nuclear medicine[18]

One of the advantages of designing iodine-based radiopharmaceuticals is that the emission properties of the agent can be varied by simply changing the isotope and not the structure of the radiopharmaceutical. Radioactive isotopes of iodine offer the rare opportunity to expand the general utility of an agent to include imaging (PET or SPECT) and targeted radiotherapy applications.[38] In

theory (assuming equivalent stability and limited impact of specific activity), a potential radiopharmaceutical could be evaluated *in vitro* using iodine-125, then imaged in small animals using the same isotope. If successful, human studies could be performed using PET (iodine-124) or SPECT (iodine-123) and a therapeutic agent created from compounds which show high target to non-target ratios (iodine-131 or iodine-125).[21]

1.6. Radioiodination methods

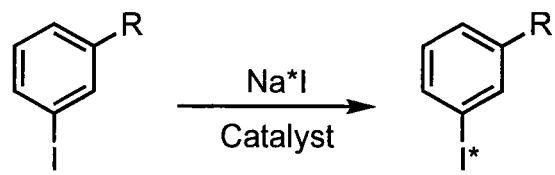
Radioisotopes of iodine are typically available as sodium iodide. As such, methods designed to incorporate this atom include nucleophilic iodine exchange, direct (*ortho*) electrophilic iodination, and demetallation strategies. An important consideration in selecting a labelling method is the nature of the target carbon-iodine bond. There are dramatic differences in the chemical and *in vivo* stabilities between compounds containing aromatic and vinylic C-I bonds compared with alkyl iodides; the latter of which are more prone to degradation *in vivo*.[15] The iodine bound to an aromatic sp^2-hybridized carbon has a bond energy of 268 kJ/mol, which is substantially higher than the sp^3-hybridized carbon-iodine bond energy of 222 kJ/mol (Table 1-3).[36, 39]

Bond-type	Bond strength (kJ/mol)
sp^3 – alkyl	222 ± 12
sp^2 – vinyl	297
sp^2 – aryl	268

Table 1-3. Carbon-iodine bond strengths[39]

1.6.1. Halogen exchange

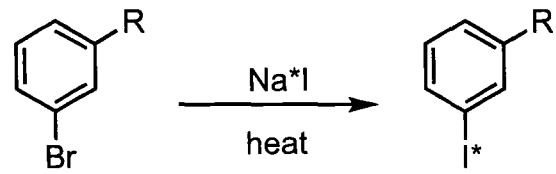
Isotope exchange is one of the simplest ways of introducing an atom of radioactive iodine into an organic molecule. Typically, procedures involve the direct substitution of radioactive iodine as I^- for an iodine atom already present in the molecule (Scheme 1-1).[8, 37]



Scheme 1-1. Isotope exchange method (I* represents radioiodine)

The major disadvantages of this method are that it requires very high temperatures to drive reactions, and products are obtained as a mixture of radio- and cold-iodinated material. Furthermore, since both the starting material and the radiolabelled product are structurally identical, they cannot be separated chromatographically. This is problematic since during a typical radiolabelling experiment, the non-radioactive starting material is in excess of the amount of radioactive sodium iodide by several orders of magnitude.[36] This means that in a given isotopic exchange labelling reaction, approximately one molecule in two thousand is radioactive[40] and therefore, the ESA of the prepared radiopharmaceutical is very low (*circa* 1-10 mCi/mg).

In order to obtain radiotracers of high effective specific activity it is necessary to alter the starting material so that it can be separated from the desired product. One approach taken has been to replace the iodine in the original starting material with another halogen such as bromine (Scheme 1-2).[39]

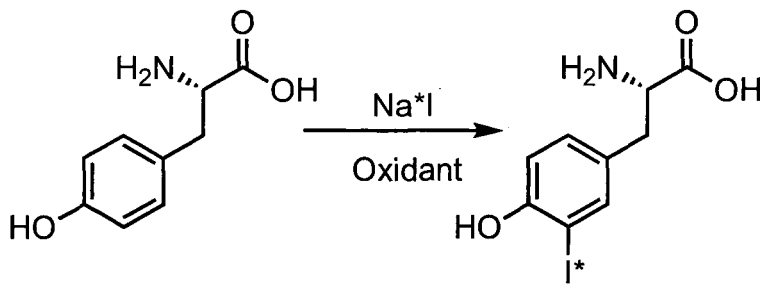


Scheme 1-2. Halogen exchange method (I* = radioiodine)

Here, the identity and chemical properties of the radioactive product are different from those of the starting material and chromatographic techniques such as High Performance Liquid Chromatography (HPLC) can be used to isolate the pure radiolabelled product. Unfortunately, HPLC is time consuming and impractical for routine clinical use, particularly when using radioisotopes with short half-lives.[41] Furthermore, it increases the risk of radiation exposure to the operator during the routine production of these radiotracers.[21] Another limitation of this approach is the harsh reaction conditions required to achieve good yields (temperatures exceeding 100 °C) which can lead to the degradation of thermally unstable compounds.[8, 39]

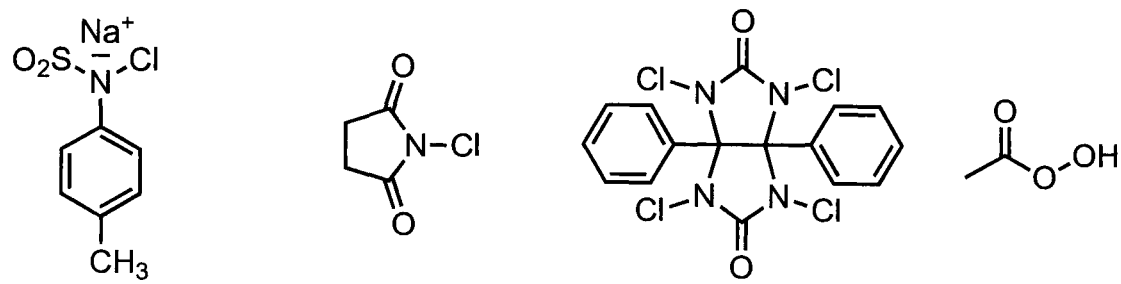
1.6.2. Direct (*ortho*) iodination

Aromatic compounds substituted with OH- or NH_2-groups (*i.e.* phenols and anilines) are strongly activated toward electrophilic substitution.[42] These compounds react rapidly with electrophilic halogen synthons. Iodide, unlike most other halides (*e.g.* fluoride, bromide) is easily oxidized to an electrophilic form, which readily reacts with the activated aromatic rings including the amino acid tyrosine in proteins (Scheme 1-3).[21]



Scheme 1-3. Direct-radioiodination method (I*= radioiodine)

There are several oxidants that are used to convert nucleophilic iodide (I^-) into an electrophilic species (I^+) at the tracer level. These include chloramine-T, N-halosuccinimides, 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (iodogen), peroxides and peracids (Figure 1-4).[43] When high specific activity radioiodide is oxidized *in situ* it generates an electropositive iodine species, where the I^+ ion forms a complex with a nucleophilic species such as water or chloride derived from the oxidizing agent.[15, 44]



Chloramine-T N-Chlorosuccinimide Iodogen Peracetic acid

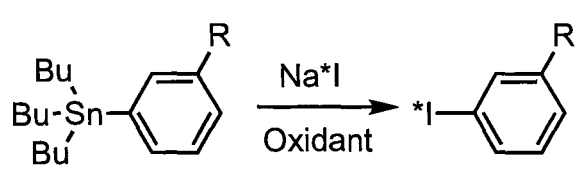
Figure 1-4. Common oxidants used for radioiodination reactions

In general, this method gives rise to high radiochemical yields, is simple to perform, and is currently the most widely used method for the radiohalogenation of proteins.[21, 36] One of the limitations of this method is that *in vivo*, radioiodinated proteins frequently undergo rapid deiodination which results in a

large accumulation of radioactivity in the thyroid gland.[15, 45] Furthermore, for ligands that contain multiple sites that can react with iodine, complex mixtures result due to poor control over regiochemistry.

1.6.3. Iododemetallation (iododestannylation)

One of the most selective methods for the synthesis of radioiodinated ligands involves demetallation reactions.[15, 36, 46] Iododemetallation represents a milder alternative to halogen exchange that has the ability to produce radiotracers in high effective specific activity.[47] This method is centered around the oxidative cleavage of a metal-carbon bond. Although a variety of metals can be used, the most common substrates are aryl-stannanes, which are treated with radioactive sodium iodide (Na*I) in the presence of an oxidizing agent to produce the desired iodo-arene (Scheme 1-4). These organometallic reagents are preferred over their trialkylsilyl-arene analogues because the silicon-carbon bond is stronger than the carbon-tin bond (bond energies are 301.5 and 226.1 kJ/mol respectively). Tin reagents display significantly higher reactivity towards electrophilic iodine and thus afford higher radiolabelling yields as compared to their silicon analogues.[36] Significant progress involving the labelling of boronate esters has also been made, however this method has not been widely used for the production of clinically applicable agents.[48]



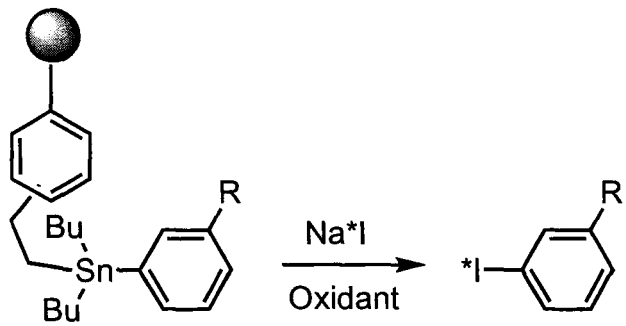
Scheme 1-4. Example of an iododestannylation reaction (I* = radioiodine)

Mechanistically, electrophilic destannylation reactions are considered to occur as a two-step process beginning with nucleophilic addition to the electropositive metal atom, which is quickly followed by electrophilic substitution at the carbon atom.[49-52] In these reactions, radioiodine is incorporated regiospecifically at the position occupied by the metal moiety with high yields.[12] An additional benefit is that these reactions are generally carried out at room temperature which prevents the possibility of thermal decomposition of sensitive molecules.[47]

The demetallation strategy commonly requires HPLC purification of the radiolabelled product both to achieve the necessary effective specific activity of the tracer and the complete separation of the potentially toxic stannyl-precursor from the radiopharmaceutical formulation.[8, 39] Regulatory guidelines specify that the quantity of metals, including tin, that can be administered to a patient must be below 10 ppm.[53]

1.6.4. Polymer-supported iododestannylation

A solid-phase variation on the iododestannylation reaction has been reported as a means of preparing radiotracers in high effective specific activity without the need for HPLC purification.[54] This is achieved through the labelling of a substrate bound to an insoluble polymer, as outlined below in Scheme 1-5.



Scheme 1-5. Example of solid-phase radiolabelling platform (I* = radioiodine)

Treatment of the polymer-bound substrate with a radiohalide and an appropriate oxidant results in the selective release of the radiohalogenated compound *via* aryl-tin bond cleavage. The unreacted polymer-bound substrate and polymer-bound side products are insoluble and thus are easily removed by filtration.[54]

The primary advantage of this radiolabelling strategy is that it avoids the need for time-consuming purification steps. There are however, a number of limitations that are primarily a consequence of using insoluble polymer supports. Firstly, despite major advances, purity analysis and characterization are not nearly as straightforward for solid-state materials as they are for small molecules;[55] often involving relatively insensitive or non-quantitative techniques such as solid-state magic-angle spinning ^{119}Sn NMR spectroscopy.[56] A related disadvantage is the inability to purify impure polymer supported precursors resulting from incomplete derivatization of reagents bound to the solid support.[57] Since there is no way to purify the resin-bound material, iodination can result in the release of a mixture of products which negates any inherent advantage of using the solid-phase approach. Furthermore, different batches of resin-bound

precursors can have variable levels precursor loading due to variation in their surface area and extent of functionalization of the parent polymer. As a result, labelling reactions using different batches of polymer can lead to inconsistent radiochemical yields.[41]

1.6.5. Designing a new radiolabelling paradigm

In comparing and contrasting the advantages and limitations of the aforementioned radiolabelling strategies, it becomes clear that an optimal labelling approach for producing targeted molecular imaging and therapy agents has not yet been developed.[4, 47] The characteristics of such a strategy would incorporate all of the advantages of the above methods including the ability to separate products from starting materials in a rapid and efficient fashion as in the solid-phase labelling strategy, and the ability to fully purify and characterize all precursors as is the case using soluble tin precursors. This thesis focuses on developing such a method through the use of fluorous chemistry.

1.7. Fluorous chemistry

1.7.1. Basic concepts

Fluorous chemistry has emerged over the last decade as an alternative to solid-phase techniques. The term "fluorous" applies to compounds or molecules bearing highly fluorinated (perfluoro) groups.[55] Perfluorinated (fluorous) liquids are both hydrophobic and lipophobic, and hence are immiscible and readily form bilayers with both aqueous systems and most common organic solvents. Thus, highly fluorinated solvents and compounds that partition into them form a

separate, orthogonal liquid phase called the "fluorous" phase into which organic and inorganic compounds have little or no tendency to dissolve.[55, 58]

The concept of using fluorous chemistry as a means of performing organic synthesis was introduced by Curran and coworkers.[59] Fluorous synthesis can be carried out in solution where perfluorocarbon chains act as a soluble support, analogous to the polymeric resins common to solid-phase synthesis. In the first stage, a fluorous tag (typically of the form $CH_2CH_2(CF_2)_nCF_3$) is attached to an organic substrate. These newly-formed fluorous molecules have two domains: a fluorous domain that controls the solubility of the molecule and an organic domain that directs the reactivity of the compound.[58]

The most common fluorous tags used are alkyl chains ranging from C_3F_7 to $C_{10}F_{21}$; "heavy" tags often contain two or more of these moieties.[58-60] The fluorous labels themselves are chemically inert; therefore the organic portion can undergo chemical transformation without interference from the fluorous tag which facilitates purification (*vide infra*). At the conclusion of the synthesis, the desired organic product is obtained after the detachment of the fluorous tag. (Figure 1-5).[58]

The most common fluorous tags have been shown to be inert to a wide range of chemical reactions and have minimal effect on the reactivity of the "non-fluorous" portion of the attached molecules.[60] Although their relative molecular weights are high (1000 ± 250 Da), the substrates in fluorous synthesis are generally soluble in common organic solvents.

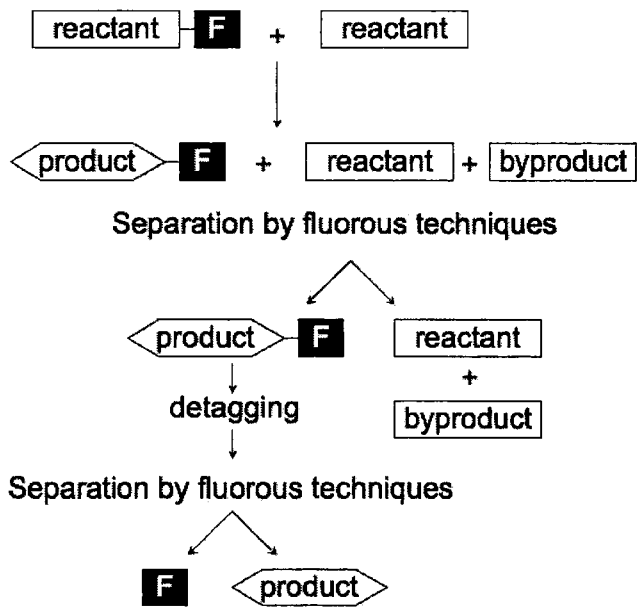


Figure 1-5. Use of fluorous-tagged reactants in organic synthesis[57]

Fluorous-tagged substrates have both physical and chemical characteristics (*e.g.* solubilities and reactivities) that resemble those of small organic molecules.[55, 59, 61] A further advantage over solid-phase methods is that conventional analytical methods for monitoring reactions and purity of products can be employed when working with fluorous compounds. These include TLC, HPLC, IR spectroscopy, solution NMR spectroscopy, X-ray crystallography and mass spectrometry.[60, 61]

1.7.2. Fluorous separations

There are several techniques that have been developed for separating fluorous and non-fluorous materials. The main methods are liquid-liquid extraction and fluorous solid-phase extraction (F-SPE).[59] Liquid-liquid extraction is a viable method for fluorous molecules bearing what are known as "heavy

fluorous tags". These compounds are generally 60 % or more fluorine by molecular weight (often made up of two or more perfluorinated alkyl chains). At these levels, fluorous compounds can be extracted efficiently into perfluorinated solvents such as perfluoroinated hexanes (C_6F_{14}, also commonly referred to as FC-72®) and perfluorobutyl methyl ether (PFBME).[61-63]

An alternative method of separating fluorous components from non-fluorous reactants and products involves fluorous solid-phase extraction (F-SPE) on silica gel, containing a bonded phase of -$SiMe_2(CH_2)_2C_8F_{17}$.[60, 61, 63] The fluorous stationary phase adsorbs fluorous molecules irrespective of the polarity of the various organic components.[61, 64] Non-fluorous components are readily eluted using a "fluorophobic" solvent; typically an alcohol-water mixture that is at least 15 % water. Regardless of the volume of the eluent passed through the cartridge, fluorous components are retained until eluted with a "fluorophilic" solvent (methanol, acetonitrile, acetone, or tetrahydrofuran). One of the major advantages of this method is applicable for compounds with modest fluorine content (between 60 % and 40 % fluorine by molecular weight) and the fact that costly and environmentally harmful perfluorinated solvents are not needed. Furthermore the F-SPE cartridges can be regenerated for further use by flushing with warm tetrahydrofuran.[64, 65]

1.7.3. General aspects of fluorous chemistry

The fluorous approach to organic synthesis resembles traditional solution-phase methods because the fluorous reactions proceed as homogeneous solutions. The main advantage of fluorous reactions becomes apparent at the

purification stage, where desired products can be selectively isolated by fluorous separation methods, which in the case of F-SPE more closely resembles filtration than traditional chromatography. These separations depend primarily on the presence (or absence) of a fluorous tag, and are not influenced by polarity or other molecular features that govern chromatography.[61, 65]

A further advantage in the case of fluorous-phase synthesis is that the reactions can be monitored *in situ* by a variety of techniques, including HPLC. Thus reactions on fluorous supports can be optimized much more readily than those on conventional solid supports.[59] A comparison of the basic features of solution-, solid-, and fluorous-phase synthesis is summarized in Table 1-4.[66] These advantages coupled with the success of the solid-phase labelling approaches[41, 56] suggest that a radiolabelling method based on fluorous chemistry would be of significant benefit with respect to purification and novel probe discovery.

	Solution-phase	Solid-phase	Fluorous
Standard lab equipment	☑		☑
Intermediate characterization	☑		☑
Ease of scale-up	☑		☑
Ease of purification		☑	☑
Use of excess reagent		☑	☑
Mixture synthesis		☑	☑
Potential for automation		☑	☑

Table 1-4. Comparison of solution-, solid- and fluorous-phase synthetic strategies[60]

1.8. Scope and summary of research goals

The objective of this thesis was to develop a novel methodology for the production of radiotracers and therapeutics derived from the principles of fluorous

chemistry. This began with the selection of a model system to test the feasibility of the labelling method and to optimize purification protocols (Chapter 2). It is imperative that the method of purifying the prepared tracers is able to remove all tin-containing species. Following this, the application of the fluorous labelling strategy to the preparation of a radiopharmaceutical that is used clinically was undertaken (Chapter 3) and the purity of the product and ease of purification compared to the conventional methods. Finally, a broader collection of synthons were prepared so that the fluorous labelling strategy can be used as a platform for the discovery of novel molecular imaging and therapy agents and for the radiolabelling of biomolecules (Chapters 4 and 5).

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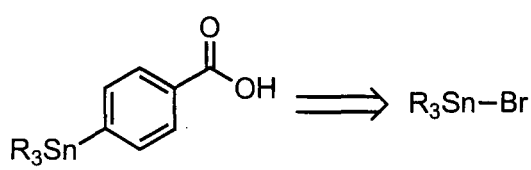
Chapter 2: Development of the fluorous labelling approach: proof of concept.

2.1. Overview

Ideal radiolabelling methods are those that are regioselective, high yielding and that produce the target molecule in high effective specific activity. The goal of this research is to develop a new radiolabelling methodology using fluorous chemistry

As an initial target, we elected to prepare and evaluate the reactivity of 3- and 4-(*tris*[2-perfluorohexylethyl]stannyl) benzoic acid (*m*-**2.1** and *p*-**2.1**). In addition to serving as a model system for developing and validating a radiolabelling methodology, this compound would also serve as a platform from which other targeted derivatives, including benzamides and protein conjugates, can be synthesized.

The synthetic strategy for preparing *m*- and *p*-**2.1** was based on the retrosynthesis outlined below (Figure 2-1), starting from the commercially available trialkyltin bromide.



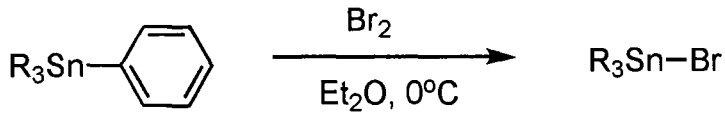
p-**2.1** **2.2**

Figure 2-1. Proposed retrosynthesis of **2.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

Once the fluorous-tin precursor was prepared, the reactivity of the product was tested using non-radioactive iodine and an iodinated analogue to serve as an authenticated reference standard for radiolabelling experiments was prepared. The non-radioactive work was used to optimize the purification protocols and to produce a well characterized standard for the radiochemical work.[1]

2.2. Synthesis and characterization of tris(2-perfluorohexylethyl)stannyl benzoic acid (2.1)

Tris(2-perfluorohexylethyl)stannyl bromide **2.2** was synthesized in excellent yield by treating *tris*(2-perfluorohexylethyl)phenyl tin with an ethereal solution of bromine at 0 °C (Scheme 2-1).[2] After warming the reaction to room temperature, the product was isolated by liquid-liquid extraction between perfluorohexanes (FC-72®) and dichloromethane.[3]



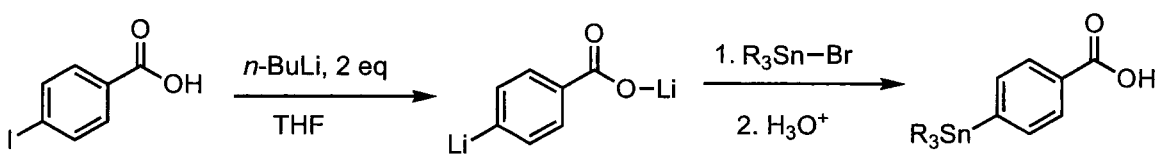
2.2

Scheme 2-1. Synthesis of **2.2** (R = $CH_2CH_2(CF_2)_5CF_3$)

The direct conversion of **2.2** to *m*-**2.1** and *p*-**2.1** was attempted by treating 4-iodobenzoic acid with two mole equivalents of *n*-butyllithium to generate the di-lithiated anion *in situ*, which was then reacted with **2.2** (Scheme 2-2).[4] This preparation resulted in low and inconsistent yields (< 35 %) of the desired product, thus an alternative approach was investigated (Scheme 2-3).[5]

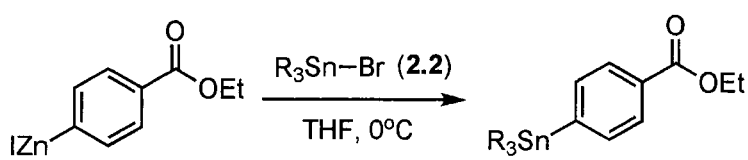
Commercially available reagents, 3- and 4-(ethoxycarbonyl)phenyl-zinc iodide (*m*-**2.3** and *p*-**2.3**), were combined in separate vessels with the fluorous tin bromide **2.2** at 0 °C in anhydrous tetrahydrofuran. After warming to room temperature, the products *m*-**2.4** and *p*-**2.4** were obtained in good yield (69 - 80 %) as colourless oils *via* liquid-liquid extraction using perfluorinated hexanes and methanol.

The increased yield was likely due to the softer nature of the aryl zinc reagents which match better with the softer tin electrophile than does the corresponding lithium anion. One cautionary note however was that some batches of the organozinc reagent contained a mixture of isomers (*m*, *p*). Prior to use, each lot of reagent was characterized by ^{1}H NMR.



p-**2.1**

Scheme 2-2. Preparation of *p*-**2.1** *via* lithium dianion (R = $CH_2CH_2(CF_2)_5CF_3$)[4]

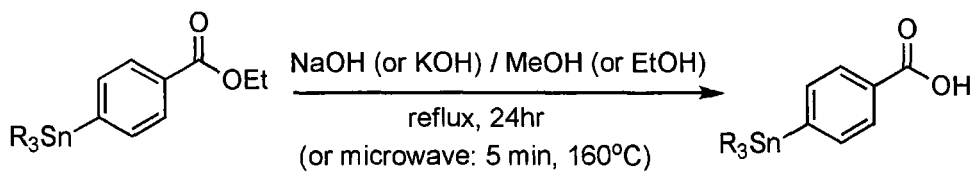


p-**2.3** *p*-**2.4**

Scheme 2-3. Alternative organometallic approach to *p*-**2.4** (R = $CH_2CH_2(CF_2)_5CF_3$)[5]

Saponification of the esters (**2.4**) using NaOH led to the formation of the desired products *m*- and *p*-**2.1** in excellent yields (81 % and 83 %, respectively) (Scheme 2-4). These were subsequently purified by silica gel chromatography

which was an important step since high purity of **2.1** was later determined to be essential for obtaining high radiochemical purity when preparing the radioiodinated compounds (Section 2.5.3) since even small impurities (*i.e.* < 5%) were found to severely impact both radiochemical yield and purity.[6]



p-**2.4** *p*-**2.1**

Scheme 2-4. Hydrolysis of *p*-**2.4** (R = $CH_2CH_2(CF_2)_5CF_3$)

Characterization of the hydrolysis products confirmed the identity of the products *m*- and *p*-**2.1** respectively. In the ^1H NMR spectrum of *p*-**2.1** for example, protons on the ethyl group were absent and the remaining signals were due to the aromatic protons (doublets at 8.01 and 7.60 ppm (J = 8.0 Hz each)) and the fluorous methylene protons (multiplets present at 2.41 and 1.34 ppm). In the ^{13}C NMR spectrum the signals associated with the carboxyl carbon appeared at 170.56 ppm and four signals appear in the aromatic region which is expected of a para-substituted aromatic ring. In addition, signals resulting from the carbon atoms of the fluorous chain are attributed to the multiplets occurring between 121.43 and 110.27 ppm while the fluorous methylene carbons are assigned to the resonances at 28.67 and -0.44 ppm. For both isomers, mass spectral analysis of the reaction product showed a major peak with a mass-to-charge ratio of 1280.9556 which is consistent with the $[M-H]^-$ ion of **2.1** (calc. 1280.9548).

Single crystals of *m*-**2.1** suitable for single crystal X-ray diffraction analysis were obtained and the structure determined (Figure 2-2). The structure is consistent with the expected product and represents a rare example of a solid-state structure of a fluorous compound. The ability to unequivocally characterize precursors is a significant advantage of solution-phase methods over solid-phase labeling methods. This is particularly true for compounds which are destined for clinical use. Crystallographic details *m*-**2.1** are provided in Appendix I.

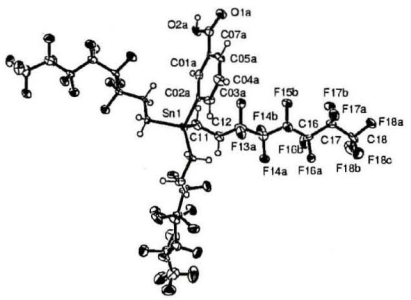


Figure 2-2. ORTEP drawing of *m*-**2.1** (30% thermal probability ellipsoids)

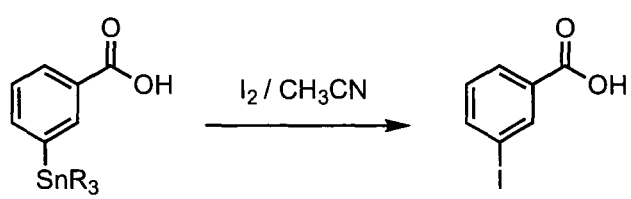
2.3. Iodination of 2.1

2.3.1. Non-radioactive iodination of 2.1

With the synthesis, purification and characterization of the fluorous benzoic acids *m*-**2.1** and *p*-**2.1** completed, the non-radioactive iodination of these species was performed with two goals in mind. First, it was necessary to investigate the reactivity of these compounds and to determine whether their fluorous tags had any effect in comparison to the tributyl tin analogues. Second, non-radioactive, or cold, iodinations were designed to evaluate the efficiency of

the Fluoro*Flash*® solid-phase extraction (F-SPE) cartridges in selectively removing starting materials and to optimize elution conditions for future radiolabelling experiments.

To probe the reactivity of the fluorous precursors, molecular iodine was selected as a source of iodine.[7-10] A typical procedure (Scheme 2-5) involved dissolving a stannyl precursor (*m*-**2.1**) in a small volume of acetonitrile to which 0.5 mole equivalents of an iodine/acetonitrile solution was added. After 15 min. the reaction was quenched by the addition of sodium metabisulfite, which converted all residual electrophilic iodine present in the reaction mixture to iodide, effectively halting the reaction. Analysis of the crude reaction mixture by HPLC showed a sharp peak at 13.9 min which is identical to the retention time of an authentic sample of 3-iodobenzoic acid under the same conditions, thereby confirming formation of the desired product. The reaction times and yields are similar to that for trialkyltin analogues[11] which indicated that the fluorous tin groups have a minimal impact on reactivity of the tin group.



m-**2.1** *m*-**2.5**

Scheme 2-5. Non-radioactive iodination of *m*-**2.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

The viability of using F-SPE cartridges as a means of selectively retaining fluorous compounds following a labelling experiment was assessed using a

series of cold iodination reactions and macroscopic amounts of *m*-**2.1**. The reactions were performed using the same reagents employed at the tracer level (sodium iodide, iodogen), but using a substantially larger amount of the tin compound (100 mg) compared to 0.5 mg traditionally used in radiolabelling experiments. This scale was employed to ensure that sufficient amounts of material were present to accurately determine by UV-HPLC whether or not breakthrough was occurring. After quenching the reaction with sodium metabisulfite, the crude reaction mixture was diluted to a volume of 1 mL using distilled water and loaded onto a F-SPE cartridge. The cartridge was first flushed with water to remove any inorganic salts, then the desired iodinated product, *m*-**2.5**, was eluted using a solution of 20 % water in methanol.

Analysis of the crude reaction mixture using HPLC revealed that both the desired iodinated products and the tin-containing starting materials were present; this was verified by comparison with authentic reference standards as described previously. The HPLC chromatograms of the iodination reaction of *m*-**2.1** (Figure 2-3) displayed peaks with retention times (R_t) of 13.9 and 20.2 min, corresponding to compounds *m*-**2.5** and *m*-**2.1**. After F-SPE purification, the chromatogram showed only the iodinated product *m*-**2.5**. Based on the detection limits of the UV detector, which were determined by generating a calibration curve using *m*-**2.1**, the purified fractions contained less than 13 ppm of the starting material. Since the aforementioned pharmaceutical guidelines are much more stringent, other more sensitive methods of detection were explored to more accurately quantify the amounts of tin present in the purified samples.

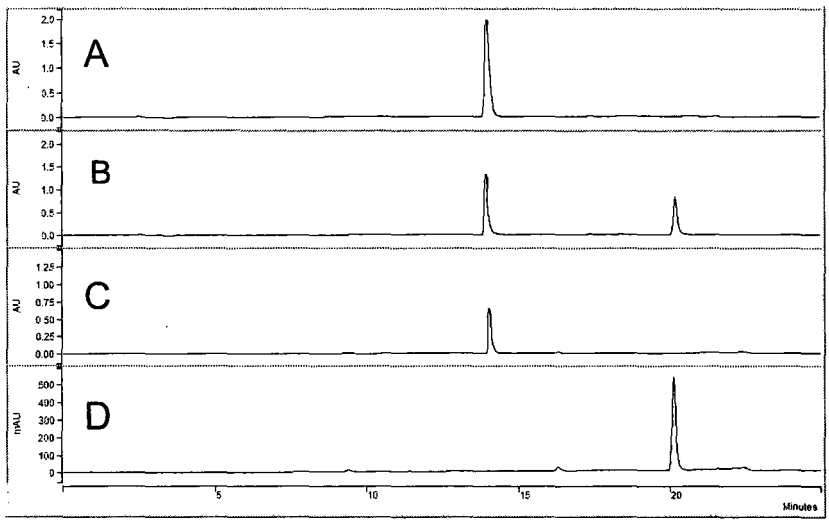


Figure 2-3. HPLC chromatograms (UV) from the non-radioactive iodination of *m*-**2.1**. 3-Iodobenzoic acid standard (*A*); reaction mixture (*B*); purified *m*-**2.5** (*C*); and compound *m*-**2.1** (*D*)

2.3.2. Alternate methods of quantifying residual tin contaminants in *m*-2.5

The need for careful purification of the prepared radiotracers is to ensure complete removal of stannyl precursor. This is a consequence of the fact that high concentrations of organotin compounds are known to exert toxic effects in man and animals.[12] With this in mind, pharmaceutical standard-setting authorities such as the US Pharmacopeia (USP) and the British Pharmacopeia (BP) have limited the amount of metal impurities, including tin, that can be administered to a patient to less than 10 ppm.[13]

In general, the maximum toxicological effects are found for the trialkyl substituted analogues of the form R_3SnX (X = Ar, halogen *etc.*). Research has revealed that although the nature of the X group has almost no effect on the

degree of toxicity of the compound, the chain length of the alkyl group (R) correlates closely with the compound's toxicity. Lower homologues in the series, where the alkyl groups are methyl or ethyl, are the most toxic while toxicity diminishes progressively to the tri-*n*-octyl tin compounds, which are sufficiently benign that they are used as stabilizers for food packaging materials.[14, 15]

Following evaluation by HPLC, electrospray mass spectrometry was investigated as a more sensitive means of quantifying the amount of tin present in F-SPE fractions containing *m*-**2.5**. Fractions eluted from the F-SPE cartridge were analyzed and showed no signal corresponding to *m*-**2.1**. The spectrum of the purified materials *m*-**2.5** showed the absence of signals corresponding to the tin containing species.

To further corroborate the ES-MS results, which can be influenced by the "ionizability" of a compound, inductively coupled plasma mass spectrometry (ICP-MS) was used. ICP-MS is one of the most sensitive techniques for the analysis and quantification of trace elements and isotopes in both liquids and solid samples.[16] ICP-MS offers higher analytical precision, and low detection limits (ranging from ppb to ppt) for most elements and the signal strength and sensitivity of the instrument is not influenced by the functional groups present.[16] Analysis of the F-SPE purified samples revealed that the total amount of tin present was less than 1 ppm (or < 2 ng).

This data demonstrated that even at loading levels that are significantly greater than those used in radiolabelling experiments, the F-SPE procedure is highly effective at removing the fluorous-tin precursor and any cleaved fluorous-

tin groups. The level of tin in all cases was below that described in the Pharmacopeia.

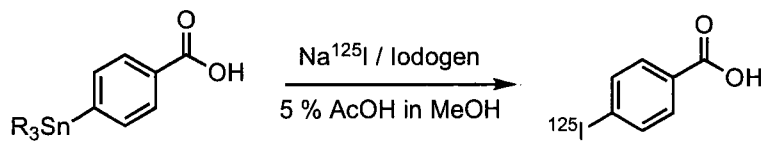
2.3.3. Radioiodination of 2.1 with iodine-125

After successfully developing a means of purifiying mixtures of fluorous benzoic acids and their iodinated derivatives, the next step was to adapt the fluorous labelling method to the tracer level and to evaluate the effectiveness of the fluorous method using ^{125}I. This isotope was selected for the optimization phase based on its long half-life and low cost.

As described earlier, there are a number of reagents used to convert radioactive iodide into a source of electrophilic iodine (Figure 1-4). For the purpose of the experiments described in this body of work, iodogen was selected as the oxidant, based on the short reaction times required to obtain high radiochemical yields and the ease with which it is separated from the desired product as compared with other agents such as chloramine-T (CAT).[17, 18]

Ultimately, the radiolabelling chemistry was based in part on the method reported by Weinreich and coworkers.[19] To a reaction vial precoated with iodogen was added *p*-**2.1**, followed by a solution of 5 % acetic acid in methanol and sodium [^{125}I]iodide (Scheme 2-6). After 3 min, the reaction was quenched with sodium metabisulfite; an aliquot was taken and co-injected with a sample of non-radioactive iodobenzoic acid (*p*-**2.5**) for HPLC analysis. The peak with a retention time of 14.2 min in the gamma trace corresponded to the non-radioactive standard (UV trace), indicating that the desired radiotracer (*p*-

[^{125}I]**2.5**) had indeed been prepared (Figure 2-4). The reaction mixture was then diluted with water and purified following the method developed for the non-radioactive analogue (Section 2.5.1).



p-**2.1** *p*-**2.5**

Scheme 2-6. Radioiodination of *p*-**2.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

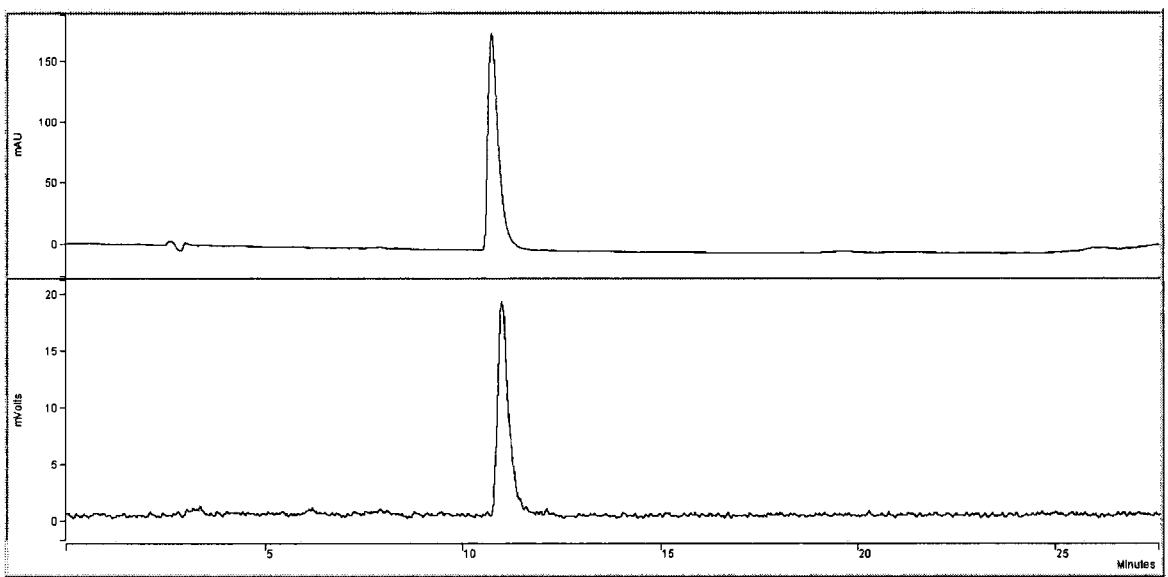


Figure 2-4. UV- and γ-HPLC chromatograms of: non-radioactive *p*-**2.5** (*top*) and F-SPE purified *p*-[^{125}I]**2.5** (*bottom*)

HPLC analysis of the purified reaction mixture revealed that greater than 99 % of the radioiodide was converted into the desired radiotracer, *p*-**2.5** and the radiochemical purity of the sample was found to be greater than 98 % which far exceeds the requirements for most radiopharmaceuticals.[13, 17] The overall yield for a typical 100 μCi reaction was 92 μCi (92 %). The amount of activity lost on the F-SPE cartridge did not exceed 7 μCi (or 7 % of total activity). It should be emphasized that the purity of radiolabelling precursors is *crucial* for obtaining

high radiochemical yields. Initial attempts at radiolabelling compound **2.1** resulted in the formation of a 50:50 mixture of products despite good purity as assessed by NMR spectroscopy and HPLC. The impurity was identified as iodobenzene, resulting from trace amounts of *tris*(2-perfluorohexylethyl)phenyl tin which is significantly more reactive than **2.1**, consequently despite being present in minute quantities (< 5 %), at the tracer level it consumed nearly 50 % of the Na[^{125}I]I. Overall, these results demonstrate that the fluorous approach to radiolabelling is in fact a viable method for producing radiotracers of high radiochemical yield and purity.

2.4. Synthesis of iodobenzamide from N-*(2-diethylaminoethyl)-4-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-2.7)*

Radioiodobenzamides are under study for the diagnosis and treatment of cutaneous melanoma (skin cancer) and its metastases.[20] The first of these compounds to enter into clinical trials as a potential diagnostic tool was *N*-(2-diethylaminoethyl)-4[^{123}I]iodobenzamide ([^{123}I]BZA) (Figure 2-5).[20, 21] Since this time, developments have been focused on improving the pharmacokinetics of the radiotracer.[21] Most of the radiotracers reported in these studies were produced using iodine exchange procedures. There is one report of a trialkyltin precursor which was purified by HPLC chromatography[22] which is suitable for research but not for routine preparation of tracers for clinical use. In addition, a more efficient means of optimizing the nature of the benzamide to improve its pharmacokinetics is needed.[23] Fluorous-mediated benzamides, derived from **2.1**, offer a means to

produce tracers in much higher effective specific activity than conventional exchange methods, while also being a platform for varying the pendent amide. To this end, the 4-(*tris*[2-perfluorohexylethyl]stannyl)-*N,N*-dimethylaminoethyl- and *N,N*-diethylaminoethyl-benzamides *p*-**2.6** and *p*-**2.7** were chosen as initial synthetic targets (Figure 2-5).

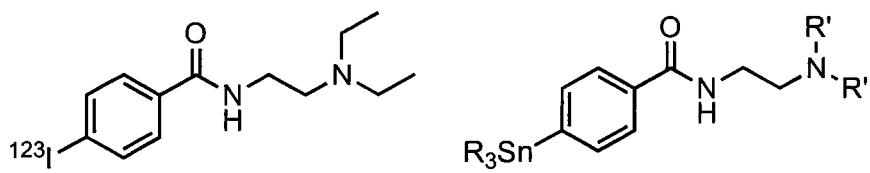
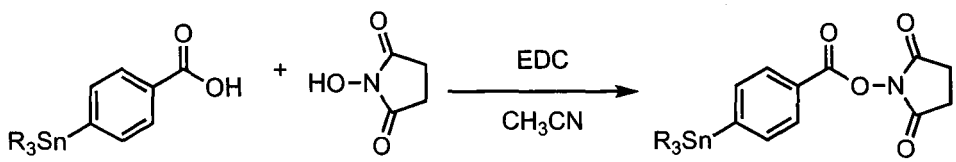


Figure 2-5. *N*-(2-diethylaminoethyl)-4[^{123}I]iodobenzamide ([^{123}I]BZA)(*left*), fluorous benzamides (*right*) *p*-**2.6** (R'=Me) *p*-**2.7** (R'=Et) (R = $CH_2CH_2(CF_2)_5CF_3$)

2.4.1. Toward the synthesis of *p*-2.6 and *p*-2.7: preparation of an active ester of 2.1

N-Hydroxysuccinimidyl active esters are one of the most common activating groups used to form amides and have been utilized extensively for labelling proteins since they are easily prepared and are highly reactive towards primary amines.[24] As such, the synthesis of the benzamides *p*-**2.6** and *p*-**2.7** was initially attempted by first converting *p*-**2.1** to its *N*-hydroxysuccinimidyl active ester *p*-**2.8** (Scheme 2-7). This was carried out by combining *p*-**2.1** with *N*-hydroxysuccinimide and *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) in acetonitrile. Unfortunately, the product *p*-**2.8** could not be obtained in sufficient purity to warrant further use. As an alternative route to *p*-**2.6** and *p*-**2.7,** attempts at direct coupling using *in situ* activating reagents such as HBTU were made,[25]

however combining *p*-**2.1** with *N,N*-dimethylethyldiamine under such conditions resulted in poor yields of the desired benzamide *p*-**2.6** (< 15 %).

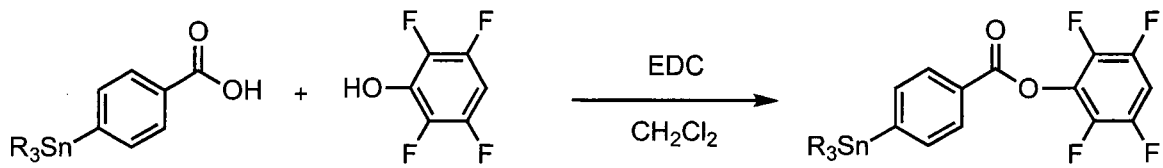


p-**2.1** *p*-**2.8**

Scheme 2-7. Synthesis of *p*-**2.8** (R = $CH_2CH_2(CF_2)_5CF_3$)

An alternative synthesis of compounds *p*-**2.6** and *p*-**2.7** was attempted *via* a tetrafluorophenol active ester (*p*-**2.9**), since such compounds are stable under a number of conditions yet react rapidly with amines.[11] In this procedure (Scheme 2-8), tetrafluorophenol was combined with *p*-**2.1** in the presence of EDC in a solution of either ethyl acetate or dichloromethane. After 16 h, the solvent was removed and the majority of the unreacted tetrafluorophenol was separated from the desired product by fluorous liquid-liquid extraction. The resulting oil was further purified by silica gel chromatography, and identified as compound *p*-**2.9** by ^1H, ^{13}C NMR and IR spectroscopy, and high resolution mass spectrometry. The IR spectrum of compound *p*-**2.9** displayed a strong absorption at 1763 cm^{-1}, which is consistent with the C=O stretch of an organic ester and significantly different from the starting material (*p*-**2.1**) whose C=O stretching bands appear at 1703 cm^{-1}. Furthermore, the ^1H NMR spectrum of *p*-**2.9** (Figure 2-7) reveals two doublets (8.21 and 7.61 ppm) resulting from aromatic protons. The characteristic signal associated with the tetrafluorophenol hydrogen atom appears as a multiplet at 7.05 ppm, its multiplicity due to coupling interactions

with the neighboring fluorine atoms.[26] In addition, the upfield proton resonances at 2.35 and 1.38 ppm are the result of the methylene groups on the fluorous chains. The complex splitting of these signals are due to coupling to adjacent fluorine and tin atoms respectively.



p-**2.1** *p*-**2.9**

Scheme 2-8. Synthesis of *p*-**2.9** (R = $CH_2CH_2(CF_2)_5CF_3$)

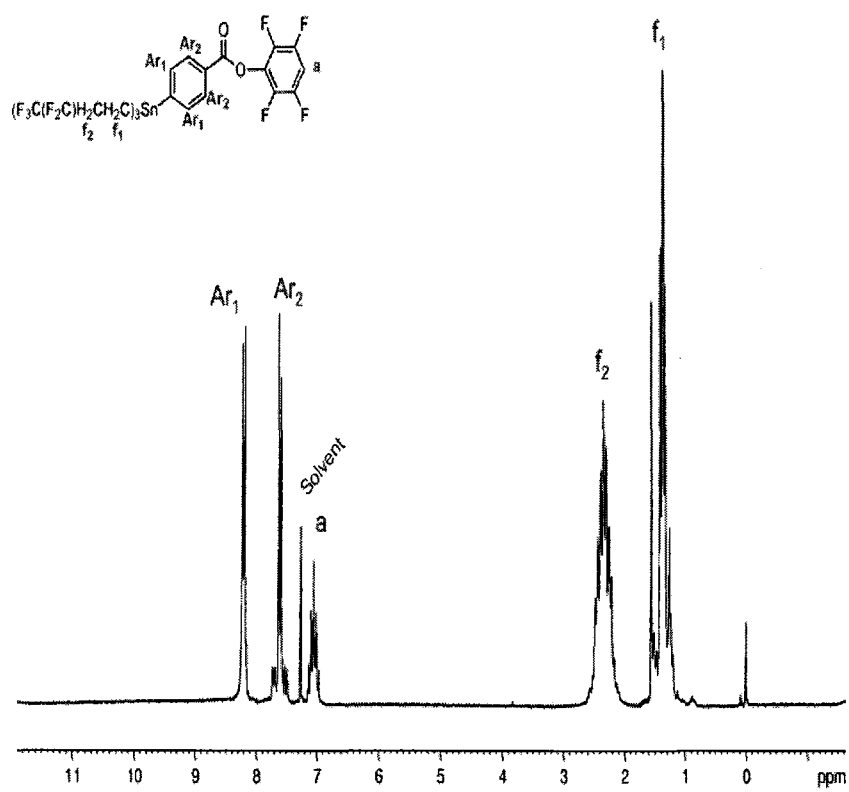
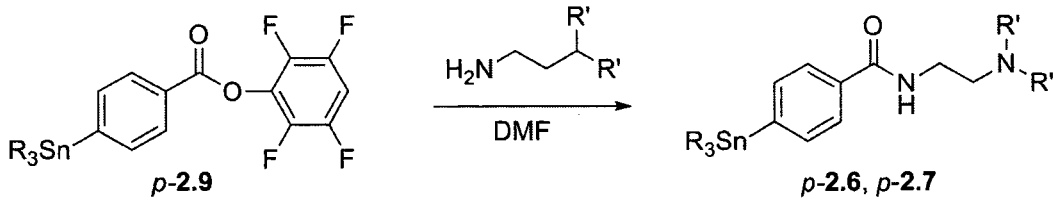


Figure 2-6. ^{1}H NMR spectrum of *p*-**2.9** (200.23 MHz, $CDCl_3$)

2.4.2. Synthesis of *p*-2.6 and *p*-2.7

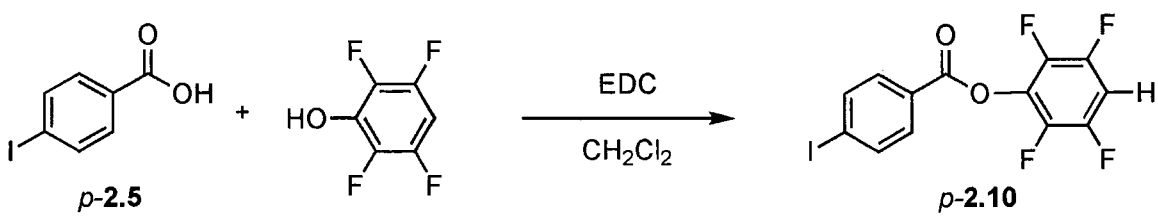
The synthesis of *p*-**2.6** (and *p*-**2.7**) was accomplished by treating *p*-**2.9** with distilled *N,N*-dimethylethyldiamine in *N,N*-dimethylformamide (DMF) (Scheme 2-9), followed by two successive liquid-liquid extractions; the first using FC-72® and the reaction solvent to get rid of the high-boiling DMF and any tetrafluorophenol byproduct, and the second involving FC-72® and water to remove any excess amine. The products, *p*-**2.6** and *p*-**2.7**, were isolated in 90 % and 80 % yields respectively.

NMR spectroscopy (^{1}H and ^{13}C), IR spectroscopy and high resolution mass spectrometry provided evidence confirming the identities of the fluorous *N,N*-dialkylbenzamides. For example, the ^{1}H NMR of *p*-**2.6** shows a pair of doublets assigned to the protons on the aromatic ring, and those adjacent to the tin atom (7.46 ppm) have ^{119}Sn satellites, where as the signals representing the protons of the methyl groups of the benzamide appear as a singlet at 2.28 ppm integrating to 6 protons. This, and the characteristic resonances of the fluorous methylene protons at 2.30 and 1.32 ppm, are consistent with the structure of the product. In addition to this, the IR spectrum provides evidence of the formation of *p*-**2.6** through the observed C=O stretch at 1654 cm^{-1}, and the broad N-H stretches at 3341 cm^{-1} which is not present in the starting material (*p*-**2.9**). Furthermore, high-resolution positive-ion mass spectrometry also support the identity of the product as *p*-**2.6** with an observed *m/z* value of 1353.0601 which is in agreement with the calculated $[M+H]^{+}$ value of 1353.0634.

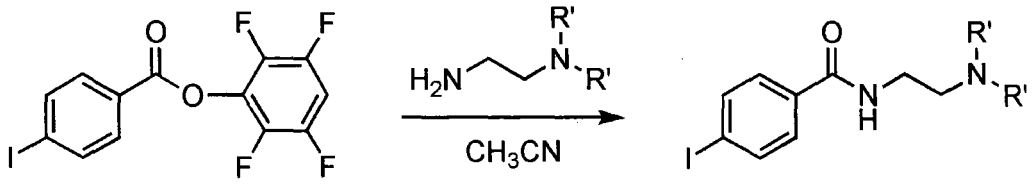


Scheme 2-9. Synthesis of *p*-**2.6** (R'=Me) and *p*-**2.7** (R' =Et) (R=$CH_2CH_2(CF_2)_5CF_3$)

The next step in the synthesis was the preparation of non-radioactive iodinated compounds to be used as reference standards for radiolabelling experiments. These standards were synthesized by first converting the respective iodobenzoic acids to their tetrafluorophenol active esters *p*-**2.10** (Scheme 2-10). This was followed by treatment of the esters with the amine in an analogous fashion to the synthesis of *p*-**2.6** and *p*-**2.7**, this time using acetonitrile as a reaction solvent. The products *p*-**2.11** and *p*-**2.12** were isolated by evaporation of the solvent and subsequent liquid-liquid extraction using chloroform and water (Scheme 2-11) both in 40 % yield. This poor isolated yields of *p*-**2.11** and *p*-**2.12** was attributed to the low extraction efficiency of the polar dialkylamino benzamides in chloroform.



Scheme 2-10. Synthesis of *p*-**2.10**



p-**2.10** *p*-**2.11**, *p*-**2.12**

Scheme 2-11. Synthesis of **2.11** (R'=Me) and **2.12** (R'=Et)

HPLC analysis of *p*-**2.6**, *p*-**2.7** and their respective iodostandards were carried out prior to radiolabelling experiments to verify purity. Even at very high concentrations (> 2 mg/mL) the signals observed in the UV chromatogram (λ = 254 nm) were only slightly above baseline, which suggested that the desired compounds were being retained on the HPLC column. The retention is attributed to strong interactions between the dialkyl amino group and the unfunctionalized silanol residues on the silica. We investigated the effects of different solvents, the pH of the mobile phases as well as the nature of the acids used to adjust the pH. Ultimately, the optimal eluent had an aqueous phase containing 0.5 % tris(hydroxymethyl) aminomethane (TRIS) adjusted to pH 2.6 with H_3PO_4. Using this eluent, the iodostandards were detected as pure products at a retention time of 8.5 minutes.[27]

2.4.3. Radioiodination of *p*-2.6 and *p*-2.7

With reference standards and a suitable HPLC method in hand, the radioiodinations of compounds *p*-**2.6** and *p*-**2.7** were performed following the method used to prepare *m*-[^{125}I]**2.5** and *p*-[^{125}I]**2.5**. The radioiodination of *p*-**2.7** took place in good radiochemical yield (78 %) and the products isolated in high

purity (98 %) (Figure 2-8). Conversely, radiolabelling experiments involving compound *p*-**2.6** revealed minor radioactive impurities which arose due to small impurities in the starting material that could not be removed.

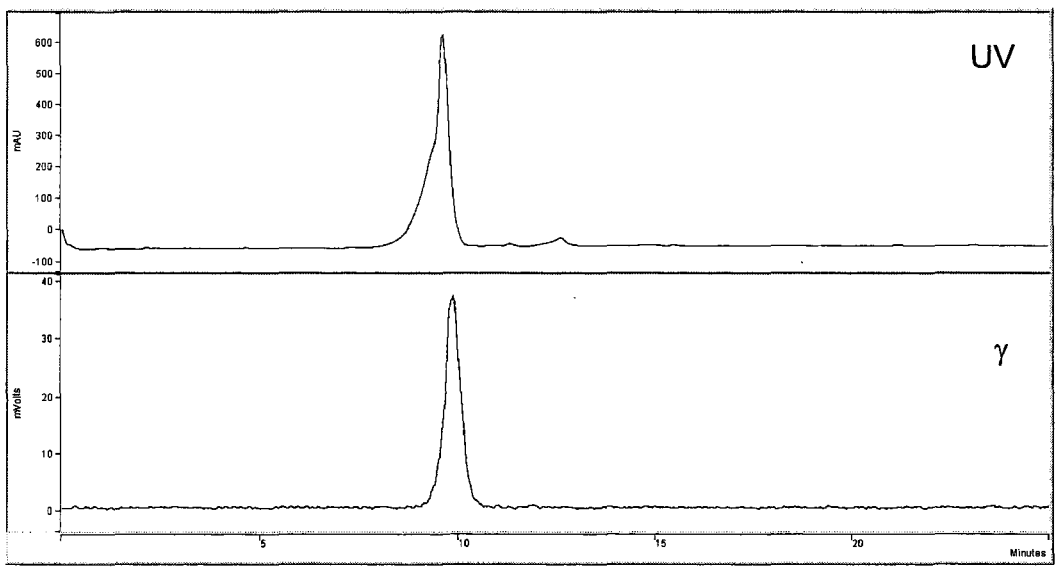


Figure 2-7. UV-(*top*) and γ-HPLC chromatograms of non-radioactive *p*-**2.12** and F-SPE purified *p*-[^{125}I]**2.12** (*below*)

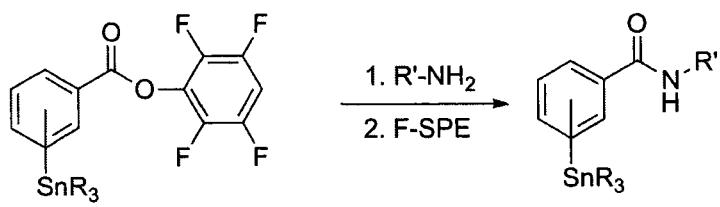
2.5. Library synthesis

In addition to producing compounds in high effective specific activity, the fluorous labeling approach is also amenable to producing libraries of precursors and radiotracers alike. Since the development, validation and subsequent advancement to the clinic of new molecular imaging probes is a time-consuming, expensive and slow process, alternative technologies such as high throughput small-molecule screening (HTS) are being explored as vehicles to accelerate the discovery phase of probe development.[28] The facile purification of the fluorous-labelled material feature can potentially be used to drastically improve the rate at

which promising lead candidates are discovered, particularly when compared to conventional one molecule at a time discovery methods.[29]

2.5.1. Synthesis and radiolabelling of library

To demonstrate the potential of fluorous supports as tools to create libraries of both precursors and labelled compounds, a collection of sample benzamides was prepared in a similar fashion used to synthesize *p*-**2.7**. A two-fold excess of a series of amines were added to a collection of reaction vials containing *m*-**2.9** and *p*-**2.9** (Scheme 2-12). After stirring at ambient temperature for 30 min, the products were isolated *via* liquid-liquid extraction. The compounds were subsequently characterized by standard spectroscopic methods and then radiolabelled using sodium [^{125}I]iodide to produce the radioiodinated derivatives in good radiochemical yield (average = 77 %) and once again in high radiochemical purity (average = 96 %).



m-**2.9**
p-**2.9**

m-**2.13-2.16**
p-**2.13-2.16**

Scheme 2-12. Synthesis of small benzamide library (**2.13** R' = *n*-Pr, **2.14** R' = *i*-Pr, **2.15** R' = *n*-Bu, **2.16** R' = *t*-Bu) (R = $CH_2CH_2(CF_2)_5CF_3$)

2.6 Conclusions

A new strategy for preparing and purifying radiotracers was developed. HPLC and ICP-MS data indicated that the desired products could be isolated free

from the excess starting materials used in the labeling reactions. Furthermore, this method was applied to the preparation of libraries of radiolabelling precursors and [^{125}I]-iodobenzamides; an approach which could be used in HTS in conjunction with novel phases of imaging probes. The results supported further development of the fluorous labeling strategy (FLS).

2.7 Experimental

Materials and Instrumentation

Analytical TLC was performed on silica gel 60-F_{254} (Merck) plates. NMR spectroscopy was performed on a Bruker Avance AV200 spectrometer at ambient temperature. The chemical shifts (δ) for ^{1}H and ^{13}C were recorded relative to residual non-deuterated solvent peaks as internal standards. Signals for C-F carbon atoms are enclosed in square brackets. Electrospray ionization (ESI) mass spectrometry experiments were performed on a Micromass Quattro Ultima instrument where samples were dissolved in either dichloromethane or methanol. High resolution MS spectra were obtained by FAB MS on a Waters-Micromass Q-TOF Ultima Global instrument. IR spectra were run on a Biorad FTS-40 FTIR spectrometer. HPLC experiments were performed on a Varian Prostar Model 230 instrument, fitted with a Varian Pro Star model 330 PDA detector and an IN/US γ-RAM gamma detector. The wavelength for detection was set at λ = 254 nm and the dwell time in the gamma detector was 5 s in a 10 μL loop. All runs were performed using a Zorbax (250 mm × 4.6 mm) SB-C18 analytical column (300 Å-5 μm, RP-C18). The elution conditions were as follows:

Method A: Solvent A = MeOH, Solvent B=H_2O containing 0.1% HOAc: Elution Conditions: 60% A 0-2 min; 60%A to 100% A 2-15 min; 100% A 15-25 min. **Method B:** Solvent A = MeOH, Solvent B = H_2O containing 0.9% TRIS, pH 2.6 (H_3PO_4): Elution Conditions: 60% A 0-2 min; 60% A to 100% A 2-15 min; 100% A 15-25 min. For both methods the flow rate was set to 1 mL/min.

X-ray crystallographic data was collected from a single crystal mounted on a glass fiber. Data was collected using a P4 Bruker diffractometer, equipped with a Bruker SMART 1K charged coupled device (CCD) area detector, using the SMART[30] program, and a rotating anode, using graphite monochromated Mo-Kα radiation (0.71073 Å). The crystal-to-detector distance was 4.987 cm, and the data collection was carried out in 512 x 512 pixel mode, utilizing 2 x 2 pixel binning. The initial unit cell parameters were determined by a least-squares fit of the angular settings of the strong reflections, collected by a 12° scan in 40 frames over three different sections of reciprocal space (160 frames total). Almost a complete sphere of data was collected at 103 K. Data were truncated using either the XPREP program or SHELXTL since the data were too weak to refine to less than 0.8 Å. Upon completion of the data collection, the first 40 frames were recollected in order to improve the decay correction analysis. Data reduction was carried out using the program SAINT,[31] which applied Lorentz and polarization corrections to the three-dimensionally integrated diffraction spots. The program SADABS,[32] was utilized for the scaling of diffraction data, the application of a decay correction, and an empirical absorption correction based on redundant reflections, for all data sets. The XPREP[32] program was used to

confirm the original cells obtained and determine that the crystal lattice was of the correct space group. The solution was initiated by first locating the tin atoms using the Patterson method. Subsequent atoms were located using direct methods, by first locating the strongest atoms, followed by a least-squares refinement of all atomic positions and isotropic thermal parameters to locate any remaining. The final refinement was obtained by refining all the atoms by full-matrix least squares methods on F^2 with anisotropic thermal parameters for all non-hydrogen atoms in all cases, giving rise to the residual R_1 values found herein. For compound *m*-**2.1**, thermal restraints were placed on some carbon atoms as well as the tin atom in molecule 1 in order to refine them anisotropically. The crystal was a weak diffractor. The data intensity statistics were reviewed in XPREP (SHELXTL software package) and were found to exhibit weak data ($I/\sigma < 1$) below 0.800 Å ($\theta = 24^\circ$). The data beyond $2\theta = 48^\circ$ were subsequently removed, resulting in an overall improvement of the residual agreement factor for the model given. Hydrogen atoms were added as fixed contributors at calculated positions with isotropic thermal parameters, based on the atom to which they were bound. Potential disorder in the perfluorocarbon chains was also noted by the location of the remaining peaks in the difference map. However, upon modeling no significant degree of partial occupancy was observed from analysis of the least squares refinement of the thermal parameters about the atoms in question.

Caution: ^{125}I is radioactive and should only be handled in a facility appropriately

equipped and licensed to handle radioactive materials.

2.7.1 Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl) ethylbenzoate (*m*-2.4)

Tris(2-perfluorohexylethyl)stannyl-bromide (3.00 g, 2.42 mmol) was dissolved in THF (10 mL) under an inert atmosphere and the reaction mixture cooled in an ice bath. A solution of 3-(ethoxycarbonyl)phenylzinc iodide in THF (0.50 M, 14.6 mL, 7.30 mmol) was added dropwise and the mixture was allowed to warm to room temperature, with stirring, overnight. The solvent was removed by rotary evaporation, and the residue dissolved in FC-72® (10 mL) and methanol (20 mL). The fluorous phase was collected and the reaction solvent removed by rotary evaporation to give a colorless oil. Yield: 2.19 g, 69%. TLC (1:10 EtOAc:Hex): R_f = 0.20. HPLC (Method A): R_t = 21.7 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.04 (m, 2H, Ar-*H*), 7.50 (m, 2H, Ar-*H*), 4.39 (q, *J* = 7.0 Hz, 2H OCH_2CH_3), 2.34 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.39 (m, OCH_2CH_3), 1.36 (m, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.4, 140.1, 136.7, 130.7, 130.6, 128.7, 61.2, 27.6, 14.1, -1.3. HRMS(-): mass calcd for $C_{33}H_{21}F_{39}O_2Sn$ = 1369.0082. Found: 1368.9972. FTIR (KBr, cm^{-1}): 2949, 1714.

2.7.2 Synthesis of 4-(*tris*[2-perfluorohexylethyl]stannyl) ethylbenzoate (*p*-2.4)

Synthesized in an analogous fashion to *m*-**2.4**, using 4-(ethoxycarbonyl)phenylzinc iodide in THF in place of 3-(ethoxycarbonyl)phenylzinc iodide. Yield: 2.53 g, 80 %. TLC (1:10 EtOAc:Hex): R_f = 0.19. HPLC (Method A): R_t = 22.2 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.48 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 4.39 (q, *J* = 7.0 Hz, 2H, OCH_2CH_3), 2.32 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.40 (m, OCH_2CH_3), 1.36 (m, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.0, 142.7, 135.5, 131.0, 129.0, [117.8, 115.9, 110.5, 109.7,] 60.7, 27.1, 13. 8, -1.8. HRMS(-): mass calcd for $C_{33}H_{21}F_{39}O_2Sn$ = 1369.0082. Found: 1369.0045. FTIR (KBr, cm^{-1}): 3003, 2943, 1716.

2.7.3. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl) benzoic acid (*m*-2.1)

Method A- conventional heating: Compound *m*-**2.5** (2.53 g, 1.93 mmol) was added to a mixture of NaOH (1M, 10 mL) and MeOH (10 mL) and the mixture heated at 80°C for 24 h. The solvent was removed by rotary evaporation, and the residue extracted three times with a mixture of FC-72® (5 mL) and CH_2Cl_2 (15 mL). The fluorous layers were combined and the solvent removed by rotary evaporation yielding a sticky white solid. This solid was then loaded onto a silica column (2.5 x 10 cm), and purified running 0-25 % MeOH *vs* CH_2Cl_2. Single

crystals were obtained by slow evaporation of a hexanes solution. Yield: 2.01 g, 81 %. TLC (1:50 MeOH:CH_2Cl_2): R_f = 0.70. HPLC (Method A): R_t = 20.1 min. ^{1}H NMR (200.23 MHz, CD_3OD) δ: 8.16 (s, 1H, Ar-*H*), 8.02 (m, 1H, Ar-*H*), 7.71 (m, 1H, Ar-*H*), 7.50 (m, 1H, Ar-*H*), 2.42 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.38 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, CD_3OD) δ: 140.3, 137.5, 131.3, 128.8, [118.3, 105.7,] 27.5, 5.9, -1.4. HRMS(-): mass calcd for $C_{31}H_{17}F_{39}O_2Sn$ = 1280.9556. Found: 1280.9523. FTIR (KBr, cm^{-1}): 2944, 1634.

Method B- microwave heating: Same as above, except compound *m*-**2.4** (2.53 g, 1.98 mmol) was added to a mixture of NaOH (or KOH) (1M, 2 mL) and 95 % EtOH (2 mL) in a sealed Emry vial (5 mL) containing a magnetic stir bar. The mixture was then heated at 160°C for 5 min in a microwave reactor; the product was isolated as described above. Yield: 2.10 g, 83 %.

2.7.4. Synthesis of 4-(*tris*[2-perfluorohexylethyl]stannyl) benzoic acid (*p*-2.1)

Compound was prepared following the same procedures described for *m*-**2.1**. (2.57 g, 2.01 mmol of p-**2.4** was used). Yield: 2.12 g, 83 %. TLC (1:50 MeOH:CH_2Cl_2): R_f = 0.70. HPLC (Method A): R_t = 20.6 min. ^{1}H NMR (200.23 MHz, CD_3OD) δ: 8.01 (d, *J* = 8.0 Hz. 2H, Ar-*H*), 7.60 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 2.41 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.34 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, CD_3OD) δ: 170.6, 145.7, 136.9, 133.1, 130.2, [120.6, 115.7, 112.5, 110.3],

28.7, -0.4. HRMS(-): mass calcd for $C_{31}H_{17}F_{39}O_2Sn$ = 1280.9556. Found: 1280.9548. FTIR (KBr, cm^{-1}): 2956, 1703.

2.7.5. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl)benzoate tetrafluorophenyl ester (*m*-2.9)

A solution of 2,3,5,6-tetrafluorophenol (162 mg, 975 μmol) and EDC (187 mg, 975 μmol) in EtOAc (1 mL) containing H_2O (10 μL) (or neat with CH_2Cl_2) was added to a suspension of *m*-**2.1** (250 mg, 195 μmol) in EtOAc (or CH_2Cl_2) (15 mL). The mixture was stirred at room temperature for 16 h before the solvent was removed by rotary evaporation. The resulting residue was dissolved in $CHCl_3$ (50 mL), extracted with 10 % $NaHCO_3$ (3 x 25 mL) and the organic layer dried over anhydrous Na_2SO_4 and the solvent removed. The resulting residue was purified using a SP1 Biotage purification system using a gradient of hexanes and EtOAc. Yield: 65 mg, 23 %. TLC (1:20 EtOAc:Hex): R_f = 0.44. HPLC (Method A): R_t= 22.2 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.22 (m, 2H, Ar-*H*), 7.65 (m, 2H, Ar-*H*), 7.06 (m, 1H, C_6HF_4), 2.35 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.38 (t, *J* = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 162.5, 141.8, 137.7, 134.6, 130.7, 128.8, 127.5, 103.3, 68.2, 27.5, -1.2. HRMS(+): mass calcd for $C_{37}H_{17}F_{43}O_2Sn$ = 1556.8617, found, 1556.8702. FTIR (KBr, cm^{-1}): 3303, 2976, 1762.

2.7.6. Synthesis of 4-(*tris*[2-perfluorohexylethyl]stannyl)benzoate tetrafluorophenyl ester (*p*-2.9)

Prepared following the same protocol as for *m*-**2.9**. Yield: 53 mg, 51 %. TLC (1:20 EtOAc:Hex): R_f = 0.44. HPLC (Method A): R_t = 22.4 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.21 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 7.61 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 7.05 (m, 1H, C_6HF_4), 2.35 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.38 (t, *J* = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 162.5, 148.3, 136.3, 130.2, 129.7, 128.1, 103.4, 27.5, -1.2. HRMS(-): mass calcd for $C_{37}H_{17}F_{43}O_2Sn$ = 1488.9706. Found: 1488.9664. FTIR (KBr, cm^{-1}): 3096, 2945, 1763.

2.7.7. General procedure for the synthesis of *tris*(2-perfluorohexylethyl)stannyl benzamides

Compound *m*-**2.9** or *p*-**2.9** (50 mg; 35 μmol) was dissolved in DMF (1.5 mL). An excess of amine was added (105 μmol) and the reaction mixture stirred at ambient temperature for 2 h. Perfluorinated hexanes was added to the reaction vessel (5 mL) and the reaction mixture was extracted with H_2O (3 x 5 mL). Evaporation of the fluorous layer afforded the products as clear oils.

*2.7.7.1. Synthesis of 4-N,N-dimethylethyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.6**)*

Yield: 43 mg, 90 %. TLC (1:9 MeOH:CH_2Cl_2): R_f = 0.30. HPLC (Method B): R_t = 19.0 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.82 (d, *J* = 7.0 Hz, 2H, Ar-*H*), 7.46 (d,

J = 7.2 Hz, 2H, Ar-*H*), 6.90 (s, 1H, N*H*$CH_2CH_2N(CH_3)_2$), 3.53 (m, 2H, NHC*H*$_2CH_2N(CH_3)_2$), 2.53 (t, *J* = 5.6 Hz, 2H, $NHCH_2CH_2N(CH_3)_2$), 2.30 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 2.28 (s, 6H, $NHCH_2CH_2N(CH_3)_2$), 1.32 (t, *J* = 8.2 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.1, 141.0, 136.0, 127.0, 57.7, 45.1, 37.2, 27.6, -1.3. HRMS(+): mass calcd for $C_{35}H_{27}F_{39}N_2SnO$ = 1353.0601. Found: 1353.0634. FTIR (KBr, cm^{-1}): 3341, 2957, 2865, 2833, 2771, 1654.

*2.7.7.2. Synthesis of 4-N,N-diethylethyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.7**).*

Yield: 39 mg, 80 %. TLC (1:9 MeOH:CH_2Cl_2): R_f = 0.42. HPLC (Method B): R_t = 18.9 min. ^1H NMR (200.23 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 7.9 Hz, 2H, Ar-*H*), 7.47 (d, *J* = 7.8 Hz, 2H, Ar-*H*) 6.93 (s, 1H, N*H*$CH_2CH_2N(CH_2CH_3)_2$), 3.49 (q, *J* = 5.4 Hz, 2H, NHC*H*$_2CH_2N(CH_2CH_3)_2$), 2.60 (m, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$), 2.31 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.33 (m, 6H, $SnCH_2CH_2C_6F_{13}$) 1.04 (t, *J* = 7.1 Hz, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.0, 141.1, 136.2, 127.1, [114.5, 110.4,] 51.4, 46.9, 37.4, 27.8, 12.2, -1.1. HRMS(+): mass calcd for $C_{37}H_{31}F_{39}N_2OSn$ $[M+H]^+$ = 1381.0922. Found: 1381.0903. FTIR (KBr, cm^{-1}): 3376, 2981, 2828, 1658.

*2.7.7.3. Synthesis of 3-n-propyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (m-**2.13**)*

Yield: 41 mg, 88 %. TLC (2:5 EtOAc:Hex): R_f = 0.53. HPLC (Method A): R_t = 20.5 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.84 (s, 1H, Ar-*H*), 7.70 (m, 1H, Ar-*H*), 7.49 (m, 2H, Ar-*H*), 6.14 (s, 1H, N*H*$CH_2CH_2CH_3$), 3.43 (q, *J* = 6.3 Hz, 2H, NHC*H*$_2CH_2CH_3$), 2.32 (m, 6H, Sn-CH_2C*H*$_2C_6F_{13}$), 1.63 (m, 2H, NHCH_2C*H*$_2CH_3$), 1.34 (t, *J* = 8.4 Hz, 6H Sn-C*H*$_2CH_2C_6F_{13}$), 0.99 (t, *J* = 7.4 Hz, 3H, NH-CH_2CH_2C*H*$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.5, 138.7, 137.7, 135.1, 134.5, 128.7, 127.4, 41.8, 27.5, 22.9, 11.3, -1.3. HRMS(+): mass calcd for $C_{34}H_{24}F_{39}NOSn$ = 1324.0342. Found: 1324.0364. FTIR (KBr, cm^{-1}): 3307, 2966, 2935, 1634.

*2.7.7.4. Synthesis of 4-n-propyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.13**).*

Yield: 38 mg, 83 %. TLC (2:5 EtOAc:Hex): R_f = 0.53. HPLC: R_t = 20.8 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 8.1 Hz, 2H, Ar-*H*), 7.47 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 6.10 (s, 1H, N*H*$CH_2CH_2CH_3$), 3.44 (q, *J* = 6.7 Hz, 2H, NHC*H*$_2CH_2CH_3$), 2.31 (m, 6H, Sn-CH_2C*H*$_2C_6F_{13}$), 1.65 (m, 2H, NHC*H*$_2CH_2CH_3$) 1.33 (t, *J* = 8.0 Hz, 6H, Sn-C*H*$_2CH_2C_6F_{13}$), 1.00 (t, *J* = 7.5 Hz, 3H, NHCH_2CH_2C*H*$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 141.1, 136.1, 126.9, 41.8, 27.6, 22.9, 11.4, -1.3. HRMS(+): mass calcd for $C_{34}H_{24}F_{39}NOSn$ = 1324.0342. Found: 1324.0303. FTIR (KBr, cm^{-1}): 3309, 2971, 2883, 1636.

*2.7.7.5. Synthesis of 3-n-butyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (m-**2.14**).*

Yield: 40 mg, 86 %. TLC (3:10 EtOAc:Hex): R_f = 0.28. HPLC (Method A): R_t = 20.6 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.83 (s, 1H, Ar-*H*), 7.70 (m, 1H, Ar-*H*), 7.47 (m, 2H, Ar-*H*), 6.04 (s, 1H, N*H*$CH_2CH_2CH_2CH_3$), 3.47 (q, *J* = 6.8 Hz, 2H, NHC*H*$_2CH_2CH_2CH_3$), 2.31 (m, 6H, Sn-$CH_2CH_2C_6F_{13}$), 1.61 (m, 4H, $NHCH_2CH_2CH_2CH_3$), 1.33 (t, *J* = 8.0 Hz, 6H, $SnCH_2CH_2C_6F_{13}$), 0.96 (t, *J* = 7.2 Hz, 3H, $NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.4, 138.7, 134.5, 128.8, 127.3, 39.9, 31.7, 27.6, 20.1, 13.7, -1.3. HRMS(+): mass calcd for $C_{35}H_{26}F_{39}NOSn$ = 1338.0499. Found: 1338.0479. FTIR (KBr, cm^{-1}): 3308, 2970, 2934, 2877, 1633.

*2.7.7.6. Synthesis of 4-n-butyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.14**).*

Yield: 37 mg, 80 %. TLC (2:5, EtOAc:Hex): R_f = 0.38. HPLC (Method A): R_t = 20.9 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.46 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 6.09 (s, 1H, N*H*$CH_2CH_2CH_2CH_3$), 3.47 (q, *J* = 6.7 Hz, 2H, NHC*H*$_2CH_2CH_2CH_3$), 2.31 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.52 (m, $NHCH_2CH_2CH_2CH_3$), 1.33 (t, *J* = 8.0 Hz, $SnCH_2CH_2C_6F_{13}$), 0.96 (t, *J* = 7.1 Hz, 3H, $NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.1, 141.1, 136.1, 126.8, 39.9, 31.7, 27.6, 20.1, 13.8, -1.3. HRMS(+): mass calcd for $C_{35}H_{26}F_{39}NOSn$ = 1338.0499. Found: 1338.0454. FTIR (KBr, cm^{-1}): 3310, 3084, 2974, 2943, 2874, 1636.

*2.7.7.7. Synthesis of 3-iso-propyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (m-**2.15**).*

Yield: 42 mg, 90 %. TLC (2:5 EtOAc:Hex): R_f = 0.50. HPLC (Method A): R_t = 20.2 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.82 (s, 1H, Ar-H), 7.70 (m, 1H, Ar-H) 7.46 (m, 2H, Ar-H), 5.86 (s, 1H, $NHCH(CH_3)_2$), 4.28 (m, 1H, $NHCH(CH_3)_2$), 2.33 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.33 (t, *J* = 8.0 Hz, 6H, $SnCH_2CH_2C_6F_{13}$), 1.26 (d, *J* = 6.6 Hz, $NHCH(CH_3)_2$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.6, 138.7, 137.6, 135.2, 134.5, 128.7, 127.4, [118.3, 114.3, 110.8], 42.0, 27.5, 22.7, -1.3. HRMS(+): mass calcd for $C_{34}H_{24}F_{39}NOSn$ = 1324.0342. Found: 1324.0389. FTIR (KBr, cm^{-1}): 3303, 2979, 2949, 1633.

*2.7.7.8. Synthesis of 4-iso-propyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.15**).*

Yield: 41 mg, 88 %. TLC (2:5 EtOAc:Hex): R_f = 0.47. HPLC (Method A): R_t = 20.3 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 8.0 Hz, 2H, Ar-*H*), 7.46 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 5.88 (s, 1H, N*H*), 4.30 (m, 1H, $NHCH(CH_3)_2$), 2.31 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.33 (t, *J* = 8.0 Hz, $SnCH_2CH_2C_6F_{13}$), 1.27 (d, *J* = 6.5 Hz, $NHCH(CH_3)_2$); ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.3, 141.0, 136.1, 126.8, 42.0, 27.5, 22.8, -1.3. HRMS(+): mass calcd for $C_{34}H_{24}F_{39}NOSn$ = 1324.0342. Found: 1324.0382. FTIR (KBr, cm^{-1}): 3323, 2975, 1652.

*2.7.7.9. Synthesis of 3-tert-butyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (m-**2.16**).*

Yield: 37 mg, 79 %. TLC (2:5 EtOAc:Hex): R_f = 0.58. HPLC (Method A): R_t = 20.6 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.80 (s, 1H, Ar-*H*), 7.64 (m, 1H, Ar-*H*), 7.45 (m, 2H, Ar-*H*), 5.90 (s, 1H, N*H*C$(CH_3)_3$), 2.30 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.50 (s, 9H, NHC$(CH_3)_3$), 1.32 (t, *J* = 8.4 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.8, 138.5, 137.6, 136.1, 134.4, 128.7, 127.3, [111.1,] 51.8, 28.8, 27.5, -1.3. HRMS(+): mass calcd for $C_{35}H_{26}F_{39}NOSn$ = 1338.0499. Found: 1338.0461. FTIR (KBr, cm^{-1}): 3331, 2979, 2938, 1644.

*2.7.7.10. Synthesis of 4-tert-butyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (p-**2.16**).*

Yield: 38 mg, 81 %. TLC (2:5 EtOAc:Hex): R_f = 0.56. HPLC (Method A): R_t = 20.4 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 7.8 Hz, 2H, Ar-*H*), 7.44 (d, *J* = 7.8 Hz, 2H, Ar-*H*), 5.93 (s, 1H, N*H*C$(CH_3)_3$), 2.31 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.48 (s, 9H, NHC$(CH_3)_3$), 1.32 (t, *J* = 8.6 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 136.0, 126.8, 51.7, 28.8, 27.1, -1.3. HRMS(+): mass calcd for $C_{35}H_{26}F_{39}NOSn$ = 1338.0499. Found: 1338.0505. FTIR (KBr, cm^{-1}): 3452, 3330 2973, 2928, 2868, 1652.

*2.7.7.11. Synthesis of 3-N,N-diethylethyl-(tris[2-perfluorohexylethyl]stannyl) benzamide (m-**2.7**).*

Yield: 37 mg, 77 %. TLC (1:9 MeOH:CH_2Cl_2): R_f = 0.43. HPLC (Method B): R_t = 19.6 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.87 (s, 1H, Ar-*H*), 7.74 (m, 1H, Ar-*H*), 7.48 (m, 2H, Ar-*H*), 7.02 (s, 1H, N*H*$CH_2CH_2N(CH_2CH_3)_2$), 3.50 (q, *J* = 5.4 Hz, 2H, NHC*H*$_2$$CH_2N(CH_2CH_3)_2$) 2.42 (m, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$), 2.31 (m, 6H, Sn-$CH_2CH_2C_6F_{13}$), 1.33 (t, *J* = 8.6 Hz, 6H, Sn-C*H*$_2$$CH_2C_6F_{13}$), 1.03 (t, *J* = 7.08 Hz, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 167.1, 138.6, 137.5, 135.1, 134.5, 128.8, 127.5, 51.3, 46.7, 37.3, 27.6, 11.9, -1.3. HRMS(+): mass calcd for $C_{37}H_{31}F_{39}N_2OSn$ = 1381.0922. Found: 1381.0953. FTIR (KBr, cm^{-1}): 3333, 2976, 2938, 2817, 1647.

2.7.8. Synthesis of 3-iodobenzoate tetrafluorophenyl ester (*m*-2.10)

3-Iodobenozic acid (400 mg, 1.60 mmol) was suspended in EtOAc (or CH_2Cl_2) (15 mL). To this was added 2,3,5,6-tetrafluorophenol (668 mg, 4.1 mmol) and EDC (768 mg, 4.1 mmol) in EtOAc (1 mL) containing H_2O (10 μL) (or CH_2Cl_2). The mixture stirred at ambient temperature overnight, after which the solvent was removed by rotary evaporation. The resulting residue was dissolved in $CHCl_3$ (25 mL), extracted with 10% sodium bicarbonate (3 x 15 mL) and the organic layer dried over anhydrous Na_2SO_4. The mixture was purified by automated silica gel chromatography using a 2-5 % gradient of EtOAc in hexanes. Yield: 490 mg, 78 %. TLC (1:20 EtOAc:Hex): R_f = 0.48. HPLC (Method A): R_t = 18.4 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.54 (s, 1H, Ar-*H*), 8.18 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 8.03 (d,

J = 8.0 Hz, 1H, Ar-*H*), 7.30 (t, *J* = 7.8 Hz, 1H, Ar-*H*), 7.06 (m, 1H, C_6HF_4). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 161.2, 148.6, 143.4, 139.4, 138.4, 130.5, 129.8, 129.0, 103.5, 94.0. ESMS(+): 376.4 $[M\text{-}HF]^+$. FTIR (KBr, cm^{-1}): 3091, 2968, 2933, 1764.

2.7.9. Synthesis of 4-iodobenzoate tetrafluorophenyl ester (*p*-2.10)

Prepared following the same protocol as for *m*-**2.10** using 4-iodobenzoic acid (400 mg, 1.60 mmol), tetrafluorophenol (666 mg, 4.1 mmol) and EDC (773 mg, 4.1 mmol). Yield: 500 mg, 80 %. TLC (1:20 EtOAc:Hex): R_f = 0.48. HPLC (Method A): R_t = 18.6 min. ^1H NMR (200.23 MHz, $CDCl_3$) δ: 7.92 (s 4H, Ar-*H*), 7.05 (m, 1H, C_6HF_4). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 162.3, 138.3, 131.9, 126.6, 103.5. ESMS(+): 376.4 $[M\text{-}HF]^+$. FTIR (KBr, cm^{-1}): 3095, 2966, 2925, 1764, 1526, 1232.

2.7.10. General procedure for the synthesis of iodobenzamides

A stock solution containing either compound *m*-**2.10** or *p*-**2.10** was prepared using CH_3CN (45 mg / mL) and aliquoted into vials (1.5 mL, 67 mg, 170 μmol). An excess of amine (1 mmol) was added and the reaction mixture stirred at ambient temperature for 2 h. The reaction solvent was removed by rotary evaporation. Liquid-liquid extraction from CH_2Cl_2 (5 mL) and H_2O (3 x 5 mL) was carried out on the sample. Organic fractions were combined, dried with $NaSO_4$ and concentrated, affording the products as clear oils.

*2.7.10.1 Synthesis of 4-N,N-dimethylethyl iodobenzamide (p-**2.11**)*

Yield: 22 mg, 40 %. TLC (3:20 MeOH: CH_2Cl_2): R_f = 0.28. HPLC (Method B): R_t = 8.5 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 8.2 Hz, 1H, Ar-*H*), 7.45 (d, *J* = 8.2 Hz, 1H, Ar-*H*), 6.80 (s, 1H, N*H*$CH_2CH_2N(CH_3)_2$), 3.43 (q, *J* = 5.6 Hz, 2H, NHC*H*$_2CH_2N(CH_3)_2$), 2.44 (t, ^3J = 6.0 Hz, 2H, $NHCH_2CH_2N(CH_3)_2$), 2.20 (s, 6H, $NHCH_2CH_2N(CH_3)_2$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.6, 137.7, 134.1, 128.7, 98.2, 57.6, 45.2, 37.2. ESMS(+): 319.2. FTIR (KBr, cm^{-1}): 3317, 3063, 2971, 2942, 2770, 1638.

*2.7.10.2. Synthesis of 4-N,N-diethylethyl iodobenzamide (p-**2.12**)*

Yield: 24 mg, 40 %; TLC (3:20 MeOH:CH_2Cl_2): R_f = 0.28; HPLC (Method B): R_t = 8.5 min. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.51 (m, *J* = 8.4 Hz, 2H, Ar-*H*), 7.07 (s, 1H, N*H*$CH_2CH_2N(CH_3)_2$), 3.48 (q, *J* = 5.3 Hz, 2H, NHC*H*$_2CH_2N(CH_2CH_3)_2$), 2.67 (t, *J* = 5.9 Hz, 2H, $NHCH_2CH_2N(CH_2CH_3)_2$), 2.59 (q, *J* = 7.1 Hz, 4H, $NHCH_2CH_2N(CH_2CH_3)_2$), 1.04 (t, *J* = 7.1 Hz, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ: 166.6, 137.9, 134.3, 128.7, 98.3, 51.4, 46.9, 37.3, 12.0. ESMS(+): 347.3. FTIR (KBr, cm^{-1}): 3291, 2967, 2930, 2812, 1638.

*2.7.10.3. Synthesis of 3-n-propyl-iodobenzamide (m-**2.17**)*

Yield: 43 mg, 88 %. TLC (1:30 MeOH:CH_2Cl_2): R_f = 0.77. HPLC (Method A): R_t = 13.7 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.06 (m, 1H, Ar-*H*), 7.81 (d, *J* = 8.0

Hz, 1H, Ar-*H*), 7.71 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 7.16 (t, *J* = 8.0 Hz, 1H, Ar-*H*), 6.18 (s, 1H, N*H*$CH_2CH_2CH_3$), 3.41 (q, *J* = 7.0 Hz, 2H, NHC*H*$_2CH_2CH_3$), 1.62 (m, 2H, NHCH$_2$C*H*$_2CH_3$), 0.98 (t, *J* = 7.4 Hz, 3H, NH-$CH_2CH_2CH_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.1, 140.2, 136.9, 135.9, 130.2, 126.1, 94.3, 41.9, 22.9, 11.6. ESMS(+): 290.1. FTIR (KBr, cm^{-1}): 3242, 3064, 2958, 2874, 1635.

*2.7.10.4. Synthesis of 4-n-propyl-iodobenzamide (p-**2.17**)*

Yield: 44 mg, 90 %. TLC (1:4 EtOAc:Hex): R_f = 0.10. HPLC (Method A): R_t = 13.9 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.77 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.48 (d, *J* = 7.2 Hz, 2H, Ar-*H*), 6.18 (s, 1H, N*H*$CH_2CH_2CH_3$), 3.40 (q, *J* = 6.0 Hz, 2H, NHC*H*$_2CH_2CH_3$), 1.63 (m, 2H, NHCH$_2$C*H*$_2CH_3$), 0.97 (t, *J* = 7.4 Hz, 3H, NHCH$_2$CH$_2$C*H*$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.8, 137.8, 134.2, 128.5, 98.2, 41.9, 22.9, 11.4. ESMS(+): 290.1 $[M+H]^+$. FTIR (KBr, cm^{-1}): 3315, 3072, 2965, 2877, 1633.

*2.7.10.5 Synthesis of 3-n-butyl-iodobenzamide (m-**2.18**)*

Yield: 44 mg, 85 %. TLC (2:5 EtOAc:Hex): R_f = 0.40. HPLC (Method A): R_t = 14.5 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.08 (s, 1H, Ar-*H*), 7.82 (d, *J* = 8.0 Hz, 1H, Ar-*H*), 7.76 (d, *J* = 7.8 Hz, 1H, Ar-*H*) 7.17 (t, *J* = 7.8 Hz, 1H, Ar-*H*), 6.05 (s, 1H, N*H*$CH_2CH_2CH_2CH_3$), 3.45 (q, *J* = 6.0 Hz, 2H, NHC*H*$_2CH_2CH_2CH_3$), 1.60 (m, 2H, NHCH$_2$C*H*$_2$C*H*$_2CH_3$), 1.42 (m, 2H, NHCH$_2$C*H*$_2$C*H*$_2CH_3$), 0.96 (t, *J* = 7.4 Hz, 3H, NHCH$_2$CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.0, 140.2, 136.9, 135.9,

130.2, 126.1, 94.2, 40.0, 31.7, 20.2, 13.8. ESMS(+): 304.1. FTIR (KBr, cm^{-1}): 3304, 3063, 2966, 2935, 2874, 1635.

*2.7.10.6 Synthesis of 4-n-butyl-iodobenzamide (p-**2.18**).*

Yield: 45 mg, 88 %. TLC (2:5, EtOAc:Hex): R_f = 0.40. HPLC (Method A): R_t = 14.9 min. ^1H NMR (200.23 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 7.41 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 6.10 (s, 1H, N*H*$CH_2CH_2CH_2CH_3$), 3.36 (q, *J* = 6.6 Hz, 2H, NHC*H*$_2CH_2CH_2CH_3$), 1.50 (m, NHCH$_2$C*H*$_2$C*H*$_2CH_3$), 1.31 (m, 2H, NHCH$_2$C*H*$_2$C*H*$_2CH_3$), 0.88 (t, *J* = 7.2 Hz, 3H, NHCH$_2CH_2CH_2$C*H*$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.7, 137.7, 134.2, 128.5, 98.2, 39.9, 31.7, 20.1, 13.8. ESMS(+): 304.1. FTIR (KBr, cm^{-1}): 3310, 3073, 2967, 2932, 2866, 1632.

2.7.10.7. Synthesis of 3-iso-propyl-iodobenzamide (*m-***2.19**)

Yield: 40 mg, 82 %. TLC (2:5 EtOAc:Hex): R_f = 0.48. HPLC (Method A): R_t = 13.0 min. ^1H NMR (200.23 MHz, $CDCl_3$) δ: 8.06 (m, 1H, Ar-*H*), 7.74 (m, 2H, Ar-*H*) 7.14 (t, *J* = 7.8 Hz, 1H, Ar-*H*), 5.96 (s, 1H, N*H*CH$(CH_3)_2$), 4.23 (m, 1H, NHC*H*$(CH_3)_2$), 1.25 (d, *J* = 6.4 Hz, 6H, NHCH(C*H*$_3)_2$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 165.2, 140.1, 137.0, 135.9, 130.2, 126.1, 94.2, 42.1, 22.8. ESMS(+): 290.1. FTIR (KBr, cm^{-1}): 3239, 3068, 2976, 1632.

*2.7.10.8. Synthesis of 4-iso-propyl-iodobenzamide (p-**2.19**)*

Yield: 42 mg, 85 %. TLC (2:5 EtOAc:Hex): R_f = 0.45. HPLC (Method A): R_t = 13.3 min. ^1H NMR (200.23 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.47 (d, *J* =

8.4 Hz, 2H, Ar-*H*), 5.91 (s, 1H, N*H*CH(CH_3)$_2$), 4.26 (m, 1H, NHC*H*(CH_3)$_2$), 1.25 (d, *J* = 6.6 Hz, 6H, NHCH(C*H*$_3$)$_2$); ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 165.9, 137.7, 134.4, 128.5, 98.1, 42.1, 22.8. ESMS(+): 290.1. FTIR (KBr, cm^{-1}): 3307, 2973, 2928, 1618.

*2.7.10.9. Synthesis of 3-tert-butyl-iodobenzamide (m-**2.20**)*

Yield: 47 mg, 92 %. TLC (2:5 EtOAc:Hex): R_f = 0.57. HPLC (Method A): R_t = 14.8 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 8.03 (s, 1H, Ar-*H*), 7.70 (m, 2H, Ar-*H*), 7.13 (t, *J* = 7.8 Hz, 1H, Ar-*H*), 5.91 (s, 1H, N*H*C(CH_3)$_3$), 1.46 (s, 9H, NHC(C*H*$_3$)$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 165.3, 139.9, 137.9, 135.8, 130.2, 126.0, 94.2, 51.9, 28.8. ESMS(+): 304.0. FTIR (KBr, cm^{-1}): 3308, 3061, 2968, 2927, 1640.

*2.7.10.10. Synthesis of 4-tert-butyl-iodobenzamide (p-**2.20**)*

Yield: 45 mg, 88 %. TLC (2:5 EtOAc:Hex): R_f = 0.57. HPLC (Method A): R_t = 14.3 min. ^{1}H NMR (200.23 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.36 (d, *J* = 8.2 Hz, 2H, Ar-*H*), 5.85 (s, 1H, N*H*CH_2(CH_3)$_3$), 1.39 (s, 9H, NHC(C*H*$_3$)$_3$). ^{13}C NMR (50.35 MHz, $CDCl_3$) δ: 166.1, 137.6, 135.3, 128.4, 97.8, 51.8, 28.3. ESMS (+): 304.1. FTIR (KBr, cm^{-1}): 3318, 3061, 2971, 2933, 2857, 1634, 1540.

2.7.11. Non-radioactive (cold) iodination of *p*-2.1

Method 1: Iodine (I_2)

To a vial charged with *p*-**2.1** (10 mg, 7.9 μmol), was added 200 μL CH_3CN followed by a solution of I_2 in CH_3CN (2 μL, 4 mmol). The reaction was stirred for 15 minutes and then quenched by the addition of sodium metabisulfite (10 μL, 0.01 M). The crude reaction mixture was diluted with water (2 mL) and loaded onto a conditioned Fluoro*Flash*® SPE cartridge. The reaction vial was subsequently rinsed with an additional 3 mL of water which was added to the F-SPE cartridge. The desired iodinated compound was then eluted using 80% methanol in water and analyzed by HPLC (Method A): R_t = 13.9 min.

Method 2: Sodium iodide (NaI)

An aliquot (100 μL, 230 μmol) of iodogen dissolved in $CHCl_3$ (1 mg / mL) was added to a PTFE vial and the solvent evaporated under reduced pressure. To this vial, a solution of *m*-**2.1** (100 mg, 76 μmol) in a solution of acetic acid in methanol (5 %, 250 μL) and sodium iodide (1 mg, 6 μmol) were added. After 3 min the reaction was quenched by the addition of aqueous sodium metabisulfite ($Na_2S_2O_5$) (100 μL, 231 mM). The reaction mixture was diluted to 2 mL and purified as described above in method 1. HPLC (Method A): R_t = 13.9 min; ESMS (+): 247.1 $[M+H]^+$.

2.7.12. Radiochemistry

General Radiolabelling 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). An aliquot (100 μL) of the fluorous material in 5 % acetic acid in methanol (1 mg/ mL) was added followed by Na[^{125}I]I (5 μL, 20 mCi/mL, pH 10). The reaction was stirred for 3 min and then quenched by the addition of aqueous sodium metabisulfite (10 μL, 44 mg/mL). The reaction mixture was diluted with water (1 mL) and loaded onto a Fluoro*Flash*® SPE cartridge. The reaction vial was subsequently rinsed with an additional 3 mL of water and this was also added to the F-SPE cartridge. The F-SPE cartridge was eluted with water (9 mL), followed by an 80:20 (*v/v*) methanol-water mixture (9 mL).

3-Iodobenzoic acid (*m*-[^{125}I]**2.5**): R_t: 14.2 min (Method A); RCY: 92 %; Radiochemical Purity: >98 %.

4-Iodobenzoic acid (*p*-[^{125}I]**2.5**): R_t: 14.2 min (Method A); RCY: 93 %; Radiochemical Purity: >98 %.

4-N,N-Diethylethyl-iodobenzamide (*p*-[^{125}I]**2.12**): R_t: 9.8 min (Method B); RCY: 79 %; Radiochemical Purity: 95 %.

3-Propyl-iodobenzamide (*m*-[^{125}I]**2.17**): R_t: 13.9 min (Method A); RCY: 86 %; Radiochemical Purity: > 98 %.

4-Propyl-iodobenzamide (*p*-[^{125}I]**2.17**): R_t: 13.9 min (Method A); RCY: 81 %; Radiochemical Purity: > 98 %.

3-Butyl-iodobenzamide (*m*-[^{125}I]**2.18**): R_t: 15.2 min (Method A); RCY: 68 %; Radiochemical Purity: > 98 %.

4-Butyl-iodobenzamide (*p*-[^{125}I]**2.18**): R_t: 14.9 min (Method A); RCY: 76 %;

Radiochemical Purity: > 98 %.

3-Iso-propyl-iodobenzamide (*m*-[^{125}I]**2.19**): R_t: 13.7 min (Method A); RCY: 80 %;

Radiochemical Purity: 95 %.

4-Iso-propyl-iodobenzamide (*p*-[^{125}I]**2.19**): R_t: 13.8 min (Method A); RCY: 75 %;

Radiochemical Purity: 96 %.

3-Tert-butyl-iodobenzamide (*m*-[^{125}I]**2.20**): R_t: 15.1 min (Method A); RCY: 81 %;

Radiochemical Purity: > 98 %.

4-Tert-butyl-iodobenzamide (*p*-[^{125}I]**2.20**): R_t: 14.8 min (Method A); RCY: 82 %;

Radiochemical Purity: >98 %.

2.8. References

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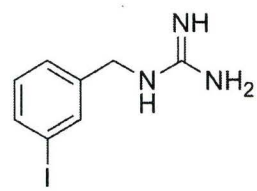
Chapter 3: Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl)benzylguanidine and the production of [$^{123/125}$I]-MIBG

3.1. Introduction: clinical utility and current routes to MIBG

A primary objective in establishing the utility and versatility of the fluorous labelling strategy was to extend the method to compounds that are of immediate clinical relevance and that would benefit from being produced in high effective specific activity. To this end, we sought to develop the means to prepare *meta*-iodobenzylguanidine (MIBG, **3.1,** Figure 3-1) from fluorous precursors.

MIBG is a radiopharmaceutical used for both imaging (^{123}I) and targeted radiotherapy (^{131}I) of neural crest-derived tumours such as pheochromocytoma and neuroblastoma.[1, 2] Cellular uptake mechanisms vary greatly between tumour and non-target tissues; the latter accumulates the pharmaceutical by passive diffusion, while active uptake has been found to be the predominate mechanism in neuroblastoma cells when the MIBG is present at low concentrations (*i.e.* high specific activity).[1] This differential uptake provides the opportunity to administer the

radiotracer at concentrations that will ensure selective uptake, resulting in a drastic improvement in the overall target to non-target ratio and with concomitant improvements both image quality (^{123}I) and therapeutic efficacy (^{131}I).[2] In fact, it is this selective uptake of MIBG in tumours that makes feasible the use of [^{131}I]-MIBG as a therapeutic agent.

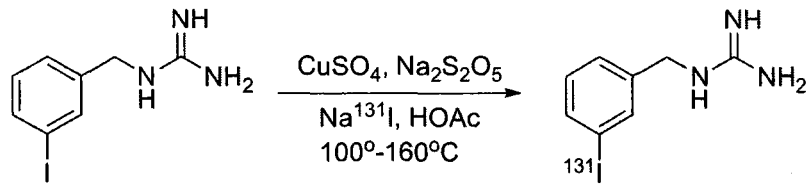


3.1

Figure 3-1. Meta-iodobenzylguandine (MIBG)

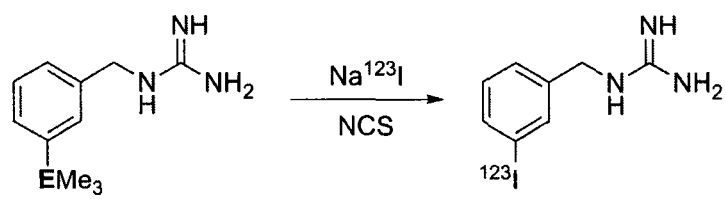
3.1.1 Preparation of radioiodinated MIBG

The most common method for producing therapeutic [^{131}I]MIBG is through an isotopic exchange strategy (Scheme 3-1). The problem inherent to this radiolabelling methodology is that the products are obtained in low specific activity (1-5 mCi/mg).[3] Although effective for diagnostic imaging, the amount of non-radioactive MIBG administered when delivering an effective therapeutic dose (100-200 mCi) is enough to cause undesirable physiological effects including nausea, vomiting and high blood pressure,[2] and perhaps even more importantly, radiation-induced bone marrow suppression.[1]



Scheme 3-1. Synthesis of [^{131}I]MIBG *via* nucleophilic exchange

Several methods for preparing radioiodinated MIBG in high effective specific activity have been investigated. These include the use of radioiododemetallation procedures on silicon- and tin-precursors to MIBG (Scheme 3-2).[3-5] Despite producing the desired radiopharmaceutical in high yield (90 % with silicon precursor), a significant disadvantage of this radiolabelling method is that HPLC purification is required to remove the unreacted starting materials.

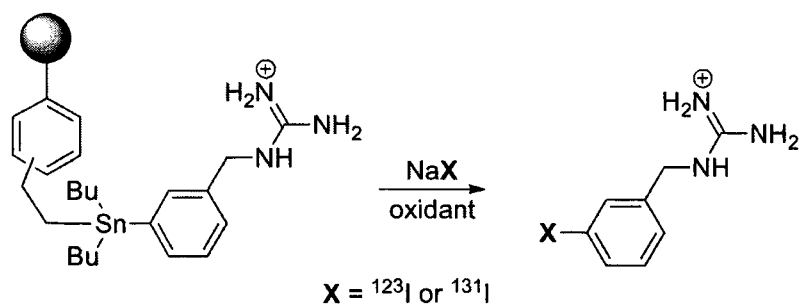


E = Si or Sn

Scheme 3-2. Radioiodemetallation route to [^{123}I]MIBG

To eliminate the need for HPLC purification, an alternative route for preparing MIBG in high effective specific activity has been developed that employs a resin-bound precursor.[6] Upon labelling, the link between the resin and the substrate is cleaved and the desired product is released into solution (Scheme 3-3) as outlined in Section 1.4.4. Radioiodinated MIBG

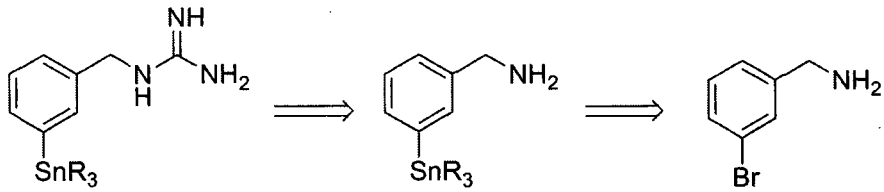
can then be conveniently isolated in very high purity and specific activity by simply filtering the reaction mixture.[5] Unfortunately, as with all reactions performed on insoluble supports, preparing the resin-bound substrates is non-trivial, particularly at the level of purity required for radiopharmaceutical products.[7] Moreover, there is often variability in precursor-loading levels from batch-to-batch which can impart variability in radiochemical yields.[8]



Scheme 3-3. Solid-phase synthesis of radioiodinated MIBG[6, 8]

3.2 Synthetic strategy to produce fluorous-MIBG

Because of its clinical importance and the significant difficulties involved in synthesizing the agent in high effective specific activity, MIBG was chosen as a target to investigate the potential utility of the fluorous labelling strategy. In order to accomplish this, a method to produce a fluorous precursor of MIBG (**3.2**) was developed. The retrosynthesic strategy employed is outlined in Figure 3-2.

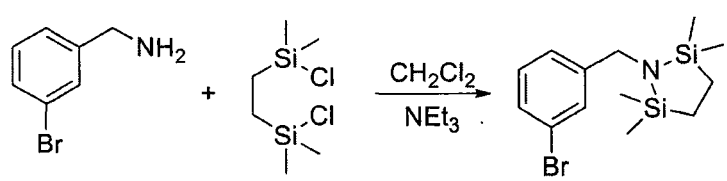


3.2 **3.3**

Figure 3-2. Retrosynthesis of **3.2** (R = $CH_2CH_2(CF_2)_5CF_3$)

3.3. Synthesis of 3-(tris[2-perfluorohexylethyl]stannyl) benzylamine

The first synthetic step towards the target **3.2** was the conversion of 3-bromobenzylamine to 3-(*tris*[2-perfluorohexylethyl]stannyl) benzylamine **3.3** (Scheme 3-4). The preparation began with the protection of the primary amine of 3-bromobenzylamine as a STABASE (2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane) derivative. This particular protecting group was selected since it is stable to strong bases such as LDA and alkyllithium reagents, which are employed in a later step, yet is easily removed under mildly acidic conditions.[9]



3.4

Scheme 3-4. Synthesis of bromobenzylamine-STABASE derivative **3.4**

The preparation of **3.4** was carried out by adding *bis*(chlorodimethylsilyl) ethane to a mixture of 3-bromobenzylamine and triethylamine, dissolved in dichloromethane at 0 °C.[10] Compound **3.4** was prone to degradation by moisture. As a result, measures were taken to minimize exposure to the atmosphere during reaction workup including filtering the reaction mixture through a Schlenk apparatus and conducting all the rotary evaporation steps under an argon atmosphere. The ^{1}H NMR spectrum of the crude product was consistent with the predicted structure of the product (Figure 3-3), with resonances in the aromatic region (multiplet at *ca.* 7.5 ppm) as well as 3 sharp singlets at 4.01, 0.91 and 0.13 ppm which are attributed to the benzylic, methylene and methyl protons respectively in **3.4**. Because of its tendency to react with silica and moisture, the product was used without further purification.

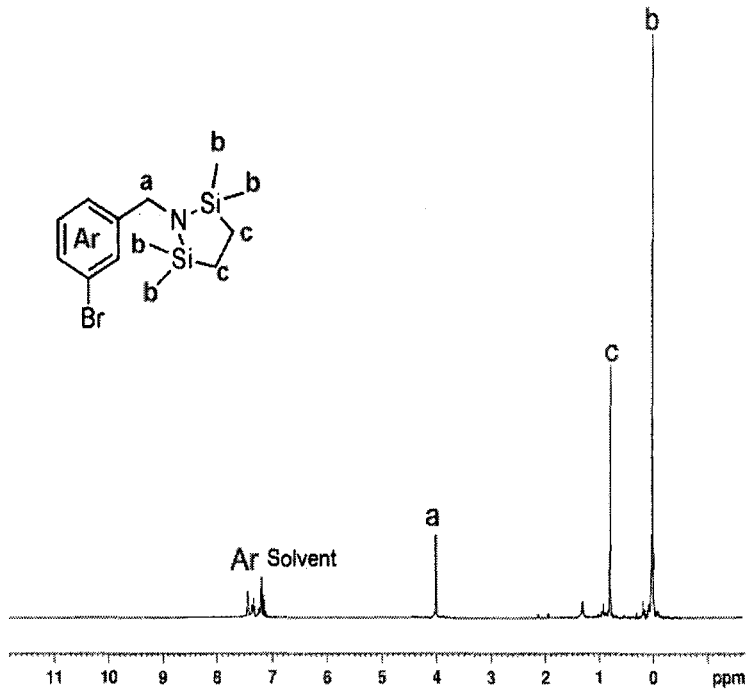
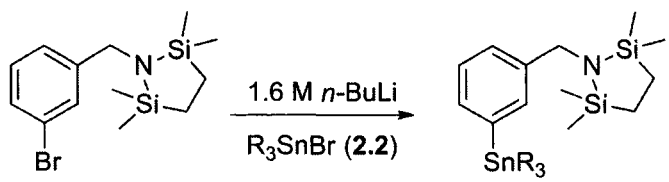


Figure 3-3. ^{1}H NMR spectrum of **3.4** (200.23 MHz, $CDCl_3$)

3.3.1. Investigations into the generation of fluorous benzylamine with alkyllithium reagents

The synthesis of **3.3** was attempted by treating the silyl-protected bromobenzylamine **3.4**, with *n*-butyllithium followed by treatment with **2.2** (Scheme 3-5). Initial efforts were conducted by first heating each reagent (**3.4** and **2.2**) in separate flasks under reduced pressure to remove all residual moisture and solvent. Following this, **3.4** was dissolved in dry THF and cooled to -78 °C prior to the dropwise addition of one equivalent of *n*-butyllithium (1.6 M in hexanes). After stirring for 30 min; a solution of

2.2 in THF was added slowly to the reaction. Reaction workup involved warming the reaction to room temperature, evaporation of the reaction solvents and isolation of the product by liquid-liquid extraction between FC-72® (perfluorinated hexanes) and dichloromethane.



3.4 **3.5**

Scheme 3-5. Synthesis of fluorous benzylamine-STABASE derivative, **3.5** (R = $CH_2CH_2(CF_2)_5CF_3$)

Using this standard procedure, reaction outcomes were highly variable and unpredictable. To demonstrate the varibility, two lithiation experiments carried out in parallel, with reagents aliquoted from the same stock solutions and partitioned between two reaction vessels. Typical ^1H NMR spectra which demonstrate the variability of the reaction outcomes are shown in Figure 3.4. For reaction A, aromatic signals in the product appear around 7.3 ppm and signals attributed to both the benzylic hydrogens in **3.5** appear at *ca.* 4.0 ppm, while those belonging to the fluorous tags appear at 1.2 and 2.3 ppm respectively. In contrast, the ^1H NMR spectrum of reaction B did not portray the characteristic proton signals of the product. The aromatic region is devoid of signals and the fluorous region (2.3 ppm – 1.3 ppm), particularly the protons on the carbon

atom adjacent to the tin atom of the tag (1.3 ppm), which is usually a triplet, has lost much of its symmetry, and appears quite different from those shown from reaction A.

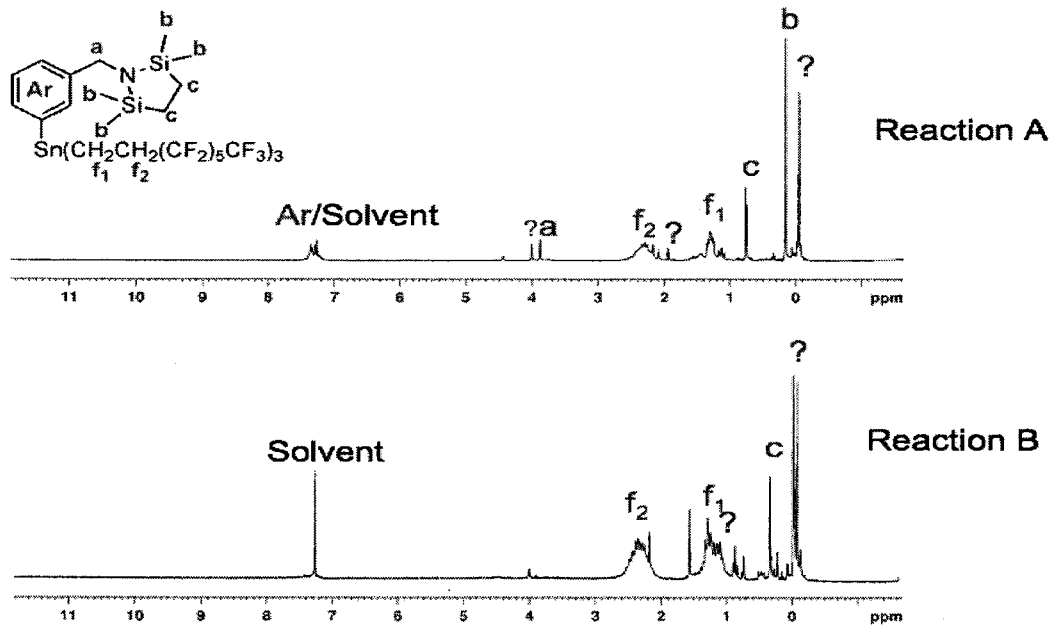
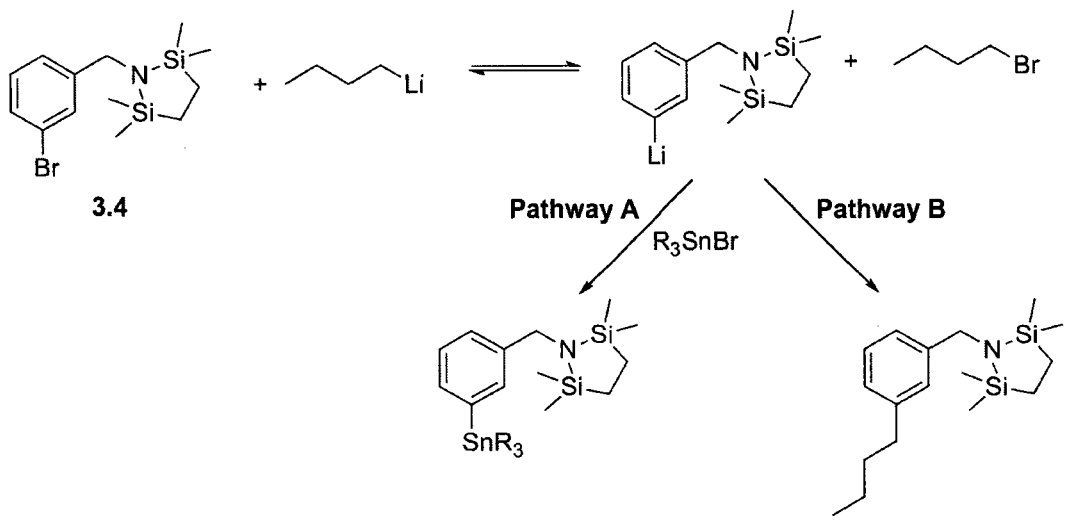


Figure 3-4. ^{1}H NMR spectra of two parallel lithiation reactions conducted simultaneously (200.23 MHz, $CDCl_3$)

Attempts were made to probe the underlying factors that cause the variability and then use that information to ultimately optimize the reaction. First the reactivity of the anion derived from the bromobenzylamine-STABASE derivative was examined by treating **3.4** with *n*-butyllithium under the conditions described above and quenching with D_2O. ^{1}H NMR analysis of the resulting reaction mixture did provide evidence of the deuterated product. Several other factors were investigated including the

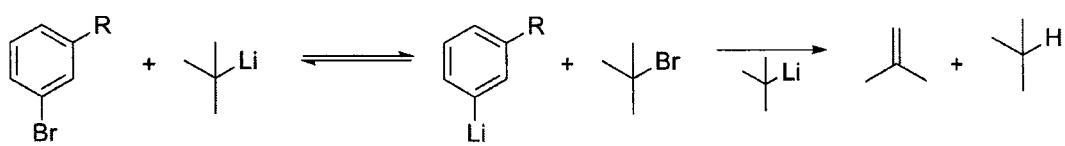
nature of the reaction solvents, the concentrations of reagents, order of addition and stoichiometry, but none were found to further improve the consistency of the reaction or the overall yield.

It has been demonstrated in the literature that halogen-lithium exchange is an equilibrium process which in most cases favors formation of the more stable, less basic organolithium reagent.[11] When considering the reaction products between an aryl halide and *n*-butyllithium, the resonance-stabilized organolithium formed is expected to be favored, and the reverse reaction, attack of ArLi on BuX, is expected to be rather slow (Scheme 3-6, Pathway A). Given the difficulties experienced in preparing the STABASE-protected fluorous benzylamine (**3.5**), it was brought into question whether or not the fluorous tin bromide (**2.2**) was indeed less reactive (electrophilic) when compared with the unhindered alkyl halide. In attempts to generate compound **3.5,** it was postulated that the observed mixture of products was due to secondary reactions of the anion in fact reacting with the alkyl bromide instead of the intended fluorous tin bromide (Scheme 3-6, Pathway B).



Scheme 3-6. Reaction of **3.4** with *n*-BuLi (R = $CH_2CH_2(CF_2)_5CF_3$)

To drive the equilibrium toward the desired anion and to eliminate secondary reactions, the use of *tert*-butyllithium was investigated. This base is well suited to difficult exchange reactions whose equilibria are only moderately favored.[11, 12] By using two equivalents of the alkyllithium, the reaction equilibrium is irreversibly shifted toward the formation of the desired aryllithium, as the first equivalent of base acts to form the lithium salt of the aryl anion and an alkyl halide; the second equivalent of base then reacts with the alkyl halide by-product to form isobutane and isobutene, rendering the reaction irreversible (Scheme 3-7).

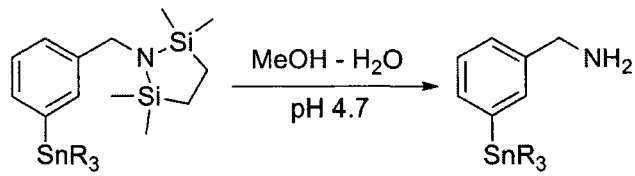


Scheme 3-7. Representative lithiation reaction using two equivalents of *tert*-butyllithium[11]

To generate **3.5**, two equivalents of *tert*-butyllithium were added to an ethereal solution of **3.4** cooled to -98°C. Following this, **2.2** was added and the reaction workup was conducted as previously described. The results of this change were dramatic. Parallel reactions could now be conducted in which the colour of the solutions and yields were similar. It is important to note that the desired product was again used without further purification because of the sensitivity of the protecting group towards silica gel.

3.3.2. Deprotection, isolation and characterization of fluorous benzylamine, 3.3

Following the development of a consistent and reliable method for generating **3.5**, the silyl-protecting group was removed by stirring the protected material in a solution of methanol adjusted to pH 4.7 with HCl for 12 hours (Scheme 3-8). Concentration of the reaction solution followed by extraction with perfluorinated hexanes from dichloromethane afforded a yellow oil which was purified by silica gel chromatography giving compound **3.3** in 35 % overall yield from **2.2**. The ^{1}H NMR spectrum of **3.3** (Figure 3-5) no longer contained signals for the protecting group (0.91 and 0.13 ppm). Other characterization techniques such as ^{13}C NMR spectroscopy and mass spectrometry were consistent with the desired product, while its purity was established as greater than 95 % by HPLC.



3.5 **3.3**

Scheme 3-8. Deprotection of **3.5** to give **3.3** (R = $CH_2CH_2(CF_2)_5CF_3$)

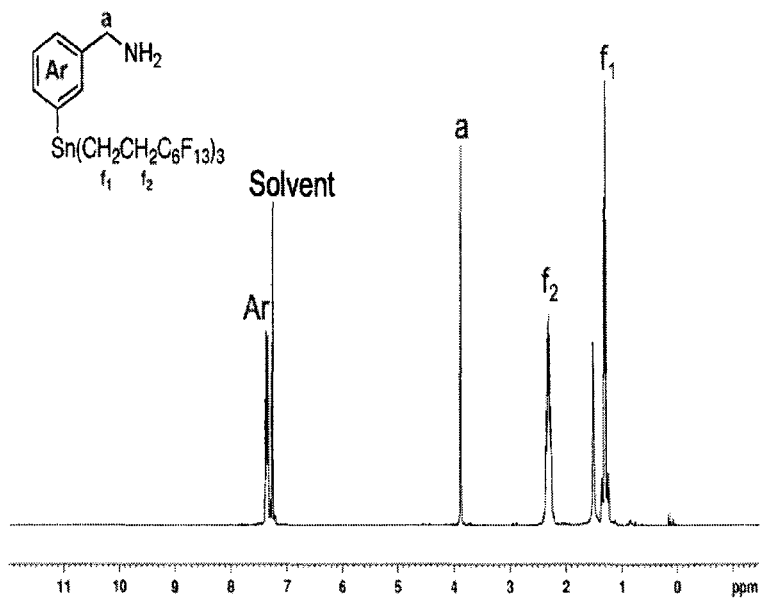


Figure 3-5. ^{1}H NMR spectrum of **3.3** (500.13 MHz, $CDCl_3$)

3.4 Synthesis of 3-(tris[2-perfluorohexylethyl]stannyl) benzylguanidine

3.4.1 Selection of guanylating agent

With the synthesis of **3.3** optimized, the subsequent step was to convert the benzylamine to the guanidine **3.2**. Prior to attempting the

derivatization of **3.3**, a synthetic methodology for the guanylation reaction was carried out using 3-iodobenzylamine (**3.6**) (Figure 3-6), which is commercially available, and far less costly than the fluorous derivatives.

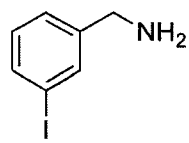


Figure 3-6. 3-Iodobenzylamine, **3.6**

There are several reagents that can promote the conversion of an amine to a guanidine; a selection of these are shown in Figure 3-7.[6, 13-16] Initial experiments were conducted using aminoiminomethane sulfonic acid **3.9** as the guanylation agent, which is reportedly a milder agent than the others which generally require heating; unfortunately, no appreciable amount of **3.1** could be isolated. 1*H*-pyrazole carboxamidine (**3.10**), was subsequently investigated which has been reported to selectively react with primary and secondary aliphatic amines.[16]

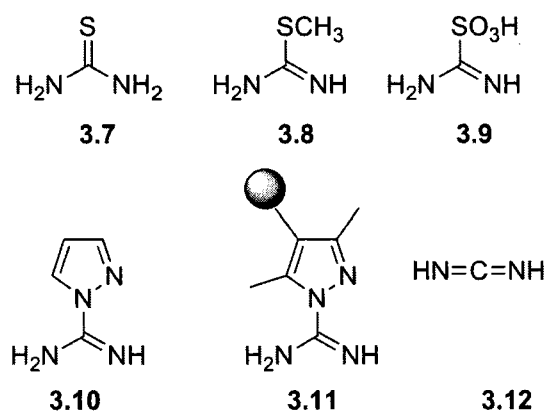
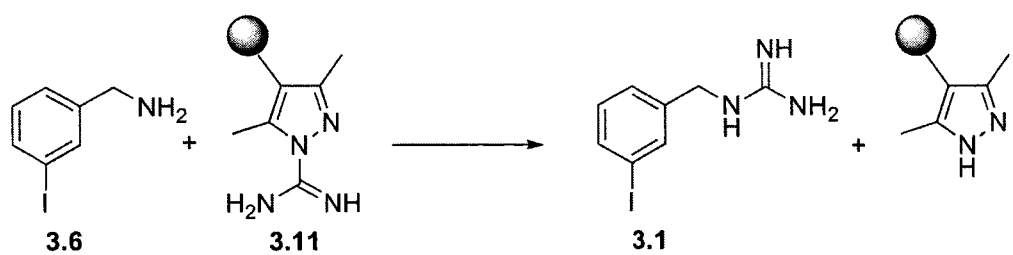


Figure 3-7. Common guanylating agents[6, 13-16]

Guanylation reactions were conducted by suspending 3-iodobenzylamine (**3.6**) hydrochloride in dichloromethane and adding two mole equivalents of compound **3.10** and three equivalents of triethylamine. The progress of the reaction was monitored by TLC using a ninhydrin stain to monitor the disappearance of the amine, and was later quantified by HPLC. After stirring overnight the reaction was determined to be 93 % complete.

To determine if isolation of the polar guanidine could be simplified without sacrificing reaction yields, the use of a polymer-supported carboxamidine reagent was then investigated (Scheme 3-9). Unfortunately, reaction yields did not exceed 50 %. Additional guanylating reagents were investigated including N,N'-bis(tert-butyoxycarbonyl)-1*H*-pyrazole carboxamidine, and Fmoc-protected thioisocyanate but in each case, the yield of **3.1** was less than that with 1*H*-pyrazole carboxamidine, therefore it was this reagent that was used in the preparation of the fluorous precursor **3.2**.



3.6 **3.11** **3.1**

Scheme 3-9. Polymer-supported guanylation reaction

3.4.2 Optimization of 1*H*-pyrazole carboxamidine reaction

The guanylation procedure described in the previous section using *1H*-pyrazole carboxamidine hydrochloride was subsequently translated for use with the fluorous amine (**3.3**). With the intention of monitoring the progress of the reaction by HPLC, a method capable of distinguishing between **3.3** and **3.2** was first developed. Finding suitable elution conditions was non-trivial, since the basic nature of the functional groups caused them to be strongly retained on the HPLC column, where retention in this case is largely dictated by an ion-exchange mechanism.[17-20] In order to overcome the ionic interaction, several amine modifiers were investigated; ultimately, a mobile phase comprised of 90 % methanol containing 10 mM octylamine, adjusted to pH 4.85 using phosphoric acid, was found to be effective at separating compounds **3.3** and **3.2**.

With an analytical HPLC method in hand, experiments directed at determining the length of time necessary to convert compound **3.3** to compound **3.2** were undertaken. The reaction was conducted as described previously, with aliquots periodically analyzed by HPLC. As can be observed from a stacked plot of chromatograms (Figure 3-8) conversion of **3.3** (R_t = 18.3 min) to **3.2** (R_t = 16.3 min) is more than 90 % complete after 3 h. The gradual shift to earlier retention times, which does

not impact the conclusions of the study is attributed to insufficient conditioning of the HPLC column at the earlier time points.

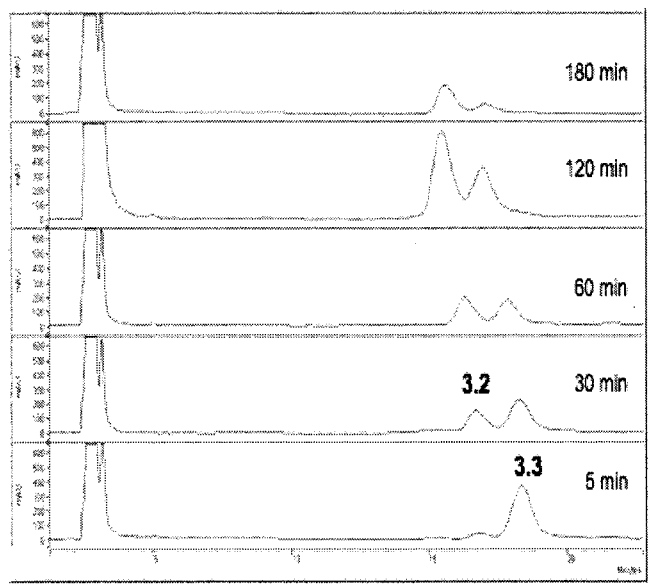


Figure 3-8. Conversion of **3.3** to **3.2** Monitored by HPLC

3.4.3 Isolation and characterization of 3.2

To isolate **3.2** from the reaction mixture containing an excess of **3.10,** the use of simple liquid-liquid extraction was explored. Several experiments that involved partitioning the reaction products between FC-72® and a variety of different solvents including the reaction solvent, dichloromethane, distilled deionized water, aqueous sodium carbonate and aqueous sodium hydroxide were attempted. Surprisingly, in no case was partitioning of the fluorous compound **3.2** observed in the fluorous layer. This result was attributed to the conflicting polarities between the

polar guanidine functionality (Figure 3-9) and the extremely hydrophobic perfluoroalkyl groups. Consequently, acetonitrile, a solvent of intermediate dielectric constant (Table 3-1) was selected for use in this process. Subsequent liquid-liquid extraction with perfluorinated hexanes and analysis of the fluorous layer by HPLC indicated that the product (**3.2**) had indeed partitioned into the fluorous layer, and was isolated in 98 % yield.

Solvent	Dielectric constant (ε)
Dichloromethane (CH_2Cl_2)	8.93
Acetonitrile (CH_3CN)	36.64
Water (H_2O)	80.10

Table 3-1. Dielectric constants of liquids[21]

The ^1H NMR spectrum of **3.2** (Figure 3-10), showed aromatic signals between 7.51 and 7.63 ppm, and a signal corresponding to the benzylic protons at 4.52 ppm. Although the ^1H NMR spectra of compound **3.2** and **3.3** were acquired in different solvent (CD_3OD and $CDCl_3$, respectively), this signal can be interpreted as distinctly different than the chemical shift of the signal from the comparable protons in the starting material (**3.3**) which appear at 3.88 ppm since the difference in chemical shift is much greater than any potential solvent effect (< 0.1 ppm). High-resolution mass spectrometry showed a group of peaks with a mass-to-charge ratio consistent with the molecular mass of **3.2** (1310.0254 Da), and a characteristic isotope pattern confirming the presence of tin.

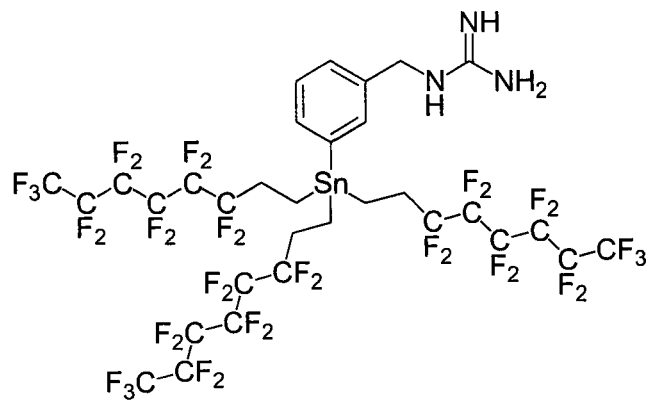


Figure 3-9. Fluorous benzylguanidine **3.2**

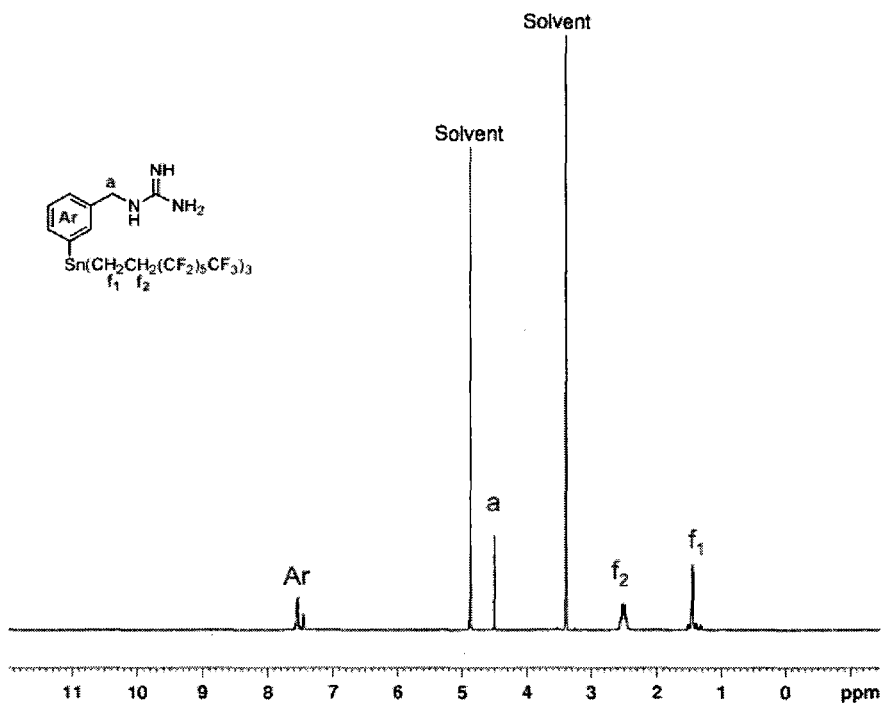


Figure 3-10. ^{1}H NMR spectrum of **3.2** (500.13 MHz, CD_3OD)

3.5. Non-radioactive iodination of 3.2

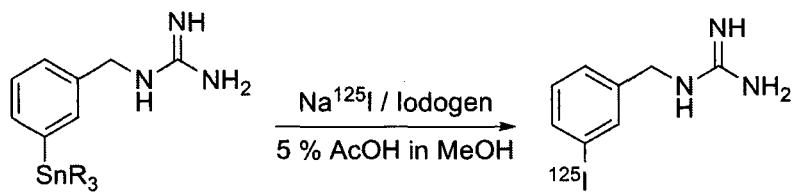
As previously discussed (Chapter 2), prior to radioactive iodination it is prudent to establish a sound methodology for the synthesis of the desired compound and subsequent F-SPE procedures using non-

radioactive iodine. Given the extreme polarity of both **3.2** and **3.1** it was important to validate the efficacy of the fluorous purification strategy with the fluorous MIBG precursor **3.2** and its radioiodinated product. To this end, in an attempt to mimic a radiolabelling experiment, a solution of **3.2** in 5 % acetic acid in methanol and 0.08 mole equivalents of sodium iodide were combined in the presence of iodogen (1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril). Following quenching with sodium metabisulfite ($Na_2S_2O_5$), the resulting mixture was analyzed by HPLC which indicated the presence of the target molecule. Purification of the reaction mixture was conducted by F-SPE, after which, the amount of tin present in the purified samples was determined in a similar fashion to that described in Chapter 2. Analysis of the isolated product was performed using HPLC which confirmed that the amount of tin present was below the limit of detection of the PDA detector (14 ppm). The sample was subjected to ICP-MS analysis which confirmed that the amount of tin present in the F-SPE purified material was less than 1 ppm.

3.6. Radioiodination: production of [$^{123/125}$I]MIBG

The radioactive iodination of **3.2** was carried out using sodium [^{125}I]iodide and iodogen (Scheme 3-10). F-SPE purification of **3.1** at the tracer level was modified to compensate once again for the extremely basic nature of the target. Under standard elution conditions (80 %

methanol in water), only 15 % of the radioactivity was released from the sorbent. The yield was increased by acidifying the aqueous component thus a radiochemical yield of 81 % was achieved using a 20 % solution of 1 M acetic acid in methanol.



3.2 **3.1**

Scheme 3-10. Radiosynthesis of [^{125}I]MIBG (R = $CH_2CH_2(CF_2)_5CF_3$)

Overall the radiochemical yield of **3.1** was 81 ± 3 % (*n* = 3) and, based on HPLC analysis, the radiochemical purity was greater than 95 %. The specific activity of the [^{125}I]MIBG prepared was determined by first preparing a calibration curve using samples of non-radioactive **3.1**, correlating the amount of material injected and the corresponding response of the PDA detector. Following this, a sample of [^{125}I]MIBG was analyzed by HPLC. The area under the curve in the UV-chromatogram was used to determine the overall mass of compound present in the sample. The specific activity was determined to be 75 GBq/μmol ± 0.3 GBq (2020 ± 9 mCi/μmol which is only slightly less than the theoretical value of 79 GBq/μmol (2125 mCi/μmol).

Furthermore, to demonstrate the suitability for this method for clinical applications, the above radiolabelling procedure was repeated using iodine-123 (62 MBq or 1.7 mCi), a commonly used clinical isotope. Here we observed no significant difference in the obtained radiochemical yield or radiochemical purity.

3.7. Conclusions

A convenient and readily scaleable method for the synthesis of a fluorous precursor to MIBG was developed. This product can be radioiodinated to produce MIBG in good radiochemical yield and in high radiochemical purity through the use of an expedient solid-phase extraction method. HPLC and ICP-MS data indicate that the desired product can be isolated in the absence of any precursor.

3.8. Experimental

Reagents and General Procedures

All chemicals were purchased from Sigma-Aldrich and Fluorous Technologies Inc. unless otherwise noted. Sodium [^{125}I] iodide with a specific activity of ~17 Ci/mg was generously provided by the McMaster Nuclear Reactor (Hamilton, ON). No carrier added sodium [^{123}I] iodide was purchased from MDS Nordion (Vancouver, BC). *Tris*(2-

perfluorohexylethyl)stannyl bromide was synthesized as previously reported.[7]

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

Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker DRX-500 spectrometer with chemical shifts reported in ppm relative to the residual proton signal of the deuterated solvent (^{1}H NMR) or the carbon signal of the solvent (^{13}C NMR). 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 ultraviolet (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 where samples were first dissolved in methanol. High-resolution mass spectral data were obtained using a Waters-Micromass Q-TOF Ultima Global spectrometer. High-pressure 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 a Supelco Discovery-C_{18} column (4.6 × 250 mm, 300 Å-5 μm). The wavelength for UV detection was set at 229 nm and the dwell time in the gamma detector was 5 s in a 10 μL loop. The delay between the UV and gamma detectors was 0.3 min. The elution conditions were as follows: **Method A:** Solvent A = CH_3CN (0.1 % H_3PO_4), Solvent B = H_2O (0.1 % H_3PO_4); 0-10 minutes 23 % A; 10-15 minutes 23 % A to 100 % A; 15-30 minutes 100 % A. **Method B:** Solvent A = CH_3CN (0.1 % H_3PO_4), Solvent B = H_2O (0.1 % H_3PO_4); 0-10 minutes 23 % A (isocratic). For both methods the flow rates were 1 mL / min. Inductively coupled-mass spectrometry (ICP-MS) analysis, in which a conductive gas is used to generate metal ions that are detected by a mass spectrometer, was conducted by Activation Laboratories Ltd. (Ancaster, ON).

Stock solutions were prepared by dissolving standards (*meta*-iodobenzylguanidine (MIBG) and *meta*-iodobenzylamine) in a solution of water (77 %, v/v) and acetonitrile; the pH was adjusted to 2.4 using H_3PO_4 to obtain a concentration of 0.1 mg/mL. Analyte solutions at 6 different concentrations of MIBG in the range of 0 μM to 15 μM containing the internal standard at a constant concentration were prepared. Each calibration solution was injected in triplicate and the calibration curves

were analyzed by the least-squares method. The limit of quantitation (LOQ) and the limit of detection (LOD) were calculated as the analyte concentration which gave rise to chromatographic peaks whose height was equal to 10- or 3-times the baseline noise, respectively. The specific activity of the radiolabelled product was subsequently determined by injecting 250 μCi of the purified fraction and analyzing the resulting signal in the UV chromatogram and correlating the detector output with the calibration curve.

3.8.1. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl) benzylamine (3.3)

3-Bromobenzylamine hydrochloride (3.60 g, 16.2 mmol) was dissolved / suspended in CH_2Cl_2 (25 mL) and the solution extracted 3 times with 10 % aqueous sodium carbonate. The organic fractions were combined and dried over Na_2SO_4, filtered and the solvent evaporated. The resulting yellow oil was redissolved in dry CH_2Cl_2 (125 mL); triethylamine (3.19 g, 31.5 mmol) was added and the mixture cooled in an ice bath for 30 min under an argon atmosphere. A solution of *bis*(chlorodimethylsilyl)ethane (2.78 g, 12.9 mmol) in dry CH_2Cl_2 (100 mL) was then transferred to the reaction mixture *via* cannula. The reaction was stirred at low temperature for 1.5 h and then at room temperature for an additional 3 h before the solvent was removed by rotary evaporation.

The resulting white solid was suspended in hexanes and filtered using a Schlenk-type filter funnel. Removal of the solvent by rotary evaporation afforded a transparent light-yellow oil. A sample of this oil (317 mg, 0.966 mmol) was subsequently dissolved in Et_2O (10 mL) and cooled in a cold bath (-98°C). To this was slowly added *tert*-butyl lithium in hexanes (1.7 M, 1.03 mL, 1.76 mmol) using a glass syringe. After stirring at low temperature for 5 min, an ethereal solution of *tris*[2-perfluorohexylethyl]stannyl bromide (**2.2**) was added (1.09 g, 0.879 mmol, in 5 mL) *via* a cannula. The resulting yellow solution was stirred at -98°C for 1 h and then warmed to room temperature over an additional hour before the solvent was removed under reduced pressure. Following liquid-liquid extraction using perfluorinated hexanes (FC-72®) (10 mL) and CH_2Cl_2, (3 x 5 mL) the fluorous fraction containing **3.3** was concentrated and the resulting yellow oil dissolved in a 9:1 methanol-water solution adjusted to pH 4.5 with 1M HCl (10 mL). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The resulting oil was dissolved in FC-72® (10 mL) and extracted with CH_2Cl_2 (3 x 5 mL). Following concentration of the fluorous fraction, the resulting yellow oil was loaded on to a silica column (1 x 10 cm) first eluting with $CHCl_3$ and then 9:1 EtOH / $CHCl_3$ to isolate the desired product which was a colorless oil. Yield: 390 mg, 35 %. TLC (10 % EtOH:$CHCl_3$): R_f = 0.17.

^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.37 (m, 3H, Ar-*H*), 7.30 (m, 1H, Ar-*H*), 3.89 (s, 2H, $C_6H_4CH_2NH_2$), 2.32 (m, 6H, Sn-$CH_2CH_2C_6F_{13}$), 1.53 (s, 2H, $C_6H_4CH_2NH_2$), 1.31 (t, *J* = 8.5 Hz, 6H, Sn-$CH_2CH_2C_6F_{13}$). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 143.6, 138.5, 136.6, 134.3, 134.3, 128.9, 128.4, [120.5, 118.3, 116.0, 113.1, 110.8, 108.6], 46.3, 27.7, -1.8. HRMS(+): mass calcd for $C_{31}H_{20}F_{39}NSn$ $[M+H]^+$: 1268.0073. Found: 1268.0072. FTIR (KBr, cm^{-1}): 2948, 1652, 1443, 1239, 1145.

3.8.2. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl) benzylguanidine (3.2)

1*H*-Pyrazole carboxamidine hydrochloride (71.5 mg, 488 μmol) and triethylamine (74.1 mg, 732 μmol) were added to compound **3.3** (310 mg, 244 μmol) in CH_2Cl_2 (5 mL). The resulting solution stirred overnight at room temperature. The reaction solvent was removed by rotary evaporation and the desired product isolated by liquid-liquid extraction using FC-72® (15 mL) and CH_3CN (3 x 5 mL). Evaporation of the fluorous solvent produced a clear yellow oil. Yield: 320 mg, 98 %. ^{1}H NMR (500.13 MHz, CD_3OD) δ: 7.61 (s, 1H, Ar-*H*), 7.51 (m, 3H, Ar-*H*), 7.44 (m, 1H, Ar-*H*), 4.52 (s, 2H, $C_6H_4CH_2NHC(NH)NH_2$), 2.49 (m, 6H, Sn-$CH_2CH_2C_6F_{13}$), 1.44 (t, *J* = 8.5 Hz, 6H, Sn-$CH_2CH_2C_6F_{13}$). ^{13}C NMR (125.76 MHz,

CD_3OD) δ: 158.9, 139.8, 137.9, 136.8, 136.2, 130.1, 129.3, [122.1, 119.9, 117.7, 114.5, 112.4, 110.1], 46.1, 28.8, -0.5. HRMS(+): mass calcd for $C_{32}H_{22}F_{39}N_3Sn$ $[M+H]^+$: 1310.0291. Found: 1310.0254. FTIR (KBr, cm^{-1}): 3329, 3171, 2931, 1652, 1243, 1146.

3.8.3. Non-radioactive (cold) iodination of 3.2 to give 3.1

Compound **3.2** (100 mg, 76 μmol) in a solution of 5 % acetic acid in methanol (250 μL) and sodium iodide (1 mg, 6 μmol) were combined in the presence of iodogen (1 mg, 2 μmol). After 3 min, the reaction was quenched by the addition of aqueous sodium metabisulfite ($Na_2S_2O_5$) (100 μL, 231 mM). The reaction mixture was diluted to 2 mL with water and was passed through a Fluoro*Flash*® solid-phase extraction cartridge. The cartridge was first eluted with water (6 mL) and then 5 x 2 mL fractions of an 80:20 (v/v) methanol / water mixture were collected. HPLC (**Method B**): R_t = 8.3 min. ESMS (+): 276.1 $[M+H]^+$, No peak due to 1310.0 [**3.2** $+H]^+$ observed.

3.8.4. Fluorous-based radiosynthesis of [^{125}I]MIBG

Compound **3.2** (0.5 mg, 0.4 μmol) was added to a sample vial coated with iodogen (20 μL, 1 mg / mL). A solution of 5% acetic acid in methanol (100 μL) was added followed by sodium [^{125}I]iodide (5 μL, 3700 Bq (100 μCi),

pH 10). After manually agitating the mixture for 3 min, the reaction was quenched by the addition of aqueous sodium metabisulfite ($Na_2S_2O_5$) (10 μL, 231 mM). The reaction mixture was diluted with water (1 mL) and loaded onto an F-SPE cartridge. The reaction vial was subsequently rinsed with water (3 mL); that was also added loaded onto the F-SPE cartridge. The cartridge was eluted with water (6 mL), followed by 80:20 (*v/v*) solution of methanol-1M acetic acid (6 mL). Radioactive fractions (1.5 mL) eluted from the F-SPE cartridge were analyzed by HPLC where one single radioactive peak was observed (R_t = 8.4 min). When co-injected with the non-radioactive MIBG standard, the peaks coeluted. The average radiochemical yield for this reaction was 81 % (*n* = 3).

3.8.5. Fluorous-based radiosynthesis of [^{123}I]MIBG

Radiochemistry was conducted in the same manner used to prepare [^{125}I]MIBG except sodium [^{123}I]iodide (5 μL, 333 mCi/mL, pH 10) was used. Yield 80% (*n* = 2). HPLC (Method A): R_t = 8.3 min.

3.9. References

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Chapter 4: The fluorous labelling approach and bioconjugate chemistry

4.1. Overview

Chapters 2 and 3 demonstrated that radiolabelling and purification using fluorous supports is an effective means of producing iodine-containing radiotracers based on the model benzoic acids, benzylamine and benzamide derivatives discussed in previous chapters. The approach has been proven effective for preparing libraries of both precursors and radiolabelled compounds. The fluorous labeling strategy can also be used to produce a clinically approved radiopharmaceutical (*meta*-iodobenzylguanidine (MIBG)) in high effective specific activity. The next objective was to extend the fluorous labelling strategy beyond small molecules and to expand the number of fluorous synthons that can be used to prepare radiolabelled bioconjugates

4.2. Bioconjugates for the radiolabelling of proteins

One facet of current cancer research focuses on applying the model of antigen-antibody recognition in the human immune system to the targeting of radionuclides to tumour cells for *in vivo* imaging and radiation therapy of malignancies.[1] Because of their diversity, specificity and

biological activity, antibodies and antibody fragments that selectively interact with specific cell types are attractive vehicles for the delivery of radioactivity to target tissues.[1, 2] In order for these vectors to be used to create viable radiopharmaceuticals, they must be radiolabelled in an efficient and expedient manner that does not destroy their biological properties. There is a need for fast and efficient chemical reactions that can be used to link the radionuclides to antibodies in a controlled manner.[3]

As stated previously (Section 1.4.), the most common route to radiohalogenated proteins is through the preparation of an *in situ* electrophilic radioiodine species which reacts with functional groups on the native protein. The most readily iodinated amino acid residues are the activated phenolic rings of tyrosine and the imidazole rings of histidine (Figure 4-1).[4] At physiological pH (7.4) the iodination reactions will predominantly occur at the tyrosine residues.[5]

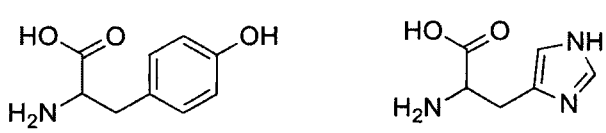


Figure 4-1. Groups present in biomolecules activated towards electrophilic iodine

Direct labelling is a convenient method for tagging proteins / antibodies with isotopes of radioiodine, resulting in relatively high specific activity preparations.[5] This approach does however, have several

limitations. For instance, amino acid residues that are directly radioiodinated frequently undergo extensive *in vivo* deiodination. This is considered to be the result of the structural similarities between the iodophenyl groups and thyroid hormones; compounds for which deiodinases of varying specificity are known to exist.[6] Secondly, since there is no way to control the regiochemistry of the iodination reaction, it is common that this approach results in the loss or reduction of the targeting ability of the antibody.

This loss of biological activity is typically overcome using a prosthetic-group approach. Here, a small molecule designed to form stable covalent bonds to both the radionuclide and the biomolecule is conjugated to the compound of interest.[3] The principal functional groups on proteins which are used in conjugation reactions with other molecules are amines, sulfhydryl groups and oxidized sugars;[4] the most common site being the ε- amino group of lysine residues. A collection of reagents for linkng "labelable" synthons to lysine residues are shown below (Figure 4-2).[4,7]

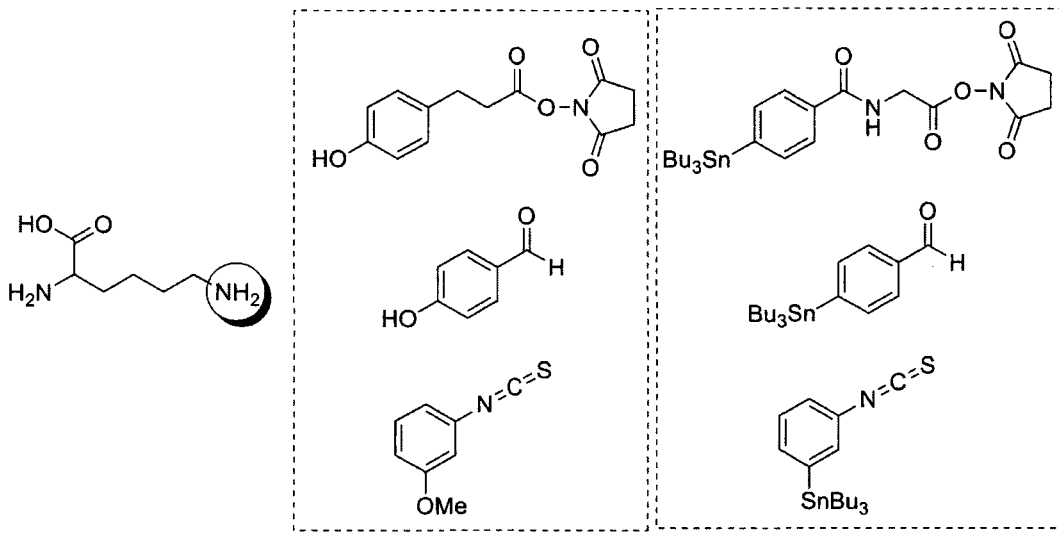


Figure 4-2. Common reagents used in conjugation reactions with lysine residues[4]

One of the limitations of existing methods for tagging macromolecules with iodine is the lack of a technique capable of rapidly separating unlabelled starting material (native substrate) from the labelled species, resulting in the administration of radiotracers with low effective specific activities. In the case of antibodies, residual unlabelled material is a safety concern as even low amounts of the proteins can cause an immunogenic response. One possible solution is to prepare fluorous derivatives of these macromolecules in which the radiolabelled products could then be isolated *via* F-SPE or fluorous HPLC.

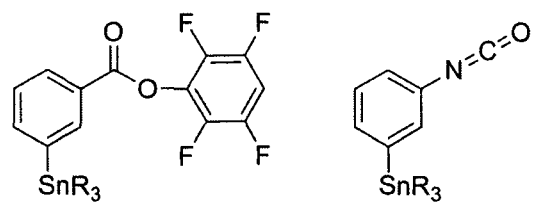
4.3. Selection of fluorous bioconjugates for protein labelling

Our objective is to use fluorous chemistry to prepare novel tin-containing "tags" (Figure 4-3), which, when conjugated to a peptide or protein, should greatly facilitate the production of such radiotracers in high effective specific activity.

It has been demonstrated that fluorous solid-phase purification of biomolecules including peptides and oligosaccharides is feasible,[8] and has been used with whole proteins to facilitate proteome characterization.[9] Of particular interest is the recent work which that demonstrated that tagged molecules bearing different numbers of fluorous groups can be selectively eluted using a gradient of increasing fluorophilic (*i.e.* organic) content.[9] This is significant because the fluorous prosthetic groups, like standard labelling synthons, will likely react with multiple reactive sites within the proteins. Purification of these conjugation products *via* fluorous separations will provide a means of controlling the extent of conjugation, a feature that is not readily accessible with other prosthetic groups.

Substrates *m*-**2.9**, *p*-**2.9** and **4.1** were selected as initial targets since both compounds react rapidly with free amines (such as the ε-amine on lysine residues). In the labelling of biomolecules, acylation reactions using active ester chemistry are the most common methods for

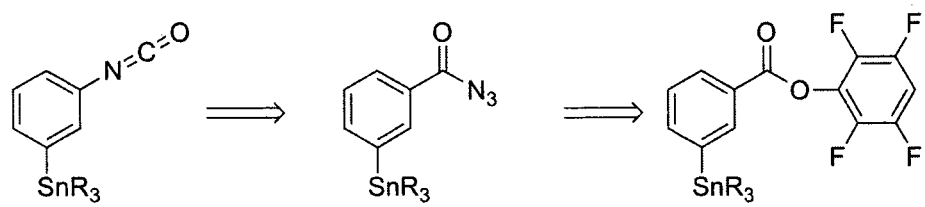
conjugating radiolabelled prosthetic groups.[2] Since the conjugation chemistry of the activated esters (*m*-**2.9** and *p*-**2.9**) was thoroughly discussed in Chapter 2, efforts focused on the preparation of a fluorous isocyanate (**4.1**) and an investigation into its conjugation chemistry.



m-**2.9** **4.1**

Figure 4-3. Proposed fluorous molecules for bioconjugation (R = $CH_2CH_2(CF_2)_5CF_3$)

Isocyanates are organic functional groups that are extremely reactive and react rapidly with amines. The associated conjugation reactions proceed with high selectivity in the presence of competing nucleophiles to form stable urea bonds.[10] Despite their reactivity, the isocyanate-based conjugation reactions have been largely overlooked in the preparation of radiohalogenated compounds. This is likely a result of how easily these compounds are hydrolyzed in aqueous media. Due to the orthogonal solubilities of fluorous compounds and aqueous systems, the fluorous isocyanate (**4.1**) could potentially be more resistant to hydrolysis thereby providing a highly selective fluorous bioconjugation synthon. The target molecule was prepared according to the following retrosynthesis (Scheme 4-1):

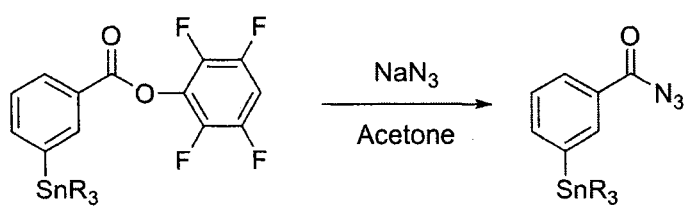


4.1 **4.2** ***m*-2.9**

Scheme 4-1. Retrosynthesis of **4.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

4.3.1. Synthesis and characterization of 3-(*tris*[2-perfluorohexylethyl]stannyl) phenyl isocyanate (4.1)

Towards the synthesis of the fluorous acyl azide **4.1**, compound **4.2** was prepared by treating ***m*-2.9** with two mole equivalents of sodium azide in a solution of acetone (Scheme 4-2).[11] The reaction solvent was removed by rotary evaporation and the product (**4.2**) was isolated quantitatively by liquid-liquid extraction using C_6F_{14} (FC-72®) and water. The resulting colourless oil was characterized by ^1H NMR, ^{13}C NMR and IR spectroscopy, where the characteristic azide stretch[11] was observed at 2140 cm^{-1} (Figure 4-4).



***m*-2.9** **4.2**

Scheme 4-2. Synthesis of **4.2** (R = $CH_2CH_2(CF_2)_5CF_3$)

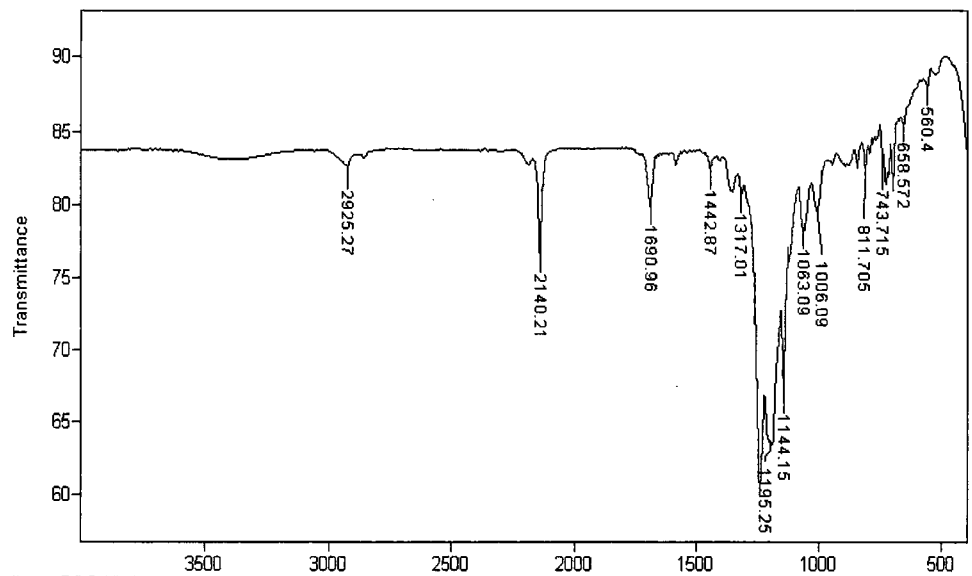
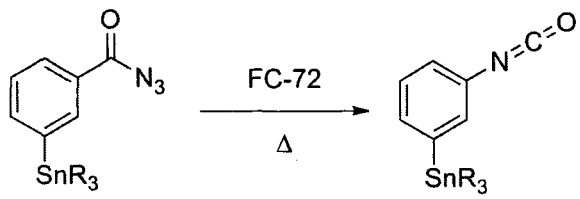


Figure 4-4. FTIR spectrum of **4.2** (KBr)

Acyl azides can be readily converted to isocyanates *via* a simple Curtius rearrangement.[12] The first attempted preparation of **4.1** involved heating **4.2** at 90°C for four hours in toluene;[11] however, no product formation was detected using IR spectroscopy. This was attributed to the limited solubility of **4.2** in toluene. In order to circumvent this issue, the synthesis of **4.1** was attempted by heating compound **4.2** in perfluorinated hexanes at reflux (~70°C) for ≥ 4 h (Scheme 4-3). Evaporation of the reaction solvent afforded a colorless oil which was immediately analyzed by ^{1}H and ^{13}C NMR spectroscopy as well as IR spectroscopy. The IR spectrum of the product showed a strong absorption at 2272 cm^{-1} (Figure 4-5) which is consistent with other aryl-isocyanates.[12]



4.2 **4.1**

Scheme 4-3. Synthesis of fluorous isocyanate (**4.1**) in fluorous solvent (R = $CH_2CH_2(CF_2)_5CF_3$)

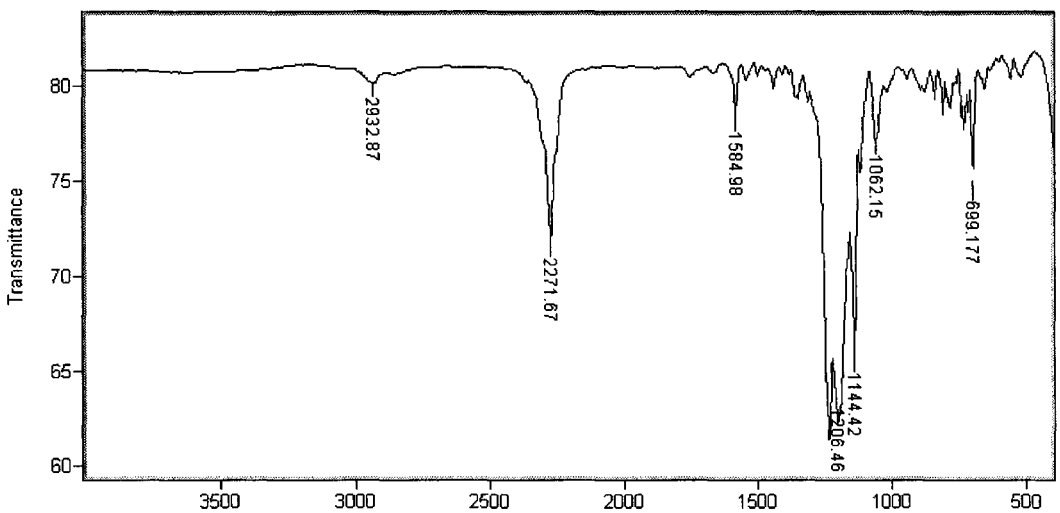
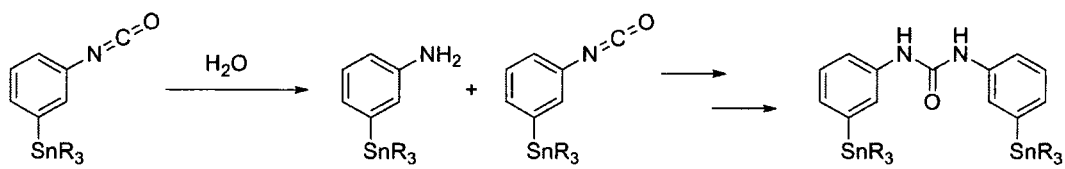


Figure 4-5. FTIR spectrum of **4.1** (KBr)

With the fluorous isocyanate (**4.1**) in hand we set out to investigate its stability with the knowledge that organic isocyanates are generally very susceptible to hydrolysis. The experiments were designed to allow us to determine whether the **4.1** would be sufficiently stable for storage, or whether it would be necessary to prepare it immediately prior to use.

Mechanistically, isocyanates react with water according to the reaction pathway outlined in Scheme 4-4. The resulting aniline derivative

is a superior nucleophile to water and as such, reacts with the aromatic isocyanate generating a bis-fluorous urea.[13] With this in mind, samples of **4.1** were analyzed by IR spectroscopy over time, looking for stretches either at 2270 cm^{-1} (N=C=O asymmetric stretch of fluorous isocyanate) or 1650 cm^{-1} (C=O stretch of bis-fluorous urea).



Scheme 4-4. Hydrolysis of **4.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

Initially, compound **4.1** was stored neat, open to the atmosphere. After 4 h, the IR spectrum indicated that no isocyanate remained, which is exactly what is observed for aryl-isocyanates. Based on the fact that the fluorous solvents are immiscible with water, the shelf-life of a solution of fluorous isocyanate (**4.1**) in perfluorinated hexanes was examined. As depicted by the IR spectra in Figure 4-6, the fluorous isocyanate was remarkably stable when stored in perfluorinated solvents, with no degradation observed even after 21 days. These results contrast to what is typically observed with an organic aryl-isocyanate dissolved in solution.

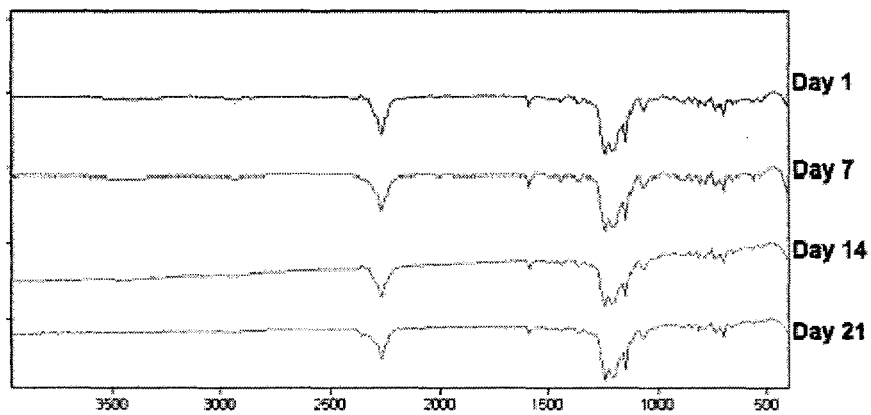


Figure 4-6. FTIR spectra r of **4.1** in FC-72® over time

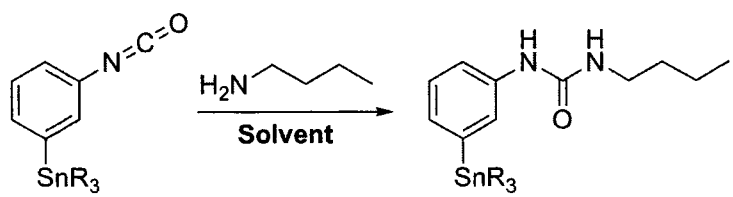
To verify the results, the stability experiment was repeated using phenyl isocyanate which was commercially available. Complete decomposition of phenyl isocyanate in hexanes was observed by IR spectroscopy after only 30 minutes. An attempt was made to establish whether the organic isocyanates would also display an increased resistance to hydrolysis if stored in perfluorinated solvents. Unfortunately phenyl isocyanate was completely insoluble in FC-72® and thus its stability in perfluorinated solution could not be measured. By comparison, the stability of **4.1** in hexanes was also attempted, however it could not be measured owing to a lack of solubility.

4.4. Conjugation chemistry of the fluorous isocyanate

With the preparation and characterization of **4.1** complete, the next objective was to establish conditions suitable for conducting

bioconjugation reactions. One of the main challenges to overcome here was the orthogonal solubilites of antibodies and other biomolecules which are typically stored (and used) in buffered aqueous systems, and that of the fluorous isocyanate **4.1** which has to be prepared (and stored) in perfluorinated hexanes.

In order to evaluate approaches to overcome this obstacle, a series of reactions were conducted between **4.1** and the highly nucleophilic amine *n*-butylamine which was selected as a simple model system. A variety of solvent systems, each biphasic, were tested by stirring **4.1** in the presence of an excess of the amine (Scheme 4-5). After 2 h, the fluorous layer was isolated, the solvent removed and the resulting oily residue analyzed by mass spectrometry and ^{1}H NMR spectroscopy (Figure 4-7). In all cases the formation of the desired urea was detected and was the major product. These results were promising and suggest that even in biphasic reaction conditions, **4.1** is sufficiently reactive to form the desired urea without reacting with water.



4.1 **4.3**

Solvent = (a) Neat/C_6H_{14}
(b) H_2O/C_6H_{14}
(c) $(NaH_2PO_4)_{aq\text{-}7.4}$ /C_6H_{14}
(d) $(Na_2CO_3)_{aq\text{-}9.5}$ /C_6H_{14}

Scheme 4-5. Conjugation of **4.1** with model amine; pH denoted as subscripts (R = $CH_2CH_2(CF_2)_5CF$

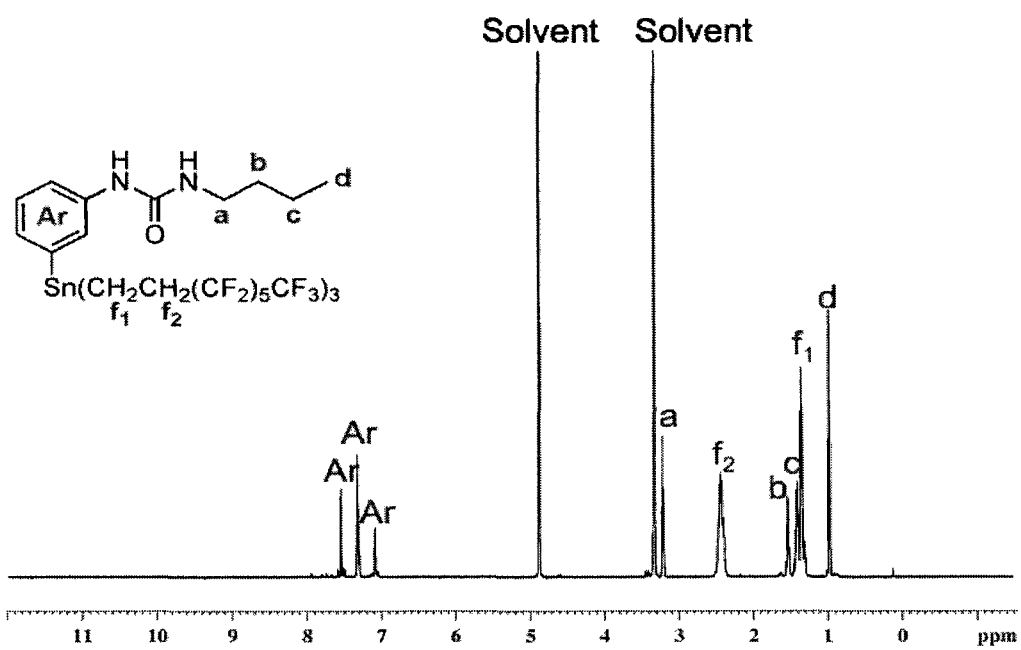


Figure 4-7. ^{1}H NMR spectrum of **4.3** (reaction conducted in $PBS_{7.4}/C_6F_{14}$) (500.13 MHz, CD_3OD)

While useful for developing reaction conditions, the highly reactive primary amine used in the model reactions (*n*-butylamine) is not representative of the reactivity or solubility of most biologically relevant

amines. In consequence reactions involving different amines were investigated. A secondary amine 1-(2 methoxyphenyl)piperazine, was chosen since it is significantly more sterically hindered than *n*-butylamine and is of interest as an established pharmacophore for imaging serotonin receptors.[14] Conjugation experiments were conducted by combining a solution of the amine in phosphate buffered saline with the isocyanate **4.1** in perfluorinated hexanes. This particular solvent system was chosen since it is one of the most common solvents in which traditional antibody / protein conjugation experiments are conducted.[15] After 2 h of vigorous stirring, the fluorous layer was removed and the product isolated as described previously. The presence of the piperazine group of the isolated product was evident from the ^{1}H NMR spectrum (Figure 4-8) with the signal for the methylene units ascribed to two sets of triplets at 3.78 ppm (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, displaying the methylene protons as a multiplet at 2.50 ppm and a triplet at 1.45 ppm (J = 8.4 Hz). These data further support the conclusion that compound **4.1** can be utilized to react efficiently with amines under biphasic reaction conditions to prepare stable fluorous-urea conjugates.

The next objective was to apply this conjugation strategy to the synthesis of compounds of clinical relevance.

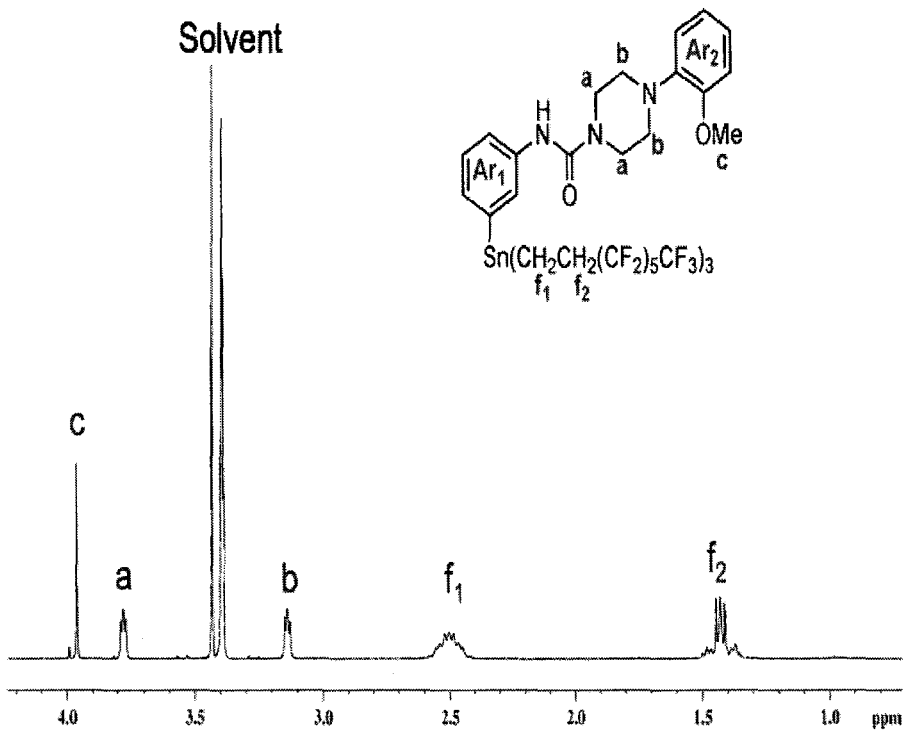


Figure 4-8. ^{1}H NMR spectrum of **4.4** (500.13 MHz, CD_3OD)

4.5 Glu-U-Lys: clinical utility and current routes to [^{125}I]Glu-U-Lys

Prostate cancer is the most prevalent cancer in men and represents the second leading cause of cancer death in Canadian males.[16] Currently research efforts are being put towards identifying and subsequently potential target sites for imaging and diagnosing prostate cancer.[17] Prostate specific membrane antigen (PSMA) is a potential target for prostate tumour imaging and therapy since it is significantly up-

regulated in prostate cancer and metastases relative to the few normal tissues in which it resides.[18] Present efforts are focused on the design of small molecule probes for PSMA to address the slow accumulation and target to non-target ratios of the clinically approved antibody-based agent Prostascan.

A series of iodinated glutamate urea heterodimers based on the basic structure below (Figure 4-9) bind to PSMA with high affinity.[19] Currently these iodinated derivatives are prepared from trialkyltin precursors through conjugation *via* the ε-amine of the lysine residue. As such, this heterodimer was selected as a good biologically relevant target for demonstrating the potential of the fluorous isocyanate. To do this we focused on the preparation of a fluorous-heterodimer (Glu-U-Lys) that is being pursued as a putative agent for diagnosing and staging prostate cancer.

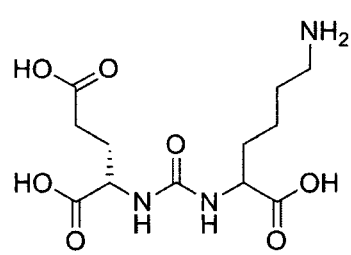
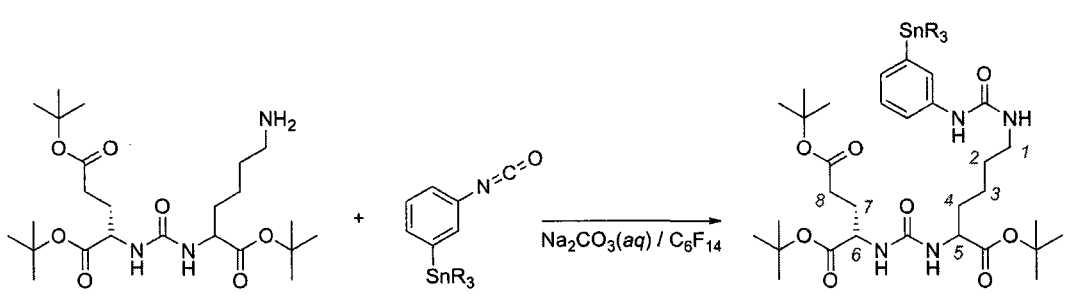


Figure 4-9. Basic structure of Glu-U-Lys heterodimer

In order to ensure regioselectivity, the tri-*tert*-butyl ester (^{t}Bu) derivative of the urea heterodimer, in which each acid is protected by a t-butyl group, was chosen as the starting material. Initial reactions were conducted as described in the previous section, dissolving Glu-U-Lys(^{t}Bu) in an aqueous sodium carbonate solution and stirring with a solution of **4.1** in perfluorinated hexanes (FC-72®) (Scheme 4-6). After reaction work-up, mass spectrometry confirmed the presence of the desired product. Despite this however, the extent of conjugation was difficult to elucidate from the ^{1}H NMR spectrum due in particular to the *tert*-butyl carboxylate protecting groups, which collectively integrate to 27, making all other proton signals hard to differentiate from baseline. This, coupled with the fact the limited quantity of Glu-U-Lys(^{t}Bu) in our possession, optimization of the conjugation reaction was done using a simpler commercially available model compound, Nα-acetyl-L-lysine-methyl ester. Unfortunately the same low yield of the desired product was observed.



4.1 **4.5**

Scheme 4-6. Attempted conjugation of **4.1** with Glu-U-Lys (^{t}Bu) (R = $CH_2CH_2(CF_2)_5CF_3$) and NMR numbering scheme for **4.5**

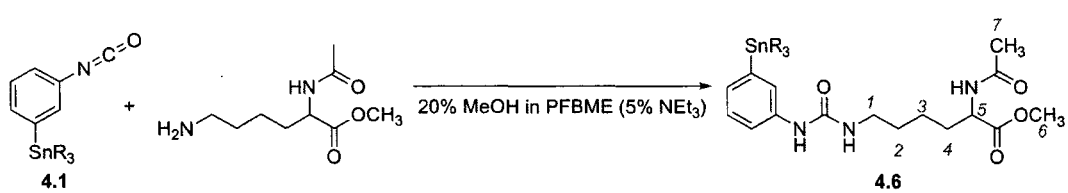
4.5.1. Toward Glu-U-Lys conjugation: reactions with Nα-acetyl-L-lysine methyl ester

In comparing the near quantitative conversions obtained using the model amines with the lack of any apparent reaction with the Glu-U-Lys derivatives, it was postulated that the model amines were more soluble in fluorous media than the amino acid / peptidyl derivatives which is what drove the equilibrium of those reactions to favour the products.

To address this issue, a solvent or biphasic catalyst that could facilitate the mixing of the orthogonal layers was required. Perfluorobutyl methyl ether (PFBME) is a polar solvent that can dissolve most fluorous compounds and it is miscible with most organic solvents (including MeOH, DMF, toluene, hexane, Et_2O, THF, CH_3CN and CH_2Cl_2) under ambient conditions.[20] Using this solvent instead of FC-72®, the conjugation experiment was repeated as depicted in Scheme 4-7. The reaction mixture was homogeneous and TLC analysis indicated complete consumption of **4.1** after 2 h. Following removal of the solvent, the fluorous components were separated by fluorous liquid-liquid extraction and the desired product was obtained as a clear oil following silica gel chromatography.

Characterization of the product was carried out using standard spectroscopic techniques. The ^1H NMR spectrum showed the diagnostic

fluorous methylene protons ascribed to the multiplet at 2.28 and the triplet at 1.27 ppm with ^{119}Sn satellites. Furthermore, the methyl protons on the methyl-ester and acetyl- protecting groups give rise to the singlets appearing at 3.72 and 2.02 ppm respectively. The remaining signals, as shown in the spectrum below, (Figure 4-10) were in agreement with the structure of **4.6**. The ^{13}C NMR spectrum was also in agreement with the assigned structure where two-dimensional techniques (COSY, HMBC, HSQC) were used to make specific assignments. IR spectroscopy and high-resolution mass spectrometry further confirmed that the isolated product was in fact **4.6**.



Scheme 4-7. Conjugation of **4.1** with Nα-acetyl lysine methyl ester (*homogeneous conditions)* (R = $CH_2CH_2(CF_2)_5CF_3$) and NMR numbering scheme for **4.6**

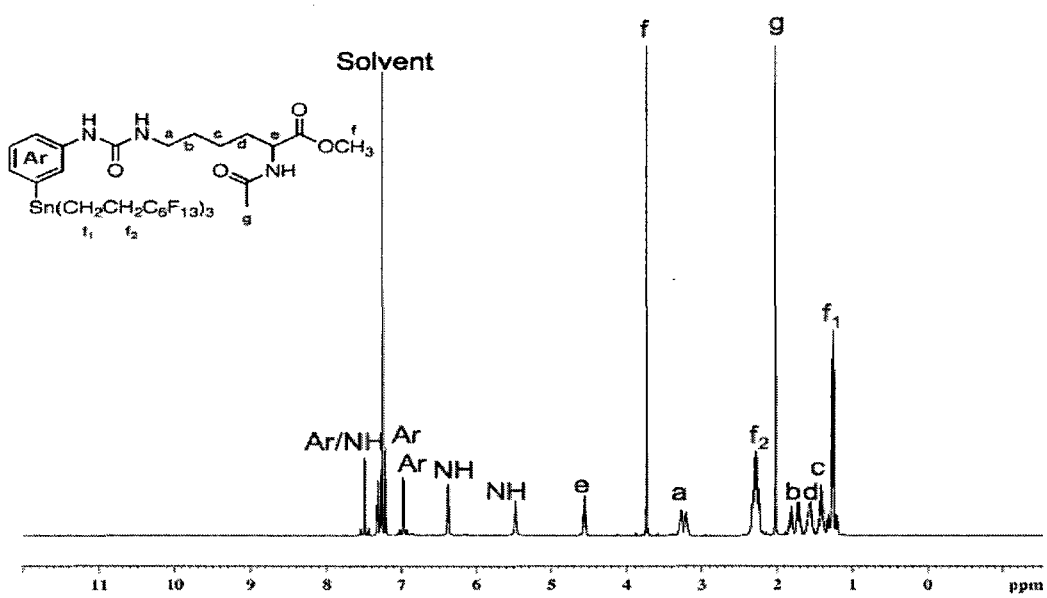


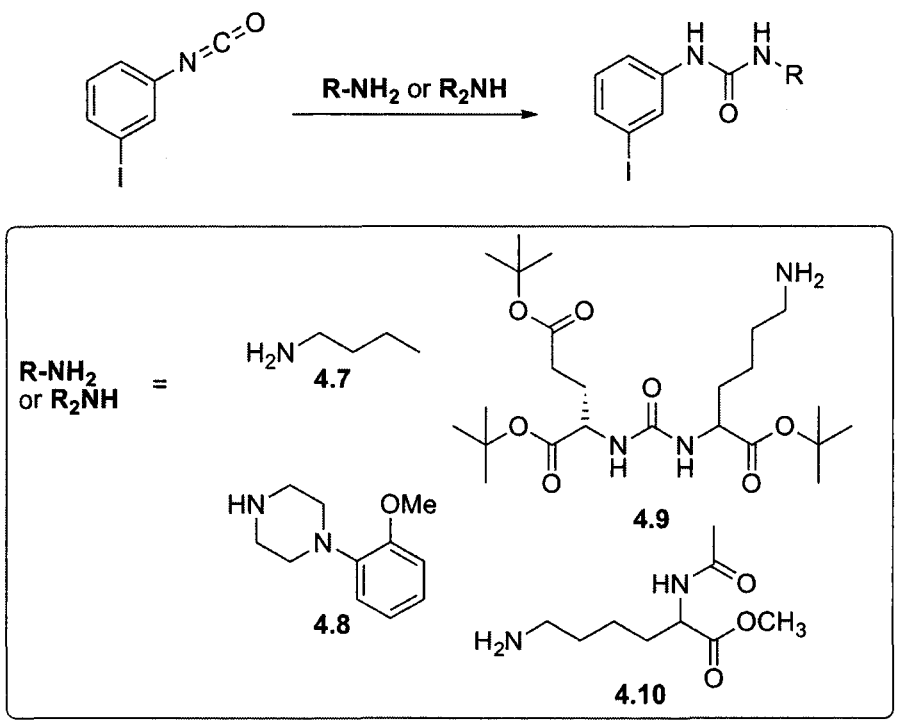
Figure 4-10. ^{1}H NMR spectrum of **4.6** (600.13 MHz, $CDCl_3$)

With the intricacies of the conjugation chemistry worked out for the preparation of compound **4.6**, the same methods were applied to the synthesis of the fluorous heterodimer **4.5**. The desired product was purified by silica gel chromatography using 30 % ethyl acetate in hexanes to afford a colorless oil. The ^{1}H NMR spectrum of the oil shows three distinct singlets (1.41, 1.39 and 1.36 ppm) which each integrate to 9 protons and are ascribed to each of the 3 *tert*-butyl ester protecting groups. Also consistent with the structure of **4.5** are the fluorous methylene signals appearing as a multiplet at 2.29 ppm and a triplet (J = 8.3 Hz). Two-dimensional NMR techniques (COSY, HSQC, HMBC) were used in conjunction with the ^{13}C NMR spectrum to assign the remaining

signals which was in good agreement with that expected for **4.5**. The high-resolution positive-ion electrospray mass spectrum of **4.5** had a peak corresponding to the $[M+H]^+$ ion at *m/z* = 1767.2902 which was in good agreement with the calculated value of 1767.2967.

4.6. Synthesis of iodinated compounds (4.7-4.10)

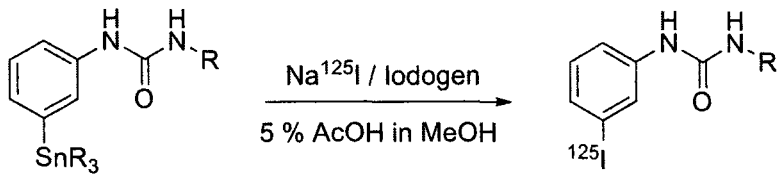
The preparation and complete characterization of non-radioactive standards are essential as they are used to verify the identity of the products resulting from radiolabelling reactions, which are prepared at the tracer level (10^{-9}- 10^{-12} M) and are not amenable to traditional methods of characterization. As such, the iodinated urea derivatives (**4.7** – **4.10**) were synthesized from iodophenyl-isocyanate and the respective amines as outlined in Scheme 4-8. Using stoichiometric amounts of the reagents afforded white solids in yields exceeding 70 % that required minimal purification. These compounds were then characterized by ^{1}H NMR, ^{13}C NMR and IR spectroscopy and mass spectrometry.



Scheme 4-8. Synthesis of iodo-urea standards

4.6.1 Radioiodination of conjugates

With the fluorous precursors and non-radioactive standards in hand, the radioiodination reactions were conducted with sodium [^{125}I]iodide and iodogen as previously described (Scheme 4-9). In all cases, the radiochemical yields of the desired iodo-ureas exceeded 87 % and good correlation was observed between the reference standard (UV) and the radio-HPLC traces (Figure 4-9), confirming the identities of the radioiodinated compounds.



4.3-4.6 **[^{125}I]4.7-[^{125}I]4.10**

Scheme 4-9. Radioiodination of urea conjugates using Na [^{125}I]I (R = $CH_2CH_2(CF_2)CF_3$)

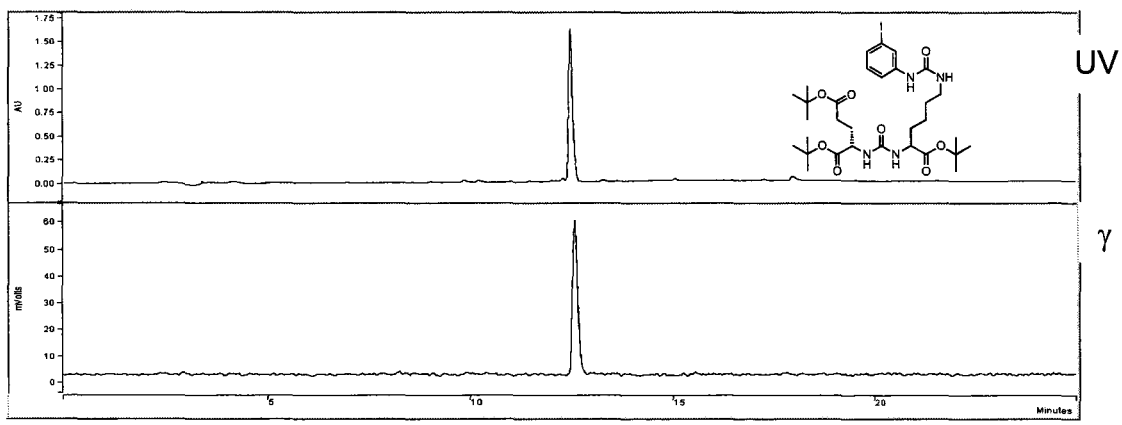


Figure 4-11. UV- and γ-HPLC chromatograms of non-radioactive **4.9** (R_t = 11.5 min) (*above*) and F-SPE purified [^{125}I]**4.9** (R_t = 11.8 min) (*below*)

Compound	RCY	RCP
4.7	88 ± 2 %	> 98 %
4.8	87 ± 4 %	> 98 %
4.9	91 ± 1 %	> 98 %
4.10	90 ± 2 %	> 98 %

Table 4-1. Summary of radioiodination yields and purities (*n = 3)*

4.7 Summary and conclusions

A fluorous arylisocyanate was prepared and its suitability as a prosthetic group for bioconjugation examined using simple model amines. Of particular interest was the increased stability of the fluorous isocyanate

synthon compared to organic isocyanates particularly when the former is dissolved in FC-72®. The potential utility of this synthon was demonstrated through the formation of small molecules currently under investigation as agents for imaging prostate cancer. Fluorous urea conjugates were radiolabelled with iodine-125 and purified using the fluorous purification methodology established in Chapter 2 to produce the desired radiotracers in good radiochemical yield and purity.

Ultimately, compound **4.1** represents a useful prosthetic group for radiolabelling biomolecules. It can be prepared in large batches, stored in fluorous solvent and used when needed and even shipped to groups interested in employing the FLS to prepare molecular imaging and therapy agents. Given the ease with which the FLS is applied, the technology can be utilized by groups that have minimal radiochemistry expertise or infrastructure.

4.8 Experimental

Reagents and General Procedures

All chemicals were purchased from Sigma Aldrich unless otherwise noted. Compound *m*-**2.9** was prepared as previously reported.[21] Sodium [^{125}I] iodide with a specific activity of ~17 Ci / mg was obtained from the McMaster Nuclear Reactor (Hamilton, ON).

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, AV 700 or AV 600 spectrometer with chemical shifts reported in ppm relative to the residual proton (^{1}H NMR) or carbon signal (^{13}C NMR) 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 where samples were first dissolved in methanol. High-resolution mass spectrometry data were obtained using a Waters-Micromass Q-TOF Ultima Global spectrometer. High-pressure 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 × 250 mm, 300 Å-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: Solvent A = CH_3CN (0.1 % H_3PO_4), Solvent B = H_2O (0.1 % H_3PO_4); 0-10 minutes 23 % A; 10-15 minutes 23 % A to 100 % A; 15-30 minutes 100 % A. The flow rate was maintained at 1 mL / min.

4.8.1. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl) benzoyl azide (4.2)

Sodium azide (30 mg, 461 μmol) was dissolved in 3 mL of a 1 % solution of water in acetone (*v/v*) and combined with 3-(*tris*[2-perfluorohexylethyl]stannyl)-tetrafluorophenol benzoate (*m*-**2.9**) (300 mg, 210 μmol) in acetone (10 mL). The resulting solution was stirred at room temperature overnight. The reaction solvent was then removed and the resulting residue dissolved in FC-72® and extracted with water (3 x 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: 273 mg, > 99 %. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 8.04 (m, 2H, Ar-*H*), 7.64 (m, 1H, Ar-*H*), 7.51 (m, 1H, Ar-*H*), 2.33 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.36 (t, *J* = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.77 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.

4.8.2. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl)phenyl isocyanate (4.1)

Compound **4.2** (200 mg, 154 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: 195 mg, > 99 %. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.37 (t, *J* = 7.6 Hz, 1H, Ar-*H*), 7.19 (d, *J* = 7.2 Hz, 1H, Ar-*H*), 7.12 (d, J = 7.9 Hz, 1H, Ar-*H*), 7.09 (s, 1H, Ar-*H*), 2.32 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.32 (t, *J* = 8.3 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 138.7, 134.3, 132.9, 131.6, 129.9, 125.8 (2C), 27.6, -1.2. FTIR (KBr, cm^{-1}): 2272, 1585.

4.8.3. 3-(*tris*[2-perfluorohexylethyl]stannyl)phenyl isocyanate: Stability Study

3-(*Tris*[2-perfluorohexylethyl]stannyl)benzoyl azide (**4.2**) (100 mg; 77μmol) was dissolved in FC-72® and heated at reflux for 16 hours. After cooling to room temperature a small sample of the reaction mixture was collected for spectroscopic analysis. 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 using IR spectroscopy. No decomposition was observed for up to 3 weeks.

4.8.4. General procedure for biphasic conjugation reactions (4.3, 4.4)

To a solution of 3-(*tris*[2-perfluorohexylethyl]stannyl)phenyl isocyanate in FC-72® (200 mg, 157 μmol) was added the amine of interest (300 μmol), dissolved 2 mL of one of the following: (A) $Na_2CO_{3(aq)}$ (0.5 M), (B) PBS (pH 7.4), (C) distilled deionized H_2O, (D) no solvent. 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 were obtained irrespective of the solvent used to dissolve the amine.

*4.8.4.1. Synthesis of 1-butyl-(3-tris[2-perfluorohexylethyl]stannyl)phenyl) urea (**4.3**)*

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, Ar-*H*), 7.32 (m, 2H, Ar-*H*), 7.09 (d, J = 6.3, 1H, Ar-*H*), 3.21 (t, J = 7.0 Hz, 2H, $NHCH_2CH_2CH_2CH_3$), 2.43 (m, 6H,

$SnCH_2CH_2C_6F_{13}$), 1.53 (m, 2H, $NHCH_2CH_2CH_2CH_3$), 1.42 (m, 2H, $NHCH_2CH_2CH_2CH_3$), 1.35 (t, J = 8.4 Hz, 6H, $SnCH_2CH_2C_6F_{13}$), 0.97 (t, J = 7.4 Hz, 3H, $NHCH_2CH_2CH_2CH_3$). ^{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.

4.8.4.2. Synthesis of 4-(2-methoxyphenyl)-N-(3-tris[2-perfluorohexylethyl]stannyl-phenyl)-piperazine-1-carboxamide ***(4.4)***

Yield: 211 mg, 92 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.78. ^1H NMR (500.13 MHz, CD_3OD) δ: 7.62 (s, 1H, Ar-*H*), 7.47 (m, 2H, Ar-*H*), 7.22 (d, *J* = 6.9 Hz, 1H, Ar-*H*), 7.05 (m, 4H, Ar-*H*), 3.96 (s, 3H, OCH_3), 3.78 (t, *J* = 5.0 Hz, 4H, $N(CH_2CH_2)_2NAr(OCH_3)$), 3.14 (t, *J* = 5.0 Hz, 4H, $N(CH_2CH_2)_2NAr(OCH_3)$), 2.51 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.43 (t, *J* = 8.4 Hz, 6H, $SnCH_2CH_2C_6F_{13}$). ^{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.

4.8.5. General procedure for single-phase conjugation reactions with 4.1 (4.5, 4.6)

To a solution of 3-(*tris*[2-perfluorohexylethyl]stannyl)phenyl isocyanate (**4.1**) (200 mg, 156 μmol) in PFBME (3 mL) was added the amine (300 μmol), dissolved in methanol (1 mL) containing 5 % triethylamine (*v/v*). The resulting mixture stirred vigorously for 2 hours when the reaction solvent was removed by rotary evaporation. Fluorous products were isolated using liquid-liquid extraction FC-72® and water. Following concentration of the fluorous layer, the desired product was immediately purified by silica gel chromatography.

*4.8.5.1. Conjugation to Nα-Acetyl-Lys-OMe: Synthesis of **4.6***

Purified using 10 % ethanol in chloroform. 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, Ar-*H*), 7.33 (s,1H, N*H*,), 7.32 (m, 1H, Ar-*H*), 7.21 (m, 1H, Ar-*H*), 6.98 (d, *J* = 7.0 Hz, 1H, Ar-*H*), 6.48 (d, *J* = 7.8 Hz, 1H, N*H*), 5.48 (m, 1H, N*H*), 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 $C_{40}H_{34}F_{39}N_3O_4Sn$ $[M+H]^+$: 1483.1105. Found: 1483.1005. FTIR (KBr, cm^{-1}): 3314, 2952, 2943, 2868, 1747, 1667, 1554.

4.8.5.2. Conjugation to Glu-U-Lys (tri-^{t}Bu ester): Synthesis of ***4.5***

Purified using 30 % ethyl acetate in hexanes. 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, Ar-*H*), 7.79 (s, 1H, N*H*), 7.26 (m, 1H, Ar-*H*), 7.18 (m, 1H, Ar-*H*), 6.92 (d, *J* = 7.1 Hz, 1H, Ar-*H*), 6.34 (m, 2H, N*H*), 5.71 (d, J = 6.6 Hz, 1H, N*H*), 4.32 (m, 1H, H-5), 4.00 (m, 1H, H-6), 3.50 (m, 1H, H-1), 3.01 (m, 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.

4.8.6. General procedure for conjugation reactions with iodophenyl-isocyanate

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 obtained that required no further purification.

*4.8.6.1. Synthesis of 1-butyl-3-(iodophenyl)urea (**4.7**)*

Yield: 92 mg, 95 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.59. HPLC: R_t = 10.3 min. ^{1}H NMR (500.13 MHz, CD_3OD) δ: 7.87 (s, 1H, Ar-*H*), 7.28 (m, 2H, Ar-*H*), 6.98 (m, *J* = 8.0 Hz, 1H, Ar-*H*), 3.18 (t, *J* = 7.0 Hz, 2H, Ar-NH-CO-NHCH_2CH$_2$CH$_2$CH$_3$), 1.51 (m, 2H, Ar-NH-CO-NHCH$_2$CH_2CH$_2$CH$_3$), 1.39 (m, 2H, Ar-NH-CO-NHCH$_2$CH$_2$CH_2CH$_3$), 0.96 (t, *J* = 7.33 Hz, 3H, Ar-NH-CO-NHCH$_2$CH$_2$CH$_2$CH_3). ^{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.

*4.8.6.2. Synthesis of 4-(2-methoxyphenyl)-N-(3-iodophenyl)-piperazine-1-carboxamide (**4.8**)*

Yield: 127 mg, 95 %. TLC (9:1 $CHCl_3$: EtOH) R_f = 0.7. HPLC: R_t = 14.1 min. ^1H NMR (600.13 MHz, CD_3OD) δ: 7.87 (s, 1H, Ar-*H*), 7.38 (m, 2H, Ar-*H*), 6.98 (m, 5H, Ar-*H*), 3.86 (s, 3H, OCH_3), 3.69 (t, *J* = 5.0 Hz, 4H, $N(CH_2CH_2)_2NAr(OCH_3)$), 3.06 (t, *J* = 5.0 Hz, 4H, $N(CH_2CH_2)_2NAr(OCH_3)$). ^{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.

*4.8.6.3. Derivatization of 3-iodophenyl isocyanate with Nα-Acetyl Lys-OMe: Synthesis of **4.9***

Nα-Acetyl-lysine methyl ester (38 mg; 204 μmol) was dissolved in methanol containing 5 % triethylamine (*v/v*) (1 mL). To this was added 3-iodophenyl isocyanate (50 mg; 204 μmol). The reaction stirred at room temperature for 6 h at which point solvent was removed by rotary evaporation. **4.9** was purified by silica gel chromatography. The product was eluted with a 10 % solution of ethanol in chloroform (*v/v*)). Yield: 59 mg, 65 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.61. HPLC: R_t = 6.5 min. ^1H NMR ($CDCl_3$, 600.13 MHz) δ: 7.80 (s, 1H, Ar-*H*), 7.76 (s, 1H, N*H*), 7.27 (m, 2H,

Ar-*H*), 6.92 (t, *J* = 8.0 Hz, 1H, Ar-*H*), 6.81 (d, *J* = 7.4 Hz, 1H, N*H*), 5.80 (m, 1H, N*H*), 4.44 (m, 1H, H-5), 3.70 (s, 3H, H-6), 3.18 (m, 2H, H-1), 2.0 (s, 3H, H-7), 1.76 (m, 2H, H-2), 1.48 (m, 2H, H-4), 1.36 (m, 2H, H-3). ^{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.

4.8.6.4. Derivatization of 3-iodophenyl isocyanate with Glu-U-Lys: Synthesis of ***4.10***

Prepared in an analogous fashion to **4.9**. Yield: 84 mg, 56 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.7. HPLC: R_t = 11.5 min. ^1H NMR ($CDCl_3$, 500.13 MHz) δ: 7.90 (s, 1H, N*H*), 7.87 (t, *J* = 2.1 Hz, 1H, Ar-*H*), 7.43 (ddd, *J* = 8.4 Hz, 2.1 Hz, 1.0 Hz, 1H, Ar*H*), 7.24 (ddd, *J* = 8.4 Hz, 2.1 Hz, 1.0 Hz, 1H, Ar*H*), 6.94 (t, *J* = 7.7 Hz, 1H, Ar*H*), 6.41 (d, *J* = 7.7 Hz, 1H, N*H*), 6.30 (br m, 1H, *NH*), 5.78 (d, *J* = 7.0 Hz, 1H, NH), 4.28 (ddd, *J* = 9.8 Hz, 7.7 Hz, 4.9 Hz, 1H, H-5), 3.98 (ddd, *J* = 10.0 Hz, 6.7 Hz, 3.5 Hz, 1H, H-6), 3.52 (dddd, *J* = 13.8 Hz, 7.4 Hz, 7.0 Hz, 4.2 Hz, 1H, H-1), 3.04 (ddd, *J* = 17.3 Hz, 8.4 Hz, 3.5 Hz, 1H, H-1), 2.39 (m, 2H, H-7), 2.08 (dddd, *J* = 14.5 Hz, 9.5 Hz, 8.4 Hz, 4.9 Hz, 1H, H-2), 1.89 (m, 1H, H-2), 1.74 (tt, *J* = 6.3 Hz, 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, H4). ^{13}C NMR (156.77 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.

4.8.7. Radiochemistry

General radioiodination procedure

Compounds **4.3**, **4.4**, **4.5**, and **4.6** (0.5 mg) were added to a sample vial coated with iodogen (20 μL, 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 minutes, the reaction was quenched by the addition of aqueous sodium metabisulfite ($Na_2S_2O_5$, 10 μL, 231 mM). 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 HPLC where in all cases, one radioactive peak was observed. When co-injected with the non-radioactive standards, the peaks co-eluted.

1-Butyl-3-([^{125}I]iodophenyl)urea ([^{125}I]**4.7**): R_t: 10.6 min; RCY: 88 %; Radiochemical Purity: >98 %.

4-(2-Methoxyphenyl)-N-(3-[^{125}I]iodophenyl)-piperazine-1-carboxamide ([^{125}I]**4.8**): R_t: 14.4 min; RCY: 87 %; Radiochemical Purity: >98 %.

3-[^{125}I]Iodophenyl isocyanate conjugation with Nα-Acetyl Lys-OMe ([^{125}I]**4.9**): R_t: 6.9 min; RCY: 91 %; Radiochemical Purity: >98 %.

3-[^{125}I]Iodophenyl isocyanate conjugation with Glu-U-Lys ([^{125}I]**4.10**): R_t: 11.8 min; RCY: 90 %; Purity: >98 %.

4.9. References

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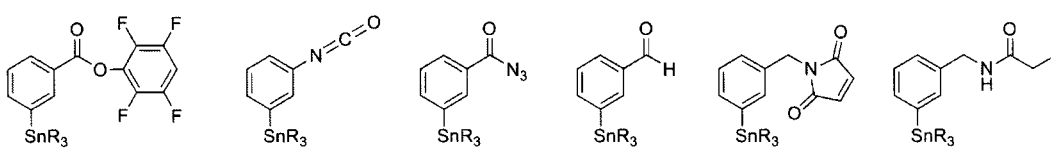
Chapter 5: Preparation of the second generation of fluorous synthons for bioconjugation

5.1. Introduction

The synthesis and conjugation of fluorous analogues of two very common prosthetic groups (active benzoic acid esters and aryl isocyanates) have been described. In order to make the FLS a truly general method for labelling biomolecules a greater number of fluorous tagging synthons are needed. This is a result of the fact that no single functional group (*e.g.* isocyanates) will react effectively with all classes of biomolecules. As a result, a more diverse collection of fluorous tagging groups was prepared (Figure 5-1) and their reactivity with model compounds investigated.



m-**2.9** **4.1** **4.2** *m*-**5.1** **5.2** **5.3**

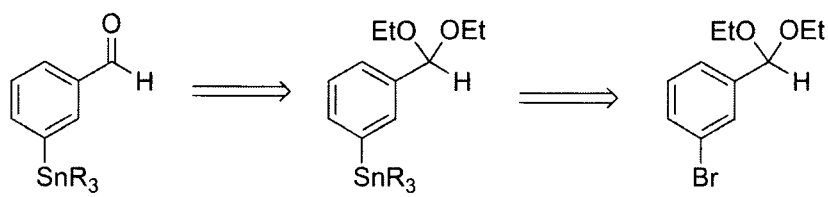
Figure 5-1. Proposed fluorous prosthetic groups (R = $CH_2CH_2(CF_2)_5CF_3$)

5.2. Tris[2-perfluorohexylethyl]stannyl) benzaldehyde

Aryl aldehydes are attractive choices for modifying small molecules and biomolecules as they are readily introduced into diverse scaffolds at free amine-sites under mild conditions. The resulting Schiff-base can be irreversibly reduced to stable amine functional groups under mild conditions compatible with a number of sensitive functional groups.[1]

5.2.1. Synthesis and characterization

The synthetic strategy for preparing *m*-**5.1** and *p*-**5.1** was based on the retrosynthesis outlined below (Figure 5-2). This particular approach was selected because it is not possible to simply reduce a benzoic acid derivative (*m*-**2.1** or *p*-**2.1**) since the aryl-tin bond is susceptible to cleavage by metal hydrides.[2]

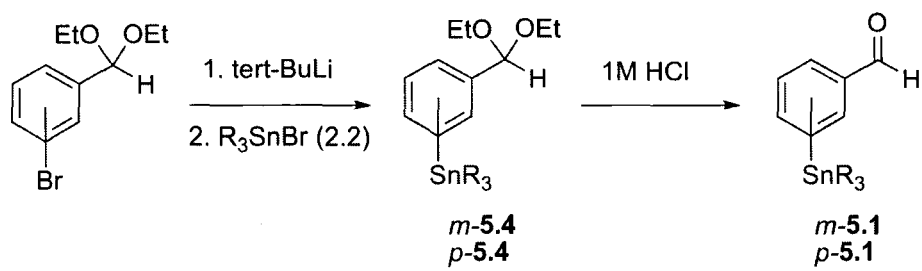


m-**5.1** *m*-**5.4**

Figure 5-2. Retrosynthesis of *m*-**5.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

3-Bromobenzaldehyde diethyl acetal (Aldrich) was dissolved in Et_2O, and treated with two mole equivalents of *tert*-butyllithium at -100 °C (Scheme 5-1). After 40 min, *tris*(2-perfluorohexylethyl)stannyl bromide (**2.2**) was added and fluorous components were subsequently extracted

into perfluorinated hexanes (FC-72®). Following evaporation, a clear colorless oil (**5.4**) was obtained and treated with 1M hydrochloric acid in order to hydrolyze the acetal protecting groups and generate compound **5.1**.[3] Despite heterogeneous reaction conditions, which was due to the poor solubility of **5.4** in the reaction solvent, deprotection was complete after 6 h as indicated by performing TLC analysis on a small amount of oil removed from the reaction.



Scheme 5-1. Synthesis of compound **5.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

Compound **5.1** was subsequently purified by automated silica gel chromatography using a gradient of ethyl acetate and hexanes to remove any residual traces of **2.2**. The product **5.1** was isolated in 50 % yield and characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, FTIR). The data collected were consistent with the structure of the desired product. The ^{13}C NMR spectrum (Figure 5-3) for instance showed a resonance at 192.2 ppm which corresponds closely to carbonyl carbon signals in analogous compounds[3] but is distinctly different from the signal in the benzoic acid, *m*-**2.1**, which appears at *ca.* 170 ppm. In addition to the appropriate number of signals in the aromatic region (6), the

characteristic signals of the fluorous methylene carbon atoms appear at 27.8 and -0.8 ppm.

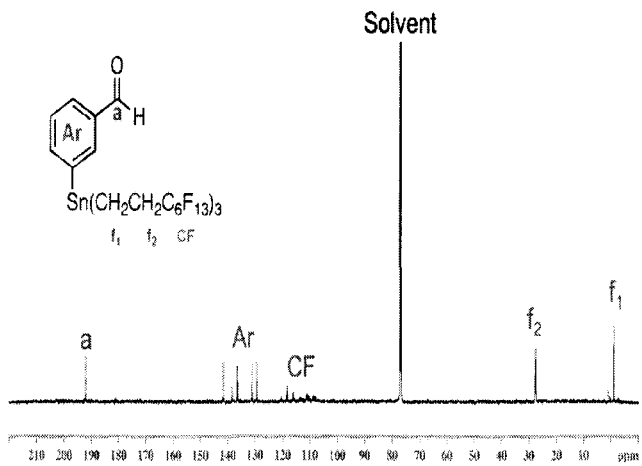
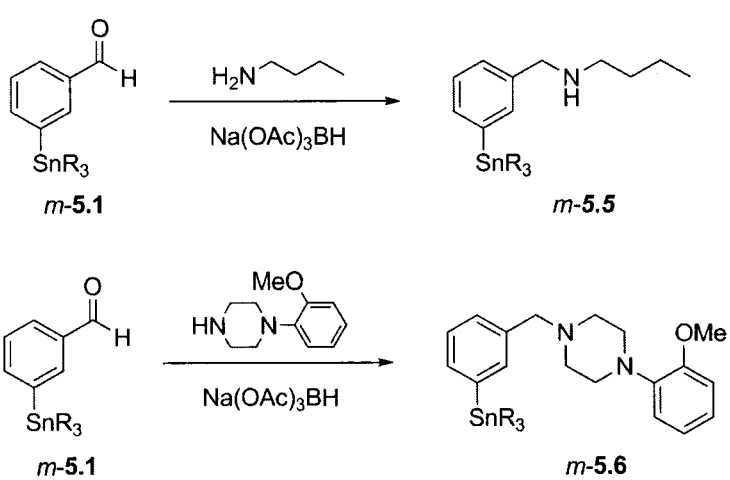


Figure 5-3. ^{13}C NMR spectrum of fluorous benzaldehyde *m*-**5.1** (125.77 MHz, $CDCl_3$) (R = $CH_2CH_2(CF_2)_5CF_3$)

5.2.2 Conjugation chemistry: model system

Reductive amination is a common method employed to conjugate an aldehyde-containing molecule to compounds such as proteins and peptides that contain amine functionalities. To establish the reactivity of *m*-**5.1** and *p*-**5.1** for reactions of this type, representative reductive aminations were conducted using *n*-butylamine and 1-(2-methoxyphenyl)piperazine (primary and secondary amines). Compound **5.1** and the desired amine were combined in chloroform in the presence of a 3-fold excess of sodium triacetoxyborohydride under an inert (argon) atmosphere.[1] Initially, the reaction mixture was stirred at room temperature for 90 min, prior to quenching the reaction (Scheme 5-2).

After liquid-liquid extraction using perfluorinated hexanes and water, ^{1}H NMR spectroscopy revealed that the imine-intermediate was the major species present where a singlet appearing at 8.3 ppm arising from the *H*-C=N proton was evident.[4] Extending the reaction time to 18 h with an additional mole equivalent of triacetoxyborohydride present resulted in the formation of the desired compound **5.5** or **5.6**. The "sluggish" reaction kinetics were likely a result of the low solubilities of the fluorous components and the heterogeneous reaction conditions. Performing the reactions in a 1:1 mixture of perfluorinated hexanes and tetrahydrofuran resulted in a small reduction in reaction time (12 h). The reductive amination product was ultimately isolated *via* liquid-liquid extraction using FC-72® and water. The resulting oil was subsequently purified by silica gel chromatography, affording products **5.5** and **5.6** in 80 and 85 % yield respectively.



m-**5.1** *m*-***5.5*** *m*-**5.1** *m*-**5.6**

Scheme 5-2. Reductive amination reactions involving compound *m*-**5.1** (R = $CH_2CH_2(CF_2)_5CF_3$)

As a representative example, the ^{1}H NMR spectrum of *m*-**5.5** (Figure 5-4) shows a multiplet centered around 7.36 ppm resulting from the multiple aromatic protons. The benzylic protons appear as a singlet at 3.79 ppm, and the aliphatic protons are identified as triplets at 2.64 and 0.90 ppm with coupling constants of 7.0 and 7.3 Hz, respectively. The characteristic fluorous methylene protons are present at 2.31 and 1.37 ppm and all the integrations are consistent with the identity of the expected product. Mass spectral analysis of the reaction product shows a major peak with a mass-to-charge ratio of 1324.0653 which is consistent with the $[M+H]^+$ ion of compound *m*-**5.5** (calc. 1324.0699).

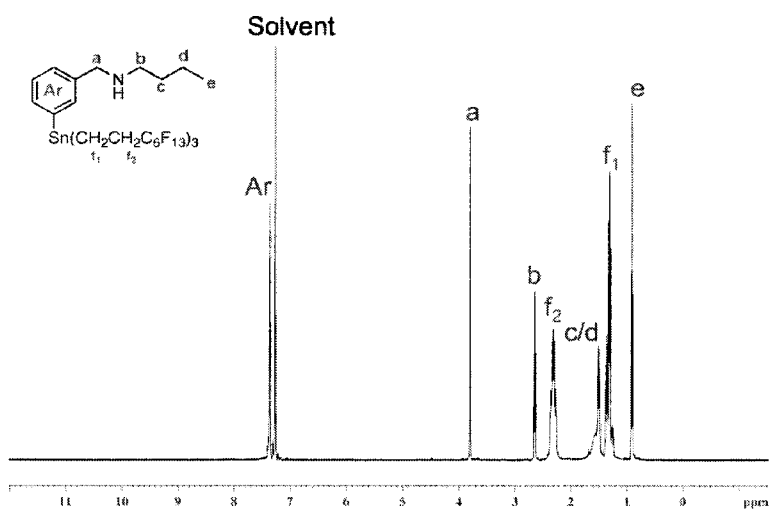
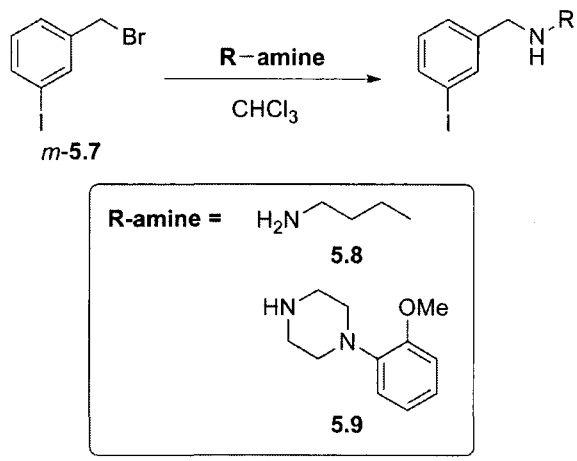


Figure 5-4. ^{1}H NMR spectrum of *m*-**5.5** (500.13 MHz, $CDCl_3$) (R = $CH_2CH_2(CF_2)_5CF_3$)

5.2.3. Synthesis and characterization of iodinated reference standards

Non-radioactive iodinated aryl amines were prepared according to Scheme 5-3. 3- and 4-iodobenzyl bromide (*m*-**5.7** and *p*-**5.7**) were dissolved in chloroform and reacted separately with two mole equivalents of the respective amine in the presence of triethylamine. The mixtures were heated at reflux overnight (70 °C). After cooling, the reaction solvent was removed and the resultant yellow residue was subsequently purified by silica gel chromatography. The desired products were obtained by eluting with a 10 % solution of ethanol in chloroform. The identities of the products were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy, IR spectroscopy and electrospray mass spectrometry. The ^{13}C NMR of compound *m*-**5.8** (Figure 5-5) contains the expected number of aromatic signals, including one at lower chemical shift (95 ppm *c.f.* 125-143 ppm for other resonances) representing the carbon atom bearing the iodine atom, and 5 aliphatic peaks including two appearing at 53 and 49 ppm attributed to the methylene carbons flanking the amine nitrogen atoms. The spectral data for *p*-**5.8** were similarly consistent with that expected for the desired product.



Scheme 5-3. Synthesis of non-radioactive reference standards

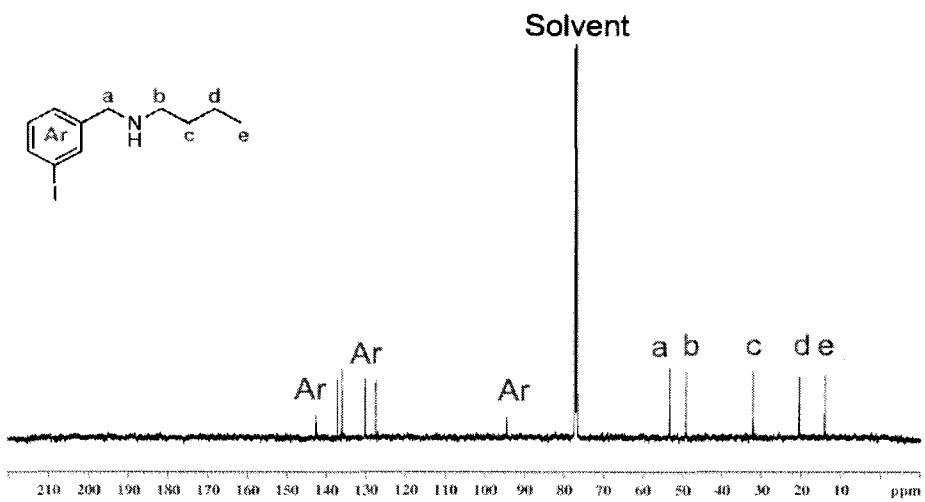
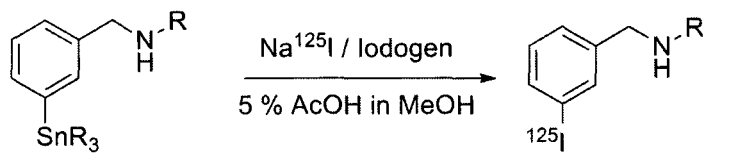


Figure 5-5. ^{13}C NMR spectrum of *m*-**5.8** (125.77 MHz, $CDCl_3$)

5.2.4. Radioiodination of fluorous arylamines

Following UV HPLC analysis which confirmed the purity of all fluorous precursors and non-radioactive reference standards, radioiodination reactions were conducted in a manner similar to those described previously (Scheme 5-4). In short, the precursor was dissolved in a methanolic soution containing 5 % acetic acid which was

subsequently added to an iodogen-coated vial. After 5 minutes the reaction was quenched by the addition of sodium metabisulfite and the reaction mixture purified by fluorous solid phase extraction. Radiochemical yields for all fluorous reductive amination products exceeded 80 % and the radiochemical purities were greater than 98 % as determined by HPLC (Figure 5-6).



m-**5.5**, *m*-**5.6**
p-**5.5**, *p*-**5.6**

m-[^{125}I]**5.7**, *m*-[^{125}I]**5.8**
p-[^{125}I]**5.7**, *p*-[^{125}I]**5.8**

Scheme 5-4. Radiolabelling of reductive amination products

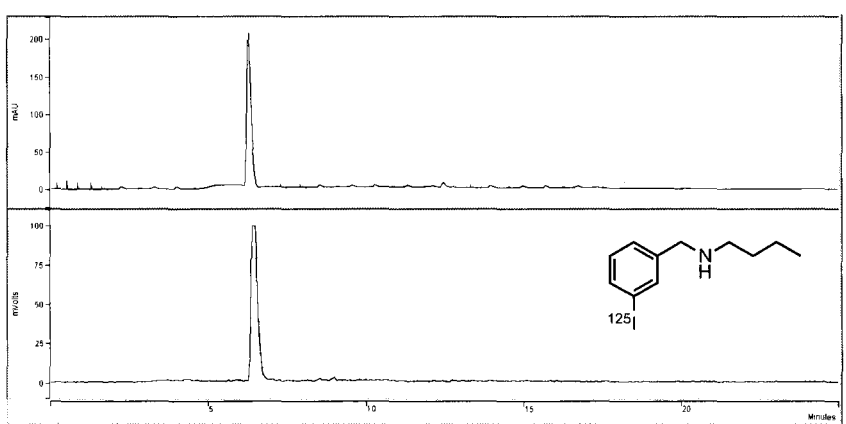


Figure 5-6. HPLC chromatograms of *m*-**5.7** (*above*) and *m*-[^{125}I]**5.7** (*below*)

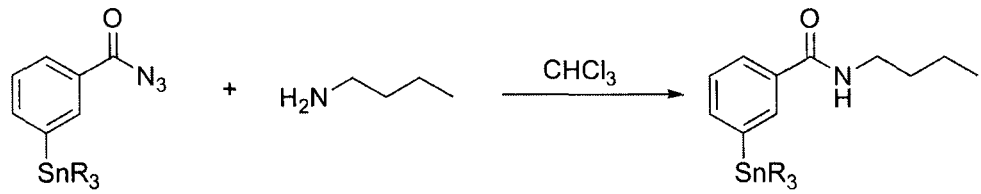
Compound	RCY	RCP
m-**5.7**	92 ± 2%	> 98 %
m-**5.8**	83 ± 5%	> 98 %

Table 5-1. Summary of radioiodination yields and purities (n = 2)

5.3. 3-(Tris[2-perfluorohexylethyl]stannyl) acyl azide (4.2)

5.3.1. Conjugation chemistry: model system

An acyl azide is an activated carboxylate group that can react with a primary amine to form an amide linkage. Coupling reactions occur by attack of the nucleophile at the electrophilic carbonyl carbon atom, and are best performed at an alkaline pH (8.5-10) in the absence of any other competing bases or nucleophiles.[5] The preparation of **4.2** was discussed previously as an intermediate formed in the synthesis of the fluorous isocyanate, **4.1**. To investigate the potential of conducting conjugation reactions with this functional group, compound **4.2** was dissolved in FC-72® and neat *n*-butylamine was added (Scheme 5-5). The reaction mixture was stirred vigorously for 1.5 h after which the resulting fluorous benzamide was isolated *via* liquid-liquid extraction from perfluorinated hexanes. Following purification by silica gel chromatography, the product **4.2** was characterized by NMR spectroscopy (^{1}H and ^{13}C), IR spectroscopy and high resolution mass spectrometry which confirmed that the structure of the product was identical to that prepared by the active ester route discussed previously (Section 2.8.7).



4.2 *m*-**2.18**

Scheme 5-5. Synthesis of *m*-**2.18** (R = $CH_2CH_2(CF_2)_5CF_3$)

5.3.2. Radiochemistry

The fluorous benzamide (*m*-**2.18**) resulting from the conjugation of **4.2** was radioiodinated using Na[^{125}I]I in the presence of iodogen. The desired radiotracer was prepared in good yields (89 %) and high purity (> 96 %) as demonstrated in Figure 5-7. Despite these positive results, this synthon was not pursued further since less byproducts were obtained for the fluorous benzamides prepared *via* tetrafluorophenol active esters as discussed earlier (Section 2.8.7).

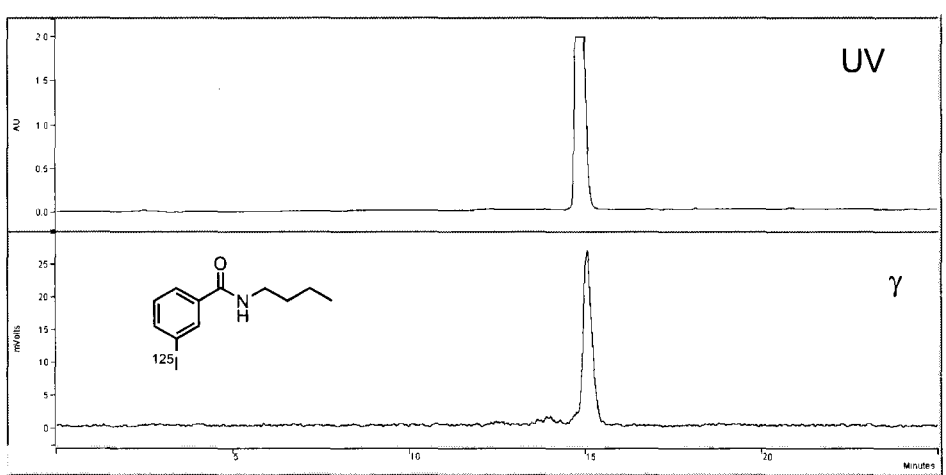
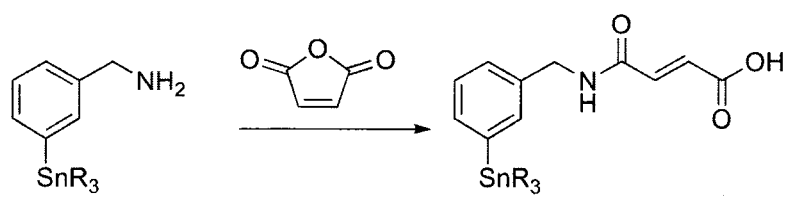


Figure 5-7. UV-and γ-HPLC chromatograms resulting from the radioiodiation of benzamide, *m*-**2.18**, reference standard (*above*), radiolabelled product (*below*).

5.4. 1-(3-(*Tris*[2-perfluorohexylethyl]stannyl)benzyl)-1H-pyrrole-2,5-dione

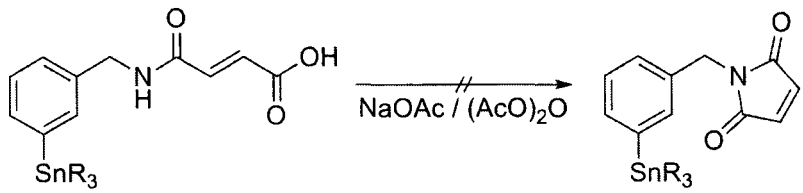
Maleimide derivatives react selectively with protein sulfhydryl groups at moderate pH values (6.5 - 8.5) and have proven useful as general conjugating reagents.[6] Literature reports propose the use of radioiodinated maleimide analogues as a suitable method for tagging either pH sensitive proteins or those whose lysine residues are unavailable for conjugation either due to chemical modification or because they are critical for protein function.[6] The first step in preparing the fluorous maleimide **5.2** was treating benzylamine **3.2** with maleic anhydride according to Scheme 5-6.[6]



3.2 **5.11**

Scheme 5-6. First step in the synthesis of fluorous maleimide **5.2** (R = $CH_2CH_2(CF_2)_5CF_3$)

Compound **3.2** was dissolved in diethyl ether and was stirred overnight in the presence of maleic anhydride. Solvent evaporation, followed by liquid-liquid extraction afforded an off-white solid; the product **5.11** was subsequently suspended in a mixture of acetic anhydride and sodium acetate (Scheme 5-7) and heated for 4 h at 90 °C prior to addition of FC-72®.



5.11 **5.2**

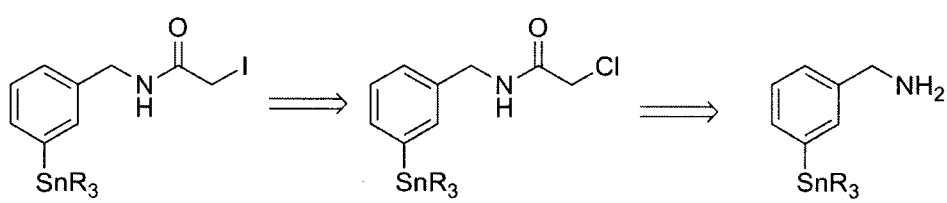
Scheme 5-7. Attempted synthesis of fluorous maleimide, **5.2** (R = $CH_2CH_2(CF_2)_5CF_3$)

After liquid-liquid extraction the fluorous layers were combined and washed with water, followed by aqueous sodium carbonate. A small amount of a yellow-orange oil was recovered and ^1H NMR spectroscopy revealed that contrary to the anticipated formation of **5.2** no aromatic signals were visible in the spectrum which contained primarily fluorous tin degradants. The reason for the observed protodestannylation of the benzylamine **3.2** is suspected to be due to a combination of trace amounts of acetic acid in the acetic anhydride, and poor solubility of the fluorous intermediate in the reaction medium. An attempt to improve the homogeneity of the reaction mixture by adding a small amount of FC-72® to the acetic anhydride mixture did not change the outcome of the reaction. For these reasons, the preparation of this prosthetic group was abandoned and we set out to synthesize another thiol-selective compound, a fluorous iodoacetamide derivative.

5.5. 2-Iodo-N-(3-(tris[2-perfluorohexylethyl]stannyl)benzyl)acetamide

Iodoacetamides can react with a number of functional groups on proteins including the thioether functionality in methionine, sulfhydryl group of cysteine, the ε-amine of lysine, histidine residues as well as N-terminal α-amines.[5] Both the specificity and rate of this conjugation depend strongly on the degree of ionization of the protein and pH of the reaction mixture; nevertheless, it has been determined that the relative rate of reactivity of α-haloacetamides such as **5.3** is as follows: sulfhydryl > imidazoyl > thioether > amine.[5] Because of this increased reactivity toward sulfhydryl cysteine residues, iodoacetamides can be directed specifically for –SH modification.

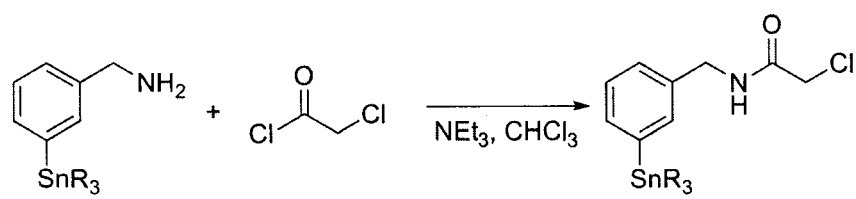
The synthetic approach to compound **5.3** was based on a simple retrosynthesis (Figure 5-8). The desired product can be prepared from the chloroacetamide which in turn is accessible from the benzylamine **3.2** reported in Chapter 3.



5.3 **5.12** **3.2**

Figure 5-8. Retrosynthesis of fluorous iodoacetamide, **5.3** (R = $CH_2CH_2(CF_2)_5CF_3$)

The fluorous benzylamine **3.2** was converted to **5.12** *via* treatment with chloroacetyl chloride in chloroform containing a 3-fold excess of triethylamine (Scheme 5-8).[7] At the end of the reaction a standard fluorous workup ensued, including liquid-liquid extraction between FC-72® and the reaction solvent (chloroform). From this, a dark brown oil was isolated and **5.12** was isolated by column chromatography in 65 % yield.



3.2 **5.12**

Scheme 5-8. Synthesis of chloroacetamide, **5.12** (R = $CH_2CH_2(CF_2)_5CF_3$)

The ^1H NMR spectrum of the isolated product (Figure 5-9) displayed a doublet at 4.5 ppm that was ascribed to the benzylic protons and a singlet of equal intensity at 4.1 ppm which arises from the methylene protons of the chloroacetyl group which helped confirm the structure of **5.12**.

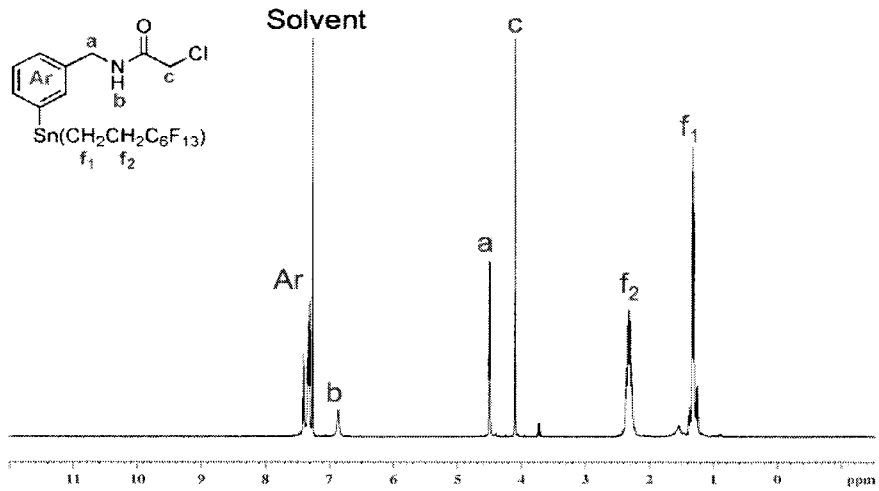
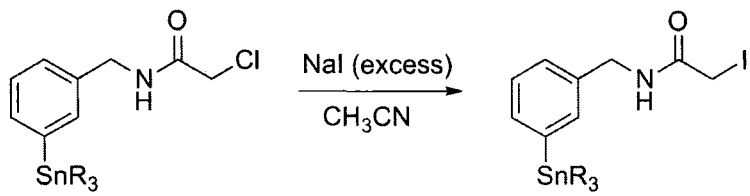


Figure 5-9. ^{1}H NMR spectrum of fluorous chloroacetamide **5.12** (500.13 MHz, $CDCl_3$) (R = $CH_2CH_2(CF_2)_5CF_3$)

5.5.1. Synthesis and characterization of 5.3

With compound **5.12** purified and completely characterized, conversion to the iodoacetamide (compound **5.3**) was achieved *via* a halogen exchange reaction. This was accomplished by heating the fluorous chloroacetamide in a solution of acetonitrile at reflux (85 °C) overnight in the presence of a large excess of sodium iodide, as depicted in Scheme 5-9. After purification by column chromatography, the product was characterized by NMR spectroscopy (^{1}H, and ^{13}C), IR spectroscopy and high-resolution mass spectrometry. There were significant differences in the aliphatic region of the ^{13}C NMR spectra of the chloro- and iodoacetamides (Figure 5-10). Specifically, the carbon atom α to the halide which resonates at 42.5 ppm in the chloracetamide **5.12**, shifts to -

1.1 ppm in the iodoacetamide. This phenomenon is due primarily to changes in inductive effects.[8]



5.12 **5.3**

Scheme 5-9. Synthesis of **5.3** (R = $CH_2CH_2(CF_2)_5CF_3$)

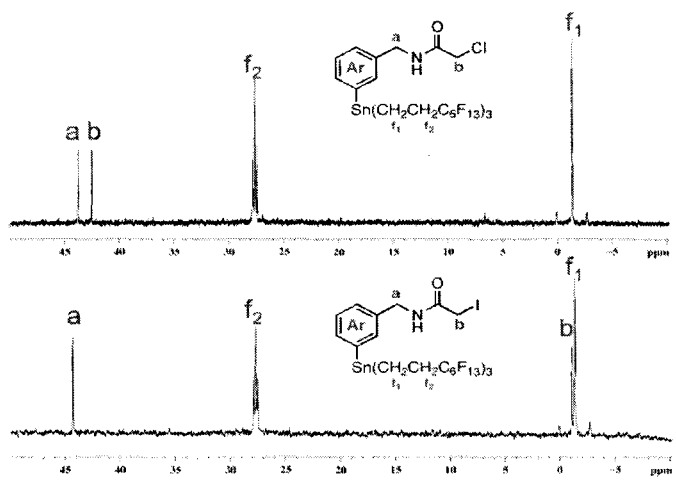
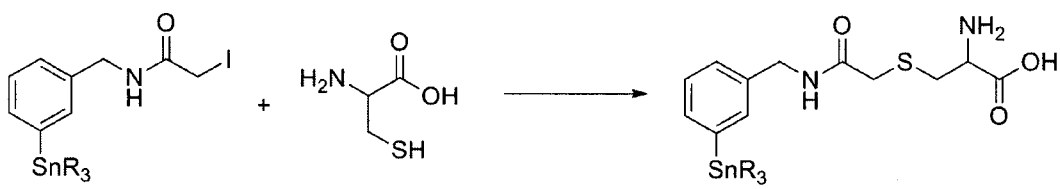


Figure 5-10. ^{13}C NMR spectra for chloro- (*above*) and iodoacetamide (*below*) (125.76 MHz, $CDCl_3$)

5.5.2. Conjugation of 3-(*tris*[2-perfluorohexylethyl]stannyl) iodoacetamide with L-cysteine

As mentioned previously, iodoacetamides react selectively with sulfhydryl residues in proteins and other biomolecules. To probe the reactivity and selectivity of this fluorous analogue, L-cysteine was chosen as a model system. Initial attempts at conjugation were using the biphasic

strategy presented in Section 4.4 were not successful. Ultimately conjugation was achieved by utilizing a 1:1 methanol / PFBME mixture, to improve the homogeneity of the reaction mixture (Scheme 5-10). After 2h, the reaction solvents were concentrated and the product isolated in excellent yield (95 %) by liquid-liquid extraction using perfluorinated hexanes and water.



5.3 **5.13**

Scheme 5-10. Conjugation of **5.3** with L-cysteine (R = $CH_2CH_2(CF_2)_5CF_3$)

The ^1H NMR data are presented in Figure 5-11. Of particular interest are the protons adjacent to the sulfur atom that originated from the amino acid component (annotated as **c** in Figure 5-11). They are diastereotopic and therefore appear as distinct signals showing both geminal coupling to each other (-13.5 Hz) and vicinal coupling (4.3 and 7.6 Hz) to the α proton.

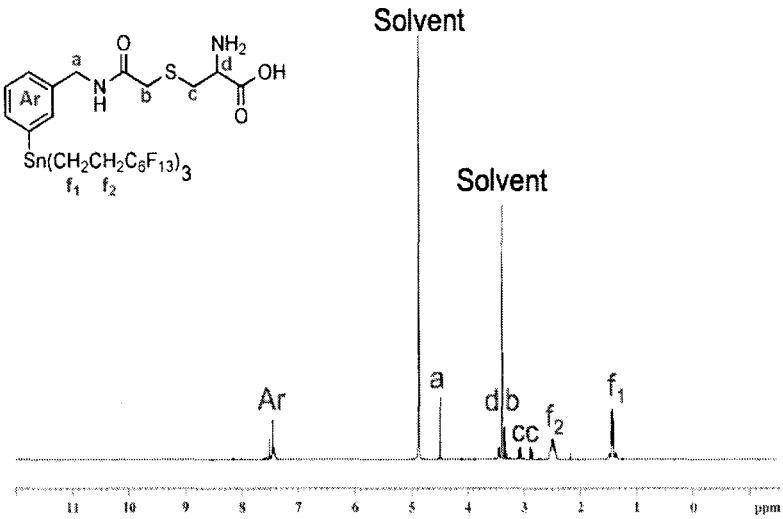
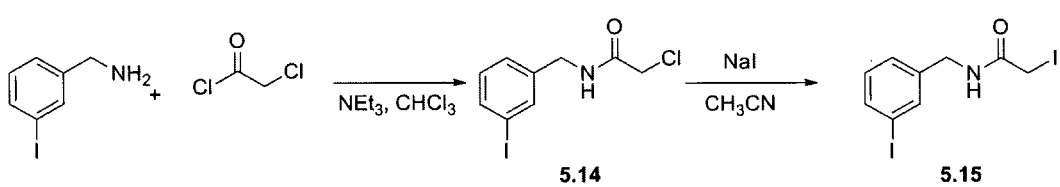


Figure 5-11. ^{1}H NMR of thioether conjugation product **5.13** (500.13 MHz, CD_3OD) (R = $CH_2CH_2(CF_2)_5CF_3$)

5.5.3. Synthesis of iodinated reference standards

The non-radioactive analogue of the iodoacetamide **5.15** was prepared as depicted in Scheme 5-11. The chloroacetamide was produced by reacting iodobenzylamine with chloroacetyl chloride in a solution of chloroform in the presence of triethylamine. Following purification *via* silica gel chromatography (61 % yield) and spectroscopic characterization, this derivative was subsequently heated to reflux with an excess of sodium iodide in acetonitrile affording, after purification, iodoacetamide **5.15** in 86 % isolated yield.



Scheme 5-11. Synthesis of non-radioactive reference standard **5.15**

Following the isolation of **5.15**, the iodoacetamide was combined with L-cysteine. Here, the iodoacetamide was stirred in the presence of the amino acid in a methanolic solution of potassium hydroxide (1M). After 1 h, HPLC analysis confirmed the complete consumption of the starting material. The reaction solvent was evaporated and the desired product isolated as a white solid. The purity of the product was determined initially by HPLC-mass spectrometry, which was consistent with the mass of **5.15**.

From the ^{1}H NMR spectrum (Figure 5-12) the cysteine protons adjacent to the sulfur atom were again diastereotopic, appearing as distinct signals showing both geminal coupling (-13.5 Hz) and vicinal coupling (4.3 and 7.6 Hz). Also apparent from the NMR spectrum are resonances due to the two benzylic protons appearing as a singlet at 4.32 ppm, and the four aromatic protons attributed to the singlet at 7.65 ppm, the doublets at 7.56 and 7.28 ppm (J = 7.7 and 7.6 Hz, respectively) and the triplet at 7.06 ppm (J = 7.8 Hz). The two remaining protons, namely the α- proton of the amino-acid and the acetamido- proton forming the linkage to sulfur are attributed to the signals at 3.34 and 3.27 ppm. These were assigned with the aid of two-dimensional NMR techniques (HSQC, HMBC).

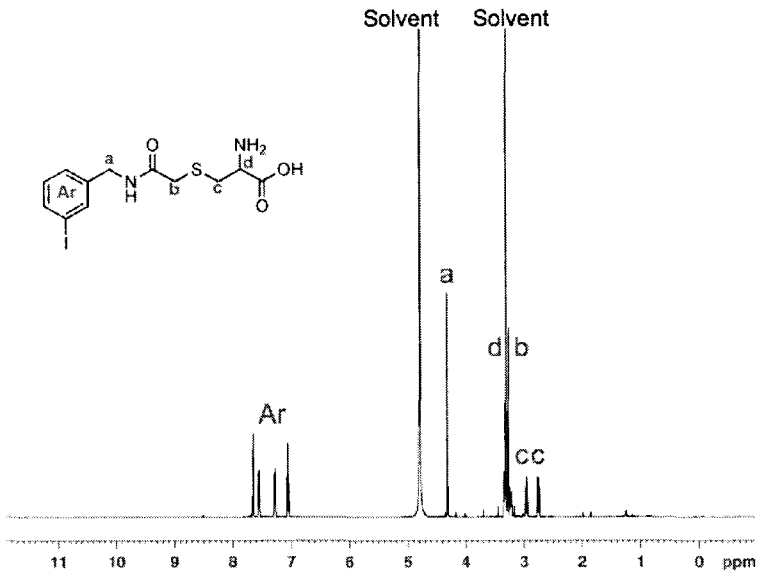


Figure 5-12. ^{1}H NMR spectrum of **5.16** (600.13 MHz, CD_3OD)

5.5.4. Radiolabelling

Radioiodination reactions and purification procedures were conducted using the previously developed methodology. Briefly, the fluorous precursor, **5.17** (0.4 μmol) was dissolved in a 5 % solution of acetic acid in methanol and was transferred to an iodogen-coated vial. Sodium [^{125}I]iodide was then added to the vessel which was agitated gently for 3 minutes prior to quenching with sodum metabisulfite. A small aliquot of the mixtures was then analyzed by UV- and γ-HPLC. The single radioactive peak correlated with its corresponding reference standard (**5.18**) (Figure 5-13). The radiochemical yield was determined to be 90 % and the purity of all products was greater than 98 % as determined by HPLC.

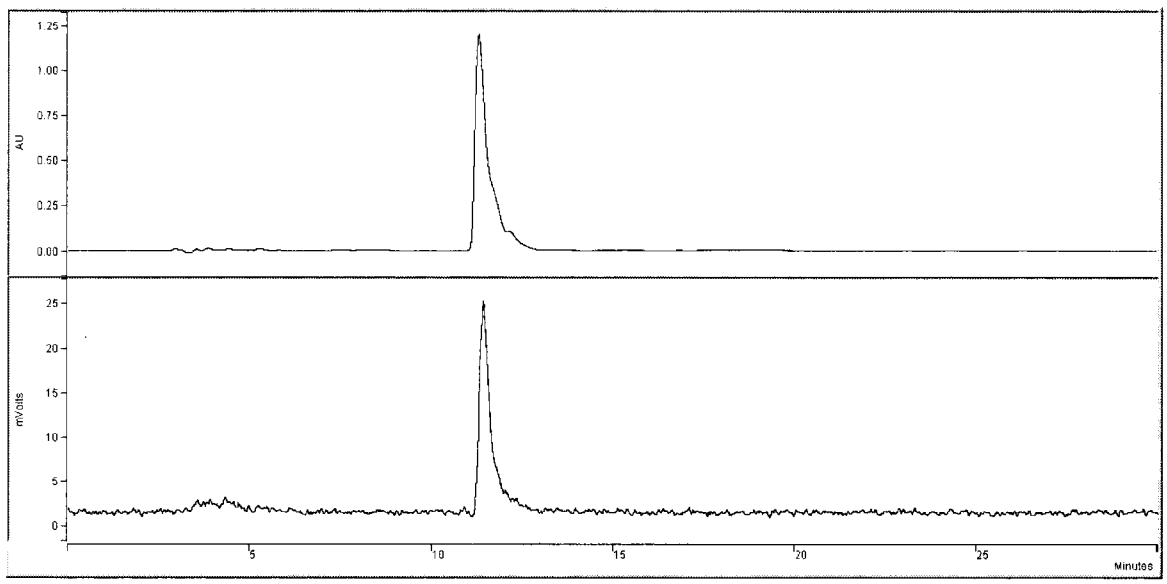


Figure 5-13. UV-(*top)* and γ-(*bottom*) HPLC chromatograms resulting from the radioiodiation of *m*-**5.18**, and *m*-[^{125}I]**5.18**.

5.6. Conclusions

To create a "tool kit" of fluorous reagents that can be used to label biomolecules, a series of new fluorous prosthetic groups were prepared and their conjugation chemistry examined using simple model systems. In all cases, conjugation reactions were conducted at room temperature and proceeded in high yields and the products readily isolated using standard methods that are compatible with biomolecules. The radiolabelling reaction similarly took place in high yield and products were readily isolated by fluorous SPE.

5.7. Experimental

Reagents and General Procedures

All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Compounds **2.2** and **3.2** were prepared as previously reported.[9, 10] Sodium [^{125}I]iodide with a specific activity of ~17 Ci / mg was obtained from the McMaster Nuclear Reactor (Hamilton, ON)

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 or AV 600 spectrometer with chemical shifts reported in ppm relative to the residual proton (^{1}H NMR) or carbon signal (^{13}C NMR) 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 where samples were first dissolved in methanol. High-resolution mass spectrometry data were obtained using a Waters-Micromass Q-TOF Ultima Global spectrometer. High-pressure 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 × 250 mm, 300 Å-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: Solvent A = CH_3CN (0.1 % H_3PO_4), Solvent B = H_2O (0.1 % H_3PO_4); 0-10 minutes 23 % A; 10-15 minutes 23 % A to 100 % A; 15-30 minutes 100 % A. The flow rate was maintained at 1 mL / min.

5.7.1. Synthesis of 3-(*tris*[2-perfluorohexylethyl]stannyl)benzaldehyde (*m*-5.1)

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 (1.00 ml, 1.7M, 1.71 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. The product was isolated by liquid-liquid extraction

using FC-72® (3 x 5mL) and dichloromethane (3x 10 mL). Purification by flash chromatography (5 % EtOAc in hexanes) afforded a clear, colorless oil. Yield: 440 mg, 35 %. TLC(CH_2Cl_2): R_f = 0.72. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 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 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.77 MHz) δ:193.0, 142.9 138.5, 137.9, 137.2, 130.3, 28.4, 0.1. FTIR (KBr, cm^{-1}): 2933, 2852, 1706, 1577.

5.7.2. Synthesis of 4-(*tris*[2-perfluorohexylethyl]stannyl) benzaldehyde (*p*-5.1)

Prepared in the same fashion as described for *m*-**5.1**. Yield: 377 mg, 30 %. TLC(CH_2Cl_2): R_f = 0.71. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 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 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.77 MHz) δ: 192.2, 146.1, 137.1, 136.5, 129.3, 27.6, -1.1. FTIR (KBr, cm^{-1}): 2933, 2831, 1707, 1592.

5.7.3. Aldehyde conjugation *via* reductive amination : general procedure

To a solution of 3- or 4-(*tris*[2-perfluorohexylethyl]stannyl) benzaldehyde (*m*-**5.1** or *p*-**5.1**) (75 mg, 61 μmol) in chloroform and was added an amine (234 μmol). The mixture was stirred for 2 h at which point sodium triacetoxyborohydride (50 mg, 234 μmol) was added to the mixture. The reaction mixture was stirred at ambient temperature for 16 h prior to removal of the reaction solvent under reduced pressure. The resulting white residue was suspended in FC-72® (10 mL) and washed once with $Na_2CO_{3(aq)}$ (1M, 20 mL) and twice with H_2O (2 x 20 mL). The fluorous layers were combined and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded a clear, colorless oil that was purified by silica gel chromatography. The desired products were eluted using a solution of 10 % ethanol in chloroform.

*5.7.3.1. Synthesis of N-butyl-3-(tris[2-perfluorohexylethy]stannyl) benzylamine (m-**5.5**)*

Yield: 140 mg, 85 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.30. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.37 (m, 4H, Ar-*H*), 3.79 (s, 2H, Ar-C*H*$_2$NHCH$_2$CH$_2$CH$_2$CH$_3$), 2.64 (t, *J* = 7.0 Hz, 2H, Ar-CH$_2$NHC*H*$_2$CH$_2$CH$_2$CH$_3$), 2.31 (m, 6H, SnC*H*$_2$CH$_2$C$_6$F$_{13}$ 1.51 (m, 3H, Ar-CH$_2$NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.37-1.27 (m, 8H, Sn(CH$_2$C*H*$_2$C$_6$F$_{13}$)$_3$, Ar-

$CH_2NHCH_2CH_2CH_2CH_3$), 0.90 (t, J = 7.3 Hz, 3H, Ar-$CH_2NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (125.77 MHz) δ: 141.0, 136.4, 135.6, 134.5, 129.2, 54.0, 49.2, 32.1, 27.7, 20.4, 13.8, -1.5. HRMS(+): mass calcd for $C_{35}H_{28}NF_{39}Sn$ $[M+H]^+$: 1324.0707. Found: 1324.0653. FTIR (KBr, cm^{-1}): 3437, 2961, 2929, 1239, 1207.

*5.7.3.2. Synthesis of N-butyl-4-(tris[2-perfluorohexylethyl]stannyl) benzylamine (p-**5.5**)*

Yield: 132 mg, 80 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.30. ^1H NMR (500.13 MHz, $CDCl_3$) δ: 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-$CH_2NHCH_2CH_2CH_2CH_3$), 2.66 (t, J = 7.3 Hz, 2H, Ar-$CH_2NHCH_2CH_2CH_2CH_3$), 2.30 (m, 6H, $SnCH_2CH_2C_6F_{13}$) 1.56 (m, 3H, Ar-$CH_2NHCH_2CH_2CH_2CH_3$), 1.33 (m, 8H, $Sn(CH_2CH_2C_6F_{13})_3$, Ar-$CH_2NHCH_2CH_2CH_2CH_3$), 0.91 (t, J = 7.3 Hz, 3H, Ar-$CH_2NHCH_2CH_2CH_2CH_3$). ^{13}C NMR (125.77 MHz) δ: 136.1, 129.0, 53.3, 48.7, 31.5, 27.7, 20.3, 13.8, -1.5. HRMS(+): mass calcd for $C_{35}H_{29}NF_{39}Sn$ $[M+H]^+$: 1324.0699. found: 1324.0653. FTIR (KBr, cm^{-1}): 3395, 2922, 1996, 1436, 1239.

*5.7.3.3. Synthesis of N-3-(tris[2-perfluorohexylethyl]stannyl)-1(2-methoxyphenyl)-piperazine (m-**5.6**)*

Yield: 142 mg, 79 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.70. ^1H NMR (500.13 MHz, $CDCl_3$) δ: 7.39 (m, 3H, Ar-*H*), 7.27 (m, 1H, Ar-*H*), 7.01-6.85 (m, 4H, Ar-*H*), 3.85 (s, 3H, Ar-CH_2-N(CH_2CH_2)$_2$N-Ar-OC*H*$_3$), 3.60 (s, 2H, Ar-C*H*$_2$-N(CH_2CH_2)$_2$N-Ar-OCH_3), 3.10 (s, 4H, Ar-CH_2-N(CH_2C*H*$_2$)$_2$N-Ar-OCH_3), 2.67 (s, 4H, Ar-CH_2-N(C*H*$_2CH_2$)$_2$N-Ar-OCH_3), 2.32 (m, 6H, Sn(CH_2C*H*$_2$$C_6F_{13}$)$_3$), 1.32 (t, *J* = 8.3 Hz, 6H, Sn(CH_2C*H*$_2$$C_6F_{13}$)$_3$). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 152.3, 141.4, 138.8, 136.6, 134.6, 130.5, 128.8, 122.9, 121.0, 118.2, 111.3, 63.0, 55.3, 53.3, 50.6, 27.7, -1.5. HRMS(+): mass calcd for $C_{42}H_{33}F_{39}N_2OSn$ $[M+H]^+$: 1443.1070. Found: 1443.1052. FTIR (KBr, cm^{-1}): 3422, 2940, 2817, 1501, 1238.

*5.7.3.4. N-4-(tris[2-perfluorohexylethyl])stannyl)-1(2-methoxyphenyl)-piperazine (p-**5.6**)*

Yield: 150 mg, 83 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.68. ^1H NMR (500.13 MHz, $CDCl_3$) δ: 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, Ar-CH_2-N(CH_2CH_2)$_2$N-Ar-OC*H*$_3$), 3.59 (s, 2H, Ar-C*H*$_2$-N(CH_2CH_2)$_2$N-Ar-OCH_3), 3.10 (s, 4H, Ar-CH_2-N(CH_2C*H*$_2$)$_2$N-Ar-OCH_3), 2.66 (s, 4H, Ar-CH_2-N(C*H*$_2CH_2$)$_2$N-Ar-OCH_3), 2.31 (m, 6H, Sn(CH_2C*H*$_2$$C_6F_{13}$)$_3$), 1.30 (t, *J* = 8.3 Hz, 6H, Sn(CH_2C*H*$_2$$C_6F_{13}$)$_3$). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 152.3, 141.5,

139.9, 135.9, 134.7, 129.8, 122.9, 121.0, 116.1, 111.2, 62.9, 55.3, 53.4, 50.7, 27.7, -1.5. HRMS(+): 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, 1239.

5.7.4. Preparation of iodo-arylamines: general procedure

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 and stirred at reflux (70 °C) overnight. Following this, the reaction solvent was removed under reduced pressure. The obtained yellow residue was subsequently purified by silica gel chromatography (10 % solution of ethanol in chloroform).

5.7.4.1 N-*butyl-3-iodobenzylamine (m-***5.7***)*

Yield: 76 mg, 78 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.5. HPLC: R_t = 11.1 min. ^1H NMR (500.13 MHz, $CDCl_3$) δ: 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-C*H*$_2$NH-$CH_2CH_2CH_2CH_3$), 2.64 (t, *J* = 7.2 Hz, 2H, Ar-CH_2NH-C*H*$_2CH_2CH_2CH_3$), 1.61 (s, 1H, Ar-CH_2N*H*-$CH_2CH_2CH_2CH_3$) 1.52 (m, 2H, Ar-CH_2NH-CH_2C*H*$_2CH_2CH_3$), 1.38 (m, 2H, Ar-CH_2NH-CH_2CH_2C*H*$_2CH_3$), 0.92 (t, *J* = 7.3 Hz, 3H, Ar-CH_2NH-$CH_2CH_2CH_2$C*H*$_3$). ^{13}C

NMR (125.76 MHz, CDCl3) δ: 142.6, 137.2, 136.0, 130.1, 127.4, 94.4, 53.2, 49.0, 32.0, 20.4, 13.9. ESMS (+): 289.9 $[M+H]^+$. FTIR (KBr, cm-1): 3448, 2961, 2935, 2865, 2796.

*5.7.4.2. Synthesis of N-butyl-4-iodobenzylamine (p-**5.7**)*

Yield: 76 mg, 78 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.5. ^1H NMR (500.13 MHz, $CDCl_3$) δ: 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-C*H*$_2$NH-$CH_2CH_2CH_2CH_3$), 2.60 (t, *J* = 7.3 Hz, 2H, Ar-CH_2NH-C*H*$_2$$CH_2CH_2CH_3$), 1.48 (tt, *J* = 7.2, 7.6 Hz, 2H, Ar-CH_2NH-CH_2C*H*$_2$$CH_2CH_3$), 1.35 (m, 2H, Ar-$CH_2$NH-$CH_2CH_2$C*H*$_2$$CH_3$), 0.91 (t, *J* = 8.3 Hz, 3H, Ar-CH_2NH-$CH_2CH_2CH_2$C*H*$_3$). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 140.5, 137.5, 130.3, 92.2, 53.6, 49.3, 32.4, 20.6, 14.2. ESMS (+): 289.9 $[M+H]^+$. Found: 290.0417. FTIR (KBr, cm^{-1}): 3448, 2962, 2935, 2796, 1197, 1123.

*5.7.4.3. Synthesis of N-(3-iodobenzyl)-1-(2-methoxyphenyl)-piperazine (m-**5.8**)*

Yield: 131 mg, 95 %. TLC ($CHCl_3$): R_f = 0.38. HPLC: R_t = 14.5 min. ^1H NMR (600.13 MHz, $CDCl_3$) δ: 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-C*H*$_2$-N(CH_2CH_2)$_2$N-C_6*H*$_4$$OCH_3$), 3.89 (s, 3H, OCH_3), 3.55 (s, 2H, Ar-C*H*$_2$-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$), 3.13 (s, 4H, Ar-CH_2-

N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$), 2.68 (s, 4H, Ar-CH_2-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$). ^{13}C NMR (150.92 MHz, $CDCl_3$) δ: 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. ESMS (+): 409.0 $[M+H]^+$. FTIR (KBr, cm^{-1}): 3063, 2935, 2816, 1500, 1241.

*5.7.4.4. Synthesis of N-(4-iodobenzyl)-1-(2-methoxyphenyl)-piperazine (p-***5.8***)*

Yield: 128 mg, 93 %. TLC ($CHCl_3$): R_f = 0.40. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 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, Ar-C*H*$_2$-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$), 3.86 (s, 3H, OCH_3), 3.52 (s, 2H, Ar-C*H*$_2$-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$), 3.10 (s, 4H, Ar-CH_2-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$), 2.64 (s, 4H, Ar-CH_2-N(CH_2CH_2)$_2$N-$C_6H_4OCH_3$). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 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. ESMS (+): 409.1 $[M+H]^+$. Found: 409.0795. FTIR (KBr, cm^{-1}): 3068, 2934, 2816, 1592, 1509, 1241, 1012.

5.7.5. Synthesis of 2-chloro-N-(3-(*tris*[2-perfluorohexylethyl]stannyl)-benzyl)acetamide (5.12)

To a solution of 3-(*tris*[2-perfluorohexylethyl]stannyl)benzylamine (80 mg, 67 μmol) in chloroform was added chloroacetyl chloride (521 mg, 188

μmol) at 0°C, followed by the 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 washed with water (3 x 15 mL). The fluorous layer was dried over anhydrous sodium sulfate. Removal of the FC-72® gave a dark yellow-brown oil which was purified by silica-gel chromatography. The product was eluted using a 10 % solution of ethanol in chloroform. Yield: 62 mg, 78 %. TLC (9:1 $CHCl_3$:EtOH): R_f = 0.70. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.41 (m, 1H, Ar-*H*), 7.31 (m, 3H, Ar-*H*), 6.89 (s, 1H, Ar-CH_2N*H*C(O)CH_2Cl), 4.50 (d, *J* = 5.9 Hz, 2H, Ar-C*H*$_2$NHC(O)CH_2Cl), 4.10(s, 2H, Ar-CH_2NHC(O)C*H*$_2$Cl), 2.32 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.32 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ: 165.9, 138.0, 137.5, 135.3, 129.3, 128.9, 43.8, 42.5, 27.7, -1.3. HRMS (+): $C_{33}H_{22}NOF_{39}SnCl$ $[M+H]^+$= 1343.9789. Found: 1343.9807. FTIR (KBr, cm^{-1}): 3298, 2943, 1666, 1550.

5.7.6. Synthesis of 2-iodo-N-(3-(*tris*[2-perfluorohexylethyl]stannyl)-benzyl)acetamide (5.3)

Compound **5.14** (45 mg, 33 μmol) was dissolved in acetonitrile (5 mL) and sodium iodide was added (50 mg, 335 μmol). The resulting solution was heated at reflux (85 °C) overnight, then allowed to cool to ambient temperature. Solvent was removed and the resulting residue was

dissolved in FC-72® and washed with water. The fluorous layers were combined and dried over anhydrous sodium sulfate. Removal of the FC-72® afforded an off-white solid. Yield: 42 mg, 86 %. TLC (9:1, $CHCl_3$:EtOH) R_f = 0.67. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.40 (m, 1H, Ar-*H*), 7.32 (m, 1H, Ar-*H*), 6.34 (s, 1H, Ar-$CH_2NHC(O)CH_2I$), 4.47 (d, *J* = 5.8 Hz, 2H, Ar-$CH_2NHC(O)CH_2I$), 3.73 (s, 2H, Ar-$CH_2NHC(O)CH_2I$), 2.32 (m, 6H, $SnCH_2CH_2C_6F_{13}$), 1.30 (m, 6H, $SnCH_2CH_2C_6F_{13}$). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 166.7, 138.2, 137.5, 135.2, 129.0, 44.3, 27.7, -1.2, -1.3. HRMS(+) 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.

5.7.7. 3-(*Tris*[2-perfluorohexylethyl]stannyl)-benzyl-iodoacetamide: conjugation with L-cysteine (5.13)

Fluorous iodoacetamide (30 mg, 21 μmol) was dissolved in a 1:1 mixture of PFBME / methanol (10 mL). A solution of L-cysteine (10 mg, 82 μmol) was dissolved in a solution of potassium hydroxide in methanol (5 mL, 1M) which was added to the iodoacetamide solution. The resulting homogeneous mixture stirred for 2 h at which point the solvent was removed and the product isolated by liquid-liquid extraction using FC-72® and water. Yield: 28 mg, 95 %. TLC (9:1, $CHCl_3$:EtOH): R_f = 0.1. ^{1}H NMR (500.13 MHz, CD_3OD) δ: 7.44 (m, 4H, Ar-*H*) 4.41 (s, 2H, Ar-

C*H*$_2$NHC(O)CH$_2$SCH$_2$CH(NH$_2$)COOH), 3.36 (m, 1H, Ar-CH$_2$NHC(O)CH$_2$SCH$_2$C*H*(NH$_2$)COOH), 3.26 (d, 2H, Ar-CH$_2$NHC(O)C*H*$_2$SCH$_2$CH(NH$_2$)COOH), 2.99 (dd, *J* = 13.5 Hz, *J* = 4.3 Hz, 2H, Ar-CH$_2$NHC(O)CH$_2$SC*H*^{a}$_2$CH(NH$_2$)COOH), (dd, *J* = 13.5 Hz, *J* = 7.6 Hz, 2H, Ar-CH$_2$NHC(O)CH$_2$SC*H*^{b}$_2$CH(NH$_2$)COOH) 2.41 (m, 6H, SnCH$_2$C*H*$_2$C$_6$F$_{13}$), 1.42 (t, *J* = 8.3 Hz, 6H, SnC*H*$_2$CH$_2$C$_6$F$_{13}$). ^{13}C NMR (125.77 MHz, CD$_3$OD) δ: 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(-): mass calcd for C$_{36}$H$_{26}$N$_2$O$_3$F$_{39}$SSn: 1427.0063 [M-H]$^-$. Found: 1427.0048. FTIR (KBr, window, cm^{-1}): 3401, 1634.

5.7.8. Synthesis of 2-chloro-*N*-(3-iodobenzyl)acetamide (5.14)

3-Iodobenzylamine (501 mg, 1.85 mmol) was dissolved in chloroform (15 mL), 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, 21.5 mmol). The resulting mixture was stirred at room temperature overnight. Solvent and excess triethylamine were removed under reduced pressure. The resulting residue was purified by silica-gel chromatography. The product was eluted using a 10 % solution of ethanol in chloroform. Yield: 348 mg, 61 %. TLC (9:1 CHCl$_3$:EtOH): R$_f$ = 0.70. ^{1}H NMR (500.13 MHz, CDCl$_3$) δ: 7.64 (m, 2H, Ar-*H*), 7.27 (m, 2H, Ar-*H*), 7.09 (m, 1H, Ar-*H*), 6.94 (s, 1H, Ar-CH$_2$N*H*C(O)CH$_2$Cl), 4.44 (d, *J*

= 6.0 Hz, 2H, Ar-C*H*$_2$NHC(O)CH$_2$Cl), 4.11 (s, 2H, Ar-CH$_2$NHC(O)C*H*$_2$Cl). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 166.0, 139.9, 137.0, 136.8, 130.6, 127.1, 94.7, 43.2, 42.7. ESMS (+): 310.0 $[M+H]^+$. FTIR (KBr, cm^{-1}): 3272, 3068, 1653, 1555.

5.7.9. Synthesis of 2-iodo-*N*-(3-iodobenzyl)acetamide (5.15)

3-iodobenzyl-chloroacetamide (53 mg, 172 μmol) was dissolved in acetonitrile (5 mL) and sodium iodide was added (258 mg, 1.72 mmol). The resulting solution was heated at reflux overnight and allowed to cool. Solvent was removed and the resulting residue was purified by silica gel chromatography. The product was eluted using a 10 % solution of ethanol in chloroform. Yield: 59 mg, 86 %. TLC ($CHCl_3$) R_f = 0.67. ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 7.64 (m, 2H, Ar-*H*), 7.27 (m, 2H, Ar-*H*), 7.09 (m, 1H, Ar-*H*), 6.38 (s, 1H, Ar-CH$_2$N*H*C(O)CH$_2$Cl), 4.41 (d, *J* = 5.9 Hz, 2H, Ar-C*H*$_2$NHC(O)CH$_2$Cl), 3.75 (s, 2H, Ar-CH$_2$NHC(O)C*H*$_2$Cl). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 167.0, 140.1, 137.0, 136.8, 130.7, 127.1, 94.7, 43.8, -0.8. ESMS (+): 399.9 $[M+H]^+$. FTIR (KBr, cm^{-1}): 3271, 2942, 2873, 1632, 1550.

5.7.10. Conjugation of 2-iodo-N-(3-iodobenzyl)acetamide with L-cysteine (5.16)

Iodoacetamide **5.15** (100 mg; 250 μmol) was dissolved in methanol (2 mL). L-cysteine (30 mg; 247 mmol) was dissolved in a methanolic solution of potassium hydroxide (5 mL, 1M) and was later added to the iodoacetamide solution. The resulting homogeneous mixture stirred for 1 hour at 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 = 6.0 min. ^{1}H NMR (500.13 MHz, CD_3OD) δ: 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-C*H*$_2$NHC(O)CH$_2$SCH$_2$CH(NH$_2$)COOH), 3.34 (m, 1H, Ar-CH$_2$NHC(O)CH$_2$SCH$_2$C*H*(NH$_2$)COOH), 3.28 (m, 2H, Ar-CH$_2$NHC(O)C*H*$_2$SCH$_2$CH(NH$_2$)COOH), 2.96 (dd, *J* = 13.5 Hz, *J* = 4.3 Hz, 2H, Ar-CH$_2$NHC(O)CH$_2$SC*H*^{a}$_2$CH(NH$_2$)COOH), 2.75 (dd, *J* = 13.5 Hz, *J* = 7.6 Hz, 2H, Ar-CH$_2$NHC(O)CH$_2$SC*H*^{b}$_2$CH(NH$_2$)COOH). ^{13}C NMR (125.77 MHz, CD_3OD) δ: 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(+): 395.1 $[M+H]^+$. (KBr, cm^{-1}): 3417, 2505, 1625, 1592, 1417.

5.7.11. Radiochemistry

General radioiodination procedure

Compounds *m*-**5.5, *m*-5.6 or 5.13** (0.5 mg) were added to a sample vial coated with iodogen (20 μL, 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, 231 mM). The reaction mixture was diluted with water (1 mL) and loaded onto a Fluoro*Flash*® 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 HPLC where in all cases, one radioactive peak was observed. When co-injected with the non-radioactive standards, the peaks co-eluted.

N-butyl-3- [^{125}I]iodobenzylamine (*m*-[^{125}I]**5.8**): R_t: 11.4 min; RCY: 92 %; Radiochemical Purity: >98 %.

N-(3-[^{125}I]iodobenzyl)-1-(2-methoxyphenyl)piperazine (*m*-[^{125}I]**5.9**): R_t: 14.8 min; RCY: 83 %; Radiochemical Purity: >98 %.

L-Cysteine conjugate ([^{125}I]**5.16**): R_t: 6.4 min; RCY: 86 %; Radiochemical Purity: >98 %.

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Chapter 6: Summary, impact and future work

6.1. Summary

The objective of this thesis was to develop a new, simple and general purpose method for the production of radiotracers and therapeutics in high effective specific activity using the principles of fluorous chemistry. Such a labelling method was developed and validated through a model system based on benzoic acid derivatives. Most significant here was that the method produced radiolabelled compounds in high radiochemical yields and the purification strategy efficiently removed all tin-containing precursors and by-products, as verified by ICP-MS analysis. Following this, the fluorous labelling strategy was applied to the preparation of a well-known radiopharmaceutical, *meta*-iodobenzylguanidine (MIBG), which has proven difficult to prepare in high effective specific activity using conventional methods. Finally, a collection of synthons for derivatizing biomolecules with fluorous groups was developed. Here a variety of fluorous synthons for tagging amines and thiols were prepared and model conjugates labelled and purified using methods that are compatible with biomolecules

6.2. Impact

With the increasing demand for targeted radiotracers and therapeutics there is a concomitant need for means of producing compounds in high effective specific activity. The described fluorous labelling methodology meets this need in that it is easy to use, can be applied to a wide range of compounds and isotopes. In addition to producing tracers and therapeutics in high effective specific activity, it can also be used to prepare libraries of compounds and the corresponding dehalogenated and even ^{3}H analogues thereby creating a new and efficient means of discovering lead agents. Because the same class of fluorous substrates are used in all cases (*i.e.* fluorous stannanes), the fluorous labeling techniques offers a means of linking early drug discovery efforts (*i.e.* library synthesis and screening and evaluation of ^{3}H analogues of leads) with biodistribution and preclinical molecular imaging studies (using radiolabelled materials). As such, this versatile technique can be used not only to enhance the production and discovery of novel molecular imaging probes and therapeutics but also to facilitate the union of molecular imaging and drug discovery.

6.3. Potential Limitations

The fluorous labeling method is an effective way of preparing radioiodinated compounds in high yield and effective specific activity. At

this stage of the development, the technique is not without limitations. It would be prudent to discuss these here as they will be the basis for future research.

Firstly, perfluorocarbons are expensive and certain derivatives are regarded as potential environmental hazards.[1] Fortunately, the quantities of precursor need to produce radiopharmaceuticals is so small that the total cost and environmental impact is negligible, particularly when compared to the cost of the isotope with respect to overall expense. The scale also mitigates any possible environmental effects, which is further supported by the fact that the reaction byproducts and starting materials associated with radiopharmaceuticals are disposed of in a controlled manner as medical waste.

A more practical limitation at present is with respect to adapting the fluorous labeling strategy to the preparation of radiolabelled analogues of large biomolecules. The first issue is whether or not there is a molecular weight limit, more specifically a percent-fluorine by weight limit, which will prevent the F-SPE cartridge from separating the labeled product from the precursor. Since the strength of the interaction is proportional to the ratio comparing the mass of fluorine present to the mass of the entire compound, it is possible that very large molecules will not be retained. Once the maximum molecular weight where effective F-SPE can be

performed is identified, it will be determined whether fluorous-HPLC could be an alternative method for purifying mixtures that exceed this limit.

In addition to challenges presented by the high molecular weight of biomolecules, an additional issue involves solvent incompatibilities. Generally, the fluorous tagging strategy described in the thesis is effective when using either fluorous or organic solvents; both of which are incompatible with most proteins. In order to adapt this labeling method to the tagging of biovectors, it will be necessary to examine alternative solvent systems (*e.g.* water/alcohol mixtures) or employ a fluorous-phase transfer catalyst,[2] which are designed to facilitate reactions at the interface of orthogonal phases.

6.4. Future work

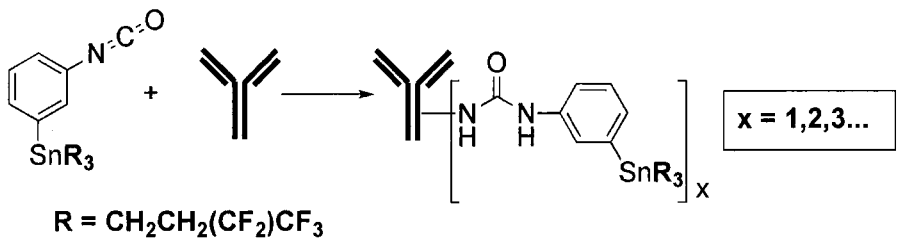
6.4.1. Biomolecules

In Chapters 4 and 5, the preparation of a variety of fluorous synthons designed for conjugation to biomolecules was described, and their conjugation chemistry optimized with model systems. The immediate future work will be to investigate the potential of reacting fluorous synthons with biomolecules, such as peptides and large proteins including antibodies to ascertain whether the fluorous labeling method can be used for the preparation of biomolecule radiopharmaceuticals (versus small molecules) in high effective specific activity.

Currently, the most common method for preparing radiolabelled peptides is direct electrophilic radioiodination *via* amino acid residues containing activated aromatic rings (*e.g.* tyrosine, histidine).[3] (Section 1.4.2.) For molecules that cannot be radioiodinated by this route (either because such residues are inaccessible or because their radiolabelling would result in a significant loss in binding activity), prosthetic groups are an excellent alternative.[3, 4] Typically, large molecules of this nature have multiple sites suitable for conjugation. As a result, one has very little control, aside from adjusting the stoichiometry of the reagents and parameters such as reaction time and temperature,[5] over the extent of conjugation (*i.e.* how many prosthetic groups are attached to each protein), much less the regioselectivity of the attachment of the prosthetic group. Furthermore, it is very difficult to obtain radiolabelled molecules of this type in high effective specific activity. Currently, in order to increase the effective specific activity of the final formulations, the amounts of starting materials (*i.e.* initial proteins) used are minimized,[3] since the most common method employed for purifying radiolabelled proteins is size exclusion chromatography, which is not effective for separating unlabeled protein from that which is radiolabelled.[6-8]

The fluorous strategy may provide an alternative avenue for purifying such molecules. If a pre-labelling approach can be used, whereby the fluorous-tin moiety is attached to the protein/antibody prior to

iodination, fluorous techniques can be used to separate any non-radioactive proteins from the mixture (Scheme 6-1).



Scheme 6-1. Synthesis of fluorous bioconjugates (*i.e.* antibody)

Fluorous separation methods are capable of resolving compounds based on their absolute fluorine content by altering the water content used to elute the fluorous solid-phase extraction cartridge.[9] This feature creates an opportunity to separate bioconjugates bearing differing numbers of fluorous groups (Figure 6-1).

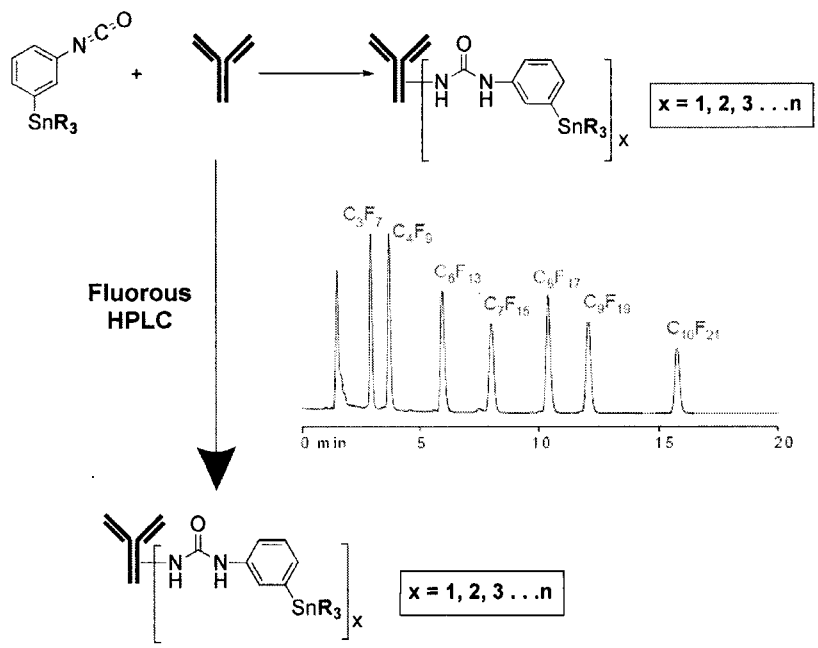
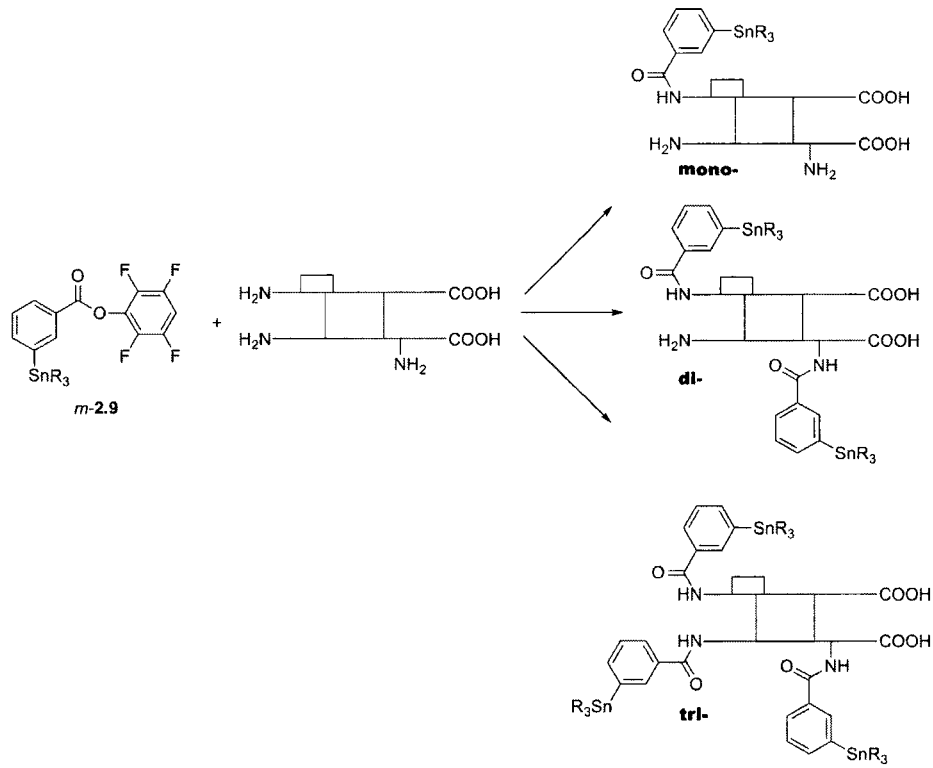


Figure 6-1. Proposed application of fluorous conjugation reactions towards protein labelling

To investigate the potential of using fluorous synthons to facilitate the radiolabelling and subsequent purification of biomolecules including peptides, and large proteins containing various numbers of reactive sites, fluorous conjugates of insulin were selected as an initial target molecule. This small hormone (MW 5800 Da) was chosen as a simplified example since it contains just 3 reactive sites, and provides a good starting point for exploring the separation and purification of complex mixtures containing compounds that are "fluorous" to differing degrees.

Preliminary experiments were conducted by dissolving human insulin in a small volume of a 5 % solution of NEt_3 in *N,N'*-dimethylformamide (DMF) (Scheme 6.2). To this was added fluorous TFP-active ester (*m*-**2.9**) dissolved in the same solvent mixture. Despite heterogeneous reaction conditions, analysis of the reaction mixture by electrospray mass spectroscopy showed that both di- and tri-functionalized insulin derivatives were produced. These results are encouraging and future work will involve optimizing the reaction conditions (solvent, temperature and concentration of reagents), and conducting iodination reactions (non-radioactive and radioactive) to optimize the solid-phase extraction conditions.



Scheme 6-2. Conjugation of insulin with *m*-**2.9**

6.4.2. Choice of oxidant

Several oxidizers are reported in the literature as being highly efficient at generating electrophilic iodine species.[10-12] In this work, iodogen was selected as the oxidant because it gave the desired radioactive products in high yields in a very short period of time which is important for method development purposes.[13, 14]

The disadvantage of using *N*-chloro oxidants, such as iodogen, is that in addition to being a powerful oxidizing agent, this class of compounds can also act as a chlorinating agent in electrophilic substitution reactions. This can result in the production of a significant

amount of non-radioactive chlorinated by-products, and thus lowering the effective specific activity of the radiopharmaceutical formulation.[5, 15] As an alternative, peracetic acid should be investigated as it appears to be the oxidant of choice for maximizing ESA.[10] It is prepared by mixing hydrogen peroxide and acetic acid, which reacts with the radiohalide to form hypohalous acid (*e.g.* HO-I in the case of radioiodide). Reaction parameters including amount of substrate, reaction time, and amount of oxidant will need to be examined in order to optimize the use of this oxidant for the fluorous radiolabelling method.

6.4.3 Automation

A desirable goal when developing a radiopharmaceutical is to be able to produce agents in a single, rapid and high yielding step that meets Good Manufacturing Practices (GMP) standards. If the process can be automated it is even more attractive. To streamline and potentially automate the fluorous labelling approach an alternative process to the purification methodology previously described should be investigated. In this new method, the fluorous radiolabelling precursors are adsorbed onto fluorous silica gel. During a radiolabelling experiment, this silica will be transferred to a vial coated with the desired oxidant and a solution of sodium iodide (or other isotope of interest). What is innovative about this

approach is that reaction work up and isolation of the desired radiotracer should involve only a straightforward filtration step since only the radiolabelling product will be in solution while the unlabelled material will remain bound to the silica.

Preliminary experiments conducted with fluorous benzoic acid, **2.1** appear promising. To adsorb the labelling precursor, a small amount was dissolved in perfluorinated hexanes. *FluoroFlash*® silica gel was then added and the solvent removed. Subsequent radiolabelling involved the addition of the a small amount of the precursor-loaded silica into an iodogen-coated vial followed by the addition of a 5 % acetic acid solution in methanol and sodium [^{125}I]iodide. The reaction was halted by the addition of sodium metabisulfite, the mixture diluted with distilled water and filtered through plug of glass wool. These reactions were successful in producing the desired radiotracer in good radiochemical yield (75 %). Unfortunately however, under these initial conditions HPLC analysis of the product mixture showed a small amount of **2.1** in the final product. Thus, reaction conditions must be optimized to ensure the retention of the fluorous-tin starting material on the silica gel. Future experiments should first attempt to address this problem by examining both reaction solvent and the sorbent used. It is possible that greater retention could be observed if the fluorous precursor is loaded onto a F-SPE cartridge instead of loose silica. Once optimized, the new technique could be used

to produce instant kits for iodinated radiopharmaceuticals akin to that used for technetium.

6.4.4. Applying the fluorous-labelling approach to drug discovery

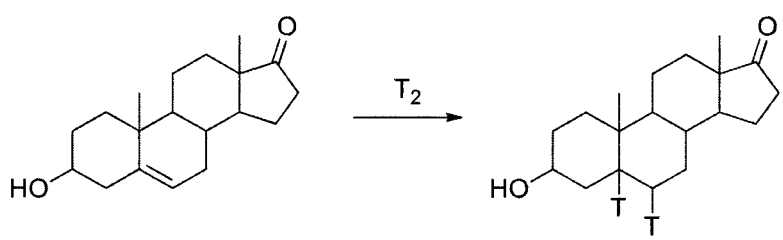
The drug discovery process involves selecting a target of interest, designing a bioassay that is suitable for measuring biological activity, constructing a high-throughput screen, profiling hits obtained through the screening and optimizing lead candidates.[16] Assays based on ^{3}H play an important part in the drug discovery process. ^{3}H compounds are used to obtain *in vitro* data through radioligand binding assays which are viewed as the "gold standard" for compound characterization and structure activity relationship (SAR) development, and help quantify the affinity of a radioligand for a receptor site (K_d), and the concentration of binding sites in a given tissue (β_{max}).[16] ^{3}H compounds are also used to look at pharmacokinetics and metabolism. Access to ^{3}H materials therefore plays an important role in characterizing new leads.

The preparation of tritium-labelled compounds with high specific activity is most typically accomplished by catalytic reduction of carbon-carbon double bonds or dehalogenation with gaseous tritium (Figure 6-2).[17] More recently however, ^{3}H-labelled compounds have been prepared

by exchange of a hydrogen atom in the target compound with tritium using gaseous tritium or tritiated water, or tritiated metal hydrides, such as sodium borotritide (NaB^3H_4).

The fluorous labelling strategy offers a convenient platform for producing new lead compounds and their tritiated analogues. The strategy can be used to tag leads with isotopes of hydrogen as a means of producing substrates in high purity for use in high-throughput screening and low throughput *in vitro* assays. Preliminary experiments were based on the work by Seitz and coworkers[18] who demonstrated that in the presence of excess trifluoroacetic anhydride, trialkylstannyl compounds undergo deuterio- and tritio-destannylation upon the addition of deuterated and tritiated water.

Catalytic Reduction



Halogen Exchange

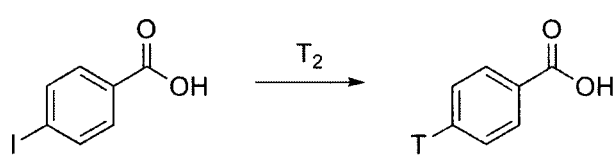


Figure 6-2. Methods for preparing ^3H-labelled compounds

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Appendix I

Table S1. Crystal data and structure refinement for compound *m*-**2.1**.

Empirical formula	$C_{62}H_{34}F_{78}O_4Sn_2$	
Formula weight	2562.27	
Temperature	103(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.961(4) Å	α= 99.038(6)°.
	b = 15.641(6) Å	β= 95.230(7)°.
	c = 24.999(9) Å	γ = 95.691(6)°.
Volume	4187(3) Å3	
Z	4	
Density (calculated)	2.032 Mg/m^3	
Absorption coefficient	0.817 mm^{-1}	
F(000)	2480	
Crystal size	0.03 x 0.08 x 0.25 mm^3	
θ range for data collection	1.33 to 24.00°.	
Index ranges	-12<=h<=12, -17<=k<=17, -28<=l<=28	
Reflections collected	22701	
Independent reflections	12047 [R(int) = 0.1154]	
Completeness to θ = 24.00°	91.5 %	
Absorption correction	Semi-empirical from equivalence	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	12047 / 54 / 1316	
Goodness-of-fit on F^2	1.020	
Final R indices [I>2σ(I)]	R1 = 0.0787, wR2 = 0.0998	
R indices (all data)	R1 = 0.1697, wR2 = 0.1219	
Extinction coefficient	0.00020(7)	
Largest diff. peak and hole	0.854 and -0.726 e.Å^{-3}	

Table S2. Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters ($Å^2x10^3$) for *m*-**2.1**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
Sn(1)	14156(1)	2858(1)	3306(1)	20(1)
Sn(2)	9086(1)	6382(1)	3041(1)	19(1)
F(53B)	6732(5)	5266(4)	2177(2)	35(2)
F(44B)	9466(4)	8977(3)	5016(2)	23(1)
F(36B)	15276(5)	387(3)	5629(2)	28(2)
F(44A)	9516(5)	7686(3)	5251(2)	25(2)
F(33B)	15407(5)	2702(3)	5215(2)	25(1)
F(16B)	8466(5)	1992(4)	981(2)	36(2)
F(56B)	2504(5)	5065(4)	1362(2)	38(2)
F(56A)	3413(6)	5566(4)	716(2)	44(2)
F(54B)	4189(5)	5458(4)	2250(2)	35(2)
F(45B)	11559(5)	8113(3)	5861(2)	29(2)
F(24B)	17356(5)	1075(4)	2168(2)	38(2)
F(53A)	6789(5)	6387(4)	1757(2)	33(2)
F(58C)	1482(5)	3270(4)	-36(2)	45(2)
F(45A)	12064(5)	9270(3)	5507(2)	27(2)
F(46B)	10422(5)	10128(3)	5948(2)	32(2)
F(43B)	11300(5)	8783(3)	4425(2)	23(1)
F(55B)	5531(5)	5104(4)	1040(2)	37(2)
F(36A)	16365(5)	1645(3)	5951(2)	24(1)
F(13B)	10631(5)	2882(4)	2745(2)	37(2)
F(55A)	4735(5)	4219(4)	1540(2)	36(2)
F(13A)	12200(5)	3184(3)	2301(2)	34(2)
F(47B)	12347(5)	9583(4)	6663(2)	32(2)
F(33A)	15511(5)	1374(3)	4820(2)	25(1)
C(03A)	13972(9)	4870(6)	3417(4)	21(3)
C(53)	6188(9)	6003(7)	2125(4)	24(3)
C(13)	11295(10)	2527(7)	2344(4)	27(3)
C(51)	7558(8)	7094(6)	2844(4)	24(3)
C(27)	16111(11)	-1497(8)	1275(4)	37(3)

C(43)	10787(9)	8064(6)	4597(4)	19(3)
C(02A)	13515(9)	4097(6)	3596(4)	19(3)
C(33)	14715(9)	1983(6)	4914(4)	16(2)
C(31)	15038(8)	2389(6)	3992(3)	14(2)
C(45)	11107(8)	8772(6)	5634(4)	16(2)
C(46)	10457(9)	9327(6)	6087(4)	20(2)
C(41)	10366(8)	7295(6)	3608(3)	17(2)
F(48C)	11157(6)	10946(4)	6958(2)	52(2)
F(14B)	9609(5)	1648(4)	1863(2)	35(2)
F(43A)	11759(5)	7623(3)	4742(2)	25(1)
F(24A)	16353(5)	1288(4)	1416(2)	39(2)
F(58B)	2371(6)	4469(4)	-208(3)	55(2)
F(58A)	1039(6)	4502(4)	384(3)	50(2)
F(54A)	4395(5)	6428(3)	1721(2)	30(2)
F(27B)	16932(6)	-1682(4)	1663(2)	47(2)
F(27A)	14969(6)	-1758(4)	1388(3)	55(2)
C(02B)	8445(9)	5363(6)	3464(4)	15(2)
C(03B)	8968(9)	4558(6)	3402(4)	23(3)
F(16A)	9822(5)	2885(4)	704(2)	31(2)
C(36)	15342(9)	1133(7)	6001(4)	21(3)
C(56)	3366(10)	4923(7)	1023(4)	25(3)
C(52)	6258(8)	6604(6)	2672(4)	23(3)
C(37)	15476(10)	852(7)	6578(4)	29(3)
C(14)	10429(10)	2315(7)	1812(4)	27(3)
C(54)	4868(9)	5715(6)	1866(4)	21(3)
C(55)	4643(9)	4986(7)	1361(4)	26(3)
C(58)	1934(11)	4090(9)	178(5)	45(4)
C(42)	9887(8)	7505(6)	4169(3)	17(2)
C(24)	16227(10)	1004(6)	1895(4)	21(3)
C(44)	10226(9)	8366(6)	5125(4)	22(3)
C(01B)	7588(8)	5450(6)	3835(4)	18(3)
F(37B)	14713(5)	128(4)	6562(2)	32(2)
F(47A)	10847(5)	8697(4)	6867(2)	33(2)
F(37A)	15167(5)	1491(4)	6954(2)	40(2)
F(28B)	15422(7)	-2013(5)	348(3)	76(3)
F(28C)	16385(7)	-2857(4)	787(2)	61(2)
C(21)	15342(8)	3147(6)	2709(4)	22(3)

F(18B)	7147(6)	2509(5)	142(3)	64(2)
F(28A)	17374(7)	-1750(5)	566(3)	64(2)
C(04B)	8647(9)	3920(6)	3700(4)	26(3)
C(06B)	7242(9)	4812(6)	4138(4)	18(3)
C(05B)	7800(9)	4043(6)	4079(4)	23(3)
F(35B)	14458(5)	2425(4)	6175(2)	31(2)
F(23B)	14448(5)	1719(4)	1893(2)	34(2)
F(23A)	15039(5)	1208(3)	2619(2)	30(2)
F(34B)	12905(5)	2191(3)	5316(2)	30(2)
F(18C)	8334(6)	3646(5)	66(3)	59(2)
C(34)	13801(9)	1645(6)	5287(4)	21(3)
C(25)	15709(10)	16(7)	1765(4)	25(3)
C(32)	14123(8)	2190(6)	4389(3)	16(2)
C(22)	16154(9)	2482(6)	2472(4)	27(3)
C(35)	14195(10)	1587(7)	5892(4)	25(3)
C(01A)	12611(9)	4128(6)	3948(4)	22(3)
C(15)	9712(9)	3055(7)	1641(4)	25(3)
C(23)	15470(10)	1616(7)	2222(4)	29(3)
F(26A)	17491(5)	-237(4)	1327(2)	40(2)
F(38C)	16740(5)	287(4)	7206(2)	32(2)
F(14A)	11107(5)	2055(3)	1404(2)	28(2)
F(17B)	7036(5)	3214(4)	1227(2)	40(2)
F(38B)	17195(5)	122(4)	6371(2)	35(2)
F(15B)	8883(5)	3225(4)	2007(2)	37(2)
F(17A)	8446(5)	4254(4)	1135(2)	39(2)
F(34A)	13245(5)	850(3)	5036(2)	27(2)
F(35A)	13243(5)	1189(3)	6083(2)	31(2)
F(15A)	10498(5)	3771(3)	1670(2)	27(2)
F(57B)	2579(5)	3460(4)	935(2)	39(2)
F(38A)	17553(5)	1386(4)	6896(2)	41(2)
F(25B)	14489(5)	-51(4)	1639(2)	37(2)
O(2B)	5897(6)	5692(4)	4597(3)	27(2)
F(48B)	9519(5)	10143(4)	7047(2)	40(2)
O(1B)	5904(6)	4316(4)	4751(3)	24(2)
F(26B)	15745(6)	-329(4)	815(2)	45(2)
C(04A)	13556(9)	5641(6)	3613(4)	30(3)
C(62)	9934(9)	6169(6)	1853(4)	22(3)

C(17)	7995(9)	3424(7)	954(4)	25(3)
C(38)	16766(10)	652(8)	6765(5)	34(3)
C(61)	9974(9)	5764(6)	2376(3)	22(3)
C(11)	12550(9)	1919(6)	3075(4)	30(3)
C(12)	11865(9)	1742(6)	2498(4)	24(3)
C(07B)	6313(10)	4912(7)	4522(4)	26(3)
C(26)	16271(11)	-504(7)	1281(5)	34(3)
C(18)	7500(11)	3342(7)	354(5)	37(3)
F(25A)	15952(5)	-325(4)	2217(2)	37(2)
F(18A)	6503(5)	3762(4)	315(2)	40(2)
C(66)	11329(11)	8741(7)	921(4)	33(3)
O(2A)	10995(6)	4248(4)	4719(3)	31(2)
C(07A)	11249(10)	4976(7)	4519(4)	24(3)
C(05A)	12656(9)	5682(6)	3964(4)	21(3)
C(06A)	12173(9)	4919(7)	4135(4)	19(3)
F(63A)	11806(5)	7077(4)	2124(2)	37(2)
F(65B)	11998(6)	8651(4)	1817(2)	42(2)
F(66B)	10552(6)	8311(4)	495(2)	51(2)
F(67B)	11863(6)	10180(4)	1390(2)	41(2)
F(65A)	10098(6)	8871(4)	1643(2)	48(2)
F(67A)	10121(6)	9897(4)	866(3)	52(2)
F(66A)	12481(6)	8562(4)	835(2)	45(2)
O(1A)	10726(6)	5632(4)	4634(3)	30(2)
F(68B)	11549(7)	9583(4)	-22(3)	59(2)
C(64)	10602(10)	7441(7)	1402(4)	32(3)
C(67)	11320(11)	9729(8)	907(5)	37(3)
C(63)	10603(10)	7057(7)	1919(4)	27(3)
C(65)	11019(10)	8440(7)	1449(4)	28(3)
C(68)	11940(13)	10062(8)	452(5)	48(4)
F(63B)	10109(5)	7626(4)	2297(2)	31(2)
F(64B)	9437(6)	7310(4)	1144(2)	52(2)
F(68C)	11655(7)	10877(4)	427(3)	66(2)
F(68A)	13157(7)	10130(5)	549(3)	62(2)
C(28)	16314(15)	-2035(8)	721(5)	51(4)
F(64A)	11341(6)	7021(4)	1069(2)	45(2)
F(57A)	3952(5)	3782(4)	396(2)	44(2)
C(57)	2979(10)	4045(7)	634(4)	28(3)

F(46A)	9305(5)	8961(3)	6089(2)	26(2)
F(48A)	11157(5)	10136(4)	7572(2)	43(2)
C(16)	8998(10)	2821(7)	1069(4)	25(3)
C(47)	11121(9)	9436(6)	6670(4)	20(2)
C(48)	10728(10)	10198(7)	7069(4)	27(3)

Table S3. Bond lengths [Å] and angles [°] for *m*-**2.1**.

Sn(1)-C(21)	2.138(9)	C(53)-C(54)	1.525(13)
Sn(1)-C(11)	2.151(9)	C(13)-C(12)	1.517(12)
Sn(1)-C(31)	2.155(8)	C(13)-C(14)	1.532(13)
Sn(1)-C(02A)	2.167(9)	C(51)-C(52)	1.540(12)
Sn(2)-C(02B)	2.143(9)	C(27)-F(27A)	1.346(12)
Sn(2)-C(41)	2.149(8)	C(27)-F(27B)	1.347(11)
Sn(2)-C(61)	2.151(9)	C(27)-C(26)	1.544(14)
Sn(2)-C(51)	2.164(8)	C(27)-C(28)	1.550(16)
F(53B)-C(53)	1.368(10)	C(43)-F(43A)	1.377(9)
F(44B)-C(44)	1.372(10)	C(43)-C(42)	1.492(11)
F(36B)-C(36)	1.363(10)	C(43)-C(44)	1.532(13)
F(44A)-C(44)	1.350(11)	C(02A)-C(01A)	1.384(13)
F(33B)-C(33)	1.366(10)	C(33)-C(32)	1.506(11)
F(16B)-C(16)	1.344(11)	C(33)-C(34)	1.540(12)
F(56B)-C(56)	1.336(11)	C(31)-C(32)	1.518(11)
F(56A)-C(56)	1.359(11)	C(45)-C(44)	1.536(12)
F(54B)-C(54)	1.352(10)	C(45)-C(46)	1.582(13)
F(45B)-C(45)	1.365(9)	C(46)-F(46A)	1.334(10)
F(24B)-C(24)	1.343(10)	C(46)-C(47)	1.547(12)
F(53A)-C(53)	1.359(11)	C(41)-C(42)	1.540(11)
F(58C)-C(58)	1.337(12)	F(48C)-C(48)	1.298(11)
F(45A)-C(45)	1.335(10)	F(14B)-C(14)	1.337(11)
F(46B)-C(46)	1.355(10)	F(24A)-C(24)	1.354(10)
F(43B)-C(43)	1.357(10)	F(58B)-C(58)	1.311(13)
F(55B)-C(55)	1.333(11)	F(58A)-C(58)	1.326(13)
F(36A)-C(36)	1.339(10)	F(54A)-C(54)	1.361(10)
F(13B)-C(13)	1.373(11)	C(02B)-C(01B)	1.380(12)
F(55A)-C(55)	1.354(11)	C(02B)-C(03B)	1.427(12)
F(13A)-C(13)	1.381(11)	C(03B)-C(04B)	1.373(12)
F(47B)-C(47)	1.342(10)	F(16A)-C(16)	1.349(10)
F(33A)-C(33)	1.362(10)	C(36)-C(35)	1.528(13)
C(03A)-C(04A)	1.366(12)	C(36)-C(37)	1.573(13)
C(03A)-C(02A)	1.416(12)	C(56)-C(57)	1.550(13)
C(53)-C(52)	1.522(12)	C(56)-C(55)	1.554(13)

C(37)-F(37B)	1.332(11)	F(17B)-C(17)	1.346(11)
C(37)-F(37A)	1.351(11)	F(38B)-C(38)	1.335(12)
C(37)-C(38)	1.525(14)	F(17A)-C(17)	1.335(11)
C(14)-F(14A)	1.350(11)	F(57B)-C(57)	1.340(11)
C(14)-C(15)	1.554(13)	F(38A)-C(38)	1.343(11)
C(54)-C(55)	1.546(13)	O(2B)-C(07B)	1.337(11)
C(58)-C(57)	1.556(14)	F(48B)-C(48)	1.315(11)
C(24)-C(23)	1.520(13)	O(1B)-C(07B)	1.236(11)
C(24)-C(25)	1.566(13)	F(26B)-C(26)	1.333(11)
C(01B)-C(06B)	1.389(12)	C(04A)-C(05A)	1.377(13)
F(47A)-C(47)	1.343(10)	C(62)-C(63)	1.482(13)
F(28B)-C(28)	1.295(13)	C(62)-C(61)	1.538(11)
F(28C)-C(28)	1.330(12)	C(17)-C(18)	1.529(14)
C(21)-C(22)	1.521(12)	C(17)-C(16)	1.554(13)
F(18B)-C(18)	1.332(12)	C(11)-C(12)	1.535(11)
F(28A)-C(28)	1.316(14)	C(18)-F(18A)	1.334(11)
C(04B)-C(05B)	1.392(13)	C(66)-F(66B)	1.344(11)
C(06B)-C(05B)	1.395(12)	C(66)-F(66A)	1.350(12)
C(06B)-C(07B)	1.465(13)	C(66)-C(65)	1.524(14)
F(35B)-C(35)	1.377(11)	C(66)-C(67)	1.553(14)
F(23B)-C(23)	1.367(11)	O(2A)-C(07A)	1.329(11)
F(23A)-C(23)	1.355(11)	C(07A)-O(1A)	1.232(11)
F(34B)-C(34)	1.364(10)	C(07A)-C(06A)	1.458(13)
F(18C)-C(18)	1.312(13)	C(05A)-C(06A)	1.402(12)
C(34)-F(34A)	1.358(10)	F(63A)-C(63)	1.366(10)
C(34)-C(35)	1.554(13)	F(65B)-C(65)	1.328(10)
C(25)-F(25B)	1.336(10)	F(67B)-C(67)	1.351(11)
C(25)-F(25A)	1.342(11)	F(65A)-C(65)	1.353(11)
C(25)-C(26)	1.557(14)	F(67A)-C(67)	1.363(12)
C(22)-C(23)	1.495(13)	F(68B)-C(68)	1.310(12)
C(35)-F(35A)	1.326(11)	C(64)-F(64A)	1.355(11)
C(01A)-C(06A)	1.399(12)	C(64)-F(64B)	1.359(11)
C(15)-F(15A)	1.330(10)	C(64)-C(63)	1.509(14)
C(15)-F(15B)	1.362(11)	C(64)-C(65)	1.567(14)
C(15)-C(16)	1.539(12)	C(67)-C(68)	1.510(16)
F(26A)-C(26)	1.350(11)	C(63)-F(63B)	1.380(11)
F(38C)-C(38)	1.321(11)	C(68)-F(68A)	1.324(13)

C(68)-F(68C)	1.352(12)	F(43B)-C(43)-F(43A)	105.5(7)
F(57A)-C(57)	1.337(11)	F(43B)-C(43)-C(42)	112.4(8)
F(48A)-C(48)	1.322(10)	F(43A)-C(43)-C(42)	111.2(8)
C(47)-C(48)	1.551(13)	F(43B)-C(43)-C(44)	107.7(8)
		F(43A)-C(43)-C(44)	106.2(7)
C(21)-Sn(1)-C(11)	119.9(4)	C(42)-C(43)-C(44)	113.4(8)
C(21)-Sn(1)-C(31)	115.0(3)	C(01A)-C(02A)-C(03A)	119.4(9)
C(11)-Sn(1)-C(31)	101.8(3)	C(01A)-C(02A)-Sn(1)	119.3(7)
C(21)-Sn(1)-C(02A)	103.9(4)	C(03A)-C(02A)-Sn(1)	121.3(8)
C(11)-Sn(1)-C(02A)	107.1(4)	F(33A)-C(33)-F(33B)	105.7(7)
C(31)-Sn(1)-C(02A)	108.6(3)	F(33A)-C(33)-C(32)	111.4(8)
C(02B)-Sn(2)-C(41)	107.3(3)	F(33B)-C(33)-C(32)	111.1(8)
C(02B)-Sn(2)-C(61)	106.0(4)	F(33A)-C(33)-C(34)	107.0(7)
C(41)-Sn(2)-C(61)	111.6(4)	F(33B)-C(33)-C(34)	106.5(8)
C(02B)-Sn(2)-C(51)	108.7(4)	C(32)-C(33)-C(34)	114.6(8)
C(41)-Sn(2)-C(51)	105.4(3)	C(32)-C(31)-Sn(1)	111.2(5)
C(61)-Sn(2)-C(51)	117.5(4)	F(45A)-C(45)-F(45B)	107.7(7)
C(04A)-C(03A)-C(02A)	119.6(10)	F(45A)-C(45)-C(44)	111.3(8)
F(53A)-C(53)-F(53B)	106.1(8)	F(45B)-C(45)-C(44)	108.2(7)
F(53A)-C(53)-C(52)	112.1(9)	F(45A)-C(45)-C(46)	109.1(8)
F(53B)-C(53)-C(52)	111.2(8)	F(45B)-C(45)-C(46)	106.9(8)
F(53A)-C(53)-C(54)	106.8(8)	C(44)-C(45)-C(46)	113.4(8)
F(53B)-C(53)-C(54)	107.4(8)	F(46A)-C(46)-F(46B)	109.0(8)
C(52)-C(53)-C(54)	113.0(8)	F(46A)-C(46)-C(47)	108.3(8)
F(13B)-C(13)-F(13A)	104.9(8)	F(46B)-C(46)-C(47)	108.3(8)
F(13B)-C(13)-C(12)	110.9(9)	F(46A)-C(46)-C(45)	109.6(8)
F(13A)-C(13)-C(12)	110.6(8)	F(46B)-C(46)-C(45)	106.8(8)
F(13B)-C(13)-C(14)	107.5(9)	C(47)-C(46)-C(45)	114.7(8)
F(13A)-C(13)-C(14)	109.1(9)	C(42)-C(41)-Sn(2)	112.1(6)
C(12)-C(13)-C(14)	113.4(9)	C(01B)-C(02B)-C(03B)	115.7(8)
C(52)-C(51)-Sn(2)	119.9(6)	C(01B)-C(02B)-Sn(2)	123.7(7)
F(27A)-C(27)-F(27B)	108.5(10)	C(03B)-C(02B)-Sn(2)	120.4(7)
F(27A)-C(27)-C(26)	110.2(9)	C(04B)-C(03B)-C(02B)	121.7(10)
F(27B)-C(27)-C(26)	108.6(9)	F(36A)-C(36)-F(36B)	108.4(8)
F(27A)-C(27)-C(28)	107.8(10)	F(36A)-C(36)-C(35)	110.8(8)
F(27B)-C(27)-C(28)	108.3(10)	F(36B)-C(36)-C(35)	109.1(8)
C(26)-C(27)-C(28)	113.3(10)	F(36A)-C(36)-C(37)	108.4(8)

F(36B)-C(36)-C(37)	106.4(8)	F(58A)-C(58)-C(57)	110.5(10)
C(35)-C(36)-C(37)	113.7(9)	F(58C)-C(58)-C(57)	107.1(11)
F(56B)-C(56)-F(56A)	107.9(9)	C(43)-C(42)-C(41)	114.8(8)
F(56B)-C(56)-C(57)	109.2(9)	F(24B)-C(24)-F(24A)	107.8(8)
F(56A)-C(56)-C(57)	107.8(8)	F(24B)-C(24)-C(23)	107.9(8)
F(56B)-C(56)-C(55)	109.3(8)	F(24A)-C(24)-C(23)	107.7(8)
F(56A)-C(56)-C(55)	107.8(9)	F(24B)-C(24)-C(25)	108.2(8)
C(57)-C(56)-C(55)	114.6(9)	F(24A)-C(24)-C(25)	108.0(8)
C(53)-C(52)-C(51)	111.8(8)	C(23)-C(24)-C(25)	117.1(9)
F(37B)-C(37)-F(37A)	109.3(9)	F(44A)-C(44)-F(44B)	107.4(8)
F(37B)-C(37)-C(38)	107.0(9)	F(44A)-C(44)-C(43)	109.0(8)
F(37A)-C(37)-C(38)	108.0(9)	F(44B)-C(44)-C(43)	106.9(7)
F(37B)-C(37)-C(36)	108.4(8)	F(44A)-C(44)-C(45)	107.8(8)
F(37A)-C(37)-C(36)	109.5(8)	F(44B)-C(44)-C(45)	107.3(8)
C(38)-C(37)-C(36)	114.5(9)	C(43)-C(44)-C(45)	118.1(8)
F(14B)-C(14)-F(14A)	108.9(8)	C(02B)-C(01B)-C(06B)	123.6(9)
F(14B)-C(14)-C(13)	106.8(8)	C(22)-C(21)-Sn(1)	120.6(7)
F(14A)-C(14)-C(13)	107.8(9)	C(03B)-C(04B)-C(05B)	120.7(9)
F(14B)-C(14)-C(15)	108.3(8)	C(01B)-C(06B)-C(05B)	119.1(10)
F(14A)-C(14)-C(15)	107.2(8)	C(01B)-C(06B)-C(07B)	122.7(9)
C(13)-C(14)-C(15)	117.6(9)	C(05B)-C(06B)-C(07B)	118.2(9)
F(54B)-C(54)-F(54A)	106.4(8)	C(04B)-C(05B)-C(06B)	119.1(9)
F(54B)-C(54)-C(53)	108.5(8)	F(34A)-C(34)-F(34B)	106.7(8)
F(54A)-C(54)-C(53)	107.7(8)	F(34A)-C(34)-C(33)	108.2(8)
F(54B)-C(54)-C(55)	107.7(8)	F(34B)-C(34)-C(33)	106.8(8)
F(54A)-C(54)-C(55)	107.4(8)	F(34A)-C(34)-C(35)	108.1(8)
C(53)-C(54)-C(55)	118.6(8)	F(34B)-C(34)-C(35)	103.8(8)
F(55B)-C(55)-F(55A)	108.3(8)	C(33)-C(34)-C(35)	122.3(8)
F(55B)-C(55)-C(54)	108.8(8)	F(25B)-C(25)-F(25A)	109.1(8)
F(55A)-C(55)-C(54)	107.3(8)	F(25B)-C(25)-C(26)	108.2(9)
F(55B)-C(55)-C(56)	109.3(8)	F(25A)-C(25)-C(26)	109.8(9)
F(55A)-C(55)-C(56)	108.1(8)	F(25B)-C(25)-C(24)	108.8(8)
C(54)-C(55)-C(56)	114.9(9)	F(25A)-C(25)-C(24)	107.4(8)
F(58B)-C(58)-F(58A)	110.9(12)	C(26)-C(25)-C(24)	113.4(9)
F(58B)-C(58)-F(58C)	108.5(10)	C(33)-C(32)-C(31)	113.6(7)
F(58A)-C(58)-F(58C)	108.9(10)	C(23)-C(22)-C(21)	114.3(9)
F(58B)-C(58)-C(57)	110.8(10)	F(35A)-C(35)-F(35B)	109.2(8)

F(35A)-C(35)-C(36)	109.7(8)	O(2B)-C(07B)-C(06B)	115.2(9)
F(35B)-C(35)-C(36)	106.2(8)	F(26B)-C(26)-F(26A)	108.9(9)
F(35A)-C(35)-C(34)	107.7(8)	F(26B)-C(26)-C(27)	109.7(9)
F(35B)-C(35)-C(34)	107.7(8)	F(26A)-C(26)-C(27)	107.9(9)
C(36)-C(35)-C(34)	116.2(9)	F(26B)-C(26)-C(25)	108.9(9)
C(02A)-C(01A)-C(06A)	120.2(9)	F(26A)-C(26)-C(25)	108.4(9)
F(15A)-C(15)-F(15B)	108.4(9)	C(27)-C(26)-C(25)	113.0(9)
F(15A)-C(15)-C(16)	109.9(8)	F(18C)-C(18)-F(18B)	108.1(10)
F(15B)-C(15)-C(16)	108.0(8)	F(18C)-C(18)-F(18A)	110.3(10)
F(15A)-C(15)-C(14)	109.1(8)	F(18B)-C(18)-F(18A)	107.0(9)
F(15B)-C(15)-C(14)	107.5(8)	F(18C)-C(18)-C(17)	111.9(10)
C(16)-C(15)-C(14)	113.8(9)	F(18B)-C(18)-C(17)	110.1(10)
F(23A)-C(23)-F(23B)	105.5(9)	F(18A)-C(18)-C(17)	109.3(9)
F(23A)-C(23)-C(22)	109.5(8)	F(66B)-C(66)-F(66A)	108.1(9)
F(23B)-C(23)-C(22)	110.6(9)	F(66B)-C(66)-C(65)	110.9(9)
F(23A)-C(23)-C(24)	107.8(9)	F(66A)-C(66)-C(65)	108.4(9)
F(23B)-C(23)-C(24)	108.3(8)	F(66B)-C(66)-C(67)	107.0(8)
C(22)-C(23)-C(24)	114.7(10)	F(66A)-C(66)-C(67)	106.0(9)
C(03A)-C(04A)-C(05A)	121.7(10)	C(65)-C(66)-C(67)	116.1(10)
C(63)-C(62)-C(61)	114.7(8)	O(1A)-C(07A)-O(2A)	123.2(10)
F(17A)-C(17)-F(17B)	109.2(8)	O(1A)-C(07A)-C(06A)	122.9(10)
F(17A)-C(17)-C(18)	108.1(9)	O(2A)-C(07A)-C(06A)	113.9(9)
F(17B)-C(17)-C(18)	107.4(9)	C(04A)-C(05A)-C(06A)	119.4(9)
F(17A)-C(17)-C(16)	109.6(8)	C(01A)-C(06A)-C(05A)	119.7(10)
F(17B)-C(17)-C(16)	107.7(9)	C(01A)-C(06A)-C(07A)	121.5(10)
C(18)-C(17)-C(16)	114.7(9)	C(05A)-C(06A)-C(07A)	118.8(9)
F(38C)-C(38)-F(38B)	109.7(9)	F(64A)-C(64)-F(64B)	108.1(8)
F(38C)-C(38)-F(38A)	107.2(8)	F(64A)-C(64)-C(63)	109.2(9)
F(38B)-C(38)-F(38A)	108.5(10)	F(64B)-C(64)-C(63)	108.7(9)
F(38C)-C(38)-C(37)	109.9(10)	F(64A)-C(64)-C(65)	106.7(9)
F(38B)-C(38)-C(37)	110.5(9)	F(64B)-C(64)-C(65)	105.5(9)
F(38A)-C(38)-C(37)	111.0(9)	C(63)-C(64)-C(65)	118.2(9)
C(62)-C(61)-Sn(2)	117.5(6)	F(67B)-C(67)-F(67A)	106.7(9)
C(12)-C(11)-Sn(1)	122.5(7)	F(67B)-C(67)-C(68)	109.0(11)
C(13)-C(12)-C(11)	113.1(8)	F(67A)-C(67)-C(68)	108.9(9)
O(1B)-C(07B)-O(2B)	121.6(10)	F(67B)-C(67)-C(66)	108.4(8)
O(1B)-C(07B)-C(06B)	123.2(10)	F(67A)-C(67)-C(66)	107.8(10)

C(68)-C(67)-C(66)	115.6(10)	F(57A)-C(57)-F(57B)	109.4(9)
F(63A)-C(63)-F(63B)	104.8(7)	F(57A)-C(57)-C(56)	109.8(8)
F(63A)-C(63)-C(62)	111.6(9)	F(57B)-C(57)-C(56)	107.5(8)
F(63B)-C(63)-C(62)	110.7(9)	F(57A)-C(57)-C(58)	108.0(9)
F(63A)-C(63)-C(64)	107.1(9)	F(57B)-C(57)-C(58)	108.5(9)
F(63B)-C(63)-C(64)	107.5(9)	C(56)-C(57)-C(58)	113.5(10)
C(62)-C(63)-C(64)	114.6(8)	F(16B)-C(16)-F(16A)	107.5(8)
F(65B)-C(65)-F(65A)	107.4(8)	F(16B)-C(16)-C(15)	110.6(8)
F(65B)-C(65)-C(66)	108.9(9)	F(16A)-C(16)-C(15)	107.5(8)
F(65A)-C(65)-C(66)	109.1(9)	F(16B)-C(16)-C(17)	108.7(8)
F(65B)-C(65)-C(64)	108.2(8)	F(16A)-C(16)-C(17)	107.8(8)
F(65A)-C(65)-C(64)	107.4(9)	C(15)-C(16)-C(17)	114.5(8)
C(66)-C(65)-C(64)	115.5(9)	F(47B)-C(47)-F(47A)	109.4(8)
F(68B)-C(68)-F(68A)	111.4(12)	F(47B)-C(47)-C(46)	110.4(8)
F(68B)-C(68)-F(68C)	107.1(10)	F(47A)-C(47)-C(46)	108.1(8)
F(68A)-C(68)-F(68C)	106.1(11)	F(47B)-C(47)-C(48)	107.5(8)
F(68B)-C(68)-C(67)	111.6(11)	F(47A)-C(47)-C(48)	107.8(9)
F(68A)-C(68)-C(67)	111.6(10)	C(46)-C(47)-C(48)	113.5(8)
F(68C)-C(68)-C(67)	108.8(11)	F(48C)-C(48)-F(48B)	109.7(9)
F(28B)-C(28)-F(28A)	110.3(12)	F(48C)-C(48)-F(48A)	109.4(9)
F(28B)-C(28)-F(28C)	109.2(11)	F(48B)-C(48)-F(48A)	107.6(9)
F(28A)-C(28)-F(28C)	106.8(11)	F(48C)-C(48)-C(47)	111.1(9)
F(28B)-C(28)-C(27)	111.7(11)	F(48B)-C(48)-C(47)	110.3(9)
F(28A)-C(28)-C(27)	110.3(11)	F(48A)-C(48)-C(47)	108.8(8)
F(28C)-C(28)-C(27)	108.5(11)		

Table S4. Anisotropic displacement parameters ($Å^2x10^6$) for *m*-**2.1**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+...+2hka^*b^*U_{12}]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Sn(1)	22(1)	12(1)	26(1)	6(1)	4(1)	6(1)
Sn(2)	21(1)	14(1)	23(1)	1(1)	4(1)	4(1)
F(53B)	36(4)	26(4)	39(4)	-8(3)	-7(3)	15(3)
F(44B)	21(3)	17(3)	32(4)	5(3)	-5(3)	9(3)
F(36B)	37(4)	13(3)	35(4)	3(3)	6(3)	6(3)
F(44A)	29(4)	20(4)	25(4)	4(3)	5(3)	-10(3)
F(33B)	28(4)	16(4)	31(4)	1(3)	3(3)	3(3)
F(16B)	39(4)	16(4)	47(4)	2(3)	-17(3)	1(3)
F(56B)	26(4)	42(4)	40(4)	-10(3)	2(3)	7(3)
F(56A)	55(5)	34(4)	40(4)	9(3)	-8(3)	-3(3)
F(54B)	34(4)	44(4)	25(4)	-3(3)	14(3)	-2(3)
F(45B)	37(4)	22(4)	30(4)	7(3)	0(3)	15(3)
F(24B)	16(4)	35(4)	61(5)	2(3)	5(3)	8(3)
F(53A)	32(4)	32(4)	33(4)	4(3)	9(3)	-6(3)
F(58C)	35(4)	49(5)	41(4)	-9(4)	0(3)	-13(4)
F(45A)	14(3)	26(4)	39(4)	1(3)	7(3)	-5(3)
F(46B)	51(4)	13(3)	38(4)	11(3)	8(3)	17(3)
F(43B)	21(3)	16(4)	35(4)	11(3)	9(3)	4(3)
F(55B)	27(4)	39(4)	39(4)	-4(3)	11(3)	-8(3)
F(36A)	17(3)	26(4)	35(4)	16(3)	9(3)	4(3)
F(13B)	42(4)	41(4)	31(4)	10(3)	5(3)	16(3)
F(55A)	35(4)	22(4)	47(4)	0(3)	-12(3)	5(3)
F(13A)	29(4)	19(4)	51(4)	9(3)	-7(3)	-2(3)
F(47B)	16(4)	45(4)	32(4)	-3(3)	-1(3)	4(3)
F(33A)	27(4)	19(4)	30(4)	5(3)	6(3)	6(3)
C(03A)	27(7)	7(6)	24(7)	-5(5)	4(5)	-6(5)
C(53)	15(7)	31(8)	24(7)	-2(6)	-1(5)	9(6)
C(13)	20(7)	24(7)	39(8)	6(6)	23(6)	-2(6)
C(51)	17(6)	23(7)	36(7)	7(5)	7(5)	6(5)
C(27)	45(9)	36(8)	26(8)	-3(6)	-7(7)	9(7)

C(43)	14(6)	8(6)	37(7)	11(5)	1(5)	1(5)
C(02A)	32(7)	11(6)	15(6)	3(5)	-5(5)	15(5)
C(33)	12(6)	7(6)	29(7)	4(5)	7(5)	1(5)
C(31)	17(5)	11(5)	15(5)	5(4)	1(4)	2(4)
C(45)	11(6)	12(6)	28(7)	16(5)	2(5)	0(5)
C(46)	17(5)	21(5)	27(5)	8(4)	11(4)	2(4)
C(41)	14(6)	17(6)	21(6)	4(5)	0(5)	3(5)
F(48C)	87(6)	13(4)	53(5)	-4(3)	17(4)	-6(4)
F(14B)	27(4)	23(4)	55(4)	14(3)	-2(3)	-2(3)
F(43A)	25(3)	18(3)	30(4)	0(3)	-1(3)	11(3)
F(24A)	46(4)	37(4)	41(4)	12(3)	21(3)	11(3)
F(58B)	53(5)	76(6)	31(4)	8(4)	1(4)	-13(4)
F(58A)	38(5)	54(5)	50(5)	-4(4)	-13(4)	4(4)
F(54A)	30(4)	23(4)	37(4)	-1(3)	-4(3)	13(3)
F(27B)	75(5)	29(4)	38(4)	1(3)	-2(4)	27(4)
F(27A)	54(5)	29(4)	77(5)	-3(4)	9(4)	-6(4)
C(02B)	21(6)	9(6)	19(6)	8(5)	8(5)	3(5)
C(03B)	33(7)	10(6)	27(7)	3(5)	7(5)	2(5)
F(16A)	25(4)	46(4)	24(4)	5(3)	3(3)	11(3)
C(36)	9(6)	22(7)	29(7)	0(6)	4(5)	3(5)
C(56)	28(7)	24(7)	21(7)	-6(6)	10(5)	2(5)
C(52)	27(7)	21(7)	20(6)	-3(5)	3(5)	7(5)
C(37)	26(7)	22(7)	35(8)	0(6)	4(6)	-3(6)
C(14)	26(7)	23(7)	29(8)	1(6)	1(6)	-2(6)
C(54)	31(7)	22(7)	17(6)	11(5)	14(5)	15(5)
C(55)	16(7)	30(8)	31(7)	5(6)	0(5)	6(5)
C(58)	31(9)	54(10)	39(9)	-12(8)	5(7)	-10(7)
C(42)	13(6)	15(6)	23(6)	6(5)	-1(5)	5(5)
C(24)	28(7)	18(7)	22(7)	2(5)	14(5)	11(5)
C(44)	15(6)	18(7)	31(7)	7(5)	-15(5)	4(5)
C(01B)	20(6)	6(6)	23(7)	-6(5)	-2(5)	3(5)
F(37B)	26(4)	28(4)	44(4)	14(3)	4(3)	-3(3)
F(47A)	41(4)	24(4)	34(4)	8(3)	4(3)	4(3)
F(37A)	58(5)	36(4)	32(4)	5(3)	12(3)	24(4)
F(28B)	104(7)	57(6)	56(5)	-13(4)	-32(5)	26(5)
F(28C)	107(6)	32(5)	43(5)	-1(4)	11(4)	19(4)
C(21)	21(6)	13(6)	34(7)	2(5)	14(5)	5(5)

F(18B)	68(5)	57(5)	54(5)	-25(4)	-31(4)	33(4)
F(28A)	82(6)	59(6)	56(5)	2(4)	27(5)	27(5)
C(04B)	24(7)	7(6)	47(8)	-1(6)	3(6)	11(5)
C(06B)	16(6)	13(6)	24(7)	8(5)	-3(5)	-2(5)
C(05B)	20(6)	14(6)	38(7)	16(5)	0(5)	5(5)
F(35B)	40(4)	21(4)	34(4)	3(3)	10(3)	10(3)
F(23B)	33(4)	36(4)	34(4)	7(3)	-4(3)	16(3)
F(23A)	37(4)	22(4)	33(4)	1(3)	18(3)	6(3)
F(34B)	30(4)	23(4)	44(4)	12(3)	11(3)	10(3)
F(18C)	37(5)	109(7)	41(5)	30(4)	12(4)	26(4)
C(34)	21(7)	14(7)	32(7)	2(5)	7(5)	11(5)
C(25)	22(7)	15(7)	37(8)	-1(6)	10(6)	1(5)
C(32)	19(5)	8(5)	21(5)	-4(4)	3(4)	10(4)
C(22)	25(7)	30(7)	25(7)	1(6)	8(5)	2(6)
C(35)	26(7)	21(7)	29(7)	0(6)	6(6)	7(6)
C(01A)	22(7)	17(7)	26(7)	10(5)	-1(5)	-2(5)
C(15)	11(6)	33(8)	30(7)	10(6)	3(5)	-8(5)
C(23)	45(8)	31(8)	13(7)	0(6)	11(6)	5(6)
F(26A)	33(4)	39(4)	49(4)	5(3)	16(3)	13(3)
F(38C)	36(4)	30(4)	32(4)	19(3)	-1(3)	1(3)
F(14A)	36(4)	20(4)	30(4)	1(3)	9(3)	9(3)
F(17B)	28(4)	56(5)	41(4)	12(3)	13(3)	14(3)
F(38B)	35(4)	35(4)	38(4)	5(3)	3(3)	19(3)
F(15B)	31(4)	50(5)	35(4)	10(3)	12(3)	17(3)
F(17A)	47(4)	28(4)	40(4)	0(3)	-8(3)	11(3)
F(34A)	24(4)	16(4)	38(4)	1(3)	4(3)	-3(3)
F(35A)	23(4)	28(4)	46(4)	17(3)	13(3)	1(3)
F(15A)	29(4)	12(3)	37(4)	-1(3)	-1(3)	2(3)
F(57B)	43(4)	27(4)	43(4)	3(3)	-7(3)	-4(3)
F(38A)	34(4)	34(4)	52(4)	17(3)	-7(3)	-8(3)
F(25B)	16(4)	37(4)	56(4)	0(3)	2(3)	0(3)
O(2B)	28(4)	17(4)	39(5)	8(4)	14(4)	1(4)
F(48B)	23(4)	51(5)	46(4)	-2(3)	0(3)	25(3)
O(1B)	29(5)	15(4)	29(5)	8(4)	8(4)	2(3)
F(26B)	68(5)	44(4)	27(4)	8(3)	2(4)	20(4)
C(04A)	34(8)	9(7)	48(8)	5(6)	8(6)	2(5)
C(62)	27(7)	20(7)	20(6)	-7(5)	6(5)	11(5)

C(17)	13(6)	29(8)	29(7)	-4(6)	0(5)	-1(5)
C(38)	31(8)	28(8)	40(8)	8(7)	-8(6)	-1(6)
C(61)	36(7)	17(6)	19(6)	3(5)	18(5)	9(5)
C(11)	36(7)	5(6)	46(8)	-3(5)	1(6)	3(5)
C(12)	20(6)	11(6)	39(7)	-1(5)	-1(5)	-2(5)
C(07B)	31(7)	18(7)	26(7)	-2(6)	-4(6)	4(6)
C(26)	35(8)	30(8)	38(8)	8(6)	8(6)	8(6)
C(18)	40(9)	19(8)	48(9)	-13(7)	-6(7)	19(6)
F(25A)	51(4)	27(4)	37(4)	9(3)	10(3)	15(3)
F(18A)	32(4)	50(5)	38(4)	2(3)	0(3)	21(3)
C(66)	41(8)	25(8)	33(8)	3(6)	-4(6)	8(6)
O(2A)	35(5)	24(5)	41(5)	16(4)	13(4)	16(4)
C(07A)	32(7)	12(7)	26(7)	0(6)	-3(6)	4(6)
C(05A)	19(7)	5(6)	38(7)	0(5)	4(5)	2(5)
C(06A)	24(7)	23(7)	12(6)	6(5)	-4(5)	13(5)
F(63A)	31(4)	37(4)	44(4)	6(3)	3(3)	9(3)
F(65B)	54(4)	34(4)	32(4)	5(3)	-10(4)	-14(3)
F(66B)	75(5)	36(4)	33(4)	0(3)	-12(4)	-12(4)
F(67B)	67(5)	22(4)	37(4)	7(3)	8(4)	8(3)
F(65A)	58(5)	41(5)	55(5)	13(4)	33(4)	25(4)
F(67A)	45(5)	50(5)	70(5)	27(4)	12(4)	21(4)
F(66A)	46(5)	40(4)	52(5)	10(3)	13(4)	19(4)
O(1A)	46(5)	13(4)	36(5)	10(4)	12(4)	14(4)
F(68B)	93(6)	55(5)	30(4)	8(4)	8(4)	10(4)
C(64)	36(8)	35(8)	20(7)	-7(6)	6(6)	-3(6)
C(67)	51(9)	36(8)	25(8)	1(6)	3(7)	20(7)
C(63)	28(7)	33(8)	12(6)	-10(6)	-5(5)	-9(6)
C(65)	22(7)	43(9)	16(7)	2(6)	-6(6)	0(6)
C(68)	74(11)	32(9)	38(9)	10(7)	0(8)	6(8)
F(63B)	42(4)	27(4)	26(4)	0(3)	16(3)	0(3)
F(64B)	47(5)	62(5)	39(4)	16(4)	-22(4)	-13(4)
F(68C)	102(6)	38(5)	60(5)	20(4)	9(5)	6(4)
F(68A)	46(5)	80(6)	71(5)	38(4)	16(4)	3(4)
C(28)	88(13)	20(9)	44(10)	-1(7)	9(9)	13(8)
F(64A)	75(5)	25(4)	43(4)	8(3)	34(4)	17(4)
F(57A)	26(4)	55(5)	44(4)	-16(3)	11(3)	0(3)
C(57)	21(7)	33(8)	26(7)	-9(6)	8(6)	2(6)

F(46A)	13(3)	34(4)	29(4)	1(3)	4(3)	-1(3)
F(48A)	46(4)	52(5)	27(4)	-12(3)	-4(3)	25(3)
C(16)	27(7)	32(8)	16(7)	-3(6)	10(5)	1(6)
C(47)	24(5)	13(5)	26(5)	11(4)	7(4)	4(4)
C(48)	28(5)	24(5)	29(6)	0(5)	2(5)	8(5)

Table S5. Hydrogen coordinates ($x10^4$) and isotropic displacement parameters ($Å^2x10^3$) for *m*-**2.1**.

	x	y	z	U(eq)
H(03A)	14563	4853	3161	25
H(51B)	7502	7540	3166	29
H(51A)	7776	7410	2546	29
H(31B)	15411	1854	3861	17
H(31A)	15707	2834	4182	17
H(41B)	11161	7050	3654	21
H(41A)	10518	7841	3458	21
H(03B)	9553	4461	3147	27
H(52B)	6031	6255	2953	28
H(52A)	5656	7031	2647	28
H(42B)	9136	7803	4125	20
H(42A)	9645	6951	4296	20
H(01B)	7214	5975	3886	21
H(21B)	14817	3293	2400	26
H(21A)	15892	3684	2871	26
H(04B)	9007	3390	3647	31
H(05B)	7604	3610	4295	27
H(32A)	13661	2698	4473	19
H(32B)	13523	1688	4212	19
H(22B)	16600	2721	2191	32
H(22A)	16778	2395	2765	32
H(01A)	12285	3609	4063	26
H(2BA)	5331	5685	4802	40
H(04A)	13897	6162	3506	36
H(62B)	10291	5782	1571	27
H(62A)	9062	6192	1720	27
H(61B)	10850	5752	2509	27
H(61A)	9594	5151	2277	27
H(11B)	12792	1354	3153	36
H(11A)	11937	2089	3328	36

H(12B)	12449	1563	2234	29
H(12A)	11206	1252	2473	29
H(2AA)	10436	4313	4926	46
H(05A)	12365	6223	4088	26