TOWARDS RADIOPHARMACEUTICAL SYNTHESIS USING FLUOROUS CHEMISTRY

TOWARDS RADIOPHARMACEUTICAL SYNTHESIS USING FLUOROUS CHEMISTRY

By

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Abstract

Nuclear medicine requires the use of radiolabelled pharmaceuticals in order to carry out imaging and therapeutic protocols.[1] Unfortunately, traditional radiolabelling approaches used in radiopharmaceutical synthesis often generate multiple products, which require exhaustive HPLC purification prior to use.[2] Chromatographic purification reduces radiochemical yields, increases exposure, and can, in certain cases, preclude the use of shorter-lived isotopes.

In light of the limitations of current radiolabelling methods, we endeavored to develop a versatile and efficient radiolabelling strategy that would avoid the need for HPLC purification. To this end, the compounds to be labelled were first bound to a highly fluorinated stannylated precursor, which, when reacted with a radiohalogen, generated the corresponding radiolabelled compound. Unlike other halodestannylation reactions, however, the radiolabelled compound could be isolated from the stannylated precursor by elution through a fluorous Sep-Pak.

As a model system, tris(perfluorohexylethyl)tin-3-benzoic acid (**1**), was synthesised and labelled. Compound **1** was prepared through a novel reaction which involved treatment of bromotris[(2-perfluorohexyl)ethyl]tin with the organozinc reagent 3-(ethoxycarbonyl)phenylzinc. Reaction of compound **1** with $[^{18}F]F_2$, followed by fluorous

[1] Wilson, M.A.; Hammes, R.J. Textbook of Nuclear Medicine, M.A. Wilson (Ed), Lippincott-Raven, Philadelphia, **1998**, 385-390.

Sep-Pak purification, generated the corresponding labelled 3-[^{18}F]fluorobenzoic acid in 27 min, in 30 % radiochemical yield, and having a specific activity of 1966 mCi/mol. Alternatively, reaction of compound **1** with Na^{125}I provided the corresponding product, 3-[^{125}I[iodobenzoic acid, in <1 hr, in 75 % radiochemical yield, and greater than 99 % radiochemical purity. Prior tests clearly showed that any excess or unreacted substrate was fully removed from the product using a fluorous Sep-Pak.

In addition to the initial validation studies, new synthetic methods were developed as a means of preparing more complex "fluorous" substrates. A coupling methodology was developed which permitted synthesis of a fluorous "tagged" benzamide, through reaction of **1** in the presence of HBTU with N,N-dimethylethylenendiamine. Subsequent labelling using F_2 and I_2 has been shown to generate the corresponding labelled benzamides, which are important agents for imaging melanoma and dopamine receptors.[3] Another relevant radiopharmaceutical precursor, tris[(2-perfluorohexyl)ethyl]tin-3-benzylamine (**2**), was synthesized though reaction of bromotris[(2-perfluorohexyl)ethyl]tin with 1-(3-bromobenzyl)-2,2,5,5-tetramethyl-1,2,5-azadisilolidine. Compound **2** was successfully coupled to the chemotactic peptide, GFLM(f), and the product subsequently labelled with iodine. Compound **2** was also used to prepare the corresponding benzylguanidine (**3**), an important precursor to m-

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[2] Bolton, R. *J. Labeled Compd. Radiopharm.* **2002**, 45, 485.
[3] Moreau, M.F.; Labarre, P.; Foucaud, A.; Seguin, H.; Bayle, M.; Papon, J.; Madelmont, J.C. *J. Labeled Compd. Radiopharm.* **1998**, *41*, 965.

iodobenzylguanidine, which is used for imaging and therapy of neural crest tumors.[4] Initial labelling results show that reaction of **3** with NaI and an oxidant generates the corresponding labelled m-iodobenzylguanidine.

Results suggest that the fluorous synthesis method will offer several advantages over traditional radiolabelling strategies. The radiolabelled products are generated in high yield, through rapid and facile reactions that avoid the need for HPLC purification.

[4] Vaidyanathan, G.; Affleck, D.J.; Zalutsky, M.R. *Bioconjugate Chem.* **1996**, *7*, 102.

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Table of Contents

List of Figures

List of Abbreviations and Symbols

ACN	Acetonitrile
Å	Angstorm
Ci	Curie
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
d	Doublet (^{1}H NMR)
DIC	Diisopropylcarbodiimide
EDC	Ethyl-3-(3-dimethylamino)-propylcarbodiimide-hydrochloride
EOB	End of bombardment
Et_2O	Diethylether
f	Formyl
FDG	2-fluoro-2-deoxy-D-glucose
Gly	Glycine
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HBTU	*N*-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophasphate *N*-oxide
HOBt	1-Hydroxylbenzotriazole
HPLC	High Performance Liquid Chromatography
IR	Infrared Spectroscopy
keV	kiloelectron volt
Leu	Leucine
m	Multiplet (^{1}H NMR)
MeOH	Methanol
Met	Methionine
MFBG	meta-fluorobenzylguanidine
MIBG	meta-iodobenzylguanidine
mp	Melting point
MS	Mass Spectrometry
PET	Positron Emission Tomography
Phe	Phenylalanine
ppm	Parts per million
s	Singlet (^{1}H NMR)
SPECT	Single Photon Emission Computed Tomography
t	Triplet (^{1}H NMR)
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography

Chapter 1

Introduction and Purpose

Nuclear medicine is a specialised subset of radiology, which involves the use of radiochemicals for both imaging and therapeutic applications. More than 50,000 nuclear medicine procedures are carried out around the world everyday, which is a testament to the utility of this field.[1] Furthermore, it is estimated that one in three hospital patients will undergo a nuclear medicine procedure in the management of their illness.[2]

In addition to serving an increasingly important role in modern medicine, radioimaging techniques are also widely used in cutting edge biochemical and pharmaceutical research. For example, between 80 and 90 % of the new drugs which receive FDA approval have been radiolabelled in order to provide biodistribution, metabolism and excretion data.[1] With respect to biochemical research, dynamic imaging using a radiolabelled glucose analogue has been used to identify pathways that protect tumour cells from apoptosis or cell mediated death.[3]

With the growing use of radioimaging techniques, there is an increasing demand for both new radiopharmaceuticals and methods for their production.

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[1] Industry Canada. R&D Innovation Success Stories in the Chemical Sector: MDS Nordion. 1997. <http://strategis.ic.gc.ca/SSG/bt01061e.html.>

1.1 Objectives

The objective of this thesis was to develop a rapid and facile method for the regioselective labelling of biomolecules. Ideally, this synthetic method would produce the desired product in high yield and specific activity in a short period of time and would not require HPLC purification. A synthetic strategy, namely the "fluorous synthesis technique", was explored as a route towards these ambitious goals.[4]

In order to demonstrate the utility, flexibility, and advantages of this approach for the development of radiopharmaceuticals, a select series of model substrates were synthesised and labelled using cold isotopes. Pending satisfactory labelling yields with the cold isotopes, development of the appropriate radiolabelling protocols for the incorporation of radiohalogens, particularly $^{18}F[F_2]$ and $^{125}I[I_2]$, into these various pharmaceutical precursors was undertaken. The ultimate gauges of success would be the ability to synthesise the labelled compounds rapidly, in high purity, and without the need for complex purification protocols.

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[2] United States Office of Nuclear Energy, Sciences & Technology. Nuclear Energy Research Advisory Committee: Subcommittee for Isotope Research & Production Planning. Washington: April 2000. <http://nuclear.gov/nerac/finalisotopereport.pdf.>

[3] Haberkorn, U.; Bellemann, M.E.; Brix, G.; Kamencic, H.; Morr, I.; Traut, U.; Altmann, A.; Doll, J.; Blatter, J.; Kinscheft, R. *Eur. J. Nucl. Med.* **2001**, *28*, 418.

1.2 Nuclear Medicine

Nuclear medicine relies on radiolabelled molecules, so called radiopharmaceuticals, which, when administered to a patient, selectively localise within certain tissues or organs. Modern radiopharmaceuticals are typically composed of two distinct components: a radionuclide and a biomolecule. The biomolecule determines the biodistribution of the radiopharmaceutical, while the decay of the radioisotope generates the signal, which is used to create images of the corresponding biochemical process under study. Unlike conventional drugs, radiopharmaceuticals are administered in minuscule quantities, and as such, do not perturb normal physiologic function.[5] **Figure 1-1** presents a selection of radiopharmaceuticals that are used for therapy,[5] diagnosis,[6] and biochemical research.[7]

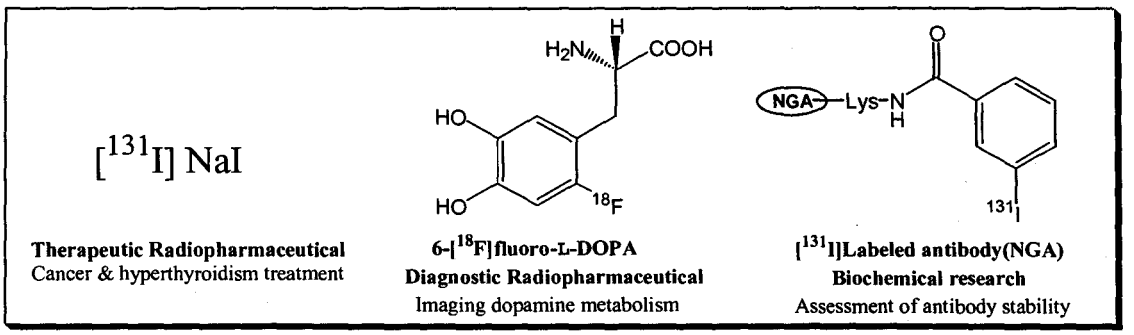

Figure 1-1. Examples of radiopharmaceuticals

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[4] Curran,D.P.; Hadida,S.; Studer,A.; He,M.; Kim,S.Y.; Luo,Z.; Larhed,M.; Hallberg,A.; Linclau,B. Combinatorial Chemistry: A Practical Approach, H.Fenniri (Ed.), Oxford University Press, Oxford. **2000**, 327-390.

[5] Wilson, M.A.; Hammes, R.J. Textbook of Nuclear Medicine, M.A. Wilson (Ed), Lippincott-Raven , Philadelphia, **1998**, 385-390.

[6] Namavari, M.; Bishop, A.; Satyamurthy, N.; Bida, G.; Barrio, J.R. *Appl. Radiat. Isot.* **1992**, *43*, 989.

[7] Arano, Y.; Wakisaka, K.; Ohmomo, Y.; Uezono, T.; Mukai, T.; Motonari, H.; Shino, H.; Sakahara, H; Konishi, J.; Tankaka, C.; Yokoyamam, A. *J. Med. Chem.* **1994**, *37*, 2609.

1.3 Nucleonics

Radionuclides decay by a number of different pathways. The nature of the decay, in conjunction with the emitted energy and half-life, determine an isotope's potential utility. Isotopes which decay via γ and β^+ pathways are most suited for imaging, while β^-, α and electron capture decaying isotopes are potentially more suited for therapeutic applications (**Figure 1-2**).

Alpha (α) Decay: $^{235}_{92}U \longrightarrow ^{231}_{90}Th + ^{4}_{2}He^{2+}$

Beta (β^-) Decay: $^{131}_{53}I \longrightarrow ^{131}_{54}Xe + \beta^- + \bar{\nu}_{e^-}$

Positron (β^+) Decay $^{18}_{9}F \longrightarrow ^{18}_{8}O + \beta^+ + \nu_{e^-}$

Electron Capture: $^{67}_{31}Ga + e^- \longrightarrow ^{67}_{30}Zn + \nu_{e^-}$

Figure 1-2. Examples of various nuclear decay processes

1.3.1 Decay modes

Nuclei which undergo alpha decay liberate a helium ion (an α particle), and as a result the daughter nuclei have a mass number four less than the parent isotope. Alpha particles deposit their energy over a short distance due to their high mass; the result is highly localised ionisation and cell damage.[5]

The three types of beta decay involve, in the simplest terms, the conversion of either a neutron to a proton or visa versa. In negatron decay, the overabundance of neutrons in the nucleus results in a neutron to proton conversion with an accompanying emission of an electron (a β^- particle) and an antineutrino (ν_{e^-}). The majority of β^- emitting radioisotopes are produced in a nuclear reactor.

In positron decay, a proton is converted into a neutron in a neutron-deficient nuclide, with the resultant emission of a positron (β^+) and neutrino (ν_{e^-}). When the emitted positron, the antiparticle of an electron, loses kinetic energy (1-2 mm), it combines with an electron and annihilates, giving off two co-linear gamma rays with an energy of 511 keV. This property, unique to positron emitting isotopes, forms the basis for positron emission tomography (PET).

In electron capture decay, an orbital electron is captured by the unstable nuclei, followed by the emission of a neutrino. This process may result in an excited state, which subsequently leads to gamma emission followed by characteristic X-ray or Auger electron emission. Several radionuclides that find application in nuclear medicine and biochemistry, such as ^{125}I, decay through electron capture.

1.4 Imaging in Nuclear Medicine

There are two main types of imaging techniques utilised in nuclear medicine, namely single photon emission computed tomography (SPECT) and positron emission tomography (PET). Radioimaging devices are largely based on the same principles used in the first gamma cameras, which were introduced in the 1950's.[8] To image γ-emitters, emitted photons first interact with scintillation crystals located in one or more cameras that surround a portion of a patient's body. Interaction of the photon with the crystal generates a small amount of light, which is converted to measurable electric pulses using photomultiplier tubes.[8] Modern advancements have resulted in the capacity to create

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[8] Saha, G.B; Fundamentals of Nuclear Pharmacy, 4th Edition, Springer, New York, **1998**, Chapter III.

three-dimensional images, or tomographic images, using several and/or rotating cameras coupled with the appropriate computer software.

1.4.1 SPECT

In SPECT, pharmaceuticals labelled with gamma-emitting nuclides such as ^{99m}Tc, ^{123}I, ^{67}Ga, and ^{111}In produce tomographic images when their single photon emissions are monitored by a rotating gamma camera. At present, SPECT imaging remains the most common nuclear medicine imaging technique, benefiting from its proven performance and low cost.[5]

1.4.2 PET

In PET, positron emitting isotopes such as ^{18}F, ^{13}N, ^{15}O, and ^{11}C decay and produce two co-linear photons of 511 keV (**Figure 1-3**). A ring of detectors surrounding the patient allows for simultaneous detection of the two photons, which in turn allows for determination of the position of the decaying isotope. Consequently, one of the advantages of PET over SPECT is that it is possible to acquire an absolute measurement of isotope concentration in tissue, permitting quantitative assessment of physiological parameters.[8]

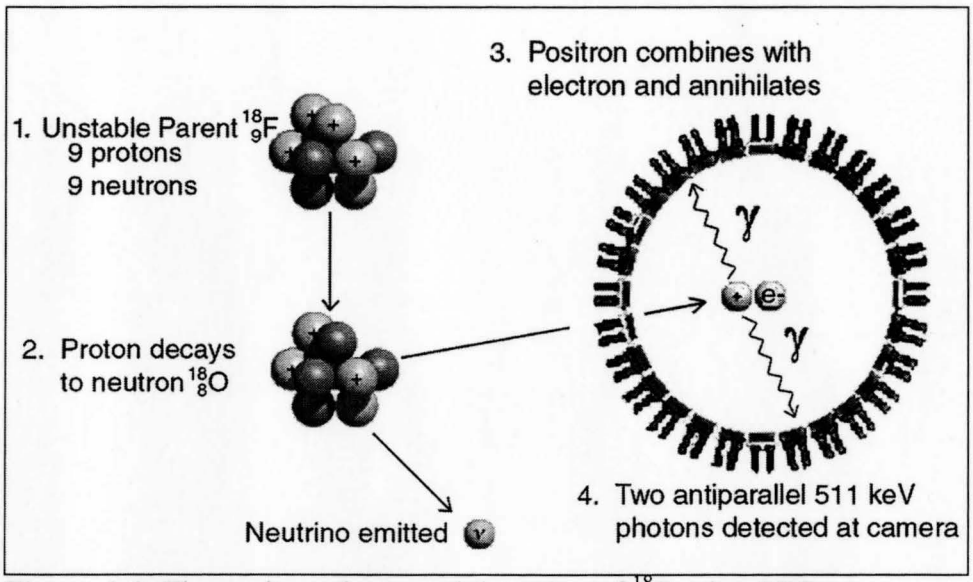

Figure 1-3. The positron decay and detection of ^{18}F using PET

A whole body PET with stacked 2-D images from front to back is shown in **Figure 1-4**.[9] These images diagnose a patient with melanoma; the cancer is identified though an accumulation of [^{18}F]fluoro-2-deoxy-D-glucose (FDG) in the ankle region.

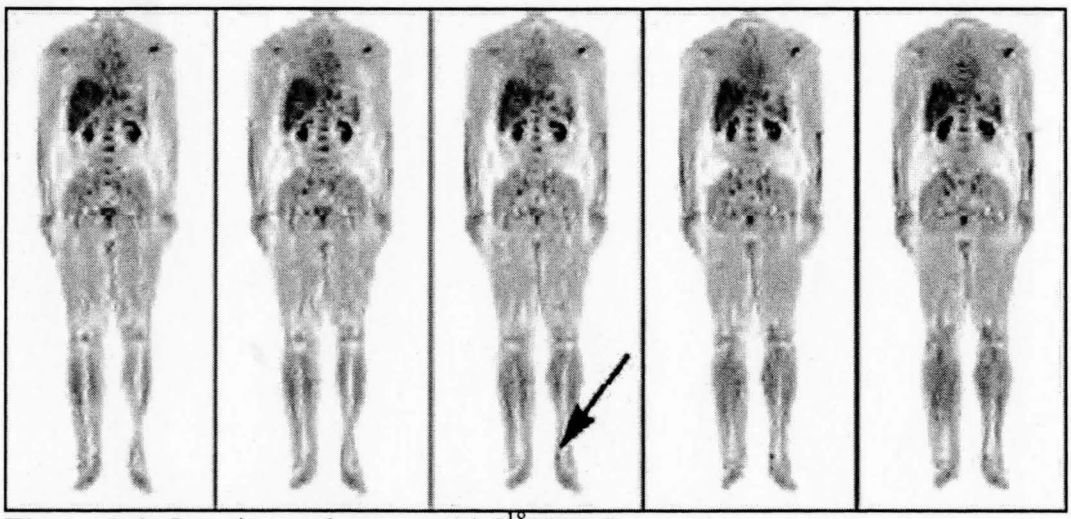

Figure 1-4. Imaging melanoma with [^{18}F]FDG

[9] Cherry, S.R. Physics web: Watching biology in action. Bristol: June 2002. <http://physicsweb.org/article/world/15/6/7#pw506072>

Despite the fact that PET is more sensitive and provides better resolution than SPECT, its growth has been hampered, until recently, by the higher cost of isotope production which typically requires an on-site cyclotron. The number of clinical PET facilities have expanded recently however, having justified the increased cost with more highly resolved images and invaluable assessment/diagnosis potential.[10]

1.5 Radiohalogens and Radiochemistry

Radiohalogens play an important role in nuclear medicine. For instance, ^{18}F ($t_{1/2}$=109 min) is the most widely utilised isotope in PET studies, largely due to oncology's widespread use of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG).[7] Incorporation of fluorine, rather than other radiohalogens into biomolecules, such as FDG and ^{18}F-fluorodopa, is less likely to perturb *in* vivo functioning because of its small size.

Several isotopes of iodine are also commonly utilised in nuclear medicine, including ^{123}I ($t_{1/2}$=13.2 hr) and ^{131}I ($t_{1/2}$=8 days). Whereas ^{123}I and ^{131}I are used in SPECT, the β^+ emitting ^{124}I ($t_{1/2}$=101 hr), though not widely available, could in the future be a valuable isotope for PET imaging. The even longer half-life and low-energy gamma of ^{125}I (35 keV, $t_{1/2}$=60 days) have led to its widespread use in biochemical research.[5]

The positron emitting isotope of bromine, ^{76}Br ($t_{1/2}$=16.2 hr), is less common largely due to its more difficult and expensive production.[11] The main advantage of using ^{76}Br,

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[10] Schiepers, C.; Hoh, C.K. *Eur. Radiol.* **1998**, *8*, 1481.
[11] Kao, C.K.; Sassaman, M.B.; Sazajek, L.P.; Waki, Y.M.; Eckelman, W.C. *J Labeled Compd. Radiopharm.* **2001**, *44*, 889.

however, is its long half-life, which permits for PET imaging of compounds with slow kinetics.[12]

^{211}At is an α-emitter with a reasonably long half-life (7.2 hr) and no β^- or γ emissions. ^{211}At-labeled compounds are therefore increasingly being applied in radioimmunotherapy roles. Zalutsky *et al.* have, for example, labelled monoclonal antibodies for cancer therapy with ^{211}At, using the precursor *N*-succinimidyl 3-(trimethylstannyl)benzoate.[13] Unfortunately, widespread use of this radionuclide is limited because only a select few research centres have the appropriate facilities to produce and handle the isotope.

There are three principle methods employed for the labelling of compounds with halogens: electrophilic halodeprotonation, nucleophilic displacement of a leaving group, and halodemetallation reactions.[14] There are often numerous synthetic approaches to the same radiopharmaceutical, and the most advantageous are determined on a case by case basis.

1.5.1 Radiofluorination strategies

The half-life of ^{18}F, 109.8 min, is sufficiently long enough to allow for the application of a large number of different incorporation strategies. **Figure 1-5** compares three synthetically different routes and yields in the production of L-6-[^{18}F]fluorodopa.

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[12] Kao, C-H. *J. Labeled Compd. Radiopharm.* **2001**, *44*, 889.
[13] Zalutsky, M.R.; Zhao, X-G; Alston, K.L.; Bigner, D. *J. Nuc. Med.* **2001**, *42*, 1508.
[14] Bolton, R. *J. Labeled Compd. Radiopharm.* **2002**, *45*, 485

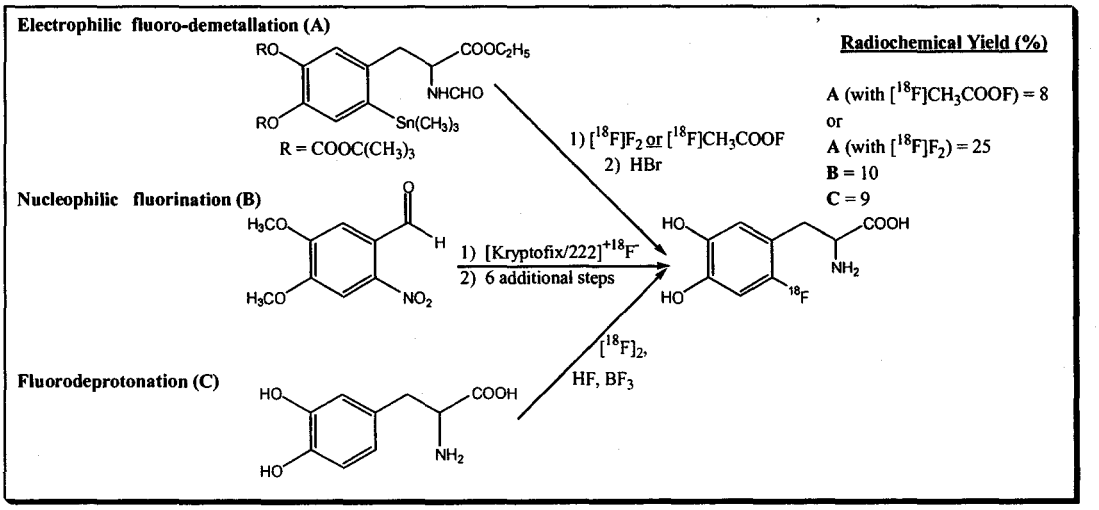

Figure 1-5. Comparing the synthetic routes to L-6-[^{18}F]fluorodopa

One of the simplest labelling strategies is direct labelling using F_2. The greater chemical reactivity of fluorine, however, often results in poor regioselectivity.[14] This is evident in the direct fluorination of L-dopa with [^{18}F]F_2 or [^{18}F]acetyl hypofluorite, which in both cases leads to mixtures of 2-, 5-, and 6-Fluoro-dopa.[15,16] These regioisomers can only be separated through exhaustive HPLC purification. In special instances, however, direct fluorination has been reported to lead to the generation of a regioselective product, such as in the synthesis of [^{18}F]-L-4-fluoro-*o*-tyrosine.[17]

Nucleophilic attack of fluoride ion on a compound containing a good leaving group has proven to be a more selective labelling strategy. For example, fluoro-arenes have been synthesised by nucleophilic attack of F^- on a phenyl derivative bearing fluorine, nitro, or trimethylammonium leaving groups.[14] One of the major advantages in using the

[15] Chirakal, R.; Firnau, G.; Garnett, E.S. *J. Nuc. Med.* **1986**, *27*, 417.
[16] Chaly, T.; Diksic, M. *J. Nuc. Med.* **1986**, *27*, 1897.
[17] DeJesus, O.T.; Nickles, R.J.; Murali, D.J. *J. Labeled Compd. Radiopharm.* **1999**, *42*, 781.

nucleophilic labelling approach is that $^{18}F^-$, unlike $^{18}F_2$, can be produced in a no-carrier-added form.

Fluorodemetallation reactions involving aryl-metal substrates are typically high yielding, regiospecific, and can be automated. [^{18}F]Fluorodemercuration and destannylation reactions have been used effectively for the production of 6-[^{18}F]fluoro-L-DOPA.[18,19] The fluorodestannylation technique can be carried out using [^{18}F]F_2 and acetyl hypofluorite (AcOF).

There are some limitations with the fluorodemetallation, the most significant being that the reactions are generally only applicable for labelling aryl rings. Demetallation reactions also give rise to toxic metal impurities, which must be removed prior to use of the radiopharmaceutical. In addition, the products are produced in low specific activity because the fluorinating species are always diluted with an amount of cold fluorine. Furthermore, in the case of destannylation reactions, the greater chemical reactivity of fluorine compared to other halogens can result in the formation of regioisomers.[20] While milder fluorinating agents, such as acetyl hypofluorite, reduce the amount of these side-products, a lower overall yield is often associated with their use.[21]

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[18] DeVries, E.F.J.; Luurtsema, G.; Brussermann, M.; Elsinga, P.H.; Vallburg, W. *Appl. Radiat. Isot.* **1999**, *51*, 389.
[19] Luxen, A., Perlmutter, M., Bida, G.T., Van Moffaert, G., Cook, J.S., Satyamurthy, N., Phelps, M.E., and Barrio, J.R. *Appl. Radiat. Isot.* **1990**, *41*, 275.
[20] Coenen, H.H., and Moerlein, S.M. *J. Fluorine Chem.* **1987**, *36*, 63.
[21] Namavari, M., Satyamurthy, N., Phelps, M.E., Barrio, J.R. *Appl. Radiat. Isot.* **1993**, *44*, 527.

1.5.2 Radioiodination strategies

The incorporation of radioiodide into radiopharmaceuticals involves methods that are similar to radiofluorinations. Electrophilic iododeprotonation, electrophilic iododemetallation (Sn, Si, Ge), and nucleophilic displacement are commonly utilised methods. **Figure 1-6** illustrates how some of these methods are applied to the synthesis of meta-[^{131}I]iodobenzylguanidine (MIBG), an important adrenal imaging agent.

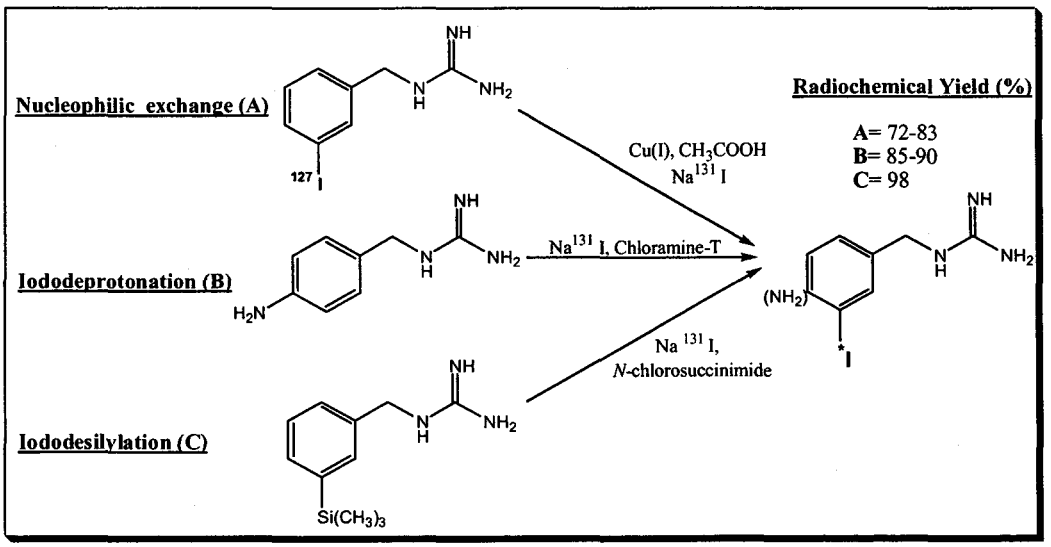

Figure 1-6. Various routes to the preparation of meta-[^{131}I]iodobenzylguanidine

In all electrophilic iodinations, NaI is first oxidised to I_2 using a range of oxidants, including chloramine-T, *N*-chlorosuccinimide, iodogen, and peracetic acid. When the sensitivity of biological molecules is not an issue, peracetic acid has been favoured because it leads to higher radiochemical yields and fewer side products.[22]

[22] Kung, M.P.; Kung, H.F. *J. Labeled Compd. Radiopharm.* **1989**, *27*, 691.

Iododemetallation reactions are popular because they proceed with high regioselectivity. The majority of aromatic iododemetallation reactions use trialkyltin derivatives, as radiochemical yields have been shown to increase in the order Si<Ge<Sn. This observation parallels the increasing carbon-metal bond lengths (Si:Ge:Sn=1.31 Å:1.36 Å:1.54 Å) and decreasing energies of the carbon-metal bond (Si:Ge:Sn = 352 kJ/mol:308 kJ/mol:257 kJ/mol).[23]

The mechanism of iododemetallation reactions is postulated to involve electrophilic attack at the ipso carbon to form a σ-complex intermediate (**Figure 1-7**).[24] Nucleophilic attack at the metal centre, by either the halide or oxidising species, leads to σ bond cleavage and regeneration of aromaticity.

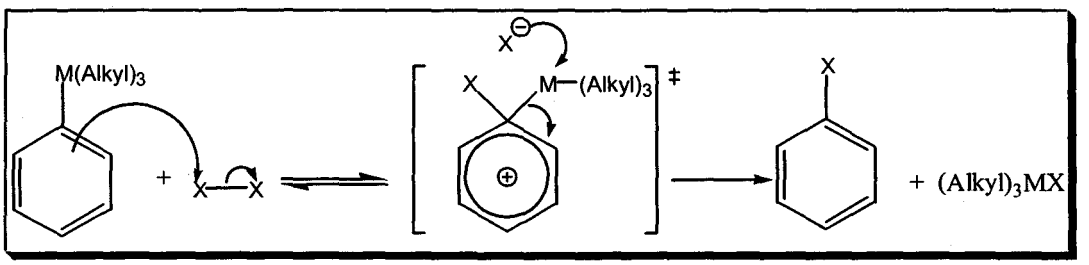

Figure 1-7. Proposed mechanism of halodemetallation reactions

One example of the application of the iododemetallation reaction is the no-carrier-added synthesis of MIBG (**Figure 1-6**). The product is formed having a high specific activity without the need for complex protecting group strategies.[25] A silicon derivative is used in place of a tin analogue in this case, due to the susceptibility of tin-aryl bonds to undergo premature cleavage under acidic conditions.

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[23] Moerlein, S.M., Coenen, H.H. *J. Chem. Soc. Perkin Trans. I* **1985**, 1941.
[24] Eaborn, C. *J. Organomet. Chem.* **1975**, *100*, 43.
[25] Vaidyanathan, G.; Zalutsky, M.R. *Appl. Radiat. Isot.* **1993**, *44*, 621.

Nucleophilic exchange reactions are a common route to radioiodine incorporation. Unlike fluorine, the lesser nucleophilic character of I^- often necessitates the addition of catalyst. For example, the ^{131}I for ^{127}I exchange reaction for MIBG synthesis (**Figure 1-6**), produces a higher radiochemical yield with the addition of either Cu(I) or ammonium sulfate.[26] This popular isotope-exchange method avoids the need for substrate/product purification, but leads to products having low specific activity.

1.6 Solid phase labelling methods

The growing popularity of PET for both clinical and biological applications has resulted in an increased demand for new radiopharmaceuticals and improved labelling methods. Despite the challenges associated with using shorter-lived, positron emitting isotopes, they are attractive for clinical imaging studies because they minimise patient exposure, allow for same day repeat studies, and provide higher resolution images. Notwithstanding, the advantages of using short half-life radioisotopes can be compromised if production of the desired radiopharmaceutical cannot all be performed within a short period of time.

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[26] Prabhakar, G.; Mehra, K.S.; Ramamoorthy, N.; Chattopadhyay, S.; Oommen, R.; Narasimhan, S.; Nair, A.; Gunasekaran, S. *Appl. Radiat. Isot.* **1999**, *50*, 1011.

The current methodologies for incorporation of a radiohalogen into a pharmaceutical, namely electrophilic aromatic deprotonation reactions, nucleophilic substitution reactions, and electrophilic demetallation reactions, all involve the use of excess substrate and the generation of several byproducts. The aforementioned labelling methods often require intensive HPLC purification to separate impurities from the desired radiopharmaceutical. In addition to concerns over radiation exposure, the time required to complete the purification reduces the overall radiochemical yield, reduces the specific activity, and shortens the available imaging time.

Recently, an alternative labelling strategy based on solid phase synthesis was proposed by Hunter *et al.*[27] It involves the use of a cross-linked tin polymer, shown in **Figure 1-8,** which can be reacted to form various tin-aryl compounds. Upon treatment with the appropriate radiohalogen, $^{125}I_2$ for example, the radiopharmaceutical is released into solution and isolated from any unreacted substrate by filtration. This approach is a polymer "bound" version of the solution phase labelling method mentioned previously, which employed trialkyltin precursors (**Figure 1-9**).

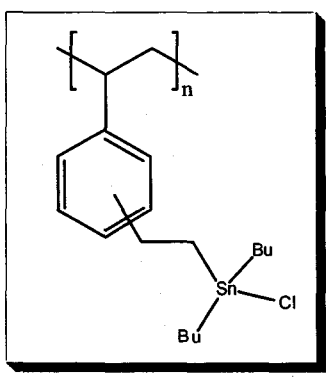

Figure 1-8. Chlorostannane polymer

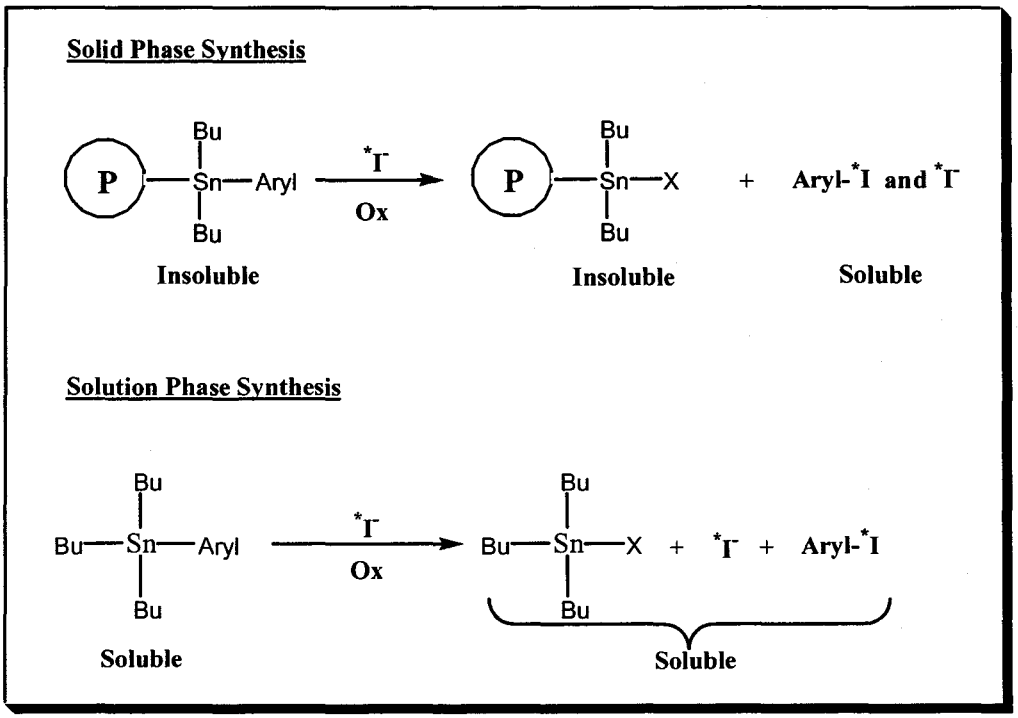

Figure 1-9. Compares the varied products of solution and solid phase synthesis

The solid phase synthesis approach was used to prepare [^{131}I]MIBG in 92% radiochemical yield with a specific activity of ≥500 Ci/mmol.[28] This is high considering the theoretical specific activity of ^{131}I (1.6 x 10^4 Ci/mmol) and the specific activity of ^{131}I[MIBG] (2.8-5.5 Ci/mmol) obtained by Prabhakar *et al.* using the Cu(I) catalysed exchange process.[26] Hunter and colleagues have also shown that this method could facilitate the use of extremely short-lived isotopes such as ^{122}I ($t_{1/2}$=3.62 min.). For example, the total synthesis and purification time for the preparation of [^{125}I]-4-iodophenyl-*N*, *N*-dimethylpiperazinium ion was less than a minute.[29]

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[27] Culbert, P.A.; Hunter, D.H. *Reactive Polymer* **1993**, *19*, 247.
[28] Hunter, D.H.; Zhu, X. *J. Labeled Compd. Radiopharm.* **1999**, *42*, 653.
[29] Dorff, P.N.; Hanrahan, S.M.; Hunter, D.H.; VanBrocklin, H.F. *Abstr.Pap.-Am. Chem. Soc.* **2001**, 221st, Nucl-177.

Another variation on the technique of solid phase radiolabelling was developed by Kawai, who prepared polymer-bound mercury precursors of L-DOPA.[30] Kawai showed that 6-mercuric TFA derivatives could be linked to insoluble resins containing pendent carboxylates. In this way, radioiododemercuration with [^{125}I]I_2 of the bound Hg-DOPA gave the corresponding 6-iodo protected DOPA in 92-97% radiochemical yield. **Figure 1-10** illustrates the synthetic scheme, where Bio-Rex cation exchange resin was used as the polymeric support.

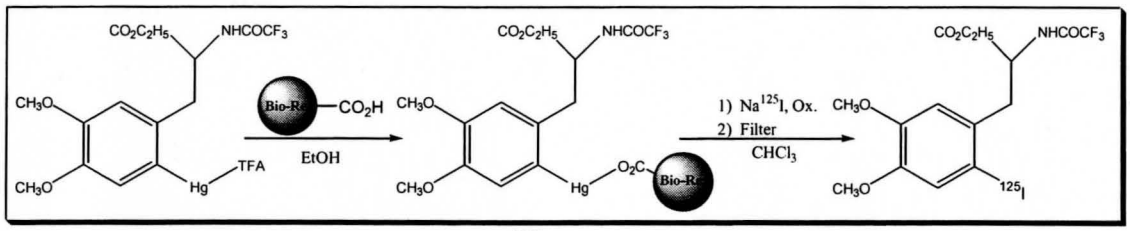

Figure 1-10. Solid phase synthesis of [^{125}I]6-iodo protected DOPA

Unfortunately, there are some inherent disadvantages to solid phase chemistry that must be considered. If reactions are not driven to completion, side products will inevitably be formed, which cannot be removed prior to labelling. Furthermore, the compound, when bound to the solid support, cannot be characterised by the normal techniques that are used in solution phase products. For example, in the case of Hunter's chlorostannane polymer, one must rely on solid state ^{119}Sn NMR and IR spectroscopy. In order to characterise the bound products by NMR, MS, and HPLC, one must first cleave the compound from the solid support.

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[30] Kawai, K.; Ohta, H.; Channing, M.A.; Kubodera, A.; Eckelman, W.C. *Appl. Radiat. Isot.* **1996**, *47*, 37.

The current limitations of traditional and solid phase synthesis labelling suggests the need for a new methodology for radiopharmaceutical synthesis. Several criteria must be met in order for this approach to improve upon current methods. The technique must be: a) suitable for rapid labelling; b) high yielding; c) regioselective; and d) amenable to simple purification.

1.7 Fluorous Chemistry

Synthetic chemistry has traditionally involved two distinct and important stages: the synthesis of the desired compound, followed by purification of product(s) from impurities. Purification is typically carried out by extraction, which is one of the oldest means of isolating a desired compound.[31] Extraction, which relies upon the differences in compounds states and solubilities, typically involves the use of polar phase (H_2O) and an immiscible organic phase (Et_2O, CH_2Cl_2). In 1994, Horvath and Rabai introduced another phase, which phase separates from solids, gases, and aqueous and organic liquids. This new phase was named the "fluorous liquid phase", and is composed of highly fluorinated solvents which preferentially extract fluorinated materials.[32]

The fluorous liquid phase was first exploited as a novel means of catalyst immobilisation and regeneration. The "fluorous biphasic catalyst" system entailed dissolution of a fluorinated catalyst within a fluorous liquid phase, which is then mixed with an organic phase. A reaction takes place at the solvent interface, and because of the

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[31] Curran, D.P. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1175.
[32] Horvath, I.T., Rabai, J. *Science*. **1994**, *266*, 72.

immisability of the fluorous components, the products are separable through liquid phase extraction.[33]

More recently, Curran and coworkers have developed the concept of "fluorous" organic chemistry as an approach to small molecule synthesis.[34] The initial step in fluorous chemistry (**Figure 1-11**) is to couple the substrate of interest to a fluorous tag, which is sufficiently fluorine rich so as to render the coupled product soluble in fluorous solvents. The fluorous tags most widely employed are either perfluorinated tin or silicon derivatives, containing three perfluorohexylethyl chains.[4] The next step involves a reaction which liberates the fluorous tag from the product, so that the product can be isolated from the fluorine containing species by simple extraction.

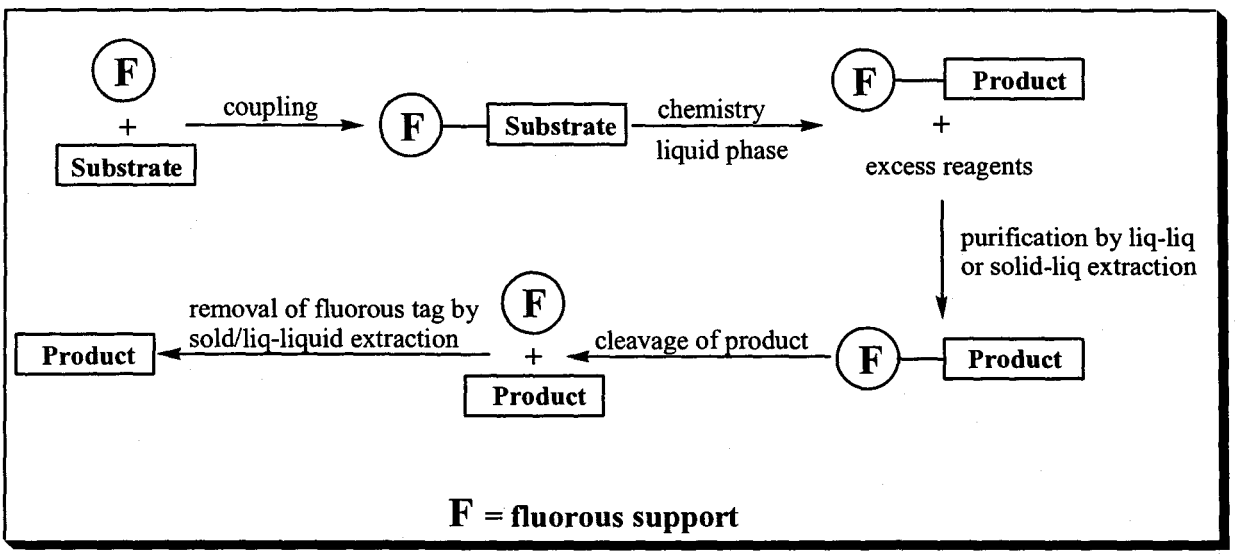

Figure 1-11. Synthesis and purification of products using the fluorous technique

[33] Cornils, B. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2057.
[34] Curran, D.P., Studer, A., Jeger, P., Wipf, P. *J. Org. Chem.* **1997**, *62*, 2917.

A useful application of the fluorous synthesis method is the modified Stille reaction, shown in **Figure 1-12.**[35] The Stille-coupling reaction is a popular carbon-carbon bond forming reaction typically utilising trialkyltin reagents.[36] In Curran's adaptation of this reaction, bromo[tris(2-perfluorohexylethyl)tin] (**1.0**) was first reacted with either the Grignard or phenyl-lithium to generate the corresponding phenyl-tin (**1.1**). Reaction of excess **1.1** with a halo-arene, such as 1-bromo-4-nitrobenzene (**1.2**), in DMF/BTF with $PdCl_2(PPh_3)_2$ /LiCl, generated a coupled product. Isolation of the organic products from the inorganic and fluorous products was facilitated by a three-phase extraction between water, dichloromethane, and FC-72®. The crude organic product, isolated from the dichloromethane layer, was purified by flash chromatography.

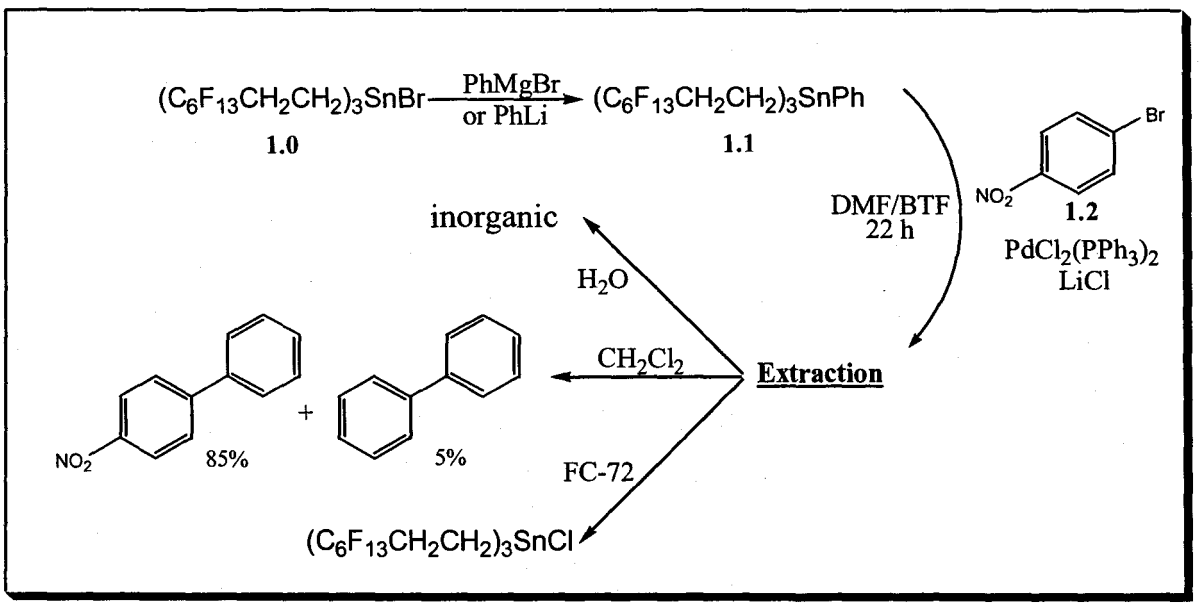

Figure 1-12. Fluorous synthesis strategy for Stille-couplings

[35] Curran, D.P., Masahide, H., Degenkolb, P. *J. Org. Chem.* **1997**, *62*, 8341.
[36] Stille, J.K. *Pure Appl. Chem.* **1985**, *57*, 1771.

As mentioned previously, purification of the fluorous compounds from organic reagents or products makes use of the phase partitioning of fluorous solvents, such as FC-72® or FC-84®, from organics and water. In this way, a two or three phase extraction isolates fluorinated materials into the fluorous phase, while excess reagents or cleaved products can be extracted into either the organic or aqueous layers. Commonly employed solvents, which result in little or no phase partitioning of fluorinated compounds, are methanol, acetonitrile, dichloromethane, or water.

As an alternative to liquid extraction, solid phase extraction can be carried out using silica gel having a highly fluorinated bonded phase, which is referred to as fluorous reverse phase (FRP) silica.[37] Synthesis of FRP silica has been described,[38] though it is also commercially available through Silicycle® and Fluorous technologies®. Ideal for automation and high throughput synthesis, elution with fluorophobic solvent (i.e. acetonitrile/water) down a small column of FRP silica, retains the fluorinated tag near the head of the column, while organic or aqueous compounds elute near the solvent front. Isolation of organic/aqueous compounds is followed by elution with a fluorophilic solvent (such as ether) to isolate the fluorinated tag and allow for the columns recycling. **Figure 1-13** illustrates the separation and isolation of materials produced using the fluorous synthesis strategy.

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[37] Curran, D.P. *Synlett.* **2001**, *9*, 1488.
[38] Berendsen, G.E., Galan, L.D. *J. Liquid Chromatogr.* **1978**, *1*, 403.

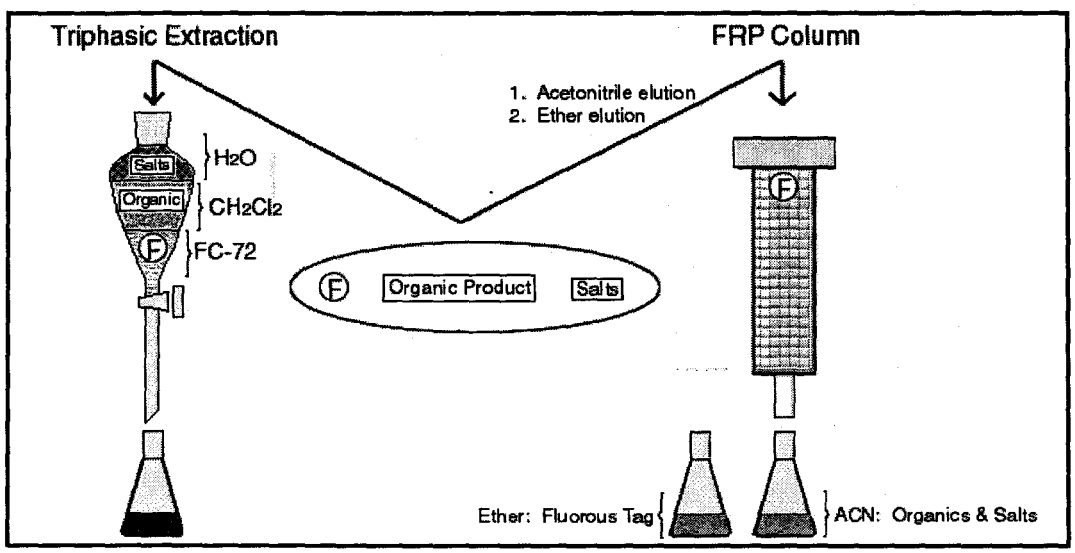

Figure 1-13. Purification strategy using fluorous synthesis technique

In principle, the fluorous synthesis technique provides the same facile purification features of solid phase synthesis while maintaining the flexibility associated with performing reactions in solution. The homogeneous reaction phase can result in higher yields, shorter reaction times, and negates the need for performing multiple coupling reactions, which are often required in solid phase synthesis.[39] If a fluorous reaction does not go to completion, unreacted substrate can, unlike in solid phase synthesis, be removed using classical separation methods. In addition, in order for products of solid phase synthesis to be characterised by standard methods, they must first be cleaved from the solid support. In fluorous synthesis, the reaction products can be characterised at all steps of the synthesis by standard methods (NMR, MS, X-ray crystallography).[35,39]

[39] Curran, D.P.; Hadida, S.; Kim, S-Y; Luo, Z. *J. Am. Chem. Soc.* **1999**, *121*, 6607.

1.8 Fluorous derived radiopharmaceuticals

This thesis explores the application of a new approach towards the development of radiopharmaceuticals using fluorous synthetic methods. The technique will be based on the established halodemetallation of trialkyltin precursors mentioned previously. If successful, this approach will offer a number of advantages over existing strategies, which will be highlighted throughout this thesis.

The following thesis contains 4 chapters. Chapter 2 details the synthesis and labelling of a fluorous "tagged" benzoic acid derivative, which was used as a model compound to validate the approach and optimise reaction conditions. Included in Chapter 2 is the coupling of fluorous "tagged" benzoic acid to form a benzamide. Chapters 3 describes the synthesis and labelling of more complex substrates, which will highlight the flexibility and benefits of applying this approach for radiopharmaceutical development. Chapter 4 summarises and concludes the research presented in this thesis.

Chapter 2

Labelling benzoic acid and benzamide using fluorous chemistry

2.1 Receptor targeted radiopharmaceuticals in nuclear medicine

The application of radiolabelled peptides, monoclonal antibodies, and oligodeoxynucleotides for diagnostic imaging has heralded a new era for nuclear medicine.[1] Because of their specificity, these compounds allow for functional imaging of specific receptor mediated biochemical processes. Synthetic oligopeptides have been particularly attractive because they can be readily synthesised, and their pharmacokinetic properties are often ideal for nuclear medicine application. For example, an indium-111 labelled somatostatin analogue consisting of 8 amino acids is now used routinely to localise and image neuroendocrine tumours.[2]

2.1.1 Labelling peptides

There are two main approaches for incorporating radiohalogens into peptides. The first approach is direct labelling of the parent molecule. Tyrosine residues, for example, can be labelled through electrophilic iodination reactions[3], via the iodogen method[4] or through the use of the Bolton-Hunter reagent.[5] The main disadvantage of these strategies is that the regioselectivity and stoichiometry of the labelling reaction is hard to control.

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[1] Blok, D.; Feitsma, R.I.J.; Vermeij, P.; Pauwels, E.J.K. *Eur J Nucl Med.* **1999**, *26*, 1511.
[2] Krenning, E.P.; Bakker, W.H.; Breeman, W.A. *Lancet.* **1989**, *I*, 242.
[3] Hunter, R.M.; Greenwood, F.C. *Nature.* **1962**, *194*, 495.

The second approach involves derivatising a pendent amino group on the peptide with an activated ester carrying a radiolabel. When attention is paid to reaction conditions, the resulting amide bonds can be formed regioselectively. Two of the most common labelling agents are, *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and *N*-succinimidyl 3-[^{131}I]iodobenzoate (SIB).[6,7] These ^{18}F- and ^{125}I- derivatives are typically synthesised by nucleophilic substitution and destannylation reactions, respectively (**Figure 2-1**).

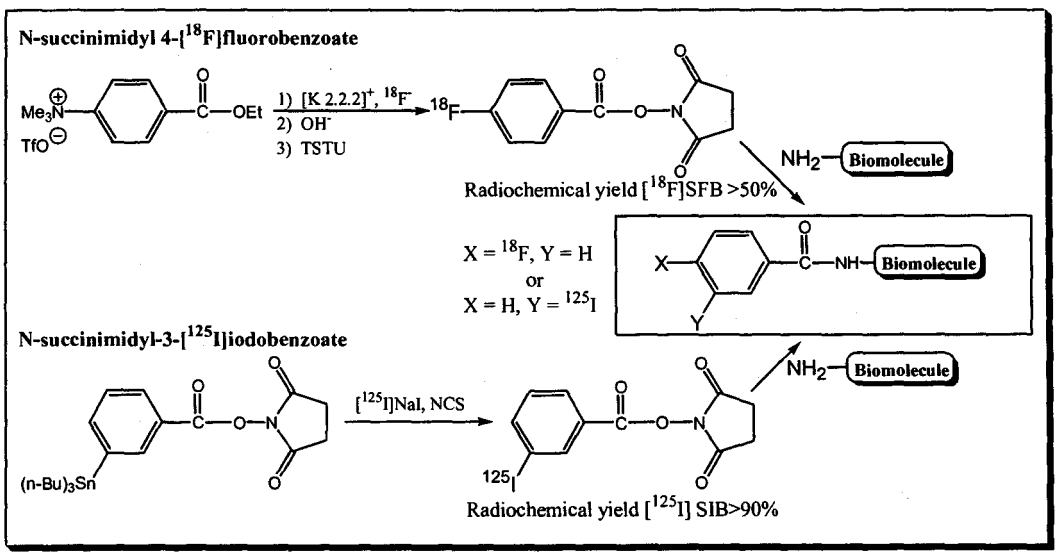

Figure 2-1. Synthesis and conjugation of [^{18}F]SFB and [^{125}I]SIB

[4] Fracker, P.J.; Speck, J.C. *Biochem. Biophys. Res. Commun.* **1987**, *80*, 849.
[5] Bolton, A.M.; Hunter, R.M. *Biochem. J.* **1973**, *133*, 529.
[6] Okarvi, S.M. *Eur. J. Nucl. Med.* **2001**, *28*, 929.

2.2 *Model System – halobenzoic acid*

In order to best illustrate the utility of the fluorous synthesis approach for radiopharmaceutical development, a model compound, which was both useful and amenable to different labelling approaches, was chosen. In this way, the target compound became tris(perfluorohexylethyl)tin-3 or 4-benzoic acid (**Figure 2-2**). It was hoped that **2.1** and/or **2.2** would facilitate the regioselective incorporation of a variety of isotopes including ($[^{18}F]F_2$ and $[^{125}I]I_2$), to generate the corresponding labelled benzoic acid. The labelled benzoic acid could then be activated and coupled to a variety of amino terminated compounds and biomolecules. Alternatively, the peptide or biomolecule could be coupled directly to **2.1** or **2.2** prior to their radiolabelling.

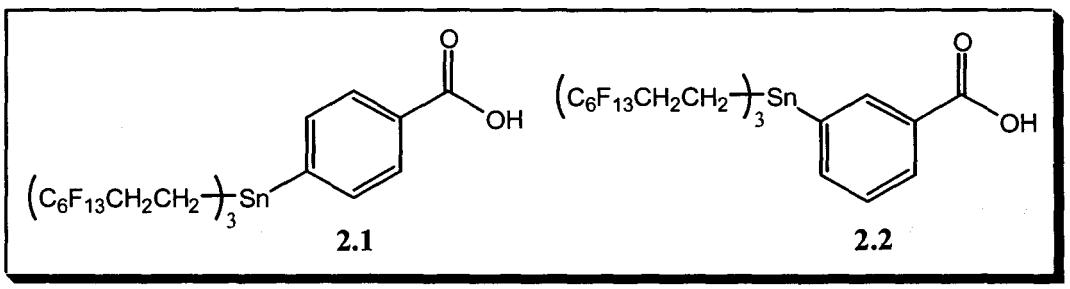

Figure 2-2. Tris(perfluorohexylethyl)tin-3 or 4-benzoic acid

2.2.1 *The "fluorous tag"*

The "fluorous tag" used throughout this research was bromo[tris(2-perfluorohexylethyl)tin] (**2.3**), which was prepared following the method of Curran *et al.*[8] Compound **2.3** was synthesised via the arylstannyl, **2.4**, which in turn was prepared using a Grignard reaction of phenyltintrichloride and 2-perfluorohexyl-1-

[7] Garg, P.K.; Alston, K.L.; Zalutsky, M.R. *Bioconj. Chem.* **1995**, *6*, 493.
[8] Hoshino, M.; Degenkolb, P.; Curran, D.P. *J. Org. Chem.* **1997**, *62*, 8341.

iodoethane (**Figure 2-3**). Removal of the homocoupled impurity by vacuum distillation and subsequent column chromatography yielded **2.4** in 75 % yield. The ^{1}H NMR spectrum of **2.4** in $CDCl_3$ showed a singlet at 7.33 ppm (5H, aromatic) along with the triplet at 1.23 ppm (J=8.4 Hz with ^{119}Sn (spin ½) satellites $^{2}J_{Sn,H}$= 51.7 Hz) and multiplet at 2.24 ppm corresponding to the methylene protons α ($CH_2\mathbf{C}H_2Sn$) and β ($\mathbf{C}H_2CH_2Sn$) to tin. The ^{13}C NMR spectrum shows signals corresponding to the aromatic ring, in addition to resonances at –1.49 ppm and triplet at 27.74 ppm ($^{2}J_{F,C}$=23.5 Hz) correspond to the carbon atoms α ($CH_2\mathbf{C}H_2Sn$) and β ($\mathbf{C}H_2CH_2Sn$) to tin respectively. The negative ion electrospray mass spectrum of compound **2.4** showed peaks at m/z = 1297 $[M+OAc-H]^-$ and m/z = 1283.0 $[M+OAc-CH_3]^-$ indicative of product formation. In addition, the IR spectrum reveals strong absorbances corresponding to sp^2 and sp^3 C-H stretches at 2962, 2928, 2874, and 2862 cm^{-1}. These findings are consistent with literature values.[8]

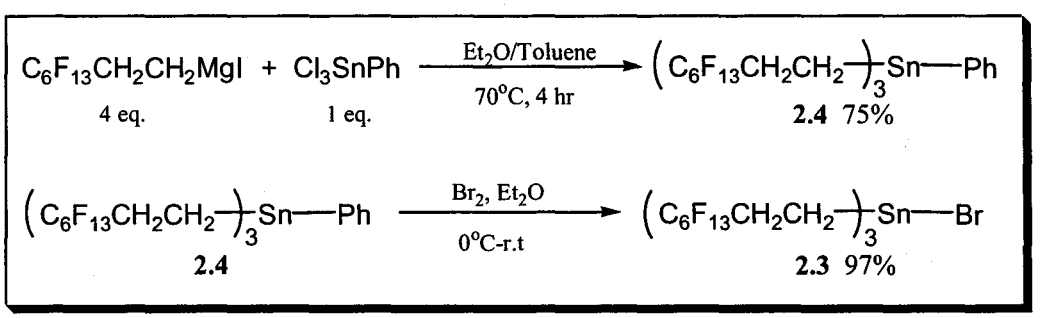

Figure 2-3. Synthesis of the "fluorous tag", compound **2.3**

Compound **2.4** was subsequently reacted with excess bromine and **2.3** was purified through vacuum distillation, yielding the desired product in 97% yield. Conversion of **2.4** to **2.3** was confirmed through disappearance of aromatic resonances in ^{1}H and ^{13}C NMR spectra. In addition, substitution of the electronegative bromine shifts ^{1}H and ^{13}C signals for the nuclei α to the tin to lower

field. The effect is quite dramatic; the α-proton chemical shift increases from 1.23 ppm to 1.57 ppm (J= 7.9 Hz with ^{119}Sn satellites $^{2}J_{Sn,H}$= 54.1 Hz), while the α-carbon signal shifts from –1.49 ppm to 6.11 ppm. The ^{13}C resonances for the fluorine bearing carbon atoms appear as highly coupled multiplets from 108.86 ppm to 121.71 ppm. The negative ion electrospray mass spectrum for **2.3** gave a peak at m/z= 1279.5 $[M+OAc]^-$. These results are also consistent with literature findings.[8]

2.2.2 Synthesis of tris(perfluorohexylethyl)tin-3 or 4-benzoic acid (2.1, 2.2)

Four strategies for the synthesis of **2.1** were undertaken (**Figure 2-4**). Each involves nucleophilic attack of an organometallic reagent onto the tin-bromide compound (**2.3**).

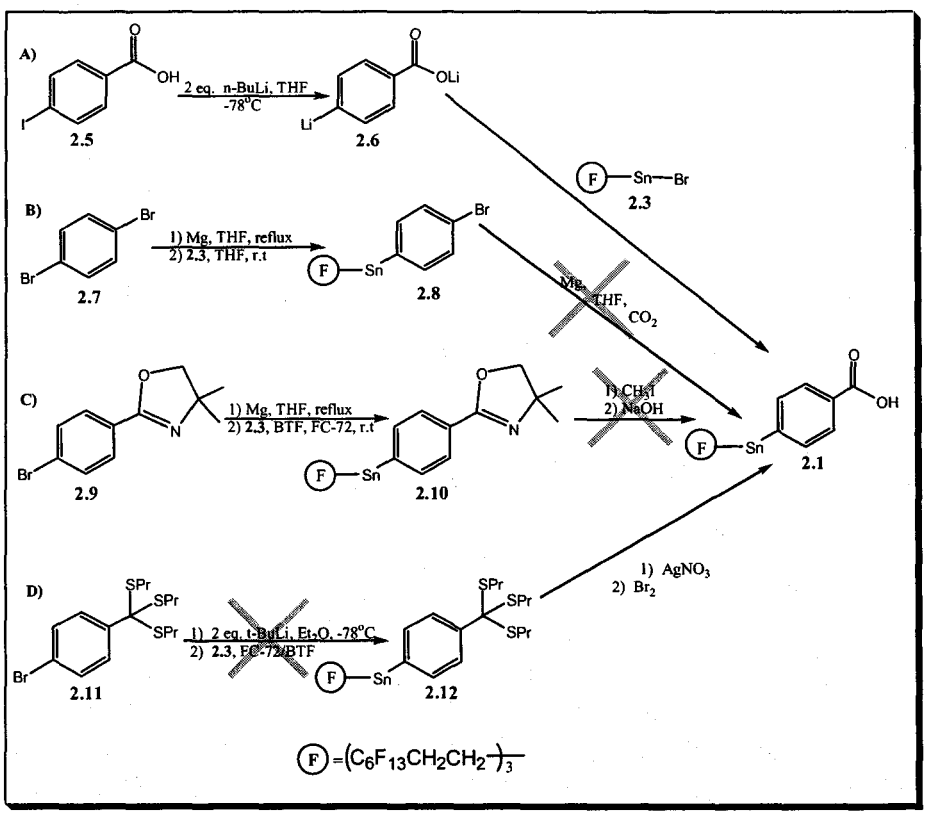

Figure 2-4. Four approaches towards tris(perfluorohexylethyl)tin-benzoic acid (**2.1**)

In the first approach, **A**, the procedure of Zalutsky *et al.*[4], which was used to prepare *N*-succinimidyl-3-(tri-n-butylstannyl)benzoate, was employed. Reaction of **2.3** with excess of the dilithiated species (**2.6**) successfully generated **2.1**. Purification of the fluorous material was facilitated through a triphasic extraction into FC-72® from dichloromethane and water. Unfortunately, the extent of benzoic acid incorporation into the final product was consistently < 35% of total available sites. The extent of product (aryl-stannane) formation vs. unreacted starting material (bromo-stannane) was determined using ^{1}H NMR spectroscopy. Integration of the α and β proton signals corresponding to the two different chemical environments provides a reasonable assessment of product formation for this and other reactions. (**Figure 2-5**).

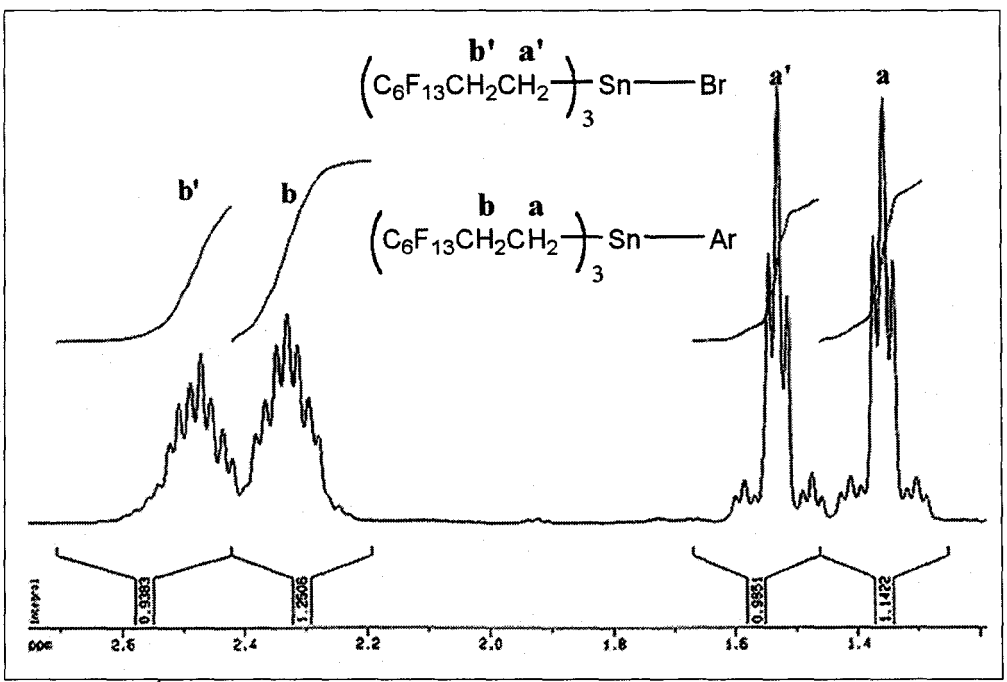

Figure 2-5. ^{1}H-NMR [$CDCl_3$, 500 MHz] spectrum of incomplete reaction

Purification was attempted though column chromatography in accordance with the methods described by Curran *et al.*[9] Due to the similarity in R_f values between **2.1** and **2.3,** no separation could be attained.

Approach **B** involved modifying the procedure described by Lequan *et al.* for the synthesis of *p*-(phenylmethylisopropylstannyl)benzoic acid.[10] The mono-anion of *p*-dibromobenzene was reacted with **2.3,** yielding **2.8** quantitatively. Unfortunately, repeated attempts to lithiate **2.8** were unsuccessful, preventing the successive reaction with CO_2.

Approach **C** was based on the method reported by Milius *et al.* for the synthesis of 4-tri-n-butylstannyl-benzoic acid oxazoline.[11] The appeal of the oxazoline protecting group was its stability to Grignard reaction conditions, and, more importantly, its ability to be deprotected under mild, non acidic conditions. The precursor, compound **2.9**, was synthesised by treatment of *p*-bromobenzoic acid with thionyl chloride to give the acid chloride. The acid chloride was subsequently reacted with 2-amino-2-methyl-propanol to afford the amide. Treatment of the amide with thionyl chloride in the absence of solvent induced cyclization to the oxazoline ring, generating **2.9** in 95 % yield.

The ^{1}H NMR spectrum of compound **2.9** showed peaks corresponding to the oxazoline group at 1.42 ppm (s, 6H, CH_3) and 4.17 ppm (s, 2H, OCH_2C), in addition to the aromatic peaks at 7.56 (AA′BB′, J=8.5 Hz, 2H) and 7.87 ppm (AA′BB′,

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[9] Studer, A; Jeger, P.; Wipf, P.; Curran, D.P. *J. Org. Chem.* **1997**, *62*, 2917.
[10] Lequan, M.; Meganem, F. *J. Organometallic Chem.* **1975**, *94*, C1-C2.
[11] Milius, R.A.; McLaughlin, W.H.; Lambrecht, R.M.; Wolk, A.P.; Carroll, J.J.; Adelstein, S.J.; Bloomer, W.D. *Appl. Radiat. Isot.* **1986**, *37*, 799.

J=8.5 Hz, 2H). The ^{13}C NMR spectrum and the electron impact mass spectrum (m/z=254) for **2.9** also agree well with the literature.[12]

Formation of the Grignard was sluggish, and necessitated the addition of 1,2-dibromoethane in order to promote the reaction. Eventually, **2.3** was quantitatively converted to **2.10**, which was purified through a triphasic extraction and isolated in a 90% yield.

The ^1H NMR spectrum of **2.10** showed the expected shift in α and β protons to higher field. The ^1H NMR spectrum also revealed peaks at 1.40 ppm (s, 6H, CH_3) and 4.14 ppm (s, 2H, OCH_2C) from the oxazoline group, as well as aromatic signals at 7.44 ppm (AA'BB', J= 8.1 Hz, 2H) and 7.97 ppm (AA'BB', J=8.1 Hz, 2H). Similarly, the ^{13}C NMR spectrum showed a shift in the signal for the α carbon to a higher field of -1.25 ppm, in addition to the appearance of the oxazoline's methyl carbons at 28.5 ppm, and aromatic resonances at 128.4 ppm and 136.0 ppm. The negative ion electrospray mass spectrum gave a peak at m/z=1394 $[M+OAc]^-$.

In order to facilitate cleavage of the oxazoline group under basic conditions, it was necessary to convert the oxazoline to the oxazolinium ion. In all instances, reaction with methyiodide under mild reaction conditions yielded none of the desired quaternerized product. Alternatively, under the vigorous reaction conditions suggested by literature, cleavage of the tin-aryl bond occurred. [13]

Approach **D** required the initial synthesis of a thiol protected intermediate, tripropyl 4-bromoorthothiobenzoate **2.11**. The reaction pathway for **D** (**Figure 2-4**) was applied originally to the synthesis of the analogous silicon fluorous compound.[9] The synthesis of the precursor **2.11** involved reaction of *p*-bromobenzoic acid with

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[12] Hughes, A.B.; Melvyn, V. *J. Chem. Soc. Perkin.* Trans. **1989**, *I*, 1787.

thionyl chloride to generate the acid chloride, which was then reacted with excess propanethiol in the presence of $AlCl_3$. Despite the fact that a great deal of attention was paid to ensuring reagent quality ($AlCl_3$ was freshly sublimed and propanethiol was freshly distilled), the crude reaction product consisted of only one or two condensed propanethiol groups. The orthothiobenzoate was never observed as it was described in the paper by Studer *et al.*[9]

The successful methodology, approach **E** (**Figure 2-6**), entailed adaptation of research reported by Xizhen, Z *et al.*, who established the feasibility of synthesising arylstannanes using organozinc reagents.[14] The use of the robust organozinc reagents, rather than organolithium reagents, facilitates the incorporation of compounds with electrophilic functionalities, such as esters, nitriles, and ketones.

Excess 3-ethoxycarbonylphenylzinc (**2.13**), which is commercially available through Rieke Metals Inc., was reacted with **2.3** overnight (**Figure 2-6**). The product **2.14** was isolated through a biphasic extraction between FC-72® and methanol in excellent yield (99%).

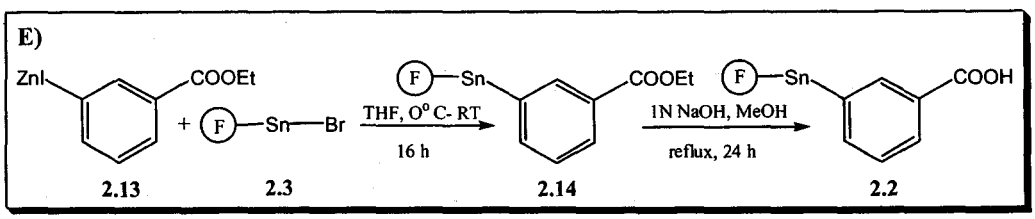

Figure 2-6. Synthesis of tris(perfluorohexylethyl)tin-3-benzoic acid (**2.2**)

Analysis of ^1H NMR for compound **2.14** revealed the appropriate shift in the signals of α and β protons to a higher field, 1.35 ppm (t, J=8.2 Hz, 6H) and 2.33 ppm

[13] Ishibashi, K.; Nakajima, K.; Nishi, T. *Heterocycles.* **1998**, *48*, 2669.
[14] Xizhen, Z.; Blough, B.E.; Carroll, F.I. *Tetrahedron Letters.* **2000**, *41*, 9222.

(m, 6H), respectively. Signals corresponding to ethylbenzoate were seen at 1.39 ppm (m, 3H, CH_2CH_3), 4.39 ppm (q, J=7.1 Hz, 2H, CH_2CH_3), and 7.47-8.07 ppm (m, 4H, aromatic). The ^{13}C NMR spectrum for **2.14** showed the appropriate α and β carbons signals at −1.12 ppm and 27.87 ppm ($^2J_{F,C}$=23.3 Hz), in addition to signals corresponding to the ethylester at 14.15 ppm (CH_2CH_3) and 61.32 ppm (CH_2CH_3). At lower field the ^{13}C NMR spectrum had resonances corresponding to carbon atoms with attached fluorines (106.46 ppm to 121.17 ppm) and those in the aromatic ring. The negative ion mass spectrum of **2.14** gave peaks indicative of the product at m/z=1279.4 [M-ethyl] and m/z=1369.5 $[M+OAc]^-$.

Saponification of **2.14** was achieved using excess base, despite the fact that the substrate was immiscible in the reaction solvent (methanol/water 4:1). Small amounts of the transesterification product were occasionally observed; however, this product was removed by way of a second hydrolysis reaction. Isolation of the product from FC-72® following several washings with water yielded **2.2,** presumably as the sodium salt, in 99% yield. Extraction of the sodium salt of **2.2** between FC-72®, dichloromethane, and a 1N HCl solution, produced the free acid.

The difference in solubility of the salt vs. the acid in $CDCl_3$ was pronounced. The acid dissolves in $CDCl_3$ to provide well resolved ^1H and ^{13}C NMR spectra, while the sodium salt was only sparingly soluble. The free carboxylic acid, **2.2**, unlike the sodium salt, crystallised over several days yielding a white solid.

The ^1H NMR spectrum of compound **2.2** (**Figure 2-7**) showed an absence of the signals corresponding to the ester group, but was otherwise unchanged from **2.14**. Signals corresponding to the α and β protons were seen at 1.34 ppm (t, J=8.2 Hz with ^{119}Sn satellites $^2J_{Sn,H}$=53.4 Hz, 6H) and 2.31 ppm (m, 6H), respectively. The aromatic

signals were consistent with a meta-disubstituted ring. Similarly, the ^{13}C NMR spectrum showed peaks corresponding to the α and β carbon atoms at -1.53 to -1.06 ppm and 27.42 (t, $^{2}J_{F,C}$=24.40 Hz), respectively; yet lacked the peaks associated with the ethylester. The peaks corresponding to the carbonyl carbon atom shifted to a lower field (172.61 ppm and 172.04 ppm) as would be expected. The all the signals in the ^{13}C NMR spectrum have a small shoulder peak similar to the carbonyl carbon, which is perhaps a reflection of the presence of a small amount of sodium salt of compound **2.2**. The negative ion electrospray mass spectrum of compound **2.2** (**Figure 2-8**) shows a peak at m/z=1279 $[M-H]^-$, which is characteristic of product formation. The IR spectrum of **2.2** importantly showed a strong O-H stretch at 3410 cm^{-1}, C=O stretch at 1632 cm^{-1}, and an sp^2 C-H stretch at 2950 cm^{-1}.

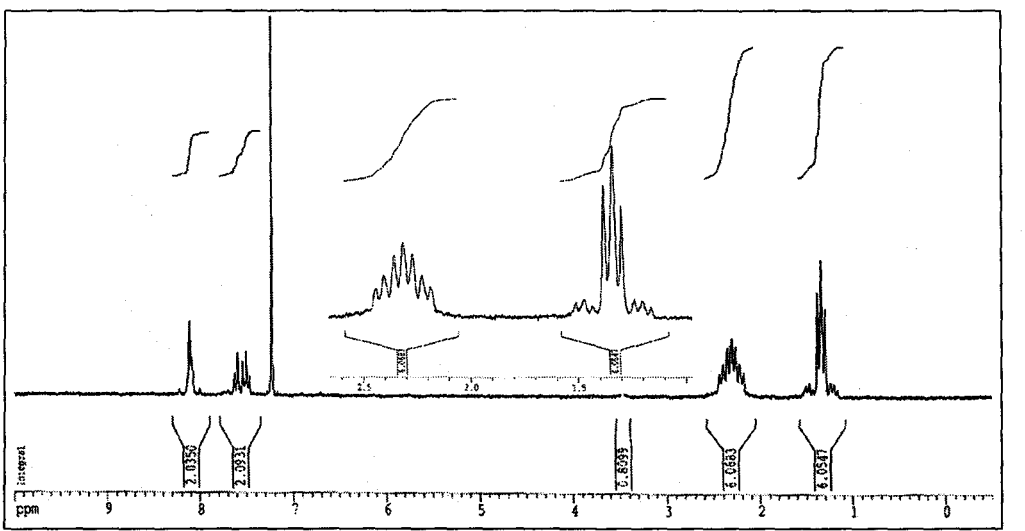

Figure 2-7. ^{1}H NMR [$CDCl_3$, 200 MHz] spectrum of compound **2.2**

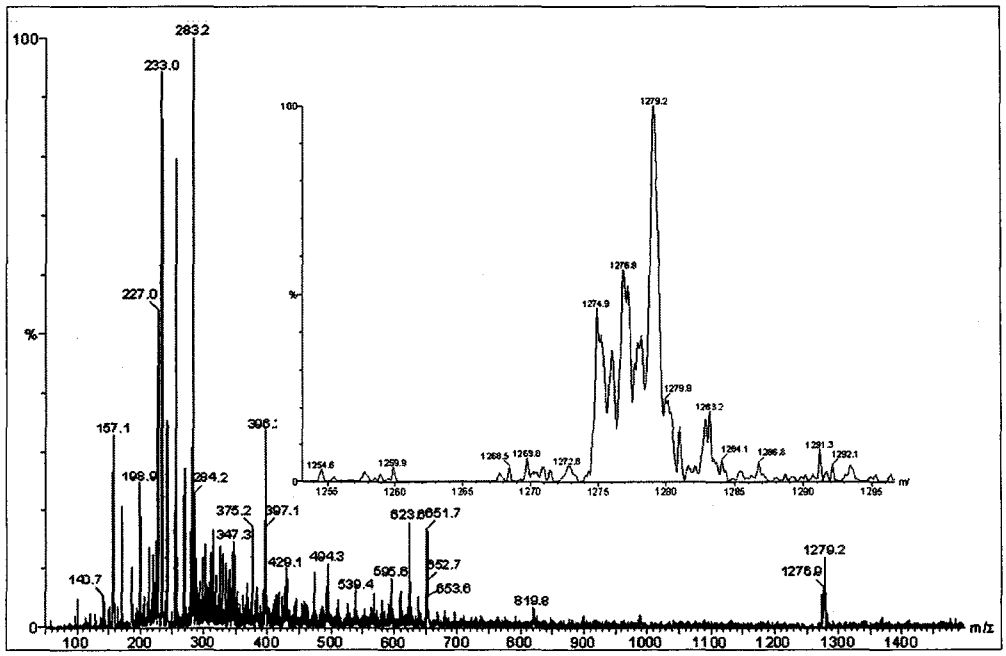

Figure 2-8. Negative ion electrospray mass spectrum of compound **2.2**

Dissolving a small quantity of **2.2** in pentane, followed by its slow evaporation, produced long needle-like crystals from which an X-ray crystal structure was obtained. The mounting of the crystal and the interpretation of the data was kindly performed by Paul Schaffer. This X-ray crystal structure is significant, as it represents the first reported crystal structure of a perfluorostannane species of any variety. Compound **2.2** crystallised in the triclinic P-1 space group with two independent molecules in the unit cell (Z=4). The structure proved difficult to solve, in large part due to the high level of disorder in one particular perfluorooctyl chain. This is reasonable considering the low barrier of rotation around the C-C bond, which typically leads to the oily property of these compounds. Though additional work is still required prior to publishing the X-ray crystal structure, the current structure verifies the presence of compound **2.2** (**Figure 2-9**).

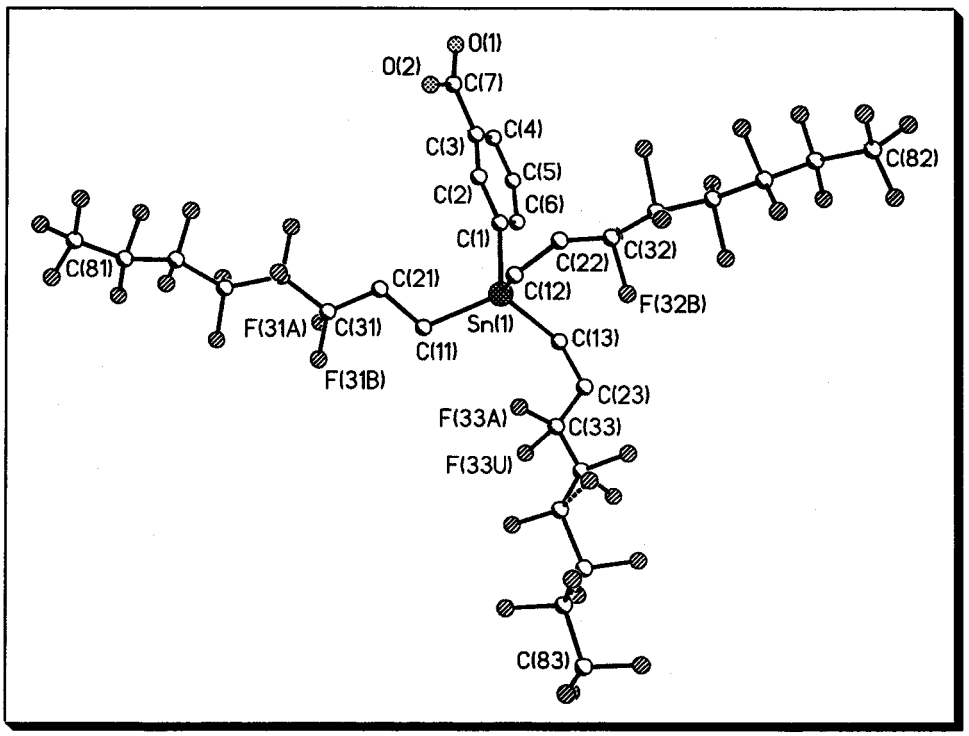

Figure 2-9. The X-ray crystal structure of compound **2.2**

2.3 Synthesis of 3-fluorobenzoic acid (**2.15**).

Fluorination of tris(perfluorohexylethyl)tin-3-benzoic acid (**2.2**) was initially performed in perfluorinated hexanes (FC-72®), rather than the more commonly employed HF, or freons such as $CFCl_3$. The use of FC-72® is advantageous, since it readily dissolves the precursor, has a suitable freezing and boiling point range (-100°C and 65°C respectively) and is not susceptible to degradation by F_2. The reaction conditions were worked out and optimised through a number of fluorination reactions, where conditions mimic those of the $[^{18}F]F_2$ reaction without having to deal with the risks of radiation-exposure. **Figure 2-10** and **2-11** illustrates the reaction and apparatus used in a general fluorination reaction, respectively.

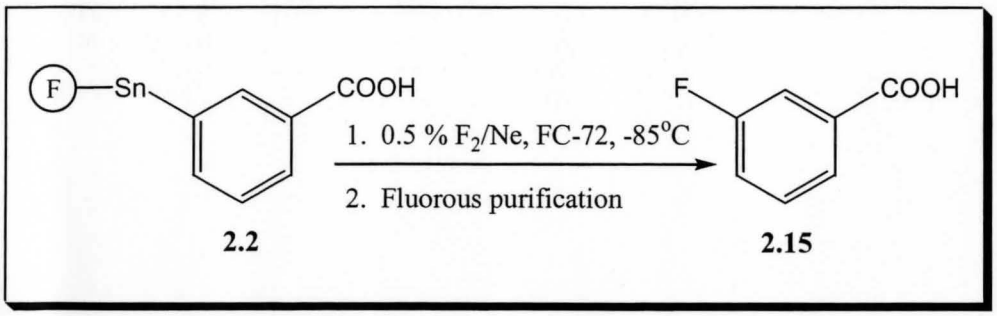

Figure 2-10. Synthesis of **2.15** from fluorous substrate **2.2**

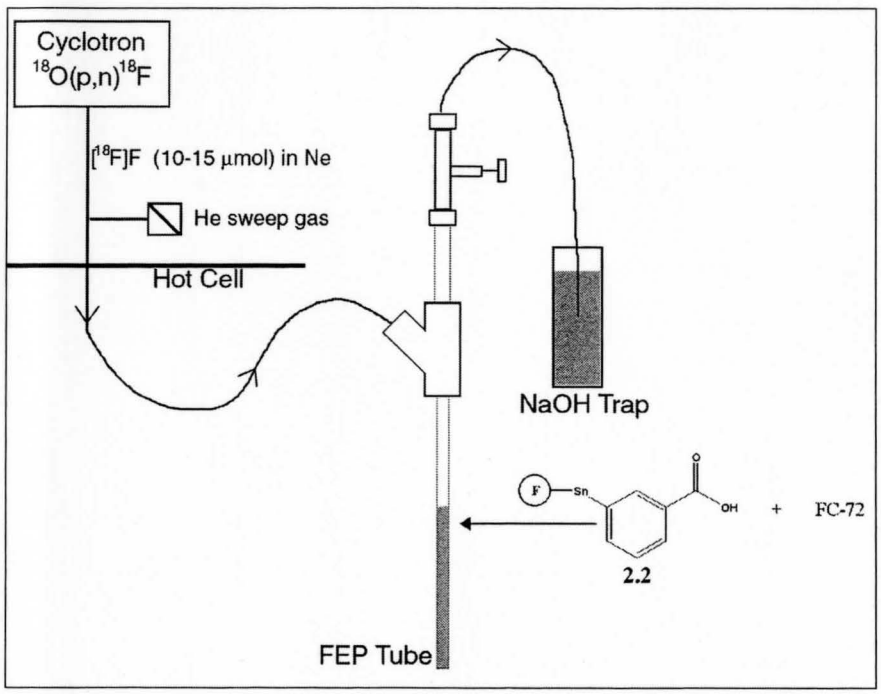

Figure 2-11. Fluorination apparatus used in both F_2 and $[^{18}F]F_2$ reactions

In general, the substrate **2.2** was diluted in FC-72® (1 mL) and transferred to a dried fluoropolymer vessel. The contents of the vessel were cooled to approximately -85°C in a MeOH/N_2 slush bath, after which a 0.5% F_2 in Ne solution (118 µmol in ~ 23 mL Ne) was bubbled through the solution over a 20-30 minute period. The solvent

was transferred to a vial along with methanol, which was used to rinse the reaction vessel. The entire mixture was evaporated by rotary evaporation, dissolved in acetonitrile:water (50:50) and passed down a fluorous column. Fractions (3 x 3 mL) were collected and characterised using ^{19}F NMR, HPLC and electrospray mass spectrometry.

The ^{19}F NMR spectrum of the reaction product **2.15** showed roughly a quartet at -112.00 ppm ($^{3}J_{F,H}$=5.76 Hz) when run in MeOH:$CHCl_3$, consistent with an authentic m-fluorobenzoic acid standard and literature values.[15,16] The negative ion electrospray mass spectrum of compound **2.15** gave the requisite peak at m/z=139.1 $[M-H]^-$. HPLC of the fluorous column purified reaction mixture produced a single peak at 4.22 min, consistent with the authentic standard.

Initially in this research, fluorous silica synthesised in our laboratory was used in the purification process. It proved, however, to be less effective at retaining fluorous material than commercially available fluorous modified silica manufactured by Silicycle®. The improved retention of the commercial variety, which was attributed to improved loadings, facilitated a more rapid purification. In the case of the "home-made" and commercial fluorous silica we also observed that the use of alcoholic solvents as a mobile phase resulted in substantial breakthough of the fluorous impurities. In order to remedy this, an acetonitrile:water (50:50) eluent system was used, and appears to have prevented any elution of the perfluorotin impurity. Elution of the product **2.15**, however occurs rapidly and is obtained (>99%) within the first 9 mL of eluent.

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[15] Gutowsky, H.S.; Hoffman, C.J. *J. Chem. Phys.* **1951**, *19*, 1259.
[16] Taft, R.W. *J. Phys. Chem.* **1960**, *64*, 1805.

In the initial reaction mixtures, two extraneous peaks were consistently found in the ^{19}F NMR spectrum (-74 ppm and -153 ppm), in addition to the product peak at -112 ppm. Initially, it was believed that these additional peaks were the result of FC-72®, which is composed of multiple isomers of perfluorinated hexanes. However, subjecting FC-72® to the same fluorination and purification conditions yielded no observable peaks in the fluorine spectrum.

It was later found that the peak at -74 ppm was not present when medical grade sterile water replaced the laboratories own distilled-deionized water. Further, the peak at -153 ppm was found to originate from the use of Silicycle® brand fluorous silica. Replacement of this brand of silica with that prepared by Fluorous Technologies® proved to remove this peak from the fluorine spectrum.

The reaction temperature also proved to influence the products generated in these reactions. When the reactions were carried out at higher temperatures, >-65°C, a small peak arose at -105 ppm (unresolved coupling) in the ^{19}F NMR spectrum. This could be the result of *ortho* substitution or a di-fluorinated ring, both of which would result in deshielding of the attached fluorine. This small impurity, however, was not seen when the reaction was carried out at lower temperatures (-85°C to -75°C).

In the course of these cold fluorinations, the yield of m-fluorobenzoic acid was optimised. The ratio of substrate to F_2 was varied between 0.7 to 3.0, in all cases using 118 μmol of a 0.5 % solution of F_2 in Ne (~ 23 mL), similar to the amount used in a $^{18}F[F_2]$ reaction. The percent yield of **2.15** with respect to F_2 decreased from 18% to 16% when 0.65 and 2.9 equivalents were used respectively. The yield analysis was based on comparison with calibration curves. It was found that the yield of **2.15** with respect to F_2 reached a maximum at approximately 24% when the ratio of substrate to

F_2 was 1.2:1. Since the reactions were run in equivalent volumes of FC-72®, the decreasing yield may be a result of a visibly increasing viscosity in the more concentrated samples.

2.4 Synthesis of 3-[^{18}F]fluorobenzoic acid (**2.16**).

The successful cold labelling and purification of **2.15** using the precursor **2.2** prompted the investigation of [^{18}F]F_2 labelling. The reaction scheme is shown in **Figure 2-12**.

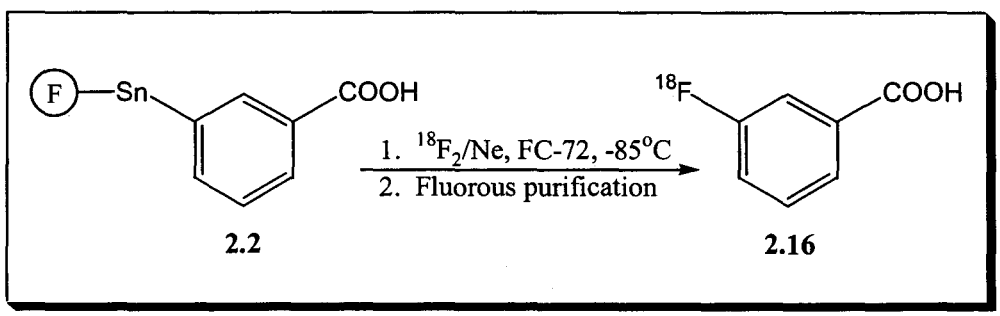

Figure 2-12. Synthesis of 3-[^{18}F]fluorobenzoic acid **2.16**

Fluorine-18 was produced at Hamilton Health Sciences - McMaster University Medical Centre by the $^{18}O(p,n)^{18}F$ nuclear reaction using a Siemens RDS 112 proton cyclotron operating at 11 MeV by the "double shoot" method.[17] The "double shoot" method entails diluting ^{18}F, which remains largely bound to the target wall following the nuclear reaction with $^{19}F_2$. Irradiation results in fluoride exchange and releases 40-50 μmol of carrier-added $^{18}F[F_2]$. The $^{18}F[F_2]$ in Ne was carried through a teflon tube and was bubbled through the 1 mL solution of **2.2** in FC-72® at –85°C.

[17] Chirakal, R.; Adams, R.M.; Firnau, G.; Schrobilgen, G.J.; Coates, G.; Garnette, E.S. *Nucl. Med.Biol.* **1995**, *22*, 111.

The fluorination reaction was carried out in a FEP (perfluoroethylenepropylene co-polymer) tube, and the outlet gas was bubbled through a 0.1 N NaOH solution.

Assessment of $[^{18}F]F_2$ consumed in the reaction was determined by measuring the total radioactivity in the vessel, compared to that in the NaOH trap. Work-up involved transferring the contents of the vessel to another vial using pressure generated by a syringe. The vessel was then rinsed with HPLC grade methanol and the combined solvents were evaporated in a hot water bath under a rapid flow of nitrogen. To the resulting residue was added 3 x 3 mL of acetonitrile:water (50:50), and each aliquot successively transferred to the fluorous column. Fractions of 3 mL were collected and characterised.

In total, five $[^{18}F]F_2$ fluorinations of **2.2** were carried out. **Figure 2-13** shows the typical HPLC chromatograms which were generated. The UV trace of compound **2.16** generated a single peak eluting at 4.18 minutes, which is identical to that of an authentic standard. Integration of the peak area and comparison to the calibration curve indicates a 19.4 % yield of labelled product (^{18}F & ^{19}F). The radioactivity detector trace for compound **2.16** shows a single peak eluting at 4.99 min. The later elution time is consistent with the time delay between the UV lamp and radiation detector.

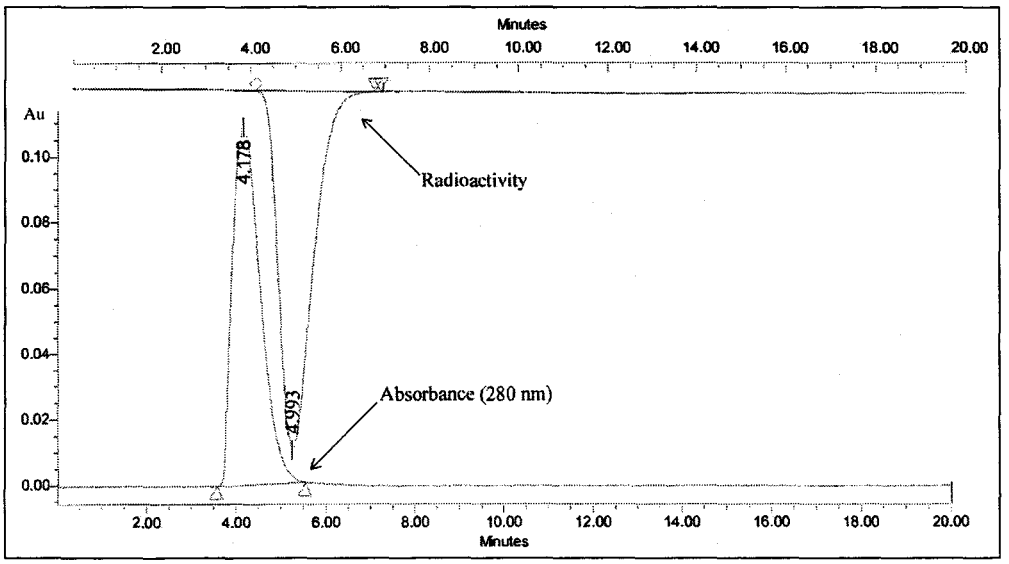

Figure 2-13. The UV and radioactivity chromatograms for the fluorous purified reaction mixture (**2.16**) (conditions: analytical C_8 (Microsorb-MV, 250 x 4.6 mm), 50:50 ACN (0.2 % formic acid):H_2O (0.2 % formic acid), flow 2 mL/min)

In the last two reactions, the radiochemical yield and specific activity of **2.16** were assessed. In these instances, the decay-corrected radiochemical yields of **2.16** were 30.2 % and 11.2 %; the lower yield was attributed to the vial walls not being rinsed effectively prior to purification. The theoretical maximum yield for this synthesis is 50 %, as half of the activity is lost as tris(perfluorohexylethyl)tin-[^{18}F]fluoride. This is comparable to the [^{18}F]F_2 destannylation reactions where 6-[^{18}F]fluoro-L-DOPA and 6-[^{18}F]fluoro-L-*m*-tyrosine were generated with radiochemical yields of 33 % and 23 % respectively.[18,19]

The specific activity of **2.16** following fluorous purification in the two experiments was 1970 and 2900 mCi/mmol, respectively. The discrepancy can, in part, be attributed to the shorter purification times of the second vs. the first (27 min. vs 49 min.). The specific activity is dependent on the amount of F_2 mixed in the

[18] DeVries, E.F.J.; Luurtsema, G.; Brussermann, M.; Elsinga, P.H.; Vallburg, W. *Appl. Radiat. Isot.* **1999**, *51*, 389.

target gas, and as such it is difficult to make a direct comparison to other fluorodestannylation reactions. However, the specific activities are reasonably high when compared to other electrophilic fluorination reactions. For example, various direct electrophilic fluorination approaches to generate 6-[^{18}F]fluoro-L-DOPA give specific activities of ≤ 2000 mCi/mmol.[20,21] Though similar specific activities were obtained, our approach did not require HPLC purification.

The ^{19}F NMR spectra of the crude reaction products from an analogous cold fluorination (**2.15**) and the purified reaction (**2.16**) products are shown in **Figure 2-14** and **2-15**, respectively. The ^{19}F NMR spectrum was obtained after allowing sufficient time for decay of ^{18}F-labeled **2.16.** In the ^{19}F NMR spectrum of crude reaction, the sensitivity of the fluorine nucleus to detection by NMR spectroscopy is evident in the clarity obtained following only a few scans. The crude spectrum shows six clearly resolved peaks corresponding to the six fluorine containing carbons atoms along three equivalent n-octyl chains. There was no discernible shift in these peaks prior to or following the fluorination reaction.

The ^{19}F NMR spectrum of the fluorous column purified reaction mixture (**Figure 2-15**) shows a pseudo-quartet at –110.10 ppm ($^{3}J_{F,H}$=7.24 Hz) when run in acetonitrile:water (50:50). The peak position and coupling is consistent with an authentic standard of m-fluorobenzoic acid in which the ^{19}F-signal appears at -109.8 ppm, and is also consistent with literature values.[16] Furthermore, it is important to note the absence of peaks associated with the fluorous "tag", which is a testament of the efficiency of the fluorous purification method.

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[19] Namavari, M.; Bishop, A.; Satyamurthy, N.; Bida, G.; Barrio, J.R. *Appl. Radiat. Isot.* **1992**, *43*, 989.
[20] Lemaire, C.; Guillaume, M.; Cantineau, R.; Plenevaux, A.; Christiaens, L. *Appl. Radiat. Isot.* **1991**, *42*, 629.

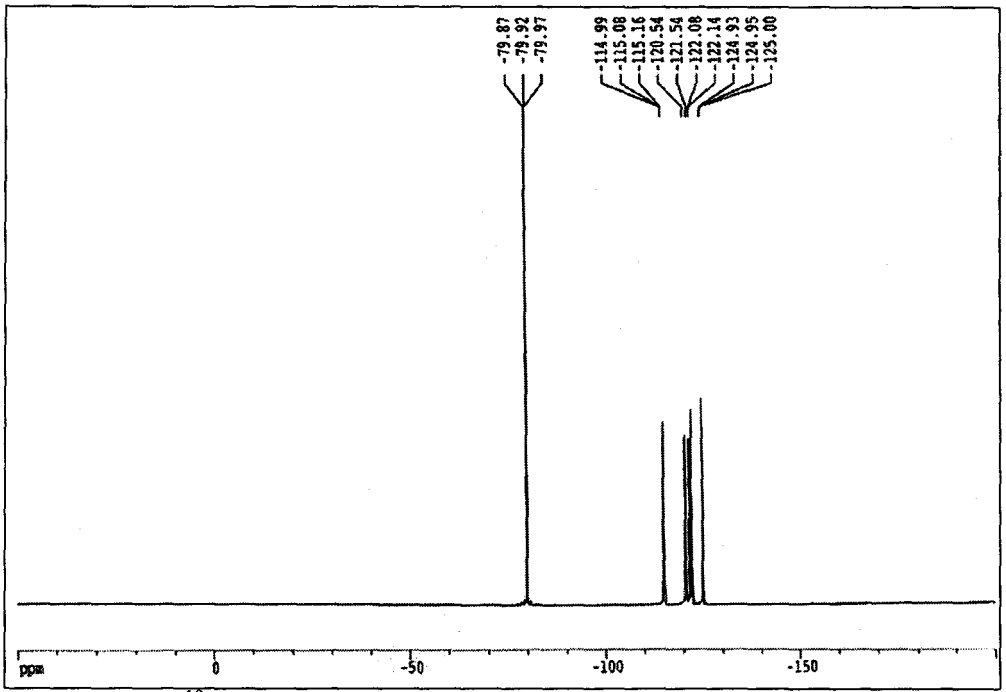

Figure 2-14. ^{19}F NMR [MeOH:ACN, 188 MHz] spectrum of the crude cold fluorination reaction (**2.15**)

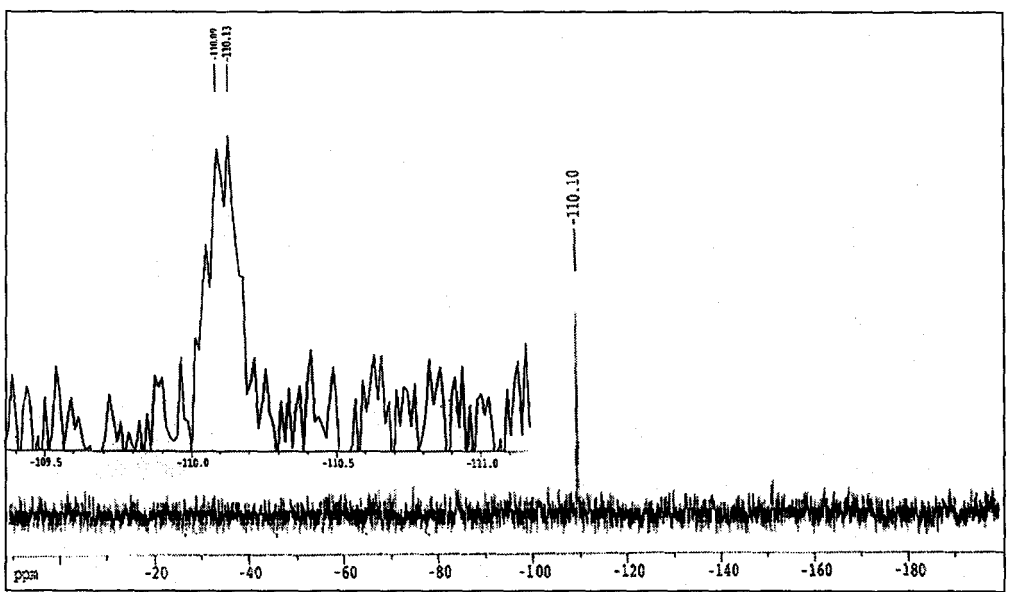

Figure 2-15. ^{19}F NMR [H_2O:ACN, 188 MHz] spectrum of fluorous purified reaction (**2.16**)

[21] Chirakal, R.; Finau, G.; Garnett, E.S. *J. Nucl. Med.* **1986**, *27*, 417.

The negative ion electrospray mass spectrum of a crude fluorination reaction and the fluorous purified reaction mixture (**2.16**) are shown in **Figure 2-16** and **Figure 2-17**, respectively. The electrospray mass spectrum of the reaction mixture prior to fluorous column purification shows the product peak at m/z=139.1 $[M-H]^-$ and the fluorous "tag" impurity around m/z=1319.2, 1345.2. However, the fluorous purified reaction mixture (**Figure 2-17**) shows the product peak at m/z=139.0 $[M-H]^-$ without any indication of the fluorous impurity.

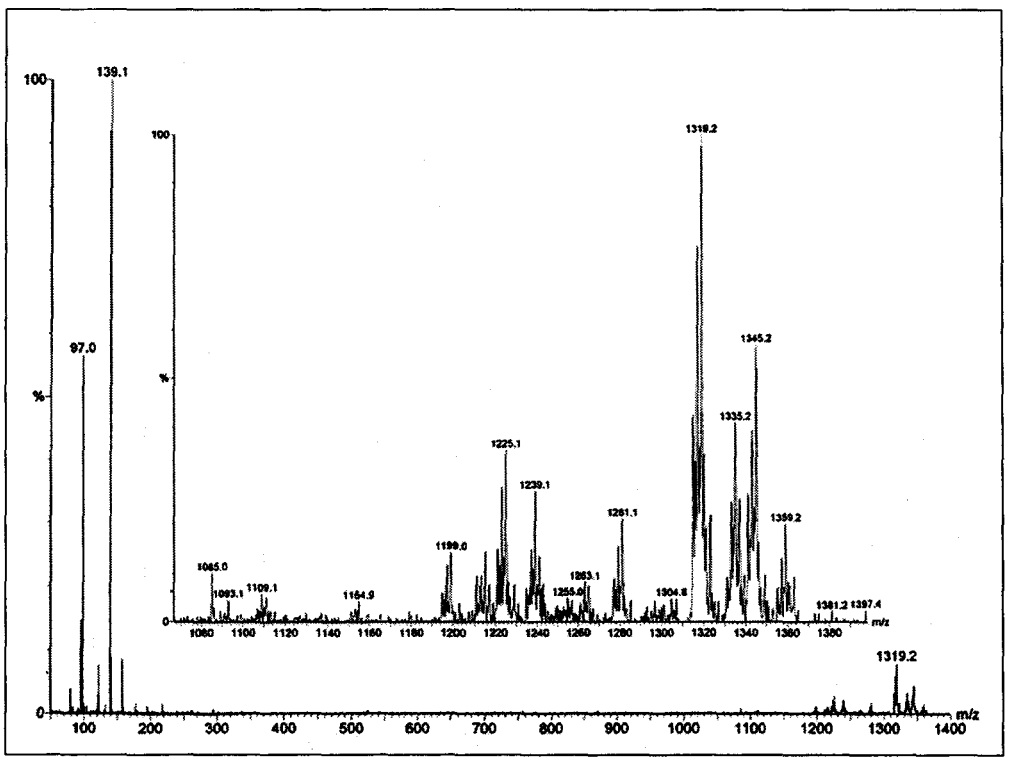

Figure 2-16. Negative ion electrospray of fluorinated **2.2** prior to purification

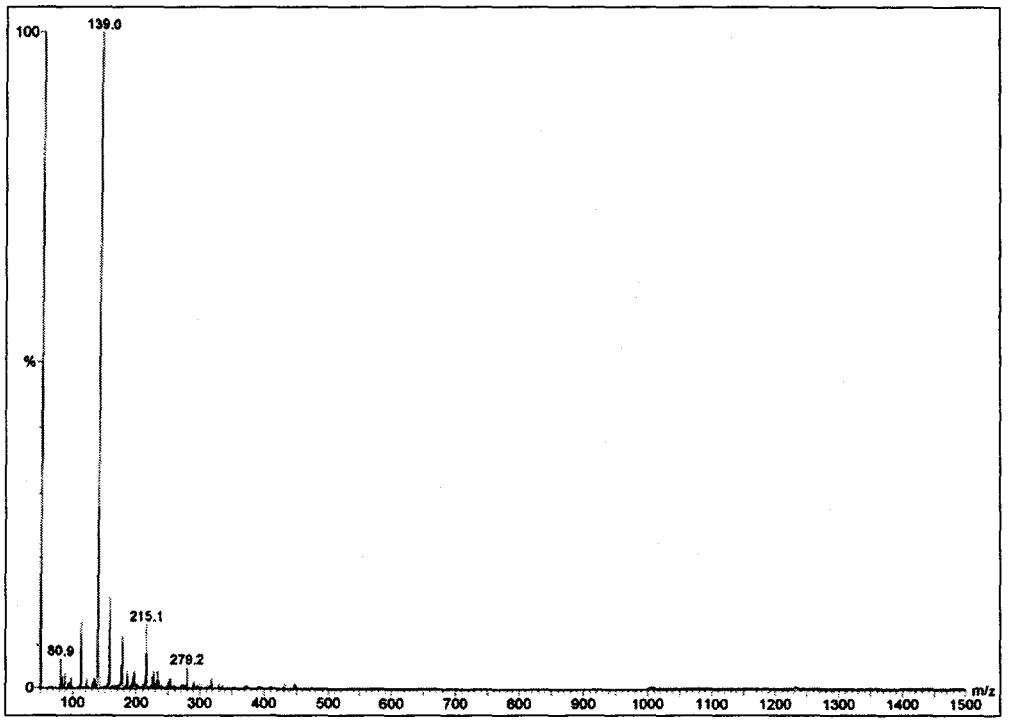

Figure 2-17. Negative ion electrospray mass spectrum of the reaction mixture after Sep-pak purification (**2.16**)

As mentioned previously, the highest radiochemical yield (EOB) obtained was 30.2 %. However, it should be noted that approximately 20 mCi of radioactivity (or ≈ 11 %) was lost during evaporation of the FC-72® solvent. It is possible that the substitution of H-atoms in FC-72® by [^{18}F]fluoride accounts for this loss of activity post evaporation. De Vries *et al.* observed a 61-73 % loss of radioactivity to the reaction solvent when they switched from $CFCl_3$ to the more environmentally appropriate $CHCl_3$ or CH_3CN.[18] This reduced the radiochemical yield of 6-[^{18}F]fluoro-L-DOPA, obtained through fluorodestannylation, from 33 % to 5 % ($CHCl_3$) and 17 % (CH_3CN). It appears, despite the loss of activity, that FC-72® permits higher overall radiochemical yields compared with other reaction solvents.

In developing these $[^{18}F]F_2$ reactions, it quickly became evident that a workup procedure needed to be devised to permit a more "hands-free" or automated approach. The challenge with this work-up is that the fluorophilic solvent (FC-72®/methanol) needed to be exchanged with a fluorophobic solvent (acetonitrile/water). Rotary evaporation required too much manual manipulation. Alternatively, solvent evaporation in a hot water bath under a rapid flow of nitrogen took too long and often dispersed the product.

In an attempt to improve upon these procedures, a U-tube like apparatus was constructed (**Figure 2-18**). Following the fluorination reaction, the vessel contents could be transferred to the U-tube using syringe pressure. Applying a weak vacuum to the top of the U-tube facilitated removal of the solvent at room temperature within a couple of minutes. Addition of 3 x 3 mL of acetonitrile:water (50:50), followed successively with applied syringe pressure, transferred the contents to the fluorous Sep-Pak and into the collection vial. In a trial cold reaction this apparatus appeared to facilitate a more suitable "hands-free" workup.

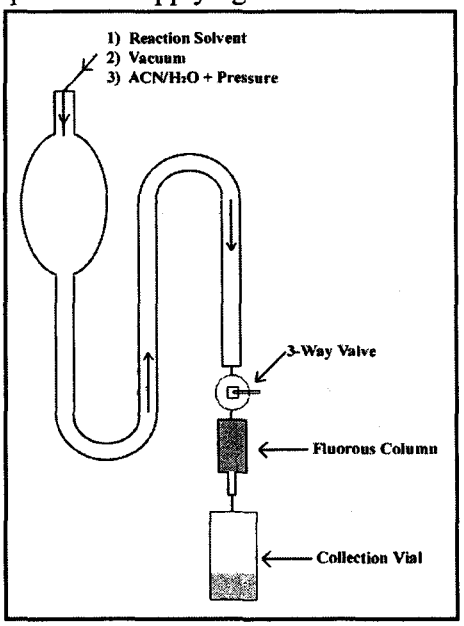

Figure 2-18. A novel workup apparatus

The facile synthesis and purification of **2.16** demonstrates that the fluorous strategy shows promise as a convenient route for the preparation of $^{18}F[F_2]$ labelled radiopharmaceuticals. There is a complete removal of the fluorous "tag" through a

quick and simple fluorous column purification, which requires less than a minute. This approach therefore would be appealing in certain applications, as it avoids time intensive purification, reduces exposure, and can increase overall specific activity when compared to standard methods.

2.5 Synthesis of 3-iodobenzoic acid (2.17).

With the success of the fluorination reactions, we explored labelling benzoic acid with iodine. The cold iodinolysis of the fluorous "tagged" model compound (**2.2**) was carried out in order to assess the capacity for introducing ^{125}I, ^{131}I, and ^{123}I. In addition to being interested in simple product generation, optimising reaction conditions was also an important goal. The iododestannylation reaction of **2.2** using excess iodine is shown in **Figure 2-19.**

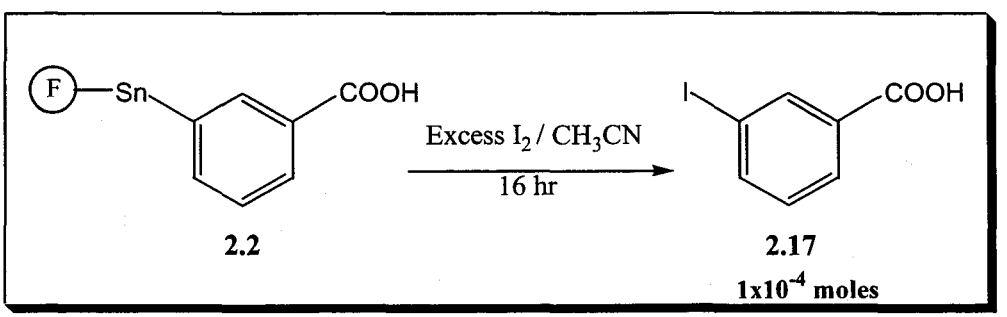

Figure 2-19. Iododestannylation of **2.2** using I_2

The iodination reaction was carried out using excess I_2 (100 μmol) dissolved in methanol, which was added to a sizeable (99 μmol) sample of **2.2**. The reaction was allowed to proceed overnight, after which sodium metabisulfite was added to quench any unreacted iodine. Methanol was removed under reduced pressure and the residue was dissolved in 5 x 5 mL volumes of HPLC grade acetonitrile:water (50:50), and

each washing was eluted through a fluorous column. In this case, fluorous column purification utilised a 3.9 g sample of loose fluorous silica (Silicycle®), packed into a 40 cm narrow column. The 5 ml aliquots were assessed for purity through HPLC (**Figure 2-20**) and electrospay mass spectrometry (**Figure 2-21**).

The HPLC chromatogram contained three peaks, corresponding presumably to salts (solvent front) and **2.17** (t_R=9.9 min). The peak at 9.9 min was shown to be **2.17** through comparison to a standard sample of 3-iodobenzoic acid.

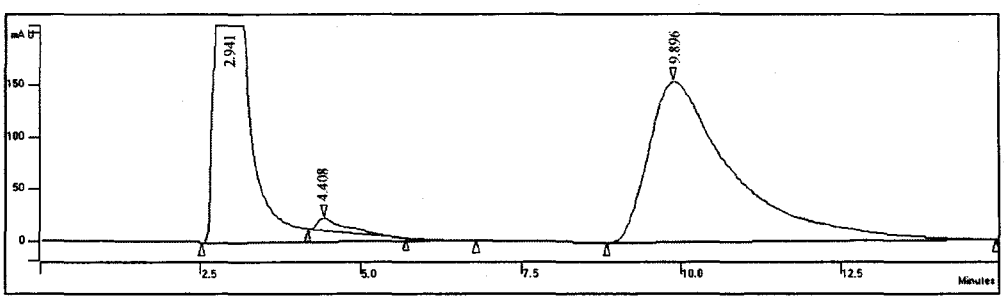

Figure 2-20. HPLC chromatogram of the reaction mixture (**2.17**) (conditions: analytical C_8 (Microsorb-MV, 250 x 4.6 mm), 80:20 H_2O (0.1 % HFBA):ACN, flow 1 mL/min)

The negative ion electrospray mass spectrum showed a peak at m/z=246.9 $[M-H]^-$, which is consistent with the formation of **2.17**. There was no evidence of the fluorous "tag" which would be seen at m/z>1000.

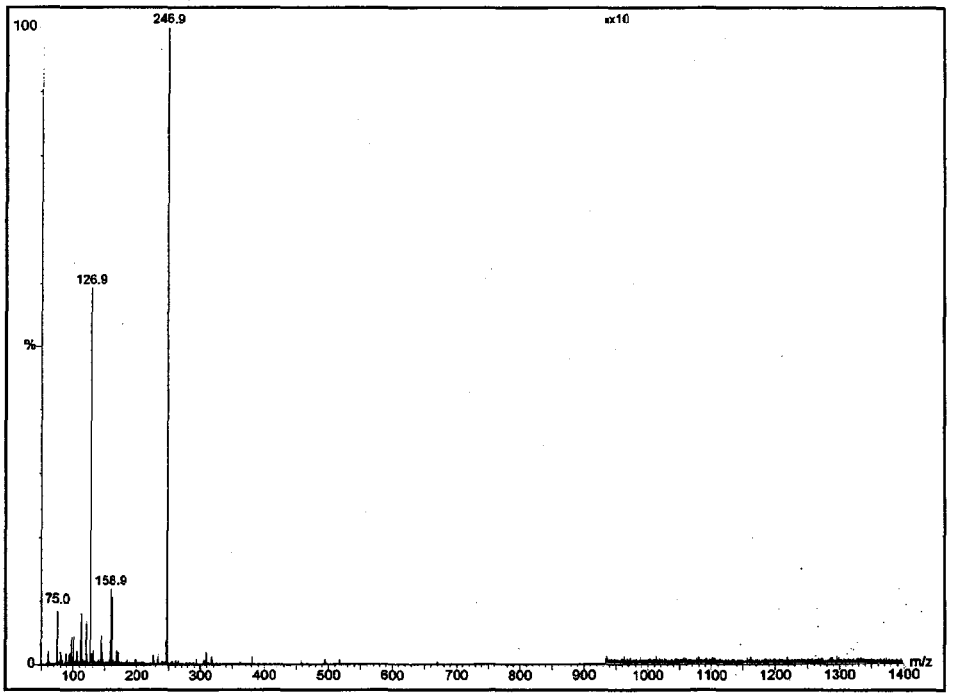

Figure 2-21. Negative ion electrospray mass spectrum of the reaction mixture (**2.17**)

2.5.1 Iododestannylation of ***2.2*** *using NaI (****2.18****).*

The iodinolysis reactions discussed above used an excess of iodine and 10^{-4} moles of substrate, and are therefore not representative of radioiodination reactions. In order to develop a labelling approach towards **2.18**, reactions with cold $Na^{127}I$ at concentrations that mimic those that would be used with iodine radionuclides were undertaken (**Figure 2-22**).

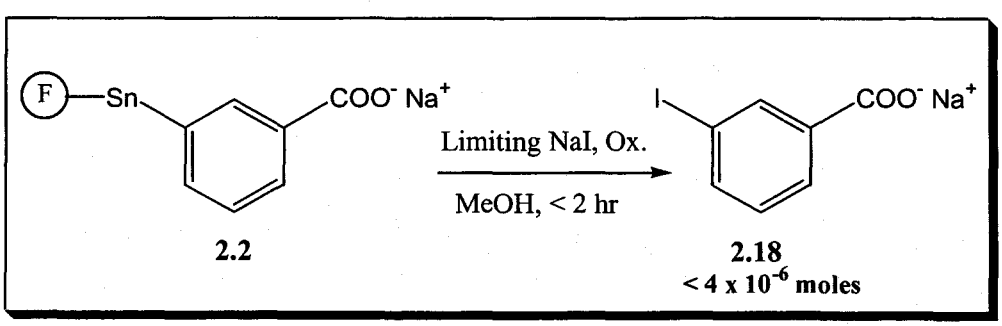

Figure 2-22. Iododestannylation of **2.2** using NaI and oxidant

In an attempt to optimise the cold iodination reaction a number of reaction conditions were investigated. First, a wide range of oxidants, which are commonly used in radioiododestannylation reactions, were screened. These included chloramine-T (*N*-monochloro-*p*-toluenesulfonamide), *N*-chlorosuccinimide, and peracetic acid. Peracetic acid showed the highest conversions, which is consistent with literature reports.[22]

The choice of solvent can also dramatically impact the radiochemical yields. For the most part, methanol was utilised because of its ability to dissolve **2.2** and has been shown to be compatible with the other reagents and reaction conditions. Iodination reactions are also highly dependent on the pH of the solvent, generally being promoted in an acetic medium and sometimes arresting when the pH increases towards neutrality. For this reason, researchers often add small quantities of HCl or acetic acid to the reaction; however, it was found that the oxidant (32 % peracetic acid in acetic acid) was adequately acidic to promote the aforementioned reaction.

In addition to optimising the reaction conditions, detection of the very small quantity of product (**2.18**) being generated necessitated optimising the HPLC conditions. It was found through lengthy trial and error that separation of **2.18** from salts in solution could not be exacted using a C_8 analytical column. This problem was rectified by switching to a C_{18} analytical column which facilitated significant separation.

In the end, the optimum reaction involved dissolving compound **2.2** (Na^+ salt) (4 x 10^{-6} mol) in methanol (200 μL) with stirring. To this solution was added NaI (4 μL, 1.8 x 10^{-7} mmol) in 0.1 N NaOH, which was followed immediately by the addition of

[22] Katsifis, A.; Mattner, F.; Zhang, Z.; Papazian, V. *J. Labeled Compd. Radiopharm.* **2000**,*43*, 385.

freshly prepared peracetic acid solution (2 μL). The reaction was quenched after 2 hours with excess sodium metabisulfite and diluted to 1 mL with distilled deionized water.

The HPLC chromatogram of the reaction mixture (**2.18**) shows peaks with earlier elution times of 4.8-6.3 min and 10.3 minutes, presumably corresponding to salts (solvent front) and **2.18** respectively (**Figure 2-23**). An authentic standard of 3-iodobenzoic acid under the same elution conditions produced a peak at 10.2 minutes, supporting the prior peak assignment.

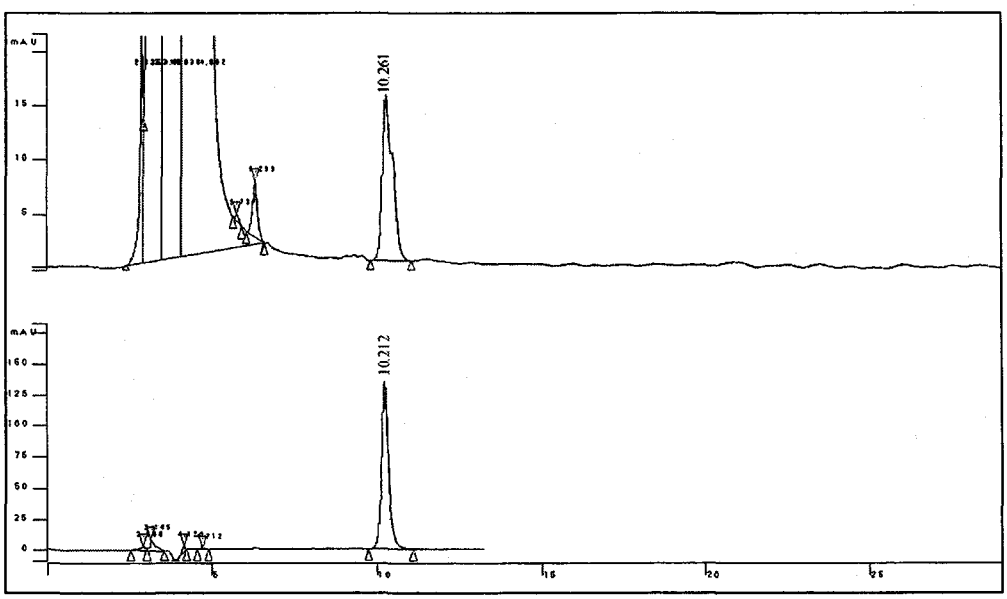

Figure 2-23. HPLC chromatogram of the reaction mixture (**2.18**) (above) and authentic 3-iodobenzoic acid (below) (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 50:50 ACN (0.2 % formic acid):H_2O (0.2 % formic acid), flow 1 mL/min)

The advantage to developing this chemistry using a cold isotope, similar to the case of fluorine, was that reactions could be conducted and handled without risk of exposure. However, the difficulty in developing radiochemical labelling procedures with representative quantities of $Na^{127}I$ for $Na^{125}I$, was that detection had to be based solely on ultraviolet absorption. Comparatively, the use of 400 μCi

(approx. 1.8 x 10^{-7} mmol) $Na^{125}I$ would result in an extremely intense peak on a gamma detector, although a very small, if visible, ultraviolet absorbance. Recall that the typical incorporation of iodine (as I_2) into the target molecule is ≤50%; therefore, in an analogous 400 μCi reaction, the maximum product yield is ≤ 9 x 10^{-8} moles.

2.5.2 Synthesis of 3-[^{125}I]iodobenzoic acid (2.19)

The successful cold labeling of **2.2** using cold NaI led to the corresponding radioiododestannylation using $Na^{125}I$ (**Figure 2-24**).

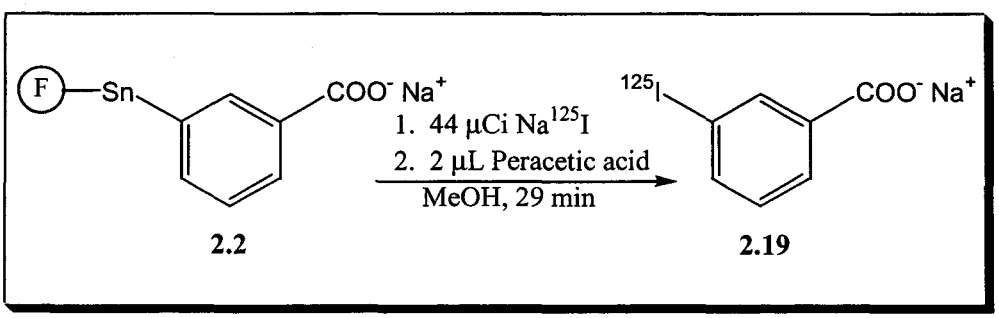

Figure 2-24. Synthesis of 3-[^{125}I]iodobenzoic acid (**2.19**)

The reaction was conducted in a similar fashion to the cold iododestannylation reactions. Compound **2.2** (Na^+ salt) (9 x 10^{-4} mol) was dissolved in 200 μL of methanol with stirring, prior to the addition of $Na^{125}I$ (44 μCi) in approximately 200 μL of 0.1 N NaOH solution, and 2 μL of fresh peracetic acid. The reaction was allowed to stir for 29 min prior to quenching with sodium metabisulfite (100 μL).

A 20 μL aliquot of the crude reaction mixture was injected onto the HPLC for analysis. The UV trace revealed only a single peak corresponding to the solvent front, while the radioactivity chromatogram showed several peaks (**Figure 2-25**).

The peak at 5.3 min is coincident with the solvent front and presumably represents free ^{125}I. The peak at 17.1 min was assigned as **2.19** through injection of the standard 3-iodobenzoic acid. However, the identity of the other extraneous peaks, particularly the large peak at 24.9 min could not be assigned at the time of the reaction. A later experiment would establish that these additional peaks were likely the result of the stock $Na^{125}I$ solution being contaminated.

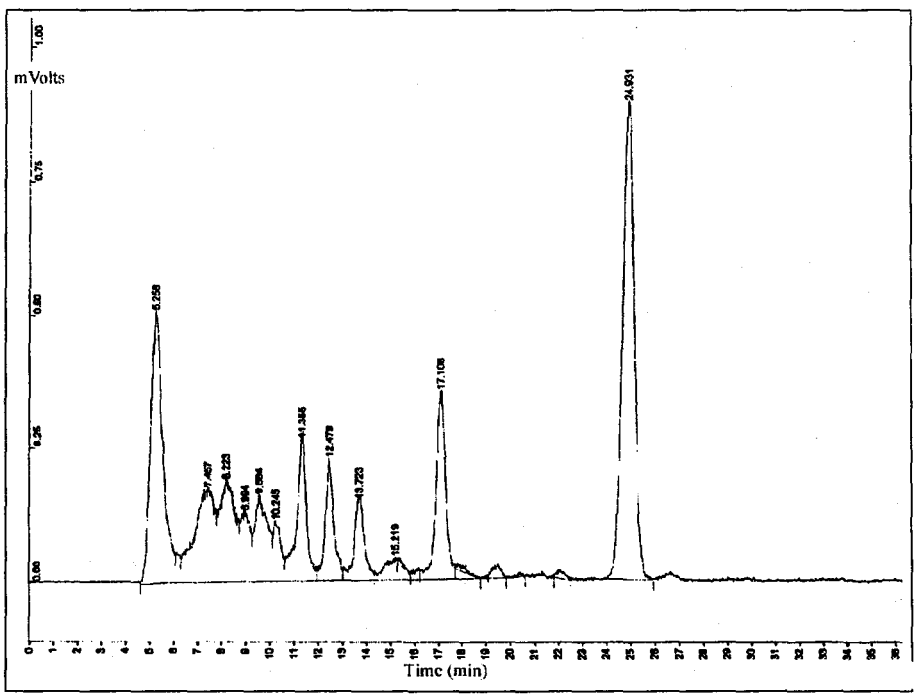

Figure 2-25. HPLC chromatogram of the crude reaction mixture for **2.19** (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 50:50 ACN (0.2 % formic acid):H_2O (0.2 % formic acid), flow 0.5 mL/min)

Although the quality of the crude reaction mixture containing **2.19** is less than ideal, a simple purification was undertaken to illustrate our capacity to remove any unreacted free ^{125}I in solution. The aforementioned crude reaction mixture was diluted with 500 μL of water and added to a conditioned C_{18} Sep-Pak. The Sep-Pak was eluted with 2 mL of distilled deionized water to remove unbound ^{125}I, followed

by elution with 1 mL of methanol. HPLC analysis of a 20 μL aliquot of the methanol fraction is shown in **Figure 2-26**. The chromatogram reveals that essentially all of the radioactive impurities up to **2.19** (t_R=16.9 min) are removed by washing the column with water. Further, taking into account dilution, most of **2.19** was eluted with the 1 mL of methanol. However, the then unidentified peak at 24.5 minutes was still present.

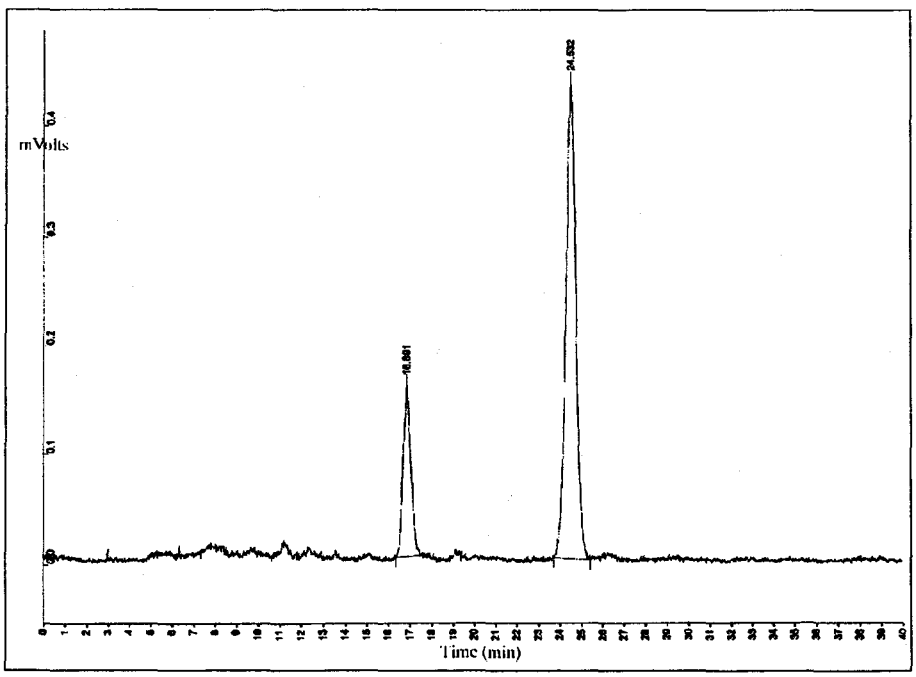

Figure 2-26. HPLC chromatogram of the reaction mixture (**2.19**) following Sep-Pak purification (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 50:50 ACN (0.2 % formic acid):H_2O (0.2 % formic acid), flow 0.5 mL/min)

The less-than favourable results obtained in the above reaction prompted another reaction with a fresh source of $Na^{125}I$. In this reaction, compound **2.2** (Na^+ salt) (1.1 x 10^{-6} mol) was dissolved in 200 μL of methanol with stirring, prior to the addition of $Na^{125}I$ (32 μCi) in approximately 5 μL of 0.1 mM NaOH solution, followed by 2 μL of a freshly prepared solution of peracetic acid. The reaction was allowed to stir for

47 min, prior to quenching with excess sodium metabisulfite (20 μL) and dilution with 300 μL of distilled-deionized water.

A 20 μL aliquot of the crude reaction mixture was injected onto the HPLC for analysis. The UV trace revealed only a peak representative of the solvent front, while the radioactivity chromatogram showed small peaks around the solvent front and a peak with a retention time of 16.91 min (**Figure 2-27**). The large peak is consistent with the formation of **2.19**, confirmed by injection of 3-iodobenzoic acid, which elutes at 15.86 min. The difference in retention times is a result of the time delay between the UV and radiation detectors.

The radioactivity chromatogram of the crude reaction mixture illustrates that **2.19** was essentially generated in quantitative yield with no significant contribution of unbound/unreacted iodine. The radiochemical purity of crude **2.19** was ≥ 90 %. This level of incorporation and purity in a crude iododestannylation reactions is exceptional, especially given the short reaction time.

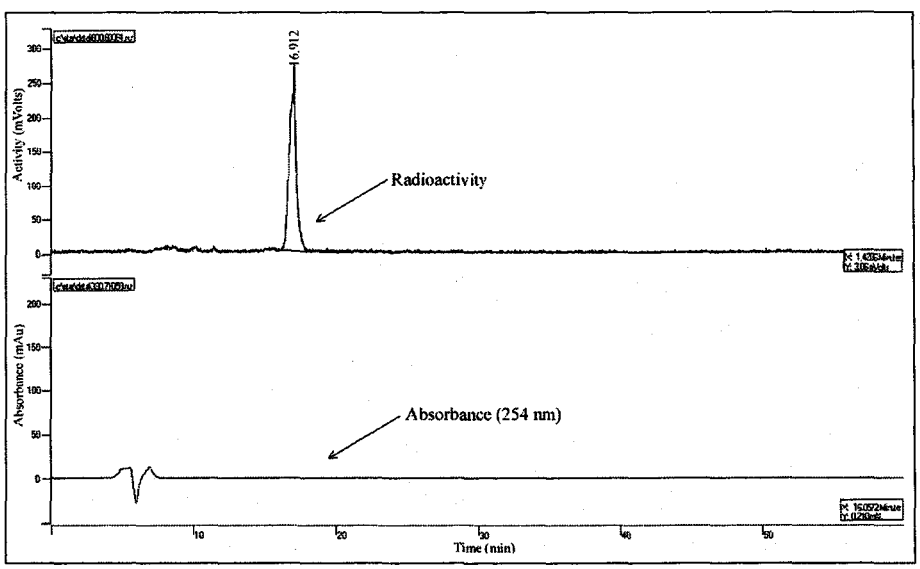

Figure 2-27. HPLC chromatogram of the crude reaction mixture for **2.19** (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 50:50 ACN (0.2 % formic acid):H_2O (0.2 % formic acid), flow 0.5 mL/min)

Although there appears to be only trace amounts of unbound iodine or radiolabelled salts in the reaction mixture, a short purification was undertaken to indicate that they could in the future be removed from the product. The reaction solution was diluted with approximately 1.5 mL of water and passed down a C_{18} Sep-Pak column, conditioned with methanol. The column was further washed with 1.5 mL of water, and these fractions combined. The Sep-Pak was then eluted with 2 mL of acetonitrile and collected into a separate vial. The acetonitrile faction contained 72 % of the activity, and further elution of the column with acetonitrile released only small additional amounts of activity. A total of (4 μCi, 12 %) was bound to the Sep-Pak column, likely the more highly retained and radiolabelled fluorous "tag" ($R_3Sn^{125}I$). The other activity was found in the water (3 μCi, 9 %), the reaction vessel (1 μCi, 3 %), and in an additional 1mL washing of the Sep-Pak with acetonitrile (1 μCi, 3 %). HPLC analysis of the fraction containing the majority of the activity displayed a single peak in the radiochromatogram corresponding to **2.19** at 16.59 minutes. The final radiochemical yield of purified **2.19** was 75 % with respect to the total $Na^{125}I$ activity added. Yields of this magnitude are uncommon, considering that often 50 % of the activity is often bound to tin. The results of Hunter *et al.* are fairly representative of a radioiododestannylation reaction. They observed a 50.8 % radiochemical yield of [^{131}I]MIBG; 44 % of the activity was bound to tin and 5.1 % was free $^{131}I^-$ in solution.[23]

Given the high radiochemical yield, it became important to quantify the purity of [^{125}I]-3-iodobenzoic acid with regards to any labelled or unlabelled precursor **2.2**. As ^{19}F NMR and mass spectrometry are not feasible for ^{125}I labelled compounds, we had

[23] Hunter, D.H.; Zhu, X. *J. Labeled Compd. Radiopharm.* **1999**, *42*, 653.

to rely on HPLC analysis. Analysis of the precursor **2.2** on a C_{18} analytical column with 100 % acetonitrile generated a UV peak at 6.61 minutes. Similarly HPLC analysis of the Sep-Pak purified faction exhibited peaks at 3.19 and 4.17 min, corresponding to the solvent front and perhaps cleaved benzoic acid, and 6.38 and 6.72 min, likely corresponding to the Na^+ salt and protonated forms of **2.2** (**Figure 2-28**). The radioactivity chromatogram showed what appears to be a single but broad peak at the solvent front, 4.46 min, corresponding presumably to **2.19**. A radioactivity peak corresponding to a labelled fluorous "tag" product would be predicted to elute in a similar position to **2.2**; however, this is not seen. This indicates that our previous radiochemical yield of 75 % is accurate, though there appears to be some unreacted **2.2** present in this reaction solution.

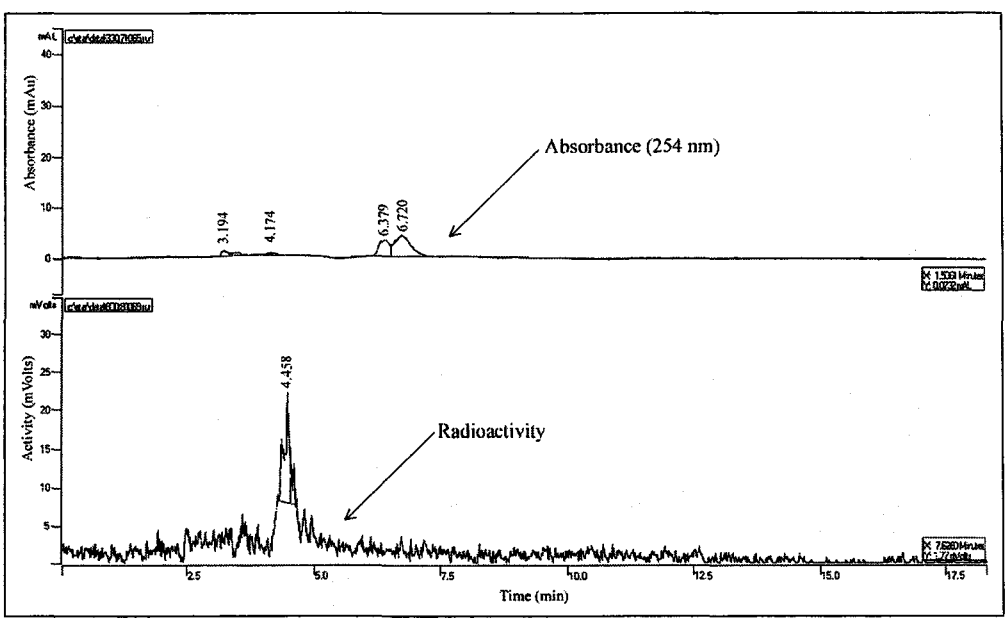

Figure 2-28. HPLC chromatograms of the reaction mixture **(2.19)** after Sep-Pak purification (conditions: C_{18} (Nucleosil, 250 x 4.6 mm), 100 % ACN, flow 1 mL/min)

It has previously been established that even large quantities (>200 mg) of the "fluorous tag" can readily be removed using a fluorous column and an

acetonitrile:water (50:50) mobile phase. This system should therefore readily facilitate the removal of the much smaller quantities of substrate (1.4 mg) used in this and other typical radioiodination reactions. In order to demonstrate this purification approach, the mixture containing **2.19** in acetonitrile was diluted with an equal volume of distilled-deionized water and passed down a conditioned fluorous column. Washing the column with an additional 4 mL of acetonitrile:water (50:50) liberated all the activity (19 μCi). Analysis of an aliquot of this solution showed, upon expansion of the chromatogram, a solvent peak at 3.055 min and a small peak at 6.53 min (**Figure 2-29**).

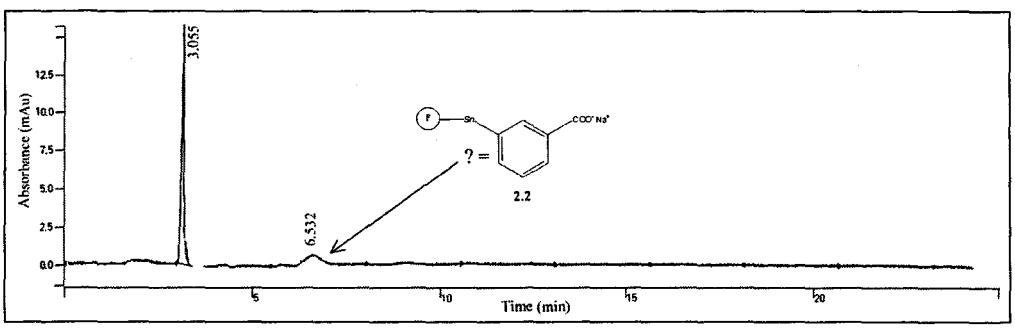

Figure 2-29. UV chromatogram of the reaction mixture (**2.19**) following purification down a fluorous column (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 100 % ACN, flow 1 mL/min)

Because we have shown that the fluorous Sep-Pak can remove large quantities of the fluorous "tag", the peak at 6 minutes likely arose through another source. One possibility is that the fluorous column, which had been recycled from another reaction, might not have been adequately cleaned. Alternatively, since fluorous material is prone to sticking to the HPLC loop, it is possible that accumulated material was released into this injection.

2.6 Summary

A method was developed to prepare tris(perfluorohexylethyl)tin-3-benzoic acid and to label this material with fluorine and iodine. The fluorous approach using both hot and cold F_2 and I_2 was effective in generating the desired products. Additional experiments are needed to optimise the reactions, particularly with respect to purification protocols.

2.7 Developing coupling procedures – Benzamide synthesis

Initially, the rationale behind the synthesis of **2.2** lay in permitting the facile radiolabelling of peptides/biomolecules through coupling to labelled benzoic acid. The successful synthesis and labelling of **2.16** and **2.19** encouraged the synthesis of more complex compounds. One such approach that would benefit from, and extend the utility of, compound **2.2** would be its conversion to biologically active derivatives.

2.7.1 Radioiodobenzamide

Radioiodobenzamides, or *N*-Alkyl-iodobenzamides, constitute a new class of important radiopharmaceuticals.[24] Exhibiting a high affinity towards σ_1 and σ_2 receptors, radioiodobenzamides are currently the best known radiopharmaceuticals for the diagnosis of cutaneous melanoma and its metastases.[24] This class of compounds have also been found to bind strongly to dopamine receptors, and are therefore

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[24] Auzeloux, P.; Papon, J.; Azim, E.M.; Borel, M.; Pasqualini, R.; Veyre, A.; Madelmont, J-C. *J. Med. Chem.* **2000**, *43*, 190.

effective imaging agents for diagnosis of Parkinson's and schizophrenia.[25] One of the most clinically relevant compounds is [^{123}I]-*N*-(2-diethylaminoethyl)-4-iodobenzamide (^{123}I-BZA) (**Figure 2-30**), which possesses ideal properties for melanoma scintigraphy.[26]

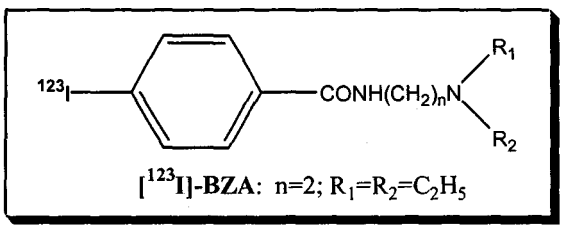

Figure 2-30. [^{123}I]-*N*-alkyl-*p*-iodobenzamides and ^{123}I-BZA

Currently, the most facile route to ^{123}I-BZA involves an isotope exchange reaction (^{123}I for ^{127}I). This method affords a carrier-added product, which leads a reduced image sensitivity due to the presence of non-radiolabelled substrate, which competes for available receptor sites. A more ideal strategy, which would lead to a no-carrier-added product, is radioiododestannylation of a trialkyltin precursor, which has been developed by Moreau *et al.*[26] With this in mind, the fluorous synthesis approach would seem suited for synthesis of radiolabelled benzamides and would avoid the need for exhaustive purification.

The aim of this project was the synthesis of iodobenzamide, **2.20**, through an iododestannylation reaction of a corresponding fluorous "tagged" precursor (**2.21**) (**Figure 2-31**). The synthesis of **2.20** required the development of a new coupling methodology.

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[25] Laulumaa, V.; Kuikka, J.T.; Soininen, H.; Bergstrom, K.; Lansimies, E.; Riekkinen, P. *Arch. Neurol.* **1993**, *50*, 509.
[26] Moreau, M.F.; Labarre, P.; Foucaud, A.; Seguin, H.; Bayle, M.; Papon, J.; Madelmont, J.C. *J. Labeled Compd. Radiopharm.* **1998**, *41*, 965.

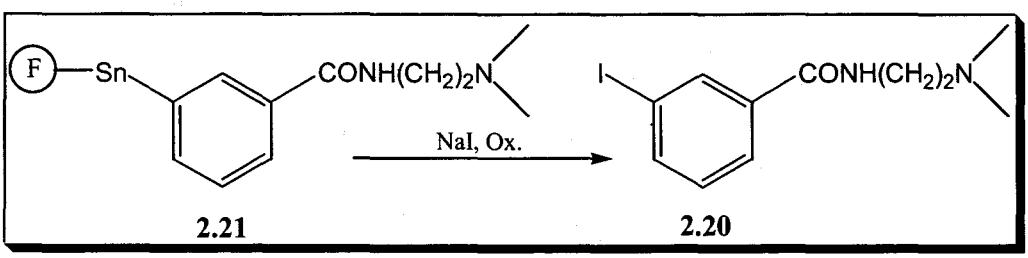

Figure 2-31. The target compound, *N*, *N*-dimethyl-*m*-iodobenzamide

2.7.2 Synthesis of tris(perfluorohexylethyl)tin-3-benzamide (2.21)

The approach towards the synthesis of **2.21** concentrated on adapting traditional peptide synthesis procedures. The success of these reactions was qualified through ^{1}H NMR and electrospray mass spectrometry. Integration of the ethylene protons (NCH_2CH_2N) with respect to the protons positioned α and β to tin served to quantify the extent of derivatization. Initially, carbodiimide activating agents such as diisopropylcarbodiimide (DIC) and EDC were employed; however, they led to little detectable product formation. It was difficult to determine if the lack of reaction was due to the reagent or the reaction solvent. In most instances, good solvents for the coupling reagents proved to be poor solvents for **2.2** and visa versa. While coupling reactions were promoted in polar aprotic solvents such as acetonitrile and DMF, compound **2.2** (acid form) was generally solvated by only extremely non-polar solvents. Solvents such as THF, which solvated both **2.2** and DIC, did not result in conversion to **2.21**. EDC had another drawback in that it contains an ammonium salt which proved acidic enough to result in the cleavage of > 30 % of the tin aryl bonds. It was later realised that in order to improve the solubility of compound **2.2** in solvents such as DMF, the Na^+ salt rather than the protonated form had to be used.

Successful synthesis of **2.21** employed the use of the coupling reagent HBTU (2-(1H-Benzotriazol-1yl)-1,1,3,3-tertramethyluronium hexaflurophosphate) in DMF (**Figure 2-33**). HBTU promotes couplings by readily generating an activated intermediate concurrent with the formation of a urea byproduct. This activated complex reacts with amines with the subsequent loss of 1-hydroxybenzotriazole (HOBt) (**Figure 2-32**).

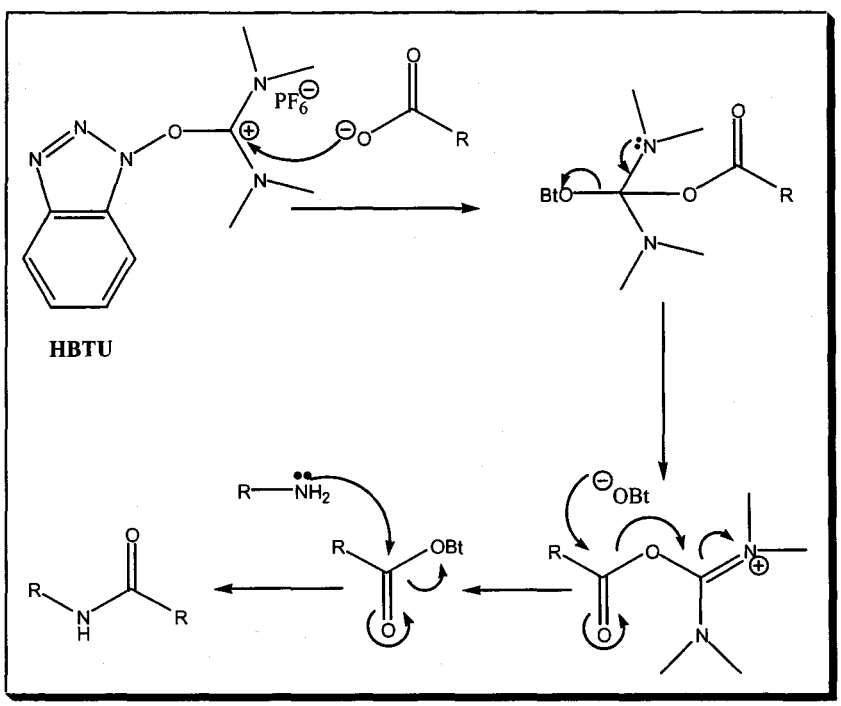

Figure 2-32. Mechanism of HBTU promoted peptide coupling. Additional forms have been omitted for clarity

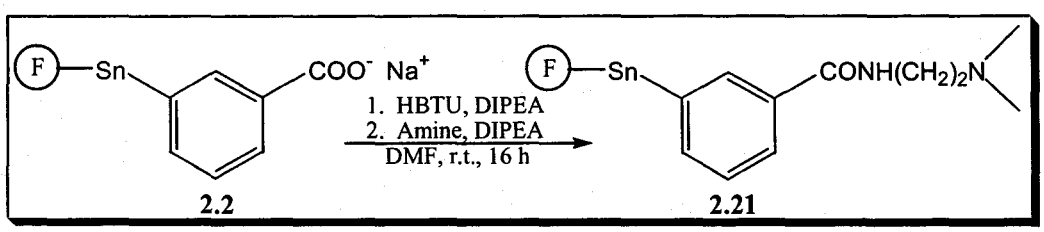

Figure 2-33. Synthesis of perfluorotin-3-benzamide (**2.21**) using HBTU

Reaction of HBTU and compound **2.2** (Na^+ salt) was carried out in DMF in the presence of DIPEA for 5 min, prior to addition of the amine. Experiments have shown that this incubation leads to a dramatic improvement in coupling rates and yields.[27] Following addition of excess *N, N*-dimethylethylenediamine in an equivalent of DIPEA, the reaction was allowed to stir for 16 hours.

Due to the high solubility of **2.21** in DMF, water was added to facilitate extraction of fluorous compounds into dichloromethane and FC-72®. The more organic **2.21** could then be selectively extracted into dichloromethane from FC-72®. Several more extractions into dichloromethane yielded pure **2.21**, while unreacted **2.2** remained in FC-72®. Compound **2.21**, a dark yellow oil, was obtained in satisfactory yield (74 %). The substantial difference in R_f values between **2.21** and **2.2** (0;0.21), suggests that chromatographic purification would likely be a more appropriate and higher yielding purification method for the future.

The ^{1}H NMR spectrum of compound **2.21** (**Figure 2-34**) revealed the expected shift in protons positioned α and β to tin to a higher field, 1.31 ppm (t, J=8.1 Hz with ^{119}Sn satellites $^2J_{Sn,H}$= 54.8 Hz, 6H) and 2.33 ppm (t, 6H), respectively. In addition, the ^{1}H NMR spectrum showed peaks corresponding to the benzamide: a broad singlet at 2.31 ppm (6H, $N(CH_3)_2$), a triplet at 2.59 ppm (J= 5.7 Hz, 2H, $CH_2CH_2N(CH_3)_2$) , a quartet at 3.55 ppm (2H, $CH_2CH_2N(CH_3)_2$), and the expected aromatic peaks from 7.39-8.01 ppm (4H). The ^{13}C NMR spectrum of **2.21** showed the predicted signal shift of the carbon atom situated α to tin to higher field, -1.43 ppm, in addition to the β carbon atom at 27.55 ppm ($^2J_{F,C}$= 23.4 Hz). The ^{13}C NMR spectrum also showed

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[27] Li, W-R.; Chou, H-H. *Synthesis.* **2000**, *1*, 84.

peaks characteristic of the amide group at 37.11 ppm ($CH_2\mathbf{C}H_2N(CH_3)_2$), 44.87 ppm ($N(\mathbf{C}H_3)_2$), and 57.75 ppm ($\mathbf{C}H_2CH_2N(CH_3)_2$). The IR spectrum of compound **2.21** showed sp^2 C-H stretches at 2900 cm^{-1}, in addition to the C=O absorption at 1650 cm^{-1}, and a N-H stretch at 3338 cm^{-1}. The electrospray mass spectrum of **2.21** (**Figure 2-35**) showed, in the positive ion mode, a peak at m/z=1353 $[M+H]^+$, representative of the product. Importantly, the negative ion electrospray mass spectrum of the same compound did not show the precursor peak at m/z=1279 $[M-H]^-$. These results are consistent with formation of the **2.21**.

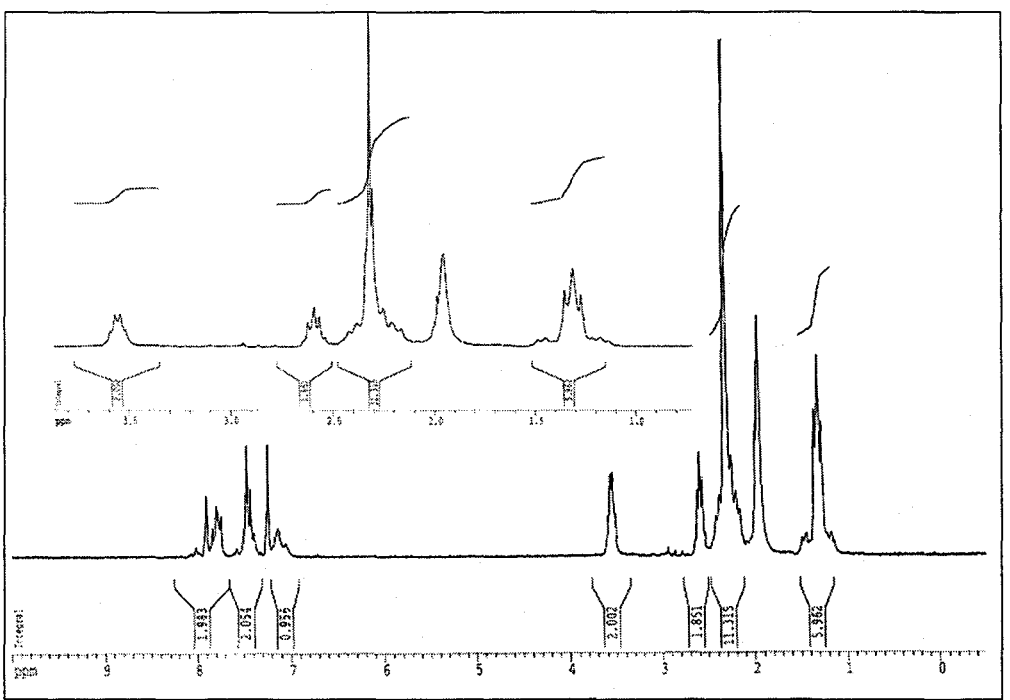

Figure 2-34. ^1H NMR [$CDCl_3$, 200 MHz] spectrum of compound **2.21**

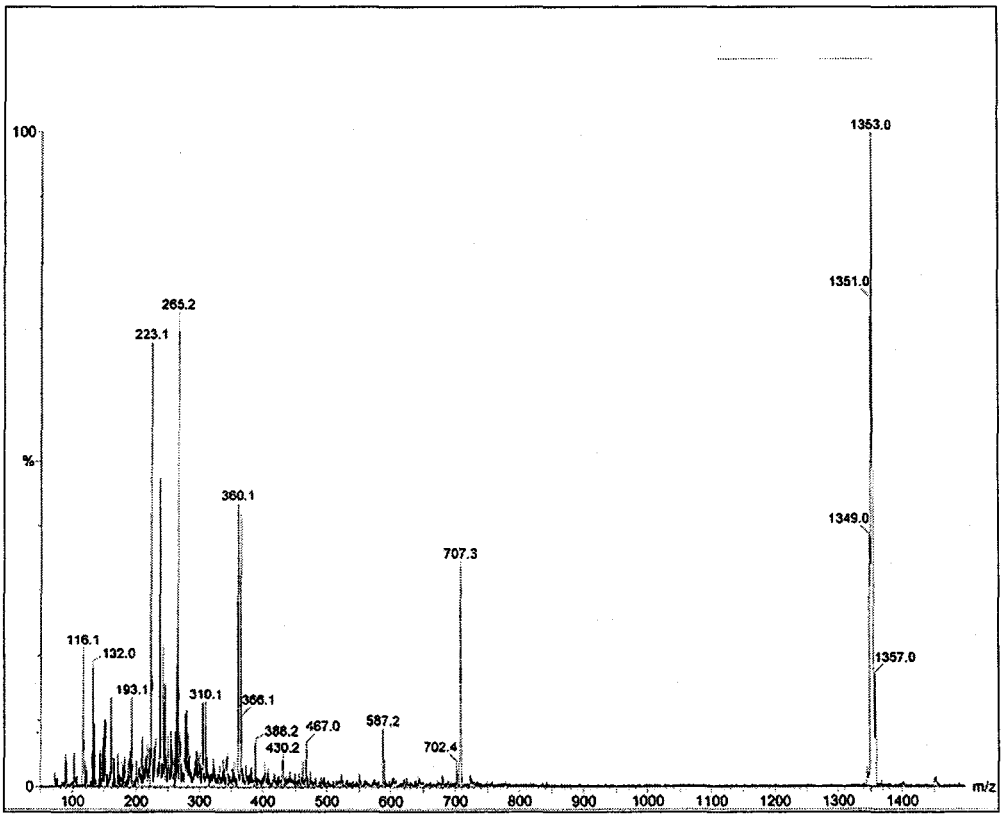

Figure 2-35. Positive ion electrospray mass spectrum of compound **2.21**

2.7.3 Synthesis of 3-iodobenzamide (2.20)

The iododestannylation of compound **2.21** and fluorous column purification of the reaction mixture (**2.20**) was carried out in a similar manner to that used for compound **2.2** (**Figure 2-36**).

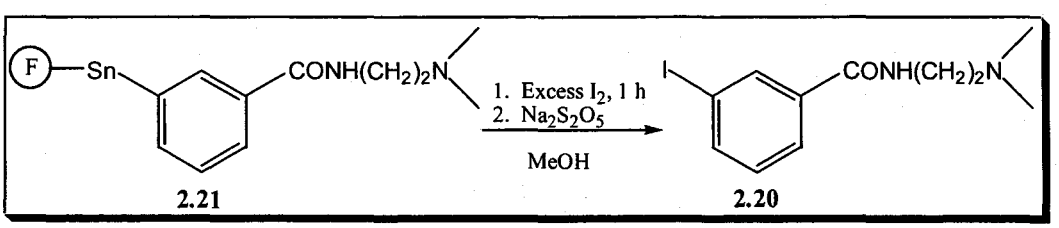

Figure 2-36. Synthesis of 3-iodobenzamide (**2.20**)

An excess of iodine was added to a small quantity (2.37 μmol) of **2.21** in methanol and the reaction was stirred for 1 hour at room temperature. The reaction

solution was quenched with sodium metabisulfite and placed on the rotary evaporator to remove methanol. The vial was washed with 1 mL of acetonitrile:water (50:50) and passed down a conditioned fluorous column. An additional 1 mL was used to rinse the vial and added to the column. The combined fractions were analysed using HPLC (**Figure 2-37**) and electrospray mass spectrometry (**Figure 2-38**).

The HPLC chromatogram of the reaction mixture (**2.20**) shows three principal peaks eluting at 6.6, 16.6, and 18.9 minutes. The earliest peak was assigned as the solvent front, while the later eluting peaks were presumably the protonated and deprotonated states of **2.20,** respectively. The other peaks have not yet been identified. The positive ion electrospray mass spectrum of the reaction mixture (**2.20**) showed a peak at m/z=319.0 $[M + H]^+$. The crude reaction mixture containing **2.20** showed no peak in the negative mode at m/z=247 $[M - H]^-$, which would be present had unreacted **2.2** existed.

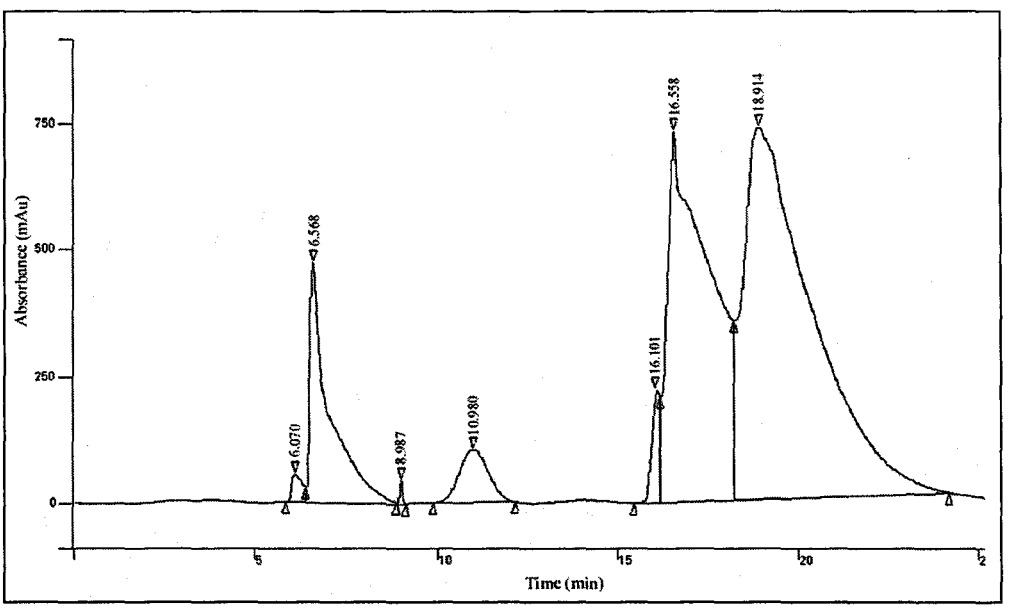

Figure 2-37. HPLC chromatogram of the crude reaction mixture (**2.20**) (conditions: analytical C_{18} (Nucleosil, 250 x 4.6 mm), 80:20 H_2O (0.01 N NaH_2PO_4):ACN, flow 2 mL/min)

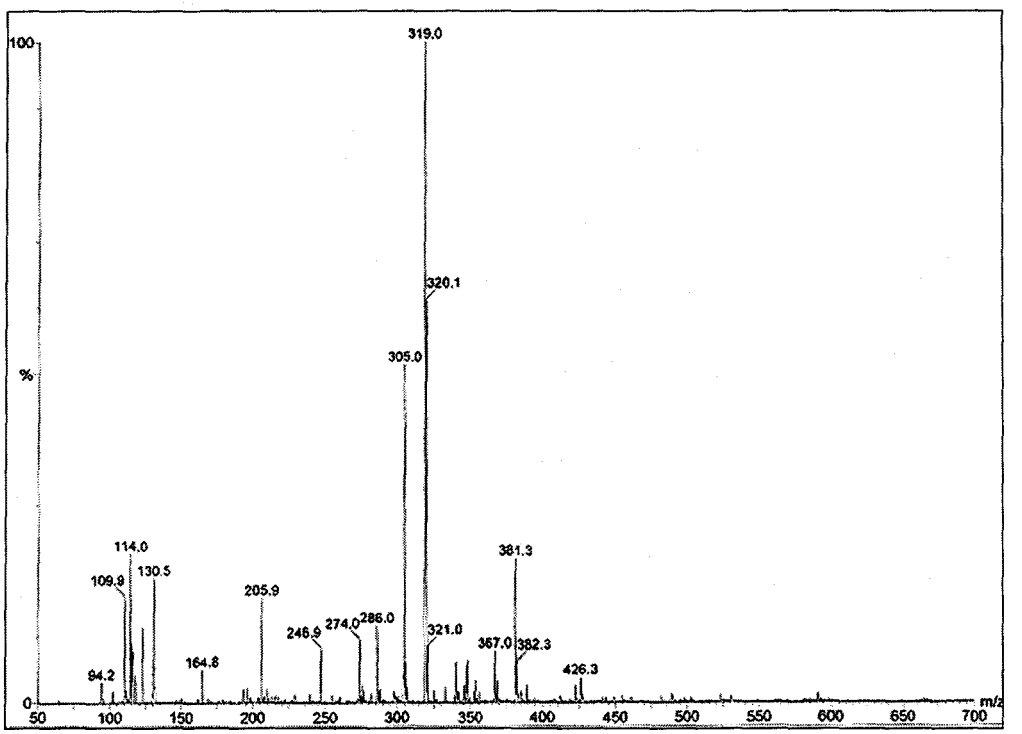

Figure 2-38. Positive ion electrospray mass spectrum of the reaction mixture (**2.20**)

The cold fluorination of **2.21** was undertaken in a similar manner employed for **2.2**. Preliminary results from the electrospray mass spectrum reveal peaks corresponding to the product at m/z=211 $[M+H]^+$ and m/z=193 $[M–F+2H]^+$. (**Figure 2-39**). The negative ion mode did not reveal any of the possible impurity, 3-fluorobenzoic acid, at m/z=139 $(M-H)^-$.

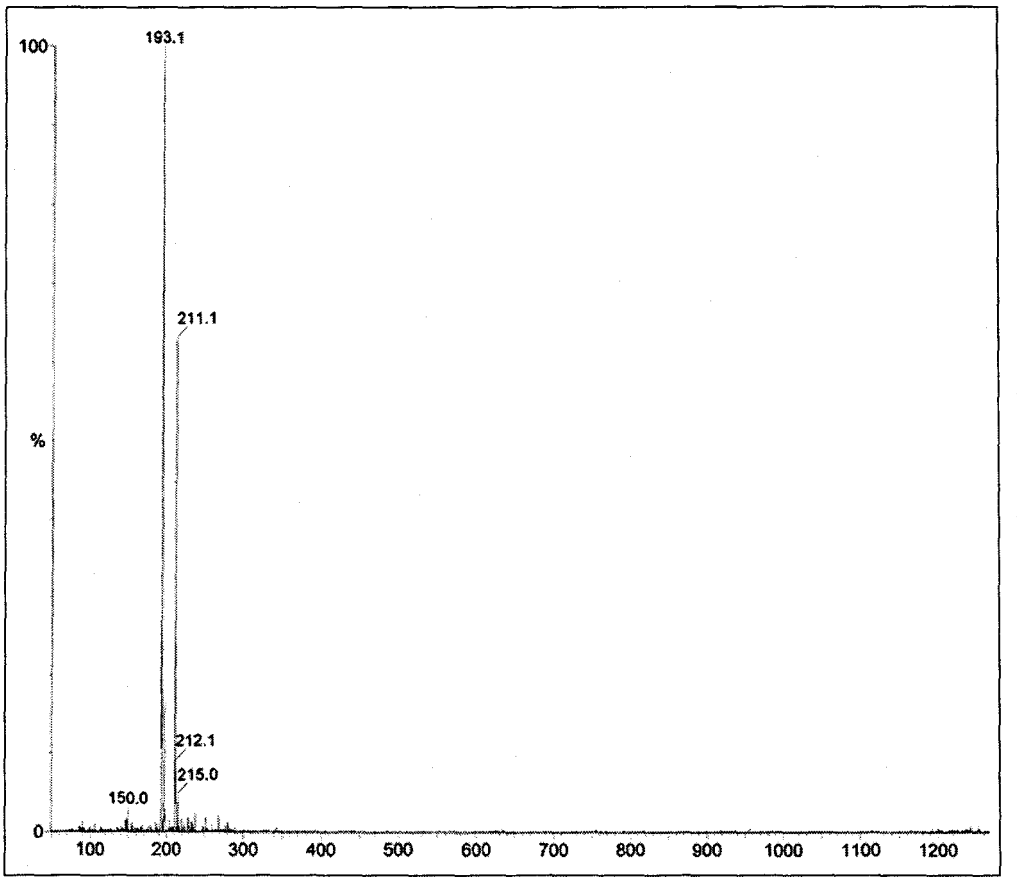

Figure 2-39. Positive ion electrospray mass spectrum from fluorination of **2.21**

2.7.4 Summary

These initial cold experiments clearly indicate the potential to label **2.21** with $^{18}F[F_2]$ and $Na^{125}I$, following the method used to label **2.2**. Success would provide a facile route to radiolabelled benzamides for both SPECT and PET, and thereby increase their clinical utility. The development of a coupling procedure will allow us to prepare a diverse array of benzamides and related compounds for future radiolabelling.

2.8 Experimental

General Procedure

Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by long wavelength ultraviolet light. HPLC experiments (cold) utilized a Varian ProSTar HPLC system with a photo diode array detector and C_8 or C_{18} reverse phase column (where mentioned). HPLC analysis of fluorine-18 labelled 3-fluorobenzoic acid employed a Waters 490E programmable multiwavelength detector and a Beckman radioisotope detector (Model 170). Gradient elution was performed as indicated with acetonitrile and distilled-deionized water as the mobile phase (buffered/acidified where indicated). ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on the Bruker Avance AC-200 or DRX-500 spectrometers. The X-ray structure was collected using Mo Kα radiation on a Siemens rotating anode instrument fitted with a CCD detector. Electrospray mass spectrometry (ESMS) were performed on a Micromass Quattro Ultima or Quatro LC. Chemical ionisation mass spectra (CIMS) were performed on a Micromass GCT instrument. IR spectra were run on a Bio-Rad FTS-40 FT FTIR spectrometer. Melting points were determined using a Fisher-John melting point apparatus. Fluorine-18 labelled F_2 was produced by the $^{18}O(p, n)^{18}F$ nuclear reaction using a Siemens RDS 112 proton cyclotron operating at 11 MeV by the "double shoot" method.[18]

Materials

All commercial reagents were used as supplied with the following exceptions: THF was distilled from sodium and benzophenone; toluene was distilled from calcium hydride. Enriched $[^{18}O]O_2$ (^{18}O, 95.87 at %, Eurisotope, St. Aubin, France),

neon (99.999%, Air products), 1% F_2 in neon (Canadian Liquid Air), HPLC grade solvents (Caledon), reagent grade FC-72® (3M corporation), and 2-(perfluorohexyl)-1-iodoethane, phenyltintrichloride, 3-(ethoxycarbonyl)phenylzin solution, and benzotrifluoride were all purchased from Aldrich.

Tris[(2-Perfluorohexyl)ethyl]phenyltin (2.4). The procedure developed by Masahide *et al.* was followed.[28] To magnesium turnings 2.308g (94.9 mmol) was added 22.501 g (47.5 mmol) of 2-(perfluorohexyl)-1-iodoethane in 10 mL of dry ether. The reaction mixture was stirred at reflux for 25 min and then 1.95 mL (11.9 mmol) phenyltintrichloride was added in 20 mL of dry toluene. The reaction was stirred at 70 °C for 4 h and then at room temperature overnight. The reaction mixture was quenched with a 40 mL of ammonium chloride solution, and washed with three 200 mL portions of a 5% sodium thiolsulfate solution. The combined aqueous layers were additionally extracted with three 100 mL portions of diethyl ether. The combined organic fractions were then dried ($MgSO_4$) and concentrated under reduced pressure. Vacuum distillation removed the homocoupled impurity at 82 °C (≈0.2 mm Hg) and the residue was purified by flash chromatography on neutral alumina. Elution with hexane gave **2.4** as a colorless oil: yield 11.031 g (75 %). TLC R_f 0.89 (6:1 hexanes-diethyl ether). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, J=8.4 Hz with ^{119}Sn satellites $^2J_{Sn,H}$=51.7 Hz, 6H), 2.24 (m, 6H), 7.33 (s, 5H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ -1.49, 27.74 (t, $^2J_{F,C}$=23.5 Hz), 129.06, 129.65, 136.08. MS (ESMS), (IPA, 2mM NH_4OAc): m/z 1297.0 $[M+OAc-H]^-$, m/z=1283.0 $[M+OAc-CH_3]^-$. IR (thin film): 2962, 2928, 2875, 2862, 1241, 1146, 497 cm^{-1}.

Bromotris[(2-Perfluorohexyl)ethyl]tin (2.3). To a solution containing 15.860 g (12.8 mmol) of **2.4** in 20 mL of dry diethyl ether at 0 °C was added slowly a solution containing 670 μL (13 mmol) of bromine in 20 mL of dry diethyl ether. The reaction solution was stirred at 0 °C for 2 h and then at room temperature overnight. The reaction solution was concentrated under diminished pressure. Vacuum distillation at 162 °C (≈0.2 mmHg) gave **2.3** as a colorless oil: yield 15.487 g (97 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.57 (t, J=7.9 Hz with Sn satellites $^2J_{Sn,H}$=54.1 Hz, 6H), 2.46 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 6.11 (with ^{119}Sn satellites $^1J_{Sn,C}$=374 Hz), 27.60 (t, $^2J_{F,C}$=22.9 Hz), 108.86-120.71 (m, CF_2, CF_3). MS (ESMS, IPA 2mM NH_4OAc): m/z 1279.5 $[M+OAc]^-$. IR (thin film): 3472, 3417, 2949, 1442, 1146 cm^{-1}.

Synthesis of Tris[2-Perfluorohexylethyl]tin-4-bromobenzene (2.8). The procedure was adapted from that used by Lequan *et al.*[29] To 37 mg (1.52 mmol) of magnesium turnings was slowly added a solution containing 390 mg (1.66 mmol) *p*-dibromobenzene in 8 mL of THF. The reaction mixture was refluxed for 2 h at which time a solution containing 820 mg (0.662 mmol) of **2.3** in 6 mL of THF was added. The reaction solution was stirred overnight and then concentrated under reduced pressure. The residue was extracted with three (3 mL) portions of FC-72® from dichloromethane and water. The combined FC-72® layers were extracted again from dichloromethane and then concentrated under reduced pressure to give **2.8** as a clear colourless oil: yield 0.538 mmol (81 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (t,

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[28] Masahide, H.; Degenkolb, P.; Curran, D.P. *J. Org. Chem.* **1997**, 62, 8342.
[29] Lequan, M.; Meganem, F. *J. Organometallic Chem.* **1975**, 94, C1-C2.

J=7.8 Hz, 6H), 2.30 (m, 6H), 7.24 (AA'BB', J=8.2 Hz, 2H), 7.56 ppm (AA'BB', J=8.2 Hz, 2H). MS (ESMS): m/z 1375.0 $[M+OAc]^+$, and 1297.1 $[M+OAc-Br]^+$.

4-Bromobenzyloxazoline (2.9). The procedure was adapted from that used by Hughes, A. *et al.*[30] A mixture of 4.00 g (19.9 mmol) of 4-bromobenzoic acid in 7.0 mL (96 mmol) thionyl chloride was refluxed for 2 h prior to concentration under reduced pressure. To the product dissolved in 10 mL of dichloromethane at 0 °C was slowly added 3.8 mL (40 mmol) of 2-amino-2-methyl-1-propanol in 10 mL of dichloromethane. The reaction solution was allowed to warm gradually overnight, filtered, and extracted from two 10 mL portions of water and dried over $MgSO_4$. The solution was concentrated under reduced pressure and to 4.850 g (17.82 mmol) of the solid was added 6 mL (80 mmol) of thionyl chloride. The reaction mixture was stirred for 45 min followed by addition of a large volume of diethyl ether to precipitate a white solid. The solid was filtered and extracted into diethyl ether from 3 N NaOH, and washed with an additional three 10 mL portions of 3 N NaOH. The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **2.9** as a clear solid: yield 4.810 g (95 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (s, 6H), 4.17 (s, 2H), 7.56 (AA'BB', J=8.5 Hz, 2H), 7.87 (AA'BB', J=8.5 Hz, 2H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 28.26, 67.58, 79.32, 125.99, 126.68, 129.80, 131.53, 161.48. Mass spectrum (CIMS): m/z 254.

Synthesis of Tris[2-Perfluorohexylethyl]tin-benzyloxazoline (2.10). The procedure was adapted from that used by Milius *et al.*[31] To 215 mg (8.83 mmol) of magnesium turning was slowly added a solution containing 1.122 g (4.415 mmol) of

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[30] Hughes, A.B., Sargent, Melvyn, V. *J. Chem. Soc. Perkin. Trans.* **1989**, I, 1787.
[31] Milius, R.A.; McLughlin, W.H.; Lambrecht, R.M.; Wolk, A.P.; Carroll, J.J.; Adelstein, S.J.; Bloomer, W.D. *Appl. Radiat. Isot.* **1986**, 37, 799.

2.9 in 18 mL of dry THF. To the stirring mixture was added 1,2-dibromoethane (20 drops) and allowed to reflux for 1 h. This solution was added to a solution containing 547 mg (4.415 mmol) of **2.3** in 3 mL of FC-72® and 14 mL of benzotrifluoride. The reaction solution was stirred overnight at room temperature, and then concentrated under reduced pressure. The residue was extracted with three (3 mL) portions of FC-$72_{®}$ from dichloromethane and water. The combined FC-$72_{®}$ layers were re-extracted with dichloromethane and concentrated under reduced pressure to give **2.10** as a clear colorless oil: yield 528 mg (90 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32 (t, 8.2 Hz, 6 H), 1.40 (s, 6H), 2.30 (m, 6H), 4.14 (s, 2H), 7.44 (AA'BB', J=8.1 Hz, 2H), 7.97 (AA'BB', J=8.1 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ -1.25, 27.68 (t, $^{2}J_{F,C}$=23.4 Hz), 28.47, 67.71, 79.46, 128.36, 135.97. MS (ESMS): m/z 1394.2 $[M+OAc]^{+}$.

Tris[2-Perfluorohexylethyl]tin-3-ethylbenzoate (2.14). To a solution containing 8.523 g (6.879 mmol) of **2.3** in 10 mL of dry THF at 0 °C was slowly added 41.2 mL (20.6 mmol) of a 0.5 M 3-(ethoxycarbonyl)phenylzinc solution in THF. The solution was warmed to room temperature over 2 h and stirred overnight at r.t. The reaction solution was concentrated under diminished pressure. The residue was extracted with four 5 mL portions of FC-72® from 20 mL of methanol. The combined FC-$72_{®}$ layers were concentrated under reduced pressure and dried under high vacuum to give **2.14** as a colorless oil : yield 8.903 g (98.9 %). TLC R_f 0.58 (6 :1 hexane : diethyl ether). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (t, J=8.2 Hz, 6H), 1.39 (m, 3H), 2.33 (m, 6H), 4.39 (q, J=7.5 Hz, 2H), 7.49 (t, J=7.5 Hz, 1H), 7.57 (d, J=7.5 Hz, 1H), 8.05 (d, J=7.5 Hz, 1H), 8.07 (s, 1H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ -1.12, 14.20, 27.87 (t, $^{2}J_{F,C}$=23.3 Hz), 61.17, 108.92-118.84 ppm (m, CF_2, CF_3),

128.90, 129.54, 130.79, 131.13, 131.84, 136.06, 136.97, 137.34, 140.30, 143.46, 166.67. MS (ESMS, IPA 2mM NH_4OAc): m/z 1369.5 $[M+OAc]^-$, m/z=1279.4 $[M-OEt]^-$.

Tris[2-Perfluorohexylethyl]tin-3-benzoic acid (2.2). A mixture of 8.903 g (6.801 mmol) of **2.14** and 34 mL of 1N NaOH in 34 mL of methanol was refluxed for 24 h. Methanol was removed under diminished pressure and the residue was extracted with four 5 mL portions of FC-72®. The combined FC-72® layers were then extracted twice from 20 mL of dichloromethane and 10 mL of 1N HCl. The combined FC-72® layers were concentrated under diminished pressure to give 2.2 as a colourless oil : yield 8.584 g (98 %). After several days **2.2** crystallised as a white solid. Dissolving approximately 100 mg of **2.2** in 1 mL of pentane followed by slow evaporation over one week gave **2.2** as colourless needles. TLC R_f 0.21 (6 :1 hexane-diethylether). m.p= 57-58°C. ^{1}H NMR (200MHz, $CDCl_3$): δ 1.34 ppm (t, J=8.2 Hz with ^{119}Sn satellites $^2J_{Sn,H}$=53.4 Hz, 6H)), 2.31 (m, 6H), 7.51 (t, 7.4 Hz,1H), 7.62 (d, J=7.4 Hz, 1H), 8.11 (d, 1H), 8.12 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ -1.53, -1.06, 27.42 (t, $^2J_{F,C}$=24.4 Hz), 108.49-118.51 (m, CF_2, CF^3), 128.66, 129.02, 129.73, 130.01, 130.39, 131.08, 131.34, 134.00, 135.906, 136.16, 137.53, 141.00, 141.23, 172.61, 172.04. MS (ESMS, IPA): m/z 1279.1 $[M-H]^-$. IR (thin film): 3410, 2981, 2950, 1631, 1610, 1593 cm^{-1}.

General procedure: 3-Fluorobenzoic acid from F_2 reaction (2.15). To 0.191 g (0.149 mmol) of **2.2** in 1 mL of FC-72® at –85°C in a FEP tube was bubbled 118 μmol of 0.5 % F_2 in Ne (~23 mL). The F_2 was steadily released into the solution over 35 min. The reaction solution along with three 3 mL portions of methanol used to rinse the vessel were concentrated in a large vial. The residue was washed with three

3 mL portions of 50:50 acetonitrile :water and eluted down a conditioned fluorous reverse phase column (1 g) to give **2.15**. Yield 28.2 μmol (24 %). HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_8 reversed-phase column. A retention time of 4.22 min. consistent with the standard was produced when conditions: 50:50 water (0.2 % TFA):acetonitrile (0.2 % TFA), λ=280 nm, flow 1 mL/min. ^{19}F NMR (188.16 MHz, MeOH:$CHCl_3$) : δ -112.00 (pseudo-quartet, $^3J_{F,H}$=5.7 Hz). MS [ESMS, 1:1 IPA:(ACN:H_2O)]: m/z 139.1 $[M-H]^-$.

General procedure : [^{18}F]3-fluorobenzoic acid (2.16). To 0.124 g (97.2 μmol) of **2.2** in 1 mL FC-72® at –85 °C in a FEP tube was bubbled [^{18}F]F_2 (15-20 μmol) in Ne over 10 min. The reaction solution and two 2 mL portions of methanol used to rinse the vessel were combined and evaporated on a hot water bath under a stream of N_2. The residue was rinsed with three 3 mL portions of 50:50 acetonitrile : water and eluted down a fluorous reverse phase column (1 g). HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_8 reversed-phase column. A retention time of 4.18 min, consistent with the standard, was produced when conditions: 50:50 water (0.2 % TFA):acetonitrile (0.2 % TFA), λ=280 nm, flow 1 mL/min. The chromatogram using a γ detector produced a single peak with a retention time of 4.99 min, which is consistent with the delay times between instruments. ^{19}F NMR (188.16 MHz, CH_3CN:H_2O) : δ -110.10 (pseudo-quartet, $^3J_{F,H}$=7.2 Hz). MS [ESMS, 50:50 IPA:(ACN:H_2O)]: m/z 139.0 $[M-H]^-$.

3-Iodobenzoic acid (I_2 reaction) (2.17). To a mixture containing 0.127g (99.1 μmol) of **2.2** in 2 mL acetonitrile was added 1 mL (0.1 mmol) iodine in methanol. The reaction mixture was stirred for 16 hr and then quenched with a crystal of sodium

metabisulfite. The reaction was diluted with 2.5 mL of distilled deionized water and the total volume added to a fluorous column (3.9 g), pre-conditioned with 50:50 acetonitrile:water. The column was eluted with 25 mL of 50:50 acetonitrile :water to give **2.17** in solution. HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_8 reversed-phase column. A retention time of 9.90 minutes, which is consistent with a standard of 3-iodobenzoic acid, was observed when conditions: 80:20 water (0.1 % HFBA):acetonitrile, λ=254 nm, flow 1 mL/min. Alternatively, varying elution conditions: 80:20 water (pH=7.4):acetonitrile, λ=254 nm, flow 1 mL/min, resulted in elution of **2.17** at 2.9 minutes, also consistent with the authentic standard. MS (ESMS), m/z 246.9 $[M-H]^+$.

3-Iodobenzoic acid ($Na^{127}I$ reaction) (2.18). To a solution containing 5.4 mg (4.15 μmol) of **2.2** in 200 μL of methanol was added 4 μL (0.184 nmol) NaI in 0.1 N NaOH, followed by 2 μL of peracetic acid (32 % in acetic acid). The reaction was quenched at 2 h with 100 μL of a 10 % sodium metabisulfite solution and diluted to 1 mL with distilled deionized water. HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_{18} reversed-phase nucleosil column. HPLC analysis of a 100 μL aliquot gave a retention of 10.2 minutes, analogous to an authentic standard when conditions: 50:50 water (0.2 % formic acid):acetonitrile (0.2 % formic acid), λ=254 nm, flow 1 mL/min.

[^{125}I]3-Iodobenzoic acid ($Na^{125}I$ reaction) (2.19 – no impurities). To a solution containing 1.4 mg (1.07 μmol) of **2.2** in 200 μL of methanol was added 5 μL (32 μCi) $Na^{125}I$ in 0.01 N NaOH, followed by 2 μL of peracetic acid (32 % in acetic acid). The reaction was stirred for 47 min followed by quenching with 20 μL of a 10

% solution of sodium metabisulfite and dilution with 300 μL of distilled-deionized water. HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_{18} reversed-phase nucleosil column. HPLC analysis of a 20 μL aliquot gave a retention time of 16.91 min on the chromatogram using the γ detector. There was no visible UV absorbance other than the solvent front. The retention time was consistent with an authentic standard of 3-iodobenzoic acid when conditions: 50:50 water (0.2 % formic acid):acetonitrile (0.2 % formic acid), λ=254 nm, flow 0.5 mL/min.

The solution was diluted with 1 mL of distilled deionized water and eluted through a Waters C_{18} Sep-Pak previously conditioned with water. The column was eluted with an additional 1.5 mL of distilled deionized water and the combined fractions showed an activity of 3 μCi. The column was then washed with 2 mL of HPLC grade acetonitrile and released 23 μCi of activity. An additional washing of the column with 1 mL of acetonitrile resulted in only 1 μCi of activity being released. The remaining activity was found in the Sep-Pak (4 μCi) and original reaction vessel (1 μCi). HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_{18} reversed-phase nucleosil column. HPLC analysis of a 20 μL aliquot gave a retention of 16.586 min on the γ detector and no visible UV peak. The retention time was consistent with an authentic standard of 3-iodobenzoic acid when conditions: 50:50 water (0.2 % formic acid):acetonitrile (0.2 % formic acid), λ=254 nm, flow 0.5 mL/min.

Modification of the elution conditions: 100 % acetonitrile, λ=254 nm, flow 1 mL/min resulted in a peak at 4.458 min on the γ detector and two peaks at 6.379 min

and 6.720 min on the UV chromatogram. These two peaks have a similar retention time as **2.2**, 6.613 min, under similar elution conditions.

The acetonitrile solution (approx. 2 mL) was diluted with 2 mL of distilled deionized water and passed down a Fluorous technologies® Sep-Pak. A total of 9 μCi was released in the eluting volume. Washing the column with an additional 4 mL of (50:50) acetonitrile:water yielded a total 19 μCi when combined with the previous fraction. No additional activity was found in either the Fluorous Sep-Pak or previous vial. HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_{18} reversed-phase nucleosil column. HPLC analysis of a 20 μL aliquot gave a small peak at 6.532 min on the UV chromatogram when conditions: 100 % acetonitrile, λ=254 nm, flow 1 mL/min.

Tris[2-Perfluorohexylethyl]tin-3-benzamide (2.21). To a reaction solution containing 294 mg (226 μmol) of **2.2** in 2.5 mL of DMF was added 0.130 g (344 μmol) of HBTU, followed by 90 μL (517 μmol) diisopropylethylamine (DIPEA). The reaction solution was stirred for 5 min prior to addition of 251 μL (2.29 mmol) of *N,N*-dimethylethylenediamine and 400 μL (2.30 mmol) of DIPEA. The reaction solution was then stirred for 16 h. The solution was diluted with 20 mL of water and extracted into 50 mL of dichloromethane and 10 mL of FC-72®. The FC-72® layer was re-extracted with three additional 10 mL portions of dichloromethane. The combined organic layers were re-extracted with 20 mL of water prior to concentration under reduced pressure to give **2.21** as a dark orange oil: yield 227 mg (74 %). TLC R_f 0.00 (6 :1 hexane : diethyl ether).^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.31 (t, J=8.1 Hz with ^{119}Sn satellites $^2J_{Sn,H}$=54.8 Hz, 6H) 2.31 (bs, 6H), 2.33 (m, 6H), 2.59 (t, J=5.7

Hz, 2 H), 3.55 (q, 2H), 7.14-7.90 (m, 4H). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ -1.43, 27.55 (t, $^2J_{F,C}$=23.4 Hz), 37.11, 44.87, 57.75, 104.80-120.03 (m, CF_2, CF_3), 127.04, 127.71, 128.66, 134.70, 134.82, 136.01, 137.53, 138.69, 167.16, 167.41. IR (thin film): 3338, 2950, 2831, 1650 cm^{-1}. MS (ESMS), m/z 1353.0 $[M+H]^+$.

3-Iodobenzamide (I_2 reaction of 2.21) (2.20). To a solution containing 3.2 mg (2.37 μmol) of **2.21** in 200 μL methanol was added 30 μL (3.0 μmol) of 0.1 M iodine. The reaction solution was stirred for 1 h prior to quenching with 100 μL of a 10 % solution of sodium metabisulfite. The solution was diluted with 700 μL of distilled-deionized water and analysed on a nucleosil C_{18} reversed-phase column. A retention time of 16.6 min and 18.9 min was observed when conditions: 80:20 H_2O (0.01 M NaH_2PO_4):acetonitrile, λ=254 nm, flow 2 mL/min. MS (ESMS), m/z 319 $[M+H]^+$.

3-Fluorobenzamide (F_2 reaction of 2.21). To 180 mg (133 μmol) of **2.21** in 1 mL of FC-72® at –90 °C in a FEP tube was bubbled 131 μmol of 0.5 % F_2 in Ne. The F_2 was steadily released into the solution over 25 min. The reaction solution along with two 3 mL portions of FC-72® used to rinse the vessel were concentrated in a large vial. The residue was washed with three 3 mL portions of acetonitrile and eluted down a conditioned fluorous reversed-phase column (1 g). MS (ESMS), m/z 211.1 $[M+H]^+$, 193.1 $[M–F+H]^+$.

Chapter 3

Synthesis of Benzylamine and Derivatives

3.1 Introduction

With the success attained at producing labelled benzoic acid and derivatives, we sought to expand the fluorous synthesis method to benzylamines and related derivatives (**Figure 3-1**). This would provide a complementary nucleophilic derivative to the electrophilic halobenzoic acids described in Chapter 2. In addition, it would expand the potential variety of compounds which could be coupled to the fluorous "tag" and then radiolabelled.

Derivatives of benzylamine have been used to label biomolecules,[1] and are precursors to the synthesis of [^{131}I] and [^{123}I] *meta*-iodobenzylguanidine (MIBG),[2] which is a valuable but synthetically challenging radiopharmaceutical.

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[1] Kuhnast, B; Dolle,F; Terrazzino, S.; Rousseau, B.; Loc'h, C.; Vaufrey, F.; Hinnen, F.; Doignon, I.; Pillon, F.; David, C; Crouzel, C.; Tavitian, B. *Bioconjugate Chem.* **2000**, *11*, 627.

[2] Wafelman, A.R.; Konings, M.C.P; Hoefnagel, C.A.; Maes, R.A.A.; Beijnen, J.H. *Appl. Radiat. Isot.* **1994**, *10*, 997.

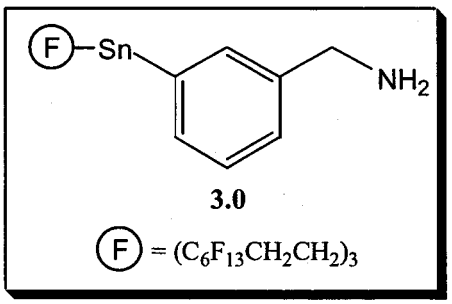

Figure 3-1. Tris(perfluorohexylethyl)tin-3-benzylamine (**3.0**)

3.2. Synthesis of fluorous "tagged" benzylamine (**3.0**)

There are scarce examples in the literature describing the synthesis and/or labelling of trialkyltin bound benzylamine. Vaidyanathan *et al.* synthesised 3-(tri-n-butylstannyl)benzylamine in a 30 % yield using n-BuLi, 3-bromobenzylamine, and a two-fold excess of tributyltin-chloride.[3] This approach was not considered for the synthesis of **3.0**, due to the poor yield obtained and the generation of a large excess of fluorous by-products. Rather, a method reported by Hunter *et al.* for the preparation of a polymer bound 3-benzylamine was adapted for the synthesis of **3.0**.[4]

Hunter's method utilised the precursor, **3.1**, an azadisilolidine protected derivative of 3-bromobenzylamine. This silicon-based protecting group is stable to n-BuLi, allowing for the synthesis of the corresponding monolithium salt, **3.2**.

Synthesis of **3.1** entailed the reaction of 3-bromobenzylamine in triethylamine with 1,1,4,4-tetramethyl-1,4-dichlorosilethylene at room temperature for 1.5 hours (**Figure 3-2**). Pouring the crude solution into aqueous sodium dihydrogen phosphate, followed by

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[3] Vaidyanathan, G.; Zalutxky, M.R.; DeGrado, T.R. *Bioconjugate Chem.* **1998**, *9*, 758.
[4] Hunter, D.H.; Zhu, Xizhen. *J. Labeled Compd. Radiopharm.* **1999**, *42*, 653.

distillation of the crude organic extract, provided the product in moderate yield (64 %). The ^{1}H NMR spectrum of compound **3.1** revealed signals at 0.00 ppm (s, 12H, C**H**$_3$), 0.78 ppm (s, 4H, C**H**$_2$-C**H**$_2$), and 4.06 ppm (s, 2H, Ar-C**H**$_2$-N), in addition to the aromatic peaks appearing from 7.20-7.48 ppm (m, 4H). The ^{13}C NMR spectrum of **3.1** had resonances at –0.26 ppm (**C**H$_3$) , 8.01 ppm (**C**H$_2$-**C**H$_2$), 45.59 ppm (Ar-**C**H$_2$-N), and those corresponding to the aromatic carbons. The electron impact mass spectrum of **3.1** gave a peak at m/z=312. These spectra are consistent with data reported in the literature.[4,5]

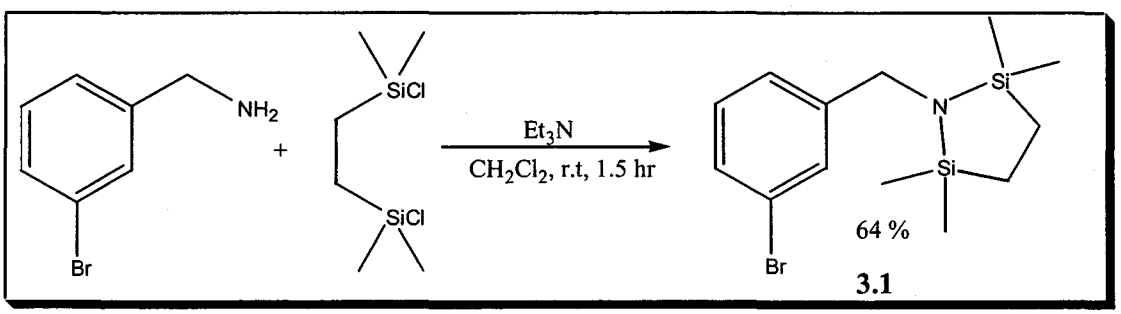

Figure 3-2. Precursor synthesis: silicon protected 3-bromobenzylamine (**3.1**)

The synthesis of **3.3** (**Figure 3-3**) involved reaction of **3.1** with n-BuLi in THF at –78°C for a period of 35 minutes to generate **3.2**. Compound **2.3** in THF was then added to **3.2** dropwise. The reaction was kept at –78°C for 2 hours, where upon FC-72® was added and the mixture stirred for 10 minutes. The reaction was subsequently quenched through the addition of methanol (30 mL). Following the addition of methanol, the reaction was extracted with FC-72®, water, and dichloromethane. The FC-72® was removed on the rotary evaporator, providing **3.3** in 89 % yield.

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[5] Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Letters*. **1981**, *22*, 1787.

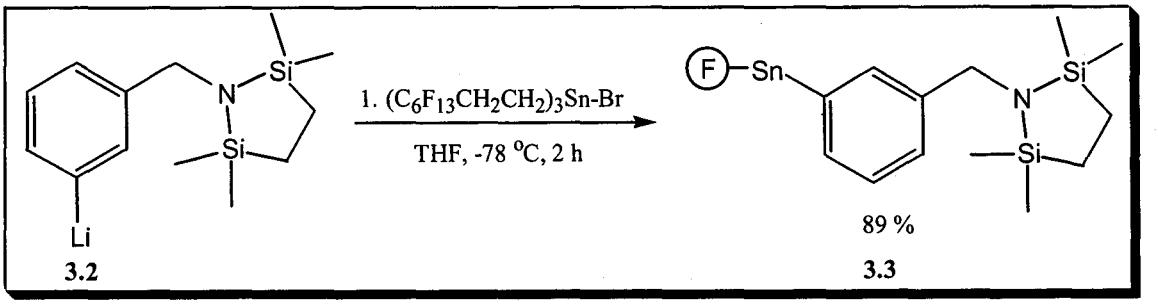

Figure 3-3. Synthesis of azadisilolidine protected perfluorotin-3-benzylamine (**3.3**)

Hydrolysis of **3.3** (**Figure 3-4**) involved stirring the compound overnight in methanol with sufficient 1 M HCl to give a pH≈3. The product was extracted into FC-72®, and concentrated to give **3.0** as a light yellow oil in 97% yield.

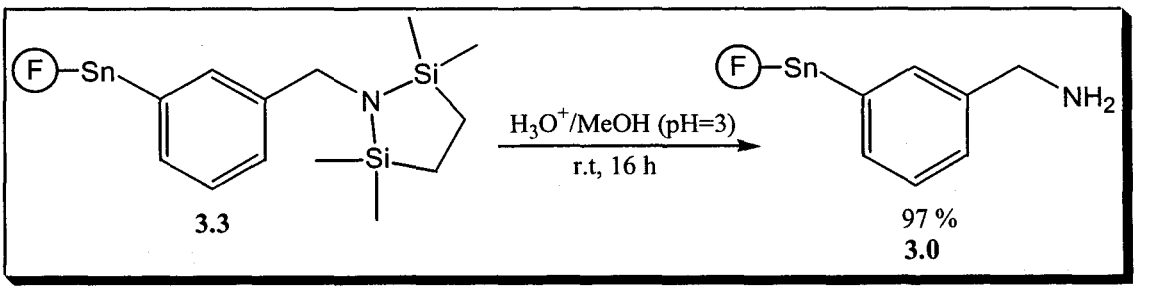

Figure 3-4. Hydrolysis of the silicon protecting group to generate **3.0**.

The ^{1}H NMR spectrum of compound **3.0** (**Figure 3-5**) showed the expected shift in the signals for the protons positioned α and β to tin, to 1.31 ppm (t, J=8.3 Hz with ^{119}Sn satellites $^2J_{Sn,H}$= 54.2 Hz, 6H), and 2.31 ppm (m, 6H) respectively. The signals corresponding to benzylamine appeared at 3.88 ppm (s, Ar-C**H**$_2$-N, 2H), in addition to aromatic peaks from 7.22-7.46 ppm (m, 4H). Trace amounts of the silicon protecting group can be seen in the baseline from 0.1-0.2 ppm. The ^{13}C NMR spectrum showed the appropriate shift in the signal corresponding the α carbon to –1.37 ppm ($^1J_{Sn,C}$= 347 Hz),

in addition to the signal for the β carbon at 27.94 ppm (t, $^2J_{F,C}$=23.4 Hz), and benzyl carbon at 46.62 ppm (Ar-CH_2-N). The multiplets corresponding to carbon atoms bonded to fluorine were seen from 106.17-121.17 ppm, and the peaks associated with the aromatic region have yet to be definitively assigned. The positive ion electrospray mass spectrum of compound **3.0** (**Figure 3-6**) shows a single peak at m/z=1268.5 $[M+H]^+$. The IR spectrum showed strong absorbances corresponding to sp^3 and sp^2 C-H stretches at 2850 and 2955 cm^{-1}, and for the primary amine at 3354 cm^{-1}. These results are all consistent with formation of the desired product.

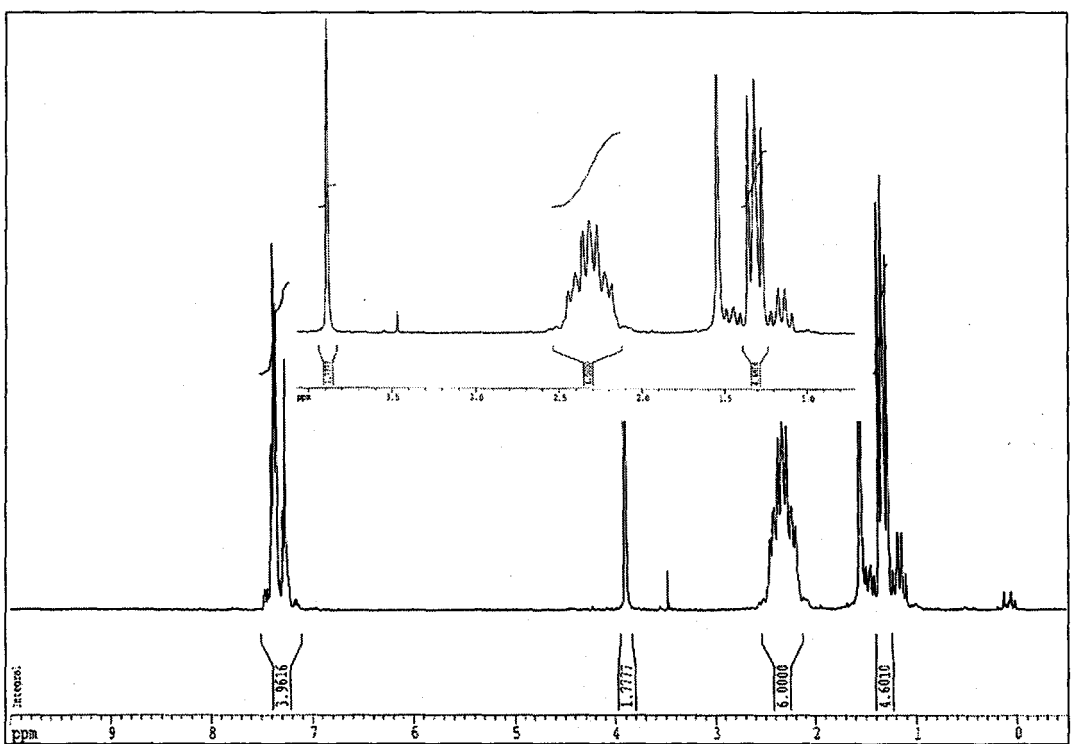

Figure 3-5. ^1H NMR [$CDCl_3$, 200 MHz] spectrum of compound **3.0**

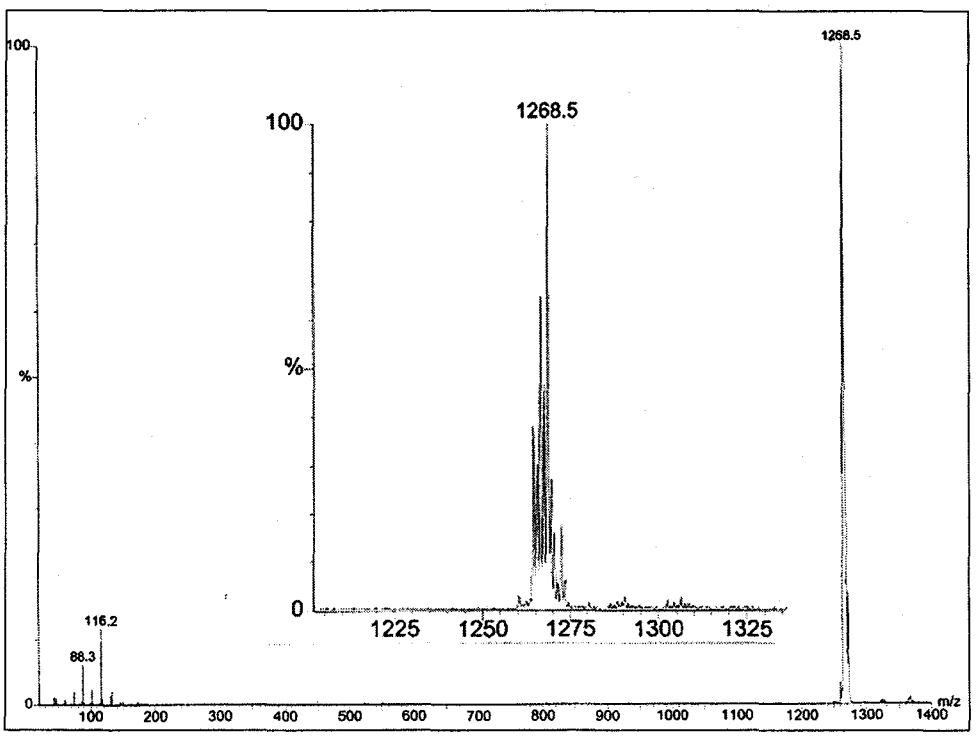

Figure 3-6. Positive ion electrospray mass spectrum of compound **3.0**

The quantitative conversion of the stannylbromide precursor (**2.3**) to **3.3** proved extremely difficult. Early on it was appreciated that the azadisilolidine protected 3-bromobenzylamine (**3.1**) was not particularly stable. Synthesis and purification of **3.1** had to be immediately followed by reaction with n-BuLi to generate **3.2**. If these measures were not taken, incomplete conversion of **2.3** would result.

Hunter and coworkers reported that reaction of **3.2** with the chlorostannane polymer for 7 hours at –78°C, followed by stirring at room temperature for 2 hours, resulted in quantitative functionalization of Sn-Cl bonds.[4] In contrast with these results, it was found that under similar reaction conditions only 50-67% of Sn-Br sites were converted to product (**3.3**). Through extensive trials it was appreciated that the product was extremely prone to decomposition if the reaction solution was allowed to warm to room

temperature. The complete conversion of **2.3** to **3.3,** therefore, could only be facilitated if the reaction was kept at –78°C, prior to immediate extraction into FC-72® and quenching with methanol.

3.2.1 Synthesis of Iodobenzyamine

Iododinolysis of **3.0** was carried out, in order to further characterise the product bound to the fluorous tag and to ensure its purity (**Figure 3-7**). Compound **3.0** was reacted with an excess of iodine in acetonitrile overnight, followed by quenching with sodium metabisulfite. The solution was diluted with water and passed down a conditioned fluorous column with an acetonitrile:water eluent (50:50). Aliquots (3 x 5 mL) were collected and the products characterised using HPLC and mass spectrometry.

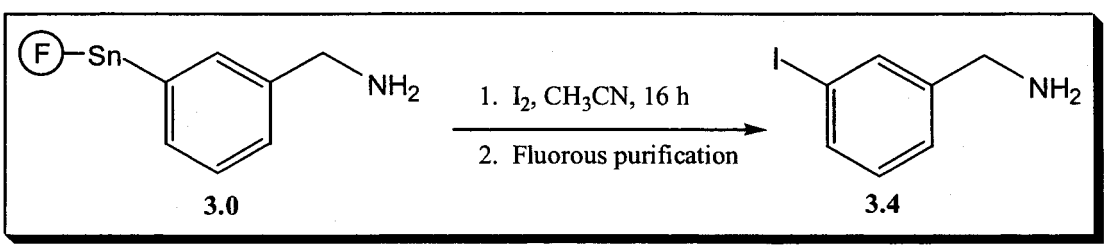

Figure 3-7. Iododestannylation of **3.0**

The HPLC chromatogram (**Figure 3-8**) of the reaction mixture (**3.4**) following fluorous purification showed three peaks at 1.78, 3.37, and 6.46 minutes. The peak at 1.78 min and 6.46 min correspond to the solvent front and 3-iodobenzylamine, respectively, while the peak at 3.37 min has not yet been identified. An authentic standard of 3-iodobenzylamine under similar elution conditions produced a peak at 6.47 minutes. The positive ion electrospray (**Figure 3-9**) mass spectrum of the reaction

mixture produced a peak at m/z=233.9 $[M+H]^+$, with no evidence of the fluorous impurity at approximately m/z>1200. These results are consistent with formation of **3.4**.

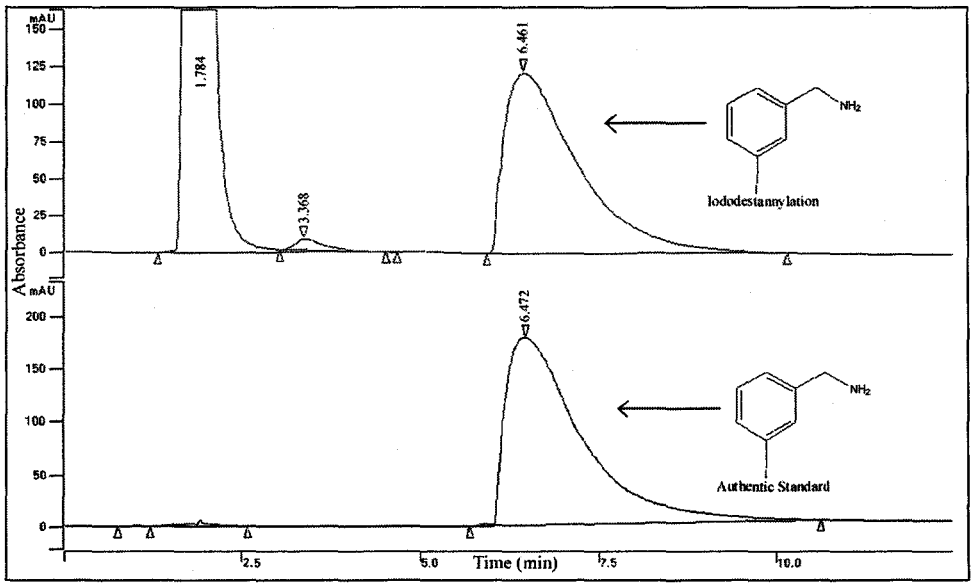

Figure 3-8. HPLC chromatogram of authentic standard (lower) and crude reaction mixture (**3.4**) (conditions: C_8 analytical (Microsorb-MV, 250 x 4.6 mm), 80:20 H_2O(pH≈7.4):ACN, flow 1.5 mL/min)

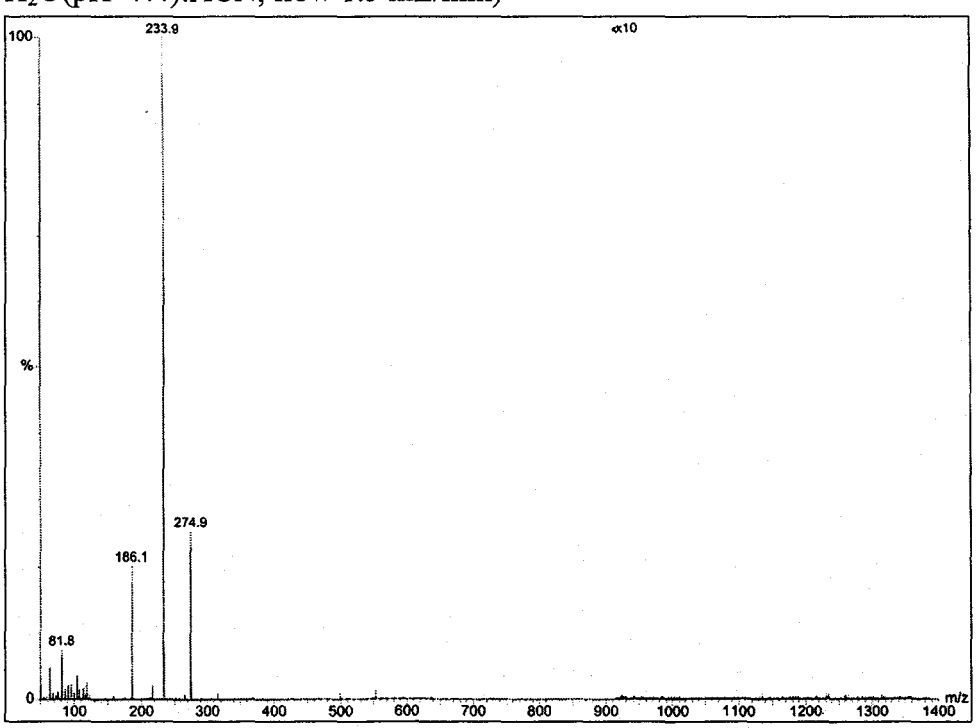

Figure 3-9. Positive ion electrospray mass spectrum of the crude reaction mixture (**3.4**)

3.3 Meta-iodobenzylguanidine

During the past two decades, radioiodinated MIBG (m-iodobenzylguanidine) has been used extensively in nuclear medicine.[6] It is used primarily for diagnostic scintigraphy and therapy of neural crest tumours such as phaeochromocytoma and neuroblastoma.[7] In addition, it is increasingly being used to assess the status of adrenergic nerves in the heart muscle.[6]

The most widely employed synthesis method for production of [^{123}I] or [^{131}I] MIBG involves the Cu^{+} catalyzed exchange process. Unfortunately, this method yields a low specific activity product (14 Ci/mmol for [^{123}I]) necessitating an increased dose, which in turn results in poorer quality images.[4] Consequently, several routes to a no-carrier-added product have been investigated; however, none have found widespread application.[8]

A fluorous strategy for the synthesis of MIBG may ameliorate the aforementioned synthetic limitations. Furthermore, if a convenient labelling method were available, there is substantial interest in generating a positron emitting MIGB-related radiopharmaceutical. For example, Zalutsky *et al.* synthesised meta-[^{18}F]fluorobenzylguanidine and para-[^{18}F]fluorobenzylguanidine in three steps with a fluoro for nitro exchange reaction. They reported lower than desirable radiochemical yields of 10-15 % ([^{18}F]MFBG) and 50-55 % [^{18}F]PFBG, and difficulty removing impurities.[9] The next section describes the development of a fluorous strategy towards the eventual preparation of [*I]MIBG and [^{18}F]MFBG.

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[6] Amartey, J.K.; Al-Jammaz, I.; Lambrecht, R.M. *Appl. Radiat. Isot.* **2001**, *54*, 711.
[7] Vaidyanathan, G.; Affleck, D.J.; Zalutsky, M.R. *Bioconjugate Chem.* **1996**, *7*, 102.
[8] Vaidyanathan, G.; Zalutsky, M.R. *Appl. Radiat. Isot.* **1993**, *3*, 621.
[9] Grag, P.K.; Garg, S.; Zalutsky, M.R. *Nucl. Med. Biol.* **1994**, *21*, 97.

3.3.1 Synthesis of tris(perfluorohexylethyl)tin-3-benzylguanidinium (3.5)

In order to produce tris(perfluorohexylethyl)tin-3-benzylguanidine, **3.5,** several synthetic routes were attempted. The first approach, approach **A** (**Figure 3-10**), applied the method developed by Wieland *et al.* for synthesis of **3.5**.[10] Wieland's method involves the reaction of m-iodobenzylamine with cyanimide at 100°C for 4 hours. Unfortunately, the synthesis of **3.5** through various adapted procedures would only yield trace amounts of the product, as indicated by electrospray mass spectrometry.

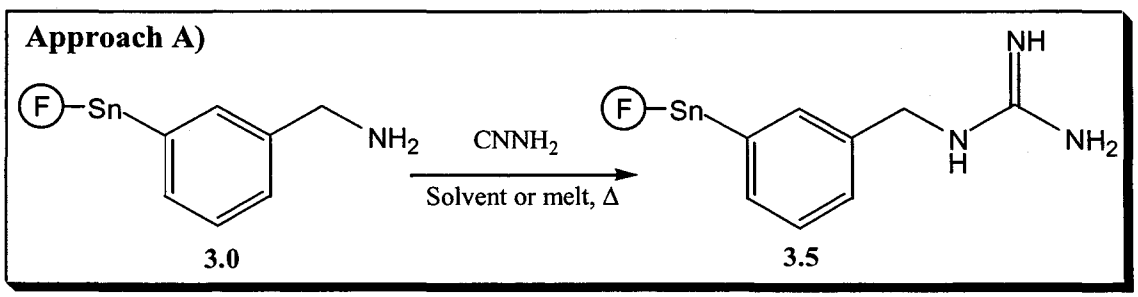


Figure 3-10. Attempted synthesis of **3.5** using cyanimide

The failure of this reaction method to generate **3.5** is likely a result of the precursor **3.0** not being protonated. Although hydrolysis of the silicon-protecting group to generate **3.0** occurred at a pH of 3, the expected benzylammonium chloride was not formed. The benzylammonium chloride is necessary in order to activate cyanimide to nucleophilic attack (**Figure 3-11**).

[10] Wieland, D.M.; Wu, J-I.; Brown, L.E.; Mangner, T.J.; Swanson, D.P.; Beirwaltes, W.H. *J. Nucl. Med.* **1980**, *21*, 349.

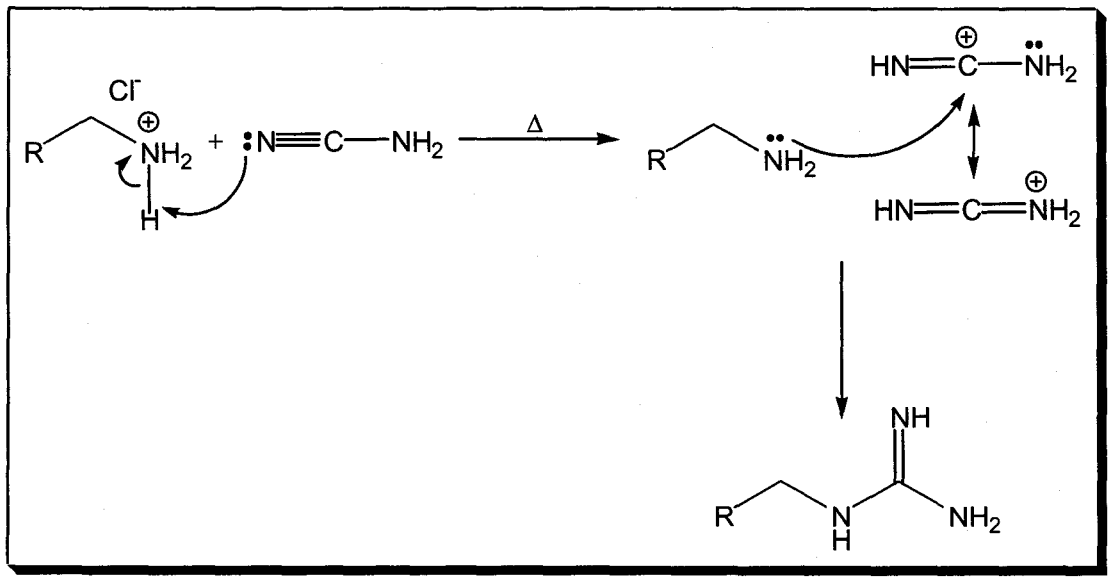


Figure 3-11. Mechanism of guanidine formation with cyanimide

Any further attempts at protonating **3.0** resulted in protodestannylation. Similarly, the addition of catalytic amounts of HCl (0.05 eq) resulted in protodestannylation under the reaction conditions (54°C). These results mirror the findings of Vaidyanathan *et al.*, who were unable to convert 3-(tri-*n*-butylstannyl)benzylamine to the guanidine.[8] Rather, they were forced to synthesise [^{131}I]MIBG from radioiododestannylation of (trialkylstannyl)benzylamine, followed by its subsequent reaction with cyanimide. Approach **B** entailed the adaptation of research by *Jursic et al.* for their preparation of *N*-formamidinylamino acids.[11] Here, the reaction of formamidinesulfinic acid [$HN=C(NH_2)SO_2H$] with a substituted amino acid (*D,L*-phenylalanine) in aqueous sodium hydroxide leads to the generation of *D,L-N*-formamidinephenylalanine (**Figure 3-12**).

[11] Jursic, B.S.; Neumann, D.; McPherson, A. *Synthesis*. **2000**, *12*, 1656.

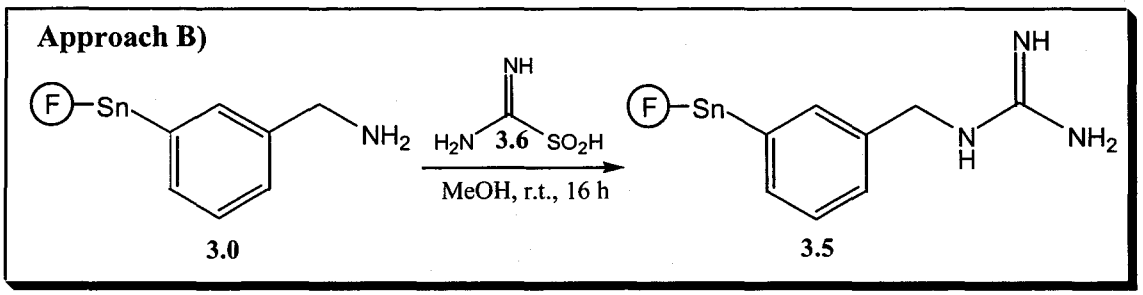


Figure 3-12. Attempted synthesis of **3.5** using foramidinesulfinic acid

Application of this approach towards **3.5** was found to be most successful when **3.0** was stirred with 2.0 equivalents of foramidinesulfinic acid in methanol overnight at room temperature. The methanol was removed on the rotary evaporator, prior to a triphasic extraction. The white viscous oil obtained following removal of FC-72® was heated in chloroform and subsequently decanted to remove any unreacted **3.0**. The product, a viscous white oil, was obtained in good yield (86%). The positive ion electrospray mass spectrum of compound **3.5** (synthesised using foramidinesulfinic acid) showed a peak at m/z=1310.2 $[M+H]^+$, in addition to peaks at m/z=1325.1 and m/z=1293.1 (**Figure 3-13**). The ^1H NMR spectrum and ^{13}C NMR spectrum for compound **3.5** could not be acquired, as no suitable solvent could be found.

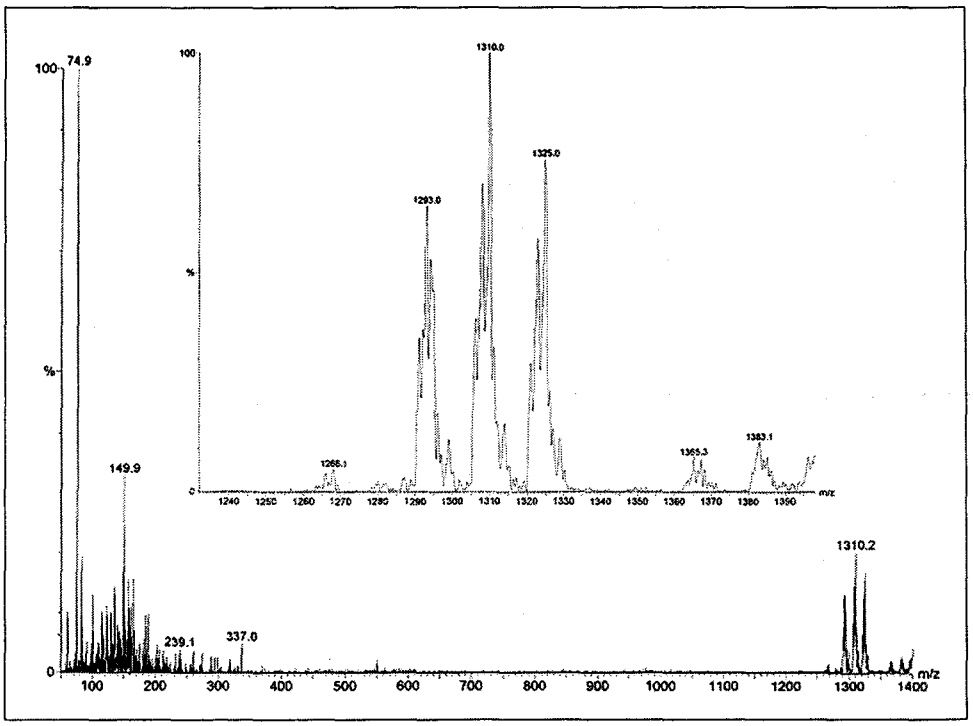


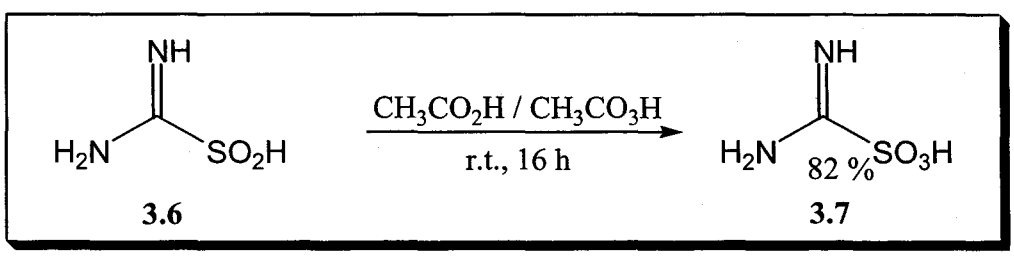
Figure 3-13. Positive ion electrospray mass spectrum of compound **3.5** (Appro.**B**)

Compound **3.5** was treated with cold I_2 and F_2, and a similar peak pattern in the electrospray mass spectrum was found for the cleaved products. The peak associated with the product was typically the most intense, flanked on either side with a peak of +/- 15 mass units. As the resulting peak pattern could not be rationalised, other routes to the synthesis of **3.5** were investigated.

3.3.2 Approach C

Approach **C** involved adaptation of the research by Mosher *et al.*, who converted several primary amines to the corresponding guanidines.[12] The conversions were accomplished by reacting aminoiminomethanesulfonic acid with a primary amine for two hours at room temperature to generate the corresponding guanidine in moderate yield (22-80 %). This method appeared applicable for the synthesis of **3.5,** as a free amine could be converted to the guanidine under mild conditions (pH=3.1).

Aminoiminomethanesulfonic acid (H_2N-C(=NH)SO_3H) (**3.7**) was synthesised in high yield through reaction of foramidinesulfinic acid (**3.6**) with peracetic acid, following the procedure of Mosher (**Figure 3-14**).[12] The melting point of compound **3.7** was consistent with literature findings of 125-126 °C.[12]



3.6 **3.7**

Figure 3-14. Synthesis of aminoiminomethansulfonic acid (**3.7**)

Compound **3.7** was first reacted with m-iodobenzylamine in order to assess the products formed and to obtain a standard sample of MIBG (**Figure 3-15**). Equivalent molar quantities of **3.7** and **3.8** were combined in methanol and refluxed overnight. The resulting product (**3.9**) was characterised without further purification. The ^{1}H NMR

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[12] Kim, K.; Lin, Y-T.; Mosher, H.S. *Tetrahedron Lett.* **1988**, *29*, 3183.

spectrum revealed a singlet at 4.22 ppm, and signals corresponding to the benzylic protons between 6.90-7.56 ppm. The ^{13}C NMR spectrum showed the appropriate peak pattern, with signals at 48.9 ppm (Ar-CH_2-N), 99.3 ppm (N-C=N), and those of the aromatic ring. The positive ion electrospray mass spectrum showed a peak at m/z=276.1 $[M+H]^+$ corresponding to **3.9,** and an extremely small peak at m/z=233.9 $[M+H]^+$ corresponding to **3.8**. The HPLC analysis of the crude reaction **3.9** generated principle peak at t_R=24.54 minutes (86 % of total peak area). This data is consistent with literature reports.[13]

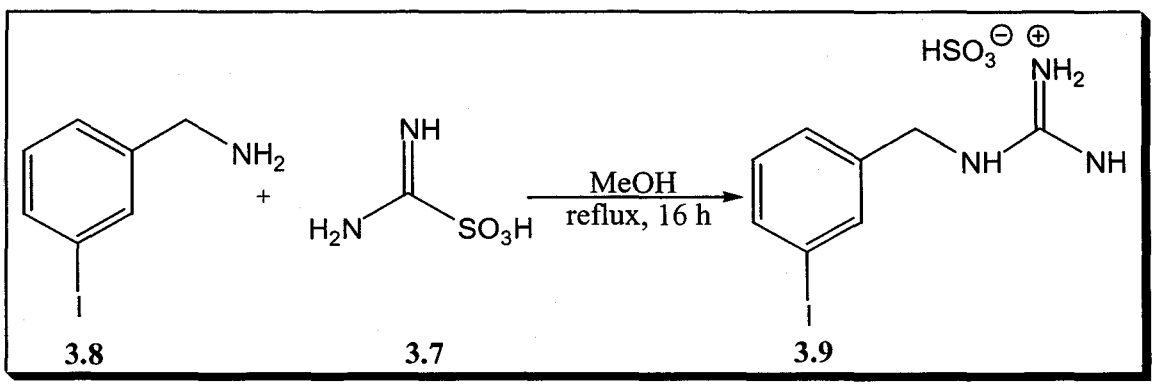

Figure 3-15. Synthesis of standard m-iodobenzylguanidine (**3.9**)

The synthesis of compound **3.9** using **3.7** prompted the application of this procedure toward the synthesis of **3.5** (**Figure 3-16**, Approach **C**). Compound **3.0** was combined with 1.1 equivalents of **3.7** in methanol and refluxed overnight. Incomplete conversion occurred if the reaction was carried out at room temperature as suggested by Mosher *et al.*[12] Extraction of the crude reaction mixture into FC-72® from methanol generated what is presumably the product as a milky white oil in acceptable yield (88 %).

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[13] Wafelman, A.R.; Konnings, M.C.P.; Hoefnagel, C.A.; Maes, R.A.A.; Beijnen, J.H. *Appl. Radiat. Isot.* **1994**, *45*, 997.

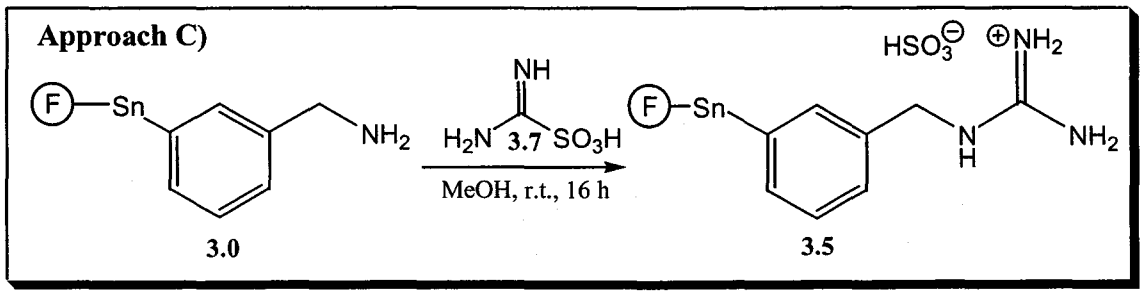


Figure 3-16. Attempted synthesis of compound **3.5** following approach **C**

Positive ion electrospray mass spectrometry (**Figure 3-17**) showed a peak at m/z=1309.9 $[M+H]^+$, which is consistent with the formation of **3.5**. The electrospray spectrum did not show any peaks that were associated with the precursor (**3.0**), which had a m/z value of 1268, or peaks corresponding to m/z+/-15, which had been seen using approach **B**. ^1H NMR and ^{13}C NMR spectra for compound **3.5** were not obtained.

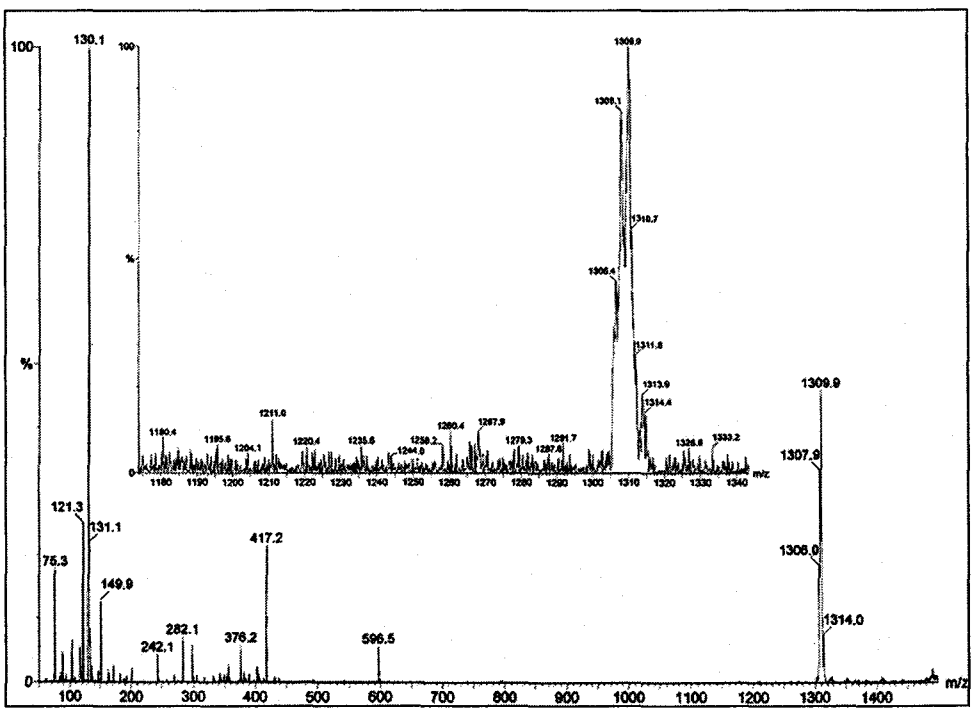


Figure 3-17. Positive ion electrospray mass spectrum of **3.5** via Approach **C**

3.4 Synthesis of labelled MIBG (3.10)

The cold iodination of **3.5** was undertaken in order to assess the products and reaction conditions for eventual use of $Na^{125}I$ (**Figure 3-18**). A sample of **3.5** (3.90 μmol), synthesised through approach **C**, was dissolved in methanol. To the stirring solution was added NaI (4.6×10^{-7} mmol), which was followed promptly by addition of the peracetic acid oxidant. The reaction was stirred for 2 hours and then quenched with 100 μL of a 10 % sodium metabisulfite solution. Purification of the dilute reaction solution was not attempted, though it has been established that fluorous material can easily be removed from the cleavage products.

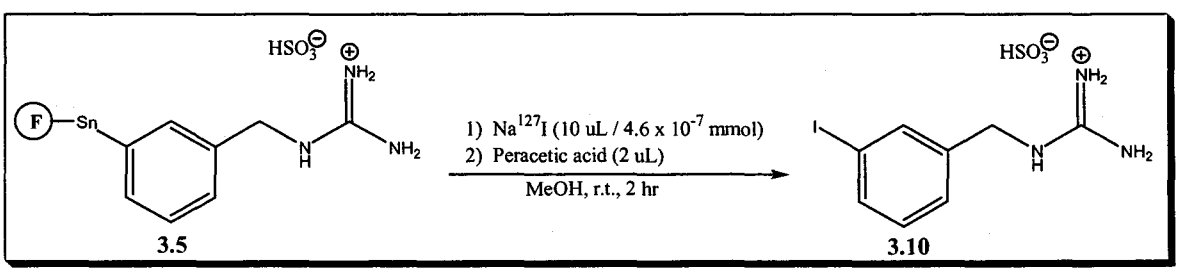


Figure 3-18. Cold labelling of MIBG with NaI (**3.10**)

The positive ion electrospray mass spectrum of the crude reaction mixture (**3.10**) revealed a peak at m/z=275.9 $[M+H]^+$ which is consistent with the product (**Figure 3-19**). HPLC analysis of a 100 μL aliquot of the crude reaction mixture (**3.10**) showed peaks with retention times of 7.2, 14.7, and 24.9 minutes (**Figure 3-20**). The peaks eluting at 7 minutes and 24.9 minutes were assigned to the solvent front and product **3.10**, respectively. The standard sample of MIBG eluted with a similar retention time of 24.5 minutes. The peak at 14.7 minutes accounted for <1 % of total MIGB and the nature of the compound giving rise to the peak remains unknown.

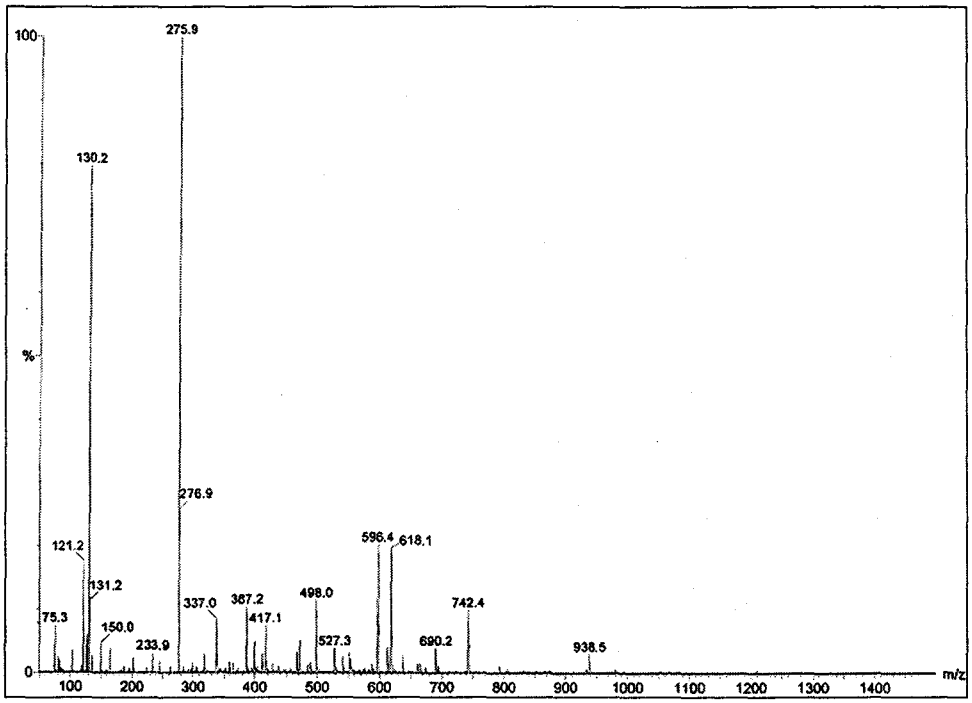


Figure 3-19. Positive ion electrospray mass spectrum of the crude reaction (**3.10**)

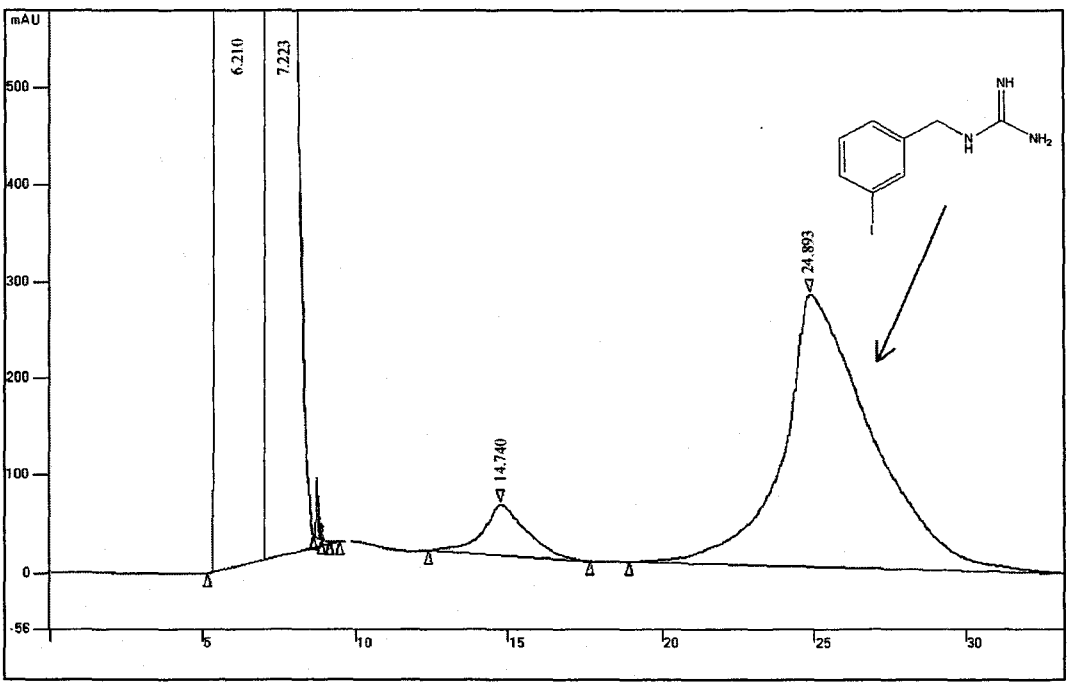
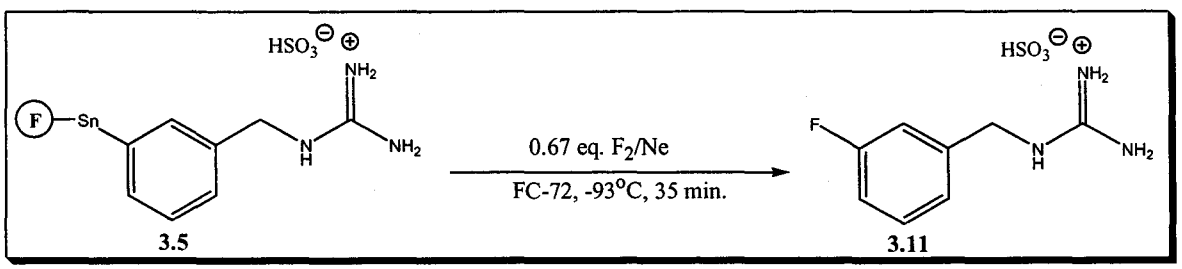


Figure 3-20. HPLC chromatogram of the crude reaction mixture (**3.10**) (conditions: C_{18} analytical (Nucleosil, 250 x 4.6 mm), 80:20 H_2O (0.01 N NaH_2PO_4):ACN, flow 2 mL/min)

3.5 Synthesis of MFBG (3.11)

The encouraging results for the iodine labelling of **3.5** prompted us to investigate the possibility of synthesising m-fluorobenzylguanidine (MFBG). The fluorodestannylation reaction for the synthesis of MFBG (**3.11**) is shown in **Figure 3-21**. The cold fluorination reaction of compound **3.5** proceeded in a manner analogous to those of previous reactions (3-fluorobenzoic acid and 3-fluorobenzamide). To an FEP tube containing **3.5** dissolved in FC-72® at –93°C was bubbled approximately 0.7 equivalents of F_2 (0.6% F_2 in ≈ 23 mL Ne). Following the reaction, the FC-72® from the reaction along with methanol used to rinse the vessel were removed on rotary evaporator, prior to diluting with acetonitrile:water (50:50) and eluting down a conditioned fluorous column.



3.5 3.11

Figure 3-21. Synthesis of MFBG (**3.11**)

The positive ion electrospray mass spectrum for the crude reaction (**3.11**) showed a peak at m/z=168.0 $[M+H]^+$, which is consistent with product formation (**Figure 3-22**). The mass spectrum showed no evidence of any fluorous impurity at m/z >1000 or the possible impurity, 3-fluorobenzylamine, at m/z=126 $[M+H]^+$.

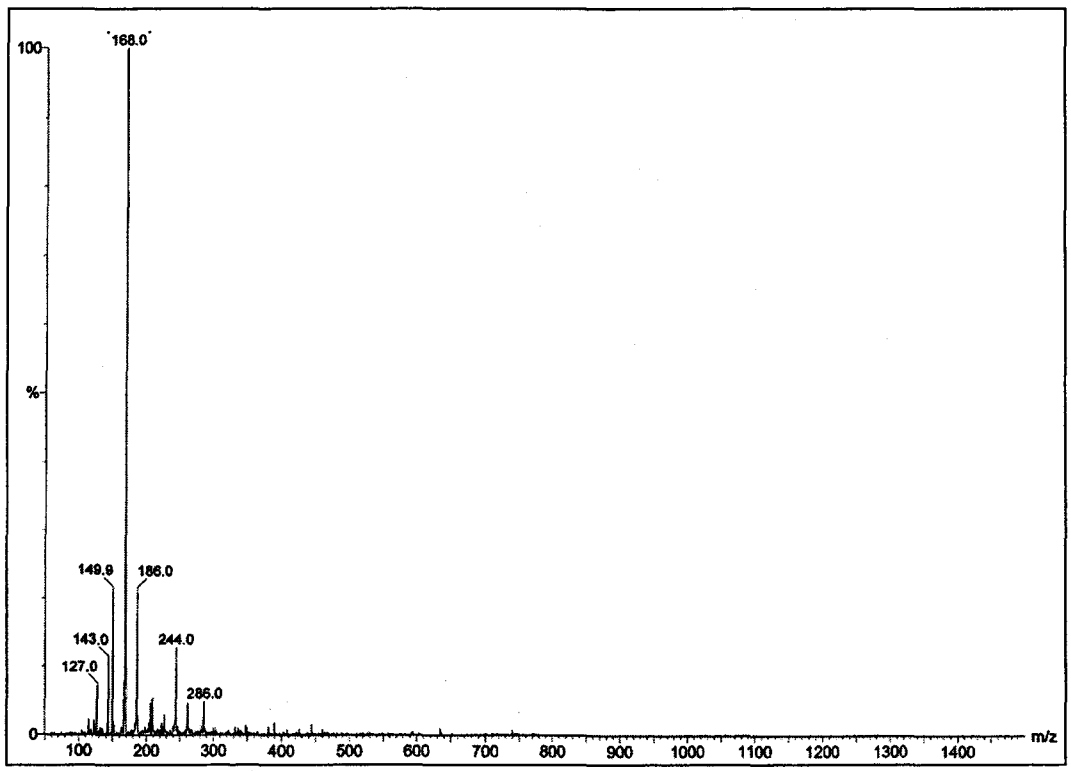


Figure 3-22. Positive ion electrospray mass spectrum of the crude reaction mixture (**3.11**)

The HPLC chromatogram of the reaction mixture (**3.11**) contains peaks at the solvent front (t_R=2-6 min.) and peaks eluting at 25.3 min., 30.3 min., and 35.0 minutes (**Figure 3-23**). There are no peaks corresponding to 3-fluorobenzylamine, which has a retention time of 15.8 minutes under these elution conditions. The elution conditions are the same as those used for MIBG, and it is therefore surprising that the principle peak (61+ %) eluting at 35 minutes is more highly retained than MIBG. The longer retention time might suggest a di-fluorinated or a bi-guanidinium species; however, peaks corresponding to these products are not found in the electrospray mass spectrum. Unfortunately, at the time of these experiments, an authentic standard of MFBG was not available to better interpret these results.

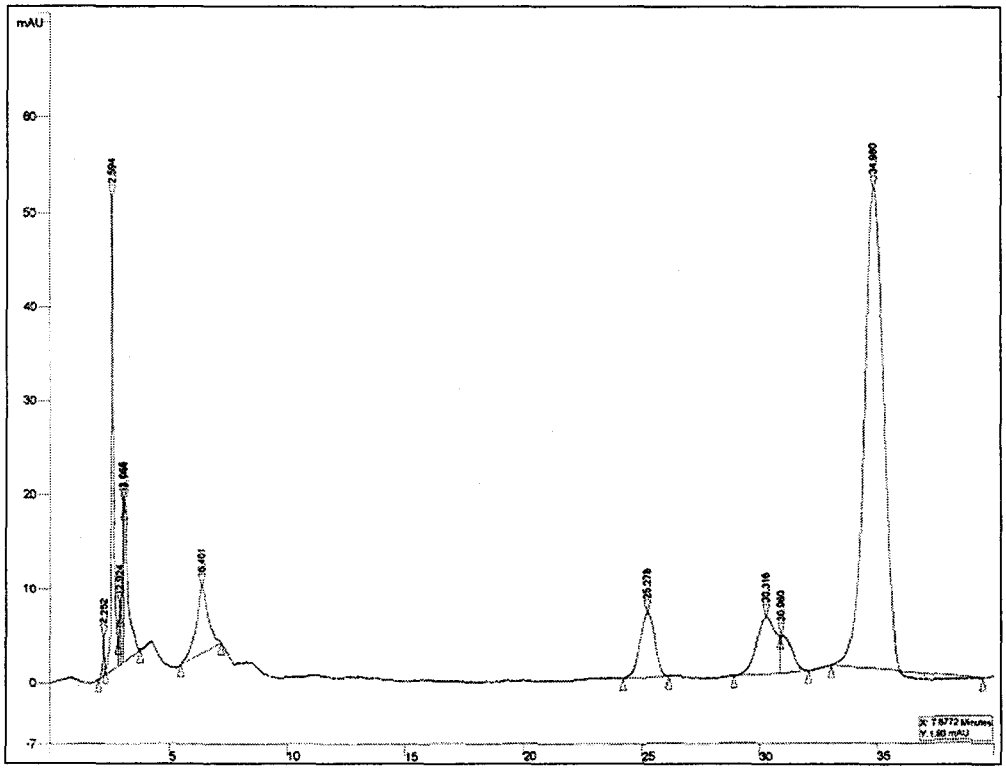


Figure 3-23. HPLC chromatogram of the crude reaction mixture (**3.11**) (conditions: C_{18} analytical (Nucleosil, 250 x 4.6 mm), 80:20 H_2O (0.01 N NaH_2PO_4):ACN, flow 2 mL/min)

The ^{19}F NMR spectrum of the crude reaction mixture (**3.11**) shows three peaks, which are all split to some extent (**Figure 3-24**). The two principle multiplets are centred at –109.5 ppm and –110.3 ppm, with apparently $^{3}J_{H,F}$ couplings of 9.2 Hz and 8.7 Hz, respectively. These peak positions and coupling constants are consistent with a meta or para-fluorinated aryl compound. The smaller coupling constants initially suggest that a 1,2 or 1,4 difluorinated species is not present. The varying peak positions, rather than being attributed to isomers, could be the results of varying protonation states, which has been shown to markedly affect fluorine shifts.[14] The poor resolution of the spectrum can

be attributed to the dilute sample used. Lyophilizing was avoided, as it appeared to result in the loss of product on several occasions.

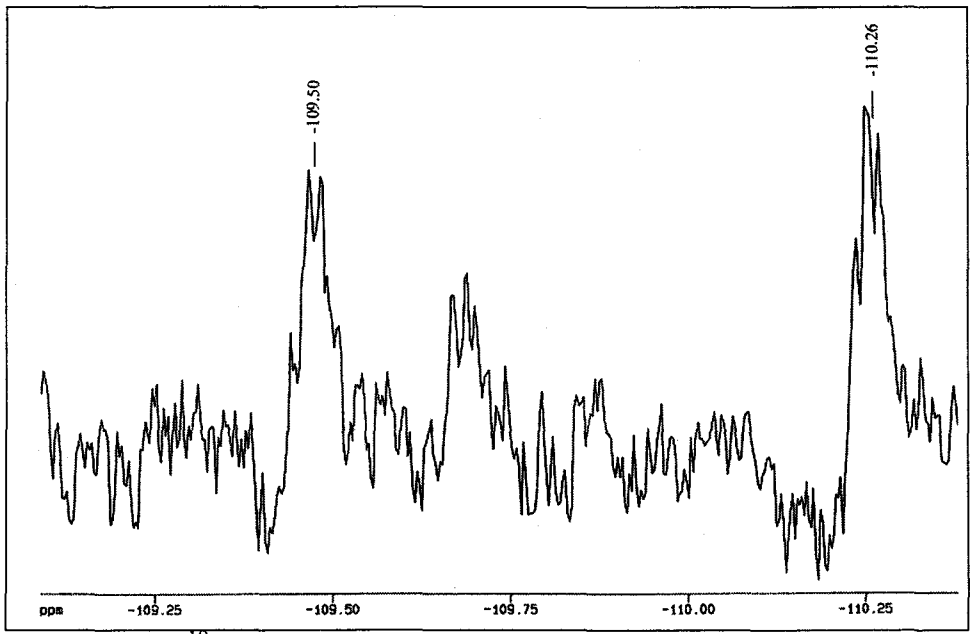


Figure 3-24. ^{19}F NMR [ACN:H_2O, 188 MHz] spectrum of the reaction mixture (**3.11**)

It is clear that the synthesis of MFBG using the fluorous strategy was not as successful as MIBG. Additional experiments are needed to optimise this approach, so that only one species is produced.

3.6 Coupling through tris(perfluorohexylethyl)tin-3-benzylamine

As mentioned in Chapter 2, short peptide sequences have been used to target radionuclides to specific receptors. For receptor specific agents of this type, it is important that all unreacted material be separated from the radiopharmaceutical. It would be advantageous therefore to develop the fluorous approach for radiolabelling peptides.

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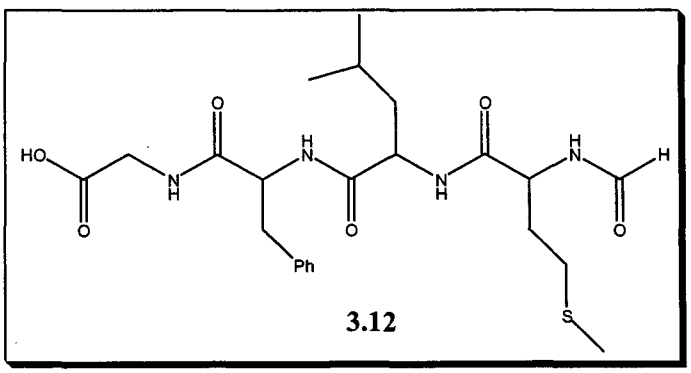
[14] Taft, R.W.; Price, E.; Fox, I.R.; Lewis, I.C.; Anderson, K.K.; Davis, G.T. *J. Am. Chem. Soc.* **1963**, *85*, 3146.

In this chapter preliminary steps towards these goals were taken. In particular, a method of coupling the carboxylic acid terminus of a model oligopeptide to the fluorous "tagged" benzylamine was developed.

The chemotactic peptide *N*-formyl-Met-Leu-Phe-Gly, **3.12**, (**Figure 3-25**) is a bacterial product which binds to polymorphonuclear leucocytes and mononuclear macrophages.[15] Fischman *et al.* have shown that radiolabelled derivatives of this peptide are effective for imaging sites of abscesses and inflammation.[16] The severe toxicity of chemotactic peptides in higher doses has hampered their clinical application; consequently it is essential that any unlabelled material be removed.



3.12

Figure 3-25. *N*-formyl-Met-Leu-Phe-Gly or GFLM(f) (**3.12**)

3.6.1 Synthesis of tris(perfluorohexylethyl)-3-benzylamine-GFLM(f) (3.13)

The coupling strategy developed in Chapter 1 for the synthesis of fluorous "tagged" benzamide should be applicable to the current objective. In this case, however, the peptides carboxylic acid terminus will be activated (HBTU) for nucleophilic attack by benzylamine (**3.0**).

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[15] Blok, D.; Feitsma, R.I.J.; Vermeij, P.; Pauwels, E.J.K. *Eur J Nucl Med.* **1999**, *26*, 1511.

Synthesis of compound **3.13** (**Figure 3-26**) entailed combining **3.0** and **3.12** in DMF, followed by addition of the acylating reagent (HBTU) and base. The reaction was stirred at room temperature overnight, diluted with water, and extracted into FC-72®. The FC-72® layer was found to contain only a small quantity of product **3.13** along with unreacted **3.0**, as determined by electrospray mass spectrometry. The majority of **3.13** was in fact partitioned between FC-72® and DMF/H_2O. Evidently, the polar nature of the peptide is significant enough to make the product no longer completely soluble in the fluorous solvent, while the fluorous "tag" prevents the peptide from dissolving in the H_2O phase. This result is somewhat favourable, as it permits facile purification of the fluorophobic product (**3.13**) from any unreacted fluorophilic precursor (**3.0**) by collecting the interfacial emulsion.

Isolating the resulting white emulsion was followed by re-extraction from FC-72® to remove any unreacted **3.0**. The yield (33 %) of the resulting thick, gummy, white solid was compromised so as to ensure the isolation of a pure sample.

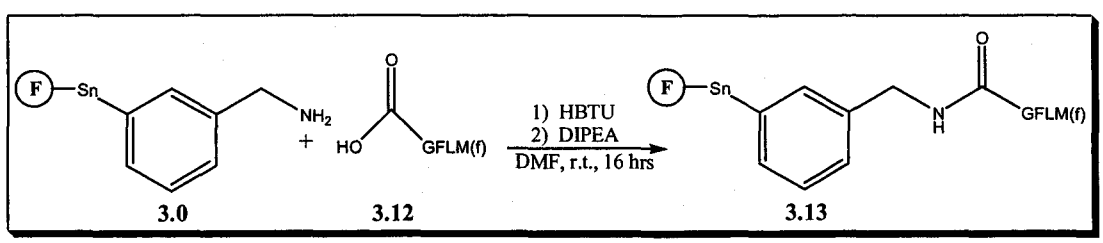

Figure 3-26. Coupling compound **3.0** to GFLM(f)

[16] Fischman, A.J.; Pike, M.C.; Kroon, D. *J Nucl Med.* **1991**, *32*, 483.

Figure 3-27 shows the positive ion electrospray mass spectrum of compound **3.13**. The peak pattern is characteristic of the product with m/z=1744 $[M+H]^+$, m/z=1761 $[M+NH_4]^+$, and m/z=1766 $[M+Na]^+$. The spectrum revealed no peak at m/z=1268 corresponding to the precursor **3.0**, although there is a small peak at m/z=1266 which was not identified.

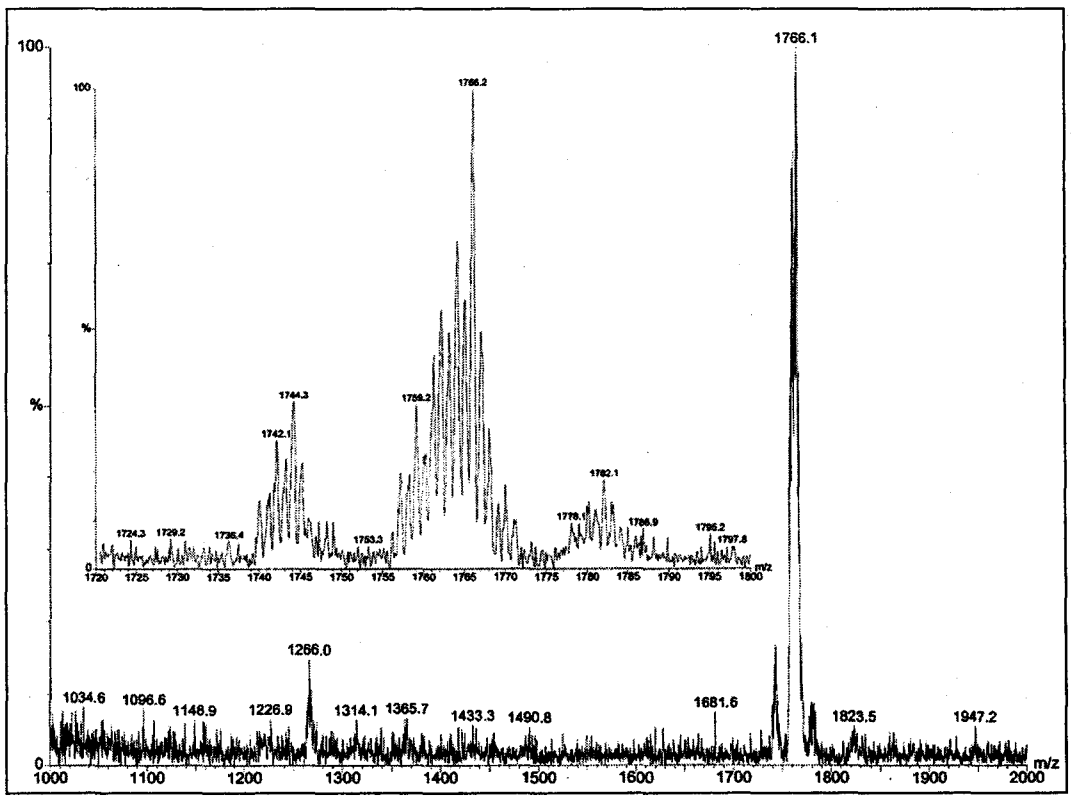

Figure 3-27. Positive ion electrospray mass spectrum of compound **3.13**

*3.6.2 Iodine labelling of compound **3.13**.*

In order to characterise the fluorous "tagged" compound (**3.13**) further, it was cleaved through an iodinolysis reaction (**Figure 3-28**). A purified sample of **3.13** was reacted with excess iodine in methanol and chloroform overnight. The excess iodine was quenched with sodium metabisulfite and the solution was concentrated. The resulting residue was diluted with acetonitrile:water (50:50) and characterised using electrospray mass spectrometry (**Figure 3-29**) and HPLC (**Figure 3-30**)

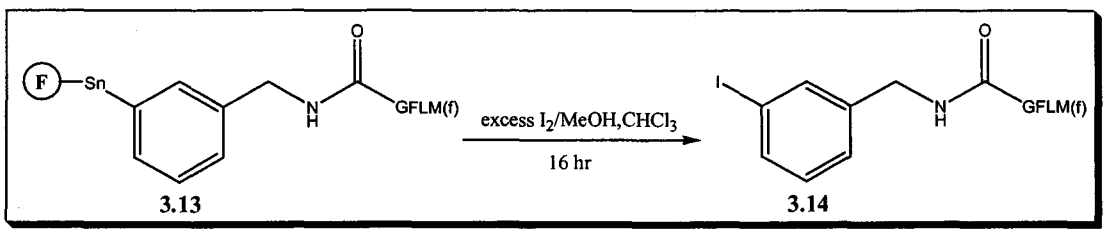

Figure 3-28. Iodinolysis of compound **3.13**

The positive ion mass spectrum of the crude reaction mixture (**3.14**) reveals peaks corresponding to the desired product at m/z=710 $[M+H]^+$, m/z=727 $[M+NH_4]^+$, and m/z=732 $[M+Na]^+$. There is no peak corresponding to the possible impurity, 3-iodobenzylamine, at m/z=234. The HPLC chromatogram of the crude reaction mixture (**3.14**) shows several peaks, the two most sizeable have retention times of 3.3 minutes and 19.4 minutes, presumably the solvent front and product, respectively. The HPLC chromatogram of the GFLM(f) under the same elution conditions has a retention time of 13.6 and 14.8 minutes, while an authentic standard of 3-iodobenzoic acid has a retention time of 6.5 minutes. The chromatogram of the crude reaction mixture (**3.14**) therefore seems to support formation of the desired product, with a longer retention time compared to GFLM(f) and no indication of the impurity at t_R=6.5 minutes.

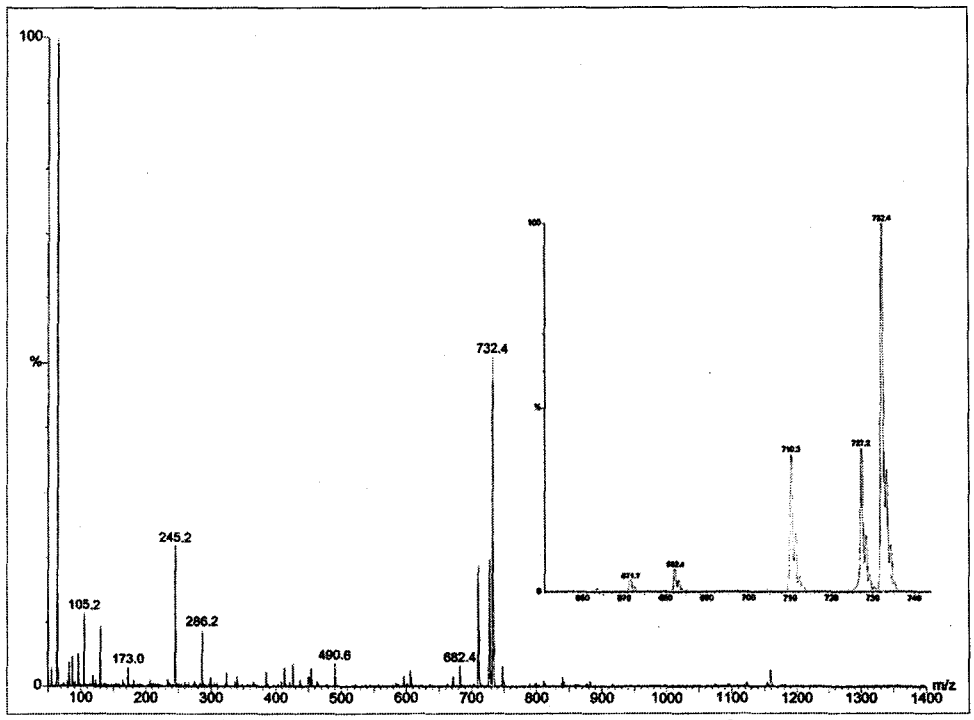

Figure 3-29. Positive ion electrospray mass spectrum of the crude reaction mixture (**3.14**)

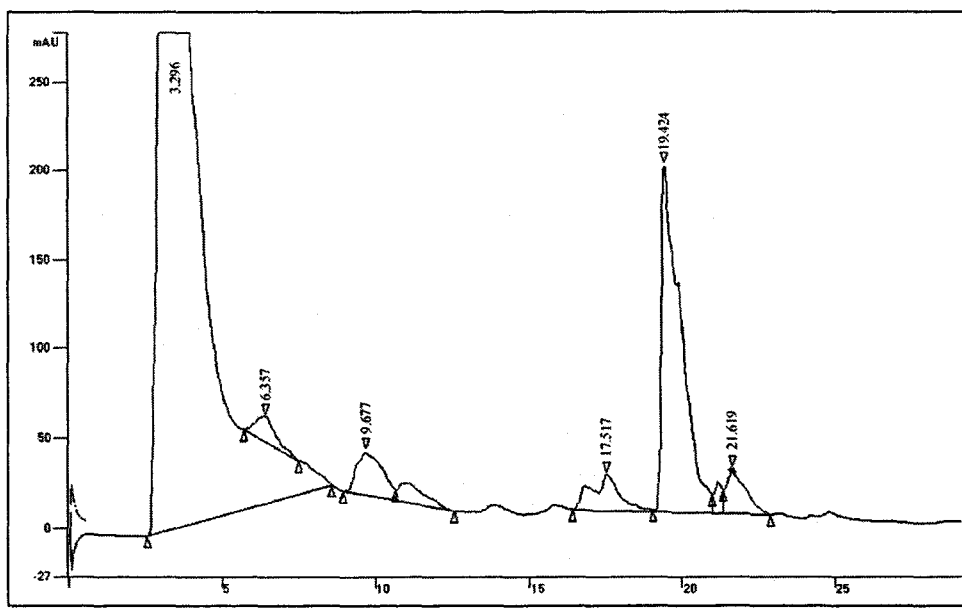

Figure 3-30. HPLC chromatogram of the crude reaction mixture (**3.14**) (conditions: C_8 analytical (Microsorb-MV, 250 x 4.6 mm), 100 % H_2O to 100 % ACN over 35 minutes, flow 1 mL/min)

3.6.3 Summary

The synthesis of tris(perfluorohexylethyl)tin-3-benzylamine (**3.0**) should facilitate the synthesis and labelling of a wider array of biomolecules. Initial results appear to confirm the successful synthesis of MIBG (**3.10**) and MFBG (**3.11**) through the corresponding fluorous "tagged" precursor (**3.5**). Further detailed characterisation of the precursor and products is required however, including expanding the labelling experiments to include $[^{18}F]F_2$ and $Na^{125}I$.

The synthesis of fluorous "tagged" peptides through compound **3.0,** has also been shown using standard coupling methodology. The differences in solubility allow for purification of the peptide coupled product (**3.13**) from any unreacted fluorous substrate by simple extraction. This coupling protocol should permit for a wide array of short peptides to be coupled to the fluorous support in the future. The products from preliminary labelling of **3.13** with iodine need to be characterised in greater detail, prior to extending this work to $[^{18}F]F_2$ and $Na^{125}I$ radiolabelling.

3.7 Experimental

1-(3-Bromobenzyl)-2,2,5,5-tetramethyl-1,2,5-azadisilolidine (3.1). The procedure developed by Magnus *et al.* was followed.[5] To a solution containing 2.228 g (11.98 mmol) of 3-bromobenzylamine in 10 mL of dry dichloromethane was added 3.4 mL (24 mmol) of triethylamine. The solution was stirred for 30 min and then treated with a solution containing 2.579 g (11.98 mmol) of 1,1,4,4-tetramethyl-1,4-dichlorosilethylene in 5 mL of dry dichloromethane. The reaction mixture was stirred for 3 h and then poured into 100 mL of saturated sodium dihydrogen phosphate. The reaction mixture was extracted with three 50 mL portions of dichloromethane, then dried ($MgSO_4$), and concentrated under reduced pressure. The residue was distilled at 160 °C to give **3.1** as a clear colourless oil: yield 2.510 g (64 %). ^{1}H NMR (200 MHz, acetone-d_6): δ 0.00 (s, 12H), 0.78 (s, 4H), 4.06 (s, 2H), 7.20-7.48 (m, 4H). ^{13}C NMR (50.3 MHz, Acetone-d6): δ -0.26, 8.01, 45.59, 122.15, 126.10, 129.35, 129.53, 130.69, 146.01. IR (thin film): 3388, 2953, 1666, 1251, and 1132 cm^{-1}. MS (CIMS): m/z=312.

Tris[2-Perfluorohexylethyl]tin-(3-bromobenzyl)-2,2,5,5-tetramethyl-1,2,5-azadisilolidine (3.3). To a solution containing 4.301 g (13.1 mmol) of **3.1** in 30 mL of dry THF at –78 °C was slowly added 5.24 mL (13.1 mmol) of 2.5 M n-BuLi. The reaction solution was stirred for 40 minutes, followed by addition of a solution containing 4.3662 g (3.521 mmol) of **2.3** in 20 mL of dry THF. The reaction solution was stirred at –78 °C for 2 h and then diluted with 5 mL of FC-72® and 30 mL of methanol. The reaction solution was extraction with three 4 mL portions of FC-72®. The combined

fluorous layers were concentrated under reduced pressure to give **3.3** as a light yellow oil: yield 4.732 g (96 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.01-0.21 (s,12H), 0.80 (s, 4H), 1.34 (t, 6H, $^{2}J_{Sn,C-H}$=27.5 Hz), 2.35 (m, 6H), 3.96-4.06 (s, 2H), 7.28-7.39 (m, 4H). IR (thin film), 3354, 2955, 2849,1256, and 442 cm^{-1}. MS (ESMS, IPA): m/z 1268.3 [M – (2,2,5,5-tetramethyl-1,2,5-azadisilolidine+H]$^{+}$.

Tris[2-Perfluorohexylethyl]tin-3-benzylamine (3.0). A mixture containing 3.990 g (2.84 mmol) of **3.3** in 125 mL of 9:1 methanol: water with sufficient 0.5 N HCl to give a pH=3.07 was stirred overnight. To the reaction mixture was added 20 mL 1 N NaOH solution, which was followed by removal of methanol under reduced pressure. The reaction mixture was subsequently extracted with four (3 mL) portions of FC-72®. The FC-72® layers were combined and re-extracted from 5 mL of dichloromethane. The solvent was concentrated under reduced pressure to give **3.0** as a light yellow oil: yield 3.482 g (97 %). TLC R_f 0.22 (6 :1 hexane-diethyl ether).^{1}H NMR (200 MHz, $CDCl_3$): δ 1.31 (t, J=8.3 Hz with ^{119}Sn satellites $^{2}J_{Sn,H}$=54.2 Hz, 6H), 2.31 (m, 6H), 3.88 (s, 2H), 7.22-7.46 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ -1.37 (with Sn satellites $^{1}J_{Sn,C}$=347 Hz), 27.94 (t, $^{2}J_{F,C}$=23.4 Hz), 46.62, 106.17-121.17 (m, CF_2, CF_3), 128.63, 129.19, 129.72, 134.60, 134.90, 135.56, 135.66, 136.96, 138.42, 140.08, 143.89, 162.09. IR (thin film), 3386, 2944, 2870, 1647, 1250 cm^{-1}. MS (ESMS, IPA): m/z 1268.5 [M+H]$^{+}$.

3-Iodobenzylamine (I_2 reaction with 3.0) (3.4). To a mixture of 0.164 g (129 μmol) of **3.0** in 2 mL of acetonitrile was added 1.5 mL of 0.1 M iodine in methanol. The reaction mixture was stirred for 16 h prior to quenching with a crystal of sodium thiosulfate and dilution with 3 mL of deionized-distilled water. The reaction mixture was

purified by flash chromatography using silicycle® fluorous silica (3.9 g). Elution with 50:50 acetonitrile-water and collection of four 5 mL fractions gave **3.4** in solution. HPLC analysis was carried out on an analytical (250 mm x 4.6 mm) C_8 reversed-phase column. A retention time of 6.461 min, consistent with a standard, was generated when conditions: 80:20 H_2O (pH≈7.4):ACN, λ=254 nm, flow 1.5 mL/min. MS (ESMS), m/z 233.9 $[M+H]^+$.

Tris[2-Perfluorohexylethyl]tin-3-benzylguanidine using formamidinesulfinic acid (3.5 – Approach B). To a mixture containing 1.964 g (1.549 mmol) of **3.0** in methanol (15 mL) was added 0.184 g (1.704 mmol) of **3.7**. The reaction mixture was stirred for 16 h and then methanol was decanted from the resulting viscous oil. The oil was washed with three (10 mL) portions of hot chloroform and then two portions of hot water. The residue was extracted into 5 mL of FC-72® from dichloromethane and residual water. The solvent was concentrated to give **3.5** as a clear orange oil: yield 1.654 g (82 %). Mass spectrum (ESMS), m/z 1310.2 $[M+H]^+$, 1293.0 $[M+H-15]^+$, and 1325.0 $[M+H+15]^+$.

Aminoiminomethanesulfonic acid (3.7). The procedure developed by Mosher *et al.* was followed.[12] To a mixture containing 0.633 g (5.85 mmol) of **3.6** in 3.0 mL of glacial acetic acid at 0 °C was slowly added 1.56 mL of 32 % peracetic acid. The reaction mixture was then stirred for 16 h at room temperature. The precipitate was filtered and washed with five 5 mL portions of absolute ethanol and dried to give 3.7 as a white crystalline solid: yield 596 mg (82 %). mp 125-126°C.

3-Iodobenzylguanidine (3.9). To a solution containing 168 mg (721 μmol) of **3.8** in 1 mL of methanol was added 90.1 mg (726 μmol) of **3.7**. The reaction solution was refluxed for 16 h and then concentrated under reduced pressure to give **3.9** as a viscous yellow gum: yield 258 mg. HPLC analysis was performed using a nucleosil C_{18} reversed-phase column. A retention time of 24.54 min was generated when conditions: 80:20 H_2O (0.01 M NaH_2PO_4):ACN, λ=231 nm, flow 2.0 mL/min. ^1H NMR (MeOH, 200 MHz): δ 4.22 (s, 2H), 6.99 (t, 1 H), 7.22 (d, 1H), 7.49 (d, 1H), 7.56 (s, 1H). ^{13}C NMR (MeOH, 50.3 MHz): δ 48.95, 99.31, 131.64, 135.72, 141.05, 141.93, 144.30, 162.65. IR (thin film): 3407, 3192, 1653, 1115 cm^{-1}. MS (ESMS, methanol), m/z 276.1 $[M+H]^+$.

Tris[2-Perfluorohexylethyl]tin-3-benzlguanidine using aminoimino-methanesulfinic acid (3.5 – Approach C). To a mixture containing 518 mg (409 μmol) of **3.0** in 1 mL of methanol was added 55.8 mg (450 μmol) of aminoiminomethane-sulfonic acid. The reaction mixture was then refluxed for 16 h. The reaction mixture was extracted into 5 mL of FC-72® from 10 mL of methanol. The solvent was concentrated under reduced pressure to give **3.5** as an orange oil: yield 468 mg (88 %). TLC R_f 0.25 (6 :1 hexane-diethylether). IR (thin film), 3349, 3197, 2946, 1647,1449, 1239, 446 cm^{-1}. Mass spectrum (ESMS), m/z 1309.9 $[M+H]^+$.

3-Iodobenzylguanidine (NaI reaction with 3.5) (3.10). To a reaction mixture containing 5.1 mg (3.90 μmol) of **3.5** in 200 μL of methanol was added 10 μL (0.460 nmol) of NaI followed by 2 μL of solution of peracetic acid (35 % in acetic acid). The

reaction mixture was stirred for 2 h and then quenched with 100 μL of sodium metabisulfite (10 %) solution, prior to dilution to 1 mL with distilled-deionized water. HPLC analysis was performed with a nucleosil C_{18} analytical column. A retention time of 24.89 min was observed when conditions: 80:20 H_2O (0.01 M NaH_2PO_4):ACN, λ=231 nm, flow 2.0 mL/min. MS (ESMS), m/z 276.0 $[M+H]^+$.

Fluorination of 3.5 using [F_2] (3.11). To 0.334 g (0.255 mmol) of **3.5** in 1 mL of FC-72® at –95°C in a FEP tube was bubbled 172 μmol of 0..63 % F_2 in Ne. The F_2 was steadily released into the solution over 35 min. The reaction solution along with two 3 mL portions of FC-72® used to rinse the vessel were concentrated in a large vial. The residue was washed with three 3 mL portions of 50:50 acetonitrile:water and eluted down a conditioned fluorous reversed-phase column (1 g) to give **3.11** in solution. HPLC analysis was carried out on a nucleosil analytical (250 mm x 4.6 mm) C_{18} reversed-phase column. A retention time of 34.98 min was observed when conditions: 80:20 H_2O (0.01 M NaH_2PO_4):ACN, λ=231 nm, flow 2.0 mL/min. ^{19}F NMR (ACN:H_2O, 470.493 Hz): δ -110.3 ($^3J_{F,H}$= 8.7 Hz), -109.5 ($^3J_{F,H}$=9.2 Hz). MS (ESMS), m/z 168.0 $[M+H]^+$.

Tris[2-Perfluorohexylethyl]-3-benzylamine-GFLM(f) (3.13). To a reaction solution containing 137 mg (108 μmol) of **3.0** and 84 mg (170 μmol) of GFLM(f) in 5 mL of DMF was added 71 mg (187 μmol) HBTU. To the reaction solution was added 97 μL of DIPEA and allowed to stir at for 16 h. The solution was diluted with 20 mL of water and extracted with 5 mL of FC-72®. The emulsion partitioning FC-72® and the aqueous layer was extracted and washed with three 3 mL portions of FC-72®. The

residual solvent was removed under reduced pressure to give **3.12** as a milky white oil: yield 63 mg (33 %). MS (ESMS), m/z 1744 $[M+H]^+$, 1761 $[M+NH_4]^+$, 1766 $[M+Na]^+$.

3-Iodobenzyl-GFLM(f) (I_2 reaction with 3.13) (3.14). To a reaction mixture containing 50 mg (28.7 μmol) of **3.13** in 3 mL of chloroform was added 1.5 mL (150 μmol). The reaction mixture was stirred for 16 h prior to quenching with a sodium thiosulfate solution. The chloroform was removed under reduced pressure, and the mixture was diluted with 10 mL of 5:1 acetonitrile:water. The reaction solution was washed with three 1.5 mL portions of FC-72® and the aqueous layer was isolated and assessed for the presence of **3.14**. HPLC analysis was carried out on a nucleosil C_{18} reversed-phase analytical column (250 mm x 4.6 mm). A retention time of 19.4 min was observed when conditions: 80:20 H_2O (0.01 M NaH_2PO_4):ACN, λ=254 nm, flow 2.0 mL/min. MS (ESMS), m/z 319 $[M+H]^+$.

Chapter 4

Conclusions

In the course of this thesis, the fluorous synthesis technique was adapted for the preparation of radiolabelled compounds. Several fluorous "tagged" substrates were synthesised using novel methodologies. A model compound, tris(perfluorohexyethyl)tin-3-benzoic acid, was labelled with both $[^{18}F]F_2$ and $Na^{125}I$ though a halodestannylation reaction. The regioselectively labelled product was readily purified from any excess or unreacted starting material through a short column of fluorous derivatized silica. The successful application of the fluorous method to synthesis of 3-$[^{18}F]$fluorobenzoic acid and 3-$[^{125}I]$iodobenzoic acid, establishes that fluorous phase synthesis is a viable method for radiopharmaceutical development.

A series of other fluorous derivatives were also synthesised and labelled using cold isotopes. Specifically, adaptation of peptide synthesis methods facilitated coupling of the fluorous "tagged" benzoic acid to amines, allowing for F_2 and I_2 labelling of benzamide. Similarly, synthesis of a fluorous "tagged" benzylamine allowed for synthesis and subsequent labelling of the clinically important compound m-iodobenzylguanidine and a short peptide (GFLM(f)).

In the case of the benzylamine derivatives, the product yields were not optimal. Further work should entail improving these labelling reactions as well as optimising the labelling of compounds **3.5** and **3.13** with $Na^{125}I$ and $[^{18}F]F_2$.