TRIALKYLSTANNYLATION OF ARYL AND VINYL HALIDES

WITH A FLUOROUS DISTANNANE

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By

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TITLE: Trialkylstannylation of Aryl and Vinyl Halides with a Fluorous Distannane

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Abstract

The development of a convenient route for the preparation of fluorous-tagged compounds for use with the fluorous labeling strategy (FLS) is described in this thesis. The FLS is a new and convenient method for the preparation of radiotracers and therapeutics in high effective specific activity (HESA) without the use of preparative HPLC. The objective of this thesis was to expand the general utility of the FLS by enabling the introduction of fluorous tags into molecules using a palladium-catalyzed cross-coupling reaction. To this end, a fluorous distannane, hexa(1H,1H,2H,2H-perfluorooctyl)distannane, was prepared from the corresponding fluorous tin hydride and used to produce trialkylarylstannanes from aryl and vinyl halides. Using the developed methodology, fluorous precursors for two radiopharmaceuticals, fialuridine (FIAU) and idoxuridine (IUdR), were prepared. The fluorous-tagged products were radiolabeled with iodine-125 to afford the desired compounds in high effective specific activity and in good radiochemical yield.

Hexa(1H,1H,2H,2H-perfluorooctyl)distannane was prepared from the corresponding tin hydride in nearly quantitative yield in the presence of $Pd(PPh_3)_4$. The

distannane was combined with a series of seventeen aryl bromides and iodides and the appropriate palladium catalyst to afford trialkylarylstannanes in 15-59% isolated yield.

The use of a phosphaadamantane ligand reported by Capretta *et al.* in the cross-coupling was also investigated, and the yields for the model compounds ranged from 13-67% Although no substantial change in yields was observed for aryl halides compared to the traditional catalyst $Pd(PPh_3)_4$, the phosphaadamantane ligands were more effective for the synthesis of precursors to [^{125}I]fialuridine (FIAU) and [^{125}I]idoxuridine (IUdR). Using this ligand system, the FIAU precursor was prepared in 38% overall yield from a dibenzoyl-protected vinyl bromide, and the IUdR precursor was prepared in 21% yield from a vinyl iodide.

Following preparation of the FIAU and IUdR precursors, direct iodinolysis using a sub-stoichiometric amount of iodine was performed and the products isolated in excellent yield and purity using fluorous solid-phase extraction (FSPE). Following these experiments, the precursors were radiolabeled with [^{125}I]NaI (50 – 500 μCi, 1.9 – 19 MBq) in the presence of Iodo-Gen® as an oxidant. Average radiochemical yields for three trials were 88% for FIAU and 94% for IUdR. The precursor was not observed in

the FSPE-purified reaction mixture by UV-HPLC within the instrument's detection limit.

The fluorous labeling strategy allows molecular imaging and associated therapy agents to be produced in high effective specific activity in a rapid and convenient manner. With the development of the fluorous distannane and the associated coupling reactions reported here, the general utility of the fluorous labeling strategy has been greatly expanded.

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List of Abbreviations and Symbols

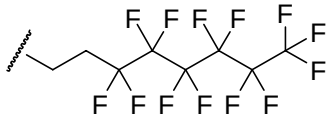
Standard abbreviations for SI units (e.g. mL, mg, cm) are not included in this list.

Ci	curie (1 Ci = 3.7×10^{10} Bq)
COSY	correlation spectroscopy
CT	computed tomography
CV	column volume
dba	dibenzylideneacetone
DMF	*N,N*-dimethylformamide
DNA	deoxyribonucleic acid
EC	electron capture
ESA	effective specific activity (activity per unit mass of total sample)
ESMS	electrospray mass spectrometry
FDG	2-fluoro-2-deoxy-D-glucose
FIAU	fialuridine
FID	flame ionization detector
FMT	fluoro-*meta*-tyrosine
FLS	fluorous labeling strategy
FSPE	fluorous solid-phase extraction
GC	gas chromatography
HMBC	heteronuclear multiple bond correlation spectroscopy
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single-quantum coherence
HSV1-tk	herpes simplex virus type 1 thymidine kinase
INEPT	insensitive nucleus enhancement by polarization transfer
IR	infrared
IUdR	5-iodo-2′-deoxyuridine
keV	kiloelectronvolt
LC/MS	liquid chromatography/mass spectrometry
LRMS	low resolution mass spectrometry
MIBG	*m*-iodobenzylguanidine
MRI	magnetic resonance imaging

MS	mass spectrometry
NMR	nuclear magnetic resonance
OAc	acetyl group
PA	phosphaadamantane
PA/Pd	phosphaadamantane/palladium
PET	position emission tomography
SPE	solid-phase extraction
SPECT	single photon emission computed tomography
TdR	2′-deoxythymidine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
T/NT	target to non-target ratio
TOF	time of flight
UdR	2′-deoxyuridine
UV	ultraviolet
Ci	microcurie (1 Ci = 37 kBq)

List of Numbered Compounds

For all compounds, R = 1H,1H,2H,2H-perfluorooctyl:

R = 

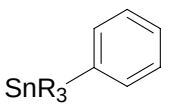
$CH_2CH_2(CF_2)_5CF_3$

1. Hexa(1H,1H,2H,2H-perfluorooctyl)distannane

$R_3Sn{-}SnR_3$

1

2. Tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzene and derivatives



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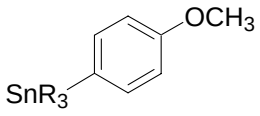

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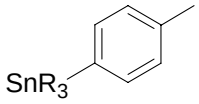

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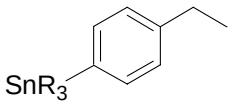

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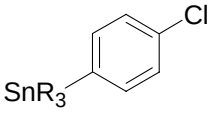

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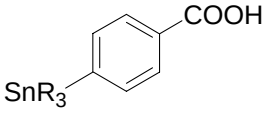

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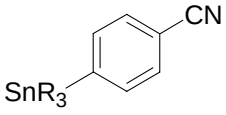
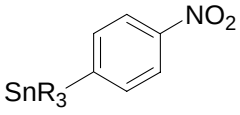


2g



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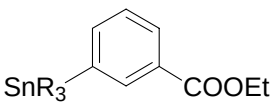

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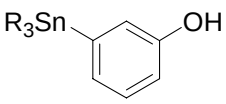

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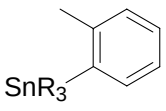

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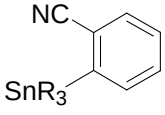
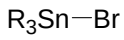


2l

3. Tris(1H,1H,2H,2H-perfluorooctyl)tin bromide



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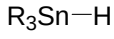
4. Tris(1H,1H,2H,2H-perfluorooctyl)tin hydride



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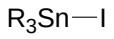
5. Aryl halide precursors to **2a-2l** – see **Table 2-1**

6. Tris(1H,1H,2H,2H-perfluorooctyl)tin iodide



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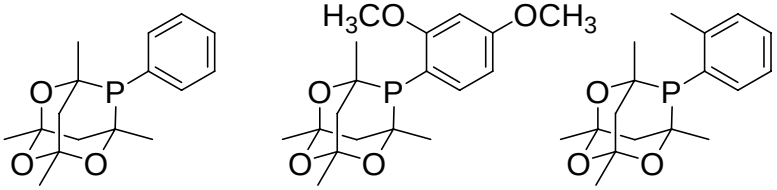
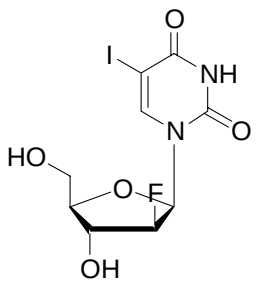
7. Phosphaadamantane ligands



7a **7b** **7c**

8. 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil



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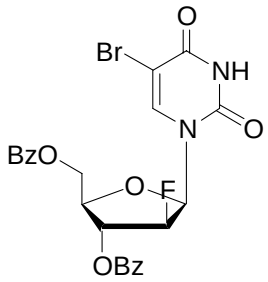
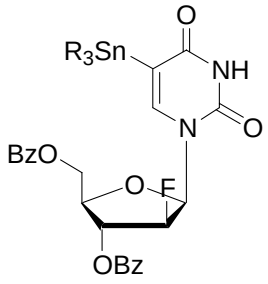
9. 1-(3′,5′-dibenzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-bromouracil



9

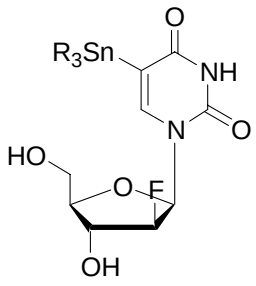
10. 1-(3′,5′-dibenzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-

tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil



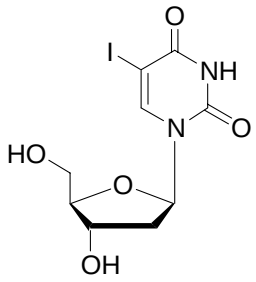
10

11. 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil



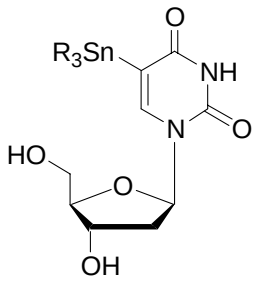
11

12. 1-(2′-deoxy-β-D-ribofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil



12

13. 1-(2′-deoxy-β-D-ribofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil



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Chapter 1

Introduction

1.1 Objectives

Trialkylarylstannanes, which are typically prepared by palladium-catalyzed cross-coupling reactions from aryl iodides, are used as precursors in the synthesis of compounds labeled with isotopes of fluorine, bromine and iodine.[1,2] Analogous compounds bearing perfluoroalkyl groups – fluorous compounds – can be used similarly while facilitating purification using perfluorinated solvents or fluorous solid-phase extraction (FSPE) techniques. This thesis describes the development of a methodology for preparing fluorous trialkylarylstannanes *via* a fluorous distannane, hexa(1H,1H,2H,2H-perfluorooctyl)distannane, and the investigation of the scope and utility of this methodology in preparing and developing radiolabeled compounds.

1.2 Radiofluorine and radioiodine in nuclear medicine

Radiopharmaceuticals labeled with ^{18}F and with isotopes of iodine such as ^{123}I and ^{131}I are important in nuclear medicine and are used for targeted radiation therapy and for molecular imaging techniques such as position emission tomography (PET) and single photon emission computed tomography (SPECT) **(Table 1-1)**. Although only compounds labeled with iodine-125 are discussed in this thesis, these imaging techniques are discussed in brief because the labeling methodology presented here can be applied to any radiohalogen.[1,2]

Isotope	Production	Decay Mode	Half-life	Application
^{18}F	Cyclotron	β^+	109.8 min.	PET
^{123}I	Cyclotron	EC	13.2 h	SPECT
^{124}I	Cyclotron	EC (75%) β^+ (25%)	4.18 d	PET
^{125}I	Nuclear reactor	EC	59.4 d	Therapy
^{131}I	Nuclear reactor	β^-	8.0 d	Therapy, SPECT

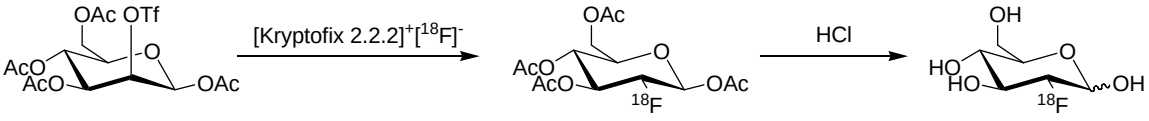
Table 1-1: Some halogen radionuclides of interest in nuclear medicine.[2]

In PET, a patient is injected with an agent labeled with a positron-emitting isotope such as fluorine-18 ($t_{½}$ = 109.8 minutes). The positrons emitted by this process travel a specific, isotope-dependent distance before they collide with an electron, thereby undergoing an annihilation reaction which emits two 511 keV photons. The

photons are separated by a 180° angle and describe a trajectory known as a coincidence line. The photons can be detected by a rotating pair of gamma cameras or a specialized instrument having a number of detectors arranged in a ring. Each detector is equipped with a collimator which permits only photons with a certain trajectory to enter; this permits the instrument to differentiate between photons traveling along different coincidence lines. The detectors contain a scintillation crystal (typically bismuth germanate, BGO), which undergoes fluorescence upon absorption of high-energy photons. The light emitted by scintillation is amplified by a photomultiplier tube and the signals from the detectors are digitally processed to generate a series of images showing the distribution of the radionuclide undergoing decay within the patient.[1-4]

PET provides functional information to clinicians which is not offered by anatomical imaging techniques such as MRI and CT.[3] For example, [^{18}F]fluorodeoxy-D-glucose (FDG, **Scheme 1-1**) is preferentially taken up by malignant tumours since these overexpress the glucose transporter and hexokinase, for which FDG is a substrate.[2,3] After phosphorylation, FDG remains in the cell and does not undergo further metabolism, leading to a high local concentration of the tracer and ultimately to im-

proved image quality. A PET image showing a high incidence of annihilation photons in a particular location suggests the presence of a tumour. PET can also be used in neurology (e.g. in the diagnosis of Parkinson's disease) and in cardiology (e.g. to measure blood flow in the myocardium).[1-4]



Scheme 1-1: Synthesis of FDG from mannosyl-2-triflate tetraacetate and $^{18}F^-$.

Radionuclides used in PET are typically short-lived elements that are commonly found in organic molecules, such as ^{11}C ($t_{½}$ = 20.3 minutes), ^{13}N ($t_{½}$ = 10.0 minutes), and ^{15}O ($t_{½}$ = 2.0 minutes). Fluorine-18 is used as an analogue of hydrogen or hydroxyl groups due to its small atomic radius and the relative strength and low reactivity of the C-F bond. As a result of the short half-lives, the radionuclide must be prepared no more than ~3 half-lives before the PET procedure, which usually necessitates production with an on-site cyclotron.[2,4] Fluorine-18, for example, can be produced by the $^{18}O(p,n)^{18}F$ reaction, which produces $[^{18}F]F^-$ from $H_2{}^{18}O$ and $[^{18}F]F_2$ from $^{18}O_2$. $[^{18}F]F_2$ can also be produced by the $^{20}Ne(d,\ )^{18}F$ reaction.[2] The radiopharmaceutical is pre-

pared immediately prior to use; an example illustrating the synthesis of FDG is shown in **Scheme 1-1**.[2,3]

In SPECT, the patient is first injected with a radiotracer labeled with a gamma-emitting radionuclide. One or more gamma cameras (which detect incoming gamma rays) are rotated about the patient, and multiple images are acquired from various angles.[4] The resulting data is transformed to provide a three-dimensional image or a series of two-dimensional 'slices' depicting the distribution of the radiopharmaceutical in the patient. At present, the resolution is generally poorer than PET. However, the radionuclides most commonly used in SPECT, including technetium-99m ($t_{1/2}$ = 6.0 h) and iodine-123 ($t_{1/2}$ = 13.2 h), have longer half-lives than those most commonly used for PET. This decreases cost and increases availability because the required radionuclides can be obtained from an off-site facility or, in the case of ^{99m}Tc, a radionuclide generator.[4]

The labeling methods proposed in this project are applicable to all radiohalogens. The advantage of the method – rapid purification of the reaction mixture following labeling – is especially relevant for the production of PET radiotracers, since the short half-life of ^{18}F means that rapid purification is highly desirable. A further ad-

vantage of the proposed approach is that it can be used to introduce modern high-throughput drug development methods such as automated parallel synthesis and combinatorial chemistry into the radiopharmaceutical discovery process.[5,6]

1.3 Need for purification

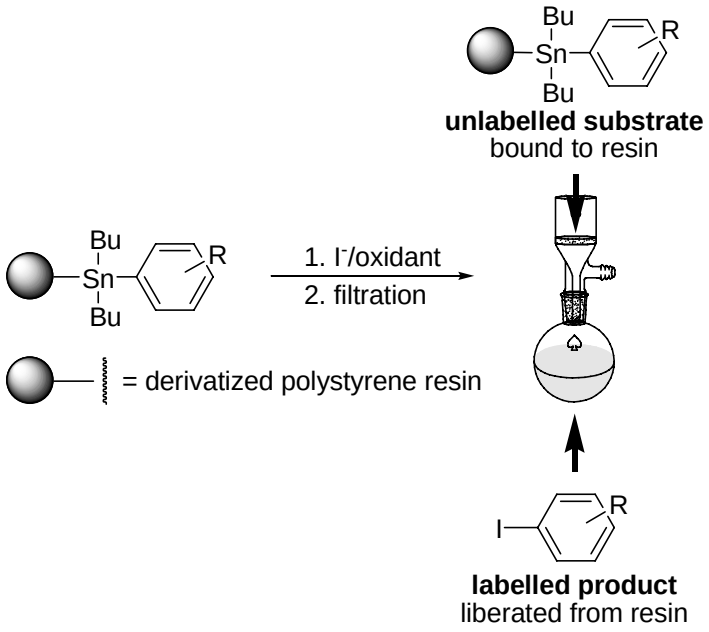
Unlike most pharmaceuticals, radiotracers for use in imaging are typically prepared on-site shortly before use. The time required for synthesis is particularly important for short-lived isotopes, because the tracer must be injected into a patient no more than 3 half-lives after production. Labeling procedures are usually rapid, but, since the precursor is always present in excess and the radionuclide source may not be completely incorporated into the product, the labeling reaction produces a mixture of the labeled product, the unlabeled substrate, and perhaps the radionuclide source or other impurities. The presence of these impurities reduces the effective specific activity of the preparation. Low effective specific activity can reduce image quality in cases where the substrate competes with the tracer at biological targets that exist in low concentrations; in extreme cases, the preparation may elicit a biological response, in violation of the tracer principle.[5,6]

Some radiopharmaceuticals, particularly technetium-99m radiotracers such as ^{99m}Tc-pyrophosphate, can be purified with liquid-liquid extraction and solid-phase (Sep-Pak®) extraction.[5] Currently, however, PET radiotracers and some SPECT radiotracers must be purified by HPLC after labeling.[6] This lengthy purification procedure decreases specific activity due to radioactive decay and increases radiation exposure. It also increases the cost of PET and SPECT procedures and consequently decreases the accessibility of these diagnostic procedures for patients and clinicians, since HPLC purification must be performed by a qualified radiopharmacist. Automated synthesizer units for select tracers such as FDG are commercially available, but their high cost and complexity creates a need for a rapid, facile labeling and purification methodology for radiohalogenated tracers.[5,6]

1.4 Solid-phase labeling strategies

There exist a select number of routes to radiopharmaceuticals in high effective specific activity that do not require HPLC purification.[5-10] One such method is the use of a solid-phase resin derivatized with a radiopharmaceutical precursor **(Scheme 1-2)**. In this method, the precursor detaches from the solid-phase support and enters the

solution phase upon reaction with the radionuclide; unreacted precursor remains bound to the resin. This method allows the radiopharmaceutical to be purified by filtration. In 1999, Hunter and Zhu described the use of this methodology to prepare *m*-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG) in 92% radiochemical yield with a specific activity of ≥ 500 Ci/mmol (18.5 GBq/mmol).[9]



Scheme 1-2: Schematic overview of an arylstannane solid-phase labeling strategy.[9]

Despite its convenience, the solid-phase labeling methodology has several notable disadvantages. Characterization of the polymer-supported precursor is generally

limited to solid-state NMR and HPLC analysis of the product following a sample labeling. The resins themselves may be subject to radiolysis during the labeling procedure, potentially leading to the release of unwanted byproducts. Of the limitations of the solid-phase methodology, the most significant is that it is not possible to remove impurities from the resin-bound precursor. Therefore, if the immobilization onto the resin of the substrate to be labeled does not proceed to completion, further purification of the radiolabeled product will be required.[5,6,9]

1.5 Fluorous-phase labeling

The chemistry of organic compounds bearing perfluorinated alkyl groups is known as fluorous chemistry. The term 'fluorous' was coined in analogy to 'aqueous', because compounds with perfluoroalkyl groups (fluorophilic compounds) partition into a perfluorinated or 'fluorous' phase in the same way hydrophilic compounds partition into an aqueous phase.[11] This allows fluorous compounds to be separated from non-fluorous compounds by liquid-liquid extraction or by fluorous solid-phase extraction (FSPE) using perfluoroalkyl-derivatized silica.[11] Fluorous chemistry was first

described in 1994 by Horváth and Rabái and was subsequently developed by others, notably D. P. Curran.[11,12] The methodology has been applied to several problems in separation, including the recovery of catalysts,[12] parallel synthesis of natural product derivatives,[13] and solid-phase peptide synthesis.[14] A monograph published in 2004 describes many of the applications of fluorous chemistry and the properties of fluorous compounds.[11]

A schematic depiction of a typical application of fluorous chemistry is shown in **Figure 1-1**.[5,15] In the first step, a substrate is coupled with a fluorous moiety to produce a fluorous compound which can undergo further reaction to produce some product, which remains fluorous. The fluorous product can be isolated from the reaction mixture by FSPE or liquid-liquid extraction. Cleavage of the fluorous moiety followed by FSPE recovers the fluorous moiety and isolates the desired product, which is no longer fluorous and remains in the organic layer during extraction or is eluted from an FSPE cartridge by a so-called fluorophobic solvent (one which contains water and does not elute fluorous compounds from the cartridge).[5,11,16] Depending on the desired application, the fluorous moiety may be either chemically inert, such as a protecting

group which is introduced at the beginning of the reaction and cleaved at the end, or it may be reactive, such as a trialkylstannyl group which is cleaved to give the product.

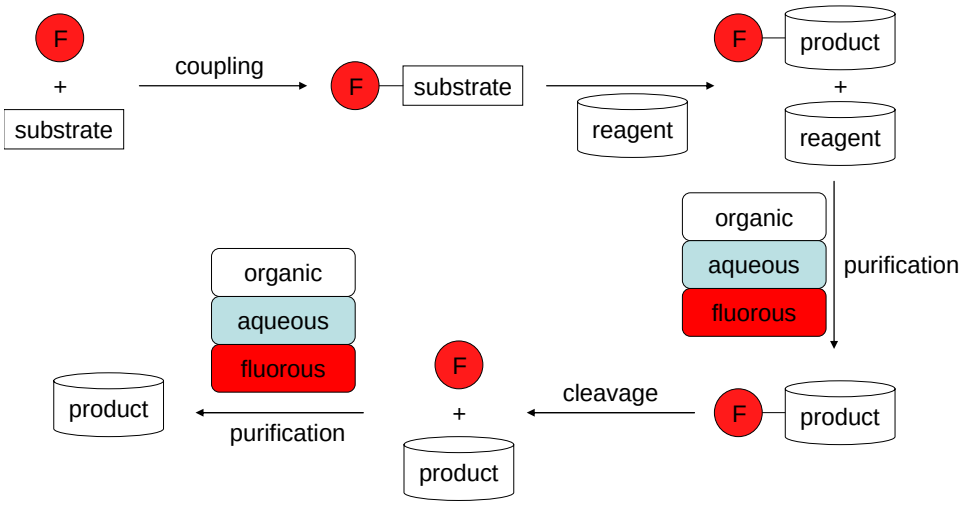


Figure 1-1: Schematic representation of a reaction using fluorous chemistry. (From Donovan *et al.,* 2007.)

A radiolabeling strategy based on fluorous chemistry (the fluorous labeling strategy, FLS) was developed in our group.[5,6] The basic procedure involved treating fluorous arylstannanes with ^{125}I and an oxidant and then purifying the reaction mixture using a fluorous solid-phase extraction procedure **(Figure 1-2)**. In this procedure, the crude reaction mixture is loaded onto an FSPE cartridge which is then washed with water to elute water-soluble salts. The radiolabeled product is recovered by elut-

ing the cartridge with 80:20 methanol-water. Fluorous compounds are retained during elution with methanol-water, but can be recovered by washing with methanol or tetrahydrofuran (THF).

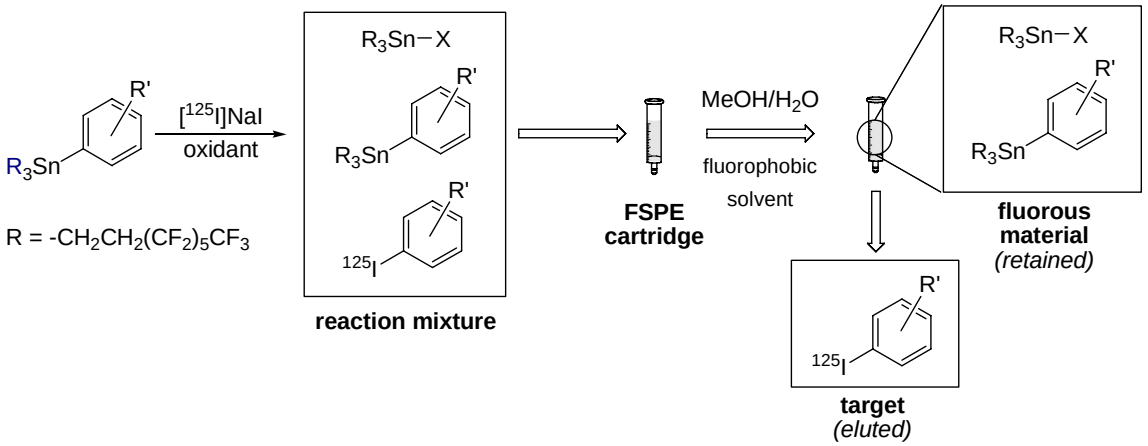
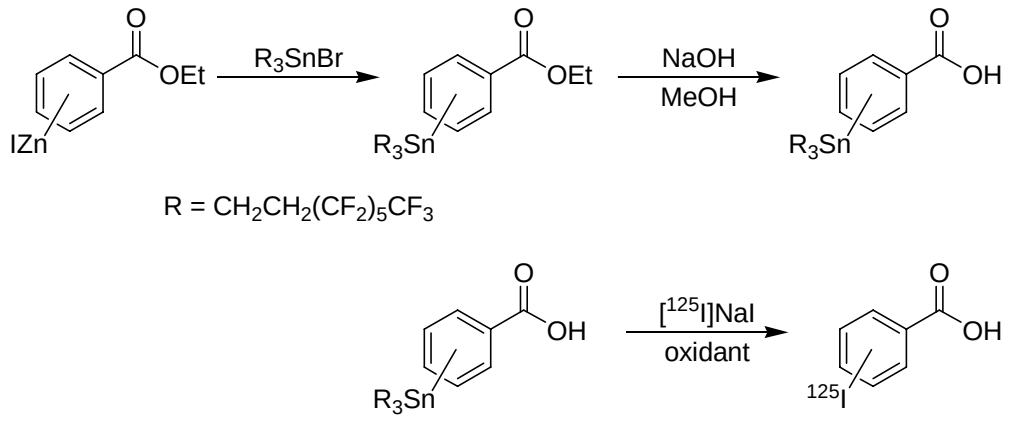


Figure 1-2: Schematic overview of the fluorous labeling strategy.

The FLS is analogous to solid-phase labeling, with perfluoroalkyl groups taking the place of the resin, but the fluorous labeling strategy offers several significant advantages. Fluorous radiopharmaceutical precursors can be characterized and purified using conventional techniques routinely employed with small molecules. The structural identity and purity of the precursors can be established using, for example, ^{1}H and ^{13}C NMR spectroscopy or LC/MS. As a result, the purity of a fluorous radiopharmaceutical precursor can be assured prior to use. An additional advantage is that

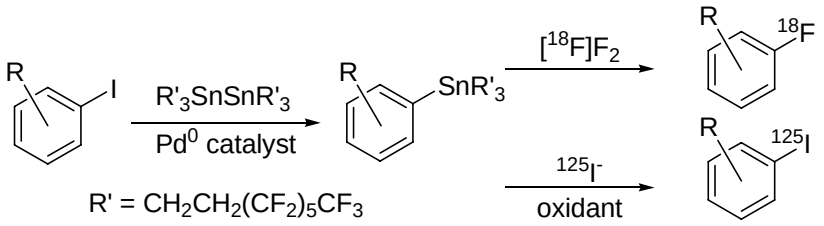
the labeled product can be eluted from the solid-phase extraction cartridge using an ethanol-water mixture to obtain a solution of the product that is ready for administration to the patient following quality assurance. This is not possible for a product purified by HPLC, since the product is usually obtained as a solution in aqueous methanol or acetonitrile; even if ethanol were used, the solution would be too dilute to inject directly.[5,6,16]

The FLS has been used to produce radioiodinated and radiofluorinated compounds. For example, *m*- and *p*-benzoic acid derivatives were prepared from tris(1H,1H,2H,2H-perfluorooctyl)tin bromide and an organozinc reagent **(Scheme 1-3)**. From the fluorous benzoic acid derivatives, a library of benzamides was prepared and labeled with iodine-125 in >85% yield and the products were isolated in exquisite purity.[6]



Scheme 1-3: Synthesis and radiolabeling of a fluorous benzoic acid derivative *via* an organozinc reagent.

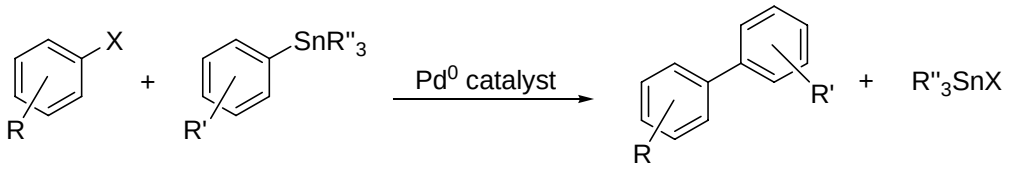
Previous work on the FLS has focused on preparing arylstannane precursors by reaction of a fluorous trialkyltin bromide with either an organozinc reagent, as described above, or with a lithium salt prepared from an aryl bromide and *n*-butyllithium. In both cases, protection of most functional groups is required. For this reason, a complementary trialkylstannylation method that is tolerant of a wide variety of functional groups is desirable. The objective of this thesis has been to develop such a method based on a fluorous analog of an established method for producing trialkylstannyl radiopharmaceutical precursors by the palladium-catalyzed cross-coupling reaction between an aryl or vinyl halide and a hexaalkyldistannane **(Scheme 1-4)**.[1,2]



Scheme 1-4: Trialkylstannylation and radiohalodestannylation reactions.

1.6 Palladium-catalyzed cross-coupling of aryl halides

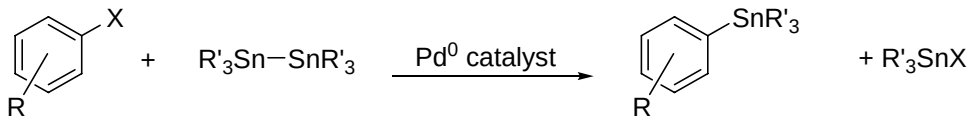
The transition metal-catalyzed reaction of organostannanes with organic electrophiles, such as aryl halides, is generally known as the Stille reaction.[17,18] The reaction is particularly valuable for the regiospecific coupling of functionalized substrates, as it is tolerant of a wide range of functional groups.[19,20] In its most common form, the Stille reaction consists of the coupling of an aryl halide with a trialkylarylstannane or a tetraarylstannane, as shown in **Scheme 1-5**:



Scheme 1-5: General scheme of the Stille reaction.

In the reaction depicted in **Scheme 1-5**, R and R′ are any substituent on an aryl group and R is usually methyl, butyl or phenyl. The R_3SnX byproduct – a trialkyltin halide – must be removed from the reaction mixture to afford the desired biaryl product, which is perhaps the reaction's most significant disadvantage, besides the toxicity of organotin compounds, which can limit the reaction's utility in pharmaceutical synthesis.[21] A fluorous analog of this type of Stille reaction was developed by Curran in 1997.[22] However, the type of palladium cross-coupling reaction of interest in radiopharmaceutical synthesis has not been previously investigated.

Instead of a trialkylarylstannane of the form R_3SnAr, the Stille reaction can also be conducted between an aryl halide and a distannane. This reaction was first investigated in 1981 by Kosugi[20] and independently by Azizian, Eaborn and Pidcock.[19] The general reaction scheme is shown below **(Scheme 1-6)**.

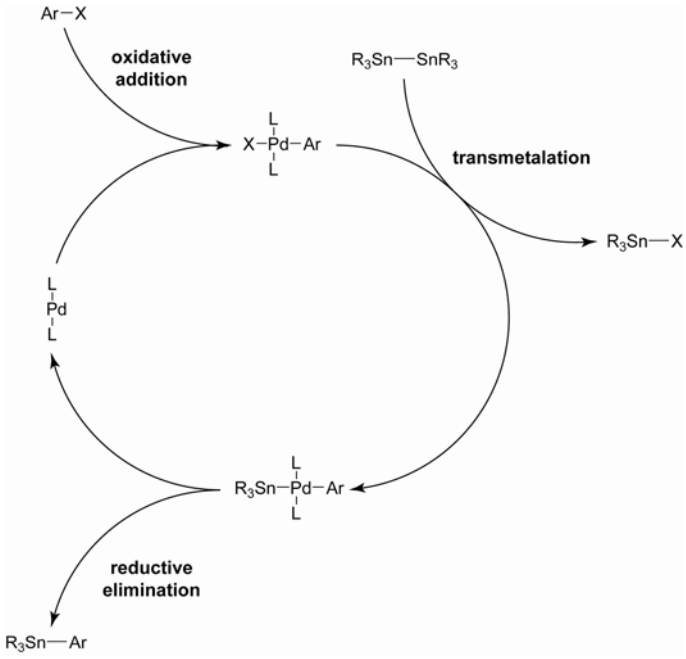


Scheme 1-6: Trialkylstannylation of an aryl halide with a distannane.

The first application suggested for this reaction was the preparation of trialkylarylstannanes which were not accessible *via* methods involving Grignard or other

organometallic reagents.[19] Later, the production of trialkylstannyl precursors for radiohalodestannylation reactions became an important application, since many desirable radiotracers contain functional groups which are incompatible with organometallic reagents but which can be prepared from the trialkylstannylation of an aryl or vinyl halide using a hexaalkyldistannane.[1,2]

A schematic depiction of the mechanism of the Stille reaction, and therefore of the trialkylstannylation reaction, is shown in **Scheme 1-7**. In this diagram, Ar is any aryl species and R is usually methyl, butyl or phenyl. The reaction consists formally of an oxidative addition of an aryl (or vinyl) halide to a palladium(0) species, followed by a transmetallation step wherein a Pd^{II}-Sn bond is formed with attendant loss of a tin halide, R_3SnX. The final step is a reductive elimination of R_3SnAr from the Pd^{II} center to give the product and regenerate the Pd^0 catalyst. The PdL_2 species depicted is formed from PdL_4; this ligand dissociation must take place before the reaction can commence.[18,23,24]



Scheme 1-7: A schematic overview of the mechanism of the Stille reaction. (After Espinet, 2004.)[18]

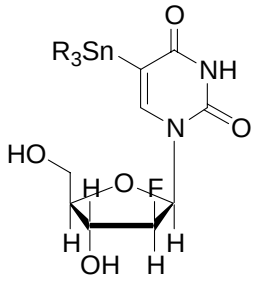
It should be stressed that **Scheme 1-7** merely provides an overview of the reaction's principal steps. The precise mechanism by which each of these steps takes place is not entirely understood, particularly because competing mechanisms exist for each step, and the reaction may proceed by entirely different pathways depending on the substrates and reaction conditions.[18] For example, the transmetallation step is usually rate-limiting, but under certain conditions the oxidative addition step or even the reductive elimination can be rate-limiting. The stereochemistry of the intermediates is also dependent on reaction conditions. Oxidative addition gives a *cis* product that

isomerizes to the more stable *trans* intermediate. Transmetallation may proceed *via* either an open or a cyclic transition state. Reductive elimination must proceed from a *cis* intermediate; the cyclic transition state gives the *cis* intermediate directly, while the open transition state gives a *trans* intermediate that must isomerize before reductive elimination can occur.[18] A thorough review published by Espinet *et al*. in 2004 describes the state of the knowledge of the reaction's mechanism in that year, and builds on the earlier work reviewed by Stille himself in 1986.[17,18]

1.7 Goals

The objective of this thesis was to develop a method of producing fluorous trialkylstannyl compounds based on a palladium cross-coupling reaction between an aryl or vinyl halide and a fluorous distannane, R_3SnSnR_3 (**1**, R = $CH_2CH_2(CF_2)_5CF_3$). This method will allow for the preparation of functionalized radiopharmaceutical precursors not easily attainable by existing methods of introducing the fluorous group. The first stage of development, described in Chapter 2, involved the synthesis of a fluorous distannane. Next, a general cross-coupling and purification protocol was developed using a small number of target compounds. Then, the scope of the reaction

was investigated by preparing a library of model trialkylarylstannanes. In Chapter 3, the application of this methodology to the synthesis of two clinically relevant radio-pharmaceutical precursors is discussed. One of these targets, a fluorous trialkylstannyl precursor of fialuridine, FIAU, is shown in **Figure 1-3**.



R = $CH_2CH_2(CF_2)_5CF_3$

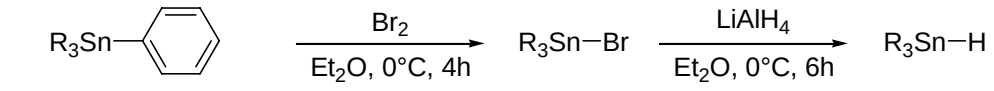
Figure 1-3: A fluorous fialuridine precursor.

Chapter 2

Synthesis of Model Compounds

2.1 Synthesis of a fluorous distannane

In order to develop a fluorous trialkylstannylation procedure, it was first necessary to develop a means of preparing a fluorous distannane. Most distannanes are prepared from trialkyl or triaryl halides or hydrides.[21] Consequently, the first objective was to prepare fluorous trialkyltin bromide **3** and tin hydride **4** from the commercially available starting material **2a**, according to a procedure reported by Curran *et al.* **(Scheme 2-1)**.[25]



R = -$CH_2CH_2(CF_2)_5CF_3$

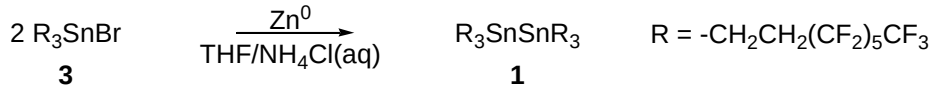
2a **3** **4**

Scheme 2-1: Synthesis of a fluorous trialkyltin hydride.

Trialkyltin bromide **3** was prepared from **2** by adding an excess of bromine as a solution in diethyl ether to a solution of **2** in diethyl ether at 0°C. Liquid-liquid extraction of a solution of the crude product using dichloromethane and FC-72 (perfluorohexanes) afforded **3** in 97% yield. The bromide can be used without further purification in the synthesis of tin hydride **4**, which is achieved by reduction of the bromide with lithium aluminum hydride. The reaction is conducted in diethyl ether at 0°C. Destruction of excess lithium aluminum hydride with isopropanol, methanol, water and sodium potassium tartrate solution followed by extraction of the aqueous layer with diethyl ether yielded a crude product which was purified by column chromatography using a gradient from 0 to 10% hexanes:EtOAc to afford **4** in 82% yield.

The first method attempted for the synthesis of a fluorous distannane was based on a literature procedure for the synthesis of asymmetric distannanes *via* a Wurtz coupling reported by Marton *et al.*[26] This procedure involved the reaction of **3** in THF and saturated ammonium chloride in the presence of zinc metal **(Scheme 2-2)**. This reaction gave a mixture of fluorous products; analysis by ^{119}Sn NMR indicated the presence of unreacted **3**, among other impurities. Because of the difficulty in

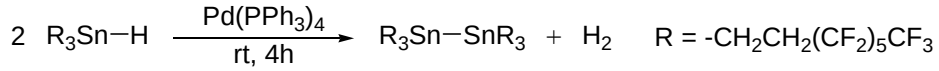
purifying mixtures of fluorous compounds, this method was abandoned in favour of a method capable of producing **1** in higher yield.



Scheme 2-2: Attempted synthesis of a fluorous distannane from trialkyltin bromide in the presence of zinc metal.

Dehydrogenative coupling of a trialkyltin hydride is perhaps the most effective and reliable method of preparing symmetric distannanes **(Scheme 2-3)**.[21,27-29] The reaction is driven by the evolution of hydrogen and quantitative yields are typically obtained.[21] It is also advantageous that a palladium catalyst is used; since these catalysts are also used for cross-coupling reactions, it is possible to prepare the distannane *in situ* before a cross-coupling reaction. The initial choice of catalyst was $Pd(PPh_3)_4$, since this compound was reported to have a higher turnover number than other catalysts such as $Pd(OAc)_2$ and $Pd(PPh_3)_2Cl_2$.[28,29] According to literature precedent, 6 mol% (with respect to **4**) was used, as optimal yields were obtained in previous experiments with amounts in this range.[21,24,27] Analysis of the reaction mixture by ^{1}H and ^{119}Sn NMR indicated complete consumption of **4** after four hours. The distannane can be isolated by extraction of the reaction mixture with FC-72. Isolation was performed to

characterize the distannane and for the first few cross-couplings. However, formation of the distannane *in situ* is more convenient and this method was used subsequently.



4 **1**

Scheme 2-3: Synthesis of **1** by dehydrogenative coupling.

The ^{1}H NMR spectrum of **1** in FC-72 shows a multiplet at 2.479 ppm corresponding to the methylene group in the β-position relative to the tin atom; a triplet with ^{119}Sn satellites at 1.594 ppm corresponds to the -methylene group. The ^{13}C NMR spectrum contains signals at 27.94 ppm and -1.47 ppm for the β-methylene and -methylene groups, respectively. These signals are all typical of 1H,1H,2H,2H-perfluorooctyl compounds in general and cannot conclusively establish the identity of the product as hexa(1H,1H,2H,2H-perfluorooctyl)distannane. However, the ^{119}Sn chemical shift of -58.8 ppm is typical of a distannane **(Figure 2-1)**.[21]

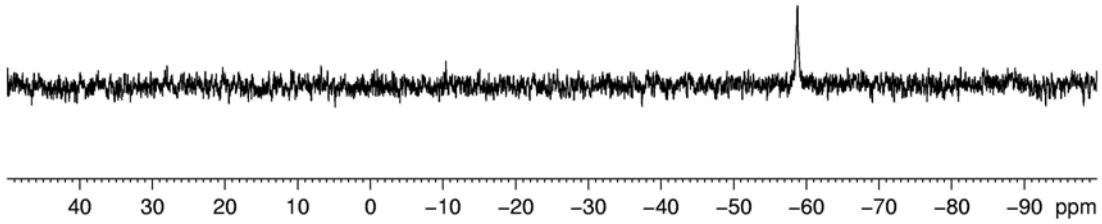
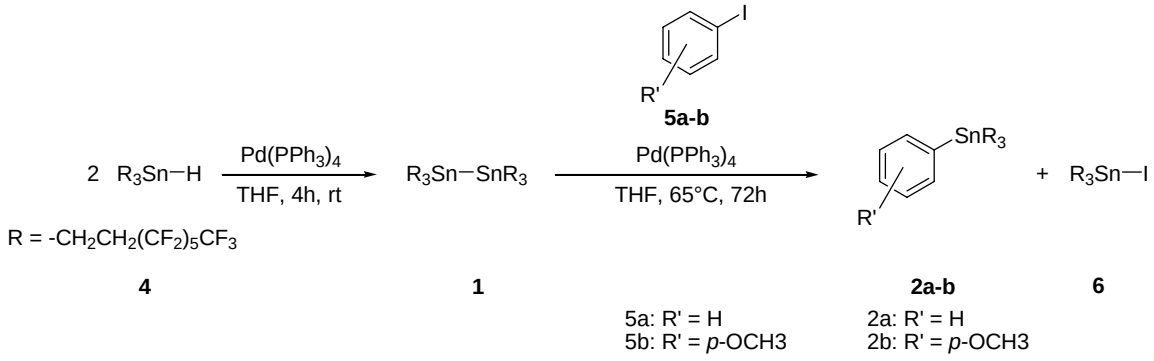


Figure 2-1: ^{119}Sn NMR spectrum of fluorous distannane **1**.

2.2 Preliminary cross-coupling reactions

Before attempting the synthesis of radiopharmaceutical precursors or a library of model compounds, it was necessary to develop a general procedure for the cross-coupling reaction. Preliminary cross-couplings were performed with **5a** and **5b** as substrates **(Scheme 2-4)**, since **2a** was commercially available and **5b** was expected to give high yield owing to its electron-donating substituent.



Scheme 2-4: Cross-coupling reactions.

The reaction conditions were based on literature precedent for cross-coupling reactions between aryl halides and hexabutyldistannane.[19,20] The catalyst chosen was $Pd(PPh_3)_4$, which offers marginally better performance than similar triarylphosphine catalysts such as $Pd(PPh_3)_2Cl_2$.[30] The amount of catalyst used, 6 mol% (with respect to the tin hydride) was taken from the literature. Tetrakis(triphenylphosphine)palladium(0) is moderately air-sensitive, and it is oxidized over time if it is handled in air. The initial supply of $Pd(PPh_3)_4$ oxidized to a brown powder without catalytic activity over several months of frequent handling. Thereafter, portions of the catalyst were transferred from the reagent bottle to 2 mL glass vials in a glove bag under nitrogen, weighed, sealed with Parafilm and stored in a freezer until use.

The choice of solvent was also based on literature precedent. Toluene was used as a solvent in the older literature, perhaps because of its higher boiling point, but it gave poor results when attempted here. There is some evidence that solvent coordination to palladium is involved in the mechanism of the Stille reaction.[31] For this reason, polar solvents such as N,N-dimethylformamide (DMF) are now generally favoured.[32] Fluorous compounds are highly lipophilic and are generally not soluble in DMF.

Tetrahydrofuran was used as the solvent for the preliminary cross-coupling reactions shown in **Scheme 2-4**. The low boiling point of THF (65°C) requires longer reaction times, but it dissolves most fluorous compounds and is easily evaporated. Conducting the reaction in toluene or 1,4-dioxane did not afford substantially higher yields than THF, despite the higher boiling points of those solvents.[24]

A final variable that was investigated was the choice of the ratio of distannane to aryl halide. Trialkylstannylation reactions can afford significant quantities of biaryl compounds produced by transmetallation of the Ar-Pd-X intermediate by R_3SnAr rather than the distannane.[18,20] Addition of excess distannane, usually in a 2:1 or 4:1 ratio, can deter formation of biaryl compounds. However, the fluorous distannane under investigation is more costly than hexabutyldistannane, and any improvement in yield resulting from the use of excess distannane would need to be justified in terms of overall economy of the fluorous reagent. The use of a twofold excess of distannane was attempted in the synthesis of **5c** (R′ = *p*-Me). With one equivalent of distannane, **5c** did not afford isolable product, but with two equivalents a 60% yield was obtained. This improvement in yield is likely associated with an increased rate of transmetallation, which is often the rate-determining step of the Stille reaction. For all other

reactions, 0.9 equivalents of the aryl iodide were used, with respect to the distannane.[18,24]

The reaction mixtures were heated at reflux temperature for 72 hours, even if the reaction mixtures underwent a colour change before this time had elapsed. It later became possible to monitor the progress of the reaction by GC, in which case experiments were worked up when complete. Upon cooling to room temperature, the reaction mixtures were extracted with three 3 mL portions of FC-72 and the fluorous layer was dried over sodium sulfate and concentrated to afford, typically, a colourless or yellow oil.

Extraction of the reaction mixture into FC-72 afforded a crude product nominally consisting of a 1:1 mixture of the desired product and the fluorous trialkyltin halide **3** or **6**. Analysis of several organic layers by GC following extraction showed that loss of product due to incomplete partitioning into the fluorous layer was negligible. Isolation of the trialkylarylstannane component of this mixture was not facile. Several methods of converting the halides to a species more readily removed by column chromatography were attempted. Conversion of the trialkyltin iodide or bromide to a polymeric trialkyltin fluoride was unsuccessful, as the desired product was destroyed

along with the byproduct. Hydroxide ion, which converts the trialkyltin halide to hexaalkylstannoxane in equilibrium with trialkyltin hydroxide, did not destroy the product, but the byproduct was not completely converted to the stannoxane even after prolonged stirring.

Once optimized, column chromatography proved to be the most practical method of purifying the products; it was particularly effective with products bearing polar substituents, since these have significantly longer retention times than the tin halide. With less polar products, breakthrough of the tin halide into the fractions containing product was sometimes still observed, necessitating repetition of the purification step. The yields of **2a** and **2b** were 59% and 51%, respectively. A more detailed discussion of the characterization of these compounds and the synthesis of a larger library of model compounds will be found in the next section.

2.3 Preparation of a library of fluorous trialkylarylstannanes

After a viable method of producing a fluorous distannane had been developed and before attempting the synthesis of fluorous radiopharmaceutical precursors, the scope of the fluorous trialkylstannylation reaction was investigated (**Scheme 2-4**,

Table 2-1). The reaction was attempted with seventeen different aryl bromides and iodides leading to fifteen potential products (for R = H and R = *p*-MeO, both the bromide and iodide were used) **(Table 2-1)**. The yields reported are the average of two trials. Where available, literature yields for the analogous tributylarylstannanes are given. In certain cases, however, only the GC yields are available in the literature.[19] In the present work, when the reaction mixtures were analyzed by GC, complete consumption of the aryl halide was typically observed. Comparison between GC yields and isolated yields for the fluorous compounds suggests that there was a significant loss of product during purification. Although the data available for comparison are limited, similar losses may have occurred during purification of the tributylstannyl analogs (for example, see **Table 2-1**, Entry 7).

Entry	Aryl halide	R =	X =	Product	Isolated yield (*n* = 2)	Literature yield (Bu_3SnAr)
1	**5a**	H	I	**2a**	59	96[a] (GC)
2	**5b**	p-OCH_3	I	**2b**	51	81[a] (GC)
3	**5c**	p-Me	I	**2c**	0 (1 equiv. **1**) 60 (2 equiv. **1**)	76[a] (GC)
4	**5d**	p-Et	I	**2d**	37	
5	**5e**	p-Cl	I	**2e**	42	59[a] (GC, ArBr)
6	**5f**	p-COOH	I	**2f**	30	
7	**5g**	p-CN	Br	**2g**	15	57[a] (GC), 22[b]
8	**5h**	p-NO_2	I	**2h**	59	0[a] (GC) 38[b] (ArBr)
9	**5i**	m-COOEt	I	**2i**	24	
10	**5j**	m-OH	I	**2j**	38	
11	**5k**	o-CH_3	I	**2k**	22	64[a] (GC, ArBr)
12	**5l**	o-CN	Br	**2l**	28	42[b]
13	**5m**	o-OH	I	**2m**	0	
14	**5n**	p-NMe_2	Br	**2n**	0	
15	**5o**	o-NH_2	Br	**2o**	0	
16	**5p**	H	Br	**2a**	27	79[a] (GC)
17	**5q**	p-OCH_3	Br	**2b**	21	

[a] GC yield from Azizian *et al.*[19] Conditions: 2 equiv. Bu_6Sn_2, 0.65 mol% $Pd(PPh_3)_4$, 15 h at 115°C in toluene. Reactions where the aryl bromide was used in the cited work are marked 'ArBr'.

[b] Isolated yield from Kosugi *et al.*[20] Conditions: 1.2 equiv. Bu_6Sn_2, 1 mol% $Pd(PPh_3)_4$, 80°C in toluene until precipitation of Pd^0. Aryl bromides were used exclusively; reactions where the aryl iodide was used in the present work are marked 'ArBr'.

Table 2-1: Substituents and yields for the library of model compounds.

Reactions were performed in parallel using a Radleys Discovery Technologies carousel, which allows up to twelve reactions to be conducted at reflux under an inert atmosphere. Initially, each set of reactions included reactions with **5b** as an internal control in the carousel and an external control in a round-bottom flask. This was done to monitor the yields between sets of experiments and establish whether decomposition of the catalyst was affecting yields. It also allowed comparison of the yields obtained with the carousel with those obtained with reactions in a round-bottom flask. The use of the control reactions was discontinued once it was demonstrated that reactions in the carousel afford yields similar to reactions in round-bottom flasks and that the catalyst's activity was not decreasing over time when the compound was taken from small vials filled under nitrogen.

Similar reaction conditions were used for all experiments. The solvent was THF freshly distilled over sodium-benzophenone and sparged with nitrogen for several minutes before use. The carousel was flushed with nitrogen for several minutes before reagents were added, and a positive pressure of nitrogen was maintained throughout the reaction. The reaction tubes were heated at reflux for 72 hours. Most

reaction mixtures changed colour from yellow to brown or black before 72 hours had elapsed. The colour change is caused by precipitation of palladium(0) resulting from ligand dissociation. This can signify complete consumption of the aryl halide, but it can also result from poisoning of the catalyst by oxygen.[19,20,22] In the attempted syntheses of **2n** and **2o**, the reaction mixture turned black almost immediately, and no product was isolated or observed by ^{1}H NMR, leading to the conclusion that the catalyst was poisoned by amino groups. During the synthesis of the model compounds, no attempt was made to replenish the catalyst following precipitation of palladium(0), as it was expected that the presence of palladium black would interfere with the active catalyst or promote its decomposition.

On completion of each set of reactions, the reaction mixtures were extracted 3 times with 3 mL of FC-72. The fluorous layer was dried with sodium sulfate and concentrated to afford, typically, a yellow or colourless oil. The crude products, consisting of the desired product and tris(1H,1H,2H,2H-perfluorooctyl)tin halide, were purified on a Biotage SP1 automated purification system. The stationary phase was silica gel contained in a cartridge (1.5 × 120 mm). Gradient elution was performed beginning with 3 column volumes (CV) of hexanes increasing to 0, 2, 5 or 10 percent ethyl ace-

tate over 10 CV, followed by 3 CV at the final solvent strength. For **2f**, diethyl ether was used as the strong solvent and the final composition was 1:1 hexanes:diethyl ether. To identify the fractions containing product, the ultraviolet absorbance of the eluent was monitored at two wavelengths corresponding to the $_{max}$ of the product being purified.

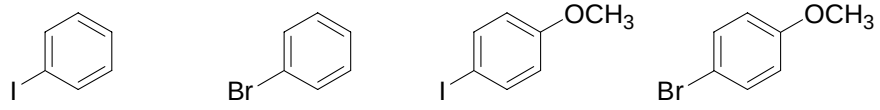
The purification method described above gave the best results, in terms of removal of the tin halide byproduct, for polar products having longer retention times. For non-polar products such as **2a** and **2c**, retention times were short even in hexanes, and further purification was sometimes necessary. This was done either by a second purification run on a Biotage SP1 automated purification system or with a Pasteur pipette packed with silica gel.

To avoid contamination of the products, only glass syringes were used and the use of silicone grease was avoided. A small impurity, consisting of small multiplets typical of alkanes at δ = 0.88 and 1.26 ppm, was occasionally evident in ^{1}H NMR spectra. The impurity could be removed by column chromatography using silica gel in a Pasteur pipette using 0-10% ethyl acetate in hexanes as the mobile phase. It could also be removed by FSPE using methanol as a fluorophilic solvent. Commercial **2b** was

used without further purification because the tin bromide **3** and tin hydride **4** produced from it were free of the impurity.

The isolated yields obtained for the library of model compounds are given in **Table 2-1**. It should be noted that it is not entirely clear whether modest yields should be attributed to the reaction itself or to the purification process, as will be discussed later.

Aryl iodides afford higher yields than aryl bromides, as demonstrated in **Figure 2-2**. The bond dissociation energy of the C-I bond in iodobenzene is 272 kJ·mol^{-1}, while that of the C-Br bond in bromobenzene is 336 kJ·mol^{-1}.[33] The weaker C-I bond promotes oxidative addition to palladium.[24] This trend, unlike the other trends observed, is quite unambiguous.



5a	**5p**	**5b**	**5q**
59%	27%	51%	21%

Figure 2-2: Yields obtained with aryl iodides vs. aryl bromides.

Yields for compounds which illustrate electronic effects are shown in **Figure 2-3**. These compounds are those with *meta* or *para* subsituents and are all prepared

from aryl iodides. The highest yields are observed for the extremes in electron-donating and electron-withdrawing properties of the substituent, although, anomalously, the yield with **5a** (R = H) is 59%, the highest yield of any compound. Slightly higher yields were obtained for electron-donating substituents compared to electron-withdrawing substituents, but compound **5h** gives a yield as high as **5a**. These observations are consistent with the trends in yields reported in the literature.[19,20]

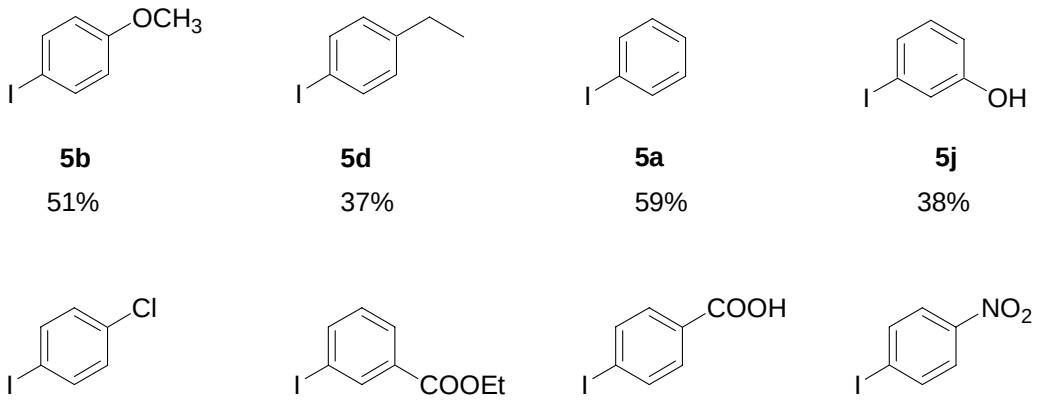


Figure 2-3: Yields illustrating the effect of the electronic properties of the substituent.

It is again important to stress that the comparison in isolated yields introduces the percent recovery of the purification method as a variable. This is particularly true in light of the relatively small differences in yields under identical reaction conditions. Electronic effects likely do contribute to yield to a certain extent, but it should be re-

membered that the worst yields were scarcely less than half the best ones. When GC yields were compared, the differences between substituents were minimal, and greater than 95% consumption of the aryl halide was observed with all substituents.

Steric effects are examined in the series shown in **Figure 2-4**. It was initially proposed that *ortho* substituents might disfavour homocoupling; however, when reactions were monitored by GC, the formation of biaryl compounds was not observed.[19,20] It is therefore not clear why the *ortho*-substituted benzonitrile and toluene derivatives afforded higher yields than the *para*-substituted compounds. Also, the *meta*-substituted compound afforded higher yields than the *ortho* compound for the hydroxyl group. Although the substituents are not exactly the same, **5f** and **5i** have a similar substituent with identical Hammett values for a given position of substitution. The yield is slightly higher for the *para*-carboxyl group than for the less electron-withdrawing ethyl *meta*-carboxylate ester.

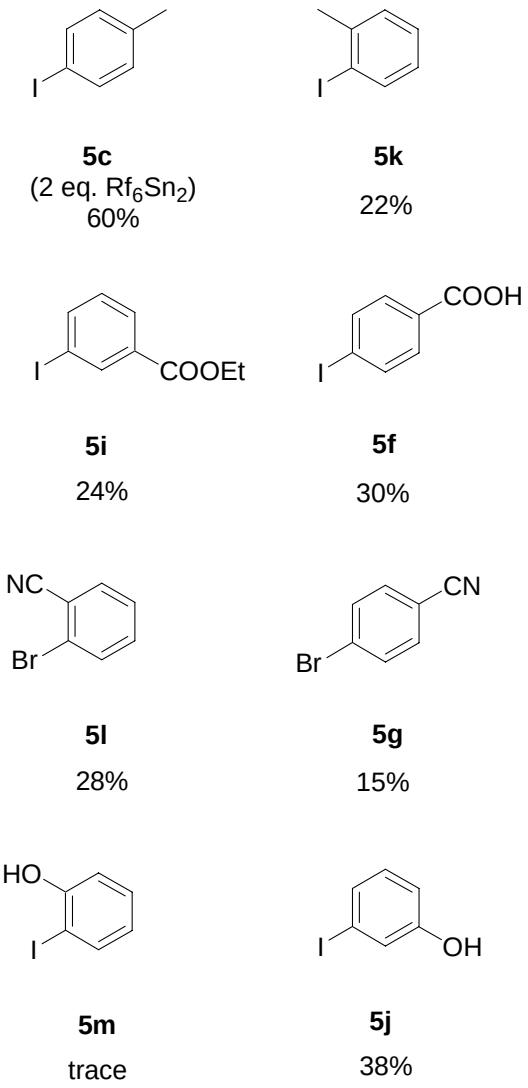


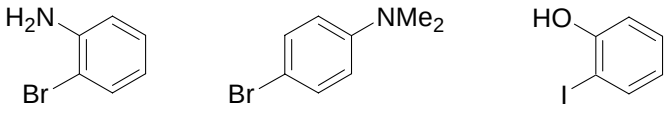
Figure 2-4: Yields illustrating the steric effect of the substituent.

It is only in the oxidative addition step that an *ortho* substituent might decrease the rate of a reaction by steric hindrance. It is possible that *ortho* substituents might favour ligand dissociation in the transmetallation step.[18,24] Alternatively, the effect of

the position of substitution may be more electronic than steric. This might explain the higher yields obtained for electron-withdrawing substituents in the *ortho* position. That is, a substituent in the *ortho* position may favour the reaction because it does not disfavour it so much as the same substituent in the *para* position. The number of compounds tested is not sufficient to provide a widely applicable conclusion on either the electronic or steric effect of the substituent, and further investigation is clearly required.

Two products, **2n** and **2o**, could not be isolated or detected in the reaction mixture by ^{1}H NMR **(Figure 2-5)**. It is possible that the catalyst was poisoned by amino compounds, at least when the catalyst is $Pd(PPh_3)_4$.[19,20] This could represent a significant limitation in the scope of the reaction, particularly because of the prevalence of amino groups in potential target molecules. However, the apparent immediate poisoning of the catalyst did not occur in later experiments with a different catalyst, and evidence for formation of the product was obtained by HPLC. Poor yields were also obtained for **2m**. Purification of reaction mixtures containing **2m** typically afforded about 5% yield. This is insufficient to verify the purity of the product by ^{1}H

NMR on the scale typically used for the reaction. Therefore, **2m** was not isolated and characterized, although there was evidence that the product was formed.

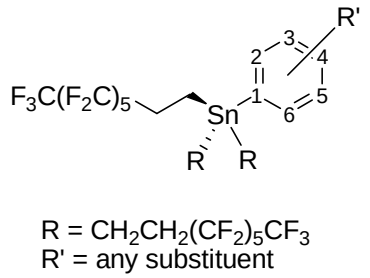


5n	**5o**	**5m**
0%	0%	trace

Figure 2-5: Substituents giving no yield or trace yield.

Characterization of the products was performed by ^{1}H, ^{13}C and ^{119}Sn NMR, IR, MS and GC and/or HPLC. The numbering scheme used is shown in **Figure 2-6**. The ^{1}H NMR spectra show two distinctive signals corresponding to the perfluoroalkyl groups. A multiplet, appearing at 2.295 ppm in the ^{1}H NMR spectrum of **2b** in $CDCl_3$, corresponds to the methylene group adjacent to the perfluorohexyl group. A triplet with ^{119}Sn satellites appears at 1.276 ppm in **2b**; this corresponds to the methylene group adjacent to tin. In *para*-substituted compounds, the signal corresponding to the 2′ and 6′-protons has ^{119}Sn satellites. This signal appears at 7.297 ppm in **2b**; satellites are also observed on the appropriate signals for *ortho-* and *meta*-substituted compounds. These satellites, along with the appearance of the perfluoroalkyl group, demonstrate conversion of the aryl halide to the expected product. The absence of tin satellites aids

in the assignment of the 3′ and 4′-proton signals in *para*-substituted compounds, which appear at 6.979 in **2b**. Those substituents which can be identified in ^{1}H NMR appear at the appropriate frequency; for example, the methoxy group in **2b** is a singlet at 3.824 ppm. When necessary, as in the case of *ortho* compounds, assignment was aided by the COSY experiment. The ^{1}H NMR spectrum of **2b** is shown below as a representative example (**Figure 2-7**).



R = $CH_2CH_2(CF_2)_5CF_3$
R' = any substituent

Figure 2-6: Numbering scheme for the model compounds.

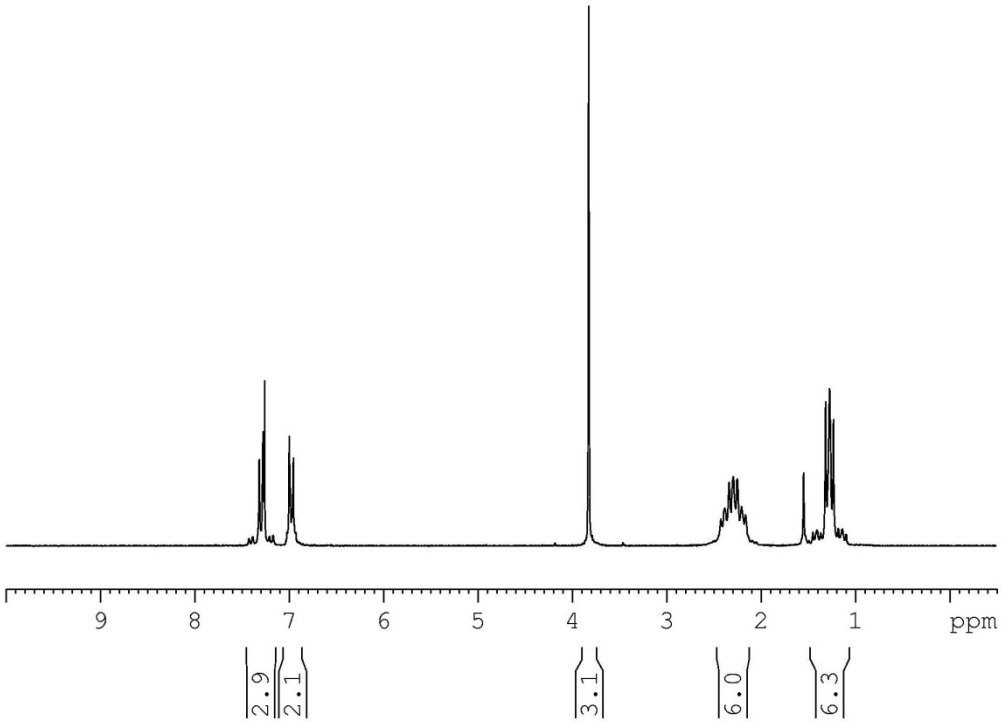


Figure 2-7: ^{1}H NMR spectrum of tris(1H,1H,2H,2H-perfluorooctyl)stannylanisole (**2b**, 200.16 MHz, $CDCl_3$).

In carbon-13 NMR, the perfluoroalkyl groups typically gave a signal below 0 ppm for C-1 (e.g. -1.32 ppm for **2b**) and around 30 ppm for C-2 (23.4 ppm for **2b**). Depending on signal intensity, $^{1}J_{13C\text{-}119Sn}$ coupling was sometimes observed for C-1. The C-2 signal is a triplet, since there is a two-bond coupling to fluorine-19. Coupling to ^{19}F is extensive for the carbon atoms in the perfluorohexyl group. In a ^{1}H-decoupled

^{13}C NMR spectrum, weak, overlapping signals are observed. As is the norm in the fluorous chemistry literature, these signals were not assigned, although it would be possible to resolve them using ^{19}F decoupled ^{13}C NMR spectroscopy or to enhance their intensity by running an INEPT experiment. The aromatic carbon atoms appeared at typical frequencies, and all quaternary carbon atoms could be identified by their relative intensities. The chemical shifts for C-1′ through C-4′ in **2b** are 137.31, 137.44, 115.11, and 161.03, respectively. When necessary, HSQC and HMBC experiments were used to aid in the assignment of signals of ambiguous identity. To eliminate undesired curvature in the baselines of ^{13}C NMR spectra, the spectra were processed using linear backward prediction as discussed in **Section 5.1**. The ^{13}C NMR spectrum of **2b** is shown below as a representative example **(Figure 2-8)**.

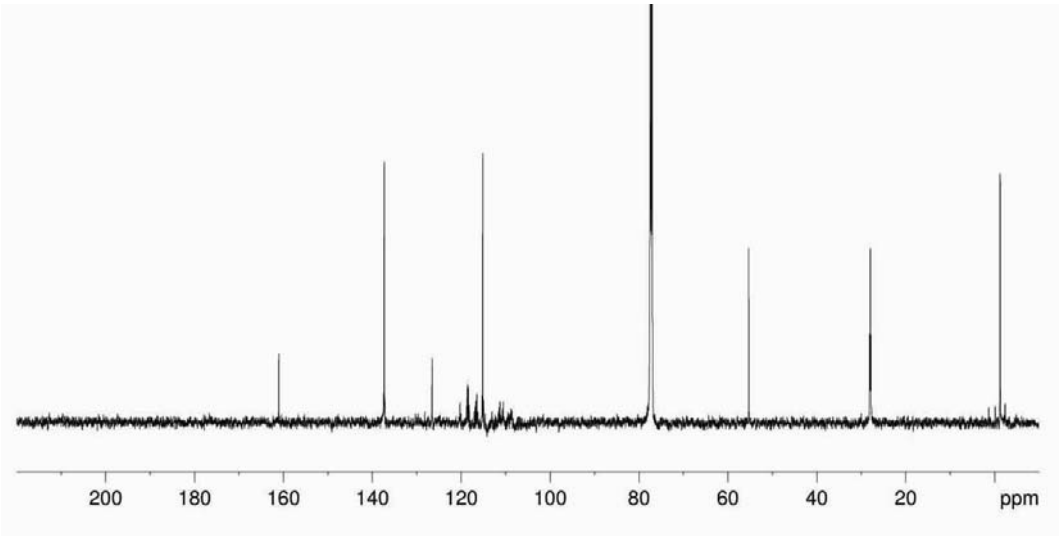


Figure 2-8: ^{13}C NMR spectrum of tris(1H,1H,2H,2H-perfluorooctyl)stannylanisole (**2b**, 150.90 MHz, $CDCl_3$)

Tin-119 NMR spectroscopy was useful in demonstrating the conversion of **4** to the product. Chemical shifts in ^{119}Sn NMR vary widely and depend on the nature of the substituents on tin. The ^{119}Sn chemical shift of tin hydride **4** is 101.3 ppm, while those of **2a-m** range from -29.4 for **2a** to -23.7 for **2l** (-25.6 ppm for **2b**). These chemical shifts fall within the expected range for trialkylarylstannanes, allowing identification of the products as compounds of that class. Tin-119 has a higher natural abundance than carbon (8.59% vs. 1.07%) and ^{119}Sn has a receptivity in NMR 26.6 times that of ^{13}C.[34] Acquisition of ^{119}Sn NMR spectra is therefore more rapid than for ^{13}C spectra, but the sensitivity of ^{119}Sn NMR spectroscopy is low enough that the technique is of

limited utility in determining the purity of a sample. The ^{119}Sn NMR spectrum of **2b** is shown below as a representative example **(Figure 2-9)**:

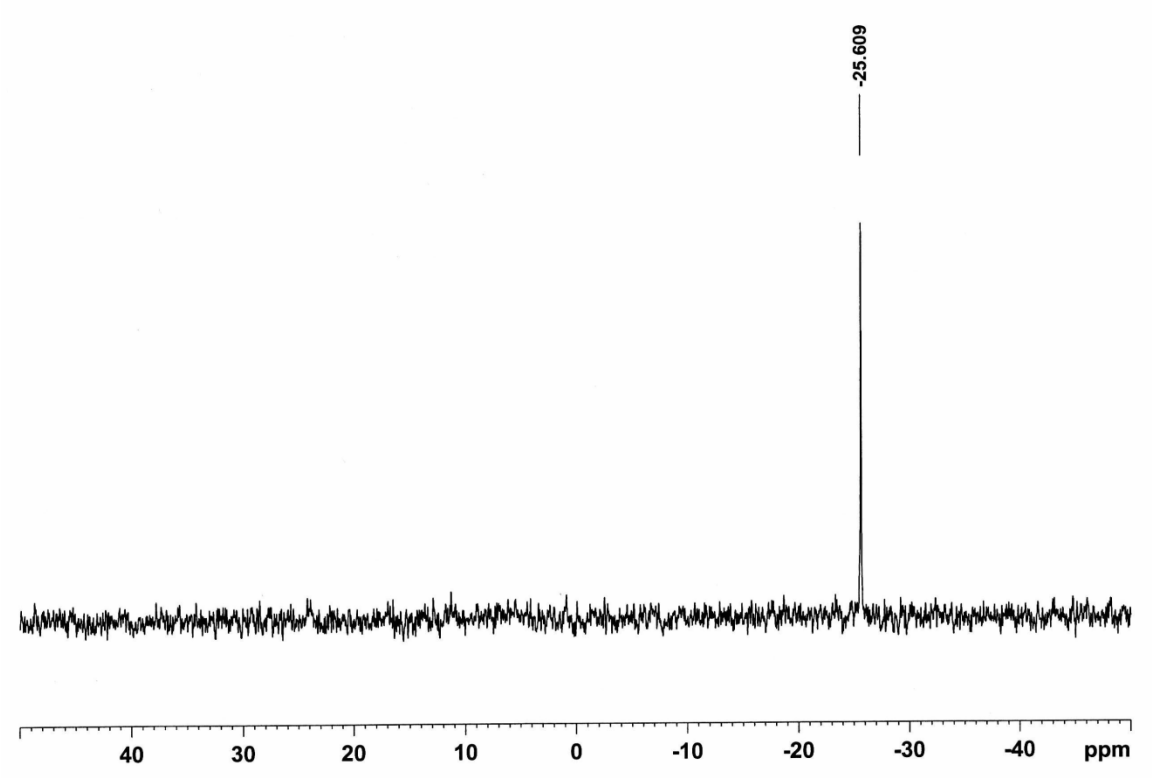
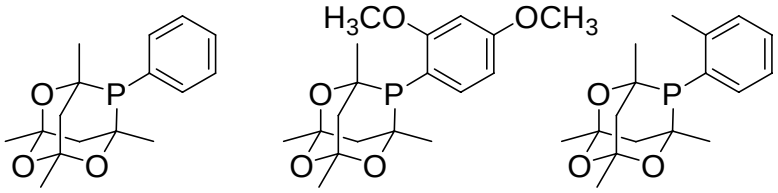


Figure 2-9: ^{119}Sn NMR spectrum of tris(1H,1H,2H,2H-perfluorooctyl)stannylanisole (**2b**, 223.79 MHz, $CDCl_3$).

Electrospray mass spectrometry (ESMS) was used to confirm the molecular weights of the products. The best results were usually obtained with the negative ion mode with ammonium acetate as an additive. Under these circumstances, the products were observed as the acetate adduct. In other cases, better results were observed

with the trifluoroacetate adducts. Occasionally, formate adducts and occasionally $[M-H]^-$ ions were observed, likely due to contamination of the instrument. For all compounds **2a-2l**, high-resolution time-of-flight (TOF) ESMS gave the expected signals within 5 ppm of the calculated *m/z* ratio.

2.4 Palladium trialkylphosphine catalysts for the Stille reaction



7a **7b** **7c**

Figure 2-10: Three of the phenylphosphaadamantane ligands developed by Capretta *et al.*

A series of sterically bulky, electron-rich, air-stable phosphine ligands based on a phenylphosphaadamantane framework were recently reported by Capretta *et al.*[35-37] Three members of the series – those which have been used in the present work – are shown in **Figure 2-10**. These ligands have been successful in catalyzing Suzuki and Sonogashira reactions. They had not been used previously in the Stille reaction, but were of interest because they are air-stable and have tunable properties.[35] By modify-

ing the substituents on the phenyl group, the electronic and steric properties of the ligand can be tuned; for example, a 2,4-dimethoxyphenyl group leads to a more electron-rich phosphine. In a Suzuki reaction, as an example, yields varied from 5% with ligand **7a** to 96% for ligand **7b**.[37]

Electron-rich ligands are favourable in palladium cross-coupling reactions, including the Stille reaction. Electron-rich phosphines enhance the rate of the reaction by accelerating the transmetallation step, which is usually rate-determining, because they undergo the necessary dissociation from palladium more rapidly.[38] Sterically hindered groups lead to a larger cone angle for the phosphine ligand, which may also accelerate ligand dissociation in the transmetallation step.[24,32] A well-known example of an electron-rich, bulky phosphine which accelerates palladium cross-coupling reactions is tris(*tert*-butyl)phosphine, $^{t}Bu_3P$.[32] This compound is pyrophoric, but Fu and Netherton have reported on the *in situ* generation of $^{t}Bu_3P$ from the air-stable tetrafluoroborate salt in the presence of base.[39] The phosphaadamantane ligands developed by Capretta *et al*. can also be safely handled under air, although reactions using the ligands must be performed under argon in dry, degassed solvents.[37]

Several phosphaadamantane ligands were donated by the Capretta group for use in the preparation of fluorous trialkylarylstannanes and radiopharmaceutical precursors. To determine the most effective catalyst, three phosphaadamantanes were used to catalyze the synthesis of **2k**: the unsubstituted **7a**, the electron-rich **7b**, and the sterically hindered **7c**. The reactions were monitored by GC, and the highest consumption of starting material was observed with **7b**, as was expected considering previous results with the Suzuki reaction.[37] This ligand was therefore used in subsequent experiments.

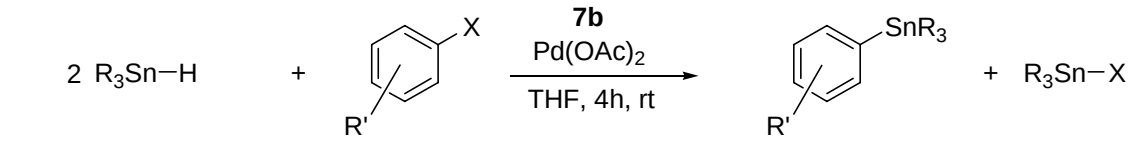
A source of palladium must be added to the reaction mixture along with the ligand. Capretta *et al.* used both tris(dibenzylideneacetone)dipalladium, $Pd_2(dba)_3$, and palladium(II) acetate, $Pd(OAc)_2$, as palladium sources.[37] There is some evidence in the literature that $Pd(OAc)_2$ may be a better source than $Pd_2(dba)_3$ because the former more readily undergoes ligand substitution to form the phosphine complex.[24] Also, the purity of commercial $Pd_2(dba)_3$ is subject to concern. Therefore, $Pd(OAc)_2$ was used primarily. Some experiments with $Pd_2(dba)_3$ were also performed after fluorous trialkyltin acetate was observed by MS, leading to the suspicion that some product was

destroyed by the acetate. However, no change in yield was observed under these conditions.

A final variable in the experiment is the palladium:ligand ratio. The ratios used vary, but x-ray crystallography of the active catalyst has shown it possesses two ligands.[35] The Capretta group has typically used less than two equivalents of ligand with respect to palladium, but a 2:1 ratio was chosen for the present work. Palladium(II) acetate was used at 3 mol% along with two equivalents (6 mol%) of the ligand.

When $Pd(PPh_3)_4$ was used as the catalyst, the tin hydride and catalyst mixture was stirred at room temperature for four hours prior to addition of the halide and the heating of the reaction mixture. With the phosphaadamantane-palladium catalyst, samples analyzed by GC several minutes after addition of the catalyst showed no presence of tin hydride. The reaction mixtures also evolved hydrogen much more vigourously than with $Pd(PPh_3)_4$. Therefore, the delay before addition of the halide was discontinued; the time required to dispense and add the halide and to heat the reaction mixture to reflux appeared to be long enough to allow the distannane to form. With the omission of the four-hour period of stirring at room temperature, the *in situ* formation of the distannane became a one-pot procedure **(Scheme 2-5)**, with all reagents

added in sequence at the beginning of the reaction. The tin hydride was added first, followed by 3 mol% of the phosphaadamantane, then 1.5 mol% of the palladium source, and finally the aryl halide. Once the reaction was at reflux temperature, second, equal portions of the phosphaadamantane and palladium source were added, for a total of 6 mol% of the former and 3 mol% of the latter. If the palladium was added before the ligand, immediate precipitation of Pd^0 was sometimes observed. Likewise, immediate decomposition of the catalyst sometimes occurred when the reaction mixtures were brought to reflux; for this reason, half the catalyst was reserved until the reaction mixture had reached reflux temperature, as described previously. The reactions were performed under an inert atmosphere, as described in more detail in the experimental section.



4 — R = $-CH_2CH_2(CF_2)_5CF_3$

5a-q — X = Br, I

2a-o

6

Scheme 2-5: One-pot trialkylstannylation with a PA/Pd catalyst.

The reactions were monitored by GC, which was superior to the colour change as an indicator of the reaction's end point. When reactions were shown to contain un-

reacted aryl halide after a colour change, further portions of **7b** and $Pd(OAc)_2$ equal to the first portion were added. Subsequent GC analyses demonstrated that the starting material continued to be consumed after replenishment of the catalyst, but not if the brown reaction mixture was heated without additional catalyst.

The isolated yields obtained with the PA-Ph catalyst are shown in **Table 2-2**. The apparent failure of **7b** to improve the yield of the model compounds should not be taken to mean that the phosphaadamantanes lack utility. The rate of the reaction was appreciably faster; most of the starting material was typically consumed within 2 hours at 65°C. Analysis of the reaction mixtures by GC did not conclusively establish the proportion of starting material that was converted to product following its consumption. It was, however, possible to exclude the formation of biaryl species, since no byproducts having retention times similar to a biaryl standard were observed. A mechanism for loss of product during purification was not established. Interestingly, the tin halide byproducts were never recovered as a separate component, although contamination of **2a-l** by the halides was sometimes observed. The reactivity of organotin halides with silanol groups in silica gel has been reported. [40]

Entry	Aryl halide	R =	X =	Product	Isolated Yield (n = 2)	
					$Pd(PPh_3)_4$	$Pd(OAc)_2$/**7b**
1	**5a**	H	I	**2a**	59	67
2	**5b**	p-OCH_3	I	**2b**	51	36
3	**5c**	p-Me	I	**2c**	0 (1 equiv. **1**) 60 (2 equiv. **1**)	24
4	**5d**	p-Et	I	**2d**	37	13
5	**5e**	p-Cl	I	**2e**	42	42
6	**5f**	p-COOH	I	**2f**	30	32
7	**5g**	p-CN	Br	**2g**	15	19
8	**5h**	p-NO_2	I	**2h**	59	54
7	**5i**	m-COOEt	I	**2i**	24	20
10	**5j**	m-OH	I	**2j**	38	34
11	**5k**	o-CH_3	I	**2k**	22	12
12	**5l**	o-CN	Br	**2l**	28	22
13	**5m**	o-OH	I	**2m**	0	0
14	**5n**	p-NMe_2	Br	**2n**	0	0
15	**5o**	o-NH_2	Br	**2o**	0	0
16	**5p**	H	Br	**2a**	27	29
17	**5q**	p-OCH_3	Br	**2b**	21	24

Table 2-2: Comparison of yields obtained with $Pd(PPh_3)_4$ and $Pd(OAc)_2$/**7b**.

The preparation of the model compounds demonstrate that the palladium cross-coupling approach to preparing fluorous trialkylstannyl compounds is viable for a range of functional groups, including those which are not compatible with previous

synthetic methods. However, the yields for the cross-coupling method are generally lower than for methods based on organometallic reagents.[5,6] The nature of the cross-coupling reaction necessitates purification, because of the formation of an equimolar quantity of a tin halide byproduct alongside the desired product. Although HPLC and GC analysis of the crude reaction mixture suggested that the observed yields resulted from loss of product during purification, the mechanism of this loss was not identified. Several techniques for flash chromatography were assessed: the Biotage SP1 purification unit, conventional open glass columns with isocratic and step-gradient elution, and Pasteur pipettes packed with a minimal amount of silica gel. Similar yields were obtained with all methods; the Biotage SP1 was most convenient and permitted the highest throughput, while pipette columns often required repetition of the purification procedure. Reverse-phase column chromatography using an octadecylsilica (ODS or C_{18}) column on the Biotage SP1 and elution conditions derived from the HPLC conditions (elution method B) was also attempted, but yields were lower than those obtained with normal-phase chromatography.

Future work on this methodology may reveal a method which affords yields that more closely resemble those suggested by chromatographic analysis of the reac-

tion mixture. It is certainly worth investigating the possibility of removing the by-product using a non-chromatographic method: a polymer-supported reagent capable of reacting with tin halides. A polymer-bound strong base, which may bind the tin halide as a trialkylstannoxy species, is one possibility. If such an approach could be developed, purification could be performed with only a small column or by filtration. This would be particularly desirable for the synthesis of combinatorial libraries.

An advantage of the intended use of the fluorous products is that extremely small amounts are required. For a typical radiolabeling reaction, the scale is 0.1-0.3 mol of ligand. Consequently, the method reported here provides ample material for the FLS. In the next chapter, the application of the cross-coupling reaction to the synthesis of fluorous radiopharmaceutical precursors will be demonstrated.

Chapter 3

Synthesis of Radiopharmaceutical Precursors

3.1 Overview

After the fluorous distannane had been developed and its reactivity investigated, attention shifted to the preparation of fluorous precursors of radiotracers currently in use. Two radiotracers, fialuridine (FIAU) and idoxuridine (IUdR) were selected because they are challenging to prepare at the tracer level and because the relevant fluorous precursors are only accessible by the palladium-catalyzed trialkylstannylation method and not by previously-reported methods.[5,6]

3.2 Fialuridine (FIAU)

The nucleoside analog 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU, **8**, **Figure 3-1**) was first investigated as an antiviral drug for treatment of herpes simplex, cytomegalovirus and hepatitis B infection. However, its hepatotoxicity caused the deaths of nine patients enrolled in clinical trials between 1991 and 1993.

The trials were discontinued and investigation of the drug as an antiviral at macroscopic doses was abandoned.[41] As a radiopharmaceutical, FIAU continues to attract interest. FIAU provides an illustration of the tracer principle: it poses unacceptable risks at therapeutic doses, but has attractive properties at the tracer level. It is also an example of an agent where high effective specific activity is essential.

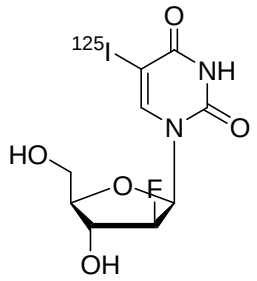


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Figure 3-1: 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-[^{125}I]iodouracil, [^{125}I]FIAU.

Viral and bacterial enzymes display a much higher affinity for FIAU than do human enzymes. For example, FIAU is a substrate for herpes simplex virus type 1 thymidine kinase (HSV1-tk), an enzyme which transfers a phosphate group from adenosine triphosphate to the 5′-hydroxyl group of the substrate.[42] In a manner analogous to [^{18}F]-fluoro-D-glucose, the phosphorylated product is retained in the cell and does not undergo catabolism. [^{124}I]-FIAU and its analog, [^{76}Br]-FBAU, have been used in the PET imaging of transfected tumours expressing HSV1-tk as a reporter

gene.[43] [^{125}I]-FIAU has also been used to image bacterial infections, as it is a substrate for *Escherichia coli* thymidine kinase and that of other pathogenic bacteria.[42] Recently, [^{125}I]-FIAU has been used to image tumours associated with the Epstein-Barr virus (EBV) after administration of bortezomib, which induces EBV thymidine kinase expression. A SPECT-CT image of a murine xenograft with an EBV-positive tumour is shown in **Figure 3-2**.[42]

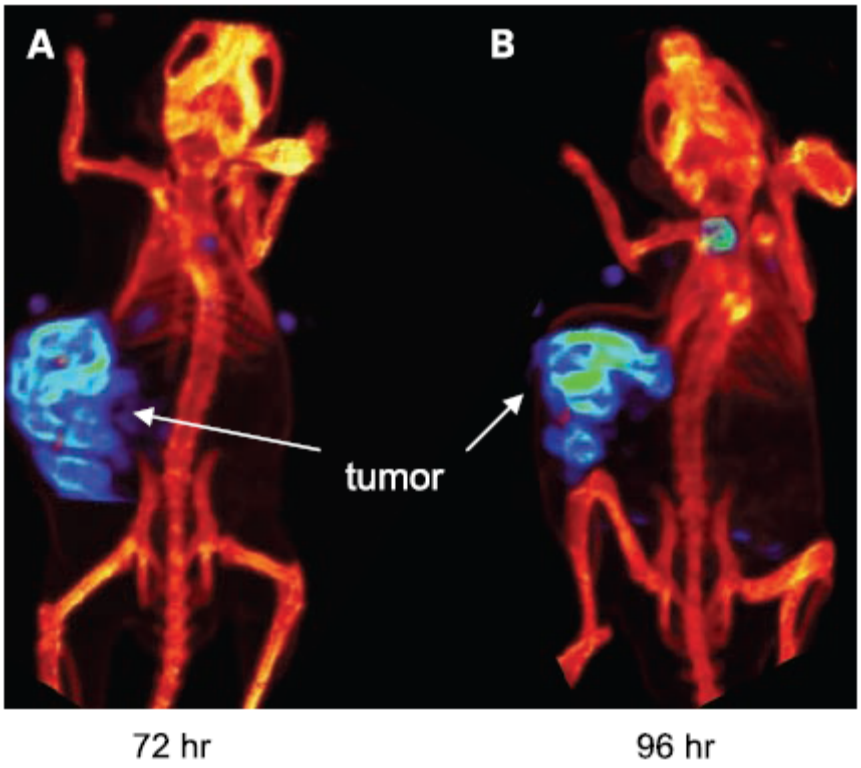
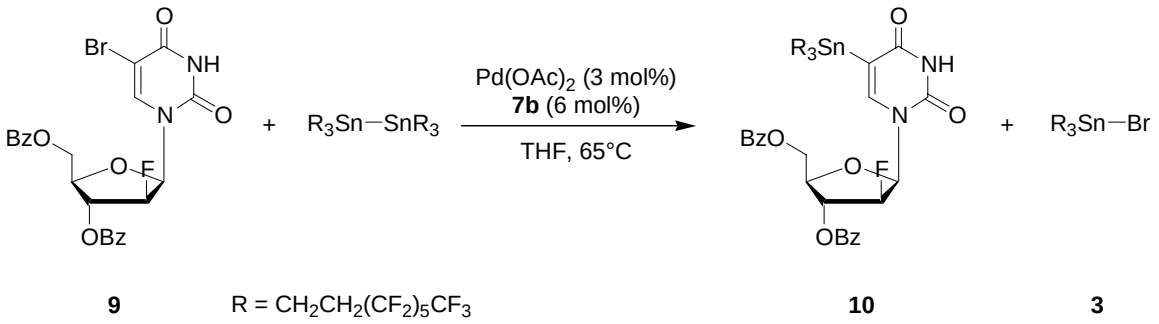


Figure 3-2: SPECT-CT image showing uptake of [^{125}I]-FIAU 72 h and 96 h after injection into a murine xenograft with Epstein-Barr virus positive Burkitt's lymphoma treated with bortezomib 24 h prior to injection of [^{125}I]-FIAU.[42]

3.3 Synthesis of an FIAU precursor

The synthesis of a fluorous precursor of FIAU is shown in **Scheme 3-1**. The synthesis was adapted from previous reports of the synthesis of the trimethylstannyl and tributylstannyl FIAU analogs.[44,45] Several syntheses of the vinyl bromide precursor, **9**, are described in the literature.[44,45] Compound **9** was provided by Dr. Michael Kovacs of the Lawson Health Research Institute at the University of Western Ontario.



Scheme 3-1: Synthesis of a fluorous FIAU precursor, 10.

Preparation of **10** failed when $Pd(PPh_3)_4$ was used as the catalyst. Although some evidence of product formation was obtained by ^1H NMR of the crude reaction mixture, only a trace of product was detected. The poor yield was likely attributable to the reactivity of the substrate. As a vinyl bromide, **9** is expected to be less reactive than an aryl bromide or iodide (see Section 2.3).[19,24] Microwave heating was attempted as a way to drive the reaction to completion in a shorter period of time.[46] A reaction

temperature of 160°C and a reaction time of 6 minutes was selected as equivalent to 72 hours at 65°C. The low boiling point and relatively small dipole moment of THF means that it was not suitable for microwave-assisted reactions, so the reactions were conducted in *N,N*-dimethylformamide (DMF) instead. Although fluorous compounds do not dissolve well in DMF at room temperature, it was expected that solubility at 160°C would be sufficient for the reaction. Following the reaction, HPLC analysis indicated a complex mixture with much unconsumed starting material and little product. Ultimately, the microwave heating approach did not permit the synthesis of **10**.

The preparation of **10** was eventually made possible by the use of a phosphaadamantane ligand in place of triphenylphosphine. These sterically demanding and electron-rich ligands offer an improvement in reaction rate, as discussed in Section 2.5. When a first attempt was made to synthesize **10** with the phosphaadamantane-palladium catalyst under nitrogen, the reaction mixture turned black before the reaction came to reflux. Interestingly, a 24% yield was still obtained after 24 hours at reflux; the reaction may have been fast enough with the phosphaadamantane catalyst to permit formation of product even after apparent decomposition of the catalyst, or the catalyst may have continued to have some activity after apparent decomposition.

When the reaction was performed under argon, the yields obtained for **10** increased to 48% after the first few attempts. It was necessary to adapt the purification protocol to accommodate the fact that the product did not partition into FC-72. This can be seen as an advantage, since the trialkyltin bromide byproduct partitioned into FC-72. Following removal of the trialkyltin bromide by extraction, most of the product remained in the THF layer. When the THF layer was concentrated and the resultant orange-brown oil was dissolved in 80:20 acetonitrile-water, a fluorophobic solvent system, extraction with FC-72 recovered most, but not all, of the product in the fluorous layer. Regardless of the extraction procedure used, column chromatography (on the Biotage SP1), using a hexanes:diethyl ether gradient beginning at 10% diethyl ether and increasing over 10 column volumes to 50% diethyl ether, was necessary to obtain the product in high purity.

Characterization of **10** was performed by the usual spectroscopic and chromatographic techniques. NMR spectroscopy confirmed the identity of the product. The ^{1}H spectrum showed the perfluoroalkyl group signals, with the H-2 signal appearing at 2.261 ppm and the H-1 signal with its ^{119}Sn satellites appearing at 1.170 ppm. The vinylic proton at H-6 on the uracil ring appears at 7.527 ppm, but its ^{119}Sn satellites are

not resolved from the benzoyl group signals even at 600 MHz. The remaining signals, as shown in the spectrum below **(Figure 3-3)**, were in agreement with the assigned structure.

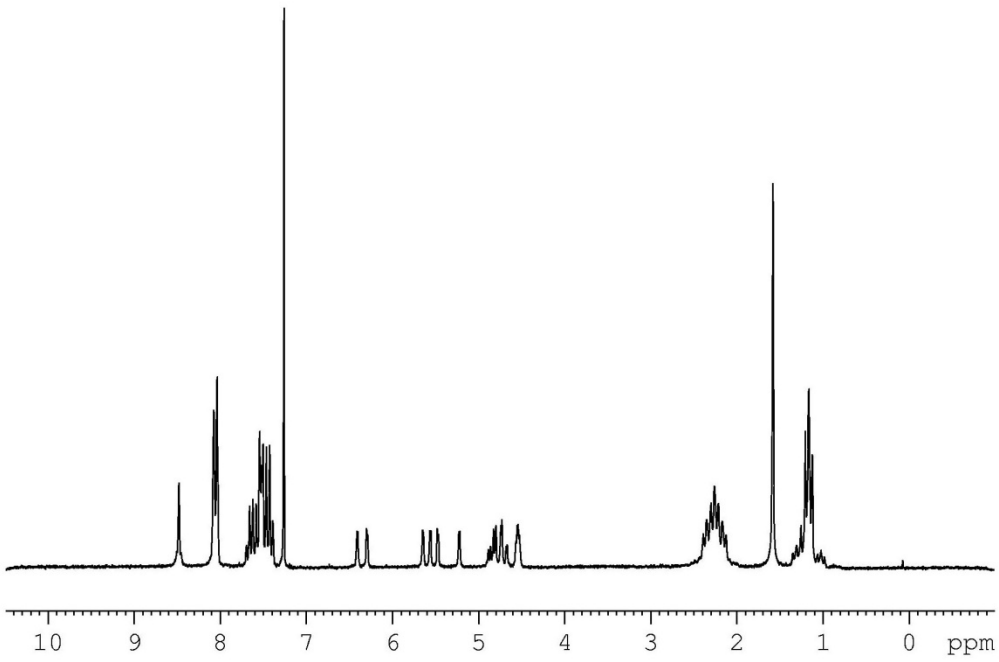


Figure 3-3: ^{1}H NMR spectrum of the protected fluorous FIAU precursor (200.13 MHz, $CDCl_3$).

The ^{13}C NMR spectrum of **10** was also in agreement with the assigned structure; HSQC and HMBC experiments were used to assign the spectrum **(Figure 3-4)**.

The ^{119}Sn NMR spectrum gives a signal at -23.32 ppm, which is typical of a trialkyl-vinylstannane and resembles the chemical shifts observed for the model compounds **(Figure 3-5)**. This, combined with the COSY spectrum, provides assurance that the fluorous trialkylstannyl group is indeed incorporated within the molecule and that it exists at the expected C-5 position. Finally, the high-resolution negative-ion electro-spray mass spectrum of **10** has a peak corresponding to the $[M - H]^-$ anion at *m/z* = 1613.0389, which is within 2 ppm of the calculated value of 1613.0358.

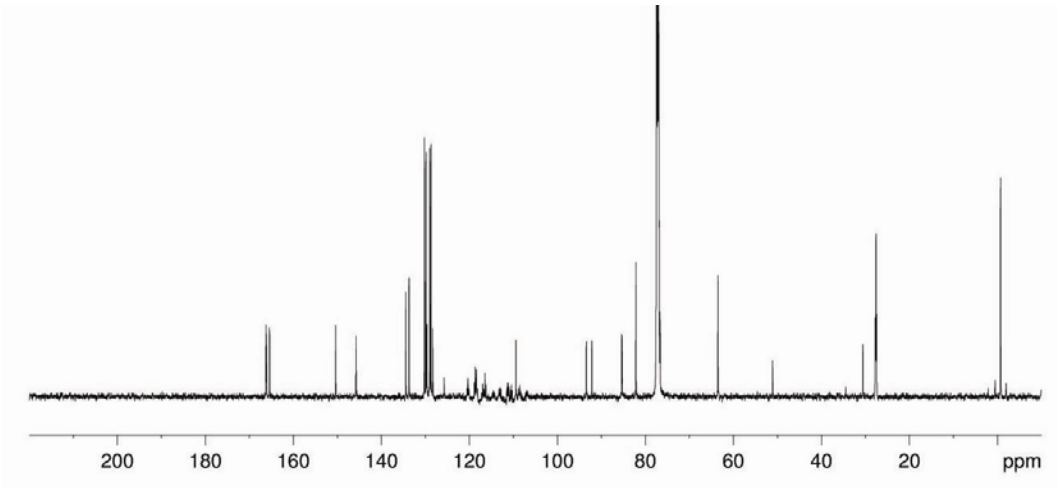


Figure 3-4: ^{13}C NMR spectrum of **10** (150.90 MHz, $CDCl_3$).

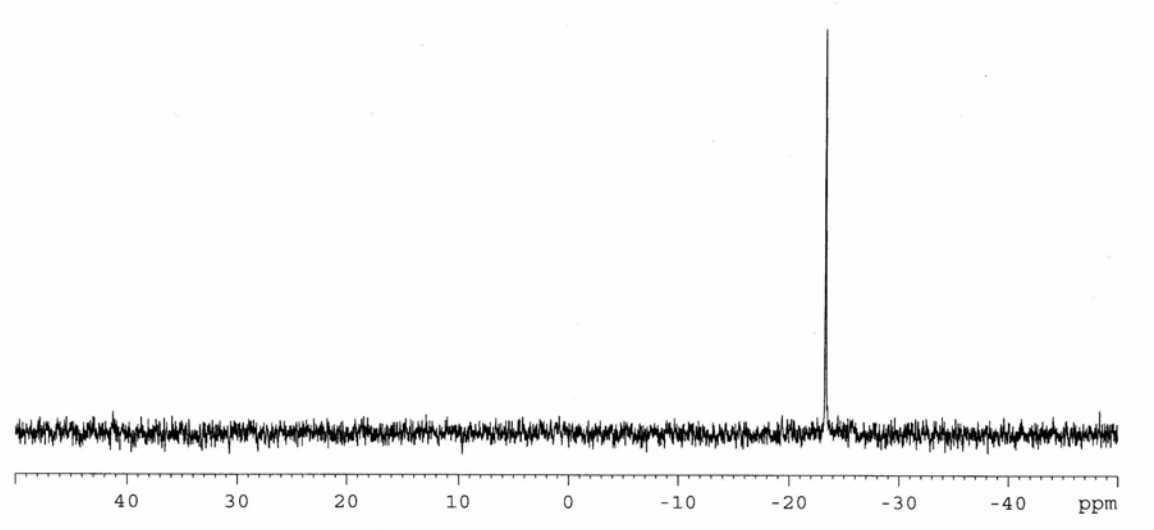
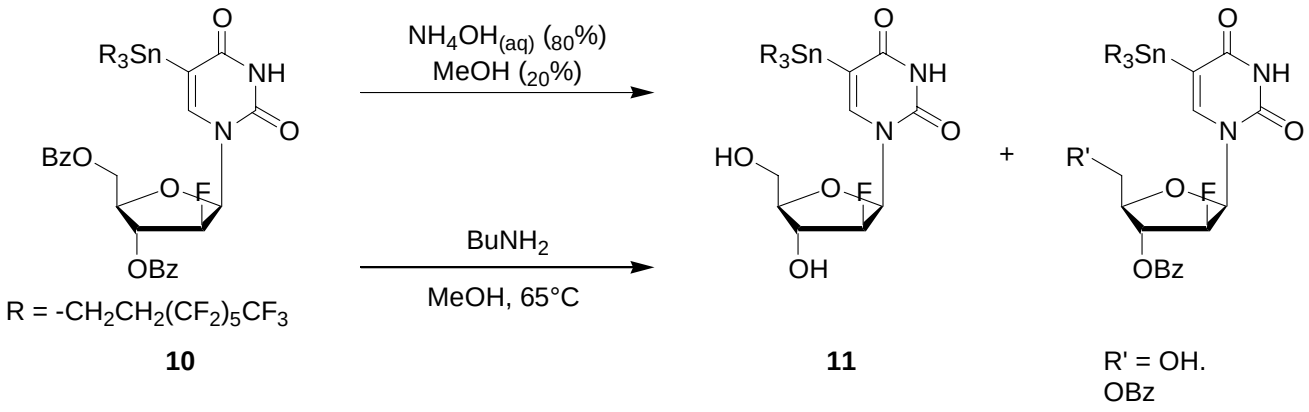


Figure 3-5: 119Sn NMR spectrum of 10 (186.5 MHz, $CDCl_3$).

The bromide precursor supplied by Dr. Kovacs was protected as the 3′,5′-di-benzoyl ester, which is necessary for the synthesis of the precursor. The product of the cross-coupling needed to be deprotected to arrive at a final product. Many methods for the deprotection of 3′,5′-dibenzoyl esters are described in the literature, and several were investigated **(Scheme 3-2)**. The first method attempted was that used for the deprotection of trimethylstannyl and tributylstannyl FIAU precursors: stirring over-night, at room temperature, in a 80:20 mixture of methanol and saturated ammonium hydroxide.[45] This method led to incomplete deprotection of the product, even when

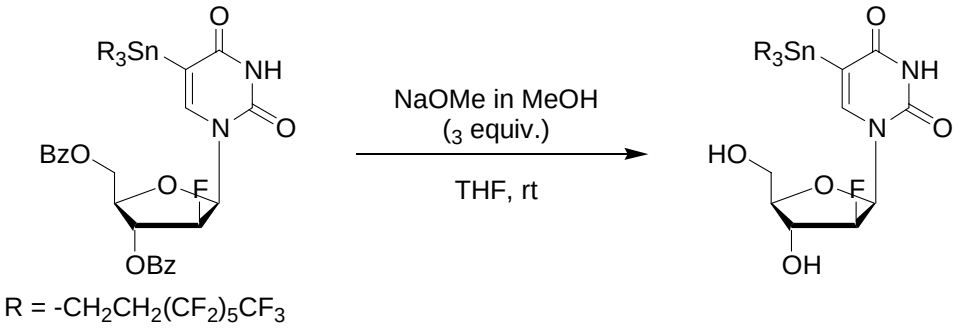
the product was stirred for up to nine days. Poor solubility of the fluorous compound in the solvent was judged to have been the cause of this failure. Another procedure involved refluxing heating **10** to reflux for 3 hours in methanol in the presence of 3 equivalents of *n*-butylamine.[47] Since the product had some solubility in hot methanol, it was hoped that this method might give better results. Unfortunately, this approach did not afford pure **11**. Mixtures of **11** and the mono- and dibenzoyl esters were obtained.



Scheme 3-2: Attempted deprotection of **10**.

Mangner *et al.* reported a synthesis of [^{18}F]FIAU and several analogs with 2′-[^{18}F]fluorine and a non-radioactive iodine, bromine, or hydrogen atom or a methyl group at the 5-position on the uracil ring.[48] In this synthesis, ^{18}F is introduced into a ribose derivative before deprotection or even addition of the uracil derivative. The

short half-life of ^{18}F necessitated the use of fast, high-yielding reactions to obtain the product. Deprotection of the 3′,5′-dibenzoyl intermediate in Mangner's procedure was achieved with sodium methoxide in methanol **(Scheme 3-3)**.



R = $-CH_2CH_2(CF_2)_5CF_3$

10 **11**

Scheme 3-3: Successful deprotection of **10**.

Compound **10** was dissolved in THF to which 3 equivalents (*i.e.* a 1.5-fold excess) of sodium methoxide was added as a 1.5 mol/L solution in methanol. The reaction was followed by HPLC and deprotection of **10** to **11** was complete after 20 minutes. HPLC analysis was performed using a C_{18} column, a flow rate of 1.0 mL/min, and a gradient of 70% acetonitrile in water (0-10 minutes) to 100% acetonitrile (10-25 minutes) (Method A). The excess sodium methoxide was neutralized with a strongly acidic ion-exchange resin. Since the reaction was quantitative, **11**, the only fluorous product, could be isolated by fluorous solid-phase extraction (FSPE) using 80:20 meth-

anol:water as the fluorophobic solvent and methanol as the fluorophilic solvent. HPLC analysis of the product after FSPE demonstrated high purity **(Figure 3-6)**. When a 2 g FSPE cartridge was used, the yield, however, was relatively modest at 35%, even when the cartridge was washed with several column volumes of THF. However, a Pasteur pipette packed with fluorous silica gel gave 80% recovery when it was washed with 5 mL each of methanol and THF.[16]

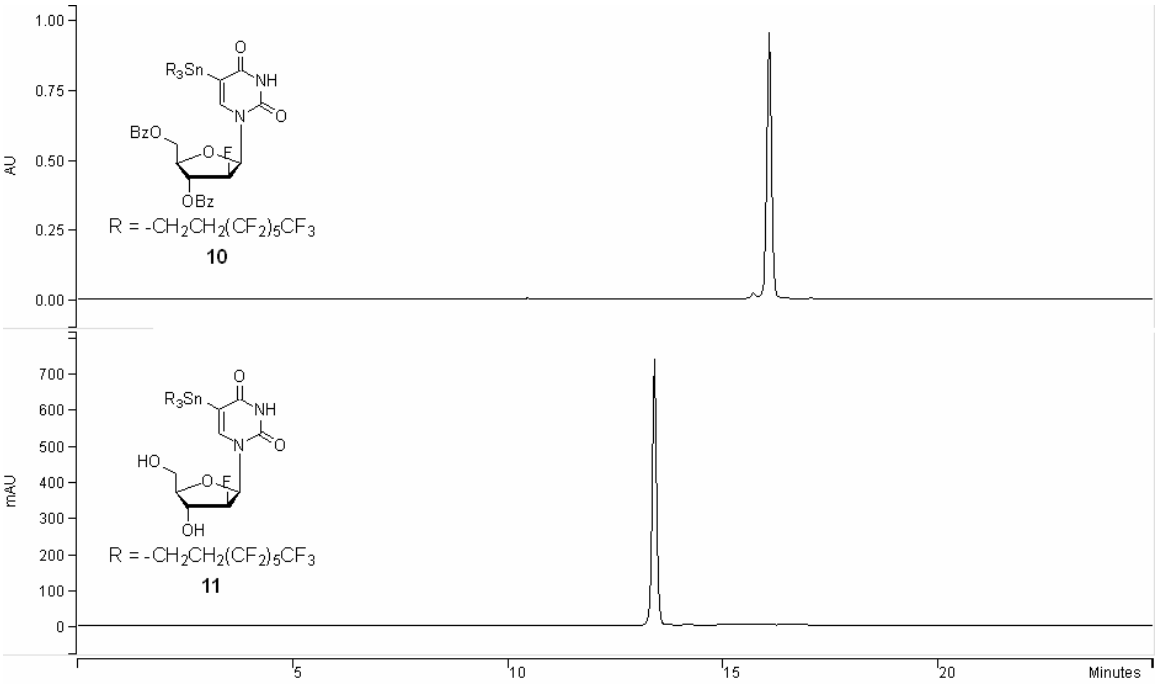


Figure 3-6: UV-HPLC (elution method A) of the protected FIAU precursor **10** and the deprotected precursor **11**.

In addition to the chromatographic evidence of the conversion of **10** to **11** compound **11** was isolated and fully characterized. The disappearance of the benzoyl

groups in the ^{1}H and ^{13}C NMR spectra clearly demonstrated the successful deprotection of **10**, as illustrated in **Figure 3-7**. The remainder of these spectra, along with the ^{119}Sn NMR spectra, were in agreement with the assigned structure. The high-resolution negative-ion electrospray mass spectrum contains a peak corresponding to the $[M - H]^-$ anion at m/z = 1404.9844, which is within 0.7 ppm of the calculated mass of 1404.9834.

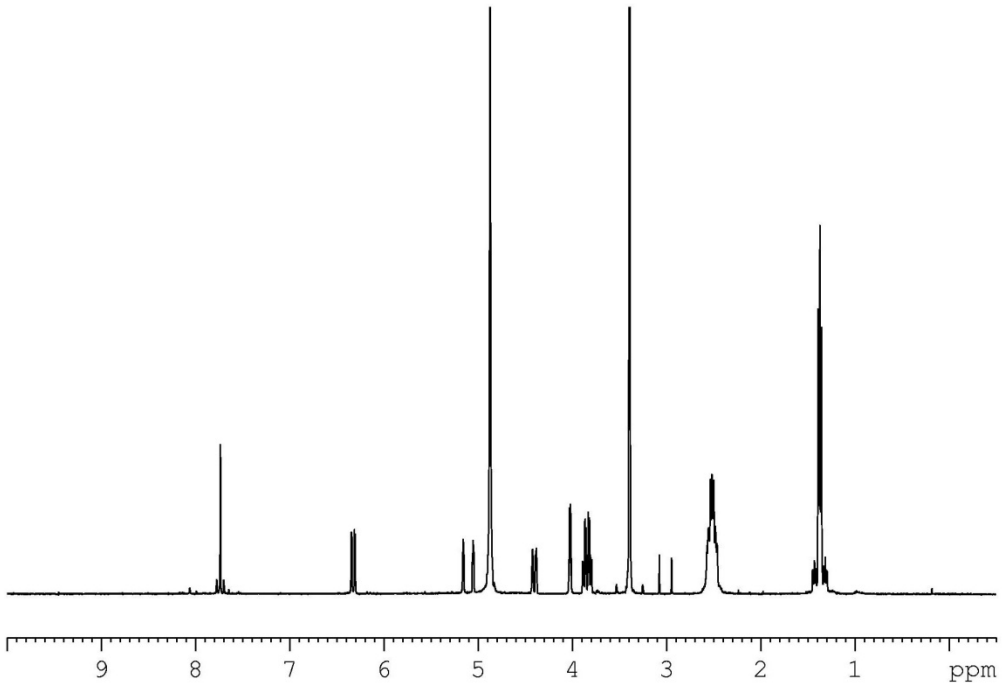
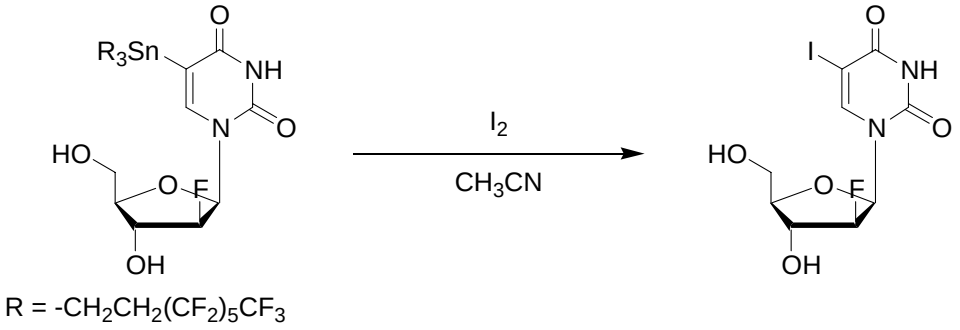


Figure 3-7: ^{1}H NMR spectrum of the deprotected FIAU precursor (200.13 MHz, CD_3OD).

3.4 Cold iodination of 11

To evaluate the reactivity of **11** and to obtain an authentic standard for the radiolabeling experiment, a cold iodination experiment was performed. Compound **8** was prepared by direct iodinolysis of **11 (Scheme 3-4)**. In this experiment, 2 mg of **11** was combined with I_2 (200 μg, 3 eq.) in acetonitrile. After 15 minutes, the reaction was quenched with sodium metabisulfite. The crude reaction mixture was analyzed by HPLC and ESMS.



Scheme 3-4: Cold iodination of the FIAU precursor.

The HPLC chromatograms obtained from the cold labeling experiment with the FIAU precursor **11** are shown below **(Figure 3-8)**. HPLC analysis was performed using a C_{18} column, a flow rate of 2.0 mL/min, and a gradient of 10% acetonitrile in water (0-3 minutes) to 100% acetonitrile (6-12 minutes) (Method B). To demonstrate

the efficiency of the FSPE purification method, it is necessary that some unreacted starting material remains after direct iodinolysis, so an excess of **11** was used, as demonstrated by the chromatogram of the crude reaction mixture. The chromatogram of the purified product demonstrates the successful removal of the unreacted starting material by FSPE.

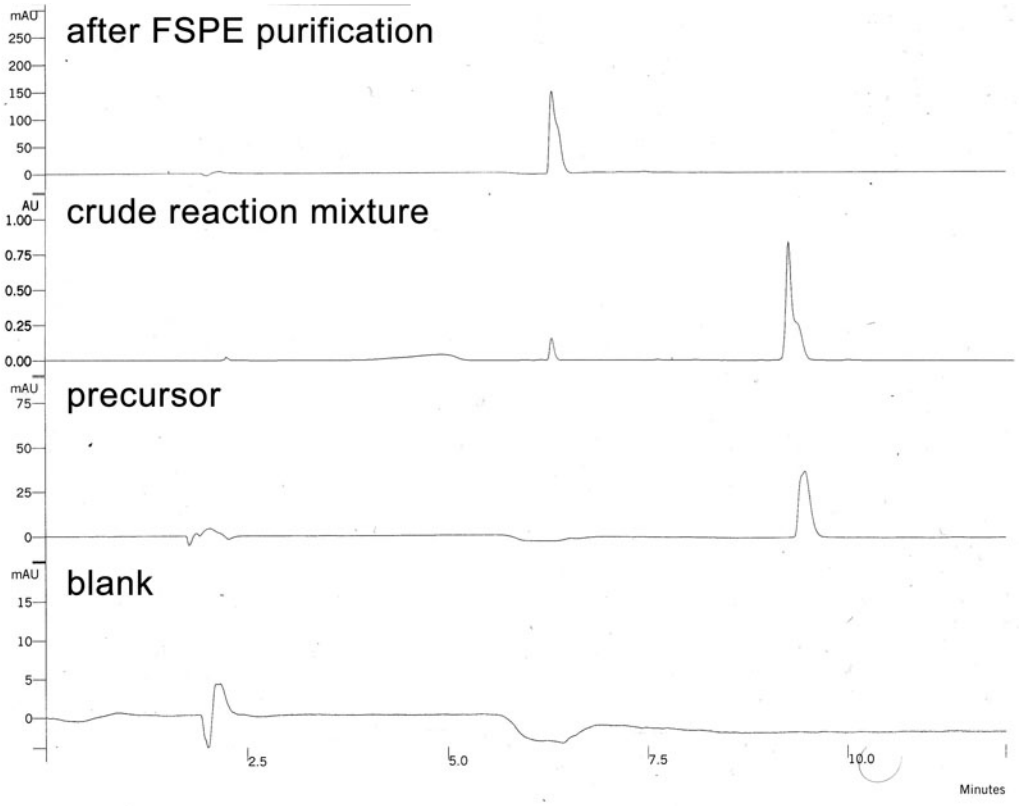
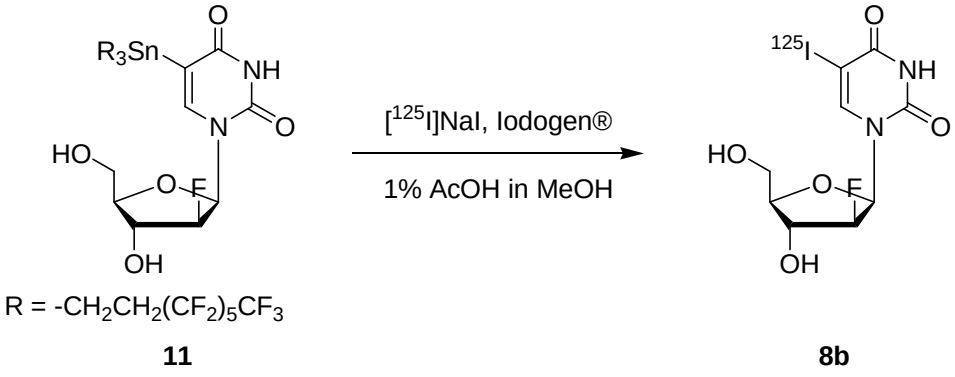


Figure 3-8: UV-HPLC chromatograms (elution method B) of the cold labeling of the FIAU precursor before (bottom) and after (top) purification by FSPE.

3.5 Synthesis of [^{125}I]FIAU

The synthesis of [^{125}I]FIAU was performed using [^{125}I]NaI obtained from the McMaster Nuclear Reactor **(Scheme 3-5)**. Iodine-125 has a half-life of 59.7 days and decays by electron capture to stable ^{125}Te *via* the first excited state of ^{125}Te with emission of a variety of Auger electrons and gamma rays having an average energy of 26.4 keV.[49,50] Production of ^{125}I is a no-carrier-added synthesis by the ^{124}Xe(n,γ)^{125}Xe reaction; the resultant ^{125}Xe decays by electron capture with a half-life of 16.9 hours to give ^{125}I. The ^{125}I is dissolved in dilute sodium hydroxide to afford a solution with nominal specific activity of ~17 Ci/mg (629 GBq/mg).[51]



R = -$CH_2CH_2(CF_2)_5CF_3$

11 **8b**

Scheme 3-5: Synthesis of [^{125}I]FIAU.

The characterization of **11** was carried out on samples purified by column chromatography and fluorous solid-phase extraction. These samples were of adequate purity for characterization and afforded satisfactory HPLC chromatograms. However, when radiolabeling was attempted on the precursor, small but undesirable extraneous peaks were evident on the UV-HPLC chromatogram. As a result, the precursor was purified by semi-preparative HPLC prior to radiolabeling (Method C: C_{18} column, flow rate: 8.0 mL/min, gradient : 10% acetonitrile in water from 0-3 minutes to 100% acetonitrile from 6-12 minutes). By removing the trace impurities, a precursor with sufficient purity for radiolabeling was obtained.

To prepare [^{125}I]FIAU from the deprotected precursor **11**, 100 μL of a 1 mg/mL solution of the precursor in methanol was transferred into a vial containing an oxidant. Since the sodium [^{125}I]iodide is furnished as a solution in dilute sodium hydroxide, 1 μL of glacial acetic acid was added to the reaction mixture to adjust the pH.

Radioiodination reactions require an oxidant to form a nominal $^{125}I^{+}$ species (which is actually present as ^{125}ICl) from the $^{125}I^{-}$ present in the sodium [^{125}I]iodide solution.[52] Three oxidants were screened by evaluating the incorporation of ^{125}I into the desired product under otherwise identical conditions. The first oxidant, chloramine-T

(sodium *N*-chloro-*para*-toluenesulfonamide), gave 74% of the total activity in the product, 12% as sodium iodide, and the remainder as two extraneous peaks. The second oxidant, peracetic acid (formed *in situ* from glacial acetic acid and hydrogen peroxide), yielded 88% of the total activity in the product, 3% as sodium iodide and the remander in two extraneous peaks. The third oxidant, Iodo-Gen®, or 1,3,4,6-tetra-chloro-3 ,6 -diphenylglycouril **(Figure 3-9)**, afforded complete incorporation of the radioactivity in the product without extraneous peaks. Across all the experiments conducted with Iodo-Gen®, greater than 95% incorporation was observed, with complete incorporation of the activity in the product being observed in most cases. To use the oxidant, 2 mL glass vials were coated with Iodo-Gen® by transferring 20 μL of a 1 mg/mL solution in chloroform into each vial; the chloroform was then immediately evaporated under vacuum and the vials were stored in a freezer until immediately before use.

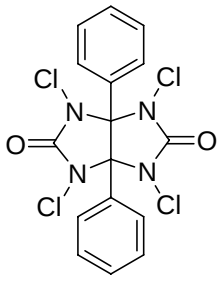


Figure 3-9: The structure of Iodo-Gen®.

Radiolabeling of precursor **11** was accomplished by adding 1 μL of a 100 mCi/mL (3.7 GBq/mL) solution of [^{125}I]NaI to the reaction vessel and allowing it to stand at room temperature with occasional swirling. After 3 minutes, the reaction was quenched by the addition of sodium metabisulfite (10 μL of a 0.01 mol/L solution) to reduce the nominal $^{125}I^{+}$ species to unreactive $^{125}I^{-}$. To prepare a sample for analysis by HPLC, a 10 μL aliquot was transferred into a second vial containing 60 μL of acetonitrile. The total activities of both these fractions were then measured using a dose calibrator. The HPLC chromatograms of the crude reaction mixture are shown below **(Figure 3-10)**.

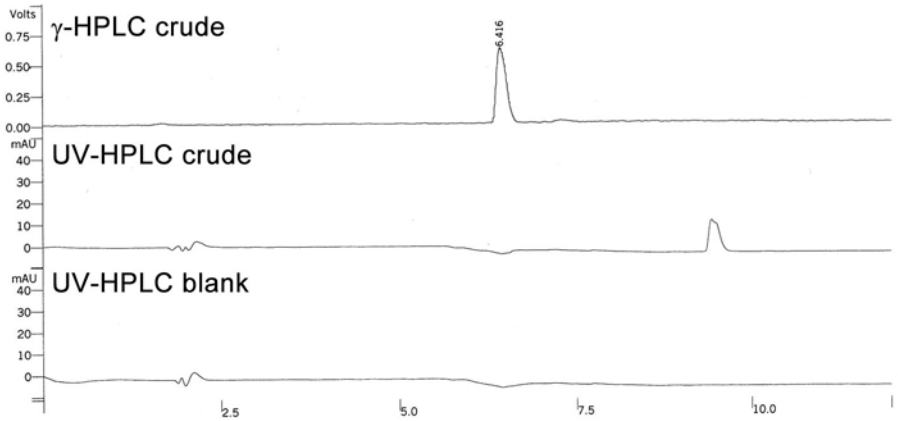


Figure 3-10: HPLC chromatograms of the crude reaction mixture from the labeling of the FIAU precursor (elution method B): UV-HPLC blank (bottom), UV-HPLC (middle) and γ-HPLC (top).

The UV-HPLC chromatogram demonstrates that an excess (about 22-fold) of starting material is used, and the principal species detected is the excess precursor. The remainder of the chromatogram matches the blank baseline. The γ-HPLC chromatogram of the labeled product contains only one signal: the labeled product **8b**, [^{125}I]FIAU. The absence of a signal corresponding to [^{125}I]NaI at the column's dead time in the γ-HPLC chromatogram suggests complete incorporation of the radioactivity into **8b**, within the instrument's detection limit (5×10^{-5} mol/L, 10^2 μg/mL).

Isolation of the labeled product from the crude reaction mixture was performed by fluorous solid-phase extraction. This was done immediately after measuring the activity of the crude reaction mixture, as extraneous peaks began to appear in the HPLC chromatogram if the reaction mixture was allowed to stand. To perform the separation, the crude reaction mixture was diluted with 1 mL of water and loaded onto a fluorous solid-phase extraction cartridge that was previously conditioned with 6 mL of 80% methanol-water. Although FIAU is not soluble in water on a macroscopic scale, the amount of [^{125}I]FIAU present in the sample is so small that it is soluble in water at the tracer level. Following collection of the first 1 mL fraction of water in an Eppendorf vial, the reaction vessel was rinsed with an additional 1 mL of water which was

also loaded onto the cartridge and collected in a separate vial. To remove water-soluble species including unreacted [^{125}I]NaI from the mixture, the cartridge was then eluted with 5 mL of water which was collected as separate 1.5 mL fractions in Eppendorf vials. To recover the labeled product, the cartridge was eluted with 6 mL of 80% methanol-water, which was collected as separate 1.5 mL fractions in Eppendorf vials. The unreacted starting material remained on the cartridge along with a portion of **8b** representing an average of 8% of the total activity. The activity of each of the fractions collected was then measured using a dose calibrator, and the fraction with the highest activity was analyzed by HPLC. The fraction having the highest activity contained, on average, 60% of the total activity. However, when all of the fractions were analyzed, all except the first aqueous fraction were shown to contain only **8b** with no evidence of **11**. Therefore, the average radiochemical yield was 88%. Example chromatograms of the purified labeled product are shown below **(Figure 3-11)**.

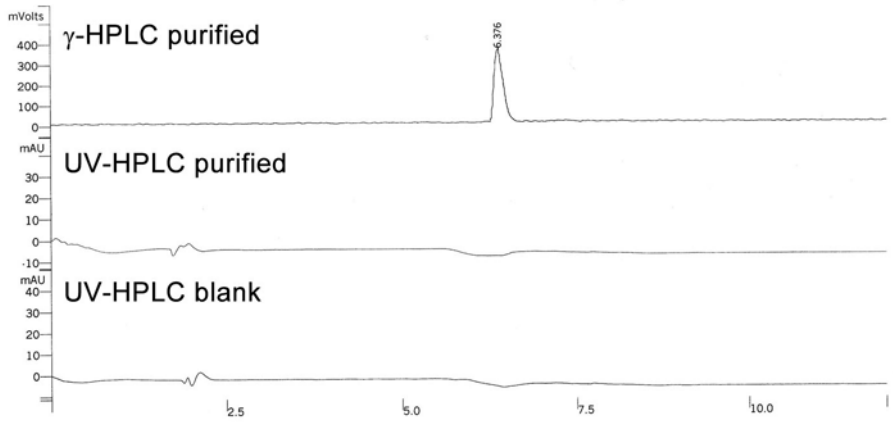


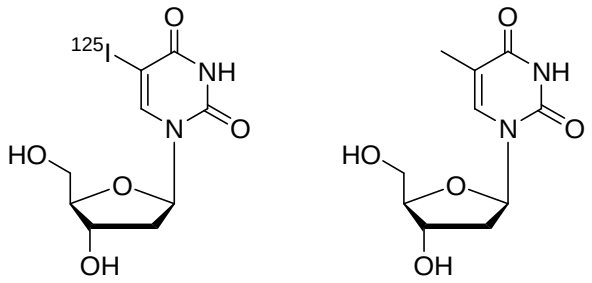
Figure 3-11: UV-HPLC chromatogram (middle) and γ-HPLC chromatogram (top) of [^{125}I]FIAU after FSPE purification.

The UV-HPLC chromatogram of the labeled product does not contain unreacted precursor, indicating that fluorous compound **11** was retained on the FSPE cartridge. The γ-HPLC chromatogram contained a single peak corresponding to the labeled product, [^{125}I]FIAU. The product has a retention time slightly longer than that observed in the UV-HPLC chromatogram of the authentic [^{127}I]FIAU standard; this is because the eluent from the HPLC column flows through the UV detector shortly before it reaches the gamma detector.

3.6 5-Iodo-2′-deoxyuridine (IUdR)

5-Iodo-2′-deoxyuridine, also known as idoxuridine or IUdR, is an analog of 2′-deoxythymidine (TdR) that has been investigated for a number of clinical uses since its

first synthesis in 1959. The abbreviation IUdR is derived from UdR, the abbreviation for 2′-deoxyuridine. The structure of [^{125}I]IUdR (**12**) is shown below, along with 2′-deoxythymidine, for comparison **(Figure 3-12)**.[53]



12 **TdR**

Figure 3-12: The structures of [^{125}I]IUdR and 2′-deoxythymidine.

Upon uptake into the cell, both IUdR and TdR are phosphorylated by thymidine kinase to give the monophosphates, called IdUMP and dTMP respectively. Both monophosphates can undergo further phosphorylation and incorporation into DNA. IUdR can also undergo dehalogenation by thymidylate synthetase to give 2′-deoxyuracil monophosphate, which is subsequently converted to dTMP and incorporated into DNA.[53] When IUdR is administered intravenously to humans, dehalogenation in the liver is rapid and the drug has a half-life of 5 minutes. However, the dehalogenation pathway can be at least partially circumvented by injecting the drug intra-

tumourally or perfusing the tumour with IUdR before liver metabolism can take place. There is a positive aspect to the rapid dehalogenation: since IUdR is rapidly converted to a non-toxic species, its incorporation into the DNA of non-tumour cells is minimal.[53,54]

The primary clinical application of radiolabeled IUdR is in therapy. Upon administration, IUdR is taken up by rapidly dividing cells such as tumour cells. Provided that adequate steps, such as intratumoural or locoregional injection of the drug, are taken to avoid dehalogenation in the liver, IUdR is incorporated into the DNA of tumour cells and rapidly clears from the rest of the body following dehalogenation. When labeled with therapeutic radionuclides, IUdR is a potent cytotoxin because it emits Auger electrons (in the case of [^{123}I]IUdR or [^{125}I]IUdR) or β^- particles ([^{131}I]IUdR) in close proximity to the cells' nuclear DNA, causing DNA damage leading to cell death.[53,55-57]

The rapid hepatic metabolism of IUdR limits its utility as a systemic imaging agent; it is necessary to know the location of the tumour prior to therapy. This is illustrated by the γ-camera scintigraphic image shown in **Figure 3-13**, which also illustrates accumulation of ^{125}I in the bladder following dehalogenation in the liver. Local-

ization of activity in the tumour is poor, but recall that the activity in the tumour represents [^{125}I]IUdR incorporated into DNA and the activity outside the tumour represents free [^{125}I]iodide.[53,54]

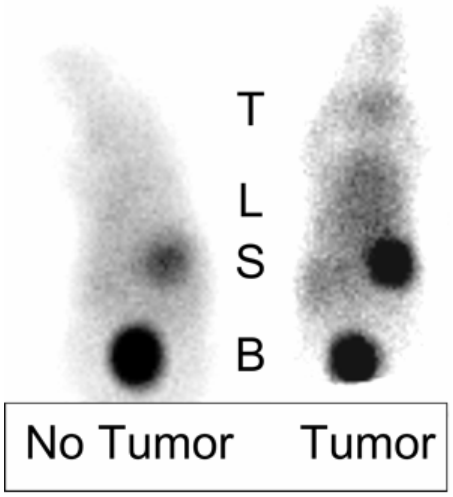


Figure 3-13: γ-Camera scintigraphic image of non-tumour-bearing (left) and lung tumour-bearing (right) mice 24 h after administration of [^{125}I]IUdR, showing distribution of the drug in the thyroid (T), lung (L), stomach (S) and bladder (B). (From Semnani *et al*., 2005.)

Much of the recent research on IUdR has focused on methods of improving the target to non-target (T/NT) distribution ratio and the uptake by tumour cells. In 2005, Semnani *et al*. prepared [^{123}I]IUdR and [^{125}I]IUdR from ~1 mCi (37 MBq) of radioactive NaI and varying amounts of a tributylstannyl IUdR precursor in the presence of Iodo-Gen® as an oxidant. An aliquot of the crude reaction mixture (0.2 mCi, 7.4 MBq)

was injected into mice with lung tumours without purification and uptake into the tumour was measured. Interestingly, the best T/NT ratios and uptake was observed when 5 μg of the stannyl precursor was present; this is in contrast to the notion that high effective specific activity is preferable. It was proposed that the stannyl precursor may inhibit thymidylate kinase and aid in the incorporation of the radiolabeled IUdR into DNA.[53] A more direct method of inhibiting thymidylate kinase was proposed by Chi *et al*. in 2004; in this method, thymidylate kinase antisense plasmids were used to interfere with the expression of the gene. Chi *et al.* also injected crude reaction mixtures containing [^{131}I]IUdR and its trialkylstannyl precursor.[57] This practice was strongly criticized in a 2005 letter in which J. Baranowska-Kortylewicz and J. Nearman claimed that, since the trialkylstannyl precursors themselves are cytotoxic, it was impossible to determine whether the observed effects arose from increased uptake of radiolabeled IUdR into the tumour or from the cytotoxicity of the trialkylstannyl precursor.[56]

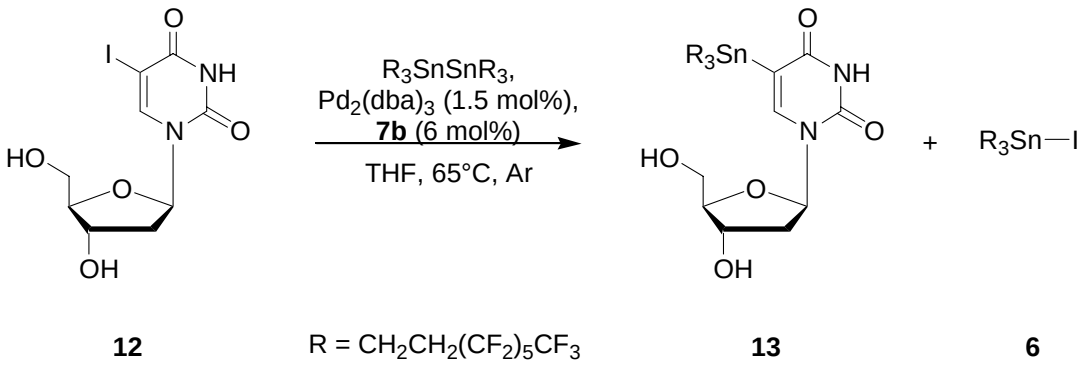
It would appear to be more responsible to administer a high-ESA radiotherapeutic agent in combination with a selective enzyme inhibitor than to inject a subject with a crude reaction mixture in the hope that a toxic tin precursor might have some

beneficial effect. Such a proposal was made in 2004 by Buchegger *et al.*, who used fluorodeoxyuridine, a known inhibitor of thymidylate synthetase, to favour incorporation of IUdR into tumour cell DNA rather than the dehalogenation reaction. In mice pre-treated with fluorodeoxyuridine, mean uptake of [^{125}I]IUdR in the tumour was approximately 20% of the injected dose, compared with 4% in non-pre-treated mice.[58]

In the literature, the usual method of preparing IUdR has been by reaction of the trialkylstannyl precursor with radioactive sodium iodide in the presence of an oxidant such as Iodo-Gen®. The trialkylstannyl precursors were prepared from [^{127}I]IUdR (**12a**) in the presence of tetrakis(triphenylphosphine)palladium(0) and hexamethyldistannane[59] or hexabutyldistannane.[60] The synthesis of the fluorous IUdR precursor proposed herein is analogous to these methods. A halodemercuration reaction was also proposed where a HgCl precursor was prepared by reaction of 2′-deoxyuracil with $Hg(OAc)_2$ and NaCl; this was labeled with NaI in the presence of an oxidant.[60]

3.7 Synthesis of a fluorous IUdR precursor

The synthesis of the fluorous IUdR precursor, **13**, was adapted from the synthesis of the FIAU precursor **(Scheme 3-6)**. Due to the structural similarity of the compounds, the synthesis was expected to be facile. However, since the vinyl iodide **12a** lacks the benzoyl protecting groups of the FIAU precursor **9**, the synthesis and purification of **13** required some modifications to the procedure.



Scheme 3-6: Synthesis of a fluorous IUdR precursor.

Unlike all previous substrates, the highly polar vinyl iodide **12a** is insoluble in THF. Following literature precedent, the reaction was first attempted in dioxane, with **12a** introduced as a solution in hot dioxane.[60] This method was unsuccessful, perhaps because of decomposition of the catalyst in the hot dioxane solution. Next, attempts

were made to exploit the solubility of **12a** in *N,N*-dimethylformamide. Owing to its high boiling point and large dipole moment, *N,N*-dimethylformamide (DMF) is an excellent solvent for microwave-assisted reactions, so several microwave-assisted reactions were attempted.[61] In these reactions, the tin hydride was added to a 2-5 mL microwave vial as a neat oil, followed by 3 mol% of tris(dibenzylideneacetone)-dipalladium and 6 mol% of the phosphaadamantane as solutions in DMF. Finally, 0.9 equivalents of **12a** were added and the volume of solvent was brought up to 3 mL. The reaction mixture was heated for three five-minute intervals at a temperature of 160°C, with replenishment of the catalyst between intervals. The reaction mixture turned brown after the first interval of heating, suggesting either completion of the reaction or decomposition of the catalyst. Concentration and attempted purification on a Biotage SP1 failed to afford the desired product, as had happened with the attempted microwave-assisted synthesis of **10**.

The successful synthesis of **13** was achieved by reverting to the original reaction conditions, with THF as solvent. The reaction vessel was charged with the tin hydride **4** as an oil and the vinyl iodide **12a** as a solid, then evacuated and purged with argon at least five times. Using a glass syringe, 3 mol% $Pd_2(dba)_3$ and 6 mol% of

phosphaadamantane **7b** were added as solutions in freshly distilled or column-dried THF and the reaction volume was brought to 5 mL with the addition of dry THF. This heterogeneous reaction mixture was stirred vigourously and allowed to reflux for 72 hours before being extracted into FC-72 (3 × 3 mL) and purified on a Biotage SP1 using silica gel as the stationary phase and a mobile phase of 0-10% methanol in dichloromethane from 0 to 10 CV, followed by 5 CV of 10% methanol in dichloromethane. In the first attempt, further purification by FSPE was required to separate a partially co-eluting byproduct from **13**. This did not appear in subsequent experiments, but sample cleanup by FSPE was still desirable to obtain a product of high purity for characterization and radiolabeling. This was performed using a Pasteur pipette packed with fluorous silica gel. The column was conditioned with 1 mL of DMF and 5 mL of 80% methanol in water; then the impure **13** was loaded onto the column in 80% methanol in water and the column was eluted with 5 mL of 80% methanol in water, then 5 mL of methanol collected in several fractions. The fractions were analyzed by TLC and those containing product were combined and evaporated to afford pure **13**.

As with other fluorous compounds, the ^{1}H NMR spectrum includes several signals that confirm the identity of the product. The most important are the

1H,1H,2H,2H-perfluorooctyl groups, which gave a multiplet at 2.646 ppm for C-2 and a triplet with ^{119}Sn satellites at 1.306 ppm for C-1, and the vinylic C-6 proton on the uracil ring, which gave a singlet with ^{119}Sn satellites at 7.937 ppm. The protons on the ribose ring were assigned with the aid of the COSY experiment. The two C-2′ protons, which confirm the 2′-deoxyribose moiety, were at 2.324 and 2.255 ppm, having the lowest frequency of the carbohydrate protons. The ^{13}C NMR spectrum was also in agreement with the assigned structure; the HSQC and HMBC experiments were very helpful in assigning the spectrum. Finally, the ^{119}Sn NMR spectrum gave a chemical shift of -25.47 ppm, which is expected for the trialkylvinylstannane product. The ^{1}H NMR and ^{13}C NMR spectra are shown in **Figure 3-14** and **Figure 3-15**, respectively.

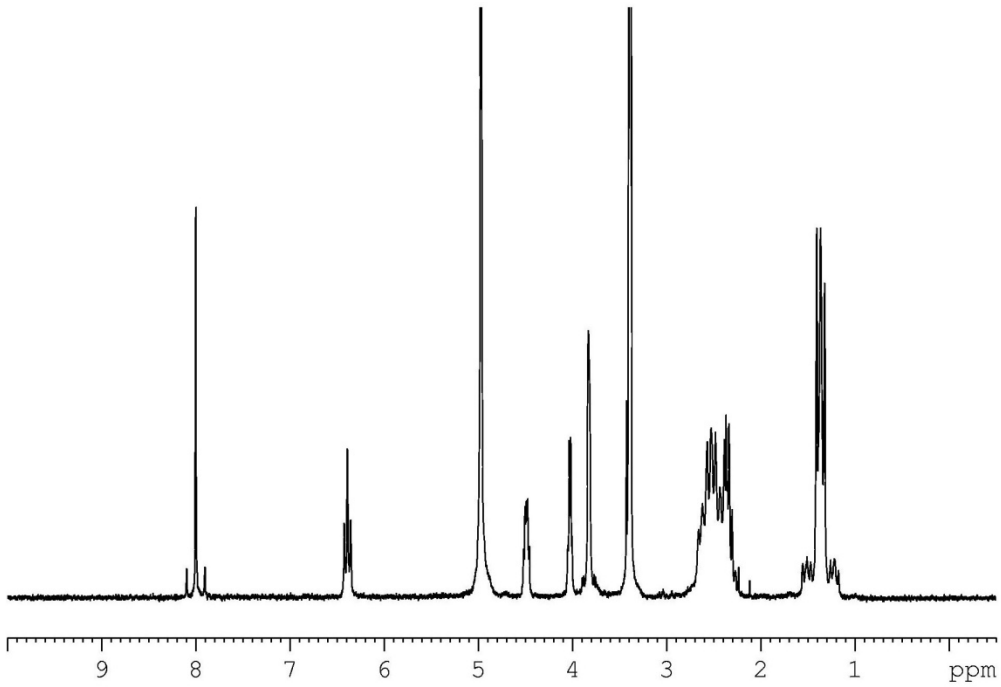


Figure 3-14: ^{1}H NMR spectrum of the fluorous IUdR precursor (200.13 MHz, CD_3OD).

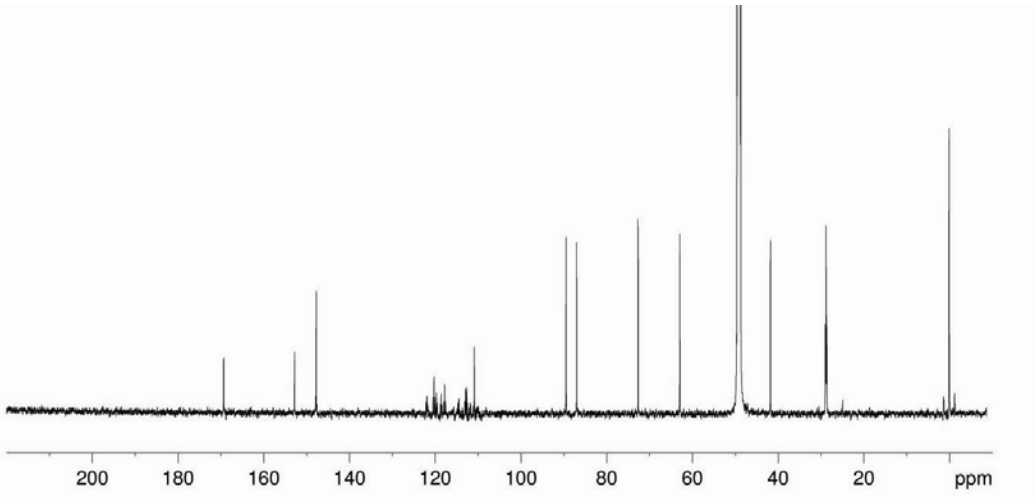
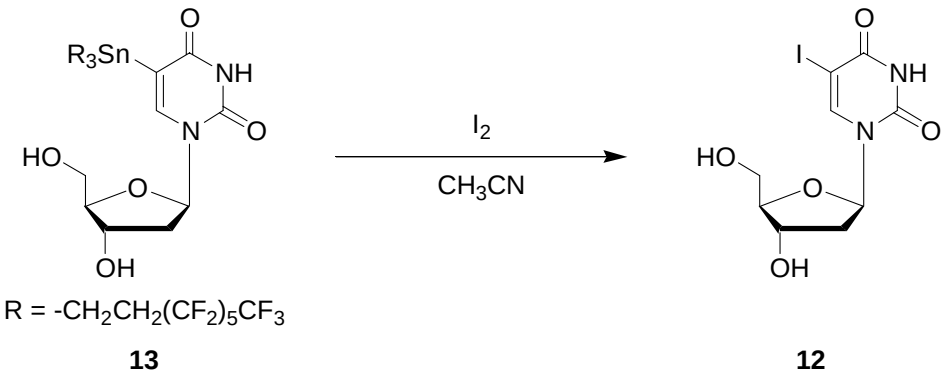


Figure 3-15: ^{13}C NMR spectrum of the fluorous IUdR precursor (150.90 MHz, CD_3OD).

In addition to the NMR spectra, the identity of the IUdR precursor was confirmed by high-resolution negative-ion electrospray mass spectrometry, which gave *m/z* = 1500.9869 for $[M + TFA]^-$, within 0.8 ppm of the calculated value of 1500.9857.

3.8 Cold labeling of the IUdR precursor

The cold labeling experiment was performed on the IUdR precursor in the same manner as described above for the FIAU precursor **(Scheme 3-7)**. A sample of the precursor was dissolved in acetonitrile and reacted with I_2 for 15 minutes prior to quenching with sodium metabisulfite. The crude reaction mixture was analyzed by HPLC (elution method B) and then purified by FSPE to isolate the cold-labeled product. Analysis of the purified product by HPLC confirmed that the unreacted precursor had been removed and demonstrated the identity of the product with authentic [^{127}I]IUdR. The HPLC chromatograms from the cold labeling reaction are shown below **(Figure 3-16)**.



13 **12**

Scheme 3-7: Cold labeling of the IUdR precursor.

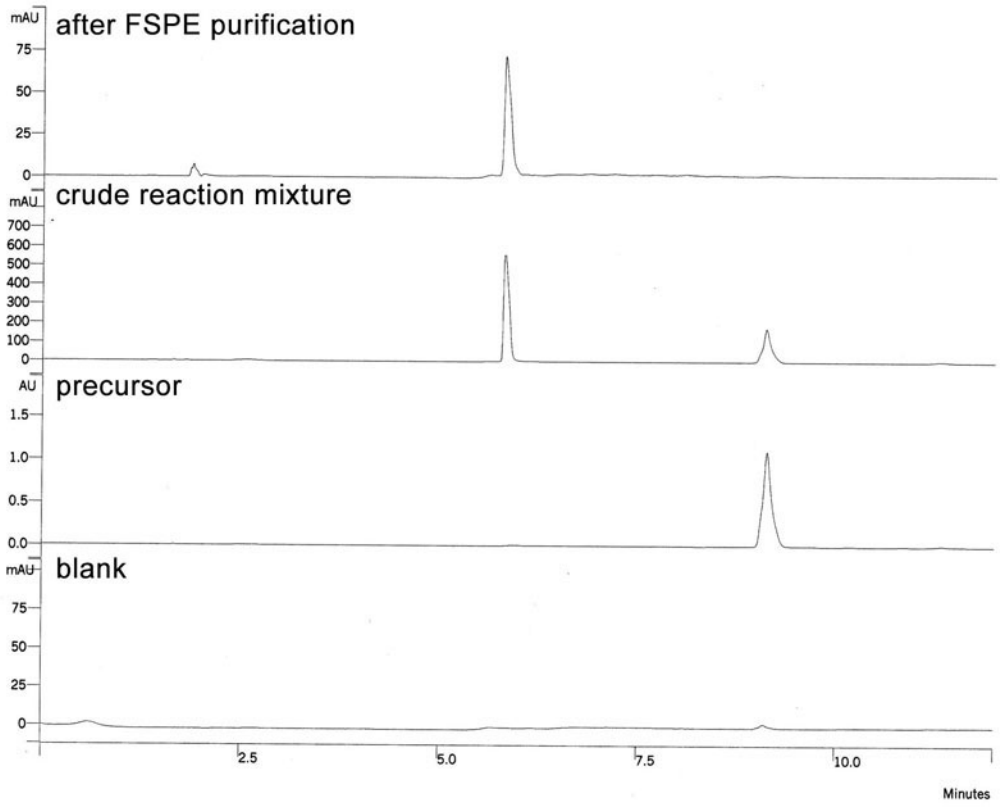
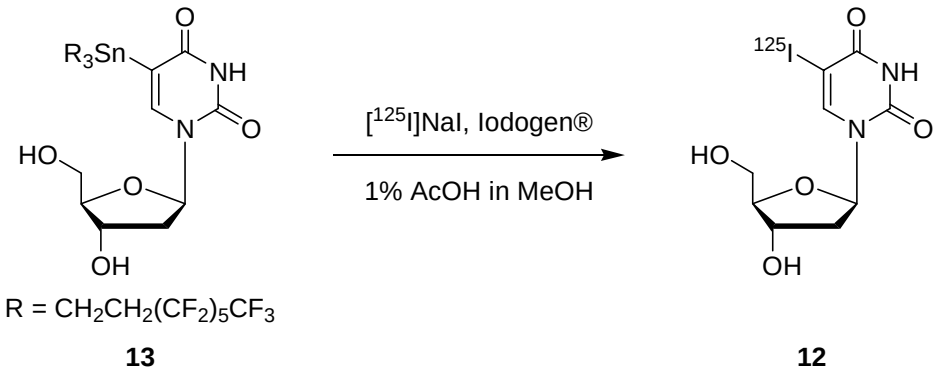


Figure 3-16: HPLC chromatograms (elution method B) from the cold labeling of IUdR precursor **13** (bottom) showing the crude reaction mixture (middle) and product after purification by FSPE (top).

3.9 Synthesis of [^{125}I]IUdR

The radiolabeling of the IUdR precursor **13** was carried out in the same manner as the FIAU precursor **11 (Scheme 3-8)**. Precursor **13** was purified by semi-preparative HPLC in the same manner as **11** to eliminate small extraneous peaks that were evident after labeling the precursor purified by column chromatography. As with FIAU, the reaction was carried out in triplicate to establish its reproducibility. For each reaction, 100 μL of the precursor was added as a 1 mg/mL solution in methanol to a vial previously filmed with 2 μg of Iodo-Gen® followed by 1 μL of glacial acetic acid and 1 μL of [^{125}I]NaI (~ 100 Ci, 3.7 MBq) as a solution in dilute NaOH. After 3 minutes, the reaction was quenched, the HPLC sample of the crude reaction mixture was prepared in the same manner as for FIAU, and the activities of both fractions were measured on the dose calibrator. The crude reaction mixture was immediately purified by FSPE in the manner described for the FIAU precursor, with the cartridge being eluted with 6 mL of water followed by 6 mL of 80% methanol-water, all collected in 1.5 mL fractions. The activity of each fraction was measured using a dose calibrator and

the methanol-water fraction having the highest activity was analyzed by HPLC. Example chromatograms before and after purification are shown below **(Figure 3-17)**.



R = $CH_2CH_2(CF_2)_5CF_3$

13 **12**

Scheme 3-8: Synthesis of [^{125}I]IUdR.

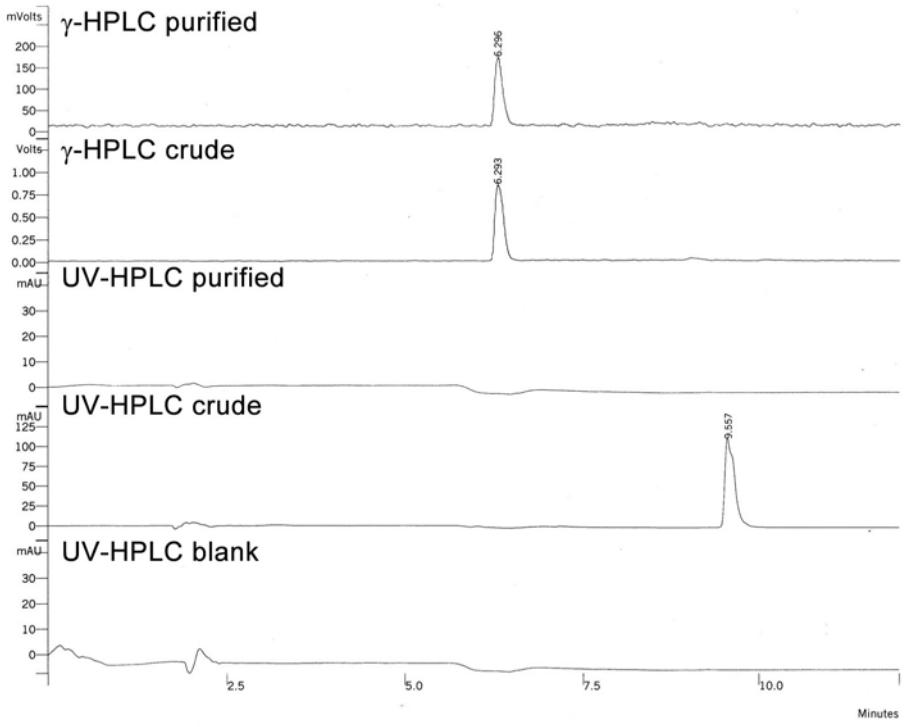


Figure 3-17: HPLC chromatograms (elution method B) from the synthesis of [^{125}I]IUdR. From top to bottom: γ-HPLC of the purified reaction mixture, γ-HPLC of the crude reaction mixture, UV-HPLC of the purified reaction mixture, UV-HPLC of the crude reaction mixture, and UV-HPLC blank.

The synthesis of **12** was performed three times with 75-85 Ci (2.8-3.1 MBq) of [^{125}I]NaI, and similar results were obtained from all three trials. As with the synthesis of **8**, the majority (an average of 54%) of the activity was in the second methanol fraction. When all fractions were analyzed, all but the first methanol-water fraction contained pure **12** and no **13**; the overall radiochemical yield was therefore 94%.

To demonstrate that the reaction can be conducted on a larger scale, one reaction was performed with 608 Ci (22.5 MBq) of [^{125}I]NaI. In this reaction, only 42% of the total activity was detected in the fraction having the highest activity, so all the fractions were analyzed by HPLC. None contained **13** within the detection limit of the instrument (5×10^{-5} mol/L, 10^2 μg/mL), and all but the first aqueous fraction (which contained some sodium [^{125}I]iodide) were found to contain only the desired labeled product, **12b**. The overall radiochemical yield was 86%.

Chapter 4

Conclusion and Future Work

A method of preparing fluorous trialkylarylstannanes and trialkylvinylstannanes was developed to complement existing methods of preparing these compounds involving organometallic reagents. The method is based on a palladium-catalyzed cross-coupling reaction between an aryl or vinyl halide and a fluorous distannane, which is prepared by palladium-catalyzed dehydrogenative coupling of a fluorous trialkyltin hydride. A library of model trialkylarylstannanes was prepared to investigate the scope of the reaction, with yields ranging from 10 to 60% when $Pd(PPh_3)_4$ was used as a catalyst. The same compounds were obtained in 12-67% yield with a phosphaadamantane-palladium catalyst. The synthesis, iodinolysis and radiolabeling with ^{125}I of fluorous precursors to two radiopharmaceuticals, FIAU and IUdR, was also performed.

One area of future work may lie in radiolabeling the existing precursors with radiohalogens other than iodine-125. Halodestannylation is possible with all radiohalogens, even astatine, although some radionuclides are more accessible and clinically desirable than others. Bromine-76, for example, is a cyclotron-produced positron emitter useful for PET that has a half-life of 16.2 hours, making it preferable to fluorine-18, which has a half-life of 109.7 minutes, for applications requiring longer periods of observation (e.g. the imaging of events involving DNA synthesis).[62,63] Isotopes of iodine other than ^{125}I could also be incorporated.

Labeling with fluorine-18 is also a possibility, and fluorous labeling precursors have been labeled with ^{18}F in the past, although labeling with fluorine-18 often poses technical problems.[5] It is possible, though, that the FLS may obviate some of these technical problems. For example, one PET radiotracer for dopaminergic activity in the brain, [^{18}F]fluoro-*meta*-tyrosine (FMT), is very difficult to prepare from the trimethylstannyl precursor. Reaction of the precursor with [^{18}F]F_2 gives an insoluble oligomeric tin fluoride byproduct which leads to blockages in the reaction vial; also, the trimethylstannyl precursor is reactive with silica gel and is difficult to purify.[64] It may prove more practical to conduct the reaction in fluorous solvents, which are inert to

F_2, and to use the rapid FSPE purification method rather than column chromatography or HPLC.

Another future objective should be the development of an efficient solution for the reaction's major disadvantage: the formation of an equimolar amount of tin halide alongside the product. Purification of the reaction mixture by column chromatography is effective for the preparation of small libraries, but it causes loss of product and complicates parallel or continuous synthesis. As mentioned earlier, it may be possible to remove the tin halide with a solid-phase scavenger resin, perhaps one based on a strong base that would bind the tin halide as a stannoxane or tin hydroxide.

Long-term possibilities for development of the palladium-catalyzed fluorous trialkylstannylation method include adapting the method to combinatorial chemistry. The convenience of the method permitted a small library of compounds to be prepared easily and with parallel synthesis. It should be possible to adapt the procedure to prepare a much larger library of compounds. For example, a library of aryl halides with one or more variable substituents could be prepared by conventional combinatorial methods. Then, the fluorous cross-coupling reaction could be conducted on the library to introduce a fluorous trialkylstannyl group. Separation of the fluorous compo-

nents of the reaction mixtures would not be difficult to achieve by fluorous solid-phase extraction. The challenge would be the removal of the tin halide byproduct. However, if it is possible to remove this byproduct with a solid-phase scavenger resin, the method may have much potential. The library could be screened with the use of, for example, a perfluoroalkyl-derivatized microscope slide printed with a microarray.[65]

With the addition of the palladium-catalyzed cross-coupling reaction to the existing methods of preparing precursors for the fluorous labeling strategy, it is now possible to prepare an FLS precursor for almost any radiotracer imaginable. This development should permit the rapid and facile synthesis of a wide range of radiotracers in high effective specific activity. With further research, the fluorous labeling strategy has the potential to accelerate the development of novel radiotracers, aid in the drug discovery process by permitting the synthesis of radiolabeled analogs of drug leads for biodistribution studies, and make accessible a wider range of targeted radiotracers for use in diagnostic imaging and therapy.

Chapter 5

Experimental

5.1 Equipment and Instrumentation

Unless otherwise specified, all reactions were conducted under an atmosphere of dry nitrogen. Glassware was flame-dried prior to use. Tris(1H,1H,2H,2H-perfluorooctyl)phenyltin was obtained from Fluorous Technologies Incorporated (970 William Pitt Way, Pittsburgh, PA). Fluorinert™ electronic liquid FC-72 (perfluorinated hydrocarbons consisting mostly of perfluorohexanes) was obtained from 3M Canada Company (London, ON). Other reagents were obtained from Sigma-Aldrich. All reagents were used without further purification. Prior to use, THF and diethyl ether were distilled over sodium/benzophenone and DCM and toluene were distilled over calcium hydride.

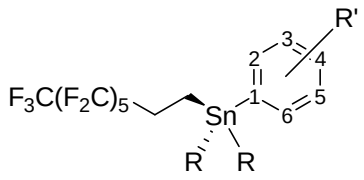
Proton NMR spectroscopy was performed on a Bruker Avance 200, DRX500, or Avance 600 spectrometer. Carbon-13 and tin-119 NMR spectroscopy were performed on a Bruker DRX500 or Avance 600 spectrometer. Carbon-13 NMR spectra

were processed by linear backward prediction on the real and imaginary data using an offset of 32 data points and 1024 coefficients. When possible, NMR spectroscopy was performed in chloroform-d or methanol-d_4 with the residual solvent signal used as a reference for ^{1}H and ^{13}C-NMR. For 500 and 600 MHz NMR spectra, sealed ampoules of deuterated solvent (Cambridge Isotope Laboratories, Andover, MA) were used. Fluorous compounds not soluble in conventional NMR solvents were dissolved in FC-72 containing tetramethylsilane as an internal reference. Chemical shifts for ^{119}Sn NMR spectroscopy were referenced to the solvent signal of a ^{1}H spectrum of the same sample acquired immediately before acquisition of the ^{119}Sn spectrum.[34]

Infrared spectroscopy was performed on a Bio-Rad FTS-40 spectrometer using thin films on KBr disks. Low-resolution mass spectrometry was performed on a Micromass Quattro Ultima triple quadrupole mass spectrometer; high-resolution mass spectrometry was performed on a Micromass Global Ultima quadrupole time-of-flight mass spectrometer. Gas chromatography was performed using an Agilent 7890A gas chromatograph with an Agilent 5975C mass spectrometric detector (MSD) and an Agilent HP-5MS column (30 m × 0.25 mm, 0.25 micron). Helium was used as the carrier gas and the injection volume was 1 μL. The initial column oven temperature of

50°C was held for one minute, followed by an increase of 20°C per minute to 250°C at 6 minutes; the final temperature was held for 10 minutes.

HPLC was performed using a Varian ProStar 230 pump and 330 PDA detector with a Nucleosil 100-5 C_{18} 250 × 4.6 mm column. For cold iodination and radiochemistry **(elution method A)**, a flow rate of 2.0 mL/min was used with a starting mobile phase composition of 90:10 water:acetonitrile which was held until 3 minutes, followed by a gradient to 100% acetonitrile at 6 minutes, with a total time of 12 minutes. For characterization of compounds **(elution method B)**, the mobile phase (flow rate 1.0 mL/min) was initially 70:30 acetonitrile:water and was increased to 100% acetonitrile from 0 to 10 minutes. The final composition was held until 25 minutes. The UV detector was set to 254, 230 or 220 nm. For column chromatography, the solvent composition used for TLC was used with a glass column and 40 – 63 m silica gel, or a Biotage SP1 automated purification system was used with a 15 × 120 mm silica cartridge and a solvent composition starting at 100% hexanes and increasing over 10 column volumes (CV) to the solvent composition used for TLC; the final composition was held from 10 to 20 CV.



R = $CH_2CH_2(CF_2)_5CF_3$
R' = any substituent

Figure 5-1: Numbering scheme for spectroscopic data.

5.2 Synthesis of tris(1H,1H,2H,2H-perfluorooctyl)tin bromide[25]

A solution of bromine (0.4 mL, 1.25 g, 7.8 mmol) in 10 mL of diethyl ether was added dropwise over 20 minutes to a stirred solution of tris(1H,1H,2H,2H-perfluoro-octyl)phenyltin (2.47 g, 2 mmol) in diethyl ether (25 mL) kept at 0°C. The mixture was allowed to warm to room temperature over 12 hours with stirring. The solvent and excess bromine, benzene and bromobenzene byproducts were removed by rotary evaporation. The orange oil thus obtained was dissolved in FC-72 (10 mL) and washed with dichloromethane (3 × 5 mL). The fluorous layer was then dried with sodium sulfate and the solvent was removed by rotary evaporation to yield a colourless oil (2.40 g, 1.9 mmol, 97%). TLC: R_f = 0.68 (5:1 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 2.516 ppm (m, 6H, $CF_2C\underline{H_2}$), 1.566 (m, 6H, $C\underline{H_2}Sn$); ^{13}C NMR (150.90 MHz, FC-72): δ 26.93 ppm ($CF_2\underline{C}H_2$), 4.28 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, FC-72): δ 102.1; IR (cm^{-1}): 2943, 1239, 1207, 1145; MS (ES-): 1320.9, ($[M+Br]^-$).

5.3 Synthesis of tris(1H,1H,2H,2H-perfluorooctyl)tin hydride[25]

A 1.0 M solution of lithium aluminum hydride in diethyl ether (2 mL, 2 mmol) was added to 10 mL of diethyl ether and the mixture was cautiously added dropwise over 30 minutes to a stirred solution of tris(1H,1H,2H,2H-perfluorooctyl)tin bromide (2.47 g, 2 mmol) in diethyl ether (25 mL) kept at 0°C under an atmosphere of nitrogen. The mixture was stirred for 6 hours then quenched by the careful addition of isopropanol (3 mL), methanol (3 mL), and deionized water (7.5 mL) to the vigourously stirred mixture. A solution of sodium potassium tartrate (20% w/w, 7.5 mL) was added and the organic layer was separated and set aside. The aqueous layer was extracted with diethyl ether (3 × 25 mL) and the combined organic layers dried over magnesium sulfate. Removal of the solvent by rotary evaporation afforded a white oil which was purified by column chromatography on silica (5:1 hexanes:EtOAc) to give a colourless oil (1.90 g, 1.6 mmol, 82%). Washing the column with diethyl ether was necessary for complete recovery of the product. TLC: R_f = 0.65 (5:1 hexanes:EtOAc); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 5.339 ppm (s with ^{119}Sn satellites, 1H, $^1J_{HSn}$ = 1807.4 Hz, Sn-H), 2.379 (m, 6H, $CF_2C\underline{H_2}$), 1.238 (m, 6H, $C\underline{H_2}Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 28.57

($CF_2\underline{C}H_2$), -2.42 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -62.9; IR (cm^{-1}): 1860; MS: (ES-): 1161.1, ($[M-H]^-$).

5.4 Synthesis of hexa(1H,1H,2H,2H-perfluorooctyl)distannane

To a stirred solution of tris(1H,1H,2H,2H-perfluorooctyl)tin hydride (120 mg, 0.10 mmol) in toluene or THF (3.8 mL) under a nitrogen atmosphere was added 1.2 mg of $Pd(PPh_3)_4$ in toluene (1.2 mL). The reaction mixture was protected from light and stirred for four hours, after which it was extracted with FC-72 (3 × 3 mL). The combined fluorous layers were dried over sodium sulfate and the solvent removed to afford a pale yellow oil which was purified by column chromatography on silica (eluent: 5:1 hexanes:EtOAc) to give a colourless oil (106 mg, 0.05 mmol, 91%). TLC: R_f = 0.70 (5:1 hexanes:EtOAc); 0.06 (99:1 hexanes:EtOAc); ^1H NMR (600.13 MHz, FC-72): δ 2.479 (m, 6H, $CF_2C\underline{H_2}$), 1.370 (m, 6H, $C\underline{H_2}Sn$); ^{13}C NMR (150.90 MHz, FC-72): δ 27.96 ($CF_2\underline{C}H_2$), -1.46 ($\underline{C}H_2Sn$); ^{119}Sn NMR (223.79 MHz, $CDCl_3$): δ -58.8; IR (cm^{-1}): 1238, 1204, 1145; HRMS (ES+): 2339.9 ($[M + NH_4]^+$).

5.5 Cross-coupling reactions

The synthesis of tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzene (**2a**) is used as a representative example. Compounds **2b-2l** were prepared in a similar manner. All compounds were isolated by column chromatography as discussed in Section 5.1.

5.5.1 Synthesis of 2a using purified distannane (Method A)

To a stirred solution of hexa(1H,1H,2H,2H-perfluorooctyl)distannane (116 mg, 50 μmol) in THF (3.9 mL) was added $Pd(PPh_3)_4$ (1.7 mg, 1.5 μmol, 3 mol%) in THF (0.6 mL) and a solution of iodobenzene (9 mg, 45 μmol, 0.9 equiv.) in THF (0.5 mL). The reaction mixture was brought to reflux and stirred for 72 hours. The reaction mixture was extracted with FC-72 (3 × 5 mL) and the combined fluorous layers were dried with sodium sulfate and concentrated by rotary evaporation. The desired product was isolated as a colourless oil (30 mg, 24 μmol, 48%) by flash chromatography on silica gel using an eluent mixture of hexanes and ethyl acetate (199:1).

5.5.2 Synthesis of 2a using distannane formed *in situ* (Method B)

To a stirred solution of tris(1H,1H,2H,2H-perfluorooctyl)tin hydride (174 mg, 150 μmol) in THF (4.2 mL) was added $Pd(PPh_3)_4$ (2.6 mg, 2.3 μmol, 3 mol%) in THF (0.85 mL) under an inert atmosphere. The reaction mixture was protected from light and stirred at room temperature. After four hours, a solution of iodobenzene (14 mg, 68 μmol, 0.9 equiv.) in THF (0.35 mL) and an additional 0.5 mg of $Pd(PPh_3)_4$ in THF (0.25 mL) were added. The mixture was heated at reflux temperature for 72 hours or until completion of the reaction had been observed by GC. Aliquots of ~ 0.1 mL were taken for analysis by GC using a glass syringe, then diluted with hexanes to 1 mL and filtered through glass wool prior to GC analysis. Upon completion of the reaction, the reaction mixture was extracted with FC-72 (3 × 3 mL). The combined fluorous layers were dried over sodium sulfate and the solvent was removed by rotary evaporation to yield a yellow oil. The product was isolated by column chromatography on silica gel (hexanes:EtOAc, 199:1). The fractions containing product were collected and the solvent was removed to afford a colourless oil (43 mg, 51%).

5.5.3 One-pot synthesis of 2a using phosphaadamantane ligand (Method C)

A solution of tris(1H,1H,2H,2H-perfluorooctyl)tin hydride (232 mg, 200 μmol) in THF (4 mL) was added to a reaction vessel which had been evacuated and purged with argon. A solution of 1,3,5,7-tetramethyl-2,4,8-trioxa-(2,4-dimethoxyphenyl)-6-phosphaadamantane (2.1 mg, 6 μmol, 6 mol%) in THF (1 mL) was added to the reaction vessel, followed by a solution of palladium acetate (0.7 mg, 3 μmol, 3 mol%) in THF (1 mL). The reaction mixture was stirred for 10 minutes, during which time evolution of hydrogen was observed. Then, iodobenzene (18 mg, 90 μmol, 0.9 equiv.) in THF (1 mL) was added. The mixture was heated at reflux temperature for 72 hours or until completion of the reaction had been observed by GC. Aliquots of ~ 0.1 mL were taken for analysis by GC using a glass syringe, then diluted with hexanes to 1 mL and filtered through glass wool prior to GC analysis. Upon completion of the reaction, the reaction mixture was extracted with FC-72 (3 × 3 mL). The combined fluorous layers were dried over sodium sulfate and the solvent was removed by rotary evaporation. The product was isolated by flash chromatography on silica gel (hexanes:EtOAc, 199:1) to afford a colourless oil (74 mg, 60 μmol, 67%).

5.6 Characterization of cross-coupling products

Tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzene (**2a**)

Colourless oil; yield 48% (from iodobenzene, method A), 51% (from iodobenzene, method B), 27% (from bromobenzene, method B), 67% (method C); TLC: R_f = 0.78 (5:1 hexanes:EtOAc); 0.37 (199:1 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ .435 (m, 5H, H-aryl), 2.310 (m, 6H, $CF_2C\underline{H}_2$), 1.305 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): 136.65 (C-1), 136.19 (C-2, C-6), 129.77 (C-4), 129.18 (C-3, C-5), 27.90 ($CF_2\underline{C}H_2$), -1.31 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -29.39; IR (cm^{-1}): 1239, 1207, 1145; HRMS (ES-): 1350.9567 ([M + TFA]$^-$, 1350.9586 calculated); HPLC (230 nm): R_t = 20.3 min.; GC/MS: R_t = 10.3 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylanisole (**2b**)

Colourless oil; yield 60% (from 4-iodoanisole, method B), 21% (from 4-bromoanisole, method B); TLC: R_f = 0.29 (99:1 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.298 (m, 2H, H-2, H-6), 6.979 (d, 2H, ^{3}J = 8.4 Hz, H-3, H-5), 3.824 (s, 3H, $OC\underline{H}_3$), 2.295 (m, 6H, $CF_2C\underline{H}_2$), 1.271 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 161.03 (C-4), 137.31 (C-2,C-6), 126.51 (C-1), 115.11 (C-3,C-5), 55.26 ($O\underline{C}H_3$), 27.91 (C

TLC: R_f = 0.65 (9:1 DCM:MeOH); ^{1}H NMR (200.13 MHz, CD_3OD): 7.900 (s, 1H, H-6), 6.288 (t, 1H, H-1′), 4.387 (m, 1H, H-3′), 3.916 (m, 1H, H-4′), 3.727 (m, 2H, H-5′), 2.467 (m, 6H, $CF_2C\underline{H}_2$), 2.333 (m, 2H, H-2′), 1.260 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, CD_3OD): δ 169.35 (C-4), 152.79 ($CF_2\underline{C}H_2$), 147.74 (C-6), 110.87 (C-5), 89.45 (C-4′), 87.00 (C-1′), 72.65 (C-3′), 62.94 (C-5′), 41.76 (C-2′), 28.81 ($CF_2\underline{C}H_2$), 0.07 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, CD_3OD): δ -25.47; IR (cm^{-1}): 3437, 1649, 1237, 1197, 1142; HRMS (ES-): 1500.9869 ([M + TFA]$^-$, 1500.9865 calculated); HPLC (254 nm): R_t = 13.7 min.$F_2\underline{C}H_2$), -1.32 ($\underline{C}H_2Sn$); ^{119}Sn NMR (223.79 MHz, $CDCl_3$): δ -25.61; IR (cm^{-1}): 1240, 1207, 1145; HRMS (ES-): 1326.9965 ([M + acetate]$^-$, 1326.9976 calculated); HPLC (230 nm): R_t = 19.8 min.; GC/MS: R_t = 11.2 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannyltoluene (**2c**)

Colourless oil; yield 60% (method B with 2 equivalents of distannane **1**), 24% (method C); TLC: R_f = 0.33 (hexanes); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.40-7.18 (m, 4H, H-aryl), 2.367 (s, 3H, $C\underline{H}_3$), 2.299 (m, 6H, $CF_2C\underline{H}_2$), 1.240 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 139.79 (C-4), 136.12 (C-2, C-6), 132.50 (C-1), 130.03 (C-3,C-5), 27.91 ($CF_2\underline{C}H_2$), 21.60 ($\underline{C}H_3$), -1.39 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -

28.33; IR (cm^{-1}): 2933, 1239, 1204, 1145; HRMS (ES-): 1311.0037 ([M + acetate]$^-$, 1311.0026 calculated); HPLC (230 nm) R_t = 21.4 min.; GC/MS: R_t = 10.6 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylethylbenzene (**2d**)

Colourless oil; yield 37% (method B), 18% (method C); TLC: R_f = 0.34 (hexanes); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.273 (m, 4H, H-aryl), 2.660 (q, 2H, ^3J = 7.5 Hz, $C\underline{H_2}CH_3$), 2.301 (m, 6H, $CF_2C\underline{H_2}$), 1.285 (m, 8H, $CH_2C\underline{H_3}$, $C\underline{H_2}Sn$; ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 146.09 (C-4), 136.26 (C-2, C-6), 132.89 (C-1), 129.00 (C-3,C-5), 29.03 ($\underline{C}H_2CH_3$), 27.93 ($CF_2\underline{C}H_2$), 15.52 ($CH_2\underline{C}H_3$), -1.37 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -28.45; IR (cm^{-1}): 2974, 2942, 1239, 1202; HRMS (ES-): 1325.0189 ([M + acetate]$^-$, 1325.0183 calculated); HPLC (230 nm): R_t = 22.0 min., GC/MS: R_t = 10.9 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylchlorobenzene (**2e**)

Colourless oil; yield 42% (method B), 41% (method C); TLC: R_f = 0.44 (hexanes); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.356 (m, 4H, H-aryl), 2.258 (m, 6H, $CF_2C\underline{H}_2$), 1.301 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 137.25 (C-2, C-6), 136.36 (C-1), 134.86 (C-4), 129.40 (C-3,C-5), 27.86 ($CF_2\underline{C}H_2$), -1.04 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -26.00; IR (cm^{-1}): 2944, 1239, 1203, 1145; HRMS (ES-): 1384.9188 ([M + TFA]$^-$, 1384.9190 calculated); HPLC (220 nm): R_t = 20.5 min.; GC/MS: R_t = 11.0 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzoic acid (**2f**)

Colourless oil; yield 30% (method B), 44% (method C); TLC: R_f = 0.19-0.40 (1:1 hexanes:diethyl ether); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 8.113 (d, 2H, ^{3}J = 8.0 Hz, H-3, H-5), 7.530 (m, 2H, H-2, H-6), 2.326 (m, 6H, $CF_2C\underline{H}_2$), 1.351 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 171.39 ($\underline{C}OOH$), 145.13 (C-1), 136.25 (C-2, C-6), 130.31 (C-4), 130.08 (C-3, C-5), 27.79 ($CF_2\underline{C}H_2$), -0.98 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -28.13; IR (cm^{-1}): 2927, 1698, 1238, 1205, 1144; HRMS (ES-): 1394.9596 ([M + TFA]$^-$, 1394.9556 calculated); HPLC (254 nm): R_t = 18.1 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzonitrile (**2g**)

Colourless oil; yield 15% (method B), 19% (method C); TLC: R_f = 0.27 (95:5 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.402 (d, 2H, ^3J = 7.9 Hz, H-3, H-5), 7.308 (m, 2H, H-2, H-6), 2.301 (m, 6H, $CF_2C\underline{H}_2$), 1.344 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 137.25 (C-2, C-6), 136.35, 136.19, 129.41 (C-4), 129.20 (C-3, C-5), 27.86 ($CF_2\underline{C}H_2$), -1.26 ($\underline{C}H_2Sn$); ^{119}Sn NMR (223.79 MHz, $CDCl_3$): δ -26.09; IR (cm^{-1}): 2943, 2364, 1576, 1239, 1145; HRMS (ES-): 1321.9824 ([M + acetate]$^-$, 1321.9822 calculated); HPLC (230 nm): R_t = 20.6 min.; GC/MS: R_t = 11.0 min.

p-Tris(1H,1H,2H,2H-perfluorooctyl)stannylnitrobenzene (**2h**)

Colourless oil; yield 59% (method B), 54% (method C); TLC: R_f = 0.23 (95:5 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 8.234 (d, 2H, ^3J = 8.5 Hz, H-3, H-5), 7.604 (m, 2H, H-2, H-6), 2.338 (m, 6H, $CF_2C\underline{H}_2$), 1.376 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 149.18 (C-4), 147.31 (C-1), 136.87 (C-2, C-6), 123.20 (C-3, C-5), 27.70 ($CF_2\underline{C}H_2$), -0.67 ($\underline{C}H_2Sn$); ^{119}Sn NMR (223.79 MHz, $CDCl_3$): δ -25.69; IR (cm^{-1}): 2932, 1523, 1351, 1239, 1202, 1145; HRMS (ES-): 1395.9430 ([M + TFA]$^-$, 1395.9437 calculated); HPLC (254 nm): R_t = 18.1 min.

Ethyl m-tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzoate (**2i**)

Colourless oil; yield 24% (method B), 20% (method C); TLC: R_f = 0.44 (9:1 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 8.067 (m, 2H, H-2, H-4), 7.480 (m, 2H, H-5, H-6), 4.389 (q, 2H, ^{3}J = 7.0 Hz, O$\underline{H_2}$CH$_3$), 2.322 (m, 6H, CF$_2$C$\underline{H_2}$), 1.301 (m, 9H, C$\underline{H_2}$Sn, OCH$_2$C$\underline{H_3}$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 166.66 (C=O), 140.33 (C-6), 137.27 (C-1), 136.92 (C-2), 130.98 (C-3), 130.80 (C-4), 128.96 (C-5), 61.40 (O$\underline{C}$H$_2$CH$_3$), 27.81 (CF$_2$$\underline{C}H_2$), 14.38 (OCH$_2$$\underline{C}H_3$), -1.04 ($\underline{C}H_2$Sn); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -27.14; IR (cm^{-1}): 2964, 1715, 1238, 1200, 1143; HRMS (ES-): 1369.0081 ([M + acetate]$^-$, 1369.0082 calculated); HPLC (220 nm): R_t = 19.9 min.; GC/MS: R_t = 11.8 min.

m-Tris(1H,1H,2H,2H-perfluorooctyl)stannylphenol (**2j**)

Colourless oil; yield 38% (method B), 34% (method C). TLC: R_f = 0.23 (9:1 hexanes:EtOAc); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.298 (m, 1H, H-5), 6.858 (m, 3H, H-2, H-4, H-6), 4.789 (s, O$\underline{H}$), 2.303 (m, 6H, CF$_2$C$\underline{H_2}$), 1.291 (m, 6H, C$\underline{H_2}$Sn); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 155.19 (C-2), 137.60 (C-1), 129.57 (C-6), 127.69 (C-5), 121.84 (C-3), 116.05 (C-4), 27.14 (CF$_2$$\underline{C}H_2$), -1.98 ($\underline{C}H_2$Sn); ^{119}Sn NMR (223.79 MHz, $CDCl_3$):

δ -27.31; IR (cm^{-1}): 1238, 1205; HRMS (ES-): 1366.9547 ($[M + TFA]^-$, 1366.9536 calculated); HPLC (230 nm): R_t = 17.3 min.; GC/MS: R_t = 11.5 min.

o-Tris(1H,1H,2H,2H-perfluorooctyl)stannyltoluene (**2k**)

Colourless oil; yield 22% (method B), 12% (method C); TLC: R_f = 0.28 (hexanes); ^1H NMR (200.13 MHz, $CDCl_3$): δ 7.275 (m, 4H, H-aryl), 2.372 (s, 3H, $C\underline{H}_3$), 2.279 (m, 6H, $CF_2C\underline{H}_2$), 1.323 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 144.12 (C-1), 136.84 (C-2), 136.27 (C-5), 130.09 (C-3, C-6), 126.10 (C-4), 28.01 ($CF_2\underline{C}H_2$), 25.15 ($\underline{C}H_3$), -0.86 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -26.24; IR (cm^{-1}): 2924, 1238, 1196, 1144; HRMS (ES-): 1364.9728 ($[M + TFA]^-$, 1364.9744 calculated); HPLC (220 nm): R_t = 20.8 min.**;** GC/MS: R_t = 10.6 min.

o-Tris(1H,1H,2H,2H-perfluorooctyl)stannylbenzonitrile (**2l**)

Colourless oil; yield 28% (method B), 22% (method C); TLC: R_f = 0.33 (9:1 hexanes:EtOAc); ^1H NMR (500.13 MHz, $CDCl_3$): δ 7.734 (d, 1H, ^3J = 7.7 Hz, H-3), 7.609 (m, 1H, H-5), 7.503 (m, 2H, H-4, H-6), 2.389 (m, 6H, $CF_2C\underline{H}_2$), 1.478 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 144.27 (C-1), 136.78 (C-6), 133.41 (C-3),

132.67 (C-5), 130.04 (C-4), 120.67 and 120.10 (CN and C-2), 27.83 (CF_2CH_2), -0.37 (CH_2Sn); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -23.65; IR (cm^{-1}): 2945, 2222, 1238, 1206, 1144; HRMS (ES-): 1307.9680 ($[M + formate]^-$, 1307.9666 calculated); HPLC (230 nm): R_t = 18.3 min.; GC/MS: R_t = 11.1 min.

5.7 Synthesis of a protected fluorous FIAU precursor, 1-(3′,5′-dibenzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl) stannyluracil (10)

Following evacuation and filling of a reaction flask with argon, a solution of tris(1H,1H,2H,2H-perfluorooctyl)tin hydride (232 mg, 200 μmol) in THF (2 mL) was added followed by solutions of 1,3,5,7-tetramethyl-2,4,8-trioxa-(2,4-dimethoxyphenyl)-6-phosphaadamantane (2.1 mg, 6 μmol, 6 mol%) in THF (1 mL) and palladium acetate (0.7 mg, 3 μmol, 3 mol%) in THF (1 mL). The reaction mixture was stirred at room temperature for 10 minutes, during which time evolution of hydrogen was observed. A solution of 1-(3′,5′-dibenzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-bromouracil (48 mg, 90 μmol, 0.9 equiv.) in 1 mL of THF was added and

the reaction mixture was heated at reflux. Progress of the reaction was monitored by HPLC (elution method B). If the progress of the reaction appeared to stop before complete consumption of the starting material or if a colour change from yellow-orange to black was observed, further portions of the phosphaadamantane ligand and palladium(II) acetate were added in the same amounts stated above. Upon completion of the reaction, the reaction mixture was filtered through a 1 cm bed of silica with 0.5 cm layers of Celite above and below, and the silica was washed with THF (10 mL). The pale brown solution was extracted with FC-72 (3 × 5 mL) to remove the fluorous tin halide and distannane byproducts, and the organic layer was concentrated by rotary evaporation to afford an orange-brown oil. The oil was purified by column chromatography using an eluent mixture of hexanes and diethyl ether. The fractions containing product were concentrated by rotary evaporation to yield a white foam (69 mg, 43 μmol, 48%). TLC: R_f = 0.54 (1:1 hexanes:Et_2O); ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 8.480 (s br, 1H, N<u>H</u>), 8.055 (m, 4H, H-2″, H-6″), 7.527 (m, H-6, H-3″, H-4″, H-5″), 6.349 (m, 1H, H-1′), 5.601 (m, H-3′), 5.346 (m, 1H, H-2′), 4.835, 4.709 (m, 1H, H-5′), 4.537 (m, 1H, H-4′), 2.300 (m, 6H, $CF_2C\underline{H}_2$), 1.170 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.91 MHz, $CDCl_3$): δ 166.24, 166.13 (<u>C</u>=O), 165.43 (C-4), 150.37 ($CF_2\underline{C}H_2$), 145.77 (C-6), 134.46

and 133.72 (C-4″), 130.20, 129.84 (C-2″, C-6″), 129.65 and 128.32 (C-1″), 128.99, 128.74 (C-3″, C-5″), 109.42 (C-5), 93.41, 92.14 (C-2′), 85.41, 85.30 (C-1′), 82.14 (C-4′), 76.67 (C-3′), 63.49 (C-5′), 27.59 ($CF_2\underline{C}H_2$), -0.73 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $CDCl_3$): δ -23.32; IR (cm^{-1}): 3438, 1726, 1239, 1207, 1145; HRMS (ES-): 1613.0389 ([M - H]$^-$, 1613.0358 calculated); HPLC (254 nm): R_t = 16.1 min.

5.8 Synthesis of a deprotected fluorous FIAU precursor, 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil (11)

To a solution of 5-tris(1H,1H,2H,2H-perfluorooctyl)-1-(3′,5′-dibenzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)uracil (81 mg, 50 μmol) in THF (2 mL) was added a 0.5 M solution of sodium methoxide in MeOH (0.3 mL, 150 μmol). The reaction was monitored by HPLC (elution method B). Upon completion, the reaction mixture was neutralized with glacial acetic acid (approx. 30 μL). The product was loaded onto an FSPE cartridge which had been conditioned with 6 mL of 80:20 MeOH:water.The cartridge was then washed with 80:20 MeOH:water (8 mL) and MeOH (8 mL). Evaporation of the MeOH fractions afforded the product as a white foam (56 mg, 40 μmol,

80%). TLC: R_f = 0.69 (9:1 DCM:MeOH); ^{1}H NMR (500.13 MHz, CD_3OD): δ 7.736 (m, 1H, H-6), 6.325 (m, 1H, H-1′), 5.103 (m, 1H, H-2′), 4.401 (m, 1H, H-3′), 4.023 (m, 1H, H-4′), 3.830 (m, 2H, H-5′), 2.515 (m, 6H, $CF_2C\underline{H}_2$), 1.352 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (125.75 MHz, CD_3OD): δ 169.16 (C-4), 152.53 ($CF_2\underline{C}H_2$), 147.97 (C-6), 109.97 (C-5), 97.15 (C-2′), 85.98 (C-4′), 85.53 (C-1′), 75.31 (C-3′), 62.01 (C-5′), 28.83 ($CF_2\underline{C}H_2$), 0.17 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, $(CD_3)_2C=O$): δ -24.87; IR (cm^{-1}): 3437, 2922, 1124; HRMS (ES-): 1465.0084 ([M + acetate]$^-$, 1465.0052 calculated); HPLC (265 nm): R_t = 13.4 min.

5.9 Synthesis of a fluorous IUdR precursor, 1-(2′-deoxy-β-D-ribofuranosyl)-5-tris(1H,1H,2H,2H-perfluorooctyl)stannyluracil (13)

Following evacuation and filling with argon of the reaction vessel containing tris(1H,1H,2H,2H-perfluorooctyl)tin hydride (232 mg, 200 μmol) and 5-iodo-1-(2′-deoxy-β-D-ribofuranosyl)uracil (33 mg, 90 μmol) under argon, THF (2 mL) was added followed by a solution of 1,3,5,7-tetramethyl-2,4,8-trioxa-(2,4-dimethoxyphenyl)-6-phosphaadamantane (2.1 mg, 6 μmol, 6 mol%) in THF (1 mL). A solution of tris(dibenzylideneacetone)dipalladium (1.4 mg, 1.5 μmol, 1.5 mol%) in THF (1 mL)

was added and the reaction mixture was heated to 65°C and stirred under argon. The progress of the reaction was monitored by HPLC (elution method B). Upon completion of the reaction, the reaction mixture was diluted with THF (10 mL) and extracted with FC-72 (3 × 5 mL). The organic layer was dried with magnesium sulfate, filtered, and concentrated to afford a brown oil from which the desired product was isolated by column chromatography on a Biotage SP1 automated chromatography unit (stationary phase: silica gel, mobile phase: gradient of 0-10% MeOH in DCM (0-10 column volumes), 10% MeOH in DCM (10-20 column volumes). After concentration, the residue was dissolved in 80% MeOH-water (250 μL) and loaded onto an FSPE cartridge that had been conditioned with DMF (1 mL) and 80% MeOH-water (5 mL). The column was washed with 80% MeOH-water (5 mL), then MeOH (5 mL) to elute the desired product which was isolated, following concentration of the methanol fraction by rotary evaporation, as a white foam (26 mg, 19 μmol, 21%). TLC: R_f = 0.65 (9:1 DCM:MeOH); ^{1}H NMR (200.13 MHz, CD_3OD): 7.900 (s, 1H, H-6), 6.288 (t, 1H, H-1′), 4.387 (m, 1H, H-3′), 3.916 (m, 1H, H-4′), 3.727 (m, 2H, H-5′), 2.467 (m, 6H, $CF_2C\underline{H}_2$), 2.333 (m, 2H, H-2′), 1.260 (m, 6H, $C\underline{H}_2Sn$); ^{13}C NMR (150.90 MHz, CD_3OD): δ 169.35 (C-4), 152.79 ($CF_2\underline{C}H_2$), 147.74 (C-6), 110.87 (C-5), 89.45 (C-4′), 87.00 (C-1′), 72.65 (C-

3′), 62.94 (C-5′), 41.76 (C-2′), 28.81 ($CF_2\underline{C}H_2$), 0.07 ($\underline{C}H_2Sn$); ^{119}Sn NMR (186.50 MHz, CD_3OD): δ -25.47; IR (cm^{-1}): 3437, 1649, 1237, 1197, 1142; HRMS (ES-): 1500.9869 ([M + TFA]$^-$, 1500.9865 calculated); HPLC (254 nm): R_t = 13.7 min.

5.10 Cold iodination[6]

To a vial containing 10 mg of the desired trialkylstannyl precursor was added acetonitrile (200 μL) and a solution of iodine in MeCN (10 mg/mL, 2 μL). After stirring for 15 minutes, the reaction was quenched by the addition of an aqueous solution of sodium metabisulfite (10 μL, 0.01 M). The crude reaction mixture was analyzed by HPLC (elution method A), then diluted with water (2 mL). The diluted reaction mixture was loaded onto a fluorous solid-phase extraction cartridge that had been conditioned with DMF (1 mL) and 80% MeOH-water (6 mL). After loading, the reaction vial was rinsed with water (2 mL) which was added to the FSPE cartridge. The product was then eluted using 80% MeOH-water (12 mL). The MeOH-water fractions were concentrated and the product was dissolved in MeCN (1 mL) for analysis by HPLC (elution method A).

5.11 General radioiodination procedure

A glass vial was coated with Iodo-Gen® (1,3,4,6-tetrachloro-3 ,6 -diphenyl-glycouril) by transferring the oxidant as a solution in chloroform (2 μL, 1 mg/mL) into the vial and removing the chloroform under reduced pressure. The vial was stored in the freezer until immediately before use. To an Iodo-Gen vial was added a solution of the trialkylstannyl precursor in methanol (100 μL, 1 mg/mL), then glacial acetic acid (1 μL) and aqueous sodium [^{125}I]iodide (1 μL, 25-45 Ci, 925-1455 kBq). The reaction mixture was allowed to stand for 3 minutes with occasional swirling, then quenched by the addition of aqueous sodium metabisulfite (10 μL, 0.01 mol/L). For HPLC analysis of the crude reaction mixture, a 10 μL aliquot was diluted with MeCN to 70 μL. The reaction mixture was immediately diluted with water (1 mL), then loaded onto a FSPE cartridge which had been conditioned with 80% MeOH-water (6 mL). The reaction vial was rinsed with water (1 mL), which was added to the FSPE cartridge. The cartridge was then eluted with additional water (5 mL) collected in 1.5 mL fractions to elute excess sodium [^{125}I]iodide. To recover the labeled product, the cartridge was eluted with 80% MeOH-water (6 mL, collected in 1.5 mL fractions). The activities of all collected fractions were measured using a dose calibrator.

The radiolabeling reaction was performed in triplicate for each precursor. For one reaction with each precursor, all fractions were analyzed. The first aqueous fraction and the first two organic fractions were analyzed separately; the remaining aqueous and organic fractions were analyzed together. For the remaining two experiments with each precursor, only the fraction having the highest activity was analyzed.

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