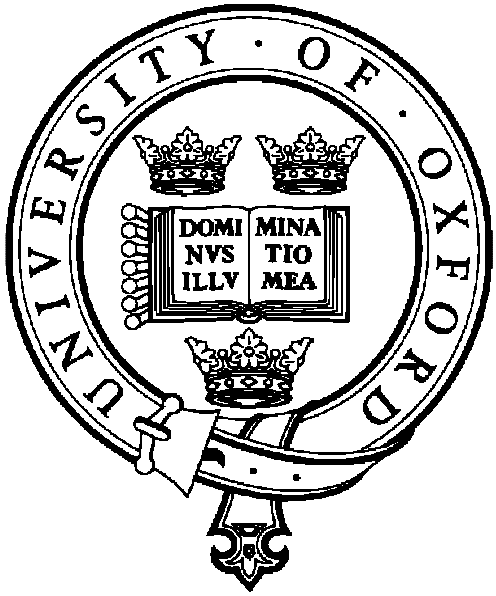
NOVEL RADIOCHEMISTRY FOR ^{18}F LABELLED AROMATICS

A thesis submitted to the Board of the Faculty of Physical Science in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford

Lei Li



Hertford College, Oxford, Trinity Term 2011

This thesis is dedicated to my parents:

Mr. Guo Li and Mrs Peirong Chen.

此论文谨献予我的父母：李果 先生，陈佩荣 女士。

Author's Declaration

The work presented in this thesis was conducted at the Chemistry Research Laboratory and the Inorganic Chemistry Laboratory at the University of Oxford and the GlaxoSmithKline Clinical Imaging Centre at Hammersmith Hospital, London between Michaelmas Term 2007 and Trinity Term 2011 under the supervision of Professor Véronique Gouverneur. All the work is my own, except where otherwise stated, and has not been submitted for any other degree at this or any other university.

Lei Li

September 2011

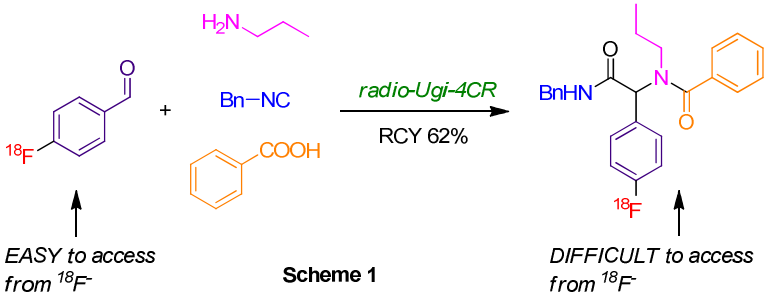
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D.Phil
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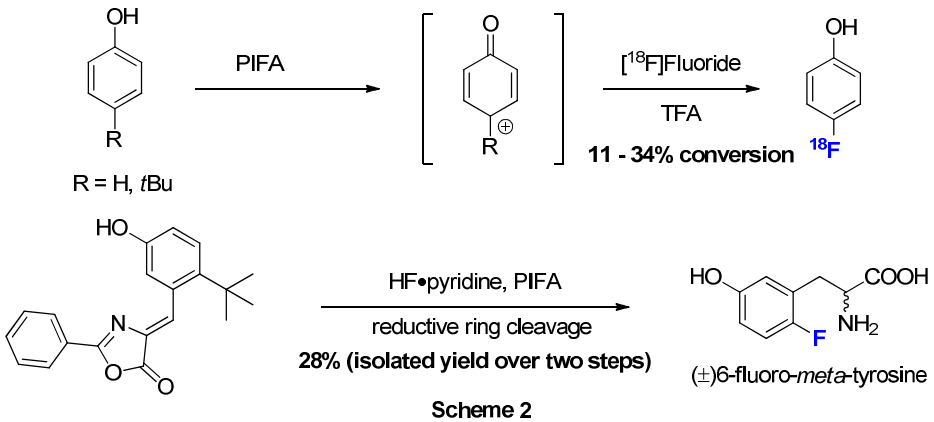
Positron emission tomography (**PET**) employs short half-life positron emitting isotopes, typically ^{18}F, for *in vivo* measurement of physiological processes. Easy access to structurally diverse radiolabelled probes would accelerate the rapid progress of PET imaging but, to date, radiochemistry is still limited by cost and efficiency. Nucleophilic fluorination with ^{18}F-fluoride is the preferred "*non-carrier-added*" methodology in the synthesis of ^{18}F-labelled pharmaceuticals because it leads to radiotracers with a high specific activity, a key feature allowing for investigations to be performed in sub-toxic doses. **Chapter 1** serves as an introduction on radiochemistry, especially focussing on current radiosynthetic methods for the synthesis of ^{18}F-labelled aromatics.

Aromatic compounds without electron-withdrawing groups are notoriously difficult to label with ^{18}F-fluoride. In this thesis, we present two novel methodologies to deliver ^{18}F-labelled aromatic compounds from nucleophilic ^{18}F-fluoride**. Chapter 2** details the experimental efforts towards "**Convergent Radiosynthesis**" (Scheme 1). We proposed a convergent synthetic tactic that allows for simultaneous reaction between three or more substrates, including an ^{18}F-labelled building block. This chemistry has been validated by the radiosynthesis of various structural scaffolds which are not responsive to direct nucleophilic fluorination.



Scheme 1

Chapter 3 presents our research into "**Oxidative Nucleophilic ^{18}F-Fluorination**" (Scheme 2). We proposed that electron-rich aromatics, such as phenols, which are not responsive to nucleophilic fluorination may undergo umpolung reactivity under oxidative conditions. This "umpolung strategy" allows for the direct transformation from ^{18}F-fluoride to 4-[^{18}F]fluorophenol. Potentially, this established oxidative fluorination strategy could be adapted to the radiosynthesis of radiotracers containing a 4-fluorophenol sub-motif, such as 6-fluoro-*meta*-tyrosine. An appropriate precursor has been validated for the prospective radiosynthesis of 6-[^{18}F]fluoro-*meta*-tyrosine.



Scheme 2

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I would like to take this opportunity to thank my Mum and Dad for all the efforts they have made to send me to one of the best Universities in the world, from such a small city in China. I also feel thankful for having a wonderful brother who always backs me up and supports everything I have done. Thank you for being the best family in the world.

Abbreviations

[^{18}F]	Fluorine-18 label
[^{18}F]EF5	2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoropropyl)acetamide
[^{18}F]FBA	4-[^{18}F]Fluorobenzaldehyde
[^{18}F]FDG	2-Deoxy-2-[^{18}F]fluoro-D-glucose
[^{18}F]FDOPA	6-[^{18}F]Fluoro-L-3,4-dihydroxyphenylalanine
[^{18}F]FET	*O*-(2-[^{18}F]fluoroethyl)-L-tyrosine
[^{18}F]FMISO	[^{18}F]Fluoromisonidazole
[^{18}F]FLT	[^{18}F]Fluorothymidine
Å	Ångström (10^{-10} metres)
Ac	Acetyl
aq	Aqueous
Bn	Benzyl
Boc	*t*-Butyloxycarbonyl
Bq	Becquerel (s^{-1})
Bu	Butyl
Bz	Benzoyl
^{o}C	Degrees Celsius
c.a.	*Carrier-Added*
CAN	Ceric Ammonium Nitrate
Cbz	Carboxybenzyl
Ci	Curie
CI	Chemical Ionisation
cm^{-1}	Wavenumber
CSA	Camphorsulfonic Acid
CT	Computed Tomography
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
d.c.	*Decay-corrected*
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DHMP	3,4-Dihydropyrimidin-2-(*1H*)-one
DIPA	Diisopropylamide
DIPEA	Diisopropylethylamine
DMA	Dimethylacetamide
DMSO	Dimethyl sulfoxide
DMAP	4-*N,N*-Dimethylammonium pyridine
DMF	*N,N*-Dimethyl formamide
dr	Diastereoisomeric Ratio
E^0	Standard Electrode Potential
E1cB	Elimination from Conjugate Base
ee	Enantiomeric Excess

Et	Ethyl
eV	Electron volts
FT-IR	Fourier Transform Infrared
HBTU	*O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HSA	Human Serum Albumin
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
hr	hour(s)
IMCR	Isocyanide-Based Multicomponent Reaction
$^{n}J_{XY}$	Coupling constant in NMR soectra, between nuclei X and Y through n bonds
K_{222}	Kryptofix 222, 1,10-Diaza-4,7,13,16,21,24-hexaoxabicyclo[8.8.8]hexacosane
LDA	Lithium diisopropylamide
LUMO	Lowest Unoccupied Molecular Orbital
M	Moles per cubic decimetre ($moldm^{-3}$)
MAO-N	Monoamine oxidase-N
mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
mol	Mole(s)
m.p.	Melting point
m/z	Mass/charge ratio
MeCN	Acetonitrile
MeOH	Methanol
MCR	Multicomponent reaction
MRI	Magnetic resonance imaging
NBS	*N*-Bromosuccinimide
n.c.a.	*non-carried-added*
n.d.c.	*non-decay-corrected*
n.r.	No reaction
NFSI	*N*-Fluorobenzenesulfonimide
NMR	Nuclear Magnetic Resonance Spectroscopy
o/n	Overnight
PDC	Pyridinium dichromate
PET	Positron emission tomography
PIDA	Phenyliodine diacetate
PIFA	Phenyliodine bis(trifluoroacetate)
PMT	Photo Multiplier Tube
QMA	Quaternary Methyl Ammonium
ppm	Parts per million
R_f	Retention factor
γ-rays	Gamma rays
RCY	Radiochemical yield

rt	Room temperature
SA	Specific activity
SI Unit	Système international d'unités (The International System of Units)
S_EAr	Electrophilic Arimatic Substitution
S_NAr	Nucleophilic Aromatic Substitution
SPECT	Single Photo Emission Computed Tomography
t	Tertiary (*tert*)
Tf	Trifluoromethanesulfonyl (Trifyl)
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Ts	*para*-Toluenesulfonyl (Tosyl)
Ugi-3-CR	Ugi Three Component Reaction (U-3-CR)
UV	Ultraviolet
VCAM-1	Vascular cell adhesion molecule-1

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Chapter 1

Introduction

1.1 Production of positron-emitting radionuclides

Radionuclides are produced in a cyclotron using neutrons or charged particles as the irradiation source. Irradiation by neutrons leads to the production of neutron-rich nuclides *via* neutron capture. Irradiation by charged particles leads to neutron-deficient nuclides. For example, ^{18}F is produced by bombarding [^{18}O]H_2O or [^{18}O]O_2 with proton beams or by bombarding [^{20}Ne]Ne with deuterium beams. The nuclear reaction can be presented as a chemical equation or a compact notation as shown in **Scheme 1.1**. The symbols *p*, *n*, *d* and α are used to represent proton, neutron, deuterium and alpha particle (He^{2+}) respectively.

$^{18}_{8}O + {}^{1}_{1}H \longrightarrow {}^{18}_{9}F + n$ or $^{18}O(p,n)^{18}F$

$^{20}_{10}Ne + {}^{2}_{1}H \longrightarrow {}^{18}_{9}F + {}^{4}_{2}He$ or $^{20}Ne(d,\alpha)^{18}F$

$^{1}_{1}H = p$ = proton; n = neutron

$^{2}_{1}H = d$ = deuterium; $^{4}_{2}He = \alpha$ = alpha particle

Scheme 1.1. Nuclear reaction for production of ^{18}F

1.2 Positron Emission Tomography (PET)

Positron emission tomography (PET) employs short half-life positron-emitting isotopes, such as ^{15}O ($t_{1/2}$ = 2.04 min), ^{13}N ($t_{1/2}$ = 9.97 min), ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 109.7 min), for *in vivo* measurement of physiological processes.[1] To date, PET imaging is a widely applied non-invasive imaging technique with extensive use in diagnostic medicine and clinical pharmaceutical research. The major clinical applications of PET are in the areas

of oncology, cardiology and neurology. PET is typically used for clinical diagnosis, to access how patients respond to therapy and to accelerate drug discovery.[2]

Radionuclides used in PET imaging emit positrons upon decay. For ^{18}F, the decay results in the formation of an ^{18}O atom and a positively charged beta particle (or positron β^+) along with a neutrino (ν, **Figure 1.1**).

$$^{18}F \longrightarrow {}^{18}O + \beta^+ + \nu$$

Figure 1.1. Decay of ^{18}F atom.

Table 1.1 summarises the production and physical properties of the most commonly used short-lived radionuclides in PET.[3]

Table 1.1 Production and physical properties of important short-lived radionuclides in PET[1]

Radionuclide	Half-life $t_{1/2}$ (min)	Nuclear Reaction	Target	Product	Decay Product
^{11}C	20.4	$^{14}N(p,\alpha)^{11}C$	$N_2(+O_2)$	$[^{11}C]CO_2$	^{11}B
			$N_2(+H_2)$	$[^{11}C]CH_4$	
^{13}N	9.97	$^{16}O(p,\alpha)^{13}N$	H_2O	$[^{13}N]NO_x$	^{13}C
			H_2O+EtOH	$[^{13}N]NH_3$	
^{15}O	2.04	$^{15}N(p,n)^{15}O$	$N_2(+O_2)$	$[^{15}O]O_2$	^{15}N
^{18}F	110	$^{18}O(p,n)^{18}F$	$[^{18}O]H_2O$	$[^{18}F]F^-$	^{18}O
		$^{20}Ne(d,\alpha)^{18}F$	$Ne(+F_2)$	$[^{18}F]F_2$	

p: proton; α: alpha particle ($^4_2He^{2+}$); *d*: deuterium (^2_1H); *n*: neutron

The positron subsequently annihilates with a nearby electron to produce two γ-quanta departing at approximately 180^o to each other (**Figure 1.2**). A cylindrical detector surrounding the imaging target collects the simultaneously emitted γ–quanta pair. Computer-aided image reconstruction of the data allows for the output of 3D-images of the regions of interest (**Figure 1.3**). The accuracy and resolution of PET images is 1.5-8 mm, which is

comparable with other clinical noninvasive imaging modalities (Computed Tomography, CT ~ 1 mm and Single-Photo Emission Computed Tomography, SPECT ~ 20 mm).[3]

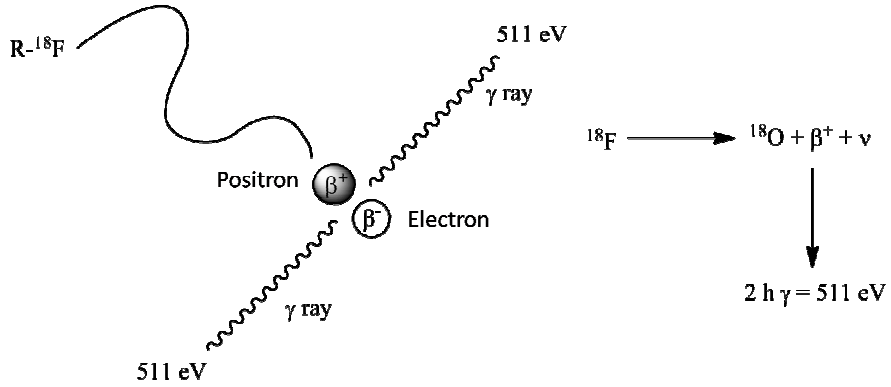


Figure 1.2. Decay of ^{18}F and subsequent annihilation.

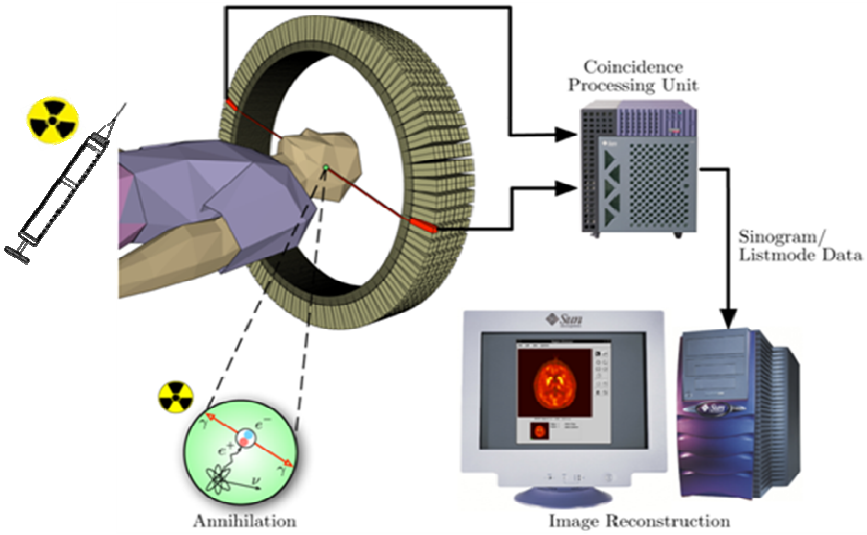


Figure 1.3. Principles of PET[2]

In addition, physiological and pharmacological parameters can be acquired by dynamic PET studies with long scan times, when combined with a suitable bio-mathematical model. As a result, PET studies provide accurate, quantitative information about transport processes, metabolic rates and receptor concentrations, depending on the specific radiotracer used.[1]

[1] The table is adapted from reference 3, with permission from John Wiley and Sons.
[2] The figure is provided by Jens Langner and reprinted with permission from its author.

1.3 Radionuclide selection

Although a large pool of radioisotopes produced by nuclear reactions in cyclotrons is currently available for PET labelling, only a few are qualified for clinical imaging *in vivo*. To select a suitable radioisotope for labelling, the intrinsic properties of the radionuclide should be taken into consideration. Positrons lose their kinetic energy in matter and travel a relatively short distance (~2 – 8 mm)[4] before annihilation with electrons (β^-). The low positron energy of ^{18}F gives the highest potential resolution in a PET camera since the positrons travel typically around 2 mm, which is similar to the highest spatial resolution achievable with modern PET cameras (**Table 1.2**).

Table 1.2 The decay process, positron energy and linear range in water of the most commonly used short-lived radionuclides in PET

Radionuclide	Decay Process	Maximum β^+ energy (MeV)	Linear range in water (mm)
^{11}C	99.8% β^+	0.96	4.12
^{13}N	100% β^+	1.19	5.39
^{15}O	99.9% β^+	1.72	8.20
^{18}F	97% β^+ + 3% EC	0.635	2.39

β+ : positron decay (see Figure 1.1); EC: Electron Capture decay ($^{18}F + e^- \longrightarrow {}^{18}O + \nu$)

The radiological half-life of the labelled radionuclide should be commensurate with the timescale of the biological process to be studied. In contrast to the radiological (physical) half-life, the biological half-life is a measure of how long it takes to eliminate half of the radioactivity taken into the body through various biological processes (e.g. excretion). For example, ^{15}O radionuclide ($t_{1/2}$ = 2 min) is suitable for the studies of blood flow, liver perfusion and myocardial perfusion (in the form of $[^{15}O]H_2O$). A ^{11}C- ($t_{1/2}$ = 20 min) or ^{18}F-labelled radiotracer ($t_{1/2}$ = 110 min) is more appropriate for the study of receptor-ligand interactions, peptide/protein synthesis and interactions. In these cases, the ^{15}O would have decayed by the time the radiotracer could reach the biological target. However, a

radionuclide with a shorter half-live, (e.g. ^{11}C) may be more suitable because the higher rate of decay reduces the overall radiation exposure of the subjects being studied.

1.4 Radiosynthesis under "*non carrier added*" conditions

The SI unit of radioactivity is the ***Becquerel*** (Bq), named after Henri Becquerel, who shared a Nobel Prize in chemistry with Pierre and Marie Curie for their pioneering work on radioactivity. One Bq is defined as the activity of a quantity of radioactive material in which one nucleus decays per second. It is therefore equivalent to s^{-1}.[5] The ***Curie*** (Ci) is a non-SI unit which is roughly the activity of 1 gram of the radium isotope ^{226}Ra. It is defined as being equal to the activity of any radionuclide decaying with a rate of 3.7×10^{10} decays per second or 3.7×10^{10} Bq (**Equation 1.1**).[5]

$$1\ Bq = 1\ s^{-1}$$

$$1\ Ci = 3.7 \times 10^{10}\ Bq = 37\ GBq$$

$$1\ Bq = 2.70 \times 10^{-11}\ Ci$$

Equation 1.1. The conversion between different units of radioactivity.

The quantity of the radioactive material is only determined by the number of decays; the activity of 30 GBq (average production at a small cyclotron) ^{18}F is equivalent to 4.7×10^{-10} mol of fluorine-18 (**Equation 1.2**). Due to the extremely insignificant quantities of radioactive species, a fundamental criterion of the quality of a radiotracer is its specific activity (SA), which reflects the amount of stable isotopes being present (i.e. *carrier*).

$$Q[mol] = \frac{Rad[Bq] \times t_{1/2}[sec]}{N_0 \times \ln(2)}$$

$N_0 = 6.022 \times 10^{23}\ mol^{-1}$ Avogadro Constant

Equation 1.2. Quantification of radioactive materials(Q = quantity)

The term *non carrier added* or *n.c.a.* production is used when no identical cold material is added during the production of the radionuclide.[5] For example, the production of ^{18}F by the $^{18}O(p, n)^{18}F$ reaction is a *n.c.a.* process which gives $[^{18}F]F^-$ in [^{18}O]water. By contrast, when ^{18}F is produced by the $^{20}Ne(d,\alpha)^{18}F$ reaction, F_2 has to be added to the Ne gas target, to coat the target chamber wall thereby preventing absorption of ^{18}F to the wall. The addition of "cold" $^{19}F_2$ carrier leads to contamination by "cold" material of the same chemical identity during preparation of the radiopharmaceutical.

By definition, the ***specific activity*** of a radiotracer is the amount of radioactivity per gram or mole[5] and requires careful consideration if the tracer is toxic or able to saturate the receptor sites (**Equation 1.3**).

$$\text{Specific Activity (SA) [GBq/}\mu\text{mol]} = \frac{\text{Amount of radioactivity [GBq]}}{\text{Mass [}\mu\text{mol]}}$$

Equation 1.3. Specific activity

The theoretical maximum specific activity is determined by the half-life of the radionuclide (**Equation 1.4**).

$$\text{Specific Activity (SA)}_{max}\text{[Bq/mol]} = N_0\lambda$$

$\lambda = \ln(2) / t_{1/2}$[sec] Decay Constant

$N_0 = 6.022 \times 10^{23}$ mol^{-1} Avogadro Constant

Equation 1.4. The equation to calculate the theoretical maximum specific activity.

In practice, the theoretical maximum specific activity is never reached. Contamination of radioactive isotope originates from radionuclide production, radioactivity transportation, chemical materials and impurities. For example, typical cyclotron production gives *carrier-added* $[^{18}F]F_2$ with specific activity ~ 55 GBq/μmol, this value is significantly lower than the specific activities typically reached using [^{18}F]fluoride (up to 4000 GBq/μmol, **Table 1.3**).

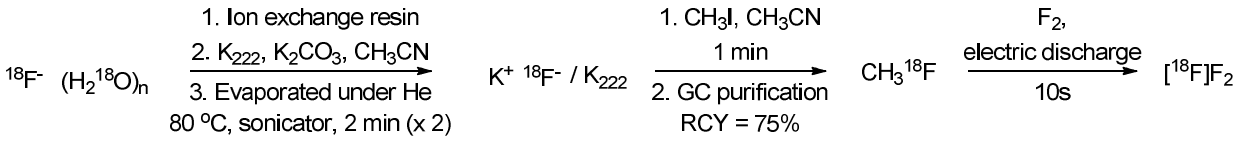
Contamination of $^{11}CO_2$ can occur easily with CO_2 from the atmosphere and lead to a dramatic decrease in the specific activity of ^{11}C-labelled compounds.

Table 1.3 The half-lives, theoretical maximum specific activities and specific activities achieved for the most commonly used short-lived radionuclides in PET

Radionuclide	Half-life $t_{1/2}$ (min)	Theoretical SA limit GBq/μmol	Maximum SA reported
^{11}C	20.4	3.4×10^5	500-2000 GBq/μmol
^{13}N	9.97	7.0×10^5	> 400 GBq/μmol
^{15}O	2.04	3.36×10^5	4 MBq/μmol
^{18}F	110	6.34×10^4	55 GBq/μmol for $[^{18}F]F_2$ 4000 GBq/μmol for $[^{18}F]F^-$

For commonly used radiotracers, the typical reported values of SA are in the order of 50-500 GBq/μmol (*ca.* 1-15 Ci/μmol), and are highly dependent on the choice of chemicals and handling process. The preparation of radiotracers with high specific activity implies that only a trace amount of the compound needs to be administered (typically in the low or sub-microgram level), and thus avoid physiological effects originating from the tracer and the need for toxicology testing.[6]

Bergman and Solin reported the "post-target" protocol for the production of high specific activity $[^{18}F]F_2$ (up to 55 GBq/μmol) from nucleophilic $[^{18}F]$fluoride (**Scheme 1.2**).[7] High specific activity $[^{18}F]$fluoromethane was produced by nucleophilic substitution of methyl iodide with $[^{18}F]$fluoride. High specific activity $[^{18}F]F_2$ was then prepared in an electrical discharge chamber by an $^{18}F/^{19}F$ exchange reaction involving $[^{18}F]$fluoromethane and a low amount of the F_2 carrier. The specific activity of the labelled product could be increased from less than 0.4 GBq/μmol to the range of 10–20 GBq/μmol with high specific activity $[^{18}F]F_2$ prepared from nucleophilic $[^{18}F]$fluoride.



Scheme 1.2. Preparation of "high specific" $[^{18}F]F_2$ from nucleophilic $[^{18}F]$fluoride

Typically, radiotracers whose production starts with $[^{18}F]$fluoride require a much lower dose to achieve the same level of radioactivity (**Table 1.4**).

Table 1.4 Labelling methods and the variation in mass required to achieve an injection of 185 MBq of radioactivity of a radiotracer (mw = 300 g/mol).

Labelling Method (^{18}F)	Specific Activity (GBq/μmol)	Mass Injected (μg)
Nucleophilic Labelling	37-1000	0.06-1.5
High SA Electrophilic Labelling	3.7-25	2.2-15
Electrophilic Labelling	0.037-0.75	74-1500

Due to the small amount of radionuclide present, the radioisotope ^{18}F is always sub-stoichiometric by a substantial margin with respect to the precursor, typically in the ratio of 10^3. Hence, the course of the reaction may differ strongly from that of a classical reaction using approximately equimolar stoichiometric ratios of reagents and reactants. For a typical nucleophilic ^{18}F-fluorination, where R is the precursor and R'^{18}F the labelled product, ^{18}F is consumed by pseudo first-order kinetics, since the concentration of the precursor is in very high excess to the *n.c.a.* ^{18}F and can be approximately assumed to be constant. If the starting concentration of ^{18}F is $[^{18}F]_0$, the concentration of R'^{18}F after the reaction time *t* can be presented as shown in **Scheme 1.3**, where k' is the rate coefficient of pseudo-first-order type and contains [R] as constant factor.[8]

$$R + {}^{18}F \longrightarrow R'{}^{18}F$$

$$[R'{}^{18}F]_t = [{}^{18}F]_0 \times (1 - e^{-k't}) \quad [mol/L]$$

k' = pseudo rate constant

Scheme 1.3. Pseudo first-order kinetics of an ^{18}F-fluorination under *n.c.a.* conditions

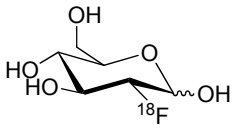
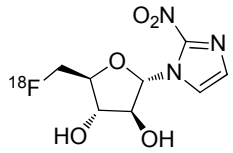
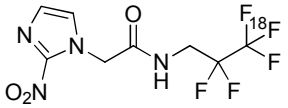
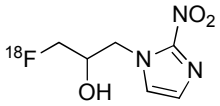
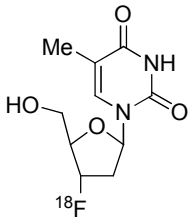
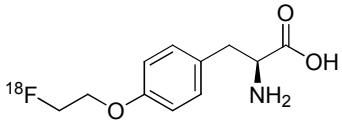
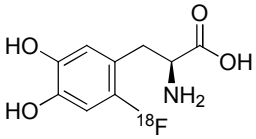
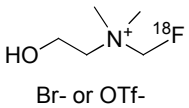
For a chemical reaction involving radioactive species, yields are typically reported either as being corrected for isotopic decay or as uncorrected yields. The *decay-corrected* radiochemical yield (RCY) is commonly used to evaluate the efficiency of a radiochemical reaction.[9] In this thesis, all quoted RCYs are *decay-corrected* radiochemical yields, unless otherwise stated.

1.5 ^{18}F-Radiotracers and Applications

As mentioned previously, ^{11}C, ^{13}N, ^{15}O and ^{18}F are the short-lived positron-emitting radionuclides that have made great impact on PET. This is understandable because the first three are isotopes of the most important elements of life. For example, replacement of a native ^{12}C atom with a radioactive ^{11}C atom allows for visualisations of the compound without any change of its chemical-properties and *in vivo* behavior. In contrast, there are only a very limited number of naturally occurring molecules that contain a fluorine atom.[10] The main feature that makes ^{18}F special is its relatively long half-life of ~110 min. From a chemical point of view, it allows for multi-step synthetic approaches that can be extended over hours and therefore facilitate the radiolabelling of complicated high-molecular-weight macromolecules such as peptides, proteins[11, 12] or oligonucleotides.[13, 14] From a biological point of view, it not only expands the time observation window for kinetic studies, but also improves the quality of metabolite and plasma analysis due to the higher count rates and signal-to-noise ratio over a longer period of time. As a consequence of the ultra short half-

lives of 2 – 20 minutes, ^{11}C, ^{13}N and ^{15}O must be produced immediately before use, normally onsite by a dedicated biomedical cyclotron, whereas ^{18}F permits offsite use. Thus more and more efforts have been devoted to developing new ^{18}F-labelled radiopharmaceuticals with the hope to find new ^{18}F-labelled products that can be distributed to patients.

There is a growing interest in developing ^{18}F-labelled radiopharmaceuticals to monitor molecular targets for ***proliferation***, ***angiogenesis***, ***apoptosis***, ***receptor expression***, ***metastasis***, ***gene transfection*** and ***hypoxia***. **Scheme 1.4** highlights the most important ^{18}F-radiotracers which are used for clinical PET imaging or preclinical microPET studies: 2-deoxy-2-[^{18}F]fluoro-$_D$-glucose ([^{18}F]FDG, **[^{18}F]1-1**),[15],[16] [^{18}F]fluoroazomycin arabinoside ([^{18}F]FAZA, **[^{18}F]1-2**),[17, 18] 2-(2-nitro-1*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-[^{18}F]pentafluoro propyl)-acetamide ([^{18}F]EF5, **[^{18}F]1-3**),[19, 20] [^{18}F]fluoromisonidazole ([^{18}F]FMISO, **[^{18}F]1-4**),[21],[22] 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT, **[^{18}F]1-5**),[23, 24] *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (L-[^{18}F]FET, L-**[^{18}F]1-6**),[25] 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine (L-[^{18}F]FDOPA, L-**[^{18}F]1-7**),[26] and [^{18}F]fluoromethylcholine (**[^{18}F]1-8**).[27]

Area	Measurement	^{18}F-Radiopharmaceuticals	
Cardiology Oncology Neurology	Glucose Metabolism	[^{18}F]FDG **[^{18}F]1-1**	
Cardiology Oncology	Hypoxia	[^{18}F]FAZA **[^{18}F]1-2**	
Cardiology Oncology	Hypoxia	[^{18}F]EF5 **[^{18}F]1-3**	
Cardiology Oncology	Hypoxia	[^{18}F]FMISO **[^{18}F]1-4**	
Oncology	Cellular Profileration	[^{18}F]FLT **[^{18}F]1-5**	
Oncology	Protein synthesis rate	L-[^{18}F]FET **L-[^{18}F]1-6**	
Neurology	Receptor Measurement	L-[^{18}F]FDOPA **L-[^{18}F]1-7**	
Oncology	Soft tissue metastases	[^{18}F]Fluorocholine **[^{18}F]1-8**	 Br- or OTf-

Scheme 1.4. Key ^{18}F-radiopharmaceuticals.[3]

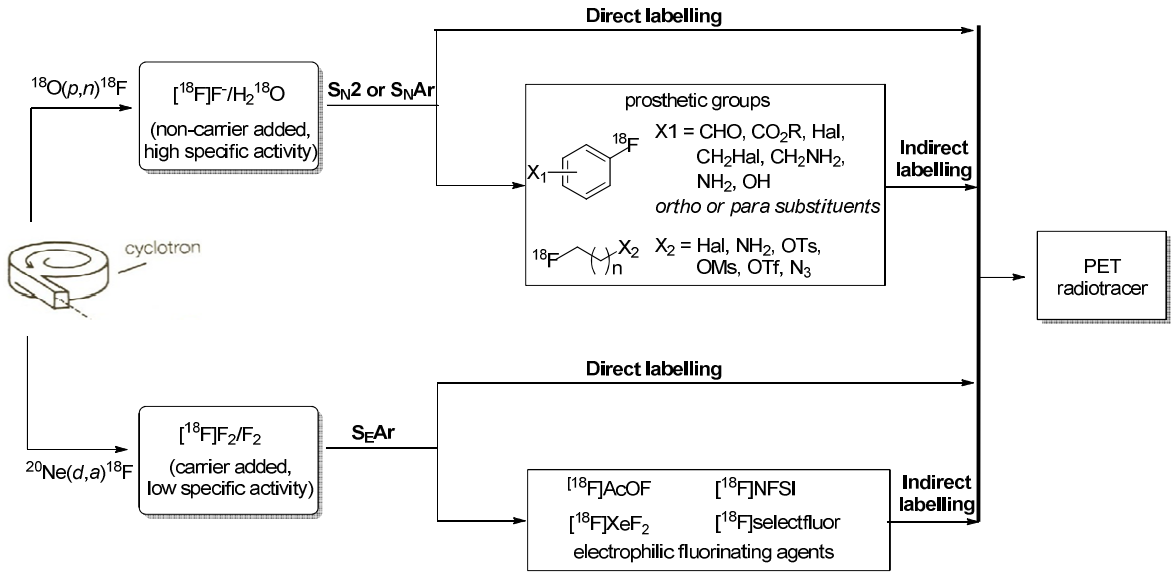
[3] The scheme is adapted from reference 1.

1.6 Overview of ^{18}F-Radiochemistry[28]

Easy access to structurally diverse radiolabelled probes can accelerate the rapid progress of PET imaging, but to date, radiochemistry is still limited by cost and efficiency. An ideal radiosynthetic protocol, as expected in a conventional organic synthetic method, should be as efficient as possible. Due to the short half-life of radioisotopes, the retro-radiosynthetic route should be designed to minimise functional group manipulations after the introduction of the radioisotope and to avoid the formation of side-products that cause complications during purification.

Chemical reactions employed for ^{18}F radiolabelling start from nucleophilic and electrophilic ^{18}F sources produced in a cyclotron *via* nuclear reactions (**Scheme 1.5**). Nucleophilic fluorination with ^{18}F-fluoride is the preferred "*no-carrier-added*" methodology because it is much easier to access and handle than [^{18}F]F_2, and leads to radiotracers with a higher specific activity, a key feature allowing for investigations to be performed at sub-toxic doses.

However, this strategy suffers from limitations. Hydrated [^{18}F]fluoride ions are not good nucleophiles and [^{18}F]fluoride based radiochemistry is relatively limited in scope. Moreover, the harsh conditions required for successful nucleophilic ^{18}F-fluorination are often not compatible with biological molecules.



Scheme 1.5. Overview of ^{18}F-radiochemistry.

Indirect ^{18}F-labelling is a method based on the bimolecular coupling of a small ^{18}F-labelled reactive precursor with the probe of interest. This strategy is very often used for the labelling of peptides or to access structural motifs not accessible by direct nucleophilic ^{18}F-fluorination.

In direct electrophilic fluorination, the drawback is the recognition that the *carrier-added* method employed for the production of [^{18}F]F$_2$ gas affords labelled products with low specific activity. In addition, F$_2$ gas often reacts unselectively and leads to a mixture of products. Side reactions not only lower RCY, but may also lead to problematic and time-consuming purification. Therefore, efforts have been devoted to ^{18}F-reagents for indirect electrophilic fluorination with tailored reactivity, easy handling and transportation.

Nucleophilic and electrophilic fluorinations are complementary processes and the method of choice depends on the reactivity profile of the precursor to be fluorinated. Independent of the chosen strategy (nucleophilic or electrophilic, direct or indirect ^{18}F-labelling), the challenge is for the total radiosynthesis time (synthesis, purification, analysis and formulation) not to exceed roughly three half-lives of the radioisotope (i.e. ~ 6 hours for ^{18}F).[29]

1.7 Fluoroaromatics in medicinal chemistry

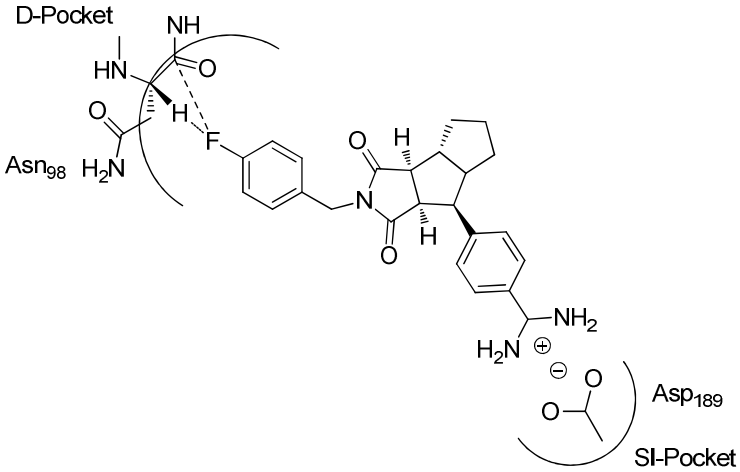
Fluorinated compounds play an important role in providing lead compounds for therapeutic applications.[10] Fluorinated aromatic compounds are of interest because they are generally more resistant to metabolism compared with fluorinated aliphatic compounds.[30] The presence of the fluorine group in aromatic systems serves many purposes, including modulation of lipophilicity, improvement of metabolic stability and more efficient protein–ligand interactions.[31]

Monofluorination and trifluoromethylation of saturated alkyl groups causes a decrease in lipophilicity, mainly due to the relatively polar character of the monofluoro- and trifluoromethylalkanes with their strong C–F and C–CF_3 bond dipoles.[32] By contrast, the replacement of a single aromatic hydrogen atom with a fluorine atom usually results in a modest increase in lipophilicity. This can be attributed to the excellent overlap between the fluorine 2s or 2p orbitals with the corresponding orbitals on carbon that makes the C–F bond highly non-polarisable.[10]

Cytochrome P450 monooxygenases are the most important group of enzymes that metabolise drugs. The electron withdrawing nature of the fluorine substituent deactivates the aromatic ring towards oxidative metabolic pathways by reducing the electron density of the ring. The fluorine substituent also blocks the site of oxidation.[33] Therefore, the presence of fluorine in a benzene ring can block oxidation at a specific position and thus improve metabolic stability.

It has been recognised that fluorine participates in electrostatic interactions and may contribute to the enhanced binding affinity of organofluorine compounds for the enzyme's active site. In a study of a series of thrombin inhibitors, a 4-fluorophenyl derivative showed a five to ten-fold higher affinity than ligands with other fluorination patterns. X-Ray crystal

structure analysis revealed the presence of dipolar C–F…H–Cα and C–F…C=O interactions between the para-fluorinated analogue and the backbone H–Cα–C=O unit of Asn98 in thrombin (**Scheme 1.6**). These interactions are believed to be the determining factor for the observed significant increase in potency. [34]



Aromatic Substitution	K_i(μM)
H	0.31
2-F	0.50
3-F	0.36
4-F	**0.057**
2,3-F_2	0.49
2,6-F_2	0.61
3,4-F_2	0.26
3,5-F_2	0.59
1,2,3,4,5-F_5	0.27
4-Cl	0.19

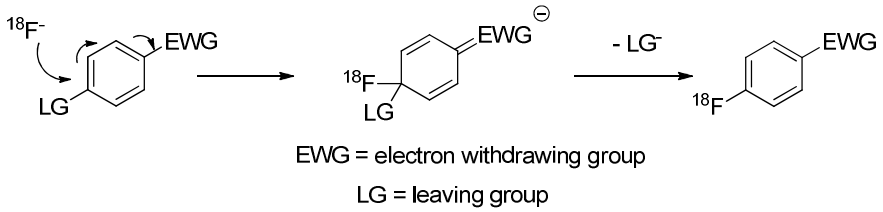
Scheme 1.6. Fluorine in electrostatic interactions in a thrombin inhibitor.

1.8 Radiochemistry for ^{18}F-labelled aromatics

1.8.1. Direct nucleophilic substitution for ^{18}F-labelled aromatics

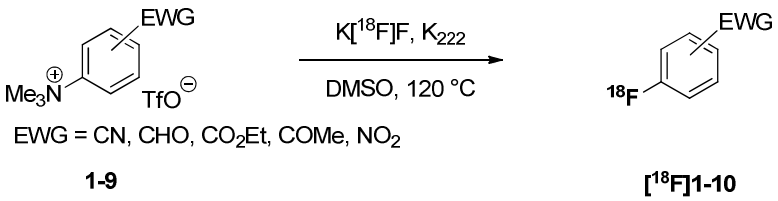
1.8.1.1 ^{18}F-Fluorination *via* S_NAr mechanism

Aromatic nucleophilic substitution with [^{18}F]fluoride in aromatic substrates are typical S_NAr reactions and proceed *via* an addition-elimination mechanism (**Scheme 1.7**).



Scheme 1.7. The S_NAr mechanism of nucleophilic aromatic fluorination

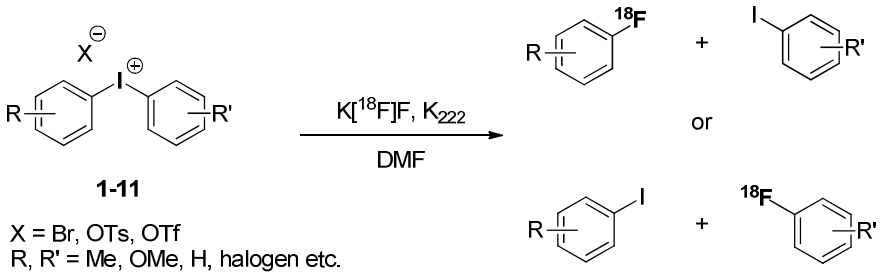
Direct nucleophilic fluorination of aromatic substrates has been well-explored during the past decade. However, the reaction is limited to aromatic substrates with an electron withdrawing group (nitro, cyano or acyl) positioned *ortho* or *para* (**1-9**) to a good leaving group. Nitro, trialkylammonium, halogen, mesylate, tosylate and triflate are the groups most commonly displaced (**Scheme 1.8**).



Scheme 1.8. Nucleophilic fluorination for [^{18}F] fluoroaromatic structures

1.8.1.2 ^{18}F-Fluorination of iodonium salts

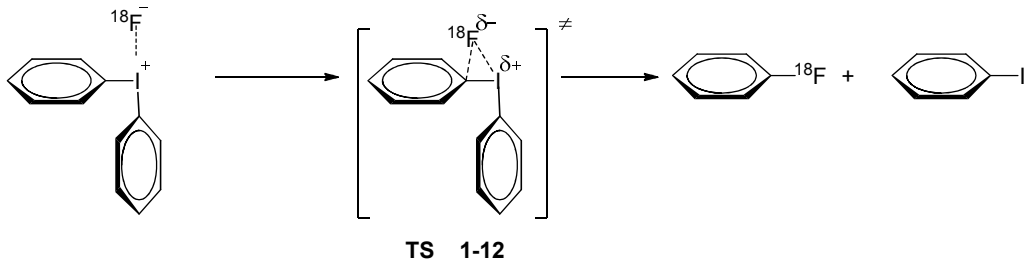
In 1995, Pike and Aigbirhio reported the *n.c.a.* nucleophilic ^{18}F-fluorination of iodonium salts **1-11** as a general route to label a series of "unactivated" electron-neutral or electron-rich aromatic compounds (aromatics without electron withdrawing groups, **Scheme 1.9**).[35]



Scheme 1.9. Nucleophilic fluorination *via* iodonium salt

X-ray crystallographic analysis[36] on iodonium salts, RR'I$^+$ X$^-$, showed that the average bond distances between the iodine atom and the anion were within a range of 2.3–2.7 Å and the bond angles R–I–R' in the iodonium salts were close to 90°. The results indicate the secondary bonding character of the I$^+$–X$^-$ bond and the pseudo-trigonal bipyramidal geometry of the structure. The mechanism for ^{18}F-fluorination of a symmetrical diaryliodonium salt was proposed by Pike.[37] The reaction is believed to proceed *via* the

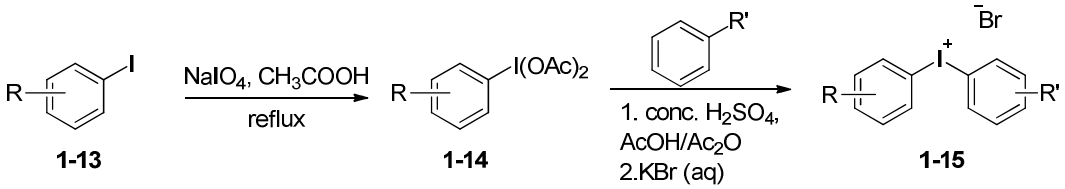
transition state **TS 1-12** (**Scheme 1.10**) and is driven by the favourable two-electron reduction of the iodine center with the entropically favourable elimination of iodobenzene.



Scheme 1.10. Mechanism of ^{18}F-fluorination of a symmetrical diaryliodonium salt[4]

The reaction affords *n.c.a.* ^{18}F-fluoroarenes and non-radioactive iodoarenes as by-products. When asymmetrical iodonium salts are used as precursors, the distribution of ^{18}F-fluorinated products was found to be highly dependent on the electronic character of the aryl groups of the salt. The most electron deficient ring is preferably attacked by [^{18}F]fluoride.

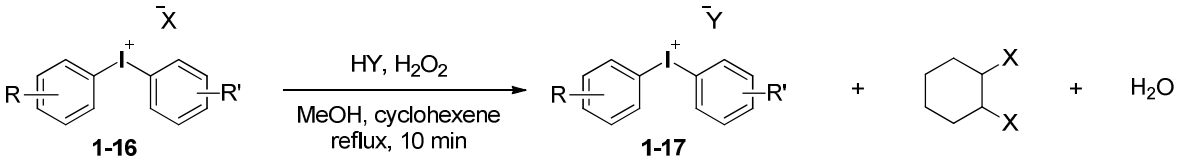
Diaryliodonium salts **1-15** can be synthesised by a variety of methods.[38, 39] The most commonly used procedure involves a typical electrophilic aromatic substitution. The iodoarene **1-13** is converted by the oxidant (e.g. sodium periodate) in acetic acid into its diacetoxyiodo analogue **1-14**. Subsequently, the (diacetoxyiodo)arene **1-14** and the appropriate aromatic compound are treated with concentrated sulphuric acid in a mixture of acetic acid and acetic anhydride. Finally, by the addition of aqueous chloride, bromide or iodide solution, the desired diaryliodonium halide **1-15** precipitates out (**Scheme 1.11**).



Scheme 1.11. General synthesis of a diaryliodonium bromide *via* oxidation to the (diacetoxyiodo)arene and S_EAr reaction catalysed by sulphuric acid

[4] The Scheme is reprinted from reference 38, with permission from American Chemistry Society.

The resulting diaryliodonium halides can be transferred in high yields into various iodonium salts with different counter ions, by an oxidative anion metathesis reaction.[40] in high yields.[38, 39] Typically, the diaryliodonium halide **1-16** is heated to reflux in the presence of hydrogen peroxide, an acid and cyclohexene for 10 min. This reaction delivers the corresponding iodonium salts **1-17** with the deprotonated acid as the counter ion. Cyclohexene traps any liberated bromine or chlorine evolving in the oxidative reaction (**Scheme 1.12**).

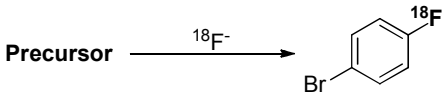


1-16
X = Br, Cl
Y = HSO_4, OTf, OTs, BF_4

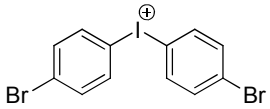
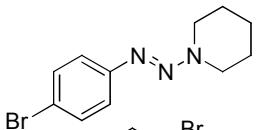
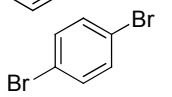
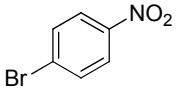
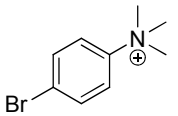
1-17

Scheme 1.12. Oxidative anion metathesis of diaryliodonium bromides and chlorides

To calibrate the values of various precursors leading to 1-bromo-4-[^{18}F]fluorobenzene **[^{18}F]1-19**, a series of representative precursors **1-18** to ^{18}F-aromatics were subjected to ^{18}F-fluorination. The result indicated that (4-bromophenyl)iodonium salts **1-18a** gave the highest RCYs (**Scheme 1.13**).[41]

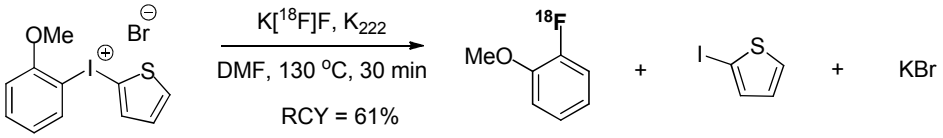


1-18 [18F]1-19

Precursor		RCY
	**1-18a**	50-60%
	**1-18b**	3-4%
	**1-18c**	1-2%
	**1-18d**	<0.5%
	**1-18e**	8-12%

Scheme 1.13. Synthesis of 1-bromo-4-[^{18}F]fluorobenzene **[^{18}F]1-19**.

The 2-thienyl group was introduced with the aim to direct the ^{18}F nucleophilic substitution. The radiosynthesis of *ortho*-[^{18}F] fluoroanisole **[^{18}F]1-21** from 2-methoxy-phenyl(2-thienyl)iodonium salt **1-20** was selected as a model reaction, to investigate the influence of the substitution pattern, counter anion and solvent on the reactivity and selectivity of the [^{18}F]fluorination (**Scheme 1.14**). DMF gave higher RCYs compared to DMSO, acetonitrile and dimethylacetamide (DMA). The optimal temperature was found to be 130 ^{o}C with the bromide counter-anion, giving the highest RCY.[42]

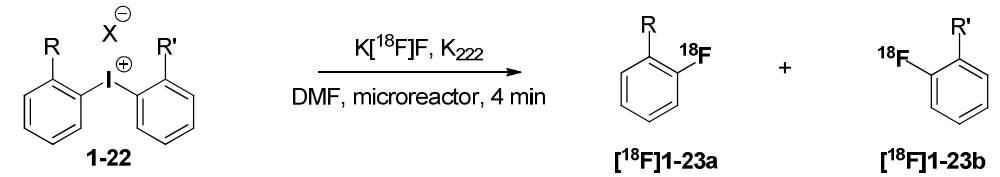


1-20 [^{18}F]1-21

Scheme 1.14. Nucleophilic fluorination for *ortho*-[^{18}F]fluoroanisole

More recently, Pike reported[37] the application of microreactors to the reactions of [^{18}F]fluoride with diaryliodonium salt **1-22** to shorten the reaction time to less than 8

minutes with moderate (R = R' = 2-MeO, 51%) to high (R = R' = Ph or Me, 85%) decay-corrected radiochemical yields (**Scheme 1.15**). In the case of unsymmetrical iodonium salts, the distribution of ^{18}F in the two possible [^{18}F]fluoroarenes **[^{18}F]1-23a/b** was controlled by the nature of the *ortho* substituents. The impact of *ortho*-substitution on regioselectivity of ^{18}F-fluorination was defined as the "*ortho*-effect" by Pike. The *ortho*-effect was observed in the following order: 2,6-di-Me>2,4,6-tri-Me>Br>Me>Et ≈ iPr >>H>OMe.



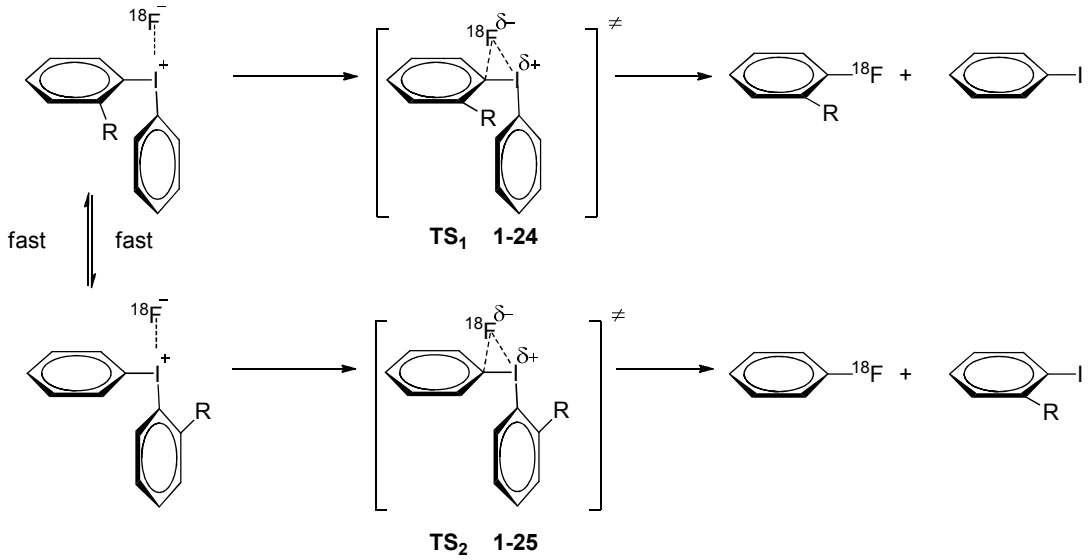
X = Br, OTs, OTf
R, R' = Me, OMe, H, halogen etc.

RCY = 51% - 85%
distribution of **[^{18}F]1-23a** : **[^{18}F]1-23b** = 1.2 – 18.8 : 1
Ortho-effect directing power:
2,6-di-Me>2,4,6-tri-Me>Br>Me>Et ~^{i}Pr >>H>OMe

Scheme 1.15. Microreactor Synthesis of *ortho*-Substituted [^{18}F]Fluoroarenes

The *ortho*-effect can be ascribed to steric and electronic influence. Iodonium cations with trigonal bipyramidal geometry are known to be fluxional and able to interconvert through conformations in which the ligands rapidly interchange equatorial and axial positions (**Scheme 1.16**). The substituted aromatic ring is preferentially oriented in an equatorial position with the two electron pairs of the iodine in order to attain maximal distance and therefore is easily accessible for attack by an apically approached ^{18}F-fluoride. Pike proposed that the fluorination proceeded in accordance with the Curtin–Hammett principle, i.e. the ratio of products formed from two critical conformations of substrates only depends on the difference in the free energy of the transition states **TS1 1-24** and **TS2 1-25** since the rates of reaction are much slower than the rates of conformational interconversion (**Scheme 1.16**). The *ortho*-effect is not only determined by the steric bulk of the substituent.

Deactivating (as for OMe) and activating (as for Br) electronic influences also need to be considered in predicting product selectivity.

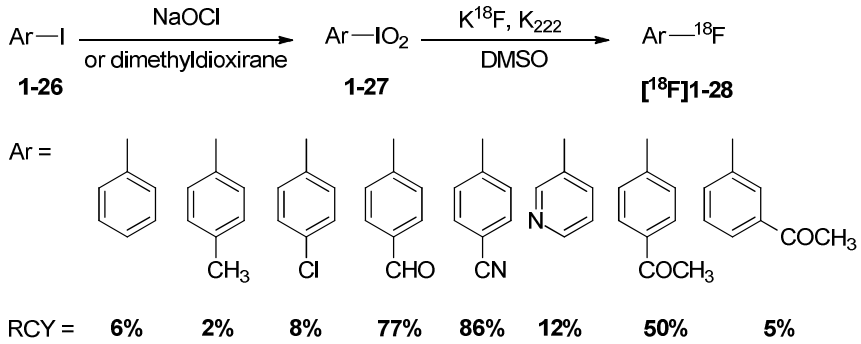


Scheme 1.16. Suggested mechanism for the ^{18}F-fluorination of an asymmetrical diaryliodonium salt, TS = Transition States[5]

1.8.1.3 ^{18}F-Fluorination of iodylbenzene derivatives

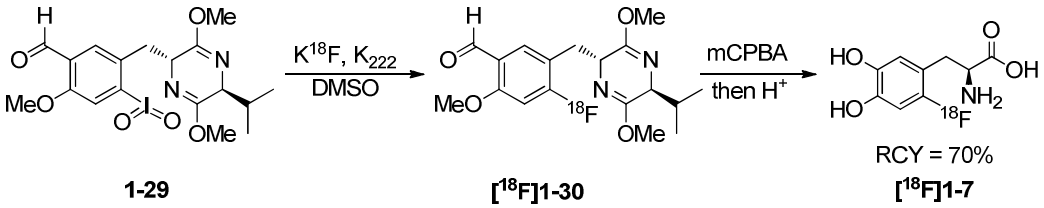
Satyamurthy and Barrio[43] reported the application of iodylbenzene derivatives **1-27**, which can be substituted with electron-donating as well as electron-withdrawing groups on the aromatic ring, as precursors in nucleophilic substitution reactions (**Scheme 1.17**). High RCY can be obtained when the precursor is substituted with *para*-positioned electron-withdrawing groups (e.g. CHO, CN).

[5] The Scheme is reprinted from reference 38, with permission from American Chemistry Society.



Scheme 1.17. Aromatic ^{18}F-fluorination of iodylbenzene derivatives

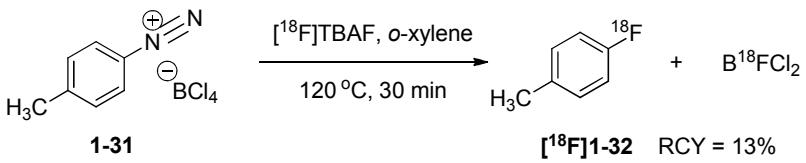
An elegant application of the radiochemistry was the preparation L-[^{18}F]FDOPA L-**[^{18}F]1-7**. Nucleophilic fluorination of iodylbenzene derivative **1-29**, activated by a *para*-positioned aldehyde, followed by functional group manipulations of **[^{18}F]1-30** gave L-[^{18}F]FDOPA L-**[^{18}F]1-7** in an overall 70% RCY (**Scheme 1.18**).[43]



Scheme 1.18. Radiosynthesis of [^{18}F]FDOPA **[^{18}F]1-7** by nucleophilic fluorination

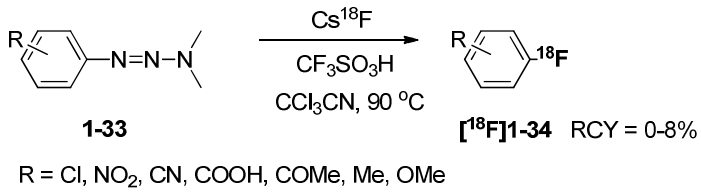
1.8.1.4 Balz-Schiemann and Wallach methods

The Balz-Schiemann reaction has been adapted to ^{18}F radiochemistry to give ^{18}F-labelled aromatics (**[^{18}F]1-32**, **Scheme 1.19**). The decomposition of an aryl diazonium salt **1-31** in the presence of [^{18}F]fluoride requires harsh conditions, under which the ion exchange between [^{18}F]fluoride and chloride is unavoidable. [44] Generally, the incorporation of ^{18}F is low.



Scheme 1.19. Nucleophilic ^{18}F-fluorination *via* Balz-Schiemann reaction

The Wallach reaction of [^{18}F]fluoride with a diazonium salt, formed by *in situ* acid-mediated decomposition of the triazene precursor **1-33**, has been used to label electron rich aromatics. (**Scheme 1.20**)[45, 46]



Scheme 1.20. Nucleophilic fluorination *via* Wallach Reaction

Both Balz-Schiemann and Wallach reactions are rarely used today in ^{18}F radiolabelling, mainly due to their low RCY. Notably, both reactions are dediazoniation reactions, where the fluoride replaces a diazonium functionality. The reactions are of the S_N1-type. Therefore, before being trapped by ^{18}F-fluoride, the reactive cations can react with any nucleophilic species nearby. Under *n.c.a.* conditions, the statistical probability of the reactive intermediates to encounter a [^{18}F]fluoride ion is marginal and only carrier addition leads to satisfying results.[47]

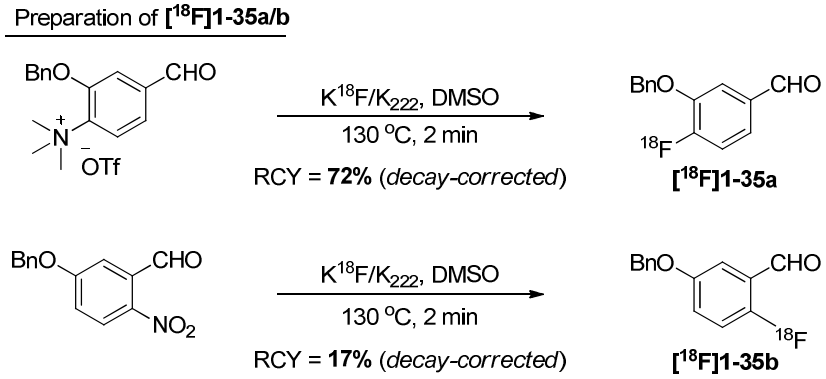
1.8.2. Prosthetic group methods for ^{18}F-labelled aromatics

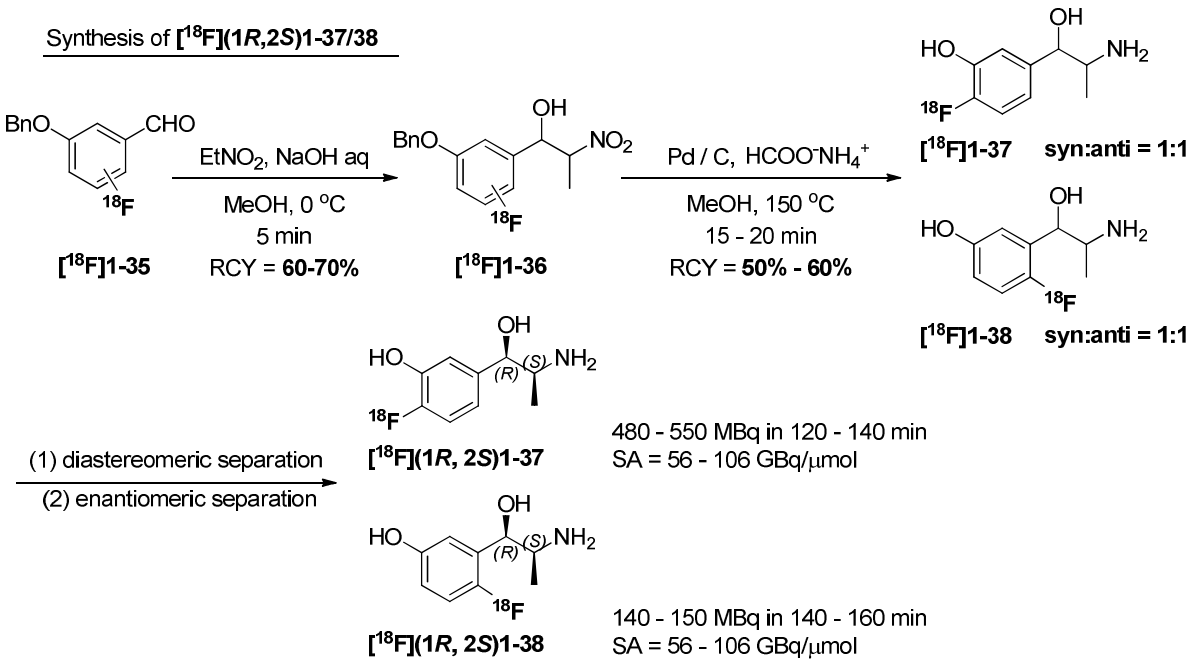
Among the known ^{18}F radiotracers for PET, most are labelled directly *via* nucleophilic fluorination of the corresponding precursors. However, the range of radiochemical methodologies for direct introduction of ^{18}F into the target molecule of interest is rather limited. Therefore, small ^{18}F-labelled alkyl or aryl groups with reactive functional groups have been synthesised as prosthetic groups. More importantly, they can be used to react with more complex biological molecules that are not suitable or stable enough for direct fluorination methods.

1.8.2.1. ^{18}F-Fluorobenzaldehyde as a prosthetic group

Due to the versatility of the aldehyde functional group in synthetic chemistry, [^{18}F]fluorobenzaldehyde ([^{18}F]FBA) has been extensively used as a prosthetic group to afford a broad range of ^{18}F-labelled aromatic compounds and ^{18}F-labelled peptides.[48, 49] Typically, 4-[^{18}F]fluorobenzaldehyde can be produced efficiently (RCY usually higher than 50%)[50] in one step by ^{18}F nucleophilic aromatic substitution of the corresponding benzaldehyde bearing a leaving group, such as a trimethylammonium functionality (**Scheme 1.21**).

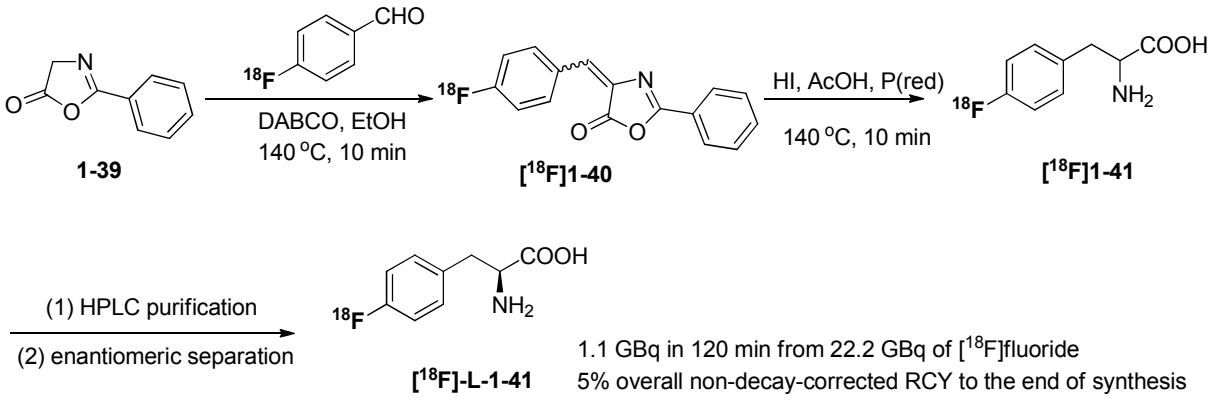
The synthesis of (1*R*, *2S*)-4- and 6-[^{18}F]fluorometaraminol (**[^{18}F](1*R*, 2*S*)1-37/38**) involved the nucleophilic addition of nitroethane to a *O*-benzyl protected ^{18}F-labelled benzaldehyde **[^{18}F]1-35**, followed by deprotection of the benzyl group and simultaneous reduction of the nitro group with Pd/C-ammonium formate. Typically, optically pure **[^{18}F](1*R*, 2*S*)1-37** could be obtained in 480 – 550 MBq (specific activity 56 – 106 GBq/μmol) within a total synthetic time 120 – 140 min (including diastereomeric separation and enantiomeric separation *via* HPLC). **[^{18}F](1*R*, 2*S*)1-38** could be obtained within 140 – 160 min *via* an analogous method. [51]





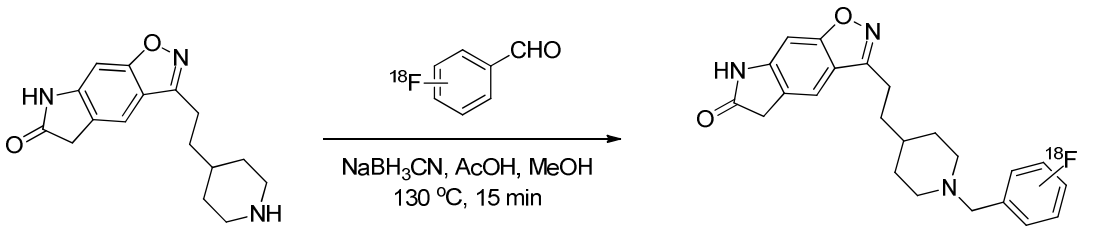
Scheme 1.21. Radiosynthesis of optically pure **[^{18}F](1R, 2S)1-37/38**.

A variety of base-mediated condensations with C–H acidic compounds and 4-[^{18}F]FBA as the carbonyl component are widely used in ^{18}F radiochemistry. An early example is the radiosynthesis of 4-[^{18}F]fluorophenylalanine (L-**[^{18}F]-1-41, Scheme 1.22**), which employed the Knoevenagel condensation of [^{18}F]FBA with 2-phenyl-5-oxazolone **1-39** as the key step. The enantiomerically pure product L-**[^{18}F]-1-41** was obtained with 5% RCY (*non-decay-corrected* to the end of synthesis) after acidic hydrolysis, HPLC purification and enantiomeric separation on a chiral stationary phase.[52]



Scheme 1.22. Radiosynthesis of L-4-[^{18}F]fluorophenylalanine **[^{18}F]-L-1-41**

Reductive amination of ketones and aldehydes is commonly used in synthetic chemistry for the formation of amines.[53], [54] The radiolabelling procedure is performed as a two-step synthetic sequence in one pot. DMSO or DMF are the preferred solvents and $NaBH_3CN$ is the preferred reducing agent with acetic acid to maintain the appropriate pH value for the reduction. 2-[^{18}F]Fluoro-CP-118,954 **[^{18}F]1-43** and 4-[^{18}F]fluoro-CP-118,954 **[^{18}F]1-44** (**Scheme 1.23**) have been synthesised *via* the reductive amination of 2 or 4-[^{18}F]FBA respectively.[55],[56]



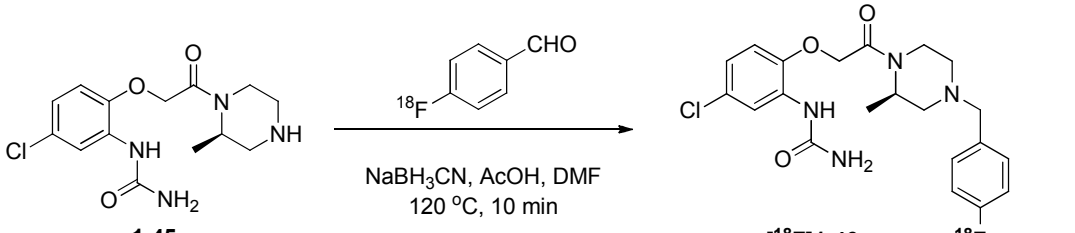
1-42

2-^{18}F **[^{18}F]1-43** RCY = 20 - 35% SA 41-53 GBq/μmol

4-^{18}F **[^{18}F]1-44** RCY = 29 - 35% SA 36-42 GBq/μmol

Scheme 1.23. Reductive amination with [^{18}F]FBA.

Similarly, 4-[^{18}F]FBA can be efficiently transformed into [^{18}F]1-(5-chloro-2-{2-[(2R)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**[^{18}F]1-46**, RCY = 5%-13%, non-decay-corrected based on [^{18}F]fluoride) within 90-95 min (**Scheme 1.24**).[57] **[^{18}F]1-46** is recognised as a promising radiotracer for the early diagnosis of Alzheimer's disease by PET.[58]



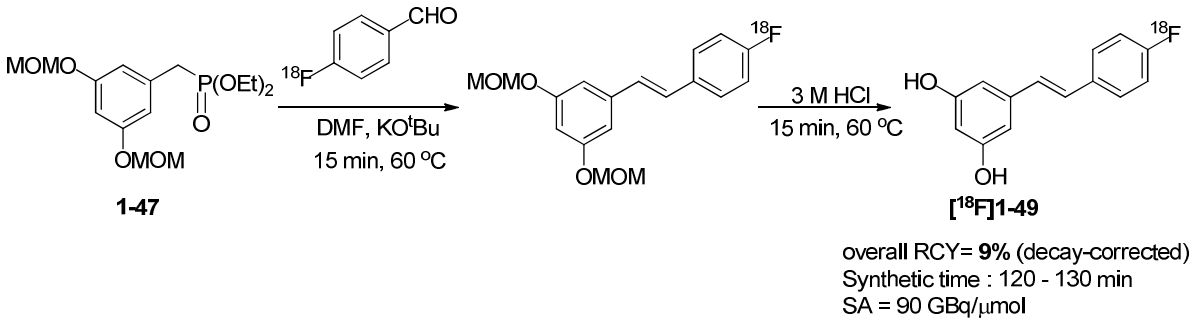
1-45

[^{18}F]1-46

RCY = **5-13%** (*non-decay corrected*)
synthetic time: 90 - 95 min
SA = 59 - 184 GBq/μmol

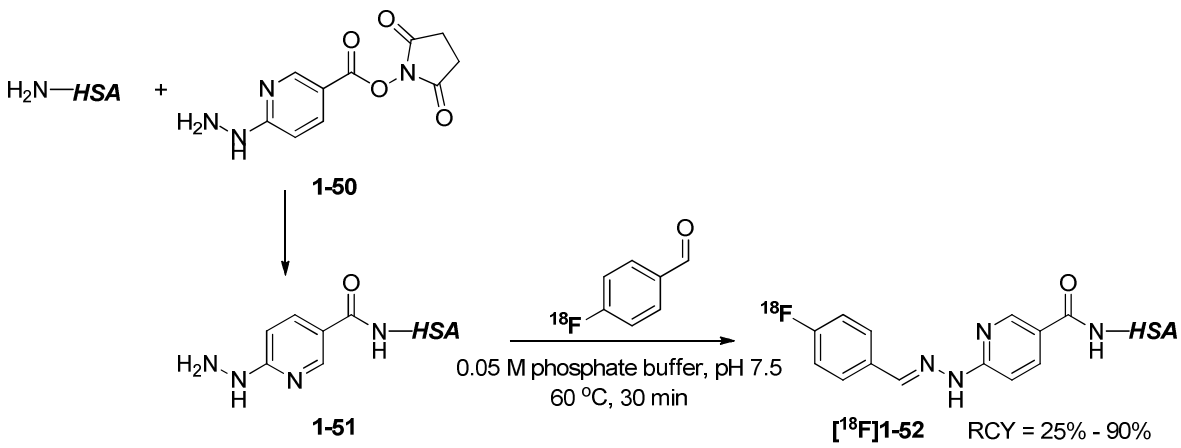
Scheme 1.24. Radiosynthesis of **[^{18}F]1-46** *via* reductive amination

4-[^{18}F]FBA can be treated with a stabilised phosphorus ylide **1-47** to afford the ^{18}F-labelled *E*-alkene with high selectivity. The desired *E*-isomer of ***E*-[^{18}F]1-49** was synthesised exclusively in three steps within 120 – 130 min, using the Horner-Wadsworth-Emmons reaction as a key transformation. The overall RCY was 9% (*decay-corrected*) with respect to [^{18}F]fluoride and the specific activity was determined to be in the range of 90 GBq/μmol (**Scheme 1.25**).[59],[60]



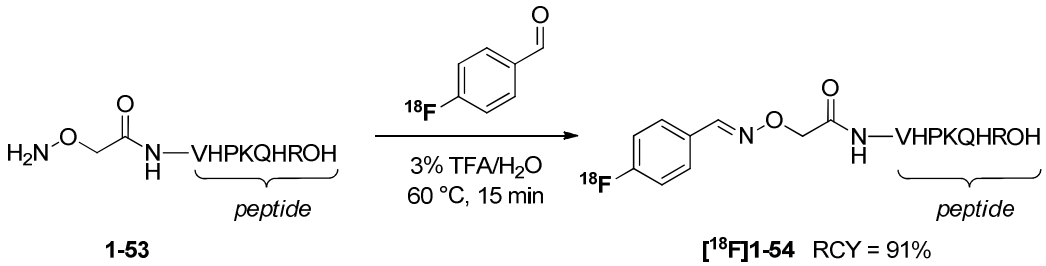
Scheme 1.25. Synthesis of ***E*-[^{18}F]1-49** *via* the Horner-Wadsworth-Emmons reaction

In addition, [^{18}F]FBA has been employed as a versatile reagent for the indirect labelling of peptides and proteins. [^{18}F]FBA reacts with peptides *via* oxime or hydrazone formation under mild conditions with high chemoselectivity and high RCYs.[61] Human serum albumin (HSA) was first reacted with hydrazinonicotinic acid **1-50** and then treated with [^{18}F]FBA to give [^{18}F]HSA **[^{18}F]1-52** in RCY 25-90%, depending on the reaction conditions (**Scheme 1.26**).[62]



Scheme 1.26. Synthesis of [^{18}F]HSA **[^{18}F]1-52** *via* hydrazone-bond formation

The short peptide sequence VHPKQHR, a known ligand for VCAM-1 (vascular cell adhesion molecule-1), has been ^{18}F-labelled by the Gouverneur group, to detect VCAM-1 expression and cancer micrometastases (**Scheme 1.27**).[63] Purified 4-[^{18}F]FBA was subjected to hydroxylamine-peptide **1-53** and heated at 60 °C for 15 min. ^{18}F-oxime-peptide **[^{18}F]1-54** was obtained with the highest RCY of 91% at a concentration of 0.008 M and 4:1 H_2O:MeOH mixture.

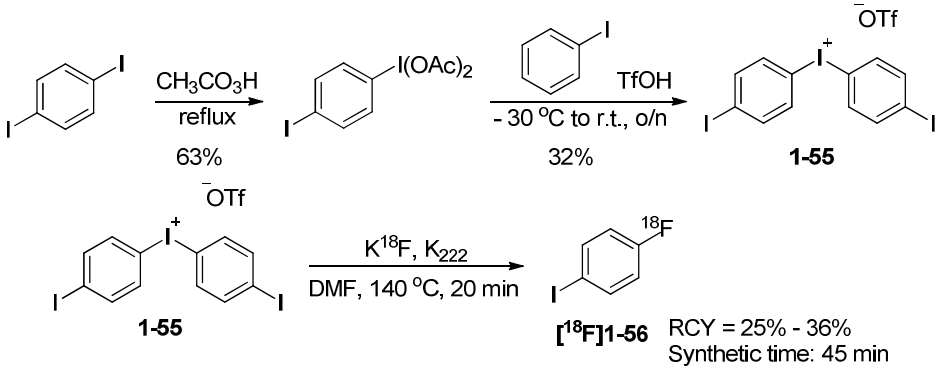


Scheme 1.27. Synthesis of ^{18}F-labelled peptide **[^{18}F]1-54** *via* oxime formation.

1.8.2.2. ^{18}F-labelled aryl halides in transition metal-mediated reactions

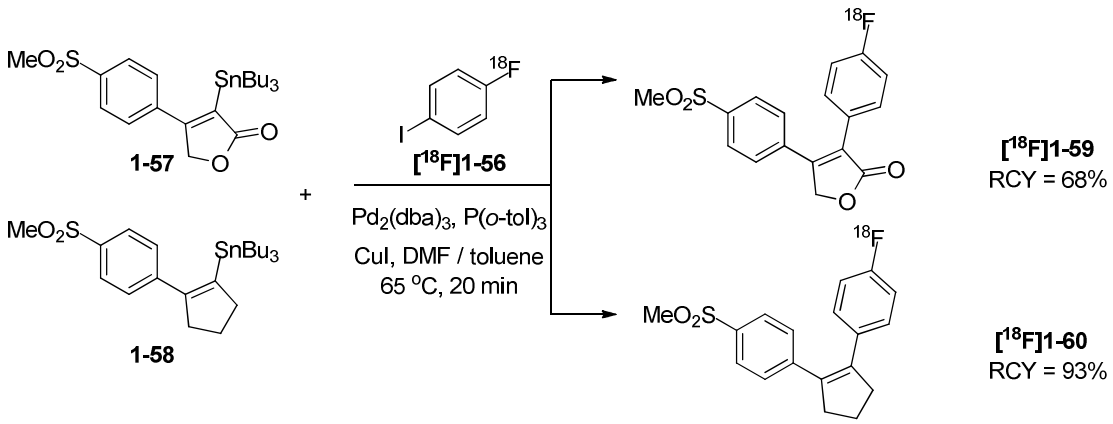
The reliability and versatility of transition metal catalysed cross coupling reactions has been well-studied during the past fifty years.[64] Palladium-mediated cross coupling reactions, such as the Stille Reaction,[65] Suzuki Reaction, Sonogashira Reaction and Buchwald-Hartwig *N*-arylation[66] reactions are among the most widely-applied in both academic research and industrial manufacturing.[67] As a general prosthetic group for palladium-

mediated cross coupling reactions,[49] 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56** can be synthesised by thermal decomposition of 4,4'-diiododiphenyliodonium triflate **1-55** in the presence of ^{18}F-fluoride within 45 min in RCY 25%–36% (**Scheme 1.28**).[68] A typical synthetic route to the symmetrical 4,4'-diiododiphenyliodonium triflate **1-55** involves refluxing 1,4-diiodobenzene with peracetic acid, to give 4-iodo-1-(diacetoxyiodo)benzene in 63% yield, followed by reaction with trifluorosulfonic acid and iodobenzene to give **1-55** in a 32% yield.[69]



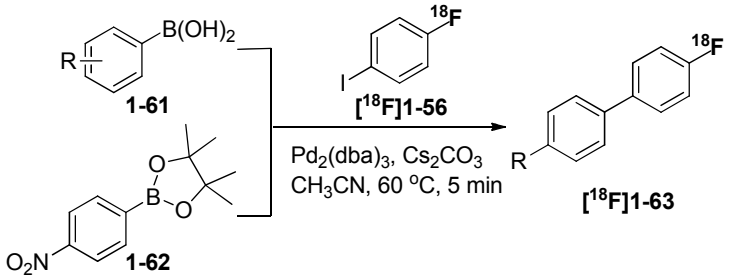
Scheme 1.28. Radiosynthesis of 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56**.

The palladium-mediated cross-coupling reactions with 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56** can be regarded as a general labelling method for the introduction of a 4-[^{18}F]fluorophenyl group, a common structural component found in many fluorinated drugs. The Stille reaction of a trialkyl tin precursor **1-57/58** with 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56** has been used to form **[^{18}F]1-59/60** as potential PET radiotracers (**Scheme 1.29**).[70] Up to 93% radiochemical yield can be obtained under optimised conditions with stannane/[$Pd_2(dba)_3$]/P(*o*-tol)$_3$/CuI (in 1:2:1.5:2 molar ratio) in a DMF/toluene (1 : 1 / *v* : *v*) mixed solvent system at 65°C for 20 minutes.



Scheme 1.29. Radiosynthesis of **[^{18}F]1-59/60** *via* Stille reaction

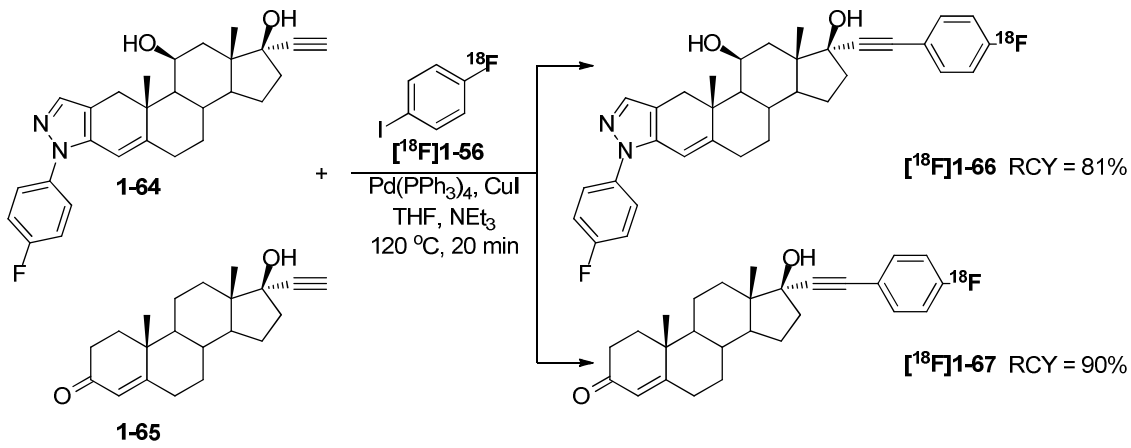
A range of *p*-tolylboronic acids **1-61** and boronic ester **1-62** have been treated with 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56** under Suzuki conditions (**Scheme 1.30**). [71] The reaction proceeded to give **[^{18}F]1-63** in excellent radiochemical yields of up to 94% within 5 minutes. In addition, the results showed that the reaction was compatible with a wide range of functional groups, including esters, carboxylic acids, methoxy and hydroxyl groups.



R	RCY (%)	Time
p-CH_3, *m*-CH_3, *o*-CH_3, F, Cl, OH OCH_3, $COOCH_3$, $SOOCH_3$, SCH_3, NO_2	80-94%	5 min
Br, COOH	30-37%	5 min

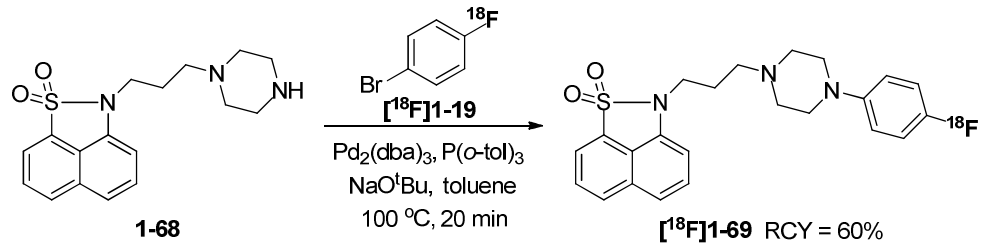
Scheme 1.30. Radiosynthesis of **[^{18}F]1-63** *via* Suzuki Reaction

The synthesis of the 4-[^{18}F]fluorophenylethynyl-substituted compound **[^{18}F]1-66/67** has been validated by the reaction of 1-[^{18}F]fluoro-4-iodobenzene **[^{18}F]1-56** and terminal alkynes **1-64/65** under classical Sonogashira reaction conditions (**Scheme 1.31**).[69] The reaction was performed in $Pd(PPh_3)_4$/CuI in THF/triethylamine at 120 °C for 20 minutes to give **[^{18}F]1-66** in RCY 81%, and **[^{18}F]1-67** in RCY 90% based on ^{18}F-fluorinated iodobenzene **[^{18}F]1-56**.



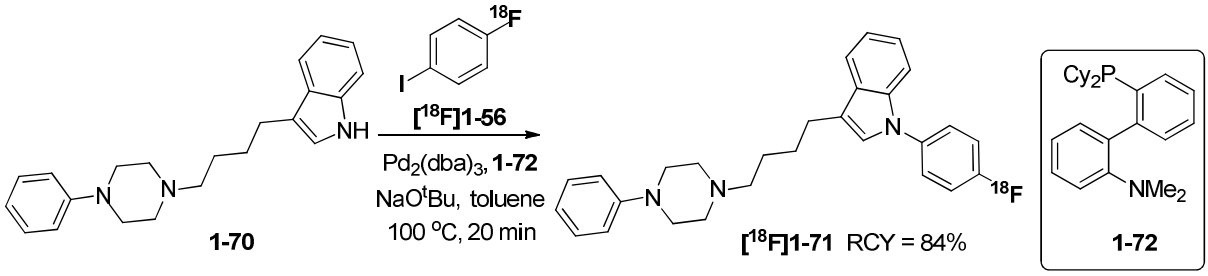
Scheme 1.31. Radiosynthesis of **[^{18}F]66/67** *via* Sonogashira Reaction

^{18}F-labelled aryl halides can be used in cross-coupling reactions involving the formation of C–N bonds (**Scheme 1.32**). The methodology was first exemplified by the synthesis of the selective 5-HT2A receptor ligand [^{18}F]RP 62203 **[^{18}F]1-69** *via* a palladium-mediated *N*-arylation of the piperazine moiety with 1-bromo-4-[^{18}F]fluorobenzene **[^{18}F]1-19** in RCY 60%.[72]



Scheme 1.32. Radiosynthesis of **[^{18}F]1-69** *via* Buchwald-Hartwig Reaction

The Buchwald *N*-arylation was later expanded to indole substrates for the formation of ^{18}F-labelled *N*-arylindoles, which are key structural motifs in many drugs and other pharmaceutically important compounds. In the radiolabelling of **[^{18}F]1-71**, the highest radiochemical yield was 84% (based upon 1-[^{18}F]fluoro-4-iodobenzene, **[^{18}F]1-56**) with $Pd_2(dba)_3$ as the palladium catalyst and **1-72** as an electron-rich phosphine ligand (**Scheme 1.33**).



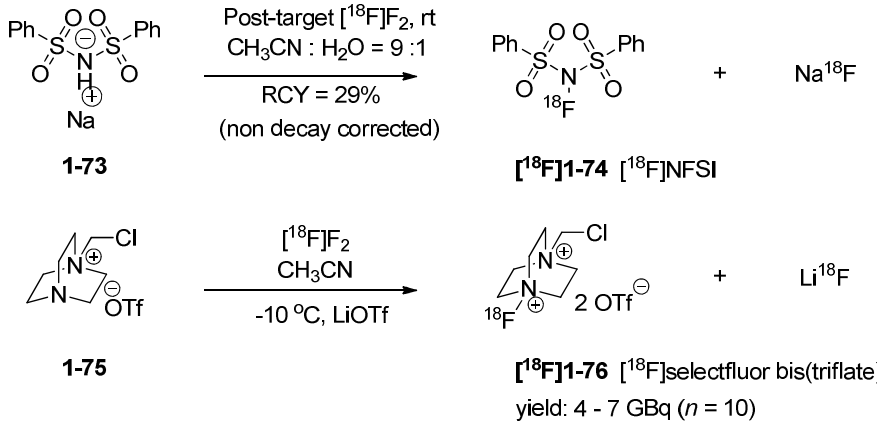
Scheme 1.33. Radiosynthesis of **[^{18}F]1-71** *via* Buchwald-Hartwig Reaction

1.8.3. Electrophilic substitution for ^{18}F-labelled aromatics

1.8.3.1 Electrophilic ^{18}F-fluorination reagents

Electrophilic fluorinations are less favoured in current labelling practice for many reasons: (1) labelled products can only be delivered with low specific activity because of the *carrier-added* method of [^{18}F]F_2 production; (2) Special equipment is usually needed for handling and labelling with [^{18}F]F_2; (3) reactions with [^{18}F]F_2 generally lack selectivity and can result in mixture of ^{18}F labelled products. Therefore, efforts have been devoted to the development of milder, selective [^{18}F]fluorination reagents, such as [^{18}F]-N-fluorobenzenesulfonimide ([^{18}F]NFSI)[73] and [^{18}F] Selectfluor *bis* (triflate).[74]

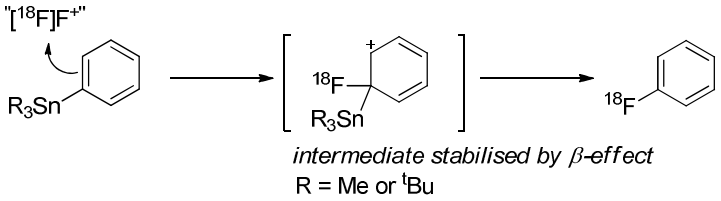
Within the Gouverneur group, much research has been carried out investigating ^{18}F-labelled reagents as a suitable source of "$^{18}F^{+}$" with tailored selectivity. The radiosynthesis of [^{18}F]NFSI **[^{18}F]1-74** and [^{18}F]Selectfluor bis(triflate) **[^{18}F]1-76** (**Scheme 1.34**) extended the scope of [^{18}F]electrophilic fluorination and facilitated the transformation from conventional fluorination chemistry to ^{18}F-radiolabelling.



Scheme 1.34. Preparation of [^{18}F]NFSI **[^{18}F]1-74** and [^{18}F]Selectfluor bis(triflate) **[^{18}F]1-76** as "$^{18}F^{+}$" reagents

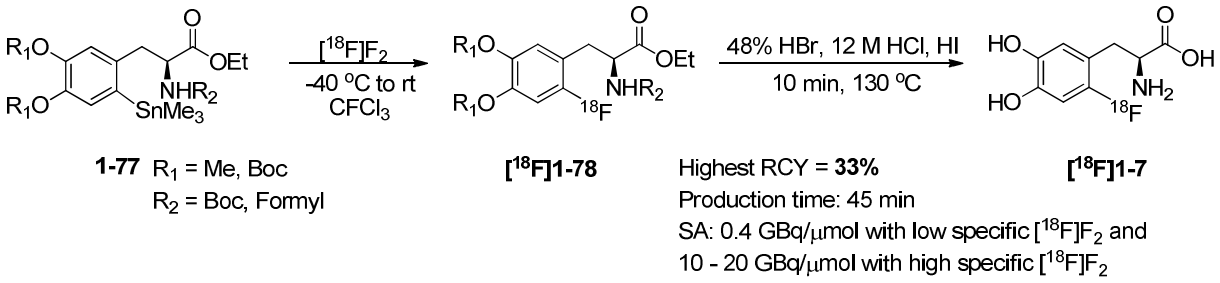
1.8.3.2 Electrophilic fluorination *via* fluorodemetalation

Electrophilic aromatic ^{18}F substitution of organometallic compounds has been used to prepare a wide range of ^{18}F-labelled aromatics which are not easily accessible *via* conventional radiochemistry with ^{18}F-fluoride. Fluorodemetalation of aromatic substrates is thought to proceed *via* an electrophilic *ipso* substitution mechanism as shown in **Scheme 1.35**. The *ipso* attack at the substituted position is promoted by the β-effect of the tin atom.



Scheme 1.35. The arenium mechanism of electrophilic fluorination of aromatics

Fully automated protocols have been established to produce L-[^{18}F]FDOPA L-**[^{18}F]1-7** *via* fluorodemetalation of the stannane precursor L-**1-77** followed by acidic hydrolysis, in 45 minutes with a RCY of 33% (**Scheme 1.36**). The specific activity of the labelled product could be increased from less than 0.4 GBq/μmol to 10-20 GBq/μmol using "high specific" [^{18}F]F_2 prepared from nucleophilic [^{18}F]fluoride, (**Scheme 1.2**) but this is still not high enough to perform brain receptor studies.



1-77 R_1 = Me, Boc; R_2 = Boc, Formyl

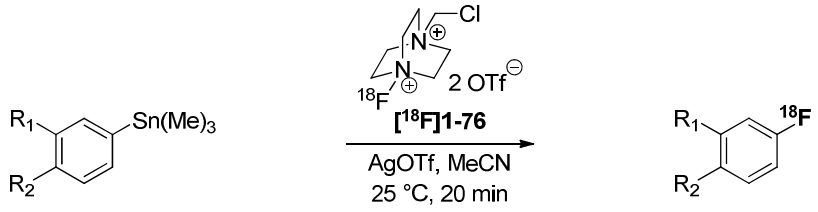
[^{18}F]1-78

Highest RCY = **33%**
Production time: 45 min
SA: 0.4 GBq/μmol with low specific [^{18}F]F_2 and
10 - 20 GBq/μmol with high specific [^{18}F]F_2

[^{18}F]1-7

Scheme 1.36. Radiosynthesis of [^{18}F]FDOPA **[^{18}F]1-7** by electrophilic fluorination

The novel "$^{18}F^{+}$" reagent [^{18}F]Selectfluor bis(triflate) (**[^{18}F]1-76**) was produced and subsequently subjected to the organometallic substrates (**1-79a/b** and **1-80**) in a solution of acetonitrile and silver triflate. The radiochemistry was demonstrated by the synthesis of [^{18}F]fluoroanisole (**[^{18}F]1-81a/b**) and [^{18}F]fluorophenol (**[^{18}F]1-82**) in RCY 14 – 18% with specific activity of 20 GBq/μmol (**Scheme 1.37**).[74]



R_1 = OMe, R_2 = OMe **1-79a**
R_1 = H, R_2 = OMe **1-79b**
R_1 = H, R_2 = OH **1-80**

R_1 = OMe, R_2 = OMe **[^{18}F]1-81a** RCY = 18%
R_1 = H, R_2 = OMe **[^{18}F]1-81b** RCY = 14%
R_1 = H, R_2 = OH **[^{18}F]1-82** RCY = 17%

specific activity = ~ 20 GBq/μmol

Scheme 1.37. Electrophilic fluorodemetalation using [^{18}F]Selectfluor *bis* (triflate)

1.9 Impetus in radiosynthesis of ^{18}F-labelled electron-rich aromatics

The research on late stage ^{18}F-fluorination of aromatics with fluorine is driven by the demand for radiotracers for positron emission tomography (PET),[75] an imaging technology that relies on robust synthetic routes to access simple or structurally complex ^{18}F-labelled molecules (when ^{18}F is the choice of radionuclide). Fluoroaromatics constitute an important

class of pharmacophores. The beneficial effect of a fluoroaryl group in drug design and development, with regard to drug metabolism, *in vivo* activity and stability has been reviewed recently.[10, 31]

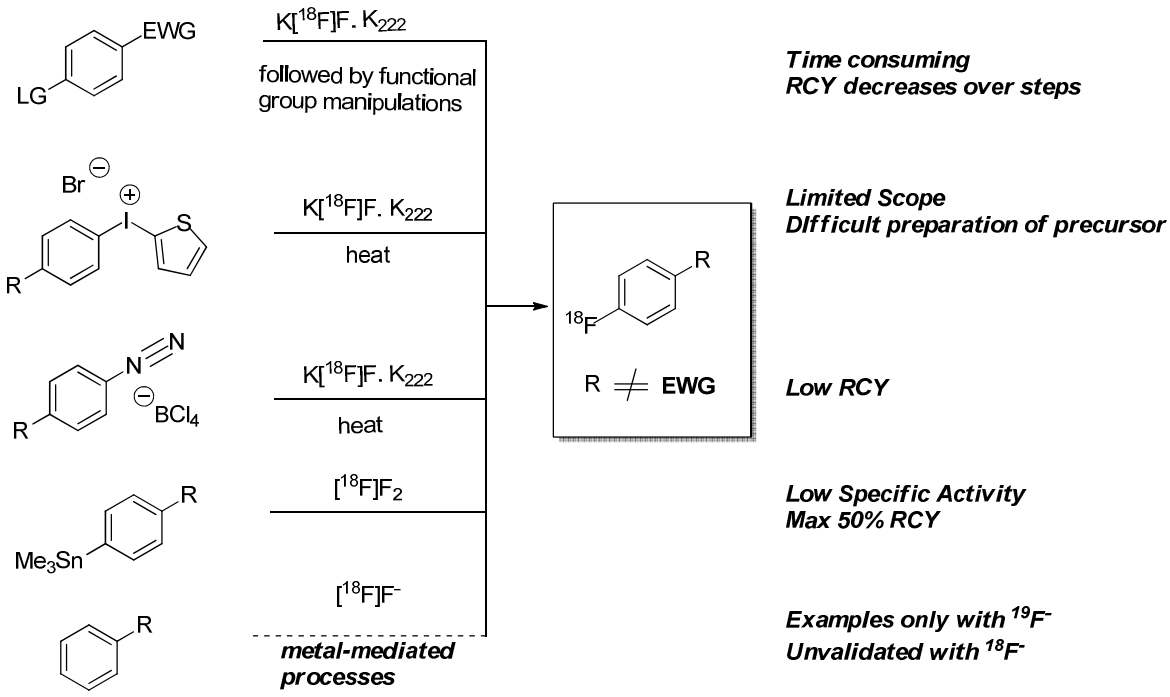
Aromatic compounds without electron-withdrawing groups are notoriously difficult to label with [^{18}F]fluoride and the search for effective methods to prepare ^{18}F labelled electro-neutral/rich aromatics remains an ongoing quest for radiochemists.

The radiosynthesis of high specific activity ^{18}F-labelled aromatics has improved significantly over the years (**Scheme 1.38**) but is still limited to the nucleophilic ^{18}F-fluorination of activated substrates and relies on stepwise functional group manipulations. These methods are always time-consuming and lead to a decrease in radiochemical yield.

Diaryliodonium salts have proven to be an efficient method for the introduction of ^{18}F fluoride onto electron-rich aromatic rings. However, the preparation of the corresponding iodonium salts requires reaction conditions that are not compatible with all substrates. To date, the scope of the method is still limited.

The low radiochemical yield and harsh reaction conditions are the major drawbacks to using Balz-Schiemann and Wallach methods in ^{18}F radiosynthesis.

Notably, the recent breakthrough in palladium-mediated C–F bond construction from fluoride sources[76] implies that transition-metal-mediated reactions in the presence of [^{18}F]fluoride may be a plausible method for introduction of ^{18}F fluoride onto electron rich aromatic rings. However, further optimisation is necessary for application to ^{18}F-PET studies.

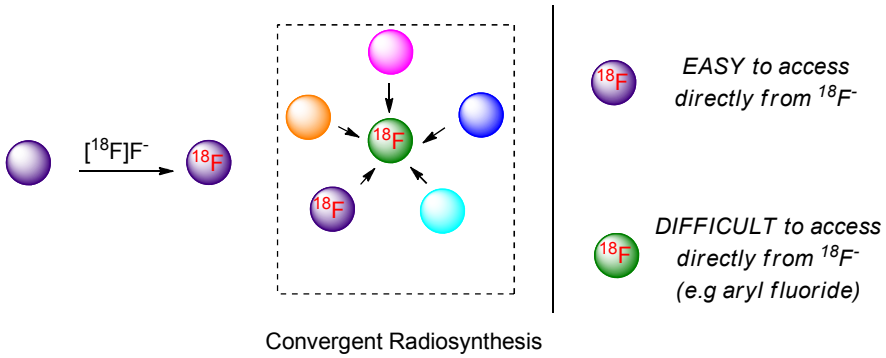


Scheme 1.38. Radiosynthesis of ^{18}F-labelled electron-neutral/rich aromatics

1.10 Aim and outline of the thesis

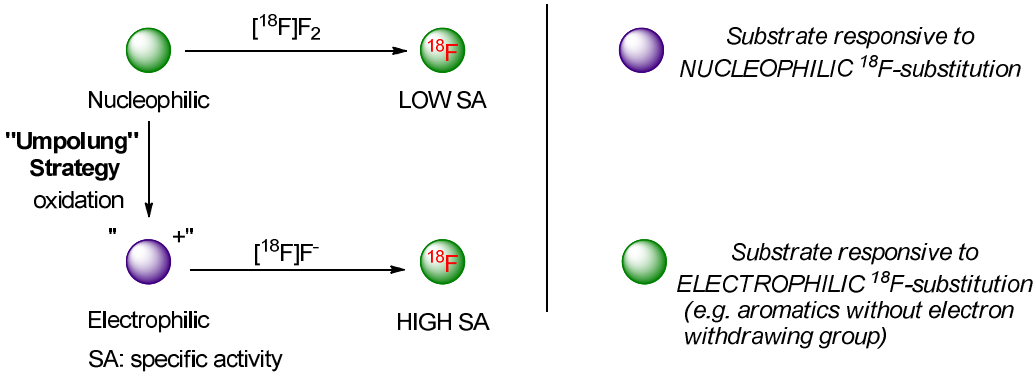
Despite the extensive studies devoted to the radiosynthesis of ^{18}F-labelled electron-rich aromatics, new methodologies are highly sought after to improve the radiosynthetic efficiency, versatility and structural diversity of the labelled products.

Two novel methodologies to deliver ^{18}F-labelled aromatic compounds from nucleophilic ^{18}F-fluoride are presented in this thesis. ***Chapter 2*** details the experimental efforts towards "Convergent Radiosynthesis". We proposed a convergent synthetic tactic that allows for the simultaneous reaction between three or more substrates, including an ^{18}F-labelled building block. The multi-component reaction was used to validate an extremely concise two-step synthetic sequence for the radiosynthesis of various structural scaffolds. Notably, the chemistry allowed for the ^{18}F-substituent to be positioned on an aryl sub-motif not responsive to direct nucleophilic fluorination. This strategy would greatly extend the scope of prosthetic group radiochemistry (**Scheme 1.39**).



Scheme 1.39. Convergent ^{18}F radiosynthesis

Chapter 3 presents the concept of "Oxidative Nucleophilic ^{18}F-Fluorination". We proposed that electron-rich aromatics, such as phenols, which are not responsive to nucleophilic fluorination, may undergo reactivity umpolung under oxidative conditions. If this is possible in the presence of fluoride, a new radiolabelling route allowing for the direct nucleophilic ^{18}F-fluorination of phenols may become possible (**Scheme 1.40**).



Scheme 1.40. Oxidative nucleophilic ^{18}F-fluorination

Chapter 2

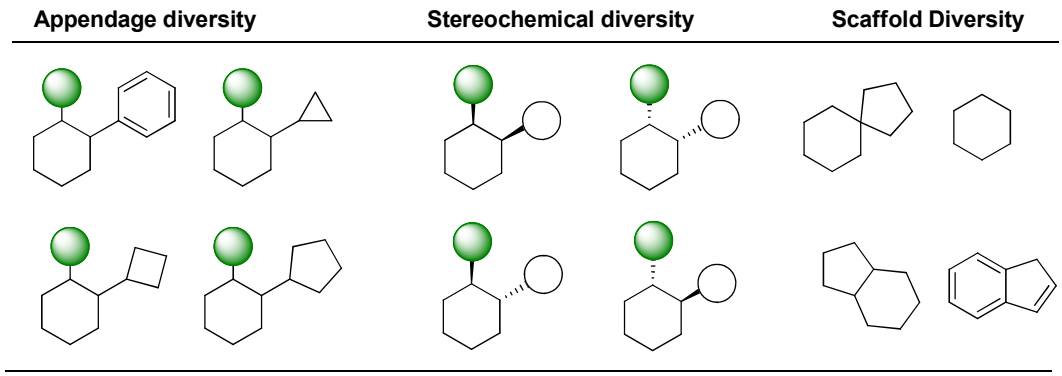
Convergent Radiosynthesis

2.1 Introduction

2.1.1 Multicomponent reactions (MCRs) as a convergent synthetic method

Multicomponent reactions (MCRs)[77, 78] are those in which three or more compounds react to form a single product with high atom economy and bond-forming efficiency. MCR strategies are highly attractive for drug discovery and medicinal chemistry research because high diversity can be efficiently achieved compared to conventional strategies. Most MCRs involve a sequence of elementary steps in equilibrium followed by an irreversible product forming step, such as a C–C bond forming reaction or a rearrangement.[79]

MCRs have been used extensively for the rapid generation of small-molecule libraries in the search for lead compounds for therapeutic applications. To increase the chances of finding a lead compound with the desired biological activity, the molecular diversity between library members should be as great as possible within the boundaries of biologically active sites. Three fundamental levels of molecular diversity were distinguished by the Orru group in a recent review:[80] a) *Appendage diversity* refers to the installation of various appendages into a common chemical motif; b) *Stereochemical diversity* refers to the generation of stereoisomers *via* stereospecific reactions and c) *Scaffold diversity* refers to the formation of a series of compounds with different skeletons (**Scheme 2.1**)



Scheme 2.1. The three fundamental levels of molecular diversity: appendage diversity, stereochemical diversity and scaffold diversity.[6]

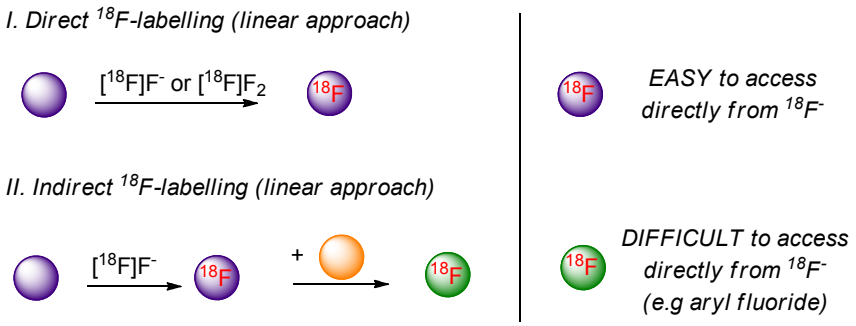
An analysis of the state-of-play of MCRs reveals two main limitations. Firstly, although post-MCR cyclisation strategies are widely used to deliver ever more complex molecular skeletons, the applications of MCRs in chemical biology are still restricted by a lack of scaffold diversity. The most straightforward solution to this problem is the rational design of novel MCRs. Secondly, the use of MCRs as synthetic tools is limited by the lack of stereocontrol. Although isocyanide-based MCRs (IMCRs) are a very important sub-class of MCRs, the stereoselectivity of many IMCRs is notoriously poor. The establishment of a robust catalytic asymmetric Ugi reaction is considered as the holy grail in MCR chemistry.[80]

2.1.2 Convergent Radiosynthesis: radio-MCR with ^{18}F-Labelled Building Blocks.

In PET chemistry, the variability of chemical reactions employed for ^{18}F-radiolabelling relies on the availability of nucleophilic and electrophilic sources of ^{18}F. ^{18}F-fluoride is the only ^{18}F source that leads to *n.c.a.* ^{18}F-radiopharmaceuticals with high specific activity.

Radiochemists typically select the site of ^{18}F-radiolabelling to fit existing radiosynthetic methods, an approach that is limited by the efficiency of these methods and could therefore be detrimental for rapid progress in PET tracer discovery. For example, the scope of conventional nucleophilic ^{18}F-fluorination on an aromatic motif *via* the S_NAr mechanism is highly dependent on the activating effect of an electron-withdrawing substituent and fluorination of unactivated aromatics remains a major problem to be solved.

The accessibility of structurally diverse radiopharmaceuticals is crucial to meet the growing demand for PET tracers. For compounds that are difficult to access directly from $^{18}F^-$, e.g. ^{18}F-labelled aryl fluoride, the ^{18}F-radio-retrosynthetic strategies applied to date are typically based on linear sequences of functional group transformations, starting with ^{18}F-labelled prosthetic groups that are easy to access directly from $^{18}F^-$. This linear approach is designed with the aim of introducing an ^{18}F-label as late as possible, but this criteria becomes increasingly difficult to meet when the probe is structurally complex or highly functionalised (**Scheme 2.2**).



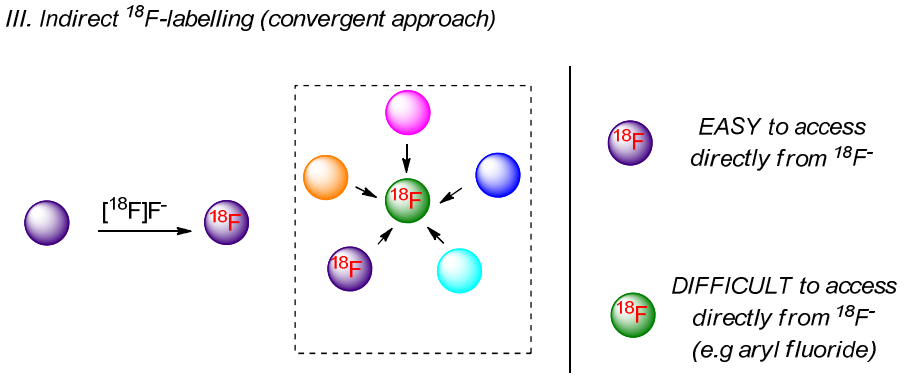
Scheme 2.2. Linear radiosynthetic approach of ^{18}F-labelling

Convergent synthetic routes, in which three or more substrates react simultaneously to deliver one molecule with structural complexity, have never been explored in the context of

[6] The scheme is adapted from reference 81, with permission from John Wiley and Sons.

^{18}F-radiochemistry. We postulated that this research area may benefit from convergent synthetic tactics that should allow for rapid assembly of ^{18}F-radiotracers from readily accessible ^{18}F-labelled prosthetic groups with high diversity and efficiency. Additionally, radiochemists will have the possibility to introduce the ^{18}F-label on different positions of the target probe without the need to redesign the overall radiosynthetic route.

To illustrate the value of this convergent radiosynthetic approach, we aim to develop new methodologies suitable for PET chemistry by introducing ^{18}F-labelled building blocks into multicomponent reactions, so as to deliver ^{18}F-labelled compounds that could not be easily accessed by a direct ^{18}F-fluorination. Moreover, this strategy will deliver structurally diverse ^{18}F-labelled compounds based on one single reaction. If feasible, convergent radiosynthesis could be a powerful strategy that expands dramatically the scope of ^{18}F-prosthetic group radiochemistry (**Scheme 2.3**).



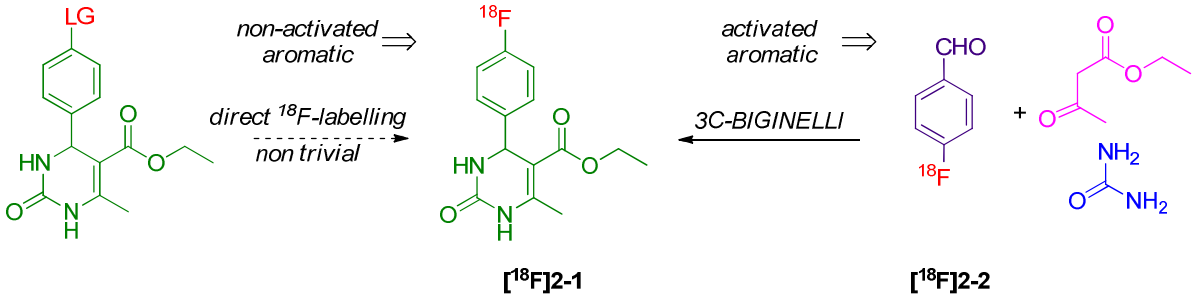
Scheme 2.3. Convergent radiosynthetic approach of ^{18}F-labelling

2.2 Conceptual validation with Radio-Biginelli MCR

We chose to validate this novel MCR-based ^{18}F-radiochemistry with the view to synthesise ^{18}F-labelled compounds that are not obvious candidates for a retro-radiosynthetic route based on direct nucleophilic ^{18}F-fluorination. ^{18}F-labelled benzaldehyde derivatives were selected as radioactive components because aldehydic components have been widely used in

many important "cold" MCRs. In addition, the preparation of 4-[^{18}F]fluorobenzaldehyde from *n.c.a.* $^{18}F^-$ and its application as a prosthetic group in radiosynthesis has been well documented.[50]

The advantages of this approach can be demonstrated with the preparation of a 3,4-dihydropyrimidin-2-(1*H*)-one (DHPM) analogue **[^{18}F]2-1** featuring the ^{18}F-labelled tag at the aryl group. Upon radio-MCR, the readily accessible ^{18}F-benzaldehyde **[^{18}F]2-2** will deliver an ^{18}F-labelled DHPM analogue. This compound is notoriously difficult to access using ^{18}F-fluoride as the necessary precursor features an aryl group that is not sufficiently activated for a classical S_NAr reaction (**Scheme 2.4**).

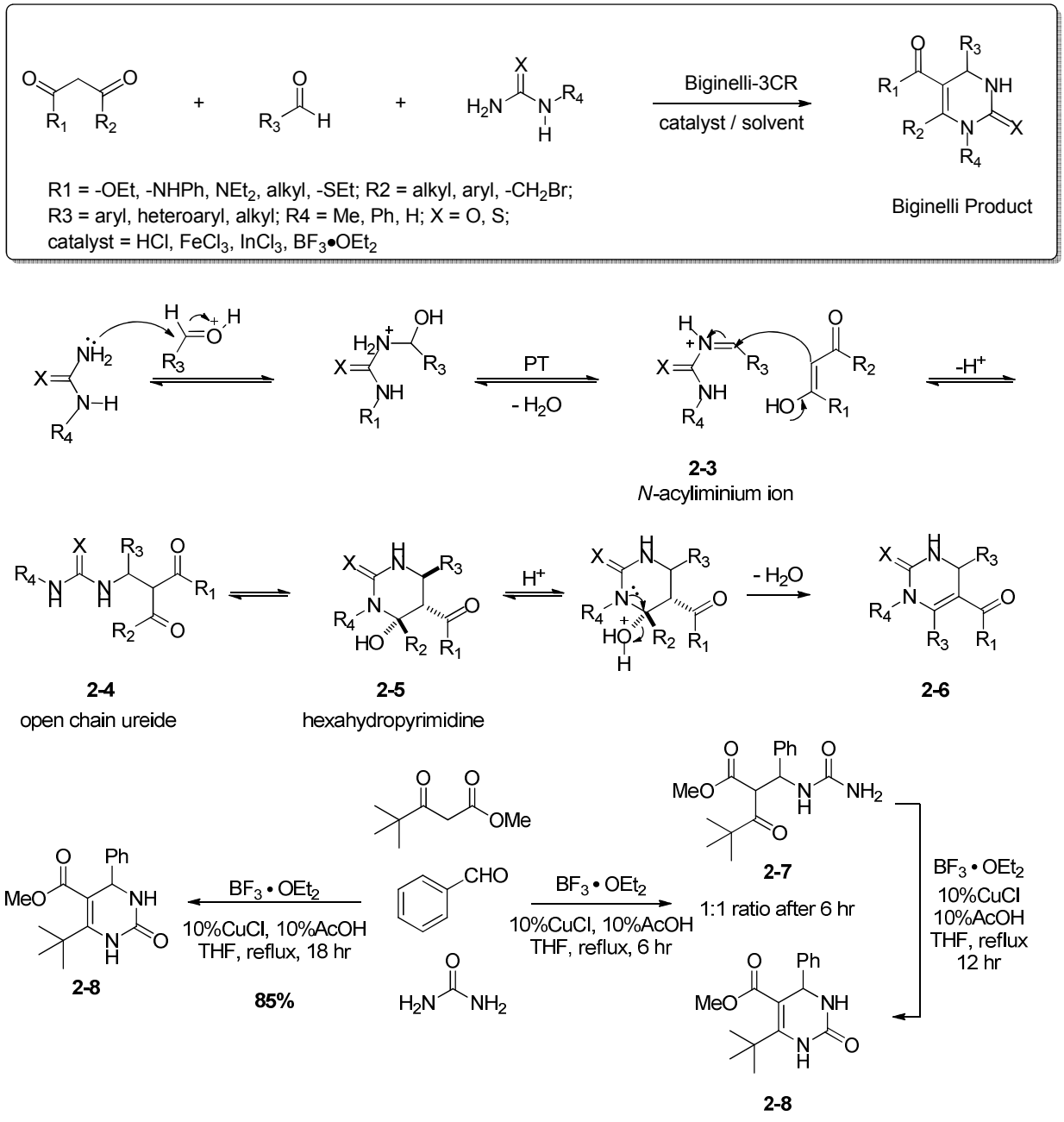


Scheme 2.4. Biginelli reaction with ^{18}F-labelled benzaldehyde (**[^{18}F]2-2**)

2.2.1 Biginelli-MCR

In 1893, the acid-catalysed cyclocondensation between ethyl acetoacetate, benzaldehyde and urea was reported by Biginelli (**Scheme 2.5**).[81] The widely accepted mechanism for Biginelli reactions was first proposed by Kappe in 1997.[82, 83] The first step involves the condensation of the aldehyde and the urea. The resulting *N*-acyliminium intermediate **2-3** acts as an electrophile for the nucleophilic addition of the ketoester enol. The ketone carbonyl of the resulting adduct **2-4** then undergoes condensation with the urea to form the hexahydropyrimidine **2-5**, which is subsequently dehydrated to afford the cyclised product **2-6**. This mechanism was later supported with experimental evidence by the Hu group.[84]

They reported the formation and isolation of intermediate **2-7** and product **2-8** in a 1:1 ratio after 6 hours when the reaction was performed with a bulky β–keto ester substituent. Isolated **2-7** was resubjected to the reaction conditions to afford the DHPM compound **2-8**.

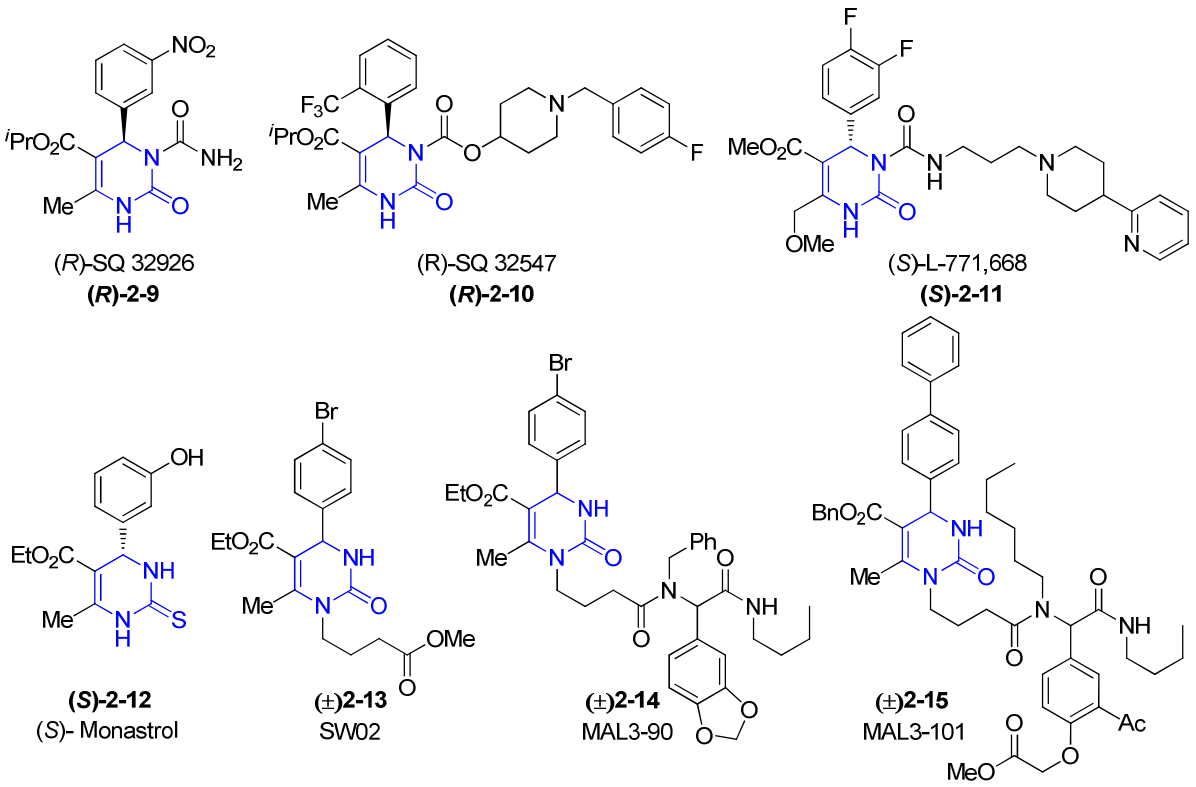


Scheme 2.5. Biginelli reaction and experimental evidence of its mechanism

A typical Biginelli reaction is carried out in a one-pot procedure by heating to reflux a mixture of the three components in ethanol, with a catalytic amount of hydrogen chloride.

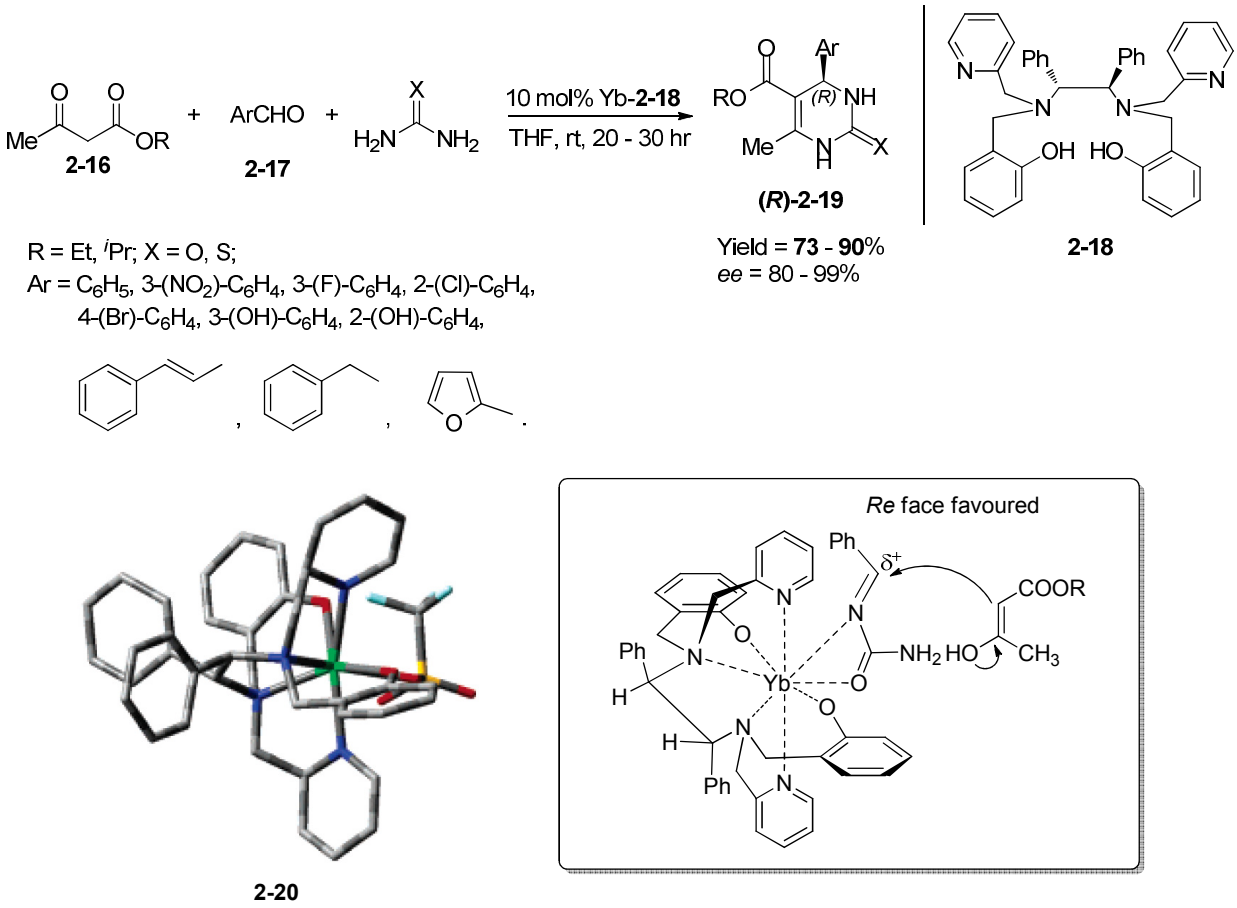
The classical Biginelli synthesis suffers from harsh reaction conditions, lengthy reaction times, and low to moderate yields. Moreover, upon prolonged refluxing in a strongly acidic medium, side processes tend to decrease the yields of desired product. The Kappe group has extensively explored the scope of the Biginelli reaction by varying all of the components, as well as the catalysts. [81, 85, 86] Dotsenko recently catalogued over 200 different conditions for the Biginelli MCR and the large range of suitable catalysts includes Brønsted and Lewis acids, in addition to non-acidic additives (e.g. NaCl, baker's yeast and ionic liquids).[87]

DHPMs and their derivatives are of growing interest due to their recently discovered biological activities. (*R*)-SQ 32926 **(*R*)-2-9** and (*R*)-SQ 32547 **(*R*)-2-10** were reported to be effective orally active antihypertensive agents.[88] (*S*)-L-771,668 **(*S*)-2-11**[89, 90] is a potent antagonist of the α_{1A}-selective adrenoceptor. (*S*)-Monastrol, 3,4-dihydropyrimidine-2(1*H*)-thione complex **(*S*)2-12**[91] is the only cell-permeable molecule currently known to selectively inhibit mitotic kinesin Eg5 and is therefore a promising lead to new anticancer drugs. In addition, SW02 (±)**2-13**, MAL3-90 (±)**2-14** and MAL3-101 (±)**2-15** have been shown to be modulators of molecular chaperone Hsp70 (heat shock protein 70), and thus trigger further interest as anti-cancer leads (**Scheme 2.6**).[92-94]



Scheme 2.6. DHMP compounds with biological activity

The acid-catalysed Biginelli reaction has benefited from the availability of various chiral Brønsted/Lewis acid catalysts[95],[96], and organocatalysts.[97],[98] Highly enantioselective Biginelli reactions have been validated using ytterbium triflate and a chiral hexadentate ligand **2-18**.[99] The asymmetric Biginelli reaction of 1,3-ketoester **2-16**, aldehyde **2-17** and urea or thiourea afforded the dihydropyrimidine-5-carboxylate complex **(*R*)-2-19** in 73 – 90% yield and 80 – 99% *ee*. In the formation of a Biginelli adduct, the stereodetermining step is the nucleophilic attack of the enol ester on the imine (or iminium). The ytterbium complex **2-20**, generated *in situ* from $Yb(OTf)_3$ and chiral ligand **2-18**, shielded the *Si*-face of the *N*-acylimine intermediate *via* coordination and allowed for nucleophilic attack of the enol ester predominatly from the *Re*-face (**Scheme 2.7**).



Scheme 2.7. Asymmetric Biginelli reaction.[7]

2.2.2 Biginelli reaction with fluorinated building blocks

In principle, MCRs could be adapted from conventional organic chemistry to *n.c.a.* radiochemistry with radioactive building blocks. It was apparent that extensive optimisation would be necessary with both "cold" and "hot" reagents to validate the concept of convergent radiosynthesis and to define the optimal reaction conditions. Firstly, MCRs with significant stoichiometric unequivalence, i.e. a large excess (mmol to μmol) of nonradioactive components relative to the amount of radioactive component (nmol), could

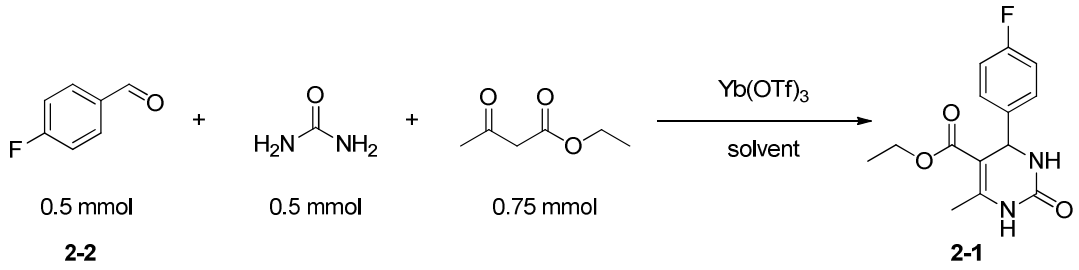
[7] The scheme is reprinted from reference 99, with permission from Ameracan Chemical Society.

display an unfavourable kinetic profile. Secondly, MCRs are lengthy reactions which will have to be optimised to meet the most important criteria for ^{18}F radiochemistry, which is for the total radiosynthesis time not to exceed approximately 6 hours (~three half-lives for ^{18}F).[29]

It has been reported that Brønsted acids and a wide range of Lewis acids including $FeCl_3$,[100] $Yb(OTf)_3$,[101] $BF_3{\cdot}OEt_2$ and CuCl[84] can be used to catalyse the Biginelli reaction. $Yb(OTf)_3$ was identified as a suitable catalyst for this transformation not only because it efficiently catalyses the Biginelli reaction but also due to its compatibility with water and harsh reaction conditions such as high temperatures or microwave irradiation. $Yb(OTf)_3$ has been employed in automatic synthetic modules for the production of a library of DHMP compounds. [102, 103]

A variety of conditions were screened for the non-isotopically labelled cyclocondensation as shown in **Table 2.1**. Neither synthetic yields nor reaction times were improved by increasing the catalyst loading (**Table 2.1,** Entries 1 – 3). The reaction was equally efficient in methanol or acetonitrile (Entry 1 *vs* 4). Entries 5 – 9 showed that the reaction was greatly accelerated by microwave irradiation. When the power of microwave irradiation was increased, higher yields were observed (Entry 5 *vs* 8). However, partial decomposition of the product was observed when the reaction mixture was kept under strong irradiation for a long period of time (Entry 9). Unfortunately, we were not able to identify any side products due to the complexity of reaction pathways.

Table 2.1 The model Biginelli reaction under different conditions



0.5 mmol 0.5 mmol 0.75 mmol

2-2 **2-1**

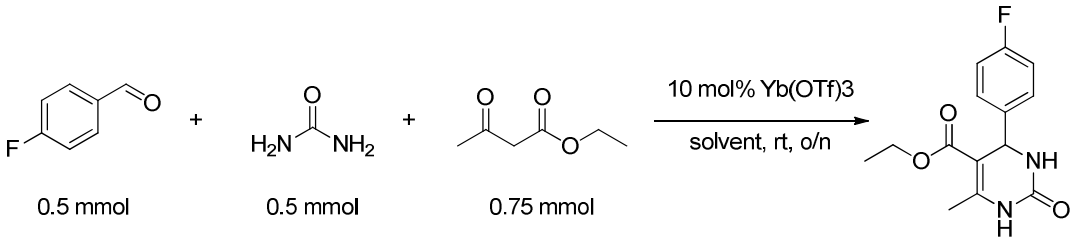
Entry	Cat. (%mol)	Solvent (ml)	Condition	Time	Yield[a] (%)
1	10	MeCN / 5	A	5 hrs	70
2	50	MeCN / 5	A	5 hrs	60
3	100	MeCN / 5	A	5 hrs	70
4	10	MeOH / 5	A	5 hrs	72
5	10	MeOH / 0.5	B	120 s	14[b]
6	10	MeOH / 0.5	C	60 s	22[b]
7	10	MeOH / 0.5	C	90 s	39[b]
8	10	MeOH / 0.5	C	120 s	46[b]
9	10	MeOH / 0.5	C	180 s	n/a[c]

Conditions: A. Heating in sealed vial at 80 ^{o}C; B. Microwave irradiation (30 W); C. Microwave irradiation (50 W); a. isolated yield; b. Yield calculated by ^{19}F NMR; c. Decomposition.

Further optimisation was performed to find out the compatibility of the Biginelli reaction with a range of organic solvents. Solutions of 4-fluorobenzaldehyde (**2-2**, 5 µl, 0.5 mmol), urea (3 mg, 0.5 mmol), ethyl acetoacetate (6 µl, 0.75 mmol) and ytterbium triflate (3 mg, 5 µmol, 10mol%) in a variety of solvents were stirred overnight at room temperature (**Table 2.2**). The amount of Biginelli product **2-1** in the reaction mixture was determined by ^{19}F NMR, using fluorobenzene as the internal reference. No reaction was observed in DMF, DMSO or diglyme (Entries 2, 3 and 7). Protic polar solvents such as 3-methyl-1-butanol and ethylene glycol gave higher efficiencies than a polar aprotic solvent such as acetonitrile (Entries 4 and 5 *vs* 1). The use of either 3-methyl-1-butanol or ethylene glycol should allow the Biginelli-MCR to be performed at a temperature higher than 130 ^{o}C. We postulated that

this would further shorten the reaction time of the MCR and thus benefit the future radiolabelling process.

Table 2.2 Biginelli-MCR in different solvents



0.5 mmol 0.5 mmol 0.75 mmol

2-2 **2-1**

Entry	Solvent	b.p. (^{0}C)	Yield[a] (%)
1	MeCN	81	5
2	DMF	153	0
3	DMSO	189	0
4	3-methyl-1-butanol	132	70
5	ethylene glycol	197	100
6	2-ethoxyethanol	135	5
7	diglyme	162	0

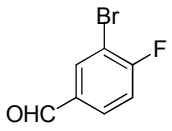
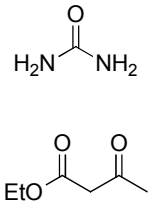
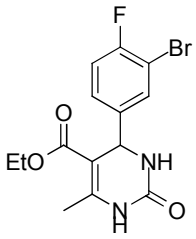
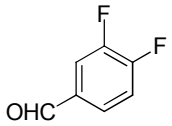
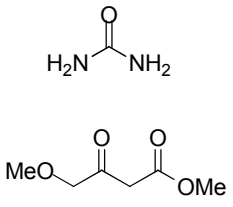
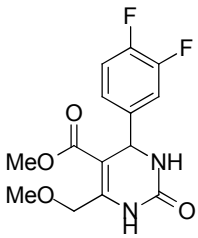
a. Yield calculated by ^{19}F NMR integration with fluorobenzene as an internal reference

In these feasibility studies, we have therefore validated the involvement of 4-fluorobenzaldehyde **2-2** in a Biginelli-MCR to afford a dihydropyrimidinone complex **2-1** with the aryl group tagged with fluorine. Next, 3-bromo-4-fluorobenzaldehyde **2-21** and 3,4-difluorobenzaldehyde **2-23** were used to deliver more fluorinated Biginelli-adducts, compounds **2-22** and **2-24** respectively.

Compound **2-24** contains the DHMP motif as found in the promising drug candidate (*S*)-L-771,668 **(*S*)-2-11** (**Scheme 2.6**). Hence the ^{18}F-labelled **2-24** is a potential intermediate to access the ^{18}F-labelled lead compound (*S*)-L-771,668 **(*S*)-2-11.**

The Biginelli reactions were performed by heating the fluorobenzaldehyde derivative (0.5 mmol), ethyl acetoacetate (0.75 mmol), urea (0.5 mmol) and ytterbium(III) trifluoromethanesulfonate (10 mol%) in a solution of acetonitrile (0.5 mL) at 80 ^{0}C for 5 hours. The DHPM compounds were obtained in moderate to excellent yields and served as references for radiosynthesis (**Table 2.3**).

Table 2.3 Biginelli reaction with fluorinated components

Fluorinated Component	Non-fluorinated Component	Product	Yield
			90%
2-21		2-22	
			80%
2-23		2-24	

All Biginelli reactions were performed by heating fluorobenzaldehyde (0.5 mmol), β-keto ester (0.75 mmol), urea (0.5 mmol) and $Yb(OTf)_3$ (10 mol%) in a solution of acetonitrile (0.5 mL) at 80 ^{0}C for 5 hours. Upon cooling down to room temperature, the residue was purified by column chromatography on silica gel to afford the DHPM compounds.

2.2.3 Equipment for ^{18}F-radiochemistry

To minimise the radiation dose to researchers, the synthesis and purification of radioactive compounds were typically performed in a customised automated radiochemical apparatus purchased from SCINTOMICS. The synthesiser operates within a hotcell shielded by lead panels (**Figure 2.1**). The synthesis unit is built around two reaction vessels (each measures 1.5 cm diameter and 3.5 cm height, with a volume of 5 mL). It also contains a heating assembly underneath each reactor and a syringe pump to precisely transfer the reagents from vessels to reactors through primed lines. The synthetic module is also equipped with a HPLC

column and UV and radiation detectors as the purification unit. The valves, pump, heater and other components of the synthesiser are controlled by software and the interface also allows for a range of operational modes, including fully-automated, semi-automated and manual.

A microwave synthesiser is housed in the hotcell. As in conventional organic chemistry, microwave reactors are widely used in radiochemistry to reduce the reaction times required for drying the ^{18}F-fluoride, for the ^{18}F-fluorination reaction or any subsequent chemical transformations. Microwave irradiations are commonly used for nucleophilic aromatic ^{18}F-fluorination, which typically requires harsh conditions, and some examples have been reported for aliphatic ^{18}F-radiolabelling.[104]

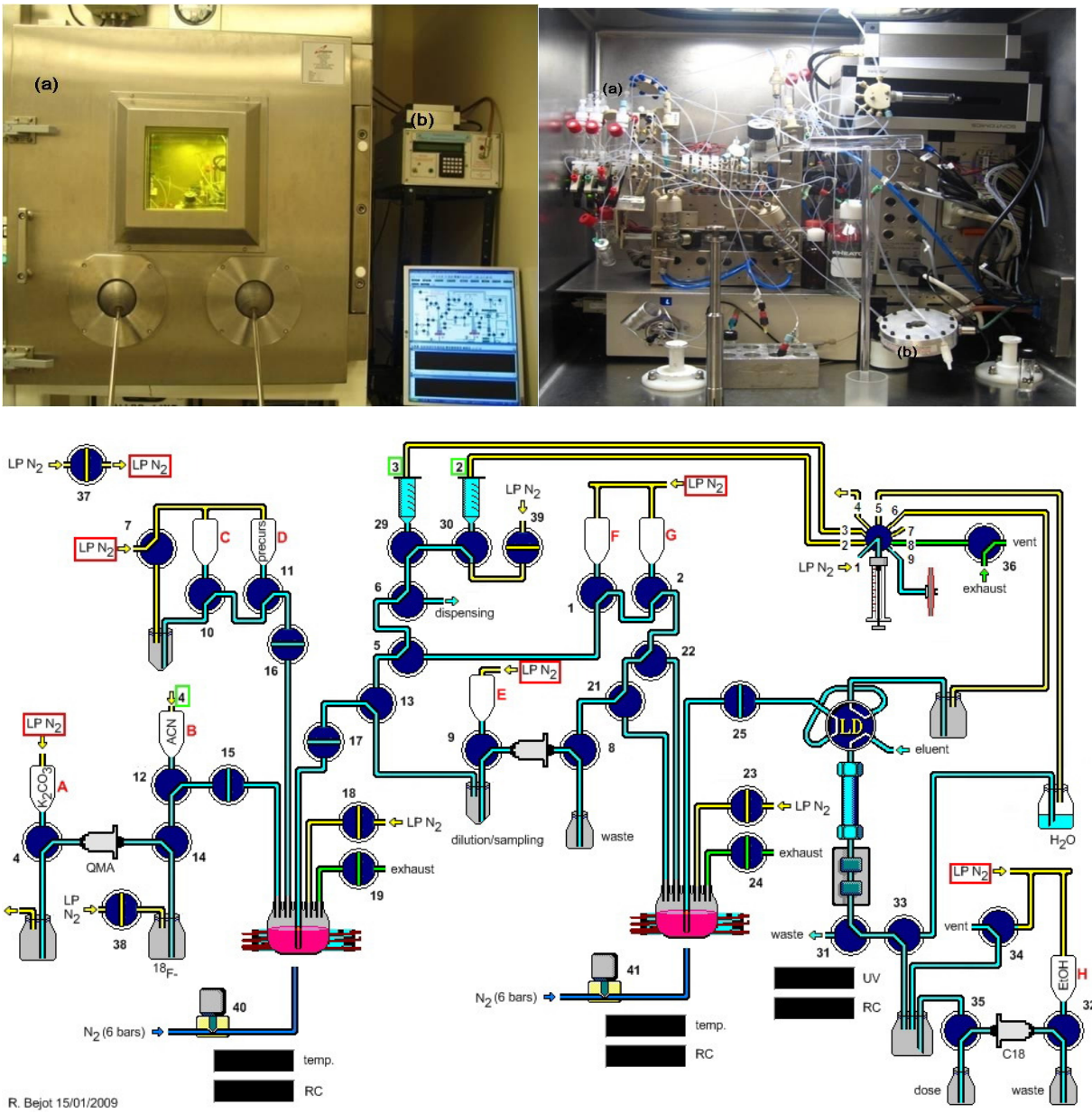


Figure 2.1 Top left: (a) view of Hotcell, (b) monitor for microwave synthesiser; top right (a) Scintomics radiosynthesiser, (b) microwave reactor; bottom: Schematic representative of Scintomics radiosynthesiser[8]

Another automated synthetic module for radiochemistry is the prototype microreactor from SIEMENS Molecular Imaging.[9] (**Figure 2.2**) The synthetic unit is constructed around a microfluidic chip. The reaction vessel centred in the chip measures 5 mm diameter and 3 mm height with a volume of 60 µL. The reactor is advantageous for ^{18}F-radiosynthesis as it

[8] The photos and schematic figure are provided by Dr. Romain Bejot and reprinted with permission.

minimises the reaction volume and therefore avoids the dilution of radioactive isotope (presented in nmol to μmol range). The lab-on-chip technology[105] also minimises the quantity of precursor needed. Compared with the conventional synthetic module previously described (SCINTOMICS), the reactor size is decreased from 5 mL to 60 μL and the typical reaction volume decreased from 300-500 μL to 40-60 μL. The required amount of precursor is reduced from ~10 mg to ~2 mg. As a result, the reaction time for ^{18}F-fluorination can be shortened from 10 – 15 min to 2 – 5 min without compromising the radiosynthetic efficiency.

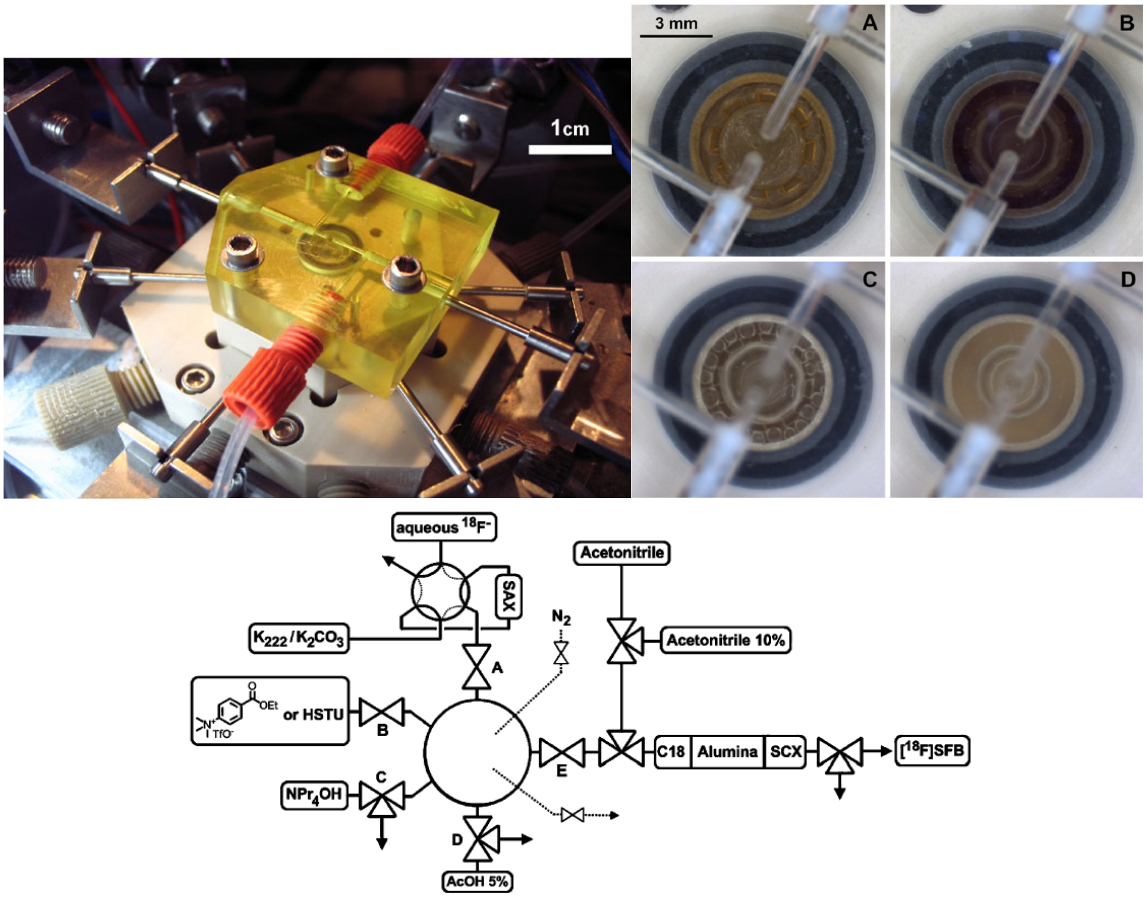
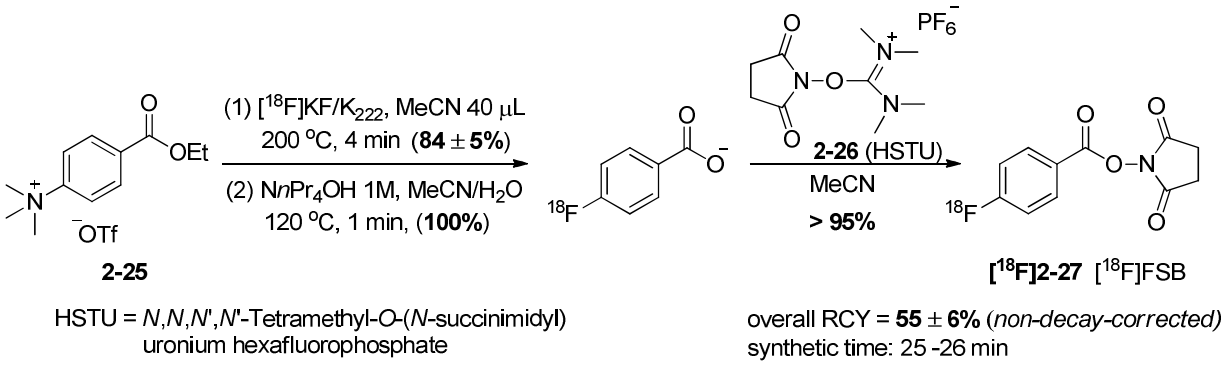


Figure 2.2 Top left: view of the reaction vessel, plungers, lid and base; top right: illustration of processes in the chip; botton: flow scheme of the microfluidic preparation of [^{18}F]FSB.[10]

[9] We thank Dr Arkadij Elizarov at SIEMENS Molecular Imaging for assistance with the microfluidic device.

[10] The figures are reprinted from reference 106, with permission from John Wiley and Sons.

A batch-mode three-step, one-pot synthesis and purification of [^{18}F]SFB **[^{18}F]2-27** has been validated using this microfluidic reactor.[106] *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB, **[^{18}F]2-27**) is an important prosthetic group widely used to label sensitive biomolecules such as peptides and proteins.



Scheme 2.8. The batch-mode microfluidic radiosynthesis of [^{18}F]SFB **[^{18}F]2-31**

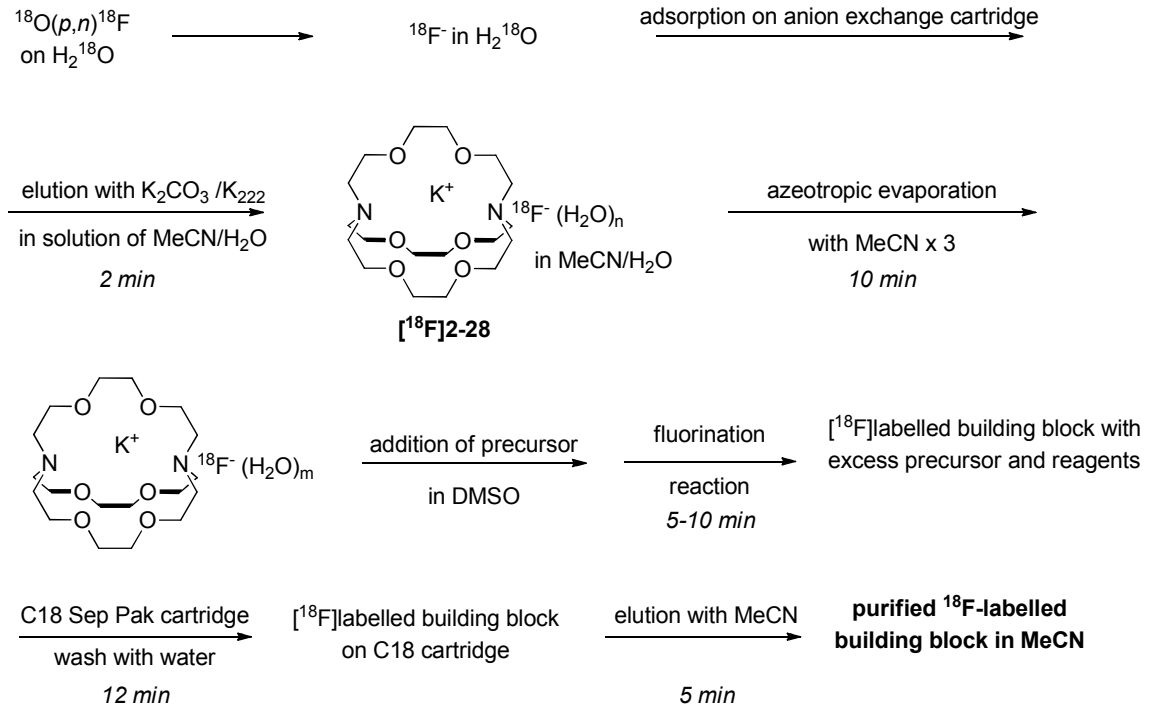
The radiosynthesis started with the direct nucleophilic ^{18}F-fluorination of the benzoic ester **2-25** in excellent RCY (84±5%). The hydrolysis of the ester group was completed in 1 min with 100% efficiency. The resulting 4-[^{18}F]fluorobenzoate salt was converted to *N*-succinimdyl ester **[^{18}F]2-27** by reacting with HSTU **2-26** in acetonitrile. This elegant radiosynthetic route afforded [^{18}F]SFB **[^{18}F]2-27** in 25 min, with 55±6% *non-decay-corrected* RCY and >98% radiochemical purity (**Scheme 2.8**). A typical synthetic procedure in a "large-scale" automated synthetic module requires a synthetic time of 60 – 100 min to give [^{18}F]SFB **[^{18}F]2-27** in comparable RCY and radiochemical purity.[50]

For the ^{18}F-radiochemistry described in this thesis, the ^{18}F-labelling reactions were analysed by both HPLC and radio-TLC. HPLC analysis was performed on a Gilson 322 system equipped with a NaI/PMT-radiodetector and a UV-detector. Radio-TLC analysis was performed on Macherey-Nagel Polygram Silica Plates, eluted with 95% aqueous acetonitrile

and analysed by a plastic scintillator/PMT detector. In this chapter, all quoted RCYs are *decay-corrected* radiochemical yields calculated by Radio-TLC, unless otherwise stated. The identity of radioactive species was determined by comparing the retention time against that of the reference compound, using HPLC methods.

2.2.4 Synthesis of ^{18}F-labelled building blocks

[^{18}F]fluoride was produced by PETNet Solutions at Mount Vernon Hospital (UK) and delivered as [^{18}F]fluoride in [^{18}O]water (1 – 2 GBq, 1 – 5 mL). Although unsolvated fluoride ion in gaseous form is a potent nucleophile, aqueous fluoride is a very poor nucleophile due to its high degree of hydration.[107, 108] Therefore, ^{18}F-fluoride needs to be dehydrated and solubilised in a suitable organic solvent before being used for nucleophilic substitution. The ^{18}F-fluoride was adsorbed on an anion exchange resin (typically a silica-based quaternary ammonium modified strong anion exchanger e.g. QMA from Waters®), eluted with a small volume of a weak base (commonly 3 – 5 mg of K_2CO_3 in 0.5 – 1.0 mL of acetonitrile / water (4 : 1 = *v* : *v*)), and further subjected to successive azeotropic evaporation with acetonitrile under a flow of inert gas. To enhance the nucleophilicity of the ^{18}F-fluoride ion, the counterion K^+ is encapsulated by a cryptand such as Kryptofix 2.2.2. (K_{222} purchased from Merck) by forming a [^{18}F]fluoride ion-K_{222}-K^+ complex **[^{18}F]2-28** (**Scheme 2.9**).

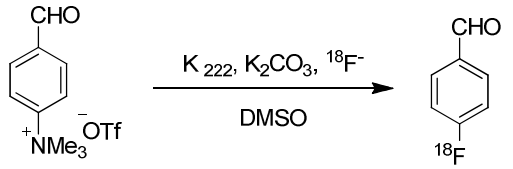


Scheme 2.9. Radiosynthetic procedure of ^{18}F-labelled building block.

We followed a well-documented protocol to prepare 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2**.[50] Upon drying of the ^{18}F-fluoride, the resulting $K^{18}F{\bullet}K_{222}$ (**[^{18}F]2-8**) was dissolved in an anhydrous solution of DMSO containing the precursor **2-29**, which possesses a trimethylammonium leaving group, and then transferred to a vial for radiolabelling reactions as shown in **Table 2.4**.

The results indicated that microwave irradiation was more efficient than conventional heating at promoting the ^{18}F-fluorination. When the precursor 4-formyl-*N*,*N*,*N*-trimethylbenzenaminium triflate **2-29** (10 mg in 300 μL of DMSO) and $K^{18}F/K_{222}$ were irradiated under 50 W microwaves for 100s, 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** was formed in a RCY of 83% (n = 3, Entry 2). These are the optimised conditions to produce 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2**. Prolonged microwave irradiation under high temperature may lead to evaporation of the volatile ^{18}F-fluorinated aldehyde **[^{18}F]2-2** and thus a loss of radioactivity.

Table 2.4 [^{18}F] fluorination under different conditions



2-29 **[^{18}F]2-2**

Entry	Condition[a]	Time	RCY[b](%)	*n*	Specific Activity
1	A	10 min	35	1	n/a
2	B	100 s	83	3	30-90 Gbq/μmol

Conditions: a. Reagents: 3 mg K_2CO_3, 15 mg K_{222} and 10 mg 4-trimethyl-ammoniumbenzaldehyde triflate; solvent: 0.3 ml DMSO; b. RCY is determined by radio-TLC (Hexane: EtOAc = 1:1); A. Heating (150 ^{o}C); B. Microwave irradiation (50 W).

Purification of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** was performed by solid-phase extraction (S-PE). The crude reaction mixture was diluted with water (with a volume 10 – 15 times to the reaction) and passed through a Sep-Pak cartridge packed with C18 silica resin. The solid phase was washed with water to remove unreacted ^{18}F-fluoride and water soluble impurities. After drying the solid phase for 3 min under a flow of nitrogen, the purified ^{18}F-labelled aldehyde **[^{18}F]2-2** was eluted with 1.0 mL acetonitrile or isoamyl alcohol and used for the subsequent multicomponent reaction.

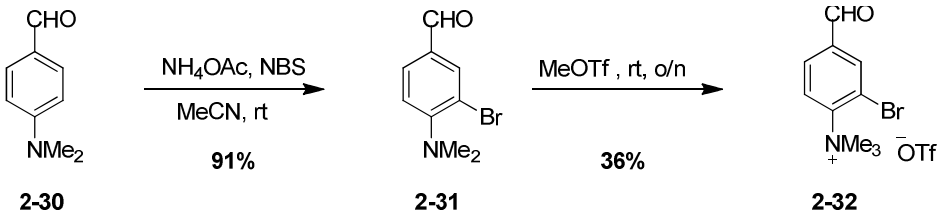
The calculation of specific activity of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** was performed based on **Equation 2.1**.

$$\text{Specific Activity (SA) [GBq/μmol]} = \frac{\text{Amount of radioactivity [GBq]}}{\text{Mass [μmol]}}$$

Equation 2.1. Specific activity

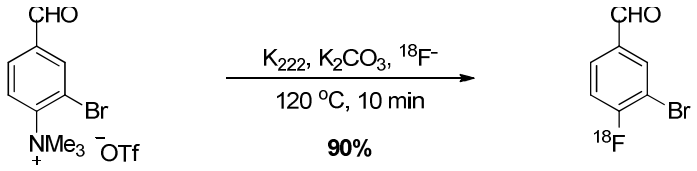
The quantities of the radioactive compounds were determined by HPLC using calibration curves obtained by analysis of the UV absorbance (254 nm) of the relevant non-radioactive 4-fluorobenzaldehyde. The specific activity of **[^{18}F]2-2** was found to be 35–90 GBq/μmol (*n* = 3), *decay-corrected* to the end of synthesis.

Precursor 2-bromo-4-formyl-*N*,*N*,*N*-trimethylbenzenaminium triflate **2-32** was prepared by bromination of 4-dimethylaminobenzaldehyde **2-30** with NBS (*N*-Bromosuccinimide)[109] followed by methylation with methyl trifluoromethanesulfonate (**Scheme 2.10**). Nucleophilic ^{18}F-fluorination afforded the novel 3-bromo-4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-21** in a high RCY within 10 min (90%, *decay-corrected*, **Scheme 2.11**).



2-30 **2-31** **2-32**

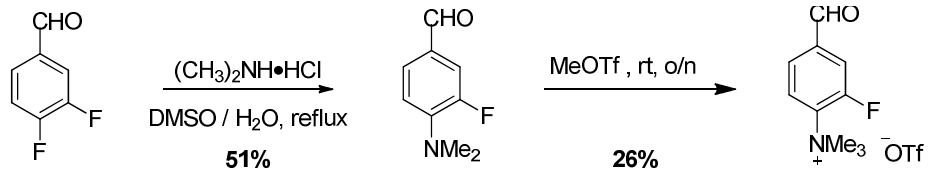
Scheme 2.10. Synthesis of precursor **2-32**.



2-32 **[^{18}F]2-21**

Scheme 2.11. Synthesis of **[^{18}F]2-21**

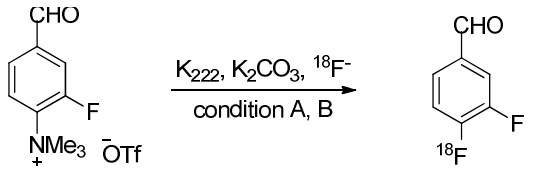
Precursor 2-fluoro-4-formyl-*N*,*N*,*N*-trimethylbenzenaminium triflate **2-34** was synthesised in an overall yield of 13% after two steps. The 3,4-difluorobenzaldehyde **2-23** was first refluxed with dimethylamine hydrochloride in an aqueous solution of DMSO/K_2CO_3 for 6 hours to give 3-fluoro-4-dimethylammoniumbenzaldehyde **2-33** (**Scheme 2.12**). The second step involves the nucleophilic reaction of **2-33** and methyl triflate to afford the trimethylammonium triflate salt **2-34** in 26% yield.



2-23 **2-33** **2-34**

Scheme 2.12. Synthesis of **2-34**

Initial studies on the synthesis of **[^{18}F]2-23**, performed in the SCINTOMICS synthesiser based on our routine ^{18}F-nucleophilic fluorination protocol, were not successful. The dehydrated ^{18}F-fluoride was heated with precursor **2-34** (10 mg in 400 μL of MeCN) at 120 ^{o}C for 10 min. The reaction afforded no target product **[^{18}F]2-23** (**Scheme 2.14**, condition A). We reasoned that the failure was due to the thermal instability of the fluorine substituted precursor **2-34**, which decomposes at 58 °C. In contrast, the melting point of its bromo analogue, 3-bromo-4-trimethylammoniumbenzaldehyde triflate **2-32** is 176 °C. Pleasingly, [^{18}F]-labelled 3,4-difluorobenzaldehyde **[^{18}F]2-23** was successfully obtained in a microreactor (SIEMENS) by heating the precursor **2-34** (2 mg in 40 μL of MeCN) at 180 ^{o}C for 4 min (condition B), in 73% RCY.[11] The dramatic decrease in reaction volume from 400 μL to 40 μL minimises the dilution of ^{18}F-fluoride and thus promotes the ^{18}F-fluorination. These results also illustrate how microfluidic batch devices are advantegeous for producing radiotracers that may not be accessible by "large-scale" synthetic modules. The specific activity of the radioactive building block was found to be 30-59 GBq/μmol (n = 3), *decay-corrected* to the end of synthesis (**Scheme 2.13**).



2-34 **[^{18}F]2-23**

Condition	2-42	Solvent	Equipment	Temperature	Time	RCY	*n*	Specific Activity
A	10 mg	MeCN, 400 μl	Heating	120 °C	10 min	0%	1	n/a
B	2 mg	MeCN, 40 μl	Microreactor	180 °C	4 min	**73%**	3	30 -59 GBq / μmol

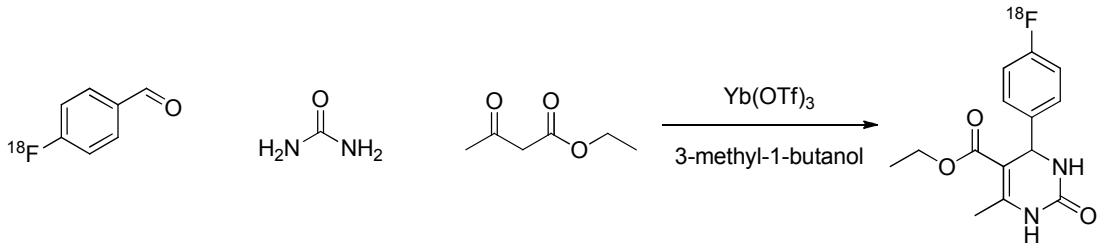
Scheme 2.13. Synthesis of **[^{18}F]2-23**.

[11] The fluorination was performed by Dr. R. Bejot in the microreactor.

2.2.5 Radio-Biginelli MCR

A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** (ca. 200 µl with a radioactivity of 5 – 15 MBq) in 3-methyl-1-butanol was loaded into a Reactivial® containing urea (3 mg, 50 µmol), ethyl acetoacetate (6 µl, 50 µmol) and ytterbium triflate (3 mg, 5 µmol). A variety of conditions for Biginelli condensation were tested (**Table 2.5**).

Table 2.5 Optimisation of Radio-Biginelli Reaction



[^{18}F]2-2 **[^{18}F]2-1**

Entry	Temp.[a] (ºC)	Time (min)	Urea (mg)	Yb(OTf)$_3$ (mg)	RCY[b] (%)
1	120	20	3	3	29
2	130	10	3	3	43
3	130	15	3	3	34
4	130	20	3	3	66 (*n*=3)
5	130	30	3	3	44
6	140	10	3	3	62
7	130	20	9	3	54
8	130	20	3	9	44
9	130	20	9	9	15
10	130[c]	20	3	3	44

[a]Conventional heating. [b]Decay corrected. Determined by radio-TLC. [c]Performed under microwave irradiation (50 W).

As shown in the radio-HPLC (**Figure 2.3**), the radioactive aldehyde component **[^{18}F]2-2** was completely consumed in 20 min (**Table 2.5**, Entry 4). HPLC analysis confirmed that the desired Biginelli-adduct **[^{18}F]2-1** was formed as the main radioactive compound in the reaction. An unidentified side product was also observed, which may plausibly be an intermediate in the reaction or a side product from a competing reaction.

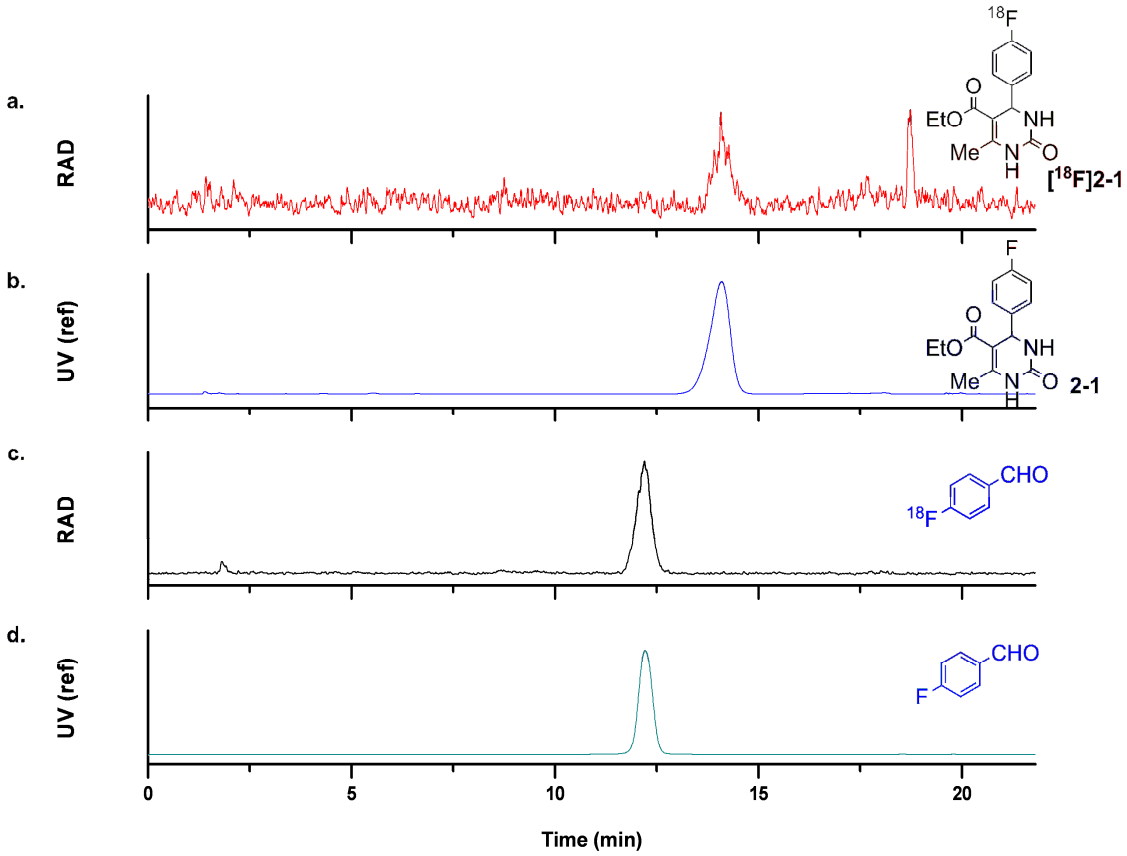


Figure 2.3 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Biginelli reaction (Entry 4); b. UV HPLC trace (254 nm) of "cold" **2-1** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]-fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde.

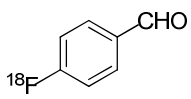
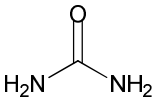
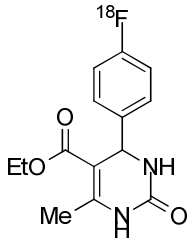
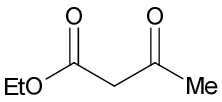
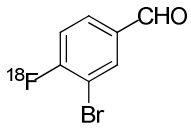
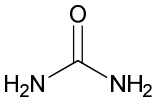
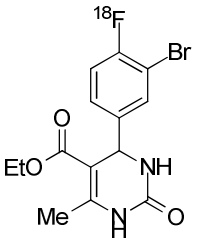
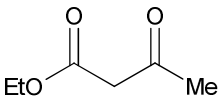
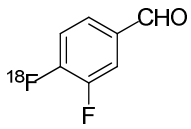
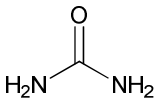
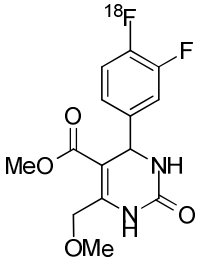
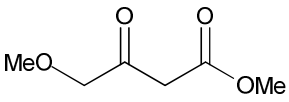
Conventional heating was found to be more efficient than heating under microwave irradiation, for reactions performed at the same temperature (**Table 2.5**, Entry 4 *vs* 10). We observed an increase in RCY from 29% to an average 66% when the temperature was increased from 120 °C to 130 °C (Entry 1 *vs* 4). The RCY cannot be improved by increasing the reaction temperature (to 140 °C, Entry 6) or using increased amounts of non-radioactive components (Entries 7, 8, 9 *vs* 4). The optimised conditions to access the ^{18}F-labelled dihydropyrimidinone **[^{18}F]2-1** therefore consist of heating a solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-1** in isoamyl alcohol (200 µL, 5 – 10 MBq) in a sealed vial containing urea (3 mg), $Yb(OTf)_3$ (3 mg) and ethyl acetoacetate (6 µL), for 20 minutes at 130 °C. The average RCY under these conditions was 66% (n = 3, Entry 4).

This protocol afforded the desired product, however, the specific activity of the ^{18}F-labelled Biginelli product **[^{18}F]2-1** cound not be measured directly. To quantify the specific activity of a labelled compound requires direct comparision of the radioactive peak and corresponding UV peak (**Section 1.4**). In the Biginelli reaction mixture, identification of the UV peak was difficult because it was overlapped by inseparable unidentified impurities. Since the Biginelli reaction does not involve [^{18}F]fluorine-carbon bond formation, it is reasonable to assume that the specific activity of **[^{18}F]2-1** remains unchanged from the corresponding decay corrected [^{18}F]-building blocks.

For the first time, we have demonstrated that multicomponent reactions are possible in the context of radiosynthesis. The scope of the radio-Biginelli reaction could be greatly expanded by varying the radioactive building blocks and nonradioactive building blocks.

The radioactive aldehyde components **[^{18}F]2-21** and **[^{18}F]2-23** were also engaged in a model Biginelli reaction, to synthesise the DHMP analogues **[^{18}F]2-22** and **[^{18}F]2-24** respectively, in moderate RCYs (38% for **[^{18}F]2-22** and 25% for **[^{18}F]2-24**, **Table 2.6**).

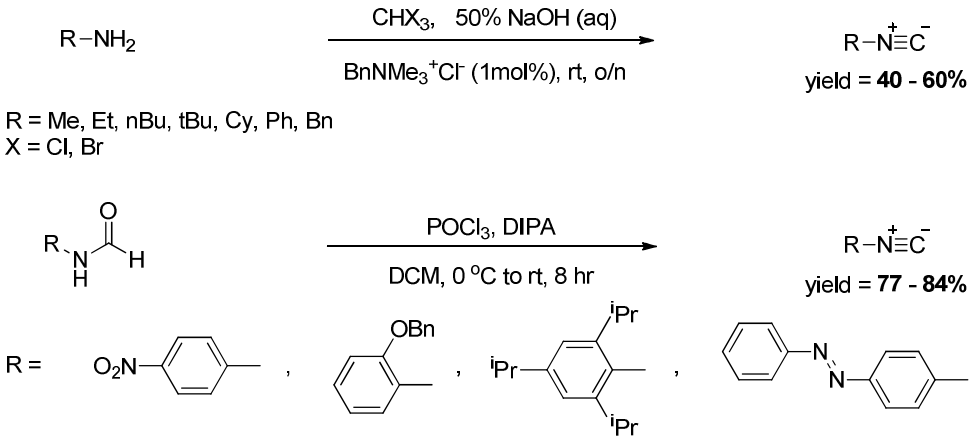
Table 2.6 Radio-Biginelli reaction

Entry	^{18}F-building block	non-radioactive reagent		^{18}F-labelled product	RCY (%)
1			3 mg		**66 (n = 3)**
		$Yb(OTf)_3$	3 mg		
	[^{18}F]2-2		6 μl	**[^{18}F]2-1**	
2			3 mg		**38 (n = 1)**
		$Yb(OTf)_3$	3 mg		
	[^{18}F]2-21		6 μl	**[^{18}F]2-22**	
3			3 mg		**25 (n = 3)**
		$Yb(OTf)_3$	3 mg		
	[^{18}F]2-23		6 μl	**[^{18}F]2-24**	

All radio-Biginelli reactions were performed by dispensing a solution of [^{18}F]-labelled benzaldehyde derivatives in 3-methyl-1-butanol or acetonitrile (200 μL, 5-15 MBq) into a sealed vial containing urea (3 mg), $Yb(OTf)_3$ (3 mg) and β-keto ester (6 μL). The mixture was heated under vigorous stirring at 130 ^{o}C for 20 minutes. Identities of labelled products were determined by radio-HPLC and the RCYs were determined by radio-TLC.

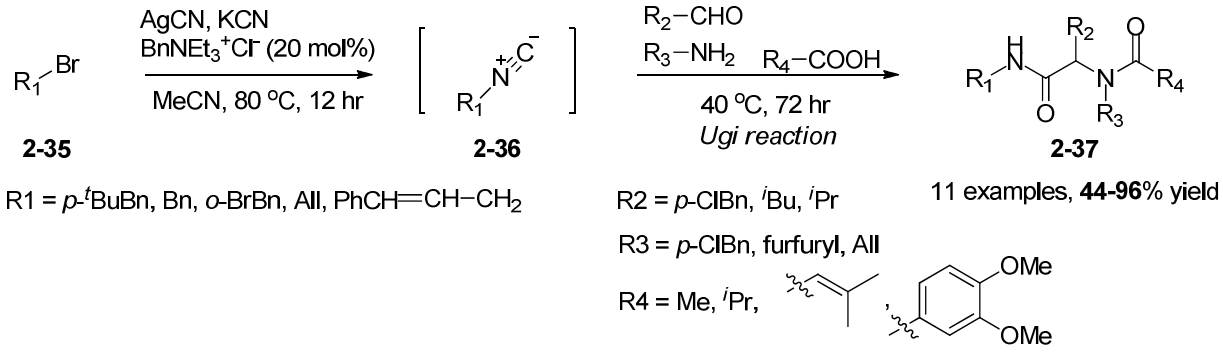
2.3 Isocyanide-based multicomponent reaction

Isocyanides can react with both nucleophiles and electrophiles at the same carbon centre. Conventional procedures for the synthesis of isocyanides involve the reaction of primary amines with dichlorocarbene[110] and dehydration of a *N*-formamide with phosphorus oxychloride (**Scheme 2.14**).[79, 111]



Scheme 2.14. Synthesis of isocyanide

Following early studies, [112, 113] El Kaim and Grimaud devised a process that involves the nucleophilic substitution of allylic or benzylic bromide derivatives **2-35** with silver cyanide and potassium cyanide in one pot to afford a range of benzylic and allylic isocyanides **2-36**.[114] The mechanism of isocyanide formation is not clear and the authors suggest that the reactive metal species involved is the silver complex $Ag(CN)_2^-NBn(Et)_3^+$. The conditions for isocyanide formation are compatible with the Ugi reaction and the *in situ* generated isocyanide **2-36** can be treated with aldehyde, amine, and carboxylic acid to give the Ugi products **2-37** in moderate to high yields (44-96%, **Scheme 2.15**).[115] Isocyanide-based MCRs (IMCRs) are particularly interesting subclasses of MCRs because they are very versatile in terms of accessible scaffolds.[78, 116, 117]

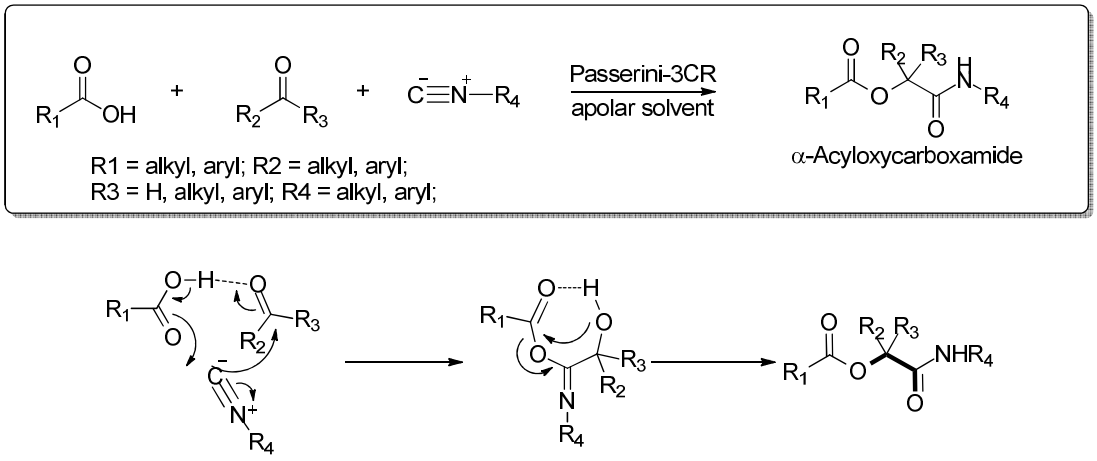


Scheme 2.15. "Isocyanide-free Ugi reaction" with isocyanides generated *in situ*

2.4 Development of radio-Passerini reaction

2.4.1 Passerini MCR

The classic multicomponent reaction between carboxylic acids, aldehydes or ketones and isocyanides to form α-acyloxycarboxamides is named after Passerini, who first reported this transformation in 1921.[118] Kinetic studies carried out in the Baker and Stanonis group showed that Passerini reactions occurred 4 times faster in tetrachloromethane than in ether, under the same reaction conditions.[119] Experimental results led to the well accepted non-ionic mechanism of the Passerini reaction as depicted in **Scheme 2.16**.[120] Hydrogen bonding is believed to play a crucial role, by activating the carbonyl towards nucleophilic attack of the isocyanide, in the formation of the presumed cyclic transition state for the reaction.



Scheme 2.16. A conventional mechanism of the Passerini Reaction

However, there are debates regarding the mechanism of the Passerini reaction. Uchiyama and Morokuma reported[121] a study on all the possible reaction pathways between the three reactants of the Passerini reaction, using quantum chemical methods. They applied the "AFIR (artificial force induced reaction)" approach[122] to find the transition states (TSs) and potential energy profile of the most preferable mechanistic scenario as presented in **Figure 2.4**.

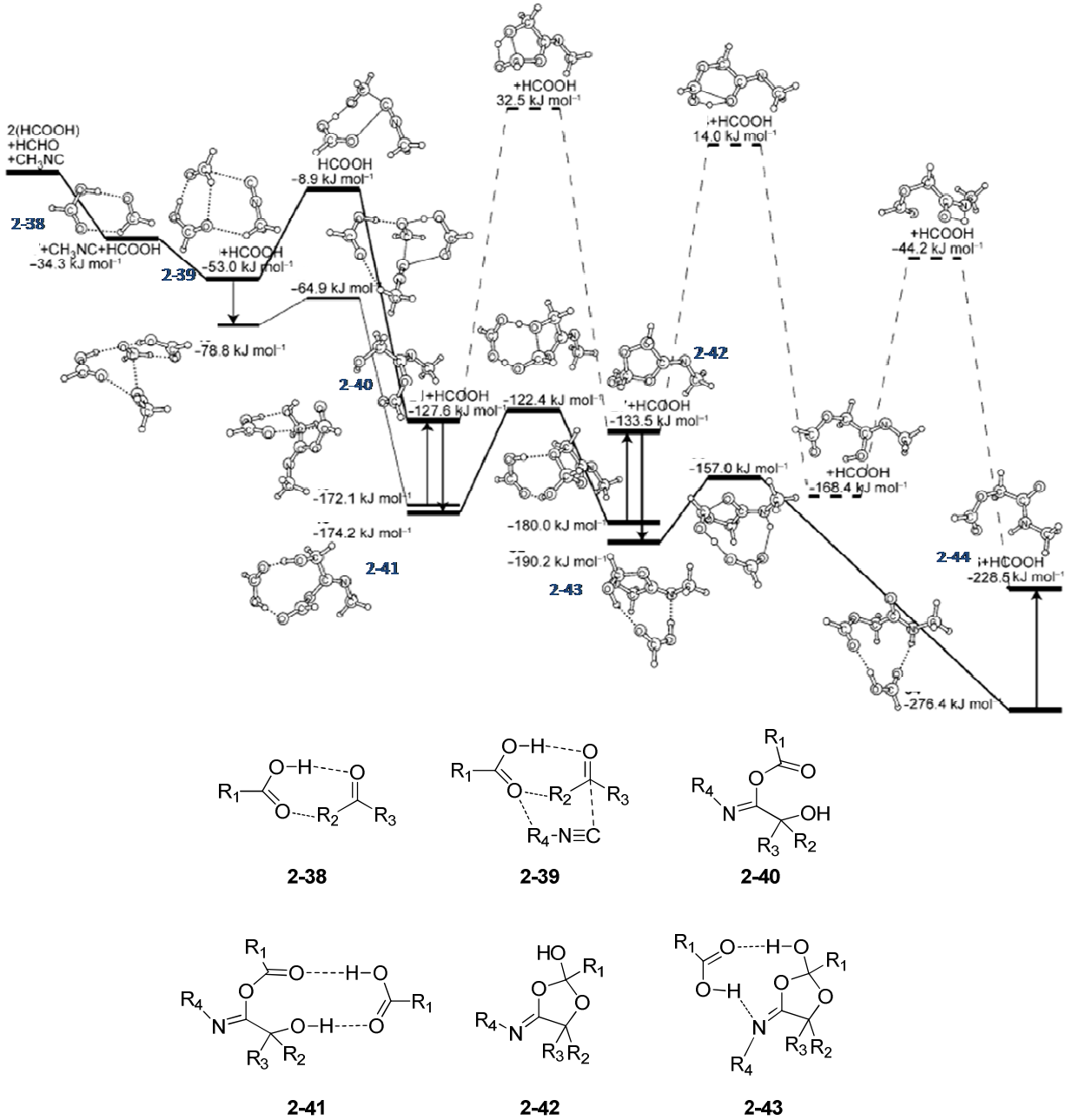


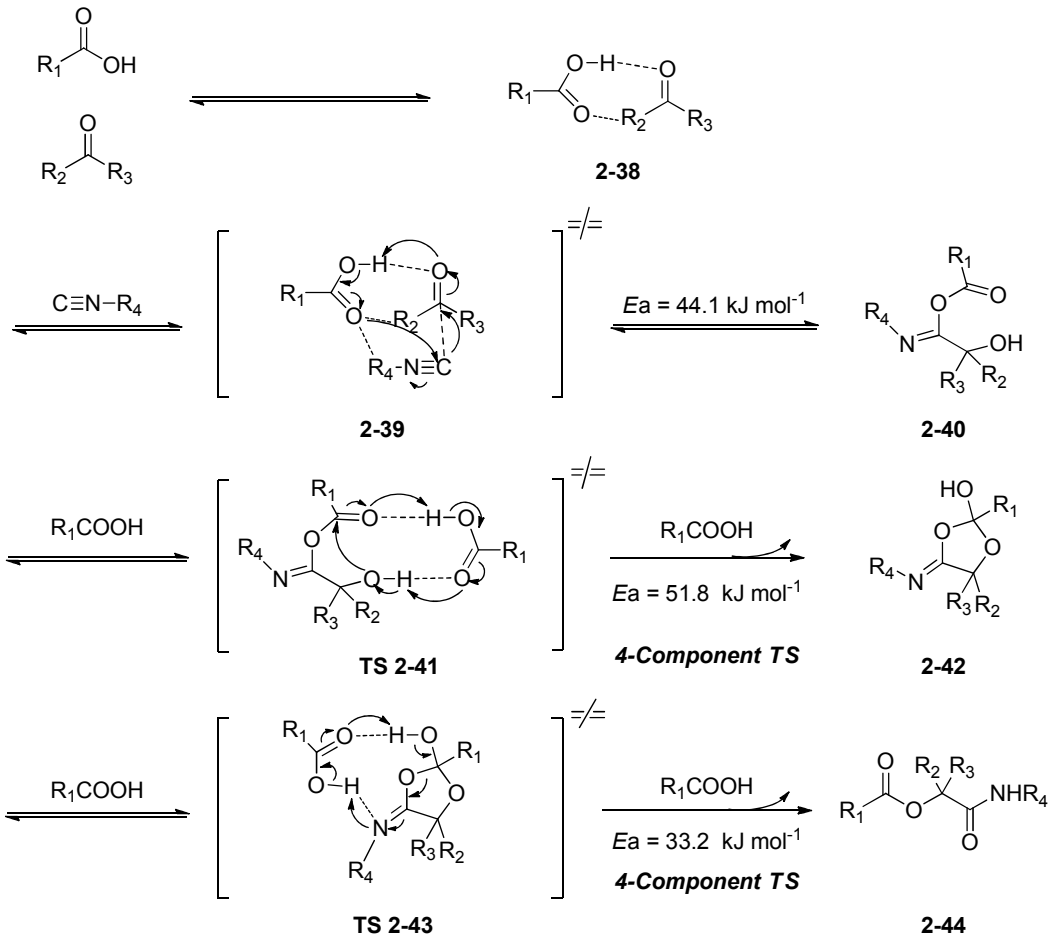
Figure 2.4 Potential profile of the most preferable pathway (solid line) leading to product **2-44** at the M06/6-31+G** level. This pathway involves four components. A minor three-component pathway is also shown (dashed line) for comparison. Energies (including ZPE corrections) are relative to the separated four reactant molecules. The most favourable route is emphasized with thick lines.(R_1 = H, R_2 = H, R_3 = H, R_4 = CH_3)[12]

The results showed that the direct transformation from **2-40** to the final product **2-44**, as suggested in the 3-component mechanism, was an unfavoured process because the first

[12] The figure is reprinted from reference 121, with permission from John Wiley and Sons.

rearrangement step from **2-40** → **2-42** has a very high barrier (160.1 kJmol^{-1}). With the participation of the extra component of HCOOH, the *Ea* value of the rearrangement from **2-41** → **2-42** is significantly lowered to 51.8 kJ mol^{-1}.

Based on these energy profiles, a 4-component pathway of the Passerini reaction was proposed. The first step of the reaction is the generation of an H-bonded cluster **2-38** which reacts with the isocyanide to produce intermediate **2-40** *via* transition state **TS 2-39** (**Scheme 2.17**).

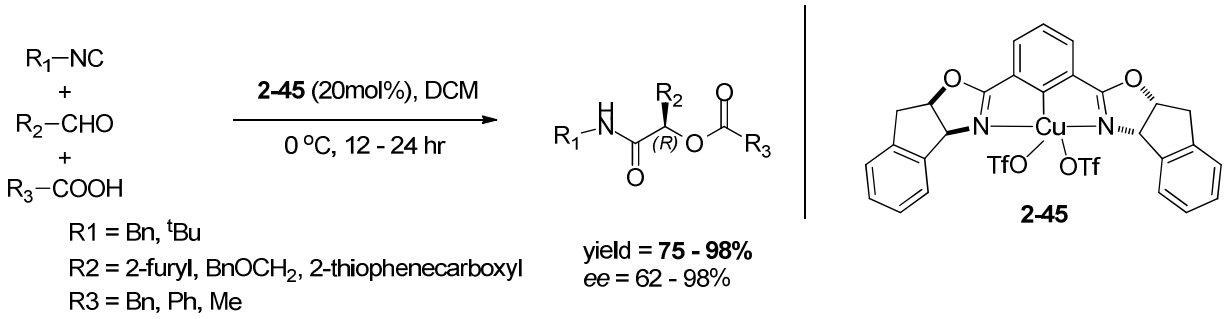


Scheme 2.17. Four-component Passerini reaction mechanism proposed by Uchiyama and Morokuma, TS = transition state, *E*a = activation energy, calculated based on the present set of reactants in gas-phase at the M06/6-31 + G** level.[13]

[13] The scheme is reprinted from reference 121, with permission from John Wiley and Sons.

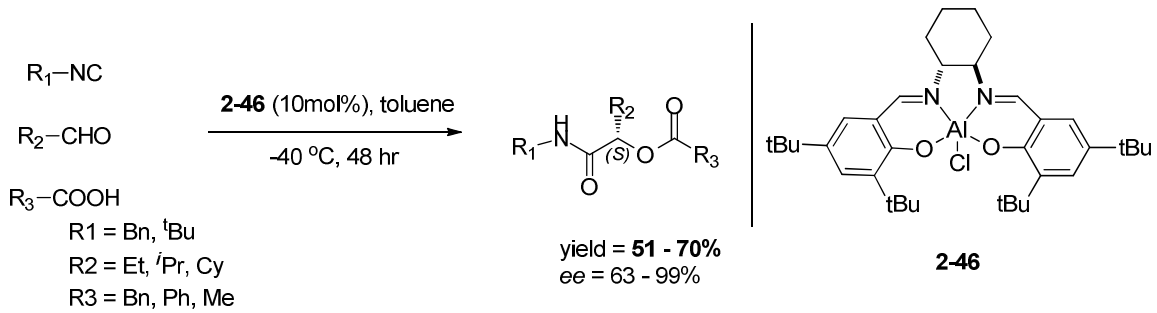
The extra carboxylic acid is involved in the rearrangement steps as a proton donor and acceptor. It plays a critical role in the formation of the final product **2-44**. Proton exchange involving the extra acid molecule occurs twice *via* **TS 2-41** and **TS 2-43**. The study suggested that the concentration of carboxylic acid may be crucial to the chemical outcome of the Passerini reaction. Noteworthly, in the context of a *n.c.a.* radio-MCR reaction, the radioactive component was present in picomolar range while the other components were present in micromolar amounts. In other words, the radioactive building block was sub-stoichiometric by a substantial margin. Therefore we postulate that a radio-Passerini reaction using ^{18}F-labelled benzoic acid as the radioactive component may have lower efficiency than with ^{18}F-labelled benzaldehyde. Reaction stoichiometry and the significant stoichiometric difference between the conventional and radiolabelling conditions should be taken into consideration in the selection of a *n.c.a* radioactive component for validation of the radio-MCR reaction.

An enantioselective Passerini reaction has been validated. The Schreiber group made use of a chiral Lewis acid, the indan-pybox-CuII (pybox = pyridinebis(oxazoline)) complex **2-45**, in Passerini MCRs with substrates capable of bidentate coordination, to control the configurational outcome of the reaction. [123] The reaction gave Passerini adducts (α-aceloxycarboxamides) in 75 – 98% yield and good enatioselectivities of 62 – 98% *ee* (**Scheme 2.18**).



Scheme 2.18. Cu^{II} complex **2-45** catalysed enantioselective Passerini reaction.

Zhu's reaction catalysed by a chiral salen-aluminum complex **2-46** extended the enantioselective Passerini MCR to broader scopes since it doesn't require a chelating aldehyde such as furan-2-carbaldehyde.[124] The catalytic Passerini reactions afforded the corresponding α-aceloxycarboxamides in 51 – 70% yield and good to excellent enantioselectivity (75 – 99% *ee*, **Scheme 2.19**). The authors suggest that the stereocontrol of this process originates from enantioselective α-addition of the isocyanide to the aldehyde, which is shielded on one face by the chiral catalyst through coordination.[125] The observed *S* enantioselectivity indicates that the isocyanide preferentially attacks the *Re* face of the aldehyde. Notably, a syringe pump is used to control the precise addition of the reagents.



Scheme 2.19. Al complex **2-46** catalysed enantioselective Passerini reaction.

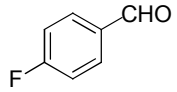
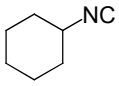
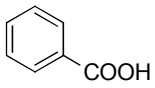
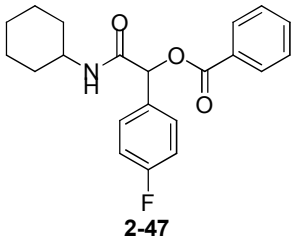
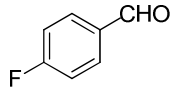
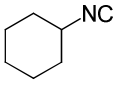
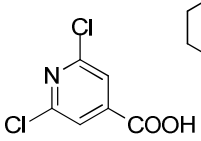
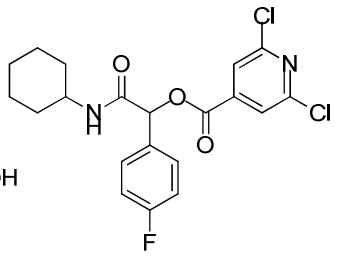
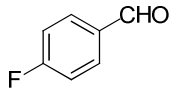
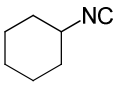
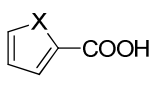
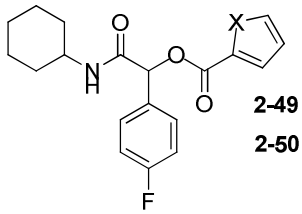
2.4.2 Passerini reaction with fluorinated building blocks

During the past two decades, many novel variations on the Passerini reaction have been reported. This facile synthetic method has delivered a large collection of structurally diverse α-acyloxycarboxamide scaffolds. However, there are not many examples of Passerini reactions with fluorinated building blocks. Therefore, we selected a series of fluorinated α-acyloxycarboxamides **2–47/50**, as prototypes to validate the compatibility of a fluorinated component in the MCR, and as references for radiolabelling experiments. These compounds **2–47/50** feature an aromatic ring without electron-withdrawing substituents and are therefore not responsive to S_NAr ^{18}F-fluorination. The fluorinated Passerini-adducts **2–47/50** are potentially accessible *via* direct ^{18}F-fluorinations on the appropriate iodonium salt precursors. However, the preparation of the required precursor, if possible, would be time-consuming and elaborate.

The Pirrung group reported that Passerini reactions were significantly accelerated (~280-fold over DCM) in the medium of concentrated aqueous lithium chloride (1.0 M to 2.5 M in water).[126] This acceleration was rationalised by the increased hydrophobic effect caused by ionic solutes. Fluorinated α-acyloxycarboxamides **2–47/50** were synthesised [14] by a Passerini reaction *via* a modified literature procedure.[126] A typical synthetic protocol involves the reaction of 4-fluorobenzaldehyde, cyclohexyl isocyanide and an acid in a 4.0 M aqueous solution of LiCl, at ambient temperature for 8 hours. Upon purification, all products **2–47/50** were afforded in good yields (63 – 98 %, **Table 2.7**).

[14] Compounds **2-47** to **2-50** were prepared by Dr. R. Leuma Yona.

Table 2.7 Passerini reaction with fluorinated component

Fluorinated Component	Non-fluorinated Component	Product	Yield
 **2-2**	 	 **2-47**	**98 %**
 **2-2**	 	 **2-48**	**63 %**
 **2-2**	  X = O X = S		**2-49** X = O **88 %** **2-50** X = S **70%**

All Passerini reactions were performed by stirring 4-fluorobenzaldehyde (1.0 mmol), carboxylic acid (1.2 mmol) and cyclohexyl isocyanide (1.0 mmol) in an aqueous solution of LiCl (4M, 5 mL) at 25 °C for 8 hours. The reaction was extracted by DCM and purified by column chromatography on silica gel.

2.4.3 Radio-Passerini reaction

Without isotopically labelled reagents, the Passerini reactions undertaken in aqueous lithium chloride (4M) solutions were found to be highly efficient. However, in the context of radio-Passerini reactions, an organic solvent is essential to allow for solid-phase extraction (S-PE) of the radioactive component.

Disappointingly, no Passerini reaction occurred when pure methanol was employed as a solvent (**Table 2.8**, Entry 1). After adding an aqueous solution of lithium chloride (4M, 600 μL) to the reaction in 100 μL of methanol, the ^{18}F-labelled Passerini-adduct **[^{18}F]2-57** was observed, albeit in only 5% RCY (**Table 2.8**, Entry 2). We postulated that the ratio by

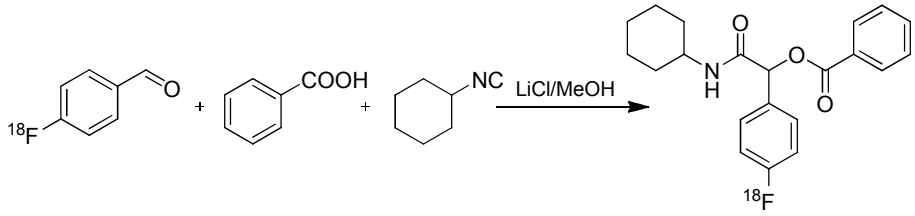
volume of LiCl aqueous solution to organic solvent was an important parameter to optimise for the radio-Passerini reaction. [15]

The efficiency of the radio-Passerini reaction is influenced by additional factors such as the concentration of the non-radioactive components, the temperature and the reaction time. Increasing the concentration of the non-radioactive components improved RCYs (Entry 7 *vs* 8). Upon prolonged heating of the reaction mixture, an ^{18}F-labelled side product appeared and led to a decrease of RCY (Entry 5 *vs* 10, Entry 8 *vs* 11 &12). Unfortunately, we were not able to identify the side product and we presumed it resulted from an undesired decomposition or a side reaction.

Methanol was found to be superior to ethanol in the radio-Passerini reaction (Entry 3 *vs* 8). Addition of acetonitrile (300 μL) to the reaction mixture after the radio-MCR is necessary to obtain an homogeneous reaction mixture for HPLC analysis. The optimised conditions to label the model Passerini compound **[^{18}F]2-47** involve heating a solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in methanol (5 – 15 MBq, 100 μL) with cyclohexyl isocyanide (4 μL) and benzoic acid (7 mg) in an aqueous solution of LiCl (4 M, 600 μL), for 30 minutes at 100 ^{o}C, to give an average radiochemical yield of 65% (n = 3, Entry 8).

[15] The 4-[^{18}F]fluorobenzaldehyde was prepared by Lei Li and radio-Passerini reactions were carried out by Dr. R. Leuma Yona and Lei Li.

Table 2.8 Optimisation of Radio-Passerini Reaction



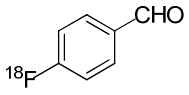
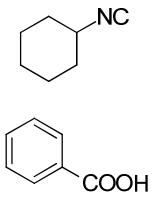
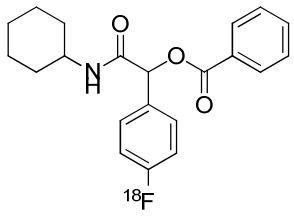
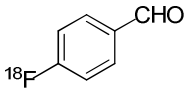
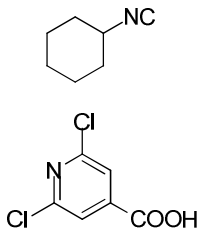
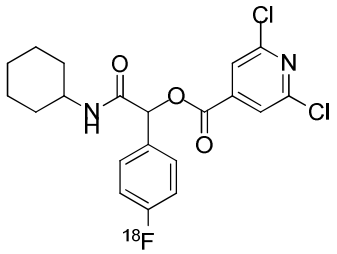
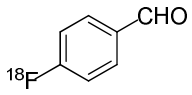
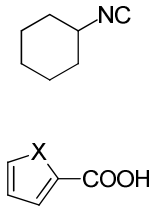
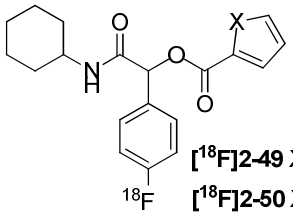
[^{18}F]2-2 [^{18}F]2-47

Entry	Temp. (°C)	Time (min)	Benzoic Acid (mg)	Cyclohexyl isocyanide (µL)	4M LiCl (aq.) (µL)	Methanol (µL)	RCY[b] [%]
1	80	30	7	4	0	500	0
2	80	30	7	4	600	100	5
3	100	30	7	4	600	100[a]	3
4	100	30	7	4	300	200	0
5	100	10	7	4	600	100	44
6	100	20	7	4	600	100	56
7	100	30	4	2	600	100	59
8	100	30	7	4	600	100	65 (n=3)
9	100	30	7	4	700	100	61
10	120	10	7	4	600	100	14
11	120	30	7	4	600	100	32
12	130	30	7	4	600	100	27

a.The organic solvent is ethanol instead of methanol; b. Decay-corrected RCY and determined by radio-TLC.

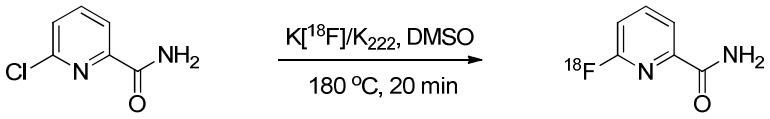
Additional radio-Passerini reactions were carried out under these optimised conditions. The introduction of various heterocyclic fragments into the α-acyloxycarboxamide scaffold was found to be possible, as illustrated with the radiosynthesis of **[^{18}F]2-47/50** in moderate RCYs ranging from 28% to 33% (**Table 2.9**).

Table 2.9 Radio-Passerini reactions

Radioactive Component	Non-radioactive reagent		Product	RCY
 **[^{18}F]2-2**		4 μl 7 mg	 **[^{18}F]2-47**	**65 % (n = 3)**
 **[^{18}F]2-2**		4 μl 7 mg	 **[^{18}F]2-48**	**33 % (n = 2)**
 **[^{18}F]2-2**		4 μl X = O 7 mg X = S 7 mg	 **[^{18}F]2-49** X = O **[^{18}F]2-50** X = S	**28 % (n = 2)** **32 % (n = 2)**

All radio-Passerini reactions were performed by dispensing a solution of **[^{18}F]2-2** in methanol (100 μL, 5-15 MBq) into a sealed vial containing cyclohexyl isocyanide (4 μL) and carboxylic acid (7 mg) in an aqueous solution of LiCl (4M, 600 μL). The reactions were heated at 100 °C for 30 minutes. Identities of labelled products were determined by radio-HPLC and RCYs were determined by radio-TLC.

The radiosynthesis of **[^{18}F]2-48** emphasised that the radio-MCR strategy could be used to synthesise an ^{18}F-labelled aromatic containing a 2,6-dichloropyridine functionality, which is labile under direct nucleophilic ^{18}F-fluorination conditions. Typically, a 2-chloropyridine derivative is used as the activated precursor in nucleophilic ^{18}F-substitution protocol to afford the 2-^{18}F-labelled pyridine functionality. The Langer group reported that 6-chloro-2-pyridinecarboxamide **2-51** could be efficiently converted to 6-[^{18}F]fluoro-2-pyridinecarboxamide **[^{18}F]2-52** in RCYs of 96 ± 2% (*n* = 4, **Scheme 2.20**).[127] The reaction occurs *via* a S_NAr mechanism.



2-51 **[^{18}F]2-52**

96 ± 2% RCY (*n* = 4)

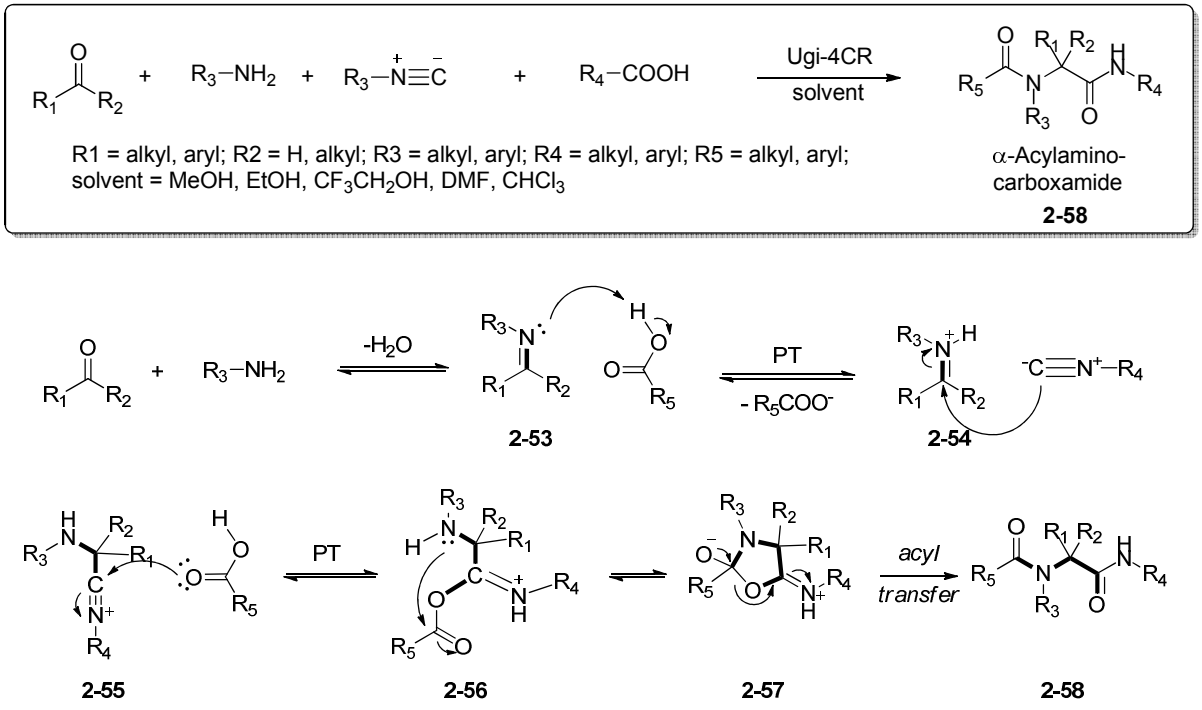
Scheme 2.20. Synthesis of 6-[^{18}F]fluoro-2-pyridinecarboxamide

2.5 Development of radio-Ugi reaction

2.5.1 Ugi-MCR

The Ugi reaction refers to "the four-component reaction of isocyanides in the presence of an amine, aldehyde or ketone and a nucleophile to provide a single condensation product". [83, 128] The reaction stands out as one of the most performed IMCRs in pharmaceutical industry today.[78]

The first step of the Ugi reaction involves the formation of an imine **2-53** *via* the condensation of the amine and aldehyde. The protonated iminium **2-54** subsequently reacts with the isocyanide and the resulting intermediate **2-55** is attacked by the carboxylic acid to afford the α-adduct **2-56**. The intramolecular acyl transfer reaction of **2-56** affords the α-acylaminocarboxamide **2-58** as the final product (**Scheme 2.21**). The mechanistic pathway of Ugi reaction consists of a series of elementary steps occurring in linear and parallel sequences. The identity of the rate-determining step remains unknown.[129]

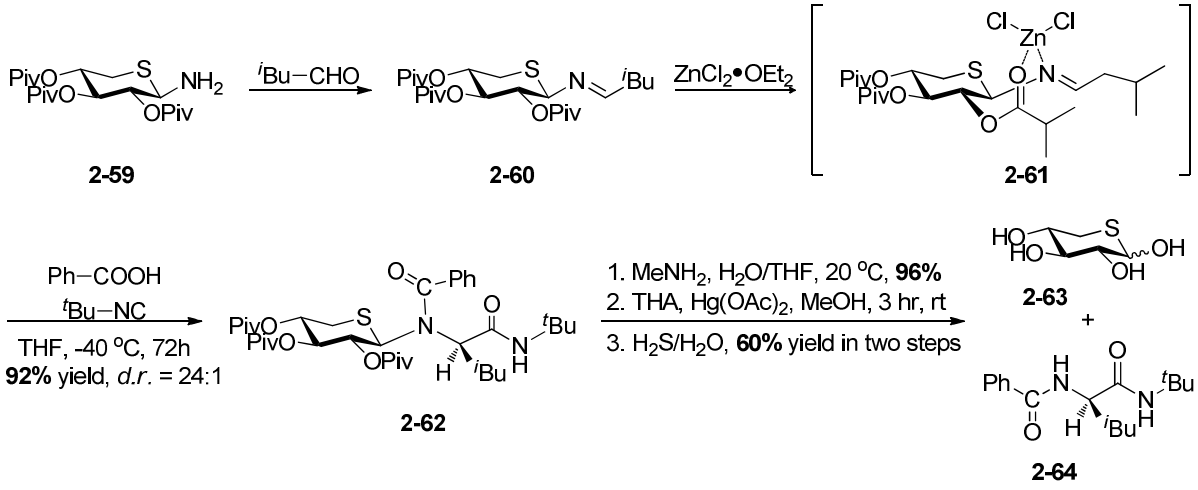


Scheme 2.21. Mechanism of the Ugi Reaction

In the absence of stereoinduction, the Ugi reaction affords racemic products. Although asymmetric Passerini reactions catalysed by chiral Lewis acids have been demonstrated (**Section 2.4.1**), these catalysts usually show little to no effect on the Ugi reaction.[130] The traditional approach to induce stereocontrol in an Ugi reaction is to use chiral starting materials. Ugi found that stereocontrol of an Ugi reaction could be achieved by using a chiral amine, whereas other starting materials gave poor or no induction.[78]

Ugi presented a sugar derived auxiliary, the xylopyranose derived peracylated thiosugar **2-59** which contains an amine functionality at the anomeric position.[131] The key transformation of the Ugi reaction involves the condensation between the imine **2-60**, *tert*-butyl isocyanide and benzoic acid at -40 ^{o}C for 72 hours. The stereodetermining step is believed to be the *a*-addition of the isocyanide to the prochiral iminium functionality. Zinc chloride was added to conformationally lock the intermediate **2-61**, allowing for the *Re*-face of the imino group to be shielded towards subsequent attack from the isocyanide. The Ugi-

adduct **2-62** was obtained with good stereoselectivity (diastereomeric ratio *d.r.* = 24:1) and 92% yield. Moreover, the auxiliary could be cleaved under mild acidic conditions using a dilute methanolic solution of trifluoroacetic acid in the presence of $Hg(OAc)_2$ at room temperature, to give the *R*-amino acid **2-64** in 60% yield (**Scheme 2.22**).

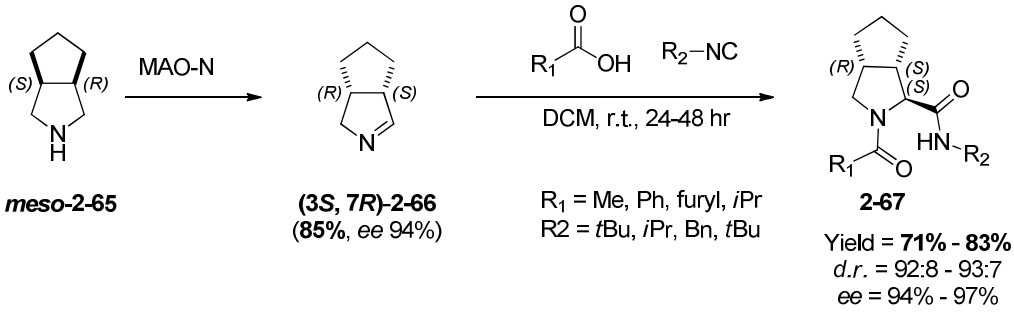


Scheme 2.22. Stereoselective Ugi reaction with **2-59**

Catalytic asymmetric methods to control the stereochemical outcome of the Ugi reaction are currently not available, presumably because the reaction is essentially uncatalysed.[80] To generate an optically pure MCR component and combine the desymmetrisation step with an Ugi MCR could be a possible solution. It has been reported[132] that the monoamine oxidase N (MAO-N) can bio-catalytically desymmetrise 3,4-substituted *meso*-pyrrolidines to 1-pyrrolines in excellent yields and *ee* values. As imines are intermediates for many common multicomponent reactions, Orru proposed that the use of these optically active 1-pyrrolines in a Ugi MCR would be able to induce diastereoselectivity during the α-addition of isocyanides to the imine.[133] The enantiopure cyclic imine **(3*S*,7*R*)-2-74** was prepared by MAO-N-catalysed desymmetrisation of the *meso*-pyrrolidine **2-73** in 84% yield and 94% *ee* (**Scheme 2.23**). The imine **(3*S*,7*R*)-2-74** was reacted with a range of carboxylic acids and

isocyanides to afford substituted prolyl peptides **2-75** in good yield (71%–83%) and *d.r.* (92:8 to 93:7) with very good *ee* (94%–97%).

Crystallographic analysis was performed to determine the absolute configurations of the products. During a typical Ugi reaction, a stereocenter is formed in the α-addition of the isocyanide to the iminium. The 2,3-*trans* relationship found in the final product **2-67** suggested that the isocyanide attacks predominantly the sterically less hindered *Re* face of the iminium. The method has been applied to synthesise a series of polycyclic alkaloid-type compounds[134], and drug candidates[135] (e.g. hepatitis C virus NS3 protease inhibitors telaprevir) containing this substructure.



Scheme 2.23. Oxidative desymmetrisation of *meso*-pyrrolidines ***meso*-2-65** by mono-amine oxidase N (MAO-N) and subsequent Ugi-type MCR.

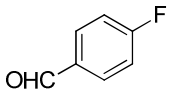
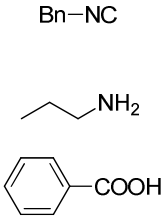
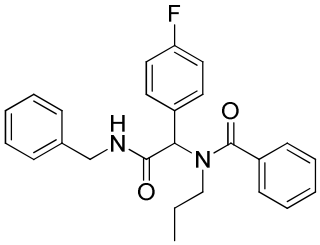
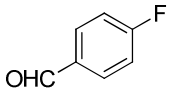
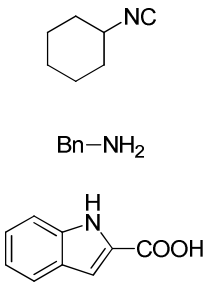
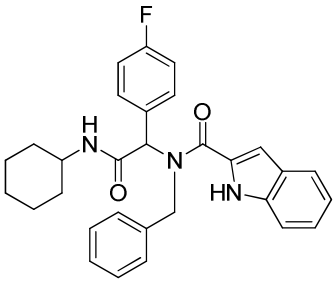
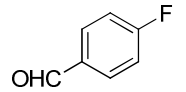
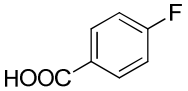
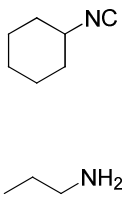
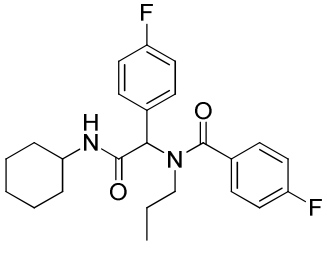
2.5.2 Ugi reaction with fluorinated building blocks

The Ugi reaction is known to be compatible with a variety of functional groups. We selected the fluorinated α-aminoacylamines **2-68/69** and **2-71** as model compounds for two reasons. Firstly, these Ugi-adducts are not obvious substrates for direct nucleophilic ^{18}F-fluorination and thus are ideal candidates to demonstrate the advantage of the proposed radio-Ugi reaction. Secondly, the relevant fluorinated or non-fluorinated components are commercially available and widely applied in the Ugi reaction. We hoped that the difluorinated bis-amide **2-71**, potentially obtained by an Ugi MCR of 4-fluorobenzaldehyde, 4-fluorobenzoic acid,

an amine and an isocyanide, could be used to validate the idea of introducing a radioisotope in different positions of the compound without changing the radiosynthetic strategy.

Ugi reactions[136] were performed to give novel dipeptide-type compounds **2-68/69** and **2-71**, in high yields ranging from 61% to 82%. A typical synthetic process involves dissolving all of the components (0.5 mmol of each) in ethanol (1 mL), and stirring at ambient temperature for 48 hours. Target products can be purified by flash column chromatography on silica gel or *via* recrystallisation (**Table 2.10**).

Table 2.10 Ugi reaction with fluorinated component

Fluorinated Component	Non-fluorinated Component	Product	Yield
 **2-2**		 **2-68**	**78%**
 **2-2**		 **2-69**	**82%**
 **2-2**  **2-70**		 **2-71**	**61%**

All Ugi reactions were performed by stirring 4-fluorobenzaldehyde (0.5 mmol), isocyanide (0.5 mmol), amine (0.5 mmol) and carboxylic acid (0.5 mmol) in a solution of ethanol (1 mL) for 48 hours at 25 °C. The residue was purified by column chromatography on silica gel to afford pure product.

2.5.3 Radio-Ugi reaction

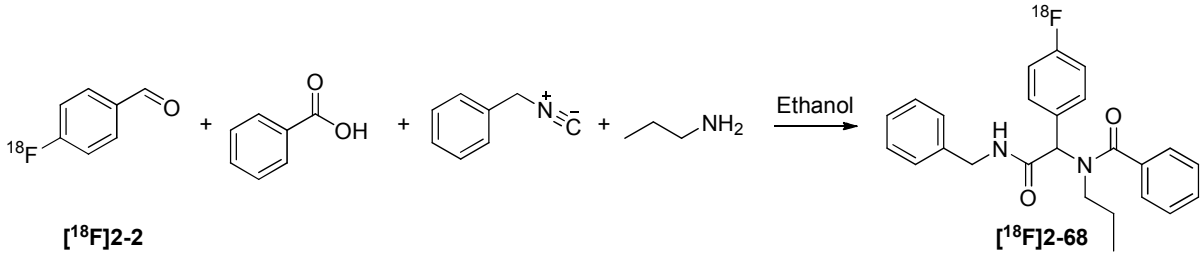
All elementary steps of the Ugi reaction are in equilibrium except for the last step, in which the intramolecular acylation and subsequent hydroxylamine to amide rearrangement (Mumm rearrangement) favours exclusively the Ugi product. The driving force of the overall reaction sequence is the oxidation of the isocyanide to the amide and the reaction rate is determined by the reactivity profile of each component, the solvent and the concentration.[137]

Therefore, to validate the radio-Ugi reaction with 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** as the radioactive building block, efforts must be devoted to shorten the reaction time from 48 hours to a reasonable time scale suitable for ^{18}F radiolabelling. A solution of **[^{18}F]2-2** in ethanol (200 μL, 5-15 MBq) was dispensed into a sealed vial containing benzoic acid, benzyl isocyanide and 1-propylamine. The mixture was heated and stirred before being allowed to cool to room temperature and analysed by radio-TLC. The results for our optimisation studies are shown in **Table 2.11.**

Extending the reaction time from 10 min to 20 min led to a limited increase in RCY from 5% to 10% (**Table 2.11**, Entry 1 *vs* 2). Moreover, a slight decrease in RCY from 10% to 8% was observed during prolonged heating for 30 min (Entry 2 *vs* 3), presumably due to undesired decomposition of the labelled product and/or emergence of side reactions. Increasing the temperature above 100 ^{o}C did not accelerate the reaction (Entry 3 *vs* 4). Pleasingly, significant improvements in radiochemical yield were observed when the concentrations of non-radioactive components were increased by 5-fold (Entry 4 *vs* 6 and Entry 3 *vs* 7). Optimised radiosynthesis of the ^{18}F-labelled α-aminoacylamide **[^{18}F]2-68** could be achieved by heating a solution of 4-^{18}F-fluorobenzaldehyde (5 – 15 MBq, 200 μL in EtOH) with cyclohexyl isocyanide (6 μL), 1-propylamine (4 μL) and 4-fluorobenzoic acid

(8 mg), for 30 minutes at 100 °C, to give an average radiochemical yield of 62% (*n* = 3, Entry 6).

Table 2.11 Optimisation of Radio-Ugi Reaction

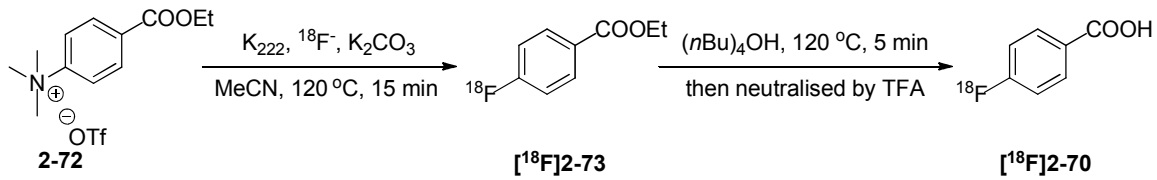


[^{18}F]2-2 [^{18}F]2-68

Entry	Temp.[a] (°C)	Time (min)	Benzoic Acid (mg)	Benzyl isocyanide (µL)	1-Propyl amine (µL)	Ethanol (µL)	RCY[b] (%)
1	120	10	3	3	2	500	5
2	120	20	3	3	2	500	10
3	120	30	3	3	2	500	8
4	100	30	3	3	2	500	7
5	130	10	3	3	2	500	5
6	100	30	6	6	4	200	62 (*n*=3)
7	120	30	6	6	4	200	59

[a]Conventional heating. [b]Decay corrected. Determined by radio-TLC.

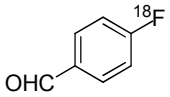
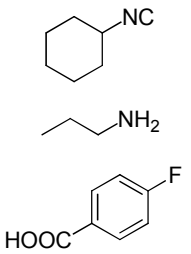
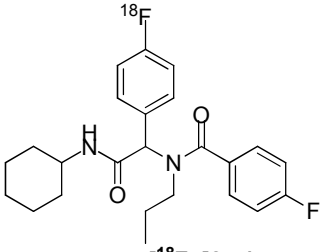
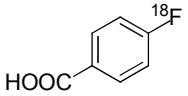
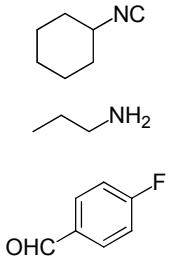
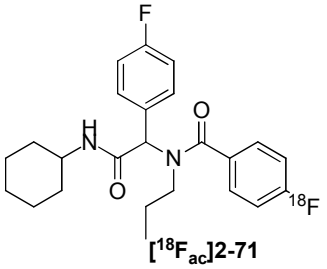
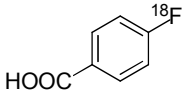
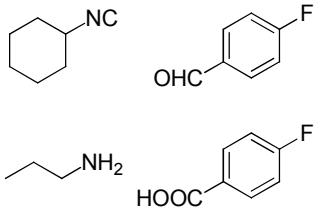
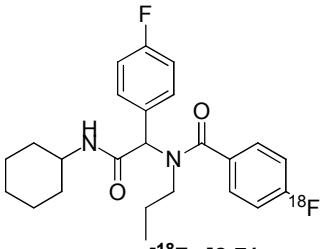
In principle, this labelling method offers the possibility to introduce the radioactive isotope at an alternative position of the Ugi-adduct, by using the corresponding ^{18}F-component instead of revising the radiosynthetic route. To compare the synthetic efficiency of a radio-MCR with two different ^{18}F-components, the difluorinated bis-amide **2-71** was chose as the prototype. The radioactive component 4-[^{18}F]fluorobenzoic acid **[^{18}F]2-70** was synthesised by a nucleophilic aromatic ^{18}F-substitution on the benzoic ester precursor **2-72** followed by hydrolysis of the ester group under basic conditions. **[^{18}F]2-70** was synthesised in 56% (*n* = 2) RCY over two steps (**Scheme 2.24**).



Scheme 2.24. Radiosynthesis of 4-[^{18}F]fluorobenzoic acid **[^{18}F]2-70**

With 4-[^{18}F]fluorobenzaldehyde as the radioactive component, ^{18}F-labelled α-aminoacylamide **[^{18}F]2-71** was afforded in a 56% RCY under the optimised conditions as mentioned below (**Table 2.12**, Entry 1). When a solution of 4-[^{18}F]fluorobenzoic acid, **[^{18}F]2-70** (5 – 15 MBq in 200 μL of ethanol), was heated with 4-fluorobenzaldehyde (5 μL), 1-propylamine (4 μL) and cyclohexyl isocyanide (6 μL) at 100 °C for 30 min, no reaction occurred (**Table 2.12**, Entry 1 *vs* 2) and only the unreacted 4-[^{18}F]fluorobenzoic acid **[^{18}F]2-70** was observed in the radio HPLC. An extra 8 milligram of "cold" 4-fluorobenzoic acid **2-70** was added to the reaction and heated for 30 min at 100 °C. Pleasingly, the "*carrier-added*" radio-Ugi reaction gave the ^{18}F-labelled α-aminoacyl amide **[^{18}F]2-71** in 10% RCY (n = 2, Entry 3).

Table 2.12 Radiosynthesis of [^{18}F]2-71 *via* Radio-Ugi reactions

Entry	Radioactive Building Block	Nonradioactive reagent	Product	RCY[a]
1	 **[^{18}F]2-2**		 **[$^{18}F_{al}$]2-71**	**56%** (n = 2)
2	 **[^{18}F]2-70**		 **[$^{18}F_{ac}$]2-71**	**0%**
3	 **[^{18}F]2-70**		 **[$^{18}F_{ac}$]2-71**	**10%** (n = 2)

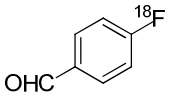
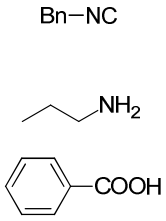
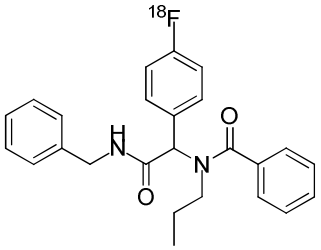
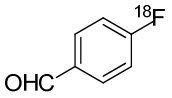
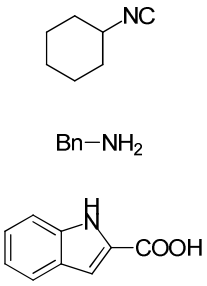
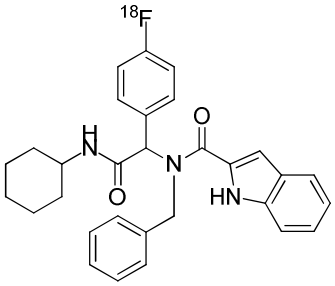
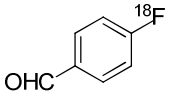
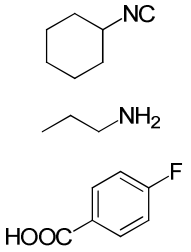
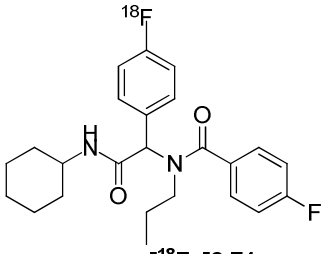
All radio-Ugi reactions were performed by dispensing a solution of **[^{18}F]2-2** or **[^{18}F]2-70** in ethanol (200 μL, 5-15 MBq) into a sealed vial containing cyclohexyl isocyanide (6 μL), 1-propylamine (4 μL), 4-fluorobenzoic acid (8 mg for Entries 1 and 3) and 4-fluorobenzaldehyde (5 μL for Entries 2 and 3). The reactions were heated at 100 ^{o}C for 30 minutes. Identities of labelled products were determined by radio-HPLC and RCYs were determined by radio-TLC.

Although the Ugi-adduct **[^{18}F]2-71** can be accessed by selecting the ^{18}F-labelled aldehyde or carboxylic acid as the radioactive component, our preliminary work revealed that all possible combinations will not necessarily be of comparable efficiency. The additional "cold" 4-fluorobenzoic acid acts as an isotopic carrier and thus reduces the specific activity of the labelled product. This result indicates that the concentration of certain components is crucial to radio-MCRs and choice of radioactive building block plays a vital role in the application of the methodology. Given that a second molecule of carboxylic acid is required

as the "extra" component, according to the recent proposed "four-component" mechanism of the Passerini reaction (**Section 2.4.1**), we hypothesise that a reasonable concentration of carboxylic acid (i.e. other than *n.c.a.* [^{18}F]fluorobenzoic acid) is required for the Ugi reaction to proceed.

The optimised conditions for the Radio-Ugi reaction were then applied to the synthesis of the ^{18}F-labelled aromatic compound **[^{18}F]2-69** in a 50% RCY. The result indicated that the radio-Ugi reaction is compatible with indole functionality. The synthesis of ^{18}F-labelled α-aminoacyl amides (bis-amides) using the radio-Ugi reaction as the key transformation are summarised in **Table 2.13**.

Table 2.13 Radio-Ugi reactions

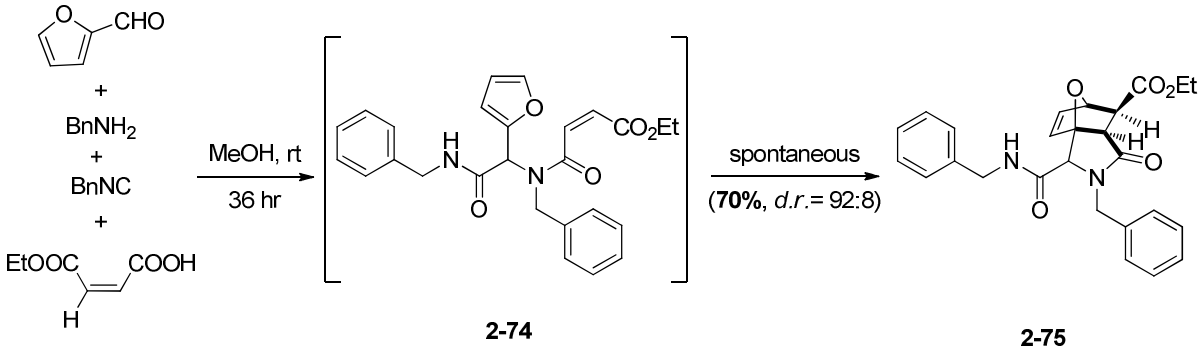
Entry	Radioactive Building Block	Nonradioactive reagent	Product	RCY
1			 **[^{18}F]2-68**	**62%** (n = 3)
2			 **[^{18}F]2-69**	**50%** (n = 3)
3			 **[$^{18}F_{al}$]2-71**	**56%** (n = 2)

All radio-Ugi reactions were performed by dispensing a solution of **[^{18}F]2-2** in ethanol (200 μL, 5-15 MBq) into a sealed vial containing carboxylic acid (6–8 μL), isocyanide (6 μL) and amine (4–6 μL) components. The reactions were heated at 100 °C for 30 minutes. Identities of labelled products were determined by radio-HPLC and RCYs were determined by radio-TLC.

The product obtained from an Ugi condensation consists of a bis-amide skeleton and thereby delivers peptidomimetics with potential pharmaceutical applications. This radio-Ugi reaction greatly expands the scope and availability of radiochemistry for ^{18}F-labelled aromatics and can potentially be applied to the generation of libraries of ^{18}F-labelled α-aminoacylamides for screening purposes.

In addition to the formation of α-aminoacylamide derivatives, Ugi reactions have been used, in combination with a series of cyclisations, to access more complex poly-cyclic

compounds. The Paulvannan group reported[138] the formation of a tricyclic lactam **2-75**, initialised by the Ugi condensation of furaldehyde, benzylamine, benzyl isocyanide and (*Z*)-4-ethoxy-4-oxobut-2-enoic acid. Upon formation of the *a*-acylamino amide **2-74**, it spontaneously undergoes the intramolecular Diels-Alder reaction to afford the final tricyclic product **2-75** in 70% yield and good diastereoselectivity (*d.r.* = 92 : 8, **Scheme 2.25**).



Scheme 2.25. Ugi/Diels-Alder reaction

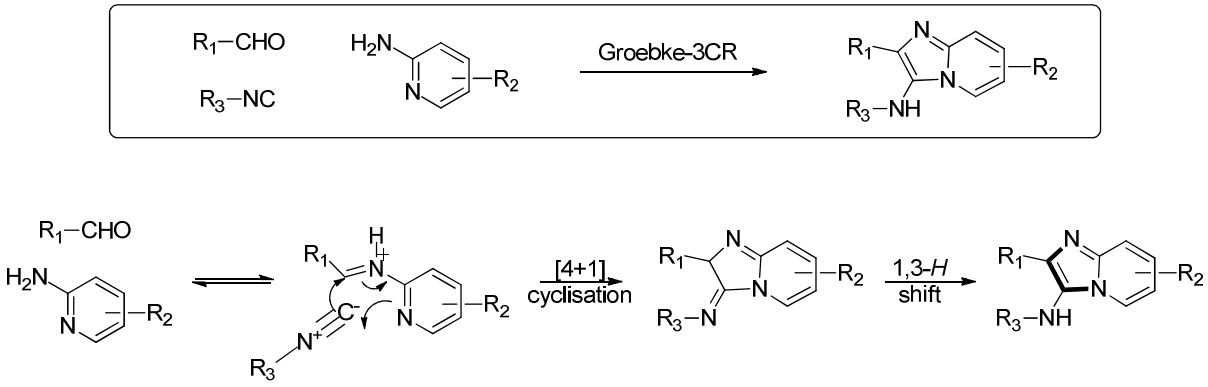
Therefore, we proposed that in addition to the synthesis of ^{18}F-labelled α-aminoacyl amides, the radio-Ugi reaction could be extended to a "radio-Ugi/Diels-Alder reaction" and deliver ^{18}F-labelled poly-cyclic scaffolds, upon careful selection of the starting components.

2.6 Development of radio-Groebke reaction

2.6.1 Groebke MCR

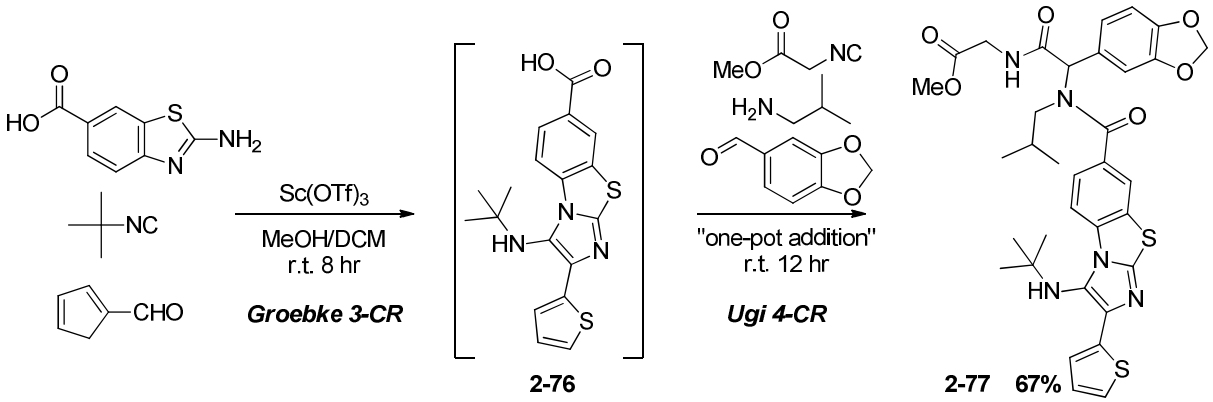
The Groebke reaction, a new variant of the Ugi-4CR, was discovered by variation of a large number of isocyanides, aldehydes, carboxylic acids and amines in the Ugi reaction. It was found that when a heteroarene (e.g. 2-aminopyridine) with an H_2N–C=N group was reacted with an aldehyde and an isocyanide, the Ugi-type condensation yielded the imidazo[1,2-*a*]pyridine scaffold.

First reported in 1998 by three independent groups, the Groebke-Bienaymé-Blackburn reaction[139-141] has received substantial attention in drug discovery and the products have been found in pharmaceutical investigations across a wide range of therapeutic areas.[142] The mechanism[143] involves the formation of an imine by condensation between a heterocyclic aromatic 2-aminopyridine and an aldehyde, in a similar manner to the Ugi reaction. The protonated Schiff base intermediate, containing both nucleophilic and electrophilic centers, then undergoes a [4+1] cyclisation with the isocyanide carbon to form a bicyclic adduct, followed by rearomatisation *via* a 1,3-*H* shift to give the final product as a imidazol[1,2-*a*]pyridine. (**Scheme 2.26**)



Scheme 2.26. Groebke Reaction

An interesting feature of the Groebke reaction is that it directly affords pharmaceutically relevant heterocyclic products. Al-Tel combined a Groebke reaction with an Ugi reaction to synthesise an impressive range of polyfunctionalised heterocycles.[144] For example, **2-76,** containing the benzo[*d*]imidazo[2,1-*b*]thiazole core, could be generated under typical Groebke conditions and subsequently react as the carboxylic component in a Ugi reaction with a concomitant addition of the isocyanide, amine and aldehyle. This one-pot six-component Groebke/Ugi reaction gave the highly complex product **2-77** in a 67% yield (**Scheme 2.27**).



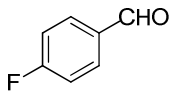
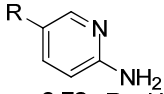
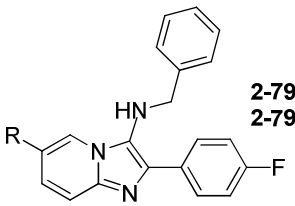
Scheme 2.27. Combination of Groebke 3-CR and Ugi 4-CR for the synthesis of **2-77**.

2.6.2 Groebke reaction with fluorinated building blocks

With the aim of illustrating the feasibility of this radio-MCR strategy to access ^{18}F-labelled imidazo[1,2-*a*]pyridine, Groebke-adducts **2–79a/b** [16] were prepared as "cold" reference compounds (**Table 2.14**). A typical synthetic procedure involves addition of benzyl isocyanide (0.58 mmol) and scandium(III) trifluoromethane-sulfonate ($Sc(OTf)_3$, 25 μmol, 5 mol%) to a solution of 4-fluorobenzaldehyde (0.75 mmol) and 2-aminopyridine **2-78a/b** (0.5 mmol) in methanol (1 mL), and stirring at ambient temperature for 24 hours. Target products were obtained in high yields after aqueous work-up and recrystallisation.

[16] Compounds **2-79a/b** were prepared by Mr. M. Hopkinson.

Table 2.14 Groebke reaction with fluorinated component

Fluorinated Component	Non-fluorinated Component	Product	Yield
 **2-2**	 **2-78a** R = H **2-78b** R = Cl		**2-79a** R = H 73 % **2-79b** R = Cl 62 %

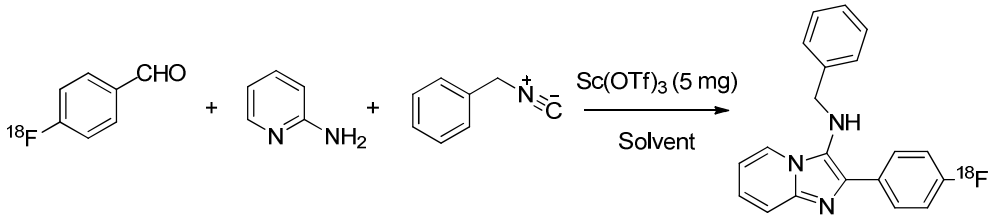
All Groebke reactions were performed by adding benzyl isocyanide (0.58 mmol) to a solution of 4-fluorobenzaldehyde (0.75 mmol) and 2-aminopyridine (0.5 mmol) in methanol (1 mL). $Sc(OTf)_3$ (25 µmol, 5 mol%) was then added and the reaction was stirred at 25 °C for 24 hours. Pure product was afforded by recrystallisation.

2.6.3 Radio-Groebke reaction

Initial studies of radio-Groebke reactions used a solution of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**, 5 – 15 MBq in isoamyl alcohol or acetonitrile), 2-aminopyridine (3 mg) and benzylisocyanide (3 µL). [17] The reactions were performed under different temperatures ranging from 100 °C to 170 °C and the synthetic outcome indicated that high temperature and protic solvents were beneficial for the condensation. By changing the solvent from acetonitrile to 3-methyl-1-butanol and increasing the reaction temperature from 100 °C to 150 °C, the RCY can be improved from 3% to 73% (**Table 2.15**, Entry 1 *vs* 4). Furthermore, prior addition of the benzyl isocyanide to 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** led to a slight decrease in RCY from 73% to 64% (Entry 4 *vs* 5). Interestingly, the sequence addition of the component did not show any influence on other Radio-MCRs according to our preliminary results. Microwave irradiation had an accelerating effect on the reaction by shortening the reaction time from 30 min to 15 min in similar RCYs (Entry 6 *vs* 7). However, the highest efficiency for the synthesis of **[^{18}F]2-79a** was obtained by adding a solution of 4-^{18}F-

fluorobenzaldehyde (5 – 15 MBq, 200 μL in isoamyl alcohol) to a vial containing 2-aminopyridine (3 mg) and scandium(III) triflate ($Sc(OTf)_3$, 3 mg). A solution of benzyl isocyanide (3 μL in 300 μL of isoamyl alcohol) was then added *via* syringe. The reaction was heated at 170 °C for 15 min to give an average RCY of 85% (n = 4, Entry 8). Similarly, **[^{18}F]2-79b** was synthesised under the optimised conditions in 76% RCY (n = 3, **Table 2.16**).

Table 2.15 Optimisation of Radio-Groebke-Bienaymé-Blackburn Reaction



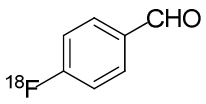
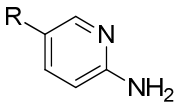
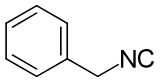
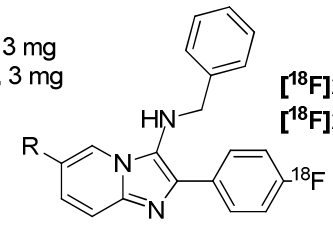
[^{18}F]2-2 3 mg 3 μL **[^{18}F]2-79a**

Entry	Temp. (ºC)[a]	Time (min)	Solvent (0.5 mL)	RCY (%)[b]
1	100	15	MeCN	3
2	100-120[c]	20	MeCN	8
3	110	15	3-Methyl-1-butanol	31
4	150	15	3-Methyl-1-butanol	73
5	150[d]	15	3-Methyl-1-butanol	64
6	150	30	3-Methyl-1-butanol	80
7	150[e]	15	3-Methyl-1-butanol	81
8	**170**	**15**	**3-Methyl-1-butanol**	**85 (*n*=4)**

a. Conventional heating. b. Decay corrected. Determined by radio-TLC. c.100 ºC for 15 minutes followed by 5 minutes at 120 ºC. [d]Benzyl isocyanide added prior to [^{18}F]4-fluorobenzaldehyde. [e]Performed under microwave irradiation (50 W).

[17] The 4-[^{18}F]fluorobenzaldehyde was prepared by Lei Li and Radio-Groebke reactions were carried out by M.Hopkinson.

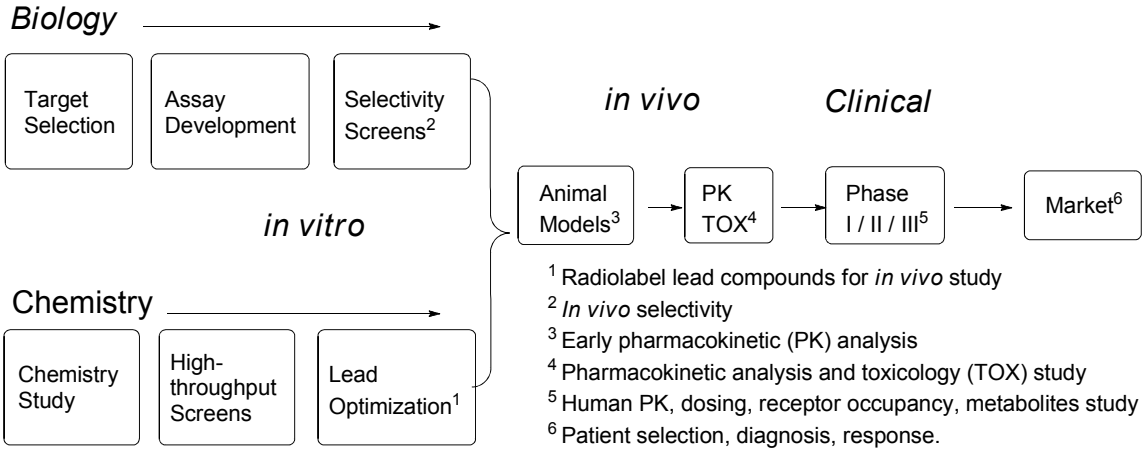
Table 2.16 Radio-Groebke reactions

Radioactive Component	Non-radioactive reagent	Product	RCY
 **[^{18}F]2-2**	 **2-78a** R = H, 3 mg **2-78b** R = Cl, 3 mg $Sc(OTf)_3$ 5 mg  3 μl		**[^{18}F]2-79a** R = H 85% (n = 4) **[^{18}F]2-79b** R = Cl 76% (n = 3)

All radio-Groebke reactions were performed by dispensing a solution of **[^{18}F]2-2** in 3-methyl-1-butanol (200 μL, 5-15 MBq) into a sealed vial containing $Sc(OTf)_3$ (5 mg) and 2-aminopyridine (3 mg). A solution of benzyl isocyanide (3 μL) in 3-methyl-1-butanol (300 μL) was then added *via* syringe. The reaction was heated at 170 °C for 15 minutes. Identities of labelled products were determined by radio-HPLC and RCYs were determined by radio-TLC.

2.7 PET in drug discovery

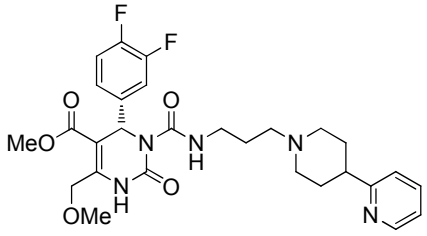
^{18}F-Labelled small molecules can provide early clinical distribution data to facilitate lead compound selection or offer valuable information by probing drug-receptor/enzyme occupancies and disease mechanisms across multiple therapeutic areas (eg. neurology, oncology, metabolic and immunological disorders, **Scheme 2.28**).[145-149] Statistical data showed that the longest periods of a drug approval process attribute to the discovery phase and Phase III testing. [147] Eckelman proposed in a recent review that the greatest contribution of PET would probably be in the discovery phase, if biodistribution, transport efficiency, occupancy of the saturable receptor sites or half-occuancy time of a drug candidate can be determined by this non-invasive *in vivo* imaging technology as early as possible. [147]



Scheme 2.28. Application of PET in different stages of drug discovery.[18]

In the context of synthesis of radiopharmaceuticals, we have validated radio-MCR methodology for four types of radio-MCR reactions: radio-Biginelli reactions, radio-Passerini reactions, radio-Ugi reactions, and radio-Groebke reactions. Encouraged by these results, we attempt to expand the practical utility of the methodology to the synthesis of some biologically active compound.

In 2000, *Merck* and *Synaptic* reported L-771,688 (SNAP 6383) **2–11** as a promising lead compound for the treatment of benign prostatic hyperplasia (**Scheme 2.29**).[90]



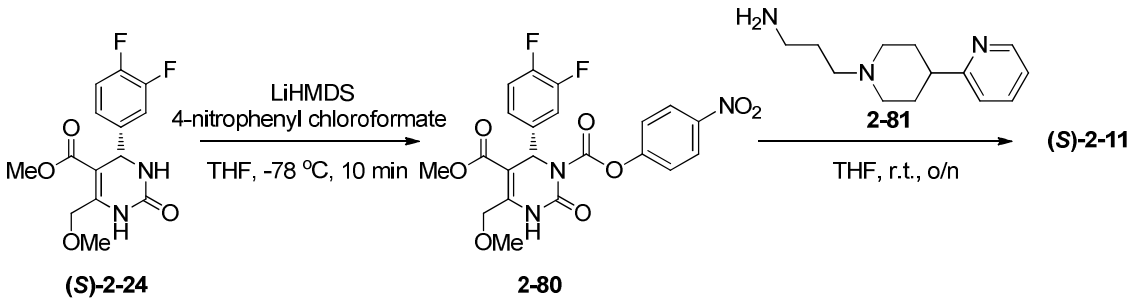
(*S*)-L-771,688 (SNAP 6383) **(*S*)-2-11**

Scheme 2.29. Lead compound for treatment of benign prostatic hyperplasia

The current synthetic procedure for the synthesis of optically active **(*S*)-2-11** is shown in **Scheme 2.30**. The 3,4-dihydropyrimidin-2-(1*H*)-one skeleton within the target compound **(*S*)-2-11** was constructed by a Biginelli MCR with difluorobenzaldehyde, methyl 4-methoxy-

[18] The scheme is adapted from renerence 146 and 149 with modifications.

3-oxobutanoate and urea which afforded a racemic mixture of **2-24**. Enantiopure **(*S*)-2–24** was obtained *via* HPLC chiral stationary phase enantiomeric separation. The fluorinated dihydropyrimidinone **(S)-2–24** was reacted with LiHMDS (Lithium bis(trimethylsilyl)amide), followed by addition of 4-nitrophenyl chloroformate to give the activated DHMP carbamate ester **(*S*)-2-80**. The reaction between **(*S*)-2–80** and amine side chain **2–81** gave the final drug candidate SNAP 6383 **(*S*)-2-11** *via* the formation of the urea linkage. The synthetic route requires a two-step transformation to form the urea linkage after Biginelli-MCR which may be time-consuming and not efficient enough for its application in ^{18}F radiochemistry (**Scheme 2.30**).

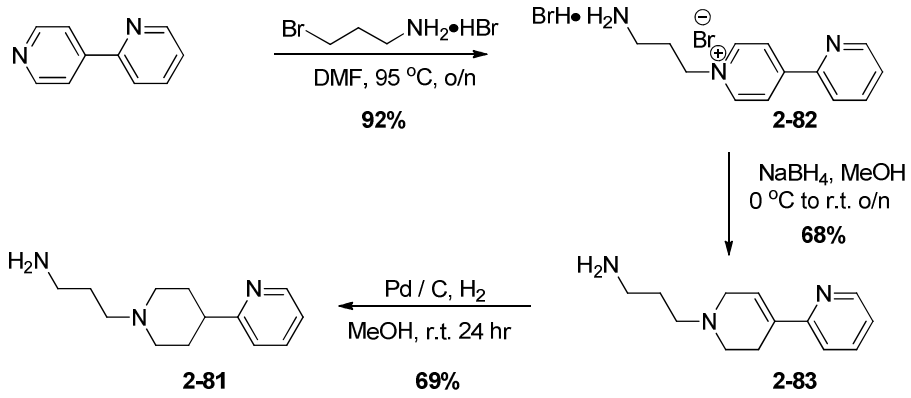


Scheme 2.30. Reported synthetic procedure for **(*S*)-2-11**.

Improved efficiency could be achieved by activating the amine group in **2-81** to facilitate the urea linkage formation and thus shorten the radiosynthetic route to only one step after the radio-Biginelli condensation.

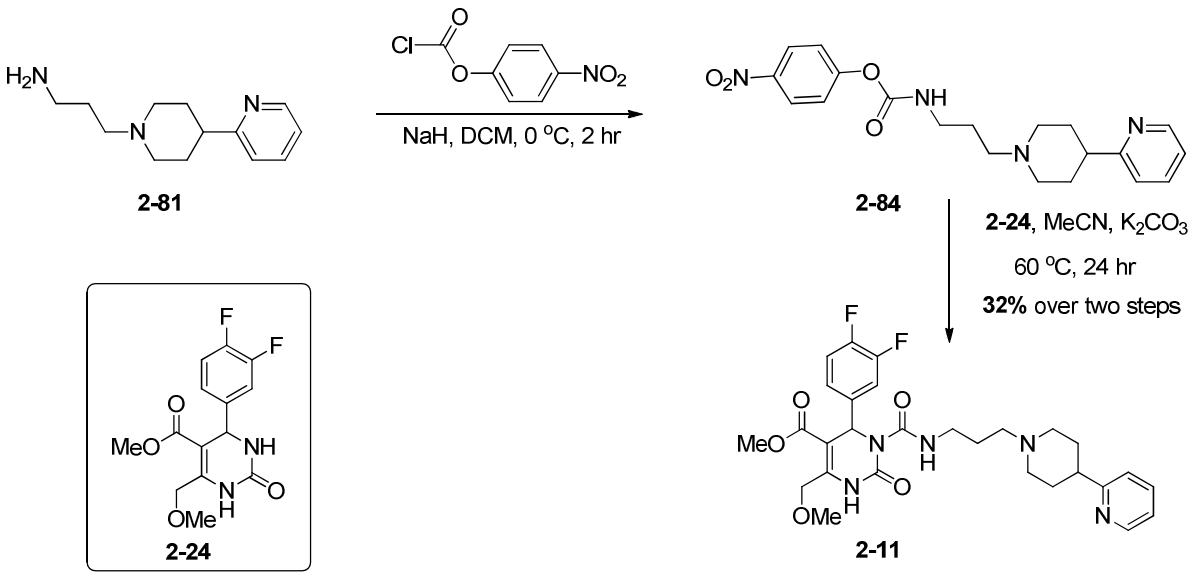
The amine side chain **2–81** was prepared according to the procedure below. Equimolar amounts of 2,4'-dipyridyl and 3-bromopropylamine hydrobromide were heated in a solution of DMF at 95 °C overnight. The resulting [2,4'-bipyridin]-1'-ium bromide salt **2-82** was obtained as a white solid in a 92% yield. Reduction of the pyridinium sub-structure in **2-82** using sodium borohydride yielded the 4-phenyl-1,2,3,6-tetrahydropyridine **2-83** in 68% efficiency. Purified tetrahydropyridine **2-83** was then hydrogenated catalytically with 10%

palladium on carbon, to afford the 4-phenylpiperidine derivative **2-81** in a 69% yield (**Scheme 2.31**).



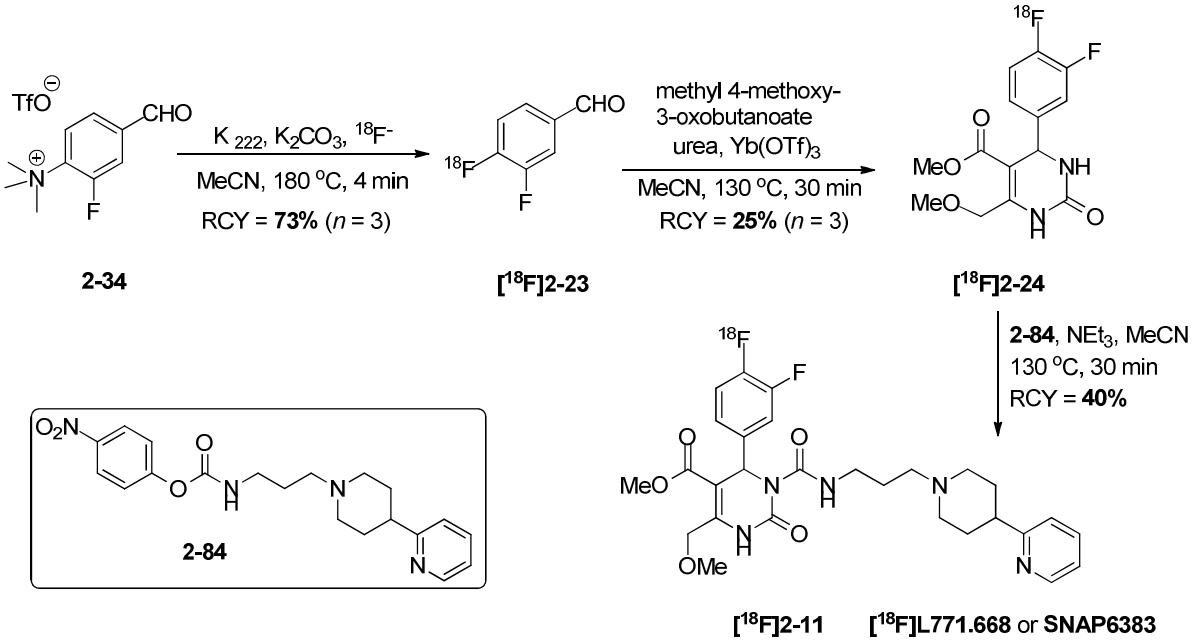
Scheme 2.31. Synthesis of **2-81**

To carry out the radiolabelling as late as possible in the radiosynthetic sequence, prosthetic group **2-84** bearing an activated ester was prepared and proved to be an efficient urea linking agent. The preparation involved deprotonation of the terminal amine **2-81** by sodium hydride and subsequent addition of 4-nitrophenyl chloroformate. The 4-nitrophenyl carbamate complex **2-84** could not be purified by flash column chromatography on silica gel, due to its instability in nucleophilic polar solvents such as methanol which is most commonly used in the purification process. Condensation of the Biginelli-adduct **2-24** and crude carbamate **2-84** gave the target drug candidate **2-11** in 32% yield over two steps (**Scheme 2.32**). Compared with the known method, this strategy is advantageous because of the late-stage introduction of the fluorinated complex in the synthetic procedure. Accordingly, we proposed that the synthesis can be adapted to a three-step radiolabelling sequence to access the SNAP 6383 **[^{18}F]2-11** in ^{18}F-labelled form.



Scheme 2.32. Synthesis of **2-11** with late stage introduction of fluorinated intermediate for adaptation to radiochemistry.

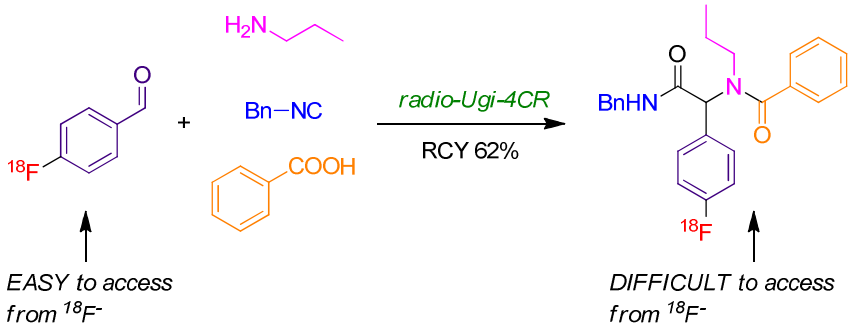
The three-step retro-radiosynthetic sequence for the ^{18}F-labelled DHMP drug candidate **[^{18}F]2-11** started from ^{18}F-nucleophilic fluorination on the aromatic precursor, 3-fluoro-4-trimethylammoniumbenzaldehyde triflate **2-34**, to give the ^{18}F-labelled 3,4-difluoro-benzaldehyde **[^{18}F]2-23** in a 74% RCY (n = 3). The ^{18}F-labelled building block **[^{18}F]2-23** was then reacted with urea and methyl 4-methoxy-3-oxobutanoate in a radio-Biginelli reaction as the key transformation, to construct the 3,4-dihydropyridin-2-(1*H*)-one skeleton of **[^{18}F]2-24** in a 25% RCY (*n* = 3). Base-mediated post-condensation between the ^{18}F-labelled DHMP complex **[^{18}F]2-24** and the pre-activated carbamate linking agent **2-84**, formed the urea linkage in 40% yield (**Scheme 2.33**). Acetonitrile was selected over an alcoholic solvent to avoid the decomposition of the carbamate **2-84** in the last step. The overall synthetic sequence delivered the desired ^{18}F-labelled aromatic drug molecule **[^{18}F]2-11** in just over one hour with an overall 7% RCY (*decay-corrected*). The convergent nature of this concise radiosynthesis could give access to various structural analogues by using alternative components (ketoester or urea) or upon attachment of other side chains.[150]



Scheme 2.33. 3-Step radiosynthesis of **[^{18}F]2-11** using a radio-Biginelli reaction as the key transformation.

2.8 Conclusion and Future Work

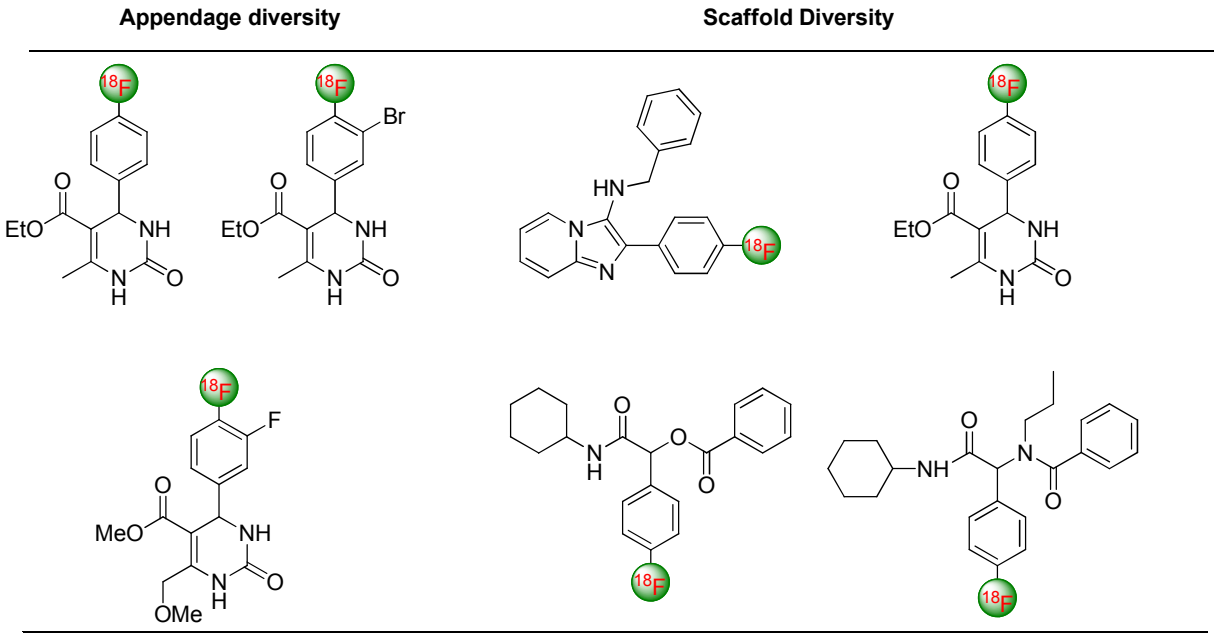
We have developed a general two-step synthetic sequence to furnish ^{18}F-labelled aromatics starting with a nucleophilic ^{18}F-fluorination and followed by a multi-component reaction involving the ^{18}F-labeled building block as the key transformation (**Scheme 2.34**)



Scheme 2.34. Radio-Ugi-4CR as an example of convergent radiosynthesis

The new radiosynthetic strategy was validated by the synthesis of various ^{18}F-labelled 3,4-dihydropyrimidin-2-(1*H*)-ones, imidazol[1,2-*a*]pyridines, α-acyloxyamides and peptide-type products, with ^{18}F on an aryl sub-motif which are notoriously difficult to obtain by direct ^{18}F-fluorination.

The established radio-MCRs have been used to deliver ^{18}F-labelled aromatics with two levels of molecular diversity: a) *Appendage diversity* involves the introduction of different appendages to a common ^{18}F-labelled molecular skeleton and b) *Scaffold diversity* involves the generation of a collection of ^{18}F-labelled aromatic compounds with different skeletons. Further studies are underway to expand the range of MCRs suitable for ^{18}F-labelling and the pool of radioactive building blocks and to map the various possible combinations of non-labelled and labelled components (**Scheme 2.35**).



Scheme 2.35. ^{18}F-labelled aromatics in three fundamental level of molecular diversity: appendage diversity, scaffold diversity and stereochemical diversity.

In principle, this methodology allows for the introduction of the radioisotope at different positions of the labelled compound based on the choice of ^{18}F-labelled components. However, our preliminary work revealed that all combinations will not necessarily be of comparable efficiency. Therefore, choice of the radioactive building block requires careful consideration in application of the methodology.

We exemplified the practical utility of the methodology in drug discovery, by ^{18}F-labelling a reported potent antagonist of the α_{1A}-selective adrenoceptor. Generally speaking,

optimisation of the model reaction is necessary, to transfer the MCR from "cold" (under conventional organic synthetic conditions) to "hot" (in the conext of radiochemistry). Our preliminary results also indicated that the microfluidic technology benefits the radiosynthetic process by avoiding the dilution of radioactivity and thus promoting the ^{18}F-substitution on the substrates.

Chapter 3

Oxidative Nucleophilic Fluorination

3.1. Radiochemistry of 4-[^{18}F]fluorophenol

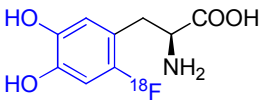
^{18}F-labelled radiopharmaceuticals with 4-[^{18}F]fluorophenol and 4-[^{18}F]fluorocatechol motif have found growing clinical applications in PET, and thus stimulated an interest in developing new radiolabelling strategies for robust synthesis to acess these motifs with high efficiency.

In **Scheme 3.1**, representative PET radiopharmaceuticals containing 4-[^{18}F]fluorophenol and 4-[^{18}F]fluorocatechol sub-motif are highlighted. As a PET tracer for neurology studies, L-6-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (L-[^{18}F]FDOPA, L-**[^{18}F]3-1**) has been used exclusively for the clinical diagnosis of Parkinson's disease. Recently, clinical studies have been carried out to investigate the potential application of L-[^{18}F]FDOPA L-**[^{18}F]3-1** as an oncologic PET tracer.[151]

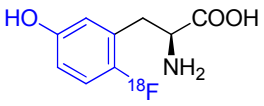
The non-catecholic L-[^{18}F]FDOPA analogue, L-6-[^{18}F]fluoro-*m*-tyrosine (L-[^{18}F]FmT, L-**[^{18}F]3-2**) showed an improved signal/noise contrast in brain imaging according to a comparative study performed in the DeJesus group.[152] In cardiology, (1*R*,2*S*)-6-[^{18}F]fluorometaraminol ((1*R*,2*S*)-6-[^{18}F]FmR, **[^{18}F](1*R*,2*S*)-3-3**)[51, 153] and (+)-6-[^{18}F]fluoro-*m*-norepinephrine ((+)-6-[^{18}F]FNE, **(+)-[^{18}F]3-4**)[154] were synthesised and evaluated for non-invasive neuronal mapping of the heart.

(±)-α-Fluoromethyl-6-fluoro-*m*-tyrosine ((±)-FM-6-[^{18}F]FmT, **(±)-[^{18}F]3-5**)[155] has been reported as a promising carcinoid tumor-imaging agent with good selectivity. In oncology, 6-[^{18}F]fluorodopamine ([^{18}F]FDA, **[^{18}F]3-6**) has been applied as a novel functional imaging

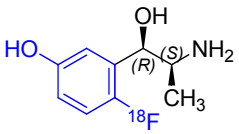
agent for the *in vivo* detection of pheochromocytoma, a type of neuroendocrine tumor.[156, 157]



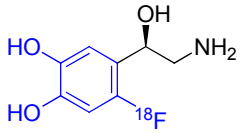
L-[^{18}F]FDOPA L-**[^{18}F]3-1**



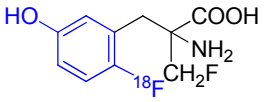
L-[^{18}F]FmT L-**[^{18}F]3-2**



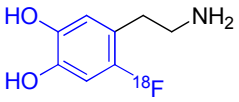
(1*R*,2*S*)-6-[^{18}F]FmR **[^{18}F](1*R*,2*S*)-3-3**



(+)-[^{18}F]FNE **(+)-[^{18}F]3-4**



(±)-FM-6-[^{18}F]FmT **(±)-[^{18}F]3-5**



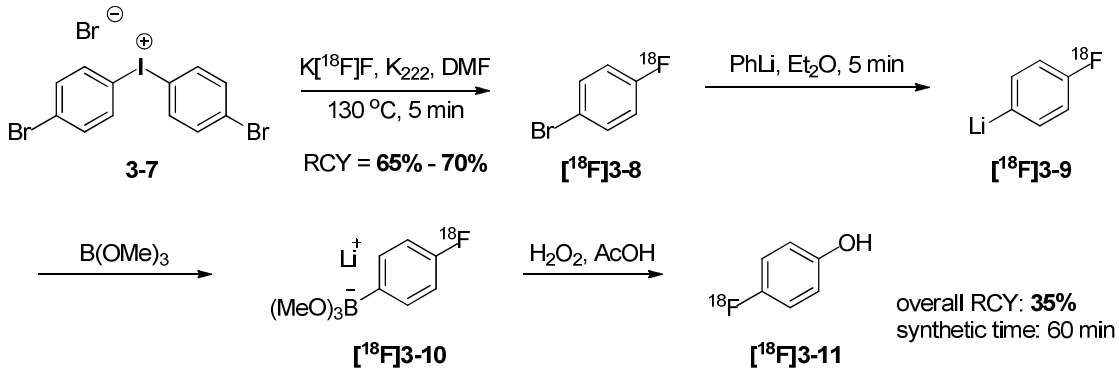
[^{18}F]FDA **[^{18}F]3-6**

Scheme 3.1. PET radiopharmaceuticals containing 4-[^{18}F]fluorophenol or 4-[^{18}F]fluorocatechol sub-motif.

3.1.1. Synthesis of 4-[^{18}F]fluorophenol

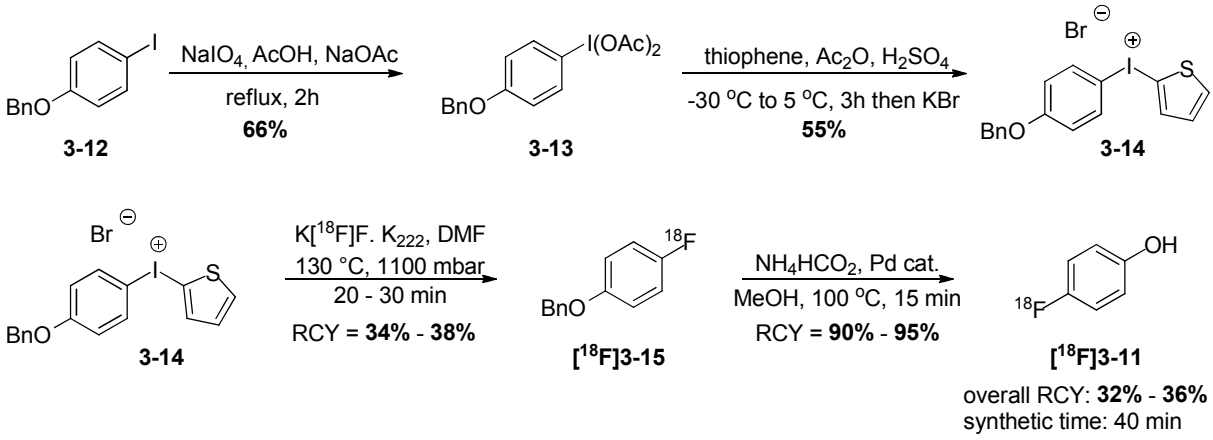
The majority of the radiosynthetic methods for electron-rich aromatics are based on regiospecific, electrophilic fluorodestannylation reactions.[3] Developing a convenient and efficient *non-carrier-added* (*n.c.a.*) synthetic protocol of 4-[^{18}F]fluorophenol (and 4-[^{18}F]fluorophenol derivatives) would facilitate access to a wide variety of radiotracers with potential applications in PET and pave the way for a novel synthetic method for 4-[^{18}F]fluorocatechol.

Multi-step synthetic strategies based on a nucleophilic ^{18}F-fluorination followed by functional group transformations, are commonly used for the synthesis of *n.c.a.* 4-[^{18}F]fluorophenol **[^{18}F]3-11**. The radiosynthesis is typically time-consuming (more than one hour) and requires harsh conditions such as high temperature and/or high pressure. The nucleophilic fluorination of a symmetrical iodonium salt **3-7** serves as the key transformation to introduce the ^{18}F-radioisotope, in the synthesis of the phenol derivative **[^{18}F]3-11**, with an overall RCY of 35% within 60 minutes (**Scheme 3.2**).[42, 158, 159]



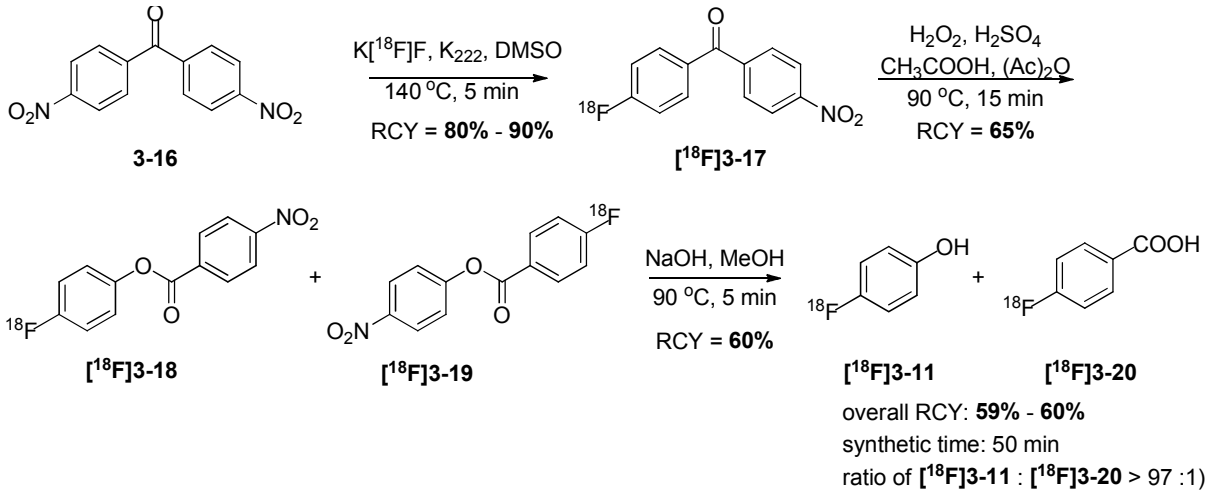
Scheme 3.2. Synthesis of 4-[^{18}F]fluorophenol **[^{18}F]3-11** *via* nucleophilic ^{18}F-fluorination of a symmetrical iodonium salt.

The synthesis was improved by using a non symmetrical aryl(2-thienyl)iodonium salt **3-14** as the precursor. It was prepared by reacting the diacetoxyiodoarene **3-13** and thiophene in excess acetic acid anhydride and a catalytic amount of sulfuric acid. The diacetoxyiodoarene **3-13** is commonly synthesised from the iodoarene **3-12** *via* oxidation using sodium periodate in acetic acid. The radiosynthesis of 4-[^{18}F]fluorophenol **[^{18}F]3-11** started with a nucleophilic ^{18}F-fluorination on the relatively electron-deficient phenyl ring of the iodonium salt **3-14** and the ^{18}F-fluorinated product **[^{18}F]3-11** was obtained upon reductive cleavage of the benzyl protecting group with an overall RCY of 32% - 36% within 40 minutes (**Scheme 3.3**). [42, 158]



Scheme 3.3. Synthesis of 4-[^{18}F]fluorophenol **[^{18}F]3-11** *via* nucleophilic ^{18}F-fluorination of an asymmetric iodonuim salt.

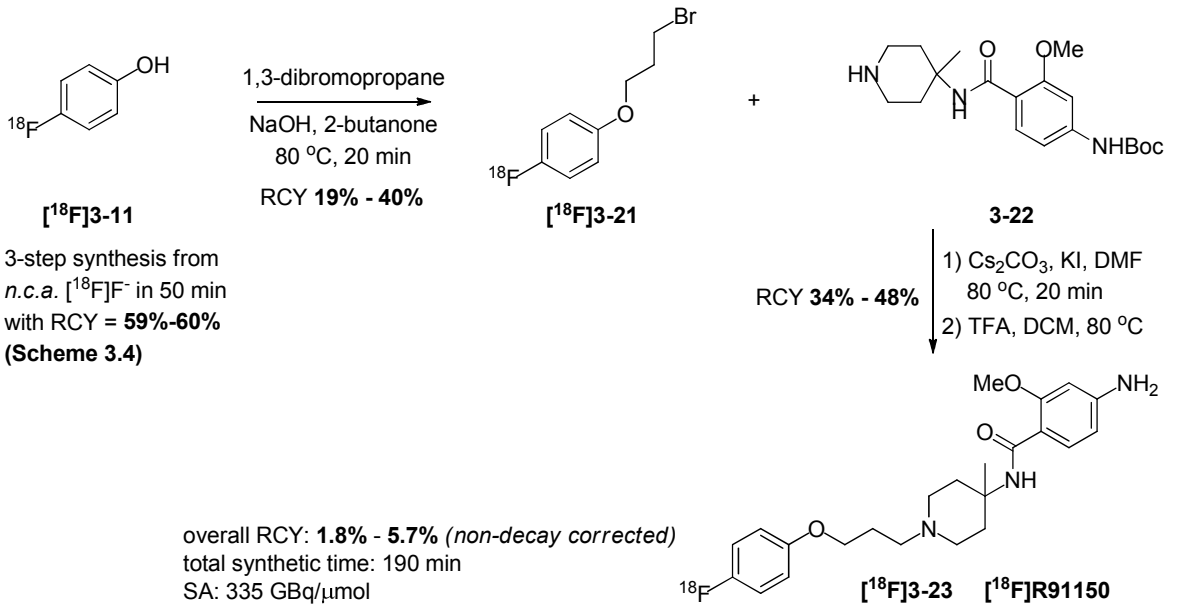
Activated by the electron-withdrawing carbonyl group, the benzophenone derivative **3-16** can be ^{18}F-fluorinated *via* an S_NAr reaction in 80% – 90% RCY, as reported by the Coenen group.[160] The ^{18}F-fluorinated benzophenone **[^{18}F]3-17** undergoes a Baeyer-Villiger oxidation followed by base-mediated hydrolysis, which leads to the selective formation of ^{18}F-fluorinated phenol **[^{18}F]3-11** in 31-35% overall RCY after 60-80 minutes (with 1% ^{18}F-labelled benzoic acid **[^{18}F]3-20** as the side product, **Scheme 3.4**).



Scheme 3.4. Synthesis of 4-[^{18}F]fluorophenol **[^{18}F]3-11** *via* S_NAr ^{18}F-fluorination followed by a Baeyer-Villiger oxidation.

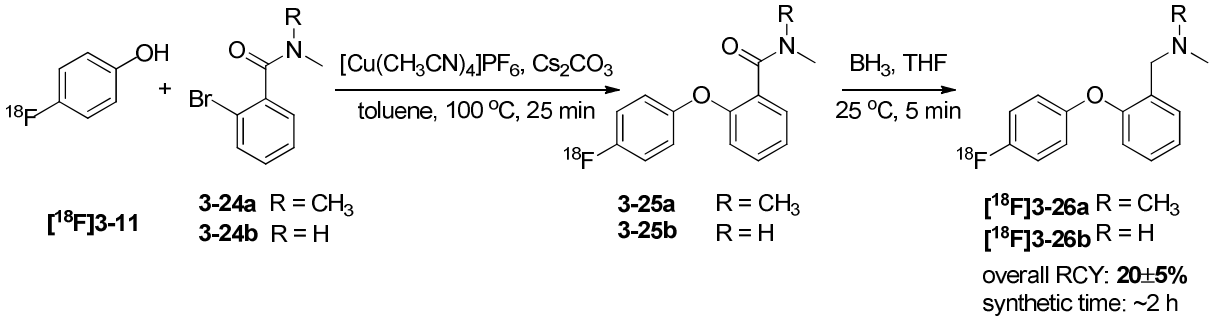
3.1.2. 4-[^{18}F]Phenol as a prosthetic group in radiosynthesis

In a follow-up study, the Coenen group applied the 4-[^{18}F]fluorophenol **[^{18}F]3-11** in the multi-step synthesis of a radiopharmaceutical, which is not accessible *via* direct nucleophilic fluorination, for example the ^{18}F-labelled aryl-alkyl ether **[^{18}F]3-23**. Starting from *n.c.a.* [^{18}F]fluoride, a six-step synthesis gave [^{18}F]R91150 **[^{18}F]3-23** after 190 min, in an overall RCY of 1.8% - 5.7% (**Scheme 3.5**). This potential PET tracer for neurology studies can be obtained in a high specific activity (SA) of 335 GBq/μmol at the end of synthesis. [161]



Scheme 3.5. Radiosynthesis of [^{18}F]R91150 **[^{18}F]3-23**, SA = specific activity.

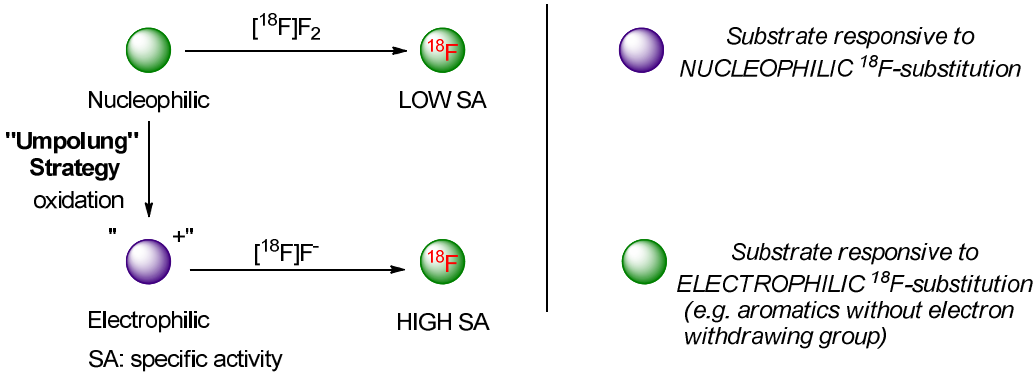
Both ^{18}F-labelled diaryl ethers **[^{18}F]3-26a/b** are potential radiotracers for *in vivo* mapping of serotonin reuptake transporter (SERT) sites.[162] Starting with 135 MBq of [^{18}F]fluoride, 14–15 MBq of SERT tracer **[^{18}F]3-26a/b** could be obtained within ~2 hours.[163] The key step involves an Ullmann biaryl ether coupling promoted by a Cu(I) complex followed by the reduction of the ^{18}F-labelled benzamide intermediates **3-25a/b** (**Scheme 3.6**). It has been recognised that the rapid progress in PET probe discovery for SERT is restricted by the time-consuming radiosynthesis of the ^{18}F-labelled prosthetic group.



Scheme 3.6. Synthesis of **[^{18}F]3-26a**/b *via* Ullmann Biaryl Ether Synthesis.

3.1.3. Conceptual advance to facilitate access to 4-[^{18}F]fluorophenol

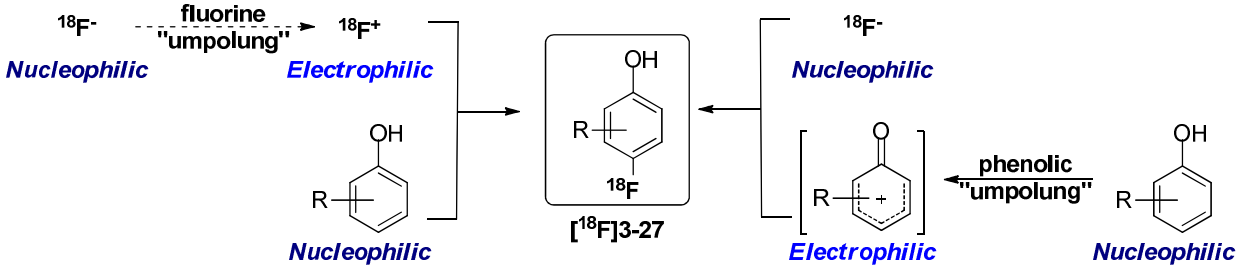
Non-carried-added formation of aryl C–F bond starts with direct nucleophilic fluorination of electron-deficient aromatic substrates (e.g. di-nitro substituted benzophenone **3-16** in **Scheme 3.4** or as previously discussed in **Section 1.5.1**). The *carrier-added* strategy usually involves electrophilic fluorination between an electron-rich substrate and an $^{18}F^{+}$ reagent (e.g. [^{18}F]F_2, [^{18}F]NFSI or [^{18}F]Selectfluor as discussed in **Section 1.5.3**). Since the conversion of nucleophilic [^{18}F]F^- to electrophilic [^{18}F]F^+ is considerably challenging ($2F^- \rightarrow F_2(g) + 2e$, $E^0_v = -2.87$ V) , we postulated that a more facile nucleophilic synthesis of the electron-rich aromatic compounds, could be *via* an *"**phenolic umpolung**"* that reversed the reactivity of the substrate (**Scheme 3.7**). We proposed that electron-rich aromatic compounds not responsive to nucleophilic ^{18}F-substitution, might undergo the desired reactivity switch *via* oxidation, and the resulting *in situ* generated intermediate could react with *n.c.a* [^{18}F]fluoride to afford ^{18}F-labelled compounds in high specific activity.



Scheme 3.7. Oxidative nucleophilic ^{18}F-fluorination.

If feasible, the proposed one-pot "oxidative nucleophilic ^{18}F-fluorination" method could greatly simplify the radiosynthesis of some important ^{18}F-labelled electron-rich aromatic compounds such as 4-[^{18}F]fluorophenol derivatives **[^{18}F]3-27**. Moreover, for the first time,

we would be able to provide a direct radiolabelling method to access ^{18}F-labelled electron-rich aromatics with nucleophilic ^{18}F-fluoride (**Scheme 3.8**).

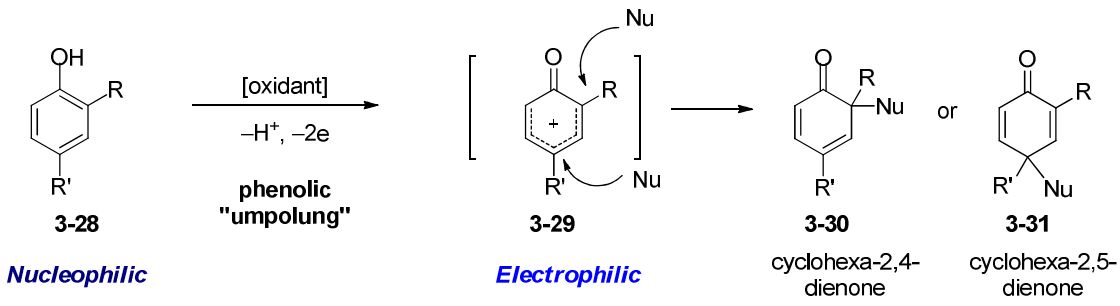


Scheme 3.8. Oxidative nucleophilic fluorination *via* "phenolic umpolung"

3.2. Inspiration and validation of "oxidative nucleophilic fluorination"

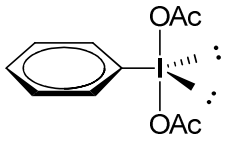
3.2.1. The concept of phenolic "umpolung"

As the key intermediate in the proposed "oxidative nucleophilic ^{18}F-fluorination", the phenolic "umpolung" has been well explored in organic chemistry.[164, 165] Under oxidising conditions, the dearomatization of *ortho*- or *para*-substituted phenols **3-28** forms cyclohexadienones **3-30/31**, which can undergo a variety of reactions and are widely used in synthetic chemistry.[166-168] Hypervalent iodine reagents such as PIDA (phenyliodine diacetate) and PIFA (phenyliodine bis(trifluoroacetate)) are known to be mild and efficient oxidants to convert phenol into cyclohexadienones in the presence of nucleophiles, suggesting that they could be applied in our proposed oxidative ^{18}F-fluorination process (**Scheme 3.9**).[166, 169]



Scheme 3.9. Phenolic "Umpolung"

The iodine atom in λ^3-iodanes (RIX_2, e.g. $PhI(OAc)_2$) has a total 10 electrons and the overall geometry of a distorted trigonal bipyramid (**Scheme 3.10**). Two heteroatom ligands X occupy the axial positions and the least electronegative carbon ligand and two electron pairs reside in equatorial positions. The linear X–I–X bond uses the non-hybridized 5p orbital of iodine. Such a linear three-center, four electron (3c–4e) bond is highly polarized and leads to the high electrophilicity of λ^3-iodanes.[170]

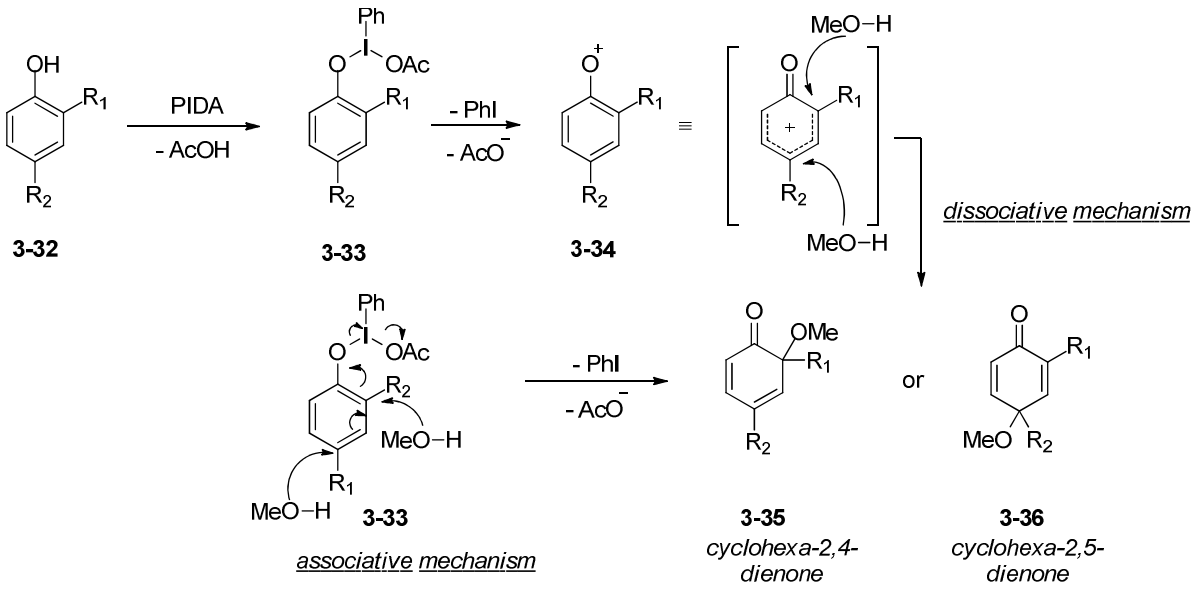


PIDA

Scheme 3.10. Structure of $PhI(OAc)_2$ or PIDA

To date, the exact mechanism of hypervalent iodine promoted oxidations of phenol remains unclear and two possibilities have been proposed. [171, 172] The discussion herein is based on the hypothesis proposed by the Antus and Pelter group.[171] The substituted phenol complex **3-32** is oxidised by PIDA to form the iodine (III) aryl complex **3-33**. The resulting intermediate can react with a nucleophile (in this case MeOH) *via* the phenoxenium (PhO^+) cation **3-34** (dissociative mechanism) or *via* an associative bimolecular mechanism (**Scheme 3.11**). In many cases it is difficult to ascertain which path a particular reaction undergoes and the product distribution between *meso*-cyclohexa-2,4- or -2,5-dienones **3-35/36** was

found to be highly dependent on the substituted pattern of the starting phenol **3-32** and electronic properties of the substituents.



Scheme 3.11. Oxidation of phenol derivatives **3-32** to *meso*-cyclohexadienones **3-35/36** in methanol

The calculated values of AM1-Mulliken charge distributions at each reaction site of substituted phenoxenium ions **3-34** is summarised in **Table 3.1** whilst **Table 3.2** lists the LUMO coefficiencies of the same ions. Experimental results indicated that when phenols with different substitution patterns were treated with PIDA in DCM containing 2 molar equivalents of methanol, the product distribution between *cyclohexa-2,4-dienone* **3-35** and *cyclohexa-2,5-dienone* **3-36** are very much in line with predictions based on charge distributions and LUMO coefficients for corresponding phenoxenium ions **3-34**. 2-Methoxyphenol reacts at C – 2 although this is the most hindered position in the substrate. Phenol and 4-methoxyphenol are attacked at C – 4. Upon oxidation, both 3-methyl and 4-methyl phenol were predicted be substituted at C – 4 and the predictions were in accordance with literature precedents.[173] The predictions for dimethylamino substituted phenol are in line with those for corresponding methoxy analogues. For all three isomers of nitrophenols,

the predicted position of attack is at C – 4 carbon. Similarly, substitutions are most likely happen on C – 4 of 2-, 3- or 4-bromophenol.

Table 3.1 Charge distributions at C-2, C-4, C-6, O-1 of substituted phenoxenium ions[19]

Substituent	C-2	C-4	C-6	O-1
H	0.030	0.213	0.030	0.067
2-Me	0.143	0.184	−0.279	−0.094
3-Me	0.004	0.207	0.029	−0.073
4-Me	0.007	0.282	0.005	0.089
2-OMe	0.235	0.121	−0.010	−0.091
3-OMe	−0.100	0.177	0.030	−0.068
4-OMe	−0.003	0.335	−0.003	−0.110
2-NMe_2	0.108	0.027	−0.156	−0.187
3-NMe_2	−0.162	0.167	0.044	−0.095
4-NMe_2	−0.065	0.217	−0.062	−0.144
2-NO_2	−0.033	0.217	0.071	−0.022
3-NO_2	0.075	0.242	0.048	−0.043
4-NO_2	0.048	0.180	0.048	−0.044
2-Br	0.052	0.189	0.007	−0.070
3-Br	0.035	0.229	0.042	−0.063
4-Br	0.015	0.181	0.015	−0.007

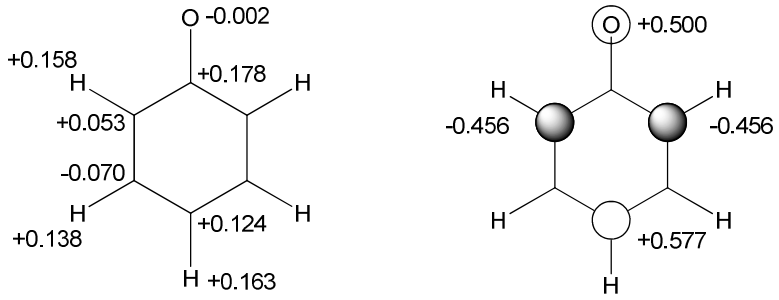
Table 3.2 LUMO coefficients for substituted phenoxenium ions[20]

Substituent	C-2	C-4	C-6
H	−0.476	0.606	−0.477
2-Me	0.535	−0.573	0.409
3-Me	0.471	0.608	0.479
4-Me	−0.451	0.618	−0.444
2-OMe	0.558	0.505	0.329
3-OMe	0.466	−0.610	0.484
4-OMe	−0.437	0.588	−0.401
2-NMe_2	−0.566	0.411	−0.267
3-NMe_2	0.424	−0.621	0.509
4-NMe_2	0.383	−0.542	0.382
2-NO_2	−0.449	0.601	−0.501
3-NO_2	−0.470	0.599	−0.482
4-NO_2	0.472	−0.600	0.472
2-Br	−0.518	0.574	−0.427
3-Br	−0.466	0.607	−0.484
4-Br	−0.446	0.618	−0.446

Ab initio calculations on the phenoxenium ion were carried out by the Shudo group based on the STO-3G basis set.[174] The calculated C–O bond length of PhO^+ is 1.241 Å and close to

[19] Table 3.1 is reprinted from reference 171, with permission from the Royal Society of Chemistry.
[20] Table 3.2 is reprinted from reference 171, with permission from Royal Society of Chemistry.

the C–O bond length of acetone (1.219 Å), indicating nearly full double-bond character and the ring charge is mostly located on the carbon in *para* position (**Scheme 3.12**).



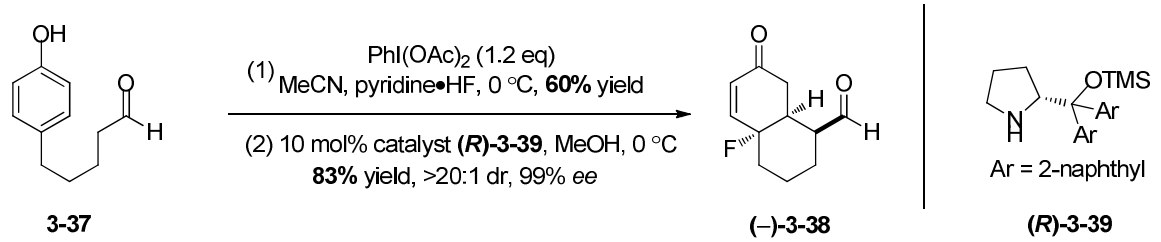
Scheme 3.12. STO-3G-caculated charge distribution (left) and LUMO coefficient (right) of phenoxenium ion.[21]

In summary, both experimental and theoretical studies support the postulation that when a resonance-stabilised PhO^+ cation forms, it reacts with a nucleophile at the site with the greatest LUMO coefficient. Therefore, examinations of the relative resonance contributors of the PhO^+ cation can provide satisfactory predictions regarding the site of nucleophile addition.[165]

3.2.2. Utilisation of phenolic "umpolung" in fluorination

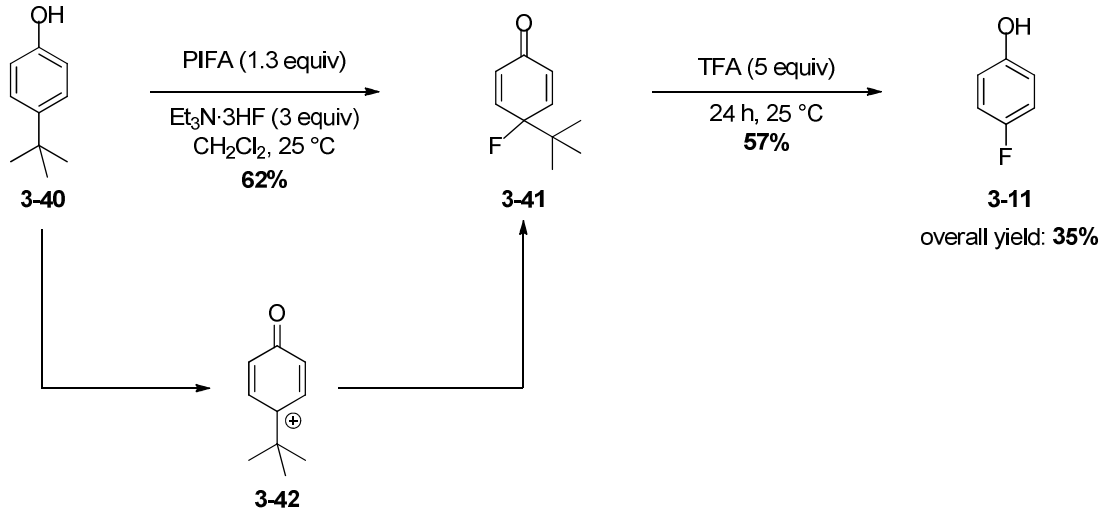
Recently, the Gaunt group developed a catalytic enantioselective strategy for the direct conversion of substituted phenols to complex non-aromatic molecular architectures.[175] The method was implemented to install a tertiary C–F bond within a complex chiral molecule (**Scheme 3.13**). The process involves oxidative dearomatisation of a *para*-substituted phenol **3-37,** followed by a pyrrolidine **(*R*)-3-39** catalysed asymmetric intramolecular Michael addition to give the fluorinated enantioenriched decalin **(–)-3-38** in 83% yield and excellent *ee* (> 99%).

[21] The Scheme is reprinted from reference 174, with permission from American Chemical Society.



Scheme 3.13. Catalytic enantioselective dearomatization for the synthesis of fluorinated decalin **(–)-3-38**.

The feasibility of the oxidative fluorination process is also underlined by literature precedent. The Langlois group reported[176] the straightforward two-step conversion of *tert*-butyl phenol **3-40** into 4-fluorophenol **3-11** under oxidising conditions. The intermediate 4-(*tert*-butyl)-4-fluorocyclohexa-2,5-dienone **3-41** was obtained, by fluorination of, presumably the tertiary carbocation **3-42**, which was generated *via* oxidation of the phenolic starting material **3-40**. Acid-mediated cleavage of the *tert*-butyl group and re-aromatisation gives 4-fluorophenol **3-11** in an overall yield of 35% (**Scheme 3.14**).



Scheme 3.14. Oxidative nucleophilic fluorination of the *tert*-butylphenol **3-40**

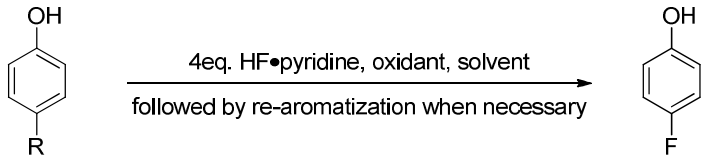
3.2.3. Oxidative fluorination of phenol and *tert*-butylphenol

Based on this precedent, we studied the reactivities of both phenol and *tert*-butylphenol as prototypical substrates, HF•pyridine as the fluoride source and polyvalent iodine compounds as oxidants, in the context of oxidative fluorination.

Phenol **3-43** was first treated with an equimolar amount of PIDA in the presence of 4 equiv. of HF•pyridine in DCM. The reaction was stirred at 0 ^{o}C for 10 minutes and subsequently quenched by the addition of solid potassium carbonate. The reaction mixture was filtered and concentrated before analysed by TLC, ^{19}F NMR and HPLC. In the ^{19}F NMR analysis, the triplet of a triplet with coupling constants of 8.6 Hz and 4.5 Hz at –127.7 ppm are in agreement with $^{3}J_{\text{F-H}}$ and $^{4}J_{\text{F-H}}$ coupling constants and the chemical shift of commercial 4-fluorophenol. Two fluorinated side products were observed by ^{19}F NMR. Unfortunately, we were unable to isolate or identify these products due to their overlapping with inseparable side products formed in deleterious side reactions. The reaction mixture was spiked with 4- and 2-fluorophenol (^{19}F NMR of 2-fluorophenol shows a broad singlet at –140.1 ppm) and ^{19}F NMR analysis indicated no formation of 2-fluorophenol in the oxidative fluorination process. The main fluorinated product was isolated from the reaction mixture by flash column chromatography. Full NMR and MS characterisation confirmed the formation of 4-fluorophenol **3-11**, which was also supported by HPLC analysis.[22]

The results suggested that the proposed "oxidative fluorination" of phenol to give the *para*-fluorinated phenol **3-11** was feasible, albeit in 2% low yield (**Table 3.3**, Entry 1).

Table 3.3 Validation of the oxidative-fluorination strategy



R = H **3-43**
R = ^{t}Bu **3-40**

3-11

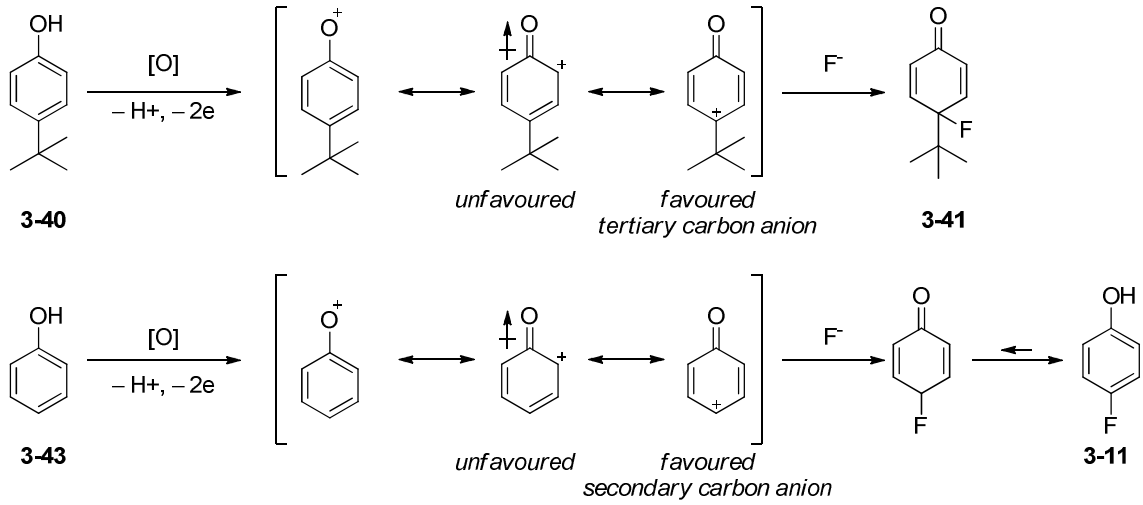
Entry	R	Oxidant	Eq.	Solvent	Temp.	Time	Yield[a]
1	H	PIDA	1	DCM	0 °C	10 min	2%
2	H	PIFA	1	DCM	0 °C	10 min	1%
3[b]	^{t}Bu	PIDA	1	DCM	0 °C	10 min	34%[c]
4[b]	^{t}Bu	PIDA	1	THF	0 °C	10 min	0%[c]
5[b]	^{t}Bu	PIDA	1	MeCN	0 °C	10 min	0%[c]
6[b]	^{t}Bu	PIDA	1	DCE	0 °C	10 min	37%[c]

a. Yield based on HPLC calibration. b. The one-pot re-aromatization was performed by subsequent addition of 5% TFA at 25 °C and stirring for 15 min at ambient temperature before quenching with K_2CO_3. c. Two-step yield (fluorination and re-aromatization) based on HPLC calibration.

The synthetic outcome was not improved by using PIFA, the more reactive oxidant (**Table 3.3**, Entry 2 *vs* 1). Notably, the two-step fluorination of *tert*-butylphenol **3-40** was successfully combined into a one-pot procedure to give 4-fluorophenol **3-11** in 34% yield (Entry 3), which is comparable with the reported value of 35% over two steps (**Scheme 3.14**). The reaction gave 34% yield in dichloromethane and 37% in dichloroethane (**Table 3.3**, Entries 3 and 6) but did not occur in THF or MeCN (Entries 4 and 5). This preliminary result indicates that *tert*-butyl group plays an essential role in the fluorination possibly by stabilising the cyclohexadienylium cationic intermediate **3-42**.

[22] The preliminary study was carried on in collaboration with Dr. Y. H. Lim and we thank Dr. M. Gao and Mr. L. Wong for repeating the reaction.

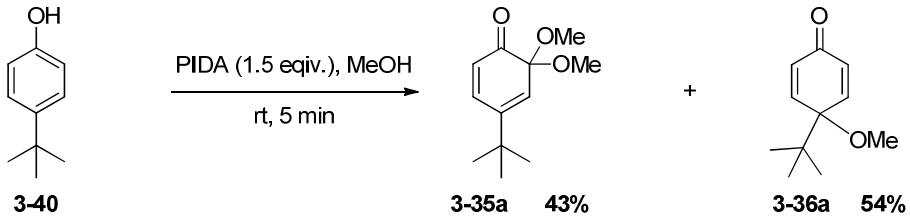
The significant difference in efficiency of the oxidative-fluorination of phenol **3-43** compared to *tert*-butylphenol **3-40** can be attributed to the formation of a *tertiary* phenoxenium intermediate by oxidising a *para*-alkylated phenol **3-40** (**Scheme 3.15**). However, upon oxidation, the reactive cationic intermediates can react with any nucleophilic species nearby, before being trapped by fluoride and thus lead to low yields of the desired fluorinated product. The fluorination of either *tert*-butylphenol **3-40** or phenol **3-43** occurs preferentially at *para*-position in our studies. The concentration of charge at C – 4 position of a phenoxenium and *para*-alkylated phenoxenium cation, as previously mentioned, suggests that to place a positive charge at C – 2 is less favourable. This is due to the destabilising effect of the adjacent carbonyl group (**Scheme 3.15**).



Scheme 3.15. Dissociative mechanism for oxidative fluorination of phenol and *tert*-butyl phenol

Notably, the Russell group reported the oxidation of 4-*tert*-butylphenol with 1.5 equivalents of PIDA in methanol to give 4-(*tert*-butyl)-6,6-dimethoxycyclohexa-2,4-dienone **3-35a** and 4-(*tert*-butyl)-4-methoxycyclohexa-2,5-dienone **3-36a** in 43% and 54% yield, respectively (**Scheme 3.16**). The results indicated that steric factors could be influential on the synthetic

outcome. When the reaction was performed on the symmetrical 2,4,6-tri-*tert*-butylphenol, the addition occurred at C – 4 exclusively.[173]



3-40 **3-35a** **43%** **3-36a** **54%**

Scheme 3.16. Formation of **3-35a** and **3-36a**

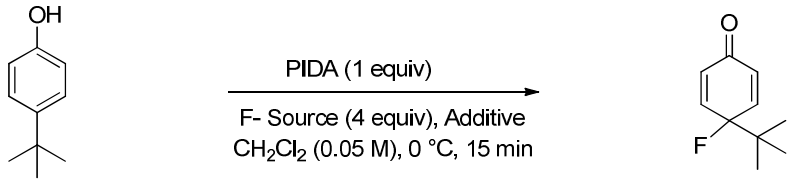
3.2.4. Optimisation of the oxidative fluorination

Although HF•pyridine is a suitable fluoride source for this reaction, its isotopic analogue [^{18}F]HF is volatile and difficult to handle in a radiolabelling process. Unfortunately, no fluorination of *tert*-butylphenol **3-40** was observed in the presence of TBAF or CsF even in the medium of AcOH (pKa = 4.76) or $KHSO_4$ (pKa = 1.99, **Table 3.4**, Entry 1 *vs* 2 – 6). When TFA (pKa = –0.25) was added to the reactions, the fluorinated dienone **3-41** could be obtained in 29% and 47% yield (**Table 3.4**, Entries 7 and 8 respectively). We attributed this significant improvement to the *in situ* generation of HF (pKa = 3.17) in the reaction mixture. Moreover, when PIFA was used as the oxidant with CsF as the fluoride source, the oxidative fluorination yielded the fluorinated cyclohexadienone **3-41** in 10% efficiency, without the need for the addition of TFA (**Table 3.4**, Entry 9). These results suggested that the TFA, which was generated *in situ* during the oxidation process, could further serve as an additive to promote the subsequent fluorination.

The combination of "CsF or TBAF plus TFA" hence can be viewed as a replacement of HF to facilitate the transformation of the conventional synthetic method to radiochemistry. Labelled [^{18}F]CsF and [^{18}F]TBAF are readily available, as previously discussed.

The result indicates that oxidative fluorination occurs exclusively in the the presence of HF but the reason remains unclear. This may be due to the poor solubility of CsF in DCM.

Table 3.4 Screening of fluoride sources



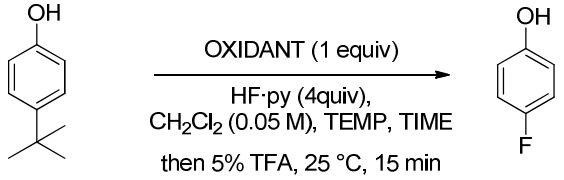
3-40 **3-41**

ENTRY	F- SOURCE	ADDITIVE	YIELD
1	HF·py	None	50 %
2	TBAF	None	0 %
3	TBAF	AcOH	0 %
4	CsF	None	0 %
5	CsF	AcOH	0 %
6	CsF	$KHSO_4$	0 %
7	TBAF	TFA	29%
8	CsF	TFA	47 %
9*	CsF	None	10 %

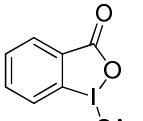
*PIFA was used as the oxidant

Hypervalent iodine (III) compounds were found to be the most efficient oxidant for this chemistry (**Table 3.5**). *Para*-fluorinated phenol **3-11** can be obtained using metal oxidants such as MnO_2 and Ag_2O, however, only trace amount of product could be observed in the ^{19}F NMR spectrum of crude reaction mixtures (Entries 5 and 6). Polyvalent iodine (III) compound **3-44** was synthesised and used for fluorination to give 4-fluorophenol **3-11** in 22% yield (Entry 7).

Table 3.5 Screening of oxidant



3-40 **3-11**

ENTRY[a]	OXIDANT	TEMP	TIME	YIELD[b]
1	CAN	0 °C	15 min	0 %
2	DDQ	0 °C	15 min	0 %
3	Oxone	25 °C	60 min	N. R.
4	H_2O_2·urea	25 °C	60 min	N. R.
5	MnO_2	25 °C	60 min	trace
6	Ag_2O	25 °C	60 min	trace
7	 **3-44**	25 °C	60 min	22 %

[a] All reactions are conducted with *tert*-butylphenol (1 mmol) scale.
[b] Yield based on HPLC area integration. N.R. = No reaction

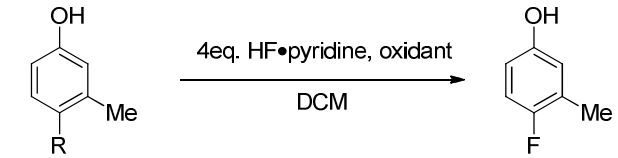
3.2.5. Oxidative-fluorination of substituted phenols

Having demonstrated the viability of the oxidative-fluorination strategy, we turned to investigate the scope of the transformation. The optimal procedure for the oxidative-fluorination involves addition of 1.0 mmol of oxidant (PIDA or PIFA) to a solution of DCM containing 1.0 mmol of phenol substrate, 4.0 mmol of HF•pyridine and 4.0 mmol of TFA when the *tert*-butyl substituted precursor was used. The reaction was quenched by addition of potassium carbonate, filtered and concentrated under reduced pressure. Isolated yields were not presented in some cases when the pure product could not be separated form the reaction mixture.

Oxidative fluorinations on the *meta*-methyl substituted phenol **3-45a** and unsubstituted phenol **3-11** were of similar efficiency (**Table 3.6**, Entry 1). No improvement in terms of

conversion or yield was observed when *meta*-cresol **3-45a** was treated with PIFA or at increased temperature (**Table 3.6**, Entry 1 *vs* 2). Notably, when 4-(*tert*-butyl)-3-methylphenol **3-45b** was subjected to fluorination followed by acidic cleavage of the *tert*-butyl group, 4-fluoro-3-methylphenol **3-46** was obtained in 39% yield (**Table 3.6**, Entry 3). The improved efficiency is attributed to the formation of corresponding tertiary phenoxenium intermediate.

Table 3.6 Oxidative Fluorination of 3-methylphenol



3-45a R = H
3-45b R = ^{t}Bu

3-46

Entry	R	Oxidant	Eq.	Temp.	Time	Conversion	Yield[a]
1	H	PIDA	1	0 °C	10 min	61%	6% (10%)
2	H	PIFA	1	25 °C	30 min	72%	5% (7%)
3	^{t}Bu	PIFA	1	25 °C	30 min	100%	39%(39%)[b]

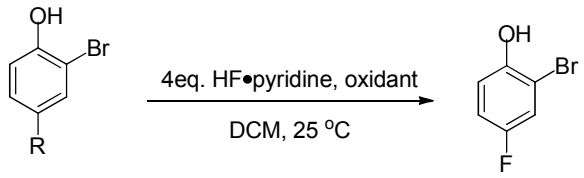
a. Yield based on column chromatography separation; bracket denotes yield based on conversion; conversion is calculated based on starting material remains. b. Yield based on fluoro *m*-cresole after cleavage of ^{t}Bu group by treating with 5% TFA at 25 °C for 10 min.

Oxidative fluorination of *o*-bromophenol **3-47a** using PIDA or PIFA as the oxidant, afforded 2-bromo-4-fluorophenol **3-48** in 9% and 4% yields, respectively (**Table 3.7**, Entries 1 and 2).[23] Encouragingly, when the *tert*-butyl substituted *o*-bromophenol **3-47b** was fluorinated, with subsequent acidic cleavage of the *tert*-butyl group, fluorinated **3-48** can be obtained in up to 80% yield within 15 min (**Table 3.7**, Entry 3). The improved efficiency in oxidative fluorination of 4-(*tert*-butyl)-2-bromophenol **3-47b** compared with 4-*tert*-butylphenol **3-40**,

[23] We thank Dr. M. Gao for repeating the reactions.

may be rationalised by the donating effect of the bromo substituent *via* conjugation to stabilise the tertiary phenoxenium intermediate.

Table 3.7 Fluorination of 2-bromophenol



3-47a R = H
3-47b R = ^{t}Bu

3-48

Entry	R	Oxidant	Eq.	Time	Yield[a]
1	H	PIDA	1	30 min	9%
2	H	PIFA	1	30 min	4%
3	^{t}Bu	PIDA	1	15 min	80%[b]
4	^{t}Bu	PIFA	1	15 min	66%[b]

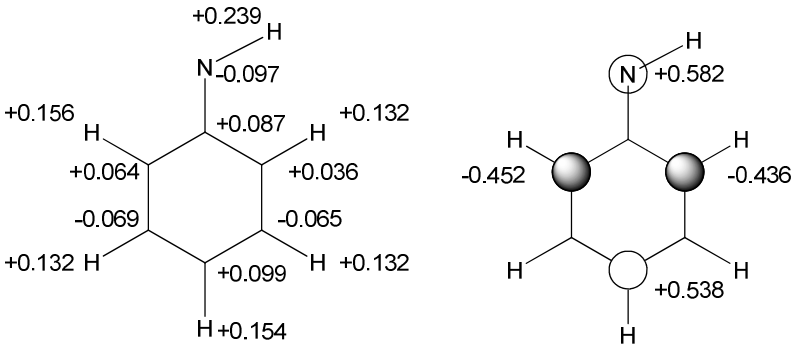
a. Yield based on HPLC separation. b. Yield based on 2-bromophenol after cleavage of ^{t}Bu group by treating with 5% TFA at 25 °C for 10 min.

3.3. Oxidative fluorination for the synthesis of 4-fluoroaniline[24]

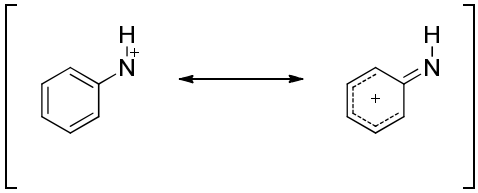
We have showed that 4-fluorophenol is accessible directly from phenol or 4-*tert*-butylphenol *via* the proposed "oxidative fluorination" method. Accordingly, we attempted to expand the scope of the chemistry to the one-pot synthesis of 4-fluoroaniline. The Shudo group reported the charge distribution and LUMO coefficients of the phenylnitrenium ion based on ab initio calculations using the STG-3G basis set. The positive charged is found to be located predominantly on the *para* carbon (**Scheme 3.17**). The results also indicate substantial contributions of the quinoid resonance structures. The C–N bond length of 1.311 Å is close

[24] Experiments in this section were carried on in collaboration with Dr. Y. H. Lim and have been presented in her Dphil thesis.

to those calculated for methylene imine (1.273 Å), indicating significant double-bond character. (**Scheme 3.18**).[174]



Scheme 3.17. STO-3G-calculated charge distribution (left) and LUMO coefficient of phenylnitrenium ion.[25]



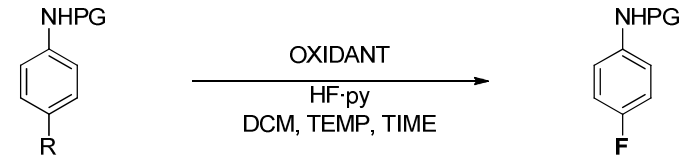
Scheme 3.18. Phenylnitrenium intermediate of the oxidative-fluorination

Addition of PIDA to a solution of aniline **3-49a** in DCM, in presence of HF•pyridine, resulted in a series of unidentified side products with no fluorinated compound being observed (**Table 3.8**, Entry 1). Pleasingly, when aniline **3-49a** was protected with a tosyl group, **3-49b**, fluorination using the established procedure gave *para*-fluorinated phenylsulfonamide **3-51b** in 10% isolated yield (**Table 3.8**, Entry 2). The preferential formation of 4-fluorophenylsulfonamide **3-51b** is in line with the reported LUMO coefficents. A range of cleavable electron-withdrawing groups were selected as the protecting group to access 4-fluoroaniline **3-51a**.

[25] The Scheme is reprinted from reference 174, with permission from American Chemical Society.

Mono-protected *N*-Boc-aniline **3-49c** was not reactive with PIDA but could be oxidised smoothly by PIFA to give *para*-fluoroaniline **3-51a** in 15% yield, after acidic deprotection of the Boc group in 10% TFA (**Table 3.8**, Entry 3 *vs* 4). Similar efficiency in the oxidative fluorination was observed when CsF and TFA were used as the fluoride source, in place of HF•pyridine (**Table 3.8**, Entry 5). When aniline **3-49a** was mono-protected by trifluoroacetate, the resulting phenylacetamide **3-49d** was inert towards oxidation. (**Table 3.8**, Entries 6 and 7). As expected, a *tert*-butyl substituent *para* to the protected aniline aids the reaction. Thus, oxidative fluorination of *N*-Boc-4-*tert*-butylaniline **3-50c**, followed by treatment of 10% TFA gave the desired 4-fluoroaniline **3-51a** in 37% yield over the two steps (**Table 3.8**, Entry 8).

Table 3.8 Extension of the two-step one-pot synthesis of 4-fluoroaniline



R = H 3-49
R = ^{t}Bu 3-50

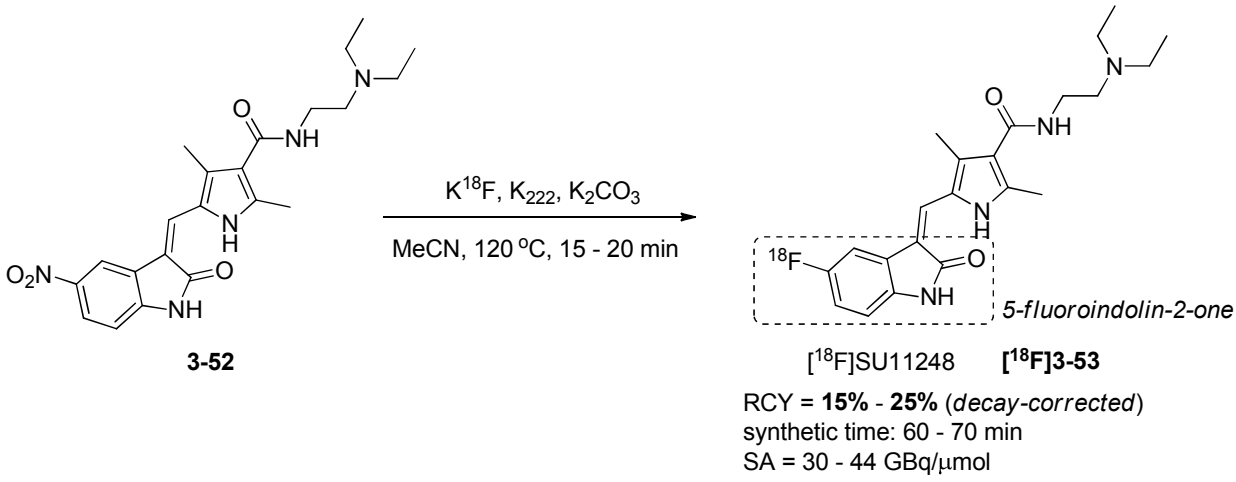
3-51

Entry	PG	R	OXIDANT (1 eq)	HF·py	CONCENTRATION	TEMP	TIME	YIELD
1	**3-49a**: H	H	PIDA	4 eq	0.05	0 °C	10 min	0 %
2	**3-49b**: Ts	H	PIDA	4 eq	0.05	0 °C	10 min	10 %
3	**3-49c**: Boc	H	PIDA	4 eq	0.05	0 °C	10 min	0 % (N.R.)
4	**3-49c**: Boc	H	PIFA	4 eq	0.05	RT	30 min	15 %*
5	**3-49c**: Boc	H	PIFA	"CsF (4 eq) + TFA (4 eq)"	0.05	RT	30 min	11 %*
6	**3-49d**: $COCF_3$	H	PIDA	4 eq	0.05	0 °C	10 min	0 % (N.R.)
7	**3-49d**: $COCF_3$	H	PIFA	4 eq	0.05	RT	30 min	0 % (N.R.)[a]
8	**3-50c**: Boc	^{t}Bu	PIFA	4 eq	0.05	RT	30 min	37 %*

* Yield based on 4-fluoroaniline **3-51a** obtained after cleavage of protecting group by treating with 10% TFA at 25 °C for 10 min. a. No reaction observed after up to 2 hours at 25 °C.

3.4. Oxidative fluorination for the synthesis of 4-fluorooxindole

Oxindole skeletons exist in a variety of natural products and are the precursors to many pharmaceuticals.[177, 178] For example, the 5-fluoroindolin-2-one motif was found in SU11248 **3-53** (**Scheme 3.19**), a potent anticancer drug marketed by Pfizer as Sutent®.[179] ^{18}F-labelled radiotracers (such as [^{18}F]FDG) have been used to monitor tumor response to antiangiogenic Sutent therapy.[180, 181] The Zheng group reported the synthesis of [^{18}F]SU11248 **[^{18}F]3-53** as a PET tracer for imaging tyrosine kinase. The key transformation involves a direct S_NAr ^{18}F-fluorination of the electron-deficient precursor **3-52** with the nitro functionality acting as the leaving group. Formulated **[^{18}F]3-53** can be obtained in 60-70 min with a specific activity of 30 – 44 GBq/μmol in 15 – 25% RCY, *decay-corrected* to the end of bombardment (**Scheme 3.19**).

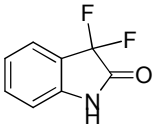
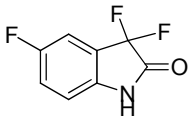
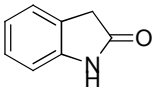
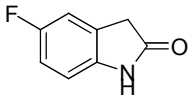


Scheme 3.19. Synthesis of [^{18}F]SU11248 **[^{18}F]3-53** for imaging cancer tyrosine kinase

Aiming to expand the scope of the oxidative fluorination strategy to access 5-fluorinated oxindolin-2-one, unsubstituted oxindoles **3-54** and **3-56** were treated with 1 equivalent of

PIFA in a solution of DCM with 4 equivalents of HF•pyridine, according to the conditions validated previously. The 3,3-difluoroindolin-2-one **3-54** was not responsive to oxidation, possibly due to the electronegativity of the gem-difluoro functionality (**Table 3.9**, Entry 1). Pleasingly, fluorine could be incorporated *para* to the acetamide functionality of oxindole **3-55** upon oxidative-fluorination, in 17% yield (Entry 2). The result suggested that an alternative ^{18}F-nucleophilic radiolabelling method for the pharmacologically important sub-motif would be feasible, after adapting the chemistry to a radiolabelling protocol.

Table 3.9 Oxidative-fluorination of oxindole

Entry	Substrate		Product		Condition	Yield
1		**3-54**		**3-55**	A	**N.R.**
2		**3-56**		**3-57**	A	**17%**[a]

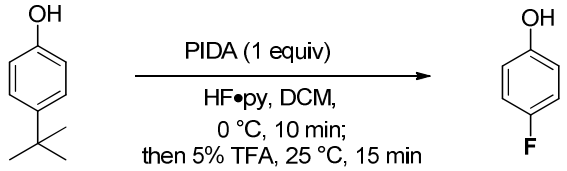
N.R. = No Reaction. a. Yield was determined by NMR integration; Identity of product was determined by comparation with the corresponding commercial compound. Condition: A: PIFA (1 eq), HF·py (4 eq), TFA (4 eq), RT, 30 min.

3.5. Investigation into the effects of quantity of HF•pyridine on the efficiency of the fluorination reaction

In the context of radiochemistry, stoichiometry differs considerably from that used in conventional synthesis. All previous results are based on an excess amount of fluoride, the isotopically labelled form of which will be sub-stoichiometric by a substantial margin. Since the generation of the carbon cationic intermediate is the key step of the oxidative-fluorination of *tert*-butyl phenol, we were curious if the kinetic profile of the reaction would change in a sub-stoichiometric amount of fluoride, hence changing the efficiency of the reaction in the radiolabelling process.

A series of reactions were carried out to investigate the effects of the quantity of HF•pyridine on the efficiency of the fluorination reaction. The yield of the fluorination reaction dropped (from 35% to 21% yield) when the stoichiometric amount of fluoride was decreased (from 4.0 equiv to 1.0 equiv, Entry 1 vs 3, **Table 3.10**). An initial 10-fold decrease in the concentration of HF•pyridine to sub-stoichiometric level (from 1.0 equivalent to 0.1 equivalent) showed no change in chemical yield (**Table 3.10**, Entry 3 vs 4) whereas further lowering of the number of equivalents HF•pyridine (from 0.1 equivalent to 0.01 equivalent) led to tremendous drop in efficiency (from 21% to 6%, Entry 4 vs 5, **Table 3.10**). These results indicate a decreasing synthetic efficiency with a decrease in the amount of fluoride. Notably, at pronounced low stoichiometric levels of fluoride (0.01 equivalent), 4 fold improvements in the yield of the fluorination can be obtained by increasing the concentration of the substrate by 20 times (from 6% to 28% Entry 5 vs 6, **Table 3.10**). However, the efficiency of the fluorination reaction did not increase at higher concentration when HF•pyridine is present in excess (Entry 1 vs 2, **Table 3.10**).

Table 3.10 Oxidative-fluorination at different concentrations of fluoride



3-40 **3-11**

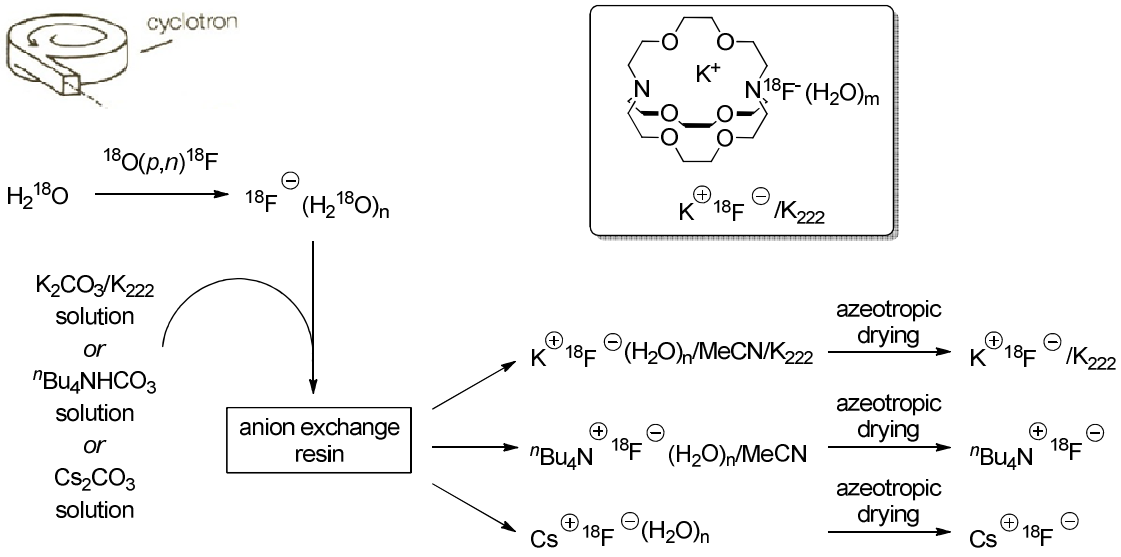
Entry[a]	HF·py	[Conc]	Yield[b]
1	4.0 equiv	0.05 M	35 %
2	4.0 equiv	1.0 M	19 %
3	1.0 equiv	0.05 M	21 %
4	0.1 equiv	0.05 M	21 %
5[c]	0.01 equiv	0.05 M	6 %
6[c]	0.01 equiv	1.0 M	28 %

a. All reactions are conducted with *tert*-butylphenol (1 mmol) scale. b. Yield based on HPLC area integration. c. 50 mL of a HF·py in DCM stock solution (26 mL in 5 mL DCM) was used.

3.6. Oxidative-[^{18}F]fluorination in ^{18}F-radiochemistry

3.6.1 Radiolabelling in a Conventional Synthetic Module[26]

The oxidative-[^{18}F]fluorination was first attempted using the three commonly used ^{18}F-fluoride sources, K[^{18}F]F/K_{222}, TBA[^{18}F]F and Cs[^{18}F]F. The [^{18}F]fluoride in [^{18}O]H_2O was firstly adsorped onto an anion exchange resin and eluted by a small volume (~0.5 – 1 mL) of a weak base in CH_3CN/H_2O, such as K_2CO_3/K_{222} (Kryptofix 2.2.2), *n*BuNHCO$_3$ or Cs_2CO_3 for the formation of K[^{18}F]F/K_{222}, TBA[^{18}F]F and Cs[^{18}F]F respectively. The eluent was further dehydrated by successive azeotropic evaporation with acetonitrile and a flow of inert gas to yield anhydrous K[^{18}F]F/K_{222}, TBA[^{18}F]F and Cs[^{18}F]F as nucleophilic reagents for ^{18}F-fluorination (**Scheme 3.20**) .



Scheme 3.20. Synthesis of the nucleophilic reagents for ^{18}F-fluorination: K[^{18}F]F/K_{222}, TBA[^{18}F]F and Cs[^{18}F]F .

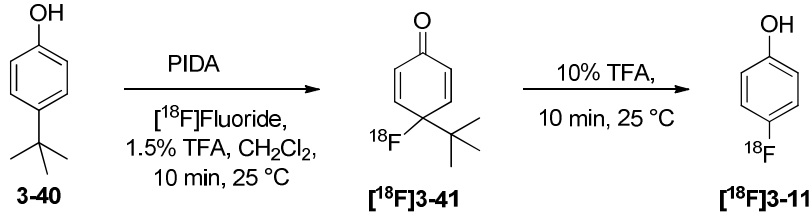
[26] Experiments in this section were carried on in collaboration with Dr. Y. H. Lim and have been presented in her Dphil thesis.

Upon drying, the ^{18}F-fluorinated reagent was dissolved in DCM (100 µL), followed by addition of a solution of 4-*tert*-butylphenol/TFA (3.0 µL in 100 µL of DCM) and PIDA as the oxidant. The reaction mixture was kept for 10 min at room temperature. Additional TFA (20 µL, ~10% v%) was added to cleave of *tert*-butyl group, by stirring the reaction mixture for a further 10 min at 25 °C. Acetonitrile (300 µL) was used to dilute the reaction mixture which was then analysed by HPLC and radio-TLC.

Unfortunately, no ^{18}F-fluorination occurred even when the concentration of the *tert*-butylphenol **3-40** substrate was increased from 0.05 M to 0.5 M (**Table 3.11**, Entries 1 – 5). Pleasingly, when the PIDA oxidant was added to a plastic vial containing [^{18}F]TBAF or [^{18}F]CsF, in presence of 1.5% TFA and 5.0 M *tert*-butylphenol **3-40**, 4-[^{18}F]fluorophenol **[^{18}F]3-11** was formed in 4% and 6% RCY respectively, upon treatment in 10% TFA for 10 min (**Table 3.11**, Entries 8 – 9). In addition, a 3% RCY was obtained when phenol **3-43** was used as the substrate (**Table 3.11**, Entry 10).

The results underlined the feasibility of the direct transformation from [^{18}F]fluoride to *para*-^{18}F-labelled phenol **[^{18}F]3-11** *via* oxidative ^{18}F-fluorination of 4-*tert*-butylphenol **3-40** or phenol **3-43**. Although in considerably low efficiency (3 – 6%), this ^{18}F-fluorination has never been reported before. [^{18}F]TBAF was the superior [^{18}F]F$^-$ source because of its good solubility in dichloromethane and dichloroethane when compared with [^{18}F]CsF. No fluorinated product was observed when K[^{18}F]F/K$_{222}$ was used as the ^{18}F-fluorinating reagent.

Table 3.11 ^{18}F-fluorination with conventional radiolabelling method



3-40 **[^{18}F]3-41** **[^{18}F]3-11**

ENTRY	[^{18}F]FLUORIDE	SUBSTRATE	PIDA	[CONC][a]	YIELD[b]
1	K[^{18}F]F/K_{222}	0.01 mmol	0.01 mmol	0.05 M	0%
2	TBA[^{18}F]F	0.01 mmol	0.01 mmol	0.05 M	0%
3	Cs[^{18}F]F	0.01 mmol	0.01 mmol	0.05 M	0%
4	TBA[^{18}F]F	0.05 mmol	0.05 mmol	0.25 M	0%
5	TBA[^{18}F]F	0.10 mmol	0.10 mmol	0.50 M	0%
6	TBA[^{18}F]F	0.20 mmol	0.20 mmol	1.0 M	0%
7	TBA[^{18}F]F	0.50 mmol	0.50 mmol	2.5 M	0%
8	TBA[^{18}F]F	1.0 mmol	1.0 mmol	5.0 M	4%
9	Cs[^{18}F]F	1.0 mmol	1.0 mmol	5.0 M	6%
10[c]	Cs[^{18}F]F	1.0 mmol	1.0 mmol	5.0 M	3%

a. Concentration refers to the substrate concentration in CH_2Cl_2; *tert*-butylphenol: PIDA = 1:1. b. Yield refers to the yield of 4-[^{18}F]fluorophenol **[^{18}F]3-11** after deprotection in 10%TFA; Yield calculated based on peak integration of radio-HPLC trace; Identification of product was confirmed by spiking the reaction mixture with reference compound. c. Phenol **3-43** was used as the substrate.

3.6.2 Radiolabelling in a Microfluidic Module[27]

Microfluidic reactors are generally defined as devices with internal structural features measured in microns (typically 10–500 μm) that are capable of performing chemical or biological reactions in a continuous-flow manner.[182],[183] By decreasing the physical size of reactor to microscale, the *surface area-to-volume* ratio will be increased dramatically. The *surface area-to-volume value* of a microfluidic reactor can be as high as 50,000 m^2 per m^{-3} whereas for conventional reactors (e.g. a round-bottom flask with stirrer), is not higher than

1000 m^2 per m^{-3}. The increase of *surface area-to-volume* value can lead to significant improvements in reactions performed in a microfluidic environment in terms of mixing rate, heat transfer efficiency, reaction rates and yields. This "Lab-on-a-chip" technology is becoming more and more important for the preparation of radiotracers. In the context of radiochemistry, reactions performed in a microfluidic synthesiser are commonly carried out in a continuous-flow manner, through a reactor chip with a typical volume of 16 – 64 μL. In a conventional batch-mode synthetic module, the reaction volume ranges from 0.3 to 1.0 mL. By decreasing the volume of the reaction, dilution of the radioactive species which are substantially sub-stoichoimetric (nmol - μmol *vs* μmol – mmol of the non-radioactive reagents), can be minimised.

We proposed that the efficiency of radiolabelling could be greatly improved by using a microfluidic synthetic module. We believed that the microfluidic strategy would give an increased concentration of ^{18}F-radioisotope (~3 GBq in 16 – 64 μL of solvent) and the high *surface area-to-volume* value of the microfluidic reactor would significantly improve the mixing efficiency, which was a key parameter for further optimisation.

Therefore, the oxidative ^{18}F-radiolabelling strategy was examined on a microfluidic synthetic module (NanoTEK®Advion). The synthesiser is built around a concentrator for drying the ^{18}F-fluoride and two microfluidic reactors. All reagents are dissolved in appropriate solvents, loaded into the system in large volumes and transported into storage loops. The precise amounts of solutions are dispensed to the chip, using three independent syringe pumps through primed tubes. The diagram of the automatic pumping module is shown in **Figure 3.1**.

[27] Experiments in this section were carried on in collaboration with Dr. M. Huiban and have been repeated by Dr. M. Gao.

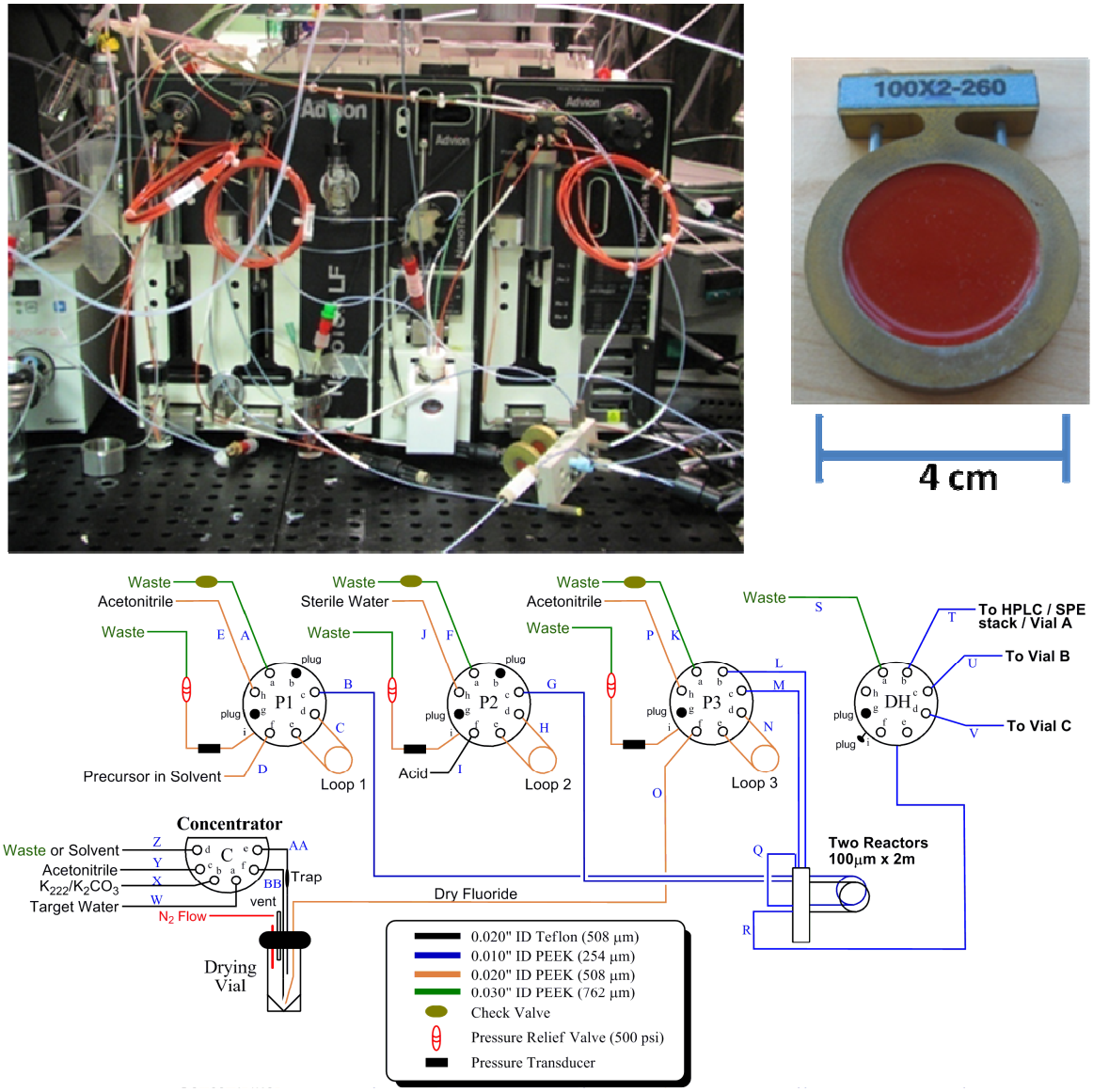


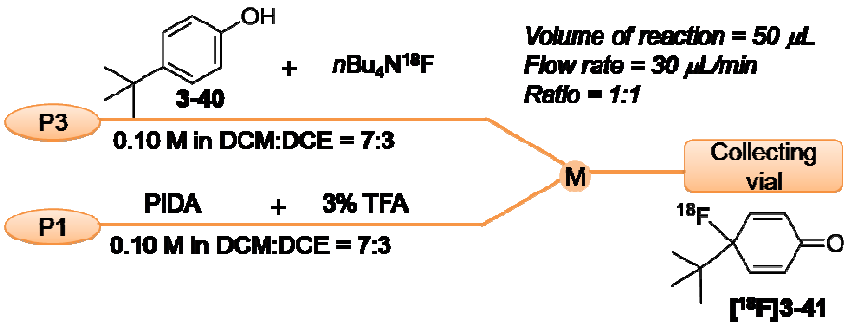
Figure 3.1. Top left: view of the synthesiser; top right: microfluidic reactor; Bottom: automatic pumping diagram of the NanoTEK microfluidic system.[28]

Non-carrier-added aqueous [^{18}F]fluoride was produced in a cyclotron, delivered to a vial (connected *via* tube **W** as in **Figure 3.1**) and adsorbed onto an anion-exchange cartridge. The [^{18}F]fluoride was subsequently eluted by a solution of nBu$_4$NHCO$_3$ (8 mg) in acetonitrile/water (4:1, 500µl) into the drying vial (*via* tube **X**). The solution was dried with

[28] The photos and diagram are provided by Dr M. Huiban and reprinted with permission.

two cycles of azeotropic drying with acetonitrile (300µl, tube **Y**), according to the standard protocol for the formation of [^{18}F]TBAF in the microfluidic module.

[^{18}F]TBAF was dissolved with the *tert*-butylphenol **3-40** substrate in a solution (of DCM : DCE = 7 : 3, 0.10 M) and loaded in Loop 3 *via* Pump 3 (**P3**, **Scheme 3.21**). PIDA in a separate solution (of DCM : DCE = 7 : 3, 0.10 M with 3% TFA) was loaded into Loop 1 *via* Pump 1 (**P1**, **Scheme 3.21**). Oxidative ^{18}F-fluorination occurs when a 50 µL solution of PIDA and TFA from Pump1 **P1** was mixed with a 50 µL solution of *tert*-butylphenol and [^{18}F]TBAF from Pump3 **P3** in the microfluidic chip **M** at the rate of 30 µL/min, in a ratio of 1:1. Subsequently, the collected reaction mixture (with total volume ~200 µL) was analysed by HPLC and the distribution profile of radioactive species was used to evaluate the efficiency of the ^{18}F-fluorination.



Scheme 3.21. Representation of the ^{18}F-fluorination on a microfluidic system, P = pump, M = microfluidic reactor.

HPLC analysis revealed that ^{18}F-labelled cyclohexadienone **[^{18}F]3-41**, with a retention time of 8.86 min was the major ^{18}F-fluorinated product in the reaction (**Figure 3.2**). The identity of ^{18}F-fluorinated **[^{18}F]3-41** was confirmed by spiking the reaction mixture with the authentic compound **3-41**.

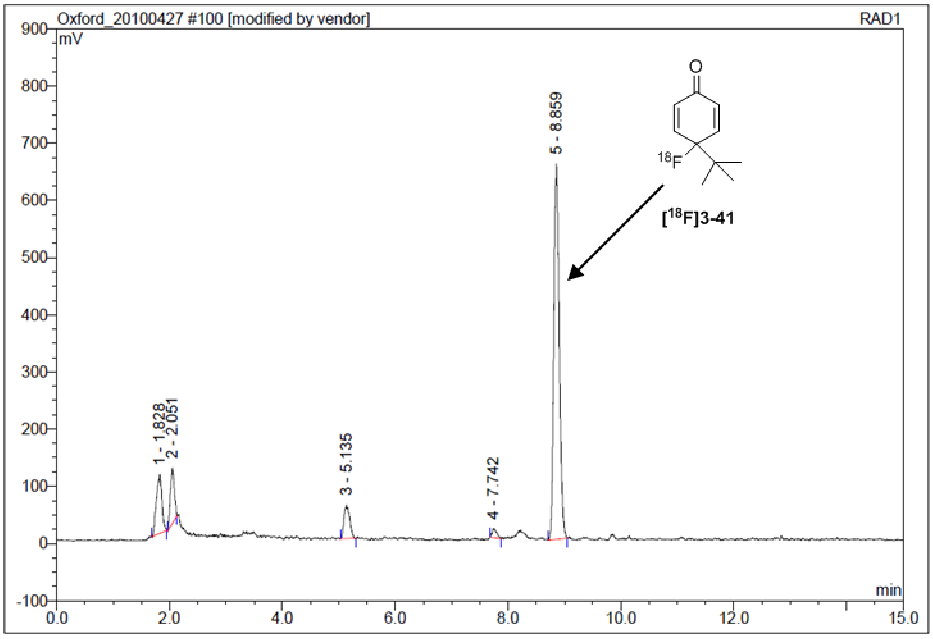


Figure 3.2. Radioactivity HPLC spectrum of crude reaction mixture of the ^{18}F-fluorination on *tert*-butylphenol **3-40** (before acid-mediated rearomatisation)

Acid-mediated rearomatisation was performed by adding TFA (10 µL) to 100 µL of the reaction mixture. The solution was kept at room temperature for 10 min and analysed by HPLC. The disappearance of the radioactive peak with a retention time of 8.86 min and appearance of a new radioactive peak with a retention time of 6.67 min, indicated the acid-mediated cleavage of *tert*-butyl group (**Figure 3.3**). Also, we observed that the proportion of a radioactive peak with the retention time of 2.00 min, was increased after the addition of TFA. This suggested that undesired side-reactions, such as decomposition of the oxidative ^{18}F-fluorinated intermediate **[^{18}F]3-41** or final product **[^{18}F]3-11** occurred during the acid-promoted transformation. Unfortunately, we were not able to identify the radioactive species at 2.00 min.

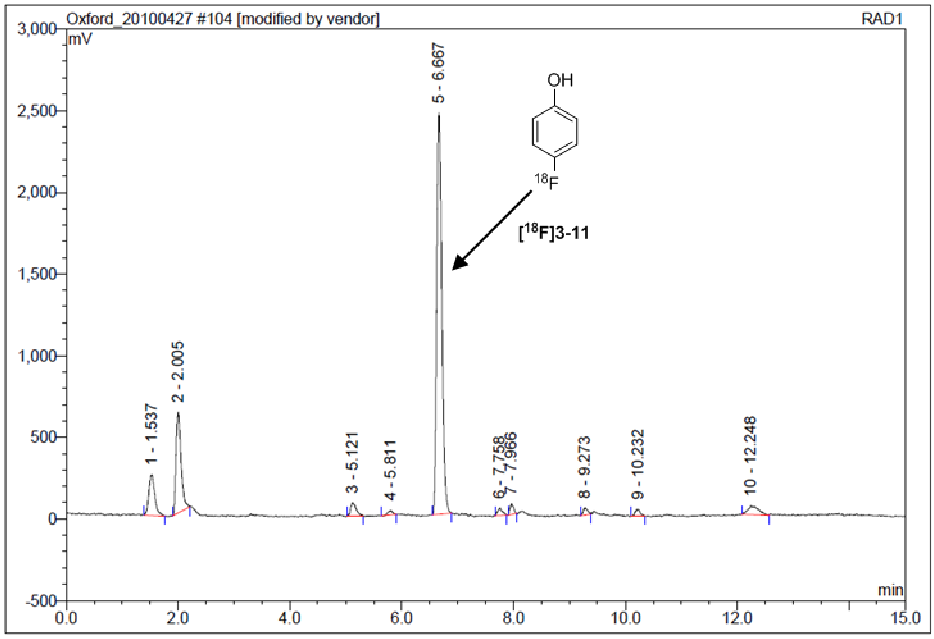
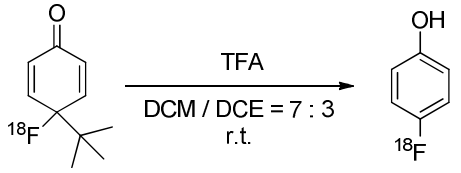


Figure 3.3. Radioactivity HPLC spectrum of crude reaction mixture of the ^{18}F-fluorination on *tert*-butylphenol (after acid-mediated rearomatisation)

With the aim to determine the opitimised conditions for the rearomatisation step after the ^{18}F-fluorination, 100 µL of the collected ^{18}F-fluorination mixture was treated by various amounts of TFA. No conversion of the ^{18}F-labelled dienone **[^{18}F]3-41** to 4-[^{18}F]fluorophenol **[^{18}F]3-11** was observed when the reaction mixture was treated by 5% TFA for 25 min (**Table 3.12**, Entry 1). Accordingly, 71% conversion from intermediate **[^{18}F]3-41** to *para*-^{18}F-labelled phenol **[^{18}F]3-11** was obtained with a higher concentration of TFA (10% v%, **Table 3.12**, Entry 3). However, extending the reaction time from 10 min to 25 min led to a decreased conversion from 71% to 18% (**Table 3.12**, Entry 3 *vs* 2).

Table 3.12 Investigation into the effect of conditions of re-aromatization on the efficiency of the oxidative ^{18}F-fluorination.



[^{18}F]3-41 **[^{18}F]3-11**

ENTRY	TFA	TIME	conversion[a]	*n*
1	5%	25 min	0%	1
2	10%	25 min	18%	1
3	10%	10 min	71%	3

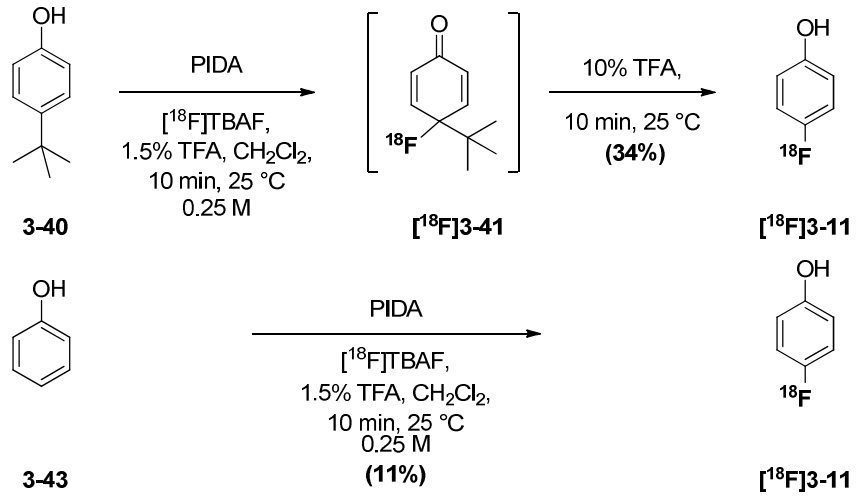
a. The decay-corrected conversion based on HPLC area integration to evaluate the efficiency of re-aromatization.

HPLC can be an efficient method for both evaluating conversion in a chemical transformation and the confirmation of the identity of the labelled product. We are aware that HPLC conversion is not a RCY in an ^{18}F-fluorination processes from [^{18}F]fluoride because of the high tendency of fluoride to stick on the reversed stationary phases.

Therefore, the radioactivities of the reaction mixture (prior to HPLC injection) and the collected radioactive product (after HPLC isolation) were measured to determine the distribution profile of radioactivity. After HPLC separation of the reaction mixture with pre-measured radioactivity, 34% of radioactivity can be collected and assigned to the labelling product 4-[^{18}F]fluorophenol **[^{18}F]3-11**.

In addition, the radiolabelling procedure proceeded well when phenol **3-43** was used as the substrate (**Scheme 3.22**). The HPLC distribution profile of radioactivity in the crude reaction mixture showed that 34% was attributed to 4-[^{18}F]fluorophenol **[^{18}F]3-11**, 18% to unreacted [^{18}F]fluoride and the rest 46% to unidentified [^{18}F]fluorinated side products.

Similarly, 11% of radioactivity can be isolated and attributed to 4-[^{18}F]fluorophenol **[^{18}F]3-11** after HPLC separation of the reaction mixture with pre-measured radioactivity.



Scheme 3.22. ^{18}F-fluorination on a microfluidic system.

The feasibility of a robust radiosynthesis of 4-[^{18}F]fluorophenol **[^{18}F]3-11** using the "oxidative-^{18}F-fluorination" was validated. The approach harnessed the advantages of the microfluidic synthesis system to improve the synthetic efficiency. With the established protocol in hand, further work was aimed to apply the radiosynthesis to ^{18}F-label a clinical radiotracer with 4-[^{18}F]fluorophenol sub-motif, such as 6-[^{18}F]fluoro-*m*-tyrosine ([^{18}F]FmT, **[^{18}F]3-2**).

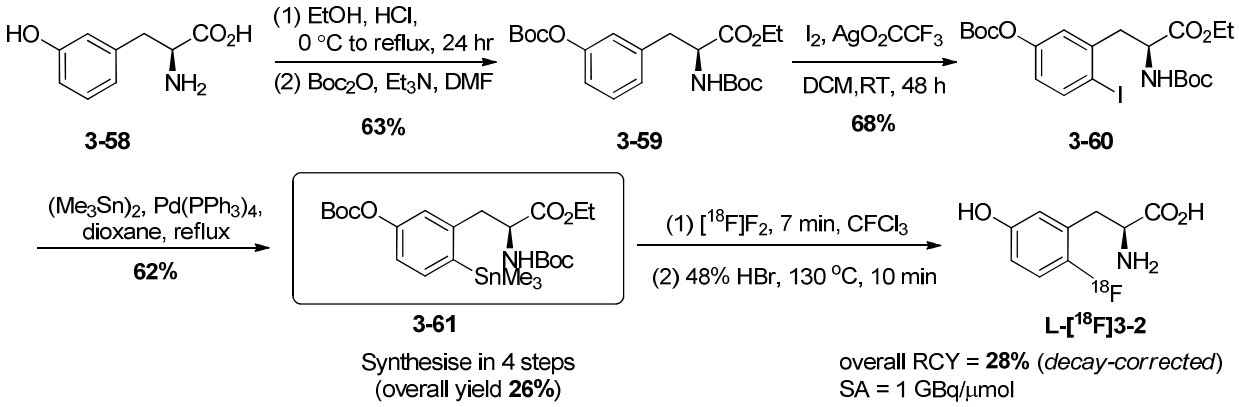
3.7. Oxidative ^{18}F-Fluorination towards a clinical radiotracer

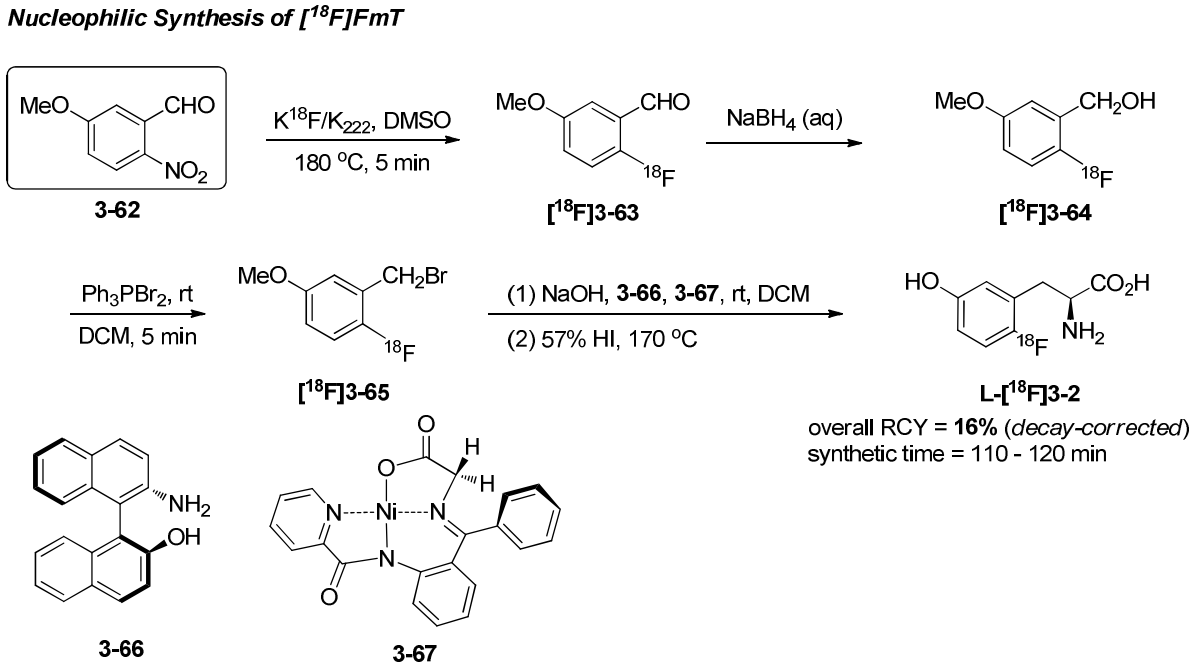
[^{18}F]FmT **[^{18}F]3-2** can be used as a PET tracer to study intracerebral dopamine metabolism, to diagnose Parkinson's disease and evaluate treatment responses.[155, 184] A preliminary clinical study[185] suggested that [^{18}F]FmT **[^{18}F]3-2** would be considerably advantageous over [^{18}F]FDOPA **[^{18}F]3-1** for the measurement of intracerebral AADC (aromatic acid decarboxylase) activity, due to its simpler peripheral metabolism. Accurate and quantitative kinetic analysis of [^{18}F]FDOPA **[^{18}F]3-1** is difficult. This is because the tracer readily forms

^{18}F-labelled methylated metabolites (*via* COMT, catechol-*O*-methyltransferase), which can also enter the brain. Hence the kinetic study of [^{18}F]FDOPA **[^{18}F]3-1** requires compartmental modeling of several physiological processes in brain and plasma. [186]

Currently preparation of [^{18}F]FmT involves either an electrophilic fluorodestannylation using [^{18}F]F_2 gas[187] or nucleophilic fluorination on an electron-deficient aromatic precursor using ^{18}F-fluoride. The major drawback of the electronic method is that the synthesis involves a toxic stannyl precursor and delivers [^{18}F]FmT with low specific activity (typically no higher than 1 GBq/μmol) whilst the nucleophilic method is usually time consuming (~ 2 hours) and suffers from a low radiochemical yield (**Scheme 3.23**).[184]

Electrophilic Synthesis of [^{18}F]FmT





Scheme 3.23. Current synthetic methods for L-[^{18}F]FmT

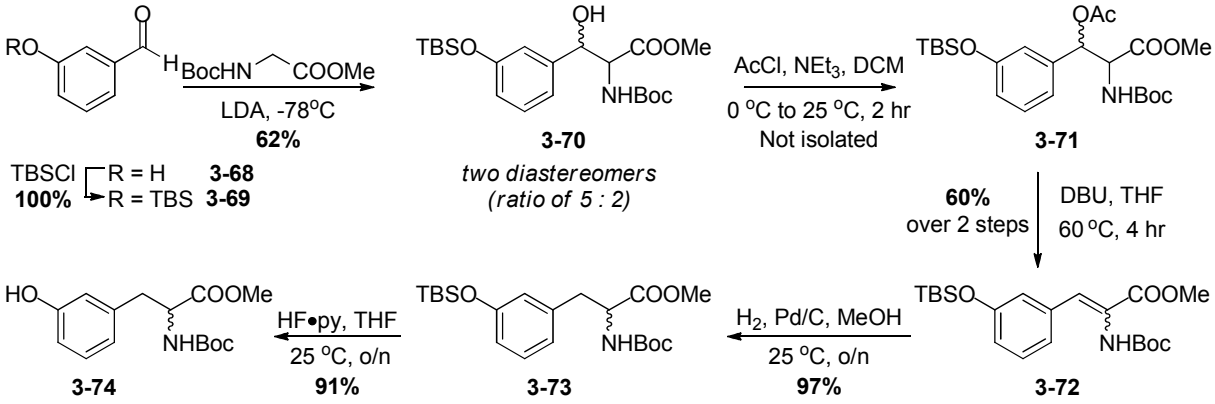
Prospectively, the established oxidative fluorination on phenol is potentially transferrable to the radiosynthesis of [^{18}F]FmT. This one-step transformation from ^{18}F-fluorine to [^{18}F]FmT, if feasible, would dramatically simplify the preparation protocol of the radiotracer. The major challenge will be to design an appropriate precursor to validate to "cold" synthesis of FmT directly from nucleophilic fluoride.

3.7.1. Protected *m*-tyrosine as precursor for FmT

We assumed that the precursor should contain the basic *meta*-tyrosine motif to give [^{18}F]FmT **3-2** in ^{18}F-labelled form. Since the amino acid functionality may interfere in the oxidative fluorination, we chose the protected *m*-tyrosine **3-74** as the 1st generation precursor.

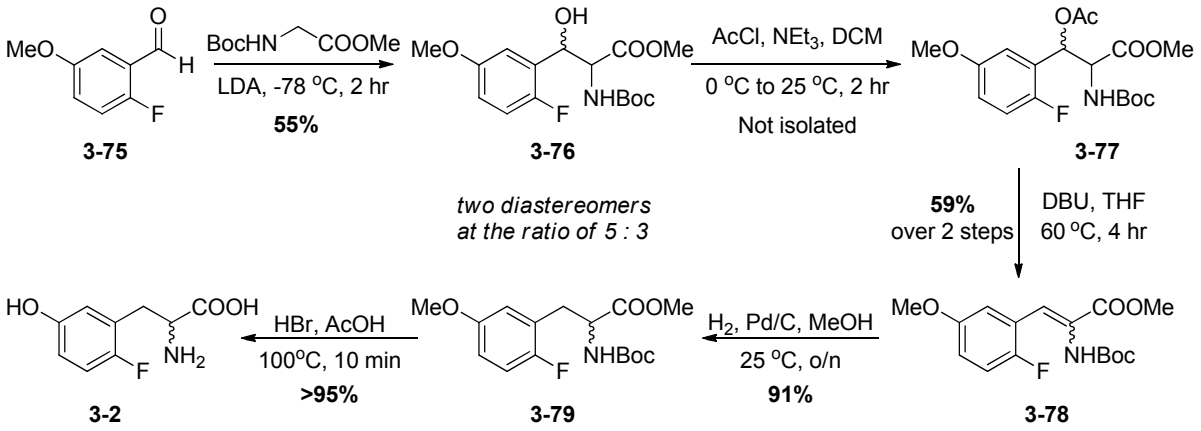
Commercial *N*-Boc-glycine methyl ester in a solution of THF was first reacted with LDA (lithium diisopropylamide) at -78 °C followed by addition of the TBS-protected benzaldehyde **3-69**. Aldolisation afforded the β-hydroxy-carbonyl compound **3-70** as two

diastereomers in a 5:2 ratio and 62% overall yield. The two diastereomers **3-70** were reacted with acetyl chloride to give the crude acetylated product **3-71** after an aqueous work-up. E1cB elimination of the acetylated aldol complex **3-71** with DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) gave the αβ-unsaturated carbonyl compound **3-72** in 60% yield over two steps. The double bond was then reduced (H_2, Pd/C (10%)) to afford the *meta*-tyrosine derivative **3-73** in 97% yield. Upon cleavage of the TBS protecting group, this 6-step synthetic route gave the protected *meta*-tyrosine **3-74** in 33% overall yield (**Scheme 3.24**).



Scheme 3.24. Synthesis of 1st generation precursor **3-74** for FmT.

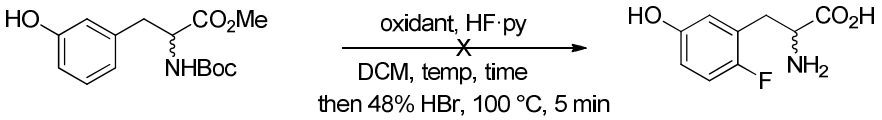
In addition, the synthetic method can also be applied to prepare FmT **3-2** "cold" using a fluorinated building block. In the last step of the synthesis, deprotection of the fluoro-m-tyrosine **3-79** was performed by dissolving it in a solution of 48% hydrogen bromide and acetic acid (1:1, *v*:*v*) and heating at 100 °C for 10 min. Purification on a semi-preparative HPLC column afforded the racemic FmT in excellent yield. This 5-step synthetic route gave FmT in 30% overall yield (**Scheme 3.25**).



Scheme 3.25. Synthesis of 6-fluoro-*m*-tyrosine (FmT, **3-2**).

Precursor **3-74** was subjected to the established oxidative fluorination protocol, in attempt to validate the oxidative fluorination strategy for the synthesis of FmT **3-2**. Based on ^{19}F-NMR and HPLC comparative analyses of the authentic product independently synthesised and the reaction mixture, we confirmed that fluorinated FmT **3-2** was not formed under all of the oxidative fluorination conditions (**Table 3.13**, Entries 1 – 4).

Table 3.13 Fluorination of 1st generation precursor 3-79



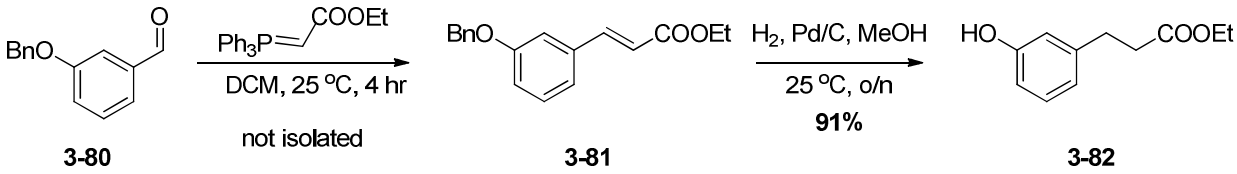
3-64 **3-2**

Entry	Oxidant (equiv)	F^- (equiv)	Temp	Time	Yield
1	PIDA (1)	HF·py (4)	0 °C	10 min	0%
2	PIFA (1)	HF·py (4)	25 °C	30 min	0%
3	PIFA (1)	HF·py (4)	25 °C	30 min	0%
4	PIFA (4)	HF·py (10)	25 °C	30 min	0%

We ascribed the failure in fluorination of protected tyrosine **3-74** to two plausible reasons. The presursor lacks a *tert*-butyl group. As shown in previous section, the existence of a *tert*-butyl group leads to significant improvement in the efficiency of fluorination. An

intramolecular nucleophile, either the nitrogen or oxygen in the amino acid substituent, compete with the intermolecular fluorination.

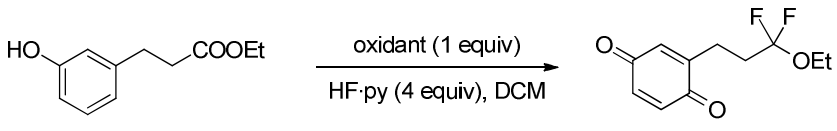
Therefore, we built up a simplified substrate **3-82** with the ester functionality as found in the target structure. Oxidative fluorination on the model compound should uncover the involvement of the ester group in the process of oxidative fluorination. The ethyl 3-(3-hydroxyphenyl)propanoate **3-82** was synthesised *via* a Wittig Reaction followed by palladium-mediated reduction. (**Scheme 3.26**).



Scheme 3.26. Synthesis of model substrate 3-82

The ester functioned **3-82** was oxidised by PIDA in the presence of HF•pyridine (**Table 3.14**, Entry 1). No aromatic fluorine was observed in ^{19}F NMR spectroscopy analysis of the crude reaction mixture and instead, a strong triplet was found at a chemical shift of -74.2 ppm with a coupling constant of 11.3 Hz, as the dominant fluorine peak. The pure fluorinated compound was carefully separated from a series of side products in 10% yield. The efficiency of fluorination decreased from 10% to 5% when the reaction was performed at 25 ^{o}C and PIFA used as the oxidant.

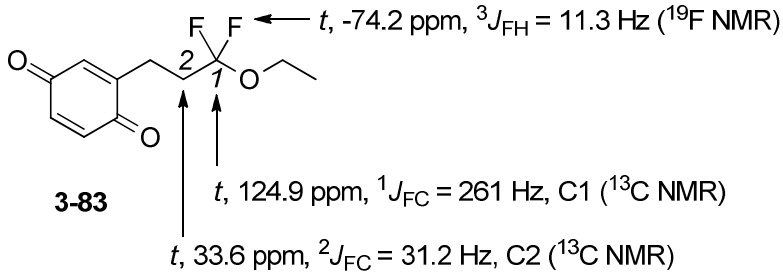
Table 3.14 Fluorination of 3-82



3-82 **3-83**

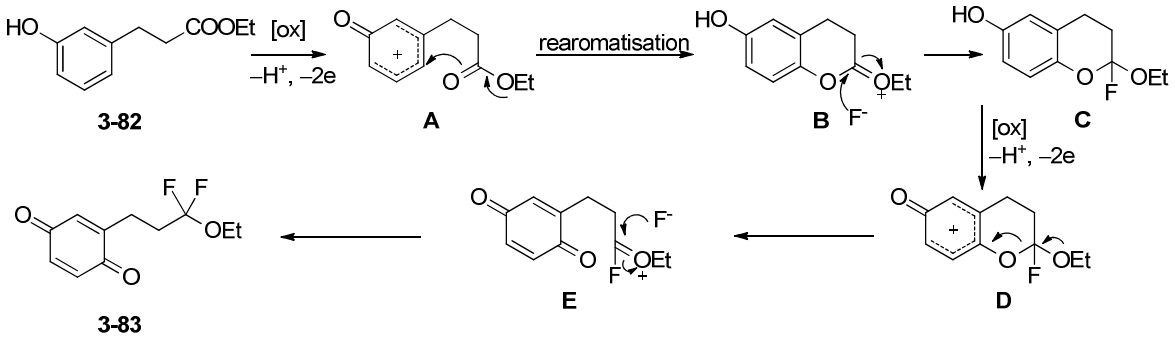
Entry	Oxidant	Temp	Time	Yield
1	PIDA	0 °C	10 min	10%
2	PIFA	25 °C	30 min	5%

The isolated product was identified as the benzoquinone structure **3-83** containing a 3-ethoxy-3,3-difluoropropyl functionality. The identification of the product was also supported by ^{13}C NMR analysis (**Scheme 3.26**). In ^{13}C NMR spectra, the triplet at 124.9 ppm with a coupling constant of 261 Hz is in agreement with a typical $^{1}J_{FC}$ coupling pattern and assigned to C1. The triplet at 33.6 ppm with a coupling constant of 31.2 Hz was assigned to C2, in correspondence with typical $^{2}J_{FC}$ coupling constants.



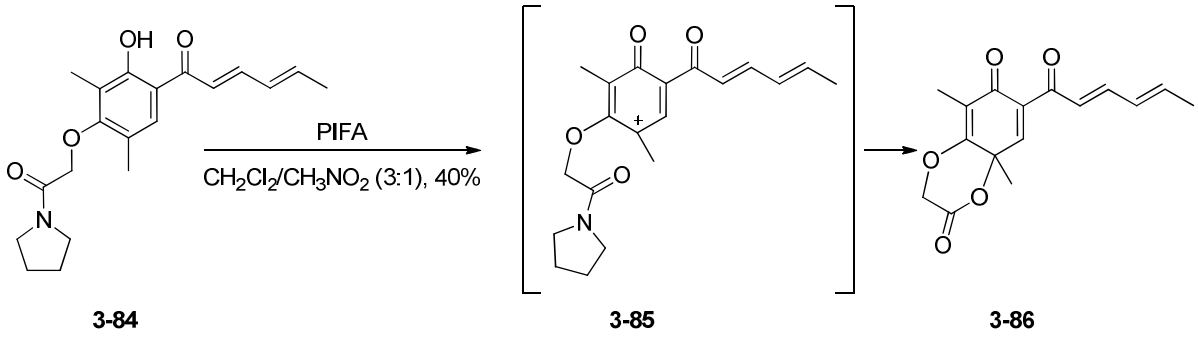
Scheme 3.27. Selected ^{19}F and ^{13}C NMR peaks for identification of **3-83**.

The formation of the CF_2 functionality in **3-83** suggested that the ester group participates in the fluorination process. A possible mechanism scenario is depicted in **Scheme 3.28**. The first step involves oxidation of the substrate **3-82**, which leads to the phenoxenium cation **A** (phenolic "umpolung"). The resulting cationic intermediate **A** rearomatises to produce the oxonium intermediate **B**, which is subsequently trapped by the fluoride. The resulting 2-ethoxy-2-fluorochroman-6-ol **C** was further oxidised to phenoxenium cation **D**, the intermediate that reacts with fluoride to give difluorinated **3-83** *via* oxonium **E**.



Scheme 3.28. Proposed mechanism for the formation of **3-83.**

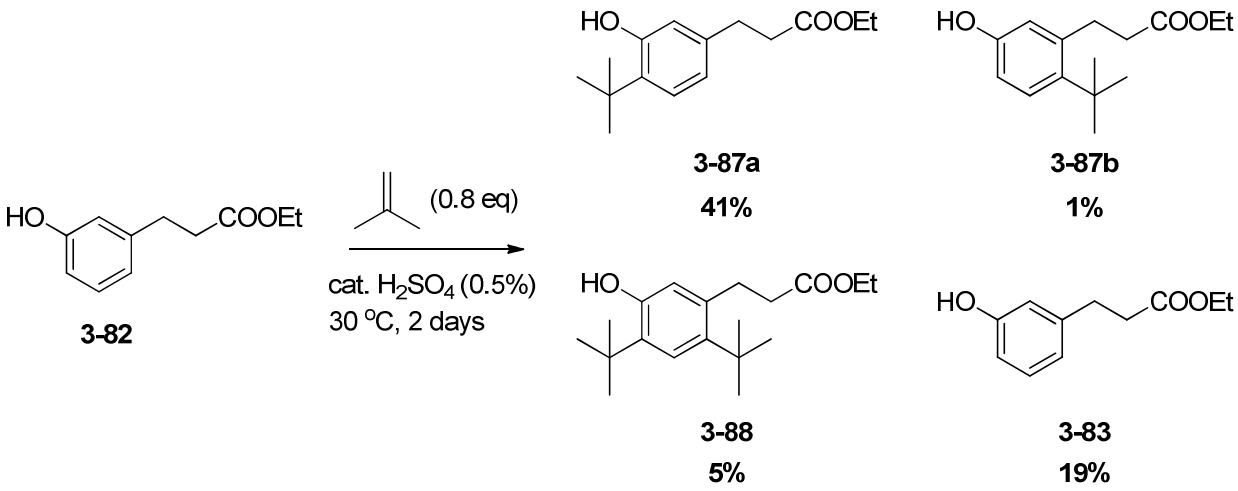
In fact, the oxidative dearomatisation of a *para*-alkylated phenol derivative, followed by an intramolecular cyclisation reaction has been well explored and used as the key transformation in the synthesis of a series of natural products. For example, oxidation of the resorcinol derivative **3-84** with equimolar amount of PIDA followed by aqueous workup gave the cyclohexa-2,5-dienone **3-86** in 40% yield, as recently reported by the Pettus group (**Scheme 3.29**).[167, 188]



3-84 **3-85** **3-86**

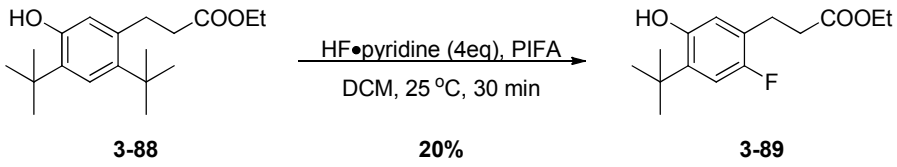
Scheme 3.29. Synthesis of **3-86** under oxidative conditions

We postulated that the installation of a bulky *tert*-butyl substituent at the *para* position of the phenol complex **3-82** may diminish the intramolecular cyclisation and thus promote the intermolecular fluorination. Thus the *meta*-substituted phenol **3-82** was treated with an excess of isobutylene and a catalytic amount of sulphuric acid. The reaction formed *ortho-tert*-butyl substituted **3-87a**, *para*-substituted product **3-87b** and di-*tert*-butylated product **3-88** in 41%, 1% and 5% isolated yield respectively (**Scheme 3.30**).



Scheme 3.30. *tert*-Butylation of **3-82** in isobutyene

The *para*- and *meta*-di-*tert*-butyl substituted precursor **3-88** was chosen to validate the oxidative fluorination of a phenol substrate containing a ester functionality. Upon fluorination of **3-88**, the *para*-fluorinated product **3-89** was obtained in 20% isolated yield. This result suggests that significant improvement in the fluorination can be achieved by the installation of a *para-tert*-butyl group (**Scheme 3.31**).



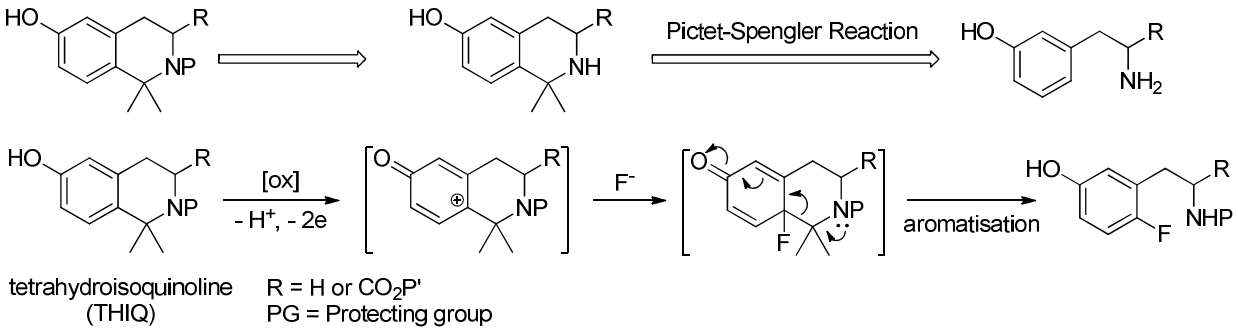
Scheme 3.31. Fluorination of **3-88**.

3.7.2. Bicyclic and Tricyclic Tetrahydroisoquinoline (THIQ) compounds as precursors for FmT

The *para-tert*-butyl group has been proven to be beneficial for the oxidative fluorination of phenol derivatives. Therefore, the prospective precursor for FmT should feature a *tert*-butyl functionality at the fluorination site. This requires installation of a *tert*-butyl group onto the

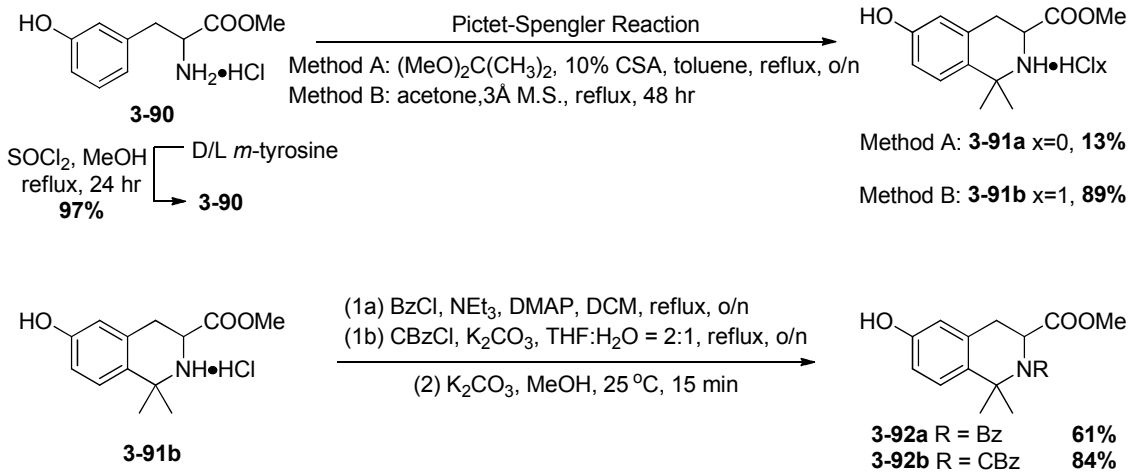
meta-tyrosine motif, *para* to the hydroxyl group with good regioselectivity and this is a challenging process.

We proposed that the precursors with a bicyclic tetrahydroisoquinoline (THIQ) motif may be an elegant solution to the problem. The complex contains a gem-dimethyl amino substituent at the fluorination site which can stabilise the cationic intermediate upon oxidation and rearomatise to give the fluorinated *meta*-tyrosine. In addition, the synthesis of THIQ compounds *via* Pictet-Spengler reaction has been well documented (**Scheme 3.32**).



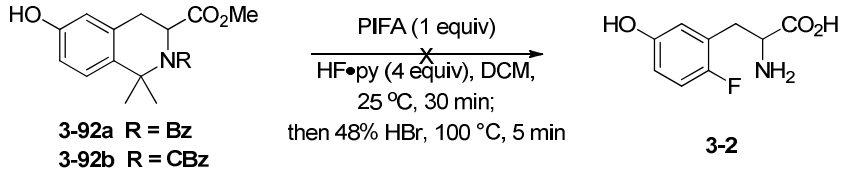
Scheme 3.32. Retro-synthesis and proposed oxidative fluorination of THIQs

The Pictet-Spengler reaction of the methyl ester protected *m*-tyrosine **3-90** was first performed in a solution of toluene with 2,2-dimethoxypropane and a catalytic amount of acid to give cyclised **3-91a** in 13% yield. The THIQ synthesis was significantly improved by refluxing the *m*-tyrosine methyl ester (**3-90** in the form of HCl salt) with acetone, in the presence of 3Å molecular sieves to give the cyclised **3-91b** in 89% yield, in the form of a hydrogen chloride salt.[29] Treating the THIQ•HCl compound **3-91b** with 2 equivalent of benzyl chloride or benzyl chloroformate and cleaving the *O*-protecting group under basic conditions, gave the phenol precursors **3-92a/b** with THIQ motif in 61% and 84% yield respectively (**Scheme 3.33**).



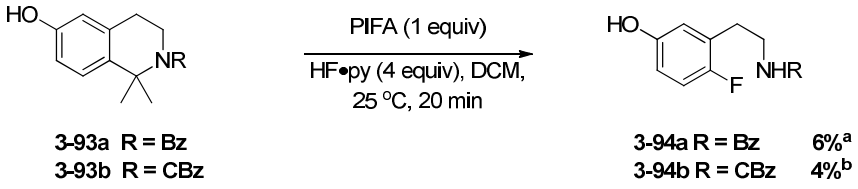
Scheme 3.33. Synthesis of 2nd generation precursor of 6-fluoro-*m*-tyrosine

We attempted to fluorinate precursors **3-92a/b** under oxidative conditions. Unfortunately, the reaction afforded no FmT **3-2**, according to ^{19}F-NMR and HPLC analysis (**Scheme 3.34**).



Scheme 3.34. Fluorination of 2nd generation precursor **3-92a/b**.

For comparatative study, the *N*-protected THIQ analogues **3-93a/b** without ester substitution were synthesised by L. Wong as model substrates. Encouragingly, the oxidative fluorination process gave *para*-fluorinated products **3-94a/b** in a 6% isolated yield (**Scheme 3.35**).[30]



a. isolated yield, b. yield determined by ^{19}F-NMR integration

Scheme 3.35. Fluorination of **3-94**.

[29] We thank Dr. Z. Gao for optimising the reaction.
[30] L.Wong Part II thesis, University of Oxford, 2011.

The results emphasised that the competing reaction induced by the ester group lead to the failure of oxidative fluorination in **3-92**. The $^{3}J_{Ha\text{-}Hc}$ and $^{3}J_{Hb\text{-}Hc}$ values were measured by NMR analysis and used to investigate the H–C–C–H dihedral angles (**Figure 3.4**).[189, 190] The $^{3}J_{H\text{-}H}$ coupling constants of H^c of the *N*-unprotected THIQ **3-91a** were found to be 11.1 Hz and 4.6 Hz, and indicated the axial orientation of H^c and equatorial orientation of the ester substituent. When the benzoyl protecting group was installed, $^{3}J_{H\text{-}H}$ coupling constants of H^c in **3-92a** were 4.6 Hz and 3.0 Hz, suggesting the pseudoaxial position of the ester group. The structure was confirmed by X-Ray crystallographic study[31] (**Figure 3.5**).

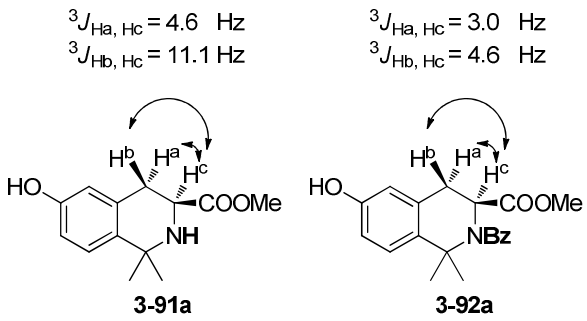


Figure 3.4. ^{3}J coupling constants of H^c in **3-91a** and **3-92a**

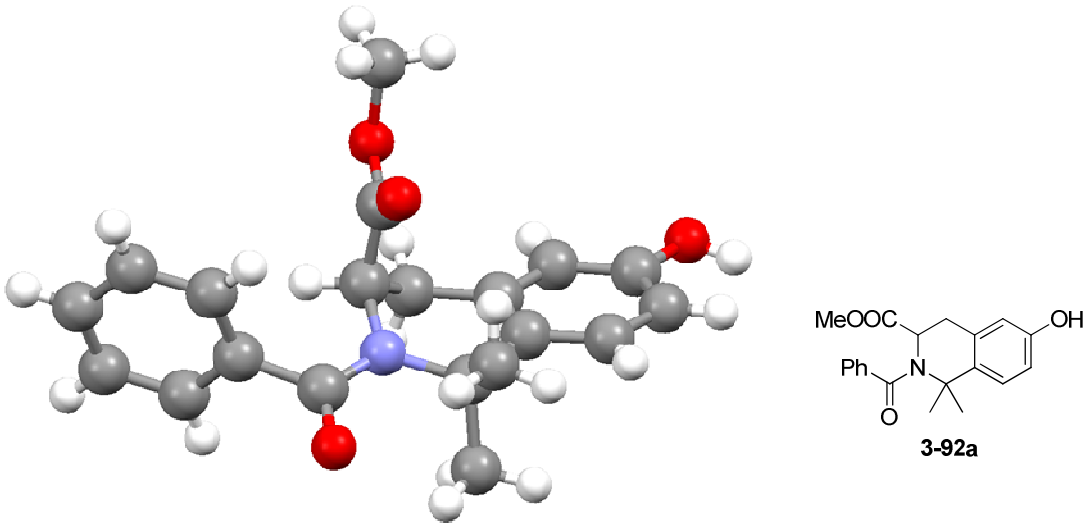
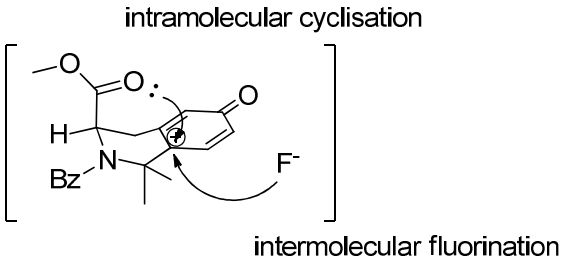


Figure 3.5. X-ray crystallography of **3-92a**.

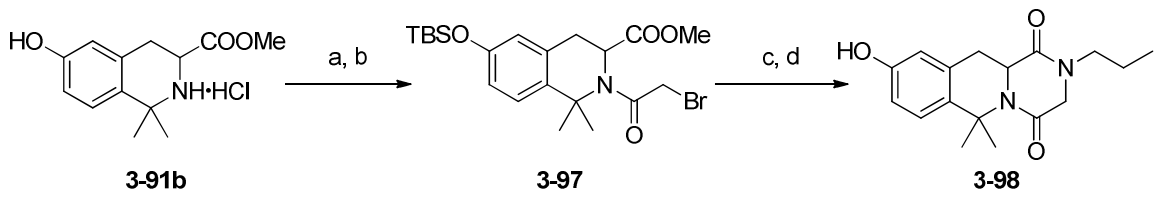
[31] We thank Mr. G. Giuffredi for the X-Ray analysis.

When the THIQ complexes **3-92a** and **b** were subjected to the oxidative conditions with fluoride, we suggested that the dominant reaction might be the intramolecular cyclisation induced by the carbonyl group at pseudoaxial position of the tetrahydroisoquinoline, as discussed above (**Scheme 3.36**).



Scheme 3.36. Competing intromolecular cyclisation and intermolecular fluorination upon oxidation of **3-92a**

Next, the tricyclic piperazine-2,5-dione complex **3-98** was synthesised, *via* an efficient four-step synthetic route in a 44% overall yield, aiming to minimise the side reaction induced by the carbonyl group (**Scheme 3.37**).



a. TBSCl, NEt_3, DMAP, r.t., o/n, **88%** yield; b. Bromo acetobromide, NEt_3, DCM, 0 °C to r.t., 2 hr, **80%** yield; c. 1-propylamine, K_2CO_3, DCM, reflux, 30 min, **82%** yield; d. 1.2 M pyridinium fluoride, THF, r.t., o/n, **76%** yield.

Scheme 3.37. Four-step synthesis of the tricyclic THIQ precursor **3-98**

The $^3J_{H\text{-}H}$ coupling constants of H^c in tricyclic piperazine-2,5-dione **3-98** were measured to be 11.9 Hz and 2.0 Hz. The coupling pattern of H^a, H^b and H^c is comparable with those for *N*-unprotected THIQ **3-91a** (**Figure 3.6**). NMR studies indicate that H^c occupies an axial position in both molecules.

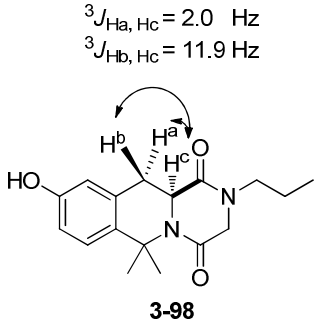
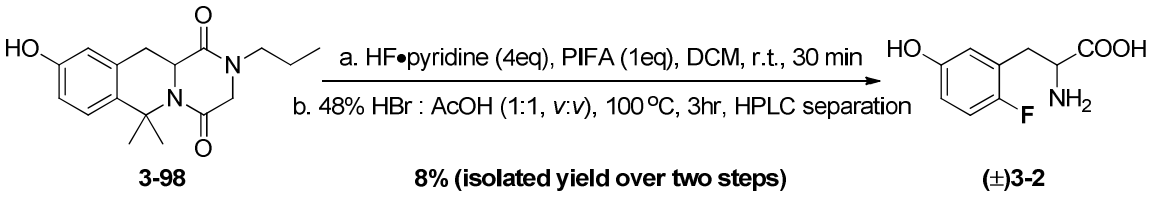


Figure 3.6. ^3J coupling constants of H^c in **3-98**.

When the tricyclic precursor **3-98** was treated with 4 equivalent of HF•pyridine and 1 equivalent of PIFA in the solution of DCM for 30 minutes at ambient temperature, racemic 6-fluoro-*m*-tyrosine **3-2** was obtained in 8% isolated yield, after acidic cleavage and separation *via* preparative HPLC chromatography (**Scheme 3.38**).



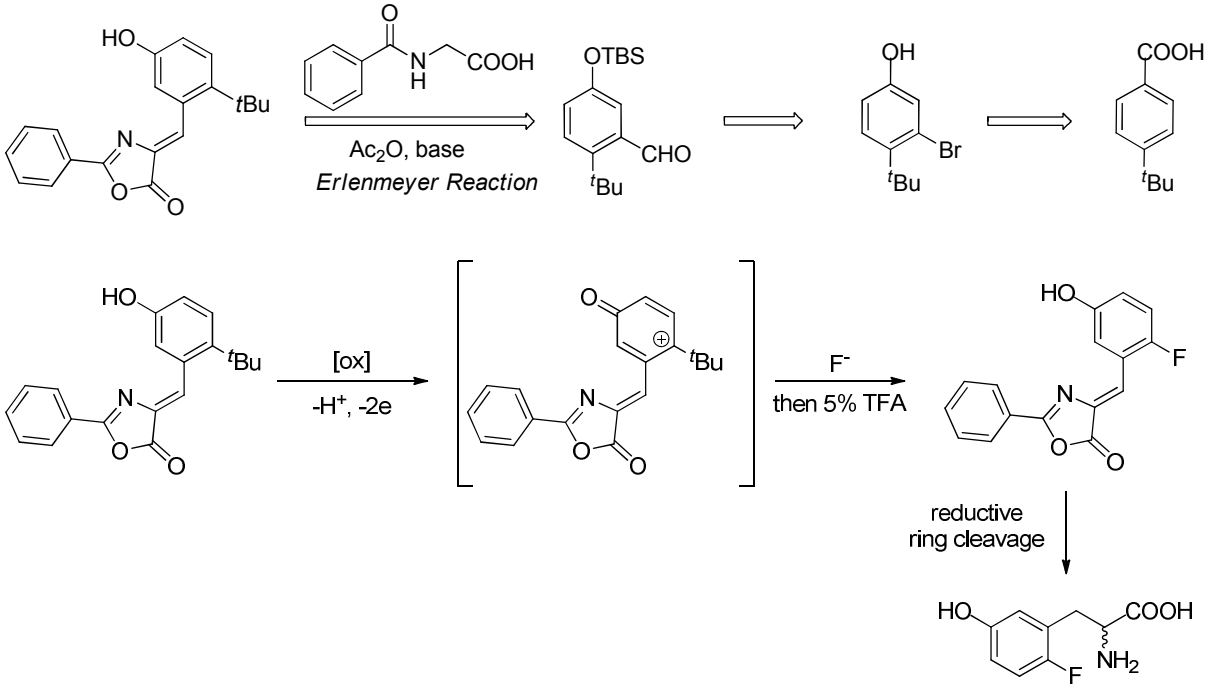
Scheme 3.38. Fluorination of 3rd generation of precursor **3-98**

3.7.3. Unsaturated 5(4*H*)-oxazolone as precursor for FmT

The encouraging results inspired us to design an FmT precursor containing a masking group for the amino acid functionality and a *tert*-butyl substituent at the fluorination site. We postulated that an oxazolone motif can be implemented as the protecting group of the amino acid functionality.

Oxazolone compounds have been well-applied in the preparation of $_{DL}$-α-amino acid and they are readily available *via* Erlenmeyer reaction.[191] The condensation involves a three-component condensation between a carbonyl compound (in most cases, an aromatic aldehyde), a *N*-acetyl- or *N*-benzoylglycine, and a catalytic amount of base (usually, NaOAc) in excess acetic anhydride. As the key reagent, the 2-(*tert*-butyl)-5-(*O*-

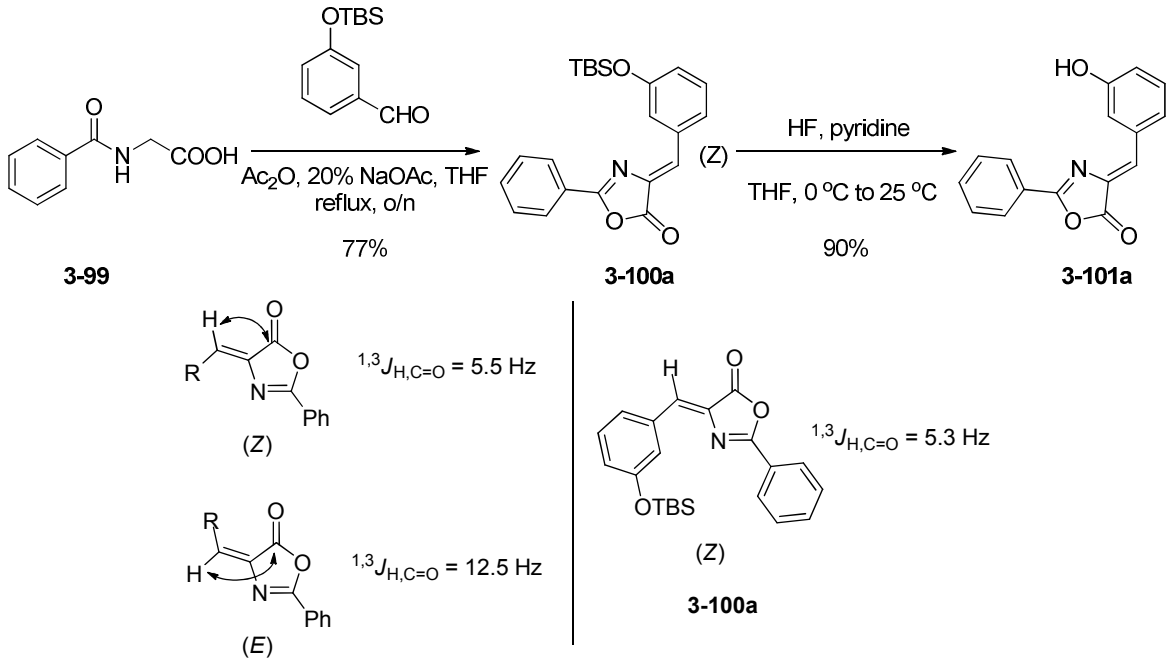
TBS)benzaldehyde could be synthesised *via* a series of functional group manipulations, starting from the commercial 4-*tert*-butylbenzoic acid. Upon oxidative fluorination, if feasible, reductive ring cleavage (Na/Hg, P(red)/HI or catalytic hydrogenation) of the resulting oxazolone would yield the target 6-fluoro-*meta*-tyrosine (**Scheme 3.39**).



Scheme 3.39. Retro-synthesis and proposed oxidative fluorination of a oxazolone

The 3-(*O*-TBS)-benzaldehyde was selected as the starting material to validate the retro-synthetic route as it is readily available. Erlenmeyer condensation between the aldehyde, hippuric acid in an excess amount of acetic anhydride are catalysed by sodium acetate (20%) to afford the unsaturated 5-(4*H*)-oxazolone **3-100a** in 77% yield (**Scheme 3.40**). The configuration of (*Z*) and (*E*)-unsaturated 5-(4*H*)-oxazolones can be assigned by the value of the $^{13}C–^{1}H$ three-bond coupling constants, between the olefinic proton and the C–5 carbonyl carbon in a fully coupled ^{13}C NMR spectra, because $^{1,3}J_{CH(trans)} > {}^{1,3}J_{CH(cis)}$. For (*Z*)-unsaturated 5-(4*H*)-oxazolones, a typical value of the $^{1,3}J_{H,\,C=O}$ coupling constant is found to be ~5.5 Hz and ~12.5 Hz for (*E*)-oxazolones.[192] The $^{1,3}J_{H,\,C=O}$ coupling constant of **3-100a**,

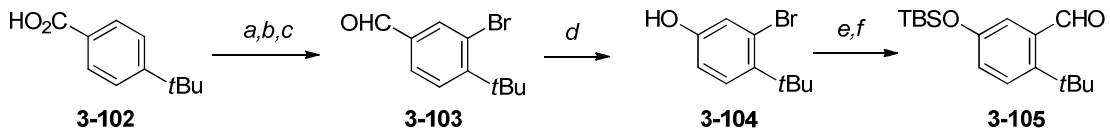
determined by NMR spectroscopy, is 5.3 Hz. This indicates the exclusive formation of the **(*Z*)-3-100a** during the condensation.



Scheme 3.40. Synthesis of **3-100/101a** and configuration assignments *via* NMR

The *o*-(*tert*-butyl)-*m*-hydroxybenzaldehyde **3-105** was synthesised in six steps (**Scheme 3.41**).[32] The preparation started with the bromination of the *tert*-butylbenzoic acid **3-102** in 49% yield. Reduction with borane followed by oxidation in MnO_2 transformed the acid substituent in **3-102** into the aldehyde function (**3-103**) in 71% yield over two steps.[193] Baeyer–Villiger oxidation of **3-103** gave corresponding phenol **3-104** in 98% yield. 3-Bromo-4-*tert*-butylphenol **3-104** was converted to **3-105** in high yield after functional group manipulations.

[32] We thank Dr. Z. Gao for preparing starting material **3-104**.

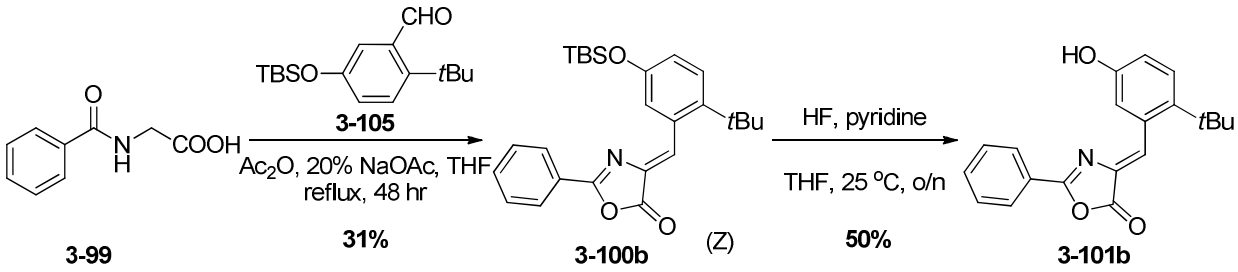


3-102 **3-103** **3-104** **3-105**

a. Br_2, $AgNO_3$, HNO_3, AcOH, **49%**; b. BH_3·THF, THF; c. MnO_2, pentane, **71%** over 2 steps; d. CF_3CO_3H, KH_2PO_4, 0 °C, 0.5 hr; then 0.2% K_2CO_3/MeOH, 10 min; **98%**; e. TBSCl, imidazole, DMAP, DCM, **92%**; f. *n*-BuLi, DMF, **72%**;

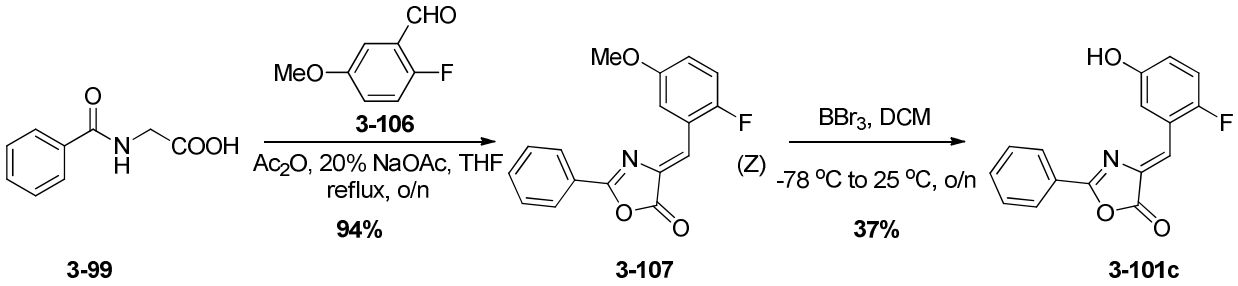
Scheme 3.41. Six-step synthesis of **3-105**.

With the *tert*-butylated aldehyde **3-105** in hand, we then synthesised the unsaturated 5-(4*H*)-oxazolone **3-101b** and its fluorinated analogue **3-101c** in satisfactory yield (**Scheme 3.42** and **3.43**).



3-99 **3-100b** (Z) **3-101b**

Scheme 3.42. Synthesis of precursor **3-101b** with *tert*-butyl substitution.

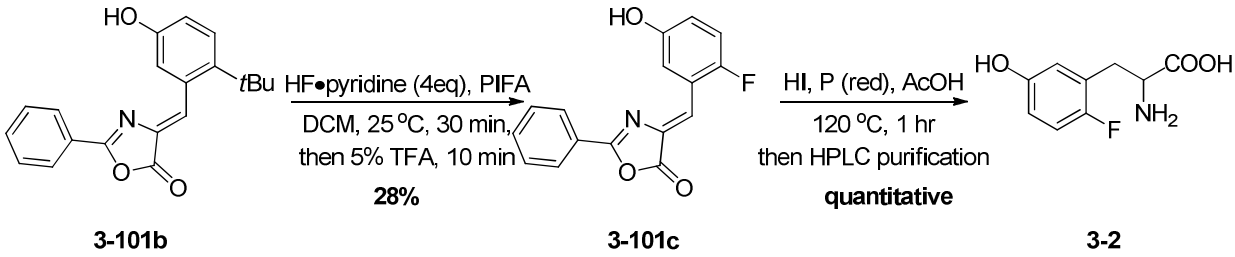


3-99 **3-107** **3-101c**

Scheme 3.43. Synthesis of fluorinated **3-101c** as reference compound

The reaction of *tert*-butyl substituted **3-101b** gave *para*-fluorinated phenolic compound **3-101c** in 28% isolated yield. In contrast, only a trace amount of product was observed by ^{19}F-NMR analysis of the crude reaction mixture, when **3-101a** without *tert*-butyl substituent was used as the precursor (**Scheme 3.44**). Upon fluorination, the isolated **3-101c** was treated by

red phosphorus in hydroiodic acid at 120 °C for 1 hour. The reaction converted the oxazolone motif in **3-101c** to amino acid and thus afford the racemic FmT **(±)3-2** in a quantitative yield, upon purification *via* semi-preparative HPLC chromatography. Protentially, the two-step synthesis can be further adapted to radiosynthesis of [^{18}F]FmT **[^{18}F]3-2**.

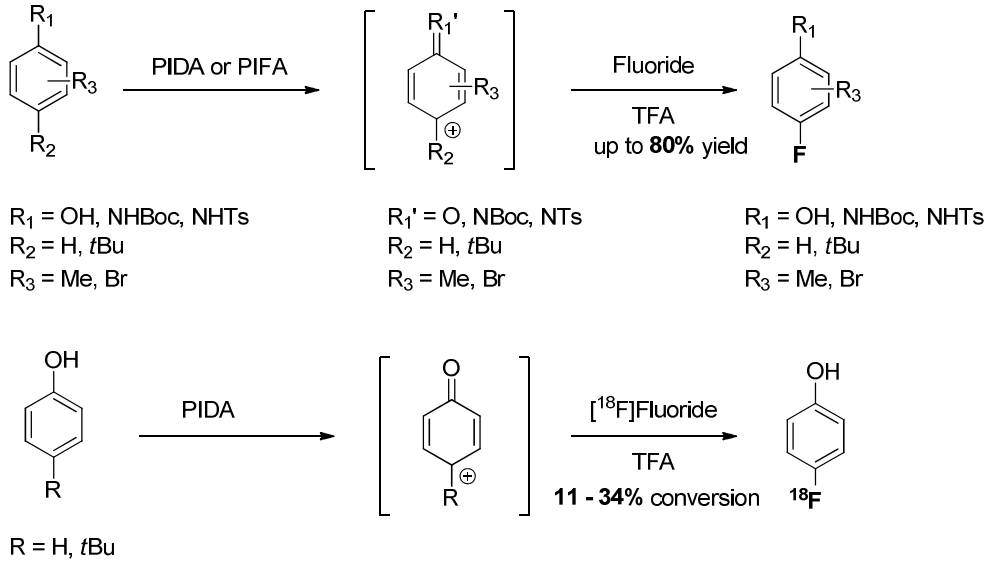


Scheme 3.44. Fluorination of **3-101b**.

3.8. Conclusion and Future Work

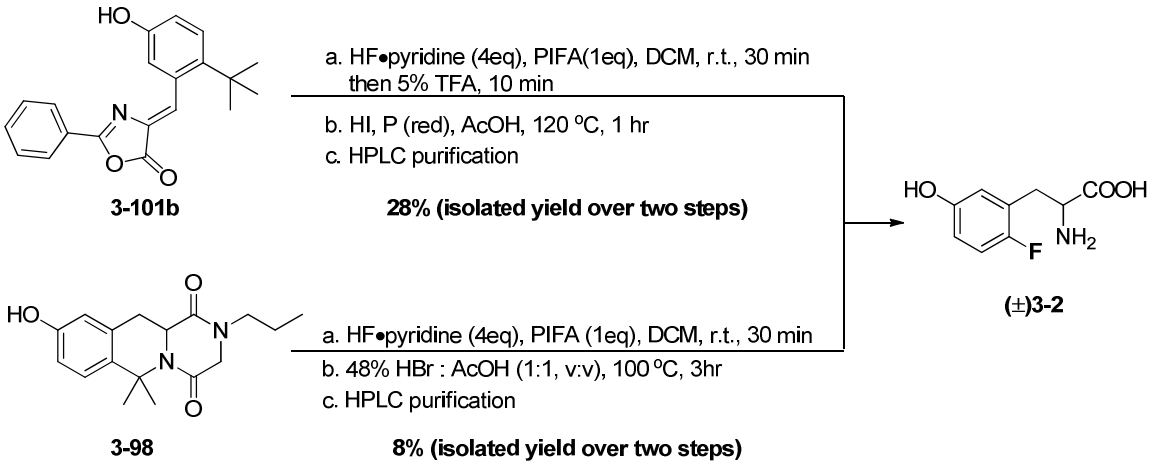
We have developed a "oxidative nucleophilic fluorination" protocol for direct fluorination of phenol and aniline, to give useful prosthetic groups such as 4-fluorophenol and 4-fluoroaniline. The installation of a *tert*-butyl group at the fluorination site significantly improved the synthetic outcome. The feasibility of the reaction in the context of ^{18}F-radiolabelling was initially validated on 4-*tert*-butylphenol and phenol, albeit in low RCY (3 – 6%). Transferring the radiolabelling protocol from a conventional radiosynthetic module to a microfluidic synthesiser resulted in significant improvements in the efficiency of the radiochemistry (11 – 34% conversion). The approach harnessed the advantages of the microfluidic synthesis system to improve the synthetic efficiency, by maximizing the concentration of radioactivity and mixing efficiency of the reagents. To the best of our knowledge, this is the first direct one-pot transformation from ^{18}F-fluoride to 4-

[^{18}F]fluorophenol (**Scheme 3.45**). The routine radiosynthetic process based on the oxidative nucleophilic ^{18}F-fluorination could potentially be of great use for the synthesis of prosthetic groups in ^{18}F radiolabelling.



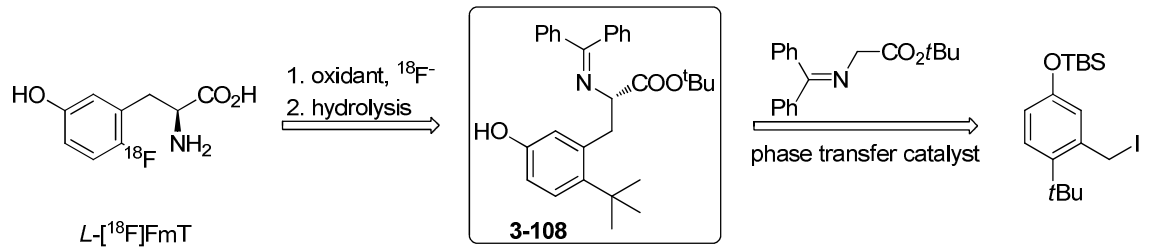
Scheme 3.45. Development of a simple, direct oxidative nucleophilic ^{18}F/^{19}F-fluorination of electro-rich aromatics.

Our efforts were dedicated to the design and synthesis of elaborate precursors for the application of the radiosynthetic process in the synthesis of a clinical PET tracer 6-fluoro-*m*-tyrosine FMT (**Scheme 3.46**). The synthesis of FMT was accomplished by oxidative-fluorination of the tricyclic tetrahydroisoquinoline (THIQ) compound **3-98** and the *tert*-butyl phenol derivative with oxazolone motif **3-101b** in 8% and 28% isolated yield, respectively.



Scheme 3.46. Potential precursors for radiosynthesis of FmT

Research is underway within the Gouverneur group, aiming to transfer the oxidative-fluorination stategy to the radiosynthesis of [^{18}F]FmT. In addition, the method could also be applied to synthesise $_{L}$-FmT with high optical purity, when a *tert*-butyl substituted phenol precursor **3-108** with a pre-installed stereocenter is used (**Scheme 3.47**).



Scheme 3.47. Plausible precursor for radiosynthesis of [^{18}F]FmT

Chapter 4

Experimental Section

All reactions were carried out under an atmosphere of argon in flame-dried glassware with magnetic stirring. Solvents were dried and purified before use according to standard procedures. All reactions were monitored by TLC using Merck Kieselgel 60 F_{254} plates. Visulisation of reaction components was achieved by using UV fluorescence (254 nm), and acidified potassium permanganate or ceric ammonium molybdate stain. "Flash" column chromatography was carried out over Merck silica gel C60 (40–60 μm) using eluent systems as described for each experiment.

Microwave assisted reactions were performed in the cavity connected to Model 521 microwave instrument supplied by Resonance Instruments, Inc with infrared temperature control.

Unless otherwise stated, ^{1}H NMR spectra were recorded on Bruker DPX200, AV400, DPX400 or AVC500 spectrometers. ^{13}C NMR spectra were recorded on Bruker AV400 or AVC500 spectrometers fitted with a ^{13}C cryoprobe. ^{19}F NMR spectra were recorded on a Bruker AV400 spectrometer. ^{1}H and ^{13}C NMR spectra are reported as chemical shifts (δ) in parts per million (ppm) relative to the solvent peak using the Bruker internal referencing procedure (edlock). ^{19}F NMR spectra are referenced relative to $CFCl_3$ in $CDCl_3$. Scalar coupling constants (*J*) are reported in units of hertz (Hz).

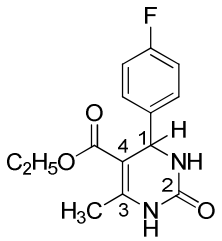
Mass spectra (m/z) were recorded on on a Micromass GCTof in Chemical Ionisation (NH_3, CI^+) or Electron Impact (EI). High resolution mass spectra (HRMS, *m/z*) were recorded on a Bruker MicroTOF spectrometer using positive (ESI^+) or negative electrospray ionization (ESI^-).

Infrared spectra were recorded using a Bruker Tensor 27 FT-IR spectrometer either on NaCl discs as a thin film in a solution in dichloromethane or in a KBr pellet which is prepared by grinding the solid sample with solid KBr and applying great pressure to the dry mixture. Absorptions are reported in wavenumbers (cm^{-1}) and only peaks of interest are included. Melting points of solids were measured on either a Griffin or Phillip Harris apparatus and were uncorrected.

4.1 Experimental data for Chapter 2

4.1.1. Synthesis data for Section 2.2.2

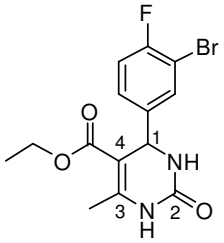
4-(4-Fluorophenyl)-5-(ethyoxycarbonyl)-6-methyl-3, 4-dihydropyrimidin-2(1*H*)-one (2-1)



4-Fluorobenzaldehyde (53.14 µL, 0.5 mmol) was added to ethyl acetoacetate (96 µL, 0.75 mmol), urea (30 mg, 0.5 mmol) and ytterbium(III) trifluoromethanesulfonate (31 mg, 10 mol%) in acetonitrile (0.5 mL) was added to a vial and sealed. The mixture was stirred, heated up to 80 ^{0}C and kept for 5 hours until TLC showed the reaction was completed. After cooled down, white precipitate appeared. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel with EtOAc / PE 40-60 2:1 (R_f = 0.2) **^{1}H NMR (200 MHz, $(CD_3)_2SO$)** δ ppm 9.24 (br.s, 1 H, N*H*), 7.76 (br.s, 1 H, N*H*), 7.07 - 7.32 (m, 4 H, Ar – *H*), 5.15 (d, *J* = 3.41 Hz, 1 H, C*H*), 3.98 (q, *J* = 7.05 Hz, 2 H, OCH_2), 2.25 (s, 3 H, CH_3), 1.08 (t, *J* = 7.17 Hz, 3 H, OCH_2CH_3); **^{13}C NMR (101 MHz, $(CD_3)_2SO$)** ppm 165.5 (*C*=O), 162.3 (d, *J* = 246.1 Hz, *C* – F), 153.6 (*C* – 2), 146.4 (*C* – 3), 139.6 (d, *J* = 3.2 Hz, Ar – *C*), 128.3 (d, *J* = 8.0 Hz, Ar – *C*), 115.5 (d, *J* = 21.6 Hz, Ar – *C*), 101.2 (*C* – 4),

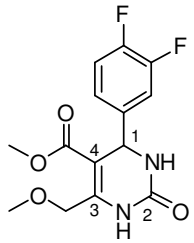
60.1 (COO<u>C</u>$H_2$$CH_3$), 55.0 (<u>C</u> – 1), 18.6 (<u>C</u>H_3C=C), 14.1 (COOCH_2<u>C</u>H_3); **^{19}F {^{1}H} NMR (376 MHz, $CDCl_3$)** ppm –114.19; m.p. = 102 – 103 ^{o}C; Other characterization data are in consistence with reported results.[101]

Ethyl 4-(3-bromo-4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2-22)



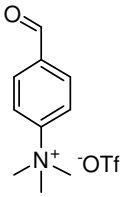
3-Bromo-4-fluorobenzaldehyde (101 mg, 0.5 mmol) was added to ethyl acetoacetate (96 µL, 0.75 mmol), urea (30 mg, 0.5 mmol) and ytterbium(III) trifluoromethanesulfonate (31 mg, 10 mol%) in acetonitrile (0.5 mL). The vial was sealed and the reaction mixture was stirred at 100^{o}C for 5 hours. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography on silica gel (50% ethyl acetate in hexane, R_f=0.2) to give the pure product as a white solid (160 mg, 0.45 mmol, 90% yield). **^{1}H NMR (400 MHz, $(CD_3)_2SO$)**: ppm 9.31 (br. s., 1 H, N<u>H</u>), 7.80 (br. s., 1 H, N<u>H</u>), 7.49 (dd, *J* = 6.7, 2.1 Hz, 1 H, Ar–<u>H</u>), 7.35 (dd, *J* = 8.8, 8.6 Hz, 1 H, Ar–<u>H</u>), 7.26 (ddd, *J* = 8.6, 4.8, 2.3 Hz, 1 H, Ar–<u>H</u>), 5.15 (d, *J* = 3.3 Hz, 1 H, C<u>H</u>–1), 3.92-4.06 (m, 2 H, OC<u>H</u>$_2$$CH_3$), 2.25 (s, 3 H, C<u>H</u>$_3$), 1.09 (t, *J* = 7.1 Hz, 3 H, OCH_2C<u>H</u>$_3$); **^{13}C NMR (125 MHz, $(CD_3)_2SO$)**: ppm 165.1 (<u>C</u>=O), 157.4 (d, *J* = 244.1 Hz, <u>C</u>–F), 151.8 (<u>C</u> – 2), 149.1 (<u>C</u> – 3), 143.0 (d, *J* = 3.8 Hz, Ar – <u>C</u>), 131.3 (Ar – <u>C</u>), 127.6 (d, *J* = 7.6 Hz, Ar – <u>C</u>), 116.9 (d, *J* = 22.9 Hz, Ar – <u>C</u>), 107.6 (d, *J* = 21.0 Hz, Ar – <u>C</u>), 98.5 (<u>C</u> – 4), 59.3 (O<u>C</u>$H_2$$CH_3$), 53.1(<u>C</u> – 1), 17.8(<u>C</u>H_3), 14.0(OCH_2<u>C</u>H_3); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm –110.4; **HRMS** (ESI$^+$, *m/z*) $C_{14}H_{15}BrFN_2O_3^+$ ([M+H]$^+$) *calc.* 357.0245, *found* 357.0245; **IR**: 3055, 2987, 2306, 1703, 1422; **m.p.** = 193 – 195 °C.

Methyl 4-(3, 4-difluorophenyl)-6-(methoxymethyl)-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (2-24)



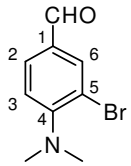
3, 4-Difluorobenzaldehyde (110 µl, 1 mmol), methyl 4-methoxyacetate (129 µl, 1.5 mmol), urea (60 mg, 1 mmol), ytterbium(III) triflate (62 mg, 10 mol%) and 1 ml acetonitrile were added to a sealed vial. The mixture was stirred, heated up to 100 ^{o}C and kept for 5 hours until the reaction was completed. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (80% ethyl acetate in hexane, R_f = 0.3) to give pure product as white solid (250 mg, 0.80 mmol, 80% yield). **^{1}H NMR (500 MHz, ($CDCl_3$)** ppm 7.58 (br. s., 1 H, N*H*), 7.02 - 7.15 (m, 3 H, Ar–H), 6.05 (br. s., 1 H, N*H*), 5.34 (d, *J* = 2.8 Hz, 1 H, C*H*–1), 4.64 (ddd, *J* = 19.68, 16.71, 0.63 Hz, 2 H, C*H*$_2$OCH$_3$), 3.65 (s, 3 H, COOC*H*$_3$), 3.47 (s, 3 H, CH$_2$OC*H*$_3$); **^{13}C NMR (125 MHz, ($CDCl_3$)** ppm 165.1 (*C*=O), 151.8 (*C* – 2), 150.3 (dd, *J* = 248.9, 12.4 Hz, *C* – F), 150.0 (dd, *J* = 248.9, 12.4 Hz, *C* – F), 147.6 (*C* – 3), 140.4 (t, *J* = 4.3 Hz, Ar – *C*), 122.4(dd, *J* = 6.7, 3.8 Hz, Ar – *C*), 117.5 (d, *J* = 18.1 Hz, Ar – *C*), 115.6(d, *J* = 17.2 Hz, Ar – *C*), 98.3 (*C* – 4), 68.5 (*C*H$_2$OCH$_3$), 59.1 (CH$_2$O*C*H$_3$), 54.7 (*C* – 1), 51.4 (COO*C*H$_3$), **^{19}F {^{1}H}NMR (377 MHz, CD_3Cl)**: ppm –136.5, –138.4. Other characterization data are in consistency with reported results.[194]

4-Formyl-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (2-29)[50]



4-(Dimethylamino)benzaldehyde (750 mg, 5 mmol) was dissolved in dichloromethane (80 mL). Methyl trifluoromethanesulfonate (610 μL, 5.4 mmol) was added and the reaction was stirred at room temperature overnight. The crude reaction mixture was concentrated at room temperature to a volume of 20 mL before being poured carefully into cold diethyl ether (200 mL). A precipitate appeared which was filtered and dried *in vacuo*. Recrystallization from dichloromethane/acetonitrile afforded the pure product as a white solid (407 mg, 1.3 mmol, 26% yield). The precursor was dried *in vacuo* for overnight before applied to radiosynthesis. **^{1}H NMR (200 MHz, $(CD_3)_2SO$)**: ppm 10.13 (s, 1 H, C*H*O), 8.15-8.27 (m, 4 H, Ar–*H*), 3.68 (s, 9 H, CH_3 × 3). Other characterization data are in consistence with reported results.[50]

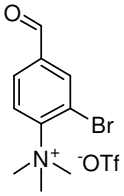
3-Bromo-4-dimethyaminobenzaldehyde (2-31)



N-bromosuccinimide (186.9 mg, 1.05 mmol) was added to a mixture of 4-dimethylamino benzaldehyde (149.2 mg, 1.0 mmol) and ammonium acetate (15 mg, 20 mol %) in 5 ml anhydrous acetonitrile. The mixture was stirred at room temperature for 45 minutes until the reaction was completed. The mixture was concentrated and extracted by ethyl acetate / water (1:1) (5 ml × 3). Then the organic portion was dried in sodium sulfate and concentrated under reduced pressure. Pure product was obtained by column chromatography on silica gel (10% ethyl acetate in hexane) as yellow oil with the yield 92%. **^{1}H NMR (200 MHz, $CDCl_3$)** . ppm 9.83 (s, 1 H, C*H*O), 8.05 (d, 1 H, *J* = 1.7 Hz, *H*-6), 7.75 (dd, *J* = 8.4, 1.9 Hz,

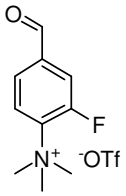
1 H, H-2), 7.08 (d, J = 8.2 Hz, 1 H, H-3), 2.97 (s, 6 H, $CH_3 \times 2$); **^{13}C NMR (101 MHz, $CDCl_3$)** ppm 189.70 (CHO), 156.88 (C-4), 135.98 (C-6), 130.99 (C-2), 129.91 (C-1), 119.40 (C-3), 116.59 (C-5), 43.48 ($CH_3 \times 2$), Other charaiterization data are in consistence with reported results.[109]

2-Bromo-4-formyl-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (2-32)



3-Bromo-4-(dimethylamino)benzaldehyde (114 mg, 0.5 mmol) was dissolved in dichloromethane (8 mL). Methyl trifluoromethanesulfonate (62 µL, 0.55 mmol) was added and the reaction was stirred at room temperature overnight. The crude reaction mixture was concentrated at room temperature to a volume of 4 mL before being poured carefully into cold diethyl ether (20 mL). A precipitate appeared which was filtered and dried *in vacuo*. Recrystallization from dichloromethane/diethyl ether afforded the pure product as a light brown solid (71 mg, 0.18 mmol, 36% yield). **^{1}H NMR (500 MHz, $(CD_3)_2CO$)**: ppm 10.17 (s, 1 H, CHO), 8.51-8.54 (m, 2 H, Ar – H), 8.23-8.25 (m, 1 H, Ar – H), 4.21 (s, 9 H, $CH_3 \times 3$); **^{13}C NMR (100 MHz, $(CD_3)_2CO$)**: ppm 190.9 (CHO), 147.8 (Ar – C), 139.5 (Ar – C), 138.2 (Ar – C), 131.0 (Ar – C), 128.4 (m, OSO_2CF_3), 126.1 (Ar – C), 115.6 (Ar –C), 57.2 ($CH_3 \times 3$); **HRMS** (ESI$^+$, *m/z*) $C_{10}H_{13}BrNO^+$ ($[M–OTf]^+$) *calc*. 242.0175, *found* 242.0177; **IR:** 3051, 2987, 2305, 1698, 1422; **m.p.** = 175 – 177 °C (decomposition observed).

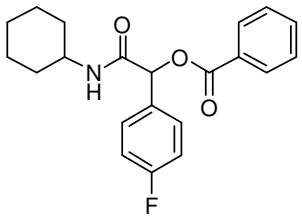
2-Fluoro-4-formyl-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate (2-34)



3-Fluoro-4-(dimethylamino)benzaldehyde (334 mg, 2.0 mmol) was dissolved in dichloromethane (15 mL). Methyl trifluoromethanesulfonate (280 µL, 2.4 mmol) was added and the reaction mixture was stirred at room temperature overnight. The crude mixture was concentrated *in vacuo* at room temperature to a volume of 4 mL before being poured carefully into cold diethyl ether (20 mL). A precipitate appeared which was filtered and dried *in vacuo*. Recrystallization from dichloromethane/diethyl ether afforded the pure product as a pale yellow solid (172 mg, 0.52 mmol, 26% yield). **^{1}H NMR (500 MHz, $(CD_3)_2CO$)**: ppm 10.18 (d, *J* = 1.9 Hz, 1 H, C*H*O), 8.41 (dd, *J* = 8.5, 8.2 Hz, 1 H, Ar–*H*), 8.09 (dd, *J* = 8.4, 1.6 Hz, 1 H, Ar–*H*), 8.09 (dd, *J* = 9.3, 1.4 Hz, 1 H, Ar–*H*), 4.08 (s, 9 H, C*H*$_3$ × 3); **^{13}C NMR (125 MHz, $(CD_3)_2CO$)**: ppm 191.0 (*C*HO), 156.4 (d, *J* = 253.7 Hz, *C* – F), 140.9 (d, *J* = 6.7 Hz, Ar – *C*), 138.3 (d, *J* = 6.7 Hz, Ar – *C*), 127.8 (d, *J* = 2.9 Hz, Ar – *C*), 125.2 (Ar – *C*), 122.3 (q, *J* = 322.3 Hz, OSO_2CF_3), 119.4 (d, *J* = 22.9 Hz, Ar – *C*), 57.6 (d, *J* = 5.7 Hz, *C*H$_3$ × 3); **^{19}F{^{1}H}NMR (377 MHz, $(CD_3)_2CO$)**: ppm –110.4, –78.9; **HRMS** (ESI$^+$, *m/z*) $C_{10}H_{13}FNO^+$ ([M–OTf]$^+$) *calc*.182.0976, *found* 182.0982; **IR**: 3056, 2988, 2306, 1707, 1594, 1500, 1435; **m.p.** = 56 – 58 °C (decomposition observed).

4.1.2. Synthesis data for Section 2.4.2[33]

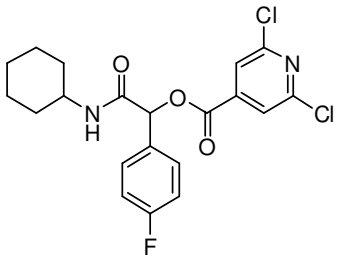
2-(Cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl benzoate (2-47)



4-Fluorobenzaldehyde (107 μL, 1.0 mmol), benzoic acid (146 mg, 1.2 mmol) and cyclohexyl isocyanide (124 μL, 1.0 mmol) were added to an aqueous solution of LiCl (4M, 5 mL). The mixture was stirred at room temperature for 8 hours. Dichloromethane was added (20 mL) and the layers were separated. The aqueous phase was washed with dichloromethane (10 mL) and the combined organic layers were dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Flash column chromatography on silica gel (20% ethyl acetate in hexane, R_f = 0.3) afforded the pure product as a white solid (350 mg, 0.98 mmol, 98% yield). **^{1}H NMR (400 MHz, $CDCl_3$)**: ppm 8.09 (dd, *J* = 8.6, 1.3 Hz, 2 H, Ar – *H*), 7.60-7.66 (m, 1 H, Ar – *H*), 7.46-7.55 (m, 4 H, Ar – *H*), 7.07 (t, *J* = 8.7 Hz, 2 H, Ar – *H*), 6.29 (s, 1 H, C*H*), 6.14 (d, *J* = 8.1 Hz, 1 H, N*H*), 3.77-3.89 (m, 1 H, Cy – *H*), 1.86-1.99 (m, 2 H, Cy – *H*), 1.57-1.76 (m, 3 H, Cy – *H*), 1.30-1.43 (m, 2 H, Cy – *H*), 1.08-1.26 (m, 3 H, Cy – *H*); **^{13}C NMR (100 MHz, $CDCl_3$)**: ppm 167.2 (*C*=O), 164.8 (*C*=O), 163.0 (d, *J* = 248.0 Hz, *C* – F), 133.7, 131.7 (d, *J* = 2.9 Hz), 129.8, 129.3 (d, *J* = 8.6 Hz), 129.1, 128.7, 115.6 (d, *J* = 21.9 Hz), 75.1 (*C*H), 48.3 (Cy – *C*), 33.0 (Cy – *C*), 32.8 (Cy – *C*), 25.4 (Cy – *C*), 24.7 (Cy – *C*), 24.7 (Cy – *C*); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm -112.5; **HRMS** (ESI$^+$, *m/z*) $C_{21}H_{22}FNNaO_3^+$ ([M+Na]$^+$) *calc.*378.1476, *found* 378.1471; **IR**: 3267, 3099, 2993, 1724, 1654, 1117; **m.p.** = 190 – 191 °C.

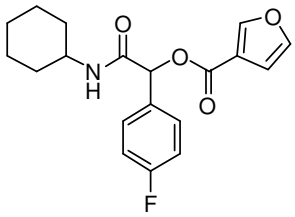
[33] The compounds in this section are provided by Dr. R. Leuma Yona.

2-(Cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl 2,6-dichloroisonicotinate (2-48)



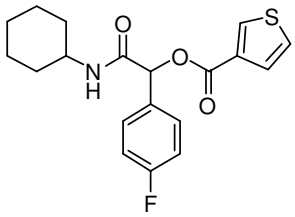
4-Fluorobenzaldehyde (107 µL, 1.0 mmol), 2,4-dichloropyridin-4-carboxylic acid (230 mg, 1.2 mmol) and cyclohexyl isocyanide (124 µL, 1.0 mmol) were added to an aqueous solution of LiCl (4M, 5 mL). The mixture was stirred at room temperature for 8 hours. Dichloromethane was added (20 mL) and the layers were separated. The aqueous phase was washed with dichloromethane (10 mL) and the combined organic layers were dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Flash column chromatography on silica gel (20% ethyl acetate in hexane, R_f=0.3) afforded the product as a white solid (270 mg, 0.63 mmol, 63% yield). **^{1}H NMR (500 MHz, $CDCl_3$)**: ppm 8.22 (d, *J* = 8.2 Hz, 1 H, Ar – *H*), 7.49 (dd, *J* = 8.7, 5.2 Hz, 2 H, Ar – *H*), 7.39 (d, *J* = 8.2 Hz, 1 H, Ar – *H*), 7.08 (t, *J* = 8.7 Hz, 2 H, Ar – *H*), 6.37 (d, *J* = 7.9 Hz, 1 H, N*H*), 6.25 (s, 1 H, C*H*), 3.73-3.89 (m, 1 H, Cy – *H*), 1.88-1.98 (m, 2 H, Cy – *H*), 1.70 (td, *J* = 8.0, 3.8 Hz, 2 H, Cy – *H*), 1.62 (ddd, *J* = 12.8, 3.6, 3.5 Hz, 1 H, Cy – *H*), 1.31-1.42 (m, 2 H, Cy – *H*), 1.10-1.24 (m, 3 H, Cy – *H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 166.4 (*C*=O), 163.2 (d, *J* = 248.9 Hz, *C* – F), 162.2 (*C*=O), 153.6, 149.0, 143.5, 130.9 (d, *J* = 3.8 Hz), 129.7 (d, *J* = 8.6 Hz), 124.6, 123.3, 115.9 (d, *J* = 21.9 Hz), 76.6 (*C*H), 48.5 (Cy – *C*), 32.9 (Cy – *C*), 32.8 (Cy – *C*), 25.3 (Cy – *C*), 24.6 (Cy – *C*); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm –111.6; **HRMS** (ESI^+, *m/z*) $C_{20}H_{19}Cl_2FN_2NaO_3^+$ ($[M+Na]^+$) *calc.* 447.0649, *found* 447.0643; **IR**: 3253, 3090, 1744, 1650, 1139, 1054; **m.p.** = 173 – 175 °C.

2-(Cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl 3-furoate (2-49)



4-Fluorobenzaldehyde (107 µL, 1.0 mmol), 3-furoic acid (134 mg, 1.2 mmol) and cyclohexyl isocyanide (124 µL, 1.0 mmol) were added to an aqueous solution of LiCl (4M, 5 mL). The mixture was stirred at room temperature for 8 hours. Dichloromethane was added (20 mL) and the layers were separated. The aqueous phase was washed with dichloromethane (10 mL) and the combined organic layers were dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Flash column chromatography on silica gel (20% ethyl acetate in hexane, $R_f = 0.4$) afforded the product as a white solid (300 mg, 0.88 mmol, 88% yield). **^{1}H NMR (400 MHz, $CDCl_3$)**: ppm 8.11 (m, 1 H, Ar – *H*), 7.44-7.49 (m, 3 H, Ar – *H*), 7.03-7.08 (m, 2 H, Ar – *H*), 6.70-6.78 (m, 1 H, Ar – *H*), 6.20 (s, 1 H, C*H*), 6.08 (d, *J* = 8.1 Hz, 1 H, N*H*), 3.76-3.86 (m, 1 H, Cy – *H*), 1.84-1.98 (m, 2 H, Cy – *H*), 1.56-1.74 (m, 3 H, Cy – *H*), 1.30-1.41 (m, 2 H, Cy – *H*), 1.11-1.22 (m, 3 H, Cy – *H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 167.0 (C=O), 163.0 (d, *J* = 248.0 Hz, *C* – F), 162.0, 161.2, 148.2, 144.2, 131.5 (d, *J* = 3.8 Hz), 129.3 (d, *J* = 8.6 Hz), 118.4, 115.7 (d, *J* = 21.0 Hz), 109.7, 74.6 (*C*H), 48.3, 32.9, 32.8, 25.4, 24.7, 24.6; **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm –112.4; **HRMS** (ESI$^+$, *m/z*) $C_{19}H_{20}FNNaO_4^+$ ([M+Na]$^+$) *calc.* 368.1269, *found* 368.1264; **IR**: 3161, 2998, 1478, 1046, 991; **m.p.** = 167 – 169 °C.

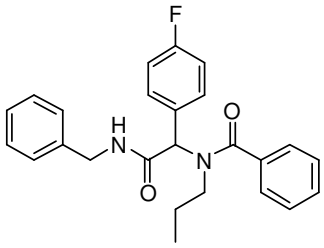
2-(Cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl thiophene-3-carboxylate (2-50)



4-Fluorobenzaldehyde (107 µL, 1.0 mmol), thiophene-3-carboxylic acid (146 mg, 1.2 mmol) and cyclohexyl isocyanide (124 µL, 1.0 mmol) were added to an aqueous solution of LiCl (4M, 5 mL). The mixture was stirred at room temperature for 8 hours. Dichloromethane was added (20 mL) and the layers were separated. The aqueous phase was washed with dichloromethane (10 mL) and the combined organic layers were dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. Flash column chromatography on silica gel (30% ethyl acetate in hexane, R_f=0.5) afforded the product as a white solid (250 mg, 0.70 mmol, 70% yield). **^{1}H NMR (400 MHz, $CDCl_3$)**: ppm 8.19-8.23 (m, 1 H, Ar – *H*), 7.57 (dt, *J* = 5.1, 1.3 Hz, 1 H, Ar – *H*), 7.49 (dd, *J* = 8.2, 5.4 Hz, 2 H, Ar – *H*), 7.34-7.40 (m, 1 H, Ar – *H*), 7.07 (t, *J* = 8.3 Hz, 2 H, Ar – *H*), 6.23 (s, 1 H, C*H*), 6.08 (d, *J* = 7.8 Hz, 1 H, N*H*), 3.77-3.89 (m, 1 H, Cy – *H*), 1.87-1.99 (m, 2 H, Cy – *H*), 1.57-1.75 (m, 3 H, Cy – *H*), 1.31-1.43 (m, 2 H, Cy – *H*), 1.09-1.27 ppm (m, 3 H, Cy – *H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 167.1 (*C*=O), 163.0 (d, *J* = 247.9 Hz, *C* – F), 160.8 (C=O), 133.7, 132.4, 131.6 (d, *J* = 3.8 Hz), 129.4 (d, *J* = 7.6 Hz), 127.7, 126.7, 115.7 (d, *J* = 21.9 Hz), 74.9 (*C* – H), 48.2, 33.0, 32.8, 25.4, 24.7, 24.6; **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm –112.5; **HRMS** (ESI$^+$, *m/z*) $C_{19}H_{20}FNNaO_3S^+$ ([M+Na]$^+$) *calc.* 384.1040, *f*ound 384.1035; **IR**: 3269, 3091, 1715, 1654, 1158; **m.p.** = 178 – 180 °C.

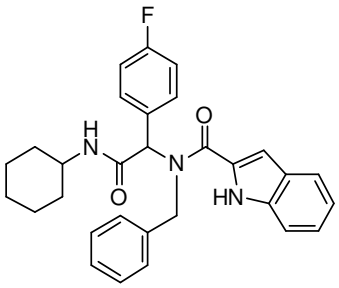
4.1.3. Synthesis data for Section 2.5.2

***N*-[2-(Benzylamino)-1-(4-fluorophenyl)-2-oxoethyl]-*N*-propylbenzamide (2-68)**



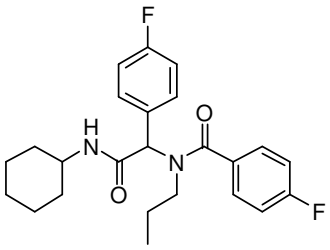
4-Fluorobenzaldehyde (54 µL, 0.5 mmol), benzyl isocyanide (66 µL, 0.5 mmol), 1-propylamine (41 µL, 0.5 mmol) and benzoic acid (61 mg, 0.5 mmol) were mixed in ethanol (1 ml) and stirred for 48 hours at room temperature. After removal of solvent *in vacuo*, the residue was purified by flash column chromatography on silica gel (33% ethyl acetate in hexane, R_f=0.3) to give the pure product as a white solid (138 mg, 0.36 mmol, 78% yield). **^{1}H NMR (200MHz, $CDCl_3$)**: ppm 6.98-7.58 (m, 14 H, Ar – *H*), 6.61 (br. s, 1 H, N*H*), 5.71 (br. s, 1 H, C*H*), 4.52 (d, *J* = 5.9 Hz, 2 H, C*H*$_2$Ph), 3.15-3.38 (m, 2 H, C*H*$_2$CH$_2$CH$_3$), 0.90-1.55 (m, 2 H, CH$_2$C*H*$_2$CH$_3$), 0.49-0.56 (m, 3 H, CH$_2$CH$_2$C*H*$_3$); **^{13}C NMR (125MHz, $CDCl_3$)**: ppm 172.8 (*C*=O), 169.5 (*C*=O), 162.6 (d, *J* = 248.0 Hz, *C*–F), 137.9 (Ar – *C*), 136.3 (Ar – *C*), 131.1 (d, *J* = 4.0 Hz, Ar – *C*), 130.9 (d, *J* = 7.6 Hz, Ar – *C*), 129.7 (Ar – *C*), 128.6 (Ar – *C*), 128.5 (Ar – *C*), 127.7 (Ar – *C*), 127.5 (Ar – *C*), 126.5 (Ar – *C*), 115.8 (d, *J* = 21.0 Hz, Ar – *C*), 62.9 (*C*H), 50.8 (*C*H$_2$CH$_2$CH$_3$), 43.7 (*C*H$_2$Ph), 22.7 (CH$_2$*C*H$_2$CH$_3$), 11.0 (CH$_2$CH$_2$*C*H$_3$); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm -113.0; **HRMS** (ESI$^+$, *m/z*) $C_{25}H_{25}FN_2NaO_2^+$ ([M+Na]$^+$) *calc*. 427.1792, *found* 427.1780; **IR:** 3055, 2986, 2306, 1682, 1628, 1510, 1421; **m.p.** = 141 – 143 °C.

***N*-benzyl-*N*-[2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl]-1*H*-indole-2-carboxamide (2-69)**



4-Fluorobenzaldehyde (54 µL, 0.5 mmol), cyclohexyl isocyanide (62.2 µL, 0.5 mmol), 1-benzylamine (54.6 µL, 0.5 mmol) and indole-2-carboxylic acid (80.6 mg, 0.5 mmol) were mixed in 1 mL ethanol and stirred for overnight at room temperature. After removal of solvent under reduced pressure, the residue was purified by column chromatography on silica gel (33% ethyl acetate in hexane, R_f = 0.5) to give pure product as a pale yellow solid (192 mg, 0.41 mmol, 82% yield). **^{1}H NMR (400MHz, $(CD_3)_2SO$)** ppm 11.75 (s, 1 H, N*H*), 8.17 (br.s, 1 H, Ar – *H*), 6.91 - 7.53 (m, 13 H, Ar – H), 6.60 (br.s, 1 H, C*H*), 6.13 (br.s, 1 H, N*H*), 4.50 - 5.36 (m, 2 H, C*H*$_2$Ph), 3.57 (br.s, 1 H, Cy – *H*), 1.57 - 1.74 (m, 4 H, Cy – *H*), 1.48 - 1.57 (m, 1 H, Cy – *H*), 0.98 - 1.30 (m, 5 H, Cy – *H*); **^{13}C NMR (126MHz, $(CD_3)_2SO$)** ppm 168.3 (*C*=O), 164.3 (*C*=O), 161.6 (d, *J* = 245.1 Hz, *C* – F), 138.5 (Ar – *C*), 136.0 (Ar – *C*), 131.6 (Ar – *C*), 129.5 (Ar – *C*), 129.4 (Ar – *C*), 128.5 (Ar – *C*), 127.7 (Ar – *C*), 126.3 (Ar – *C*), 126.3 (Ar – *C*), 123.6 (Ar – *C*), 121.5 (Ar – *C*), 119.8 (Ar – *C*), 114.8 (d, *J* = 20.1 Hz, Ar – *C*), 112.1 (Ar – *C*), 103.7 (Ar – *C*), 62.0 (*C*HPh-4F), 56.4 (*C*H$_2$Ph), 47.7 (Cy – C), 32.1 (Cy – *C*), 32.0 (Cy – *C*), 25.2 (Cy – *C*), 24.5 (Cy – *C*), 24.4 (Cy – *C*); **^{19}F NMR (376 MHz, $(CD_3)_2SO$)** ppm -114.4; **HRMS** (ESI^+, *m/z*) $C_{30}H_{29}FN_3O_2^-$ ($[M-H]^-$) *calc*. 482.2249, *found* 482.2252; **IR:** 3055, 2987, 2306, 1510, 1422; **m.p.**=195 – 197 °C

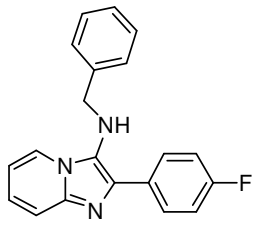
***N*-[2-(Cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl]-4-fluoro-*N*-propylbenzamide (2-71)**



4-Fluorobenzaldehyde (54 µL, 0.5 mmol), cyclohexyl isocyanide (62 µL, 0.5 mmol), 1-propylamine (41 µL, 0.5 mmol) and 4-fluorobenzoic acid (70 mg, 0.5 mmol) were mixed in ethanol (1 mL) and stirred for 48 hours at room temperature. After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography on silica gel (50% ethyl acetate in hexane, R_f=0.5) to give the pure product as a white solid (124 mg, 0.30 mmol, 61% yield). **^{1}H NMR (500 MHz, $CDCl_3$)**: ppm 7.45 (dd, *J* = 8.5, 5.4 Hz, 4 H, Ar–*H*), 7.10 (dt, *J* = 11.0, 8.7 Hz, 4 H, Ar–*H*), 6.17 (br. s, 1 H, C*H*), 5.64 (br. s, 1 H, N*H*), 3.78-3.92 (m, 1 H, Cy–*H*), 3.24 (dd, *J* = 10.1, 5.4 Hz, 2 H, C*H*$_2$CH$_2$CH$_3$), 1.87-2.01 (m, 2 H, Cy–*H*), 1.70 (td, *J* = 8.4, 3.9 Hz, 2 H, CH$_2$C*H*$_2$CH$_3$), 1.61 (dd, *J* = 9.0, 3.9 Hz, 1 H, Cy–*H*), 1.33-1.46 (m, 3 H, CH$_2$CH$_2$C*H*$_3$), 1.03-1.23 (m, 4 H, Cy–*H*), 0.57 (br. s, 3 H, Cy–*H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 171.9 (*C*=O), 168.5 (*C*=O), 163.3 (d, *J* = 249.8 Hz, *C*–F), 162.6 (d, *J* = 248.9 Hz, *C*–F), 132.5 (d, *J* = 2.9 Hz, Ar–*C*), 131.3 (d, *J* = 2.9 Hz, Ar–*C*), 130.9 (d, *J* = 7.6 Hz, Ar–*C*), 128.8 (d, *J* = 7.6 Hz, Ar–*C*), 115.8 (d, *J* = 21.0 Hz, Ar–*C*), 115.7 (d, *J* = 21.0 Hz, Ar–*C*), 63.0 (*C*H), 51.8 (*C*H$_2$CH$_2$CH$_3$), 48.6 (Cy–*C*), 32.8 (Cy–*C*), 25.4 (Cy–*C*), 24.7 (CH$_2$*C*H$_2$CH$_3$), 24.6 (CH$_2$CH$_2$*C*H$_3$), 22.6 (Cy–*C*), 11.1 (Cy–*C*); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm -113.0, -110.1; **HRMS** (ESI$^+$, *m/z*) $C_{24}H_{28}F_2N_2NaO_2^+$ ([M+Na]$^+$) *calc*. 437.2011, *found* 437.2001; **IR:** 3055, 2987, 2306, 1422; **m.p.** = 183 – 184 °C.

4.1.4. Synthesis data for Section 2.6.2[34]

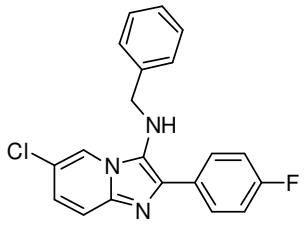
N-Benzyl-2-(4-fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (2-79a)



Benzyl isocyanide (70 µL, 0.58 mmol) was added to a solution of 4-fluorobenzaldehyde (81 µL, 0.75 mmol) and 2-aminopyridine (47 mg, 0.5 mmol) in methanol (1 mL). Scandium(III) trifluoromethanesulfonate (12 mg, 25 µmol, 5 mol%) was then added and the reaction was stirred at room temperature for 24 hours. The mixture was diluted with dichloromethane (5 mL) and quenched with water (5 mL). The layers were separated and the organic fraction was washed with saturated aqueous glutamic acid solution (5 mL, pH 10) and brine (5 mL). After drying over anhydrous sodium sulfate and filtration, the solvents were removed *in vacuo*. Recrystallization from dichloromethane/hexane afforded the product as an orange/brown solid (116 mg, 0.37 mmol, 73% yield). **^{1}H NMR (400 MHz, $CDCl_3$)**: ppm 7.94-7.99 (m, 3 H, Ar–H), 7.54-7.57 (m, 1 H, Ar – *H*), 7.27-7.36 (m, 5 H, Ar – *H*), 7.10-7.17 (m, 3 H, Ar – *H*), 6.76 (m, 1 H, Ar – *H*), 4.19 (d, *J* = 6.1 Hz, 2 H, NC*H*$_2$), 3.43 (t, *J* = 6.1 Hz, 1 H, N*H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 162.3 (d, *J* = 247.0 Hz, *C*F), 141.1, 138.8, 134.8, 129.6 (d, *J* = 2.9 Hz), 128.8, 128.7, 128.1, 127.7, 125.2, 124.7, 122.4, 117.0, 115.6 (d, *J* = 21.0 Hz), 112.1, 52.3 (N*C*H$_2$); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm -114.5; **HRMS** (ESI$^+$, *m/z*) $C_{20}H_{17}FN_3^+$ ([M+H]$^+$) *calc.* 318.1401, *found* 318.1400; **IR:** 3054, 2987, 2306, 1422; **m.p.** = 109 – 110 ºC.

[34] The compounds in this section are provided by Mr M. Hopkinson.

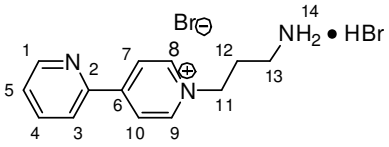
_N_-Benzyl-6-chloro-2-(4-fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine (2-79b)



Benzyl isocyanide (70 µL, 0.58 mmol) was added to a solution of 4-fluorobenzaldehyde (81 µL, 0.75 mmol) and 2-amino-5-chloropyridine (64 mg, 0.5 mmol) in methanol (1 mL). Scandium(III) trifluoromethane-sulfonate (12 mg, 25 µmol, 5 mol%) was then added and the reaction was stirred at room temperature for 24 hours. The mixture was diluted with dichloromethane (5 mL) and quenched with water (5 mL). The layers were separated and the organic fraction was washed with saturated glutamic acid solution (5 mL, pH 10) and brine (5 mL). After drying over anhydrous sodium sulfate and filtration, the solvents were removed *in vacuo*. Recrystallization from dichloromethane/hexane afforded the product as a pale yellow solid (112 mg, 0.32 mmol, 62% yield). **^{1}H NMR (400 MHz, $CDCl_3$)**: ppm 7.89-7.96 (m, 3 H, Ar–H), 7.45-7.48 (m, 1 H, Ar–H), 7.27-7.36 (m, 5 H, Ar–H), 7.06-7.16 (m, 3 H, Ar–H), 4.17 (d, *J* = 5.8 Hz, 2 H, NC*H*$_2$), 3.47 (t, *J* = 5.8 Hz, 1 H, N*H*); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 162.4 (d, *J* = 247.0 Hz, *C*F), 139.6, 138.5, 136.5, 129.6 (d, *J* = 2.9 Hz), 128.8, 128.7, 128.2, 127.9, 125.7, 125.6, 120.4, 120.4, 117.6, 115.6 (d, *J* = 21.9 Hz), 52.4 (N*C*H$_2$); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm -113.9; **HRMS** (ESI$^+$, *m/z*) $C_{20}H_{15}ClFN_3Na^+$ ([M+Na]$^+$) *calc.* 374.0831, *found* 374.0830; **IR**: 3055, 2983, 2333, 1422; **m.p.** = 177 – 179 ºC.

4.1.5. Synthesis data for Section 2.7

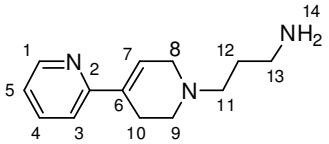
1-(3-Aminopropyl)-2, 4'-bipyridinium bromide monohydrobromide (2-82)



2-4'-Dipyridyl (176 mg, 1.13 mmol) and 3-bromopropylamine hydrobromide (297 mg, 1.36 mmol) were dissolved in 1 mL dimethylformamide. The mixture was heated at 95 °C for overnight before cooling down. Ether was added to the mixture to afford the crude product as pale yellow solid. The crude product was filtered, washed by methanol / ether (1:1, v:v) and dried *in vacuum.* It was used in next step without further purification (390mg, 1.0 mmol, 90% yield).

^{1}H NMR (400 MHz, ($(CD_3)_2SO$) δ ppm 9.25 (d, *J* = 7.1 Hz, 2 H, *H* – 8 and *H* – 9), 8.89 (dd, *J* = 3.8, 1.0 Hz, 1 H, *H* – 1), 8.86 (d, *J* = 7.1 Hz, 2 H, *H* – 7 and *H* – 10), 8.48 (d, *J* = 8.1 Hz, 1 H, *H* – 3), 8.14 (td, *J* = 7.8, 1.8 Hz, 1 H, *H* – 4), 7.95 (br. s., 3 H, *H* – 14), 7.69 (ddd, *J* = 7.6, 4.8, 1.0 Hz, 1 H, *H* – 5), 4.77 (t, *J* = 7.2 Hz, 2 H, *H* – 11), 2.86 - 2.95 (m, 2 H, *H* – 13), 2.28 ppm (qd, *J* = 7.3, 7.1 Hz, 2 H, *H* – 12); **^{13}C NMR** (125 MHz, ($(CD_3)_2SO$) δ ppm 153.0 (*C* – 1), 150.7 (*C* – 2), 149.7 (*C* – 8 and *C* – 9), 145.3 (*C* – 4), 138.3 (*C* – 6), 126.7 (*C* – 5), 124.5 (*C* – 7 and *C* – 10), 123.4 (*C* – 3), 57.2 (*C* – 11), 35.6 (*C* – 13), 28.6 (*C* – 12). Other characterization data are in agreement with reported results.[194]

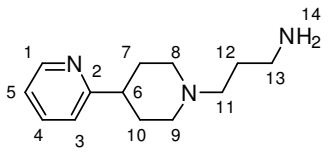
3-(3',6'-Dihydro-2,4'-bipyridin-1'(2'*H*)-yl)propan-1-amine (2-83)



2-82 (344 mg, 1 mmol) was dissolved in methanol (4 mL) and coolled to 0 °C. Sodium borohydride (151 mg, 4 mmol) was added carefully. The reaction mixture was stirred for 2 hours at 0 °C before being allowed to warm to room temperature overnight. The solvent was

removed *in vacuo* and the crude residue was dissolved in diethyl ether (30 mL). After washing with 20% NaOH (aq., 15 mL), the aqueous phase was extracted with diethyl ether (2×30 mL). The combined organic fractions were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford the crude product as a yellow oil. Flash column chromatography on silica gel (10% methanol in dichloromethane with 1% ammonia, R_f = 0.2) afforded the pure product as a pale yellow oil (135 mg, 0.68 mmol, 68% yield). **^{1}H NMR (500 MHz, $CDCl_3$)**: ppm 8.56 (ddd, *J* = 4.9, 1.7, 0.9 Hz, 1 H, *H* – 1), 7.63 (td, *J* = 7.7, 1.9 Hz, 1 H, *H* – 4), 7.38 (d, *J* = 10.1 Hz, 1 H, *H* – 3), 7.13 (ddd, *J* = 7.5, 4.8, 0.9 Hz, 1 H, *H* – 5), 6.64 (ddd, *J* = 3.4, 2.0, 1.8 Hz, 1 H, *H* – 7), 3.23 (dd, *J* = 6.3, 2.8 Hz, 2 H, *H* – 8), 2.82 (t, *J* = 6.8 Hz, 2 H, *H* – 13), 2.73 - 2.76 (m, 2 H, *H* – 10), 2.71-2.69 (m, 2 H, *H* – 9), 2.57(t, *J* = 7.2 Hz, 2 H, *H* – 11), 1.71 - 1.79 ppm (m, 2 H, *H* – 12); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 157.4 (*C* – 2), 148.9 (*C* – 1), 136.3 (*C* – 4), 135.0 (*C* – 6), 125.4 (*C* – 7), 121.8 (*C* – 5), 119.0 (*C* – 3), 56.3 (*C* – 11), 53.4 (*C* – 8), 50.3 (*C* – 10), 40.8 (*C* – 13), 30.4 (*C* – 12), 26.7 (*C* – 9); **HRMS** (ESI$^+$, *m/z*) $C_{13}H_{20}N_3^+$ ([M+H]$^+$) *calc*. 218.1652, *found* 218.1655; **IR**: 3385, 2909, 1641, 1632, 1469, 1433, 1384.

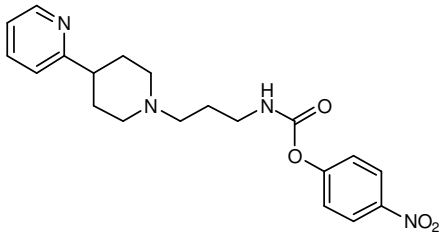
3-[4-(Pyridin-2-yl)piperidin-1-yl]propan-1-amine (2-81)



2-83 (360 mg, 1.8 mmol) and palladium on carbon (36 mg, 10% wt loading) were dissolved in methanol (5 mL). The reaction was purged with hydrogen and stirred overnight at room temperature. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. Flash column chromatography on silica gel (10% methanol in dichloromethane with 1% ammonia, R_f = 0.2) afforded the pure product as a pale yellow oil (251 mg, 1.2 mmol, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$): ppm 8.50 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1 H, *H* – 1), 7.61 (td, *J* = 7.7, 1.8 Hz, 1 H, *H* – 4), 7.18 (dt, *J* = 7.8, 0.9 Hz, 1 H, *H* – 3), 7.11 (ddd, *J* = 7.5, 4.9,

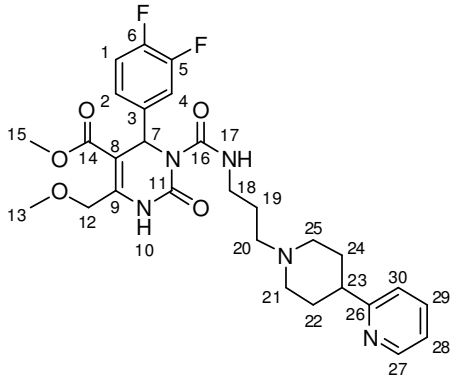
1.0 Hz, 1 H, H – 5), 3.04 - 3.11 (m, 2 H, H – 8 and H – 9), 2.76 (t, *J* = 6.8 Hz, 2 H, H – 13), 2.66 - 2.74 (m, 1 H, H – 6), 2.43 (t, *J* = 7.2 Hz, 2 H, H – 11), 2.05 (td, *J* = 11.9, 2.5 Hz, 2 H, H – 8' and H – 9'), 1.90 - 1.98 (m, 2 H, H – 7 and H – 10), 1.84 (td, *J* = 12.3, 3.2 Hz, 2 H, H – 7' and H – 10'), 1.68 (tt, *J* = 7.2, 6.8 Hz, 2 H, H – 12); ^{13}C NMR (125 MHz, $CDCl_3$): ppm 165.1 (C – 2), 149.1 (C – 1), 136.4 (C – 4), 121.2 (C – 5), 120.6 (C – 3), 56.8 (C – 11), 54.2 (C – 8 and C – 9), 44.67 (C – 6), 41.0 (C – 13), 32.0 (C – 7 and C – 10), 30.6 (C – 12); **HRMS** (ESI$^+$, *m/z*) $C_{13}H_{22}N_3^+$ ([M+H]$^+$) *calc*. 220.1808, *found* 220.1810; **IR**: 3357, 2939, 1592, 1474, 1435, 1215, 1125

4-Nitrophenyl {3-[4-(pyridin-2-yl)piperidin-1-yl]propyl}carbamate (2-84)



2-81 (185 mg, 0.84. mmol) was dissolved in dichloromethane (20 mL) and cooled to 0^{o}C. After addition of sodium hydride (60% dispersion in mineral oil, 41 mg, 1 mmol), the mixture was stirred for 1 hour at 0^{o}C. 4-Nitrophenyl chloroformate (205 mg, 1 mmol) was then carefully added and the reaction was stirred for another 1 hour at 0^{o}C. The reaction mixture was filtered through Celite® and concentrated *in vacuo* at room temperature to a volume of 3 mL. The concentrated mixture was then added dropwise to cold diethyl ether (25 mL). A white precipitate was formed which was filtered and washed with diethyl ether. The crude product was dried under *vacuum* and used directly in the next step without further purification.

Methyl 4-(3,4-difluorophenyl)-6-(methoxymethyl)-2-oxo-3-({3-[4-(pyridin-2-yl)piperidin-1-yl]propyl}carbamoyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (2-11)



2-24 (62 mg, 0.2 mmol) was dissolved in acetonitrile (1 mL). **2-84** (77 mg, 0.2 mmol) and potassium carbonate (83 mg, 0.6 mmol) were added and the reaction mixture was stirred for 24 hours at 60°C. After removal of the solvent *in vacuo*, flash column chromatography column on silica gel (10% methanol in dichloromethane, R_f=0.2) afforded the pure product as a yellow oil (35 mg, 0.06 mmol, 32% yield over two steps); ^{1}H NMR (500 MHz, $CDCl_3$): ppm 8.88 (t, *J* = 5.4 Hz, 1 H, *H* – 17), 8.52 (dd, *J* = 4.7, 0.9 Hz, 1 H, *H* – 27), 7.71 (br. s., 1 H, *H* – 10), 7.61 (td, *J* = 7.7, 1.9 Hz, 1 H, *H* – 1), 7.16 - 7.21 (m, 2 H, *H* – 2 and *H* – 4), 7.05 - 7.12 (m, 3 H, *H* – 28, *H* – 29 and *H* – 30), 6.68 (s, 1 H, *H* – 7), 4.68 (s, 2 H, *H* – 12), 3.71 (s, 3 H, *H* – 15), 3.48 (s, 3 H, *H* – 13), 3.41 (qd, *J* = 13.0, 6.6 Hz, 1 H, *H* – 18), 3.32 (qd, *J* = 12.9, 6.6 Hz, 1 H, *H* – 18'), 3.05 (d, *J* = 9.8, 2 H, *H* – 21 and *H* – 25), 2.66 - 2.76 (m, 1 H, *H* – 23), 2.44 (t, *J* = 6.3, 1 H, *H* – 20), 2.10 (m, 2 H, *H* – 21' and *H* – 25'), 1.83 - 2.00 (m, 4 H, *H* – 22/22' and *H* – 24/24'), 1.78 (m, 2 H, *H* – 19); **^{13}C NMR (125 MHz, $CDCl_3$)**: ppm 164.9 (*C* – 14 or *C* – 26), 164.6 (*C* – 14 or *C* – 26), 153.1 (*C* – 11 or *C* – 16), 152.3 (*C* – 11 or *C* – 16), 150.2 (dd, *J* = 248.9, 12.4 Hz, *C* – 5 or *C* – 6), 149.1 (*C* – 27), 149.0 (dd, *J* = 248.9, 12.4 Hz, *C* – 5 or *C* – 6), 146.3 (*C* – 9), 137.5 (t, *J* = 4.3 Hz, *C* – 3), 136.5 (*C* – 29), 123.0 (dd, *J* = 6.7, 3.8 Hz, *C* – 2), 121.3 (*C* – 28 or *C* – 30), 120.6 (*C* – 28 or *C* – 30), 117.3 (d, *J* = 11.2 Hz, *C* – 1 or *C* – 4), 116.1 (d, *J* = 18.1 Hz, *C* – 1 or *C* – 4) , 101.3 (*C* – 8), 68.1

(C – 12), 59.2 (C – 13), 56.1 (C – 20), 54.0 (C – 21 and C – 25), 52.9 (C – 7), 51.7 (C – 15), 44.4 (C – 23), 39.2 (C – 18), 31.7 (C – 22 and C – 24), 26.6 (C – 19); **^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$)**: ppm –138.4, –136.8; **HRMS** (ESI^+, *m/z*) $C_{28}H_{34}F_2N_5O_5^+$ ($[M+H]^+$) *calc*. 558.2523, *found* 558.2531; **IR**: 3316, 2941, 1714, 1648, 1518, 1436, 1395.

4.2 Radiosynthesis results for Chapter 2

Radiolabelling experiments were performed in a customized automated radiochemical apparatus purchased from SCINTOMICS and controlled by Scintomics Control Center version 5.03.053.

Microwave assisted reactions were performed in the cavity connected to Model 521 microwave instrument supplied by Resonance Instruments, Inc with infrared temperature control.

[^{18}F]Fluoride was produced at the cyclotron of PETNET Solutions at Mont Vernon Hospital (UK) *via* the $^{18}O(p,n)^{18}F$ reaction (1-2 GBq, 1-5 mL). [^{18}F]Fluoride was separated from ^{18}O-enriched-water using anion exchange cartridges supplied by Synthra (^{18}F-Separation, 45 mg, activated by water (3 mL)) and eluted with a solution of Kryptofix® 222 (15 mg) and potassium carbonate (3 mg) in acetonitrile/water (4:1, 1 mL). The complex was dried under a gentle stream of nitrogen at 120 ^{o}C with successive addition of acetonitrile (3×0.5 mL). The resulting K^{18}F/Kryptofix® 222 complex was dissolved in an anhydrous solvent for use in nucleophilic radiofluorination reactions.

Solid-Phase-Extraction (SPE) was performed on C-18 Sep-Pak cartridges which were purchased from the Waters Corporation and activated by washing with methanol (2 mL) followed by water (2×5 mL). The crude reaction mixture was diluted with water (10-15 times volume) and loaded on a C-18 Sep-Pak cartridge. A further 3 mL of water was passed through and the cartridge was then dried under a gentle stream of nitrogen for 5 minutes.

The purified ^{18}F-labeled prosthetic group was eluted from the cartridge with an organic solvent and dispensed into vials for use in 'hot' multicomponent reactions.

Microwave assisted reactions were performed in the cavity of a Model 521 microwave instrument supplied by Resonance Instruments, Inc. with infrared temperature control. Radio-TLC was conducted using Merck silica plates on a Mini-Scan TLC scanner system supplied by LabLogic Systems Ltd. All quoted radiochemical yields (RCYs) are decay corrected.

^{18}F-labeled compounds were identified by comparison of their radioactive HPLC peaks with the UV traces of their non-radioactive reference compounds. Radio-HPLC was conducted on a Gilson HPLC instrument with a NaI-radiodetecter fitted. Unless otherwise stated, the HPLC separations were carried out on a Phenomenex NX 5u C18 column (150×4.60 mm) at room temperature using acetonitrile/water as the mobile phase. The flowing gradients were applied at a flow rate of 1 mL/min:

Gradient A: start with and hold at 10% acetonitrile for 1 minute, linear increase to 30% acetonitrile in 3 minutes, then hold for next 9 minutes before increase to 75% acetonitrile in next 2 minutes, holds for 2 minutes before decrease to 10% acetonitrile.

Gradient B: start with and hold at 10% acetonitrile for 1 minute, linear increase to 45% acetonitrile in 3 minutes, then increase to 55% acetonitrile in next 12 minutes, to 75% acetonitrile in next 3 minutes and hold for 3 minutes before decrease to 10% acetonitrile.

Gradient C: start with and hold at 10% acetonitrile for 1 minute, linear increase to 40% acetonitrile in 3 minutes, then increase to 50% acetonitrile in next 12 minutes, to 75% acetonitrile in next 3 minutes and hold for 3 minutes before decrease to 10% acetonitrile.

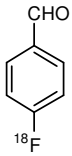
Gradient D: start with and hold at 10% acetonitrile for 1 minute, linear increase to 30% acetonitrile in 3 minutes and hold for 6 minutes, then increase to 55% acetonitrile in next 6 minutes and hold for 3 minutes before decrease to 10% acetonitrile.

Gradient E: linear increase from 35% acetonitrile to 40% acetonitrile in 10 minutes, then increase to 75% acetonitrile in next 5 minutes, and hold for 15 minutes before decrease to 35% acetonitrile.

Gradient F: linear increase from 5% acetonitrile to 95% acetonitrile in 10 minutes, then hold for next 5 minutes before decrease to 5% acetonitrile.

4.2.1 ^{18}F-Labelled building blocks (Section 2.2.4)

4-[^{18}F]Fluorobenzaldehyde ([^{18}F]2-2)



K^{18}F/Kryptofix® 222 in dimethyl sulfoxide (0.5 mL) was added to a sealed reaction vial containing **2-29** (10 mg) and heated under 50 W microwave irradiation for 100 seconds. The crude reaction mixture was diluted with water (10 mL). Analysis by HPLC (MeCN/H_2O **Gradient A**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-2** at a retention time of 12.2 min which was consistent with the non-radioactive 4-fluorobenzaldehyde. Analysis by radio-TLC (5% water in acetonitrile) indicated a radiochemical yield of 83% (*n*=3). Purification of **[^{18}F]2-2** was achieved by solid-phase-extraction on a C-18 Sep-Pak cartridge in a solution of an organic solvent.

Specific activities of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**) was measured after nucleophilic radiofluorination of **2-29** (2 mg of precursor at 160 ^{o}C for 240 seconds). The reaction mixtures were analysed by HPLC (MeCN/H_2O, 1 mL/min, 20 µL injected, linear increase from 25% acetonitrle to 30% acetonitrile in 20 minutes). The concentrations of the radioactive compounds were determined using calibration curves obtained by analysis of the UV absorbance (254 nm) of the relevant non-radioactive 4-fluorobenzaldehyde.

The specific activity of 4-[^{18}F]fluorobenzaldehyde was 35-90 GBq/μmol (*n*=3) *decay-corrected* to the end of synthesis. The calibration curve (absorbance to concentration) for 4-fluorobenzaldehyde is shown in **Figure 4.1**.

log(Absorbance)=3.79+0.92 log(Concentration)

R^2=0.9994

Concentration: mg/mL

Absorbance: AU

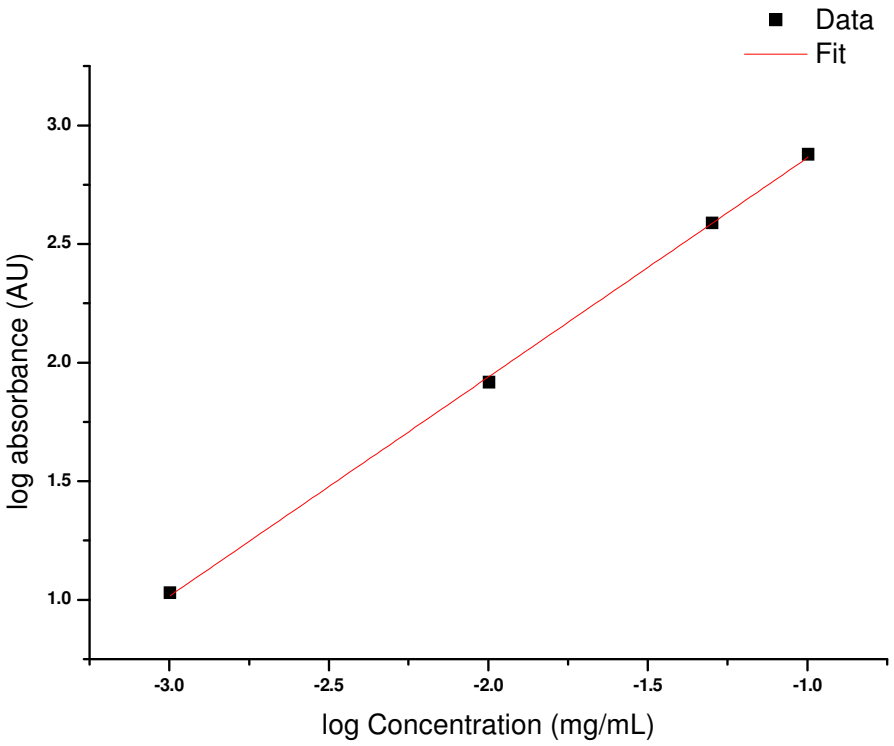
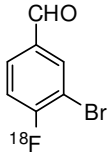


Figure 4.1 Calibration Curve for 4-Fluorobenzaldehyde

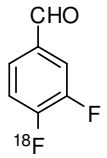
3-Bromo-4-[^{18}F]fluorobenzaldehyde ([^{18}F]2-21)



K^{18}F/Kryptofix® 222 in acetonitrile (0.3 mL) was added to a sealed reaction vial containing **2-32** (10 mg) and heated at 100 °C for 10 minutes. The crude reaction mixture was diluted with water (6 mL). Analysis by HPLC (MeCN/H_2O **Gradient B**, 1 mL/min, 20 μL injected)

indicated the formation of **[^{18}F]2-21** at a retention time of 10.9 min which was consistent with the non-radioactive 3-bromo-4-fluorobenzaldehyde. Analysis by radio-TLC (5% water in acetonitrile) indicated a radiochemical yield of 90%. Purification of **[^{18}F]2-21** was achieved by solid-phase-extraction on a C-18 Sep-Pak cartridge in a solution of 3-methyl-1-butanol.

3,4-[4-^{18}F]Difluorobenzaldehyde ([^{18}F]2-23)



Using an experimental batch microfluidic device [6] (Siemens Molecular Imaging), the aqueous [^{18}F]fluoride solution was passed through an strong anion exchanger column (AG1-X8). [^{18}F]Fluoride was eluted into a reaction cavity with a solution of K_2CO_3 (13 µL, 22.5 mg/mL) followed by Kryptofix® 222 (30 µL, 30 mg/mL). The complex was dried under 2 psi N_2 stream at 130 °C. The resulting dry complex of K^{18}F/Kryptofix® 222 was used for further nucleophilic ^{18}F-fluorination: **2-34** (2 mg) in acetonitrile (40 µL) was added and heated for 4 min at 180 °C. Analysis by HPLC (MeCN/H_2O **Gradient C**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-23** at a retention time of 9.2 min which was consistent with the non-radioactive 3,4-difluorobenzaldehyde. Elution with water (1 mL) and analysis by radio-TLC (5% water in acetonitrile) indicated a radiochemical yield of 73% (*n*=3). Purification of **[^{18}F]2-23** was achieved by solid-phase-extraction on a C-18 Sep-Pak cartridge in a solution of acetonitrile.

Specific activities of 3,4-[4-^{18}F]difluorobenzaldehyde (**[^{18}F]2-23**) was measured after nucleophilic radiofluorination of **2-34** (2 mg of precursor at 200 °C for 200 seconds). The reaction mixtures were analyzed by HPLC (MeCN/H_2O, 1 mL/min, 20 µL injected, linear increase from 25% acetonitrle to 30% acetonitrile in 20 minutes). The concentrations of the

radioactive compounds were determined using calibration curves obtained by analysis of the UV absorbance (254 nm) of the relevant non-radioactive 3,4-difluorobenzaldehyde.

The specific activity of 3,4-[4-^{18}F]difluorobenzaldehyde was 30-59 GBq/μmol (*n*=3) decay-corrected to end of synthesis. The calibration curve (absorbance to concentration) for 3,4-difluorobenzaldehyde is shown in **Figure 4.2**.

log(Absorbance)=3.64 + 0.99 log(Concentration)

R^2=0.9999

Concentration: mg/mL

Aborbance: AU

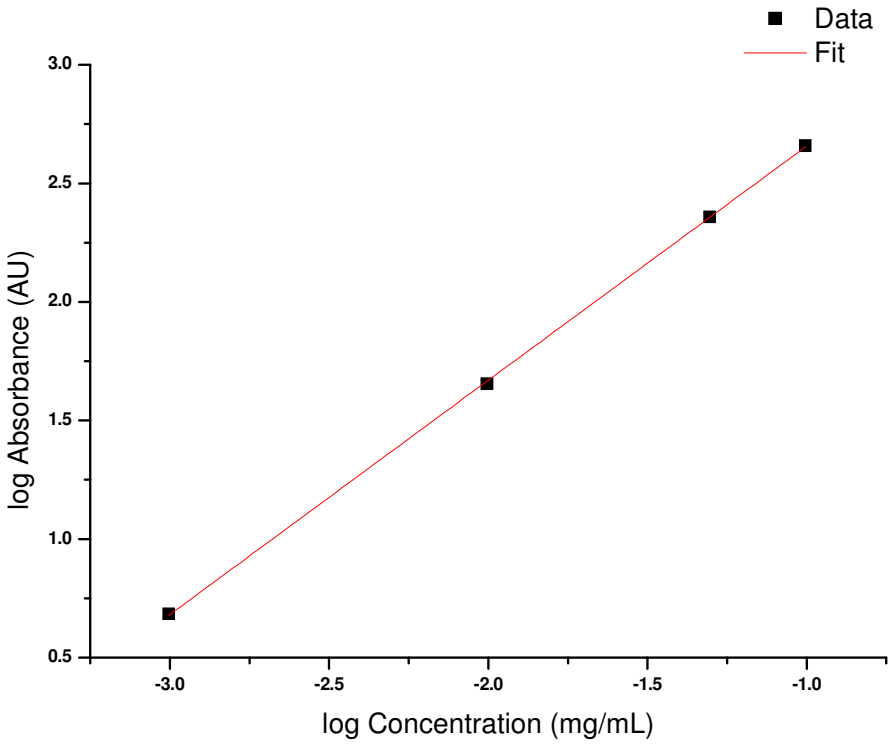


Figure 4.2 Calibration Curve for 3,4-Difluorobenzaldehyde

4.2.2 Radio- Biginelli Reaction (Section 2.2.5)

Optimisation of Radio–Biginelli Reaction

A solution of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**) in 3-methyl-1-butanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing urea, ytterbium(III) trifluoromethanesulfonate and ethyl acetoacetate (6 µL). The mixture was heated before being allowed to cool to room temperature, diluted with methanol (1 mL) and analyzed by radio-TLC. The results for the optimisation are shown in **Table 4.1**.

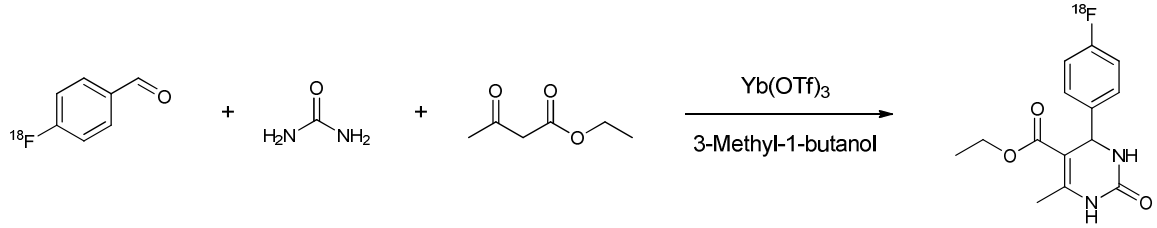
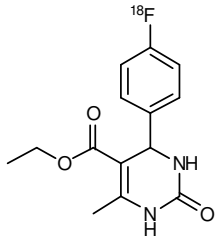


Table 4.1 Optimisation of Radio-Biginelli Reaction

Entry	Temp.[a] (ºC)	Time (min)	Urea (mg)	Yb(OTf)$_3$ (mg)	RCY[b] (%)
1	120	20	3	3	29
2	130	10	3	3	43
3	130	15	3	3	34
4	130	20	3	3	66 (*n*=3)
5	130	30	3	3	44
6	140	10	3	3	62
7	130	20	9	3	54
8	130	20	3	9	44
9	130	20	9	9	15
10	130[c]	20	3	3	44

[a]Conventional heating. [b]Decay corrected. Determined by radio-TLC. [c]Performed under microwave irradiation (50 W).

Ethyl 4-(4-[^{18}F]fluorophenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate ([^{18}F]2-1)



A solution of **[^{18}F]2-2** in 3-methyl-1-butanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing urea (3 mg), ytterbium(III) trifluoromethanesulfonate (3 mg) and ethyl acetoacetate (6 µL). The mixture was heated under vigorous stirring at 130 ^{o}C for 20 minutes before being allowed to cool to room temperature and diluted with methanol (1 mL). Analysis by HPLC (MeCN/H_2O **Gradient A**, 1 mL/min, 20 µL injected) indicated 100% consumption of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**, retention time=12.2 min) with corresponding formation of **[^{18}F]2-1** at a retention time of 14.1 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (66% ethyl acetate in hexane, R_f=0.3) indicated a radiochemical yield of 66% (*n*=3).

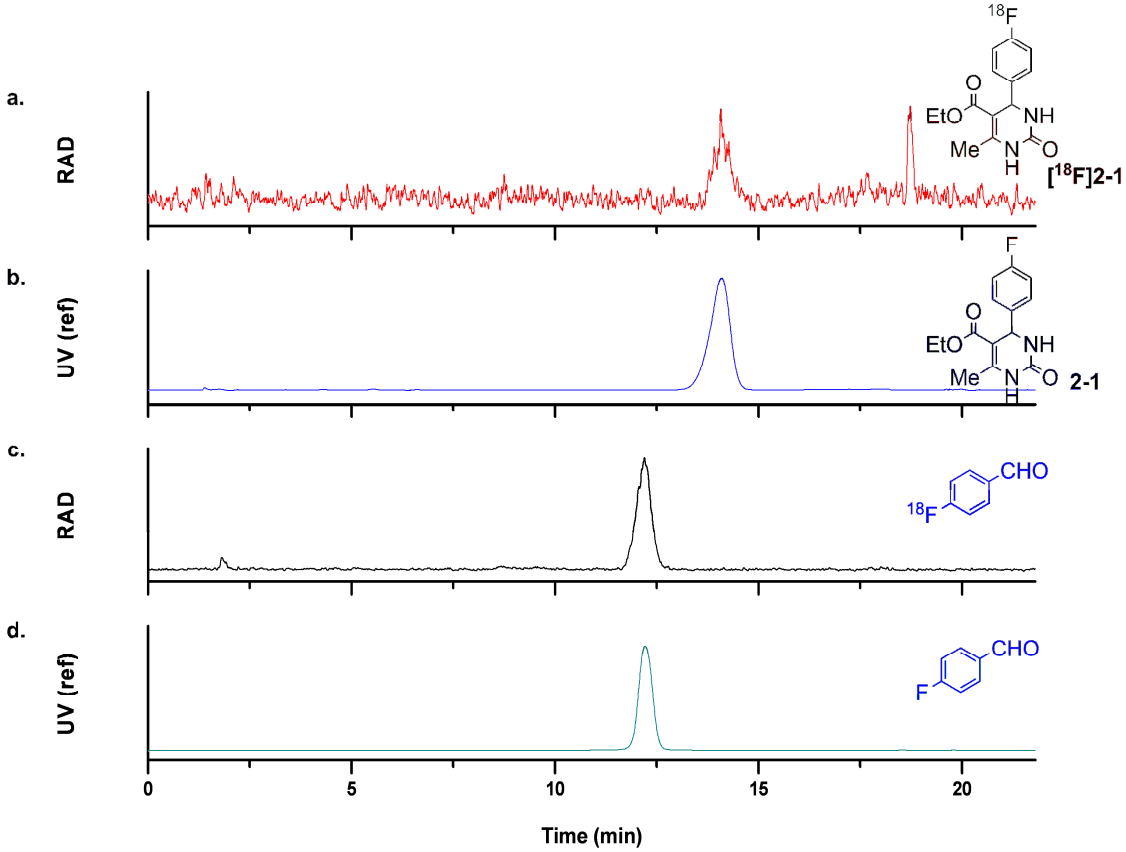
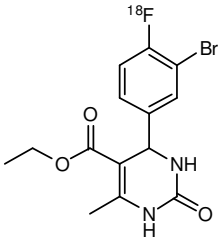


Figure 4.3 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Biginelli reaction; b. UV HPLC trace (254 nm) of "cold" **2-1** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient A, 1 mL/min, 20 µL injected]

Ethyl-4-(3-bromo-4-[^{18}F]fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate ([^{18}F]2-22)



A solution of **[^{18}F]2-21** in 3-methyl-1-butanol (200 µL, 8.8 MBq) was dispensed into a sealed vial containing urea (3 mg), ytterbium(III) trifluoromethanesulfonate (3 mg) and ethyl acetoacetate (6 µL). The mixture was heated to 130 °C for 20 minutes before being allowed to cool to room temperature and diluted with methanol (1 mL). Analysis by HPLC (MeCN/H_2O **Gradient B**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-22** at

a retention time of 8.8 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (50% ethyl acetate in hexane, R_f=0.2) indicated a radiochemical yield of 38%.

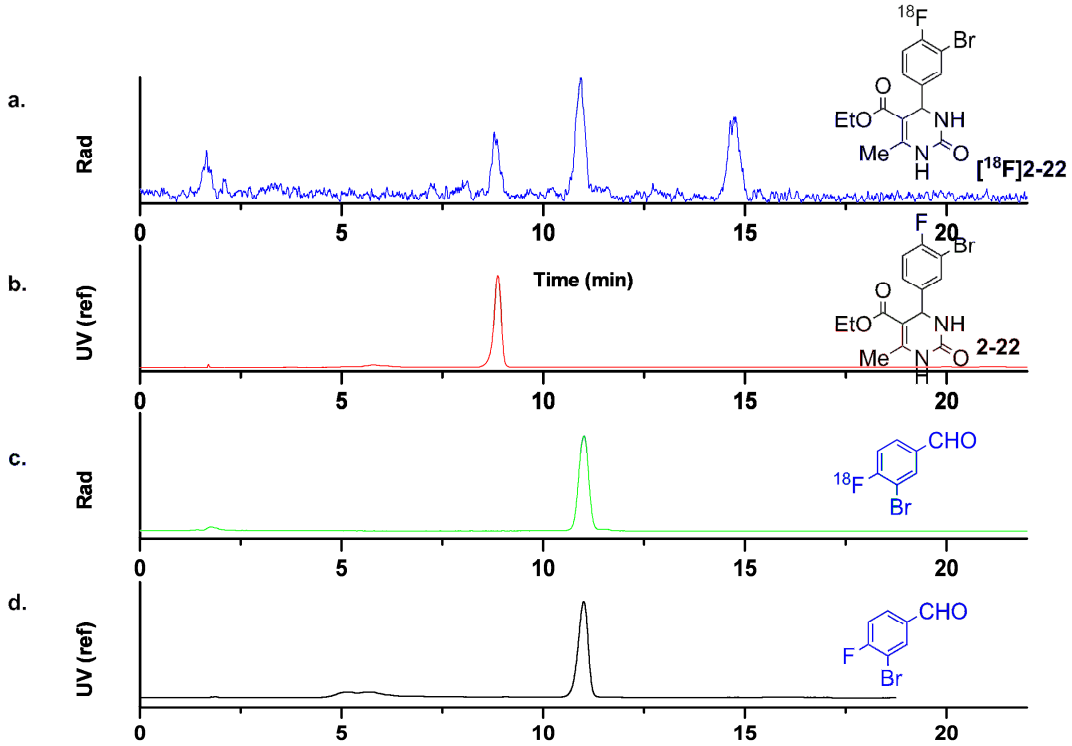
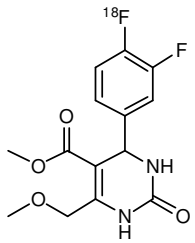


Figure 4.4 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Biginelli reaction; b. UV HPLC trace (254 nm) of "cold" **2-22** reference compound; c. Radioactivity HPLC trace of purified 3-bromo-4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 3-bromo-4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient B, 1 mL/min, 20 µL injected]

Methyl 4-(3,4-[4-^{18}F]difluorophenyl)-6-(methoxymethyl)-2-oxo-1,2,3,4 tetrahydropyrimidine-5-carboxylate ([^{18}F]2-24)



A solution of **[^{18}F]2-23** in acetonitrile (200 µL, 9.8 MBq) was dispensed into a sealed vial containing urea (3 mg), ytterbium(III) trifluoromethanesulfonate (3 mg) and methyl 4-methoxy-3-oxobutanoate (6 µL). The mixture was heated to 130 °C for 30 minutes before

being allowed to cool to room temperature. Analysis by HPLC (MeCN/H_2O **Gradient C**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-24** at a retention time of 8.8 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (80% ethyl acetate in hexane, R_f=0.3) indicated a radiochemical yield of 25% (*n*=3).

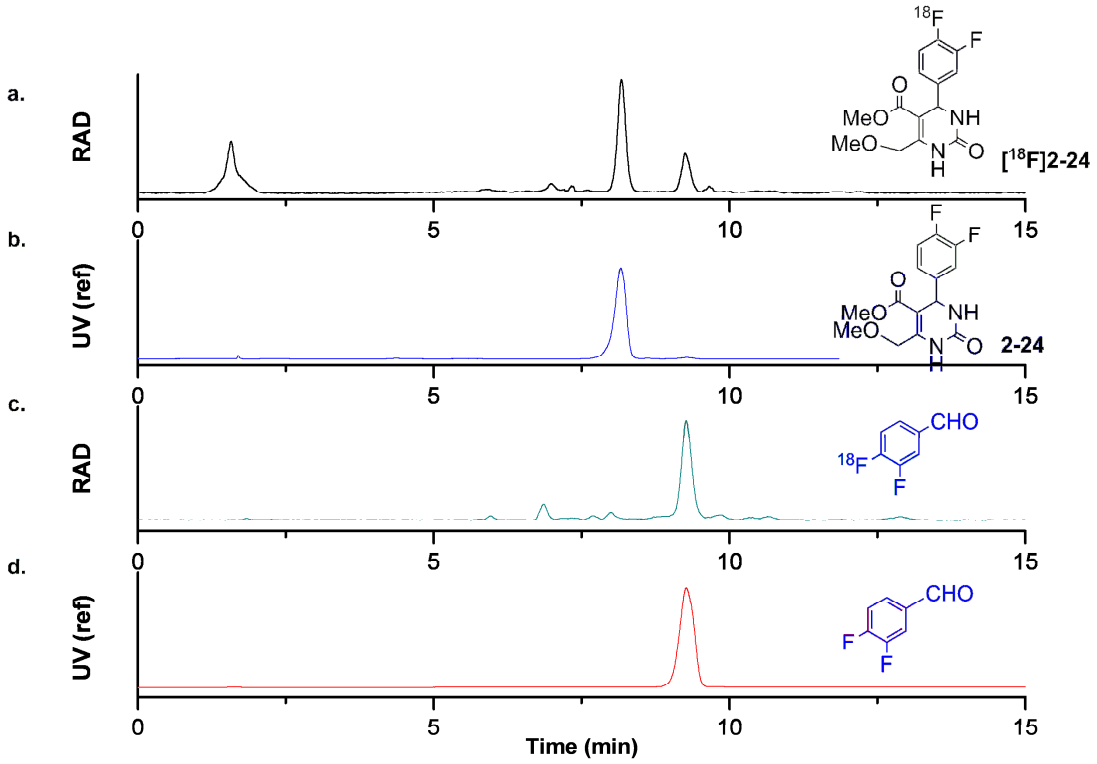


Figure 4.5 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Biginelli reaction; b. UV HPLC trace (254 nm) of "cold" **2-24** reference compound; c. Radioactivity HPLC trace of purified 3,4-[^{18}F]-difluorobenzaldehyde; d. UV HPLC trace of "cold" 3,4-difluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient C, 1 mL/min, 20 µL injected]

4.2.3 Radio–Passerini Reaction (Section 2.4.3)[35]

Optimisation of Radio–Passerini Reaction

A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in methanol (100 µL, 5-15 MBq) was dispensed into a sealed vial containing benzoic acid and cyclohexyl isocyanide in an aqueous solution of lithium chloride (4M). The mixture was heated before being allowed to

[35] The experiments in this section were performed in collaboration with Dr. R. Leuma Yona.

cool to room temperature, diluted with acetonitrile (300 µL) and analyzed by radio-TLC. The results for the optimisation are shown in **Table 4.2**.

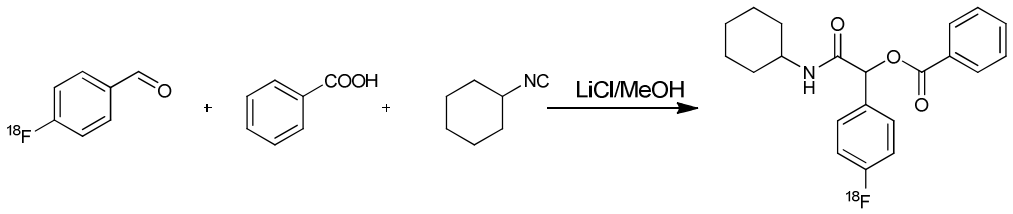
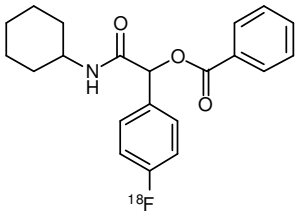


Table 4.2 Optimisation of Radio-Passerini Reaction

Entry	Temp. (°C)	Time (min)	Benzoic Acid (mg)	Cyclohexyl isocyanide (µL)	4M LiCl (aq.) (µL)	Methanol (µL)	RCY[a] [%]
1	80	30	7	4	0	500	0
2	80	30	7	4	600	100	5
3	100	30	7	4	600	100	3
4	100	30	7	4	300	200	0
5	100	10	7	4	600	100	44
6	100	20	7	4	600	100	56
7	100	30	4	2	600	100	59
8	100	30	7	4	600	100	65 (*n*=3)
9	100	30	7	4	700	100	61
10	120	10	7	4	600	100	14
11	120	30	7	4	600	100	32
12	130	30	7	4	600	100	27

[a]Decay-corrected. Determined by radio-TLC

2-(Cyclohexylamino)-1-(4-[^{18}F]fluorophenyl)-2-oxoethylbenzoate ([^{18}F]2-47)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in methanol (100 µL, 5-15 MBq) was dispensed into a sealed vial containing benzoic acid (7 mg) and cyclohexyl isocyanide (4 µL) in an aqueous solution of LiCl (4M, 600 µL). The mixture was heated at 100 °C for 30 minutes before being allowed to cool to room temperature and diluted with acetonitrile (300 µL). Analysis by HPLC (MeCN/H_2O **Gradient F**, 1 mL/min, 20 µL injected) indicated the

formation of **[^{18}F]2-47** at a retention time of 10.5 minutes, which was consistent with the cold reference compound, in a radiochemical yield of 65% (*n*=3).

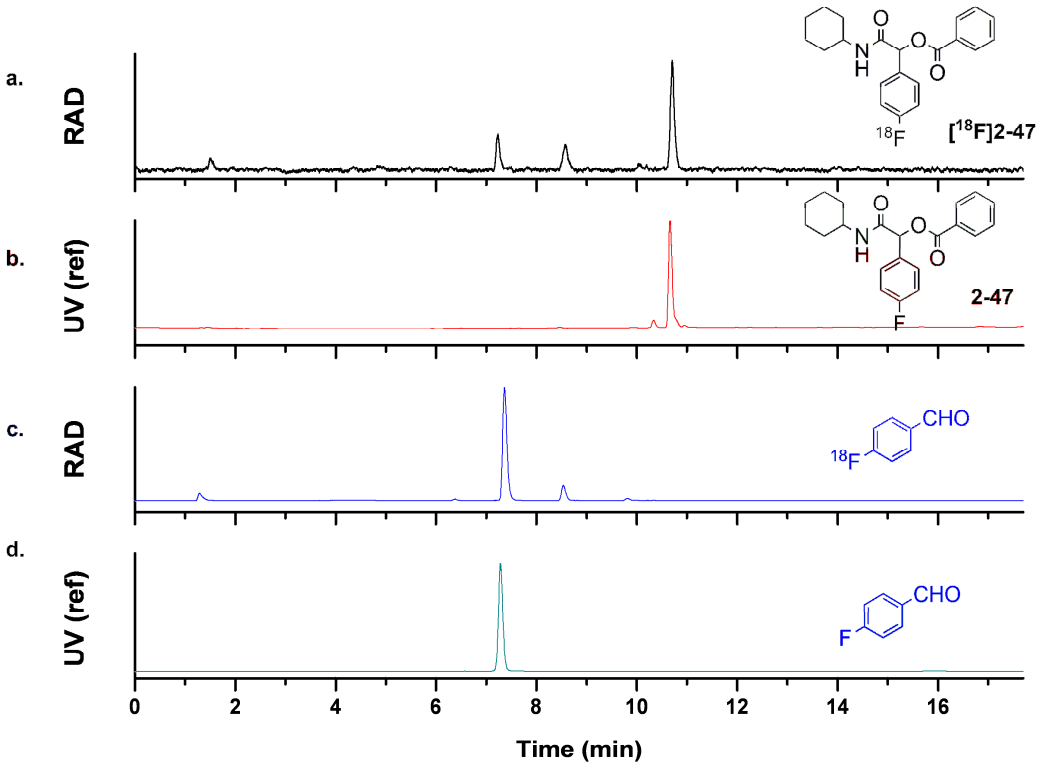
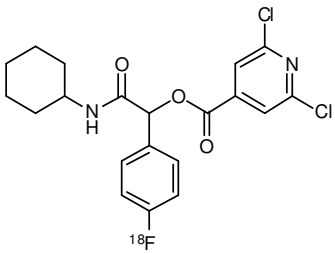


Figure 4.6 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Passerini reaction; b. UV HPLC trace (254 nm) of "cold" **2-47** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient F, 1 mL/min, 20 µL injected]

2-(Cyclohexylamino)-1-(4-[^{18}F]fluorophenyl)-oxoethyl 2,6-dichloropyridine-4-carboxylate ([^{18}F]2-48)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in methanol (100 µL, 5-15 MBq) was dispensed into a sealed vial containing 2,4-dichloropyridin-4-carboxylic acid (7 mg) and cyclohexyl isocyanide (4 µL) in an aqueous solution of LiCl (4M, 600 µL). The mixture was

heated at 100 °C for 30 minutes before being allowed to cool to room temperature and diluted with acetonitrile (300 µL). Analysis by HPLC (MeCN/H_2O **Gradient F**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-48** at a retention time of 10.8 minutes, which was consistent with the cold reference compound, in a radiochemical yield of 33% (*n*=2).

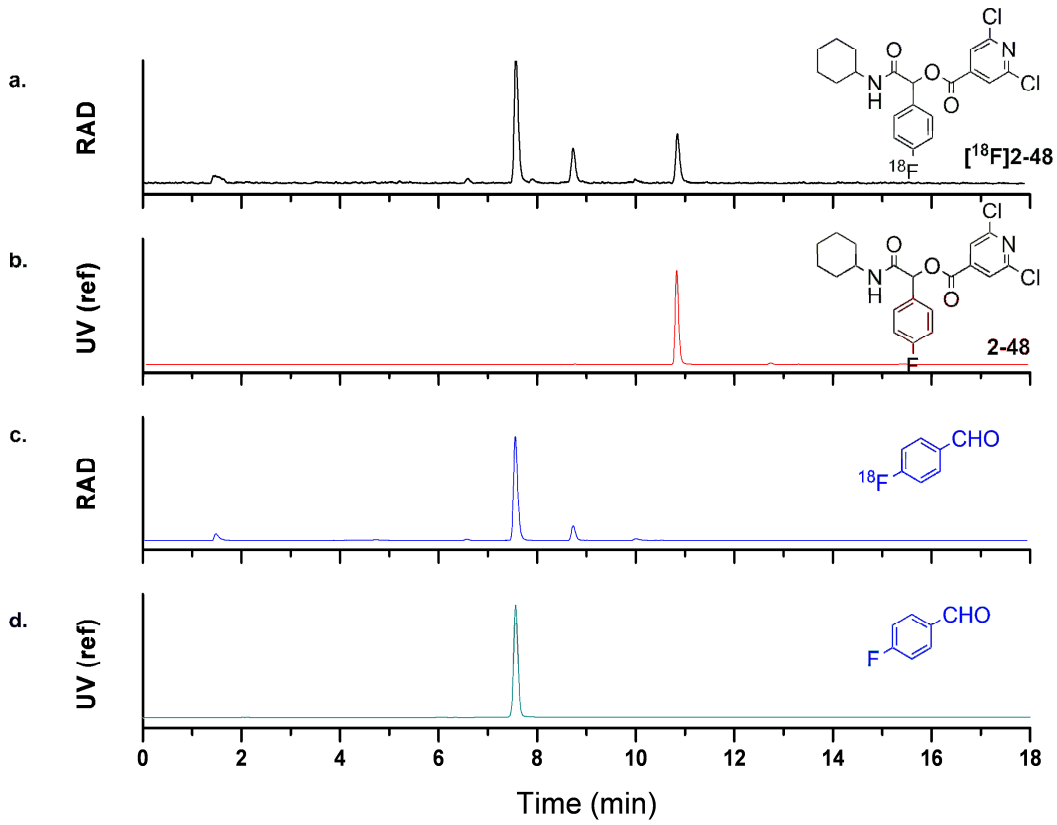
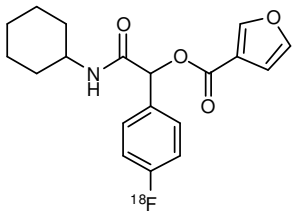


Figure 4.7 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Passerini reaction; b. UV HPLC trace (254 nm) of "cold" **2-48** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient F, 1 mL/min, 20 µL injected]

2-(Cyclohexylamino)-1-(4-[^{18}F]fluorophenyl)-2-oxoethylfuran-3-carboxylate ([^{18}F]2-49)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in methanol (100 µL, 5-15 MBq) was dispensed into a sealed vial containing furan-3-carboxylic acid (7 mg) and cyclohexyl isocyanide (4 µL) in an aqueous solution of LiCl (4M, 600 µL). The mixture was heated at 100 ^{o}C for 30 minutes before being allowed to cool to room temperature and diluted with acetonitrile (300 µL). Analysis by HPLC (MeCN/H_2O **Gradient F**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-49** at a retention time of 9.8 minutes, which was consistent with the cold reference compound, in a radiochemical yield of 28% (*n*=2).

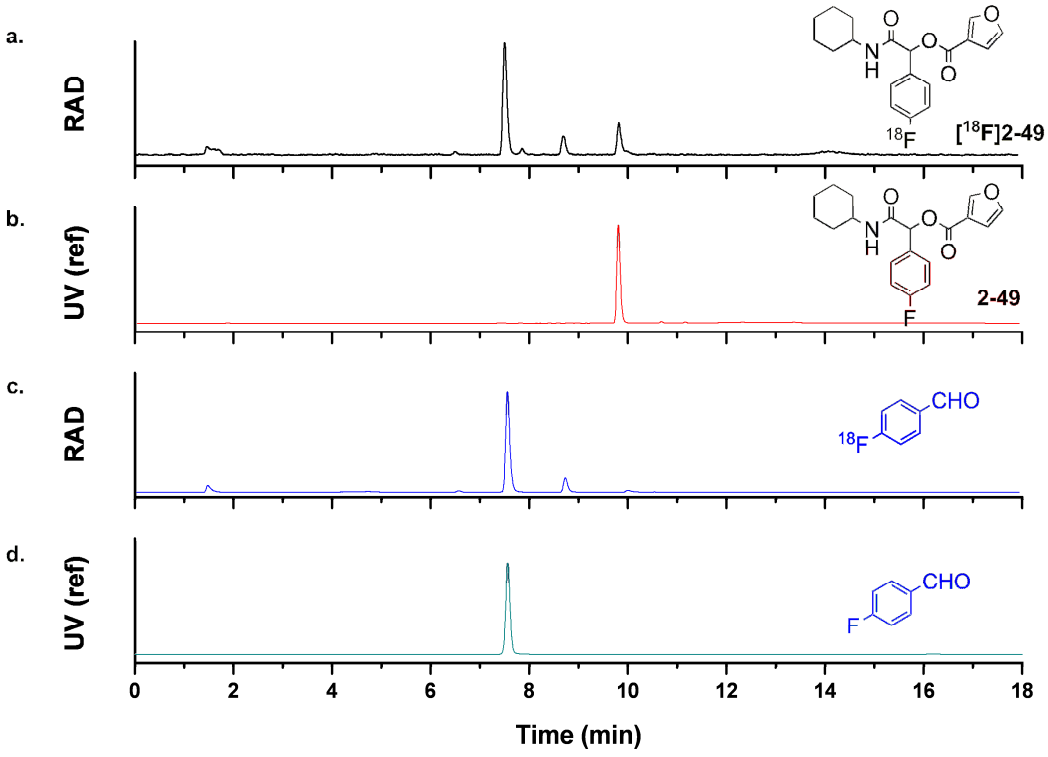
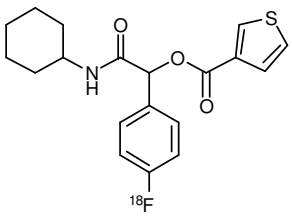


Figure 4.8 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Passerini reaction; b. UV HPLC trace (254 nm) of "cold" **2-49** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient F, 1 mL/min, 20 µL injected]

2-(Cyclohexylamino)-1-(4-[^{18}F]fluorophenyl)-2-oxoethylthiophene-3-carboxylate ([^{18}F]2-50)



A solution of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**) in methanol (100 µL, 5-15 MBq) was dispensed into a sealed vial containing thiophene-3-carboxylic acid (7 mg) and cyclohexyl isocyanide (4 µL) in an aqueous solution of LiCl (4M, 600 µL). The mixture was heated at 100 °C for 30 minutes before being allowed to cool to room temperature and diluted with acetonitrile (300 µL). Analysis by HPLC (MeCN/H_2O **Gradient F**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-50** at a retention time of 10.2 minutes, which was consistent with the cold reference compound, in a radiochemical yield of 32% (*n*=2).

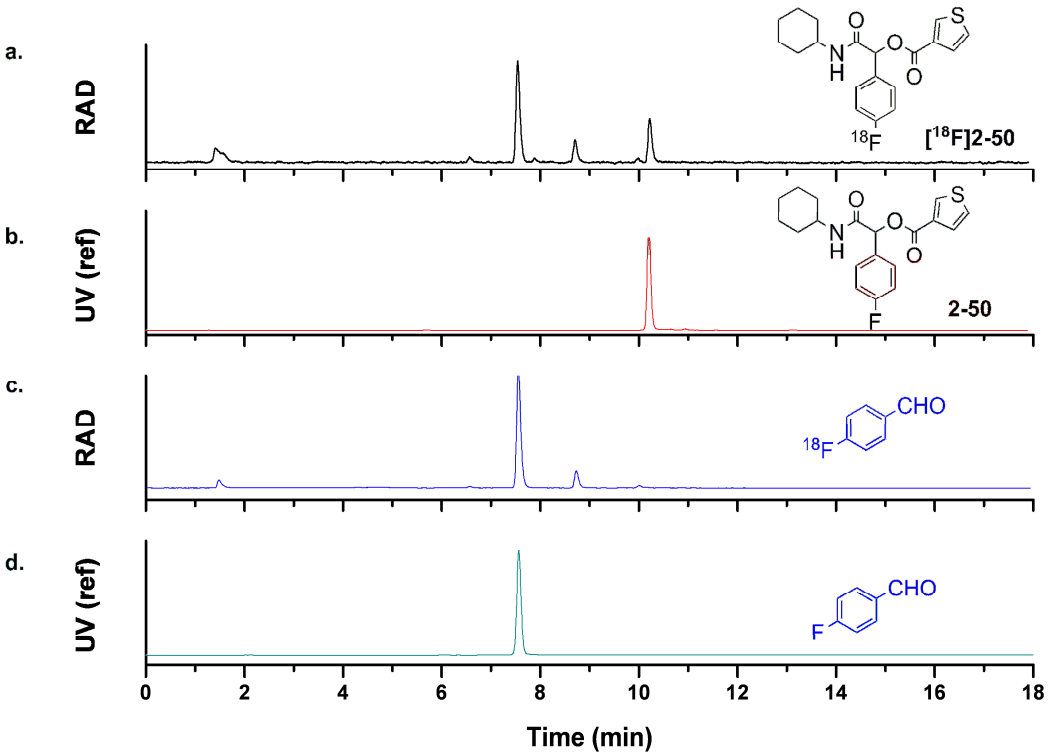


Figure 4.9 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Passerini reaction; b. UV HPLC trace (254 nm) of "cold" **2-50** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient F, 1 mL/min, 20 µL injected]

4.2.4 Radio-Ugi Reaction (Section 2.5.3)

Optimisation of Radio–Ugi Reaction

A solution of 4-[^{18}F]fluorobenzaldehyde (**[^{18}F]2-2**) in ethanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing benzoic acid, benzyl isocyanide and 1-propylamine. The mixture was heated and stirred before being allowed to cool to room temperature and analyzed by radio-TLC. The results for the optimisation are shown in **Table 4.3**.

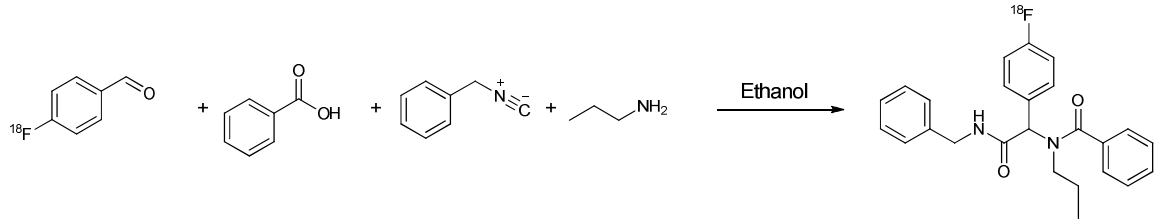
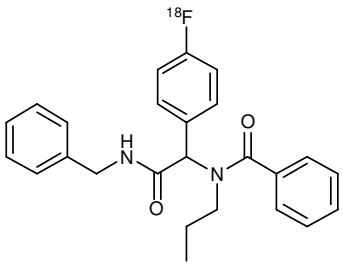


Table 4.3 Optimisation of Radio-Ugi Reaction

Entry	Temp.[a] (°C)	Time (min)	Benzoic Acid (mg)	Benzyl isocyanide (µL)	1-Propyl amine (µL)	Ethanol (µL)	RCY[b] (%)
1	120	10	3	3	2	500	5
2	120	20	3	3	2	500	10
3	120	30	3	3	2	500	8
4	100	30	3	3	2	500	7
5	130	10	3	3	2	500	5
6	100	30	6	6	4	200	62 (*n*=3)
7	120	30	6	6	4	200	59

[a]Conventional heating. [b]Decay corrected. Determined by radio-TLC.

***N*-[2-(Benzylamino)-1-(4-[^{18}F]fluorophenyl)-2-oxoethyl]-*N*-propylbenzamide ([^{18}F]2-68)**



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in ethanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing benzoic acid (6 mg), benzyl isocyanide (6 µL), and 1-propylamine (4 µL). The mixture was heated to 100 °C and stirred for 30 minutes before

being allowed to cool to room temperature. Analysis by HPLC (MeCN/H_2O **Gradient D**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-68** at a retention time of 16.7 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (33% ethyl acetate in hexane, R_f=0.3) indicated a radiochemical yield of 62% (*n*=3).

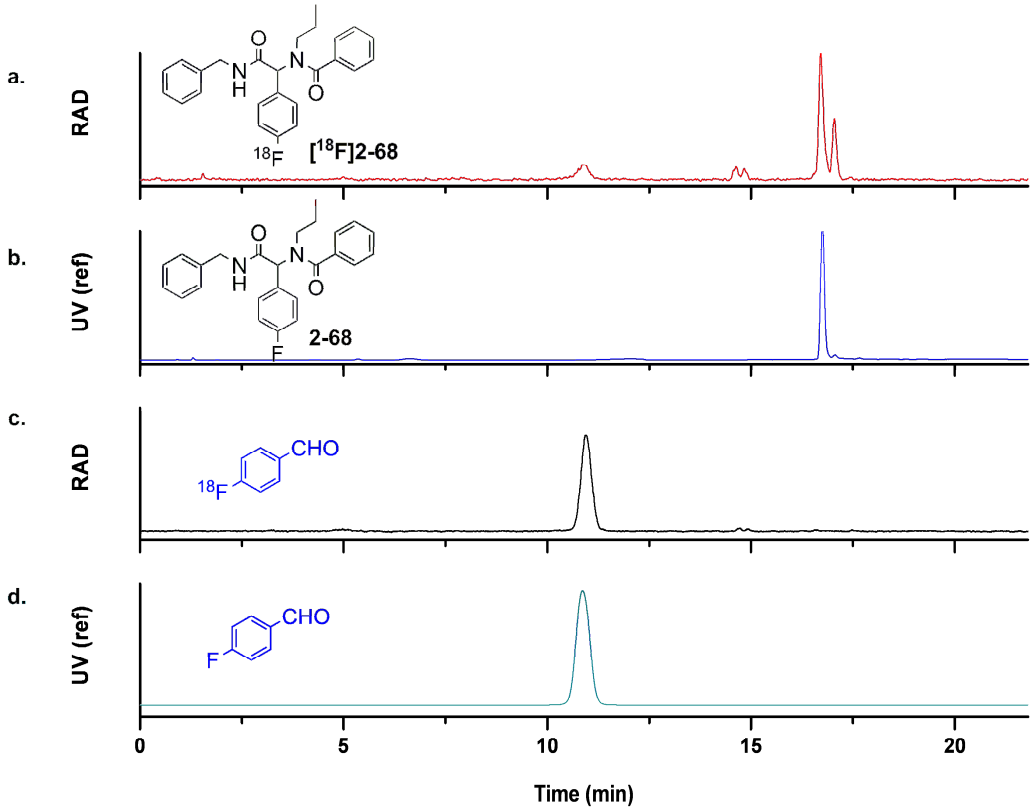
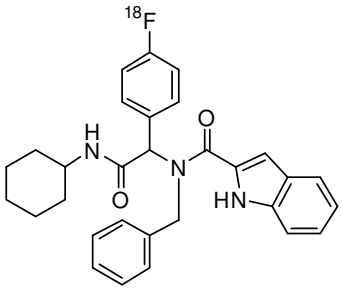


Figure 4.10 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Ugi reaction; b. UV HPLC trace (254 nm) of "cold" **2-68** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient D, 1 mL/min, 20 µL injected]

[^{18}F]-*N*-benzyl-*N*-[2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethyl]-1*H*-indole-2-carboxamide ([^{18}F]2-69)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in ethanol (200 µL, 13.4 MBq) was dispensed in a sealed vial containing cyclohexyl isocyanide (6.2 µL), indole-2-carboxylic acid (8 mg) and benzyl amine (5.5µL). The mixture was heated at 100 °C for 30 minutes under stirring. Analysis by HPLC (MeCN/H2O gradient C, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-69** in a retention time of 18.12 min, which was consistent with the 'cold' reference compound. Anylysis by radio-TLC (33% ethyl acetate in hexane, R_f = 0.2) indicated 26% radiochemistry yield (*n*=3).

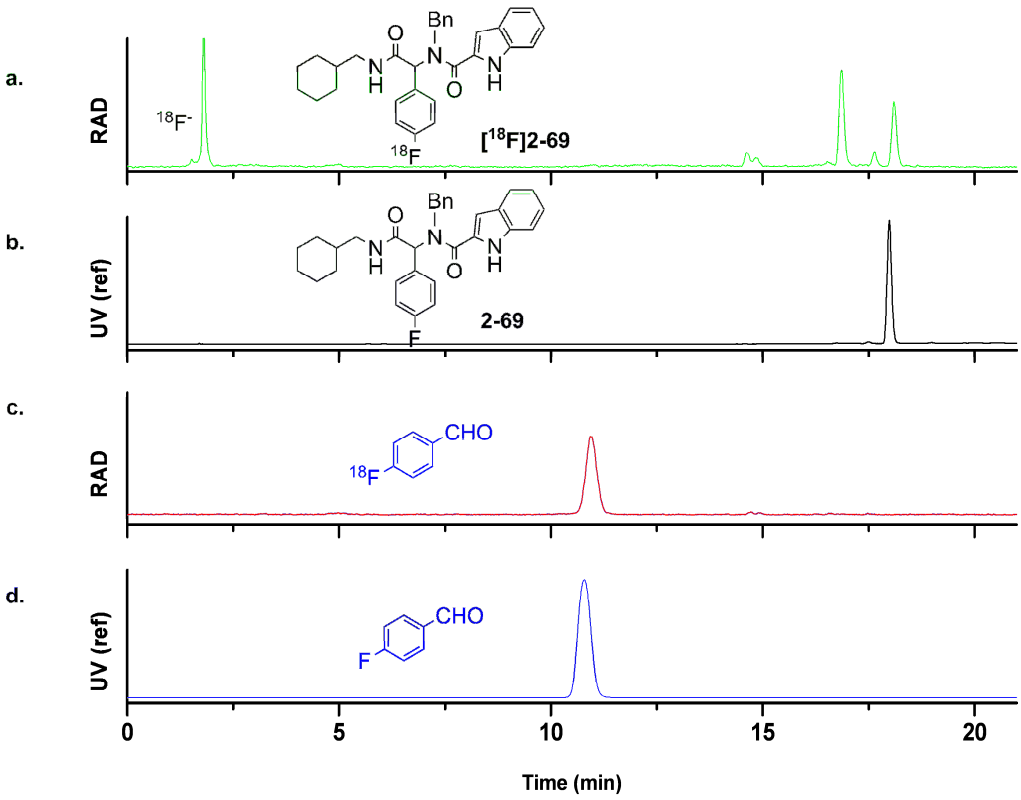
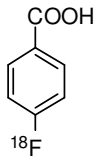


Figure 4.11 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Ugi reaction; b. UV HPLC trace (254 nm) of "cold" **2-69** reference compound; c.

Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient D, 1 mL/min, 20 µL injected]

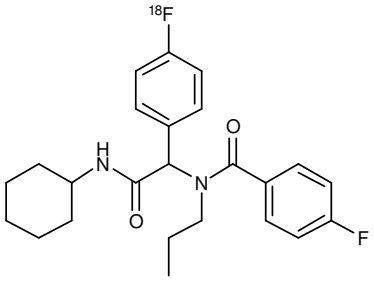
4-[^{18}F]Fluorobenzoic acid ([^{18}F]2-70)



K^{18}F/Kryptofix® 222 in acetonitrile (0.3 mL) was added to a sealed reaction vial containing 4-(ethoxycarbonyl)-*N*,*N*,*N*-trimethylbenzenaminium trifluoromethane-sulfonate [36] (10 mg) and heated to 120 °C for 15 minutes. Tetrapropylammonium hydroxide (200 µL, 1 M in H_2O) was added to the crude reaction mixture and heated at 120°C for a further 5 minutes. The reaction was then neutralized by stirring with trifluoroacetic acid (200 µL, 1 M in H_2O) at room temperature for 5 minutes. The crude reaction mixture was diluted with water (6 mL). Analysis by HPLC (MeCN/H_2O **Gradient D**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-70** at a retention time of 8.8 min which was consistent with the non-radioactive 4-fluorobenzoic acid. Analysis by radio-TLC (5% H_2O in acetonitrile, R_f=0.5) indicated 56% radiochemistry yield (*n*=2). Purified **[^{18}F]2-70** was obtained by solid-phase-extraction on a C-18 Sep-Pak cartridge in a solution of ethanol.

***N*-[2-(Cyclohexylamino)-1-([4-[^{18}F]fluorophenyl)-2-oxoethyl]-4-fluoro-*N*-propylbenzamide ([$^{18}F_{al}$]2-71)**

[36] This precursor is a kind offer from Dr. L. Carroll.



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in ethanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing 4-fluorobenzoic acid (8 mg), cyclohexyl isocyanide (6 µL) and 1-propylamine (4 µL). The mixture was heated to 100 °C and stirred for 30 minutes before being allowed to cool to room temperature. Analysis by HPLC (MeCN/H_2O **Gradient D**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-71** at a retention time of 17.2 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (20% ethyl acetate in hexane, R_f=0.3) indicated a radiochemical yield of 56% (*n*=2).

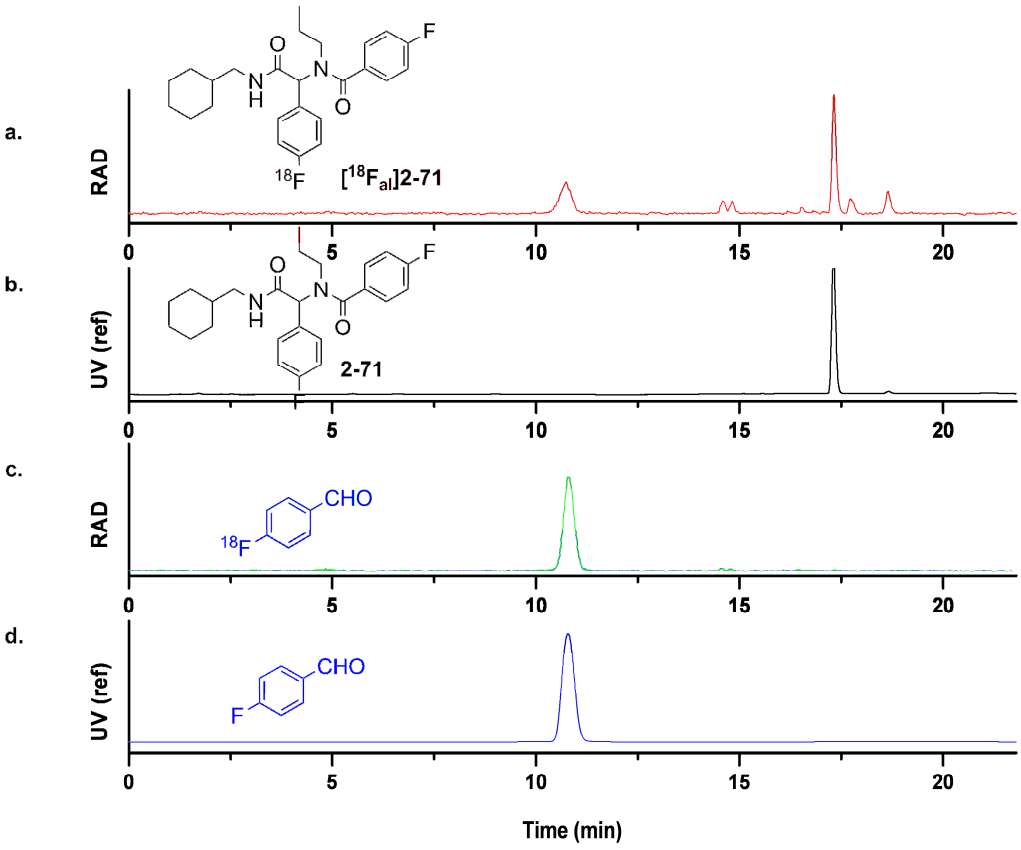
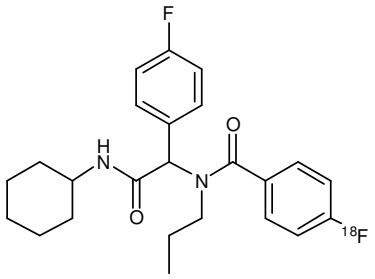


Figure 4.12 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Ugi reaction; b. UV HPLC trace (254 nm) of "cold" **2-71** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of

"cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient D, 1 mL/min, 20 µL injected]

_N_-[2-(Cyclohexylamino)-1-(4-[^{18}F]fluorophenyl)-2-oxoethyl]-4-fluoro-_N_-propylbenzamide ([$^{18}F_{ac}$]2-71)



A solution of 4-[^{18}F]-fluorobenzoic acid **[^{18}F]2-70** in ethanol (200 µL, 6.3 MBq) was dispensed into a sealed vial containing 4-fluorobenzaldehyde (5 µL), cyclohexyl isocyanide (6 µL), 'cold' 4-fluorobenzoic acid (8 mg) and 1-propylamine (4 µL). The mixture was heated at 100 ^{o}C for 30 minutes. Analysis by HPLC (MeCN/H_2O **Gradient D**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-71** at a retention time of 17.3 min, which was consistent with the 'cold' reference compound. Analysis by radio-TLC (33% ethyl acetate in hexane, R_f=0.5) indicated a radiochemical yield of 10% (*n*=2).

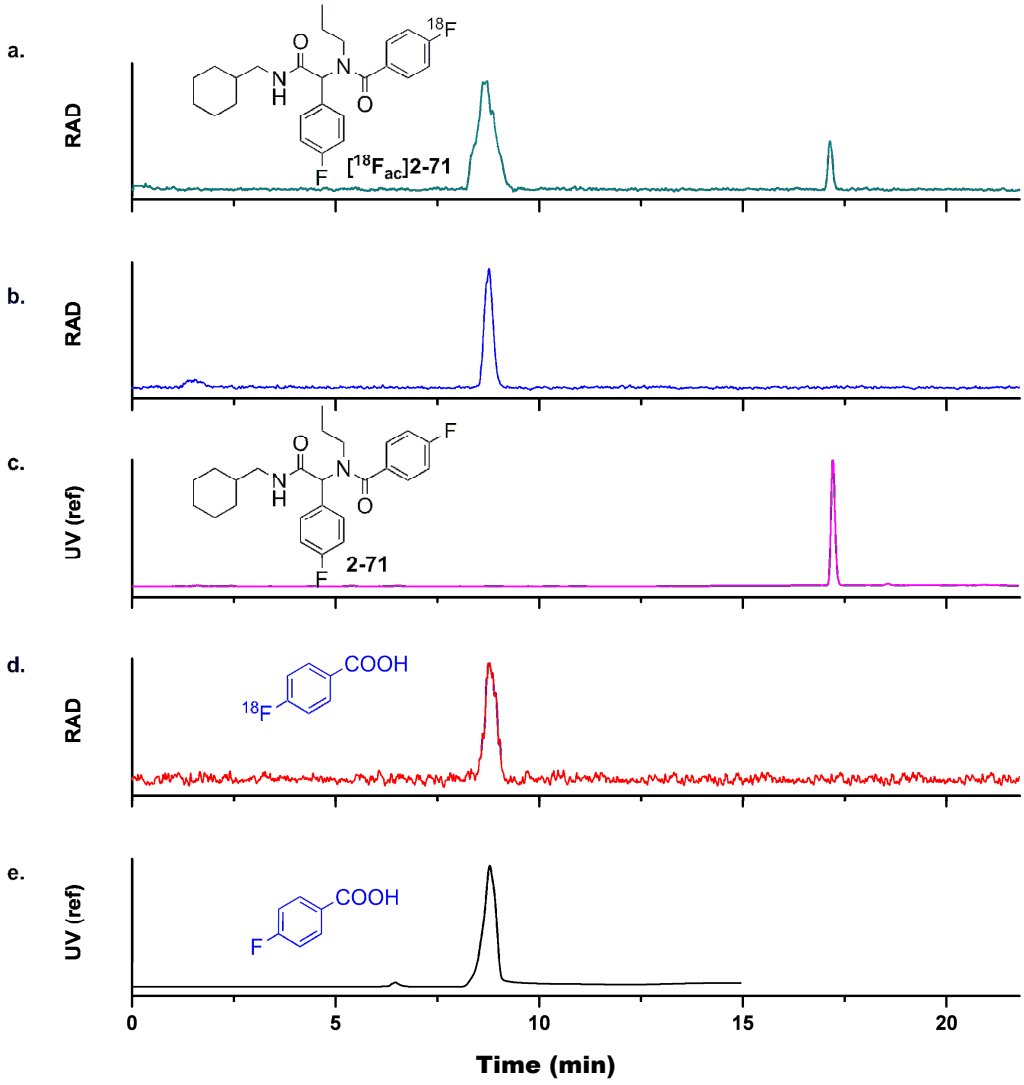


Figure 4.13 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Ugi reaction with addition of "cold" 4-fluorobenzoic acid; b. Radioactivity HPLC trace of crude reaction mixture of the radio-Ugi reaction without addition of "cold" 4-fluorobenzoic acid; c. UV HPLC trace (254 nm) of "cold" **2-71** reference compound; d. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzoic acid; e. UV HPLC trace of "cold" 4-fluorobenzoic acid. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient D, 1 mL/min, 20 µL injected]

4.2.5 Radio-Groebke Reaction (Section 2.6.3)[37]

Optimisation of Radio–Groebke Reaction

A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in a solvent (200 µL, 5-15 MBq) was dispensed into a sealed vial containing scandium(III) trifluoromethanesulfonate (5 mg) and

2-aminopyridine (3 mg). A solution of benzyl isocyanide (3 µL) in the solvent (300 µL) was then added *via* syringe. The reaction was heated before being allowed to cool to room temperature, diluted with acetonitrile (1 mL) and analyzed by radio-TLC. The results for the optimisation are shown in **Table 4.4**.

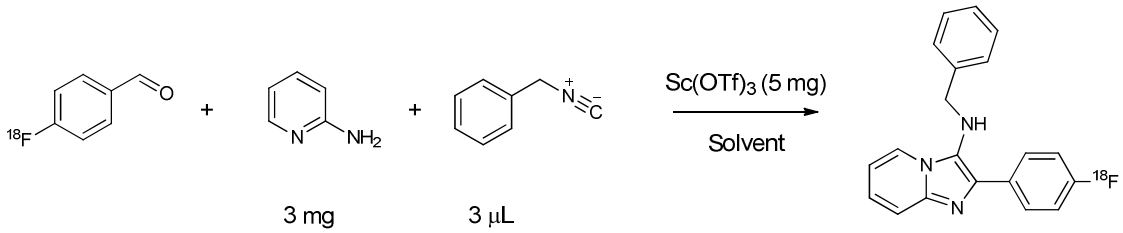
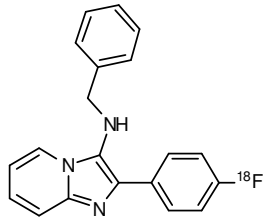


Table 4.4 Optimisation of Radio-Groebke-Bienaymé-Blackburn Reaction

Entry	Temp. (ºC)[a]	Time (min)	Solvent (0.5 mL)	RCY (%)[b]
1	100	15	MeCN	3
2	100-120[c]	20	MeCN	8
3	110	15	3-Methyl-1-butanol	31
4	150	15	3-Methyl-1-butanol	73
5	150[d]	15	3-Methyl-1-butanol	64
6	150	30	3-Methyl-1-butanol	80
7	150[e]	15	3-Methyl-1-butanol	81
8	170	15	3-Methyl-1-butanol	85 (*n*=4)

[a]Conventional heating. [b]Decay corrected. Determined by radio-TLC. [c]100 ºC for 15 minutes followed by 5 minutes at 120 ºC. [d]Benzyl isocyanide added prior to [^{18}F]4-fluorobenzaldehyde. [e]Performed under microwave irradiation (50 W).

N-Benzyl-2-(4-[^{18}F]fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine ([^{18}F]2-79a)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in 3-methyl-1-butanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing scandium(III) trifluoro-methanesulfonate (5 mg) and 2-aminopyridine (3 mg). A solution of benzyl isocyanide (3 µL) in 3-methyl-1-butanol (300 µL) was then added *via* syringe. The reaction was heated at 170 ^{o}C for 15 minutes

[37] The experiments in this section were performed in collaboration with Mr M. Hopkinson.

before being allowed to cool to room temperature. The mixture was then diluted with acetonitrile (1 mL). Analysis by radio-HPLC (MeCN/Phosphate Buffer (10mM, pH 7.0) **Gradient E**, 1 mL/min, 20 µL injected) indicated 100% consumption of the **[^{18}F]2-2** (retention time=7.5 min) with corresponding formation of **[^{18}F]2-79a** at a retention time of 16.2 min, which was consistent with the 'cold' reference compound. Radio-TLC (50% ethyl acetate in hexane, R_f=0.2) indicated a radiochemical yield of 85% (*n*=4).

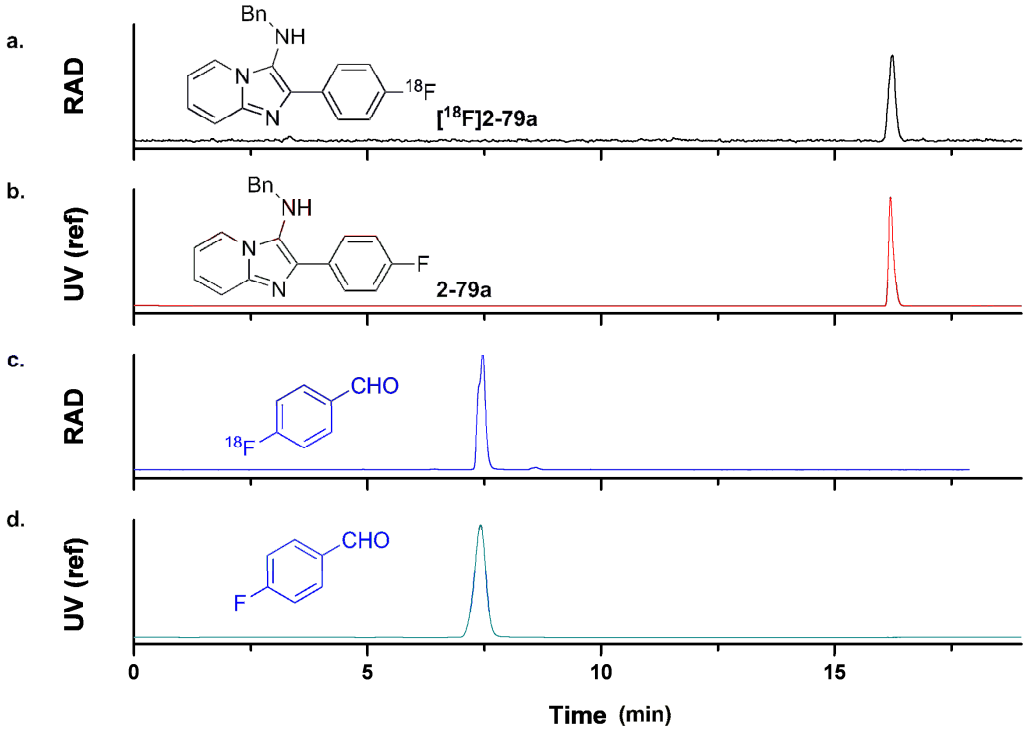
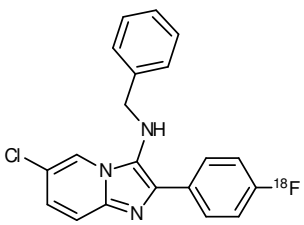


Figure 4.14 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Groebke reaction; b. UV HPLC trace (254 nm) of "cold" **2-79a** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient E, 1 mL/min, 20 µL injected]

_N_-Benzyl-6-chloro-2-(4-[^{18}F]fluorophenyl)imidazo[1,2-*a*]pyridin-3-amine ([^{18}F]2-79b)



A solution of 4-[^{18}F]fluorobenzaldehyde **[^{18}F]2-2** in 3-methyl-1-butanol (200 µL, 5-15 MBq) was dispensed into a sealed vial containing scandium(III) trifluoromethanesulfonate (5 mg) and 2-amino-5-chloropyridine (3 mg). A solution of benzyl isocyanide (3 µL) in 3-methyl-1-butanol (300 µL) was then added *via* syringe. The reaction was heated at 170 ºC for 15 minutes before being allowed to cool to room temperature. The mixture was then diluted with acetonitrile (1 mL). Analysis by radio-HPLC (MeCN/Phosphate Buffer (10mM, pH 7.0) **Gradient E**, 1 mL/min, 20 µL injected) indicated 100% consumption of the **[^{18}F]2-2** (retention time=7.5 min) with corresponding formation of **[^{18}F]2-79b** at a retention time of 18.4 min, which was consistent with the 'cold' reference compound. Radio-TLC (50% ethyl acetate in hexane, R_f=0.4) indicated a radiochemical yield of 76% (*n*=3).

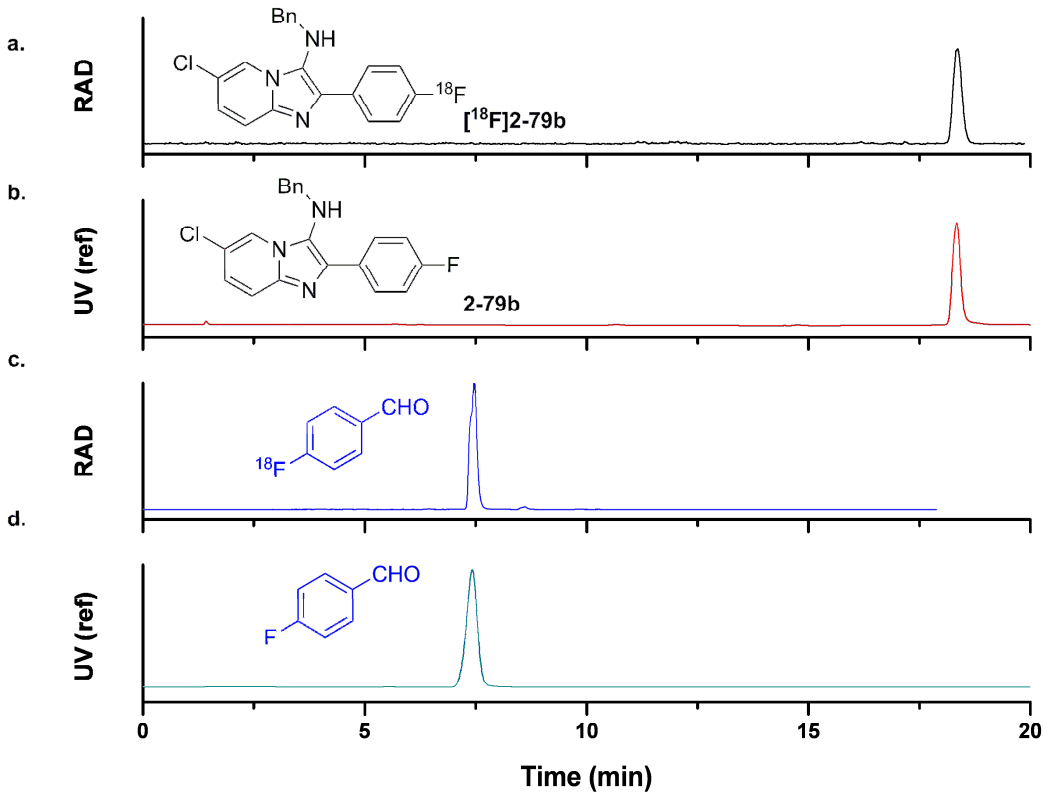
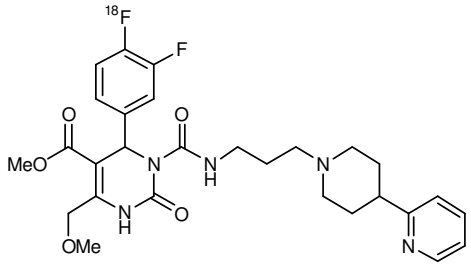


Figure 4.15 a. Radioactivity HPLC trace of crude reaction mixture of the radio-Groebke reaction; b. UV HPLC trace (254 nm) of "cold" **2-79b** reference compound; c. Radioactivity HPLC trace of purified 4-[^{18}F]fluorobenzaldehyde; d. UV HPLC trace of "cold" 4-fluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient F, 1 mL/min, 20 µL injected]

4.2.6 Radiosynthesis of [^{18}F]L771.668 (Section 2.7)

Methyl 4-(3, 4-[4-^{18}F]difluorophenyl)-6-(methoxymethyl)-2-oxo-3-({3-[4-(pyridin-2-yl)piperidin-1-yl]propyl}carbamoyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate ([^{18}F]2-11)



A solution of **2-84** (10 mg) and triethylamine (5 µL) in aceronitrile (200 µL) was added to the crude reaction mixture of **[^{18}F]2-24** (200 µL, 6.8 MBq) and heated to 130 ^{o}C for 30 minutes before being allowed to cool to room temperature. Analysis by HPLC (MeCN/H_2O **Gradient C**, 1 mL/min, 20 µL injected) indicated the formation of **[^{18}F]2-11** at a retention time of 5.4 min, which was consistent with the 'cold' reference compound. Analysis by radio-HPLC by integration of peak areas indicated a radiochemical yield of 18% in two steps and a radiochemical yield of 40% for the coupling step. The overall RCY from [^{18}F]fluoride of **[^{18}F]2-11** is 7% over three steps (75 min overall radiosynthesis time).

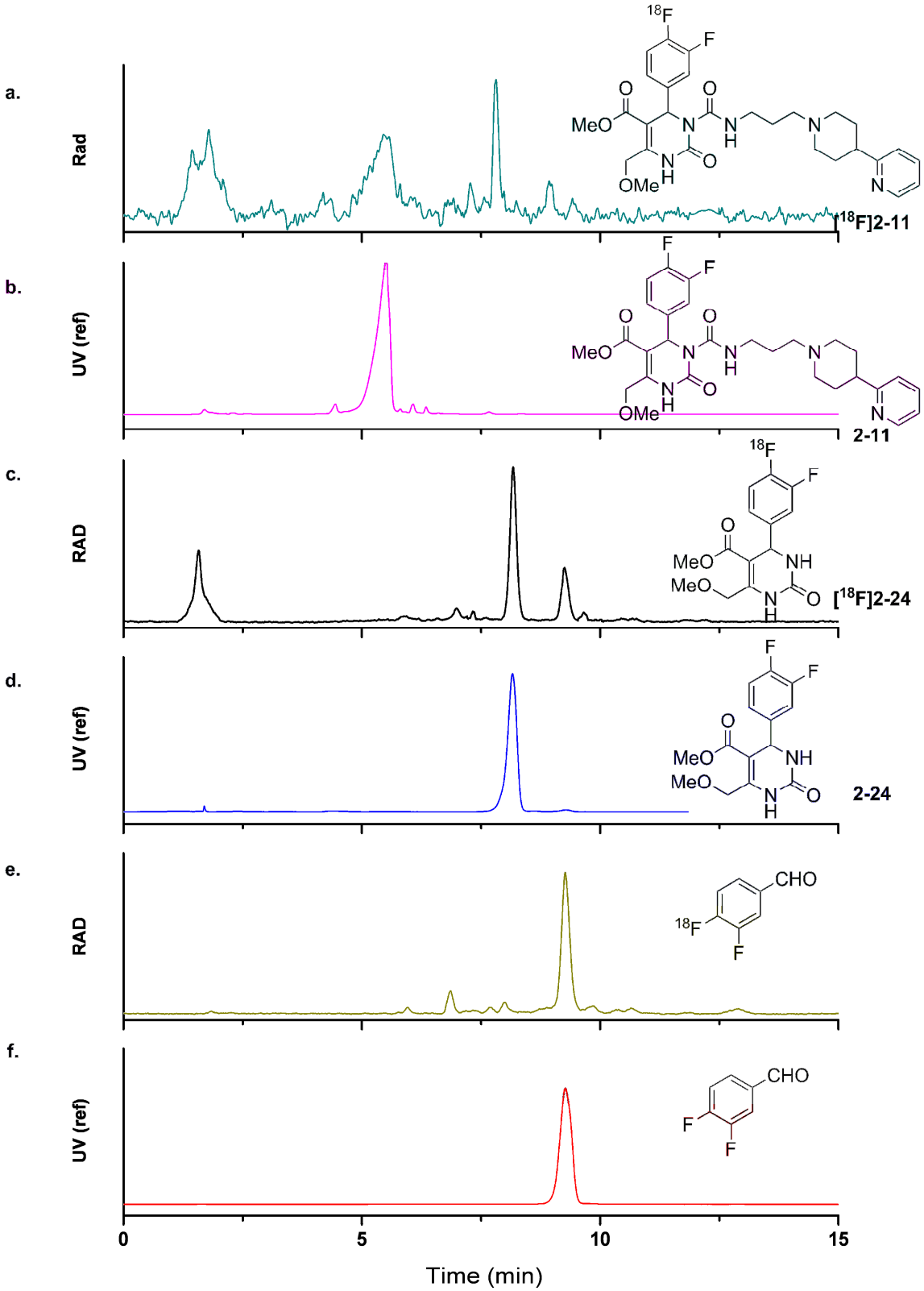


Figure 4.16 a. Radioactivity HPLC trace of crude reaction mixture of the condensation for **[^{18}F]2-11**; b. UV HPLC trace (254 nm) of "cold" **2-11** reference compound; c. Radioactivity HPLC trace of crude reaction mixture of the radio-Beginelli reaction; d. UV HPLC trace of "cold" 2-24 reference compound; e. purified 3,4-[^{18}F]-difluorobenzaldehyde; f. UV HPLC trace of "cold" 3,4-difluorobenzaldehyde. [Phenomenex NX 5 µm C18 (150 ×4.60 mm), MeCN/H_2O Gradient C, 1 mL/min, 20 µL injected]

4.3 Experimental Data for Chapter 3

4.3.1 General Procedure for Oxidative Fluorination

In a plastics sealed tube, to the substrate (1.0 mmol) in a solution of anhydrous CH_2Cl_2 (20 mL) at 0 °C or 25 °C was added HF•pyridine (4.0 mmol) followed by an oxidant (1.0 mmol). Reaction mixture was stirred for 10 min – 2 hr before quenching with K_2CO_3 (solid), filtered and concentrated *in vacuo* at 25 ^oC. When deprotection was needed, typically 100 μL TFA (5%) was added to reaction mixture, kept for 30 min before quenching with K_2CO_3 (solid).

Unless otherwise stated, fluorinated compounds were identified by their commercial reference compounds *via* comparison of their UV signals and ^{19}F NMR signals. The HPLC separations were carried out on a Phenomenex NX 5u C18 column (150 × 4.60 mm) at room temperature, using acetonitrile / water as mobile phase (gradient 5 → 95% acetonitrile over 10 min; flow rate: 1 mL/min). The semi-preparative HPLC purifications were carried out on a Waters SunFire Prep C18 10 μm column (10 × 250 mm) at room temperature, using acetonitrile / water (with 0.1% TFA) as mobile phase (gradient 5 → 50% acetonitrile over 10 min; flow rate: 4.0 mL/min)

4.3.2 General Procedure for Oxidative ^{18}F-fluorination

For radiolabelling with conventional apparatus (e.g. Scintomics) [38]

[^{18}F]Fluoride was produced by the cyclotron of PETNET Solutions at Mount Vernon Hospital (UK) *via* the $^{18}O(p,n)^{18}F$ nuclear reaction and delivered as [^{18}F]fluoride in [^{18}O]H_2O (2 – 4 GBq, 1 – 3 mL). This target solution was passed through an anion exchange resin cartridge. [^{18}F]Fluoride adsorbed on the charged-resin was eluted into a reaction vial with a solution of nBu_4NHCO_3 (8 mg) in 1.0 mL acetonitrile/water (4:1) or Cs_2CO_3 (8 mg) in water (500 µL). Excess water was removed under nitrogen stream at 120 °C, and the resulting complex was azeotropically dried with acetonitrile (0.5 mL × 3) under nitrogen stream. The resulting dry [^{18}F]TBAF or [^{18}F]CsF was further dissolved in the appropriate organic solvent, in a plastics tube and used for further reactions.

High performance liquid chromatography (HPLC) analysis was performed with the Gilson 322 system, equipped with a NaI/PMT radiodetector and a UV-detector using the analytical Phenomenex Gemini-NX C18 column (150 × 4.6 mm, 5 µm).

To dried [^{18}F]TBAF or [^{18}F]CsF in CH_2Cl_2 at 25 °C was added the substrate (1 mmol) and trifluoroacetic acid (1.5% v/v) in CH_2Cl_2 followed by an oxidant (1 mmol) and the reaction mixture was stirred for 10 min. Trifluoroacetic acid (10% v/v) was added to the reaction mixture and stirred for a further 10 min at 25 °C, should re-aromatization be necessary. Acetonitrile was added to dilute the reaction mixture. Reaction mixture was analyzed by HPLC and radio-TLC. Authenticity of the radiolabelled product was confirmed by spiking the reaction mixture with reference product. Radiochemical yield was calculated by area-integration of HPLC spectra.

4-[^{18}F]fluorophenol ([^{18}F]3-11, synthesised from 4-*tert*-butylphenol)

4-*Tert*-butylphenol **3-40** (150 mg) and TFA (3 μL) in CH_2Cl_2 (100 μL) were added To dried TBA[^{18}F]F or Cs[^{18}F]F in CH_2Cl_2 (100 μL) at 25 °C followed by PIDA (320 mg) and the reaction mixture was stirred for 10 min. More TFA (20 μL) was added and the reaction mixture was stirred for a further 10 min at 25 °C. Reaction mixture was diluted by MeCN (300 μL) and analyzed by HPLC. Authenticity of the radiolabelled product was confirmed by spiking the reaction mixture with reference product. Analysis by radio-HPLC (gradient 5 → 95% MeCN/H_2O over 10 min, 1 mL/min, 20 μL injected) indicated **[^{18}F]3-11** in a retention time of 6.27 min with RCY = 4 – 6% (n = 2) (decay-corrected RCY based on HPLC integration).

4-[^{18}F]fluorophenol ([^{18}F]3-11, synthesised from phenol 3-43)

Phenol **3-43** (94 mg) and TFA (3 μL) in CH_2Cl_2 (100 μL) were added to dried TBA[^{18}F]F or Cs[^{18}F]F in 100 μL CH_2Cl_2 at 25 °C followed by PIDA (320 mg) and the reaction mixture was stirred for 10 minutes. Reaction mixture was then diluted by MeCN (300 μL) and analysed by HPLC. Authenticity of the radiolabelled product was confirmed by spiking the reaction mixture with reference product. Analysis by radio-HPLC (gradient 5 → 95% MeCN/H_2O over 10 min, 1 mL/min, 20 μL injected) indicated **[^{18}F]3-11** in a retention time of 6.27 min with RCY = 3% (decay-corrected RCY based on HPLC integration).

[38] The reactions were performed in Siemens Oxford Molecular Imaging Laboratory, University of Oxford

For radiolabelling with microfluidic apparatus (e.g. NanoTek ®, Advion)[39]

The radiosynthesis and azeotropic drying was automatically performed on a commercial microreactor device (NanoTek ®, Advion). Cyclotron-produced *non-carrier-added* aqueous [^{18}F]fluoride was first adsorbed onto an anion-exchange cartridge and subsequently released with 500µl solution of nBu_4NHCO_3 (8 mg) in acetonitrile/water (4:1) into the reactor. The solution was dried with two cycles of azeotropic drying with acetonitrile (300µl) and dissolved in CH_2Cl_2/$ClCH_2CH_2Cl$ (7:3 v/v, 1000µl) containing the substrate (0.1 – 0.5 M). In a separate solution, the oxidant (0.1 – 0.5 M) was dissolved in DCM/DCE (7:3 *v/v*) containing trifluoroacetic acid (3%). Both solutions were delivered at various flow rates (2 – 30µl/min) through the microfluidic reactor at room temperature. The reaction mixture was treated with trifluoroacetic acid (10% *v/v*) for a further 10 min at 25 °C, with tert-butylphenol was used as the precursor, and diluted by acetonitrile (100 µL, 1:1 *v/v*) before HPLC analysis.

Chemical identity was verified with radio-HPLC using the Gilson 322 system, equipped with a NaI/PMT radiodetector and a UV-detector using the analytical Phenomenex Gemini-NX C18 column (150 × 4.6 mm, 5 µm). Conversion of the radiochemistry was calculated by area-integration of HPLC spectra.

4-[^{18}F]fluorophenol ([^{18}F]3-11, synthesised from 4-*tert*-butylphenol 3-40)

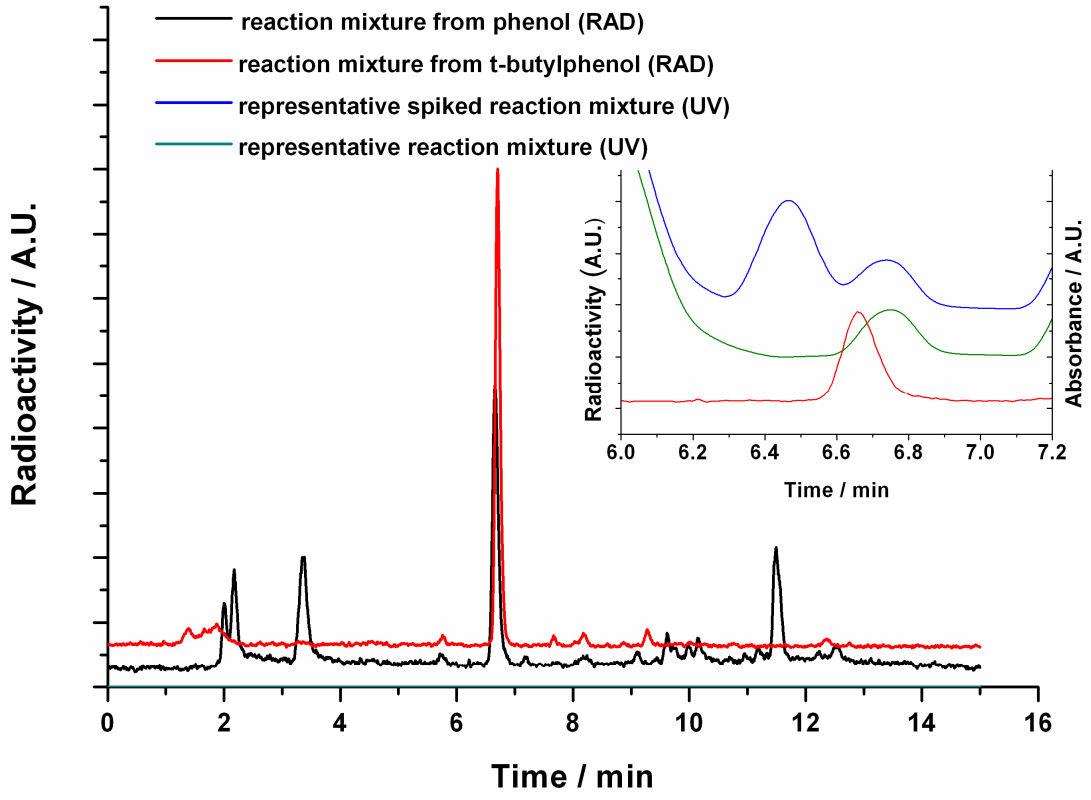
TBA[^{18}F]F was dissolved in CH_2Cl_2/$ClCH_2CH_2Cl$ (7:3 v/v, 1000µl) containing the substrate (0.25 – 0.5 M). In a separate solution, the oxidant (0.25 – 0.5 M) was dissolved in CH_2Cl_2/$ClCH_2CH_2Cl$ (7:3 *v/v*) containing trifluoroacetic acid (3%). Both solutions (30 µL of each) were delivered at a flow rate of 30µl/min through the microfluidic reactor at 25 °C.

[39] The reactions were performed in Clinical Imaging Centre, GlaxoSmithKline in London under the supervision from Dr. M. Huiban.

Trifluoroacetic acid (20 µL, 10% *v/v*) was added to the reaction mixture and kept for a further 10 min at 25 °C for re-aromatization. Reaction mixture was diluted by MeCN (100 µL) and analyzed by HPLC. Authenticity of the radiolabelled product was confirmed by spiking the reaction mixture with reference product. Analysis by radio-HPLC (gradient 5 → 95% MeCN/H_2O over 10 min, 1 mL/min, 20 µL injected) indicated **[^{18}F]3-11** at a retention time of 6.27 min with 34 % conversion.

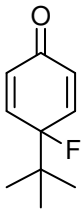
4-[^{18}F]fluorophenol ([^{18}F]3-11, synthesised from phenol 3-43)

Using the same procedure, 4-[^{18}F]fluorophenol (**[^{18}F]3-11**) was synthesised from phenol in 11 % conversion.



4.3.3 Experimental Data for Section 3.2

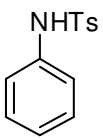
4-(*tert*-Butyl)-4-fluorocyclohexa-2,5-dien-1-one (3-41) [176]



To a solution of *t*-butylphenol (150 mg, 1.0 mmol) and HF•pyridine (70% wt, 104 μL, 4.0 mmol) in DCM (20 mL) was added phenyliodine diacetate (PIDA, 322 mg, 1.0 mmol). The reaction was stirred at ambient temperature for 15 min and quenched by K_2CO_3 (1.0 g). The reaction was filtered and concentrated under reduced pressure. The isolated product was obtained by column chromatography on silica gel (100% DCM, Rf = 0.5) as a colourless oil (78 mg, 0.46 mmol, 46% yield). **^{1}H NMR (400 MHz, CO(CD$_3$)$_2$)**: ppm 7.15 (dd, *J* = 10.4, 7.3 Hz, 2 H, C*H*C=O × 2), 6.27 (d, *J* = 10.1 Hz, 2 H, CFC*H* × 2), 1.07 ppm (d, *J* = 1.0 Hz, 9 H, C(C*H*$_3$)$_3$); **^{13}C NMR (101 MHz, CO(CD$_3$)$_2$)**: ppm 184.7 (d, *J* = 4.8 Hz, *C*=O), 145.8 (d, *J* = 22.4 Hz, CF*C*H), 130.9 (d, *J* = 8.0 Hz, *C*HC=O), 93.9 (d, *J* = 167.8 Hz, *C*F), 39.4 (d, *J* = 22.4 Hz, *C*(CH$_3$)$_3$), 25.4 (d, *J* = 4.8 Hz, C(*C*H$_3$)$_3$); **^{19}F NMR (376 MHz, CO(CD$_3$)$_2$)**: ppm -161.5 (t, *J* = 6.9 Hz); Other characterization data are in agreement with the literature.[176]

4.3.4 Experimental Data for Section 3.3

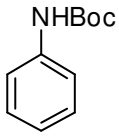
4-Methyl-*N*-phenylbenzenesulfonamide (3-49b) [195]



To a solution of aniline (4.65 mL, 50 mmol) and pyridine (8 mL, 100 mmol) in DCM (250 mL) was added *p*-toluenesulfonyl chloride (11.4 g, 60 mmol) at 0 ^{o}C. The reaction was warmed up to 25 ^{0}C, stirred for one hour. The reaction was quenched by satd. $NaHCO_3$ and extracted with DCM (150 mL × 2). The combined organic phase was washed with satd.

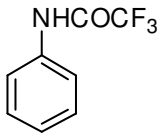
$NaHCO_3$, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Purified product was obtained by column chromatography on silica gel (25% EtOAc in Hexane, Rf = 0.4) as a white solid (12.12 g, 49 mmol, 98% yield). **^{1}H NMR (400MHz, $CDCl_3$)**: ppm 7.67 (d, *J* = 8.3 Hz, 2 H, Ar – *H*), 7.20 - 7.28 (m, 4 H, Ar – *H*), 7.05 - 7.15 (m, 3 H, Ar – *H*), 6.80 (br. s., 1 H, N*H*), 2.38 ppm (s, 3 H, C*H*$_3$); **^{13}C NMR (75MHz, $CDCl_3$)**: ppm 143.8 (Ar – *C*), 136.6 (Ar – *C*), 135.9 (Ar – *C*), 129.6 (Ar – *C*), 129.2 (Ar – *C*), 127.2 (Ar – *C*), 125.0 (Ar – *C*), 121.2 (Ar – *C*), 21.4 (*C*H$_3$); **MS** (ESI, *m/z*) $C_{13}H_{13}NNaO_2S$ ($M+Na^+$) *calc.* 270.06, *found* 270.08; Other characterization data are in agreement with the literature.[195]

***tert*-Butyl phenylcarbamate (3-49c)** [196]



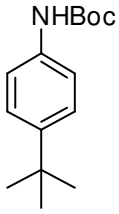
To di-*tert*-butyl dicarbonate (4.26 g, 20 mmol) that was being stirred in a round bottom flask was added aniline (1.86 mL, 20 mmol) slowly. The reaction was stirred at ambient temperature for 30 min. The neat reaction mixture was recrystallized in diethyl ether / hexane to afford pure product as white needles (1.8 g, 9.3 mmol, 47% yield). **^{1}H NMR (300 MHz, $CDCl_3$)**: ppm 7.23 - 7.34 (m, 4 H, Ar – *H*), 7.00 (tt, *J* = 7.16 Hz, 1.32 Hz, 1 H, Ar – *H*), 6.46 (br. s, 1 H, N*H*), 1.49 (s, 9 H, $CH_3 \times 3$); **^{13}C NMR (75 MHz, $CDCl_3$)** ppm 152.8 (*C*=O), 138.3 (Ar – *C*), 129.0 (Ar – *C*), 123.0 (Ar – *C*), 118.5 (Ar – *C*), 80.5 (*C*$(CH_3)_3$), 28.4 (C(*C*$H_3)_3$); **MS** (ESI, *m/z*) $C_{11}H_{15}NNaO_2$ ($M+Na^+$) *calc.* 216.10, *found* 216.10**;** The characterization data are in agreement with the literature.[196]

2,2,2-Trifluoro-*N*-phenylacetamide (3-49d) [197]



To a solution of aniline (1.86 mL, 20.0 mmol) and pyridine (3.2 mL, 40.0 mmol) in DCM (50 mL) was added trifluoroacetic anhydride (2.8 mL, 20.0 mmol) at 0 ^{o}C. The reaction was stirred for three hours at 0 ^{o}C, quenched by satd. $NaHCO_3$ and extracted with DCM (50 mL × 2). The combined organic phase was washed with satd. $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Purified product was obtained by column chromatography on silica gel (25% EtOAc in Hexane, Rf = 0.7) as a pale yellow solid (2.8 g, 14.8 mmol, 74% yield). **^{1}H NMR (300MHz, $CDCCl_3$)**: ppm 8.21 (br. s., 1 H, N*H*), 7.57 (d, *J* = 7.9 Hz, 2 H, Ar – *H*), 7.38 (t, *J* = 7.8 Hz, 2 H, Ar – *H*), 7.24 (t, *J* = 7.2 Hz, 1 H, Ar – *H*); **^{13}C NMR (75 MHz, $CDCl_3$)**: ppm 155.0 (q, *J* = 37.9 Hz, *C*=O), 135.0 (Ar – *C*), 129.3 (Ar – *C*), 126.4 (Ar – *C*), 120.6 (Ar – *C*), 115.7 (q, *J* = 288.7 Hz, CO*C*F_3); **^{19}F NMR (376MHz, $CDCl_3$)**: ppm -75.74; **MS** (ESI, *m/z*) $C_8H_6F_3NNaO^+$ ($M+Na^+$) *calc.* 212.03, *found* 212.02**;** Other characterization data are in agreement with the literature.[197]

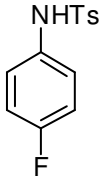
***tert*-Butyl (4-(*tert*-butyl)phenyl)carbamate (3-50c)** [198]



To di-*tert*-butyl dicarbonate (4.26 g, 20.0 mmol) that was being stirred in a round bottom flask was added *ter*t-butylaniline (3.19 mL, 20.0 mmol) slowly. The reaction was stirred at ambient temperature for 30 min. The neat reaction mixture was recrystallised in hexane to afford the pure product as white needles (3.6 g, 14.4 mmol, 72% yield). **^{1}H NMR (250 MHz, $CDCl_3$)**: ppm 7.36 (br. s, 4 H, Ar – *H*), 6.59 (br. s, 1 H, N*H*), 1.59 (s, 9 H, C*H*$_3$ × 3), 1.36 (s, 9 H, C*H*$_3$ × 3); **^{13}C NMR (63MHz, $CDCl_3$)**: ppm 152.9 (*C*=O), 145.9 (Ar – *C*),

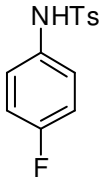
135.6 (Ar – <u>C</u>), 125.7 (Ar – <u>C</u>), 118.4 (Ar – <u>C</u>), 80.2 (<u>C</u>(CH_3)$_3$ in Boc), 34.2 (<u>C</u>(CH_3)$_3$ in *t*Bu), 31.3 (C(<u>C</u>H_3)$_3$ in *t*Bu), 28.3 (C(<u>C</u>H_3)$_3$ in Boc); **MS** (ESI, *m/z*) $C_{15}H_{23}NNaO_2^+$ ($M+Na^+$) *calc.* 272.16, *found* 272.17**;** The characterisation data are in agreement with the literature.[198]

***N*-(4-fluorophenyl)-4-methylbenzenesulfonamide (3-51b)** [199]



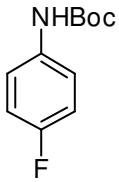
To a solution of 4-fluoroaniline (0.48 mL, 5.0 mmol) and pyridine (0.8 mL, 10.0 mmol) in DCM (25 mL) was added *p*-toluenesulfonyl chloride (953 mg, 5.0 mmol) at 0 ^oC. The reaction was warmed up to 25 ^0C, stirred for one hour. The reaction was quenched by satd. $NaHCO_3$ and extracted with DCM (25 mL × 2). The combined organic phase was washed with satd. $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Purified product was obtained by column chromatography on silica gel (33% EtOAc in Hexane, Rf = 0.4) as a pale yellow solid (1.33 g, 5.0 mmol, quantative yield). **^{1}H NMR (400MHz, $CDCl_3$)**: ppm 7.62 - 7.70 (m, 3 H, N<u>H</u> and Ar – <u>H</u>), 7.17 - 7.24 (m, 2 H, Ar – <u>H</u>), 7.05 - 7.12 (m, 2 H, Ar – <u>H</u>), 6.85 - 6.93 (m, 2 H, Ar – <u>H</u>), 2.36 (s, 3H, C<u>H</u>$_3$); **^{13}C NMR (75MHz, $CDCl_3$)**: ppm 160.4 (d, *J* = 244.8 Hz, <u>C</u> – F), 143.0 (Ar – <u>C</u>), 135.5 (Ar – <u>C</u>), 132.4 (d, *J* = 2.7 Hz, Ar – <u>C</u>), 129.6 (Ar – <u>C</u>), 127.2 (Ar – <u>C</u>), 124.2 (d, *J* = 8.2 Hz, Ar – <u>C</u>), 115.9 (d, <u>J</u> = 22.5 Hz, Ar – <u>C</u>), 21.5 (<u>C</u>H_3); **^{19}F NMR (376 MHz, $CDCl_3$)**: ppm -116.6 (dddd, *J* = 12.6, 9.2, 4.6, 3.4 Hz); **MS** (ESI, *m/z*) $C_{13}H_{12}FNNaO_2S$ ($M+Na^+$) *calc.* 288.05, *found* 288.06**;** The characterisation data are in agreement with the literature.[199]

***N*-(4-fluorophenyl)-4-methylbenzenesulfonamide** (**3-51b**, Synthesised *via* oxidative fluorination)



To a solution of 4-Methyl-*N*-phenylbenzenesulfonamide (**3-49b**, 247.3 mg, 1.0 mmol) and HF•pyridine (70% wt, 104 μL, 4.0 mmol) in DCM (20 mL) was added phenyliodine diacetace (PIDA, 322 mg, 1.0 mmol). The reaction was stirred at 0 ^{o}C for 15 min and quenched by K_2CO_3 (1.0 g). The reaction was filtered and concentrated under reduced pressure. The isolated product was obtained by column chromatography on silica gel (100% DCM, Rf = 0.5) as pale yellow oil (27 mg, 0.1 mmol, 10% yield). Characterisation data are in agreement with the reference compound synthesised independently.

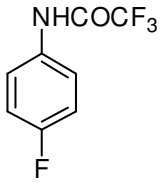
***tert*-Butyl (4-fluorophenyl)carbamate (3-51c)** [200]



To di-*tert*-butyl dicarbonate (4.26 g, 20 mmol) that was being stirred in a round bottom flask was added 4-fluoroaniline (1.92 mL, 20 mmol) slowly. The reaction was stirred at ambient temperature for 30 min. The neat reaction mixture was recrystallised in diethyl ether / hexane to afford pure product as white crystal (2.5 g, 12 mmol, 60% yield). **^{1}H NMR (200 MHz, $CDCl_3$)**: ppm 7.32 (dd, *J* = 9.0, 4.8 Hz, 2 H, Ar – *H*), 6.99 (dd, *J* = 8.8, 8.5 Hz, 2 H, Ar – *H*), 6.43 (br. s, 1 H, N*H*), 1.52 (s, 9 H, CH_3 × 3); **^{13}C NMR (75 MHz, $CDCl_3$)**: 158.7 (d, *J* = 242.1 Hz, C – *F*), 152.9 (*C*=O), 134.3 (d, *J* = 2.7 Hz, Ar – *C*), 120.3 (d, *J* = 7.7 Hz, Ar – *C*), 115.5 (d, *J* = 22.5 Hz, Ar – *C*), 80.6 (*C*(CH_3)$_3$), 28.3 (C(*C*H_3)$_3$); **MS** (ESI, *m/z*)

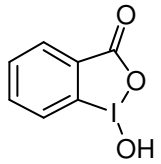
$C_{11}H_{14}FNNaO_2^+$ ($M+Na^+$) *calc.* 234.09, *found* 234.11**;** The characterization data are in agreement with the literature.[200]

2,2,2-Trifluoro-*N*-(4-fluorophenyl)acetamide (3-51d) [201]



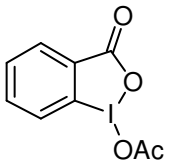
To a solution of 4-fluoroaniline (1.92 mL, 20.0 mmol) and pyridine (3.2 mL, 40.0 mmol) in DCM (50 mL) was added trofluoroacetic anhydride (2.8 mL, 20.0 mmol) at 0 ^oC. The reaction was stirred for three hours at 0 ^oC, quenched by satd. $NaHCO_3$ and extracted with DCM (50 mL × 2). The combined organic phase was washed with satd. $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Purified product was obtained by column chromatography on silica gel (25% EtOAc in Hexane, Rf = 0.8) as a pale yellow solid (3.7 g, 17.8 mmol, 89% yield). **^{1}H NMR (300MHz, $CDCl_3$)**: ppm 8.05 (br. s., 1 H, N*H*), 7.51 - 7.59 (m, 2 H, Ar – *H*), 7.05 - 7.14 (m, 2 H, Ar – *H*); **^{13}C NMR (75MHz, $CDCl_3$)**: ppm 160.5 (d, *J* = 246.2 Hz, C – *F*), 155.0 (q, *J* = 37.3 Hz, *C*=O), 131.0 (d, *J* = 2.7 Hz), 122.6 (d, *J* = 8.2 Hz), 116.2 (d, *J* = 23.1 Hz), 115.7 (q, *J* = 288.2 Hz, *C*F_3); **^{19}F NMR (376MHz, $CDCl_3$)**: ppm -75.6 (s, 3 F, C*F*$_3$), -114.6 (tt, *J* = 8.6, 4.3 Hz, 1 F, Ar – *F*); **MS** (ESI, *m/z*) $C_8H_4F_4NO$ ($M–H^–$) *calc.* 206.02, *found* 206.03**;** The characterisation data are in agreement with the literature.[201]

1-Hydroxy-1,2-benziodoxol-3-(1*H*)-one[202]



The suspension of 2-iodobenzoic acid (2.0 g, 8.06 mmol) and sodium periodate (1.78 g, 8.3 mmol) in acetic acid (3.6 mL) and water (3.6 mL) was refluxed for 4 hours under vigorous stirring. The reaction was cooled to ambient temperature and diluted with cold water (30 mL). The precipitate was filtered, washed by cold water (5 mL × 2), acetone (3 mL) and dried *in vacuum* in dark to afford pure product as white solid (1.95 g, 7.3 mmol, 91% yield). **^{1}H NMR (300MHz, SO(CD$_3$)$_2$)**: ppm 8.05 (br. s, 1 H, O*H*), 8.01 (dd, *J* = 7.5, 1.4 Hz, 1 H, Ar – *H*), 7.96 (td, *J* = 7.3, 1.6 Hz, 1 H, Ar – *H*), 7.84 (br. d, *J* = 7.6 Hz, 1 H, Ar – *H*), 7.70 ppm (td, *J* = 7.3, 1.0 Hz, 1 H, Ar – *H*); **^{13}C NMR (75MHz, SO(CD$_3$)$_2$)**: ppm 167.7, 134.5, 131.5, 131.1, 130.4, 126.3, 120.4; **MS** (ESI, *m/z*) $C_7H_5INaO_3$ ($M+Na^+$) *calc.* 286.92, *found* 286.94**;** The characterisation data are in agreement with the literature. [203]

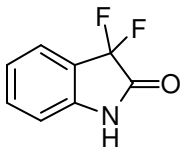
1-Acetoxy-1,2-benziodoxol-3-(1*H*)-one (3-44) [204]



1-Hydroxy-1,2-benziodoxol-3-(1*H*)-one (1.63 g, 6.2 mmol) was refluxed in acetic anhydride (5 mL) until a clear solution was obtained. Upon cooling, white crystals precipitated and the cooling was continued at -20 ^{o}C for 4 hours. The resulting white powder was collected by filtration and dried under reduced pressure to afford pure product (1.54 g, 5.1 mmol, 82% yield). **^{1}H NMR (300MHz, CDCl$_3$)**: ppm 8.23 (dd, *J* = 7.6, 1.5 Hz, 1 H, Ar – *H*), 7.96 - 8.02 (m, 1 H, Ar – *H*), 7.88 - 7.96 (m, 1 H, Ar – *H*), 7.70 (td, *J* = 7.3, 1.0 Hz, 1 H, Ar – *H*), 2.25 (s, 3 H, C*H*$_3$); **^{13}C NMR (75MHz, CDCl$_3$)**: ppm 176.3 (*C*=O), 168.1 (*C*=O), 136.1 (Ar – *C*), 133.1 (Ar – *C*), 131.2 (Ar – *C*), 129.2 (Ar – *C*), 128.9 (Ar – *C*), 118.3 (Ar – *C*), 20.2 (*C*H$_3$);

MS (ESI, *m/z*) $C_7H_5INaO_3$ (M+OH–OAc+Na^+) *calc.* 286.92, *found* 286.93**;** Other characterisation data are in agreement with the literature.[205]

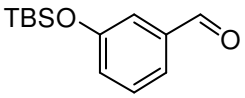
3,3-Difluoroindolin-2-one (3-54)[206]



To the suspension of isatin (735 mg, 5.0 mmol) in DCM (50 mL) was added diethylaminosulfur trifluoride (DAST, 1.3 mL, 10.0 mmol) slowly. The reaction was stirred for overnight at ambient temperature, quenched by satd. NH_4Cl at 0 ^oC and extracted with DCM (30 mL × 2). The combined organic phase was washed with satd. NH_4Cl, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Crude product was washed by diethyl ether (5 mL) and hexane (20 mL) and dried *in vacuum* to afford pure product as a yellow solid (584 mg, 3.7 mmol, 75% yield). **^{1}H NMR (300MHz, $CDCl_3$)**: ppm 9.06 (br. s., 1 H, N*H*), 7.54 (d, *J* = 7.5 Hz, 1 H, Ar – *H*), 7.46 (td, *J* = 7.8 × 2, 1.2 Hz, 1 H, Ar – *H*), 7.17 (t, *J* = 7.6 Hz, 1 H, Ar – *H*), 7.02 (d, *J* = 7.9 Hz, 1 H, Ar – *H*); **^{13}C NMR (75MHz, $CDCl_3$)**: ppm 167.5 (t, *J* = 30.2 Hz, *C*=O), 141.1 (t, *J* = 7.4 Hz, Ar – *C*), 133.7 (Ar – *C*), 125.0 (Ar – *C*), 124.0 (Ar – *C*), 120.2 (t, *J* = 23.1 Hz, Ar – *C*), 111.9 (Ar – *C*), 111.0 (t, *J* = 249.7 Hz, *C*F_2); **^{19}F NMR (376MHz, $CDCl_3$)**: ppm -111.95; **MS** (ESI, *m/z*) $C_8H_5F_2NNaO$ (M+Na^+) *calc.* 192.02, *found* 192.03**;** The characterisation data are in agreement with the literature.[206]

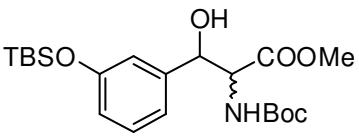
4.3.5 Experimental Data for Section 3.5

3-((*tert*-Butyldimethylsilyl)oxy)benzaldehyde (**3-69**) [207]



To a solution of 3-hydroxylbenzaldehyde (5.2 g, 42 mmol), imidazole (3.5 g, 51 mmol) and *N*,*N*-dimethylaminopyridine (51 mg, 0.4 mmol) in DCM (100 mL) was added *tert*-butyldimethylsilyl chloride (6.7 g, 44 mmol) and the reaction mixture was stirred at ambient temperature for overnight. The reaction was quenched by satd. $NaHCO_3$ and extracted by DCM (100 mL × 2). The combined organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as light yellow oil. Purified product was obtained by column chromatography on silica gel (10% diethyl ether in hexane, Rf = 0.5) to give the pure product as a colourless oil (9.9 g, 42 mmol, 100% yield). **^{1}H NMR (200MHz, $CDCl_3$)** ppm 9.95 (s, 1 H, C*H*O), 7.44 - 7.50 (m, 1 H, Ar – *H*), 7.39 (t, *J* = 7.5 Hz, 1 H, Ar – *H*), 7.31 - 7.34 (m, 1 H, Ar – *H*), 7.01 - 7.18 (m, 1 H, Ar – *H*), 0.99 (s, 9 H, *C*H_3 × 3 in TBS), 0.22 (s, 6 H, C*H*$_3$ × 2 in TBS); **^{13}C NMR (50MHz, $CDCl_3$)** ppm 192.1 (*C*HO), 156.4 (Ar – *C*); 137.9 (Ar – *C*), 130.1 (Ar – *C*), 126.5 (Ar – *C*), 123.5 (Ar – *C*), 119.8 (Ar – *C*), 25.6 (C(*C*H_3)$_3$ in TBS), 18.2 (*C*H_3 × 3 in TBS), -4.5 (*C*H_3 × 2 in TBS); **MS** (ESI, *m/z*) $C_{13}H_{20}NaO_2Si$ ($M+Na^+$) *calc.* 259.11, *found* 259.11**;** Other characterization data are in agreement with the literature. [207]

(±)Methyl-2-((tert-butoxycarbonyl)amino)-3-(3-((tert-butyldimethylsilyl)oxy) phenyl)-3-hydroxypropanoate (**3-70a**)

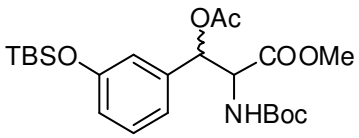


Diisopropylamine (9 mL, 60 mmol) was dissolved in a solution of anhydrous THF (100 mL) and treated with n-BuLi (55 mmol, 22.5 mL, 2.5 M in hexane) at -78 ^{o}C. The reaction

mixture was stirred for 30 min and subjected with a solution of methyl 2-((tert-butoxycarbonyl)amino)acetate (3.7 g, 25 mmol) in anhydrous THF (10 mL). The reaction was kept for 1 hour at -78 ^{o}C before the addition of **3-69** (5.5 g, 25 mmol) and warmed up to ambient temperature. The reaction was stirred at ambient temperature for 5 hours, carefully quenched by satd. NH_4Cl (20 mL) and extracted by ethyl acetate (150 mL × 3). The combined organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as yellow oil. ^{1}HNMR indicated the alkylated product **3-70** was formed as two diastereomers **3-70a/b** at the ratio of 5:2. Separation of the diastereomers was achieved by careful column chromatography on silica gel (20% ethyl acetate in hexane, $Rf_{3\text{-}70a}$ = 0.4, $Rf_{3\text{-}70b}$ = 0.3) to give the pure product as colourless oil (5.75g, 15.5 mmol, 62% overall yield). The characterisation data of **3-70a** are: **^{1}H NMR (500 MHz, $CDCl_3$)**: ppm 7.15 (t, *J* = 7.9 Hz, 1 H, Ar – *H*), 6.76 - 6.83 (m, 2 H, Ar – *H*), 6.74 (dd, *J*=8.0, 1.7 Hz, 1 H, Ar – *H*), 5.30 (d, *J*=7.6 Hz, 1 H, N*H*), 5.10 (br. s., 1 H, OHC*H*), 4.67 (dd, *J* = 7.4, 3.6 Hz, 1 H, NHC*H*), 4.15 (br. s, 1 H, O*H*), 3.65 (s, 3 H, COOCH_3), 1.41 (s, 9 H, CH_3 × 3 in Boc), 0.97 (s, 9 H, (CH_3 × 3 in TBS), 0.18 ppm (s, 6 H, CH_3 × 2 in TBS); **^{13}C NMR (125MHz, $CDCl_3$)** ppm 170.4 (*C*$OOCH_3$), 156.4 (Ar – *C* or *C*=O in Boc), 155.7 (Ar – *C* or *C* = O in Boc), 140.9 (Ar – *C*), 129.3 (Ar – *C*), 119.7 (Ar – *C*), 119.0 (Ar – *C*), 118.0 (Ar – *C*), 80.6 (*C*$(CH_3)_3$ in Boc), 74.6 (OH*C*H), 59.7 (NH*C*H), 52.4 (COO*C*H_3), 28.3 (*C*H_3 × 3 in Boc), 25.7 (*C*H_3 × 3 in TBS), 18.2 (*C*$(CH_3)_3$ in TBS), -4.3 (*C*H_3 × 2 in TBS); **IR** 3437, 2955, 1716, 1251, 839, 781, 696; **HRMS** (ESI, *m/z*) $C_{21}H_{35}NNaO_6Si$ ($M+Na^+$) *calc.* 448.2126, *found* 448.2120. The characterisation data of **3-70b** are: **^{1}H NMR (500MHz, $CDCl_3$)** ppm 7.19 (t, *J*=7.9 Hz, 1 H, Ar – *H*), 6.93 (br. d, *J*=7.8 Hz, 1 H, Ar – *H*), 6.86 (s, 1 H, Ar – *H*), 6.75 (dd, *J*=7.9, 1.9 Hz, 1 H, Ar – *H*), 5.31 - 5.38 (m, 1 H, N*H*), 5.16 (br. s., 1 H, C*H*OH), 4.46 - 4.53 (m, 1 H, NHC*H*), 3.74 (s, 3 H, COOCH_3), 3.07 (br. s, 1 H, O*H*), 1.35 (s, 9 H, CH_3 × 3 in Boc), 0.98 (s, 9 H, CH_3 × 3 in TBS), 0.19 (s, 6 H, CH_3 × 2 in TBS); **^{13}C NMR (125MHz, $CDCl_3$)** ppm 171.4 (*C*$OOCH_3$), 155.7 (Ar – *C* and *C*=O in Boc), 141.4 (Ar

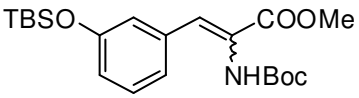
– C), 129.3 (Ar – C), 119.6 (Ar – C), 118.9 (Ar – C), 117.8 (Ar – C), 80.0 (C(CH$_3$)$_3$ in Boc), 73.5 (OHCH), 59.4 (NHCH), 52.5 (COOCH$_3$), 28.1 (C(CH$_3$)$_3$ in Boc), 25.6 (C(CH$_3$)$_3$ in TBS), 18.1 (C(CH$_3$)$_3$ in TBS), -4.4 (CH$_3$ × 2 in TBS); **IR** 3442, 2955, 1723, 1254, 840, 781, 695; **HRMS** (ESI, *m/z*) $C_{21}H_{35}NNaO_6Si$ ($M+Na^+$) *calc.* 448.2126, *found* 448.2120

Methyl-3-acetoxy-2-((tert-butoxycarbonyl)amino)-3-(3-((tert-butyldimethylsilyl)oxy) phenyl)propanoate (3-71)



3-70 (5.4 g, 13.3 mmol) was dissolved in DCM (100 mL) with triethylamine (8.5 mL, 53 mmol) and dimethylaminopyridine (81mg, 5mol %) and chilled to 0 ^oC. Acetyl chloride (1.9 mL, 26.6 mmol) was added slowly and the reaction mixture was kept stirring for 2 hours. The reaction was quenched by satd. $NaHCO_3$ and extracted with DCM (100 mL × 2). The combined organic phase was washed with satd. $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was subjected to next step without further purification.

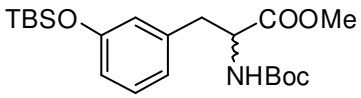
Methyl-2-((tert-butoxycarbonyl)amino)-3-(3-((tert-butyldimethylsilyl)oxy) phenyl)acrylate (3-72)



Crude **3-71** (13.3 mmol) was dissolved in THF (50 mL) in the presence of 1,8-diazabicycloundec-7-ene (DBU, 2 mL, 13.3 mmol) and the solution was heated at 60 ^oC for 1 hour. The reaction mixture was cooled down, diluted by ethyl acetate (100 mL) and washed by satd. NH_4Cl. The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as light yellow oil. Purified product was

obtained by careful column chromatography on silica gel (20% ethyl acetate in hexane, Rf = 0.2) to give pure product as colourless oil (2.7 g, 6.9 mmol, 52% yield over two steps). **^{1}H NMR (500MHz, $CDCl_3$)** ppm 7.23 (t, *J* = 7.9 Hz, 1 H, Ar – *H*), 7.16 (s, 1 H, Ar – *H*), 7.12 (br. d, *J* = 7.6 Hz, 1 H, Ar – *H*), 7.05 (t, *J* = 1.9 Hz, 1 H, Ar – *H*), 6.81 (ddd, *J* = 7.9, 2.2, 0.9 Hz, 1 H, C*H*=C), 3.85 (s, 3 H, COOC*H*$_3$), 1.42 (s., 9 H, , C*H*$_3$ × 3 in Boc), 0.99 (s, 9 H, C*H*$_3$ × 3 in TBS), 0.21 (s, 6 H, C*H*$_3$ × 2 in TBS); **^{13}C NMR (126MHz, $CDCl_3$)**: ppm 166.2 (*C*OOCH$_3$), 155.9 (Ar – *C* or *C*OO^{t}Bu), 153.1 (Ar – *C* or *C*OO^{t}Bu), 135.4 (Ar – *C*), 130.1 (Ar – *C*), 129.7 (Ar – *C*), 125.2 (CH=*C*), 123.1 (Ar – *C*), 121.2 (*C*H=C or Ar – *C*), 121.2 (*C*H=C or Ar – *C*), 81.2 (*C*(CH$_3$)$_3$ in Boc), 52.7 (COO*C*H$_3$), 28.3 (C(*C*H$_3$)$_3$ in Boc), 25.8 (C(*C*H$_3$)$_3$ in TBS), 18.4 (*C*(CH$_3$)$_3$ in TBS), -4.2 (*C*H$_3$ × 2 in TBS); **IR** 2954, 1709, 1643, 1485, 840, 782, 690**; HRMS** (ESI, *m/z*) $C_{21}H_{33}NNaO_5Si$ ($M+Na^+$) *calc.* 430.2020, *found* 430.2011**.**

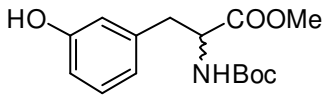
(±)Methyl-2-((*tert*-butoxycarbonyl)amino)-3-(3-((*tert*-butyldimethylsilyl)oxy)phenyl) propanoate (3-73)



3-72 (1.82 g, 4.7 mmol) was dissolved in a solution of methanol (60 mL) with 182 mg of 10% palladium on carbon (10 wt%). The solution was purged with argon for 30 min then with hydrogen for 30 min before stirring under hydrogen atmosphere for overnight at room temperature. The reaction was filtered through Celite and concentrated under reduced pressure to afford the crude product as colourless oil. The purified product was obtained by column chromatography on silica gel (20% ethyl acetate in hexane, Rf = 0.6) to give the pure product as colourless oil (1.78 g, 4.5 mmol, 97% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.13 (t, *J* = 7.8 Hz, 1 H, Ar – *H*), 6.67 - 6.74 (m, 2 H, Ar – *H*), 6.61 (s, 1 H, Ar – *H*), 5.00 (d, *J* = 7.8 Hz, 1 H, N*H*), 4.56 (dd, *J* = 13.1, 5.8 Hz, 1 H, CH_2C*H*), 3.70 (s, 3 H,

COOC$\underline{H}_3$), 3.05 (dd, *J* =13.9, 5.8 Hz, 1 H, C$\underline{H}_2$CH), 3.00 (dd, *J* =13.9, 6.3 Hz, 1 H, C$\underline{H}_2$CH), 1.42 (s, 9 H, C$\underline{H}_3$ × 3 in Boc), 0.98 (s, 9 H, C$\underline{H}_3$ × 3 in TBS), 0.18 (s, 6 H, C$\underline{H}_3$ × 2 in TBS); **^{13}C NMR (126MHz, CDCl$_3$)**: ppm 172.2 ($\underline{C}$OOCH$_3$), 155.7 (Ar – $\underline{C}$ or $\underline{C}$OO^{t}Bu), 155.0 (Ar – $\underline{C}$ or $\underline{C}$OO^{t}Bu), 137.4 (Ar – $\underline{C}$), 129.4 (Ar – $\underline{C}$), 122.2 (Ar – $\underline{C}$), 121.2 (Ar – $\underline{C}$), 118.7 (Ar – $\underline{C}$), 79.8 ($\underline{C}$(CH$_3$)$_3$ in Boc), 54.3 (CH$_2\underline{C}$H), 52.1 (COO$\underline{C}$H$_3$), 38.0 ($\underline{C}$H$_2$CH), 28.3 (C($\underline{C}$H$_3$)$_3$ in Boc), 25.6 (C($\underline{C}$H$_3$)$_3$ in TBS), 18.1 ($\underline{C}$(CH$_3$)$_3$ in TBS), -4.4 ($\underline{C}$H$_3$ × 2 in TBS); **IR** 1716, 1603, 1162, 838, 781, 697; **HRMS** (ESI, *m/z*) $C_{21}H_{35}NNaO_5Si$ ($M+Na^+$) *calc.* 432.2177, *found* 432.2171**.**

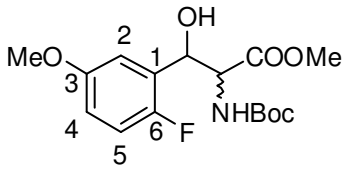
Methyl 2-((tert-butoxycarbonyl)amino)-3-(3-hydroxyphenyl)propanoate (3-74)



Commercial HF•pyridine (70%, 0.8 mL) was dissolved in anhydrous THF (20 mL) with anhydrous pyridine (3.18 mL), to give a stock solution of pyridinium fluoride in the concentration of 1.2 M. To the solution of **3-73** (100 mg, 0.25 mmol) in THF (1 mL) was added the stock solution (2.5 mL, 3 mmol) and stirred for overnight at ambient temperature. The reaction was carefully quenched by the addition of methoxytrimethylsilane (0.4 mL, 3 mmol) and stirring for 10 min at ambient temperature. The solution was concentrated under reduced pressure to afford the crude product as a white solid. Purified product was obtained by column chromatography on silica gel (30% ethyl acetate in hexane, Rf = 0.3) to give the pure product as a white solid (68 mg, 0.23 mmol, 91% yield). **^{1}H NMR (500MHz, CDCl$_3$)** ppm 7.15 (t, *J* = 7.9 Hz, 1 H, Ar – $\underline{H}$), 6.73 (dd, *J* = 8.0, 1.7 Hz, 1 H, Ar – $\underline{H}$), 6.68 (d, *J* = 7.6 Hz, 1 H, Ar – $\underline{H}$), 6.63 - 6.65 (m, 1 H, Ar – $\underline{H}$), 5.94 (br. s., 1 H, O$\underline{H}$), 5.05 (d, *J* = 7.9 Hz, 1 H, N$\underline{H}$), 4.58 (dd, *J* =13.9, 6.3 Hz, 1 H, CH$_2$C$\underline{H}$NH), 3.72 (s, 3 H, COOC$\underline{H}_3$), 3.06 (dd, *J*=13.9, 5.7 Hz, 1 H, C$\underline{H}_2$CH), 2.99 (dd, *J*=13.9, 6.6 Hz, 1 H, C$\underline{H}_2$CH), 1.43 (s, 9 H, C$\underline{H}_3$ × 3); **^{13}C NMR (126MHz, CDCl$_3$)** ppm 172.7 ($\underline{C}$OOCH$_3$), 156.2 ($\underline{C}$OO^{t}Bu or Ar – $\underline{C}$), 155.5

(<u>C</u>OO^{t}Bu or Ar – <u>C</u>), 137.8 (Ar – <u>C</u>), 130.0 (Ar – <u>C</u>), 121.6 (Ar – <u>C</u>), 116.3 (Ar – <u>C</u>), 114.3 (Ar – <u>C</u>), 80.4 (<u>C</u>(CH$_3$)$_3$), 54.5 (CH$_2$<u>C</u>HNH), 52.5 (COO<u>C</u>H$_3$), 38.4 (<u>C</u>H$_2$CHNH), 28.5 (C(<u>C</u>H$_3$)$_3$); **HRMS** (ESI, *m/z*) $C_{15}H_{21}NNaO_5$ (M+Na$^+$) *calc.* 318.1312, *found* 318.1297**;** Other characterisation data are as reported.[208]

(±)Methyl-2-((tert-butoxycarbonyl)amino)-3-(2-fluoro-5-methoxyphenyl)-3-hydroxypropanoate (3-76)

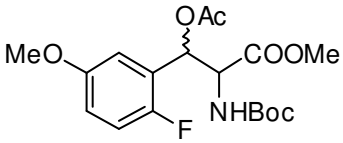


Diisopropylamine (3.6 mL, 24 mmol) was dissolved in a solution of anhydrous THF (50 mL) and treated with n-BuLi (22 mmol, 9 mL, 2.5 M in hexane) at -78 ^{o}C. The reaction mixture was stirred for 30 min and subjected with a solution of methyl 2-((tert-butoxycarbonyl)amino)acetate (1.25 mL, 10 mmol) in anhydrous THF (10 mL). The reaction was kept for 1 hour at -78 ^{o}C before addition of 2-fluoro-5-methoxybenzaldehyde (1.25 mL, 10 mmol) and warmed up to ambient temperature. The reaction was stirred at ambient temperature for 5 hours, carefully quenched by satd. NH_4Cl (30 mL) and extracted by ethyl acetate (80 mL × 3). The combined organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as yellow oil. ^{1}HNMR indicated the alkylated product **3-76** was formed as two diastereomers **3-76a/b** at the ratio of 5:3. Separation of the diastereomers was achieved by careful column chromatography on silica gel (30% ethyl acetate in hexane, $Rf_{3\text{-}76a}$ = 0.3, $Rf_{3\text{-}76b}$ = 0.2) to give pure products as colourless oil (1.89g, 5.5 mmol, 55% overall yield). The characterization data for **3-76a**: **^{1}H NMR (500 MHz, CDCl$_3$)**: ppm 6.95 (dd, *J*=5.0, 3.2 Hz, 1 H, H–2), 6.88 (t, *J*=9.3 Hz, 1 H, H–5), 6.74 (dt, *J*=8.9, 3.6 Hz, 1 H, H–4), 5.33 - 5.45 (m, 2 H, C<u>*H*</u>OH and CHN<u>*H*</u>), 4.69 (d, *J*=6.3 Hz, 1 H, C<u>*H*</u>NH), 4.60 (br. s., 2 H, CHO<u>*H*</u>), 3.75 (s, 3 H, C<u>*H*</u>$_3$), 3.70 (s, 3 H, C<u>*H*</u>$_3$),

1.41 (s, 9 H, C*H*$_3$ × 3); **^{13}C NMR (125 MHz, CDCl$_3$)**: ppm 170.1 (*C*OOMe), 156.3 (*C*–3 or *C*OO^{t}Bu), 155.6 (*C*–3 or *C*OO^{t}Bu), 153.8 (d, J = 237.5 Hz, *C*–6), 127.0 (d, J = 16.2 Hz, *C*–1), 115.4 (d, J = 23.8 Hz, *C*–5), 114.6 (d, J = 7.6 Hz, *C*–4), 112.5 (d, J = 3.8 Hz, *C*–2), 80.6 (*C*(CH$_3$)$_3$), 69.7 (*C*HOH), 58.9 (NH*C*H), 55.6 (O*C*H$_3$), 52.5(COO*C*H$_3$), 28.1 (*C*H$_3$ × 3); **IR** cm^{-1} 3433, 2978, 1717, 1497, 1200, 1162, 857, 815, 778; **HRMS** (ESI, *m/z*) $C_{16}H_{22}FNNaO_6$ (M+Na$^+$) *calc.* 366.1323, *found* 366.1314**.**

The characterization data for **3-76b**: **^{1}H NMR (500MHz, CDCl$_3$)**: ppm 7.01 (br. s., 1 H, Ar–*H*), 6.94 (t, *J* = 9.3 Hz, 1 H, Ar–*H*), 6.73 - 6.79 (m, 1 H, Ar–*H*), 5.53 (br. s., 1 H, OHC*H*), 5.34 (br. d, *J* = 8.5 Hz, 1 H, N*H*CH), 4.63 (br. d, *J* = 8.5 Hz, 1 H, NHC*H*), 3.80 (s, 3 H, C*H*$_3$), 3.78 (s, 3 H, C*H*$_3$), 2.9 (br. s, 1H, O*H*CH) 1.31 (s, 9 H, C*H*$_3$ × 3); **^{13}C NMR (125MHz, CDCl$_3$)**: 171.0 (*C*OOMe), 155.7 (*C*–3 or *C*OO^{t}Bu), 155.6 (*C*–3 or *C*OO^{t}Bu), 153.8 (d, *J* = 237.5 Hz, *C*–6), 127.9 (d, *J* = 14.3 Hz, *C*–1), 115.6 (d, *J* = 23.8 Hz, *C*–5), 114.2 (d, *J* = 7.6 Hz, *C*–4), 112.5 (d, *J* = 3.8 Hz, *C*–2), 80.8 (*C*(CH$_3$)$_3$), 68.3 (*C*HOH), 58.0 (NH*C*H), 55.7 (O*C*H$_3$), 52.6(COO*C*H$_3$), 28.0 (*C*H$_3$ × 3); **IR** 3400, 2978, 1694, 1496, 1207, 1163, 855, 817, 710; **HRMS** (ESI, *m/z*) $C_{16}H_{22}FNNaO_6$ (M+Na$^+$) *calc.* 366.1323, *found* 366.1318**.**

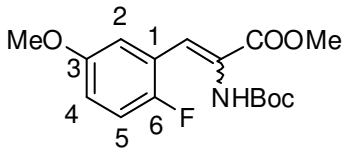
Methyl-3-acetoxy-2-((tert-butoxycarbonyl)amino)-3-(2-fluoro-5-methoxyphenyl) propanoate (3-77)



3-76 (1.6 g, 4.7 mmol) was dissolved in DCM (50 mL) with triethylamine (1.5 mL, 9.4 mmol) and dimethylaminopyridine (28mg, 5mol%) and chilled to 0 ^{o}C. Acetyl chloride (0.67 mL, 9.4 mmol) was added slowly and the reaction mixture was kept stirring for 2 hours. The reaction was quenched by satd. $NaHCO_3$ and extracted with DCM (50 mL × 2). The combined organic phase was washed with satd. $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was subjected to next step without

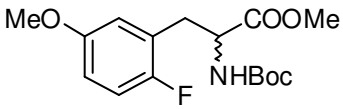
further purification.

Methyl 2-((tert-butoxycarbonyl)amino)-3-(2-fluoro-5-methoxyphenyl)acrylate (3-78)



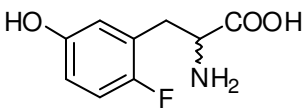
Crude **3-77** (4.7 mmol) was dissolved in THF (50 mL) in the presence of 1,8-diazabicycloundec-7-ene (DBU, 0.7 mL, 4.7 mmol) and the solution was heated at 60 ^{o}C for 1 hour. The reaction mixture was cooled down, diluted by ethyl acetate (80 mL) and washed by satd. NH_4Cl. The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as light yellow oil. The purified product was obtained as a colourless oil by careful column chromatography on silica gel (30% ethyl acetate in hexane, Rf = 0.6, 890 mg, 2.8 mmol, 59% yield over two steps). **^{1}H NMR (500MHz, $CDCl_3$)**: ppm 7.25 (br.s. 1 H, C*H*=C), 7.07 - 7.16 (m, 1 H, Ar–*H*), 6.98 (t, *J* = 9.3 Hz, 1 H, Ar–*H*), 6.81 (dt, *J* = 8.9, 3.6 Hz, 1 H, Ar–*H*), 6.50 (br. s., 1 H, N*H*), 3.85 (s, 3 H, CH_3), 3.75 (s, 3 H, CH_3), 1.37 (s, 9 H, (CH_3) × 3); **^{13}C NMR (126MHz, $CDCl_3$)**: ppm 165.5 (*C*$OOCH_3$), 155.2 (d, *J* = 1.9 Hz, *C*–3), 154.9 (d, *J* = 243.2 Hz, *C*–6), 152.3 (*C*OO^{t}Bu), 126.2 (CH=*C*), 122.5 (d, *J* = 14.3 Hz, *C*–1), 120.8 (d, *J* = 3.8 Hz, *C*H=C), 116.3 (d, *J* = 8.6 Hz, *C*–4), 116.0 (d, *J* = 23.8 Hz, *C*–5), 113.5 (d, *J* = 1.9 Hz, *C*–2), 80.9, *C*$(CH_3)_3$), 55.5 (*C*H_3), 52.5 (*C*H_3), 27.9 (C(*C*$H_3)_3$); **IR** 3325, 2955, 1719, 1645, 1495, 815, 777, 718; **HRMS** (ESI, *m/z*) $C_{16}H_{20}FNNaO_5$ ($M+Na^+$) *calc.* 348.1218, *found* 348.1207.

(±)Methyl 2-((tert-butoxycarbonyl)amino)-3-(2-fluoro-5-methoxyphenyl)propanoate (3-79)



3-78 (849 mg, 2.6 mmol) was dissolved in a solution of methanol (30 mL) with 85 mg 10% palladium on carbon (10 wt%). The solution was purged with argon for 30 min then with hydrogen for 30 min before stirring under hydrogen atmosphere for overnight at room temperature. The reaction was filtered through Celite and concentrated under reduced pressure to obtain the crude product as colourless oil. Purified product was obtained as a colourless oil by column chromatography on silica gel (20% ethyl acetate in hexane, Rf = 0.4, 776 mg, 2.4 mmol, 91% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 6.94 (t, *J* = 9.1 Hz, 1 H, Ar – H), 6.70 - 6.76 (m, 1 H, Ar – H), 6.66 (dd, *J* = 5.7, 2.9 Hz, 1 H, Ar – H), 5.08 (d, *J* =7.6 Hz, 1 H, N*H*), 4.57 (dd, *J* = 13.4, 6.8 Hz, 1 H, NHC*H*), 3.76 (s, 3 H, CH_3), 3.73 (s, 3 H, CH_3), 3.15 (dd, *J* = 13.9, 5.3 Hz, 1 H, CH_2CH), 3.04 (dd, *J* = 13.9, 6.6 Hz, 1 H, CH_2CH), 1.41 (s, 9 H, (CH_3)$_3$); **^{13}C NMR (100MHz, $CDCl_3$)** ppm 172.1 (*C*OOMe), 155.7 (d, J = 238.1 Hz, *C* – F), 155.5 (Ar – *C* or *C*OO(CH$_3$)$_3$), 155.0 (Ar – *C* or *C*OO(CH$_3$)$_3$)123.8 (d, J = 17.6 Hz, Ar – *C*), 116.4 (d, J = 3.2 Hz, Ar – *C*), 115.7 (d, J = 24.0 Hz, Ar – *C*), 113.6 (d, J = 8.0 Hz, Ar – *C*), 79.9 (*C*(CH$_3$)$_3$), 55.6 (*C*H$_3$), 53.6 (NH*C*H), 52.3 (*C*H$_3$), 32.0 (*C*H$_2$CH), 28.2 (C(*C*H$_3$)$_3$); **^{19}F{^{1}H}NMR (376 MHz, $CDCl_3$)** ppm -128.6; **IR** 3368, 2971, 1715, 1501, 813, 779, 710; **HRMS** (ESI, *m/z*) $C_{16}H_{22}FNNaO_5$ (M+Na$^+$) *calc.* 350.1374, *found* 350.1362**.**

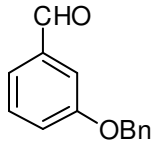
(±)2-amino-3-(2-fluoro-5-hydroxyphenyl)propanoic acid (3-2) [209]



3-79 (66.8 mg, 0.2 mmol) was dissolved in 1 mL hydrogen bromide (48% aqueous solution) and 1 mL acetic acid. The solution was heated at 100 ^{o}C for 10 min before cooling down and

neutralized by 5 M aqueous solution of NaOH. The reaction mixture was diluted by water, dried azeotropically with acetonitrile and purified by HPLC on a semi-preparative C18 column. Pure product was obtained as a white solid (40 mg, 0.2 mmol) in 100% yield. **^{1}H NMR (500MHz, D_2O)** ppm 7.01 (dd, *J* = 9.5, 9.1 Hz, 1 H, Ar – *H*), 6.76 - 6.81 (m, 1 H, Ar – *H*), 6.73 - 6.76 (m, 1 H, Ar – *H*), 3.97 (dd, *J* = 7.9, 5.7 Hz, 1 H, NH_2CH), 3.26 (dd, *J* = 14.5, 5.4 Hz, 1 H, CH_2CH), 3.03 (dd, *J* = 14.8, 7.9 Hz, 1 H, CH_2CH); **^{13}C NMR (126MHz, D_2O)** ppm 173.3 (*C*OOH), 155.4 (d, *J* = 235.6 Hz, *C* – F), 151.7 (d, *J* = 2.9 Hz, Ar – *C*), 122.8 (d, *J* = 17.2 Hz, Ar – *C*), 117.7 (d, *J* = 4.8 Hz, Ar – *C*), 116.4 (d, *J* = 24.8 Hz, Ar – *C*), 116.0 (d, *J* = 8.6 Hz, Ar – *C*), 54.9 (NH_2CH), 30.1 (CH_2CH); **^{19}F NMR (376MHz, D_2O)** ppm – 129.0 (dt, *J* = 9.2, 4.6 × 2 Hz, 1F); **MS** (ESI, *m/z*) $C_9H_{11}FNO_3$ ($M+H^+$) *calc.* 200.07, *found* 200.07**;** Other characterisation data are in agreement with the literature.[209]

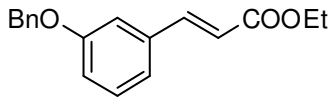
3-(Benzyloxy)benzaldehyde (3-80) [210]



To a solution of 3-hydroxylbenzaldehyde (6.1 g, 50.0 mmol) and potassium carbonate (20.7 g, 100.0 mmol) in anhydrous DMF (100 mL) was added benzyl bromide (7.2 mL, 60.0 mmol) slowly. The reaction was stirred at ambient temperature for overnight, quenched by water and extracted with diethyl ether (150 mL × 2). The combined organic phase was washed with satd. NaCl, dried ($MgSO_4$), filtered and concentrated in reduced pressure. Purified product was obtained by column chromatography on silica gel (10% ethyl acetate in hexane, Rf = 0.3) to give pure product as white solid (10.06 g, 50.0 mmol, quantitative yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 9.99 (s, 1 H, C*H*O), 7.44 - 7.51 (m, 5 H, Ar – *H*), 7.39 - 7.44 (m, 2 H, Ar – *H*), 7.34 - 7.39 (m, 1 H, Ar – *H*), 7.27 (dt, *J* = 7.3, 2.3 Hz, 1 H, Ar – *H*), 5.14 ppm (s, 2 H, CH_2); **^{13}C NMR (100MHz, $CDCl_3$)** ppm 192.0 (*C*HO), 159.3 (Ar – *C*), 137.8 (Ar – *C*), 136.3 (Ar – *C*), 130.1 (Ar – *C*), 128.6 (Ar – *C*), 128.2 (Ar – *C*), 127.5

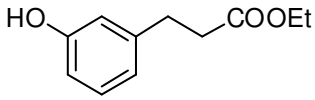
(Ar – <u>C</u>), 123.6 (Ar – <u>C</u>), 122.1 (Ar – <u>C</u>), 113.2 (Ar – <u>C</u>), 70.2 (<u>C</u>H_2); Other characterisation data are as reported.[210]

Ethyl 3-(3-(benzyloxy)phenyl)acrylate (3-81)



To a solution of 3-(benzyloxy)benzaldehyde (**3-80**, 3.2g, 15 mmol) in DCM (100 mL) was added ethyl 2-(triphenylphosphoranylidene)acetate (5.5 g, 15.7 mmol) and the reaction was stirred at amnient temperature for 4 hours. The solvent was removed under reduced pressure and the residue was dissolved and filtered through Celite. The filtrate was concentrated under reduced pressure to afford the crude product as colourless oil. The crude product was subjected to next step without further purification.

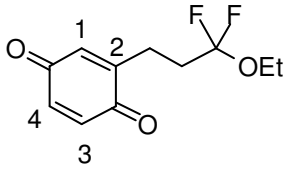
Ethyl 3-(3-hydroxy-phenyl)-propionate (3-82)



3-81 (15 mmol) was dissolved in a solution of methanol (60 mL) with 400 mg of 10% palladium on carbon (~10% wt). The solution was purged with argon for 30 min then with hydrogen for 30 min before stirring under hydrogen atmosphere for overnight at room temperature. The reaction was filtered through Celite and concentrated under reduced pressure to afford the crude product. The purified product was obtained by column chromatography on silica gel (20% EtOAc in hexane, Rf = 0.3) as a colourless oil (2.65 g, 13.6 mmol, 91% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.13 - 7.18 (m, 1 H, Ar – <u>H</u>), 6.75 - 6.79 (m, 1 H, Ar – <u>H</u>), 6.67 - 6.71 (m, 2 H, Ar – <u>H</u>), 5.35 – 5.47 (m, 1 H, O<u>H</u>), 4.14 (q, *J* = 7.2 Hz, 2 H, OC<u>H_2</u>CH_3), 2.91 (t, *J* = 7.8 Hz, 2 H, C<u>H_2</u>), 2.62 (t, *J* = 7.8 Hz, 2 H, C<u>H_2</u>), 1.25 (t, *J*=7.2 Hz, 3 H, OCH_2C<u>H_3</u>); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 173.6 (<u>C</u>=O), 155.9 (Ar –

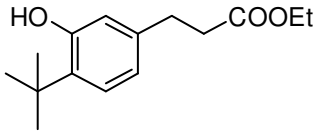
C), 142.1 (Ar – C), 129.6 (Ar – C), 120.4 (Ar – C), 115.3 (Ar – C), 113.3 (Ar – C), 60.7 (OCH_2CH_3), 35.8 (CH_2), 30.7 (CH_2), 14.1 (OCH_2CH_3); **MS** (ESI, *m/z*) $C_{11}H_{14}NaO_3$ ($M+Na^+$) *calc.* 217.08, *found* 217.08**;** Other characterisation data are as reported. [211]

2-(3-Ethoxy-3,3-difluoropropyl)cyclohexa-2,5-diene-1,4-dione (3-83)



To a solution of **3-82** (194 mg, 1 mmol) and 70% HF•pyridine (104 μL, 4 mmol) in DCM (20 ml) was added PIDA (322 mg, 1 mmol) at 0 °C. The reaction was kept stirring for 10 min before quenching by K_2CO_3. The reaction was filtered and concentrated at 26 °C under reduced pressure. Purified product was obtained by careful column chromatography on silica gel (100% DCM, Rf = 0.6) to give pure product as a yellow oil (22 mg, 0.22 mmol, 10% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 6.70 - 6.81 (m, 2 H, H–3 and H–4), 6.58 - 6.60 (m, 1 H, H–1), 3.90 (q, *J* = 7.2 Hz, 3 H, OCH_2CH_3), 2.66 (td, *J* = 7.8, 1.3 Hz, 2 H, $CH_2CH_2CF_2$), 2.14 - 2.25 (m, 2 H, $CH_2CH_2CF_2$), 1.23 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 187.5 (C=O), 187.0 (C=O), 147.6 (C–2), 136.8 (C–3), 136.3 (C–4), 133.0 (C–1), 124.9 (t, *J* = 261 Hz, CF_2), 59.0 (t, *J* = 6.9 Hz, OCH_2CH_3), 33.6 (t, *J* = 31.5 Hz, $CH_2CH_2CF_2$), 23.3 (t, *J* = 3.8 Hz, $CH_2CH_2CF_2$), 14.9 (OCH_2CH_3); **^{19}F NMR (376 MHz, $CDCl_3$)** ppm -74.17 (t, *J* = 11.3 Hz); **IR** 2981, 1769, 1672, 1187, 1139; **HRMS** (FI) $C_{11}H_{12}F_2O_3$ *calc.* 230.0755, *found* 230.0757.

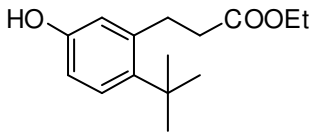
Ethyl 3-(4-(*tert*-butyl)-3-hydroxyphenyl)propanoate (3-87a)



To a mixture of **3-82** (1.7 g, 8.75 mmol) and conc. H_2SO_4 (10 μL, 0.1%) was added 2-

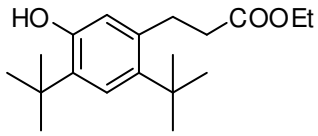
methylprop-1-ene (0.66 mL, 7 mmol) at -78 °C. The reaction was warmed up and kept at 30 °C for two days. Careful column chromatography on silica gel (5% →20% EtOAc in Hexane) gave **3-88** (99 mg, in 5% yield, Rf = 0.64 in 20% EtOAc / Hexane), **3-87a** (713 mg, in 41% yield, Rf = 0.55 in 20% EtOAc / Hexane), **3-87b** (11 mg, in 1% yield, Rf = 0.5 in 20% EtOAc / Hexane) and recovered starting material (323 mg, 19%, Rf = 0.4 in 20% EtOAc / Hexane), as colourless oil. **^{1}H NMR (500MHz, $CDCl_3$)** ppm 7.18 (d, *J* = 7.9 Hz, 1 H, , Ar – *H*), 6.71 (dd, *J* = 7.9, 1.9 Hz, 1 H, , Ar – *H*), 6.55 (d, *J* = 1.6 Hz, 1 H, , Ar – *H*), 5.14 (s, 1 H, O*H*), 4.15 (q, *J* = 7.2 Hz, 2 H, OC*H*$_2$CH$_3$), 2.87 (t, *J* = 7.7 Hz, 2 H, C*H*$_2$CH$_2$), 2.61 (t, *J* = 7.9 Hz, 2 H, C*H*$_2$CO), 1.40 (s, 9 H, C*H*$_3$ × 3), 1.25 (t, *J* = 7.2 Hz, 3 H, OCH$_2$C*H*$_3$); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 173.3 (*C*=O), 154.3 (Ar – *C*), 139.5 (Ar – *C*), 134.0 (Ar – *C*), 127.1 (Ar – *C*), 120.1 (Ar – *C*), 116.5 (Ar – *C*), 60.5 (O*C*H$_2$CH$_3$), 35.7 (*C*H$_2$CO), 34.2 (*C*(CH$_3$)$_3$), 30.1 (*C*H$_2$), 29.6 (C(*C*H$_3$)$_3$), 14.2 (OCH$_2$*C*H$_3$); **IR** 3442, 3080, 1710, 1199, 861, 816; **HRMS** (ESI, *m/z*) $C_{15}H_{22}NaO_3$ (M+Na$^+$) *calc.* 273.1461, *found* 273.1457.

Ethyl 3-(2-(*tert*-butyl)-5-hydroxyphenyl)propanoate (3-87b)



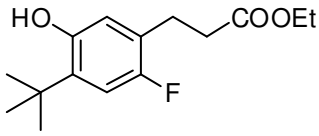
^{1}H NMR (500MHz, $CDCl_3$) ppm 7.23 (d, *J* = 8.5 Hz, 1 H, , Ar – *H*), 6.68 (d, *J* = 2.8 Hz, 1 H, , Ar – *H*), 6.62 (dd, *J* = 8.8, 2.8 Hz, 1 H, , Ar – *H*), 5.11 (s, 1 H, O*H*), 4.19 (q, *J* = 7.1 Hz, 2 H, OC*H*$_2$CH$_3$), 3.16 (t, *J* = 7.8 Hz, 2 H, C*H*$_2$CH$_2$), 2.62 (t, *J* = 7.8 Hz, 2 H, C*H*$_2$CO), 1.40 (s, 9 H, C*H*$_3$ × 3), 1.24 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 173.3 (*C*=O), 153.6 (Ar – *C*), 140.8 (Ar – *C*), 139.9 (Ar – *C*), 127.6 (Ar – *C*), 117.1 (Ar – *C*), 112.7 (Ar – *C*), 60.6 (O*C*H$_2$CH$_3$), 36.9 (*C*H$_2$CO), 35.1 (*C*(CH$_3$)$_3$), 31.8 (C(*C*H$_3$)$_3$), 29.4 (*C*H$_2$), 14.2 (OCH$_2$*C*H$_3$); **IR** 3401, 2958, 1709, 1248, 864, 816; **HRMS** (ESI, *m/z*) $C_{15}H_{22}NaO_3$ (M+Na$^+$) *calc.* 273.1461, *found* 273.1460.

Ethyl 3-(2,4-di-*tert*-butyl-5-hydroxyphenyl)propanoate (3-88)



^{1}H NMR (500MHz, $CDCl_3$) ppm 7.26 (s, 1 H, Ar – *H*), 6.49 (s, 1 H, Ar – *H*), 4.81 (s, 1 H, O*H*), 4.18 (q, *J* = 7.2 Hz, 2 H, OC*H*$_2$CH$_3$), 3.11 (t, *J* = 7.8 Hz, 2 H, C*H*$_2$CH$_2$), 2.62 (t, *J* = 7.8 Hz, 2 H, C*H*$_2$CO), 1.40 (s, 18 H, CH_3 × 6), 1.28 ppm (t, *J* = 7.3 Hz, 3 H, OCH$_2$C*H*$_3$); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 173.3 (*C*=O), 151.9 (Ar – *C*), 139.2 (Ar – *C*), 137.6 (Ar – *C*), 132.9 (Ar – *C*), 125.4 (Ar – *C*), 119.0 (Ar – *C*), 60.5 (O*C*H$_2$CH$_3$), 36.9 (*C*H$_2$), 35.5 (*C*(CH$_3$)$_3$), 34.6 (*C*(CH$_3$)$_3$), 31.9 (C(*C*H$_3$)$_3$), 29.7 (C(*C*H$_3$)$_3$), 28.8 (*C*H$_2$), 14.2 (OCH$_2$*C*H$_3$); **IR** 3446, 2955, 1710, 1274, 861; **HRMS** (ESI, *m/z*) $C_{19}H_{30}NaO_3$ ($M+Na^+$) *calc.* 329.2087, *found* 329.2081.

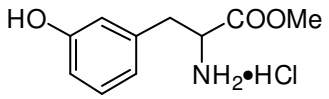
Ethyl 3-(4-(*tert*-butyl)-2-fluoro-5-hydroxyphenyl)propanoate (3-89)



To a solution of **3-88**, 76.6 mg, 0.25 mmol) and HF•pyridine (70% wt, 26 μL, 1.0 mmol) in DCM (5 mL) was added phenyliodine di(trifluoroacetace) (PIFA, 107 mg, 0.25 mmol). The reaction was stirred at 25 ^{o}C for 30 min and concentrated under reduced pressure. The purified product was obtained by careful column chromatography on silica gel (20% diethyl ether in petane, Rf = 0.2) as a yellow oil (13 mg, 0.05 mmol, 20% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 6.93 (d, *J* = 11.9 Hz, 1 H, Ar – *H*), 6.51 (d, *J* = 6.6 Hz, 1 H, Ar – *H*), 4.72 (s, 1 H, O*H*), 4.14 (q, *J* = 7.2 Hz, 2 H, COOC*H*$_2$CH$_3$), 2.87 (t, *J* = 7.6 Hz, 2 H, C*H*$_2$), 2.60 (t, *J* = 7.7 Hz, 2 H, C*H*$_2$), 1.38 (s, 9 H, CH_3 × 3), 1.24 ppm (t, *J* = 7.2 Hz, 3 H, COOCH$_2$C*H*$_3$); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 172.9, 155.1 (d, *J* = 236.0 Hz), 149.8 (d, *J* = 2.2 Hz), 136.0 (d, *J* = 6.0 Hz), 124.8 (d, *J* = 18.1 Hz), 117.8 (d, *J* = 5.5 Hz), 113.9 (d, *J* =

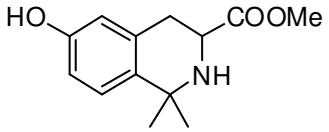
24.7 Hz), 60.5, 34.4, 34.4, 29.4, 24.1, 14.2; **^{19}F NMR (377MHz, $CDCl_3$)** ppm -129.2 (dd, *J* = 11.5, 6.9 Hz, 1 F);

Methyl 2-amino-3-(3-hydroxyphenyl)propanoate hydrogen chloride (3-90)



Thionyl chloride (0.8 mL, 11 mmol) was slowly added to a solution of (±) *m*-tyrosine (1 g, 5.5 mmol) in anhydrous methanol (50 mL) at 0 °C. The solution was refluxed for 24 hours before cooling down. The solvent was removed under reduced pressure. Co-evaporation of the residue with toluene *in vacuum* to afford the product as a light yellow solid in the form of chlorohydrate salt (1.24 g, 5.3 mmol, 97% yield). **^{1}H NMR (200MHz, D_2O)** ppm 7.22 (t, *J* = 8.0 Hz, 1 H, Ar – *H*), 6.72 - 6.83 (m, 2 H, Ar – *H*), 6.67 - 6.72 (m, 1 H, Ar – *H*), 4.29 - 4.39 (m, 1 H, CH_2CH), 3.76 (s, 3 H, $COOCH_3$), 3.22 (dd, *J* =14.7, 5.8 Hz, 1 H, CH_2CH), 3.09 (dd, *J* =14.3, 7.5 Hz, 1 H, CH_2CH); **^{13}C NMR (75MHz, D_2O)** ppm 170.0 (*C*=O), 156.0 (Ar – *C*), 135.5 (Ar – *C*), 130.6 (Ar – *C*), 121.4 (Ar – *C*), 116.1 (Ar – *C*), 115.0 (Ar – *C*), 54.4 (CH_2CH), 53.5 ($COOCH_3$), 35.4 (CH_2CH); **MS** (ESI, *m/z*) $C_{10}H_{14}NO_3$ ($M+H^+$) *calc.* 196.10, *found* 196.12; Other characterization data are as reported.[212]

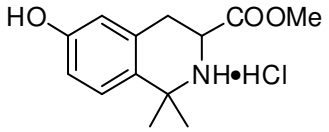
Methyl 6-hydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (3-91a)



3-90 (655 mg, 2.8 mmol) was dissolved in satd. $NaHCO_3$ and extracted by ethyl acetate (25 mL × 2). The combined organic phase was concentrated under reduced pressure and dried *in vacuum*. The residue product was dissolved in a solution of toluene (40 mL) with dimethoxylisopropane (1.74 mL, 14.1 mmol) and camphorsulfonic acid (65 mg, 10 mol%). The reaction was refluxed in the presence of 3Å molecular sieves for overnight. The reaction

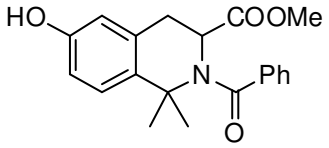
mixture was filtered, concentrated and work up with ethyl acetate. Purified product was obtained by careful column chromatography on silica gel (5% methanol in DCM, Rf = 0.3) as a yellow solid (88 mg, 0.36 mmol, 13% yield). **^{1}H NMR (300MHz, $CDCl_3$)** ppm 7.07 (d, *J* = 8.5 Hz, 1 H, Ar – *H*), 6.68 (dd, *J* = 8.5, 2.6 Hz, 1 H, Ar – *H*), 6.51 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 3.90 (dd, *J* = 11.1, 4.7 Hz, 1 H, CH_2CH), 3.78 (s, 3 H, $COOCH_3$), 2.95 (dd, *J* = 16.1, 4.7 Hz, 1 H, CH_2CH), 2.86 (dd, *J* = 16.1, 11.1 Hz, 1 H, CH_2CH), 1.52 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 173.7 ($COOCH_3$), 154.0 (Ar – *C*), 135.0 (Ar – *C*), 133.7 (Ar – *C*), 126.9 (Ar – *C*), 115.2 (Ar – *C*), 114.2 (Ar – *C*), 53.7 ($C(CH_3)_2$), 52.3 ($COOCH_3$), 51.8 (CH_2CH), 33.3 (CH_2CH), 31.8 (CH_3), 30.9 (CH_3);

Methyl 6-hydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride (3-91b)



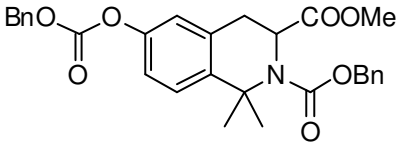
3-90 (9.0 g, 38.8 mmol) was dispensed in acetone (100 mL) and refluxed through activated 3Å molecular sieves for 48 hours until ^{1}H NMR shows full conversion of the starting material to product. The reaction mixture was filtered, concentrated and washed by diethyl ether to afford pure product as a white powder (9.38 g, 34.5 mmol, 89% yield). **m.p.** = 238-240 ºC. **^{1}H NMR** (400 MHz, D_2O) δ: 7.29 (d, *J* = 8.8 Hz, 1 H, Ar–*H*), 6.86 (dd, *J* = 2.5, 8.6 Hz, 1 H, Ar–*H*), 6.72 (d, *J* = 2.5 Hz, 1 H, Ar–*H*), 4.61 (dd, *J* = 5.1, 12.6 Hz, 1 H, CH_2CH), 3.91 (s, 3 H, CO_2CH_3), 3.39 (dd, *J* = 5.1, 17.4 Hz, 1 H, CH_2CH), 3.21 (dd, *J* = 12.6, 17.2 Hz, 1 H, CH_2CH), 1.81 (s, 3 H, $C(CH_3)_2$), 1.65 (s, 3 H, $C(CH_3)_2$). **^{13}C NMR** (101 MHz, D_2O) δ: 170.3 (*C*=O), 155.8 (Ar–*C*), 130.9 (Ar–*C*), 129.5 (Ar–*C*), 127.5 (Ar–*C*), 116.1 (Ar–*C*), 115.3 (Ar–*C*), 59.7 ($C(CH_3)_2$), 54.4 (CH_2CH), 51.3 (CO_2CH_3), 29.2 (CH_2), 28.3 (CH_3), 27.6 (CH_3). **IR** (Neat): 3216, 1757, 1615, 1227, 894, 860, 819. **HRMS** (ESI^+, m/z): *calc*. $C_{13}H_{18}NO_3$ ($[M+H]^+$) 236.1281, *found* 236.1275.

Methyl 2-benzoyl-6-hydroxy-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (3-92a)



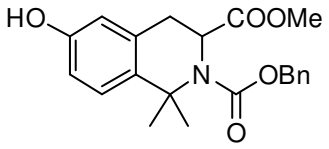
To a solution of **3-91b** (543 mg, 2.0 mmol) , triethylamine (1.4 mL, 10 mmol) and DMAP (5 mg) in a solution of DCM (30 mL) was added benzyl chloride (813 μL, 7 mmol) and the reaction mixture was heated to reflux for overnight. The reaction mixture was cooled down, diluted by DCM (70 mL) and washed by water (50 mL × 2). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was dissolved in MeOH (50 mL) and K_2CO_3 (138 mg, 1.0 mmol) was added. The reaction was stirred at 25 ^oC for 15 min, quenched by 2 M HCl and concentrated under reduced pressure. The residue was dissolved in water (30 mL) and extracted by DCM (50 mL × 3). The combined organic phase was washed with satd. $NaHCO_3$ (30 mL), brine (30 mL), dried in Na_2SO_4, filtered and concentrated to give the crude product. Purified product was obtained by column chromatography on silica gel (30% EtOAc in Hexane, Rf = 0.2) as a white solid (568 mg, 1.67 mmol, 84% yield). **^{1}H NMR (400MHz, MeOD)** ppm 7.42 - 7.49 (m, 3 H, Ar – *H*), 7.36 (dt, *J* = 4.0, 2.8 Hz, 2 H, Ar – *H*), 7.24 (d, *J* = 8.8 Hz, 1 H, Ar – *H*), 6.71 (dd, *J* = 8.7, 2.4 Hz, 1 H, Ar – *H*), 6.52 (d, *J* = 2.3 Hz, 1 H, Ar – *H*), 4.72 (dd, *J* = 3.0, 4.0 Hz, 1 H, CH_2CH), 3.16 (dd, *J* = 15.4, 4.5 Hz, 1 H, CH_2CH), 3.08 (dd, *J* = 15.7, 3.0 Hz, 1 H, CH_2CH), 2.12 (s, 3 H, CH_3), 1.81 (s, 3 H, CH_3); **^{13}C NMR (101MHz, MeOD)** ppm 174.8 (*C*=O), 172.8 (*C*=O), 157.0 (Ar – *C*), 140.4 (Ar – *C*), 136.4 (Ar – *C*), 133.8 (Ar – *C*), 130.6 (Ar – *C*), 130.2 (Ar – *C*), 127.5 (Ar – *C*), 126.5 (Ar – *C*), 116.0 (Ar – *C*), 115.5 (Ar – *C*), 62.2 ($C(CH_3)_2$), 60.9 (CH_2CH), 52.9 ($COOCH_3$), 33.3 (CH_2CH), 32.0 ($C(CH_3)_2$), 24.8 ($C(CH_3)_2$);

2-Benzyl 3-methyl 6-(((benzyloxy)carbonyl)oxy)-1,1-dimethyl-3,4-dihydroisoquino-line-2,3(1*H*)-dicarboxylate



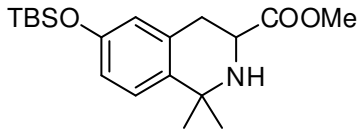
To a solution of **3-91b** (407 mg, 1.5 mmol) and K_2CO_3 (1.65 g, 8 mmol) in THF/H_2O (2:1, 6 mL) was added benzyl chloroformate (1.07 mL, 5 mmol) and the reaction mixture was heated to reflux for overnight. The reaction mixture was cooled down and neutralized by 1 M HCl (aq., ~ 15 mL) and extracted by EtOAc (30 mL × 2). The combined organic layer was washed by satd. NH_4Cl (aq. 40 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purified product was obtained by column chromatography on silica gel (20% EtOAc in Hexane, Rf = 0.2) as a colourless oil (507 mg, 1.0 mmol, 67% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.32 - 7.49 (m, 11 H, Ar – *H*), 7.10 (dd, *J* = 8.8, 2.5 Hz, 1 H, Ar – *H*), 6.98 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 5.39 (br. s., 1 H, C*H*), 5.16 - 5.31 (m, 4 H, CH_{Bn2} × 2), 3.53 (s, 3 H, COOCH_3), 3.28 (dd, *J* = 15.7, 2.5 Hz, 1 H, CH_2CH), 3.17 (dd, *J* = 15.7, 5.3 Hz, 1 H, CH_2CH), 2.06 (s, 3 H, CH_3), 1.72 (s, 3 H, CH_3); **^{13}C NMR (100MHz, $CDCl_3$)** ppm 171.5 (*C*=O), 153.4 (*C*=O), 149.0 (Ar – *C*), 141.5 (Ar – *C*), 136.4 (Ar – *C*), 134.6 (Ar – *C*), 132.2 ((Ar – *C*)), 128.6 (Ar – *C*), 128.5 (Ar – *C*), 128.4 (Ar – *C*), 128.4 (Ar – *C*), 127.8 (Ar – *C*), 127.7 (Ar – *C*), 126.5 (Ar – *C*), 119.9 (Ar – *C*), 119.8 (Ar – *C*), 70.2 (*C*H_2Ph), 67.1 (*C*H_2Ph), 59.0 (*C*$(CH_3)_2$), 54.8 (CH_2*C*H), 52.0 (COO*C*H_3), 31.9 (*C*H_2CH), 31.4 (*C*H_3), 24.8 (*C*H_3); **IR** 1757, 1706, 1225, 910, 744, 698; **HRMS** (ESI, *m/z*) $C_{29}H_{29}NNaO_7$ ($M+Na^+$) *calc.* 526.1836, *found* 526.1831.

2-Benzyl 3-methyl 6-hydroxy-1,1-dimethyl-3,4-dihydroisoquinoline-2,3(1*H*)-dicarboxylate (3-92b)



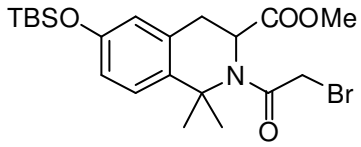
To a solution of 2-benzyl 3-methyl 6-(((benzyloxy)carbonyl)oxy)-1,1-dimethyl-3,4-dihydroisoquino-line-2,3(1*H*)-dicarboxylate (358 mg, 0.71 mmol) in MeOH (5 mL) was added K_2CO_3 (200 mg, 1.42 mmol). The reaction was stirred at 25 ^oC for 15 min, quenched by 2M HCl (aq., ~ 2mL) and concentrated under reduced pressure. The residue was dissolved in water (30 mL) and extracted by EtOAc (30 mL × 2). The combined organic phase was washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the crude product. Purified product was obtained by column chromatography on silica gel (33% EtOAc in Hexane, Rf = 0.1) as a white foam (238 mg, 0.65 mmol, 91% yield). **^{1}H NMR (500MHz, $CDCl_3$)** ppm 7.30 - 7.42 (m, 5 H, Ar – *H*), 7.20 (d, *J* = 8.5 Hz, 1 H, Ar – *H*), 6.72 (dd, *J* = 8.7, 2.7 Hz, 1 H, Ar – *H*), 6.56 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 5.63 (br. s., 1 H, O*H*), 5.30 (s, 1 H, CH_2CH), 5.23 (br. s., 2 H, CH_2Ph), 3.53 (s, 3 H, $COOCH_3$), 3.20 (dd, *J* = 15.4, 1.6 Hz, 1 H, CH_2CH), 3.11 (dd, *J* = 15.4, 5.0 Hz, 1 H, CH_2CH), 1.99 (s, 3 H, CH_3), 1.66 (s, 3 H, CH_3); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 172.2 (*C*=O), 154.2 (*C*=O), 154.0 (Ar – *C*), 136.5 (Ar – *C*), 135.9 (Ar – *C*), 132.3 (Ar – *C*), 128.5 (Ar – *C*), 128.0 (Ar – *C*), 127.8 (Ar – *C*), 126.7 (Ar – *C*), 114.8 (Ar – *C*), 114.3 (Ar – *C*), 67.0 (CH_2Ph), 59.1 ($C(CH_3)_2$), 55.2 (CH_2CH), 52.2 ($COOCH_3$), 32.1 (CH_2CH), 32.0 ($C(CH_3)_2$), 24.9 ($C(CH_3)_2$); **IR** cm^{-1} 3375, 1749, 1706, 1237, 917, 737, 699; **HRMS** (ESI, *m/z*) $C_{21}H_{23}NNaO_5$ ($M+Na^+$) *calc.* 392.1468, *found* 392.1456.

Methyl 6-((*tert*-butyldimethylsilyl)oxy)-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (3-91(*O*-TBS))



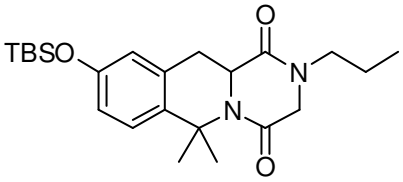
To the solution of **3-91b** (407 mg, 1.5 mmol), triethylamine (502 μL, 3.6 mmol) and *N*,*N*-dimethylpyridine (18 mg, 0.15 mmol) in anhydrous DCM (10 mL) was added *tert*-butylchlorodimethylsilane (249 mg, 1.65 mmol). The reaction was stirred at 25 ^{o}C for overnight, quenched by satd. $NaHCO_3$ (aq. 20 mL) and extracted by DCM (30 mL × 2). The combined organic phase was washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the crude product. Purified product was obtained by column chromatography on silica gel (33% EtOAc in Hexane, Rf = 0.4) as a colourless oil (448 mg, 1.3 mmol, 85% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.06 (d, *J* = 8.4 Hz, 1 H, Ar – *H*), 6.66 (dd, *J* = 8.4, 2.6 Hz, 1 H, Ar – *H*), 6.54 (d, *J* = 2.2 Hz, 1 H, Ar – *H*), 3.88 (dd, *J* = 11.4, 4.1 Hz, 1 H, CH_2CH), 3.80 (s, 3 H, $COOCH_3$), 2.97 (dd, *J* = 16.0, 4.1 Hz, 1 H, CH_2CH), 2.86 (dd, *J* = 16.0, 11.6 Hz, 1 H, CH_2CH), 1.50 (s, 3 H, CCH_3), 1.41 (s, 3 H, CCH_3), 0.97 (s, 9 H, $C(CH_3)_3$), 0.19 ppm (s, 6 H, $Si(CH_3)$ × 2); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 173.8 (*C*=O), 153.4 (Ar – *C*), 136.0 (Ar – *C*), 133.6 (Ar – *C*), 126.6 (Ar – *C*), 119.6 (Ar – *C*), 118.4 (Ar – *C*), 53.5 ($C(CH_3)_2$), 52.2 ($COOCH_3$), 51.9 (CH_2CH), 33.5 (CH_2CH), 31.9 ($C(CH_3)_2$), 31.0 ($C(CH_3)_2$), 25.6 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), -4.4 ($Si(CH_3)_2$); **IR** 2956, 1744, 1608, 1253, 851, 780; **HRMS** (ESI, *m/z*) $C_{19}H_{32}NO_3Si$ ($M+H^+$) *calc.* 350.2146, *found* 350.2140.

Methyl 2-(2-bromoacetyl)-6-((*tert*-butyldimethylsilyl)oxy)-1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (3-97)



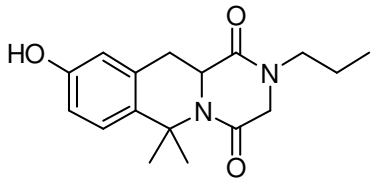
To a solution of **3-91(*O*-TBS)** (445 mg, 1.3 mmol) and triethylamine (280 μL, 2 mmol) in anhydrous DCM (10 mL) was added 2-bromoacetyl bromide (140 μL, 1.6 mmol) at 0 ^{o}C. The reaction was warmed up to ambient temperature and stirred for 3 hours, quenched by satd. $NaHCO_3$ (aq. 20 mL) and extracted by DCM (30 mL × 2). The combined organic phase was washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated to give the crude product. Purified product was obtained by column chromatography on silica gel (20% EtOAc in hexane, Rf = 0.3) as yellow oil (490 mg, 1.0 mmol, 80% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.17 (d, *J* = 8.8 Hz, 1 H, Ar – *H*), 6.72 (dd, *J* = 8.6, 2.5 Hz, 1 H, Ar – *H*), 6.59 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 4.85 - 4.92 (m, 1 H, CH_2CH), 3.93 (d, *J* = 10.6 Hz, 1 H, CH_2Br), 3.84 (d, *J* =10.9 Hz, 1 H, CH_2Br), 3.54 (s, 3 H, $COOCH_3$), 3.27 (dd, *J* = 15.4, 2.8 Hz, 1 H, CH_2CH), 3.17 (dd, *J* = 15.2, 4.5 Hz, 1 H, CH_2CH), 1.97 (s, 3 H, CCH_3), 1.67 (s, 3 H, CCH_3), 0.98 (s, 9 H, $C(CH_3)_3$), 0.20 (s, 3 H, $Si(CH_3)_2$), 0.19 (s, 3 H, $Si(CH_3)_2$); **^{13}C NMR (100MHz, $CDCl_3$)** ppm 170.7 (*C*=O)**,** 166.4 (*C*=O), 153.8 (Ar – *C*), 136.6 (Ar – *C*), 131.8 (Ar – *C*), 126.3 (Ar – *C*), 119.4 (Ar – *C*), 119.1 (Ar – *C*), 61.0 ($C(CH_3)_2$), 57.4 (CH_2CH), 52.5 ($COOCH_3$), 32.0 (CH_2CH), 30.8 ($C(CH_3)_2$), 30.0 (CH_2Br), 25.6 ($C(CH_3)_3$), 24.1 ($C(CH_3)_2$), 18.2 ($C(CH_3)_3$), -4.4 ($Si(CH_3)_2$); **IR** 1740, 1657, 1260, 841, 782**; HRMS** (ESI, *m/z*) $C_{21}H_{32}BrNNaO_4Si$ ($M+Na^+$) *calc.* 492.1176, *found* 492.1190.

9-((*tert*-Butyldimethylsilyl)oxy)-6,6-dimethyl-2-propyl-2,3,11,11a-tetrahydro-1*H*-pyrazino[1,2-b]isoquinoline-1,4(6*H*)-dione



3-97 (470 mg, 1.0 mmol) was dissolved in a solution of DCM (20 mL) with 1-propylamine (100 μL, 1.2 mmol) and potassium carbonate (226 mg, 1.6 mmol). The reaction was refluxed for 3 hours, cooled down and washed with brine (20 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to give the crude product. The purified product was obtained by column chromatography on silica gel (33% EtOAc in Hexane, Rf = 0.3) as pale yellow oil (334 mg, 0.82 mmol, 82% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.12 (d, *J* = 8.6 Hz, 1 H, Ar – *H*), 6.75 (dd, *J* = 8.7, 2.7 Hz, 1 H, Ar – *H*), 6.61 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 4.04 (dd, *J* = 17.4, 1.5 Hz, 1 H, COC*H*$_2$), 4.02 (dd, *J* = 11.1, 1.8 Hz, 1 H, CH_2C*H*), 3.93 (dd, *J* = 16.9, 1.3 Hz, 1 H, COC*H*$_2$), 3.44 (dd, *J* = 15.4, 2.0 Hz, 1 H, C*H*$_2$CH), 3.40 - 3.52 (m, 1 H, NC*H*$_2$$CH_2CH_3$), 3.28 - 3.36 (m, 1 H, NC*H*$_2$$CH_2CH_3$), 2.94 (dd, *J* = 15.4, 11.9 Hz, 1 H, C*H*$_2$CH), 1.87 (s, 3 H, CC*H*$_3$), 1.86 (s, 3 H, CC*H*$_3$), 1.60 - 1.67 (m, 2 H, NCH_2C*H*$_2$$CH_3$), 0.99 (s, 9 H, C(C*H*$_3$)$_3$), 0.96 (t, *J* = 7.6 Hz, 3 H, NCH_2CH_2C*H*$_3$), 0.21 ppm (s, 6 H, Si(C*H*$_3$)$_2$); **^{13}C NMR (100MHz, $CDCl_3$)** ppm 164.5 (*C*=O), 162.3 (*C*=O), 153.6 (Ar – *C*), 135.9 (Ar – *C*), 134.0 (Ar – *C*), 126.8 (Ar – *C*), 119.3 (Ar – *C*), 118.8 (Ar – *C*), 62.5 (*C*$(CH_3)_2$), 55.9 (CH_2*C*H), 50.5 (C(O)*C*H_2), 47.4 (N*C*$H_2CH_2CH_3$), 35.9 (*C*H_2CH), 31.8 (C(*C*$H_3)_2$), 25.6 (C(*C*$H_3)_3$), 24.8 (C(*C*$H_3)_2$), 19.6 (NCH_2*C*H_2CH_3), 18.1 (*C*$(CH_3)_3$), 11.2 (NCH_2CH_2*C*H_3), -4.4 (Si(*C*$H_3)_2$); **IR** cm $^{-1}$1662, 1501, 1255, 841, 781**; HRMS** (ESI, *m/z*) $C_{23}H_{37}N_2O_3Si$ ($M+H^+$) *calc.* 417.2568 , *found* 417.2552.

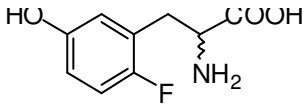
9-Hydroxy-6,6-dimethyl-2-propyl-2,3,11,11a-tetrahydro-1*H*-pyrazino[1,2-b]isoquinoline-1,4(6*H*)-dione (3-98)



Commercial HF•pyridine (70%, 0.8 mL) was dissolved in anhydrous THF (20 mL) with anhydrous pyridine (3.18 mL), to give a stock solution of pyridinium fluoride in the concentration of 1.2 M. To 9-((*tert*-Butyldimethylsilyl)oxy)-6,6-dimethyl-2-propyl-2,3,11,11a-tetrahydro-1*H*-pyrazino[1,2-b]isoquinoline-1,4(6*H*)-dione (315 mg, 0.76 mmol) in an solution of anhydrous THF (2.5 mL) was added the stock solution (2.5 mL, 3.0 mmol) and stirred for overnight at ambient temperature. The reaction was carefully quenched by the addition of methoxytrimethylsilane (0.62 mL, 3.8 mmol) and stirring for 10 min at ambient temperature. The solution was concentrated under reduced pressure to afford the crude product as a yellow solid. Purified product was obtained by column chromatography on silica gel (66% ethyl acetate in hexane, Rf = 0.3) to give pure product as a yellow solid (173 mg, 0.58 mmol, 76% yield). **^{1}H NMR (400MHz, $CDCl_3$)** ppm 7.15 (d, *J* = 8.6 Hz, 1 H, Ar – *H*), 6.80 (dd, *J* = 8.6, 2.5 Hz, 1 H, Ar – *H*), 6.76 (d, *J* = 2.5 Hz, 1 H, Ar – *H*), 6.73 (br. s., 1 H, O*H*), 4.07 (dd, *J* = 11.9, 2.0 Hz, 1 H, CH_2CH), 4.07 (dd, *J* = 17.4, 1.5 Hz, 1 H, $COCH_2$), 3.97 (dd, *J* = 17.4, 1.8 Hz, 1 H, $COCH_2$), 3.49 (dd, *J* = 15.6, 2.2 Hz, 1 H, CH_2CH), 3.42 - 3.56 (m, 1 H, $NCH_2CH_2CH_3$), 3.30 - 3.40 (m, 1 H, $NCH_2CH_2CH_3$), 2.99 (dd, *J* = 15.5, 11.7 Hz, 1 H, CH_2CH), 1.88 (s, 3 H, CH_3), 1.86 (s, 3 H, CH_3), 1.60 - 1.71 (m, 2 H, $NCH_2CH_2CH_3$), 0.97 (t, *J* = 7.5 Hz, 3 H, $NCH_2CH_2CH_3$); **^{13}C NMR (126MHz, $CDCl_3$)** ppm 164.9 (*C*=O), 162.1 (*C*=O), 154.5 (Ar – *C*), 134.8 (Ar – *C*), 133.9 (Ar – *C*), 127.1 (Ar – *C*), 115.2 (Ar – *C*), 114.3 (Ar – *C*), 62.7 ($CH_3)_2$), 56.0 (CH_2CH), 50.4 (CH_2), 47.7 ($NCH_2CH_2CH_3$), 36.2 (CH_2CH), 31.8 ($CH_3)_2$), 24.8 ($CH_3)_2$), 19.6 ($NCH_2CH_2CH_3$), 11.2 ($NCH_2CH_2CH_3$); **IR** cm^{-1} 3276, 1649, 1604, 1314, 863, 756; **HRMS** (ESI, *m/z*) $C_{17}H_{23}N_2O_3$

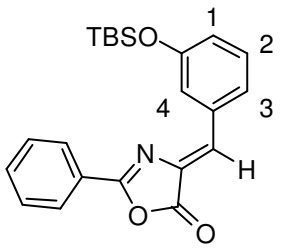
$(M+H^+)$ *calc.*303.1703, *found* 303.1696.

(±)6-F-*m*-tyrosine (Synthesised from 3-98)



To a solution of **3-98** (76 mg, 0.25 mmol) and HF•pyridine (70% wt, 26 μL, 1 mmol) in DCM (5 mL) was added phenyliodine di(trifluoroacetace) (PIFA, 107 mg, 0.25 mmol). The reaction was stirred at 25 ^{o}C for 30 min before being quenched by methoxytrimethylsilane (0.18 mL). The reaction was concentrated under reduced pressure. The residue was heated in a solution of HBr (~48% in water, 1.0 mL) and acetic acid (1.0 mL) at 100 ^{o}C for 3 hour before cooling down and neutralized by 20% aqueous solution of NaOH. The reaction mixture was diluted by 50% MeOH in water, dried azeotropically with acetonitrile and purified by HPLC on a semi-preparative C18 column. Pure product was obtained as a white solid (4 mg, 0.02 mmol) in 8% yield over two steps. Characterisation data are in agreement with the reference compound synthesised independently.

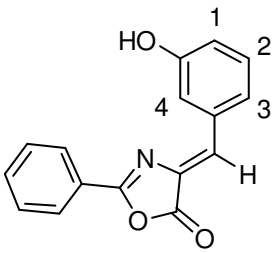
(4*Z*)-4-(3-{[*tert*-butyl(dimethyl)silyl]oxy}benzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one (3-100a)



To a solution of 3-((*tert*-butyldimethylsilyl)oxy)benzaldehyde (**3-69**, 2.36 g, 10 mmol) and hyppuric acid (2.15g, 12 mmol) in anhydrous THF (40 mL) was added sodium acetate (196 mg, 2.4 mmol, 24%, mol%) and acetic anhydride (2.4 mL, 24 mmol). The reaction was refluxed for 18 hours before being cooled down, diluted by ethyl acetate and washed by satd.

$NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as yellow oil. Purified product was obtained by column chromatography on silica gel (3% ethyl acetate in hexane, Rf = 0.5) to give pure product as yellow solid (2.94 g, 7.7 mmol, 77% yield). **^{1}H NMR (500MHz, $CO(CD_3)_2$)** ppm 8.18 - 8.20 (m, 2 H, Ph – *H*), 8.10 (dd, *J* = 2.2, 1.9 Hz, 1 H, *H*–4), 7.79 (br. d, *J* = 7.6 Hz, 1 H, *H*–3), 7.73 (tt, *J* = 6.9, 1.6 Hz, 1 H, Ph – *H*), 7.62 - 7.66 (m, 2 H, Ph – *H*), 7.41 (t, *J* = 7.9 Hz, 1 H, *H*–2), 7.26 (s, 1 H, C*H*=C), 7.04 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1 H, *H*–1), 1.05 (s, 9 H, CH_3 × 2 in TBS), 0.33 (s, 6 H, CH_3 × 2 in TBS); **^{13}C NMR (126MHz, $CO(CD_3)_2$)** ppm 167.7, 164.6, 156.9, 135.9, 134.5, 134.4, 131.5, 130.9, 130.1, 129.0, 127.1, 126.7, 124.2, 123.6, 26.1, 18.9, -4.2; **IR** 1794, 1655, 1598, 1558, 913, 841, 780, 702; **HRMS** (ESI, *m/z*) $C_{22}H_{25}NNaO_3Si$ ($M+Na^+$) *calc.* 402.1496, *found* 402.1482; **m.p. =** 109 – 11 1 °C

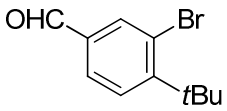
(4*Z*)-4-(3-hydroxybenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one (3-101a)



Commercial HF•pyridine (70%, 0.8 mL) was dissolved in anhydrous THF (20 mL) with anhydrous pyridine (3.18 mL), to give a stock solution of pyridinium fluoride in the concentration of 1.2 M. To **3-100a** (178 mg, 0.47 mmol) was added the stock solution (5 mL, 6 mmol) and stirred for overnight at ambient temperature. The reaction was carefully quenched by the addition of methoxytrimethylsilane (0.85 mL, 8 mmol) and stirring for 10 min at ambient temperature. The solution was concentrated under reduced pressure to afford the crude product as a yellow solid. Purified product was obtained by column chromatography on silica gel (5% ethyl acetate in hexane, Rf = 0.4) to give pure product as a yellow solid (112 mg, 0.42 mmol, 90% yield). **^{1}H NMR (500MHz, $CO(CD_3)_2$)** ppm 8.74 (s,

1 H, O*H*), 8.18 - 8.21 (m, 2 H, Ph – *H*), 8.04 (t, *J* = 1.9 Hz, 1 H, *H* – 4), 7.72 (tt, *J* = 7.6, 1.6 Hz, 1 H, Ph – *H*), 7.69 (br. d, *J* = 7.6 Hz, 1 H, *H* – 3), 7.64 (tt, *J* = 7.6, 1.3 Hz, 2 H, Ph – *H*), 7.36 (t, *J* = 7.9 Hz, 1 H, *H* – 2), 7.21 (s, 1 H, C*H*=C), 7.01 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1 H, *H* – 1); **^{13}C NMR (126MHz, CO(CD$_3$)$_2$)** ppm 167.8, 164.5, 158.6, 135.9, 134.5, 134.3, 132.0, 130.8, 130.1, 129.1, 126.7, 119.6, 119.3; **IR** 1739, 1654, 1595, 1557, 778, 701; **HRMS** (ESI, *m/z*) $C_{16}H_{11}NNaO_3$ ($M+Na^+$) *calc.* 288.0631, *found* 288.0624**; m.p. =** 174 – 176 °C

3-Bromo-4-(tert-butyl)benzaldehyde (3-103) [193]40

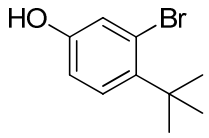


3-Bromo-4-*tert*-butylbenzoic acid (18.3 g, 71.2 mmol) was dissolved in anhydrous THF (100 mL) and cooled to 0°C. Borane-THF complex (1.0 M solution in THF, 142 mL, 142 mmol) was added over 1 hour. The reaction mixture was stirred at RT for 12 hours before carefully quenched with 2 M HCl (200 mL). The mixture was extracted with diethyl ether (2 x 200 mL). The combined organic layer was washed with 2 M HCl (100 mL), water (100 mL) and brine (100 mL), dried, filtered and concentrated to give product as a yellow oil (18.2 g, crude yield 105%). This product was used to the next step without further purification.

The crude 3-bromo-4(*tert*-butyl)benzaldehyde (maximum 95%, 20 g, 78.4 mmol) was dissolved in petroleum ether (200 mL). Activated manganese dioxide (43.5 g, 500 mmol) was added. The suspension was stirred at RT for 18 h. A 2nd portion of activated manganese dioxide (22 g, 250 mmol) was added. The suspension was stirred for 4 hours. After centrifugation, the supernatant was separated and filtered through a small silica pad (2 g), eluted with petroleum ether (80 mL). The clear filtrate was concentrated to give product as yellow oil (13.4 g, 55.6 mmol) which is NMR pure. Yield over 2 steps 71%. **^{1}H NMR**

(**200MHz**, **$CDCl_3$**) ppm 9.93 (s, 1 H, C*H*O), 8.08 (d, *J* = 1.8 Hz, 1 H, Ar – *H*), 7.75 (dd, *J* = 1.8, 8.2 Hz, 1 H, Ar – *H*), 7.61 (d, *J* = 8.2 Hz, 1 H, Ar – *H*), 1.55 (s, 9 H, CH_3 x 3). **^{13}C NMR** (**50MHz**, **$CDCl_3$**) δ = 190.5 (*C*=O), 154.7 (Ar – *C*), 136.8 (Ar – *C*), 135.5 (Ar – *C*), 128.6 (Ar – *C*), 128.1 (Ar – *C*), 123.2 (Ar – *C*), 37.3 (*C*$(CH_3)_3$), 29.4 (C(*C*$H_3)_3$); Other characterisation data are in agreement with the literature.[193]

3-Bromo-4-(*tert*-butyl)phenol (3-104)

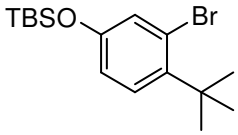


Trifluoroacetic anhydride (20.8 mL, 150 mmol) was slowly added to a suspension of 30% hydrogen peroxide (3.1 mL, 30 mmol) and dichloromethane (40 mL) at 0°C. The mixture was stirred at 0°C for 1 hour to give the peracid solution. A 1-L flask was charged with potassium phosphate monobasic (54.4 g, 400 mmol), 3-bromo-4-*tert*-butylbenzaldehyde (4.82 g, 20 mmol) and dichloromethane (200 mL) and cooled to 0°C. The above peracid solution was added over 30 minute with vigorous stirring. The resulting suspension was stirred for another 30 minutes before brine (200 mL) was added followed by sodium sulphite (2.5 g). The mixture was stirred for 5 minutes and diluted with ether (800 mL). The organic layer was washed with water (2 x 200 mL) and brine (200 mL), dried, filtered and concentrated. The residue was dissolved in methanol (100 mL) containing potassium carbonate (2 mg/mL) and stirred at RT for 10 minutes. The solvent was removed under reduced pressure. The residue was suspended in diethyl ether (200 mL), water (100 mL) and small amount of HCl (2 M, 2 mL). The organic layer was washed with water and brine, dried and concentrated. The crude product was purified by column chromatography (15-20% Et_2O in petroleum ether) to give pale yellow oil (4.49 g, yield 98%). **^{1}H NMR** (**400MHz** ,

[40] This compound is provided by Dr. M. Gao.

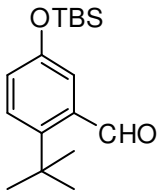
$CDCl_3$) ppm 7.31 (d, *J* = 8.6 Hz, 1 H, Ar – *H*), 7.13 (d, *J* = 2.8 Hz, 1 H, Ar – *H*), 6.73 (dd, *J* = 2.8, 8.8 Hz, 1 H, Ar – *H*), 1.49 (s, 9 H, C*H*$_3$ x 3). **^{13}C NMR** (**101MHz**, **$CDCl_3$**) δ = 153.6 (Ar – *C*), 140.1 (Ar – *C*), 128.6 (Ar – *C*), 122.6 (Ar – *C*), 122.4 (Ar – *C*), 113.9 (Ar – *C*), 36.0 (*C*$(CH_3)_3$), 30.0 (C(*C*$H_3)_3$).

(3-Bromo-4-(*tert*-butyl)phenoxy)(*tert*-butyl)dimethylsilane (3-104(*O*-TBS))



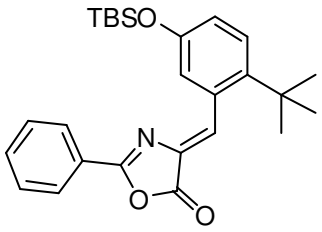
To a solution of 3-bromo-4-(*tert*-butyl)phenol (**3-104**, 2.29 g, 10.0 mmol), imidazole (1.63 g, 24.0 mmol) and *N*,*N*-dimethylaminopyridine (61 mg, 0.5 mmol) in DCM (50 mL) was added *tert*-butyldimethylsilyl chloride (3.16 g, 21.0 mmol) and the reaction mixture was stirred at ambient temperature for overnight. The reaction was quenched by satd. $NaHCO_3$ and extracted by DCM (50 mL × 2). The combined organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as light yellow oil. Pure product was obtained by column chromatography on silica gel (pure hexane, Rf = 0.7) as a colourless oil (2.63 g, 7.7 mmol, 77% yield). **^{1}H NMR (300MHz, $CDCl_3$)** ppm 7.06 (d, *J* = 8.6 Hz, 1 H, Ar – *H*), 6.90 (d, *J* = 2.6 Hz, 1 H, Ar – *H*), 6.50 (dd, *J* = 8.8, 2.6 Hz, 1 H, Ar – *H*), 1.27 (s, 9 H, C*H*$_3$ × 3 *t*Bu), 0.78 (s, 9 H, C*H*$_3$ × 3 TBS), 0.00 (s, 6 H, C*H*$_3$ × 2 TBS); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 153.9 (Ar – *C*), 140.4 (Ar – *C*), 128.1 (Ar – *C*), 127.1 (Ar – *C*), 122.4 (Ar – *C*), 118.3 (Ar – *C*), 36.0 (*C*$(CH_3)_3$ *t*Bu), 33.0 (*C*H_3 × 3 *t*Bu), 25.6 (*C*$(CH_3)_3$ TBS), 18.1 (*C*H_3 × 3 TBS), -4.4 (*C*H_3 × 2 TBS);

2-(*tert*-butyl)-5-((*tert*-butyldimethylsilyl)oxy)benzaldehyde (3-105)



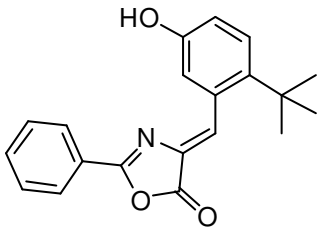
n-Butyllithium (2.5 M solution in hexane, 2.1 mL, 5.3 mmol) was added slowly to the solution of **3-104(*O*-TBS)** (1.51 g, 4.4 mmol) in anhydrous THF (50 mL) at -78 ^{o}C over 2 min. Anhydrous *N*,*N*-dimethylformamide (0.68 mL, 8.8 mmol) was added to the reaction after 30 min at -78 ^{o}C and kept for another 30 min before quenching by 1 M HCl (5 mL). The reaction was warmed up to ambient temperature, diluted with diethyl ether (100 mL) and washed by satd. NH_4Cl (50 mL × 2). The organic phase was dried in anhydrous $MgSO_4$, filtered and concentrated in reduced pressure to afford the crude product as light yellow oil. Purified product was obtained by column chromatography on silica gel (2% diethyl ether in hexane, R_f = 0.2) as colourless oil (924 mg, 3.2 mmol, 72% yield). **^{1}H NMR (300MHz, $CDCl_3$)** ppm 10.86 (s, 1 H, C*H*O), 7.45 (d, *J* = 2.7 Hz, 1 H, Ar – *H*), 7.38 (d, *J* = 8.8 Hz, 1 H, Ar – *H*), 7.00 (dd, *J* = 8.8, 2.7 Hz, 1 H, Ar – *H*), 1.54 (s, 9 H, C*H*$_3$ × 3 *t*Bu), 1.04 (s, 9 H, C*H*$_3$ × 3 TBS), 0.27 (s, 6 H, C*H*$_3$ × 2 TBS); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 192.3 (*C*HO), 153.9 (Ar – *C*), 145.1 (Ar – *C*), 136.4 (Ar – *C*), 127.7 (Ar – *C*), 124.7 (Ar – *C*), 120.6 (Ar – *C*), 35.1 (*C*$(CH_3)_3$ *t*Bu), 33.3 (*C*H_3 × 3 *t*Bu), 25.6 (*C*$(CH_3)_3$ TBS), 18.1 (*C*H_3 × 3 TBS), -4.5 (*C*H_3 × 2 TBS); **HRMS** (ESI, *m/z*) $C_{17}H_{28}NaO_2Si$ ($M+Na^+$) *calc.* 315.1751, *found* 315.1744;

(Z)-4-(2-(*tert*-butyl)-5-((*tert*-butyldimethylsilyl)oxy)benzylidene)-2-phenyloxazol-5(4*H*)-one (3-100b)



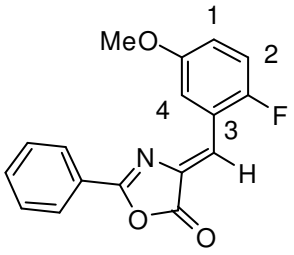
To a solution of **3-105** (802 mg, 2.8 mmol) and hippuric acid (590 mg, 3.3 mmol) in anhydrous THF (20 mL) was added sodium acetate (54 mg, 0.7 mmol, 24%, mol%) and acetic anhydride (0.66 mL, 6.6 mmol). The reaction was refluxed for 48 hours. The reaction mixture was cooled down, diluted by ethyl acetate and washed by satd. $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and concentrated in reduced pressure to afford the crude product as yellow oil. The purified product was obtained by column chromatography on silica gel (2% diethyl ether in hexane, Rf = 0.2) to give pure product as yellow oil (448 mg, 1.0 mmol, 31% yield). **^{1}H NMR (250MHz, $CDCl_3$)** ppm 8.19 - 8.26 (m, 2 H, Ar – *H*), 8.16 (d, *J* = 3.0 Hz, 1 H, Ar – *H*), 8.06 (s, 1 H, C=C*H*), 7.66 (d, *J* = 7.0 Hz, 1 H, Ar – *H*), 7.54 - 7.62 (m, 2 H, Ar – *H*), 7.40 (d, *J* = 8.8 Hz, 1 H, Ar – *H*), 6.93 (dd, *J* = 8.8, 2.7 Hz, 1 H, Ar – *H*), 1.55 (s, 9 H, C*H*$_3$ × 3 in *t*Bu), 1.10 (s, 9 H, C*H*$_3$ × 3 in TBS), 0.38 (s, 6 H, C*H*$_3$ × 2 in TBS); **^{13}C NMR (63MHz, $CDCl_3$)** ppm 167.9, 163.8, 153.4, 144.3, 133.2, 132.2, 132.1, 132.0, 128.8, 128.2, 127.4, 125.7, 124.7, 122.4, 35.3 (*C*$(CH_3)_3$ in *t*Bu), 32.4 (*C*H$_3$ × 3 in *t*Bu), 25.7 (*C*$(CH_3)_3$ in TBS), 18.2 (*C*H$_3$ × 3 in TBS), -4.3 (*C*H$_3$ × 2 in TBS); **HRMS** (FI, *m/z*) $C_{26}H_{33}NO_3Si$ (M^+) *calc.* 435.2226, *found* 435.2226; **IR** cm^{-1} 2956, 1800, 1650, 866.

(Z)-4-(2-(*tert*-butyl)-5-hydroxybenzylidene)-2-phenyloxazol-5(4*H*)-one (3-101b)



Commercial HF•pyridine (70%, 0.8 mL) was dissolved in anhydrous THF (20 mL) with anhydrous pyridine (3.18 mL), to give a stock solution of pyridinium fluoride in the concentration of 1.2 M. To **3-100b** (338 mg, 0.78 mmol) was added the stock solution (3.25 mL, 3.9 mmol) and stirred for overnight at ambient temperature. The reaction was carefully quenched by the addition of methoxytrimethylsilane (0.65 mL, 4.7 mmol) and stirring for 10 min at ambient temperature. The solution was concentrated under reduced pressure to afford the crude product as yellow oil. Purified product was obtained by column chromatography on silica gel (40% diethyl ether in hexane, Rf = 0.4) to give pure product as a yellow solid (126 mg, 0.39 mmol, 50% yield). **^{1}H NMR (300MHz, $CDCl_3$)** ppm 8.07 - 8.12 (m, 2 H), 7.94 (d, *J* = 2.9 Hz, 1 H), 7.91 (s, 1 H), 7.50 - 7.58 (m, 1 H), 7.41 - 7.48 (m, 2 H), 7.29 (d, *J* = 8.6 Hz, 1 H), 7.19 (s, 1 H), 6.79 (dd, *J* = 8.7, 2.9 Hz, 1 H), 1.40 ppm (s, 9 H, C$\underline{H}_3$ × 3); **^{13}C NMR (75MHz, $CDCl_3$)** ppm 167.8, 164.1, 153.3, 143.9, 133.4, 132.6, 132.3, 131.8, 128.9, 128.4, 127.7, 125.5, 120.2, 117.5, 35.4, 32.4; **HRMS** (FI, *m/z*) $C_{20}H_{19}NO_3$ (M^+) *calc.* 321.1371, *found* 321.1365; **IR** cm^{-1} 2924, 2854, 1796, 1650**; m.p. =** 129 – 131 ^{o}C**.**

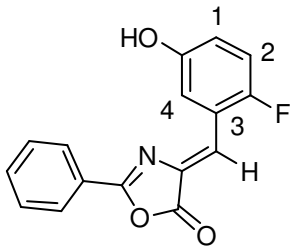
(4*Z*)-4-(2-fluoro-5-methoxybenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one (3-107)



To a solution of 2-fluoro-5-methoxylbenzaldehyde (**3-75**, 1.54 g, 10 mmol) and hyppuric acid (2.15g, 12 mmol) in anhydrous THF (40 mL) was added sodium acetate (196 mg, 2.4

mmol, 24%, mol%) and acetic anhydride (2.4 mL, 24 mmol). The reaction was refluxed for overnight until thin layer chromatography showed that the reaction was finished. The reaction mixture was cooled down, diluted by ethyl acetate and washed by satd. $NaHCO_3$. The organic phase was dried in anhydrous $MgSO_4$, filtered and concentrated in reduced pressure to afford the crude product as yellow solid. Purified product was obtained by recrystallization of the crude product in ethyl acetate to give pure product as yellow solid (2.78 g, 9.1 mmol, 91% yield). **^{1}H NMR (500MHz, CO(CD$_3$)$_2$)** ppm 8.52 (dd, *J* = 6.0, 3.2 Hz, 1 H, *H*–4), 8.21 (dd, *J* = 8.5, 1.3 Hz, 2 H, Ph – *H*), 7.76 (tt, *J* = 7.6, 1.3 Hz, 1 H, Ph – *H*), 7.66 (dd, *J* = 8.5, 7.6 Hz, 2 H, Ph – *H*), 7.36 (s, 1 H, C=C*H*), 7.22 (dd, *J* = 9.8, 9.1 Hz, 1 H, *H*–2), 7.13 (ddd, *J* = 9.1, 4.3, 3.3 Hz, 1 H, *H*–1), 3.94 (s, 3 H, OC*H*$_3$); **^{13}C NMR (126MHz, CO(CD$_3$)$_2$)** ppm 167.3 (*C*=O), 165.5 (*C*=N), 157.3 (d, *J* = 247 Hz, *C*–F), 157.0 (d, *J* = 1.9 Hz, *C*OCH$_3$), 135.6 (*C*=CH), 134.8 (Ar – *C*), 130.2 (Ar – *C*), 129.2 (Ar – *C*), 126.3 (Ar – *C*), 122.7 (d, *J* = 12.4 Hz, *C*–3), 121.2 (d, *J* = 7.6 Hz, C=*C*H), 120.3 (d, *J* = 7.6 Hz, *C*–1), 117.1 (d, *J* = 23.8 Hz, *C*–2), 116.4 (*C*–4), 56.2 (O*C*H$_3$); **^{19}F{^{1}H}NMR (376 MHz, CO(CD$_3$)$_2$)** ppm -127.1; **HRMS** (ESI, *m/z*) $C_{17}H_{12}FNNaO_3$ ($M+Na^+$) *calc.* 320.0693, *found* 320.0683**; IR** 1796, 1653, 1597, 1556, 880, 811, 692, 681; **m.p. =** 155 – 157 °C.

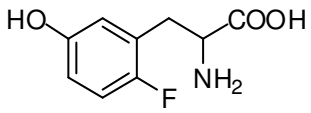
(4*Z*)-4-(2-fluoro-5-hydroxybenzylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one (3-101c)



To a solution of **107** (835 mg, 2.8 mmol) in anhydrous DCM (40 mL) was added tribromoborane (0.35 mL, 3.4 mmol) at –78 °C. The reaction mixture was warmed up to 0 °C after one hour then warmed up to room temperature for overnight. The reaction was quenched by careful addition of water at 0 °C and extracted by DCM (50 mL × 2). The

organic phase was dried in anhydrous $MgSO_4$, filtered and concentrated in reduced pressure to afford the crude product as a orange solid. Purified product was obtained by column chromatography on silica gel (20% ethyl acetate in hexane, Rf = 0.3) to give pure product as yellow solid (293 mg, 1.0 mmol, 37% yield). **^{1}H NMR (500MHz, $CO(CD_3)_2$)** ppm 8.75 (s, 1 H, O*H*), 8.50 (dd, *J* = 6.0, 3.2 Hz, 1 H, *H*–4), 8.19 - 8.23 (m, 2 H, Ph – *H*), 7.74 (tt, *J* = 7.6, 1.3 Hz, 1 H, Ph – *H*), 7.63 - 7.67 (m, 2 H, Ph – *H*), 7.35 (s, 1 H, C=C*H*), 7.12 (dd, *J* = 9.9, 9.0 Hz, 1 H, *H*–2), 7.01 (ddd, *J* = 9.1, 4.4, 3.2 Hz, 1 H, *H*–1); **^{13}C NMR (126MHz, $CO(CD_3)_2$)** ppm 167.5 (*C*=O), 165.3 (*C*=N), 156.8 (d, *J* = 245.1 Hz, *C*–F), 154.8 (d, *J* = 1.9 Hz, *C* – OH), 135.5 (*C*=CH), 134.8 (Ph – *C*), 130.2 (Ph – *C*), 129.2 (Ph – *C*), 126.5 (Ph – *C*), 122.8 (d, *J* = 12.4 Hz, *C*–3), 121.5 (d, *J* = 7.6 Hz, C=*C*H), 121.2 (d, *J* = 8.6 Hz, *C*–1), 118.4 (*C*–4), 117.1 (d, *J* = 23.8 Hz, *C*–2); **^{19}F{^{1}H}NMR (376 MHz, $CO(CD_3)_2$)** ppm -128.6; **HRMS** (ESI, *m/z*) $C_{16}H_{10}FNNaO_3$ ($M+Na^+$) *calc.* 306.0537, *found* 306.0527; **IR** 3398, 1793, 1655, 1599, 1560, 818, 801, 741**; m.p. =** 126 – 128 °C .

6-F-*m*-tyrosine (Synthesis from 3-101b)



To a solution of **3-101b**, 64.3 mg, 0.2 mmol) and HF•pyridine (70% wt, 20.8 μL, 0.8 mmol) in DCM (4 mL) was added phenyliodine di(trifluoroacetace) (PIFA, 86 mg, 0.2 mmol). The reaction was stirred at 25 °C for 30 min. Trifluoroacetic acid (225 μL, 5%) was added and the reaction was stirred for another 30 min before being quenched by methoxytrimethylsilane (0.1 mL). The reaction was concentrated under reduced pressure. Isolated intermediate of oxidative fluorination was obtained by column chromatography on silica gel (50% diethyl ether, Rf = 0.5) as pale yellow oil (16 mg, 0.06 mmol, 28% yield). Characterization data are in agreement with the reference compound synthesised independently (**3-101c**). The isolated compound was heated with red phosphorus (100 mg)

in a solution of HI (~66%, 1 mL) and acetic acid (1.5 mL) at 120 ^{o}C for 1 hour before cooling down and neutralized by 20% aqueous solution of NaOH. The reaction mixture was diluted by water, dried azeotropically with acetonitrile and purified by HPLC on a semi-preparative C18 column. Pure product was obtained as a white solid (11 mg, 0.06 mmol) in 28% yield over two steps. Characterisation data are in agreement with the reference compound synthesised independently.

References

[1] A. M. J. Paans, A. van Waarde, P. H. Elsinga, A. T. M. Willemsen, W. Vaalburg, *Methods* **2002**, *27*, 195–207.

[2] P. H. Elsinga, *Methods* **2002**, *27*, 208–217.

[3] P. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew. Chem. Int. Ed.* **2008**, *47*, 8998–9033.

[4] M. E. Phelps, E. I. Hoffman, S. C. Huang, M. M. Ter-Pogossian, *J. Nucl. Med.* **1975**, *16*, 649–652.

[5] R. Van Grieken, M. De Bruin, *Pure & Appl. Chem.* **1994**, *66*, 2513–2526.

[6] M. Bergström, A. Grahnén, B. Långström, *Eur. J. Clin. Pharmacol* **2003**, *59*, 357–366.

[7] J. Bergman, O. Solin, *Nucl. Med. Biol.* **1997**, *24*, 677–683.

[8] P. Atkins, J. de Paula, *ATKINS' Physical Chemistry*, 8th ed., Oxford University Press, Oxford, UK, **2006**.

[9] G. Antoni, T. Kihlberg, B. Långström, *Aspects on the Synthesis of ^{11}C-labelled Compounds*, in *Handbook of Radiopharmaceuticals* (Eds.: M. J. Welch, C. S. Redvanly), John Wiley & Sons Ltd., Chichester, UK, **2005**.

[10] S. Purser, P. R. Moore, S. Swallow, V. Gouverneur, *Chem. Soc. Rev.* **2008**, *37*, 320–330.

[11] D. S. Wilbur, *Bioconjugate Chem.* **1992**, *3*, 433–470.

[12] S. M. Okarvi, *Eur. J. Nucl. Med.* **2001**, *28*, 929–938.

[13] B. Tavitian, *Gut* **2001**, *52*, iv40–iv47.

[14] B. Tavitian, S. Terrazzino, B. Kuhnast, S. Marzabal, O. Stettler, F. Dollé, J. R. Deverre, A. Jobert, F. Hinnen, B. Bendriem, C. Crouzel, L. DiGiamberardino, *Nat. Med.* **1998**, *4*, 467–471.

[15] E. K. J. Pauwels, M. J. Ribeiro, J. H. M. B. Stoot, V. R. McCready, M. Bourguignon, B. Maziére, *Nucl. Med. Biol.* **1998**, *25*, 317–322.

[16] G. Lucignani, K. C. Schmidt, R. M. Moresco, G. Striano, F. Colombo, L. Sokoloff, F. Fazio, *J. Nucl. Med.* **1993**, *34*, 360–369.

[17] P. Kumar, D. Stypinski, H. Xia, A. J. B. McEwan, H.-J. Machulla, L. I. Wiebe, *J. Label. Compd. Radiopharm* **1999**, *42*, 3–16.

[18] G. Reischla, W. Ehrlichmanna, C. Biega, C. Solbacha, P. Kumarb, L. I. Wiebeb, H.-J. Machullaa, *Appl. Radiat. Isot.* **2005**, *62*, 897–901.

[19] W. R. Dolbier, A.-R. Li, C. J. Koch, C.-Y. Shiue, A. V. Kachur, *Appl. Radiat. Isot.* **2001**, *54*, 73–80.

[20] A. V. Kachur, W. R. DolbierJr., W. Xu, C. J. Koch, *Appl. Radiat. Isot.* **2010**, *68*, 293–296.

[21] D. R. Hwang, C. S. Dence, T. A. Bonasera, M. J. Welch, *Inter.J. Radiat. Appl. Instru. (A) Appl. Radiat. Isot.* **1989**, *40*, 117–126.

[22] G. Tang, M. Wang, X. Tang, M. Gan, L. Luo, *Nucl. Med. Biol.* **2005**, *32*, 553–558.

[23] A. F. Shields, J. R. Grierson, B. M. Dohmen, H. J. Machulla, J. C. Stayanoff, J. M. Lawhorn-Crews, J. E. Obradovich, O. Muzik, T. J. Manger, *Nat. Med.* **1998**, *4*, 1334–1336.
[24] S. J. Martina, J. A. Eisenbartha, U. Wagner-Utermanna, W. Mierb, M. Henzeb, H. Pritzkowc, U. Haberkornb, M. Eisenhuta, *Nucl. Med. Biol.* **2002**, *29*, 263–273.
[25] K. Hamacher, H. H. Coenen, *Appl. Radiat. Isot.* **2002**, *57*, 853–856.
[26] G. Firnau, E. S. Garnett, R. Chirakal, S. Sood, C. Nahmias, G. Schrobilgen, *Inter. J. Radiat. Appl. Instru. (A) Appli. Radiat. Isot.* **1986**, *37*, 669–675.
[27] R. Iwata, C. Pascalic, A. Bognic, S. Furumotob, K. Terasakid, K. Yanaie, *Appl. Radiat. Isot.* **2002**, *57*, 347–352.
[28] L. Cai, S. Lu, V. W. Pike, *Eur. J. Org. Chem.* **2008**, *2008*, 2853–2873.
[29] J. S. Fowler, A. P. Wolf, *Acc. Chem. Res.* **1997**, *30*, 181–188.
[30] S. D. Nelson, W. F. Trager, *Drug Metab. Dispos.* **2003**, *31*, 1481–1497.
[31] B. K. Park, N. R. Kitteringham, P. M. O'Neill, *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443–470.
[32] S. Bruce E, *J. Fluorine Chem.* **2001**, *109*, 3–11.
[33] S. Barnard, R. Storr, P. O'Neill, B. Park, *J. Pharm. Pharmacol.* **1993**, *45*, 736–744.
[34] J. A. Olsen, D. W. Banner, P. Seiler, B. Wagner, T. Tschopp, U. Obst-Sander, M. Kansy, K. Mueller, F. Diederich, *ChemBioChem* **2004**, *5*, 666–675.
[35] V. W. Pike, F. I. Aigbirhio, *J. Chem. Soc., Chem. Commun.* **1995**, *21*, 2215–2216.
[36] V. V. Zhdankin, P. J. Stang, *Chem. Rev.* **2008**, *108*, 5299–5358.
[37] J.-H. Chun, S. Lu, Y.-S. Lee, V. W. Pike, *J. Org. Chem* **2010**, *75*, 3332–3338.
[38] A. McKillop, D. Kemp, *Tetrahedron* **1989**, *45*, 3299–3306.
[39] P. Kazmierczak, L. Skulski, L. Kraszkiewicz, *Molecules* **2001**, *6*, 881–891.
[40] P. Kazmierczak, L. Skulski, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 219–224.
[41] J. Ermert, C. Hocke, T. Ludwig, R. Gail, H. H. Coenen, *J. Label. Compd. Radiopharm.* **2004**, *47*, 429–441.
[42] T. L. Ross, J. Ermert, C. Hocke, H. H. Coenen, *J. Am. Chem. Soc.* **2007**, *129*, 8018–8025.
[43] N. Satyamurthy, J. R. Barrio, *Vol. WO 2010/008522 A2*, C07B 59/00 ed., USA, **2010**.
[44] A. Knoechel, O. Zwernemann, *Appl. Radiat. Isot.* **1991**, *42*, 1077–1080.
[45] T. Pages, B. R. Langlois, *J. Fluorine Chem.* **2001**, *107*, 321–327.
[46] T. Pages, B. R. Langlois, D. L. Bars, P. Landais, *J. Fluorine Chem.* **2001**, *107*, 329–335.
[47] H. H. Coenen, *No-carrier-added ^{18}F-chemistry of Radiopharmaceuticals*, in *Synthesis and Application of Isotopically Labelled Compounds* (Eds.: T. A. Baille, J. R. Jones), Elsevier Publ., Amsterdam **1989**, pp. 433–448.
[48] C. Lemaire, P. Damhaut, A. Plenevaux, R. Cantineau, L. Christiaens, M. Guillaume, *Appl. Radiat. Isot.* **1992**, *43*, 485–494.
[49] F. Wüst, *Fluorine-18 Labeling of Small Molecules: The Use of ^{18}F-Labeled Aryl Fluorides Derived from No-Carrier-Added [^{18}F]Fluoride as Labeling Precursors*, in *PET Chemistry: The Driving Force in Molecular Imaging* (Eds.: P. A. Schubiger, L. Lehmann, M. Friebee), Springer, New York, **2006**, pp. 51–78.

[50] G. Vaidyanathan, M. R. Zalutsky, *Nat. Protoc.* **2006**, *1*, 1655–1661.
[51] O. Langer, F. Dollé, H. Valette, C. Halldin, F. Vaufrey, C. Fuseau, C. Coulon, M. Ottaviani, K. Någren, M. Bottlaender, B. Mazière, C. Crouzel, *Bioorg. Med. Chem.* **2001**, *9*, 677–694.
[52] C. Lemaire, M. Guillaume, L. Christiaens, A. J. Palmer, R. Cantineau, *Appl. Radiat. Isot.* **1987**, *38*, 1033–1038.
[53] E. W. Baxter, A. B. Reitz, *Reductive Aminations of Carbonyl Compounds with Borohydride and Borane Reducing Agents*, in *Organic Reactions, Vol. 59* (Ed.: L. E. Overman), John Wiley & Sons, Inc., Hoboken, New Jersey, **2004**.
[54] A. A. Wilson, R. F. Dannals, H. T. Ravert, H. N. Wagner, *J. Label. Compd. Radiopharm.* **1990**, *28*, 1189–1199.
[55] E. Kyoung Ryu, Y. S. Choe, E. Y. Park, J.-Y. Paik, Y. R. Kim, K.-H. Lee, Y. Choi, S. E. Kim, B.-T. Kim, *Nucl. Med. Biol.* **2005**, *32*, 185–191.
[56] S.-Y. Lee, Y. S. Choe, Y. R. Kim, J.-Y. Paik, B. W. Choi, S. E. Kim, K.-H. Lee, Y. Choi, B.-T. Kim, *Nucl. Med. Commun.* **2004**, *25*, 591–596.
[57] P. Mäding, F. Füchtner, C. S. Hilger, M. Halks-Miller, R. Horuk, *J. Label. Compd. Radiopharm.* **2004**, *47*, 1053.
[58] M. Liang, C. Mallari, M. Rosser, H. P. Ng, K. May, S. Monahan, J. G. Bauman, I. Islam, A. Ghannam, B. Buckman, K. Shaw, G.-P. Wei, W. Xu, Z. Zhao, E. Ho, J. Shen, H. Oanh, B. Subramanyam, R. Vergona, D. Taub, L. Dunning, S. Harvey, R. M. Snider, J. Hesselgesser, M. M. Morrissey, H. D. Perez, R. Horuk, *J. Biol. Chem.* **2000**, *275*, 19000–19008.
[59] S. Gester, F. R. Wüst, B. Pawelke, R. Bergmann, J. Pietzsch, *Amino Acids* **2005**, *29*, 415–428.
[60] S. Gester, J. Pietzsch, F. R. Wüst, *J. Label. Compd. Radiopharm.* **2007**, *50*, 105–113.
[61] T. Poethko, M. Schottelius, G. Thumshirn, U. Hersel, M. Herz, G. Henriksen, H. Kessler, M. Schwaiger, H.-J. Wester, *J. Nucl. Med.* **2004**, *45*, 892–902.
[62] Y. S. Chang, J. M. Jeong, Y.-S. Lee, H. W. Kim, G. B. Rai, S. J. Lee, D. S. Lee, J.-K. Chung, M. C. Lee, *Bioconjugate Chem.* **2005**, *16*, 1329–1333.
[63] Y. H. Lim, *D.phil Thesis*, University of Oxford, UK, **2010**.
[64] L. Yin, J. Liebscher, *Chem. Rev.* **2007**, *107*, 133–173.
[65] B. Carsten, F. He, H. J. Son, T. Xu, L. Yu, *Chem. Rev.* **2011**, *111*, 1493–1528.
[66] S. L. Buchwald, C. Mauger, G. Mignani, U. Scholz, *Adv. Synth. Catal.* **2006**, *348*, 23–39.
[67] J. Magano, J. R. Dunetz, *Chem. Rev.*, *111*, 2177–2250.
[68] F. R. Wüst, T. Kniess, *J. Label. Compd. Radiopharm* **2004**, *47*, 457–468.
[69] F. R. Wüst, T. Kniess, *J. Label. Compd. Radiopharm.* **2003**, *46*, 699–713.
[70] F. R. Wüst, A. Hohne, P. Metz, *Org. Biomol. Chem.* **2005**, *3*, 503–507.
[71] B. Steiniger, F. R. Wüst, *J. Label. Compd. Radiopharm.* **2006**, *49*, 817–827.
[72] E. Marriere, L. Chazalviel, M. Dhilly, J. Toutain, C. Perrio, F. Dauphin, M.-C. Lasne, *J. Label. Compd. Radiopharm.* **1999**, *42*, S69–71.
[73] H. Teare, E. G. Robins, E. Årstad, S. K. Luthra, V. Gouverneur, *Chem. Commun.* **2007**, *23*, 2330–2332.

[74] H. Teare, E. G. Robins, A. Kirjavainen, S. Forsback, G. Sandford, O. Solin, S. K. Luthra, V. Gouverneur, *Angew. Chem. Int. Ed.* **2010**, 6821–6824.
[75] J. Brown, V. Gouverneur, *Angew. Chem. Int. Ed.* **2009**, *48*, 8610–8614.
[76] D. A. Watson, M. Su, G. Teverovskiy, Y. Zhang, J. Garcia-Fortanet, T. Kinzel, S. L. Buchwald, *Science* **2009**, *325*, 1661–1664.
[77] J. Zhu, H.Bienayme, *Multicomponent Reactions*, Wiley–VCH, Weiheim, **2005**.
[78] A. Dömling, *Chem. Rev.* **2006**, *106*, 17–89.
[79] A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, *39*, 3168–3210.
[80] E. Ruijter, R. Scheffelaar, Romano V. A. Orru, *Angew. Chem. Int. Ed.* **2011**, *50*, 6234–6246.
[81] C. O. Kappe, *The Biginelli Reaction*, in *Multicomponent Reactions* (Eds.: J. Zhu, H.Bienayme), Wiley–VCH, Weiheim, **2005**.
[82] C. O. Kappe, *J. Org. Chem* **1997**, *62*, 7201–7204.
[83] L. Kürti, B. Czakó, *Strategic Applications of Named Reactions in Organic Synthesis*, Elsevier Inc., Burlington, MA, USA, **2005**.
[84] E. H. Hu, D. R. Sidler, U.-H. Dolling, *J. Org. Chem.* **1998**, *63*, 3454–3457.
[85] C. O. Kappe, *Acc. Chem. Res.* **2000**, *33*, 879–888.
[86] C. O. Kappe, A. Stadler, *Methods Enzymol.* **2003**, *369*, 197–223.
[87] M. Kolosov, V. Orlov, D. Beloborodov, V. Dotsenko, *Mol. Diversity* **2009**, *13*, 5–25.
[88] G. J. Grover, S. Dzwonczyk, D. M. McMullen, C. S. Normadinam, P. G. Sleph, S. Moreland, *J. Cardiovasc. Pharmacol.* **1995**, *26*, 289–294.
[89] T. G. Murali Dhar, D. Nagarathnam, M. R. Marzabadi, B. Lagu, W. C. Wong, G. Chiu, S. Tyagarajan, S. W. Miao, F. Zhang, W. Sun, D. Tian, Q. Shen, J. Zhang, J. M. Wetzel, C. Forray, R. S. L. Chang, T. P. Broten, T. W. Schorn, T. B. Chen, S. O'Malley, R. Ransom, K. Schneck, R. Bendesky, C. M. Harrell, K. P. Vyas, K. Zhang, J. Gilbert, D. J. Pettibone, M. A. Patane, M. G. Bock, R. M. Freidinger, C. Gluchowski, *J. Med. Chem.* **1999**, *42*, 4778–4793.
[90] R. S. L. Chang, T.-B. Chen, S. S. O'Malley, D. J. Pettibone, J. DiSalvo, B. Francis, M. G. Bock, R. Freidinger, D. Nagarathnam, S. W. Miao, Q. Shen, B. Lagu, T. G. Murali Dhar, S. Tyagarajan, M. R. Marzabadi, W. C. Wong, C. Gluchowski, C. Forray, *Eur. J. Pharmacol.* **2000**, *409*, 301–312.
[91] T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. L. Schreiber, T. J. Mitchison, *Science* **1999**, *286*, 971–974.
[92] S.W. Fewell, C. M. Smith, M. A. Lyon, T. P. Dumitrescu, P. Wipf, B. W. Day, J. L. Brodsky, *J. Biol. Chem.* **2004**, *279*, 51131–51140.
[93] A. Rodina, M. Vilenchik, K. Moulick, J. Aguirre, J. Kim, A. Chiang, J. Litz, C. C. Clement, Y. Kang, Y. She, N. Wu, S. Felts, P. Wipf, J. Massague, X. Jiang, J. L. Brodsky, G. W. Krystal, G. Chiosis, *Nat. Chem. Biol.* **2007**, *3*, 498–507.
[94] S. Wisen, J. Androsavich, C. G. Evans, L. Chang, J. E. Gestwicki, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 60–65.
[95] X. H. Chen, X. Y. Xu, H. Liu, L. F. Cun, L. Z. Gong, *J. Am. Chem. Soc.* **2006**, *128*, 14802–14803.
[96] N. Li, X.-H. Chen, J. Song, S.-W. Luo, W. Fan, L.-Z. Gong, *J. Am. Chem. Soc.* **2009**, *131*, 15301–15310.

[97] Y. Wang, H. Yang, J. Yu, Z. Miao, R. Chen, *Adv. Synth. Catal.* **2009**, *351*, 3057–3062.
[98] J. Xin, L. Chang, Z. Hou, D. Shang, X. Liu, X. Feng, *Chem. Eur. J.* **2008**, *14*, 3177–3181.
[99] Y. Huang, F. Yang, C. Zhu., *J. Am. Chem. Soc.* **2005**, *127*, 16386–16387.
[100] J. Lu, H. Ma, *Synlett* **2000**, 63–64.
[101] Y. Ma, C. Qian, L. Wang, M. Yang, *J. Org. Chem.* **2000**, *65*, 3864–3868.
[102] D. Dallinger, C. O. Kappe, *Nat. Protoc.* **2007**, *2*, 317–321.
[103] D. Dallinger, C. O. Kappe, *Nat. Protoc.* **2007**, *2*, 1713–1721.
[104] S. Stone-Elander, N. Elander, *J. Label. Compd. Radiopharm.* **2002**, *45*, 715–746.
[105] C.-C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y.-S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. C. Kolb, O. N. Witte, N. Satyamurthy, J. R. Heath, M. E. Phelps, S. R. Quake, H.-R. Tseng, *Science* **2005**, *310*, 1793–1796.
[106] R. Bejot, A. M. Elizarov, E. Ball, J. Zhang, R. Miraghaie, H. C. Kolb, V. Gouverneur, *J. Label. Compd. Radiopharm.* **2011**, *54*, 117–122.
[107] T. L. Ross, H. J. Wester, *Radiochemistry and Radiopharmaceutical Chemistry in Life Sciences*, in *Handbook of Nuclear Chemistry*, 2nd ed. (Eds.: A. Vértes, S. Nagy, Z. Klencsár, R. G. Lovas, F. Rösch), Springer Science+Business Media B.V., Dordrecht, **2011**, p. 2021.
[108] D. O'Hagan, *Chem. Soc. Rev.* **2008**, *37*, 308–319.
[109] B. Das, K. Venkateswarlu, A. Majhi, V. Siddaiah, K. R. Reddy, *J. Mol. Catal. A: Chem.* **2007**, *267*, 30–33.
[110] W. P. Weber, G. W. Gokel, I. K. Ugi, *Angew. Chem. Int. Ed.* **1972**, *11*, 530–531.
[111] R. Obrecht, R. Herrmann, I. Ugi, *Synthesis* **1985**, *1985*, 400–402.
[112] J. Songstad, L. J. Stangeland, T. Austad, *Acta. Chem. Scand.* **1970**, *24*, 355–356.
[113] L. B. Engemyr, A. Martinsen, J. Songstad, *Acta. Chem. Scand. Ser. A.* **1974**, *28*, 255–266.
[114] L. El Kaim, L. Grimaud, A. Schiltz, *Synlett* **2009**, *2009*, 1401–1404.
[115] L. El Kaim, L. Grimaud, A. Schiltz, *Org. Biomol. Chem.* **2009**, *7*, 3024–3026.
[116] I. Akritopoulou-Zanze, *Curr. Opin. Chem. Biol.* **2008**, *12*, 324–331.
[117] A. Dömling, *Curr. Opin. Chem. Biol.* **2000**, *4*, 318–323.
[118] M. Passerini, *Gazz. Chim. Ital.* **1921**, *51*, 181–189.
[119] R. H. Baker, D. Stanonis, *J. Am. Chem. Soc.* **1951**, *73*, 699–702.
[120] I. Ugi, R. Meyr, *Chem. Ber.* **1961**, *94*, 2229–2233.
[121] S. Maeda, S. Komagawa, M. Uchiyama, K. Morokuma, *Angew. Chem. Int. Ed.* **2011**, *50*, 644–649.
[122] S. Maeda, K. Morokuma, *J. Chem. Phys.* **2010**, *132*, 241102–241105.
[123] P. R. Andreana, C. C. Liu, S. L. Schreiber, *Org. Lett.* **2004**, *6*, 4231–4233.
[124] S.-X. Wang, M.-X. Wang, D.-X. Wang, J. Zhu, *Angew. Chem. Int. Ed.* **2008**, *47*, 388–391.
[125] S.-X. Wang, M.-X. Wang, D.-X. Wang, J. Zhu, *Org. Lett.* **2007**, *9*, 3615–3618.
[126] M. C. Pirrung, K. D. Sarma, *J. Am. Chem. Soc.* **2003**, *126*, 444–445.
[127] A. Abrahim, P. Angelberger, K. Kletter, M. Müller, C. Joukhadar, T. Erker, O. Langer, *J. Label. Compd. Radiopharm.* **2006**, *49*, 345–356.
[128] I. Ugi, C. Steinbruckner, *Angew. Chem.* **1960**, *72*, 267–268.

[129] I. Ugi, K. Offermann, *Angew. Chem. Int. Ed.* **1963**, *2*, 624.
[130] T. Godet, Y. Bonvin, G. Vincent, D. Merle, A. Thozet, M. A. Ciufolini, *Org. Lett.* **2004**, *6*, 3281–3284.
[131] G. F. Ross, E. Herdtweck, I. Ugi, *Tetrahedron* **2002**, *58*, 6127–6133.
[132] V. Köhler, K. R. Bailey, A. Znabet, J. Raftery, M. Helliwell, N. J. Turner, *Angew. Chem. Int. Ed.*, *49*, 2182–2184.
[133] A. Znabet, E. Ruijter, F. J. J. de Kanter, V. Köhler, M. Helliwell, N. J. Turner, R. V. A. Orru, *Angew. Chem. Int. Ed.* **2010**, *49*, 5289–5292.
[134] A. Znabet, J. Zonneveld, E. Janssen, F. J. J. De Kanter, M. Helliwell, N. J. Turner, E. Ruijter, R. V. A. Orru, *Chem. Commun.* **2010**, *46*, 7706–7708.
[135] A. Znabet, M. M. Polak, E. Janssen, F. J. J. de Kanter, N. J. Turner, R. V. A. Orru, E. Ruijter, *Chem. Commun.* **2010**, *46*, 7918–7920.
[136] M. Shoda, T. Harada, Y. Kogami, R. Tsujita, H. Akashi, H. Kouji, F. L. Stahura, L. Xue, J. Bajorath, *J. Med. Chem.* **2004**, *47*, 4286–4290.
[137] S. Marcaccini, T. Torroba, *Nat. Protoc.* **2007**, *2*, 632–639.
[138] K. Paulvannan, *Tetrahedron Lett.* **1999**, *40*, 1851–1854.
[139] K. Groebke, L. Weber, F. Mehlin, *Synlett* **1998**, *6*, 661–663.
[140] H. Bienaymé, K. Bouzid, *Angew. Chem. Int. Ed.* **1998**, *37*, 2234–2237.
[141] C. Blackburn, B. Guan, P. Fleming, K. Shiosaki, S. Tsai, *Tetrahedron Lett.* **1998**, *39*, 3635–3638.
[142] A. R. Katritzky, Y.-J. Xu, H. Tu, *J. Org. Chem.* **2003**, *68*, 4935–4937.
[143] V. A. Chebanov, K. A. Gura, S. M. Desenko, *Aminoazoles as Key Reagents in Multicomponent Heterocyclizations*, in *Synthesis of Heterocycles Via Multicomponent Reactions I* (Eds.: R. V. A. Orru, E. Ruijter), Springer, Heidelberg, **2010**, p. 67.
[144] T. H. Al-Tel, R. A. Al-Qawasmeh, W. Voelter, *Eur. J. Org. Chem.*, *2010*, 5586–5593.
[145] M. T. Klimas, *Mol. Imaging Biol.* **2002**, *4*, 311–337.
[146] M. Rudin, R. Weissleder, *Nat. Rev. Drug Discov.* **2003**, *2*, 123–131.
[147] W. C. Eckelman, *Drug Discov. Today* **2003**, *8*, 404–410.
[148] J. W. Dalley, T. D. Fryer, F. I. Aigbirhio, L. Brichard, H. K. Richards, Y. T. Hong, J. C. Baron, B. J. Everitt, T. W. Robbins, *Neuropharmacology* **2009**, *56*, 9–17.
[149] D. Maclean, J. P. Northrop, H. C. Padgett, J. C. Walsh, *Mol. Imaging Biol.* **2003**, *5*, 304–311.
[150] C. G. Wermuth, *J. Med. Chem.* **2004**, *47*, 1303–1314.
[151] S. Hoegerle, C. Altehoefer, N. Ghanem, I. Brink, E. Moser, E. Nitzsche, *Eur. J. Nucl. Med. Mol. Imaging* **2001**, *28*, 64–71.
[152] O. T. DeJesus, C. J. Endres, S. E. Shelton, R. J. Nickles, J. E. Holden, *J. Nucl. Med.* **1997**, *38*, 630–636.
[153] D. M. Wieland, K. C. Rosenspire, G. D. Hutchins, M. Van Dort, J. M. Rothley, S. G. Mislankar, H. T. Lee, C. C. Massin, D. L. Gildersleeve, *J. Med. Chem.* **1990**, *33*, 956–964.
[154] Y. S. Ding, J. S. Fowler, S. John Gatley, S. L. Dewey, A. P. Wolf, *J. Med. Chem.* **1991**, *34*, 767–771.

[155] D. Murali, L. G. Flores, A. D. Roberts, R. J. Nickles, O. T. DeJesus, *Appl. Radiat. Isot.* **2003**, *59*, 237–243.
[156] I. Ilias, J. Yu, J. A. Carrasquillo, C. C. Chen, G. Eisenhofer, M. Whatley, B. McElroy, K. Pacak, *J. Clin. Endocrinol. Metab.* **2003**, *88*, 4083–4087.
[157] P. Kaji, J. A. Carrasquillo, W. M. Linehan, C. C. Chen, G. Eisenhofer, P. A. Pinto, E. W. Lai, K. Pacak, *Eur. J. Endocrinol.* **2007**, *156*, 483–487.
[158] T. L. Ross, *Dissertation*, Institut fuer Nuklearchemie, Universität zu Köln, **2005**.
[159] T. Ludwig, *Dissertation*, Institut fuer Nuklearchemie, Universität zu Köln, **2001**.
[160] T. Ludwig, J. Ermert, H. H. Coenen, *Nucl. Med. Biol.* **2002**, *29*, 255–262.
[161] U. Muhlhausen, J. Ermert, H. H. Coenen, *J. Label. Compds. Radiopharm* **2008**, *52*, 13–22.
[162] S. Oya, M.–P. Kung, P. D. Acton, Mu, C. Hou, H. F. Kung, *J. Med. Chem.* **1999**, *42*, 333–335.
[163] T. Stoll, J. Ermert, S. Oya, H. F. Kung, H. H. Coenen, *J. Label. Compd. Radiopharm.* **2004**, *47*, 443–455.
[164] L. Pouységu, D. Deffieux, S. Quideau, *Tetrahedron* **2010**, *66*, 2235–2261.
[165] D. Magdziak, S. J. Meek, T. R. R. Pettus, *Chem. Rev.* **2004**, *104*, 1383–1430.
[166] A. Pelter, S. M. A. Elgendy, *J. Chem. Soc., Perkin Trans. 1* **1993**, 1891–1896.
[167] L. H. Mejorado, T. R. R. Pettus, *J. Am. Chem. Soc.* **2006**, *128*, 15625–15631.
[168] S. P. Cook, A. Polara, S. J. Danishefsky, *J. Am. Chem. Soc.* **2006**, *128*, 16440–16441.
[169] V. V. Zhdankin, P. J. Stang, *Chem. Rev.* **2002**, *102*, 2523–2584.
[170] V. V. Zhdankin, *ARKIVOC* **2009**, *i*, 1–62.
[171] L. Kurti, P. Herczegh, J. Visy, M. Simonyi, S. Antus, A. Pelter, *J. Chem. Soc., Perkin Trans. 1* **1999**, 379–380.
[172] Y. Kita, T. Takada, H. Tohma, *Pure Appl. Chem.* **1996**, *68*, 627–630.
[173] A. S. Mitchell, R. A. Russell, *Tetrahedron* **1997**, *53*, 4387–4410.
[174] T. Ohwada, K. Shudo, *J. Am. Chem. Soc.* **1989**, *111*, 34–40.
[175] N. T. Vo, R. D. M. Pace, F. O'Har, M. J. Gaunt, *J. Am. Chem. Soc.* **2007**, *130*, 404–405.
[176] A. Bienvenu, A. Barthelemy, S. Boichut, B. Marquet, T. Billard, B. R. Langlois, *Collect. Czech. Chem. Commun.* **2002**, *67*, 1467–1478.
[177] S. R. S. Rudrangi, V. K. Bontha, V. R. Manda, S. Bethi, *Asian J. Research Chem.* **2011**, *4*, 335–338.
[178] H. Xu, M. Lv, *Curr. Pharm. Des.* **2009**, *15*, 2120–2148.
[179] M. E. Revheim, A. K. Winge–Main, G. Hagen, J. G. Fjeld, S. D. Fosså, W. Lilleby, *Clin. Oncol.* **2011**, *23*, 339–343.
[180] M. R. Battle, J. L. Goggi, L. Allen, J. Barnett, M. S. Morrison, *J. Nucl. Med.* **2011**, *52*, 424–430.
[181] W. B. Nagengast, M. N. Lub-de Hooge, S. F. Oosting, W. F. A. Den Dunnen, F. J. Warnders, A. H. Brouwers, J. R. De Jong, P. M. Price, H. Hollema, G. A. P. Hospers, P. H. Elsinga, J. W. Hesselink, J. A. Gietema, E. G. E. De Vries, *Cancer Res.* **2011**, *71*, 143–153.
[182] P. W. Miller, *J. Chem. Technol. Biotechnol.* **2009**, *84*, 309–315.

[183] F. Brady, S. K. Luthra, J. M. Gillies, N. T. Geffery, *Vol. PCT, WO 03/078358 A2*, **2003**.
[184] R. N. Krasikova, V. V. Zaitsev, S. M. Ametamey, O. F. Kuznetsova, O. S. Fedorova, I. K. Mosevich, Y. N. Belokon, S. Vyskocil, S. V. Shatik, M. Nader, P. A. Schubiger, *Nucl. Med. Biol.* **2004**, *31*, 597–603.
[185] C. Nahmias, L. Wahl, R. Chirakal, G. Firnau, E. S. Garnett, *Mov. Disord.* **1995**, *10*, 298–304.
[186] M. Okada, R. Nakao, R. Hosoi, M.-R. Zhang, T. Fukumura, K. Suzuki, O. Inoue, *J. Cereb. Blood Flow Metab.* **2010**, *1*, 1–8.
[187] H. F. VanBrocklin, M. Blagoev, A. Hoepping, J. P. O'Neil, M. Klose, P. A. Schubiger, S. Ametamey, *Appl. Radiat. Isot.* **2004**, *61*, 1289–1294.
[188] L. H. Pettus, R. W. Van De Water, T. R. R. Pettus, *Org. Lett.* **2001**, *3*, 905–908.
[189] M. Karplus, *J. Chem. Phys.* **1959**, *30*, 11–15.
[190] M. J. Minch, *Concept Magnetic Res.* **1994**, *6*, 41–56.
[191] C. E. Humphrey, M. Furegati, K. Laumen, L. L. Vecchia, T. Leutert, J. C. D. Müller-Hartwieg, M. Vögtle, *Org. Process Res. Dev.* **2007**, *11*, 1069–1075.
[192] C. Cativiela, M. D. Diaz-de-Villegas, *5(2H)-Oxazolones and 5(4H)-Oxazolones*, in *The Chemistry of Heterocyclic Compounds, Oxazoles: Synthesis, Reactions and Spectroscopy, Part B* (Ed.: D. C. Palmer), John Wiley & Sons, Inc., Hoboken, New Jersey, **2004**, p. 285.
[193] T. W. Hambley, S. Sternhell, C. W. Tansey, *Aust. J. Chem.* **1990**, *43*, 807–814.
[194] D. R. Sidler, N. Barta, W. Li, E. Hu, L. Matty, N. Ikemoto, J. S. Campbell, M. Chartrain, K. Gbewonyo, R. Boyd, E. G. Corley, R. G. Ball, R. D. Larsenan, P. J. Reider, *Can. J. Chem.* **2002**, *80*, 646–652.
[195] S. Jammi, S. Sakthivel, L. Rout, T. Mukherjee, S. Mandal, R. Mitra, P. Saha, T. Punniyamurthy, *J. Org. Chem.* **2009**, *74*, 1971–1976.
[196] S. V. Chankeshwara, A. K. Chakraborti, *Org. Lett.* **2006**, *8*, 3259–3262.
[197] S. Darvesh, R. S. McDonald, K. V. Darvesh, D. Mataija, S. Mothana, H. Cook, K. M. Carneiro, N. Richard, R. Walsh, E. Martin, *Bioorg. Med. Chem.* **2006**, *14*, 4586–4599.
[198] S. Bhagwanth, A. G. Waterson, G. M. Adjabeng, K. R. Hornberger, *J. Org. Chem.* **2009**, *74*, 4634–4637.
[199] C. Pan, J. Cheng, H. Wu, J. Ding, M. Liu, *Synth. Commun.* **2009**, *39*, 2082 – 2092.
[200] F. Bellezza, A. Cipiciani, R. Ruzziconi, S. Spizzichino, *J. Fluorine Chem.* **2008**, *129*, 97–107.
[201] J. Salazar, S. E. López, O. Rebollo, *J. Fluorine Chem.* **2003**, *124*, 111–113.
[202] L. Kraszkiewicz, L. Skulski, *ARKIVOC* **2003**, *6*, 120–125.
[203] S. Nicolai, S. Erard, D. F. González, J. Waser, *Org. Lett.* **2009**, *12*, 384–387.
[204] K. Stanek, R. Koller, A. Togni, *J. Org. Chem.* **2008**, *73*, 7678–7685.
[205] P. Eisenberger, S. Gischig, A. Togni, *Chem. Euro. J.* **2006**, *12*, 2579–2586.
[206] J. Torres, S. J. Garden, A. C. Pinto, F. S. Q. da Silva, N. Boechat, *Tetrahedron* **1999**, *55*, 1881–1892.
[207] C. A. Faler, M. M. Joullié, *Org. Lett.* **2007**, *9*, 1987–1990.
[208] E. M. Carreira, J. Du Bois, *J. Am. Chem. Soc.* **1995**, *117*, 8106–8125.

[209] M. Monclus, C. Masson, A. Luxen, *J. Fluorine Chem.* **1995**, *70*, 39–43.
[210] S. Huang, T. B. Petersen, B. H. Lipshutz, *J. Am. Chem. Soc.* **2010**, *132*, 14021–14023.
[211] P. Sauerberg, G. S. Olsen, L. Jeppesen, J. P. Mogensen, I. Pettersson, C. B. Jeppesen, J. R. Daugaard, E. D. Galsgaard, L. Ynddal, J. Fleckner, V. Panajotova, Z. Polivka, P. Pihera, M. Havranek, E. M. Wulff, *J. Med. Chem.* **2007**, *50*, 1495–1503.
[212] S. Aubry, S. Pellet-Rostaing, M. Lemaire, *Eur. J. Org. Chem.* **2007**, *2007*, 5212–5225.

Appendix

A1. X-Ray Crystallographic Data

In each case, a typical crystal was chosen and mounted on a hair using perfluoropolyether oil and cooled rapidly to 150 °K in a stream of cold N_2 using an Oxford Cryosystems Cryostream N_2 open flow cooling device.[1] Diffraction data were measured using an Enraf-Nonius KappaCCD diffractometer (graphite-monochromated MoKα radiation, λ = 0.71073 Å) to a maximum resolution of 0.77 Å. Intensity data were processed using the DENZO-SMN package and were corrected for absorption and other effects using SCALEPACK.[2]

The systematic absences in the intensity data were examined to determine the space group. In each case, the structure was solved using the direct-methods program SIR92,[3] which located all non-hydrogen atoms. Subsequent full-matrix least-squares refinement was carried out using the CRYSTALS[4] program suite to refine coordinates and anisotropic thermal parameters of all non-hydrogen atoms. Hydrogen atoms were located in the difference map and refined before being added to the model using a riding constraint.

A1.1 X-Ray data for 3-92a

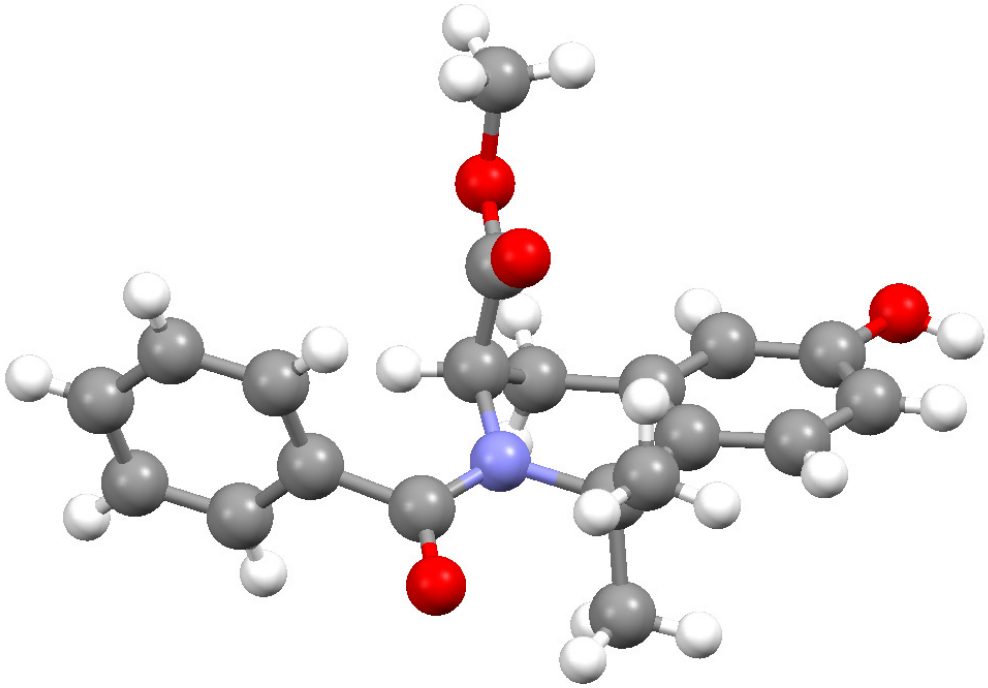


Figure A1 X-ray crystal structure of **3-92a** only the (*R*) enantiomer is depicted.

Table 1: Crystal data and refinement details

Crystal identification	050GGxxxZG02
Compound number	**3-92a**
Empirical formula	C20 H21 N1 O4
Formula weight	339.39
Temperature	150 K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 1 21/c 1
Unit cell dimensions	a = 9.0738(2) Å
	b = 17.6672(4) Å
	c = 10.9288(3) Å
	α = 90°
	β = 98.9867(9)°
	γ = 90°

Volume	1730.47(7) $Å^3$
Z	4
Density (calculated)	1.303 Mg/m^3
Absorption coefficient	0.091 mm^{-1}
F(000)	720
Crystal shape	block
Crystal colour	colourless
Crystal size	0.12 × 0.10 × 0.08 mm^3
Crystallization solvent	EtOAc / Hexane
Theta range for data collection (θ)	4.1 to 27.9°
Index ranges	−11<=h<=11, −21<=k<=22, −14<=l<=14
Reflections collected	22278
Independent reflections	4091
Completeness to theta max. (θ)	99.2%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.95 and 0.99
Refinement method	Full-matrix least-squares on F^2
Data/ restraints/ parameters	4091 / 0 / 226
Goodness-of-fit on F^2	0.9924
Final R indices [I>2sigma(I)]	R1 = 0.0459, wR2 = 0.0861
R indices (all data)	R1 = 0.0721, wR2 = 0.1098
Largest dif. peak and hole	–0.39 and 0.41 e. $Å^{-3}$

Table 2: Atomic coordinates and equivalent isotropic thremal parameters ($Å^2$) of non-hydrogen atoms

Atom	x	y	z	U_{equiv}
O1	0.53456 (16)	0.22873 (8)	0.97027 (13)	0.0307
C2	0.4875 (2)	0.29162 (11)	0.90209 (18)	0.0244
C3	0.4099 (2)	0.34662 (11)	0.95664 (17)	0.0245

C4	0.3616 (2)	0.41243 (10)	0.89227 (17)	0.022
C5	0.2782 (2)	0.47231 (11)	0.95160 (17)	0.0236
C6	0.1665 (2)	0.51158 (10)	0.85305 (17)	0.0225
N7	0.24319 (17)	0.54503 (9)	0.75772 (14)	0.0229
C8	0.3550 (2)	0.49859 (11)	0.70129 (17)	0.0247
C9	0.3880 (2)	0.42368 (11)	0.77102 (17)	0.0229
C10	0.4630 (2)	0.36659 (11)	0.71666 (18)	0.0259
C11	0.5125 (2)	0.30151 (11)	0.78076 (18)	0.0261
C12	0.5045 (2)	0.54171 (12)	0.7165 (2)	0.0309
C13	0.2925 (3)	0.48435 (13)	0.56400 (18)	0.0338
C14	0.2057 (2)	0.61518 (11)	0.71426 (18)	0.0259
O15	0.26470 (17)	0.64675 (9)	0.63300 (14)	0.035
C16	0.0866 (2)	0.65765 (11)	0.76708 (19)	0.0273
C17	0.1266 (2)	0.71148 (12)	0.8581 (2)	0.0337
C18	0.0182 (3)	0.75522 (14)	0.9011 (2)	0.0419
C19	–0.1307 (3)	0.74536 (14)	0.8517 (3)	0.045
C20	–0.1710 (3)	0.69202 (15)	0.7604 (3)	0.0451
C21	–0.0635 (2)	0.64770 (13)	0.7178 (2)	0.0366
C22	0.0396 (2)	0.46036 (11)	0.79517 (18)	0.0249
O23	–0.02156 (17)	0.42550 (9)	0.88380 (13)	0.0335
C24	–0.1509 (3)	0.37882 (15)	0.8404 (2)	0.0423
O25	–0.00502 (19)	0.45451 (10)	0.68679 (14)	0.0441

Table 3: Atomic coordinates and equivalent isotropic thermal parameters (Å^2) of hydrogen atoms

Atom	x	y	z	U_{iso}
H31	0.3902	0.3395	1.0392	0.0289*
H51	0.3501	0.5106	0.9921	0.0280*
H52	0.2243	0.4494	1.0149	0.0280*
H61	0.1183	0.5527	0.8933	0.0266*

H101	0.4817	0.372	0.6334	0.0310*
H111	0.5662	0.264	0.7435	0.0312*
H123	0.5812	0.5079	0.6906	0.0455*
H122	0.5353	0.5543	0.8046	0.0454*
H121	0.4954	0.5886	0.6662	0.0466*
H131	0.3671	0.4582	0.5249	0.0509*
H132	0.2673	0.5321	0.5217	0.0507*
H133	0.2027	0.4533	0.5576	0.0504*
H171	0.2288	0.7186	0.8911	0.0402*
H181	0.0464	0.7918	0.9647	0.0506*
H191	−0.2046	0.7753	0.8804	0.0546*
H201	−0.2731	0.6862	0.7245	0.0550*
H211	−0.0895	0.6108	0.6556	0.0442*
H243	−0.2021	0.3687	0.9102	0.0640*
H242	−0.2163	0.405	0.7746	0.0643*
H241	−0.1177	0.3322	0.8076	0.0641*
H11	0.596	0.2058	0.9338	0.0470*

Table 4: Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0362 (8)	0.0255 (7)	0.0324 (7)	0.0058 (6)	0.0114 (6)	0.0024 (6)
C2	0.0225 (9)	0.0223 (9)	0.0285 (9)	−0.0027 (7)	0.0040 (7)	0.0001 (7)
C3	0.0251 (9)	0.0269 (9)	0.0221 (9)	−0.0011 (7)	0.0053 (7)	−0.0010 (7)
C4	0.0194 (8)	0.0243 (9)	0.0223 (8)	−0.0009 (7)	0.0033 (7)	−0.0022 (7)
C5	0.0228 (9)	0.0266 (9)	0.0219 (8)	0.0020 (7)	0.0051 (7)	−0.0011 (7)
C6	0.0234 (9)	0.0219 (9)	0.0226 (9)	−0.0013 (7)	0.0055 (7)	−0.0001 (7)
N7	0.0227 (8)	0.0229 (8)	0.0237 (7)	−0.0015 (6)	0.0050 (6)	0.0017 (6)
C8	0.0259 (9)	0.0275 (9)	0.0215 (9)	0.0001 (7)	0.0061 (7)	0.0011 (7)
C9	0.0219 (9)	0.0241 (9)	0.0228 (9)	−0.0030 (7)	0.0040 (7)	−0.0011 (7)
C10	0.0270 (9)	0.0280 (10)	0.0243 (9)	−0.0026 (8)	0.0085 (7)	−0.0024 (7)

C11	0.0247 (9)	0.0252 (9)	0.0297 (10)	−0.0011 (7)	0.0088 (7)	−0.0046 (8)
C12	0.0250 (10)	0.0343 (11)	0.0343 (11)	−0.0016 (8)	0.0078 (8)	0.0068 (9)
C13	0.0406 (12)	0.0381 (12)	0.0223 (9)	0.0033 (9)	0.0037 (8)	−0.0004 (8)
C14	0.0231 (9)	0.0265 (9)	0.0269 (9)	−0.0039 (7)	0.0006 (7)	0.0012 (8)
O15	0.0340 (8)	0.0342 (8)	0.0384 (8)	−0.0022 (6)	0.0101 (6)	0.0113 (7)
C16	0.0277 (10)	0.0233 (9)	0.0305 (10)	−0.0004 (7)	0.0032 (8)	0.0040 (8)
C17	0.0305 (11)	0.0318 (11)	0.0383 (11)	−0.0046 (9)	0.0043 (9)	−0.0025 (9)
C18	0.0478 (13)	0.0327 (12)	0.0471 (13)	−0.0013 (10)	0.0131 (11)	−0.0064 (10)
C19	0.0401 (13)	0.0363 (12)	0.0604 (16)	0.0102 (10)	0.0135 (11)	0.0023 (11)
C20	0.0271 (11)	0.0441 (14)	0.0618 (16)	0.0068 (10)	−0.0003 (10)	0.0040 (12)
C21	0.0298 (11)	0.0330 (11)	0.0445 (13)	−0.0009 (9)	−0.0027 (9)	−0.0010 (10)
C22	0.0240 (9)	0.0234 (9)	0.0278 (9)	0.0000 (7)	0.0055 (7)	0.0021 (7)
O23	0.0339 (8)	0.0377 (8)	0.0298 (7)	−0.0147 (6)	0.0081 (6)	0.0002 (6)
C24	0.0404 (13)	0.0473 (14)	0.0404 (12)	−0.0221 (11)	0.0107 (10)	−0.0040 (10)
O25	0.0430 (9)	0.0586 (11)	0.0283 (8)	−0.0221 (8)	−0.0018 (7)	0.0036 (7)

Table 5: Bond Length (Å)

O1—C2	1.368 (2)	C12—H122	0.985
O1—H11	0.839	C12—H121	0.991
C2—C3	1.388 (3)	C13—H131	0.972
C2—C11	1.391 (3)	C13—H132	0.972
C3—C4	1.394 (3)	C13—H133	0.975
C3—H31	0.955	C14—O15	1.239 (2)
C4—C5	1.505 (2)	C14—C16	1.503 (3)
C4—C9	1.397 (2)	C16—C17	1.383 (3)
C5—C6	1.525 (2)	C16—C21	1.395 (3)
C5—H51	0.995	C17—C18	1.390 (3)
C5—H52	0.993	C17—H171	0.95
C6—N7	1.465 (2)	C18—C19	1.386 (3)
C6—C22	1.522 (3)	C18—H181	0.954

C6—H61	0.986	C19—C20	1.380 (4)
N7—C8	1.509 (2)	C19—H191	0.945
N7—C14	1.352 (2)	C20—C21	1.387 (3)
C8—C9	1.533 (3)	C20—H201	0.953
C8—C12	1.543 (3)	C21—H211	0.944
C8—C13	1.540 (3)	C22—O23	1.339 (2)
C9—C10	1.400 (3)	C22—O25	1.195 (2)
C10—C11	1.384 (3)	O23—C24	1.452 (2)
C10—H101	0.956	C24—H243	0.97
C11—H111	0.951	C24—H242	0.974
C12—H123	0.991	C24—H241	0.965

Table 6: Bond Angles (°)

C2—O1—H11	107.9	H123—C12—H122	108.1
O1—C2—C3	118.07 (17)	C8—C12—H121	110.9
O1—C2—C11	122.53 (17)	H123—C12—H121	110.3
C3—C2—C11	119.39 (18)	H122—C12—H121	109.9
C2—C3—C4	120.52 (17)	C8—C13—H131	109.3
C2—C3—H31	119.8	C8—C13—H132	110.1
C4—C3—H31	119.7	H131—C13—H132	109.4
C3—C4—C5	120.57 (16)	C8—C13—H133	109.8
C3—C4—C9	120.68 (17)	H131—C13—H133	109.4
C5—C4—C9	118.75 (17)	H132—C13—H133	108.9
C4—C5—C6	109.80 (15)	N7—C14—O15	123.44 (19)
C4—C5—H51	109.3	N7—C14—C16	118.66 (17)
C6—C5—H51	108.8	O15—C14—C16	117.89 (18)
C4—C5—H52	110.5	C14—C16—C17	119.72 (18)
C6—C5—H52	109.4	C14—C16—C21	120.37 (19)
H51—C5—H52	109.1	C17—C16—C21	119.7 (2)
C5—C6—N7	110.43 (15)	C16—C17—C18	120.4 (2)

C5—C6—C22	113.51 (15)	C16—C17—H171	119.7
N7—C6—C22	110.90 (15)	C18—C17—H171	119.9
C5—C6—H61	108.2	C17—C18—C19	119.8 (2)
N7—C6—H61	108.3	C17—C18—H181	120
C22—C6—H61	105.3	C19—C18—H181	120.2
C6—N7—C8	119.70 (15)	C18—C19—C20	120.0 (2)
C6—N7—C14	119.89 (16)	C18—C19—H191	120
C8—N7—C14	120.23 (15)	C20—C19—H191	120
N7—C8—C9	110.97 (14)	C19—C20—C21	120.5 (2)
N7—C8—C12	108.88 (16)	C19—C20—H201	120.1
C9—C8—C12	105.80 (15)	C21—C20—H201	119.3
N7—C8—C13	108.56 (16)	C16—C21—C20	119.6 (2)
C9—C8—C13	110.92 (16)	C16—C21—H211	119
C12—C8—C13	111.70 (16)	C20—C21—H211	121.4
C8—C9—C4	123.02 (16)	C6—C22—O23	110.18 (16)
C8—C9—C10	118.77 (16)	C6—C22—O25	125.50 (18)
C4—C9—C10	117.83 (17)	O23—C22—O25	124.22 (18)
C9—C10—C11	121.64 (17)	C22—O23—C24	115.45 (16)
C9—C10—H101	120.1	O23—C24—H243	108
C11—C10—H101	118.3	O23—C24—H242	109.9
C2—C11—C10	119.90 (18)	H243—C24—H242	110.9
C2—C11—H111	119.4	O23—C24—H241	108.9
C10—C11—H111	120.6	H243—C24—H241	110.5
C8—C12—H123	108.4	H242—C24—H241	108.7
C8—C12—H122	109.1		

Table 7: Hydrogen-bond geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
C13—H132···O15	0.97	2.36	2.987 (3)	121
C13—H133···O25	0.98	2.53	3.240 (3)	130

C17—H171···O1[i]	0.95	2.6	3.505 (3)	160
O1—H11···O15[ii]	0.84	1.87	2.708 (3)	174

Symmetry codes: (i) −*x*+1, −*y*+1, −*z*+2; (ii) −*x*+1, *y*−1/2, −*z*+3/2.

A1.2 References

1 Cosier, J.; Glazer, A. M. *J. Appl. Cryst.* **1986**, 19, 105.

2 Otwinowski, Z.; Minor, W., "Processing of X-ray Diffraction Data Collected in Oscillation Mode" *Methods Enzymol.* **1997**, 276, Eds. Carter, C. W.; Sweet, R. M. Academic Press.

3 Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994**, 27, 435.

4 Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Cryst.* **2003**, 36, 1487.