Sulfonium Salts for the Synthesis of PET Tracers

A dissertation presented by
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Declaration

I, Klaudia Aleksandra Cybulská, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.
Abstract

Positron Emission Tomography (PET) is a non-invasive medical imaging technique, which allows for quantification of biochemical processes in vivo by employing positron-emitting radiopharmaceuticals. Fluorine-18 is the most widely used radionuclide due to its favourable physical properties. Despite impressive technological advancements and large investments in PET instrumentation, its full medical potential has not been realised. The lack of broadly applicable, versatile and robust 18F-labelling strategies hampers development of PET tracers.

This work describes development and optimisation of dibenzothiophene sulfonium salts as leaving groups for efficient [18F]fluorination of aromatic molecules. A novel ring-closing reaction for sulfonium salt formation is described, which allows access to structurally diverse N-heterocyclic scaffolds, such as indoles, imidazoles and pyridines. Insights into the cyclisation mechanism and factors which orchestrate reactivity of the system are provided. A scope of common pharmacological motifs was selected to demonstrate the potential of dibenzothiophene sulfonium salts for accessing drug-like molecules.

The presented strategy was exploited to simplify and enhance radiosynthesis of a tracer for the imaging for the mGluR5 receptor, [18F]FPEB. A concise and practical approach is presented, which afforded the radiolabelled product in a high radiochemical yield, offering evident advantages over existing strategies. Synthesis of a sulfonium salt precursor to a novel tracer for the imaging of aldosterone-producing adenomas is described. Radiolabelling proceeded in an excellent RCY, allowing biological evaluation in vivo.

The results of this work constitute a major advancement in the field of PET chemistry,
rendering dibenzothiophene sulfonium salt as one of the most attractive strategies for $^{18}$F-labelling in the multitude of novel approaches. The research has the potential to provide tools for early preclinical imaging and ultimately, might lead to development of new tracers for improving patient care.
Acknowledgements

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Abbreviations

2D  Two-dimensional
AIBN  Azobisisobutyronitrile
AMT  \(\alpha\)-methyl-L-tryptophan
APA  Aldosterone producing adenoma
AVS  Adrenal vein sampling
Bq  Becquerel
CDCl\(_3\)  Deuterated chloroform
Ci  Curie
CI  Chemical ionisation
CNS  Central nervous system
CT  Computer Tomography
CYP11B1  11\(\beta\)-Hydroxylase
CYP11B2  Aldosterone synthase
dba  Dibenzylideneacetone
d.c.  Decay-corrected
DCE  Dichloroethane
DCM  Dichloromethane
dec  Decomposition
DFT  
Density functional theory

DIPEA  
$N,N$-Diisopropylethylamine

DMF  
Dimethylformamide

DMSO  
Dimethyl sulfoxide

DPEphos  
Bis[(2-diphenylphosphino)phenyl] ether

E  
Electrophile

EI  
Electron ionisation

EOS  
End of synthesis

equiv.  
Equivalent

ESI  
Electrospray ionisation

$[^{18}F]FDG$  
2-$[^{18}F]$Fluoro-2-deoxy-D-glucose

GMP  
Good Manufacturing Practice

h  
Hours

HATU  
1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate

HMDS  
Hexamethyldisilazane

HOBt  
1-Hydroxybenzotriazole hydrate

HPLC  
High performance liquid chromatography

HRMS  
High-resolution mass spectrometry

IDO  
Indoleamine 2,3-dioxygenase

$K_{222}$  
Kryptofix 222

LC-MS  
Liquid chromatography mass spectrometry

M  
Molarity
mGlu<sub>5</sub>  Metabotropic glutamate receptor subtype 5
mp  Melting point
MPO  Myeloperoxidase
MRI  Magnetic Resonance Imaging
MTO  Metomidate
N  Normality
NBS  N-Bromosuccinimide
n.d.c.  Non-decay-corrected
NIS  N-Iodosuccinimide
NCS  N-Chlorosuccinimide
NMR  Nuclear magnetic resonance
Nu  Nucleophile
<sup>o</sup>  Ortho
<sup>p</sup>  Para
Palau'Chlor<sup>®</sup>  2-Chloro-1,3-bis(methoxycarbonyl)guanidine
<sup>p</sup>-TSA  Para-toluenesulfonic acid
PE  Petroleum ether
Pd/C  Palladium on carbon
PET  Positron Emission Tomography
PA  Primary hyperaldosteronism
PK  Pharmacokinetics
ppm  Parts per million
Py  Pyridine
RAAS  Renin-angiotensin-aldosterone system
RCC  Radiochemical conversion
RCY  Radiochemical yield
rt  Room temperature
SA  Specific activity
SAR  Structure-activity relationship
S_N2  Nucleophilic substitution
S_NAr  Nucleophilic aromatic substitution
SUV  Standardised uptake value
TBAF  Tetrabutylammonium fluoride
TEAB  Triethylammonium bicarbonate
TEMPO  2,2,6,6-Tetramethylpiperidine 1-oxyl
t  Tert
TCCA  Trichloroisocyanuric acid
TFA  Trifluoroacetic acid
TfOH  Triflic acid
TLC  Thin layer chromatography
TMS  Trimethylsilyl
Trp  Tryptophan
Ts  Tosyl
Xantphos  4,5-Bis(diphenylphosphinoo)-9,9-dimethylxanthene
1 Radiolabelling with Fluorine-18 for Positron Emission Tomography

1.1 Introduction to Positron Emission Tomography

Positron Emission Tomography (PET) is a non-invasive medical imaging technique which relies on the use of radiolabelled tracers to quantify biochemical and physiological processes in vivo. PET allows for tracking molecular events before they develop into anatomical abnormalities. It is used in early-stage diagnosis, as guidance for surgical procedures and in research, where it can provide insights into the pharmacokinetics of novel therapeutics and drug target interactions.

1.1.1 Principles of PET

PET tracers are biologically active molecules labelled with a positron emitter, which decays spontaneously via $\beta^+$ emission, releasing a positron. This positively-charged particle is the antimatter of an electron. The distance it travels is proportional to its kinetic energy, falling in the range of a few millimetres. Encounter with an electron in the surrounding tissue results in an annihilation event, in which two anti-parallel high-energy photons (511 keV) are produced. Simultaneous detection of these energy quanta allows to construct a PET image. The number of individual annihilation events is proportional to the signal-to-noise ratio. This is simplistically illustrated in Figure 1.1 using $^{18}$FFDG, one of the most important PET radiopharmaceuticals.
Figure 1.1: Simplified illustration of the principles of PET imaging using [\(^{18}\text{F}\)]FDG, an \(^{18}\text{F}\)-labelled glucose analogue. [\(^{18}\text{F}\)]FDG is a PET tracer, which enables imaging of glucose metabolism \textit{in vivo}.

Figure 1.2: A PET-CT image of a mouse\(^{[1]}\).

A typical PET image is a grayscale or colour-coded map of time-dependent distribution of radioactivity in the body. With technological advancements in the field of multimodal imaging, it is now possible to superimpose biochemical information obtained in a PET scan onto functional anatomical detail, provided by, for instance MRI (Magnetic Resonance Imaging) or Computer Tomography (CT). PET-CT imaging, a union of PET and CT modalities, is now routinely used in preclinical
and clinical imaging for the evaluation of novel PET tracers, as well as diagnosis of various pathologies, including cancer. An example of a mouse PET-CT image is shown in Figure 1.2.

Conveniently, several positron emitters are isotopes of atoms found abundantly in biologically active molecules. They are produced in cyclotron facilities by bombardment of the appropriate radionuclide with a beam of protons or deuterons. Carbon-11, oxygen-15 and nitrogen-13 are short-lived radionuclides which have been used in PET imaging, enabling study and visualisation of biochemical processes without disrupting normal bioactivity. Fluorine-18 is by far the most widely used positron emitter for clinical applications (Section 1.1.2). The main focus of UCL Radiochemistry lies in designing efficient methods of $^{18}$F-incorporation for the synthesis of $^{18}$F-radiotracers. Half-lives and nuclear reactions for production of the aforementioned radionuclides are shown in Table 1.1.

### Table 1.1: Positron emitters commonly used in PET radiopharmaceuticals, with their half-lives and nuclear reactions. Based on Miller and co-workers[2].

<table>
<thead>
<tr>
<th>Positron emitter</th>
<th>Half-life (min)[2]</th>
<th>Nuclear reaction</th>
<th>Product</th>
<th>Decay product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{15}$O</td>
<td>2.04</td>
<td>$^{15}$N($d$, n)$^{15}$O</td>
<td>$[^{15}$O]O$_2$</td>
<td>$^{15}$N</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>9.97</td>
<td>$^{16}$O($p$, $\alpha$)$^{13}$N</td>
<td>$[^{13}$N]NO$_x$ or $[^{13}$N]NH$_3$</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>20.4</td>
<td>$^{14}$N($p$, $\alpha$)$^{11}$C</td>
<td>$[^{11}$C]CO$_2$ or $[^{11}$C]CH$_4$</td>
<td>$^{11}$B</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>110</td>
<td>$^{18}$O($p$, n)$^{18}$F</td>
<td>$[^{18}$F]fluoride or $[^{18}$F]F$_2$</td>
<td>$^{18}$O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{20}$Ne($d$, $\alpha$)$^{18}$F</td>
<td>$[^{18}$F]F$_2$</td>
<td>$^{18}$O</td>
</tr>
</tbody>
</table>

#### 1.1.2 Radiolabelling with $^{18}$F

**Properties of $^{18}$F**

Fluorine-18 possesses the most favourable decay properties among other positron emitters. Its half-life (110 min) is: 1) short enough to allow convenient radiolabelling and 2) long enough for the tracer to accumulate in target tissue. The use of fluorine-18 is not limited to centres located in close proximity to a cyclotron, making it more available than other radionuclides (Table 1.1). The low positron energy of fluorine-18 (0.635 MeV) translates to high resolution PET images. In addition, positron emission accounts for 97% of its decay profile, yielding a stable oxygen
isotope, $^{18}$O, which can be metabolised without biological consequences.\(^6\)

Organofluorine compounds are generally not found in nature. Recent decades, however, have witnessed an increase in the number of fluorinated therapeutics. In 2010 O’Hagan estimated that at least 20% of drugs on the market contained a fluorine atom. In 2016 Zhou et al. predicted this number to have increased to 30%. The importance of fluorine in contemporary medicinal chemistry is ascribed to its unique chemical properties. Fluorine is the most electronegative element in the periodic table. Consequently, the carbon-fluorine bond is highly polarised and metabolically stable.\(^7\, 10\, 11\) Its van der Waals radius is only slightly larger than that of hydrogen (1.35 Å versus 1.20 Å), allowing fluoride substitution without severe steric repercussions.\(^11\,12\) Incorporation of fluorine impacts hydrophobicity of drug-like molecules. Replacement in aliphatic molecules decreases their lipophilic character. The opposite effect is observed for aromatic compounds. It is therefore possible to refine membrane permeability properties and solubility of biologically active molecules.\(^13\)

Fluorine substitution has found uses in pharmaceuticals, agrochemicals and PET chemistry, in line with advancements in the field of PET imaging. One of the most remarkable bioisosteric replacement by a fluorine-18 atom gave rise to an important PET tracer, $[^{18}$F]$FDG$ (Figure 1.3).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{2-$[^{18}$F]$Fluoro-2-deoxy-D-glucose or $[^{18}$F]$FDG.$}
\end{figure}

2-$[^{18}$F]$Fluoro-2-deoxy-D-glucose is undoubtedly one of the gold standard PET tracers. $[^{18}$F]$FDG$ has been used to image glucose metabolism in various disease states, e.g. cancer: 1) melanoma, 2) breast cancer and 3) colorectal cancer. It has also been used to diagnose various forms of neurodegeneration, such as Alzheimer’s and Parkinson’s disease. Its mode of action relies on the substitution of the 2-hydroxyl moiety by fluorine-18. After injection, the tracer is transported into cells, where it undergoes phosphorylation by the hexokinase enzyme to form $[^{18}$F]$FDG-$
phosphate. However, unlike for regular glucose, this metabolite cannot participate in further glycolysis. Consequently, it is accumulated in cells, enabling its visualisation by PET. It is therefore possible to assess the activity of glucose transporters and hexokinase, which provide a reflection of glucose metabolism in various pathologies.

Remarkably, imaging of glucose activity by isotopic substitution with carbon-11 would not be as efficient. Although an excellent diagnostic tool for early stage cancer detection and mapping response to therapy, $[^{18}\text{F}]\text{FDG}$ lacks specificity to distinguish between tumour types. Accumulation can also occur in areas of usually-enhanced metabolic activity, such as inflammations, myocardium and the urinary tract.

**Preparation and Considerations of $^{18}\text{F}$**

Production of radionuclides, as mentioned in Section 1.1.1, is performed in a cyclotron. Depending on further use, fluorine-18 can be provided in aqueous or gaseous form. This is done through irradiation of heavy water, $[^{18}\text{O}]\text{H}_2\text{O}$, or oxygen gas, $[^{18}\text{O}]\text{O}_2$, respectively (Table 1.1).

Water-bound $[^{18}\text{F}]$fluoride cannot usually be used directly in radiolabelling. The anion is strongly bound to water molecules by a hydrogen bonding network, which inhibits its nucleophilicity and ability to participate in most chemical transformations. To restore its reactivity, water is azeotropically evaporated with acetonitrile under a stream of inert gas. A mild base, such as potassium carbonate or bicarbonate, is added to neutralise any remaining hydrofluoric acid. Complexation of the potassium cation with a macrocyclic phase-transfer reagent, *e.g.* Kryptofix 222 ($K_{222}$), leaves $[^{18}\text{F}]$fluoride "naked" and highly nucleophilic (Figure 1.4). An alternative $^{18}\text{F}$-source, $^{18}\text{F}$-tetrabutylammonium fluoride ($[^{18}\text{F}]\text{TBAF}$), can be prepared by elution with tetrabutylammonium bicarbonate. Radiolabelling can also be performed using $[^{18}\text{F}]\text{F}_2$ but it is usually transformed into less cumbersome reagents (Section 2.2.1).
“Naked” $^{18}$F-fluoride anion for labelling

Figure 1.4: Trapping of potassium cations by Kryptofix 222, leaving behind highly reactive $^{18}$F-fluoride anions for radiolabelling. Adapted from Miller and co-workers[2].

1.1.3 Nomenclature for Radiochemistry

Efficiency of $^{18}$F-labelling is expressed in terms of radiochemical yield (RCY). It is a function of the chemical yield of a reaction and radioactive decay[2]. RCY is obtained by dividing the amount of radioactivity remaining in the sample after purification by the initial value at the beginning of radiosynthesis. RCY can be reported with decay correction in order to account for the loss of radioactivity during manipulations.

Radioactivity is measured in becquerels (Bq), where 1 Bq is equal to one radioactive decay per second. Radiosyntheses for clinical uses usually start with GBq quantities, whereas for preclinical and clinical injections, MBqs of activity are used. Historically, radioactivity was expressed in curies (Ci), where 1 Ci corresponds to $3.7 \times 10^{10}$ Bq. Quantities used for PET imaging are usually reported in the micro and millicurie range[20].

Figure 1.5: Radiochemical yield is a function of the chemical yield (red) and radioactive decay (blue). Decay- and non-decay-corrected RCYs are also shown.
Specific activity (SA) is defined as radioactivity per unit mass of a radiotracer and it is reported in GBq per micromol. The theoretical value of SA is calculated using the following formula:

\[
SA = (\ln 2/t_{1/2})N
\]  

(1.1)

where \( t_{1/2} \) is half-life of the radionuclide (e.g. 110 min for fluorine-18) and \( N \) is the number of fluorine-18 atoms in the sample. This value is never reached because of isotopic dilution with fluorine-19 atoms. High specific activity is desired to achieve a good signal-to-noise ratio. Low specific activity results not only in target saturation (binding sites, etc.) but also presents a toxicity hazard\(^{21}\).

1.1.4 \( ^{18}\text{F}\)-Radiochemistry: Analytical Techniques

This section gives a brief introduction to modern instrumental chromatography techniques used to confirm the identity of \( ^{18}\text{F}\)-labelled compounds by UCL Radiochemistry.

Unlike non-radioactive drug-like compounds synthesised in research laboratories, identity of PET radiopharmaceuticals cannot be confirmed using standard NMR spectroscopic techniques. This is because: 1) tracers are synthesised in subnanomolar quantities which cannot be detected by the inherently insensitive NMR spectroscopy and 2) \( ^{18}\text{F}\)-labelled samples must be appropriately shielded. For RCY and SA calculations, precise radioactivity measurements are required. Confirmation of product identity and quality control for chromophores are performed routinely using high-performance liquid chromatography (HPLC) with an integrated radioactivity detection system, known collectively as radio-HPLC. Detectors used by UCL Radiochemistry are scintillation-based. Emitted radioactivity hits the scintillation crystal and interacts with its atoms, exciting them to higher molecular orbitals, which leads to emission of light. Light is then converted into electrical energy and consequently, a signal on the detector output\(^{22/23}\).

All radiolabelled compounds prepared and described in this thesis are aromatic molecules and all analytical work was performed using radio-HPLC. Non-radioactive
compounds were analysed using NMR spectroscopy and/or HPLC/LC-MS.

**High-Performance Liquid Chromatography**

High-Performance Liquid Chromatography is a modern analytical technique used to separate chemical mixtures into their constituents. Designed on the foundations of traditional liquid chromatography, HPLC employs significantly smaller sorbent particles (5 to 250 µm) to increase the surface area available for interaction and achieve the maximum resolving power. It is a powerful and versatile technique and a gold standard purification method in pharmaceutical industry and research\(^{222}\). The key components are outlined in Figure 1.6.

\[\text{Figure 1.6: Schematic illustration of HPLC principles.}\]

HPLC uses a wide range of separation methodologies, however, those pertinent to this work are: normal and reverse-phase. In the traditional normal-phase mode, the stationary phase is hydrophilic (silica- or alumina-based). Strong interaction with
hydrophilic analytes causes the lipophilic ones to elute more rapidly. The mobile phase is usually significantly less polar than the packing material in the column. Typical solvents include ethyl acetate and hexanes. In the reverse-phase, the opposite phenomenon occurs, however, the stationary phase is non-polar and usually contains silica with adsorbed long alkyl chains (8 and 18 carbons). Significantly more polar solvents are required, e.g. water, methanol and acetonitrile. Reverse-phase HPLC is commonly used in pharmaceutical research.

Parameters of the column packing material are selected to provide the highest separating power. Several factors contribute to the strength of interactions between the stationary phase and components of the mixture. These include: 1) type of particle backbone, e.g. silica, alumina, 2) bonded material, e.g. C-8 or C-18 alkyl chains, 3) particle size and surface area for resolution, as well as 4) column dimensions.

For certain compounds, adjustments of pH of the mobile phase must be made to ensure good resolution. When pH of the solvent carrier is equal to the pK\textsubscript{a} of the compound, it will exist as both its protonated and neutral form, resulting in two peaks. Trifluoroacetic acid is a common additive in reverse-phase HPLC which rectifies this problem\textsuperscript{26}. Elution can be performed in two modes: gradient and isocratic. Gradient elution relies on a changing composition of the solvent mixture to achieve separation, whereas in the isocratic mode a single solvent percentage is used from the outset and throughout. There are several detection types available, depending on the nature of compound, including UV (specific wavelengths can be set), fluorescence and radioactivity, etc.

**Radio-HPLC** Chromatography analysis of reaction mixtures is performed on a radio-HPLC, which is equipped with a gamma-ray detector. Preparative and semi-preparative radio-HPLC machines are used in radiopharmaceutical production for preclinical and clinical applications\textsuperscript{27,28}.

Identification of the radiolabelled product is performed by co-elution with its non-radioactive reference compound (Figure 1.7). Comparison of the radioactive and UV traces allows to indirectly infer identity of the radioactive compound. Specific
activity calculations are performed using a calibration curve, based on concentrations of the non-radioactive analogue and the area under the peak corresponding to the radiolabelled compound on the UV trace. This is usually close to the limit of quantification of the machine. The amount of the $[^{19}\text{F}]$fluorinated product can be established using its molar mass and concentration (calibration curve), while for the $[^{18}\text{F}]$fluorinated product a radioactivity detector is needed.

**Figure 1.7:** Co-injection of the radioactive trace corresponding to the $[^{18}\text{F}]$fluorinated product with the UV trace of its $^{19}\text{F}$-analogue on radio-HPLC. Note the small delay due to the sequential use of radioactivity and UV detectors.

**Liquid Chromatography Mass Spectrometry**

Liquid chromatography mass spectrometry (LC-MS) is a union of HPLC and mass spectrometry instrumentation that provides analysis, identification and purification options for reaction mixtures. Research presented in this thesis relied on the use of LC-MS to confirm the mass of reaction intermediates.

In an LC-MS sequence, after HPLC separation (Figure 1.6), the components are passed through an ion source which produces various gaseous ionised species. These are then arranged according to their mass to charge ratio ($m/z$). Superimposition onto the HPLC trace allows to identify the masses of resolved peaks.

There are several ionisation sources available in mass spectrometry, the most common
one being Electrospray Ionisation (ESI). Strong electric field is applied to transfer ions in the solution into the gaseous phase. Large charge droplets are formed and evaporation of solvent results in the release of ions due to strong repulsion forces.\textsuperscript{30}
PET imaging is interdisciplinary. Its success is proportional to the degree of collaboration between different fields. Engineers and computer scientists work towards improving PET sensitivity by designing better detectors and image reconstruction algorithms. Biologists and clinicians perform biological studies, while chemists and radiochemists design PET tracers and efficient routes for radionuclide incorporation. Despite major advancements in PET chemistry, certain tracers cannot be radiosynthesised efficiently or prepared according to Good Manufacturing Practice (GMP) standards. For widespread clinical use, production of PET tracers must be scalable to quantities > 200 MBq. Radiopharmaceuticals must exhibit high specific activity values, as well as pass quality control tests for the presence of impurities, such as metallic residues. The quest for practical, efficient and scalable $^[18]F$-fluorination is ongoing. The main challenge lies in C-F bond formation, which imposes severe synthetic restrictions. Extensive reviews of $^[18]F$-labelling strategies have been published, with the most recent by Preshlock and van der Born, giving an overview of the most prominent late-stage direct $^[18]F$-fluorinations. This chapter gives an outlook of the most recent and/or interesting approaches to $^[18]F$-labelling, with the main focus on aromatic molecules.

2.1 $^[18]F$-Labelling of Aliphatic Molecules

There are several approaches to $^[18]F$-incorporation into aliphatic molecules, with the most prominent utilising nucleophilic $^[18]F$-fluoride in an S$_2$N$_2$ substitution reaction. Gold standard radiopharmaceuticals, such as $^[18]F$FDG and $^[18]F$FMISO (imaging of hypoxia), are prepared using this method. Similarly to other nucleophilic transformations, a good leaving group is required. Tosylate offers a favourable combination
of stability and reactivity but triflate and mesylate are also used. Halides are less favoured. Facility of substitution increases with decreasing steric bulk of the substrate, so $[^{18}\text{F}]$fluoride attack is most efficient at primary benzylic positions\[^{10}\]. Reactions proceed in polar aprotic solvents, \textit{e.g.} DMF, DMSO and MeCN, and usually require moderate heat.

![Scheme 2.1: Nucleophilic aliphatic $[^{18}\text{F}]$fluorination proceeds via the $S_N$2 mechanism. $[^{18}\text{F}]$FDG is radiosynthesised using this strategy.][1]

**2.2 $^{18}$F-Labelling of Aryls: Traditional Approach**

Aromatic rings are vastly abundant in biological molecules, \textit{e.g.} as ligands. Incorporation of arenes into drug molecules increases their metabolic stability, in comparison with unsaturated hydrocarbons. It can also induce strong interactions with proteins through $\pi-\pi$ stacking, hydrogen bonding and stabilisation of functional groups\[^{34,35}\].

PET radiopharmaceuticals, like therapeutics, interact with specific targets \textit{in vivo}. They can be $[^{18}\text{F}]$fluorinated analogues of biologically active molecules or drugs. Direct late-stage labelling of aromatic molecules is desired, however as already mentioned, C-F bond formation is troublesome, and even more so aromatic fluorination. A multitude of impressive $^{18}$F-labelling strategies have been published in the last decade. One of the main problems of fluorine-18 chemistry is, however, the lack of broadly applicable, practical and robust methods which could be adapted to GMP environments. Consequently, in order to utilise the enormous potential of PET and the ever-increasing pool of bioactive molecules, practical and elegant $[^{18}\text{F}]$fluorination methods need to be developed.
2.2.1 Electrophilic Aromatic Substitution

A review of $^{18}$F-fluorination techniques for aromatic systems would not be complete without a brief introduction to electrophilic aromatic substitution. Historically, it was a common strategy for radiosynthesis of electron-rich and electron-neutral aromatic systems, including important PET radiopharmaceuticals, such as $^{18}$F-DOPA (Scheme 2.2). The use of electrophilic $^{18}$F-fluorination of PET tracers is currently outdated and replaced by technologies employing nucleophilic $^{18}$F-fluoride.

 Traditiona...
Scheme 2.3: Electrophilic aromatic $^{18}\text{F}$-fluorination of the organotin precursor was a common radiosynthetic route to clinical $^{18}\text{F}$-DOPA. The strategy was optimised for automation by de Vries and co-workers.\textsuperscript{39}

Milder electrophilic $^{18}\text{F}$-fluorine sources include xenon difluoride ($^{18}\text{F}$XeF\textsubscript{2}), acetyl hypofluorite and a recent invention by the Gouverneur group, $^{18}\text{F}$Selectfluor (Scheme 2.4).\textsuperscript{40-42} Its cumbersome preparation limits its widespread use.\textsuperscript{43} As these reagents still do not provide enough regioselectivity and require complicated purification procedures, the search for mild, specific and efficient radiosynthetic routes for the labelling of electron-rich and electron-neutral aromatic scaffolds is still ongoing.

Scheme 2.4: Radiosynthesis of $^{18}\text{F}$Selectfluor, a milder electrophilic alternative to $^{18}\text{F}$fluorine gas.\textsuperscript{43}

2.2.2 Nucleophilic Aromatic Substitution

Nucleophilic aromatic substitution is routinely used to incorporate fluorine-18 into aromatic molecules. Radiolabelling is performed with $^{18}\text{F}$fluoride. This poses restrictions on the type of aromatic scaffolds which can be labelled efficiently. Radiolabelling proceeds in high RCYs for aromatics decorated with strongly electron-withdrawing substituents, such as trimethylammonium or nitro groups, located ortho or para to the leaving group (Scheme 2.5). Nucleophilic attack at these positions leads to the
formation of a negatively charged Meisenheimer complex, which is best stabilised by substituents capable of accepting electron density.\(^{1044}\)

**Scheme 2.5:** Nucleophilic aromatic substitution with \([^{18}\text{F}]\)fluoride occurs via a Meisenheimer complex, stabilised by electron-deficient substituents in the *ortho* - or *para*-positions.

Typical radiolabelling using nucleophilic \([^{18}\text{F}]\)fluoride requires a polar aprotic solvent, such as DMF, DMSO or MeCN, thermal activation (> 100 °C) or microwaves, a phase-transfer catalyst, *e.g.* Kryptofix 222, and a mild base (Scheme 2.6).

**Scheme 2.6:** Typical nucleophilic aromatic \([^{18}\text{F}]\)fluorination of activated aromatic systems.

Perhaps the most prominent example of nucleophilic aromatic \([^{18}\text{F}]\)fluorination is the GMP radiosynthesis of \(^{18}\text{F}\)-DOPA. To date, this is the most efficient strategy for clinical production of this tracer. The precursor, 6-nitroveratraldehyde, is substituted by \([^{18}\text{F}]\)fluoride in the presence of K\(_{222}\) and K\(_2\)CO\(_3\). Reduction of the carbonyl moiety with NaBH\(_4\) is followed by iodination, and the resulting \([^{18}\text{F}]\)fluorinated benzyl iodide reacts with a Schiff’s base and a chiral catalyst to afford \(^{18}\text{F}\)-DOPA in at least 35% non-decay-corrected RCY. This route is shown in Scheme 2.7.
Scheme 2.7: Radiosynthesis of $^{18}$F-DOPA performed in GMP laboratories. The first step is a nucleophilic aromatic substitution using $[^{18}\text{F}]$fluoride with NO$_2$ as a leaving group.
2.2.3 Indirect Labelling via Prosthetic Groups

In radiochemistry, a prosthetic group is a bifunctional labelling agent incorporated into molecules of interest to act as an $^{18}\text{F}$-tag\(^{45}\). It is a common way of labelling aromatic molecules which, for various reasons, cannot be decorated with fluorine-18 directly. The use of prosthetic groups is not always ideal, as they might interfere with biological activity of PET tracers. Radiosynthesis of biomolecules, such as peptides and proteins, relies largely on indirect $^{18}\text{F}$-incorporation, as their temperature- and base-sensitive nature often precludes the use of $[^{18}\text{F}]$fluoride\(^{42}\). Fortunately, installation of prosthetic groups is normally performed under mild conditions. An arsenal of such entities is now available to radiochemists as a result of concerted efforts to increase the pool of biologically active molecules for PET imaging. Attachment usually occurs via the NH\(_2\)- or SH-moieties\(^{45}\). New generation reagents can be installed using mild and elegant solutions such as "click reactions". A remarkable example is 2-$[^{18}\text{F}]$fluoroethylazide, developed by Glaser and Årstad, which enables formation of $^{18}\text{F}$-labelled 1,2,3-triazoles using Cu(I)-catalysed Huisgen cycloadditions (Scheme 2.8\(^{46}\)).

![Scheme 2.8: 2-$[^{18}\text{F}]$Fluoroethylazide is a versatile prosthetic group, which can be used to label biologically active molecules. It reacts with terminal alkynes in elegant "click transformations"\(^{46}\).]

2.3 $^{18}\text{F}$-Labelling of Aryls: Recent Advances

The ongoing search for milder and high yielding radiolabelling strategies has led to ground-breaking advances, particularly in the last few years. Focus has been placed on engineering versatile precursors for practical and regioselective $^{18}\text{F}$-labelling of electron-neutral and electron-rich aromatics. Particularly favoured are routes which avoid the use of highly reactive and low specific activity gaseous species, toxic metals, such as tin, or lengthy multi-step procedures. Although an impressive repertoire of
strategies is available, the majority cannot be readily adapted to automated GMP radiosynthesis.

This section provides a brief overview of the most recent and attractive radiosynthetic avenues for labelling of non-activated and deactivated aryls with nucleophilic $[^{18}\text{F}]$fluoride. Such transformations can be achieved using hypervalent iodine(III) species, sulfur-based precursors or metal-assisted reductive elimination.

### 2.3.1 Metal-Free $^{18}\text{F}$-Chemistry

This section discusses recent advances in $[^{18}\text{F}]$fluorination of aromatic systems employing metal-free precursors.

**Diaryliodonium Salts: Chemistry**

Diaryliodonium salts (Figure 2.1) have been used in synthetic transformations for decades. The "soft" nature of the iodine(III) core makes it a good leaving group and its electron-poor character renders it prone to nucleophilic attack.$^{47}$

![Figure 2.1: A functionalised diaryliodonium salt.](image)

**Scheme 2.9:** Efficient and practical iodonium salt formation using $m$CPBA.$^{48}$
To date, there exist several synthetic routes to diaryiodonium salts. However, it was the recent work of Olofson et al. which has given access to structurally sophisticated variants. Asymmetrical diaryiodonium salts can be prepared from the corresponding aryls in the presence of mCPBA and TfOH or TsOH in dichloromethane at room temperature. For symmetrical analogues, molecular iodine is required (Scheme 2.9). For more challenging regiospecific syntheses, arylboronic acids can be employed. A broad scope of diaryiodonium salts has been prepared using the aforementioned strategies.

Among the wide range of nucleophilic species that react with diaryiodonium salts are thiols, thioethers, amines and phenoxides. This methodology was expanded by Pike and Aigbirhio, who realised the potential of diaryiodonium chemistry for direct 18F-functionalisations of arenes in mid-1990s. Asymmetrical precursors decorated with electron-rich methoxy and methyl substituents reacted in excellent radiochemical yields. The most remarkable example features formation of [18F]fluoroanisole from 4-methoxydiphenyliodonium bromide in 88% decay-corrected RCY. While the triflate counterion remained most explored, labelling also proceeded in the presence of chloride, bromide and trifluoroacetate. The generic radiolabelling reaction of [18F]fluoride is shown in Scheme 2.10.

Reactivity towards nucleophiles is governed by the molecular geometry exhibited by diaryiodonium salts. Trigonal bipyramidal arrangement of substituents (Scheme 2.11) is commonly found in hypervalent iodine species, however conventional depiction with the 109° bond angle (Figure 2.1) prevails. The degree of dissociation between the counterion and the iodine(III) centre depends on the strength of the solvent interaction.
Generic reaction of an asymmetrical diaryliodonium salt with a nucleophile is presented in Scheme 2.11. Firstly, an exchange occurs between the counterion and the nucleophile, the latter occupying the axial position. Pseudorotation around the iodine core gives rise to two intermediates, A and B. The corresponding transition state At is favoured and nucleophilic substitution occurs preferentially at the ipso-carbon of the more electron-withdrawing ring. This pattern can, however, be overridden when one of the aryl rings possesses an ortho-substituent. T-shaped geometry forces the more sterically hindered aryl to occupy the spacious equatorial position, leading to nucleophilic substitution ortho to the substituent, even if it is more electron-rich by nature. This selectivity is referred to as the ortho-effect.

![Scheme 2.11: Reactivity of asymmetrical diaryliodonium salts towards nucleophiles. Adapted from Yusubov and co-workers](image)

Chun et al. reported that in certain instances, radioactive product distribution cannot be explained in terms of the ortho-effect and substituent bulk. The fact that
[¹⁸F]fluoride incorporation on the methoxy-decorated ring of (2-methoxyphenyl)phenyl iodonium tosylate did not occur, brought Chun and co-workers to the conclusion that other factors could perhaps influence the fate of the nucleophilic attack, e.g. lipophilicity and electronics.

[¹⁸F]Fluorinations of asymmetrical diaryliodonium salts performed by Gail et al. confirmed the observations made by Chun et al. RCY increased with the number of methyl substituents on the aryl ring, which cannot be explained solely by ring electronics. Figure 2.2 illustrates the ortho-effect observed in ¹⁸F-labelling experiments.

Figure 2.2: The ortho-effect was observed during radiolabelling of asymmetrical diaryliodonium salts by Gail and co-workers. Adapted from Coenen.

Ross et al. investigated nucleophilic ¹⁸F-labelling of heteroaromatic diaryliodonium salts with a selection of counterions and substituents. A thiophene ring was selected to represent an electron-deficient arene, while the phenyl ring was decorated with electron-rich methoxy and benzyl substituents or halides (Scheme 2.12).

Scheme 2.12: ¹⁸F-Labelling of heteroaromatic diaryliodonium salts to investigate the influence of substituents and counterions on RCY, was performed by Ross and co-workers.

As postulated earlier, ortho-substituted diaryliodonium salts exhibited higher RCYs, furthering evidence towards the ortho-effect. While 2-[¹⁸F]fluoroanisole was accessed
in 60% RCY, labelling of the meta- and para-regiomers proceeded less efficiently, reaching 20% and 29% RCY, respectively. Ross and co-workers also studied the effect of counterions on the fate of radiolabelling. Highest radiochemical yields were obtained with the bromide counterion, followed by triflate, iodide and tosylate. Triflate diaryliodonium salts exhibited higher reaction rates with $[^{18}\text{F}]$fluoride. NMR studies of inorganic counterions provided evidence against full dissociation of the salt in solution. Bromide was observed to be least strongly associated with iodine(III), in line with the highest reactivity of the corresponding diaryliodonium salt towards $[^{18}\text{F}]$F$^-$. 

**Applications of Diaryliodonium Salts to PET Tracers**

Diaryliodonium salts were successfully employed by Telu et al. in the radiosynthesis of $[^{18}\text{F}]$FSP203, a radiotracer for the imaging of the mGluR5 receptor subtype (Scheme 2.13). The $^{18}$F-labelled product was obtained in 33% isolated RCY, in the presence of TEMPO, a radical scavenger. TEMPO addition has been critical for radiofluorinations of certain diaryliodonium salts, which were described as prone to photodecomposition by Irving and Reid in the 1960s.

Radiosynthesis of $[^{18}\text{F}]$flumazenil, a tracer for the imaging of the central benzodiazepine receptor in the CNS, has been substantially enhanced with the use of a diaryliodonium salt precursor (Scheme 2.14). High RCY of 67 ± 3% (99% radiochemical purity) was obtained, showcasing a major improvement on the 15-20% decay-corrected RCY afforded using a nitro-precursor.
Scheme 2.14: Application of diaryliodonium salts: radiosynthesis of $[^{18}\text{F}]$flumazenil$^{[60]}$. 

Chemistry of Iodonium Ylides

Iodonium ylides, like diaryliodonium salts, belong to the family of hypervalent iodine(III) precursors for $^{18}$F-labelling. These spirocyclic reagents were designed by Rotstein et al. to address photostability and decomposition issues of diaryliodonium salts, and to provide stabilisation for non-activated substrates during $^{18}$F-incorporation$^{[62]}$. After an exhaustive optimisation process, a stable spiroiodine(III) was identified, which proved reactive towards $[^{18}\text{F}]$fluoride (Scheme 2.15).

Scheme 2.15: One-pot synthesis of an optimised spirocyclic iodonium ylide precursor for $^{18}$F-labelling, developed by Rotstein and co-workers$^{[62]}$. 

Iodonium ylides can be accessed from the corresponding aryl iodide in a convenient one-pot oxidation with $m$CPBA or Selectfluor, the spirocyclic ligand and sodium carbonate at room temperature (Scheme 2.15). Selectfluor is a strong oxidant, commonly employed in preparation of hypervalent iodine species from arenes$^{[63]}$. The range of precursor which can be accessed using this method is therefore limited to oxidation-resistant moieties.

Complementary routes were designed for substrates prone to oxidation, such as
quinolines and indoles. The former can be afforded by $N$-protonation, followed by treatment with Oxone®. Indole-based substrates require prior $N$-Boc protection. Oxidation to the corresponding iodonium ylides proceeds in the presence of dimethyldioxirane (DMDO) in acidic media.

The substrate scope for $^{18}$F-labelling included non-activated aromatic moieties, decorated with iso-propyl, methyl, methoxy and iso-propanoxy substituents in the ortho-position. Enhanced RCYs were obtained for hindered alkyl-substitution patterns. The $N$-tosyl-protected 6-$^{18}$F-fluoroindoline was accessed in 34 ± 8% RCY, while incorporation at the 3-position on the pyridine afforded the corresponding $^{18}$F-fluorinated product in 65 ± 11% RCY. Labelling of non-activated meta-substituted arenes proceeded with good to excellent RCYs. Representative examples from Rotstein’s scope study are shown in Scheme 2.16.

Scheme 2.16: Radiolabelling of structurally diverse aryls using iodonium ylides as precursors, performed by Rotstein and co-workers. Analytical RCYs were established using radio-TLC and radio-HPLC.
Rotstein and co-workers also presented a mechanistic interpretation of iodonium ylide reactivity towards $^{18}$F-fluoride. Using *in silico* methods and NMR spectroscopy, the authors were able to compare the reactivity of iodonium ylides versus diaryliodonium salts in radiolabelling and to explain the excellent regiospecificity of $^{18}$F-incorporation at the aryl rather than the spirocyclic auxiliary. It was hypothesised that the transition state involving coordination of the nucleophile to the hypervalent iodine centre, and subsequent reductive elimination to form the $^{18}$F-fluorinated aryl, has a lower activation energy, due to the stabilising effect of the auxiliary. The latter also plays a key role in enhancing thermal stability of the precursor during high temperature radiolabelling, often required for electron-rich scaffolds. As expected, iodonium ylides decorated with electron-deficient substituents, such as NO$_2$ or CN, can be labelled even at ambient temperatures. It was postulated that reductive elimination is likely to be the rate-determining step, with the activation barrier lower for electron-poor substrates, in line with radiosynthetic findings. Through the design of stable iodonium ylides for $^{18}$F-fluorination of functionalised drug-like molecules, Rotstein *et al.* addressed the issues associated with iodonium salts precursors, such as decomposition and lower radioefficiency for deactivated aromatics.

**Applications of Iodonium Ylides to PET Tracers**

Several PET tracers have been prepared using the iodonium ylide strategy since its emergence in 2015. Stephenson *et al.* validated practicality of the method on $^{18}$F-FPEB, a tracer for the imaging of the mGluR5 receptor (Chapter 5). The iodonium ylide precursor to $^{18}$F-FPEB was synthesised in 40% yield over 6 steps. With optimisation of the labelling conditions, Stephenson *et al.* arrived at the combination of $^{18}$F-fluoride and tetraethylammonium carbonate in DMF at 80 °C for 5 min, to give $^{18}$F-FPEB in 49 ± 6% RCY, as determined by radio-TLC. A high radioactivity scale radiosynthesis for clinical use afforded the tracer in 20% non-decay-corrected (n.d.c) RCY at the end of synthesis (Scheme 2.17).
Scheme 2.17: Clinical-scale radiosynthesis of $[^{18}\text{F}]$FPEB, starting from the iodonium ylide precursor, developed by Stephenson and co-workers\textsuperscript{65}.

Labelling with Sulfonium Salts

Sulfonium salts have long been considered as promising precursors for $^{18}\text{F}$-labelling, owing to their excellent leaving group character in nucleophilic substitution reactions\textsuperscript{66}. In the late 1980s, Maeda et al. reported successful $^{18}\text{F}$-incorporation into aromatic molecules using aryldimethylsulfonium mesylates\textsuperscript{67}. The scope was limited to aryls activated by the strongly electron-deficient nitro group in the ortho- or para-position, which reacted with $^{18}\text{F}$-TBAF in 11-77% RCYs (n.d.c). Low yields were reported for $p$-nitrile substitution and no reaction was observed for $p$-aniline (Scheme 2.18). One of the drawbacks of this strategy is facile formation of methyl $[^{18}\text{F}]$fluoride and demethylation of the precursor to the corresponding sulfide.

Scheme 2.18: Labelling of aryldimethylsulfonium salts with $^{18}\text{F}$-TBAF, performed by Maeda and co-workers\textsuperscript{67}.
Nearly 30 years later, Mu et al. developed a radiolabelling protocol employing triarylsulfonium triflate salts, in response to the growing demand for alternative strategies to access non-activated and deactivated $^{18}$F-fluoroaryl. The authors explored the influence of para-substitution by non-activating and deactivating groups on the RCY. A generic radiolabelling reaction of triarylsulfonium salts and the scope investigated by Mu et al. are shown in Scheme 2.19. High radiochemical conversions (RCCs) were reported for $^{18}$F-fluoride substitution of an aryl iodide: 1) 90-91% in MeCN, 2) 62-81% in DMF and 3) 48-61% in DMSO. Regioselectivity issues were experienced, with the highest percentage of side products in DMF reactions. Decent conversion was also observed for an aryl bromide, consistent with its less activating nature, as noted from Hammett constants ($\sigma$: $p$-Br = 0.232, $p$-I = 0.276). $^{18}$F-Fluorobenzene formation from triphenylsulfonium triflate proceeded in $70 \pm 7\%$ RCC in DMF, dropping to $48 \pm 3\%$ in MeCN. By-product formation was favoured for electron-donating substituents. Simple peptide scaffolds were also prepared.

**Scheme 2.19:** $^{18}$F-Fluorination of triarylsulfonium salts, performed by Mu and co-workers. The scope and radiolabelling conversions are shown.
Mu et al. performed density functional theory (DFT) calculations to gain more understanding into the fate of sulfonium salt precursors decorated with substituents of varied activating properties. Having assumed a nucleophilic substitution pathway, the authors compared energies of the transition states arising from $^{18}$F-fluoride attack at the ipso-position of the substituted ring or the phenyls. Activation barrier for incorporation into an electron-rich ring is higher than for $^{18}$F-fluorobenzene formation. It decreases with increasing electron-withdrawing character of the para-substituent.

Although triarylsulfonium salts demonstrate great potential, there are synthetic limitations associated with this strategy, such as harsh reagents and reaction conditions. In addition, demonstration of applications to more sophisticated drug-like scaffolds is required.

### 2.3.2 Metal-Mediated $^{18}$F-Chemistry

Transition metals have been used widely in $^{18}$F-fluorination strategies, owing to their ability to promote carbon-heteroatom bond formation. Palladium- and copper-mediated radiosyntheses have been particularly explored. Although favoured in organic chemistry, metal-catalysis poses health hazards in drug synthesis, and is best avoided for the synthesis of molecules for biomedical purposes, if at all possible. This section briefly highlights the most important advances in the area of metal-mediated radiofluorination.

**Palladium-Mediated $^{18}$F-Chemistry**

Palladium involvement in $^{18}$F-fluorination has been studied by various researchers in the field, with the generally accepted pathway relying on a Pd(II)/Pd(IV) catalytic cycle. Lee et al. proposed a high-oxidation state Pd(IV) complex as a source of electrophilic $^{18}$F-fluorine. The uniqueness of this strategy lies in the convenient in situ formation of the oxidative palladium-$^{18}$F-complex, in which the $^{18}$F-species behaves as an electrophile. Reductive elimination results in the release of the $^{18}$F-fluorinated product. Starting with nucleophilic $^{18}$F-fluoride, it is therefore possible to induce umpolung and access electron-rich $^{18}$F-fluorinated aryls without
the use of fluorine gas carrier and consequently, without compromising on specific activity. This strategy requires anhydrous conditions due to the sensitive nature of the Pd core. Radiosynthesis using the palladium(IV)-$^{18}$F-complex is shown in Scheme 2.20 (top).

![Scheme 2.20](image)

**Scheme 2.20:** Top: labelling of aryls using Pd(IV) complex, which acts as a source of electrophilic $^{18}$F-fluorine, developed by Lee and co-workers.\(^{69}\) Bottom: Radiosynthesis of $^{18}$F-paroxetine using the Pd(IV) complex, performed by Kamlet and co-workers.\(^{70}\)
The aforementioned Pd(IV)-mediated approach was employed by Kamlet et al. in the radiosynthesis of $[^{18}\text{F}]$paroxetine, an existing antidepressant medication. The molecule served as a proof-of-concept for the applicability of this strategy to radiolabelling of electron-rich aromatics. The authors did not provide RCYs or RCCs, instead, success of the reaction was measured in units of radioactivity (Ci, in this case) and specific activity (Ci/µmol) (Scheme 2.20, bottom). Radio-HPLC was used for quality control. Suitable purity was obtained for further biological evaluation in baboons.

**Copper-Mediated $^{18}\text{F}$-Chemistry**

Copper-promoted radiolabelling has been studied by numerous researchers worldwide. The Gouverneur group in Oxford has been particularly interested in strategies employing this versatile transition metal. Relying on the previously reported copper(II)-mediated formation of carbon-heteroatom bonds (Chan-Lam reaction) and fluorination of aryl stannanes and trifluoroborates via a copper(II) complex, Tredwell et al. proposed a strategy combining the best of both worlds. Using approximately 10 mol% loading of a copper pyridine complex, $[\text{Cu(OTf)}_2(\text{py})_4]$, a broad scope of electronically-diverse aromatics was labelled (Scheme 2.21). 4-$[^{18}\text{F}]$Fluoro-1,1-biphenyl was formed in an excellent RCY of 74 ± 5%. $[^{18}\text{F}]$Fluorination meta to a formyl group proceeded in 59 ± 8% RCY. Lower yields were obtained for substrates with activating substituents para to the leaving group (39 ± 7% for CN and 41 ± 4% for NO$_2$). N-tosyl-protected indoles reacted with $[^{18}\text{F}]$fluoride in 19 ± 4% RCY, while placement of a bulky and electron-rich tert-butyl and bromide meta to the boronic ester afforded the corresponding $[^{18}\text{F}]$fluoroaryl in 28 ± 5% RCY. Substrates with electron-donating groups, such as anisoles, reacted efficiently with $[^{18}\text{F}]$F$^-$. With ortho-methoxy substitution, 11 ± 2% RCY was reached. 3,4-Dianisole reacted with $[^{18}\text{F}]$fluoride in 54 ± 3% RCY. Addition of a methyl substituent ortho to the leaving group resulted in an enhanced RCY of 62 ± 4%. 

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Scheme 2.21: Top: copper-mediated $^{18}$F-labelling of boronic esters. Most of the presented substrates are deactivated and non-activated arenes. RCYs are decay-corrected. Bottom: radiosynthesis of $^{18}$F-L-DOPA, performed by Tredwell and co-workers.73
Tredwell et al. applied their strategy to prepare $^{18}$F-DOPA, a well-established PET tracer for the imaging of various neurological pathologies, e.g. Parkinson’s disease, and oncological targets, such as neuroendocrine tumours. Protection of the hydroxyl and amine moieties by methyl ethers and $N,N$-diBoc, respectively, was required. The protected intermediate was labelled enantioselectively in $55 \pm 23\%$ RCY. Subsequent deprotection in aqueous HI afforded $^{18}$F-L-DOPA quantitatively. The loading of $[\text{Cu(OTf)}_2(\text{py})_4]$ was increased, however, to 25 mol%. The route is presented in Scheme 2.21 (bottom).

**Copper-Assisted Diaryliodonium Salt $^{18}$F-Labelling**

Inspired by advances in the development of practical diaryliodonium salt chemistry for fluorine incorporation, Ichiishi et al. tested the potential impact of copper catalysis on the efficiency of C-F bond formation. Endeavours to facilitate fluorination of aromatic molecules are driven not only by the increasing proportion of fluorine-containing therapeutics, but also the growing demand for elegant solutions for fluorine-18 chemistry. Ichiishi and co-workers employed catalytic copper(II) triflate, in conjunction with KF, to effectuate fluorination of asymmetric diaryliodonium salts. The scope embraced aryls decorated with electron-withdrawing and electron-rich substituents, the latter being of particular interest. Fluorination of mono- to tri-methoxy-substituted phenyls proceeded more efficiently in the presence of copper. Surprisingly, for activated aryls possessing nitrile, carbonyl or methylsulfonyl moieties, fluorination was hampered when assisted by $\text{Cu(OTf)}_2$. The authors complemented their investigation with *in silico* methods to uncover the reaction mechanism. Early findings pointed towards a catalytic cycle, in which Cu(II) is oxidised to Cu(III) by the iodonium salt. Subsequent reductive elimination of the fluorinated aryl was thought to occur via a low-lying transition state. Copper-catalysed transformations investigated by Ichiishi et al. are shown in Scheme 2.22.
Scheme 2.22: Copper-catalysed fluorination of diaryliodonium precursors, developed by Ichiishi and co-workers. Representative electron-rich substrates are shown, together with yields with and without Cu-mediation. Ratio of desired product to side-product (2-fluoro-1,3,5-trimethylbenzene) is shown in parentheses.

In the following publication, Ichiishi and co-workers utilised their newly-developed copper-mediated approach to incorporate $^{18}$F fluoride into aromatic systems. $^{18}$F (MeCN)$_4$CuOTf was found to mediate radiofluorination of anisoles more efficiently. A scope encompassing non-activated and deactivated substrates was incorporated into (mesityl)arylodonium salts. Radiolabelling conditions and examples of successfully labelled scaffolds are shown in Scheme 2.23.

Scheme 2.23: Copper-catalysed $^{18}$F fluorination of diaryliodonium precursors, performed by Ichiishi and co-workers. Representative electron-rich substrates are shown with RCYs.
2.4 Summary Review of $^{18}$F-Labeling Strategies for Aryls

This chapter summarised the most important strategies for $^{18}$F-incorporation into drug-like molecules for PET imaging. For aromatic molecules decorated with leaving groups, such as NO$_2$ or NMe$_3^+$, S$_N$2 substitution usually affords radiolabelled products in good RCYs. Biomolecules, such as as proteins and peptides, require intricate handling, hence $^{18}$F-based prosthetic groups are used instead of $[^{18}$F]fluoride. Conveniently, installation proceeds under mild conditions without affecting sensitive entities. Performing organofluorine chemistry on aromatic scaffolds with $[^{18}$F]F$^-$ is particularly challenging and only scaffolds decorated with strong electron-withdrawing groups in the ortho- or para-positions react efficiently. Good leaving groups are required. Labelling of electron-rich and electron-neutral scaffolds has, for that reason, remained inaccessible. This, in turn, has hampered clinical manufacture of promising radiopharmaceuticals. A multitude of novel $[^{18}$F]fluorination strategies have emerged in the last decade. Reviews by Preshlock and van den Born provide an extensive outlook on the state-of-the-art radiolabelling technologies, up until mid-2017. The routes presented in Scheme 2.24 were chosen based on their applications to radiolabelling of electron-rich and electron-neutral aromatics. They represent some of the most attractive and practical approaches to date.

One of the main challenges that researchers face is the lack of consistency in reporting experimental findings. This is particularly evident in the field of PET chemistry and radiochemistry. In addition, despite there being a plethora of extensive reviews, a critical perspective is often lacking. Essential information about the way RCYs are established is frequently omitted. Some authors do not discuss limitations of their approach or provide information about potential for GMP production. As a result, it is often difficult to make a fair comparison of the available technologies.
Scheme 2.24: Various strategies for late-stage aromatic $[^{18}\text{F}]$fluorination of electron-neutral and electron-poor scaffolds with $[^{18}\text{F}]$fluoride. Only the most recent or innovative routes are presented. Adapted from van der Born and co-workers.\textsuperscript{32}
The potential of new $^{18}$F-fluorination strategies is measured by: 1) RCY and ease of synthesis and purification, 2) precursor stability and 3) specific activity, among others. The method should be broadly applicable, automatable and adaptable to GMP environments.\(^{77}\)

Metal-based $^{18}$F-fluorination is generally disfavoured and metallic residue-directed quality control must be performed to ensure that regulatory limits have been met. Although the emergence of palladium-mediated radiolabelling represents a major advancement in the field, preparation of precursors is challenging and expensive. Palladium complexes are air and moisture sensitive and must be synthesised fresh before each labelling experiment. Moderate RCYs are obtained for electron-rich and electron-neutral precursors.

Copper-mediated $^{18}$F-fluorination using boronic esters is perhaps the most attractive strategy among the metal-based routes, however there are severe limitations associated with its use. Firstly, the presence of copper residues must be quantified for every tracer production, which is not only cumbersome and time-consuming, but could also suffer from reproducibility issues. This can be particularly problematic for large scale syntheses. In addition, high precursor loadings are employed (0.02-0.06 mmol $\approx$ 14-40 mg), requiring robust and high yielding preparations.\(^{78}\) Moderate RCYs were obtained for electron-rich and electron-poor precursors.

Iodine(III)-based radiochemistry employs diaryliodonium salts or iodonium ylides to label scaffolds decorated with electron-donating substituents. Moderate RCYs were obtained with (hetero)aryliodonium salts. Precursors are prone to decomposition and have limited shelf-stability, requiring frequent resynthesis. Harsh reaction conditions ($>\ 150\ ^\circ\ C$) are required for their preparation, which poses severe restrictions on the type of scaffolds which can be labelled, particularly with regards to functional and protecting groups. Iodonium salts suffer from regioselectivity issues and the outcome of radiolabelling is often difficult to predict. Purification of undesired radioactive products is often challenging.
On the other hand, iodonium ylides offer a mild route to labelling non-activated aromatics. They address photostability issues of diaryliodonium salts and exhibit enhanced selectivity for $[{^{18}}F]$fluorination. Moderate RCYs were generally reported, favouring electron-deficient substrates. Applicability of the strategy to highly-functionalised drug-like molecules was exemplified using $[{^{18}}F]$FPEB. Precursors have long shelf-stability, however their preparation is restricted to non-oxidisable functional groups, which might limit practicality of the method in favour of simpler transformations.

Sulfonium salts have also been used as leaving groups in $[{^{18}}F]$-labelling. The substrate scope investigated by Mu et al. was, however, confined to simple deactivated and non-activated $[{^{18}}F]$fluoroarenes. One of the major limitations lies in poor synthetic access to precursors.

Despite remarkable developments in the field, there is little information about scalability of these methods to GMP environments. Applicability to clinical radiopharmaceutical production is the end goal of PET chemistry research. Novel alternative strategies are very much in demand, however most of the currently adopted protocols still rely on conventional methods, such as the GMP radiosynthesis of $^{18}$F-DOPA (Section 2.2.2 Scheme 2.7 page 29). A more flexible platform is needed to access clinically attractive targets and sadly, the scope of robust, automatable and adaptable technologies remains insufficient.
3 Sulfonium Salts as $^{18}\text{F}$-Labelling Precursors

3.1 Triarylsulfonium Salts

Building on the pioneering work by Mu et al. (Chapter 2, Section 2.3.1, page 39), the Årstad group realised the potential of sulfonium salt chemistry for radiofluorination. Efforts were directed towards exploring the capacity of triarylsulfonium salts to act as leaving groups in radiolabelling, with the focus on non-activated and deactivated aromatic molecules. Mu and co-workers demonstrated that good RCYs can be obtained for $[^{18}\text{F}]$fluorobenzene and simple aromatics decorated with para-amide or para-halide substituents. The strategy proved inadequate for electron-rich methoxy and methyl groups. Scope elaboration to more sophisticated scaffolds was also required.

Sander et al. adopted and optimised the protocol for sulfonium salt synthesis, established by Crivello and Lam, to afford a para-ketone functionalised triarylsulfonium salt. The precursors were prepared by coupling of diaryliodonium triflate with appropriate thioethers using copper(II) benzoate catalysis. Ketones substituted with aliphatic amine entities, such as piperidine, were protonated with TfOH prior to addition of the copper salt. Generic sulfonium salt formation reaction and the substrate scope, developed by Sander and co-workers, are shown in Scheme 3.1. Moderate to excellent yields of precursors were obtained in DMSO. Pleasingly, radiolabelling proceeded with satisfactory RCYs at 110 °C, in the presence of unprotected hydroxyl and amine moieties, representing a major breakthrough in nucleophilic $[^{18}\text{F}]$fluorination.
Scheme 3.1: First attempts at the synthesis and labelling of highly-functionalised triarylsulfonium salts, performed by Sander and co-workers.

Figure 3.1: Left: optimised triarylsulfonium salt structure with methoxy groups in the *para*-position to the sulfur atom. Right: electron-neutral aromatic system successfully labelled using the optimised scaffold.

Installation of methoxy groups *para* to the sulfur atom of the sulfonium salt scaffold improved radiolabelling of an electron-neutral piperidin-4-ylidene-based scaffold (from 10 ± 1% to 16 ± 3% decay-corrected isolated RCY) by directing [*18*F]fluoride to the less electron-dense ring (Figure 3.1). Labelling of other *N*-heterocyclic sulfonium salts using this method is described further in later chapters. [*18*F]Fluorination of the pyridine moiety at the 2-position proceeded with excellent RCYs in the range of 68-84% (Chapter 7). An imidazole-decorated substrate underwent [*18*F]-labelling in
a moderate decay-corrected isolated RCY of 20 ± 2% (Chapter 8).

Efforts of the Årstad group have opened opportunities for the synthesis of PET radiopharmaceuticals, previously unrealisable by conventional labelling methods. Despite improvements, the synthetic route to triarylsulfonium salts has its limitations. Substrates must be able to withstand high temperatures (up to 125 °C), which increases the risk of decomposition and side reactions, e.g. polymerisations. Whilst a versatile strategy has been established for electron-deficient systems, nucleophilic \(^{18}\text{F}\)fluorination at electron-rich aromatics still remains a challenge. Consequently, to expand the scope of PET radiotracers, it was essential to develop a new generation sulfonium salt which would complement the existing strategy.

3.2 New Generation Dibenzothiophene Sulfonium Salts

Preparation of challenging radiotracers can be facilitated by a different class of sulfonium salts designed and developed by Dr Thibault Gendron (UCL Radiochemistry, unpublished work). Dibenzothiophene sulfonium salts could address those labelling issues which cannot be solved by the acyclic counterpart (Section 3.1, Figure 3.1). It was speculated that with these structural modifications, it would be possible to expand the scope of \(^{18}\text{F}\)-labelled aromatics. A dibenzothiophene sulfonium salt is a tricyclic scaffold, where the central sulfur atom is substituted by the molecular structure of the desired PET radiotracer (Figure 3.2, right). Once the labelling target is incorporated into the generic diaryl thioether scaffold, ring closure results in the formation of the desired sulfonium salt. A small library of diaryl thioether scaffolds was synthesised to elucidate the most appropriate leaving group for \(^{18}\text{F}\)-labelling. The lead candidate offered the most favourable combination of: 1) regioselective radiolabelling of a model aromatic system (\([^{18}\text{F}]\)fluorobenzene) and 2) yield of dibenzothiophene formation.
Dibenzothiophene sulfonium salts are accessed from the corresponding diaryl thioethers. Formation of the latter is achieved through a 2-step sequence of Pd-mediated transformations. Protection of the sensitive thiol moiety using an aliphatic thiol surrogate, 2-ethylhexyl 3-mercaptopropionate (Scheme 3.2), is required to avoid palladium catalyst poisoning. The protecting group was designed by Itoh and Mase in an effort to circumvent issues arising from palladium reactivity with standard carbonyl protecting groups, for example in Suzuki-Miyaura couplings. Conveniently, it is commercially available and affordable. The first step of the diaryl thioether synthesis involves installation of the aforementioned alkyl chain into 2-bromo-1-iodo-4-methylbenzene in a Pd-mediated coupling. Using conditions established by Itoh and Mase, the desired thioether can be accessed in quantitative yields using Pd$_2$(dba)$_3$ and xantphos (ligand). The next step involves addition of the dimethoxybenzene moiety via Suzuki coupling, employing 3,5-dimethoxyphenylboronic acid and Pd(PPh$_3$)$_4$. The protected diaryl thioether, ready for further functionalisations, can be obtained in 84% yield. The entire synthetic route (Scheme 3.2) is practical and robust on a multigram scale and the protected diaryl entity has potential as a commercial product.
Scheme 3.2: Synthesis of a diaryl thioether, a precursor to dibenzothiophene sulfonium salts, using a sequence of Pd-catalysed reactions. The reagent can be prepared conveniently on a multigram scale.

Ring-closing sulfonium salt formation was reported in literature by several researchers, mainly in the interest to synthesise sulfur-containing polymers for electronic purposes. Haryono et al. described formation of dibenzothiophene sulfonium salts from TfOH-promoted condensation of arylsulfoxides. Recently, Vasu et al. investigated metamorphosis of dibenzothiophenes into triphenylenes via a cyclic sulfonium salt intermediate. Cyclisation was effectuated through a AgBF₄-mediated substitution of a bromoalkyl at the sulfur centre. The authors described that the substrates underwent rapid dealkylative decomposition as a result of excellent leaving group ability of dibenzothiophenes. The reaction is thought to follow a similar mechanism to that of the Pummerer rearrangement of sulfoxides, which proceeds via a reactive sulfonium salt intermediate. Chen et al. proposed a route to 4H-thiopyran-4-ones, in which a chlorosulfonium salt intermediate was formed from tetrahydro-4H-thiopyran-4-one.
using N-chlorosuccinimide (NCS). Similar intermediates can also be observed in the chlorination of unsymmetrical dialkyl sulfides with NCS, sulfuryl chloride or Cl₂, to afford α-chloro sulfides. Building on the described work, Gendron investigated analogous ring-closing reactions of diaryl thioethers, mediated by an electrophilic chlorine source.

NCS was chosen as the most convenient electrophilic reagent for the cyclisation. It is affordable and easy to handle, as opposed to molecular chlorine or SO₂Cl₂. AgOTf was employed to drive the reaction forward by forming AgCl precipitate, as well as to provide the triflate counterion. Encouragingly, an excellent yield of 79% was obtained with a non-activated model compound in refluxing 1,4-dioxane (Scheme 3.3). Interestingly, both NCS and AgOTf were required for the cyclisation to occur. Dibenzothiophene sulfonium salts can be purified by silica gel flash column chromatography using a DCM/MeOH eluent system. The protocol is straightforward, owing to the large difference in Rf values between the product and the starting material.

![Scheme 3.3](image)

**Scheme 3.3:** Successful cyclisation of a model diaryl thioether mediated by AgOTf and NCS in refluxing 1,4-dioxane. The desired sulfonium salt was accessed in 79% yield.

These observations served as a proof-of-concept and foundations for further investigation. To demonstrate practical applications, the novel strategy required substantial substrate scope elaboration, with the focus on highly-functionalised non-activated and deactivated aromatics.
4 | Project Description: Aims and Objectives

This thesis builds on the knowledge gained in the investigation of sulfonium salts as leaving groups for $^{18}$F-labelling, performed by Sander and Gendron of the Årstad group (UCL Radiochemistry).

The aim of this work was to explore the potential of dibenzothiophene sulfonium salts as a technically-simple, robust and versatile method for $^{18}$F-fluorination of highly-functionalised small drug-like molecules. It was evaluated through extension of the substrate scope, encompassing non-activated and deactivated aromatic systems with $N$-heterocyclic moieties. The strategy was exploited to: 1) simplify radiosyntheses of established and existing tracers and improve their radiochemical properties, 2) develop novel and more sophisticated PET tracers.

The potential of dibenzothiophene sulfonium salts as versatile precursors for $^{18}$F-labelling was first exemplified using $[^{18}\text{F}]$FPEB in Chapter 5 in a proof-of-concept radiolabelling, preceded by an optimisation process of sulfonium salt formation. Insights into the cyclisation mechanism are provided in Chapter 6, which describes the quest for a mild and robust reaction system using a simplified phenyl model compound. In Chapter 7 the scope is expanded to simple structures decorated with $N$-heterocycles (indole and pyridines), motifs commonly found in drug molecules and PET tracers. Efforts towards finding a compatible dibenzothiophene formation strategy are described.

Building on these results, Chapters 8 and 9 focus on the practical aspect of dibenzothiophene sulfonium chemistry work and present applications of this method to the synthesis of novel PET tracers for the imaging of aldosterone-producing adenomas
(APAs), one of the main causes of hyperaldosteronism. The scaffold described in Chapter [is a phenylimidazole-based small molecule inhibitor of aldosterone synthase. Synthetic challenges associated with formation of the corresponding sulfonium salts are described. Several approaches are also presented in an effort to obtain the desired product, followed by insights into $^{18}$F-labelling. Chapter [presents an alternative pyridine-based tracer structure for the imaging of APAs. Successful cyclisation to the sulfonium salt, subsequent high-yielding radiolabelling and preliminary biological evaluation are described.
5 Synthesis of Dibenzothiophene Sulfonium Salt Precursor to $^{18}$F|FPEB: Proof-of-Concept

This chapter presents early stages of the research, based on the findings by Sander et al., with insights into new generation dibenzothiophene sulfonium salts provided by Dr Thibault Gendron (UCL Radiochemistry). Successful formation of a cyclic sulfonium salt decorated with a phenyl moiety and subsequent labelling of $^{18}$F|fluorobenzene inspired further investigation into their potential as versatile precursors for $^{18}$F-labelling of highly-functionalised drug-like molecules. To demonstrate applicability of the method to structurally-sophisticated scaffolds, 3-$^{18}$F|fluoro-5-(2-pyridinylethynyl)benzonitrile ($^{18}$F|FPEB) was selected as a proof-of-concept molecule. This chapter summarises efforts towards the synthesis of a dibenzothiophene sulfonium salt precursor to $^{18}$F|FPEB. Insights into reactivity of the sulfur centre are provided. Subsequent $^{18}$F|fluorination allowed for the assessment of practicality and robustness of this strategy to access highly-functionalised deactivated molecules, such as $^{18}$F|FPEB.

5.1 $^{18}$F|FPEB: PET Tracer for Imaging of mGluR5 Receptor

$^{18}$F|FPEB is a promising tracer candidate for the imaging of the metabotropic glutamate receptor 5 (mGluR5), implicated in various neurological pathologies (Figure 5.1). Its clinical applications have been impeded by the lack of efficient $^{18}$F-incorporation strategies.
Figure 5.1: $^{18}$F-FPEB is a PET tracer candidate for the imaging of the mGlu5 receptor.

The mGluR5 receptor is a subtype of G-protein coupled mGluR receptors, responsible for signal transmission by glutamate, the key excitatory neurotransmitter in mammals. MGlur5 belongs to class I metabotropic glutamate receptors, which are known to activate phospholipase C - an enzyme responsible for lipid hydrolysis. MGlur5 receptors orchestrate various physiological processes in the brain, e.g. signal transduction, neuronal development and growth, dysregulation of which have been implicated in various pathologies, including Parkinson’s disease, epilepsy and amyotrophic lateral sclerosis (ALS). Expressed in various human cancer types, mGluR5 has also been involved in tumour development, i.e. glutamate has been shown to promote tumour cell growth.

Design of specific mGluR5 receptor agonists and antagonists proved challenging due to high homology of the glutamate binding site across the mGluR receptor family. It was the development of high-affinity diaryl alkyne mGluR5 ligands (Figure 5.2) that opened up opportunities for PET imaging of the mGluR5 receptor. Hamill and co-workers radiolabelled promising mGluR5 antagonists and performed in vitro and in vivo analysis in rhesus monkeys.
Figure 5.2: Existing mGluR5 ligands labelled with carbon-11 and fluorine-18 for PET studies.\(^{03}\)

Conveniently substituted with fluorine, \(^{18}\text{F}\)FPEB displayed high potency for human mGluR5 (IC\(_{50}\)=0.66 nM), measured using calcium ion flux assays with glutamate, as well as suitable lipophilicity (log\(P\)=2.8)\(^{03}\). PET images in monkeys showed efficient brain-blood-barrier entry and specific signal, with significant uptake in the stratum, where mGluR5 is expressed. In 2013 Wong et al. reported the first PET study, in which \(^{18}\text{F}\)FPEB was administered to healthy human subjects in an effort to establish the whole-body pharmacokinetics. The tracer accumulated in mGluR5-rich locations and was judged safe and tolerable for human use\(^{04}\). Pleasingly, lower radiation doses were recorded than for similar scans with \(^{18}\text{F}\)FDG.

Recently, Felts et al. reported a blocking study using \(^{18}\text{F}\)FPEB in their clinical evaluation of a novel mGluR5 allosteric modulator. Their observations provided further evidence towards potential suitability of the tracer for the imaging of mGluR5, and implications there-of in neurodegenerative diseases and cancer\(^{05}\). Unfortunately, routine clinical use of \(^{18}\text{F}\)FPEB has been impeded by the lack of efficient radiosynthetic routes.
The first $^{18}$F-labelling was performed by Wang et al. A one-step nucleophilic aromatic substitution using a nitro-precursor (Scheme 5.1) afforded $[^{18}\text{F}]$FPEB in a low RCY of 5\%. The lack of efficient radiochemical routes has hampered clinical use of $[^{18}\text{F}]$FPEB.

**Scheme 5.1:** Radiolabelling strategy for $[^{18}\text{F}]$FPEB, starting from a nitro-substituted precursor, performed by Wang and co-workers$^{96}$. The isolated RCY was 5\%.

Radiosynthesis was significantly improved with the emergence of iodonium ylides (Chapter 1, Section 2.3.1, page 38) as precursors for $^{18}$F-labelling (Scheme 5.2), developed by Stephenson et al.$^{65}$.

**Scheme 5.2:** Radiolabelling strategy for $[^{18}\text{F}]$FPEB, starting from an iodonium ylide precursor, developed by Stephenson and co-workers$^{65}$. The isolated non-decay-corrected RCY is 20 ± 5\%.
5.1.1 Synthesis of Sulfonium Salt Precursor to $[^{18}\text{F}]$FPEB

Scheme 5.3: Synthesis of sulfonium salt 7.
Reagents and conditions: (i) Pd$_2$(dba)$_3$, xantphos, Et$_3$N, toluene, reflux, 4.5 h; (ii) 3,5-Dimethoxyphenylboronic acid, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene/water, reflux, 5 h; (iii) 3,5-Dibromobenzonitrile, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 15 min; (iv) Pyridine 9, Pd(PPh$_3$)$_4$, toluene, reflux, 2 h; (v) Optimisation of cyclisation conditions; (vi) nBuLi, Me$_3$SnCl, -78 °C → rt, 1 h.
Dibenzothiophene sulfonium salt precursor to $[^{18}\text{F}]$FPEB 7 was synthesised from
diaryl thioether 4 (Chapter 3, Section 3.2, page 55), which was coupled to 3,5-
dibromobenzonitrile in a Pd-mediated transformation with DPEphos as a ligand
(Scheme 5.3). Subsequent Stille coupling to 3-((trimethylstannyl)ethynyl)pyridine
afforded thioether precursor 6 in 73% yield. Subsequent treatment with AgOTf and
NCS in refluxing 1,4-dioxane (analogously to Scheme 3.3) afforded dibenzothiophene
sulfonium salt precursor 7 in only 6% analytical yield (HPLC).

Scheme 5.4: Conditions established by Dr Thibault Gendron were initially used to
effectuate formation of product 7. It was obtained in poor yield.

$[^{18}\text{F}]$Fluorination of precursor 7 (performed by Dr Thibault Gendron) proceeded
with an excellent non-corrected isolated RCY of 43 ± 2% (Scheme 5.5). This was the
first experimental application of dibenzothiophene sulfonium salts to the synthesis of
a PET tracer. In addition, to the best of my knowledge, this is the highest yielding
radiolabelling of $[^{18}\text{F}]$FPEB to date, offering radiosynthetic advantages over the
route proposed by Stephenson et al. (Scheme 2.17): (i) higher RCY and (ii) lower
precursor load (1.6 µmol vs 8 µmol).

Scheme 5.5: Optimised $^{18}\text{F}$-labelling of sulfonium salt 7, carried out by Dr Thibault
Gendron. The isolated non-decay-corrected yield (n=5) was 43 ± 2%.

With this successful labelling in hand, it was imperative to develop an efficient
cyclisation strategy to increase the potential of dibenzothiophene sulfonium salts as precursors for $^{18}$F-labelling. Low cyclisation efficiency renders the synthesis of sulfonium salt 7 impractical.

It was speculated that by increasing sulfur nucleophilicity, formation of the chloro-sulfonium intermediate would proceed more readily. The role of Lewis acid in the cyclisation was considered. As demonstrated by Gendron on a simplified phenyl scaffold, both NCS and AgOTf were required for dibenzothiophene formation (Chapter 1, Section 3.2). Non-toxic and inexpensive Lewis acids, commonly employed in synthetic organic transformations, were selected for further investigation. A series of cyclisation experiments was designed, the results of which are presented in Table 5.1.
Table 5.1: Screening of Lewis acids and reaction conditions of the cyclisation reaction to form $[^{18}F]FPEB$ sulfonium salt precursor 7.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lewis Acid</th>
<th>Solvent</th>
<th>Temperature $[^{°}C]$</th>
<th>Time [h]</th>
<th>Reaction Conditions</th>
<th>Analytical Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AgOTf</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>6%</td>
</tr>
<tr>
<td>2</td>
<td>Ag(phen)OTf</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>La(OTf)$_3$</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>7%</td>
</tr>
<tr>
<td>4</td>
<td>TiCl$_4$</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>Side product$^a$</td>
</tr>
<tr>
<td>5</td>
<td>Bi(OTf)$_3$</td>
<td>1,4-dioxane</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>48%</td>
</tr>
<tr>
<td>6</td>
<td>Bi(OTf)$_3$</td>
<td>DMF</td>
<td>120</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>7%</td>
</tr>
<tr>
<td>7</td>
<td>Bi(OTf)$_3$</td>
<td>DCE</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>Mixture of products$^b$</td>
</tr>
<tr>
<td>8</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>7%</td>
</tr>
<tr>
<td>9</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>Reflux</td>
<td>0.5</td>
<td>Anhydrous</td>
<td>76%</td>
</tr>
<tr>
<td>10</td>
<td>10 mol% Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>Reflux</td>
<td>2.5</td>
<td>Anhydrous</td>
<td>2%</td>
</tr>
<tr>
<td>11</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>10 min</td>
<td>Anhydrous</td>
<td>53%</td>
</tr>
<tr>
<td>12</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>1</td>
<td>Anhydrous</td>
<td>62%</td>
</tr>
<tr>
<td>13</td>
<td>50 mol% Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>10 min</td>
<td>Anhydrous</td>
<td>13%</td>
</tr>
<tr>
<td>14</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>10 min</td>
<td>Ambient$^c$</td>
<td>65%</td>
</tr>
<tr>
<td>15$^d$</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>0.5</td>
<td>Ambient</td>
<td>0%</td>
</tr>
<tr>
<td>16$^e$</td>
<td>Bi(OTf)$_3$</td>
<td>MeCN</td>
<td>rt</td>
<td>0.5</td>
<td>Ambient</td>
<td>0%</td>
</tr>
</tbody>
</table>

$^a$ Mixture of products, co-elution on HPLC complicated identification of reaction components.  
$^b$ Mono- and dichlorinated sulfonium salts, later confirmed by LC-MS analysis.  
$^c$ Ambient refers to non-anhydrous conditions and no air exclusion.  
$^d$ Control experiment: no NCS.  
$^e$ Control experiment: no Bi(OTf)$_3$. 
Ag(phen)OTf, a phenanthroline-based silver complex, has shown superior catalytic properties to AgOTf in various intramolecular transformations. Sadly, no sulfonium salt formation was observed (Table 5.1, Entry 2). La(OTf)₃ performed only slightly better, and sulfonium salt 7 was obtained in 7% yield (Entry 3). Titanium tetrachloride reacted aggressively with thioether 6, giving rise to a mixture of products. The hygroscopic nature of this reagent makes its handling cumbersome (Entry 4).

The high catalytic potential of bismuth salts in organic synthesis has been highlighted by many researchers in the field. Convenient transformations mediated by bismuth(III) complexes were reported for cyclisation-type reactions, for example Diels-Alder. In many instances, bismuth compounds outperformed the usually high-yielding scandium- or titanium-based catalysts. The recent review by Ondet et al. classifies Bi(OTf)₃-mediated cyclisations based on their mode of activation. Of particular interest is its mode of heteroatom activation. The [3+2]-type cycloaddition of N-tosylaziridine and benzonitrile is thought to proceed via activation of aziridine nitrogen by the bismuth centre, resulting in enhancement of electrophilicity (Scheme 5.6). Bi(OTf)₃-mediated carbon-sulfur bond formations, such as sulfonylation of aromatics, were discussed by Leonard and co-workers.

When stoichiometric Bi(OTf)₃ was employed in the cyclisation reaction of thioether 6, a significantly enhanced yield of 48% was reported (Table 5.1, Entry 5). To further explore the cyclisation potential of bismuth triflate, a range of high-boiling polar aprotic solvents was screened. Based on the assumption that a solvent with a higher dielectric constant would stabilise the charged transition state better, dichloroethane (DCE), DMF and MeCN were selected (Entries 6, 7 and 8). Although a promising HPLC trace was obtained with DCE, additional LC-MS and NMR analysis revealed the presence of mono- and dichlorinated side products (Figure 5.3). MeCN proved to be the most suitable solvent. Formation of desired sulfonium salt 7 (as the sole...
product) proceeded with an excellent analytical yield of 79%. Nearly full consumption of starting material 6 was observed.

Figure 5.3: Mono and dichlorinated side products identified in the cyclisation reaction in refluxing DCE.

With these excellent results at hand, a kinetic study was performed to investigate (qualitatively) the reaction rate of Bi(OTf)$_3$-mediated cyclisations. Aliquots of the reaction mixture in refluxing MeCN were collected at 10, 20, 60, 90, 120 and 150 min and analysed by HPLC. Plotting HPLC conversion versus time revealed that a plateau had been reached between 10 and 30 min (Figure 5.4). An excellent analytical yield of 76% was obtained from a 30 min reaction in MeCN at 90 °C and the product was isolated by flash column chromatography in 68% yield (Table 5.1 Entry 9).

Figure 5.4: Plot of HPLC conversion to product 7 versus time with NCS and Bi(OTf)$_3$ in refluxing MeCN.
In the next step, the effect of reaction temperature was investigated. An attempt to carry out the transformation at room temperature was met with success. A satisfactory analytical yield of 53% was obtained after 10 min. After 1 h, this increased to 62% (Table 5.1, Entries 11 and 12). Minimum losses occurred during purification of the reaction mixture by flash column chromatography. Precursor 7 was obtained in 61% yield.

In an endeavour to make dibenzothiophene sulfonium salts a competitive $^{18}$F-labelling strategy, it was important to consider stoichiometry of Bi(OTf)$_3$ in the cyclisation. Although bismuth triflate is not considered toxic, attempts were made to reduce its loading, with potential translation to GMP environments in mind. Disappointingly, the reaction proved less efficient when 10 and 50 mol% loadings were used (Table 5.1, Entries 10 and 13). It was therefore elucidated that cyclisation required stoichiometric quantities of the Lewis acid. Conveniently, the reaction also proved insensitive to air and moisture, yielding 65% of sulfonium salt 7 when performed in non-anhydrous MeCN at room temperature (Entry 14). Control experiments confirmed that both NCS and the Lewis acid are required for the reaction to take place (Entries 15 and 16).

### 5.2 Conclusion

Building on the pioneering work in the Årstad group, the dibenzothiophene sulfonium salt precursor to $[^{18}\text{F}]$FPEB was prepared as a proof-of-concept for $^{18}$F-fluorination of highly-functionalised drug-like molecules.

In the first instance, emphasis was placed on establishing a robust, practical and efficient cyclisation route to $[^{18}\text{F}]$FPEB. Several Lewis acids and solvents were screened in an endeavour to obtain sulfonium salt precursor 7. Best results were obtained with stoichiometric Bi(OTf)$_3$ and NCS in refluxing MeCN (68% isolated yield). Gratifyingly, the yield was closely reproduced when the reaction was carried out for 1 h at room temperature (61% isolated yield). A practical and robust protocol for the synthesis of sulfonium salt precursors has been developed, which allows to access highly-functionalised drug-like molecules in a rapid one-pot transformation.
at room temperature, without the need for air or moisture exclusion. Conveniently, purification of precursor 7 can be performed by silica gel column chromatography, which allows for the isolation of the desired compound as a stable, non-hygroscopic, crystalline solid.

$[^{18}\text{F}]$Fluorination of sulfonium salt 7 proceeded with an excellent isolated RCY of 43 ±2% (n=5, n.d.c.), representing an improvement on the state-of-art radiosyntheses employing nitro- or iodonium ylide-based leaving groups.

Dibenzothiophene sulfonium salts have the potential to become a practical route for clinical manufacture of $[^{18}\text{F}]$FPEB. A scope study employing drug-abundant motifs is imperative to further ascertain the practical aspect and robustness of the presented strategy.
6 | Insights into Mechanism of Sulfonium Salt Formation

Building on the innovative work on dibenzothiophene sulfonium salts, this chapter summarises further investigation into the putative reaction mechanism. It was imperative to gain a more in-depth understanding of the factors orchestrating the cyclisation in order to expand the scope of substrates for $^{18}$F-labelling. The sulfonium salt precursor to $[^{18}$F]FPEB was initially used as substrate for NMR studies, later to be complemented by a simplified model compound.

6.1 Putative Mechanism of Sulfonium Salt Formation

In the early design stages, Gendron formulated a working hypothesis about the mechanism of dibenzothiophene sulfonium salt formation (Chapter 3, Section 3.2). It was proposed that activation of the nucleophilic sulfur atom by $N$-chlorosuccinimide resulted in the formation a chlorosulfonium intermediate. It was also thought to be the rate-determining step. Breaking of the S-Cl bond triggered intramolecular cyclisation. This is simplistically illustrated in Scheme 6.1.

**Scheme 6.1**: Sulfur acts as a nucleophile towards NCS, resulting in the formation of a chlorosulfonium intermediate, which then collapses to generate a dibenzothiophene sulfonium salt.
6.2 Early Mechanistic Study with NMR Spectroscopy

In an effort to probe reaction intermediates, a preliminary mechanistic study was performed using thioether 6 (Figure 6.1), NCS and Bi(OTf)$_3$ in deuterated MeCN (Figure 6.1).

![Figure 6.1](image_url)

**Figure 6.1:** Thioether 6 and sulfonium salt precursor to [18F]FPEB.

$^1$H NMR spectra were acquired of the following premixed combinations at room temperature: 1) thioether 6 and Bi(OTf)$_3$, 2) thioether 6 and NCS and 3) NCS and Bi(OTf)$_3$. Remarkably, no intermediates were observed in the spectrum of premixed Bi(OTf)$_3$ and NCS. Similarly, peak shifting/disappearance did not occur for the mixtures of thioether 6 and NCS or Bi(OTf)$_3$. A prominent triflic acid peak was, however, present in the latter. Various small multiplets were also observed in all Bi(OTf)$_3$ mixtures. This can be explained by the well-known interaction of TfOH and acetonitrile, resulting in the formation of various aliphatic derivatives.$^{103}$ These NMR experiments provide further evidence that both the Lewis acid and the electrophile are required for cyclisation to occur.

The prominent presence of TfOH in the NMR spectra of Bi(OTf)$_3$ mixtures raised questions about its role in the cyclisation reaction. A small-scale control experiment was set up, in which triflic acid was added to a stirring solution of NCS and thioether 6 in MeCN. Three equivalents of acid were used: 1 equivalent is used to protonate the pyridyl moiety and an excess is available for the transformation. Gratifyingly, complete conversion to sulfonium salt 7 was achieved in merely 5 minutes at room temperature, as judged by TLC analysis. Identity of the product was then confirmed using HPLC. NMR experiments were set up at room temperature and -50 °C, in order to probe intermediate species, however the rate of cyclisation was significantly

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faster than the NMR timescale and instantaneous full conversion to product 7 was observed.

6.2.1 NCS/TfOH System for Cyclisation: Formation of Superelectrophilic Species

Thioether 6 did not make a suitable substrate for mechanistic studies using NMR spectroscopy. Cyclisation occurred too rapidly to allow for observation of intermediates. Furthermore, these preliminary NMR studies uncovered that a Lewis acid might not be necessary to effect cyclisation - similar results can be achieved with a Brønsted acid at significantly lower temperatures. In an ongoing debate about the role of the Bi\textsuperscript{III} centre and triflic acid in organic synthesis, the following scenarios were previously outlined by Ondet \textit{et al.} (Scheme 6.2).

\[
\begin{align*}
\text{Bi(OTf)}_3 + \text{H}_2\text{O} &\xrightarrow{\text{Hydrolysis}} \text{Bi(OTf)}_2(\text{OH}) + \text{TfOH} \\
\text{Bi(OTf)}_2(\text{OH}) + \text{TfOH} &\xrightarrow{\text{Hydration}} \text{(TfO)}_3\text{Bi} \cdots \text{O} \cdots \text{H} \\
\end{align*}
\]

\textbf{Scheme 6.2:} Hydrolysis and hydration of bismuth triflate were identified as possible modes of its interaction with water. Adapted from Ondet and co-workers\textsuperscript{101}.

For reactions catalysed by Bi(OTf)\textsubscript{3}, the true catalytic species could be, according to the hydrolysis scenario, triflic acid. This process requires energy. Conversely, hydration is exothermic and the resulting complexation of water to the bismuth centre increases acidity of water protons. Versatility of Bi(OTf)\textsubscript{3} as a catalyst can be ascribed to its oxo- and carbophilic character. Lambert \textit{et al.} carried out Bi(OTf)\textsubscript{3}- and TfOH-mediated epoxide rearrangements to elucidate their role in the transformation. Remarkably, filtration and consequent removal of suspended Bi(OTf)\textsubscript{3} solids from the reaction mixtures hindered reaction progress. The authors suggested that TfOH, the true catalytic species in the reaction, was released following association of reagents with bismuth triflate salts. The metal centre was thought to facilitate this transformation, owing to its high Lewis acidity. For bismuth, it is the availability of $\sigma^*$ and d-orbitals, in addition to complexation by electron-withdrawing triflate
residues, which are considered to increase its Lewis acidic character.

Olah and co-workers investigated the role of triflic acid in iodination reactions of electron-deficient aromatics with N-iodosuccinimide\textsuperscript{104}. Using \textsuperscript{13}C NMR studies of NIS in triflic acid at -20 °C, they speculated that iodine(I) triflate, formed \textit{in situ}, could be the effective superelectrophilic species (Scheme 6.3).

\begin{center}
\begin{tikzpicture}
\node (a) at (-2,0) {$\text{NIS}$};
\node (b) at (2,0) {$\text{TfOH}$};
\node (c) at (2,-2) {$\text{EWG}$};
\node (d) at (-2,-2) {$\text{EWG}$};
\node (e) at (0,2) {$\text{HO}$};
\node (f) at (0,-2) {$\text{OH}$};
\node (g) at (-2,2) {$\text{N}$};
\node (h) at (2,2) {$\text{N}$};
\node (i) at (-2,4) {$\text{O}$};
\node (j) at (2,4) {$\text{O}$};
\node (k) at (-2,6) {$\text{I}$};
\node (l) at (2,6) {$\text{I}$};
\node (m) at (0,4) {$\text{TfO}$};
\node (n) at (0,2) {$\text{TfOH}$};
\node (o) at (0,0) {$\text{NH}$};
\node (p) at (0,-4) {$\text{I}$};
\node (q) at (0,-6) {$\text{OTf}$};
\node (r) at (2,-4) {$\text{S}$};
\node (s) at (2,-6) {$\text{F}_2$};
\node (t) at (2,-8) {$\text{SO}_2$};
\node (u) at (2,-10) {$\text{I}$};
\node (v) at (-2,-4) {$\text{S}$};
\node (w) at (-2,-6) {$\text{F}_2$};
\node (x) at (-2,-8) {$\text{SO}_2$};
\node (y) at (-2,-10) {$\text{I}$};
\node (z) at (2,-10) {$\text{I}$};
\node (aa) at (-2,-12) {$\text{EWG}$};
\node (bb) at (2,-12) {$\text{EWG}$};
\node (cc) at (0,-12) {$\text{EWG}$};
\node (dd) at (-2,-14) {$\text{EWG}$};
\node (ee) at (2,-14) {$\text{EWG}$};
\node (ff) at (0,-14) {$\text{EWG}$};
\node (gg) at (-2,-16) {$\text{EWG}$};
\node (hh) at (2,-16) {$\text{EWG}$};
\node (ii) at (0,-16) {$\text{EWG}$};
\node (jj) at (-2,-18) {$\text{EWG}$};
\node (kk) at (2,-18) {$\text{EWG}$};
\node (ll) at (0,-18) {$\text{EWG}$};
\node (mm) at (-2,-20) {$\text{EWG}$};
\node (nn) at (2,-20) {$\text{EWG}$};
\node (oo) at (0,-20) {$\text{EWG}$};
\node (pp) at (-2,-22) {$\text{EWG}$};
\node (qq) at (2,-22) {$\text{EWG}$};
\node (rr) at (0,-22) {$\text{EWG}$};
\node (ss) at (-2,-24) {$\text{EWG}$};
\node (tt) at (2,-24) {$\text{EWG}$};
\node (uu) at (0,-24) {$\text{EWG}$};
\node (vv) at (-2,-26) {$\text{EWG}$};
\node (ww) at (2,-26) {$\text{EWG}$};
\node (xx) at (0,-26) {$\text{EWG}$};
\node (yy) at (-2,-28) {$\text{EWG}$};
\node (zz) at (2,-28) {$\text{EWG}$};
\node (aaa) at (0,-30) {$\text{EWG}$};
\node (bbb) at (-2,-30) {$\text{EWG}$};
\node (ccc) at (2,-30) {$\text{EWG}$};
\node (ddd) at (0,-32) {$\text{EWG}$};
\node (eee) at (-2,-32) {$\text{EWG}$};
\node (fff) at (2,-32) {$\text{EWG}$};
\node (ggg) at (0,-34) {$\text{EWG}$};
\node (hhh) at (-2,-34) {$\text{EWG}$};
\node (iii) at (2,-34) {$\text{EWG}$};
\node (jjj) at (0,-36) {$\text{EWG}$};
\node (kkk) at (-2,-36) {$\text{EWG}$};
\node (lll) at (2,-36) {$\text{EWG}$};
\node (mmm) at (0,-38) {$\text{EWG}$};
\node (nnn) at (-2,-38) {$\text{EWG}$};
\node (ooo) at (2,-38) {$\text{EWG}$};
\node (ppp) at (0,-40) {$\text{EWG}$};
\node (qqq) at (-2,-40) {$\text{EWG}$};
\node (aaa) at (2,-40) {$\text{EWG}$};
\node (bbb) at (0,-42) {$\text{EWG}$};
\node (ccc) at (-2,-42) {$\text{EWG}$};
\node (ddd) at (2,-42) {$\text{EWG}$};
\node (eee) at (0,-44) {$\text{EWG}$};
\node (fff) at (-2,-44) {$\text{EWG}$};
\node (ggg) at (2,-44) {$\text{EWG}$};
\node (hhh) at (0,-46) {$\text{EWG}$};
\node (iii) at (-2,-46) {$\text{EWG}$};
\node (jjj) at (2,-46) {$\text{EWG}$};
\node (kkk) at (0,-48) {$\text{EWG}$};
\node (lll) at (-2,-48) {$\text{EWG}$};
\node (mmm) at (2,-48) {$\text{EWG}$};
\node (nnn) at (0,-50) {$\text{EWG}$};
\node (ooo) at (-2,-50) {$\text{EWG}$};
\node (ppp) at (2,-50) {$\text{EWG}$};
\node (qqq) at (0,-52) {$\text{EWG}$};
\node (aaa) at (-2,-54) {$\text{EWG}$};
\node (bbb) at (2,-54) {$\text{EWG}$};
\node (ccc) at (0,-56) {$\text{EWG}$};
\node (ddd) at (-2,-58) {$\text{EWG}$};
\node (eee) at (2,-58) {$\text{EWG}$};
\node (fff) at (0,-60) {$\text{EWG}$};
\node (ggg) at (-2,-60) {$\text{EWG}$};
\node (hhh) at (2,-60) {$\text{EWG}$};
\node (iii) at (0,-62) {$\text{EWG}$};
\node (jjj) at (-2,-62) {$\text{EWG}$};
\node (kkk) at (2,-62) {$\text{EWG}$};
\node (lll) at (0,-64) {$\text{EWG}$};
\node (mmm) at (-2,-64) {$\text{EWG}$};
\node (nnn) at (2,-64) {$\text{EWG}$};
\node (ooo) at (0,-66) {$\text{EWG}$};
\node (ppp) at (-2,-66) {$\text{EWG}$};
\node (qqq) at (2,-66) {$\text{EWG}$};\end{tikzpicture}
\end{center}

\textbf{Scheme 6.3:} Interaction of NIS with TfOH was proposed to lead to the formation of a powerful superelectrophilic species, iodine(I) triflate, capable of iodinating deactivated aromatics. Scheme adapted from Olah and co-workers\textsuperscript{104}.

This protosolvated iodinating agent is particularly useful for severely deactivated aromatics, giving significantly higher yields than those reported with NIS and AgOTf at elevated temperatures\textsuperscript{105}. In their extensive \textit{in silico} investigation, Prakash \textit{et al}. generalised that all \textit{N}-halosuccinimides (Cl, Br, I) are activated in the presence of triflic acid to form multiprotonated superelectrophiles, capable of halogenating aromatic systems decorated with electron-withdrawing substituents\textsuperscript{106}. The degree of protonation of NCS is directly related to its destabilisation. The release of chlorine(I) species is thought to reduce Coulombic repulsion, originating from the multiplicity of positive charges. This effect is likely to act as a driving force in electrophilic aromatic
chlorination, as depicted in Scheme 6.4. The authors speculated that at high acidities, multiprotonation produces enough Coulombic repulsion to force ring opening and formation of the corresponding acylium ion. Acid pK\textsubscript{a} values are considered to be a good measure of reactivity of the superelectrophilic species in aromatic halogenations. Interestingly, Prakash et al. observed that deactivated aromatic systems failed to react in the presence of trifluoroacetic acid.

Scheme 6.4: Chlorination of aromatic systems mediated by protonated NCS species, resulting from the interaction of NCS with TfOH. The driving force of the reaction is thought to be extreme charge repulsion, which causes destabilisation and release of "Cl\textsuperscript{+}". The true nature of the "superelectrophile" has not been confirmed\textsuperscript{106}.

The NCS/TfOH reagent pair is extremely efficient at cyclising thioether 6 to the corresponding sulfonium salt 7 (Figure 6.1); however, undesired ring chlorination could also be expected. In fact, the chlorinated counterpart (Figure 6.2) constituted less than 10% of the isolated sample, as elucidated using NMR spectroscopy, HPLC and LC-MS. Furthermore, it was noted that when an NMR tube containing thioether 6, NCS and TfOH was left overnight, the spectrum obtained thereafter revealed a 50:50 ratio of sulfonium salt 7 to its chlorinated analogue 10. This was later confirmed by LC-MS and HPLC. Surprisingly, a chlorinated analogue of thioether 6 has never been observed. These findings raised fundamental questions:

1. Could weaker acids promote the cyclisation step?

2. Is acid-free sulfonium salt formation possible?

3. Which factors influence the final product distribution?

4. Can ring chlorination be retarded or eliminated?
**Figure 6.2:** The structure of chlorinated sulfonium salt 10, identified as a minor product in the reactions of 6 with NCS and TfOH/Bi(OTf)$_3$, was elucidated using proton NMR spectroscopy.

### 6.3 Design of Model Compound to Investigate Cyclisation Mechanism

To address multiple questions raised during the investigation, a simplified model compound was designed. Although thioether 6 was an interesting substrate for such studies, conversion to sulfonium salt 7 occurred almost instantly, rendering it unsuitable for mechanistic studies using NMR spectroscopy. No intermediates were observed, even at lower NMR operating temperatures (Section 6.2).

It was proposed that the model substrate should possess a fluorine atom, that would allow for additional analysis using $^{19}$F NMR spectroscopy. The advantage of having a fluorine tag is associated with its large spectral window, which eliminates issues of ambiguous peak identities, frequently observed in proton NMR. In addition, protons in close proximity to the fluorine atom display large $J_{HF}$ coupling values and complex splitting patterns. This could perhaps be helpful in identifying intermediates formed in the cyclisation reaction. The position of the fluorine tag was selected based its proximity to the sulfur atom. Fluorine in the ortho-position is expected to experience pronounced changes in response to the formation of the chlorosulfonium intermediate. Thioether 13, shown in Scheme 6.5, is the new substrate in the cyclisation mechanism investigation. It was obtained in 69% yield over two palladium-catalysed steps.
**Scheme 6.5:** Synthesis of sulfonium salt 14.
Reagents and conditions: (i) 2-Bromo-1-iodo-4-methylbenzene, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 2 h; (ii) 3,5-Dimethoxyphenylboronic acid, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene/water, reflux, 5 h; (iii) Optimisation of cyclisation conditions.

### 6.3.1 Expansion of Scope of Cyclisation Reagents: Acids

The presence of strong acid in the cyclisation reaction raised potential incompatibility issues, particularly for dibenzothiophene sulfonium salts decorated with acid-sensitive protecting groups or N-heterocycles. The next step involved assessment of weaker Brønsted acids as NCS activators to form the superelectrophilic species advocated by Prakash et al. Common laboratory acids were selected based on their pK$_a$ values. The aim of the investigation was to provide rapid semi-quantitative analysis. Experiments was performed on a small scale (10-50 mg of thioether 13) and reaction progress was monitored using HPLC and/or LC-MS. Precise analytical yields were of less importance than qualitative conclusions. The results are presented in Table 6.1. A graphical illustration is shown in Figure 6.3.
Table 6.1: The results of a Brønsted acid screen, performed in an effort to increase the practicality of dibenzothiophene sulfonium salt formation. All reactions were carried out with NCS in ambient MeCN, in the presence of NaOTf to provide the triflate counterion. Analytical yields were established using HPLC.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>$pK_a$ (MeCN)</th>
<th>Product [%]</th>
<th>Starting Material [%]</th>
<th>Side Products [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bi(OTf)$_3$</td>
<td>2.6</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>TfOH</td>
<td>2.6</td>
<td>88</td>
<td>8</td>
<td>4$^b$</td>
</tr>
<tr>
<td>3</td>
<td>$p$-TSA</td>
<td>8.0</td>
<td>33</td>
<td>66</td>
<td>1$^c$</td>
</tr>
<tr>
<td>4</td>
<td>TFA</td>
<td>12.7</td>
<td>4</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Benzoic acid</td>
<td>21.5</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Source of TfOH.  
$^b$ Chlorinated sulfonium salt: 1%. Unknown impurity: 3%.
$^c$ Chlorinated sulfonium salt.

Cyclisation of thioether 13 was attempted using NCS and Bi(OTf)$_3$ and afforded 90% conversion to sulfonium salt 14 within 10 minutes (Table 6.1, Entry 1). This result was closely reproduced with NCS and TfOH, however, negligible presence of the corresponding chlorinated sulfonium salt was observed (Entry 2). Subsequent flash column chromatography purification afforded sulfonium salt 14 in 66% yield. Both reactions proceeded too rapidly to allow for intermediates to be observed by NMR spectroscopy. In the next attempt, cyclisation was performed with $p$-toluenesulfonic acid ($p$-TSA). $p$-TSA is a solid and more convenient to handle than fuming TfOH. Sadly, only 33% of product 14 was obtained (Entry 3). Both TFA and benzoic acid gave unsatisfactory results (Entries 4 and 5). It can be inferred that efficiency of dibenzothiophene sulfonium salt formation is closely related to the strength of the Brønsted acid. It has been demonstrated that dibenzothiophene sulfonium salt formation can be achieved without the bismuth cation. The use of triflic acid, although less practical, eliminates concerns about trace metal residues in the precursor, paramount for potential GMP compliance of the strategy to the manufacture of, for instance $[^{18}\text{F}]$FPEB. The optimised route to dibenzothiophene sulfonium salt 14 is shown in Scheme 6.6.
6.3.2 Expansion of Scope of Cyclisation Reagents: Hypochlorites

In a parallel study, the use of alternative reagents to achieve sulfonium salt formation was explored. To implement dibenzothiophene precursors for practical widespread applications, it was imperative to investigate milder chlorinating agents that could overcome the use of strongly acidic TfOH.

It was speculated that hypochlorites could be employed as electrophiles to effectuate ring closure. The range of commercially available hypochlorite reagents is limited. Organic hypochlorites are explosive and hazardous to handle, while most inorganic hypochlorites exist as solutions in water, e.g. sodium hypochlorite (bleach). Small-scale reactions of thioether 13 with hypochlorites, as the sole electrophilic reagent,
were analysed by HPLC and/or LC-MS.

The first candidate, tBuOCl, is a highly reactive organic hypochlorite, commonly employed in chlorination reactions of nitrogenous compounds, e.g. benzylamines and N-chloramines. Interestingly, and perhaps pertinent to the current investigation, tBuOCl does not chlorinate anisoles at low temperatures, unless assisted by an acid catalyst. It is synthesised using commercial grade bleach, acetic acid and tert-butanol (Scheme 6.7). Due to its inherent instability, it was immediately employed in cyclisation reactions. Fresh batches of tBuOCl were prepared on every occasion.

\[
\text{NaOCl} + \text{AcOH} \xrightarrow{0 \, ^\circ \text{C}, 3 \, \text{min}} \text{tBuOCl}
\]

**Scheme 6.7:** Synthesis of tBuOCl.

Sodium hypochlorite exists as a mixture of species in water (Scheme 6.8). Bleach can be used to chlorinate phenols under basic conditions.

\[
\text{NaOCl} \rightleftharpoons \text{Na} + \text{OCl} \\
\text{OCl} + \text{H}_2\text{O} \rightleftharpoons \text{HClO} + \text{OH}
\]

**Scheme 6.8:** Active species in an aqueous solution of sodium hypochlorite.

Calcium hypochlorite is another member of the hypochlorite family, used in organic synthesis as an oxidant and chlorinating agent. Successful aromatic chlorinations mediated by Ca(OCl)\(_2\) were reported for electron-rich substrates at 0 °C in aqueous acetone or AcOH. The results of the hypochlorite investigation with thioether are shown in Table 6.2.
Table 6.2: Hypochlorite-based reagents were screened in an effort to overcome the use of TfOH. All reactions were carried out in non-anhydrous MeCN, in the presence of NaOTf to provide the triflate counterion. Analytical yields were established using HPLC.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hypochlorite Reagent</th>
<th>Product [%]</th>
<th>Starting Material [%]</th>
<th>Side Products [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tBuOCl</td>
<td>48</td>
<td>14</td>
<td>38(a)</td>
</tr>
<tr>
<td>2</td>
<td>NaOCl (&lt; 5%)</td>
<td>76</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>NaOCl (10-15%)(^c)</td>
<td>11</td>
<td>0</td>
<td>89(^d)</td>
</tr>
<tr>
<td>4</td>
<td>NaOCl (10-15%)(^e)</td>
<td>51</td>
<td>47</td>
<td>2(^f)</td>
</tr>
<tr>
<td>5</td>
<td>Ca(OCl)(_2)</td>
<td>34</td>
<td>63</td>
<td>3(^g)</td>
</tr>
</tbody>
</table>

\(^a\) Chlorinated sulfonium salt: 31%, chlorinated sulfoxides: 7%. \(^b\) Commercial bleach. \(^c\) Reagent grade bleach, used in approximately 25-37 equivalents. \(^d\) Chlorinated sulfonium salt: 76%, chlorinated sulfoxides: 13%. \(^e\) Reagent grade bleach, used in approximately 5-7 equivalents. \(^f\) Chlorinated sulfonium salt: 1%. \(^g\) Unknown impurities.

When thioether 13 was treated with 1 equivalent of tBuOCl, 48% conversion to sulfonium salt 14 was observed after 10 minutes. The chlorinated counterpart constituted 31% of the reaction mixture (Table 6.2, Entry 1). A small amount of the starting material was still present in the reaction mixture. In addition, an extra peak appeared on the trace, later to be identified using LC-MS as a mixture of various chlorinated sulfoxide species. Hypochlorites are strong oxidising agents, hence formation of various oxidation forms, such as sulfoxides, is not surprising. In addition, HCl, the by-product of sulfonium salt formation, can react with a hypochlorite ion to generate chlorine gas, thus increasing the chance of competing ring chlorination. It was assumed that by introducing an antioxidant into the reaction mixture, this process could be inhibited. Addition of ascorbic acid did not alter the HPLC profile. Another idea involved addition of tert-butanol. It was speculated that the alcohol could act as a trap for chloride ions to prevent their re-oxidation. Unfortunately, no changes in reaction composition were observed. With limited control over tBuOCl, the cyclisation was not robust and did not offer any practical advantages over the NCS/TfOH reagent pair.

Commercial bleach (> 5% active chlorine) was employed in a trial cyclisation reaction of thioether 13. Encouragingly, after 10 min at room temperature, HPLC
analysis revealed 76% analytical yield of sulfonium salt 14, with no chlorinated side products (Table 6.2, Entry 2). Furthermore, it was observed that the reaction was tolerant of substantial (50%) water content. The investigation was repeated with more concentrated reagent-grade bleach (10-15% available chlorine), which was used in approximately 25-37 fold excess. Sulfonium salt 14 constituted 11% of the reaction mixture, whereas the chlorinated counterpart accounted for nearly 76% (Entry 3). Starting material was consumed after 10 min. Reduction in the amount of NaOCl (5-7 fold excess) had an impact on the rate of chlorination. Conversion to sulfonium salt 14 was reduced to 51% (Entry 4). Disappointingly, attempts to reproduce these results in subsequent experiments were futile. Low reproducibility of aqueous hypochlorite reagents precluded their use in the cyclisation reaction. Despite the shortcomings, it has been demonstrated that dibenzothiophene sulfonium salt formation does not require the use of Brønsted acid. This was a turning point in the investigation, which inspired further efforts to optimise the cyclisation, in the hope to make it a versatile and robust strategy for the synthesis of radiolabelling precursors.

Encouraged by these results, NaOCl was replaced by Ca(OCl)₂. The reagent is sold as a technical-grade solid, with a higher percentage of available chlorine (65%). Similarly to other calcium salts, it is insoluble in organic solvents, with very limited water solubility. Cyclisation of thioether 13 was first attempted with 1 equivalent of Ca(OCl)₂. Gratifyingly, sulfonium salt 14 was obtained, as the sole product, in 34% analytical yield after 10 minutes (Table 6.2, Entry 5). Consequently, attempts were made to optimise this reaction in the hope of enhancing the yield. The results are presented in Table 6.3.

An analogous cyclisation to that of Entry 5 in Table 6.2 was set up. A 30 min reaction of thioether 13 with 1 equivalent of Ca(OCl)₂ at room temperature afforded the desired sulfonium salt in 30% analytical yield. The reaction was shown to reach a plateau after 1 h (Table 6.3, Entries 1 and 2). It was proposed that better conversions could perhaps be obtained on increasing Ca(OCl)₂ solubility. According to Le Châtelier’s principle - if more hypochlorite reacts with the thioether, more will dissolve in MeCN. The impact of methanol as a solvent was tested, but only
a poor conversion of 8% was observed (Entry 3). The reaction proceeded slowly in 10% water/acetonitrile. Slow eluting impurities were observed in all described experiments. It was proposed that addition of water increases solubility of Ca(OCl)$_2$ and in turn, hydrolysis to Ca(OH)$_2$. Hydroxide could act as a nucleophile and cause ring opening of the newly-formed sulfonium salt (Entry 4). Increasing the number of equivalents of Ca(OCl)$_2$ or sonication had little effect on the rate of cyclisation (Entries 5, 6 and 7).

**Table 6.3:** Further optimisation of cyclisation conditions using CaOCl$_2$. All reactions were carried out in MeCN (unless stated otherwise) in the presence of NaOTf to provide the triflate counterion. Analytical yields were established using HPLC.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time [min]</th>
<th>Product [%]</th>
<th>Starting Material [%]</th>
<th>Side Products [%]</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>20</td>
<td>78</td>
<td>2</td>
<td>New reaction$^a$</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>19</td>
<td>78</td>
<td>3</td>
<td>Reaction mixture from Entry 1</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>8</td>
<td>82</td>
<td>10</td>
<td>In MeOH</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1</td>
<td>89</td>
<td>10</td>
<td>In 9:1 MeCN:H$_2$O$^b$</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>4</td>
<td>91</td>
<td>5</td>
<td>2 eq of Ca(OCl)$_2$ with sonication$^c$</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>10</td>
<td>87</td>
<td>3</td>
<td>Reaction mixture from Entry 5</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>19</td>
<td>78</td>
<td>3</td>
<td>10 eq of Ca(OCl)$_2$ with sonication$^c$</td>
</tr>
</tbody>
</table>

$^a$ Analogous to Table 6.2, Entry 5, but stirred for 30 min.  
$^b$ Cloudy reaction mixture.  
$^c$ The reaction vessel was placed in an ultrasound bath.

Ca(OCl)$_2$ offers certain advantages over other hypochlorite reagents: 1) ease of handling and 2) reduction of chlorinated sulfonium salt formation. Nevertheless, the use of Ca(OCl)$_2$ significantly reduced reactivity of thioether 13, and the desired product was obtained in low analytical yields. Increasing the solubility of Ca(OCl)$_2$ in MeCN could address this issue. Further optimisation could include the use of an appropriate buffer for maintaining neutral pH in order to limit base-mediated ring opening of the dibenzothiophene ring. Although hypochlorite-mediated cyclisations showed limited practicality, the results constituted an advancement in sulfonium salt chemistry for the Årstad group. With further refinement, Ca(OCl)$_2$-mediated cyclisations could be rendered broadly applicable to a wider scope of substrates,
particularly for those with acid-sensitive moieties.

6.3.3 Expansion of Scope of Cyclisation Reagents: Alternative Chlorine Sources

Cyclisation of thioether 13 was also attempted with alternative reagents: 1) tungsten hexachloride and 2) sulfuryl chloride. WCl$_6$ was found to promote ring expansion-chlorination reactions of 1,3-dithiolanes and 1,3-dithanes$^{112}$. When employed in the cyclisation reaction with thioether 13 in non-anhydrous MeCN, in the presence of triethylamine and sodium triflate, a low analytical yield of 16% was obtained after 10 min. Sulfuryl chloride is another source of electrophilic chlorine and easier to handle than Cl$_2$. Remarkably, nearly quantitative conversion to sulfonium salt 14 was observed after 10 min. Unfortunately, the ratio of sulfonium salt 14 to its chlorinated analogue could not be elucidated, owing to co-elution on the HPLC. Despite promoting fast product formation, reactivity of sulfuryl chloride is difficult to control. Significant side product formation, hygroscopicity and instability preclude its use as a practical reagent in dibenzothiophene sulfonium salt formation.

6.4 Conclusion

In this investigation insights into the cyclisation mechanism were presented. Synthetic route towards the sulfonium salt precursor to $[^{18}\text{F}]$FPEB and the simplified model compound was explored with alternative chlorinating reagents.

TfOH activation of NCS has been shown to promote dibenzothiophene sulfonium formation in an efficient and robust manner. The highly acidic nature of the reaction has inherent limitations. Compatibility issues can be expected for acid-labile protecting groups or other acid-sensitive moieties, e.g. N-heterocycles. Functionalities protonated during the cyclisation must be deprotonated before $[^{18}\text{F}]$-labelling to avoid $[^{18}\text{F}]$fluoride deactivation. The NCS/Bi(OTf)$_3$ reagent pair works in an analogous manner to NCS/TfOH. Bi(OTf)$_3$ acts as a source of Brønsted acid (in the presence of moisture), believed to promote formation of a superelectrophile with NCS. The strength of the acid affects reactivity of the multiprotonated NCS species. Conversion to the desired sulfonium salt increases as p$K_a$ decreases. Disappointingly, the range
of useful $pK_a$ values is narrow and cyclisation is significantly less efficient with weaker acids, such as $p$-TSA or TFA.

Hypochlorite reagents, $t$BuOCl, NaOCl and Ca(OCl)$_2$, all promote dibenzothiophene sulfonium salt formation at room temperature. They have limited shelf-stability and reproducibility issues were faced throughout. Calcium hypochlorite is a promising cyclisation reagent, however it was thought that low solubility in MeCN hampered its reactivity towards the $S$-centre.

Chlorination of the electron-rich methoxy-substituted ring could become problematic on scope expansion. Understanding and appreciation of the factors which orchestrate side product formation is essential.
7 Expansion of Structural Scope of Sulfonium Salts for $^{18}$F-Labelling

The main challenge of sulfonium salt chemistry for $^{18}$F-labelling lies in the inherent reactivity conflict of the sulfur centre towards: 1) electrophilic chlorine, during the cyclisation reaction and 2) nucleophilic $[^{18}$F$]$fluoride, during radiolabelling.

The study into the cyclisation mechanism and cyclisation reagents, presented in the previous chapter, deepened our understanding of the factors that influence sulfonium salt formation. The best reagent pair, NCS in conjunction with Bi(OTf)$_3$ or TfOH, proved extremely effective at mediating dibenzothiophene sulfonium salt formation. In comparison to triflic acid, bismuth triflate presents a more controlled and practical source of Brønsted acid. The presence of strongly acidic reaction environment is perhaps most concerning when it comes to assessing the applicability and limitations of the system with respect to acid-sensitive entities, for instance $N$-heterocycles.

This chapter describes efforts towards evaluation of $N$-heterocycles as substrates for $^{18}$F-labelling with dibenzothiophene sulfonium salt precursors. Sulfonium salts bearing indole and ortho- and meta-pyridyl moieties were prepared. The choice was dictated by their: 1) complex reactivities towards electrophiles and nucleophiles and 2) increasing presence in PET tracers and drug-like molecules.

7.1 $^{18}$F-AMT: PET Tracer for Imaging Tryptophan Metabolism

Indoles are commonly found in biologically active molecules and therapeutics. They can participate in both nucleophilic and electrophilic transformations, however, it is the former that have been more thoroughly investigated. The indole motif is found in tryptophan (Trp), one of the 22 essential amino acids. In addition to
being a building block in the synthesis of proteins, tryptophan serves as a substrate for two metabolic pathways: 1) serotonin and 2) kynurenine (Figure 7.2, page 88). Palego et al., along with other researchers, reported that impairment of tryptophan homeostasis has been linked to pathologies of the immune and central nervous systems. A carbon-11 analogue of tryptophan, α-methyl-L-tryptophan (AMT), has been used as a PET tracer for the imaging of both metabolic pathways (Figure 7.1). Conveniently, AMT cannot be incorporated into proteins.

Figure 7.1: The structures of tryptophan and AMT.

The metabolic fate of tryptophan is depicted in Figure 7.2. The serotonin pathway, implicated in neurotransmission and insulin secretion, begins with hydroxylation of the Trp benzene ring by tryptophan hydroxylase (TPH), followed by decarboxylation. Studies in the 1990s, performed by Diksic, utilised AMT to measure brain serotonin production in rats and dogs. AMT metabolism follows that of Trp, with the end product being α-methylserotonin (α-M5HT) which, unlike serotonin, is not a substrate for monoamine oxidase (MAO), so it is accumulated in serotogenic cell bodies, making it a suitable tool for PET. Encouraged by autoradiography results that allowed calculation of the rates of α-methylserotonin synthesis in rat brains, Diksic extended their investigation to dogs with elevated plasma Trp levels. PET was utilised to measure brain serotonin levels. Consistent with expectations, an increase in serotonin synthesis was observed. Diksic et al. advocated applicability of the method for PET imaging of the human brain. Of particular interest is the implication of serotonin in epilepsy - the link between the two has been highlighted by several researchers. Interestingly, further exploration of AMT in PET revealed increased uptake of AMT in epileptogenic tubers in patients with tuberous sclerosis, a genetic condition which frequently leads to the development of epilepsy. It develops through formation of benign hamartomas, for example in the brain. Kumar et al. further ascertained the imaging properties of AMT with regards to
Figure 7.2: Tryptophan: the simplified kynurenine and serotonin pathways, with structures of metabolites. AMT is metabolised in the same way as Trp. Enzymes are marked in blue. Adapted from Guastella et al. and Chugani.\cite{116,117}
epileptogenic brain by comparing it against a PET gold standard, $[^{18}\text{F}]$FDG, and MRI imaging. Both provide useful information about: hypometabolism (PET) or anatomical detail of tubers (MRI), however only AMT allows epileptogenic and nonepileptogenic tubers to be distinguished$^{[124]}$.

The alternative metabolic pathway of tryptophan is orchestrated by indoleamine 2,3-dioxygenase (IDO), and is referred to as the kynurenine pathway (Figure 7.2). According to Peters, it accounts for over 95% of Trp metabolism$^{[125]}$. Tryptophan is a substrate for IDO1, IDO2 and TDO2 (all are dioxygenase enzymes), which catalyse formation of $N$-formylkynurenine, followed by kynurenine and other toxic downstream metabolites. Many publications have been devoted to implications of kynurenine imbalance in various pathologies, e.g. Alzheimer’s disease, epilepsy and inflammation. Efforts have been directed towards investigating IDO1 expression and its links to the aforementioned disease states. IDO1 is expressed in many organs, including the brain, where it is produced by various cell types (dendritic cells, monocytes, macrophages, etc.), as well as tumours. Dounay et al. expressed interest in utilising IDO1 as a therapeutic for cancer and immunosuppressive disorders, calling it a "gatekeeper" enzyme$^{[126]}$. IDO1 is involved in the defence mechanism against pathogen infections through mopping up tryptophan and subsequently handicapping protein synthesis channels, yet simultaneously impeding the host’s immune system. Two mechanisms of action have been proposed: 1) tryptophan concentration is reduced by IDO1, affecting proliferation in cells and promoting apoptosis and 2) increased IDO1 activity triggers enhanced downstream synthesis of toxic kynurenines. T-lymphocytes have been shown to respond to increased IDO1 levels. Fox et al. studied the response of T-cells to influenza virus, which acts by upregulating IDO levels in the host, leading to tryptophan depletion and loss of immunity$^{[127]}$. Kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic and quinolinic acid are neurotoxic and changes in the concentration of these intermediates have been implicated in various immune system disorders$^{[127]}$. They have been shown to disrupt T-cell functioning and promote apoptosis$^{[128]}$.

Disruption of the kynurenine pathway has been implicated in various pathologies.
Recent publications describe involvement of dysregulated T-cell response in various cancer forms, e.g. melanoma and glioma. It has been hypothesised that IDO expression in cancer cells leads to a local depletion of tryptophan and consequent impediment of T-lymphocytes and increased concentration of toxic kynurenines. Although evidence is currently insufficient, it is thought that excessive presence of kynurenine metabolites in the brain, particularly 3-hydroxykynurenine and quinolinic acid, leads to neuronal degeneration. AMT has also been employed to image the kynurenine pathway via monitoring of IDO activity to provide insights into cancer immunotherapy response.

The main limitation of $^{11}$C-labelled AMT is the short half-life of carbon-11, limiting its clinical availability. Recent publications highlight the growing potential of $^{18}$F-fluorinated AMT analogues in tryptophan imaging (Figure 7.3).

Xin and Cai optimised radiolabelling of two enantiomers of $^{18}$F-FETrp (Figure 7.3, (a), left) and demonstrated potential use of the L-form as a PET tracer. Favourable uptake was seen in breast cancer cells, while significant reduction occurred in the presence of NLG919 (Figure 7.3, (a), right), an IDO inhibitor. Ex vivo findings were consistent with PET imaging results. Michelhaugh *et al.* assessed $^{18}$F-FETrp using patient-derived brain tumour xenografts in mice with regards to tumour uptake against the $^{11}$C-analogue. Higher standardised uptake value was obtained for the investigated cancer types, glioblastoma and metastasised brain tumours (lung and breast cancer), for the $^{18}$F-fluorinated counterpart, further ascertaining its potential for the imaging of tryptophan metabolism in cancer.
Conveniently, structure-activity relationship analysis identified potential analogues of AMT as substrates for IDO. Derivatives substituted with fluorine in the 4-, 5- and 6-position on L-tryptophan (Figure 7.3, (b), right) exhibited high affinity binding (as judged by \( V_{\text{max}} \) and \( K_{\text{m}} \) values) for IDO, rendering them attractive for \(^{18}\text{F}\)-labelling\(^{135}\). \( V_{\text{max}} \) is the maximum rate of catalysis when an enzyme is saturated with its substrate. \( K_{\text{m}} \) represents the required substrate concentration for the enzyme to reach its \( V_{\text{max}} \). \( K_{\text{m}} \) values of all tryptophan analogues are similar to unsubstituted Trp, however a slightly higher concentration of the 6-fluorinated form is required for IDO. The aforementioned regiomer has the highest \( V_{\text{max}}/K_{\text{m}} \) ratio among substituted tryptophans (including methyl, methoxy and hydroxy substituents in the 5- or 6-position), rendering it the best substrate for IDO. The values are presented in Table 7.1.
Table 7.1: $V_{\text{max}}$, $K_m$, and $V_{\text{max}}/K_m$ values (pH=7.0) for ring-fluorinated L-tryptophan analogues as substrates for IDO, adapted from Sono et al.\textsuperscript{135}

<table>
<thead>
<tr>
<th></th>
<th>L-Trp</th>
<th>4-F</th>
<th>5-F</th>
<th>6-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ [µM]</td>
<td>13</td>
<td>13.2</td>
<td>13.2</td>
<td>15.3</td>
</tr>
<tr>
<td>$V_{\text{max}}$ [nmol/min/nmol IDO]</td>
<td>122</td>
<td>18</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>$V_{\text{max}}/K_m$ [(µMmin)$^{-1}$]</td>
<td>9.38</td>
<td>1.36</td>
<td>3.46</td>
<td>4.44</td>
</tr>
</tbody>
</table>

The recent study by Giglio et al. presents the synthesis and labelling of aromatically substituted $[^{18}\text{F}]$fluorinated variants of L-Trp\textsuperscript{134}. Their preliminary small animal PET experiments uncovered that 5-$[^{18}\text{F}]$F-AMT (Figure 7.3, (b), middle) exhibited significant uptake in B16F10, a murine melanoma cell line. Labelling at the 5-position of AMT is shown in Scheme 7.1, and $[^{18}\text{F}]$fluorination at the 6-position of tryptophan in Scheme 7.2.

Scheme 7.1: Radiosynthesis of 5-$[^{18}\text{F}]$F-AMT using different precursors, from Giglio and co-workers\textsuperscript{133}.
Labelling: $^{18}$F-TBAF, (Py)$_4$Cu(OTf)$_2$
Deprotection: 3M H$_2$SO$_4$

**Scheme 7.2:** Radiosynthesis of 5- and 6-$^{18}$F-Trp, from Giglio and co-workers.$^{134}$

Based on the above literature review, $^{18}$F fluorinated AMT variants make suitable PET tracer candidates for the imaging of tryptophan metabolism, both through the serotonin and kynurenine pathways. As pointed out by Giglio and co-workers, there are several structural modification sites at a tryptophan residue which could be probed to obtain a suitable PET tracer candidate (Figure 7.4).

**Figure 7.4:** Structural modifications of tryptophan analogues that should be investigated to design a promising $^{18}$F-labelled PET tracer candidate.

### 7.2 Synthesis of Sulfonium Salt with Indole Moiety

A simple indole-based model compound was selected to test its compatibility to undergo cyclisation to the dibenzothiophene sulfonium salt with the current reaction system: NCS and Bi(OTf)$_3$/TfOH. The synthetic route towards the compound is shown in Scheme 7.3.
**Scheme 7.3:** Syntheses of sulfonium salt 18 and fluorinated reference 20.

Reagents and conditions: (i) KOH, p-TsCl, Aliquat® 336, toluene/water, rt, 1 h; (ii) Thioether 4, Pd₂(dba)₃, DPEphos, KOTBu, toluene, reflux, 1 h; (iii) NCS, Bi(OTf)₃, MeCN, rt, 20 min; (iv) KOH, p-TsCl, Aliquat® 336, DCM, rt, 1 h.

N-tosyl protected bromoindole 16 was coupled to diaryl thioether 4 (Chapter 5, Scheme 5.3) in a palladium-catalysed reaction to afford thioether 17 in 44% yield. It was then employed in a cyclisation reaction with Bi(OTf)₃ and NCS, which reached full conversion within 20 min. The corresponding sulfonium salt 18 was isolated in 73% yield. Fluorinated reference compound 20, for HPLC co-injection after radiolabelling, was obtained in 36% yield.

Preliminary ¹⁸F-labelling of sulfonium salt 18, performed by Dr Thibault Gendron, proceeded with a low analytical RCY of 2-5% (Scheme 7.4). Nucleophilic substitution on indoles is challenging due to their electron-rich nature. This issue could perhaps be resolved with further optimisation of the dibenzothiophene substitution pattern to enhance its leaving group ability and consequently, improve radiolabelling efficiency.
Scheme 7.4: Radiosynthesis of 6-[\(^{18}\)F]fluoroindole using sulfonium salt 18 (performed by Dr Thibault Gendron).

7.3 Radiotracers with Pyridyl Moiety

The pyridine moiety can be found in over 100 drugs on the market\(^{136}\). It is a bioavailable motif - nicotinic acid (niacin) and nicotinamide (niacinamide) are part of the vitamin B\(_3\) complex. Pyridine itself is notorious for its toxicity, however an increase in the proportion of pyridine-containing drugs can be rationalised by its metabolic stability\(^{137,138}\). Vast functionalisations of the pyridine moiety in drug-like molecules have seen a reflection in the number of PET tracer candidates containing this heterocycle\(^{139}\). A prominent representative of this category is \(^{18}\)F-FPEB, discussed in Chapter 5. Examples of other pyridine-based PET tracers are shown in Figure 7.5.

Figure 7.5: Existing pyridine-based PET tracers.

Direct \(^{18}\)F-fluorination of the pyridyl moiety using traditional leaving groups, such as NMe\(_3^+\) and NO\(_2\), proceeds efficiently at the ortho position (Scheme 7.5). RCYs in the range of 82-91% can be obtained after 20 min at 120 °C with trimethylammonium and nitropyridines. Halopyridines require temperatures up to 180 °C to achieve efficient radiolabelling (19-87%)\(^{130}\).
**Scheme 7.5:** Generic radiolabelling of activated *ortho*-pyridines with $^{18}$F-fluoride.

Sander *et al.* attempted radiolabelling of pyridines in the *ortho*-position using triarylsulfonium salts (Chapter 3, Section 3.1), which proceeded in good isolated decay-corrected RCYs (Scheme 7.6).

**Scheme 7.6:** Radiosynthesis of functionalised 2-$^{18}$F-fluoropyridines using first generation triarylsulfonium salts, developed by Sander and co-workers.

One of the most recent examples of promising 2-$^{18}$F-fluoropyridine-based structures is $^{18}$F-DCFPyL (Figure 7.6), a PET tracer candidate for the imaging of metastatic prostate cancer. It is a low-molecular weight inhibitor designed to bind to the prostate-specific membrane antigen (PSMA), which is overexpressed in prostate cancer epithelial cells even up to 80 times higher than in healthy prostate. Elucidation of this structure represents a great achievement in the field. Chen *et al.* argued that issues associated with development of high quality tracers for prostate cancer imaging are caused by: 1) slow growth and metabolism of cancer cells, hence limited suitability for $^{18}$F-FDG studies, 2) close location of prostate and urinary bladder, requiring rapid scanning and 3) lack of markers for differentiation of slow from fast developing cancer.
Bouvet et al. published an optimised automated synthesis of $[^{18}F]$DCFPyL, which proceeded in $23 \pm 5\%$ isolated decay-corrected RCY\textsuperscript{[144]}. The RCY had to be compromised, in comparison to the original synthesis by Chen et al. (36-53\%, decay corrected, Scheme \textsuperscript{[7.7]}, to avoid the use of prosthetic groups.

**Figure 7.6:** PET tracer candidate for the imaging of prostate cancer, $[^{18}F]$DCFPyL\textsuperscript{[111]}.  

**Scheme 7.7:** Radiosynthesis of $[^{18}F]$DCFPyL by Chen and co-workers\textsuperscript{[141]}.  

**Scheme 7.8:** Automated radiosynthesis of $[^{18}F]$DCFPyL by Bouvet and co-workers\textsuperscript{[144]}.
The relative ease of $^{18}$F-fluorination at the ortho-position of the pyridine is reflected in the growing number of PET tracer structures thereof, in comparison to 3-$^{18}$F-fluoropyridine. Nucleophilic substitution at the 3-position of the pyridine ring is challenging, owing to its electron-withdrawing character. During nucleophilic attack, both the 2- and 4-positions are targeted preferentially, due to greater stability of the resulting Meisenheimer complexes, in which the negative charge is placed on the nitrogen atom. This effect is strengthened in the presence of electron-deficient substituents, e.g. NO$_2$, NMe$_3$$^+$. 

**Scheme 7.9:** Nucleophilic attack at the pyridine. Resonance forms are shown for the ortho-, meta- and para-positions. Most favourable resonance forms (structures in blue) result in the negative charge on the nitrogen. Fluoride incorporation is therefore favoured at the 2- and 4-positions.

Unsuccessful attempts at $^{18}$F-labelling at the meta-position by nucleophilic aromatic substitution of bromo- and nitro-precursors were described by Karramkam et al. (Scheme 7.10). 

**Scheme 7.10:** Failed attempts to incorporate fluorine-18 at the meta to the pyridyl nitrogen. Adapted from Karramkam and Preshlock. 

\[ X = \text{Br, NO}_2 \] 

Maximum RCY: 2% 
(2 min 100 W microwaves)
There is the only example of direct labelling of a PET tracer scaffold at the 3-position (Figure 7.11), dating back to the 1990s. Beer et al. labelled a promising radiotracer for the imaging of monoamine oxidase-B (MAO-B) in 40% decay-corrected RCY\textsuperscript{146}. Activation of the 3-position towards the nucleophile was achieved by placing an electron-withdrawing amide \textit{para} to the nitro leaving group.

Scheme 7.11: The only documented case of successful $^{18}$F-incorporation at the \textit{meta}-pyridine entity of a PET tracer candidate, by Beer and co-workers\textsuperscript{146}.

Recently Brugarolas et al. described direct $[^{18}\text{F}]$fluorination at the 3-position of pyridine \textit{N}-oxides as a way of accessing \textit{meta}-$^{18}$F-fluoropyridines\textsuperscript{147}. The main limitation lies in the post-labelling reduction step using Pd/C (Scheme 7.12). The decay-corrected RCY before reduction was established at 10.4 ± 1.8% (n=8), however, interestingly, co-injection of the crude reaction mixture with the non-radioactive reference compound resulted in a significant RCY increase to 25 ± 4%. This phenomenon was hypothesised to occur via exchange of fluorine-19 of the cold reference with unreacted $[^{18}\text{F}]$fluoride. The total synthesis time was under 2 hours, however the final RCY was reduced to 5.4% after decay-correction. Despite efforts from Brugarolas and co-workers, the method does not alleviate the challenging task of labelling at the \textit{meta}-position. Activation by a nitro substituent \textit{ortho} to the leaving group is still required to promote labelling. Masking of the pyridyl moiety using \textit{N}-oxide adds an extra synthetic step during radiolabelling, severely affecting the practical aspects of the method.
Scheme 7.12: Direct $^{18}$F-incorporation at the meta-position of N-oxide pyridines by Brugarolas and co-workers\cite{Brugarolas}. Subsequent palladium-mediated hydrogenation allows for simultaneous reduction of the N-oxide back to pyridine and nitro group to amine (for further functionalisations).

Perhaps the most elegant solution, to date, is offered by Rotstein et al\cite{Rotstein}. Radiolabelling using an iodonium ylide precursor afforded 3-$^{18}$F-fluoropyridine in an analytical RCY of 65%, without the need for an activating group (Chapter 2, Section 2.3.1, page 36). No information about isolated RCY or ease of purification was, however, given by the authors, making it difficult to assess the practicality of the method. To the best of my knowledge, a PET tracer scaffold has not yet been accessed using this method.

7.4 Synthesis of Sulfonium Salts with 2-Pyridyl Moiety

Labelling of the ortho-pyridine position using triarylsulphonium salts by Sander and co-workers (Section 7.3, Figure 7.6) proceeded efficiently. Implementation of the novel dibenzothiophene sulphonium salt strategy to direct labelling of pyridines remains yet to be undertaken. The PSMA selective radiotracer, $^{18}$F-DCFPyL (Section 7.3, Figure 7.6), was published in 2011 and remains an attractive candidate for the imaging of prostate cancer using PET, despite challenges with the original radiosynthesis, requiring prosthetic groups (Scheme 7.7). A simplified analogue of $^{18}$F-DCFPyL makes an ideal case study for $^{18}$F-labelling using dibenzothiophene sulphonium salts. Model compound (Scheme 7.13, structure 29) was designed to provide an adequate mimic of the target tracer. The scaffold was simplified to include basic 2-pyridine and amide functionalities, expected to provide further insights into the potential of cyclic sulphonium salt precursors for radiolabelling of aromatic scaffolds decorated with such entities. The terminal amine moiety of urea on the $^{18}$F-DCFPyL scaffold is predicted to undergo a straightforward coupling to a 2-nicotinic acid in later stages.
The synthetic route is presented in Scheme 7.13.

Scheme 7.13: Syntheses of sulfonium salt 27 and fluorinated reference 29.
Reagents and conditions: (i) But-1-ylamine, HATU, HOBT, DIPEA, DCM, rt, 1 h; (ii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 2.5 h; (iii) NCS, TfOH, MeCN, rt, 3 h; (iv) But-1-ylamine, HATU, HOBT, DIPEA, DCM, rt, 2 h.

Amide 25 was synthesised using standard peptide coupling procedure with HATU, HOBT and DIPEA in DMF in 67% yield. It was then employed in the Pd-catalysed thioetherification with thioether 4 (Scheme 5.3) and the desired sulfonium salt precursor 26 was obtained in 65% yield. Cyclisation mediated by NCS and TfOH delivered sulfonium salt 27 in a poor yield of 5%, with no signs of side chlorination. A simple ortho-pyridine thioether scaffold failed to undergo cyclisation with NCS and TfOH (Scheme 7.14).

Scheme 7.14: Syntheses of sulfonium salt 32.
Reagents and conditions: (i) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 24 h; (ii) NCS, TfOH, rt, 2 h.
Challenging cyclisation to ortho-pyridine scaffolds 27 and 32 can be explained by the electron-withdrawing effect of the nitrogen atom, leading to a decrease in the nucleophilicity of sulfur and consequently, low reactivity towards the electrophilic reagent pair. Pyridine is protonated during the cyclisation reaction, giving rise to a positive charge ortho to the thioether, which further deactivates the substrates towards the electrophile. As discussed on page 86 an electron-deficient S-centre is expected to react readily with nucleophilic $[^{18}\text{F}]$fluoride but electrophilic transformations proceed sluggishly.

Inherent problems arising from highly acidic reaction conditions were highlighted in Chapter 6. Although it has been demonstrated that a simple phenyl-based sulfonium salt can be cyclised under basic conditions with hypochlorite reagents, the method lacks robustness and suffers from decomposition issues due to the release of hydroxide ions. The search for mild and neutral cyclisation reagents arrived at N-chlorosulfonamides as chlorinating agents (described fully in Section 7.5, Scheme 7.17 and Figure 7.8). Reactions of thioether 31 with N-chlorosulfonamides did not yield sulfonium salt 32. Interestingly, NMR analysis of crude reaction mixtures revealed the presence of 2 side-products - chlorinated and dichlorinated analogues of thioether 31 (Figure 7.7). Perhaps it can be inferred that for electron-deficient substrates, such as ortho-pyridines, the rate of diaryl thioether chlorination surpasses the rate of chlorosulfonium intermediate formation, leading to the observed product distribution.

![Figure 7.7: Chlorinated and dichlorinated analogues of thioether 31.](image)

At the time of writing this thesis, Dr Thibault Gendron optimised reaction conditions for the cyclisation of acid-sensitive substrates. Building on the foundations of hypochlorite-mediated reagents for sulfonium salt formation (Chapter 6, Section 6.3.2, 102)
Table 6.3, he established that efficient and robust cyclisation of thioether 31 could be achieved with Ca(OCl)$_2$, which became soluble in MeCN, when used in conjunction with acetate buffer (pH=4). The latter ensured mopping up of hydroxide anions to prevent base-mediated decomposition. The method elegantly avoided the use of TfOH or Bi(OTf)$_3$, hence offering a mild, inexpensive and complementary solution for substrates which do not tolerate the acidic environment. Using this method, Gendron accessed sulfonium salt 32 in a gratifying yield of 54%. Radiolabelling proceeded with an analytical RCY of 85 ± 3% (n=3). Both reactions are shown in Scheme 7.15. In comparison, with the same precursor load (2 mg), Rotstein et al. labelled the ortho-pyridine position using an iodonium ylide precursor with 65 ± 11% RCY (n=3).

Scheme 7.15: Formation of sulfonium salt 32 proceeded efficiently in the presence of Ca(OCl)$_2$ and acetate buffer, a newly proposed hypochlorite-based cyclisation system for acid-sensitive substrates. Radiolabelling afforded 2-[¹⁸F]fluoropyridine in an excellent analytical RCY of 85 ± 3%. Both reactions were performed by Dr Thibault Gendron at the time of writing this thesis.

7.5 Synthesis of Sulfonium Salt with 3-Pyridyl Moiety

Synthesis of a meta-pyridine dibenzothiophene sulfonium salt was expected to proceed more readily than for the ortho-regioisomer 32, owing to the electron-withdrawing nature of the nitrogen atom in the 3-position. The synthetic route to 3-pyridine
thioether 36 is shown in Scheme 7.16.

Scheme 7.16: Synthesis of sulfonium salt 37.
Reagents and conditions: (i) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 2 h; (ii) Optimisation of cyclisation conditions.

Thioether 36 was obtained through a palladium-mediated coupling of 3-iodopyridine to thioether 4 (Scheme 5.3) in 81% yield. Efforts were then directed to finding suitable cyclisation conditions. These results are summarised in Table 7.2.

Table 7.2: Optimisation of cyclisation with thioether 36. All reactions were performed in MeCN at room temperature. Chlorinated sulfonium salt (structure 41) is shown in Scheme 7.19.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>Chlorinating Agent</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TfOH</td>
<td>NCS</td>
<td>overnight</td>
<td>52% yield$^a$</td>
</tr>
<tr>
<td>2</td>
<td>TfOH</td>
<td>NCS</td>
<td>30 min</td>
<td>14% yield$^b$</td>
</tr>
<tr>
<td>3</td>
<td>BiOTf$_3$$^c$</td>
<td>NCS</td>
<td>overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>Sulfonamide 40</td>
<td>3 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>Sulfonamide 38</td>
<td>2 h</td>
<td>Trace product$^d$</td>
</tr>
<tr>
<td>6</td>
<td>TfOH</td>
<td>Sulfonamide 38</td>
<td>1.5 h</td>
<td>29% yield$^e$</td>
</tr>
<tr>
<td>7</td>
<td>Bi(OTf)$_3$</td>
<td>Sulfonamide 38</td>
<td>2.5 h</td>
<td>35% yield$^f$</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>Palau‘Chlor$^{®}$</td>
<td>overnight</td>
<td>No reaction</td>
</tr>
<tr>
<td>9</td>
<td>-$^g$</td>
<td>NCS</td>
<td>10 min</td>
<td>Decomposition</td>
</tr>
<tr>
<td>10</td>
<td>-$^h$</td>
<td>NCS</td>
<td>overnight</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

$^a$ Yield calculation based on NMR data. Isolated sample: 3:1 Sulfonium salt: chlorinated sulfonium salt.  
$^b$ 15.7:1 Sulfonium salt: chlorinated sulfonium salt. Also isolated from the reaction mixture was a mixture of starting material derivatives.  
$^c$ Source of TfOH.  
$^d$ LC-MS revealed a mixture of sulfonium salt and its chlorinated counterpart.  
$^e$ Yield calculation based on NMR data. 3.5:1 Sulfonium salt: chlorinated sulfonium salt. Incomplete conversion.  
$^f$ Yield calculation based on NMR data. 4.6:1 Sulfonium salt: chlorinated sulfonium salt.  
$^g$ 10 mol% 2,4,6-Trimethylaniline.  
$^h$ 10 mol% Triphenylphosphine sulfide.
With NCS and TfOH, sulfonium salt formation was observed early on, however a significant amount of starting material remained even after prolonged stirring. (Table 7.2, Entry 1). Sulfonium salt \( \text{37} \) was afforded in 52% analytical yield, however separation from its chlorinated analogue was not achieved. Both products have similar \( R_f \) values, which makes flash column chromatography separation arduous. An analogous attempt, with a significantly shorter reaction time, afforded the desired sulfonium salt with a reproducible yield and pleasingly, with a more favourable ratio of the sulfonium salt to its chlorinated derivative (Entry 2). Interestingly, NMR analysis of the recovered starting material revealed the presence of both the mono- and dichlorinated thioether derivatives. No product was formed when the cyclisation was mediated by Bi(OTf)\(_3\) and NCS (Entry 3).

At the time of the investigation, Heuger and Göttlich published an elegant strategy of electrophilic addition to unsaturated compounds, mediated by copper(I)\(^{148}\). \( N \)-chlorosulfonamides were described as a mild yet reactive source of chlorine without the need for acid activation. Inspired by these findings, it was speculated that \( N \)-chlorosulfonamides could act as electrophiles for sulfonium salt formation, perhaps offering better regioselectivity over ring chlorination due to increased steric bulk on the sulfonamide moiety. Their formation and a representative reaction are shown in Scheme 7.17. A selection of \( N \)-chlorosulfonamides was prepared according to the method by Heuger and Göttlich is presented in Figure 7.8.

**Scheme 7.17:** Preparation of \( N \)-chlorosulfonamides and their representative high-yielding addition to styrene, both from Heuger and Göttlich\(^{148}\).
Figure 7.8: Scope of N-chlorosulfonamides prepared for further optimisation of the cyclisation protocol, synthesised using the method from Heuger and Göttlich (Scheme 7.17).

*N*-chlorosulfonamides 38 and 40, used as the sole reagent, were unable to effectuate dibenzothiophene sulfonium salt formation (Table 7.2, Entries 4 and 5). Gratifyingly, when employed in conjunction with TfOH or Bi(OTf)₃, sulfonium salt 37 was obtained in 29% yield or 35% yield (based on NMR), respectively, nevertheless its chlorinated analogue constituted approximately 20% of the sample (Entries 6 and 7).

Several chlorination technologies were recently introduced as practical alternatives to highly reactive reagents such as chlorine gas or sulfuryl chloride, yet exhibiting greater potential than conventional NCS. Rodriguez *et al.* have commercialised a guanidine-based chlorinating reagent, CBMG, also known as Palau’chlor® (Figure 7.9, (a)). It has shown superior properties for direct electrophilic chlorinations of various heteroaromatic systems, in comparison with NCS or tBuOCl. A wide scope of aromatic heterocycles was screened, including benzimidazoles, indoles and pyrroles. Examples of substrates which underwent elegant chlorination with Palau’chlor® are shown in Figure 7.9.
Figure 7.9: (a) Palau’chlor®. (b) Representative compounds chlorinated using Palau’chlor®, with yields compared against NCS.

Shortly after Rodriguez et al. published their Palau’chlor® chlorination strategy, Samanta and Yamamoto proposed the use of aniline catalysis to achieve selective electrophilic halogenations. 2,4,6-Trimethylaniline was employed in conjunction with NCS in electrophilic chlorination of various aromatic systems, including anisoles, which were of immediate interest to this study. The proposed mechanism assumes formation of an N-haloaniline adduct, which then acts as a halogen donor to the structure of interest. The optimum catalyst loading was established to be between 5 and 10 mol%. The route is particularly attractive due to the absence of Brønsted acid. Maddox et al. also performed a mild and efficient chlorination of aromatic systems using the concept of Lewis base catalytic activation of N-halosuccinimides, in lieu of conventional Lewis or Brønsted acid activation. They demonstrated that catalytic triphenylphosphine sulfide was capable of activating NCS, hence boosting its reactivity towards arenes. Both technologies are presented in Scheme 7.18.
Scheme 7.18: Novel strategies for electrophilic chlorination: 1) using NCS and an aniline-based catalyst, proposed by Samanta and Yamamoto and 2) triphenylphosphine sulfide, proposed by Maddox and co-workers. Excellent yields were obtained for anisole substrates.

Disappointingly, none of the presented alternative approaches afforded sulfonium salt 37 (Table 7.2 Entries 8, 9 and 10). Scheme 7.19 presents the most successful cyclisation of thioether 36 to sulfonium salt 37 using NCS and TfOH.

Scheme 7.19: Most successful cyclisation of thioether 36 with NCS and TfOH in MeCN overnight. NMR yield of sulfonium salt 37 was 52%, however it could not be isolated from its chlorinated analogue 41.

The mixture of sulfonium salts 37 and 41 (Scheme 7.19) was subjected to $^{18}$F-labelling. The chlorinated analogue was still expected to undergo labelling, although the leaving group ability of dibenzothiophene could perhaps be affected. Radiolabelling was carried out in DMSO at 110 °C for 15 min (Scheme 7.20). The isolated decay-corrected RCY was 41-50% (n=2).
Scheme 7.20: Successful direct $^{18}\text{F}$-incorporation into the pyridyl *meta*-position with a mixture of sulfonium salts 37 and 41.

This is an elegant example of direct one-step incorporation of fluorine-18 into the *meta*-position of pyridine, offering advantages over the recently published findings by Brugarolas *et al.*\(^{147}\). The pyridine nitrogen does not have to be activated by *N*-oxide transformation or possess strongly-electron withdrawing groups *ortho* or *para* to the leaving group. Brugarolas *et al.* obtained 3-$^{18}\text{F}$fluoro-4-nitropyridine *N*-oxide in 10.4 ± 1.8% RCY, compared to 41-50% RCY of unsubstituted 3-$^{18}\text{F}$fluoropyridine presented in this work. Rotstein *et al.* obtained compound 23 using an iodonium ylide precursor in an analytical RCY of 65% (Chapter 2, Section 2.3.1, Scheme 2.16).

As described in Section 7.4 (Scheme 7.15), the *ortho*-pyridine-based thioether was successfully cyclised using Ca(OCl)$_2$ and acetate buffer (pH=4) in MeCN at 0 °C (Dr Thibault Gendron). At the time of writing this thesis, cyclisation was also attempted with *meta*-pyridine isomer 36. Gratifyingly, sulfonium salt 37 was obtained as the sole product in 72% yield. Subsequent labelling afforded 3-$^{18}\text{F}$fluoropyridine in 65 ± 5% analytical RCY. Both reactions are shown in Scheme 7.21. Interestingly, both dibenzothiophene sulfonium salts (developed in the Årstad group) and iodonium ylides delivered the radiolabelled product with the same analytical RCY. As no information on the ease of isolation is available, both strategies constitute practical, and to the best of my knowledge, competitive solutions to direct radiolabelling of the 3-pyridyl moiety, to date.

The aforementioned radiolabelling of sulfonium salt 37 uncovered an important finding, which provides further evidence towards the robust nature of dibenzothio-
phene sulfonium salts as precursors for $^{18}\text{F}$-labelling. The presence of the chlorinated sulfonium salt does not affect the RCY. Provided successful isolation and similar end of synthesis time, the isolated RCY expected from radiolabelling of 37 by Gendron oscillates in the same range as that obtained from radiolabelling of the mixture of sulfonium salts 37 and 41.

**Scheme 7.21:** Successful cyclisation to sulfonium salt 37 as the sole product. Subsequent radiolabelling proceeded very efficiently, reaching a high analytical RCY. Both reactions were performed by Dr Thibault Gendron.

### 7.6 Conclusion

Synthesis of dibenzothiophene sulfonium salts bearing $N$-heterocyclic aromatic moieties allowed for further verification of their use as versatile and robust precursors for $^{18}\text{F}$-labelling. Indole and pyridine-decorated sulfonium salts were constructed as model compounds of highly-functionalised drug-like molecules.

Indole, due to its electron-rich nature, is a better substrate for the cyclisation with NCS and Bi(OTf)$_3$/TfOH than 2- or 3-pyridines. The lower cyclisation yield of pyridine thioethers results from protonation of the nitrogen atom in the presence of triflic acid, leading to a reduction of electron density on the sulfur centre. This effect is more pronounced for the 2-pyridyl moiety. NMR spectra of crude reaction mixtures revealed significant progress of side chlorination (and dichlorination) of
the electron-rich dibenzothiophene. This is not surprising, as ring chlorination is a competing reaction which depletes the available electrophile (Chapter 6). It is believed that by taking certain measures, such as: 1) reducing reaction time and temperature or 2) changing the order of addition, ring chlorination could perhaps be controlled, to an extent. The rate of side chlorination is a function of electrophile strength and sulfur nucleophilicity. Generally, the rate of sulfonium salt formation is expected to outpace ring chlorination for electron-rich scaffolds. This is illustrated in Figure 7.10.

Figure 7.10: Illustration of factors orchestrating reactivity of the sulfur centre in the cyclisation step.

Reactivity trend for the $S$-centre can be elucidated using the cyclisation yields obtained for all substrates afforded using NCS and TfOH or Bi(OTf)$_3$. This is a qualitative illustration, as direct comparison of substituent effects or yields cannot be made without a deeper investigation and at least 3 repetitions for statistical significance. Electron-poor scaffolds are expected to react slowly towards electrophilic chlorine sources and highest cyclisation yields were obtained for scaffolds with electron-rich indole or phenyl entities. Cyclisation of the 2-pyridine-decorated thioether was eventually possible with Ca(OCl)$_2$, as shown in Section 7.4. In the presence of triflic acid, the proximal pyridine nitrogen is protonated, which leads to a significant decrease in thioether nucleophilicity. The 3-pyridyl dibenzothiophene sulfonium salt was also synthesised using the newly-established non-acidic reaction conditions but a higher yield was observed (72% vs 54%).
Figure 7.11: Experimentally established reactivity pattern of investigated thioethers towards the NCS and TfOH/Bi(OTf)$_3$ electrophilic system. Reactivity of synthesised sulfonium salts towards $[^{18}\text{F}]{\text{F}}^-$ is inversely proportional to the ease of cyclisation. The highest isolated (where possible) cyclisation yields are shown. Whenever possible, isolated decay-corrected RCY as shown.

As described on page 86, the inherent challenge of dibenzothiophene sulfonium salts lies in an attempt to make the sulfur centre reactive towards an electrophile in the cyclisation, and a nucleophile in the radolabelling. Synthesis of sulfonium salts of electron-rich substrates proceed readily, however labelling is challenging, and vice versa. On balance, sulfonium salts as a strategy for $[^{18}\text{F}]$fluorination is most practical and robust for electron-neutral and mildly-activated substrates. Compromises in cyclisation and radiochemical yields might be necessary for certain strongly electron-
rich or electron-poor systems. This is illustrated in Figure 7.11.
Applications of Dibenzothiophene Sulfonium Salts: PET Tracer 1

This chapter presents efforts towards the synthesis of an imidazole-based PET tracer for the imaging of aldosterone-producing adenomas (APAs), using dibenzothiophene sulfonium salts as precursors for $^{18}$F-labelling.

8.1 Towards Novel Tracer for Imaging of Aldosterone-Producing Adenomas

8.1.1 Aldosterone-Producing Adenomas

Aldosterone, a steroid hormone, is the third pillar of the renin-angiotensin-aldosterone system (RAAS), a controlling hormonal cascade for arterial blood pressure (Figure 8.3, bottom).

An overproduction of aldosterone under low renin conditions, for instance through APAs, has a major impact on the blood pressure regulatory system. Although hypertension has been associated with excessive aldosterone secretion, clinical and experimental evidence demonstrated that even in cases of balanced aldosterone activity, a hypertensive condition might occur. Among several APA-related pathologies is primary hyperaldosteronism (PA), a type of drug-resistant hypertension. According to Mattson and Young, PA affects 10% of the US population. In their recent study Monticone et al. described that patients diagnosed with PA exhibited cardiovascular related issues and organ damage, in comparison to other hypertensive patients.

APAs can be recognised and differentiated non-invasively using PET tracers that have been structurally optimised to interact with an enzyme involved in the overexpression of aldosterone. CYP11B2, also known as aldosterone synthase or steroid
18-hydroxylase (Figure 8.1), is a mitochondrial protein of the cytochrome P450 family expressed in the zona glomerulosa in the adrenal cortex. It acts as a catalyst for the conversion of 11-deoxycorticosterone to aldosterone. The intermediates and enzymes involved in this transformation are presented in Figure 8.3.

**Figure 8.1:** Co-crystal structure of CYP11B2 with a steroid substrate (11-deoxycorticosterone) at its active site. Image courtesy of Strushkevich and co-workers.

Aldosterone synthase shares 93% homology with steroid 11-β-hydroxylase (CYP11B1), an enzyme which participates in cortisol biosynthesis. The corresponding genes for the proteins, *CYP11B2* and *CYP11B1*, are both present on the human chromosome 8, located approximately 40 kilobase pairs apart from each other, very likely due to an evolutionary gene duplication (Figure 8.2).

**Figure 8.2:** Visualisation of structural homology of CYP11B2 and CYP11B1. Differing residues are labelled. Access channels, through which the enzyme active site can be reached, are shown in mesh. Image courtesy of Strushkevich and co-workers.
Figure 8.3: Enzymatic pathway of CYP11B2 (including CYP11B1), showing intermediate product structures. Chemical transformations are marked in red. Adapted from Shojaati and co-workers.[159]

8.1.2 PET Imaging of Aldosterone-Producing Adenomas

A small difference of 29 residues between mature CYP11B2 and CYP11B1 handicaps design of potent CYP11B2 inhibitors for PET imaging of APAs. Table 8.1 summarises CYP11B2 inhibitors that have been extensively studied in the last two decades, owing to their potential therapeutic usage in the treatment of aldosterone-
related pathologies. $[^{18}F]Fluorinated candidates for PET imaging of APAs are also presented. The key properties that define potential value of these compounds for preliminary clinical applications are: 1) $IC_{50}$ CYP11B2, 2) $IC_{50}$ CYP11B1 and 3) CYP11B2/CYP11B1 selectivity.

**Table 8.1:** PET tracer structures based on clinically attractive CYP11B2 inhibitors, with inhibition potencies. Compound **42** was a potential therapeutic.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$[^{11}C]MTO^{160}$</th>
<th>$[^{18}F]FETO^{160}$</th>
<th>$[^{18}F]LCI699^{161}$</th>
<th>42$^{162}$ (this work)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP11B2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$IC_{50}$ [nM]</td>
<td>16.7</td>
<td>20.2</td>
<td>0.7</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>CYP11B1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$IC_{50}$ [nM]</td>
<td>4.6</td>
<td>2.9</td>
<td>2.5</td>
<td>28</td>
</tr>
<tr>
<td><strong>Selectivity</strong> (B1/B2)</td>
<td>0.28</td>
<td>0.15</td>
<td>3.57</td>
<td>16.5</td>
</tr>
</tbody>
</table>

An imidazole-based CYP11B2 and CYP11B1 inhibitor, metomidate (MTO, Figure 8.4), previously serving as a veterinary anaesthetic, gained significant attention in 1998, when its $^{11}C$-labelled analogue was first used as a PET tracer for the imaging of tumours of adrenocortical origin in primates$^{163}$.

**Figure 8.4:** Metomidate its carbon-11 analogue, $[^{11}C]MTO$. 

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It exhibits high and selective uptake in the adrenal cortex, while having low affinity towards noncortical masses (the standardised uptake value (SUV) of 30.7 versus 18.4, respectively). PET imaging with $^{11}$C-MTO offered a non-invasive route for diagnosis of primary hyperaldosteronism. It was not only a more cost-effective but also less practically-demanding alternative to the current state-of-art invasive diagnosis via adrenal vein sampling (AVS). AVS, a clinical procedure for the assessment of unilaterality or bilaterality of hormonal production, is not straightforward. Issues have been reported, particularly with the small-sized right adrenal vein, which suffers from a surprisingly low success rate for cannulation (50-80%) even in the most experienced institutions. Complications following AVS include blood vessel rupture and haemorrhage, which could have a disruptive effect on laparoscopic surgery. In addition to its non-invasive nature, the applicability of PET in diagnostic tumour imaging extends to distinguishing not only its origin, cortical (such as adrenal adenomas) or noncortical (such as cyst or lipomas), but also malignancy. Although implementation of $^{11}$C-metomidate for the imaging of APAs represented a major advancement in PHA diagnosis, its widespread clinical use is inherently handicapped by its far from perfect selectivity towards aldosterone synthase in comparison with $^{11}$β-hydroxylase (B1/B2, Table 8.1). The study by Bergström et al. unveiled that high uptake of $^{11}$C-MTO was also observed in adenomas that were not hormone-secreting, hence limiting its use as an APA tracer. Evaluation of the imaging powers of $^{11}$C-MTO and $^{18}$F-FDG for adrenal incidentalomas, performed by Minn et al., confirmed the superior properties of the former in characterising tumour origin and malignancy. $^{11}$C-MTO displayed lower uptake in noncortical tumours in contrast to $^{18}$F-FDG. The short half-life of carbon-11 (20 min) and challenging radiosynthesis played a major role in the assessment of its practicality for widespread use. The use of carbon-11 is limited to institutions with an on-site cyclotron. The cost adds up quickly as only one patient can be scanned per tracer batch. Researchers have expressed moderate excitement for the future of $^{11}$C-MTO as a diagnostic tool for incidentalomas. Efforts have been directed towards the synthesis of its $^{18}$F-fluorinated analogue.
Figure 8.5: $^{18}$F\text{FETO}.

Wadsak and Mitterhauser designed $^{18}$F\text{FETO} as an alternative to $^{11}$C\text{MTO}, based on the higher affinity of the ethyl ester for CYP11B1, compared to the methyl analogue (metomidate versus etomidate). In addition, $^{18}$F-incorporation via aliphatic nucleophilic substitution is more facile than radiosynthesis of $^{11}$C\text{MTO}. Despite advantages associated with the half-life of fluorine-18 (110 min), such as wider availability, $^{18}$F\text{FETO} exhibits a similar binding profile to CYP11B2 to $^{11}$C\text{MTO} (Table 8.1), as well as equally fast metabolism. An investigation of biological properties of $^{18}$F\text{FETO} analogues was performed by Erlandsson et al., who introduced halogen substituents at the para-position and/or increased the ester chain size to 3 carbons. They concluded that better tracer uptake in the adrenals was observed for the para-substituted analogues than for $^{11}$C\text{MTO}, with decreased uptake in other organs (kidneys, liver, pancreas and lungs). The 4-chloro derivative was considered the most suitable candidate for further studies, based on: 1) favourable uptake ratio between the adrenals and liver, 2) smaller degree of defluorination and 3) highest specific binding in the series, despite relatively faster metabolism.

The search for potent CYP11B2 inhibitors arrived at fadrozole (Figure 8.6), a cyanated derivative of metomide, originally a CYP19 (aromatase) inhibitor. Interestingly, chiral separation led to the differentiation into enantiomers of nearly opposing inhibition with respect to CYP11B2 and CYP11B1.
Figure 8.6: Fadrozole, CYP11B2 inhibitor, and its enantiomers.

*R*-Fadrozole, FAD286, was shown to exhibit superior aldosterone synthase inhibition in male Chinese hamster cell lines, in comparison to 11β-hydroxylase (B1/B2 selectivity of 19.8). *S*-Fadrozole is less selective for B2 than B1 (B1/B2=0.23), similarly to [11C]MTO, however with significantly less potency for both enzymes. Simultaneously, structure-activity relationship studies of FAD286, carried out by Meredith *et al.* at Novartis, aimed to investigate its biological potential and evaluate its use as an inhibitor of aldosterone synthase. Inspired by the intriguing reversal of B2/B1 selectivity on inversion of configuration, efforts were made to explore the substitution pattern at the chiral carbon, as well as at the ortho-position on the phenyl ring. Decreasing the size of the cyclohexane by one carbon and introduction of an ortho-fluorine atom gave rise to LCI699 (Figure 8.7), with a significantly higher recombinant human CYP11B2 inhibition potency than FAD286 (1.8 nM vs 0.7 nM). Good inhibition was also obtained for CYP11B1. LCI699 is favoured over MTO and FETO based on: 1) higher potency against CYP11B2 and 2) better selectivity for B2.

Figure 8.7: LCI699, a derivative of FAD286.

In 2013 Schumacher *et al.* described their findings on the behaviour of LCI699
in vivo, following phase II clinical trials for patients with 4 types of hypertension: essential, controlled, resistant and secondary. Particularly relevant to the subject of this research were results of the proof-of-concept study with patients diagnosed with primary hyperaldosteronism. The impact of LCI699 on the activity of CYP11B2 was investigated. The levels of 11-deoxycorticosterone (a substrate for aldosterone synthase, Figure 8.3) were significantly elevated, more than it was observed with fadrozole. Plasma aldosterone levels were depleted, ascertaining the inhibitory action of LCI699 on aldosterone biosynthesis. The compound also exhibited good metabolic stability and it was well tolerated at the employed doses (0.25-2 mg). Despite promising results, the route to LCI699 becoming a therapeutic agent is impeded by its insufficient selectivity for aldosterone synthase versus 11β-hydroxylase. This manifests itself in elevated levels of 11-deoxycortisol (a substrate for CYP11B1), which triggers a hypothalamic-pituitary-adrenal feedback axis in response to the shortage of cortisol. Novartis expressed concerns about the suitability of LCI699 as a selective CYP11B2 inhibitor. Despite clear limitations of LCI699 as a therapeutic strategy, the presence of a fluorine atom offers an opportunity for quantification of aldosterone-secreting adenomas using PET imaging with fluorine-18. Tracers for PET studies are employed in subpharmacological doses (cf. nanomolar for PET to mmol for therapeutic doses) with the intention to image biochemical changes without homeostatic disruption. An [18F]fluorinated analogue of LCI699 makes a suitable tracer candidate for the imaging of APAs.

Encouragingly, an analogue of LCI699 has already been prepared by the Årstad group (Figure 8.8). The findings published were by Sander et al. in the proof-of-concept investigation of functionalised triarylsulfonium salts as precursors for 18F-labelling (Chapter 3). An imidazole-based scaffold (Scheme 8.1) was prepared in 52% yield over 5 steps. The sulfonium salt was labelled in 20 ± 2% decay-corrected isolated RCY. This example represented a major advancement in the field of fluorine-18 chemistry. A mildly-activated aromatic system was successfully labelled directly, in the presence of a basic moiety.
Figure 8.8: \([^{18}\text{F}]\text{LCI699}\) (left) and its imidazole-based structural derivative (right), investigated by Sander and co-workers\(^{[3]}\).

Scheme 8.1: Synthesis and radiolabelling of the imidazole scaffold performed by Sander and co-workers.  
Reagents and conditions:  
(i) NaBH\(_4\), MeOH, 0 °C - rt, 2 h;  
(ii) PBr\(_3\), DMC, 0 °C - rt, 6 h;  
(iii) NaH, imidazole, DMF, rt, 16 h;  
(iv) Bis(4-methoxyphenyl)iodonium, Cu(II) benzoate, TfOH, chlorobenzene, reflux, 12 h;  
(v) \([^{18}\text{F}]\text{F}^-\), K\(_{222}\), KHCO\(_3\) DMSO, 110 °C, 15 min.  

LCI699, among many others, is a product of extensive structural modifications of \(R\)-fadrozole. They were carried out in the hope of arriving at a more potent CYP11B2 inhibitor for the study and treatment of aldosterone-dependant pathologies, including primary hyperaldosteronism. These alterations involved basic heterocycles, such as
imidazoles, pyridines or isoquinolines, owing to their affinity for the central Fe$^{2+}$ ion of hemoproteins, e.g. CYP11B1 and CYP11B2. Roumen et al. employed molecular docking to investigate the structure-activity relationship of fadrozole analogues. The list of possibilities was condensed to 1-benzyl-1H-imidazoles, devoid of chiral centres or bicyclics, based on the interaction of 18-hydroxycorticosterone, a CYP11B2 substrate, with the active site of the enzyme. The results of in silico research were complemented by in vitro screening in hamster cells modified to express human cortisol and aldosterone synthases. Substitution pattern at the phenyl ring was explored through F, Br, Cl, CN, methoxy, amine and hydroxy substituents in the meta- and para-positions, as well as the 5-position on the imidazole. Structure 42 proposed by Roumen and co-workers possesses a nitrile group in the 4-position and a phenylimidazole moiety (Figure 8.9).

Figure 8.9: Compound 42, investigated by Roumen and co-workers, is a structural derivative of fadrozole.

Imidazole 42 was found to exhibit high potency for CYP11B2 and the highest selectivity for B2 versus B1 (Table 8.1 page 117). The para-CN substituent and its interaction with arginine Arg123 was found to be essential for achieving desired selectivity for aldosterone synthase. The bulky 5-phenyl substituent introduces steric hindrance and reduces conformational freedom experienced by the 1-benzyl-1H-imidazole skeleton of fadrozole.
8.1.3 Synthesis of Imidazole-Based Dibenzothiophene Sulfonium Salt Precursor to Novel PET Tracer Candidate

First Attempt at Cyclisation and $^{18}$F-Labelling

Having explored numerous avenues in the search for promising PET tracers, high hopes were placed on candidate 43 as a potential CYP11B2 inhibitor for the imaging of aldosterone-producing adenomas.

\[
\text{CN} \quad \text{F}
\]

Figure 8.10: Compound 43, with a fluorine-18 tag at the 2-position of the phenyl ring, was proposed as the new lead structure for the imaging of APAs.

Encouraged by the successful labelling of metomidate derivative 44 by Sander et al. (Scheme 8.1), a synthetic route towards compound 43 was designed (Figure 8.10). It was speculated that radiolabelling efficiency could be improved, given the more electron-withdrawing power of the nitrile group meta to the $^{18}$F substituent (Hammett constant for CN, $\sigma_{\text{meta}} = 0.56$) in comparison with chlorine in the 4-position ($\sigma_{\text{para}} = 0.23$). The synthetic route to sulfonium salt precursor 49 is shown in Scheme 8.2.
Scheme 8.2: Synthesis of sulfonium salt 49.
Reagents and conditions: (i) HMDS, reflux, 20 h; (ii) 4-(Bromomethyl)-3-iodobenzonitrile, K$_2$CO$_3$, DMF, 100 °C, 3 h; (iii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 3.5 h; (iv) Optimisation process of cyclisation conditions.

Initial stages of the investigation were performed by Dr Vincent Gray (formerly UCL Radiochemistry), who synthesised the non-radioactive fluorinated counterpart to compound 43 and established the synthetic route to thioether 48. Phenylimidazole was first protected with a trimethylsilyl group in an overnight reaction with hexamethyldisilazene (HMDS) at 100 °C. The resulting compound was used without purification in the next step, where it underwent an S$_N$2 reaction with 4-(bromomethyl)-3-iodobenzonitrile to form imidazole 47. Coupling to thioether 4 (Scheme 5.3), mediated by Pd$_2$(dba)$_3$, afforded thioether 48 in 75% yield. It was then employed in the cyclisation reaction to form sulfonium salt 49, a precursor for $^{18}$F-labelling.

Cyclisation was first attempted with NCS and Bi(OTf)$_3$ in MeCN at room temperature for 2 h. Disappointingly, only a negligible yield of the desired product was obtained. NMR analysis revealed the presence of a chlorinated sulfonium salt analogue (Figure 8.11), resulting from the competitive side chlorination, as observed for sulfonium salt 37 (Chapter 7, Section 7.5, Table 7.2). HPLC analysis revealed
that the chlorinated product constituted as much as 83% of the sample. Its mass was confirmed by LC-MS analysis. Similar results were obtained when NCS and TfOH were used in the cyclisation. In addition, it was observed that any efforts towards deprotonation of the imidazole moiety failed or resulted in decomposition. In the presence of strong bases such as NaH or NaOH decomposition occurred, while milder bases, such as potassium carbonate, failed to accomplish deprotonation. In contrast, the imidazole-decorated triarylsulfonium salt (Scheme 8.1) was washed with 3 M NaOH, before column chromatography purification, without decomposition. When subjected to $[^{18}\text{F}]\text{F}^{-}$, the mixture of sulfonium salt 49 and its chlorinated analogue 50, failed to deliver the radiolabelled product. Although both are expected to undergo radiolabelling in a similar manner, $[^{18}\text{F}]\text{F}^{-}$ is deactivated by the extra proton as a result of hydrogen bonding. Precursors for labelling are used in large excess, hence no radiolabelling is expected to occur. This is illustrated in Figure 8.11.

![Inhibition of labelling](image)

**Figure 8.11:** The major product of the cyclisation reaction of thioether 48 with NCS/Bi(OTf)$_3$ and NCS/TfOH is chlorinated sulfonium salt 50.

Taking over the investigation from Dr Vincent Gray, an initial explanation for his results was formulated. It was based on a hydrogen-bond interaction of the proton with a neighbouring methoxy group on the dibenzothiophene scaffold. This could result in stabilisation of the protonated form and unsuccessful deprotonation attempts (Figure 8.12). To test this hypothesis, a radiolabelling experiment was set up, in which the sulfonium salt precursor to $[^{18}\text{F}]\text{FPEB}$ (Chapter 5) was labelled in the presence of sulfonium salt 49 and 50. Remarkably, the reaction failed and no $[^{18}\text{F}]\text{FPEB}$ formation was observed, leading to the conclusion that $[^{18}\text{F}]$fluorination of the corresponding sulfonium salt 6 was blocked. Protonation of the imidazole
moiety of sulfonium salts 49 and 50 could be a potential explanation for the findings.

![Chemical structure](image)

**Figure 8.12:** Hypothetical "proton sponge". Interaction of the OMe substituent with the protonated imidazole leads to the formation of a stabilised structure.

It was proposed that the problem could be approached from two angles by: 1) by performing the cyclisation with hypochlorite-based reagents to avoid protonation (Chapter 6) or 2) removing the problematic methoxy group to help deprotonation.

In the first instance, hypochlorite reagents were employed to effect ring closure. Both NaOCl and Ca(OCl)$_2$ dissociate in water to form HOCl and strong bases, NaOH or Ca(OH)$_2$, respectively. For a sulfonium salt to be formed from the corresponding thioether, a proton needs to be eliminated to restore aromaticity. In basic conditions, undesired imidazole protonation would not occur. Small-scale cyclisations of thioether 48 were set up with NaOCl (10-15%, reagent grade) and Ca(OCl)$_2$.

HPLC analysis of a 10 min reaction of thioether 48 with NaOCl at room temperature revealed the presence of sulfonium salt 49 with a relatively small proportion of the chlorinated side product. A significant proportion of the starting material was still observed in the reaction profile, with prominent lipophilic impurities. It was difficult to quantify conversion due to peak tailing and the presence of shoulders. When the reaction was repeated, the profile could not be reproduced and only decomposition was observed. This scenario was already seen in the optimisation process with thioether 14 (Chapter 6, Section 6.3.2). Cyclisation with Ca(OCl)$_2$ did not afford any product at room temperature, yet prominent decomposition peaks appeared after 10 min. Despite the underlying potential of hypochlorite-mediated dibenzothiophene sulfonium salt formation, the method suffers from reproducibility and robustness.
issues and an alternative approach was required.

**Modifications of Dibenzothiophene Sulfonium Salt Scaffold**

In order to prove the existence of the "proton sponge" effect (Figure 8.1), the proximal ortho-methoxy group was removed from the dibenzothiophene scaffold. There were two potential benefits associated with this modification: 1) interruption of the hypothetical stabilisation effect caused by H-bonding between the protonated imidazole and the ortho-methoxy group, 2) reduction of electron density on the diaryl thioether scaffold, thus lowering probability of side chlorination.

The modified thioether 52 was accessed in an analogous synthetic route to thioether 48 (Scheme 8.2). Suzuki coupling of thioether 3 with 3-methoxyphenylboronic acid afforded thioether 51 in an excellent yield of 87%. Thioether 52 was obtained in 77% yield. The entire synthetic strategy is presented in Scheme 8.3.

![Scheme 8.3: Synthesis of sulfonium salt 53.](image)

Reagents and conditions: (i) 3-Methoxyphenylboronic acid, Pd(PPh₃)₄, K₂CO₃, toluene/water, reflux, 16 h; (ii) Thioether 51, Pd₂(dba)₃, DPEphos, KOTBu, toluene, reflux, 3.5 h; (iii) NCS, TfOH, MeCN, rt, 2 h.

The first cyclisation was performed with NCS and Bi(OTf)₃ in MeCN. After 10 min at room temperature, a sample of the reaction mixture was analysed by HPLC, however only starting material was observed. Another aliquot was collected after 2 h but no changes occurred. With 1 equivalent of NCS and 3 equivalents of TfOH,
the cyclisation reached full conversion after 2 h. Only 20% analytical yield was obtained from a 10 min reaction but no other side products were identified. It can thus be inferred that the rate of chlorination was significantly impeded. Flash column chromatography was performed with an addition of ammonia in the eluent. As judged from a 2D TLC plate, no decomposition occurred. Sulfonium salt 53 was isolated in 74% yield. Conveniently, only one regioisomer was formed in the cyclisation, as elucidated from the splitting pattern of the aromatic protons on the dibenzo thiophene scaffold, depicted in Figure 8.13.

Figure 8.13: Two possible regioisomers of sulfonium salt 53, expected from the cyclisation reaction of thioether 52. Only the left-hand side structure was observed by proton NMR.

NMR analysis of the purified product showed a noticeable chemical shift difference of the methylene bridging moiety, compared to the parent thioether spectrum. A shift of 1.1 ppm is too large to result from temperature or pH fluctuations. The likely explanation is the persistent presence of the protonated imidazole form. The change in the methylene peak multiplicity (from singlet to doublet) results from chirality at the sulfur core, which induces In thioether 52, CH₂ exists as singlet, in the case of sulfonium salt 53, two well resolved doublets are observed due to chirality at the S-centre. Interestingly, no nitrogen-bonded protons were observed by NMR spectroscopy. This is shown in Figure 8.14. A similar picture was also observed by Dr Vincent Gray, with thioether 48 and sulfonium salt 49 (Scheme 8.2). The protonated sulfonium salt form was also identified in the ESI mass spectrum. Sulfonium salt 53, similarly to sulfonium salt 49, failed to undergo [¹⁸F]fluorination.
Figure 8.14: Superimposition of proton NMR spectra of thioether 52 (red) and sulfonium salt 53 (black) in d$_6$-DMSO. The difference in chemical shift for the methylene protons between the compounds is approximately 1.1 ppm.

Further Modifications to Dibenzothiophene Sulfonium Salt Scaffold

Removing the ortho-methoxy group allowed for the following observations. Firstly, electron density on the dibenzothiophene scaffold influences the rate of sulfonium salt formation as well as the rate of chlorination. Secondly, there are other factors implicated in the unusual stability of the protonated form of sulfonium salt 53, which cannot be ascribed solely to the hydrogen bonding interaction between the ortho-methoxy substituent. It was proposed that by replacing the dibenzothiophene methyl group with a methoxy, electron density of the system would increase, resulting in a more efficient cyclisation. It was speculated that sulfonium salt formation could perhaps be achieved in the presence of hypochlorite reagents, such as Ca(OCl)$_2$. Consequently, the problem of imidazole protonation would be eliminated. The described scenario is illustrated in Figure 8.15. Another benefit of this approach could include deconvolution of NMR spectra analysis of sulfonium salt, as a result of formation of a symmetrical dibenzothiophene scaffold. The proposed synthetic route to sulfonium salt 58 is presented in Scheme 8.4.
Figure 8.15: Replacing the methyl group with a methoxy is expected to increase electron density on the dibenzothiophene scaffold and facilitate cyclisation, even with milder reagents.

Scheme 8.4: Synthesis of sulfonium salt 58.
Reagents and conditions: (i) NBS, DMF, -5 °C, 1 h; (ii) Pd$_2$(dba)$_3$, xantphos, Et$_3$N, toluene, reflux, 18 h; (iii) Thioether 56, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 18 h; (iv) Optimisation process of cyclisation conditions.
Aromatic bromination of 3,3’-dimethoxy-1,1’-biphenyl at the para-position afforded the desired bromoarene 55 quantitatively. Installation of the alkyl protecting group was completed after refluxing overnight, however flash chromatography purification proved more challenging due to the formation of impurities, which co-eluted with product 56. As a result, two consecutive chromatography purifications were required. A 0-10% DCM/toluene elution allowed for a seemingly successful separation, however as the presence of impurities was revealed by NMR spectroscopy, a 10-30% ether/petroleum ether gradient was required to obtain a high-purity sample of thioether 56. Eventually, it was isolated in 41% yield. Subsequent coupling with imidazole 47 afforded thioether 57 in 71% yield.

Cyclisation of thioether 57 was attempted with Ca(OCl)₂ in MeCN at room temperature, yet no sulfonium salt formation was observed. LC-MS analysis of the reaction mixture revealed the presence of starting material and its chlorinated analogue. Perhaps the substantial increase in electron density, resulting from the replacement of the methyl entity by methoxy, favoured aromatic chlorination over S-Cl bond formation.

Further Insights into Dibenzothiophene Sulfonium Salt Formation

An alternative hypothesis was proposed to explain the unusual stability of the protonated imidazole towards deprotonation. It was based on its interaction with the soft triflate counterion. Replacement with hydroxide caused decomposition of the sulfonium salt scaffold. This is illustrated in Figure 8.16. One piece of evidence towards this assumption was provided by Dr Vincent Gray, who at the beginning of the investigation, attempted deprotonation of sulfonium salt 49 with NaOH. Decomposition was observed as an almost instantaneous darkening of the reaction mixture during liquid-liquid extraction.
To test this concept, imidazole 47 (Scheme 8.4) was protonated with 1 equivalent of TfOH and subsequently washed with 2 M NaOH. Proton NMR spectrum of the resulting organic extracts showed no signs of decomposition or peak shifting (Figure 8.17).

This issue could perhaps be resolved with replacement of the triflate counterion during sulfonium salt formation. Crivello et al. successfully synthesised triarylsulfonium salts with fluoride-based counterions: BF$_4^-$, AsF$_6^-$, SbF$_6^-$ and PF$_6^-$ [79]. The
aforementioned anions are soft and non-nucleophilic, thus very good at stabilising the positive charge on the sulfur. Limitations of this method lie in the potential significant decrease of specific activity of $[^{18}\text{F}]\text{F}^-$ due to isotopic exchange by the counterion. Small nucleophilic anions, such as $\text{Cl}^-$ or $\text{Br}^-$ are not suitable for this application. Swain and Kaiser reported decomposition of trialkyl- and triaryl sulfonium halides\[173\].

**Modifications of PET Tracer Structure**

Findings described in previous sections inspired further investigation into 1-phenyl-$1^H$-imidazoles as inhibitors of aldosterone synthase. Migration of the phenylimidazole moiety from the *ortho*-position, with respect to the nitrile, to the *meta*, gave rise to a novel PET tracer candidate for the imaging of APAs (Figure 8.18). Substituent shift is expected to: 1) change electronics of the system, 2) affect the rate of sulfonium salt formation and 3) reactivity towards $[^{18}\text{F}]\text{F}^-$. This investigation also served as a practical approach for further validation of the counterion effect hypothesis (Figure 8.16).

![Figure 8.18](image)

**Figure 8.18:** Target tracer 43 has not been obtained. Regioisomer 59 could address the limitations of its *ortho*-analogue during radiolabelling.

Although *in vitro* binding affinities of CYP11B2 and CYP11B1 were established for various structurally related 1-phenyl-$1^H$-imidazoles, for most of these compounds, the imidazole moiety is situated *para* to the phenyl substituent\[162\]. Although no potencies were available for compound 59 at the time of this investigation, the structure made an interesting case study from the chemistry and radiochemistry point of view. It was thought to complement the scope of highly-functionalised drug-like molecules in the ongoing evaluation of dibenzothiophene sulfonium salts as
precursors for $^{18}$F-labelling. The synthetic route to sulfonium salt 64 is presented in Scheme 8.5.

**Scheme 8.5:** Synthesis of sulfonium salt 64.
Reagents and conditions: (i) AIBN, NBS, chlorobenzene, reflux, 4 h; (ii) Phenylimidazole 46, K$_2$CO$_3$, DMF, 100 °C, 1 h; (iii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 2 h; (iv) NCS, TfOH, MeCN, rt, 2 h.

Benzyl bromide 61 was accessed in 48% yield from a Wohl-Ziegler bromination of arene 60 with NBS and a radical initiator AIBN$^{174}$. It was then employed in a regioselective N-alkylation with the TMS-protected imidazole 46 (Scheme 8.2). Imidazole 62 was synthesised in 72% yield. Palladium-mediated thioetherification with thioether 4 (Scheme 5.3) afforded compound 63 in a surprisingly low yield of 17%. Pleasingly, sulfonium salt 64 was formed in 48% yield.

Analysis of proton NMR shifts of the bridging CH$_2$ protons of thioether 63 and sulfonium salt 64, revealed a difference of approximately 0.4 ppm (Figure 8.19), much smaller than observed for thioether 52 and sulfonium salt 53 (Figure 8.14). For the latter, unsuccessful $^{18}$F-labelling attempts, together with NMR data, served as evidence towards protonation of the imidazole moiety and formation of a highly-stable complex.

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Figure 8.19: Superimposition of proton NMR spectra of thioether 63 (red) and sulfonium salt 64 (black) in CDCl$_3$. The difference in chemical shift is calculated as approximately 0.4 ppm.

The cold reference compound for co-injections, imidazole 67, was accessed in a 2-step route, starting from 3-fluoro-5-methylbenzonitrile. The brominated intermediate 66 was obtained in 48% yield. Finally, coupling to imidazole 46 yielded reference compound 67 in 50% yield (Scheme 13.11).

Scheme 8.6: Synthesis of reference compound 67.
Reagents and conditions: (i) AIBN, NBS, chlorobenzene, reflux, 4 h; (ii) Phenylimidazole 46, K$_2$CO$_3$, DMF, 100 °C, 2 h.

Radiolabelling of Novel Imidazole-Based Dibenzothiophene Precursor

Sulfonium salt 64 was employed in a series of $^{18}$F-labelling experiments, however poor RCYs were obtained, as shown in Table 8.2. When $^{18}$F-fluorination was performed in DMSO, radio-HPLC purification of the entire reaction mixture was not possible due to precipitation after quenching with water. As a result, only an approximate analytical RCY of 7-12% was obtained. In MeCN, tracer 59 was isolated in 6-7%
RCY (Scheme 8.7).

**Table 8.2:** Radiolabelling experiments with sulfonium salt 64. All isolated yields are decay-corrected and represent the end of synthesis HPLC yields. Whenever the radiolabelled product was not isolated, analytical radio-HPLC yields are provided.

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<tr>
<td>1</td>
<td>2</td>
<td>DMSO</td>
<td>110</td>
<td>15</td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>DMSO</td>
<td>110</td>
<td>15</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>MeCN</td>
<td>80</td>
<td>15</td>
<td>7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>MeCN</td>
<td>80</td>
<td>15</td>
<td>6</td>
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<sup>a</sup> Analytical radio-HPLC yield. Only half of the crude reaction mixture analysed due to precipitation.  
<sup>b</sup> Only half of the crude reaction mixture was analysed.

**Scheme 8.7:** Most successful labelling of sulfonium salt 64 with [18F]F<sup>-</sup>. Isolated decay-corrected RCY was 6-7%.

Despite low radiochemical efficiency, the results of the labelling experiment represent a significant advancement in the study of imidazole-based dibenzothiophene sulfonium salts. Following a long investigation, it was possible to achieve [18F]fluorination of sulfonium salt 64. It served as a learning curve experiment, through which better understanding of sulfonium salt chemistry was gained. Firstly, the dibenzothiophene scaffold can be modified to modulate the rate of chlorination at the methoxy-decorated ring. Secondly, the sulfur centre is highly sensitive to changes in the electronic structure of the PET scaffold, and both cyclisation and [18F]fluorination efficiencies will be affected.
The striking difference in the ease of deprotonation for sulfonium salt 64 versus sulfonium salt 53 remains unaccounted for. The counterion stability hypothesis (Figure 8.16) cannot be backed up by this observation. It can be speculated that sulfonium salt 49 (Figure 8.20) adopts a stable conformation, in which the protonated imidazole is stabilised by the methoxy-substituent and/or the dibenzothiophene as a result of π-π stacking. This could perhaps be the consequence of rotational freedom around the methylene bridge. This interaction might not possible when the imidazole substituent is moved to the meta-position. Structural evidence, for example, crystallography data or in silico methods, are required to gain more insights into the factors orchestrating reactivity of these sulfonium salts towards $[^{18}\text{F}]\text{F}^-$. A crystal structure of the allegedly protonated forms could help confirm protonation of the imidazole moiety. Given unsuccessful labelling experiments of sulfonium salts 49 and 53, even in the presence of $[^{18}\text{F}]\text{FPEB}$ (labelled in good RCYs, Chapter 5), the unusual proton stability and consequences thereof are the most likely explanation for the above findings. All imidazole-based sulfonium salts described in this chapter are shown in Figure 8.20.

**Figure 8.20:** All imidazole-based sulfonium salt precursors to the novel PET tracer candidate for the imaging of APAs. The difference in the position of the imidazole substituent could affect the ease of deprotonation, decomposition under basic conditions and radiolabelling efficiency.
8.2 Conclusion

The investigation presented in this chapter has deepened knowledge into the factors orchestrating formation, stability and reactivity of imidazole-based dibenzothiophene sulfonium salts.

Removal of the ortho-methoxy group (Figure 8.20) caused significant retardation of the rate of competitive aromatic chlorination. The corresponding sulfonium salt was afforded as the sole product, however, it was obtained in its protonated form due to the acidic nature of the cyclisation with NCS and TfOH. Efforts to deprotonate the product by addition of ammonia to the eluent during flash chromatography proved futile. Disappointingly, alternative reagents did not mediate sulfonium salt formation.

Attempts to explain the unusual stability of the protonated sulfonium salt form were unsuccessful. The triflate counterion was initially thought to stabilise the imidazolium moiety as deprotonation with strong bases resulted in decomposition. This effect was not observed when the imidazole precursor (Figure 8.17) was protonated with triflic acid and subsequently successfully deprotonated with sodium hydroxide. Unsurprisingly, sulfonium salt 53 did not undergo $^{18}$F-labelling.

The meta-isomer 64 (Figure 8.20) was successfully deprotonated with sodium hydroxide before being subjected to $^{18}$F-labelling. A surprisingly low isolated RCY of 6-7% was obtained. It had initially been speculated that radiolabelling of sulfonium salt 64 could proceed with similar RCY to that of sulfonium salt 7, the precursor to $[^{18}\text{F}]\text{FPEB}$ (Chapter 5, Section 5.1.1, Scheme 5.5). At this moment, it is difficult to rationalise the difference in reactivity towards $[^{18}\text{F}]\text{F}^-$ of these structurally-similar substrates.

In addition to the limited practicality of the radiolabelling, there currently is no biological data, specifically binding affinities for CYP11B2 and CYP11B1, to back up further development of precursor 64.
9 | Applications of Dibenzothiophene Sulfonium Salts: PET Tracer 2

This chapter presents efforts towards the synthesis of a meta-pyridine-based PET tracers for the imaging of aldosterone-producing adenomas (APAs), using dibenzothiophene sulfonium salts as precursors for $^{18}$F-labelling. It builds on the foundations of Chapter 7, which provides insights into the synthesis and radiolabelling of a simplified meta-pyridine sulfonium salt scaffold.

9.1 Synthesis of Dibenzothiophene Sulfonium Salt Based on Aldosterone Synthase Inhibitor

9.1.1 Selection of Novel PET Tracer Candidate for Imaging of APAs

A selection of small molecule CYP11B2 inhibitors were described in Chapter 8. LCI699 was shown to lower aldosterone levels and blood pressure in the clinic, thereby validating this mechanism as a treatment for hypertension. In cell-based in vitro assays, LCI699 inhibited CYP11B2 with a modest 4-fold selectivity versus CYP11B1. It also produced an undesired, dose-limiting impairment of cortisol response, presumably as a result of CYP11B1 inhibition. Clinical candidates with higher B2/B1 selectivity are required in antihypertensive therapies.

In 2015 Merck published their discovery of benzimidazole-based CYP11B2 inhibitors, which demonstrated good pharmacokinetic properties in rat and rhesus monkeys. This extensive optimisation involved screening the Merck compound library to search for a candidate with a high CYP11B2 inhibition potency and selectivity versus CYP11B1. A selection of Merck’s benzimidazole series is shown in Figure 9.1.
Figure 9.1: Selected benzimidazole inhibitors of CYP11B2 investigated by Merck, with reported IC\textsubscript{50} values for CYP11B2 (a) and B1/B2 selectivity (b).

Initially N-substitution pattern on the benzimidazole moiety was studied. A correlation was found between potency deterioration and increasing the size of the aliphatic substituent. Interestingly, N-cyclopropyl-substituted benzimidazole 69 outperformed its isopropyl counterpart 68 in terms of CYP11B2 inhibition. Subsequent optimisation of benzimidazole phenyl ring substitution identified 5,6-disubstituted analogues, such as compound 70, with favourable IC\textsubscript{50} values for CYP11B2 and good B1/B2 inhibition ratios. Investigation of pyridine substitution lead to the refinement of CYP11B2 potencies, particularly with electron-donating groups, such as methyl or alkoxy. Structure 71, exhibited excellent inhibition of CYP11B2, however its B1/B2 selectivity was compromised. Inhibitor 72 was identified as the benchmark compound, which owing to a good inhibition and convenient pharmacokinetics, was evaluated in rhesus monkeys. Structure 73 displayed decent CYP11B2 potency (IC\textsubscript{50} of 4.7 nM), with favourable selectivity over B1. A comparison of its inhibition profile with LCI699 is shown in Table 9.1. Despite a lower CYP11B2 inhibition value, LCI699 is significantly less selective for B2 than benzimidazole 73.
Table 9.1: Inhibition profiles of benzimidazole 73 (Merck) and LCI699 (Novartis).

<table>
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<tr>
<th>Inhibitor</th>
<th>LCI699 (^{[161]})</th>
<th>Benzimidazole 73 (^{[175]})</th>
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<tr>
<td>CYP11B2</td>
<td>0.7</td>
<td>4.7</td>
</tr>
<tr>
<td>IC(_{50}) [nM]</td>
<td>2.5</td>
<td>435</td>
</tr>
<tr>
<td>Selectivity (B1/B2)</td>
<td>3.57</td>
<td>93</td>
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With a good \textit{in vivo} PK profile, plasma clearance and half-life, the molecule represents a suitable radioligand candidate for PET imaging of aldosterone-producing adenomas. Substitution of the \textit{meta}-fluoro substituent on the pyridine with fluorine-18 was chosen as a proof-of-concept case study for dibenzothiophene sulfonium salts as leaving groups in \[^{18}\text{F}\]fluorination. Encouraged by the successful labelling of a simplified \textit{meta}-pyridyl moiety (Chapter \[7\] Section \[7.5\]), the strategy was then implemented to access more structurally demanding drug-like scaffolds. Target PET tracer candidate 74 is shown in Figure 9.2.

![Figure 9.2](image)

\textbf{Figure 9.2:} Benzimidazole 74 was selected as the novel tracer candidate for PET imaging of APAs.

### 9.1.2 Synthesis of 3-Pyridyl-Based Dibenzothiophene Sulfonium Salt Precursor to Novel PET Tracer

Syntheses of the sulfonium salt precursor to tracer 74 proved less facile than initially anticipated. It was based on patent procedures published by Merck, often lacking
full method descriptions and characterisation\textsuperscript{175}. The early efforts are presented in Scheme 9.1.

**Scheme 9.1:** First attempt at the synthesis of benzimidazole 79 and cold reference compound for co-injections 73, based on the patent by Merck\textsuperscript{175}.  
Reagents and conditions: (i) Cyclopropylamine, KF, K$_2$CO$_3$, MeCN, reflux, 1 h; (ii) Pd/C, H$_2$, MeOH, rt, 20 h; (iiia) 5-Bromonicotinic acid, HATU, HOBT, DIPEA, DCM, rt, 2 h; (iiib) 5-Fluoronicotinic acid, HATU, HOBT, DIPEA, DCM, rt, 17 h; (iv) AcOH, 100 °C, 1 h.

Formation of amine 76 was perhaps the most challenging transformation. Hoyt et al. accessed this compound via a nucleophilic aromatic substitution using microwaves. Due to equipment restrictions, a thermal reaction was attempted instead. S$_{N}$$\text{Ar}$ reactions favour polar aprotic solvents, so acetonitrile was chosen, based on its convenient boiling point. The reaction was expected to give 2 regiomers (Figure 9.3) as the nitro group is both an ortho- and para-activator.
Figure 9.3: Two products were obtained in the substitution reaction of 1,2,4-trifluoro-5-nitrobenzene with cyclopropylamine. Compound 76 is required for further transformations.

The following variables were investigated: 1) order of addition, 2) stoichiometry with respect to KF and 3) temperature. The reaction proved challenging to optimise and it suffered from reproducibility issues. Yields between 4 and 43% were obtained, with undesired regiomer 81 as the major product. To achieve the maximum potential of this reaction, it was essential to use at least 1.5 equivalents of KF, while feeding cyclopropylamine dropwise into the refluxing reaction mixture. For best results, uniform heat distribution was ensured through the use of a sand bath. In addition, frequent monitoring of reaction progress was essential. Product 76 (orange solid) was easily separated from its regiomer (yellow solid) by flash column chromatography, as the fast eluting fraction. Full consumption of starting material is critical as it has a similar Rf value to amine 75.

The nitro group of amine 76 was reduced to form dianiline 77 in 86% yield, in an overnight reaction with Pd/C and hydrogen gas. Subsequent coupling to 5-fluoronicotinic acid using HATU, HOBT and DIPEA afforded the corresponding amide 80 in 65% yield. Formation of non-radioactive reference compound 73 was achieved by heating the former in refluxing acetic acid, which proceeded quantitatively. Analogously, 5-bromonicotinic acid was coupled to diamine 77 in 65% yield and subsequent dehydration afforded the corresponding benzimidazole 79 in 74% yield.

Benzimidazoles 79 and 73 can also be obtained directly from diamine 77 in a one-step convenient Oxone®-mediated condensation with the corresponding aldehydes. 5-Fluoro- and 5-bromonicotinaldehyde were coupled to diamine 77 in 65% and 59% yield, respectively. Although the yields are slightly lower than those obtained in Scheme 9.1, the new route is quicker and more practical. Subsequent
palladium-catalysed coupling of thioether 4 (Scheme 5.3) and benzimidazole 79 afforded thioether 82 in 88% yield. Reference compound 73 (for co-injections) was isolated in 65% yield. Sulfonium salt 82 was accessed in 66% yield. A short optimisation process with alternative milder reagents, such as hypochlorites, did not produce satisfactory results. Reproducible yields were obtained when the appropriate amount of NCS was added from stock solution of NCS to thioether 82, followed by neat TfOH. The synthesis is summarised in Scheme 9.2.

Scheme 9.2: Syntheses of sulfonium salt 83 and cold reference compound 73. Reagents and conditions: (i) Cyclopropylamine, KF, K$_2$CO$_3$, MeCN, reflux, 1 h; (ii) Pd/C, H$_2$, MeOH, rt, 20 h; (iii) 5-Bromonicotinaldehyde, Oxone®, DMF/H$_2$O, rt, 1 h; (iv) 5-Fluoronicotinaldehyde, Oxone®, DMF/H$_2$O, rt, 1 h; (v) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 2 h; (v) NCS (stock solution), TfOH, MeCN, rt, 30 min.
9.2 Radiolabelling of Novel PET Tracer Candidate

Substantial efforts were directed towards establishing optimum $^{18}$F-labelling conditions for sulfonium salt 83. The results are presented in Table 9.2.

**Table 9.2:** Radiolabelling experiments with sulfonium salt 83. All reactions employed 2 mg of the precursor. All isolated yields are isolated (unless stated otherwise), decay-corrected and represent the end of synthesis HPLC yields.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Temperature [$^\circ$C]</th>
<th>Time [min]</th>
<th>Radio-HPLC Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMSO</td>
<td>110</td>
<td>15</td>
<td>18-87</td>
</tr>
<tr>
<td>2</td>
<td>DMSO</td>
<td>110</td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>DMSO</td>
<td>50</td>
<td>15</td>
<td>-a</td>
</tr>
<tr>
<td>4</td>
<td>MeCN</td>
<td>80</td>
<td>15</td>
<td>24</td>
</tr>
</tbody>
</table>

*a* Not isolated. Analytical radio-HPLC yield: 56%.

The first radiolabelling attempt, with $K_{222}$ and KHCO$_3$ in DMSO at 110 $^\circ$C, afforded a high analytical RCY of 72%. The tracer was isolated with 46% RCY. In subsequent experiments, RCYs in the range of 18 to 87% were obtained (Table 9.2, Entry 1). A decrease in reaction time to 5 min had little impact on the RCY - 43% of the labelled product was isolated (Entry 2). The reaction proceeded remarkably well at 50 $^\circ$C for 15 min and an analytical RCY of 56% was obtained (Entry 3). Radiolabelling was also evaluated with MeCN, a preferred solvent under Good Manufacturing Practice synthesis of PET tracers, however a significantly lower isolated RCY were obtained (Entry 4). Benchmark labelling conditions were established as: DMSO at 110 $^\circ$C for 15 min (Scheme 9.3). The average decay-corrected isolated RCY obtained for this reaction system was 53 ± 18% (n=12). The average specific activity was established at 14.7 ± 9.6 GBq/µmol. Considerable amount of spread in the data can be expected for manual labelling experiments. It is speculated that large temperature fluctuations in the radiochemistry lab had a major impact on radio-HPLC retention times, often causing co-elution of the tracer with impurities, resulting in lower RCYs. Some discrepancies can also be expected as two different sulfonium salt batches were used for radiolabelling. At this stage of the investigation, no efforts were made to control these variables. To the best of my knowledge, this is currently the most efficient direct $^{18}$F-labelling of a highly-functionalised tracer scaffold at the meta-pyridine moiety without the use of activating groups.
Scheme 9.3: Optimised $^{18}$F-labelling conditions for sulfonium salt 83. Average isolated RCY was established at 53 ± 18% (decay-corrected, n=12).

9.3 Preliminary Biological Evaluation of Novel PET Tracer Candidate

With radiolabelling of precursor 83 proceeding in high isolated RCYs, preliminary biological evaluation was initiated without further delay. All animal experiments were performed by Dr Kerstin Sander (UCL Radiochemistry). Tracer 74 was evaluated in healthy wildtype mice. Radioactivity distribution was assessed at 5 different time intervals (from 0 to 60 min) after injection of 5-8 MBq of the tracer. In 2-5 minutes post-injection, the tracer was shown to accumulate in the liver and in the next 10 minutes, excretion process began. After 15 min, the tracer began accumulating in the joints and skull, potentially due to defluorination. Reassuringly, no uptake was detected in the adrenal region, hence no background signal in that region is expected for subjects overexpressing aldosterone synthase.

Figure 9.4: Preliminary biological evaluation of tracer 74 in healthy wildtype mice. PET experiments were performed by Dr Kerstin Sander. Pharmacokinetic profile was established using 5-8 MBq of tracer candidate after intravenous injection.
Additional scans, together with biodistribution and metabolite analysis, are required to confirm these preliminary results. Further evaluation includes autoradiography using adenoma-affected adrenal masses. This investigation has now been taken over by fellow members of the Årstad group at UCL Radiochemistry.

9.4 Conclusion

In the presented investigation, a dibenzothiophene sulfonium salt precursor to a novel PET tracer candidate was synthesised. Its structure is based on a potent aldosterone synthase inhibitor structure, developed by Merck. The tracer is a potential candidate for the imaging of aldosterone-producing adenomas, one of the causes of drug-resistant hypertension.

Dibenzothiophene sulfonium salt 83 was of particular interest to the Årstad group for the following reasons. Firstly, it is a precursor to a potent CYP11B2 inhibitor, displaying good selectivity versus CYP11B1. Secondly, it is a highly-functionalised drug-like molecule, based on basic N-heterocycles - benzimidazole and pyridine. Thirdly, it represents a deactivated aromatic system, with a pyridyl moiety situated meta to the sulfur atom. In this investigation, an attempt was made to label a more complex scaffold without the use of directing groups.

Building on the work of Chapter 7, sulfonium salt precursor 83 was obtained in a gratifying yield of 66% from a cyclisation reaction mediated by NCS and TfOH. Conveniently, no chlorinated sulfonium salt was observed. Successful $^{18}$F-labelling allowed for isolation of tracer 74 in an average decay-corrected RCY of 53 ± 18%.

The presented work constitutes a ground-breaking advancement in radiolabelling of deactivated aromatic scaffolds. It is, to the best of my knowledge, the first example of direct one-step incorporation of fluorine-18 at the meta-pyridyl position of a biologically active drug-like molecule, without the use of activating groups. The use of dibenzothiophene sulfonium salt precursors opens up an opportunity to access various 3-$[^{18}\text{F}]$fluorinated scaffolds.
Conclusion

Despite remarkable advances in fluorine-18 incorporation into aromatic scaffolds for PET imaging, the lack of broadly applicable methods renders labelling of biologically active compounds challenging. As a result, the selection of established tracers for clinical imaging is limited. Frequently, their radiosyntheses are not operationally simple, RCYs and specific activities are low and cannot be adapted to clinical manufacture. Sadly, despite impressive technological advances, the potential of PET to revolutionise experimental medicine is not fully realised.

A novel strategy employing dibenzothiophene sulfonium salts as leaving groups for $[^{18}F]$fluorinations of highly-functionalised molecules has been developed. Building on the ground-breaking work by Sander et al. and Dr Thibault Gendron (UCL Radiochemistry), an efficient and robust system to access dibenzothiophene precursors for labelling was established. The compounds can be accessed in rapid one-pot transformation, their purification is straightforward and can be achieved by flash column chromatography. The resulting products exhibit remarkable stability, yet are very reactive towards $[^{18}F]$fluoride, and radiolabelling occurs with high regioselectivity. $[^{18}F]$fluorinations were performed on non-activated and electron-deficient aromatic systems, in the presence of $N$-heterocycles, such as pyridines, indoles and imidazoles.

Dibenzothiophene sulfonium salts were accessed from the corresponding diaryl thioethers in a novel ring-closing reaction mediated by NCS (electrophile) and TFOH (activator of NCS) in acetonitrile at room temperature. Electron-rich aromatics reacted most readily with the resulting superprotonated NCS species ("superelectrophile") as they increase nucleophilicity of the $S$-centre. Good cyclisation yields were obtained for $N$-protected indole and mildly-deactivated scaffolds, such as the
precursor to $[^{18}F]$FPEB. Sulfonium salts were also formed in the presence of basic pyridine and imidazole moieties. One of the main challenges of this chemistry was protonation of these entities by TfOH. Firstly, formation of positive charge on the 2-pyridyl moiety resulted in loss of reactivity of the sulfur centre. Secondly, unusual stabilisation of the protonated imidazole resulted in radiolabelling inhibition. Efforts to find milder, non-acidic cyclisation reagents were moderately successful. Although sulfonium salt formation is possible with standard hypochlorite-based reagents, the method was not robust enough to allow broad implementation. At the time of writing this thesis, the cyclisation system was further optimised by Dr Thibault Gendron. Refining of the $\text{Ca(OCl)}_2$-mediated dibenzothiophene sulfonium salt formation protocol resulted in a remarkably mild and robust strategy.

$[^{18}F]$Fluorinations of dibenzothiophene sulfonium salts proceeded most efficiently for deactivated scaffolds, such as 2-pyridine moieties, however excellent RCYs were also obtained for non-activated aromatics. A prominent example of this is radiolabelling of $[^{18}F]$FPEB, which offers certain advantages over existing technologies. Preparation of the corresponding sulfonium salt precursor is high yielding, technically simple and delivers a highly pure product.

The major achievement of this work is the successful synthesis and radiolabelling of a dibenzothiophene sulfonium salt precursor to a novel PET tracer candidate. The structure is based on a potent aldosterone inhibitor investigated by Merck. Cyclisation proceeded in a good yield and subsequent labelling at the 3-pyridyl position afforded the tracer in high RCY. This work constitutes, to the best of my knowledge, the first direct incorporation of fluorine-18 at the meta-pyridyl position of a highly-functionalised drug-like scaffold without the need for activating groups. The candidate is currently undergoing biological evaluation in mice.

These results illustrate the enormous potential of dibenzothiophene sulfonium salts as precursors for $[^{18}F]$fluorination of aromatic molecules. Precursor synthesis does not involve the use of toxic metals, harsh reaction conditions or intricate equipment. Radiolabelling can also be tailored to GMP environment. As a result, the
method exhibits great potential for broad applications in clinical radiopharmaceutical manufacture for PET imaging.
General Procedures

11.1 General Procedures: Synthetic Chemistry

**Solvents and Reagents:** Anhydrous solvents were purchased from Sigma Aldrich. Reagents were purchased from Sigma Aldrich, Fluorochem, Acros Organics and Alfa Aesar and were used without additional purification. **Flash Column Chromatography:** Purification was performed using Merck Geduran Silica Gel 60 40-63 µm. TLC was performed on aluminium-backed plates pre-coated with silica (0.2 mm, 60 F254). The plates were developed using the following visualising agents: UV fluorescence (254 nm), KMnO₄, p-anisaldehyde and Draggendorff, as appropriate. **¹H NMR Spectra:** These were recorded at 300, 400, 500 or 600 MHz on respective Bruker Avance machines. All samples were prepared as solutions in 0.7 mL CDCl₃, d₃-MeCN or d₆-DMSO. Chemical shifts (δ_H) are quoted in ppm, referenced to an appropriate residual solvent peak and rounded up to the nearest 0.01 ppm. Coupling constants (J) are rounded up to the nearest 0.1 Hz. **¹³C NMR Spectra:** These were recorded at 150 MHz on a Bruker AV-600 instrument. Chemical shifts (δ_C) are reported in ppm, referenced to an appropriate residual solvent peak and rounded up to the nearest 0.1 ppm. **¹⁹F NMR Spectra:** These were recorded at 282 MHz on a Bruker AV-300 instrument. Chemical shifts (δ_F) are reported in ppm, referenced to an appropriate residual peak and rounded up to the nearest 0.1 ppm. **Infrared Spectra:** These were recorded as thin films on a Bruker Alpha FTIR spectrometer. Only selected absorbances (ν_max) are reported to the nearest cm⁻¹. **Mass Spectra:** These were performed by the Mass Spectrometry Facility of the Chemistry Department of University College London. **Melting Points:** These were measured on a Gallenkamp melting point apparatus to the nearest 0.1°C.
11.2 General Procedures: Radiochemistry

All radiolabelling experiments were performed manually using $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]\text{H}_2\text{O}$. Radio-HPLC was performed with an Agilent 1200 HPLC system equipped with a 1200 Series Diode Array Detector and a GABI Star NaI(Tl) scintillation detector (energy window 400-700 keV). The system was used for purification as well as characterisation of radiotracers. Column specifications are provided.

Radiolabelling:
A solution of $[^{18}\text{F}]$fluoride (50 MBq-1.5 GBq) in $[^{18}\text{O}]\text{H}_2\text{O}$ was trapped on a Sep-Pak® QMA cartridge, released with a solution of KHCO$_3$ (30 mM) and Kryptofix 222 (30 mM) in 15% MeCN/H$_2$O solution (0.5 mL). The solvent was removed by azeotropic distillation at 90 °C under a stream of nitrogen. Anhydrous MeCN was added and distillation was continued. This was repeated once more until a dry residue was observed and the reaction vial was capped. The appropriate sulfonium salt precursor (2-5 mg) was dissolved in anhydrous MeCN or DMSO (0.5 mL) and added to the reaction vial. The resulting mixture was stirred at an appropriate temperature for the specified duration. After cooling, the reaction was quenched by addition of water (1.0-1.5 mL) and purified by semi-preparative radio-HPLC using either a Luna® 5µm C18(2) (10 × 250 mm, Phenomenex) or a Chromolith® Performance RP18-e column. The analytical yield was determined by integrating the area under the curve in the radio-HPLC chromatogram. The decay-corrected isolated RCY was calculated by measuring the amount of radioactivity of the purified radiotracers with respect to the initial amount of $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]\text{H}_2\text{O}$, accounting for the total synthesis time (from $[^{18}\text{F}]$fluoride in water to the end of purification or formulation). The analytical RCY was determined by integrating the area under the curve in the semi-preparative radio-HPLC chromatogram. The identity of the $^{18}\text{F}$-labelled product was confirmed by co-injection with its non-radioactive analogue. Quality control was performed using either a Zorbax® C18 (4.5 × 250 mm) or a Zorbax® Eclipse Plus 5 µm C18 column. Together with the eluent, they were checked for residual radioactivity to ensure all of the $^{18}\text{F}$-compounds/products were eluted.
12 | Radiolabelling

12.1 Preparation of Tracer 74

Radiolabelling was performed with 2 mg of precursor 83 in anhydrous DMSO at 110 °C for 15 min. After cooling, the reaction mixture was quenched with H$_2$O (1.5 mL) and the resulting solution was purified on a Luna® 5 µm C18(2) column (10 × 250 mm, Phenomenex) at room temperature using H$_2$O and MeOH (each containing 0.5% TFA). Isocratic elution was performed at 41% MeOH content and with a flow rate of 5 mL/min. Retention time was ≈ 22 min. Quality control was performed on a Zorbax® Eclipse Plus 5 µm C18 column (4.6 × 150 mm) at room temperature and at a flow rate of 1.5 mL/min using H$_2$O and MeOH. Gradient elution started with 50% MeOH content that was increased to 75% in 10 min and further to 90% in 2 min. Retention time was ≈ 6.3 min. The decay-corrected isolated RCY at the end of HPLC purification was 53 ± 18% (n=12). The specific activity was 14.7 ± 9.6 GBq/µmol. The total synthesis time, from [$^{18}$F]fluoride in [$^{18}$O]H$_2$O to the end of HPLC purification, was < 90 min. The column and eluent were checked for residual radioactivity to ensure all of the $^{18}$F-compounds/products were eluted.
Preclinical studies:

1.0 GBq of $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]{\text{H}}_{2}\text{O}$ was used. Purification was performed using the previously described conditions. The tracer solution was diluted with H$_2$O to a final solution of 20 mL, passed through a Sep-Pak® Alumina N cartridge and then trapped on a Sep-Pak® SPE C-18 light cartridge, from which the labelled product was released with EtOH (0.5 mL). Having reduced the volume to < 0.1 mL under a stream of nitrogen, the solution was diluted with saline (1.0 mL), to give the final ethanol concentration of < 5%, and sterilised by filtration. Quality control was performed using the previously described conditions. Specific activity was 30.6 GBq/µmol. The identity of the tracer was confirmed by co-injection with the non-radioactive analogue 73 (Figure 12.1). The total production time, from $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]{\text{H}}_{2}\text{O}$ to the end of the tracer preparation for injection, was 80 min. The column and eluent were checked for residual radioactivity to ensure all of the $^{18}\text{F}$-compounds/products were eluted.

**Figure 12.1:** HPLC chromatogram of isolated tracer 74, co-injected with non-radioactive reference compound 73. UV detection at 254 nm.
12.1.1 Calculation of Specific Activity of Tracer 74

![Calibration curve of reference compound 73 for specific activity calculation of tracer 74.](image)

**Figure 12.2:** Calibration curve of reference compound 73 for specific activity calculation of tracer 74.
12.2 Preparation of 3-[\textsuperscript{18}F]fluoropyridine

Radiolabelling was performed with 2 mg of mixture of compounds 37 and 41 in anhydrous DMSO at 110 °C for 15 min. After cooling, the reaction mixture was quenched with H\textsubscript{2}O (1.0 mL) and the resulting solution was purified using a Luna\textsuperscript{®} 5 µm C18(2) column (10 × 250 mm, Phenomenex) at room temperature using H\textsubscript{2}O and MeOH (each containing 0.5% TFA) and with a flow rate of 5 mL/min. Gradient elution started with 5% MeOH content that was increased to 95% in 10 min. Retention time was \(\approx\) 9.1 min. The decay-corrected isolated RCY at the end of HPLC purification was 41-50%. Quality control was performed on a Zorbax\textsuperscript{®} C18 column (4.5 × 250 mm) at room temperature and at a flow rate of 3.0 mL/min using H\textsubscript{2}O and MeOH (each containing 0.5% TFA). Gradient elution started with 3% MeOH content that kept constant for 7 min, increased to 95% in 7 min and kept at that for 6 min. Retention time was \(\approx\) 7.1 min. The total production time, from [\textsuperscript{18}F]fluoride in [\textsuperscript{18}O]H\textsubscript{2}O to the end of HPLC purification, < 60 min. The column and eluent were checked for residual radioactivity to ensure all of the \textsuperscript{18}F-compounds/products were eluted.
12.3 Preparation of Tracer 59

The most successful radiolabelling was performed with 5 mg of precursor 64 in anhydrous MeCN at 80 °C for 15 min. After cooling, the reaction mixture was quenched with 20% EtOH/H$_2$O (1.0 mL) and the resulting solution was purified on a Chromolith® Performance RP18-e column (100 × 10 mm) at room temperature using H$_2$O and MeOH (each containing 0.5% TFA) and a flow rate of 1.5 mL/min. Gradient elution started with 20% MeOH content kept constant for 5 min. It was increased to 50% in 10 min and then further to 90% in 5 min. Retention time was ≈ 12.8 min. Quality control was performed on a Zorbax® Eclipse Plus 5 µm C18 column (4.6 × 150 mm) at room temperature and at a flow rate of 1.5 mL/min using H$_2$O and MeOH (each containing 0.5% TFA). Gradient elution started with 30% MeOH that was increased to 90% in 13 min. Retention time was ≈ 6.7 min. The identity of the tracer was confirmed by co-injection with the non-radioactive analogue 67 (Figure 12.4). The decay-corrected isolated RCY at the end of HPLC purification was 6%. The total synthesis time, from $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]$H$_2$O to the end of HPLC purification, was 60 min. The column and eluent were checked for residual radioactivity to ensure all of the $^{18}$F-compounds/products were eluted.
Figure 12.3: HPLC chromatogram of crude $\text{3-[^{18}\text{F}]fluoropyridine}$, co-injected with 3-fluoropyridine. UV detection at 254 nm.

Figure 12.4: HPLC chromatogram of isolated tracer 59, co-injected with non-radioactive reference compound 67. UV detection at 254 nm.
13 | Characterisation of Synthesised Compounds

13.1 Synthetic Route Towards Sulfonium Salt 7 (Chapter 5)


Reagents and conditions: (i) Pd$_2$(dba)$_3$, xantphos, Et$_3$N, toluene, reflux, 4.5 h; (ii) 3,5-Dimethoxyphenylboronic acid, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene/water, reflux, 5 h; (iii) 3,5-Dibromobenzonitrile, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 15 min; (ivb) Pyridine 9, Pd(PPh$_3$)$_4$, toluene, reflux, 2 h; (v) NCS, Bi(OTf)$_3$, MeCN, rt, 1 h; (vi) nBuLi Me$_3$SnCl, -78 °C → rt, 1 h.

DPEphos:
Octan-3-y1-3-((2-bromo-4-methylphenyl)thio)propanoate 3

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (88.0 mL), 2-bromo-1-iodo-4-methylbenzene (2.88 g, 9.70 mmol, 1.0 equiv.) and Et₃N (1.27 g, 12.6 mmol, 1.3 equiv.). The reaction vessel was degassed by argon purging before Pd₂(dba)₃ (266 mg, 0.291 mmol, 0.03 equiv.) was added, followed by xantphos (337 mg, 0.582 mmol, 0.06 equiv.) and 2-ethylhexyl 3-mercaptopropanoate (2.12 g, 9.70 mmol, 1.0 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 4.5 h. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (10 → 50% DCM/PE) of the resulting yellow oil afforded the title compound as a colourless oil (3.57 g, 95%). νmax/cm⁻¹: 2924, 1730, 1461, 1238. ¹H NMR (600 MHz, CDCl₃) δH: 7.42 (1H, d, J 2.1, H1), 7.24 (1H, d, J 7.9, H4), 7.08 (1H, dd, J 7.9, 2.1, H3), 4.01 (2H, 2 × m, H15), 3.17 (2H, t, J 7.5, H10), 2.64 (2H, t, J 7.5, H11), 2.31 (3H, s, H7), 1.60-1.54 (1H, m, H16), 1.38-1.26 (8H, m, H17, H18, H19 and H21), 0.90-0.88 (6H, m, H20 and H22). ¹³C NMR (150 MHz, CDCl₃) δC: 171.9 (C), 138.2 (C), 133.9 (CH), 132.8 (C), 130.4 (CH), 128.9 (CH), 125.4 (C), 67.4 (CH₂), 38.8 (CH), 34.1 (CH₂), 30.1 (CH₂), 29.0 (CH₂), 28.8 (CH₂), 23.9 (CH₂), 23.1 (CH₂), 20.7 (CH₃), 14.2 (CH₃), 11.1 (CH₃). HRMS (EI) Calculated for C₁₈H₂₇O₂BrS [M⁺]: 386.0910; found: 386.0909.
Octan-3-yl 3-((3′,5′-dimethoxy-5-methyl-[1,1′-biphenyl]-2-yl)thio)propanoate 4

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (22.0 mL) and thioether 3 (4.09 g, 10.6 mmol, 1.0 equiv.), followed by a solution of K$_2$CO$_3$ (5.86 g, 42.4 mmol, 4.0 equiv.) in H$_2$O (22 mL) and 3,5-dimethoxyphenylboronic acid (2.41 g, 13.3 mmol, 1.25 equiv.). The reaction vessel was degassed by argon purging with vigorous stirring to ensure efficient removal of O$_2$ trapped in the aqueous layer. Pd(PPh$_3$)$_4$ (612 mg, 0.530 mmol, 0.05 equiv.) was added and after additional degassing, the resulting mixture was plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 5 h. After cooling to room temperature, it was washed with a saturated aqueous solution of K$_2$CO$_3$. The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (3 → 7% EtOAc/PE) of the resulting yellow oil afforded the title compound as a colourless oil (4.08 g, 84%). $\nu_{\text{max}}$/cm$^{-1}$ 2923, 1730, 1461. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.32 (1H, d, $J$ 8.0, H4), 7.13 (1H, dd, $J$ 8.0, 2.3, H3), 7.10 (1H, d, $J$ 2.3, H1), 6.54 (2H, d, $J$ 2.3, H9 and H13), 6.47 (1H, t, $J$ 2.3, H11), 3.96 (2H, 2 × m, H24), 3.82 (6H, s, H16 and H17), 2.97 (2H, t, $J$ 7.7, H19), 2.51 (2H, t, $J$ 7.7, H20), 2.35 (3H, s, H7), 1.54 (1H, m, H25), 1.35-1.24 (8H, m, H26, H27, H28 and H30), 0.90-0.86 (6H, m, H29 and H31). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 172.1 (C), 160.4 (C), 143.0 (C), 142.8 (C), 136.2 (C), 131.3 (CH), 130.3 (C), 129.6 (CH), 128.9 (CH), 107.6 (CH), 99.7 (CH), 67.2 (CH$_2$), 55.5 (CH$_3$), 38.8 (CH), 34.3 (CH$_2$), 30.5 (CH$_2$), 29.0 (CH$_2$), 28.8 (CH$_2$), 23.8 (CH$_2$), 23.1 (CH$_2$), 21.0 (CH$_3$), 14.2 (CH$_3$), 11.1 (CH$_3$). HRMS (EI) Calculated for C$_{26}$H$_{36}$O$_4$S [M]$^+$: 444.2329; found: 444.2329.
3-Bromo-5-((3',5'-dimethoxy-5-methyl-[1,1'-biphenyl]-2-yl)thio)benzonitrile 5

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (27.0 mL), 3,5-dibromobenzonitrile (1.92 g, 7.36 mmol, 1.4 equiv.) and KOtBu (958 mg, 8.54 mmol, 1.6 equiv.). The reaction vessel was degassed by argon purging before Pd$_2$(dba)$_3$ (49.0 mg, 53.4 µmol, 0.01 equiv.) was added, followed by DPEphos (58.0 mg, 0.107 mmol, 0.02 equiv.) and thioether 4 (2.37 g, 5.34 mmol, 1.0 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 15 min. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (5 → 10% Et$_2$O/PE) of the resulting brown oil afforded the title compound as a yellow oil (1.51 g, 65%). $\nu_{\text{max}}$/cm$^{-1}$ 1728, 1589, 1546, 1201. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.45 (1H, d, $J$ 7.9, H4), 7.44 (1H, dd, $J$ 1.7, 1.4, H13) 7.34 (1H, t, $J$ 1.7, H11), 7.25 (1H, d, $J$ 1.9, H1), 7.22 (1H, dd, $J$ 7.9, 1.9, H3), 7.12 (1H, dd, $J$ 1.7, 1.4, H15), 6.42 (1H, t, $J$ 2.3, H18), 6.36 (2H, d, $J$ 2.3, H16 and H20), 3.75 (6H, s, H23 and H24), 2.48 (3H, s, H7). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 160.3 (C), 146.0 (C), 143.3 (C), 142.3 (C), 140.4 (C), 135.6 (CH), 134.9 (CH), 132.2 (CH), 131.2 (CH), 129.8 (CH), 129.8 (CH), 126.0 (C), 122.9 (C), 117.1 (C), 114.2 (C), 107.4 (CH), 99.7 (CH), 55.5 (CH$_3$), 21.4 (CH$_3$). HRMS (EI) Calculated for C$_{22}$H$_{18}$NO$_2$BrS [M]$^+$: 439.0236; found: 439.0235.
To a flame-dried round-bottom flask equipped with an argon inlet were added anhydrous THF (2.0 mL) and 2-ethynylpyridine (710 mg, 6.89 mmol, 1.0 equiv.). The mixture was cooled to -78 °C and a 1.6 M solution of n-butyllithium in hexane (4.31 mL, 6.89 mmol, 1.0 equiv.) was introduced dropwise. After stirring for 1 h at -78 °C, a 1 M solution of Me₃SnCl in THF (8.27 mL, 8.27 mmol, 1.2 equiv.) was added. The reaction mixture was allowed to warm up to room temperature and stirred for 1 h. It was then quenched with a saturated solution of NH₄Cl, extracted with EtOAc and concentrated in vacuo to afford the title compound as a black oil (2.00 g) of ≈ 70% purity as determined by ¹H NMR spectroscopy. The crude product was employed in the next step without further purification. ¹H NMR (600 MHz, CDCl₃) δ_H 8.56-8.55 (1H, m, H2), 7.62 (1H, m, H6), 7.44-7.30 (1H, m, H1), 7.21-7.19 (1H, m, H5), 0.38 (9H, s, H9).
3-((3’,5’-Dimethoxy-5-methyl-[1,1’-biphenyl]-2-yl)thio)-5-(pyridin-2-ylethynyl)benzonitrile 6

To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (5.0 mL), thioether 5 (1.09 g, 2.48 mmol, 1.0 equiv.) and tin precursor 9 (70%, 1.32 g, 3.72 mmol, 1.5 equiv.). The reaction vessel was degassed by argon purging before Pd(PPh₃)₄ (143 mg, 0.124 mmol, 0.05 equiv.) was added. The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 2 h. After cooling to room temperature, it was diluted with EtOAc, washed with an aqueous solution of KF, followed by brine. The organic layer was dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (25 → 40% EtOAc/PE) of the resulting brown oil afforded the title compound as an orange oil (835 mg, 73%).

υₓmax/cm⁻¹ 1583, 1460, 1420, 1201. ¹H NMR (600 MHz, CDCl₃) δH 8.63 (1H, dd, J 5.0, 1.7, H30), 7.71 (1H, td, J 7.8, 1.7, H32), 7.53 (1H, t, J 1.7, H22), 7.51 (1H, d, J 7.8, H33), 7.45 (1H, d, J 7.9, H4), 7.43 (1H, t, J 1.7, H24), 7.29 (1H, dd, J 7.8, 5.0, H31), 7.25 (1H, d, J 2.3, H1), 7.21 (1H, dd, J 7.9, 2.3, H3), 7.20 (1H, t, J 1.7 H20), 6.42 (1H, t, J 2.3, H11), 6.38 (2H, d, J 2.3, H9 and H13), 3.74 (6H, s, H16 and H17), 2.44 (3H, s, H8). ¹³C NMR (150 MHz, CDCl₃) δC 160.3 (C), 150.4 (CH), 145.9 (C), 142.4 (C), 141.8 (C), 140.1 (C), 136.5 (CH), 135.5 (CH), 135.2 (CH), 132.2 (CH), 132.0 (CH), 131.3 (CH), 129.9 (CH), 127.6 (CH), 126.3 (C), 124.2 (C), 123.6 (CH), 117.7 (CN), 113.3 (C), 107.4 (CH), 99.8 (CH), 90.8 (C), 86.1 (C), 55.5 (CH₃), 21.4 (CH₃). HRMS (ESI) Calculated for C₂₉H₂₃N₂O₂S [M+H]⁺: 463.1480; found: 463.1462.
To a round-bottom flask were added MeCN (2.0 mL), thioether 6 (116 mg, 0.250 mmol, 1.0 equiv.), NCS (34.0 mg, 0.250 mmol, 1.0 equiv.) and Bi(OTf)$_3$ (164 mg, 0.250 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 1 h. It was then concentrated *in vacuo* and the residue resuspended in DCM and washed with H$_2$O. The organic layer was dried over MgSO$_4$ and concentrated *in vacuo*. Flash column chromatography purification (5 → 10% 1 NNH$_3$ in MeOH/DCM) afforded the title compound as an off-white solid (98.0 mg, 61%), mp 252 °C (dec). $\nu_{\text{max}}$/cm$^{-1}$ 1589, 1248. $^1$H NMR (600 MHz, d$_6$-DMSO) $\delta_{H}$ 8.64 (1H, ddd, $J$ 4.8, 1.8, 1.0, H30), 8.49 (1H, t, $J$ 1.4, H22), 8.42 (1H, s, H2), 8.29 (1H, dd, $J$ 1.8, 1.4, H20), 8.26 (1H, d, $J$ 8.3, H15), 8.15 (1H, t, $J$ 1.8, H24), 7.91 (1H, td, $J$ 7.7, 1.8, H32), 7.74 (1H, d, $J$ 2.2, H2), 7.69 (1H, dt, $J$ 7.7, 1.0, H33), 7.60 (1H, d, $J$ 8.3, H14), 7.49 (1H, ddd, $J$ 7.7, 4.9, 1.0, H31), 6.94 (1H, d, $J$ 2.1, H6), 4.01 (3H, s, H11), 3.95 (3H, s, H9), 2.55 (3H, s, H17). $^{13}$C NMR (150 MHz, d$_6$-DMSO) $\delta_{C}$ 167.2 (C), 158.0 (C), 150.5 (CH), 145.1 (C), 142.5 (C), 141.0 (C), 140.5 (CH), 139.7 (C), 137.1 (CH), 136.3 (CH), 133.6 (CH), 132.5 (CH), 130.6 (C), 129.5 (C), 128.2 (CH), 128.0 (CH), 125.8 (CH), 125.1 (C), 124.6 (CH), 116.1 (C), 114.8 (C), 107.3 (C), 102.5 (CH), 100.8 (CH), 92.8 (C), 84.2 (C), 57.5 (CH$_3$), 56.8 (CH$_3$), 21.3 (CH$_3$). $^{19}$F (282 MHz, d$_6$-DMSO) $\delta_{F}$ -78.2. HRMS (ESI) Calculated for C$_{29}$H$_{21}$N$_2$O$_2$S [M]$^+$: 461.1324; found: 461.1320.
13.2 Synthetic Route Towards Sulfonium Salt 14 (Chapter 6)

Reagents and conditions: (i) 2-Bromo-1-iodo-4-methylbenzene, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 2 h; (ii) 3,5-Dimethoxyphenylboronic acid, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene/water, reflux, 5 h; (iii) NCS, Bi(OTf)$_3$, MeCN, rt, 1.5 h.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (94.0 mL), 2-bromo-1-iodo-4-methylbenzene (3.47 g, 11.7 mmol, 1.0 equiv.) and KOtBu (1.54 g, 12.9 mmol, 1.1 equiv.). The reaction vessel was degassed by argon purging before Pd$_2$(dba)$_3$ (107 mg, 0.117 mmol, 0.01 equiv.), DPEphos (135 mg, 0.234 mmol, 0.02 equiv.) and 2-fluorothiophenol (1.50 g, 11.7 mmol, 1.0 equiv.) were added. The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 2 h. After cooling to room temperature, it was washed with a 2 M aqueous NaOH solution, filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (2 → 8% DCM/PE) of the resulting brown oil afforded the title compound as a colourless oil (2.92 g, 84%).ν$_\text{max}$/cm$^{-1}$ 1591, 1253, 1169.  

$^1$H NMR (600 MHz, CDCl$_3$) δH 7.45 (1H, d, J 1.9, H1), 7.35-7.31 (1H, m, H13), 7.29-7.27 (1H, m, H12), 7.16-7.11 (2H, m, H11 and H14), 7.01 (1H, dd, J 7.8, 1.9, H3), 6.97 (1H, d, J 7.8, H4), 2.31 (3H, s, H8). $^{13}$C NMR (150 MHz, CDCl$_3$) δC 162.5 & 160.8 (d, J 249.0, C), 138.8 (C), 134.2 (CH), 133.9 (CH), 132.3 (C), 131.2 (CH), 130.2 & 130.1 (d, J 7.8, CH), 129.1 (CH), 125.1 & 125.0 (d, J 3.9, CH), 124.6 (C), 121.4 & 121.3 (d, J 17.9, C), 116.4 & 116.2 (d, J 21.9, CH), 20.8 (CH$_3$). $^{19}$F NMR (282 MHz, CDCl$_3$) δF -108.1. HRMS (CI) Calculated for C$_{13}$H$_{10}$BrFS [M]$: 295.9665; found: 295.9964.
(3′,5′-Dimethoxy-5-methyl-[1,1′-biphenyl]-2-yl)(2-fluorophenyl)sulfane 13

To a three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (12.0 mL) and thioether 12 (1.89 g, 6.36 mmol, 1.0 equiv.), followed by a solution of K₂CO₃ (3.52 g, 25.4 mmol, 4.0 equiv.) in H₂O (12.0 mL) and 3,5-dimethoxyphenylboronic acid (1.45 g, 7.95 mmol, 1.25 equiv.). The reaction vessel was degassed by argon purging with vigorous stirring to ensure efficient removal of O₂ trapped in the aqueous layer. Pd(PPh₃)₄ (367 mg, 0.318 mmol, 0.05 equiv.) was added and after additional degassing, the resulting mixture was plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 5 h. After cooling to room temperature, it was washed with a saturated aqueous solution of K₂CO₃. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (3 → 12% EtOAc/PE) of the resulting yellow oil afforded the title compound as a colourless oil (1.54 g, 69%).

ν<sub>max</sub>/cm<sup>-1</sup> 1611, 1583, 1467, 1334. ¹H NMR (600 MHz, CDCl₃) δ<sub>H</sub> 7.22-7.18 (2H, m, H12 and H22), 7.18 (1H, d, J 7.9, H15), 7.12 (1H, td, J 7.8, 1.6, H21), 7.09 (1H, dd, J 7.9, 1.2, H14), 7.04-7.01 (2H, m, H20 and H23), 6.52 (2H, d, J 2.3, H2 and H4), 6.45 (1H, t, J 2.3, H6), 3.76 (6H, s, H10 and H11), 2.37 (3H, s, H17). ¹³C NMR (150 MHz, CDCl₃) δ<sub>C</sub> 161.9 & 160.3 (d, J 246.5, C), 160.3 (C), 143.8 (C), 142.7 (C), 137.6 (C), 133.3 (CH), 132.3 (CH), 131.5 (CH), 129.2 (CH), 129.1 (C), 128.9 (d, J 7.8, CH), 124.7 & 124.6 (d, J 3.7, CH), 124.0 & 123.9 (d, J 17.5, C), 115.9 & 115.8 (d, J 22.1, CH), 107.4 (CH), 100.0 (CH), 55.4 (CH₃), 21.2 (CH₃). ¹⁹F NMR (282 MHz, CDCl₃) δ<sub>F</sub> -109.0. HRMS (ESI) Calculated for C₂₁H₂₀O₂FS [M+H]+: 355.1168; found: 355.1161.
5-(2-Fluorophenyl)-2,4-dimethoxy-8-methyl-5H-dibenzo[b,d]thiophen-5-ium trifluoromethanesulfonate

To a round-bottom flask were added MeCN (2.0 mL), thioether 13 (100 mg, 0.282 mmol, 1.0 equiv.), NCS (37.0 mg, 0.282 mmol, 1.0 equiv.) and Bi(OTf)$_3$ (185 mg, 0.282 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 1.5 h. It was then diluted with DCM and washed with H$_2$O. The organic layer was dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (1 → 5% MeOH/DCM) afforded the title compound as a white solid (94.0 mg, 66%), mp 210-212 °C (dec). $\nu_{\text{max}}$/cm$^{-1}$ 1590, 1252, 1219. $^1$H NMR (600 MHz, d$_6$-DMSO) $\delta$H 8.43 (1H, d, J, 2.0, H12), 8.29 (1H, d, J 8.2, H15), 7.80-7.77 (1H, m, H22), 7.72 (1H, d, J 2.2, H2), 7.63-7.59 (2H, m, H14 and H23), 7.34-7.31 (1H, m, H20), 7.09 (1H, td, J 7.4, 1.6, H21), 6.9 (1H, d, J 2.2, H6), 3.98 (3H, s, H10 or H11), 3.92 (3H, s, H10 or H11), 2.56 (3H, s, H17). $^{13}$C NMR (150 MHz, d$_6$-DMSO) $\delta$C 166.8 (C), 162.3 & 160.6 (d, J 256.7, C), 157.6 (C), 145.1 (C), 141.6 (C), 140.4 (C), 137.3 & 137.2 (CH), 132.5 (CH), 130.0 (CH), 128.5 (CH), 128.3 (C), 127.3 (d, J 3.0, CH), 125.5 (CH), 119.6 (C), 118.1 & 117.9 (d, J 19.8, CH), 114.7 & 114.6 (d, J 14.3, C), 102.1 (CH), 100.7 (CH), 57.4 (CH$_3$), 56.8 (CH$_3$), 21.3 (CH$_3$). $^{19}$F NMR (282 MHz, d$_6$-DMSO) $\delta$F -77.8, -109.8. HRMS (Cl) Calculated for C$_{21}$H$_{19}$O$_2$FS [M]$^+$$^+$: 353.1084; found: 353.1083.
13.3 Synthetic Route Towards Sulfonium Salt 18 (Chapter 6)

Scheme 13.3: Syntheses of sulfonium salt 18 and fluorinated reference 20.
Reagents and conditions: (i) KOH, pTsCl, Aliquat® 336, toluene/water, rt, 1 h; (ii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 1 h; (iii) NCS, Bi(OTf)$_3$, MeCN, rt, 20 min; (iv) KOH, pTsCl, Aliquat® 336, DCM, rt, 1 h.
To a round-bottom flask were added toluene (20.0 mL) and 6-bromoindole (2.00 g, 10.2 mmol, 1.0 equiv.), followed by a solution of KOH (1.72 g, 30.6 mmol, 3.0 equiv.) in H₂O (10.0 mL), p-toluenesulfonyl chloride (2.33 g, 12.2 mmol, 1.2 equiv.) and a few droplets of Aliquat® 336. The resulting biphasic mixture was stirred at room temperature for 1 h. After dilution with H₂O, the organic layer was washed with more H₂O brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (5 → 15% Et₂O/PE) of the resulting brown solid afforded the title compound as a white solid (2.35 g, 66%), mp 134-135 °C. ν_max/cm⁻¹ 3137, 3108, 2927, 1595, 1523, 1420, 1354. ¹H NMR (600 MHz, CDCl₃) δ_H 8.17 (1H, d, J 1.7, H3), 7.77 (2H, d, J 8.4, H13 and H17), 7.53 (1H, d, J 3.7, H8), 7.38 (1H, d, J 8.4, H6), 7.34 (1H, dd, J 8.4, 1.7, H1), 7.26-7.25 (2H, m, H14 and H16), 6.62 (1H, dd, J 3.7, 0.8, H9), 2.37 (3H, s, H20). ¹³C NMR (150 MHz, CDCl₃) δ_C 145.4 (C), 135.6 (C), 135.1 (C), 130.2 (CH), 129.9 (C), 126.9 (CH), 126.9 (CH), 126.8 (CH), 122.6 (CH), 118.4 (C), 116.7 (CH), 108.9 (CH), 21.8 (CH₃). HRMS (Cl) Calculated for C₁₅H₁₂BrNO₂S [M+H]^+: 348.9767; found: 348.9766.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (1.3 mL), bromoindole 16 (100 mg, 0.286 mmol, 1.0 equiv.) and KOtBu (42.0 mg, 0.372 mmol, 1.3 equiv.). The reaction vessel was degassed by argon purging before Pd₂(dba)₃ (2.60 mg, 2.86 µmol, 0.01 equiv.), DPEphos (3.10 mg, 5.72 µmol, 0.02 equiv.) and thioether 4 (127 mg, 0.286 mmol, 1.0 equiv.) were added. The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 1 h. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (15 → 20% EtOAc/PE) of the resulting brown oil afforded the title compound as a yellow oil (62.0 mg, 44%). νmax/cm⁻¹ 2932, 2837, 1590, 1493, 1452, 1419. ¹H NMR (600 MHz, CDCl₃) δH 7.83 (1H, d, J0.8, H24), 7.61 (2H, d, J8.4, H30 and 34), 7.52 (1H, d, J3.7, H26), 7.38 (1H, d, J8.2, H11), 7.21 (1H, d, J2.0, H8), 7.16 (2H, d, J8.4, H31 and H33), 7.12-7.10 (2H, m, H20 and H21), 7.08 (1H, dd, J8.2, 2.0, H10), 6.59 (1H, d, J3.7, H25), 6.48 (2H, d, J2.3, H2 and H4), 6.44 (1H, t, J2.3, H6), 3.68 (6H, s, H15 and H17), 2.41 (3H, s, H13), 2.34 (3H, s, H35). ¹³C NMR (150 MHz, CDCl₃) δC 160.3 (C), 145.1 (C), 143.6 (C), 142.8 (C), 137.2 (C), 135.5 (C), 135.3 (C), 135.0 (C), 133.1 (C), 132.4 (CH), 131.3 (CH), 131.3 (C), 130.0 (CH), 129.7 (C), 129.1 (CH) 127.0 (CH), 126.7 (CH), 121.8 (CH), 116.3 (CH), 108.9 (CH), 108.8 (CH), 107.4 (CH), 107.3 (CH), 100.0 (CH), 55.4 (CH₃), 21.7 (CH₃), 21.2 (CH₃). HRMS (ESI) Calculated for C₃₀H₂₈NO₄S₂ [M+H]⁺: 530.1460; found: 530.1434.
2,4-Dimethoxy-8-methyl-5-(1-tosyl-1\textit{H}-indol-6-yl)-5\textit{H}-dibenzo\textit{b,d}thiophen-5-ium trifluoromethanesulfonate 18

To a round-bottom flask were added MeCN (1.0 mL), thioether 17 (62.0 mg, 0.114 mmol, 1.0 equiv.), NCS (15.0 mg, 0.114 mmol, 1.0 equiv.) and Bi(OTf)$_3$ (75.0 mg, 0.114 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 20 min. After concentration the residue was resuspended in DCM and washed with H$_2$O. The organic layer was dried over MgSO$_4$ and concentrated \textit{in vacuo}. Flash column chromatography purification (5 → 10% MeOH/DCM) afforded the title compound as an off-white solid (62.0 mg, 73%), mp 125-127 °C. $\nu_{\text{max}}$/cm$^{-1}$ 1591, 1253. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.33 (1H, d, $J$ 1.8, H$_2$4), 8.13 (1H, d, $J$ 8.3, H15), 8.05 (1H, d, $J$ 1.8, H12), 7.73-7.71 (3H, m, H$_2$6, H$_3$2 and H$_3$6), 7.63 (1H, d, $J$ 8.6, H21), 7.47 (1H, dd, $J$ 8.3, 1.8, H14), 7.36 (1H, d, $J$ 2.1, H2), 7.31 (2H, d, $J$ 8.3, H33 and H35), 7.22 (1H, dd, $J$ 8.6, 1.9, H20), 6.70 (1H, d, $J$ 3.7, H25), 6.60 (1H, d, $J$ 2.1, H6), 4.07 (3H, s, H8), 3.89 (3H, s, H10), 2.60 (3H, s, H17), 2.40 (3H, s, H37). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ $^c$ 167.8 (C), 158.6 (C), 146.4 (C), 145.7 (C), 142.2 (C), 138.9 (C), 135.4 (C), 134.4 (C), 134.2 (C), 132.8 (CH), 130.6 (CH), 130.5 (CH), 130.1 (C), 128.9 (CH), 127.2 (CH), 125.2 (CH), 124.4 (CH), 122.9 (CH), 122.1 (C), 120.0 (C), 116.5 (CH), 108.8 (CH), 101.4 (CH), 100.6 (CH), 57.1 (CH$_3$), 21.9 (CH$_3$), 21.8 (CH$_3$). $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$ $^f$ -78.1. HRMS (ESI) Calculated for C$_{30}$H$_{26}$NO$_4$S$_2$ [M]$^+$: 528.1303; found: 528.1287.
6-Fluoro-1-tosyl-1\textit{H}-indole 20

To a flame-dried round-bottom flask equipped with an argon inlet were added DCM (3.0 mL) and 6-fluoroindole (100 mg, 0.740 mmol, 1.0 equiv.), followed by powdered NaOH (130 mg, 3.25 mmol, 4.4 equiv.), \textit{p}-toluenesulfonyl chloride (169 mg, 0.890 mmol, 1.2 equiv.) and a droplet of Aliquat\textsuperscript{®} 336. The resulting mixture was stirred at room temperature for 1 h. It was then diluted with DCM and washed with H\textsubscript{2}O. The aqueous layer was extracted with DCM and the combined organic extracts were washed with brine, dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. Flash column chromatography purification (15 \rightarrow 20\% Et\textsubscript{2}O/PE) afforded the title compound as a white solid (77.0 mg, 78\%), mp 105-106 °C. ν\textsubscript{max}/cm\textsuperscript{-1} 3143, 3118, 2927, 1614, 1591, 1530, 1479, 1429. \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}) δ\textsubscript{H} 7.77 (2H, d, J 8.4, H15 and H19), 7.72 (1H, dd, J 9.6, 2.4, H3), 7.54 (1H, d, J 3.7, H8), 7.44 (1H, dd, J 8.9, 5.3, H6), 7.25 (2H, d, J 8.4, H16 and H18), 6.99 (1H, td, J 8.9, 2.4, H1), 6.62 (1H, dd, J 3.7, 0.8, H9), 2.36 (3H, s, H20). \textsuperscript{13}C NMR (150 MHz, CDCl\textsubscript{3}) δ\textsubscript{C} 161.7 & 160.1 (d, J 242.1, C), 145.3 (C), 135.1 (C), 135.0 (C), 130.1 (CH), 127.0 (CH), 126.7 (d, J 4.1, CH), 122.2 (d, J 9.9, CH), 112.0 & 111.8 (d, J 24.4, CH), 108.9 (CH), 101.1 & 101.0 (d, J 28.1, CH), 21.7 (CH\textsubscript{3}). \textsuperscript{19}F NMR (282 MHz, CDCl\textsubscript{3}) δ\textsubscript{F} -116.5. HRMS (ESI) Calculated for C\textsubscript{15}H\textsubscript{13}FNO\textsubscript{2}S [M+H\textsuperscript{+}]: 290.0651; found: 290.0649.
13.4 Synthetic Route Towards Sulfonium Salt 27 (Chapter 7)

Scheme 13.4: Syntheses of sulfonium salt 27 and fluorinated reference 29.
Reagents and conditions: (i) But-1-ylamine, HATU, HOBt, DIPEA, DCM, rt, 1 h; (ii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 2.5 h; (iii) NCS, TfOH, MeCN, rt, 3 h; (iv) But-1-ylamine, HATU, HOBt, DIPEA, DCM, rt, 2 h.
6-Bromo-N-butynicotinamide 25

To a stirring solution of but-1-ylamine (808 µL, 8.18 mmol, 1.1 equiv.) in DCM (25.0 mL) were added 6-bromonicotinic acid (1.50 g, 7.43 mmol, 1.0 equiv.), HATU (3.12 g, 8.21 mmol, 1.1 equiv.), HOBt (1.10 g, 8.14 mmol, 1.1 equiv.) and DIPEA (5.18 mL, 29.7 mmol, 4.0 equiv.). The resulting yellow solution was stirred at room temperature for 1 h. The reaction mixture was then diluted with DCM, washed with H₂O and brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 50% EtOAc/PE) afforded the title compound as a shiny white solid (1.28 g, 67%), mp 110-111 °C. νmax/cm⁻¹ 3304, 3046, 2932, 2868, 1636, 1577, 1538, 1454. ¹H NMR (600 MHz, CDCl₃) δH 8.69 (1H, d, J 2.7, H4), 7.97 (1H, dd, J 8.2, 2.7, H6), 7.58 (1H, d, J 8.2, H1), 6.10 (1H, bs, H9), 3.47 (2H, td, J 7.1, 5.7, H11), 1.62 (2H, m, H12), 1.42 (2H, m, H13), 0.97 (3H, t, J 7.4, H14). ¹³C NMR (150 MHz, CDCl₃) δC 164.7 (C), 148.2 (CH), 145.2 (C), 137.7 (CH), 129.8 (C), 128.4 (CH), 40.2 (CH₂), 31.7 (CH₂), 20.3 (CH₂), 13.9 (CH₃). HRMS (ESI) Calculated for C₁₀H₁₄BrN₂O [M+H]⁺: 257.0290; found: 257.0292.
N-Butyl-6-((3’,5’-dimethoxy-5-methyl-[1,1’-biphenyl]-2-yl)thio)nicotinamide

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (7.0 mL), Pd_2(dba)_3 (178 mg, 0.194 mmol, 0.1 equiv.) and DPEphos (209 mg, 0.388 mmol, 0.2 equiv.), followed by amide 25 (500 mg, 1.94 mmol, 1.0 equiv.), KOtBu (239 mg, 2.13 mmol, 1.1 equiv.) and thioether 4 (949 mg, 2.13 mmol, 1.1 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C and refluxed for 2.5 h. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (50 → 100% Et_2O/PE) afforded the title compound as a yellow oil (551 mg, 65%). ν_max/cm⁻¹ 3306, 2956, 2932, 1588, 1494, 1452. ¹H NMR (600 MHz, CDCl₃) δ_H 8.65 (1H, d, J 2.5, H23), 7.82 (1H, dd, J 8.4, 2.5, H21), 7.56 (1H, d, J 7.8, H15), 7.3 (1H, d, J 2.2, H12), 7.24 (1H, dd, J 7.8, 2.2, H14), 6.87 (1H, dd, J 8.4, 0.6, H20), 6.44 (2H, d, J 2.3, H2 and H4), 6.39 (1H, t, J 2.3, H6), 6.01 (1H, t, J 5.7, H26), 3.68 (6H, s, H9 and H11), 3.43 (2H, m, H29), 2.44 (3H, s, H17), 1.58 (2H, m, H29), 1.4 (2H, m, H3), 0.95 (3H, t, J 7.4, H31). ¹³C NMR (150 MHz, CDCl₃) δ_C 166.2 (C), 165.4 (C), 160.2 (C), 147.3 (CH), 146.9 (C), 142.7 (C), 140.5 (C), 137.2 (CH), 135.5 (CH), 132.1 (CH), 129.7 (CH), 126.2 (C), 124.7 (C), 120.6 (CH), 107.4 (CH), 99.8 (CH), 55.4 (CH₃), 40.0 (CH₂), 31.8 (CH₂), 21.5 (CH₃), 20.3 (CH₂), 13.9 (CH₃). HRMS (ESI) Calculated for C_{25}H_{29}N_{2}O_{3}S [M+H]^+: 437.1899; found: 437.1895.
To a stirring solution of thioether 26 (71.0 mg, 0.163 mmol, 1.0 equiv.) in MeCN (1.5 mL) at -10 °C were added NCS (21.7 mg, 0.163 mmol, 1.0 equiv.) and TfOH (43 µL, 0.489 mmol, 3.0 equiv.). The reaction mixture was stirred at -10 °C for 20 min but as no conversion was observed, it was allowed to warm to room temperature, at which it was stirred for 3 h. As the reaction did not progress, the mixture was concentrated in vacuo, the residue dissolved in DCM, washed with 2 M NaOH solution, dried over MgSO\(_4\) and concentrated in vacuo. Flash column chromatography purification (0 → 10% MeOH/DCM) afforded the title compound as a yellow residue (5.0 mg, 5%). The sample was not clean enough to allow for mass spectrometry so \(^1\)H, \(^{13}\)C and \(^{19}\)F NMR spectroscopy were used to confirm identity of the title compound. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\)H 9.03 (1H, dd, J 2.4, 0.6, H23), 8.58 (1H, dd, J 8.3, 2.4, H21), 8.02 (1H, d, J 8.2, H11), 7.89 (1H, d, J 1.8, H8), 7.78 (1H, dd, J 8.3, 0.6, H20), 7.49 (1H, dd, J 8.2, 1.8, H10), 7.23 (1H, d, J 2.0, H2), 6.68 (1H, d, J 2.0, H6), 4.05 (3H, s, H14), 4.00 (3H, s, H16), 3.39 (2H, dt, J 7.7, 6.1, H28), 2.58 (3H, s, H17), 1.59 (2H, m, H29), 1.36 (2H, m, H30), 0.91 (3H, t, J 7.4, H31). \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\)C 168.3 (C), 163.9 (C), 159.4 (C), 152.8 (CH), 149.4 (C), 146.0 (C), 143.6 (C), 139.7 (CH), 139.4 (C), 135.4 (C), 133.1 (CH), 128.1 (CH), 127.4 (C), 125.1 (CH), 125.0 (CH), 103.2 (C), 101.9 (CH), 100.4 (CH), 57.3 (CH\(_3\)), 56.8 (CH\(_3\)), 40.3 (CH\(_2\)), 31.2 (CH\(_2\)), 22.1 (CH\(_3\)), 20.3 (CH\(_2\)), 13.8 (CH\(_3\)). \(^{19}\)F NMR (282 MHz, CDCl\(_3\)) \(\delta\)F -78.4.
To a stirring solution of but-1-ylamine (154 µL, 1.56 mmol, 1.1 equiv.) in DCM (7.0 mL) were added 6-fluoronicotinic acid (200 mg, 1.42 mmol, 1.0 equiv.), HATU (597 mg, 1.57 mmol, 1.1 equiv.), HOBt (211 mg, 1.56 mmol, 1.1 equiv.) and DIPEA (989 µL, 5.68 mmol, 4.0 equiv.). The resulting yellow solution was stirred at room temperature for 2 h. The reaction mixture was then diluted with DCM, washed with H₂O and brine, dried over MgSO₄ and concentrated *in vacuo*. Flash column chromatography purification (20 → 60% EtOAC/PE) afforded the title compound as a white solid (246 mg, 88%), mp 112-115 °C. νₑ鸰max/cm⁻¹ 3309, 2977, 2958, 2872, 1636, 1593, 1535, 1474, 1465. ¹H NMR (600 MHz, CDCl₃) δH 8.58 (1H, d, J 2.5, H5), 8.24 (1H, ddd, J 8.5, 7.5, 2.5, H3), 7.01 (1H, ddd, J 8.5, 2.5, 0.7, H2), 6.12 (1H, bs, H9), 3.48 (2H, td, J 7.4, 5.7, H11), 1.62 (2H, p, J 7.4, H12), 1.42 (2H, sex, J 7.4, H13), 0.97 (3H, t, J 7.4, H14). ¹³C NMR (150 MHz, CDCl₃) δC 165.9 & 164.2 (d, J 244.0, C), 164.6 (C), 146.5 & 146.4 (d, J 16.0, CH), 141.0 & 140.9 (d, J 8.8, CH), 129.0 & 128.9 (d, J 4.4, C), 110.0 & 109.8 (d, J 37.4, CH), 40.1 (CH₂), 31.8 (CH₂), 20.3 (CH₂), 13.9 (CH₃). ¹⁹F NMR (282 MHz, CDCl₃) δF -63.7. HRMS (ESI) Calculated for C₁₀H₁₄N₂OF [M+H]⁺: 197.1090; found: 197.1091.
Scheme 13.5: Synthesis of sulfonium salt 32.
Reagents and conditions: (i) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOtBu, toluene, reflux, 24 h; (ii) No reaction.
2-((3’,5’-Dimethoxy-5-methyl-[1,1’-biphenyl]-2-yl)thio)pyridine 31

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (5.0 mL), Pd$_2$(dba)$_3$ (124 mg, 0.135 mmol, 0.1 equiv.) and DPEphos (145 mg, 0.269 mmol, 0.2 equiv.), followed by 2-bromopyridine (278 mg, 1.76 mmol, 1.3 equiv.), KOtBu (167 mg, 1.49 mmol, 1.1 equiv.) and thioether 4 (600 mg, 1.35 mmol, 1.0 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C and refluxed for 24 h. After cooling to room temperature, the mixture was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (0 → 12% EtOAc/toluene) afforded the title compound as a white solid (283 mg, 62%), mp 85-88 °C. $\nu_{\text{max}}$/cm$^{-1}$ 2956, 2927, 2830, 1591, 1571, 1445, 1413. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 8.36 (1H, ddd, $J$ 4.9, 2.0, 1.0, H23), 7.57 (1H, d, $J$ 7.9, H11), 7.41 (1H, ddd, $J$ 8.2, 7.5, 2.0, H21), 7.28 (1H, d, $J$ 2.1, H8), 7.21 (1H, dd, $J$ 7.9, 2.1, H10), 6.93 (1H, ddd, $J$ 7.5, 4.9, 1.2, H22), 6.86 (1H, ddd, $J$ 8.2, 1.2, 1.0, H20), 6.47 (2H, d, $J$ 2.3, H2 and H4), 6.40 (1H, t, $J$ 2.3, H6), 3.67 (6H, s, H15 and H17), 2.43 (3H, s, H13). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 162.2 (C), 160.2 (C), 149.6 (CH), 146.6 (C), 143.0 (C), 139.7 (C), 137.0 (CH), 136.5 (CH), 131.8 (CH), 129.5 (CH), 125.9 (C), 121.5 (CH), 119.6 (CH), 107.4 (CH), 100.0 (CH), 55.4 (CH$_3$), 21.4 (CH$_3$). HRMS (ESI) Calculated for C$_{20}$H$_{20}$NO$_2$S [M+H]$^+$: 338.1215; found: 338.1208.
13.6 Synthetic Route Towards Sulfonium Salt 37 (Chapter 7)

Scheme 13.6: Synthesis of sulfonium salt 37.
Reagents and conditions: (i) Thioether 4, Pd\textsubscript{2}(dba)\textsubscript{3}, DPEphos, KOtBu, toluene, reflux, 2 h; (ii) Bi(OTf)\textsubscript{3}, Sulfonamide 38, MeCN, 2.5 h, rt.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (15.0 mL), Pd$_2$(dba)$_3$ (223 mg, 0.244 mmol, 0.1 equiv.) and DPEphos (263 mg, 0.488 mmol, 0.2 equiv.), followed by 3-iodopyridine (500 mg, 2.44 mmol, 1.0 equiv.), KOtBu (301 mg, 2.68 mmol, 1.1 equiv.) and thioether 4 (1.19 g, 2.68 mmol, 1.1 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C and refluxed for 2 h. After cooling to room temperature, the mixture was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (50 → 70% Et$_2$O/PE) afforded the title compound as an orange oil (663 mg, 81%). $\nu_{\max}$/cm$^{-1}$: 2998, 2936, 2835, 1589, 1453, 1421. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 8.38-8.37 (2H, m, H20 and H22), 7.43-7.41 (1H, m, H23), 7.28 (1H, d, J 7.8, H11), 7.19 (1H, d, J 2.3, H8), 7.14-7.11 (2H, m, H10 and H24), 6.45-6.44 (3H, m, H2, H4 and H6), 3.75 (6H, s, H15 and H17), 2.38 (3H, s, H13). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 160.4 (C), 150.9 (CH), 147.4 (CH), 144.5 (C), 142.6 (C), 138.4 (C), 137.6 (CH), 134.7 (C), 133.4 (CH), 131.7 (CH), 129.4 (CH), 128.8 (C), 123.7 (CH), 107.5 (CH), 99.8 (CH), 55.5 (CH$_3$), 21.2 (CH$_3$). HRMS (ESI) Calculated for C$_{20}$H$_{20}$NO$_2$S [M+H]$^+$: 338.1214; found: 338.1215.
2,4-Dimethoxy-8-methyl-5-(pyridin-3-yl)-5H-dibenzo[b,d]thiophen-5-ium trifluoromethanesulfonate 37

To a stirring solution of Bi(OTf)$_3$ (194 mg, 0.296 mmol, 1.0 equiv.) and $N$-chloro-$N$-methylbenzenesulfonamide 38 (61.0 mg, 0.296 mmol, 1.0 equiv.) in MeCN (0.5 mL) was added a solution of thiother 36 (100 mg, 0.296 mmol, 1.0 equiv.) in MeCN (0.5 mL). The resulting solution was stirred at room temperature for 2.5 h. The mixture was then concentrated in vacuo, diluted with DCM, washed with a solution of EDTA in saturated aqueous K$_2$CO$_3$, followed by H$_2$O, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 10% 1 N NH$_3$ in MeOH/DCM) to afford a mixture of the title compound and its chlorinated analogue (62 mg). A pure sample could not be obtained, owing to the challenging separation of sulfonium salt 37 and its chlorinated analogue 41. $^1$H, $^{13}$C and $^{19}$F NMR spectroscopy were used to confirm the molecular structure. NMR yield of the title compound corresponds to 35% (51 mg). $^1$H NMR (600 MHz, d$_6$-DMSO) $\delta$H 9.01 (1H, d, $J$ 2.5, H20), 8.85 (1H, dd, $J$ 4.7, 1.5, H24), 8.42 (1H, d, $J$ 1.7, H8), 8.26 (1H, d, $J$ 8.3, H11), 7.73 (1H, d, $J$ 2.0, H2), 7.69 (1H, ddd, $J$ 8.4, 2.5, 1.5, H22), 7.61 (1H, dd, $J$ 8.3, 1.7, H10), 7.56 (1H, dd, $J$ 8.4, 4.7, H23), 6.92 (1H, d, $J$ 2.0, H6), 3.99 (3H, s, H15), 3.93 (3H, s, H17), 2.55 (3H, s, H13). $^{13}$C NMR (150 MHz, d$_6$-DMSO) $\delta$C 166.9 (C), 157.7 (C), 154.5 (CH), 150.6 (CH), 145.0 (C), 141.9 (C), 139.7 (C), 136.4 (CH), 132.5 (CH), 129.6 (C), 128.2 (CH), 126.4 (CH), 126.3 (C), 125.6 (CH), 108.5 (C), 102.2 (CH), 100.8 (CH), 57.4 (CH$_3$), 56.8 (CH$_3$), 21.3 (CH$_3$). $^{19}$F NMR (282 MHz, d$_6$-DMSO) $\delta$F -77.8.
Scheme 13.7: Synthesis of sulfonium salt 49.
Reagents and conditions: (i) HMDS, reflux, 20 h; (ii) 4-(Bromomethyl)-3-iodobenzonitrile, \( \text{K}_2\text{CO}_3 \), DMF, 100 °C, 3 h; (iii) Thioether 4, \( \text{Pd}_2(\text{dba})_3 \), DPEphos, \( \text{KOTBu} \), toluene, reflux, 3.5 h; (iv) TfOH/\( \text{Bi(OTf)}_3 \), NCS, MeCN, 2.5 h, rt (isolated and analysed by Dr Vincent Gray).
4-Phenyl-1-(trimethylsilyl)-1H-imidazole 46

![Chemical Structure Image]

To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added 4-phenyl-1H-imidazole (1.00 g, 6.94 mmol, 1.0 equiv.) and hexamethyldisilazane (7.30 mL, 34.8 mmol, 5.0 equiv.). The reaction vessel was degassed by argon purging before being plunged into a preheated block maintained at 130 °C. The reaction mixture was refluxed for 20 h. The reaction progress was monitored by 1H NMR spectroscopy. The title compound was obtained in quantitative yield and was used immediately in the next step without further purification. 1H NMR (600 MHz, CDCl3) δH 7.81 (2H, dd, J 8.1, 1.6, H1 and H5), 7.60 (1H, d, J 1.4, H10), 7.37 (2H, t, J 7.7, H2 and H4), 7.24-7.22 (2H, m, H3 and H8), 0.49 (9H, s, H13, H14 and H15).
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added DMF (10.0 mL), phenylimidazole 46 (1.50 g, 6.94 mmol, 1.0 equiv.), followed by 4-(bromomethyl)-3-iodobenzonitrile (2.68 g, 8.33 mmol, 1.2 equiv.) and K$_2$CO$_3$ (1.05 g, 7.63 mmol, 1.1 equiv.). The reaction vessel was degassed by argon purging before being plunged into a preheated block maintained at 100 °C. The reaction mixture was heated for 3 h. After cooling to room temperature, it was diluted with DCM, washed with H$_2$O and a saturated aqueous solution of LiCl. The organic layer was dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 20% EtOAc/DCM) of the resulting brown oil afforded the title compound as a yellow solid (1.39 g, 52%), mp 148-151 °C. $\nu_{\text{max}}$/cm$^{-1}$ 2225, 1605, 1553, 1487, 1439. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 8.17 (1H, d, $J$ 1.7, H2), 7.79 (2H, dd, $J$ 7.8, 1.5, H16 and H20), 7.77 (1H, bs, H14), 7.61 (1H, dd, $J$ 8.1, 1.7, H4), 7.40 (2H, t, $J$ 7.8, H17 and H19), 7.28 (1H, t, $J$ 7.8, H18), 7.21 (1H, d, $J$ 1.4, H12), 6.93 (1H, d, $J$ 8.1, H5), 5.24 (2H, s, H9). $\delta$C (150 MHz, CDCl$_3$) $\delta$C 144.0 (C), 143.1 (C), 142.7 (CH), 138.0 (CH), 133.2 (C), 132.6 (CH), 128.9 (CH), 128.4 (CH), 127.6 (CH), 125.0 (CH), 116.6 (C), 115.0 (C), 114.1 (CH), 97.3 (C), 55.7 (CH$_2$). HRMS (CI) Calculated for C$_{17}$H$_{13}$IN$_3$ [M+H]$^+$: 386.0154; found: 386.0136.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (7.0 mL), Pd$_2$(dba)$_3$ (30.0 mg, 32.5 µmol, 0.025 equiv.) and DPEphos (35.0 mg, 65.0 µmol, 0.05 equiv.). The reaction vessel was degassed by argon purging before thioether 4 (694 mg, 1.56 mmol, 1.2 equiv.) and KOtBu (175 mg, 1.56 mmol, 1.2 equiv.) were added. The resulting suspension was stirred at room temperature for 10 min and imidazole 47 (500 mg, 1.30 mmol, 1.0 equiv.) was added. The reaction vessel was degassed by argon purging once more before being plunged into a preheated block maintained at 125 °C. It was refluxed for 3.5 h. After cooling to room temperature, it was diluted with DCM, washed with brine, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 12% Et$_2$O/DCM) afforded the title compound as a yellow oil (535 mg, 75%). $\nu_{\text{max}}$/cm$^{-1}$ 2228, 1588, 1452, 1421. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.76 (2H, dd, $J$ 8.2, 1.6, H33 and H37), 7.58 (1H, d, $J$ 1.4, H31), 7.42-7.38 (3H, m, H15, H34 and H36), 7.34 (1H, dd, $J$ 8.0, 1.6, H22), 7.28-7.25 (2H, m, H12 and H20), 7.23-7.22 (2H, m, H14 and H35), 7.06 (1H, d, $J$ 1.4, H29) 6.77 (1H, d, $J$ 8.0, H23), 6.43 (1H, t, $J$ 2.4, H6), 6.32 (2H, d, $J$ 2.4, H2 and H4), 5.03 (2H, s, H26), 3.72 (6H, s, H9 and H11), 2.44 (3H, s, H17). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 160.4 (C), 145.4 (C), 142.8 (C), 142.5 (C), 140.6 (C), 140.2 (C), 138.4 (C), 138.0 (CH), 135.0 (CH), 134.1 (CH), 133.5 (C), 132.4 (CH), 130.1 (CH), 129.9 (CH), 128.8 (CH), 127.6 (CH), 127.4 (CH), 126.7 (C), 124.0 (CH), 118.1 (C), 115.2 (CH), 112.6 (C), 107.3 (CH), 107.3 (CH), 100.0 (CH), 55.5 (CH$_3$), 48.5 (CH$_2$), 21.4 (CH$_3$). HRMS (ESI) Calculated for C$_{32}$H$_{28}$N$_3$O$_2$S [M+H]$^+$: 518.1897; found: 518.1893.
13.8 Synthetic Route Towards Sulfonium Salt 53 (Chapter 8)

Scheme 13.8: Synthesis of sulfonium salt 53.

Reagents and conditions: (i) 3-Methoxyphenylboronic acid, Pd(PPh₃)₄, K₂CO₃, toluene/water, reflux, 16 h; (ii) Thioether 51, Pd₂(dba)₃, DPEphos, KOtBu, toluene, reflux, 3.5 h; (iii) NCS, TfOH, MeCN, rt, 2 h.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (10.0 mL) and thioether 3 (2.00 g, 5.16 mmol, 1.0 equiv.), followed by a solution of K$_2$CO$_3$ (3.57 g, 25.8 mmol, 5.0 equiv.) in H$_2$O (10.0 mL) and 3-methoxyphenylboronic acid (980 mg, 6.45 mmol, 1.25 equiv.). The reaction vessel was degassed by argon purging with vigorous stirring to ensure efficient removal of O$_2$ trapped in the aqueous layer. Pd(PPh$_3$)$_4$ (373 mg, 0.323 mmol, 0.06 equiv.) was added and after additional degassing, the resulting mixture was plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 16 h. After cooling to room temperature, it was washed with a saturated aqueous solution of K$_2$CO$_3$. The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 10% Et$_2$O/PE) of the resulting brown oil afforded the title compound as a colourless oil (1.86 g, 87%).

$\nu_{\text{max}}$/cm$^{-1}$ 2957, 2927, 1732, 1597, 1579, 1462. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.35 (1H, d, $J$ 8.0, H4), 7.32 (1H, t, $J$ 7.8, H11), 7.14 (1H, dd, $J$ 8.0, 2.1, H3), 7.11 (1H, d, $J$ 2.1, H1), 6.99-6.97 (1H, m, H10), 6.95 (1H, dd, $J$ 2.6, 1.6, H14), 6.92-6.90 (1H, m, H12), 3.99-3.94 (2H, 2 ×m, H22), 3.84 (3H, s, H16), 2.95 (2H, t, $J$ 7.6, H17), 2.50 (2H, t, $J$ 7.6, H18), 2.36 (3H, s, H7), 1.56-1.51 (1H, m, H23), 1.35-1.25 (8H, m, H24, H25, H26 and H28), 0.90-0.86 (6H, m, H27 and H29). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 172.1 (C), 159.2 (C), 143.1 (C), 142.3 (C), 136.4 (C), 131.4 (CH), 130.3 (C), 129.9 (CH), 129.1 (CH), 128.9 (CH), 121.9 (CH), 115.0 (CH), 113.1 (CH), 67.2 (CH$_2$), 55.4 (CH$_3$), 38.8 (CH), 34.3 (CH$_2$), 30.5 (CH$_2$), 29.0 (CH$_2$), 28.9 (CH$_2$), 23.9 (CH$_2$), 23.1 (CH$_2$), 21.0 (CH$_3$), 14.2 (CH$_3$), 11.1 (CH$_3$). HRMS (EI) Calculated for C$_{25}$H$_{34}$O$_3$S [M]$^+$: 414.2223; found: 414.2222.
3-((3’-Methoxy-5-methyl-[1,1’-biphenyl]-2-yl)thio)-4-((5-phenyl-1H-imidazol-1-yl)methyl)benzonitrile 52

To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (3.0 mL), Pd₂dba₃ (59.4 mg, 64.9 µmol, 0.01 equiv.) and DPEphos (69.9 mg, 0.130 mmol, 0.02 equiv.). The reaction vessel was degassed by argon purging before thioether 51 (377 mg, 0.909 mmol, 1.4 equiv.) and KOtBu (102 mg, 0.909 mmol, 1.4 equiv.) were added. The resulting suspension was stirred at room temperature for 10 min and imidazole 47 (250 mg, 0.649 mmol, 1.0 equiv.) was added. The reaction vessel was degassed by argon purging once more before being plunged into a preheated block maintained at 125 °C. It was refluxed for 3.5 h. After cooling to room temperature, it was diluted with DCM, washed with brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 5% Et₂O/PE) of the resulting brown oil afforded the title compound as a yellow oil (243 mg, 77%). νmax/cm⁻¹ 2228, 1595, 1578, 1445. ¹H NMR (600 MHz, d₆-DMSO) δH 7.71 (4H, m, H20, H27, H31 and H35), 7.44 (1H, d, J 1.4, H29), 7.39 (1H, d, J 1.7, H18), 7.35-7.30 (4H, m, H5, H11, H32 and H34), 7.27-7.26 (2H, m, H8 and H10), 7.19 (1H, t, J 7.4, H33), 7.04 (1H, d, J 8.0, H21), 6.93-6.91 (2H, m, H4 and H6), 6.84 (1H, dd, J 2.6, 1.5, H2), 5.18 (2H, s, H24), 3.71 (3H, s, H15), 2.37 (3H, s, H13). ¹³C NMR (150 MHz, d₆-DMSO) δC 158.8 (C), 144.0 (C), 142.4 (C), 141.4 (C), 141.1 (C), 138.9 (C), 138.4 (CH), 136.7 (C), 134.3 (C), 134.2 (CH), 133.5 (CH), 131.8 (CH), 131.2 (CH), 129.8 (CH), 129.2 (CH), 128.8 (CH), 128.5 (CH), 127.4 (C), 126.4 (CH), 124.2 (CH), 121.3 (CH), 118.0 (C), 115.9 (CH), 114.4 (CH), 113.3 (CH), 111.6 (C), 55.1 (CH₃), 47.6 (CH₂), 20.7 (CH₃). HRMS (ESI) Calculated for C₃₁H₂₆N₃OS [M+H]⁺: 488.1791; found: 488.1794.
To a round-bottom flask were added MeCN (1.0 mL), thioether 52 (49.0 mg, 0.100 mmol, 1.0 equiv.), NCS (13.0 mg, 0.100 mmol, 1.0 equiv.) and TfOH (26.5 µL, 0.300 mmol, 3.0 equiv.). The reaction mixture was stirred at room temperature for 2 h. After concentration, the residue was dissolved in DCM and washed with H2O. The organic layer was dried over MgSO4 and concentrated in vacuo. Flash column chromatography purification (1 → 10% 1 N NH3 in MeOH/DCM) afforded the title compound as an off-white solid (47.0 mg, 74%), mp 150-152 °C (dec). νmax/cm⁻¹ 1591, 1482. 1H NMR (600 MHz, d₆-DMSO) δH 8.46 (1H, d, J 1.4, H10), 8.19 (1H, dd, J 8.1, 1.6, H20), 8.13 (1H, d, J 2.6, H2), 8.06 (1H, bs, H29) 7.91 (1H, d, J 8.9, H6), 7.85 (1H, bs, H27), 7.82 (2H, dd, J 8.1, 1.6, H31 and 35), 7.61 (1H, d, J 8.3, H13), 7.58 (1H, d, J 8.1, H21), 7.48 (1H, dd, J 8.3, 1.4, H12), 7.41-7.38 (2H, m, H32 and H34), 7.35 (1H, d, J 1.6, H18), 7.28-7.24 (2H, m, H6 and H33), 6.26 (1H, d, J 16.3, H24), 6.19 (1H, d, J 16.3, H24), 3.99 (3H, s, H9), 2.53 (3H, s, H17). 13C NMR (150 MHz, d₆-DMSO) δC 164.4 (C), 148.2 (C), 147.7 (C), 146.1 (C), 145.1 (C), 142.6 (C), 141.7 (C), 139.5 (C), 138.7 (CH), 138.5 (CH), 132.3 (CH), 132.2 (CH), 130.5 (C), 129.5 (CH), 128.7 (CH), 127.3 (CH), 126.9 (CH), 125.7 (CH), 124.5 (CH), 123.6 (CH), 121.5 (C), 118.0 (CH), 116.4 (C), 116.3 (CH), 114.2 (C), 109.9 (CH), 56.6 (CH₃), 47.6 (CH₂), 21.3 (CH₃). 19F NMR (282 MHz, d₆-DMSO) δF -77.8. HRMS (ESI) Calculated for C₃₁H₂₄N₃OS [M]+: 486.1637; found: 486.1740.
13.9 Synthetic Route Towards Sulfonium Salt 58 (Chapter 8)

Scheme 13.9: Synthesis of sulfonium salt 58.
Reagents and conditions: (i) NBS, DMF, -5 °C, 1 h; (ii) Pd$_2$(dba)$_3$, xantphos, Et$_3$N, toluene, reflux, 18 h; (iii) Thioether 56, Pd$_2$(dba)$_3$, DPEphos, KOrBu, toluene, reflux, 18 h; (iv) Not isolated.
To a stirring solution of 3,3'-dimethoxy-1,1'-biphenyl (750 mg, 3.50 mmol, 1.0 equiv.) in DMF (10.0 mL) maintained -5 °C was added dropwise over 1 h a solution of NBS (685 mg, 3.85 mmol, 1.1 equiv.) in DMF (10.0 ml). The reaction mixture was diluted with water and extracted with Et₂O. The combined organic extracts were washed with an aqueous solution of 1 M Na₂S₂O₃, then a saturated solution of LiCl, dried over MgSO₄ and concentrated in vacuo to afford the title compound as an off-white solid in quantitative yield. It was employed used in the next step without further purification. \( ^1H \) NMR (600 MHz, CDCl₃) \( \delta_H \) 7.54 (1H, d, \( J \) 8.8, H11), 7.36-7.33 (1H, m, H5), 7.00-6.98 (1H, m, H4), 6.96-6.93 (2H, m, H2 and H6), 6.88 (1H, d, \( J \) 3.0, H8), 6.78 (1H, dd, \( J \) 8.8, 3.0, H10), 3.85 (3H, s, H14 or H16), 3.81 (3H, s, H14 or H16).
To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (14.0 mL), anisole 55 (1.00 g, 3.41 mmol, 1.0 equiv.) and NEt$_3$ (0.950 mL, 6.82 mmol, 2.0 equiv.). The reaction vessel was degassed by argon purging before Pd$_2$(dba)$_3$ (94.0 mg, 0.102 mmol, 0.03 equiv.) was added, followed by xanthphos (118 mg, 0.204 mmol, 0.06 equiv.) and thiol 2 (745 mg, 3.41 mmol, 1.0 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 18 h. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (10 → 30% Et$_2$O/PE) of the resulting yellow oil afforded the title compound as a colourless oil (600 mg, 41%).

$\nu_{\text{max}}$/cm$^{-1}$ 2957, 2929, 1731, 1591, 1463. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.44 (1H, dd, $J$ 8.1, 0.8, H12), 7.33 (1H, dd, $J$ 8.2, 7.5, H5), 6.99-6.98 (1H, m, H4), 6.96 (1H, dd, $J$ 2.5, 1.6, H2), 6.92 (1H, dd, $J$ 8.2, 25, H6), 6.88-6.86 (2H, m, H10 and H13), 3.97-3.91 (2H, 2 × m, H22), 3.84 (3H, s, H8 or H30), 3.82 (3H, s, H8 or H30), 2.82 (2H, t, $J$ 7.5, H17), 2.42 (2H, t, $J$ 7.5, H18), 1.55-1.51 (1H, m, H23), 1.34-1.25 (8H, m, H24, H25, H26 and H28), 0.90-0.85 (6H, m, H27 and H29). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 172.1 (C), 158.9 (C), 158.8 (C), 145.8 (C), 142.3 (C), 133.9 (CH), 129.1 (CH), 124.0 (C), 121.9 (CH), 116.2 (CH), 115.1 (CH), 113.9 (CH), 113.2 (CH), 67.2 (CH), 55.6 (CH$_3$), 55.4 (CH$_3$), 38.8 (CH$_2$), 34.5 (CH$_2$), 30.5 (CH$_2$), 30.0 (CH$_2$), 29.0 (CH$_2$), 23.8 (CH$_2$), 23.1 (CH$_2$), 14.2 (CH$_3$), 11.1 (CH$_3$).

HRMS (EI) Calculated for C$_{25}$H$_{34}$O$_4$S [M]$^+$: 430.2172; found: 430.2171.
3-((3’,5-Dimethoxy-[1,1’-biphenyl]-2-yl)thio)-4-((5-phenyl-4,5-dihydro-1H-imidazol-1-yl)methyl)benzonitrile 57

To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added toluene (2.0 mL), Pd$_2$(dba)$_3$ (24.0 mg, 26.0 µmol, 0.02 equiv.) and DPEphos (28.0 mg, 52.0 µmol, 0.04 equiv.). The reaction vessel was degassed by argon purging before thioether 56 (268 mg, 0.623 mmol, 1.2 equiv.) and KOtBu (70.0 mg, 0.623 mmol, 1.2 equiv.) were added. The resulting suspension was stirred at room temperature for 10 min and imidazole 47 (200 mg, 0.519 mmol, 1.0 equiv.) was added. The reaction vessel was degassed by argon purging once more before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 18 h. After cooling to room temperature, it was diluted with DCM, washed with brine, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (1 → 16% Et$_2$O/DCM) of the resulting brown oil afforded the title compound as an orange oil (243 mg, 71%). $\nu_{\text{max}}$/cm$^{-1}$ 2228, 1589, 1561, 1463. $^1$H NMR (600 MHz, d$_6$-DMSO) $\delta$H 7.71-7.70 (2H, m, H30, H32 and H36), 7.63 (1H, dd, J 8.0, 1.7, H21), 7.52 (1H, d, J 8.6, H13), 7.46 (1H, d, J 1.3, H28), 7.36-7.33 (2H, m, H33 and H35), 7.29 (1H, dd, J 8.4, 7.6, H5), 7.21-7.18 (2H, m, H19 and H34), 7.08 (1H, dd, J 8.6, 3.0, H12), 7.02 (1H, d, J 3.0, H10), 6.99 (1H, d, J 8.0, H22), 5.15 (2H, s, H24), 6.92-6.90 (2H, m, H4 and H6), 6.83 (1H, dd, J 2.6, 1.7, H2), 3.85 (3H, s, H8 or H16), 3.69 (3H, s, H8 or H16). $^{13}$C NMR (150 MHz, d$_6$-DMSO) $\delta$C 160.2 (C), 158.7 (C), 146.8 (C), 141.4 (C), 141.1 (C), 141.0 (C), 138.5 (CH), 138.2 (C), 137.0 (CH), 134.3 (C), 132.2 (CH), 130.3 (CH), 129.1 (CH), 128.5 (CH), 126.4 (CH), 124.3 (CH), 121.3 (CH), 119.9 (C), 118.1 (C), 116.7 (CH), 115.9 (CH), 115.1 (CH), 114.4 (CH), 113.4 (CH), 111.4 (C), 109.9 (CH), 55.6 (CH$_3$), 55.1 (CH$_3$), 47.4 (CH$_2$). HRMS (ESI) Calculated for C$_{31}$H$_{26}$N$_3$O$_2$S [M+H]$^+$: 504.1746; found: 504.1744.
13.10 Synthetic Route Towards Sulfonium Salt 64 (Chapter 8)

Scheme 13.10: Synthesis of sulfonium salt 64.
Reagents and conditions: (i) AIBN, NBS, chlorobenzene, reflux, 4 h; (ii) Phenylimidazole 46, K$_2$CO$_3$, DMF, 100 °C, 1 h; (iii) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 2 h; (iv) NCS, TfOH, MeCN, rt, 2 h.

Reagents and conditions: (i) AIBN, NBS, chlorobenzene, reflux, 4 h; (ii) Phenylimidazole 46, K$_2$CO$_3$, DMF, 100 °C, 2 h.
3-Bromo-5-(bromomethyl)benzonitrile 61

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added chlorobenzene (25.0 mL) and AIBN (167 mg, 1.02 mmol, 0.2 equiv.), followed by 3-bromo-5-methylbenzonitrile (1.00 g, 5.10 mmol, 1.0 equiv.) and NBS (998 mg, 5.61 mmol, 1.1 equiv.). The resulting solution was purged with argon before being plunged into a preheated block maintained at 125 °C and refluxed for 4 h. After cooling to room temperature, the mixture was diluted with DCM, washed with a 1 M aqueous Na₂S₂O₃ solution, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 8% Et₂O/PE) afforded the title compound as a white solid (672 mg, 48%), mp 108-110 °C. νmax/cm⁻¹ 2236, 1567, 1434, 1424, 1251, 1221. ¹H NMR (600 MHz, CDCl₃) δH 7.78 (1H, t, J 1.7, H6), 7.73 (1H, t, J 1.7, H2), 7.63 (1H, t, J 1.7, H4), 4.42 (2H, s, H9). ¹³C NMR (150 MHz, CDCl₃) δC 141.2 (C), 136.6 (CH), 134.6 (CH), 131.2 (CH), 123.9 (C), 116.9 (C), 114.7 (C), 30.1 (CH₂). HRMS (CI) Calculated for C₈H₇Br₂N₂ [M + NH₄⁺]: 290.9127; found: 290.9128.
3-Bromo-5-((5-phenyl-1H-imidazol-1-yl)methyl)benzonitrile 62

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condensers were added DMF (4.0 mL), phenylimidazole 46 (472 mg, 2.18 mmol, 2.0 equiv.), benzyl bromide 61 (300 mg, 1.09 mmol, 1.0 equiv.) and K₂CO₃ (377 mg, 2.73 mmol, 2.5 equiv.). The resulting solution was purged with argon before being plunged into a preheated block maintained at 100 °C and heated for 1 h. After cooling to room temperature, the mixture was diluted with DCM, washed with a saturated aqueous solution of LiCl, followed by H₂O, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (5 → 60% EtOAc/PE) afforded the title compound a yellow solid (267 mg, 72%), mp 148-150 °C. ν max/cm⁻¹ 3113, 3065, 2235, 1602, 1586, 1500, 1350. ¹H NMR (600 MHz, CDCl₃) δH 7.78-7.77 (3H, m, H₂, H₁₆ and H₂₀), 7.61 (1H, d, J 1.5, H₁₄), 7.55 (1H, t, J 1.8, H₆), 7.41 (1H, t, J 1.8, H₄), 7.39 (2H, t, J 7.8, H₁₇ and H₁₉), 7.28-7.26 (1H, m, H₁₈), 7.17 (1H, d, J 1.5, H₁₂), 5.17 (2H, s, H₉). ¹³C NMR (150 MHz, CDCl₃) δC 143.8 (C), 139.9 (C), 137.7 (CH), 134.8 (CH), 134.6 (CH), 133.6 (C), 129.2 (CH), 128.8 (CH), 127.4 (CH), 125.1 (CH), 124.0 (C), 116.8 (C), 115.1 (C), 114.7 (CH), 49.6 (CH₂). HRMS (ESI) Calculated for C₁₇H₁₃BrN₃ [M+H]⁺: 338.0293; found 338.0292.
3-((3’,5’-Dimethoxy-5-methyl-[1,1’-biphenyl]-2-yl)thio)-5-((5-phenyl-1H-imidazol-1-yl)methyl)benzonitrile

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (12.0 mL), Pd$_2$(dba)$_3$ (104 mg, 0.114 mmol, 0.1 equiv.) and DPEphos (123 mg, 0.228 mmol, 0.2 equiv.), followed by imidazole 62 (400 mg, 1.14 mmol, 1.0 equiv.), thioether 4 (735 mg, 1.63 mmol, 1.4 equiv.) and KOtBu (183 mg, 1.63 mmol, 1.4 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. It was refluxed for 2 h. After cooling to room temperature, it was filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (0 → 50% Et$_2$O/DCM) afforded the title compound as a yellow oil (53 mg, 17%). ν$_{\text{max}}$/cm$^{-1}$ 3310, 2957, 2931, 1635, 1585, 1542, 1454. $^1$H NMR (600 MHz, CDCl$_3$) δ$_H$ 7.77-7.76 (2H, m, H$_{33}$ and H$_{37}$), 7.53 (1H, d, $J$ 1.4, H$_{31}$), 7.41 (1H, d, $J$ 8.2, H$_{11}$), 7.39 (2H, tt, $J$ 7.8, 2.4, H$_{34}$ and H$_{36}$), 7.28-7.25 (1H, m, H$_{35}$), 7.20 (1H, d, $J$ 2.1, H$_8$), 7.17-7.15 (1H, dd, $J$ 8.2, 2.1, H$_{10}$), 7.15 (1H, t, $J$ 1.8, H$_{20}$), 7.11 (1H, t, $J$ 1.8, H$_{22}$), 7.09 (1H, d, $J$ 1.4, H$_{29}$), 6.98 (1H, t, $J$ 1.8, H$_{24}$), 6.40 (1H, t, $J$ 2.3, H$_6$), 6.33 (2H, d, $J$ 2.3, H$_2$ and H$_4$), 5.03 (2H, s, H$_{26}$), 3.71 (6H, s, H$_{14}$ and H$_{16}$), 2.37 (3H, s, H$_{17}$). $^{13}$C NMR (150 MHz, CDCl$_3$) δ$_C$ 160.3 (C), 145.9 (C), 143.4 (C), 142.6 (C), 142.4 (C), 140.2 (C), 138.1 (C), 137.7 (CH), 135.5 (CH), 133.8 (C), 132.1 (CH), 131.0 (CH), 130.6 (CH), 129.8 (CH), 128.8 (CH), 127.2 (CH), 127.0 (CH), 126.3 (C), 125.0 (CH), 117.9 (C), 114.7 (CH), 113.7 (C), 107.4 (CH), 99.6 (CH), 55.5 (CH$_3$), 49.9 (CH$_2$), 21.3 (CH$_3$). HRMS (ESI) Calculated for C$_{32}$H$_{28}$N$_3$O$_2$S [M+H]$^+$: 518.1902; found 518.1909.
5-(3-Cyano-5-((5-phenyl-1H-imidazol-1-yl)methyl)phenyl)-2,4-dimethoxy-8-methyl-5H-dibenzo[b,d]thiophen-5-ium trifluoromethanesulfonate 64

To a round-bottom flask were added MeCN (1.0 mL), thioether 63 (42.0 mg, 81.1 µmmol, 1.0 equiv.), NCS (10.8 mg, 81.1 µmmol, 1.0 equiv.) and TfOH (21.0 µL, 0.243 mmol, 3.0 equiv.). The reaction mixture was stirred at room temperature for 2 h. It was then concentrated in vacuo, diluted with DCM and washed with a 2 M NaOH solution. The organic layer was stirred vigorously with saturated NaOTf aqueous solution for 10 min, separated, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 14% MeOH/DCM) afforded the title compound as an off-white solid (26.0 mg, 48%), mp 165-167 °C (dec). ν_{max}/ cm⁻¹ 3060, 2950, 2929, 1591, 1485, 1447, 1427. ¹H NMR (600 MHz, CDCl₃) δ_H 8.60 (1H, t, J 1.8, H24), 8.36 (1H, d, J 8.3, H15), 7.86 (1H, d, J 2.0, H12), 7.77-7.76 (2H, m, H33 and H37), 7.72 (1H, d, J 1.5, H31), 7.59 (1H, t, J 1.8, H22), 7.47 (1H, dd, J 8.3, 2.0, H14), 7.42 (1H, d, J 1.5, H29), 7.38 (2H, t, J 7.8, H34 and H36), 7.28-7.25 (1H, m, H35), 7.18 (1H, d, J 2.1, H6), 7.07 (1H, t, J 1.8, H20), 6.54 (1H, d, J 2.1, H2), 5.48 (1H, d, J 16.4, H26), 5.40 (1H, d, J 16.4, H26), 4.01 (3H, s, H9), 3.83 (3H, s, H17), 2.56 (3H, s, H11). ¹³C NMR (150 MHz, CDCl₃) δ_C 168.2 (C), 158.8 (C), 146.6 (C), 143.0 (C), 142.4 (C), 142.3 (C), 139.0 (C), 137.7 (CH), 136.5 (CH), 135.1 (CH), 133.8 (CH), 133.3 (C), 130.2 (C), 129.8 (CH), 129.5 (CH), 128.8 (CH), 128.1 (C), 127.4 (CH), 125.0 (CH), 121.6 (C), 116.1 (C), 115.9 (C), 115.4 (CH), 106.1 (CH), 101.6 (C), 100.5 (CH), 57.2 (CH₃), 56.7 (CH₃), 49.4 (CH₂), 22.04 (CH₃). ¹⁹F NMR (282 MHz, CDCl₃) δ_F -78.3. HRMS (ESI) Calculated for C₃₂H₂₆N₃O₂S [M]⁺: 516.1740; found: 516.1742.
3-(Bromomethyl)-5-fluorobenzonitrile 66

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added chlorobenzene (37.0 mL) and AIBN (49.0 mg, 0.30 mmol, 0.2 equiv.), followed by 3-fluoro-5-methylbenzonitrile (200 mg, 1.48 mmol, 1.0 equiv.) and NBS (290 mg, 1.63 mmol, 1.1 equiv.). The resulting solution was purged with argon before being plunged into a preheated block maintained at 125 °C. It was refluxed for 4 h. After cooling to room temperature, the mixture was diluted with DCM, washed with a 1 M aqueous Na₂S₂O₃ solution, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 12% Et₂O/PE) afforded the title compound as a shiny white solid (152 mg, 48%), mp 48-50 °C. νmax/cm⁻¹ 3079, 2229, 1600, 1587, 1444, 1435, 1306. ¹H NMR (600 MHz, CDCl₃) δH 7.50 (1H, t, J 1.6, H₄), 7.38 (1H, ddd, J 9.0, 2.5, 1.6, H₆), 7.31 (1H, ddd, J 7.7, 2.5, 1.6, H₂), 4.44 (2H, s, H₈). ¹³C NMR (150 MHz, CDCl₃) δC 163.1 & 161.5 (d, J 250.5, C), 142.0 & 141.9 (d, J 8.3, C), 128.6 & 128.5 (d, J 3.2, CH), 121.2 & 121.1 (d, J 21.9, CH), 119.2 & 119.1 (d, J 24, CH), 117.2 & 117.1 (d, J 3.3, C), 114.5 & 114.4 (d, J 9.9, C), 30.3 & 30.2 (d, J 1.7, CH₂). ¹⁹F NMR (282 MHz, CDCl₃) δF -108.8. HRMS (CI) Calculated for C₈H₉BrFN₂ [M + NH₄⁺]: 230.9928; found: 230.9928.
3-Fluoro-5-((5-phenyl-1H-imidazol-1-yl)methyl)benzonitrile 67

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added DMF (1.5 mL) and phenylimidazole 46 (205 mg, 0.947 mmol, 1.6 equiv.), followed by benzyl bromide 66 (123 mg, 0.575 mmol, 1.0 equiv.) and K$_2$CO$_3$ (144 mg, 1.04 mmol, 1.8 equiv.). The resulting solution was purged with argon before being plunged into a preheated block maintained at 100 °C and heated for 2 h. After cooling to room temperature, the mixture was diluted with DCM and washed with a saturated aqueous solution of LiCl, followed by H$_2$O, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 60% EtOAc/DCM) afforded the title compound as a yellow solid (80.0 mg, 50%), mp 135-137 °C. $\nu_{\text{max}}$/cm$^{-1}$ 3079, 3047, 3007, 1676, 1552, 1500, 1481. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.77 (2H, m, H16 and H20), 7.62 (1H, d, $J$ 1.5, H14), 7.39 (2H, tt, $J$ 7.6, 3.6, H17 and H19), 7.35 (1H, dd, $J$ 7.8, 1.9, H2), 7.31 (1H, d, $J$ 1.9, H4), 7.27 (1H, tt, $J$ 7.6, 3.6, H18), 7.18 (1H, d, $J$ 1.5, H12), 7.11 (1H, dt, $J$ 8.7, 1.9, H6), 5.20 (2H, s, H9). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 163.6 & 161.9 (d, $J$ 252.2, C), 143.8 (C), 140.8 (d, $J$ 7.1, C), 137.7 (CH), 133.6 (C), 128.8 (CH), 127.4 (CH), 126.4 (d, $J$ 3.3, CH), 125.0 (CH), 119.4 & 119.2 (d, $J$ 24.9, CH), 119.2 & 119.0 (d, $J$ 22.0, CH), 117.0 (C), 115.0 & 114.9 (d, $J$ 9.8, C), 114.7 (CH), 49.8 (CH$_2$). $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$F -107.8. HRMS (ESI) Calculated for C$_{17}$H$_{15}$FN$_3$ [M+H]$^+$: 278.1094; found: 278.1091.
13.11 Synthetic Route Towards Sulfonium Salt 83 (Chapter 9)

Scheme 13.12: Synthesis of sulfonium salt 83 and cold reference compound 73.
Reagents and conditions: (i) Cyclopropylamine, KF, K$_2$CO$_3$, MeCN, reflux, 1 h; (ii) Pd/C, H$_2$, MeOH, rt, 20 h; (iii) 5-Bromonicotinaldehyde, Oxone®, DMF/H$_2$O, rt, 1 h; (iiiia) 5-Fluoronicotinaldehyde, Oxone®, DMF/H$_2$O, rt, 1 h; (iiib) Thioether 4, Pd$_2$(dba)$_3$, DPEphos, KOTBu, toluene, reflux, 2 h; (v) NCS (stock solution), TfOH, MeCN, rt, 30 min.
To a solution of KF (988 mg, 17.0 mmol, 1.5 equiv.), K$_2$CO$_3$ (1.56 g, 11.3 mmol, 1.0 equiv.) and 2,4,5-trifluoronitrobenzene (2.00 g, 11.3 mmol, 1.0 equiv.) in MeCN (5.0 mL) at reflux was added dropwise over 30 min a solution of cyclopropylamine (783 µL, 11.3 mmol, 1.0 equiv.) in MeCN (5.0 mL). The resulting deep-yellow suspension was refluxed for 1 h. After cooling to room temperature, the mixture was concentrated in vacuo. The residue was dissolved in DCM, washed with H$_2$O, brine, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 → 5% EtOAc/PE) afforded the title compound (fast eluting component) as a bright orange solid (1.03 g, 43%), mp 73-74 ºC. $\nu$$_{\text{max}}$/cm$^{-1}$ 3371, 3078, 3007, 1651, 1579, 1514, 1233. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 8.10 (1H, bs, H10), 8.04 (1H, dd, J 10.6, 8.3, H6), 7.10 (1H, dd, J 12.6, 7.0, H3), 2.57-2.53 (1H, m, H11), 0.97-0.94 (2H, m, H12 or H13), 0.69-0.67 (2H, m, H12 or H13). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 157.0-155.2 (dd, J 257.0, 14.3, C), 144.9 & 144.8 (d, J 10.5, C), 142.2-140.5 (dd, J 240.9, 14.1, C) 126.8 & 126.7 (d, J 6.0, C), 115.1-114.9 (dd, J 21.5, 3.3, CH), 102.9 & 102.8 (d, J 22.5, CH), 24.9 (CH), 8.0 (CH$_2$). $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$F -122.1 & -122.2 (1F, d, J 23.4), -150.0 & -150.1 (1F, d, J 23.4). HRMS (CI) Calculated for C$_9$H$_7$F$_2$N$_2$O$_2$ [M+H]$^+$: 215.0627; found: 215.0628.
To a flame-dried three-neck round-bottom flask equipped with an argon inlet were added 10 mol% Pd/C (72.0 mg, 0.667 mmol, 0.1 equiv.) and MeOH (35 mL), followed by nitrobenzene 76 (1.45 g, 6.77 mmol, 1.0 equiv.). The reaction vessel was thoroughly evacuated before H₂ balloon insertion and the resulting black suspension was stirred at room temperature for 20 h. It was then diluted with DCM, filtered through a pad of Celite and concentrated in vacuo. Flash column chromatography purification (0 → 50% EtOAc/PE) afforded the title compound as a red solid (1.07 g, 86%), mp 54-56 °C. ν max/cm⁻¹ 3422, 3332, 3263, 2954, 1634, 1518, 1375. ¹H NMR (600 MHz, CDCl₃) δ H 6.83 (1H, dd, J 12.4, 7.8, H6), 6.52 (1H, dd, J 11.2, 7.7, H3), 3.83 (1H, bs, H10), 3.17 (2H, bs, H9), 2.39 (1H, m, H11), 0.77-0.74 (2H, m, H12 or H13), 0.52-0.49 (2H, m, H12 or H13). ¹³C NMR (150 MHz, CDCl₃) δ C 143.6-143.9 (dd, J 234.6, 13.2, C), 144.0-142.2 (dd, J 234.6, 13.1, C), 134.8-134.8 (dd, J 234.6, 13.1, C), 129.3-129.2 (dd, J 7.4, 2.3, C), 105.5 & 105.4 (d, J 20.3, CH), 101.8 & 101.7 (d, J 21.9, CH), 26.7 (CH₂), 7.2 (CH₂). ¹⁹F NMR (282 MHz, CDCl₃) δ F -148.7 & -148.8 (1F, d, J 22.8), -151.9 & -152.0 (1F, d, J 22.8). HRMS (ESI) Calculated for C₉H₁₁F₂N₂ [M+H]⁺: 185.0890; found: 185.0887.
To a stirring solution of diamine 77 (130 mg, 0.706 mmol, 1.0 equiv.) in 97% DMF/H$_2$O were added 5-fluoronicotinaldehyde (97.0 mg, 0.775 mmol, 1.1 equiv.), followed by Oxone® (141 mg, 0.469 mmol, 0.65 equiv.). The resulting brown mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, diluted with EtOAc and H$_2$O, followed by addition of solid K$_2$CO$_3$ until the aqueous layer reached pH of $\approx 9$. The organic layer was separated, washed with H$_2$O and brine, dried over MgSO$_4$ and concentrated in vacuo. Flash column chromatography purification (0 $\rightarrow$ 30% EtOAc/DCM) afforded the title compound as an orange solid (133 mg, 65%), mp 185-188 °C. $\nu$$_{\text{max}}$/cm$^{-1}$ 3030, 1605, 1573, 1472, 1417. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ H 9.05 (1H, bs, H4), 8.62 (1H, d, J 2.7, H6), 8.02 (1H, ddd, J 8.8, 2.7, 1.7, H2), 7.58 (1H, dd, J 10.2, 7.3, H13 or H16), 7.42 (1H, dd, J 9.7, 7.0, H13 or H16), 3.58 (1H, tt, J 6.9, 3.8, H19), 1.27-1.24 (2H, m, H20 or H21), 0.83-0.80 (2H, m, H20 or H21). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ C 160.0 & 158.3 (d, J 256.5, C), 151.1 (d, J 2.1, C), 149.7-149.3 (ddd, J 48.0, 16.0, C), 148.0-147.7 (dd, J 48.0, 16.0, C), 145.6 & 145.5 (d, J 4.4, CH), 139.3 & 139.2 (d, J 22.5, CH), 137.9 & 137.8 (d, J 11.0, C), 132.7 & 132.6 (d, J 10.4, C), 127.9 & 127.8 (d, J 4.4, C), 123.3 & 123.2 (d, J 19.8, CH), 107.7 & 107.6 (d, J 18.6, CH), 99.3 & 99.2 (d, J 23.1, CH), 26.6 (CH$_2$), 9.2 (CH). $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$ F -125.8 (1F, s), -139.4 & -139.5 (1F, d, J 20.6), -142.2 & -142.3 (1F, d, J 20.6). HRMS (CI) Calculated for C$_{15}$H$_{11}$F$_3$N$_3$ [M+H]$^+$: 290.0900; found: 290.0899.
To a stirring solution of diamine 77 (1.07 g, 5.81 mmol, 1.0 equiv.) in 97% DMF/H₂O were added 5-bromonicotinaldehyde (1.19 g, 6.40 mmol, 1.1 equiv.), followed by Oxone® (1.16 g, 3.77 mmol, 0.65 equiv.). The resulting brown mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, diluted with EtOAc and H₂O and solid K₂CO₃ was added until the aqueous layer reached pH of ≈ 9. The organic layer was separated and washed with H₂O and brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 30% EtOAc/DCM) of the resulting yellow oil afforded the title compound as a light pink solid (1.20 g, 59%), mp 150-151 °C. ν max/cm⁻¹ 3024, 1481, 1467, 1446, 1405, 1397. ¹H NMR (600 MHz, CDCl₃) δH 9.13 (1H, d, J 2.2, H4), 8.80 (1H, d, J 2.2, H2), 8.45 (1H, t, J 2.2, H6), 7.57 (1H, dd, J 10.2, 7.3, H13 or H16), 7.41 (1H, dd, J 9.7, 7.0, H13 or H16), 3.58 (1H, tt, J 6.8, 3.9, H17), 1.27-1.24 (2H, m, H20 or H21), 0.83-0.80 (2H, m, H20 or H21). ¹³C NMR (150 MHz, CDCl₃) δC 151.7 (CH), 151.0 (C), 149.7-149.3 (dd, J 23.6, 15.3, C), 148.0-147.6 (dd, J 22.4, 15.9, C), (CH), 138.9 (CH), 132.7 & 132.6 (d, J 10.4, C), 137.9 & 137.8 (d, J 12.1, C), 127.9 (C), 120.8 (C), 107.7 & 107.6 (d, J 19.8, CH), 99.3 & 99.1 (d, J 23.0, CH), 26.6 (CH), 9.17 (CH₂). ¹⁹F NMR (282 MHz, CDCl₃) δF -139.4 & -139.5 (1F, d, J 20.8, -142.2 & -142.3 (1F, d, J 20.8). HRMS (ESI) Calculated for C₁₅H₁₁BrF₂N₃ [M+H]+: 350.0104; found: 350.0109.
1-Cyclopropyl-2-(5-((3′,5′-dimethoxy-5-methyl-[1,1′-biphenyl]-2-yl)thio)pyridin-3-yl)-5,6-difluoro-1\textit{H}-benzo[\textit{d}]imidazole 82

To a flame-dried three-neck round-bottom flask equipped with an argon inlet and condenser were added toluene (12.0 mL), \textit{Pd}_2(\textit{dba})_3 (104 mg, 0.114 mmol, 0.1 equiv.) and DPEphos (123 mg, 0.228 mmol, 0.2 equiv.), followed by bromopyridine 79 (400 mg, 1.14 mmol, 1.0 equiv.), thioether 4 (735 mg, 1.63 mmol, 1.4 equiv.) and \textit{KO}_{tBu} (183 mg, 1.63 mmol, 1.4 equiv.). The resulting mixture was purged with argon before being plunged into a preheated block maintained at 125 °C. The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was filtered through a pad of Celite and concentrated \textit{in vacuo}. Flash column chromatography purification (0 \rightarrow 50\% \text{Et}_2\text{O/DCM}) of the resulting green oil afforded the title compound as a light orange solid (498 mg, 83\%), mp 74-75 °C. \\(\nu_{\text{max}}/\text{cm}^{-1}\) 3080, 2932, 2837, 1590, 1478, 1466. \textit{\textit{H}} NMR (600 MHz, CDCl\textsubscript{3}) \(\delta_H\) 8.91 (1H, d, \(J = 2.0\), H22), 8.41 (1H, d, \(J = 2.2\), H20), 7.94 (1H, t, \(J = 2.2\), H24), 7.54 (1H, dd, \(J = 10.2\), 7.3, H30 or H33), 7.40 (1H, d, \(J = 7.9\), H15), 7.37 (1H, d, \(J = 9.8\), 6.9, H30 or H33), 7.22 (1H, d, \(J = 2.2\), H12), 7.16 (1H, dd, \(J = 7.9\), 2.2, H14), 6.47 (2H, d, \(J = 2.3\), H2 and H4), 6.41 (1H, t, \(J = 2.3\), H6), 3.74 (6H, s, H10 and H11), 3.40 (1H, tt, \(J = 6.9\), 3.9, H36), 2.39 (3H, s, H13) 1.12-1.08 (2H, m, H37 or H38), 0.72-0.70 (2H, m, H37 or H38). \textit{\textit{13C}} NMR (150 MHz, CDCl\textsubscript{3}) \(\delta_C\) 160.4 (C), 152.0 (C), 150.9 (CH), 149.5-149.1 (dd, \(J = 19.5\), 14.5, C), 147.8-147.5 (dd, \(J = 18.1\), 15.3, C), 147.0 (CH), 145.1 (CH), 142.5 (C), 139.1 (C), 137.9 & 137.8 (d, \(J = 9.8\), C), 136.9 (CH), 135.1 (C), 134.3 (CH), 132.6 (d, \(J = 10.4\), C), 131.9 (CH), 129.5 (CH), 129.4 (C), 127.7 (C), 126.5 (C), 107.6 (CH), 107.6 & 107.4 (d, \(J = 19.7\), CH), 99.7 (CH), 99.1 & 99.0 (d, \(J = 23.0\), CH), 55.5 (CH\textsubscript{3}), 26.5 (CH), 21.2 (CH\textsubscript{3}), 9.0 (CH\textsubscript{2}). \textit{\textit{19F}} NMR (282 MHz, CDCl\textsubscript{3}) \(\delta_F\) -140.0 & -140.1 (1F, d, \(J = 21.0\), -142.6 & -142.7 (1F, d, \(J = 21.0\)). HRMS (ESI) Calculated for C\textsubscript{30}H\textsubscript{26}F\textsubscript{2}N\textsubscript{3}O\textsubscript{2}S [M+H]\textsuperscript{+}:
530.1714; found: 530.1709.
To a round-bottom flask were added thioether 82 (50 mg, 0.0944 mmol, 1.0 equiv.), NCS (12.6 mg, 0.0944 mmol, 1.0 equiv.) in MeCN (2.0 mL), followed by TfOH (25.0 µL, 0.285 mmol, 3.0 equiv.). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo and the resulting yellow residue dissolved in DCM, washed with a 2 M NaOH solution, then H₂O, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 10% MeOH/DCM) afforded the title compound as a light yellow solid (42.0 mg, 66%), mp 138-140 °C. ν_max/cm⁻¹ 3057, 2931, 1591, 1468, 1411. ¹H NMR (600 MHz, CDCl₃) δ_H 9.38 (1H, d, J 2.1, H22), 9.30 (1H, t, J 2.1, H24), 8.39 (1H, d, J 8.3, H11), 8.21 (1H, d, J 2.1, H20), 8.01 (1H, d, J 2.0, H8), 7.54 (1H, dd, J 10.1, 7.1, H30 or H33), 7.47-7.42 (2H, m, H30 or H33 and H10), 7.36 (1H, d, J 2.1, H2), 6.58 (1H, d, J 2.1, H6), 4.07 (3H, s, H15), 3.97 (1H, tt, J 6.9, 3.8, H36), 3.91 (3H, s, H17), 2.56 (3H, s, H13), 1.44-1.39 (1H, m, H37 or H38), 1.34-1.29 (1H, m, H37 or H38), 0.81-0.77 (1H, m, H37 or H38), 0.65-0.60 (1H, m, H37 or H38). ¹³C NMR (150 MHz, CDCl₃) δ_C 168.2 (C), 158.6 (C), 154.4 (CH), 149.8-147.8 (2C) 148.1 (CH), 146.2 (C), 142.8 (C), 139.5 (CH), 138.8 (C), 137.8 & 137.7 (d, J 11.0, C), 133.2 (CH), 132.9 & 132.8 (d, J 11.0, C), 129.6 (CH), 129.3 (C), 128.5 (C), 125.4 (C), 125.2 (CH), 121.9 (C), 107.6 & 107.5 (d, J 19.7, CH), 106.2 (C), 101.4 (CH), 100.7 (CH), 99.7 & 99.5 (d, J 23.0, CH), 57.2 (CH₃), 57.0 (CH₃), 26.6 (CH), 21.9 (CH₃), 9.5 (CH₂), 8.9 (CH₂). ¹⁹F NMR (282 MHz, CDCl₃) δ_F -78.4, -138.5 & -138.6 (1F, d, J 20.3), -141.8 & -141.9 (1F, d, J 20.3). HRMS (Cl) Calculated for C₃₀H₂₆F₂N₃O₂S [M]⁺: 

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528.1552; found: 528.1553.
A regioisomer of 76 was isolated during flash column chromatography purification (slow eluting component) as a bright yellow solid. $^1$H NMR (600 MHz, CDCl$_3$) $\delta_H$ 7.79 (1H, dd, $J$ 11.1, 6.2, H6), 6.80 (1H, dd, $J$ 13.3, 7.1, H3), 5.11 (1H, bs, H8), 2.56-2.52 (1H, m, H11), 0.94-0.91 (2H, m, H12 or H13), 0.67-0.64 (2H, m, H12 or H13). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta_C$ 155.8 & 154.1 (d, $J$ 261.6, C), 145.7 & 144.1 (d, $J$ 238.5, C), 144.5 & 144.4 (d, $J$ 25.2, C), 125.1 & 125.0 (d, $J$ 6.7, C), 112.1 & 112.0 (d, $J$ 26.0, CH), 100.2 & 100.0 (d, $J$ 23.8, CH), 24.5 (CH), 7.69 (CH$_2$). $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta_F$ -116.8 & -116.9 (1F, d, $J$ 13.0), -140.2 & -140.3 (1F, d, $J$ 13.0). HRMS (ESI) Calculated for C$_9$H$_9$F$_2$N$_2$O$_2$ [M+H]$^+$: 215.0627; found: 215.0616.
To a stirring solution of diamine 77 (300 mg, 1.63 mmol, 1.0 equiv.) in DCM (8 mL) were added 5-bromonicotinic acid (362 mg, 1.79 mmol, 1.1 equiv.), HATU (685 mg, 1.80 mmol, 1.1 equiv.), HOBt (242 mg, 1.79 mmol, 1.1 equiv.) and DIPEA (1.14 mL, 6.55 mmol, 4.0 equiv.). The resulting dark red solution was stirred at room temperature for 2 h. It was then diluted with DCM, washed with H₂O and brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 30% Et₂O/DCM) afforded the title compound as an off-white solid (391 mg, 65%), mp 183-185 °C (dec). νmax/cm⁻¹: 3265, 3021, 1643, 1518, 1421. 

¹H NMR (600 MHz, CDCl₃) δH: 8.98 (1H, d, J 2.1, H17), 8.87 (1H, d, J 2.1, H19), 8.37 (1H, t, J 2.1, H21), 7.54 (1H, bs, H13), 7.30 (1H, dd, J 11.2, 8.6, H3 or H6), 7.04 (1H, dd, J 11.8, 7.8, H3 or H6), 4.20 (1H, bs, H9), 2.45 (1H, m, H10), 0.80-0.78 (2H, m, H11 and H12), 0.53-0.50 (2H, m, H11 and H12). 

¹³C NMR (150 MHz, CDCl₃) δC: 162.9 (C), 154.2 (CH), 154.0 (C), 148.9 (CH), 140.2 & 140.1 (d, J 6.0, C), 138.3 (CH), 130.9 (CH), 121.4 (CH), 118.2 & 118.1 (d, J 6.6, C), 118.0 (C), 114.6-114.5 (dd, J 19.0, 1.1, CH), 103.6-103.5 (dd, J 20.0, 1.7, CH), 25.8 (CH), 7.51 (CH₂). 

¹⁹F NMR (282 MHz, CDCl₃) δF: -137.4 & -137.5 (d, J 23.0), -149.3 & -149.4 (d, J 23.0). Calculated for C₁₅H₁₃BrF₂N₃O [M+H]⁺: 368.0210; found: 368.0203.
To a stirring solution of diamine 77 (70.0 mg, 0.380 mmol, 1.0 equiv.) in DCM (2.0 mL) were added 5-fluoronicotinic acid (59.0 mg, 0.418 mmol, 1.1 equiv.), HATU (160 mg, 0.421 mmol, 1.1 equiv.), HOBt (57.0 mg, 0.422 mmol, 1.1 equiv.) and DIPEA (265 µL, 1.52 mmol, 4.0 equiv.). The resulting black solution was stirred at room temperature for 17 h. The reaction mixture was then diluted with DCM, washed with H₂O and brine, dried over MgSO₄ and concentrated in vacuo. Flash column chromatography purification (0 → 30% Et₂O/DCM) afforded the title compound as an off-white solid (76.0 mg, 65%), 145-147 °C. \( \nu_{\text{max}} / \text{cm}^{-1} \) 3266, 1647, 1526, 1512, 1425. \(^1\)H NMR (600 MHz, CDCl₃) \( \delta_H \) 8.90 (1H, bs, H17), 8.68 (1H, d, \( J \) 2.7, H19), 7.97 (1H, dd, \( J \) 8.5, 2.2, H21), 7.57 (1H, bs, H13), 7.33 (1H, dd, \( J \) 11.1, 7.9, H3 or H6), 7.04 (1H, dd, \( J \) 12.5, 7.6, H3 or H6), 4.20 (1H, bs, H9), 2.46 (1H, m, H10), 0.81-0.79 (2H, m, H11 and H12), 0.53-0.51 (2H, m, H11 and H12). \(^{13}\)C NMR (150 MHz, CDCl₃) \( \delta_C \) 162.9 (C), 160.4 & 158.7 (d, \( J \) 262, C), 143.3 (d, \( J \) 3.9, CH), 141.9 & 141.8 (d, \( J \) 22.5, CH), 140.1 (C), 140.0 (dd, \( J \) 2.8, 1.1, C), 131.2 (d, \( J \) 3.9, C), 122.8 & 122.6 (d, \( J \) 18.8, CH), 118.3 (C), 114.5 & 114.4 (d, \( J \) 21.6, CH), 103.7 & 103.5 (d, \( J \) 21.6, CH), 25.8 (CH), 7.5 (CH₂). \(^{19}\)F NMR (282 MHz, CDCl₃) \( \delta_F \) -124.9, -138.9 & -139.0 (d, \( J \) 23.0), -139.6 & -139.7 (d, \( J \) 23.0). HRMS (ESI) Calculated for C₁₅H₁₃F₃N₃O [M+H]⁺: 308.1011; found: 308.0644.
13.13  *N*-Chlorosulfonamides as Reagents for Cyclisation

*N*-Chloro-*N*-methylbenzenesulfonamide 38

To a stirring solution of technical grade calcium hypochlorite (65 ww%, 167 mg, 0.762 mmol, 1.3 equiv.) and alumina (300 mg, 2.94 mmol, 5.0 equiv.) in CHCl$_3$ (1.5 mL) at 40 °C was added *N*-methylbenzenesulfonamide (100 mg, 0.584 mmol, 1.0 equiv.). The resulting suspension was stirred at 40 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with CHCl$_3$, filtered and concentrated *in vacuo*. Flash column chromatography purification (0 → 40% Et$_2$O/PE) afforded the title compound as a white solid (112 mg, 93%). Data in agreement with literature$^{149}$.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$H 7.96 (2H, dd, 8.3, 1.3, H4 and H6), 7.74 (1H, tt, $J$ 7.6, 1.3, H2), 7.63 (2H, dd, $J$ 8.3, 7.6, H1 and H3), 3.12 (3H, s, H11).

$^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$C 134.5 (CH), 131.6 (C), 130.0 (CH), 129.2 (CH), 45.5 (CH$_3$).
To a stirring solution of technical grade calcium hypochlorite (65 ww%, 772 mg, 3.51 mmol, 1.3 equiv.) and alumina (1.40 g, 13.7 mmol, 5.0 equiv.) in CHCl₃ (7.0 mL) at 40 °C was added N,N₄-dimethylbenzenesulfonamide (500 mg, 2.70 mmol, 1.0 equiv.). The resulting suspension was stirred at 40 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with CHCl₃, filtered and concentrated in vacuo. Flash column chromatography purification (50% Et₂O/PE) afforded the title compound as a white solid (512 mg, 88%). Data in agreement with literature[148].

^1H NMR (600 MHz, CDCl₃) δH 7.84 (2H, d, J 8.3, H₄ and H₆), 7.41 (2H, d, J 8.3, H₁ and H₃), 3.11 (3H, s, H₇), 2.49 (3H, s, H₁₃). ^13C NMR (150 MHz, CDCl₃) δC 145.7 (C), 130.0 (CH), 129.8 (CH), 128.6 (C), 45.5 (CH₃), 21.9 (CH₃).
To a stirring solution of technical grade calcium hypochlorite (65 ww%, 403 mg, 1.83 mmol, 1.3 equiv.) and alumina (719 mg, 7.05 mmol, 5.0 equiv.) in CHCl₃ (3.5 mL) at 40 °C was added N-(tert-butyl)benzenesulfonamide (300 mg, 1.41 mmol, 1.0 equiv.). The resulting suspension was stirred at 40 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with CHCl₃, filtered and concentrated in vacuo. Flash column chromatography purification (50% Et₂O/PE) afforded the title compound as a colourless oil (326 mg, 93%). Data in agreement with literature. ¹H NMR (600 MHz, CDCl₃) δH 7.99 (2H, dd, J 8.5, 1.3, H4 and H6), 7.65 (1H, tt, J 7.8, 1.3, H2), 7.55 (2H, dd, J 8.5, 7.8, H1 and H3), 1.40 (9H, s, H13, H14 and H15). ¹³C NMR (150 MHz, CDCl₃) δC 138.5 (C), 133.7 (CH), 129.0 (CH), 129.0 (CH), 68.3 (C), 29.3 (CH₃).
13.14 Miscellaneous Compounds

(1,10-Phenanthroline)silver trifluoromethanesulfonate

\[
\text{N} \quad \text{N} \quad \text{Ag} \quad \text{OTf}
\]

To a round-bottom flask were added MeOH (7.0 mL), 10-phenanthroline (200 mg, 1.11 mmol, 1.0 equiv.) and a solution of AgOTf (285 mg, 1.11 mmol, 1.0 equiv.) H2O (7.0 mL). The resulting suspension was stirred for 15 min at room temperature. The title compound was isolated by filtration, washed with cold MeOH and dried over P2O5 overnight (286 mg, 55%).

*tert*-Butyl hypochlorite

To an ice-cooled commercial bleach solution (< 5% chlorine content, 40 mL) in a round-bottom flask were added *t*-butanol (176 µL, 18.4 mmol, 1.0 equiv.) and glacial acetic acid (104 µL, 18.4 mmol, 1.0 equiv.). The reaction mixture was stirred in darkness at 0 °C for 3 min. The mixture was washed with a 10% solution of K2CO3, H2O and brine. The resulting yellow oil was stored over CaCl2 in the freezer for a short period of time.
13.15 Calibration Curve for Sulfonium Salt 7

This calibration curve was prepared in order to determine the concentration of sulfonium salt 7 in cyclisation reactions performed in Chapter 5 Table 5.1 (page 66).

![Calibration curve for sulfonium salt 7](image)

**Figure 13.1:** Calibration curve for sulfonium salt 7.
References


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