The Development of a Simple Process for Producing Medicinal Diagnostic $^{18}\text{F}$ Agents for Molecular Imaging using Positron-Emission-Tomography

Amy Clare Topley

Thesis for the degree of Doctor of Philosophy

June 2010
THE DEVELOPMENT OF A SIMPLE PROCESS FOR PRODUCING MEDICINAL DIAGNOSTIC $^{18}$F AGENTS FOR MOLECULAR IMAGING USING POSITRON-EMISSION-TOMOGRAPHY

By Amy Clare Topley

A range of resin bound sulfonate ester linkers were developed for use in the resin-linker-vector (RLV) approach for the synthesis of $[^{18}$F]-fluoride radiotracers for use as imaging agents in positron-emission-tomography (PET). The RLV strategy immobilises a precursor to the desired radiotracer on a solid support which is cleaved on exposure to $[^{18}$F]-fluoride ion to give the $^{18}$F-labelled radiotracer in solution. The RLV methodology allows for easier purification of the $^{18}$F-labelled radiotracer as a simple filtration step removes the unreacted starting material and the cleaved resin.

Synthetic routes to a 4-alkylphenylsulfonate linker and a 4-nitrophenylsulfonate linker were developed and these were shown to work as desired as part of the RLV construct in $[^{19}$F]-fluoridolysis reactions. $[^{18}$F]-Fluoridolysis reactions using the 4-alkylphenylsulfonate linker gave the desired $^{18}$F-labelled product in excellent radiochemical purity. The RLV strategy with this linker type was applied in a new synthetic route to $O$-(2-$[^{18}$F]-fluoroethyl)-L-tyrosine.

A fluorinated analogue of Alzheimer’s disease drug (−)-galanthamine was synthesised with complete stereocontrol as a potential new imaging agent for use in PET.
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Declaration

I declare that the work presented in this thesis is of my own composition and has been generated by me as a result of my own original research while in candidature at this university for the degree of Doctor of Philosophy, with exception of the radiochemistry experiments which were carried out by Imtiaz Khan of GE Healthcare and are acknowledged. Where I have consulted the published work of others, this is clearly attributed. Where I have quoted from the work of others, the source is always given and with exception of such quotations this thesis is entirely my own work. No part of this work part has previously been submitted for a degree or other qualification, or has been published before submission.

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Amy Clare Topley, June 2010
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Many thanks to GE Healthcare for providing funding for this project, with special thanks to Duncan Wynn for his assistance as my industrial supervisor and Imtiaz Khan for carrying out the radiochemistry experiments.

A big thank you to Richard for his supervision of the project, who provided endless ideas and enlightenment as well as boundless enthusiasm through the challenging times.

I would like to thank my parents and my sister for their unwavering support throughout my seemingly endless education.

Finally, my greatest thanks go to Alex, without whose love and support, especially in the last few months, this would not have been possible.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tr>
<td>ADDP</td>
<td>1,1’-(Azodicarbonyl) dipiperidine</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetate</td>
</tr>
<tr>
<td>apt</td>
<td>Apparent</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>BBN</td>
<td>Borabicyclo[3.3.1]nonane</td>
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<tr>
<td>BPB</td>
<td>(S)-[N-2-(N’-Benzylprolyl)amino]benzophenone</td>
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<td>BTEAC</td>
<td>Benzyltriethylammonium chloride</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyl carbonate</td>
</tr>
<tr>
<td>BP</td>
<td>Boiling point</td>
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<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoate</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionisation (mass spectrosopy)</td>
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<td>Cyclopentadieneyl ring</td>
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<tr>
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<td>diethylamine sulfurtrifluoride</td>
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<tr>
<td>DBU</td>
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<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
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<td>DIBAL-H</td>
<td>Diisobutyl aluminium hydride</td>
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<td>DIC</td>
<td>1,3-Diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
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<tr>
<td>DMAC</td>
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<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
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<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
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</tr>
<tr>
<td>dppp</td>
<td>1,3-Bis(diphenylphosphino)propane</td>
</tr>
<tr>
<td>ee</td>
<td>Enantiomeric excess</td>
</tr>
<tr>
<td>er</td>
<td>Enantiomeric ratio</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact (mass spectrosopy)</td>
</tr>
<tr>
<td>eq</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ES</td>
<td>Electrospray (mass spectrosopy)</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>FSPE</td>
<td>Fluorous solid phase extraction</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectroscopy</td>
</tr>
<tr>
<td>i</td>
<td>Iso</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>MAS</td>
<td>Magic Angle Spinning (NMR spectroscopy)</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
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<tr>
<td>mL</td>
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<tr>
<td>MP</td>
<td>Melting point</td>
</tr>
<tr>
<td>MS</td>
<td>Molecular sieves</td>
</tr>
<tr>
<td>MTPA</td>
<td>α-Methoxy-α-trifluoromethyl phenyl acetic acid</td>
</tr>
<tr>
<td>Mw</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>NaHMDS</td>
<td>Sodium hexamethyldisilazide</td>
</tr>
<tr>
<td>NMO</td>
<td>N-Methyl morpholine-N-oxide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>p</td>
<td>Para</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PFP</td>
<td>Pentafluorophenyl</td>
</tr>
<tr>
<td>PMA</td>
<td>Phosphomolybdic acid</td>
</tr>
<tr>
<td>Pr</td>
<td>Propyl</td>
</tr>
<tr>
<td>pyr</td>
<td>Pyridine</td>
</tr>
<tr>
<td>quant</td>
<td>Quantitative</td>
</tr>
<tr>
<td>RCM</td>
<td>Ring Closing Metathesis</td>
</tr>
<tr>
<td>RLV</td>
<td>Resin-Linker-Vector</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>sat</td>
<td>Saturated</td>
</tr>
<tr>
<td>sol</td>
<td>Solution</td>
</tr>
<tr>
<td>t</td>
<td>Tertiary</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>TADDOL</td>
<td>Tartaric Acid Derived Diol</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-Butyl dimethyl silyl</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethane sulfonate</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>Ts</td>
<td>para-Toluene sulfonil</td>
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Chapter 1 – Introduction

Positron-emission-tomography (PET) is a nuclear imaging technique which is used to produce three-dimensional images to map functional processes in vivo and has both medical and research applications. The work of this project involved the development of a solid phase approach to the synthesis of $[^{18}\text{F}]$-fluoride imaging agents for medicinal diagnostic applications using PET. This chapter is composed of a general overview of PET as an imaging technique and the $^{18}\text{F}$-labelled radiopharmaceuticals used in PET, as well as covering the background to this work and the scope of the project. Further introductory material for more specific areas of the project can be found in the relevant chapters.

To ensure the reader is familiar with the radiochemical terminology used in this report the common radiochemical terms are defined at this juncture.

**Specific activity** – The activity of a radioactive element (the number of decays per unit time) divided by the mass of the material in which it exists. It is used to describe the amount of a sample that is the radioactive element and non-radioactive element, high specific activity meaning a high proportion of $^{18}\text{F}$ with a low proportion of $^{19}\text{F}$, for example.

**Incorporation** – Calculated from the integration of the radioactive HPLC trace of a sample and in this work it is used describes the percentage of total $^{18}\text{F}$ present that is part of the desired product. The rest of $^{18}\text{F}$ could be present as unreacted $[^{18}\text{F}]$-fluoride or as other $^{18}\text{F}$-containing products.

**Radiochemical yield** – The yield of the radiochemical product of a reaction. This is calculated from the starting $^{18}\text{F}$ activity and the product activity and can be quoted as either non decay corrected (calculated directly from the starting $^{18}\text{F}$ activity and the product activity measurements) or decay corrected (where the product activity is corrected based on the time elapsed since the starting activity was measured and the product isolated and the half-life of the radioactive element). The decay corrected yield will always be higher than the non decay corrected yield.
Radiochemical purity – The amount of desired radiochemical product compared to any other radiochemical products formed in the reaction. High radiochemical purity would be the result of the formation of the desired radiochemical product only.

1.1 History of PET
The use of positrons in medicinal applications was first reported in 1951 when a simple brain probe was developed to localise brain tumours.¹ The first PET tomograph was built in 1973 by Phelps and co-workers who carried out studies to establish the principles of PET and were the first to image blood flow and metabolism in animals.² The following year, the first PET tomograph for studies with human subjects was constructed.³⁴ It was made up of a hexagonal array of 48 sodium iodide (NaI(T1)) detectors and it was used to produce the first human PET images of blood flow, oxygen and glucose metabolism and [¹⁸F]-fluoride bone scans. The first commercial PET scanner became available only a few years later and so provided the means for PET to be established in research programs worldwide. This tomograph, trade named ECAT II (which stood for Emission Computed Axial Tomograph), was made up of 96 NaI(T1) crystal detectors and sold for approximately US$600,000 in 1978.¹ The late 1970s saw the development of the scintillation material used in the detectors and bismuth-germanate (BGO) replaced NaI(T1) as the material of choice. The mid-eighties saw the development of radio-pharmaceutical delivery systems (RDS) which incorporated mini-cyclotrons and allowed the automated synthesis of the radiotracers. In 1991, Phelps and co-workers obtained the first whole-body oncology images⁵ which could be used to detect primary and metastatic disease, differentiate benign from malignant lesions and access therapeutic responses by being able to image all of the organs of the body in a single examination.¹ Developments in PET are ongoing with tomographs in the early 2000s typically having in the order of 120,000 detectors, now made of lutetium oxyorthosilicate (LSO). PET imaging is now a well established and important diagnostic tool in modern medicine.

1.2 The principles of PET
PET imaging involves a radioactive tracer molecule or radiotracer which is usually a small, biologically active compound that contains a positron-emitting radionuclide. The
A radiotracer is injected into the bloodstream of the living subject and after a short time the active molecule becomes concentrated in the tissues of interest and can be detected by an imaging scanner. The radionuclide undergoes positron decay (also known as positive beta decay) and emits a positron, which is the antimatter component to an electron. When this positron collides with an electron in the surrounding tissue it forms a positronium which promptly annihi lates producing two γ-rays (photons). These γ-rays have identical energies (511 keV$^6$) but travel in opposite directions and are detected by an external imaging device (Figure 1.1). The detector is made of a scintillator material (NaI(T1) or LSO) which absorbs the γ-rays to produce bursts of light which are detected by photomultiplier tubes and the data is then processed to give an image of the subject.


The imaging device must completely surround the subject, either as a single ring of detectors (for two-dimensional images) or multiple rings effectively making a cylinder of detectors (for three-dimensional images), as the technique depends on the simultaneous detection of both γ-rays of the pair. Those not arriving within a few nanoseconds of each other are ignored when the data is processed.

Single photon emission computerised tomography (SPECT) is an imaging technique related to PET which similarly uses a radiotracer and the detection of γ-rays to produce an image of the subject. In contrast to PET, the radiotracers used in SPECT emit γ-
radiation directly, which is then detected by a γ-camera that is rotated around the subject. Although PET gives higher resolution images, SPECT is a simpler, cheaper and more widely available technique that can be applied to clinical populations more easily than PET.7

1.3 Radionuclides

The radionuclides used in PET and SPECT are typically isotopes with short half lives so as to limit the exposure to radiation of the patient. The radionuclide is incorporated into the radiotracer which is either a compound normally used by the body (water, ammonia, glucose or a glucose analogue) or a biologically active molecule which binds to a drug receptor. The radionuclides most commonly used in SPECT are $^{99m}$Tc, $^{123}$I and $^{133}$Xe although these can be hard to incorporate into compounds that behave in a natural biochemical fashion in the body.7 Radiotracers for use in PET most commonly contain $^{11}$C, $^{13}$N, $^{15}$O or $^{18}$F although the use of $^{64}$Cu, $^{68}$Ga, $^{86}$Y and $^{124}$I is becoming increasingly prevalent.8 This list is by no means exhaustive and many other isotopes are currently under development, such as $^{74}$As.9 The four positron-emitting radioisotopes, $^{11}$C, $^{13}$N, $^{15}$O and $^{18}$F, are used more than any of the others because they can be easily substituted directly onto biomolecules. Substitution of the so called “elements of life” (carbon, nitrogen and oxygen) with their radioactive isotope is easy to achieve and does not significantly affect the properties of a molecule. Due to the steric similarities between a fluorine atom and an oxygen atom, $^{18}$F may be incorporated by the replacement of a hydroxyl group as it has a similar bond length to carbon and has similar polarity. The presence of the more electronegative fluorine atom may affect one or more aspect of the biological behaviour of the molecule (for example distribution, metabolism or protein binding) although this change may be advantageous resulting in a molecule with enhanced properties compared to the original compound. Since the van der Waals radius of the fluorine atom is similar to that of hydrogen (1.20 Å and 1.47 Å, respectively), $^{18}$F may also be substituted for a hydrogen atom with very little affect on the sterics of the molecule. Shown in Table 1.1 is data for the characteristics of the four most common PET radionuclides.
<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Half Life</th>
<th>Decay Mode</th>
<th>Maximum Energy</th>
<th>Maximum Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}$C</td>
<td>20.4 min</td>
<td>100% $\beta^+$</td>
<td>0.96 MeV</td>
<td>4.1 mm</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>9.98 min</td>
<td>100% $\beta^+$</td>
<td>1.19 MeV</td>
<td>5.4 mm</td>
</tr>
<tr>
<td>$^{15}$O</td>
<td>2.03 min</td>
<td>100% $\beta^+$</td>
<td>1.7 MeV</td>
<td>8.0 mm</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>109.8 min</td>
<td>97% $\beta^+$ 3% EC</td>
<td>0.69 MeV</td>
<td>2.4 mm</td>
</tr>
</tbody>
</table>

**Table 1.1** Characteristics of the four most common PET radionuclides. $^{10}$ $\beta^+$ = positron emission. EC = electron capture. Maximum range in water.

The comparatively short half lives of $^{13}$N and $^{15}$O pose problems in the synthesis of radiotracers containing these isotopes and so their use has been limited to the production of radiolabelled ammonia, oxygen, carbon dioxide, carbon monoxide and water. Due to the short half lives of $^{11}$C, $^{13}$N and $^{15}$O, it is normally necessary to produce these isotopes using a cyclotron (a compact particle accelerator) which is in very close proximity to the scanning facility. In general, a PET radiotracer needs to be synthesised, purified, analysed and formulated ready for injection into the subject before 2-3 half lives of the radionuclide have passed. $^{11}$ Radiotracers containing $^{11}$C are typically prepared in around 30 min to retain enough radioactivity for the PET scan and so the longer half life of $^{11}$C allows more time for the synthesis of radiolabelled compounds. The use of $^{18}$F in radiotracers is becoming increasingly popular due to the advantages in its characteristics over those of the other commonly used radionuclides. The significantly longer half life of $^{18}$F (over five times that of $^{11}$C) allows for a more complex synthesis of the radiotracer within the decay time of the radioisotope and permits the investigation of biological processes with slower kinetics as PET scans can be acquired over a few hours. $^{6}$ This also means that the radiotracers can be transported over considerable distances and so in-house cyclotrons at each scanning facility can be replaced with a central production centre covering many facilities. Upon decay, $^{18}$F emits a quite low energy positron (up to 0.69 MeV) which has a short path in vivo before its annihilation. The maximum range of a positron emitted from $^{18}$F is shorter than a positron emitted from $^{11}$C (which has a higher maximum energy) leading to superior resolution of the resulting PET image with a radiotracer containing $^{18}$F.

There are a number of nuclear reactions which can be used to produce $^{18}$F, $^{12}$ the most common of which is the proton irradiation of $[^{18}$O]-enriched water. $^{13}$ In this nuclear reaction ($^{18}$O(p,n)$^{18}$F, which stands for the bombardment of $^{18}$O with protons causing...
the ejection of a neutron to give $^{18}\text{F}$), the $^{18}\text{F}$ is obtained as a solution of [$^{18}\text{F}$]-fluoride in irradiated target water and is obtained “no-carrier-added” (NCA). This means that the ratio of [$^{18}\text{F}$]-fluoride to the total fluoride ion content is high and so the [$^{18}\text{F}$]-fluoride ion produced is said to have a very high specific radioactivity. The reaction is high yielding and can be carried out using small, compact cyclotrons to give several Curies of product from a single irradiation, of which only 5-15 mCi is required for injection into a human subject for a single PET examination. The other commonly used reaction, particularly for the production of electrophilic $^{18}\text{F}$, is the irradiation of neon gas ($^{20}\text{Ne}(d,\alpha)^{18}\text{F}$, which stands for the bombardment of $^{20}\text{Ne}$ with deuterium ions causing ejection of an $\alpha$-particle to give $^{18}\text{F}$). Although the yield obtained from this reaction is substantially lower than that of the previous method the natural abundance of the target material is advantageous.

### 1.4 Applications of PET

PET is seen as the most specific and sensitive means of quantitatively imaging molecular pathways and interactions in vivo as it is regarded as having a level of sensitivity that is unmatched by other imaging techniques. Hence, it is unsurprising that PET imaging is now routinely used in medicinal diagnostics both to locate and assess abnormalities in a wide range of medical fields and also to research physiological processes. PET is one of the techniques used in neurology to further the understanding of the pathophysiological mechanisms of neurological diseases and to aid the assessment of neurological patients. The areas of neurology which have been studied using PET include: the recovery mechanism after a stroke; the pathophysiological aspects of migraine headaches and epilepsy; Parkinson’s and Huntington’s disease; Alzheimer’s disease and other forms of dementia; and multiple sclerosis. In cardiology, PET has been used to image blood flow using [$^{13}\text{N}$]-ammonia and for investigating glucose metabolism using a $^{18}\text{F}$-labelled glucose analogue and also as an aid in cardiac surgery. PET is a very versatile and powerful imaging method in oncology where it is used to image tumours using $^{18}\text{F}$-labelled glucose analogues to investigate glucose metabolism and $^{18}\text{F}$-labelled steroids for imaging hormone-responsive cancers.
1.5 \(^{18}\text{F}\)-labelled radiopharmaceuticals for used in PET

A large number of radiotracers containing \(^{18}\text{F}\) for use in PET have been synthesised, which include \(^{18}\text{F}\)-labelled aromatic amino acids, phenethylamines, steroids, tropanes and antibodies as well as other small molecules.\(^{20}\) The chemical reactions used for the labelling of these organic molecules with \(^{18}\text{F}\) can be categorised as either electrophilic or nucleophilic, based on the form of \(^{18}\text{F}\) employed. Historically, electrophilic fluorination was used although as the field has developed the use of higher yielding, higher specific activity nucleophilic routes have dominated. The electrophilic fluorination uses \([^{18}\text{F}]-\text{F}_2\) in aliphatic or aromatic addition-elimination reactions, although the latter tend to be non-regioselective leading to a mixture of \([^{18}\text{F}]-\text{fluorinated}\) products. Electrophilic fluorination reactions also suffer from low radiochemical yield since the maximum achievable radiochemical yield is only 50% as only one of the two fluorine atoms in \([^{18}\text{F}]-\text{F}_2\) are incorporated into the product. An example of electrophilic fluorination is the synthesis of \([^{18}\text{F}]-5\)-fluorouracil (\([^{18}\text{F}]-\text{FU}\), Figure 1.1, Scheme 1) which is a radiolabelled analogue of the commonly used cancer chemotherapeutic 5-fluorouracil and was synthesised by the reaction of \([^{18}\text{F}]-\text{F}_2\) with uracil (1.01).\(^{21}\) The glucose analogue \([^{18}\text{F}]-2\)-fluoro-2-deoxy-\(\beta\)-glucose (\([^{18}\text{F}]-\text{FDG}\)) and the \(^{18}\text{F}\)-labelled phenylalanine analogue \(\text{L}-3,4\)-dihydroxy-6-[\(^{18}\text{F}\)]-fluorophenylalanine (\([^{18}\text{F}]-\text{FDOPA}\)) can both be synthesised by either electrophilic or nucleophilic methods (Figure 1.1, Schemes 2 and 3). The synthetic routes to \([^{18}\text{F}]-\text{FDG}\) are discussed in the next section of this chapter. An electrophilic fluorination approach to \([^{18}\text{F}]-\text{FDOPA}\) involved the fluorodestannylation of the trimethylstannyli precursor (1.02) followed by removal of the protecting groups and this pathway has been applied in an automated synthesis of the radiotracer.\(^{22}\) In the nucleophilic approach to \([^{18}\text{F}]-\text{FDOPA}, [^{18}\text{F}]-\text{fluoride}\) displaces the nitro group of nitroaldehyde 1.03 followed by reaction with 2-phenyl-5-oxazolone and hydrolysis to remove the protecting groups to give the product as a racemic mixture of the \(\text{L}\) and \(\text{D}\) forms. Chiral HPLC was then used to separate the enantiomers and the whole procedure was reported to take 100 min to complete.\(^{23}\)
The synthesis of $[^{18}F]$-FDG is discussed in detail in the next section of this chapter.

Figure 1.1 The fluorination step in the synthesis of some $[^{18}F]$-labelled radiotracers for use in PET. Reagents and conditions: a) $[^{18}F]$-F$_2$, TFA; b) (i) $[^{18}F]$-F$_2$, CFCl$_3$, (ii) HBr; c) (i) $[^{18}F]$-F$_2$, CFCl$_3$, (ii) 2-phenyl-5-oxazolone, DABCO, EtOH, (iii) AcOH, red phosphorus; d) (i) TBA$[^{18}F]$, DMSO, (ii) AlCl$_3$, LiAlH$_4$, (iii) EtSH, AlBr$_3$; e) $[^{18}F]$-F$_2$, TFA.
The nucleophilic reactions use $[^{18}\text{F}]$-fluoride ion to displace a leaving group on the precursor molecule. This is generally carried out in dipolar aprotic solvents and in the presence of a cationic counterion (typically a soft metal ion complexed by a cryptand such as Kryptofix [2.2.2]) to increase the reactivity of the $[^{18}\text{F}]$-fluoride ion. Nucleophilic fluorination was used in the synthesis of the $^{18}\text{F}$-labelled steroid 2-$[^{18}\text{F}]$-fluoroestradiol (2-$[^{18}\text{F}]$-FES, Figure 1.1, Scheme 4) from a precursor $(1.04)$ containing a quaternary ammonium salt as the leaving group.$^{19}$ The promising dopamine D$_2$ antagonist $[^{18}\text{F}]$-fallypride (Figure 1.1, Scheme 5) has also been synthesised using a nucleophilic fluorination reaction to displace an alkyl tosylate leaving group on the precursor $(1.05).^{24}$

1.6 $[^{18}\text{F}]$-2-Fluoro-2-deoxy-$\alpha$-glucose

Glucose analogue $[^{18}\text{F}]$-2-fluoro-2-deoxy-$\alpha$-glucose ($[^{18}\text{F}]$-FDG, Figure 1.1, Scheme 2) is by far the most widely used radiotracer in clinical use in PET. This is because it has been shown to be a multipurpose radiotracer with applications in a variety of diagnostic fields including neurology, cardiology and oncology.$^{16}$ For example, the use of $[^{18}\text{F}]$-FDG in conjunction with whole body PET scanners enables the detection of broad varieties of tumour tissue and its metastases at an early stage of the disease with a single scan of the whole body. This is due to the increased glucose metabolism in tumour cells and so the uptake of $[^{18}\text{F}]$-FDG is significantly higher in tumour tissue compared to “normal” tissue since the biochemical behaviour and the initial metabolism of glucose and $[^{18}\text{F}]$-FDG are very similar.

The first synthesis of $[^{18}\text{F}]$-FDG was published by Ido et al. in 1977$^{25,26}$ and the subsequent development of the synthetic route has been driven by the need for reliable, high yielding reactions which give the desired product in high purity with minimal preparation time and at low cost. This first synthesis of $[^{18}\text{F}]$-FDG was carried out by the addition of $[^{18}\text{F}]$-F$_2$ across the double bond of 3,4,6-tri-$O$-acetyl-$\alpha$-glucal $(1.06).$ This electrophilic fluorination reaction formed two isomers, the 1,2-difluoro-glucose isomer $(1.07)$ and the 1,2-difluoro-mannose isomer $(1.08),$ which were formed in a ratio of 3:1 (Scheme 1.1). Hydrolysis of isomer $1.07$ using hydrochloric acid gave the desired $[^{18}\text{F}]$-FDG in approximately 10% overall radiochemical yield.
**Scheme 1.1** The first published synthesis of $[^{18}F]$-FDG using electrophilic fluorination.$^{25}$ *Reagents and conditions:* a) $[^{18}F]$-F$_2$; b) HCl.

A modification to this route, published in 1982, improved the overall radiochemical yield to around 20% by the use of $^{18}$F-labelled acetyl hypofluorite ($\text{CH}_3\text{CO}_2^{18}$F, generated *in situ* from $^{18}$F-F$_2$ and ammonium acetate) as the electrophilic fluorinating reagent.$^{27}$ This reagent was also found to improve the selectivity of the fluorination reaction which was thought to be due to the larger size of acetyl hypofluorite relative to elemental fluorine, resulting in the selective addition to the less hindered side of the molecule.$^{27}$ The highest selectivity for the fluorination reaction was achieved through the reaction of 3,4,6-tri-$O$-acetyl-$\alpha$-glucal (1.06) with $\text{CH}_3\text{CO}_2^{18}$F in CFCl$_3$ which gave a mixture of $[^{18}F]$-FDG and $[^{18}F]$-FDM in a ratio of 95:5.$^{28}$

**Scheme 1.2** Synthesis of $[^{18}F]$-FDG by nucleophilic fluorination.$^{29}$ *Reagents and conditions:* a) [K/K2.2.2]$^{+}$ $^{18}$F$^{-}$, MeCN; b) HCl.

The low yields and limited stereoselectivity of the electrophilic fluorination addition reaction were overcome by the development of a synthetic route using nucleophilic fluorination and it is this method that is now routinely used in the majority of the world’s PET facilities.$^{16}$ This route utilises 1,3,4,6-tetra-$O$-acetyl-2-
trifluoromethanesulfonyl-β-α-manno-pyranose (1.09), which is prepared selectively in two steps from α-mannose, as the precursor (Scheme 1.2). The combination of the powerful triflate leaving group and the use of no-carrier-added [18F]-fluoride of very high specific activity leads to high yields for the fluorination reaction, giving fluoride 1.10 with a 95% incorporation of [18F]-fluoride. The precursor is combined with the aminopolyether potassium complex [K/K2.2.2] {+} 18F as a phase-transfer agent which generates a highly reactive [18F]-fluoride ion due to the masking of the potassium counterions with Kryptofix [2.2.2]. The cleavage of the protecting groups is carried out rapidly under mild conditions using hydrochloric acid as before. The radiochemical yield for this strategy is 60% (non-decay corrected) over the two steps which represents a significant improvement over the electrophilic fluorination approach.

Automated procedures for the production of [18F]-FDG have been developed to minimise the radiation risk during the synthesis and to improve the reliability and reproducibility of the fluorination reaction. The use of polymer-supported reagents in [18F]-fluorination reactions can be easily adapted for automated radiosynthesis and has the advantage of simplifying the purification of the final product. Two solid-phase strategies have been explored for use in the synthesis of [18F]-FDG, one approach traps the [18F]-fluoride ion on a polymer support with a weakly basic counterion and the other approach attaches the precursor to the polymer-support (Scheme 1.3).

The resin-supported [18F]-fluoride ion approach uses 4-aminopyridinium (1.11) or tris-(n-butyl)-phosphonium salts which are attached to a polymer support and are used for on-column fluorination. The quaternary salts immobilise the [18F]-fluoride ions (1.12) and act as the phase-transfer catalyst negating the need for Kryptofix [2.2.2], which can appear as a contaminant in the final product. This approach has been used to synthesise [18F]-FDG in radiochemical yields of >70% (Scheme 1.3). The alternative strategy was recently developed in collaboration between the Brown group at the University of Southampton and GE Healthcare in Amersham. This approach immobilised the precursor on a solid support which liberated protected [18F]-FDG into solution upon treatment with [18F]-fluoride ions. A perfluoroalkylsulfonate linker was used to attach the precursor to a solid support consisting of polystyrene resin beads. When this resin (1.13) was subjected to the normal conditions used in nucleophilic fluorination, [18F]-fluoride in the presence of Kryptofix [2.2.2], the [18F]-fluoride
cleaved the precursor from the solid support. This method has been used to obtain $[^{18}\text{F}]-\text{FDG}$ in excellent chemical purity and in an average radiochemical yield of 73% (decay corrected). Both of these strategies could be applied in the preparation of other $^{18}\text{F}$-labelled radiotracers.

Scheme 1.3 Solid-phase routes to $[^{18}\text{F}]-\text{FDG}$ using polymer-supported $[^{18}\text{F}]-\text{fluoride}$ ion$^{31}$ (top) or polymer supported precursor$^{33,34}$ (bottom). Reagents and conditions: a) (i) $^{18}\text{F}^-$/H$_2^{18}$O, (ii) MeCN; b) (i) 1,3,4,6-tetra-$O$-acetyl-2-trifluoromethanesulfonyl-$\beta$-D-manno-pyranose (1.09), MeCN, 100 °C, (ii) 1 N HCl, 100 °C; c) (i) [K/K2.2.2]+ $^{18}$F$^-$, MeCN, 86 °C, (ii) 6 M HCl, 125 °C.

1.7 Recent strategies for the production of $^{18}\text{F}$-labelled radiopharmaceuticals

In addition to the solid phase approaches developed for the synthesis of $[^{18}\text{F}]-\text{FDG}$ discussed previously, a couple of other strategies have been recently developed for the synthesis of $^{18}$F-labelled radiotracers. Bejot et al. reported the use of a fluorous tag attached to the precursor of the $^{18}$F-labelled radiotracer which was displaceable upon nucleophilic fluorination with $[^{18}\text{F}]-\text{fluoride}$ ion.$^{35}$ This novel application of fluorous chemistry paralleled the use of solid phase chemistry by Brown et al.$^{33,34}$ discussed earlier as the highly perfluorinated region on the fluorous tag allows for easy purification of the crude reaction mixtures using fluorous solid phase extraction (FSPE) in much the same way as the solid support allows easy purification by filtration.$^{35}$
When compared to solid phase chemistry, fluorous chemistry is seen as a solution phase synthesis technique and so has more favourable reaction kinetics than solid phase synthesis although the benefit of separation tagging is retained. The use of nucleophilic fluorination in the oxidative de-tagging of a molecule containing a fluorous tag and the subsequent FSPE purification was developed by Boldon et al. in the novel strategy for the synthesis of allylic fluorides from fluorous allylsilanes\(^ {36}\) and this approach was also used in the oxidative de-tagging of fluorous organosilanes to give alcohols.\(^ {37}\) This application of fluorous organosilanes was combined with the use of sulfonate leaving groups, which are generally employed in the synthesis of \(^{18}\)F-labelled radiotracers, to give a sulfonate leaving group with a fluorous tag made up of a polyfluorinated alkyl chain.\(^ {35}\)

![Scheme 1.4](image)

**Scheme 1.4** The fluorous synthesis of \([^{18}\text{F}\text{-}cis-4\text{-fluoro-}\text{l-}\text{proline}}\).\(^ {35}\) *Reagents and conditions:* a) (i) \([K/K2.2.2]^+\) \(^{18}\text{F}^−\), MeCN, 100-120 °C, (ii) FSPE, (iii) TfOH, MeCN/H\(_2\)O.

This fluorous protocol, using nucleophilic fluorination for the removal of a fluorous tag, was applied in the synthesis of \([^{18}\text{F}\text{-}cis-4\text{-fluoro-}\text{l-}\text{proline}}\) (Scheme 1.4). Fluorous precursor 1.14 was subjected to the normal conditions used in nucleophilic fluorination, \([^{18}\text{F}]\text{-fluoride}\) in the presence of Kryptofix [2.2.2], and the \([^{18}\text{F}]\text{-fluoride\,ion}\) removed the fluorous tag whilst fluorinating the precursor. Purification using FSPE separated the unreacted fluorous precursor 1.14 and the cleaved fluorous tag from the reaction mixture and deprotection with triflic acid afforded \([^{18}\text{F}\text{-}cis-4\text{-fluoro-}\text{l-}\text{proline}}\) in a radiochemical yield of 42%.\(^ {35}\) However, it was found that the presence of the fluorous tag caused a decrease in the specific activity of the product compared to a non-fluorous precursor, possibly due to leaching of \([^{19}\text{F}]\text{-fluoride\,ion}\) from the tag.
There is a continuing drive to broaden the spectrum of molecular scaffolds that can be used as $^{18}$F-labelled radiotracers for imaging with PET. Some substrates are incompatible with $[^{18}F]$-fluoride ion most commonly used in the labelled step making it necessary to separate the $^{18}$F-C bond formation step from the molecule to be labelled.\(^{38}\)

The approach recently developed for this strategy is the synthesis of $^{18}$F-labelled reagents that chemoselectively bind to substrates under mild conditions and the use of “click labelling” in this application has rapidly grown in popularity in recent years. “Click” chemistry is the term used for the 1,3-dipolar cycloaddition reaction of a terminal alkyne with a substituted azide, first discovered by Michael in 1893,\(^{39}\) and developed in the early twenty-first century after the discovery of the use of copper(I) to catalyse the reaction (Scheme 1.5).\(^{40-42}\) “Click labelling” utilises click chemistry in the synthesis of $^{18}$F-labelled radiotracers by attaching a $^{18}$F-labelling reagent, containing an alkyne or azide functional group, to a substrate molecule with the opposite group. The 1,4-disubstituted 1,2,3-triazole formed in the cycloaddition reaction is stable under \textit{in vivo} conditions and can result in an improvement in the pharmacological properties of the resulting radiotracer.\(^{38}\)

\[
\begin{align*}
R^1\equiv + & \quad \begin{array}{c}
\varnothing \\
N=\equiv N
\end{array} & \overset{\text{Cu(I)}}{\longrightarrow} & N^{-} \equiv N^{-} \equiv N^{-} R^2
\end{align*}
\]

\textbf{Scheme 1.5} “Click” chemistry: the copper(I) catalysed 1,3-dipolar cycloaddition of substituted alkynes and azides.

A range of alkynes and azides have been radiolabelled for use as $^{18}$F-labelling reagents in click labelling and these have been shown to be easily reacted with the appropriate substrate to give the desired $^{18}$F-labelled small molecule (a selection of $^{18}$F-labelled small molecules and the $^{18}$F-labelling reagents used in their synthesis are shown in Figure 1.2). Among the $^{18}$F-labelled small molecules synthesised using click labelling are the glucose derivative \textbf{1.20} and $[^{18}F]$-FDG derivative \textbf{1.24}. Glucose derivative \textbf{1.20} was obtained from the reaction of a fluorinated alkyne \textbf{1.15}\(^{43}\) with a glucose molecule containing an azide functionality.\(^{44}\) Conversely, the synthesis of $[^{18}F]$-FDG derivative \textbf{1.24} was carried out using a protected $[^{18}F]$-FDG molecule containing an azide functional group (\textbf{1.19}) and a substituted glycine derivative.\(^{45}\)
Figure 1.2 A selection of $^{18}$F-labelling reagents for use in “click labelling” and some of the $^{18}$F-labelled small molecules obtained from the reaction with an appropriate substrate (a complete list of the $^{18}$F-labelling reagents and $^{18}$F-labelled small molecules published between 2006 and 2009 can be found in the review by Glaser et al.).$^{38}$

$^{18}$F-Labelled triazole 1.21 was synthesised from azide 1.16 and was used as a model compound to study the “click labelling” chemistry.$^{46}$ The purification of either of the $^{18}$F-alkyne or $^{18}$F-azide reagents is generally found not to be necessary and the synthesis of the $^{18}$F-labelled small molecules can be carried out by a simple one-pot chemistry technique, as shown in the synthesis of $^{18}$F-labelled triazole 1.22 from alkyne 1.17.$^{47}$
Azide 1.18 was used in the synthesis of $^{18}$F-labelled phenylalanine derivative 1.23 in a model reaction to investigate the click labelling of peptides. A variety of $^{18}$F-labelled peptides have also been synthesised by click labelling which has been shown to be a chemoselective and high-yielding coupling method that does not require the use of protecting groups making it advantageous over other $^{18}$F-labelling methods.38

1.8  Resin-linker-vector strategy

The approach used by Brown in collaboration with GE Healthcare for the synthesis of $[^{18}\text{F}]$-FDG from a solid-supported precursor (Scheme 1.3) utilised the resin-linker-vector (RLV) strategy. The idea behind the RLV strategy is that the precursor to the labelled product (known as the vector) is attached to a solid support via a linker of appropriate length and reactivity, forming a functionalised resin that makes up the RLV construct. When this resin is used in the fluorination reaction, $[^{18}\text{F}]$-fluoride ions cleave the vector molecule from the linker to give the $^{18}$F-labelled vector molecule in a solution containing a suspension of the resin. Since the unreacted precursor remains attached to the resin it can be easily removed from the reaction mixture, along with the spent resin, by a simple filtration to give pure $^{18}$F-labelled vector in solution and so making lengthy purification processes unnecessary (Figure 1.3).

Figure 1.3  Schematic of the resin-linker-vector (RLV) strategy.
The developed RLV strategy uses a sulfonate ester functionality for the attachment of the vector molecule to the linker portion. This can be easily constructed by the reaction of a sulfonyl chloride (or a shelf-stable alternative such as a pentafluorophenyl (PFP) sulfonate\textsuperscript{50}) on the end of the linker with a hydroxyl group on the vector molecule at the position where the $[\^{18}\text{F}]$-fluorine is required on the $^{18}\text{F}$-labelled vector. When reacted with a nucleophile, the linker part of RLV construct behaves as a good leaving group aiding the modifying cleavage reaction of the vector molecule.

Perfluoroalkylsulfonate linkers, like the one used in the Brown group synthesis of $[\^{18}\text{F}]$-FDG (section 1.6), have been used previously as traceless linkers in solid-phase synthesis to carry out simple transformations on small organic molecules. Pan \textit{et al.} used them as traceless linkers in a solid-phase approach to carry out the deoxygenation of phenols\textsuperscript{51} and for the carbon-carbon bond forming reaction in the synthesis of biaryls through Suzuki cleavage (Figure 1.4, Scheme 1).\textsuperscript{52} The use of traceless linkers in solid-phase synthesis is advantageous as the functional group present in the linkage is modified upon cleavage and so does not remain on the vector molecule.\textsuperscript{53} A wide range of solid-phase sulfonate linkers have been developed showing them to be very useful for the attachment and subsequent modifying cleavage of small molecules using nucleophiles – a few examples are discussed here. A tetrafluoroarylsulfonate linker that behaves like a triflate leaving group was developed simultaneously by two separate groups in 2004 and has also been shown to be of use as a traceless linker in deoxygenation and cross-coupling reactions (Figure 1.4, Scheme 2).\textsuperscript{54,55} An arylsulfonate linker, first used for the synthesis of arginine-containing peptides by a guanidine attachment to a solid support,\textsuperscript{56} was further developed for use in the preparation of amine libraries (Figure 1.4, Scheme 3).\textsuperscript{57} In this work the amine functionality was formed from an immobilised alkyl chain, attached \textit{via} a sulfonate ester linkage, by cleavage with amines, thiolates or imidazoles. Cyclic molecules can also be formed from solid-supported precursors as shown by Holte \textit{et al.} in their solid-phase synthesis of 3,5-disubstituted 1,3-oxazolidin-2-ones (Figure 1.4, Scheme 4).\textsuperscript{58} In this work the solid-phase linker acts as both a protecting group and an activated leaving group. The authors attached 1,2-diols to a solid support \textit{via} a sulfonate ester linkage to the primary alcohol functionality of the diol. This allowed the selective activation of the secondary alcohol by an on-resin reaction with $p$-toluenesulfonyl isocyanate and the subsequent cyclo-elimination then occurred with concurrent detachment from the resin.
Figure 1.4  Other applications of solid-supported sulfonate linkers. Reagents and conditions: a) R\textsubscript{1}C\textsubscript{6}H\textsubscript{4}OH, K\textsubscript{2}CO\textsubscript{3}, DMF\textsuperscript{51,52} b) Pd(OAc)\textsubscript{2}, dppp, Et\textsubscript{3}N, HCO\textsubscript{2}H, DMF\textsuperscript{51,54,55} c) R\textsuperscript{2}C\textsubscript{6}H\textsubscript{4}B(OH)\textsubscript{2}, PdCl\textsubscript{2}(dppf), Et\textsubscript{3}N, DMF\textsuperscript{52,55} or Pd(dppf), K\textsubscript{2}CO\textsubscript{3}, THF/H\textsubscript{2}O\textsuperscript{54} d) R\textsuperscript{1}C\textsubscript{6}H\textsubscript{4}OH, Et\textsubscript{3}N\textsuperscript{54} or i-Pr\textsubscript{2}NEt\textsuperscript{55}, CH\textsubscript{2}Cl\textsubscript{2} e) R\textsuperscript{3}ZnI, Ni(PPh\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}, PPh\textsubscript{3}, LiCl, THF\textsuperscript{54} f) R\textsuperscript{1}OH\textsuperscript{57} g) R\textsuperscript{3}R\textsuperscript{2}NH neat or in DMF, THF or MeCN h) R\textsuperscript{2}S\textsuperscript{-}Na\textsuperscript{+}, MeCN; i) Imidazole, MeCN j) RCH(OH)CH\textsubscript{2}OH, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}\textsuperscript{58} k) Tosyl isocyanate, CH\textsubscript{2}Cl\textsubscript{2} l) DBN, CH\textsubscript{2}Cl\textsubscript{2}. 
The use of enantiopure 1,2-diols in this solid phase system gave oxazolidinones of high enantiopurity.

1.9 Scope of this project

The objective of this project, which was sponsored by GE Healthcare, was to develop the synthesis of a range of linker types that mimic sulfonate leaving groups for use as part of the RLV construct. This would allow the RLV strategy to be applied in the synthesis of a variety of $^{18}$F-labelled radiotracers. This involved the synthesis of a selection of linker types which had differing reactivity so that the leaving group ability of the linker could be tuned for different vector molecules. The developed linkers were attached to a suitable vector molecule and immobilised on a solid support to give a collection of RLV constructs. Fluoridolysis to give the cleaved vector molecule was carried out for each of the RLV constructs synthesised to establish if the linker methodology was successful.

![Linker types](image)

**Figure 1.5** Linker types of interest for use as part of the RLV construct: 4-alkylphenylsulfonate (1.25), 4-alkoxyphenylsulfonate (1.26), 4-nitrophenylsulfonate (1.27) and alkylsulfonate (1.28). In each case, n denotes variation in the alkyl chain length of the linker.

In the first instance, we were interested in the development of a 4-alkylphenylsulfonate linker type which would mimic a toluene sulfonate leaving group (1.25, Figure 1.5). A number of other possible linker types that would mimic nosylate or mesylate leaving groups were also desired (1.26, 1.27 and 1.28) and solution model studies were carried
out to determine which of these linkers were worthy of investigation. Each linker contained a different sulfonate ester type, an alkyl chain spacer and appropriate functionality to allow the attachment to a solid support. The optimum length of the alkyl chain of the linkers (n) was determined by evaluation of the efficiency of fluoridolysis of the RLV construct. $[^{19}\text{F}]$-Fluoridolysis was carried out at The University of Southampton and $[^{18}\text{F}]$-fluoridolysis by scientists from GE Healthcare at The Grove Centre, Amersham. Small organic molecules were used as the vector molecule to establish that the linkers performed as desired and then the newly developed linker was applied in a novel synthetic pathway for the synthesis of a known radiotracer.
Chapter 2 – Linkers

This chapter covers the development of the synthesis of the 4-alkylphenylsulfonate linker. This was used to synthesise RLV constructs of varying linker lengths. Solution phase model studies determined that the second linker type to be developed was the 4-nitrophenylsulfonate linker and the synthesis of this linker is presented. RLV constructs of both linker types were synthesised with multiple vector molecules.

2.1 Synthesis of the 4-alkylphenylsulfonate linker

2.1.1 Development of linker synthesis

The 4-alkylphenylsulfonate linker was synthesised using 6-phenylhexanoic acid (2.01) as the starting material, which would produce a linker with an alkyl chain length of five methylene units. This commercially available starting material was chosen as it contained a carboxylic acid group for attachment to the solid support and a benzene ring which would permit the introduction of the sulfonyl chloride functionality, and thence the vector molecule.

![Scheme 2.1](image)

Scheme 2.1  Reagents and conditions: a) TMSCl, MeOH, 0 °C; b) i) ClSO2OH, CH2Cl2, 0 °C to rt, ii) AcCl, MeOH, CH2Cl2, 0 °C; c) Neopentyl alcohol, NaHMDS, THF.

The first step in the synthesis was the protection of 6-phenylhexanoic acid (2.01) to give methyl ester 2.02 which was carried out in quantitative yield using chlorotrimethylsilane in methanol following the procedure of Hanessina et al. (Scheme 2.1).59 This reaction was shown to proceed very cleanly by 1H NMR and so crude methyl ester 2.02 could be
used in subsequent reactions without further purification. Methyl ester 2.02 was converted directly into sulfonyl chloride 2.03 using excess chlorosulfonic acid following the procedure of Shirley et al. although the yield was only 45%. The use of an excess of chlorosulfonic acid in this reaction is important as the intermediate sulfonic acid can be prepared using the same reagent and is then converted to the chloride with the extra equivalents of the reagent. In an attempt to improve the yield of this reaction a different strategy was tested whereby methyl ester 2.02 was first transformed into the corresponding sulfonic acid using a single equivalent of chlorosulfonic acid or using concentrated sulfuric acid and then converted to sulfonyl chloride 2.03 using phosphorus pentachloride. The sulfonic acid was found to be very difficult to handle and so was not isolated before the second step and the crude reaction mixture was carried through. Although an overall yield of 19% of the desired sulfonyl chloride 2.03 was achieved over the two steps, this result was not an improvement on the original method.

On re-examination of the original reaction conditions it was found that the reaction had given 45% of the desired sulfonyl chloride 2.03 along with 30% of the desired product where the methyl ester had been hydrolysed to the free acid. Acetyl chloride in methanol had been used in the literature for carboxylate ester formation from the corresponding free acid and so a second step was added to the transformation whereby the crude reaction mixture from the first step was exposed to acetyl chloride and methanol. This converted the free acid by-product to the methyl ester and gave the desired sulfonyl chloride 2.03 in an improved 68% yield over two steps. Sulfonyl chloride 2.03 was then reacted with neopentyl alcohol to give sulfonate ester 2.04, albeit in very poor yield. This result showed the attachment of a vector molecule to this linker to be viable although it indicated that the conditions for the sulfonate ester formation needed some work.

Sulfonyl chloride 2.03 was then reacted with 3-phenyl propan-1-ol (2.05) which was chosen because it represented a more realistic example of vector molecule that could be investigated in the cleavage reaction with [19F]-fluoride. The reaction was carried out using varying equivalents of sulfonyl chloride 2.03 and alcohol 2.05 as well as a range of reagents and conditions (Table 2.1). Alcohol 2.05 and the desired product, sulfonate ester 2.06, were found to be extremely difficult to separate by chromatography (entry 3)
and so subsequent reactions were carried out using the alcohol as the limiting reagent. The best conditions were found to be entry 5 using sulfonyl chloride \textbf{2.03} (2 eq), alcohol \textbf{2.05} (1 eq) and triethylamine (2.5 eq) at reflux which gave the highest yield of product whilst limiting the amount of excess sulfonyl chloride \textbf{2.03} used. Attempts to further reduce the number of equivalents of sulfonyl chloride \textbf{2.03} used in the reaction gave either a decrease in yield (entry 6) or inseparable mixtures where the reaction had not gone to completion (entries 7 & 8).

![Reaction diagram]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sulfonyl chloride \textbf{2.03}</th>
<th>Alcohol \textbf{2.05}</th>
<th>Reagents and conditions</th>
<th>Yield of \textbf{2.06}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 eq</td>
<td>1 eq\textsuperscript{a}</td>
<td>Et\textsubscript{3}N/1 eq, 3.25 h then NaHMDS/1 eq, rt, 21 h</td>
<td>17%</td>
</tr>
<tr>
<td>2</td>
<td>1 eq</td>
<td>1.2 eq\textsuperscript{a}</td>
<td>Pyridine/4 eq, 20 h</td>
<td>32%</td>
</tr>
<tr>
<td>3</td>
<td>1 eq</td>
<td>1 eq\textsuperscript{a}</td>
<td>Pyridine/4 eq, DMAP/0.1 eq, rt</td>
<td>38 mg mixture of \textbf{2.05} and \textbf{2.06} plus 2% \textbf{2.03}</td>
</tr>
<tr>
<td>4</td>
<td>3 eq</td>
<td>1 eq\textsuperscript{c}</td>
<td>Et\textsubscript{3}N/2.5 eq 3 h then at 50 °C for 5.5 h</td>
<td>85% plus 40% of starting \textbf{2.03}</td>
</tr>
<tr>
<td>5</td>
<td>2 eq</td>
<td>1 eq\textsuperscript{b}</td>
<td>Et\textsubscript{3}N/2.5 eq at 50 °C for 7 h</td>
<td>82% plus 11% of starting \textbf{2.03}</td>
</tr>
<tr>
<td>6</td>
<td>1.5 eq</td>
<td>1 eq\textsuperscript{a}</td>
<td>Et\textsubscript{3}N/2.5 eq at 50 °C for 7.5 h</td>
<td>36% plus 15% of starting \textbf{2.03}</td>
</tr>
<tr>
<td>7</td>
<td>1.2 eq</td>
<td>1 eq\textsuperscript{b}</td>
<td>Et\textsubscript{3}N/2.5 eq at 50 °C for 24 h</td>
<td>13 mg mixture of \textbf{2.06} &amp; \textbf{2.03}</td>
</tr>
<tr>
<td>8</td>
<td>1 eq</td>
<td>1 eq\textsuperscript{b}</td>
<td>Et\textsubscript{3}N/2.5 eq at 50 °C for 7 h</td>
<td>60 mg mixture of \textbf{2.06} &amp; \textbf{2.05} plus 4% \textbf{2.06} and 3% of starting \textbf{2.03}</td>
</tr>
</tbody>
</table>

\textbf{Table 2.1} Effect of number of equivalents of starting materials and the reagents and conditions on the outcome of the sulfonate ester formation reaction.

\textsuperscript{a}Reaction carried out on a <25 mmol scale. \textsuperscript{b}Reaction carried out on a 0.25-0.50 mmol scale. \textsuperscript{c}Reaction carried out on a >0.50 mmol scale.
An enzyme mediated method using Novozym 435® was employed for the hydrolysis of methyl ester 2.06 to give free acid 2.07 in moderate yield along with 22% recovered unreacted ester (Scheme 2.2). The procedure was developed previously in the group by Thomas Logothetis as a mild method for the orthogonal deprotection of carboxylic acids. A neutral phosphate buffer was present in the reaction mixture to prevent the solution becoming acidic on generation of the free acid which would cause degradation of the enzyme and eventually stop the reaction.

**Scheme 2.2 Reagents and conditions**: a) Novozym 435®, aq phosphate buffer (pH 7), CH₂Cl₂/acetone/Et₂O, 50 °C; b) Benzylamine, HOBt, DIC, CH₂Cl₂; c) Amino methyl polystyrene resin, HOBt, DIC, DMF/CH₂Cl₂.

*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.

A solution phase equivalent to the RLV construct was then prepared by reacting acid 2.07 with benzylamine (a solution phase analogue of amino methyl polystyrene resin) under standard coupling conditions to give amide 2.08. This showed the attachment of the linker-vector portion to the solid phase to be viable. The poor yield of this
unoptimised reaction was of no great concern as when this step is transferred to the solid phase it will employ an excess of reagents relative the amount of resin. This would drive the reaction to completion and the excess reagents could then be easily removed during the workup by a simple filtration. Acid 2.07 was then attached to a solid support in the form of amino methyl polystyrene resin using the same conditions employed in the solution phase but using excess reagents. After gentle stirring for 19 h at room temperature a ninhydrin test on a small sample of resin was negative indicating that the coupling had been successful as there was no primary amine functionality remaining on the resin. The MAS $^1$H and $^{13}$C NMR spectra of the resin were obtained to confirm that resin 2.09 had been formed.

Scheme 2.3  Reagents and conditions: a) ClSO$_2$OH, CH$_2$Cl$_2$, 0 °C to rt; b) 3-Phenyl propan-1-ol, pyridine, CH$_2$Cl$_2$.

The synthesis of acid 2.07 was also investigated using unprotected 6-phenylhexanoic acid (2.01) as this route would save two transformations in the synthetic pathway. The chlorosulfonation reaction of acid 2.01 to form acid 2.10 proceeded in a reduced yield (54% down from 68% with the methyl ester equivalent) although the second step using acetyl chloride was of course not necessary (Scheme 2.3). The sulfonate ester formation reaction gave greatly reduced yields and was less clean due to the formation of by-products including the product of the reaction of the carboxylic acid group with the alcohol to give ester 2.11. The lower yields and extra by-products minimised the
potential advantage of losing the protection/deprotection steps and so this route was not pursued further.

Cleavage of the vector molecule from sulfonate ester 2.06 and amide 2.08 was attempted using potassium fluoride as the source of [19F]-fluoride in the presence of the phase-transfer agent Kryptofix [2.2.2] (1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo[8.8.8]hexacosan) following the procedure described previously by the Brown group (Scheme 2.4). In both cases, an inseparable mixture of the corresponding sulfonic acid (2.12 or 2.13) and Kryptofix [2.2.2] were isolated from the crude product but the labelled vector, 3-fluoropropylbenzene, was not isolated. The boiling point of 3-fluoropropylbenzene has been reported to be 45-46 °C/3 mm so it was thought that any fluorinated product that may have formed had been lost either during the reaction or when the crude or purified product was dried under vacuum.

\[
\text{Scheme 2.4 Reagents and conditions: a) KF, Kryptofix [2.2.2], MeCN, reflux.}
\]

Due to the volatility of 3-fluoropropylbenzene, alcohol 2.05 had clearly been a poor choice of model to test the RLV approach. So that the fluoridolysis could be investigated, a different vector molecule with a higher molecular weight was required so that the fluorinated vector could be more easily detected.

2.1.2 (4-Hydroxy butyl) phenyl carbamic acid tert-butyl ester as vector molecule

(ω-Fluoroalkyl)phenylamines have been used as model systems by GE Healthcare when investigating tracers for functional imaging with 18F PET scanners. Accordingly, these less volatile targets were selected as vector molecules to use in the development and testing of the new linkers for the RLV strategy. The synthesis of (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14) was developed previously by Thomas
Logothetis as a straightforward, two step procedure starting from aniline and 4-chlorobutanol, affording product in moderate yield.\(^6\)

The reaction of sulfonyl chloride 2.03 with alcohol 2.14 using the previously optimised conditions gave a disappointing yield of 53\% of sulfonate ester 2.15. After investigating this reaction further it was found that the yield could be greatly improved by the addition of a catalytic amount of DMAP to the reaction mixture. Test reactions also showed there to be no need to reflux the reaction mixture as the same yield was achieved when the reaction was carried out at room temperature and these new conditions resulted in an improved yield of 81\% of sulfonate ester 2.15 (Scheme 2.5). The fluoridolysis was first attempted in solution phase to establish whether the new vector molecule could be detected and pleasingly the desired fluoride 2.18 was easily isolated in moderate yield.

\[ 
\text{Scheme 2.5 } \text{Reagents and conditions: a) Sulfonyl chloride 2.03 (1.2 eq), Et}_3\text{N, DMAP, CH}_2\text{Cl}_2; \text{ b) Novozym 435®, aq phosphate buffer (pH 7), CH}_2\text{Cl}_2/\text{acetone, 50 °C; c) Amino methyl polystyrene resin, HOBt, DIC, DMF/CH}_2\text{Cl}_2; \text{ d) KF, Kryptofix [2.2.2], MeCN, reflux.} \\
\text{*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.} \\
\text{The enzymatic hydrolysis was carried out as before to give free acid 2.16 which was then coupled to amino methyl polystyrene resin using the standard conditions to give} 
\]
resin 2.17. The solid phase fluoridolysis was carried out using resin 2.17, the resin was then removed by filtration and the crude reaction mixture was passed through a plug of silica to remove the Kryptofix and gave the desired fluorinated vector, fluoride 2.18 (the low yield of this reaction would be optimised during the radiochemistry experiments). This showed the 4-alkylphenylsulfonate linker to work as required in regards to the attachment of the vector molecule to the solid phase and the subsequent cleavage to give the labelled vector molecule.

2.1.3 Synthesis of shorter 4-alkylphenylsulfonate linkers

Previous studies as part of the synthesis of a solid supported precursor to $[^{18}\text{F}]$-FDG showed that altering the chain length of the linker had an effect on the radiochemical yield of the fluorinated vector. The 4-alkylphenylsulfonate linker synthesised to date had an alkyl chain of five methylene units so we wanted to synthesise linkers with shorter alkyl spacers from commercially available starting materials by the same pathway. The efficiency of fluoridolysis of these linkers could then be compared and the optimum linker length could be determined based on the fluorination yield and efficiency of synthesis.

![Scheme 2.6](image)

**Scheme 2.6** *Reagents and conditions:* a) TMSCl, MeOH, 0 °C; b) i) ClSO$_2$OH, CH$_2$Cl$_2$, 0 °C to rt, ii) AcCl, MeOH, CH$_2$Cl$_2$ 0 °C; c) Alcohol 2.14, Et$_3$N, DMAP, CH$_2$Cl$_2$; d) Novozym 435®, aq phosphate buffer (pH 7), CH$_2$Cl$_2$/acetone, 50 °C; e) Amino methyl polystyrene resin, HOBT, DIC, DMF/CH$_2$Cl$_2$.

*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.
4-Alkylphenylsulfonate linkers with shorter alkyl chain lengths of three and one methylene unit were synthesised from commercially available acids 2.19 and 2.25, respectively, following the synthetic pathway developed for the longer linker (Scheme 2.6 and Scheme 2.7).

Firstly, the carboxylic acid groups were protected as the corresponding methyl esters in good to quantitative yields to give methyl esters 2.20 and 2.26, respectively. These were then converted into the sulfonyl chlorides using the optimised two step procedure which gave sulfonyl chloride 2.21 in good yield, although the yield for sulfonyl chloride 2.27 was significantly lower. This was thought to be due to the increased reactivity of sulfonyl chloride 2.27 because of the shorter alkyl chain length, which made the product less stable. However, the increased reactivity of sulfonyl chloride 2.27 meant that the original conditions (without the use of DMAP) were used in the formation of sulfonate ester 2.28 and a yield of 83% was obtained, while sulfonate ester 2.22 was formed in a slightly higher yield with the addition of DMAP to the reaction mixture. The methyl esters were deprotected in moderate to good yields and the resulting free acids 2.23 and 2.29 were coupled to the solid support to give resins 2.24 and 2.30.

Scheme 2.7  Reagents and conditions: a) TMSCl, MeOH, 0 °C; b) i) ClSO₂OH, CH₂Cl₂, 0 °C to rt, ii) AcCl, MeOH, CH₂Cl₂, 0 °C; c) Alcohol 2.14, Et₃N, CH₂Cl₂, reflux; d) Novozym 435®, aq phosphate buffer (pH 7), CH₂Cl₂/acetone, 50 °C; e) Amino methyl polystyrene resin, HOBt, DIC, DMF/CH₂Cl₂.

*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.
2.2 Solution phase model studies

Solution phase model studies were carried out to determine the identity of the next linker type for synthesis. The efficiency of the sulfonate ester forming reaction and the subsequent fluoridolysis of the developed 4-alkylphenylsulfonate linker was compared to some model solution phase linker types. Sulfonate esters with tosylate, nosylate and mesylate functionality were synthesised from the appropriate sulfonyl chloride using (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14) as the vector molecule. These linker models were chosen as they were expected to have varying reactivity’s. The tosylate group was expected to have a similar reactivity to the 4-alkylphenylsulfonate linker. The nosylate group was expected to be more reactive than the 4-alkylphenylsulfonate linker due to the electron withdrawing effect of the nitro group in the para position on the phenyl ring. The mesylate group was chosen so that a comparison could be made with a linker that did not contain a phenyl ring.

![Chemical Structure](image)

**Table 2.2** Reaction details for the solution phase model studies.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sulfonate ester</th>
<th>Sulfonate Ester Formation</th>
<th>Fluoridolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reaction time</td>
<td>Yield</td>
</tr>
<tr>
<td>1</td>
<td>2.15</td>
<td>16 h</td>
<td>35%</td>
</tr>
<tr>
<td>2</td>
<td>2.31</td>
<td>16 h</td>
<td>47%</td>
</tr>
<tr>
<td>3</td>
<td>2.32</td>
<td>1.5 h</td>
<td>49%</td>
</tr>
<tr>
<td>4</td>
<td>2.33</td>
<td>1.5 h</td>
<td>69%</td>
</tr>
</tbody>
</table>
Tosylate 2.31 (Table 2.2, entry 2) was formed in a similar reaction time as the 4-alkylphenylsulfonate linker, sulfonate ester 2.15 (Table 2.2, entry 1), while nosylate 2.32 and mesylate 2.33 required significantly shorter reaction times (Table 2.2, entries 3 & 4). The yields for the sulfonate ester formation were slightly better with the solution models than with the developed linker, with the best yield occurring with the mesylate. The recovery of the excess sulfonyl chloride starting material was reasonable in most cases.

Fluoridolysis reactions using the standard conditions of potassium fluoride and Kryptofix [2.2.2] were carried out with each sulfonate ester, using HPLC to monitor the rates of the reactions. Unfortunately, a reliable HPLC method could not be developed and so the reactions were compared on the basis of reaction time and yield only. Tosylate 2.31 was expected to give similar results for the fluoridolysis to those obtained for sulfonate ester 2.15 and gave fluoride 2.18 in a slightly higher yield after a similar reaction time. Nosylate 2.32 gave the same yield of fluoride 2.18 as the tosylate but required a shorter reaction time, as anticipated, due the electron withdrawing effect of the nitro group. Mesylate 2.33 gave the best yield of fluoride 2.18 although it required a longer reaction time of three and a half hours, which could be impractical if the reaction was to be carried out under radiochemical conditions due to the half-life of $^{18}\text{F}$ ($t_{1/2} = 109.8 \text{ min}$).

It was decided that the next linker type to be investigated as part of this project should be the nosylate type linker on the basis of the increased rates of fluoridolysis and yield. This was because a linker mimicking a nosylate leaving group would offer a more reactive alternative to the 4-alkylphenylsulfonate linker already developed.

### 2.3 Synthesis of the 4-nitrophenylsulfonate linker

#### 2.3.1 Development of linker synthesis

The synthesis of a 4-nitrophenylsulfonate linker, with the nitro group in the para position and the carbonyl functionality in the meta position relative to the sulfonate group, was developed. A similar nosylate linker with the nitro group and carbonyl functionality in the ortho and para positions, respectively, had been previously
synthesised by Thomas Logothetis on an earlier project for GE Healthcare and some of the same reaction conditions were used in the synthesis of this new nosylate type linker.\textsuperscript{65} Earlier work showed that the conversion of the disulfide species to the corresponding sulfonyl chloride gave a greatly reduced yield in comparison with starting with the methyl sulfide and so the perhaps more efficient use of 5,5'-dithiobis(2-nitrobenzoic acid) (2.34) as the starting material was discounted. Instead, 5-chloro-2-nitrobenzoic acid (2.35) was chosen as the starting material for the synthesis (Figure 2.1) and it was thought that the sulfonyl chloride functionality could be easily introduced at the desired 5-position via the methyl sulfide.

Figure 2.1 Possible starting materials in the synthesis of the 4-nitrophenylsulfonate linker, 5,5'-dithiobis(2-nitrobenzoic acid) (2.34) and 5-chloro-2-nitrobenzoic acid (2.35).

6-Aminohexanoic acid (2.36) was converted into methyl ester 2.37 in quantitative yield following the procedure of Lin \textit{et al.} (the hydrochloric acid salt was formed in the same step to prevent cyclisation of the product).\textsuperscript{68} Benzoic acid 2.35 was converted in excellent yield to acid chloride 2.38 using the standard conditions of thionyl chloride in benzene. The use of benzene as a reaction solvent is to be avoided if possible, so the reaction was also carried out in dichloromethane although a reduced yield of 68\% was obtained. Both these high yielding reactions were found to give very clean conversions and the products could be used either without purification or after recrystallisation (Scheme 2.8). The coupling between methyl ester 2.37 and acid chloride 2.38 using Hünig’s base gave the nosylate linker backbone in chloride 2.39 in high yield after recrystallisation.

Alternatively, chloride 2.39 could be synthesised directly by the coupling of methyl ester 2.37 with benzoic acid 2.35 using carbodiimide coupling conditions. This direct reaction was found to give the same yield of chloride 2.39 as the two-step pathway
although it was necessary to purify the product by lengthy column chromatography to remove the close running urea by-product. The use of a water soluble coupling agent could possibly simplify the purification of chloride 2.39 although this was not attempted.

Scheme 2.8 Reagents and conditions: a) SOCl₂, MeOH, 0 °C to rt; b) SOCl₂, benzene, reflux; c) DIPEA, dioxane, 70 °C; d) Methyl ester 2.37, HOBt, DIC, DIPEA, DMF/CH₂Cl₂.

Chloride 2.39 was reacted with sodium thiomethylate under phase transfer conditions to give methyl sulfide 2.40 in good yield (Scheme 2.9). Efforts to convert methyl sulfide 2.40 into the corresponding sulfonyl chloride using an excess of chlorine gas and glacial acetic acid only gave a trace of the desired product although the intermediates that were isolated from the reaction give an insight into a possible alternative mechanism for the oxidative chlorination reaction which will be discussed later. The addition of small quantities of distilled water to the reaction mixture, which some authors employed in this reaction, was found not to improve the outcome and was suspected to cause hydrolysis of the methyl ester. It was thought that the electron withdrawing effect of the para nitro group was making the methyl sulfide less nucleophilic towards oxidative chlorination.

Benzyl sulfides have been used under these reaction conditions to give sulfonyl chlorides in excellent yields and so the corresponding benzyl sulfide 2.41 was synthesised and was obtained in high yield from chloride 2.39 using benzyl mercaptan
and sodium hydroxide under phase transfer conditions. It was suspected that poisoning of the phase transfer catalyst, tetrabutylammonium chloride, was occurring during the course of the reaction as \(^1\)H NMR monitoring of the reaction showed the ratio of starting chloride 2.39 and product benzyl sulfide 2.41 to remain constant after several days in the presence of the other reagents. This was overcome by working the reaction up and then subjecting the crude product to the same reaction conditions using fresh reagents. The oxidative chlorination of benzyl sulfide 2.41 was successful to yield 62% of the desired sulfonyl chloride 2.42.

Scheme 2.9  Reagents and conditions: a) NaSMe, Bu_4NCl, CH_2Cl_2/H_2O, 30-35 °C; b) BnSH, NaOH, Bu_4NCl, CH_2Cl_2/H_2O, 30-35 to 55 °C; c) Cl_2, AcOH.

Sulfonyl chloride 2.42 was successfully reacted with vector molecule alcohol 2.14 to give sulfonate ester 2.43 in good yield (Scheme 2.10). The enzyme mediated hydrolysis using Novozym 435®, as used previously for the analogous transformation with the 4-alkylphenylsulfonate linker in moderate to good yields, worked less satisfactory with the 4-nitrophenylsulfonate linker. The hydrolysis of sulfonate ester 2.43 under the established conditions gave only 25% of the desired free acid 2.44 and 57% recovered starting material. The conversion of the reaction was found to be no better when the reaction time was increased to four days. A range of different esterases, sourced from Amano Enzyme Inc (Lipase AY “Amano” 30, Lipase PS “Amano” and Lipase AK “Amano” 20) were trialled in the hydrolysis reaction under the same conditions to see if
an improvement in the yield could be achieved. However, analysis of the reaction mixtures (by TLC) found no product to have formed with any of these other enzymes. An alternative procedure for the use of Novozym 435® using acetonitrile instead of CH₂Cl₂/acetone as the organic solvent was tested. Once again these conditions were found not to give the desired product and isolated from the reaction mixture was alcohol 2.14 from the undesired hydrolysis of the vector molecule. The reasons behind the lower yield for the enzyme mediated hydrolysis of the 4-nitrophensulfonate linker remain unknown. Acid 2.44 was successfully coupled to amino methyl polystyrene resin to give resin 2.45.

![Reaction Scheme](image)

**Scheme 2.10** Reagents and conditions: a) Alcohol 2.14, Et₃N, DMAP, CH₂Cl₂; b) Novozym 435®, aq phosphate buffer (pH 7), CH₂Cl₂/acetone, 50 °C; c) Amino methyl polystyrene resin, HOBt, DIC, DMF/CH₂Cl₂.

*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.

### 2.3.2 Oxidative chlorination mechanism

The specific mechanism for the formation of sulfonyl chlorides by oxidative chlorination using chlorine gas in glacial acetic acid has not been published in the literature although the identity of the likely intermediates in the mechanistic pathway has been discussed in some detail by Douglass et al. They found that when forming.
sulfinyl chlorides from mercaptans or disulfides the stoichiometry of the chloride and the acetic acid was very important in determining the outcome of the reaction (two equivalents of chlorine and one equivalent of acetic acid are required for each equivalent of mercaptan or disulfide). If less then the required amount of chloride was used then the product was found to be contaminated with the sulfenyl chloride (Langler\textsuperscript{71} also proposed that sulfenyl chloride was an intermediate in the formation of sulfonyl chloride from benzyl methyl sulfide although, unlike Douglass, was not able to provide experimental evidence to support this theory). If less than one equivalent of acetic acid was used then some sulfur trichloride was formed, which implies that the oxygen in the sulfinyl chloride is derived from the acetic acid used in the reaction. If more than two equivalents of chlorine and an excess of acetic acid were used then some of the sulfinyl chloride was converted to the sulfonyl chloride. If wet or aqueous acetic acid is used then it is likely that water rather than acetic acid may provide the final oxygen atom to give the sulfonyl chloride.

Taking account of all of these observations, it is likely that the mechanism for the oxidative chlorination of alkyl sulfides to give sulfonyl chlorides goes by the stepwise formation of the sulfenyl chloride and the sulfinyl chloride. The alkyl sulfide first reacts with one equivalent of chlorine to give the sulfenyl chloride with loss of the methyl group by attack of chloride in a manner similar to that seen in the Arbuzov reaction. The sulfenyl chloride then reacts with the second equivalent of chlorine which, in the absence of acetic acid, forms the sulfur trichloride. In the presence of acetic acid, the charged dichloro species reacts with the acetic acid to give the sulfinyl chloride. This reacts with a third equivalent of chlorine and then with either a second equivalent of acetic acid or water to give the sulfonyl chloride. The suggested mechanism for the formation of sulfonyl chlorides by oxidative chlorination of methyl sulfides based on these literature observations is shown in Scheme 2.11.
Scheme 2.11 Proposed mechanism for oxidative chlorination based on information from literature sources.69,71

However, this mechanism does not account for the formation of the intermediates isolated from the reaction of methyl sulfide 2.40 under oxidative chlorination conditions (Figure 2.2). The first intermediate was isolated from a clean spot to spot reaction of methyl sulfide 2.40 with chlorine in acetic acid was identified as the chloromethylsulfoxide (2.46). This was previously thought to be a by-product of the oxidative chlorination reaction65 but it was found that when re-exposed to the reactions conditions some sulfonyl chloride was isolated. Hence, sulfoxide 2.46 is proposed as an intermediate in an alternative mechanistic pathway. The second intermediate, sulfonyl dichloromethane 2.47, was formed when sulfoxide 2.46 was again re-exposed to chlorine in acetic acid and continued exposure led to the formation of the sulfonyl chloride. Therefore, sulfonyl dichloromethane 2.47 is thought to be another
intermediate rather than a by-product. The discovery of these intermediates implies that the mechanism of the oxidative chlorination of the methyl sulfide in this system proceeds via the formation of the chloromethylsulfoxide followed by the sulfonyl dichloromethane and from there forms the sulfonyl chloride.

![Figure 2.2 Intermediates isolated from the reaction of methyl sulfide 2.40 with chlorine in acetic acid.](image_url)

A proposed alternative mechanism that accounts of the formation of the isolated intermediates is shown in Scheme 2.12. First, chlorination of the methyl sulfide occurs to form a chlorosulfonium ion as before but instead of the loss of the methyl group to give the sulphenyl chloride chlorination of the methyl group occurs. The chlorosulfonium ion loses a proton to form a sulfonium ylid. The chloride ion is then transferred from the sulfur atom to the adjacent carbon atom by the Pummerer rearrangement. This species then reacts with acetic acid give the chloromethylsulfoxide. This reacts with a second equivalent of chlorine and then a molecule of acetic acid or water, followed by a second chlorination of the methyl group to give the sulfonyl dichloromethane. A third chlorination of the methyl group then takes place and then chloride displaces a trichloromethane ion in an iodoform type reaction to give the sulfonyl chloride.
Scheme 2.12 Proposed alternative mechanism for oxidative chlorination accounting for the formation of the isolated intermediates, the chloromethylsulfoxide and the sulfonyl dichloromethane (highlighted in boxes).

The key difference between the two mechanisms is seen in the early steps of the mechanistic pathway, the difference being whether the initially formed chlorosulfonium ion loses the methyl group to give the sulphenyl chloride or chlorination of the methyl
group occurs via the loss of a proton to give the sulfonium ylid followed by chloride transfer by the Pummerer rearrangement. The conditions of the mechanism shown in Scheme 2.11 support the $S_N1$ loss of the methyl group. Since methyl groups are very poor in $S_N1$ reactions due to the formation of the unstable methyl cation this would be very unfavourable. Conversely, with the benzyl sulfide, the benzyl group is much more prone to loss under $S_N1$ conditions and so this pathway would be favourable. Benzyl ether protecting groups are removed with a nucleophilic conjugate base, normally hydrogen bromide in acetic acid. The conditions for the oxidative chlorination reaction are essentially the same as for the deportection of a benzyl group so it would be reasonable to presume that the benzyl sulfide reacts via the initial removal of the benzyl group and so by the mechanism shown in Scheme 2.11. The methyl sulfide would more readily form the sulfonium ylid, as shown in the mechanism in Scheme 2.12. Whilst the benzyl sulfide would form a stabilised sulfonium ylid (due to the carbanion being conjugated into the benzene ring) and so be less reactive by this pathway, the methyl sulfide forms an unstabilised sulfonium ylid. This would be more reactive and so would readily undergo a Pummerer rearrangement to chlorinate the methyl group, as shown in the mechanism in Scheme 2.12. While the evidence suggests that the methyl sulfide reacts via the mechanism shown in Scheme 2.12, no intermediates were isolated in the oxidative chlorination reaction of the benzyl sulfide so we can only speculate that it would be more likely to react via the mechanism shown in Scheme 2.11.

2.4 3-(2-Naphthlenoxy)-1-propanol as vector molecule

The mesylate of 3-(2-naphthlenoxy)-1-propanol has been used as a model compound by Chi and co-workers in a range of studies investigating nucleophilic fluorination with respect to $^{18}$F labelling\textsuperscript{73-76} and so the use 3-(2-naphthlenoxy)-1-propanol (2.48) as a vector molecule with the linkers developed as part of the RLV construct would enable our results to be compared with solution studies from the literature. Therefore, resins with the 4-alkylphenylsulfonate linker and the 4-nitrophenylsulfonate linker and with alcohol 2.48 as the vector molecule were prepared.

Alcohol 2.48 was synthesised in good yield from 2-naphthol and 3-bromo-1-propanol following the published procedure.\textsuperscript{77} For the 4-alkylphenylsulfonate linker construct, alcohol 2.48 was reacted with sulfonyl chloride 2.03 to give sulfonate ester 2.49 in
quantitative yield (Scheme 2.13). Sulfonate ester 2.49 was hydrolysed using the Novozym 435® to give acid 2.50 in good yield which was coupled to the solid support to give resin 2.51. Similarly for the 4-nitrophenylsulfonate linker construct, alcohol 2.48 was reacted with sulfonyl chloride 2.42 to give sulfonate ester 2.52 in good yield. This was hydrolysed to give acid 2.53 in moderate yield (which was an improvement on the yield obtained previously for the enzyme mediated hydrolysis with this linker type, Scheme 2.10). The acid was coupled to the solid support to give resin 2.54.

Scheme 2.13  
Reagents and conditions: a) Sulfonyl chloride 2.03, Et3N, DMAP, CH2Cl2; b) Novozym 435®, aq phosphate buffer, CH2Cl2/acetone, 50 °C; c) Amino methyl polystyrene resin, N-hydrosuccinimide, DIC, DMF/CH2Cl2; d) Sulfonyl chloride 2.42, Et3N, DMAP, CH2Cl2; e) Amino methyl polystyrene resin, HOBt, DIC, DMF/CH2Cl2.

*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.

As the enzyme mediated hydrolysis of the 4-nitrophenylsulfonate linkers gave poor yields of the free acids, more standard hydrolysis conditions of sodium hydroxide in methanol/water were tested using sulfonate ester 2.52. None of the desired acid 2.53 was isolated from the crude reaction mixture which was found to contain alcohol 2.52 as a result of the cleavage of the vector molecule and acid 2.55 where the methyl ester had been hydrolysed as required but the nitro group had been replaced with a methoxy
group (Figure 2.3). Hence, the necessity of the orthogonal enzyme mediated hydrolysis reaction was shown in the synthesis of the 4-nitrophenylsulfonate linkers.

![Image](2.48)

**Figure 2.3** Undesired products isolated from the hydrolysis the methyl ester of sulfonate ester 2.52 under standard conditions.

The observation of the substitution of the nitro group via a $S_N$Ar reaction to give acid 2.55 highlighted a possible problem with the 4-nitrophenylsulfonate linker when used to immobilise a precursor for use in $[^{18}F]$-fluoridolysis reactions. The nitro group has been shown to be a better leaving group than halogens in nucleophilic displacement reactions using $[^{18}F]$-fluoride to give labelled aryl fluorides.\(^{78}\) This transformation was used in the synthesis of $[^{18}F]$-FDOPA (discussed in Chapter 1, Section 1.5) and $[^{18}F]$-flumazenil which used a precursor containing an aryl nitro group to give the desired product with an $[^{18}F]$-fluoride incorporation of 55-60% (Scheme 2.14).\(^{79}\) If the nucleofugicity of the nitro group on the linker is similar to that of the sulfonate then the fluoridolysis reaction may be complicated by fluorination on the linker itself rather than cleavage of the vector.

![Image](2.56)

**Scheme 2.14** The synthesis of $[^{18}F]$-flumazenil via the nucleophilic fluorination of the nitro analogue.\(^{79}\) Reagents and conditions: a) $[K/K2.2.2]^+ ^{18}F^-$, DMF, 160 °C.

### 2.5 Conclusions

The synthesis of a 4-alkylphenylsulfonate linker was developed and applied to produce a range of resins containing this linker type with varying lengths of alkyl spacer and (4-
hydroxy-butyl)-phenyl-carbamic acid tert-butyl ester (2.14) as the vector molecule. A solution model study was carried out to investigate the efficiency of the sulfonate ester formation and subsequent $[^{19}\text{F}]-$fluoridolysis for various sulfonate ester types to determine the second linker type for development. The nosylate gave the most promising results and so the synthesis of a 4-nitrophenylsulfonate linker was developed. Difficulties with the oxidative chlorination reaction to give the sulfonyl chloride were overcome by the replacement of a methyl sulfide with a benzyl sulfide as the starting material in this reaction and the mechanism for this transformation was discussed. Resins containing the 4-alkylphenylsulfonate and the 4-nitrophenylsulfonate linker type and 3-(2-naphthlenoxy)-1-propanol (2.48) as the vector molecule were prepared so that the results of the fluoridolysis reaction could be compared with those published for this vector molecule.

The results of the $[^{19}\text{F}]-$ and $[^{18}\text{F}]-$fluoridolysis of all the of the resins synthesised in this chapter, along with a comparison of the results using the 3-(2-naphthlenoxy)-1-propanol vector with the literature, are presented in Chapter 3.
Chapter 3 – [\\textsuperscript{19}F] Experiments and [\\textsuperscript{18}F] Radiolabelling

This chapter covers all of the [\\textsuperscript{19}F]-fluoride experiments carried out with the range of resins developed as part of this project, the synthesis of which was discussed in Chapter 2. Following this, the results of the [\\textsuperscript{18}F]-fluoridolysis reactions are presented and discussed for which we gratefully acknowledge Imtiaz Khan of GE Healthcare for carrying out these experiments.

3.1 Solid phase fluoridolysis of RLV constructs containing the two linker types

The [\\textsuperscript{19}F]-fluoridolysis of the resins utilising the RLV construct was carried out using potassium fluoride as the source of [\\textsuperscript{19}F]-fluoride and Kryptofix [2.2.2] in acetonitrile at 80 °C to give [\\textsuperscript{19}F]-2.18 or [\\textsuperscript{19}F]-2.57 after a reaction time of 30 min. Purification of the fluorinated products involved filtration of the reaction mixture to remove the resin followed by elution through a short silica column to remove the Kryptofix [2.2.2]. The yields of the fluorinated products were calculated based on the loading of the resin used (the loadings were estimated from the results of the elemental analyses of sulfur for the individual resins).

All of the resins were found to yield the desired fluorinated product as the only major product isolated from the reaction mixture showing the RLV construct to work as intended. Resins 2.17, 2.24 and 2.30, with the 4-alkylphenylsulfonate linker, gave consistent yields of [\\textsuperscript{19}F]-2.18 for the three linker lengths (Table 3.1). The yields were congruent with what would be expected for nucleophilic fluorination reactions. Radiochemistry experiments would be carried out using a large excess of resin relative to the amount of [\\textsuperscript{18}F]-fluoride and so would result in good radiochemical yields for the fluoridolysis. Resin 2.45 with the 4-nitrophenylsulfonate linker gave a lower yield of [\\textsuperscript{19}F]-2.18 than with the other linker type which was surprising as the nosylate mimicking linker was expected to be more reactive. Similarly, for the 3-(2-naphthlenoxy)-1-propanol vector molecule, resin 2.51, with the 4-alkylphenylsulfonate linker, yielded about twice as much fluorinated product ([\\textsuperscript{19}F]-2.57) than resin 2.54, with the 4-nitrophenylsulfonate linker (Table 3.2). Higher yields were obtained for this vector than the (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester vector for both
linker types which may be due to the structure of the vector molecule being more lipophilic and easier to manipulate.

![Diagram of [19F]-2.18]

<table>
<thead>
<tr>
<th>Resin</th>
<th>Structure and loading</th>
<th>Yield of [19F]-2.18</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.17</td>
<td><img src="image" alt="Structure 2.17" /></td>
<td>0.80 mmolg⁻¹</td>
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<tr>
<td></td>
<td></td>
<td>28%</td>
</tr>
<tr>
<td>2.24</td>
<td><img src="image" alt="Structure 2.24" /></td>
<td>0.81 mmolg⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28%</td>
</tr>
<tr>
<td>2.30</td>
<td><img src="image" alt="Structure 2.30" /></td>
<td>0.95 mmolg⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27%</td>
</tr>
<tr>
<td>2.45</td>
<td><img src="image" alt="Structure 2.45" /></td>
<td>0.58 mmolg⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13%</td>
</tr>
</tbody>
</table>

**Table 3.1** Results of the [19F]-fluoridolysis of the resins with the (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester vector molecule.
Chi and co-workers used the mesylate of 3-(2-naphthlenoxy)-1-propanol (2.58) as a model compound in studies investigating new methods of nucleophilic fluorination using ionic liquids,75,76 protic solvents73 and tertiary alcohols.74 They carried out the fluorination of mesylate 2.58 using standard conditions so that a comparison could be made with results using their new reaction conditions. They reported that the reaction of mesylate 2.58 with potassium fluoride and 18-crown-6 (a crown ether reagent, similar to Kryptofix [2.2.2]) in acetonitrile at 100 °C for 24 h gave [19F]-2.57 in a 40% yield after extraction of the reaction mixture into organic solvent followed by column chromatography (Scheme 3.1).75 This result is comparable to the yield obtained from the fluoridolysis of resin 2.51, although our solid phase reaction required a much shorter reaction time (30 min) and a far simpler workup and purification procedure.

### Table 3.2 Results of the [19F]-fluoridolysis of the resins with the 3-(2-naphthlenoxy)-1-propanol vector molecule.

<table>
<thead>
<tr>
<th>Resin</th>
<th>Structure and loading</th>
<th>Yield of [19F]-2.57</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.51</td>
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<td>37%</td>
</tr>
<tr>
<td>2.54</td>
<td><img src="image2" alt="Structure 2.54" /></td>
<td>22%</td>
</tr>
</tbody>
</table>

**Scheme 3.1** Fluorination of mesylate 2.58 using standard conditions as carried out by Chi et al.75 **Reagents and conditions:** a) KF, 18-crown-6, MeCN, 100 °C, 24 h.
3.2 $^{19}$F NMR Kinetic study

A $^{19}$F NMR kinetic study was carried out to compare the relative rates of fluoridolysis for resins containing the RLV constructs with the three different length 4-alkylphenylsulfonate linkers (resins 2.17, 2.24 and 2.30) and the resin with the 4-nitrophenylsulfonate linker (resin 2.45). Previous work in the group on a RLV construct designed to release protected $[^{18}$F]-fluoro-2-deoxy-D-glucose ($[^{18}$F]-FDG) from a perfluoroalkylsulfonate linker found that the radiochemical yield was affected by altering the chain length of the linker. A steady rise in radiochemical yield was observed with increasing linker length up to a point where any further increase in linker length resulted in no additional improvement in the yield of the product.33,34 Hence, we wanted to investigate if the linker chain length of resins with the 4-alkylphenylsulfonate linker had an effect on the rate of cleavage of the fluorinated product and to see if changing the linker type to the 4-nitrophenylsulfonate linker (which was thought to be more reactive) would have an effect on the rate of cleavage.

To establish the feasibility of the use of $^{19}$F NMR spectroscopy to monitor the fluoridolysis and to investigate the conditions for the reactions, a series of trial reactions was first carried out. A reaction mixture containing 50 mg of resin 2.30 and an excess of potassium fluoride and Kryptofix [2.2.2] in deuterated acetonitrile was made up in an NMR tube and $^{19}$F NMR spectra were acquired as the reaction progressed. The fluoridolysis reactions are usually carried out at 80 °C but were also shown in test reactions to proceed at room temperature, albeit at a reduced rate. As a temperature of 80 °C was too high to be maintained by the NMR probe the first trial reaction was carried out at room temperature. The concentration of $[^{19}$F]-fluoride 2.18 formed in the reaction was calculated by comparison of the size of the $^{19}$F NMR peak due to $[^{19}$F]-fluoride 2.18 (−218 ppm) with that of the internal standard (hexafluorobenzene, −162 ppm), which was present in the deuterated acetonitrile at a known concentration.
The results of the first trial reaction (Figure 3.1) showed an increase in product concentration with time up to 3090 min (51.5 h) at which point it was assumed that the reaction had gone to completion. This showed that it was feasible to investigate the kinetics of the reaction using $^{19}\text{F}$ NMR spectroscopy although some optimisation of the procedure was necessary. As the reaction took over two days for the product concentration to remain constant it was clear that the use of an elevated temperature would be necessary so that the reaction was complete within a more reasonable timescale. This would also allow the acquisition of $^{19}\text{F}$ NMR spectra at more regular intervals during the reaction giving a more even data set. The hexafluorobenzene internal reference peak occurs at −162 ppm which coincided with an unavoidable bump in the baseline of the $^{19}\text{F}$ NMR spectrum. This broad bump in the baseline, occurring over the range −150 to −200 ppm, appears in the blank spectra so is not related to the reaction mixture. It originates from components within either the spectrometer or the probe which contain fluorine nuclei. These fluorine containing components are solid and so result in an unavoidable broad bump in the baseline of the spectra (similar issues are observed with boron NMR as the tubes and other glass components are made of borosilicate glass). This bump in the baseline would affect the accuracy of the integration of the reference peak and so an alternative internal reference compound was
sought. Commercially available trifluorotoluene has a $^{19}$F NMR chemical shift of $-63$ ppm$^{80}$ which did not coincide with the bump on the baseline although it was considered to be too far away from the chemical shift of $[^{19}$F]-fluoride 2.18 ($-218$ ppm) as this would result in a large spectral window for acquisition. Benzyl fluoride (3.02) has a $^{19}$F NMR chemical shift of $-207$ ppm$^{81}$ and so was chosen as the internal reference as it has a similar shift to the product meaning a small spectral window for acquisition could be used, over a region that had a nice flat baseline.

Benzyl fluoride was not available from commercial sources so the synthesis was attempted in one step from benzyl bromide (3.01) following the procedure of Clark et al.$^{82}$ This method used potassium fluoride and tetraphenylphosphonium bromide in acetonitrile at 80 °C using conventional heating and required over seven days for the reaction to approach completion (monitored by $^1$H NMR). In order the speed up the reaction the use of microwave heating was investigated as it had been shown to greatly accelerate halogen exchange reactions.$^{83}$ Using microwave heating, the reaction time was significantly reduced to one hour at 120 °C followed by six hours at 150 °C to give the desired benzyl fluoride (3.02) in a 16 % isolated yield. The poor yield achieved for this reaction was attributed to the low boiling point of the product (139.9 °C at 780 mm/Hg$^{84}$) which caused difficulties in handling and purification. Further work on this reaction showed it to proceed as desired using simply benzyl bromide and an excess of potassium fluoride in acetonitrile, negating the need for the tetraphenylphosphonium bromide. Using these modified conditions microwave heating at 150 °C for two hours gave the desired benzyl fluoride (3.02) in an 18% isolated yield (Scheme 3.2).

![Scheme 3.2 Reagents and conditions: a) KF, MeCN, 150 °C (microwave heating).](image)

To check the stability of benzyl fluoride (3.02) under the fluoridolysis reaction conditions, a $^{19}$F NMR spectrum of a sample of deuterated acetonitrile containing a small amount of benzyl fluoride (3.02) and hexafluorobenzene (known to be stable) was acquired. The sample was then incubated at 50 °C for three days before a second
A second trial reaction was carried out using 50 mg of resin 2.30 and an excess of potassium fluoride and Kryptofix [2.2.2] in deuterated acetonitrile containing benzyl fluoride (3.02) as the internal reference (2.7 \( \mu \text{molL}^{-1} \)). The \(^{19}\text{F} \) NMR probe was maintained at 50 °C and spectra were acquired at regular intervals (Figure 3.2, left). The product concentration was observed to have levelled out after only one hour which, when you consider it takes around 15 min to acquire one spectrum, was considered to be too fast to get adequate data points for analysis. The internal reference peak was observed to be too small which would make measuring the integration less accurate and so a higher concentration of benzyl fluoride (3.02) in the deuterated acetonitrile was deemed necessary. The resin itself appeared visually to contain two layers in the bottom of the NMR tube at the end of the experiment implying only the upper portion had reacted due to poor mixing. To improve on these issues, the third trial reaction was carried out using less resin (20 mg), the spectra were acquired at 40 °C and the reaction
mixture was removed from the spectrometer and agitated between each acquisition (Figure 3.2, right). The concentration of the internal reference was also increased to 27.0 μmolmL⁻¹ which gave a larger reference peak for more accurate integration. At this lower reaction temperature the rate of the reaction was reduced and the product concentration levelled off after around three hours. The combination of using a smaller amount of resin and agitating the reaction mixture gave much better results. Both data sets from these two trial reactions (Figure 3.2) showed a slower reaction rate at the start of the reaction, up until around 20 min, which gave a slightly sigmoidal shape to the data. This is thought to be due to the initial swelling of the resin in the reaction solvent as the resins were not pre-swollen prior to use.

The [¹⁹F]-fluoridolysis reactions for the kinetic study were carried out using a stock solution of deuterated acetonitrile containing benzyl fluoride (3.02) as the internal reference at a concentration of 10.6 μmolmL⁻¹ and were carried out in 0.5 mL of solvent. Each reaction used 19 μmol of resin-bound substrate (based on the loadings of the resin calculated by elemental analysis of sulfur) along with 1.5 eq of potassium fluoride and Kryptofix [2.2.2]. The spectra were acquired with the temperature of the NMR probe at 40 °C, which was checked prior to commencing the reaction by analysis of the peak separation in the ¹H NMR of 80% glycol (1,2-ethanediol) in DMSO. Agitation of the reaction mixture took place between each spectral acquisition to ensure ample mixing of the resin suspension. ¹⁹F NMR spectra were acquired at regular intervals (every 12-15 min) until the amount of product in the reaction mixture remained constant, indicating that the cleavage was complete. The reaction was repeated for each resin (Figure 3.3) until consistent results had been achieved. Graphs of amount of product cleaved (μmol) against time (min) were plotted for each reaction and the data were found to give good kinetic curves. The gradient of the linear portion of the kinetic curves over the region 10-85 min were used to calculate the rate of cleavage of the fluorinated product from the RLV construct (Figure 3.4).
Figure 3.3 The resins analysed as part of the $^{19}$F NMR kinetic study.

Figure 3.4 Graph to show the results of the kinetic study fluoridolysis reactions for resin 2.17 (light blue), resin 2.24 (pink), resin 2.30 (dark blue) and resin 2.45 (green). Shown are the linear portion of the kinetic curves over the region 10-85 min which were used to calculate the rate of cleavage of the fluorinated product from the RLV construct.

The data showed the rate of cleavage of the RLV constructs containing the 4-alkylphenylsulfonate linker to increase with linker chain length, as the rate for resin 2.30 was the slowest at 0.136 $\mu$molmin$^{-1}$, resin 2.24 was 0.202 $\mu$molmin$^{-1}$ and resin
2.17 was the fastest at 0.216 \( \mu \text{mol min}^{-1} \). This was the expected trend as the increasing chain length would allow the sulfonate ester group to have a more “solution-like” character and to be more easily accessed by the fluoride ion nucleophile and hence the reaction would proceed quicker. The results for the 4-nitrophenylsulfonate linker were unexpected as this linker was thought to be more electron withdrawing and so was expected to have a faster rate of fluoridolysis. The rate for resin 2.45 was found to be just 0.027 \( \mu \text{mol min}^{-1} \) which was significantly lower than the rates for the 4-alkylphenylsulfonate linkers. Further examination of the complete kinetic curves found the graphs for resins 2.17, 2.24 and 2.30 to level out at 15-20 \( \mu \text{mol} \) of product showing that, within experimental error, all the 19 \( \mu \text{mol} \) of immobilised vector had been cleaved. The graphs for resin 2.45 were found to level out at around 2 \( \mu \text{mol} \) of product which was significantly less than the expected amount and indicated the possibility of a side reaction taking place.

A possible complication with the 4-nitrophenylsulfonate linker was thought to be fluorination of the phenyl ring of the linker by displacement of the nitro group with \([^{19}\text{F}]\)-fluoride (as discussed in Chapter 2). If this side reaction was taking place then it would be quantified as an additional peak in the \(^{19}\text{F}\) NMR spectrum in the region characterised by aryl fluorides (around −100 to −125 ppm). No additional peak, in this region or elsewhere, was detected during the kinetic study experiments with the 4-nitrophenylsulfonate linker (resin 2.45) and only peaks for the benzyl fluoride internal reference and the fluorinated product were seen which implied that the displacement of the nitro group with \([^{19}\text{F}]\)-fluoride was not taking place, nor were any side reactions involving \([^{19}\text{F}]\)-fluoride occurring. On examination of the reaction mixture at the end of the experiment, the resin was observed as being “clumpy” and the solution had turned pale brown. At the end of the experiments with the 4-alkylphenylsulfonate linkers the resins remained free flowing and the solutions were colourless and so it was assumed that degradation of the 4-nitrophenylsulfonate linker had taken place during or prior to the reaction. The resin was removed from the reaction mixture by filtration and a \(^1\text{H}\) NMR spectrum of the concentrated filtrate was acquired. The resulting spectrum contained peaks for the fluorinated product as expected but also contained other peaks in the aromatic region which were thought to originate from the phenyl ring in the 4-nitrophenylsulfonate linker. Although the speculations that fluorination of the linker
itself may occur were unsupported, as the sulfonate ester was found to perform better in the nucleophilic fluorination reaction than the nitro group, this work supported the assumption that degradation of the 4-nitrophenylsulfonate linker had occurred.

3.3 Repeat cleavage experiments using resin 2.17

A series of fluoridolysis reactions using limited fluoride were carried out on a batch of resin to demonstrate the ability of the RLV construct to release multiple portions of the fluorinated product. The use of limiting fluoride also more closely resembled the conditions that would be employed in the radiochemistry experiments involving the resins.

A sample of resin 2.17 was reacted under the usual fluoridolysis conditions with a limited amount of potassium fluoride and Kryptofix [2.2.2] in acetonitrile at 80 °C for 30 min after which time the resin was removed by filtration and the reaction mixture was passed through a short silica column (Scheme 3.3). The recovered resin was re-exposed to the reaction conditions and was shown to release further fluorinated product [\(^{19}\)F]-2.18 for a total of seven cycles. The first five cleavage reactions gave consistent yields of 30-45% of \([^{19}\text{F}]\)-fluoride 2.18 and subsequent reactions gave significantly lower yields (Figure 3.5).

These results clearly demonstrate that the unreacted vector molecule remained attached to the solid phase \(\text{via}\) 4-alkylphenylsulfonate linker during the fluoridolysis reactions and could be released upon further exposure to fluoride ions. The RLV approach clearly showed that the crude product was easy to separate from both the unreacted starting material and from the reagents used in the cleavage reaction. The release of multiple portions of fluorinated product from one batch of resin means that the RLV construct could be easily adapted for automated radiosynthesis. A cartridge containing
some appropriately functionalised resin could, in principle, be used in an automated setup to produce multiple batches of radiotracer.

![Graph showing yield of $[^{19}\text{F}]-2.18$ obtained from successive fluoridolysis reactions carried out using the same batch of resin 2.17.](image)

**Figure 3.5** Graph to show the yield of $[^{19}\text{F}]-2.18$ obtained from successive fluoridolysis reactions carried out using the same batch of resin 2.17.

### 3.4 Radiochemistry results

We gratefully acknowledge Imtiaz Khan of GE Healthcare, White Lion Road, Amersham, UK, for carrying out the radiochemistry experiments with our resins. These experiments are described and discussed in the ensuing section.

The radiolabelling experiments were carried out manually using potassium $[^{18}\text{F}]-$fluoride, potassium carbonate and Kryptofix [2.2.2] in acetonitrile with a reaction time of 15 min and the radiochemical yields were established by reverse-phase HPLC analysis of the crude reaction mixture using UV (254 nm) and $\gamma$-detection (a full experimental procedure can be found in Chapter 6). The reactions used a severely limiting amount of $[^{18}\text{F}]-$fluoride compared to the amount of vector immobilised on the resin, the latter being in excess by an order of a few thousand. The unreacted resin was easily removed from the reaction mixture by filtration and so the HPLC traces were not complicated by the presence of large peaks due to the starting material.
Table 3.3  Results of the \(^{18}\text{F}\)-fluoridolysis reactions. *Reagents and conditions:* a) \(\text{K}^{18}\text{F}, \text{K}_2\text{CO}_3, \text{Kryptofix [2.2.2]}, \text{MeCN, 100-110 °C.}\)

The results of experiments with resins 2.17, 2.24 and 2.30 (which were made up of the 4-alkylphenylsulfonate linker with varying lengths and with the (4-hydroxy-butyl)-phenyl-carbamic acid tert-butyl ester vector molecule) are shown in Table 3.3. In each case, the desired product \(^{18}\text{F}\)-2.18 was formed as the only radiochemical product and its identity was confirmed by comparison of the retention time with that of the cold standard \(^{19}\text{F}\)-2.18. Resin 2.17, with the longest linker length, resulted in the highest incorporation of \(^{18}\text{F}\)-fluoride (Table 3.3, entry 1) which correlates with the fastest rate of cleavage seen in the kinetic study and is thought to be due to the increased “solution-like” character of the sulfonate ester with the longer linker length. Resins 2.24 and 2.30 were expected to show a sequential reduction in the incorporation of \(^{18}\text{F}\)-fluoride although their results were found to be comparable (Table 3.3, entries 2 & 3). This was attributed to the higher loading of resin 2.30 which would be expected to yield slightly more product than resin 2.24 as the same mass of resin was used for the reactions. Stirring of the resin suspension during the reaction was found to greatly improve the outcome of the reaction, in the reaction of resin 2.17 without stirring a reduced 34% incorporation of \(^{18}\text{F}\)-fluoride was observed (14% non-decay corrected yield).
Figure 3.6 Reverse phase HPLC analyses of the $[^{18}F]$-fluoridolysis reaction with resin 2.24 of the crude reaction mixture (top) and the formulated reaction mixture (bottom). In both cases the red line is the radioactive trace and the blue line is the UV trace (254 nm).

The HPLC analyses of the $[^{18}F]$-fluoridolysis reaction with resin 2.24 are shown in Figure 3.6, where, in both analyses, the radioactive trace is shown as the red line and the UV trace is shown as the blue line. The top trace is the HPLC analysis of the crude reaction mixture which was acquired after filtration of the reaction mixture through an acrodisc to remove the resin from the solution. The radioactivity trace showed only two peaks, the first peak had a retention time of less than two min and is due to unreacted $[^{18}F]$-fluoride, the second peak had a retention time of 16 min and is due to the $[^{18}F]$-fluorinated product, $[^{18}F]$.2.18. The $[^{18}F]$-fluoride incorporation of 31% for this reaction was calculated from the relative areas of the unreacted $[^{18}F]$-fluoride peak and the $[^{18}F]$-fluorinated product peak. The reaction mixture was then formulated into a form that would be injectable into a patient (a solution containing a mixture of
phosphate buffered saline and ethanol). The sample was passed through a C18 Sep-Pak cartridge, eluting with water and ethanol before phosphate buffered saline was added to the filtrate and the HPLC analysis was repeated. The radioactive trace now had only one peak due to the $[^{18}\text{F}]$-fluorinated product as the unreacted $[^{18}\text{F}]$-fluoride has been removed from the sample. This shows that $[^{18}\text{F}]-2.18$ had been formed in excellent radiochemical purity as it was the only radiochemical product formed in the reaction.

The UV traces, the blue lines, show the chemical make-up of the crude and formulated reaction mixtures, from which information can be obtained regarding the chemical purity of the reaction. Since the unreacted precursor remained attached to the resin and was removed by filtration, the UV traces don’t contain a large peak due to the excess of precursor used in the reaction. Due to the tiny amount of $[^{18}\text{F}]$-fluoride used in the reaction, a chemical peak due to the $[^{18}\text{F}]$-fluorinated product was not expected and so all of the UV peaks seen are a result of side reactions or degradation of the RLV construct or the resin beads themselves. The UV traces show chemical impurities with retention times of around 4, 8 and 16 min, with the latter being the most significant (although the peak at 16 min appears close to the product peak on the radioactivity trace it is not the chemical peak for the product). Attempts were made to identify these chemical impurities but with limited success. The impurity giving the peak at 8 min is thought to be the product of hydrolysis of the vector molecule from a comparison of the HPLC analysis of a sample of the alcohol. The more significant impurity resulting in the peak at 16 min was thought to most likely be the elimination product, although this was not confirmed. It should be noted that the elimination product was not obtained in the $[^{19}\text{F}]$-fluorination reactions, highlighting the difference in the reaction conditions for the $[^{19}\text{F}]$- and $[^{18}\text{F}]$-fluoridolysis reactions.

The $[^{18}\text{F}]$-fluoridolysis reaction of resin 2.17 also resulted in the formation of some chemical impurities (Figure 3.7, top). HPLC purification of the reaction mixture removed most of the chemical impurities although the impurity at 16 min that was close-running to the $[^{18}\text{F}]$-fluorinated product was not separated under the HPLC conditions and setup employed by the radiochemists (Figure 3.7, bottom).
Figure 3.7 Reverse phase HPLC analyses of the $[^{18}\text{F}]$-fluoridolysis reaction with resin 2.17 of the crude reaction mixture (top) and the HPLC purified reaction mixture (bottom). In both cases the red line is the radioactive trace and the blue line is the UV trace (254 nm).

A $[^{18}\text{F}]$-fluoridolysis reaction was carried out with resin 2.45 (with the 4-nitrophenylsulfonate linker and the (4-hydroxy-butyl)-phenyl-carbamic acid tert-butyl ester vector molecule) although no $[^{18}\text{F}]$-fluorinated products were observed. The radioactivity level of the filter, containing the resin after the reaction, was checked and found to be of a comparable low level as for the resins with the 4-alkylphenyl sulfonate linkers. This indicated that displacement of the nitro group on the linker by $[^{18}\text{F}]$-fluoride was not taking place. However, the lack of $[^{18}\text{F}]$-fluorinated product formed in the reaction implied that the RLV construct had undergone significant degradation during storage at room temperature prior to the $[^{18}\text{F}]$-fluoridolysis reaction. $[^{18}\text{F}]$-Fluoridolysis results were not obtained for resins 2.51 and 2.54 with the 3-(2-
naphthalenoxy)-1-propanol vector due to the reverse phase HPLC conditions and setup employed by the radiochemists not being appropriate for this vector molecule.

3.5 Conclusions

All of the resins synthesised as part of this project (discussed in Chapter 2) successfully gave the desired $[^{19}\text{F}]$-fluorinated product when exposed to the conditions of $[^{19}\text{F}]$-fluoridolysis. A kinetic study was carried out using $^{19}\text{F}$ NMR to investigate the effect of altering the alkyl chain length of the 4-alkylphenylsulfonate linker and concluded that the rate of cleavage of the $[^{19}\text{F}]$-fluorinated product increased with linker length. The 4-nitrophenylsulfonate linker, thought to be the more reactive linker type, was found to have a lower cleavage rate than the shortest 4-alkylphenylsulfonate linker which was thought to be due to degradation of the 4-nitrophenylsulfonate linker rather than a true comparison of the relative reaction rates. A repeat cleavage experiment was carried out on a RLV construct containing the 4-alkylphenylsulfonate linker and showed that the same batch of resin was able to give consistent yields of the $[^{19}\text{F}]$-fluorinated product for up to five cycles before a significant drop in yield was observed. This showed that an appropriately functionalised resin, with the developed RLV construct, could be used to produce multiple batches of radiotracer and could, in principle, be easily adapted for use in automated radiosynthesis of $^{18}\text{F}$-labelled radiotracers. $[^{18}\text{F}]$-Fluoridolysis reactions were carried out successfully on resins with the 4-alkylphenylsulfonate linker to give the desired $^{18}\text{F}$-labelled vector molecule as the only radiochemical product, the highest yield of which was obtained with the resin with the longest linker length. The $[^{18}\text{F}]$-fluorinated products were formed in excellent radiochemical purity although were found to contain some chemical impurities most of which could be removed by HPLC purification although a close-running impurity was not separated. The results of the $[^{19}\text{F}]$-fluoridolysis reactions, kinetic study and lack of product formed in the $[^{18}\text{F}]$-fluoridolysis reaction implied that the 4-nitrophenylsulfonate linker had degraded since the completion of the synthesis which indicated there were problems with the stability of this linker type. Hence, of the two linker types developed, the 4-alkylphenylsulfonate linker was concluded as being the superior linker for use as part of the RLV construct. The 4-alkylphenylsulfonate linker with the longest linker length was found to have the fastest reaction kinetics and gave the best yields in the $[^{18}\text{F}]$-fluoridolysis reactions.
Chapter 4 – \(O\)-(2-\([^{18}\text{F}]\)-Fluoroethyl)-\(\text{L}\)-tyrosine

This chapter discusses the application of the 4-alkylphenylsulfonate linker as part of the RLV construct in a novel synthetic route to \(O\)-(2-\([^{18}\text{F}]\)-fluoroethyl)-\(\text{L}\)-tyrosine.

4.1 Introduction

4.1.1 Background

While the glucose analogue (2-deoxy-2-\([^{18}\text{F}]\)-fluoro)-\(\text{D}\)-glucose (\([^{18}\text{F}]\)-FDG) is routinely used in PET imaging for tumour diagnosis, the high uptake of \([^{18}\text{F}]\)-FDG in brain and non-malignant, inflammatory tissue can lead to a loss of contrast when imaging brain tumours and peripheral tumours (such as lymphoma, lung tumours and breast cancer).\(^{86}\) Hence it is possible that false-positive results could be obtained when imaging with \([^{18}\text{F}]\)-FDG if tumours and inflammatory processes are both present in the patient.\(^{87}\) The normal excretory route of \([^{18}\text{F}]\)-FGD is via the kidneys which also makes it of limited use for the investigation of renal and bladder cancers.\(^{88,89}\) Amino acids have a much lower uptake in normal brain tissue and so the contrast obtained when imaging brain tumours could be greatly improved by the use of positron-labelled amino acids instead of \([^{18}\text{F}]\)-FDG.\(^{90}\)

\[
\begin{align*}
\text{\([^{18}\text{F}]\)-FET} & \\
\begin{array}{c}
\text{O} \\
\text{CH}_2 \text{C} \text{H} \text{N} \text{H}_2 \\
\text{O} \\
\end{array}
\end{align*}
\]

\textbf{Figure 4.1} \(O\)-(2-\([^{18}\text{F}]\)-fluoroethyl)-\(\text{L}\)-tyrosine (\([^{18}\text{F}]\)-FET).

In 1999, Wester \textit{et al.} developed the synthesis of the tyrosine derivative \(O\)-(2-\([^{18}\text{F}]\)-fluoroethyl)-\(\text{L}\)-tyrosine (\([^{18}\text{F}]\)-FET, Figure 4.1) as a tracer for the imaging of cancer.\(^{90,91}\) Tyrosine derivatives could potentially give very good tracer molecules since tyrosine shows a high brain uptake and fluoroalkylation of the hydroxyl group gives a metabolically stable product.\(^{90}\) \([^{18}\text{F}]\)-FET is a tracer for the imaging of amino acid transport (a higher demand for which is related to increased needs for tumour growth\(^{91}\)) and should not incorporate into newly formed proteins and so no significant
accumulation of the tracer in peripheral organs would be expected.\textsuperscript{90} \[^{18}\text{F}\]-FET has been shown to be superior to \[^{18}\text{F}\]-FGD for brain tumour imaging in studies using rats\textsuperscript{92} and mice\textsuperscript{93} and is now in clinical use for tumour imaging.\textsuperscript{20}

4.1.2 Previous Syntheses

The synthesis of \[^{18}\text{F}\]-FET developed by Wester et al.\textsuperscript{90} involved a two-step reaction pathway consisting of the \[^{18}\text{F}\]-fluorination of ethylene glycol-1,2-ditosylate (4.01) and the subsequent \[^{18}\text{F}\]-fluoroethylation of unprotected \(\text{L-tyrosine}\) (Scheme 4.1). Reverse phase HPLC purification was required after each step of the synthesis and the product was passed through a strong cation exchange cartridge before formulation. The synthesis was completed in around 60 min with a radiochemical yield of 40\% (based on \[^{18}\text{F}\]-fluoride) and a radiochemical purity of 97-99\%. In 2006, the purification of 1-[\(^{18}\text{F}\)]-2-tosyloxyethane (4.02) was reported using disposable SPE cartridges instead of HPLC.\textsuperscript{94} The mono-fluorination of ditosylate 4.01 was carried out and the reaction mixture was then passed through a Sep-Pak silica cartridge, using \(n\)-hexane/ether (3:1) as eluent. However, the use of a range of solvents in the different purification steps was thought to make this method unsuitable for automated production.\textsuperscript{95}

\[
\text{TsO} - \text{OTs} \xrightarrow{a} ^{18}\text{F} - \text{OTs} \xrightarrow{b} ^{18}\text{F} - \text{[18F]-FET}}
\]

Scheme 4.1 The synthesis of \[^{18}\text{F}\]-FET by the \[^{18}\text{F}\]-fluoroethylation of \(\text{L-tyrosine}\).\textsuperscript{90} Reagents and conditions: a) \([\text{K/K2.2.2}]^+ \ ^{18}\text{F}^-\), MeCN, 90 °C; b) \(\text{L-Tyrosine (di-potassium salt)}\), DMSO, 90 °C.

In 2002, an alternative synthesis of \[^{18}\text{F}\]-FET was reported by Hamacher et al. which implemented a direct nucleophilic radiofluorination of protected alkyl tyrosine derivative \(O-(2\text{-tosyloxyethyl})-N\text{-trityl-\(\text{L-tyrosine}\)}\) (4.03).\textsuperscript{96} The synthesis (Scheme 4.2) involves the \[^{18}\text{F}\]-fluorination of tosylate 4.03, using the tetra butyl ammonium cation as the phase transfer agent rather than Kryptofix [2.2.2] as this gave higher radiochemical yields (the average radiochemical yield obtained for this reaction was >80\%). Deprotection of the trityl and tertiary butyl ester groups was carried out using
TFA and was followed by a solid phase extraction using a Si60 silica gel cartridge. The eluted $[^{18}\text{F}]-\text{FET}$ was then purified by reverse phase HPLC to give a radiochemical yield of 55-60% over the two steps and a radiochemical purity of $>99\%$, with a total synthesis time of about 80 min.

Further studies on a similar precursor to tosylate 4.03 but with different protecting groups were reported by Wang et al. in 2005. In this work, a self-modified automated PET tracer synthesiser was used following the same synthetic pathway to $[^{18}\text{F}]-\text{FET}$ that was previously outlined (Scheme 4.2) with the starting tosylate containing $N$-Boc and $O$-benzyl protecting groups. After $[^{18}\text{F}]-\text{fluoroethyla}tion$, the reaction mixture was purified using Sep-Pak Silica Plus cartridges before the deprotection, which was carried out using 1 N HCl rather than TFA and final HPLC purification was not required. The synthesis took approximately one hour to complete and gave $[^{18}\text{F}]-\text{FET}$ in a radiochemical yield of 50-55% (decay corrected) and with a radiochemical purity of $>98\%$.

An automated commercial synthesis of $[^{18}\text{F}]-\text{FET}$, following the original synthetic pathway (Scheme 4.1), was developed by Tang et al. in 2003. This fully automated synthesis utilised the commercial PETtrace FDG Microlab which is a computer controlled automated radiochemistry system that is routinely used for the synthesis of $[^{18}\text{F}]-\text{FDG}$ for clinical use. In this system, the chemical transformations and the final production of $[^{18}\text{F}]-\text{FDG}$ take place within a disposable cassette (one cassette per batch.
production run of $[^{18}\text{F}]$-FDG) and the same cassettes were used for the synthesis of $[^{18}\text{F}]$-FET. The synthetic strategy involves first the trapping of $[^{18}\text{F}]$-fluoride ions on a solid phase extraction column, where the trapping agent is 4-(4-methylpiperidinyl)pyridinium cations. Di-tosylate 4.01 is then passed through the column where it reacts with the trapped $[^{18}\text{F}]$-fluoride and is eluted as 1-$[^{18}\text{F}]$-2-tosyloxyethane (4.02), which is transferred to the $[^{18}\text{F}]$-fluoroethylation vessel along with L-tyrosine, sodium hydroxide and DMSO. Heating for 20 min at 100°C followed by elution through silica, C-18 and Al$_2$O$_3$ Sep-Pak cartridges gave $[^{18}\text{F}]$-FET in an overall radiochemical yield of 8-10% (non-decay corrected) and with a radiochemical purity of >95%. The synthesis time of 52 min is comparable to the original method although HPLC purification of the intermediate and the final product is not required and no operator handling is necessary except to remove the final product vial.

![Scheme 4.3](image)

**Scheme 4.3** The synthesis of $[^{18}\text{F}]$-FET from Ni$^{II}$ Schiff’s base precursor 4.05. 95

*Reagents and conditions:* a) Bu$_4$N$^+$-$[^{18}\text{F}]$, MeCN, 80°C; b) 0.5 M HCl, 120°C.

Most recently, in 2008 Krasikova et al. pursued a different approach to the synthesis of $[^{18}\text{F}]$-FET by elaborating the structure of a new labelling precursor. 95 Their precursor was based on a Ni$^{II}$ complex of a Schiff’s base of ($S$)-[N-2-(N’-benzylprolyl)amino]benzophenone (BPB) with alkylated L-tyrosine, Ni-(S)-BPB-(S)-Tyr-OCH$_2$CH$_2$OTs (4.05). Tosylate 4.05 was synthesised from the reaction of (S)-BPB and racemic tyrosine in the presence of a nickel nitrate salt to form the Ni$^{II}$ complex followed by O-alkylation of the hydroxyl group of tyrosine and then tosylation of the O-hydroxyethyl group. The one-pot radiosynthesis of $[^{18}\text{F}]$-FET is then accomplished in two steps (Scheme 4.3), the nucleophilic fluorination again using the tertiary butyl ammonium cation followed by hydrolysis/deprotection using moderate aqueous
conditions (only minimal intermediate purification steps were required). The crude product was then purified by semi-preparative HPLC to give $[^{18}\text{F}]-\text{FET}$ in a radiochemical yield of 57% and a radiochemical purity of >99% with an overall synthesis time of 55 min.

### 4.1.3 The Brown group approach to the synthesis of $\text{O}(2-[^{18}\text{F}]-\text{fluoroethyl})\text{-L-tyrosine}$

Our strategy for the synthesis of $[^{18}\text{F}]-\text{FET}$ utilises a solid supported precursor, based on the resin-linker-vector (RLV) construct, which can be used in a direct nucleophilic radiofluorination reaction followed by a quick deprotection step to give the product. Our 4-alkylphenylsulfonate linker, which mimics the tosylate leaving group, was used to synthesise a solid supported FET precursor analogous to the solution phase tosylate precursors discussed previously. A solid supported precursor to $[^{18}\text{F}]-\text{FET}$ has not been reported in the literature and has the potential to simplify the purification of the product through the use of filtration to remove the solid supported leaving group in the fluorination reaction and the unreacted precursor. Ideally, the use of this precursor would make HPLC purification unnecessary. The strategy involves the synthesis of alcohol **4.07** (an alcohol precursor to FET, Figure 4.2) which uses a different combination of protecting groups to those previously reported. This would be attached to a solid support via the 4-alkylphenylsulfonate linker in the manner developed as part of this work.

![Figure 4.2 Alcohol 4.07 for attachment to the solid phase to give the solid supported precursor to $[^{18}\text{F}]-\text{FET}$.

### 4.2 Synthesis of solid supported FET precursor

The alcohol precursor to FET for use as part of the RLV construct was synthesised from commercially available $N-(\text{tert-butoxycarbonyl})\text{-L-tyrosine (4.08)}$. The first step in the synthesis was the protection of the free acid as the tertiary butyl ester (4.09) which was
carried out following a modified procedure to that described by Chevallet et al. in excellent yield (Scheme 4.5). The O-alkylation of ester 4.09 to give alcohol 4.07 (the alcohol precursor to FET) turned out to be much harder than anticipated due to the consistent low conversion from this reaction when carried out directly.

A series of test reactions were carried out using a range of bases, additives and solvents in order to establish the best conditions for the O-alkylation reaction (Table 4.1). None of the reactions were found to go to completion and some of the conditions tried were found not to give any of the desired product (entries 9 and 12). The use of potassium carbonate gave a better conversion than caesium carbonate with the same solvent (entries 1 and 2 compared with 10 and 11). The use of BTEAC or Bu4NI as additives resulted in an increase in the conversion of the reaction although the final yield was still less than 50% (entries 6-8). Of the solvents tested, DMF gave the best conversion and was found suppress the multiple additions of the O-ethoxy unit which was observed as a by-product with other solvents (namely acetonitrile, tetrahydrofuran, acetone and

### Table 4.1 Test reactions for step (b), Scheme 4.4.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Bromoethanol</th>
<th>Base</th>
<th>Additive</th>
<th>Solvent</th>
<th>Outcome (ratio 4.09:4.07)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>-</td>
<td>DMF</td>
<td>5.5:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>-</td>
<td>Acetone&lt;sup&gt;98&lt;/sup&gt;</td>
<td>7.9:1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>-</td>
<td>Benzene&lt;sup&gt;99&lt;/sup&gt;</td>
<td>10:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>-</td>
<td>MeCN</td>
<td>5.8:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>-</td>
<td>THF</td>
<td>7.5:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>3 eq</td>
<td>K2CO3</td>
<td>BTEAC</td>
<td>DMF</td>
<td>1.2:1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>1 eq</td>
<td>K2CO3</td>
<td>BTEAC</td>
<td>DMAC</td>
<td>1.7:1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>1.1 eq</td>
<td>K2CO3</td>
<td>Bu4NI</td>
<td>DMF&lt;sup&gt;100&lt;/sup&gt;</td>
<td>2:1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>5 eq</td>
<td>K2CO3</td>
<td>NaI</td>
<td>Acetone&lt;sup&gt;101&lt;/sup&gt;</td>
<td>1:0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>3 eq</td>
<td>Cs2CO3</td>
<td>-</td>
<td>DMF</td>
<td>9.8:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>3 eq</td>
<td>Cs2CO3</td>
<td>-</td>
<td>Acetone</td>
<td>11:1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>1 eq</td>
<td>NaOH</td>
<td>-</td>
<td>MeOH&lt;sup&gt;102&lt;/sup&gt;</td>
<td>1:0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Yields were determined from the <sup>1</sup>H NMR of the crude products. <sup>b</sup>Yields of compounds isolated by chromatography.
methanol). The best conversion was seen with potassium carbonate and BTEAC in DMF (entry 6) although isolation of the product resulted in only a 33% yield of the desired product after a reaction time of 48 h.

Scheme 4.4 Reagents and conditions: a) Ethylbromoacetate, Cs₂CO₃, dioxane, 80 °C; b) LiOH, MeOH/H₂O; c) Alcohol 4.13, tri-n-butylphosphine, ADDP, CH₂Cl₂/THF, 0 °C to rt; d) 1% aq NaOH/MeOH.

An alternative method for the O-alkylation of ester 4.09 was investigated involving the formation of ethyl ester 4.10 and then the successive hydrolysis to carboxylic acid 4.11 and then reduction to alcohol 4.07 in an attempt to improve the overall yield of this transformation (Scheme 4.4). The formation of ethyl ester 4.10 was successful albeit in only moderate yield but the subsequent hydrolysis to give carboxylic acid 4.11 failed. This was thought to be due to the acidic workup causing the cleavage of the N-Boc and tertiary butyl protecting groups. A two-step method via protected alcohol 4.12 was found to be much more successful. Mono-protected ethylene glycol (4.13) (synthesised in good yield following the procedure described by Wiseman et al.¹⁰⁴) was coupled to ester 4.09 in a moderately yielding Mitsunobu reaction using the modified conditions of tri-n-butylphosphine and 1,1’-(azodicarbonyl) dipiperidine (ADDP) which had been
reported to be good reagents for the $O$-alkylation of tyrosine\textsuperscript{105} (more standard Mitsunobu reagents, triphenylphosphine and DIAD, gave lower yields). Removal of the benzoyl protecting group under standard conditions gave alcohol 4.07 in good yield. The yield over the two steps was only 27\% which is less than the one step method and although the total reaction time was considerably shorter (less than six hours versus forty-eight hours) the two step route did not prove to be a better strategy than the direct method.

![Scheme 4.5 Reagents and conditions](image)

Gratifyingly, when the direct $O$-alkylation reaction was performed on a much larger scale (>10 mmol) using bromoethanol (1.1 eq, followed by 0.5 eq after forty-eight hours), K$_2$CO$_3$ (3 eq) and BTEAC (2 eq) in DMF at 50 °C for a total reaction time of 72 h the conversion was greatly improved and a 73\% yield of alcohol 4.07 was obtained, along with 9\% recovered phenol 4.09 (Scheme 4.5). This reaction completed the synthesis of the FET precursor which followed a two step pathway with an overall yield of 67\%.

Alcohol 4.07 was reacted in excellent yield with sulfonyl chloride 2.03 (the 4-alkylphenylsulfonate linker type with the longest chain length of five methylene units) to give sulfonate ester 4.14 (Scheme 4.6). The solution phase fluoridolysis was carried out using sulfonate ester 4.14 to make sure the reaction worked before it was transferred to the solid phase and a 48\% yield of protected [$^{19}$F]-FET ([$^{19}$F]-4.15) was obtained which was very encouraging. Enzyme mediated hydrolysis of sulfonate ester 4.14 gave
free acid 4.16 in moderate yield which was then coupled to the solid support to give resin 4.17.

Scheme 4.6  
Reagents and conditions: a) Sulfonyl chloride 2.03, Et₃N, DMAP, CH₂Cl₂; b) KF, Kryptofix [2.2.2], MeCN, 80 °C; c) Novozym 435®, aq phosphate buffer (pH 7), CH₂Cl₂/acetone, 50 °C; d) Amino methyl polystyrene resin, HOBT, DIC, DMF/CH₂Cl₂.  
*Calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin.

A small scale test reaction to investigate the removal of the N-Boc and tertiary butyl protecting groups was carried out using the standard reagent, trifluoroacetic acid. TLC of the reaction showed a clean, spot to spot conversion of the starting material after a reaction time of only 10 min and mass spectrometry confirmed the presence of the desired molecular ion indicating the formation of the product. No further work was carried out on this reaction as the deprotection step would need to be optimised as part of the radiochemical studies with resin 4.17 and some tyrosine derivative deprotection strategies have been previously reported.⁹²,⁹⁵,⁹⁶  This work showed the feasibility of the synthesis of [¹⁸F]-FET by the cleavage and subsequent deprotection of a solid supported precursor using our developed RLV strategy.
4.3 Solid phase fluoridolysis

$[^{19}\text{F}]-\text{Fluoridolysis}$ of resin 4.17 was carried out using potassium fluoride and Kryptofix [2.2.2] in acetonitrile at 80 °C to give $[^{19}\text{F}]-4.15$ in a 50% yield after a reaction time of 45 min (Scheme 4.7). The purification of $[^{19}\text{F}]-4.15$ involved filtration of the reaction mixture which was then passed through a short silica column. The yield of product was comparable to that obtained from the analogous solution phase reaction and significantly higher than the yields achieved for the (4-hydroxy-butyl)-phenyl-carbamic acid tert-butyl ester (2.14) vector molecule with the same linker type.

![Scheme 4.7 Reagents and conditions: a) KF, Kryptofix [2.2.2], MeCN, 80 °C.](image)

4.4 Radiochemistry results

We gratefully acknowledge Imtiaz Khan of GE Healthcare, White Lion Road, Amersham, UK, for carrying out the radiochemistry experiments with our resins.

The $[^{18}\text{F}]-\text{fluoridolysis}$ reaction using resin 4.17 was carried out as discussed in Chapter 3 to give $[^{18}\text{F}]-4.15$ with a 53% incorporation of $[^{18}\text{F}]-\text{fluoride}$ and a non-decay corrected yield of 23% and was formed in excellent radiochemical purity. These results were comparable with those obtained for resin 2.17 which was made up of the same length 4-alkylphenylsulfonate linker and the (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester vector molecule.

The HPLC analyses of the $[^{18}\text{F}]-\text{fluoridolysis}$ reaction with resin 4.17 are shown in Figure 4.3, where, in both analyses, the radioactive trace is shown as the red line and the
UV trace is shown as the blue line. The top trace is the HPLC analysis of the crude reaction mixture which was acquired after filtration of the reaction mixture through an acrodisc to remove the resin from the solution. The radioactivity trace showed only two peaks due to the unreacted $^{18}$F-fluoride and the $^{18}$F-fluorinated product, $[^{18}$F]-4.15. The reaction mixture was then formulated (as outlined in Chapter 3) and the HPLC analysis was repeated. The radioactive trace now showed only one peak due to the desired $^{18}$F-fluorinated product as the only radiochemical product of the reaction, demonstrating that it had been formed in high radiochemical purity.

![Figure 4.3](image.png)

**Figure 4.3** Reverse phase HPLC analyses of the $[^{18}$F]-fluoridolysis reaction with resin 4.17 of the crude reaction mixture (top) and the formulated reaction mixture (bottom). In both cases the red line is the radioactive trace and the blue line is the UV trace (254 nm).

The UV traces, the blue lines, show the chemical make-up of the crude and formulated reaction mixtures and give an idea of the chemical purity of the solutions. Since the unreacted precursor remained attached to the resin and was removed by filtration, the
UV traces don’t contain a large peak due to the excess of precursor used in the reaction. Due to the tiny amount of $[^{18}\text{F}]-\text{fluoride}$ used in the reaction, a chemical peak due to the $[^{18}\text{F}]-\text{fluorinated}$ product was not expected and so all of the UV peaks seen are a result of side reactions or degradation of the RLV construct or the resin beads themselves. The UV traces showed some chemical impurities to be present in both the crude and formulated reaction mixtures. At the solvent front, at around the same retention time as the $[^{18}\text{F}]-\text{fluoride}$ ion peak (around two min), often a response is seen when the solvent hits the UV flow cell which may be the cause of the UV peaks with retention times between one and three min. Alternatively, these may be due to hydrophilic by-products of the $[^{18}\text{F}]-\text{fluoridolysis}$ reaction. None of the chemical impurities shown in the UV traces were identified. Since there were no chemical impurities with a similar retention time to the $[^{18}\text{F}]-\text{fluorinated}$ product, HPLC purification of the reaction mixture was carried out to remove the chemical impurities (Figure 4.4). This gave a solution of $[^{18}\text{F}]-4.15$ with excellent chemical purity (only one very minor impurity just before 17 min remained).

**Figure 4.4** Reverse phase HPLC analysis of the HPLC purified reaction mixture from the $[^{18}\text{F}]-\text{fluoridolysis}$ reaction with resin 4.17. The red line is the radioactive trace and the blue line is the UV trace (254 nm).

### 4.5 Conclusions

The RLV construct was applied in a new synthetic route to $[^{18}\text{F}]-\text{FET}$. A precursor to FET, alcohol 4.07, was synthesised and was attached to a solid support as part of a RLV construct using the developed 4-alkylphenylsulphonate linker to give resin 4.17. The resin was shown to release protected $[^{18}\text{F}]-\text{FET}$ in good yield and excellent
radiochemical purity when exposed to $[^{18}\text{F}]$-fluoride during radiolabelling experiments. HPLC purification of the reaction mixture gave the product in excellent chemical purity.
Chapter 5 – Galanthamine Analogue

This chapter covers the synthesis of a fluorinated analogue of (−)-galanthamine which has the potential for use as a new imaging agent for use in PET.

5.1 Introduction to galanthamine

5.1.1 Background

(−)-Galanthamine (−-5.01, Figure 5.1) is a naturally occurring Amaryllidaceae alkaloid which can be isolated from the Caucasian snowdrop (Galanthus woronowii)

and from the bulbs of daffodils (Pseudonarcisses L.).

It is a centrally acting, selective, reversible and competitive inhibitor of acetylcholinesterase (AChE) as well as an allosteric modulator of the neuronal nicotinic receptor for acetylcholine and so is used for the symptomatic treatment of Alzheimer’s disease.

The key feature of Alzheimer’s disease is a loss in cognitive function which is characterised by loss of short-term memory and learning ability, impaired attention associated with relentlessness, disturbances of language and emotional instability. In early studies, patients with Alzheimer’s disease who were given (−)-galanthamine (−-5.01) showed significant improvement in performance and attention and the compound was well tolerated. Today, (−)-galanthamine (−-5.01), which is commercially available as galanthamine hydrobromide (Razadyne® or Reminyl®), has become one of the top drugs for the symptomatic treatment of early on-set Alzheimer’s disease.
The low yield (0.1-2% dry weight)\textsuperscript{106} and high cost (~$50,000/kg)\textsuperscript{107} of extracting (−)-galanthamine ((−)-\textbf{5.01}) from natural sources coupled with the biological interest of (−)-galanthamine ((−)-\textbf{5.01}) for clinical use has led to significant activity in the development of a synthetic process for its large scale commercial production.\textsuperscript{109,110}

\section{5.1.2 Previous syntheses of galanthamine}

The structure of (−)-galanthamine ((−)-\textbf{5.01}) is complex incorporating four fused rings, three of which are bridged with a quaternary stereocentre, and so provides the synthetic chemist with a challenging target molecule. A number of syntheses of racemic galanthamine have been published since the early 1960s,\textsuperscript{106} the latest of which was reported in 2006.\textsuperscript{111} The first asymmetric synthesis of enantiomerically pure galanthamine was reported in the late 1970s and a number of other syntheses have been published since that time.\textsuperscript{106,107,112-118} The pharmacological and synthetic strategies to (−)-galanthamine ((−)-\textbf{5.01}) have been discussed in detail in a review by Marco-Contelles \textit{et al.} published in 2006\textsuperscript{106} and in the thesis of Miller in 2008\textsuperscript{119} and so will only be discussed briefly herein, along with an overview of the more recent work on the synthesis of (−)-galanthamine ((−)-\textbf{5.01}).

The synthetic approaches to galanthamine may be categorised into two groups based on the method employed for the formation of the stereogenic quaternary centre of the molecule: biomimetic approaches and other approaches.\textsuperscript{106} The biomimetic approach is characterised by the use of phenolic oxidative coupling in the presence of a metal oxidant and was first reported by Barton \textit{et al.} in 1962.\textsuperscript{120} In this synthetic pathway, a phenolic oxidative coupling is used to close the D ring followed by a Michael addition of the phenolic oxygen to the dienone system to give narwedine (\textbf{5.04}) which is then reduced to give galanthamine (\textbf{5.01}). Node and co-workers investigated this strategy in 2001\textsuperscript{113} which led to their total synthesis of (−)-galanthamine ((−)-\textbf{5.01}) in an overall yield of 23% over 14 steps in 2004\textsuperscript{112} which was further elaborated in 2006 (22% over 13 steps).\textsuperscript{114} More recently, in 2008 this approach was used by Reddy \textit{et al.} in an alternative synthesis of (−)-galanthamine hydrobromide.\textsuperscript{115} The pilot scale process for the kilogram synthesis of (−)-galanthamine ((−)-\textbf{5.01}) was developed utilising the phenolic oxidative coupling to give racemic narwedine (\textpm\textbf{5.04}) which then underwent
a crystallisation-induced chiral conversion to give (−)-narwedine ((−)-5.04). This second order asymmetric transformation is achieved without the use of a chiral auxiliary other than the presence of seed crystals of (−)-narwedine (−)-5.04. Reduction using L-Selectride® gives (−)-galanthamine ((−)-5.01) which is finally reacted with hydrogen bromide to give (−)-galanthamine hydrobromide, the active component in drugs used in the treatment of Alzheimer’s disease. This large scale, commercial synthesis of (−)-galanthamine hydrobromide obtained yields of up to 19% over nine steps.

Scheme 5.1 Biomimetic synthesis of (−)-galanthamine ((−)-5.01).

The non-biomimetic approaches utilise several alternative strategies for the construction of the stereogenic quaternary centre. The use of an intermolecular Heck reaction was employed in the asymmetric synthesis of (−)-galanthamine ((−)-5.01) by Trost et al. in 2005 (10 steps from commercially available materials, 8% overall yield, 96% ee) and was also used in 2007 by Satcharoen et al. (11 linear steps). Hu et al. generated the quaternary centre in their synthesis of (±)-galanthamine using a semipinacol rearrangement (13 steps, 12% overall yield). Finally, the asymmetric synthesis of (+)-galanthamine using a Claisen rearrangement was reported by Tanimoto et al. in 2007. The stereoselective total synthesis of (+)-galanthamine, the enantiomer of the
natural product, could be applied in the synthesis of structural analogues that are not available by the chemical modification of (−)-galanthamine ((−)-5.01).\textsuperscript{117}

5.1.3 Structural analogues of galanthamine

The desire to synthesise structural analogues of (−)-galanthamine ((−)-5.01) originates from the need to develop more potent drug molecules. Structure-activity studies had identified four sites on the structure of (−)-galanthamine ((−)-5.01) which could undergo chemical modification: the hydroxyl group, the B ring, the tertiary amine and the methoxy group.\textsuperscript{121} This work led to the synthesis of a number of galanthamine analogues of which galanthamine \textit{n}-butyl carbamate (5.05) was found to have greater AChE inhibitory activity than (−)-galanthamine ((−)-5.01) (Figure 5.2).\textsuperscript{121}

![Figure 5.2](attachment:figure52.png)

\textbf{Figure 5.2} (−)-Galanthamine analogues which were found to have increased AChE inhibitory activity compared to the parent compound.

The crystallographic structure of AChE from electric fish (\textit{Torpedo californica}) was determined in 1991 to contain an active site composing of a catalytic triad located in the bottom of a narrow gorge along with peripheral binding sites at the bottom of the cavity.
and at the opening of the gorge.\textsuperscript{122} This information provided the rationale behind the synthesis of a series of \emph{bis}-ligand galanthamine analogues which were designed to simultaneously interact with the active site of AChE and the peripheral sites and thereby optimise the inhibition potency.\textsuperscript{123} Compounds \textbf{5.07}, \textbf{5.08} and \textbf{5.09} (Figure 5.2) were found to be more active than (−)-galanthamine ((−)-\textbf{5.01}) in inhibiting AChE. The simpler iminium salt of galanthamine, compound \textbf{5.06} (Figure 5.2), was confirmed as able to cross the blood-brain barrier (thought to be due to the presence of an equilibrium between the iminium functionality and the neutral carbinol amine) as it was shown to enhance learning and memory in young and old rats.\textsuperscript{124,125}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{galanthamine_analogues.png}
\caption{\textbf{5.07} X = CH\textsubscript{2}Cl, Y = CO\textsubscript{2}Et \hfill \textbf{5.08} X = CH\textsubscript{2}OH, Y = H \hfill \textbf{5.09} X = CHO, Y = H \hfill \textbf{5.10} \hfill \textbf{5.11} \hfill \textbf{5.12} \hfill \textbf{5.13} \hfill \textbf{5.14} \hfill \textbf{5.15}}
\end{figure}

\textbf{Figure 5.3} (−)-Galanthamine analogues which were found to have lower or no inhibitory activity compared to the parent compound.

The synthesis of a series of (−)-galanthamine analogues with open D rings (compounds \textbf{5.10}, \textbf{5.11} and \textbf{5.12}, Figure 5.3) was reported by Herem \textit{et al.} although these were found to have lower activity than the parent compound.\textsuperscript{126} Treu and co-workers synthesised a sulfur analogue (\textbf{5.13})\textsuperscript{127} and an analogue with a crinine-type alkaloid structure (\textbf{5.14}),\textsuperscript{128} both of which showed no inhibitory activity toward AChE. (−)-Galanthamine analogue \textbf{5.15} was synthesised by Liang \textit{et al.} but it was found to have very poor
activity which highlighted the importance of the C ring in the structure of the parent compound.\textsuperscript{129}

5.1.4 Labelled analogues of galanthamine

In 2001, the US Food and Drug Administration approved the use of the hydrobromic acid salt of (−)-galanthamine (\textit{[(−)-5.01]}) for the treatment of mild to moderate Alzheimer’s disease (under the name Razadyne\textsuperscript{®} or Reminyl\textsuperscript{®}). In the process leading to the final approval, \textit{[14C]}-carbon-, tritium- and stable-isotope-labelled galanthamine analogues were synthesised for use in pharmacokinetic research.\textsuperscript{130} The \textit{[14C]}-carbon-containing galanthamine analogues were synthesised by the selective N- or O-demethylation of galanthamine followed by reaction with \textit{[14C]}-methyl iodide to give compounds \textit{5.16} and \textit{5.17}, respectively (Figure 5.4). The stable-isotope-labelled galanthamine analogue \textit{5.18} was obtained likewise by \textit{13CD3OD}-methylation of O-demethylated galanthamine under Mitsunobu conditions. A tritium-containing (−)-galanthamine analogue \textit{5.19} was synthesised by the reduction of racemic (±)-1-bromonarwedine using L-Selectride\textsuperscript{®} and then bromo-tritium exchange followed by a final resolution.

Also in 2001, Linnemann \textit{et al.} synthesised a tritium-containing (−)-galanthamine analogue \textit{(5.20)} to facilitate further investigation of AChE inhibition by galanthamine and to analyse the interaction between galanthamine and the nicotinic acetylcholine-receptor (Figure 5.4).\textsuperscript{131} The authors used tritiated L-Selectride\textsuperscript{®} to reduce (−)-narwedine \textit{((−)-5.04)} to give tritiated (−)-galanthamine \textit{(5.20)} in good yield. This compound was subsequently used as a photoaffinity probe to aid the localisation of the allosteric binding site of (−)-galanthamine \textit{((−)-5.01)} at the nicotinic acetylcholine receptor (nAChR) in studies \textit{in vitro}.\textsuperscript{132} To examine the mechanism of the interaction of (−)-galanthamine \textit{((−)-5.01)} derivatives with nicotinic acetylcholine receptors, Schildan and co-workers synthesised tritium labelled 10-methylgalanthamine hydroiodide \textit{(5.21)} (Figure 5.4).\textsuperscript{133,134} Galanthamine was reacted with tritritium methyl iodide to give tritiated 10-methylgalanthamine hydroiodide \textit{(5.21)} in a radiochemical yield of >70%.
More recently in 2008, Rouleau et al. reported the synthesis of deuterium labelled (−)-galanthamine (5.22) for use in biological studies (Figure 5.4).\textsuperscript{135} Their synthesis, starting with (−)-galanthamine ((−)-5.01), involved the selective $O$-demethylation and then the previously unknown $N$-demethylation via the $N$-oxide. The secondary amine was then protected and alkylation of the phenol using dimethylsulfate-$d_6$ was carried out. Removal of the protecting group and treatment with deuterated sodium borohydride gave hexadeuterated (−)-galanthamine 5.22 in an overall yield of 25% over seven steps. The inclusion of six deuterium atoms would allow better detection of this compound by a method based on deuterium analysis.\textsuperscript{135}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.4.png}
\caption{Labelled analogues of (−)-galanthamine.}
\end{figure}

Schildan and co-workers also mentioned, in an earlier symposium abstract, the synthesis of [$^{18}$F]-fluoride containing (−)-galanthamine derivatives 5.23 and 5.24, that had the
potential for use in PET studies and psychopharmacological examinations. Their synthesis involved the fluoroalkylation of O-demethylated and N-demethylated (−)-galanthamine using 2-[18F]-fluoroethyltosylate in the same manner as in the first synthesis of [18F]-FET (see Chapter 4). The [18F]-fluoride (−)-galanthamine derivatives 5.23 and 5.24 were found to have an allosteric potentiating affect (albeit weak) and an increased nicotinic response, although this was only 50% of that seen with (−)-galanthamine. However, this showed that some activity was retained when the fluoroethoxy units were added to the basic structure of (−)-galanthamine (−)-5.01 making them promising imaging agents for use in PET.

The fluorinated (−)-galanthamine analogues 5.23 and 5.24 were of much interest to us due to the Brown group’s previous work on the total synthesis of (−)-galanthamine and our work on the RLV concept for the synthesis of labelled drug molecules for PET. Hence, we decided to undertake the synthesis of (−)-galanthamine analogue 5.23.

5.1.5 Our strategy for the synthesis of galanthamine analogue 5.23

The Brown group’s asymmetric synthesis of (−)-galanthamine (−)-5.01 was published in 2007, with a second generation synthesis completed a year later by Miller. Our synthesis of (−)-galanthamine analogue 5.23 follows the synthetic pathway developed by Miller, using a modified starting material, to give diol 5.25. Instead of starting with iso-vanillin, 3,4-dihydroxybenzaldehyde (5.26) would be selectively alkylated on the 4-position using O-Boc protected bromoethanol (5.27) and then undergo a series of transformations, as previously described, to give aromatic fragment 5.28 (Scheme 5.2). Enantiomerically enriched propargylic fragment 5.29 would be coupled to aromatic fragment 5.28 before oxidative cleavage of the terminal olefin to give aldehyde 5.30. Asymmetric allylation would give alcohol 5.31 and enyne RCM would then be used to close the B ring. Hydroboration, Heck coupling to close the C ring and finally azepine ring formation would provide alcohol 5.33. Activation of the free hydroxyl group, either in solution phase via the formation of the tosylate or by attachment to the solid phase using the developed 4-alkylphenylsulfonate linker, followed by fluoridolysis would give fluorinated (−)-galanthamine analogue 5.23.
Scheme 5.2 Retrosynthetic analysis of (−)-galanthamine analogue 5.23.

5.2 Synthesis of galanthamine analogue (−)-5.25

5.2.1 Synthesis of aromatic fragment 5.28

The O-hydroxy ethyl group, replacing the methoxy group on galanthamine, was installed at the beginning of the synthesis of aromatic fragment 5.28 as the O-Boc derivative. Aromatic fragment 5.28 was synthesised from cheap, commercially available starting materials bromoethanol (5.34) and 3,4-dihydroxybenzaldehyde. A range of reagents and conditions were tested in the reaction to form the O-Boc protected
bromoethanol (5.27). The best yield was obtained following a modified procedure to that described by Mejorado et al. which used di-tert-butyldicarbonate, catalytic DMAP and DIPEA and gave bromoalkane 5.27 in good yield (Scheme 5.3).\textsuperscript{136}

![Scheme 5.3](image)

**Scheme 5.3** Reagents and conditions: a) Di-tert-butyldicarbonate, DMAP, DIPEA, THF; b) 3,4-Dihydroxybenzaldehyde (5.26), NaH, DMF, 0 to 40 °C; c) ICl, pyridine, dioxane, 0 °C to rt; d) i) MeNH\textsubscript{2}, MeOH, ii) NaBH\textsubscript{4}, MeOH, molecular sieves, iii) Di-tert-butyldicarbonate, sat aq NaHCO\textsubscript{3}, brine, CHCl\textsubscript{3}, 50 °C.

The selective addition of bromoalkanes to the 4-position of 3,4-dihydroxybenzaldehyde (5.26) using sodium hydride in DMF had been reported previously by Nicolaou et al.\textsuperscript{137} In our hands, the reaction of bromoalkane 5.27 with 3,4-dihydroxybenzaldehyde (5.26) gave, in moderate yield, the desired aldehyde 5.35 as the major product, with addition to the 4-position. The reaction also gave addition to the 3-position and the double addition product as minor by-products. The double addition product was found to be an oil and so could be easily separated from the white solid of aldehyde 5.35 by recrystallisation. The 3-position addition product was found to be a solid of similar polarity to the product and so was very difficult to separate by column chromatography. However, it was found not to react under the conditions of iodination, the next step in the synthesis, and so small amounts could be carried through and removed after the next step. The \textit{O}-alkylation reaction to give aldehyde 5.35 was found not to have gone to completion even after reaction times of up to 5 days (\textsuperscript{1}H NMR of the crude reaction mixture showed 30% of the starting 3,4-dihydroxybenzaldehyde to still be present). Efforts to improve
the conversion of the reaction either by using more than one equivalent of sodium hydride or by increasing the reaction temperature above 40 °C were found to give predominately the undesired double addition product. The use of sodium bis(trimethylsilyl)amide instead of sodium hydride was tested but upon addition this was observed to give an insoluble precipitate and TLC of the reaction mixture after 24 h showed no product to have formed.

The regioselective iodination of aldehyde 5.35 was achieved using iodine monochloride according to the procedure described by Markovich et al.\textsuperscript{138} The reaction is extremely slow requiring five days at room temperature to give aldehyde 5.36 in a yield of 51%. Aldehyde 5.36 was converted to N-Boc protected amine 5.28 via a three step condensation, reduction and N-Boc protection sequence. The intermediate compounds were not isolated and the crude mixtures were carried through to give protected amine 5.28 in a yield of 80% over three steps. The conditions for the N-Boc protection were adjusted from those used by Miller (di-tert-butyldicarbonate, sodium hydroxide, dioxane)\textsuperscript{119} because there was evidence to show that the phenol was also being protected. The milder conditions of di-tert-butyldicarbonate in chloroform and a mixture of aqueous sodium carbonate and brine were successful to give protected amine 5.28 with the free phenol group.

### 5.2.2 Synthesis of enantiomerically enriched propargylic alcohol (+)-5.29

The synthesis of enantiomerically enriched alcohol (+)-5.29 was achieved via the enzymatic resolution of racemic alcohol (±)-5.29 following the procedure described by Burova et al.\textsuperscript{139}. A Mitsunobu reaction was included to recycle the undesired enantiomer, transforming alcohol (−)-5.29 into acetate (+)-5.39 (Scheme 5.4).

Commercially available TMS acetylene (5.37) was formylated in good yield following the procedure described by Journet et al.\textsuperscript{140} The low boiling point of aldehyde 5.38 (40 °C, 25 mm/Hg\textsuperscript{141}) can make isolation and purification of the product difficult and lead to lower yields. Miller reported a yield of 56% for this reaction carried out on a 56 mmol scale and isolating the product by careful distillation at atmospheric pressure followed by purification by Kugelrohr distillation.\textsuperscript{119} In our hands, the reaction was carried out on a 145 mmol scale, the bulk of the solvent was removed by distillation at
atmospheric pressure and the remaining solvent was removed under reduced pressure to give an increased yield of 81% of aldehyde 5.38 which was used without further purification. Aldehyde 5.38 was then treated with allylmagnesium bromide to give racemic propargylic alcohol (±)-5.29 in good yield.

![Chemical structure](image)

**Scheme 5.4** *Reagents and conditions:* a) n-BuLi, Et₂O, −40 °C, then DMF; b) Allylmagnesium bromide, Et₂O, −50 to −10 °C; c) Amano® AK20 lipase, vinyl acetate, molecular sieves, hexane; d) DIAD, PPh₃, AcOH, pyridine, THF, −50 °C to rt; e) DIBAL-H, CH₂Cl₂, −78 °C.

The enzymatic resolution of racemic alcohol (±)-5.29 was achieved using Amano® AK20 lipase in the presence of vinyl acetate¹³⁹ which acylated only the desired (+)-enantiomer to give acetate (+)-5.39 in excellent yield (94% theoretical maximum yield). The undesired enantiomer (−)-5.29 was inverted to give acetate (+)-5.39 in good yield through a Mitsunobu reaction using acetic acid as described by Jin et al.,¹⁴² using DIAD as the azodicarboxylic coupling agent rather than DEAD as was published. Deprotection using DIBAL-H selectively cleaved the O-Ac protecting group and left the C-TMS protection in place to give alcohol (+)-5.29 in quantitative yield.
5.2.3 Mosher ester analysis to determine the absolute configuration and enantiomeric excess of alcohols (+)-5.29 and (−)-5.29

The stereochemistry of alcohol (+)-5.29 fixes an important stereogenic centre in the final galanthamine analogue 5.23 and so we wanted to check that we had made the desired enantiomer and to establish enantiomeric purity. The assignment of absolute configuration can be carried out in a number of ways including: the comparison of the optical rotation with synthetic analogues with known configuration; X-ray crystallographic or optical rotary dispersion methods; the use of NMR spectroscopy.\textsuperscript{143}

Out of the NMR based methods, the one now most commonly used for the determining the absolute configuration of the stereogenic carbon centre in secondary alcohols was first reported in 1973\textsuperscript{144} and has come to be known as the Mosher ester analysis. This method involves the reaction of the alcohol of unknown configuration with both the \( R \) and \( S \) enantiomers of Mosher’s acid, \( \alpha \)-methoxy-\( \alpha \)-trifluoromethyl phenylacetic acid (MTPA). This gives a pair of diastereomeric Mosher esters which will have differing \(^1\text{H}\) NMR spectra and from the comparison of the chemical shifts of certain protons the absolute configuration of the original alcohol can be deduced.

![Mosher esters](image)

**Figure 5.5** Relationship between Mosher esters: dashed line – diastereoisomers; solid line – enantiomers.

In order to determine the absolute configuration of alcohol (+)-5.29, the synthesis of the corresponding Mosher’s esters would be required. To check the selectivity of the
enzymatic resolution using lipase Amano® AK20 the synthesis of Mosher esters of alcohol \((-)-5.29\) would also be required. Additionally, this would give us a way of deducing the enantiomeric purity of alcohol \((+)-5.29\) by the comparison of the \(^1\)H NMR spectra of the resulting Mosher esters of the two alcohols. Mosher ester analysis normally requires the synthesis of both of the \(R\) and \(S\) Mosher esters of both alcohols. However, because of the relationship between diastereoisomers and enantiomers (enantiomers having identical physical and spectroscopic properties) for our strategy it was only necessary to synthesise one Mosher ester of each enantiomeric alcohol (see Figure 5.5).

Mosher esters \((+)-5.40\) and \((-)-5.40\) were synthesised in moderate yields following the procedure of Fos et al.\(^{145}\) using the \(R\) enantiomer of Mosher’s acid, \((R)-(+)\)-MTPA. The Mosher’s acid was first converted, using excess thionyl chloride, to the Mosher acid chloride, \((S)-(+)\)-MTPA chloride, which was used without further purification in the formation of the \(R\) Mosher esters \((+)-5.40\) and \((-)-5.40\) (note the change in the stereochemical description for the absolute configuration in the transformation from the \(R\) Mosher acid to the \(S\) Mosher acid chloride and back to the \(R\) Mosher ester due to a change in relative priority of the substituent groups). The \(^1\)H NMR spectra of the Mosher esters \((+)-5.40\) and \((-)-5.40\) were then obtained and fully assigned. The difference in chemical shifts for analogues pairs of protons were calculated according to the convention: \(\Delta\delta_{(S-R)} = \delta_S - \delta_R\), the shift for the \(R\) Mosher ester is subtracted from the shift of the \(S\) Mosher ester.

To assign the absolute configuration of alcohol \((+)-5.29\), the chemical shifts for analogues pairs of protons from Mosher ester \((+)-5.40\) (the \(R\) Mosher ester) were subtracted from those of Mosher ester \((-)-5.40\) (the enantiomer of the \(S\) Mosher ester, see Figure 5.5) and the results were tabulated (Table 5.1). Protons with positive \(\Delta\delta_{(S-R)}\) values are found on the same side of the molecule as the phenyl ring of the MTPA portion and so are at the front of the molecule as drawn with Table 5.1. This is due to the shielding effect of the phenyl ring which causes a more upfield chemical shift in the spatially proximal protons (indicated by the red arrow) and gives rise to the positive \(\Delta\delta_{(S-R)}\) value. Protons with negative \(\Delta\delta_{(S-R)}\) values are found on the same side as the methoxy group of the MTPA and so are at the back of the molecule.

87
Proton  |  S-(MTPA) ester (analogous to \((-\text{5.40})\)  |  R-(MTPA) ester \((+\text{-5.40})\)  |  \(\Delta\delta_{(S-R)}\)  |  Configuration  
---|---|---|---|---
CH=CH\(_2\) | 5.81 ppm  | 5.72 ppm  | +0.09  |  
CH\(_2\) | 2.62 ppm  | 2.57 ppm  | +0.05  |  
TMS  | 0.19 ppm  | 0.20 ppm  | −0.01  |  
CH(O) | 5.58 ppm  | 5.62 ppm  | −0.04  |  

**Table 5.1** \(^1\)H NMR data for the assignment of the absolute configuration of Mosher ester \((+\text{-5.40})\). The red arrow in the figure represents the shielding effect of the phenyl group.

Proton  |  S-(MTPA) ester (analogous to \((+\text{-5.40})\)  |  R-(MTPA) ester \((-\text{-5.40})\)  |  \(\Delta\delta_{(S-R)}\)  |  Configuration  
---|---|---|---|---
CH(O) | 5.62 ppm  | 5.58 ppm  | +0.04  |  
TMS  | 0.20 ppm  | 0.19 ppm  | +0.01  |  
CH\(_2\) | 2.57 ppm  | 2.62 ppm  | −0.05  |  
CH=CH\(_2\) | 5.72 ppm  | 5.81 ppm  | −0.09  |  

**Table 5.2** \(^1\)H NMR data for the assignment of the absolute configuration of Mosher ester \((-\text{-5.40})\). The red arrow in the figure represents the shielding effect of the phenyl group.
Hence, the stereogenic centre in \((+)-5.40\) was assigned as \(R\) and that the absolute configuration of alcohol \((+)-5.29\) was inferred as being \(R\) as expected. The same analysis was carried out for Mosher ester \((-)-5.40\) and the absolute configuration was found to be \(S\) (Table 5.2).

The enantiomeric purity of alcohols \((+)-5.29\) and \((-)-5.29\) was determined by comparing the \(^1\)H NMR spectra of the corresponding Mosher esters \((+)-5.40\) and \((-)-5.40\). In both spectra, the minor Mosher ester derivative was not observed (within the limits of the 300 MHz spectrometer used to acquire the spectra) and so the Mosher esters were deduced to be enantiomerically pure. From this it was concluded that alcohols \((+)-5.29\) and \((-)-5.29\) were formed with an enantiomeric purity of \(>99:1\).

![Figure 5.6](Image)

*Figure 5.6* \(^1\)H NMR spectra of Mosher esters \((+)-5.40\) (top) and \((-)-5.40\) (bottom). The peak at 3.51 ppm is due to the methoxy group in traces of unreacted MTPA.
This analysis has shown the enzymatic resolution using lipase Amano® AK20 to be very efficient as all of alcohol (+)-5.29 was converted to acetate (+)-5.39 during the reaction and all of alcohol (−)-5.29 was left untouched. Alcohol (+)-5.29 was shown to have the desired absolute configuration and very high enantiomeric purity and so would give the galanthamine analogue 5.23 with the correct stereochemistry of the tertiary stereogenic centre connecting the B and D rings.

5.2.4 Synthesis and asymmetric allylation of aldehyde 5.30

The Mitsunobu coupling of phenol 5.28 and alcohol (+)-5.29 was carried out using DIAD as the coupling reagent following the modified conditions reported by Miller119 to give olefin (+)-5.41 in quantative yield (Scheme 5.5). A two-step dihydroxylation/oxidative cleavage was then employed to afford aldehyde 5.30, as the direct conversion had previously been shown not to work for this system.119

Scheme 5.5 Reagents and conditions: a) DIAD, PPh₃, THF, 55 °C; b) OsO₄, NMO, citric acid, t-BuOH/H₂O; c) NaIO₄, acetone/H₂O.
Dihydroxylation of olefin (+)-5.41 was carried out under acidic conditions as reported by Dupau et al. (OsO4, NMO, citric acid)146 to give a moderate yield of diol 5.42 along with 12% recovered starting material. The oxidative cleavage of diol 5.42 was mediated by sodium periodate and gave the desired aldehyde 5.30 in excellent yield.

The enantioselective allylation of aldehyde 5.30 was carried out using TiCpCl-(R,R)-TADDOL ((R,R)-5.43) as this was found by Miller119 to be superior to alternative asymmetric allylation reagents for this reaction. The TADDOL complex was prepared in one step from commercially available starting materials in good yield following the procedure of Hafner et al.147 The enantioselective allylation reaction afforded alcohol (+)-5.31 in excellent yield (Scheme 5.6). The unmasking of the enyne was necessary before the RCM reaction to give the cyclohexene ring B could be performed. Miller employed potassium carbonate in methanol for this transformation119 although this was found to also remove the more labile O-Boc group. The use of TBAF in this reaction left the O-Boc group untouched while successfully cleaving the TMS group to give enyne (+)-5.44 in excellent yield.

5.2.5 Closure of the B and C rings
The next step was a RCM reaction using the Grubbs’ I catalyst (5.45) to afford the closure of the B ring of the galanthamine backbone. Miller previously found the
Grubbs’ I catalyst (5.45) mediated enyne RCM reaction to be incompatible with the substrate containing the secondary alcohol, possibly due to chelation of the Lewis-basic oxygen of the free hydroxyl group to the ruthenium of the catalyst.\(^{119}\) Hence, it was necessary to protect the free hydroxyl group before carrying out the RCM reaction. Alcohol (+)-5.44 was protected as the O-TBS ether using standard conditions and in excellent yield. The RCM reaction of enyne (−)-5.46 gave diene (−)-5.32 in good yield with low catalyst loading (Scheme 5.7), using two separate additions of Grubbs’ I catalyst (5.45). The hydroboration of the terminal double bond of diene (−)-5.32 was carried out using 9-BBN to form the borane species which was then oxidised with sodium hydroxide and hydrogen peroxide to give the desired alcohol (−)-5.47 in good yield.

Scheme 5.7 Reagents and conditions: a) TBSOTf, 2,6-lutidine, CH\(_2\)Cl\(_2\), −78 to 0 °C; b) Grubbs’ I (5.45) (6 mol%), CH\(_2\)Cl\(_2\), 40 °C; c) 9-BBN, THF then NaOH/H\(_2\)O\(_2\); d) Pd(OAc)\(_2\), dppp, Ag\(_2\)CO\(_3\), toluene, 90 °C.

Gratifyingly, the O-Boc protecting group was unaffected by this reagent combination. Closure of the C ring was achieved \textit{via} an intermolecular Heck reaction to give tricyclic
alcohol (+)-5.48 in good yield, the stereochemistry at the newly formed quaternary centre being controlled by the adjacent stereocentre.

5.2.6 Closure of the D ring to give galanthamine analogue (−)-5.25

The closure of the D ring turned out to be much more troublesome than initially anticipated. Ideally, we wanted to activate the primary alcohol in (+)-5.48, remove both the N-Boc and O-Boc protecting groups and close the D ring but leave the O-TBS protecting group in place. This would then allow us to selectively fluorinate the primary alcohol on the O-ethoxy chain without complications arising from reactions of the secondary alcohol. Miller’s strategy for the activation of the primary alcohol was to form the sulfonate ester although both the tosylate and mesylate were formed in lower than expected yields (53% and 59% respectively). So alternative strategies for the activation of the primary alcohol and the subsequent ring closure were investigated.

The first strategy investigated was the activation of alcohol (+)-5.48 as the corresponding bromide following the procedure described by Baughman et al. (Scheme 5.8). The reaction gave a complex mixture of products that, with careful chromatography, gave a poor yield of bromide 5.49 which was found to have lost the O-TBS protecting group. The cyclisation of bromide 5.49 was successful to form the D ring and give diol (−)-5.25 in moderate yield although the loss of the O-TBS protecting group in the first step made this route less attractive. The second strategy to be investigated was the conversion of alcohol (+)-5.48 to the aldehyde followed by deprotection of the N-Boc group and cyclisation via imine formation and finally reduction to give the tertiary amine (this strategy had been investigated in a earlier total synthesis of galanthamine in the Brown group by Satcharoen). Aldehyde 5.50 was formed in good yield by the treatment of alcohol (+)-5.48 with Dess-Martin periodinane. However, the two-step deprotection/cyclisation and then reduction reaction resulted in a very poor mass recovery and none of the desired cyclised product was detected (with or without the O-TBS protecting group present).
Since the alternative strategies failed to achieve the desired outcome, attention was turned back to the original method of alcohol activation via the formation of the tosylate. This was a problem step in Miller’s synthesis of galanthamine, who employed tosyl chloride and pyridine for this reaction and obtained a disappointing 53% yield of the tosylate and 27% recovered starting material.\textsuperscript{119} Using our experience in performing sulfonate ester forming reactions from the work involving the RLV constructs, we modified conditions to tosyl chloride and triethylamine with a catalytic amount of DMAP. These conditions gave a much improved 86% yield of tosylate (\textit{\textbf{+5.51}}) with no recovered alcohol starting material (Scheme 5.9). The deprotection/cyclisation reaction was attempted using TFA to remove the Boc protecting groups, followed by aqueous sodium hydrogen carbonate to facilitate the $S_N2$ displacement of the tosylate group by the free secondary amine. Again, the reaction gave the cyclised product diol (\textit{\textbf{-5.25}}) with undesired loss of the $O$-TBS protecting group, although it had been formed in a 59% yield. Examination of the intermediates of the reaction indicated that...
the O-TBS group was the most labile of the protecting groups under these conditions, as compounds that had only lost the O-TBS group and cyclised compounds that had lost the O-TBS group but still contained the O-Boc group were isolated. These finding were contrary to what would be expected and contrasted with the findings of Miller who reported the O-TBS group as being the least labile under similar reaction conditions of AcCl in MeOH.\textsuperscript{119} Unfortunately, we were unable to find conditions that retained the O-TBS group during the formation of the D ring.

![Diagram](image)

**Scheme 5.9** *Reagents and conditions: a) TsCl, Et\textsubscript{3}N, DMAP, CH\textsubscript{2}Cl\textsubscript{2}; b) TFA, CH\textsubscript{2}Cl\textsubscript{2} then K\textsubscript{2}CO\textsubscript{3}, THF.*

Diol (−)-\textsuperscript{5.25} was found to have a significant solubility water, requiring a large number (>10) of extractions with organic solvent for it to be completely removed from the aqueous phase during workup. This was impractical and could lead to loss of material and so reaction conditions which did not require use of an aqueous base or an aqueous workup were sought. The reaction was tried using DBU as the base and although diol (−)-\textsuperscript{5.25} was formed in the reaction, we were not able to remove all of the DBU from the product even after column chromatography and drying under high vacuum. Gratifyingly, an alternative method whereby the TFA/CH\textsubscript{2}Cl\textsubscript{2} from the deprotection step were removed *in vacuo* and the residue was re-dissolved in THF and treated with potassium carbonate to afford the ring closure gave the desired product after filtration of the reaction mixture. Thus, diol (−)-\textsuperscript{5.25} was obtained in an improved 65% yield without the need for an aqueous workup.
5.3 Fluorination to give galanthamine analogue (−)-5.23

Due to constraints on the amount of material available, it was decided not to attempt to attach diol (−)-5.25 to the solid phase using our developed linker system but to carry out the fluorination to give galanthamine analogue (−)-5.23 in solution phase only. The loss of the O-TBS protecting group from the secondary alcohol when closing the D ring meant that, to give the desired fluorinated galanthamine analogue (−)-5.23, the selective fluorination of the primary alcohol in the presence of the secondary alcohol was required.

![Figure 5.7 Reagents that mediate dehydroxyfluorination reactions.](image)

There are a number of reagents available for the dehydroxyfluorination of alcohols, from diethylamine sulfur trifluoride (DAST) which has been commercially available since the 1980s, Deoxo-Fluor™ (a second generation DAST reagent) and more recently tetrafluoroethylidimethylamine (TFEDMA) and FLUOLEAD™ (Figure 5.7). Since primary alcohols are more reactive than secondary alcohols, it was postulated that the reaction of diol (−)-5.25 with one equivalent of DAST would lead to the desired product, fluoride (−)-5.23. However, due to the small scale of the reaction (<0.02 mmol because of constraints in the amount of material remaining) it was extremely difficult to accurately add one equivalent of the DAST reagent to the reaction mixture which, upon workup, was found to contain both mono- and di-fluorination products.

As the direct fluorination was found not to be selective for fluorination on the primary alcohol an alternative strategy involving the activation of the primary alcohol was investigated. The mono-tosylation of diol (−)-5.25 was attempted using the same conditions as employed previously for the activation of the alcohol in the formation of the D ring and encouragingly, tosylation was observed exclusively on the primary alcohol to give tosylate 5.52 (Scheme 5.10). However, there was evidence to show that nitrogen of the tertiary amine was sufficiently nucleophilic to be able to displace a
tosylate group on another molecule to form dimer 5.53. It is believed that this
dimerisation occurs predominately when the solution of tosylate 5.52 is concentrated
and so this complication could be avoided by the in situ formation and displacement of
the tosylate leaving group. Shimizu et al. reported the use of tosyl fluoride and TBAF
in THF for the chemoselective fluorination of primary alcohols in the presence of
secondary and tertiary alcohols.151 Diol (−)-5.25 was reacted under these conditions and
although the desired fluoride (−)-5.23 was formed it was not possible to separate the
TBAF reagent from the product due to the similarity in the polarity of these compounds.

\[ \text{Scheme 5.10 Reagents and conditions: a) TsCl, Et}_3\text{N, DMAP, CH}_2\text{Cl}_2. \]

TLC investigations showed the alternative fluoride source of potassium fluoride and
Kryptofix [2.2.2] to be separable from fluoride (−)-5.23 and these were also found to be
soluble in THF. Hence, the use of TBAF as the fluorinating agent was replaced with
potassium fluoride and Kryptofix [2.2.2] and the reaction was repeated to give fluoride
(−)-5.23 which was easily separated from the Kryptofix [2.2.2] by column
chromatography on alumina. However, the \(^1\)H NMR spectrum of fluoride (−)-5.23
showed it to contain impurities with tosyl-like signals and these were still found to be
present after multiple purification attempts by column chromatography on alumina.
Finally, passage through an Isolute SCX-2 cation exchange cartridge removed these
impurities to give fluoride (−)-5.23, albeit in a low 9\% isolated yield (Scheme 5.11).
Unfortunately, there was insufficient material for this reaction to be optimised.
However, we are confident that the losses occurred during the purification stage and that the knowledge of the new purification strategy would lead to a significantly higher yield being obtained if this reaction was to be repeated.

Scheme 5.11  
Reagents and conditions: a) TsF, KF, Kryptofix, THF, 70 °C.

Figure 5.8  $^1$H NMR of fluorinated (−)-galanthamine analogue, fluoride (−)-5.23.
5.4 Conclusions

An analogue of (−)-galanthamine with a fluoro-ethoxy group in place of the methoxy group was synthesised, with the backbone of the parent structure constructed following the synthetic pathway previously developed by Miller. An O-ethoxy chain was installed early in the synthesis of aromatic fragment 5.28. The synthesis of the enantiomerically enriched alcohol (+)-5.29 fragment was achieved using an enzymatic resolution and a Mosher ester analysis confirmed that the desired R enantiomer had been formed in an enantiomeric purity of >99:1. The aromatic and alcohol fragments were coupled together and underwent a series of transformations to give a (−)-galanthamine analogue, diol (−)-5.25. As part of this work, the previously low yielding tosylation reaction as part of the formation of the D ring was optimised. The selective mono-fluorination of diol (−)-5.25 gave fluorinated (−)-galanthamine analogue (−)-5.23.

The galanthamine analogues diol (−)-5.25 and fluoride (−)-5.23 have been sent for testing to establish whether the activity of the parent compound is retained with the structural modifications.

5.5 Further work

The loss of the O-TBS group during the closure of the D ring made the subsequent fluorination more difficult due to complications involving the displacement of the protecting group on the secondary alcohol. Hence, the use of a different protecting group on the secondary alcohol would have been desirable. A series of small scale (<0.01 mmol) test reactions were carried out to investigate changing the O-TBS protecting group for a benzoyl protecting group (Scheme 5.12). Due to the small scale of these reactions, full data was not obtained for each product although data to support the formation of the desired product was collected at each step (1H NMR or MS). Tosylate (+)-5.51, prior to closure of the D ring, was reacted with TBAF which selectively removed the O-TBS protecting group and the free secondary alcohol (5.54) was then reprotected as the benzoyl ester (5.55). The deprotection/cyclisation reaction was then carried out using TFA followed by sodium hydrogen carbonate to close the D ring and crucially the benzoyl protecting group was retained to give alcohol 5.56. Fluorination of the free primary alcohol was then achieved using DAST as there were...
no longer any issues with the selectivity of the reaction. The benzoyl protecting group was then removed to give fluorinated \((-\)-galanthamine analogue \((-\)-5.23). This reaction pathway, although less elegant due to the additional steps for the protecting group manipulations, avoids the risk of formation of dimer 5.53 and makes the fluorination reaction much simpler. It could also be applied in the solution phase synthesis of \([^{18}\text{F}]\)-fluoride labelled galanthamine analogue (\([^{18}\text{F}]\)-\((-\)-5.23) by the use of \([^{18}\text{F}]\)-DAST (first reported by Straatmann et al. in 1977)\(^{152}\).

\[
\begin{align*}
\text{Scheme 5.12} & \quad \text{A series of test reactions carried out on a <0.01 mmol scale.} \quad \text{Reagents and conditions:} \\
a) & \quad \text{TBAF, THF; } b) \quad \text{BzCl, Et}_3\text{N, DMAP, CH}_2\text{Cl}_2; \quad c) \quad \text{TFA, CH}_2\text{Cl}_2 \text{ then sat aq NaHCO}_3; \quad d) \quad \text{DAST, CH}_2\text{Cl}_2; \quad e) \quad \text{K}_2\text{CO}_3, \text{MeOH.}
\end{align*}
\]
A more elegant strategy would be to install the benzoyl protecting group earlier in the synthesis of the parent structure before the closure of the B ring by RCM. Assuming it was tolerated through the subsequent transformations this approach would require less protecting group manipulations near the end of the synthesis.
6.1 General experimental

All non-aqueous reactions were carried out under an inert atmosphere (nitrogen or argon) in oven or flame-dried glassware. Reaction solvents were distilled before use: CH₂Cl₂ and MeOH were distilled over CaH₂; THF and Et₂O over sodium/benzophenone; toluene and hexane over sodium. All other solvents and reagents were used as received. The enzymes used were kindly donated by the manufacturer: Novozym 435® from NovoNordisk A/S and Amano® AK20 Lipase from Amano Enzyme Inc. Column chromatography was carried out using Merck Kieselgel 60A (particle size 35-70 microns) unless stated otherwise. Thin layer chromatography was carried out on Merck silica gel 60 F₂₅₄, visualised under UV illumination (254 nm) and stained with potassium permanganate or phosphomolybdic acid (PMA). Both ¹H and ¹³C NMR spectra were recorded on either a Bruker AV-300 spectrometer at 300 MHz and 75 MHz respectively or a Bruker DPX-400 spectrometer at 400 MHz and 100 MHz respectively in CDCl₃ or acetone-d₆ at 300 K. Chemical shifts for proton and carbon spectra are reported on the δ scale in ppm and were referenced to residual solvent (CDCl₃: 7.27 ppm for ¹H and 77.0 ppm for ¹³C; acetone-d₆: 2.05 ppm for ¹H and 29.92 ppm for ¹³C). ¹⁹F NMR spectra were recorded on a Bruker AV-300 spectrometer at 282 MHz in CDCl₃ at 300 K (decoupled) and were referenced to C₆F₆ (−162.9 ppm). Infrared data were collected on a Thermo Nicolet 380 FT-IR spectrometer with a Smart Orbit Goldengate attachment using OMNIC software. The IR spectra are reported in wavenumbers (cm⁻¹). Absorption peaks are reported as either: weak (w), medium (m), strong (s) or broad (br). Optical rotations were collected on an Optical Activity PolAAr 2001 machine. Melting points were collected on a Gallenkamp Electrothermal® melting point apparatus and are uncorrected. All electrospray low resolution mass spectra were recorded on a Waters ZMD quadrupole spectrometer. EI and CI low resolution mass spectroscopic data were collected on a ThermoQuest TraceMS single quadrupole GC-MS. High resolution mass spectroscopic data were collected on a Bruker Apex III FT-ICR-MS. Yields for the resin coupling reactions describe the mass increase of the resin and are calculated from the theoretical mass gain using the loading of the unfunctionalised resin and the observed mass gain of the product resin. In some cases these yields are >100% which may be due to
inaccuracies in the resin loading quoted by the supplier (Novabiochem) or to traces of reagent or solvent molecules in the resin matrix due to incomplete washing and/or drying of the product resin.

6.2 Experimental detail

6-Phenylhexanoic acid methyl ester (2.02)

C\textsubscript{13}H\textsubscript{18}O\textsubscript{2}

\begin{align*}
\text{Mw} & = 206.28 \text{ g mol}^{-1} \\
\text{Colourless oil}
\end{align*}

Following the procedure of Hanessian et al.,\textsuperscript{59} to a solution of 6-phenylhexanoic acid (2.01, 0.50 mL, 2.6 mmol) in MeOH (15 mL) at 0 °C was added TMSCl (1.30 mL, 10.4 mmol). The solution was stirred for 4 h at rt before it was quenched with water (2 mL) and concentrated \textit{in vacuo}. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2}, dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 0-10% EtOAc/hexane) to give a colourless oil (550 mg, 2.6 mmol, 100%). Spectroscopic data were in agreement with those published in the literature.\textsuperscript{59}

\textbf{IR} \quad \nu_{\text{max}} \text{ (neat)} 3026 \text{ (w)}, 2933 \text{ (w)}, 2857 \text{ (w)}, 1736 \text{ (s)}, 1170 \text{ (m)}, 698 \text{ (s)} \text{ cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} \quad (300 \text{ MHz, CDCl}_3) \delta 7.32-7.25 \text{ (2H, m, C\textsubscript{Ar}H ortho to CH\textsubscript{2})}, 7.22-7.15 \text{ (3H, m, C\textsubscript{Ar}H meta and para to CH\textsubscript{2})}, 3.67 \text{ (3H, s, C(O)OCH\textsubscript{3})}, 2.63 \text{ (2H, t, } J = 7.7 \text{ Hz, C\textsubscript{Ar}CH\textsubscript{2}}), 2.32 \text{ (2H, t, } J = 7.5 \text{ Hz, C(O)CH\textsubscript{2}}), 1.73-1.60 \text{ (4H, m, C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2})}, 1.44-1.32 \text{ (2H, m, C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2})}.

\textbf{\textsuperscript{13}C NMR} \quad (75 \text{ MHz, CDCl}_3) \delta 174.2 \text{ (C(O)OCH\textsubscript{3})}, 142.5 \text{ (C\textsubscript{Ar}CH\textsubscript{2})}, 128.4 \text{ (C\textsubscript{Ar}H meta to alkyl group)}, 127.9 \text{ (C\textsubscript{Ar}H ortho to alkyl group)}, 125.6 \text{ (C\textsubscript{Ar}H para to alkyl group)}, 51.4 \text{ (OCH\textsubscript{3})}, 35.7 \text{ (C\textsubscript{Ar}CH\textsubscript{2})}, 34.0 \text{ (C(O)CH\textsubscript{2})}, 31.0 \text{ (C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2})}, 28.7 \text{ (C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2})}, 24.8 \text{ (C\textsubscript{Ar}CH\textsubscript{2}CH\textsubscript{2})}.
6-(4-Chlorosulfonyl-phenyl) hexanoic acid methyl ester (2.03)

To a solution of methyl ester 2.02 (6.55 g, 31.8 mmol) in CH₂Cl₂ (60 mL) at 0 °C was added ClSO₂OH (6.40 mL, 95.3 mmol). The solution was allowed to slowly warm to rt and was stirred for 17.5 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (50 mL) and quenched with water. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The residue was dissolved in CH₂Cl₂ (40 mL) and cooled to 0 °C. MeOH (20 mL) and AcCl (1.2 mL, 15.9 mmol) were added and the solution was stirred for 3.25 h. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (eluent 10-50% EtOAc/hexane) to give the title compound as a colourless oil (6.57 g, 21.6 mmol, 68%).

IR

νₑₓ所提供的 (neat) 2943 (w), 2861 (w), 1735 (m), 1375 (m), 1174 (s), 573 (m) cm⁻¹.

¹H NMR

(300 MHz, CDCl₃) δ 7.95 (2H, d, J = 8.6 Hz, C₆H₄H ortho to SO₂Cl), 7.42 (2H, d, J = 8.6 Hz, C₆H₄H meta to SO₂Cl), 3.67 (3H, s, C(O)OC₃H₃), 2.75 (2H, t, J = 7.7 Hz, C₆H₄CH₂), 2.33 (2H, t, J = 7.5 Hz, C(O)CH₂), 1.75-1.63 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.45-1.34 (2H, m, C(O)CH₂CH₂CH₂).

¹³C NMR

(75 MHz, CDCl₃) δ 173.9 (C(O)OCH₃), 151.2 (C₆H₄CH₂), 142.0 (C₆SO₂Cl), 129.6 (C₆H₄H meta to SO₂Cl), 127.1 (C₆H₄H ortho to SO₂Cl),
51.5 (OCH₃), 35.8 (C₆H₃), 33.8 (C(O)CH₂), 30.5 (C(O)CH₂CH₂CH₂), 28.6 (C(O)CH₂CH₂), 24.6 (C₆H₃).

**LRMS**  

**HRMS**  
(ES+) calcd for C₁₃H₁₇O₄S³⁵ClNa [M + Na]⁺ 327.0428, found 327.0427.

---

6-[4-(2,2-Dimethyl-propoxysulfonyl) phenyl] hexanoic acid methyl ester (2.04)

![Chemical Structure](image)

C_{18}H_{28}O_{5}S  
Mw = 356.48 gmol⁻¹  
Colourless oil

To a solution of neopentyl alcohol (12 mg, 0.14 mmol) in THF (2 mL) at rt was added NaHMDS (1.0 M sol in THF, 0.14 mL, 0.14 mmol). A solution of sulfonyl chloride 2.03 (43 mg, 0.14 mmol) in THF (1 mL) was added and the mixture was stirred for 5 h. The reaction mixture was diluted with EtOAc (5 mL) and washed with sat aq NH₄Cl (2 x 5 mL), water (2 x 5 mL) and sat aq NaCO₃H (1 x 5 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 5-20% EtOAc/hexane) to give the title compound as a colourless oil (4 mg, 0.01 mmol, 7%).

**IR**  
ν<sub>max</sub> (neat) 2956 (w), 2866 (w), 1736 (m), 1359 (m), 1176 (s), 967 (m) cm⁻¹.

**¹H NMR**  
(300 MHz, CDCl₃) δ 7.81 (2H, d, J = 8.4 Hz, C₆H ortho to SO₂O), 7.35 (2H, d, J = 8.4 Hz, C₆H meta to SO₂O), 3.70 (2H, s, SO₂OCH₂), 3.67 (3H, s, C(O)OCH₃), 2.72 (2H, t, J = 7.9 Hz, C₆HCH₂), 2.33 (2H, t, J = 7.5 Hz, C(O)CH₂), 1.68 (4H, quin, J = 7.5 Hz, C(O)CH₂CH₂CH₂CH₂), 1.44-1.32 (2H, m, C(O)CH₂CH₂CH₂), 0.90 (9H, s, C(CH₃)₃).
**13C NMR** (100 MHz, CDCl$_3$) δ 174.0 (C(O)OCH$_3$), 149.0 (C$_{Ar}$CH$_2$), 133.5 (C$_{Ar}$SO$_2$O), 129.1 (C$_{Ar}$H _meta_ to SO$_2$O), 128.0 (C$_{Ar}$H _ortho_ to SO$_2$O), 79.5 (SO$_2$OCH$_2$), 51.5 (OCH$_3$), 35.6 (C$_{Ar}$CH$_2$), 33.8 (C(O)CH$_2$), 31.7 (C(CH$_3$)$_3$), 30.6 (C(O)CH$_2$CH$_2$CH$_2$), 28.6 (C(O)CH$_2$CH$_2$), 26.0 (C(CH$_3$)$_3$), 24.6 (C$_{Ar}$CH$_2$CH$_2$).


**HRMS** (ES+) calcd for C$_{18}$H$_{28}$O$_5$SNa [M + Na]$^+$ 379.1550, found 379.1555.

6-[4-(3-Phenyl propoxysulfonyl) phenyl] hexanoic acid methyl ester (2.06)

![Chemical Structure](image)

C$_{22}$H$_{28}$O$_5$S

Mw = 404.52 gmol$^{-1}$

Colourless oil

To a solution of 3-phenyl propan-1-ol (0.062 mL, 0.46 mmol) in CH$_2$Cl$_2$ (1 mL) was added Et$_3$N (0.16 mL, 1.15 mmol) and sulfonyl chloride 2.03 (279 mg, 0.92 mmol) in CH$_2$Cl$_2$ (2 mL) and the solution was heated to reflux for 7 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (5 mL), washed with 2N HCl (1 x 10 mL), brine (1 x 10 mL) and water (1 x 10 mL), dried over MgSO$_4$ and concentrated _in vacuo_. The residue was purified by column chromatography (eluent 5-15% EtOAc/hexane) to give the title compound as a colourless oil (152 mg, 0.38 mmol, 82%) and recovered sulfonyl chloride 2.03 (32 mg, 0.11 mmol, 11%).

**IR** $\nu_{\text{max}}$ (neat) 2935 (w), 2860 (w), 1735 (m), 1360 (m), 1174 (s), 928 (m) cm$^{-1}$.

**1H NMR** (300 MHz, CDCl$_3$) 7.82 (2H, d, $J = 8.4$ Hz, C$_{Ar}$H _ortho_ to SO$_2$O), 7.35 (2H, d, $J = 8.4$ Hz, C$_{Ar}$H _meta_ to SO$_2$O), 7.29-7.13 (3H, m, C$_{Ar}$H _meta_ & _para_ to CH$_2$), 7.18 (2H, d, $J = 6.6$ Hz, C$_{Ar}$H _ortho_ to CH$_2$), 4.06 (2H, t, $J = 6.3$ Hz, SO$_2$OCH$_2$), 3.69 (3H, s, C(O)OCH$_3$), 2.75-2.63 (4H, m, 2
x C_{Ar}\text{CH}_2), 2.33 (2H, t, J = 7.4 Hz, C(O)\text{CH}_2), 2.03-1.90 (2H, m, SO_3\text{CH}_2\text{CH}_2), 1.74-1.62 (4H, m, C(O)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2), 1.45-1.32 (2H, m, C(O)\text{CH}_2\text{CH}_2\text{CH}_2).

^{13}\text{C NMR} \quad (75 \text{ MHz, CDCl}_3) \: \delta \ 174.0 \ (\text{C(O)OCH}_3), 149.1 \ (\text{C}_{Ar}\text{CH}_2), 140.4 \ (\text{C}_{Ar}\text{CH}_2\text{CH}_2\text{CH}_2\text{O}), 133.5 \ (\text{C}_{Ar}\text{SO}_2\text{O}), 129.2 \ (\text{C}_{Ar}\text{H meta to SO}_2\text{O}), 128.5 \ (\text{C}_{Ar}\text{H meta to CH}_2), 128.4 \ (\text{C}_{Ar}\text{H ortho to CH}_2), 128.0 \ (\text{C}_{Ar}\text{H ortho to SO}_2\text{O}), 126.1 \ (\text{C}_{Ar}\text{H para to CH}_2), 69.6 \ (\text{SO}_2\text{OCH}_2), 52.0 \ (\text{SO}_2\text{OCH}_3), 35.7 \ (\text{C}_{Ar}\text{CH}_2), 33.9 \ (\text{C(O)CH}_2), 31.5 \ (\text{SO}_2\text{OCH}_2\text{CH}_2), 30.6 \ (\text{C(O)CH}_2\text{CH}_2\text{CH}_2), 30.5 \ (\text{SO}_2\text{OCH}_2\text{CH}_2\text{CH}_2), 28.6 \ (\text{C(O)CH}_2\text{CH}_2), 24.6 \ (\text{C}_{Ar}\text{CH}_2\text{CH}_2).

LRMS \quad (\text{ES}+) \ m/z \ 405 \ [\text{M + H}]^+, \ 422 \ [\text{M + NH}_4]^+, \ 427 \ [\text{M + Na}]^+, \ 831 \ [\text{2M + Na}]^+.

HRMS \quad (\text{ES}+) \ \text{calcd for} \ C_{22}\text{H}_{28}\text{O}_5\text{SNa} \ [\text{M + Na}]^+ \ 427.1550, \ \text{found} \ 427.1549.

6-[4-(Phenyl propoxysulfonyl) phenyl] hexanoic acid (2.07)

To a suspension of Novozym 435® (260 mg) in an aq phosphate buffer (pH 7, 1 mL) was added a solution of methyl ester 2.06 (262 mg, 0.65 mmol) in CH_2Cl_2/acetone/EtO (3:1:1, 2.5 mL) and the mixture was stirred at 50 °C for 23.5 h. The cold suspension was filtered through Celite®, the pad was washed with CHCl_3 and EtOAc and the organic phase was concentrated in vacuo. The residue was purified by column chromatography (eluent 10-50% EtOAc/hexane) to give the title compound as a colourless oil (125 mg, 0.32 mmol, 49%) and recovered methyl ester 2.06 (46 mg, 0.11 mmol, 22%).
IR

$\nu_{\text{max}}$ (neat) 3028 (w), 2935 (br), 2860 (w), 1705 (s), 1358 (m), 1174 (s), 926 (m) cm$^{-1}$.

$^1$H NMR

(300 MHz, CDCl$_3$) $\delta$ 7.83 (2H, d, $J = 8.4$ Hz, C$_{Ar}$H ortho to SO$_2$O), 7.36 (2H, d, $J = 8.4$ Hz, C$_{Ar}$H meta to SO$_2$O), 7.28-7.14 (3H, m, C$_{Ar}$H meta & para to CH$_2$), 7.18 (2H, d, $J = 6.6$ Hz, C$_{Ar}$H ortho to CH$_2$), 4.08 (2H, t, $J = 6.2$ Hz, SO$_2$OCH$_2$), 2.76-2.62 (4H, m, 2 x C$_{Ar}$CH$_2$), 2.37 (2H, t, $J = 7.3$ Hz, C(O)CH$_2$), 2.03-1.92 (2H, m, SO$_2$OCH$_2$CH$_2$), 1.75-1.63 (4H, m, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.47-1.36 (2H, m, C(O)CH$_2$CH$_2$CH$_2$CH$_2$).

C$_{13}$ NMR

(75 MHz, CDCl$_3$) $\delta$ 179.4 (C(O)OH), 149.1 (C$_{Ar}$CH$_2$), 140.4 (C$_{Ar}$CH$_2$CH$_2$CH$_2$O), 133.5 (C$_{Ar}$SO$_2$O), 129.2 (C$_{Ar}$H meta to SO$_2$O), 128.5 (C$_{Ar}$H meta to CH$_2$), 128.4 (C$_{Ar}$H ortho to CH$_2$), 128.0 (CH ortho to SO$_2$O), 126.1 (C$_{Ar}$H para to CH$_2$), 69.6 (SO$_2$OCH$_2$), 35.6 (C$_{Ar}$CH$_2$), 33.7 (C(O)CH$_2$), 31.5 (SO$_2$OCH$_2$CH$_2$), 30.6 (C(O)CH$_2$CH$_2$CH$_2$), 30.5 (SO$_2$OCH$_2$CH$_2$CH$_2$), 28.5 (C(O)CH$_2$CH$_2$), 24.3 (C$_{Ar}$CH$_2$CH$_2$).


HRMS (ES+) calcd for C$_{21}$H$_{26}$O$_5$SNa [M + Na]$^+$ 413.1393, found 413.1399.

4-(5-Benzylcarbamoyl phenyl) benzenesulfonic acid 3-phenyl propyl ester (2.08)

![4-(5-Benzylcarbamoyl phenyl) benzenesulfonic acid 3-phenyl propyl ester](image)

C$_{28}$H$_{33}$NO$_4$S  
Mw = 479.63 gmol$^{-1}$  
Colourless oil

Carboxylic acid 2.07 (43 mg, 0.11 mmol) and HOBt (14 mg, 0.11 mmol) were dissolved in CH$_2$Cl$_2$ (1 mL) and stirred for 10 min at rt. DIC (0.02 mL, 0.11 mmol) was added and the solution was stirred for 1.5 h. Benzyamine (0.12 mL, 0.11 mmol) was added and the solution was stirred for 21 h. The reaction mixture was diluted with
EtOAc (5 mL), washed with sat aq NaCO₃H (1 x 10 mL), water (1 x 10 mL) and brine (1 x 10 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20-50% EtOAc/hexane) to give the title compound as a colourless oil (17 mg, 0.36 mmol, 33%).

**IR**

\(\nu_{\text{max}}\) (neat) 3297 (br), 3063 (w), 3029 (w), 2932 (w), 2859 (w), 1647 (m), 1540 (w), 1360 (m), 1174 (s), 929 (m) cm⁻¹.

**¹H NMR**

(300 MHz, CDCl₃) \(\delta\) 7.81 (2H, d, \(J = 8.4\) Hz, \(\text{C}_{\text{Ar}}\text{H}_{\text{ortho}}\) to SO₂O), 7.39-7.15 (10H, m, \(\text{C}_{\text{Ar}}\text{H}\)), 7.09 (2H, d, \(J = 8.2\) Hz, \(\text{C}_{\text{Ar}}\text{H}\)), 5.65 (1H, s, NH) 4.45 (2H, d, \(J = 5.7\) Hz, CH₂NH), 4.06 (2H, t, \(J = 6.2\) Hz, SO₂OCH₂), 2.75-2.62 (4H, m, 2 x \(\text{C}_{\text{Ar}}\text{CH}_2\)), 2.21 (2H, t, \(J = 7.5\) Hz, C(O)CH₂), 2.05-1.92 (2H, m, SO₂OCH₂CH₂), 1.78-1.63 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.45-1.37 (2H, m, C(O)CH₂CH₂CH₂CH₂).

**¹³C NMR**

(75 MHz, CDCl₃) \(\delta\) 172.5 (C(O)NH), 149.2 (C₆H₅CH₂), 140.4 (C₆H₅CH₂CH₂CH₂O), 138.3 (C₆H₅CH₂NH), 133.4 (C₆H₅SO₂O), 129.2 (C₆H₅ meta to SO₂O), 128.7 (C₆H₅ meta to CH₂NH), 128.5 (C₆H₅ meta to CH₂), 128.4 (C₆H₅ ortho to CH₂), 127.9 (C₆H₅ ortho to SO₂O), 127.8 (C₆H₅ ortho to CH₂NH), 127.5 (C₆H₅ para to CH₂NH), 126.1 (C₆H₅ para to CH₂), 69.6 (SO₂OCH₂), 43.6 (CH₂NH), 36.5 (C₆H₅CH₂), 35.6 (C(O)CH₂), 31.4 (SO₂OCH₂CH₂), 30.7 (C(O)CH₂CH₂CH₂), 30.4 (SO₂OCH₂CH₂CH₂), 28.7 (C(O)CH₂CH₂), 25.3 (C₆H₅CH₂CH₂).

**LRMS**


**HRMS**

(ES+) calcd for C₂₈H₃₄NO₄S [M + H]⁺ 480.2203, found 480.2197.
Solid supported 6-[4-(phenyl propoxysulfonyl) phenyl] hexanoic acid (2.09)

Theoretical loading = 0.90 mmolg$^{-1}$

Resin beads

To a solution of HOBt (65 mg, 0.48 mmol) and DIC (0.08 mL, 0.48 mmol) in DMF/CH$_2$Cl$_2$ (2:3, 1 mL) was added carboxylic acid 2.07 (120 mg, 0.25 mmol) in CH$_2$Cl$_2$ (1 mL) and amino methyl polystyrene resin (107 mg, loading 1.5 mmolg$^{-1}$, 0.16 mmol). The mixture was stirred at rt for 19 h after which time a ninhydrin test performed on a small sample of the resin beads was negative, indicating that all of the amino groups had reacted. The resin was removed by filtration, washed with CH$_2$Cl$_2$ (3 x 10 mL), MeOH (3 x 10 mL) and Et$_2$O (3 x 10 mL) and dried in vacuo at 40 $^\circ$C for 50 h to give the product resin (177 mg, theoretical loading 0.90 mmolg$^{-1}$, 117%).

IR $\nu_{\text{max}}$ (neat) 2924 (w), 1738 (s), 1366 (m), 1217 (m), 696 (m) cm$^{-1}$.

$^1$H NMR MAS (400 MHz, CDCl$_3$) $\delta$ 7.87 (s, C$_{Ar}$H ortho to SO$_2$O), 7.40-7.10 (m, C$_{Ar}$H meta to SO$_2$O & C$_{Ar}$H ortho, meta & para to CH$_2$), 6.66 (amino methyl resin), 5.50 (s, NH), 4.45 (CH$_3$NH), 4.11 (s, OCH$_2$), 2.77-2.70 (m, 2 x C$_{Ar}$CH$_2$), 2.26 (s, C(O)CH$_2$), 2.02 (SO$_2$OCH$_2$CH$_2$), 1.75 (s, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.46 (s, C(O)CH$_2$CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR MAS (100 MHz, CDCl$_3$) $\delta$ 172.6 (C(O)NH), 149.3 (C$_{Ar}$CH$_2$), 145.3 (amino methyl resin), 140.4 (C$_{Ar}$CH$_2$), 133.5 (C$_{Ar}$SO$_2$O), 129.3 (C$_{Ar}$H meta to SO$_2$O), 128.5 (C$_{Ar}$H meta to CH$_2$), 128.1 (C$_{Ar}$H ortho to CH$_2$), 126.2 (C$_{Ar}$H ortho to SO$_2$O), 125.8 (C$_{Ar}$H para to CH$_2$), 69.8 (SO$_2$OCH$_2$), 43.4 (NCH$_2$), 40.5 (amino methyl resin), 36.5 (C$_{Ar}$CH$_2$), 35.8 (C(O)CH$_2$), 31.5 (SO$_2$OCH$_2$CH$_2$), 30.8 (C(O)CH$_2$CH$_2$CH$_2$), 30.5 (C$_{Ar}$CH$_2$), 28.9 (C(O)CH$_2$CH$_2$), 25.5 (C$_{Ar}$CH$_2$CH$_2$).
6-(4-Chlorosulfonyl phenyl) hexanoic acid (2.10)

\[
\text{\begin{tabular}{c}
\includegraphics[width=0.3\textwidth]{structure.png} \\
C_{12}H_{15}ClO_4S \\
Mw = 290.76 \text{ gmol}^{-1} \\
Colourless oil
\end{tabular}}
\]

To a solution of 6-phenylhexanoic acid (2.01, 150 mg, 0.78 mmol) in CHCl₃ (2 mL) at 0 °C was added ClSO₂OH (0.16 mL, 2.34 mmol). The reaction was slowly allowed to warm to rt and stirred for 17 h. The reaction mixture was diluted with CHCl₃ (5 mL) and poured onto an excess of ice. The organic layer was separated and the aqueous layer was extracted with CHCl₃ (2 x 10 mL). The combined organic layers were washed with 2 N HCl (1 x 10 mL), dried over MgSO₄ and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 20-50% EtOAc/hexane) to give a colourless oil (123 mg, 0.42 mmol, 54%).

\begin{itemize}
\item \textbf{IR} \vmax (neat) 2939 (br), 2860 (w), 1706 (s), 1593 (w), 1375 (m), 1174 (s), 573 (s) cm\(^{-1}\).
\item \textbf{\textsuperscript{1}H NMR} (300 MHz, CDCl₃) \(\delta\) 7.96 (2H, d, \(J = 8.6\) Hz, \(\text{C}_{\text{Ar}}\text{H} \text{ortho} \) to \(\text{SO}_2\text{Cl}\)), 7.42 (2H, d, \(J = 8.6\) Hz, \(\text{C}_{\text{Ar}}\text{H} \text{meta} \) to \(\text{SO}_2\text{Cl}\)), 2.76 (2H, d, \(J = 7.7\) Hz, \(\text{C}_{\text{Ar}}\text{CH}_2\)), 2.38 (2H, t, \(J = 7.3\) Hz, \(\text{C(O)CH}_2\)), 1.79-1.64 (4H, m, \(\text{C(O)CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\)), 1.48-1.36 (2H, m, \(\text{C(O)CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\)).
\item \textbf{\textsuperscript{13}C NMR} (75 MHz, CDCl₃) \(\delta\) 179.0 (C(O)OH), 151.0 (C\(_{\text{Ar}}\text{CH}_2\)), 142.0 (C\(_{\text{Ar}}\text{SO}_2\text{Cl}\)), 129.6 (C\(_{\text{Ar}}\text{H} \text{meta} \) to \(\text{SO}_2\text{Cl}\)), 127.2 (C\(_{\text{Ar}}\text{H} \text{ortho} \) to \(\text{SO}_2\text{Cl}\)), 35.7 (C\(_{\text{Ar}}\text{CH}_2\)), 33.6 (C(O)CH₂), 30.4 (C(O)CH₂CH₂CH₂), 28.4 (C(O)CH₂CH₂), 24.3 (C\(_{\text{Ar}}\text{CH}_2\text{CH}_2\)).
\end{itemize}

\begin{itemize}
\item \textbf{LRMS} (ES+) \textit{m/z} 313 [M + Na]⁺, 315 (\(^{37}\)Cl isotope peak).
\item \textbf{HRMS} (ES+) calcd for \(\text{C}_{12}\text{H}_{15}\text{ClO}_4\text{SNa} \) [M + Na]⁺ 313.0272, found 313.0271.
\end{itemize}
6-(4-(2-[4-(tert-Butoxycarbonyl phenyl amino) butoxy] sulfonyl) phenyl) hexanoic acid methyl ester (2.15)

\[
\text{C}_28\text{H}_{39}\text{NO}_7\text{S} \\
\text{Mw} = 533.68 \text{ gmol}^{-1}
\]

Orange oil

A solution of sulfonyl chloride 2.03 (5.94 g, 19.5 mmol), (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14, 4.31 g, 16.3 mmol), Et₃N (6.8 mL, 49.0 mmol) and DMAP (500 mg, 4.1 mmol) in CH₂Cl₂ (100 mL) was stirred at rt for 3.25 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL), washed with 2 N HCl (1 x 100 mL), water (1 x 100 mL) and brine (1 x 100 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 10-60% EtOAc/hexane) to give the title compound as an orange oil (7.08 g, 13.3 mmol, 81%).

\[\text{IR } \nu_{\text{max}}\text{ (neat) 2935 (w), 2862 (w), 1736 (m), 1693 (s), 1597 (w), 1363 (m), 1172 (s), 1146 (m) cm}^{-1}.\]

\[\text{H NMR}\]
(400 MHz, CDCl₃) \(\delta\) 7.78 (2H, d, \(J = 8.5\) Hz, \(\text{C}_\text{Ar} \text{H} \text{ortho}\) to SO₂O), 7.37-7.30 (4H, m, \(\text{C}_\text{Ar} \text{H} \text{meta}\) to SO₂O & \text{meta}\) to N), 7.22 (1H, t, \(J = 7.5\) Hz, \(\text{C}_\text{Ar} \text{H} \text{para}\) to N), 7.12 (2H, d, \(J = 7.5\) Hz, \(\text{C}_\text{Ar} \text{H} \text{ortho}\) to N), 4.03 (2H, t, \(J = 6.5\) Hz, SO₂OCH₂), 3.67 (3H, s, C(O)OC₃H₃), 3.60 (2H, t, \(J = 7.2\) Hz, NCH₂), 2.70 (2H, t, \(J = 7.8\) Hz, C₃H₂CH₂), 2.32 (2H, t, \(J = 7.4\) Hz, C(O)CH₂), 1.72-1.63 (6H, m, C(O)CH₂CH₂CH₂CH₂ & SO₂OCH₂CH₂), 1.60-1.52 (2H, m, NCH₂CH₂) 1.43-1.34 (11H, m, C(O)OC(CH₃)₃ & C(O)CH₂CH₂CH₂).

\[\text{C NMR}\]
(100 MHz, CDCl₃) \(\delta\) 174.0 (C(O)OCH₃), 154.7 (C(O)OC(CH₃)₃), 149.1 (C₃H₂CH₂), 142.2 (C₃N), 133.4 (C₃H₂SO₂O), 129.1 (C₃H₂SO₂O), 128.8 (C₃H₂SO₂O), 127.9 (C₃H₂CO₂O), 127.1 (C₃H₂CO₂O), 126.1 (C₃H₂CO₂O), 80.2 (C(O)OC(CH₃)₃), 70.1 (SO₂OCH₂), 51.5 (C(O)CH₃), 49.0 (CH₂N), 35.6 (C₃H₂CH₂), 33.8 (C(O)CH₂), 30.6
(C(O)CH₂CH₂CH₂), 28.6 (C(O)CH₂CH₂), 28.2 (C(O)OC(CH₃)₃), 26.1 (SO₂OCH₂CH₂), 24.6 (C₆H₅CH₂CH₂), 24.1 (CH₂CH₂N).

LRMS  (ES+) m/z 556 [M + Na]+.

HRMS  (ES+) calcd for C₂₈H₃₉NO₇SNa [M + Na]+ 556.2339, found 556.2330.

**General procedure A for sulfonate ester formation as part of the solution model studies**

To a solution of the sulfonyl chloride (2 eq) and the alcohol (1 eq) in CH₂Cl₂ was added Et₃N (2.5 eq) and the solution was heated under reflux at 50 °C. The cooled reaction mixture was diluted with CH₂Cl₂, washed with 2 N HCl, water, and brine, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (eluent 5-40% EtOAc/hexane) to give the title compound.

**6-(4-(2-[4-(tert-Butoxycarbonyl phenyl amino) butoxy] sulfonyl) phenyl) hexanoic acid methyl ester (2.15)**

\[
\text{C}_{28}\text{H}_{39}\text{NO}_{7}\text{S} \\
M_w = 533.68 \text{ gmol}^{-1} \\
\text{Orange oil}
\]

Sulfonyl chloride **2.03** (500 mg, 1.64 mmol) and alcohol **2.14** (218 mg, 0.82 mmol) were used in general procedure A and the reaction was stirred for 16 h to give the title compound as orange oil (155 mg, 0.29 mmol, 35%). Spectroscopic data were consistent with those previously collected.
6-(4-(2-[4-(tert-Butoxycarbonyl phenyl amino) butoxy] sulfonyl) phenyl) hexanoic acid (2.16)

\[
\text{C}_{27}\text{H}_{37}\text{NO}_7\text{S} \\
\text{Mw} = 519.65 \text{ gmol}^{-1}
\]

Colourless oil

To a suspension of Novozym 435® (200 mg) in an aq phosphate buffer (pH 7, 4 mL) was added methyl ester 2.15 (197 mg, 0.37 mmol) in CH\textsubscript{2}Cl\textsubscript{2}/acetone (2:1, 1.5 mL) and the mixture was heated at 50 °C for 18 h. The cooled suspension was filtered through Celite® and the pad was washed with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 mL) and EtOAc (3 x 10 mL). The filtrate was concentrated \textit{in vacuo} and the residue was purified by column chromatography (eluent 10-100% EtOAc/hexane) to give the title compound as a colourless oil (113 mg, 0.22 mmol, 59%) and recovered methyl ester 2.15 (22 mg, 0.04 mmol, 11%).

**IR** \( \nu_{\text{max}} \) (neat) 2934 (br), 2862 (w), 1705 (w), 1693 (br), 1597 (w), 1364 (m), 1172 (s), 1148 (m) cm\(^{-1}\).

**\(^1\)H NMR** (300 MHz, CDCl\textsubscript{3}) \( \delta \) 7.77 (2H, d, \( J = 8.4 \) Hz, C\textsubscript{Ar}H \textit{ortho} to SO\textsubscript{2}O), 7.37-7.28 (4H, m, C\textsubscript{Ar}H \textit{meta} to SO\textsubscript{2}O & \textit{meta} to N), 7.20 (1H, t, \( J = 7.3 \) Hz, C\textsubscript{Ar}H \textit{para} to N), 7.12 (2H, d, \( J = 7.3 \) Hz, C\textsubscript{Ar}H \textit{ortho} to N), 4.02 (2H, t, \( J = 6.3 \) Hz, SO\textsubscript{2}OCH\textsubscript{2}), 3.59 (2H, t, \( J = 7.0 \) Hz, NCH\textsubscript{2}), 2.70 (2H, t, \( J = 7.6 \) Hz, C\textsubscript{Ar}CH\textsubscript{2}), 2.35 (2H, t, \( J = 7.3 \) Hz, C(O)CH\textsubscript{2}), 1.74-1.60 (6H, m, C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2} & SO\textsubscript{2}OCH\textsubscript{2}CH\textsubscript{2}), 1.60-1.49 (2H, m, NCH\textsubscript{2}CH\textsubscript{2}) 1.45-1.33 (11H, m, C(O)OC(CH\textsubscript{3})\textsubscript{3} & C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}).

**\(^{13}\)C NMR** (75 MHz, CDCl\textsubscript{3}) \( \delta \) 178.9 (C(O)OH), 154.8 (C(O)OC(CH\textsubscript{3})\textsubscript{3}), 149.0 (C\textsubscript{Ar}CH\textsubscript{2}), 142.1 (C\textsubscript{Ar}N), 133.3 (C\textsubscript{Ar}SO\textsubscript{2}O), 129.1 (C\textsubscript{Ar}H \textit{meta} to SO\textsubscript{2}O), 128.7 (C\textsubscript{Ar}H \textit{ortho} to N), 127.9 (C\textsubscript{Ar}H \textit{ortho} to SO\textsubscript{2}O), 127.0 (C\textsubscript{Ar}H \textit{meta} to N), 126.2 (C\textsubscript{Ar}H \textit{para} to N), 80.3 (C(O)OC(CH\textsubscript{3})\textsubscript{3}), 70.1 (SO\textsubscript{2}OCH\textsubscript{2}), 49.0 (CH\textsubscript{2}N), 35.6 (C\textsubscript{Ar}CH\textsubscript{2}), 33.7 (C(O)CH\textsubscript{2}), 30.5 (C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}).
28.4 (C(O)CH₂CH₂), 28.2 (C(O)OC(CH₃)₃), 26.0 (SO₂OCH₂CH₂), 24.3 (C₆H₅CH₂CH₂ & CH₂CH₂N).

**LRMS** (ES+) m/z 542 [M + Na]⁺.

**HRMS** (ES+) calcd for C₂₇H₃₇NO₇SNa [M + Na]⁺ 542.2183, found 542.2176.

**Solid supported 6-(4-(2-[4-(tert-butoxycarbonyl phenyl amino) butoxy] sulfonyl) phenyl) hexanoic acid (2.17)**

![Chemical structure](image)

Loading = 0.80 mmolg⁻¹

Resin beads

To a solution of HOBt (53 mg, 0.39 mmol) and DIC (0.06 mL, 0.39 mmol) in DMF (0.6 mL) was added carboxylic acid 2.16 (100 mg, 0.19 mmol) in CH₂Cl₂ (1.4 mL) and amino methyl polystyrene resin (87 mg, loading 1.5 mmolg⁻¹, 0.13 mmol). The mixture was stirred at rt for 17 h after which time a ninhydrin test performed on a small sample of the resin beads was negative, indicating that all of the amino groups had reacted. The resin was removed by filtration, washed with CH₂Cl₂ (3 x 10 mL), MeOH (3 x 10 mL) and Et₂O (3 x 10 mL) and dried *in vacuo* at 40 °C for 24 h to give the product resin (157 mg, theoretical loading 0.83 mmolg⁻¹, actual loading (S elemental analysis) 0.80 mmolg⁻¹, 108%).

**IR**

ν max (neat) 2970 (w), 1739 (m), 1365 (m), 1217 (m), 669 (m) cm⁻¹.

**¹H NMR**

MAS (400 MHz, CDCl₃) δ 7.77 (s, C₆H₅ ortho to SO₂O), 7.32 (s, C₆H₅ meta to SO₂O & ortho to N), 7.20 (s, C₆H₅ para to N), 7.12 (s, C₆H₅ meta to N), 6.66 (amino methyl resin), 4.02 (s, SO₂OCH₂), 3.59 (s, NCH₂), 2.70 (s, C₆H₅CH₂), 2.35 (s, C(O)CH₂), 1.70 (s, C(O)CH₂CH₂CH₂CH₂ & SO₂OCH₂CH₂), 1.55 (s, NCH₂CH₂), 1.38 (s,
C(O)OC(CH$_3$)$_3$ & C(O)CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR MAS (100 MHz, CDCl$_3$) δ 172.8 (C(O)NH), 154.8 (C(O)OC(CH$_3$)$_3$), 149.4 (C$_{Ar}$CH$_2$), 145.3 (amino methyl resin), 142.3 (C$_{Ar}$N), 133.4 (C$_{Ar}$SO$_2$O), 129.4 (C$_{Ar}$H meta to SO$_2$O), 129.0 (C$_{Ar}$H ortho to N), 128.0 (C$_{Ar}$H ortho to SO$_2$O), 127.2 (C$_{Ar}$H meta to N), 126.3 (C$_{Ar}$H para to N), 80.3 (C(O)OC(CH$_3$)$_3$), 70.3 (SO$_2$OCH$_2$), 49.1 (CH$_2$N), 43.3 (amino methyl resin), 40.6 (amino methyl resin), 36.4 (C$_{Ar}$CH$_2$), 35.4 (C(O)CH$_2$), 30.8 (C(O)CH$_2$CH$_2$CH$_2$), 28.9 (C(O)CH$_2$CH$_2$), 28.4 (C(O)OC(CH$_3$)$_3$), 26.2 (SO$_2$OCH$_2$CH$_2$), 25.6 (C$_{Ar}$CH$_2$CH$_2$), 24.6 (CH$_2$CH$_2$N).

(4-Fluoro butyl) phenyl carbamic acid tert-butyl ester (2.18)

![Chemical Structure](image)

C$_{15}$H$_{22}$FNO$_2$
Mw = 267.34 gmol$^{-1}$
Colourless oil

**Solution Phase Method:** A solution of sulfonate ester 2.15 (181 mg, 0.34 mmol), KF (24 mg, 0.41 mmol) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (154 mg, 0.41 mmol) in MeCN (7 mL) was heated under reflux for 15 min. The cooled reaction mixture was concentrated *in vacuo* and the residue was dissolved in CH$_2$Cl$_2$ and was washed with water. The aqueous phase was re-extracted with CH$_2$Cl$_2$ (3 x 20 mL) and the combined organic layers were washed with brine (1 x 20 mL), dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (eluent 5-10% EtOAc/hexane) to give a colourless oil (47 mg, 0.18 mmol, 52%).

**Solid Phase Method – General Procedure B:** A suspension of the resin (1.0 eq), KF (1.2 eq) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (1.2 eq) in MeCN (1 mL/40 μmol of resin) was heated to reflux for 30 min. The resin was removed from the cooled suspension by filtration and was washed with CH$_2$Cl$_2$ (10 mL/40 μmol of
The filtrate was concentrated in vacuo and the residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a colourless oil.

Resin \textbf{2.17} (84 mg, 0.80 mmol\textsuperscript{−1}, 67 \mu mol) was used in general procedure \textbf{B} to give the title compound (5 mg, 19 \mu mol, 28%).

Resin \textbf{2.24} (95 mg, 0.81 mmol\textsuperscript{−1}, 77 \mu mol) was used in general procedure \textbf{B} to give the title compound (6 mg, 22 \mu mol, 28%).

Resin \textbf{2.30} (100 mg, 0.95 mmol\textsuperscript{−1}, 95 \mu mol) was used in general procedure \textbf{B} to give the title compound (7 mg, 26 \mu mol, 27%).

Resin \textbf{2.45} (50 mg, 0.58 mmol\textsuperscript{−1}, 29 \mu mol) was used in general procedure \textbf{B} to give the title compound (1 mg, 3.7 \mu mol, 13%).

\textbf{IR} \quad \nu_{\max} \text{ (neat)} 2970 \text{ (w)}, 1738 \text{ (w)}, 1694 \text{ (s)}, 1391 \text{ (m)}, 1366 \text{ (m)}, 1147 \text{ (m)}, 698 \text{ (m)} \text{ cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} \quad (300 MHz, CDCl\textsubscript{3}) \delta 7.45-7.29 \text{ (2H, m, C\textsubscript{Ar}H meta to N)}, 7.26-7.11 \text{ (3H, m, C\textsubscript{Ar}H ortho \& para to N)}, 4.44 \text{ (2H, dt,} \, ^{2}J_{HF} = 47.4, \, J = 5.7 \text{ Hz, FCH\textsubscript{2})}, 3.68 \text{ (2H, t,} \, J = 7.0 \text{ Hz, NCH\textsubscript{2})}, 1.82-1.64 \text{ (4H, m, FCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}}), 1.43 \text{ (9H, s, C(O)OC(CH\textsubscript{3})\textsubscript{3})}.

\textbf{\textsuperscript{13}C NMR} \quad (75 MHz, CDCl\textsubscript{3}) \delta 154.7 \text{ (C(O)OC(CH\textsubscript{3})\textsubscript{3})}, 142.3 \text{ (C\textsubscript{Ar}N)}, 128.8 \text{ (C\textsubscript{Ar}H ortho to N)}, 127.1 \text{ (C\textsubscript{Ar}H meta to N)}, 126.1 \text{ (C\textsubscript{Ar}H para to N)}, 83.7 \text{ (d,} \, J = 164.8 \text{ Hz, FCH\textsubscript{2})}, 80.1 \text{ (C(O)OC(CH\textsubscript{3})\textsubscript{3})}, 49.4 \text{ (NCH\textsubscript{2})}, 28.3 \text{ (C(O)OC(CH\textsubscript{3})\textsubscript{3})}, 27.3 \text{ (d,} \, J = 19.9 \text{ Hz, FCH\textsubscript{2}CH\textsubscript{2})}, 24.3 \text{ (d,} \, J = 4.4 \text{ Hz, NCH\textsubscript{2}CH\textsubscript{2})}.

\textbf{\textsuperscript{19}F NMR} \quad (282 MHz, CDCl\textsubscript{3}) \delta -218.4 \text{ (FCH\textsubscript{2})}.
LRMS  

HRMS  
(ES+) calcd for C$_{15}$H$_{22}$FNO$_2$Na [M + Na]$^+$ 290.1527, found 290.1521.

General procedure C for the solution phase fluoridative cleavage to give (4-fluorobutyl) phenyl carbamic acid tert-butyl ester (2.18)

A solution of the sulfonate ester (1 eq), KF (1.2 eq) and Kryptofix 2.2.2 (1.2 eq) in MeCN was heated under reflux at 80 °C. The cooled reaction mixture was concentrated in vacuo and the residue was dissolved in CH$_2$Cl$_2$, washed with water and the aqueous phase extracted was with CH$_2$Cl$_2$. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (eluent 10% EtOAc/hexane) to give the title compound as a colourless oil. Spectroscopic data were consistent with those previously collected.

Sulfonate ester 2.31 (140 mg, 0.33 mmol) was used in general procedure C and the reaction was stirred for 45 min to give the title compound (56 mg, 0.21 mmol, 64%).

Sulfonate ester 2.32 (149 mg, 0.33 mmol) was used in general procedure C and the reaction was stirred for 30 min to give the title compound (55 mg, 0.21 mmol, 62%).

Sulfonate ester 2.33 (113 mg, 0.33 mmol) was used in general procedure C and the reaction was stirred for 3.5 h to give the title compound (66 mg, 0.25 mmol, 75%).
4-Phenyl butyric acid methyl ester (2.20)

Following the procedure of Hanessian et al.,$^{59}$ to a solution of 4-phenyl-butyric acid (2.19, 5.00 g, 30.5 mmol) in MeOH (80 mL) at 0 ºC was added TMSCl (15.50 mL, 121.8 mmol) and the solution was stirred for 1.5 h. The reaction mixture was quenched with water (6 mL) and concentrated in vacuo. The residue was dissolved in CH$_2$Cl$_2$, dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (elucent 20% EtOAc/ hexane) to give the title compound as a colourless liquid (4.17 g, 23.4 mmol, 77%). Spectroscopic data were in agreement with those published in the literature.$^{153}$

**IR** $\nu_{\text{max}}$ (neat) 3027 (w), 2950 (w), 1733 (s), 1454 (m), 1435 (m), 1201 (m), 1143 (m), 745 (m), 699 (s) cm$^{-1}$.

**$^1$H NMR** (300 MHz, CDCl$_3$) $\delta$ 7.37 (2H, m C$_{Ar}$H ortho to CH$_2$), 7.30-7.23 (3H, m, C$_{Ar}$H meta & para to CH$_2$), 3.74 (3H, s, OCH$_3$), 2.73 (2H, t, $J = 7.5$ Hz, C$_{Ar}$CH$_2$), 2.41 (2H, t, $J = 7.5$ Hz, C(O)CH$_2$), 2.04 (2H, quin, $J = 7.5$ Hz, C(O)CH$_2$CH$_2$).

**$^{13}$C NMR** (75 MHz, CDCl$_3$) $\delta$ 173.9 (C(O)OCH$_3$), 141.3 (C$_{Ar}$CH$_2$), 128.5 (C$_{Ar}$H meta to CH$_2$), 128.4 (C$_{Ar}$H ortho to CH$_2$), 126.0 (C$_{Ar}$H para to CH$_2$), 51.5 (C(O)CH$_3$), 35.1 (C$_{Ar}$CH$_2$), 33.4 (C(O)CH$_2$), 26.5 (C(O)CH$_2$CH$_2$).

**LRMS** (EI) 178 (M$^+$, 30%), 104 (C$_6$H$_5$CH$_2$CH$_2$CH$^+$, 89%), 91 (C$_6$H$_5$CH$_2$,$^+$, 100%).
4-(4-Chlorosulfonyl phenyl) butyric acid methyl ester (2.21)

To a solution of methyl ester 2.20 (3.10 g, 17.4 mmol) in CH₂Cl₂ (60 mL) at 0 °C was added ClSO₂OH (3.50 mL, 52.2 mmol). The solution was allowed to slowly warm up to rt and it was stirred for 19 h. The reaction mixture was concentrated in vacuo and the residue dissolved in Et₂O (40 mL). The solution was quenched with ice and the aqueous layer was separated and extracted with Et₂O (2 x 20 mL). The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The residue was dissolved in CH₂Cl₂ (40 mL) and cooled to 0 °C before MeOH (1 mL) and acetyl chloride (0.60 mL, 8.0 mmol) was added and the solution was stirred for 1 h. Further MeOH (5 mL) and acetyl chloride (0.30 mL, 4.0 mmol) were added and the solution stirred for an additional 1 h. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (eluent 10% EtOAc/hexane) to give the title compound as a colourless oil (2.90 g, 10.5 mmol, 60%).

IR

νₓₓₓ (neat) 2952 (w), 1732 (m), 1593 (w), 1374(m), 1172 (s), 571 (s) cm⁻¹.

¹H NMR

(300 MHz, CDCl₃) δ 7.97 (2H, d, J = 8.4 Hz, C₆H ortho to SO₂Cl), 7.44 (2H, d, J = 8.4 Hz, C₆H meta to SO₂Cl), 3.69 (3H, s, OC₃H₃), 2.80 (2H, t, J = 7.5 Hz, C₆CH₂), 2.38 (2H, t, J = 7.5 Hz, C(O)CH₂), 2.01 (2H, quin, J = 7.5 Hz, C(O)CH₂CH₂).

¹³C NMR

(75 MHz, CDCl₃) δ 173.3 (C(O)OCH₃), 150.1 (C₆CH₂), 142.2 (C₆SO₂Cl), 129.7 (C₆H meta to SO₂Cl), 127.2 (C₆H ortho to SO₂Cl), 51.7 (OCH₃), 35.1 (C₆CH₂), 33.1 (C(O)CH₂), 25.9 (C(O)CH₂CH₂).

LRMS

(ES+) m/z 299 [M + Na]⁺, 301 (¹⁷Cl isotope peak).
4-(4-[4-(tert-Butoxycarbonyl phenyl amino) butoxysulfonyl] phenyl) butyric acid methyl ester (2.22)

A solution of sulfonyl chloride 2.21 (2.22 g, 8.0 mmol), (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14, 1.78 g, 6.7 mmol), Et₃N (2.30 mL, 16.8 mmol) and DMAP (208 mg, 1.7 mmol) in CH₂Cl₂ (30 mL) was stirred at rt for 2.5 h. The reaction mixture was diluted with CH₂Cl₂ (10 mL), washed with 2 N HCl (1 x 30 mL), water (1 x 30 mL) and brine (1 x 30 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20-40% EtOAc/hexane) to give the title compound as a colourless oil (2.92 g, 5.8 mmol, 86%).

IR ν max (neat) 2952 (w), 1736 (m), 1694 (m), 1597 (w), 1364 (m), 1175 (s) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 7.80 (2H, d, J = 8.3 Hz, C_ArH ortho to SO₂O), 7.38-7.30 (4H, m, C_ArH meta to SO₂O & meta to N), 7.20 (1H, tt, J = 7.5, 2.1 Hz, C_ArH para to N), 7.13 (2H, d, J = 7.5 Hz, C_ArH ortho to N), 4.02 (2H, t, J = 6.2 Hz, SO₂OCH₂), 3.69 (3H, s, OCH₃), 3.60 (2H, t, J = 7.0 Hz, NCH₂), 2.74 (2H, t, J = 7.5 Hz, C_ArCH₂), 2.36 (2H, t, J = 7.3 Hz, C(O)CH₂H₂), 1.98 (2H, tt, J = 7.5, 7.3 Hz, C(O)CH₂H₂), 1.71-1.53 (4H, m, SO₂OCH₂H₂CH₂H₂), 1.51 (9H, s, C(O)OC(C₃H₃)).

¹³C NMR (75 MHz, CDCl₃) δ 176.1 (C(O)CH₃), 154.7 (NC(O)O), 148.0 (C_ArCH₂), 138.6 (C_ArN), 133.8 (C_ArSO₂O), 129.3 (C_ArH meta to SO₂O), 128.8 (C_ArH ortho to N), 128.0 (C_ArH ortho to SO₂O), 127.1 (C_ArH meta...
to N), 126.2 (C<sub>A</sub>H <i>para</i> to N), 80.2 (C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 70.1 (SO<sub>2</sub>OCH<sub>2</sub>), 51.6 (OCH<sub>3</sub>), 49.0 (CH<sub>2</sub>N), 35.0 (C<sub>A</sub>CH<sub>2</sub>), 33.2 (C(O)CH<sub>2</sub>), 28.3 (C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 26.2 (SO<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>), 26.0 (C(O)CH<sub>2</sub>CH<sub>2</sub>), 24.5 (CH<sub>2</sub>CH<sub>2</sub>N).

**LRMS**  
(ES+) <i>m/z</i> 528 [M + Na]<sup>+</sup>, 1034 [2M + Na]<sup>+</sup>.

**HRMS**  
(ES+) calcd for C<sub>26</sub>H<sub>35</sub>NOC<sub>7</sub>SNa [M + Na]<sup>+</sup> 528.2026, found 528.2027.

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4-(4-[4-(<i>tert</i>-Butoxycarbonyl phenyl amino) butoxysulfonyl] phenyl) butyric acid (2.23)

![Chemical structure](image)

**C<sub>25</sub>H<sub>33</sub>NOC<sub>7</sub>S**  
**Mw = 491.60 gmol<sup>-1</sup>**  
**Colourless oil**

To a suspension of Novozym 435® (250 mg) in aq phosphate buffer (pH 7, 5 mL) was added methyl ester 2.22 (228 mg, 0.45 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/acetone (4:3, 3.5 mL) and the mixture was stirred at 50 °C for 22 h. Further Novozym 435® (100 mg) was added and the suspension stirred for an additional 7 h. The cooled reaction mixture was filtered through a pad of Celite® and the pad was washed with CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The filtrate was acidified with 2N HCl, the organic layer separated and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated <i>in vacuo</i>. The residue was purified by column chromatography (eluent 20-40% EtOAc/hexane) to give the title compound as a colourless oil (128 mg, 0.26 mmol, 58%).

**IR**  
<i>ν<sub>max</sub></i> (neat) 2974 (w), 2934 (w), 1733 (w), 1693 (m), 1597(w), 1363 (m), 1173 (s), 935 (m) cm<sup>-1</sup>.

**<sup>1</sup>H NMR**  
(300 MHz, CDCl<sub>3</sub>) δ 7.80 (2H, d, <i>J</i> = 8.2 Hz, C<sub>A</sub>H <i>ortho</i> to SO<sub>2</sub>O),
7.39-7.31 (4H, m, C>H meta to SO2O & meta to N), 7.21 (1H, t, J = 7.5 Hz, C>H para to N), 7.12 (2H, d, J = 7.5 Hz, C>H ortho to N), 4.05 (2H, t, J = 6.3 Hz, SO2OCH2), 3.60 (2H, t, J = 7.1 Hz, NCH2), 2.77 (2H, t, J = 7.3 Hz, CArCH2), 2.40 (2H, t, J = 7.3 Hz, C(O)CH2), 2.00 (2H, quin, J = 7.3 Hz, C(O)CH2CH2), 1.72-1.50 (4H, m, SO2OCH2CH2CH2), 1.40 (9H, s, C(O)OC(CH3)3).

\[^{13}\text{C} \text{ NMR} \]
(75 MHz, CDCl3) δ 178.2 (C(O)OH), 154.8 (NC(O)O), 147.8 (CArCH2), 142.1 (CArN), 133.8 (CArSO2O), 129.3 (CArH meta to SO2O), 128.8 (CArH meta to CN), 128.0 (CArH ortho to SO2O), 127.1 (CArH meta to N), 126.2 (CArH para to N), 80.3 (C(O)OC(CH3)3), 70.2 (SO2OCH2), 48.9 (CH2N), 34.9 (CArCH2), 33.0 (C(O)CH2), 28.3 (C(O)OC(CH3)3), 26.2 (SO2OCH2CH2), 25.6 (C(O)CH2CH2), 24.3 (CH2CH2N).

\[^{13}\text{C} \text{ NMR} \]
(75 MHz, CDCl3) δ 178.2 (C(O)OH), 154.8 (NC(O)O), 147.8 (CArCH2), 142.1 (CArN), 133.8 (CArSO2O), 129.3 (CArH meta to SO2O), 128.8 (CArH meta to CN), 128.0 (CArH ortho to SO2O), 127.1 (CArH meta to N), 126.2 (CArH para to N), 80.3 (C(O)OC(CH3)3), 70.2 (SO2OCH2), 48.9 (CH2N), 34.9 (CArCH2), 33.0 (C(O)CH2), 28.3 (C(O)OC(CH3)3), 26.2 (SO2OCH2CH2), 25.6 (C(O)CH2CH2), 24.3 (CH2CH2N).


Solid supported 4-(4-[[4-(tert-butoxycarbonyl phenyl amino) butoxysulfonyl] phenyl) butyric acid (2.24)

To a solution of HOBt (97 mg, 0.72 mmol) in DMF (1.2 mL) was added carboxylic acid 2.23 (119 mg, 0.24 mmol) in CH2Cl2 (2.8 mL) and the solution was stirred at rt for 10 min. DIC (0.11 mL, 0.72 mmol) was added and the solution was stirred for a further 10 min. Amino methyl polystyrene resin (110 mg, 1.5 mmolg⁻¹, 0.16 mmol) was added and the suspension was stirred for 18 h after which time a ninhydrin test on a sample of resin beads was negative. The resin was removed by filtration, washed with CH2Cl2 (3 x 10 mL), MeOH (3 x 10 mL) and Et2O (3 x 10 mL) and dried in vacuo at 40 °C for 35
h to give the product resin (174 mg, theoretical loading 0.92 mmolg\(^{-1}\), actual loading (S elemental analysis) 0.81 mmolg\(^{-1}\), 84%).

**IR**

\(\nu_{\text{max}}\) (neat) 3360 (br), 3023 (w), 2924 (w), 1694 (m), 1598 (w), 1493 (m), 1363 (m), 1173 (m), 697 (s) cm\(^{-1}\).

**\(^1\)H NMR**

MAS (400 MHz, CDCl\(_3\)) \(\delta\) 7.76 (s, C\(_{\text{Ar}}\)H \text{ortho} to SO\(_2\)O), 7.30 (s, C\(_{\text{Ar}}\)H \text{ortho} to N), 7.11 (s, C\(_{\text{Ar}}\)H \text{para} to N), 7.10 (s, C\(_{\text{Ar}}\)H \text{meta} to N), 6.54 (amino methyl resin), 4.28 (amino methyl resin), 4.01 (s, SO\(_2\)OCH\(_2\)), 3.58 (s, NCH\(_2\)), 2.71 (s, C\(_{\text{Ar}}\)CH\(_2\)), 2.16 (s, NC(O)CH\(_2\)), 1.98 (s, NC(O)CH\(_2\)CH\(_2\)), 1.82 (amino methyl resin), 1.64 (s, CH\(_2\)CH\(_2\)N), 1.54 (s, SO\(_3\)CH\(_2\)CH\(_2\)), 1.39 (s, C(O)OC(CH\(_3\))\(_3\)).

**\(^{13}\)C NMR**

MAS (100 MHz, CDCl\(_3\)) \(\delta\) 172.3 (NC(O)CH\(_2\)), 155.1 (NC(O)O), 148.7 (C\(_{\text{Ar}}\)CH\(_2\)), 145.5 (amino methyl resin), 142.5 (C\(_{\text{Ar}}\)N), 134.1 (C\(_{\text{Ar}}\)SO\(_2\)O), 129.8 (C\(_{\text{Ar}}\)H \text{meta} to SO\(_2\)O), 129.3 (C\(_{\text{Ar}}\)H \text{ortho} to N), 128.4 (C\(_{\text{Ar}}\)H \text{ortho} to SO\(_2\)O), 127.5 (C\(_{\text{Ar}}\)H \text{meta} to N), 126.6 (C\(_{\text{Ar}}\)H \text{para} to N), 80.6 (C(O)OC(CH\(_3\))\(_3\)), 70.7 (SO\(_2\)OCH\(_2\)), 49.4 (CH\(_2\)N), 43.8 (amino methyl resin), 40.8 (amino methyl resin), 35.8 (C\(_{\text{Ar}}\)CH\(_2\)), 35.5 NC(O)CH\(_2\)), 28.7 (C(O)OC(CH\(_3\))\(_3\)), 27.0 (SO\(_2\)OCH\(_2\)CH\(_2\)), 26.5 (C(O)CH\(_2\)CH\(_2\)), 24.9 (CH\(_2\)CH\(_2\)N).

**Phenyl acetic acid methyl ester (2.26)**

\[
\begin{array}{c}
\text{MeO} \\
\text{O} \\
\text{C} \\
\text{O} \\
\text{C} \\
\text{C} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\end{array}
\]

\(C_9H_{10}O_2\)

Mw = 150.17 gmol\(^{-1}\)

Pale yellow liquid

Following the procedure of Hanessian et al.,\(^{59}\) to a solution of phenyl acetic acid (2.25, 20.00 g, 146.9 mmol) in MeOH (200 mL) at 0 °C was added TMSCl (19.00 mL, 146.9 mmol) and the solution was stirred for 2.25 h. The solvent was removed and the residue was diluted with ether (150 mL) and washed with sat aq NaCO\(_3\)H (1 x 50 mL). The
aqueous layer was extracted with ether (2 x 50 mL). The combined organic layers were
dried over MgSO₄ and concentrated in vacuo to give the crude product as a pale yellow
liquid (21.14 g, 140.8 mmol, 96%). The crude product was used in subsequent
reactions without further purification. Spectroscopic data were in agreement with those
published in the literature.¹⁵⁴

**IR**

\[ \nu_{\text{max}} \text{ (neat)} = 3031 \text{ (w), } 2952 \text{ (w), } 1733 \text{ (s), } 1435 \text{ (m), } 1252 \text{ (m), } 1139 \text{ (m), } 696 \text{ (s) cm}^{-1}. \]

**¹H NMR**

(300 MHz, CDCl₃) \( \delta = 7.40-7.25 \) (5H, m, C₆H₅), 3.71 (3H, s, OCH₃), 3.65
(2H, s, C(O)CH₂).

**¹³C NMR**

(75 MHz, CDCl₃) \( \delta = 172.0 \) (C(O)CH₃), 134.0 (C₆H₅CH₂), 129.2 (C₆H₅ ortho to CH₂), 128.8 (C₆H₅ meta to CH₂), 127.1 (C₆H₅ para to CH₂), 52.0 (OCH₃), 41.2 (C(O)CH₂).

**LRMS**

(ES⁺) \( m/z = 173 \) [M + Na]⁺.

(4-Chlorosulfonyl phenyl) acetic acid methyl ester (2.27)

\[ \begin{align*}
\text{C₉H₉ClO₄S} \\
\text{Mw} = 248.68 \text{ gmol}^{-1} \\
\text{Colourless liquid}
\end{align*} \]

To a solution of methyl ester 2.26 (1.93g, 12.8 mmol) in CH₂Cl₂ (40 mL) at 0 °C was
added ClSO₂OH (2.60 mL, 38.4 mmol) and the solution was allowed to slowly warm to
rt. Further ClSO₂OH (0.90 mL, 12.8 mmol) was added after 17 h and the solution was
stirred for a total of 21 h. The reaction mixture was concentrated in vacuo and the
residue was dissolved in Et₂O (40 mL) and quenched with water (30 mL). The aqueous
layer was separated and extracted with Et₂O (2 x 30 mL). The combined organic layers
were dried over MgSO₄ and concentrated in vacuo. The residue was dissolved in
CH₂Cl₂ (30 mL) and cooled to 0 °C. MeOH (5 mL) and AcCl (0.70 mL, 9.6 mmol)
were added and the solution was stirred for 1.5 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (eluent 10% EtOAc/hexane) to give the title compound as a colourless liquid (1.08 g, 4.3 mmol, 34%).

**IR**

\[ \nu_{\text{max}} \text{ (neat)} 2954 \text{ (w)}, 1733 \text{ (m)}, 1372 \text{ (m)}, 1164 \text{ (s)}, 567 \text{ (s)} \text{ cm}^{-1}. \]

**\(^1\)H NMR**

(300 MHz, CDCl\(_3\)) \[ \delta \] 8.01 (2H, d, \( J = 8.5 \text{ Hz} \), C\(_{\text{Ar}}\)H \text{ortho} to SO\(_2\)Cl), 7.55 (2H, d, \( J = 8.5 \text{ Hz} \), C\(_{\text{Ar}}\)H \text{meta} to SO\(_2\)Cl), 3.77 (2H, s, C(O)CH\(_2\)), 3.75 (3H, s, OCH\(_3\)).

**\(^{13}\)C NMR**

(75 MHz, CDCl\(_3\)) \[ \delta \] 170.3 (C(O)CH\(_3\)), 142.0 (C\(_{\text{Ar}}\)CH\(_2\)), 135.4 (C\(_{\text{Ar}}\)SO\(_2\)Cl), 130.7 (C\(_{\text{Ar}}\)H \text{meta} to SO\(_2\)Cl), 127.3 (C\(_{\text{Ar}}\)H \text{ortho} to SO\(_2\)Cl), 52.5 (OCH\(_3\)), 40.9 (C(O)CH\(_2\)).

**LRMS**

(ES+) \( m/z \) 271 [M + Na]\(^+\), 273 (\(^{35}\)Cl isotope peak).

**HRMS**

(ES+) calcd for C\(_9\)H\(_9\)\(^{35}\)ClO\(_4\)SNa [M + Na]\(^+\) 270.9802, found 270.9804.

(4-[4-(tert-Butoxycarbonyl phenyl amino) butoxysulfonyl] phenyl) acetic acid methyl ester (2.28)

![Chemical Structure](image)

\( \text{C}_{24}\text{H}_{31}\text{NO}_{7}\text{S} \)

Mw = 477.57 g mol\(^{-1}\)

Orange oil

To a solution of sulfonyl chloride 2.27 (3.76 g, 15.1 mmol) and (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14, 2.00 g, 7.5 mmol) in CH\(_2\)Cl\(_2\) (50 mL) was added Et\(_3\)N (2.60 mL, 18.8 mmol) and the mixture was stirred at 50 °C for 3 h to give a yellow/green solution. The cooled reaction mixture was diluted with CH\(_2\)Cl\(_2\), washed with 2N HCl (1 x 50 mL), water (1 x 50 mL) and brine (1 x 50 mL), dried over MgSO\(_4\) and concentrated *in vacuo*. The residue was purified by column chromatography
(eluent 10-40% EtOAc/hexane) to give the title compound as an orange oil (2.94 g, 6.2 mmol, 83%).

IR

\[
\nu_{\text{max}} \text{ (neat) } 2976 \text{ (w), 1737 (m), 1690 (s), 1364 (m), 1145 (s), 729 (m), 697 (m) cm}^{-1}.
\]

\[\text{^1H NMR} \]

(300 MHz, CDCl\textsubscript{3}) \(\delta\) 7.82 (2H, d, \(J = 8.4\) Hz, \(\text{C}_\text{Ar}\) \(\text{H} \) ortho to SO\textsubscript{2}O), 7.46 (2H, d, \(J = 8.4\) Hz, \(\text{C}_\text{Ar}\) \(\text{H} \) meta to SO\textsubscript{2}O), 7.32 (2H, t, \(J = 7.5\) Hz, \(\text{C}_\text{Ar}\) \(\text{H} \) meta to N), 7.20 (1H, t, \(J = 7.5\) Hz, \(\text{C}_\text{Ar}\) \(\text{H} \) para to N), 7.13 (2H, d, \(J = 7.5\) Hz, \(\text{C}_\text{Ar}\) \(\text{H} \) ortho to N), 4.03 (2H, t, \(J = 6.2\) Hz, SO\textsubscript{2}OC\textsubscript{H}\textsubscript{2}), 3.73 (5H, s, \(\text{CH}_3\text{OC(O)CH}_3\)), 3.60 (2H, t, \(J = 7.0\) Hz, NCH\textsubscript{2}), 1.70-1.50 (4H, m, SO\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}), 1.40 (9H, s, C(O)OC(CH\textsubscript{3})\textsubscript{3}).

\[\text{^13C NMR} \]

(75 MHz, CDCl\textsubscript{3}) \(\delta\) 170.7 (C(O)OC\textsubscript{H}\textsubscript{3}), 154.7 (NC(O)O), 142.1 (\(\text{C}_\text{Ar}\)CH\textsubscript{2}), 140.1 (\(\text{C}_\text{Ar}\)N), 134.8 (\(\text{C}_\text{Ar}\)SO\textsubscript{2}O), 130.2 (\(\text{C}_\text{Ar}\) \(\text{H} \) meta to SO\textsubscript{2}O), 128.8 (\(\text{C}_\text{Ar}\) \(\text{H} \) ortho to N), 128.0 (\(\text{C}_\text{Ar}\) \(\text{H} \) ortho to SO\textsubscript{2}O), 127.0 (\(\text{C}_\text{Ar}\) \(\text{H} \) meta to N), 126.1 (\(\text{C}_\text{Ar}\) \(\text{H} \) para to N), 80.2 (C(O)OC(CH\textsubscript{3})\textsubscript{3}), 70.3 (SO\textsubscript{2}OCH\textsubscript{2}), 52.3 (OCH\textsubscript{3}), 48.9 (CH\textsubscript{2}N), 40.8 (C(O)CH\textsubscript{2}), 28.2 (C(O)OC(CH\textsubscript{3})\textsubscript{3}), 26.1 (SO\textsubscript{2}OCH\textsubscript{2}CH\textsubscript{2}), 24.4 (CH\textsubscript{2}CH\textsubscript{2}N).

LRMS

(ES+) \text{m/z} 500 [M + Na]+.

HRMS

(ES+) calcd for C\textsubscript{24}H\textsubscript{31}NO\textsubscript{7}SNa [M + Na]+ 500.1713, found 500.1721.

\((\text{4-[4-}\text{(tert-Butoxycarbonyl phenyl amino) butoxysulfonyl} \text{ phenyl)acetic acid (2.29)}})\)

\[
\begin{align*}
\text{C}_2\text{H}_2\text{NO}_7\text{S} \\
\text{Mw} = 463.54 \text{ gmol}^{-1} \\
\text{Colourless oil}
\end{align*}
\]

To a suspension of Novozym 435® (2.80 g) in aq phosphate buffer (pH 7, 60 mL) was added methyl ester 2.28 (2.84 g, 6.0 mmol) in CH\textsubscript{2}Cl\textsubscript{2}/acetone (4:3, 46 mL) and the
solution was stirred at 50 °C for 23 h. Further Novozym 435® (1.00 g) was added and
the suspension stirred for a further 24 h. The cooled reaction mixture was filtered
through a pad of Celite® and the pad was washed with CH₂Cl₂. The filtrate was
acidified with 2N HCl, the organic layer separated and the aqueous layer was extracted
with CH₂Cl₂ (4 x 100 mL). The combined organic layers were dried over MgSO₄ and
concentrated in vacuo. The residue was purified by column chromatography (eluend 20-
60% EtOAc/hexane) to give the title compound as a colourless oil (1.25 g, 2.7 mmol,
45%) and recovered methyl ester 2.28 (522 mg, 1.0 mmol, 17%).

IR ν<sub>max</sub> (neat) 2970 (w), 1738 (m), 1625 (m), 1365 (m), 1174 (m), 912 (m),
726 (s) cm<sup>−1</sup>.

<sup>1</sup>H NMR (300 MHz, CDCl₃) δ 7.85 (2H, d, <i>J</i> = 8.4 Hz, C<sub>Ar</sub>H <i>ortho</i> to SO₂O), 7.47
(2H, d, <i>J</i> = 8.4 Hz, C<sub>Ar</sub>H <i>meta</i> to SO₂), 7.33 (2H, t, <i>J</i> = 7.3 Hz, C<sub>Ar</sub>H
<i>meta</i> to N), 7.22 (1H, t, <i>J</i> = 7.3 Hz, C<sub>Ar</sub>H <i>para</i> to N), 7.12 (2H, d, <i>J</i> = 7.3
Hz, C<sub>Ar</sub>H <i>ortho</i> to N), 4.04 (2H, t, <i>J</i> = 6.2 Hz, SO₂OCH₂), 3.74 (2H, s, C(O)CH₂),
3.59 (2H, t, <i>J</i> = 7.1 Hz, NCH₂), 1.75-1.46 (4H, m, SO₂OCH₂CH₂CH₂), 1.40 (9H, s, C(O)OC(CH₃)₃).

<sup>13</sup>C NMR (75 MHz, CDCl₃) δ 174.5 (C(O)OH), 154.8 (NC(O)O), 142.2 (C<sub>Ar</sub>CH₂),
139.6 (C<sub>Ar</sub>N), 135.2 (C<sub>Ar</sub>SO₂O), 130.3 (C<sub>Ar</sub>H <i>meta</i> to SO₂O), 128.8
(C<sub>Ar</sub>H <i>ortho</i> to N), 128.1 (C<sub>Ar</sub>H <i>ortho</i> to SO₂O), 127.1 (C<sub>Ar</sub>H <i>meta</i> to
N), 126.2 (C<sub>Ar</sub>H <i>para</i> to N), 80.4 (C(O)OC(CH₃)₃), 70.4 (SO₂OCH₂),
49.0 (CH₂N), 40.6 (C(O)CH₂), 28.3 (C(O)OC(CH₃)₃), 26.1
(SO₂OCH₂CH₂), 24.4 (CH₂CH₂N).

LRMS (ES+) <i>m/z</i> 486 [M + Na]<sup>+</sup>, 950 [2M + Na]<sup>+</sup>.

HRMS (ES+) calcd for C₂₃H₂₉NO₇S [M + Na]<sup>+</sup> 486.1557, found 486.1560.
Solid supported \((4-[4-\text{(tert-butoxycarbonyl phenyl amino) butoxysulfonyl]} \text{ phenyl) acetic acid (2.30)\}^\text{\textsuperscript{\textcopyright}}\)

\[
\begin{align*}
\text{Loading} &= 0.95 \text{ mmolg}^{-1} \\
\text{Resin beads}
\end{align*}
\]

To a solution of HOBt (703 mg, 5.2 mmol) in DMF (12 mL) was added carboxylic acid \textbf{2.29} (1.23 g, 2.6 mmol) in \(\text{CH}_2\text{Cl}_2\) (28 mL) and the solution was stirred at rt for 10 min. DIC (0.80 mL, 5.2 mmol) was added and the solution was stirred for a further 10 min. Amino methyl polystyrene resin (1.13 g, 1.5 mmolg\(^{-1}\), 1.7 mmol) was added and the suspension was stirred for 21 h after which time a ninhydrin test on a small sample of resin beads was negative, indicating that all of the amino groups had reacted. The resin was removed by filtration, washed with \(\text{CH}_2\text{Cl}_2\) (3 x 100 mL), MeOH (3 x 100 mL) and \(\text{Et}_2\text{O}\) (3 x 100 mL) and dried in vacuo at 40 °C for 35 h to give the product resin (1.98 g, theoretical loading 0.86 mmolg\(^{-1}\), actual loading (S elemental analysis) 0.95 mmolg\(^{-1}\), 112%).

\[
\begin{align*}
\textbf{IR} & \quad \nu_{\text{max}} \text{(neat)} & 3308 \text{ (br)}, & 3025 \text{ (w)}, & 2923 \text{ (w)}, & 1684 \text{ (m)}, & 1598 \text{ (w)}, & 1493 \text{ (m)}, & 1363 \text{ (m)}, & 1173 \text{ (m)}, & 696 \text{ (s)} \text{ cm}^{-1}.
\end{align*}
\]

\[
\begin{align*}
\textbf{\textsuperscript{1}H NMR} & \quad \text{MAS (400 MHz, CDCl}_3) & \delta & 7.83 \text{ (s, C}_\text{Ar} \text{H ortho to SO}_2\text{O)}, & 7.45 \text{ (s, C}_\text{Ar} \text{H meta to SO}_2\text{O)}, & 7.35 \text{ (s, C}_\text{Ar} \text{H ortho to N)}, & 7.22 \text{ (s, C}_\text{Ar} \text{H para to N)}, & 7.17 \text{ (s, C}_\text{Ar} \text{H meta to N)}, & 6.63 \text{ (amino methyl resin)}, & 4.05 \text{ (s, SO}_2\text{OC}_2\text{H}_2), & 3.63 \text{ (s, CH}_2\text{N)}, & 3.52 \text{ (s, NC(O)CH}_2), & 1.68 \text{ (s, SO}_2\text{OCH}_2\text{CH}_2), & 1.60 \text{ (s, CH}_2\text{CH}_2\text{N)}, & 1.44 \text{ (s, C(O)OC(CH}_3)_3).
\end{align*}
\]

\[
\begin{align*}
\textbf{\textsuperscript{13}C NMR} & \quad \text{MAS (100 MHz, CDCl}_3) & \delta & 169.1 \text{ (NC(O)CH}_2), & 154.8 \text{ (NC(O)O),} & 145.2 \text{ (amino methyl resin),} & 142.2 \text{ (C}_\text{Ar}\text{CH}_2), & 141.7 \text{ (C}_\text{Ar}N), & 134.7 \text{ (C}_\text{Ar}\text{SO}_2\text{O),} & 130.2 \text{ (C}_\text{Ar}\text{H meta to SO}_2\text{O),} & 128.9 \text{ (C}_\text{Ar}\text{H ortho to N),} & 128.1 \text{ (C}_\text{Ar}\text{H ortho to SO}_2\text{O),} & 127.2 \text{ (C}_\text{Ar}\text{H meta to N),} & 126.3 \text{ (C}_\text{Ar}\text{H para to N),} & 80.3 \text{ (C(O)OC(CH}_3)_3), & 70.5 \text{ (SO}_2\text{OCH}_2), & 49.1 \text{ (CH}_2\text{N),} & 43.1 \text{ (amino methyl}
\end{align*}
\]
resin), 40.8 (NC(O)CH₂), 28.4 (C(O)OC(CH₃)₃), 26.2 (SO₂OCH₂CH₂), 24.5 (CH₂CH₂N).

**Toluene-4-sulfonic acid 4-(**tert**-butoxycarbonyl phenyl amino) butyl ester (2.31)**

![Structural formula]

C₂₂H₂₉NO₅S  
Mw = 419.53 gmol⁻¹  
Colourless oil

Toluene sulfonyl chloride (500 mg, 2.62 mmol) and alcohol 2.14 were used general procedure A and the reaction was stirred for 16 h to give the title compound as a colourless oil (261 mg, 0.62 mmol, 47%).

**IR**  
νₘₐₓ (neat) 2975 (w), 1692 (s), 1597 (m), 1495 (m), 1363 (s), 1174 (s), 935 (m), 663 (m), 554 (m) cm⁻¹.

**¹H NMR**  
(300 MHz, CDCl₃) δ 7.75 (2H, d, J = 8.2 Hz, C₆H ortho to SO₂O), 7.37-7.29 (4H, m, C₆H meta to SO₂O & meta to N), 7.20 (1H, t, J = 7.3 Hz, C₆H para to N), 7.12 (2H, d, J = 7.3 Hz, C₆H ortho to N), 4.04 (2H, t, J = 6.2 Hz, SO₂OCH₂), 3.60 (2H, t, J = 7.1 Hz, NCH₂), 2.45 (3H, s, C₆H₃), 1.75-1.49 (4H, 2 x m, SO₂OCH₂CH₂CH₂), 1.40 (9H, s, C(O)OC(CH₃)₃).

**¹³C NMR**  
(75 MHz, CDCl₃) δ 154.7 (C(O)OC(CH₃)₃), 144.7 (C₆H₃), 142.2 (C₆N), 133.1 (C₆SO₂O), 129.8 (C₆H meta to SO₂O), 128.8 (C₆H ortho to N), 127.8 (C₆H ortho to SO₂O), 127.1 (C₆H meta to N), 126.1 (C₆H para to N), 80.2 (C(O)OC(CH₃)₃), 70.1 (SO₂OCH₂), 49.0 (NCH₂), 28.3 (C(O)OC(CH₃)₃), 26.1 (SO₂OCH₂CH₂), 24.4 (NCH₂CH₂), 21.6 (C₆H₃).

**LRMS**  
4-Nitro benzenesulfonic acid 4-(tert-butoxycarbonyl phenyl amino) butyl ester (2.32)

4-Nitrobenzene sulfonyl chloride (500 mg, 2.26 mmol) and alcohol 2.14 (300 mg, 1.13 mmol) were used general procedure A and the reaction was stirred for 1.5 h to give the title compound as an orange oil (248 mg, 0.55 mmol, 49%).

IR \( \nu_{\text{max}} \) (neat) 2977 (w), 1692 (s), 1533 (s), 1366 (s), 1185 (s) cm\(^{-1}\).

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.39 (2H, d, \( J = 8.8 \) Hz, C\(_{Ar}\)H ortho to SO\(_2\)O), 8.08 (2H, d, \( J = 8.8 \) Hz, C\(_{Ar}\)H meta to SO\(_2\)O), 7.33 (2H, t, \( J = 7.3 \) Hz, C\(_{Ar}\)H meta to N), 7.22 (1H, tt, \( J = 7.3, 2.0 \) Hz, C\(_{Ar}\)H para to N), 7.11 (2H, d, \( J = 7.3 \) Hz, C\(_{Ar}\)H ortho to N), 4.15 (2H, t, \( J = 7.0 \) Hz, SO\(_2\)OCH\(_2\)), 3.62 (2H, t, \( J = 7.0 \) Hz, NCH\(_2\)), 1.75-1.67 (2H, m, NCH\(_2\)CH\(_2\)), 1.58-1.51 (2H, m, SO\(_2\)OCH\(_2\)CH\(_2\)), 1.39 (9H, s, C(O)OC(CH\(_3\))\(_3\)).

\(^13\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 154.7 (C(O)OC(CH\(_3\))\(_3\)), 150.7 (C\(_{Ar}\)NO\(_2\)), 142.1 (C\(_{Ar}\)N), 141.9 (C\(_{Ar}\)SO\(_2\)O), 129.2 (C\(_{Ar}\)H ortho to SO\(_2\)O), 128.9 (C\(_{Ar}\)H ortho to N), 127.1 (C\(_{Ar}\)H meta to N), 126.2 (C\(_{Ar}\)H para to N), 124.4 (C\(_{Ar}\)H meta to SO\(_2\)O), 80.3 (C(O)OC(CH\(_3\))\(_3\)), 71.3 (SO\(_2\)OCH\(_2\)), 48.8 (NCH\(_2\)), 28.3 (C(O)OC(CH\(_3\))\(_3\)), 26.1 (SO\(_2\)OCH\(_2\)CH\(_2\)), 24.3 (NCH\(_2\)CH\(_2\)).

LRMS (ES+) \( m/z \) 473 [M + Na]\(^+\), 924 [2M + Na]\(^+\).

HRMS (ES+) calcd for C\(_{21}\)H\(_{26}\)N\(_2\)O\(_7\)SNa [M + Na]\(^+\) 473.1353, found 473.1349.
Methanesulfonic acid 4-(tert-butoxycarbonyl phenyl amino) butyl ester (2.33)

![Chemical structure](image)

C<sub>16</sub>H<sub>25</sub>NO<sub>5</sub>S
Mw = 343.44 gmol<sup>−1</sup>
Colourless oil

Methanesulfonyl chloride (0.34 ml, 3.46 mmol) and alcohol 2.14 (459 mg, 1.73 mmol) were used in general procedure A and the reaction was stirred for 1.5 h to give the title compound as a colourless oil (410 mg, 1.19 mmol, 69%).

**IR**

ν<sub>max</sub> (neat) 2975 (w), 1689 (s), 1597 (w), 1352 (s), 1170 (s), 936 (m), 699 (m), 528 (m) cm<sup>−1</sup>.

**<sup>1</sup>H NMR**

(300 MHz, CDCl<sub>3</sub>) δ 7.35 (2H, t, <i>J</i> = 7.5 Hz, C<sub>Ar</sub>H<sub>meta</sub> to N), 7.22 (1H, t, <i>J</i> = 7.5 Hz, C<sub>Ar</sub>H<sub>para</sub> to N), 7.17 (2H, d, <i>J</i> = 7.5 Hz, C<sub>Ar</sub>H<sub>ortho</sub> to N), 4.23 (2H, t, <i>J</i> = 6.2 Hz, SO<sub>2</sub>OCH<sub>2</sub>H), 3.69 (2H, t, <i>J</i> = 7.1 Hz, NCH<sub>2</sub>), 3.00 (3H, s, CH<sub>3</sub>SO<sub>2</sub>O), 1.83-1.71 (2H, m, NCH<sub>2</sub>CH<sub>2</sub>), 1.70-1.60 (2H, m, SO<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>), 1.42 (9H, s, C(O)OC(CH<sub>3</sub>)<sub>3</sub>).

**<sup>13</sup>C NMR**

(75 MHz, CDCl<sub>3</sub>) δ 154.7 (C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 142.1 (C<sub>Ar</sub>N), 128.8 (C<sub>Ar</sub>H<sub>ortho</sub> to N), 127.1 (C<sub>Ar</sub>H<sub>meta</sub> to N), 126.2 (C<sub>Ar</sub>H<sub>para</sub> to N), 80.2 (C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 69.5 (SO<sub>2</sub>OCH<sub>2</sub>H), 48.9 (NCH<sub>2</sub>), 37.3 (CH<sub>3</sub>SO<sub>2</sub>O), 28.3 (C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 26.3 (SO<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>), 24.4 (NCH<sub>2</sub>CH<sub>2</sub>).

**LRMS**

(ES+) m/z 366 [M + Na]<sup>+</sup>.

**HRMS**

(ES+) calcd for C<sub>16</sub>H<sub>25</sub>NO<sub>5</sub>SNa [M + Na]<sup>+</sup> 366.1346, found 366.1346.
**6-Aminocaproic acid methyl ester (2.37)**

![Chemical structure](image)

C\textsubscript{7}H\textsubscript{16}ClNO\textsubscript{2}

Mw = 181.66 gmol\textsuperscript{-1}

White solid

Following the procedure of Lin et al.,\textsuperscript{68} a colourless solution of SOCl\textsubscript{2} (12.50 mL, 167.6 mmol) in MeOH (80 mL) was stirred at 0 °C for 10 min. 6-Aminohexanoic acid (2.36, 10.00 g, 76.2 mmol) was added to give a cloudy white mixture which was stirred at rt and turned colourless after 15 min. The solution was stirred for a further 4 h and was then concentrated in vacuo to give the title compound as a cream solid (13.69 g, 75.4 mmol, 99%) which was sufficiently pure (\textsuperscript{1}H NMR) to be used in subsequent reactions without further purification. Spectroscopic data were in agreement with those published in the literature.\textsuperscript{68}

**MP**

124-127 °C (lit 120.0-121.0 °C).\textsuperscript{68}

**IR**

\(\nu_{\text{max}}\) (neat) 3440 (br), 2931 (m), 2896 (m), 2868 (m), 1728 (s), 1621 (w), 1581 (w), 1517 (m), 1175 (m) cm\textsuperscript{-1}.

**\textsuperscript{1}H NMR**

(300 MHz, CDCl\textsubscript{3}) \(\delta\) 8.25 (3H, broad s, NH\textsubscript{3}), 3.67 (3H, s, OCH\textsubscript{3}), 3.02 (2H, t, \(J = 7.5\) Hz, CH\textsubscript{2}NH\textsubscript{3}), 2.34 (2H, t, \(J = 7.3\) Hz, C(O)CH\textsubscript{2}), 1.82 (2H, tt, \(J = 7.7, 7.5\) Hz, CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{3}), 1.68 (2H, tt, \(J = 7.7, 7.3\) Hz, C(O)CH\textsubscript{2}CH\textsubscript{2}), 1.48-1.43 (2H, m, C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}).

**\textsuperscript{13}C NMR**

(75 MHz, CDCl\textsubscript{3}) \(\delta\) 173.9 (C(O)OCH\textsubscript{3}), 51.6 (OCH\textsubscript{3}), 39.7 (CH\textsubscript{2}NH\textsubscript{3}), 33.6 (C(O)CH\textsubscript{2}), 27.2 (CH\textsubscript{2}CH\textsubscript{2}NH\textsubscript{3}), 25.9 (C(O)CH\textsubscript{2}CH\textsubscript{2}), 24.2 (C(O)CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}).

**LRMS**

(ES+) \(m/z\) 146 [M – 35Cl]\textsuperscript{+}.
5-Chloro-2-nitrobenzoyl chloride (2.38)

![Chemical Structure of 5-Chloro-2-nitrobenzoyl Chloride]

C$_7$H$_3$Cl$_2$NO$_3$

Mw = 220.01 gmol$^{-1}$

Orange oil

To a suspension of 5-chloro-2-nitrobenzoic acid (2.35, 10.00 g, 48.2 mmol) in benzene (150 mL) was added SOCl$_2$ (7.00 mL, 96.4 mmol) and the solution was heated under reflux for 3 h. The cooled reaction mixture was concentrated *in vacuo* and the residue was dissolved in CH$_2$Cl$_2$ (50 mL), washed with water (1 x 50 mL) and sat aq NaCO$_3$H (1 x 50 mL), dried over MgSO$_4$ and concentrated *in vacuo*. The residue was dissolved in hot CH$_2$Cl$_2$ and filtered to remove the solid impurities. Removal of the solvent afforded the title compound as an orange oil (9.60 g, 43.6 mmol, 91%).

**IR**

$\nu_{\text{max}}$ (neat) 3103 (w), 1783 (m), 1530 (s), 1339 (s), 1194 (s), 916 (m), 842 (s), 736 (s) cm$^{-1}$.

**$^1$H NMR**

(300 MHz, CDCl$_3$) $\delta$ 8.10 (1H, d, $J = 8.6$ Hz, C$_{Ar}$H *ortho* to NO$_2$), 7.69 (1H, dd, $J = 8.6$, 2.2 Hz, C$_{Ar}$H *ortho* to Cl), 7.66 (1H, d, $J = 2.2$ Hz, C$_{Ar}$H *ortho* to C(O)Cl).

**$^{13}$C NMR**

(75 MHz, CDCl$_3$) $\delta$ 164.5 (C(O)Cl), 143.1 (C$_{Ar}$NO$_2$), 141.0 (C$_{Ar}$Cl), 133.9 (C$_{Ar}$C(O)Cl), 132.4 (C$_{Ar}$H *ortho* to C(O)Cl), 128.0 (C$_{Ar}$H *ortho* to Cl), 126.1 (C$_{Ar}$H *ortho* to NO$_2$).

Methyl 6-(5-chloro-2-nitrobenzamido)hexanoate (2.39)

![Chemical Structure of Methyl 6-(5-chloro-2-nitrobenzamido)hexanoate]

C$_{14}$H$_{17}$ClN$_2$O$_5$

Mw = 328.75 gmol$^{-1}$

Cream solid
Method 1: To a solution of methyl ester 2.37 (5.20 g, 28.6 mmol) and acid chloride 2.38 (6.29 g, 28.6 mmol) in dioxane (90 mL) was added DIPEA (10.00 mL, 57.2 mmol) and the solution was stirred at 70 °C for 3.5 h. The cooled reaction mixture was concentrated in vacuo and the residue was dissolved in CH₂Cl₂ (100 mL), washed with 2 N HCl (1 x 75 mL) and brine (1 x 50 mL), dried over MgSO₄ and concentrated in vacuo. The residue was by recrystallisation from hot CH₂Cl₂ to give the title compound as a cream solid (8.41 g, 25.6 mmol, 89%).

Method 2: A solution of HOBt (67 mg, 4.96 mmol) in DMF (12 mL) and 5-chloro-2-nitrobenzoic acid (1.00 g, 4.96 mmol) in CH₂Cl₂ (28 mL) was stirred at rt before DIC (0.77 mL, 4.96 mmol) was added and the solution was stirred for 5 min. Methyl ester 2.37 (900 mg, 4.96 mmol) and DIPEA (2.6 mL, 14.88 mmol) were added and the solution was stirred for 19 h. The reaction mixture was diluted with CH₂Cl₂, washed with 2 N HCl (1 x 100 mL) and brine (1 x 50 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the product as a white solid (1.32 g, 4.00 mmol, 81%).

MP 82-84 ºC.

IR νₚₑₘₐₓ (neat) 3272 (br), 3079 (w), 2944 (w), 2863 (w), 1732 (m), 1643 (m), 1527 (s), 1342 (s), 1304 (m), 1163 (m), 839 (m) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 8.02 (1H, d, J = 8.8 Hz, C₆H₄ortho to NO₂), 7.52 (1H, dd, J = 8.8, 2.2 Hz, C₆H₄ortho to Cl), 7.48 (1H, d, J = 2.2 Hz, C₆H₄ortho to C(O)NH), 6.12 (1H, br m, NH), 3.66 (3H, s, OCH₃), 3.45 (2H, apt q, J = 7.0 Hz, CH₂NH), 2.34 (2H, t, J = 7.3 Hz, C(O)CH₂), 1.75-1.59 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.51-1.35 (2H, m, C(O)CH₂CH₂CH₂).

¹³C NMR (75 MHz, CDCl₃) δ 174.1 (C(O)OCH₃), 165.1 (C(O)NH), 144.5 (C₆NO₂), 140.3 (C₆Cl), 134.6 (C₆C(O)NH), 130.3 (C₆Hortho to Cl), 129.0 (C₆Hortho to C(O)NH), 126.0 (C₆Hortho to NO₂), 51.5 (OCH₃), 40.0 (CH₂NH), 33.7 (C(O)CH₂), 28.6 (CH₂CH₂NH), 26.2
(C(O)CH₂CH₂), 24.2 (C(O)CH₂CH₂CH₂).


HRMS (ES+) calcd for C₁₄H₁₇³⁷Cl N₂O₅Na [M + Na]⁺ 351.0718, found 351.0718.

Methyl 6-(5-methylthio-2-nitrobenzamido) hexanoate (2.40)

\[
\begin{align*}
\text{MeO} & \quad \text{N} \\
\text{O} & \quad \text{N} \\
\text{O} & \quad \text{MeS} \\
\end{align*}
\]

C₁₅H₂₀N₂O₅S
Mw = 340.39 gmol⁻¹
Pale yellow solid

To a solution of chloride 2.39 (1.13 g, 3.4 mmol) in CH₂Cl₂/water (1:1, 54 mL) was added Bu₄NCl (47 mg, 0.2 mmol) followed by NaSMe (2.10 mL, 6.8 mmol) and the solution was stirred vigorously at 30-35 °C. Further Bu₄NCl (77 mg, 0.3 mmol)/NaSMe (1.00 mL, 3.4 mmol) were added after 7 h followed by the addition of Bu₄NCl (47 mg, 0.2 mmol)/NaSMe (2.00 mL, 6.8 mmol) after 23 h. The solution was stirred for a total reaction time of 25 h. The reaction mixture was partitioned between CH₂Cl₂ and water, the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (2 x 25 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20-60% EtOAc/hexane) to give the title compound as a pale yellow solid (834 mg, 2.5 mmol, 74%).

MP 100-102 °C.

IR ν_max (neat) 3271 (br), 3078 (w), 2944 (w), 2862 (w), 1736 (s), 1644 (m), 1566 (m), 1519 (s), 1337 (s) cm⁻¹.

¹H NMR (300 MHz, CDCl₃) δ 8.02 (1H, d, J = 8.8 Hz, C₆H ortho to NO₂), 7.29 (1H, dd, J = 8.8, 2.2 Hz, C₆H ortho to SCH₃), 7.22 (1H, d, J = 2.2 Hz,
CArH ortho to C(O)NH, 5.91 (1H, br m, NH), 3.66 (3H, s, OCH3), 3.45 (2H, td, J = 7.0, 6.2 Hz, CH2NH), 2.56 (3H, s, SCH3), 2.35 (2H, t, J = 7.3 Hz, C(O)CH2), 1.75-1.58 (4H, m, C(O)CH2CH2CH2CH2), 1.50-1.35 (2H, m, C(O)CH2CH2CH2).

13C NMR (75 MHz, CDCl3) δ 174.1 (C(O)OCH3), 166.5 (C(O)NH), 148.6 (CArSMe), 142.2 (CArNO2), 133.8 (CArC(O)NH), 125.7 (CArH ortho to SCH3), 125.0 (CArH ortho to C(O)NH), 124.4 (CArH ortho to NO2), 51.5 (OCH3), 40.0 (CH2NH), 33.8 (C(O)CH2), 28.7 (CH2CH2NH), 26.2 (C(O)CH2CH2), 24.3 (C(O)CH2CH2CH2), 14.8 (SCH3).


Methyl 6-(5-benzylthio-2-nitrobenzamido) hexanoate (2.41)

![Chemical structure](image)

C21H24N2O5S
Mw = 416.49 g mol⁻¹
Pale yellow solid

To a solution of chloride 2.39 (6.50 g, 19.8 mmol) in CH2Cl2/water (1:1, 240 mL) was added NaOH (1.58 g, 39.6 mmol), Bu4NCl (550 mg, 2.0 mmol) and benzyl mercaptan (4.70 mL, 39.6 mmol). The solution was stirred vigorously at 30-35 ºC for 70 h. A solution of NaOH (792 mg, 19.8 mmol), Bu4NCl (275 mg, 1.0 mmol) and benzyl mercaptan (2.3 mmol, 19.8 mmol) in water (5 mL) was added, the temperature was increased to 55 ºC and the mixture was stirred vigorously for 5 h after which time further Bu4NCl (275 mg, 1.0 mmol) was added. After a total of 97 h the reaction mixture was partitioned between CH2Cl2 and water, the organic layer was separated and the aqueous layer was extracted with CH2Cl2 (2 x 25 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over MgSO4 and concentrated in vacuo. The residue was dissolved in CH2Cl2 (30 mL), added to a solution of NaOH
(792 mg, 19.8 mmol), Bu₄NCl (550 mg, 2.0 mmol) and benzyl mercaptan (2.33 mL, 19.8 mmol) in water (25 mL) and the mixture was stirred vigorously at 30-35 ºC for 20 h. The reaction mixture was worked up as before and the residue was purified by column chromatography (eluent 20-50% EtOAc/hexane) to give the title compound as a pale yellow solid (6.91 g, 16.6 mmol, 84%).

**MP** 83-85 ºC.

**IR** \( \nu_{\text{max}} \) (neat) 3273 (br), 3066 (w), 2942 (w), 2861 (w), 1732 (m), 1643 (m), 1564 (m), 1518 (m), 1336 (s) cm\(^{-1}\).

**\(^1\)H NMR** (300 MHz, CDCl₃) \( \delta \) 7.96 (1H, d, \( J = 8.8 \) Hz, C\(_{Ar}\)H ortho to NO₂), 7.45-7.19 (7H, m, C\(_{Ar}\)H ortho to SCH₂, C\(_{Ar}\)H ortho to C(O)NH & benzyl C\(_{Ar}\)H), 5.83 (1H, br m, NH), 4.25 (2H, s, SCH₂), 3.66 (3H, s, OCH₃), 3.44 (2H, apt q, \( J = 6.8 \) Hz, CH₂NH), 2.35 (2H, t, \( J = 7.3 \) Hz, C(O)CH₂), 1.76-1.57 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.50-1.37 (2H, m, C(O)CH₂CH₂CH₂).

**\(^{13}\)C NMR** (75 MHz, CDCl₃) \( \delta \) 174.1 (C(O)OCH₃), 166.2 (C(O)NH), 146.8 (C\(_{Ar}\)SCH₂), 142.8 (C\(_{Ar}\)NO₂), 135.1 (C\(_{Ar}\)CH₂), 133.7 (C\(_{Ar}\)C(O)NH), 128.9 & 128.8 (C\(_{Ar}\)H ortho & meta to CH₂), 127.9 (C\(_{Ar}\)H para to CH₂), 127.3 (C\(_{Ar}\)H ortho to SCH₂), 126.1 (C\(_{Ar}\)H ortho to C(O)NH), 125.0 (C\(_{Ar}\)H ortho to NO₂), 51.5 (OCH₃), 39.9 (CH₂NH), 37.0 (SCH₂), 33.8 (C(O)CH₂), 28.8 (CH₂CH₂NH), 26.2 (C(O)CH₂CH₂), 24.3 (C(O)CH₂CH₂).

**LRMS** (ES+) \( m/z \) 439 [M + Na]⁺, 855 [2M + Na]⁺.

**HRMS** (ES+) calcd for C\(_{21}\)H\(_{24}\)N\(_2\)O\(_5\)SNa [M + Na]⁺ 439.1298, found 439.1288.
Methyl 6-(5-chlorosulfonyl-2-nitrobenzamido) hexanoate (2.42)

Benzyl sulfide 2.41 (5.53 g, 13.3 mmol) was dissolved in glacial acetic acid (40 mL) and molecular chlorine was bubbled through the solution for 1 h, during which time the temperature of solution was maintained below 30 °C with an ice-water bath. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (eluent 35% EtOAc/hexane) to give the title compound as a white solid (3.27 g, 8.3 mmol, 62%).

**MP** 100-103 °C.

**IR** $\nu_{\text{max}}$ (neat) 3292 (m), 3083 (w), 2948 (w), 1736 (s), 1646 (s), 1541 (m), 1379 (m), 1182 (m), 628 (m), 545 (m) cm$^{-1}$.

**$^1$H NMR** (300 MHz, CDCl$_3$) $\delta$ 8.25-8.15 (3H, m, C$_{Ar}$H), 6.39 (1H, br m, NH), 3.66 (3H, s, OCH$_3$), 3.50 (2H, apt q, $J = 6.7$ Hz, CH$_2$NH), 2.36 (2H, t, $J = 7.1$ Hz, C(O)CH$_2$), 1.77-1.60 (4H, m, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.54-1.39 (2H, m, C(O)CH$_2$CH$_2$CH$_2$).

**$^{13}$C NMR** (75 MHz, CDCl$_3$) $\delta$ 174.2 (C(O)OCH$_3$), 163.5 (C(O)NH), 150.3 (C$_{Ar}$SO$_2$Cl), 147.3 (C$_{Ar}$NO$_2$), 134.4 (C$_{Ar}$C(O)NH), 129.1 (C$_{Ar}$H *ortho* to SO$_2$Cl), 127.6 (C$_{Ar}$H *ortho* to C(O)NH), 126.0 (C$_{Ar}$H *ortho* to NO$_2$), 51.6 (OCH$_3$), 40.1 (CH$_2$NH), 33.6 (C(O)CH$_2$), 28.5 (CH$_2$CH$_2$NH), 26.0 (C(O)CH$_2$CH$_2$), 24.0 (C(O)CH$_2$CH$_2$CH$_2$).


**HRMS** (ES+) calcd for C$_{14}$H$_{17}$ClN$_2$O$_7$SNa [M + Na]$^+$ 415.0337, found 415.0341.
6-(5-[4-(tert-Butoxycarbonyl phenyl amino) butoxysulfonyl]-2-nitro benzoylelamino) hexanoic acid methyl ester (2.43)

C_{29}H_{39}N_{3}O_{10}S  
Mw = 621.70 gmol^{-1}  
Orange oil

To a solution of sulfonyl chloride 2.42 (1.30 g, 3.3 mmol), (4-hydroxy butyl) phenyl carbamic acid tert-butyl ester (2.14, 732 mg, 2.8 mmol) and DMAP (84 mg, 0.7 mmol) in CH_{2}Cl_{2} (20 mL) was added Et_{3}N (0.96 mL, 6.9 mmol). The solution was stirred at rt for 2.5 h. The reaction mixture was diluted with CH_{2}Cl_{2} (20 mL), washed with 2 N HCl (1 x 40 mL), water (1 x 20 mL) and brine (1 x 20 mL), dried over MgSO_{4} and concentrated in vacuo. The residue was purified by column chromatography (eluent 10-35% EtOAc/petroleum ether) to give the title compound as an orange oil (1.28 g, 2.1 mmol, 75%).

IR  
\( \nu_{\text{max}} \) (neat) 3298 (br), 2970 (w), 2940 (w), 2866 (w), 1736 (m), 1692 (s), 1670 (s), 1536 (m), 1366 (s), 1165 (s), 698 (w) cm\(^{-1} \).

\(^1\)H NMR  
(300 MHz, CDCl_{3}) \( \delta \) 8.15 (1H, d, \( J = 8.9 \) Hz, C_{Ar}H ortho to NO_{2}), 8.08-8.03 (2H, m, C_{Ar}H ortho to C(O)NH & ortho to SO_{2}O), 7.33 (2H, td, \( J = 7.4, 1.9 \) Hz, C_{Ar}H meta to N), 7.22 (1H, tt, \( J = 7.4, 1.9 \) Hz, C_{Ar}H para to N), 7.09 (2H, d, \( J = 7.4 \) Hz, C_{Ar}H ortho to N), 6.81 (1H, br m, NH), 4.18 (2H, t, \( J = 6.4 \) Hz, SO_{2}OCH_{2}), 3.66 (3H, s, OCH_{3}), 3.56 (2H, t, \( J = 6.8 \) Hz, CH_{2}NC(O)O), 3.46 (2H, apt q, \( J = 7.0 \) Hz, CH_{2}NH), 2.35 (2H, t, \( J = 7.0 \) Hz, C(O)CH_{2}), 1.73-1.38 (10H, 2 x m, C(O)CH_{2}CH_{2}CH_{2}CH_{2} & SO_{2}OCH_{2}CH_{2}CH_{2}), 1.34 (9H, s, C(O)OC(CH_{3})_{3}).

\(^{13}\)C NMR  
(75 MHz, CDCl_{3}) \( \delta \) 174.0 (C(O)OCH_{3}), 164.4 (C(O)NH), 154.8 (NC(O)O), 149.4 (C_{Ar}NO_{2}), 140.7 (C_{Ar}N), 134.1 (C_{Ar}C(O)NH), 130.7 (C_{Ar}SO_{2}O), 129.6 (C_{Ar}H ortho to SO_{2}O), 128.9 (C_{Ar}H ortho to N), 128.5 (C_{Ar}H ortho to C(O)NH), 126.8 (C_{Ar}H meta to N), 126.5 (C_{Ar}H para to
N), 125.4 (C₆H₄ ortho to NO₂), 80.4 (OC(CH₃)₃), 71.6 (SO₂OCH₂), 51.6 (OCH₃), 49.0 (CH₂NC(O)O), 40.0 (CH₂NH), 33.7 (C(O)CH₂), 28.7 (CH₂CH₂NH), 28.2 (OC(CH₃)₃), 26.2 (SO₂OCH₂CH₂), 25.7 (C(O)CH₂CH₂), 24.2 (CH₂CH₂NC(O)O), 23.7 (C(O)CH₂CH₂CH₂).

**LRMS** (ES+) m/z 644 [M + Na]+.


6-(5-[4-(tert-Butoxycarbonyl phenyl amino) butoxysulfonyl]-2-nitro benzoylamino) hexanoic acid (2.44)

![Chemical Structure](image)

C₂₈H₃₇N₃O₁₀S  
Mw = 607.67 gmol⁻¹  
Orange oil

To a solution of methyl ester 2.43 (222 mg, 0.36 mmol) in CH₂Cl₂/acetone (4:1, 10 mL) was added aq phosphate buffer (pH 7, 15 mL) followed by Novozym 435® (300 mg) and the suspension was stirred vigorously at 50 °C. Further Novozym 435® (300 mg) was added after 23 and 28 h, and the reaction was stirred for a total of 48 h. The cooled reaction mixture was acidified with HCl and filtered through a pad of Celite®. The pad was washed with CH₂Cl₂ (20 mL) and MeOH (20 mL). The organic phase of the filtrate was separated and the aqueous phase was extracted with CH₂Cl₂ (2 x 10 mL). The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 60-100% EtOAc/hexane then 10% MeOH/CH₂Cl₂) to give the title compound as an orange oil (54 mg, 0.089 mmol, 25%) and recovered methyl ester 2.43 (128 mg, 0.21 mmol, 57%).

**IR**  
νmax (neat) 3292 (br), 3078 (w), 2931 (m), 2866 (w), 1652 (s), 1538 (s), 1366 (s), 1188 (s), 1152 (s), 934 (m) cm⁻¹.
**¹H NMR** (300 MHz, CDCl₃) δ 8.13 (1H, d, J = 9.1 Hz, C₆H ortho to NO₂), 8.08-8.01 (2H, m, C₆H ortho to C(O)NH & ortho to SO₂O), 7.33 (2H, td, J = 7.3, 1.7 Hz, C₆H meta to N), 7.21 (1H, tt, J = 7.3, 1.7 Hz, C₆H para to N), 7.09 (2H, d, J = 7.3 Hz, C₆H ortho to N), 6.92 (1H, br s, NH), 4.17 (2H, t, J = 6.4 Hz, SO₂OCH₂), 3.56 (2H, t, J = 7.0 Hz, CH₂NC(O)O), 3.51-3.38 (2H, m, CH₂NH), 2.38 (2H, t, J = 7.0 Hz, C(O)CH₂), 1.76-1.37 (10H, 2 x m, C(O)CH₂CH₂CH₂CH₂ & SO₂OCH₂CH₂CH₂), 1.34 (9H, s, C(O)OC(CH₃)₃).

**¹³C NMR** (75 MHz, CDCl₃) δ 177.8 (C(O)OH), 164.4 (C(O)NH), 155.0 (NC(O)O), 149.5 (C₆NO₂), 141.7 (C₆SO₂O), 140.6 (C₆N), 134.0 (C₆C(O)NH), 129.7 (C₆H ortho to SO₂O), 128.9 (C₆H ortho to N), 128.4 (C₆H ortho to C(O)NH), 127.0 (C₆H meta to N), 126.4 (C₆H para to N), 125.5 (C₆H ortho to NO₂), 80.5 (OC(CH₃)₃), 71.7 (SO₂CH₂), 40.0 (CH₂NH), 33.5 (C(O)CH₂), 30.9 (CH₂NC(O)O), 28.5 (CH₂CH₂NH), 28.2 (OC(CH₃)₃), 26.1 (SO₂OCH₂CH₂), 25.6 (C(O)CH₂CH₂), 24.0 (CH₂CH₂NC(O)O), 23.7 (C(O)CH₂CH₂CH₂).

**LRMS** (ES−) m/z 606 [M − H]⁺.

**HRMS** (ES+) calcd for C₂₈H₃₇N₃O₁₀SNa [M + Na]⁺ 630.2092, found 630.2098.

Solid supported 6-(5-[4-(tert-butoxycarbonyl phenyl amino) butoxysulfonyl]-2-nitro benzoylelamino) hexanoic acid (2.45)

![Chemical structure](image)

A solution of HOBt (174 mg, 1.29 mmol) and carboxylic acid 2.44 (312 mg, 0.51 mmol) in CH₂Cl₂/DMF (7:3, 10 mL) was stirred at rt for 10 min. DIC (0.20 mL, 1.29
mmol) was added and the solution was stirred for 10 min. Amino methyl polystyrene resin (360 mg, 1.20 mmol g\(^{-1}\), 0.43 mmol) was added and the resin was stirred gently. A ninhydrin test on a small sample of resin beads after 18 h was negative, indicating that all of the amino groups had reacted. The resin was removed by filtration, washed with CH\(_2\)Cl\(_2\) (50 mL), MeOH (50 mL) and Et\(_2\)O (50 mL) and dried \textit{in vacuo} to give the product resin (574 mg, theoretical loading 0.75 mmol g\(^{-1}\), actual loading (S elemental analysis) 0.58 mmol g\(^{-1}\), 84%).

\textbf{IR} \quad \nu_{\text{max}} \text{ (neat)} 3307 (br), 3025 (w), 2992 (w), 2855 (w), 1651 (w), 1531 (m), 756 (m), 696 (s) cm\(^{-1}\).

\textbf{\(^1\)H NMR} \quad \text{MAS (400 MHz, CDCl}_3\text{)} \quad \delta \ 8.03-7.83 \ (m, \text{C}_A\text{H ortho to NO}_2, \text{ortho to C(O)NH & ortho to SO}_2O), \ 7.30 \ (s, \text{C}_A\text{H ortho to N}), \ 7.26 \ (s, \text{C}_A\text{H para to N}), \ 7.10 \ (s, \text{C}_A\text{H meta to N}), \ 6.59 \ (\text{amino methyl resin}), \ 4.11 \ (s, \text{SO}_2\text{OCH}_2), \ 3.65 \ (s, \text{CH}_2\text{NC(O)O}), \ 3.56 \ (s, \text{CH}_2\text{NH}), \ 1.97 \ (s, \text{C(O)CH}_2), \ 1.87-1.41 \ (m, \text{C(O)CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \text{ & SO}_2\text{OCH}_2\text{CH}_2\text{CH}_2), \ 1.35 \ (s, \text{NC(O)OC(CH}_3)_3\text{)}.

\textbf{\(^{13}\)C NMR} \quad \text{MAS - not acquired due to low resin loading.}

\textbf{3-(2-Naphthylenoxy)-1-propanol (2.48)}

\[
\begin{align*}
\text{C}_{13}\text{H}_{14}\text{O}_2 \\
\text{Mw} = 202.25 \text{ gmol}^{-1} \\
\text{Off white solid}
\end{align*}
\]

Following the procedure of Shi \textit{et al.},\textsuperscript{77} to a suspension of 2-naphthol (3.00 g, 20.8 mmol) in water (60 mL) was added NaOH (2.00 g, 50.0 mmol) and the solution was stirred for 30 min until clear. 3-Bromo-1-propanol (2.90 mL, 20.8 mmol) was added and the solution was stirred at rt for 72 h. The reaction mixture was extracted with CH\(_2\)Cl\(_2\) (3 x 100 mL) and the combined organic layers were washed with water (1 x 100
mL) and concentrated in vacuo. The residue was recrystallised from hot EtOAc to give the title compound as an off white solid (2.79 g, 13.8 mmol, 66%).

**MP**

102-104 °C (lit 99-100 °C).\(^{155}\)

**IR**

\(\nu_{\text{max}} \text{ (neat)}\) 3272 (br), 2955 (w), 2878 (w), 1629 (m), 1600 (m), 1509 (m), 1467 (m), 1390 (w), 1260 (m), 1217 (m), 1184, (m), 1054 (m), 1038 (s), 837 (s), 750 (m) cm\(^{-1}\).

**\(^1\)H NMR**

(300 MHz, CDCl\(_3\)) \(\delta\) 7.81-7.70 (3H, m, H\(_4\), H\(_5\) & H\(_8\)), 7.45 (1H, ddd, \(J = 8.1, 6.9, 1.2\) Hz, H\(_6\)), 7.35 (1H, ddd \(J = 8.1, 6.9, 1.2\) Hz, H\(_7\)), 7.19-7.12 (2H, m, H\(_1\) & H\(_3\)), 4.26 (2H, t, \(J = 6.1\) Hz, C\(_{\text{ArOCH}_2}\)), 3.93 (2H, t, \(J = 5.8\) Hz, CH\(_2\)OH), 2.13 (2H, tt, \(J = 6.1, 5.8\) Hz, CH\(_2\)CH\(_2\)OH).

**\(^{13}\)C NMR**

(75 MHz, CDCl\(_3\)) \(\delta\) 156.5 (C\(_{\text{ArOCH}_2}\)), 134.5 (C\(_{10}\)), 129.4 (C\(_9\)), 129.1 (C\(_5\)), 127.6 (C\(_4\)), 126.7 (C\(_7\)), 126.4 (C\(_8\)), 123.7 (C\(_6\)), 118.8 (C\(_3\)), 106.6 (C\(_1\)), 65.7 (C\(_{\text{ArOCH}_2}\)), 60.6 (CH\(_2\)OH), 31.9 (CH\(_2\)CH\(_2\)OH).

**LRMS**

(ES+) \(m/z\) 225 [M + Na]\(^+\).

**HRMS**

(ES+) calcd for C\(_{13}\)H\(_{14}\)O\(_2\)Na [M + Na]\(^+\) 255.0886, found 225.0890

**6-(4-[3-(2-Naphthalenyloxy) propoxysulfonyl] phenyl) hexanoic acid methyl ester (2.49)**

![Chemical structure](image)

\(\text{C}_{26}\text{H}_{30}\text{O}_{6}\text{S}\)

Mw = 470.58 g mol\(^{-1}\)

Orange oil

To a solution of sulfonyl chloride \textbf{2.03} (1.36 g, 4.5 mmol), alcohol \textbf{2.48} (750 mg, 3.7 mmol) and DMAP (113 mg, 0.9 mmol) in CH\(_2\)Cl\(_2\) (20 mL) was added Et\(_3\)N (1.30 mL, 9.3 mmol) and the solution was stirred at rt for 1.5 h. The reaction mixture was diluted
with CH₂Cl₂ (5 mL), washed with 2 N HCl (1 x 20 mL), water (1 x 10 mL) and brine (1 x 10 mL), dried over MgSO₄ and concentrated *in vacuo* to give an orange oil (1.73 g, 3.7 mmol, 100%) which was used in subsequent reactions without further purification.

**IR**  \( \nu_{\text{max}} \) (neat) 3057 (w), 2938 (w), 2859 (w), 1733 (m), 1359 (m), 1216 (m), 1174 (s) cm⁻¹.

**¹H NMR** (300 MHz, CDCl₃)  δ 7.80-7.68 (5H, m, C₆H ortho to SO₂O, H₄, H₅ & H₆), 7.45 (1H, td, J = 8.0, 1.2 Hz, H₆), 7.35 (1H, td, J = 8.0, 1.2 Hz, H₇), 7.19 (2 H, d, J = 8.3 Hz, C₆H meta to SO₂O), 7.04-6.98 (2H, m, H₁ & H₃), 4.31 (2H, t, J = 6.1 Hz, CH₂OC₆H₆), 4.08 (2H, t, J = 5.8 Hz, SO₂OCH₂), 3.68 (3H, s, OCH₃), 2.52 (2H, t, J = 7.7 Hz, CH₂C₆H₆), 2.30 (2H, t, J = 7.4 Hz, C(O)CH₂), 2.20 (2H, tt, J = 6.1, 5.8 Hz, SO₂OCH₂CH₂), 1.70-1.50 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.45-1.20 (2H, m, C(O)CH₂CH₂CH₂).

**¹³C NMR** (75 MHz, CDCl₃)  δ 174.0 (C(O)OCH₃), 156.3 (C₂), 149.2 (C₆H₂CH₃), 134.4 (C₁₀), 133.0 (C₆SO₂O), 129.3 (C₉), 129.1 (C₆H-meta to SO₂O), 129.0 (C₅), 127.8 (C₆H ortho to SO₂O), 127.6 (C₄), 126.7 (C₇), 126.4 (C₈), 123.7 (C₆), 118.6 (C₇), 106.5 (C₁), 106.1 (SO₂OCH₂), 63.0 (CH₂OC), 51.5 (OCH₃), 35.4 (CH₂C₆H₆), 33.8 (C(O)CH₂), 30.4 (C(O)CH₂CH₂CH₂), 28.8 (SO₂OCH₂CH₂), 28.6 (C(O)CH₂CH₂), 24.6 (CH₂CH₂C₆H₆).

**LRMS** (ES⁻) \( m/z \) 493 [M + Na]⁺, 963 [2M + Na]⁺.

6-(4-[3-(2-Naphthalenyloxy) propoxysulfonyl] phenyl) hexanoic acid (2.50)

\[
\begin{align*}
\text{C}_25\text{H}_{28}\text{O}_6\text{S} \\
\text{Mw} = 456.55 \text{ gmol}^{-1} \\
Pale \text{ yellow solid}
\end{align*}
\]

To a suspension of Novozym 435® (2.00 g) in aq phosphate buffer (pH 7, 100 mL) was added a solution of methyl ester \textbf{2.49} (1.73 g, 3.7 mmol) in CH\textsubscript{2}Cl\textsubscript{2}/acetone (4:1, 50 mL) and the suspension was stirred vigorously at 50 °C for 46 h. The cooled reaction mixture was filtered through a pad of Celite® and the pad was washed with CH\textsubscript{2}Cl\textsubscript{2} (30 mL) and MeOH (30 mL). The aqueous layer was separated and extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 x 25 mL). The combined organic layers were dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 10-70% EtOAc/petroleum ether) to give the title compound as a pale yellow solid (1.21 g, 2.7 mmol, 73%).

\textbf{MP} \hspace{1cm} 85-88 °C.

\textbf{IR} \hspace{1cm} \nu_{\text{max}} \text{ (neat)} 3056 \text{ (w)}, 2936 \text{ (w)}, 2860 \text{ (w)}, 1704 \text{ (m)}, 1356 \text{ (m)}, 1174 \text{ (s)} \text{ cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} \hspace{1cm} (300 MHz, CDCl\textsubscript{3}) \delta 7.81-7.67 \text{ (5H, m, C}_{Ar}\text{H} \text{ ortho to SO}_2\text{O, H}_4, H_5 \text{ & } H_8), 7.45 \text{ (1H, td, } J = 8.0, 1.2 \text{ Hz, H}_6), 7.35 \text{ (1H, td, } J = 8.0, 1.2 \text{ Hz, H}_7), 7.19 \text{ (2H, d, } J = 8.3 \text{ Hz, C}_{Ar}\text{H} \text{ meta to SO}_2\text{O), 7.04-6.95 \text{ (2H, m, H}_1 \text{ & } H_3), 4.31 \text{ (2H, t, } J = 6.1 \text{ Hz, CH}_2\text{OCAr), 4.07 \text{ (2H, t, } J = 5.8 \text{ Hz, SO}_2\text{OCH}_2), 2.51 \text{ (2H, t, } J = 7.7 \text{ Hz, CH}_2\text{CAr), 2.35 \text{ (2H, t, } J = 7.4 \text{ Hz, C(O)CH}_2), 2.20 \text{ (2H, tt, } J = 6.1, 5.8 \text{ Hz, SO}_2\text{OCH}_2\text{CH}_2), 1.72-1.48 \text{ (4H, m, C(O)CH}_2\text{CH}_2\text{CH}_2\text{CH}_2), 1.40-1.22 \text{ (2H, m, C(O)CH}_2\text{CH}_2\text{CH}_2\text{CH}_2).}

\textbf{\textsuperscript{13}C NMR} \hspace{1cm} (75 MHz, CDCl\textsubscript{3}) \delta 179.0 \text{ (C(O)OH), 156.3 \text{ (C}_2), 149.1 \text{ (C}_{Ar}\text{CH}_2), 134.4 \text{ (C}_10), 133.0 \text{ (C}_{Ar}\text{SO}_2\text{O), 129.3 \text{ (C}_9), 129.1 \text{ (C}_{Ar}\text{H} \text{ meta to SO}_2\text{O), 128.9 \text{ (C}_5), 127.9 \text{ (C}_{Ar}\text{H} \text{ ortho to SO}_2\text{O), 127.6 \text{ (C}_4), 126.7 \text{ (C}_7), 126.4 \text{ (C}_8), 123.7 \text{ (C}_6), 118.6 \text{ (C}_3), 106.6 \text{ (C}_1), 67.1 \text{ (SO}_2\text{OCH}_2), 63.0 \text{ (CH}_2\text{OCAr),}

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35.4 (CH₂CAr), 33.7 (C(O)CH₂), 30.3 (C(O)CH₂CH₂CH₂), 28.8 (SO₂OCH₂CH₂), 28.5 (C(O)CH₂CH₂), 24.3 (C(O)CH₂CH₂CH₂).


HRMS (ES+) calcd for C₂₅H₂₈O₆SNa [M + Na]⁺ 479.1499, found 479.1499.

Solid supported 6-(4-[3-(2-naphthalenylmethoxy) propoxysulfonyl] phenyl) hexanoic acid (2.51)

![Chemical Structure]

Loading = 0.67 mmolg⁻¹

Resin beads

To a solution of N-hydroxysuccinimide (543 mg, 4.72 mmol) in DMF (10 mL) was added carboxylic acid 2.50 (1.08 g, 2.36 mmol) in CH₂Cl₂ (35 mL) and the solution was stirred at rt for 10 min. DIC (0.73 mL, 4.72 mmol) was added and the solution was stirred for a further 10 min. Amino methyl polystyrene resin (1.05 g, 1.5 mmolg⁻¹, 1.57 mmol) was added and the suspension was stirred gently. A ninhydrin test carried out on a small sample of the resin after 68 h was negative, indicating that all of the amino groups had reacted. The resin was removed by filtration, washed with CH₂Cl₂ (3 x 100 mL), MeOH (3 x 100 mL) and Et₂O (3 x 100 mL) and dried in vacuo to give the product resin (1.62 g, theoretical loading 0.97 mmolg⁻¹, actual loading (S elemental analysis) 0.67 mmolg⁻¹, 83%).

IR νmax (neat) 3306 (br), 3025 (w), 2923 (w), 1738 (s), 1651 (w), 1365 (m), 1216 (m), 697 (s) cm⁻¹.

¹H NMR MAS (400 MHz, CDCl₃) δ 7.72-7.63 (m, C₆H ortho to SO₂O, H₄, H₅ & H₈), 7.36 (s, H₆), 7.26 (s, H₇), 7.11 (s, C₆H meta to SO₂O), 7.02-6.95 (m, H₁ & H₃), 6.55 (amino methyl resin), 4.23 (s, CH₂OC₆H₅), 3.97 (s, SO₂OCH₂), 2.44 (s, CH₂CAr), 2.08 (s, C(O)CH₂ & SO₂OCH₂CH₂), 1.57-
1.47 (m, C(O)CH$_2$CH$_2$CH$_2$H), 1.27 (s, C(O)CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR MAS (100 MHz, CDCl$_3$) δ 172.5 (C(O)N), 156.3 (C$_2$), 149.2 (C$_{Ar}$CH$_2$), 145.2 (amino methyl resin), 135.4 (amino methyl resin), 134.4 (C$_{10}$), 133.0 (C$_{Ar}$SO$_2$O), 129.1 (C$_6$), 129.0 (C$_{Ar}$H meta to SO$_2$O), 127.8 (C$_3$), 127.6 (C$_{Ar}$H ortho to SO$_2$O), 126.7 (C$_4$), 126.4 (C$_7$), 125.7 (C$_8$), 123.7 (C$_6$), 118.7 (C$_3$), 106.6 (C$_1$), 67.2 (SO$_2$OCH$_2$), 63.0 (CH$_2$OC$_{Ar}$), 43.3 (amino methyl resin), 40.3 (amino methyl resin), 36.4 (CH$_2$C$_{Ar}$), 35.4 (C(O)CH$_2$), 30.5 (C(O)CH$_2$C$_2$H$_2$CH$_2$), 28.8 (SO$_2$OCH$_2$CH$_2$ & C(O)OCH$_2$CH$_2$), 25.4 (CH$_2$CH$_2$C$_{Ar}$).

6-(5-[3-(2-Naphthalenyloxy) propoxysulfonyl]-2-nitro benzolyarnino) hexanoic acid methyl ester (2.52)

![6-(5-[3-(2-Naphthalenyloxy) propoxysulfonyl]-2-nitro benzolyarnino) hexanoic acid methyl ester (2.52)](image)

C$_{27}$H$_{30}$N$_2$O$_9$S

Mw = 558.60 g mol$^{-1}$

Cream solid

To a solution of sulfonyl chloride 2.42 (500 mg, 1.27 mmol), alcohol 2.38 (215 mg, 1.06 mmol) and DMAP (33 mg, 0.27 mmol) in CH$_2$Cl$_2$ (10 mL) was added Et$_3$N (0.37 mL, 2.65 mmol) and the solution was stirred at rt for 3 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (10 mL), washed with 2 N HCl (1 x 10 mL), water (1 x 10 mL) and brine (1 x 10 mL), dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (elucent 10-70% EtOAc/petroleum ether) to give the title compound as a cream solid (384 mg, 0.69 mmol, 65%).

MP 106-109 °C.

IR $\nu_{\text{max}}$ (neat) 3308 (br), 3063 (w), 2941 (w), 2865 (w), 1735 (m), 1653 (m), 1539 (s), 1366 (m), 1188 (s) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.01-7.89 (3H, m, C$_{Ar}$H ortho to NO$_2$, ortho to
C(O)NH & ortho to SO₂O), 7.77 (1H, d, J = 8.0 Hz, H₅), 7.70 (1H, d, J = 8.0 Hz, H₈), 7.69 (1H, d, J = 9.0 Hz, H₄), 7.45 (1H, ddd, J = 8.0, 7.1, 1.2 Hz, H₁), 7.36 (1H, ddd, J = 8.0, 7.1, 1.2 Hz, H₇), 7.02 (1H, d, J = 2.7 Hz, H₆), 6.89 (1H, dd, J = 9.0, 2.7, H₃), 5.90 (1H, br t, J = 5.8 Hz, NH), 4.40 (2H, t, J = 5.8 Hz, CH₂OC₆H₄), 4.07 (2H, t, J = 5.5 Hz, SO₂OCH₂), 3.66 (3H, s, OCH₃), 3.30-3.20 (2H, m, CH₂NH), 2.32 (2H, t, J = 7.4 Hz, C(O)CH₂), 2.22 (2H, tt, J = 5.8, 5.5 Hz, SO₂OCH₂CH₂), 1.63 (2H, tt, J = 7.7, 7.4 Hz, C(O)CH₂CH₂), 1.55-1.43 (2H, m, CH₂CH₂NH), 1.30-1.27 (2H, m, C(O)CH₂CH₂CH₂).

¹³C NMR (75 MHz, CDCl₃) δ 174.1 (C(O)OCH₃), 164.1 (C(O)NH), 156.0 (C₂), 149.2 (C₆ArNO₂), 140.2 (C₆ArSO₂O), 134.2 (C₁₀), 133.9 (C₆Ar(C(O)N)), 129.7 (C₉), 129.6 (C₆Ar ortho to SO₂O), 129.0 (C₅), 128.3 (C₆ArH ortho to C(O)NH), 127.7 (C₄), 126.7 (C₇), 126.6 (C₈), 125.3 (C₆ArH ortho to NO₂), 124.0 (C₆), 118.3 (C₃), 106.3 (C₁), 68.4 (SO₂OCH₂), 62.4 (CH₂OC₆H₄), 51.5 (OCH₃), 39.9 (CH₂NH), 33.7 (C(O)CH₂), 28.48 (SO₂OCH₂CH₂), 28.45 (CH₂CH₂NH), 26.1 (C(O)CH₂CH₂), 24.2 (C(O)CH₂CH₂CH₂).


6-(5-[3-(2-Naphthalenloyloxy) propoxysulfonyl]-2-nitro benzoylamino) hexanoic acid (2.53)

To a solution of methyl ester 2.52 (285 mg, 0.51 mmol) in CH₂Cl₂/acetone (4:1, 10 mL) was added aq phosphate buffer (pH 7, 15 mL) followed by Novozym 435® (300 mg)
and the suspension was stirred vigorously at 50 °C for 48 h. The cooled suspension was filtered through a pad of Celite® and the pad was washed with CH₂Cl₂ (20 mL). The filtrate was acidified with HCl and the aqueous phase was extracted with CH₂Cl₂ (2 x 10 mL). The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20-60% EtOAc/hexane) to give the title compound as a cream solid (152 mg, 0.28 mmol, 55%) and recovered methyl ester 2.52 (64 mg, 0.11 mmol, 22%).

**MP**

118-120 ºC.

**IR**

ν_max (neat) 3271 (br), 3066 (w), 2937 (w), 2865 (w), 1708 (m), 1642 (s), 1630 (s), 1536 (s), 1346 (m), 1182 (s), 840 (m) cm⁻¹.

**¹H NMR**

(300 MHz, acetone-d₆) δ 8.24-8.03 (3H, m, C₆H ortho to NO₂, ortho to C(O)NH & ortho to SO₂O), 7.84-7.75 (3H, m, H₄, H₅ & H₈), 7.44 (1H, ddd, J = 8.1, 7.0, 1.1 Hz, H₆), 7.34 (1H, ddd, J = 8.1, 7.0, 1.1 Hz, H₇), 7.25 (1H, d, J = 2.6 Hz, H₁), 7.05 (1H, dd, J = 9.0, 2.6, H₃), 4.48 (2H, t, J = 6.1 Hz, CH₂OC₆H), 4.19 (2H, t, J = 6.0 Hz, SO₂OCH₂), 3.44-3.31 (2H, m, CH₂NH), 2.39-2.20 (4H, m, C(O)CH₂ & SO₂OCH₂CH₂), 1.75-1.55 (4H, m, C(O)CH₂CH₂CH₂CH₂), 1.54-1.37 (2H, m, C(O)CH₂CH₂CH₂). NH not observed.

**¹³C NMR**

(75 MHz, acetone-d₆) δ 174.7 (C(O)OCH₃), 164.5 (C(O)NH), 157.4 (C₂), 140.8 (C₆SO₂O), 139.7 (C₅NO₂), 135.6 (C₁₀), 134.7 (C₆C(O)N), 131.0 (C₉), 130.3 (C₆H ortho to SO₂O), 129.8 (C₃), 128.2 (C₆H ortho to C(O)NH), 128.5 (C₄), 127.7 (C₇), 127.3 (C₈), 126.5 (C₆H ortho to NO₂), 124.6 (C₆), 119.5 (C₃), 107.6 (C₁), 70.0 (SO₂OCH₂), 64.1 (CH₂OC₆H), 40.4 (CH₂NH), 34.2 (C(O)CH₂), 30.7 (SO₂OCH₂CH₂), 29.1 (CH₂CH₂NH), 27.1 (C(O)CH₂CH₂), 25.3 (C(O)CH₂CH₂CH₂).

**LRMS**

(ES−) m/z 543 [M − H]^+.

**HRMS**

Solid supported 6-(5-[3-(2-naphthalenyl)oxy] propoxysulfonyl]-2-nitro benzoylamino) hexanoic acid (2.54)

![Chemical Structure](image)


c

c

A solution of HOBt (101 mg, 0.75 mmol) and carboxylic acid 2.53 (167 mg, 0.30 mmol) in CH$_2$Cl$_2$/DMF (7:3, 10 mL) was stirred at rt for 10 min. DIC (0.12 mL, 0.75 mmol) was added and the solution was stirred for 5 min. Amino methyl polystyrene resin (208 mg, 1.20 mmol$^{-1}$, 0.25 mmol) was added and the suspension was stirred gently. A ninhydrin test carried out on a small sample of the resin after 22 h was negative, indicating that all of the amino groups had reacted. The resin was removed from the suspension by filtration and was washed with CH$_2$Cl$_2$ (50 mL), MeOH (50 mL) and Et$_2$O (50 mL) and dried *in vacuo* to give the title resin (324 mg, theoretical loading 0.77 mmol$^{-1}$, actual loading (S elemental analysis) 0.66 mmol$^{-1}$, 88%).

IR  
$\nu_{\text{max}}$ (neat) 3306 (br), 3025 (w), 2922 (w), 2852 (w), 1651 (w), 1532 (m), 1452 (m), 755 (m), 696 (s) cm$^{-1}$.

$^1$H NMR  
MAS (400 MHz, CDCl$_3$) $\delta$ 8.01 (s, C$_{Ar}$H ortho to NO$_2$, ortho to C(O)NH & ortho to SO$_2$O), 7.83-7.66 (m, H$_4$, H$_5$ & H$_8$), 7.40 (s, H$_6$), 7.26 (s, H$_7$), 7.06 (amino methyl resin), 7.00 (s, H$_1$), 6.93 (s, H$_3$), 6.59 (amino methyl resin), 4.33 (s, CH$_2$OC$_{Ar}$), 4.01 (s, SO$_2$OCH$_2$), 3.29 (s, CH$_2$NH), 2.13 (s, C(O)CH$_2$ & SO$_2$OCH$_2$CH$_2$), 1.56 (s, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.27 (s, C(O)CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR  
MAS (100 MHz, CDCl$_3$) $\delta$ 172.5 (C(O)NH), 166.0 (C$_{Ar}$C(O)NH), 156.5 (C$_2$), 149.7 (C$_{Ar}$NO$_2$), 145.7 (amino methyl resin), 140.4 (C$_{Ar}$SO$_2$O), 134.7 (C$_{10}$ & C$_{Ar}$C(O)N), 130.0 (C$_0$), 129.4 (C$_{Ar}$H ortho to SO$_2$O), 128.4 (C$_5$ & C$_4$), 127.1 (C$_{Ar}$H ortho to C(O)NH), 126.1 (C$_7$, C$_8$ & C$_{Ar}$H ortho to NO$_2$), 124.4 (C$_0$), 118.8 (C$_3$), 106.9 (C$_1$), 66.2 (SO$_2$OCH$_2$), 63.0 (CH$_2$OC$_{Ar}$), 44.2 (amino methyl resin), 42.3 (amino methyl resin), 40.8
(CH$_2$NH), 36.4 (C(O)CH$_2$ & SO$_2$OCH$_2$CH$_2$), 28.9 (CH$_2$CH$_2$NH), 26.6 (C(O)CH$_2$CH$_2$), 25.0 (C(O)CH$_2$CH$_2$CH$_2$).

2-(3-Fluoropropoxy) naphthalene (2.57)

![Chemical Structure](image)

$C_{13}H_{13}FO$

Mw = 204.24 gmol$^{-1}$

Colourless oil

**Solid Phase Method – General Procedure D**: A suspension of the resin (1 eq), KF (1.2 eq) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (1.2 eq) in MeCN (1 mL/40 μmol of resin) was heated under reflux for 30 min. The resin was removed from the cooled suspension by filtration and was washed with CH$_2$Cl$_2$ (10 mL/40 μmol of resin). The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a colourless oil. Spectroscopic data were in agreement with those published in the literature.$^{74}$

Resin 2.51 (100 mg, 0.67 mmolg$^{-1}$, 67 μmol) was used in general procedure D to give the title compound (5 mg, 25 μmol, 37%).

Resin 2.54 (50 mg, 0.66 mmolg$^{-1}$, 33 μmol) was used in general procedure D to give the title compound (1.5 mg, 7.3 μmol, 22%).

**IR**

$\nu_{\text{max}}$ (neat) 3057 (w), 2967 (w), 1629 (m), 1600 (m), 1464 (m), 1389 (m), 1257 (s), 1215 (s), 1181 (s), 1051 (m), 837 (s), 746 (m), 472 (m) cm$^{-1}$.

**$^1$H NMR**

(300 MHz, CDCl$_3$) $\delta$ 7.80-7.71 (3H, m, H$_4$, H$_5$ & H$_8$), 7.45 (1H, td, $J = 8.0$, 1.2 Hz, H$_6$), 7.35 (1H, td, $J = 8.0$, 1.2 Hz, H$_7$), 7.12-7.18 (2H, m, H$_1$ & H$_3$), 4.71 (2H, dt, $^2J_{HF} = 47.2$, $J = 5.8$ Hz, FCH$_2$), 4.24 (2H, t, $J = 6.1$ Hz, CH$_2$O), 2.25 (2H, dtt, $^3J_{HF} = 26.0$, $J = 6.1$, 5.8 Hz, FCH$_2$CH$_2$).
\( ^{13} \text{C NMR} \) (100 MHz, CDCl\(_3\)) \( \delta \) 156.7 (C\(_{10}\)), 134.3 (C\(_9\)), 129.4 (C\(_8\)), 129.0 (C\(_7\)), 127.6 (C\(_6\)), 126.7 (C\(_5\)), 126.4 (C\(_4\)), 123.7 (C\(_3\)), 118.8 (C\(_2\)), 106.7 (C\(_1\)), 80.8 (d, \( J = 164.3 \) Hz, FC\(_2\)H), 63.6 (d, \( J = 4.9 \) Hz, C\(_{\text{ArOCH}}\)), 30.4 (d, \( J = 19.4 \) Hz, FCH\(_2\)CH\(_2\)).

\( ^{19} \text{F NMR} \) (282 MHz, CDCl\(_3\)) \( \delta \) -222.4 (FCH\(_2\)).

\( \text{LRMS} \) (EI) 204 (M\(^+\), 67%), 144 ([M – FC\(_3\)H\(_5\)]\(^+\), 100%), 115 ([M – FC\(_4\)H\(_6\)O]\(^+\), 89%).

**Benzyl Fluoride (3.02)**

\[
\text{C}_7\text{H}_7\text{F} \\
\text{Mw} = 110.13 \text{ g mol}^{-1} \\
\text{Colourless liquid}
\]

To a stirred solution of KF (674 mg, 11.60 mmol) in MeCN (4 mL) was added benzyl bromide (3.01, 0.70 mL, 5.89 mmol). The solution was heated using microwave irradiation at 150 °C for 2 h. The reaction mixture was partitioned between water and ether and the aqueous layer was extracted with ether (1 x 5 mL). The combined organic layers were washed with water (1 x 10 mL) and brine (1 x 10 mL), dried over MgSO\(_4\) and the solvent was carefully removed in vacuo. The residue was purified by column chromatography (eluent 100% pentane) to give the title compound as a colourless liquid (114 mg, 1.04 mmol, 18%). Spectroscopic data were in agreement with those published in the literature\(^{81,156}\).

**IR** \( \nu_{\text{max}} \) (neat) 3017 (w), 2970 (w), 1738 (s), 1434 (w), 1366 (m), 1217 (m) cm\(^{-1}\).

\( ^{1} \text{H NMR} \) (300 MHz, CDCl\(_3\)) \( \delta \) 7.53-7.29 (5H, m, C\(_{\text{ArH}}\)), 5.40 (2H, d, \( ^2J_{\text{HF}} = 47.9 \) Hz, CH\(_2\)F).
\(^{13}\)C NMR \((75\ \text{MHz, CDCl}_3)\ \delta\ 136.3\ (C_{Ar}\text{CH}_2), 128.7\ (d, \ J = 3.3\ \text{Hz, } C_{Ar}\text{H meta to CH}_2), 128.6\ (C_{Ar}\text{H para to CH}_2), 127.5\ (d, \ J = 5.5\ \text{Hz, } C_{Ar}\text{H ortho to CH}_2), 84.6\ (d, \ J = 165.9\ \text{Hz, CH}_2F).\n
\(^{19}\)F NMR \((282\ \text{MHz, CDCl}_3)\ \delta\ -207.9\ (\text{FCH}_2).\n
LRMS \((\text{EI})\ 110\ (M^{+*}, 52\%), 109\ ([M - H]^+, 100\%).\n
\((S\)-(+)\-2\-tert\-Butoxycarbonylamino\-3\-[4\-(2\-hydroxy\ ethoxy)\ phenyl] propionic\ acid\ tert\-butyl\ ester\ \text{(4.07)}\)

\[
\begin{align*}
\text{HO} & \text{O} \\
\text{NHBOc} & \\
\text{OH} & \text{O} \\
\text{C}_{20}\text{H}_{31}\text{NO}_6 & \\
\text{Mw} & = 381.46\ \text{gmol}^{-1} \\
\text{White solid} & 
\end{align*}
\]

\text{Method 1: A solution of phenol 4.09 (3.85 g, 11.4 mmol) and BTEAC (5.20 g, 22.8 mmol) in DMF (50 mL) was warmed at 50 °C for 10 min. K}_2\text{CO}_3 (4.45 g, 34.2 mmol) was added and the solution was stirred for 30 min. Bromoethanol (0.90 mL, 12.5 mmol) was added dropwise and the solution was stirred. After 48 h, bromoethanol (0.45 mL, 6.3 mmol) was added dropwise and the solution was stirred for a further 24 h. The cooled reaction mixture was diluted with CH}_2\text{Cl}_2 (100 mL), washed with water (1 x 150 mL) and brine (1 x 150 mL), dried over MgSO}_4 and concentrated \text{in vacuo}. The residue was purified by column chromatography (eluent 40% EtOAc/hexane) to give the title compound as a white solid (3.17 g, 8.3 mmol, 73%) and recovered phenol 4.09 (364 mg, 1.1 mmol, 9%).

\text{Method 2: Benzoic ester 4.12 (265 mg, 0.55 mmol) was stirred in a 1% aq solution of NaOH/MeOH (25 mL) at rt for 4 h. The reaction was added to water (10 mL) and extracted with CH}_2\text{Cl}_2 (3 x 25 mL). The combined organic layers were dried over MgSO}_2 and concentrated \text{in vacuo}. The residue was purified by column chromatography (eluent 30% EtOAc/hexane) to give the title compound as a colourless oil (139 mg, 0.36 mmol, 66%).
MP 87-90 °C.

$\left[ \alpha \right]_D$ +17.8 (c 1.06, CHCl$_3$, 23 °C).

IR $\nu_{\text{max}}$ (neat) 3435 (br), 2977 (w), 2932 (w), 1701 (m), 1510 (m), 1366 (m), 1246 (m), 1151 (s) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.09 (2H, d, $J = 8.7$ Hz, C$_{Ar}$H meta to OCH$_2$), 6.85 (2H, d, $J = 8.7$ Hz, C$_{Ar}$H ortho to OCH$_2$), 4.98 (1H, br d, $J = 7.7$ Hz, NH), 4.41 (1H, ddd, $J = 7.7$, 6.4, 6.0 Hz, C(O)CH), 4.07 (2H, t, $J = 4.0$ Hz, OCH$_2$CH$_2$OH), 3.95 (2H, t, $J = 4.0$ Hz, OCH$_2$CH$_2$OH), 3.10-2.86 (2H, m, C$_{Ar}$CH$_2$), 2.09 (1H, br s, OH), 1.43 & 1.42 (18H, 2 x s, 2 x C(O)OC(CH$_3$)$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.0 (CHC(O)OC(CH$_3$)$_3$), 157.6 (NHC(O)OC(CH$_3$)$_3$), 155.1 (C$_{Ar}$OCH$_2$), 130.6 (C$_{Ar}$H meta to OCH$_2$), 128.9 (C$_{Ar}$CH$_2$), 114.4 (C$_{Ar}$H ortho to OCH$_2$), 82.0 & 79.6 (2 x OC(CH$_3$)$_3$), 69.2 (OCH$_2$CH$_2$OH), 61.4 (OCH$_2$CH$_2$OH), 54.9 (C(O)CH), 37.5 (C$_{Ar}$CH$_2$), 28.3 & 27.9 (2 x OC(CH$_3$)$_3$).


HRMS (ES+) calcd for C$_{20}$H$_{31}$NO$_6$Na [M + Na]$^+$ 404.2044, found 404.2044.
(S)-(+)\textbf{-2-tert-Butoxycarbonylamino-3-(4-hydroxy phenyl) propionic acid tert-butyl ester (4.09)}

\[
\text{C}_{18}\text{H}_{27}\text{NO}_{5} \\
\text{Mw }= 337.41 \text{ gmol}^{-1} \\
\text{Cream solid}
\]

Following a modified procedure to that described by Chevallet \textit{et al.},\textsuperscript{97} to a solution of \textit{N-(tert-butoxycarbonyl)-L-tyrosine (4.08, 2.0 g, 7.1 mmol), BTEAC (1.6 g, 7.1 mmol) and K}_{2}\text{CO}_{3} (24.0 g, 185.0 mmol) in DMAC (22 mL) was added 2-bromo-2-methyl propane (38.0 mL, 340.0 mmol) and the solution was stirred at 55 \textdegree C for 48 h. The cooled reaction mixture was poured onto water and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluuent 10-50% EtOAc/hexane) to give the title compound as a cream solid (2.2 g, 6.5 mmol, 92\%). Spectroscopic data were in agreement with those published in the literature.\textsuperscript{157}

\textbf{MP} \hspace{1cm} 117-120 \textdegree C (lit 112.8-113.0 \textdegree C).\textsuperscript{157}

\textbf{[\alpha]D} \hspace{1cm} +36.2 (c 1.00, CHCl\textsubscript{3}, 24 \textdegree C) (lit +8.0 (c 1.00, EtOH, 20 \textdegree C).\textsuperscript{157}

\textbf{IR} \hspace{1cm} \nu_{\text{max}} \text{(neat)} 3351 \text{(br)}, 2978 \text{(w)}, 2932 \text{(w)}, 1716 \text{(shoulder)}, 1686 \text{(m)}, 1515 \text{(m)}, 1366 \text{(m)}, 1159 \text{(s)} \text{cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} \hspace{1cm} (300 MHz, CDCl\textsubscript{3}) \delta 7.03 \text{(2H, d, J = 8.1 Hz, C}_{\text{Ar}}\text{H meta to OH), 6.74 (2H, d, J = 8.1 Hz, C}_{\text{Ar}}\text{H ortho to OH), 5.43 (1H, s, OH), 5.01 (1H, br d, J = 7.9 \text{ Hz, NH), 4.43-4.37 (1H, m, C(O)CH), 3.00-2.94 (2H, m, C}_{\text{Ar}}\text{CH}_{2}), 1.43 \& 1.42 (18H, 2 x s, 2 x C(O)OC(CH\textsubscript{3})_{3}).}

\textbf{\textsuperscript{13}C NMR} \hspace{1cm} (75 MHz, CDCl\textsubscript{3}) \delta 171.1 \text{(CHC(O)OC(CH\textsubscript{3})_{3})}, 155.2 (NHC(O)OC(CH\textsubscript{3})_{3}), 154.7 \text{(C}_{\text{Ar}}\text{OH), 130.6 (C}_{\text{Ar}}\text{H meta to OH), 128.2 (C}_{\text{Ar}}\text{CH}_{2}), 115.2 (C}_{\text{Ar}}\text{H ortho to OH), 82.1 \& 79.8 (2 x OC(CH\textsubscript{3})_{3}), 55.0 (C(O)CH), 37.7 (C}_{\text{Ar}}\text{CH}_{2}), 28.3 \& 28.0 (2 x OC(CH\textsubscript{3})_{3}).

(S)-2-tert-Butoxycarbonylamino-3-(4-ethoxycarbonylmethoxy phenyl) propionic acid tert-butyl ester (4.10)

\[
\begin{align*}
C_{22}H_{33}NO_7 & \quad \text{Mw} = 423.50 \text{ gmol}^{-1} \\
\text{Colourless oil} & 
\end{align*}
\]

Following the procedure of Thompson et al.,\textsuperscript{103} a vigorously stirred solution of phenol 4.09 (120 mg, 0.36 mmol) and Cs\textsubscript{2}CO\textsubscript{3} (352 mg, 1.08 mmol) in dioxane (5 mL) was aged at rt for 19 h. To this solution was added ethyl bromoacetate (1.80 mL, 16.20 mmol) and the reaction mixture was heated to 80 °C for 6.5 h. The cooled reaction mixture was diluted with CHCl\textsubscript{3} (5 mL), filtered and the filtrate was concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 5-40% EtOAc/hexane) to give the title compound as a colourless oil (72 mg, 0.17 mmol, 47%).

IR \( \nu_{\text{max}} \) (neat) 3388 (br), 2978 (w), 2933 (w), 1712 (m), 1511 (m), 1366 (m), 1152 (s) cm\textsuperscript{−1}.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) 7.10 (2H, d, \( J = 8.8 \) Hz, C\textsubscript{Ar}H meta to OCH\textsubscript{2}), 6.84 (2H, d, \( J = 8.8 \) Hz, C\textsubscript{Ar}H ortho to OCH\textsubscript{2}), 4.97 (1H, br d, \( J = 7.1 \) Hz, NH), 4.60 (2H, s, OCH\textsubscript{2}C(O)O), 4.42-4.38 (1H, m, C(O)CH), 4.26 (2H, q, \( J = 7.1 \) Hz, C(O)OCH\textsubscript{2}), 3.02-2.99 (2H, m, C\textsubscript{Ar}CH\textsubscript{2}), 1.43 & 1.41 (18H, 2 x s, 2 x C(O)OCH\textsubscript{3}), 1.30 (3H, t, \( J = 7.1 \) Hz, C(O)OCH\textsubscript{2}CH\textsubscript{3}).

\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \( \delta \) 170.9 (CHC(O)OC(CH\textsubscript{3})\textsubscript{3}), 168.9 (C(O)OCH\textsubscript{2}CH\textsubscript{3}), 156.8 (NHC(O)OC(CH\textsubscript{3})\textsubscript{3}), 155.0 (C\textsubscript{Ar}OCH\textsubscript{2}), 130.6 (C\textsubscript{Ar}H meta to OCH\textsubscript{2}), 129.6 (C\textsubscript{Ar}CH\textsubscript{2}), 114.6 (C\textsubscript{Ar}H ortho to OCH\textsubscript{2}), 82.0 & 79.6 (2 x OC(CH\textsubscript{3})\textsubscript{3}), 65.6 (OCH\textsubscript{2}C(O)O), 61.3 (C(O)OCH\textsubscript{2}CH\textsubscript{3}), 54.9
(C(O)CH), 37.6 (C\textsubscript{A1}CH\textsubscript{2}), 28.3 & 27.9 (2 x OC(CH\textsubscript{3})\textsubscript{3}), 14.1 (C(O)OCH\textsubscript{2}CH\textsubscript{3}).

**LRMS**

(ES+) m/z 446 [M + Na]\textsuperscript{+}, 870 [2M + Na]\textsuperscript{+}.

**HRMS**

(ES+) calcd for C\textsubscript{22}H\textsubscript{33}NO\textsubscript{7}Na [M + Na]\textsuperscript{+} 446.2149, found 446.2143.

*(S)-Benzoic acid 2-[4-(2-\textit{tert}-butoxycarbonyl-2-\textit{tert}-butoxycarbonylamino ethyl)phenoxy] ethyl ester (4.12)*

\[
\text{C}_{27}\text{H}_{35}\text{NO}_{7} \\
\text{Mw = 485.57 gmol}^{-1} \\
\text{White solid}
\]

Following the general procedure of Chen et al.,\textsuperscript{105} to a solution of phenol 4.09 (431 mg, 1.30 mmol), alcohol 4.13 (216 mg, 1.30 mmol) and tri-\textit{n}-butylphosphine (0.65 mL, 2.60 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (30 mL) at 0 °C was added dropwise a solution of ADDP (656 mg, 2.60 mmol) in THF (10 mL). The solution was stirred at 0 °C for 1 h and then allowed to warm to rt and was stirred for a further 18 h. The reaction mixture was concentrated \textit{in vacuo} and the residue was purified by column chromatography (eluent 5-30% EtOAc/hexane) to give the title compound as a white solid (265 mg, 0.55 mmol, 42%).

**MP**

65-70 °C.

**IR**

ν\textsubscript{max} (neat) 3369 (br), 2977 (w), 2890 (w), 1713 (s), 1511 (m), 1366 (m), 1243 (s), 1150 (s), 712 cm\textsuperscript{-1}.

**\textsuperscript{1}H NMR**

(300 MHz, CDCl\textsubscript{3}) δ 8.06 (2H, d, J = 7.8 Hz, C\textsubscript{A1}H \textit{ortho} to C(O)O), 7.58 (1H, t, J = 7.4 Hz, C\textsubscript{A1}H \textit{para} to C(O)O), 7.45 (2H, dd, J = 7.8, 7.4 Hz, C\textsubscript{A1}H \textit{meta} to C(O)O), 7.10 (2H, d, J = 8.6 Hz, C\textsubscript{A1}H \textit{meta} to OCH\textsubscript{2}), 6.87 (2H, d, J = 8.6 Hz, C\textsubscript{A1}H \textit{ortho} to OCH\textsubscript{2}), 4.97 (1H, br d, J = 7.5


$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.0 (CHC(O)OC(CH$_3$)$_3$), 166.5 (C(O)C$_{Ar}$), 157.5 (NHCC(O)OC(CH$_3$)$_3$), 155.1 (C$_{Ar}$OCH$_2$), 133.1 (C$_{Ar}$ H para to C(O)O), 130.6 (C$_{Ar}$H meta to OCH$_2$), 129.7 (C$_{Ar}$H ortho to C(O)O), 129.0 (C$_{Ar}$CH$_2$), 128.3 (C$_{Ar}$C(O)O), 128.2 (C$_{Ar}$H meta to C(O)O), 114.6 (C$_{Ar}$H ortho to OCH$_2$), 82.0 & 79.6 (2 x OC(CH$_3$)$_3$), 66.0 (C(O)OCH$_2$C$_H$), 63.3 (C(O)OCH$_2$CH$_2$), 54.9 (C(O)CH), 37.5 (C$_{Ar}$CH$_2$), 28.3 & 28.0 (2 x OC(CH$_3$)$_3$).


HRMS (ES+) calcd for C$_{27}$H$_{35}$NO$_7$Na [M + Na]$^+$ 508.2306, found 508.2305.

**Benzoic acid 2-hydroxy ethyl ester (4.13)**

\[
\text{C$_9$H$_{10}$O$_3$}
\]

Mw = 166.17 gmol$^{-1}$

Colourless liquid

Following the procedure of Wiseman *et al.*,\textsuperscript{104} to a solution of ethylene glycol (1.70 mL, 30.0 mmol) and pyridine (0.90 mL, 11.0 mmol) in CH$_2$Cl$_2$ (10 mL) at 0 °C was slowly added benzyol chloride (1.16 mL, 10.0 mmol). The reaction mixture was allowed to warm to rt and was stirred for 20.5 h. The reaction mixture was diluted with EtOAc (10 mL), washed with water (2 x 25 mL) and brine (1 x 50 mL), dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (eluent 5-50% EtOAc/hexane) to give the title compound as a colourless liquid (1.31 g, 7.9 mmol, 79%). Spectroscopic data were in agreement with those published in the literature.\textsuperscript{104}

**IR** $\nu_{\text{max}}$ (neat) 3413 (br), 2952 (w), 2890 (w), 1699 (m), 1269 (s), 1068 (m),
1H NMR (300 MHz, CDCl₃) δ 8.10-8.04 (2H, m, C₆H ortho to C(O)O), 7.57 (1H, tt, J = 7.4, 1.4 Hz, C₆H para to C(O)O), 7.45 (2H, dd, J = 7.7, 7.3 Hz, C₆H meta to C(O)O), 4.50-4.45 (2H, m, C(O)OC₂H₅), 4.00-3.95 (2H, m, C(O)OCH₂C₂H₅), 2.39 (1H, br s, OCH₃).

13C NMR (75 MHz, CDCl₃) δ 166.9 (C(O)O), 133.2 (C₆H para to C(O)O), 129.8 (C₆C(O)O), 129.7 (C₆H ortho to C(O)O), 128.4 (C₆H meta to C(O)O), 66.6 (C(O)OC₂H₅), 61.4 (C(O)OCH₂C₂H₅).

LRMS (ES+) m/z 189 [M + Na]⁺.

(5S)-(+)-6-(4-(2-[4-(2-tert-Butoxycarbonyl-2-tert-butoxycarbonylamino phenoxy] ethoxysulfonyl) phenyl) hexanoic acid methyl ester (4.14)

C₃₃H₄₇NO₁₀S
Mw = 649.79 gmol⁻¹
Colourless oil

To a solution of alcohol 4.07 (1.53 g, 4.0 mmol), sulfonyl chloride 2.03 (1.46 g, 4.8 mmol) and DMAP (122 mg, 1.0 mmol) in CH₂Cl₂ (25 mL) was added Et₃N (1.40 mL, 10.0 mmol) and the solution was stirred at rt for 1.75 h. The reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with 2 N HCl (1 x 40 mL), water (1 x 40 mL) and brine (1 x 40 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 30% EtOAc/hexane) to give the title compound as a colourless oil (2.15 g, 3.3 mmol, 83%).

[α]D +7.2 (c 1.10, CHCl₃, 24 °C).

IR
νₘₐₓ (neat) 3393 (br), 2977 (w), 2935 (w), 1735 (m), 1716 (m), 1512 cm⁻¹.
(m), 1365 (m), 1249 (m), 1176 (s) cm$^{-1}$.

$^1$H NMR

(300 MHz, CDCl$_3$) $\delta$ 7.85 (2H, d, $J = 8.3$ Hz, C$_{Ar}$H ortho to SO$_2$O), 7.35 (2H, d, $J = 8.3$ Hz, C$_{Ar}$H meta to SO$_2$O), 7.06 (2H, d, $J = 8.6$ Hz, C$_{Ar}$H meta to OCH$_2$), 6.72 (2H, d, $J = 8.6$ Hz, C$_{Ar}$H ortho to OCH$_2$), 4.97 (1H, br d, $J = 7.1$ Hz, NH), 4.40-4.33 (3H, m, SO$_2$OCH$_2$ & CHC(O)), 4.17-4.09 (2H, m, SO$_2$OCH$_2$CH$_2$), 3.67 (3H, s, C(O)OCH$_3$), 3.09-2.89 (2H, m, C$_{Ar}$CH$_2$CH), 2.71 (2H, t, $J = 7.7$ Hz, C$_{Ar}$CH$_2$CH$_2$), 2.32 (2H, t, $J = 7.4$ Hz, C(O)CH$_2$), 1.76-1.61 (4H, m, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.53-1.31 (20H, m, 2 x C(O)OC(CH$_3$)$_3$ & C(O)CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR

(75 MHz, CDCl$_3$) $\delta$ 174.0 (C(O)OCH$_3$), 170.9 (CHC(O)OC(CH$_3$)$_3$), 157.0 (NHC(O)OC(CH$_3$)$_3$), 155.0 (C$_{Ar}$OCH$_3$), 149.4 (C$_{Ar}$CH$_2$), 139.0 (C$_{Ar}$H meta to SO$_2$O), 133.2 (C$_{Ar}$SO$_2$O), 130.6 (C$_{Ar}$H meta to OCH$_2$), 129.2 (C$_{Ar}$CH$_2$CH), 128.1 (C$_{Ar}$H ortho to SO$_2$O), 114.4 (C$_{Ar}$H ortho to OCH$_3$), 82.0 & 79.6 (2 x OC(CH$_3$)$_3$), 68.1 (SO$_2$OCH$_2$), 65.5 (SO$_2$CH$_2$CH$_2$), 54.9 (C(O)CH), 51.5 (C(O)CH$_3$), 37.6 (C$_{Ar}$CH$_2$CH), 35.7 (C$_{Ar}$CH$_2$CH$_2$), 34.8 (C(O)CH$_2$), 30.6 (C(O)CH$_2$CH$_2$CH$_2$), 28.6 (C(O)CH$_2$CH$_2$), 28.3 & 28.0 (2 x OC(CH$_3$)$_3$), 24.6 (C$_{Ar}$CH$_2$CH$_2$).

LRMS

(ES+) m/z 672 [M + Na]$^+$.  

HRMS

(ES+) calcd for C$_{33}$H$_{47}$NO$_{10}$SNa [M + Na]$^+$ 672.2813, found 672.2826.

(S)-(+)-2-tert-Butoxycarbonylamino-3-[4-(2-fluoro ethoxy) phenyl] propionic acid tert-butyl ester (4.15)

$\text{C}_2\text{H}_{30}\text{FNO}_5$

$\text{Mw} = 383.45\text{ gmol}^{-1}$

Colourless oil
**Solution Phase Method:** A solution of sulfonyl ester **4.14** (16 mg, 0.025 mmol), KF (2 mg, 0.030 mmol) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (11 mg, 0.030 mmol) in MeCN (1 mL) was heated under reflux for 1 h. The cooled reaction mixture was concentrated *in vacuo* and the residue was dissolved in CH$_2$Cl$_2$ and was washed with water. The aqueous phase was extracted with CH$_2$Cl$_2$ (3 x 2 mL) and the combined organic layers were dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (eluent 2-40% EtOAc/hexane ) to give the title compound as a colourless oil (4 mg, 0.012 mmol, 48%).

**Solid Phase Method:** Resin **4.17** (100 mg, 0.78 mmol$^{-1}$, 0.078 mmol), KF (5 mg, 0.084 mmol) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (32 mg, 0.084 mmol) were heated under reflux in MeCN (2 mL) for 45 min. The resin was removed by filtration and was washed with CH$_2$Cl$_2$ (10 mL). The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography (eluent 40% EtOAc/hexane) to give the title compound as a colourless oil (15 mg, 0.039 mmol, 50%).

|α|d| +4.0 (c 0.70, CHCl$_3$, 23 °C) |
|---|---|

**IR**

$\nu_{\text{max}}$ (neat) 3367 (br), 2978 (w), 1708 (m), 1511 (m), 1366 (m), 1248 (m), 1150 (s), 1953 (m), 801 (m) cm$^{-1}$.

**$^1$H NMR**

(300 MHz, CDCl$_3$) $\delta$ 7.10 (2H, d, $J = 8.6$ Hz, C$_{Ar}$H *meta* to OCH$_2$), 6.86 (2H, d, $J = 8.6$ Hz, C$_{Ar}$H *ortho* to OCH$_2$), 4.97 (1H, br d, $J = 7.7$ Hz, NH), 4.75 (2H, dt, $^2J_{HF} = 47.4$, $J = 4.2$ Hz, FCH$_2$), 4.41 (1H, dt, $J = 7.7$, 6.2 Hz, C(O)CH), 4.20 (2H, dt, $^3J_{HF} = 27.8$, $J = 4.2$ Hz, FCH$_2$CH$_2$), 3.05-3.95 (2H, m, C$_{Ar}$CH$_2$), 1.43 & 1.42 (18H, 2 x s, 2 x C(O)OC(CH$_3$)$_3$).

**$^{13}$C NMR**

(100 MHz, CDCl$_3$) $\delta$ 171.0 (CHC(O)OC(CH$_3$)$_3$), 157.4 (NHC(O)OC(CH$_3$)$_3$), 155.1 (C$_{Ar}$OCH$_2$), 130.6 (C$_{Ar}$H *meta* to OCH$_2$), 129.1 (C$_{Ar}$CH$_2$), 114.5 (C$_{Ar}$H *ortho* to OCH$_2$), 81.98 (OC(CH$_3$)$_3$), 81.95 (d, $J = 170.0$ Hz, FCH$_2$), 81.1 (OC(CH$_3$)$_3$), 67.2 (d, $J = 20.4$ Hz,
FCH₂CH₂), 54.9 (C(O)CH), 37.6 (C₆H₅CH₂), 28.3 & 28.0 (2 x OC(CH₃)₃).

¹⁹F NMR (282 MHz, CDCl₃) δ –225.1 (FCH₂).


(S)-(+)-6-(4-[2-[4-(2-tert-Butoxycarbonyl-2-tert-butoxycarbonylamino ethyl)phenoxy] ethoxysulfonyl] phenyl) hexanoic acid (4.16)

A solution of methyl ester 4.14 (1.92 g, 3.0 mmol) and Novozym 435® (2.00 g) in CH₂Cl₂/acetone (5:1, 30 mL) and aq phosphate buffer (pH 7, 80 mL) was heated at 50 ° C for 28 h. Further Novozym 435® (1.00 g) was added and the reaction mixture was stirred for an additional for 24 h. The cooled reaction mixture was filtered through Celite® and the pad was washed with CH₂Cl₂. The filtrate was acidified with HCl, the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 40% EtOAc/hexane) to give the title compound as a colourless oil (969 mg, 1.5 mmol, 50%) and recovered methyl ester 4.14 (475 mg, 0.7 mmol, 23%).

[α]D +6.8 (c 1.12, CHCl₃, 24 °C).

IR ν max (neat) 3395 (br), 2977 (w), 2933 (w), 1708 (m), 1512 (m), 1365
(m), 1248 (m), 1174 (s), 1153 (s), 927 (m) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.84 (2H, d, $J = 8.2$ Hz, $C_{Ar}$H ortho to SO$_2$O), 7.35 (2H, d, $J = 8.2$ Hz, $C_{Ar}$H meta to SO$_2$O), 7.06 (2H, d, $J = 8.3$ Hz, $C_{Ar}$H meta to OCH$_2$), 6.70 (2H, d, $J = 8.3$ Hz, $C_{Ar}$H ortho to OCH$_2$), 5.01 (1H, br d, $J = 7.9$ Hz, NH), 4.54-4.24 (3H, m, SO$_2$OCH$_2$ & $C_{Ar}$CH$_2$CH), 4.23-3.92 (2H, m, SO$_2$OCH$_2$CH$_2$), 3.02-2.93 (2H, m, $C_{Ar}$CH$_2$CH), 2.71 (2H, t, $J = 7.6$, $C_{Ar}$CH$_2$CH$_2$), 2.36 (2H, t, $J = 7.3$ Hz, C(O)CH$_2$), 1.88-1.55 (4H, m, C(O)CH$_2$CH$_2$CH$_2$CH$_2$), 1.52-1.29 (20H, m, 2 x C(O)OC(CH$_3$)$_3$ & C(O)CH$_2$CH$_2$CH$_2$).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 178.6 (C(O)OH), 171.0 (CHC(O)OC(CH$_3$)$_3$), 157.0 (NHC(O)OC(CH$_3$)$_3$), 155.1 ($C_{Ar}$OCH$_2$), 149.3 ($C_{Ar}$CH$_2$CH$_2$), 133.2 ($C_{Ar}$SO$_2$O), 130.5 ($C_{Ar}$H meta to OCH$_2$), 129.2 ($C_{Ar}$H meta to SO$_2$O), 129.2 ($C_{Ar}$CH$_2$CH), 128.1 ($C_{Ar}$H ortho to SO$_2$O), 114.4 ($C_{Ar}$H ortho to OCH$_2$), 82.0 & 79.7 (2 x OC(CH$_3$)$_3$), 68.2 (SO$_2$OCH$_2$), 65.4 (SO$_2$OCH$_2$CH$_2$), 54.9 (C(O)CH), 37.6 ($C_{Ar}$CH$_2$CH), 35.6 ($C_{Ar}$CH$_2$CH$_2$), 33.7 (C(O)CH$_2$), 30.5 (C(O)CH$_2$CH$_2$CH$_2$), 28.5 (C(O)CH$_2$CH$_2$), 28.3 & 27.9 (2 x OC(CH$_3$)$_3$), 24.4 ($C_{Ar}$CH$_2$CH$_2$).

LRMS (ES+) m/z 658 [M + Na]$^+$.  

HRMS (ES+) calcd for C$_{32}$H$_{45}$NO$_{10}$SNa [M + Na]$^+$ 658.2656, found 658.2640.

Solid supported (S)-6-(4-[2-[4-(2-tert-butoxycarbonyl-2-tert-butoxycarbonylamino ethyl) phenoxy] ethoxysulfonyl] phenyl) hexanoic acid (4.17)

Loading = 0.78 mmolg$^{-1}$
Resin beads
To a solution of HOBt (523 mg, 3.87 mmol) and DIC (0.60 mL, 3.87 mmol) in DMF (5 mL) was added carboxylic acid 4.16 (823 mg, 1.29 mmol) in CH₂Cl₂ (20 mL) and amino methyl polystyrene resin (573 mg, 1.5 mmol g⁻¹, 0.86 mmol). The reaction mixture was stirred at rt for 18 h after which time a ninhydrin test carried out on a small sample of the resin was negative. The resin was removed by filtration, washed with CH₂Cl₂ (3 x 100 mL), MeOH (3 x 100 mL) and Et₂O (3 x 100 mL) and dried in vacuo at 40 °C for 24 h to give the product resin (1.22 g, theoretical loading 0.70 mmol g⁻¹, actual loading (S elemental analysis) 0.78 mmol g⁻¹, 122%).

**IR**

\[ \nu_{\text{max}} \text{ (neat)} \ 3399 \text{ (w), 2927 \text{ (w), 1708 \text{ (m), 1361 \text{ (m), 1175 \text{ (m), 729 \text{ (s), 699 \text{ (s) cm}^{-1}}})}} \]

**¹H NMR**

MAS (400 MHz, CDCl₃) \( \delta \ 7.84 \text{ (s, C₆H ortho to SO₂O), 7.34 \text{ (s, C₆H meta to SO₂O), 7.07 \text{ (d, C₆H meta to OCH₂), 6.73 \text{ (d, C₆H ortho to OCH₂), 6.66 \text{ (amino methyl resin), 5.03 \text{ (s, NH(C(O)O), 4.40 \text{ (s, C₆HCH₂CH), 4.36 \text{ (s, SO₂OCH₂), 4.12 \text{ (s, SO₂OCH₂CH₂), 3.50 \text{ (m, amino methyl resin), 3.00 \text{ (s, C₆HCH₂CH), 2.70 \text{ (s, C₆HCH₂CH₂), 2.20 \text{ (s, C(O)CH₂), 1.69 \text{ (s, C(O)CH₂CH₂CH₂CH₂), 1.43 \text{ (s, 2 x OC(CH₃)₃ & C(O)CH₂CH₂CH₂)}}})}})}}} \]

**¹³C NMR**

MAS (100 MHz, CDCl₃) \( \delta \ 172.6 \text{ (NH(C(O)), 171.0 \text{ (CHC(O)O), 157.0 \text{ (NH(C(O)O), 155.1 \text{ (C₆HCH₂CH), 149.5 \text{ (C₆HCH₂CH₂), 145.3 \text{ (amino methyl resin), 135.5 \text{ (amino methyl resin), 133.2 \text{ (C₆HSO₂O), 130.6 \text{ (C₆H meta to OCH₂), 129.3 \text{ (C₆H meta to SO₂O), 128.1 \text{ (C₆H ortho SO₂O & C₆HCH₂CH), 114.5 \text{ (C₆H ortho to OCH₂), 82.0 & 79.7 \text{ (2 x C(O)OC(CH₃)₃), 68.3 \text{ (SO₂OCH₂), 65.5 \text{ (SO₂OCH₂CH₂), 55.0 \text{ (C₆HCH₂CHC(O)), 43.4 \text{ (amino methyl resin), 40.4 \text{ (amino methyl resin), 37.6 \text{ (C₆HCH₂CH), 36.4 \text{ (C₆HCH₂CH₂), 35.7 \text{ (C(O)CH₂), 30.8 \text{ (C(O)CH₂CH₂CH₂), 28.9 \text{ (C(O)CH₂CH₂), 28.4 & 28.0 \text{ (2 x C(O)OC(CH₃)₃), 25.5 \text{ (C₆HCH₂CH₂)}})}}})}}}}}} \]

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A solution of diol (–)-5.25 (33 mg, 0.10 mmol), TsF (36 mg, 0.21 mmol), KF (12 mg, 0.21 mmol) and 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (39 mg, 0.10 mmol) in THF (2.5 mL) was heated under reflux for 43 h. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (alumina, Brockman grade III, 6% H2O, 50-200 mesh, eluent EtOAc then 4% MeOH/CH2Cl2). MeOH (2 mL) was passed through a Isolute SCX-2 cation exchange cartridge before the residue from the column was dissolved in MeOH (0.5 mL) and loaded onto the cartridge. The cartridge was washed with MeOH (5 mL) before being eluted with NH4/MeOH (2 mL). The solvent was removed in vacuo to give the title compound as a colourless oil (3 mg, 9.4 μmol, 9%).

[α]D –49.3 (c 0.20, CHCl3, 25 °C).

IR νmax (neat) 2918 (w), 2027 (w), 1506 (w), 1443 (w), 1286 (w), 1175 (w), 1043 (m) cm⁻¹.

1H NMR (400 MHz, CDCl3) δ 6.70 (1H, d, J = 8.3 Hz, CAH ortho to OCH2), 6.61 (1H, d, J = 8.3 Hz, CAH ortho to CH2N), 6.07 (1H, d, J = 10.2 Hz, CCH=CH), 6.01 (1H, dd, J = 10.2, 5.0 Hz, CCH=CH), 4.73 (2H, dt, J = 47.4, 4.2 Hz, CH2F), 4.62 (1H, apt br s, CHOCO), 4.37-4.18 (2H, m, CH2CH2F), 4.15 (1H, apt br s, CHOH), 4.09 (1H, d, J = 15.3 Hz, NCHH), 3.69 (1H, d, J = 15.3 Hz, NCHH), 3.27 (1H, t, J = 13.3 Hz, CH2CHHN), 3.06 (1H, d, J = 14.0 Hz, CH2CHHN), 2.69 (1H, dt, J =
15.7, 1.6 Hz, C\text{HCHOC}_{\text{Ar}}, 2.42 (4H, br s, NCH$_3$ & O\text{H}), 2.09 (1H, td, J = 13.3, 3.0 Hz, C\text{HHCH}_{2}\text{N}), 2.02 (1H, ddd, J = 15.7, 5.0, 2.3 Hz, C\text{HHCHOC}_{\text{Ar}}), 1.59 (1H, dd, J = 14.0, 2.1 Hz, C\text{HHCH}_{2}\text{N}).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 146.5 (\text{C}_{\text{ArOCH}_2}), 142.7 (\text{C}_{\text{ArOCH}}), 133.6 (\text{C}_{\text{ArC}}), 130.6 (\text{C}_{\text{ArCH}_2\text{N}}), 127.7 (\text{CCH}=\text{CH}), 126.9 (\text{CCH}=\text{CH}), 122.1 (\text{C}_{\text{ArH ortho to OCH}_2}), 114.2 (\text{C}_{\text{ArH ortho to CH}_2\text{N}}), 88.7 (\text{CHOC}_{\text{Ar}}), 82.0 (d, J = 171.1 Hz, \text{CH}_2\text{F}), 68.6 (d, J = 21.4 Hz, \text{CH}_2\text{CH}_2\text{F}), 62.1 (\text{CHOH}), 60.7 (\text{NCH}_2), 53.9 (\text{CH}_2\text{CH}_2\text{N}), 48.2 (\text{CCH}=\text{CH}), 42.4 (\text{NCH}_3), 33.9 (\text{C}_{\text{ArOCHCH}_2}), 29.9 (\text{CH}_2\text{CH}_2\text{N}).

$^{19}$F NMR (282 MHz, CDCl$_3$) δ −224.8 (F\text{CH}_2).

LRMS (ES+) m/z 320 [M + H]$^+$, 342 [M + Na]$^+$.

HRMS (ES+) calcd for C$_{18}$H$_{23}$FNO$_3$ [M + H]$^+$ 320.1656, found 320.1656.

$^{(4aS,6R,8aS)}$-$^{(−)}$-$^{4a,5,9,10,11,12}$-\text{Hexahydro-3-(2’-hydroxy ethoxy)-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol ((−)-5.25)}

\[
\text{C$_{18}$H$_{23}$NO}_4
\]
\[
\text{Mw} = 317.38 \text{ gmol}^{-1}
\]
Colourless oil

\textit{Method 1}: Bromide 5.49 (13 mg, 0.022 mmol) was dissolved in CH$_2$Cl$_2$ (0.5 mL) and was treated with TFA (0.2 mL). The solution was stirred at rt for 45 min before sat aq NaHCO$_3$ (1 mL) was added and stirring was continued for a further 15 min. The organic phase was separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (4 x 5 mL). The combined organic phase was dried over MgSO$_4$ and concentrated \textit{in vacuo} to give the title compound as a colourless oil (4 mg, 0.013 mmol, 57%).

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Method 2: Tosylate (+)-5.51 (66 mg, 0.082 mmol) was dissolved in CH$_2$Cl$_2$ (1 mL) and was treated with TFA (0.10 mL). The solution was stirred at rt for 3.5 h before the solvent was removed in vacuo. The residue was dissolved in THF (1 mL), K$_2$CO$_3$ (100 mg, 0.72 mmol) was added and the colourless opaque solution was stirred at rt for 4 h. The reaction mixture was filtered, the filtrate was dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (eluent 4-10% MeOH/CH$_2$Cl$_2$) to give the title compound as a colourless oil (17 mg, 0.054 mmol, 65%).

[α]$_D$ = $-57.4$ (c 0.70, CHCl$_3$, 27 °C).

IR $\nu_{\text{max}}$ (neat) 3365 (br), 3027 (w), 2924 (w), 1738 (m), 1680 (m), 1505 (m), 1444 (m), 1366 (m), 1284 (m), 1202 (s), 1175 (s), 1043 (s), 730 (s) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.70 (1H, d, $J = 8.4$ Hz, C$_{Ar}$H ortho to OCH$_2$), 6.61 (1H, d, $J = 8.4$ Hz, C$_{Ar}$H ortho to CH$_2$N), 6.06 (1H, d, $J = 10.2$ Hz, CCH=CH), 5.99 (1H, dd, $J = 10.2$, 4.5 Hz, CCH=CH), 4.60 (1H, apt br s, CHO$_{Ar}$), 4.20-4.01 (4H, m, C$_{Ar}$OCH$_2$CH$_2$), NCHH & CHO$_{OH}$, 3.92-3.87 (2H, m, C$_{Ar}$OCH$_2$), 3.72 (1H, d, $J = 15.3$ Hz, NCHH), 3.29 (1H, t, $J = 13.2$ Hz, CH$_3$CHH$_2$N), 3.08 (1H, d, $J = 14.0$ Hz, CH$_2$CHH$_N$), 2.75 (2H, br s, 2 x OH), 2.70-2.62 (1H, m, CHHCHOC$_{Ar}$), 2.43 (3H, s, NCH$_3$), 2.10 (1H, td, $J = 13.2$, 3.0 Hz, CHHCH$_2$N), 2.00 (1H, ddd, $J = 15.7$, 5.0, 2.4 Hz, CHHCHOC$_{Ar}$), 1.60 (1H, dd, $J = 14.0$, 2.0 Hz, CHHCH$_2$N).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 146.5 (C$_{Ar}$OCH$_2$), 143.0 (C$_{Ar}$OCH), 133.2 (C$_{Ar}$C), 129.6 (C$_{Ar}$CH$_2$N), 127.6 (CCH=CH), 126.8 (CCH=CH), 122.2 (C$_{Ar}$H ortho to OCH$_2$), 113.9 (C$_{Ar}$H ortho to CH$_2$N), 88.4 (CHO$_{OC}$)$_3$, 70.9 (C$_{Ar}$OCH$_2$CH$_2$), 61.8 (CHO$_{OH}$), 61.1 (NCH$_2$), 60.5 (C$_{Ar}$OCH$_2$), 53.8 (CH$_2$CH$_2$N), 48.1 (CCH=CH), 42.0 (NCH$_3$), 33.6 (C$_{Ar}$OCHCH$_2$), 29.9 (CH$_2$CH$_2$N).

**HRMS** (ES+) calcd for C₁₈H₂₄NO₄ [M + H]⁺ 318.1700, found 318.1699.

*tet*-Butyl 2-bromoethyl carbonate (5.27)

![Chemical Structure]

**C₇H₁₃BrO₃**

Mw = 225.08 gmol⁻¹

Colourless oil

Following a modified procedure to that described by Mejorado *et al.*,¹³⁶ a solution of bromoethanol (5.34, 0.73 mL, 9.60 mmol), di-*tet*-butyldicarbonate (698 mg, 3.20 mmol), DMAP (39 mg, 0.32 mmol) and DIPEA (0.11 mL, 0.64 mmol) in THF (5 mL) was stirred at rt for 5 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by column chromatography (eluent 10% EtOAc/hexane) to give the title compound as a colourless oil (528 mg, 2.35 mmol, 73%).

**IR**  νₘₐₓ (neat) 2980 (w), 2937 (w), 1739 (s), 1369 (m), 1272 (s), 1252 (s), 1154 (s), 1114 (m), 861 (m) cm⁻¹.

**¹H NMR** (300 MHz, CDCl₃) δ 4.37 (2H, t, J = 6.4 Hz, BrCH₂CH₂), 3.52 (2H, t, J = 6.4 Hz, BrCH₂), 1.50 (9H, s, OC(CH₃)₃).

**¹³C NMR** (75 MHz, CDCl₃) δ 152.9 (C(O)O), 82.8 (C(CH₃)₃), 66.0 (BrCH₂CH₂), 28.3 (BrCH₂), 27.7 (C(CH₃)₃).

**LRMS** (EI) 124/126 ([M – Boc + H]⁺, 5%), 107/109 ([M – BocOH]⁺, 59%), 93/95 ([M – BocOCH₂]⁺, 16%), 79/81 (Br⁺, 6%), 44 ([CH₂CH₂O]⁺, 100%).

**HRMS** (ES+) calcd for C₇H₁₃BrO₃Na [M + Na]⁺ 246.9940, found 246.9939.
**tert-Butyl-3-hydroxy-2-iodo-(4-ethyl tert-butyl carbonate)benzylmethylcarbamate (5.28)**

![Chemical Structure](image)

C_{20}H_{30}INO_{7}

Mw = 523.36 g mol^{-1}

White solid

Aldehyde **5.36** (14.58 g, 35.7 mmol) was dissolved in MeOH (50 mL) and treated with MeNH₂ (35.71 mL, 2.0 M sol in MeOH, 71.4 mmol) and the yellow solution was stirred at rt for 21.5 h. The solvent was removed *in vacuo* and the residue was dissolved in MeOH (180 mL). 4 Å Molecular sieves (5 g) were added and the reaction was stirred for 15 min before the portionwise addition of NaBH₄ (1.49 g, 39.3 mmol) over 10 min. The reaction mixture was stirred at rt for 3.5 h before the sieves were removed by filtration and the solvent was removed *in vacuo*. The residue was dissolved in CHCl₃ (80 mL) and sat. aq NaHCO₃ (40 mL) and brine (40 mL) were added before di-tert-butyldicarbonate (7.79 g, 35.2 mmol). The emulsion was stirred vigorously at 50 °C for 16.5 h. The cooled reaction mixture was separated and the aqueous layer was extracted with CHCl₃ (1 x 100mL). The combined organic layers were washed with water (1 x 150mL) and brine (1 x 150 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a white solid (14.93 g, 28.5 mmol, 80%).

**MP** 93-95 °C.

**IR** ν max (neat) 3413 (br), 2977 (w), 2932 (w), 1739 (m), 1664 (m), 1479 (m), 1367 (m), 1276 (s), 1252 (s), 1151 (s), 1064 (m) cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃) δ 6.81, (1H, d, J = 8.3 Hz, C₆H, ortho to OCH₂), 6.63 (1H, br s, C₆H ortho to CH₂N), 6.56 (1H, s, OH), 4.62-4.33 (4H, m, CH₂N & C₆OCH₂CH₂), 4.33-4.10 (2H, m, C₆OCH₂), 2.85 (3H, br
s, NCH₃), 1.50-1.35 (18H, m, 2 x C(O)OC(CH₃)₃). Spectrum exhibited broadening of peaks due to restricted rotation.

**¹³C NMR** (100 MHz, CDCl₃) δ 155.9 (NC(O)O), 153.5 (OC(O)O), 146.2 (C₆H₅OCH₂), 143.7 (C₆H₅OH), 133.4 (C₆H₅CH₂N), 117.9 (C₆H₅ ortho to CH₂N), 112.1 (C₆H₅ ortho to OCH₂), 85.8 (C₆H₅I), 82.8 (OC(O)C(CH₃)₃), 79.7 (NC(O)OC(CH₃)₃), 68.1 (C₆H₅OCH₂CH₂), 64.7 (C₆H₅OCH₂), 57.1 (NCH₂), 34.2 (NCH₃), 28.4 (NC(O)C(CH₃)₃), 27.7 (OC(O)C(CH₃)₃).

**LRMS** (ES−) m/z 522 [M − H]+.


**Anal** Calcd for C₂₀H₃₀INO₇ C = 45.90, H = 5.78, N = 2.68. Found C = 46.41, H = 5.83, N = 2.54.

(±)-1-Trimethylsilyl hex-5-en-1-yn-3-ol ((±)-5.29)

Aldehyde 5.38 (8.72 g, 69.1 mmol) in Et₂O (7 mL) was added dropwise to a solution of allylmagnesium bromide (76 mL, 1.0 M sol in Et₂O, 76.0 mmol) at −50 °C. The reaction was stirred for 2 h over which time it was allowed to warm to −10 °C. The reaction was quenched by pouring onto a mixture of a sat aq solution of NH₄Cl (300 mL) and Et₂O (60 mL). The mixture was stirred rapidly for 10 min before it was separated and the aqueous layer was extracted with Et₂O (3 x 75 mL). The combined organic layers were washed with water (1 x 100 mL) and brine (1 x 100 mL), dried over MgSO₄ and concentrated in vacuo. The resulting oil was purified by distillation (110-120 °C, 25 mm/Hg) to give the title compound as a colourless liquid (8.82 g, 52.4
mmol, 76%). Spectroscopic data were in agreement with those published in the literature.158

\[ \text{IR} \quad \nu_{\text{max}} \text{(neat)} 3336 \text{ (br), 3080 (w), 2960 (w), 2901 (w), 2175 (w), 1250 (m), 1028 (m), 837 (s), 759 (m) cm}^{-1}. \]

\[ \text{\textsuperscript{1}H NMR} \] (300 MHz, CDCl\textsubscript{3}) \( \delta \) 6.20-5.62 (1H, m, CH=CH\textsubscript{2}), 5.31-5.07 (2H, m, CH=CH\textsubscript{2}), 4.41 (1H, apt q, \( J = 6.0 \text{ Hz}, \text{CH(OH)} \)), 2.48 (2H, br t, \( J = 6.0 \text{ Hz}, \text{CH} \)), 1.95 (1H, d, \( J = 6.0 \text{ Hz}, \text{OH} \)), 0.18 (9H, s, Si(CH\textsubscript{3})\textsubscript{3}).

\[ \text{\textsuperscript{13}C NMR} \] (75 MHz, CDCl\textsubscript{3}) \( \delta \) 132.9 (CH=CH\textsubscript{2}), 119.0 (CH=CH\textsubscript{2}), 105.9 (SiC≡C), 89.8 (SiC≡C), 61.9 (CH(OH)), 42.1 (CH\textsubscript{2}), −0.2 (Si(CH\textsubscript{3})\textsubscript{3}).

\[ \text{LRMS} \quad (\text{EI}) 127 ([M – C\textsubscript{3}H\textsubscript{5}]^+, 90\%), 99 ([M – C\textsubscript{3}H\textsubscript{5}CO]^+, 100\%). \]

\( (R)-(+)\)-1-Trimethylsilyl hex-5-en-1-yn-3-ol ((+)-5.29)

\[
\begin{align*}
\text{TMS} & \quad \text{C}_9\text{H}_{10}\text{OSi} \\
\equiv & \quad \text{Mw} = 168.31 \text{ g mol}^{-1} \\
\equiv & \quad \text{Colourless liquid}
\end{align*}
\]

Following the procedure of Burova et al.,139 acetate (+)-5.39 (3.41 g, 16.2 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (16 mL) and cooled to −78 °C before DIBAL-H (28 mL, 1.0 M soln in hexanes, 28.0 mmol) was added dropwise. The resulting pale yellow solution was stirred for 30 min before EtOAc (10 mL) was added. The reaction mixture was stirred for a further 20 min at −78 °C before it was poured onto a sat aq solution of Rochelle’s salt (150 mL) and stirred for 1 h at rt. The layers were separated and the aqueous layer was extracted with Et\textsubscript{2}O (2 x 100 mL) and EtOAc (3 x 75 mL). The combined organic layers were dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (elucent 15% EtOAc/hexane) to give the title compound as a colourless liquid (2.648 g, 15.7 mmol, 97%). Spectroscopic data were consistent with those previously collected.
$[\alpha]_D ^0$ : $+43.3$ (c 1.05, CHCl$_3$, 24 °C) (lit: $+34$, c 0.940, rt).$^{139}$

(S)-tert-Butyl-3-((S)-1-formyl-4-(trimethylsilyl)but-3-yn-2-yloxy)-2-iodo-(4-ethyl tert-butyl carbonate) benzylmethylcarbamate (5.30)

$\text{C}_{28}\text{H}_{42}\text{INO}_8\text{Si}$

Mw = 675.62 g mol$^{-1}$

Pale yellow oil

Diol ($\pm$)-5.42 (6.82 g, 9.6 mmol) was dissolved in acetone (40 mL) and water (20 mL) before NaIO$_4$ (4.12 g, 19.3 mmol) was added in one portion. The mixture was stirred vigorously at rt for 2 h during which time it became cloudy. The solvent was removed in vacuo before EtO$_2$ (100 mL) and water (50 mL) were added. The aqueous layer was extracted with EtO$_2$ (3 x 50 mL). The combined organic layers were washed with water (1 x 100 mL) and brine (1 x 100 mL), dried over MgSO$_4$ and concentrated in vacuo to give the title compound as a pale yellow oil (5.98 g, 8.9 mmol, 93%) which required no subsequent purification.

IR $\nu_{\text{max}}$ (neat) 2975 (w), 2930 (w), 1741 (m), 1693 (m), 1476 (m), 1391 (m), 1273 (m), 1250 (s), 1153 (m), 845 (m) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 10.12 (1H, t, $J = 2.2$ Hz, CHO), 7.03-6.70 (2H, m, 2 x C$_{Ar}$$\text{H}$), 5.71 (1H, br s, CHOC$_{Ar}$), 4.60-4.30 (4H, m, CH$_2$N & C$_{Ar}$OCH$_2$CH$_2$), 4.22 (2H, t, $J = 4.3$ Hz C$_{Ar}$OCH$_2$), 2.98 (2H, dd, $J = 5.6$, 2.2 Hz, CH$_2$CHOC$_{Ar}$), 2.91-2.69 (3H, m, NCH$_3$), 1.60-1.30 (18H, m, 2 x C(O)OC(CH$_3$)$_3$), 0.05 (9H, s, Si(CH$_3$)$_3$). Spectrum exhibited broadening of peaks due to restricted rotation.

$^{13}$C NMR (100MHz, CDCl$_3$) $\delta$ 200.3 (CHO), 155.9 (NC(O)O), 153.3 (OC(O)O), 150.0 (C$_{Ar}$OCH$_2$), 146.1 (C$_{Ar}$OCH), 133.5 (C$_{Ar}$CH$_2$N), 123.6 & 122.6
(C\textsubscript{A-H} ortho to OCH\textsubscript{2}), 114.0 & 113.8 (C\textsubscript{A-H} ortho to CH\textsubscript{2}N), 101.4 (SiC\equivC), 94.5 (SiC\equivC), 82.6 (OC(O)C(CH\textsubscript{3})\textsubscript{3}), 79.8 (NC(O)OC(CH\textsubscript{3})\textsubscript{3}), 67.2 (C\textsubscript{A}OCH\textsubscript{2}CH\textsubscript{2}), 67.1 (CHOC\textsubscript{A}), 64.8 (C\textsubscript{A}OCH\textsubscript{2}), 57.7 & 56.6 (NCH\textsubscript{2}), 48.6 (CH\textsubscript{2}CHOCA\textsubscript{A}), 34.2 (NCH\textsubscript{3}), 28.4 (NC(O)C(CH\textsubscript{3})\textsubscript{3}), 27.7 (OC(O)C(CH\textsubscript{3})\textsubscript{3}), –0.5 (Si(CH\textsubscript{3})\textsubscript{3}). No C\textsubscript{A-I} peak observed.

LRMS (ES+) m/z 698 [M + Na]\textsuperscript{+}.

HRMS (ES+) calcd for C\textsubscript{28}H\textsubscript{42}INO\textsubscript{8}SiNa [M + Na]\textsuperscript{+} 698.1617, found 698.1621.

(S)-(+-)\textit{tert}-Butyl-3-((3S,5S)-5-hydroxy-1-(trimethylsilyl)-oct-7-en-1-yn-3-yloxy)-2-iodo-(4-ethyl \textit{tert}-butyl carbonate) benzyl methyl carbamate ((+-)-5.31)

TiCpCl-(R,R)-TADDOL complex ((R,R)-5.43, 3.35 g, 5.6 mmol) was suspended in Et\textsubscript{2}O (80 mL) and cooled to 0 °C before allyl magnesium bromide (5.60 mL, 1.0 M sol in Et\textsubscript{2}O, 5.6 mmol) was added dropwise. The brown reaction mixture was stirred for 1.5 h before it was cooled to –78 °C and a solution of aldehyde 5.30 (3.16 g, 4.7 mmol) in Et\textsubscript{2}O (10 mL) was added dropwise. The reaction mixture was stirred for 5.5 h then quenched by the addition of water (50 mL). The reaction mixture was allowed to warm to rt and stirred for 16 h. Filtration through Celite\textsuperscript{®} and concentration \textit{in vacuo} gave a yellow solid which was purified by column chromatography (eluent 15-40% EtOAc/hexane) to give the title compound as a pale yellow oil (3.18 g, 4.4 mmol, 94%).

[\alpha]_D \text{D} +7.1 (c 1.00, CHCl\textsubscript{3}, 24 °C).
IR

\[ \nu_{\text{max}} \text{ (neat)} \ 3463 \text{ (br w)}, 2976 \text{ (w)}, 2930 \text{ (w)}, 1742 \text{ (m)}, 1476 \text{ (m)}, 1392 \text{ (m)}, 1273 \text{ (m)}, 1249 \text{ (s)}, 1152 \text{ (m)}, 842 \text{ (m)} \text{ cm}^{-1}.\]

\(^1\text{H NMR}\)

(300 MHz, CDCl\(_3\)) \(\delta \ 7.01-6.68 \text{ (2H, m, 2 x } C_{\text{Ar}}H)\), 5.92 \(\text{ (1H, ddt, } J = 17.2, 10.1, 6.9 \text{ Hz, } CH=CH_2\)\), 5.46 \(\text{ (1H, br s, } CHOC_{\text{Ar}}\)\), 5.24-4.99 \(\text{ (2H, m, } CH=CH_2\)\), 4.57-4.30 \(\text{ (4H, m, } CH_2N \& C_{\text{Ar}}OCH_2CH_2\)\), 4.27-4.11 \(\text{ (3H, m, } C_{\text{Ar}}OCH_2 \& CHOH\)\), 2.94-2.66 \(\text{ (4H, m, NCH}_3 \& OH\)\), 2.36 \(\text{ (2H, t, } J = 6.9 \text{ Hz, } CH_2CH=CH_2\)\), 2.26-2.05 \(\text{ (2H, m, } CH_2CHOC_{\text{Ar}}\)\), 1.66-1.29 \(\text{ (18H, m, 2 x } COOC(CH_3)_3\)\), 0.03 \(\text{ (9H, s, } Si(CH_3)_3\)\).

Spectrum exhibited broadening of peaks due to restricted rotation.

\(^{13}\text{C NMR}\)

(100MHz, CDCl\(_3\)) \(\delta \ 155.9 \text{ (NC(O)O)}, 153.2 \text{ (OC(O)O)}, 149.9 \text{ (C}_{\text{Ar}}OCH_2), 146.2 \text{ (C}_{\text{Ar}}OCH), 134.8 \text{ (CH=CH}_2\), 133.5 \text{ (C}_{\text{Ar}}CH_2N), 123.3 \& 122.3 \text{ (C}_{\text{Ar}}H \text{ ortho to OCH}_2\), 117.5 \text{ (CH=CH}_2\), 113.6 \& 113.4 \text{ (C}_{\text{Ar}}H \text{ ortho to CH}_2N\), 103.1 \text{ (SiC=C)}, 93.5 \text{ (SiC=C)}, 82.7 \text{ (OC(O)C(CH}_3}_3), 79.8 \text{ (NC(O)OC(CH}_3}_3), 70.8 \text{ (CHOH)}, 68.9 \text{ (CHOC}_{\text{Ar}}), 67.0 \text{ (C}_{\text{Ar}}OCH}_2CH_2), 64.9 \text{ (C}_{\text{Ar}}OCH}_2, 57.6 \& 56.5 \text{ (NCH}_2\), 42.5 \text{ (CH}_2CH=CH}_2), 41.9 \text{ (CH}_2CHOC_{\text{Ar}}), 34.1 \text{ (NCH}_3\), 28.4 \text{ (NC(O)C(CH}_3}_3), 27.7 \text{ (OC(O)C(CH}_3}_3), -0.4 \text{ (Si(CH}_3)_3\)\).

No C\(_{\text{Ar}}\)I peak observed.

LRMS

\(\text{(ES+) } m/z \ 740 \text{ [M + Na]}^+.\)

HRMS

\(\text{(ES+) calcd for } C_{31}H_{48}INO_8SiNa \text{ [M + Na]}^+ \ 740.2086, \text{ found 740.2096.}\)
(S)-(−)-tert-Butyl-3-(((1S,5S)-5-[(tert-butyldimethylsilyl)oxy]-2-ethenylcyclohex-2-en-1-yloxy)-2-iodo-4-(ethyl tert-butyl carbonate) benzylmethylcarbamate (−)-5.32

Enyne (−)−5.46 (5.03 g, 6.6 mmol) was dissolved in CH₂Cl₂ (140 mL) and degassed for 5 min. Grubbs’ I catalyst (5.45, 273 mg, 0.33 mmol, 5 mol %) was added and the solution was heated to reflux. Further Grubb’s I catalyst (5.45, 54 mg, 0.07 mmol, 1 mol %) was added after 6 h. After 21 h the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (eluent 10-20% EtOAc/hexane) to give the title compound as an orange oil (3.24 g, 4.3 mmol, 65%).

[α]D −61.8 (c 1.04, CHCl₃, 22 °C).

IR
νmax (neat) 2953 (w), 2929 (w), 2856 (w), 1743 (m), 1697 (m), 1474 (m), 1391 (m), 1276 (s), 1252 (s), 1155 (s), 837 (m) cm⁻¹.

¹H NMR
(300 MHz, CDCl₃) δ 6.98-6.66 (2H, m, 2 x C₆H), 6.44 (1H, dd, J = 17.6, 11.2 Hz, CH=CH₂), 5.85 (1H, br d, J = 3.7 Hz, CHO₆Ar), 5.72-5.45 (2H, m, CH=CH₂), 5.05 (1H, d, J = 11.3 Hz, C=CH), 4.52-4.30 (4H, m, CH₃N & C₆OCH₂CH₂), 4.30-4.15 (2H, m, C₆OCH₂), 3.78 (1H, tt, J = 9.8, 5.1 Hz, CHOTBS), 3.01-2.63 (3H, br m, NCH₃), 2.47-2.12 (2H, m, C=CHCH₂), 2.07-1.82 (2H, m, CH₂CHO₆Ar), 1.65-1.30 (18H, m, 2 x C(O)OC(CH₃)₃), 0.84 (9H, s, Si(C(CH₃)₃)), 0.02 (3H, s, SiCH₃), 0.00 (3H, s, SiCH₃). Spectrum exhibited broadening of peaks due to restricted rotation.

¹³C NMR
(75 MHz, CDCl₃) δ 155.9 (NC(O)O), 153.3 (OC(O)O), 149.6
(C\textsubscript{Ar}OCH\textsubscript{2}), 146.0 (C\textsubscript{Ar}OCH), 137.5 (C=CH), 135.8 (CH=CH\textsubscript{2}), 133.6 (C\textsubscript{Ar}CH\textsubscript{2}N), 126.2 (C=CH), 122.1 & 121.4 (C\textsubscript{Ar}H ortho to OCH\textsubscript{2}), 114.1 (CH=CH\textsubscript{2}), 113.2 & 113.0 (C\textsubscript{Ar}H ortho to CH\textsubscript{2}N), 82.3 (OC(O)C(CH\textsubscript{3})\textsubscript{3}), 79.7 (NC(O)OC(CH\textsubscript{3})\textsubscript{3}), 76.1 (CHOTBS), 67.1 (CHOC\textsubscript{Ar}), 66.6 (C\textsubscript{Ar}OCH\textsubscript{2}CH\textsubscript{2}), 64.8 (C\textsubscript{Ar}OCH\textsubscript{2}), 57.8 (NCH\textsubscript{2}), 38.9 (C\textsubscript{Ar}OCHCH\textsubscript{2}), 35.9 (C=CHCH\textsubscript{2}), 34.3 (NCH\textsubscript{3}), 28.4 (NC(O)C(CH\textsubscript{3})\textsubscript{3}), 27.7 (OC(O)C(CH\textsubscript{3})\textsubscript{3}), 25.8 (SiC(CH\textsubscript{3})\textsubscript{3}), 18.1 (SiC(CH\textsubscript{3})\textsubscript{3}), –4.6 (Si(CH\textsubscript{3})\textsubscript{2}). No C\textsubscript{Ar}I peak observed.

LRMS  (ES+) m/z 782 [M + Na]\textsuperscript{+}.

HRMS  (ES+) calcd for C\textsubscript{34}H\textsubscript{54}INO\textsubscript{8}SiNa [M + Na]\textsuperscript{+} 782.2556, found 782.2555.

2-(4-Formyl-2-hydroxyphenoxy) ethyl tert-butyl carbonate (5.35)

\[
\text{C}_{14}\text{H}_{18}\text{O}_6 \\
\text{Mw = 282.29 gmol}^{-1} \\
\text{White solid}
\]

Following the procedure of Nicolaou et al.,\textsuperscript{137} NaH (4.54 g, 113.5 mmol, 60% dispersion in mineral oil) was added portionwise to a solution of 3,4-dihydroxybenzaldehyde (5.26, 15.68 g, 113.5 mmol) in DMF (80 mL) at 0 °C. The solution was stirred for 15 min before a solution of bromide 5.27 (25.55 g, 113.5 mmol) in DMF (60 mL) was added. The ice bath was removed and the solution was warmed at 40 °C for 5 days. Water was added to the cooled reaction mixture and the pH was adjusted to pH 5 using 2 N HCl. The solution was extracted with Et\textsubscript{2}O (3 x 200 mL). The combined organic layers were dried over MgSO\textsubscript{4} and concentrated in vacuo. The residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a white solid (17.11 g, 60.6 mmol, 53%).
MP \quad 93-98 ^\circ \text{C}.

IR \quad \nu_{\text{max}} \text{ (neat)} \quad 3415 \text{ (br)}, \quad 2980 \text{ (w)}, \quad 2937 \text{ (w)}, \quad 1738 \text{ (m)}, \quad 1685 \text{ (m)}, \quad 1609 \text{ (m)}, \quad 1587 \text{ (m)}, \quad 1508 \text{ (m)}, \quad 1271 \text{ (s)}, \quad 1253 \text{ (s)}, \quad 1157 \text{ (s)} \text{ cm}^{-1}.

^{1}H \text{ NMR} \quad (300 \text{ MHz, CDCl}_3) \quad \delta \quad 9.85 \text{ (1H, s, C(O)H)}, \quad 7.45 \text{ (1H, d, } J = 1.9 \text{ Hz, C}_p\text{H ortho to OH)}, \quad 7.41 \text{ (1H, dd, } J = 8.2, 1.9 \text{ Hz, C}_p\text{H ortho to C(O)H)}, \quad 6.94, \quad (1H, d, J = 8.2 \text{ Hz, C}_p\text{H, orth to OCH}_2), \quad 6.00 \text{ (1H, s, OH), 4.55-4.48 \text{ (2H, m, C}_p\text{OCH}_2\text{CH}_2), 4.38-4.31 \text{ (2H, m, C}_p\text{OCH}_2), 1.51 \text{ (9H, s, C(CH}_3)_3}).

^{13}C \text{ NMR} \quad (75 \text{ MHz, CDCl}_3) \quad \delta \quad 190.9 \text{ (C(O)H)}, \quad 153.4 \text{ (OC(O)O)}, \quad 150.6 \text{ (C}_p\text{OCH}_2), \quad 146.5 \text{ (C}_p\text{OH), 131.2 \text{ (C}_p\text{C(O)H), 124.0 \text{ (C}_p\text{H ortho to C(O)H), 114.7 \text{ (C}_p\text{H ortho to OH), 111.2 \text{ (C}_p\text{H ortho to OCH}_2), 83.1 \text{ (C(CH}_3)_3), 67.6 \text{ (C}_p\text{OCH}_2\text{CH}_2), 64.4 \text{ (C}_p\text{OCH}_2), 27.7 \text{ (C(CH}_3)_3)).}

LRMS \quad (ES+) \text{ m/z 305 [M + Na]^+}.

HRMS \quad (ES+) \text{ calcd for C}_{14}H_{18}O_{6}Na \text{ [M + Na]^+ 305.0996, found 305.0999}.

2-(4-Formyl-2-hydroxy-3-iodophenoxy) ethyl tert-butyl carbonate (5.36) 

\[ \text{C}_{14}H_{17}IO_{6} \]

\[ \text{M}_{w} = 408.19 \text{ gmol}^{-1} \]

Pale yellow solid

Following the procedure of Markovich et al.,\textsuperscript{138} alcohol 5.35 (9.60 g, 34.0 mmol) was dissolved in pyridine (20 mL) and cooled to 0 \textdegree C before a solution of ICl (1.80 mL, 35.7 mmol) in dioxane (35 mL) was added. The solution was stirred for 1 h before the ice bath was removed and the solution was allowed to warm to rt and stirred for 5 days.
The solvent was removed in vacuo and water (50 mL) was added. The aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with 5% aq Na₂S₂O₃ (2 x 100 mL), water (2 x 100 mL) and brine (1 x 100 mL), dried over MgSO₄ and concentrated in vacuo. The residue was recrystallised from EtOAc to give the title compound as a pale yellow solid (6.53g, 16.0 mmol, 47%). The filtrate from the recrystallisation was purified by column chromatography (eluent 10% EtOAc/hexane) to give further product (557 mg, 1.4 mmol, 4%).

**MP** 155-156 °C.

**IR** $\nu_{\text{max}}$ (neat) 3415 (br), 2979 (w), 2935 (w), 1740 (m), 1583 (m), 1277 (s), 1255 (m), 1158 (m) cm$^{-1}$.

**$^1$H NMR** (300 MHz, CDCl$_3$) $\delta$ 10.05 (1H, s, C(O)H), 7.35 (1H, d, $J = 8.5$ Hz, C$_{Ar}$H ortho to C(O)H), 6.90, (1H, d, $J = 8.5$ Hz, C$_{Ar}$H, ortho to OCH$_2$), 6.60 (1H, s, OH), 4.55-4.48 (2H, m, C$_{Ar}$OCH$_2$C$_H$), 4.39-4.32 (2H, m, C$_{Ar}$OCH$_2$C$_H$), 1.51 (9H, s, C(C$_H$)$_3$).

**$^{13}$C NMR** (75 MHz, CDCl$_3$) $\delta$ 194.8 (C(O)H), 153.6 (OC(O)O), 149.4 (C$_{Ar}$OCH$_2$), 146.2 (C$_{Ar}$OH), 129.3 (C$_{Ar}$C(O)H), 123.4 (C$_{Ar}$H ortho to C(O)H), 111.0 (C$_{Ar}$H ortho to OCH$_2$), 88.1 (C$_{Ar}$I), 83.2 (C(CH$_3$)$_3$), 68.1 (C$_{Ar}$OCH$_2$CH$_2$), 64.3 (C$_{Ar}$OCH$_2$), 27.7 (C(CH$_3$)$_3$).


**HRMS** (ES+) calcd for C$_{14}$H$_{17}$IO$_6$Na [M + Na]$^+$ 430.9962, found 430.9969.

3-(Trimethylsilyl) propioaldehyde (5.38)

![Diagram](image)

C$_6$H$_{10}$OSi  
Mw = 126.23 g mol$^{-1}$  
Yellow oil
Following the procedure of Journet et al., TMS acetylene (5.37, 20.50 mL, 145.0 mmol) was dissolved in Et₂O (200 mL) and cooled to −40 °C before n-BuLi (58 mL, 2.5 M solution in hexanes, 145.0 mmol) was added dropwise. The solution was stirred for 5 min before DMF (21 mL, 261.0 mmol) was added. The cold bath was removed and the reaction was stirred vigorously for 30 min. The reaction mixture was quenched by pouring into a mixture of 10% aq NaH₂PO₄ (70.0 g, 580.0 mmol) and Et₂O (150 mL). The mixture was stirred rapidly for 10 min over which time the aqueous layer turned bright yellow. The layers were separated and the organic layer was washed with water (100 mL). The combined aqueous layers were extracted with Et₂O (2 x 50 mL). The combined organic layers were dried over MgSO₄ and the bulk of the solvent was removed by distillation (50 °C, atm pressure). The remaining solvent was removed by distillation (40 °C, 25 mm/Hg) to give the title compound as a yellow free flowing oil (14.91 g, 118.1 mmol, 81%) which was used in subsequent reactions without further purification. Spectroscopic data were in agreement with those published in the literature.

**IR**

ν_{max} (neat) 2964 (w), 2859 (w), 2154 (w), 1666 (s), 1388 (w), 1252 (m), 995 (s), 839 (s), 760 (m) cm⁻¹.

**¹H NMR**

(300 MHz, CDCl₃) δ 9.17 (1H, s, C(O)H), 0.27 (9H, s, Si(CH₃)₃).

**¹³C NMR**

(75 MHz, CDCl₃) δ 176.7 (C(O)H), 103.0 (SiC≡C), 102.2 (SiC≡C), −0.9 (Si(CH₃)₃).

**LRMS**

(EI) 125 ([M – H]⁺, 3%), 111 ([M – CH₃]⁺, 100%).

(R)-(+) -1-(Trimethylsilyl) hex-5-en-1-yn-3-acetate ((+)-5.39)

![Chemical Structure](image)

C_{11}H_{18}O_2Si  
Mw = 210.34 gmol⁻¹  
Colourless oil
Method 1: Following the procedure of Burova et al.,\textsuperscript{139} to a solution of racemic alcohol (\(\pm\))-5.29 (5.54 g, 32.9 mmol) in hexane (275 mL) was added 4 Å molecular sieves (10 g), vinyl acetate (11.60 mL, 126.5 mmol) and Amano® AK20 Lipase enzyme (6.50 g). The suspension was stirred at rt for 23 h before it was filtered through Celite® and the pad was washed with Et₂O (2 x 100 mL). The filtrate was concentrated \textit{in vacuo} and the residue was purified by column chromatography (eluent 9-20\% Et₂O/hexane) to give the title compound as a colourless oil (3.25 g, 15.4 mmol, 47\%) and alcohol (\(-\))-5.29 as a pale yellow oil (2.08 g, 12.4 mmol, 38\%).

\((+)-5.39 [\alpha]_D : +91.7 (c 1.10, CHCl₃, 24 °C) (lit: +86, c 0.970, rt, 99\% ee).\textsuperscript{139}

\((-)-5.29 [\alpha]_D : -41.4 (c 1.15, CHCl₃, 24 °C) (lit: -29, c 0.850, rt, 98\% ee).\textsuperscript{139}

Method 2: Following the procedure of Jin \textit{et al.},\textsuperscript{142} a solution of alcohol (\(-\))-5.29 (3.77 g, 22.4 mmol), PPh₃ (23.49 g, 89.6 mmol), pyridine (3.62 mL, 44.8 mmol) and acetic acid (6.4 mL, 112.0 mmol) in THF (140 mL) at \(-50 °C\) was added DIAD (17.63 mL, 89.6 mmol). The solution was stirred for 10 min at \(-50 °C\) and then was warmed to 0 °C and stirred for 22 h, during which time it was allowed to warm to rt. The reaction mixture was concentrated \textit{in vacuo} and the residue was dissolved in Et₂O (200 mL) and was washed with sat aq NaHCO₃ (1 x 200 mL), 2 N HCl (1 x 150 mL) and brine (1 x 150 mL), dried over MgSO₄ and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 5-10\% EtOAc/hexane) to give the title compound as a colourless oil (3.58 g, 17.0 mmol, 76\%).

\((+)-5.39 [\alpha]_D : +99.9 (c 1.00, CHCl₃, 24 °C) (lit: +86, c 0.970, rt, 99\% ee).\textsuperscript{139}

Spectroscopic data were in agreement with those published in the literature.\textsuperscript{139}

\textbf{IR} \quad \nu_{\text{max}} \, (\text{neat}) \, 3080 \, (w), \, 2960 \, (w), \, 2902 \, (w), \, 1745 \, (s), \, 1371 \, (w), \, 1227 \, (s), \, 1021 \, (m), \, 841 \, (s), \, 760 \, (m) \, \text{cm}^{-1}.

\textbf{1H NMR} \quad (400 MHz, CDCl₃) \delta \, 5.82 \, (1H, ddt, J = 17.2, 10.2, 6.6 Hz, CH=CH₂), \, 5.43 \, (1H, t, J = 6.6 Hz, CH(OC(O)CH₃)), \, 5.22-5.08 \, (2H, m, CH=CH₂),
2.52 (2H, t, $J = 6.6$ Hz, CH$_2$), 2.08 (3H, s, C(O)CH$_3$), 0.18 (9H, s, Si(CH$_3$)$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.7 (C(O)CH$_3$), 132.2 (CH=CH$_2$), 118.6 (CH=CH$_2$), 102.1 (Si=C=C), 90.9 (Si=C=C), 63.6 (CH(OH)), 39.4 (CH$_2$), 21.0 (C(O)CH$_3$), −0.2 (Si(CH$_3$)$_3$).

LRMS (EI) 169 ([M – C$_3$H$_5$]$^+$, 82%), 43 ([M – C$_2$H$_3$]$^+$, 100%).

General Procedure E for the Preparation of Mosher Esters of Alcohols

$(R)$-$(+)$-1-Trimethylsilyl hex-5-en-1-yn-3-ol ($(+)\text{-}5.29$) and $(S)$-$(−)$-1-Trimethylsilyl hex-5-en-1-yn-3-ol ($(−)\text{-}5.29$)

Following the procedure of Fos et al.,$^{145}$ $(R)$-$(+)$-α-methoxy-α-trifluoromethyl phenyl acetic acid ($(R)$-$(+)$-MTPA, 500 mg, 2.14 mmol) was dissolved in SOCl$_2$ (20 mL) and heated to reflux for 4 h. The excess SOCl$_2$ was removed in vacuo to give the Mosher acid chloride ($(S)$-$(+)$-MTPA chloride) as an orange liquid which was used without further purification. To a solution of the appropriate alcohol (50 mg, 0.30 mmol) in CH$_2$Cl$_2$ (1 mL) was added $(S)$-$(+)$-MTPA chloride (150 mg, 0.60 mmol) and pyridine (3 drops). The solution was stirred at room temperature for 27 h and then heated to 40 °C for a further 3 h (total reaction time 30 h). The cooled reaction mixture was diluted with CH$_2$Cl$_2$ (5 mL), washed with 0.1 N HCl (10 mL), dried over MgSO$_4$ and concentrated in vacuo. The resulting orange liquid was purified by column chromatography (eluent 5% EtOAc/hexane) to give the Mosher ester as a colourless liquid.

IR (neat) 3078 (w), 2969 (w), 2850 (w), 1753 (s), 1231 (m), 1169 (s), 844 (s) cm$^{-1}$


(3R)-(2'R)-(+)1-(Trimethylsilyl) hex-5-en-1-yn-3-yl 3',3',3'-trifluoro-2'-methoxy-2'-phenylpropanoate ((+)-5.40)

C_{19}H_{23}F_3O_3Si  
Mw = 384.46 gmol^{-1}

Colourless oil

Alcohol (+)-5.29 was used in general procedure E to give the title compound (73 mg, 0.19 mmol, 63%).

\[ \alpha \]D +51.0 (c 0.98, CHCl₃, 24 °C)

$^1$H NMR (300 MHz, CDCl₃) δ 7.60-7.54 (2H, m, C₆H), 7.44-7.36 (3H, m, C₆H), 5.72 (1H, ddt, $J = 16.8, 9.7, 6.8$ Hz, CH=CH₂), 5.62 (1H, t, $J = 6.8$ Hz, CH(O)), 5.17-5.00 (2H, m, CH=CH₂), 3.61 (3H, s, OCH₃), 2.57 (2H, t, $J = 6.8$ Hz, CH₂), 0.20 (9H, s, Si(CH₃)₃).

$^{13}$C NMR (75 MHz, CDCl₃) δ 165.5 (C(O)O), 138.9 (C₆H), 132.3 (C₆H), 131.4 (CH=CH₂), 129.6 (CF₃), 128.9 (C₆H para to alkyl group), 128.4 (C₆H ortho to alkyl group), 127.4 (C₆H meta to alkyl group), 119.2 (CH=CH₂), 100.6 (SiC=C), 92.3 (SiC=C), 65.6 (CH(O)), 55.5 (OCH₃), 38.9 (CH₂), −0.4 (Si(CH₃)₃).

$^{19}$F NMR (282 MHz, CDCl₃) δ −72.4 (CF₃).
(3S)-(2'R)-(−)-1-(Trimethylsilyl) hex-5-en-1-yn-3-yl 3',3',3'-trifluoro-2’-methoxy-2'-phenylpropanoate ((−)-5.40)

\[
\begin{align*}
\text{C}_{19}\text{H}_{23}\text{F}_{3}\text{O}_{3}\text{Si} \\
\text{Mw} &= 384.46 \text{ g mol}^{-1} \\
\text{Colourless oil}
\end{align*}
\]

Alcohol (−)-5.29 was used in general procedure E to give the title compound (70 mg, 0.18 mmol, 61%).

\[\alpha\] _D \quad -5.1 (c 0.97, CHCl₃, 24 °C)

\[\text{^1H NMR}\]
(300 MHz, CDCl₃) \(\delta\) 7.59-7.50 (2H, m, C₂ArH), 7.44-7.37 (3H, m, C₂ArH), 5.81 (1H, ddt, \(J = 16.9, 10.2, 6.8\) Hz, CH=CH₂), 5.58 (1H, t, \(J = 6.8\) Hz, CH(O)), 5.29-5.06 (2H, m, CH=CH₂), 3.57 (3H, s, OCH₃), 2.62 (2H, t, \(J = 6.8\) Hz, CH₂), 0.19 (9H, s, Si(CH₃)₃).

\[\text{^13C NMR}\]
(75 MHz, CDCl₃) \(\delta\) 165.5 (C(O)O), 139.8 (C₂ArC), 132.0 (C₂ArC), 131.8 (CH=CH₂), 129.6 (CF₃), 128.9 (C₂Ar para to alkyl group), 128.3 (C₂Ar ortho to alkyl group), 127.5 (C₂Ar meta to alkyl group), 119.2 (CH=CH₂), 100.4 (SiC≡C), 92.1 (SiC≡C), 65.9 (CH(O)), 55.6 (OCH₃), 38.9 (CH₂), −0.4 (Si(CH₃)₃).

\[\text{^19F NMR}\]
(282 MHz, CDCl₃) \(\delta\) −73.1 (CF₃).
(S)-(+-)-tert-Butyl-3-(1-(trimethylsilyl) hex-5-en-1-yn-3-yloxy)-2-iodo-(4-ethyl tert-butyl carbonate) benzylmethylcarbamate ((+-)-5.41)

C_{29}H_{44}INO_{7}Si
Mw = 673.65 gmol

Pale pink oil

To a solution of alcohol (+)-5.29 (2.37 g, 14.1 mmol) in THF (60 mL) was added a solution of phenol 5.28 (7.74 g, 14.8 mmol) and PPh\(_3\) (7.39 g, 28.2 mmol) in THF (40 mL). The solution was stirred for 5 min before DIAD (5.54 mL, 28.2 mmol) was added dropwise to give a dark orange solution. The solution was heated to 55 ºC for 6 h before being cooled to rt and concentrated in vacuo. The resulting orange oil was purified by column chromatography (eluent 10% EtOAc/hexane) to give the title compound as a pale pink oil (9.20 g, 13.7 mmol, 97%).

\[\alpha\]D +7.7 (c 1.04, CHCl\(_3\), 23 ºC).

IR \(\nu_{\text{max}}\) (neat) 3081 (w), 2976 (w), 2932 (w), 1742 (m), 1476 (m), 1367 (m), 1274 (m), 1250 (s), 1154 (m), 843 (m) cm\(^{-1}\).

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.13-6.47 (2H, m, 2 x C\(_A\)H), 6.05 (1H, ddt, \(J = 17.1, 10.2, 6.8\) Hz, CH=CH\(_2\)), 5.30-5.03 (3H, m, CHOC\(_A\) & CH=CH\(_2\)), 4.72-4.31 (4H, m, CH\(_2\)N & C\(_A\)OCH\(_2\)CH\(_2\)), 4.20 (2H, t, \(J = 4.6\) Hz, C\(_A\)OCH\(_2\)), 3.14-2.56 (5H, m, CH\(_2\)CHOC\(_A\) & NCH\(_2\)), 1.55-1.35 (18H, m, 2 x C(O)OC(CH\(_3\))\(_3\)), 0.04 (9H, s, Si(CH\(_3\))\(_3\)). Spectrum exhibited broadening of peaks due to restricted rotation.

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 155.9 (NC(O)O), 153.3 (OC(O)O), 150.0 (C\(_A\)OCH\(_2\)), 146.7 (C\(_A\)OCH), 133.5 (CH=CH\(_2\)), 133.3 (C\(_A\)CH\(_2\)N), 123.0 & 122.1 (C\(_A\)H ortho to OCH\(_2\)), 117.8 (CH=CH\(_2\)), 113.7 (C\(_A\)H

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ortho to CH$_2$N), 103.1 (SiC=), 92.7 (SiC=), 82.4 (OC(O)C(CH$_3$)$_3$), 79.7 (NC(O)OC(CH$_3$)$_3$), 72.2 & 71.6 (CHOCAr), 67.0 (CArOCH$_2$CH$_2$), 64.9 (CArOCH$_2$), 57.7 (NCH$_2$), 40.4 (CH$_2$CHOCAr), 34.1 (NCH$_3$), 28.4 (NC(O)C(CH$_3$)$_3$), 27.7 (OC(O)C(CH$_3$)$_3$), –0.4 (Si(CH$_3$)$_3$). No C$_h$I peak observed.

LRMS (ES+) $m/z$ 696 [M + Na]$^+$.  

HRMS (ES+) calcd for C$_{29}$H$_{44}$INO$_7$SiNa [M + Na]$^+ 696.1824$, found 696.1808.

(3S,5S)- and (3S,5R)-tert-Butyl-3-(5,6-dihydroxy-1-(trimethylsilyl) hex-1-yn-3-yl-oxo)-2-iodo-(4-ethyl tert-butyl carbonate) benzylmethylcarbamate (5.42)

Following the procedure of Dupau et al.,$^{146}$ alkene (+)-5.41 (8.05 g, 12.0 mmol) was dissolved in $t$-BuOH/water (1:1, 16 mL) and treated with citric acid (2.76 g, 14.3 mmol). The solution was stirred for 5 min before Os$_4$ (2.3 mL, 2.5 wt% sol in $t$-BuOH, 0.18 mmol) and NMO (1.68 g, 14.4 mmol) were added. The reaction was stirred at rt for 26 h. The reaction was quenched by the addition of sodium dithionite (4.0 g, 23.0 mmol) and the reaction was stirred for 20 min. The reaction mixture was added to EtOAc (50 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 50 mL) and CH$_2$Cl$_2$ (6 x 50 mL). The separate organic layers were washed with water (1 x 10 mL) and brine (1 x 10 mL), dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography (elucent 15-50% EtOAc/hexane) to give the title compound as a colourless oil (5.55 g, 7.8 mmol, 65%) and recovered alkene (+)-5.41 (989 mg, 1.4 mmol, 12%). The (3S,5S) and (3S,5R) epimers were formed in a 1:1 ratio (1H NMR).
IR $\nu_{\text{max}}$ (neat) 3446 (br w), 2975 (w), 2930 (w), 1742 (m), 1696 (m), 1476 (m), 1393 (m), 1275 (m), 1251 (s), 1154 (m), 844 (m) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.06-6.67 (2H, m, 2 x C$_{Ar}$H), 5.47 (1H, br s, CHOC$_{Ar}$), 4.59-4.36 (4H, m, CH$_2$N & C$_{Ar}$OCH$_2$CH$_2$), 4.35-4.27 (1H, m, OH), 4.26-4.13 (2H, m, C$_{Ar}$OCH$_2$), 3.81-3.68 (1H, m, CHHOH), 3.68-3.52 (1H, m, CHHOH), 3.34 (0.5 H, d, $J = 3.8$ Hz, CHO), 3.13 (0.5H, d, $J = 3.8$ Hz, CHOH), 2.95-2.70 (3H, m, NCH$_3$), 2.43-2.05 (3H, m, CH$_2$CHOCH$_2$Ar & OH), 1.60-1.35 (18H, m, 2 x C(O)OC(CH$_3$)$_3$), 0.03 (9H, s, Si(CH$_3$)$_3$). Spectrum exhibited broadening of peaks due to restricted rotation.

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.9 (NC(O)O), 153.2 (OC(O)O), 149.4 (C$_{Ar}$OCH$_2$), 146.0 (C$_{Ar}$OCH), 133.3 (C$_{Ar}$CH$_2$N), 122.3 (C$_{Ar}$H ortho to OCH$_2$), 113.2 (C$_{Ar}$H ortho to CH$_2$N), 102.8 (SiC≡C), 93.4 (SiC≡C), 82.9 (OC(O)C(CH$_3$)$_3$), 79.8 (NC(O)OC(CH$_3$)$_3$), 70.2 & 69.9 (CHOH), 69.5 (CHOCH$_2$), 68.7 (C$_{Ar}$OCH$_2$CH$_2$), 66.9 & 66.5 (CH$_2$OH), 64.8 (C$_{Ar}$OCH$_2$), 57.6 (NCH$_3$), 39.4 & 39.3 (CH$_2$CHOCH$_2$), 34.1 (NCH$_3$), 28.4 (NC(O)C(CH$_3$)$_3$), 27.7 (OC(O)C(CH$_3$)$_3$), −0.4 (Si(CH$_3$)$_3$). No C$_{Ar}$I peak observed.

LRMS (ES+) $m/z$ 730 [M + Na]$^+$. 

HRMS (ES+) calcd for C$_{29}$H$_{46}$INO$_9$SiNa [M + Na]$^+$ 730.1879, found 730.1885.
(3S,5S)-(+)\textit{-}tert-Butyl-3-(5-hydroxyoct-7-en-1-yn-3-yloxy)-2-iodo-4-(ethyl \textit{\textit{tert}-butyl carbonate)} benzylmethylcarbamate ((+)\textit{-}5.44)

\[
\begin{align*}
\text{C}_{28}\text{H}_{40}\text{INO}_{8} \\
\text{Mw} = 645.52 \text{ gmol}^{-1}
\end{align*}
\]

Orange oil

Following the procedure of Marshall \textit{et al.},\textsuperscript{159} protected alkyne ((+)\textit{-}5.31 (4.11 g, 5.7 mmol) was dissolved in THF (20 mL) and cooled to 0 °C before TBAF (11.40 mL, 1.0 M sol in THF, 11.4 mmol) was added dropwise turning the colourless solution a dark red colour. The ice bath was removed and the solution was stirred for 30 min. The reaction was re-cooled to 0 °C and ice water (20 mL) was added. The aqueous layer was extracted with Et\textsubscript{2}O (3 x 50 mL). The combined organic layers were washed with water (1 x 50 mL) and brine (1 x 50 mL), dried over MgSO\textsubscript{4} and concentrated \textit{in vacuo}. The residue was purified by column chromatography (eluent 30% EtOAc/hexane) to give the title compound as an orange oil (3.34 g, 5.2 mmol, 91%).

\[\left[\alpha\right]_{D}^{\circ} +2.7 \,(c \,1.00, \text{CHCl}_{3}, 23 ^{\circ}\text{C}).\]

\textbf{IR} \quad \nu_{\text{max}} \,(\text{neat}) \,3452 \,(\text{br w}), \,3290 \,(w), \,2977 \,(w), \,2930 \,(w), \,1741 \,(m), \,1690 \,(m), \,1476 \,(m), \,1392 \,(m), \,1274 \,(s), \,1253 \,(s), \,1154 \,(s) \,\text{cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} \quad (300 \text{ MHz, CDCl}_{3}) \,\delta \,7.08-6.61 \,(2\text{H, m, 2} \times \text{C}_{\text{Ar}}\text{H}), \,5.90 \,(1\text{H, ddt, } J = 17.2, 10.1, 7.0 \text{ Hz, CH=CH}_{2}), \,5.50 \,(1\text{H, td, } J = 6.9, 2.1 \text{ Hz, CHOC}_{\text{Ar}}), \,5.25-4.99 \,(2\text{H, m, CH=CH}_{2}), \,4.63-4.31 \,(4\text{H, m, CH}_{2}\text{N} \text{& CH}_{2}\text{OCH}_{2}\text{CH}_{2}), \,4.22 \,(2\text{H, t, } J = 4.5 \text{ Hz, CH}_{3}\text{OCH}_{2}), \,4.17-4.03 \,(1\text{H, m, CHOH}), \,2.85 \,(3\text{H, br s, NCH}_{3}), \,2.68 \,(1\text{H, d, } J = 4.0 \text{ Hz, OH}), \,2.41 \,(1\text{H, d, } J = 2.1 \text{ Hz, C=CH}), \,2.35 \,(2\text{H, t, } J = 7.0 \text{ Hz, CH}_{2}\text{CH=CH}_{2}), \,2.27-2.08 \,(2\text{H, m, CH}_{2}\text{CHOCA}_{\text{Ar}}), \,1.65-1.29 \,(18\text{H, m, 2} \times \text{C(O)OC(CH}_{3})_{3}).

Spectrum exhibited broadening of peaks due to restricted rotation.
**13C NMR**

(75 MHz, CDCl₃) δ 155.9 (NC(O)O), 153.2 (OC(O)O), 149.7 (C₆H₅OCH₂), 146.0 (C₆H₅OCH), 134.6 (CH=CH₂), 133.6 (C₆H₅CH₂N), 123.0 & 122.0 (C₆H₅ ortho to OCH₂), 117.7 (CH=CH₂), 113.4 (C₆H₅ ortho to CH₂N), 82.8 (OC(O)C(CH₃)₃), 81.5 (C≡CH), 79.8 (NC(O)OC(CH₃)₃), 76.1 (C≡CH), 70.3 (CHOH), 68.6 (CHOC₆H₅), 66.9 (C₆H₅OCH₂CH₂), 64.9 (C₆H₅OCH₂), 57.6 & 56.7 (NCH₂), 42.7 (CH₃CH=CH₂), 42.0 (CH₂CHOCAr), 34.3 (NCH₃), 28.4 (NC(O)C(CH₃)₃), 27.7 (OC(O)C(CH₃)₃). No C₆H₅I peak observed.

**LRMS**

(ES+) m/z 668 [M + Na]⁺.

**HRMS**

(ES+) calcd for C₂₈H₄₀INO₈Na [M + Na]⁺ 668.1691, found 668.1686.

**(3S,5S)-(−)-tert-Butyl-3-(5-[(tert-butyldimethylsilyl)oxy]-7-en-1-yn-3-yloxy)-2-iodo-4-(ethyl tert-butyl carbonate)benzylmethylcarbamate ((−)-5.46)**

\[
\begin{align*}
\text{(−)-5.46} & \\
\text{C}_{34}\text{H}_{54}\text{INO}_8\text{Si} \\
\text{Mw = 759.78 gmol}^{-1} \\
\text{Pale yellow oil}
\end{align*}
\]

Alcohol (+)-5.44 (2.33 g, 3.6 mmol) was dissolved in CH₂Cl₂ (10 mL), treated with 2,6-lutidiene (0.84 mmol, 7.2 mmol) and cooled to −78 °C. TBSOTf (0.87 mL, 3.8 mmol) was added dropwise and the resulting solution was stirred at −78 °C for 5 min before it was warmed to 0 °C and stirred for 20 min. The reaction was quenched by the addition of a sat aq NH₄Cl (50 mL). The reaction mixture was extracted with Et₂O (3 x 50 mL). The combined organic layers were washed with NaHCO₃ (1 x 75 mL) and brine (1 x 75 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 10-20% EtOAc/hexane) to give the title compound as a pale yellow oil (2.49 g, 3.3 mmol, 92%).
$[\alpha]_D$  

–4.4 (c 0.98, CHCl$_3$, 23 °C).

IR  

$\nu_{\text{max}}$ (neat) 3290 (br w), 2929 (w), 2856 (w), 1742 (m), 1694 (m), 1474 (m), 1391 (m), 1273 (s), 1252 (s), 1154 (s), 836 (m) cm$^{-1}$.

$^1$H NMR  

(300 MHz, CDCl$_3$) $\delta$ 6.98-6.65 (2H, m, 2 x C$_{Ar}$H), 5.87 (1H, ddt, $J =$ 17.1, 10.1, 7.1 Hz, CH=CH$_2$), 5.25 (1H, br t, $J =$ 6.5 Hz, CHOC$_{Ar}$), 5.17-4.98 (2H, m, CH=CH$_2$), 4.61-4.31 (4H, m, CH$_2$N & C$_{Ar}$OCH$_2$CH$_2$), 4.21 (2H, t, $J =$ 4.8 Hz, C$_{Ar}$OCH$_2$), 4.14-4.02 (1H, m, CHOTBS), 3.06-2.61 (3H, br m, NCH$_3$), 2.51-2.19 (4H, m, C=CH, CH$_2$CHOC$_{Ar}$ & CHHCH=CH$_2$), 2.16-2.04 (1H, m, CHHCH=CH$_2$), 1.65-1.29 (18H, m, 2 x C(O)OC(CH$_3$)$_3$), 0.88 (9H, s, Si(C(CH$_3$)$_3$)), 0.09 (6H, s, Si(CH$_3$)$_2$). Spectrum exhibited broadening of peaks due to restricted rotation.

$^{13}$C NMR  

(75 MHz, CDCl$_3$) $\delta$ 155.9 (NC(O)O), 153.3 (OC(O)O), 150.1 (C$_{Ar}$OCH$_2$), 146.6 (C$_{Ar}$OCH), 134.5 (CH=CH$_2$), 133.6 (C$_{Ar}$CH$_2$N), 123.0 & 122.5 (C$_{Ar}$H ortho to OCH$_2$), 117.4 (CH=CH$_2$), 114.2 & 114.0 (C$_{Ar}$H ortho to CH$_2$N), 82.4 (OC(O)C(CH$_3$)$_3$), 81.7 (C=CH), 79.7 (NC(O)OC(CH$_3$)$_3$), 75.9 (C=CH), 70.2 (CHOTBS), 68.8 (CHOC$_{Ar}$), 67.0 (C$_{Ar}$OCH$_2$CH$_2$), 64.8 (C$_{Ar}$OCH$_2$), 57.6 & 56.8 (NCH$_2$), 42.9 (CH$_2$CH=CH$_2$), 41.9 (CH$_2$CHOC$_{Ar}$), 34.3 (NCH$_3$), 28.4 (NC(O)C(CH$_3$)$_3$), 27.7 (OC(O)C(CH$_3$)$_3$), 25.9 (SiC(CH$_3$)$_3$), 18.0 (SiC(CH$_3$)$_3$), –4.3 & –4.6 (2 x SiCH$_3$). No C$_{Ar}$I peak observed.

LRMS  

(ES+) m/z 782 [M + Na]$^+$.  

HRMS  

(ES+) calcd for C$_{34}$H$_{54}$INO$_8$SiNa [M + Na]$^+$ 782.2556, found 782.2550.
(1S,5S)-(--)-**tert-**-Butyl-3-(5-[(**tert**-butyldimethylsilyl)oxy]-2-(2-hydroxyethyl)cyclohex-2-en-1-yloxy)-2-iodo-4-(ethyl **tert**-butyl carbonate) benzylmethylcarbamate ((--)-5.47)

Diene ((--)-5.32 (1.51 g, 2.0 mmol) was treated with 9-BBN (12.0 mL, 0.5 M sol in THF, 6.0 mmol) and stirred at rt for 42 h. 2 N NaOH (10 mL) was added followed cautiously by H₂O₂ (4 mL, 30% sol in H₂O) over 15 min. Stirring continued for 5 min before the layers were partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O (2 x 50 mL). The combined organic layers were washed with brine (1 x 75 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 25% EtOAc/hexane) to give the title compound as a colourless oil (1.19 g, 1.5 mmol, 75%).

$$\alpha_D$$ –9.0 (c 1.27, CHCl₃, 24 °C).

**IR**  
\[\nu_{ \text{max} } (\text{neat}) \] 3468 (br w), 2929 (w), 2857 (w), 1743 (m), 1686 (m), 1474 (m), 1391 (m), 1274 (s), 1251 (s), 1153 (s), 1103 (s), 836 (s), 730 (s) cm⁻¹.

**¹H NMR**  
(300 MHz, CDCl₃) \(\delta\) 6.87 (1H, d, \(J = 8.4\ \text{Hz}\), C\text{Ar}\text{H }\text{ortho} \text{to OCH}_2), 6.84-6.75 (1H, m, C\text{Ar}\text{H }\text{ortho} \text{to CH}_2N), 5.55 (1H, br d, \(J = 4.6\ \text{Hz}\), CHOC\text{Ar}), 5.41 (1H, br m, C=CH), 4.68-4.29 (4H, m, CH\text{2N }\text{& }C\text{ArOCH}_2CH\text{2}), 4.28-4.11 (2H, m, C\text{ArOCH}_2), 3.99-3.62 (3H, m, CHOTBS \& CH\text{2OH}), 2.99-2.76 (3H, br m, NCH\text{3}), 2.76-2.60 (1H, m, CHHCH\text{2OH}), 2.50 (1H, ddd, \(J = 14.0, 6.9, 6.8\ \text{Hz}\), CHHCH\text{2OH}), 2.37-2.20 (1H, m, CH\text{HCHOC\text{Ar}}), 2.14 (1H, br d, \(J = 9.7\ \text{Hz}\), CHH\text{CHOC\text{Ar}}),
2.04 (1H, br t, \( J = 5.3 \) Hz, \( \text{OH} \)), 1.88 (2H, dd, \( J = 10.1, \) 8.4 Hz, C=CHCH\(_2\)), 1.65-1.30 (18H, m, 2 x C(O)OC(CH\(_3\))\(_3\)), 0.82 (9H, s, Si(C(CH\(_3\)))\(_3\)), 0.00 (3H, s, SiCH\(_3\)), −0.02 (3H, s, SiCH\(_3\)). Spectrum exhibited broadening of peaks due to restricted rotation.

\( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)) \( \delta \) 155.8 (NC(O)O), 153.2 (OC(O)O), 149.5 (C\(_{Ar}\)OCH\(_2\)), 145.6 (C\(_{Ar}\)OCH), 136.0 (C=CH), 133.8 (C\(_{Ar}\)CH\(_2\)N), 123.9 (C=CH), 122.3 & 121.7 (C\(_{Ar}\)H ortho to OCH\(_2\)), 113.3 (C\(_{Ar}\)H ortho to CH\(_2\)N), 82.7 (OC(O)C(CH\(_3\))\(_3\)), 79.7 (NC(O)OC(CH\(_3\))\(_3\)), 77.7 (CHOTBS), 67.3 (CHOC\(_{Ar}\)), 66.8 (C\(_{Ar}\)OCH\(_2\)CH\(_2\)), 64.9 (C\(_{Ar}\)OCH\(_2\)), 61.6 (CH\(_2\)OH), 57.8 & 56.9 (NCH\(_2\)), 38.9 (C\(_{Ar}\)OCHCH\(_2\)), 36.1 (CH\(_2\)CH\(_2\)OH), 35.9 (C=CHCH\(_2\)), 34.1 (NCH\(_3\)), 28.4 (NC(O)C(CH\(_3\))\(_3\)), 27.7 (OC(O)C(CH\(_3\))\(_3\)), 25.8 (SiC(CH\(_3\))\(_3\)), 18.1 (SiC(CH\(_3\))\(_3\)), −4.6 (Si(CH\(_3\))\(_2\)). No C\(_{Ar}\)I peak observed.

LRMS  (ES+) \( m/z \) 800 [M + Na]⁺.

HRMS  (ES+) calcd for C\(_{34}\)H\(_{56}\)INO\(_9\)SiNa [M + Na]⁺ 800.2661, found 800.2647.

\((+)-\text{tert-Butyl-N-[((1aS,9aS,11aR)-11a-[(\text{tert-butyl(dimethyl)silyl)}\text{oxy}]-1-(hydroxyethyl)-6-(ethyl \text{tert-butyl carbonate}-1,9a,10,11a-tetrahydrodibenzo[b,d]furan-12-yl)-3-methyl]-N-methylcarbamate ((+)-5.48)\)

\[
\begin{align*}
\text{C}_{34}\text{H}_{55}\text{NO}_{9}\text{Si} \\
\text{Mw} = 649.89 \text{ gmol}^{-1} \\
\text{Yellow oil}
\end{align*}
\]

To a solution of iodide \((-)-5.47\) (1.19 g, 1.5 mmol) in toluene (45 mL) was added Ag\(_2\)CO\(_3\) (1.27 g, 4.6 mmol), dppp (95 mg, 0.2 mmol) and Pd(OAc)\(_2\) (45 mg, 0.2 mmol). The reaction mixture was stirred at rt for 10 min and then heated to 90 °C for 28 h. The
solvent was removed in vacuo and the residue was purified by column chromatography (eluent 25-40% EtOAc/hexane) to give the title compound as a yellow oil (777 mg, 1.2 mmol, 78%).

$$[\alpha]_D$$ +7.2 (c 1.15, CHCl$_3$, 24 °C).

IR $\nu_{\max}$ (neat) 3435 (br w), 2929 (w), 2857 (w), 1742 (m), 1681 (m), 1392 (m), 1367 (m), 1252 (s), 1147 (s), 1092 (s), 836 (s), 730 (s) cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.74 (1H, d, $J = 8.3$ Hz, C$_{Ar}$H ortho to OCH$_2$), 6.56 (1H, d, $J = 8.3$ Hz, C$_{Ar}$H ortho to CH$_2$N), 5.79 (1H, d, $J = 10.1$ Hz, CCH=CH), 4.84 (1H, dd, $J = 11.5$, 4.8 Hz, CHOC$_{Ar}$), 4.62 (1H, br s, NCHH), 4.39 (2H, t, $J = 4.7$ Hz, C$_{Ar}$OCH$_2$CH$_2$), 4.35-4.27 (2H, m, NCHH & CHOTBS), 2.79 (3H, br s, NCH$_3$), 2.37-2.17 (1H, m, CHHCHOC$_{Ar}$), 1.61-1.29 (18H, m, Si(C(CH$_3$)$_3$)), 0.07 (3H, s, SiCH$_3$), 0.06 (3H, s, SiCH$_3$). Spectrum exhibited broadening of peaks due to restricted rotation.

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 155.9 (NC(O)O), 153.3 (OC(O)O), 147.1 (C$_{Ar}$OCH$_2$), 143.2 (C$_{Ar}$OCH), 134.0 (CCH=CH), 130.4 (C$_{Ar}$C), 128.0 (CCH=CH), 127.1 (C$_{Ar}$CH$_2$N), 121.1 & 119.9 (C$_{Ar}$H ortho to OCH$_2$), 114.1 (C$_{Ar}$H ortho to CH$_2$N), 84.8 (CHOC$_{Ar}$), 82.3 (OC(O)C(CH$_3$)$_3$), 79.8 (NC(O)OC(CH$_3$)$_3$), 67.0 (C$_{Ar}$OCH$_2$CH$_2$), 65.3 (CHOTBS), 59.4 (CH$_2$OH), 50.3 (CCH=CH), 48.9 (NCH$_2$), 41.8 (C$_{Ar}$OCHCH$_2$), 37.0 (CH$_2$CH$_2$OH), 34.0 (NCH$_3$), 28.4 (NC(O)C(CH$_3$)$_3$), 27.7 (OC(O)C(CH$_3$)$_3$), 25.7 (SiC(CH$_3$)$_3$), 18.1 (SiC(CH$_3$)$_3$), $-4.6$ (SiCH$_3$), $-4.8$ (SiCH$_3$).

LRMS (ES+) $m/z$ 672 [M + Na$^+$].

HRMS (ES+) calcd for C$_{34}$H$_{55}$NO$_9$SiNa [M + Na$^+$] 672.3538, found 672.3534.
**tert-Butyl-N-[(1aS,9aS,11aR)-11a-[(tert-butyldimethylsilyl)oxy]-1-(bromoethyl)-6-(ethyl tert-butyl carbonate)-1,9a,10,11a-tetrahydrodibenzo[b,d]furan-12-yl]-3-methyl]-N-methylcarbamate (5.49)**

C$_{28}$H$_{40}$BrNO$_8$  
M$_w$ = 598.52 gmol$^{-1}$  
Colourless oil

Following the procedure of Baughman et al.,$^{148}$ alcohol (+)-5.48 (50 mg, 0.077 mmol) and CBr$_4$ (28 mg, 0.085 mmol) were dissolved in CH$_2$Cl$_2$ (3 mL) and cooled to 0 °C before PPh$_3$ (22 mg, 0.085 mmol) was added turning the colourless solution pale yellow. The solution was stirred at rt for 2 h. The solvent was removed in vacuo and the residue was purified by column chromatography (eluent 20-100% EtOAc/hexane) to give the title compound as a colourless oil (13 mg, 0.022 mmol, 28%).

**IR**  
$\nu_{\text{max}}$ (neat) 3435 (br w), 2955 (w), 2926 (m), 2853 (w), 1742 (m), 1695 (m), 1275 (s), 1255 (s), 1158 (s) cm$^{-1}$.

**$^1$H NMR**  
(300 MHz, CDCl$_3$) $\delta$ 6.78 (1H, d, $J$ = 8.4 Hz, C$_{Ar}$H ortho to OCH$_2$), 6.63 (1H, d, $J$ = 8.4 Hz, C$_{Ar}$H ortho to CH$_2$N), 6.00 (1H, dd, $J$ = 9.9, 3.8 Hz, CCH=CH), 5.88 (1H, br s, CCH=CH), 4.78 (1H, t, $J$ = 5.3 Hz, CHO$_{C_{Ar}}$), 4.54 (1H, br s, NCH$_3$), 4.46-4.33 (3H, m, C$_{Ar}$OCH$_2$CH$_2$ & NCH$_3$), 4.28-4.17 (3H, m, C$_{Ar}$OCH$_2$ & CHO$_3$), 3.30 (1H, dt, $J$ = 11.7, 5.1 Hz, CHHBr), 3.11 (1H, ddd, $J$ = 11.7, 10.2, 5.1 Hz, CHHBr), 2.81 (3H, br s, NCH$_3$), 2.55-2.40 (1H, m, CHHCH$_2$Br), 2.36-2.11 (4H, m, CHHCH$_2$Br, CH$_2$CHOC$_{Ar}$ & OH), 1.61-1.41 (18H, m, 2 x C(O)OC(CH$_3$)$_3$). Spectrum exhibited broadening of peaks due to restricted rotation.

**LRMS**  
(ES+) $m/z$ 620 [M + Na]$^+$, 622 (Br isotope peak).
**tert-Butyl-N-[(1aS,9aS,11aR)-11a-[(tert-butyldimethylsilyloxy]-1-(oxoethyl)-6-(ethyl tert-butyl carbonate)-1,9a,10,11a-tetrahydrodibenzo[b,d]furan-12-yl]-3-methyl]-N-methylcarbamate (5.50)**

![](image)

C_{34}H_{53}NO_{9}Si  
M_w = 647.87 gmol^{-1}  
Colourless oil

Alcohol (+)-5.48 (27 mg, 0.042 mmol) was dissolved in CH₂Cl₂ (1 mL) and was treated with Dess-Martin periodinan e (23 mg, 0.054 mmol). The solution was stirred at rt for 45 min during which time it became cloudy. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a colourless oil (18 mg, 0.028 mmol, 67%).

**IR**  
\[ \nu_{\text{max}} \text{ (neat)} \text{ 2956 (w), 2928 (w), 2857 (w), 1742 (m), 1730 (m), 1694 (m), 1367 (m), 1273 (m), 1254 (s), 1146 (m), 1091 (m), 837 (m), 777 (m) cm}^{-1} \]

**¹H NMR** 
(300 MHz, CDCl₃) δ 9.56 (1H, t, \( J = 2.3 \text{ Hz, C(O)H} \)), 6.76 (1H, d, \( J = 8.4 \text{ Hz, CₐH ortho to OCH₂} \)), 6.60 (1H, d, \( J = 8.4 \text{ Hz, CₐH ortho to CH₂N} \)), 6.16 (1H, br s, CCH=CH), 5.87 (1H, d, \( J = 10.2 \text{ Hz, CCH=CH} \)), 4.87 (1H, dd, \( J = 11.7, 4.9 \text{ Hz, CHOCA} \)), 4.73 (1H, br s, NCHH), 4.45-4.38 (2H, m, CₐOCH₂CH₂), 4.37-4.31 (1H, m, NCHH), 4.28-4.22 (2H, m, CₐOCH₂), 4.21-4.15 (1H, m, CHOTBS), 2.79 (3H, br s, NCH₃), 2.68 (2H, d, \( J = 2.3 \text{ Hz, CH₂(OH)} \)), 2.36-2.18 (1H, m, CHHCHOCₐ), 1.85-1.66 (1H, m, CHHCHOCₐ), 1.55-1.43 (18H, m, 2 x C(O)OC(CH₃)₃), 0.88 (9H, s, Si(C(CH₃)₃)), 0.09 (3H, s, SiCH₃), 0.08 (3H, s, SiCH₃). Spectrum exhibited broadening of peaks due to restricted rotation.
(+)-tert-Butyl-N-[(1aS,9aS,11aR)-11a-[(tert-butyldimethylsilyl)oxy]-1-(ethyl(4-methyl benzenesulfonylate))-6-(ethyl tert-butyl carbonate)-1,9a,10,11a-tetrahydrodibenzo[b,d]furan-12-yl)-3-methyl]-N-methylcarbamate ((+)-5.51)

\[
\text{C}_{41}\text{H}_{61}\text{NO}_{11}\text{SSi} \\
\text{Mw} = 803.37 \text{ gmol}^{-1} \\
\text{Colourless oil}
\]

To a solution of alcohol (+)-5.48 (372 mg, 0.57 mmol), TsCl (218 mg, 1.15 mmol) and DMAP (17 mg, 0.14 mmol) in CH₂Cl₂ (4 mL) was added Et₃N (0.20 mL, 1.43 mmol) and the solution was stirred at rt for 2 h. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and was washed with HCl (1 x 5 mL) and brine (1 x 5mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (eluent 20% EtOAc/hexane) to give the title compound as a colourless oil (394 mg, 0.49 mmol, 86%).

\[\alpha\]D \text{ +23.7 (c 1.30, CHCl₃, 28 °C).}

\textbf{IR} \quad \nu_{\text{max}} \text{ (neat) } 2952 \text{ (w), 2929 (w), 2857 (w), 1741 (m), 1689 (m), 1391 (m), 1366 (m), 1252 (s), 1174 (s), 1095 (s), 836 (s), 731 (s), 554 (s) cm}^{-1}.

\textbf{¹H NMR} \quad (300 MHz, CDCl₃) δ 7.72 (2H, d, \text{J = 8.2 Hz, } C_{\text{Ar}}H \text{ ortho to SO₂O}), 7.33 (2H, d, \text{J = 8.2 Hz, } C_{\text{Ar}}H \text{ meta to SO₂O}), 6.73 (1H, d, \text{J = 8.4 Hz, } C_{\text{Ar}}H \text{ ortho to OCH₂}), 6.54 (1H, d, \text{J = 8.4 Hz, } C_{\text{Ar}}H \text{ ortho to CH₂N}), 6.03 (1H, br s, CCH=CH), 5.81 (1H, d, \text{J = 10.2 Hz, } CCH=CH), 4.71 (1H, dd, \text{J = 11.7, 4.9 Hz, } CH\text{OC}_{\text{Ar}}), 4.64 (1H, br s, NCHH), 4.45-4.35 (2H,
m, C<sub>Ar</sub>OCH<sub>2</sub>CH<sub>2</sub>), 4.33-4.17 (4H, m, NCH<sub>3</sub>, CHOTBS & C<sub>Ar</sub>OCH<sub>2</sub>), 4.08-3.95 (1H, m, SO<sub>2</sub>OCHH), 3.88 (1H, dt, J = 10.2, 7.1 Hz, SO<sub>2</sub>OCHH), 2.75 (3H, br s, NCH<sub>3</sub>), 2.44 (3H, s, C<sub>Ar</sub>CH<sub>3</sub>), 2.28-2.16 (1H, m, CHHCHOCAr), 2.15-2.01 (1H, m, SO<sub>2</sub>OCH<sub>2</sub>CHH), 1.94-1.80 (1H, m, SO<sub>2</sub>OCH<sub>2</sub>CHH), 1.77-1.61 (1H, m, CHHCHOCAr), 1.60-1.31 (18H, m, 2 x C(O)OC(CH<sub>3</sub>)<sub>3</sub>), 0.87 (9H, s, Si(C(CH<sub>3</sub>)<sub>3</sub>)), 0.08 (3H, s, SiCH<sub>3</sub>), 0.07 (3H, s, SiCH<sub>3</sub>). Spectrum exhibited broadening of peaks due to restricted rotation.

**<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>) δ 155.8 (NC(O)O), 153.3 (OC(O)O), 147.0 (C<sub>Ar</sub>OCH<sub>2</sub>), 144.8 (C<sub>Ar</sub>CH<sub>3</sub>), 143.3 (C<sub>Ar</sub>OCH), 135.2 (CCH=CH), 132.9 (C<sub>Ar</sub>SO<sub>2</sub>O), 130.8 (CCH=CH), 129.8 (C<sub>Ar</sub>H <em>meta</em> to SO<sub>2</sub>O), 127.8 (C<sub>Ar</sub>H <em>ortho</em> to SO<sub>2</sub>O), 126.5 (C<sub>Ar</sub>CH<sub>2</sub>N), 121.1 & 119.7 (C<sub>Ar</sub>H <em>ortho</em> to OCH<sub>2</sub>), 114.4 (C<sub>Ar</sub>H <em>ortho</em> to CH<sub>2</sub>N), 84.2 (CHOC<sub>Ar</sub>), 82.4 (OC(O)C(CH<sub>3</sub>)<sub>3</sub>), 79.9 (NC(O)OC(CH<sub>3</sub>)<sub>3</sub>), 67.0 (C<sub>Ar</sub>OCH<sub>2</sub>CH<sub>2</sub>), 66.9 (SO<sub>2</sub>OCH<sub>2</sub>), 65.2 (CHOTBS), 65.1 (C<sub>Ar</sub>OCH<sub>2</sub>), 49.7 (CCH=CH), 48.7 (NCH<sub>2</sub>), 37.3 (SO<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>), 36.3 (C<sub>Ar</sub>OCH<sub>2</sub>CH<sub>2</sub>), 33.8 (NCH<sub>3</sub>), 28.4 (NC(O)C(CH<sub>3</sub>)<sub>3</sub>), 27.7 (OC(O)C(CH<sub>3</sub>)<sub>3</sub>), 25.7 (SiC(CH<sub>3</sub>)<sub>3</sub>), 21.6 (CH<sub>3</sub>C<sub>Ar</sub>), 18.1 (SiC(CH<sub>3</sub>)<sub>3</sub>), −4.6 (SiCH<sub>3</sub>), −4.8 (SiCH<sub>3</sub>). One C<sub>Ar</sub> quaternary centre was not observed.

**LRMS** (ES+) m/z 826 [M + Na]<sup>+</sup>.

**HRMS** (ES+) calcd for C<sub>41</sub>H<sub>61</sub>N<sub>1</sub>O<sub>1</sub>SSiNa [M + Na]<sup>+</sup> 826.3627, found 826.3624.
6.3 Radiochemistry

Procedure for the $[^{18}\text{F}]$-fluoridolysis of the RLV constructs

$[^{18}\text{F}]$-Fluoride (<400 MBq) in water (100-300 $\mu$mL) was drawn into a Wheaton vial followed by 1,10-diazo-4,7,13,16,21,24-hexaoxabicyclo [8.8.8] hexacosan (4 mg, 10.6 $\mu$mol) in MeCN (200 $\mu$mL) and aq $\text{K}_2\text{CO}_3$ (50 $\mu$L, 0.1 moldm$^{-3}$, 5 $\mu$mol) which were added through the dip tube inlet. The mixture was azeotropically dried with a flow of nitrogen and heating at 100 $^\circ$C for 20 min. The reaction vessel was cooled to rt and resin (30-40 mg) was added followed by MeCN (500 $\mu$mL). The stirred reaction mixture was heated to 100-110 $^\circ$C for 15 min before being cooled and filtered through an acrodisc. The Wheaton vial was rinsed with water (1.5 mL) and MeCN (500 $\mu$mL), which was also filtered. The sample was analysed by reverse-phase HPLC (Luna C18(2) column, 150-4.6 mm, 100 $\mu$L loop size, 1 mL/min pump speed, 254 nm wavelength, eluent 40-95% MeCN/water). To formulate the sample it was diluted to a volume of 10 mL with H$_2$O, loaded onto a conditioned light C18 Sep-Pak cartridge and flushed with H$_2$O (2 mL) and EtOH (0.5 mL) into a P6 vial before phosphate buffered saline (4.5 mL) was added and HPLC analysis was repeated.
References


65. Logothetis, T. A., Project report for GE Healthcare and the University of Southampton, 2005.


