Development of a New Synthetic Biology Tool: Synthetic Sub-Cellular Compartments

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Abbreviations

°C  Degrees celcius
\(^{13}\text{C} \)  Carbon-13
\(^{1}\text{H} \)  Proton-1
Boc  Tert-butyloxy carbonyl
CDCl\(_3\)  Deuterated chloroform
CHCl\(_3\)  Chloroform
COSY  Correlation spectroscopy
DCM  Dichloromethane
DMF  \(N,N\)-Dimethylformamide
DMSO  Dimethylsulfoxide
eq  Equivalents
ES  Electrospray
Et\(_2\)O  Diethyl ether
EtOAc  Ethyl acetate
h  Hours
H\(_2\)O  Water
HCl  Hydrochloric acid
HPLC  High performance liquid chromatography
HMQC  Heteronuclear multiple quantum coherence
Hz  Hertz
\(^{3}\text{PrOH} \)  iso-Propanol
IR  Infrared
\(J \)  Coupling constant value
K  Kelvin
LCMS  Liquid chromatography-mass spectrometry
LED  Light-emitting diode
\(m/z\)  Mass to charge ration
MeOH  Methanol
mg  Milligram
mins  Minutes
mL  Millilitre
M  Molar
NMR  Nuclear magnetic resonance
NPPOC  2-(2-Nitrophenyl)propoxycarbonyl
NVOC  6-Nitroveratryloxy carbonyl
ppm  Parts per million
Py-\(d_5\)  Deuterated pyridine
TFA  Trifluoroacetic acid
TFP  Tetrafluorophenol
THF  Tetrahydrofuran
TIPS  Triisopropylsilyl
UV  Ultraviolet
\(\delta\)  Chemical shift (delta)
Abstract

Development of a New Synthetic Biology Tool: Synthetic Sub-Cellular Compartments

Rebecca Kathryn Booth

2015

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Engineering and Physical Sciences

Methods for the remotely triggered release of biologically active molecules has been an area of intense research in recent years, as it would allow hard-to-obtain biomolecules to be tested in cell-like environments. The removal of polar functional groups from hydrophilic structures upon the application of an external stimulus would generate unfunctionalised molecules, which could then participate in amphiphilic self-assembly. This could be used in the release of bioactive molecules and therefore in the in vitro investigation of drug activity.

In this PhD project, diazene chemistry was identified as the optimal method for the generation of unfunctionalised compounds through the removal of polar groups since fragmentation takes place rapidly and without the formation of potentially toxic intermediates or by-products.

Scheme 1: Removal of polar functionality upon cleavage of a protecting group

The synthesis of a range of protected hydrazines is described in this thesis (Scheme 1), in addition to the investigation of the rates of decomposition of various simple sulfonyl hydrazides, which led to the isolation of the product of the decomposition of a sulfonyl hydrazide. The photolysis of NVOC- and NPPOC-protected hydrazine derivatives is discussed.
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For my Grandad

*John Michael Rhodes*

*1933 – 2014*
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Chapter 1 - Introduction

1.1 Background

1.1.1 Triggered uncaging techniques for biomedical applications

The lack of specificity of small molecule therapeutics, their tendency to form undesirable and potentially toxic side products and the rapid removal of such molecules through endogenous clearing mechanisms have prohibited their use in biological systems. In order to target the difficulties encountered in the use of small molecule therapeutics, many compounds have been developed that can act as vehicles that can transport their cargo (drug molecules) to the site of action, enabling spatial and temporal delivery of the therapeutic agent. The development of such systems has increased significantly in recent years as the need for an alternative approach to drug delivery has become more urgent, in order to reduce the often harsh side effects of cytotoxic agents.

The active compounds are released upon recognition of an endogenous or exogenous stimulus to effect targeted drug delivery. Endogenous stimuli such as a change in cellular concentration of small molecules, proteins, nucleic acids and osmotic pressure, have advantages over exogenous stimuli, such as ultrasound, electromagnetism, light and temperature, in environments where there are a number of binding sites due to the requirement for a high degree of selectivity and specificity. Exogenous stimuli are applied externally so there is a greater level of control over the site of drug release, hence reducing their toxicity to surrounding healthy cells/tissue. Biologically active molecules can be released from a caged structure by direct release or activation, expansion, gatekeeping strategies, switches in morphology, autonomous motion, disassembly/degradation and assembly/aggregation of nanostructures (Figure 1, Table 1). The aim of the project is to develop a method to remotely trigger the activation of drug molecules so that they can be tested in a cell-like environment using an uncaging technique.
Direct release/activation
Expansion
Gatekeeping strategies
Morphology switches
Autonomous motion
Disassembly/degradation
Assembly/aggregation

Figure 1: Cartoons depicting the methods of stimuli-responsive release of bioactive molecules\textsuperscript{1}
<table>
<thead>
<tr>
<th>Drug delivery technique</th>
<th>Stimuli</th>
<th>Features</th>
<th>Advantages/disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct release/activation(^2)</td>
<td>pH, light, enzymes/proteins</td>
<td>Cleavage of a covalent linkage releases active molecule</td>
<td>Agent can be effectively transferred to active site on release though excretion of cytotoxic agent is possible since few mechanisms for cellular absorption are known.</td>
</tr>
<tr>
<td>Expansion(^3)</td>
<td>pH, temperature</td>
<td>Nanostructure swells or contracts upon recognition of a trigger to release agent</td>
<td>Encapsulation/release efficiencies are hard to quantify, as agent can be released (leaked) unintentionally</td>
</tr>
<tr>
<td>Gatekeeping(^2)</td>
<td>pH, redox processes, enzymes/protein, nucleic acids/small molecules, light</td>
<td>Sterically bulky shell encapsulates the drug molecule</td>
<td>Applicable to a range of stimuli though a lack of universal specificity may lead to toxic side effects</td>
</tr>
<tr>
<td>Morphological switches(^4)</td>
<td>pH, enzymes/protein, temperature, light</td>
<td>Changes to structural morphology modifies molecular/cellular interactions</td>
<td>Morphological changes alters pharmacokinetics, stability, bioavailability and biodistribution but lack of imaging techniques that can efficiently track changes in morphology on the nanoscale leads to difficulties in monitoring the structural changes</td>
</tr>
<tr>
<td>Autonomous motion(^5)</td>
<td>Enzymes/protein, nucleic acids/small molecules, ultrasound, magnetic field</td>
<td>Achieved through asymmetric placement of an appropriate motor, driven by an adequate fuel</td>
<td>Allows controllable localisation, percutation and deep tissue penetration, though difficulties have been encountered in synthesis and positioning of motors at long distances</td>
</tr>
<tr>
<td>Disassembly/degradation(^6,7)</td>
<td>pH, redox processes, enzymes/protein, nucleic acids/small molecules, temperature, light</td>
<td>Nanostructure breaks into fragments by single event(^5) or multi-step(^6) disassembly upon stimuli recognition</td>
<td>Potentially provide a higher degree of specificity through multi-step degradation though additional optimisation is required before application in vivo due to the complexity of the process</td>
</tr>
<tr>
<td>Assembly/aggregation(^1,8)</td>
<td>pH, enzymes/protein, nucleic acids/small molecules, temperature, light</td>
<td>Monomers aggregate into discrete structures upon recognition of a stimulus</td>
<td>Thermo-, pH- and light-triggered(^1) assembly has been demonstrated in vitro though few in vivo applications have been developed</td>
</tr>
</tbody>
</table>

*Table 1: Stimuli responsive drug release techniques*
1.1.2 Amphiphilic self-assembly

Much research has taken place in recent years into amphiphilic self-assembly. In contrast with conventional materials, which contain covalent bonds that are formed irreversibly, and often require lengthy synthetic strategies to obtain, supramolecular materials are held together by reversible interactions. As such, these supramolecular materials are adaptive, allowing them to be easily synthesised and recycled. Such materials can also be stimuli responsive and self-healing. Thermodynamically controlled polymerisation of supramolecular materials often occurs as a result of these reversible interactions, differing from macromolecular polymerisation in that the chain length can be changed reversibly by temperature changes that create dynamic rheological behaviour, hence having notable advantages in processing.

Since amphiphilic self-assembly is most studied in aqueous environments, due to the strong polarity of water, any chemistry in which amphiphilic self-assembly is a required process must be water-compatible. Water is readily available, inexpensive and environmentally friendly. It allows the formation of exceptionally strong hydrophobic interactions, which drive the formation of supramolecular structures. Hydrophobic moieties group together in aqueous solutions to reduce the disturbance of the three-dimensional network of hydrogen bonds, decreasing the entropically unfavourable interaction between water and hydrophobic molecules. When water comes into contact with non-hydrogen-bond accepting molecules, a minimum of one of the charges on each water molecule must face towards the inert solute molecule, hence disturbing the network of hydrogen-bonds. Water can be positioned around the non-polar solute to minimise the number charges pointing towards the inert molecule, leaving the other charges pointing towards the water phase and thus being able to participate in hydrogen-bonding. However, this has been shown, both theoretically and experimentally, to be entropically unfavourable since the water molecules, when packed around the inert solute, are more ordered than in the bulk liquid, coordinating to four other water molecules through hydrogen-bonds compared to three in the bulk liquid. As a result of the hydrophobic effect, the solubility of non-polar solutes such as hydrocarbons is extremely low in water.

Such solvophobic effects are important in biological processes including protein folding and aggregation, substrate binding, and membrane formation. Hydrogen-bonding cannot be utilised to hold supramolecular structures together in water as the stability of such electrostatic interactions is significantly reduced in aqueous solutions compared to organic solvents, because
the solvent also participates in hydrogen bonding competing with the interactions within the structure.\textsuperscript{20} Similarly, ion-pair interactions are significantly weakened in water due to the inversely proportional relationship between the polarity of the solvent and the strength of electrostatic interactions. Water molecules interact with the ions to stabilise them, thus weakening any intermolecular interactions within the structures.\textsuperscript{21} Self-assembly of supramolecular systems in water, via hydrophobic and/or electrostatic interactions, has shown significant advantages over conventional covalently-bonded materials because the hydrophobic forces can be ‘switched off’ by changing the solvent or the polarity of hydrophobic groups.\textsuperscript{20}

As discussed above, hydrophobic and \(\pi-\pi\)-stacking interactions can be applied in aqueous environments in the formation of stable aggregates, a process that is driven by unfavourable interaction of the binding pairs with water. The non-specificity and -directionality of such hydrophobic interactions can be overcome by the use of aromatic compounds and extended \(\pi\)-conjugated systems which display \(\pi\)-stacking interactions. The rigid planar structure of such groups interacts unfavourably with the aqueous interface through the disruption of the complex network of hydrogen-bonds present in the structure of water. In addition, \(\pi,\pi\)-stacking interactions assists self-assembly in amphiphiles and have been shown to display electronic transitions in the UV/visible region during aggregation.\textsuperscript{18}

Hydrophilic groups must be incorporated for effective self-assembly of an amphiphile, although the main role of such groups is to increase water solubility.\textsuperscript{19} The rate and extent of aggregation of amphiphiles is largely dependent on the relative number of hydrophilic and hydrophobic groups, combined with the solvation of the hydrophilic group and the geometry of the hydrophobic structure.\textsuperscript{19} Amphiphilic self-assembly is controlled by the forces between the amphiphilic molecules within the aggregates, which are affected by changes in solution conditions. Inter-aggregate forces in more concentrated systems also contribute to the control over self-assembly. The forces that control the rate and extent of self-assembly can be categorised as hydrophobic attraction and hydrophilic, ionic or steric repulsion. The attractive forces, originating mainly from hydrophobic interactions or interfacial tension forces at the lipid-water interface result in the association of the molecules. The repulsive forces of the hydrophilic headgroups are due to a combination of steric and hydration force contributions, with electrostatic double layer contributions supplementing the effect when charged headgroups are present, causing the molecules to remain in contact with water. Double layer interactions are interaction forces of intermediate energy, which are formed when solids are immersed in ionic
solutions, with ions of one charge being preferentially adsorbed at the solid liquid interface. The subsequent interaction between the two layers supplements the hydrophobic forces present. The strength of amphiphilic self-assembly is also increased by specific headgroup interactions, e.g. ionic bridging between amphiphiles, specific chain-chain interactions and the effect of surface curvature on the chemical potential of a molecule in an aggregate, all of which are ignored by theoretical estimations of the interaction energy but are important contributing effects. Surface curvature can have a significant effect on the properties of self-assembled mono-layers as a result of differences in the density of the headgroups compared to that of the surface-attachment sites, which is dependent on the curvature of the underlying surface. On a curved surface, the density of the headgroups is smaller than that of the surface-attachment sites, with the density difference increasing with increased curvature. When particles are sufficiently small, the spacing between the headgroups of amphiphiles on a curved surface is significantly larger compared to headgroup spacing on a flat surface. The increased spacing of headgroups on a curved surface has a considerable effect on the interaction between the groups.

The geometry of an amphiphilic structure is dependent on the optimum surface area, the volume of the hydrocarbon chain and the critical chain length. The critical chain length of the lipid chain denotes the length beyond which the chain is no longer fluid. The structure with the smallest aggregation number is favoured entropically, i.e. smaller structures. However, small structures are enthalpically unfavourable because packing constraints force the surface area to increase above the optimum level, which requires the input of energy. Amphiphilic self-assembly is also controlled by the ratio of hydrophilic to hydrophobic groups, the solvation of the hydrophilic group and the shape of the hydrophobic group. Self-assembly of amphiphiles in water leads to the formation of one- and three-dimensional structures. One-dimensional supramolecular structures, micelles and vesicles contain surfactants and a hydrophilic headgroup. Amphiphiles generally assemble into spherical, globular or rod-like micelles at low concentrations. Vesicles are formed at higher amphiphile concentration; lamellar bilayers are formed initially, which then close (depending on the length of the chain, the hydrophobic volume of the amphiphile and the interfacial area). In order to form hydrogels, micelles and vesicles (Figure 2) interact to form three-dimensional networks throughout the solution, resulting in gelation.
1.1.3 Externally triggered amphiphilic self-assembly

Recent research has shown that amphiphilic self-assembly can be triggered by external stimuli, such as a change in pH, temperature or interaction with light. Minkenberg and coworkers demonstrated the self-assembly of amphiphiles formed from simple non-amphiphilic water-soluble precursors is responsive to a pH gradient. A dynamic covalent bond is formed in the non-amphiphilic precursors, in this case polar aromatic aldehydes and apolar aliphatic primary amines to form imines (Scheme 2).

![Scheme 2: Dynamic formation of imine amphiphiles](image)

The position of the equilibrium can be moved by modifying the aforementioned conditions. Imine chemistry was chosen since both starting reactants have only one reactive site that can form a covalent bond on reaction, thus preventing the formation of alternative products. Imine formation was monitored using NMR spectroscopy and was shown to be relatively slow on the NMR timescale, while self-assembly of the imines into micelles was shown to be relatively fast on the NMR timescale. Aldehydes and amines are not surface-active under the conditions used and as such remain soluble at low concentration, leading to a fully reversible system. The encapsulation and release of hydrophobic probe Nile Red by the aggregate was shown to be fully reversible upon changes to the pH level of the solution, through changes in the emission as monitored by fluorescent emission spectroscopy.

Thermally triggered self-assembly of folded proteins into vesicles in aqueous solutions was reported by Park and Champion. Three proteins were mixed at 4 °C to form amphiphilic complexes that contained α-helix coiled coil motifs, as shown by circular dichroism spectroscopy. Upon incubation at room temperature for an hour, scanning electron microscopy (SEM)
demonstrated that the protein complexes self-assembled in aqueous solution into hollow vesicles with walls of thickness of approximately 20 nm. Vesicle formation was only observed above the critical salt concentration, which is dependent on the composition of the protein complex. Cargo of a range of sizes could be encapsulated in the vesicles ranging from approximately 1-100 nm.\(^9\)

Very recently, gold nanoparticles decorated with polyethylene glycol (PEG) and photoresponsive polymethacrylate ligands containing spiropyran units (PSPMA) have been developed that can reversibly self-assemble into aggregates upon irradiation with light (Scheme 3).\(^{11}\) The evolution of a new peak in the UV-vis absorption spectrum indicated that aggregates were formed following the conversion of the spiropyran units to the zwitterionic merocyanine isomer (PMCMA) upon irradiation with UV light in a solution of \(N,N\)-dimethylformamide (DMF) and water. Upon irradiation with visible light, disassembly was indicated by the gradual diminution of the newly formed peak in the absorption spectrum, demonstrating the reversibility of the process.\(^{11}\)

\begin{center}
\textit{Scheme 3: Reversible self-assembly/disassembly processes of PSPMA to PMCMA brushes induced by UV/visible light}
\end{center}

Instead of reversible photoswitching, irreversible photocleavage of photochemical protecting groups is often used in synthetic biology to reveal the biologically active functionality, thus activating the molecule.

1.1.4 Light responsive water soluble compounds

Since the first caged molecule was developed by Kaplan \textit{et al.} in the 1970s,\(^{25}\) a variety of light-sensitive probes have been developed in which the biological activity of the molecule is completely masked prior to its exposure to a stimulus. Generally a covalently bonded photosensitive protecting group cages the biologically active group on the compound, hence making it biologically inert.\(^{26}\) Irradiation releases the biologically active molecules through the photolytic cleavage of the protecting group, thereby leading to a sudden increase in
concentration of the bioactive molecule, allowing it to carry out its function in the cell. Light can pass through cell membranes and un cage biomolecules in otherwise inaccessible intracellular compartments or extracellularly to an extent that can be carefully controlled by uniform or localised irradiation inside or outside the cell, with release of the biomolecules only occurring where the light is incident. Biomolecules can also be uncaged in rapid bursts or gradually through control of the time and amplitude of the irradiation. Light is an optimal trigger for uncaging bioactive molecules since cells do not otherwise react upon interaction with light unless highly specialised, e.g. the photoreceptors in the eye or the zebrafish.  

Caged compounds can be synthesised through multistep or one-step processes, though the former is a more common technique due to the complexity of most natural products. The caging group must fully remove the biological activity from the molecule. A high quantum yield of uncaging is desirable, though high uncaging efficiency is not always essential, as caged compounds with only moderate uncaging efficiencies have been shown to be useful in a number of biological processes, e.g. 1- (ortho-nitrophenyl)-ethyl (NPE)-caged ATP (1) and inositol-1,4,5-trisphosphate (IP$_3$, 2, Figure 3).

![Figure 3: NPE-caged ATP (1) and NPE-caged IP$_3$ (2)](image)

The uncaging process must be faster than the reaction being studied for the study of light-induced kinetics. The photochemical reaction should also take place without the formation of intermediates, which could be toxic to the biological system, and at a wavelength that is not so short (> 300 nm) as to cause damage to the cells. The caged compound should be soluble in aqueous solutions, for use in biological applications. Therefore, the final step of the synthesis of a caged molecule is generally to add a solubilising group. The caged biomolecule must also be stable in aqueous conditions, since the caging group should only be removed upon irradiation with light.

Derivatives of the ortho-nitrobenzyloxy group (3) have emerged as the most useful caging groups for use in biological processes, due to their compatibility with a number of functional groups including phosphates, carboxylates, alcohols, amines and amides, in addition to metal ions when
accompanied by photolabile chelators. They are also easily synthesised and can be removed in photochemical reactions at a reasonably high rate. ortho-Nitroveratryloxy-caged ethers (NVOC) have faster rates of photodeprotection than the corresponding ortho-nitrobenzyl derivatives (NBOC, Scheme 4). Uncaging reactions of ortho-nitrobenzyl-caged compounds (3) generally proceed without competing side reactions.

![Scheme 4: Nitrobenzyl- and nitroveratryloxy-caged ethers](image)

Most uncaging processes utilise one-photon excitation with wavelengths that are in the near-UV region (300-400 nm) which is efficient enough for most experimental work at the majority of concentrations required. However, there has been increasing use of two-photon excitation, which utilises photons at longer wavelengths, due to the toxicity associated with one-photon excitation. Since two-photon excitation generally uses excited photons of near-IR wavelengths, the photons scatter to a lesser extent in biological tissues compared to one-photon excitation. In the absence of scattered photons, which can also excite the chromophore, there is a more defined focal spot. Therefore, greater accuracy is observed deeper within live tissues. Despite these advantages, two-photon absorption and excitation is much less efficient than the corresponding one-electron technique. ortho-Nitrobenzyl-caged compounds give poor yields of the uncaged product upon irradiation using two-photon excitation. No photosensitive protecting groups have yet been developed that are suitable for use in a biological environment and can generate a high uncaging yield upon two-photon excitation.

Tamoxifen 5 (Figure 4) is used in the treatment of cancer and has been modified to allow photochemical protection and subsequent release of the drug upon irradiation. Since tamoxifen 5 does not have a functional group handle, an analogue, 4-hydroxytamoxifen 6 which is also bioactive, was developed to enable the biological activity to be caged. An ortho-nitroveratryloxy-group is added to the phenol group (Scheme 5). It is known that the phenol group in 4-hydroxytamoxifen 6 fits in to the binding pocket that tamoxifen binds to. As such, by the modification of the hydroxyl functionality in the addition of the ortho-nitroveratryloxy-group to form caged compound 7, the biological function is caged and the molecule is biologically silent until it is liberated upon irradiation.
Photochemical control over amphiphilic structures has been demonstrated through the addition of azo-benzene\textsuperscript{32–35} and pyrene\textsuperscript{36} derivatives. Molecules can be encapsulated within micelles which are then released upon irradiation as the structure of the micelle is dissociated by the change in polarity of the structures. Jinqiang et al. synthesised a photosensitive amphiphilic block copolymer incorporating the \(\alpha\)-nitrobenzyl group (3) which formed light dissociable polymer micelles (Scheme 6).\textsuperscript{37} Nile Red was used as a model hydrophobic guest and its encapsulation and release from the micelles was observed upon one- or two-photon excitation with ultraviolet (UV) and near infrared light (NIR), respectively.

**Scheme 5: NVOC-protection of 4-hydroxytamoxifen 6\textsuperscript{31}**

The initial aim of the project is to develop chemical methods of removing polar functionality from molecules to release lipophilic molecules in aqueous solution (Scheme 7). The polar functional group must be removed and replaced by an apolar hydrocarbon linkage (C-H bond) in water at room temperature and pressure, without the use of metal catalysts, which can often be toxic.

**Scheme 6: Photolysis of NBOC-protected amphiphilic block co-polymers\textsuperscript{37}**

**1.2 Project aims**

The initial aim of the project is to develop chemical methods of removing polar functionality from molecules to release lipophilic molecules in aqueous solution (Scheme 7). The polar functional group must be removed and replaced by an apolar hydrocarbon linkage (C-H bond) in water at room temperature and pressure, without the use of metal catalysts, which can often be toxic.
In current biological applications that involve the use of caged molecules, molecules undergo uncaging after absorption of the compound into the outer layer of the cell membrane has taken place, before entering the inner extremes of the membrane. The aim of the project in hand is to develop a caged compound that will be chemically unmasked prior to absorption into the cell membrane to reveal a lipid, hence differing from current research. The molecule will be efficiently transported to the site of action in an aqueous medium due to its associated hydrophilicity added by the solubilising substituent. Following the cleavage of the polar functional group by a photochemical trigger, the molecule will be absorbed into the cell membrane due to its increased lipophilicity and consequent hydrophobicity, and subsequently will pass into the cell. The uncaging of biomolecules by this method will be spatially controlled through focussed illumination, instead of sudden and short-lived increases in the concentration of biomolecules in highly localised sites. Once established, this method will be applied to the caging of compounds with known biological activity, including drugs and pheromones.

The reaction to remove polar functionality must take place quickly, without the formation of intermediates that can often participate in side-reactions to form by-products that can have adverse side effects. Therefore, a number of known literature methods were investigated for the chemical ‘unmasking’ of hydrophilic molecules, including protodecarboxylation, diazotisation, boron chemistry, light-catalysed deoxygenation and diazene chemistry (Table 2). Of these methods, only the light-catalysed deoxygenation takes place by a photochemical process. The addition of photochemical protecting groups to the molecule would allow the removal of polar functionality to be promoted by irradiation with UV light via the alternative techniques.

\[ \text{X} \xrightarrow{\text{Conditions}} \text{H} \]

Scheme 7: Removal of polar functionality
<table>
<thead>
<tr>
<th>Technique</th>
<th>Scheme</th>
<th>Advantages/disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proto-decarboxylation</td>
<td><img src="image.png" alt="Image" /></td>
<td>Use of transition metal catalysis, e.g. copper, mercury, silver, high boiling, polar solvents and high temperatures and pressures.</td>
</tr>
<tr>
<td>Diazotisation</td>
<td><img src="image.png" alt="Image" /></td>
<td>Formation of highly reactive cationic intermediates could form toxic side products on reaction with water, etc.</td>
</tr>
<tr>
<td>Boron chemistry</td>
<td><img src="image.png" alt="Image" /></td>
<td>Organoboranes are stable, easy to prepare and mild conditions are required for the coupling. Use of palladium catalysts, free radical intermediates, insolubility of organoboranes in aqueous solutions and high sensitivity to aerobic oxidation make the process incompatible.</td>
</tr>
<tr>
<td>Light-catalysed deoxygenation</td>
<td><img src="image.png" alt="Image" /></td>
<td>Externally-triggered process, though radical formation could result in the formation of toxic side products.</td>
</tr>
<tr>
<td>Diazene chemistry</td>
<td><img src="image.png" alt="Image" /></td>
<td>Conversion to the unfunctionalised product takes place rapidly, without the formation of intermediates or side toxic products, under mild conditions.</td>
</tr>
</tbody>
</table>

Table 2: Synthetic methods for the removal of polar functionalities to reveal unfunctionalised lipids

Diazene chemistry is commonly used in the removal of polar groups from molecules, including in the deamination of aliphatic amines, the deoxygenation of alcohols and in the Wolff-Kishner reaction of hydrazones. The deamination of benzylic amines by Nickon and Hill gave higher yields of the reduced products than that of non-benzylic amines due to a combination of steric and electronic factors.

Azo and diazo derivatives, in which both or one nitrogen of the diazene functionality are substituted with an atom or group other than hydrogen respectively, are commercially available as both the alkyl and aryl derivatives. The majority of such commercially available compounds are adjacent or neighbouring an electron-withdrawing substituent such as a carbonyl/sulfonyl or aromatic group, or are isolated as a salt, indicating the low stability of the group. Azo compounds are commonly used as oxidising agents and synthetic dyes, in addition to their use in drug delivery. Azobenzenes have been used in photochemistry due to the photoisomerisation of the diazene bond. Diazo compounds are predominantly used in chemical synthesis, demonstrating their versatility, and can be used to couple biomolecules onto surfaces, indicating
their biological applicability. Theoretical studies have been carried out into the stability of substituted diazene derivatives. The reaction enthalpies (ΔH) for the hypothetical isodesmic reactions between a substituted diazene and a primary imine were calculated for various substituents (Scheme 8) to determine the effect on the stability of diazenes.

\[
\text{Scheme 8: Hypothetical isodesmic reaction used to determine stability of substituted diazenes}
\]

Negative reaction enthalpies were calculated where R = CN, CF₃ and CHO, indicating that the three groups destabilise the diazene, giving an exothermic reaction. All other substituents on the diazene (R = H, F, OH, OCH₃, NH₂, CH₃) led to a positive ΔH value, indicating that they stabilise the structure since the reaction is endothermic. This can be explained by the inductive and resonance electronic effects of the substituents on the electron density on each nitrogen atom. This shows that diazenes are unstable without the use of additional stabilising substituents and can therefore be used effectively as intermediates in the fragmentation of more complex molecules.

1.3 Loss of polar functionality through diazene chemistry

Substituted hydrazines have been shown to decompose via the unstable diazene intermediate. The instability of substituted hydrazines was initially investigated by Dann and Davies as they found that nitrobenzenesulfonyl hydrazide 11 (formed from the reaction between nitro-benzene sulfonyl chloride 10 and hydrazine monohydrate) decomposed in hot solvent (Scheme 9). Upon increasing the reaction temperature following the synthesis of sulfonyl hydrazide 11, the corresponding sulfinic acid 12 and nitrogen were generated on addition of hot aqueous hydrazine or dilute hydrochloric acid.

\[
\text{Scheme 9: Formation and decomposition of nitro-benzene sulfonyl hydrazide 11}
\]

The rapid decomposition at high temperatures is due to the electronic effect of the nitro group on the aromatic ring. This has been demonstrated by the evolution of sulfur dioxide from nitro-benzene sulfinic acids in hot solvents to form the elimination products in high yields. For example,
5-nitro-p-toluenesulfonic acid 12 is stable at room temperature but loses sulfur dioxide to give 2-chloro-5-nitrotoluene 12 in 77% yield in hot pyridine (Scheme 10).

\[
\text{Scheme 10: Loss of sulfur dioxide from 5-nitro-p-toluenesulfonic acid 12 in hot pyridine}
\]

This contrasts with aromatic sulfinic acids that do not have a nitro substituent which form the elimination products in low yields, e.g. naphthalene-β-sulfinic acid 13, which decomposed to form naphthalene 14 in 6.2% yield (Scheme 11).

\[
\text{Scheme 11: Loss of sulfur dioxide from naphthalene-β-sulfinic acid 13 in hot pyridine}
\]

It was also found that the synthesis of meta- and para-substituted nitro-benzene sulfonyl hydrazides by the combination of sulfonyl chlorides and hydrazine monohydrate required a greater reaction time than the ortho-substituted analogues. Subsequent decomposition of the resulting sulfonyl hydrazides to form the nitrosulfinic acids was also shown to take place at different temperatures, indicating the difference in stabilities of the ortho-, para- and meta-substituted sulfonyl hydrazides. The order of stability is meta > para > ortho as shown by the decomposition of the sulfonyl hydrazides at increasingly high temperatures, demonstrating the effect of the nitro group on the stability of the compounds.

Myers et al. examined the application of diazene chemistry to the removal of polar functional groups, i.e. hydroxyl groups (Scheme 12). Providing an alternative to the Barton deoxygenation reaction, the saturated alcohol is converted to a monosubstituted diazene on reaction with o-nitrobenzenesulfonylhydrazine (NBSH).

\[
\text{Scheme 12: Deoxygenation of an alcohol on reaction with NBSH}
\]

NBSH is easily prepared from the reaction between o-nitrobenzenesulfonyl chloride and an excess of hydrazine monohydrate. The reaction and work-up are carried out in apolar solvents such as ethyl acetate and tetrahydrofuran (THF) to prevent premature decomposition of the product.
hydrazine, while subambient temperatures are used for the same reason.\textsuperscript{56} Elimination of o-nitrobenzenesulfinic acid from the Mitsunobu displacement product (14, Scheme 13, 18, Scheme 14) forms the deoxygenated products by either a free-radical (Scheme 13) or sigmatropic rearrangement mechanism (Scheme 14).\textsuperscript{57} The method was found to be highly effective for the cleavage of polar functionality from unhindered alcohols to form alkanes, though sterically bulky and β-oxygenated alcohols do not undergo Mitsunobu displacement. Since the latter reagent can be recovered from the reaction mixture unchanged, this technique can be used for the selective removal of an unhindered alcohol group in the presence of sterically encumbered or β-oxygenated alcohol functionality. Less reactive functional groups can be cleaved when neopentyl alcohol is added to the reaction mixture, with the displacement being carried out in N-methylmorpholine (NMM).

In order to prove that a free radical mechanism was followed in some cases, the reaction between NBSH and 2-naphthaleneethanol 13 to form the deoxygenated product, 2-ethynaphthalene 16 (Scheme 13), was monitored by variable temperature NMR.\textsuperscript{43}

\textbf{Scheme 13: Deoxygenation of 2-naphthaleneethanol 13}

The displacement of the hydroxyl group by NBSH was shown to take place in 90 minutes at -30 °C, forming 1-alkyl-1-sulfonylhydrazine 14, which was stable at temperatures below -15 °C. As the temperature was increased to 0 °C, o-nitrobenzene sulfinic acid was slowly eliminated from the molecule. The formation of a diazene intermediate (15) was demonstrated by the evolution of a peak at δ 15.6 ppm in the NMR spectrum, which coupled to the adjacent methylene group at δ 2.2 ppm. As expected, the rate of formation of the diazene intermediate increased significantly at 20 °C. At this temperature, the subsequent decomposition to 2-ethynaphthalene 16 of diazene 15 occurred at the same rate as the generation of the intermediate, with the deoxygenation process completed within 40 minutes at 20 °C.\textsuperscript{43}
Other work by the Myers group showed that the deoxygenation of unsaturated allylic and propargylic alcohols to alkenes and allenes followed a sigmatropic rearrangement mechanism upon displacement of the alcohol functional group by NBSH. The deoxygenation reaction preferentially proceeds via a sigmatropic rearrangement mechanism wherever possible, as demonstrated by the stereospecificity of the above transformation. To further investigate this, the deoxygenation of aromatic alcohols was examined through the deoxygenation of 1-napthalenemethanol 17 (Scheme 14). Such reagents should in theory favour the free radical mechanism due to benzylic stabilisation of the radical and the unfavourable loss of aromaticity. However, experimental evidence showed that the unstable naphthalene derivative 21 was formed in 65% yield by the sigmatropic rearrangement of 1-napthalenemethanol 17. The sigmatropic rearrangement proceeds by the rate-limiting step of trans to cis isomerisation of the trans-propargylic alcohol followed by elimination of dinitrogen from the cis-isomer.

\[ \text{Scheme 14: Deoxygenation of 1-napthalenemethanol 17} \]

\( N\text{-iso-Propylidene-N'\text{-2-nitrobenzenesulfonyl hydrazine (IPNBSH) has been introduced by Movassaghi and Ahmad as an alternative reagent to NBSH, which can be synthesised by the dissolution of NBSH in acetone. It is used in the Mitsunobu displacement of alcohols, providing a greater choice of solvents than NBSH. The reaction can be carried out at a greater range of temperatures, and it increases the flexibility with respect to order of addition and concentration of substrate and reagent. In the deoxygenation of an alcohol following reaction with IPNBSH (Scheme 15), the intermediate hydrazone is stable and can therefore be isolated. It is then hydrolysed under mild conditions. IPNBSH can be stored for several months at room temperature, due to its higher thermal stability than NBSH, hence making its use advantageous compared to NBSH.} \)
The alcohol is converted to the hydrazone upon reaction with IPNBSH in the presence of diethylazodicarboxylate (DEAD) and triphenylphosphine in tetrahydrofuran in a fast and high yielding Mitsunobu inversion reaction. Direct hydrolysis of the hydrazone in a 1:1 mixture of trifluoroethanol and water leads to the elimination of acetone, forming the diazene. Loss of dinitrogen \( \text{via} \) a sigmatropic rearrangement or a free radical mechanism forms the unfunctionalised product.\(^{58}\)

Diazene chemistry has been identified as the most effective and advantageous method of removing hydrophilic functional groups from organic molecules since the extrusion of nitrogen occurs at a fast rate, in contrast to the loss of carbon dioxide in protodecarboxylation, often without the formation of intermediates. This again contrasts with decarboxylation, diazotisation and deoxygenation, which form either carbocationic or free radical intermediates, subsequently generating undesirable polar functional groups on reaction with water, or with potentially toxic side-effects. The compounds involved in diazene chemistry are water soluble, with the reaction schemes being water compatible, which allows potential biological applications.

1.4 Biological activity of hydrazines

In recent years, interest has grown in the development of synthetic routes towards substituted hydrazines. This is largely due to the significant biological activity of some hydrazines against diseases such as tuberculosis, Parkinson’s disease and hypertension.\(^{59,60}\) Many compounds containing the hydrazine functionality demonstrate neuroprotective properties and can be used as antidepressant drugs.\(^{61}\) Substituted hydrazines have also been used in peptidomimetics (Figure 5), which have been shown to be particularly active against hepatitis, AIDS and SARS.\(^{60}\)
Peptidomimetics that include a nitrogen atom in place of the α-carbon that carries the side chain (Figure 5) have increased in popularity due to the problems associated with the use of conventional peptides as therapeutic agents, such as low uptake, low bioavailability and rapid excretion following enzymatic breakdown. The replacement of the CH$_2$ with an aza group to form aza-peptides has little effect on the polarity of the compound, though it results in significant changes to the chemical and biological properties due to the change in conformation. Aza-peptides may display improved metabolic stability, bioavailability and biological absorption while peptides suffer from poor membrane permeability and can degrade upon enzymatic metabolism. Rapid stereoinversion of the nitrogen centre at room temperature effectively removes stereogenicity in aza-peptides, hence reducing the number of stereocentres in the molecules compared to the analogous carbon peptides. Aza-peptides have been developed as inhibitors of several serine and cysteine proteases, which have been identified as targets for the treatment of hepatitis C.

Phenelzine (22, Figure 6) can be used as a treatment for depression and other psychiatric disorders as it is an inhibitor of monoamine oxidase (MAO), which oxidises primary amines including neurotransmitters such as noradrenaline, dopamine and 5-hydroxytryptamine. In addition, phenelzine inhibits the activity of semicarbazide-sensitive amine oxidase (SSAO) which also converts primary amines into aldehydes, generating ammonia and hydrogen peroxide. In this capacity, phenelzine provides a treatment of inflammatory diseases including rheumatoid arthritis, inflammatory bowel disease, and type 1 and 2 diabetes, since the level of SSAO in the blood plasma or membrane typically increases in patients with inflammation-associated diseases.

There are few examples of light-sensitive hydrazine derivatives but β-lactam 23 (Scheme 16) has been described as a ‘light inactivated antibiotic’. An o-nitrobenzyloxycarbonyl (NBOC)
protecting group is attached to the hydrazine functionality and is removed over a time period of several hours, following irradiation with light. Artificial light or sunlight in the UV-visible wavelength range with an average \( \lambda_{\text{max}} \) for the chromophore at 320 nm, results in the decomposition of the \( \beta \)-lactam through the removal of the protecting group, leaving a highly reactive hydrazine moiety.\(^{67,68} \) The unprotected hydrazine (24) acts as a supernucleophile\(^ {69,70} \) instigating a rapid intramolecular reaction with the lactam carbonyl group at low concentrations, effecting the fission of the \( \beta \)-lactam moiety and removal of all antibacterial activity.

Scheme 16: Photocleavage of the NBOC-protecting group followed by opening of the cephalosporin and subsequent degradation

1.5 Synthesis of substituted hydrazines

A variety of methods have been developed for the preparation of substituted hydrazines and/or hydrazides in response to the increased demand in the literature, those most predominant can be categorised under two headings; alkylation and reductive amination.
1.5.1 Alkylation of a polyanion

In the synthesis of substituted hydrazines via the alkylation of a polyanion by Bredikhin et al. (Scheme 17), Boc-carbazate was chosen as a starting reagent. The tert-butyl ester (Boc) protecting group was shown in previous work to be a superior protecting group in preference to, for example, the carboxybenzyl (Cbz) protecting group, due to its increased stability in the presence of organolithium reagents. The three NH groups in Boc-carbazate have different acidities (difference of approximately 10 $K_a$ units, $pK_a = 15.1-17.3$) and hence have different reactivity of corresponding anions, which enables stepwise deprotonation of the molecule. Consequently, the use of fewer equivalents of butyl lithium and alkyl halide can be used to form mono- and di-substituted hydrazines in addition to the tri-substituted derivative illustrated above.

In alkyling the anion, methyl iodide and allyl bromide effectively formed the desired substituted hydrazine derivative, without using excess alkyl bromide. However, synthesis of the tri-substituted hydrazine with bromobenzene was unsuccessful, affording only the di-substituted product even with excess alkylating agent, as a result of steric effects. In addition, when Boc-carbazate was treated with two equivalents of $n$-butyl lithium followed by alkylation, the 2,2-dialkylated product was formed instead of the 1,2-dialkylated derivative as would be expected with respect to the varying acidities of the protons on the hydrazine moiety. This could be due to the rapid intramolecular equilibration of the anion in the reaction mixture (Scheme 18) or self-lithiation of the anion formed on deprotonation of Boc-carbazate leading to the formation of a dianion (Scheme 19).

![Scheme 17: Stepwise alkylation of a polyanion](image1.png)

![Scheme 18: Equilibration of the anion](image2.png)

![Scheme 19: Self-lithiation of the anion](image3.png)
1.5.2 Alkylation of di-tert-butyl-carbazate-1,2-dicarboxylate

Mono-, di- and trisubstituted hydrazines have been synthesised via the alkylation of di-tert-butyl carbazate-1,2-dicarboxylate 26 under mild conditions, with the selective removal of the two Boc groups by thermolysis. Previously, substituted hydrazine derivatives have been prepared using harsh conditions, i.e. strong bases (butyl lithium or sodium hydride) or high temperatures (80-100°C). The selective mono-allylation of di-tert-butyl carbazate-1,2-dicarboxylate 26 has been carried out using excess allyl bromide 27 under mildly basic conditions in moderate yield (Scheme 20).

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{O} \\
26 & \quad + \quad \text{Br} \quad \text{Br} \\
\text{K}_2\text{CO}_3 \text{(5eq)} & \quad \text{DMF, rt, 16h} \\
\text{O} & \quad \text{N} \\
27 & \quad \text{28} \\
\end{align*}
\]

Scheme 20: Alkylation of di-tert-butyl carbazate-1,2-dicarboxylate with an allylbromide

The use of potassium carbonate to deprotonate the hydrazide functionality resulted in the formation of very little of the disubstituted product, despite the use of excess allyl bromide 27. However, in changing the base to caesium carbonate, the disubstituted product 29 has been formed as the major product when the reaction was carried out under the same conditions (Scheme 21).

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{O} \\
26 & \quad + \quad \text{Br} \quad \text{Br} \\
\text{Cs}_2\text{CO}_3 \text{(2eq)} & \quad \text{DMF, rt, 14h} \\
\text{O} & \quad \text{N} \\
27 & \quad \text{29} \\
\end{align*}
\]

Scheme 21: Formation of disubstituted hydrazide 29

Conversely, alkylation of the hydrazide has been less successful, with alkylation reactions taking significantly longer than allylations. Phase transfer conditions were employed to overcome these problems, forming alkylated hydrazines in high yields (Scheme 22). Alkylation reactions with less powerful alkylating agents have been achieved in high yields by using a slight excess of the alkyl bromide with caesium carbonate in DMF.
1.5.3 Alkylation of Boc-carbazate acetone hydrazone

Phase transfer conditions have been utilised in order to provide a mild and non-anhydrous procedure for the alkylation of tert-butyl iso-propylidene carbazate that enabled the use of base-sensitive alkyl bromides (Scheme 23). A small excess of potassium hydroxide can be used to initiate the alkylation reaction as a substitute for sodium hydride, making it much safer and suitable for large scale synthesis. To obtain the alkylated product, the mixture is washed with water until a neutral pH is achieved, and then the solvent is removed in vacuo. This methodology is, however, limited to the use of primary alkyl bromides and activated chlorides. The Boc protecting group can be then cleaved using literature methods, i.e. 2 N hydrochloric acid in refluxing tetrahydrofuran (THF), which also hydrolyses the iso-propylidene group, liberating the monoalkylhydrazine.

1.5.4 Alkylation of Boc-carbazate

In this example, Boc-carbazate 32 is used as the starting reagent in the preparation of the Boc-protected mono-substituted alkyl hydrazide 33. Boc-carbazate 32 was reacted with bromononane in refluxing THF for 18 hours, leading to the formation of the product in 25% yield (Scheme 24). The low yield is due to the absence of a base, which would allow the reaction to go
to completion by removing the hydrobromic acid produced. In the absence of a base, the bromide anions are not removed preventing the progression of the reaction, and giving a low yield of the hydrazide.\textsuperscript{77} Due to the extremely low-yielding nature of the reaction, this methodology was excluded from consideration in the first instance.

1.5.5 Reductive amination of aldehydes/ketones

The preparation of substituted hydrazines has been described \textit{via} the reductive amination of aldehydes and ketones in a number of literature examples (Scheme 25).\textsuperscript{63,78–80} Indeed, it has emerged as the most efficient method of hydrazine formation. Initially, the mono-protected hydrazide is converted to the hydrazone through condensation with an aldehyde or ketone in the rate-determining step of the process, which proceeds \textit{via} nucleophilic attack of the nitrogen on the electron-deficient carbonyl carbon. Elimination of water leads to the formation of a hydrazone (Scheme 26). The protected hydrazone is then reduced to a hydrazine \textit{via} palladium-catalysed hydrogenation or by reaction with a reducing agent, e.g. sodium cyanoborohydride.\textsuperscript{78,79}

In the hydrazone formation step, Boc-carbazate has been combined with the appropriate aldehyde or ketone in a variety of organic solvents of differing polarities, depending on the solubility of the corresponding carbonyl compound. The hydrazones were obtained in poor to excellent yields, with short-chain aliphatic and aromatic aldehydes/ketones generally forming the products in higher yields than long-chained/bulky compounds.\textsuperscript{63} The significantly lower yields can be attributed to steric hindrance and solubility issues of bulky compounds, both of which would hinder the progress of the reaction. Slight variations were made to the conditions under which the hydrazone was formed in the literature examples observed; molecular sieves were used in one such case to remove water due to the volatility of the aldehydes used, though generally the reaction was simply described as the condensation of an aldehyde/ketone and Boc-carbazate.\textsuperscript{80} The reaction was variably carried out at room temperature or in refluxing solvent, depending on
the solubility and the rate of reaction, with sterically bulkier compounds generally requiring higher reaction temperatures. In some cases, a catalytic amount of acetic acid was added to promote the formation of the hydrazone by increasing the electrophilicity of the carbonyl carbon, through protonation of the oxygen. Hydrazones are most hydrolytically stable at neutral and slightly acidic pH values.\textsuperscript{81}

The reduction of the hydrazone has generally been carried out by reaction with sodium cyanoborohydride, a reagent commonly used in the reductive amination of aldehydes and ketones.\textsuperscript{79,80,82} The cyanohydridoborate anion can be used to reduce a variety of organic functional groups with high selectivity.\textsuperscript{82} In the presence of sodium cyanoborohydride, aldehydes and ketones are reduced to primary/secondary alcohols most rapidly under strongly acidic conditions (pH 3-4), while the reductive amination of carbonyl compounds with ammonia, primary or secondary amines is quickest under mildly acidic conditions (pH 6). The pH-dependence of the reduction reaction gives a highly effective method of controlling the chemical transformation, while minimising the generation of side-products, such as the alcohol. The pH-control concurs efficiently with the acid stability of the cyanohydridoborate anion, which is stable in acidic solutions as low as pH 3.\textsuperscript{82}

The reduction of hydrazones is more difficult than the reduction of imines as a result of the delocalisation of the nitrogen lone pair which increases the stability of the C-N double bond, while reducing its electrophilicity. The addition of a carbazate protecting group to the terminal nitrogen of the hydrazone functional group increases the ease of reduction. The level of delocalisation is reduced due to the participation of the protecting group that withdraws electron density from the adjacent nitrogen and, in turn, the C-N double bond. In this way, the $\delta^+$ charge on the hydrazone carbon is increased, in comparison to unprotected hydrazones, lowering the energy of the LUMO, hence making it more susceptible to nucleophilic attack.

The reduction initially proceeds \textit{via} reversible protonation of the hydrazone nitrogen, followed by hydride transfer to the adjacent carbon, subsequently quenching the cationic intermediate (Scheme 27). Hydride transfer to the protonated structure has been previously shown to be the rate-determining step for the reduction process.\textsuperscript{82} Although the optimal pH for the reductive amination of carbonyl compounds is pH 6-8, the reaction has been found to proceed at pH 4-10 since the only requirement is the availability of a sufficient proton source to generate a cationic
intermediate imine due to protonation. Analogous to the reduction of ketones and aldehydes to alcohols by sodium borohydride, all three of the hydride ions in the cyanoborohydride moiety can be utilised in the reductive amination. Typically, a five-fold excess of the reducing agent is used to speed up the reaction, while preventing over-reduction when carbonyl side groups were used on the hydrazone, since sodium cyanoborohydride selectively reduces hydrazones at certain pH values.

Scheme 27: Reduction of a hydrazone with the cyanohydridoborate anion

The cyanoborane adduct formed on reaction of the hydrazine with sodium cyanoborohydride was hydrolysed under acidic or basic conditions, either in the same or a separate step to the reduction of the hydrazone, depending on the stability of the hydrazone to reaction with both reagents.

Alternatively, Dutta et al. have carried out the hydrogenation of the hydrazone moiety over 5% palladium-carbon to form the hydrazine functional group, although the use of this technique resulted, in some cases, in over-reduction to the unfunctionalised molecule. Diisobutylaluminium hydride (DIBAL) can be used as an alternative reducing agent, which is normally used for the reduction of esters and cyano derivatives to aldehydes. Electron-rich hydrazines were found to react at a faster rate than electron-deficient analogues, and were often reduced further to allylic alcohols. This would be a significant difficulty in the project if hydrazine derivatives with ester side groups were synthesised.

It was decided that sodium cyanoborohydride reduction of hydrazones would be the best method for synthesising substituted hydrazines as this reagent appears to give the highest yields with fewest by-products.

1.6 Identification of a test protecting group

The tert-butyloxycarbonyl (Boc) protecting group was identified as the best protecting group for the hydrazine group due to its stability towards basic and nucleophilic reagents, and also the
increased solubility of Boc-protected compounds compared to fluorenylmethyloxycarbonyl (Fmoc) protected analogues. However, in the solid phase synthesis of mixed peptidomimetics, the Fmoc-protecting group is instead employed due to the ease of removal of the Fmoc-group under basic conditions and its stability to acidic conditions which enables the addition of acid-labile protecting groups to side chains. The use of Fmoc-protecting groups also lends itself most readily to the synthesis of peptidomimetics since other standard Fmoc-protected building blocks can be incorporated into the scheme.

1.7 Project outline: Synthesis

To develop a chemical method for the removal of polar functional groups from molecules (Scheme 7, see page 42), a synthetic route to sulfonyl hydrazides will be developed that will decompose to form unfuctionalised lipids.

1.7.1 Acid-triggered decomposition

Initially, the reductive amination of a range of aliphatic and aromatic aldehydes with a number of electron-donating and -withdrawing substituents will be attempted to monitor the steric and electronic effects on the rate of reaction. Reductive amination will be carried out with Boc-carbazate and sodium cyanoborohydride with an accompanying proton source using methods established in the literature (Scheme 29).

Subsequently, procedures for the addition of substituted aromatic sulfonyl-containing compounds will be investigated following analysis of the relative rates of decomposition of the corresponding sulfonyl hydrazides. The Boc-protected hydrazines were chosen as test molecules since conditions for the cleavage of the Boc-protecting group are well known in the literature. Cleavage of the Boc-protecting group will trigger the decomposition of the sulfonyl hydrazide via a
sigmatropic rearrangement or free radical mechanism resulting in the formation of the reduced form of the product upon elimination of the sulfonyl group and nitrogen via an unstable diazene intermediate (Scheme 30).

Scheme 30: Decomposition of Boc-protected sulfonyl hydrazide via a diazene intermediate

1.7.2 Identification of an aromatic sulfonyl leaving group

Remotely triggered decomposition of the sulfonyl hydrazide would increase the potential for the biological applications of the library of hydrazine derivatives. The substituted hydrazide, formed on reaction of the compound with benzene disulfonic anhydride, is highly polar due to the anionic character of the compound. The hydrophilicity of the molecule would result in its inability to cross the cell membrane, which is highly lipophilic, though it would be efficiently transported to the active site in an aqueous solution, i.e. blood, due to the high water-solubility of the sulfonyl-substituted molecule. Following photolysis of the structure and subsequent generation of the active molecule, which would be membrane permeable and so absorbed into the cell, the anionic by-product would be quickly excreted from the biological system, avoiding any adverse side-effects.

The decomposition of the deprotected sulfonyl hydrazide should progress at a relatively fast rate to prevent the formation of intermediates, which could participate in side reactions, preventing the formation of the unfunctionalised product. Studies of the fragmentation of nitrobenzene sulfonyl hydrazides (35) to form the corresponding sulfinic acid (36) following the elimination of nitrogen were carried by Davies et al. (Scheme 31). It was found that the order of stability of substituted sulfonyl hydrazides decreased in the order meta > para > ortho, due to the electronic effect of the nitro substituent on the aromatic ring.

Scheme 31: Fragmentation of nitrobenzene sulfonyl hydrazides
In the reductive deamination of aliphatic and aromatic amines, benzene-, p-toluene- and methane sulfonyl chloride were reacted with amines to initiate fragmentation to the unfunctionalised product (Scheme 32). The yields of the deaminated product were not significantly affected by the nature of the sulfonyl chloride used, though the rates of reaction are not mentioned. It is stated that in some cases, a significant amount of starting material was recovered following the reaction which was recycled to obtain the products in high yields, indicating a slow reaction rate.

![Scheme 32: Reductive deamination of amines](image)

Therefore, an electron-withdrawing aromatic sulfonyl group will be used in the project, since the deprotected sulfonyl hydrazide must decompose quickly to avoid the formation of intermediates which may participate in side reactions. Preliminary studies by Dr Simon Webb indicated that the benzene sulfonyl hydrazide generated on reaction of an aromatic hydrazide derivative with 1,2-benzene disulfonic anhydride decomposed rapidly in water (Scheme 33).

![Scheme 33: Formation and decomposition of sulfonyl hydrazide synthesised using 1,2-benzene disulfonic anhydride](image)

1.7.3 Light triggered decomposition

In order to make the project biologically applicable, photosensitive protecting groups will need to be included in the hydrazines following the development of a successful synthetic route to Boc-protected sulfonyl hydrazides. By using light to cleave the protecting group and hence trigger the decomposition of the molecule, harsh chemical conditions, i.e. strong acid as is required for the deprotection of Boc-protected compounds, will no longer be required, making the process suitable for use in biological environments. The synthesis and subsequent photodeprotection of photochemically-protected hydrazines and sulfonyl hydrazides will be investigated, to examine the respective rates and yields of formation of the reduced products.
1.7.4 Selection of suitable photosensitive protecting groups

Photocleavable protecting groups, such as 2-nitrobenzyloxycarbonyl (NBOC, 37, Scheme 34) and 6-nitroveratryloxycarbonyl (NVOC, 38, Scheme 34) have been used in organic syntheses involving amino acids and peptides, etc. These protecting groups have been shown to be stable towards a variety of chemical conditions and readily cleavable via an internal redox reaction that is initiated by a photochemical reaction. The photoisomerisation of NBOC-protected carbonates, carbamates and esters leads to the conversion of the structures to an acetal derivative via the abstraction of the benzylic proton ortho to the nitro group.

The mechanism of the photocleavage of NBOC- and NVOC-protected compounds proceeds by initial transfer of the benzylic proton to an oxygen on the nitro group to form Z-nitronic acid 39/40 (Scheme 34). Reprotonation of dianion 41/42 forms E-nitronic acid 43/44, which undergoes cyclisation to form the N-hydroxybenzisoxazoline 45/46, which decays immediately to form nitrosobenzaldehyde by-product 47/48 and the deprotected product, after elimination of carbon dioxide. It has been suggested, based on calculations that the transformation of anionic intermediate 41/42 to cyclic intermediate 45/46 is prohibitively endothermic, thereby explaining the geometric isomerisation of the acids that proceeds via the formation of anion 41/42. The reprotonation of the anion also explains the dependence of the decay rate on proton concentration. No spectral evidence has been obtained for the formation of the cyclic intermediate so it is assumed that it decays at the same rate as it is formed.

Scheme 34: Mechanism of NBOC/NVOC-photocleavage

NBOC-, NVOC and carboxybenzyl (Cbz) protected sugars have been synthesised with reagents 49-51 (Figure 7) to compare the stability of the protecting groups in the presence of hydrogen chloride in acetic acid or a mixture of acetic acid and trifluoroacetic acid (when there was reduced
solubility. The results showed that the order of stability was as expected, Cbz < NVOC < NBOC due to the effect of the aromatic nitro or methoxy substituents on the benzyl intermediate.

![Figure 7: NBOC-chloride (49), NVOC-chloride (50) and Cbz-chloride (51)](image)

In a similar study of NBOC- and NVOC-derivatives of amino acids and peptides, photoremoval of the protecting groups has been judged to be quantitative by measurement of carbon dioxide released, with irradiation times of between one and 24 hours. The NBOC- and NVOC-protecting groups were cleaved from the amino sugars at wavelengths of 320-400 nm. The photolysis of the protecting group did not affect additional functionality in the molecule, giving the protecting groups the potential to be used in a variety of molecules with a vast range of prospective applications. However, it was found that the resulting amino functionality underwent further reaction with the nitrosobenzaldehyde by-product of the photochemical reaction (Scheme 35).

![Scheme 35: Formation of imine condensation product with nitrosobenzaldehyde by-product](image)

These nitroso side-products have been shown to be problematic, since they are reactive towards common functional groups in biologically active molecules and are toxic towards living systems. Aldehyde reagents or sulfuric acid have been added in order to improve the yield of deprotected amino derivatives by avoiding the occurrence of side-reactions. It was found that the product and the sacrificial agents were difficult to separate, so polymeric aldehyde reagents were utilised in the majority of cases, in order to simplify the purification process. Furthermore, the addition of the polymeric aldehyde reagents did not affect additional functionality within the molecule, including acetate, glycoside and hydroxyl groups. Where competing side reactions did occur between the aldehyde by-product and the free amine, hydroxylamine hydrochloride, semicarbazide hydrochloride or hydrazine also increased the yield and purity of generated products by acting as aldehyde scavengers.

The NVOC-protecting group (38) has considerable advantages over NBOC (37) since the two methoxy groups on the phenyl ring effectively shift the absorption maximum to longer
wavelengths, allowing NVOC-protected substrates to be used in syntheses involving tryptophan residues, which deteriorate after irradiation at 320 nm.\textsuperscript{83} In addition, 4,5-dimethoxy-2-nitrosobenzaldehyde by-product 48 is less reactive towards the deprotected amine than nitrosobenzaldehyde 47 that is generated following the photolysis of NBOC-protected amines. The presence of the two methoxy groups on by-product 48 reduces the electrophilicity of the by-product, thus reducing its reactivity.\textsuperscript{83}

In order to improve the efficiency of photolabile protecting groups such as NBOC (37) and NVOC (38), a number of modifications have been made to the benzylic site to increase the quantum yields of photolysis. As substitution at this site leads to the formation of a chiral centre unless the substituent is identical to the aromatic core of the compound, it can be problematic in cases where chiral molecules are being caged, e.g. in the protection of amino acids and peptides. Substitution at the benzylic position also avoids the formation of the nitrosobenzaldehyde side product, forming instead a ketone of lower reactivity, thereby eliminating the condensation reaction between the side product and the free amine.

Alternative photolabile protecting groups include 2-nitrophenyl(phenyl)methoxycarbonyl (Ndmoc, 52), 2,2'-dinitrodiphenylmethoxycarbonyl (Dnboc, 53) and methyl nitropiperonylcarbonyl (Menpoc, 54) groups (Figure 8). The additional substitution at the benzylic carbon increases the rate of deprotection via abstraction of the proton on the α-methylene carbon by the photoexcited nitro group.\textsuperscript{85} The increased substitution at the benzylic carbon is additionally advantageous because fewer side-reactions take place since the ketone by-products generated in the photolysis reactions are considerably less reactive than the aldehyde photoproducts formed in the photolysis of NBOC- (37) and NVOC-protected (38) substrates. However, NDMOC- (52), DNBOC- (53) and MENPOC-protected (54) substrates were found to be harder to synthesise due to a combination of steric and electronic effects, and thus are not as popular as NVOC (38) or 2-nitrophenylpropyloxycarbonyl (NPPOC, 56, Figure 9).\textsuperscript{87}

Figure 8: NDMOC (52), DNBOC (53) and MENPOC (54) protecting groups
Replacement of the methyl group in the NBOC group with an ethylene link to form the o-nitro-2-phenethyloxycarbonyl group (NPEOC, 55, Figure 9) was found to increase the rate of photolysis. The substitution of a methyl group at the benzylic position of NPEOC (55), developed by Hasan and co-workers to improve the quantum yield of the NBOC-group (38), resulted in the formation of the NPPOC-group (56). In studies carried out by the same group, the NPEOC (55) protecting group was shown to undergo photocleavage at a rate approximately 1.8-fold greater than the NBOC-group (37). Methylation of the benzylic position resulted in a 9.4-fold increase in the rate of photolysis of the NPPOC-group (56) compared to NPEOC (55). Addition of a second benzylic o-nitrobenzyl group led to an 8.4-fold increase in the rate of deprotection of the DNBOC protecting group (53) due to an increase in the rate of hydrogen abstraction as a result of the addition of a hydrogen abstraction unit. The modification of the aromatic ring in NPEOC (55) was also studied, though addition of a fluorine atom in the ortho position relative to the alkyl group led to a slower rate of photolysis, while o-substitution of the larger halogen atoms and a second nitro group all led to a small increase in photolysis rates due to steric and/or inductive effects. Substitution of chloro- and methoxy-groups at the meta and/or para positions had less effect on the rates of photolysis.

The mechanism of deprotection of the NPPOC-group (56) proceeds by the deprotonation of the benzylic site by the nitro group, similar to the photocleavage of the NVOC-group (37). This initiates loss of the aromaticity via photoenolisation, which is regained following decarboxylation to form styrene by-product 58 (Scheme 36). By-product 58 is significantly less toxic than nitrosobenzaldehyde by-product 48 that is formed by the photolysis of NVOC-caged compounds since it is an alkene, which is significantly less susceptible to side reactions with amine nucleophiles. The presence of the nitro group also lowers the reactivity of by-product 58 compared to the nitroso functionality in by-product 48.
Due to the high quantum yield and efficiency of photorelease of NPPOC-protected substrates, the group has become increasingly popular and is used in automated light-directed oligonucleotide synthesis. In the synthesis of DNA chips, NPPOC-protected amino acids and carbohydrates attached to the chip and are selectively uncaged upon irradiation by using a mask to expose only the desired compounds to irradiation. The uncaged compounds are then reacted with desired nucleotide in order to extend the oligomer. Repeated caging, masking, selective uncaging and synthesis led to the development of a long-chain oligonucleotide through photolithographic synthesis. However, as shown in recent examples, sensitisers are required to photodeprotect NPPOC-protected derivative using two photon excitation, since these are inefficient in two-photon deprotections.

1.8 Summary and Outlook

In summary, after reviewing the related literature in the field, it has been found that there has been little research previously undertaken on the remote generation of unfunctionalised amphiphiles. There has been no research carried out describing the use of diazene chemistry to unmask lipids, e.g. through the removal of polar, hydrophilic functional groups in water triggered by light. Therefore, the potential outcome of the project will be unique and highly applicable to synthetic biology.
Chapter 2 – Development of a Synthetic Route towards Boc-Protected Hydrazides

Boc-protected 4-methoxybenzylhydrazine 60 was identified as an initial synthetic target (Scheme 37), which would be used to develop a synthetic strategy towards more complex molecules in the project. 4-Methoxybenzaldehyde 59, which would be used as the starting material in the reductive amination, is commercially available and analogous amphiphilic compounds could be synthesised, e.g. by replacing the methyl group with a longer alkyl chain.

A benzene sulfonyl chloride or anhydride derivative would be added to the hydrazide which would act as the solubilising agent in the sulfonyl hydrazides. Such molecules have been used in previous examples to increase the water-solubility of compounds due to their high polarity and consequent hydrophilicity, e.g. in the synthesis of aminosulfonamide ligands to be used in enantioselective transfer hydrogenation.\textsuperscript{91} Substituted benzenesulfonyl hydrazides have been shown to rapidly fragment in polar solvents such as water and methanol, so it was hypothesised that the cleavage of a protecting group to reveal a free hydrazine moiety would trigger this decomposition.\textsuperscript{43,51,53} The tert-butyloxycarbonyl (Boc) protecting group was used in the initial development of a synthetic strategy towards protected sulfonyl hydrazides due to its stability to basic conditions, therefore having the ability to withstand a variety of chemical transformations prior to cleavage.

Scheme 37: Proposed synthesis and decomposition of sulfonyl hydrazide 61

A benzene sulfonyl chloride or anhydride derivative would be added to the hydrazide which would act as the solubilising agent in the sulfonyl hydrazides. Such molecules have been used in previous examples to increase the water-solubility of compounds due to their high polarity and consequent hydrophilicity, e.g. in the synthesis of aminosulfonamide ligands to be used in enantioselective transfer hydrogenation.\textsuperscript{91} Substituted benzenesulfonyl hydrazides have been shown to rapidly fragment in polar solvents such as water and methanol, so it was hypothesised that the cleavage of a protecting group to reveal a free hydrazine moiety would trigger this decomposition.\textsuperscript{43,51,53} The tert-butyloxycarbonyl (Boc) protecting group was used in the initial development of a synthetic strategy towards protected sulfonyl hydrazides due to its stability to basic conditions, therefore having the ability to withstand a variety of chemical transformations prior to cleavage.
2.1 Reductive amination of aldehydes

2.1.1 Reductive amination of 4-methoxybenzaldehyde 59

Boc-protected 4-methoxybenzylhydrazide 60 was obtained via the reductive amination of 4-methoxybenzaldehyde 59. Initially, aldehyde 59 was reacted with Boc-carbazate 32 in refluxing methanol in the presence of molecular sieves, which served to remove the water generated by the reaction (Scheme 38).\textsuperscript{80} NMR spectroscopy showed that aldehyde 59 had not been converted to hydrazone 65.

 Succesful conversion of aldehyde 59 to hydrazone 65 was achieved on combination of the starting materials in refluxing iso-propanol.\textsuperscript{79} The efficient conversion of aldehyde 59 to hydrazone 65 in iso-propanol may be a result of higher solvation of the aldehyde in iso-propanol compared with methanol. Methanol could also compete with Boc-carbazate 32 as it is less sterically hindered so the electron deficient carbonyl carbon could undergo nucleophilic attack by the methanol oxygen lone pair. The hydrazone was purified by column chromatography on silica, eluting with varying ratios of petroleum ether and ethyl acetate, demonstrating the stability of compound. The formation of hydrazone 65 was confirmed by NMR spectroscopy, mass spectrometry and infrared spectroscopy.

Reduction of resulting hydrazone 65 by a fivefold excess of sodium cyanoborohydride (1 M solution in THF) in the presence of acetic acid in methanol led to the efficient conversion of hydrazone 65 to hydrazide 60, following hydrolysis of the cyanoborane adduct on reaction with sodium hydroxide in methanol (Scheme 39).\textsuperscript{80} \textit{para}-Toluenesulfonic acid was also used as a proton source to promote the reduction of hydrazone 65 to hydrazide 60,\textsuperscript{79} though acetic acid was shown to more efficiently promote the reduction step. This is because \textit{para}-toluene sulfonic acid is a stronger acid than acetic acid. The optimum pH for the reduction of hydrazones with sodium cyanoborohydride is pH 6, making acetic acid a more suitable proton donor.\textsuperscript{82}
The formation of hydrazone 65 proceeds by an $S_{N}Ac$ mechanism. Initially, the electrophilic carbonyl carbon of aldehyde 59 undergoes nucleophilic attack by the terminal nitrogen on Boc-carbazate 32 (Scheme 40). The lone pair of electrons on the nitrogen atom is then donated into the adjacent bond, leading to the formation of the hydrazone moiety. The formation of the hydrazone is driven by the elimination of water.

Initially, the reduction reaction was carried out in anhydrous THF, as in the literature example. However, hydrazone 65 did not dissolve completely in THF. Therefore, the solvent was replaced with anhydrous methanol, which, as a polar solvent, resulted in improved solubility and subsequently improved the yield from 54% to 63%. Hydrazide 60 was formed in sufficient purity to be used in the next step of the synthesis without further purification.

The reduction of hydrazone 65 proceeds initially by the protonation of the hydrazone nitrogen, hence increasing the electron deficiency of the hydrazone carbon (Scheme 41), which increases its susceptibility to attack by a hydride anion. Subsequent hydrolysis of the cyanoborane adduct resulted in the formation of the hydrazide in 46% overall yield.
2.1.2 Reductive amination of 4-hexadecyloxybenzaldehyde 68

Following the development of the above synthetic strategy, the analogous Boc-protected 4-hexadecyloxybenzyl hydrazine 70 was synthesised. The increased lipophilicity of hydrazide 70 due to the long alkyl chain of the ether substituent would increase its biological suitability, since it would form a non-polar organic compound following deprotection and decomposition. Aldehyde 68 was also selected because the corresponding hydrazide was significantly easier to purify.

4-Hexadecylbenzaldehyde 68 was synthesised via the Williamson ether synthesis (Scheme 42), through the reaction of 4-hydroxybenzaldehyde 68 with hexadecylbromide 66 in the presence of potassium carbonate and potassium iodide in N,N-dimethylformamide (DMF).

\[
\text{CH}_3\text{(CH}_2\text{)_{15}}\text{Br} + \text{C}_6\text{H}_4\text{(OH)}\text{CH}_2\text{OH} \xrightarrow{\text{K}_2\text{CO}_3, \text{KI}} \text{C}_6\text{H}_4\text{(CH}_2\text{)_{15}}\text{CHO} \quad \text{DMF, 70°C, 16h, 77%}
\]

Scheme 42: Synthesis of 4-hexadecyloxybenzaldehyde 68

The potassium iodide salt is used to catalyse the transformation through the formation of the much more reactive alkyl iodide via halogen exchange with alkyl bromide 66 (Scheme 43). The alkyl iodide has a faster associated rate of reaction than the alkyl bromide due to the increased leaving group ability of the iodide ion due to its increased ionic radius, which results in lower basicity and consequently pK_a. The reaction follows an S_n2 mechanism in which the alkyl halide undergoes nucleophilic attack by the oxygen atom on the aromatic ring in aldehyde 68, leading to the formation of aldehyde 68 upon elimination of the iodide leaving group. DMF is used as the solvent in the reaction since, as an aprotic and polar solvent, it increases the availability of the nucleophile.

Scheme 43: Mechanism of Williamson ether synthesis of 4-hexadecyloxybenzaldehyde 68

Aldehyde 68 was then reacted with Boc-carbazate 32 to form hydrazone 69 (Scheme 44). Subsequent reduction with sodium cyanoborohydride and acetic acid in THF, followed by hydrolysis of the cyanoborane adduct with sodium hydroxide, led to the generation of hydrazide.
70 in an overall yield of 43%. THF was found to be a suitable solvent for the reduction of hydrazone 69 as it led to the efficient formation of hydrazide 70 in moderate yield, differing from the reduction of hydrazone 65, which was carried out in methanol due to solubility issues. The long alkyl chain on hydrazone 69 increases its lipophilicity, thus increasing its solubility in THF.

\[ \text{Scheme 44: Reductive amination of 4-hexadecyloxybenzaldehyde 68} \]

The yield of formation of hydrazone 69 (94%, Scheme 44) compares favourably with that of methoxy analogue 65 (73%, Scheme 39). The increased yield could be attributed to the increased solvation in iso-propanol of aldehyde 68 and subsequently hydrazone 69 due to their increased lipophilicity compared to aldehyde 59 and hydrazone 65, which led to increased reactivity. Hydrazides 60 and 70 were obtained in very similar overall yields of 46% and 43% respectively.

2.1.3 Reductive amination of 4-carboxymethylbenzaldehyde 71

The reactivity of an aromatic aldehyde with an electron-withdrawing substituent on the aromatic ring was investigated through the reductive amination of 4-carboxymethylbenzaldehyde 71. The use of an ester group in the synthetic scheme would also enable subsequent hydrolysis of the ester group to expose a carboxylic acid group, thereby adding a polar group to the structure.

\[ \text{Scheme 45: Reductive amination of 4-carboxymethylbenzaldehyde 71} \]

Hydrazone 72 was formed in high yield and purity on reaction of aldehyde 71 with Boc-carbazate 32 (Scheme 45), comparing favourably with the yields of formation of alkoxy-substituted hydrazones 65 (Scheme 39) and 69 (Scheme 44). This could be due to the increased electrophilicity at the carbonyl carbon due to the effect of the electron-withdrawing substituent on the aromatic ring. The rate of nucleophilic substitution at the electron-deficient aldehyde site
by the nitrogen lone pair is subsequently increased. The formation of imines and therefore hydrazones is generally the rate-determining step in reductive amination processes. Furthermore, hydrazone 72 would be more thermodynamically favourable than the alkoxy derivative 65 and 69 due to resonance stabilisation in the hydrazone structure (Scheme 46).

Scheme 46: Resonance stabilisation of hydrazone 72

However, reaction of hydrazone 72 with sodium cyanoborohydride and acetic acid in THF, followed by methanolysis of the cyanoborane adduct generated impure hydrazide 73 in low yield (16%, Scheme 45). The electron-withdrawing effect of the ester substituent on the hydrazone moiety can explain the low yielding nature of the reaction. The availability of the hydrazone nitrogen lone pair is reduced significantly, which reduces its ability to accept a proton, therefore prohibiting hydride transfer to form hydrazide 73. In addition, the equilibrium would lie towards the left in the reaction since hydrazone 71 is more thermodynamically favourable than hydrazide 73 due to resonance stabilisation (Scheme 46). Due to the slow reaction rate and low yield and purity of the hydrazide synthesised, it was decided that the ester functionality was incompatible with the synthetic scheme so research into this ester functionality was discontinued.

2.1.4 Reductive amination of decanal 75

The reductive amination of decanal was investigated in order to test the reactivity of aliphatic aldehydes towards Boc-carbazate 32 in the established synthetic scheme. The formation of aliphatic hydrazines has been described in the literature, though the reactions shown had low associated yields, e.g. 25 – 46%, and used aldehydes of different carbon chain lengths, e.g. 8 – 9. The reductive amination of decanal 75 was therefore investigated to optimise the reaction conditions for the conversion of decanal 75 to hydrazide 77 (Scheme 47).
Scheme 47: Reductive amination of decanal 75

Hydrazone 76 was formed in poor yield following the reaction of aldehyde 75 and Boc-carbazate 32 in refluxing iso-propanol. Due to the lack of conjugation in aldehyde 75 and thus in hydrazone 76, the equilibrium lies towards the left in the reaction as a result of the instability of the product. This explains the slower reaction and lower yield of formation of alkyl hydrazone 76 compared to the aromatic analogues 65, 69 and 72. A large amount of aldehyde 75 was identified in the NMR spectrum, despite being shown by thin layer chromatography to have been consumed by the reaction. This indicates that the hydrazone was unstable and underwent hydrolysis upon isolation due to the lack of conjugation in the structure and therefore its low stability. The hydrazone was formed in moderate purity though could not be purified due to limited solubility in solvents other than methanol. Therefore, the crude product was reduced directly, forming hydrazide 77 in low yield and moderate purity, though column chromatography proved an ineffective method of purifying the hydrazide since unidentifiable decomposition products were observed, indicating hydrazide 77 is unstable on silica.

As a result, an alternative literature method for the hydrazone formation step was utilised, in which aldehyde 75 was reacted with Boc-carbazate 32 in refluxing methanol in the presence of acetic acid.93

Scheme 48: Modified reductive amination of decanal 75

The carbonyl oxygen is protonated by the acid, generating a cationic charge and subsequently increasing the electrophilicity of the carbonyl carbon (Scheme 49). The susceptibility of the carbonyl carbon to undergo nucleophilic attack is subsequently increased, hence increasing the rate of addition of Boc-carbazate 32.

Scheme 49: Mechanism of the preparation of hydrazone 76
The yield and purity of hydrazone 76 were moderately increased compared to the previous method (Scheme 47), though reduction to hydrazide 77 was extremely low yielding and gave an impure product, which again proved difficult to purify. The addition of molecular sieves succeeded in increasing the purity of hydrazide 77, though the yield remained low (18%). Therefore, research into the synthesis of hydrazide 77 was discontinued.

### 2.2 Deprotection of Boc-protected hydrazine derivatives

Hydrazides 60 and 70 were deprotected to determine the stability of the free hydrazines and their reactivity towards sulfonyl chlorides or anhydrides. This would enable the determination of the optimal order of reaction, i.e. whether the hydrazide should be deprotected before or after the addition of the sulfonyl solubilising group (Scheme 50 A and B).

![Scheme 50: Alternate synthetic schemes for deprotection/substitution of hydrazide derivatives](image)

The deprotection reaction proceeds by the protonation of the carbonyl oxygen generating a cationic charge on the oxygen (Scheme 51). This initiates elimination of the Boc-group through decarboxylation in an E1 elimination mechanism.
2.2.1 Deprotection of Boc-protected 4-methoxybenzylhydrazine 60

Initially, traditional methods of Boc-deprotection using trifluoroacetic acid (TFA) in dichloromethane were utilised. The reactions were carried out under nitrogen to prevent oxidation. Although various concentrations of TFA (10-50%) were used in the deprotection of Boc-protected hydrazine 60, the protecting group was not cleanly removed (Scheme 52). Partial degradation of the compound was observed, possibly due to the high sensitivity of the hydrazine moiety to the acid, as is indicated by the growth of indistinguishable resonances in the NMR spectrum after treatment with strong acid. The free hydrazine may have been formed upon initial reaction with TFA though the structure degraded, which could be due to attack of the acid on the unstable hydrazine moiety. Therefore, it was concluded that milder conditions were required to promote the cleavage of the Boc protecting group, while avoiding degradation of the hydrazine.

Thus, alternative conditions that used hydrochloric acid (anhydrous, i.e. gaseous HCl or in solution of diethyl ether or dioxane) for the cleavage of Boc groups from hydrazides were researched. Boc-protected hydrazine 60 was reacted with hydrochloric acid (2 M solution in diethyl ether) in methanol at room temperature (Scheme 53) under nitrogen. The reaction was extremely slow, taking several days for the starting hydrazide to be consumed even though a fivefold excess of hydrochloric acid was used. After seven days, hydrazide 60 was shown to have been consumed by thin layer chromatography (TLC) and the solvent was removed under vacuum. The product

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Scheme 51: Mechanism of Boc-deprotection of the hydrazide functionality

Scheme 52: Deprotection of hydrazide 60 with TFA
was shown to have degraded, possibly due to the effect of the acid on the reactive hydrazine group of the deprotected hydrazine.

Scheme 53: Attempted deprotection of hydrazide 60

The reaction time was reduced to 48 hours by carrying out the deprotection process in refluxing methanol (Scheme 54), generating a cleaner product. The deprotection was monitored by TLC and the product was isolated as the hydrochloride salt by the removal of the solvent in vacuo in almost quantitative yield.

Scheme 54: Deprotection of hydrazide 60 with HCl

2.2.2 Deprotection of Boc-protected 4-hexadecyloxybenzylhydrazine 70

Hydrazide 70 was deprotected using hydrochloric acid in diethyl ether in refluxing methanol under nitrogen, forming HCl salt 83 in high yield after 24 hours (Scheme 55). The deprotection process took place at a higher rate than methoxy analogue 60, possibly due to the increased solvation as a result of the long alkyl chain. However, hydrazine 83 was obtained in a slightly lower yield than 82. If the reaction was left for 48 hours to increase the yield as for the deprotection of hydrazide 60, degradation was observed upon isolation of hydrazine 83. Indeed,
The decomposition to ether 78 was inferred (Scheme 56) by mass spectrometry if hydrazine 83 remained in solution for an extended period.

**Scheme 56: Decomposition of free hydrazine 83 to ether 78**

Ether 78 may have formed from the oxidation of hydrazine 83 to corresponding hydrazone 84 after being left in solution for an extended period. The hydrazone could tautomerise to form diazene 85, from which nitrogen would be eliminated to form ether 78 (Scheme 57).

**Scheme 57: Possible decomposition of hydrazine 83**

The deprotected hydrazides 82 and 83 were deprotonated by the addition of a molar equivalent of a weak base such as potassium carbonate or sodium bicarbonate to the NMR sample in deuterated chloroform (Scheme 58). NMR spectroscopy showed that the free hydrazine had been formed due to the slight change in the chemical shifts of the aromatic and benzylic protons compared to hydrazines 82 and 83. However, the products decomposed upon removal of the solvent in vacuo due to the instability of the hydrazine functionality and its sensitivity to air.

**Scheme 58: Deprotonation of the HCl salts 82 and 83**

Due to their apparent instability in addition to the difficulty in their preparation, i.e. decomposition following isolation of the deprotonated hydrazines, it was decided that this series of reactions was inappropriate for the project, since the decomposition of the hydrazine derivative should be remotely triggered by the removal of a protecting group.
As the substitution of the sulfonyl anhydride on the hydrazine group would increase the water solubility of the compounds, it was determined that the reaction scheme in which the substitution of the sulfonyl group on the hydrazine group takes place prior to deprotection would be more suitable. However, the above research into deprotection showed that decomposition occurs on removal of a protecting group, which will be vital in the continuation of the project and its application in a biological context.

2.3 Substitution of aryl sulfonyl groups on to the Boc-protected hydrazide derivatives

An aromatic sulfonyl headgroup was added to the structure, in order to increase the solubility of the alkoxy-substituted hydrazides in water (a property essential for the potential biological applications of the project). 1,2-Benzenedi sulfonic anhydride 87 was selected as an initial investigative target based on previous research. The sulfonyl hydrazide resulting from reaction of 1,2-benzenedi sulfonic anhydride with hydrazides had been demonstrated to significantly increase the water solubility of organic compounds. In addition, sulfonyl hydrazides had been shown to decompose rapidly in water upon deprotection, e.g. in the use of o-nitrobenzenesulfonyl hydrazide (NBSH) by Myers and coworkers in the deoxygenation of alcohols.

2.3.1 Synthesis of 1,2-benzenedi sulfonic anhydride 87

1,2-Benzenedi sulfonic anhydride 87 was synthesised by heating the dipotassium salt of 1,2-benzenedi sulfonic acid 86 and chlorosulfonic acid at 120°C for four hours, followed by work-up with ice-cold water, in the method established by Hurtley et al.

\[ \text{Scheme 59: Formation of 1,2-benzenedi sulfonic anhydride 87} \]
2.3.2 Substitution of benzene disulfonic anhydride 87 on Boc-protected 4-methoxybenzylhydrazine 60

Scheme 60: Addition of anhydride 87 to hydrazide 60

Hydrazide 60 was reacted with an equimolar quantity of 1,2-benzene disulfonic anhydride 87 in deuterated chloroform on a small scale in an NMR tube (Scheme 60). The progress of the reaction was monitored by NMR spectroscopy, which showed that sulfonyl hydrazide 88 had been formed after two hours. However, it was evident that an equal proportion of the hydrolysed sulfonyl anhydride 89 (Figure 10) was present in the solution, which was easily removed by an aqueous wash and chloroform extraction.

Figure 10: By-product 89

The formation of sulfonyl hydrazide 88 proceeds by an S_N2-like mechanism. A sulfur atom in sulfonyl anhydride 87 undergoes nucleophilic attack by the β-nitrogen in hydrazide 60, which is deprotonated by triethylamine (Scheme 61). A non-nucleophilic base was used to avoid prematurely opening the anhydride moiety by nucleophilic attack on the sulfur atom (as would occur with nucleophilic bases such as sodium hydroxide). As a result, the sulfonyl anhydride moiety is opened while generating the anionic product, which forms a salt with the protonated base.

Scheme 61: Mechanism for the formation of substituted hydrazide 88

Due to the very polar character of sulfonyl hydrazide 88, normal phase column chromatography could not be used as a method of purification. Repeated aqueous washes removed a small proportion of impurities, i.e. hydrolysed anhydride 89. Reversed-phase solid phase extraction on
a polymeric reversed-phase sorbent was instead used, though it failed to completely purify the product, providing sulfonyl hydrazide 88 in only 70% purity.

2.3.3 Substitution of benzene disulfonic anhydride 87 on Boc-protected 4-hexadecyloxybenzylhydrazide 70

Scheme 62: Addition of anhydride 87 to hydrazide 70

Sulfonyl hydrazide 90 was formed on reaction of hydrazide 70 with sulfonyl anhydride 87 after four hours, as shown by NMR spectroscopy (Scheme 62). Hydrolysed by-product 89 was observed in the NMR spectrum and was similarly removed by an aqueous wash and chloroform extraction. Reversed-phase solid phase extraction on a polymeric reversed phase sorbent generated the product in approximately 80% purity. Subsequent high-performance liquid chromatography (HPLC) failed to further purify the compound and the generation of decomposition products was observed by mass spectrometry, which could have been formed by the elimination of sulfinic acid 92 due to the instability of the sulfonyl headgroup and its sensitivity to air and moisture (Scheme 63).

Scheme 63: Possible decomposition of sulfonyl hydrazide 90

Therefore, the relative rates of various substituted sulfonyl hydrazides were studied to identify a potential solubilising group to be used in the synthesis.
2.4 Summary

The synthesis of a number of aliphatic and aromatic hydrazides has been attempted via the reductive amination of aldehydes in a two-step process,\textsuperscript{79,80} using the Boc-protecting group as a model for the later application of photochemical protecting groups. Aromatic aldehydes were found to generate hydrazides in higher yields than aliphatic analogues as a result of electronic effects. Aldehydes with electron-donating substituents on the aromatic ring were found to undergo reductive amination more efficiently than their electron-withdrawing equivalents due to the difference in relative stabilities of the hydrazones and hydrazides. Hydrazide 70 was synthesised from aldehyde 68 (Scheme 64) since the longer alkyl chain increased the lipophilicity of the molecule, thus increasing its suitability for the potential biological applications of the project.

![Scheme 64: Synthesis of Boc-protected hydrazine 70](image)

Following the successful synthesis of Boc-protected hydrazines 60 (methoxy substituent) and 70 (hexadecyloxy substituent), the deprotection of the compounds was investigated to test the stability of the free hydrazines.\textsuperscript{61} The deprotected products, although isolated as the hydrochloride salts, were shown by mass spectrometry to decompose after a short period of time in solution or in air. Therefore, it was concluded that the sulfonyl headgroup should be added while the stability of the molecule was maintained by a protecting group.

1,2-Benzene disulfonic anhydride 87 was initially selected as a solubilising agent to be used in the project due to previous work that had demonstrated the rapid decomposition of corresponding sulfonyl hydrazides.\textsuperscript{84} However, sulfonyl hydrazides 88 and 90 (Figure 11) could not be purified due to their instability, and were obtained in a maximum of 80% purity following the application of a number of purification techniques, including reversed-phase extraction and HPLC. It was therefore concluded that an alternative solubilising headgroup should be identified that displays a greater level of stability than anhydride 87 to allow time for purification.
Figure 11: Sulfonyl hydrazides 88 and 90
Chapter 3 - Investigation of Solubilising Groups

3.1 Investigation of the rate of decomposition of simple sulfonyl hydrazides

The rate of decomposition of various sulfonyl hydrazides incorporating different aromatic substituents was investigated due to the difficulty in purifying Boc-protected sulfonyl hydrazides 88 and 90 (Figure 12) as a result of the high reactivity and instability of the sulfonate headgroup ($\text{SO}_3^-$). The decomposition studies were carried out to determine an alternative sulfonyl headgroup that was stable enough to be purified, but reactive enough to trigger the decomposition of more complex sulfonyl hydrazide derivatives via a sigmatropic rearrangement or free radical mechanism.

![Figure 12: Sulfonyl hydrazides 88 and 90](image)

3.1.1 Formation of simple sulfonyl hydrazides

In addition to 1,2-benzene disulfonic anhydride 87 (Scheme 65), sulfonyl hydrazides were synthesised by combining commercially available ethyl hydrazinoacetate hydrochloride 93 with para-substituted benzenesulfonyl chlorides (methyl, nitro, carboxyl and cyano substituents). Since it had previously been shown to promote rapid decomposition, anhydride 87 was included in the reaction to compare the rates of decomposition of the other sulfonyl hydrazides with the sulfonyl hydrazide resulting from the reaction between hydrazino ester 93 and anhydride 87. The reactions were carried out on a small scale in an NMR tube at room temperature (approximately 21 °C) in a solution of deuterated pyridine or triethylamine in deuterated chloroform (0.3 M).
Scheme 65: Reaction between ethyl hydrazinoacetate hydrochloride 93 and sulfonyl anhydride 87/sulfonyl chlorides, followed by decomposition

The nitro, carboxyl and cyano functional groups were chosen due to their electron-withdrawing effects on the aromatic ring, which increases the leaving group ability of the aromatic sulfonate anion. An o-nitro group has also been incorporated into a similar hydrazine molecule by Myers et al. The cyano group is slightly less electron withdrawing than the nitro group, as shown by its Hammett coefficient ($\sigma_p$) of 0.66 compared to that of 0.78 for the nitro group. The sulfonate group was selected since it had previously been shown to promote rapid decomposition of sulfonyl hydrazides. The methyl group is conversely apolar, thus demonstrating the effect of an apolar substituent on the headgroup on the rate of reaction and subsequent decomposition.

para-Substituted benzenesulfonyl hydrazides were selected as it has been shown that para-nitro substituted benzenesulfonyl hydrazides are more stable than ortho-substituted analogues, but less stable than meta-substituted derivatives. The rate of decomposition of compounds should be fast enough to be synthetically useful though the molecules should be synthesised and isolated in high purity, i.e. before the initiation of the decomposition process. Previous attempts to purify sulfonyl hydrazides 88 and 90 (Figure 12) that incorporated an ortho-substituted aromatic sulfonate group had been unsuccessful. This was due to the initiation of the decomposition process before the pure compound had been obtained as a result of the high reactivity (and therefore instability) of the sulfonyl headgroup. Therefore, para-substituted aromatic sulfonyl chlorides were chosen in preference to ortho-substituted analogues.

Deuterated pyridine was used in preference to triethylamine since pyridine-$d_5$ does not interfere in the NMR spectrum. Pyridine also acts as a nucleophilic catalyst in acylation reactions which increases the rate of formation of the sulfonyl hydrazides. Pyridine is able to act as a
nucleophile since the lone pair of electrons on the nitrogen atom is in an sp^2 orbital orthogonal to the p orbitals in the ring which are therefore not delocalised around the ring.

Clean NMR spectra were obtained for the synthesis of the sulfonyl hydrazides incorporating the sulfonate (98a/b), methyl (99a/b), nitro (100a/b) and cyano (102a/b), when the reactions were carried out in the pyridine/chloroform solution (Scheme 66).

The reactions proceed by an SN_Ac-like mechanism, in which pyridine acts as a nucleophilic catalyst. Nucleophilic attack of the pyridine nitrogen atom on an electron-deficient sulfur atom in the sulfonyl anhydride/chloride leads to the opening of the anhydride ring in sulfonyl hydrazides 98a/b (Scheme 67) or elimination of the chloride leaving groups in sulfonyl hydrazides 99a/b, 100a/b and 102a/b (Scheme 68). Nucleophilic attack of the sulfur atom of the pyridine complex by hydrazinoacetate 93 leads to the elimination of pyridine, generating the sulfonyl hydrazide while regenerating the catalyst.

The use of a pyridine/chloroform solution generated complex NMR spectra in the synthesis and decomposition of the sulfonyl hydrazides incorporating the carboxyl substituent (101, Scheme 69), due to solubility issues. As a result, triethylamine was used as the base which generated clean NMR spectra that allowed measurement of the relative integrals of the resonances.
Similarly, the formation of sulfonyl hydrazide $101a/b$ follows an $S_N$Ac-like mechanism (Scheme 70). The reaction is not catalysed due to the absence of pyridine. Thus it would be expected that the rate of formation of the sulfonyl hydrazide $101a/b$ will be slower than that of alternative sulfonyl hydrazides. In the absence of pyridine, one of the nitrogen atoms of ethyl hydrazinoacetate hydrochloride $93$ attacks at the electron deficient sulfur atom in sulfonyl chloride $96$, leading to the elimination of the chloride anion to form sulfonyl hydrazide $101a/b$.

**Scheme 70: Possible mechanism of the formation of sulfonyl hydrazide 101a**

### 3.1.2 Decomposition of simple sulfonyl hydrazides

A proposed mechanism for the decomposition of the sulfonyl hydrazides is illustrated in Scheme 71. The nitrogen β to the sulfonyl group is deprotonated by the base present in the reaction mixture (pyridine or triethylamine), which causes the migration of a pair of electrons to form the nitrogen-nitrogen double bond in the diazene intermediate $108$. The sulfinate anion is eliminated, which is protonated to form the sulfinic acid. Loss of dinitrogen via a sigmatropic rearrangement leads to the formation of 1-ethoxyethen-1-ol $109$, which undergoes tautomerisation to form ethyl acetate $103$. 

**Scheme 69: Synthesis of sulfonyl hydrazides 101a/b**
The formation of the diazene intermediate 108 is dependent on the acidity of the hydrazide N-H and on the leaving group ability of the sulfinate anion. The decomposition is most likely to proceed via a sigmatropic rearrangement mechanism as suggested by Myers and coworkers depending on the nature of the substrate.

3.1.3 Kinetics of the formation and decomposition of sulfonyl hydrazide derivatives

The formation and decomposition of the sulfonyl hydrazide derivatives is suggested to take place in a multistep reaction as shown by the above mechanisms (Scheme 67, Scheme 68, Scheme 70, Scheme 71). Five chemical transformations may occur in the transition from the sulfonyl anhydride/chloride to ethyl acetate 103 during the formation of sulfonyl hydrazides 98a/b, 99a/b, 100a/b and 102a/b in the presence of pyridine (Scheme 72).
Using sulfonyl anhydride 87 as an example, the respective rates of the increasing concentration of sulfonyl hydrazides 98a and 98b could be expressed as differential equations (Equation 1, Equation 2 also apply to sulfonyl hydrazides 99a/b, 100a/b and 102a/b), making the assumption that the steady state approximation applies for the formation of the pyridine complex and the intermediate sulfonyl hydrazides:

\[
\frac{d[98a]}{dt} = k_1 k_2 [87][93][Py] \\
\text{Equation 1: Rate of concentration change of sulfonyl hydrazide 98a}
\]

\[
\frac{d[98b]}{dt} = k_1 k_3 [87][93][Py] \\
\text{Equation 2: Rate of concentration change of sulfonyl hydrazide 98b}
\]

If the second step is rate-limiting, i.e. formation of sulfonyl hydrazide 98a and 98b, then these become:

\[
\frac{d[98a]}{dt} = K_1 k_2 [87][93][Py] \\
\text{where } K_1 = \frac{k_1}{k_1 + k_2} \\
\text{Equation 3: Rate of concentration change of sulfonyl hydrazide 98a if the second step is rate-limiting}
\]

\[
\frac{d[98a]}{dt} = K_2 k_3 [87][93][Py] \\
\text{where } K_2 = \frac{k_1}{k_1 + k_3} \\
\text{Equation 4: Rate of concentration change of sulfonyl hydrazide 98a if the second step is rate-limiting}
\]

The rate of formation of ethyl acetate 103 is dependent on the combined rates of decomposition of sulfonyl hydrazides 98a and 98b and can be expressed as a differential equation (Equation 5), if the steady state approximation is assumed for the pyridine complex and the sulfonyl hydrazide intermediates. Using sulfonyl anhydride 87 as an example:

\[
\frac{d[103]}{dt} = k_4 ([98a]+[98a][Py]) + k_5 ([98b]+[98b][Py]) \\
\text{Equation 5: Rate of concentration change of ethyl acetate 103}
\]

In the synthesis of sulfonyl hydrazides 101a/b (carboxyl substituent), which is carried out in the presence of triethylamine instead of pyridine, ethyl acetate 103 is again formed through a multistep reaction, comprising of four chemical transformations (Scheme 73), differing from the above schemes (Scheme 72) due to the absence of a catalyst.
The respective rates of changing concentration of the sulfonyl hydrazides could be expressed as differential equations (Equation 6, Equation 7), since the mechanism for the formation of sulfonyl hydrazides 101a and 101b include both starting reagents, i.e. hydrazinoacetate 93 and sulfonyl chloride 96:

\[
\frac{d[101a]}{dt} = k_1[96][93]
\]

*Equation 6: Rate of concentration increase of sulfonyl hydrazide 98a*

\[
\frac{d[101b]}{dt} = k_2[96][93]
\]

*Equation 7: Rate of concentration change of sulfonyl hydrazide 98b*

The rate of formation of ethyl acetate 103 is dependent on the combined rates of decomposition of sulfonyl hydrazides 98a and 98b and can be expressed as a differential equation (Equation 8), if the steady state approximation is assumed for the generation of the sulfonyl hydrazide intermediates and there is no catalysis by triethylamine.

\[
\frac{d[103b]}{dt} = \frac{k_3k_4[93][96]}{k_1+k_3} \quad + \quad \frac{k_2k_4[93][96]}{k_2+k_4}
\]

*Equation 8: Rate of concentration change of ethyl acetate 103*

Accurate rate coefficients cannot be calculated for the changes in relative concentrations of the sulfonyl hydrazides and ethyl acetate from the data collected due to the number of processes taking place simultaneously and the assumptions made about the reactions. In some cases, the steady state approximation does not apply as the formation of the intermediates was observed, i.e. the rate of formation of the sulfonyl hydrazide intermediates was greater than their rate of decomposition. Therefore, the apparent rate constants for the decomposition of the sulfonyl hydrazides and the generation of ethyl acetate, \(k_{\text{obs}}\), were calculated by assuming first order kinetics, which showed the general trends in the decomposition rates. In this way, the rates of
decomposition were calculated from the time when a steady state concentration had been reached and decomposition of the sulfonyl hydrazides had been initiated. The rate of decomposition was shown to depend on the different headgroups and specifically on the polarity and electron-withdrawing nature of the substituent on the aromatic ring as detailed below.

### 3.1.4 Decomposition studies of sulfonyl hydrazide derivatives

The synthesis and subsequent decomposition of the sulfonyl hydrazides (Scheme 65) was monitored in situ by NMR spectroscopy through the changes in the respective integrals. The decomposition was most noticeable by the evolution of ethyl acetate 103, the concentration of which was measured by the relative integration of the resonance of the acetyl methyl group at 1.96ppm (Figure 13) in the NMR spectrum compared to the signals in the range 1-1.25ppm which corresponded to the terminal methyl group of hydrazine 93, the intermediate sulfonyl hydrazides and the decomposition product ethyl acetate 103, and so would remain constant throughout the reaction.

![Figure 13: Ethyl acetate 103](image)

The resonance of the acetyl methyl group at 1.96 ppm was distinct in all of the spectra, which served as an effective method of monitoring the decomposition process. The formation and decomposition of the two isomeric sulfonyl hydrazides could be compared using the integrals of the distinct singlets corresponding to the CH₂ group adjacent to the carbonyl and hydrazine groups at approximately 3.5 ppm and 4.3 ppm for isomers a and b, respectively (Figure 14).

![Figure 14: Chemical shifts of CH₂ groups in the isomeric sulfonyl hydrazides](image)

The reactions were initiated at t=0 minutes by combining the reactants in an NMR tube. The time points were calculated from the times when the NMR spectra were run. The data was plotted using the graph drawing software Origin. The data was fitted using the Levenberg-Marquardt
algorithm,\textsuperscript{99} which provided an estimation of the apparent rates of formation and decomposition of the sulfonyl hydrazide derivatives and of ethyl acetate generation. The Levenberg-Marquardt algorithm combines the Gauss-Newton and the steepest descent methods to select the parameters that minimise the deviation of the theoretical curve from the experimental points.

3.1.5 Synthesis and subsequent decomposition of sulfonyl hydrazides 98a/b

As discussed previously, the formation of sulfonyl hydrazides 98a/b proceeds via an S\textsubscript{N}Ac-like mechanism (Scheme 67, also applies to isomer b) in the presence of pyridine, which acts as a nucleophilic catalyst in the process. Decomposition of resulting sulfonyl hydrazides 98a/b to form ethyl acetate 103 takes place by the deprotonation/sigmatropic rearrangement/tautomerisation mechanism discussed previously (Scheme 71).
The formation of sulfonyl hydrazides \(98a/b\) takes place via a multistep reaction, as illustrated by the mechanism (Scheme 67), though this takes place almost instantaneously (within 15 minutes of the reagents being combined before the first NMR spectrum was recorded). Therefore, the initiation of the decomposition process is immediate. Since the sum of the initial intensities of the plots for \(98a\) (0.5) and \(98b\) (0.5) do not equal 2 as would be expected as the relative integrals were calculated from the CH\(_2\) groups in the sulfonyl hydrazides, it is possible that an alternative process is taking place by which ethyl acetate is generated. However, as no other data was recorded, the alternative process cannot be identified. Ethyl hydrazinoacetate hydrochloride \(93\) may be hydrolysed by the presence of base in solution. The sulfonate anion on sulfonyl hydrazides \(98a/b\) would also act as an internal base, hence increasing the rate of hydrolysis of hydrazinoacetate \(93\) to ethyl acetate \(103\). In addition, the first order fit of ethyl acetate formation gives an apparent rate of formation that is lower than the sum of the rates of the two decomposition processes. This could be due to the continued formation of the intermediate sulfonyl hydrazides during the decomposition process as the product was not isolated from residual starting materials before the decomposition process was initiated.

Sulfonyl hydrazide \(98b\) decomposes at a rate approximately one order of magnitude greater than the isomeric product \(98a\). However, the apparent rate constant, \(k_{\text{app}}\), is calculated from the relative concentration of sulfonyl hydrazide \(98b\) at two time points so it does not accurately represent the rate of decomposition. The faster decomposition of sulfonyl hydrazide \(98b\) compared to isomer \(98a\) could be due to the increased availability of the lone pair on the terminal...
nitrogen atom which may be donated to the adjacent nitrogen, forming the diazene intermediate **108** prior to the removal of a proton, hence initiating the decomposition process (Scheme 75).

![Scheme 75: Possible mechanism of decomposition of sulfonyl hydrazide 98a/b](image)

**3.1.6 Synthesis and subsequent decomposition of sulfonyl hydrazides 99a/b**

The formation of sulfonyl hydrazides **99a/b** follow an $S_N$Ac-like mechanism, catalysed by pyridine, as described previously (Scheme 68). The decomposition of sulfonyl hydrazides **99a/b** follows the same deprotonation/sigmatropic rearrangement/tautomerisation mechanism discussed previously (Scheme 71).
In this example, the sum of the apparent rates of decomposition of sulfonyl hydrazides 99a/b is approximately equal to the apparent rate of generation of ethyl acetate 103, suggesting that the sulfonyl hydrazide intermediates decompose cleanly to form ethyl acetate, without competing reactions. Isomer 99a decomposes at a rate approximately the same as isomer 99b within error. Any difference in the rates of decomposition may be due to the difference in the pKₐ values of the protons that are cleaved to form the diazene intermediate in the decomposition process, which affects the rate of deprotonation of the nitrogen β to the sulfonyl group to form the diazene intermediate 108. The hydrogen atom on the nitrogen atom in sulfonyl hydrazide 99a that is in a β position in relation to the sulfonyl and carbonyl groups would have a lower associated pKₐ value than the hydrogen atom on the terminal nitrogen in sulfonyl hydrazide 99b. The higher acidity of the hydrogen in sulfonyl hydrazide 99a is due to the electron-withdrawing power of the sulfonyl and carbonyl groups, which weaken the N-H bond. Therefore, less energy is required to deprotonate sulfonyl hydrazide 99a than the isomeric product 99b, increasing the rate of the deprotonation of sulfonyl hydrazide 99a, which initiates the decomposition process through the formation of diazene intermediate 108.
3.1.7 Synthesis and subsequent decomposition of sulfonyl hydrazides 100a/b

The formation and decomposition of sulfonyl hydrazides 100a/b (Scheme 77) proceed by the same mechanisms discussed previously (Scheme 68, Scheme 71).

![Scheme 77: Reaction between hydrazine 93 and sulfonyl chloride 95](image)

The sum of the apparent rates of decomposition of the two sulfonyl hydrazides 100a/b is greater than the apparent rate of ethyl acetate generation, suggesting that a competing reaction is taking place during the decomposition process, i.e. the sulfonyl hydrazides do not cleanly decompose to form ethyl acetate. However, since the starting materials ethyl hydrazinoacetate hydrochloride 93 and sulfonyl chloride 95 were not removed from the reaction vessel, continued reaction of the starting materials could explain the difference in the apparent rates of decomposition of the sulfonyl hydrazides and formation of ethyl acetate.

![Figure 17: Rate of production of ethyl acetate 103 over time by the decomposition of sulfonyl hydrazine 100a/b](image)
As in the previous decomposition studies of sulfonyl hydrazides 98a/b and 99a/b, sulfonyl hydrazide 100a decomposes at a higher apparent rate than the isomeric product 100b, though the difference in rates is not as great in this case. Sulfonyl hydrazide 100a decomposes at a rate 1.2 times greater than isomer 100b. This is again due to differences in the relative acidities of the acidic protons that are cleaved to form the diazene intermediate in the decomposition process, as discussed previously (see page 91).

3.1.8 Synthesis and subsequent decomposition of sulfonyl hydrazides 101a/b

The formation of sulfonyl hydrazide 101a/b proceeds by an S_N_Ac-like reaction as discussed previously (Scheme 70). The resulting sulfonyl hydrazides 101a/b decompose by the mechanism discussed previously (Scheme 71).
Figure 18: Rate of production of ethyl acetate 103 over time in the decomposition of sulfonyl hydrazine 101a/b

In this case, a low relative concentration of ethyl acetate 103 was generated after a week as the decomposition of sulfonyl hydrazides 101a/b is extremely slow. The relative concentration of ethyl acetate in the reaction mixture does not begin increasing until the reaction has been progressing for 3000 minutes (50 hours). This could be due to the slow formation of the sulfonyl hydrazides as a result of the absence of the pyridine catalyst. The stable relative concentrations of sulfonyl hydrazides 101a/b and slow apparent rate of ethyl acetate generation may be due to the continued synthesis of the intermediates and their slow decomposition. The slow decomposition could be a result of the low leaving group ability of the carboxylate group, which would be formed following the deprotonation of the carboxyl group by triethylamine. Initially, there is a significant decrease in the relative concentration of sulfonyl hydrazide 101b which is not matched by an increase in the relative concentration of ethyl acetate. This indicates that another process is taking place in the reaction, i.e. the sulfonyl hydrazides are not only converted to ethyl acetate. However, since ethyl acetate was the only decomposition product measured in the NMR spectra, it is not possible to determine the alternative decomposition mechanism. As a result of the difference in rates of formation of the sulfonyl hydrazides due to the absence of the catalytic pyridine, the decomposition study of sulfonyl hydrazides 101a/b cannot be compared with the other decomposition studies carried out in the presence of pyridine.
3.1.9 Synthesis and subsequent decomposition of sulfonyl hydrazide 102a/b

The formation of sulfonyl hydrazide 102a/b proceeds by an $S_N$Ac-like mechanism as discussed previously (Scheme 68). Resulting sulfonyl hydrazides 102a/b decompose by the mechanism discussed previously (Scheme 71).

![Scheme 79: Reaction between hydrazine 93 and sulfonyl chloride 97](image)

Figure 19: Rate of production of ethyl acetate 103 over time in the decomposition of sulfonyl hydrazide 102a/b

The sum of the apparent rates of decomposition of the two sulfonyl hydrazides 102a/b is greater than the apparent rate of ethyl acetate generation as in the formation and decomposition of sulfonyl hydrazides 100a/b, suggesting that a competing reaction is taking place during the decomposition process, i.e. the sulfonyl hydrazides do not cleanly decompose to form ethyl acetate in a single step.
The isomeric product decompose at approximately the same rate within error, possibly due to the relatively weaker electron-withdrawing effect of the cyano group compared to the nitro group.

### 3.1.10 Discussion of results of decomposition studies

The decomposition of sulfonyl hydrazides 98a/b ($X = \text{SO}_3^-$, Scheme 74, Figure 15), measured by the apparent rate of ethyl acetate 103 generation is seven times greater than the decomposition of sulfonyl hydrazides 100a/b ($X = \text{NO}_2^-$, Scheme 76, Figure 17). The decomposition of sulfonyl hydrazides 100a/b ($X = \text{NO}_2^-$) is fivefold faster than that of sulfonyl hydrazides 102a/b ($X = \text{CN}$, Scheme 79, Figure 19). Sulfonyl hydrazides 99a/b ($X = \text{Me}$, Scheme 76, Figure 16) and 101a/b ($X = \text{CO}_2\text{H}$, Scheme 78, Figure 18) decompose at rates approximately ten and 30 times slower than sulfonyl hydrazides 100a/b ($X = \text{NO}_2^-$).

The difference in rates of decomposition is due to the leaving group abilities of the aromatic substituents and the $pK_a$ values of the acidic proton on the $\beta$-nitrogen atom in relation to the sulfonyl group.\(^{98}\) Electron-withdrawing groups such as the nitro and cyano groups promote a relatively fast rate of decomposition since they increase the leaving group ability of the sulfinate anion. The presence of an electron-withdrawing substituent on the aromatic ring may also increase the acidity of the proton on the $\beta$-nitrogen by reducing the electron density on the nitrogen atom, therefore weakening the bond to the hydrogen atom. The methyl substituent is conversely mildly electron-donating, therefore stabilising sulfonyl hydrazide 99a/b, leading to its low rate of decomposition. The carboxylic acid group on sulfonyl hydrazide 101a/b is deprotonated by triethylamine, generating the carboxylate group which, due to its anionic charge, does not destabilise the sulfonyl hydrazide, again leading to its low rate of decomposition.

The difference in the apparent rates of decomposition of the sulfonyl hydrazides incorporating the nitro (100a/b), cyano (102a/b), methyl (99a/b) and carboxylate (101a/b) groups correlate with the Hammett coefficients ($\sigma_p$) of 0.78, 0.66, -0.17 and 0 respectively.\(^{95}\) This demonstrates that the rates of decomposition of these sulfonyl hydrazides are dependent on the electron-withdrawing or -donating character of the substituents. The effect of the sulfinate substituent on the rate of decomposition of sulfonyl hydrazide 98a/b does not correspond with the Hammett coefficients for the para ($\sigma_p$) or the meta ($\sigma_m$) Hammett substituent values of 0.09 and 0.05
respectively, since the presence of an anionic substituent on the aromatic ring should not destabilise the sulfonyl hydrazide. As the sulfinate group is substituted at the ortho position, it can be assumed that it would have a greater electron-withdrawing effect on the sulfonyl group due to orbital overlap. The sulfinate anion may also act as an internal base (deprotonating the β-nitrogen in addition to pyridine) leading to increased conversion of sulfonyl hydrazides 98a/b to the diazene intermediate, which would initiate the decomposition process.

Since it had been previously shown that sulfonate-substituted sulfonyl hydrazides 88 and 90 could not be purified due to the high sensitivity and instability of the headgroup, further study was carried out into sulfonyl hydrazides 100a/b.

### 3.2 Synthesis and Isolation of Sulfonyl Hydrazides 100a and 100b

As the decomposition studies had shown that sulfonyl hydrazides 100a/b decomposed at the fastest rate after sulfonyl hydrazides 98a/b (X = SO$_3^-$), the addition of 4-nitrobenzenesulfonyl chloride 95 to ethyl hydrazinoacetate hydrochloride 93 was carried out on a larger scale. This would allow the isolation of the isomers from the reaction mixture. The reaction was carried out in deuterated solvents such that the progress of the reaction could be monitored by NMR spectroscopy. It was assumed that the rate of decomposition would be increased by a higher concentration of base by increasing the rate of deprotonation to generate the diazene intermediate. The concentration of base in the reaction solution was reduced slightly from 0.3 M to 0.2 M such that sulfonyl hydrazides 100a/b (Scheme 80) could be isolated prior to the onset of decomposition. After allowing the reaction to stir at room temperature for two hours, the NMR spectrum of the sample indicated that decomposition had been initiated as indicated by the growth of the resonance of the acetyl methyl group at 1.96 ppm (Figure 13, see page 88). The solvent was removed by evaporation and the crude sample was purified by column chromatography on silica gel, eluting with varying ratios of petroleum ether and ethyl acetate.
Two products which were identified in the NMR and mass spectra as isomers 100a and 100b. Isomer 100a was identified to be the major product by a 2:1 ratio of isomer 100a: isomer 100b, as calculated from the analysis of the NMR spectra and comparison of the integrals of the isomeric products. Sulfonyl hydrazide 100a was identified by a peak at 3.57 ppm (singlet) and isomeric product 100b by a peak at 4.31 ppm (singlet) in the NMR spectrum. Comparison of these integrals allowed the calculation of the ratio of products (Figure 14, see page 88). The generation of ethyl acetate was monitored by the growth of the singlet at 1.96 ppm. The time points were calculated from the times when the NMR spectra were run after the isolation of the product.

Sulfonyl hydrazide 100a was identified in a higher yield than isomer 100b which may be a result of the increased nucleophilicity of the terminal nitrogen despite it being less basic, in addition to the steric bulk of the aromatic headgroup. Sulfinic acid 105 was identified in the NMR and mass spectra (m/z = 187.1), in addition to a product formed by the displacement of the sulfinic acid group (SO₂H) by pyridine, possibly by a nucleophilic substitution mechanism (Scheme 81).

By-product 110 could have also formed through the reaction between pyridine and 4-nitrobenzenesulfonyl chloride 95 or the corresponding sulfonic acid (formed by the oxidation of
sulfonyl chloride 95 or sulfinic acid 105) to give a more stable leaving group, since the sulfoxylate anion is highly reducing.

3.2.1 Decomposition of sulfonyl hydrazides in the presence of pyridine in chloroform (0.3 M)

The decomposition of sulfonyl hydrazides 100a/b was monitored by NMR spectroscopy in a solution of deuterated pyridine in deuterated chloroform (0.3 M, Figure 20, Figure 22) and in deuterated chloroform (Figure 23, Figure 25). The process was monitored by NMR spectroscopy over a period of several days.

![Figure 20: Change of NMR spectrum over time on decomposition of sulfonyl hydrazides 100a/b in the presence of pyridine in chloroform (0.3 M)](image)

The resonances in the NMR spectrum were labelled for sulfonyl hydrazides 100a and 100b, ethyl acetate 103 and sulfinic acid 105 according to proton and COSY NMR spectra. The remaining resonances in the NMR spectra were shown to couple by the COSY spectrum so it was speculated that they could be assigned to by-product 105a since it was identified in the mass spectrum.
Figure 22: Decomposition of isomers $100a$ and $100b$ in the presence of pyridine (0.3 M) in chloroform over time

The stacked NMR spectra (Figure 20) indicate that sulfonyl hydrazides $100a$ and $100b$ decompose in the presence of a base to form ethyl acetate $103$. The diazene intermediate in the proposed mechanism for the decomposition via a sigmatropic rearrangement mechanism is not observed (Scheme 71), though this would be formed and consumed instantaneously due to its instability. It has been shown previously that such reactions proceed preferentially by a sigmatropic rearrangement mechanism.\(^{43}\)

The stacked NMR spectra (Figure 20) suggest that the intermediates are cleanly converted to the product. The decomposition of sulfonyl hydrazides $100a/b$ may proceed by pseudo first order kinetics, since the rate of decomposition is dependent on the concentration of the sulfonyl hydrazide and pyridine, the latter of which acts as a catalyst in this step. Pseudo first-order kinetics fit with the proposed mechanism for the decomposition step (Scheme 82). However, since the rate of ethyl acetate evolution is dependent on the combination of two potentially pseudo first-order processes (the decomposition of sulfonyl hydrazides $100a$ and $100b$ to form ethyl acetate), it is not possible to calculate an accurate rate constant for the processes taking place in the reaction. The observed rate constant calculated through the assumption of first order kinetics shows the general trend in the rates of decomposition of the two isomeric products.
Based on the trend that has been observed in previous decomposition studies, sulfonyl hydrazide 100a has an apparent rate of decomposition eightfold greater than that of isomer 100b. This is due to the difference in the acidity of the protons that are cleaved to form the diazene intermediate, which rapidly decomposes to generate ethyl acetate (Scheme 71). As discussed previously, the proton on the nitrogen atom that is in a beta position in relation to the sulfonyl and carbonyl groups in isomer a has a lower pKₐ value than the protons on the terminal nitrogen in isomer b. This is due to the increased electron deficiency of the nitrogen in isomer a due to its proximity to the sulfonyl and carbonyl groups, which leads to a weaker N-H bond, increasing the ease of deprotonation.
3.2.2 Decomposition of sulfonyl hydrazides 100a/b in chloroform

The stacked NMR spectra (Figure 23) indicate that sulfonyl hydrazides 100a and 100b decompose cleanly to form ethyl acetate 103. The diazene intermediate in the proposed mechanism for the
decomposition via a sigmatropic rearrangement mechanism is again not observed (Figure 25),
though this would be formed and consumed instantaneously due to its instability. It has been
shown previously that such reactions proceed preferentially by a sigmatropic rearrangement
mechanism.\textsuperscript{43}

The stacked NMR spectra (Figure 23) suggest that the intermediates are cleanly converted to the
product. The decomposition of sulfonyl hydrazides 100a/b may proceed by first order kinetics,
since the rate of decomposition is dependent solely on the concentration of the sulfonyl
hydrazide. First order kinetics fit with the proposed mechanism for the decomposition step
(Scheme 83). However, since the rate of ethyl acetate evolution is dependent on the combination
of two potentially first order processes (the decomposition of sulfonyl hydrazides 100a and 100b
to form ethyl acetate), it is not possible to calculate an accurate rate constant for the processes
taking place in the reaction. The observed rate constant calculated through the assumption of
first order kinetics shows the general trend in the rates of decomposition of the two isomeric
products.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme_83}
\caption{Scheme 83: Decomposition of sulfonyl hydrazides 100a/b}
\end{figure}

The rate of decomposition of sulfonyl hydrazide 100a is fivefold greater than that of isomer 100b,
due to the differences in the $pK_a$ values of the acidic protons, as discussed in the previous section.

3.2.3 Discussion

The apparent rate of decomposition of sulfonyl hydrazides 100a/b in the presence of pyridine,
measured by the apparent rate of ethyl acetate formation is threefold greater than that in neat
chloroform. The slower rate of decomposition in neat chloroform is due to the absence of base,
confirming the role of pyridine in the reaction. This indicates that the deprotonation is the rate
determining step for the formation of the diazene intermediate, which initiates the
decomposition process. There is a greater difference in the observed rates of decomposition of sulfonyl hydrazide 100a and the isomeric product 100b in the presence of pyridine than in neat chloroform. There is an eightfold difference between the relative rates of decomposition of 100a and 100b in the presence of pyridine compared to a fivefold difference in neat chloroform. This confirms that the ease of deprotonation leads to the difference in the rates of decomposition of the isomeric products. The decomposition of sulfonyl hydrazides 100a/b in both pyridine-\textit{d}_5 in chloroform (0.3 M) and in neat chloroform fit to first order kinetics, suggesting that the decomposition of the sulfonyl hydrazides relies on the concentration of one compound, i.e. the sulfonyl hydrazide. The rate of decomposition is increased in the presence of a base, i.e. pyridine, which increases the ease of deprotonation of the β-nitrogen compared to the case where a base is not incorporated in the reaction mixture. The decomposition process is subject to general base catalysis when carried out in the presence of pyridine in chloroform (0.3 M) because the deprotonation takes place during the rate-determining step instead of before, as it would in specific base catalysis. This indicates that a base would be needed to trigger the decomposition of molecules in a cell-like environment in potential biological applications.

### 3.3 Investigation of an alternative headgroup, 2-nitrobenzenesulfonyl chloride

2-Nitrobenzenesulfonyl chloride 111 was added to ethyl hydrazinoacetate hydrochloride 93 (Scheme 84) to investigate the rate of decomposition of sulfonyl hydrazides 112a/b compared to that of the \textit{para}-substituted derivatives 100a/b. \textit{o}-Nitrobenzenesulfonyl hydrazide (NBSH) has been developed by Myers and coworkers and has been shown to rapidly decompose in solution, and is used in the deoxygenation of alcohols.\textsuperscript{43,56} It was assumed that the \textit{ortho} nitro-substituted sulfonyl hydrazide would decompose at a higher rate than the \textit{para} nitro-substituted analogue. Earlier studies by Davies and coworkers had demonstrated that \textit{ortho}-substituted nitrobenzene derivatives decomposed at a faster rate than \textit{para}- and \textit{meta}-substituted analogues.\textsuperscript{51,53}
The decomposition study was carried out as in previous examples where the two reagents were reacted in a 0.3 M solution of deuterated pyridine in deuterated chloroform, while being monitored at regular intervals by NMR spectroscopy over several days. The reactions were initiated at t = 0 minutes when the reagents were combined in the NMR tube. As in previous decomposition studies, accurate rate coefficients could not be calculated due to the complexity of the reaction which included multiple steps (Scheme 85). First order kinetics were assumed in order to calculate the observed rate constants of the processes taking place in the reaction, which showed the general trend in the rates of decomposition of sulfonyl hydrazides 112a/b and formation of ethyl acetate 103.

The respective rates of increasing concentration of sulfonyl hydrazides 112a and 112b could be expressed as differential equations making the assumption that the steady state approximation applies for the formation of the pyridine complex and the intermediate sulfonyl hydrazides (Equation 9 and Equation 10):
\[
\frac{d[112a]}{dt} = \frac{k_1 k_2 [111][93][\text{Py}]}{k_{1+}+k_2}
\]

*Equation 9: Rate of concentration change of sulfonil hydrazides 112a*

\[
\frac{d[112b]}{dt} = \frac{k_1 k_2 [111][93][\text{Py}]}{k_{1+}+k_3}
\]

*Equation 10: Rate of concentration change of sulfonil hydrazides 112b*

The rate of formation of ethyl acetate 103 is dependent on the combined rates of decomposition of sulfonil hydrazides 112a and 112b and can be expressed as a differential equation (Equation 11), if the steady state approximation is assumed for the pyridine complex and the sulfonil hydrazide intermediates.

\[
\frac{d[103]}{dt} = k_4 ([112a]+[112a][\text{Py}]) + k_5 ([112b]+[112b][\text{Py}])
\]

*Equation 11: Rate of concentration change of ethyl acetate 103*

Figure 26: Change of NMR spectrum over time on decomposition of sulfonil hydrazide 112a/b

Figure 27: Labelled structures in decomposition mixture
Figure 28: Decomposition of sulfonyl hydrazide 112a/b to form ethyl acetate 103

As demonstrated by the stacked NMR spectra (Figure 26) sulfonyl hydrazides 112a/b decompose cleanly to form ethyl acetate 103. The graph (Figure 28) demonstrates that the decomposition of sulfonyl hydrazides 112a/b occurs at an apparent rate threefold greater than that of the para-nitro substituted derivative 100a/b. The sulfonyl group is more electron-deficient in ortho-nitro substituted sulfonyl hydrazides 112a/b than para nitro substituted analogues 100a/b due to increased orbital overlap with the nitro group in the ortho nitro substituted sulfonyl hydrazide, which reduces the electron density on the sulfur atom. This increases the leaving group ability of the headgroup.

The steady state approximation applies in this example as the rates of decomposition of sulfonyl hydrazides 112a and 112b is much greater than their apparent rate of formation. This results in a consistently low concentration of sulfonyl hydrazides 112a/b as it is formed and consumed almost instantaneously.

Sulfonyl hydrazide 112a is shown to decompose at an apparent rate 1.3 times faster than the isomeric product 112b. This trend agrees with that observed in previous decomposition studies, i.e. isomer a decomposes at a faster rate than isomer b. Differences in the pKₐ values of the protons that are cleaved to form the diazene intermediate (Scheme 86) account for the difference in the rates of decomposition of the two isomers. The proton on the β-nitrogen in 112a is more acidic than the equivalent proton in 112b due to its proximity to the carbonyl and sulfonyl groups,
which result in lower electron density on the β-nitrogen. Consequently, the N-H bond is weaker, making the cleavage of the proton easier.

![Scheme 86: Mechanism of decomposition of sulfonyl hydrazides 112a/b](image)

The sulfonyl headgroup should be added only to one nitrogen atom of the hydrazine group as the two isomeric products decompose at different rates. This would reduce the rate of formation of the lipophilic molecule which may be problematic for the potential biological applications of the project.

### 3.4 Decomposition studies of sulfonyl hydrazides of higher complexity

Previously, the apparent rates of decomposition of simple sulfonyl hydrazides incorporating different aromatic groups were measured empirically and compared in order to identify the optimum headgroup for use in the project (Scheme 87). Ethyl acetate 103 was generated to varying degrees at different rates upon decomposition of the compounds and was identified spectroscopically though, due to its volatility, could not be isolated. Sulfonyl hydrazide 100a/b, incorporating the para-nitro substituted headgroup, was shown to have a relatively high rate of decomposition, while stable enough to be isolated. Therefore, 4-nitrobenzenesulfonyl chloride 95 was selected as the basis from which to synthesise more complex sulfonyl hydrazides.
The isolation of such products from a decomposition reaction, the progress of which could be monitored spectroscopically, would demonstrate that the reaction could be used to remove polar functional groups from amphiphilic molecules. Thus, analogues of sulfonyle hydrazide were synthesised that would generate products with higher boiling points, to assist the isolation of the ester decomposition products.

### 3.4.1 Synthesis of aminoglycinate derivatives

Initially, amino glycinate derivatives were synthesised through the acid-catalysed transesterification of commercially available ethyl hydrazinoacetate hydrochloride with long chained alcohols (six to eight carbons). Acid-catalysed transesterification was selected over base-catalysed transesterification since the presence of base would cause the starting reagent, ethyl hydrazinoacetate hydrochloride, to decompose to ethyl acetate. The boiling points of the expected acetate esters are 171.5 °C, 192.5 °C, 211 °C for hexyl acetate, heptyl acetate and octyl acetate respectively, which should allow their isolation.

![Scheme 88: Transesterification of ethyl hydrazinoacetate hydrochloride 93](image)

Hydrazine was reacted with the alcohols at 72 °C for 48 hours in the presence of one equivalent of trifluoroacetic acid (TFA, Scheme 88). This effectively reduces the energy barrier to the substitution of the alcohols at the carbonyl carbon which follows an $S_N$Ac mechanism. The aminoglycinate derivatives were obtained in mixtures with the starting material ethyl hydrazinoacetate hydrochloride since the transesterification reaction had not gone to completion.

---

110
completion. Identical reactions were left for longer time periods (up to five days), though conversion to the product was not significantly increased. Higher reaction temperatures may have promoted increased conversion to the aminoglycinate product though the reaction temperature could not be increased above 72 °C since this is the boiling point of TFA. Elemental analysis of the products showed that hexyl- and heptyl aminoglycinate (114 and 115 respectively) were isolated in 70% purity while octyl aminoglycinate (116) was isolated in 50% purity. It was assumed that purification by column chromatography on silica would be an inefficient method of purification of the products due to the presence of the charge on the HCl salts. Therefore the crude products were used in the next step of the reaction (synthesis and decomposition of the sulfonyl hydrazides) directly. The decomposition of sulfonyl hydrazides formed from the reaction between ethyl hydrazinoacetate hydrochloride 93 and sulfonyl chloride 95 would form ethyl acetate, which is volatile. Therefore, the longer chained decomposition products would only be isolated from the reaction mixtures following decomposition. Despite the presence of ethyl hydrazinoacetate hydrochloride 93 and subsequently sulfonyl hydrazides 100a/b in the decomposition mixtures, the general trends in the rates of decomposition of the longer chained sulfonyl hydrazides would still be evident in the decomposition studies.

The transesterification of hydrazinoacetate 93 with arylalkyl alcohols was attempted though the products did not crystallise from the solutions as did the aliphatic derivatives. Products of low purity were obtained following removal of the solvent in vacuo, indicating partial conversion to the amino glycinate and decomposition of the products that had been obtained, possibly due to the temperatures required to remove toluene. Therefore, efforts were concentrated on the aliphatic amino glycinate derivatives isolated previously (114 – 116).

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{NH}_2\cdot\text{HCl} \\
\text{93} & \quad \text{R} & \quad \text{OH} \\
\end{align*}
\]

\[
\begin{align*}
\text{TFA} & \\
\text{Toluene, 78 °C, 72 h} & \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{NH}_2\cdot\text{HCl} \\
\text{R} & \quad \text{OH} & \quad \text{R} \\
\end{align*}
\]

Scheme 89: Attempted transesterification of ethyl hydrazinoacetate hydrochloride 93 with aromatic alcohols 117 and 118

### 3.4.2 Synthesis and decomposition of sulfonyl hydrazides

4-Nitrobenzenesulfonyl chloride (95) was reacted with an equimolar concentration of the glycinate derivatives in a solution of pyridine in chloroform (0.3 M), replicating the conditions
used in previous decompositions studies (Scheme 90). The reactions were monitored regularly with NMR spectroscopy to determine the respective rates of decomposition of the sulfonyl hydrazides and evolution of the esters.

\[ \text{Scheme 90: Synthesis and decomposition of sulfonyl hydrazides} \]

3.4.3 Synthesis and decomposition of hexyl ((4-nitropheryl)sulfonyl)glycinate 121a/b

Sulfonyl hydrazides 121a/b were synthesised on a small scale in an NMR tube (Scheme 91) using conditions identical to those employed in previous decomposition studies. The formation and subsequent decomposition of the sulfonyl hydrazides were monitored by NMR spectroscopy. The starting material, sulfonyl chloride 95 was not immediately consumed in the reaction as it was present in the initial NMR spectrum. This suggests that the rate of reaction was slower than in previous decomposition studies incorporating ethyl hydrazinoacetate, possibly due to the longer alkyl chain of hexyl aminoglycinate, which may have had an effect on solvation and reactivity.

\[ \text{Scheme 91: Synthesis and decomposition of sulfonyl hydrazide 121a/b} \]
Figure 29: Synthesis and decomposition of sulfonyl hydrazide 121a/b to form hexyl acetate 124

Figure 30: Labelled structures present in decomposition mixture

The resonances in the NMR spectrum were labelled for sulfonyl hydrazides 121a and 121b, hexyl acetate 124 and sulfinic acid 105 according to proton and COSY NMR spectra. The remaining resonances in the NMR spectra were shown to couple by the COSY spectrum so it was speculated that they could be assigned to by-product 110 since it was identified in the mass spectrum.
As illustrated by the stacked NMR plots (Figure 29), sulfonyl hydrazides 121a/b are converted to hexyl acetate 124 following the loss of nitrogen, most likely through a sigmatropic rearrangement mechanism. Sulfinic acid 105 and compound 110 were identified in the NMR and mass spectra ($m/z = 187.1$ and $201.1$), which was also observed in earlier decomposition studies. The sum of the apparent rates of decomposition of sulfonyl hydrazides 121a/b, shown in the graph (Figure 31), is greater than the rate of generation of hexyl acetate, indicating that sulfonyl hydrazides 121a/b are not converted directly to the ester product. The diazene intermediate could be more stable than in earlier decomposition studies (Scheme 65), having a longer lifetime due to the longer alkyl chain. However, the diazene is not observed in the NMR spectrum so the difference in the apparent rates is most likely due to the synthesis of the sulfonyl hydrazides, which may continue throughout the reaction, i.e. after decomposition has begun.

The graph indicates that the general trend of the rates of decomposition of sulfonyl hydrazides 121a/b is the same as has been observed in previous decomposition studies, i.e. isomer 121a decomposes faster than 121b. Sulfonyl hydrazide 121a decomposes 1.5 times faster than 121b, due to the difference in acidity of the proton that is cleaved as the diazene intermediate is formed, as has been discussed previously. The rate of decomposition of sulfonyl hydrazides 121a/b, measured by the rate of hexyl acetate generation, is threefold lower than that of sulfonyl hydrazides 100a/b due to the difference in acidity of the proton on the β-nitrogen as a result of the inductive effect of the long alkyl chain.
3.4.4 Synthesis and decomposition of heptyl ((4-nitrophenyl)sulfonamido)glycinate 122a/b

As above, sulfonyl hydrazides 122a/b (Scheme 92) were synthesised on a small scale in an NMR tube in order to monitor their formation and subsequent decomposition by NMR spectroscopy (Figure 32). The starting material, sulfonyl chloride 95 was not immediately consumed in the reaction as it was present in the initial NMR spectrum. This suggests that the rate of reaction was slower than in previous decomposition studies incorporating ethyl hydrazinoacetate 93, possibly due to the longer alkyl chain of heptyl aminoglycinate, which may have an effect on solvation.

Figure 32: Synthesis and decomposition of sulfonyl hydrazide 122a/b to form heptyl acetate 125
The resonances in the NMR spectrum were labelled for sulfonyl hydrazides 122a and 122b, heptyl acetate 125 and sulfinic acid 105 according to proton and COSY NMR spectra. The remaining resonances in the NMR spectra were shown to couple by the COSY spectrum so it was speculated that they could be assigned to by-product 110 since it was identified in the mass spectrum.

As illustrated by the stacked NMR plots (Figure 32), sulfonyl hydrazides 122a/b were converted to heptyl acetate 125 following the loss of nitrogen, most likely through a sigmatropic rearrangement mechanism. Sulfinic acid 105 was identified in the NMR and mass spectra ($m/z = 187.1$), as was pyridine complex 110 ($m/z = 201.1$), which was also observed in earlier decomposition studies. The sum of the apparent rates of decomposition of sulfonyl hydrazides 122a/b, shown in the graph (Figure 34), is greater than the rate of generation of heptyl acetate,
indicating that sulfonyl hydrazides 122a/b are not converted directly to the ester product. The diazene intermediate could be more stable than in the case of earlier decomposition studies (Scheme 65), having a longer lifetime due to the longer alkyl chain. However, the diazene is not observed in the NMR spectrum so the difference in the apparent rates is most likely due to the synthesis of the sulfonyl hydrazides, which may continue throughout the reaction, i.e. after decomposition has begun.

The graph indicates that the general trend of the rates of decomposition of sulfonyl hydrazides 122a/b is the same as has been observed in previous decomposition studies, i.e. isomer 122a decomposes at a slightly faster rate than 122b. Sulfonyl hydrazide 122a decomposes 1.1 times faster than 122b, due to the difference in acidity of the proton that is cleaved as the diazene intermediate is formed, as has been discussed previously.

The rate of decomposition of sulfonyl hydrazides 122a/b, measured by the apparent rate of heptyl acetate generation, is threefold lower than that of sulfonyl hydrazides 100a/b due to the difference in acidity of the proton on the β-nitrogen. The rate of heptyl acetate generation from the decomposition of sulfonyl hydrazides 122a/b is approximately the same as the rate of hexyl acetate generation from the decomposition of sulfonyl hydrazides 122a/b. This indicates that the length of the alkyl chain affects the rate of decomposition when the chain length is increased by four/five carbons, through its effect on the acidity of the proton on the β-nitrogen, but not when the carbon chain is increased by one carbon. This may be due to the inductive effect of the alkyl chain, which increases the electron density on the β-nitrogen, so increasing the strength of the N-H bond.
3.4.5 Synthesis and decomposition of octyl ((4-nitrophenyl)sulphonamido)glycinate 123a/b

Octyl aminoglycinate 116 was reacted with sulfonyl chloride 95 to form sulfonyl hydrazides 123a/b on a small scale in an NMR tube using the same conditions as used previously (Scheme 93), in order to calculate the apparent rate of decomposition of resulting sulfonyl hydrazide 123a/b and subsequent generation of octyl acetate 126. The starting material, sulfonyl chloride 95 was not immediately consumed in the reaction as it was present in the initial NMR spectrum. This suggests that the rate of reaction was slower than in previous decomposition studies incorporating ethyl hydrazinoacetate 93, possibly due to the longer alkyl chain of octyl aminoglycinate, which may have an effect on solvation.

Figure 35: Synthesis and decomposition of sulfonyl hydrazide 123a/b to form octyl acetate 126
The resonances in the NMR spectrum were labelled accurately for sulfonyl hydrazides 123a and 123b, octyl acetate 126 and sulfinic acid 105 according to proton and COSY NMR spectra. The remaining resonances in the NMR spectra were shown to couple by the COSY spectrum so it was speculated that they could be assigned to by-product 110 since it was identified in the mass spectrum.

As illustrated by the stacked NMR plots (Figure 35), sulfonyl hydrazides 123a/b are converted cleanly to octyl acetate 126 following the loss of nitrogen, most likely through a sigmatropic rearrangement mechanism. Sulfinic acid 105 has been identified in the NMR (m/z = 187.1) and mass spectra, as has pyridine complex 110 (m/z = 201.1), which was also observed in earlier decomposition studies. The sum of the apparent rates of decomposition of sulfonyl hydrazides 123a/b, shown in the graph (Figure 37), is greater than the rate of generation of octyl acetate,
indicating that sulfonyl hydrazides 123a/b are not converted directly to the ester product. The diazene intermediate could be more stable than in the case of earlier decomposition studies (Scheme 71) so having a longer life time due to the longer alkyl chain. However, the diazene is not observed in the NMR spectrum so the difference in the apparent rates is most likely due to the synthesis of the sulfonyl hydrazides, which may continue throughout the reaction, i.e. after decomposition has begun.

The graph indicates that the general trend of the rates of decomposition of sulfonyl hydrazides 123a/b is the same as has been observed in previous decomposition studies, i.e. isomer 123a decomposes at a faster than 123b. Sulfonyl hydrazide 123a decomposes at a rate tenfold faster than 123b, due to the difference in acidity of the proton that is cleaved as the diazene intermediate is formed, as has been discussed previously. Due to the reduced rate of decomposition of sulfonyl hydrazide 123b, the rate of formation of octyl acetate 126 is lower than previous examples, due to the higher proportion of isomer 123b present in the decomposition mixture. In addition, since ethyl hydrazinoacetate hydrochloride 93 was converted to octyl aminoglycinate 116 to a lesser extent (50 %) than in the formation of the hexyl and heptyl analogues (70 %), there was a reduced amount of octyl acetate generated in the decomposition process compared to the hexyl and heptyl analogues. Ethyl acetate 103, generated on decomposition of sulfonyl hydrazides 100a/b, formed on reaction of hydrazine 93, which was present in the decomposition mixture, with sulfonyl chloride 95, would evaporate after an extended period, hence reducing the integral of the acetate peak in the NMR spectrum at 2.1 ppm. Ethyl acetate 103 was indistinguishable in the NMR spectrum from octyl acetate 126. As a result, the apparent rate of generation of octyl acetate, k_{obs}, is lower.

The rate of decomposition of sulfonyl hydrazides 123a/b, measured by the apparent rate of octyl acetate generation, is fivefold lower than that of sulfonyl hydrazides 100a/b due to the difference in acidity of the proton on the β-nitrogen. The rate of octyl acetate generation from the decomposition of sulfonyl hydrazides 123a/b is approximately half the rate of hexyl/heptyl acetate generation from the decomposition of sulfonyl hydrazides 121a/b/122a/b. This indicates that the length of the alkyl chain may affect the rate of decomposition, through its effect on the acidity of the proton on the β-nitrogen. This may be due to the inductive effect of the alkyl chain, which increases the electron density on the β-nitrogen, so increasing the strength of the N-H bond. The lower apparent rate of generation of octyl acetate compared to those of hexyl acetate and heptyl acetate may also be due to the lower percentage conversion of ethyl hydrazinoacetate
hydrochloride 93 to octyl aminoglycinate 116 in the transesterification step, since this would lead to a lower proportion of sulfonyl hydrazide 123a/b than in the case of the heptyl and hexyl analogues.

3.4.6 Isolation of the decomposition product, octyl acetate 126

The synthesis and decomposition of sulfonyl hydrazide 123a/b was repeated on a large scale in order to isolate the decomposition product, octyl acetate 126. Sulfonyl hydrazide 123a/b was selected in preference to the hexyl and heptyl analogues 121a/b and 122a/b due to the faster decomposition of isomer 123a. This resulted in a less complex mixture as the solution contained two compounds after the solution was allowed to decompose for eight days, rather than three as in the decomposition of 121a/b and 122a/b.

Difficulties were experienced in the isolation of octyl acetate 126. Ester 116 was observed in the NMR spectra of the decomposition solution, as confirmed by comparison with the NMR spectrum of commercially sourced octyl acetate (Figure 38, see page 122). Purification was attempted using column chromatography on silica, and neutral and basic alumina, in addition to chloroform extraction with aqueous washing. However, all methods were unsuccessful and generated a mixture of products. It was assumed that the presence of residual TFA in the reaction mixture may be preventing the isolation of the acetate product. Therefore, the product mixture was recrystallised from dichloromethane and the TFA-pyridine salt that crystallised out of solution was filtered off. The filtrate was purified by column chromatography on silica gel leading to the isolation of a pure sample of octyl acetate 126 (Figure 38) albeit in low yield (29%). The low yield of isolated product was due to the co-elution of the decomposition product with the ester coordinated to the TFA salt.
Scheme 94: Isolation of octyl acetate 126 following decomposition of sulfonyl hydrazide 123a/b

Figure 38: Overlaid spectra of decomposition mixture, isolated octyl acetate 126 and commercially sourced octyl acetate
3.5 Addition of 4-Nitrobenzenesulfonyl chloride (95) to Boc-protected 4-Hexadecyloxybenzylhydrazine (70)

Following the identification of 4-nitrobenzenesulfonyl chloride (95) as a viable alternative to sulfonyl anhydride 87 as a suitable headgroup to promote rapid decomposition, sulfonyl chloride 95 was added to more complex Boc-protected hydrazine 70 (Scheme 95), in order to investigate its compatibility in the project.

Scheme 95: Substitution of sulfonyl chloride 95 on Boc-protected hydrazine 70

Sulfonyl chloride 95 was reacted with hydrazide 70 in the presence of pyridine in chloroform (0.2 M) to form sulfonyl hydrazide 127 in a nucleophilic addition reaction, using the conditions identified previously where sulfonyl chloride 95 was added to ethyl hydrazinoacetate hydrochloride 93 and more complex analogues. The product was purified by column chromatography to form sulfonyl hydrazide 127 in moderate yield.

Scheme 96: Mechanism of formation of sulfonyl hydrazide 127

The addition of sulfonyl chloride 95 to hydrazide 70 follows an S<sub>n</sub>Ac-like mechanism, proceeding by the formation of a pyridine complex, as pyridine acts as a nucleophilic catalyst (Scheme 96). Nucleophilic attack of the unprotected nitrogen lone pair on the electron-deficient sulfur atom leads to regeneration of the pyridine catalyst and formation of sulfonyl hydrazide 127.
3.6 Synthesis of ether 78

Ether 78 was synthesised via the Williamson ether synthesis\(^{92}\) from \(p\)-cresol 128 (Scheme 97) in order to compare the resulting NMR spectrum to the spectra generated on decomposition of sulfonyl hydrazide 127. \(p\)-Cresol 128 was reacted with 1-bromohexadecane 66 in \(N,N\)-dimethylformamide (DMF) at 70°C in the presence of potassium carbonate and a catalytic amount of potassium iodide. After the reaction had gone to completion, as shown by thin layer chromatography (TLC), the potassium salts were removed by an aqueous wash, followed by an extraction with dichloromethane. The remaining residue was redissolved in diethyl ether and washed with water and brine, dried over magnesium sulfate, filtered and concentrated \textit{in vacuo} to remove the DMF. Following purification of the crude sample by column chromatography, ether 78 was obtained in moderate yield as colourless crystals.

\[ \text{CH}_3(CH_2)_n \text{Br} + \text{K}_2\text{CO}_3, \text{KI} \rightarrow \text{H}_3\text{C}((\text{CH}_2)_n\text{CH}_3) \]

\textit{Scheme 97: Williamson ether synthesis of ether 78}

The potassium iodide salt is used to catalyse the transformation through the formation of the much more reactive alkyl iodide via halogen exchange with alkyl bromide 66 (Scheme 98). The alkyl iodide has a faster rate of reaction than the alkyl bromide due to the increased leaving group ability of the iodide ion as a result of its increased ionic radius resulting in lower basicity and consequently \(pK_a\). Therefore, the iodide ion is a better nucleophile. The reaction follows an \(S_{N2}\) mechanism in which the alkyl halide undergoes nucleophilic attack by the deprotonated oxygen atom on the aromatic ring in \(p\)-cresol 128, leading to the formation of ether 78 upon elimination of the iodide leaving group. DMF is used as the solvent in the reaction since it is an aprotic and polar solvent that it increases the availability of the nucleophile.

\[ \text{H}_3\text{C}((\text{CH}_2)_n\text{CH}_3) \rightarrow \text{H}_3\text{C}((\text{CH}_2)_n\text{CH}_3) \text{I} \]

\textit{Scheme 98: Mechanism of Williamson ether synthesis of ether 78}

The proton NMR spectrum of the ether product has several distinguishing features from that of sulfonyl hydrazide 127. The chemical shifts of the aromatic protons in ether 78 are shifted upfield by approximately 0.5 ppm compared to hydrazide 127 due to the absence of the electron-withdrawing effect of the hydrazine functionality which reduces the electron density in the
aromatic ring in ether 78, reducing the level of deshielding experienced by the aromatic protons. The methyl group on the aromatic ring appears in the NMR spectrum as a singlet at 2.28 ppm. As there are no resonances in this region of the NMR spectrum from sulfonyl hydrazide 127, this peak can be used as a clear indicator of the transformation of sulfonyl hydrazide 127 to ether 78. Because of this, the complexity of monitoring the deprotection and decomposition of sulfonyl hydrazide 127 in addition to photocleavable analogues is reduced significantly.

3.7 Boc-deprotection of Sulfonyl Hydrazide 127

To test the theory that sulfonyl hydrazide derivatives decompose upon deprotection, sulfonyl hydrazide 127 was treated with deuterated trifluoroacetic acid in deuterated chloroform (10-50%) to cleave the Boc-protecting group (Scheme 99).

Scheme 99: Boc-deprotection of sulfonyl hydrazide 127

It was hoped that decomposition would proceed as shown in Scheme 100, although elimination of ArSO₂⁻ might be slowed by protonation of the NH₂ group in compound 129.

Scheme 100: Proposed mechanism of Boc-deprotection of sulfonyl hydrazide 127
The elimination of the headgroup could also proceed by deprotonation of the benzylic site, which would lead to the formation of hydrazone 84 (Scheme 101). The hydrazone structure would be more stable than diazene intermediate 85 (which would be formed as a mixture of isomers), formed in Scheme 100, though would not decompose to form the reduced product, ether 78, but would be hydrolysed to form aldehyde 68.

Scheme 101: Alternative possible mechanism for the elimination of the sulfonyle headgroup

This also allowed the investigation of the sensitivity of the structure to acidic conditions since the protecting group should be cleaved without causing further decomposition to the products of the sigmatropic rearrangement by the protonation of the NH₂ group. Initially, 10 equivalents of TFA were added to a solution of sulfonyle hydrazide 127 in deuterated chloroform to cleave the Boc-group. The reaction was monitored over a period of several hours by NMR spectroscopy. After a period of 18 hours, no change was observed in the NMR spectrum which showed that deprotection had not taken place. A slight excess of pyridine was added to the solution to drive the decomposition to completion, since it was assumed that the acidity of the reaction solution might prevent the progression of the decomposition process. However, this immediately resulted in the complete decomposition of the compound, leading to an indistinguishable accumulation of signals in the aromatic region of the NMR spectrum.
Subsequently, Boc-protected sulfonyl hydrazide 127 was treated with solutions ranging from 10-100% TFA in chloroform to investigate the deprotection further. The NMR spectra indicated that the compound had decomposed, though none of the signals present in the spectra could be distinguished as either the reduction product, ether 78, or the by-product, 4-nitrobenzene sulfinic acid 105. Deprotection was also attempted on reaction with an excess of hydrochloric acid (2 M) in ether. However, this also proved unsuccessful, generating similar NMR spectra to the attempted deprotection with TFA. This could be due to the interaction of the strong acid with the deprotected hydrazine, which results in degradation of the molecule due to its high sensitivity and instability.

3.8 Synthesis of Paracetamol via the Removal of Hydrophilic Groups on Hydrazine Derivatives

In order to test the central hypothesis behind the project, i.e. that biologically active, membrane permeable molecules can be released by the removal of polar functional groups from hydrazine derivatives by a sigmatropic rearrangement or free radical mechanism, a retrosynthetic analysis of paracetamol was undertaken (Scheme 102). In the determined synthetic route to the drug molecule, 4-aminophenol 136 should be initially coupled to tri-Boc-hydrazinoacetic acid 135. Following the production of amide 134, the Boc-protecting groups would be cleaved under acidic conditions. The incorporation of a polar headgroup into the molecule would initiate decomposition to the reduction product 131, upon elimination of nitrogen.
3.8.1 Coupling of 4-aminophenol 136 to tri-Boc-hydrazinoacetic acid 135

The acetic acid derivative 135 was activated by dicyclohexylcarbodiimide (DCC) to form the amide 134 through coupling of tri-Boc-hydrazinoacetic acid 135 with 4-aminophenol 136. The activated ester then underwent substitution with trifluorophenol (TFP, 137, Scheme 103). The substitution of the hydroxyl functionality for the TFP group would improve its leaving group ability, which would lower the activation energy for amide formation. Ester 138 was isolated by removal of the urea by-product by gravity filtration and column chromatography.

Scheme 103: Formation of TFP-ester 138

The aromatic proton in ester 138 appears as a quintet in the $^1$H NMR spectrum due to the coupling of the proton with the fluorine atoms on the aromatic ring (which both have a spin of a half). Splitting is also evident in the $^{13}$C NMR spectrum. Fluorine displays very strong coupling to both $^1$H and $^{13}$C and couples to atoms up to three bonds away.

The ester coupling reaction proceeded by activation of the carboxylic acid by DCC, in which the oxygen anion, formed on deprotonation of carboxylic acid 135, attacked the carbodiimide (Scheme 104). The hydroxyl oxygen of TFP then attacked the carbonyl carbon, eliminating dicyclohexylurea as activated ester 138 was formed.

Scheme 104: Mechanism of coupling reaction

Due to the presence of two nucleophilic positions on 4-aminophenol 136, which could result in the formation of two products on condensation with activated ester 138, the alcohol functional group on amine 136 was protected with a triisopropylsilyl (TIPS) group to prevent formation of
the corresponding ester. 4-Aminophenol 136 was reacted with TIPS-chloride under basic conditions at room temperature in dichloromethane to form TIPS-protected amine 139 in high yield and purity following purification by column chromatography (Scheme 105).\(^\text{103}\)

\[ \text{acetophenone} + \text{TIPS-Cl} \xrightarrow{\text{DCM, rt, 24h, 64\%}} \text{TIPS-protected amine 139} \]

Scheme 105: Formation of amine 139

The reaction proceeds via the formation of an intermediate imidazole complex following nucleophilic attack of the nitrogen lone pair on imidazole on TIPS-chloride (Scheme 106). A pentavalent silicon complex is initially formed, which is allowed due to hybridisation of the vacant d orbitals of silicon.\(^\text{104}\) A chloride anion is eliminated to form the imidazole complex which is a very reactive silylating agent. Nucleophilic attack of the hydroxyl oxygen leads to the regeneration of the imidazole catalyst.\(^\text{104}\)

TIPS-protected amine 139 was then reacted with TFP-activated ester 138 under basic conditions at room temperature in dichloromethane to form amide 140 in 14% yield (Scheme 107). The low yield is due to difficulties in purification. Amide 140 was purified by column chromatography on silica, though the process had to be repeated a number of times since the crude mixture contained impurities that were eluted with amide 140. This was followed by the cleavage of the TIPS-protecting group from the resulting amide (Scheme 107). The deprotection was carried out using tetra-n-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) at temperatures ranging from 0 °C to room temperature.
The amide coupling reaction proceeds by nucleophilic attack of the amine on the carbonyl carbon of activated ester 138, leading to the elimination of TFP (Scheme 108). The cleavage of the TIPS-protecting group proceeds via a pentavalent intermediate followed by the elimination of the silyl group. The process is driven by the formation of the Si-F bond, which is approximately 126 kJ mol\(^{-1}\) stronger than the Si-O bond, which promotes the fragmentation of the unstable intermediate.\(^{104}\)

Amide 134 was obtained in a very low yield due to the low yielding coupling step as a result of purification issues. Upon purification of the crude product by column chromatography on silica, it was found that the product and TFP eluted at very similar rates, resulting in the loss of a significant amount of product.

Amide 134 was therefore synthesised though direct coupling of amine 136 with the TFP-activated ester 138 (Scheme 109) in order to increase the ease of purification.
Amide 134 was obtained in moderate yield following purification by column chromatography. As the direct coupling of ester 138 and amine 136 gave a significantly higher yield of amide 134 which was easier to purify due to the greater difference in the rates of elution of the product and impurities compared to the TIPS-protected analogue, the direct coupling procedure was found to be preferential. Amide 134 exists as two rotameric products, as shown by the resonances in the NMR spectrum of the products, i.e. each signal was split into two resonances of equal intensity, due to the restricted rotation around the N-N bond or the amide bond as a result of the steric bulk of the two Boc-protecting groups on the terminal nitrogen.

3.8.2 Boc-deprotection of amide 134

Deprotection of amide 134 was initially attempted using different concentrations (10-50%) of deuterated trifluoroacetic acid in deuterated dichloromethane. In all cases, amide 134 was shown by NMR analysis to be consumed in three to five hours. Incomplete deprotection was observed in each reaction, though the reaction did not progress any further on leaving the reaction for longer periods of time. Deprotection was also attempted by the treatment of hydrazide 134 with hydrochloric acid in diethyl ether (2 M), but identical results were obtained as in the treatment of the hydrazide with TFA. Following treatment of the protected amide with acid, one Boc-group was removed as shown by the decrease in the corresponding integral on the NMR spectrum by nine protons. Only one rotameric product was present in the NMR spectrum after partial deprotection, indicating that a Boc-group from the terminal nitrogen had been cleaved, increasing the ease of rotation around the N-N or amide bond through the reduction of steric bulk. Previously, Boc groups have been cleaved from tri-Boc hydrazino derivatives under acidic conditions using TFA\textsuperscript{105} or hydrochloric acid.\textsuperscript{102} However, decomposition of deprotected amide 133 was observed when excess acid was added to the reaction mixture or heat was applied, in order to attempt to push the reaction to completion. This may be due to the sensitivity of the free hydrazine moiety towards acidic conditions and reduced stability of deprotected amide 133 in comparison to similar analogues previously synthesised by other groups.\textsuperscript{102,105}
3.9 Summary

A number of simple sulfonyl hydrazides were synthesised by the reaction of ethyl hydrazinoacetate hydrochloride 93 with aromatic sulfonyl compounds incorporating different aromatic substituents (sulfonyl, methyl, nitro, carboxyl and cyano) in the presence of a base in chloroform (0.3 M). The formation and decomposition of the resulting sulfonyl hydrazides were monitored by NMR spectroscopy to determine the effect of the substituents on the apparent rate of decomposition. The sulfonyl hydrazide derivatives are suggested to be formed in a multistep reaction involving a combination of first and second order processes, which, in the case of sulfonyl hydrazides 98a/b, 100a/b and 102a/b (o-SO$_2$, p-NO$_2$ and p-CN), takes place rapidly. It was therefore not possible to calculate accurate values for the rate constants. However, the general trend in the rates of decomposition was shown by assuming first order kinetics and fitted with the proposed mechanism (Scheme 111).

Scheme 111: Mechanism of decomposition of the sulfonyl hydrazides

It emerged that the rate of decomposition of the sulfonyl hydrazide derivative and subsequent evolution of ethyl acetate 103 decreased in the order illustrated in Figure 39.

Figure 39: Order of rates of decomposition of substituted sulfonyl hydrazide and evolution of ethyl acetate

Faster rates of decomposition were observed in sulfonyl hydrazides containing substituents of higher electron-withdrawing character (p-NO$_2$ and p-CN) due to their higher leaving group ability. In addition, the acidic protons on the β-nitrogen in sulfonyl hydrazides 100a/b and 102a/b (p-NO$_2$ and p-CN) have lower relative $pK_a$ values due to the higher electron-withdrawing character of p-NO$_2$ and p-CN, compared to sulfonyl hydrazides 99a/b and 101a/b (p-Me and p-CO$_2$).
Consequently, there is a higher rate of formation of the diazene intermediate by the deprotonation of sulfonyl hydrazides 98a/b, 100a/b and 102a/b, which initiates the decomposition of the structures to form ethyl acetate 103, following elimination of nitrogen. The methyl-substituted sulfonyl hydrazides 99a/b decomposed at a low rate due to its lack of polarity, while carboxylate-substituted sulfonyl hydrazides 101a/b decomposes at the slowest apparent rate, due to the anionic charge on the substituent. Sulfonyl hydrazides 98a/b (o-SO₃⁻) had a significantly faster rate of decomposition than would be expected due to its anionic character. The sulfonate headgroup possibly acts as an internal base in sulfonyl hydrazides 98a/b that catalyses the conversion of the sulfonyl hydrazides to the diazene intermediate, hence triggering the decomposition process.

The two isomeric products formed on reaction of ethyl hydrazinoacetate 93 with the aromatic sulfonyl compounds (Figure 40) were shown to decompose at different rates, with isomer a decomposing at a higher rate than isomer b in the case of sulfonyl hydrazides 99a/b, 100a/b, 101a/b and 102a/b. This is due to the difference in the relative pKₐ values of the proton on the nitrogen β to the sulfonyl group that is removed to form the diazene intermediate (Scheme 111). The proton in isomer a is more acidic than in isomer b due to its proximity to the sulfonyl and carbonyl groups, which both withdraw electrons from the nitrogen so weakening its bond to hydrogen.

![Figure 40: Isomeric sulfonyl hydrazide derivatives](image)

\[ X = o-\text{SO}_2^- (98a/b), p-\text{Me} (99a/b), p-\text{NO}_2 (100a/b), p-\text{CO}_2\text{H}^+ (101a/b), p-\text{CN} (102a/b) \]

\( \text{o-Nitrobenzenesulfonyl hydrazide had been synthesised by Myers and Movassaghi who showed that it decomposed rapidly in solution via the elimination of nitrogen, sulfonyl hydrazides 112a/b were synthesised (Scheme 112). The formation and decomposition of sulfonyl hydrazides 112a/b were monitored by NMR spectroscopy. The apparent rate of decomposition was calculated and compared with that calculated previously for the formation and decomposition of para-nitro substituted sulfonyl hydrazide 100a/b. Ethyl acetate 103 was generated from the decomposition of sulfonyl hydrazides 112a/b at a rate threefold faster than isomeric sulfonyl hydrazides 100a/b due to the increased leaving group ability of the o-nitro substituted headgroup, which was proposed to be a result of increased orbital overlap.}
Due to the intermediate rate of decomposition, clean conversion to the reduced product, ethyl acetate 103, and ease of purification of sulfonyl hydrazides 100a/b, as demonstrated by their isolation by column chromatography, 4-nitrobenzenesulfonyl chloride 95 was added to more complex hydrazine derivatives. Aminoglycinate derivatives were synthesised with alkyl chains varying from six to eight carbons in length through the acid-catalysed transesterification of ethyl hydrazinoacetate hydrochloride 93, which was converted to the amino glycinate (50-70% conversion) (Scheme 113). The corresponding sulfonyl hydrazides were synthesised by the addition of 4-nitrobenzenesulfonyl chloride 95 to the aminoglycinate derivative and the respective rates of decomposition were estimated though NMR spectroscopy. The apparent rates of decomposition of the longer chained sulfonyl hydrazides were significantly lower than those of sulfonyl hydrazides 100a/b. The two isomeric products of the sulfonyl hydrazides also had different rates of decomposition due to the difference in the pKₐ values of the acidic protons on the nitrogen β to the sulfonyl group. The variable rates of decomposition may also be due to the difference in solvation of the sulfonyl hydrazides due to the length of the carbon chain.
Octyl acetate was isolated in 29% yield following the synthesis and decomposition of sulfonyl hydrazides \(123a/b\) (Scheme 114). This result demonstrates that the products of such sigmatropic rearrangement reactions can be isolated. Therefore, hydrazine chemistry is a viable method to promote the removal of polar functional groups to generate lipophilic structures.

Boc-protected sulfonyl hydrazide \(127\) was obtained in moderate yield (61%) following the reaction of 4-nitrobenzenesulfonyl chloride \(95\) with Boc-protected hydrazine \(70\) (Scheme 115). Deprotection with TFA and hydrochloric acid both proved inefficient methods for the cleavage of the Boc-group, resulting in degradation of sulfonyl hydrazide \(127\), possibly due to the effect of the strong acids on the sensitive free hydrazine group instead of decomposition to the unfunctionalised product, ether \(78\) (Scheme 115). However, this indicates that protected sulfonyl hydrazide derivatives can be efficiently synthesised by the methods outlined. Removal of a
protecting group from an analogous sulfonyl hydrazide derivative without the use of harsh chemical conditions may be more effective.

Scheme 115: Substitution of sulfonyl chloride 95 on Boc-protected hydrazine 70

The formation of a biologically active molecule, paracetamol, through the removal of polar functional groups was investigated to test the biological compatibility of the project. Substituted hydrazine 134 was synthesised from tri-Boc-hydrazino acetic acid 135 via TFP-activated ester 138 in moderate yield following coupling with 4-aminophenol (Scheme 116). However, only one of the three protecting groups was efficiently removed following treatment with TFA or HCl. The addition of excess acid resulted in the isolation of a highly impure structure. As a result, hydrazine 133 and therefore paracetamol 131 could not be isolated. Based on these results, it was concluded that the hydrazine functionality is versatile to structural modifications, though the free hydrazine is unstable to isolation following deprotection.

Scheme 116: Attempted formation of paracetamol via removal of polar functional groups
Chapter 4 – Photosensitive Protecting Groups – The NVOC and NPPOC Groups

The reaction conditions established in the development of a synthetic route towards Boc-protected hydrazines were applied to the synthesis of NVOC- and NPPOC-protected hydrazines to develop a range of remotely activated molecules. The 6-nitroveratryl (NVOC) and 2-(2-nitrophenyl)propoxycarbonyl (NPPOC) protecting groups can be cleaved photolytically using UV light at wavelengths between 320 nm and 400 nm, hence triggering the decomposition reaction. The photodeprotection process removes the need for harsh chemical triggers, e.g. TFA, strong base or heat, and increases its suitability for use in the project, considering the potential biological applications.

Having established conditions for the successful synthesis of Boc-protected sulfonyl hydrazide 127, the key new reagents needed were NVOC- and NPPOC-hydrazine, to be used in place of Boc-carbazate.

4.1 Preparation of NVOC-protected sulfonyl hydrazide derivatives

4.1.1 Synthesis of NVOC-hydrazine

Initially, 4,5-dimethoxy-2-nitrobenzyl chloroformate 50 (6-nitroveratryl chloroformate, NVOC-chloride) was reacted with hydrazine monohydrate to form NVOC-hydrazine 141 (Scheme 117).106

![Scheme 117: Synthesis of NVOC hydrazine 141](image)

The two reagents were allowed to stir at room temperature for one hour, in which time NVOC-chloride 50 was consumed by the reaction. A tenfold excess of hydrazine monohydrate was used in order to prevent diprotection from occurring (where the NVOC-group is added to both nitrogen atoms). Upon formation, NVOC-hydrazine 141 precipitated out of solution. In order to remove
the excess hydrazine monohydrate after the reaction had gone to completion, the reaction mixture was washed with a saturated solution of sodium bicarbonate with additional solvent being added to dissolve the precipitate, and the resulting aqueous layer was extracted with dichloromethane. The product was obtained in high yield and purity following recrystallisation from ethyl acetate.

The transformation proceeds by an $S_N$Ac mechanism in which the electron-deficient carbonyl carbon in NVOC-chloride 50 undergoes nucleophilic attack by hydrazine monohydrate, leading to the elimination of the chloride anion via a tetrahedral intermediate (Scheme 118).

![Scheme 118: Mechanism of formation of NVOC-hydrazine 141](image)

4.1.2 Reductive amination of aromatic aldehydes with NVOC-hydrazine 141

4.1.2.1 Reductive amination of 4-hexadecyloxybenzaldehyde 68

NVOC hydrazine 141 was then reacted with 4-hexadecyloxybenzaldehyde 68 in refluxing $iso$-propanol to form NVOC-protected hydrazone 142 (Scheme 119). The conditions identified previously for the synthesis of Boc-protected hydrazones were used in the synthesis of NVOC-protected hydrazone 142. However, NVOC-hydrazine 141 was insoluble in $iso$-propanol which prevented the reaction from taking place efficiently.

![Scheme 119: Attempted synthesis of hydrazone 142](image)

Therefore, the solvent was changed to chloroform in which both reagents were known to be soluble (Scheme 120). A slight excess of NVOC-hydrazine 141 was used (in comparison to
aldehyde 68) in the hydrazone formation step to push the reaction to completion. The mechanism proceeds by nucleophilic attack of the terminal nitrogen of hydrazine 141 on the carbonyl carbon, followed by the elimination of water upon formation of a carbon-nitrogen double bond, as in the mechanism for the formation of Boc-protected hydrazones discussed earlier.

Scheme 120: Formation of hydrazone 142

The progress of the reaction was monitored by TLC that showed the reaction had gone to completion after 48 hours. However, resonances corresponding to aldehyde 68 were always present in the NMR spectrum of hydrazone 142 following removal of the solvent. Column chromatography was shown to be an inefficient method of purification as the relative concentration of aldehyde 68 in the sample increased compared to hydrazone 142 in the NMR spectrum. A larger excess of hydrazine 141 was used to push the reaction to completion. However, the aldehyde was still present in the NMR spectrum of the product, indicating that it was a result of hydrolysis of the hydrazone upon reaction work-up. Due to the mild acidity of silica, column chromatography of the crude product on silica resulted in increased hydrolysis of hydrazone 142, leading to an increased proportion of aldehyde 68 in the sample. Since the by-products would be removed during the purification of the final product, the crude product was taken directly on to the reduction step without purification. Sodium cyanoborohydride has previously been used to selectively reduce imines at pH 6-7 during the reductive amination of aldehydes and ketones,82 with no observable reaction with the carbonyl compounds at this pH. It was therefore concluded that the presence of aldehyde 68 would not lead to generation of the corresponding alcohol upon reduction.

Hydrazone 142 was reduced using the same reaction conditions as in the reduction of Boc-protected hydrazones 65 and 69 (Scheme 121). Solubility issues were encountered on carrying out the reaction in THF as hydrazone 142 was insoluble in THF, leading to the use of chloroform as a replacement solvent. Similarly, the formation of NVOC-protected hydrazine 143 proceeds by the same mechanism as outlined for the reduction of the Boc-protected hydrazones.
Issues were encountered in the purification of hydrazide 143; it was shown to be unstable on silica so alternative chromatographic methods were attempted, including column chromatography on alumina and silica impregnated with ammonia. However, spectroscopic analysis of the product showed that hydrazide 143 had decomposed in all cases, possibly due to hydrolysis. Recrystallisation from ethyl acetate emerged as the only efficient method for the purification of hydrazide 143, generating the pure product albeit in a low yield (36%).

4.1.2.2 Reductive amination of 4-(ethylhexanoate)oxybenzaldehyde 144

Aldehyde 144 was chosen as a second test molecule because the ester group could be hydrolysed, after adding NVOC-hydrazine 141 to form hydrazide 145 via reductive amination to reveal a polar functional group, i.e. a carboxylic acid group (Scheme 122). The molecule would decompose to form an amphiphilic molecule following photolytic cleavage of the protecting group.

Scheme 122: Formation of amphiphilic molecule 147
Therefore, aldehyde 144 was synthesised via the Williamson ether synthesis. 4-Hydroxybenzaldehyde 67 was reacted with ethyl 6-bromohexanoate 148 in the presence of a catalytic amount of potassium iodide under basic conditions in an Sn2 reaction to form aldehyde 144 (Scheme 123). The reaction proceeds by the same mechanism as outlined for the synthesis of aldehyde 68 and ether 78 (see pages 68 and 124).

Scheme 123: Synthesis of aldehyde 144

NVOC-protected hydrazone 149 was synthesised from aldehyde 144 using the same conditions as utilised in the preparation of NVOC-protected hydrazone 142 (Scheme 124). Similarly, problems were encountered in the isolation and purification of hydrazone 149 due to hydrolysis of the product, which generated the starting aldehyde 144. Therefore, the crude product was reduced directly with an excess of sodium cyanoborohydride in solution of THF and chloroform in the presence of acetic acid, followed by ethanalysis of the cyanoborane adduct with sodium ethoxide in ethanol to prevent the premature hydrolysis of the ester functionality (Scheme 124).

Scheme 124: Reductive amination of aldehyde 144 with NVOC-hydrazine 141

Issues were encountered in the purification of hydrazide 145. Recrystallisation of the crude product from ethyl acetate and petroleum ether was extremely low yielding (23%) and did not fully purify the product. Chromatographic purification techniques proved inefficient, leading to
the degradation of the hydrazide. Therefore, as hydrazide 143 had already been isolated, the synthesis of hydrazide 145 was deprioritised.

4.1.3 Addition of 4-nitrobenzenesulfonyl chloride 95 to NVOC-protected hydrazine 143

Sulfonyl chloride 95 had previously been identified through decomposition studies as a potential headgroup to be used to increase the solubility of hydrazine derivatives, while promoting their decomposition (Chapter 3, Scheme 77).

![Scheme 125: Addition of sulfonyl chloride 95 to NVOC-protected hydrazine 143](image)

NVOC-protected hydrazine 143 and sulfonyl chloride 95 were stirred at room temperature for 6.5 hours in a mildly basic solution of pyridine in chloroform (0.2 M) to form sulfonyl hydrazide 150 (Scheme 125). The progress of the reaction was monitored by NMR spectroscopy, which showed that the reaction took 6.5 hours at 25 °C. The formation of sulfonyl hydrazide 150 follows the same mechanism as in the formation of Boc-protected sulfonyl hydrazide 127 (see page 123).

Problems were encountered when attempting to purify sulfonyl hydrazine 150 since the compound decomposed on silica, alumina and ammonia-impregnated silica during column chromatography. As in the case of hydrazide 143, recrystallisation emerged as the only effective method for the purification of sulfonyl hydrazide 150. Recrystallisation of the crude product from dichloromethane generated the product in high purity but in low yield, giving an overall yield for the conversion of aldehyde 68 to sulfonyl hydrazide 150 of 12%.

The low yielding nature of the overall transformation may be due to a number of reasons; the high susceptibility of hydrazone 142 towards hydrolysis led to the loss of a significant quantity of hydrazone 142. The steric bulk of the NVOC-group may have impacted on the reduction of
hydrazone 142 to hydrazide 143. In addition, a significant amount of the product could have been lost through repeated recrystallisation processes. Since recrystallisation was the only method for efficiently purifying the products, there was no way of increasing the yield through the application of alternative purification techniques. However, sufficient material was obtained for photolysis studies.

4.2 Preparation of NPPOC-protected sulfonyl hydrazide derivatives

As an alternative to NVOC-protected sulfonyl hydrazide 150, the NPPOC-protected analogue was synthesised. The photolysis of NPPOC-protected compounds generates styrene derivative 58 (Figure 41) upon photolysis alongside the deprotected product. This is significantly less reactive than the by-product of the photolysis of NVOC-protected derivative, benzaldehyde derivative 48 (Figure 41) that could react with any unmasked hydrazines. It is therefore less susceptible to taking part in side reactions to form undesirable side products. In addition, NPPOC-caged compounds have been shown to have a higher quantum yield of uncaging than NVOC-protected analogues, typically generating a higher yield of deprotected product following photolysis.89

Figure 41: Photoby-products 48 and 58

Consequently, the synthetic strategy developed to synthesise NVOC-protected sulfonyl hydrazide 150 was applied to the synthesis of the analogous NPPOC-protected derivative.

4.2.1 Synthesis of NPPOC-hydrazine

NPPOC-hydrazine 152 was prepared by the reaction between NPPOC-chloroformate 151 and hydrazine monohydrate (Scheme 126). The same reaction and isolation conditions as previously used in the synthesis of NVOC-hydrazine 141 (see page 137).106 Similarly, an $S_{N}$Ac mechanism was followed as the electron-deficient carbonyl carbon in NPPOC-chloroformate 151 underwent nucleophilic attack by hydrazine monohydrate and the chloride leaving group was eliminated to form NPPOC-hydrazine 152.
The crude product was purified by column chromatography on silica using various ratios of petroleum ether and ethyl acetate as the eluent, generating the product in high yield and purity. The increased ease of purification of NPPOC-hydrazine 152 compared to NVOC-hydrazine 141 demonstrates that the NPPOC-group has an increased level of stability towards flash silica gel column chromatography.

### 4.2.2 Reductive amination of 4-hexadecyloxybenzaldehyde 68 with NPPOC-hydrazine 152

Following the preparation of NPPOC-hydrazine 152, hydrazone 153 was synthesised through the reaction of NPPOC-hydrazine 152 with aldehyde 68 in refluxing chloroform (Scheme 127). Following purification by column chromatography on silica gel, hydrazone 153 was isolated in high yield and purity. The extended reaction time of 96 hours that was needed for the formation of hydrazone 153 may be due to the difference in the solvation of NPPOC-hydrazine 152 compared to NVOC-hydrazine 141. The absence of an aldehyde peak in the NMR spectra of the crude and purified samples demonstrated that hydrazone 153 is more stable than corresponding NVOC-protected analogue 142.

**Scheme 127: Synthesis of hydrazone 153**

Hydrazone 153 was subsequently reduced by the method established previously in the synthesis of Boc- and NVOC-protected analogues. As in the synthesis of NVOC-protected hydrazine 143, solubility issues were experienced when the reaction was carried out in THF, as NPPOC-hydrazine 152 was insoluble in THF. The solvent was therefore changed to chloroform, in which the reaction
proceeded efficiently. NPPOC-protected hydrazine 154 was isolated in moderate yield following purification of the crude sample by column chromatography on silica gel (Scheme 128).

![Scheme 128: Synthesis of hydrazide 154](image)

The increased yield of formation of NPPOC-protected hydrazine 154, which was formed in 47% overall yield, in comparison to the NVOC-protected analogue (formed in 36% overall yield, Scheme 121) is most likely due to the increased stability of the NPPOC-protected derivatives. The increased stability allows purification of the crude mixtures by column chromatography instead of recrystallisation, which was used in the purification of the NVOC-protected analogue. As a result, a smaller proportion of product is lost during purification of the NPPOC-protected derivatives.

4.2.3 Addition of solubilising group sulfonyl chloride 95

Sulfonyl chloride 95 was then reacted with hydrazide 154 in a mildly basic solution of pyridine in chloroform (0.2 M) at room temperature (Scheme 129). After two days, NMR analysis showed that the starting materials had been consumed by the reaction and converted to product 155. The solvent was removed in vacuo and the product was purified by column chromatography on silica gel to obtain hydrazide 155 in moderate yield.

![Scheme 129: Synthesis of sulfonyl hydrazide 155](image)
The compatibility of the NPPOC-protected sulfonyl hydrazide 155 towards purification on silica again demonstrates the increased stability of the NPPOC-protecting group towards mildly acidic conditions during column chromatography on silica compared to the NVOC-protecting group.

The broadened resonances in the NMR spectrum (recorded at 27 °C/300 K) of sulfonyl hydrazide 155 corresponding to the aromatic protons 5 and 18 in Figure 43 indicate that there is restricted rotation about the amide bond due to the partial double bond character of the C-N as a result of conjugation. The restricted rotation is also possibly due to the steric bulk of the NPPOC- and sulfonyl groups. This was confirmed by VT-NMR, in which spectra were measured at temperatures ranging from 243 – 383 K (-30 – 110 °C) in deuterated tetrachloroethane (Figure 42).

![Figure 42: VT-NMR of sulfonyl hydrazide 155](image)

For temperatures between 323 – 383 K (50 – 110 °C), the resonances corresponding to protons numbered 18 appears sharper, whereas at temperatures of 303 K (30°C) and below, the peak is broad, showing the restricted rotation at lower temperatures. At 323 K (50 °C), resonances
corresponding to protons 7 and 9 are broadened while the amine protons were broadened at temperatures of 343 K (70 °C). In the NMR spectrum measured at 283 K (10 °C), the splitting of all other resonances was significantly reduced and had been eliminated entirely as the sample temperature was reduced further to 273 K (0 °C) and below.

The dynamic NMR (DNMR) program in Spinworks 4 was used to simulate the exchanging resonances in the NMR spectra, which was used to determine the rate constants ($k_r$) for the rotation of the amide bond at different temperatures. The activation energy ($E_a$) for the rotation around the bond was calculated from the Arrhenius plot (Figure 44).

![Arrhenius plot of ln($k_r$) against $T^{-1}$]

**Figure 44: Arrhenius plot of ln($k_r$) against $T^{-1}$**

Arrhenius equation:

$$k_r = A \exp(-E_a/RT)$$

where $A$ = pre-exponential factor, $R$ = gas constant = 8.3144 J mol$^{-1}$, $T$ = coalescence temperature

Slope = -$E_a/R$ = -2973.42

$E_a = 24.72$ kJ mol$^{-1}$

The enthalpy and entropy of activation ($\Delta H^\ddagger$ and $\Delta S^\ddagger$) for the rotation around the amide bond were calculated from the Eyring plot (Figure 45).
Figure 45: Eyring plot of ln(k_r/T) against T⁻¹

Slope = -ΔH°/R = -2678.64

ΔH° = 5.32 kJ mol⁻¹

Intercept = ΔS°/R + ln(K_b/h) = 9.24

ΔS° = -53.07 J mol⁻¹ K⁻¹

The Eyring equation was used to calculate the Gibbs free energy of activation (ΔG°) for the amide bond rotation at each temperature, assuming that the transmission coefficient (K) was equal to 1.

Eyring equation:

\[ k = K \left( k_b T / h \right) \exp(-\Delta G°/RT) \]

\[ \Delta G° = RT \left[ \ln(k_b T / h) - \ln(k_r) \right] \]

\[ \Delta G° = RT \left[ \ln(k_b / h) - \ln(T/k_r) \right] \]

where \( R = \) gas constant = 8.3144 J mol⁻¹, \( k_b = \) Boltzmann constant = 1.3806×10⁻²³ J mol⁻¹,

\( h = \) Planck’s constant = 6.6261×10⁻³⁴ J s, \( T = \) coalescence temperature

<table>
<thead>
<tr>
<th>Temperature/K</th>
<th>ΔG°/kJ mol⁻¹</th>
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<td>363.00</td>
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<td>243.00</td>
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</table>

Average Gibbs free energy of activation (ΔG°) = 58.85 kJ mol⁻¹
This value correlates with the Gibbs free energy of activation for restricted rotation around an amide bond in a carbamate since Rablen et al. calculated values from computational analyses in the range 64.0-65.3 kJ mol\(^{-1}\) for the restricted rotation about the amide bond in carbamate 156 (Figure 46) at 25 °C (298 K) in carbon tetrachloride, acetonitrile, methanol and methanol-water mixtures.\(^{107}\)

![Figure 46: Methyl N-benzyl-N-methyl-carbamate 156](image)

The Gibbs free energy of activation (\(\Delta G^\ddagger\)) does not vary a large amount at different temperatures because the enthalpy of activation (\(\Delta H^\ddagger\)) is much greater than the entropy of activation (\(\Delta S^\ddagger\)).

The Gibbs free energy of activation (\(\Delta G^\ddagger\)) differs from the activation energy (\(E_a\)) since \(\Delta G^\ddagger\) is the difference in Gibbs energy of the ground state of the reactants and the transition state, which is dependent on temperature, whereas activation energy (\(E_a\)) is the minimum energy required for a chemical reaction to occur and is temperature independent.\(^{108}\)

Similar broadening was observed of resonances in the NMR spectra of analogous NVOC- and NPPOC-protected hydrazines and sulfonyl hydrazides 143, 150, 154 and 157. The compounds were submitted to the NMR service in School of Chemistry at the University of Manchester for variable temperature NMR (VT-NMR) to calculate the Gibbs free energy of activation for the rotation to compare the values obtained for the derivatives. However, by the time the samples had been run, the compounds had degraded in solution so it was not possible to obtain clean spectra. The VT-NMR of sulfonyl hydrazide 155 gives a representative value for the Gibbs free energy of rotation for the above compounds.

### 4.2.4 Synthesis of sulfonyl hydrazide 157

Since 2-nitrobenzene substituted sulfonyl hydrazides 112a/b had previously been shown to decompose at a faster rate than the 4-nitrobenzene substituted analogue 100a/b (see page 105),
2-nitrobenzenesulfonyl chloride 111 was added to NPPOC-protected hydrazine 154 (Scheme 130), to increase the rate of decomposition of the molecules following photolysis of the NPPOC-group.

Scheme 130: Synthesis of sulfonyl hydrazide 157

Sulfonyl hydrazide 157 was prepared via the method used previously to synthesise sulfonyl hydrazides 127, 150 and 155, and the product was isolated in moderate yield following purification by column chromatography, eluting with various ratios of petroleum ether and ethyl acetate.

4.2.5 Synthesis of sulfonyl hydrazide 158

Sulfonyl hydrazide 158 was synthesised through the reaction between NPPOC-protected hydrazine 154 and 1,2-benzene disulfonic anhydride 87 (Scheme 131). It was hypothesised that the ortho-sulfonate group relative to the sulfur linkage in the product would result in a higher rate of decomposition, as was observed in the decomposition of sulfonyl hydrazide 98a/b to ethyl acetate 103 in earlier decomposition studies (see page 89).

Scheme 131: Synthesis of sulfonyl hydrazide 158

NPPOC-protected hydrazine 154 was reacted with an excess of 1,2-benzene disulfonic anhydride 87 in a mildly basic solution of pyridine in chloroform (0.3 M) at room temperature. The solvent was removed in vacuo after NMR analysis showed that the reaction had progressed to completion. The residue was redissolved in dichloromethane and the hydrolysed form of sulfonyl anhydride 87 was removed from solution by repeated aqueous washes. Following removal of
residual water, the product was isolated in approximately 90% purity, though the product could not be purified further due to the anionic charge on the molecule, as shown previously in the synthesis and attempted purification of sulfonyle hydrazides 88 and 90 (see pages 77 and 78).

4.2.6 Synthesis of a photosensitive aliphatic sulfonyle hydrazide derivative

An aliphatic analogue was synthesised to investigate the steric and electronic effects of the structure on the reaction. Undecane is the alarm pheromone for several species of ants (*Lasius umbratus* and *Acanthomyops claviger*), which would be produced following the uncaging of sulfonyle hydrazide 160 (Scheme 132).

Undecanal 163 was reacted with a small excess of NPPOC-hydrazine 152 in refluxing methanol (Scheme 133) to prepare hydrazone 162. The reaction was carried out in the presence of acetic acid in order to protonate the carbonyl carbon, thus increasing its electrophilicity and subsequently its susceptibility to nucleophilic attack by the nitrogen lone pair in NPPOC-hydrazine 152. This method was used instead of the combination of the reagents in refluxing *iso-*propanol/chloroform used in the synthesis of aromatic hydrazones, since it had been previously shown to increase the yield of Boc-protected hydrazone 76 obtained on reaction of decanal 75 with Boc-carbazate 32. Resulting hydrazone 162 was isolated in approximately 75% purity, though it was shown to be unstable on silica so the crude product was reduced directly with a
A fivefold excess of sodium cyanoborohydride and acetic acid in methanol (Scheme 133). Following work-up of the reaction, the cyanoborane adduct was hydrolysed with 2 M sodium hydroxide in methanol. NPPOC-protected hydrazine 161 was isolated following extraction with dichloromethane and purified by column chromatography to generate 161 in 30% overall yield.

Scheme 133: Synthesis of NPPOC-protected hydrazine 161

Sulfonyl hydrazide 160 was synthesised via the reaction between hydrazide 161 with sulfonyl chloride 95 in a solution of pyridine in chloroform (0.3 M, Scheme 134). The substitution reaction occurred at a significantly slower rate than the aromatic analogues (127, 150 and 155) and generated sulfonyl hydrazide 160 in significantly lower yield than previous reactions.

Scheme 134: Synthesis of sulfonyl hydrazide 160

The extended reaction times and low yield of formation of aliphatic derivative 161 is due to the lower stability of the structure compared to the aromatic analogues, which are resonance stabilised. The equilibrium lies towards the aldehyde starting material in the synthesis of hydrazone 162 since aldehyde 163 is more thermodynamically stable, thus leading to a slower and lower yielding reaction, which subsequently affects the overall yield of sulfonyl hydrazide 160.
4.2.7 Synthesis of NPPOC-protected hydrazine 164

Phenelzine 22 is a drug that can be used in the treatment of depression and inflammatory diseases due to the inhibition of monoamine oxidase (MAO) and semicarbazide-sensitive amine oxidase (SSAO).\textsuperscript{61,65} Phenelzine 22 was protected with the NPPOC-group to investigate the release of a biologically active molecule upon interaction with light (Scheme 135).

\textit{Scheme 135: Retrosynthesis of phenelzine 22 via photolysis of NPPOC-protected hydrazine 164}

NPPOC-protected hydrazone 165 was synthesised through the reaction between 2-phenylacetaldehyde 166 and NPPOC-hydrazine 152 in refluxing chloroform (Scheme 136), as in the synthesis of other NVOC- and NPPOC-protected aromatic hydrazone derivatives.\textsuperscript{79} The longer reaction times than for NPPOC-protected analogue 154 may be due to the higher electron density at the reaction site, i.e. the carbonyl carbon, due to its relatively greater distance from the aromatic ring. This would result in lower electrophilicity in comparison to aldehyde 68, where the electron density is reduced due to conjugation within the aromatic ring, thus increasing its electrophilicity and susceptibility to nucleophilic attack.

NPPOC-hydrazine 165 was shown to be unstable on silica. Therefore, the crude sample, isolated in moderate yield, was reduced directly using an excess of sodium cyanoborohydride and acetic acid in chloroform, as in previous examples.\textsuperscript{79,80} Similarly, the cyanoborane adduct was hydrolysed with sodium hydroxide (2 M) and methanol to form NPPOC-protected hydrazine 164 in 36% overall yield (Scheme 136).\textsuperscript{80}

\textit{Scheme 136: Synthesis of NPPOC-protected hydrazine 164}
The low yield may be due to the increased electron density on the reactive carbon, i.e. the carbonyl carbon in aldehyde 166 and the hydrazone carbon in 165, as discussed previously.

4.3 Summary

Seven photochemically protected hydrazine and sulfonyl hydrazide derivatives incorporating the NVOC- and NPPOC-groups have been synthesised for photolysis studies. The synthesis of NVOC-protected hydrazines 143 and 145 (Figure 47) has been investigated through the reductive amination of aldehydes 68 and 144. Problems were encountered with isolation of the products, particularly in the purification of hydrazone 145 that was obtained in low yield (23%) following recrystallisation which failed to completely purify the product. Hydrazide 143 was effectively purified by recrystallisation from ethyl acetate, forming the product in a yield of 36%.

![Figure 47: NVOC-protected hydrazines 143 and 145](image)

Sulfonyl hydrazide 150 was synthesised and isolated following recrystallisation from dichloromethane, generating the product in 12% overall yield from aldehyde 68 (Scheme 137). The low yield is due to the instability of the NVOC-protecting group, which leads to the loss of product at all stages of the synthetic route.
NPPOC-protected hydrazine and sulfonil hydrazide derivatives have been synthesised with relative ease and isolated in higher yields compared to the NVOC-protected analogues. This can be attributed to the increased stability of the NPPOC-group, which allows purification by column chromatography on silica. Sulfonil hydrazide 155 was subsequently synthesised and isolated in moderate yield, giving an overall yield of 28% for its formation from aldehyde 68 (Scheme 138).
Since the ortho-nitrobenzenesulfonyl group had been shown to promote a faster rate of decomposition in hydrazinoacetate derivative 112a/b in earlier decomposition studies compared to the para-nitro substituted derivative 100a/b (see page 105), 2-nitrobenzenesulfonyl chloride 90 was added to NPPOC-protected hydrazine 154. Sulfonyl hydrazide 157 was synthesised in 54% yield under identical conditions to those used in the synthesis of sulfonyl hydrazide 155 (Scheme 139).

![Scheme 139: Synthesis of sulfonyl hydrazide 157](image)

Since the ortho-sulfonate benzenesulfonyl headgroup had been shown to give the fastest rate of decomposition in earlier decomposition studies (98a/b, see page 89), 1,2-benzene disulfonic anhydride 87 was reacted with hydrazide 154. Sulfonyl hydrazide 158 was obtained in approximately 90% purity following repeated aqueous washes but it could not be purified further due to the negative charge on the headgroup (Scheme 140).

![Scheme 140: Synthesis of sulfonyl hydrazide 158](image)

An aliphatic NPPOC-protected sulfonyl hydrazide derivative was synthesised that would release undecane, the alarm pheromone of an ant, following photolysis and subsequent decomposition. 79,80,109 Sulfonyl hydrazide 160 was generated in a lower yield from aldehyde 163 than the aromatic analogues 155 and 157, giving an overall yield of 11% (Scheme 141).
A photochemically protected drug molecule was then synthesised to investigate the release of a drug molecule upon photolysis. NPPOC-protected phenelzine 164 was synthesised in 36% yield (Scheme 142).

Scheme 141: Synthesis of sulfonyl hydrazide 160

Scheme 142: Synthesis of NPPOC-protected phenelzine 164
Chapter 5 – Photolysis of NVOC- and NPPOC-protected Hydrazine Derivatives

The 6-nitroveratryloxycarbonyl (NVOC, 38, mechanism of photocleavage shown in Scheme 143) protecting group was developed as an alternative to the 2-nitrobenzyloxycarbonyl (NBOC, 37, Scheme 143) group. As the NVOC-group is cleaved upon irradiation at wavelengths longer than 320 nm, the NVOC group can be used to protect light sensitive residues such as tryptophan.\(^{83,86}\) In addition, the photobyl-product formed on photocleavage of NVOC-protected structures, nitroso benzaldehyde 48, is less nucleophilic than the by-product of NBOC-photocleavage (47).\(^{83}\) Therefore, it has a lower tendency to participate in reactions with the deprotected products.

Scheme 143: Mechanism of NBOC/NVOC-photocleavage

Modifications were made to the structure of the NBOC- (37) and NVOC-groups (38) to overcome the disadvantages associated with the nitrobenzyl cages, such as the toxicity of the by-products, which also act as internal light filters, and slow rates of release upon excitation.\(^{87,110}\) In studies carried out by Hasan and co-workers, the rates of photodeprotection of nucleotides incorporating modified forms of the NBOC-group were measured.\(^{88}\) Replacement of the benzylic methylene group in the NBOC-group with an ethylene link to form the o-nitro-2-phenethylxocarbonyl group (NPEOC, 55, Figure 48) was found to increase the rate of photolysis by a factor of 1.8 compared to the NBOC-group. The substitution of a methyl group at the benzylic position of the NPEOC-group resulted in the formation of 2-nitrophenylpropyloxycarbonyl (NPPOC, 56, Figure 48), which succeeded in increasing the rate of photolysis by a factor of 9.4 compared to the NPEOC-protected derivative.\(^{88}\)
In addition, the by-product that is formed on photocleavage of the NPPOC-group (mechanism of photocleavage shown in Scheme 144), styrene 58 is considerably less reactive than the by-product formed on cleavage of NVOC-protected derivatives, nitrosobenzaldehyde 48 (Scheme 143), so has a significantly lower tendency to participate in condensation reactions with the deprotected products, hence trapping them.

Scheme 144: Mechanism of NPPOC deprotection

5.1 Photolysis of photochemically protected sulfonil hydrazide derivatives

The photochemically protected sulfonil hydrazides synthesised and discussed in Chapter 4 were dissolved in a solution of deuterated pyridine in deuterated chloroform (0.3 M) and irradiated with UV light from an LED source. The use of an LED source has several advantages over mercury lamps, which are traditionally used to generate UV light. Mercury lamps are hazardous to health and the environment, while having a limited lifetime and choice of spectral lines of monochromaticity, whereas a range of spectral lines are available using an LED source, which has a significantly longer lifetime and less associated hazards. In addition, heat is not generated by LED sources which could affect the reaction and make it difficult to distinguish between reactions triggered solely by light or heat. Different wavelengths (330 nm, 360 nm and 375 nm) were used to irradiate the samples for varying time periods (15 minutes – 30 hours) to determine the optimal conditions for the photolysis of the sulfonil hydrazides. A 0.3 M solution of pyridine in chloroform was selected since it had promoted a relatively fast rate of sulfonil hydrazide decomposition in previous decomposition studies (Chapter 3, Scheme 65, see page 82). The reactions were carried out in deuterated solvents so that the progress of the photocleavage reactions could be monitored by NMR analysis. It was hoped that the decomposition process
would be initiated spontaneously following the cleavage of the protecting group due to the instability of the deprotected hydrazine.

It was anticipated that the decomposition process would proceed by a rapid electrocyclic rearrangement mechanism in the case of benzylic sulfonyl hydrazides 150, 155 and 157 to form the reduced product following the cleavage of the protecting group (Scheme 145) due to the conjugation in the aromatic molecule (which would stabilise the transition states in the mechanism).

Scheme 145: Photolysis and decomposition of aromatic sulfonyl hydrazides via a sigmatropic rearrangement

However, the decomposition of aliphatic analogue 160 following photolysis (Scheme 146) is most likely to proceed via a free radical mechanism since the transition states of an electrocyclic rearrangement mechanism would not be resonance stabilised in the case of intermediate 168.¹¹¹

Scheme 146: Photolysis and decomposition of aliphatic sulfonyl hydrazide 160 via a free radical mechanism

Tautomerisation of the diazene intermediate to the more stable hydrazone structure would compete with decomposition to form the unfunctionalised product.¹¹² No kinetic data is available for the conversion of monosubstituted diazenes to hydrazones, presumably as it takes

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¹¹¹

¹¹²
place rapidly due to the instability of the diazene group as a result of the low charge density on the unsubstituted (terminal) nitrogen and the higher stability of the hydrazone structure due to resonance stabilisation. Monosubstituted alkyl diazenes have been synthesised by Ackermann et al.\textsuperscript{113} and by Tsuji and Kosower,\textsuperscript{114} demonstrating the stability of the diazenes. However, both groups found that the alkyl diazenes decomposed rapidly and reacted vigorously with oxygen.\textsuperscript{115} The mechanism of decomposition of alkyl diazenes in the presence of water to form aldehydes or ketones is thought to proceed via the formation and hydrolysis of the hydrazone (Scheme 147).\textsuperscript{115}

\[ \text{Scheme 147: Decomposition of alkyldiazenes in the presence of water} \]

In an interesting example, Lemal et al. showed that the thermal decomposition of 1,1-dialkyl-2-benzenesulfonylhydrazide salts (169) in hot aqueous or alcoholic alkali to corresponding hydrazone 171 proceeds via the formation of diazene 170 upon elimination of a sulfinic acid derivative (Scheme 148).\textsuperscript{116} The diazene undergoes tautomerisation to form the hydrazone before or after the migration of the alkyl fragment to the unsubstituted nitrogen atom.

\[ \text{Scheme 148: Proposed mechanism for the formation of hydrazones on decomposition of sulfonyl hydrazides} \]

The reaction led to the rapid formation of hydrazones, though the rate of decomposition was reduced by electron-withdrawing substituents on the nitrogen atoms that stabilise the diazene. Conversely, electron-withdrawing substituents on the sulfonyl group increase the rate of decomposition, since these destabilise the diazene while stabilising the sulfonate anion and increasing the rate of elimination of the sulfonate group in the rate determining step of the decomposition process.\textsuperscript{117}
5.1.1 Photolysis of NVOC-sulfonyl hydrazide 150

In order to investigate the biological applicability of the project, the photolysis of NVOC-protected sulfonyl hydrazide 150 was investigated. Photolysis experiments were carried out in which a dilute solution of sulfonyl hydrazide 150 in a mildly basic solution of deuterated pyridine in deuterated chloroform was irradiated with UV light. Chloroform was selected as the solvent in which to carry out the photolysis experiments since it has been used in literature examples of photolysis experiments and has been reported to increase the rate of photolysis through the formation of radicals. The concentration of the pyridine/chloroform solution was chosen since it had given a relatively fast rate of reaction in earlier decomposition studies (Chapter 3). The growth of a singlet at 2.3 ppm would indicate the formation of the ether 78, the product of the sigmatropic rearrangement of free sulfonyl hydrazide 129 (Scheme 149). This would therefore demonstrate that photocleavage of the NVOC-group had taken place successfully and the resulting deprotected product had decomposed to form the unfunctionalised product, ether 78.

\[
\text{Scheme 149: Photolysis of sulfonyl hydrazide 150 at 330 nm for 30 minutes}
\]

Initially, a dilute solution of sulfonyl hydrazide 150 in deuterated pyridine and deuterated chloroform (0.3 M) was placed in a quartz cuvette, degassed under nitrogen and irradiated with light at 330 nm. The sample was degassed to remove the oxygen from the solution, which could interfere with the photolysis due to the potential formation of oxygen radicals. The reaction was run for an extended period (up to 30 hours) while monitoring at regular intervals by NMR spectroscopy. No change was observed in the spectra measured after photolysis to those recorded prior, demonstrating that the photolysis and subsequent decomposition had not taken place. It was therefore concluded that a longer wavelength was required to efficiently cleave the NVOC-protecting group of sulfonyl hydrazide 150, since the NVOC-protecting group can be cleaved between 320 and 400 nm.

Therefore, the sample was photolysed with light at 360 nm, which demonstrated more effective deprotection of sulfonyl hydrazide 150, since the NMR spectra showed that the sample composition had changed after two hours of irradiation at 360 nm (Figure 49). Resonances
evolved in the aromatic region of the NMR spectra slightly downfield (approximately 0.1 ppm) compared to those of the starting material, though these were very small compared to the resonances corresponding to the aromatic protons in sulfonyl hydrazide 150. This indicated that the NVOC-group had been partially cleaved from the compound, which increased slowly up to a maximum of 20% conversion after 2.5 hours, as demonstrated by the gradual increase in the integrals of the resonances corresponding to the deprotected product. A colour change was observed in the reaction mixture, as the solution turned yellow from colourless. This discolouration is a typical indicator of a photolysis reaction, as the evolution of the nitrosobenzaldehyde by-product 48 causes a darkening of the solution, which can act as an internal light filter.

Figure 49: Photolysis of NVOC-protected sulfonyl hydrazide 150 at 360 nm for 2.5 hours
However, the resonances expected for the decomposition product, ether 78, did not appear in the spectrum despite leaving the reaction for a longer period. In order to ensure that decomposition had not taken place, ether 78, that had previously been synthesised via the Williamson ether synthesis\textsuperscript{92} (see page 124), was spiked into the reaction mixture, which showed that no product was formed.

Following irradiation for periods longer than one hour, the sample was converted to aldehyde 68 (Figure 50), as demonstrated by the growth of a singlet at approximately 9.8 ppm (Figure 50). Comparison of the NMR and mass spectra of the photolysis mixture with those of aldehyde 68 synthesised via the Williamson ether synthesis (see page 68) confirmed the presence of the aldehyde.

![Figure 50: Expanded NMR spectrum showing the formation of aldehyde 68 following photolysis of sulfonil hydrazide 150](image)

This could be due to the formation of the diazene intermediate upon photocleavage of the NVOC-protecting group and subsequent elimination of sulfinic acid 105 (Scheme 150). The diazene may then undergo tautomerisation to form the more stable hydrazone structure, which would then be hydrolysed by residual water to form aldehyde 68.
A similar tautomerisation process has been observed to take place easily by the formation of a hydrazone side product (178) in the condensation of quinone monoketals and aliphatic hydrazines (Scheme 151). The hydrazone was most likely formed through the tautomerisation of corresponding diazene 177 due to the high acidity of the benzylic protons.

Scheme 151: Condensation of quinone monoketal 175 and aliphatic hydrazine 176

Alternatively, hydrazone 84 could be formed directly as a benzylic proton is cleaved to eliminate sulfinic acid 105 (Scheme 152). The benzylic site is prone to deprotonation due to the acidity of the benzylic protons, as electron density is withdrawn from the benzylic carbon by the conjugation in the aromatic ring and by the adjacent nitrogen atom. As a result, the C-H bonds are easily cleaved to form hydrazone 84. Hydrazine 84 would then be hydrolysed to form aldehyde 68.
Scheme 152: Alternative possible mechanism of formation of aldehyde 68

The sulfonyl headgroup could also be eliminated through a free radical mechanism, forming a radical intermediate that would be resonance stabilised (Scheme 153).\textsuperscript{52}

Another possibility is that sulfonyl hydrazide 129 is oxidised by an oxygen radical in the reaction solution following photolysis of the protecting group. Oxygen may have redissolved in the
solution after an extended period and could be excited by UV radiation. Aldehyde 68 could then be formed through a modification of the McFadyen-Stevens reaction (Scheme 154).\textsuperscript{121}

\begin{scheme}
\begin{center}
\includegraphics[width=\textwidth]{scheme154.png}
\end{center}
\caption{McFadyen-Steven conversion of sulfonyl hydrazide 150 to aldehyde 68}
\end{scheme}

Originally, the McFadyen-Stevens reaction (Scheme 155) employed harsh conditions to convert aromatic sulfonyl hydrazides (prepared from the condensation of aromatic carboxylic acids and sulfonyl chloride) to aldehydes.\textsuperscript{121} The decomposition of the sulfonyl hydrazides took place at 160 °C in the presence of sodium carbonate in ethylene glycol.\textsuperscript{121}

\begin{scheme}
\begin{center}
\includegraphics[width=\textwidth]{scheme155.png}
\end{center}
\caption{Original McFadyen-Stevens reaction conditions}
\end{scheme}

Alternative conditions have been developed that have utilised different bases and lower temperatures that have allowed the conversion of aliphatic carboxylic acids to the corresponding aldehydes, whereas the reaction was initially restricted to aromatic substrates.\textsuperscript{122–124} Substituted aromatic sulfonyl hydrazides have been converted to aldehydes at lower temperatures, such as the conversion of sulfonyl hydrazide 183 to aldehyde 184, which took place at room temperature in the presence of imidazole (Scheme 156).\textsuperscript{123}
This suggests that the formation of aldehyde 68 following the photolysis of sulfonyl hydrazide 150 via a modified version of the McFadyen-Stevens reaction is a feasible possibility if the deprotected sulfonyl hydrazide was oxidised in situ.

It was assumed that the formation of hydrazone 185 (Scheme 157), which was indicated by the evolution of a peak at 7.8 ppm in the NMR spectrum, was preventing the onset of the decomposition process. In order to inhibit the condensation reaction, scavengers were added to the photolysis solution including semicarbazide, hydroxylamine hydrochloride, and polymer-bound ethylene diamine and para-tosyl hydrazide.

On average, the hydrazone peak appeared in the spectrum after three hours when scavengers were present rather than two hours in their absence, i.e. the condensation reaction took place at a faster rate when scavengers were not added to the photolysis reaction. Therefore, the solution was irradiated for two hours in the presence of scavengers, in order to cleave the protecting group while avoiding the formation of hydrazone 185 (Scheme 157). NMR spectroscopy showed that partial deprotection of the sample had taken place, due to the presence of resonances in the aromatic region of the NMR spectrum downfield from those corresponding to the starting material.

The addition of scavengers to the photolysis solution did not give an improved yield of the deprotected product. Solubility issues were experienced with scavengers such as semicarbazide.
and hydroxylamine hydrochloride, thus explaining their low efficiency in preventing the condensation reaction. The reduced yield of photolysis could also be due to Rayleigh scattering as a result of the low solubility of the scavengers, as the photons would lose energy when they collide with insoluble particles so have insufficient energy to effect cleavage of the NVOC-group.

The effect of heating the photolysis solution to 45 °C was investigated to assess whether the loss of sulfinic acid 105 could be initiated by the application of heat. This temperature was selected since higher temperatures caused deprotected sulfonil hydrazide 129 to degrade immediately, as shown by NMR spectroscopy. Deuterated methanol was added to samples since the decomposition of hydrazine derivatives such as NBSH had been shown to take place at a higher rate in polar solvents such as methanol or water. However, neither higher temperatures nor the addition of methanol initiated the decomposition of sulfonil hydrazide 129 to ether 78. Instead, the resulting samples were shown to contain a mixture of products, with aldehyde 68 being the major product. This indicated that the rate of conversion to aldehyde 68 is increased by the application of heat, which possibly takes place by via the formation of hydrazone 84 (Scheme 150, Scheme 152 or Scheme 153) or oxidation followed by a modified McFadyen-Stevens conversion 121 (Scheme 154), as discussed previously.

Photolysis of sulfonil hydrazide 150 was attempted using light of wavelength 375 nm, in order to investigate the effect of a longer wavelength on the yield of deprotection. However, irradiation of the sample with light at this wavelength resulted in partial deprotection accompanied by the formation of hydrazone 185 and aldehyde 68, as in previous attempts. It was concluded that the formation of the by-product, nitrosobenzaldehyde 48, was preventing the initiation of the decomposition process through the condensation reaction with the free hydrazine. When scavengers were added to the reaction mixture to prevent the abovementioned condensation reaction, the yield of photolysis was further reduced possibly through Rayleigh scattering. Heating to 45 °C and adding deuterated methanol to the photolysis solution generated similar results to those obtained in previous attempts. No sign of decomposition was observed in the spectrum.

Since the formation of hydrazone 185 would have continued to be a possible problem, the photolysis of photochemically protected hydrazine derivatives incorporating the alternative
photosensitive protecting group, the 2-(2-nitrophenyl)propyl group (NPPOC, 56), was investigated.

5.1.2 Photolysis of NPPOC-protected sulfonyl hydrazide 155

NPPOC-sulfonyl hydrazide 155 was irradiated with light at 360 nm and 375 nm in order to investigate the optimal conditions for the photocleavage reaction (Scheme 158).

Initially, a dilute solution of sulfonyl hydrazide 155 in deuterated pyridine and deuterated chloroform (0.3 M) was degassed with nitrogen and irradiated with 360 nm light, since irradiation of light at this wavelength had been shown to effect the photolysis of analogous NVOC-protected derivative 150. A slight colour change was observed in the photolysis solution, which indicated the formation of light-sensitive by-product 58. However, NMR analysis of the photolysis solution at regular intervals showed very slow conversion to the deprotected product, as demonstrated by comparison of the resonances corresponding to sulfonyl hydrazide 155 with adjacent resonances corresponding to deprotected product 129.

Sulfonyl hydrazide 155 was instead irradiated with light at wavelength 375 nm, which resulted in a faster colour change, indicating a higher rate of deprotection. The resonances corresponding to the deprotected product grew into the NMR spectrum at a faster rate than previous attempts using light at 360 nm and in the photolysis of NVOC-protected sulfonyl hydrazide 150 (Scheme 149). Although a higher proportion of NPPOC-sulfonyl hydrazide 155 was deprotected than NVOC-protected analogue 150, no further deprotection was observed after 30 minutes of irradiation at 375 nm. Instead, conversion to aldehyde 68 was observed as in the photolysis of NVOC-protected sulfonyl hydrazide 150, possibly via the formation of hydrazone 84, which would
then be hydrolysed by residual water to form aldehyde 68 or a modified McFadyen-Stevens conversion to the aldehyde. The conversion of NPPOC-protected sulfonyl hydrazide 155 to aldehyde 68 may proceed by the same mechanisms as suggested for the conversion of NVOC-protected analogue 150 to aldehyde 68 (Scheme 150, Scheme 152 or Scheme 154).

The photolysis of NPPOC-protected sulfonyl hydrazide 155 generated deprotected product 129, identified by resonances slightly downfield in the NMR spectrum from those in sulfonyl hydrazide 155, in approximately 40% yield after 30 minutes (Figure 51), compared with 20% conversion of NVOC-protected sulfonyl hydrazide 150 to deprotected product 129 in 2.5 hours (Figure 49). This was calculated from the comparison of the integrals in the NMR spectrum of the respective photolysis solutions. The other resonances in the NMR spectrum of the photolysis mixture were assigned with reference to the COSY spectrum.
No signs of decomposition were observed in the spectra obtained after photolysis (Scheme 159). Decomposition of the deprotected product, sulfonyl hydrazide 129, was not initiated by heating the solution to 45 °C or by the addition of deuterated methanol. It was hypothesised that the addition of thermal energy may trigger the decomposition process and the decomposition of hydrazine derivatives such as NBSH has been shown to take place at a higher rate in polar solvents such as methanol or water. Ether 78, that had previously been synthesised via the Williamson ether synthesis (see page 124), was spiked into the reaction mixture, which confirmed that no product was formed.

Scheme 159: Conversion of sulfonyl hydrazide 155 to sulfonyl hydrazide 129
Instead, aldehyde 68 was formed following the application of heat as in the photolysis of NVOC-protected sulfonyl hydrazide 150. Similarly, this could be due to the formation and hydrolysis of hydrazone 84 (Scheme 150, Scheme 152 or Scheme 153) or the modified McFadyen-Stevens reaction of sulfonyl hydrazide 129, following oxidation by an oxygen radical (Scheme 154) as discussed previously. The rate of formation of aldehyde 68 is increased following the application of heat or addition of methanol, compared to the conversion from sulfonyl hydrazide 129 to aldehyde 68 following photolysis, as was observed in the photolysis experiments of NVOC-protected sulfonyl hydrazide 150.

5.1.3 Photolysis of sulfonyl hydrazide 157

The photolysis of sulfonyl hydrazide 157 in a solution of deuterated pyridine and deuterated chloroform (0.3 M, Scheme 160) generated the deprotected product in a higher yield than the photolysis of sulfonyl hydrazide 155, obtaining approximately 50% conversion of NPPOC-protected sulfonyl hydrazide 157 to deprotected product 186. This slight increase in yield could be due to the higher reactivity of the sulfonyl headgroup due to the ortho-substituted nitro group on the aromatic ring. The process occurred at a slightly higher rate, as the partial deprotection peaked after 15 minutes of irradiation with light of wavelength 375 nm (Figure 52).
After irradiating the sample for longer than 20 minutes, the product was converted to aldehyde 68 as observed by the evolution of resonances corresponding to aldehyde 68 in the NMR spectrum. The conversion of sulfonyl hydrazide 157 to aldehyde 68 could take place by the mechanisms as suggested for the formation of aldehyde 68 from sulfonyl hydrazides 150 and 155 as discussed previously (Scheme 150, Scheme 152, Scheme 153 or Scheme 154).
Although the evolution of new resonances in the NMR spectrum of the sample after photolysis of sulfonyl hydrazide 157 indicated that partial deprotection had taken place, the reduction product was not generated as there was no peak observed at 2.3 ppm, despite the addition of methanol and heat (45 °C) to initiate the decomposition process. Ether 78, that had previously been synthesised via the Williamson ether synthesis\textsuperscript{92} (see page 124), was spiked into the reaction mixture, which showed that no product was formed. Extended irradiation of the sample at 375 nm led to the generation of aldehyde 68, as observed previously, which may proceed by the mechanisms suggested earlier (Scheme 150, Scheme 152, Scheme 153 or Scheme 154).

\[ \text{Scheme 161: Conversion of sulfonyl hydrazide 157 to sulfonyl hydrazide 186 following photolysis} \]

The composition of the solution was modified to investigate the effect of varying the pH on the photolysis and decomposition processes. The photolysis was carried out in pure chloroform and in a solution of deuterated TFA in deuterated chloroform (5%) but the deprotection did not proceed in either case. This may have been due to the increased concentration of protons in the acidic solutions, which may have reduced the ability of the nitro group on the protecting group to deprotonate the benzylic site and initiate the deprotection mechanism.

Since this demonstrated that the presence of a base is essential for the process to proceed, the concentration of base was increased to investigate its effect on the rates of photolysis and decomposition. However, the increased basicity of the photolysis solution led to the immediate degradation of sulfonyl hydrazide 157 prior to photolysis, as shown by the diminution of all resonances in the aromatic region of the NMR spectra. It was concluded that the solution of
deuterated pyridine in deuterated chloroform (0.3 M) was of the optimum basicity to promote photodeprotection. Therefore, further modifications were made to the structure to increase its proficiency in the conversion to the deprotected sulfonyl hydrazide via photolysis.

5.1.4 Photolysis of sulfonyl hydrazide 158

![Scheme 162: Attempted photolysis of sulfonyl hydrazide 158](image)

Since sulfonyl hydrazide 158 could not be purified further due to the negative charge on the headgroup as demonstrated by earlier investigations (Chapter 2, Scheme 60), the sample, obtained following aqueous washes (90% purity), was photolysed directly. However, solubility issues prevented photolysis of the molecule in the solution of deuterated pyridine/chloroform.

5.1.5 Photolysis of sulfonyl hydrazide 160

![Scheme 163: Photolysis of sulfonyl hydrazide 160 at 375 nm for 15 minutes](image)

A dilute solution of sulfonyl hydrazide 160 in a solution of deuterated pyridine in deuterated chloroform (0.3 M) was degassed with nitrogen and irradiated with light of wavelength 375 nm (Scheme 163). Partial deprotection was observed which peaked after 15 minutes of irradiation at 375 nm (Figure 54).
No evidence was observed for the generation of the corresponding oxidation product as in previous photolysis experiments. However, degradation was evident after 20 minutes of irradiation as indistinguishable resonances appeared in the NMR spectra of the sample. Approximately 60% conversion from NPPOC-protected sulfonyl hydrazide 160 to deprotected product 162 was achieved following irradiation as indicated by the growth of new peaks in the NMR spectrum. This suggests that aliphatic compounds have a slightly higher reactivity upon interaction with light in comparison with aromatic analogues, which may be due to the lower electron density in the alkyl chain compared to in the aromatic ring as in the other structures photolysed. However, as the NMR spectra of undecane would only differ from that of sulfonyl hydrazide 160 by one proton, the product of decomposition could not be clearly identified in the NMR spectra of the photolysis mixture since it could not be purified by column chromatography or extraction. Therefore, without further evidence, the conclusion was drawn that the decomposition of sulfonyl hydrazide 160 to undecane 159 had not taken place following irradiation of the sample (Scheme 164).
5.1.6 GC-MS analysis of photolysis mixtures

In the GC-MS spectra of all photolysis solutions, a peak was present that corresponded to the unfunctionalised product, i.e. ether 78 in the case of the photolysis of sulfonyl hydrazides 150, 155 and 157 (m/z = 332.1) and undecane 159 in the case of sulfonyl hydrazide 160 (m/z = 156.3). Since unfunctionalised products 66 and 159 had not been observed in the NMR spectra, it was assumed that fragmentation had occurred in the mass spectrometer. The unfunctionalised product was also observed in the spectrum following GC-MS analysis of a photolysis reaction run by a colleague.\(^{125}\)

In order to establish whether the unfunctionalised products 78 and 159 were the products of fragmentation of photochemically protected sulfonyl hydrazides 150, 155, 157 and 160 or the deprotected analogues, sulfonyl hydrazides 150, 155, 157 and 160 were analysed by GC-MS. The peak corresponding to the unfunctionalised product was present in each spectrum, indicating that the protected sulfonyl hydrazides are able to undergo fragmentation. This indicates that the deprotected product formed by photolysis is too stable to fragment without an additional trigger. The fragmentation may occur via the thermal decomposition of the protected sulfonyl hydrazide.

\[ \text{Scheme 164: Conversion of sulfonyl hydrazide 160 to sulfonyl hydrazide 167} \]

\[ \text{Figure 55: Unfunctionalised products 78 and 159} \]
(Scheme 165) since high temperatures (350-400 °C) were employed to convert the solid sulfonyl hydrazides into gases for analysis.

\[
\begin{align*}
\text{PO}_\text{NH} & \quad \text{NO}_2 \\
\text{R}_1 & \quad \text{R}_2 \\
\text{Ng} & \quad \text{Py-d}_{9} \text{ in CDCl}_3 (0.3 \text{ M})
\end{align*}
\]

\[R_1 = \text{C}_2\text{H}_5\text{O} \quad (150, 155, 157), \quad \text{C}_1\text{H}_2\text{O} \quad (156), \quad \text{PG} = \text{NVOC} \quad (150), \quad \text{NPPOC} \quad (155, 157, 160) \]

\[R_2 = \text{C}_2\text{H}_5\text{H}_2 \quad (78), \quad \text{C}_1\text{H}_2\text{H}_2 \quad (159)\]

*Scheme 165: Possible fragmentation of sulfonyl hydrazides 150, 155, 157 and 160 via thermal decomposition*

5.2 Photolysis of NPPOC-protected hydrazine derivatives

From the photolysis experiments carried out on the NVOC- and NPPOC-protected sulfonyl hydrazides, it was apparent that irradiation of the compounds with UV light at various wavelengths did not trigger their decomposition to form unfunctionalised products. It was therefore concluded that the sulfonyl headgroups incorporated in photochemically protected sulfonyl hydrazides 150, 155, 157 and 160 may competitively absorb the UV light upon irradiation. This would in turn affect the rate of decomposition of the deprotected structure via a sigmatropic rearrangement or free radical mechanism (Scheme 166).

\[
\begin{align*}
\text{PO}_\text{NH} & \quad \text{NO}_2 \\
\text{R} & \quad \text{NH}_2 \quad \text{NO}_2 \\
\text{Ng} & \quad \text{Py-d}_{9} \text{ in CDCl}_3 (0.3 \text{ M}), 15-30 \text{ mins}
\end{align*}
\]

\[R = \text{C}_2\text{H}_5\text{H}_2 \quad (150, 155, 157), \quad \text{C}_1\text{H}_2\text{H}_2 \quad (156), \quad \text{PG} = \text{NVOC} \quad (150), \quad \text{NPPOC} \quad (155, 157, 160) \]

*Scheme 166: Possible effect of UV light on the structure of photochemically protected sulfonyl hydrazides*

The stability of the hydrazine functionality and photolysis of NPPOC-protected hydrazine derivatives were therefore investigated to examine this hypothesis.
5.2.1 Investigation of the stability of the hydrazine functionality upon irradiation

To investigate the sensitivity and stability of the hydrazine functionality towards irradiation with UV light, a dilute solution of Boc-protected hydrazine 70 in deuterated pyridine and deuterated chloroform (0.3 M) was degassed with nitrogen and irradiated with light at 375 nm for ten minutes.

![Scheme 167: Photolysis of Boc-protected hydrazine 70 at 375 nm for ten minutes](image)

As expected, no change was observed in the NMR spectrum of the solution after photolysis compared to that recorded prior to irradiation, demonstrating that the hydrazine functionality is stable to irradiation.

To confirm this conclusion and ascertain that the outcome was not a result of the added stability due to the presence of the Boc-protecting group, Boc-protected hydrazine 70 was deprotected using a previously identified method\(^{65}\) (see page 74) and the resulting HCl salt was deprotonated to liberate the free hydrazine (Scheme 168). A solution of hydrazine 80 in deuterated pyridine and deuterated chloroform (0.3 M) was then degassed with nitrogen and irradiated with light at 375 nm for ten minutes to replicate the conditions used in previous photolysis experiments.
Scheme 168: Deprotection and deprotonation of Boc-protected hydrazine 70 followed by irradiation of 80

As in the photolysis of Boc-protected hydrazine 70, there was no change in the NMR spectrum of free hydrazine 80 following irradiation compared to the initial spectrum. Degradation of the structure was observed in the NMR spectrum measured after the solvent had been removed from the sample in vacuo as shown by the diminution of all resonances in the NMR spectrum. This demonstrated that the hydrazine functionality is stable to interaction with light in solution. However, the resulting free hydrazine cannot be isolated due to the high reactivity and sensitivity of the hydrazine moiety to air.

5.2.2 Photolysis of NPPOC-protected hydrazine 154

Scheme 169: Photolysis of NPPOC-protected hydrazide 154

A dilute solution of NPPOC-protected hydrazine 154 in deuterated pyridine in deuterated chloroform (0.3 M) was degassed with nitrogen and irradiated with light at 375 nm (Scheme 169), leading to approximately 30% conversion to deprotected hydrazine 80 (Figure 56). The composition of the solution was monitored by NMR spectroscopy to identify the optimum reaction time. Resonances adjacent to those of the aromatic protons in the starting material,
Hydrazide 154 began growing in the spectrum after 5 minutes of irradiation, indicating that deprotection was taking place. After fifteen minutes of irradiation, a peak at 9.8 ppm grew in the spectrum, indicating the conversion of the deprotected hydrazine to aldehyde 68 possibly via the formation and hydrolysis of hydrazone 84. The deprotected product, hydrazine 80, cannot be clearly identified in the spectrum since the resonances of the starting material, which is not completely consumed in the reaction, overlap with those that would allow the identification of the product.

![NMR spectrum](image)

**Figure 56: Partial photolysis of NPPOC-protected hydrazine 154**

Upon isolation of the crude sample in vacuo, decomposition was observed as the peak corresponding to an aromatic proton in styrene by-product 58 at 6.03 ppm disappeared from the spectrum, in addition to the diminution of the aromatic resonances corresponding to deprotected product 80 as well as all other resonances in the NMR spectrum apart from those corresponding to the starting material. Column chromatography on silica gel was unsuccessful, only isolating a small proportion of the starting material. Other purification techniques were utilised in an effort to isolate the deprotected hydrazine 80, including extraction, preparative TLC and column chromatography on alumina. However, in all cases the compounds isolated consisted of NPPOC-
protected hydrazine 154 or structures unrecognisable from the starting material or products. This indicates that the free hydrazine 80 is unstable when isolated.

5.2.3 Isolation of the hydrochloride salt of hydrazine 80

Since it had been found that the product of photolysis, hydrazine 80, could not be isolated, the stability of the protonated form of the deprotected product was investigated. A fivefold excess of hydrochloric acid was added to the solution following photolysis (Scheme 170), in order to generate the HCl salt of the deprotected product.

Additional resonances that were observed in the NMR spectra adjacent to those of hydrazide 154 following photolysis remained in the NMR spectrum after the addition of hydrochloric acid and removal of the solvent. This indicated that the stability of the photolysis mixture was increased through the addition of an excess of hydrochloric acid in diethyl ether. However, due to the presence of at least three compounds in the mixture, it was not possible to identify the resonances corresponding to hydrazine 83. In addition, as a result of the presence of residual pyridine that could not be removed under high vacuum, the resonances were shifted by 0.1 - 0.2 ppm. Thus, the spectrum of the photolysis mixture could not be overlaid by the spectrum obtained following deprotection of Boc-protected hydrazine 70 to form hydrazine 83 (see page 74).
The COSY spectrum indicated the presence of three compounds in the resulting mixture. Cross peaks were identified as corresponding to NPPOC-protected hydrazine 154. Other resonances were identified in the same region as the product of the deprotection of Boc-protected hydrazine 70 (Scheme 55, page 74), though these were overlapped with other resonances so could not be clearly identified. Liquid chromatography mass spectrometry (LCMS) was carried out on the crude sample and a peak corresponding to hydrazine salt 83 ([M + Na]$^+$, m/z = 423.1) was identified in the spectrum. However, the peak identified as the product was relatively small compared to the other products of the reaction.
In an effort to isolate hydrazine 83 from the photolysis mixture, the crude residue was dissolved in dichloromethane and repeatedly washed with deuterium oxide to remove the hydrazine from the organic phase, since it was assumed that the HCl salt would be water soluble. Both aqueous and organic layers were analysed by NMR spectroscopy, but the product was not present in either sample. Therefore it was concluded that the sample had decomposed on contact with water if it was present in the crude sample, possibly via hydrolysis due to the high reactivity and sensitivity of the free hydrazine group. Hydrazine 83 could not be isolated through column chromatography due to the charged nature of the product.

Further research is required into the development of an efficient method for the generation of HCl salt 83 via the photolysis of NPPOC-protected hydrazine 154 and its subsequent purification, possibly by reverse phase high performance liquid chromatography (HPLC). In addition, further modifications must be made to the structure to ensure that complete conversion to the deprotected product takes place upon irradiation to increase the suitability of the technique for biological applications.

5.2.4 Photolysis of NPPOC-protected phenelzine 164

NPPOC-protected hydrazine 164 was dissolved in a solution of deuterated pyridine in deuterated chloroform (0.3 M), which was then degassed under nitrogen and irradiated with light of wavelength 375 nm (Scheme 171). The photolysis solution was analysed by NMR spectroscopy at five minute intervals to investigate the progress of deprotection.

After irradiating the sample for ten minutes, approximately 40% conversion was observed from NPPOC-protected hydrazine 164 to phenelzine 22, which was estimated through the comparison of the integrals of resonances corresponding to the starting material and product (Figure 58). Upon irradiation of the solution for longer time periods, resonances corresponding to aldehyde 166 evolved into the NMR spectrum, indicating that deprotected hydrazine 22 was oxidised as in previous examples.
However, as in the photolysis of NPPOC-protected hydrazine 154, deprotected product 22 could not be isolated. The NMR spectrum of the crude photolysis solution indicated that the deprotection had occurred due to the presence of resonances corresponding to protons in phenelzine 22. Upon removal of the solvent in vacuo, degradation was observed in the spectrum below 4.5 ppm, and the resonances corresponding to deprotected phenelzine 22 diminished significantly, leaving only the starting material unchanged. Silica gel column chromatography of the crude sample led to the isolation of the starting material in addition to compounds that were unrecognisable from the starting material or products, confirming that degradation of the products had taken place.

5.2.5 Isolation of the protonated form of photolysis product, phenelzine 22

Following the synthesis of the hydrochloride salt of deprotected hydrazine 80, it was hypothesised that phenelzine 22 could be protonated in the same way (Scheme 172), to allow its isolation.
The stability of the photolysis mixture was increased through the addition of an excess of hydrochloric acid in diethyl ether, as the new peaks that had grown into the spectrum following irradiation of the sample were still present in the spectrum after the solvent had been removed in vacuo. However, due to the presence of at least three compounds in the mixture, it was difficult to identify the resonances corresponding to hydrazine 188 (Figure 59). In addition, as a result of the presence of residual pyridine in the sample that could not be removed under high vacuum, the resonances were shifted by 0.1 - 0.2 ppm. Thus, the spectrum of the photolysis mixture could not be easily compared with NMR data of phenelzine hydrochloride obtained by Zhang and coworkers.  

Scheme 172: Photolysis and isolation of product as HCl salt 188
The presence of resonances shifted slightly from the resonances representative of the two CH$_2$ groups in NPPOC-protected hydrazine 164 that were shown to couple by cross peaks in the COSY NMR spectrum suggest that the deprotection had taken place. The identified resonances were positioned in the same region as the corresponding protons in the literature example. Liquid chromatography mass spectrometry (LCMS) was carried out on the crude sample and resonances corresponding to the hydrazine salt 188 ([M + Na]$^+$, m/z = 195.1) and the unfunctionalised product 189 (m/z = 106) were identified in the resulting spectrum. It was assumed that compound 189 was formed through the fragmentation of hydrazine 188 upon ionisation in the mass spectrometer. However, there were a number of resonances present in the spectrum, of which the resonances identified as products 188 and 189 were relatively small. This suggests that hydrazine 188 was only formed as a minor product. Combined with the NMR spectra of the crude mixture, the LCMS result suggests that the NPPOC-group was partially cleaved from hydrazide 164 photolytically.

Figure 59: Stacked NMR spectra of NPPOC-protected phenelzine 164, the photolysis mixture and the photolysis mixture after addition of HCl

Figure 60: Fragmentation product 189
Isolation of hydrazine 188 was attempted by washing the sample with deuterium oxide since it was assumed that the molecule would be water soluble due to the charge on the molecule. However, HCl salt 188 was not contained in either the aqueous or organic phases, as shown by NMR spectroscopy. Therefore it was concluded that the sample had decomposed on contact with water via hydrolysis due to the high reactivity and sensitivity of the hydrazine functionality, hence leading to its instability.

Further research is required into the development of an efficient method for the purification of HCl salt 188, possibly by reverse phase high performance liquid chromatography (HPLC). However, this study has suggested that a biologically active drug compound may be uncaged by light and is stable as the protonated form, though further research is required to confirm this and to increase the efficiency of the technique.

5.3 Summary

The photolysis of NVOC- and NPPOC-protected sulfonyl hydrazide and hydrazine derivatives was investigated using UV light of various wavelengths (330 nm, 360 nm and 375 nm) from an LED source.

Irradiation of photochemically protected sulfonyl hydrazides 150, 155, 157 and 160 with UV light of various wavelengths (320 nm, 360 nm and 375 nm) led to partial deprotection as indicated by the growth of resonances in the NMR spectrum slightly shifted (0.1 ppm) from resonances in the aromatic region corresponding to the protected sulfonyl hydrazides. However, decomposition was not observed in any of the photolysis experiments despite the application of heat (45 °C) and the addition of deuterated methanol to the photolysis mixture in an effort to initiate decomposition. Decomposition of sulfonyl hydrazides, e.g. NBSH, has been shown to be faster in polar solvents such as water or methanol. Following prolonged irradiation of aromatic sulfonyl
hydrazides 150, 155 and 157, aldehyde 68 was generated as shown by the growth of resonances in the NMR spectrum corresponding to the aldehyde. This could be due to the formation and hydrolysis of hydrazone 84 (Scheme 150, Scheme 152 or Scheme 153) or a modified form of the McFadyen-Stevens rearrangement (Scheme 154).

Each of the photolysis solutions were analysed by GC-MS and a peak corresponding to the unfunctionalised products, ether 78 in the photolysis of sulfonyl hydrazides 150, 155 and 157 ($m/z = 332.1$) and undecane 159 in the photolysis of sulfonyl hydrazide 160 ($m/z = 156.3$), was identified. It was assumed this was a result of fragmentation in the spectrometer since the unfunctionalised products were not present in the spectra. Sulfonyl hydrazides 150, 155, 157 and 160 were also analysed by GC-MS and the peaks corresponding to the unfunctionalised products were present in the spectra, demonstrating that the protected sulfonyl hydrazides are able to undergo fragmentation (possibly via thermal decomposition in the mass spectrometer). This suggests that the conditions of the photolysis experiments are not harsh enough to initiate decomposition.

It was therefore concluded that the two features of the reaction, i.e. photolysis of the photochemically protected sulfonyl hydrazides and decomposition of the resulting deprotected sulfonyl hydrazides, were incompatible. The interaction of the headgroup with light may have led to modifications to the nitro group on the aromatic headgroup in the sulfonyl hydrazides. This could have resulted in the reduced ability to promote the decomposition of the free sulfonyl hydrazide via a sigmatropic rearrangement or free radical mechanism. This could be due to the formation of free radicals or the abstraction of protons from the aromatic ring by the nitro substituent on the ring, possibly leading to the generation of a nitroso group.

The stability of the hydrazine functionality was investigated, demonstrating that it was stable to irradiation with UV light but could not be isolated since the free hydrazine degraded upon removal of the solvent in vacuo. Therefore, the photolysis of NPPOC-protected hydrazine derivatives 154 and 164 was investigated. Following the photolysis of NPPOC-protected hydrazine 154, it was found that irradiation of the NPPOC-protecting group at 375 nm for a period of ten minutes possibly led to partial deprotection (Scheme 174) (though the product of deprotection could not be clearly identified in the NMR spectrum since the resonances of the starting material and product overlapped). However, the growth of additional resonances in the spectra suggested...
that deprotection had taken place. The stability of the sample was increased through the addition of hydrochloric acid in diethyl ether, as the new resonances that had evolved in the spectrum following irradiation were still present after the removal of the solvent in vacuo. Attempts were made to isolate the hydrochloride salt of the deprotected product from the crude photolysis mixture through aqueous washes to confirm its presence in the photolysis mixture. However, this method proved ineffective. Therefore, further research is required to develop a method for the formation of the deprotected hydrazine through photolysis and its subsequent purification, possibly through reversed phase HPLC.

![Scheme 174: Photolysis of NPPOC-protected hydrazine 154 and protonation of the resulting deprotected product](image.png)

NPPOC-protected phenelzine 164 was photolysed to form phenelzine 22 (Scheme 175), as indicated by the growth of new resonances in the NMR spectrum. Similarly, the sample degraded upon removal of the solvent in vacuo. The stability of the deprotected hydrazine was increased by the generation of the protonated form. The resulting HCl salt 188 could not be isolated by washing the solution with water. Therefore further research is required into the development of a method for the purification of the deprotected product, possibly through reversed phase HPLC.
Scheme 175: Photolysis of NPPOC-protected phenelzine 164 and protonation of the resulting deprotected product
A small library of protected hydrazine and sulfonyl hydrazide derivatives have been synthesised incorporating the Boc-, NVOC- and NPPOC-protecting groups. The hydrazine derivatives were formed via the reductive amination of aldehydes.\textsuperscript{79,80} Problems were experienced with low yields and extended reaction times, in particular with aliphatic hydrazides, which were formed slowly and in low yield. Therefore, research was concentrated initially on the formation of aromatic hydrazides with electron-donating groups in the para position with respect to the hydrazine functionality, which were formed significantly faster and in higher yield. The NVOC-group was also shown to be unstable, with hydrazones readily undergoing hydrolysis upon isolation and chromatographic purification, leading to problems in the isolation of the corresponding hydrazides. Consequently, NVOC-protected hydrazines were purified by recrystallisation, but this led to a significant loss of product. The synthetic route towards protected sulfonyl hydrazides was optimised and can be used in future.

Decomposition studies were carried out using aromatic sulfonyl groups with different electronic properties, which allowed the identification of a headgroup that led to the generation of unfunctionalised products in reasonable times. Octyl acetate was generated and isolated from the decomposition of the corresponding aminoglycinate derivative, demonstrating that the technique can be used to reveal unfunctionalised products.

Photolysis experiments identified that NVOC- and NPPOC-groups can be cleaved photolytically, though problems were experienced with the photolysis of NVOC-protected sulfonyl hydrazide\textsuperscript{150} due to the formation of the nitrosobenzaldehyde photoby-product that can undergo condensation reactions with the deprotected product. Scavengers were added to the photolysis solution to prevent this but no improvement in the yield of photolysis was observed. The NPPOC-group was photolysed more efficiently since the styrene photoby-product did not participate in condensation reactions with the product of deprotection. The synthesis of NPPOC-protected sulfonyl hydrazides\textsuperscript{155} and\textsuperscript{157} was also significantly less challenging than that of the NVOC-protected analogue\textsuperscript{150} due its increased stability. Therefore, the NPPOC-group was found to be preferable compared to the NVOC-group for the protection of hydrazines.
Scheme 176: Photolysis of photochemically protected sulfonyl hydrazides

However, decomposition to the unfunctionalised products following photolysis was not observed in any of the experiments. Persistent formation of aldehyde 68 following photolysis of aromatic sulfonyl hydrazides indicated adventitious oxidation or hydrolysis are potential problems (Scheme 176).

Photolysis of NPPOC-protected hydrazines suggested that the structures are not stable to photolysis conditions, although the hydrazine functionality itself was shown to be stable to irradiation. The photolysis of NPPOC-protected phenelzine 164 appeared to generate phenelzine 22 marginally more efficiently though the NMR spectrum was not clear due to overlapping of signals of the starting material and product, which could not be isolated (Scheme 177). Therefore, further research is required into the development of a method for the generation of unfunctionalised molecules by photolysis, their isolation and purification.

Scheme 177: Photolysis of NPPOC-protected phenelzine 164 at 375 nm for 10 minutes

The generation of aldehyde 68 could be avoided by the addition of two methyl groups to the terminal nitrogen (Compound 190 in Figure 61) prior to the protection of the molecule, which would prevent the oxidation process. However, initial studies have shown the synthesis of such molecules to be exceptionally challenging. Decomposition of sulfonyl hydrazides in the solid state may also be more efficient since it would eliminate the risk of hydrolysis.
As it stands, the photolysis of NPPOC-protected hydrazines could be used to expose aldehydes on a surface, since such carbonyl compounds have demonstrated biological activity, e.g. 11-cis-retinal.\textsuperscript{126} Two-photon excitation is an alternative technique that could be used to deprotect hydrazide 154. Near-IR photons that are utilised in two-photon excitation carry a smaller risk to UV-sensitive biomolecules than conventional one-photon excitation.\textsuperscript{127} The focal point of irradiation is more defined due to reduced scattering of the photons upon near-IR irradiation.\textsuperscript{30} However, two-photon excitation has been shown to be less efficient than one-photon excitation, especially in the case of nitrobenzyl-derived protected compounds. The uncaging efficiency of the NPPOC-protecting group can be improved by the addition of a triplet sensitiser with a large two-photon absorption cross-section.\textsuperscript{127} Therefore, two-photon excitation may be used to increase the yield of deprotection of NPPOC-protected hydrazine 154 in the presence of a sensitiser (Scheme 178), while avoiding degradation of deprotected hydrazine 80.

Scheme 178: Two-photon excitation of NPPOC-protected hydrazine 154 in the presence of a triplet sensitiser, isopropyl thioxanthone\textsuperscript{127}

Alternatively, the photolysis of NPPOC-protected aliphatic hydrazine derivatives could be investigated further using one-photon excitation since sulfonyl hydrazide 160 gave the highest yield of conversion to the deprotected product.
Chapter 7 - Experimental

7.1 General methods

All experiments were carried out in oven-dried glassware and, in cases where it is stated that a
dry atmosphere is required, the reaction was carried out under a nitrogen atmosphere.
Anhydrous THF was obtained after distillation over sodium. Other solvents were obtained from
the manufacturer. Solvents were removed under reduced pressure using a Büchi rotary
evaporator and remaining traces of water were removed from samples under high vacuum.
Photolysis experiments were carried out on an Atlas Photonics instrument (Lumos 43) with a UV-
LED light source.

Analytical thin layer chromatography (TLC) was performed using silica gel pre-coated plates and
flash chromatography was carried out using silica gel (Sigma-Aldrich) 40 – 63 µm 60 Å. \(^1\)H NMR
were run at 300 MHz, 400 MHz or 500 MHz ; \(^{13}\)C NMR at 75, 100 and 125 MHz respectively.
Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane (TMS)
and coupling constants (J) are given in Hertz (Hz). Chemical shifts of resonances in \(^1\)H and \(^{13}\)C
NMR spectra were reported relative to the chemical shift of residual protio solvent in the
samples.\(^{128}\) \(^1\)H assignments were supported by 2D one-bond \(^1\)H-\(^1\)H COSY spectra and \(^{13}\)C
assignments by 2D one-bond \(^{13}\)C-\(^1\)H HMQC spectra. When reporting the \(^1\)H NMR data, the
following abbreviations will be used: s, singlet; d, doublet; t, triplet; q, quartet; dd, double
doublet; td, triplet of doublets; m, multiplet; br. s, broad singlet; quint, quintet; o/l, overlapping.

High and low resolution mass spectrometry (LRMS) was carried out by staff in the Mass
Spectrometry Laboratory in the School of Chemistry at the University of Manchester using
electrospray ionisation (ES) or gas chromatography. Electrospray mass mass spectrometry was
carried out on an Acquity UPLC instrument. GC-MS was carried out on an Agilent technologies
7890A spectrometer with an Agilent technologies 5975C inert XL EI/Ci MSD Triple-Axis Detector.
Elemental analysis was also carried out by staff of the School of Chemistry on a Thermo Scientific
instrument (Flash 2000). Infrared (IR) spectroscopy was carried out on a Bruker FT-IR
spectrometer (Alpha 11915496) in the School of Chemistry at the University of Manchester. The
recorded absorption maxima (\(v_{\text{max}}\)) are expressed in wavenumbers (cm\(^{-1}\)).
7.2 Synthesis

7.2.1 tert-Butyl (E)-2-(4-methoxybenzylidene)hydrazine-1-carboxylate$^{129}$

Boc-carbazate 32 (1.89 g, 0.014 mol) was added to a solution of 4-methoxybenzaldehyde 59 (1.89 g, 1.68 mL, 0.014 mol) in iso-propanol (70 mL) and the mixture was allowed to stir at reflux for 21 h. The solvent was removed in vacuo, leaving the product as a colourless viscous oil which solidified to give colourless plate-like crystals which required no further purification (2.57 g, 0.01 mmol, 73%), $R_f = 0.45$ (3:1 petroleum ether: ethyl acetate). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.06 (br. s, 1H, H$_4$), 7.79 (br. s, 1H, H$_5$), 7.60 (d, $J = 8.8$ Hz, 2H, H$_7$), 6.85 (d, $J = 8.8$ Hz, 2H, H$_8$), 3.80 (s, 3H, H$_{10}$), 1.52 (s, 9H, H$_1$); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 161.4 (C$_3$), 153.5 (C$_9$), 144.3 (C$_5$), 129.3 (C$_7$), 127.1 (C$_6$), 114.6 (C$_8$), 81.6 (C$_2$), 55.7 (C$_{10}$), 28.9 (C$_1$); IR cm$^{-1}$ 3294 (br.), 2860 (br.), 1686 (C=O, str.), 1607 (C=N, 1541, 1507, 1369, 1275, 1244, 1154; $m/z$ (LRMS, ES$^+$) 273 (100%, [M + Na$^+$]); HRMS (ESI) $m/z$ [M + H]$^+$, Calculated for C$_{13}$H$_{19}$N$_2$O$_3^+$, 251.1391; Found, 251.1400.
7.2.2 tert-Butyl 2-(4-methoxybenzyl)hydrazine-1-carboxylate

Sodium cyanoborohydride (1 M solution in THF, 6 mL, 6 mmol) was added to a solution of Boc-protected hydrazone 65 (400 mg, 1.6 mmol) in methanol (6 mL) and acetic acid (12 mL), then the reaction mixture was allowed to stir at room temperature for 96 h. The reaction mixture was diluted with a saturated solution of sodium bicarbonate (24 mL) and ethyl acetate (24 mL). The organic phase was separated, washed with brine and sodium bicarbonate (saturated solution), dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure to give a yellow oil. The intermediate cyanoborane adduct was then hydrolysed with sodium hydroxide (2 M, 7.6 mL) in methanol (12 mL) and the mixture was allowed to stir for 18 h. The solvent was evaporated under reduced pressure and water (20 mL) and DCM (20 mL) were added. The organic phase separated then the aqueous phase was further extracted with DCM (3 × 20 mL). The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated to give Boc-protected hydrazide as a colourless oil, which solidified on scratching the insides of the flask with a spatula to form colourless viscid crystals, which required no further purification (280 mg, 1.1 mmol, 69%), Rf = 0.19 (3:1 petroleum ether: ethyl acetate). 1H NMR (400 MHz, CDCl3) δ 7.27 (d, J = 8.5 Hz, 2H, H8), 6.85 (d, J = 8.5 Hz, 2H, H9), 6.17 (br. s, 1H, H4), 3.92 (s, 2H, H6), 3.79 (s, 3H, H11), 1.53 (br. s, 1H, H5), 1.46 (s, 9H, H1); 13C NMR (101 MHz, CDCl3) δ 159.7 (C3), 152.8 (C10), 130.9 (C7), 129.3 (C8), 114.5 (C9), 81.1 (C2), 55.9 (C6 and C11, o/l), 29.0 (C1); IR cm⁻¹ 2922 (br.), 2851 (br.), 1715 (C=O, str.), 1513, 1367, 1249, 1159, 1034; m/z (LRMS, ES+) 275 (100%, [M + Na]+), 253 (40%, [M + H]+); HRMS (ESI) m/z [M + H]+, Calculated for C13H21N2O3+, 253.1547; Found, 253.1540. 
7.2.3 (4-(Methoxy)benzyl)hydrazine hydrochloride

Hydrochloric acid (2M solution in diethyl ether, 1.2 mL, 0.375 mmol) was added to a solution of tert-butyl 2-(4-(methoxy)benzyl)hydrazine-1-carboxylate 60 (120 mg, 0.48 mmol) in methanol (5 mL) and the solution was allowed to stir at reflux for 48 h. The solvent was removed in vacuo and the product was isolated as a colourless powder (85 mg, 0.45 mmol, 95%). $^1$H NMR (300 MHz, MeOD) $\delta$ 7.37 (d, $J = 8.5$ Hz, 2H, H$_5$), 6.98 (d, $J = 8.5$ Hz, 2H, H$_6$), 4.12 (s, 2H, H$_3$), 3.81 (s, 3H, H$_8$); $^{13}$C NMR (101 MHz, MeOD) $\delta$ 162.0 (C$_7$), 139.2 (C$_5$), 132.4 (C$_4$), 115.5 (C$_6$), 62.8 (C$_8$), 55.7 (C$_3$); IR cm$^{-1}$, 3243 (br.), 2893 (br.), 1514, 1248, 1113; m/z (LRMS, ES+) 122 (100%, [M – HCl – N$_2$H$_2$]$^+$), 153 (25%, [M - Cl]$^+$).

7.2.4 tert-Butyl (E)-2-(4-(methoxycarbonyl)benzylidene)hydrazine-1-carboxylate

Boc-carbazate 32 (81 mg, 0.61 mmol) was added to a solution of 4-carboxymethylbenzaldehyde 71 (100 mg, 0.61 mmol) in iso-propanol (3 mL) and the reaction mixture was allowed to stir at reflux for 48 h. The reaction mixture was then allowed to cool to room temperature and the solvent was removed under reduced pressure to give tert-Butyl (E)-2-(4-(methoxycarbonyl)benzylidene)hydrazine-1-carboxylate as a colourless crystalline solid which required no further purification (166 mg, 0.596 mmol, 98%), $R_f$= 0.43 (2:1 petroleum ether: ethyl acetate). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.14 (br. s, 1H, H$_5$), 8.03 (d, $J = 8.0$ Hz, 2H, H$_6$), 7.88 (br. s, 1H, H$_4$), 7.74 (d, $J = 8.0$ Hz, 2H, H$_8$), 3.91 (s, 3H, H$_{11}$), 1.54 (s, 9H, H$_1$); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 207.4 (C$_{10}$), 167.0 (C$_7$), 142.5 (C$_9$), 138.5 (C$_8$), 131.3 (C$_3$), 130.2 (C$_9$), 127.3 (C$_5$), 82.2 (C$_4$), 52.6 (C$_{11}$), 28.6 (C$_1$); IR cm$^{-1}$ 3243 (br.), 2979 (br.), 2930, 1717 (C=O), 1534, 1436, 1368, 1247, 1149, 1111, 1057, 859, 770, 700; m/z (LRMS, ES-) 277 (100%, [M-H$^-$]); HRMS (ESI) m/z [M + Na]$^+$ Calculated for
C_{14}H_{18}N_{2}O_{4}Na^+, 301.1159; Found, 301.1147; Anal. Calculated for C_{14}H_{18}N_{2}O_{4}·0.66H_{2}O; C 57.92, H 6.71, N 9.65; Found, C 57.77, H 6.51, N 9.12;

7.2.5 tert-Butyl (E)-2-decylidenehydrazine-1-carboxylate

Decanal 75 (0.12 mL, 95.3 mg, 0.61 mmol) was added to a solution of Boc-carbazate 32 (81 mg, 0.61 mmol) and acetic acid (35 µL, 0.61 mmol) in methanol (3 mL) and the reaction mixture was allowed to stir at reflux for five days. The solvent was removed in vacuo and the hydrazone was isolated as a colourless glass-like solid that required no further purification (92 mg, 0.34 mmol, 56%), R_f = 0.44 (6:1 petroleum ether: ethyl acetate). ^1H NMR (400 MHz, CDCl_3) δ 7.58 (s, 1H, H_11), 7.13 (t, J = 5.0 Hz, 1H, H_10), 2.28 (td, J = 13.2 Hz, 5.0 Hz, 2H, H_9), 1.54 – 1.44 (m, 13H, H_7,8,14), 1.36 – 1.19 (m, 10H, H_2-6), 0.87 (t, J = 6.8 Hz, 3H, H_1); ^13C NMR (101 MHz, CDCl_3) δ 163.2 (C_12), 147.7 (C_10), 81.2 (C_13), 32.4 (C_9), 29.6, 29.4, 28.4, 26.9, 26.4, 26.1, 22.8 (7CH_2, C_2 to C_8), 14.2 (C_1); IR cm⁻¹ 3236 (br.), 2925 (br.), 2855, 1708 (C=O), 1535, 1457, 1366, 1250, 1168, 1046; m/z (LRMS, ES−) 269.2 (100%, [M − H]−); HRMS (ESI) m/z [M + Na]^+ Calculated for C_{15}H_{30}N_{2}O_{2}Na^+, 293.2199; Found, 293.2201; Anal. Calculated for C_{15}H_{30}N_{2}O_{2}·0.25DCM; C 63.52, H 10.66, N 9.75; Found, C 63.46, H 10.55, N 9.64.

7.2.6 4-Hexadecyloxybenzaldehyde

A mixture of 4-hydroxybenzaldehyde 67 (305.3 mg, 2.5 mmol), hexadecylbromide 66 (763.3 mg, 0.76 mL, 2.5 mmol), potassium carbonate (1.73 g, 12.5 mmol) and potassium iodide (8 mg, 0.05 mmol) in DMF (5 mL) was allowed to stir at 70°C for 18 h. The reaction was cooled to room
temperature and mixed with water (approximately 5 mL). The resulting aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure to give a colourless oil. To remove the remaining DMF the residue was redissolved in diethyl ether and washed with water and brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure to give 4-hexadecyloxybenzaldehyde as an off-white solid. This solid was used in the next step of the reaction without further purification (669 mg, 1.93 mmol, 77%), Rf = 0.72 (4:1 petroleum ether: ethyl acetate). The characterisation data corresponds with that found by Brun and Etemad-Moghadam.\textsuperscript{132} \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 9.87 (s, 1H, H\textsubscript{1}), 7.82 (d, J = 9 Hz, 2H, H\textsubscript{3}), 6.98 (d, J = 9 Hz, 2H, H\textsubscript{4}), 4.03 (t, J = 6.6 Hz, 2H, H\textsubscript{6}), 1.81 (m, 2H, H\textsubscript{7}), 1.46 (m, 2H, H\textsubscript{8}), 1.39 – 1.20 (m, 22H, H\textsubscript{9} - H\textsubscript{20}), 0.87 (t, J = 7.0 Hz, 3H, H\textsubscript{21}); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ 172.2 (C\textsubscript{1}), 164.6 (C\textsubscript{5}), 132.3 (C\textsubscript{4}), 130.0 (C\textsubscript{2}), 115.1 (C\textsubscript{3}), 68.8 (C\textsubscript{6}), 32.3, 30.0, 29.9, 29.8, 29.7, 29.4, 26.3, 23.0 (14C, CH\textsubscript{2}, C\textsubscript{7} to C\textsubscript{20}), 14.5 (C\textsubscript{21}); IR cm\textsuperscript{-1} 214 (br.), 2847, 1688 (C=O), 1601, 1578, 1469, 1396, 1166, 1046, 1026; m/z (LRMS, ES+) 369 (100%, [M + Na]\textsuperscript{+}); HRMS (ESI) m/z [M + H]\textsuperscript{+} Calculated for C\textsubscript{23}H\textsubscript{39}O\textsubscript{2}+, 347.2945; Found, 347.2934; Anal. Calculated for C\textsubscript{23}H\textsubscript{38}O\textsubscript{2}, C 79.71, H 11.05; Found, C 79.40, H 10.99.

7.2.7 tert-Butyl (E)-2-(4-(hexadecyloxy)benzylidene)hydrazine-1-carboxylate

Boc-carbazate 32 (81 mg, 0.61 mmol) was added to a solution of 4-hexadecyloxybenzaldehyde 68 (208 mg, 0.61 mmol) in iso-propanol (3 mL) and the reaction mixture was allowed to stir at reflux for 48 h. tert-Butyl (E)-2-(4-(hexadecyloxy)benzylidene)hydrazine-1-carboxylate 69 was collected in \textit{vacuo} and purified by column chromatography on silica gel (4:1 to 2:1 petroleum ether/ethyl acetate) to give a purified product as a colourless crystalline solid (265 mg, 0.58 mmol, 94%), Rf = 0.64 (4:1 petroleum ether: ethyl acetate). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 7.87 (br. s, 1H, H\textsubscript{3}), 7.76 (br. s, 1H, H\textsubscript{4}), 7.58 (d, J = 8.5 Hz, H\textsubscript{7}), 6.88 (d, J = 8.5 Hz, H\textsubscript{8}), 3.95 (t, J = 6.4 Hz, 2H, H\textsubscript{10}), 1.77 (m, 2H, H\textsubscript{11}), 1.53 (s, 9H, H\textsubscript{1}), 1.44 (m, 2H, H\textsubscript{12}), 1.40-1.25 (m, 22H, H\textsubscript{13-24}), 0.87 (t, J = 6.4 Hz, H\textsubscript{25}); \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}) δ 161.0(C\textsubscript{3}), 152.8 (C\textsubscript{5}), 144.0 (C\textsubscript{4}), 129.0 (C\textsubscript{2}), 126.7 (C\textsubscript{1}), 114.9 (C\textsubscript{6}), 81.6
(C₂), 68.4 (C₁₀), 32.3, 30.0, 29.9, 29.7, 29.5, 28.6, 26.3, 23.0 (17C, CH₂, C₁, C₁₁ to C₂₄), 14.5 (C₂₅); IR cm⁻¹ 3294 (br.), 2916 (br.), 2850, 1709 (C=O), 1608, 1534, 1509, 1473, 1363, 1244, 1166, 1054; m/z (LRMS, ES⁺) 483 (100%, [M + Na]⁺); HRMS (ESI) m/z [M + H]⁺ Calculated for C₂₈H₄₉N₂O₃⁺, 461.3738; Found 461.3734; Anal. Calculated for C₂₈H₄₉N₂O₃, C 72.68, H 10.89, N 6.05; Found, C 72.82, H 10.48, 5.96.

7.2.8 tert-Butyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate

Sodium cyanoborohydride (1 M solution in THF, 1.5 mL, 1.5 mmol) was added to a solution of tert-butyl (E)-2-(4-(hexadecyloxy)benzylidene)hydrazine-1-carboxylate 69 (130 mg, 0.28 mmol) in THF (1.5 mL) and acetic acid (3 mL), then the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with a saturated solution of sodium bicarbonate (6 mL) and ethyl acetate (6 mL). The organic phase was separated and washed with brine, then a saturated solution of sodium bicarbonate. The organic phase was dried with magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The intermediate cyanoborane adduct was hydrolysed by reaction with sodium hydroxide (2 M, 1.9 mL) in methanol (3 mL) and THF (1 mL). The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed and water (5 mL) and DCM (5 mL) were added. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. tert-Butyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate was collected as a crystalline colourless solid which required no further purification (59 mg, 0.13 mmol, 46%), Rf = 0.53 (5:1 petroleum ether: ethyl acetate). ¹H NMR (500 MHz, CDCl₃) δ 7.25 (d, J = 9.0 Hz, 2H, H₈), 6.86 (d, J = 9.0 Hz, 2H, H₉), 6.07 (br. s, 1H, H₄), 3.98 – 3.90 (m, 4H, H₆,1₁₁), 1.77 (m, 2H, H₁₂), 1.51 – 1.40 (m, 11H, H₁,1₃), 1.38 – 1.24 (m, 24H, H₁₄₋₂₅), 0.88 (t, J = 6.9 Hz, 3H, H₂₆); ¹³C NMR (126 MHz, CDCl₃) δ 159.0 (C₉), 157.0 (C₁₀), 130.6 (C₈), 129.6 (C₇), 114.8 (C₆), 80.9 (C₅), 68.4 (C₄), 55.63 (C₃), 32.3, 30.0, 29.9, 29.8, 29.7, 29.6, 28.7, 26.4, 23.0 (17C, CH₃, C₁, C₁₂ to C₂₅), 14.5 (C₂₆); IR cm⁻¹ 3223 (br.), 2894 (br.), 1691 (C=O), 1608, 1534, 1509, 1473, 1363, 1244, 1166, 1054; m/z (LRMS, ES⁺) 483 (100%, [M + Na]⁺); HRMS (ESI) m/z [M + H]⁺ Calculated for C₂₈H₄₉N₂O₃⁺, 461.3738; Found 461.3734; Anal. Calculated for C₂₈H₄₉N₂O₃, C 72.68, H 10.89, N 6.05; Found, C 72.82, H 10.48, 5.96.
7.2.9 (4-(Hexadecyloxy)benzyl)hydrazine hydrochloride

Hydrochloric acid (2 M solution in diethyl ether, 0.19 mL, 0.375 mmol) was added to a solution of tert-butyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate (35 mg, 0.075 mmol) in methanol (3 mL) and the solution was allowed to stir at reflux for 24 h. The solvent was removed in vacuo and the product was isolated as a colourless powder (28 mg, 0.069 mmol, 92%). \(^1\)H NMR (400 MHz, MeOD) \(\delta 7.35 (d, J = 8.7\ Hz, 2H, H_5), 6.95 (d, J = 8.7\ Hz, 2H, H_6), 4.11 (s, 2H, H_3), 1.76 (m, 2H, H_9), 1.45 (m, 2H, H_{10}), 1.40 - 1.22 (m, 24H, H_{11-22}), 0.89 (t, J = 6.9\ Hz, 3H, H_{23}); \(^{13}\)C NMR (126 MHz, CDCl_3) \(\delta 160.9\ (C_4), 133.2\ (C_5), 129.5\ (C_6), 115.5\ (C_7), 68.4\ (C_8), 53.6\ (C_9), 32.3, 30.0, 29.9, 29.8, 29.7, 29.6, 28.7, 26.4, 23.0\ (14C, CH_2, C_9 to C_{22}), 14.3\ (C_{23}); IR \ cm^{-1} 3309\ (br.), 2903\ (br.), 1583, 1248, 1183; m/z (LRMS, ES+) 332 (100%, [M - HCl - N_2H_2]^+) , 364 (25%, [M - Cl]^+).

7.2.10 Benzene disulfonic anhydride

Benzene disulfonic anhydride was synthesised according to the method of Hurtley.\(^{94}\) A mixture of dipotassium 1,2-benzenedisulfonate (200 mg, 0.64 mmol) and chlorosulfonic acid (1.4 g, 0.8 mL, 0.012 mol) was heated at 120°C for 4 h. The reaction mixture was cooled to room temperature followed by the dropwise addition of ice-cold water to the reaction mixture until the

1514, 1366, 1245, 1172; m/z (LRMS, ES+) 491 (100%, [M + Na + Li]^+); HRMS (ESI) m/z [M + H]^+ Calculated for C_{28}H_{51}N_2O_3^+, 463.3895; Found, 463.3885; Anal. Calculated for C_{28}H_{50}N_2O_3, C 72.68, 10.89, 6.05; Found, C 72.80, H 10.50, N 5.73.
acid was quenched. The solid precipitate was then collected by filtration under reduced pressure and washed with ice-cold water and dried in vacuo. Benzene disulfonic anhydride was collected as off-white granular crystals (59.3 mg, 0.27 mmol, 42%). \(^1\)H NMR (400 Hz, CDCl\(_3\)) \(8.05 (4H, m, H_{2,3}); \)\(^{13}\)C NMR (126 MHz, DMSO) \(\delta 139.8 (C_1), 131.7 (C_2), 128.6 (C_3); IR (cm\(^{-1}\)) 1582, 1246, 1180.

7.2.11 tert-Butyl 2-(4-(hexadecyloxy)benzyl)-2-((4-nitrophenyl)sulfonyl)hydrazine-1-carboxylate

tert-Butyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate 70 (14 mg, 0.031 mmol) and 4-nitrobenzenesulfonyl chloride (7 mg, 0.031 mmol) were dissolved in a solution of pyridine-\(d_5\) in CDCl\(_3\) (0.2M, 1 mL) and the reaction mixture was allowed to stir for 5 h at room temperature. The solvent was removed in vacuo to give the crude product as a colourless oil, which was purified by column chromatography on silica gel (4:1 petroleum ether:ethyl acetate) to give the product as a colourless solid (12.3 mg, 0.019 mmol, 61%). \(R_f = 0.60 (4:1\) petroleum ether:ethyl acetate). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 8.36 (d, J = 9.0 Hz, 2H, H_7), 8.09 (d, J = 9.0 Hz, 2H, H_6), 7.22 (d, J = 8.6 Hz, 2H, H_12), 6.87 (d, J = 8.6 Hz, 2H, H_12), 6.31 (br. s, 1H, H_4), 4.68 (br. s, 1H, H_6), 3.94 (t, J = 6.6 Hz, 2H, H_13), 1.77 (m, H_15), 1.43 (m, 2H, H_16), 1.34 - 1.15 (m, 24H, H_17-28), 0.88 (t, J = 6.9 Hz, 3H, H_29); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta 159.6 (C_3), 153.4 (2C o/l, aromatic, C_8 and C_13), 148.4 (C_9), 132.2 (C_{11}), 131.9 (C_6), 130.8 (C_{10}), 124.7 (C_7), 115.0 (C_{12}), 82.3 (C_2), 68.4 (C_{14}), 53.5 (C_9), 32.3, 30.0, 29.9, 29.7, 29.6, 28.1, 26.4, 23.0 (15C, alkyl, C_1, C_{15} to C_{28}), 14.5 (C_{29}); IR cm\(^{-1}\) 2922 (br.), 2852, 1705 (C=O), 1611, 1532, 1512, 1347, 1248, 1156, 1090, 854; \(m/z\) (LRMS, ES+) 670 (100%, \([M + Na]^+\)); HRMS (ESI) \(m/z\) \([M + Na]^+\) Calculated for \(C_{34}H_{53}N_3O_7Na^+\), 670.3496; Found, 670.3488; Anal. Calculated for \(C_{34}H_{53}N_3O_7\cdot 2\text{EtOAc}, C 61.21, H 8.44, N 5.10, S 3.89; Found, C 61.21, H 8.51, N 5.10, S 3.62.

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7.2.12 Tri-tert-butyl 2-(2-oxo-2-(2,3,5,6-tetrafluorophenoxy)ethyl)hydrazine-1,1,1-tricarboxylate

Tri-(boc)hydrazinoacetic acid 135 (90 mg, 0.23 mmol) and tetrafluorophenol 137 (42 mg, 0.25 mmol) were dissolved in CHCl₃ (5 mL). DCC (52 mg, 0.25 mmol) in CHCl₃ (5 mL) was added dropwise to the reaction mixture and was allowed to stir at room temperature for 3 h. The dicyclohexylurea was removed by filtration and the filtrate was concentrated in vacuo. The residue was suspended in petroleum ether (10 mL) and the remaining urea was removed by filtration. The filtrate was concentrated in vacuo to give the crude product as a colourless oil. The crude sample was purified by column chromatography on silica gel (4:1 petroleum ether: ethyl acetate) to obtain the pure product as a colourless oil. The product was obtained as a mixture of inseparable rotamers (1:1) (75 mg, 0.14 mmol, 55%). Rₐ = 0.26 (4:1 petroleum ether: ethyl acetate). 

¹H NMR (500 MHz, CDCl₃) δ 7.01 (tt, J = 10 Hz, 3.5 Hz, 1H, H₁), 4.50 (s, 1H, H₆), 4.45 (s, 1H, H₆), 1.51 (s, 9H, H₁₂), 1.50 (s, 9H, H₁₅), 1.47 (s, 9H, H₉); ¹³C NMR (126 MHz, CDCl₃) δ 163.8 (C₅), 153.7 (C₄), 152.8 (C₈), 150.4 (C₁₀), 150.1 (C₁₃), 147.1 (dd, J = 7.4 Hz, 5 Hz, C₃), 145.2 (dd, J = 11.7 Hz, 5.3 Hz, C₃), 141.6 (dt, J = 15.6 Hz, 2.3 Hz,C₂), 139.6 (dt, J = 15.4 Hz, 1.5 Hz, C₁), 103.6 (C₁), 84.2 (C₈), 83.5 (C₁₁), 82.8 (C₁₄), 53.1 (C₆), 28.2 (C₉), 28.0 (2C o/l, CH₃, C₁₂ and C₁₅); IR cm⁻¹ 2980 (br.), 2935 (br.), 1804, 1767 (C=O), 1727 (C=O), 1524, 1488, 1369, 1248, 1145, 1096; m/z (LRMS, ES+) 561.2 (100%, [M + Na]⁺); HRMS (ESI) m/z [M + Na]⁺ Calculated for C₂₃H₂₉N₂O₈F₄Na⁺, 561.1837; Found, 561.1831.
7.2.13 4-((Triisopropylsilyl)oxy)aniline

Triisopropylsilyl chloride (0.58 g, 0.64 mL, 3 mmol) was added to a solution of 4-aminophenol (0.24 g, 2 mmol) and imidazole (0.20 g, 3 mmol) in DCM (5 mL) and the reaction mixture was allowed to stir for 26 h at room temperature. The reaction mixture was diluted with water (9 mL) and the resulting aqueous layer was extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The crude sample was purified by column chromatography on silica gel (6:1 petroleum ether: ethyl acetate) to give the product (340 mg, 1.28 mmol, 64%). Rf = 0.13 (4:1 petroleum ether: ethyl acetate). The characterisation corresponds with that previously collected by Tietze and Ma. 1H NMR (400 MHz, CDCl$_3$) δ 6.72 (d, $J$ = 8.8 Hz, 2H, $H_3$), 6.65 (d, $J$ = 8.8 Hz, 2H, $H_4$), 1.28 – 1.09 (m, 3H, $H_6$), 1.08 (d, $J$ = 7.0 Hz, 18H, $H_7$); 13C NMR (400 MHz, CDCl$_3$) δ 148.6 (C$_2$), 139.9 (C$_5$), 120.3 (C$_3$), 116.2 (C$_4$), 17.9 (C$_6$), 12.5 (C$_7$); IR cm$^{-1}$ 2943 (br.), 2865, 1611, 1506, 1463, 1384, 1245; m/z (LRMS, ES+) 266 (100%, [M + H]$^+$); HRMS (ESI) m/z [M + H]$^+$ Calculated for C$_{15}$H$_{28}$NOSi$, 266.1935, Found, 266.1927.

7.2.14 tri-tert-Butyl 2-(2-oxo-2-((4-((triisopropylsilyl)oxy)phenyl)amino)ethyl)hydrazine-1,1,2-tricarboxylate

Triethylamine (43.6 µL, 31.6 mg, 0.31 mmol) was added to a solution of TFP-activated tri(boc)hydrazinoacetic acid (55.3 mg, 0.2085 mmol) and TIPS-protected 4-aminophenol (74.8 mg, 0.139 mmol) in acetonitrile (5 mL). The reaction mixture was allowed to stir at room temperature for 18 h. The solvent was removed in vacuo and the crude sample was purified by column chromatography on silica gel (5:1 - 3:1 petroleum ether: ethyl acetate). The product was
isolated as a mixture of inseparable rotamers (1:1) which took the form of a colourless oil (14.6 mg, 0.02 mmol, 14%). Rf = 0.31 (5:1 petroleum ether: ethyl acetate). 1H NMR (500 MHz, CDCl3) δ 10.06 (s, 0.5H, H1), 9.82 (s, 0.5H, H2), 7.48 (d and d o/l, J = 9.0 Hz, 2H, H3), 6.83 (d and d o/l, J = 9.0 Hz, 2H, H4), 4.18 (s, 1H, H5), 4.08 (s, 2H, H6), 1.55 (s, 18H, H15,18), 1.45 (s and s o/l, 9H, H12) 1.24 (m, 3H, H3), 1.10 – 1.07 (s and s o/l, 18H, H1); 13C NMR (126 MHz, CDCl3) δ 166.8 (2C o/l, aromatic, C8 and C10), 166.5 (2C o/l, carbonyl, C13 and C16), 154.1 (C1), 154.1 (C4), 153.4 (C6), 152.6 (C9), 121.2 (C4), 120.9 (C6), 120.2 (C5), 120.0 (C3), 85.4 (C11), 83.7 (C14), 82.9 (C17), 57.2 (C5), 55.1 (C8), 28.1 (3C o/l, CH3, C12, C15 and C18), 18.1 (C1), 12.7 (C2); IR cm⁻¹ 2981 (br.), 2943, 2865, 1611, 1710 (C=O), 1513, 1463, 1368, 1234, 1143, 996, 881; m/z (LRMS, ES+) 660.4 (100%, [M + Na])}; HRMS (ESI) m/z [M + Na]⁺ Calculated for C32H55N3O8SiNa⁺, 660.3656; Found, 660.3648.

7.2.15 1-(p-Hydroxyphenylamino)-2-[(tert-butyl)3,3-bis[(2,2-dimethylloxapropyl)oxamethylenemethyl]-2-carboxyhydrazino]-1-ethanone

Triethylamine (68 µL, 49 mg, 0.49 mmol) was added to a solution of the tri-tert-butyl 2-(2-oxo-2-(2,3,5,6-tetrafluorophenoxy)ethyl)hydrazine-1,1,1-tricarboxylate 138 (117 mg, 0.22 mmol) and 4-aminophenol 136 (36 mg, 0.33 mmol) in acetonitrile (5 mL), then the reaction mixture was allowed to stir at room temperature for 18 h. The solvent was removed in vacuo to give the crude sample as a brown oil, which was purified by column chromatography on silica gel (3:1 - 1:1 petroleum ether: ethyl acetate). The purified product was obtained as a brown oil which contained two inseparable rotameric product (1:1) (68 mg, 0.14 mmol, 64%). Rf = 0.24 (3:1 petroleum ether: ethyl acetate); Rf = 0.24 (3:1 petroleum ether: ethyl acetate); 1H NMR (500 MHz, CDCl3) δ 10.10 (s, 0.5H, H1), 9.85 (s, 0.5H, H2), 7.42 (d and d o/l, J = 8.8 Hz, 2H, H3), 6.79 (d and d o/l, J = 8.8 Hz, 2H, H4), 6.39 (br. s, 1H, H8), 4.18 (s, 1H, H6), 4.09 (s, 1H, H8), 1.54 (s, 18H, H15,17), 1.44 (s and s o/l, 9H, H11); 13C NMR (126 MHz, CDCl3) δ 167.2 (C7), 166.9 (C1), 154.0 (C9), 153.4 (C12), 153.2 (C15), 151.6 (C6), 130.7 (C2), 122.0 (C3), 121.7 (C13), 115.8 (C4), 115.7 (C4), 85.5 (C10), 83.9 (C13), 83.0 (C16), 57.1 (C8), 55.1 (C8), 28.2 (C11) 28.1 (2C o/l, CH3, C14 and C17); IR cm⁻¹ 3352 (br.),
2981 (br.), 1710 (C=O), 1513, 1368, 1234, 1143; m/z (LRMS, ES+) 482 (100%, [M + H]^+); HRMS (ESI) m/z [M + H]^+ Calculated for C_{23}H_{36}N_{3}O_{8}^+, 482.2491; Found, 482.2502.

7.2.16 1-(p-Hydroxyphenylamino)-2-{{(tert-butyl)3,3-bis[(2,2-dimethyloxapropyl)oxamethylenemethyl]-2-carboxyhydrazino}-1-ethanone

TBAF (1 M solution in THF, 11.58 µL, 10.46 mg, 0.04 mmol) was added to a solution of the tri-tert-butyl 2-(2-oxo-2-((4-((triisopropylsilyl)oxy)phenyl)amino)ethyl)hydrazine-1,1,2-tricarboxylate 140 (14.6 mg, 0.02 mmol) in THF (1 mL) at 0°C. The solution was stirred at 0°C for 1 h then at room temperature for 2 h. The solvent was removed in vacuo to give the crude product as a colourless oil, which was purified by column chromatography (4:1 petroleum ether: ethyl acetate, 2-5% methanol in DCM). The purified sample was isolated as a colourless oil and contained two inseparable rotamers (1:1) (24.8 mg, 0.03 mmol, 60%). R_f = 0.24 (3:1 petroleum ether: ethyl acetate); ^1H NMR (500 MHz, CDCl_3) δ 10.09 (s, 0.5H, H_1), 9.84 (s, 0.5H, H_1), 7.47 (d and d o/l, J = 8.9 Hz, 2H, H_3), 6.79 (d and d o/l, J = 8.9 Hz, 2H, H_4), 5.40 (br. s, 1H, H_6), 4.18 (s, 1H, H_8), 4.08 (s, 1H, H_8), 1.54 (s, 18H, H_14,17), 1.45 (s and s o/l, 9H, H_13). The ^1H characterisation data corresponded with that collected for the same compound synthesised by the direct coupling of 4-aminophenol and the TFP-activated acetic acid derivative (6.2.13) so no further characterisation data was collected.
7.2.17 Ethyl 6-(4-formylphenoxy)hexanoate

A mixture of 4-hydroxybenzaldehyde 67 (610 mg, 5 mmol), ethyl 6-bromohexanoate 148 (1.12 g, 0.88 mL, 5 mmol), potassium carbonate (3.36 g, 25 mmol) and potassium iodide (16 mg, 0.1 mmol) in DMF (10 mL) was allowed to stir at 70°C for 18 h. The reaction was cooled to room temperature and diluted with water (approximately 19 mL). The resulting aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The residue was extracted with diethyl ether (3 x 10 mL), washed with water and brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The crude product was collected as an off-white solid which required no further purification (911 mg, 3.45 mmol, 69%). Rf = 0.27 (4:1 petroleum ether: ethyl acetate). 1H NMR (400 MHz, CDCl3) δ 9.88 (s, 1H, H1), 7.82 (d, J = 8.8 Hz, 2H, H4), 6.98 (d, J = 8.8 Hz, 2H, H3), 4.12 (q, J = 7.2 Hz, 2H, H11), 4.04 (t, J = 6.4 Hz, 2H, H10), 2.34 (t, J = 7.2 Hz, 2H, H6), 1.82 (m, 2H, H9), 1.71 (m, 2H, H7), 1.59 - 1.47 (m, 2H, H8), 1.26 (t, J = 7.2 Hz, 3H, H12); 13C NMR (101 MHz, CDCl3) δ 191.2 (C1), 173.9 (C11), 164.4 (C6), 132.4 (C4), 130.1 (C2), 115.0 (C4), 68.4 (C10), 60.6 (C12), 34.5 (C6), 29.1 (C7), 25.9 (C9), 25.0 (C8), 14.6 (C12); IR cm⁻¹ 3627 (br.), 2982 (br.), 2941, 2866, 2745, 2254, 2210, 2018, 1897, 1726 (C=O), 1687 (C=O), 1599, 1577, 1509, 1465, 1391, 1109; m/z (LRMS, ES+) 287.1 (100%, [M + Na]+), 401.2 (90%); HRMS (ESI) m/z [M + Na]+ Calculated for C15H20O4Na, 287.1254; Found, 287.1259; Anal. Calculated for C15H20O4, C 68.16, H 7.63; Found, C 68.56, 7.87.

7.2.18 4,5-Dimethoxy-2-nitrobenzyl hydrazinecarboxylate

Hydrazine monohydrate (43 µL, 45 mg, 0.90 mmol) was added to a solution of 4,5-dimethoxy-2-nitrobenzyl chloroformate 50 (50 mg, 0.18 mmol) in DCM (5 mL), and the mixture was stirred for 2 h at room temperature. The reaction mixture was washed with a saturated solution of sodium bicarbonate (5 mL) and the resulting aqueous layer was extracted with DCM (3 x 5 mL).
The combined organic layers were washed with brine and dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure to give the product as a pale yellow solid, which required no further purification (50 mg, 0.18 mmol, 95%). \( R_f = 0.19 \) (2:1 petroleum ether: ethyl acetate). \(^1\)H NMR (500 MHz, DMSO) \( \delta \) 8.50 (br. s, 1H, H\(_2\)), 7.74 (s, 1H, H\(_3\)), 7.22 (br. s, 1H, H\(_4\)), 5.39 (s, 2H, H\(_5\)), 4.18 (br. s, 2H, H\(_6\)), 3.94 (s, 3H, H\(_7\)), 3.91 (s, 3H, H\(_8\)); \(^13\)C NMR (126 MHz, DMSO) \( \delta \) 158.7 (C\(_3\)), 154.3 (C\(_10\)), 148.6 (C\(_9\)), 140.2 (C\(_8\)), 111.5 (C\(_7\)), 109.1 (C\(_6\)), 63.4 (C\(_5\)), 57.2 (C\(_{12}\)), 57.0 (C\(_{11}\)); IR cm\(^{-1}\) 3411 (br.), 1736 (C=O), 1578, 1524, 1321, 1269, 1224; m/z (LRMS, ES+) 294.1 (100%, [M + Na]+); HRMS (ESI) m/z [M + Na]+ Calculated for C\(_{10}\)H\(_{13}\)O\(_6\)N\(_3\)Na+, 294.0697; Found, 294.0695; Anal. Calculated for C\(_{10}\)H\(_{13}\)O\(_6\)N\(_3\), C 44.28, H 4.83, N 15.49; Found, C 44.01, H 4.66, N 15.16.

7.2.19 4,5-Dimethoxy-2-nitrobenzyl 2-[(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate

![Chemical Structure](image)

NVOC-hydrazine 141 (778 mg, 2.23 mmol) was added to a solution of 4-hexadecyloxybenzaldehyde (664 mg, 2.45 mmol) in CHCl\(_3\) (20 mL) and the reaction mixture was allowed to heat at reflux for 48 h. The mixture was allowed to cool to room temperature and the solvent was removed \textit{in vacuo}. The residue was taken directly on to the next step of the reaction without further purification. Sodium cyanoborohydride (1 M solution in THF, 2.5 mL, 2.5 mmol) was added to a solution of the crude product (302 mg) in CHCl\(_3\) (5 mL) and acetic acid (5 mL) and the reaction mixture was allowed to stir at room temperature for 16 h. The residue was then diluted with a saturated solution of sodium bicarbonate (6 mL) and DCM (6 mL). The organic phase was separated and washed with brine and a saturated solution of sodium bicarbonate. It was dried with magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The cyanoborane adduct was hydrolysed by reaction with sodium hydroxide (2 M, 2.5 mL) in methanol (5 mL). The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed and water (5 mL) and DCM (5 mL) were added. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered through...
a fluted filter paper and concentrated under reduced pressure. The crude product was
recrystallised from ethyl acetate to give the product as a pale green crystalline solid (110 mg,
0.18 mmol, 36%). Rf = 0.23 (2:1 petroleum ether: ethyl acetate); 1H NMR (500 MHz, CDCl3) δ 7.72
(s, 1H, H2), 7.25 (d, J = 7.5 Hz, 2H, H13), 6.97 (br. s, 1H, H4), 6.86 (d, J = 7.5 Hz, 2H, H16), 5.56 (br. s,
2H, H8), 4.01 (br. s, 1H, H12), 3.96 - 3.90 (m, 10H, H1,13,18), 1.77 (m, 2H, H39), 1.44 (m, 2H, H20), 1.38
- 1.22 (m, 24H, H21,32), 0.88 (t, J = 6.5 Hz, 3H, H33); 13C NMR (126 MHz, CDCl3) δ 159.1 (C10), 156.6
(C9), 153.7 (C17), 148.4 (C8), 139.9 (Cj), 130.5 (3C o/l, aromatic, C5, C15 and C14), 114.7 (C16), 110.2
(C7), 108.3 (C4), 68.2 (C18), 64.3 (C9), 56.6 (3C o/l, alkyl, C1, C2 and C13), 32.1, 29.8, 29.7, 29.6, 29.5,
29.4, 26.2, 22.8 (14C, CH2, C19 to C32), 14.3 (C33); IR cm⁻¹ 3411 (br.), 1736 (C=O), 1578, 1524, 1321,
1269, 1224; m/z (LRMS, ES+) 624.4 (100%, [M + Na]+); HRMS (ESI) m/z [M + H]⁺ Calculated for
C33H53N3O7⁺, 602.3800, Found, 602.3794; Anal. Calculated for C33H31N3O7, C 44.28, H 4.83, N 15.49;
Found, C 44.01, H 4.66, N 15.16.

7.2.20 4,5-Dimethoxy-2-nitrobenzyl 2-(4-(hexadecyloxy)benzyl)-2-((4-nitrophenyl)sulfonyl)hydrazine-1-carboxylate

![Diagram of the compound]

4-Nitrobenzenesulfonyl chloride 95 (92 mg, 0.42 mmol) was added to a solution of 4,5-dimethoxy-
2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate 143 (250 mg, 0.42 mmol) in
pyridine-d5 in CDCl3 (0.2 M, 5 mL) and the reaction mixture was allowed to stir at room
temperature for 7 h. The solvent was removed in vacuo and the crude product was recrystallised
from DCM to give the product as a yellow granular solid (105 mg, 0.13 mmol, 32%). 1H NMR
(400 MHz, CDCl3) δ 8.31 (d, J = 9.0 Hz, 2H, H14), 8.05 (d, J = 9.0 Hz, 2H, H13), 7.69 (s, 1H, H3), 7.21 (d,
J = 7.9 Hz, 2H, H18), 6.82 (br. m, 3H, H4,10), 5.32 (br. s, 2H, H8), 4.65 (br. s, 1H, H11), 4.02 – 3.85 (m,
10H, H1,2,16,21), 1.76 (m, 2H, H22), 1.43 (m, 2H, H23), 1.38 – 1.20 (m, 22H, H24-35), 0.87 (t, J = 6.8 Hz,
3H, H36); 13C NMR (101 MHz, CDCl3) δ 159.8 (C10), 155.0 (Cj), 153.9 (2C o/l, aromatic, C15 and C20),
150.8 (C8), 148.7 (C12), 140.4 (C9), 131.0 (2C o/l, aromatic, C5 and C18), 129.8 (2C o/l, aromatic, C33
and C17), 126.4 (C14), 115.0 (C13), 110.9 (C3), 108.4 (C4), 68.4 (C21), 65.2 (C6), 56.8 (2C, CH3O, C1
and C2), 53.8 (C16), 32.2, 30.0, 29.9, 29.7, 29.5, 26.3, 23.0 (14C, CH2, C22 to C35), 14.5 (C36); IR cm⁻¹ 3340

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(br.), 3089, 2918 (br.), 2850, 1768 (C=O), 1613, 1512, 1376; m/z (LRMS, ES+) 809.1 (100%, [M + Na]+); HRMS (ESI) m/z [M + Na]+ Calculated for C₃₉H₅₄N₄O₁₁SNa, 809.3402; Found, 809.3404; Anal. Calculated for C₃₉H₅₄N₄O₁₁SNa, 809.3402; Found, 809.3404.

7.2.21 1-(Hexadecyloxy)-4-methylbenzene

A mixture of 4-hydroxytoluene 128 (42 mg, 0.33 mmol), 1-bromohexadecane 66 (0.1 mL, 100 mg, 0.33 mmol), potassium carbonate (228 mg, 1.65 mmol) and potassium iodide (8 mg, 0.05 mmol) in DMF (5 mL) was allowed to stir at 70°C for 18 h. The reaction mixture was allowed to cool to room temperature and diluted with water (5 mL). The resulting aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. To remove the remaining DMF the residue was redissolved in diethyl ether and washed with water and brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The crude product was obtained as a colourless oil which was purified by column chromatography on silica gel (20:1-10:1 petroleum ether: ethyl acetate). The ether was obtained as a crystalline colourless solid (49 mg, 0.15 mmol, 45%). Rᵣ = 0.42 (10:1 petroleum ether:ethyl acetate); ¹H NMR (500 MHz, CDCl₃) δ 7.07 (d, J = 8.5 Hz, 2H, H₄), 6.80 (d, J = 8.5 Hz, 2H, H₃), 3.92 (t, J = 6.6 Hz, 2H, H₂), 2.28 (s, 3H, H₁), 1.76 (m, 2H, H₇), 1.44 (m, 2H, H₆), 1.39 – 1.19 (m, 22H, H₉-20), 0.89 (t, J = 7.0 Hz, 3H, H₂₁); ¹³C NMR (126 MHz, CDCl₃) δ 157.4 (C₅), 130.2 (C₆), 130.0 (C₇), 114.7 (C₈), 68.4 (C₉), 32.3, 30.0, 29.9, 29.8, 29.7, 26.4, 23.1 (14C, CH₂, C₉ to C₂₀), 20.8 (C₁), 14.5 (C₂₁); IR 3249 (br.), 2955, 2916, 2849, 1709, 1512, 1246, 1172, 1025.05; m/z (LRMS, GCMS) 332.3 (100%, M); HRMS (GCMS) [M] Calculated for C₂₃H₄₀O, 332.3074; Found 332.3088; Anal. Calculated for C₂₃H₄₀O.0.06 DCM, C 81.91, H 11.96; Found, C 81.98, H 11.56.
7.2.22 4,5-Dimethoxy-2-nitrobenzyl 2-(2-ethoxy-2-oxoethyl)hydrazine-1-carboxylate

Ethyl hydrazinoacetate hydrochloride 93 (500 mg, 3.2 mmol) was added to a solution of sodium bicarbonate (1.31 g, 15.6 mmol) in water (35 mL). 4,5-Dimethoxy-2-nitrobenzyl chloroformate 50 (89 mg, 0.32 mmol) in methanol (5 mL) was added to the rapidly stirred solution and the reaction mixture was allowed to stir for 30 minutes at room temperature. The solvent was removed in vacuo and the aqueous layer was extracted with ethyl acetate (3 × 10 mL), washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The product was isolated as a yellow oil which solidified on scratching with a spatula, requiring no further purification (98 mg, 0.28 mmol, 86%). Rf = 0.19 (2:1 petroleum ether: ethyl acetate); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.68 (s, 2H, H\(_{13}\)), 7.00 (s, 1H, H\(_{10}\)), 6.94 (br. s, 1H, H\(_6\)), 5.55 (s, 2H, H\(_8\)), 4.33 (br. s, 1H, H\(_5\)), 4.17 (q, J = 7.2 Hz, 2H, H\(_2\)), 3.97 (s, 3H, H\(_15\)), 3.92 (s, 3H, H\(_16\)), 3.89 (t, J = 7.2 Hz, 3H, H\(_1\)); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 169.7 (C\(_7\)), 157.8 (C\(_3\)), 153.9 (C\(_{14}\)), 148.3 (C\(_{12}\)), 139.4 (C\(_{11}\)), 128.1 (C\(_9\)), 110.7 (C\(_{13}\)), 110.1 (C\(_{10}\)), 65.2 (C\(_8\)), 61.5 (C\(_6\)), 56.8 (C\(_{16}\)), 56.5 (C\(_{15}\)), 53.2 (C\(_4\)), 14.2 (C\(_1\)); IR cm\(^{-1}\) 3348 (br.), 2932, 2851, 1741 (C=O), 1710 (C=O), 1617, 1580, 1522, 1429, 1326, 1276, 1066, 872; m/z (LRMS, ES+) 380.1 (100%, [M + Na]\(^+\)); HRMS (ESI) m/z [M + Na]\(^+\) Calculated for C\(_{14}\)H\(_{19}\)O\(_8\)N\(_3\)Na\(^+\), 380.1064; Found, 380.1075; Anal. Calculated for C\(_{14}\)H\(_{19}\)O\(_8\)N\(_3\).0.14DCM, C 45.98, H 5.26, N 11.37; Found; C 45.85, H 5.67, N 11.49.

7.2.23 2-(2-Nitrophenyl)propyl hydrazinecarboxylate\(^{135}\)

Hydrazine monohydrate (410 mg, 0.4 mL, 8.2 mmol) was added to a solution of NPPOC-chloride 151 (200 mg, 0.82 mmol) in DCM (10 mL) and the solution was allowed to stir at room temperature for 1 h. The solution was then diluted with DCM and washed with a saturated solution of sodium bicarbonate. The aqueous phase was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with brine, dried over magnesium sulfate, filtered through...
a fluted filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (1:1 petroleum ether: ethyl acetate). The product was isolated as a pale yellow solid (170 mg, 0.71 mmol, 87%). Rf = 0.18 (1:1 petroleum ether: ethyl acetate); 1H NMR (500 MHz, CDCl3) δ 7.71 (d, J = 8.0 Hz, 1H, H11), 7.55 (t, J = 8.0 Hz, 1H, H8), 7.44 (d, J = 8.0 Hz, 1H, H6), 7.35 (t, J = 8.0 Hz, 1H, H10), 6.09 (br. s, 1H, H2), 4.26 (dd, J = 10.4, 6.1 Hz, 1H, H4), 4.17 – 3.64 (m, 1H, H5), 3.54 (br. s, 2H, H1), 1.32 (d, J = 6.9 Hz, 3H, H3); 13C NMR (126 MHz, CDCl3) δ 158.6 (C4), 150.9 (C10), 137.3 (C7), 132.9 (C9), 128.2 (C8), 127.7 (C10), 124.3 (C11), 69.6 (C3), 33.4 (C4), 17.6 (C2); IR cm−1: 2959, 2924, 2854, 1720 (C=O), 1524, 1355, 1272, 1181, 1064; m/z (LRMS, ES+) 262.1 (100%, [M + Na]+), 164.0 (90%); HRMS (ESI) m/z [M + Na]+ Calculated for C10H13N2O4Na, 262.0803; Found, 262.0804; Anal. Calculated for C10H13N2O4.025H2O, C 49.28, H 5.58, N 17.24; Found, C 49.16, H 5.30, N 17.25.

7.2.24 2-(2-Nitrophenyl)propyl (E)-2-(4-hexadecyloxy)benzylidene)hydrazine-1-carboxylate

A solution of 2-(2-nitrophenyl)propyl hydrazinecarboxylate 152 (100 mg, 0.4 mmol) and 4-hexadecyloxybenzaldehyde 68 (134 mg, 0.38 mmol) in CHCl3 (10 mL) was allowed to reflux for four days. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (4:1 petroleum ether: ethyl acetate) to give the product as a yellow solid (191 mg, 0.34 mmol, 89%). Rf = 0.24 (4:1 petroleum ether: ethyl acetate); 1H NMR (500 MHz, CDCl3) δ 7.86 (d, J = 8.5 Hz, 1H, H14), 7.74 (d, J = 8.0 Hz, 1H, H2), 7.58 (m, 3H, H5,14, 7.51 (br. m, 1H, H4), 7.37 (t, J = 8.0 Hz, 1H, H3), 6.88 (d, J = 8.5 Hz, 2H, H15), 4.36 (br. m, 1H, H9), 4.26 (br. m, 1H, H8), 3.96 (t, J = 6.5 Hz, 2H, H11), 3.79 (m, 1H, H7), 1.88 (m, 2H, H16), 1.48 – 1.19 (m, 27H, H8,19-31), 0.88 (t, J = 5.8 Hz, 3H, H32); 13C NMR (126 MHz, CDCl3) δ 161.2 (C10), 157.6 (C10), 151.1 (C1), 144.0 (C12), 137.4 (C3), 133.1 (C4), 129.2 (2C o/l, aromatic, C13 and C14), 127.8 (C3), 126.3 (C5), 124.4 (C2), 114.9 (C16), 69.7 (C6), 68.4 (C17), 32.3 (C4), 30.0, 29.9, 29.7, 29.5, 26.4, 23.0 (14C, CH2, C18 to C31), 17.6 (C8), 14.5 (C32); IR cm−1: 3222 (br.), 2923, 2853, 1712 (C=O), 1606, 1509, 1354, 1245, 1169, 1049; m/z (LRMS, ES+) 568.63 (100%, [M + H]+), 590.63 (50%, [M + Na]+); HRMS (ESI) m/z [M + H]+ 214
Calculated for C_{33}H_{50}N_{3}O_{5}^+, 568.3745; Found, 568.3757; Anal. Calculated for C_{33}H_{49}N_{3}O_{5}, 0.25DCM, C 67.80, H 8.47, N 7.13; Found, C 67.69, H 8.42, N 7.14.

7.2.25 2-(2-Nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate

Sodium cyanoborohydride (1 M solution in THF, 1.7 mL, 1.7 mmol) was added to a solution of the NPPOC-protected hydrazone 153 (191 mg, 0.34 mmol) in CHCl_{3} (1.7 mL) and acetic acid (3.4 mL) and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction mixture was diluted with a saturated solution of sodium bicarbonate (6 mL) and DCM (6 mL) and the organic phase was separated. The organic layer was washed with brine and a saturated solution of sodium bicarbonate, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The cyanoborane adduct was hydrolysed by reaction with sodium hydroxide (2 M, 3 mL) in methanol (6 mL). The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed and water (5 mL) and DCM (5 mL) were added. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure to give the product as a colourless crystalline solid, which required not further purification (100 mg, 0.18 mmol, 52%). R_{f} = 0.26 (3:1 petroleum ether: ethyl acetate); \textsuperscript{1}H NMR (500 MHz, CDCl_{3}) \delta 7.73 (d, J = 8.0 Hz, 1H, H_{2}), 7.56 (t, J = 8.0 Hz, 1H, H_{4}), 7.46 (d, J = 8.0 Hz, 1H, H_{5}), 7.36 (t, J = 8.0 Hz, 1H, H_{3}), 7.20 (br. d, J = 8.5 Hz, 1H, H_{15}), 6.84 (d, J = 8.5 Hz, 2H, H_{16}), 6.12 (br. s, 1H, H_{11}), 4.29 (br. m, 1H, H_{9}), 4.18 (br. m, 1H, H_{6}), 4.07 (br. s, 1H, H_{12}), 3.93 (t, J = 6.6 Hz, 2H, H_{18}), 3.87 (br. s, 2H, H_{13}), 3.71 (m, 1H, H_{3}), 1.77 (m, 2H, H_{19}), 1.44 (m, 2H, H_{20}), 1.37 – 1.22 (m, 26H, H_{8,21-32}), 0.88 (t, J = 6.9 Hz, 3H, H_{33}); \textsuperscript{13}C NMR (126 MHz, CDCl_{3}) \delta 159.0 (C_{10}), 157.2 (C_{21}), 151.0 (C_{1}), 137.5 (C_{4}), 133.0 (C_{6}), 130.5 (C_{13}), 129.4 (C_{14}), 128.3 (C_{5}), 127.8 (C_{3}), 124.4 (C_{7}), 114.8 (C_{18}), 69.3 (C_{6}), 68.4 (C_{19}, C_{21}), 55.5 (C_{13}), 33.6 (C_{7}), 30.0, 29.9, 29.7, 29.5, 26.4, 23.0 (14C, CH_{2}, C_{19} to C_{31}), 17.8 (C_{6}), 14.5 (C_{33}); IR cm\textsuperscript{-1} 2984 (br.), 2940, 2906, 1736 (C=O), 1465, 1447, 1372, 1234, 1043.36; m/z (LRMS, ES+) 570.6 (60%, [M + H]+), 592.6 (100%,
[M + Na]⁺; HRMS (ESI) m/z [M + H]⁺ Calculated for C₃₉H₅₈N₄O₅S⁺, 772.3950; Found, 772.3939; Anal. Calculated for C₃₉H₅₄N₄O₅S.0.33EtOAc, C 61.85, H 7.33, N 7.27; Found, C 61.74, H 7.66, N 7.27.

7.2.26 2-(2-Nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)-2-((4-nitrophenyl)sulfonyl)hydrazine-1-carboxylate

4-Nitrobenzenesulfonyl chloride 95 (66 mg, 0.30 mmol) was added to a solution of 2-(2-nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate 154 (168 mg, 0.30 mmol) in CDCl₃ with pyridine-d₅ (0.2 M, 5 mL). The solution was allowed to stir at room temperature for 36 h. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (4:1 - 3:1 petroleum ether: ethyl acetate) to give the product as a yellow solid (138 mg, 0.18 mmol, 61%). Rᵣ = 0.26 (3:1 petroleum ether: ethyl acetate).

¹H NMR (500 MHz, CDCl₃) δ 8.33 (d, J = 8 Hz, 2H, H₁₄), 8.03 (d, J = 8 Hz, 2H, H₁₃), 7.75 (d, J = 8 Hz, 1H, H₂), 7.56 (t, J = 8 Hz, 1H, H₄), 7.43 – 7.32 (m, 2H, H₁₃), 7.14 (br. d, J = 8.5 Hz, 2H, H₁₈), 6.82 (d, J = 8.5 Hz, 2H, H₁₉), 6.44 (br. s, 1H, H₁₁), 4.60 (br. s, 2H, H₁₂), 4.11 (dd, J = 14.3, 7.1 Hz, 1H, H₉), 3.93 (t, J = 6.5 Hz, 2H, H₁₂), 3.56 (br. m, 1H, H₁), 1.77 (m, 2H, H₁₂), 1.44 (m, 2H, H₁₃), 1.38 – 1.19 (m, 27H, H₈-3₅), 0.88 (t, J = 6.9 Hz, 3H, H₃₆); ¹³C NMR (126 MHz, CDCl₃) δ 159.6 (C₁₀), 153.8 (2C o/l, aromatic, C₂₀ and C₁₅), 150.5 (C₁), 143.9 (C₁₂), 136.6 (C₆), 132.9 (2C o/l, aromatic, C₄ and C₁₈), 130.7 (C₁₇), 129.6 (C₁₃), 127.8 (C₉), 125.1 (C₁₀), 124.4 (C₁₄), 124.2 (C₁₂), 114.9 (C₁₉), 69.9 (C₉), 68.2 (C₂₁), 52.9 (C₁₈), 33.2 (C₁₁), 32.4, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 26.2, 22.8 (14C, CH₂, C₂₂ to C₂₅), 17.5 (C₂₃), 14.3 (C₃₆); IR cm⁻¹ 2984 (br.), 1736 (C=O), 1372, 1234, 1043, 918; m/z (LRMS, ES⁺) 777.3 (100%, [M + Na]⁺); HRMS (ESI) m/z [M + NH₄]⁺ Calculated for C₃₉H₅₈N₄O₅S⁺, 772.3950; Found, 772.3939; Anal. Calculated for C₃₉H₅₆N₄O₅S.0.33EtOAc, C 61.85, H 7.33, N 7.27; Found, C 61.74, H 7.66, N 7.27.
7.2.27 2-(2-Nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)-2-((2-nitrophenyl)sulfonyl)hydrazine-1-carboxylate

2-Nitrobenzenesulfonyl chloride 111 (38 mg, 0.18 mmol) was added to a solution of 2-(2-nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate 54 (100 mg, 0.18 mmol) in pyridine-δ5 in CDCl3 (0.2 M, 3 mL) and the solution was allowed to stir at room temperature for 36 h. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (4:1 - 3:1 petroleum ether: ethyl acetate) to give the product as a powder-like yellow solid (72 mg, 0.095 mmol, 54%). Rf = 0.26 (3:1 petroleum ether: ethyl acetate); 1H NMR (400 MHz, CDCl3) δ 8.05 (d, J = 8 Hz, 1H, H16), 7.78 – 7.64 (m, 4H, H2,13,14,15), 7.55 (t, J = 8 Hz, 1H, H4), 7.36 (m, 2H, H3,5), 7.14 (d, J = 8.6 Hz, 2H, H20), 6.81 (d, J = 8.6 Hz, 2H, H21), 6.57 (br. s, 1H, H11), 4.77 (br. s, 2H, H18), 4.11 (dd, J = 14.3, 7.1 Hz, 2H, H9), 3.94 (t, J = 6.6 Hz, 2H, H22), 3.54 (m, 1H, H7), 1.77 (m, 2H, H24), 1.44 (m, 2H, H25), 1.38 – 1.19 (m, 27H, H8,26-37), 0.87 (t, J = 6.8 Hz, 3H, H38); 13C NMR (101 MHz, CDCl3) δ 159.6 (C10), 154.40 (2C o/l, aromatic, C17 and C22), 150.53 (C1), 148.38 (C12), 136.99 (C6), 134.64 (C13), 133.06 (C16), 132.12 (C20), 132.0 (C4), 130.9 (2C o/l, aromatic, C5 and C19), 128.4 (2C o/l, aromatic, C2 and C14), 125.9 (2C o/l, aromatic, C2 and C15), 115.0 (C21), 69.8 (C8), 68.4 (C23), 60.7 (C18), 33.6 (C7), 32.0, 29.8, 29.7, 29.5, 29.4, 26.2, 22.8 (14C, CH2, C24 to C37), 17.8 (C8), 14.4 (C38); IR cm⁻¹ 2923, 2853, 1741 (C=O), 1612, 1545, 1441, 1371, 1248, 1173, 1044; m/z (LRMS, ES+) 777.4 (100%, [M + Na]+); HRMS (ESI) m/z [M + NH4]+ Calculated for C39H58N3O9S⁺, 772.3950; Found, 772.3948; Anal. Calculated for C39H58N3O9S, C 62.05, H 7.21, N 7.42, S 4.25; Found, C 62.29, H 7.64, N 7.33, S 4.19.
7.2.28 2-((1-(Hexadecyloxy)benzyl)-2-((2-(2-
nitrophenyl)propoxy)carbonyl)hydrazilyn)sulfonyl)benzenesulfonate

1,2-Benzene disulfonic anhydride 87 (354 mg, 1.6 mmol) was added to a solution of 2-(2-nitrophenyl)propyl 2-(4-(hexadecyloxy)benzyl)hydrazine-1-carboxylate 54 (215 mg, 0.38 mmol) in pyridine-$d_5$ in CDCl$_3$ (0.2 M, 5 mL) and the solution was allowed to stir at room temperature for 18 h. The reaction mixture was concentrated in vacuo and the residue was redissolved in DCM (5 mL) and washed with deionised water (3 × 5 mL). The organic layer was washed with brine (5 mL), dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The product was obtained in approximately 90% purity and was taken directly on to the photolysis step. Spectral evidence for the formation of sulfonyl hydrazide: $^1$H NMR (500 MHz, MeOD, key resonances) δ 8.34 (d, $J = 7.3$ Hz, 1H, H$_{16}$), 8.19 (m, 2H, H$_{14,15}$), 7.81 – 7.68 (m, 2H, H$_{20}$), 7.65 – 7.56 (m, 1H, H$_5$), 7.54 – 7.48 (m, 1H, H$_{13}$), 7.41 (m, 1H, H$_3$), 7.11 (d, $J = 8.1$ Hz, 2H, H$_{21}$), 6.74 (d, $J = 8.1$ Hz, 2H, H$_{21}$), 4.46 (br. s, 1H, H$_{11}$), 4.17 (m, 2H, H$_9$), 3.91 (t, $J = 5.7$ Hz, 2H, H$_{23}$), 3.45 (m, 1H, H$_1$), 1.79 – 1.67 (m, 2H, H$_{3a}$), 1.45 (m, 2H, H$_{25}$), 1.39 – 1.21 (m, 27H, H$_{8,26-37}$), 0.90 (t, $J = 7.0$ Hz, 3H, H$_{38}$); IR cm$^{-1}$ 2923, 2853, 1712 (C=O), 1524, 1464, 1353, 1257, 1155, 1039; m/z (LRMS, ES-) 788.6 (100%, [M]).

7.2.29 2-(2-Nitrophenyl)propyl 2-undecylhydrazine-1-carboxylate

Undecanal 163 (0.21 mL, 170 mg, 1 mmol) was added to a solution of NPPOC-hydrazine 152 (370 mg, 1.54 mmol) and acetic acid (57 µL, 60 mg, 1 mmol) in methanol (5 mL), then the reaction mixture was allowed to stir at reflux for four days. The solvent was removed in vacuo and hydrazone 162 was isolated in approximately 90% purity (259 mg). Sodium cyanoborohydride
(1 M solution in THF, 3.3 mL, 3.3 mmol) was added to a solution of the NPPOC-protected hydrazone isolated above (259 mg) in CHCl₃ (3 mL) and acetic acid (6 mL) and the reaction mixture was allowed to stir at room temperature for 18 h. The reaction mixture was then diluted with a saturated solution of sodium bicarbonate (10 mL) and DCM (10 mL). The organic phase was separated and washed with brine and a saturated solution of sodium bicarbonate. The organic layer was then dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The intermediate cyanoborane adduct obtained was hydrolysed on reaction with sodium hydroxide (2 M, 6 mL) in methanol (12 mL). The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed and water (10 mL) and DCM (10 mL) were added. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 10 mL). The organic layers were combined then washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (6:1-3:1 petroleum ether: ethyl acetate) to give the product as a colourless oil (78 mg, 0.20 mmol, 30%). Rᵣ = 0.35 (3:1 petroleum ether: ethyl acetate). ³¹H NMR (400 MHz, CDCl₃) δ 7.66 (d, J = 8 Hz, 1H, H₂), 7.49 (t, J = 8 Hz, 1H, H₃), 7.39 (d, J = 8 Hz, 1H, H₄), 7.29 (t, J = 8, 1H, H₅), 6.11 (br. s, 1H, H₁₁), 4.21 (dd, J = 14.1, 6.1 Hz, 1H, H₉), 4.08 (dd, J = 14.1, 6.1 Hz, 1H, H₉), 3.64 (dq, J = 14.1, 6.9 Hz, 1H, H₁₁), 2.71 (t, J = 6.8 Hz, 2H, H₁₃), 1.38 – 1.14 (m, 21H, H₈,1₄-2₂); ¹³C NMR (101 MHz, CDCl₃) δ 157.0 (C₁₀), 150.8 (C₁), 137.3 (C₆), 132.7 (C₄), 128.1 (C₅), 127.5 (C₃), 124.2 (C₂), 69.1 (C₉), 53.6 (C₁₃), 33.3 (C₇), 32.0, 29.7, 29.6, 29.5, 27.8, 27.1, 22.8, 22.71, (9C α/l, CH₂, C₁₄ to C₂₂), 17.5 (C₈), 14.2 (C₂₃); IR cm⁻¹ 3310, 2923, 2853, 1712 (C=O), 1523, 1464, 1464, 1353, 1257, 1155, 1039; m/z (LRMS, ES+) 394.5 (100%, [M + H]^+), 416.4 (60%, [M + Na]^+), 787.8 (70%, [2M + H]^+), 809.7 (70%, [2M + Na]^+); HRMS (ESI) m/z [M + Na]^+ Calculated for C₂₁H₃₅N₃O₄Na⁺, 416.2525; Found, 416.2521; Anal. Calculated for C₂₁H₃₅N₃O₄·0.16H₂O, C 63.61, H 8.98, N 10.60; Found C 63.69, H 8.48, N 10.61.
4-Nitrobenzenesulfonyl chloride 95 (66 mg, 0.30 mmol) was added to a solution of 2-(2-nitrophenyl)propyl 2-undecylhydrazine-1-carboxylate 161 (78 mg 0.20 mmol) in CDCl₃ with pyridine-d₅ in (0.2 M, 3 mL) and the solution was allowed to stir at room temperature for 36 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (5:1 petroleum ether: ethyl acetate) to give the product as a yellow oil (42 mg, 0.07 mmol, 36%). Rᵣ = 0.26 (3:1 petroleum ether: ethyl acetate); ¹H NMR (400 MHz, CDCl₃) δ 8.31 (d, J = 8.5 Hz, 2H, H₁₄), 8.01 (d, J = 8.5 Hz, 2H, H₁₃), 7.75 (d, J = 8.5 Hz, 1H, H₂), 7.58 (t, J = 8.5 Hz, 1H, H₄), 7.39 (m, 2H, H₃,5), 6.61 (s, 1H, H₁₁), 4.14 (dd, J = 14.1, 6.1 Hz, 1H, H₉), 4.00 (dd, J = 14.1, 6.1 Hz, 1H, H₉), 3.65 (dq, J = 14.1, 6.9 Hz, 1H, H₇), 3.44 (t, J = 6.8 Hz, 2H, H₁₆), 1.63 (m, 2H, H₁₇), 1.51 (m, 1H, H₁₈), 1.35 – 1.18 (m, 17H, H₈,19-25), 0.88 (t, J = 6.8 Hz, 3H, H₂₆); ¹³C NMR (101 MHz, CDCl₃) δ 161.8 (C₁₀), 150.6 (2C o/l, aromatic, C₁ and C₁₅), 143.6 (C₁₂), 136.7 (C₆), 132.9 (C₄), 129.7 (C₁₃), 127.8 (2C o/l, aromatic, C₉ and C₁₆), 124.3 (2C o/l, aromatic, C₂ and C₁₄), 70.1 (2C o/l, CH₂X (X = N, O) C₉ and C₁₆), 50.2 (C₁₇), 33.1 (C₁₈), 32.0, 29.8, 29.7, 29.6, 29.5, 29.3, 22.8 (8C, CH₂, C₁₉ to C₂₅), 17.6 (C₈), 14.3 (C₂₆); IR cm⁻¹ 2925, 2854, 1729 (C=O), 1528, 1466, 1351, 1253, 1167, 1063; m/z (LRMS, ES⁺) 344.05 (60%, [M + H]+), 366.02 (100%, [M + Na]+); HRMS (ESI) m/z [M + Na]+ Calculated for C₁₈H₂₁N₃O₄Na⁺, 366.1430; Found, 366.1432; Anal. Calculated for C₁₈H₂₁N₃O₄, C 56.04, H 6.62, N 9.68, S 5.54; Found, C 56.54, H 6.57, N 9.62, S 5.10.

2-Phenylacetaldehyde 166 (0.1 mL, 100 mg, 0.83 mmol) was added to a solution of NPPOC-hydrazine 152 (218 mg, 0.91 mmol) in CHCl₃ (7 mL) and the reaction mixture was allowed to stir at reflux for 72 h. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica gel (3:1 - 1:1 petroleum ether: ethyl acetate) to give hydrazone 165 in
approximately 80% purity (168 mg), which was taken directly on to the next step of the reaction. Sodium cyanoborohydride (1 M solution in THF, 2.45 mL, 2.45 mmol) was added to a solution of the hydrazone isolated above (168 mg) in CHCl$_3$ (2.5 mL) and acetic acid (5 mL) and the reaction mixture was allowed to stir at room temperature for 4 h. The residue was diluted with a saturated solution of sodium bicarbonate (10 mL) and DCM (10 mL) and the organic phase was separated and washed with brine and a saturated solution of sodium bicarbonate. The combined organic layer was dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The cyanoborane adduct was hydrolysed on reaction with sodium hydroxide (2 M, 3 mL) in methanol (6 mL). The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed in vacuo and water (10 mL) and DCM (10 mL) were added. The organic phase was separated and the aqueous phase was extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered through a fluted filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (2:1 - 1:1 petroleum ether: ethyl acetate) to give the product as a colourless oil (61 mg, 0.18 mmol, 36%). $R_f = 0.26$ (1:1 petroleum ether: ethyl acetate). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.72 (d, $J = 8$ Hz, 1H, $H_2$), 7.55 (t, $J = 8$ Hz, 1H, $H_4$), 7.45 (d, $J = 8$ Hz, 1H, $H_5$), 7.35 (t, $J = 8$ Hz, 1H, $H_3$), 7.31 – 7.24 (m, 3H, $H_{16,18}$), 7.20 (t, $J = 7.0$ Hz, 2H, $H_{17}$), 6.15 (br. s, 1H, $H_{12}$), 4.28 (dd, $J = 10.4, 6.0$ Hz, 1H, $H_9$), 4.13 (dd, $J = 10.4, 6.0$ Hz, 1H, $H_8$), 3.95 (dq, $J = 14.1, 6.9$ Hz, 1H, $H_7$), 3.87 (br. s, 1H, $H_12$), 3.17 - 3.01 (m, 2H, $H_{13}$), 2.81 - 2.68 (m, 2H, $H_{14}$), 1.34 (d, $J = 7.0$ Hz, 3H, $H_8$); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 157.0 (C$_{10}$), 150.8 (C$_1$), 139.4 (C$_{15}$), 137.2 (C$_6$), 132.7 (C$_4$), 128.8 (C$_{17}$), 128.6 (C$_{16}$), 128.0 (C$_3$), 127.6 (C$_2$), 126.4 (C$_{18}$), 124.2 (C$_{19}$), 69.2 (C$_9$), 52.9 (C$_{13}$), 34.3 (C$_{14}$), 33.3 (C$_7$), 17.4 (C$_8$); IR cm$^{-1}$ 3306 (br.), 2934, 2358, 1715 (C=O), 1524, 1497, 1454, 1355, 1260, 1158, 1047.10; m/z (LRMS, ES+) 366.0 (100%, [M + Na]$^+$), 344.0 (60%, [M + H]$^+$); HRMS (ESI) m/z [M + Na]$^+$ Calculated for C$_{18}$H$_{21}$N$_3$O$_4$Na$^+$, 366.1430; Found 366.1432; Anal. Calculated for C$_{18}$H$_{21}$N$_3$O$_4$.H$_2$O, C 59.82, H 6.42, N 11.63; Found; C 59.63, H 6.12, N 11.74.
7.3 Decomposition studies

7.3.1 Synthesis and decomposition of sulfonil hydrazides 98a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and 1,2-benzene disulfonic anhydride 87 (21 mg, 0.1 mmol) were dissolved in a solution of pyridine-5 in CDCl₃ (0.3 M solution, 1 mL) in an NMR tube. The progress of the reaction and subsequent decomposition of sulfonil hydrazides 98a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.

7.3.2 Synthesis and decomposition of sulfonil hydrazides 99a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and para-toluenesulfonyl chloride 94 (19 mg, 0.1 mmol) were dissolved in a solution of deuterated pyridine-5 in CDCl₃ (0.3 M solution, 1 mL) in an NMR tube. The progress of the reaction and subsequent decomposition of sulfonil hydrazides 99a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.
7.3.3 Synthesis and decomposition of sulfonyl hydrazides 100a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and 4-nitrobenzenesulfonyl chloride 95 (22 mg, 0.1 mmol) were dissolved in a solution of pyridine-$d_5$ in CDCl$_3$ (0.3 M solution, 1 mL) in an NMR tube. The progress of the reaction and subsequent decomposition of sulfonyl hydrazides 100a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.

7.3.4 Synthesis and decomposition of sulfonyl hydrazides 101a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and 4-(chlorosulfonyl)benzoic acid 96 (22 mg, 0.1 mmol) were dissolved in a solution of triethylamine in CDCl$_3$ (0.3 M solution, 1 mL) in an NMR tube. The progress of the reaction and subsequent decomposition of sulfonyl hydrazides 101a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.
7.3.5 Synthesis and decomposition of sulfonyl hydrazides 102a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and 4-cyanobenzenesulfonyl chloride 97 (20.2 mg, 0.1 mmol) were dissolved in a solution of pyridine-d5 in CDCl3 (0.3 M solution, 1 mL) in an NMR tube. The progress of the reaction and subsequent decomposition of sulfonyl hydrazide 102a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.

7.3.6 Synthesis and decomposition of sulfonyl hydrazides 112a/b

Ethyl hydrazinoacetate hydrochloride 93 (15.4 mg, 0.1 mmol) and 2-nitrobenzenesulfonyl chloride 111 (22 mg, 0.1 mmol) were dissolved in a solution of pyridine-d5 in CDCl3 (0.3 M solution, 1 mL) in an NMR tube. The formation and subsequent decomposition of sulfonyl hydrazides 112a/b was monitored by NMR spectroscopy at regular intervals for a period of several days while the reaction mixture was kept at approximately 21 °C.
7.3.7 Ethyl N-amino-N-((4-nitrophenyl)sulfonyl)glycinate (100a) and ethyl ((4-nitrophenyl)sulfonamido)glycinate (100b)

![Structural diagram]

Ethyl hydrazinoacetate hydrochloride 93 (156 mg, 1 mmol) and 4-nitrobenzenesulfonyl chloride 95 (222 mg, 1 mmol) were dissolved in a solution of pyridine-$d_5$ in CDCl$_3$ (0.2 M, 10 mL). The reaction mixture was allowed to stir at room temperature for 2 h and the solvent was removed in vacuo to give an orange solid. The crude product was purified by column chromatography on silica gel (5:1 - 1:1 petroleum ether: ethyl acetate). The product was isolated as a combination of inseparable isomers 100a and 100b in the ratio 2:1. R$_f$ = 0.22 (1:1 petroleum ether: ethyl acetate); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.42 – 8.30 (m, 3H, H$_9$,17), 8.13 (d, J = 8.9 Hz, 2H, H$_8$), 8.07 (d, J = 8.9 Hz, 1H, H$_{14}$), 6.92 (br. s, 1H, H$_6$), 4.31 (s, 1H, H$_{14}$), 4.19 (q, J = 7.2 Hz, 1H, H$_3$), 4.09 (q, J = 7.2 Hz, 2H, H$_{12}$), 3.76 (br. s, 2H, H$_{19}$), 3.57 (s, 2H, H$_4$), 1.27 (t, J = 7.2 Hz, 3H, H$_1$), 1.21 (t, J = 7.2 Hz, 1.5H, H$_{11}$); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 172.0 (C$_{13}$), 168.5 (C$_3$), 150.5 (2C o/l, aromatic, C$_{10}$ and C$_{18}$), 144.1 (C$_{15}$), 142.7 (C$_7$), 129.61 (2C, aromatic, C$_8$ and C$_{16}$), 124.6 (2C, aromatic, C$_9$ and C$_{17}$), 61.8 (2C, CH$_2$, C$_2$ and C$_{12}$), 53.1 (2C, CH$_2$, C$_4$ and C$_{14}$), 14.2 (2C, CH$_3$, C$_1$ and C$_{11}$); IR cm$^{-1}$ 3141 (br.), 2921, 1735 (C=O), 1607, 1531, 1350, 1310, 1214, 1168, 1092, 1022, 855, 738; m/z (LRMS,ES-) 302 (100%, [M - H$^-$]), 489 (20%, [M + C$_6$H$_4$NO$_4$S$^-$]).

7.3.8 Hexyl aminoglycinate

![Structural diagram]

Trifluoroacetic acid (34 µL, 0.32 mmol) was added to a solution of ethyl hydrazinoacetate hydrochloride 93 (50 mg, 0.32 mmol) in 1-hexanol (3 mL) and the reaction solution was allowed to stir at 72 °C for 48 h. The reaction mixture was allowed to cool to room temperature and crystallisation was completed by cooling in iced water. The crystals were collected by reduced pressure filtration and the crystals were washed with petroleum ether. The product was collected as silver platelike crystals (47 mg). The product was isolated in approximately 70% purity since all the starting material had not been consumed in the transesterification reaction, as demonstrated
by NMR and elemental analysis. The characterisation data that enabled the identification of hexyl aminoglycinate is listed: $^1$H NMR (500 MHz, MeOD) $\delta$ 4.21 (t, $J$ = 6.8 Hz, 2H, H$_6$), 3.77 (s, 2H, H$_8$), 1.68 (m, 2H, H$_5$), 1.43 – 1.27 (m, 6H, H$_2$-4), 0.92 (t, $J$ = 6.8 Hz, 3H, H$_1$); $^{13}$C NMR (126 MHz, MeOD) $\delta$ 171.4 (C$_7$), 66.8 (C$_6$), 50.7 (C$_8$), 32.6 (C$_9$) 29.6 (C$_4$), 26.6 (C$_3$), 23.6 (C$_2$), 14.3 (C$_1$); IR cm$^{-1}$ 2944 (br.), 2831, 1735 (C=O), 1631, 1448, 1415, 1253, 1112, 1021; m/z (LRMS, ES+) 175.3 (100%, [M - Cl]$^+$), 197.3 (20%, [M – HCl + Na]$^+$); HRMS (ESI) m/z [M - Cl]$^+$ Calculated for C$_8$H$_{19}$N$_2$O$_2$, 175.1447; Found, 175.1444; Anal. Calculated for C$_8$H$_{19}$ClN$_2$O$_2$, C 42.75, H 8.71, N 14.24; Found, C 42.96, H 9.12, N 13.84.

### 7.3.9 Heptyl aminoglycinate

Trifluoroacetic acid (48 µL, 0.45 mmol) was added to a solution of ethyl hydrazinoacetate hydrochloride 93 (75 mg, 0.45 mmol) in 1-heptanol (3 mL) and the reaction solution was allowed to stir at 72 °C for 48 h. The reaction mixture was allowed to cool to room temperature and crystallisation was completed by cooling in iced water. The crystals were collected by reduced pressure filtration and were washed with petroleum ether. The product was collected as silver platelike crystals (70 mg). The product was isolated in approximately 70% purity since all the starting material had not been consumed in the transesterification reaction, as demonstrated by NMR and elemental analysis. The characterisation data that enabled the identification of heptyl aminoglycinate is listed: $^1$H NMR (400 MHz, MeOD) $\delta$ 4.21 (t, $J$ = 6.8 Hz, 2H, H$_7$), 3.78 (s, 2H, H$_9$), 1.68 (m, 2H, H$_6$), 1.44 – 1.22 (m, 8H, H$_5$-2), 0.91 (t, $J$ = 6.8 Hz, 3H, H$_1$); $^{13}$C NMR (101 MHz, MeOD) $\delta$ 171.3 (C$_8$), 66.8 (C$_7$), 50.7 (C$_9$), 32.9 (C$_6$), 30.0 (C$_4$), 29.6 (C$_3$), 26.9 (C$_2$), 23.6 (C$_1$), 14.4 (C$_1$); IR cm$^{-1}$ 2940 (br.), 2842, 1985, 1745 (C=O), 1628, 1453, 1418, 1116, 1028; m/z (LRMS, ES+) 189.3 (100%, [M - Cl]$^+$), 211.3 (20%, [M – Cl + Na]$^+$); HRMS (ESI) m/z [M - Cl]$^+$ Calculated for C$_9$H$_{21}$ClN$_2$O$_2$, 189.1603; Found, 189.1596; Anal. Calculated for C$_9$H$_{21}$ClN$_2$O$_2$.03C$_4$H$_6$ClN$_2$O$_2$, C 44.93, H 9.00, N 13.52; Found, C 44.52, H 9.22, N 13.03.
7.3.10 Octyl aminoglycinate

Trifluoroacetic acid (48 µL, 0.45 mmol) was added to a solution of ethyl hydrazinoacetate hydrochloride 93 (75 mg, 0.45 mmol) in 1-octanol (3 mL) and the reaction solution was allowed to stir at 72 °C for 48 h. The reaction mixture was allowed to cool to room temperature and crystallisation was completed by cooling in iced water. The product was collected by reduced pressure filtration and the crystals were washed with petroleum ether and collected as silver platelike crystals (56 mg). The product was isolated in approximately 50% purity since all the starting material had not been consumed in the transesterification reaction, as demonstrated by NMR and elemental analysis. The characterisation data that enabled the identification of octyl aminoglycinate is listed: ¹H NMR (400 MHz, MeOD) δ 4.21 (t, J = 6.8 Hz, 2H, H₈), 3.76 (s, 2H, H₁₀), 1.68 (m, 2H, H₇), 1.30 (m, 10H, H₂₋₆), 0.90 (t, J = 6.4 Hz, 3H, H₁); ¹³C NMR (101 MHz, MeOD) δ 171.3 (C₉), 66.8 (C₈), 50.8 (C₁₀), 32.9 (C₇), 30.3 (C₆₋₅), 29.6 (C₆), 26.9 (C₄), 23.7 (C₃), 14.4 (C₁); IR cm⁻¹ 2955 (br.), 2927, 2856, 1731 (C=O), 1634, 1379, 1239, 1098.35, 1019; m/z (LRMS, ES+) 203.3 (100%, [M - Cl⁺]), 225.3 (30%, [M - Cl + Na⁺]); HRMS (ESI) m/z [M - Cl⁺] Calculated for C₁₀H₂₃N₂O₂⁺, 203.1760; Found, 203.1758; Anal. Calculated for C₁₀H₂₃N₂O₂⁺Cl⁻, C 42.75, H 8.71, N 14.24; Found, C 42.61, H 9.17, N 13.77.

7.3.11 Formation and decomposition of longer chained sulfonyl hydrazides

4-Nitrobenzenesulfonyl chloride 95 (22 mg, 0.1 mmol) was added to a solution of the amino glycinate derivative (0.1 mmol) in pyridine-d₅ in CDCl₃ (0.3 M, 1 mL) in an NMR tube. NMR spectra were recorded at regular intervals for several days while the reaction was kept at approximately 21 °C.
7.3.12 Formation of octyl acetate via the decomposition of sulfonyle hydrazide 123

4-Nitrobenzenesulfonyl chloride 95 (358 mg, 1.61 mmol) was added to a solution of octyl aminoglycinate (326 mg, 1.61 mmol) in pyridine-d$_5$ in CDCl$_3$ (0.3 M, 7 mL) and the solution was allowed to stir at room temperature for 5 days. The solvent was removed in vacuo and the crude product was recrystallised from DCM and methanol and the pyridine-TFA salt was removed from solution by gravity filtration. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel (15:1 - 6:1 petroleum ether: ethyl acetate). The product was isolated as a colourless oil (80 mg, 0.47 mmol, 29%). $R_f = 0.65$ (15:1 petroleum ether: ethyl acetate). $^1$H NMR (400 MHz, CDCl$_3$) δ 4.04 (t, $J = 6.8$ Hz, 2H, H$_3$), 2.04 (s, 3H, H$_1$), 1.61 (mz, 2H, H$_4$), 1.37 – 1.21 (m, 10H, H$_5$-9), 0.87 (t, $J = 6.8$ Hz, 3H, H$_{10}$); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.4 (C$_2$), 64.8 (C$_3$), 31.9 (C$_4$) 29.8 (C$_5$) 29.4 (C$_6$), 28.7 (C$_7$), 26.1 (C$_8$), 22.8 (C$_9$), 21.2 (C$_1$), 14.2 (C$_{10}$); IR cm$^{-1}$ 2925.74, 2856.03, 1741.04, 1466.76, 1364.81, 1232.49, 1038.42; m/z (LRMS, ES+) 195.1 (100%, [M + Na]$^+$); HRMS (ESI) m/z [M + Na]$^+$ Calculated for C$_{10}$H$_{20}$O$_2$Na$^+$, 195.1361; Found, 195.1355; Anal. Calculated for C$_{10}$H$_{20}$O$_2$, C 69.72, H 11.70; Found, C 69.85, H 11.75.

7.4 Photolysis experiments

7.4.1 Photolysis of NVOC- and NPPOC-protected hydrazines/sulfonyl hydrazides

The NVOC/NPPOC-protected compounds (3 mg) were dissolved in a solution of pyridine-d$_5$ in CDCl$_3$ (0.3 M, 3 mL) and the solution was irradiated with light at a wavelength of 330/360/375 nm in a quartz cuvette for 10-30 minutes. The solution was analysed by $^1$H NMR, IR spectroscopy and mass spectrometry, which showed that a mixture of products had been formed.
7.4.2 Irradiation of (4-(hexadecyloxy)benzyl)hydrazine

Triethylamine (9 µL, 6.36 mg, 0.063 mmol, 5 equivalents) was added to a solution of (4-(hexadecyloxy)benzyl)hydrazine hydrochloride 83 (5 mg, 0.013 mmol) in CDCl$_3$ (1 mL) in an NMR tube. The NMR spectrum showed that the free hydrazine had been formed so the solution was diluted with CDCl$_3$ (2 mL) and irradiated at with light of wavelength 375 nm for 10 min. No change was observed in the NMR spectrum of the solution after irradiation compared to that obtained prior to the irradiation, demonstrating that the hydrazine is stable to irradiation, therefore the decomposition is caused by the isolation of the product and not by the photolysis.
References


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