Inhibition of the Aspartate-Histidine-Histidine-Cysteine (DHHC) Superfamily: Development of Chemical Tools

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Flyleaf

This thesis is the results of the author's original research. It has been composed by the author and has not been previously submitted for examination, which has led to the award of a degree.

Signed: Jayde McLellan

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Abstract

Acylation, the attachment of fatty acids onto cysteine residues, is a major post-translational modification of cellular proteins, catalysed by the DHHC superfamily. The actions of acylation impact on a number of important physiological processes, and defects in these processes have been linked to a range of diseases and disorders. A major effort has been invested in identifying the substrates that DHHC enzymes are active against, however, there is still a lack of understanding of the specific substrate profiles of individual enzymes and how DHHC-substrate specificity is achieved. To begin to assess the downstream effects of palmitoylation by this enzyme superfamily, and in turn assess the possibility of targeting palmitoylation and interrogating its therapeutic potential, chemical tools are required.

The University of Strathclyde has established a partnership with Ono Pharmaceuticals, in Japan, and Professor Luke Chamberlain from the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS). The overall objective from this partnership is to provide chemical probes to elucidate DHHC fatty acid selectivity and DHHC-substrate specificity profiles, and to prepare selective inhibitors for members of the DHHC superfamily.

The first short-term aim of the project was to establish selectivity between two DHHC enzymes, DHHC3 and DHHC7, and to develop selective inhibitors of S-acylation. In order to bring this project forward, six series of compounds have been synthesised, and tested within a cell-based assay. Two 'hit' compounds have been identified as 'semi-pan' inhibitors of S-acylation by the DHHC superfamily. The second part of the project was to develop a tool compound capable of elucidating DHHC-substrate profiles. One such compound was proposed and synthetic routes towards it are under development. The final aim of the project was to provide inhibitors of DHHC2. Three series of compounds have been targeted, synthesised and tested within an *in vitro* assay. One hit compound has been identified as a potential cysteine selective warhead and an SAR has been carried out.

Contents

Abbreviations – Biological terms

ABE -	Acyl-biotin exchange
ADP -	Adenosine diphosphate
AMPA -	α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APT -	Acyl protein thioesterase
ATP -	Adenosine triphosphate
BK -	Big potassium
BSA -	Bovine serum albumin
Cbl -	Cereblon
CNS -	Central nervous system
CoA -	Coenzyme A
CRD -	Cysteine rich domain
DHHC -	Aspartate-Histidine-Histidine-Cysteine
DMEM -	Dulbecco's modified eagle medium
DNA -	Deoxyribonucleic acid
ELISA -	Enzyme-linked immunosorbent assay
ER -	Endoplasmic reticulum
Gα -	G protein α subunit
GPCR -	G-protein coupled receptor
HEK -	Human embryonic kidney
MudPIT -	Multi-dimensional protein identification technology
NMT -	*N*-myristoyl transferase
N-Ras -	Neuroblastoma Ras
PAGE -	Polyacrylamide gel electrophoresis
Pal-OH -	Palmitic acid
Pal-CoA -	Palmitoyl-Coenzyme A
PAT -	Palmitoyl acyltransferase
PTM -	Post-translational modification
RAC -	Resin-assisted capture
Ras -	Rat sarcoma
SDS -	Sodium dodecyl sulfate

SNAP25 -	Synaptosome associated protein 25
SNARE -	SNAP Receptor
TEM -	Tumour endothelial marker
TMD -	Transmembrane domain

Abbreviations – Chemical and miscellaneous terms

Ac -	Acetyl
n-BuLi -	*n*-Butyllithium
dH_2O -	Distilled water
DMF -	*N*,*N*-Dimethylformamide
DMPU -	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO -	Dimethylsulfoxide
Et -	Ethyl
Eq. -	Equivalents
GC-MS -	Gas chromatography – mass spectrometry
HA -	Hydroxylamine
HRMS -	High resolution mass spectrometry
Me -	Methyl
MES -	2-(*N*-Morpholino)ethanesulfonic acid
Mp -	Melting point
Min -	Minutes
NMR -	Nuclear magnetic resonance
o/n -	Overnight
Pyr -	Pyridine
rt -	Room temperature
S_N2 -	Bimolecular nucleophilic substitution reaction
T -	Temperature
TBTA -	Tris((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amine
TCEP -	Tris(2-carboxyethyl)phosphine hydrochloride solution
THF -	Tetrahydrofuran
TLC -	Thin layer chromatography

1. Introduction

1.1 Post-Translational Modifications

Post-translational modifications (PTMs) are covalent chemical modifications involved in increasing a proteins functional diversity, by the introduction of new functional groups. They can occur at all stages of the protein "life-cycle", for example, immediately after translation to mediate proper protein folding or to direct the protein to distinct cellular compartments. PTMs also play a crucial role in the regulation of activity and interaction with other cellular molecules, such as proteins, nucleic acids, lipids and co-factors. Therefore, identifying and understanding PTMs has been outlined as critical in the study of cell biology and disease treatment/ prevention. An example of the importance of PTMs in drug discovery is the study of phosphorylation in relation to the treatment of cancer.

1.1.1 Phosphorylation

Phosphorylation is one of the most ubiquitous and well-studied PTMs. It is characterised by the reversible addition of a phosphate group to one of three amino acid side chains, serine, threonine or tyrosine, mediated by kinase and phosphatase enzymes (Figure 1).[1] Kinases catalyse the transfer of a phosphate group, from adenosine triphosphate (ATP), to the substrate, with release of adenosine diphosphate (ADP). Phosphatases catalyse the reverse process, the removal of a phosphate group from the substrate. Phosphorylation therefore frequently acts as a method for catalytic activation or deactivation, regulating protein function through conformational effects.

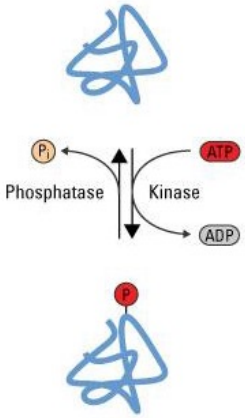


Figure 1: Phosphorylation mode of action.[2]

Phosphorylation plays a critical role in the regulation of many cellular processes including cell cycle progression, cell growth and apoptosis through its influence on signal transduction pathways.[3] More importantly, the modification of signal transduction (e.g. *via* oncogenic mutation) can lead to aberrant functioning of a cell, and in turn cancer.[4] The potential to establish a degree of control over these processes has resulted in kinases and phosphatases becoming attractive in drug discovery initiatives.[4] The importance of phosphorylation as a PTM and potential therapeutic target is clearly evident and illustrates the fundamental need to investigate other PTMs and identify their potentials as drug targets.

1.1.2 Lipidation

Lipidation, another example of a PTM, involves the introduction of unfunctionalised alkyl and acyl chains, which are hydrophobic in character, *via* covalent linkage to a protein. These alkyl and acyl chains can be added through N-, S- or O- onto amino acid side-chains.

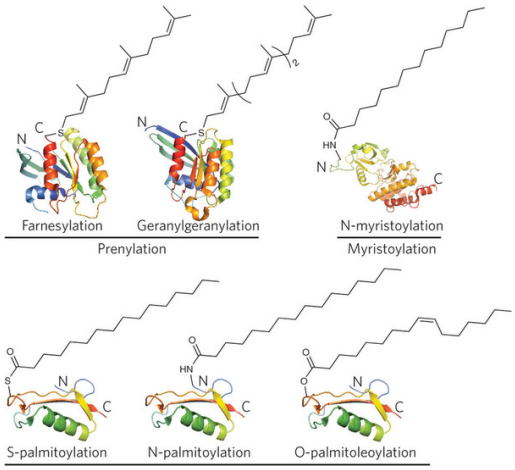


Figure 2: Common lipidation PTMs.[5]

Lipidation encompasses a wide variety of PTMs (Figure 2), including *N*-myristoylation and prenylation, and all are catalysed by specific enzymes. Lipidation of a protein affects the activity and sub-cellular location. It is used to target the protein to the membrane of an organelle, such as the endoplasmic reticulum (ER), exploiting the different types of modification to give distinct membrane affinities. Interestingly, lipidation PTMs are not mutually exclusive, therefore two or more lipids can be attached to a given protein, thus increasing membrane affinity.

Almost all lipidation PTMs are irreversible and so, unlike phosphorylation, cannot be 'switched' on and off, with the exception being S-acylation.[6]

1.2 S-Acylation

S-acylation is a form of lipidation that involves the addition of a long-chain fatty acid, as its co-enzyme A ester **2** (the structure of co-enzyme A is shown in Figure 4) to a cysteine residue **1** through thioesterification **5**, Figure 3.

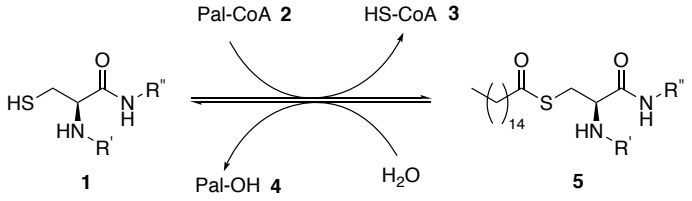


Figure 3: Acylation of a cysteine residue.

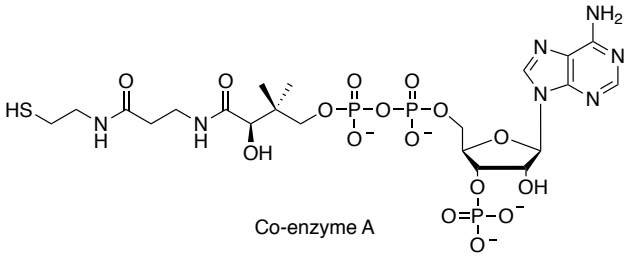


Figure 4: Structure of Co-enzyme A.

This modification is frequently referred to as palmitoylation, due to the fact that palmitate is the predominant fatty acid attached to S-acylated proteins, although other fatty acids such as stearic and oleic acid may also be added, Figure 5.[7]

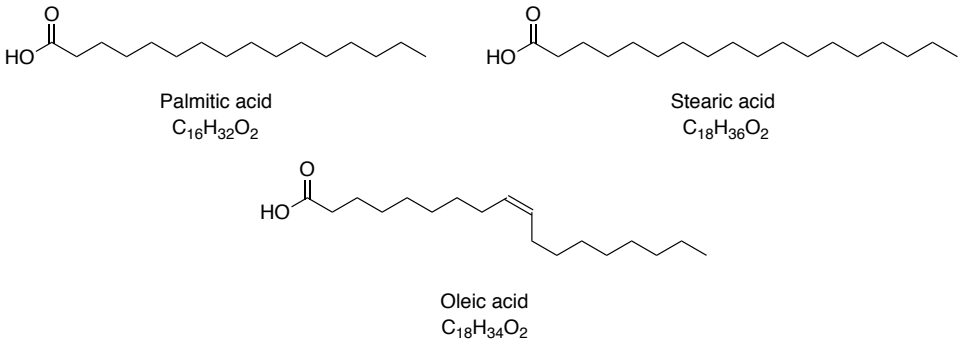


Figure 5: Examples of fatty acids.

1.2.1 Reversibility

The distinct feature that separates S-acylation from its lipidation counterparts is its reversibility. This is proposed to be due to the labile nature of the thioester linkage within an intracellular environment, allowing many proteins to undergo rapid cycles of acylation and de-acylation.[8] The reversibility of S-acylation has

been studied using radiolabelling with [^{3}H]palmitic acid, which is converted into [^{3}H]palmitoyl-Coenzyme A in cells and incorporated into palmitoylated proteins. Initially pulse-chase experiments were used to study the dynamics of S-acylation and demonstrated that the half-life of [^{3}H]palmitic acid incorporation into N-Ras was ~20 minutes.[9] However, more recent studies using an indirect reporter suggested that de-acylation of N-Ras may actually occur 10–20 times faster than that calculated by pulse-chase experiments, aligning the turnover rate of S-acylation with that of phosphorylation.[10] It is important to note that rapid cycling of palmitoylation is not universal and the turnover rates of some proteins may be very low, or completely non-existent.[11] Interestingly, turnover rates can vary across different palmitoylation sites within the same protein.[12] Therefore some major questions within S-acylation research relate to how the cycle is regulated, and why different proteins exhibit a marked difference in turnover rates.

1.2.2 Roles of S-acylation

The roles of palmitoylation are varied and it exerts a number of important effects on proteins at multiple stages of the life cycle, including, but not limited to, regulating membrane attachment, mediating intracellular trafficking, regulating membrane micro-localisation and modulating protein stability.[13]

1.2.2.1 Membrane attachment

One of the most important functions of S-acylation is to regulate the membrane attachment of peripheral membrane proteins,[8] thus affecting their sub-cellular localisation and activity. The addition of a fatty acid essentially acts as a 'lipid anchor' to the hydrophobic segment of the phospholipid bilayer, as shown in Figure 6.

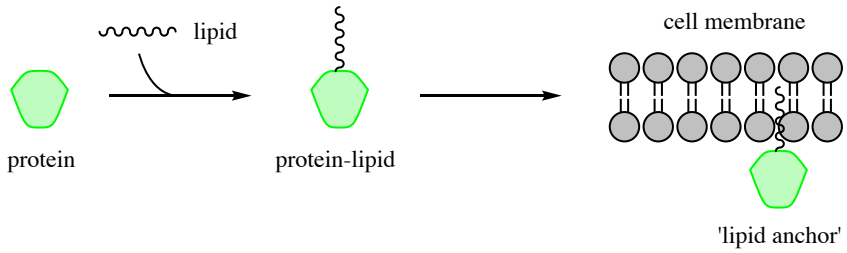


Figure 6: Sub-cellular localisation of protein *via* 'lipid anchor'.

S-acylation is often coupled with N-myristoylation or prenylation, as single myristoyl or prenyl groups are not sufficient enough to provide stable membrane attachment. Seminal studies exploring these interactions demonstrated that single myristoyl or prenyl groups only provided weak membrane affinity, sufficient for transient binding.[14] Whereas, dual lipid anchors, such as myristoyl/palmitoyl or farnesyl/palmitoyl, provided strong, essentially irreversible membrane attachment, Figure 7.

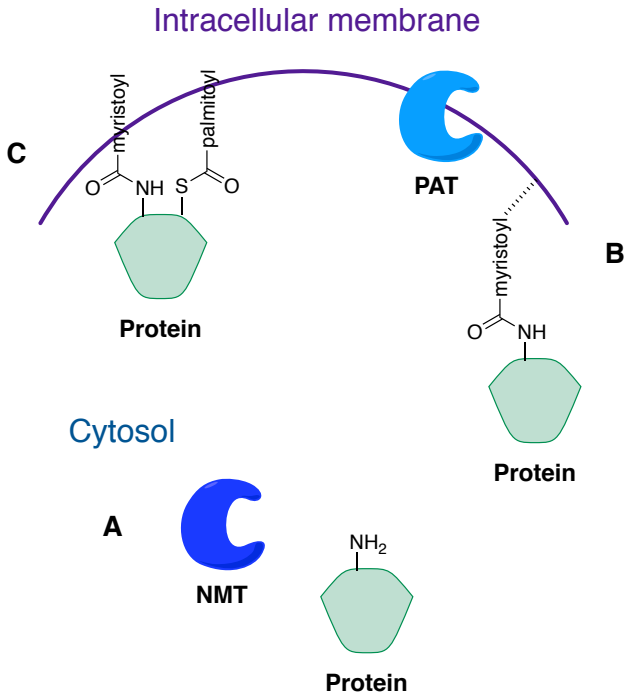


Figure 7: Effect of dual lipidation on proteins.

Considering the intracellular distribution of lipidating enzymes, dual lipidation is highly relevant as prenyl and N-myristoyl transferases (NMTs) are localised in the cell cytosol (**A**),[15-16] whereas protein S-acyl transferases (PATs) are

exclusively membrane associated. Therefore, the N-myristoylation or prenylation of proteins allows them to transiently connect with intracellular membranes (**B**), which in turn allows them to interact with membrane bound S-acyl transferases, and the subsequent S-acylation leads to stable membrane attachment (**C**). This has been demonstrated in Ras proteins, where mutation of the S-acylation site led to weak association with membranes, however mutation of the farnesylation site led to a loss of both S-acylation and membrane binding.[17]

1.2.2.2 Protein stability

S-acylation also has a strong link with ubiquitination, and therefore protein stability. Increased ubiquitination and degradation has been observed when the S-acylation of specific proteins is blocked, for example, within the yeast SNARE protein Tlg1.[18] S-acylation of Tlg1 is proposed to regulate the orientation of it's single transmembrane domain (TMD) to prevent contact of acidic residues with the membrane surface, which has been shown to promote recognition by the ubiquitin ligase Tul1. Similarly, anthrax toxin receptor TEM8 ubiquitination is prevented by S-acylation, Figure 8.[19]

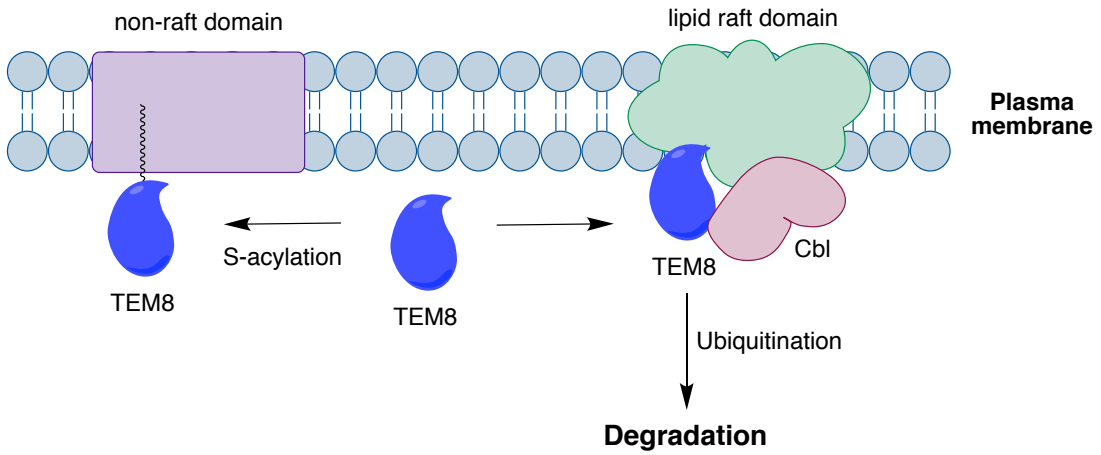


Figure 8: S-acylation of TEM8 prevents degradation.

TEM8 is restricted to non-raft domains of the plasma membrane by S-acylation and is not recognised for ubiquitination. Whereas, S-acylation mutants of TEM8

associate with lipid rafts and undergo ubiquitination mediated by Cereblon (Cbl) before being degraded *via* the proteasome. These two examples emphasize the importance of S-acylation and how it can protect against premature degradation.

1.2.3 Substrate diversity

A recent systems-level analysis has suggested that more than 10% of the proteome is S-acylated;[20] therefore the complexity of protein S-acylation approaches that of protein phosphorylation and ubiquitination.

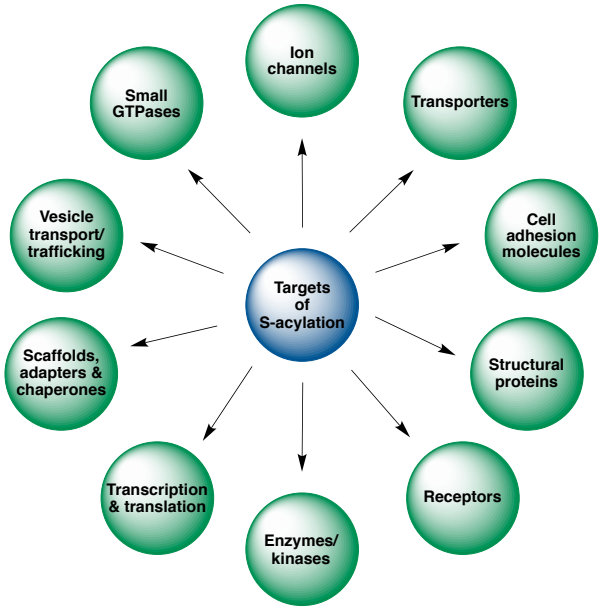


Figure 9: Diversity of S-acylated proteins.

These S-acylated substrates are extremely diverse, as highlighted in Figure 9, and include G protein-coupled receptors,[21] Ras proteins,[22] neurotransmitter receptors[23] and Src family kinases.[24]

1.2.3.1 G protein-coupled receptors

The majority of examined G protein-coupled receptors (GPCRs) have been shown to be S-acylated, usually at one to three residues in the COOH-terminal cytoplasmic tail, following the last transmembrane domain.[25-26] Despite the fact

that S-acylated cysteines are conserved across the GPCRs, the reported functional effects are as diverse as the family of GPCRs and their agonists themselves. Investigation of the β1-adrenoreceptor, a GPCR critical for heart function, has shown that different sites of palmitoylation turnover at different rates and therefore, control distinct functions, Figure 10.[12]

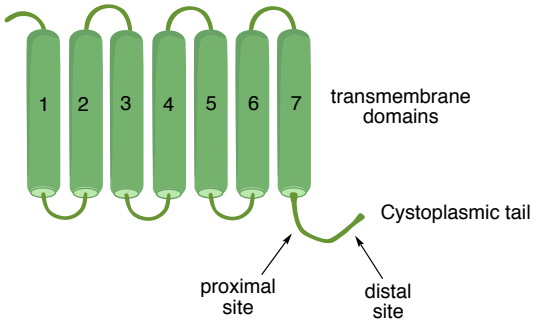


Figure 10: Topology of the β1-adrenoreceptor with highlighted palmitoylation sites.

Palmitoylation of the C-terminal cytoplasmic tail, proximal to the seventh transmembrane domain, has a low turnover rate and is proposed to contribute to proper folding. Whereas palmitoylation of the distal site, further downstream, was found to be highly dynamic and mutation of this site impaired agonist stimulated internalisation. For several GPCRs, S-acylation is required for maturation in the endoplasmic reticulum, subsequent trafficking to the plasma membrane, and targeting to 'lipid raft' domains.[27] While for other GPCRs, S-acylation can act as a switch to allow differential coupling with different effectors, for example, in the V2 vasopressin receptor.[28]

As shown, in this brief investigation of S-acylation within the GPCR family, it is apparent that the functions of S-acylation are complex and further studies are required to fully interrogate this important class of cell-surface receptors.

1.2.3.2 Ion channels

The S-acylation of ion channels has been reported to control all aspects of the ion channel life cycle from assembly, trafficking, kinetics, regulation by other PTMs, degradation and sensitivity to toxins and other pharmacological

agents.[29-30] To date, over 50 different ion channel subunits that undergo S-acylation have been identified, including the glutamate activated α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors and the large-conductance calcium and voltage-activated potassium (BK) channels.[31] AMPA receptors are ligand-gated cation channels that mediate fast synaptic transmissions in the central nervous system (CNS). Similarly to the β1-adrenoreceptor, AMPA receptors are palmitoylated at two distinct sites, each of which displays divergent functionality, Figure 11.[23]

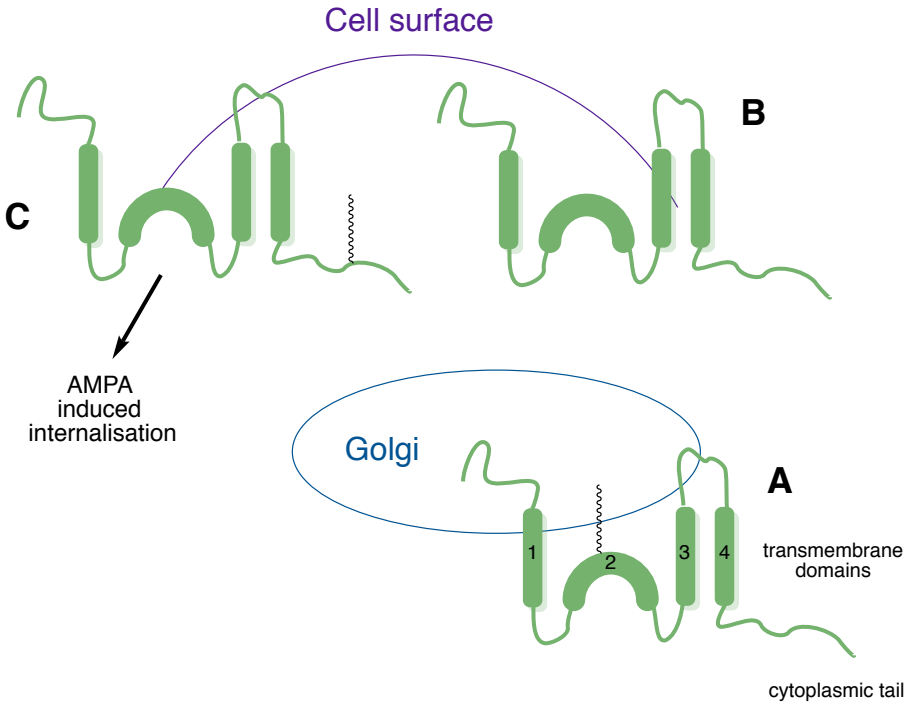


Figure 11: Roles of AMPA receptor palmitoylation.

Palmitoylation of the TMD2 site (**A**) promotes accumulation of the receptor in the Golgi apparatus. De-palmitoylation of TMD2, regulates the release of the AMPA receptor, from the Golgi, for surface delivery (**B**), where it is stabilised by various interactions. Subsequently, palmitoylation of the C-terminal site (**C**) is proposed to weaken membrane affinity, and regulate agonist-induced internalisation of the receptor by AMPA.

1.2.4 Sites of S-acylation

The proteins modified by S-acylation have divergent functions and although there is no general consensus sequence specifying an S-acylation site, the proximity to the membrane is undoubtedly an important factor that decides whether or not a cysteine is acylated.

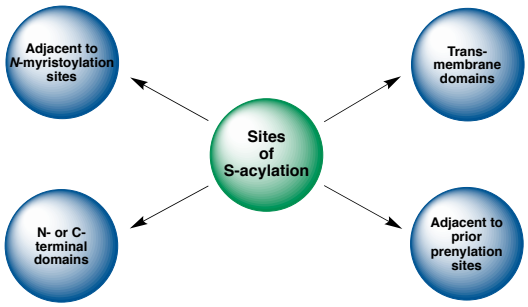


Figure 12: Sites of S-acylation.

Similarities between the sites of acylation have been sorted into four classes, Figure 12 and Figure 13.[32]

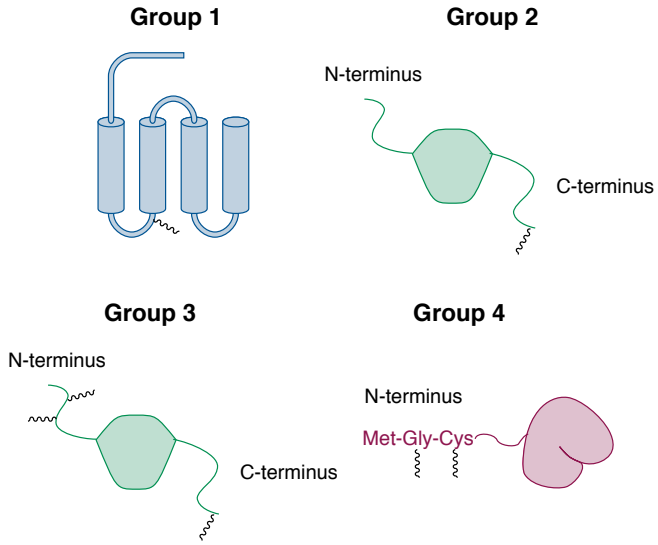


Figure 13: Four different groups of S-acylation sites.

One group comprises transmembrane proteins acylated on cysteine residues at or near the transmembrane domain. The second group is made up of proteins in which S-acylation takes place within the C-terminal region. This class of acylation is also dependent on prior prenylation of the protein at the cysteine residue within the "CAAX" box, consistent with the Ras family. A third group are

acylated at one or more cysteine residues within the N- or C-terminus. The fourth class of proteins consists of members of the Src family of tyrosine protein kinases. The majority of Src family members contain a consensus sequence for dual acylation within their N-terminal domain: Met-Gly-Cys, where the glycine residue is *N*-myristoylated and the cysteine is palmitoylated.[33-34]

1.2.5 Mechanism of palmitoylation

In contrast to other lipid modifications of proteins, palmitoylation is a reversible process; therefore, many cellular proteins can undergo continuous cycles of palmitoylation and de-palmitoylation. The first step in the palmitoylation process is the activation of the palmitic acid **4** (or other fatty acid) to its Coenzyme A (CoA) intermediate **2** (Figure 14).

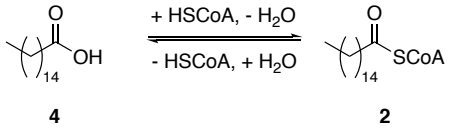


Figure 14: Activation of fatty acids.

This active intermediate, palmitoyl-CoA (Pal-CoA) **2**, is now primed for attack by the thiol of a cysteine residue **1**, releasing a molecule of SCoA **3**, which has a higher leaving group ability than the parent carboxylic acid **4**. In the reverse process, the newly formed thioester bond is readily hydrolysed, releasing the cysteine residue **1** and palmitic acid (Pal-OH) **4** (Figure 15).

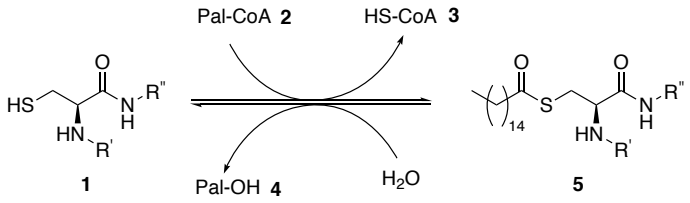


Figure 15: Process of palmitoylation.

Palmitoylation can be mediated by non-enzymatic or enzymatic processes. Certain proteins can bind to Pal-CoA directly, which results in transfer to a proximal cysteine residue in the protein. This non-enzymatic process can be

termed auto-palmitoylation and has been shown to occur in both SNAP25 protein[35] and G protein α subunit (Gα).[36] Enzymatic palmitoylation involves a family of highly conserved protein palmitoyl acyltransferases (PATs). Generally these enzymes are transmembrane proteins with a conserved Aspartate-Histidine-Histidine-Cysteine (DHHC) domain in the active site.[37] The reverse process, termed de-palmitoylation, is catalysed by acyl protein thioesterases (APTs).[38]

1.2.6 APT enzymes

APT enzymes, members of the serine hydrolase family, catalyse the process of de-palmitoylation.[38] The catalytic cycle of palmitoylation and subsequent de-palmitoylation is shown below in Figure 16.

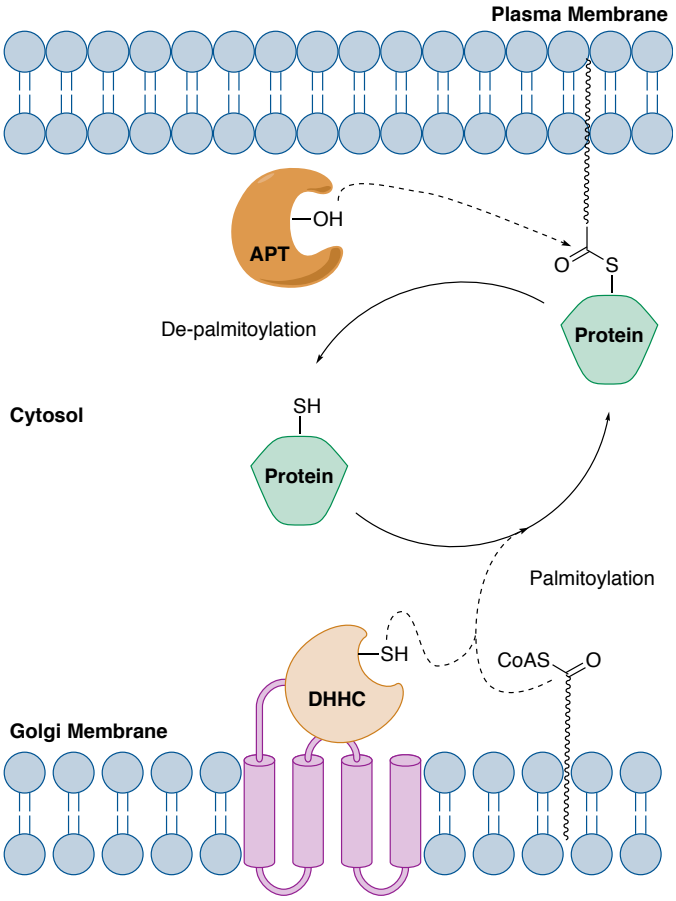


Figure 16: Catalytic palmitoylation and de-palmitoylation.

The nucleophilic serine residue of the APT enzyme (dark orange, Figure 16) attacks the carbonyl group of the thioester bond, forming an APT-palmitoyl intermediate and releasing the substrate protein (green). Upon subsequent hydrolysis with a molecule of water, the APT enzyme is regenerated and palmitic acid is released, ready to repeat the cycle.[39]

1.3 DHHC proteins

The identity of the enzymes involved in palmitoylation has only recently been elucidated. The initial breakthrough originated from studies in the yeast *Saccharomyces cerevisiae*, where seven DHHC proteins were discovered,[40-41] and since then, twenty-four DHHC proteins have been identified in the mammalian genome. The defining feature of these proteins is the presence of a highly conserved ~60 amino acid DHHC cysteine-rich-domain (CRD) (Figure 17).[42]

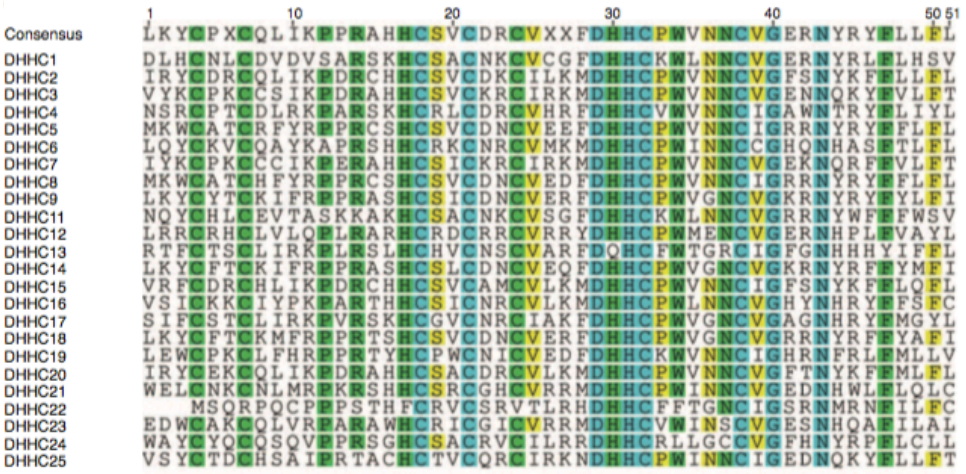


Figure 17: Sequence alignment and consensus of mouse DHHC proteins.[13]

It should be noted that DHHC10 does not exist based on the current standard nomenclature. It is also interesting to note that DHHC13 does not have the typical Aspartate-Histidine-Histidine-Cysteine (DHHC) domain, but instead an Aspartate-Glutamine-Histidine-Cysteine (DQHC) domain. Despite this unconventional tetra-peptide motif, DQHC13 is still considered a DHHC protein, and is thought to function in the same manner as the other family members.

1.3.1 Membrane topology and intracellular localisation

This family of proteins are predicted to be polytopic membrane proteins containing between four and six transmembrane domains, with the N- and C-termini present on the cytosolic face of the membrane. The catalytic DHHC CRD of the protein also lies in a cytosolic loop (Figure 18). [13]

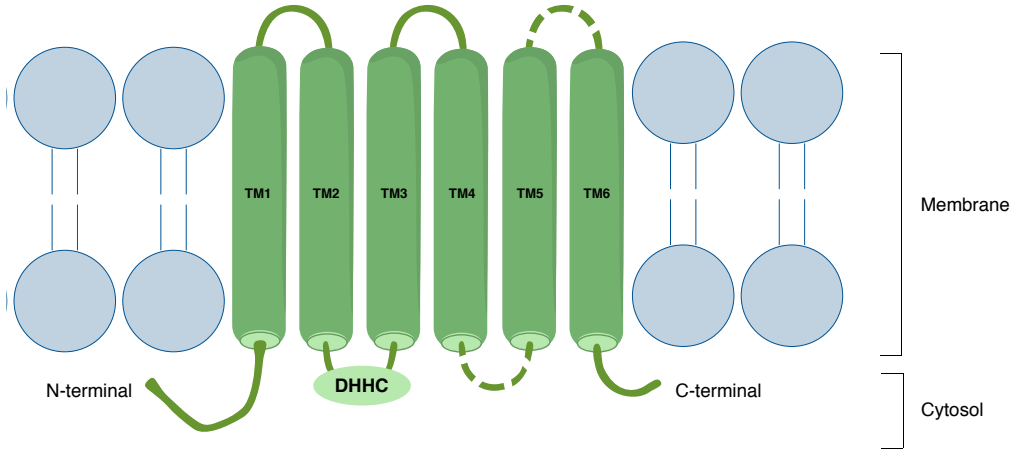


Figure 18: Topology of DHHC proteins.

The intracellular localisation of DHHC proteins has been found to be quite diverse. The majority are present at the ER and Golgi, however, some are associated with the plasma membrane (DHHC5)[43] and endosomal membranes.[44] These findings came from DHHC proteins ectopically expressed in human embryonic kidney (HEK)-293T cells, it would therefore be important to confirm these findings in different cell types. Studies on DHHC2 have shown that while it is Golgi-localised in HEK cells,[43] it is associated with mobile dendritic vesicles in cultured hippocampal neurons[45] and the plasma membrane in neuroendocrine cells.[35] These findings illustrate the complexity of DHHC proteins and how divergent a role they play within biological systems.

1.3.2 DHHC mode of action

The proposed mechanism through which DHHC proteins catalyse the transfer of palmitate, to a cysteine residue of their protein substrate, is described as a

'ping-pong' mechanism.[46] The DHHC protein is itself palmitoylated, acting as a transient acyl enzyme intermediate, before the palmitoyl group is transferred to the protein substrate, hence a two-step ping-pong mechanism. Each amino acid residue of the DHHC motif has a role to play in this transfer, surrounding the palmitoyl-CoA unit and creating a catalytic complex as shown Figure 19.

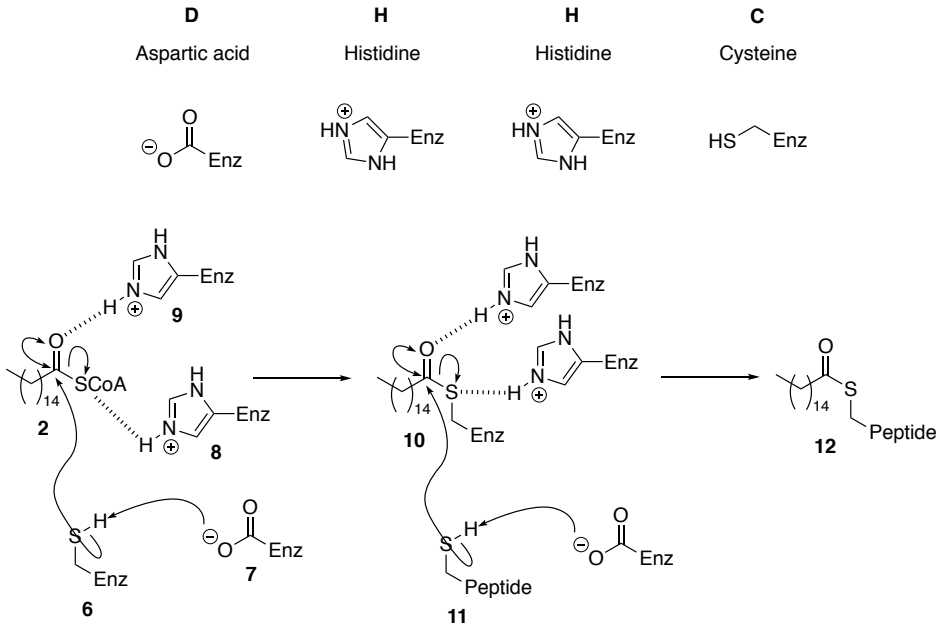


Figure 19: Mechanism of acyl transfer.

The transfer of the palmitoyl group to the catalytic cysteine is a concerted process, where the palmitoyl-CoA **2** undergoes nucleophilic attack by the catalytic cysteine residue **6**, where the carbonyl is activated by one of the histidine residues **9**. This creates a tetrahedral intermediate, from which the thiol proton of **6** is removed by the aspartic acid residue **7,** which collapses to give the palmitoylated DHHC **10**. The second histidine residue **8** serves as a hydrogen bond donor for the released SCoA, acting to improve its leaving group ability. The palmitoylated DHHC **10** is then ready to transfer the palmitoyl group to the incoming substrate **11**, following the same mechanistic course, resulting in the release of the DHHC enzyme **6** for interaction with subsequent substrates.

Although we can propose a suitable mechanism for the transfer of the palmitoyl group to the DHHC enzyme, there is still uncertainty over the method of approach of the fatty acid itself, Figure 20.

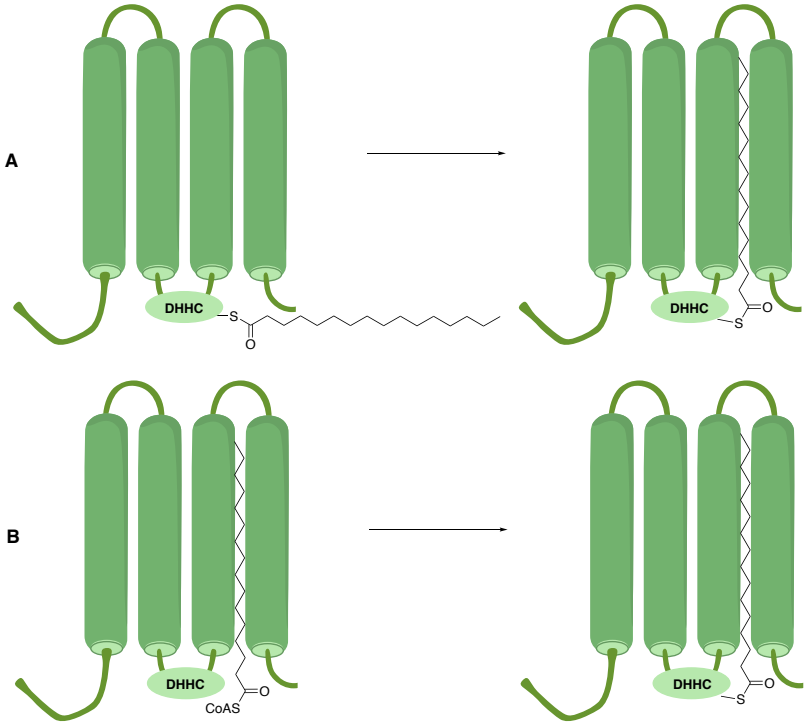


Figure 20: Methods of fatty acid recognition by DHHC enzymes: (A) Recognition by catalytic DHHC domain; (B) Recognition by transmembrane domain.

Studies have indicated that the hydrophobic chain of the fatty acid will lodge into the transmembrane channel, providing recognition and stabilisation. We propose two possible methods for this interaction. The first option (**A**) suggests that the fatty acid will initially react with the DHHC catalytic cysteine, and then the chain will move into the transmembrane channel. The second option (**B**) proposes that the chain will bind into the channel before reaction with the cysteine. On the basis of recognition, it would seem more likely for the chain to initially insert into the channel before cysteine attachment. The amino acid composition of said channel may provide the selectivity between different fatty acids, as well as potentially orientating the molecule, in the optimal alignment, for interaction with the catalytic domain.

1.3.3 DHHCs and their role in disease

It is evident, from genetic and cell-based studies, that individual DHHC proteins are crucial to normal physiological function. A vast number of studies have been undertaken to examine DHHC protein function and almost all DHHCs have been linked to specific human disorders, Table 1.[47]

Table 1: Intracellular localisation and associated diseases of human DHHCs.

DHHC isoform	Subcellular location	Physiological functions/phenotypes	Associated Disease
DHHC1	ER	Oncogenic, involved in endosomal targeting, mediates immune response	Prostate cancer, colon cancer, cardiometabolic traits
DHHC2	ER, Golgi	Tumour suppressor, mediates cell-cell contacts	Ovarian cancer, hepatocellular carcinoma
DHHC3	Golgi	Tumour suppressor	Cancer metastasis
DHHC4	ER	*not reported yet*	Autoimmune encephalitis
DHHC5	Cytoplasm	Plays a role in sorting	Schizophrenia
DHHC6	ER	Stable expression of receptors	*not reported yet*
DHHC7	Golgi	Tumour suppressor	Cystic fibrosis
DHHC8	Golgi	Neuronal growth, cortical volume	Schizophrenia
DHHC9	Cytosol, ER, Golgi	Prognostic marker for multiple myeloma	X-linked intellectual disability, speech/ language impairment
DHHC11	ER	Potential biomarker for bladder cancer	Burkitt Lymphoma, hepatoblastoma
DHHC12	ER, Golgi	Regulates alpha-secretase activity	Huntington disease, Alzheimer's, Schizophrenia
DHHC13	ER, Golgi	Regulator of Bone Morphogenetic Protein	Involved in anxiety related behaviours, amyloidosis, liver anomalies, hypermetabolism
DHHC14	ER	Tumour suppressor	Gastric cancer, acute biphenotypic leukaemia
DHHC15	Golgi	Neuronal differentiation	X-chromosome inactivation
DHHC16	ER	Mediates DNA damage response, regulates ATM	*not reported yet*
DHHC17	Cytosol, Golgi	Anti-apoptotic, required in axonal growth	Huntington disease, paralysis
DHHC18	Golgi	*not reported yet*	Schizophrenia

DHHC19	ER	Enhances viability of transfected cells *via* R-Ras	*not reported yet*
DHHC20	Plasma membrane	Oncogenic, involved in cellular transformation	Ovarian, breast and prostate cancer
DHHC21	Golgi, Plasma membrane	Hair follicle differentiation, required for gut permeability	Implication in lung pathology
DHHC22	ER, Golgi	Cell surface expression of BK channels	*not reported yet*
DHHC23	*not reported yet*	*not reported yet*	Leukaemia

Several DHHC proteins have been linked with mental retardation and cancer.[48] DHHC8 has been linked with schizophrenia,[49] mutations in DHHC9 and DHHC15 have been associated with X-linked mental retardation,[50] DHHC13 with osteoporosis, alopecia and amyloidosis[51] and DHHC17 with Huntington's disease.[52] Potential tumour suppressor genes for various cancers have been mapped to a specific chromosomal band that contains the DHHC2 gene.[53] Mutations in DHHC2 have been detected in multiple different cancers.[54] DHHC11 has also been located on a region of chromosome 5 found to be upregulated in bladder cancers with high malignant potential[55] and non-small cell lung cancers.[56] Similarly, the DHHC9 transcript is reportedly overexpressed in the majority of micro-satellite stable colorectal tumours but not in other cancers.[57]

While the impact of DHHCs on disease pathways is abundantly clear, the issue of substrate specificity still needs to be addressed, and comprehensive substrate identification remains a necessity. Only when these problems have been sufficiently solved will we be able to unlock the true therapeutic potential of this protein family.

1.3.4 Substrate specificity

Co-expression studies have shown that while some DHHC proteins are substrate specific, others palmitoylate a wide range of substrates. There are several hypotheses to explain these differences in substrate specificity. One explanation is that the protein specificity is connected to the sequence of the DHHC domain,

however, currently there is no evidence to support this.[13] Another possibility is that regions out with the DHHC domain are responsible for the specificity.[58] A third idea is that intracellular localisation of the DHHC proteins is important in specifying DHHC-substrate interactions.[13, 59] There is indeed evidence to support the latter two hypotheses. Studies have shown that certain DHHC proteins preferred a specific type of substrate; supporting the idea that localisation plays an important role. For example, some were shown to target only transmembrane proteins with juxtamembrane cysteine residues, while others targeted soluble proteins.[60] Another study reported that a specific protein, huntingtin, was palmitoylated by DHHC17 (A) but not by DHHC3 (B).[61] However, it was reported that when the ankyrin-repeat region (a common protein motif found to mediate protein-protein interactions), located at the N-terminus of DHHC17 was added to DHHC3, it allowed DHHC3 to bind and palmitoylate huntingtin (C), Figure 21. This experiment clearly confirmed that regions out with the DHHC domain could contribute to substrate specificity.

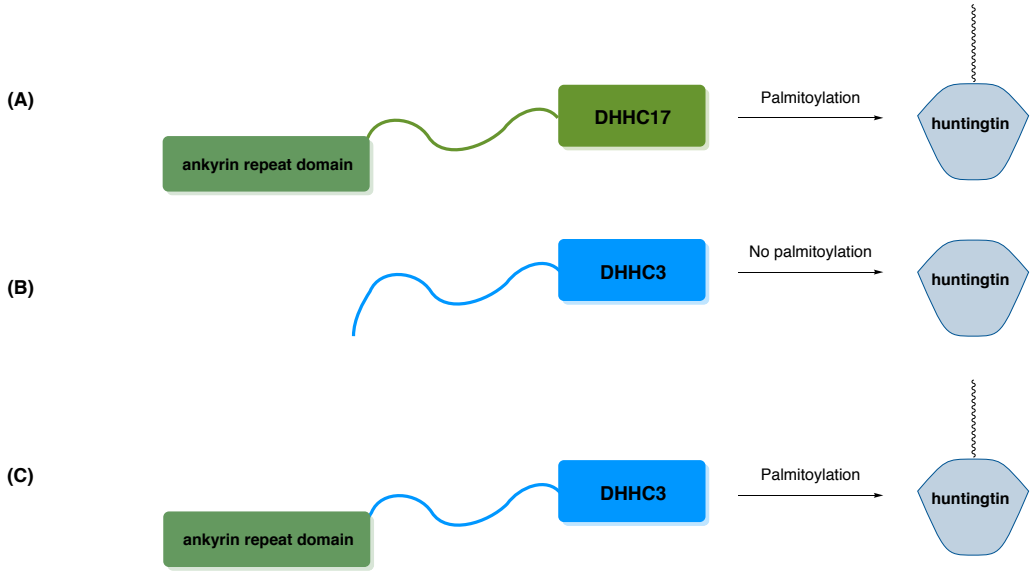


Figure 21: Abilities of DHHC17 and DHHC3 to palmitoylate huntingtin.

Several more co-expression studies have been carried out to profile the interactions between DHHC proteins and palmitoylated substrates. While this analysis is important for mapping out the potential DHHC-substrate interactions, it does contain some pitfalls. Substrates have been shown to exhibit varying degrees of promiscuity in their interactions with DHHC proteins

and so it is unclear whether a specific DHHC would play a dominant role in the palmitoylation of a specific substrate or if the expression of multiple DHHCs results in a degree of redundancy.[13] Similarly, several DHHC proteins have been shown to palmitoylate a number of substrates, leading to the same question. Therefore, DHHC depletion studies are necessary in order to decipher the role of specific DHHC proteins in cellular palmitoylation dynamics.[6]

1.3.4.1 Synaptosomal nerve-associated protein 25

One particular substrate of interest is the synaptosomal nerve-associated protein 25 (SNAP25), which has been shown to be palmitoylated by five different DHHC enzymes, DHHC2, DHHC3, DHHC7, DHHC15 and DHHC17.[35]

SNAP25 is a SNAP Receptor (SNARE) protein highly expressed in the brain, where it performs essential functions in presynaptic neurotransmitter release. SNAP25 is present at the presynaptic plasma membrane where it forms a complex with other SNARE proteins.[62] The formation of this complex is a critical step for subsequent membrane fusion (exocytosis) and secretion of neurotransmitters into the synaptic cleft.

SNAP25 is a soluble protein, which becomes membrane-associated through palmitoylation of its cysteine-rich domain, where there are four cysteine residues.[63] Different levels of palmitoylation of these residues has been shown to regulate the intracellular distribution of SNAP25, where it can associate with either recycling endosomes or the Golgi network. Interestingly, there is no evidence to suggest that SNAP25 cycles on and off membrane which may reflect the fact that it is less likely to be in a fully depalmitoylated state, due to the increased number of palmitoylation sites.[64]

While it is known that SNAP25 is palmitoylated by five different DHHC enzymes, it is still unclear whether one of these takes on a dominant role in palmitoylation, or whether palmitoylation of the four different cysteine residues is governed by different DHHCs. However, it is clear that SNAP25 is a good

model substrate with which to study palmitoylation, due to its high levels of palmitoylation, and has therefore been chosen for use within the biological studies of this project.

1.4 Current techniques to study DHHC enzymes

In order to study S-acylation and the DHHC superfamily, several techniques have been developed. However, relative to other PTMs, such as phosphorylation, the range of pharmacological, proteomic, and genetic tools to investigate the functional role of S-acylation remains somewhat limited. There is therefore a significant need to develop improved tools to interrogate and manipulate S-acylation, in order to expand our understanding of the physiological function of this PTM in disease.

1.4.1 Radiolabelling

Detection of protein S-acylation was initially performed using radiolabelled palmitate, typically ^{3}H or ^{14}C.[32] While this technique has been instrumental in the study of S-acylation with individual proteins, it has some major drawbacks. Radiolabelling is not a time effective technique, and can require weeks to visualise S-acylated proteins.[65] Iodinated fatty acids (^{125}I) were developed to improve these time constraints, but these reagents are hazardous, difficult to handle and are not readily available.[66-67] Radiolabelling also suffers from low specific activity and is not readily amenable to higher throughput proteomic analysis. [6]

1.4.2 Acyl-biotin exchange protocol

To circumvent the limitations of radiolabelled fatty acids, the acyl-biotin exchange (ABE) protocol was developed.[68] The ABE protocol exploits the labile nature of the thioester bond formed during S-acylation, which can be cleaved with hydroxylamine (HA).[69] The newly formed free sulfhydryl group can then

be labelled with a variety of constructs containing a biotin motif, for example, BMCC-biotin, Figure 22.[70]

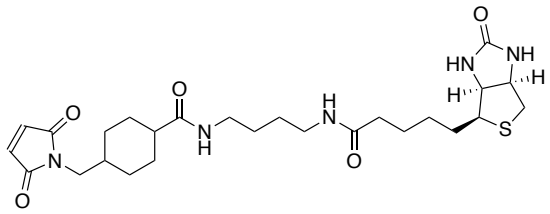


Figure 22: BMCC-biotin.

A streptavidin blot can then be performed for visualisation and quantification, or the protein may be purified using streptavidin-agarose.[70]

More recently, ABE has been combined with multi-dimensional protein identification technology (MudPIT) mass spectrometry to identify the proteins.[60] This method allowed for the identification of previously unknown S-acylated proteins. Such an analysis would not have been possible using radiolabelling approaches.

1.4.3 Acyl-resin-assisted capture

Acyl-resin-assisted capture (acyl-RAC) works in a similar manner to ABE, however it shortens the protocol by pulling down the HA-treated proteins directly using a thioreactive sepharose, such as thiopropyl sepharose® 6B (Figure 23).[71]

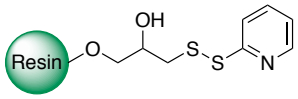


Figure 23: Thiopropyl sepharose® 6B.

As this technique involves a reduction in the number of steps and reactions, it enables S-acylated proteins to be more efficiently purified and therefore may increase the sensitivity over ABE.

Both ABE and acyl-RAC techniques have been exploited to determine the extent of S-acylation within the proteome, and both have served to increase our knowledge. However, it should be noted that those proteins identified using ABE were not always identified using acyl-RAC, and vice versa.[72] It would therefore be pertinent to use both techniques, in tandem, when investigating S-acylated proteins. Another caveat of either technique is that they are generally used to study S-acylation within animal-derived tissues, such as brain, instead of living mammalian cells. Both techniques also suffer from limitations associated with false-positives, as these methods are not specific for S-acylated cysteines but rather all cysteines within your chosen protein.

1.4.4 Bioorthogonal labelling

While ABE and acyl-RAC protocols represent cysteine-centric approaches, metabolic labelling, using bioorthogonal palmitate analogues, offers a palmitate-centric method, which allows for the labelling of living cells.

An assortment of azide and alkyne tagged fatty acid analogues have been developed, which are incorporated into S-acylated proteins by the DHHC enzymes. These bioorthogonal probes can then be reacted with a variety of chemoselective detection tags, such as biotinylated or fluorescent azides/ alkynes. Using azide or alkyne labelled biotin tags (Figure 24) for detection, offers a complementary method to that of ABE, whilst avoiding false-positives.[73]

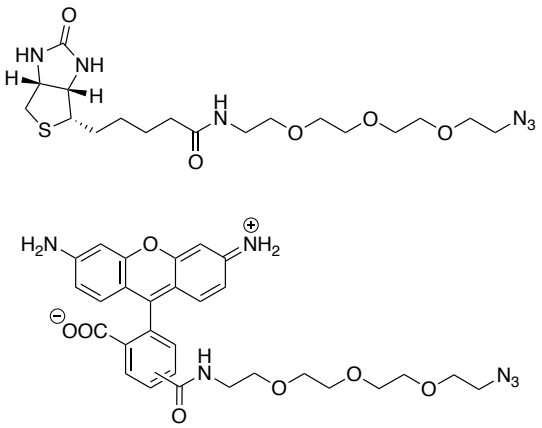


Figure 24: Biotin-azide (Top), Azido-rhodamine (Bottom).

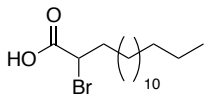
However, in-gel fluorescence detection, using alkynyl/ azido-rhodamine (Figure 24), circumvents the need for immunoblotting, thus providing a more direct and quantitative means to analyse S-acylated proteins.[74] This method also allows imaging and analysis of protein acylation in cells *via* fluorescence microscopy and flow cytometry, which could provide new opportunities to study the dynamics of S-acylation using pulse-chase experiments.

The development of bioorthogonal probes has provided novel and highly sensitive chemical tools for the interrogation of S-acylation *via* click chemistry. However, current studies have merely 'scratched the surface' of potential uses for these techniques.

1.4.5 Pharmacological manipulation of S-acylation

While a substantial effort has been made with regard to generating assays for detecting S-acylated proteins, and studying the dynamics of S-acylation, the pharmacological toolkit to interrogate S-acylation *in vitro* and *in vivo* remains incredibly limited, compared to other PTMs.

2-Bromopalmitate (2BP) **13** emerged more than 50 years ago as a non-selective inhibitor of lipid metabolism, Figure 25.[75] More recently, it has re-appeared as a general irreversible inhibitor of S-acylation,[76] and as a tool for functional analysis.



13

Figure 25: 2-Bromopalmitate.

Like palmitic acid, 2BP **13** is converted to 2-bromopalmitoyl-CoA, although with much less efficiency, before irreversibly binding to DHHC proteins. It is interesting to note that both 2BP and 2-bromopalmitoyl-CoA are incorporated into DHHC proteins, owing to the fact that the leaving group ability of the carbonyl substituent does not mitigate the reactivity of the α-bromo group.

Studies exploiting click chemistry have illustrated >450 other targets inhibited by 2BP, highlighting this compound's extreme promiscuity, Figure 26.[76]

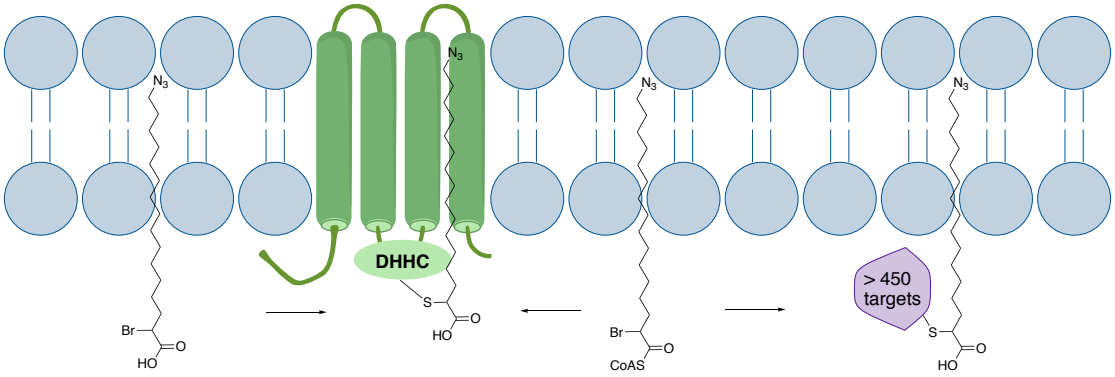


Figure 26: Inhibition by 2-bromopalmitate.

Although it has been used in several studies, 2BP is not well-tolerated by cultured cells and causes cell death even after brief exposure.[32] This promiscuity, and resulting toxicity, renders 2BP an almost useless tool to determine anything specific regarding S-acylation or DHHC proteins.

Other inhibitors that have been used extensively in the study of S-acylation, such as tunicamycin **14** and cerulenin **15**, exhibit similar issues that plague 2BP, Figure 27.[77] Tunicamycin also inhibits *N*-linked glycosylation, and cerulenin has been shown to affect many aspects of lipid metabolism.[32]

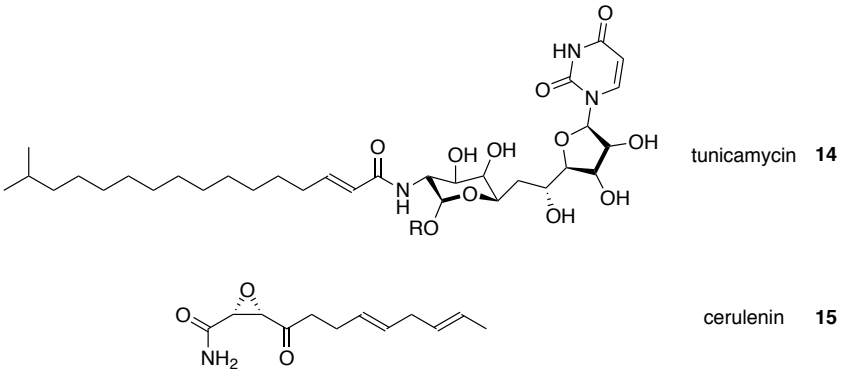


Figure 27: Tunicamycin and Cerulenin.

In an attempt to identify more selective and potent inhibitors of palmitoylation Smith *et al.*[78] screened a library of compounds, which identified single

compounds from five chemical classes that inhibited the cellular processed associated with palmitoylation, Figure 28.

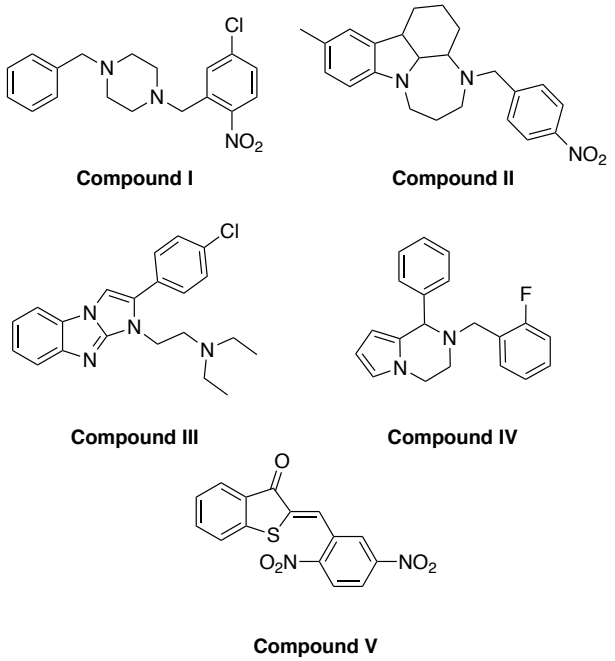


Figure 28: Five compound classes identified as inhibitors of palmitoylation.

Subsequent studies by Linder *et al.*[79] showed that none of the compounds were selective for DHHC proteins, and no substantial information has been gathered from the use of these compounds as chemical tools. Although it is interesting to note that the Michael-acceptor functionality of Compound V means it is acting as a reversible inhibitor of palmitoylation. This reversibility has not been seen with previous inhibitors, therefore the discovery of this compound, while ineffective as a chemical tool, shows good precedence for the potential to develop reversible inhibitors of palmitoylation. Selectivity is therefore the key property that remains to be addressed.

Although there are clearly several compounds in the literature that inhibit S-acylation, none have been found to have any specificity between DHHCs or in fact between other cysteine containing proteins.[80] Thus, once again, illustrating the need for selective chemical tools to not only inhibit DHHCs but to explore the S-acylation pathway and gain an insight into selectivity between DHHCs and their corresponding substrates.

1.4.6 Crystallographic data

Although our knowledge of protein S-acylation, DHHC enzymes and their substrates has been vastly improved by many of these techniques, fundamental aspects of DHHC enzymes, including their mechanism of catalysis and acyl-CoA binding and recognition, have been challenging to elucidate without structural information. Crystallographic data can be used to not only answer these questions, but also aid in the development of structure-based chemical tools.

In 2018, Banerjee *et al.*[81] solved the crystal structure of DHHC20, the first crystal structure obtained for the DHHC superfamily. Using this structure they gained valuable insight into what governs fatty acid selectivity, lending insights into the recent report by Chamberlain *et al.*[82] They showed that DHHC20's four transmembrane domains formed a tepee-like structure, coming together on the lumenal side and opening apart at the cytosolic face, Figure 29.

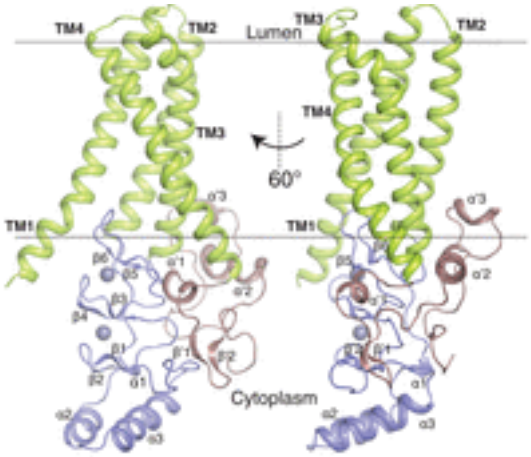


Figure 29: Cartoon representation of DHHC20. The four TM helices are shown in green, the cysteine-rich domain in blue and the C-terminal domain in brown.

The acyl chains insert into this tepee-like cavity, and cavity-lining residues have been shown to be determinants of fatty acid recognition and chain length selectivity within DHHC20, consistent with that seen for DHHC3 and DHHC7. This study noted that toward the tapering end of the acyl-binding cavity, a tyrosine residue forms a hydrogen-bonding interaction with a serine residue on the opposite transmembrane domain, effectively closing off the cavity.

Wild-type DHHC20 was shown to have a marked preference for palmitoyl (C16)-CoA as a result of this. Mutation of the tyrosine residue to the less bulky alanine resulted in a marked increase in preference for stearoyl (C18)-CoA, while mutation of the serine to a bulkier phenylalanine increased the preference for short-chain acyl-CoA. These results indicate that it is the pairing of amino acid residues within the transmembrane cavity that causes chain length selectivity, and not just one individual residue, as proposed within the Chamberlain study.

These crystallographic studies also aided in the elucidation of the mechanism of palmitoylation. They showed that the aspartic acid and one of the histidine residues form a hydrogen-bonded pair that can accept a proton from the catalytic cysteine residue, enabling nucleophilic attack on the carbonyl thioester of palmitoyl-CoA, to generate the acyl-enzyme intermediate, Figure 30. However, they could only propose the method by which the palmitoyl-CoA enters the binding cavity.

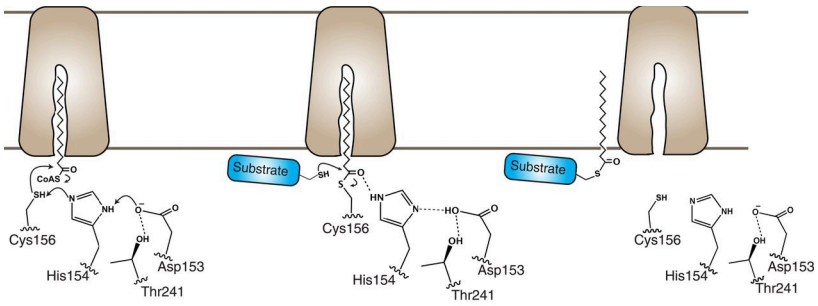


Figure 30: Proposed reaction mechanism and substrate approach.

With a crystal structure for DHHC20 now in hand and vast quantities of structural data obtained, this will inevitably lead to the generation of structure-based chemical probes, which will benefit the field of palmitoylation immensely. Solving the crystal structures for the remaining DHHC superfamily members will also be of paramount importance.

1.5 Small molecule probes

In recent years, small molecule probes have become important tools used to investigate fundamental biological mechanisms, and to explore the role of a protein in a broader biological context. As there is currently a lack of reversible inhibitors, or probes, available for DHHCs, the development of novel tools would represent a substantial advancement in the interrogation of S-acylation.

A chemical probe is a selective small organic molecule, which modulates a protein's function and is used to interrogate physiological and pathological processes.[83] The application of chemical probes can determine the tractability of a specific target. This can be its biological tractability, where they can be used to interrogate the relationship between a target and its phenotype. Or they can be used to examine the chemical tractability, by modulating that phenotype.[84]

While chemical probes have the ability to expand our knowledge of the genome and increase the number of druggable targets, it is important to note that only 'high quality' chemical probes generate meaningful data.

1.5.1 Key properties of chemical probes

To assess the viability of small molecule probes, guidelines have been introduced, which are intended to give a user confidence that their chemical probe is 'fit-for-purpose', by evaluating key properties in relation to their intended use. These 'fitness factors' can be split into four main categories, as shown in Figure 31.[85]

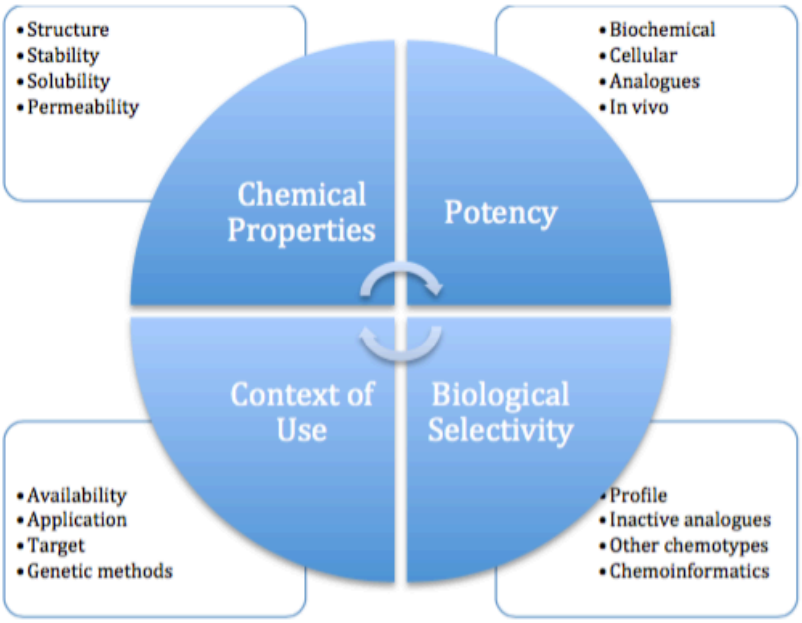
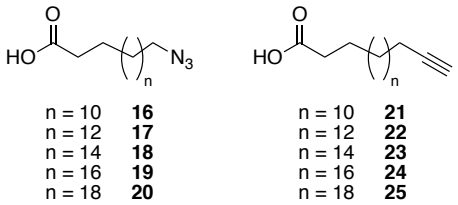


Figure 31: Fitness factors for chemical probes.

Although these guidelines provide a useful indicator as to what a 'good probe' should be, strict adherence to these criteria could stifle innovation, especially in the early stages of work on a new target, where probes are lacking. A large amount of data may still be extracted from a target using sub-optimal probes; however this is obviously dependent on the target of interest.

1.6 Previous work

Work has been undertaken within the Tomkinson group to provide the Chamberlain group with chemical tools to gain valuable insight into the subtleties of specificity, within the DHHC superfamily. These chemical tools have been based upon the natural ligand, palmitic acid, with varying chain lengths. In their study, they employ click chemistry assays; therefore all probes have been appropriately labelled with both azide and alkyne tags, Figure 32.



n = 10	**16**	n = 10	**21**
n = 12	**17**	n = 12	**22**
n = 14	**18**	n = 14	**23**
n = 16	**19**	n = 16	**24**
n = 18	**20**	n = 18	**25**

Figure 32: Chemical probes

The basis of this work was to understand if DHHC proteins would display significant differences in fatty acid selectivity profiles for both auto-palmitoylation and substrate palmitoylation. Initially this work looked to distinguish selectivity profiles between five chosen DHHC proteins; DHHC2, DHHC3, DHHC7, DHHC15 and DHHC17, which are all active against the same substrate (SNAP25), using competition-based experiments. These results (Figure 33) have shown that DHHC3 has a marked preference for smaller chain length fatty acids (C14 and C16) over longer chains (C18). Interestingly, when looking at DHHC7, which is highly related to DHHC3 at the primary sequence level, this enzyme incorporated C18 into SNAP25 more efficiently. However, C18 incorporation was still lower relative to C14/C16 incorporation for DHHC7.

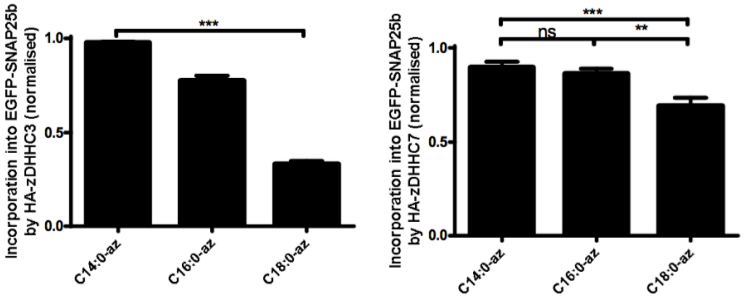


Figure 33: DHHC3 and DHHC7 selectivity profiles.[82]

DHHC2 and DHHC15, another two proteins highly related at the amino acid level, showed significant differences in fatty acid selectivity, Figure 34. DHHC2 exhibited no selectivity, whereas DHHC15 incorporated C14/C16 more readily than the C18 fatty acid.

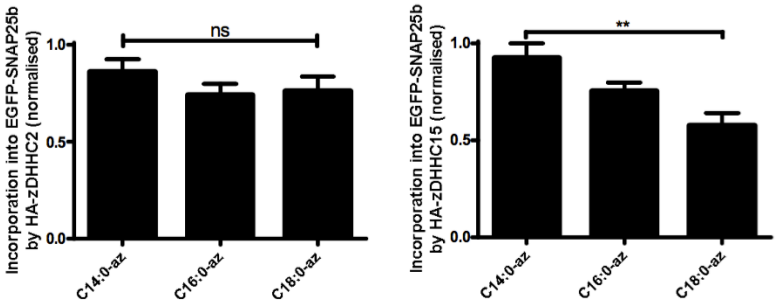


Figure 34: DHHC2 and DHHC15 selectivity profiles.[82]

Finally, DHHC17 was the first to show a marked preference for longer chains (C16/C18) over the C14 fatty acid, Figure 35. These results were consistent when using either the alkyne or azide probes.

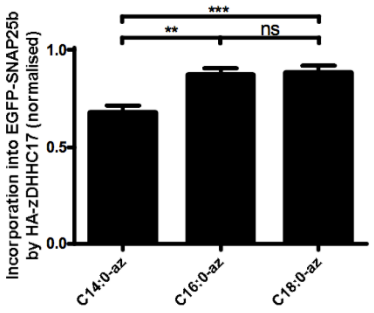


Figure 35: DHHC17 selectivity profile.[82]

Following this initial success, C20 and C22 probes were developed to further understand the limits of DHHC fatty acid selectivity. DHHC3 showed no preference between the C18 to C22 fatty acids, whereas DHHC7 and DHHC17 showed a gradual decline in incorporation as chain length was increased, Figure 36.

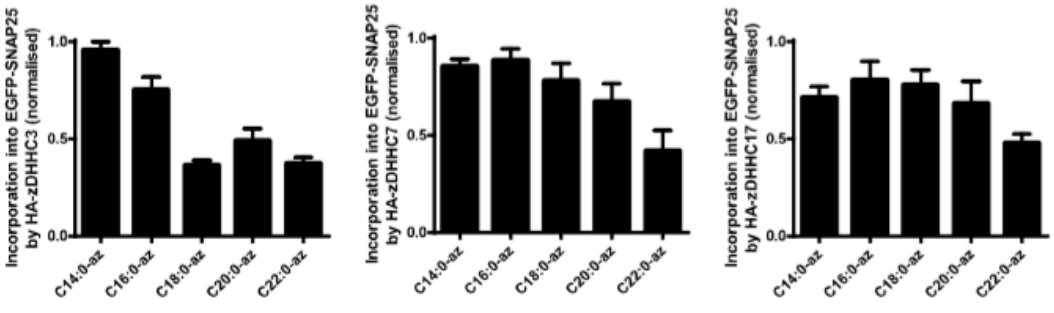


Figure 36: DHHC-3, -7 and -17 incorporation of C20/C22.[82]

As the reaction proceeds *via* a ping-pong mechanism, the study also looked to determine whether the substrate palmitoylation profiles were consistent with auto-palmitoylation of the DHHC. Interestingly, results showed that the auto-palmitoylation profiles were identical to the profiles observed for DHHC mediated palmitoylation of SNAP25.

To understand how these differences in selectivity profiles might be encoded at the molecular level, domain swapping experiments were employed. Following substantial analysis, these studies identified that the third transmembrane domain (out of four) was the key determinant limiting the ability of DHHC3 to incorporate longer chain fatty acids. Further targeted mutagenesis revealed that replacing isoleucine-182 in this third transmembrane domain with a serine residue, present at the same position in DHHC7, led to an increase in the ability of DHHC3 to use longer chain fatty acids as substrates, Figure 37.

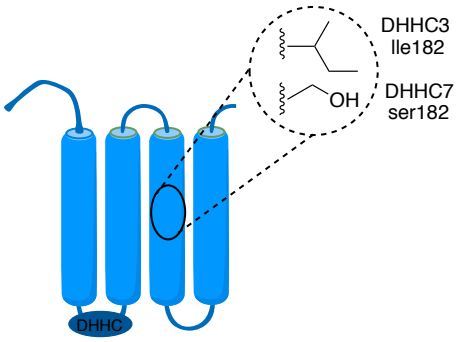


Figure 37: Transmembrane domain mutagenesis.

The steric bulk of the isoleucine residue was proposed to impede the incorporation of longer chain lengths (C18–C22) by DHHC3. DHHC7, however, with the significantly less bulky serine, was able to incorporate all chain lengths to a reasonable degree. It is incredibly interesting that the subtle change of one amino acid resulted in such a large functional change, and suggests we may be able to target specific amino acids in order to gain selectivity across this enzyme superfamily.

These were the first studies to uncover differences in the fatty acid selectivity profiles of DHHC proteins and to map molecular determinants governing this selectivity.[82]

1.7 Proposal

A recent systems-level analysis has indicated that more than 10% of the proteome is S-acylated;[81] therefore the complexity of S-acylation approaches that of protein phosphorylation. While phosphorylation has been extensively studied, with substantial clinical success being achieved with kinases as drug targets, addressing DHHC enzymes with small molecules has received significantly less attention, despite their high therapeutic potential as drug targets.

Elucidating the fundamental aspects of DHHC enzymes, including their recognition of protein substrates and their fatty acid selectivity profiles, has been extremely challenging. However, although there are major gaps in our understanding of DHHC's, it is evident from genetic and cell-based studies that individual DHHC enzymes are crucial to normal physiological function, with almost all DHHC's being linked to prominent disease states, especially cancers and neuropsychiatric disorders. Since there are currently no reversible/ non-covalent probes for the elucidation of DHHC-substrate specificity and selectivity profiles, and the interrogation of their therapeutic potential, the requirement to fill this gap served as the starting point for this investigation.

Initial studies within the Chamberlain group have provided the first instance of chain length selectivity within the DHHC superfamily, specifically between DHHC3 and DHHC7, which are highly conserved at the amino acid level. Within this project we will design small molecules to further elucidate this selectivity, exploiting the difference between the isoleucine-182 and serine-182 residues. These chemical probes will be used to dissect the fundamental biology associated with these proteins, specifically in a cell-based assay developed within the Chamberlain laboratory.

An *in vitro* assay has also been established within the Chamberlain group with the aim of developing inhibitors of palmitoylation, using DHHC2 and SNAP25 as the acylation substrate. It was envisaged that an *in vitro* assay would be preferential to a cell-based assay for this task, as lower activity compounds would not be discounted and could provide an initial starting point for investigation.

This work has the potential to provide the wider research community with the tools to interrogate this important superfamily of enzymes, and answer the many fundamental questions surrounding it.

1.8 Aims

The primary aim of this project is to develop chemical tools to elucidate any selectivity between DHHC3 and DHHC7. This work will follow on from the initial findings of the Chamberlain group, where we will attempt to increase affinity for DHHC7 by exploiting a hydrogen bonding interaction with serine182. Various hydrogen bond donating and accepting groups will be used to modify fatty acids of different chain lengths, Figure 38. Four chain lengths, between 12 and 18, have been chosen in order to ascertain the optimal carbon chain length for interaction at the 182 position.

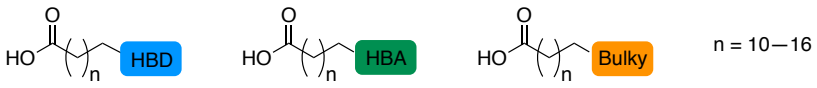
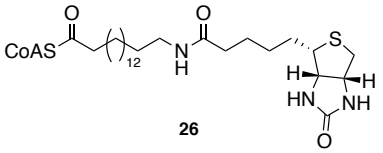


Figure 38: Fatty acid modifications.

Fatty acid analogues will be synthesised containing alcohols and thiols as hydrogen bond donating groups. Methoxy, trifluoromethoxy and acetate containing groups will be assessed as hydrogen bond accepting groups, and finally modification with a phenyl group will provide essential steric information. Once synthesised, each compound will be evaluated using a cell-based assay, where their inhibitory effect on the palmitoylation of SNAP25,

our chosen S-acylation substrate, will be assessed. Providing selective inhibitors for each of DHHC3 and DHHC7 is the main objective for this investigation.

The secondary aim is to develop a chemical tool that can be used to interrogate DHHC-substrate profiles, and has the potential to elucidate new substrates to expand the DHHC substrate library. This tool will be based upon the most common fatty acid used to modify substrates, palmitic acid, Figure 39.



26

Figure 39: Proposed chemical tool for interrogation of DHHC-substrate profiles.

It was proposed that modification with a biotin moiety would allow us to generate a multi-purpose tool. Biotin can be used as a recognition element, which could allow the levels of palmitoylation on various substrates to be calculated, but it can also be used for protein pull-down experiments. Protein pull-down has the potential to allow us to identify new substrates for each DHHC enzyme. Finally it is proposed to generate the coenzyme A ester of our fatty acid analogue, in order for this tool to be used both in cells and *in vitro*.

The final aim of this project is to develop inhibitors of DHHC2, using an *in vitro* assay, with the purpose of interrogating the roles/functions of this enzyme. No structural data for DHHC2 has been identified to have an effect on fatty acid modification to date; therefore inhibitor design has been based around our mechanistic understanding of the DHHC superfamily. Palmitoylation of the DHHC enzyme itself proceeds through a tetrahedral intermediate.

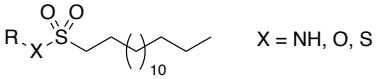


Figure 40: General structure of proposed tetrahedral compounds.

We therefore proposed a series of compounds containing tetrahedral head groups to mimic this interaction, Figure 40. We have also based a series of compounds around the structure of known palmitoylation inhibitor 2-bromopalmitate **13**, Figure 41. 2-bromopalmitate is a non-selective, covalent inhibitor of palmitoylation.

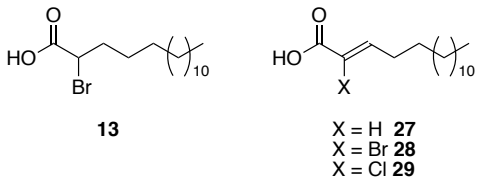


Figure 41: 2-bromopalmitate and proposed modifications.

Our modifications have been based around generating a reversible and selective compound.

2. Results and Discussion

2.1 Elucidating selectivity

As discussed in the previous work and proposal sections, we aimed to generate chemical probes to further elucidate the selectivity profiles established between DHHC3 and DHHC7. A natural starting point for this investigation was to exploit an interaction with the serine-182 residue of DHHC7. We proposed that the addition of a hydrogen bonding functionality, to fatty acids of varying chain lengths, could allow us to specifically target DHHC7 over DHHC3.

2.1.1 Alcohol series

2.1.1.1 Design rationale

The first series of probes were designed for potential interaction with the alcohol functionality of the serine-182 residue in DHHC7. Modification of fatty acids with an alcohol moiety should allow these probes to hydrogen bond with the serine-182 residue, increasing their affinity for DHHC7. However, as the functionality is discreet enough, it was envisaged that DHHC3 would also be able to incorporate these probes to some extent.

From the initial chain length selectivity profiles, Figure 33, we can conclude that C16 is the maximum chain length tolerated by DHHC3. Therefore we can infer that the 16th carbon of said chain should be in close enough proximity to the Ile-182 residue to cause this observed selectivity, and thus a C16 chain appended with an alcohol moiety should interact with serine-182. However, the initial studies used probes affixed with azide and alkyne functionalities, which although bioorthogonal, could still have an associated steric consequence. We therefore cannot deduce which chain length would be optimal for interaction with position 182 of the DHHC enzyme, and so a range of chain lengths were proposed to be synthesised, ranging from C12 to C18, Figure 42.

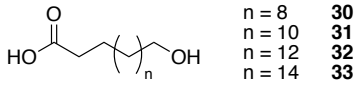
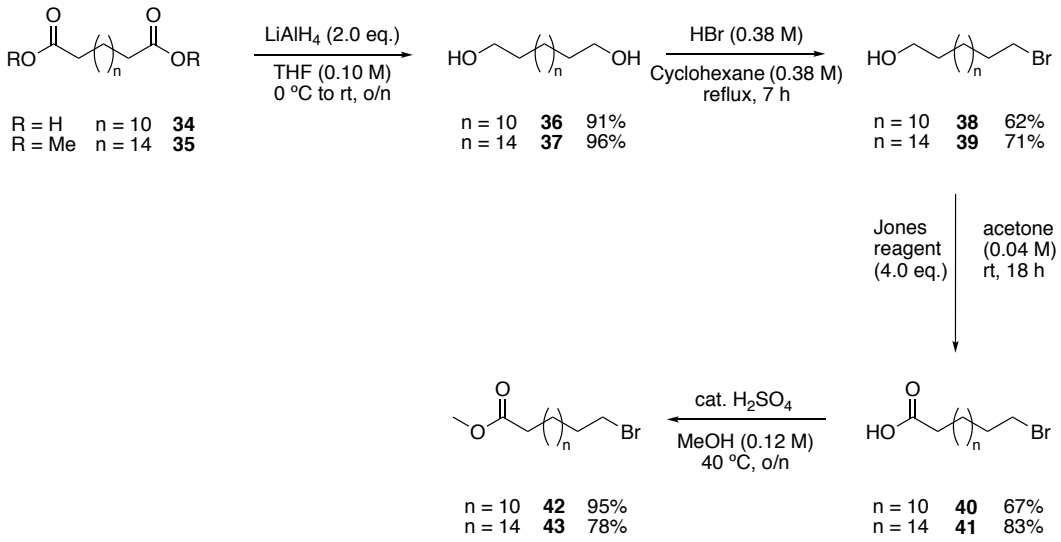


Figure 42: Proposed alcohol series.

2.1.1.2 Synthetic route

Fortuitously, two of the proposed compounds, C12-OH **30** and C16-OH **32** were commercially available and therefore only the C14-OH **31** and C18-OH **33** probes were required to be synthesised. The established synthetic route within the Tomkinson group,[82] for the generation of azide/ alkyne probes, proceeds *via* a chemically tractable bromide intermediate, which provided the first half of our synthetic route, Scheme 1.

Scheme 1: Synthesis of C14 42 and C18 43 bromide intermediates.

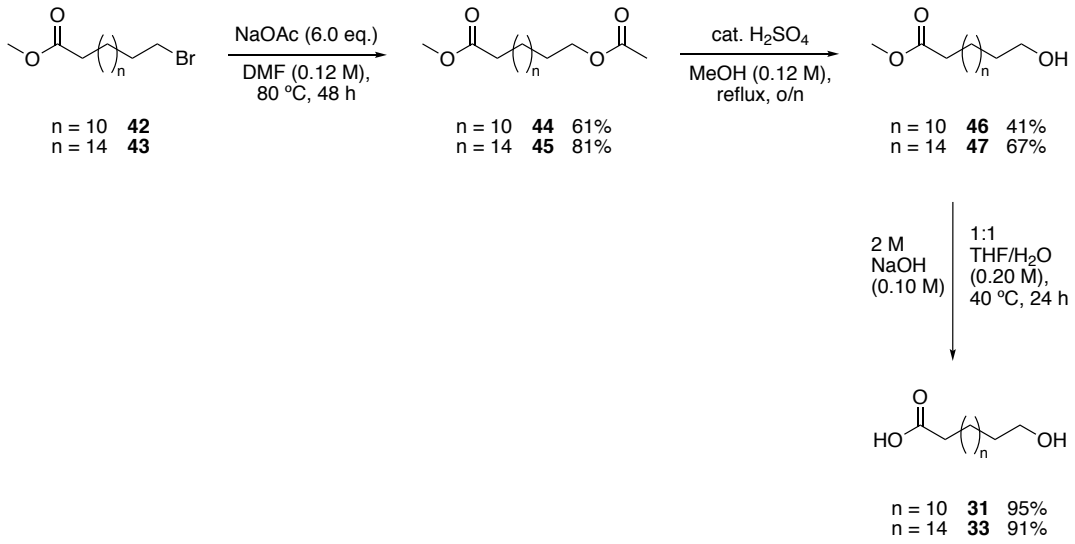


Starting from the commercially available di-acid (C14) **34** or di-ester (C18) **35**, diols **36** and **37**, were prepared in 91% and 96% yields, respectively, *via* lithium aluminium hydride ($LiAlH_4$) reduction, with no further purification required. Compounds **36** and **37** were mono-brominated under reflux conditions with hydrobromic acid (HBr), to give the bromo-alcohols **38** and **39** in 62% and 71% yields after purification by column chromatography, which were then oxidised to the bromo-acids **40** and **41** using the Jones reagent, to give **40** and **41** in 67%

and 83% yields following purification. Within the original literature procedure, the bromo-acids were used as the common intermediates. However, in subsequent reactions poor yields were observed, and this was proposed to be due to the ability of the free acid head group to form micelles in solution. It was therefore decided that protection of the acid as a methyl ester would improve yields, and aid subsequent purification. From **40** and **41**, the bromo-esters **42** and **43** were synthesised *via* an esterification with catalytic amounts of sulfuric acid (H_2SO_4) in methanol (MeOH). No further purification was required and intermediates **42** and **43** were afforded in 95% and 78% yields, respectively.

From the bromo-ester intermediates **42** and **43** it was proposed that an acetylation followed by hydrolysis would provide us with the desired alcohol functionality, as shown in Scheme 2.

Scheme 2: Synthesis of C14-OH 31 and C18-OH 33.



The acetylation of bromo-esters **42** and **43** *via* an S_N2 reaction with sodium acetate (NaOAc) in *N*,*N*–dimethylformamide (DMF) at 80 °C afforded intermediates **44** and **45** in 61% and 81% yields, respectively, following purification by flash column chromatography. This procedure was a modification of the S_N2 reaction performed to generate azides in the original paper.[82] The next step in the synthesis was the cleavage of the acetate group to reveal the alcohol. This was initially attempted using the standard method of

potassium carbonate (K_2CO_3) in MeOH at room temperature. While this method proved successful for cleavage of the acetate group, the ^1H NMR spectrum indicated that the methyl ester had also been hydrolysed in the process. It was therefore decided that de-acetylation using H_2SO_4 in MeOH would circumvent this issue, and compounds **46** and **47** were synthesised in 41% and 67% yields, without the need for purification. Hydrolysis of esters **46** and **47** using a solution of sodium hydroxide (NaOH) afforded the final compounds **31** and **33** in 91% and 95% yields, respectively.

2.1.1.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 was used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7.

Screening results for the alcohol functionalised compounds within this series are displayed in Figure 43.

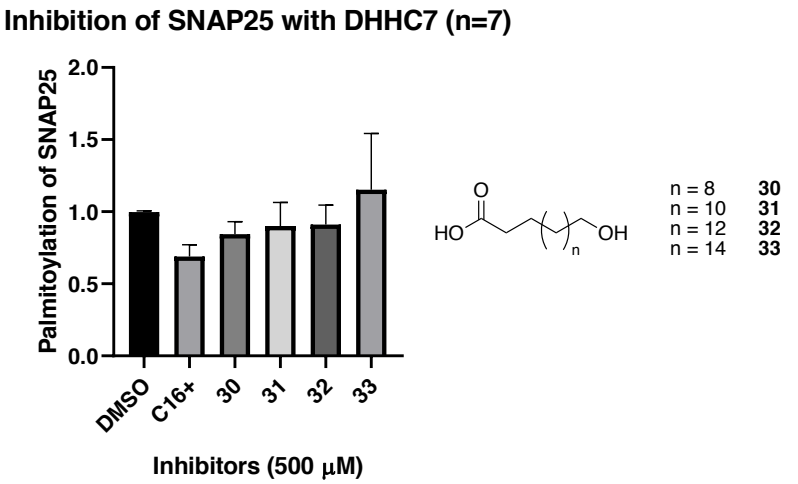


Figure 43: Graph of single point assay results for alcohol series.

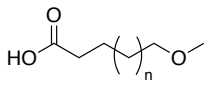
In the above graph, DMSO acts as the negative control, where 100% palmitoylation is expected. The given value of 1 is the baseline value for this assay, meaning that percentage inhibition of each compound is calculated relative to this number. The C16+ represents palmitic acid, and acts as the positive control, where we should see up to 70% inhibition.* For this particular series we can see that not one of the varying chain lengths inhibit palmitoylation of SNAP25 to any reasonable extent. A slight trend can be observed, however, where the 12-carbon chain alcohol (C12OH) inhibits palmitoylation at ~15% and this number steadily decreases as we add carbon units. Interestingly, the C18OH appears to be activating palmitoylation to ~15%, which was an unexpected result. The generally poor inhibitory effects of the alcohol series was initially surprising, however, solubility may have been an issue, as compounds were observably difficult to dissolve in DMSO. The formation of micelles with polar compounds has been proposed to have caused issues during the synthesis phase, therefore this may also contribute to the poor solubility observed.

* Lower percentage seen due to error in stock solution of cold palmitate.

2.1.2 Ether series

2.1.2.1 Design rationale

While it was proposed that the alcohol series had the potential to interact with both DHHC3 and DHHC7, it was hypothesised that modification of this functionality as a methyl ether (Figure 44) could increase the steric bulk enough that it would not be accepted by DHHC3. However, with the significantly smaller serine residue of DHHC7, this was not foreseen to be a problem.



n = 8 **48**
n = 10 **49**
n = 12 **50**
n = 14 **51**

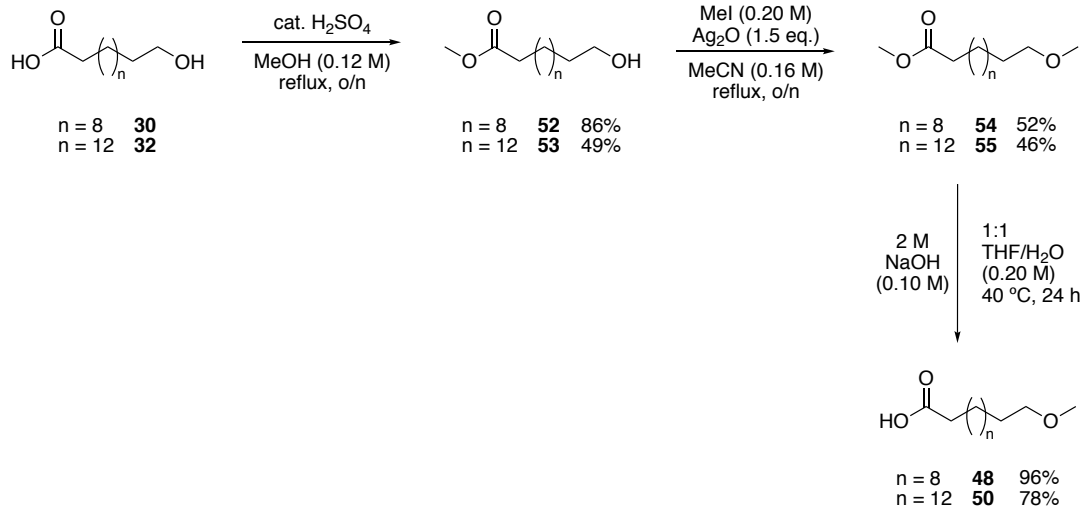
Figure 44: Proposed ether series.

As serine residues can behave as both a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), it was unclear how serine-182 within the third transmembrane domain of DHHC7 would interact with probe compounds. It was therefore essential to generate compounds that can behave as both a HBA and a HBD (alcohol series) but also compounds that can behave solely as a HBA (ether series) or HBD. The methyl ethers should only have an increased affinity for DHHC7 if the serine residue is acting as a HBD.

2.1.2.2 Synthetic route

It was determined that these compounds could be prepared *via* a simple etherification of the alcohol series generated in Section 2.1.1.2. The commercially available C12 **30** and C16 **32** alcohols would initially be esterified, while the C14 and C18 ester intermediates **46** and **47** would be used as prepared above. The synthetic route for the C12 **48** and C16 **50** ethers can be viewed in Scheme 3.

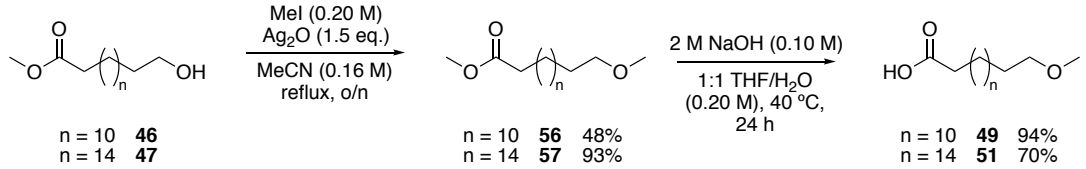
Scheme 3: Synthetic route for C12 48 and C16 50 ethers.



Starting from commercially available acids **30** and **32**, methyl esters **52** and **53** were generated by refluxing in H_2SO_4 and MeOH overnight. Compounds **52** and **53** were generated in 86% and 49% yield respectively, with no further purification required. At this stage compounds **52** and **53** were treated with methyl iodide (MeI) under basic conditions (sodium hydride (NaH)), however ^{1}H NMR spectroscopic analysis indicated only starting material remained after 24 hours. It was unclear why this procedure had been unsuccessful and therefore instead of attempting optimisation of these conditions, an alternative method was sought out. Taber *et al*. illustrated that MeI in the presence of silver(I) oxide (Ag_2O) was a superior methylating agent, due to the ability of the silver to attract the iodine away from the methyl portion, rendering it more electrophilic.[86] Therefore compounds **52** and **53** were methylated using MeI and Ag_2O in acetonitrile (MeCN), under reflux conditions. Filtration of the solids and concentration *in vacuo* afforded compounds **54** and **55** in 52% and 46% yields, respectively, without the need for further purification. The final step in the synthesis was the hydrolysis of compounds **54** and **55** using standard conditions to generate compounds **48** and **50** in 78% and 96% yields, respectively.

The synthetic route towards the C14 **49** and C18 **51** ethers is detailed below in Scheme 4.

Scheme 4: Synthetic route for C14 49 and C18 51 ethers



Starting from the previously synthesised esters **46** and **47**, ether intermediates **56** and **57** were generated in 48% and 93%, respectively,† using MeI and Ag_2O under reflux conditions. Final compounds **49** and **51** were afforded, following hydrolysis in NaOH, in 94% and 70% yields.

2.1.2.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 is used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7. The results are illustrated in Figure 45.

† The significant variation in yields was attributed to the use of different bottles of Ag_2O.

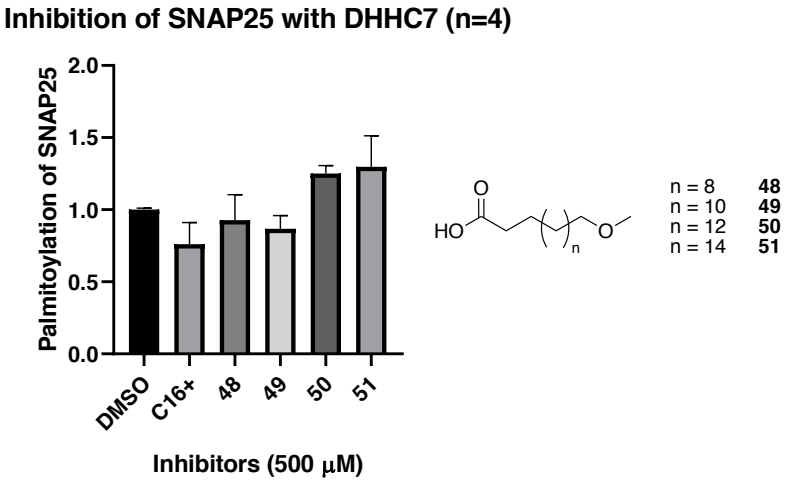


Figure 45: Graph of single point assay results for ether series.

Once again, the majority of the compounds synthesised were not effective as inhibitors of palmitoylation. Only the 14-carbon methyl ether (C14OMe) displayed any measurable activity at ~15%. This could potentially be due to the fact that serine-182 preferentially behaves as a HBA, and therefore is unable to interact with the OMe group in the proposed manner. Interestingly, two compounds from this series, C16OMe and C18OMe appear to be activating palmitoylation, this time to almost 30%. While these unexpected results are incredibly interesting, our assay was not set up to measure activation, and therefore we cannot say for certain whether these are true activators at this stage. It was interesting to observe inhibition of palmitoylation by the C14OMe at ~15%, when in the alcohol series we only observed this inhibition level for the C12OH. The difference in optimal chain length for each of these series provides an exciting insight into the behaviour of Ser-182. The optimal chain length for inhibition by the alcohol series was observed to be 12 carbons, in comparison to the 14-carbon preference observed for the ether series. This would suggest that it is the proton of the alcohol moiety that is interacting with the serine residue in this case. This would indicate that Ser-182 behaves as a hydrogen bond acceptor in the presence of another alcohol functionality. Whereas, the preferred 14-carbon chain length of the ether series would indicate interaction of the oxygen with the serine in a hydrogen bond accepting manner, suggesting that Ser-182 behaves as a hydrogen bond donor in the

presence of a hydrogen bond acceptor group. The difference in chain length selectivity observed suggests an optimal trajectory for each type of interaction.

2.1.3 Thiol series

2.1.3.1 Design rationale

As discussed within the ether series, serine residues have the ability to behave as both a HBD and HBA. Due to the slightly weaker nature of an S—H bond compared to an O—H bond, it was proposed that a thiol (Figure 46) might serve as a better HBD than the comparative alcohol. Indeed, studies have shown that the thiol moiety preferentially behaves as a HBD instead of as a HBA.[87]

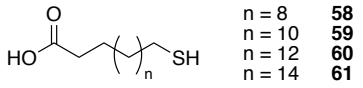


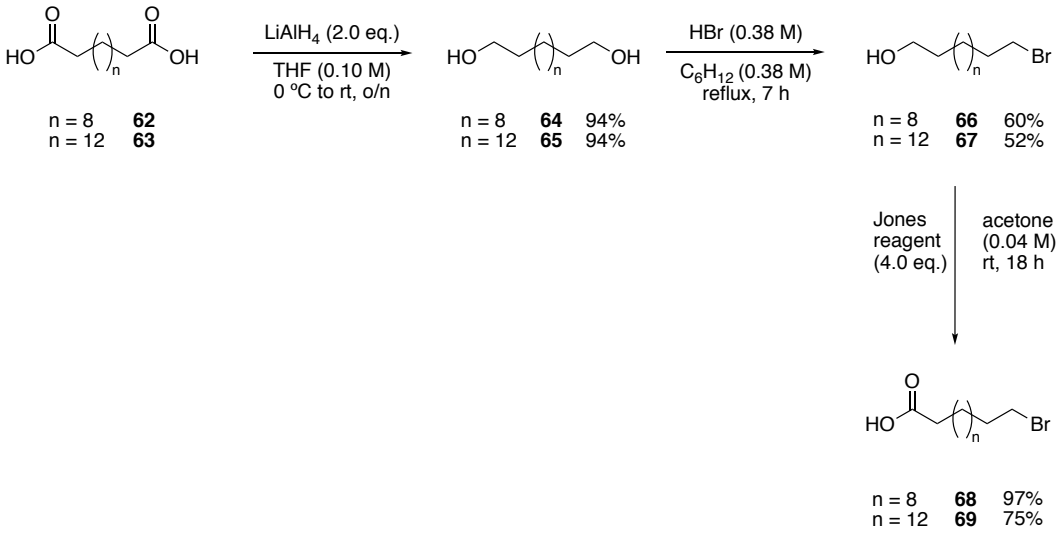
Figure 46: Proposed thiol series.

2.1.3.2 Synthetic route

Initially, routes originating from the C14 and C18 bromo-ester intermediates, **42** and **43**, were sought, as over 5 g of each had already been synthesised. Two procedures were attempted, one proceeding through a thiourea intermediate[88] and another *via* a silyl intermediate,[89] however, neither transformation proved successful. The ^{1}H NMR spectra for both attempts showed only starting material. A further search of the literature revealed Bunte salts to be a valuable intermediate in thiol chemistry. Bunte salts are prepared *via* an S_N2 reaction with sodium thiosulfate, and can either undergo acid hydrolysis to provide the desired thiols,[90] or they can be further reacted with Grignard reagents to generate sulfides.[91] While synthesis of probes with a thiol moiety was the main aim, probes containing a methyl sulfide would provide us with a direct comparison to the ether series. Due to the use of a Grignard reagent at a later stage, it was proposed to alter the methyl ester to a *tert*-butyl ester before

continuing with the generation of the Bunte salts. However, only the C14 **40** and C18 **41** bromo-acid intermediates had been generated previously, and therefore the C12 **68** and C16 **69** bromo-acids were initially required to be synthesised, Scheme 5.

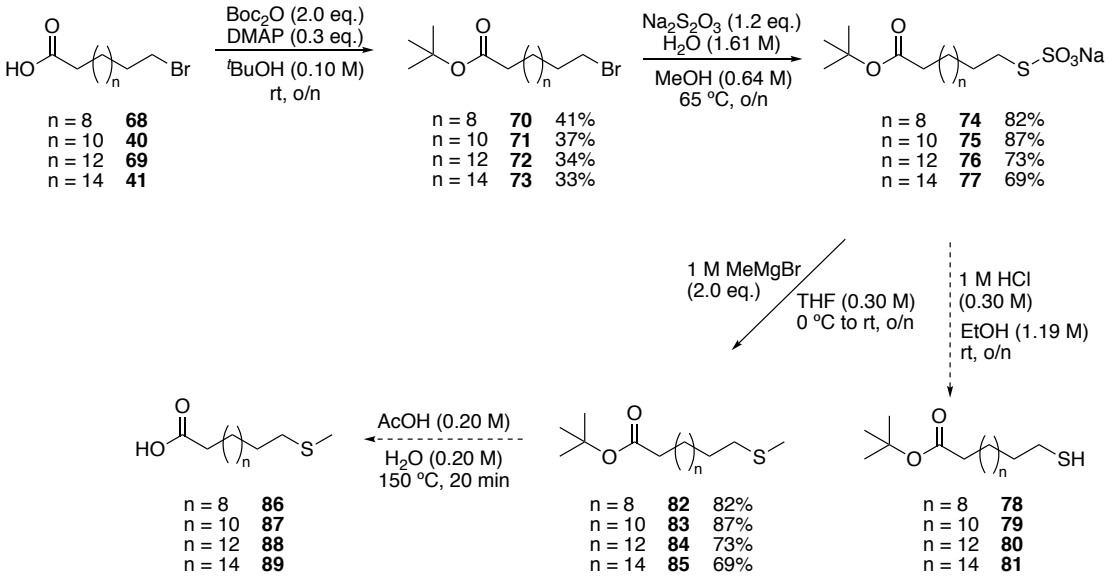
Scheme 5: Synthesis of C12 68 and C16 69 bromo-acids.



Starting from the commercially available diacids **62** and **63**, diols **64** and **65** were generated in 94% yields following $LiAlH_4$ reduction. De-symmetrisation with HBr, afforded bromo-alcohols **66** and **67** in 60% and 52% yields respectively, following purification by flash column chromatography. A final Jones oxidation afforded the desired intermediates **68** and **69** in 97% and 75% yields, after purification *via* flash column chromatography.

With all four acid intermediates (**40**, **41**, **68** and **69**) in hand, synthesis of the desired thiols was attempted. The proposed synthetic route is illustrated in Scheme 6.

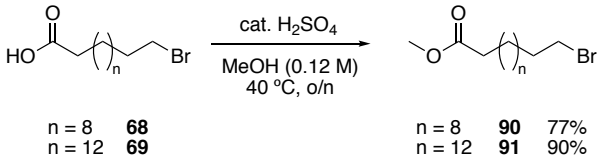
Scheme 6: Synthesis and use of Bunte salts.



Starting from the previously synthesised bromo-acids **68**, **40**, **69** and **41**, *tert*-butyl esters **70–73** were prepared *via* reaction with di-*tert*-butyl dicarbonate (Boc_2O) and 4-(dimethylamino)pyridine (DMAP) at room temperature overnight in the presence of *tert*-butanol (^{t}BuOH).[92] Further purification *via* flash column chromatography afforded the desired compounds in 33–41% yields. Bunte salts **74–77** were then synthesised by reaction of sodium thiosulfate ($Na_2S_2O_3$) in MeOH, and trituration with hexane generated **74–77** in 69–87% yields. The acid hydrolysis of **74–77**, however, did not prove successful. ^{1}H NMR spectroscopic analysis revealed a number of unknown by-products with no indication of the desired products **78–81**. The generation of methyl sulfides **82–85** by reaction of Bunte salts **74–77** with methyl magnesium bromide (MeMgBr), on the other hand, proceeded smoothly, albeit in low yields (25–31%). Upon acid hydrolysis of the sulfides **82–85**, however, a number of unidentified products were observed by ^{1}H NMR spectroscopy, alongside the desired compounds **86–89**, and purification, by flash column chromatography, recrystallisation and trituration, proved unsuccessful. At this stage the use of Bunte salts was abandoned and alternative methods were sought out.

Since acetylation, followed by hydrolysis, had proven to be a successful method for the generation of alcohols from the corresponding bromides, it was proposed that this procedure could also be utilised for the synthesis of thiols. However, initially the C12 **90** and C16 **91** methyl esters were required to be synthesised, as shown in Scheme 7.

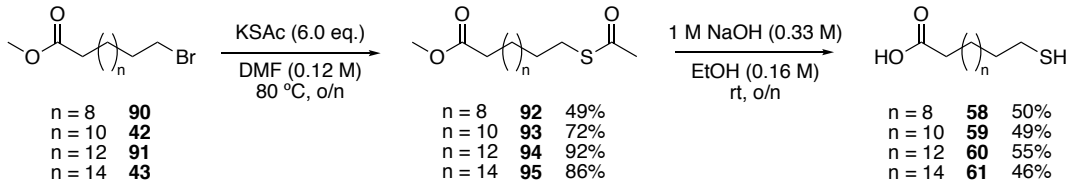
Scheme 7: Synthesis of C12 90 and C16 91 ester intermediates.



Bromo-acids **68** and **69** were esterified using MeOH and catalytic H_2SO_4, to afford the ester bromide intermediates **90** and **91** in 77% and 90% yields respectively.

The synthetic route towards the desired thiols **58**–**61** is outlined in Scheme 8.

Scheme 8: Synthetic route for thiol series.



Treatment of bromo-esters **90**, **42**, **91** and **43** with potassium thioacetate (KSAc) in DMF afforded compounds **92**–**95** in 49–92% yields, following purification *via* flash column chromatography. Acid hydrolysis of the thioacetate group followed by basic hydrolysis of the methyl ester was then attempted, as described for the alcohol series (Section 2.1.1; Scheme 2). The initial thioacetate cleavage was performed and the product isolated, however the second hydrolysis was unsuccessful. The product proved difficult to isolate from the organic layer during an acidic work up and instead had to be filtered from the aqueous layer, alongside an impurity that could not be separated during column

chromatography. Alternatively, dual hydrolysis of both the ester and thioester functionalities with NaOH solution provided the desired thiols **58**–**61** in 46–55% yields, with no need for further purification.[93] The generation of methyl sulfides was not attempted at this point.

2.1.3.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 is used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7. The results are illustrated in Figure 47.

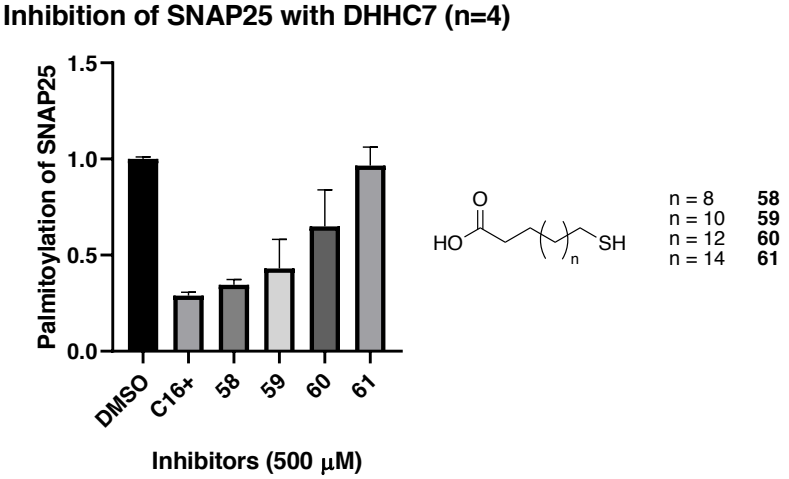


Figure 47: Graph of single point assay results for thiol series.

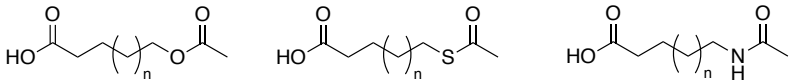
In Figure 47 we can observe a good inhibitory level (~70%) when using our positive control palmitic acid (C16+), when compared to Figure 43 and Figure 45, as fresh stock solution was prepared and used. For this series of compounds

we were pleased to see that both the 12- and 14-carbon thiols (C12/C14SH) inhibited palmitoylation of SNAP25 by up to ~65%. We propose that this inhibition is due to a HBD-HBA relationship between the thiol and serine respectively. This could also be supported by the fact that we saw lower inhibition levels with the alcohol series, which are weaker HBDs, and the ether series, which are HBAs. A nice trend can also be observed, where the extent of inhibition decreases as chain length increases. This could be explained by the proximity of the thiol moiety to the serine-182 residue, as the longer chain lengths may have too large a distance between them and the alcohol moiety for an interaction to take place. Sceptically, however, this could also be due to the decrease in solubility of the compounds as chain length increases. Solubility in the DMSO medium decreased as chain length increased, from 12-carbons to 18-carbons, for this series of compounds. The stock solution was diluted (from 50 mM to 25 mM) in an attempt to overcome this issue, however the compounds remained poorly soluble. Further dilution of the stock solution was not possible, as the volume added to the assay would have become too great. Heating of the stock solution prior to addition of the inhibitors to the assay buffer alleviated the solubility issues in DMSO, however upon cooling in the assay buffer, partial precipitation was observed.

2.1.4 Acetylated series

2.1.4.1 Design rationale

To expand our library of chemical probes, the synthesis of compounds containing an acetate, thioacetate or acetamide functional group was proposed, Figure 48. These compounds also have an increased steric bulk and therefore serve to interrogate the steric allowance of the DHHC enzymes.



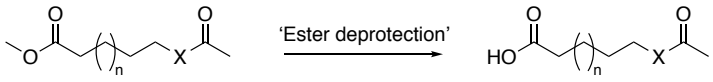
n = 8	**96**	n = 8	**100**	n = 8	**104**
n = 10	**97**	n = 10	**101**	n = 10	**105**
n = 12	**98**	n = 12	**102**	n = 12	**106**
n = 14	**99**	n = 14	**103**	n = 14	**107**

Figure 48: Proposed acetylated series.

2.1.4.2 Synthetic route

As acetate and thioacetate functionalities had been used as intermediates in the synthesis of the alcohol and thiol series, respectively, the simple ester deprotection of acetates‡ **44** and **45** and thioacetates **92–95** was proposed to provide us with the desired compounds **97**, **99** and **100–103**, Scheme 9**.**

Scheme 9: Ester deprotection of acetate and thioacetates.



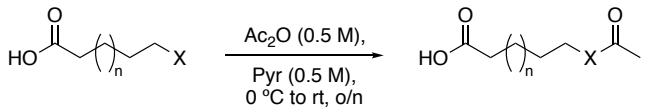
X = O	n = 10	**44**	X = O	n = 10	**97**
	n = 14	**45**		n = 14	**99**
X = S	n = 8	**92**	X = S	n = 8	**100**
	n = 10	**93**		n = 10	**101**
	n = 12	**94**		n = 12	**102**
	n = 14	**95**		n = 14	**103**

Deprotection of methyl esters is generally carried out using acidic or basic hydrolysis conditions, however, we have shown that either method would also hydrolyse the acetate and thioacetate functional groups. Therefore, a non-hydrolytic method was sought, such as the use of boron tribromide (BBr_3).[94] However, even after extended reaction times (up to 72 hours), BBr_3 deprotection did not go to completion, and isolation of the desired products **97**, **99** and **100–103** *via* flash column chromatography was unsuccessful. It was proposed that the acetate and thioacetate groups may have cleaved during purification, as the addition of acetic acid was required to move the compound on silica, due to the carboxylic acid head group. Deprotection of the methyl esters was abandoned at this stage and an alternative synthetic strategy, that

‡ C12 and C16 acetates had not been generated at this stage.

would not require purification of the final compound by column chromatography, was designed, Scheme 10.

Scheme 10: Synthetic route for acetate and thioacetates.

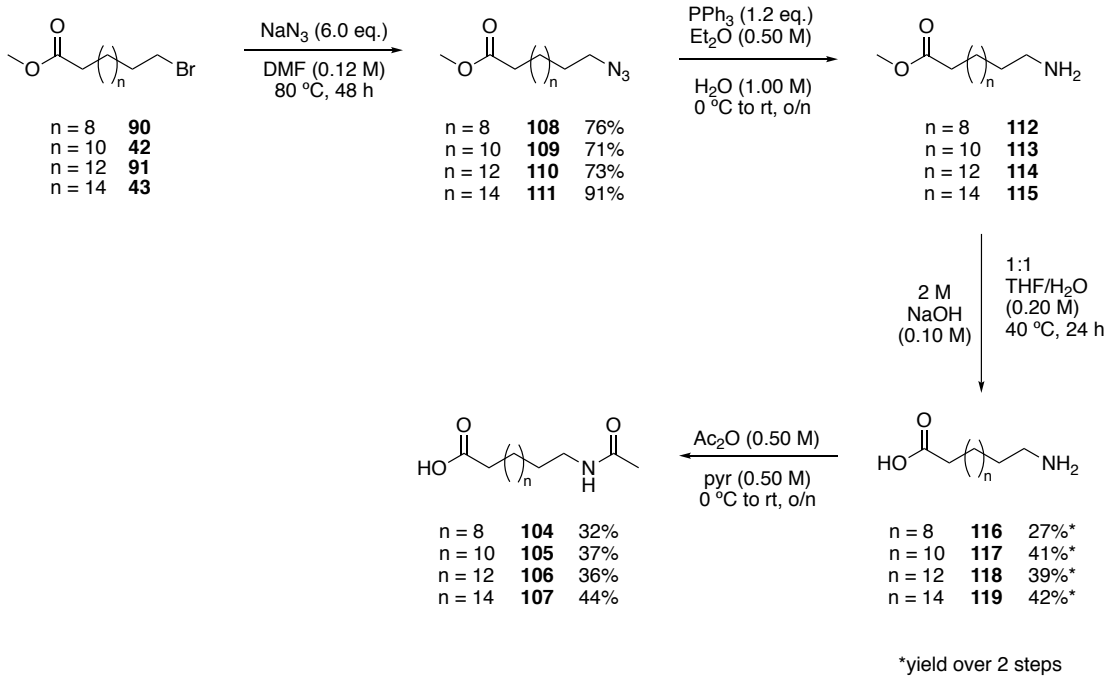


X = OH	n = 8	**30**		X = O	n = 8	**96**	90%
	n = 10	**31**			n = 10	**97**	85%
	n = 12	**32**			n = 12	**98**	98%
	n = 14	**33**			n = 14	**99**	96%
X = SH	n = 8	**58**		X = S	n = 8	**100**	70%
	n = 10	**59**			n = 10	**101**	93%
	n = 12	**60**			n = 12	**102**	97%
	n = 14	**61**			n = 14	**103**	33%

Starting from the alcohols **30–33** or thiols **58–61**, final compounds **96–103** were synthesised by reaction with acetic anhydride (Ac_2O) in pyridine (pyr). Complete product conversion was observed before Ac_2O and pyr were removed by azeotroping with cyclohexane, and no further purification was required. Acetates **96–99** were afforded in 85–98% yields, and thioacetates **100–103** in 33–97% yields. The lower yield observed for thioacetate **103** was due to the insolubility of thiol **61** in the reaction medium.

The synthetic route towards the acetamide series was again based around the already synthesised ester bromide intermediates, **90**, **42**, **91** and **43**. Addition of an azide followed by reduction could impart the necessary amine functionality, which could then be acetylated using the procedure described above. The full synthetic sequence is illustrated in Scheme 11.

Scheme 11: Synthesis of acetamide compounds.



The first step in the synthetic route was the addition of sodium azide (NaN_3) in DMF to bromides, **90**, **42**, **91** and **43**, to generate azides **108–111** in 71–91% yields, following purification *via* flash column chromatography. The subsequent reduction of azides **108–111** proved more challenging. Several reduction methods were examined using the C14 azide **109** and their efficiency is illustrated in Table 2.

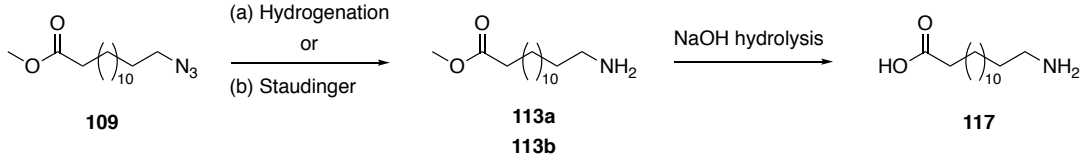
Table 2: Tested conditions for the reduction of azides.

Entry	Method	Solvent	Product (113)	Yield
1	PPh_3	Et_2O	✓	-
2	$CeCl_3$, NaI	MeCN	✓	17%
3	Pd/C, H_2	MeOH	✓	72%
4	Pd/C, H_2	EtOAc	✓	90%
5	Pd/C, H_2	THF/H_2O	✗	-

Entry 1 applied general Staudinger reduction conditions and full reduction was observed after 16 hours.[95] However, the triphenylphosphine oxide (PPh_3=O) co-product could not be separated from the otherwise clean product. A mild

cerium chloride/ sodium iodide method was then attempted, as shown in Entry 2,[96] which provided clean product but was very low yielding (<20%). Entries 3–5 illustrate attempts made using another standard azide reduction method: hydrogenation. The most common solvent used for hydrogenation reactions tends to be either MeOH or ethanol (EtOH). Entry 3 shows the reaction in MeOH, which gave product **113** in a reasonable yield (72%). This was only obtained, however, after re-subjection to the hydrogenation conditions over a second night. Therefore, other solvent systems were investigated. Entry 4 illustrates the hydrogenation in ethyl acetate (EtOAc),[97] which gave the best yield of 90%, with less than 10% baseline impurities. Entry 5 shows the result of a tetrahydrofuran/ water solvent system, which was unsuccessful, as determined by a lack of distinguishable peaks in the ^{1}H NMR spectra.[98]

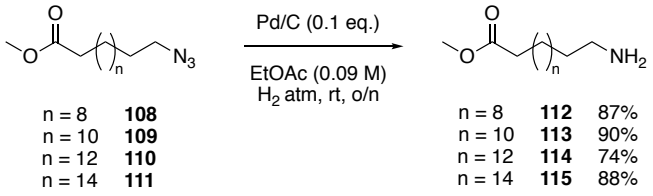
Scheme 12: Formation of acid amine 117.



From the results shown in Table 2, it was decided to continue with the hydrogenation in EtOAc, as it afforded the highest yield of clean product. However, due to the fact that the PPh_3 reduction was spotless other than the PPh_3=O, which would not affect the next reaction, the products from this step were also taken forward. The NaOH hydrolysis was therefore attempted on both reaction products **113a** and **113b** (Scheme 12), however this did not proceed as expected, as no product was isolated from the organic extracts when using **113a**. Interestingly though, PPh_3=O, only, was isolated when using **113b** and it was therefore proposed that due to the highly polar nature of the resulting compound **117**, it might be more soluble in the aqueous layer. Indeed, after leaving the aqueous layer to stand overnight, a white precipitate formed. This was filtered and ^{1}H NMR spectroscopy determined the solid was clean product. The hydrolysis of both **113a** and **113b**, following precipitation and filtration of the aqueous layer, afforded clean product **117**. Due to the more experimentally simplistic set-up of the Staudinger reduction, it was determined that this would

be the method carried forward for future reductions. Compounds **116–119** were formed in 27–42% yields after 2 steps. These were then acetylated, using the standard conditions (Ac_2O, pyr) to form final compounds **104–107** in 32–44% yields. However, in order to obtain full characterisation of the ester amine intermediates **112–115**, ester azides **108–111** were reduced using hydrogenation conditions in EtOAc to afford **112–115** in 74–90% yields, Scheme 13.

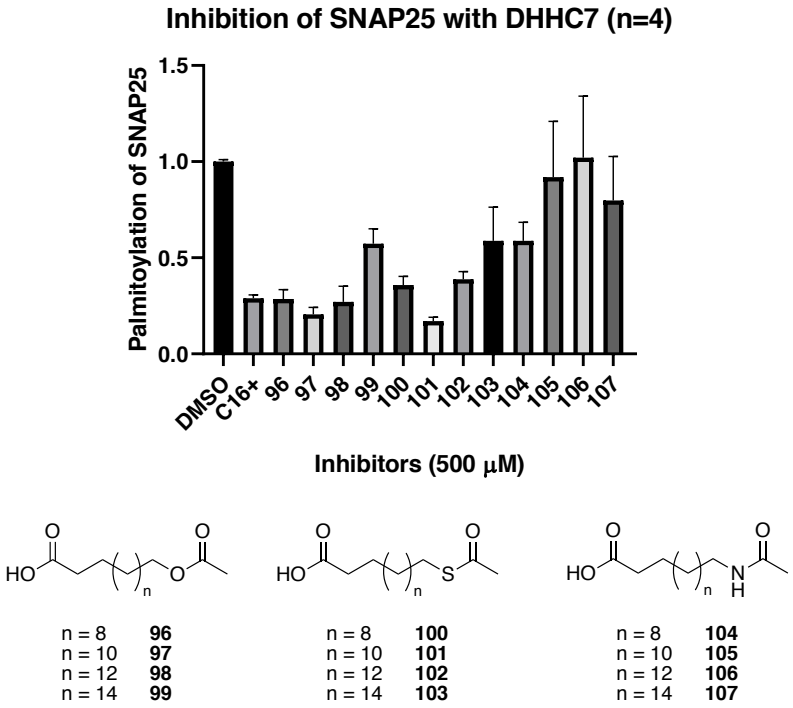
Scheme 13: Isolation of ester amine intermediates.



n = 8	**108**		n = 8	**112** 87%
n = 10	**109**		n = 10	**113** 90%
n = 12	**110**		n = 12	**114** 74%
n = 14	**111**		n = 14	**115** 88%

2.1.4.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 is used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7. The results are displayed in Figure 49.



n = 8	**96**	n = 8	**100**	n = 8	**104**
n = 10	**97**	n = 10	**101**	n = 10	**105**
n = 12	**98**	n = 12	**102**	n = 12	**106**
n = 14	**99**	n = 14	**103**	n = 14	**107**

Figure 49: Graph of single point assay results for acetylated series.

From the results obtained for this series of compounds, we can see that the C14 acetate **97** and C14 thioacetate **101** are displaying strong inhibitory behaviour (~80% inhibition). We can also observe an interesting trend for each of the acetate and thioacetate series. The C12 chain lengths (**96** and **100**) appear to be inhibiting to a lesser extent than the C14 chains, which is directly opposed to what we have seen with other series of compounds (e.g. the thiol series). This could suggest that a 14-carbon chain length affords the best proximity to the serine-182 residue for this form of functional group. The high level of inhibition observed with these compounds was somewhat surprising, due to the previously low inhibition observed with the ether series. All 3 series of acetylated compounds should behave as HBAs; so these differences in inhibition levels may suggest that a better HBA, i.e. an ester/thioester as opposed to an alkoxy, is required for interaction with serine-182. Although it is important to note that the carbonyl lone pairs are in a different position to the corresponding oxygen of the ether series for the preferred 14-carbon chain length. A more direct comparison could be made between the 14-carbon acetate/thioacetate and the 16-carbon ether, as the lone pairs would be in a more similar position. However, the 16-carbon ether appeared to activate palmitoylation by up to 30%, which is a currently unexplained phenomenon. Although, the lack of

inhibition does support our theory that a better HBA is required for inhibition. It is also worth noting that these compounds have the ability to act as dual HBAs, which could account for the higher levels of inhibition observed. For the C16 and then C18 chains we see a drop off in activity, similar to what we have observed for other compounds. The acetamide series is shown to have the lowest activity across the entire acetylated series of compounds. However, we propose that this is due to the extremely poor solubility witnessed with these compounds, and potentially the presence of a HBD within this series prevents interaction with the serine residue.

2.1.5 Trifluoromethyl ether series

2.1.5.1 Design rationale

In the last decade, the number of pharmaceutically relevant compounds containing a trifluoromethoxy group has risen dramatically.[99] The addition of a trifluoromethyl groups serves to increase the lipophilicity of a compound, and therefore increase the ability of a compound to penetrate the cell membrane, and also affords an increase in the metabolic stability. Interestingly, trifluoromethoxy groups can exist as a bonding/ non-bonding structure, which can be formally expressed by the superposition of a covalent and an ionic limiting structure, Figure 50.

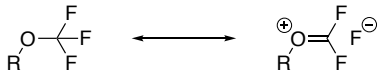
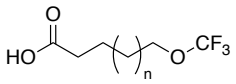


Figure 50: Neutral and ionic representations of trifluoromethoxy group.

We proposed that the trifluoromethyl ethers and the serine residue in DHHC7 could demonstrate an increased hydrogen bonding interaction, compared to previous derivatives. Therefore, a series of compounds containing this important functional group were targeted, Figure 51.

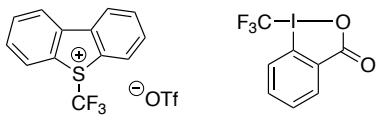


n = 8 **120**
n = 10 **121**
n = 12 **122**
n = 14 **123**

Figure 51: Proposed trifluoromethyl ether series.

2.1.5.2 Synthetic route

Although several methods for the generation of aromatic trifluoromethyl ethers exist, methodologies for the general and efficient synthesis of alkyl trifluoromethyl ethers are extremely underdeveloped and limited. However, Umemoto[100] and Togni[101] reported that direct trifluoromethylation of alcohols was possible using electrophilic trifluoromethylating agents, Figure 52.

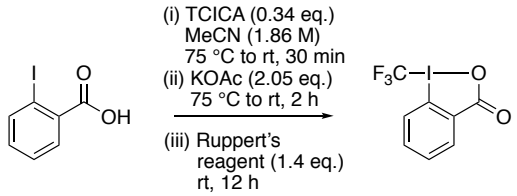


124 **125**

Figure 52: Umemoto's reagent 124 and Togni's reagent 125.

While Umemoto's reagent provides a conceptually important approach, this method requires extremely low temperatures and the majority of reagents need to be generated *in situ* by photochemical decomposition. Togni's milder methodology afforded a more convenient approach, and Togni's reagent was synthesised using a convenient one-pot procedure, as shown in Scheme 14.[102]

Scheme 14: Synthesis of Togni's Reagent 125.

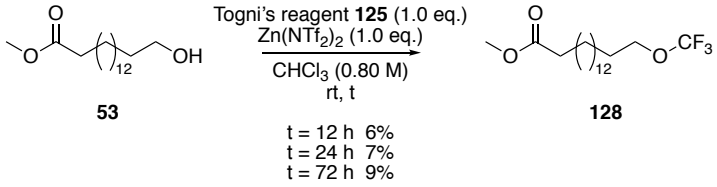


127 **125**
65%

Starting from the commercially available iodobenzoic acid **127**, Togni's reagent **125** was generated following convenient one-pot procedure. This was achieved using trichloroisocyanuric acid (TCICA), potassium acetate (KOAc) and Ruppert's reagent, and the final compound **125** was afforded in a 65% yield, after filtration through celite® and recrystallization at -20 °C in MeCN.

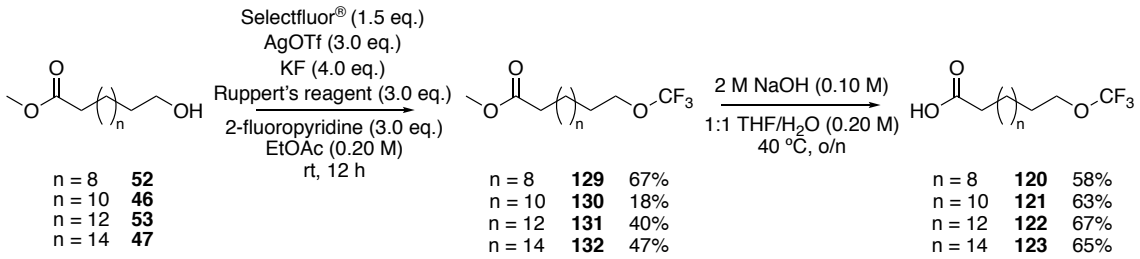
Togni's trifluoromethylation[101] procedure was then attempted using the C16 ester alcohol **53**, Scheme 15.

Scheme 15: Togni's reagent as trifluoromethylating agent.



Reaction of the previously synthesised **53** with Togni's reagent[102] **125** and Zinc di[*bis*(trifluoromethylsulfonyl)imide] ($Zn(NTf_2)_2$) at room temperature for 12 hours, afforded the trifluoromethylated alcohol **128** in a 6% yield following purification by flash column chromatography. The low yield of this step was attributed to incomplete reaction. Unfortunately, even after 72 hours the reaction was not complete and no marked improvement in the yield was observed. An alternative method using a silver-mediated oxidative approach was therefore investigated,[103] Scheme 16.

Scheme 16: Synthetic route for trifluoromethyl ether series.



The reaction of ester alcohols **52**, **46**, **53** and **47** with silver triflate (AgOTf), Ruppert's reagent as the nucleophilic source of trifluoromethyl, Selectfluor® as

the oxidant and 2-fluoropyridine as the ligand afforded the desired trifluoromethoxy species **129–132** in moderate to good yields (18–67%), following purification by flash column chromatography. **129–132** were then converted into final compounds **120–123** *via* NaOH hydrolysis. No further purification was required after hydrolysis and good yields, 58–67%, of the fatty acid derivatives were obtained.

2.1.5.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 is used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7. The results are displayed in Figure 49.
The results are illustrated in Figure 53.

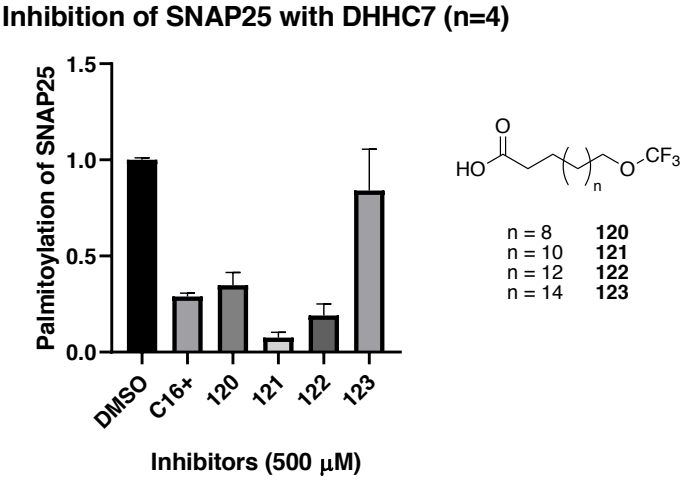


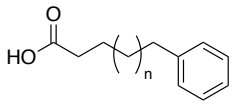
Figure 53: Graph of single point assay results for trifluoromethyl series.

We were incredibly pleased to see a ~90% inhibition with the 14-carbon trifluoromethyl ether ($C14OCF_3$) and ~80% inhibition with the $C16OCF_3$. It is possible this high level of inhibition is due to a favourable hydrogen-bonding interaction between the CF_3 group and serine-182. Although, the increased lipophilicity of this series of compounds may also aid in their ability to access the hydrophobic tunnel of DHHC7. It is interesting to note that once again we observed a drop off in inhibition with the $C12OCF_3$ and then negligible inhibition with the $C18OCF_3$. These results may confirm that a 14-carbon chain is the ideal chain length for activity with DHHC7, and interaction with serine-182.

2.1.6 Phenyl series

2.1.6.1 Design rationale

During the course of our investigations, the largest group we have appended onto our fatty acid probes had been an acetate moeity. To further interrogate the steric limitations of DHHC3 and DHHC7, we proposed the design of a series of compounds affixed with a phenyl ring, Figure 54.



n = 8 **133**
n = 10 **134**
n = 12 **135**
n = 14 **136**

Figure 54: Proposed phenyl series.

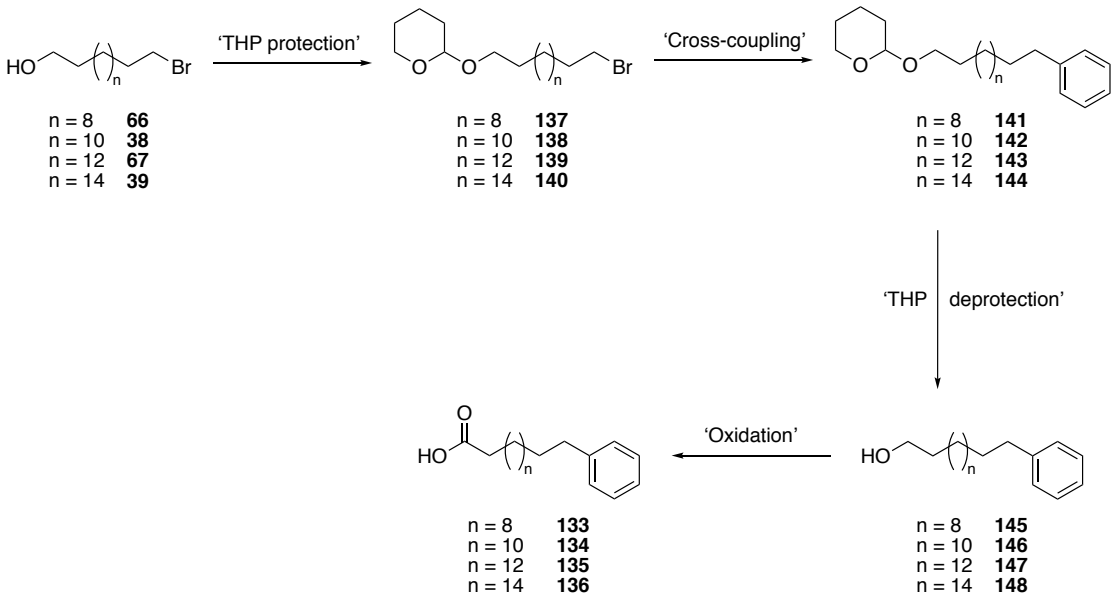
If tolerated by the DHHC's, further functionalisation of these aromatic rings could expand our chemical toolbox dramatically, and allow us to pick up additional interactions within the hydrophobic tunnel.

2.1.6.2 Synthetic route

To install the phenyl group, it was proposed to start from an alkyl bromide intermediate, as these had proven to be chemically tractable intermediates. Despite the inherent difficulties associated with metal-catalysed cross-coupling reactions with alkyl halides, this served as the best starting point for our synthetic route. In 2005, Frisch and Beller compiled a mini review highlighting the various methods available to perform these types of reactions, and to encourage chemists to exploit the potential of these approaches.[104]

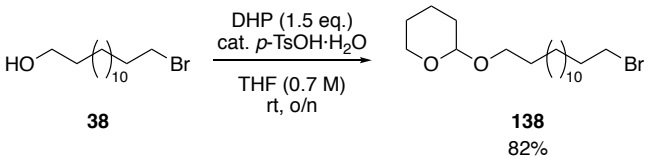
For all previously synthesised series, methyl ester bromides **90**, **42**, **91** and **43** have been the common alkyl bromide intermediates used, however, as the majority of cross-coupling methods utilise Grignard reagents as the phenyl source, it was decided that an alternative intermediate was required. Due to previous difficulties with *tert*-butyl ester protection, it was thought that a tetrahydropyran (THP) protected alcohol would better serve our purpose. The proposed synthetic route is illustrated in Scheme 17.

Scheme 17: Proposed synthetic route towards phenyl series.



As an initial starting point, only the C14 bromo-alcohol **38** was THP protected for use in the subsequent cross-coupling reaction. THP protection was carried out using 3,4-dihydro-2*H*-pyran (DHP) and catalytic *p*-toluenesulfonic acid (*p*TsOH) to afford **138** in an 82% yield following purification, Scheme 18.[105]

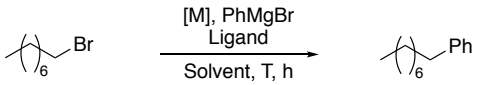
Scheme 18: THP protection of C14 bromo-alcohol 38.



38 **138** 82%

Several cross-coupling methods, detailed in the highlighted review,[104] were initially attempted on a model substrate **149**, Table 3.

Table 3: Tested cross-coupling conditions.

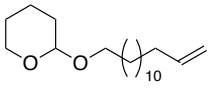


149 **150**

Entry	[M]	Ligand	Solvent	T (°C)	Time (h)	Conversion (%)
1	$Pd(dppf)Cl_2$	-	Et_2O	40	48	0
2	$Pd(OAc)_2$	PCy_3	NMP	25	16	0
3	$Fe(acac)_3$	-	Et_2O	40	0.5	100
4	$Fe(acac)_3$	TMEDA	THF	-78–0	0.5	100

Entry 1, illustrates a method using [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$),[106] refluxing in diethyl ether (Et_2O) for 48 hours, where 0% conversion was observed from the ^1H NMR spectrum of the crude reaction mixture. A palladium acetate ($Pd(OAc)_2$) method using tricyclohexylphosphine (PCy_3) as a ligand was attempted next,[107] Entry 2, however once again only starting material was observed *via* ^1H NMR spectroscopic analysis. Entry 3 details an iron-catalysed approach,[108] using tris(acetylacetonato)iron(III) ($Fe(acac)_3$) refluxing in Et_2O for 30 minutes. Pleasingly, no starting material was observed by ^1H NMR spectroscopy and further purification, by flash column chromatography, afforded **150** in a 42% yield. At the same time, another iron-catalysed method was attempted, Entry 4.[109] This procedure again used $Fe(acac)_3$ as the iron source, but used *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as an additive and was carried out at a much lower temperatures (-78 °C). Entry 4 proved to

be just as effective as Entry 3 and 100% conversion to **150** was achieved, with an isolated yield of 45%. Due to its experimentally more simplistic procedure, Entry 3 was chosen moving forward. However, upon reaction with the actual substrate **138** an impurity was detected in the ^{1}H NMR spectrum of the crude reaction mixture that unfortunately could not be separated from the desired compound **142**. This impurity was determined to be the olefin by-product **151**, formed by the loss of hydrogen bromide, Figure 55.

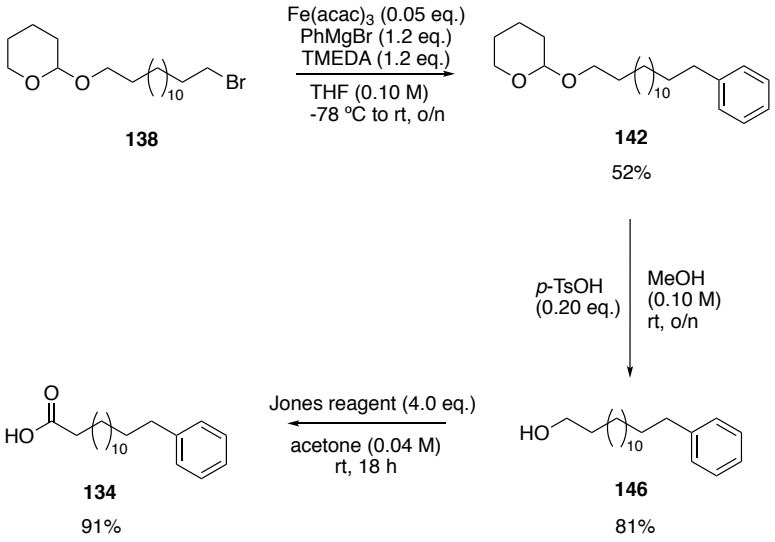


151

Figure 55: Olefin by-product.

The procedure detailed in Entry 4, was then carried out, where TMEDA was proposed to supress the formation of this olefin by-product.[109] Indeed, **142** was isolated in a 52% yield, following reaction at room temperature overnight. No evidence of the impurity **151** was seen by ^{1}H NMR spectroscopy. Scheme 19 illustrates the final synthetic route for the phenyl series.

Scheme 19: Synthetic route for phenyl series.



Deprotection of **142** was achieved using *p*-TsOH in MeOH to provide **146** in an 81% yield. Jones oxidation of **146** afforded the final compound **134** in an excellent 91% yield. No further purification was required.

2.1.6.3 Initial biological evaluation

Each of the compounds synthesised within this sub-project (Elucidating Selectivity) were screened in the cell-based assay described in Section 5.1.1, where SNAP25 is used as the substrate. As the initial objective was to find a compound with reasonable activity (>70%), for use as a chemical probe, the assay was run at a single concentration. All compounds were screened at a concentration of 500 μM, which was determined to be a reasonable concentration for an initial hit compound in a cellular assay. Initial biological evaluation was carried out testing only against DHHC7. Selected compounds then underwent further biological evaluation, using both DHHC7 and DHHC3, see Section 2.1.7. The results are displayed in Figure 49.
The results are shown in Figure 56.

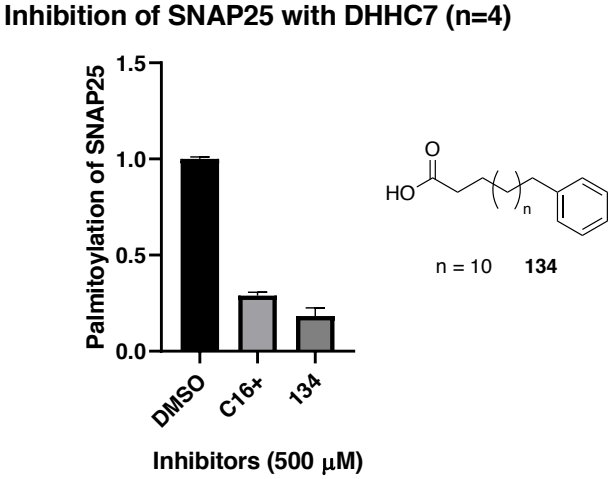


Figure 56: Graph of single point assay results for phenyl series.

We were very surprised to observe such high inhibition (~80%) from this more sterically demanding compound. The increased lipophilicity of this functional group may shed some light on this. Nevertheless, we now have indication that a

higher steric bulk is tolerated by DHHC7, thus widening the possibilities for future compounds and the generation of a selective chemical probe.

2.1.7 Further biological evaluation

From the initial biological evaluation carried out for each series of synthesised compounds, we could draw the conclusion that the 14-carbon chain afforded the consistently highest inhibition of DHHC7. We proposed that this chain length allows each of the functional groups to be in proximity to serine-182 for optimal interaction. We therefore carried out further biological evaluation to solely interrogate the compounds with 14-carbon chain lengths. The results from these studies are illustrated in Figure 57.

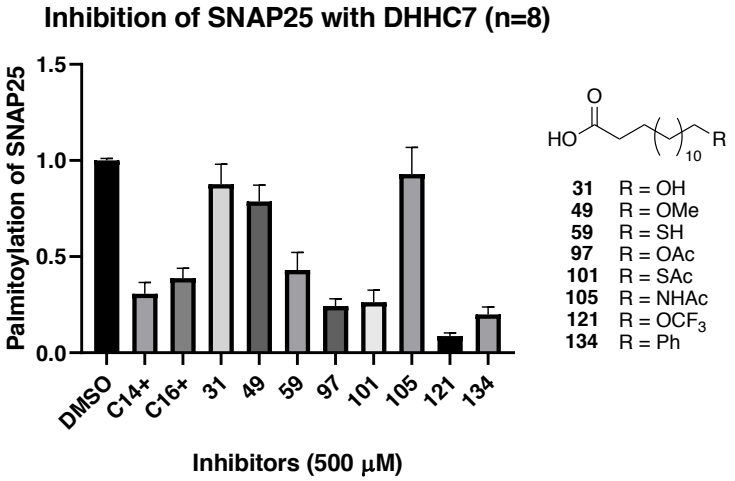


Figure 57: Interrogation of 14-carbon compounds with DHHC7.

At this stage, we also chose to add in another positive control, unlabelled myristic acid (C14+), as a direct comparison to the 14-carbon chain lengths we were investigating. For this positive control we observed ~75%, while for the C16+ control we observed ~70%, which was consistent with previous experiments. These results confirm that the OCF_3 group affords the best inhibitory effect (~90%) across all of the series. While the C14OAc, C14SAc and C14Ph show very similar inhibition, around 75–80%. We also observed a lack of

inhibition for the C14OH/OMe and NHAc compounds, which was consistent with previous results.

As we now had several compounds showing inhibition of palmitoylation of DHHC7, we wanted to evaluate whether these compounds were inhibiting due to a specific interaction with serine-182. We therefore carried out biological evaluation of our compounds within DHHC3. These results are illustrated in Figure 58.

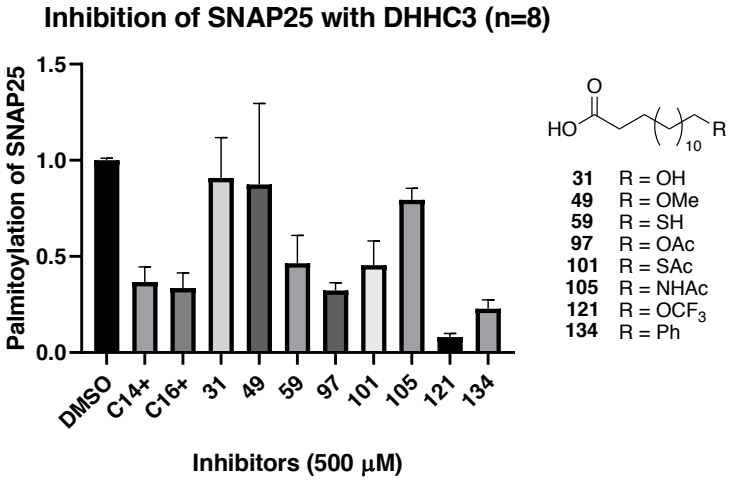
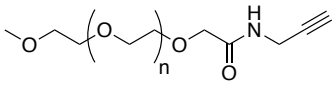


Figure 58: Interrogation of 14-carbon compounds with DHHC3.

From these results we can see that C14OCF_3 and C14Ph also inhibit palmitoylation of DHHC3 to an extent comparable with DHHC7, ~90% and 80% respectively. Unfortunately this indicates that we do not have selectivity between DHHC7 and DHHC3, suggesting that these compounds are not specifically interacting with serine-182. However, both compounds do inhibit palmitoylation to a greater extent than the parent acids, myristic and palmitic acid.

During the time frame of these experiments a new technique was developed within the Chamberlain lab, to interrogate palmitoylation of SNAP25 by various DHHC enzymes. SNAP25 has 4 cysteines that are known to be palmitoylated, and by using a pegylated alkyne **152**, Figure 59, the number of palmitoylated

cysteines can be determined, *via* separation by gel electrophoresis. This technique is more sensitive than the cell-based assay currently in use and therefore may indicate more discreet selectivity.



152

Figure 59: mPEG-alkyne.

This technique involves the simple modification of the current cell-based assay in use, where a pegylated alkyne is used in place of the usual alkyne dye. The alkyne dye is used for the quantification of the percentage of palmitoylation of SNAP25, while the pegylated alkyne is used to quantify the number of palmitoylated cysteines within SNAP25. All other aspects of this assay are consistent with the cell-based assay described in Section 5.1.1, although in this case the LI-COR® cannot be used to quantify the signal as there is no signal from the pegylation click. Instead, the LI-COR® is simply used to obtain an image of the western blot, from which we can see the number of palmitoylated cysteines. The results from this experiment with DHHC7 are illustrated by the western blot shown in Figure 60.

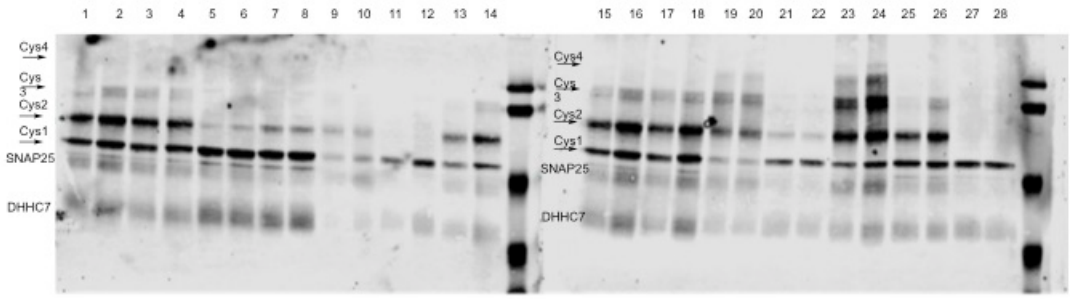


Figure 60: DHHC7 pegylation experiments.

Annotated on the western blot we can see the band representing DHHC7 and we can see SNAP25, along with the 4 cysteine residues that can undergo palmitoylation. Channels 1–4 and 15–18 represent the DMSO negative control, where 3 out of the 4 cysteines have been palmitoylated. Channels 5–6 and 7–8

show positive controls, myristic acid and palmitic acid, respectively. Here we can see that 1 cysteine has been palmitoylated to a high extent and the 2nd has been partially palmitoylated. Interestingly, the C14SH and C14SAc compounds (channels 9–10 and 11–12, respectively) show a decrease in the amount of both DHHC7 and SNAP25, suggesting that they are toxic in some manner. We can see clearly that C14OCF_3 (channels 21–22) is inhibiting the palmitoylation of SNAP25; the results show slight palmitoylation of the 2nd cysteine and reduced palmitoylation of the 1st cysteine, relative to the control channels (1–4). Channels 27–28 represent C14Ph, which appears to be inhibiting palmitoylation of the 2nd cysteine fully. These experiments suggest that C14Ph is a superior inhibitor, of palmitoylation with DHHC7, compared to C14OCF_3, which is no consistent to the inhibition figures we have gathered with the cell-based assay. This could suggest that the margin of error for the cell-based assay is higher than anticipated, and the degree of difference between 80–90% inhibition is much smaller than we initially expected. However, in general these two experimental techniques support each other nicely.

We repeated this experiment using DHHC3 to further interrogate this anomaly. The results are shown in Figure 61.

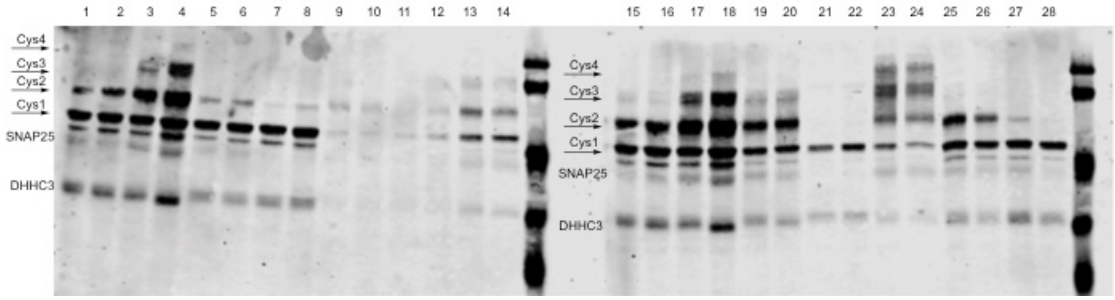


Figure 61: DHHC3 pegylation experiments.

We can observe the levels of DHHC3 and SNAP25, along with the 4 cysteine residues that can undergo palmitoylation. Channels 1–4 and 15–18 represent the DMSO negative control, which indicates the maximum level of palmitoylation observed within this assay, where 3 out of the 4 cysteines are palmitoylated to different extents. Positive controls, myristic and palmitic acid

are represented in channels 5–6 and 7–8 respectively. Here we observe a reduction in the level of palmitoylation, where only the 1st cysteine is fully palmitoylated and we observe only partial palmitoylation of the 2nd. Channels 9–10 and 11–12 represent the 14-carbon SH and SAc compounds respectively, where almost complete inhibition of palmitoylation is observed. However, extremely low levels of DHHC3 and SNAP25 are also observed, suggesting that there may be toxicity associated with these compounds. From these results, we can also observe that C14OCF_3 is inhibiting palmitoylation of the 2nd cysteine fully (channels 21–22), in contrast to the partial inhibition observed for DHHC7. Whereas, C14Ph is inhibiting palmitoylation to a lesser extent (channels 27–28), in comparison with DHHC7, as there is partial palmitoylation of the 2nd cysteine here with DHHC3.

These results are incredibly interesting and suggest that there may be discreet selectivity between the two DHHC enzymes that was not observed in the original cell-based assay results. The sensitivity of this technique has allowed us to observe palmitoylation down to individual cysteine residues, which was not previously possible. Our original technique measured and compared the inhibition of palmitoylation as a percentage of palmitoylation of SNAP25, and the results for DHHC3 and DHHC7 were within error of one another, therefore compounds **121** and **134** were deemed non-selective. However, this new technique suggests that inhibition is more complex, and some compounds may inhibit the palmitoylation of more cysteines than others, creating more discreet selectivity profiles. This higher sensitivity technique may allow us to more finely tune the selectivity of future generations of compounds.

Following on from these results, we decided to carry out dose response experiments on C14OCF_3 **121** and C14Ph **134**. Each compound was tested at 500, 250, 100 and 50 μM using DHHC7 and DHHC3. The results for DHHC7 are illustrated in Figure 62.

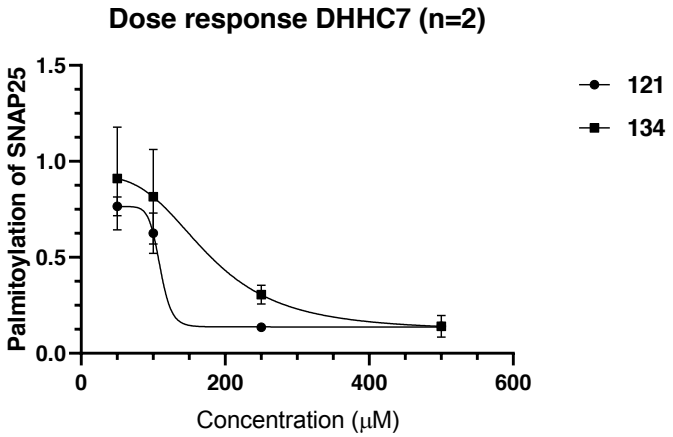


Figure 62: Dose response experiments for DHHC7.

From this curve we can see that there is a negligible difference in inhibition between each compound at 500 μM, however at 250 μM, C14OCF$_3$ **121** is still inhibiting at ~90%, whilst there is a drop off in inhibition for C14Ph **134**, from ~90% to ~75%. Using the results from the pegylation experiments, we may have expected C14Ph to perform better across the concentration range, however these experiments have only been carried out for an n of 2 across 4 concentrations. More data points and repeats would be necessary in order to confirm these results.

The results from the dose response experiments performed using DHHC3 are shown in Figure 63.

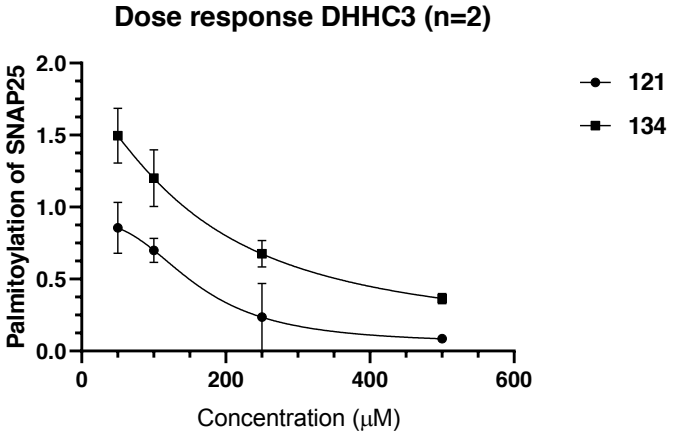


Figure 63: Dose response experiments for DHHC3.

We can observe a marked difference in inhibition for each compound at each concentration tested; C14OCF$_3$ **121** is a superior inhibitor to C14Ph **134** across the entire concentration range. These results are consistent with the results obtained from the pegylation experiments with DHHC3. However, as for the dose response experiments for DHHC7, these have only been carried out for an n of 2 across 4 concentrations. Therefore, more data points and repeats would be necessary to confirm these findings.

The results obtained, across all experiments performed, indicate that C14OCF$_3$ **121** is a non-selective inhibitor of DHHC3 and DHHC7. As these enzymes are highly conserved at the amino acid level this is not overly surprising. We therefore decided to test C14OCF$_3$ **121** (at 500 μM) across several other DHHCs known to palmitoylate SNAP25: DHHC2, DHHC15 and DHHC17. The results from this experiment are illustrated in Figure 64.

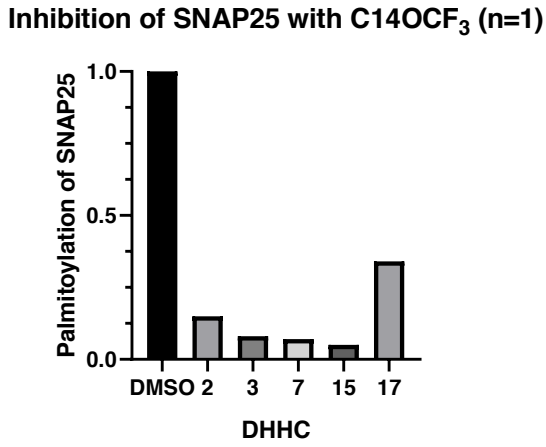


Figure 64: Interrogation of DHHCs 2,3,7,15 and 17.

From this graph, we can determine that C14OCF$_3$ **121** inhibits the palmitoylation of SNAP25 across DHHCs 2, 3, 7 and 15, at 80%, 85%, 87% and 90% respectively. Although it is important to note that expression of DHHC15 is lower compared to the other enzymes. Interestingly, inhibition of DHHC17 is much lower (65%).

Table 4: DHHC 2/3/7/15/17 and amino acids at 182 position.

DHHC	Residue at 182 position
2	Leucine
3	Isoleucine
7	Serine
15	Leucine
17	Phenylalanine

If we compare the amino acid sequences of the 3rd transmembrane domain of each enzyme, Table 4, they reveal some stimulating differences. DHHC2 and DHHC15 contain leucine residues at a similar position to the isoleucine and serine residues observed for DHHC3 and DHHC7 respectively. We propose that these residues are all small enough in size to permit C14OCF$_3$ into the channel, and allow an interaction to take place. However, DHHC17 contains a phenylalanine residue at the same position, suggesting that a steric encumbrance could prevent inhibition of palmitoylation to the same level as that observed for the other enzymes. These findings suggest that we may have a 'semi-pan' inhibitor of palmitoylation, where the extent of inhibition will be determined by the steric bulk within the channel adjacent to the DHHC domain of the protein.

Although we had initially proposed the generation of a fully selective inhibitor for DHHC7, the discovery of any reversible inhibitor of palmitoylation is still incredibly promising. Considering that the current first in class probe, 2-bromopalmitate, is toxic to cells, an irreversible inhibitor and completely non-selective for any cysteine residues, we believe that this chemical probe is a superior tool with which to study palmitoylation.

It is of obvious importance to confirm the results observed within the dose response experiments, before continuing on with further biological evaluation of these compounds. A larger concentration range should be used in order to tease out the selectivity profiles initially observed and generate more accurate profiles. It would also be important to repeat the experiments shown in Figure

64, and also repeat this entire experiment using C14 Ph **134**. It would be of particular interest to observe the inhibition of palmitoylation of SNAP25 using DHHC17 with **134**. The steric bulk of the phenyl ring compared with the OCF_3 group should theoretically prevent any inhibition by **134**, and we could expect full palmitoylation to be observed. It would also be interesting to test the entire chain length range (12–18) of both compounds **121** and **134** within this experiment. We have proposed that the decrease in inhibition of **121** when using DHHC17 is due to the more sterically encumbering transmembrane domain 3, which contains a phenylalanine residue at the 182 position. Reduction in the chain length from 14 to 12 may cause an increase in inhibition, while an increase to a 16 or 18 carbon chain would be expected to decrease the inhibition of palmitoylation of SNAP25 more dramatically, which would aid in the confirmation of our hypothesis.

From a synthesis perspective, it would be important to generate the remaining phenyl chain lengths (12, 16 and 18) and test these within all biological methods used above, in order to fully complete our analysis.

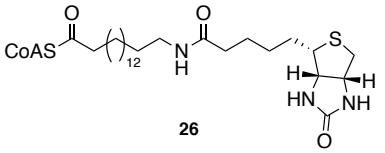
2.2 Elucidating substrates for the DHHC superfamily

Although the number of substrates that have been identified for the DHHC superfamily is continuously growing, thanks to pioneering work carried out using various techniques, such as ABE and acyl-RAC (see Sections 1.4.2 and 1.4.3), there remains a fundamental need for chemical tools to further this progress.

2.2.1 Design rationale

As fatty acids are actually converted into their CoA counterparts before incorporation into the DHHC enzymes, we proposed that a starting point to developing a chemical tool could involve mimicking this CoA head group. Therefore they could also be utilised in a non-cell-based environment and the

development of an *in vitro* assay. To serve as the recognition element, we chose a biotin moiety due to its utility in pull-down assays, which have been used extensively in the determination of substrates for various targets. The proposed chemical tool is illustrated in Figure 65.



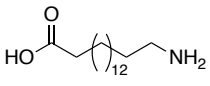
26

Figure 65: CoA-biotin chemical tool.

Within the Chamberlain group, development of an *in vitro* assay for DHHC2 is underway. A C12 CoA-biotin construct, similar to **26**, has been used within this assay to provide an easy means of recognition, by way of a neutravidin blot. Although this compound has been useful as a chemical tool, it would be more desirable to have a probe more closely related to the natural substrate, as in **26**, which contains a C16 construct.

2.2.2 Synthetic route 1

It was envisaged that the CoA head group would be added in the final step of the synthesis, through direct addition to a carboxylic acid derivative. The highly polar nature of this head group would make it incredibly challenging to carry through additional steps, as purification would be extremely difficult. Installation of the biotin moiety was proposed to take place through the addition of a biotin succinimide ester to an amine group in a 16-carbon linker molecule. Fortunately this acid-amine intermediate **118**, Figure 66, had already been synthesised for use in the generation of the acetamide series, as shown in Scheme 11 on page 56.



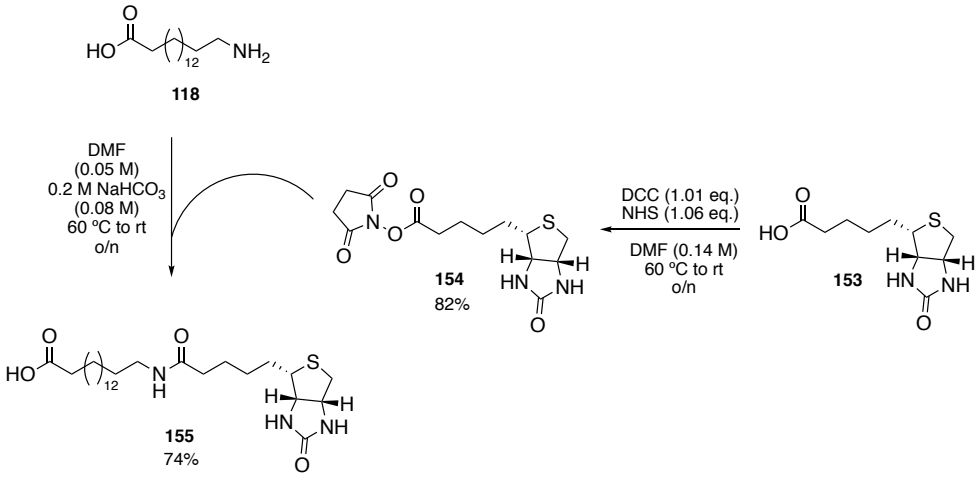
118

Figure 66: C16 acid-amine intermediate 118.

Intermediate **118** was generated in a 40% yield starting from the commercially available diacid, following a reduction, mono-bromination, oxidation, esterification, azide addition, azide reduction, deprotection sequence.

The synthesised amine intermediate **118**, was then biotinylated as shown in Scheme 20.

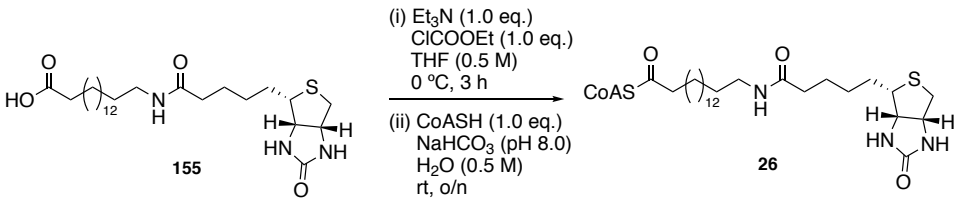
Scheme 20: Synthesis of biotin intermediate 155.



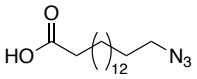
Initially, commercially available biotin **153** was converted into the *N*-hydroxysuccinimide (NHS) ester **154** using dicyclohexylcarbodiimide (DCC) in DMF.[110] Following trituration in hexane, **154** was isolated in an 82% yield. Intermediate **118** was then biotinylated using freshly prepared biotin-NHS **154** and sodium hydrogen carbonate ($NaHCO_3$) solution, to afford **155** in a 74% yield.[111]

The final step in the synthesis of this valuable tool compound **26** was the formation of the CoA thioester, Scheme 21.

Scheme 21: Synthesis of CoA thioester 26.



Initially, a procedure where the carboxylic acid precursor **155** is converted to the mixed anhydride *in situ* was examined.[112] However, due to the valuable nature of **155** it was determined that a test substrate should be used for the initial testing of conditions.

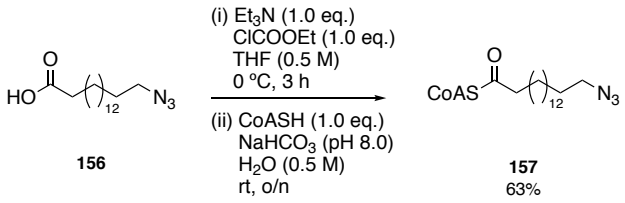


156

Figure 67: Test substrate 156.

Acid-azide **156** was chosen as the test substrate and was treated with Et_3N and ethyl chloroformate (ClCOOEt) to provide the intermediate anhydride *in situ*. Treatment of this intermediate with co-enzyme A (CoASH) in a $NaHCO_3$ solution at pH 8.0, afforded the desired compound **157** in a 63% yield, following lyophilisation of solvent, Scheme 22.

Scheme 22: CoA ester formation of test substrate 157.

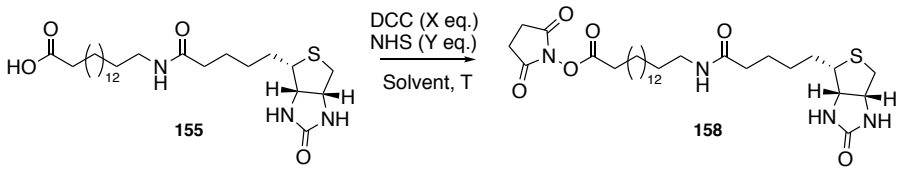


Following the success of these reaction conditions with **156**, they were then attempted on the desired substrate **155**. However, it was unclear from ^1H NMR spectroscopic and LC-MS analysis whether the desired compound **26** had been formed upon reaction. Even if the compound had been formed, LC-MS analysis indicated that a large portion of un-reacted CoA salt was present in the mixture, which would have been almost impossible to separate, although the molecular ion of the product was not observed.

Following the success of the succinimide ester chemistry, Scheme 23, and the ease of isolation and purification, it was proposed that we first convert

intermediate **155** to the succinimide ester **158** before reaction with CoASH, Scheme 23.

Scheme 23: Succinimide ester formation of 158.



This did not proceed as smoothly as expected and various conditions were employed in an attempt to synthesise the desired intermediate **158**, Table 5.

Table 5: Conditions for succinimide ester formation.

Entry	DCC (X eq.)	NHS (Y eq.)	Solvent (M)	T (°C)	Conversion (%)*
1	1.01	1.06	DMF (0.14)	60	0
2	1.1	1.2	DMF (0.14)	60	0
3	1.1	1.2	DMSO (0.14)	60	0
4	1.01	1.06	DMF (0.09)	110	0
5	1.01	1.06	DMSO (0.14)	85	0
6	1.01	1.06	THF/ DMSO (0.04)	60	0
7	1.01‡	1.06	DMSO (0.14)	85	0

*Determined by ^{1}H NMR spectroscopy. ‡ EDC was used instead of DCC.

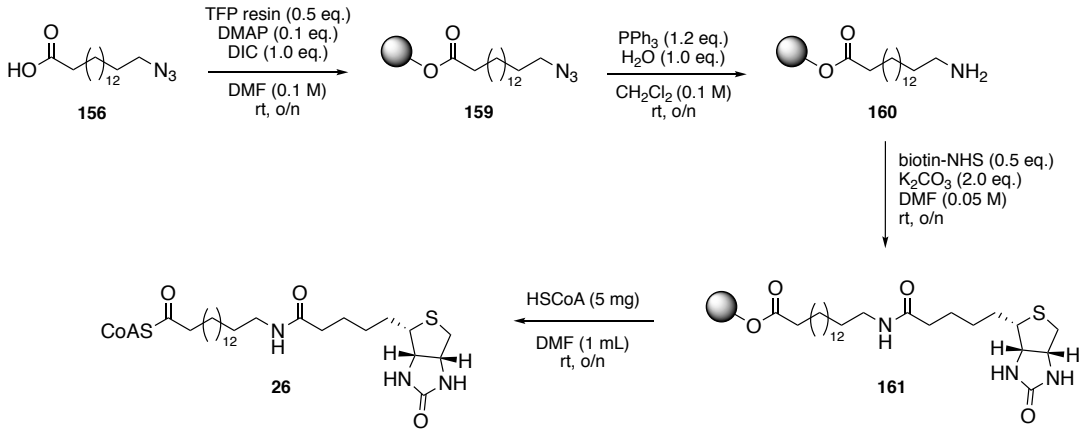
Entry 1 used the original conditions for succinimide ester formation; however, only starting material was isolated after 24 hours, as determined by ^{1}H NMR spectroscopy. It was proposed that an increase in the number of DCC and NHS equivalents used, Entry 2, might afford conversion to the desired product, however 0% conversion was still observed. Poor solubility was observed for reactions in DMF, therefore another attempt, Entry 3, was made in DMSO, using 1.1 equivalents of DCC and 1.2 equivalents of NHS. Once again, this failed to provide any conversion to product. It was postulated that an increase in temperature may improve the solubility of the substrate in DMF, and therefore the temperature was increased from 60 °C to 110 °C, Entry 4. Within this

experiment the dilution was also increase from 0.14 M to 0.09 M to facilitate solubilisation. These changes proved ineffective, and no conversion to **158** was observed. Increasing the temperature in DMSO, Entry 5, and the addition of THF as a co-solvent, Entry 6, also proved unsuccessful. A final attempt was made, Entry 7, where *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) was used as the coupling agent instead of DCC, however, this also failed to afford any of the desired product. After numerous attempts, it was decided that intermediate **155** was too insoluble and this synthetic route was abandoned.

2.2.3 Synthetic route 2

In order to circumvent the solubility issues associated with the biotin intermediate **155** a solid-support strategy was proposed, Scheme 24.

Scheme 24: Solid-supported strategy for CoA-biotin 26.



It was decided that azide intermediate **156** could be loaded onto a polymeric 4-hydroxy-2,3,5,6-tetrafluorobenzamido (TFP) resin.[113] The percentage loading was calculated to be 85%[§] and the presence of an azide was confirmed by IR analysis. The polymer-supported azide **159** was then reduced using the standard Staudinger conditions to provide amine **160**. The disappearance of the azide and appearance of an amine were confirmed using IR spectroscopy. The solid-supported amine **160** was then biotinylated **161**, using biotin-NHS and

[§] Cleavage of **157** from 100 mg of resin allowed calculation of percentage loading.

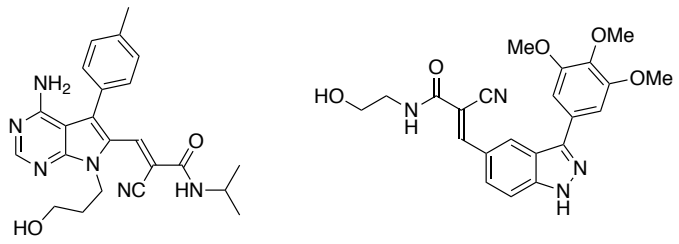
K_2CO_3, a slight modification to the original conditions used, which was confirmed by the appearance of C=O stretching absorptions attributed to the amide and urea carbonyls in the IR spectrum. Cleavage of the biotinylated intermediate **161** with CoASH was then performed in an attempt to generate the desired final compound **26**. Although the mass recovery from the reaction supported cleavage from the TFP resin by CoASH, ^{1}H NMR spectroscopic analysis was inconclusive. The majority of the peaks from the Co-enzyme A portion of the molecule were present, as confirmed by overlaying the ^{1}H NMR spectrum of Co-enzyme A with the ^{1}H NMR spectrum of the recovered solid. While this could suggest that unreacted Co-enzyme A had simply been recovered from the reaction mixture, the mass recovered was higher than that of the CoA added to the reaction (11 mg recovered versus 5 mg added) and there were several additional peaks present in the ^{1}H NMR spectra. However, while some of these additional peaks could potentially be attributed to the remaining fatty acid biotin portion of the molecule, there were too many missing peaks to confirm the presence of the desired substrate **26**. Due to the insolubility of this compound, however, a significant portion of the compound tended to precipitate from solution and therefore this could account for the missing peaks. It was determined that mass spectrometry could help elucidate the presence of **26**, however, standard LC-MS analysis was also inconclusive. A major mass peak was attributed to the CoA salt, 785.13, however this could have been from the starting material or it could have been cleaved from the desired substrate during analysis. MALDI-TOF analysis was then used in an attempt to observe the desired mass of 1265.05, however once again only the mass from the CoA salt was observed, alongside several other masses that could not be attributed to **26** or any of the reaction additives.

2.3 Inhibition

2.3.1 α,β-Unsaturated series

2.3.1.1 Design rationale

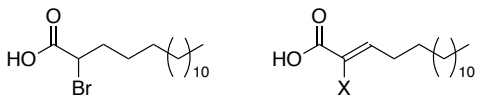
2-Bromopalmitate **13** is a known inhibitor of palmitoylation, however it has been shown to be toxic to cells and highly promiscuous in nature. Therefore chemical tools with more discreet reactivity are required to probe the inhibition of the DHHC superfamily. One of the issues surrounding 2BP **13** is its irreversible binding mode. It was proposed that modification of the structure of **13** to include unsaturation could circumvent this problem. Indeed, two kinase inhibitors **162** and **163** have been shown in the literature to be reversible covalent inhibitors that selectively target the non-catalytic cysteine p90 ribosomal protein S6 kinase RSK2, Figure 68.[114] The reversible nature of these compounds has been attributed to the presence of their common α,β-unsaturated moiety.



162 **163**

Figure 68: Reversible covalent kinase inhibitors.

An α,β-unsaturated series of compounds **27–29,** based upon the structure of 2-bromopalmitate **13**, has therefore been proposed, Figure 69.



13

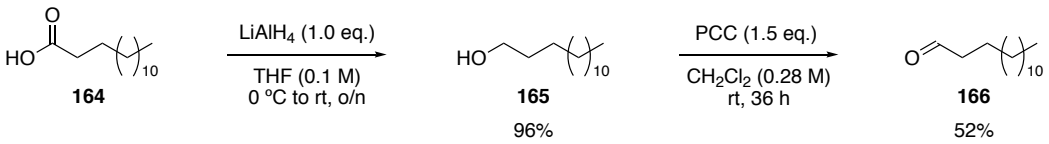
X = H **27**
X = Br **28**
X = Cl **29**

Figure 69: 2-Bromopalmitate 13 and proposed α,β-unsaturated compounds 27–29.

2.3.1.2 Synthetic route

It was proposed that a Horner-Wadsworth-Emmons reaction (HWE) could be used to install the key α,β-unsaturated component into this series of compounds.[115] An aldehyde precursor was therefore necessary for this transformation and the synthetic route towards this crucial functional group is detailed in Scheme 25

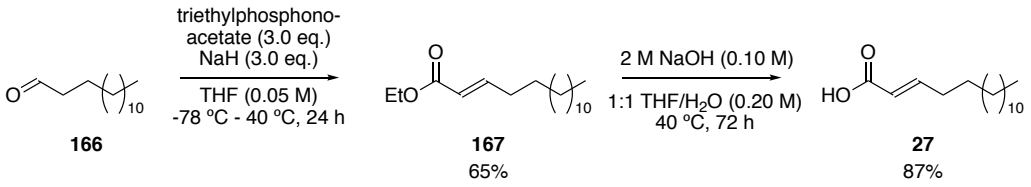
Scheme 25: Synthesis of C14 aldehyde 166.



Treatment of the commercially available myristic acid **164**, with $LiAlH_4$ afforded the C14 alcohol **165** in a 96% yield with no need for further purification. Compound **165** was then oxidised to the corresponding aldehyde **166** using pyridinium chlorochromate (PCC),[116] which was isolated in a 52% yield following purification by flash column chromatography.

The non-halogenated α,β-unstaurated carbonyl compound **27** was then prepared in 2-steps, as shown in Scheme 26.

Scheme 26: Synthesis of compound 27.

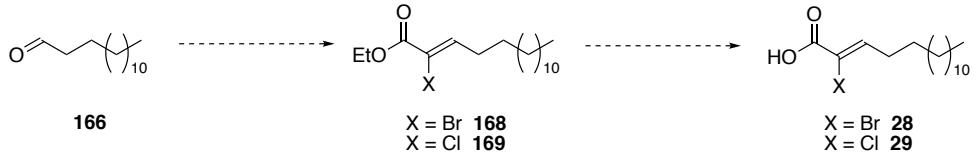


Aldehyde intermediate **166** was treated with triethylphosphonoacetate and sodium hydride (NaH) to provide **167** in a 65% yield following purification. Ester **167** was then hydrolysed under basic conditions to afford **27** in an 87%yield, with no need for further purification.

Although the conditions illustrated for the HWE reaction may appear extreme, due to difficulties separating the starting material from the product, the reaction had to proceed to completion. Following a brief optimisation it was determined that 3.0 equivalents of both the triethylphosphonoacetate and base were required and the temperature had to be raised to a minimum of 40 °C for 24 hours for the reaction to reach completion.

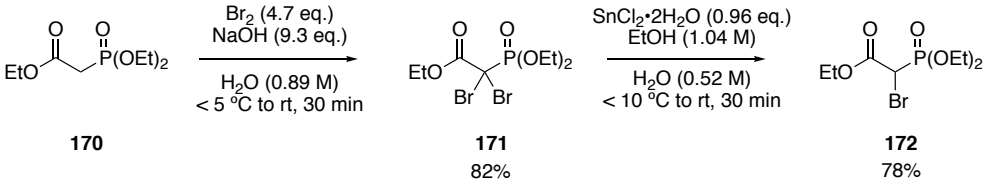
Following the successful preparation of **27**, a method to prepare the brominated and chlorinated derivatives, **28** and **29**, was investigated, Scheme 27.

Scheme 27: Proposed synthesis of halogenated-unsaturated-2BP derivatives.



While the halogens could be introduced following the initial HWE reaction, Olpp and Bruckner had previously investigated the preparation of brominated triethylphosphonoacetate derivatives and their subsequent use in HWE reactions.[117] Their described methodology would allow us to introduce the bromine and alkene in a single step. The method used for the preparation of the desired reagent **172** is outlined in Scheme 28.

Scheme 28: Synthesis of brominated HWE reagents.

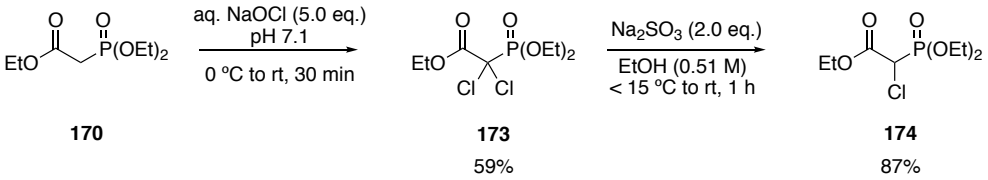


HWE reagent **170** was reacted with bromine under basic conditions to afford the di-brominated HWE reagent **171** in 82% yield. The mono-brominated HWE reagent **172** was isolated in 78% yield following reduction of **171** using tin(II) chloride dehydrate ($SnCl_2 \cdot 2H_2O$). Both steps detailed in Scheme 28 were temperature sensitive and therefore the temperature had to be strictly

controlled to within a margin of <5 °C. Compound **172** was used without further purification in subsequent HWE reactions.[118] Compounds **171** and **172** could be stored at room temperature for several months with no sign of decomposition by ^{1}H NMR spectroscopy.

The chlorinated HWE reagents were synthesised in a similar manner, as shown in Scheme 29.

Scheme 29: Synthesis of chlorinated HWE reagents.



HWE reagent **170** was reacted with sodium hypochlorite solution, adjusted to pH 7.1, to afford the di-chloro product **173** in 59% yield. Treatment of **173** with sodium sulfite provided the mono-chlorinated HWE reagent **174**, in 87% yield.[119] Both **173** and **174** were used without further purification and could be stored at room temperature for a number of months without decomposition.

Although the brominated and chlorinated HWE reagents **172** and **174** were successfully prepared, the subsequent HWE reactions failed. A number of attempts were made using the brominated HWE reagent **172**, as shown in Table 6.

Table 6: Conditions for reaction with brominated HWE reagent 172.

Entry	Triethylphosphonoacetate (eq.)	NaH (eq.)	T (°C)	Time (h)	Product 168*
1	3	3	40	24	✗
2	3	3	40	48	✗
3	3	3	40	72	✗
4	5	5	60	72	✗

* As determined by ^{1}H NMR spectroscopy.

Entry 1 shows the initial attempt made using the conditions employed for the standard HWE, shown in Scheme 26, however the ^{1}H NMR spectrum showed

only starting material **166** after the 24 hour reaction period. Two further attempts, Entries 2 and 3, were made with extended reaction times of 48 and 72 hours respectively. Once again, analysis of the ^{1}H NMR spectra showed only starting material. A final attempt was made, Entry 4, where the equivalents of triethylphosphonoacetate and NaH were increased to 5, the temperature to 60 °C and the reaction time extended to 72 hours. However, even under these much harsher reaction conditions, no product **28** was observed in the ^{1}H NMR spectra and only starting material **166** was recovered. No further attempts were made. Due to the difficulties experienced during the synthesis of **27**, when using the standard HWE conditions, this is perhaps unsurprising. Thus far, synthesis of compounds **28** and **29** has not yet been completed.

2.3.1.3 Biological evaluation

Compounds **167** and **27** were evaluated using the *in vitro* assay described in Section 5.1.2, which uses DHHC2 and SNAP25. The results are illustrated in Figure 70.

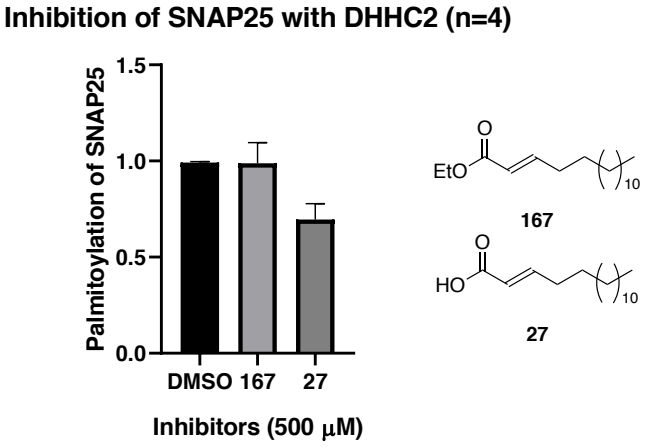


Figure 70: Graph of inhibition of palmitoylation with α,β-unsaturated series.

These results show that compound **167** has no effect on the inhibition of palmitoylation. This is not surprising as a carboxylic acid head group is required for thioester formation by the catalytic cysteine of DHHC2. Compound **27**

showed moderate inhibition, ~30%, suggesting that unsaturation alone is not enough for a compound to out-compete the natural substrate. As we were not able to synthesise the brominated and chlorinated derivatives, no further examination of this series of compounds was carried out. However, several compounds in the literature have been shown to inhibit cysteine residues to a high extent when appended with electron withdrawing groups (EWGs), such as nitriles, as shown in Figure 68. It would therefore be of interest to generate a further series of compounds containing EWGs in order to examine this, and potentially increase inhibition of palmitoylation.

2.3.2 Sulfonamide series

2.3.2.1 Design rationale

As detailed in Section 1.3.2, the mechanism of S-acylation is proposed to proceed through a tetrahedral intermediate, which is stabilised by the catalytic residues of the DHHC enzyme. We hypothesised that compounds with tetrahedral head groups, Figure 71, such as sulfonamides, may be recognised by the DHHC catalytic domain and therefore interact accordingly. These compounds are expected to bind non-covalently to the DHHC enzyme, out-competing the natural substrate, palmitic acid CoA. For this series of compounds we chose to mimic the most common natural substrate, palmitic acid. Therefore we elected to synthesise compounds containing a 15-carbon tail, where the sulfonyl head group is a mimic for the standard carboxylic acid, giving an overall 16-unit chain attached to the appropriate amine.

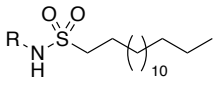
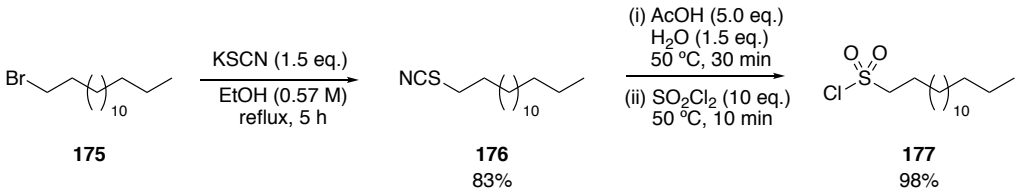


Figure 71: Proposed tetrahedral moiety.

2.3.2.2 Synthetic route

It was proposed that the various sulfonamide compounds could be prepared from a common sulfonyl chloride intermediate **177**, which could be generated from the commercially available 1-bromopentadecane **175** (Scheme 30).

Scheme 30: Synthesis of sulfonyl chloride intermediate 177.

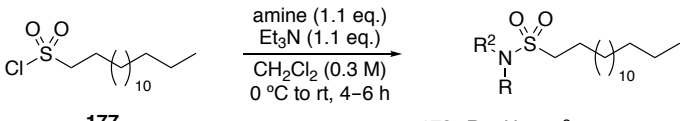


175 **176** 83% **177** 98%

Treatment of **175** with potassium thiocyanate afforded the alkyl thiocyanate **176** in 83% yield. Compound **176** was then stirred in aqueous acetic acid, followed by the addition of sulfuryl chloride to provide the alkyl sulfonyl chloride **177** in an excellent 98% yield, without further purification.[120] This synthetic route provided an excellent means to generate over 5 g of intermediate for use in subsequent reactions. It is important to note, however, that the rate of addition of sulfuryl chloride must be strictly controlled, especially for larger scale reactions. If the rate was too fast the reaction would not go to completion and the starting material **176** could not be separated from the desired product **177**.

Sulfonyl chloride intermediate **177** was then used in a late stage diversification strategy, through reaction with a number of amines, to provide a variety of compounds with a tetrahedral head group moiety. Sulfonamides **178–181** were prepared initially, Scheme 31.

Scheme 31: Sulfonamide forward synthesis.



177

178	R = H	$R^2 = CH_2C_6H_4$-*p*-OCH_3	35%
179	R = H	$R^2 = CH_2C_6H_5$	34%
180	R = CH_3	$R^2 = CH_2C_6H_5$	65%
181	R = H	$R^2 = (CH_2)_2NHC(O)CH_3$	33%

Treatment of **177** with a variety of amines afforded sulfonamides **178–181** in reasonable yields, 33–65%, after purification *via* column chromatography.[121]

Compounds **178** and **179** were synthesised initially to develop the chemistry; both contain proton environments easily distinguishable by ^{1}H NMR spectroscopy. Sulfonamide **180** was prepared to assess whether the sulfonamide nitrogen could be primary, secondary or both. Finally, compound **181** was prepared as this amine motif mimics the initial portion of the CoA group shown in green in Figure 72.

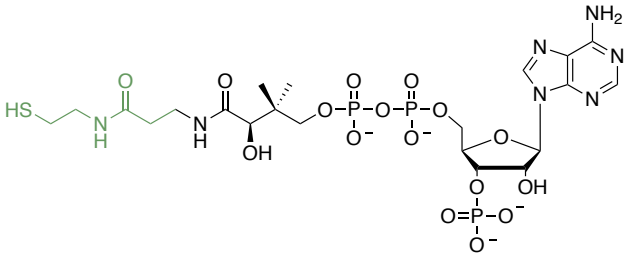
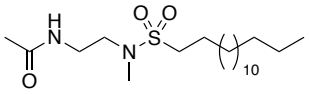


Figure 72: Coenzyme A.

Further to the synthesis of these compounds, it was determined that the sulfonamide amine of **181** should also be methylated, to asses the necessity of a free amine upon binding, leading to **182** as a target molecule, Figure 73.

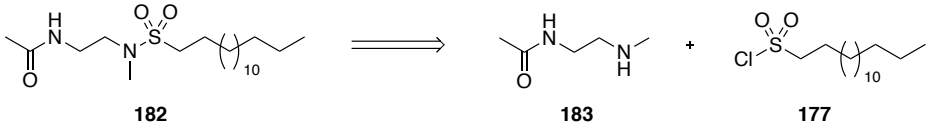


182

Figure 73: Target molecule 182.

It was decided that **182** could be prepared from amine **183** and sulfonyl chloride **177**, Scheme 32.

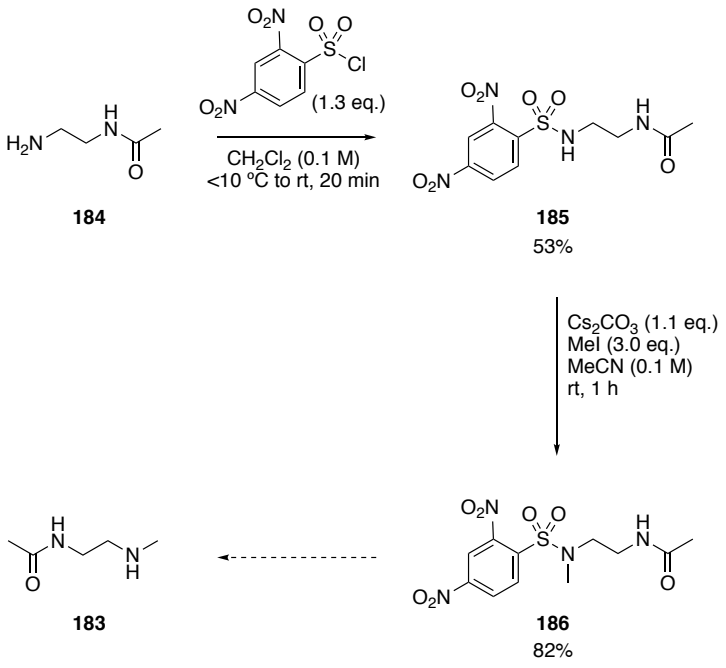
Scheme 32: Sulfonamide 53 retrosynthesis.



182 **183** **177**

Compound **183** was not commercially available and therefore had to be prepared from amine **184**, which had already been used in the preparation of sulfonamide **181**. The following synthesis, detailed in Scheme 33, used chemistry developed by Fukuyama to facilitate mono-methylation of the primary amine **184**.[122]

Scheme 33: Fukuyama synthesis.



Reaction of amine **184** with 2,4-dinitrobenzenesulfonyl chloride afforded sulfonamide **185** in 53% yield, following purification by flash column chromatography.[123] Compound **185** underwent methylation using methyl iodide under basic conditions to provide sulfonamide **186** in an 82% yield, which did not require further purification. It was envisaged that the methylated sulfonamide should then be easily deprotected to provide the corresponding methylated amine **183**. The general procedure for removal of the dinitrophenylsulfonyl group involves the reaction of an external nucleophile at the *ipso* carbon of the aromatic ring, with subsequent release of sulfur dioxide and the desired amine. A co-product from the reaction with the general structure **187**, as detailed in Figure 74, is also prepared in this sequence, which may be used to determine the success of the deprotection procedure.

Figure 74: Mechanism for the formation of expected co-product 187.

Several methods of deprotection have been described in the literature and were applied to compound **186**, Table 7.

Table 7: Optimisation of Fukuyama deprotection.

Entry	Nucleophile	Base	Work-up	Formation of co-product **187**	Isolation of amine **183**
1	PhSH	Cs_2CO_3	basic	✓	✗
2	$HSCH_2CO_2H$	$NaOCH_3$	acidic then basic	✓	✗
3	$HSCH_2CO_2H$	$NaOCH_3$	basic	✓	✗
4	PhSH	$NaOCH_3$	none	n/a	✗
5	propylamine	none	none	✓	✗
6	pyrrolidine	none	none	✓	✗

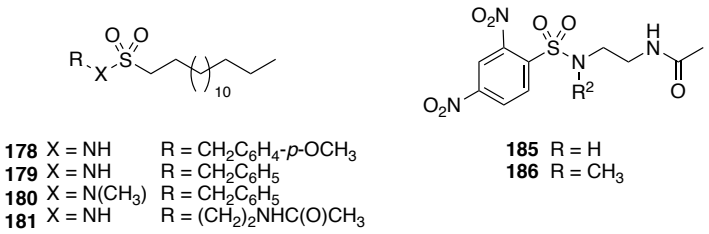
Entry 1 adopted the standard method of deprotection, using thiophenol,[123] and although the presence of both the co-product **187** and desired amine **183** were evident by ^{1}H NMR spectroscopic analysis, they were not in the expected 1:1 ratio. The ratio of **183** to **187** was 1:5 which was significantly lower than expected and isolation by flash column chromatography failed to provide any product. It was proposed that the amine may have been retained by the column, due to its polar nature. The next attempt (Entry 2), used mercaptoacetic acid to remove the protecting group,[124] where the co-product **187** from the reaction should be easily removed in the aqueous layer, following basic work-up, removing the need for column chromatography. However, no amine was recovered following work-up, which was proposed to be due to the miscibility of the amine **183** with the aqueous layer. Entry 3 shows the repeat of Entry 2, however, with the absence of an acidic wash to avoid loss of the amine to the aqueous layer. Unfortunately this also failed, as the amine **183** could not be

isolated by column chromatography. It was then proposed that thiophenol should be examined in the presence of an alternative base, sodium methoxide ($NaOCH_3$) (Entry 4), where the product would be isolated immediately *via* chromatography. Isolation of the amine **183** failed and no material was recovered from the column, despite the presence of a number of potential spots on the TLC. It was at this point that other deprotection methods were sought out. Entries 5 and 6 illustrate deprotection attempts using amine nucleophiles.[122] In these procedures excess amine and solvents could easily be removed *in vacuo* facilitating purification. ^{1}H NMR spectroscopy indicated the presence of the co-product **187** for both attempts, however, little to no amine **183** was seen, <5%. This led to the final hypothesis that not only was the amine miscible in water but was also volatile, meaning it was easily lost under reduced pressure. Despite numerous and varied attempts, the synthesis of compound **183** was not completed. Although compound **183** was commercially available, it was deemed too expensive for purchase.

Although difficulties had been encountered during the synthesis of several of the desired compounds, an array of sulfonamides **178–181** had been prepared for biological evaluation. It was determined that the two dinitrosulfonamides, **185** and **186**, prepared during the Fukuyama synthesis should also be biologically evaluated, as these had the ability to react with the catalytic cysteine residue present in the DHHC superfamily.

2.3.2.3 Biological evaluation

All synthesised compounds, Figure 75, were evaluated using the *in vitro* assay detailed in Section 5.1.2.



178 X = NH R = $CH_2C_6H_4$-*p*-OCH_3
179 X = NH R = $CH_2C_6H_5$
180 X = N(CH_3) R = $CH_2C_6H_5$
181 X = NH R = $(CH_2)_2NHC(O)CH_3$

185 R = H
186 R = CH_3

Figure 75: Compounds submitted for biological evaluation.

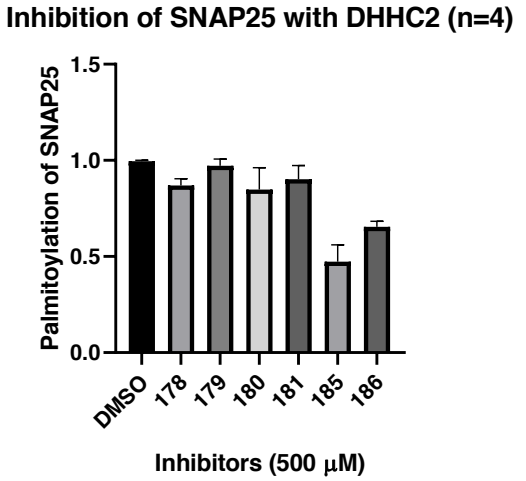


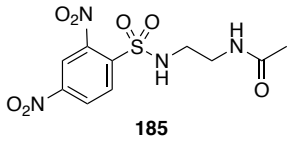
Figure 76: Inhibition of palmitoylation by sulfonamide series.

Figure 76 illustrates the results of inhibition of palmitoylation of SNAP25 with a series of inhibitors, for DHHC2. In the above graph, DMSO acts as the negative control, where 100% palmitoylation is expected. The given value of 1 is the baseline value for this assay, meaning that percentage inhibition of each compound is calculated relative to this number. From these results we can see compounds **178–181** have a negligible effect on inhibition, while compounds **185** and **186** show around 50% and 35% inhibition respectively. Interestingly, compound **186** with its methylated amine, inhibits to a lesser extent than its free amine counter part **185**. This suggests that a free amine may be necessary for binding within the active site of DHHC enzymes. Although nitrobenzene **185** was not originally intended to be tested as an inhibitor, it was the first compound to show any reasonable inhibition on this sub-project. Therefore we decided that the nitrobenzene motif required further investigation.

2.3.3 Nitrobenzene series

2.3.3.1 Design rationale

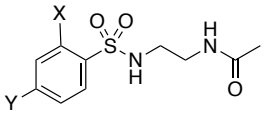
Compound **185**, Figure 77, was found to inhibit the palmitoylation of SNAP25 by ~50%. We therefore decided that an SAR study would be carried out surrounding the nitrobenzene motif.



185

Figure 77: Inhibitor 185.

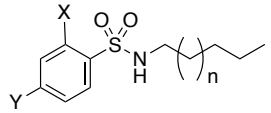
We initially proposed to simply modify the number and position of nitro-substituents around the aromatic core, as shown in Figure 78.



X = NO_2 Y = NO_2 **185**
X = NO_2 Y = H **188**
X = H Y = NO_2 **189**

Figure 78: Nitro substitution SAR.

As it was unlikely at this stage that these compounds would be selective for the catalytic cysteine of the DHHC family, replacement of the ethylacetamide functionality with a simple alkyl chain, to mimic the natural substrate, may serve to increase recognition by the DHHC enzyme, Figure 79.



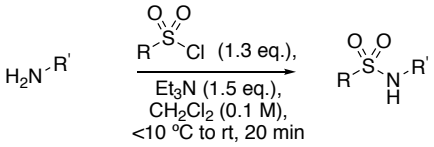
X = NO_2 Y = NO_2 n = 10 **190**
X = NO_2 Y = H n = 10 **191**
X = H Y = NO_2 n = 10 **192**
X = NO_2 Y = NO_2 n = 12 **193**
X = NO_2 Y = H n = 12 **194**
X = H Y = NO_2 n = 12 **195**

Figure 79: Alkyl chain SAR.

2.3.3.2 Synthetic route

Compounds were synthesised by treatment of the desired amine with the appropriate sulfonyl chloride, Scheme 34. Nitrobenzenesulfonamides **188–195** were prepared in moderate to good yields, 20–76%, following purification by flash column chromatography.[123]

Scheme 34: Synthesis of nitrobenzene series.

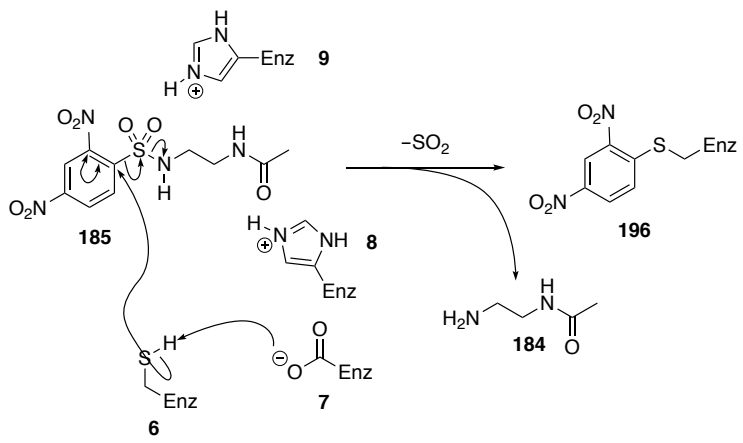


188	R = C_6H_4-2-NO_2	R' = $(CH_2)NHC(O)CH_3$	66%
189	R = C_6H_4-4-NO_2	R' = $(CH_2)NHC(O)CH_3$	20%
190	R = C_6H_3-2,4-$(NO_2)_2$	R' = $C_{14}H_{29}$	66%
191	R = C_6H_3-2-NO_2	R' = $C_{14}H_{29}$	76%
192	R = C_6H_3-4-NO_2	R' = $C_{14}H_{29}$	65%
193	R = C_6H_3-2,4-$(NO_2)_2$	R' = $C_{16}H_{33}$	46%
194	R = C_6H_3-2-NO_2	R' = $C_{16}H_{33}$	69%
195	R = C_6H_3-4-NO_2	R' = $C_{16}H_{33}$	74%

2.3.3.3 Mechanistic investigation

Initially we decided to interrogate the binding mode of the original nitrobenzene compound **185**. Evaluation of the mechanism, led us to believe that **185** may be covalently modifying the catalytic cysteine of DHHC2, as illustrated in Scheme 35.

Scheme 35: Mechanistic hypothesis.

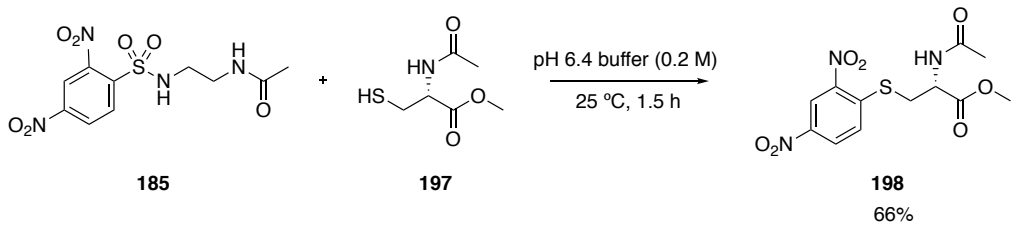


We expect that the nucleophilic cysteine **6** within the active site will attack the *ipso* carbon of **185** and eliminate sulfur dioxide and the free amine **184**, generating an irreversibly modified cysteine **196**.

Due to the lengthy process that would be necessary to precisely elucidate this interaction in a biological setting, a series of reactions were performed to investigate this phenomenon further. By allowing the nitrobenzenesulfonamide **185** and an appropriately protected cysteine (to mimic the catalytic cysteine present in the DHHC enzyme) to react under similar conditions to the assay we could potentially determine the binding mode for this series. If we were able to isolate a reaction product, after an extended period of time, it would suggest that compound **185** had the potential to covalently modify the cysteine in an irreversible manner.

The reaction of compound **185** with **197** was set up at pH 6.4 at 25 °C, in order to mimic the assay conditions, as shown in Scheme 36 and samples were taken every 15 minutes for LC-MS analysis. This analysis indicated the formation of product **198** and reaction completion was identified after 1.5 hours. Following purification, **198** was isolated in a 66% yield. This result is a clear indication that **185** has the potential to behave as a covalent inhibitor rather than a reversible binder.

Scheme 36: Synthesis of covalent inhibition product.



185 **197** **198** 66%

At pH 6.4, the thiol of cysteine would be protonated (i.e. comparable to physiological conditions); therefore, the electrophilicity of the *ipso* carbon was enough to facilitate the reaction. This high reactivity may mean that **185** would

not be selective for cysteine, and could react with other nucleophilic amino acids, such as protected lysine **199** and protected serine **200**, Figure 80.

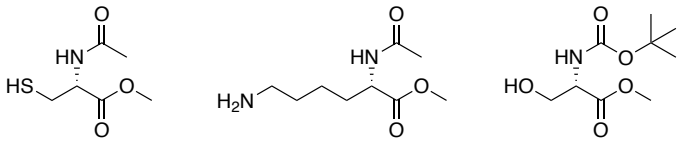


Figure 80: Protected amino acids.

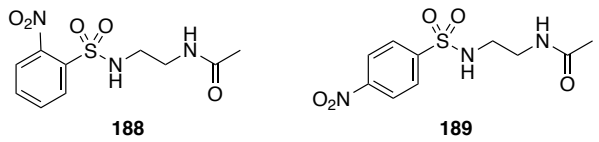
To investigate this possibility, the reaction of compound **185** and lysine derivative **199** was set up at pH 6.4 at 25 °C (Table 8, Entry 1). Samples were taken every 15 minutes for 2 hours with a final sample after 24 hours, examining the aliquot by LC-MS analysis. The results of this analysis showed 0% conversion after a 24 hour period, indicating that compound **185** was potentially selective for cysteine over lysine residues. The reaction of serine derivative **200** and compound **185** was then set up at pH 6.4 at 25 °C (Entry 2). Results from the LC-MS analysis showed 0% conversion after 24 hours, indicating the possibility that compound **185** is also selective for cysteine over serine residues.

Table 8: Covalent inhibition experiments.

Entry	Compound	Amino acid derivative	pH	% conversion after 24 hours
1	**185**	Lysine **199**	6.4	0
2	**185**	Serine **200**	6.4	0
3	**188**	Cysteine **196**	6.4	0
4	**189**	Cysteine **196**	6.4	0

The reactivity of compound **185** with cysteine was proposed to be due to the electrophilicity of the *ipso* carbon. During the course of our investigations, we had synthesised two compounds containing only one nitro species, Figure 81. The *ipso* position electrophilicity of these should be lower and therefore we

decided to test these with protected cysteine **196**, in order to determine whether these would still be viable compounds to test within the *in vitro* assay.



188 **189**

Figure 81: Compound 30 analogues.

The reaction of compound **188** with cysteine **196** was set up at pH 6.4 at 25 °C (Entry 3). After 24 hours, LC-MS analysis indicated 0% conversion, suggesting that compound **188** is not electrophilic enough for the reaction to proceed. Compound **189** was also reacted with cysteine **196** at pH 6.4 at 25 °C (Entry 4) and after 24 hours no conversion was seen.

Following these initial results, it was proposed that an increase in pH to 8.4 might facilitate the reaction of cysteine **196** with the less electrophilic compounds **188** and **189**, Table 9. It was also suggested that pH 8.4 might enable the reaction of compound **185** with lysine derivative **199**. The reaction of compound **185** with lysine derivative **199** was set up at pH 8.4 at 25 °C (Entry 5). After 24 hours, ~10% conversion was indicated by LC-MS analysis, this suggests that increasing the nucleophilicity of lysine allowed the reaction to proceed to a small extent. Compound **188** was reacted with cysteine **196** at pH 8.4 at 25 °C (Entry 6). LC-MS analysis indicated ~50% conversion after 24 hours. The reaction of compound **189** with cysteine **196** was set up at pH 8.4 at 25 °C (Entry 7). After 24 hours 0% conversion was achieved for this reaction, suggesting that that an increase to pH 8.4 was not enough to allow this transformation to proceed.

Table 9: Covalent inhibition experiments 2.

Entry	Compound	Amino acid derivative	pH	% conversion after 24 hours
5	**185**	Lysine **199**	8.4	~10
6	**188**	Cysteine **196**	8.4	~50
7	**189**	Cysteine **196**	8.4	0

From this second set of results we generated a reactivity profile for the nitrobenzenesulfonamides where: 2,4-dinitro > 2-nitro > 4-nitro, as would be expected. The remaining nitrobenzenesulfonamide compounds **190–195** were not examined.

From these results we can confirm that compound **185** may be acting as a covalent binder and more importantly, is selective for cysteine over both lysine and serine. This is a very promising result as selectivity for such a reactive compound is imperative to its potential use as a chemical tool and as a prospective cysteine-targeting warhead.

There are currently a wide number of cysteine-reactive probes allowing the design of 'tailor-made' chemical proteomic experiments, Figure 82.

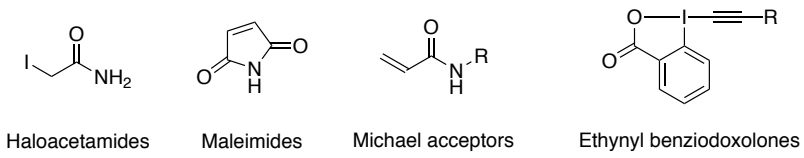


Figure 82: General scaffolds of cysteine-reactive probes.

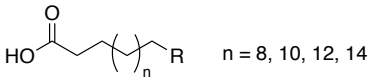
However, the promiscuity of some probes remains a concern, and high probe concentrations and longer incubation times unsurprisingly lead to side reactions with other nucleophilic residues. Therefore the reactivity of any chosen cysteine-directed probes must be considered before their use. Highly reactive probes tend to be more promiscuous in nature, while less reactive probes are not ideal for experiments that require the largest possible proteomic coverage. The haloacetamides, maleimides and Michael acceptor probes have

been widely used in the study of proteomics, however they are not fully cysteine selective and have been shown to react with other nucleophilic residues, such as lysine. Ethynyl beniodoxolones among others have shown a much greater selectivity for cysteine (97% vs 91% for iodoacetamide), however there remains room for improvement. With the current library of warheads it has become more common to use a multiple probe approach, in order to gain a fuller coverage of the subject area, and to avoid the number of false positive results, however this obviously requires a larger number of experiments, which is not ideal. The introduction of an additional recognition element to these cysteine-targeting warheads is also an interesting possibility; bi-functional probes could be very useful in the study of enzyme pathways and substrate identification. The generation of novel chemical probes with high reactivity, high cysteine selectivity and the possibility for further functionalisation is key to furthering our understanding of chemical proteomics. It is therefore of great interest to further develop compound **185** as a novel cysteine-warhead.

3. Conclusions

S-acylation, the attachment of fatty acids onto cysteine residues, is a major post-translational modification of cellular proteins, catalysed by the DHHC superfamily. The actions of S-acylation impact on a number of important physiological processes, and defects in these processes have been linked to a range of major diseases and disorders. A significant effort has been invested in identifying the substrates for the DHHC enzymes, however, there is a lack of understanding of the specific substrate profiles of individual enzymes and how DHHC-substrate specificity is achieved. To begin to assess the downstream effects of S-acylation by this enzyme superfamily, and in turn assess the possibility of targeting S-acylation and interrogating its therapeutic potential, chemical tools are required.

Previous work within our collaboration identified the first chain length selectivity profiles for a set of DHHC enzymes. This selectivity was investigated and found to be caused by a difference of one amino acid in the third transmembrane domain. DHHC3 has an isoleucine residue at position 182 while DHHC7 has a serine. This difference means that DHHC7 has a greater ability to incorporate longer chain length fatty acids (C14–C22) while DHHC3 is limited to shorter chain lengths (C14–C16). Several series of compounds have been designed based upon our mechanistic understanding of S-acylation with the aim of further interrogating this selectivity and providing a selective inhibitor of S-acylation, Figure 83.



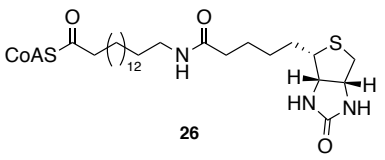
30—33	R = OH	
48—51	R = OMe	
58—61	R = SH	
96—99	R = OAc	
100—103	R = SAc	
104—107	R = NHAc	
120—123	R = OCF_3	
134	R = Ph	n = 10

Figure 83: Synthesised compounds for interrogation of selectivity.

Various R groups were added to increasing chain length fatty acids (C12–C18) with the aim of specifically targeting the serine residue of DHHC7 through a hydrogen bonding interaction. As serine has the ability to behave as a hydrogen bond acceptor (HBA) or as a hydrogen bond donor (HBD), different R groups were introduced to either be HBA's (OMe/OAc/SAc/OCF_3) or both (OH/SH/NHAc). A final compound with the addition of a phenyl ring was developed to interrogate the steric tolerance of the DHHC active channel.

These compounds were all biologically evaluated against DHHC3 and 7 using a cell-based assay developed within the Chamberlain (SIPBS) laboratory. Results indicated that the 14-carbon OCF_3 (90%) and Ph (80%) compounds had the best inhibitory effect on palmitoylation. However, these compounds were also found to be active against DHHC2/3/7 and 15. We believe this non-selective inhibition is due to the lipophilicity of these compounds. Interestingly we saw lower activity (65%) with the 14-carbon OCF_3 against a fifth enzyme, DHHC17. We propose that this slight selectivity is due to the higher degree of steric encumbrance within the DHHC17 hydrophobic channel: a phenylalanine residue is present at the 182 position. We therefore believe we have a 'semi-pan' inhibitor of S-acylation, where inhibition will be dependent upon the degree of steric hindrance within the hydrophobic channel. Although a fully selective inhibitor was the intended aim of this project, a reversible inhibitor, which is non-toxic to cells, and selective for the DHHC superfamily, is more efficient than the current state of the art, 2-bromopalmitate.

A secondary aim of this project was to develop a tool compound **26** that could be used to investigate DHHC-substrate profiles, Figure 84.

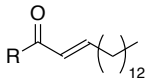


26

Figure 84: DHHC-substrate chemical tool.

This compound was designed based upon the active form of the fatty acids used for S-acylation. Fatty acids are first converted to their CoA counterparts before uptake by the DHHC enzymes. A biotin motif was envisaged to allow protein pull-down to tease out new substrates for the DHHC superfamily. Synthesis of this compound has not been completed due to the insoluble nature of the precursor to thioester formation. A solid-supported strategy was attempted but confirmation of the desired product was not achieved.

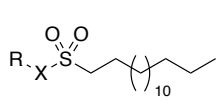
Finally, two different series of compounds were designed to look at the inhibition of DHHC2 within an *in vitro* assay also developed within the Chamberlain laboratory. The α,β-unsaturated series, Figure 85, was proposed to explore the possibility of a reversible inhibitor based on the known non-selective irreversible inhibitor, 2-bromopalmitate.

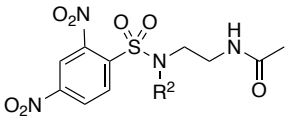


R = OEt **167**
R = OH **27**

Figure 85: α,β-unsaturated series.

Biological evaluation of these compounds revealed low inhibitory effect and no further examination of this series was carried out.





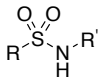
178 X = NH R = $CH_2C_6H_4$-*p*-OCH_3
179 X = NH R = $CH_2C_6H_5$
180 X = N(CH_3) R = $CH_2C_6H_5$
181 X = NH R = $(CH_2)_2NHC(O)CH_3$

185 R = H
186 R = CH_3

Figure 86: Sulfonamide series.

A series based on tetrahedral head groups was suggested to mimic the tetrahedral intermediate formed during S-acylation, Figure 86. Compounds **178–181** are 15-carbon chains with a sulfonyl head group and various side-chains (R), while compounds **185** and **186** are nitrobenzene containing

compounds. Biological evaluation indicated that **185** inhibited palmitoylation at 50%, while all other compounds showed no interesting levels of inhibition. An SAR study of **185** was carried out to further investigate this inhibition, Figure 87.



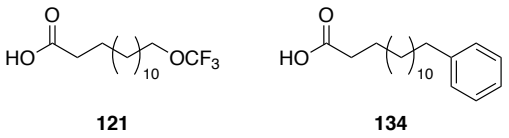
188	R = C_6H_4-2-NO_2	R' = $(CH_2)NHC(O)CH_3$
189	R = C_6H_4-4-NO_2	R' = $(CH_2)NHC(O)CH_3$
190	R = C_6H_3-2,4-$(NO_2)_2$	R' = $C_{14}H_{29}$
191	R = C_6H_3-2-NO_2	R' = $C_{14}H_{29}$
192	R = C_6H_3-4-NO_2	R' = $C_{14}H_{29}$
193	R = C_6H_3-2,4-$(NO_2)_2$	R' = $C_{16}H_{33}$
194	R = C_6H_3-2-NO_2	R' = $C_{16}H_{33}$
195	R = C_6H_3-4-NO_2	R' = $C_{16}H_{33}$

Figure 87: Nitrobenzene SAR.

The degree and position of nitration around the ring was altered (**188** and **189**) and various alkyl amines were added (**190–195**) to increase affinity for the DHHC enzymes. However, it was proposed that these nitrobenzene compounds were covalently modifying the cysteine of the DHHCs and would not be reversible inhibitors. Prior to any biological evaluation, investigation of this binding mode was carried out using protected cysteine, as well as protected lysine and serine. Our experiments indicated that nitrobenzene **185** was indeed an irreversible inhibitor; however it was specific to cysteine over other nucleophilic amino acids. While this binding mode made this an insufficient tool for interrogation of the DHHC superfamily, a separate project was initiated to evaluate nitrobenzene as a warhead for cysteine residues.

4. Future work

Initial future work within this project will be based around further biological evaluation of the C14OCF_3 **121** and C14Ph **134** compounds, Figure 88.



121 **134**

Figure 88: Current lead compounds.

Preliminary dose response experiments have been carried out but it would be necessary to repeat these with additional data points. While early experiments have indicated that these compounds are not selective across 4 of the 5 DHHCs tested, repeats would be essential to place full confidence in our findings. It would also be interesting to test these compounds across a wider range of DHHCs in order to examine their full inhibitory/ selectivity profile. Further to this, we have proposed that the lower inhibition observed with DHHC17 is due to a steric encumbrance within the channel. It would be interesting to test compound **134** and also the 12-carbon (**120** and **133**) and 16-carbon chain (**122** and **135**) lengths to further elucidate this hypothesis, Figure 89.

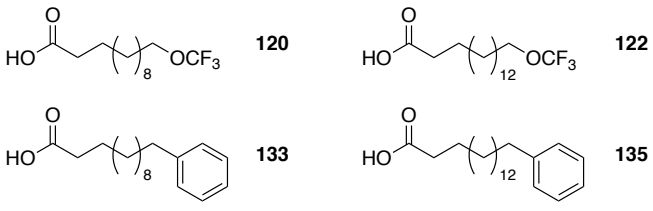


Figure 89: Compounds to interrogate sterics of DHHC17 channel.

From a chemistry perspective, future work would involve combining the key functionalities of these compounds to increase inhibition at lower concentrations, Figure 90.

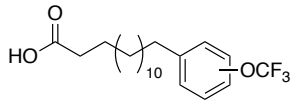


Figure 90: Hybrid inhibitor.

This next generation of compounds would involve functionalisation of the phenyl ring with a trifluoromethyl ether, at different positions around the ring in order to inform us of the appropriate positioning for inhibition. Introducing functionality to the phenyl ring could also allow us to exploit alternative interactions within the active channel, as we can add a secondary functional group, Figure 91. Exploitation of additional interactions within the channel should drive the potency of theses compounds towards desired levels of inhibition.

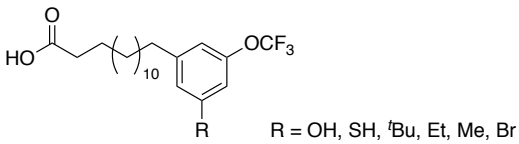


Figure 91: Second generation of inhibitors.

It would also be interesting to alter the position of the phenyl ring within the chain Figure 92. Movement of the phenyl ring along the chain could allow for optimal hydrophobic/ π-stacking/ π-cation interactions with neighbouring residues, to be adopted. Again, this should heavily influence the potency of the compounds and increase their affinity for the target.

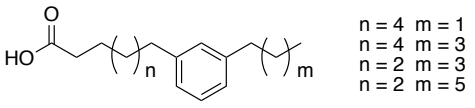


Figure 92: Alteration of phenyl ring position.

The implementation of all of these ideas will hopefully culminate in the production of a potent, selective chemical probe, which can be used to interrogate and elucidate the biological profile of our chosen DHHC targets.

5. Experimental

5.1 Biological assays

5.1.1 Cell-based assay

Within the Chamberlain group, a cell-based assay has been developed. This is a competition assay employing click chemistry. For the purposes of this biological evaluation, DHHC3 and SNAP25, with appropriate tags, have been employed as the enzyme and substrate respectively. The general protocol is detailed in Figure 93.

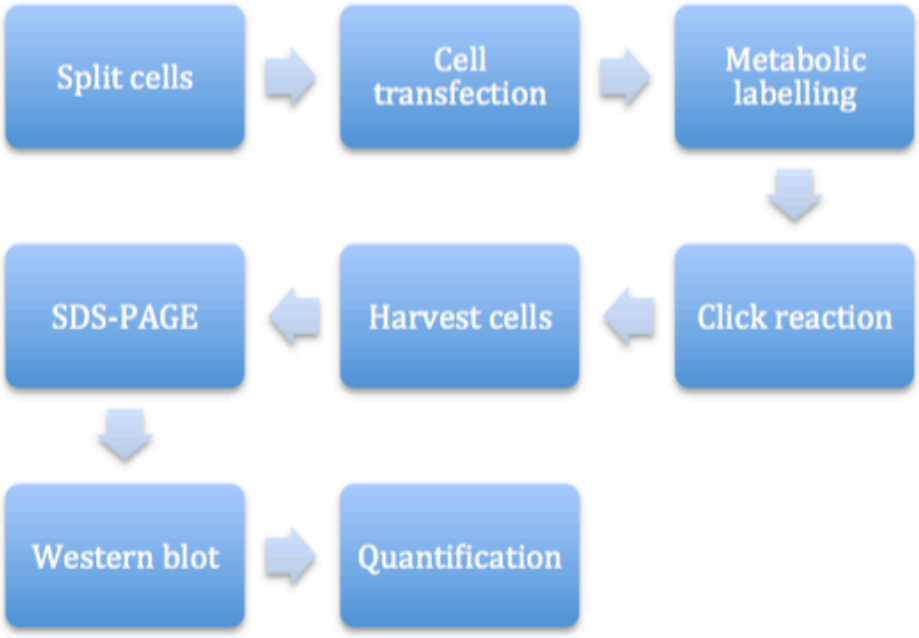


Figure 93: General protocol for cell-based assay.

Splitting of HEK293T cells[**]

1. Media was removed and discarded from the flask containing previously cultured HEK293T cells.

[**] This was carried out by another member of the Chamberlain group.

2. Cells were washed briefly with trypsin (1 × 3 mL), stored at 37 °C, which was then discarded.
3. Trypsin (3 mL) was then added and the cells incubated for 5 minutes.
4. Cells were detached from the flask and transferred to a falcon tube.
5. DMEM supplemented with 10% fetal bovine serum (7 mL), at 37 °C, was added to the falcon tube.
6. Cells were seeded 1:10 for flasks and 1:5 for 24-well plates (lysine coated, 0.5 mL/well).
7. Cells were then incubated at 37 °C, in a humidified atmosphere containing 5% CO_2.
8. Cells were split weekly.

Cell transfection[††]

Cells were transfected approximately 24 hours after plating using Lipofectamine 2000 reagent (Invitrogen).

1. 2 falcon tubes were set up as follows:
    a. DMEM (50 µL) + plasmid DNA (0.8 µg EGFP-SNAP25B, 1.6 µg HA-zDHHC3)
    b. DMEM (50 µL) + Lipofectamine reagent (4.8 mL)
2. Each tube was incubated for 5 minutes at room temperature (RT) before being mixed together.
3. The combined mixture was then incubated for a further 20 minutes at RT.
4. The transfection reaction was added to the cells (100 µL/well).
5. Cells were incubated at 37 °C/ 5% CO_2 overnight.

[††] This was carried out by another member of the Chamberlain group.

Metabolic labelling

1. Cells were serum-starved in DMEM containing 1% fatty acid free BSA for 30 minutes at 37 °C.
2. Cells were then incubated in DMEM/1% fatty acid free BSA containing 100 μM C16-azide (2 mL/well) with inhibitors/ controls for 4 hours at 37 °C.

Controls

- Negative control: DMSO (20 μL, 500 μM)
- Positive control: unlabelled C16 (20 μL, 500 μM)

Inhibitors

Samples were initially made up in DMSO at 50 mM. They were then added to the cells at a final concentration of 500 μM (20 μL/well).

3. Cells were washed with ice-cold PBS (1 mL).
4. Cells were then lysed on ice in 100 μL lysis buffer (50 mM Tris pH 8.0 containing 0.5% SDS and protease inhibitors).
5. Lysates were stored in Eppendorf® tubes.

Click chemistry

Stocks were made up as follows: 4 mM IR800 alkyne dye (DMSO, -20 °C); 40 mM $CuSO_4$ (dH_2O, RT); 100 mM tris((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amine (TBTA) (DMSO, -20 °C); 40 mM ascorbic acid (dH_2O, RT).

1. To each cell lysate was added the click reaction mixture (0.125 μL dye, 10 μL $CuSO_4$ and 0.4 μL TBTA in 69.5 μL dH_2O).
2. The mixture was vortexed before addition of ascorbic acid (20 μL).
3. The final reaction mixture was incubated for 1 hour at RT with end-over-end rotation.

4. The proteins were then precipitated: ice–cold acetone was added to the reaction (600 µL), the mixture was then vortexed and placed in the freezer (-20 °C) for 30 minutes.
5. Each tube was centrifuged at 1300 ppm at 4 °C for 5 minutes.
6. The supernatant was removed and ice-cold acetone added (1 mL).
7. The pellet was re-suspended before further centrifugation, after which the supernatant was removed and the pellet allowed to air dry for 5 minutes.

Cell harvest and SDS-PAGE

1. The pellet was re-suspended in SDS sample buffer containing 25 mM DTT (100 µL/pellet).
2. Samples were boiled at 95 °C for 5 minutes.
3. 10 µL of each sample was loaded onto 12% polyacrylamide gels alongside 10 µL of pre-stained protein marker. 2 gels were used.
4. Samples were resolved electrophoretically at 80 V through the stacking gel and then 150 V through the resolving gel.
5. Gels were then transferred onto nitrocellulose membranes at 120 mA overnight.

Western blotting

1. Nitrocellulose membranes were rinsed in PBS + 0.02% Triton X100 (PBST).
2. Membranes were then blocked in PBST + 5% defatted milk for 45 minutes, with agitation.
3. The milk solution was removed and the membranes washed with PBST until no milk solution remained.
4. Each membrane was incubated in a different primary antibody solution (diluted in PBST) for 1 hour, with agitation. Concentration of primary antibodies:
    a. anti-GFP (JL8; mouse) 1:3000

   b. anti-HA (rat) 1:1000
5. Membranes were washed with PBST (5 × 10 mL) for 5 minutes each.
6. Each membrane was then incubated in the secondary antibody (diluted in PBST) for 45 minutes, with agitation. LICOR secondary antibodies were used at a concentration of 1:10,000.
7. Membranes were washed with PBST (5 × 10 mL) for 5 minutes each, followed by a final rinse in PBS.

Quantification

As stated above, 2 separate gels were run and 2 separate western blots carried out. One western blot used antibodies specific to the EGFP tagged SNAP25. The purpose of this blot was to assess that the levels of substrate were consistent throughout the experiment, so as to ensure there was no bias between results. The second western blot used antibodies specific to the HA tagged DHHC3. The purpose of this blot was to ensure that the levels of DHHC enzyme also remained consistent throughout the experiment, again to guarantee no bias.

A click reaction will only take place between the alkyne dye and the C16-azide, if the C16-azide has been incorporated into the enzyme or substrate. This reaction exerts a signal that can be quantified. As the assay is a competition assay, the level of C16 incorporation can be used to measure the level of inhibition by the compounds tested. This level was measured by assigning the result from the positive control as 1, indicating 100% incorporation of the palmitic azide, from which all other results were measured. A percentage of incorporation of C16, in each experiment, was generated. From this, the level of inhibition was deduced. Inhibition of palmitoylation at the substrate was measured using the GFP western blot and inhibition of auto-palmitoylation was quantified from the HA western blot.

A LI-COR® machine is then used to scan the membranes from the western blot, this uses two different coloured channels, therefore signals from both the click reaction and the HA or GFP tags can be visualised at the same time. The click

reaction uses a green dye and can be seen in the 800 nm channel, the secondary antibodies for recognition of HA and GFP tags use a red dye that can be seen in the 680 nm channel, as shown in Figure 94.

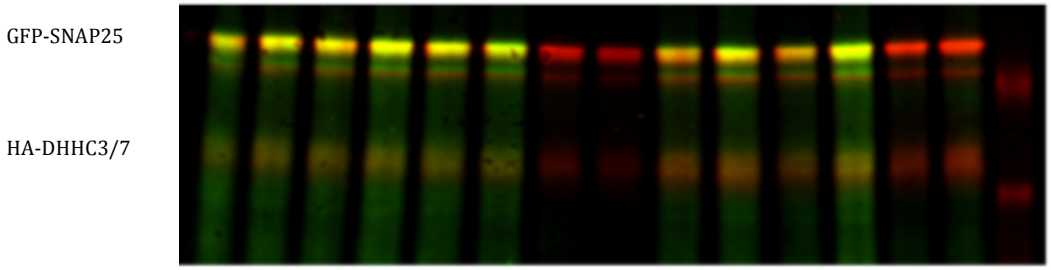


Figure 94: LI-COR image of western blot showing 'click' signal in green and HA/ GFP signals in red.

Each lane represents a different reaction, with duplicates or triplicates adjacent to one another. The LI-COR® can then be used to quantify each reaction by measuring the level of signal observed for each channel (680 or 800 nm). The level of palmitoylation, as shown in green, is then calculated relative to the level of SNAP25, in red, so as to remove any bias from lower or higher levels of expression of SNAP25 for different experiments. The level of palmitoylation of SNAP25 for the DMSO positive control is then assigned as 1 and the remaining experiments are calculated relative to this. The given values are then displayed in a bar graph depicting the level of palmitoylation of SNAP25 against each experiment.

5.1.2 In-vitro assay

This *in vitro* assay has been designed for use in 96-well plates, using DHHC2 and SNAP25 as the enzyme and substrate respectively. The general protocol is detailed in Figure 95.

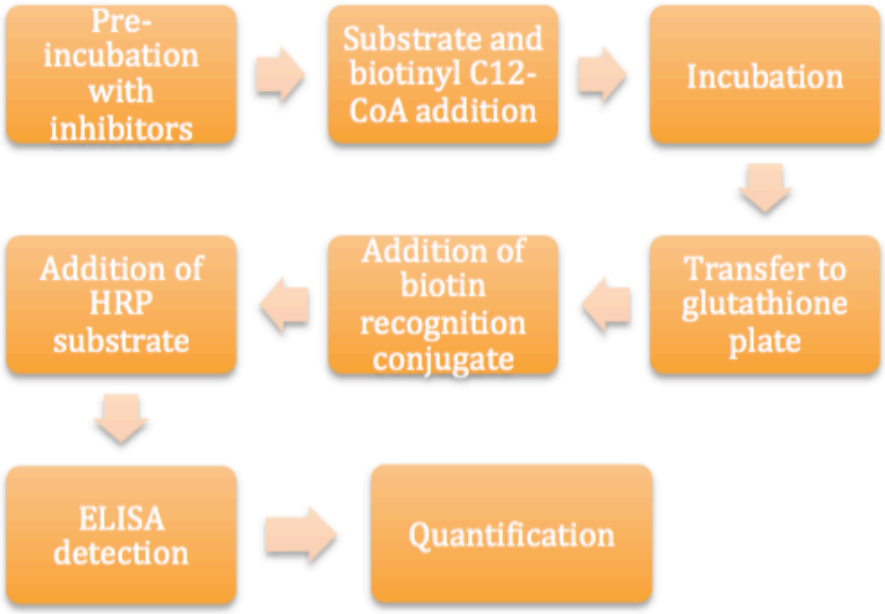


Figure 95: *In-vitro* protocol.

Reactions

In-vitro reactions were set up according to Table 10.

Table 10: *In-vitro* reactions.

Reaction	DHHC2	Substrate (1 µM)	Biotinyl C12-CoA (1 µM)	Inhibitors (100 µM)
1	-	GST	+	-
2	0.0375 µM	GST	+	-
3	-	GST-SNAP25	+	-
4	0.0375 µM	GST-SNAP25	+	+/-

Reactions 1, 2 and 3 act as negative controls for the experiment while reaction 4 in the absence of a potential inhibitor acts as a positive control.

Assay buffer

- 1 mL 50 mM MES pH 6.4.
- 100 mM NaCl.
- 0.1% DDM.
- 1 mM TCEP.

Sample preparation

1. Stock solutions of each sample are made up at 50 mM in DMSO.
2. Samples are diluted to 5 mM in DMSO (1 µL of stock + 9 µL DMSO).
3. Samples are then further diluted to 500 µM in assay buffer (2 µL of previous dilution + 18 µL assay buffer).

Substrate + biotinyl C12-CoA mix

Stock solutions are as follows: 251 µM GST, 146 µM GST-SNAP25, 1 mM biotinyl C12-CoA.

- GST diluted to 5 µM in assay buffer (1.99 µL stock + 100 µL assay buffer).
- GST-SNAP25 diluted to 5 µM in assay buffer (4.8 µL stock + 140 µL assay buffer).
- Biotinyl C12-CoA diluted to 5 µM in assay buffer (1 µL stock + 200 µL assay buffer).

1. GST and biotinyl C12-CoA dilutions mixed in 1:1 ratio.
2. GST-SNAP25 and biotinyl C12-CoA dilutions mixed in 1:1 ratio.

ELISA substrate mix

Supersignal® ELISA Pico Luminol enhancer and Supersignal® ELISA Pico stable peroxide solutions were mixed in a 1:1 ratio.

Protocol

1. To an uncoated plate was aliquoted 5 µL of DHHC2 (excluding reactions 1 and 3).
2. 2.5 µL of inhibitors/ controls were added to the appropriate wells and pre-incubated at RT for the desired amount of time.

Controls

- Negative control: DMSO.
- Positive control: 2-bromopalmitate.

3. The substrate/ biotinyl C12-CoA mixtures were then added to the appropriate wells (5 µL/well) and incubated for 45 minutes at RT.
4. The glutathione plates were washed in PBS + 0.025% TWEEN (2 × 200 µL/well) followed by PBS (1 × 200 µL/well).
5. PBS was then added to the uncoated plate (115 µL/well).
6. 19.25 µL/well of this dilution was dispensed in the glutathione plate containing PBS (85 µL/well) in duplicate.
7. Glutathione plates were incubated for 2 hours at 25 °C with slight agitation.
8. Plates were then washed according to Step 4.
9. NeutrAvidin HRP (diluted to 1:20,000 in PBS + 0.025% TWEEN) was added to each plate (100 µL/well) and incubated for 45 minutes at 25 °C.
10. Plates were then washed according to Step 4.
11. ELISA substrate mix was added (100 µL/well) and the plates read using a PolarStar Omega.

Quantification

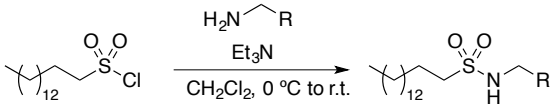
The PolarStar Omega measures the level of palmitoylation by measuring the level of signal from the reaction of the NeutrAvidin HRP and the ELISA substrate mix. NeutrAvidin has a strong affinity for biotin and therefore will bind to any biotinyl C12-CoA that has been incorporated by SNAP25, following palmitoylation by DHHC2. The data obtained from the PolarStar Omega can then be quantified by assigning the DMSO positive control as 1, and then calculating the level of palmitoylation of each experiment relative to this number. The results are then displayed in a bar graph depicting the level of palmitoylation of SNAP25 for each experiment.

5.2 General Experimental:

All reagents were obtained from commercial sources, such as Sigma-Aldrich and Alfa Aesar, and used without purification. All solvents were obtained through the SPS system at the University of Strathclyde. Reactions monitored by TLC were done so using Machery-Nagel pre-coated TLC sheets coated in 0.20 mm silica gel 60 with UV_{254} fluorescent indicator. Compounds that were not UV active were developed in $KMnO_4$, anisaldehyde or bromo-cresol dips, with gentle heating. Purification was performed by flash column chromatography, with chromatography grade silica 60 Å particle size 35–70 micron from Fisher Scientific, using the solvent systems stated. ^{1}H and ^{13}C NMR were carried out using a Bruker Avance 3 (^{1}H 400 MHz and ^{13}C 101 MHz) and a Bruker Avance 500 (^{1}H 500 MHz and ^{13}C 125 MHz) as stated. The spectra were recorded in the deuterated solvents $CDCl_3$ (chloroform) and $(CD_3)_2SO$ (dimethylsulfoxide). Multiplicities were indicated as follows: s (singlet); d (doublet); t (triplet); dd (doublet of doublets); m (multiplet) *etc*… Coupling constants (*J*) were given in Hertz (Hz). Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) ($\delta = 0.00$). Peaks with δ values of 7.26, 2.50 ppm (^{1}H NMR) and 77, 40 ppm (^{13}C NMR) correspond to the residual solvent peak for $CDCl_3$ and $(CD_3)_2SO$ respectively. Mass spectrometry was carried out on an Agilent 6130 liquid chromatograph with electrospray mass spectrometer using a 5–100% acetonitrile in water with ammonium hydroxide solvent system. Infrared spectra were recorded in the range 4000–600 cm^{-1} on a Shimadzu IRAffinity-1 equipped with an ATR accessory. Melting points were determined on a Stuart SMP11 and are uncorrected.

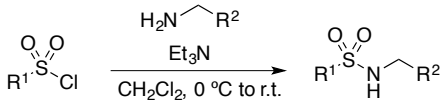
5.3 General procedures

General procedure A: Preparation of sulfonamides



To a solution of the amine (1.1 eq.) in dichloromethane (CH_2Cl_2) (0.3 M) was added triethylamine (Et_3N) (1.1 eq.) at 0 °C. The resulting solution was allowed to stir at 0 °C for 15 minutes before drop-wise addition of pentadecane-1-sulfonyl chloride (1.0 eq.). The reaction was then allowed to warm to room temperature and reaction completion was monitored by TLC. Upon completion the reaction was quenched with saturated aqueous ammonium chloride (NH_4Cl) solution and the mixture extracted with CH_2Cl_2. The combined organics were dried over magnesium sulfate ($MgSO_4$), filtered and concentrated *in vacuo*. Further purification by flash column chromatography afforded the *title compound*.

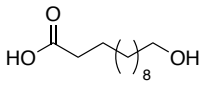
General procedure B: Preparation of nitrobenzenesulfonamides



To a solution of the amine (1.0 eq.) in CH_2Cl_2 (0.1 M) was added Et_3N (1.5 eq.) at room temperature. The mixture was then cooled to 0 °C and the sulfonyl chloride (1.3 eq.) was added portion-wise, at a rate such that the temperature remained below 10 °C. After stirring for 20 minutes at room temperature the reaction was quenched with saturated aqueous NH_4Cl solution. The mixture was separated and the aqueous layer extracted with CH_2Cl_2. The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography afforded the *title compound*.

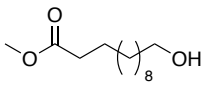
5.4 Preparation of alcohol and ether Series

12-Hydroxydodecanoic acid 30:[125]



This compound was purchased for use in subsequent reactions but was also biologically evaluated and therefore all analysis has been carried out. **M.pt**: 79–81 °C; **IR** (ATR/cm^{-1}): 3233 (br), 2911, 2846, 2630 (br), 1680; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 11.93 (br s, 1H, -COO<u>H</u>), 4.29 (br s, 1H, -O<u>H</u>), 3.37 (t, *J* 6.6 Hz, 2H, -C<u>H</u>$_2$OH), 2.18 (t, *J* 7.4 Hz, 2H, -C<u>H</u>$_2$COOH), 1.49–1.45 (m, 2H, -C<u>H</u>$_2$CH$_2$OH), 1.41–1.37 (m, 2H, -C<u>H</u>$_2$CH$_2$COOH), 1.30–1.20 (m, 14H, 7 × -C<u>H</u>$_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.5, 60.7, 33.6, 32.5, 29.1, 28.9, 28.9, 28.7, 28.5, 25.5, 24.5 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{24}O_3$ 216, found 217 $[M+H]^+$.

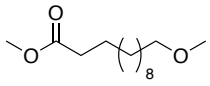
Methyl 12-hydroxydodecanoate 52:[126]



To a stirred solution of 12-hydroxydodecanoic acid **30** (1.0 g, 4.6 mmol, 1.0 eq.) in MeOH (39 mL, 0.12 M) was added a few drops of sulfuric acid (H_2SO_4). The resulting solution was allowed to stir at 40 °C overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with saturated $NaHCO_3$ solution (20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (922 mg, 0.40 mmol, 86%). **M.pt**: 38–40 °C; **IR** (ATR/cm^{-1}): 3389 (br), 2919, 2850, 1738; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.66 (s, 3H, -COOC<u>H</u>$_3$), 3.64 (t, *J* 6.5 Hz, 2H, -C<u>H</u>$_2$OH), 2.30 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOCH$_3$), 1.64–1.53 (m, 4H, 2 × -C<u>H</u>$_2$), 1.34–1.26 (m, 14H, 7 × -C<u>H</u>$_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ174.5, 63.2,

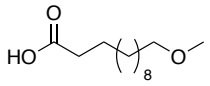
51.6, 34.3, 29.7, 29.6, 29.5, 29.4, 29.3, 25.9, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{13}H_{26}O_3$ 230, found 231 $[M+H]^+$.

Methyl 12-methoxydodecanoate 54:[127]



To a stirred solution of methyl 12-hydroxydodecanoate **52** (500 mg, 2.2 mmol, 1.0 eq.) in MeCN (14 mL, 0.16 M) was added MeI (11 mL, 0.20 M). Silver(I) oxide (755 mg, 3.3 mmol, 1.5 eq.) was then added and the mixture allowed to reflux overnight. The solids were removed *via* filtration and the filtrate concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a clear oil (276 mg, 0.11 mmol, 52%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2922, 2852, 1740; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.66 (s, 3H, -COOC$\underline{H}_3$), 3.36 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.63–1.55 (m, 4H, 2 × -C$\underline{H}_2$), 1.32–1.27 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5, 73.1, 58.7, 51.6, 34.3, 29.8, 29.7, 29.6, 29.6, 29.4, 29.3, 26.3, 25.1 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{28}O_3$ 244, found 245 $[M+H]^+$.

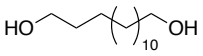
12-Methoxydodecanoic acid 48:[128]



To a 1:1 solution of THF/H_2O (2 mL, 0.20 M) was added methyl 12-methoxydodecanoate **54** (100 mg, 0.41 mmol, 1.0 eq.). A solution of 2 M NaOH (3.3 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear oil that solidified on standing (90 mg, 0.04 mmol,

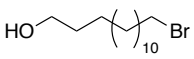
96%). **M.pt**: <30 °C; **IR** (ATR/cm^{-1}): 3084 (br), 2915, 2852, 1729; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.37 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.63 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$OCH$_3$), 1.56 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.35–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.8, 73.1, 58.7, 34.0, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.1, 26.2, 24.8; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{13}H_{26}O_3$ 230, found 231 $[M+H]^+$.

1,14–Dihydroxytetradecane 36:[82]



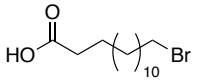
To a flame-dried three-neck flask, attached with an overhead stirrer, was added 1,14-tetradecanedioic acid (10.0 g, 38.7 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (THF) (387 mL, 0.1 M) at 0 °C under an inert atmosphere. Solid lithium aluminium hydride ($LiAlH_4$) (2.94 g, 77.5 mmol, 2.0 eq.) was then added portion-wise and the reaction allowed to stir at room temperature overnight. The reaction was monitored *via* TLC. Upon completion the reaction mixture was cooled to 0 °C and wet sodium sulfate (Na_2SO_4) was added portion-wise until the visible grey precipitate became white. The reaction was left to stir for 1 hour at room temperature to allow the reaction mixture to become homogeneous. The suspension was then dried with $MgSO_4$, filtered, washed with diethyl ether (Et_2O) (3 × 200 mL) and concentrated *in vacuo*. A white solid was afforded and used without further purification (8.1 g, 35.3 mmol, 91%). **M.pt**: 86–88 °C; **IR** (ATR/cm^{-1}): 3410 (br), 3351 (br), 2921, 2850, 1463; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 4.30 (t, *J* 5.2 Hz, 2H, 2 × -O$\underline{H}$), 3.34–3.39 (m, 4H, 2 × -C$\underline{H}_2$OH), 1.39 (app p, *J* 6.8, 4H, 2 × -C$\underline{H}_2$CH$_2$OH), 1.22–1.26 (m, 20H, 10 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 60.5, 33.2, 28.9, 28.8, 28.8, 28.7, 25.3; **LRMS** (GC-MS) *m/z* calc. for $C_{14}H_{30}O_2$ 230, found 231 $[M+H]^+$.

14–Bromotetradecanol 38:[82]



To a stirred solution of 1,14–dihydroxytetradecane **36** (5.0 g, 21.7 mmol, 1.0 eq.) in cyclohexane (57 mL, 0.38 M) was added hydrobromic acid (57 mL, 0.38 M). The resultant solution was stirred at reflux for 7 hours, before cooling to room temperature. The reaction was then quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 40 mL). The combined organics were washed with saturated sodium hydrogen carbonate ($NaHCO_3$) solution (4 × 30 mL) and brine (30 mL), then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 95:5 EtOAc, petroleum ether 70:30 EtOAc) afforded the *title compound* as an off–white solid (3.9 g, 13.4 mmol, 62%). **M.pt**: 46–48 °C; **IR** (ATR/cm^{-1}): 3274 (br), 2919, 2850, 1465; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.64 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$OH), 3.41 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$Br), 1.85 (app p, *J* 7.2 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.57 (app p, *J* 6.9 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.42 (app p, *J* 7.1 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.36–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 63.3, 34.2, 33.0, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{29}{}^{79}BrO$ 292, found 275 $[M\text{-}H_2O]^+$.

14–Bromotetradecanoic acid 40:[82]



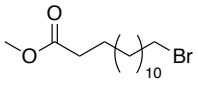
Preparation of Jones Reagent:

Chromium(VI) oxide (4.10 g, 40.8 mmol, 4.0 eq.) was dissolved in H_2SO_4 (7.5 mL, 1.36 M). Ice-cold H_2O (17 mL, 0.6 M) was added portion-wise with stirring and the reagent was allowed to stir for 10 minutes before addition to the reaction.

To a stirred solution of 14–bromotetradecanol **38** (3.0 g, 10.2 mmol, 1.0 eq.) in acetone (255 mL, 0.04 M) was added Jones Reagent, preparation as described above, drop-wise over 10 minutes at room temperature. The resultant solution was stirred at room temperature for 18 hours. The reaction was quenched with H_2O (50 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2

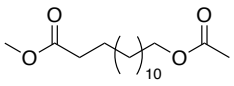
(4 × 30 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc + 0.1% acetic acid (AcOH)) afforded the *title compound* as a white solid (2.1 g, 6.84 mmol, 67%). **M.pt**: 62–64 °C; **IR** (ATR/cm^{-1}): 3036 (br), 2917, 2852, 1696, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$Br), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.85 (app p, *J* 7.2 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.64 (app p, *J* 7.4 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.42 (app p, *J* 7.1 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.35–1.25 (m, 16H, 8 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.8, 34.2, 33.9, 33.0 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{27}{}^{79}BrO_2$ 306, found 307 $[M+H]^+$.

Methyl 14-bromotetradecanoate 42:



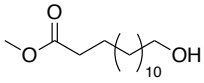
To a stirred solution of 14–bromotetradecanoic acid **40** (500 mg, 1.63 mmol, 1.0 eq.) in MeOH (14 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to stir at 40 °C overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear liquid that solidified on standing (494 mg, 0.15 mmol, 95%). **M.pt**: <30°C; **IR** (ATR/cm^{-1}): 2922, 2852, 1737, 1460, 1168; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.62 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOCH$_3$), 1.42 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.29–1.26 (m, 16H 8 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 51.6, 34.3, 34.2, 33.0, 29.7, 29.7, 29.6, 29.4, 29.3, 28.9, 28.3, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{29}{}^{79}BrO_2$ 320, found 321 $[M+H]^+$.

Methyl 14-acetoxytetradecanoate 44:



To a stirred solution of methyl 14-bromotetradecanoate **42** (200 mg, 0.6 mmol, 1.0 eq.) in DMF (5.0 mL, 0.12 M) was added sodium acetate (307 mg, 3.7 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, the mixture was diluted with H_2O (10 mL) and extracted with EtOAc (4 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a clear liquid that solidified on standing (114 mg, 0.04 mmol, 61%). **M.pt**: 29–31°C; **IR** (ATR/cm^{-1}): 2922, 1852, 1728, 1470; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OC(O)CH_3), 3.66 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH_3), 2.04 (s, 3H, -OC(O)C$\underline{H}_3$), 1.61 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$$CH_2$OC(O)$CH_3$), 1.34–1.25 (m, 20H 10 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 171.4, 64.8, 51.6, 34.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 28.8, 26.1, 25.1 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{32}O_4$ 300, found 259 $[M\text{-}OAc+H]^+$.

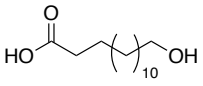
Methyl 14-hydroxytetradecanoate 46:[129]



To a stirred solution of methyl 14-acetoxytetradecanoate **44** (500 mg, 1.67 mmol, 1.0 eq.) in MeOH (14 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to reflux overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (176 mg, 0.07 mmol, 41%). **M.pt**: 42–44 °C; **IR**

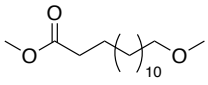
(ATR/cm^{-1}): 3391, 2917, 2848, 1738; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC<u>H</u>$_3$), 3.64 (t, *J* 6.5 Hz, 2H, -C<u>H</u>$_2$OH), 2.30 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOCH$_3$), 1.63–1.57 (m, 4H, 2 × -C<u>H</u>$_2$), 1.33–1.25 (m, 18H, 9 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.3, 73.2, 58.6, 34.1, 29.8, 29.8, 29.7, 29.6, 29.6, 29.6, 29.4, 29.2, 26.3, 24.9 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{30}O_3$ 258, found 259 $[M+H]^+$.

14-Hydroxytetradecanoic acid 31:[130]



To a 1:1 solution of THF/H_2O (3.0 mL, 0.20 M) was added methyl 14-hydroxytetradecanoate **46** (150 mg, 0.58 mmol, 1.0 eq.). A solution of 2 M NaOH (6.0 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL of each 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (144 mg, 0.06 mmol, 95%). **M.pt**: 81–83 °C; **IR** (ATR/cm^{-1}): 3231, 2911, 2846, 1682, 1470; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.65 (t, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$OH), 2.35 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOH), 1.64 (app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$OH), 1.57 (app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$COOH), 1.35–1.27 (m, 18H, 9 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.5, 60.7, 33.6, 32.5, 29.1, 29.0, 29.0, 28.9, 28.9, 28.7, 28.5, 25.5, 24.5 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{28}O_3$ 244, found 245 $[M+H]^+$.

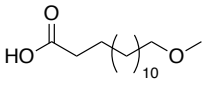
Methyl 14-methoxytetradecanoate 56:



To a stirred solution of methyl 14-hydroxytetradecanoate **46** (300 mg, 1.2 mmol, 1.0 eq.) in MeCN (7.5 mL, 0.16 M) was added MeI (6.0 mL, 0.20 M).

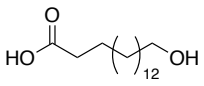
Silver(I) oxide (539 mg, 2.3 mmol, 2.0 eq.) was then added and the mixture allowed to reflux overnight. The solids were removed *via* filtration and the filtrate concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a clear oil (150 mg, 0.06 mmol, 48%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2911, 2848, 1732, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.36 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.65–1.55 (m, 4H, 2 × -C$\underline{H}_2$), 1.34–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 73.1, 58.7, 51.6, 34.3, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 26.3, 25.1 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{32}O_3$ 272, found 271 $[M-H]^+$.

14-Methoxytetradecanoic acid 49:



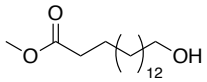
To a 1:1 solution of THF/H_2O (2.0 mL, 0.20 M) was added methyl 14-methoxytetradecanoate **56** (100 mg, 0.37 mmol, 1.0 eq.). A solution of 2 M NaOH (3.7 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (89 mg, 0.03 mmol, 94%). **M.pt**: 50–52 °C; **IR** (ATR/cm^{-1}): 3086, 2911, 2848, 1729, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.37 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.34 (s, 3H, -OC$\underline{H}_3$), 2.35 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$COOH), 1.64 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$OCH$_3$), 1.57 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.34–1,26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.9, 73.1, 58.6, 34.2, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 26.3, 24.8 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{30}O_3$ 258, found 259 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{15}H_{30}O_3$ 257.2122, found 257.2127 $[M-H]^+$.

16-Hydroxyhexadecanoic acid 32:[131]



This compound was purchased for use in subsequent reactions but was also biologically evaluated and therefore all analysis has been carried out. **M.pt**: 95–97 °C; **IR** (ATR/cm^{-1}): 3230 (br), 2913, 2848, 1683, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.66 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OH), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.63 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.57 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.38–1.26 (m, 22H, 11 × -CH$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.8, 63.3, 34.1, 33.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 25.9, 24.9 (3 carbons missing); **LRMS** (GC-MS) *m/z* calc. for $C_{16}H_{32}O_3$ 272, found 273 $[M+H]^+$.

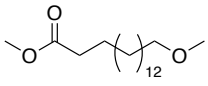
Methyl 16-hydroxyhexadecanoate 53:



To a stirred solution of 16-hydroxyhexadecanoic acid **32** (500 mg, 1.84 mmol, 1.0 eq.) in MeOH (15 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to reflux for 8 hours. The reaction was monitored by TLC. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a white solid (257 mg, 0.90 mmol, 49%). **M.pt**: 56–58 °C; **IR** (ATR/cm^{-1}): 3393 (br), 2921, 2850, 1740; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.65–3.62 (m, 2H, -C$\underline{H}_2$OH), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.62 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.59–1.54 (m, 2H, -C$\underline{H}_2$CH$_2$COOCH$_3$), 1.36–1.26 (m, 22H, 11 × -CH$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 63.3, 51.6, 34.3, 33.0, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 25.9, 25.1 (4 carbons missing); **LRMS** (GC-MS) *m/z* calc. for $C_{17}H_{34}O_3$

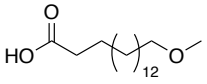
286, found 287 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{17}H_{34}O_3$ 287.3511, found 304.2817 $[M+NH_4]^+$.

Methyl 16-methoxyhexadecanoate 55:



To a stirred solution of methyl 16-hydroxyhexadecanoate **53** (50 mg, 0.18 mmol, 1.0 eq.) in acetonitrile (MeCN) (1.1 mL, 0.16 M) was added methyl iodide (MeI) (0.87 mL, 0.20 M). Silver(I) oxide (45 mg, 0.19 mmol, 1.1 eq.) was then added and the mixture allowed to reflux overnight. The solids were removed *via* filtration and the filtrate concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 95:5 EtOAc) afforded the *title compound* as a white solid (24 mg, 0.08 mmol, 46%). **M.pt**: 39–41 °C; **IR** (ATR/cm^{-1}): 2915, 2850, 1737, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.66 (s, 3H, -COOC$\underline{H}_3$), 3.35 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.32 (s, 3H, -OC$\underline{H}_3$), 2.29 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.64–1.52 (m, 4H, 2 × -CH_2), 1.28–1.24 (m, 22H, 11 × -CH_2); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 73.1, 58.7, 51.5, 34.3, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 29.4, 29.3, 29.3, 26.3, 25.1 (1 carbon missing); **LRMS** (GC-MS) *m/z* calc. for $C_{18}H_{36}O_3$ 300, found 301 $[M+H]^+$.

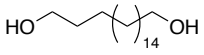
16-Methoxyhexadecanoic acid 50:[132]



To a 1:1 solution of THF/H_2O (2 mL, 0.17 M) was added methyl 16-methoxyhexadecanoate **55** (100 mg, 0.33 mmol, 1.0 eq.). A solution of 2 M NaOH (3.3 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. The reaction was monitored by TLC. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered

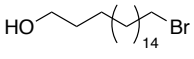
and concentrated *in vacuo* to afford the *title compound* as a white solid (74 mg, 0.26 mmol, 78%). **M.pt**: 59–61 °C; **IR** (ATR/cm^{-1}): 3392 (br), 2917, 2850, 1699, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.37 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.63 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$OCH$_3$), 1.56 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.32–1.26 (m, 22H, 11 × -CH$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.7, 73.2, 58.6, 34.0, 29.8, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 26.3, 24.8 (4 carbons missing); **LRMS** (GC-MS) *m/z* calc. for $C_{17}H_{34}O_3$ 286, found 287 $[M+H]^+$.

Octadecane-1,18-diol 37:[82]



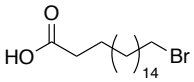
To a flame-dried three-neck flask, attached with an overhead stirrer, was added dimethyl octadecanedioate (20.0 g, 58.4 mmol, 1.0 eq.) in anhydrous THF (583 mL, 0.1 M) at 0 °C under an inert atmosphere. $LiAlH_4$ (5.76 g, 152 mmol, 2.6 eq.) was then added portion-wise and the reaction allowed to stir at room temperature overnight.. Upon completion the reaction mixture was cooled to 0 °C and wet Na_2SO_4 was added portion-wise until the visible grey precipitate became white. The reaction was left to stir for 1 hour at room temperature to allow the reaction mixture to become homogeneous. The suspension was then dried with $MgSO_4$, filtered, washed with Et_2O (4 × 200 mL) and concentrated *in vacuo*. A white solid was afforded and used without further purification (16.1 g, 56.02 mmol, 96%). **M.pt**: 98–100 °C; **IR** (ATR/cm^{-1}): 3416, 3353, 2919, 2891; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 4.29 (t, *J* 5.5 Hz, 2H, 2 × -O$\underline{H}$), 3.36 (app q, *J* 6.5 Hz, 4H, 2 × -C$\underline{H}_2$OH), 1.39 (app p, *J* 6.5 Hz, 4H, 2 × -C$\underline{H}_2$CH$_2$OH), 1.29–1.24 (m, 28H, 14 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, DMSO-d_6) δ 60.6, 32.3, 28.8, 28.8, 28.7, 25.3 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{38}O_2$ 286, found 287 $[M+H]^+$.

18-Bromooctadecan-1-ol 39:[82]



To a stirred solution of octadecane-1,18-diol **37** (16.0 g, 28.0 mmol, 1.0 eq.) in cyclohexane (74 mL, 0.38 M) was added hydrobromic acid (74 mL, 0.38 M). The resultant solution was stirred at reflux for 7 hours, before cooling to room temperature. The reaction was then quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 70 mL). The combined organics were washed with saturated aqueous $NaHCO_3$ solution (4 × 40 mL) and brine (40 mL), then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 90:10 EtOAc, petroleum ether 70:30 EtOAc) afforded the *title compound* as an off–white solid (13.9 g, 39.8 mmol, 71%). **M.pt**: 56–58 °C; **IR** (ATR/cm^{-1}): 3274, 2917, 2850; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.64 (app q, *J* 6.0 Hz, 2H, -C$\underline{H}_2$OH), 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 1.85 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.55 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.44 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.36–1.26 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 63.3, 34.2, 33.0, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9 (7 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{37}{}^{81}BrO$ 349, found 332 $[M\text{-}H_2O+H]^+$.

18-Bromooctadecanoic acid 41:[82]

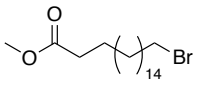


Preparation of Jones Reagent:

Chromium(VI) oxide (8.0 g, 80 mmol, 4.0 eq.) was dissolved in H_2SO_4 (15 mL, 1.36 M). Ice-cold H_2O (34 mL, 0.6 M) was added portion-wise with stirring and the reagent was allowed to stir for 10 minutes before addition to the reaction.

To a stirred solution of 18-bromooctadecan-1-ol **39** (7.0 g, 20 mmol, 1.0 eq.) in acetone (500 mL, 0.04 M) was added Jones Reagent, preparation as described above, drop-wise over 10 minutes at room temperature. The resultant solution was stirred at room temperature for 18 hours. The reaction was quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 100 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc + 0.1% AcOH) afforded the *title compound* as a white solid (6.1 g, 17 mmol, 83%). **M.pt**: 69–71 °C; **IR** (ATR/cm^{-1}): 3034, 2915, 2850, 1696; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.85 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.62 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.45–1.39 (m, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.34–1.26 (m, 24H, 12 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 179.6, 34.2, 34.2, 33.0, 29.8, 29.7, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{35}{}^{81}BrO_2$ 364, found 365 $[M+H]^+$.

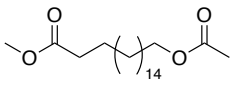
Methyl 18-bromooctadecanoate 43:[133]



To a stirred solution of 18-bromooctadecanoic acid **41** (6.0 g, 17 mmol, 1.0 eq.) in MeOH (140 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to stir at 40 °C overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ solution (3 × 80 mL) then brine (80 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as an oil that solidified on standing (4.9 g, 13 mmol, 78%). **M.pt**: 38–40 °C; **IR** (ATR/cm^{-1}): 2915, 2846, 1732, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.62 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOCH$_3$), 1.42 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.32–1.25 (m, 24H, 12 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 51.6, 34.3, 34.2, 33.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4,

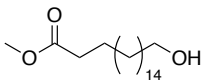
29.3, 28.9, 28.3, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{37}{}^{81}BrO_2$ 378, found 376 $[M-H]^+$.

Methyl 18-acetoxyoctadecanoate 45:[134]



To a stirred solution of methyl 18-bromooctadecanoate **43** (4.8 g, 13 mmol, 1.0 eq.) in DMF (110 mL, 0.12 M) was added sodium acetate (6.3 g, 76 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, the mixture was diluted with H_2O (80 mL) and extracted with EtOAc (4 × 50 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as clear liquid that solidified on standing (3.7 g, 10 mmol, 81%). **M.pt**: 45–47°C; **IR** (ATR/cm^{-1}): 2913, 1846, 1732, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OC(O)CH$_3$), 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 2.04 (s, 3H, -OC(O)C$\underline{H}_3$), 1.64–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.35–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 171.4, 64.8, 51.6, 34.3, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.3, 29.3, 28.8, 26.1, 26.0, 25.1, 21.2; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{21}H_{40}O_4$ 356, found 355 $[M-H]^+$.

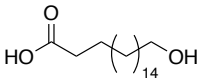
Methyl 18-hydroxyoctadecane 47:[135]



To a stirred solution of methyl 18-acetoxyoctadecanoate **45** (3.5 g, 9.8 mmol, 1.0 eq.) in MeOH (82 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to reflux overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL), then brine (20 mL), dried over

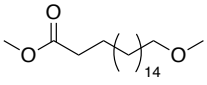
$MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (2.1 g, 6.6 mmol, 67%). **M.pt**: 56–58 °C; **IR** (ATR/cm^{-1}): 3395, 2915, 1836, 1738, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.64 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OH), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.63–1.54 (m, 4H, 2 × -C$\underline{H}_2$), 1.34–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.5, 63.3, 51.6, 34.3, 33.0, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 25.9, 25.1 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{38}O_3$ 314, found 315 $[M+H]^+$.

18-Hydroxyoctadecanoic acid 33:[136]



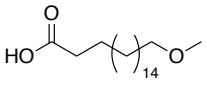
To a 1:1 solution of THF/H_2O (1.6 mL, 0.20 M) was added methyl 18-hydroxyoctadecanoate **47** (100 mg, 0.32 mmol, 1.0 eq.). A solution of 2 M NaOH (3.2 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (87 mg, 0.29 mmol, 91%). **M.pt**: 84–86 °C; **IR** (ATR/cm^{-1}): 3307, 2911, 2846, 1699, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.65 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OH), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.64 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.57 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.35–1.26 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ178.9, 62.8, 34.7, 32.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 25.8, 24.9; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{36}O_3$ 300, found 299 $[M-H]^+$.

Methyl 18-methoxyoctadecanoate 57:



To a stirred solution of methyl 18-hydroxyoctadecanoate **47** (1.5 g, 4.8 mmol, 1.0 eq.) in MeCN (31 mL, 0.16 M) was added MeI (25 mL, 0.20 M). Silver(I) oxide (2.2 g, 9.6 mmol, 2.0 eq.) was then added and the mixture allowed to reflux overnight. The solids were removed *via* filtration and the filtrate concentrated *in vacuo* to afford the *title compound* as a clear oil (1.5 g, 4.5 mmol, 93%). **M.pt**: 42–44 °C; **IR** (ATR/cm^{-1}): 2913, 2846, 1736, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.36 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.65–1.55 (m, 4H, 2 × -C$\underline{H}_2$), 1.34–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 73.1, 58.7, 51.6, 34.3, 29.8, 29.8, 29.7, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 26.3, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{40}O_3$ 328, found 329 $[M+H]^+$.

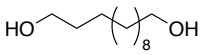
18-Methoxyoctadecanoic acid 51:



To a 1:1 solution of THF/H_2O (3.0 mL, 0.20 M) was added methyl 18-methoxyoctadecanoate **57** (200 mg, 0.61 mmol, 1.0 eq.). A solution of 2 M NaOH (6.1 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (134 mg, 0.43 mmol, 70%). **M.pt**: 55–57 °C; **IR** (ATR/cm^{-1}): 2913, 1846, 1699, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.37 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCH$_3$), 3.33 (s, 3H, -OC$\underline{H}_3$), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.64 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$OCH$_3$), 1.57 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.34–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.6, 73.2, 58.6, 34.1, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.2, 26.3, 24.8 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{38}O_3$ 314, found 315 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{19}H_{38}O_3$ 313.2748, found 313.2754 $[M-H]^+$.

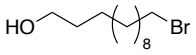
5.5 Preparation of thiol series

Dodecane-1,12-diol 64:[137]



To a flame-dried three-neck flask, attached with an overhead stirrer, was added dodecanedioic acid (10.0 g, 43.4 mmol, 1.0 eq.) in anhydrous THF (434 mL, 0.1 M) at 0 °C under an inert atmosphere. $LiAlH_4$ (3.30 g, 86.8 mmol, 2.0 eq.) was then added portion-wise and the reaction allowed to stir at room temperature overnight. Upon completion the reaction mixture was cooled to 0 °C and wet Na_2SO_4 was added portion-wise until the visible grey precipitate became white. The reaction was left to stir for 1 hour at room temperature to allow the reaction mixture to become homogeneous. The suspension was then dried with $MgSO_4$, filtered, washed with Et_2O (4 × 200 mL) and concentrated *in vacuo*. A white solid was afforded and used without further purification (8.24 g, 40.7 mmol, 94%). **M.pt**: 79–81 °C; **IR** (ATR/cm^{-1}): 3404, 3341, 2915, 2848, 1693, 1474; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 4.30 (t, *J* 5.2 Hz, 2H, 2 × -O<u>H</u>), 3.36 (app q, *J* 6.4 Hz, 4H, 2 × -C<u>H</u>$_2$OH), 1.43–1.36 (m, 4H, 2 × -C<u>H</u>$_2$CH$_2$OH), 1.29–1.24 (m, 16H, 8 × -C<u>H</u>$_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 60.7, 32.5, 29.1, 29.0, 28.9, 25.5; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{26}O_2$ 202, found 203 $[M+H]^+$.

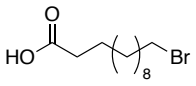
12-Bromododecan-1-ol 66:[138]



To a stirred solution of dodecane-1,12-diol **64** (8.0 g, 39.6 mmol, 1.0 eq.) in cyclohexane (100 mL, 0.38 M) was added hydrobromic acid (100 mL, 0.38 M). The resultant solution was stirred at reflux for 7 hours, before cooling to room temperature. The reaction was then quenched with H_2O (150 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 70 mL). The combined organics were washed with saturated aqueous $NaHCO_3$ solution

(4 × 40 mL) and brine (40 mL), then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 90:10 EtOAc, petroleum ether 70:30 EtOAc) afforded the *title compound* as an off–white solid (6.24 g, 23.5 mmol, 60%). **M.pt**: 30–32 °C; **IR** (ATR/cm^{-1}): 3263, 2915, 2852, 1474; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.64 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OH), 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.58 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.44–1.39 (m, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.36–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 63.2, 34.2, 33.0, 33.0, 29.7, 29.7, 29.6, 28.9, 28.3, 25.9 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{25}{}^{79}BrO$ 264, found 263 $[M-H]^+$.

12-Bromododecanoic acid 68:[139]



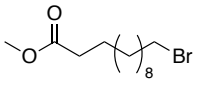
Preparation of Jones Reagent:

Chromium(VI) oxide (9.1 g, 91 mmol, 4.0 eq.) was dissolved in H_2SO_4 (17 mL, 1.36 M). Ice-cold H_2O (38 mL, 0.6 M) was added portion-wise with stirring and the reagent was allowed to stir for 10 minutes before addition to the reaction.

To a stirred solution of 12-bromododecan-1-ol **66** (6.0 g, 23 mmol, 1.0 eq.) in acetone (570 mL, 0.04 M) was added Jones Reagent, preparation as described above, drop-wise over 10 minutes at room temperature. The resultant solution was stirred at room temperature for 18 hours. The reaction was quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 100 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc + 0.1% AcOH) afforded the *title compound* as a white solid (6.1 g, 22 mmol, 97%). **M.pt**: 46–48 °C; **IR** (ATR/cm^{-1}): 3002, 2915, 2850, 1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.64

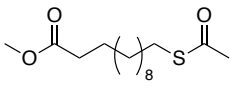
(app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$COOH), 1.42 (app p, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH$_2$CH$_2$Br), 1.35–1.26 (m, 12H, 6 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.5, 34.2, 33.9, 33.0, 29.6, 29.5, 29.5, 29.3, 29.3, 28.9, 28.3, 24.8; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{23}{}^{81}BrO_2$ 280, found 281 $[M+H]^+$.

Methyl 12-bromododecanoate 90:[140]



To a stirred solution of 12-bromododecanoic acid **68** (1.00 g, 3.58 mmol, 1.0 eq.) in MeOH (30 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to stir at 40 °C overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ solution (3 × 20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear oil (807 mg, 2.75 mmol, 77%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2922, 2852, 1738, 1437; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC<u>H</u>$_3$), 3.40 (t, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$Br), 2.30 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOCH$_3$), 1.85 (app p, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH$_2$Br), 1.62 (app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$COOCH$_3$), 1.42 (app p, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH$_2$CH$_2$Br), 1.28–1.24 (m, 12H, 6 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 51.6, 34.2, 34.2, 33.0, 29.6, 29.5, 29.5, 29.4, 29.3, 28.9, 28.3, 25.1; (LC-MS-ESI) *m/z* calc. for $C_{13}H_{25}{}^{79}BrO_2$ 292, found 293 $[M+H]^+$.

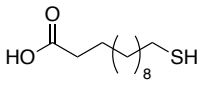
Methyl 12-(acetylthio)dodecanoate 92:



To a solution of methyl 12-bromododecanoate **90** (700 mg, 3.0 mmol, 1.0 eq.) in DMF (25 mL, 0.12 M) was added potassium thioacetate (2.27 g, 18.3 mmol, 6.0 eq.). The resulting solution was allowed to stir at 80 °C overnight. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 × 40 mL).

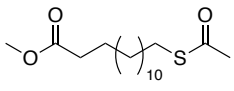
The combined organics were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a white solid (432 mg, 1.50 mmol, 49%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 1736, 1690, 1465; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.66 (s, 3H, -COOC$\underline{H}_3$), 2.86 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.32 (s, 3H, -C(O)C$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH_3), 1.64–1.55 (m, 4H, 2 × -C$\underline{H}_2$), 1.36–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 196.2, 174.5, 51.6, 34.3, 30.8, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 20.3, 29.2, 29.0, 25.1; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{28}O_3S$ 288, found 286 $[M-H]^+$.

12-Mercaptododecanoic acid 58:[141]



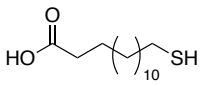
To a solution of methyl 12-(acetylthio)dodecanoate **92** (350 mg, 1.22 mmol, 1.0 eq.) in degassed ethanol (EtOH) (7.6 mL, 0.16 M) was added 1M NaOH (3.7 mL, 0.33 M). The resulting solution was allowed to stir at room temperature overnight. The reaction was then neutralised with 1M HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were washed with H_2O (2 × 20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (hexane 6:1 Et_2O + 0.1% AcOH) afforded the *title compound* as a white solid (142 mg, 0.61 mmol, 50%). **M.pt**: 44–46 °C; **IR** (ATR/cm^{-1}): 3030, 2915, 2850, 1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.92 (br s, 1H, -COO$\underline{H}$), 2.53 (app q, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SH), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.67–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.27 (m, 15H, 7 × -C$\underline{H}_2$, -S$\underline{H}$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.2, 34.2, 33.8, 29.6, 29.5, 29.4, 29.2, 28.5, 24.8, 24.8 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{24}O_2S$ 232, found 231 $[M-H]^+$.

Methyl 14-(acetylthio)tetradecanoate 93:



To a solution of methyl 14-bromotetradecanoate **42** (500 mg, 1.56 mmol, 1.0 eq.) in DMF (13 mL, 0.12 M) was added potassium thioacetate (1.07 g, 9.35 mmol, 6.0 eq.). The resulting solution was allowed to stir at 80 °C overnight. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 × 40 mL). The combined organics were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a white solid (355 mg, 1.12 mmol, 72%). **M.pt**: 32–34 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 1736, 1690, 1470; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.86 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH_3), 1.65–1.53 (m, 4H, 2 × -C$\underline{H}_2$), 1.36–1.25 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 196.2, 174.5, 51.6, 34.3, 30.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.3, 29.0, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{32}O_3S$ 316, found 314 $[M-H]^+$.

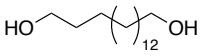
14-Mercaptotetradecanoic acid 59:



To a solution of methyl 14-(acetylthio)tetradecanoate **93** (500 mg, 1.58 mmol, 1.0 eq.) in degassed EtOH (9.9 mL, 0.16 M) was added 1M NaOH (4.8 mL, 0.33 M). The resulting solution was allowed to stir at room temperature overnight. The reaction was then neutralised with 1M HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were washed with H_2O (2 × 20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (hexane 6:1 Et_2O + 0.1% AcOH) afforded the *title compound* as a white solid (201 mg, 0.77 mmol, 49%). **M.pt**: 55–57 °C;

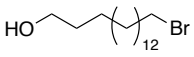
IR (ATR/cm^{-1}): 3030, 2913, 2848, 1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.53 (app q, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SH), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.67–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.26 (m, 19H, 9 × -C$\underline{H}_2$, -S$\underline{H}$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.5, 34.2, 33.9, 29.7, 29.7, 29.6, 29.4, 29.2, 28.5, 24.8, 24.8 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{28}O_2S$ 260, found 259 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{14}H_{28}O_2S$ 259.1737, found 259.1737 $[M-H]^+$.

1,16–Dihydroxyhexadecane 65:[82]



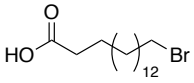
To a flame-dried three-neck flask, attached with an overhead stirrer, was added 1,16-hexadecanedioic acid (5.0 g, 17.5 mmol, 1.0 eq.) in anhydrous THF (175 mL, 0.1 M) at 0 °C under an inert atmosphere. $LiAlH_4$ (1.33 g, 35.0 mmol, 2.0 eq.) was then added portion-wise and the reaction allowed to stir at room temperature overnight.. Upon completion the reaction mixture was cooled to 0 °C and wet Na_2SO_4 was added portion-wise until the visible grey precipitate became white. The reaction was left to stir for 1 hour at room temperature to allow the reaction mixture to become homogeneous. The suspension was then dried with $MgSO_4$, filtered, washed with Et_2O (4 × 200 mL) and concentrated *in vacuo*. A white solid was afforded and used without further purification (4.2 g, 1.64 mmol, 94%). **M.pt**: 83–85 °C; **IR** (ATR/cm^{-1}): 3414, 3353, 2919, 2891, 2848; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 4.29 (t, *J* 5.0 Hz, 2H, 2 × -O$\underline{H}$), 3.36 (app q, 6.5 Hz, 4H, 2 × -C$\underline{H}_2$OH), 1.43–1.37 (m, 4H, 2 × C$\underline{H}_2$CH$_2$OH), 1.30–1.24 (m, 24H, 12 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 60.7, 32.5, 29.1, 29.0, 28.9, 25.5 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{34}O_2$ 258, found 281 $[M+Na]^+$.

16–Bromohexadecan-1-ol 67:[82]



To a stirred solution of 1,16–dihydroxyhexadecane **65** (4.5 g, 17.4 mmol, 1.0 eq.) in cyclohexane (46 mL, 0.38 M) was added hydrobromic acid (46 mL, 0.38 M). The resultant solution was stirred at reflux for 7 hours, before cooling to room temperature. The reaction was then quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with CH_2Cl_2 (4 × 40 mL). The combined organics were washed with saturated aqueous $NaHCO_3$ solution (4 × 30 mL) and brine (30 mL), then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 90:10 EtOAc, petroleum ether 70:30 EtOAc) afforded the *title compound* as an off-white solid (2.9 g, 0.91 mmol, 52%). **M.pt**: 56–58 °C; **IR** (ATR/cm^{-1}): 3274, 2917, 2850; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.64 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$OH), 3.41 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$Br), 1.85 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.57 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$OH), 1.42 (app p, *J* 6.5 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.36–1.26 (m, 22H, 11 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 63.3, 334.2, 33.0, 29.8, 29.8, 29.7, 29.6, 28.9, 28.3, 25.9 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{33}{}^{79}BrO$ 320, found 320 $[M+H]^+$.

16–Bromohexadecanoic acid 69:[82]



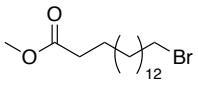
Preparation of Jones Reagent:

Chromium(VI) oxide (3.7 g, 36.5 mmol, 4.0 eq.) was dissolved in H_2SO_4 (7.0 mL, 1.36 M). Ice-cold H_2O (15 mL, 0.60 M) was added portion-wise with stirring and the reagent was allowed to stir for 10 minutes before addition to the reaction.

To a stirred solution of 16–bromohexadecan-1-ol **67** (2.9 g, 9.12 mmol, 1.0 eq.) in acetone (228 mL, 0.04 M) was added Jones Reagent, preparation as described above, drop-wise over 10 minutes at room temperature. The resultant solution was stirred at room temperature for 18 hours. The reaction was quenched with H_2O (100 mL), the layers separated and the aqueous phase extracted with

CH_2Cl_2 (4 × 100 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 90:10 EtOAc + 0.1% AcOH) afforded the *title compound* as a white solid (2.3 g, 6.9 mmol, 75%). **M.pt**: 66–68 °C; **IR** (ATR/cm^{-1}): 3034, 2917, 2850, 1696; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.64 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.42 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.35–1.26 (m, 20H, 10 × -C$\underline{H}_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.2, 34.2, 34.1, 33.0, 29.7, 29.7, 29.6, 29.4, 29.2, 28.9, 28.3, 24.8 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{31}{}^{79}BrO_2$ 334, found 335 $[M+H]^+$.

Methyl 16-bromohexadecanoate 91:[142]



Route 1:

To a stirred solution of methyl 16–bromohexadecanoic acid **69** (2.4 g, 7.2 mmol, 1.0 eq.) in MeOH (60 mL, 0.12 M) was added a few drops of H_2SO_4. The resulting solution was allowed to stir at 40 °C overnight. Upon completion the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ solution (3 × 20 mL) then brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as an oil that solidified on standing (2.2 g, 6.4 mmol, 90%).

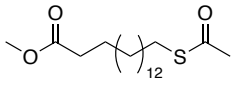
Route 2:

To a solution of methyl 16-hydroxyhexadecanoate (200 mg, 0.70 mmol, 1.0 eq.) in CH_2Cl_2 (5 mL, 0.14 M) was added carbon tetra-bromide (325 mg, 0.98 mmol, 1.4 eq.) then triphenylphosphine (PPh_3) (238 mg, 0.91 mmol, 1.3 eq.). The reaction mixture was allowed to stir overnight at room temperature. Upon completion, H_2O (5 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and

concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as an oil that solidified on standing (195 mg, 0.56 mmol, 80%).

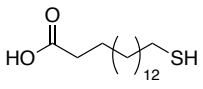
M.pt: 31–33 °C; **IR** (ATR/cm^{-1}): 2913, 2848, 1744, 1472, 1167; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.85 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$Br), 1.62 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOH), 1.42 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$Br), 1.28–1.26 (m, 20H, 10 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5, 51.6, 34.3, 34.2, 33.0, 29.8, 29.7, 29.6, 29.4, 29.3, 28.9, 28.3, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{33}{}^{81}BrO_2$ 350, found 351 $[M+H]^+$.

Methyl 16-(acetylthio)hexadecanoate 94:



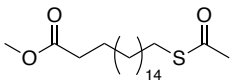
To a solution of methyl 16-bromohexadecanoate **91** (300 mg, 0.86 mmol, 1.0 eq.) in DMF (7.2 mL, 0.12 M) was added potassium thioacetate (589 mg, 5.2 mmol, 6.0 eq.). The resulting solution was allowed to stir at 80 °C overnight. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a white solid (273 mg, 0.79 mmol, 92%). **M.pt**: 41–43 °C; **IR** (ATR/cm^{-1}): 2913, 2848, 1736, 1692, 1470; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.86 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SC(O)CH$_3$), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.65–1.53 (m, 4H, 2 × -C$\underline{H}_2$), 1.36–1.25 (m, 22H, 11 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 196.2, 174.5, 51.6, 34.3, 30.8, 29.8, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.0, 25.1 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{36}O_3S$ 344, found 343 $[M-H]^+$.

16-Mercaptohexadecanoic acid 60:



To a solution of methyl 16-(acetylthio)hexadecanoate **94** (400 mg, 1.16 mmol, 1.0 eq.) in degassed EtOH (7.3 mL, 0.16 M) was added 1M NaOH (3.5 mL, 0.33 M). The resulting solution was allowed to stir at room temperature overnight. The reaction was then neutralised with 1M HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were washed with H_2O (2 × 20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (hexane 6:1 Et_2O + 0.1% AcOH) afforded the *title compound* as a white solid (186 mg, 0.65 mmol, 55%). **M.pt**: 61–63 °C; **IR** (ATR/cm^{-1}): 3028, 2913, 2848, 1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.53 (app q, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SH), 2.35 (t, *J* 7.5 Hz, 2H -C$\underline{H}_2$COOH), 1.67–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.26 (m, 23H, 11 × -C$\underline{H}_2$, -S$\underline{H}$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.8, 34.2, 33.9, 29.8, 29.7, 29.7, 29.6, 29.4, 29.2, 28.5, 24.8 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{32}O_2S$ 288, found 287 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{16}H_{32}O_2S$ 287.2048, found 287.2047 $[M-H]^+$.

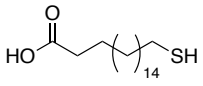
Methyl 18-(acetylthio)octadecanoate 95:



To a solution of methyl 18-bromooctadecanoate **43** (400 mg, 1.06 mmol, 1.0 eq.) in DMF (8.8 mL, 0.12 M) was added potassium thioacetate (727 mg, 6.37 mmol, 6.0 eq.). The resulting solution was allowed to stir at 80 °C overnight. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a white solid (340 mg, 0.91 mmol, 86%). **M.pt**: 49–51 °C; **IR** (ATR/cm^{-1}): 2913, 2848, 1738,

1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.86 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.32 (s, 3H, -S(O)C$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH_3), 1.65–1.53 (m, 4H, 2 × -C$\underline{H}_2$), 1.36–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 196.2, 174.5, 51.6, 34.3, 30.8, 29.8, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.3, 29.0, 25.1 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{21}H_{40}O_3S$ 372, found 371 $[M-H]^+$.

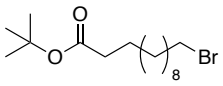
18-Mercaptooctadecanoic acid 61:



To a solution of methyl 16-(acetylthio)hexadecanoate **95** (350 mg, 0.94 mmol, 1.0 eq.) in degassed EtOH (5.9 mL, 0.16 M) was added 1M NaOH (2.9 mL, 0.33 M). The resulting solution was allowed to stir at room temperature overnight. The reaction was then neutralised with 1M HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were washed with H_2O (2 × 20 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (hexane 6:1 Et_2O + 0.1% AcOH) afforded the *title compound* as a white solid (136 mg, 0.43 mmol, 46%). **M.pt**: 69–71 °C; **IR** (ATR/cm^{-1}): 3034, 2913, 2848, 1693, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.53 (app q, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SH), 2.36 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.67–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.37–1.26 (m, 27H, 13 × -C$\underline{H}_2$, -S$\underline{H}$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.2, 34.2, 33.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.2 28.5, 24.9, 24.8 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{36}O_2S$ 316, found 315 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{18}H_{36}O_2S$ 315.2363, found 315.2360 $[M-H]^+$.

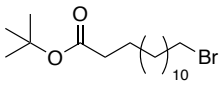
5.5.1 Preparation of Bunte salts

***tert*-Butyl 12-bromododecanoate 70:**



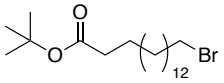
To a solution of 12-bromododecanoic acid (1.00 g, 3.58 mmol, 1.0 eq.) in *tert*-butanol (^{t}BuOH) (36 mL, 0.1 M) was added di-*tert*-butyl dicarbonate (Boc_2O) (1.56 g, 7.17 mmol, 2.0 eq.) and 4-(dimethylamino)pyridine (DMAP) (131 mg, 1.07 mmol, 0.3 eq.). The reaction was allowed to stir overnight at room temperature. Upon completion the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 8:2 toluene) afforded the *title compound* as a clear oil (494 mg, 1.47 mmol, 41%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.20 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.90–1.81 (m, 2H, -C$\underline{H}_2$Br), 1.61–1.55 (m, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.44 (s, 9H, ^{t}Bu), 1.31–1.28 (m, 14H, 7 × -C$\underline{H}_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{31}{}^{79}BrO_2$ 334, found 335 $[M+H]^+$.

***tert*-Butyl 14-bromotetradecanoate 71:**



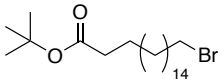
To a solution of 14-bromotetradecanoic acid (1.00 g, 3.26 mmol, 1.0 eq.) in ^{t}BuOH (33 mL, 0.1 M) was added Boc_2O (1.42 g, 6.51 mmol, 2.0 eq.) and DMAP (119 mg, 0.98 mmol, 0.3 eq.). The reaction was allowed to stir overnight at room temperature. Upon completion the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 8:2 toluene) afforded the *title compound* as a clear oil (385 mg, 1.06 mmol, 33%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.20 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.92–1.81 (m, 2H, -C$\underline{H}_2$Br), 1.61–1.55 (m, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.44 (s, 9H, ^{t}Bu), 1.35–1.23 (m, 18H, 9 × -C$\underline{H}_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{35}{}^{79}BrO_2$ 362, found 363 $[M+H]^+$.

tert-Butyl 16-bromohexadecanoate 72:



To a solution of 16-bromohexadecanoic acid (1.00 g, 2.99 mmol, 1.0 eq.) in ^{t}BuOH (30 mL, 0.1 M) was added Boc_2O (1.30 g, 5.97 mmol, 2.0 eq.) and DMAP (109 mg, 0.90 mmol, 0.3 eq.). The reaction was allowed to stir overnight at room temperature. Upon completion the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 8:2 toluene) afforded the *title compound* as a clear oil which solidified on standing (399 mg, 1.02 mmol, 34%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.20 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.89–1.81 (m, 2H, -C$\underline{H}_2$Br), 1.64–1.56 (m, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.45 (s, 9H, ^{t}Bu), 1.34–1.23 (m, 22H, 11 × -C$\underline{H}_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{39}{}^{79}BrO_2$ 390, found 391 $[M+H]^+$.

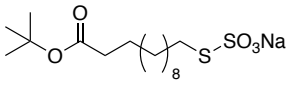
tert-Butyl 18-bromooctadecanoate 73:



To a solution of 18-bromohexadecanoic acid (1.00 g, 2.76 mmol, 1.0 eq.) in ^{t}BuOH (28 mL, 0.1 M) was added Boc_2O (1.20 g, 5.51 mmol, 2.0 eq.) and DMAP (101 mg, 0.83 mmol, 0.3 eq.). The reaction was allowed to stir overnight at room temperature. Upon completion the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 8:2 toluene) afforded the *title compound* as a clear oil which solidified on standing (397 mg, 0.95 mmol, 35%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.41 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$Br), 2.20 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.89–1.81 (m, 2H, -C$\underline{H}_2$Br), 1.62–1.55 (m, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.44 (s,

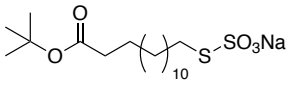
9H, ^{t}Bu), 1.33–1.26 (m, 26H, 13 × -C$\underline{H}_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{22}H_{43}{}^{79}BrO_2$ 418, found 419 $[M+H]^+$.

Sodium *S*-(12-(*tert*-butoxy)-12-oxododecyl) sulfurothioate 74:



To a solution of *tert*-butyl 12-bromododecanoate (200 mg, 0.60 mmol, 1.0 eq.) in H_2O (0.37 mL, 1.61 M) and MeOH (0.93 mL, 0.64 M) was added sodium thiosulfate (178 mg, 0.72 mmol, 1.2 eq.). The reaction was allowed to stir at 65 °C overnight. Upon completion the mixture was concentrated *in vacuo*. The resulting residue was dissolved in MeOH (2 mL), heated to 50 °C and filtered through a frit. Further purification *via* trituration in hexane afforded the *title compound* as a white solid (178 mg, 0.46 mmol, 76%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.80 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SSO$_3$Na), 2.16 (t, *J* 7.2 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.64–1.56 (m, 2H, -C$\underline{H}_2$SSO$_3$Na), 1.49–1.44 (m, 2H, -C$\underline{H}_2$COO^{t}Bu), 1.39 (s, 9H, ^{t}Bu), 1.32–1.20 (m, 14H, 7 × -C$\underline{H}_2$).

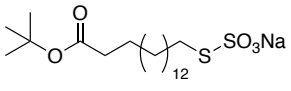
Sodium *S*-(14-(*tert*-butoxy)-14-oxotetradecyl) sulfurothioate 75:



To a solution of *tert*-butyl 14-bromotetradecanoate (200 mg, 0.55 mmol, 1.0 eq.) in H_2O (0.34 mL, 1.61 M) and MeOH (0.86 mL, 0.64 M) was added sodium thiosulfate (164 mg, 0.66 mmol, 1.2 eq.). The reaction was allowed to stir at 65 °C overnight. Upon completion the mixture was concentrated *in vacuo*. The resulting residue was dissolved in MeOH (2 mL), heated to 50 °C and filtered through a frit. Further purification *via* trituration in hexane afforded the *title compound* as a white solid (159 mg, 0.38 mmol, 69%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.80 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$SSO$_3$Na), 2.16 (t, *J* 7.2 Hz, 2H, -C$\underline{H}_2$COO^{t}Bu),

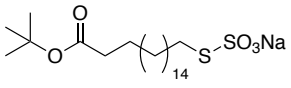
1.64–1.56 (m, 2H, -C<u>H</u>$_2$SSO$_3$Na), 1.52–1.44 (m, 2H, -C<u>H</u>$_2$COO^{t}Bu), 1.39 (s, 9H, ^{t}Bu), 1.33–1.21 (m, 18H, 9 × -C<u>H</u>$_2$).

Sodium *S*-(16-(*tert*-butoxy)-16-oxohexadecyl) sulfurothioate 76:



To a solution of *tert*-butyl 16-bromohexadecanoate (300 mg, 0.77 mmol, 1.0 eq.) in H_2O (0.48 mL, 1.61 M) and MeOH (1.20 mL, 0.64 M) was added sodium thiosulfate (229 mg, 0.92 mmol, 1.2 eq.). The reaction was allowed to stir at 65 °C overnight. Upon completion the mixture was concentrated *in vacuo*. The resulting residue was dissolved in MeOH (3 mL), heated to 50 °C and filtered through a frit. Further purification *via* trituration in hexane afforded the *title compound* as a white solid (286 mg, 0.64 mmol, 84%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.80 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$SSO$_3$Na), 2.16 (t, *J* 7.2 Hz, 2H, -C<u>H</u>$_2$COO^{t}Bu), 1.64–1.56 (m, 2H, -C<u>H</u>$_2$SSO$_3$Na), 1.49–1.44 (m, 2H, -C<u>H</u>$_2$COO^{t}Bu), 1.39 (s, 9H, ^{t}Bu), 1.32–1.22 (m, 22H, 11 × -C<u>H</u>$_2$).

Sodium *S*-(18-(*tert*-butoxy)-18-oxooctahexadecyl) sulfurothioate 77:

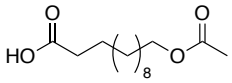


To a solution of *tert*-butyl 18-bromooctadecanoate (400 mg, 0.96 mmol, 1.0 eq.) in H_2O (0.60 mL, 1.61 M) and MeOH (1.50 mL, 0.64 M) was added sodium thiosulfate (284 mg, 1.15 mmol, 1.2 eq.). The reaction was allowed to stir at 65 °C overnight. Upon completion the mixture was concentrated *in vacuo*. The resulting residue was dissolved in MeOH (3 mL), heated to 50 °C and filtered through a frit. Further purification *via* trituration in hexane afforded the *title compound* as a white solid (413 mg, 0.83 mmol, 87%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.80 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$SSO$_3$Na), 2.16 (t, *J* 7.2 Hz, 2H, -C<u>H</u>$_2$COO^{t}Bu),

1.65–1.57 (m, 2H, -C<u>H</u>$_2$SSO$_3$Na), 1.49–1.44 (m, 2H, -C<u>H</u>$_2$COO^{t}Bu), 1.39 (s, 9H, ^{t}Bu), 1.32–1.21 (m, 16H, 8 × -C<u>H</u>$_2$).

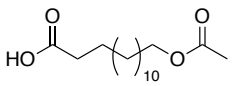
5.6 Preparation of acetylated series

12-Acetoxydodecanoic acid 96:



A flask was charged with 12-hydroxydodecanoic acid **30** (500 mg, 2.3 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (4.6 mL, 0.5 M) and acetic anhydride (4.6 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 20 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (535 mg, 2.1 mmol, 90%). **M.pt**: 39–41°C; **IR** (ATR/cm^{-1}): 2914, 1847, 1729, 1694, 1430, 1240; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 6.5 Hz, 2H, -C<u>H</u>$_2$OC(O)CH$_3$), 2.34 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOH), 2.04 (s, 3H, -OC(O)C<u>H</u>$_3$), 1.66–1.58 (m, 4H, 2 × -C<u>H</u>$_2$), 1.32–1.27 (m, 14H, 7 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.9, 171.5, 64.8, 34.1, 29.6, 29.5, 29.4, 29.3, 29.2, 28.7, 26.0, 24.8, 21.2 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{26}O_4$ 258, found 259 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{14}H_{26}O_4$ 257.1758 found 257.1757 $[M-H]^+$.

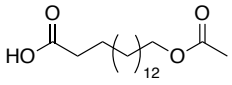
14-Acetoxytetradecanoic acid 97:



A flask was charged with 14-hydroxytetradecanoic acid **31** (50 mg, 0.2 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.4 mL, 0.5 M) and acetic anhydride (0.4 mL, 0.5 M) were added. The

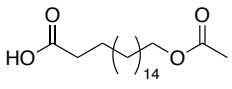
reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (50 mg, 0.18 mmol, 85%). **M.pt**: 44–46 °C; **IR** (ATR/cm^{-1}): 2914, 2847, 1731, 1703, 1465, 1257; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OC(O)CH_3), 2.34 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.04 (s, 3H, -OC(O)C$\underline{H}_3$), 1.68–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.33–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 178.4, 171.4, 64.8, 35.4, 33.9, 29.7, 29.6, 29.5, 29.4, 29.4, 29.2, 29.0, 28.8, 26.1, 24.8, 21.2; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{30}O_4$ 286, found 285 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{16}H_{30}O_4$ 285.2071, found 285.2067 $[M-H]^+$.

16-Acetoxyhexadecanoic acid 98:



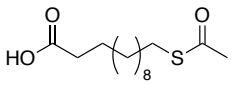
A flask was charged with 16-hydroxyhexadecanoic acid **32** (50 mg, 0.18 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.37 mL, 0.5 M) and acetic anhydride (0.37 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (57 mg, 0.18 mmol, 98%). **M.pt**: 47–49°C; **IR** (ATR/cm^{-1}): 2914, 2847, 1729, 1703, 1465, 1249; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OC(O)CH_3), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.04 (s, 3H, -OC(O)C$\underline{H}_3$), 1.68–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.33–1.25 (m, 22H, 11 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 177.9, 171.4, 64.8, 35.5, 33.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.4, 29.2, 29.0, 28.8, 26.1, 24.9, 24.4, 21.2; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{34}O_4$ 314, found 313 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{18}H_{34}O_4$ 313.2384, found 313.2381 $[M-H]^+$.

18-Acetoxyoctadecanoic acid 99:



A flask was charged with 18-hydroxyoctadecanoic acid **33** (40 mg, 0.13 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.27 mL, 0.5 M) and acetic anhydride (0.27 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (44 mg, 0.13 mmol, 96%). **M.pt**: 61–63°C; **IR** (ATR/cm^{-1}): 2916, 2847, 1731, 1703, 1474, 1246; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.05 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OC(O)CH_3), 2.34 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.04 (s, 3H, -OC(O)C$\underline{H}_3$), 1.66–1.60 (m, 4H, 2 × -C$\underline{H}_2$), 1.33–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.8, 171.5, 64.8, 35.5, 33.5, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.4, 29.2, 29.0, 28.8, 26.1, 24.9, 24.4, 21.2 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{38}O_4$ 342, found 341 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{38}O_4$ 341.2697, found 341.2694 $[M-H]^+$.

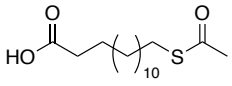
12-(Acetylthio)dodecanoic acid 100:



A flask was charged with 12-mercaptododecanoic acid **58** (50 mg, 0.2 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.4 mL, 0.5 M) and acetic anhydride (0.4 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 20 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (41 mg,

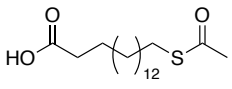
0.15 mmol, 70%). **M.pt**: 56–58°C; **IR** (ATR/cm^{-1}): 3061, 2916, 2851, 1694, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.86 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 1.63 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$$CH_2$SC(O)$CH_3$), 1.56 (app p, 7.5 Hz, 2H, -C$\underline{H}_2$$CH_2$COOH), 1.35–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 196.3, 178.5, 33.9, 30.8, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.2, 29.2, 28.9, 24.8; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{26}O_3S$ 274, found 275 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{14}H_{26}O_3S$ 273.1530, found 273.1532 $[M-H]^+$.

14-(Acetylthio)tetradecanoic acid 101:



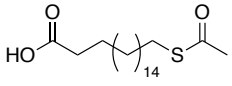
A flask was charged with 14-mercaptotetradecanoic acid **59** (50 mg, 0.19 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.39 mL, 0.5 M) and acetic anhydride (0.39 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (54 mg, 0.18 mmol, 93%). **M.pt**: 59–61 °C; **IR** (ATR/cm^{-1}): 2914, 1849, 1800, 1740, 1688, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.86 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 1.65 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$$CH_2$SC(O)$CH_3$), 1.56 (app p, 7.5 Hz, 2H, -C$\underline{H}_2$$CH_2$COOH), 1.35–1.25 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 196.2, 177.9, 33.8, 30.8, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 29.3, 29.2, 29.0, 29.0, 28.9, 24.9; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{30}O_3S$ 302, found 301 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{16}H_{30}O_3S$ 301.1843, found 301.1841 $[M-H]^+$.

16-(Acetylthio)hexadecanoic acid 102:



A flask was charged with 16-mercaptohexadecanoic acid **60** (50 mg, 0.17 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.35 mL, 0.5 M) and acetic anhydride (0.35 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (55 mg, 0.17 mmol, 97%). **M.pt**: 65–67 °C; **IR** (ATR/cm^{-1}): 2914, 2849, 1798, 1740, 1688, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.86 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 1.66 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$$CH_2$SC(O)$CH_3$), 1.56 (app p, 7.5 Hz, 2H, -C$\underline{H}_2$$CH_2$COOH), 1.36–1.25 (m, 22H, 11 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 196.2, 169.8, 35.5, 33.8, 30.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 29.3, 29.2, 29.0, 29.0, 24.9, 24.4; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{34}O_3S$ 330, found 331 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{18}H_{34}O_3S$ 329.2156, found 329.2152 $[M-H]^+$.

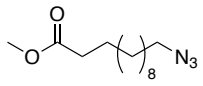
18-(Acetylthio)octadecanoic acid 103:



A flask was charged with 18-mercaptooctadecanoic acid **61** (50 mg, 0.16 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.32 mL, 0.5 M) and acetic anhydride (0.32 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (19 mg,

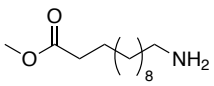
0.05 mmol, 33%). **M.pt**: 74–76 °C; **IR** (ATR/cm^{-1}): 2914, 2849, 1800, 1742, 1688, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.86 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$SC(O)CH_3), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 2.32 (s, 3H, -SC(O)C$\underline{H}_3$), 1.66 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$$CH_2$SC(O)$CH_3$), 1.56 (app p, 7.5 Hz, 2H, -C$\underline{H}_2$$CH_2$COOH), 1.36–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 196.2, 169.8, 35.5, 33.8, 30.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.0, 29.0, 24.4 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{38}O_3S$ 358, found 357 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{38}O_3S$ 357.2469, found 357.2467 $[M-H]^+$.

Methyl 12-azidododecanoate 108:



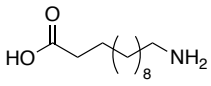
To a stirred solution of methyl 12-bromododecanoate **90** (2.00 g, 6.8 mmol, 1.0 eq.) in anhydrous DMF (57 mL, 0.12 M) was added sodium azide (2.66 g, 41 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, H_2O (25 mL) was added and the reaction mixture was extracted with EtOAc (4 × 40 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 97:3 EtOAc), afforded the *title compound* as a clear oil that solidified on standing (1.32 g, 5.2 mmol, 76%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2924, 2854, 2091, 1738, 1437; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.25 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$$N_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH_3), 1.65–1.57 (m, 4H, 2 × -C$\underline{H}_2$), 1.38–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5, 51.6, 51.6, 34.3, 29.8, 29.6, 29.5, 29.4, 29.3, 29.0, 26.9, 25.1 (1 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{13}H_{25}N_3O_2$ 255, found 256 $[M+H]^+$.

Methyl 12-aminododecanoate 112:[143]



A microwave vial was charged with methyl 12-azidododecanoate **108** (200 mg, 0.78 mmol, 1.0 eq.) and palladium on carbon (8.00 mg, 0.08 mmol, 0.1 eq.), flame dried and placed under Ar. Anhydrous EtOAc (9.2 mL, 0.09 M) was added and H_2 was bubbled through the solution for 10 minutes. The reaction mixture was allowed to stir overnight at room temperature, under a H_2 atmosphere. Upon completion, the resultant solution was filtered through Celite ® and washed with EtOAc (4 × 20 mL). Evaporation under reduced pressure afforded the *title compound* as a white solid (156 mg, 0.68 mmol, 87%). **M.pt**: 81–83 °C; **IR** (ATR/cm^{-1}): 3390, 3306, 2916, 2849, 1731, 1437; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.70 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$NH$_2$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.63–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.28–1.25 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.4, 51.6, 42.4, 34.3, 34.0, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 27.0, 25.1; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{13}H_{27}NO_2$ 229, found 230 $[M+H]^+$.

12-Aminododecanoic acid 116:

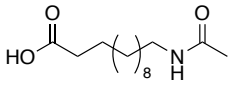


To a solution of methyl 12-azidododecanoate **108** (500 mg, 1.96 mmol, 1.0 eq.) in Et_2O (20 mL, 0.1 M) was added triphenylphosphine (617 mg, 2.35 mmol, 1.2 eq.) in Et_2O (4.0 mL, 0.5 M) drop-wise at 0°C. After 1 hour at 0°C, H_2O (2.0 mL, 1.0 M) was added and the resultant solution allowed to stir overnight at room temperature. Upon completion the mixture was poured over ice and extracted with Et_2O (4 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford methyl 12-aminododecanoate as a crude mixture that was used in the next step without purification.

To a 1:1 solution of THF/H_2O (18 mL, 0.2 M) was added methyl 12-aminododecanoate **112** (840 mg, 3.67 mmol, 1.0 eq.). A solution of

2.0 M NaOH (37 mL, 0.1 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (40 mL) and washed with 10 mL each of 1.0 M HCl, H_2O and brine. The combined aqueous layers were allowed to stand overnight until a white precipitate formed. The precipitate was filtered to afford the *title compound* as a white solid (113 mg, 0.53 mmol, 27% over 2 steps). **M.pt**: 158–160 °C; **IR** (ATR/cm^{-1}): 3145, 3133, 1713, 1556, 1467; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.31 (br s, 2H, -N$\underline{H}_2$), 2.74 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$NH$_2$), 2.18 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.53–1.48 (m, 4H, 2 × -C$\underline{H}_2$), 1.25 (br s, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 175.0, 34.0, 29.1, 29.1, 29.0, 28.9, 28.7, 28.7, 27.2, 26.0, 24.7 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{25}NO_2$ 215, found 216 $[M+H]^+$.

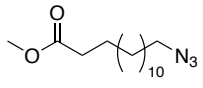
12-Acetamidododecanoic acid 104:



A flask was charged with 12-aminododecanoic acid **116** (50 mg, 0.23 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.47 mL, 0.5 M) and acetic anhydride (0.47 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 with 2.0 M HCl and the resultant solution extracted with EtOAc (4 × 10 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (19 mg, 0.07 mmol, 32%). **M.pt**: 97–99 °C; **IR** (ATR/cm^{-1}): 3347, 3321, 2918, 2851, 1800.1742, 1633; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 5.45 (br s, 1H, -N$\underline{H}$), 3.23 (app q, *J* 6.5 Hz, 2H, -C$\underline{H}_2$NHC(O)CH$_3$), 2.44 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.97 (s, 3H, -NHC(O)C$\underline{H}_3$), 1.65 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$NHC(O)CH$_3$), 1.51–1.46 (m, 2H, -C$\underline{H}_2$COOH), 1.35–1.27 (m, 14H, 7 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.5, 168.8, 38.5, 33.6, 29.1, 29.0, 28.9, 28.8, 28.7, 28.5, 26.4, 24.5, 22.6 (1 carbon missing); **LRMS** (LC-MS-ESI)

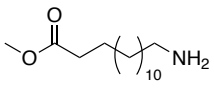
m/z calc. for $C_{14}H_{27}NO_3$ 257, found 258 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{14}H_{27}NO_3$ 256.1918, found 256.1919 $[M-H]^+$.

Methyl 14-azidotetradecanoate 109:



To a stirred solution of methyl 14-bromotetradecanoate **42** (1.50 g, 4.7 mmol, 1.0 eq.) in anhydrous DMF (39 mL, 0.12 M) was added sodium azide (1.82 g, 28 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, H_2O (25 mL) was added and the reaction mixture was extracted with EtOAc (4 × 40 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 97:3 EtOAc), afforded the *title compound* as a clear oil that solidified on standing (933 mg, 3.3 mmol, 71%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2924, 2852, 2092, 1737, 1435; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.25 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$N$_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.65–1.57 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.5, 51.7, 51.6, 34.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.0, 26.9, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{29}N_3O_2$ 283, found 284 $[M+H]^+$.

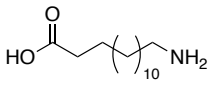
Methyl 14-aminotetradecanoate 113:[144]



A microwave vial was charged with methyl 14-azidotetradecanoate **109** (100 mg, 0.35 mmol, 1.0 eq.) and palladium on carbon (4.00 mg, 0.04 mmol, 0.1 eq.), flame dried and placed under Ar. Anhydrous EtOAc (4.2 mL, 0.085 M) was added and H_2 was bubbled through the solution for 10 minutes. The reaction mixture was allowed to stir overnight at room temperature, under a H_2

atmosphere. Upon completion, the resultant solution was filtered through Celite® and washed with EtOAc (4 × 10 mL). Evaporation under reduced pressure afforded the *title compound* as a white solid (82 mg, 0.32 mmol, 90%). **M.pt**: 84–86 °C; **IR** (ATR/cm^{-1}): 3352, 3342, 2914, 2847, 1731, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.68 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$NH$_2$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.62 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$NH$_2$), 1.43 (app p, *J* 7.5 Hz, 2H, -C$\underline{H}_2$CH$_2$COOCH$_3$), 1.33–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.5, 51.6, 42.5, 34.3, 34.1, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 27.1, 25.1; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{31}NO_2$ 257, found 258 $[M+H]^+$.

14-Aminotetradecanoic acid 117:[145]

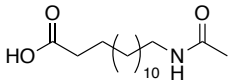


To a solution of methyl 14-azidotetradecanoate **109** (500 mg, 1.77 mmol, 1.0 eq.) in Et_2O (18 mL, 0.1 M) was added triphenylphosphine (556 mg, 2.12 mmol, 1.2 eq.) in Et_2O (3.5 mL, 0.5 M) drop-wise at 0°C. After 1 hour at 0°C, H_2O (2.0 mL, 1.0 M) was added and the resultant solution allowed to stir overnight at room temperature. Upon completion the mixture was poured over ice and extracted with Et_2O (4 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford methyl 14-aminotetradecanoate as a crude mixture that was used in the next step without purification.

To a 1:1 solution of THF/H_2O (10 mL, 0.20 M) was added methyl 14-aminotetradecanoate **113** (454 mg, 1.77 mmol, 1.0 eq.). A solution of 2.0 M NaOH (18 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (40 mL) and washed with 10 mL each of 1.0 M HCl, H_2O and brine. The combined aqueous layers were allowed to stand overnight until a white precipitate formed. The precipitate was filtered to afford the *title compound* as a

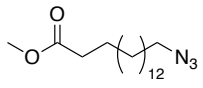
white solid (178 mg, 0.73 mmol, 41% over 2 steps). **M.pt**: 161–163 °C; **IR** (ATR/cm^{-1}): 3055, 3024, 2914, 2849, 1729, 1473; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.31 (br s, 2H, -N$\underline{H}_2$), 2.74 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$NH$_2$), 2.18 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.57–1.49 (m, 4H, 2 × -C$\underline{H}_2$), 1.26 (br s, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.8, 34.1, 29.2, 29.1, 29.0, 29.0, 28.9, 28.7, 28.7, 27.2, 26.0, 24.7 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{29}NO_2$ 243, found 244 $[M+H]^+$.

14-Acetamidotetradecanoic acid 105:



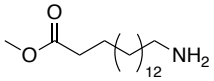
A flask was charged with 14-aminotetradecanoic acid **117** (50 mg, 0.21 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.41 mL, 0.5 M) and acetic anhydride (0.41 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 with 2.0 M HCl and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (22 mg, 0.08 mmol, 37%). **M.pt**: 98–100 °C; **IR** (ATR/cm^{-1}): 3044, 3027, 2914, 2851, 1731, 1603, 1473; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.44 (br s, 1H, -N$\underline{H}$), 3.23 (app q, *J* 6.5 Hz, 2H, -C$\underline{H}_2$NHC(O)CH$_3$), 2.44 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.97 (s, 3H, -NHC(O)C$\underline{H}_3$), 1.66 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$NHC(O)CH$_3$), 1.52–1.46 (m, 2H, -C$\underline{H}_2$ CH$_2$COOH), 1.37–1.26 (m, 18H, 9 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 175.7, 170.8, 34.3, 29.5, 29.4, 29.4, 29.3, 29.2, 29.2, 29.0, 26.9, 25.0, 23.0 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{31}NO_3$ 285, found 286 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{16}H_{31}NO_3$ 284.2231, found 284.2230 $[M-H]^+$.

Methyl 16-azidohexadecanoate 110:



To a stirred solution of methyl 16-bromohexadecanoate **91** (2.0 g, 5.7 mmol, 1.0 eq.) in anhydrous DMF (48 mL, 0.12 M) was added sodium azide (2.2 g, 34 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, H_2O (25 mL) was added and the reaction mixture was extracted with EtOAc (4 × 40 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 97:3 EtOAc), afforded the *title compound* as a clear oil that solidified on standing (1.3 g, 4.2 mmol, 73%). **M.pt**: <30 °C; **IR** (ATR/cm^{-1}): 2920, 2849, 2092, 1735, 1430; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.25 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$N$_3$), 2.30 (t, *J* 7.5 Hz, 2H, C$\underline{H}_2$COOCH$_3$), 1.63–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.36–1.25 (m, 22H, 11 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 51.7, 51.6, 34.3, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.0, 26.9, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{33}N_3O_2$ 311, found 310 $[M-H]^+$.

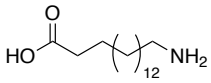
Methyl 16-aminohexadecanoate 114:[146]



A microwave vial was charged with methyl 16-azidohexadecanoate **110** (200 mg, 0.64 mmol, 1.0 eq.) and palladium on carbon (7.00 mg, 0.06 mmol, 0.1 eq.), flame dried and placed under Ar. Anhydrous EtOAc (7.6 mL, 0.085 M) was added and H_2 was bubbled through the solution for 10 minutes. The reaction mixture was allowed to stir overnight at room temperature, under a H_2 atmosphere. Upon completion, the resultant solution was filtered through Celite® and washed with EtOAc (4 × 20 mL). Evaporation under reduced pressure afforded the *title compound* as a white solid (136 mg, 0.48 mmol, 74%). **M.pt**: 87–89 °C; **IR** (ATR/cm^{-1}): 3349, 3306, 2914, 2849, 1731, 1471; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.68 (t, *J* 7.0 Hz,

2H, -C<u>H</u>$_2$NH$_2$), 2.30 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOCH$_3$), 1.62 (app p, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH$_2$NH$_2$), 1.43–1.40 (m, 2H, -C<u>H</u>$_2$CH$_2$COOCH$_3$), 1.32–1.25 (m, 22H, 11 × -C<u>H</u>$_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.5, 51.6, 42.4, 34.3, 34.0, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 27.0, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{35}NO_2$ 285, found 286 $[M+H]^+$.

16-Aminohexadecanoic acid 118:[147]

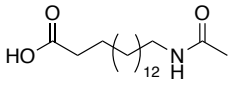


To a solution of methyl 16-azidohexadecanoate **110** (500 mg, 1.61 mmol, 1.0 eq.) in Et_2O (16 mL, 0.1 M) was added drop-wise a solution of triphenylphosphine (422 mg, 1.61 mmol, 1.0 eq.) in Et_2O (3 mL, 0.54 M) at 0 °C. The resultant solution was stirred at 0 °C for 2 hours. H_2O (1 mL, 1.0 M) was then added and the reaction mixture stirred overnight at room temperature. The mixture was poured over ice H_2O (20 mL) and extracted with Et_2O (3 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford methyl 14-aminotetradecanoate as a crude mixture that was used in the next step without purification.

To a 1:1 solution of THF/H_2O (8.0 mL, 0.20 M) was added methyl 16-aminohexadecanoate **114** (450 mg, 1.6 mmol, 1.0 eq.). A solution of 2 M NaOH (16 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 24 hours. Upon completion the reaction mixture was diluted with EtOAc (10 mL) and washed with 10 mL each of 2.0 M HCl, H_2O and brine. The combined aqueous layers were allowed to stand overnight until a white precipitate formed. The precipitate was filtered to afford the *title compound* as a white solid (120 mg, 0.44 mmol, 39% over 2 steps). **M.pt**: 158–160 °C; **IR** (ATR/cm^{-1}): 3063, 3031, 2912, 2847, 1727, 1473; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.91 (br s, 1H, -COO<u>H</u>), 3.31 (br s, 2H, -N<u>H</u>$_2$), 2.74 (t, *J* 7.2 Hz, 2H, -C<u>H</u>$_2$NH$_2$), 2.18 (t, *J* 7.6 Hz, 2H, -C<u>H</u>$_2$COOH), 1.54–1.46 (m, 4H, 2 × -C<u>H</u>$_2$), 1.34–1.24 (m, 22H, 11 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.5, 33.6,

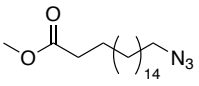
29.0, 29.0, 28.9, 28.9, 28.8, 28.7, 28.5, 28.5, 26.9, 25.8, 24.5 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{33}NO_2$ 271, found 272 $[M+H]^+$.

16-Acetamidohexadecanoic acid 106:



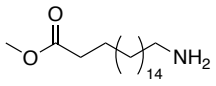
A flask was charged with 16-aminohexadecanoic acid **118** (50 mg, 0.19 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.37 mL, 0.5 M) and acetic anhydride (0.37 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 with 2.0 M HCl and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (21 mg, 0.07 mmol, 36%). **M.pt**: 110–112°C; **IR** (ATR/cm^{-1}): 3059, 3033, 2914, 2849, 1802, 1729, 1473; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.55 (br s, 1H, -NH), 2.99 (app q, *J* 6.5 Hz, 2H, -CH_2NHC(O)CH_3), 2.18 (t, *J* 7.5 Hz, 2H, -CH_2COOH), 1.77 (s, 3H, -NHC(O)CH_3), 1.50–1.45 (m, 2H, -CH_2CH_2NHC(O)CH_3), 1.38–1.33 (m, 2H, -CH_2COOH), 1.23 (br s, 22H, 11 × -CH_2); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 175.1, 170.0, 34.0, 29.3, 29.2, 29.2, 29.1, 29.0, 29.0, 28.8, 26.7, 24.8, 22.8 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{35}NO_3$ 313, found 314 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{18}H_{35}NO_3$ 312.2544, found 312.2542 $[M-H]^+$.

Methyl 18-azidooctadecanoate 111:



To a stirred solution of methyl 18-bromooctadecanoate **43** (2.00 g, 5.3 mmol, 1.0 eq.) in anhydrous DMF (44 mL, 0.12 M) was added sodium azide (2.07 g, 32 mmol, 6.0 eq.). The resultant solution was stirred at 80 °C for 48 hours. Upon completion, H_2O (25 mL) was added and the reaction mixture was extracted with EtOAc (4 × 40 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether 97:3 EtOAc), afforded the *title compound* as a clear oil that solidified on standing (1.64 mg, 4.8 mmol, 91%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 2095, 1738, 1437; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 3.25 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$N$_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.63–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 51.7, 51.6, 34.3, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.0, 26.9, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{37}N_3O_2$ 339, found 338 $[M-H]^+$.

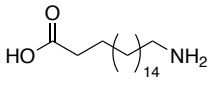
Methyl 18-aminooctadecanoate 115:



A microwave vial was charged with methyl 18-azidooctadecanoate **111** (200 mg, 0.59 mmol, 1.0 eq.) and palladium on carbon (6.00 mg, 0.06 mmol, 0.1 eq.), flame dried and placed under Ar. Anhydrous EtOAc (6.9 mL, 0.085 M) was added and H_2 was bubbled through the solution for 10 minutes. The reaction mixture was allowed to stir overnight at room temperature, under a H_2 atmosphere. Upon completion, the resultant solution was filtered through Celite® and washed with EtOAc (4 × 20 mL). Evaporation under reduced pressure afforded the *title compound* as a white solid (163 mg, 0.52 mmol, 88%). **M.pt**: 88–90 °C; **IR** (ATR/cm^{-1}): 3349, 3306, 2914, 2849, 1731, 1471; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.68 (t, *J* 7.0 Hz, 2H, -C$\underline{H}_2$NH$_2$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.63 (app p, *J* 7.0 Hz, 2H, -C$\underline{H}_2$CH$_2$NH$_2$), 1.45–1.41 (m, 2H, -C$\underline{H}_2$CH$_2$COOCH$_3$), 1.32–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 174.5, 51.6, 42.4, 34.3, 34.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3,

27.1, 25.1 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{39}NO_2$ 313, found 314 $[M+H]^+$.

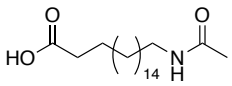
18-Aminooctadecanoic acid 119:[135]



To a solution of methyl 18-azidooctadecanoate **111** (500 mg, 1.48 mmol, 1.0 eq.) in Et_2O (15 mL, 0.10 M) was added triphenylphosphine (464 mg, 1.77 mmol, 1.2 eq.) in Et_2O (3.0 mL, 0.50 M) drop-wise at 0°C. After 1 hour at 0°C, H_2O (1.5 mL, 1.0 M) was added and the resultant solution allowed to stir overnight at room temperature. Upon completion the mixture was poured over ice and extracted with Et_2O (4 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford methyl 18-aminooctadecanoate as a crude mixture that was used in the next step without purification.

To a 1:1 solution of THF/H_2O (8.0 mL, 0.20 M) was added methyl 18-aminooctadecanoate **115** (462 mg, 1.48 mmol, 1.0 eq.). A solution of 2.0 M NaOH (15 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (40 mL) and washed with 10 mL each of 1.0 M HCl, H_2O and brine. The combined aqueous layers were allowed to stand overnight until a white precipitate formed. The precipitate was filtered to afford the *title compound* as a white solid (185 mg, 0.62 mmol, 42% over 2 steps). **M.pt**: 162–164 °C; **IR** (ATR/cm^{-1}): 3063, 3031, 2912, 2847, 1727, 1473; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.31 (br s, 2H, -N$\underline{H}_2$), 2.78–2.74 (m, 2H, -C$\underline{H}_2$NH$_2$), 2.18 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.54–1.46 (m, 4H, 2 × -C$\underline{H}_2$), 1.30–1.24 (m, 26H, 13 × -C$\underline{H}_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.4, 33.6, 29.0, 29.0, 28.9, 28.9, 28.8, 28.7, 28.5, 28.5, 26.9, 25.8, 24.5 (5 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{37}NO_2$ 299, found 298 $[M-H]^+$.

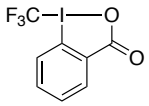
18-Acetamidooctadecanoic acid 107:



A flask was charged with 18-aminooctadecanoic acid **119** (50 mg, 0.17 mmol, 1.0 eq.), flame-dried and placed under Ar. The flask was then cooled to 0 °C and pyridine (0.33 mL, 0.5 M) and acetic anhydride (0.33 mL, 0.5 M) were added. The reaction mixture was stirred at room temperature overnight. Upon completion, the pH was adjusted to 3 with 1.0 M HCl and the resultant solution extracted with EtOAc (4 × 5 mL). The combined organics were dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (25 mg, 0.07 mmol, 44%). **M.pt**: 118–120 °C; **IR** (ATR/cm^{-1}): 3318, 3039, 2914, 2849, 1804, 1729, 1473; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 3.55 (br s, 1H, -NH), 2.99 (app q, *J* 6.5 Hz, 2H, -CH_2NHC(O)CH_3), 2.18 (t, *J* 7.5 Hz, 2H, -CH_2COOH), 1.77 (s, 3H, -NHC(O)CH_3), 1.50–1.45 (m, 2H, -CH_2CH_2NHC(O)CH_3), 1.37–1.33 (m, 2H, -CH_2COOH), 1.23 (br s, 26H, 13 × -CH_2); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.6, 169.0, 33.7, 29.1, 29.0, 29.0, 28.9, 28.8, 28.8, 28.6, 26.5, 24.5, 22.6 (7 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{39}NO_3$ 341, found 342 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{39}NO_3$ 340.2857, found 340.2854 $[M-H]^+$.

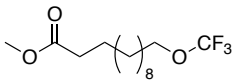
5.7 Preparation of Trifluoromethyl Series

1-(trifluoromethyl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one:[102]



To a 3-neck flask fitted with a condenser, under an atmosphere of Ar, was added 2-iodobenzoic acid (1.0 g, 4.03 mmol, 1.0 eq.), followed by anhydrous MeCN (9.0 mL, 0.46 M). The resultant solution was heated to 75 °C before the addition of TCICA (319 mg, 1.37 mmol, 0.34 eq.) in anhydrous MeCN (2.0 mL, 1.86 M). Following addition, the reaction was allowed to cool to room temperature. Dry KOAc was then added, and the resultant suspension heated to 75 °C for 2 hours. After 2 hours, the mixture was allowed to cool to room temperature. Ruppert's reagent was then added and the mixture allowed to stir at room temperature for 12 hours. A further portion of anhydrous MeCN was added (4.0 mL, 1.10 M) and the suspension brought to reflux before filtration through a 1 cm thick pad of celite®. The brown filtrate was then concentrated *in vacuo* to 1/3 of its volume before cooling to -20 °C with stirring. The resultant crystals were filtered and washed with cold MeCN (3 × 10 mL) to afford the *title compound* as a white solid (707 mg, 2.24 mmol, 56%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.44 (dd, *J* 8.0, 2.0 Hz, 1H, Ar<u>H</u>), 7.83–7.74 (m, 3H, 3 × Ar<u>H</u>); **^{19}F NMR** (125 MHz, $CDCl_3$) δ -33.8 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 166.1, 135.8, 133.8, 132.0, 127.4 (q, J_{FC} 3.25 Hz, 1C, Ar<u>C</u>), 114.9, 107.1 (q, J_{FC} 377.9 Hz, 1C, -<u>C</u>F_3); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{25}{}^{19}F_3O_3$ 315, found 314 $[M-H]^+$.

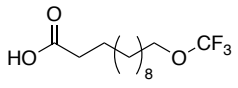
Methyl 12-(trifluoromethoxy)dodecanoate 129:



A microwave vial was charged with methyl 12-hydroxydodecanoate **52** (100 mg, 0.44 mmol, 1.0 eq.), potassium fluoride (KF) (101 mg, 1.7 mmol, 4.0

eq.), Selectfluor® (231 mg, 0.65 mmol, 1.5 eq.) and silver triflate (AgOTf) (335 mg, 1.3 mmol, 3.0 eq.), flame-dried and placed under Ar. Anhydrous EtOAc (2.2 mL, 0.2 M) was added, followed by 2-fluoropyridine (0.11 mL, 1.3 mmol, 3.0 eq.) and Ruppert's reagent (0.19 mL, 1.3 mmol, 3.0 eq.). The resulting solution was stirred at room temperature for 12 hours before filtration through a plug of silica. Further purification *via* flash column chromatography (petroleum ether 100:1 EtOAc) afforded the *title compound* as a pale yellow oil (87 mg, 0.29 mmol, 67%) **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2924, 2854, 1740, 1262, 1136; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.71–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.43–1.33 (m, 2H, -C$\underline{H}_2$), 1.33–1.26 (m, 12H, 6 × -C$\underline{H}_2$); **^{19}F NMR** (125 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.4, 121.9 (q, J_{FC} 252.1 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 3.1 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 51.6, 34.3, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 28.9, 25.6, 25.1; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{25}{}^{19}F_3O_3$ 298, found 299 $[M+H]^+$.

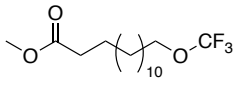
12-(Trifluoromethoxy)dodecanoic acid 120:



To a 1:1 solution of THF/H_2O (0.8 mL, 0.20 M) was added methyl 12-(trifluoromethoxy)dodecanoate **129** (50 mg, 0.17 mmol, 1.0 eq.). A solution of 2.0 M NaOH (1.7 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1.0 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (28 mg, 0.10 mmol, 58%). **M.pt**: 35–37 °C; **IR** (ATR/cm^{-1}): 2915, 2850, 1699, 1474, 1221, 1141; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.4 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 2.35 (t, *J* 7.6 Hz, 2H, -C$\underline{H}_2$COOH), 1.71–1.60 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.26 (m, 14H, 7 × -C$\underline{H}_2$); **^{19}F NMR** (100 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.1, 121.9 (q, J_{FC} 252.1 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.6 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 34.0, 29.9, 29.6, 29.5, 29.5, 29.3, 29.2, 28.9, 25.6,

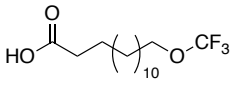
24.8; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{13}H_{23}{}^{19}F_3O_3$ 284, found 283 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{13}H_{23}{}^{19}F_3O_3$ 283.1527, found 283.1527 $[M-H]^+$.

Methyl 14-(trifluoromethoxy)tetradecanoate 130:



A microwave vial was charged with methyl 14-hydroxytetradecanoate **46** (100 mg, 0.39 mmol, 1.0 eq.), KF (90 mg, 1.6 mmol, 4.0 eq.), Selectfluor® (206 mg, 0.58 mmol, 1.5 eq.) and AgOTf (299 mg, 1.2 mmol, 3.0 eq.), flame-dried and placed under Ar. Anhydrous EtOAc (1.9 mL, 0.2 M) was added, followed by 2-fluoropyridine (0.10 mL, 1.2 mmol, 3.0 eq.) and Ruppert's reagent (0.17 mL, 1.2 mmol, 3.0 eq.). The resulting solution was stirred at room temperature for 12 hours before filtration through a plug of silica. Further purification *via* flash column chromatography (petroleum ether 100:1 EtOAc) afforded the *title compound* as a pale yellow oil (23 mg, 0.07 mmol, 18%) **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 1732, 1465, 1264, 1130; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.2 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.71–1.60 (m, 4H 2 × -C$\underline{H}_2$), 1.33–1.24 (m, 18H, 9 × -C$\underline{H}_2$); **^{19}F NMR** (100 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 174.5, 121.9 (q, J_{FC} 252.0 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.6 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 51.6, 34.3, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 28.9, 25.6, 25.1 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{29}{}^{19}F_3O_3$ 326, found 325 $[M-H]^+$.

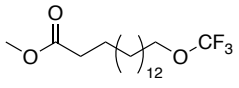
14-(Trifluoromethoxy)tetradecanoic acid 121:



To a 1:1 solution of THF/H_2O (0.3 mL, 0.20 M) was added methyl 14-(trifluoromethoxy)tetradecanoate **130** (20 mg, 0.06 mmol, 1.0 eq.). A solution of 2.0 M NaOH (0.6 mL, 0.10 M) was added and the reaction allowed to

stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1.0 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (12 mg, 0.04 mmol, 63%). **M.pt**: 42–44 °C; **IR** (ATR/cm^{-1}): 3057, 2814, 2849, 1731, 1473, 1220; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 2.35 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOH), 1.71–1.61 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.27 (m, 18H, 9 × -C$\underline{H}_2$); **^{19}F NMR** (125 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 178.1, 121.9 (q, J_{FC} 252.4 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.8 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 33.8, 29.9, 29.7, 29.6, 29.6, 29.4, 29.2, 28.9, 25.6, 24.9 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{27}{}^{19}F_3O_3$ 312, found 311 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{15}H_{27}{}^{19}F_3O_3$ 311.1840, found 311.1838 $[M-H]^+$.

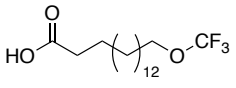
Methyl 16-(trifluoromethoxy)hexadecanoate 131:



A microwave vial was charged with methyl 16-hydroxyhexadecanoate **53** (200 mg, 0.70 mmol, 1.0 eq.), KF (163 mg, 2.8 mmol, 4.0 eq.), Selectfluor® (372 mg, 1.1 mmol, 1.5 eq.) and AgOTf (539 mg, 2.1 mmol, 3.0 eq.), flame-dried and placed under Ar. Anhydrous EtOAc (3.5 mL, 0.2 M) was added, followed by 2-fluoropyridine (0.18 mL, 2.1 mmol, 3.0 eq.) and Ruppert's reagent (0.31 mL, 2.1 mmol, 3.0 eq.). The resulting solution was stirred at room temperature for 12 hours before filtration through a plug of silica. Further purification *via* flash column chromatography (petroleum ether 100:1 EtOAc) afforded the *title compound* as a pale yellow oil (98 mg, 0.28 mmol, 40%) **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 1732, 1465, 1262, 1130; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.6 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.72–1.58 (m, 4H, 2 × -C$\underline{H}_2$), 1.40–1.26 (m, 22H, 11 × -C$\underline{H}_2$); **^{19}F NMR** (100 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 121.9 (q, J_{FC} 252.2 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.8 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 51.6, 34.3,

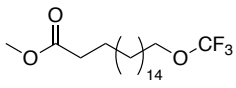
31.6, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 25.6, 25.1; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{18}H_{33}{}^{19}F_3O_3$ 354, found 355 $[M+H]^+$.

16-(Trifluoromethoxy)hexadecanoic acid 122:



To a 1:1 solution of THF/H_2O (0.7 mL, 0.20 M) was added methyl 16-(trifluoromethoxy)hexadecanoate **131** (50 mg, 0.14 mmol, 1.0 eq.). A solution of 2.0 M NaOH (1.4 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1.0 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (32 mg, 0.09mmol, 67%). **M.pt**: 52–53 °C; **IR** (ATR/cm^{-1}): 2914, 2849, 1698, 1473, 1246, 1142; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.8 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 2.35 (t, *J* 7.2 Hz, 2H, -C$\underline{H}_2$COOH), 1.71–1.60 (m, 4H, 2 × -C$\underline{H}_2$), 1.40–1.26 (m, 2H, 11 × -C$\underline{H}_2$); **^{19}F NMR** (100 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.6, 121.9 (q, J_{FC} 252.3 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.7 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 34.1, 31.6, 30.4, 29.9, 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 25.6, 24.8 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{17}H_{31}{}^{19}F_3O_3$ 340, found 339 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{17}H_{31}{}^{19}F_3O_3$ 339.2153, found 339.2150 $[M-H]^+$.

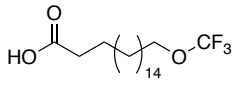
Methyl 18-(trifluoromethoxy)octadecanoate 132:



A microwave vial was charged with methyl 18-hydroxyoctadecanoate **47** (200 mg, 0.64 mmol, 1.0 eq.), KF (148 mg, 2.6 mmol, 4.0 eq.), Selectfluor® (339 mg, 0.96 mmol, 1.5 eq.) and AgOTf (491 mg, 1.9 mmol, 3.0 eq.), flame-dried and placed under Ar. Anhydrous EtOAc (3.2 mL, 0.2 M) was added, followed by

2-fluoropyridine (0.16 mL, 1.9 mmol, 3.0 eq.) and Ruppert's reagent (0.28 mL, 1.9 mmol, 3.0 eq.). The resulting solution was stirred at room temperature for 12 hours before filtration through a plug of silica. Further purification *via* flash column chromatography (petroleum ether 100:1 EtOAc) afforded the *title compound* as a pale yellow oil (113 mg, 0.30 mmol, 47%) **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2915, 2848, 1729, 1463, 1279, 1134; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.5 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 3.67 (s, 3H, -COOC$\underline{H}_3$), 2.30 (t, *J* 7.5 Hz, 2H, -C$\underline{H}_2$COOCH$_3$), 1.71–1.59 (m, 4H, 2 × -C$\underline{H}_2$), 1.39–1.25 (m, 26H, 13 × -C$\underline{H}_2$); **^{19}F NMR** (125 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.5, 121.9 (q, J_{FC} 252.1 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.5 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 51.6, 34.3, 30.5, 29.8, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 25.6, 25.1 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{37}{}^{19}F_3O_3$ 382, found 381 $[M-H]^+$.

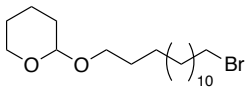
18-(Trifluoromethoxy)octadecanoic acid 123:



To a 1:1 solution of THF/H_2O (0.7 mL, 0.20 M) was added methyl 18-(trifluoromethoxy)octadecanoate **132** (50 mg, 0.13 mmol, 1.0 eq.). A solution of 2.0 M NaOH (1.3 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C overnight. Upon completion the reaction mixture was diluted with EtOAc (5 mL) and washed with 5 mL each of 1.0 M HCl, H_2O and brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (31 mg, 0.08mmol, 65%). **M.pt**: 59–61 °C; **IR** (ATR/cm^{-1}): 2915, 2850, 1699, 1474, 1275, 1141; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.95 (t, *J* 6.4 Hz, 2H, -C$\underline{H}_2$OCF$_3$), 2.35 (t, *J* 7.6 Hz, 2H, -C$\underline{H}_2$COOH), 1.71–1.60 (m, 4H, 2 × -C$\underline{H}_2$), 1.38–1.26 (m, 26H, 13 × -C$\underline{H}_2$); **^{19}F NMR** (100 MHz, $CDCl_3$) δ -60.6 (s, 3F); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 179.5, 121.9 (q, J_{FC} 252.2 Hz, 1C, -$\underline{C}$F$_3$), 67.7 (q, J_{FC} 2.7 Hz, 1C, -$\underline{C}$H$_2$OCF$_3$), 34.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.2, 28.9, 25.6, 24.8 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{35}{}^{19}F_3O_3$ 368, found 366 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{19}H_{35}{}^{19}F_3O_3$ 367.2466, found 367.2464 $[M-H]^+$.

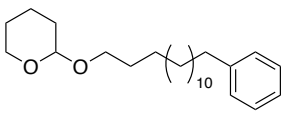
5.8 Preparation of Phenyl Series

2-((14-Bromotetradecyl)oxy)tetrahydro-2*H*-pyran 138:[148]



To a solution of 14-bromotetradecan-1-ol **38** (1.0 g, 3.4 mmol, 1.0 eq.) in THF (4.9 mL, 0.7 M) was added 3,4-dihydro-2*H*-pyran (DHP) (0.47 mL, 5.1 mmol, 1.5 eq.) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O). The resultant solution was allowed to stir overnight at room temperature. Upon completion the mixture was concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 97:3 EtOAc) afforded the *title compound* as a clear oil (1.06 g, 2.8 mmol, 82%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2918, 2849, 1454; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.58 (app t, *J* 3.0 Hz, 1H, -OC<u>H</u>O), 3.89–3.85 (m, 1H, -C<u>H</u>(H)OCHO), 3.75–3.70 (m, 1H, -CH(<u>H</u>)OCHO), 3.52–3.48 (m, 1H, -OCHOC<u>H</u>(H)), 3.41 (t, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$Br), 3.39–3.36 (m, 1H, -OCHOCH(<u>H</u>)), 1.88–1.82 (m, 3H, -C<u>H</u>(H), -C<u>H</u>$_2$$CH_2$Br), 1.74–1.69 (m, 1H, -CH(<u>H</u>)), 1.62–1.53 (m, 6H, 3 × -C<u>H</u>$_2$), 1.45–1.39 (m, 2H, -C<u>H</u>$_2$), 1.36–1.26 (m, 18H, 9 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 99.0, 67.9, 62.5, 34.2, 33.0, 31.0, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 28.9, 28.3, 26.4, 25.7, 19.9 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{37}{}^{79}BrO_2$ 376, found 375 $[M-H]^+$.

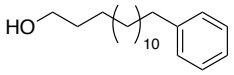
2-((14-Phenyltetradecyl)oxy)tetrahydro-2*H*-pyran 142:



A microwave vial was charged with 2-((14-bromotetradecyl)oxy)tetrahydro-2*H*-pyran **138** (250 mg, 0.66 mmol, 1.0 eq.) and tris(acetylacetonato)iron(III) (12 mg, 0.03 mmol, 0.05 eq.), flame-dried and placed under Ar. Anhydrous THF (6.7 mL, 0.10 M) was added and the resultant solution cooled to -78°C. Phenylmagnesium bromide (3.0 M in

Et_2O) solution (0.26 mL, 0.80 mmol, 1.2 eq.) was then added followed by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.12 mL, 0.80 mmol, 1.2 eq.). The reaction mixture was allowed to stir overnight t room temperature. Upon completion the reaction was quenched with NH_4Cl (5 mL) and extracted with Et_2O (4 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (petroleum ether 100:1 Et_2O) afforded the *title compound* as a clear oil (130 mg, 0.35 mmol, 52%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2921, 2850, 1467, 1033; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H, 2 × -ArH), 7.18–7.15 (m, 3H, 3 × -ArH), 4.58 (app t, *J* 4.5 Hz, 1H, -OCHO), 3.90–3.85 (m, 1H, -CH(H)OCHO), 3.75–3.71 (m, 1H, -CH(H)OCHO), 3.52–3.47 (m, 1H, -OCHOCH(H)), 3.41–3.36 (m, 1H, -OCHOCH(H)), 2.60 (t, *J* 7.5 Hz, 2H, -CH_2Ph), 1.85–1.81 (m, 1H, -CH(H)), 1.74–1.69 (m, 1H, -CH(H)), 1.62–1.35 (m, 8H, 4 × -CH_2), 1.36–1.26 (m, 20H, 10 × -CH_2); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 143.1, 128.5, 128.4, 125.7, 99.0, 67.9, 62.5, 36.1, 31.7, 31.0, 29.9, 29.8, 29.8, 29.7, 29.7, 29.5, 26.4, 25.7, 19.9 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{25}H_{42}O_2$ 374 , found 375 $[M+H]^+$.

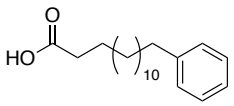
14-Phenyltetradecan-1-ol 146:



To a solution of 2-((14-phenyltetradecyl)oxy)tetrahydro-2*H*-pyran **142** (150 mg, 0.40 mmol, 1.0 eq.) in MeOH (4.0 mL, 0.10 M) was added *p*-TsOH·H_2O (15 mg, 0.08 mmol, 0.2 eq.). The resultant solution was allowed to stir at room temperature overnight. Upon completion, the mixture was concentrated *in vacuo*, the residue dissolved in EtOAc (10 mL) and washed with 10 mL each of $NaHCO_3$, H_2O and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (95 mg, 0.33 mmol, 81%). **M.pt**: 40–42 °C; **IR** (ATR/cm^{-1}): 3320, 3062, 2913, 2846, 1465; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H, 2 × -ArH), 7.18–7.15 (m, 3H, 3 × -ArH), 3.65 (app q, *J* 6.5 Hz, 2H, -CH_2OH), 2.60 (t, *J* 7.5 Hz, 2H, -CH_2Ph), 1.64–1.55 (m, 4H, 2 × -CH_2), 1.36–1.26 (m, 20H, 10 × -CH_2); **^{13}C NMR** (125 MHz,

$CDCl_3$) δ 143.1, 128.5, 128.4, 125.7, 66.3, 36.1, 33.0, 31.7, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 25.9 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{34}O$ 290, found 289 $[M-H]^+$.

14-Phenyltetradecanoic acid 134:[149]



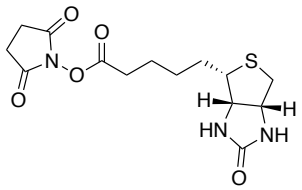
Preparation of Jones Reagent:

Chromium(VI) oxide (100 mg, 1.1 mmol, 4.0 eq.) was dissolved in H_2SO_4 (0.20 mL, 1.36 M). Ice-cold H_2O (0.46 mL, 0.6 M) was added portion-wise with stirring and the reagent was allowed to stir for 10 minutes before addition to the reaction.

To a stirred solution of 14-phenyltetradecan-1-ol **146** (80 mg, 0.28 mmol, 1.0 eq.) in acetone (6.9 mL, 0.04 M) was added Jones Reagent, preparation as described above, drop-wise over 10 minutes at room temperature. The resultant solution was stirred at room temperature for 18 hours. The reaction was quenched with H_2O (5 mL), the layers were separated and the aqueous phase extracted with CH_2Cl_2 (4 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a white solid (76 mg, 0.25 mmol, 91%). **M.pt**: 56–58 °C; **IR** (ATR/cm^{-1}): 3026, 2913, 2846, 1699, 1465; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28–7.26 (m, 2H, 2 × -Ar<u>H</u>), 7.18–7.15 (m, 3H, 3 × -Ar<u>H</u>), 2.60 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$Ph), 2.35 (t, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$COOH), 1.67–1.58 (m, 4H, 2 × -C<u>H</u>$_2$), 1.31–1.26 (m, 18H, 9 × -C<u>H</u>$_2$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 178.5, 143.1, 128.5, 128.4, 125.7, 36.1, 33.9, 31.7, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 24.8 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{32}O_2$ 304, found 306 $[M+H]^+$.

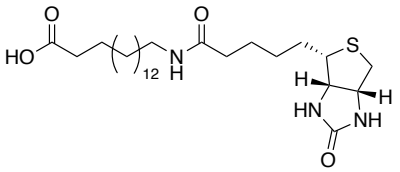
5.9 Preparation of CoA-Biotin Chemical Tool

2,5-Dioxopyrrolidin-1-yl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl) pentanoate 154:[110]



To a solution of biotin (500 mg, 2.05 mmol, 1.00 eq.) in DMF (15 mL, 0.14 M) was added *N*,*N*′-dicyclohexylcarbodiimide (427 mg, 2.07 mmol, 1.01 eq.) then *N*-hydroxysuccinimide (250 mg, 2.17 mmol, 1.06 eq.). The reaction mixture was allowed to stir at 60 °C for 2 hours, then overnight at room temperature. The resulting precipitate was filtered and the filtrate concentrated *in vacuo*. The residue was then dissolved in acetone and the precipitate filtered. Further purification *via* trituration in isopropanol (IPA) afforded the *title compound* as a white solid (405 mg, 1.19 mmol, 58%). **M.pt**: 199–201 °C; **IR** (ATR/cm^{-1}): 3286, 2916, 2847, 1692, 1634, 1564; **^{1}H NMR** (500 MHz, DMSO-d_6) δ 6.40 (s, 1H, -NH), 6.35 (s, 1H, -NH), 4.32–4.19 (m, 1H, -CHNH), 4.16–4.13 (m, 1H, -CHNH), 3.13–3.09 (m, 1H, -CH$_2$CHS), 2.89–2.83 (m, 1H, -CH(H)S), 2.81 (s, 4H, 2 × -CH$_2$C(O)N), 2.67 (t, *J* 7.0 Hz, 2H, -CH$_2$COON), 2.58 (d, *J* 12 Hz, 1H, -CH(H)S), 1.68–1.61 (m, 2H, -CH$_2$CH$_2$COON), 1.53–1.40 (m, 4H, 2 × -CH$_2$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 170.4, 169.1, 162.9, 61.1, 59.3, 55.3, 30.1, 27.9, 27.7, 25.5, 24.4 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{19}N_3O_5S$ 341, found 342 $[M+H]^+$.

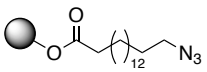
16-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl)pentanamido)hexadecanoic acid 155:



To a solution of biotin-succinimide **154** (503 mg, 1.48 mmol, 1.0 eq.) in DMF (24 mL, 0.05 M) was added a solution of 16-aminohexadecanoic acid **118** (400 mg, 1.48 mmol, 1.0 eq.) in 0.2 M $NaHCO_3$ (15 mL, 0.08 M) at 60 °C. The reaction was allowed to stir for 4 hours at 60 °C then overnight at room temperature. The resulting precipitate was filtered and washed with H_2O (20 mL) and 0.01 M HCl (20 mL) to afford the *title compound* as a white solid (545 mg, 1.10 mmol, 74%). **M.pt**: 200–202 °C; **IR** (ATR/cm^{-1}): 3026, 2917, 2850, 1699, 1692, 1634, 1564; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 11.96 (br s, 1H, -COO<u>H</u>), 7.70 (t, *J* 5.6 Hz, 1H, -CH_2N<u>H</u>), 6.40 (s, 1H, -CHN<u>H</u>), 6.34 (s, 1H, -CHN<u>H</u>), 4.30 (dd, *J* 7.7, 4.6 Hz, 1H, -C<u>H</u>NH), 4.12 (dd, *J* 7.6, 4.6 Hz, 1H, -C<u>H</u>NH), 3.11–3.06 (m, 1H, -CH_2C<u>H</u>S), 3.00 (app q, *J* 6.8 Hz, 2H, -C<u>H_2</u>NH), 2.81 (dd, *J* 12.4, 5.1, 1H, -C<u>H</u>(H)S), 2.57 (d, *J* 12.4 Hz, 1H, -CH(<u>H</u>)S), 2.18 (t, *J* 7.2 Hz, 2H, -C<u>H_2</u>COOH), 2.03 (t, *J* 7.6 Hz, 2H, -C<u>H_2</u>C(O)NH), 1.62–1.58 (m, 2H, -C<u>H_2</u>CH_2NH), 1.51–1.43, m, 6H, 3 × -C<u>H_2</u>), 1.38–1.34 (m, 2H, -C<u>H_2</u>), 1.32–1.23 (m, 22H, 11 × -C<u>H_2</u>); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.5, 171.8, 162.7, 61.0, 59.2, 55.4, 38.3, 35.2, 33.6, 29.1, 29.0, 29.0, 28.9, 28.7, 28.5, 28.2, 28.0, 26.4, 25.3, 24.5 (6 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{26}H_{47}N_3O_4S$ 497, found 496 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{26}H_{47}N_3O_4S$ 496.3215, found 496.3221 $[M-H]^+$.

5.9.1 Solid supported strategy

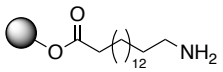
TFP polymer supported 16-azidohexadecanoic acid 159:



To a 12 mL vial was added TFP resin (200 mg, 0.202 mmol, 1.01 mmol/g) at room temperature. The resin was swelled with DMF (4 mL, 0.1 M) for 10 minutes with agitation. 16-azidohexadecanoic acid **156** (120 mg, 0.404 mmol, 2.0 eq.) was added and the mixture agitated until all acid had dissolved. DMAP (5 mg, 0.040 mmol, 0.2 eq.) followed by *N*,*N*'-diisopropylcarbodiimide (DIC) (63 µL, 0.404 mmol, 2.0 eq.) were then added and the mixture agitated at room temperature overnight. The resin was washed with DMF (3 × 10 mL),

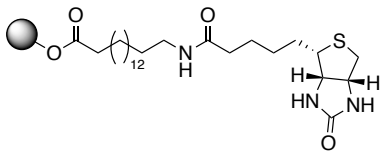
CH_2Cl_2 (3 × 10 mL) and THF (3 × 10 mL) and dried *in vacuo*. Percentage loading calculated to be 85%. **IR** (ATR/cm^{-1}): 3024 (br), 2921, 2851, 2094, 1787.

TFP polymer supported 16-aminohexadecanoic acid 160:



To a 12 mL vial was added TFP polymer supported-16-azidohexadecanoic acid **159** (200 mg), PPh_3 (220 mg) and CH_2Cl_2 (4 mL) and the mixture agitated at room temperature overnight. H_2O (1 mL) was then added and the mixture agitated for a further 2 hours at room temperature. The resin was then washed with CH_2Cl_2 (3 × 10 mL) and dried *in vacuo*. **IR** (ATR/cm^{-1}): 3053, 3024, 2921, 2849, 1785.

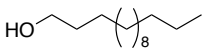
TFP polymer supported 16-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl)pentanamido)hexadecanoic acid 161:



To a 12 mL vial was added TFP polymer supported 16-aminohexadecanoic acid **160** (200 mg) in DMF (8 mL). Biotin-succinimide **154** (300 mg) and K_2CO_3 (500 mg) were then added and the mixture agitated at room temperature overnight. The resin was then washed DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) then dried *in vacuo*. **IR** (ATR/cm^{-1}): 3286 (br), 2916, 2847, 1692, 1634, 1564.

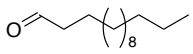
5.10 Preparation of α,β-unsaturated Series

Tetradecan-1-ol 165:[150]



To a flame-dried three-neck flask, attached with an overhead stirrer, was added tetradecanedioic acid (5.00 g, 21.9 mmol, 1.0 eq.) in anhydrous THF (219 mL, 0.1 M) at 0 °C under an inert atmosphere. Solid $LiAlH_4$ (832 mg, 21.9 mmol, 1.0 eq.) was then added portion-wise and the reaction allowed to stir at room temperature overnight. The reaction was monitored *via* TLC. Upon completion the reaction mixture was cooled to 0 °C and wet Na_2SO_4 was added portion-wise until the visible grey precipitate became white. The reaction was left to stir for 1 hour at room temperature to allow the reaction mixture to become homogeneous. The suspension was then dried with $MgSO_4$, filtered, washed with Et_2O (3 × 100 mL) and concentrated *in vacuo*. A white solid was afforded and used without further purification (4.51 g, 21.1 mmol, 96%). **M.pt**: 44–46 °C; **IR** (ATR/cm^{-1}): 3304 (br), 2958, 2917, 2850, 1465; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 4.30 (t, *J* 5.2 Hz, 1H, -O<u>H</u>), 4.39–3.34 (m, 2H, -C<u>H</u>$_2$OH), 1.39 (app p, *J* 6.9 Hz, 2H, -C<u>H</u>$_2$CH$_2$OH), 1.29–1.24 (m, 22H, 11 × -C<u>H</u>$_2$), 0.85 (t, *J* 6.8 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 60.7, 32.5, 31.3, 29.1, 29.0, 28.9, 28.7, 25.5, 22.1, 13.9 (4 carbons missing); **LRMS** (GCMS) *m/z* calc. for $C_{14}H_{30}O$ 214, found 213 $[M-H]^+$.

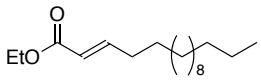
Tetradecanal 166:[151]



To a stirred solution of pyridinium chlorochromate (10.1 g, 46.7 mmol, 2.0 eq.) in CH_2Cl_2 (83.6 mL, 0.28 M) was added tetradecan-1-ol **165** (5.0 g, 23.4 mmol, 1.0 eq.). The resulting reaction mixture was stirred at room temperature for 48 hours. The mixture was filtered through a plug of silica, washed with CH_2Cl_2

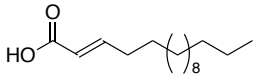
(4 × 100 mL) and concentrated *in vacuo*. Further purification *via* flash column chromatography (petroleum ether 95:5 EtOAc) afforded the *title compound* as a white solid (2.6 g, 12.1 mmol, 52%). **M.pt**: 43–45 °C; **IR** (ATR/cm^{-1}): 2954, 2913, 2850, 1699, 1472; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.77 (t, *J* 1.5 Hz, 1H, -C<u>H</u>O), 2.42 (td, *J* 7.5 Hz, *J* 1.5 Hz, 2H, -C<u>H</u>$_2$CHO), 1.63 (app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$CHO), 1.30–1.26 (m, 20H, 10 × -C<u>H</u>$_2$), 0.88 (t, *J* 7.0 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 203.2, 44.1, 32.1, 29.8, 29.7, 29.6, 29.6, 29.5, 29.3, 22.8, 22.4, 14.3 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{14}H_{28}O$ 212, found 211 $[M-H]^+$.

Ethyl (*E*)-hexadec-2-enoate 167:[152]



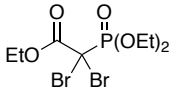
Sodium hydride (NaH) (170 mg, 7.1 mmol, 3.0 eq.) was dispersed in anhydrous THF (55 mL, 0.04 M) under an inert atmosphere. The resultant solution was cooled to 0 °C and triethylphosphonoacetate (1.59 g, 7.1 mmol, 1.5 eq.) was added drop-wise. The mixture was allowed to warm to room temperature before stirring for 30 minutes. The solution was cooled to -78 °C and tetradecanal **166** (500 mg, 2.4 mmol, 1.0 eq.) added drop-wise. The reaction was then allowed to stir at 40 °C overnight. The reaction mixture was diluted with EtOAc (100 mL) and washed with H_2O (2 × 50 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Further purification *via* flash column chromatography (hexane 98:2 Et_2O) afforded the *title compound* as a clear oil (434 mg, 1.54 mmol, 65%). **IR** (ATR/cm^{-1}): 2956, 2922, 2854, 1724; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.96 (dt, *J* 16.0 Hz, *J* 7.0 Hz, 1H, -C<u>H</u>CHCOOEt), 5.79 (d, *J* 16.0 Hz, 1H, -CHC<u>H</u>COOEt), 4.18 (q, *J* 7.5 Hz, 2H, -OC<u>H</u>$_2$CH$_3$), 2.19 (app qd, *J* 7.0 Hz, *J* 1.0 Hz, 2H, -C<u>H</u>$_2$CH), 1.44 (app p, *J* 7.5 Hz, 2H, -C<u>H</u>$_2$CH$_2$CH), 1.29–1.25 (m, 23H, 10 × -C<u>H</u>$_2$, -OCH$_2$C<u>H</u>$_3$), 0.88 (t, *J* 7.0 Hz, 3H, -CH$_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 166.9, 149.6, 121.4, 60.2, 32.3, 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 29.3, 28.2, 22.8, 14.4 (3 carbons missing); **LRMS** (GC-MS) *m/z* calc. for $C_{18}H_{34}O_2$ 282, found 283 $[M+H]^+$.

(*E*)-Hexadec-2-enoic acid 27:[153]



To a 1:1 solution of THF/H_2O (7.0 mL, 0.2 M) was added ethyl (*E*)-hexadec-2-enoate **167** (400 mg, 1.42 mmol, 1.0 eq.). A solution of 2 M sodium hydroxide (NaOH) (14.2 mL, 0.10 M) was added and the reaction allowed to stir at 40 °C for 72 hours. The reaction was monitored by TLC. Upon completion the reaction mixture was diluted with EtOAc (20 mL) and washed with 1 M HCl (20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a colourless solid (313 mg, 1.23 mmol, 87%). **M.pt**: 46–48°C; **IR** (ATR/cm^{-1}): 2960, 2915, 2848, 1712; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.09 (dt, *J* 16.0 Hz, *J* 7.0 Hz, 1H, -C<u>H</u>CHCOOH), 5.82 (d, *J* 16.0 Hz, 1H, -CHC<u>H</u>COOH), 2.23 (app q, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH), 1.44 (app p, *J* 7.0 Hz, 2H, -C<u>H</u>$_2$CH$_2$CH), 1.29–1.26 (m, 20H, 10 × -C<u>H</u>$_2$), 0.88 (t, *J* 6.5 Hz, 3H, -CH_3); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.7, 152.7, 120.6, 32.5, 32.1, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 28.0, 22.8, 14.3 (1 carbon missing); **LRMS** (GC-MS) *m/z* calc. for $C_{16}H_{30}O_2$ 254, found 255 $[M+H]^+$.

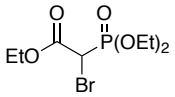
Ethyl 2,2-dibromo-2-(diethoxyphosphoryl)acetate 171:[117]



To a solution of NaOH (20.0 g, 500 mmol, 9.3 eq.) in H_2O (60 mL, 0.89 M) at 0 °C, was added bromine (40.0 g, 250 mmol, 4.7 eq.) at a rate such that the temperature did not exceed 5 °C. To the freshly prepared sodium hypobromite was added triethylphosphonoacetate (12.0 g, 53.5 mmol, 1.0 eq.) at a rate such that the temperature did not exceed 10 °C. After stirring for 5 minutes at room temperature the reaction mixture was extracted with chloroform ($CHCl_3$) (4 × 100 mL). The combined organics were washed with H_2O (2 × 20 mL), dried

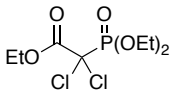
over $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil (16.9 g, 44.1 mmol, 82%). **IR** (ATR/cm^{-1}): 2984, 2937, 2911, 1735, 1264; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.41–4.26 (m, 6H, 2 × -POC$\underline{H}_2$$CH_3$, -OC$\underline{H}_2$$CH_3$), 1.36 (td, *J* 6.8 Hz, J_{PH} 0.8 Hz, 6H, 2 × -POCH$_2$C$\underline{H}_3$), 1.32 (t, *J* 7.2 Hz, 3H, -OCH$_2$C$\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.8 (d, J_{PC} 3.8 Hz), 66.4 (d, J_{PC} 6.8 Hz), 66.6, 47.0 (d, J_{PC} 159 Hz), 16.4 (d, J_{PC} 5.9 Hz), 13.8; **^{31}P NMR** (202 MHz, $CDCl_3$) δ 7.7 (p, J_{PH} 8.1 Hz, 1P, -P(OEt)$_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_8H_{15}{}^{79}Br^{81}BrO_5P$ 381, found 382 $[M+H]^+$.

Ethyl 2-bromo-2-(diethoxyphosphoryl)acetate 172:[118]



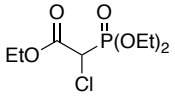
To a solution of ethyl 2,2-dibromo-2-(diethoxyphosphoryl)acetate **171** (2.00 g, 5.2 mmol, 1.0 eq.) in EtOH (5 mL, 1.04 M) was added tin(II) chloride dihydrate (1.12 g, 5.0 mmol, 0.96 eq.) in H_2O (10 mL, 0.52 M) at a rate such that the temperature did not exceed 10 °C. The resulting reaction mixture was stirred at room temperature for 5 minutes and then extracted with $CHCl_3$ (4 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a pale yellow oil (1.24 g, 4.1 mmol, 78%). **IR** (ATR/cm^{-1}): 2984, 2937, 2911, 1738, 1258; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.34 (d, J_{PH} 14 Hz, 1H, -C$\underline{H}$Br), 4.29–4.21 (m, 6H, 2 × -POC$\underline{H}_2$$CH_3$, -OC$\underline{H}_2$$CH_3$), 1.35 (td, *J* 7.2 Hz, J_{PH} 0.8 Hz, 6H, 2 × -POCH$_2$C$\underline{H}_3$), 1.29 (t, *J* 7.2 Hz, 3H, -OCH$_2$C$\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.1, 64.7 (d, J_{PC} 6.7 Hz, 2C, 2 × -PO$\underline{C}$H$_2$CH$_3$), 63.1, 35.9 (d, J_{PC} 145 Hz, 1C, -$\underline{C}$HBr), 16.4 (d, J_{PC} 6.0 Hz, 2C, 2 × -POCH$_2$$\underline{C}H_3$), 14.0; **^{31}P NMR** (202 MHz, $CDCl_3$) δ 12.6-12.4 (m, 1P, -P(OEt)$_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_8H_{16}{}^{81}BrO_5P$ 304, found 305 $[M+H]^+$.

Ethyl 2,2-dichloro-2-(diethoxyphosphoryl)acetate 173:[119]



To a solution of sodium hypochlorite (158 g, 111.5 mmol, 5.0 eq.), adjusted to pH 7.1 with 3 M HCl, was added triethylphosphonoacetate (5.00 g, 22.3 mmol, 1.0 eq.) drop-wise at 0 °C, with vigorous stirring. The reaction mixture was stirred for 5 minutes at room temperature and then the resulting turbid solution was extracted with hexane (5 × 20 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear oil (3.82 g, 13.0 mmol, 59%). **IR** (ATR/cm^{-1}): 2986, 2913, 1937, 1748, 1271; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.36–4.23 (m, 6H, 2 × -POC$\underline{H}_2$CH$_3$, -OC$\underline{H}_2$CH$_3$), 1.33–1.27 (m, 9H, 2 × -POCH$_2$C$\underline{H}_3$, -OCH$_2$C$\underline{H}_3$); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 163.5 (d, J_{PC} 4.8 Hz), 74.8 (d, J_{PC} 166 Hz), 66.2 (d, J_{PC} 7.0 Hz), 64.7, 16.4 (d, J_{PC} 5.6 Hz), 13.9; **^{31}P NMR** (500 MHz, $CDCl_3$) δ 7.6 (p, J_{PH} 7.9 Hz, 1P, -P(OEt)$_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_8H_{15}{}^{35}Cl^{37}ClO_5P$ 294, found 295 $[M+H]^+$.

Ethyl 2-chloro-2-(diethoxyphosphoryl)acetate 174:[119]

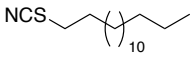


To a solution of ethyl 2,2-dichloro-2-(diethoxyphosphoryl)acetate **173** (2.00 g, 6.8 mmol, 1.0 eq.) in EtOH (13 mL, 0.51 M), at 0 °C, was added sodium sulfite (1.72 g, 13.6 mmol, 2.0 eq.) at a rate such that the temperature did not exceed 15 °C. The resulting reaction mixture was stirred for 20 minutes at room temperature and then extracted with $CHCl_3$ (5 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear oil (1.53 g, 5.9 mmol, 87%). **IR** (ATR/cm^{-1}): 2984, 2937, 1751, 1262; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.46 (d, J_{PH} 16 Hz, 1H, -C$\underline{H}$Cl), 4.27–4.19 (m, 6H, 2 × -POC$\underline{H}_2$CH$_3$, -OC$\underline{H}_2$CH$_3$), 1.34–1.25 (m, 9H,

2 × -$POCH_2C\underline{H}_3$, -$OCH_2C\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 164.7, 64.4 (d, J_{PC} 4.8 Hz, 2C, 2 × -$PO\underline{C}H_2CH_3$), 62.9, 50.1 (d, J_{PC} 144 Hz, 1C, -$\underline{C}HCl$), 16.1, 13.8; **^{31}P NMR** (202 MHz, $CDCl_3$) δ 12.4-12.2 (m, 1P, -$P(OEt)_2$); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_8H_{16}{}^{35}ClO_5P$ 258, found 259 $[M+H]^+$.

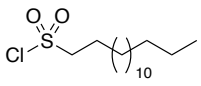
5.11 Preparation of Sulfonamide Series

1-Thiocyanatopentadecane 176:[154]



To a solution of 1-bromopentadecane (1.00 g, 3.4 mmol, 1.0 eq.) in EtOH (6 mL, 0.57 M) was added potassium thiocyanate (500 mg, 5.2 mmol, 1.5 eq.) and the reaction mixture stirred at reflux for 5 hours. After cooling to room temperature the reaction was concentrated *in vacuo* to remove all volatiles. The resultant residue was dissolved in EtOAc (10 mL) and washed with H_2O (5 mL) and brine (5 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a clear oil which solidified on standing (767 mg, 2.9 mmol, 83%). **M.pt**: <25 °C; **IR** (ATR/cm^{-1}): 2952, 2915, 2848, 2155, 1472; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.96 (t, *J* 7.6 Hz, 2H, -C$\underline{H}_2$SCN), 1.82 (app p, *J* 7.2 Hz, 2H, -C$\underline{H}_2$CH_2SCN), 1.44 (app p, *J* 6.8 Hz, 2H, -C$\underline{H}_2$CH_2CH_2SCN), 1.34–1.24 (m, 22H, 11 × -C$\underline{H}_2$) 0.88 (t, *J* 6.8 Hz, 3H, -C$\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 112.6, 34.2, 32.1, 30.0, 29.8, 28.7, 29.6, 29.5, 29.0, 28.1, 22.8, 14.3 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{16}H_{31}NS$ 269, found 268 $[M-H]^+$.

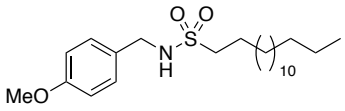
Pentadecane-1-sulfonyl chloride 177:



A solution of 1-thiocyanatopentadecane **176** (700 mg, 2.6 mmol, 1.0 eq.) in AcOH (0.75 mL, 13.0 mmol, 5.0 eq.) and H_2O (70 µL, 3.9 mmol, 1.5 eq.) was stirred for 30 minutes at 50 °C, followed by drop-wise addition of sulfuryl chloride (3.51 g, 26.0 mmol, 10.0 eq.). The addition of sulfuryl chloride was accompanied by a strong emission of chlorine and sulphur dioxide gas, which were neutralised with a 1 M solution of NaOH. After stirring for 10 minutes at 50 °C, excess sulfuryl chloride was hydrolysed by the drop-wise addition of H_2O (5 mL) and the resultant mixture extracted with EtOAc (3 × 5 mL). The

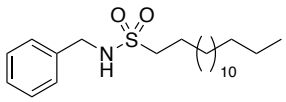
combined organics were washed with H_2O (3 × 5 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title compound* as a clear oil which solidified on standing (790 mg, 2.5 mmol, 98%). **M.pt**: 37–39 °C; **IR** (ATR/cm^{-1}): 2950, 2915, 2848, 1470, 1355, 1152; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.68–3.64 (m, 2H, -C$\underline{H}_2$SO$_2$Cl), 2.08–2.00 (m, 2H, -C$\underline{H}_2$CH$_2$SO$_2$Cl), 1.49 (app p, *J* 6.8 Hz, 2H, -C$\underline{H}_2$CH$_2$CH$_2$SO$_2$Cl), 1.37–1.26 (m, 22H, 11 × -C$\underline{H}_2$) 0.88 (t, *J* 6.8 Hz, 3H, -C$\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 65.7, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 29.0, 27.7, 24.4, 22.8, 14.3 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{15}H_{31}{}^{37}ClO_2S$ 310, found 391 $[M+DMSO+H]^+$.

***N*-(4-Methoxybenzyl)pentadecane-1-sulfonamide 178:**



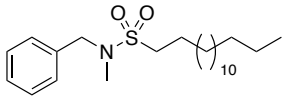
Following General Procedure A, 4-methoxybenzylamine (97 mg, 0.71 mmol), pentadecane-1-sulfonyl chloride **177** (200 mg, 0.64 m mol) and Et_3N (99 µL, 0.71 mmol) in CH_2Cl_2 (2.1 mL) were reacted for 4 hours. Further purification by flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a white solid (92 mg, 0.2 mmol, 35%). **M.pt**: 106–108 °C; **IR** (ATR/cm^{-1}): 3293, 3263, 2954, 2917, 2848, 1517, 1128; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.26 (d, *J* 8.8 Hz, 2H, 2 × Ar$\underline{H}$), 6.89 (d, *J* 8.8 Hz, 2H, 2 × Ar$\underline{H}$), 4.32 (t, *J* 6.0 Hz, 1H, -N$\underline{H}$), 4.24 (d, *J* 6.0 Hz, 2H, -C$\underline{H}_2$NH), 3.81 (s, 3H, -OC$\underline{H}_3$), 2.93–2.89 (m, 2H, -C$\underline{H}_2$SO$_2$), 1.78–1.71 (m, 2H, -C$\underline{H}_2$CH$_2$SO$_2$), 1.36–1.26 (m, 24H, 12 × -C$\underline{H}_2$), 0.88 (t, *J* 6.8 Hz, 3H, -C$\underline{H}_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 159.6, 129.5, 129.1, 114.4, 55.5, 53.5, 46.9, 39.2, 32.1, 31.4, 29.9, 29.8, 29.8, 29.7, 29.5, 29.4, 29.2, 28.4, 23.8, 22.8, 14.3; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{23}H_{41}NO_3S$ 411, found 412 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{23}H_{41}NO_3S$ 410.2734, found 410.2738 $[M-H]^+$.

***N*-Benzylpentadecane-1-sulfonamide 179:**



Following General Procedure A, benzylamine (76 mg, 0.71 mmol), pentadecane-1-sulfonyl chloride **177** (200 mg, 0.64 mmol) and Et_3N (99 µL, 0.71 mmol) in CH_2Cl_2 (2.1 mL) were reacted for 6 hours. Further purification by flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a white solid (82 mg, 0.22 mmol, 34%). **M.pt**: 96–98 °C; **IR** (ATR/cm^{-1}): 3284 (br), 2954, 2917, 2848, 1472, 1135; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.39–7.30 (m, 5H, 5 × Ar<u>H</u>), 4.42 (t, *J* 6.0 Hz, 1H, -N<u>H</u>), 4.30 (d, *J* 6.0 Hz, 2H, -C<u>H</u>$_2$NH), 2.93–2.90 (m, 2H, -C<u>H</u>$_2$$SO_2$), 1.78–1.72 (m, 2H, -C<u>H</u>$_2$$CH_2SO_2$), 1.35–1.25 (m, 24H, 12 × -C<u>H</u>$_2$), 0.88 (t, *J* 7.0 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 137.1, 129.0, 128.3, 128.1, 53.5, 47.4, 32.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 28.4, 23.8, 22.8, 14.3 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{22}H_{39}NO_2S$ 381, found 382 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{22}H_{39}NO_2S$ 380.2629, found 380.2634 $[M-H]^+$.

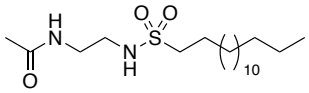
***N*-Benzyl-*N*-methylpentadecane-1-sulfonamide 180:**



Following General Procedure A, *N*-benzylmethylamine (72 mg, 0.71 mmol), pentadecane-1-sulfonyl chloride **177** (200 mg, 0.64 mmol) and Et_3N (99 µL, 0.71 mmol) in CH_2Cl_2 (2.1 mL) were reacted for 5 hours. Further purification by flash column chromatography (petroleum ether 90:10 EtOAc) afforded the final compound as a white solid (165 mg, 0.42 mmol, 65%). **M.pt**: 62–64 °C; **IR** (ATR/cm^{-1}): 2956, 2919, 2850, 1472, 1133; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.38–7.30 (m, 5H, 5 × Ar<u>H</u>), 4.34 (s, 2H, -C<u>H</u>$_2$N), 2.99–2.95 (m, 2H, -C<u>H</u>$_2$$SO_2$), 2.78 (s, 3H, -C<u>H</u>$_3$N), 1.86–1.80 (m, 2H, -C<u>H</u>$_2$$CH_2SO_2$), 1.43–1.39 (m, 2H, -C<u>H</u>$_2$$CH_2CH_2SO_2$), 1.33–1.26 (m, 22H, 11 × -C<u>H</u>$_2$), 0.88 (t, *J* 6.5 Hz, 3H, -C<u>H</u>$_3$);

^{13}C NMR (100 MHz, $CDCl_3$) δ 136.2, 128.9, 128.4, 128.1, 54.0, 50.7, 34.5, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 28.7, 23.4, 22.8, 14.3 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{23}H_{41}NO_2S$ 395, found 396 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{23}H_{41}NO_2S$ 396.2931, found 396.2936 $[M+H]^+$.

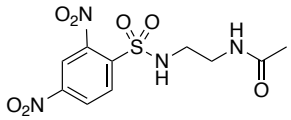
***N*-(2-(Pentadecylsulfonamido)ethyl)acetamide 181:**



Following General Procedure A, *N*-(2-aminoethyl)acetamide (72 mg, 0.71 mmol), pentadecane-1-sulfonyl chloride **177** (200 mg, 0.64 mmol) and Et_3N (99 µL, 0.71 mmol) in CH_2Cl_2 (2.1 mL) were reacted for 5 hours. Further purification by flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a white solid (80 mg, 0.21 mmol, 33%). **M.pt**: 116–118 °C; **IR** (ATR/cm^{-1}): 3313, 3269, 2952, 2917, 2848, 1644, 1549, 1102; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.05 (app s, 1H, -CON<u>H</u>), 4.82 (t, *J* 6.0 Hz, 1H, -SO_2N<u>H</u>), 4.42 (app q, *J* 5.5 Hz, 2H, -CONHC<u>H_2</u>), 3.26 (app q, *J* 6.0 Hz, 2H, -SO_2NHC<u>H_2</u>), 3.02–2.99 (m, 2H, -C<u>H_2</u>SO_2), 2.01 (s, 3H, -COC<u>H_3</u>), 1.80–1.76 (m, 2H, -C<u>H_2</u>CH_2SO_2), 1.43–1.38 (m, 2H, -C<u>H_2</u>$CH_2CH_2SO_2$), 1.30–1.26 (m, 22H, 11 × -C<u>H_2</u>), 0.88 (t, *J* 7.0 Hz, 3H, -C<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.4, 53.0, 43.3, 40.3, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 28.4, 23.8, 23.4, 22.8, 14.3 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{19}H_{40}N_2O_3S$ 376, found 377 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{19}H_{40}N_2O_3S$ 375.2687, found 375.2693 $[M-H]^+$.

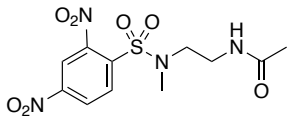
5.12 Nitrobenzene Series

N-(2-((2,4-Dinitrophenyl)sulfonamido)ethyl)acetamide 185:



Following General Procedure B, *N*-(2-aminoethyl)acetamide (500 mg, 4.9 mmol), 2,4-dinitrobenzenesulfonyl chloride (1.70 g, 6.4 mmol) and Et_3N (1.0 mL, 7.4 mmol) were reacted in CH_2Cl_2 (49 mL). Further purification *via* flash column chromatography (CH_2Cl_2 95:5 MeOH) afforded the *title compound* as a yellow solid (857 mg, 2.6 mmol, 53%). **M.pt**: 121–123 °C; **IR** (ATR/cm^{-1}): 3397 (br), 3345, 3105, 2941, 1655, 1537, 1346; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.67 (d, *J* 2.0 Hz, 1H Ar<u>H</u>), 8.56 (dd, *J* 8.5 Hz, *J* 2.0 Hz, 1H, Ar<u>H</u>), 8.35 (d, *J* 8.5 Hz, 1H, Ar<u>H</u>), 6.12 (app s, 1H, -SO_2N<u>H</u>), 5.92 (app s, 1H, -CON<u>H</u>), 3.43 (app q, *J* 5.5 Hz, 2H, -SO_2NHC<u>H_2</u>), 3.31 (app t, *J* 6.0 Hz, 2H, -CONHC<u>H_2</u>), 2.00 (s, 3H, -COC<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.8, 150.0, 148.3, 139.1, 132.7, 127.3, 120.8, 43.9, 39.7, 23.2; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{10}H_{12}N_4O_7S$ 332, found 331 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{10}H_{12}N_4O_7S$ 333.0499, found 333.0496 $[M+H]^+$.

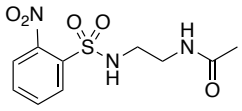
N-(2-((*N*-Methyl-2,4-dinitrophenyl)sulfonamido)ethyl)acetamide 186:



To a solution of *N*-(2-((2,4-dinitrophenyl)sulfonamido)ethyl)acetamide **185** (500 mg, 1.5 mmol, 1.0 eq.) in MeCN (15 mL, 0.10 M) was added cesium carbonate (Cs_2CO_3) (540 mg, 1.7 mmol, 1.1 eq.) at room temperature. After stirring for 15 minutes, MeI (641 mg, 4.5 mmol, 3.0 eq.) was added and the reaction allowed to stir for 1 hour. The reaction was then quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (4 × 10 mL). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the *title*

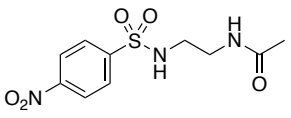
compound as a pale yellow solid (426 mg, 1.2 mmol, 82%), which was used without further purification. **M.pt**: 118–120 °C; **IR** (ATR/cm^{-1}): 3306 (br), 3107, 2924, 1651, 1558, 1536, 1349; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.51 (dd, *J* 8.5 Hz, *J* 2.0 Hz, 1H, Ar<u>H</u>), 8.47 (d, *J* 2.0 Hz, 1H Ar<u>H</u>), 8.23 (d, *J* 8.5 Hz, 1H, Ar<u>H</u>), 5.85 (app s, 1H, -CON<u>H</u>), 3.49 (app q, *J* 5.5 Hz, 2H, -SO_2NHC<u>H_2</u>), 3.41 (app t, *J* 6.0 Hz, 2H, -CONHC<u>H_2</u>), 2.97 (s, 3H, -NC<u>H_3</u>), 1.99 (s, 3H, -COC<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.9, 150.0, 148.2, 137.8, 132.9, 126.3, 119.9, 49.6, 36.9, 34.9, 23.3; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{11}H_{14}N_4O_7S$ 346, found 347 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{11}H_{14}N_4O_7S$ 347.0656, found 347.0655 $[M+H]^+$.

N-(2-((2-Nitrophenyl)sulfonamido)ethyl)acetamide 188:



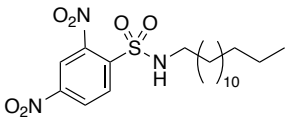
Following General Procedure B, *N*-(2-aminoethyl)acetamide (500 mg, 4.9 mmol), 2-nitrobenzenesulfonyl chloride (1.41 g, 6.4 mmol) and Et_3N (1.0 mL, 7.4 mmol) were reacted in CH_2Cl_2 (49 mL). Further purification *via* flash column chromatography (CH_2Cl_2 95:5 MeOH) afforded the *title compound* as a yellow solid (934 mg, 3.3 mmol, 66%). **M.pt**: 124–126 °C; **IR** (ATR/cm^{-1}): 3367 (br), 3341 (br), 3185, 3092, 2882, 1666, 1563, 1361; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.14–8.11 (m, 1H Ar<u>H</u>), 7.88–7.85 (m, 1H, Ar<u>H</u>), 7.77–7.74 (m, 2H, 2 × Ar<u>H</u>), 6.01 (app s, 1H, -SO_2N<u>H</u>), 5.76 (t, *J* 5.5 Hz, 1H, -CON<u>H</u>), 3.42 (app q, *J* 6.0 Hz, 2H, -SO_2NHC<u>H_2</u>), 3.25 (app q, *J* 6.0 Hz, 2H, -CONHC<u>H_2</u>), 1.99 (s, 3H, -COC<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.0, 148.1, 133.8, 133.4, 132.9, 131.1, 125.4, 43.4, 39.6, 23.2; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{10}H_{13}N_3O_5S$ 287, found 288 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{10}H_{13}N_3O_5S$ 288.0649, found 288.0651 $[M+H]^+$.

_N_-(2-((4-Nitrophenyl)sulfonamido)ethyl)acetamide 189:



Following General Procedure B, *N*-(2-aminoethyl)acetamide (500 mg, 4.9 mmol), 4-nitrobenzenesulfonyl chloride (1.41 g, 6.4 mmol) and Et_3N (1.0 mL, 7.4 mmol) were reacted in CH_2Cl_2 (49 mL). Further purification *via* flash column chromatography (CH_2Cl_2 95:5 MeOH) afforded the *title compound* as a yellow solid (284 mg, 0.99 mmol, 20%). **M.pt**: 148–150 °C; **IR** (ATR/cm^{-1}): 3416, 3379, 3103 (br), 2859, 1646, 1550, 1355; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.36 (d, *J* 8.5 Hz, 2H, 2 × Ar<u>H</u>), 8.05 (d, *J* 8.5 Hz, 2H, 2 × Ar<u>H</u>), 5.85 (app s, 1H, -SO_2N<u>H</u>), 5.72 (t, *J* 4.5 Hz, 1H, -CON<u>H</u>), 3.38 (app q, *J* 5.5 Hz, 2H, -SO_2NHC<u>H_2</u>), 3.17 (app q, *J* 5.5 Hz, 2H, -CONHC<u>H_2</u>), 1.99 (s, 3H, -COC<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 172.1, 150.0, 146.0, 128.4, 124.6, 44.3, 39.8, 23.3; **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{10}H_{13}N_3O_5S$ 287, found 288 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{10}H_{13}N_3O_5S$ 288.0649, found 288.0651 $[M+H]^+$.

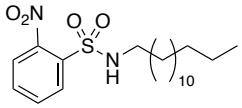
2,4-Dinitro-*N*-tetradecylbenzenesulfonamide 190:



Following General Procedure B, 1-aminotetradecane (500 mg, 2.3 mmol), 2,4-dinitrobenzenesulfonyl chloride (812 mg, 3.0 mmol) and Et_3N (0.5 mL, 3.5 mmol) were reacted in CH_2Cl_2 (23 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a yellow solid (683 mg, 1.5 mmol, 66%). **M.pt**: 96–98 °C; **IR** (ATR/cm^{-1}): 3304 (br), 3083, 3102, 2919, 2852, 1554, 1541, 1359, 1338; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.68 (d, *J* 2.0 Hz, 1H Ar<u>H</u>), 8.56 (dd, *J* 8.5 Hz, *J* 2.0 Hz, 1H, Ar<u>H</u>), 8.37 (d, *J* 8.5 Hz, 1H, Ar<u>H</u>), 5.28 (t, *J* 6.0 Hz, 1H, -SO_2N<u>H</u>), 3.15 (app q, *J* 7.0 Hz, 2H, -SO_2NHC<u>H_2</u>), 1.53 (app p, *J* 7.0 Hz, 2H, -SO_2NHCH_2C<u>H_2</u>), 1.32–1.22 (m, 22H,

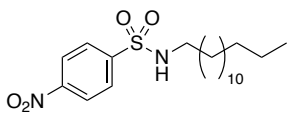
11 × -C<u>H</u>$_2$), 0.88 (t, *J* 6.5 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 149.9, 148.5, 139.6, 132.8, 127.2, 120.9, 44.2, 32.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.1, 26.6, 22.8, 14.3 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{33}N_3O_6S$ 443, found 444 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{33}N_3O_6S$ 442.2017, found 442.2022 $[M-H]^+$.

2-Nitro-*N*-tetradecylbenzenesulfonamide 191:



Following General Procedure B, 1-aminotetradecane (500 mg, 2.3 mmol), 2-nitrobenzenesulfonyl chloride (675 mg, 3.0 mmol) and Et_3N (0.5 mL, 3.5 mmol) were reacted in CH_2Cl_2 (23 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a yellow solid (710 mg, 1.8 mmol, 76%). **M.pt**: 93–95 °C; **IR** (ATR/cm^{-1}): 3287 (br), 2952, 2919, 2850, 1541, 1364; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.16–8.13 (m, 1H Ar<u>H</u>), 7.87–7.85 (m, 1H, Ar<u>H</u>), 7.76–7.72 (m, 2H, 2 × Ar<u>H</u>), 5.22 (t, *J* 6.0 Hz, 1H, -SO_2N<u>H</u>), 3.10 (app q, *J* 6.5 Hz, 2H, -SO_2NHC<u>H</u>$_2$), 1.51 (app p, *J* 6.0 Hz, 2H, -SO_2NHCH_2C<u>H</u>$_2$), 1.30–1.21 (m, 22H, 11 × -C<u>H</u>$_2$), 0.88 (t, *J* 7.0 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 148.4, 134.1, 133.6, 132.9, 131.3, 125.5, 44.0, 32.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.2, 26.6, 22.8, 14.3 (1 carbon missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{34}N_2O_4S$ 398, found 397 $[M-H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{34}N_2O_4S$ 397.2167, found 397.2172 $[M-H]^+$.

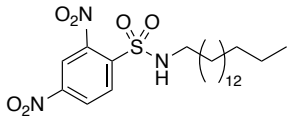
4-Nitro-*N*-tetradecylbenzenesulfonamide 192:



Following General Procedure B, 1-aminotetradecane (500 mg, 2.3 mmol), 4-nitrobenzenesulfonyl chloride (675 mg, 3.0 mmol) and Et_3N (0.5 mL,

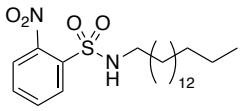
3.5 mmol) were reacted in CH_2Cl_2 (23 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a yellow solid (511 mg, 1.3 mmol, 55%). **M.pt**: 97–99 °C; **IR** (ATR/cm^{-1}): 3302 (br), 3118, 2921, 2850, 1530, 1346; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.37 (d, *J* 9.0 Hz, 2H, 2 × ArH), 8.05 (d, *J* 8.5 Hz, 2H, 2 × ArH), 4.49 (t, *J* 5.5 Hz, 1H, -SO_2NH), 3.02 (app q, *J* 7.0 Hz, 2H, -SO_2NHCH_2), 1.48 (app p, *J* 7.0 Hz, 2H, -SO_2NHCH_2CH_2), 1.31–1.22 (m, 22H, 11 × -CH_2), 0.88 (t, *J* 6.5 Hz, 3H, -CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 150.2, 146.3, 128.4, 124.5, 43.6, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 26.5, 22.8, 14.3 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{20}H_{34}N_2O_4S$ 398, found 399 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{20}H_{34}N_2O_4S$ 397.2167, found 397.2170 $[M-H]^+$.

2,4-Dinitro-*N*-hexadecylbenzenesulfonamide 193:



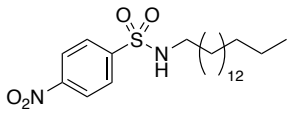
Following General Procedure B, 1-aminohexadecane (500 mg, 2.1 mmol), 2,4-dinitrobenzenesulfonyl chloride (718 mg, 2.7 mmol) and Et_3N (0.43 mL, 3.1 mmol) were reacted in CH_2Cl_2 (21 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title com*pound as a yellow solid (445 mg, 0.94 mmol, 46%). **M.pt**: 101–103 °C; **IR** (ATR/cm^{-1}): 3306 (br), 3083, 2919, 2852, 1554, 1541, 1359, 1342; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.68 (d, *J* 2.0 Hz, 1H ArH), 8.56 (dd, *J* 8.5 Hz, *J* 2.0 Hz, 1H, ArH), 8.37 (d, *J* 8.5 Hz, 1H, ArH), 5.28 (t, *J* 5.5 Hz, 1H, -SO_2NH), 3.15 (app q, *J* 7.0 Hz, 2H, -SO_2NHCH_2), 1.53 (app p, *J* 7.0 Hz, 2H, -SO_2NHCH_2CH_2), 1.32–1.22 (m, 26H, 13 × -CH_2), 0.88 (t, *J* 7.0 Hz, 3H, -CH_3); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 149.9, 148.5, 139.6, 132.8, 127.2, 120.9, 44.2, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.1, 26.6, 22.8, 14.3 (4 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{22}H_{37}N_3O_6S$ 471, found 472 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{22}H_{37}N_3O_6S$ 470.2330, found 470.2334 $[M-H]^+$.

2-Nitro-*N*-hexadecylbenzenesulfonamide 194:



Following General Procedure B, 1-aminohexadecane (500 mg, 2.1 mmol), 2-nitrobenzenesulfonyl chloride (597 mg, 2.7 mmol) and Et_3N (0.43 mL, 3.1 mmol) were reacted in CH_2Cl_2 (21 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a yellow solid (610 mg, 1.4 mmol, 69%). **M.pt**: 95–97 °C; **IR** (ATR/cm^{-1}): 3289 (br), 2954, 2919, 2850, 1541, 1366; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.17–8.11 (m, 1H Ar<u>H</u>), 7.88–7.84 (m, 1H, Ar<u>H</u>), 7.77–7.71 (m, 2H, 2 × Ar<u>H</u>), 5.23 (t, *J* 6.0 Hz, 1H, -SO_2N<u>H</u>), 3.09 (app q, *J* 6.8 Hz, 2H, -SO_2NHC<u>H_2</u>), 1.51 (app p, *J* 7.2 Hz, 2H, -SO_2NHCH_2C<u>H_2</u>), 1.33–1.21 (m, 26H, 13 × -C<u>H_2</u>), 0.88 (t, *J* 6.8 Hz, 3H, -C<u>H_3</u>); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 148.3, 134.0, 133.6, 132.9, 131.3, 125.5, 44.0, 32.1, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.2, 26.6, 22.8, 14.3 (3 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{22}H_{38}N_2O_4S$ 426, found 427 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{22}H_{38}N_2O_4S$ 425.2480, found 425.2485 $[M-H]^+$.

4-Nitro-*N*-hexadecylbenzenesulfonamide 195:

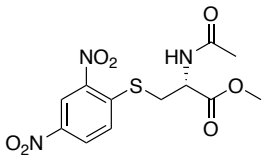


Following General Procedure B, 1-aminohexadecane (500 mg, 2.1 mmol), 4-nitrobenzenesulfonyl chloride (597 mg, 2.7 mmol) and Et_3N (0.43 mL, 3.1 mmol) were reacted in CH_2Cl_2 (21 mL). Further purification *via* flash column chromatography (petroleum ether 90:10 EtOAc) afforded the *title compound* as a yellow solid (654 mg, 1.5 mmol, 74%). **M.pt**: 101–103 °C; **IR** (ATR/cm^{-1}): 3302 (br), 3107, 2919, 2850, 1523, 1344; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.37 (d, *J* 8.0 Hz, 2H, 2 × Ar<u>H</u>), 8.05 (d, *J* 7.5 Hz, 2H, 2 × Ar<u>H</u>), 4.47 (app s, 1H, -SO_2N<u>H</u>), 3.02 (app q, *J* 6.0 Hz, 2H, -SO_2NHC<u>H_2</u>), 1.48 (app p, *J* 7.0 Hz, 2H, -SO_2NHCH_2C<u>H_2</u>),

1.25–1.22 (m, 26H, 13 × -C<u>H</u>$_2$), 0.88 (t, *J* 6.0 Hz, 3H, -C<u>H</u>$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 150.2, 146.3, 128.4, 124.5, 43.6, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.2, 26.6, 22.8, 14.3 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{22}H_{38}N_2O_4S$ 426, found 427 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{22}H_{38}N_2O_4S$ 425.2480, found 425.2486 $[M-H]^+$.

5.13 Amino Acid Selectivity

Methyl *N*-acetyl-*R*-(2,4-dinitrophenyl)-*L*-cysteinate 198:



To a solution of *N*-(2-((2,4-dinitrophenyl)sulfonamido)ethyl)acetamide **185** (100 mg, 0.3 mmol, 1.0 eq.) in pH 6.4 buffer (1.5 mL, 0.2 M) was added *N*-acetyl-L-cysteine methyl ester (53 mg, 0.3 mmol, 1.0 eq.) at 25 °C. The reaction was allowed to stir for 2 hours, with samples taken every 15 minutes for LC-MS analysis. LC-MS analysis indicated 100% completion as ~1.5 hours. The reaction was then extracted with EtOAc (3 × 5 mL). Further purification *via* flash column chromatography (CH_2Cl_2 95:5 MeOH) afforded the *title compound* as an off-white solid (68 mg, 0.2 mmol, 66%). **M.pt**: 173–175 °C; **IR** (ATR/cm^{-1}): 3299 (br), 3112, 3094, 2958, 1761, 1653, 1515, 1344; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.06 (d, *J* 2.5 Hz, 1H, ArH), 8.41 (dd, *J* 9.0 Hz, *J* 2.5 Hz, 1H, ArH), 7.81 (d, *J* 9.0 Hz, 1H, ArH), 6.32 (d, *J* 6.0 Hz, 1H, -NH), 4.93 (app q, *J* 6.0 Hz, 1H, -CHNH), 3.82 (s, 3H, -COOCH_3), 3.63 (dd, *J* 13.5 Hz, *J* 6.0 Hz, 1H, -SCH), 3.52 (dd, *J* 13.5 Hz, *J* 4.5 Hz, 1H, -SCH), 2.04 (s, 3H, -COCH_3); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.4, 170.3, 144.9, 127.7, 127.4, 121.8, 53.5, 51.4, 34.5, 23.2 (2 carbons missing); **LRMS** (LC-MS-ESI) *m/z* calc. for $C_{12}H_{13}N_3O_7S$ 343, found 344 $[M+H]^+$; **HRMS** *m/z* calc. for $C_{12}H_{13}N_3O_7S$ 344.0547, found 344.0546 $[M+H]^+$.

6. References

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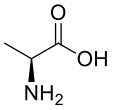
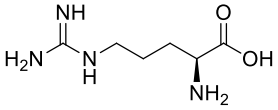
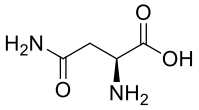
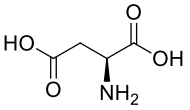
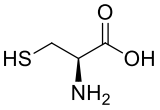
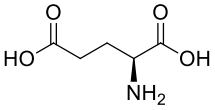
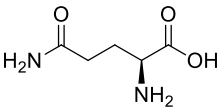
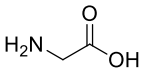
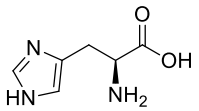
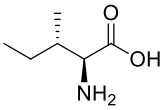
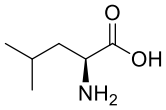
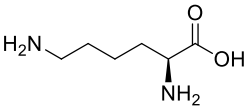
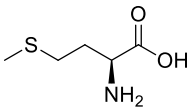
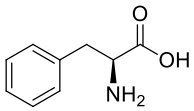
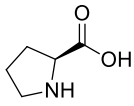
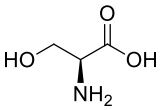
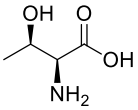
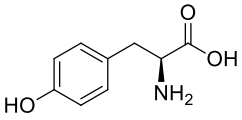
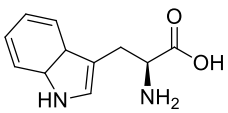
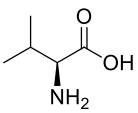
Appendix

Table 11: Table of Amino Acids

		
Alanine, Ala, A	Arginine, Arg, R	Asparagine, Asn, N
		
Aspartic acid, Asp, D	Cysteine, Cys, C	Glutamic acid, Glu, E
		
Glutamine, Gln, Q	Glycine, Gly, G	Histidine, His, H
		
Isoleucine, Ile, I	Leucine Leu, L	Lysine, Lys, K
		
Methionine, Met, M	Phenylalanine, Phe, F	Proline, Pro, P
		
Serine, Ser, S	Threonine, Thr, T	Tyrosine, Tyr, Y
		
Tryptophan, Trp, W	Valine, Val, V	