Syntheses of Probes for the Elucidation of Biosynthetic Pathways and Interactions of Natural Products

by

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Abstract

A new platform for the semi-synthetic preparation of a known nanomolar inhibitor of the *Plasmodium falciparum* lysyl-tRNA synthetase, cladosporin, from *Cladosporium cladosporioides* and its analogues was developed. Our work has shown that it is produced biosynthetically by a highly reducing (HR) and a non-reducing (NR) iterative type I polyketide synthase (PKS) pair, namely the **Cla2** and **Cla3**. The heterologous expression of these enzymes in *Saccharomyces cerevisiae* was shown to produce cladosporin, thus confirming the identity of the putative gene cluster. *In vitro* incubation of the purified **Cla3** enzyme with the synthesized natural pentaketide intermediate (or analogues) demonstrated the recognition and further elaboration of the intermediate to the completed product, cladosporin (or analogues). Incorporation of the natural pentaketide intermediate by **Cla3** indicated a 5+3 assembly between the HR-PKS, **Cla2** and the NR-PKS, **Cla3** during cladosporin biosynthesis.

Probes for the study of Coenzyme A (CoA) dependent natural product biosynthetic machinery were developed. Four CoA analogues were made in single steps through a transamidation reaction. The transformation was catalyzed by boric acid in water. This transformation offers access to useful compounds for the study of enzyme-catalyzed reactions, especially processes involving acyl/peptidyl carrier proteins (A/PCP) in polyketide synthases (PKS), fatty acid synthases (FAS), and nonribosomal peptide synthetases (NRPS). Currently work is ongoing to use these probes in the study of the **Cla2/Cla3** interaction in cladosporin biosynthesis.

A new Dess-Martin periodinane mediated oxidative rearrangement reaction was uncovered for the production of α-keto thioesters. The reaction proceeds through the

oxidation of a β-hydroxy thioester to a β-keto thioester, followed by an α-hydroxylation and further oxidation to form a vicinal tricarbonyl thioester. This product then rearranges with loss of CO_2, to form the α-keto product. The mechanism of the rearrangement was elucidated using ^{13}C labelling and analysis of proposed intermediates as well as the products of the reaction. This process allows easy preparation of α-keto thioesters, which are vital intermediates in the preparation of pharmaceutically important heterocyclic scaffolds such as the quinoxalinones.

The enantioselective preparation of spin-labelled α-amino acids for electron paramagnetic resonance (EPR) spectroscopy was studied. Spin labelled amino acids which are currently utilized are mostly racemic, labile or difficult to couple. Our work provides a simple approach towards the enantioselective preparation of two new spin labelled amino acids. This was achieved through alkylation of the chiral Ni(II) complex of the Schiff base of (*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide and glycine with the spin-labelled electrophile of choice.

Preface

Sections of this thesis have been published. However there are still several aspects of ongoing and unpublished research. Parts of Chapter 2 have been published as: [†]Cochrane, R. V., [†]Sanichar, R., Lambkin, G. R., Reiz, B., Xu, W., Tang, Y. and Vederas, J. C. (**2016**), Production of New Cladosporin Analogues by Reconstitution of the Polyketide Synthases Responsible for the Biosynthesis of this Antimalarial Agent. *Angew. Chem. Int. Ed*., 55: 664-668. This was a collaborative project with equal contributions from myself and Dr. Rachel Cochrane. Dr. Cochrane was responsible for the production of the **Cla2/Cla3** enzymes required for the study, whereas I synthesized all the advanced precursors (substrates) and potential products (standards). The bioassays were jointly done.

Parts of Chapter 3 have been published as: Sanichar, R., and Vederas, J. C. (**2017**), One-Step Transformation of Coenzyme A into Analogues by Transamidation. *Org. Lett.* 19 (8), 1950-1953. I completed all the experimental work and wrote the manuscript.

Parts of Chapter 4 have been published as: Sanichar, R., Carroll, C., Kimmis, R., Reiz, B., Vederas, J. C. (**2018**), Dess-Martin periodinane oxidative rearrangement for preparation of α-keto thioesters. *Org. Biomol. Chem.* 16 (4), 593-597. I did most of the experimental work and wrote the manuscript. Mr. Ciaran Carroll and Mr. Ryan Kimmis (both undergraduate students) assisted with the preparation of starting materials and substrate scope testing.

At the time of this thesis preparation, Chapter 5 is still unpublished.

"We live on an island surrounded by a sea of ignorance. As our island of knowledge grows, so does the shore of our ignorance."

— John Archibald Wheeler

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While the research commitment is of vital importance to a graduate student, other aspects such the relationships with family and friends takes precedence. Without the support of my amazing family and friends, this body of work would not be possible. I would like to thank my siblings for their unwavering support, especially considering the distance apart, my mom Vidya, for being the amazing woman she is, and all her sacrifices. I would to thank my late father Deo for his incredible insights and

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List of Abbreviations

$[\alpha]^{25}_{D}$	specific rotation
Å	Ångström
AcOH	acetic acid
ACP	acyl carrier protein
AIBN	2,2′–azobis(2–methylpropionitrile)
Aro	aromatase
AT	acyl transferase
ATP	adenosine–5'–triphosphate
c	concentration in g mL^{-1} (for optical rotation)
CD	circular dichroism
CHS	chalcone synthase
CMeT	C–methyltransferase domain
CoA	coenzyme A
Con	condensation domain
Cyc	cyclase
d	doublet (in NMR)
DAL	dihydroxyphenylacetic acid lactone
DBU	1,8–diazabicyclo[5.4.0]undec–7–ene
DCC	1,3–dicyclohexylcarbodiimide
DEBS	6–deoxyerythronolide synthase
DEER	double electron-electron resonance
DH	dehydratase

DHC	dehydrocurvularin
DHML	dihydromonacolin L
DIBAL	diisobutylaluminum hydride
DIPEA	N, N–diisopropylethylamine
DMAP	4–(dimethylamino)pyridine
DMF	dimethylformamide
DMP	Dess–Martin periodinane
DMSO	Dimethyl sulfoxide
DPP	dimethyl pyrophosphate
EPR	electron paramagnetic resonance
eq	equivalent(s)
ER	enoyl reductase
ESI	electrospray ionization
ESR	electron spin resonance
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FAS	fatty acid synthase
FTIR	Fourier transform infrared spectroscopy
GC–MS	gas chromatography coupled with liquid chromatography
Hex	hexane
HRMS	high–resolution mass spectrometry
HR-PKS	highly reducing polyketide synthase

HSNAC/SNAC	*N*-acetyl cysteamine
IBCF	Isobutyl chloroformate
IPP	isopentyl pyrophosphate
IR	infrared
J	coupling constant in hertz
kDa	kilodaltons
KR	ketoreductase
KS	ketosynthase
LC–MS	liquid chromatography-mass spectrometry
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazane
LysRS	lysyl tRNA synthetase
m	multiplet
m/z	mass to charge ratio
MAT	malonyl–CoA:ACP transacylase
Me	methyl
MS	mass spectrometry
MSAS	6–methylsalicylic acid synthase
MT	methyl transferase
NADPH	β–nicotinamide adenine dinucleotide phosphate
NaHMDS	sodium hexamethyldisilazane
n–BuLi	*n*–butyllithium
ncAA	non-canonical amino acid

NMR	nuclear magnetic resonance
NR-PKS	non reducing polyketide synthase
NRPS	non ribosomal peptide synthetase
PAL	phenylalanine ammonia-lyase
Ph	phenyl
PKS	polyketide synthase
Ppant	phosphopantetheine
ppm	parts per million
PR–PKS	partially reducing polyketide synthase
PT	product template
q	quartet
quant.	quantitative yield
RAL	resorcylic acid lactone
RP-HPLC	reverse phase-high performance liquid chromatography
s	singlet
SAM	*S*–adenosyl–L–methionine
SAT	starter unit:ACP transacylase
SDSL	site directed spin-labelling
STS	stilbene synthase
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*–butyldimethylsilyl
t–BuOH	*tert*–butanol

TE	thioesterase
TFA	trifluoroacetic acid
TH	thioester hydrolase domain
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
UV	ultraviolet spectroscopy
δ	chemical shift in parts per million downfield from TMS

List of Schemes

Chapter 1: Introduction

1.1 Natural Products and their Classifications

Natural products can be broadly described as any organic compound formed by a living organism.[1,2] However, this may be refined by describing them as the end products of metabolic processes, where they are not i) a structural feature of the cell, ii) used as a monomer in the production of biological polymers. These compounds may be divided into three broad categories. The first group is termed primary metabolites; these are compounds which occur in cells and play a critical role in their metabolism and reproduction.[1] Examples include the nucleic acids, common amino acids and the simple sugars.[1] The second group comprises of compounds that form cellular structures; these include the high molecular weight polymeric materials such as cellulose, lignin, and structural proteins.[1] The final group consists of the secondary metabolites; compounds that are characteristic of a limited range of species, and not necessary for the life of the organism by itself but useful for interaction with others (chemical communication, chemical defences).[1]

Secondary metabolites may be further divided into sub-groups based on the structural similarities in their assembly. These secondary metabolite sub-groups are:[1]

- Polyketides and fatty acids
- Terpenoids and steroids
- Shikimate metabolites
- Alkaloids

- Specialized amino acids and peptides
- Specialized carbohydrates

Polyketides are commonly formed in nature by the head to tail condensation of acetate units derived from the biochemical "building block" acetyl coenzyme A (CoA).[1] As this thesis is partially focused on the study of polyketides, the following chapters will delve deeper into the biosynthesis of this class of natural products.

Terpenoids and steroids have a characteristic branched chain structure and are assembled in nature by the condensation of activated C5 units; isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DPP) in a head to tail fashion.[1]

Unlike, polyketides and terpenoids, the shikimate metabolites are synthesized from the amino acids phenylalanine and tyrosine or their precursors, by the action of phenylalanine ammonia-lyase (PAL, phenylalanine/tyrosine ammonia-lyase) which transform L-phenylalanine and tyrosine into *trans*-cinnamic acid and *p*-coumaric acid, respectively.[3] These are later converted into 4-coumaroyl-CoA; the building blocks for more complex phenylpropanoids.[3]

While amino acids are normally considered primary metabolites, they are the building blocks for peptides and proteins. Some peptides and proteins do not serve a critical role in the metabolism and reproduction of cells, such as penicillins formed from small peptides.[1]

The alkaloids are perhaps the most chemically diverse group of natural products. In addition to the commonly encountered elements such as carbon, hydrogen, oxygen and nitrogen, alkaloids may also contain sulfur and, more rarely, elements such as chlorine,

bromine, and phosphorus.[1] The nitrogenous portion is derived from amino acids such as ornithine, lysine, tyrosine or tryptophan.[1]

Carbohydrates such as glucose are typically considered primary metabolites. However, the glycosides are a unique class of carbohydrate-terpenoid/alkaloid/polyketide hybrids.[1] Where the non-sugar portion of the molecules is called an aglycone.

Natural products are of interest to us for many reasons. Over 40% of pharmaceutically available compounds have their origins in these secondary metabolites. This success has further led to numerous screening programmes for other bioactive compounds that exists in nature.[1] However, if we can determine how these compounds are assembled in nature, then we could rationally manipulate these systems for the production of other potent analogues. This would allow us the use of combinatorial biosynthesis of molecules for the biosynthetic production of novel compounds with improved and/or new pharmacological properties. In addition, natural products themselves also present challenging opportunities for total synthesis, which in turn drives the development of new synthetic methodologies.[4] Figure 1.1 presents selected examples of natural products and their inspired analogues used as pharmaceuticals.

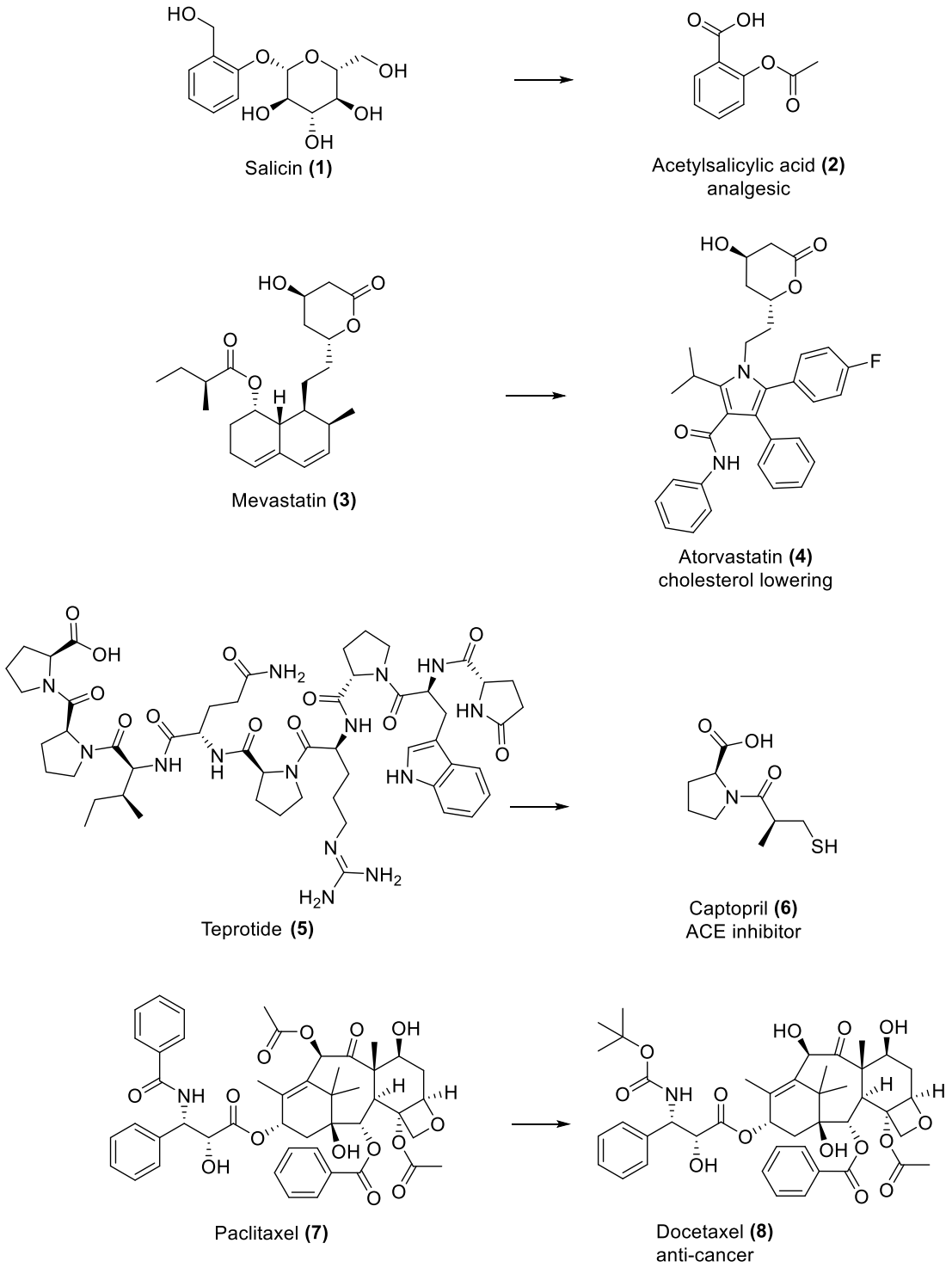


Figure 1.1 Structures of natural product pharmaceuticals and their inspired synthetic analogues.

1.2 Polyketides

Polyketides are a structurally diverse group of secondary metabolites, despite being constructed from a common monomer (Figure 1.2). They occur in various organisms, including bacteria, fungi, plants and protists, and display a wide variety of biological activities.[4-8] This class of natural products has influenced human medicine in a profound manner. Some examples include anti-malarial compounds (cladosporin **(9)**), kinase inhibitors (hypothemycin **(10)**), anti-angiogenic drugs (cytochalasin E **(11)**), antibiotics (erythromycin A **(13)**), anticancer drugs (doxorubicin **(14)**), cholesterol-lowering agents (lovastatin **(15)**), immunosuppressant drugs (rapamycin (**16)**), and antifungals (amphotericin B **(17)**), (Figure 1.2).[5,7,9]

In 2005 it was estimated that polyketide-based therapeutics represented 20% of the top-selling drugs on the global market with total annual revenues of over US $20 billion.[7] Perhaps the most attractive aspect of polyketides is that they are naturally produced in readily usable forms for pharmaceutical applications. With more than 20 commercialized polyketide drugs of the ~7000 structures known; the "hit rate" of polyketides is ~ 0.3%, which is considerably higher than that of typical high-throughput screening of synthetic compound libraries.[7]

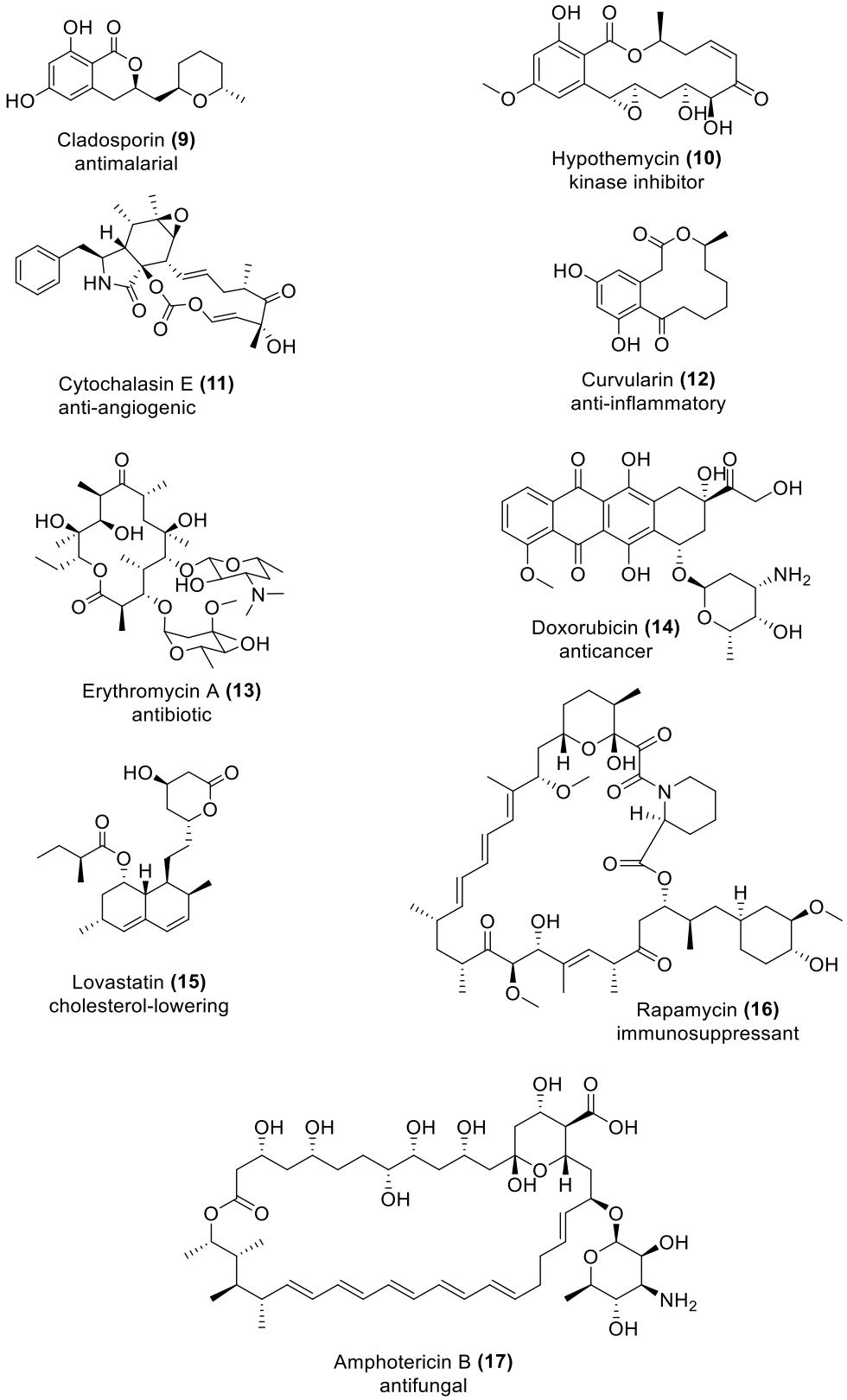
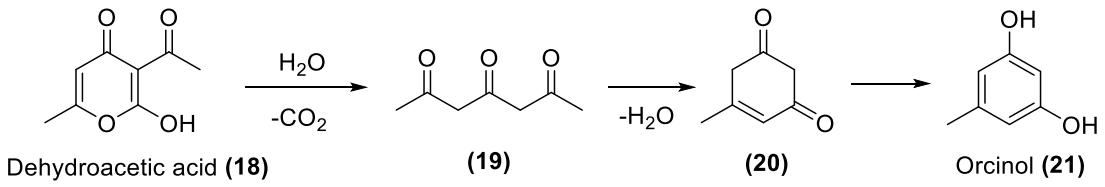


Figure 1.2 Select examples of pharmaceutically relevant polyketides.

The term "polyketide" arose from John N. Collie's early work, where he refers to them as "multiple keten group" representing the polyketomethylene group, $-(CH_2\text{-}CO)_n$.[10,11] In 1893, Collie attempted to degrade dehydroacetic acid **(18)** through treatment with barium hydroxide; and obtained orcinol **(21)** as the principal product (Scheme 1.1).[11] He postulated that the dehydroacetic acid **(18)** was hydrolysed by water to form a polyketone intermediate **19**, which was later condensed and aromatized to give the aromatic compound orcinol **(21)**.[11] Notably, he later proposed, after much probing into the reactivity of the $-(CH_2\text{-}CO)_n$- group, that fats and oils might also be derived from these simple ketides.[10,12] However, it was not until the 1950s that this proposal was supported by Arthur Birch, who demonstrated that polyketides are generated by the repeated condensation of acetate units.[13] Birch *et al.* incorporated ^{14}C-labelled acetate units into 6-methylsalicylic acid. This pioneering work along with other stable isotope-labelling methodologies (^{13}C, ^{2}H, ^{3}H, ^{18}O), in concert with the developments in NMR and mass spectrometry, has paved the way for biosynthetic studies of polyketides and what is now accepted as common practice.[4,14,15]



*Scheme 1.1 Proposed formation of orcinol **(21)**, through degradation of dehydroacetic acid **(18)**.*

1.2.1 Programming of Polyketide Synthases

The fundamentals of polyketide biosynthesis are best understood through comparison to fatty acid biosynthesis, as they share the same precursor. Both polyketides and fatty acids are usually produced from acetate units. As such their biosynthetic pathway can be described as the acetate pathway (Figure 1.3).

1. With limited exceptions, the first step in the pathway for the assembly of polyketides and fatty acids is activation of the acyl carrier protein (ACP). This activation is accomplished by the phosphopantetheinylation of a serine residue of the ACP by a phosphopantetheine (Ppant) transferase enzyme.[16-18] This 18 Å long Ppant moiety provides an anchor and the requisite flexibility allowing the growing polyketide chain to sample the various active sites of other domains for the required modifications and elongations.[19]
2. The acyltransferase (AT) domain catalyzes the priming of the starter unit (commonly acetyl-CoA) or the growing chain to the ketosynthase (KS) domain as well as the extension unit (commonly malonyl-CoA) to the ACP domain. In fungal fatty acids synthases (FASs), a single AT domain promotes both acetyl- and malonyl-priming, while bacterial polyketide synthases (PKSs) typically use different AT domains to facilitate the priming of the starter and extension units.[20] Notably, the starter units are competitively loaded to the Ppant thiol of the ACP domain. Therefore only the acetyl unit or growing polyketide chain can be transferred to the KS domain through transthioesterification.[21,22]

3. The KS domain catalyzes the decarboxylative Claisen-condensation of the extender unit, providing the nucleophile, which attacks the thioester of the growing chain furnishing a β-keto intermediate tethered to the ACP domain.[23]
4. The major difference between PKSs and FASs is the fourth step. In the biosynthesis of fatty acids, the β-keto intermediate from step 3 has to undergo three sequential reductions (4a, b, c), exerted by the ketoreductase (KR), the dehydratase (DH), and the enoyl reductase (ER) domains, finally yielding the fully reduced product which is either offloaded (6c) by the thioesterase (TE) domain or returned to the KS domain for further elongation *via* (5d).[4] However, in the biosynthesis of polyketides, the β-keto intermediate from step 3 can skip any of the reductive steps (4a, b, or c) and return to the KS domain *via* (5a, b, c, or d) for further chain elongation.[4] This variation in the programming between PKSs and FASs is responsible for the abundant diversity (not just limited to chain length) seen in polyketides, relative to fatty acids.
5. Following the reductive modifications, the growing chain is returned to the KS domain as a new starter unit. Importantly this also makes the ACP domain available for loading of another malonyl-CoA as a new extender unit for a repeated round of Claisen-condensation (step 3). From here, a production cycle is established (step 3 - step 4 - step 5 - step 3…) for further growth and elaboration of the growing chain.
6. When the desired product is generated, the thioesterase (TE) domain terminates chain elongation by offloading the product from the ACP. If all β-keto intermediates skip the reductive steps (4a, b, c) and only take pathway 5a, then

unmodified polyketides are formed (6a). Further cyclization of the unmodified polyketide chain can lead to aromatic polyketide products. If the β-keto intermediates skip some of the reductions (combinations of pathway 5a, b, c, or d), partially reduced polyketides are generated with great diversity (6b). Finally, if all β-keto intermediates undergo all the reductive steps (4a, b, c) and take pathway 5d, then the fully saturated fatty acids are produced (6c).

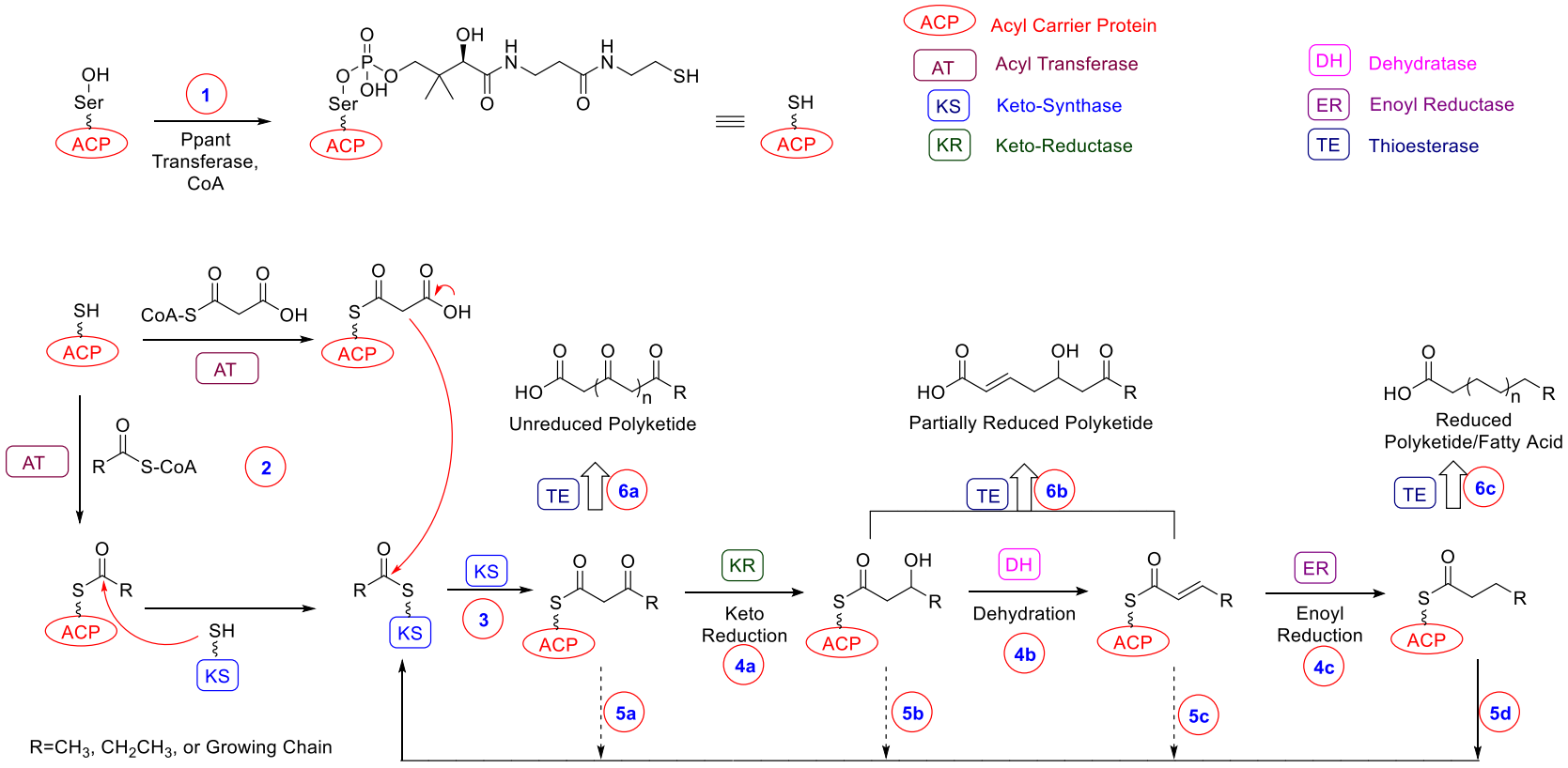


Figure 1.3 General biosynthesis of polyketides and fatty acids.

While it is known that PKSs employ more complex programming rules than FASs, it is still not entirely clear how PKSs manage to generate such structurally diverse natural products with such great efficiency. The term "programmed PKS" was introduced to describe "*control of the variables that determine the structure of the product of a specific PKS*".[24]

As an example, consider the biosynthesis of 6-deoxyerythronolide B **(22)** by the 6-deoxyerythronolide PKS (DEBS) (Figure 1.4). This is perhaps the most extensively studied PKS. Like all PKSs and FASs, the first programming feature of concern is the selection of the starter units and extender units. The DEBS specifically uses propionyl-CoA instead of acetyl-CoA as the starter unit and (2*S*)-methylmalonyl-CoA as the extender unit.[25] The second feature is the specific control of the reductive modifications of β-keto groups in the growing chain. During the first round of chain extension, DEBS selectively reduces the β-keto group to the (*R*)-β-hydroxyl moiety, which then serves as a starter unit for the second chain extension. Of note here is (i) stereochemical control of the ketoreduction, as only the (*R*)-β-hydroxyl intermediate is formed, and (ii) control of the extent of reduction, as the intermediate is not further reduced to a fully saturated fatty acid. Another feature is regulation of cyclization of the growing carbon chain; while there are several hydroxyl groups available to be cyclized and form a lactone, only a 14-membered macrolactone **22** is produced as a single isomer.

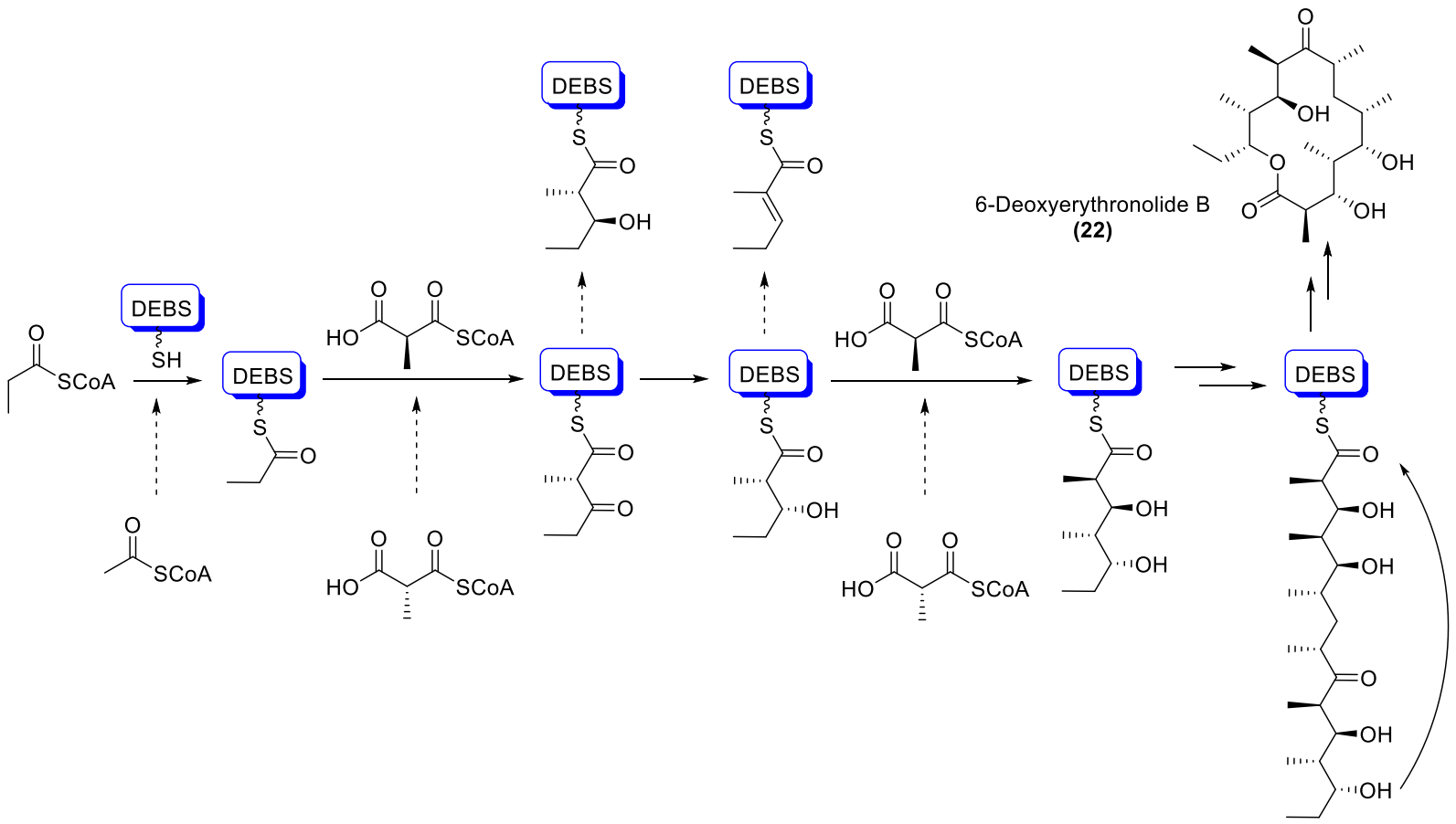


Figure 1.4 PKS programming by DEBS towards the production of 6-deoxyerythronolide B ***(22).*** *Dashed arrows indicate possible pathways, while solid arrows represent the mechanistically confirmed pathways.*

While our understanding of PKSs programming has grown over the years with the aid of crystal structures of individual PKS domains,[20,26-28] there are still numerous questions left unanswered regarding their operation as a single entity. As will be highlighted in chapters 2 and 3, efforts towards the elucidation of these still unknown details are underway in our research lab.

1.2.2 Classifications of Polyketide Synthases

Polyketide synthases are separated into three major types: type I,[28-30] type II,[31] and type III PKSs[32], with the type I PKSs further divided into two sub-classes: modular type I PKSs and iterative type I PKSs.[28,30] Modular PKSs possess multiple catalytic domains organized into 'modules', where each enzyme subunit may be comprised of several modules.[33] Conversely, iterative PKS only consists of one set of domains, utilized repetitively during each round of chain elongation.

1.2.2.1 Type I Polyketide Synthases

Type I PKSs are multimeric complexes, comprised of large, multifunctional subunits possessing all the active sites required for polyketide biosynthesis within discrete catalytic domains.[34] The type I PKSs can act in either an iterative[28] or modular[30] fashion.

1.2.2.1.1 Modular Type I Polyketide Synthases

Modular PKSs, synthesize the growing polyketide chain in an assembly line-like process, with each module participating in a single round of chain elongation and modification. At a minimum, all modules possess a KS, AT, and ACP domain. Generally, specialized modules exist at the N-terminus of specific subunits to facilitate starter molecule loading. They also feature additional AT and ACP domains, as well as TE domains associated with the last module to facilitate product offloading. A good example of the modular type I PKS is the 6-deoxyerythronolide B synthase (DEBS) from *Saccharopolyspora erythraea*, responsible for the biosynthesis of the aglycone core of the clinically important macrolide antibiotic erythromycin A **(13)**.[35] The heteromultimeric 6-deoxyerythronolide B synthase (DEBS) - complex is comprised of three approximately 330 kDa subunits (designated DEBS1, 2, and 3), each comprised of two modules, that contain additional ketoreductase, enoylreductase, or dehydratase catalytic domains (Figure 1.5).[35] Once released from the complex by the TE domain, the product 6-deoxyerythronolide B **(22)** is subsequently modified by tailoring enzymes to yield the final macrolide antibiotic **13**.

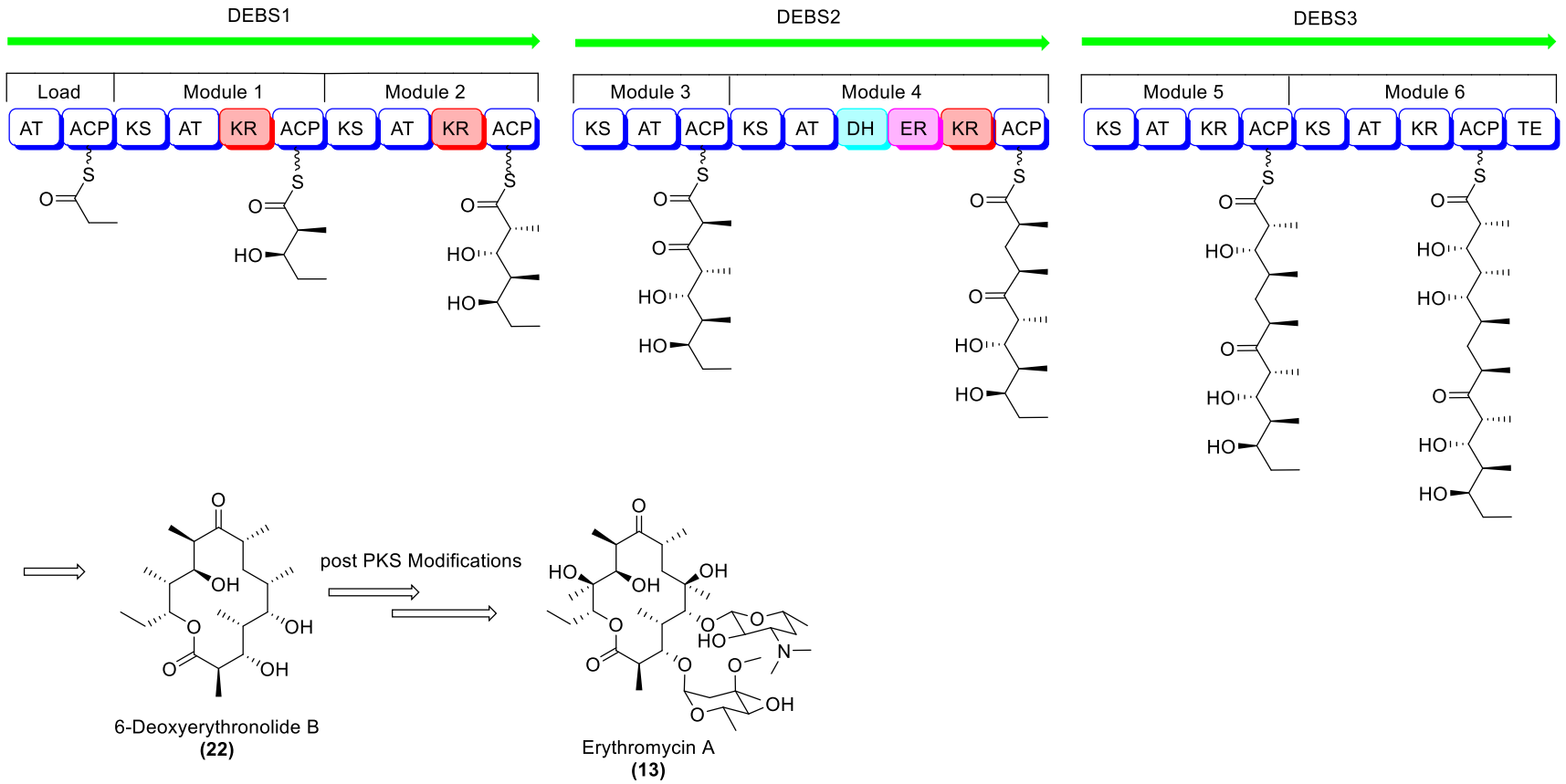


Figure 1.5 Biosynthesis of erythromycin A ***(13).***

1.2.2.1.2 Iterative Type I Polyketide Synthases

While the domain architecture of iterative PKSs resembles that of modular PKSs, iterative PKS only consists of one set of all the required domains, that are utilized repetitively during each round of chain elongation. They are predominantly employed by fungi.[28] Each domain is reused during the chain extension, in stark contrast to the bacterial type I system where the growing chain is only passed on downstream. Importantly, it is this iterative process that renders the prediction of PKS products at the genetic level impractical for iterative type I PKSs.

Iterative type I PKSs can be further subdivided into three groups based on the presence or absence of the various β-position-reducing domains (KR, DH, and ER). These sub-groups are highly reducing (HR)-PKSs, partially reducing (PR)-PKSs and non-reducing (NR)-PKSs. HR-PKSs possesses a full set of β-position-reducing domains, while the NR-PKSs have no β-position-reducing domains. Importantly, it has been shown that the HR-PKSs and NR-PKSs can work collaboratively to synthesize polyketides, with examples such as cladosporin **(9),** hypothemycin **(10)**, and radicicol **(28)**.[36-39]

The PR-PKSs are very rare, and usually, contain a KR domain or KR-DH didomain. Incidentally, the first cloned and purified fungal PKS was a PR-PKS; the 6-methylsalicylic acid **(23)** synthase (6-MSAS).[40-43] With only one ketoreduction and dehydration required to form this product, the 6-MSAS was initially believed to contain two reducing domains (KR and DH), along with a non-catalytic core domain (Figure 1.6). However, based on recent literature it is unclear whether the dehydration is enzyme-catalyzed, it suggests that the DH and core domain are better described as a

single domain; this being a thioesterase/interdomain (THID), believed to be involved in product templating and release.[44]

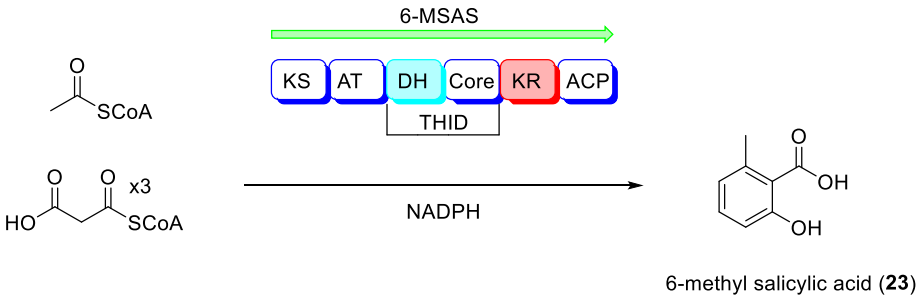


Figure 1.6 Biosynthesis of 6-methylsalicylic acid ***(23).***

The biosynthesis of aflatoxin B_1 **(25)** is a good example of an NR-PKSs. Aflatoxin B_1 **(25)**, is a well known environmental carcinogen produced after post-PKS modifications are made on the PKS product, noranthrone **(24)**.[45] It is produced in *Aspergillus spp.* by the PksA (Figure 1.7).[45] The PksA PKS contains the basic minimum domain requirement for polyketide production. These are the AT, ACP, and KS, along with others common to this class. It bears an SAT domain at the N-terminus, exclusively responsible for the loading of the hexanoyl-CoA starter unit.[46] The AT domain as expected is responsible for the loading of the extender unit, malonyl-CoA.[46] N-Terminal to the ACP is the product template (PT) domain, responsible for orienting the poly-β-keto chain and catalyzing the aldol cyclizations which eventually lead to the aromatized product **24**.[47] PT domains are integral in the production of aromatic polyketides, as they fold the growing chain into a favourable conformation thus allowing the cyclizations that lead to the product.[47] Lastly, the C-terminus of the PksA carries the TE/cyclase (CYC) domain, responsible for the Claisen/Dieckmann

condensation thus releasing the NR-PKS product noranthrone **(24)**. This NR-PKS product then undergoes a series of post-PKS modifications to finally furnish the finished product aflatoxin B_1 **(25)**.

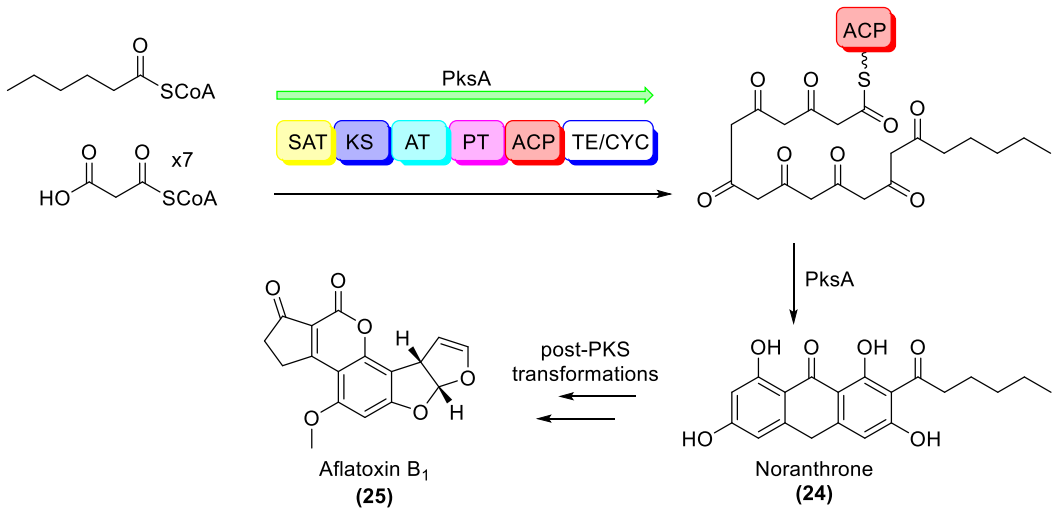


Figure 1.7 Biosynthesis of aflatoxin B_1 ***(25).***

The HR-PKSs contain KR, ER and DH domains, as well as the supplementary tailoring domains such as methyltransferases (MTs). In addition to catalyzing a specific round of chain elongations by a single set of domains, the HR-PKSs also selectively use processing domains (C-methyltransferase (CMeT), KR, DH, and ER) to modify β-keto intermediates during each round of chain elongation. Each chain length is tailored to a specific degree, creating a diversity of functional groups in a single polyketide chain. While this is a similar concept to the modular type I PKSs, the mechanism and enzyme structures are significantly different. Given that there is only one set of domains that is used iteratively, it is not entirely clear how each HR-PKS can be "programmed" to use the tailoring domains on specific intermediates in the production process to make such

structurally diverse natural products. Work by Cacho *et al*. in 2015, helped to clarify some of these cryptic transformations.[48] Through the use of lovastatin nonaketide synthase (LovB) as a model system, and a variety of acyl substrates, the substrate specificity of the LovB methyltransferase (MT) domain was characterized.[48] It was found that, while the MT domain displays methylation activity towards various β-ketoacyl groups, it is extremely selective towards its naturally programmed β-keto-dienyltetraketide substrate, when both chain length and functionalization is considered.[48] These studies showed that the selective modifications by tailoring domains (in this case the MT), may be achieved by higher kinetic efficiencies on a particular substrate relative to the rate of transformation by other competing domains on that same substrate.[48]

The lovastatin PKS (LovB) assembles the polyketide product dihydromonacolin L **(27)** (DHML), which then undergoes a series of post-PKS modification to provide the bioactive compound, lovastatin **(15)** (Figure 1.8).[27] The LovB has all the domains found in a mammalian FAS, with only a few key differences. The first difference being, C-terminal to the DH domain is a MT domain, which installs the methyl group residing at the 6 position of the decalin structure at the tetraketide stage.[48] This MT domain relies on the co-factor *S*-adenosylmethionine (SAM).[48] The second difference is that the constituent enoyl reductase in LovB is inactive. Thus the role is performed by a *trans*-acting enoyl reductase known as LovC.[27] Perhaps the most notable difference is the presence of a non-ribosomal peptide synthetase module (NRPS), truncated after the condensation (CON) domain.[49] This appears to be the remnant of a PKS-NRPS hybrid system. Whereas the CON domain is known to be required for DHML **(27)** production,

its specific function has yet to be identified.[50] Lastly, due to the absence of the domain to release the finished polyketide product, a *trans*-acting TE (LovG) has to perform this function.[51]

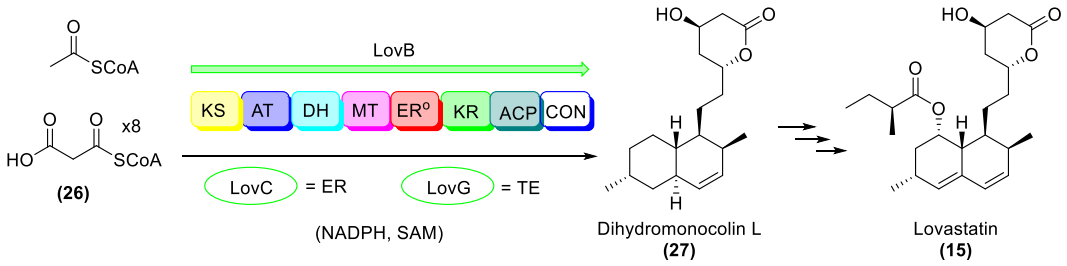


Figure 1.8 Biosynthesis of lovastatin ***(15).***

Importantly, while these iterative type I PKSs may be divided into subclasses, they often do not conform to the simple model of NR-, PR- and HR-PKSs. Many of these PKSs exist as hybrids, and work with other biosynthetic machineries to produce a diverse set of natural products (Figure 1.9). Three important groups of these hybrids include HR/NR-PKS partners, the PKS/NRPS hybrids and the meroterpenoids. As the name suggests, the HR/NR-PKS are hybrids formed by the co-operative action of a HR-PKS and a NR-PKS to produce a single polyketide product. Some well-known polyketides produced by HR/NR-PKS hybrids are hypothemycin **(10)**, radicicol **(28)**, and dehydrocurvularin **(32)** (Figure 1.9).[36,37,52-55] PKS/NRPS hybrids consist of a PKS and a NRPS module that is capable of installing an amino acid at the end of the polyketide chain before offloading. The bioactive natural products made by this class of megasynthases are highly diverse, as illustrated by the structures of cytochalasin E **(11)**, equisetin **(29)** and fusarin C **(33)** (Figure 1.9).[6] Meroterpenoids are hybrid natural

products partially derived from the terpenoid pathway.[56] The other portion may be of polyketide origin or other non-polyketide starters.[56] Examples of some polyketide-terpenoid hybrid molecules are berkeleydione **(30)** (anti-cancer drug),[57] α-tocopherol **(34)** ("vitamin E"),[58] and tetrahydrocannabinol **(31)** (component of marijuana)[59] (Figure 1.9).

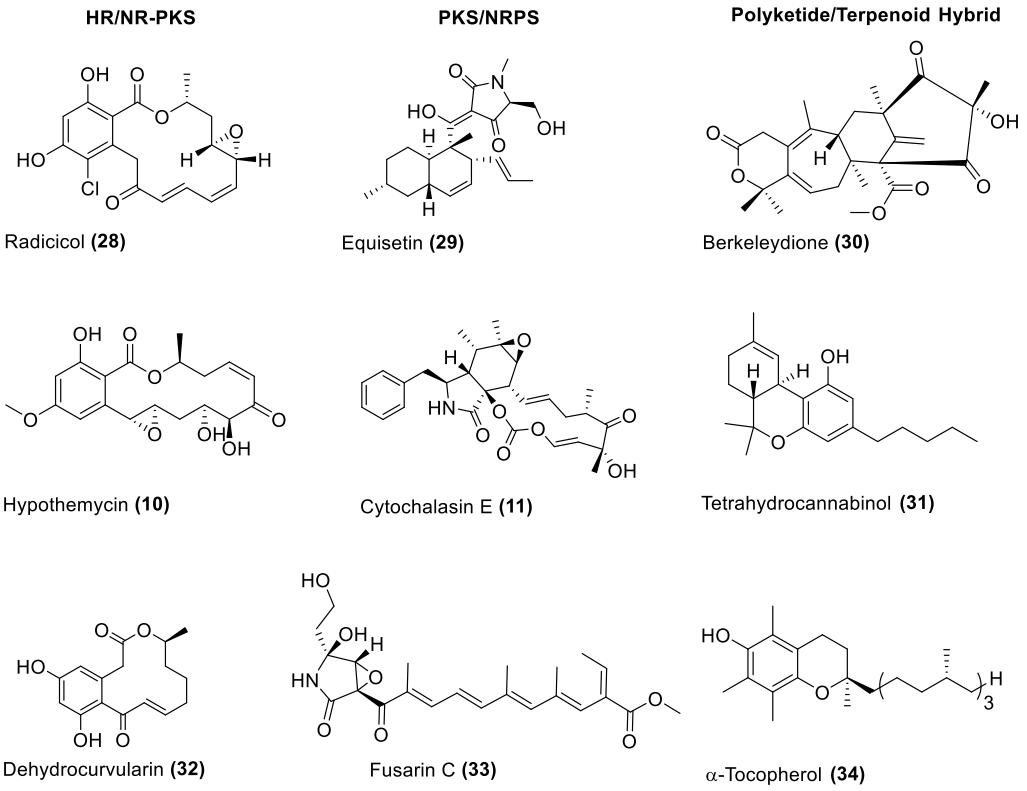


Figure 1.9 Examples of natural products made by polyketide hybrid systems.

1.2.2.2 Type II Polyketide Synthases

Unlike the type I PKS megasynthases, type II PKS enzymes are relatively small dissociable proteins (domains) analogous to type II bacterial and plant FASs.[60] They are also referred to as bacterial aromatic polyketide synthases and are involved in the

biosynthesis of some clinically important bacterial aromatic polyketide products exhibiting antitumor or antibiotic activity, such as doxorubicin and oxytetracycline.[34]

Type II PKS complexes consist of at least the KSα, KSβ, MAT, and ACP domains (Figure 1.10).[34] Additional subunits containing KR, CYC, or aromatase (ARO) activity may also occur in more complex type II synthases. Typically, the four core subunits participate in the iterative series of condensation reactions until a specified polyketide chain length is achieved. The associated ARO and CYC domains then catalyse the folding and cyclization reactions yielding the final aromatic polyketide (Figure 1.10).[34]

In contrast to the active KSα domain, the KSβ domain possesses a glutamine instead of a cysteine in the active site. KSβ forms a heterodimer with KSα to catalyze the iterative decarboxylative Claisen-condensation of malonyl-CoA extender units.[61] While inactive by itself, the KSβ has been shown to govern the chain length of the growing polyketide chain,[62] and as such it has been called the "chain length factor" (CLF).[63] Given that the AT domain is absent in most type II PKSs, the ACP domain is believed to recruit the extender unit by self-priming of malonyl-CoA. The ACP-tethered malonyl-CoA then undergoes decarboxylation to form an acetyl unit, which can then be transferred to KSα as starter units, similar to that of HR-PKSs.

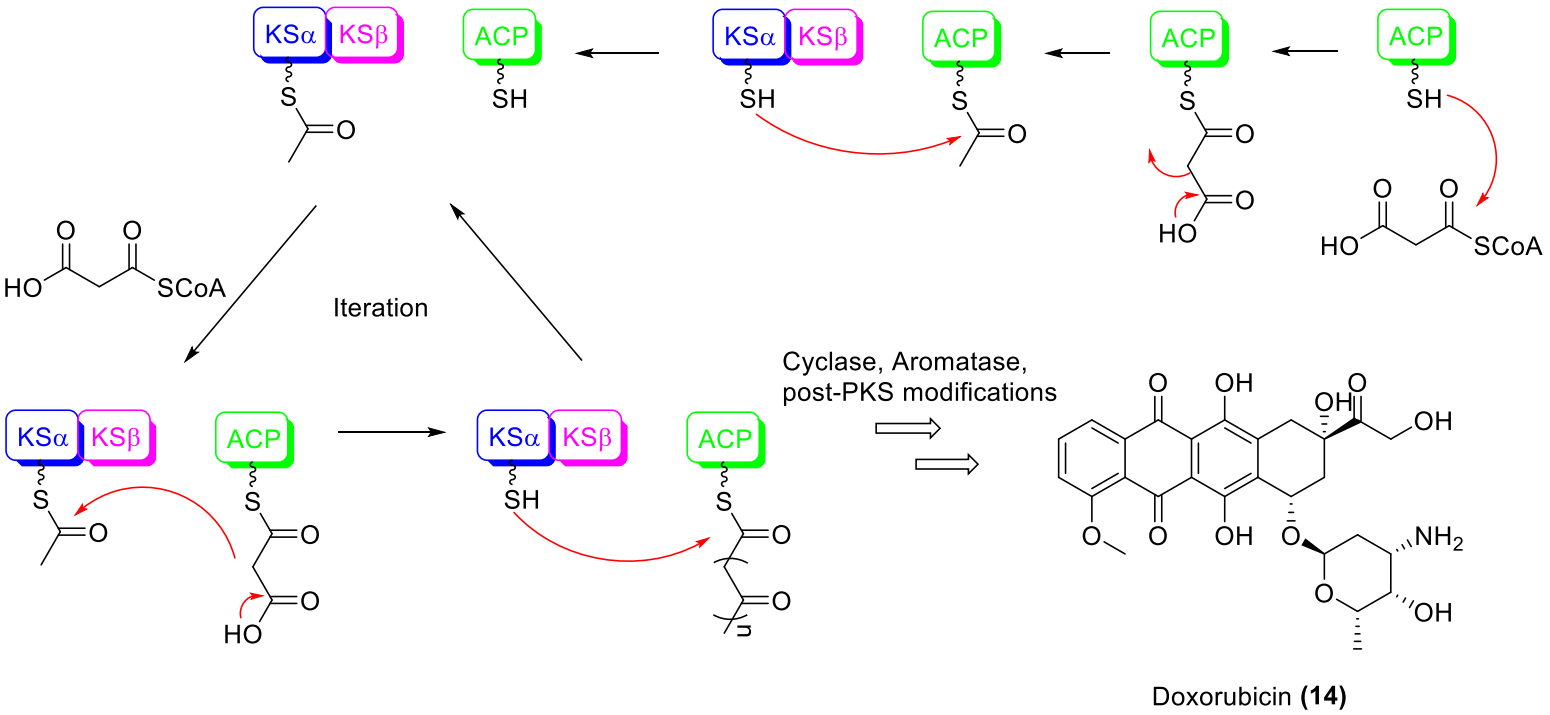


Figure 1.10 Biosynthesis of doxorubicin ***(14).***

1.2.2.3 Type III Polyketide Synthases

The type III PKSs are responsible for the biosynthesis of a large number of plant-derived natural products, including flavonoids derived from the important branched metabolite 4',2',4',6'-tetrahydroxychalcone, the product of the enzyme chalcone synthase.[64] The type III PKSs are collectively referred to as the chalcone synthase/stilbene synthase superfamily', because the chalcone synthase (CHS) was the first type III enzyme discovered, followed by a second flavonoid pathway type III enzyme, the stilbene synthase (STS).[32,65]

Of the three major types of PKS, the type III PKS is the least complex, and occurs as comparatively small homodimers, with subunits between 40-45 kDa in size.[34] As in the case for type II enzymes, type III PKSs catalyze iterative decarboxylative condensation reactions typically using malonyl-CoA extender units. However, in contrast to type II synthases, the subsequent cyclization and aromatization of the growing polyketide chains occur within the same enzyme active site.[32] Unique to this family of PKSs, is the absence of the ACP. Type III PKSs use free CoA thioesters directly as substrates for both starter and extender units. Generally type III synthases have a wider spectrum of physiological starter molecules compared to their type I and II counterparts, including a variety of aromatic and aliphatic CoA esters such as coumaryl-CoA, methyl-anthraniloyl-CoA, as well as the recently identified medium- and long-chain fatty acyl-CoA ester starters used by certain bacterial and plant type III enzymes involved in the biosynthesis of phenolic lipids.[34,65,66] Perhaps the most well studied and characterized of the type III PKSs is the chalcone synthase. It uses 4-coumaryl-CoA as the starter unit and catalyzes three successive condensation reactions with malonyl-

CoA as the extender.[67,68] Cyclization and aromatization of the linear tetraketide intermediate **(35)** is performed within the same active site, yielding the final chalcone **(36)** product.[67,68] In the case of the stilbene synthase, the variation occurs at the cyclization step, instead of a C6/C1 cyclization as seen in the CHS, the STS displays a C7/C2 cyclization, leading to the stilbene **(37)** product (Figure 1.11).

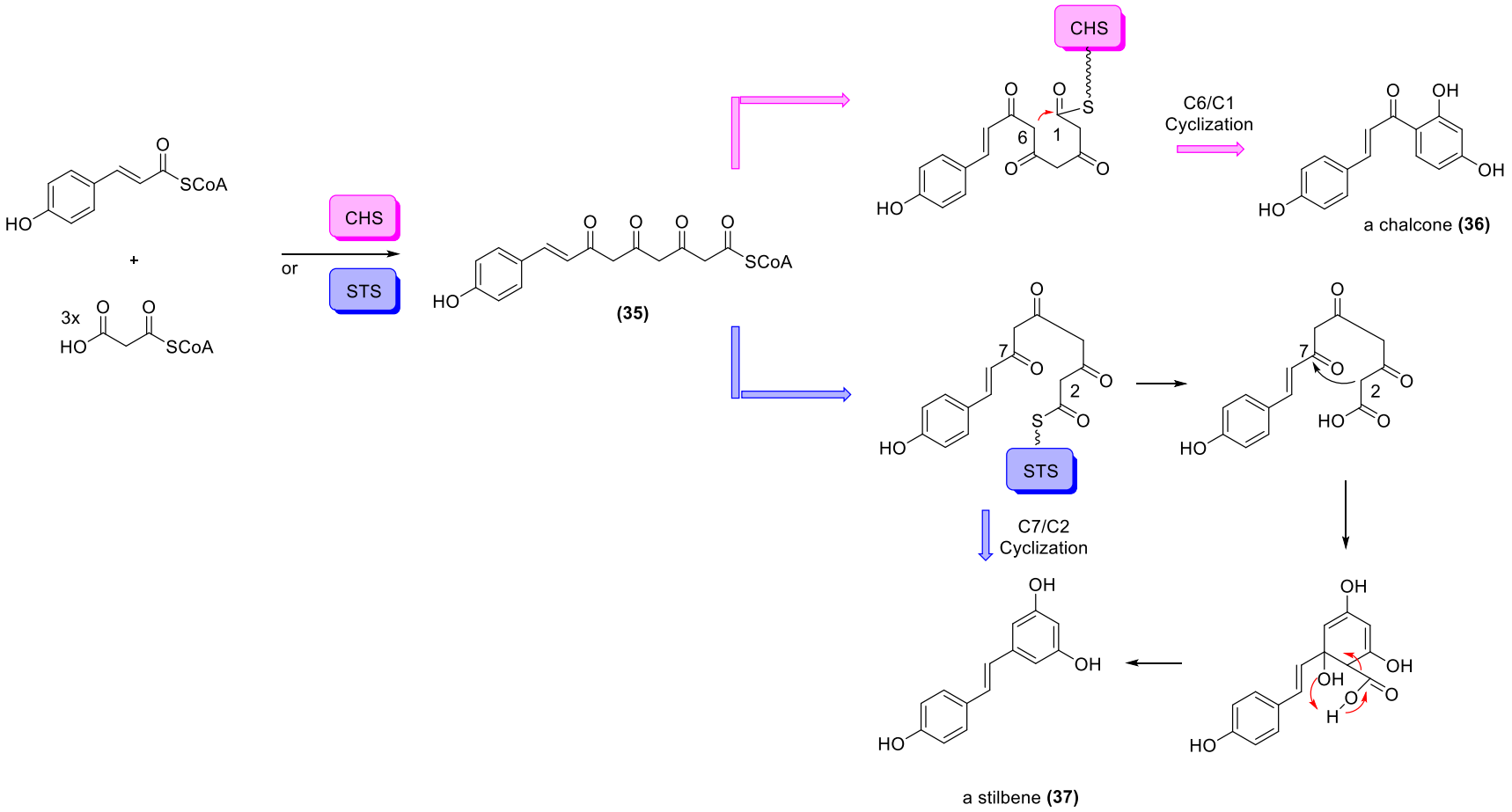


*Figure 1.11 Biosynthesis of chalcone **(36)** and stilbene **(37)** by CHS and STS, respectively.*

1.3 Probing Biological Interfaces

Chemical reporters and probes are "high-value" reagents for biological discoveries because they are orthogonal to numerous molecular biology and genetic approaches. They allow for the molecular-level interrogation of biological targets and their related pathways. Furthermore, they connect basic and translational research by providing effective pharmacological tools for validating potential clinical targets. Additionally, they offer chemical starting points that can be later optimized by medicinal chemists. In this light, site-directed spin labelling (SDSL) has become a successful method to study structural properties and conformational changes of biomolecules, especially in proteins.

Site-directed spin labelling (SDSL) allows for the systematic study of paramagnetically silent biomacromolecule of interest at user-defined positions (Figure 1.12). This is achieved by introduction of spin labelled probes into the naturally occurring material. This ultimately allows the study of structure-activity relationships of spin labelled macromolecules by electron paramagnetic resonance (EPR) spectroscopy. While EPR has been in existence for over five decades, the SDSL technique was only developed in the last 25 years, pioneered by Hubbell *et al*.[69,70] The technique has shown great utility in the study of protein interactions, but has also been used for probing the behaviour of synthetic polymers,[71,72] nucleic acids[73] and lipids[74]. Combination of EPR and SDSL provides insights into protein structure and dynamics at sites of interest almost without background. The main sources of information typically exploited are label dynamics, solvent accessibility, the polarity of the microenvironment, and perhaps most important; distance distributions between two spin labels in the

nanometer range.[75] While a broad range of spin labels can be utilized for such studies, the stable nitroxide radical is seemingly the most popular class.[76]

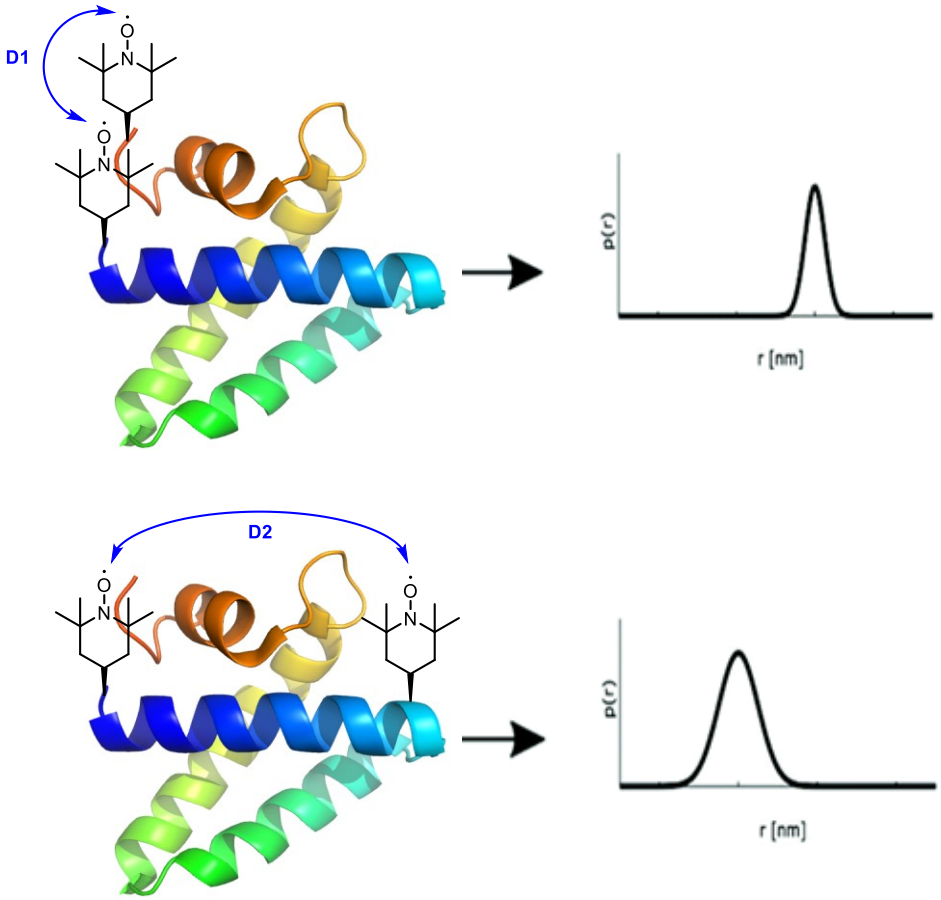


Figure 1.12 Cartoon depiction of EPR distance measurements between two nitroxide spin labels.

1.3.1 Electron Paramagnetic Resonance Spectroscopy

The EPR spectra represent a superposition of all the dynamic processes occurring. The three major factors that contribute to the global dynamics are dynamics of the spin label linker, dynamics of the proteins secondary structure at the site of incorporation, and the rotation of the entire complex.[75]

The molecular environment of the probe can be monitored, by manipulation of the linker dynamics. These changes can arise from conformational perturbations, ligand binding, or differences in the exposure to membrane lipids or intra/extracellular environments as in the case of transmembrane proteins. Notably, spectral characteristics can also be assigned to different types of secondary structural elements.[77,78]

The solvent accessibility of a labelled site can also be measured. It is worth noting that spin labels exposed to paramagnetic quencher result in a significant increase of spin-lattice relaxation.[79] Since this interaction is closely related the interface of the labelled site with the quencher, a wisely chosen reagent polarity can be employed to visualize membrane-bound regions of a molecule when the system is treated with nonpolar molecular oxygen.[75] However, polar quenchers will only interact with the solvent-exposed regions of the target molecule.[80] Through SDSL and subsequent labelling and mapping of the local polarity of a protein chain, the polarity of the microenvironment can be determined.

1.3.2 Site-Directed Spin Labelling

Even though most proteins are intrinsically diamagnetic, using SDSL double electron-electron resonance (DEER) distance measurements are made possible. Considerations must be made regarding the chemical and spectroscopic properties of the probe used, and the corresponding strategy of introduction into the compound of interest. Unfortunately, there is no general strategy that satisfactorily fulfills all the

requirements. This makes it of absolute importance to customize the SDSL to the specific demands of the desired experiment.[81,82]

A very important property of spin labels is the conformational flexibility of both the label scaffold and the linker connecting the scaffold to the protein backbone. This conformational flexibility contributes to the overall dynamics of the paramagnetic centre, which could complicate the analysis of protein dynamics. Another critical property is chemical stability in biological environments, for example, the cytoplasm of living cells. The nitroxide radicals most commonly used are stabilized by steric shielding from the bulky quaternary carbon centres. These are usually α to the nitrogen atom, and bears four methyl, ethyl or even larger alkyl substituents.[75] Notably, care must be taken that the spin labels themselves are not too large as to perturb the native structure of the labelled protein.

Aside from the properties of the spin label, the strategies used for its introduction into the target protein also plays a very important role. Perhaps the most useful method of incorporating the label is through peptide synthesis, allowing for a variety of labels that can be introduced into the peptide backbone. These include labels with improved spectroscopic properties that cannot be introduced by other methods.[81] However, the applications are limited by the inherent difficulties of solid phase synthesis in the production of large proteins, hence the need for ligation, Figure 1.13.

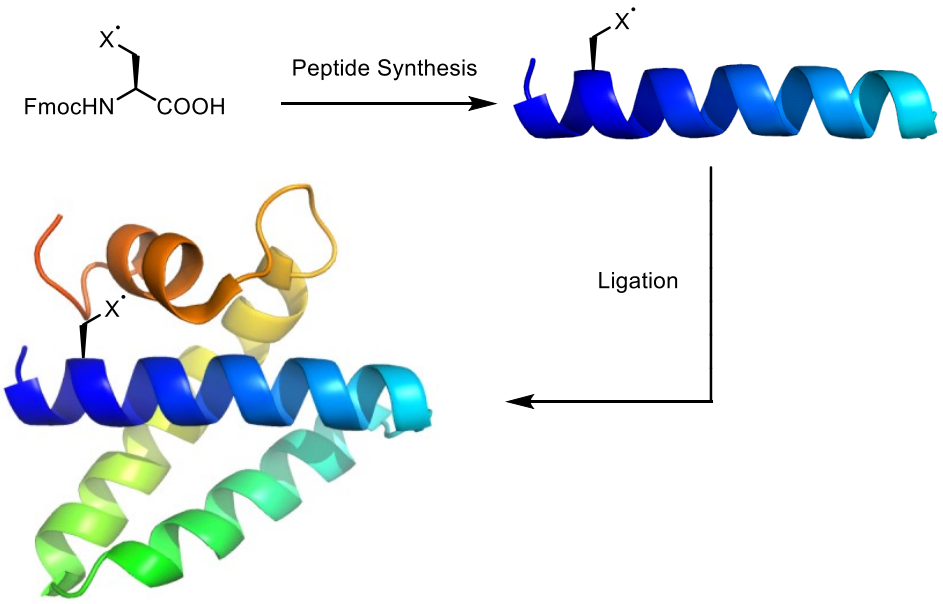


Figure 1.13 Incorporation of spin-labels into mature peptides through solid phase peptide synthesis, and peptide-protein ligation.

The most used method is the labelling of expressed proteins by chemoselective conjugation reactions at specific amino acids. This offers the potential to modify fully natural, endogenous proteins. However, this method is rather problematic as the conjugations may be non-selective and labels could be randomly distributed, unlike incorporation through peptide synthesis. Additionally, care must be taken not to unintentionally label residues required for activity or conformational stability. In such instances, mutations are required to introduce cysteines at user-defined sites to be labelled, Figure 1.14. Targeting amino acids like lysine also suffers from similar limitations. Additionally, the selective labelling of a specific amino acid becomes problematic in cases where there are several of the same amino acid present in the peptide/protein. Fortunately, this non-selectivity can be alleviated by incorporation of

noncanonical amino acids with bio-orthogonal handles, which can be latter used to conjugate to the spin-label of choice, Figure 1.15.

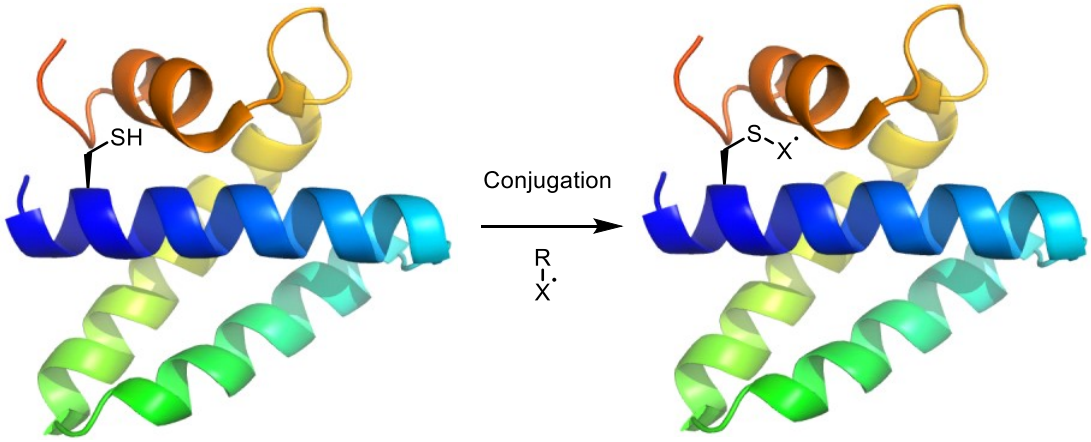


Figure 1.14 Incorporation of spin-labels into peptides, through conjugation to reactive side chains of canonical amino acids.

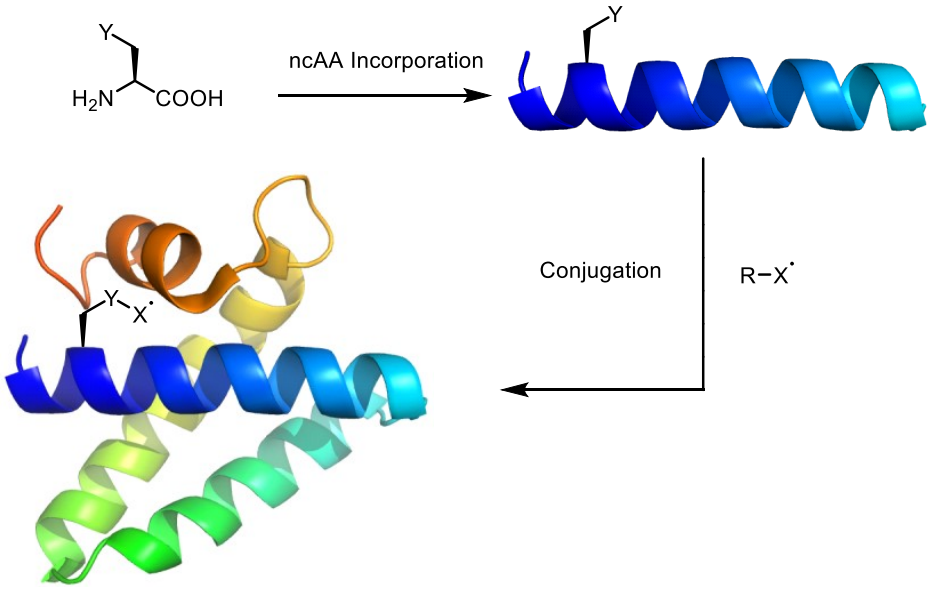


Figure 1.15 Incorporation of spin-labels into peptides, through conjugation to reactive side chains of noncanonical amino acids.

Incorporation of noncanonical amino acids (ncAA) bearing unique functional groups through translation with an expanded genetic code[83] offers chemoselective labelling of endogenous proteins irrespective of the presence of cysteines or lysines and without the introduction of large changes into the protein.[84,85] Even more impressive are the recent improvements that have allowed spin labels to be genetically encoded as ncAA during translation *in vivo*[86-88] thus removing the need for conjugation reactions at the ncAA, Figure 1.16.

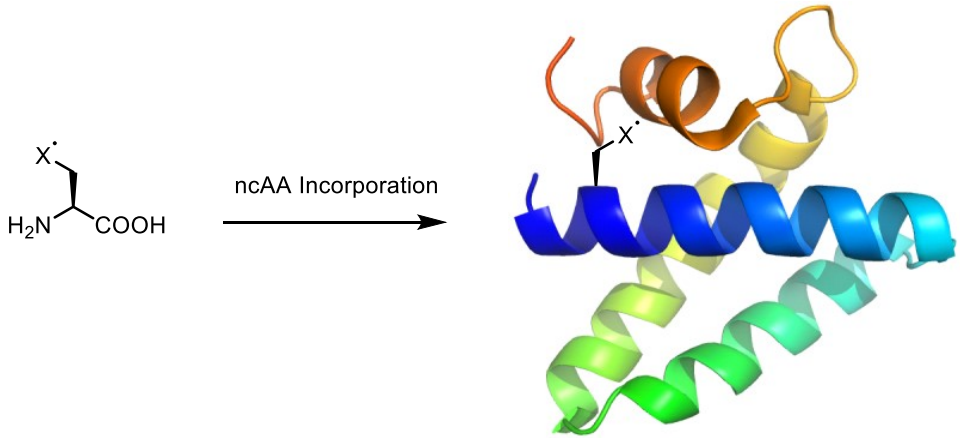


Figure 1.16 Direct genetic encoding of spin labelled ncAA.

While there have been significant improvements in the methods used for the incorporation of these spin labels as well as the EPR techniques; the spin labelled amino acids utilized remain mostly racemic, labile or difficult to couple even under ideal situations. As such, there is a need for an easy route towards the enantioselective preparation spin labelled amino acids; a proposal which will be covered in chapter 5.

1.4 Thesis Overview

Our civilization has been reliant on nature; its biosynthetic machineries and the products thereof. However, much of how nature assembles these compounds is still unclear. The efforts outlined in this thesis seek to provide both methods of probing these pathways as well as answers to some of the pending questions.

Chapter 2 will discuss a new platform for the semi-synthetic preparation of a known nanomolar inhibitor of the *Plasmodium falciparum* lysyl-tRNA synthetase, cladosporin, from *Cladosporium cladosporioides,* and its analogues. Our work will show that cladosporin is indeed biosynthesized by a highly reducing (HR) and a non-reducing (NR) iterative type I polyketide synthase (PKS) pair, namely the **Cla2** and **Cla3**. Additionally, it will show that the **Cla3** enzyme can act alone, converting both natural and unnatural pentaketide intermediates into cladosporin or analogues. This incorporation of the natural pentaketide intermediate by **Cla3** will define a 5+3 assembly between the HR-PKS, **Cla2** and the NR-PKS, **Cla3** during cladosporin biosynthesis.

Chapter 3 will outline the preparation of a set of ubiquitous probes for the study of CoA dependent natural product biosynthetic machineries. It will highlight the preparation of four different CoA analogues in single steps through a transamidation reaction. This work will offer access to useful compounds for the study of enzyme-catalyzed reactions, especially processes involving acyl/peptidyl carrier proteins (A/PCP) as in the polyketide synthases (PKS), fatty acid synthases (FAS), and nonribosomal peptide synthetases (NRPS). It will also briefly discuss some current applications of the probes,

focused towards improving our understanding of the **Cla2-Cla3** interactions for the biosynthesis of cladosporin.

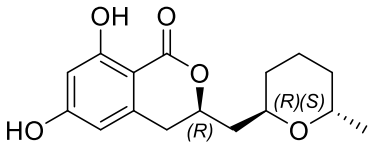
Chapter 4 will focus on a new Dess-Martin periodinane mediated oxidative rearrangement reaction that was uncovered. Through a series of labelling experiments and intermediate testing, it will be shown that the reaction proceeds through a series of oxidative steps, converting a β-hydroxy thioester to an α-keto thioester, with the loss of CO_2. It will also be shown that these α-keto thioesters react with diamines in excellent yields, under mild conditions to form quinoxalinones, a pharmaceutically important heterocyclic scaffold.

Chapter 5 will outline the enantioselective preparation of spin-labelled α-amino acids for electron paramagnetic resonance (EPR) spectroscopy. It will show that by using the chiral nickel (II) Schiff base complex of glycine, these spin-labelled α-amino acids can be prepared in a 9:1 ratio. It will also show that the enantiomerically pure L-spin-labelled amino acid can be obtained through degradation of the minor D-enantiomer by D-amino acid oxidase.

Chapter 2: Biosynthetic Studies of Cladosporin

2.1 Introduction

Cladosporin **(9)** (Figure 2.17) is an isocoumarin based fungal polyketide produced in nature by several species such as *Cladosporium*,[89-92] *Chaetomium*,[93] *Penicillium*,[94] *Eurotium*,[95,96] and *Aspergillus*.[97] This tricyclic octaketide was first discovered by Scott *et al*. in 1971, who assigned the structure to be 3,4-dihydro-6,8-dihydroxy-3-(tetrahydro-6-methyl-2*H*-pyran-2-yl)methylisocoumarin based on their spectroscopic data.[89] The absolute stereochemistry remained unassigned until 1988 when the Vederas group assigned it as (3*R*)-6,8-dihydroxy-3-[[(2*R*,6*S*)-6-methyloxan-2-yl]methyl]-3,4-dihydroisochromen-1-one.[90] This was accomplished by the use of stable isotope labelling, 2D NMR spectrometry and a degradative study.



Cladosporin **(9)**

*Figure 2.17 Structure of cladosporin **(9).***

Cladosporin **(9)** exhibits an interesting array of biological activities including antibiotic, antifungal, and plant growth inhibitory properties, as well as anti-inflammatory effects in mouse lung tissues.[98] However, of greatest significance to our research was the latest discovery where cladosporin was shown to be a potent

nanomolar (IC_{50} = 40 nM) inhibitor of *Plasmodium falciparum* blood- and liver stage proliferation.[9] Through a series of experiments Hoepfner *et al*. found that cladosporin inhibited the *P. falciparum* cytosolic lysyl tRNA synthetase (**LysRS**) and showed that cladosporin is >100-fold more selective for the parasitic enzyme than that of *Homo sapiens* (Table 2.1).[9]

Through sequence alignment and comparison of the active site residues in a series of organisms, they suggested that the activity of cladosporin was related to the specific amino acid residues present at positions 324 and 340 (Table 2.1). They later did site-specific mutations in *Saccharomyces cerevisiae* (which exhibited resistance to cladosporin), and showed that the cladosporin resistance was related to the homologous positions in the active site within the ATP binding pocket of the **LysRS**; these being Gln_{324} and Thr_{340}.[9] Systematically replacing these two residues with either valine or serine respectively, led to a 5.7- and 10.4-fold increase in cladosporin activity, while the double mutant (both amino acid replaced by valine and serine respectively) was demonstrated to be 38.7-fold more sensitive to cladosporin.[9] These results suggested that the tolerance for cladosporin appears to hinge on the presence of a small polar group at position 324 and a more bulky group at position 340. This perhaps also explains the selectivity of cladosporin to *P. falciparum* **LysRS** over *H. sapiens* **LysRS**, considering the amino acid residues at the homologous positions in the *P. falciparum* **LysRS** are Val_{324} and Ser_{340}, and those present in *H. sapiens* are Gln_{324} and Thr_{340} (Table 2.1).

Table 2.1 Summary of amino acid conservation at key residues in the ATP pocket of the LysRS and corresponding IC_{50} values of cladosporin

Organism	**IC_{50} (µM)**	**Key active site residues**	
Plasmodium falciparum	0.04-0.08	Val	Ser
Plasmodium yoelii	0.4	Val	Ser
Trypanosoma brucei	2.05	Val	Thr
Leishmania donovani	2.56	Val	Thr
Toxoplasma gondii	2.63	Asn	Ala
Homo sapiens	>10	Gln	Thr
Saccharomyces cerevisiae	30-110	Gln	Thr

These findings were further supported by the work of Khan *et al*. who published the co-crystal structure of *P. falciparum* **LysRS** and cladosporin.[99] It showed cladosporin **(9)** occupying the ATP binding site in the **LysRS**, specifically that the isocoumarin moiety of cladosporin occupies a similar orientation as that of the adenine in ATP, with its aromatic ring in a hydrophobic interaction with Val_{324}.[99] Importantly, the tetrahydropyran (THP) ring of cladosporin was observed to be located directly adjacent to Ser_{340},[99] suggesting that any increase in steric bulk would likely clash with the methyl substituent on the THP ring, and prevent cladosporin binding; possibly explaining the selectivity observed in the work published by Hoepfner *et al*.[9] The authors also conducted a sequence-based search for other pathogenic organisms containing **LysRS** enzymes with the respective cladosporin accommodating residues and found that the results included the trypanosomiasis causing pathogens: *Trypanosoma cruzi*, *Trypanosoma vivax*, *Trypanosoma congolense;* schistosomiasis causing pathogen,

Schistosoma mansoni; and loaiasis causing pathogen, *Loa loa;*[99] all of which could be potential targets of cladosporin **(9).**

While there are numerous commercially available antimalarial agents, the endoperoxides currently represent the only class of molecules for which resistance has not yet significantly developed. However, even these do not inhibit the asymptomatic liver-stage of the infection. The discovery of cladosporin's **(9)** activity against both liver- and blood-stage malaria parasites, along with the experimental dissection of its exclusive targeting of the *P. falciparum* lysyl tRNA synthetase is a pivotal achievement in anti-malarial target validation/drug development.

This new bioactivity of cladosporin **(9)** represents a promising lead for treatment of malaria. Thus, efforts to produce multitarget specific cladosporin derivatives, fine-tuned to improve the drug-like properties of cladosporin were embarked upon in our lab. We hope to capitalise on the promiscuity of the natural biosynthetic machinery to develop a semisynthetic approach in the production of new analogues.

2.2 Biosynthetic Studies of Cladosporin Production

Previously, research was conducted by the Vederas group on the biosynthesis of several related fungal polyketides belonging to the resorcylic acid lactone (RAL)- and dihydroxyphenyl acetic acid lactone (DAL)-containing polyketides, this included hypothemycin **(10)** (RAL type),[37] radicicol **(28)** (RAL type),[36] and dehydrocurvularin **(32)** (DAL type).[52-55] Studies of these compounds showed that the biosynthesis of these polyketides is achieved by the HR/NR-PKS hybrids, as they require the cooperative

action of two iterative type I polyketide synthases (PKSs): a highly reducing (HR) PKS and a non-reducing (NR) PKS.[52-55] Based on structural similarities, we hypothesized that cladosporin **(9)** was also biosynthesized by a HR- and NR-PKS hybrid (Figure 2.18). In our hypothesis, we proposed that the first five ketide units forming the pentaketide were assembled by a HR-PKS, which is then transferred to a NR-PKS for three more extensions and finally cyclization to form the final product (Figure 2.18).

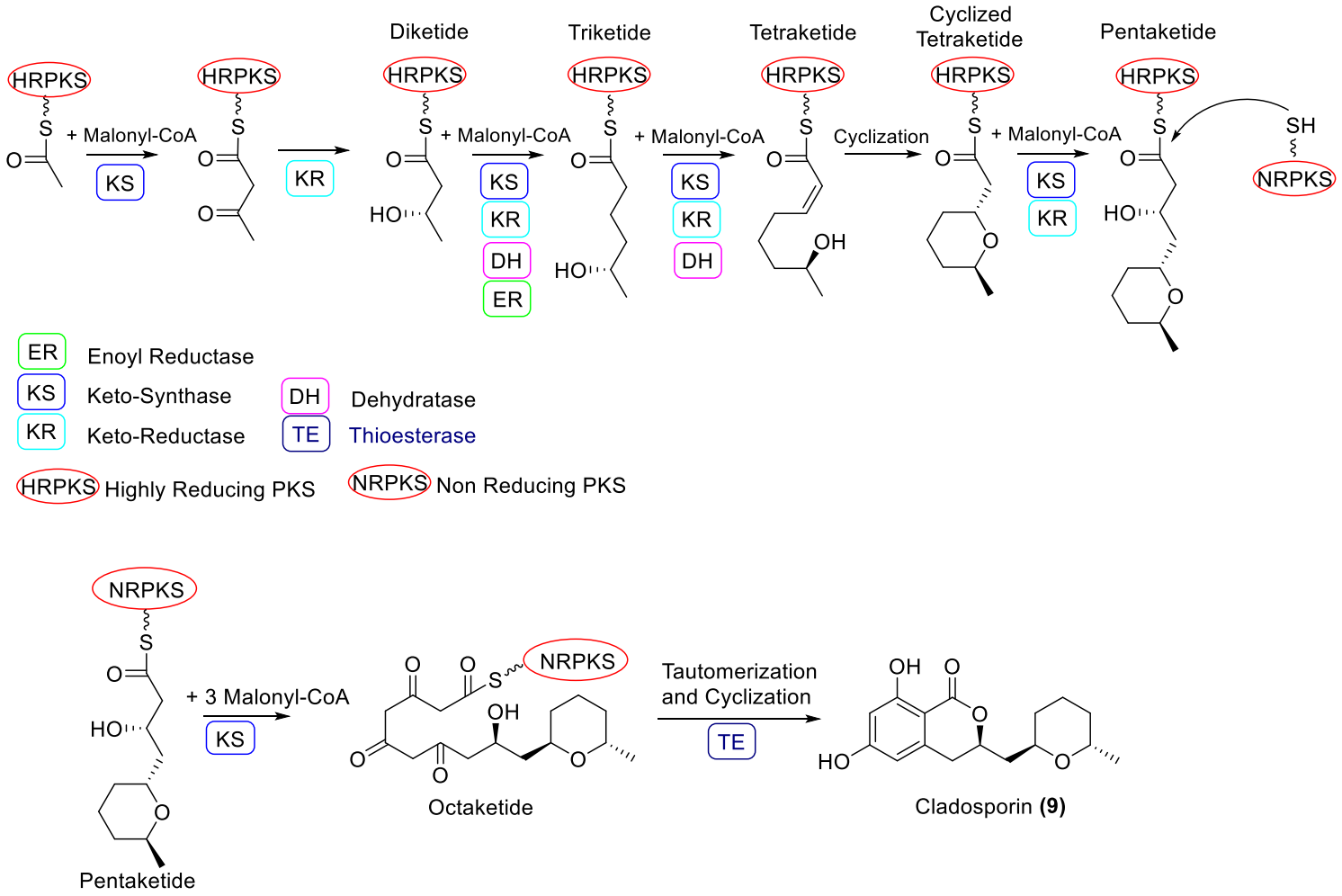


Figure 2.18 Simplified depiction of the hypothesized biosynthesis of cladosporin, by a HR/NR-PKS hybrid.

To better understand PKS assembly and enable analogue production *via* a chemo-biosynthetic approach, we sought to heterologously express the cladosporin production machinery and reconstitute cladosporin production in *Saccharomyces cerevisiae*. To this end Dr. Rachel Cochrane, a former graduate student in the Vederas Lab, sequenced the genome of the producer organism *Cladosporium cladosporioides,* UAMH 5063, resulting in 30Mb of genomic information over a total of 764 contigs. Dr. Cochrane identified 50 putative secondary metabolite gene clusters in the genome of *C. cladosporioides*, seven of which encode type I iterative PKSs.[39] Of the list, only one of these gene clusters possessed a high sequence homology to those of hypothemycin and zearalenone (Figure 2.19).

Dr. Cochrane then cloned and expressed the HR and NR-PKS contained within the gene cluster, **Cla2** (257 kDa) and **Cla3** (226 kDa) respectively in *S. cerevisiae* BJ5464-NpgA.[39] The proteins were successfully expressed from single transformants, and with minimal optimization, cladosporin was isolated from double transformants at a yield of 10 mg/L after RP-HPLC purification.[39] The identity of cladosporin was later confirmed by LC-ESI-MS, using combined retention time matching with accurate mass matching, and NMR analysis. This confirmed the identification of the cladosporin gene cluster in *C. cladosporioides*. Noting that *S. cerevisiae* is a well-studied organism for heterologous production of other important natural products such as artemisinic acid[100] and lovastatin,[51] this result constituted a significant step toward large-scale production of cladosporin.

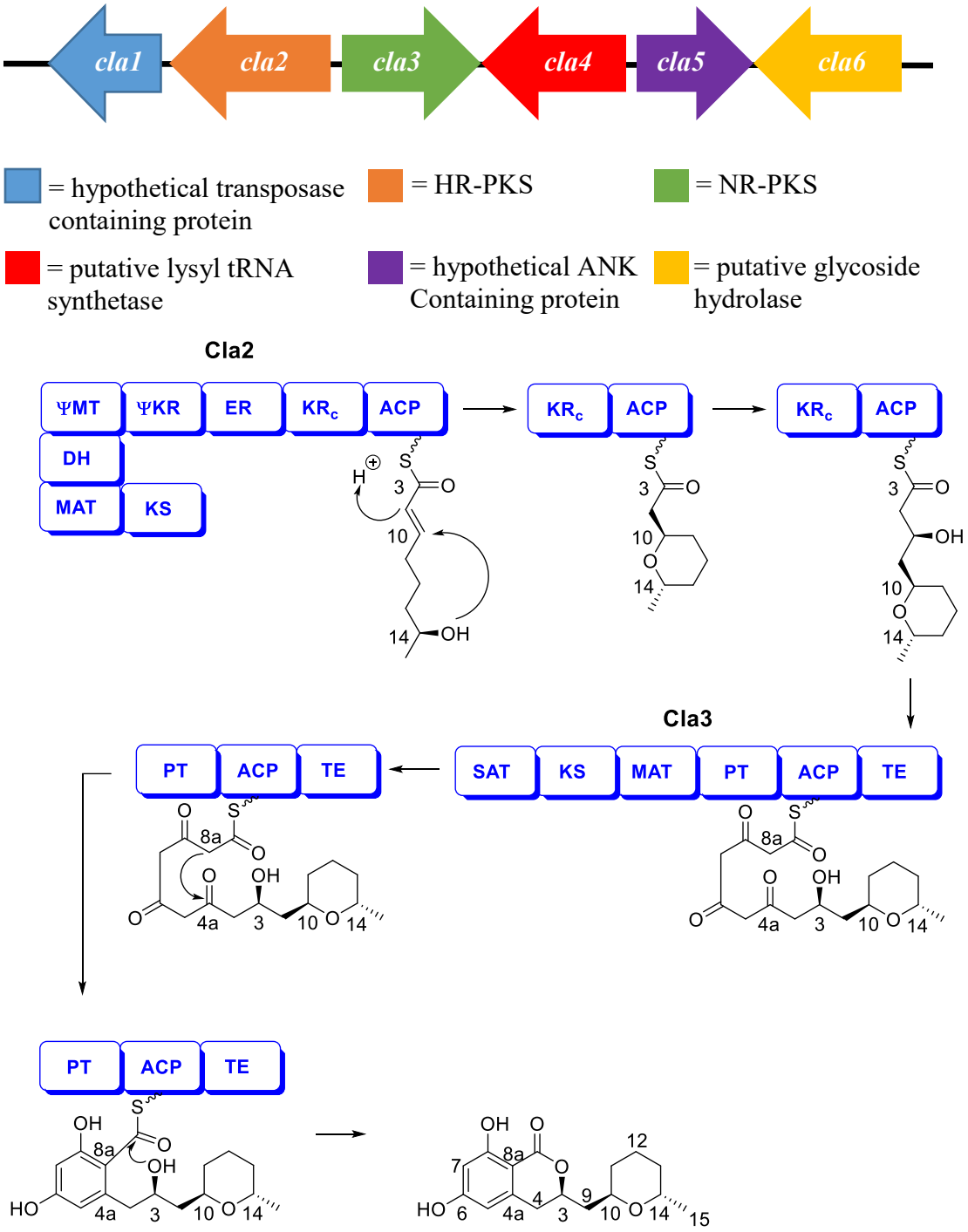


Figure 2.19 Cladosporin gene cluster in Cladosporium cladosporioides and the putative biosynthesis by the Cla2 and Cla3. KS: ketosynthase; MAT: malonyl-CoA: ACP acyltransferase; DH: dehydratase; ΨMT: pseudo-C-methyltransferase; ΨKR: structural ketoreductase; ER: enoylreductase; KRc: catalytic ketoreductase; ACP: acyl carrier protein; SAT: starter unit: ACP transacylase; PT: product template; TE: thioesterase

With this initial success, we then proceeded to probe the biosynthesis of cladosporin *in vitro* and hence my involvement in this project. The desired substrates and expected products were synthesized for feeding assays. As was hypothesized earlier, the HR-PKS, **Cla2** is responsible for the biosynthesis up to the pentaketide stage, inclusive of the tetrahydropyran (THP) ring formation, given the necessary reduction of the C3 position of cladosporin **(9)**. The three subsequent ketide extensions with no reductions are catalyzed by **Cla3**. Similar to observations from other NR-PKSs that require an HR-PKS partner, **Cla3** was unable to produce the product on its own from just malonyl-CoA. Importantly, all of our proposed advanced precursors were prepared in the SNAC form, given that SNAC mimics the interactive arm of Ppant attached to the ACP domain.[101] It serves both as a recognition element and as a labile carrier of the growing ketide chain.

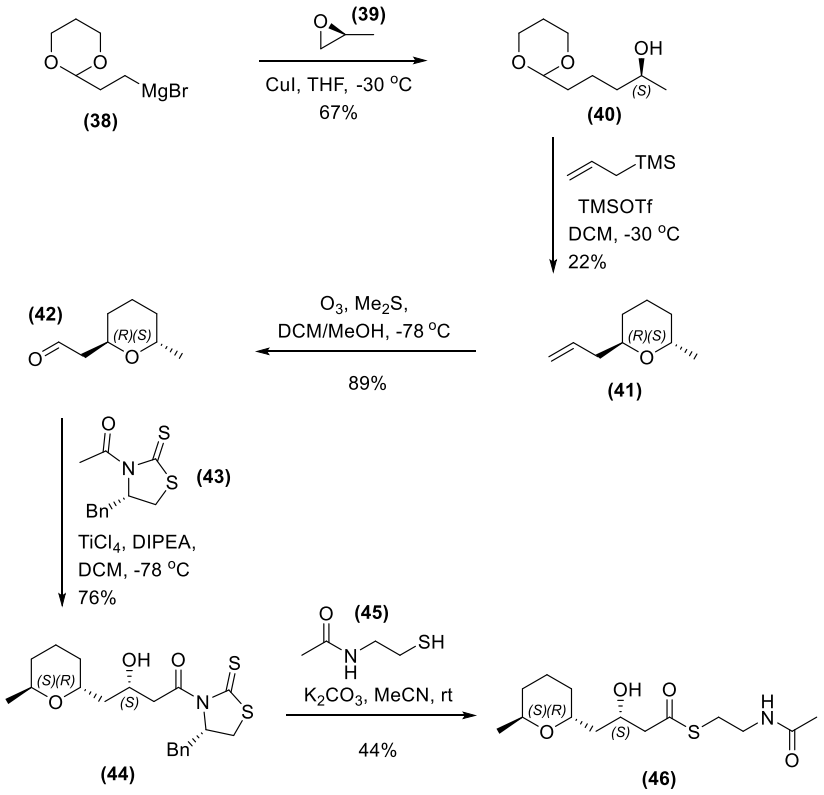
2.2.1 Results and Discussion

2.2.1.1 Synthesis of Advanced Precursors

To probe the level of substrate promiscuity, and the recognition elements of the **Cla3** PKS, several advanced precursor analogues were proposed. The analogues varied by the presence or absence of the THP ring, the stereochemistry of the β-hydroxyl group, and the chain length.

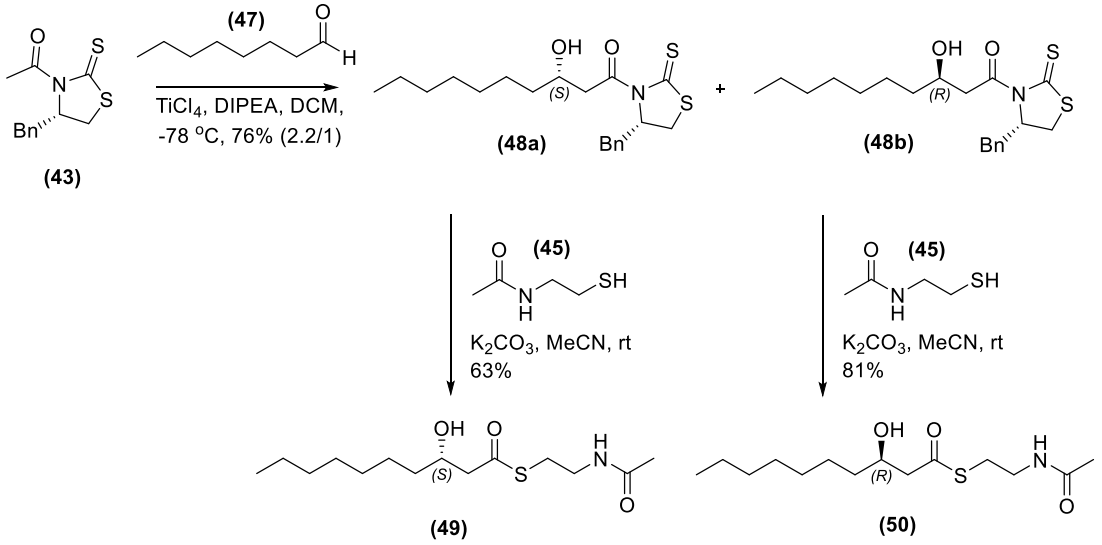
The first synthetic target was the proposed natural pentaketide as its SNAC thioester (Scheme 2.2). This was synthesized by a reaction with the commercially available Grignard reagent **38** and (*S*)-propylene oxide **(39)**, catalysed by copper(I) iodide at –30 ^{o}C to hydroxy dioxane **40** in good yields. Hydroxy dioxane **40** was then treated with

allyltrimethylsilane in the presence of catalytic amounts of TMSOTf at –30 ºC to give the 2,6-*trans* THP **41** selectively. The 2,6-*trans* THP **41** was then reacted under standard ozonolysis conditions to provide aldehyde **42** in excellent yields. The previously prepared acylated (*S*)-4-benzylthiazolidine-2-thione **(43)**, at –78 ºC was treated with $TiCl_4$ transforming the colour from yellow to a brilliant orange, indicating the complexation to the titanium. To this mixture, DIPEA was added, and the colour of the mixture changed to a dark yellow-almost black in appearance indicating the formation of the enolate. This was reacted with aldehyde **42** to provide compound **44** in good yield. The desired SNAC-pentaketide **46** was prepared by displacement of the Crimmins auxiliary by HSNAC **(45)**, in good yield.



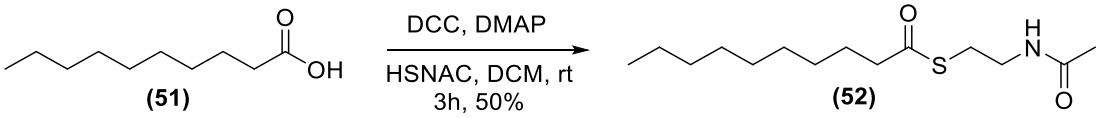
*Scheme 2.2 Synthesis of the natural SNAC-pentaketide analogue **(46).***

Compounds **48a** and **48b** were prepared from the reaction of acylated (*S*)-4-benzylthiazolidine-2-thione **(43)** with octanal **(47)** under standard conditions in a titanium catalysed aldol condensation (Scheme 2.3). These diastereomers were separated on silica column chromatography to give each product as a single spot by TLC. Compounds **49** and **50** were both accessed by reaction of **48a** and **48b** respectively with HSNAC **(45)**. While these compounds retained the original ten-carbon chain length, they lacked the THP ring, allowing for comparison of the effect of the stereochemistry of the β-hydroxyl group and the absence of the THP ring on recognition and incorporation to the final product by the NR-PKS, ***Cla3***. Compound **49** has (*S*) stereochemistry (natural stereochemistry) whereas compound **50** possesses the (*R*) configuration.



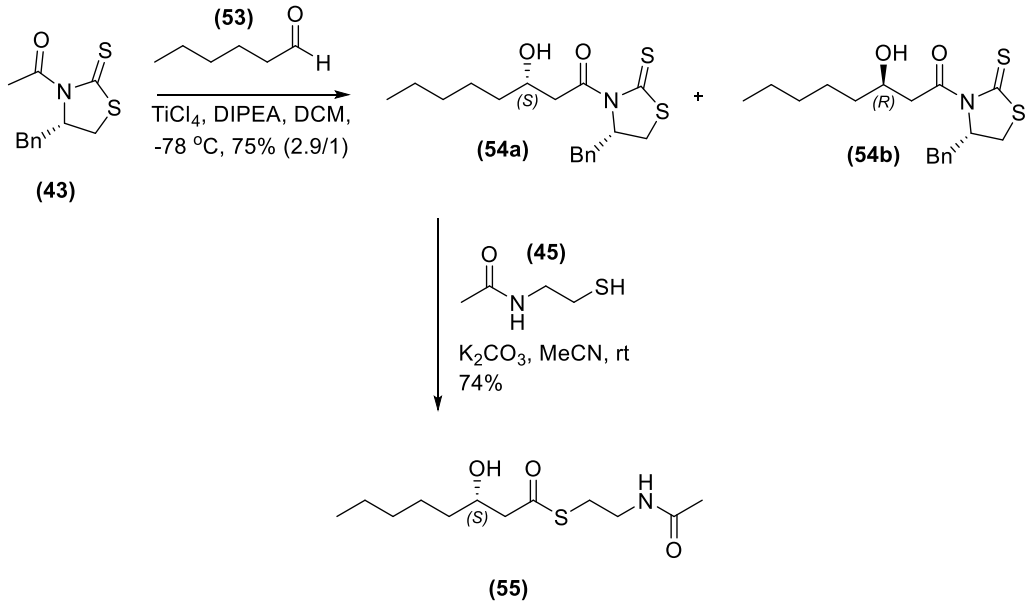
*Scheme 2.3 Synthesis of SNAC-pentaketide analogues **(49)** and **(50).***

Compound **52** was prepared by a modified Steglich coupling reaction,[102] using a thiol (HSNAC **(45)**) in the place of an alcohol, to provide thioester product **52** in good yield (Scheme 2.4). This analogue would probe the importance of the hydroxyl group for recognition and incorporation by the NR-PKS, ***Cla3***. This product retained the ten-carbon chain length but lacked the THP ring and the β-hydroxyl group.



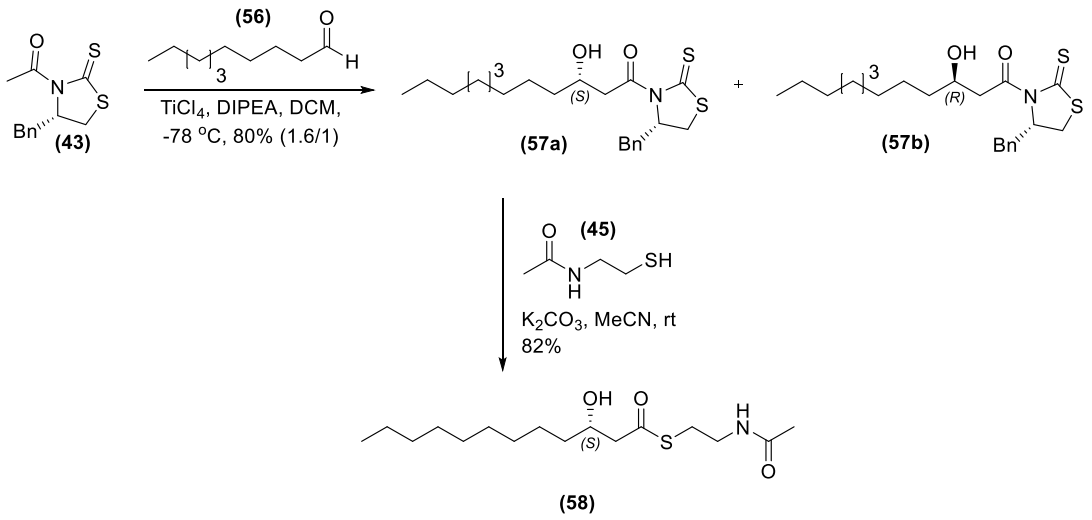
*Scheme 2.4 Synthesis of SNAC-pentaketide analogue **(52).***

Compound **54a** and **54b** were prepared in a similar fashion to **48a** and **48b**. In this case, only the major diastereomer compound **54a** was desired, incorporating the (*S*) stereochemistry (natural stereochemistry) at the β-hydroxyl group. Nonetheless both compounds **54a** and **54b** were isolated and fully characterized (Scheme 2.5). Compound **55** was prepared by reaction of HSNAC **(45)** with compound **54a** to provide the product in good yield. This analogue would probe the impact of the chain length on recognition and incorporation by the NR-PKS.



*Scheme 2.5 Synthesis of SNAC-pentaketide analogue **(55).***

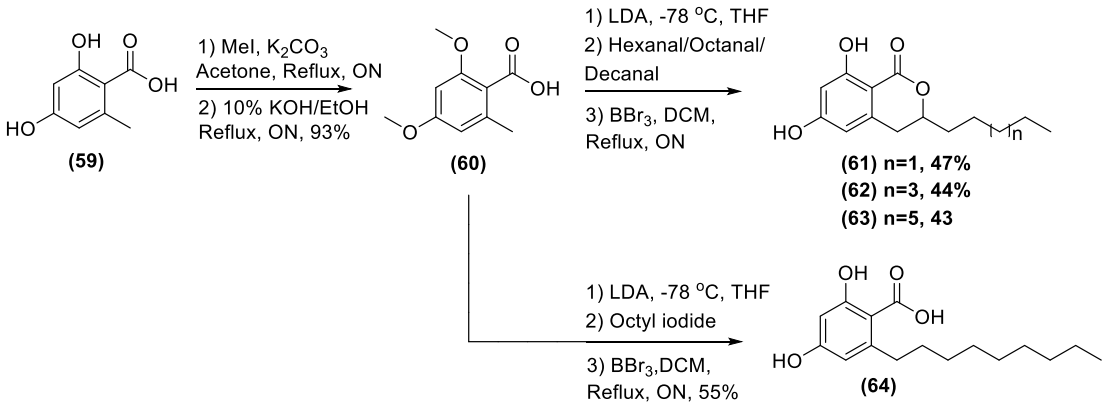
Like compound **55**, compound **58** was designed with the intention of probing the effect of chain length (bearing two extra carbons compared to the natural analogue) on recognition and incorporation by the NR-PKS. As such, in the reaction of compound **45** and **56**, only the major diastereomer compound **57a** was desired of the two diastereomers (nonetheless, both products were isolated and characterized), as it possesses the natural stereochemistry at the β-hydroxyl group (Scheme 2.6). Compound **58** was prepared by reaction of HSNAC **(45)** with **57a,** providing the desired product in good yield.



Scheme 2.6 Synthesis of SNAC-pentaketide analogue ***(58).***

Based on the analogues synthesised to probe the biosynthesis of cladosporin, the proposed product standards were also synthesized. These standards were used to confirm recognition and incorporation of analogues by accurate mass and retention time-matching using the LC-ESI-MS. The product standards were synthesized from a common intermediate, compound **60** (Scheme 2.7). Compound **60** was prepared by global protection of orsellinic acid **59**, followed by saponification of the ester to produce the intermediate acid product **60**, in good yields over two steps. Noting that the LC-ESI-MS assays were not done on a chiral column, there was no need to prepare the standards as the single enantiomers. Compound **60** was treated with LDA, prepared *in situ* to form the vinylogous enolate, to which hexanal **53** was added. The intermediate product was then globally deprotected using BBr_3 which also catalysed a lactonization to provide the desired product **61** in good yield. Compounds **62** and **63** were also prepared following the same protocol, with the only difference being the chain length

of the aldehyde used. Compound **64** was prepared by a small modification to the above protocol, using octyl iodide instead of an aldehyde, as the lactone product was not desired; all other steps were the same.



Scheme 2.7 Synthesis of proposed product standards ***(61, 62, 63, 64).***

2.2.1.2 Advanced Precursor Feeding

With the advanced precursors and analogues now available, we conducted an advanced precursor feeding study (in collaboration with Dr. Rachel Cochrane). This was achieved by incubation of the SNAC-analogues with purified **Cla3** and malonyl-CoA at room temperature for 24 hours (Figure 2.20). After the incubation period, the metabolites were extracted and analyzed by LC-ESI-MS (Figures 2.21, 2.22, 2.23).

Figure 2.20 Model for the advanced precursor feeding with SNAC-pentaketide intermediates and Cla3.

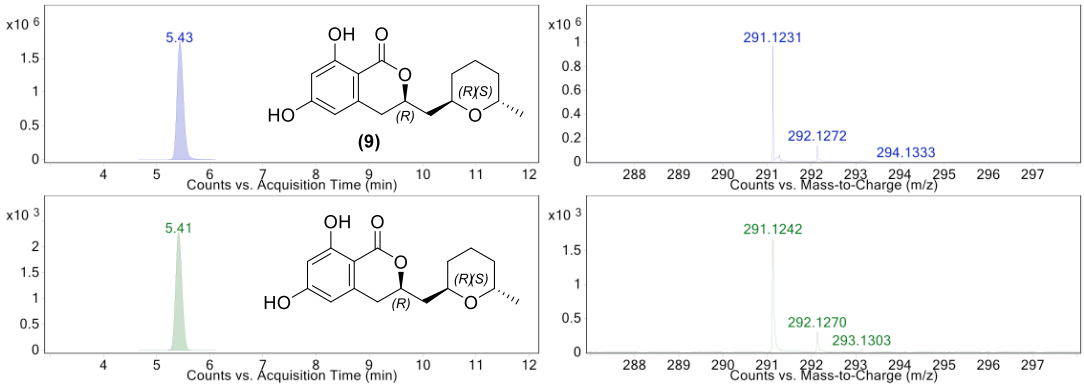


*Figure 2.21 EIC and mass spectrum of the synthetic cladosporin standard **(9)** (blue) compared to extract from in vitro assay conducted with Cla3 and pentaketide **(46)** (green).*

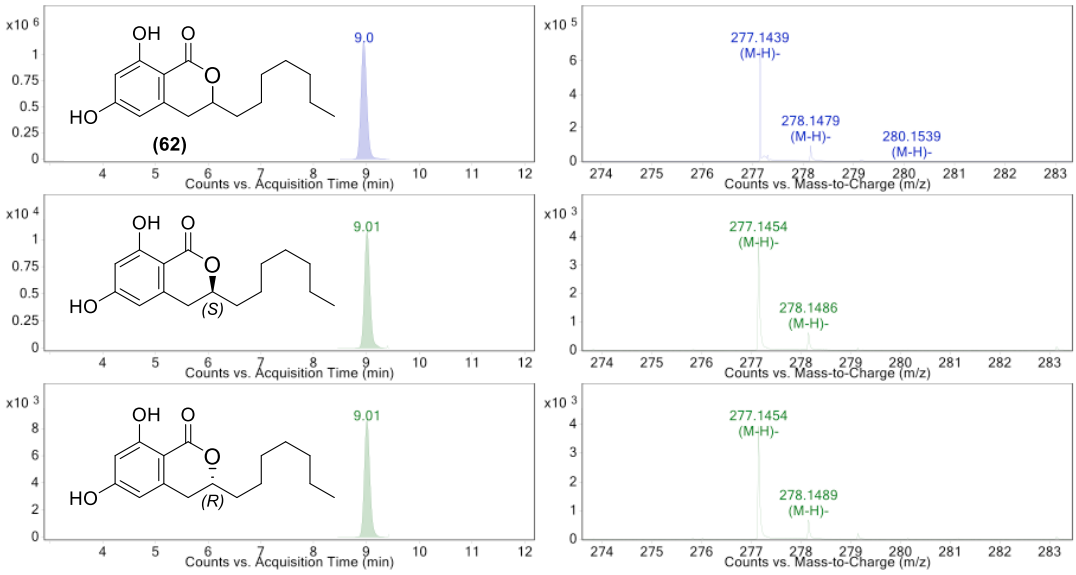


*Figure 2.22 EIC and mass spectrum of synthetic standard **(62)** (blue) in comparison to extract from in vitro assay conducted with Cla3 and analogues **(49)** and **(50)** (green).*

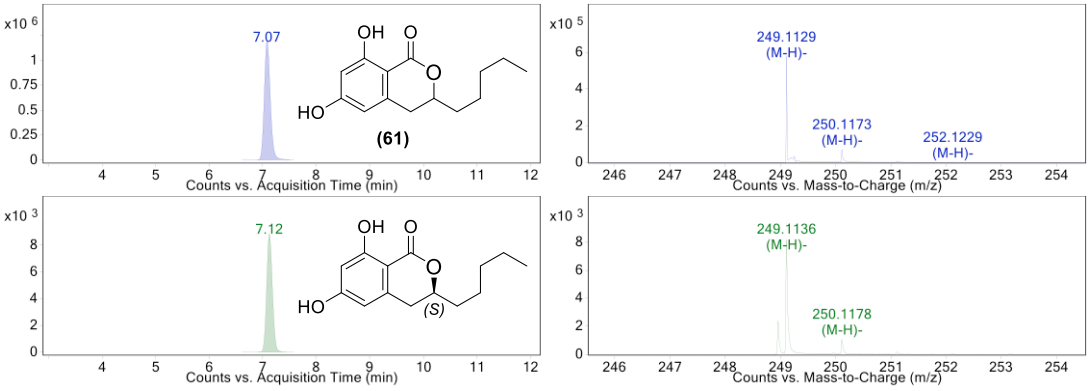


*Figure 2.23 EIC and mass spectrum of synthetic standard **(61)** (blue) in comparison to extract from in vitro assay conducted with Cla3 and analogue **(55)** (green).*

The product obtained from the feeding studies using the SNAC-pentaketide intermediate **46** was found to be cladosporin **(9)** (Figure 2.24). The identity of the product was determined using combined retention time and accurate mass matching. The production of cladosporin **(9)** confirmed that the SNAC-pentaketide intermediate

46 was recognized by **Cla3** and elaborated to produce cladosporin **(9)** (Figure 2.24). This result supported our hypothesis that the pentaketide is likely the final product of the HR-PKS **Cla2** that remains covalently bound as a thioester until transfer to the **SAT** domain of the NR-PKS **Cla3**. Assuming that this is indeed correct, then it also implies that the THP ring formation is catalyzed by **Cla2,** likely through an oxa-Michael-type cyclization on an unsaturated thioester intermediate at the tetraketide stage. The result also illustrates a "5+3" ketide assembly of cladosporin; representing the first example of its type, with other DAL- and RAL-type polyketides assembled in a "4+4" (dehydrocurvularin)[53-55] "6+3" (hypothemycin, zearalenone)[36,37,102] and "5+4" (radicicol)[36] fashion.

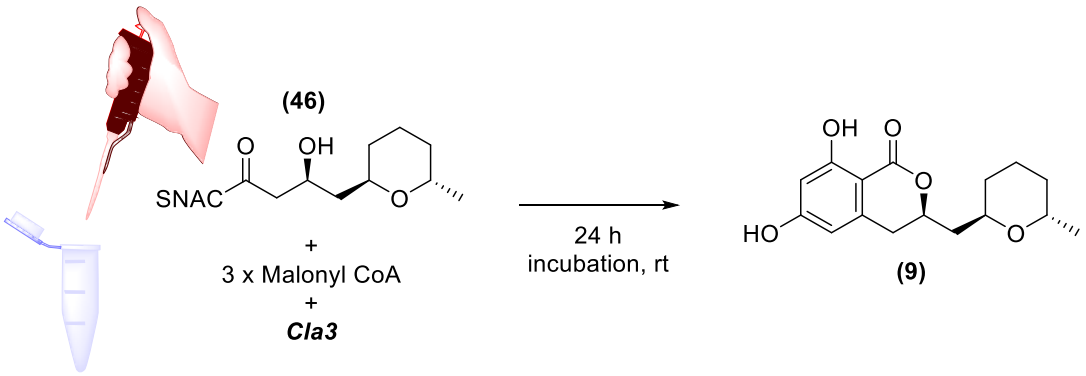
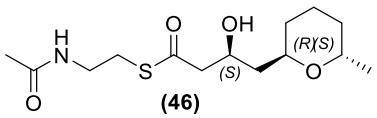
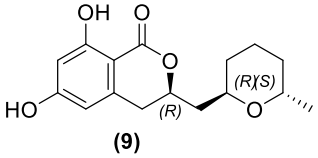
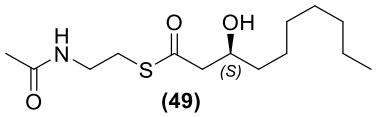
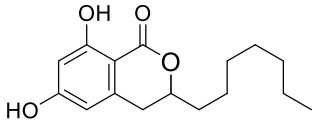
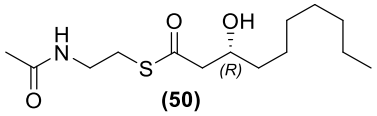
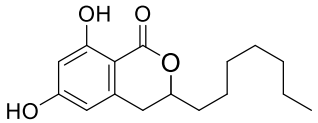
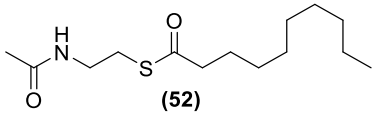
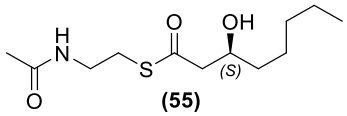
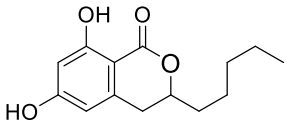
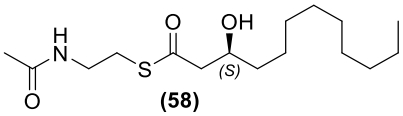


*Figure 2.24 Semisynthesis of cladosporin by **Cla3** and SNAC-pentaketide intermediate.*

Encouraged by the early success, the other analogues were tested in similar fashion. The results of these feeding studies with **Cla3** are shown in Table 2.2. Quite surprisingly, **Cla3** accepts several unnatural substrate analogues that do not contain the THP ring. It recognised and accepted analogues that are ten carbons or shorter, bearing

a β-hydroxyl group regardless of the stereochemistry in that position. To confirm that the ten-carbon analogue compound **52**, lacking the β-hydroxyl group was indeed not recognised and incorporated to a product that was still covalently bond to the **Cla3,** after extraction of the metabolites from the assay, the aqueous layer was then treated with NaOH to hydrolyse the possible thioester linkage to the substrate/product, then neutralized and extracted again. Even in this extract neither the substrate nor the expected product was observed. Importantly, even though the presence of a β-hydroxyl group seems vital for recognition, its stereochemical configuration apparently is not critical. However, the length of the carbon chain seems to play an important role as carbon chains longer than ten carbons bearing the β-hydroxyl group were not accepted. This suggested a possible hydrophobic binding pocket of limited size in the enzyme's active site. While the results of this study showed that there are some limitation to the length of the carbon chain and the requirement of the β-hydroxyl group, **Cla3** remains quite promiscuous and could allow for the semi-synthesis of new antimalarial analogues.

Table 2.2 Results from advanced precursor feeding assay with ***Cla3***

Intermediates	Products
 **(46)**	 **(9)**
 **(49)**	 **(62)**
 **(50)**	 **(62)**
 **(52)**	No product observed
 **(55)**	 **(61)**
 **(58)**	No product observed

2.3 Rational Design of Cladosporin Analogues

2.3.1 Introduction

While cladosporin **(9)** shows significant potency and selectivity towards inhibition of the *P. falciparum* **LysRS**,[9] it is not without flaws as a potential drug. Cladosporin shows poor bioavailability, and this limits its use. This has increased the need to design closely

related compounds to investigate the structure-activity relationship while improving the *in vivo* stability and bioavailability. To this end, we proposed a rational model towards the design and preparation of novel cladosporin analogues (Figure 2.25). The analogues are designed and synthesized based on potential favourable interactions, then tested for activity against the *P. falciparum* lysyl tRNA synthetase. If no activity is observed, then these modifications are reconsidered, and further changes will be made. However, if the activity is observed, then the SNAC-pentaketide form of this cladosporin analogue is synthesised and tested for conversion by the **Cla3** enzyme. We envisioned that this approach allows us to rationally identify beneficial changes in the structure while also preparing a synthetic and chemobiosynthic route for any analogue with promising activity.

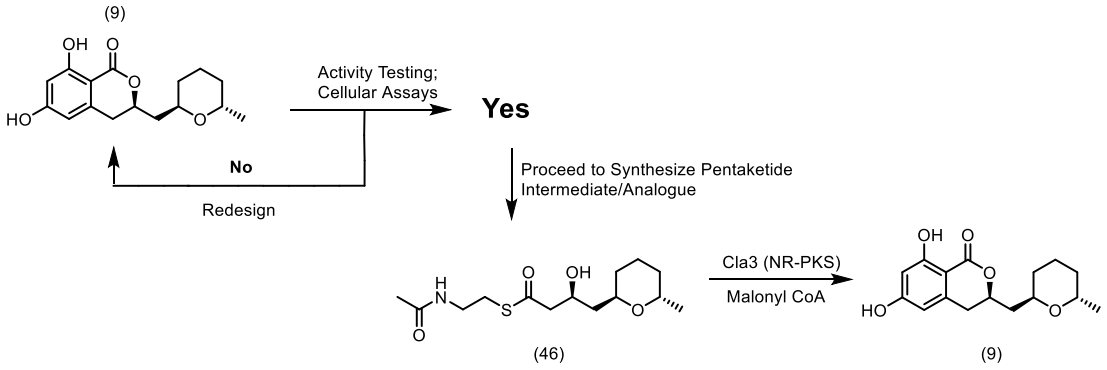


Figure 2.25 Rational model for the preparation and testing of new cladosporin analogues.

Analysis of the crystal structure of cladosporin in the **LysRS** of the *P. falciparum* and *H. sapiens*[99] shows very close similarities, the major structural difference being a Thr_{337} in place of Ser_{344} as seen in *P. falciparum*. In both the **LysRS** of *P. falciparum* and the *H. sapiens,*[99] the isocoumarin moiety of cladosporin occupies a similar orientation as

the adenine of ATP, with its aromatic ring engaging in a hydrophobic interaction with (Glu_{325}, Asn_{332}), and (Glu_{332}, Asn_{339}) respectively.

Table 2.3 Amino acid alignment of the ATP binding site residues between P. falciparum and H. sapiens. Highlighted in red are the key residues that contribute to cladosporin selectivity

P. falciparum	328	VFRNEGIDNTHNPEFTSCE	346	500	EVLN	503
H. sapiens	321	QFRNEGIDLTHNPEFTTCE	339	494	EICN	497
P. falciparum	554	GLGIDR	559	570	I	-
H. sapiens	548	GMGIDR	553	561	I	-

In both the *P. falciparum* and the *H. sapiens* **LysRS,** the isocoumarin moiety is involved in three hydrogen bonds between (i) a water molecule and the carbonyl in the lactone ring, (ii) hydroxyl at C8 and $Asn_{332/339}$, and (iii) hydroxyl at C6 and $Glu_{325/332}$ (Figure 2.26 A, B). Given that these are all beneficial interactions, modification on the isocoumarin moiety could reduce the binding efficiency to the *P. falciparum* **LysRS**. The THP ring of cladosporin is located directly adjacent to Ser_{344} and Thr_{337}, in *P. falciparum* and *H. sapiens* respectively, where any increase in steric bulk of the methyl substituent on the THP ring will clash with Ser_{344} and render that analogue less selective (Figure 2.26 C, D). Furthermore, surrounding the THP ring are several potential hydrogen bond donors and acceptors within 2-5 Å of the hydrogens on the THP ring (Figure 2.27). If these methylenes are substituted with heteroatoms such as nitrogen or oxygen, one can imagine that it would increase the binding of the analogues through hydrogen bonding.

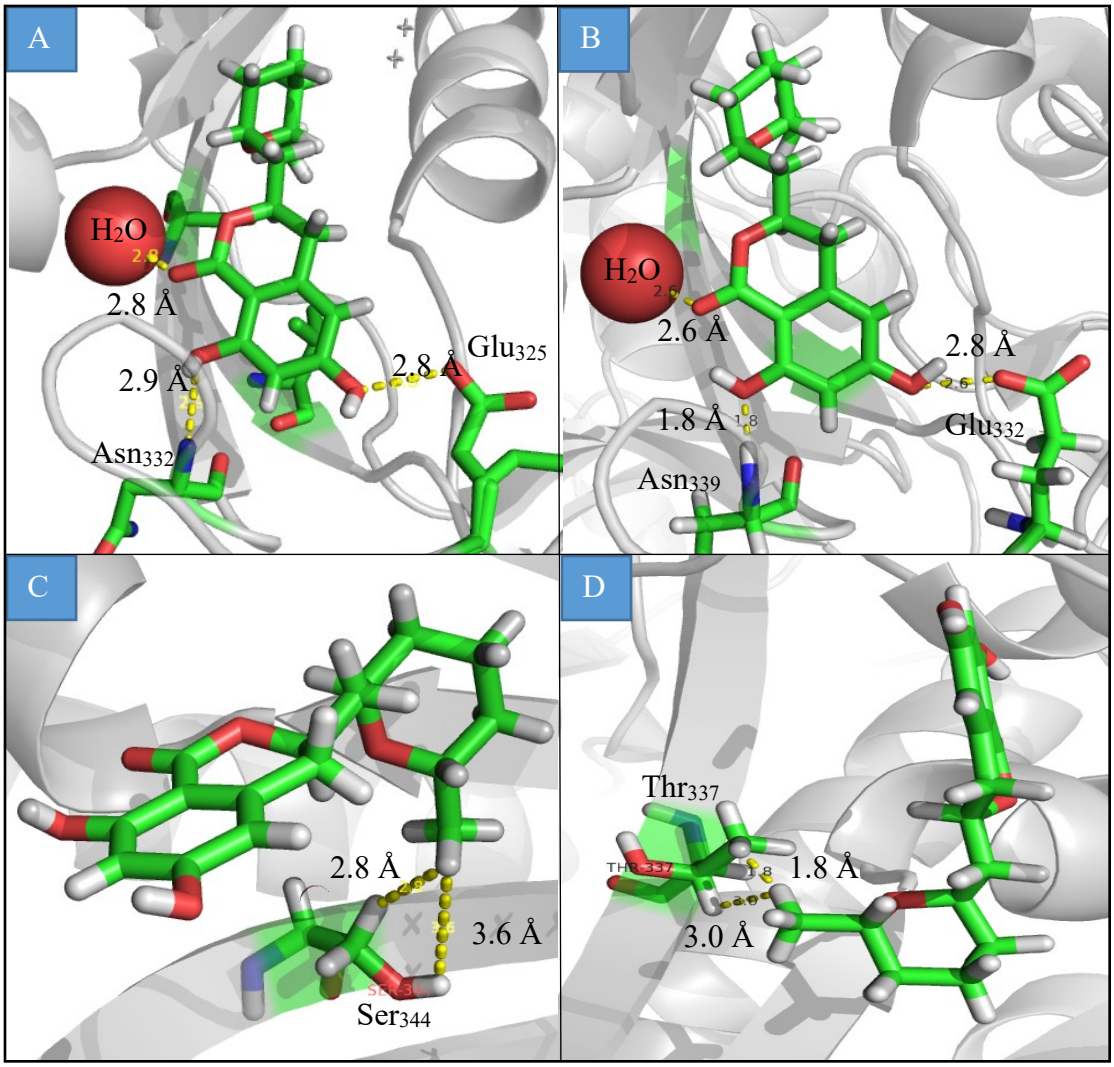


*Figure 2.26 A) Crystal structure of cladosporin in P. falciparum **LysRS**, H-bonds to the isocoumarin moiety highlighted B) Crystal structure of cladosporin in H. sapiens **LysRS**, H-bonds to the isocoumarin moiety highlighted C) Crystal structure of cladosporin in P. falciparum **LysRS**; distance of Ser344 to THP methyl group D) Crystal structure of cladosporin in H. sapiens **LysRS**; distance of Thr337 to THP methyl group.*

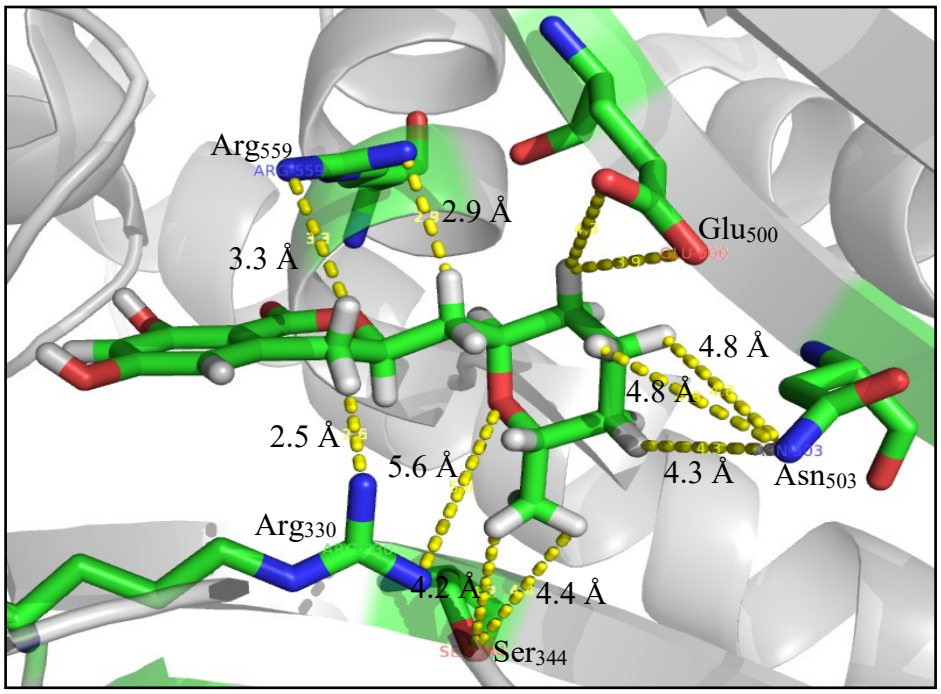


Figure 2.27 Potential hydrogen bond donors and acceptors within 2-5 Å of the hydrogens on the THP ring and neighbours.

Considering the information presented in the crystal structures and the lessons learnt from the advanced precursors feeding study, a series of analogues were proposed. These analogues were designed with the intention of increasing bioavailability through improved solubility or lipophilicity, increasing the binding by targeting specific hydrogen bond donors and acceptors, and all the while maintaining the selectivity for the *P. falciparum* **LysRS** over that of *H. sapiens.*

2.3.2 AutoDock Vina Studies and Rational Analogue Design

The proposed analogues were subjected to docking studies using AutoDock Vina[103] to determine the likelihood of any beneficial interactions. Gratifyingly, analogues

modelled into the binding site took up the same orientation as that of cladosporin in the crystal structure. The proposed modification on cladosporin analogue **ClaA1 65** attempts to marginally increase the steric bulk by replacing the methyl group with CF_3. The rationale being that it further improves selectivity towards the *Pf* **LysRS** by reducing any binding to the human enzyme. Based on the AutoDock measurements, the fluorine atom is 2.8 Å to its closest neighbour (Ser_{344}), which is the same distance as the methyl group of cladosporin to Ser_{344} (Figure 2.28). While it does not improve the solubility in water, it will potentially improve the lipophilicity and may improve the bioavailability of the compound. Also, it could potentially aid in improving hydrophobic interactions with the binding site.

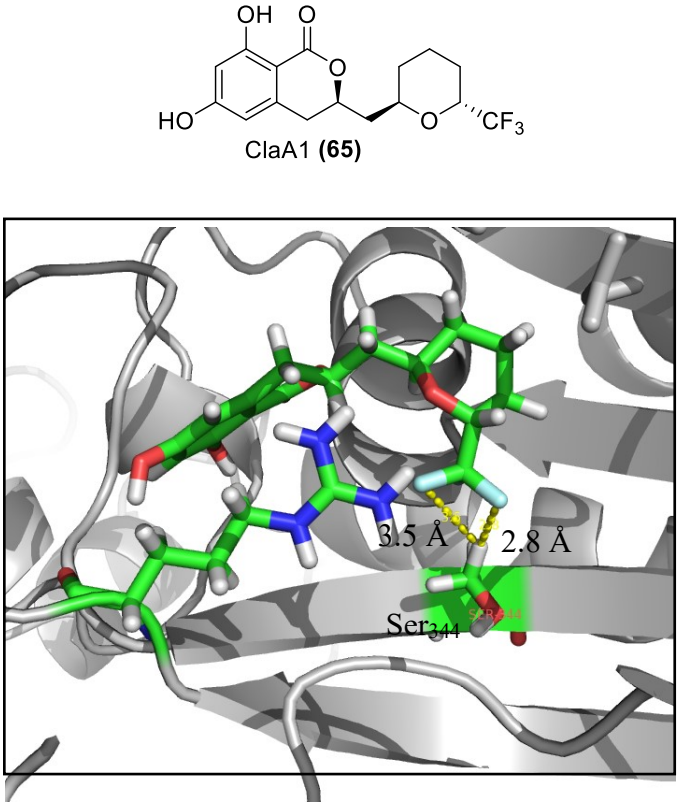


Figure 2.28 Cladosporin analogue ClaA1 ***(65)*** *autodocked into the crystal structure of P. falciparum* ***LysRS****.*

Cladosporin analogue **ClaA2 66** modifications seek to retain as much of the native cladosporin structure while improving the solubility at physiological conditions. Here, only the oxygen in the THP ring has been replaced with a nitrogen atom. Under physiological conditions, this should become protonated and enhance the bioavailability through better solubility. In addition to improved solubility, the amino group shows favourable interactions with Arg_{330}, where the distance between the nitrogen of the analogue and the hydrogens on arginine is ~3 Å, and *vice versa* (Figure 2.29). This analogue combines an attempt in improving both solubility and increased binding interactions.

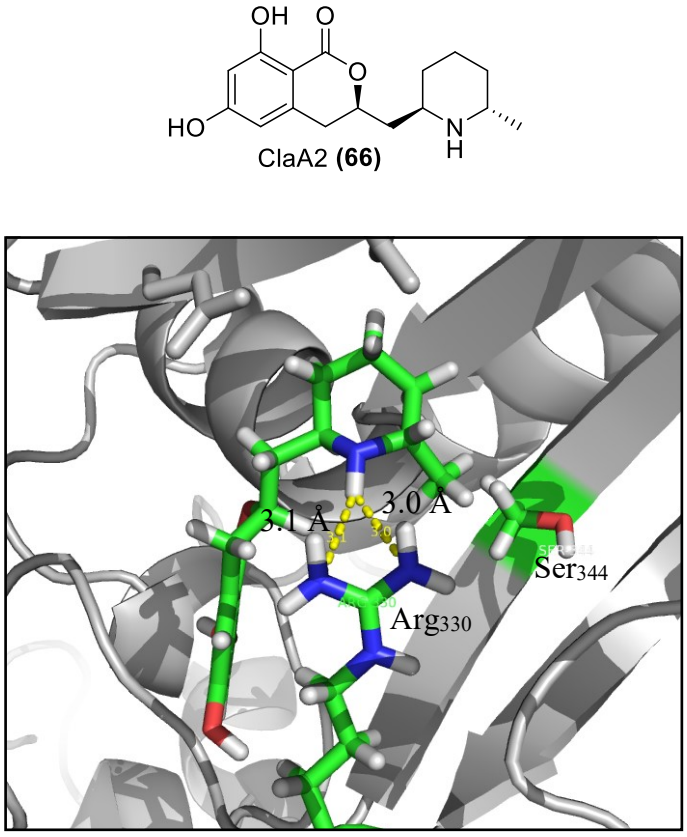
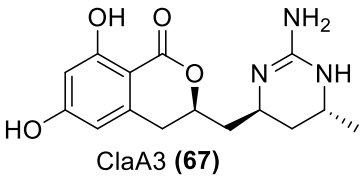


Figure 2.29 Cladosporin analogue ClaA2 ***(66)*** *autodocked into the crystal structure of P. falciparum* ***LysRS****.*

Cladosporin analogue **ClaA3 67** attempts to improve solubility of the compound under physiological conditions while strengthening hydrogen bonding interactions in the active site by addition of the guanidinyl moiety. Based on the docking study, several favourable interactions were observed between; (i) primary amine and Glu500, ii) secondary amine in the ring and Glu500, (iii) secondary amine in the ring and Asn 503, all within 4-5 Å of each other, Figure 2.30.



ClaA3 **(67)**

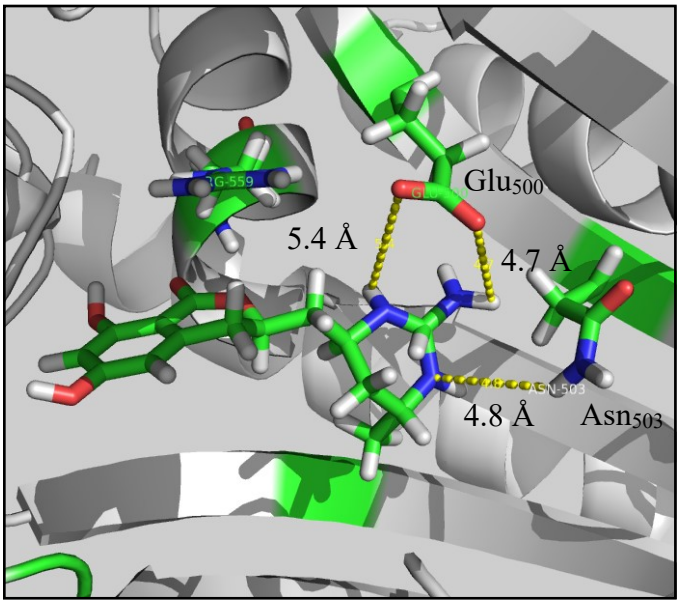


Figure 2.30 Cladosporin analogue ClaA3 ***(67)*** *autodocked into the crystal structure of P. falciparum* ***LysRS****.*

2.4 Conclusion

In this chapter, the identity and activity of the HR-PKS and NR-PKS responsible for the production of cladosporin from *Cladosporium cladosporioides* was verified through *in vivo* and *in vitro assays*. Through advanced precursor feeding studies our hypothesis that the HR-PKS, **Cla2** forms the highly reduced pentaketide which is then completed by the NR-PKS, **Cla3** was confirmed. The NR-PKS, ***Cla3*** was shown to be rather promiscuous based on the variations in the advanced precursors that are recognized and incorporated. This promiscuity has led to the chemobiosynthetic production of several cladosporin analogues. While these analogues were not specifically designed to improve the activity and bioavailability of cladosporin, this work has created a platform upon which new and improved, rationally designed semi-synthetic analogues can be produced.

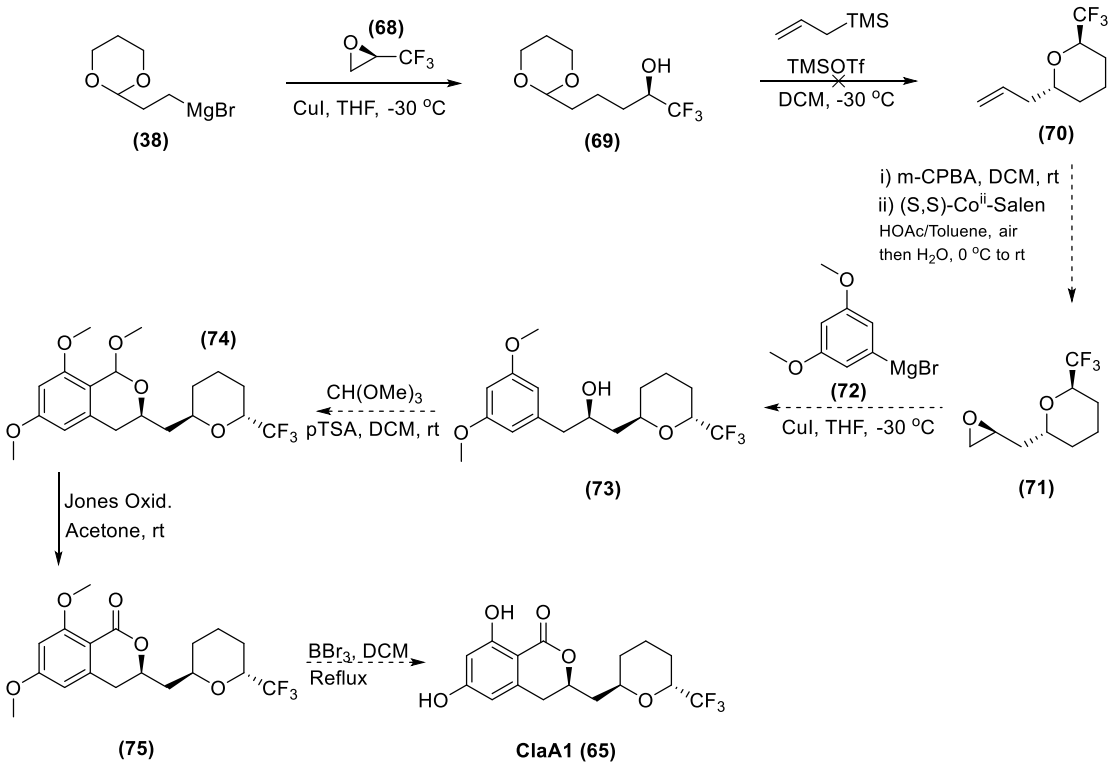
The future directions of this project will evolve around the synthesis and physiological testing of the proposed analogues from the AutoDock Vina study.

2.5 Future Directions

2.5.1 Synthesis of Analogues

Synthesis of **ClaA1 65** and **ClaA2 66** are underway however due to time constraints these were passed on to another graduate student in our research group. Two undergraduate students, Mr. Tyler McDonald and Mr. Chan-Ju Baek also worked on the synthesis of these analogues. Mr. McDonald worked on **ClaA1 65**, and Mr. Baek worked on **ClaA2 66.**

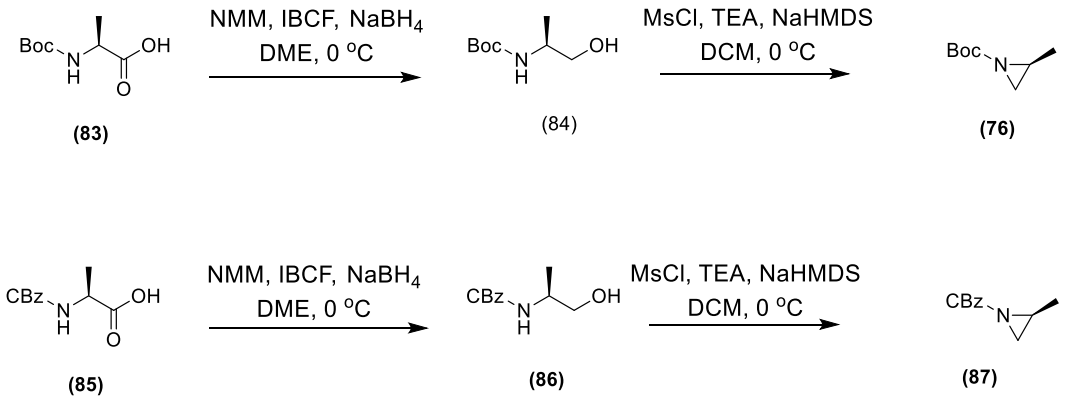
The proposed synthetic scheme for the preparation of **ClaA1 65** was designed to diverge at compound **70** (Scheme 2.8). At this point, the material could be taken onwards to the preparation of the SNAC-pentaketide form of the compound if the activity is observed. This could be achieved much like Scheme 2.2, for the transformation of compound **41** through to the SNAC-pentaketide **46**. The synthesis of the **ClaA1 65** began by reaction of the commercially available Grignard reagent **38** with (*R*)-trifluoromethylpropylene oxide **(68)** to yield hydroxy dioxane **69** in good yields. However, even after several different modifications, the cyclized product **70** could not be obtained. Initial modifications included increasing the reaction time, then temperature, neither of which led to product formation. Working under the assumption that the trifluoromethyl group was pulling electrons away from the hydroxyl group, thus making it less nucleophilic, we then tried the addition of an inorganic bases. Both potassium carbonate and cesium carbonate were tried, and neither yielded the product. We then tried a different Lewis acid ($BF_3.Et_2O$) in the hope that this may have a stronger influence on the cyclization, however this also failed. Not having success with any of the earlier attempts, we then tried to create a negatively charged oxygen by preparing the TMS protected hydroxyl group which would be deprotected *in-situ* by the fluoride anion (F^-) from the Lewis acid, however, this also failed to cyclize. These different experiments showed that perhaps the electron withdrawing effect of the trifluoromethyl group is too strong and thus a different approach to form this intermediate may be needed (Scheme 2.8).



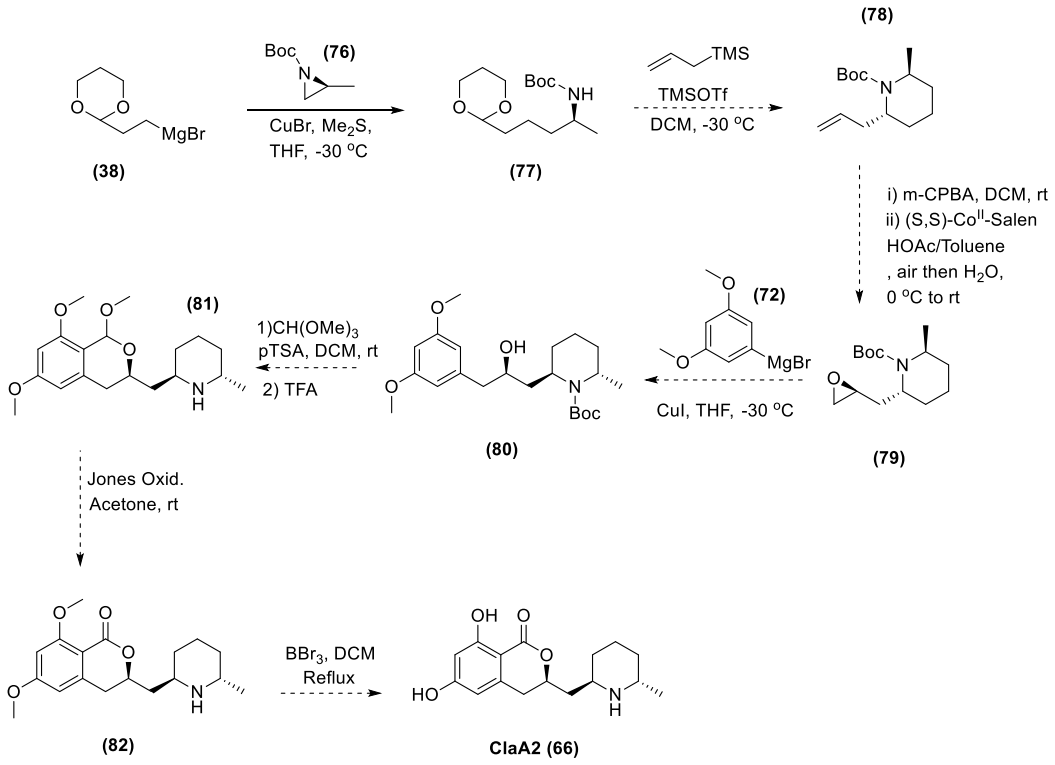
Scheme 2.8 Proposed synthetic route to cladosporin analogue ClaA1 ***(65).***

Like **ClaA1 65**, **ClaA2 66** was designed to diverge at intermediate **78** for the preparation of the SNAC-pentaketide form if this analogue showed promising activity. The synthesis of **ClaA2 66** started with the reaction of the commercially available Grignard reagent **38** with the prepared Boc-protected (*S*)-2-methylaziridine **76** (Scheme 2.10). However due to the extremely high cost for the aziridine ($ 2354.00 USD/g), we sought to optimize a protocol for the preparation in the laboratory (Scheme 2.9). Preparation of the Boc-protected (*S*)-2-methylaziridine **76** was done by the reduction of Boc-protected L-alanine **(83)** forming the amino alcohol **84**, which was then treated with MsCl and base to allow for cyclization to the product **76** in excellent yield. We also found that the Cbz-protected aziridine **87** could also be prepared readily by this

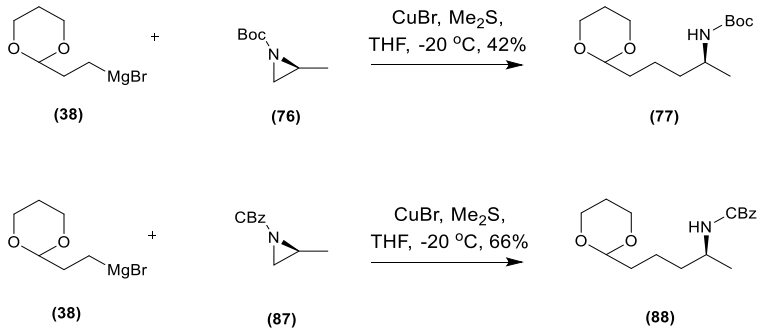
method in good yields. With a method of accessing the aziridine we then studied the reaction between the commercially available Grignard reagent **38** and Boc-protected (*S*)-2-methyl aziridine **(76)** and obtained product **77** in decent yields (Scheme 2.11). When the reaction was done using the CBz- protected aziridine **87** the yield was much improved (Scheme 2.11). Given that the difference in yield for the aziridine preparation is not as great as that of the aziridine ring opening, the use of the CBz-protected aziridine is highly recommended. Unfortunately, cyclization of compound **77** to **78** failed. Minor modifications such as increasing the reaction time, and temperature were attempted however the product was not observed. Further work on these analogues is pending.



Scheme 2.9 Synthesis of Boc- and Cbz-protected aziridines.

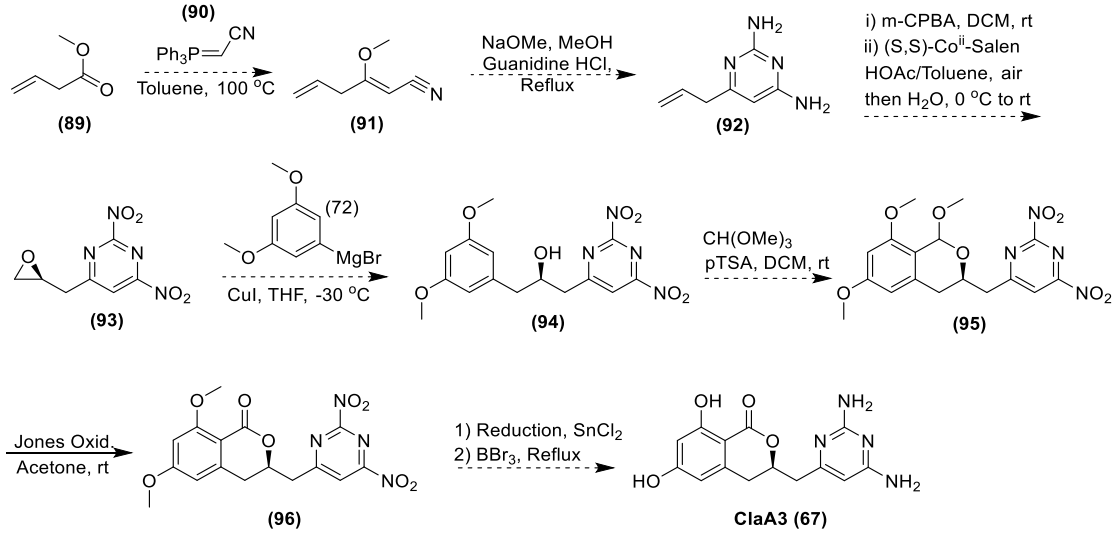


Scheme 2.10 Proposed synthetic route to cladosporin analogue ClaA2 ***(66).***



Scheme 2.11 Aziridine opening by Grignard reagent.

While no work has been done on analogue **ClaA3 67**, the proposed scheme is as follows (Scheme 2.12). Reaction of the commercially available compound **89** with (Triphenylphosphoranylidene)acetonitrile (**90)** will yield nitrile **91**, as shown by Tsunoda *et al.*.[104] This is then cyclized with guanidine to form intermediate **92**, at this point material should be saved for preparation of the SNAC-pentaketide form if the analogue shows activity. A Jacobson epoxidation on compound **92** will yield epoxide **93**, which is reacted with commercially available Grignard **72** to give compound **94**. Compound **94** is then treated with trimethyl orthoformate and *p*-toluenesulfonic anhydride to give the tricyclic compound **95**. A Jones oxidation of compound **95** will yield compound **96**, which upon reduction of the nitro group and deprotection provides the **ClaA3 67** analogue.



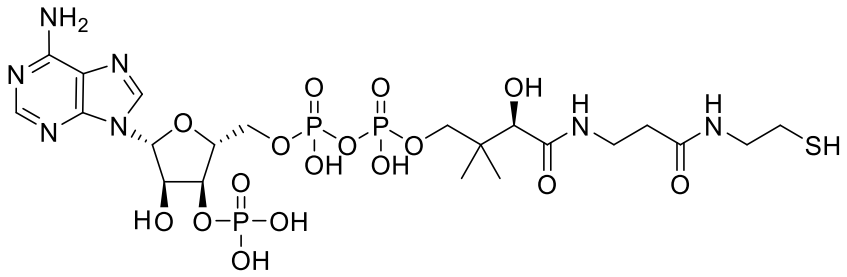
Scheme 2.12 Proposed synthetic route to cladosporin analogue ClaA3 ***(67).***

Chapter 3: Synthetic Preparation of Amino Coenzyme A

3.1 Introduction

Coenzyme A (CoA) **(97)** (Figure 3.31) is perhaps one of the most important cofactors in nature, as it is utilized in the activation and transfer of acyl groups in enzymatic reactions.[105,106] Some of these enzymatic reactions include fatty acid synthesis and degradation, polyketide assembly, nonribosomal peptide biosynthesis, and the oxidation of pyruvate in the citric acid cycle.[105,107-111] CoA is also involved in biochemical reactions as a major regulator of cellular energy metabolism in several different ways, such as substrate/product concentration, allosteric regulation of enzymes, and gene expression regulation *via* acetylation/deacetylation of histones and transcription factors.[112]

Although CoA **(97)** is highly functionalized, many of its functional groups serve primarily as recognition elements for binding to specific enzymes.[105] One key reactive feature of CoA (97) is its nucleophilic thiol group that is readily acylated under physiological conditions. This subsequently allows for the facile transfer of the acyl group to other nucleophiles. Another important property is its ability to donate its phosphopantetheinyl group to ACPs/PCPs; covalently binding the growing chains to the fatty acid synthases (FASs), polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs).[113,114] Replacement of this reactive thioester moiety of acyl CoA with a more chemically (physiological conditions) stable functionality to prevent acyl transfer has received significant attention. Notably, the amide bond has emerged as a good isostere that is physiologically stable to cleavage.[113,114]



Coezyme A **(97)**

*Figure 3.31 Structure of Coenzyme A **(97).***

Several different strategies have been developed to synthesize CoA **(97)** and its analogues, with considerable work following the original synthesis of CoA by Moffat and Khorana in 1959.[115] These methods involved connecting the adenosine bridge between the pyrophosphate and previously synthesised pantetheine portions, followed by regioselective phosphorylation of the 3' hydroxyl group on the ribose. Michelson later modified and improved this approach by the addition of the adenosine diphosphate followed by subsequent enzymatic hydrolysis of the 2',3'-cyclic phosphate to form CoA **(97)**.[116] Further improvements were made by the research group of Wright,[117] Burkart,[118,119] and Bruner,[113,120] who all employed chemoenzymatic methods for preparation of CoA **(97)** and CoA analogues (Figure 3.25). Common to these efforts is the use of synthetically modified pantetheine moieties which are further elaborated by three enzymes, to produce CoA derivatives. These enzymes are the pantothenate kinase (PanK); phosphopantetheine adenylyltransferase (PPAT); and dephosphocoenzyme A kinase (DPCK), (Figure 3.32).[113,117,118] In particular, the Bruner group used these

enzymes to synthesize "amino CoA" (CoA-NH_2) **(98)** in which the sulfhydryl group is replaced with a primary amine, which could then be acylated with an amino acid.[113,120]

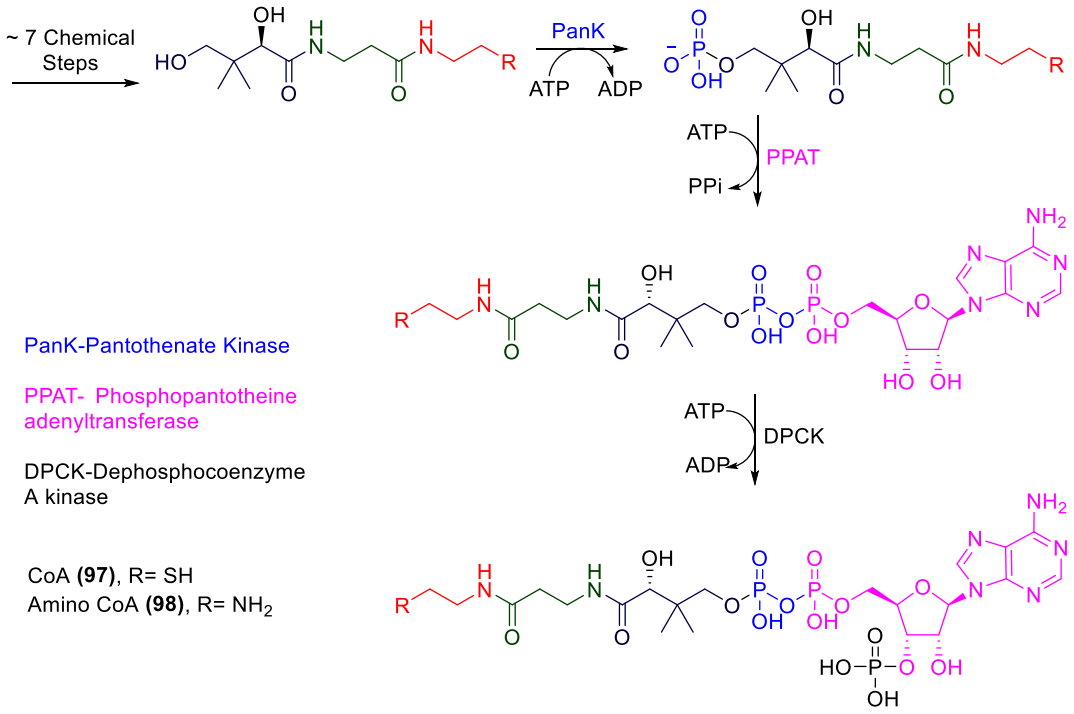


Figure 3.32 Chemoenzymatic synthesis of CoA and analogues.

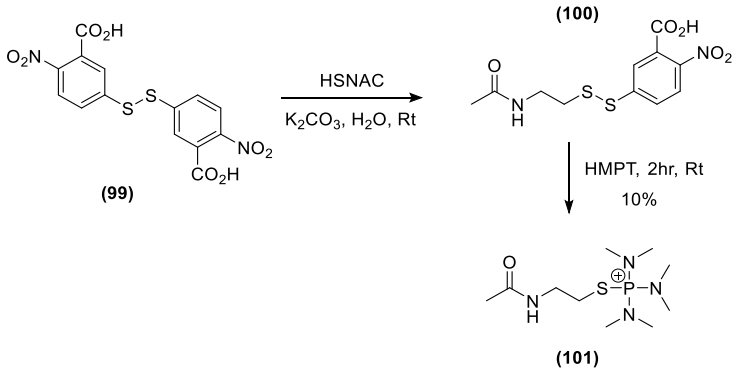
Mishra *et al*. in their review noted that, except desulfurized CoA, unnatural analogues cannot be readily prepared from CoA itself due to its sensitivity to acid, bases and high temperatures.[121] Because of the labile nature of the phosphate bonds, the diverse functionalities within the molecule, and the poor solubility of CoA in organic solvents, modifications by conventional chemistry are generally not feasible.

3.2 Results and Discussion

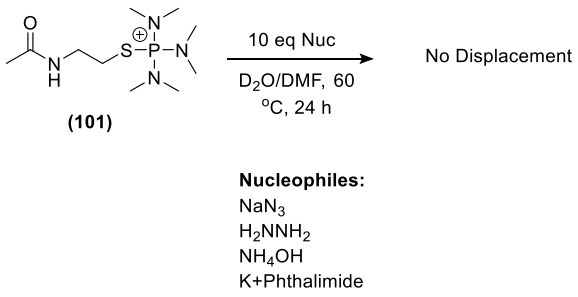
The objective of this project was to convert CoA to "amino CoA" **(98)** by the simplest manner possible, i.e., conversion of the sulfhydryl group of CoA **(97)** to a primary amine. However, we needed a suitable mimic to develop and test our reactions before using the more expensive CoA. N-Acetyl cysteamine (HSNAC) **(45)** was chosen to be this mimic, as HSNAC bears the essential functional groups of interest and is much less expensive. Additionally, given the issues with solubility and stability, any transformation envisioned must be viable in water and mild conditions (~ neutral pH, temperature, time).

Considering the transformation of CoA **(97)** to amino CoA **(98)** was a replacement of the sulfhydryl group with a primary amine, the first attempt was to transform the free thiol into a good leaving group to be displaced by a nitrogen nucleophile. In the literature, Krafft *et al.* showed that they were able to stereospecifically displace sulfur from chiral centres through activation of the sulphur leaving group as a thiophosphonium salt species, all in good yields.[122] Following their success, We synthesized the SNAC-hexamethylphosphorous triamide (HMPT) thiophosphonium adduct **(101)** by reaction of HSNAC with Ellman's reagent **(99)**, to obtain the mixed disulfide **100**. Upon reaction with HMPT, SNAC-HMPT adduct **101**, was obtained unfortunately in poor yields (Scheme 3.13). With intentions of later optimizing this reaction, the available material was then tested with several different nitrogen nucleophiles in H_2O/DMF mixture at room temperature, then at 60 ^oC, and monitored over time (Scheme 3.14). The nucleophiles included: liquid NH_3, NH_4OH, H_2NNH_2, NaN_3, and potassium phthalimide. Unfortunately, the displacement did not work for

any of these nucleophiles, and the intact thiophosphonium salt **101** was recovered. Given that these reactions were conducted in H_2O/DMF mixture, it is likely that protonation of the nucleophiles was affecting the reaction. Therefore, a reaction in dry DMF was tested, keeping the other variables the same, unfortunately, no product was observed, and again the starting material was recovered unchanged.



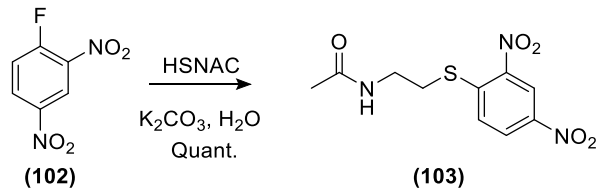
*Scheme 3.13 Synthesis of SNAC-HMPT adduct **(101).***



*Scheme 3.14 Attempts at nucleophilic displacement of the activated sulfur **(101).***

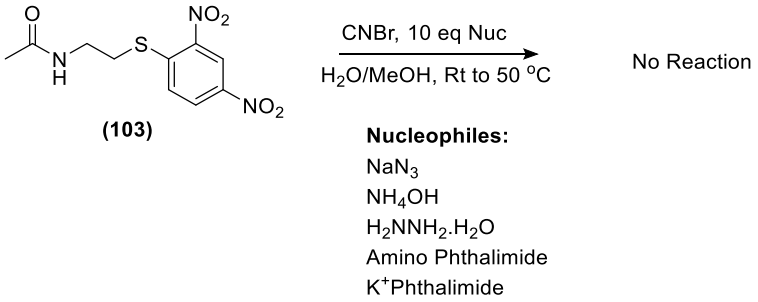
Having failed to activate the sulphur by thiophosphonium formation, activation through the use of cyanogen bromide was then attempted.[123] HSNAC **(45)** was reacted with Sanger's reagent **(102)** in water to quantitatively form compound **103** (Scheme 3.15).

With compound **103** in hand, the reactivity towards the same list of nitrogen nucleophiles as previously used was tested in the presence of cyanogen bromide (Scheme 3.16). Unfortunately, the starting material was generally recovered, possibly due to the deactivation of sulphur by the electron withdrawing effect of the two nitro groups thus reducing the nucleophilicity of the lone pair of electrons on the sulphur. Attempts to make the aromatic ring more electron rich by reduction of the nitro groups to amines[124] resulted in the formation of cyanamides and dicyanamides upon reaction with cyanogen bromide, again, without sulfur displacement from the starting material.



(102) **(103)**

*Scheme 3.15 Synthesis of compound **(103).***



(103)

*Scheme 3.16 Attempts at nucleophilic displacement of the activated sulfur **(103).***

Unable to activate the sulphur into a good leaving group, we considered an entirely different approach. Rather than simply exchanging sulphur for nitrogen, we considered exchanging the entire 2-aminoethane thiol terminal for ethylene diamine. While there

have been several methods published for transamidation,[125-128] none had existed for transamidation under aqueous conditions. Modifying literature protocols[125] by lowering the temperature to 55 ºC (CoA decomposes on heating much above that temperature) and replacing organic solvents with water (CoA is primarily water soluble), we then tested a list of Lewis acids that were known to be used in transamidation reaction (Table 3.4). Gratifyingly, boric acid ($B(OH)_3$) showed significant activity, even after correcting for any background reaction. Boric acid was found to be 10-fold more effective than $FeCl_3$, 20-fold more effective than the $Fe(NO_3)_3$, and 100-fold more than $Fe_2(SO_4)_3$ in the formation of the desired product.

Table 3.4 Lewis acid catalyst screening

Catalyst	**Background Corrected Integration***	**Relative Effectiveness (%)**
No Catalyst (Background Rxn.)	NA	NA
$Fe(NO_3)_3$	21617	5
$Fe(SO_4)_3$	2548	1
$FeCl_3$	42523	10
$ZrCl_3$	-46742	-11
$NH_2OH.HCl$	-30154	-7
$SnCl_2$	-48245	-11
$Eu(NO_3)_3$	-57336	-13
$CeCl_3$	-15563	-3
$B(OH)_3$	444704	100

*Values corrected for background reaction, i.e. in the absence of any catalyst.

Without ignoring this success, it was interesting that even in the absence of any catalyst the desired product was also formed. This could be explained by an N to S acyl transfer, and the reaction of the transient thioester with the incoming nucleophile, thus forming the product; essentially a reverse chemical ligation (Figure 3.33). Given that when

$B(OH)_3$ was used the yield improved, this suggested that the N to S acyl transfer was also being catalyzed by the addition of a Lewis acid and that transamidation was as a result of the incoming nucleophile reacting with the transient thioester. To show that this was indeed the case, a series of NMR experiments were done.

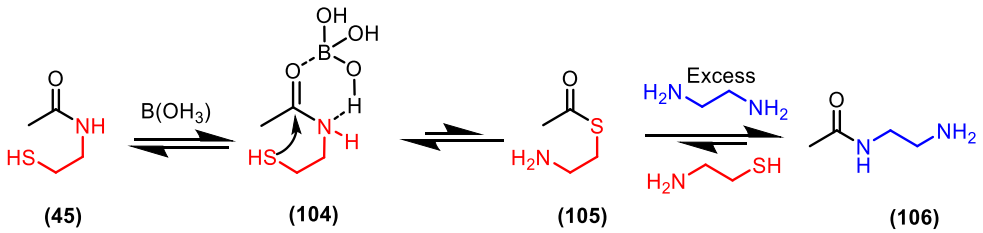
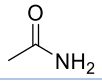
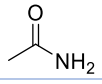
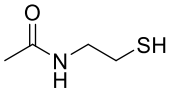
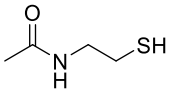
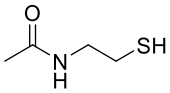
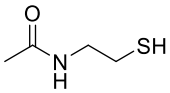


Figure 3.33 Proposed mechanism for boric acid catalyzed N to S acyl migration, followed by nucleophilic displacement of the thiol by the external nitrogen nucleophile.

Attempts to transamidate acetamide with ethylenediamine (10 equiv) in water at 55 °C showed no measurable transformation to the expected product after 48 h, in accord with the literature [125] (Table 3.5, Entry 1). However, upon addition of $B(OH)_3$ (1.0 equiv) using the same conditions, the reaction gave 9% conversion to the desired product, monoacetylated ethylenediamine, thereby supporting the beneficial effect of $B(OH)_3$ as a catalyst (Table 3.5, Entry 2). To study the impact of any potential intramolecular N to S acyl migration, HSNAC was used as the amide in water at 55 °C with ethylenediamine (10 equiv) in the absence of $B(OH)_3$. This reaction produced 23% of monoacetylated ethylenediamine (Table 3.5, Entry 4). To confirm this transformation was reliant on an intramolecular thiol transacylation, the acetamide reaction was repeated with $B(OH)_3$, and cysteamine as an external nucleophilic additive (Table 3.5, Entry 3). However, the transamidation yield was merely 9%, as seen in the earlier

reaction with acetamide and no additive. Combining the best approaches into a single reaction using SNAC as the amide, with 1.0 equiv of $B(OH)_3$ led to an increase in the conversion, 35% (Table 3.5, Entry 5). This confirmed that the N to S acyl migration was indeed catalyzed by the $B(OH)_3$, and suggested the transamidation product **106** was formed through the reaction between the transient thioester intermediate **105**, and the external nitrogen nucleophile. This was because the intramolecular reaction between the pendant thiol and the $B(OH)_3$ activated amide **104** was outcompeting the intermolecular reaction between the external nucleophile and the same activated amide.

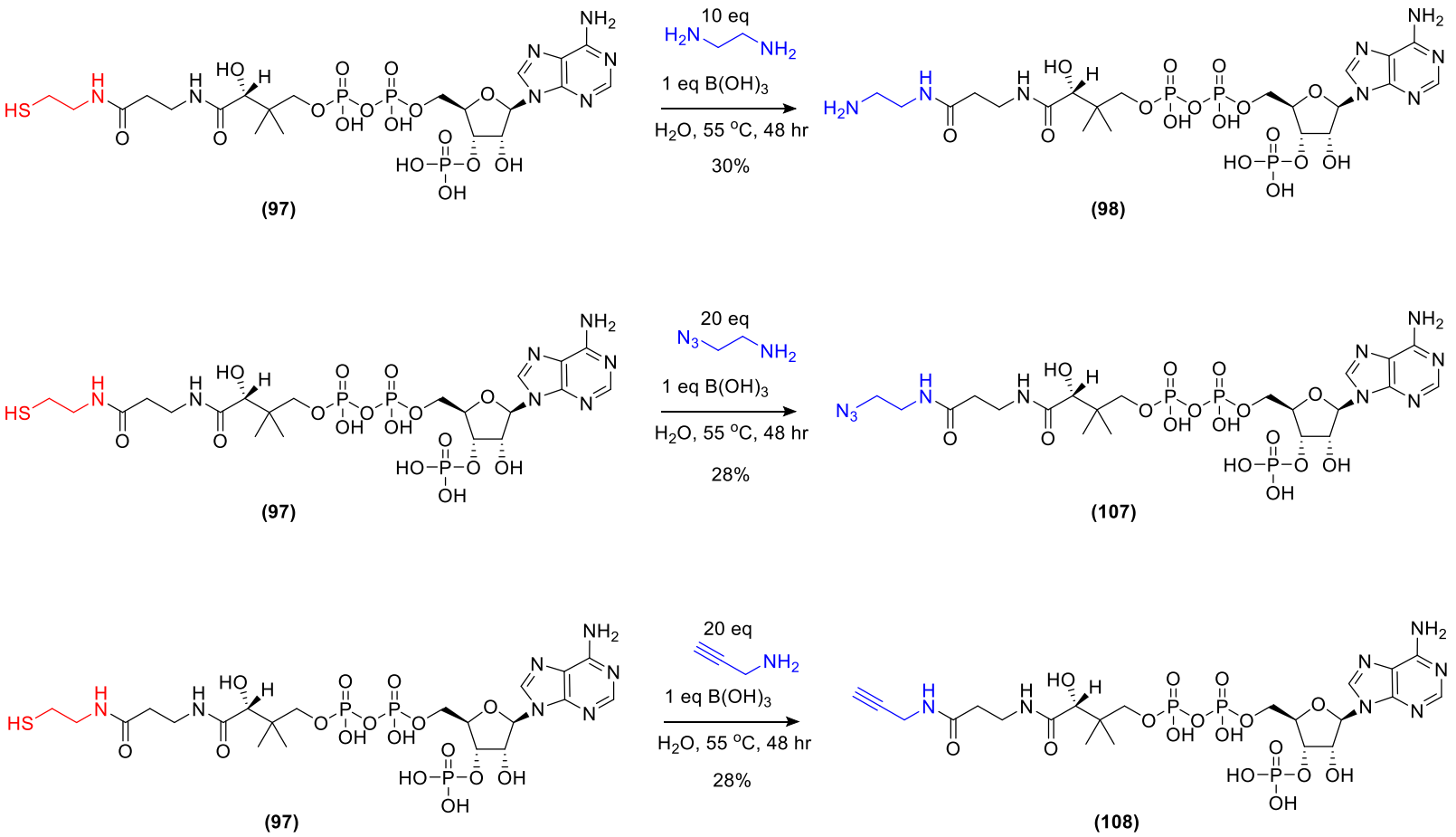
Table 3.5 Screening of reaction conditions for transamidation with ethylenediamine, in water at 55 °C

Entry	Amide	Amine equiv	$B(OH)_3$ equiv	Conversion %
1		10	-	0
2		10	1	9
3*		10	1	9
4		10	-	23
5		10	1	35
6		10	30	23
7		40	1	26

*Cysteamine was used as an additive

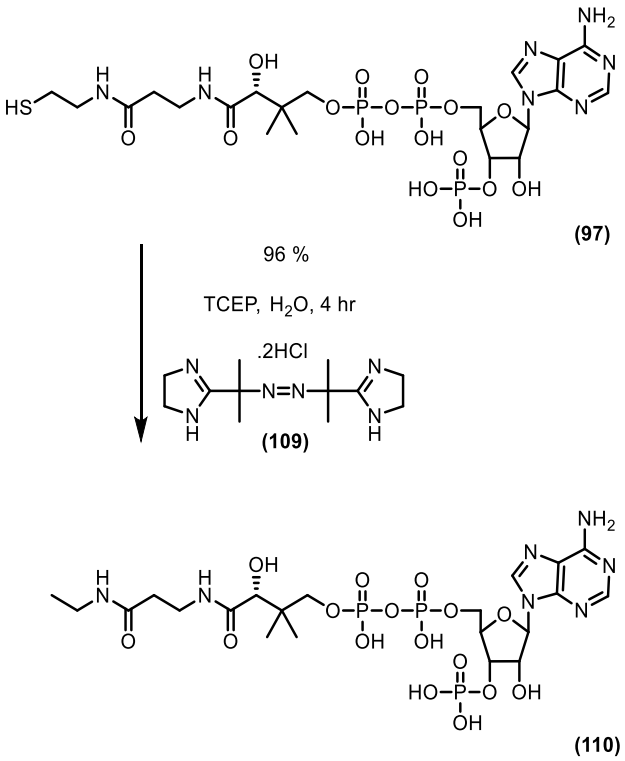
Attempts to improve the transformation by increasing the amount of $B(OH)_3$ or the amount of amine failed and reduced the overall conversion significantly. With the conditions developed in the HSNAC model system, we were able to prepare the "amino

CoA **(98)**"[113] (CoA-NH_2), as well as previously unpublished "azido CoA **(107)**" (CoA-N_3) and "alkynyl CoA **(108)**" in single steps from commercially available CoA **(97)** with reasonable yields, given both the complexity of the molecule and its sensitive nature (Scheme 3.17).



*Scheme 3.17 Synthesis of CoA analogues; amino CoA **(98)**, azido CoA **(107)** and alkynyl CoA **(108)**.*

Even though attempts were made to remove oxygen from the reaction vessels the formation of CoA disulfide could not be entirely prevented. And since the free thiol plays an important role in the transformation, water-soluble disulfide reducing agents were added. These reducing agents included the more commonly used 1,4-dithiothreitol (DTT) and tris(2-carboxyethyl)phosphine (TCEP). Although both are excellent disulfide reducing agents, the TCEP was faster at cleaving the disulfide bond at room temperature. Unfortunately, TCEP gives a side reaction with the free thiols at elevated temperature (55 ºC), namely radical desulfurization of CoA **(97)** to form a small amount of desulfurized CoA **(110)**. Given that the "desulfo" (des-thio) CoA **(110)** is also an important compound used in crystallography and as an inhibitor in several cases,[116,129,130] we decided to examine this desulfurization reaction. It was observed, that upon the addition of the commercially available water-soluble radical initiator, 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride **(109)**, we were able to transform CoA **(97)** using TECP in water at 55 ºC into desulfo CoA **(110)** in 96% yield in 4 h (Scheme 3.18). As shown earlier by Wan et al., this radical-based approach was also used in the post-ligation desulfurization of cysteines, likewise almost quantitatively.[131]



Scheme 3.18 Synthesis of desulfo CoA ***(110).***

While it was proposed that the trialkyl phosphine species are responsible for the desulfurization process,[131] we further studied the reaction to confirm that it was the sole phosphorous species present in the reaction mixture that effects this radical reaction. This was accomplished by replacing the TCEP with TCEP oxide, and TCEP sulfide, respectively, under the same reaction conditions using HSNAC as the thiol. These results showed that neither the TCEP oxide nor TCEP sulfide led to any measurable desulfurization. This confirmed that the TCEP species was required for the formation of the thiophosphine-adduct, which can then undergo a β-scission to produce the primary alkyl radical, and TCEP sulfide as a by-product. The final step being an intermolecular radical hydrogen exchange from nearby thiols, quenching the carbon

radical and producing the desulfurized product while propagating another thiyl radical (Figure 3.34).

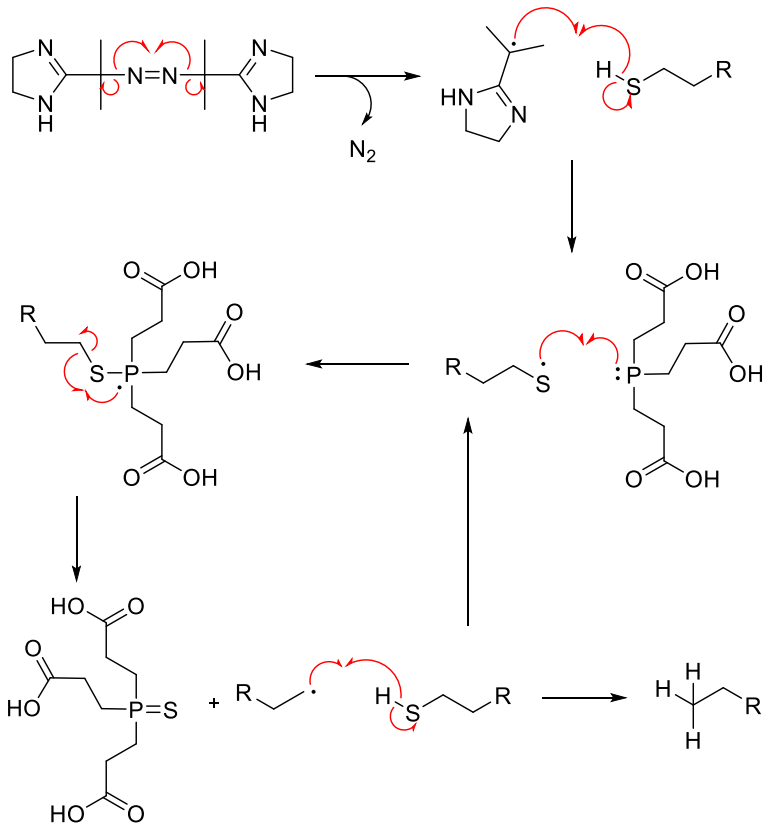
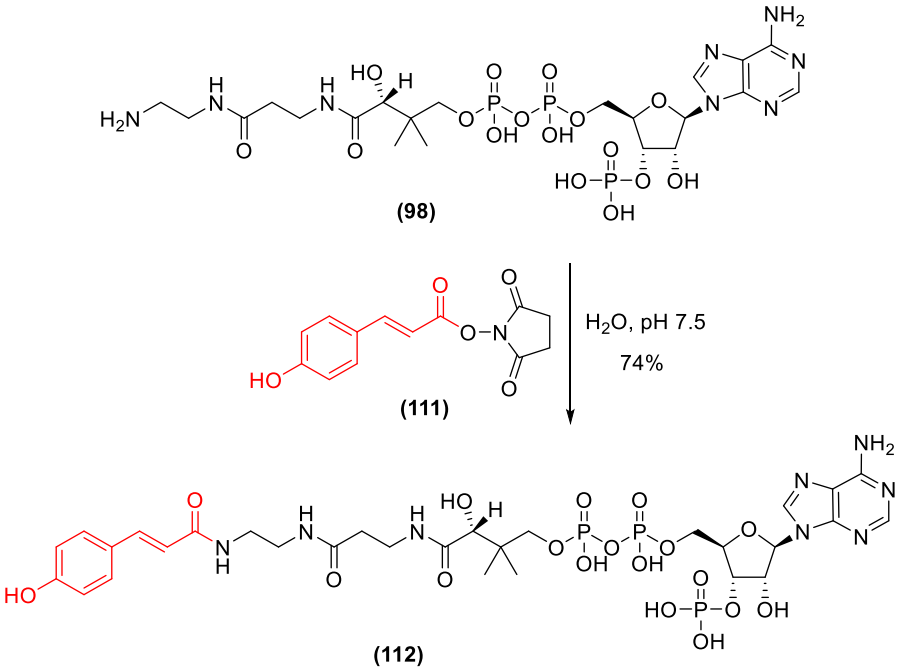


Figure 3.34 Proposed mechanism of the TCEP radical desulfurization reaction.

Thioester replacement with an amide functionality has become quite common in the study of PKS, FAS and NRPS enzymes. Normally, this is accomplished by the synthesis of the corresponding acylated pantetheine derivative followed by enzymatic transformation,[132] as shown above in Figure 3.25. The Bruner group has demonstrated that acylation of amino CoA **(98)** in water with activated amino acids works quite well.[113] Given its potential widespread application, we briefly examined the ease with which amino CoA **(98)** may be elaborated under aqueous conditions and attached to

activated acyl groups. *p*-Coumaroyl CoA amide **(112)** was readily prepared by the reaction of amino CoA **(98)** and *p*-coumaric acid *N*-hydroxysuccinimide (NHS) ester **(111)** in 74 % isolated yield. The reaction was carried out at room temperature in phosphate buffer at pH 7.5 and was completed in 14 hours - quite mild conditions (Scheme 3.19). As *p*-coumaroyl CoA is a key precursor for type III polyketide synthases,[133] the corresponding amide analogue **112** should be a useful probe for biochemical and structural studies.



*Scheme 3.19 Synthesis of p-coumaroyl CoA amide **(112)***

3.3 Conclusion

The boric acid catalysed transamidation approach discussed in this chapter allows for the rapid production of four important CoA analogues. These compounds were synthesized using a "green method", under mild conditions with catalysis by boric acid

in water. The CoA analogues accessible are easily functionalized or extended by well-precedented transformations for the particular application that may be required. It provides rapid access to compounds that are vital for the study of a broad variety of enzyme-catalyzed reactions. Particularly, processes involving acyl carrier proteins (ACP) of polyketide synthases (PKS), fatty acid synthases (FAS) and nonribosomal peptide synthetases (NRPS). There is work already in progress in our research lab towards the application of this method towards the study of the interaction between the Cla2 and Cla3 PKSs.

3.4 Future Directions

3.4.1 Probing the Interaction Between Cla2 and Cla3

Previously mentioned in Chapter 2, the HR-PKS, **Cla2** and NR-PKS, **Cla3** of the *C. cladosporioides* are believed to work in concert. The **Cla2** synthesizes the highly reduced pentaketide attached to its ACP domain and then passes it onto the SAT domain of **Cla3**, which if recognized and accepted is then further elongated and transformed into the final product, cladosporin (Figure 3.35). Each of these two independent PKSs having their own sets of domains that are iteratively used throughout the production of the natural product. While this much is understood, we have not yet been able to trap any such dual PKSs in the act of this manufacturing process. However, with ready access to amino CoA **(98),** we can now prepare probes that could selectively trap each of these domains by covalent linkages and potentially map the exact sequence of events occurring during the production process by crystallographic studies.

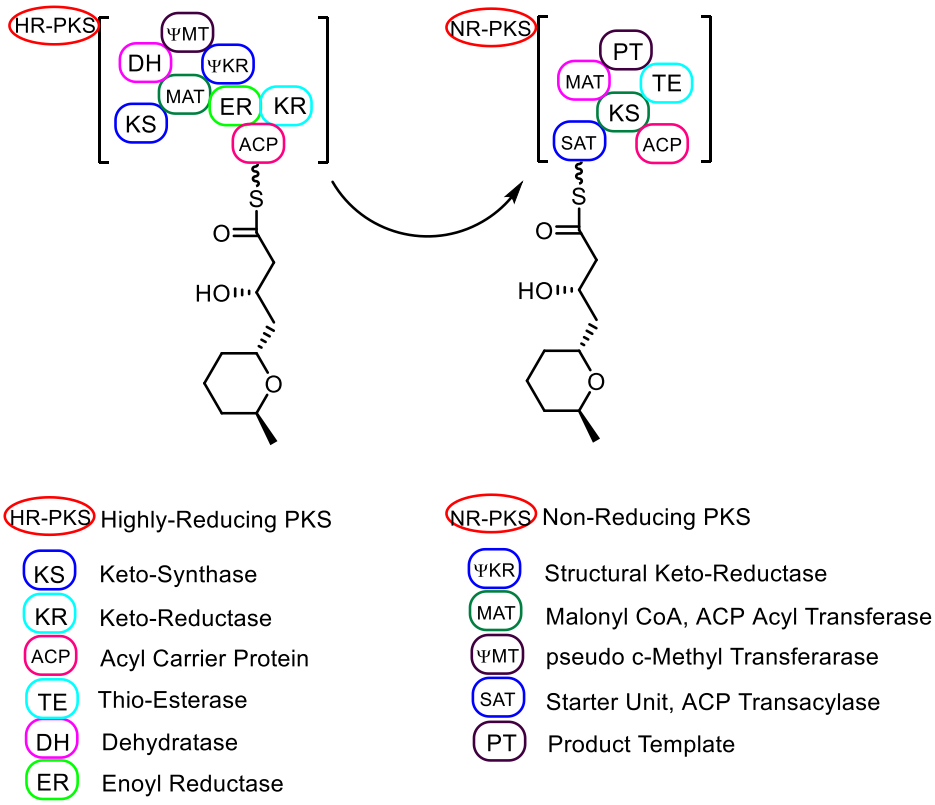
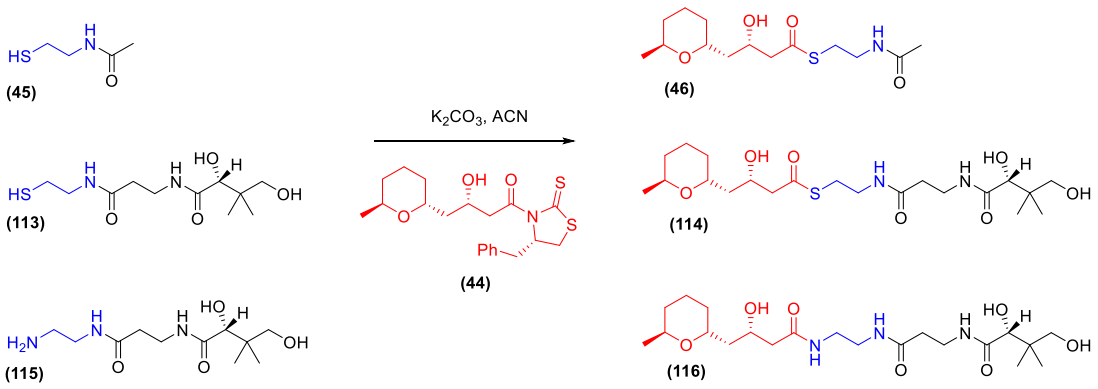


Figure 3.35 HR/NR-PKS interaction towards the production of cladosporin.

While our main objective is to map the entire sequence of events, we need to develop a method by which we can access stable crystals of these complex enzymes. We hypothesised that by covalently linking the two PKS enzymes to each other, we would be able to irreversibly trap them in a complex which could lead to stable crystals. Our plan to achieve this goal is to start by working with smaller portions of the PKSs and work our way outwards including more and more complexity. The starting point we have chosen is the ACP domain of the **Cla2** and the SAT domain of **Cla3**. These two domains are believed to be in direct communication while not covalently linked to each other. As pointed out the product of the **Cla2** enzyme connected to the ACP domain is believed to be transferred to the SAT domain of the **Cla3** for completion. We plan to

begin by co-crystallization of the **Cla3** SAT domain with several substrates (Scheme 3.20). These include the natural SNAC-pentaketide **46**, pantetheine-pentaketide **114** and amino pantetheine-pentaketide **116**. Compounds **114** and **116** can be prepared from the common intermediate **44**. These studies will provide us with an idea of the impact towards protein crystallization as well as the interaction of the substrate analogues in the active site of the SAT domain.

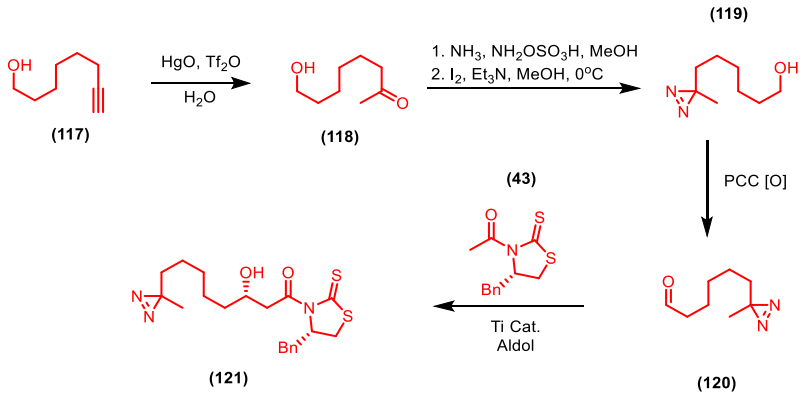
Given that both **46** and **114** are thioesters, there is the possibility of observing the orientation of the pentaketide once loaded onto the SAT domain. However, since **116** is an amide, this isostere should inhibit the loading and provide us with an image of the orientation of the substrate in the active site before loading.



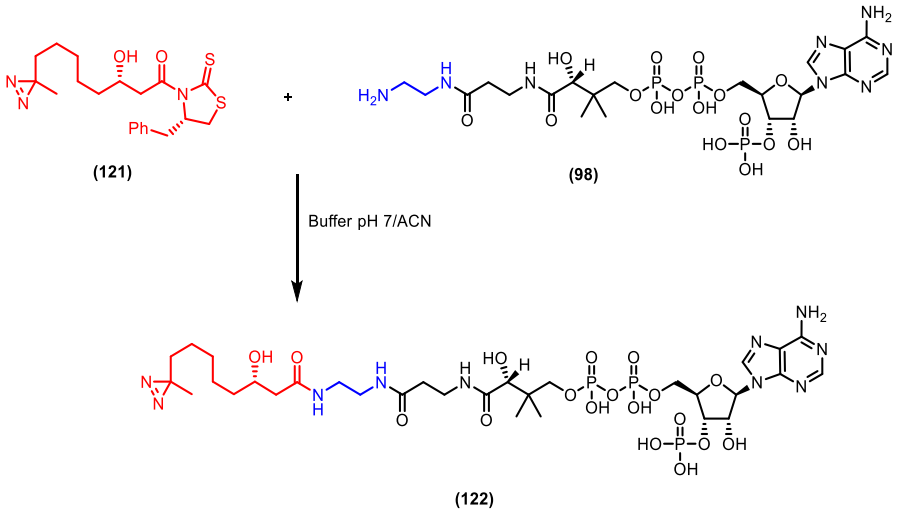
*Scheme 3.20 Synthesis of pentaketide probes for crystallographic studies of **Cla2** and **Cla3** interaction.*

With any success there, we will then synthesize an amino CoA analogue **122** bearing a pentaketide mimic that possesses a diazirine moiety. This can be prepared by the reaction of the terminal alkyne **117** with mercuric triflate which will lead to the ketone product **118**. When treated with ammonia and hydroxylamine O-sulphonic acid,

followed by iodine and base this will provide the diazirine **119** (Scheme 3.21). Oxidation of the terminal hydroxyl group will furnish aldehyde **120** which when reacted with the preformed enolate of **43** will give the final product **121**. After separation of the diastereomers, the desired pentaketide mimic can then be transferred to amino CoA **(98)** forming the diazirine probe **122** for crosslinking studies (Scheme 3.22).

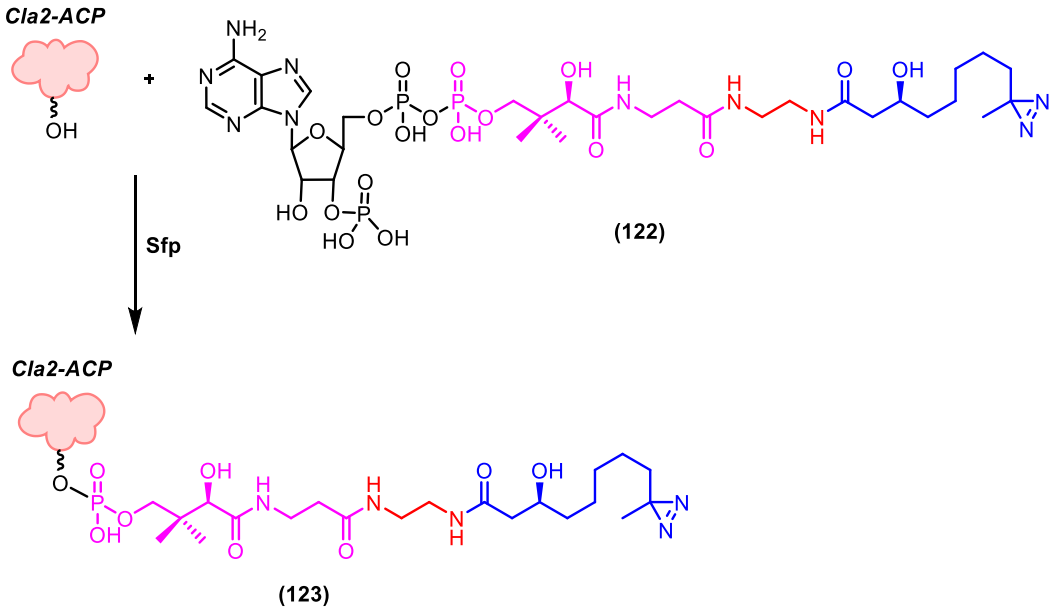


*Scheme 3.21 Synthesis of activated diazirine-pentaketide analogue **(121).***



*Scheme 3.22 Synthesis of diazirine probe **(122)** for crosslinking studies.*

After expression of the **Cla2-***apo* ACP domain, a promiscuous phophospantheteine transferase (Sfp) can be used to transfer the phosphopantetheine arm of the diazirine-CoA probe **122** onto the ACP, essentially loading the probe onto the protein (Scheme 3.23). This ACP-diazirine probe **123** will then be incubated with the **Cla3**-SAT domain, then irradiated with 350 nm UV light, forming the carbene species from the diazirine, which can then covalently link to any polar functionality nearby. If the mimic sits in the active site of the SAT during the incubation, then we will have a covalently linked probe in the active site connecting the **Cla2**-ACP and the **Cla3**-SAT. At this point attempts at crystalizing this moiety will be undertaken. Providing that this approach works, then attempts at using the entire **Cla2** and the **Cla3**-SAT, **Cla2**-ACP and entire **Cla3**, and finally, the **Cla2** and **Cla3** moiety are envisioned.



Scheme 3.23 Preparation of the diazirine labelled ***Cla2****-ACP* ***(123).***

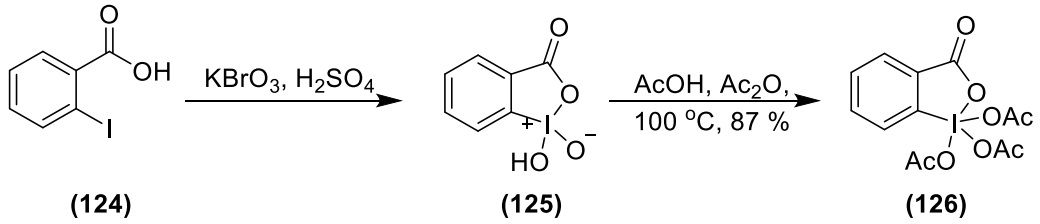
Chapter 4: Dess–Martin Periodinane Oxidative Rearrangement

4.1 Introduction

Polyvalent iodine reagents emerged in the early 1980's as a selective, mild, and environmentally friendly set of oxidants in organic chemistry.[134] One subgroup of these reagents is the periodinanes, which are pentacoordinated iodine(V) reagents.[135,136] Of these the most commonly used are 2-iodoxybenzoic acid (IBX)[137] and 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one, known as Dess-Martin Periodinane (DMP).[138] DMP is noted to be one of the mildest reagents for oxidation of primary and secondary alcohols to aldehydes and ketones, respectively.[139] In addition to oxidation of primary and secondary alcohols, DMP has been shown to effect α-oxidations of β-keto esters and amides, forming the corresponding vicinal tricarbonyl compound.[139] Some other lesser known applications of DMP include: conversion of allylic alcohols to α,β-unsaturated carbonyls;[140] cleavage of aldoximes and ketoximes to aldehydes and ketones respectively;[141] *N*-acyl hydroxylamines to acyl nitroso compounds;[142] 4-substituted anilides to *p*-quinones;[143] β-amino alcohol to α-amino aldehydes without epimerization;[144] and γ,δ-unsaturated aromatic amides to complex heterocycles.[145] However, there were no reports to show that DMP is involved in the rearrangement of β-hydroxy thioesters to α-keto thioesters, which will be described in the following.

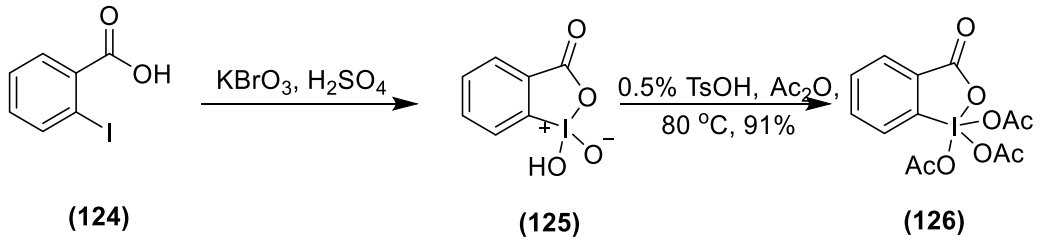
DMP **(126)** was first prepared by Dess and Martin in 1983 as a result of the poor solubility of IBX **(125)** in most organic solvents.[138] They synthesized this oxidant by treatment of 2-iodobenzoic acid **(124)** with $KBrO_3$ in H_2SO_4 to yield IBX **(125)**, which

was further treated with a mixture of acetic anhydride and acetic acid to yield what is now known as DMP **(126)** in 87% overall yield (Scheme 4.24).[138]



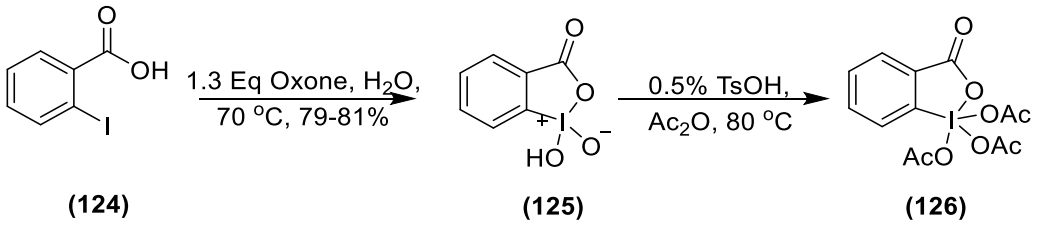
Scheme 4.24 Dess and Martin's original synthesis of the DMP reagent (126), 1983.

However, due to the inconsistency in the yield, stability of the product, and efficiency of the oxidation reaction, improvements were sought. Ireland and Liu in 1993 addressed some of these inconsistencies in the preparation of DMP **(126)** by replacing acetic acid with catalytic amounts of *p*-toluenesulfonic acid in the second step of the preparation (Scheme 4.25).[146] This modification allowed for clean and efficient acetylation of the hydroxyiodinane intermediate **(125)**, which apparently was the problem in the original preparation. This allowed for preparation of reproducible batches of the DMP reagent at consistently higher yields.[146]



*Scheme 4.25 Synthesis of DMP **(126)** by Ireland and Liu, 1993.*

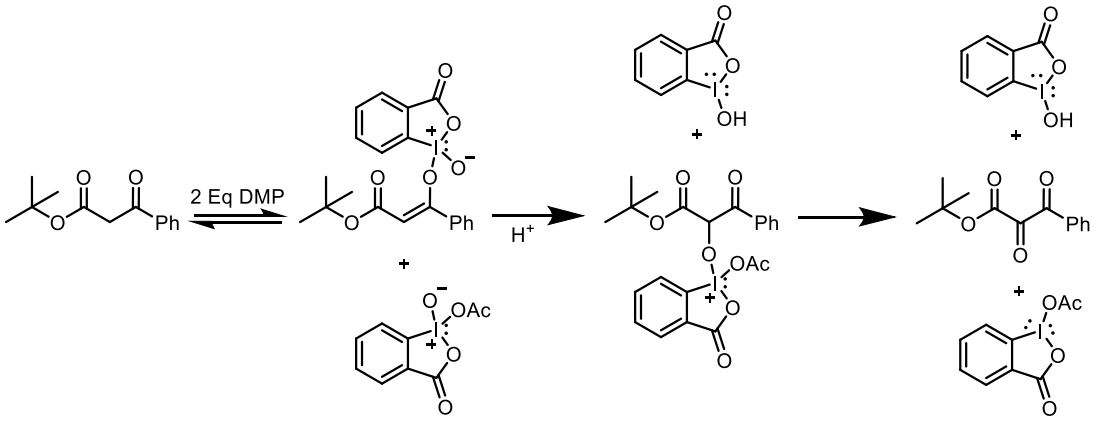
Even further improvements were later made by Frigerio *et al*. who replaced the oxidant used to prepare the IBX with oxone, reducing the safety risk compared to the earlier preparations (Scheme 4.26).[147]



Scheme 4.26 Synthesis of DMP ***(126)*** *by Frigerio et al., 1999.*

However, there were other issues regarding the inconsistent behaviour of the DMP reagent. These were addressed by Meyer and Schreiber in 1994, who showed that through exposure to the atmosphere or the addition of an equivalent of water, rates of oxidations were vastly accelerated compared to employing the pure DMP reagent under anhydrous conditions.[139] A rate increase was also seen when 2 equivalents of alcohol was used relative to DMP. The authors proposed that the water or alcohol replaces one of the acetoxy groups on DMP, and this makes the remaining iodine-alkoxy bond more labile, thereby accelerating the reaction.[139]

The work of Batchelor *et al*., showed that DMP might be used to transform β-diketones, β-ketoesters and β-ketoamides to produce the corresponding vicinal tricarbonyl compounds by α-oxidation in varying yields.[148] Subsequently, Meyer and Schreiber proposed that the α-methylene oxidation of β-keto esters is facilitated by the acetoxyiodinane oxide reacting with the enol form of the β-keto ester product, as depicted in Scheme 4.27.[139]

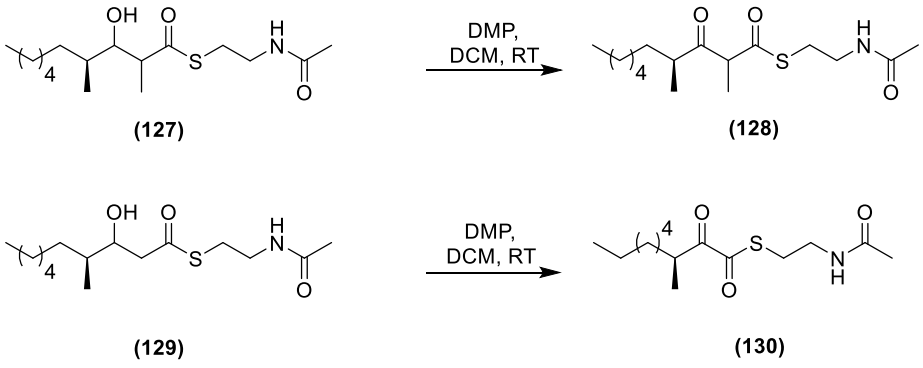


Scheme 4.27 α-Oxidation of β-keto esters to vicinal tricarbonyls.

Interestingly there were no reports of analogous transformations with β-keto thioesters. Moreover, our group had oxidized β-hydroxy thioesters to β-keto thioesters with DMP in good yields (>75%) without detecting significant over-oxidations.[39,48,92,149,150] However, as will be highlighted in this chapter, DMP can also form vicinal tricarbonyl thioesters. With excess DMP, these thioesters are susceptible to an unexpected rearrangement reaction to form α-keto thioesters. α-Keto carbonyl compounds are intermediates in the preparation of heterocyclic derivatives.[151] Although the use of α-keto acids to form heterocycles is quite common, the conditions required for transformation tend to be harsh.[152] It will be shown in this chapter that reactions of α-keto thioesters with diamines proceed under very mild conditions to give quinoxalinones in excellent yields. The quinoxalinone moiety is considered a privileged structure, often used as a core scaffold for a combinatorial library synthesis.[153]

4.2 Results and Discussion

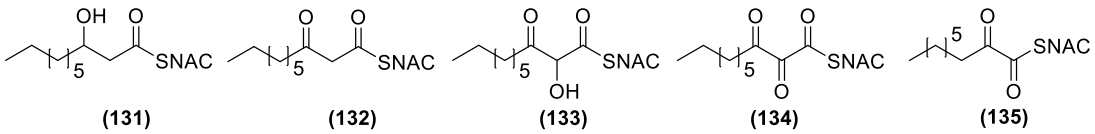
Although oxidation of α-methyl β-hydroxy thioester **(127)** with excess DMP gives the expected product **(128)**,[48] identical conditions transform compound **(129)** to α-keto thioester **(130)** rather than the expected β-keto derivative. This compound contains one less carbon, and its formation was accompanied by the production of a gas. (Scheme 4.28). Interestingly, we did not find any literature precedent for this transformation. However, literature examples of DMP oxidations of β-hydroxy thioesters to β-keto thioesters did sometimes result in low yields,[39,48,149,150] whereas the same reaction on β-hydroxy esters or amides has been reported with higher yields.[148] N-acetylcysteamine (SNAC) thioesters were used in this work due to their utility for studies with polyketide synthases,[39,48,150]as well as the fact that they are relatively non-volatile. However, the reaction works similarly with other thioesters as shown in Scheme 4.29. Since DMP is already known to convert β-hydroxy esters and amides to the vicinal tricarbonyl compounds, it seemed likely that the β-keto thioester was further oxidized to the vicinal tricarbonyl, which was then transformed to the α-keto thioester.



Scheme 4.28 Transformation of β-hydroxy thioester to different products under the same conditions.

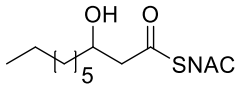
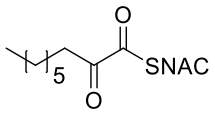
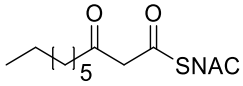
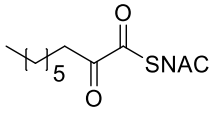
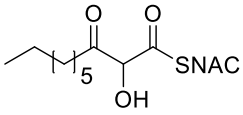
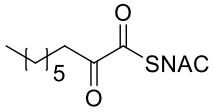
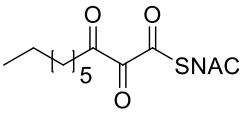
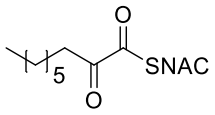
To test this, the conversion of **131** to **135** was investigated by the synthesis of proposed intermediates **132** to **134** and examination of the effect of varying equivalents of DMP **(126)** (10% excess was used to account for the purity of the reagent supplied, 95% purity) on the product distribution. (Table 4.6). The identities of the intermediates were confirmed by retention time and high-resolution liquid chromatography coupled to mass spectrometry (LCMS) and comparison to synthesized standards. Complete rearrangement starting from **131** requires 5 equivalents of DMP **(126)**. Product ratios resulting from the use of less DMP suggest that **133** and **134** are rapidly transformed to subsequent products if the additional oxidizing agent is present. To confirm that the species observed in the reaction were true intermediates, each intermediate was reacted with the requisite equivalents of DMP. It was observed that all of the proposed intermediates were transformed into the α-keto thioester product as shown in Table 4.7. Under the same conditions, the vicinal tricarbonyl compound **134** was treated with 1-acetoxybenziodoxol-3-one, the by-product from the DMP reagent, but this did not give any rearrangement. Similarly, nucleophiles with heteroatom bonds such as hydrogen peroxide or *N*-hydroxyphthalimide did not lead to a rearrangement of the vicinal tricarbonyl compound **134**. With the intermediate species in the rearrangement established, the mechanism of transformation of the vicinal tricarbonyl to the α-keto thioester was then probed.

Table 4.6 Effect of DMP equivalents on the relative distribution of products (total equivalents of 131-135 set to 100%)



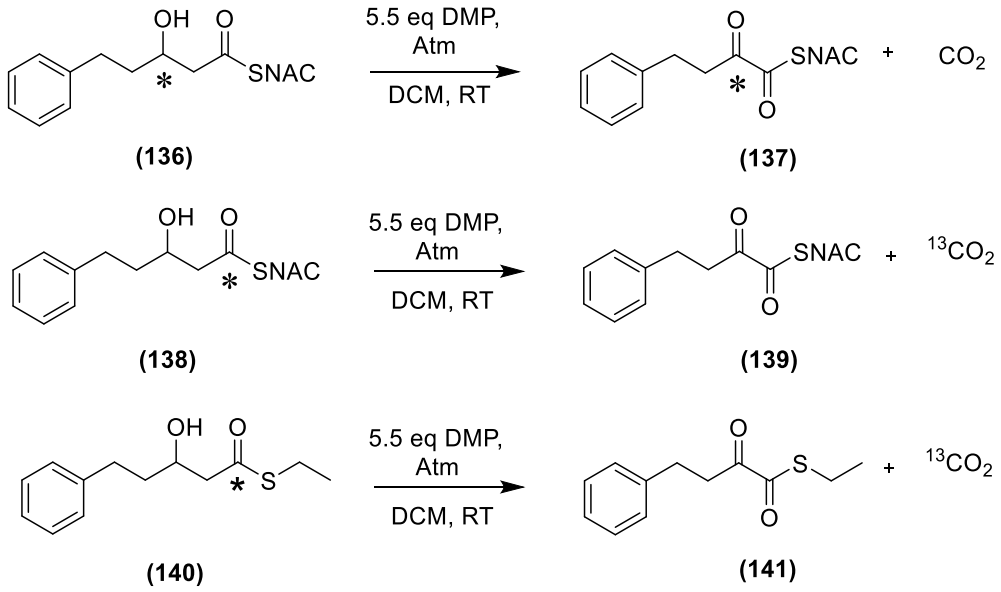
eq of DMP	% 131	% 132	% 133	% 134	% 135
1.1	0	73	1	1	25
2.2	0	10	0	7	82
3.3	0	1	0	8	91
5.5	0	0	0	1	99

Table 4.7 Rearrangement of proposed intermediates to α-keto thioesters by treatment with DMP

Substrate	eq of DMP	Product
(127) 	5.5	(131) 
(128) 	4.4	(131) 
(129) 	2.2	(131) 
(130) 	1.1	(131) 

A series of substrates labelled with ^{13}C at the β-carbon or at the thioester carbonyl (Scheme 4.29), with varied thioester was then synthesized. These were used to ascertain which carbon was being removed and to determine the identity of the gas produced.

The reaction of β-hydroxy thioester **(136)**, bearing the ^{13}C label at the β-carbon gave ^{13}C-labelled α-keto thioester **(137),** and m/z 44.1 was observed for unlabelled CO_2 in the headspace gas. This was confirmed by the gas phase IR spectra (Figure 4.36). The reaction of β-hydroxy thioester **(138)** bearing the ^{13}C label at the thioester carbonyl produced unlabelled **(139),** and m/z 45.1 was observed for the $^{13}CO_2$. The corresponding shift in the IR spectra of the resulting $^{13}CO_2$ gas produced in the reaction vessel headspace is shown in Figure 4.36. These results showed that the thioester carbonyl carbon is extruded in the form of CO_2 gas. Compound **(140)** was prepared to confirm that the N-acetyl group on the thioester played no roll in the transformation.



*Scheme 4.29 Products formed from ^{13}C labelled substrates. * Represents ^{13}C.*

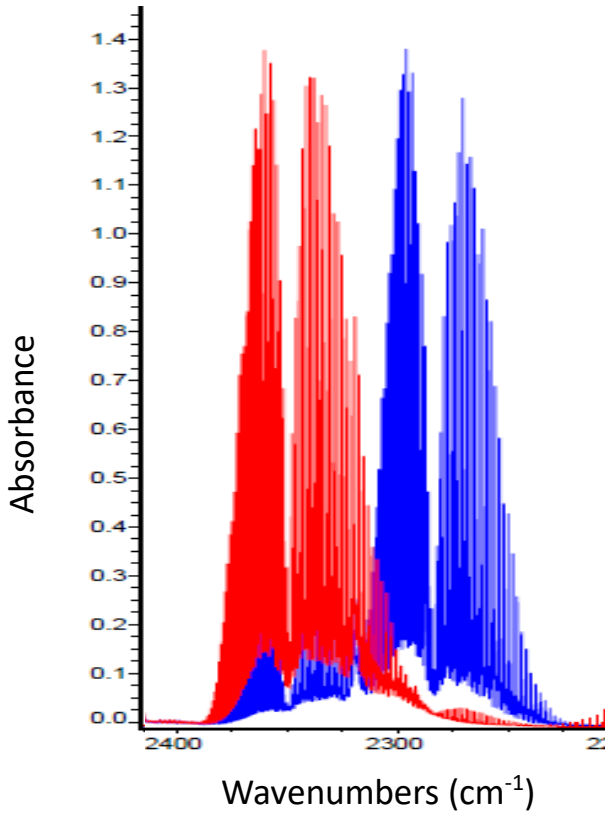
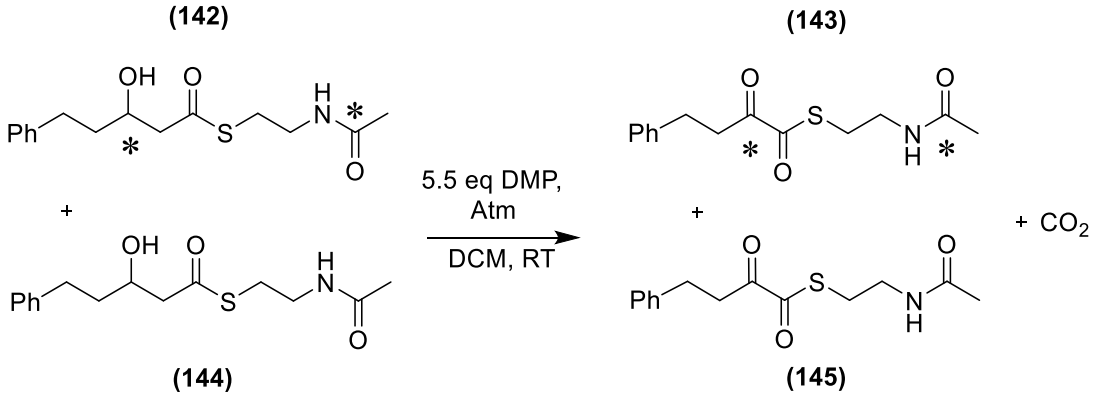


Figure 4.36 Overlaid gas phase IR spectra from gases produced by rearrangement of ***(136)*** *and* ***(138)****.* *$^{12}CO_2$ (red trace) vs. $^{13}CO_2$ (blue trace).*

Given that the thioester carbonyl carbon was being extruded, the reaction could have been proceeding through an intermolecular or intramolecular rearrangement. To determine which of the two possibilities was occurring, a crossover experiment was done. Equal amounts of unlabelled compound **144** and doubly ^{13}C-labelled compound **142** were mixed and then oxidized. An intramolecular process would give α-keto thioester products that are either completely unlabelled or doubly labelled, whereas an intermolecular reaction would generate a mixture of unlabelled, singly labelled, and doubly labelled products. Through the use of high-resolution LCMS analysis, it was

observed that only unlabelled compound **145** and doubly labelled compound **143** were formed, thereby demonstrating an intramolecular rearrangement.



Scheme 4.30 Crossover experiment, between doubly labelled and unlabelled substrates (ratio 1:1).

Based on the results obtained from our mechanistic studies, the rearrangement mechanism of the vicinal tricarbonyl thioester to the corresponding α-keto thioester may occur as outlined in Figure 4.37. Overall the transformation proceeds by oxidation of the β-hydroxy thioester to a β-keto thioester, which then undergoes an α-hydroxylation followed by another oxidation to form the vicinal tricarbonyl thioester. This vicinal tricarbonyl then undergoes an intramolecular rearrangement using a molecule of acetoxyiodinane oxide to release CO_2 and the α-keto thioester product.

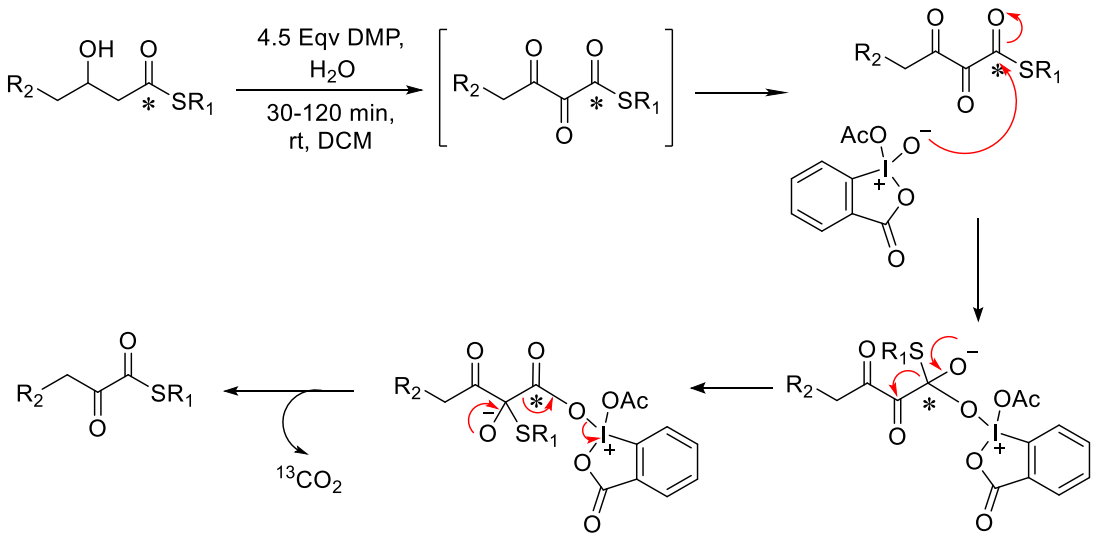
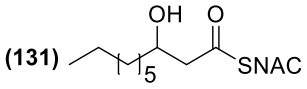
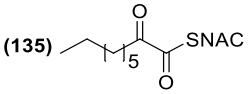
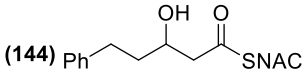
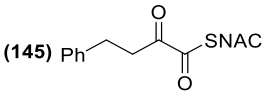
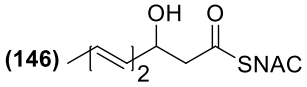
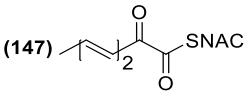
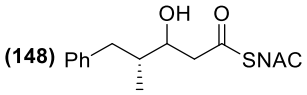
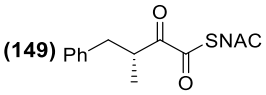
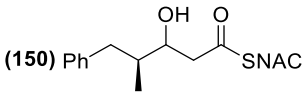
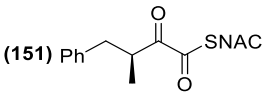


Figure 4.37 Proposed mechanism for the DMP oxidative rearrangement of β-hydroxy thioesters to α-keto thioester.

The substrate scope was briefly explored with variations at the γ–carbon of the thioester. The reaction does not seem to be affected by steric bulk at the γ–position, but a methyl substituent at the α-position (relative to thioester) does prevent the rearrangement. It appears that very little racemization at the α-substituent of the products occurred. This was determined by direct comparison of the optical rotations of enantiomeric compounds, both after rearrangement and condensation. The values were observed to be quite similar but in the opposite direction. While full conversion of the starting material is always observed, the average isolated yield is 65 % (Table 4.8). This is believed to be as a result of the polar nature of the products, making them harder to separate from the 2-iodobenzoic acid formed in the work-up of the reaction. Increasing the number of sodium bicarbonate washes aids in the removal of much of this impurity, but at the cost of a lower yield. However, if a stronger, more nucleophilic base is used, the targeted α-keto thioesters are destroyed. Other attempts were made to

further improve the overall yield of the reaction. These included the addition of a base (DBU, K_2CO_3, $CsCO_3$) to the reaction, and increasing of the reaction temperature (room temperature to 50 ^oC). However, these attempts provided no improvements to the yield. The elevated temperatures led to the decomposition of the product, and the addition of base resulted in lowered yields.

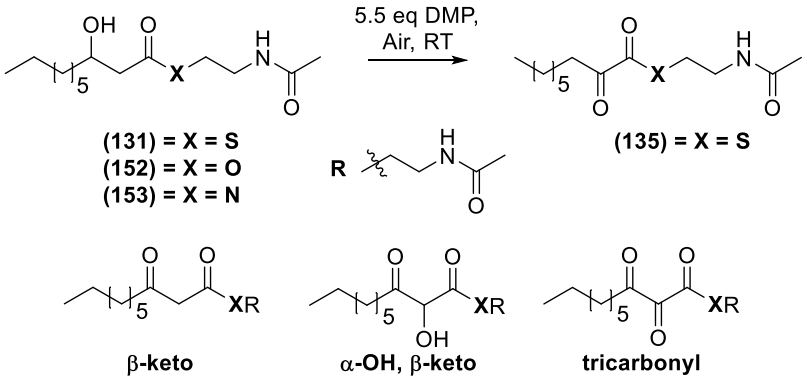
Table 4.8 Summary of substrates tested and their corresponding products. (5.5 eq DMP, RT, Atm, DCM)

Substrate	Yield %	Product
	**74**	
	**63**	
	**66**	
	**60**	
	**62**	

The reactivities of thioester, ester and amide functionalities for the rearrangement were also compared. As expected, the thioester functionality is the most susceptible of the three to the rearrangement (Table 4.9). Under the conditions used (5.5 eq DMP, RT, Atm, DCM), the rearrangement does not occur to a significant extent during oxidation of β-hydroxy amides. However, it does proceed with esters to some extent. These results may explain as to why this reaction was not previously observed, as there are

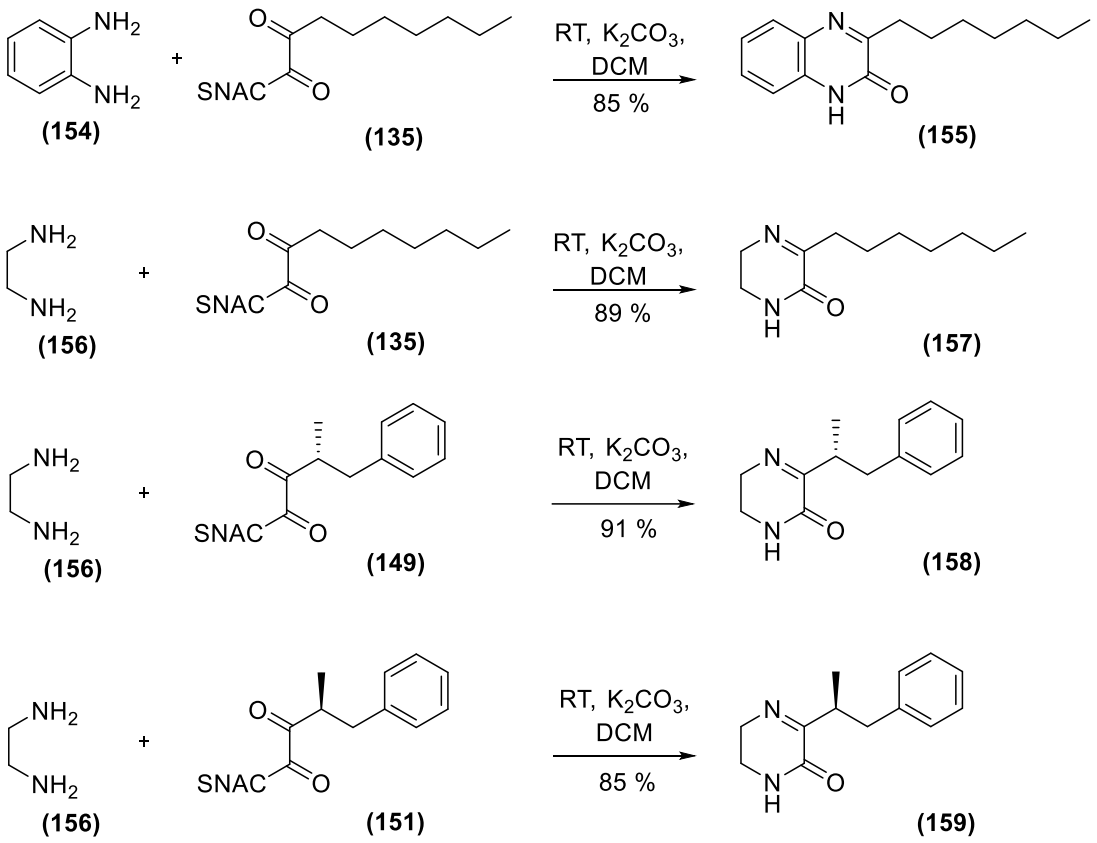
numerous reports of the use of DMP to oxidize β-hydroxy esters and amides to their corresponding β-keto and vicinal tricarbonyl compounds.[139,148]

Table 4.9 Relative distribution of products of rearrangement reaction tested on β-hydroxy- thioesters, esters and amides (total equivalents of starting material-products, set to 100%)



X	starting material	β-keto	α-OH, β-keto	tri-carbonyl	Product
S	0.0	0.0	0.0	1	99
O	0.0	2.0	34	52	11
N	2.0	0.0	2.0	95	1

The reactivity of α-keto thioesters with diamines was also briefly examined. They react readily at room temperature to form the corresponding quinoxalinones in good yields (Scheme 4.31). One of the most common approaches to prepare quinoxalinones is the treatment of α-keto acids with diamines under harsh conditions. However, our results show that α-keto thioesters may be used effectively in place of α-keto acids, thereby reducing the reaction time and temperatures, while being compatible with thermo labile functionalities.



Scheme 4.31 Preparation of quinoxalinones from α-keto thioesters.

4.3 Conclusion

In this chapter, a new Dess-Martin Periodinane (DMP) mediated intramolecular rearrangement reaction was described. Through a series of ^{13}C labelling studies and intermediate testing, it was found that the reaction converts β-hydroxy thioesters to α-keto thioesters, extruding the starting thioester carbon as CO_2 in the process. The shift of the thiol functionality to the α-carbonyl was determined to be a 1,2-migration. This mild and efficient reaction allows for easy preparation of α-keto thioesters, which can be used to prepare valuable intermediates for the synthesis of pharmaceutically important heterocyclic compounds.

Chapter 5: Enantioselective Preparation of Spin Labelled α-Amino Acids

5.1 Introduction

Electron paramagnetic resonance (EPR), is also commonly referred to as electron spin resonance (ESR).[154] This is a spectroscopic technique that allows for the study of paramagnetic compounds, meaning compounds with unpaired electrons.[81] The demand is growing for the development of more selective and accurate methods for visualizing biomolecules. This is as a direct relation to the scientific need to understand the relationship between structure and activity in biological systems. As a research tool, EPR spectroscopy is rapidly growing to satisfy these requirements.[81,155] With numerous improvements already, the pulsed EPR technique; double electron-electron resonance (DEER) in particular, has already shown great utility in the studies of biopolymers, where in addition to spin label orientations, measurement of interspin distances of 15 to >100 Å between paramagnetic centres are now possible.[156-161]

EPR detects free radicals of both organic and inorganic nature, in chemical and biological systems. While nuclear magnetic resonance (NMR) spectroscopy and EPR and quite similar, they measure different spin sources.[162] In NMR spectroscopy, it is the atomic nuclei that is perturbed whereas, in EPR spectroscopy, it is the electron spins that are studied.[162] While this phenomenon was first noted by Yevgeny Zavoisky in 1944,[163] it was independently developed by Brebis Bleaney in the same year.

It is quite uncommon to see natural products with stable, unpaired electrons, and as such are deemed ESR silent compounds. This inherent flaw can, however, be

circumvented through the use of spin-labels. Spin-labels can be loosely described as molecules that possess a stable, unpaired electron, thus being paramagnetic in nature.[164] The nitroxide family, particularly TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) **(160)** is commonly utilized as the spin label probe in EPR studies.[165] They can be considered as the standard to which all spin labels are measure, the reason being that they are very stable. It should be noted that the higher the stability of the radical species, the greater the sensitivity of the EPR measurement acquired will be. The most common of the TEMPO type spin-label are the TOAC (2,2,6,6-tetramethyl-N-oxyl-4-amino-4-carboxylic acid) **(161)**, and the methanethiosulfonate spin label (MTSL) (**162**) (Figure 5.38).

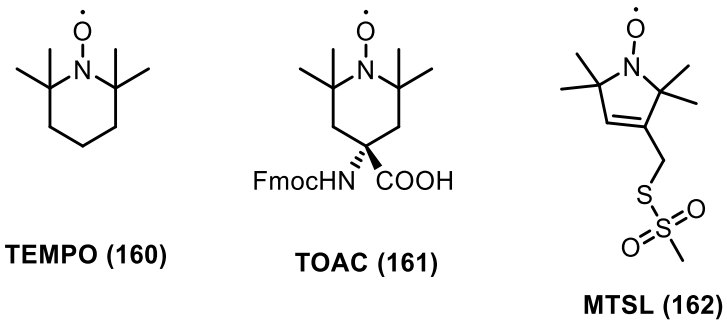


Figure 5.38 Chemical structures of nitroxide radicals.

Much like spin-labelling reagents, spin-trapping reagents are useful in EPR spectroscopy. They are used to trap and study the identity of short-lived radicals. They work by reacting with the existing spin-label on the molecule of interest, forming an even more stable radical species in the process.[166,167] This newly formed radical species can then be detected through EPR studies or other applicable techniques.[167] Common spin trapping reagents are nitrones, as they are quite stable. Of the nitrones: DMPO

(5,5-dimethyl-1-pyrroline *N*-oxide) **(163)**, PBN (*N*-tert-butyl-α-phenylnitrone) **(164)** or 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (**165**) (Figure 5.39) are most commonly used. Alternatively, nitroso compounds could also be utilized for this purpose.

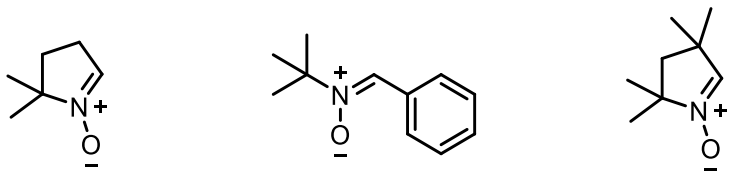


Figure 5.39 Chemical structures of spin trapping reagents.

EPR spectroscopy allows for the study of molecular motion in macromolecules.[168] Particularly since EPR is sensitive to molecular orientation in regard to the magnetic field.[168] This trait makes EPR particularly useful in probing anisotropic systems, which renders them incredibly useful for studies of anisotropic systems such as membranes.

Stone *et al*. in 1965, pioneered the use of nitroxide radicals in biological systems, showing that conformational information could be obtained *via* EPR spectroscopy.[169] They reported the study of the paramagnetic resonance of a nitroxide radical bonded to bovine serum albumin and to poly-*L*-lysine, where they noted the potential of this method to be applied to other biological systems.[169] This work showed that a paramagnetic reporter group could be introduced into an EPR silent compound. Regardless of the simplicity of the radical species, providing that it is sufficiently stable and bonded to the macromolecule, both structural and kinetic inferences can be made from an EPR spectra.[169] The nitroxide group has continued to perform this role quite

adequately for over five decades, with its use in EPR studies of spin-labelled proteins being extensively reported.[168]

While the nitroxide radical has been the common feature in these studies, the nature of the attachment and flexibility has varied quite significantly (Figure 5.40). In the majority of these studies, the targets were labeled by attachment of nitroxide-containing labels to reactive amino acid side chains, particularly cysteines, (Figure 5.40, B).[168] A possible reason for this widespread use was the introduction of site-directed spin labelling (SDSL) pioneered by Todd *et al*.[170] and Altenbach *et al*.[69] where through mutagenesis, specific amino acids residues were replaced with cysteines. This led to investigations of the interactions at specific amino acid residues between two discrete entities.

The most common form of attachment of the spin-labelled probes to cysteines has been based on the formation of a disulfide bond with the probe.[171-173] While this is a convenient reaction, the product formed is not entirely stable, mostly due to disulfide exchange, leading to crosslinked protein dimers.[174] Alternatively, the formation of the thioether bond with the probe is irreversible due to the inherent stability of the C-S-C bond, and thus advantageous in this regard.[175,176] Unfortunately, the products formed from these reactions are often non-specific, given that the preparation of these thioethers employs the use of iodo- and bromoacetamide which can also react with histidine, methionine, and lysine residues.[174] In addition, the presence of multiple cysteine residues in a protein makes selective incorporation of a paramagnetic group rather difficult, especially if such cysteine residues are functionally important and therefore cannot be mutated to serine or alanine.

Direct incorporation of a spin-labelled amino acid in solid-phase peptide synthesis is also feasible.[177-181] This method is potentially very useful, as it it permits site-specific incorporation of the radical species. It also allows control of the specific position of insertion in every round of chain extension. However, even with this improvement, the use of the thioether-linked side-chain attached spin-label is still plagued by the flexibility and free rotation of the probe, thus reducing the effectiveness and sensitivity of the measurements.

More recently, the incorporation of noncanonical amino acids (ncAA) with unique reactivity by translation with an expanded genetic code has become available.[83] It offers chemoselective labelling of the endogenous protein. This removes the complications imposed by the presence of cysteine(s) and does not introduce large changes into the protein.[84,85] Even more impressive are the recent improvements that have allowed spin labels to be directly genetically encoded as ncAA during translation *in vivo*[86-88], thereby removing the need for any conjugation reactions to the ncAA, Figure 5.40, C.

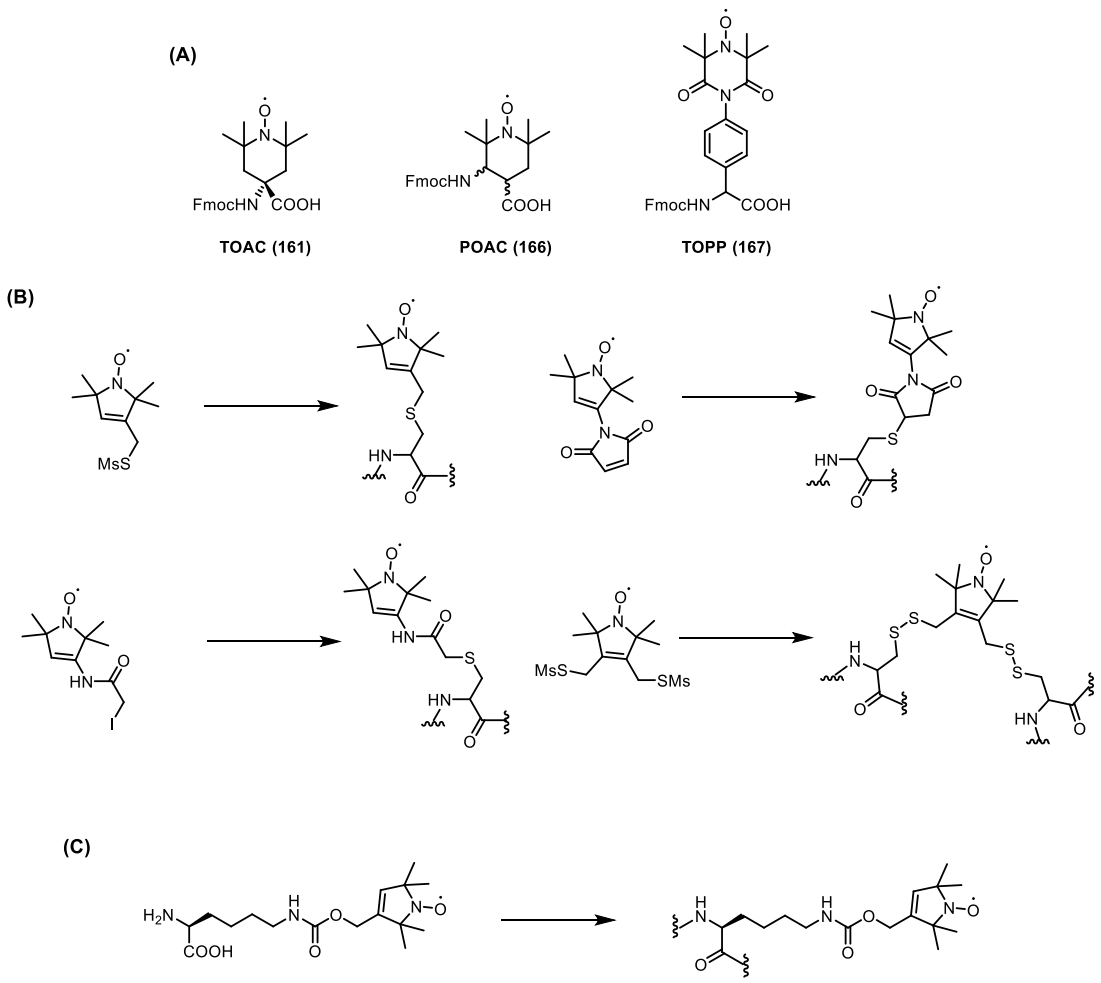


Figure 5.40 Variations of nitroxide spin labels commonly used. (A) Spin-labels for peptide labelling through solid phase synthesis. (B) Sulfhydryl-reactive spin-labels for protein labelling by cysteine conjugation reactions. (C) Spin-labelled ncAA for protein labelling by direct genetic encoding.

While there have been significant improvements in the installation of these spin labels, the spin labelled amino acids utilized remain mostly racemic, labile or difficult to couple, even under ideal situations, with the TOAC (2,2,6,6-tetramethyl-N-oxyl-4-amino-4-carboxylic acid) **(161)** being the most commonly used. As such, there is a need

for an easy route towards the enantioselective preparation of these spin labelled amino acids, which will be covered in this chapter.

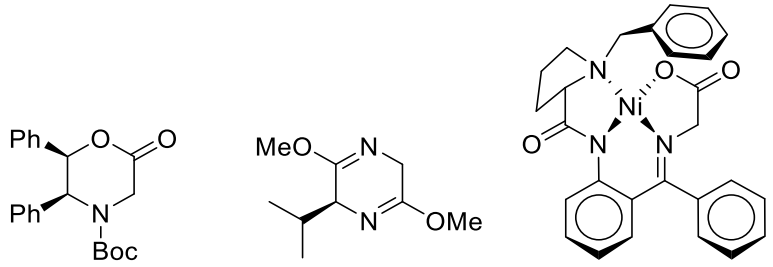
In 1981, Nakaie *et al*. introduced the use of the rigid TOAC (2,2,6,6-tetramethyl-N-oxyl-4-amino-4-carboxylic acid) **(161)**, for the preparation of peptides where the unnatural spin-labelled amino acid was incorporated into the peptide backbone *via* an amide bond.[182] As shown in the literature TOAC-labelled molecules, display a high level of crystallinity and generally takes on a helical backbone torsion angle.[183-186] EPR solution studies showed that TOAC also leads to molecules taking on a 3_{10}-α-helical backbone conformation owing to the rigidity of the spin label.[168] In view of TOAC's rigid cyclic structure, studies of backbone dynamics of peptides and proteins are not influenced by rotations of bonds in side chain residues, or those between side chains and the nitroxide group in side chain-attached spin labels, thus giving it an advantage over side chain-attached spin labels.

While the TOAC spin-label incorporated into the peptide bears advantage over the side-chain attached spin-label, it is not without its own shortcomings. It is worth noting that the incorporation of TOAC into a peptide backbone leads to a preference in α-helical, 3_{10}-α-helical and β-turn conformations, which could directly affect the nature of substrate and receptors binding in biological systems.[187] This is demonstrated by the deactivation of biologically active peptides (with flexible solution conformation) when TOAC was incorporated.[188,189] This phenomenon is quite possibly a result of a change in conformation induced by the TOAC residue. TOAC and its related analogues are also known for their difficulty in coupling during SPPS, along with the coupling of

amino acids following these unnatural residues.[190] This low reactivity is associated with the reduced nucleophilicity of the amine function of TOAC when bound to a peptide sequence.[191]

Considering these existing pitfalls in the currently available unnatural spin-labelled α-amino acids, we have developed a new approach towards the enantioselective preparation of spin-labelled α-amino acids.

Currently, several different methodologies exist to synthesize unnatural amino acids through the alkylation of chiral glycine amino acid scaffolds. These include the contributions by Williams *et al.,*[192] Schöllkopf *et al.,*[193] and Belokoń *et al.,*[194] towards the preparation of chiral glycine auxiliaries (Figure 5.41). However, the Ni-Schiff base complex **(170)** developed by Belokoń *et al.,*[194] is perhaps the most convenient due to its relative ease of preparation, the ability to recover and reuse the chiral ligand, and the reported ease of purification and crystallization of individual diastereomers.

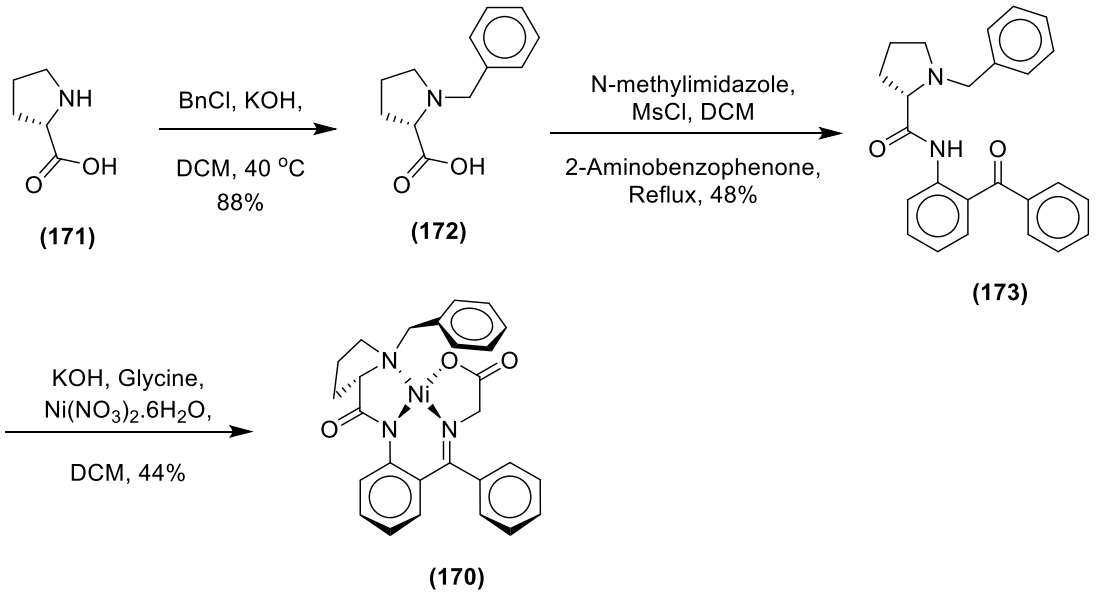


(168) **(169)** **(170)**

Figure 5.41 Chemical structures of chiral glycine auxiliaries.

5.2 Results and Discussion

Preparation of the Ni-Schiff base complex **170** was accomplished following the protocol, with minor modifications (Scheme 5.32).[194] The amino group of L-proline **171** was benzylated in the presence of benzyl chloride and potassium hydroxide, then acidified to precipitate the resultant N-benzylproline hydrochloride salt **172**. The free carboxylic acid was then converted into a mesylate through reaction with MsCl, and amidated with 2-aminobenzophenone. L-Benzylproline-2-aminobenzophenone (*S*-BPB) ligand **173** was heated under basic conditions in the presence of nickel (II) nitrate hexahydrate and excess glycine, to afford Gly-Ni-(*S*)-BPB **170** as a bright red solid.

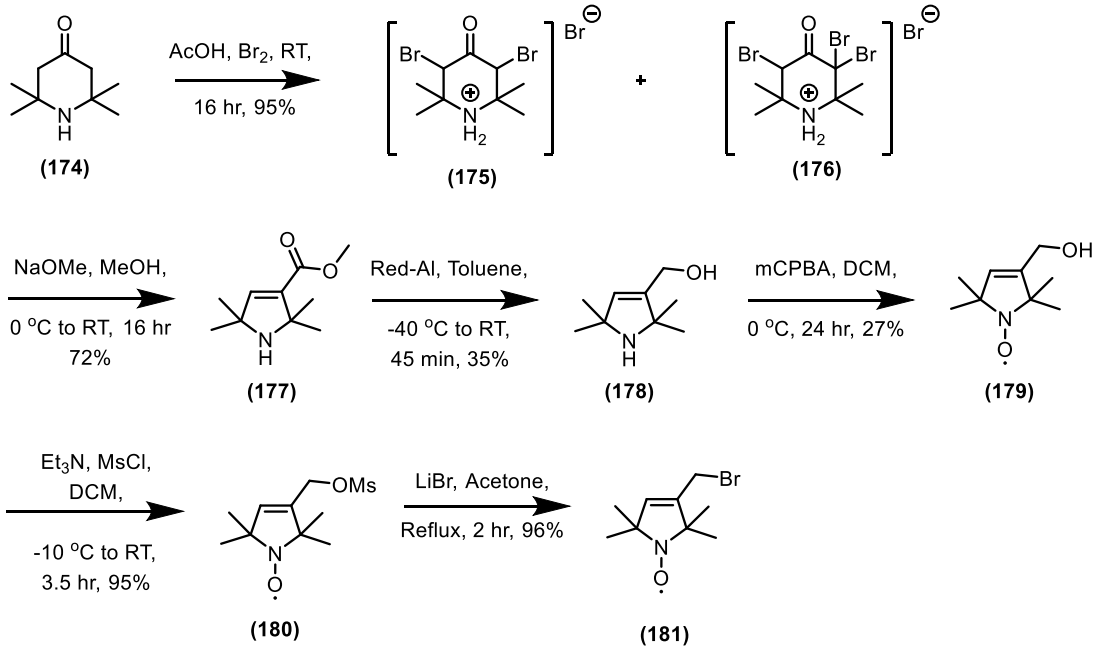


(171) **(172)** **(173)** **(170)**

*Scheme 5.32 Preparation of Ni-glycinate complex **(170).***

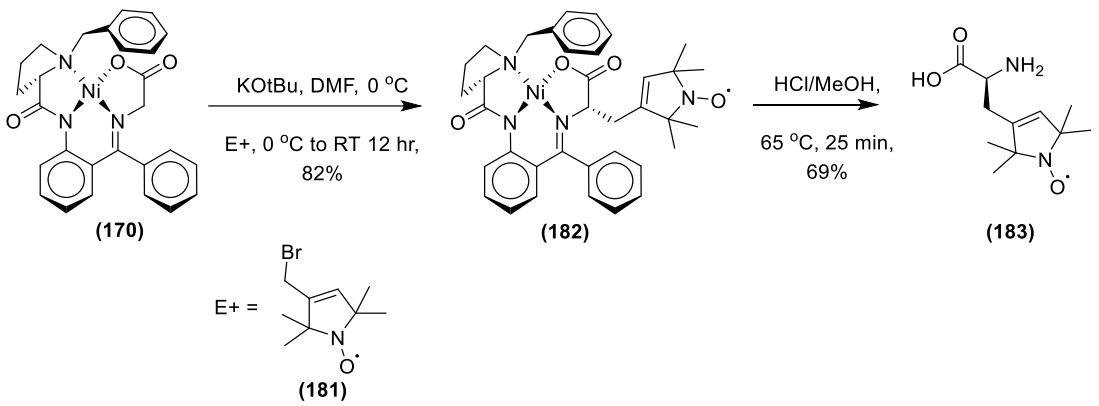
With the Ni-Schiff base complex **170** in hand, the preparation of the spin-labelled electrophile was the next focus (Scheme 5.33). The commercially available ketone **174** was treated with acetic acid and bromine, followed by treatment with NaOMe to yield the rearranged product **177**. The methyl ester **177** was then reduced with Red-Al to

yield the primary alcohol **178**, which was treated with mCPBA to give the nitroxide radical species **179**. Mesylation of the primary hydroxyl group of compound **179**, followed by displacement with LiBr gave the spin-labelled electrophile **181** in good yields. The preparation of this electrophile was completed by my summer student Mr. Tyler McDonald.



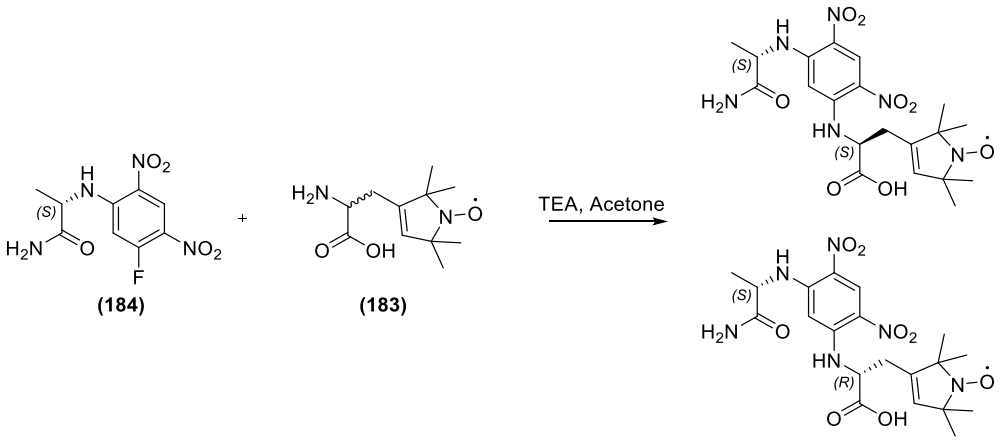
*Scheme 5.33 Synthesis of spin labelled electrophile **(181)***

Having both the Ni-Schiff base complex **170** and the spin-labelled electrophile **181**, the alkylation of the Ni-Schiff base complex **170** was conducted**.** Alkylation of the complex **170** gave the desired product **182** (Scheme 5.34). The spin labelled α-amino acid was accessed by treatment of the alkylated complex with MeOH/HCl at 65 °C, followed by purification *via* ion-exchange. After regeneration of the radical species and HPLC purification, compound **183** was obtained as a hygroscopic red-brown solid in good yields.



Scheme 5.34 Synthesis of spin labelled glycine analogue ***(183).***

To determine the extent of the selectivity for the L version of the spin-labelled amino acid, the Marfey's assay was employed. This method relies on derivatization of the spin-labelled product **183** with Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide **184**. Marfey's reagent adducts of L-**183** and D-**183** were cleanly separated chromatographically, allowing Marfey's adducts of L-**183** and D-**183** to be separated before mass spectrometry detection and quantitation. The Marfey's reagent **(184)** was reacted with the purified spin labelled product **183** in the presence of triethylamine forming the two diastereomers SS and SR, (Scheme 5.35). Based on the integration of the two peaks, the ratio of SS/SR was determined to be 9/1, indicating that the L form of the spin-labelled amino acid **183** was being favoured, Figure 5.42.



*Scheme 5.35 Marfey's assay of compound (**183**).*

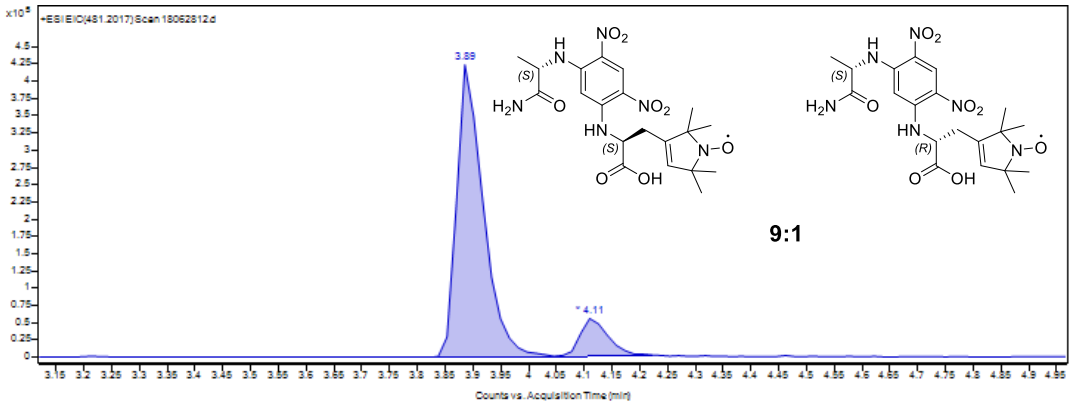


Figure 5.42 Ratio of the SS/SR diastereomers as determined by the Marfey's assay.

To access the enantiomerically pure L-form of spin label product **183,** the purified amino acid was treated with D-amino acid oxidase (DAAO) to degrade away the D-form and provide the single L-enantiomer upon purification. The Marfey's assay was again applied to confirm the product recovered from the DAAO assay was a single enantiomer. As shown in Figure 5.43, only a single peak was observed in the Marfey's assay, indicating that it was a single enantiomer remaining from the DAAO treatment.

Using circular dichroism studies, the DAAO treated **(183)** was confirmed to be the L-amino acid form, Figure 5.44.

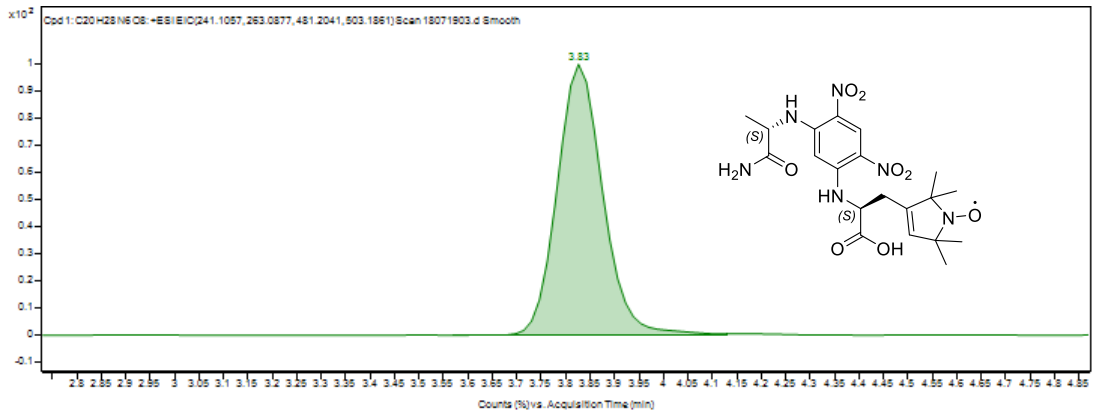


*Figure 5.43 Single peak observed after DAAO treatment of spin-labelled product **(185)**.*

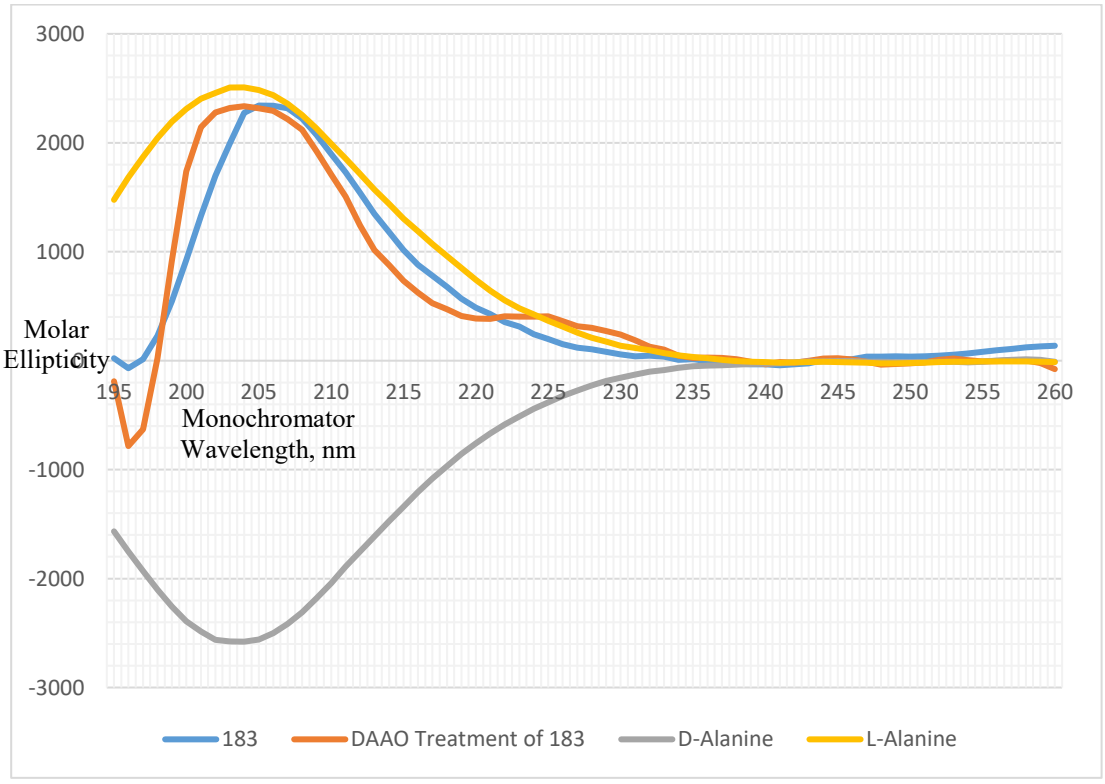


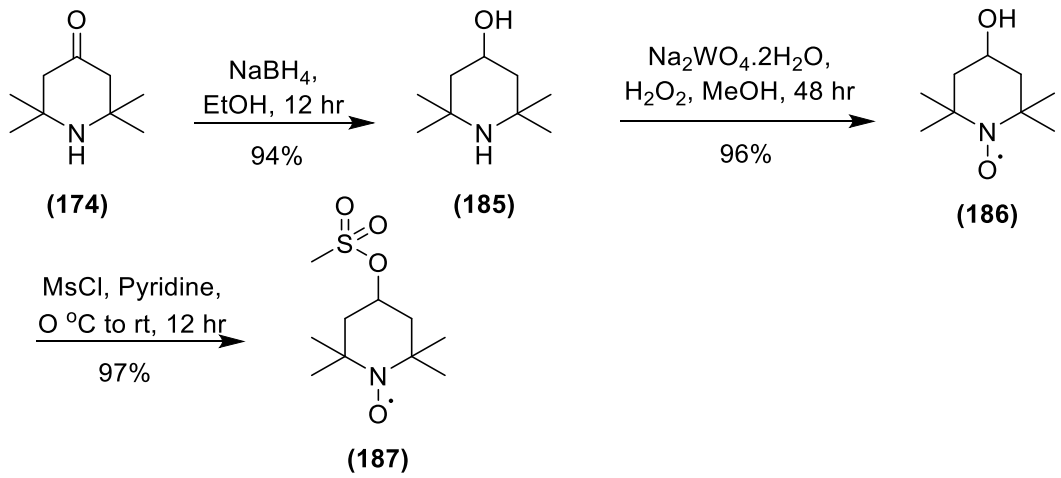
*Figure 5.44 CD results of spin labelled **(183).***

5.2 Conclusion and Future Directions

Spin labelled amino acid **183** was selectively prepared as the L-form (9/1 ratio) in excellent yields. However, as shown in the results, the pure L-form can be accessed through treatment of the L/D mixture with DAAO, followed by purification to recover the unchanged L-form of the product. It displays an unsaturated bond in the five-membered ring and is connected to the α-carbon of the amino acid by a methylene. While this may allow free rotation, it is much more controlled than a thioether and even more so than the disulfide connection previously used in the literature. This analogue will offer side chain flexibility when needed while being chemically stable. Given that the analogue is not sterically crowded at the α-position, there should be no complications in the peptide coupling for solid phase peptide syntheses or other techniques used for its incorporation into peptides/proteins.

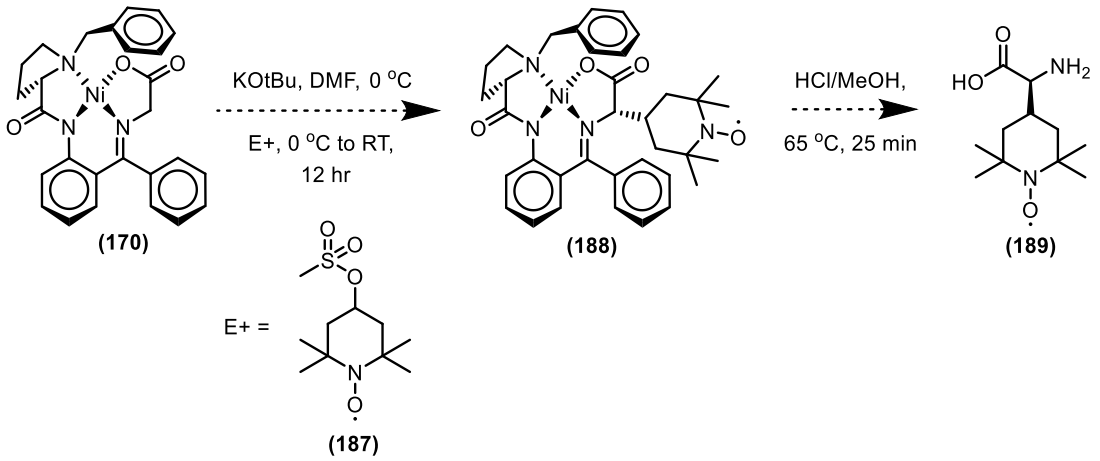
5.2.1 Future Directions

Following the success with the spin-labelled α-amino acid **183**, the synthesis of a second analogue was undertaken (Scheme 5.36). This synthesis began by treatment of the commercially available ketone **174** with sodium borohydride, which provided the secondary alcohol **185** in excellent yield. The secondary amine of **185** was then oxidized using sodium tungstate dihydrate and hydrogen peroxide furnishing the nitroxide radical **186** as a red solid in excellent yield. The alcohol **186** was then mesylated and purified to give the spin label electrophile **187** in excellent yields.



*Scheme 5.36 Synthesis of spin labelled electrophile **(187)***

Following the same approach as for the spin-labelled amino acid **183**, the spin-labelled product **189** will be prepared (Scheme 5.36).



*Scheme 5.37 Synthesis of spin labelled α-amino acid **(189).***

Analogue **189** is expected to be more rigid but not as restricted as the TOAC family to affect the native structure and potential functions. This analogue is connected to the α-

carbon of the amino acid by a secondary carbon in the spin-labelled side chain. Unlike the previous analogue, there will not be as much free rotation in the spin label and as such the sensitivity of the EPR measurement should be improved. While this analogue confers more stability, it should prevent unnatural structural changes to the labelled macromolecule of interest and allow for ease in coupling to the peptide backbone.

Chapter 6: Experimental Section

6.1 General Synthetic Procedures

6.1.1 Reagents, Solvents, and Purifications

Reactions involving either air or moisture sensitive reactants were conducted under a positive pressure of dry Ar. All solvents and chemicals were reagent grade and used as supplied unless otherwise stated. ^{13}C labelled reagents were purchased from Cambridge Isotope Laboratories. All other chemical reagents were purchased from Sigma–Aldrich Chemical Company, Alfa Aesar, AK Scientific, Tokyo Chemical Industry, Carboynth Limited and Chem Impex International. Anhydrous solvents required were dried according to the procedures outlined in Perrin and Armarego.[195] Both tetrahydrofuran and Et_2O were distilled over sodium and benzophenone under a dry Ar atmosphere. Acetonitrile, dichloromethane, methanol, pyridine, and triethylamine were distilled over calcium hydride and used immediately or stored over activated molecular sieves. Deionized water was obtained from a Milli–Q reagent water system (Millipore Co., Milford, MA). Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, HCl, NaOH, and $Na_2S_2O_3$ refer to aqueous solutions. Brine refers to a saturated solution of NaCl. Removal of organic solvents was performed under reduced pressure, below 40 °C, using a Büchi rotary evaporator. Water was removed by lyophilization. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 3 cm) pre-coated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Compounds were visualized by exposure to UV light and/ by exposing the plates to the $KMnO_4$ solution, followed by heating. Flash chromatography was performed on silica gel (EM Science, 60 Å pore

size, 230–400 mesh). Compounds were visualized by exposure to UV light and by staining with $KMnO_4$, followed by heating with a heat gun.

6.1.2 Compound Characterization

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Inova 400, Varian Mercury 400, Varian Inova 500 or Agilent VNMRS 700 MHz spectrometer respectively. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of the solvent as reference: $CDCl_3$ δ 7.26, CD_2Cl_2 δ 5.32, and CD_3OD δ 3.31. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.06, CD_2Cl_2 δ 53.8, and CD_3OD δ 49.0. Infrared spectra (IR) were recorded on a Nicolet Magna 750. Cast film refers to the evaporation of a solution on a NaCl plate. Gas Phase IR–spectra was obtained using a 10 cm gas cell, with KBr window on a Thermo Nicolet 8700 (Madison WI) equipped with a liquid nitrogen cooled MCT/B detector. The spectral resolution was 0.250 wavenumbers from 400 to 4000 wavenumbers with 128 co–added scans for both the sample and background. Optical rotations were measured on Perkin Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at 23 ºC. Mass spectra were recorded on a Kratos IMS–50 (high resolution, electron impact ionization (EI)) or by using an Agilent Technologies 6220 orthogonal acceleration TOF instrument equipped with +ve and –ve ion ESI ionization source, and full–scan MS (high resolution analysis) with two–point lock mass correction operating mode. The instrument inlet was an Agilent Technologies 1200 SL HPLC system. GC–MS analysis of headspace gas was performed using a Bruker Scion 456–GC–TQ GC–MS instrument (Billerica, Massachusetts, United States). The column used was a Phenomenex (Zebron ZB–5 fused silica capillary column (30 m x 0.25 mm ID, 0.25

μm film thickness). The method used was as follows: manual injection of 100 μL headspace gas, Injector at 200 °C, split rate 50:1, constant flow rate at 1 mL/min, helium as carrier gas, isocratic column oven temperature at 50 ºC; mass range 10 – 200 Da, total run time 10 min.

6.2 Biological Assays for Advanced Precursor Feeding

Advanced intermediate *in vitro* assays: For incorporation of cladosporin precursor assays, purified ***Cla3*** was dissolved in Buffer A (50 mM Tris-HCl, pH = 7.9, 2 mM EDTA, 2 mM DTT, 10% glycerol) to a final concentration of 10 μM. To this (1 mL) solution was added 2 mM malonyl-CoA and 2 mM of each advanced intermediate, and the reaction was incubated overnight at room temperature. In parallel and under the same reaction conditions, two negative controls were performed in either the absence of ***Cla3*** or the absence of pentaketide **(46)**. The reactions were quenched and extracted with an equal volume of 99% ethyl acetate 1% acetic acid solution, concentrated, and analyzed by LC-MS using an Agilent 1200 SL HPLC System with a Kinetex 2.6 μ XB-C18 100Å, 100 x 2.1 mm reverse phase column with guard (Phenomenex, Torrance, USA), thermostated at 55 °C and an Agilent 6220 Accurate-Mass TOF HPLC/MS system (Santa Clara, CA, USA) with dual sprayer. Presence of each expected product in the samples was confirmed by NMR and LC-ESI-MS using combined retention time matching with accurate mass matching with chemically synthesized authentic standards.

6.3 Preparation of CoA Analogues: NMR Experiments

6.3.1 Reaction Conditions Screening

Entry (1) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of acetamide (20 mg, 0.34 mmol). To the reaction mixture, ethylene diamine (0.23 ml, 3.39 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred into a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55^o C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^1H and ^1H-^1H COSY NMR, Figure 6.45.

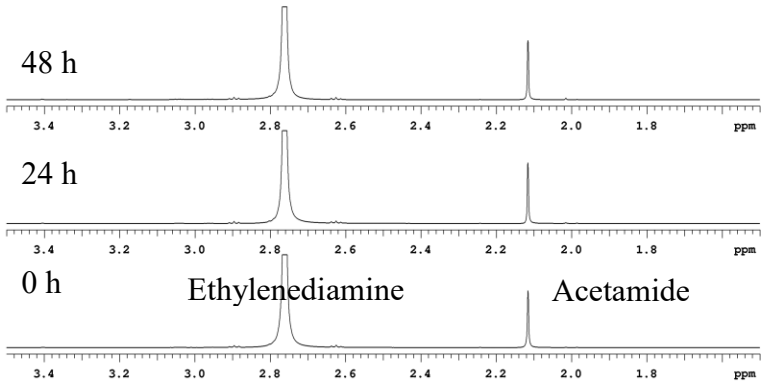


Figure 6.45 ^1H NMR spectra of entry 1- no $B(OH)_3$, absence of N-acetyl ethylenediamine after 48 hours.

Entry (2) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of acetamide (20 mg, 0.34 mmol). To the reaction mixture, boric acid (21 mg, 0.34 mmol), and ethylene diamine (0.23 ml, 3.39

mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55° C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.46.

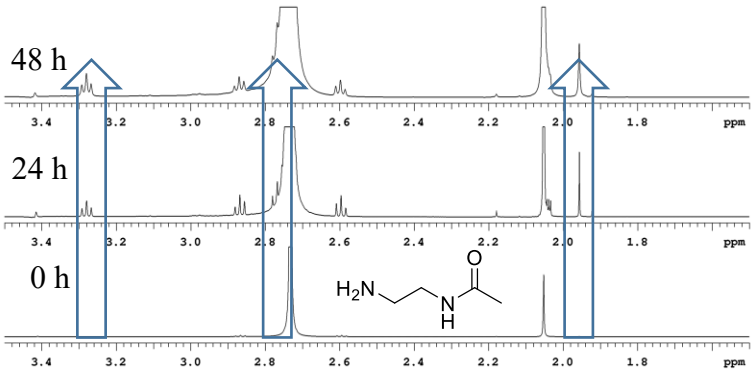


Figure 6.46 ^{1}H NMR spectra of entry 2- 1 eq $B(OH)_3$, appearance of N-acetyl ethylenediamine over time.

Entry (3) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of acetamide (20 mg, 0.34 mmol). To the reaction mixture, cysteamine (26 mg 0.34 mmol), boric acid (21 mg, 0.34 mmol), and ethylene diamine (0.23 ml, 3.39 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55° C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of

the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.47.

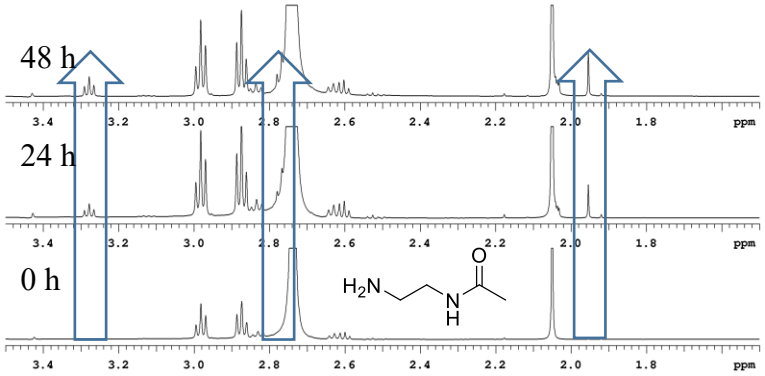


Figure 6.47 ^{1}H NMR spectra of entry 3- cysteamine additive, appearance of N-acetyl ethylenediamine over time.

Entry (4) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of N-acetylcysteamine (20 mg, 0.17 mmol). To the reaction mixture, ethylene diamine (100 mg, 1.68 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55^{o} C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.48.

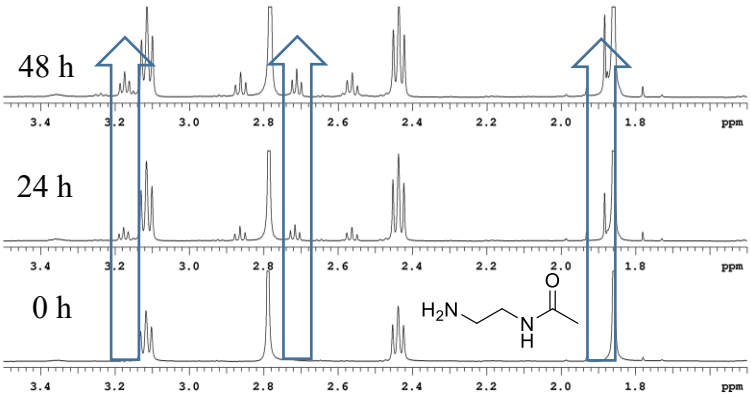


Figure 6.48 ^{1}H NMR spectra of entry 4- SNAC, no $B(OH)_3$, appearance of N-acetyl ethylenediamine over time.

Entry (5) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of N-acetylcysteamine (20 mg, 0.17 mmol). To the reaction mixture, boric acid (10 mg, 0.17 mmol), and ethylene diamine (100 mg, 1.68 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55° C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.49.

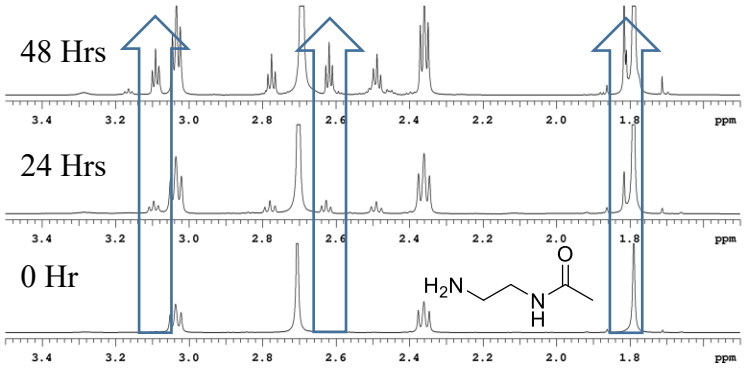


Figure 6.49 [1]H NMR spectra of entry 5- SNAC, 1 eq $B(OH)_3$, appearance of N-acetyl ethylenediamine over time.

Entry (6) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of N-acetylcysteamine (20 mg, 0.17 mmol). To the reaction mixture, boric acid (311 mg, 5.03 mmol), and ethylene diamine (100 mg, 1.68 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55° C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.50.

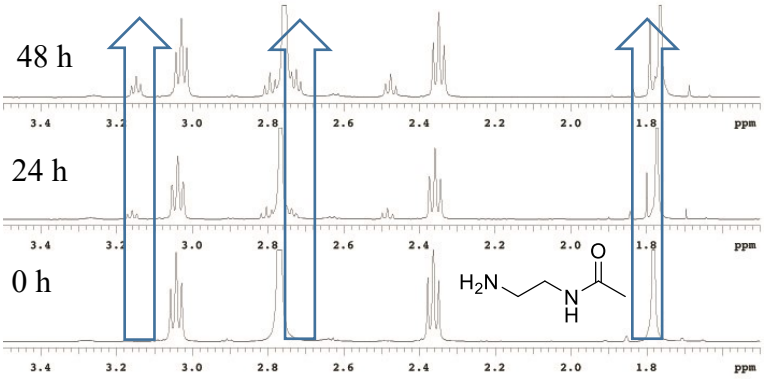


Figure 6.50 [1]H NMR spectra of entry 6- SNAC, 30 eq B(OH)3, appearance of N-acetyl ethylenediamine over time.

Entry (7) NMR Experiment. To a screw cap glass vial, 0.5 ml of D_2O was added and purged with argon, followed by the addition of N-acetylcysteamine (20 mg, 0.17 mmol). To the reaction mixture, boric acid (10 mg, 0.17 mmol), and ethylene diamine (403 mg, 6.71 mmol) was added, then the vial was capped under an argon environment and purged further with argon. The reaction mixture was transferred to a clean NMR tube and sealed under argon. The reaction mixture was monitored by proton NMR (zero time) then heated to 55° C for 48 hours, with measurements taken at 24 and 48 hours. Percentage conversion was calculated based on integral values of the methylene protons adjacent to the amide nitrogen of N-acetyl ethylenediamine, determined from ^{1}H and ^{1}H-^{1}H COSY NMR, Figure 6.51.

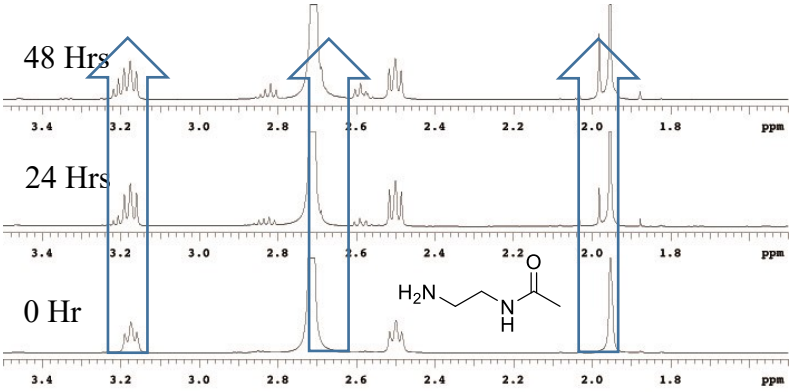


Figure 6.51 [1]H NMR spectra of entry 7- SNAC, 40 eq Amine, appearance of N-Acetyl ethylenediamine over time.

For ease of comparison the proton NMR for the 48-hour time point in each experiment was stacked. Figure 6.52 highlights the methylene protons adjacent to the amide nitrogen of the expected product.

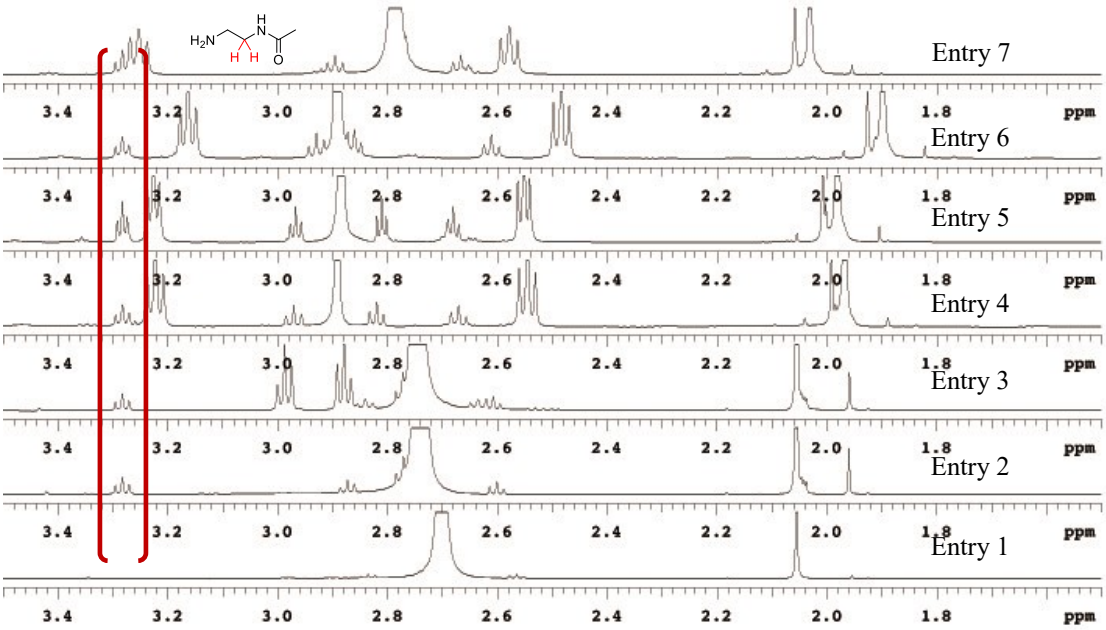


Figure 6.52 Stacked [1]H NMR spectra for the different model experiments done (Entry 1-7).

6.3.2 TCEP Desulfurization

This NMR study was conducted to confirm the identity of the phosphorous species involved in the radical desulfurization process.

1) **TCEP**

In a vial, SNAC (2.0 mg, 0.02 mmol) was added followed by 1.0 ml of D_2O. TCEP (23.5 mg, 0.0839 mmol) was then added followed by the radical initiator 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (0.8 mg, 0.003 mmol), thoroughly mixed, and transferred to an NMR tube for analysis. The ^{1}H NMR, ^{31}P, ^{1}H-^{1}H COSY spectra were obtained at zero time then heated at 55 ºC. NMR analysis was conducted at 1, 2 and 15 hours respectively. SNAC was 91% converted to the desulfurized product (ethyl acetamide) after two hours, and completely converted after 15 hours (Figure 6.53).

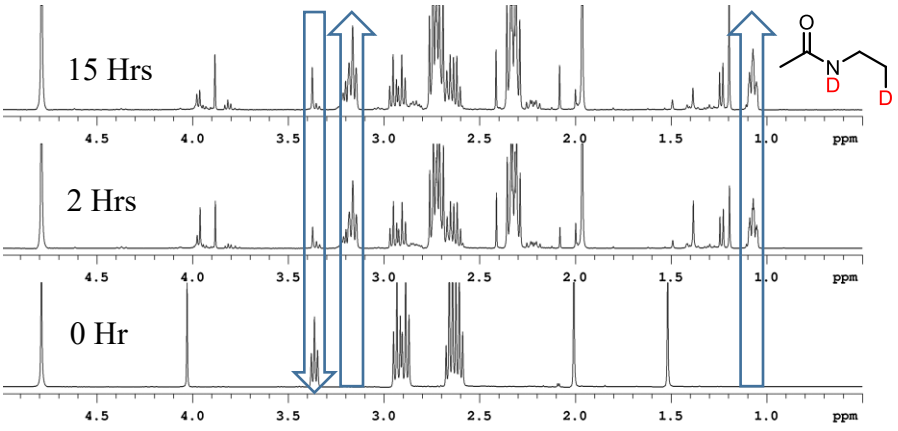


Figure 6.53 Stacked ^{1}H NMR spectra showing the disappearance of SNAC and the appearance of the desulfurized product when TCEP was used.

2) TCEPO

In a vial, SNAC (2.0 mg, 0.02 mmol) was added followed by 1.0 ml of D_2O. TCEP (24.9 mg, 0.0839 mmol) was then added followed by the radical initiator 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (0.8 mg, 0.003 mmol), thoroughly mixed, and transferred to an NMR tube for analysis. The ^{1}H NMR, ^{31}P, ^{1}H-^{1}H COSY spectra were obtained at zero time then heated at 55 ºC. NMR analysis was conducted at 1, 2 and 15 hours respectively. Even after 15 hours the desulfurized product was not observed (Figure 6.54).

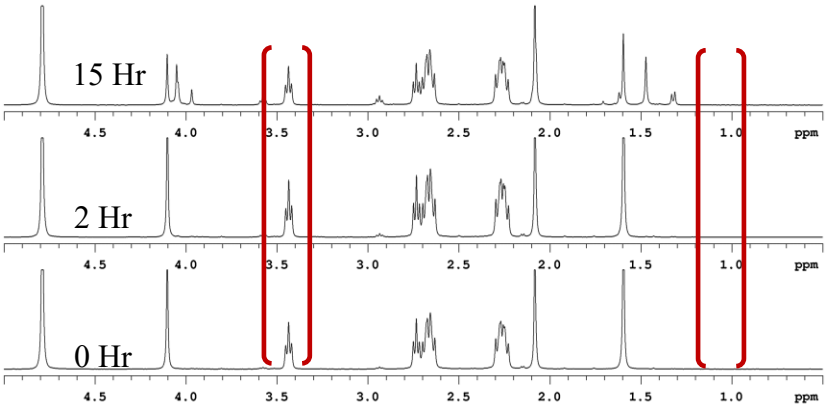


Figure 6.54 Stacked ^{1}H NMR spectra showing the presence of SNAC after 15 hours when TCEPO was used.

3) TCEPS

In a vial, SNAC (2.0 mg, 0.02 mmol) was added followed by 1.0 ml of D_2O. TCEPS (26.2 mg, 0.0839 mmol) was then added followed by the radical initiator 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (0.8 mg, 0.003 mmol), thoroughly mixed, and transferred to an NMR tube for analysis. The ^{1}H NMR, ^{31}P, ^{1}H-^{1}H COSY spectra were obtained at zero time then heated at 55 ºC. NMR analysis was conducted at 1, 2

and 15 hours respectively. Even after 15 hours the desulfurized product was not observed (Figure 6.55).

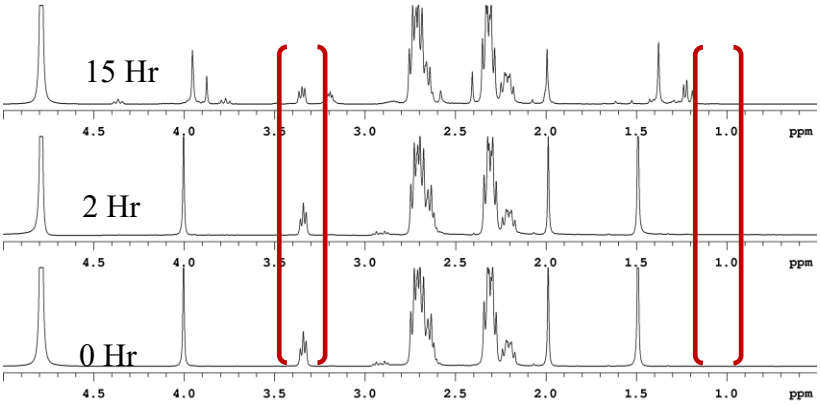


Figure 6.55 Stacked ^{1}H NMR spectra showing the presence of SNAC after 15 hours when TCEPS was used.

6.3.3 Catalyst Screening

Catalyst Screening Experiments: To a screw cap glass vial, 1.0 ml of deionized water was added and purged with argon, followed by the addition of Coenzyme A (100.0 mg, 0.127 mmol). To the reaction mixture ethylene diamine (85.0 µL, 1.3 mmol) was added, then the contents were stirred to ensure homogeneity. The contents were then divided into 10 equal portions (0.1 ml) and the respective catalyst (0.013 mmol, 1.00 Equiv) was added. The vials were then capped under an argon environment. The reaction mixtures were then heated at 55^{o} C for 48 hours and analysed by LCMS for the expected product, Amino CoA $[M+H]^{+}$ of 751.1. Given that the same concentration of starting materials was used for all the experiments and the only variable was the catalyst used, it is assumed that any differences observed after correction for background reaction in product formation is directly resulting from the catalyst. Relative Effectiveness was

calculated to correct for the background conversion to the product in the absence of any catalyst, following the equation;

Background Corrected Integration = Catalytic Conversion – Background Reaction

Relative Effectiveness % = [(Catalytic Conversion – Background Reaction)/444704]*100

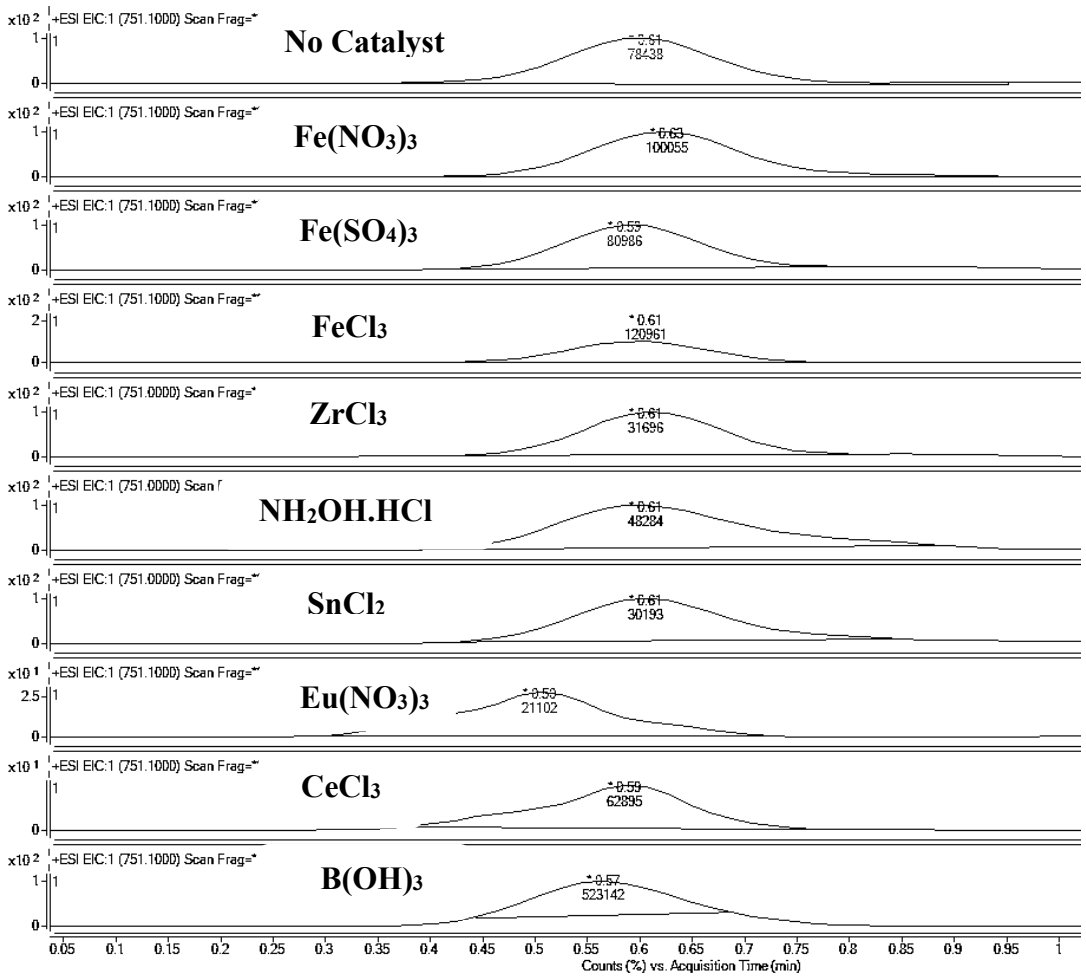


Figure 6.56 Extracted ion chromatographs for amino CoA $[M+H]^{+\cdot}$ Mz = 751.1 from the catalyst screening experiments.

6.4 DMP Oxidative Rearrangement Mechanistic Studies

6.4.1 RP–HPLC–HiRes MS Experiment Methods

RP–HPLC–Hi–ResMS was performed using an Agilent 1200 SL HPLC System with a Kinetex C8 reverse phase analytical column (2.1 x 50 mm), 100 Å pore size, 1.7 µm particle size (Phenomenex, Torrance, CA, USA), thermostated at 50 °C followed by mass spectrometric detection. The buffer gradient system was composed of 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. Methods for Separation of Analytes:

i. For the separation of analytes an aliquot was loaded onto the column at a flow rate of 0.50 $mLmin^{-1}$ and an initial buffer composition of 98% mobile phase A and 2% mobile phase B. Elution of the analytes was done by use of a linear gradient from 2% to 95% mobile phase B over a period of 5 min, held at 95% mobile phase B for 3 min to remove all analytes from the column and back to 2% mobile phase B within 1 minute.

ii. For the separation of analytes an aliquot was loaded onto the column at a flow rate of 0.50 $mLmin^{-1}$ and an initial buffer composition of 98% mobile phase A and 2% mobile phase B. Elution of the analytes was done by using a linear gradient from 2% to 50% mobile phase B over a period of 5 min, 50% to 95% mobile phase B for 3 min and back to 2% mobile phase B within 1 minute.

Mass spectra were acquired in positive mode of ionization using an Agilent 6220 Accurate–Mass TOF HPLC/MS system (Santa Clara, CA, USA) equipped with a dual sprayer electrospray ionization source with the second sprayer providing a reference mass solution. Mass correction was performed for every individual spectrum using

peaks at m/z 121.0509 and 922.0098 from the reference solution. Mass spectrometric conditions were drying gas 10 L/min at 325 °C, nebulizer 35 psi, mass range 100 – 1100 Da, acquisition rate of ~1.03 spectra/sec, fragmentor 150 V, skimmer 65 V, capillary 3200 V, instrument state 4 GHz High Resolution. Data analysis was performed using the Agilent MassHunter Qualitative Analysis software package version B.07.00 SP1.

6.4.2 RP-HPLC-HiRes MS Experiments Standards

1) **Intermediate Standards Preparation**: For each of the proposed intermediates synthetic standards were prepared by diluting 1.0 mg of the respective substrate into 10 mL of DCM, then further diluting 0.1 mL aliquots to 1.0 mL using dichloromethane (DCM). These standards were then analysed using Method (i) for separation of analytes and the retention time and mass were noted.

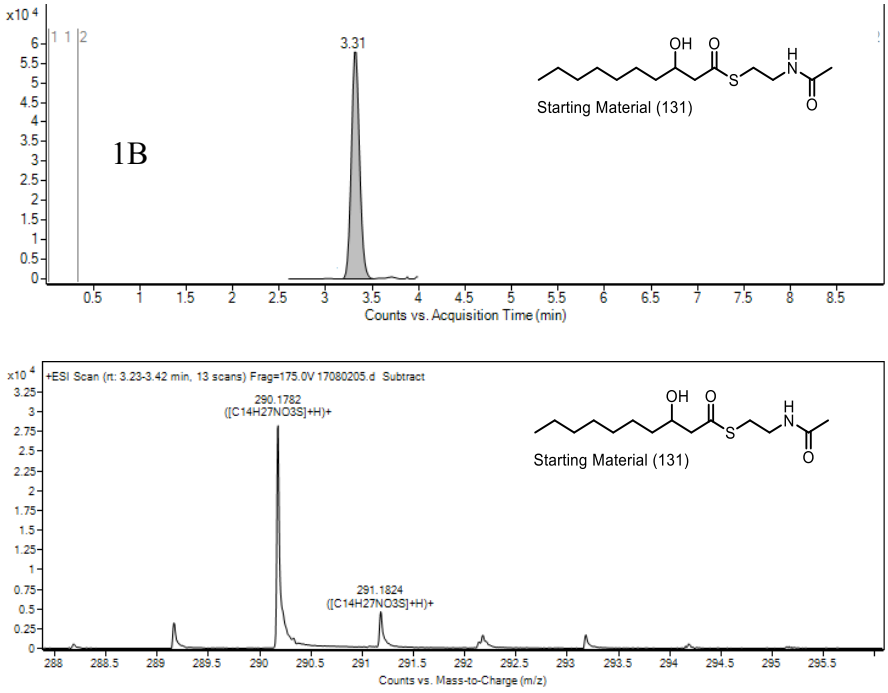


Figure 6.57 Retention time (1A) and high-resolution mass (1B) for the substrate ***(131).***

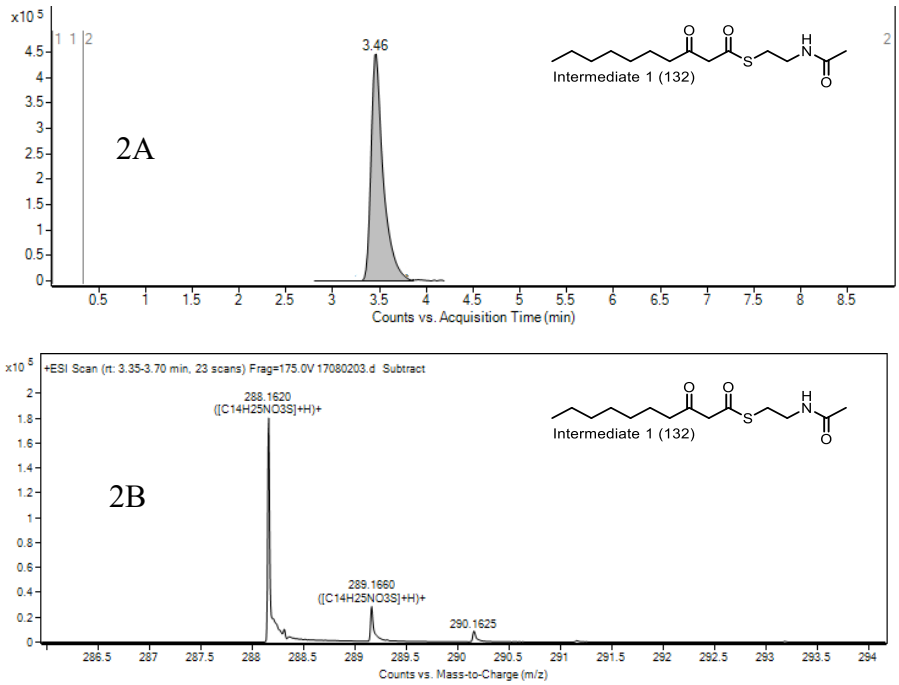


Figure 6.58 Retention time (2A) and high-resolution mass (2B) for the proposed intermediate 1 ***(132).***

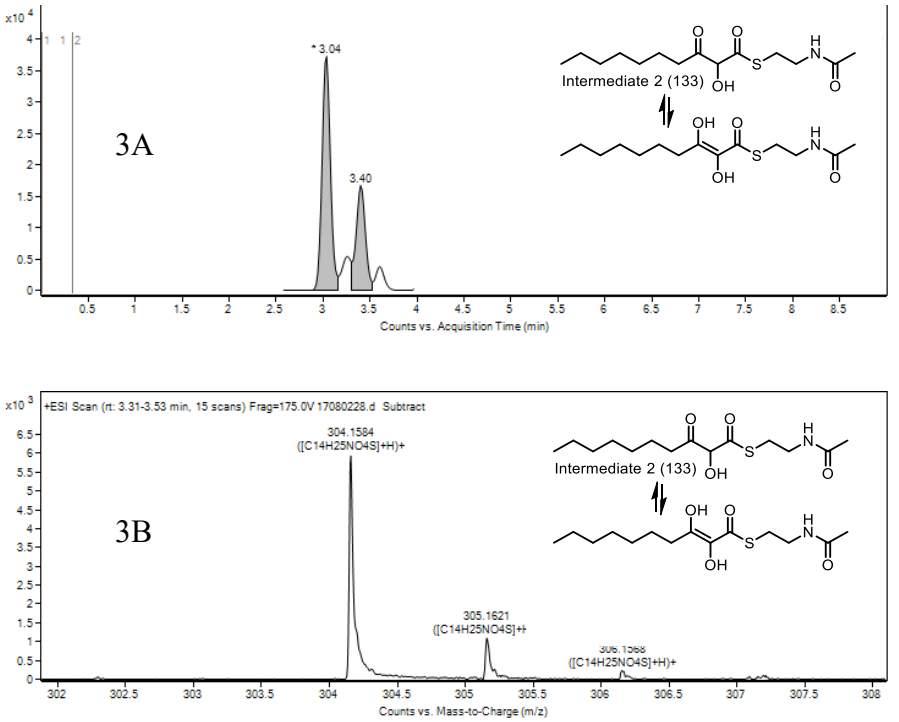


Figure 6.59 Retention time (3A) and high-resolution mass (3B) for the proposed intermediate 2 ***(133).***

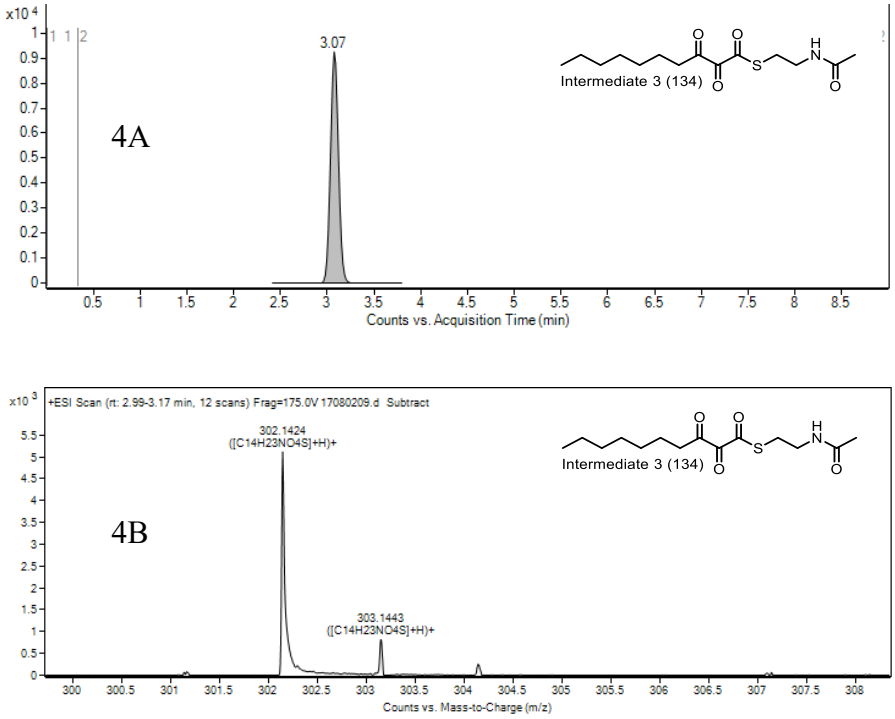


Figure 6.60 Retention time (4A) and high-resolution mass (4B) for the proposed intermediate 3 ***(134).***

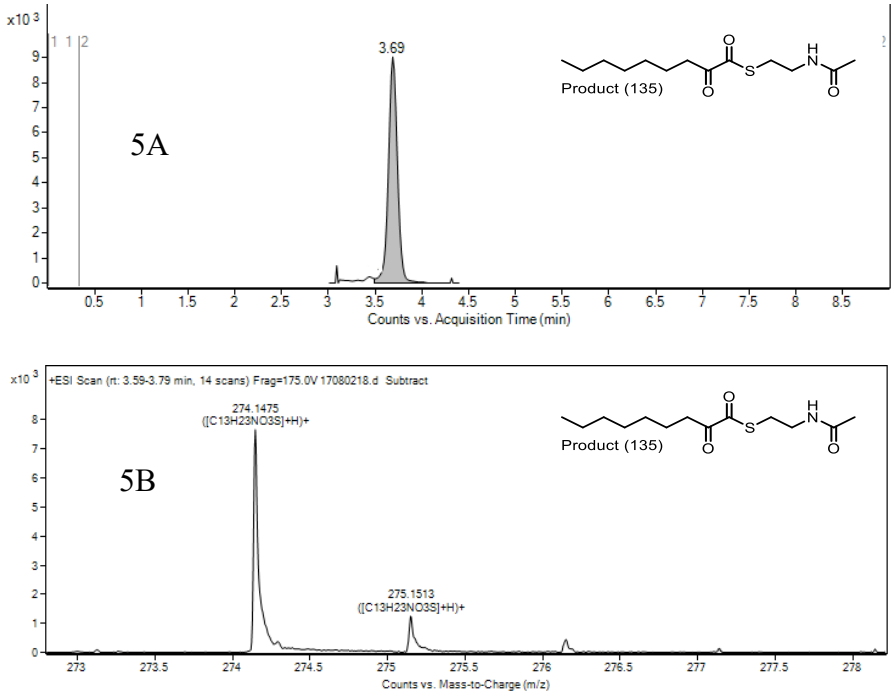


Figure 6.61 Retention time (5A) and high-resolution mass (5B) for the rearranged product ***(135).***

6.4.3 Effect of DMP Equivalents on the Relative Distribution of Intermediates

To four vials containing 10 mL of DCM each, compound **131** (6.00 mg, 0.021 mmol) was added to each vial, followed by the outlined equivalents of DMP (1.1, 2.2, 3.3 and 5.5). The DMP sourced from AK Scientific was noted to be 95% pure. As a result, a 10% excess of the reagent was used to account for this, leaving a 5% overall excess of the reagent. The reaction mixtures were stirred at room temperature for 1 h, before 0.1 mL aliquot was taken and further diluted to 6 mL using DCM, then analysed for the distribution of intermediates and product using Method (i) for the separation of analytes. The standards were used to confirm the identities of intermediates and products.

6.4.4 Rearrangement of Reaction Intermediates

To four vials containing 10 mL of DCM each, 0.003 mmol of the proposed intermediates were calculated and then added to each vial, followed by the outlined equivalents of DMP. The reaction mixtures were stirred at room temperature for 1 h, before 0.1 mL aliquot was taken and further diluted to 1 mL using DCM, then analysed for the product using Method (i) for the separation of analytes.

6.4.5 ^{13}C Labelled Substrate Study

To a vial containing 10 mL of DCM, labelled compound **(136)** (6.13 mg, 0.021 mmol) was added, followed by DMP (48.3 mg, 0.114 mmol, 5.5 eq). The reaction mixture was stirred at room temperature for 1 h before 0.1 mL aliquot was taken and further diluted to 6 mL using DCM, then analysed for the product using Method (i) for the separation of analytes. This was then repeated using labelled compound **(138)**.

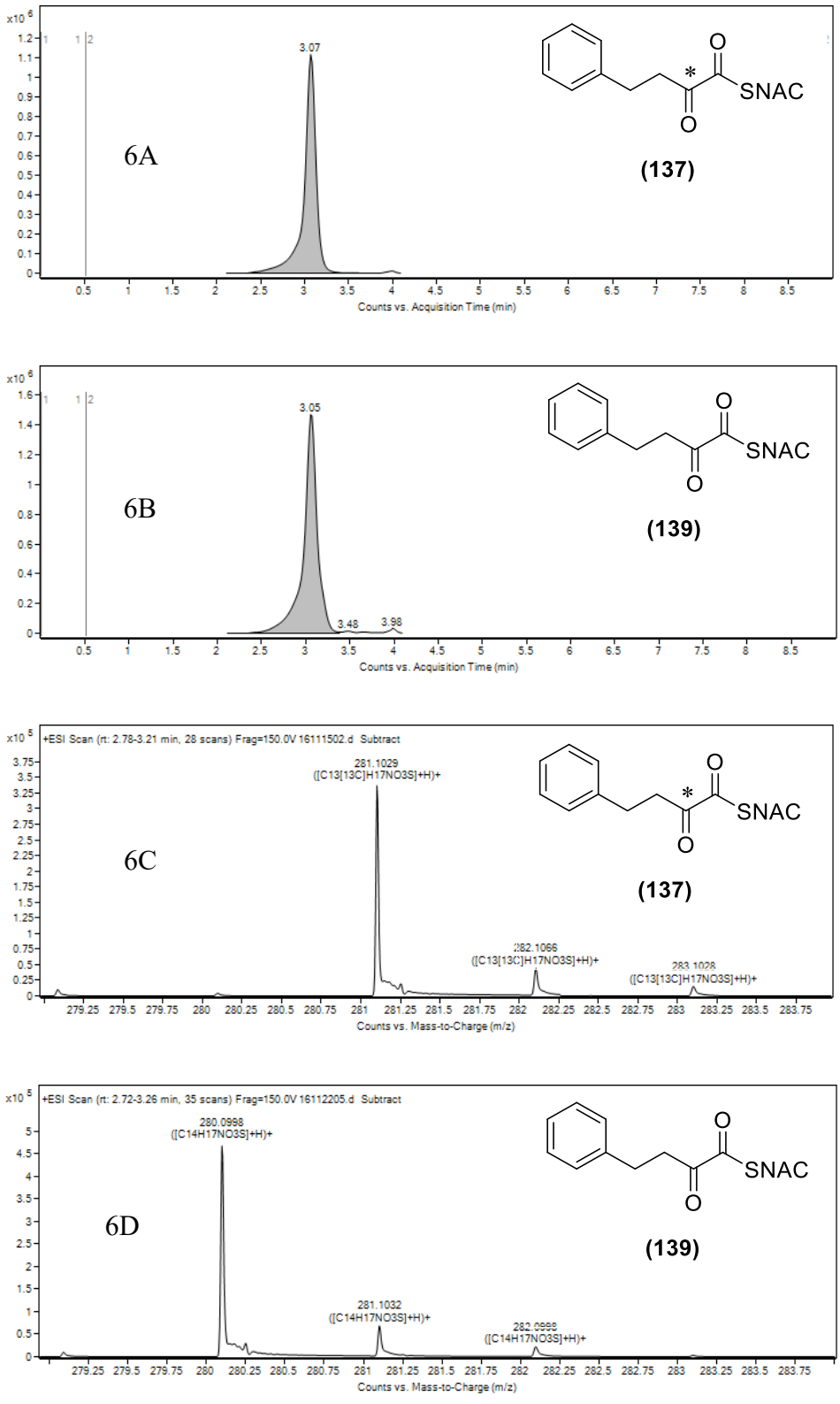


Figure 6.62 Retention time matching (6A, B) and the mass shift for the labelled and unlabelled rearrangement products (6C, D).

6.4.6 Crossover Experiment with the Labelled Substrates

To a vial containing 10 mL of DCM, 4.00 mg, 0.014 mmol each of labelled compound **(142)** and unlabelled **(144)** was added, followed by 63.0 mg, 0.150 mmol, 5.5 equiv of DMP. The reaction mixture was stirred at room temperature for 1 h, before 0.1 mL aliquot was taken and further diluted to 8 mL using DCM, then analysed for the product using Method (ii) for the separation of analytes.

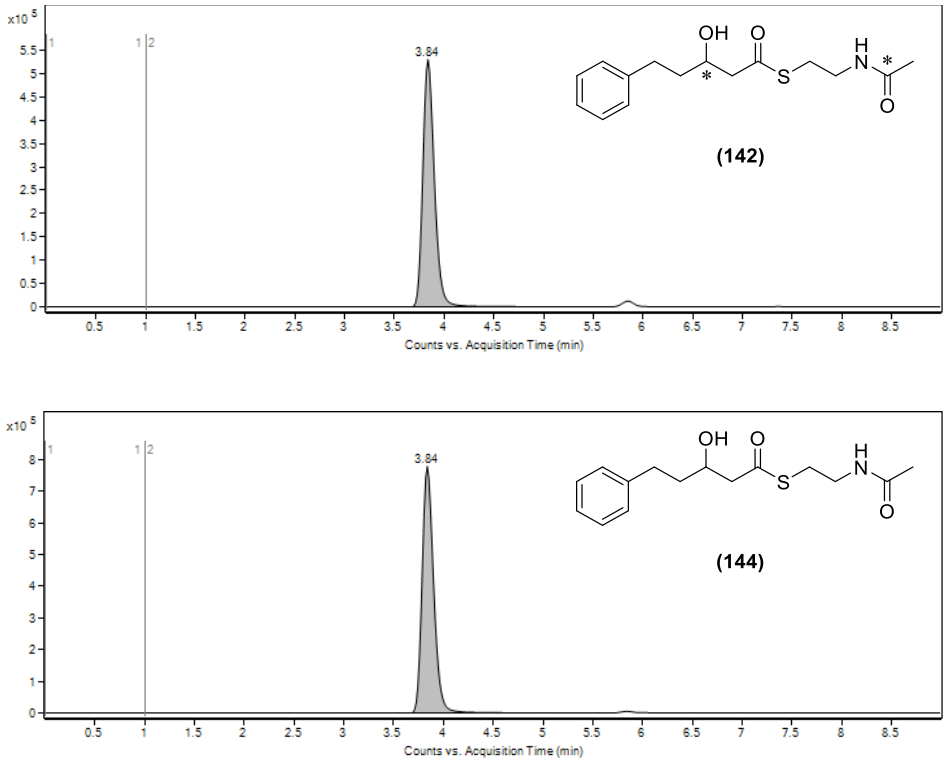


Figure 6.63 Retention times for the doubly labelled and unlabelled substrates.

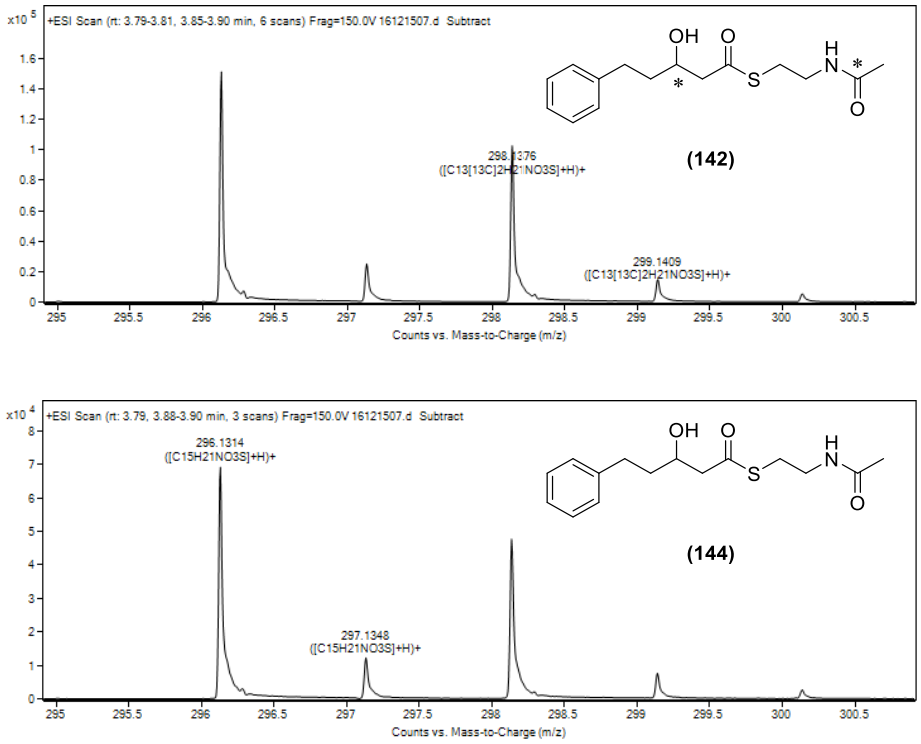


Figure 6.64 High resolution mass for the doubly labelled and unlabelled substrates.

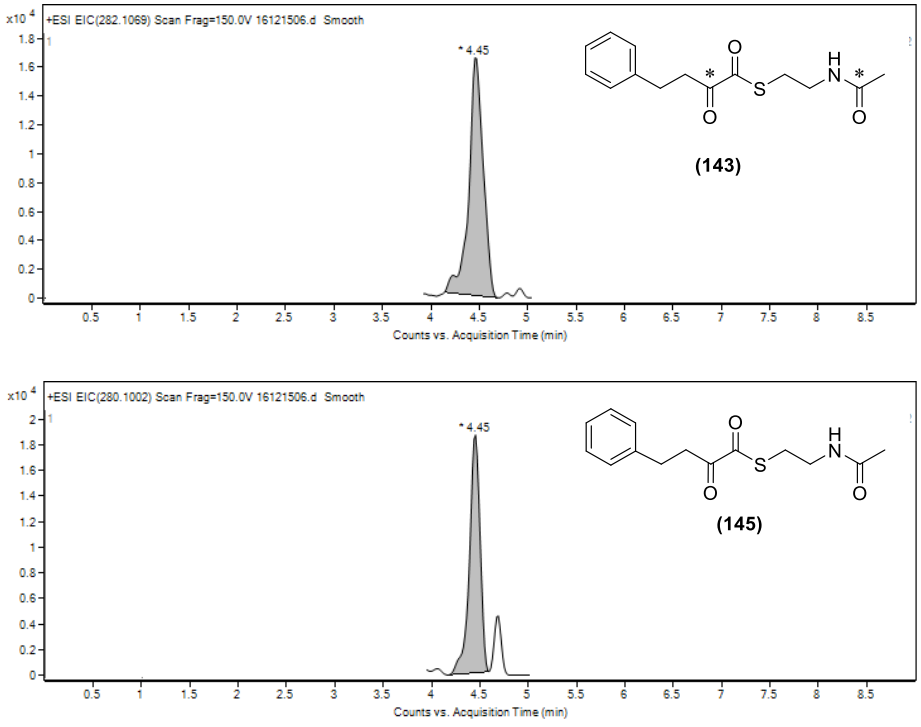


Figure 6.65 Retention times for the doubly labelled and unlabeled products.

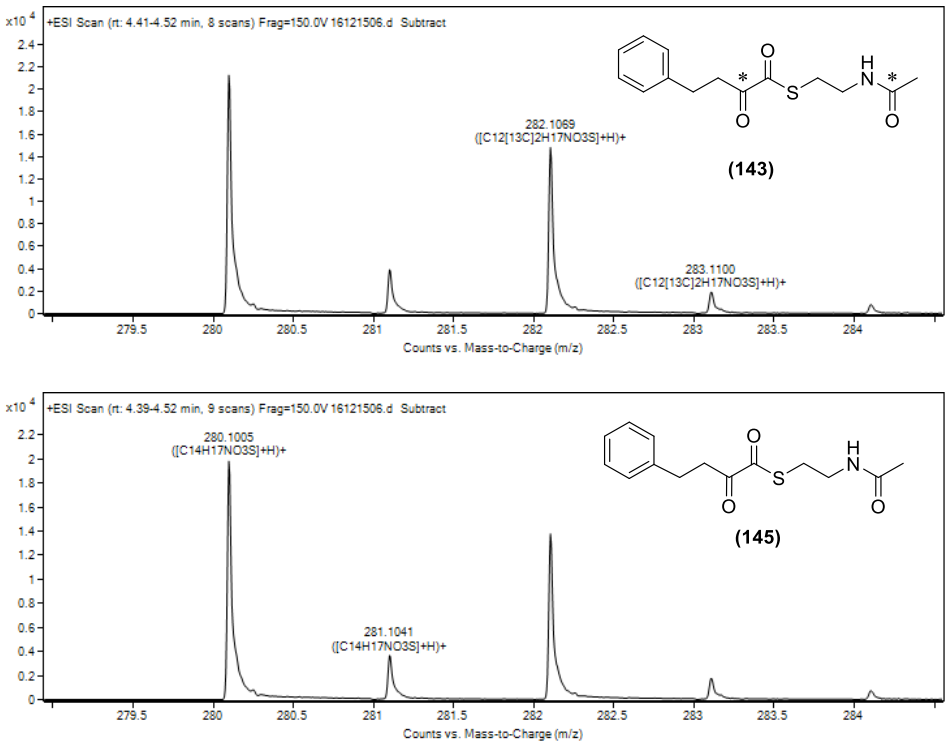


Figure 6.66 High resolution mass for the doubly labelled and unlabelled products.

6.4.7 Comparison of Thioester vs. Ester vs. Amide, by the Distribution of Intermediates and Product

To a vial containing 10 mL of DCM, compound **(131)** (6.0 mg, 0.2 mmol) was added, followed by 5.5 equivalents of DMP. The reaction mixture was stirred at room temperature for 1 h before 0.1 mL aliquot was taken and further diluted to 6 mL using DCM, then analysed for the distribution of intermediates and products using Method (i) for the separation of analytes. This experiment was repeated using compound **(154)** and **(155)** to compare the distribution of intermediates and product.

6.4.8 Analysis on Headspace Gases

To a vial containing 10 mL of DCM, labelled compound **(136)** (6.13 mg, 0.021 mmol) was added, followed by DMP (48.3 mg, 0.114 mmol, 5.5 Equiv). The reaction mixture was stirred at room temperature for 1 h before the headspace gas was analysed by gas phase IR and GC-MS. This was then repeated using labelled compound **(138)**.

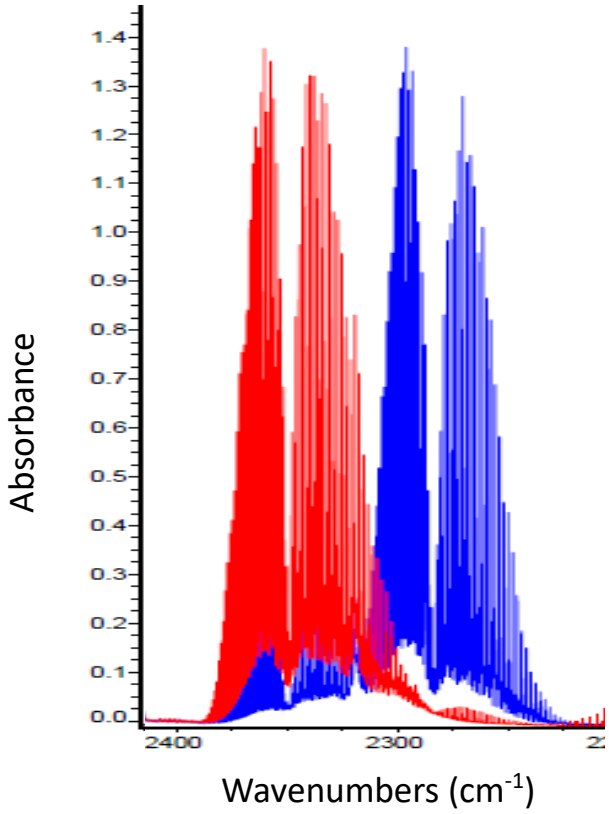


Figure 6.67 Showing the overlaid results of the gas phase IR for $^{12}CO_2$ (red trace) vs $^{13}CO_2$ (blue trace). Residual unlabelled CO_2 in the latter is background from air.

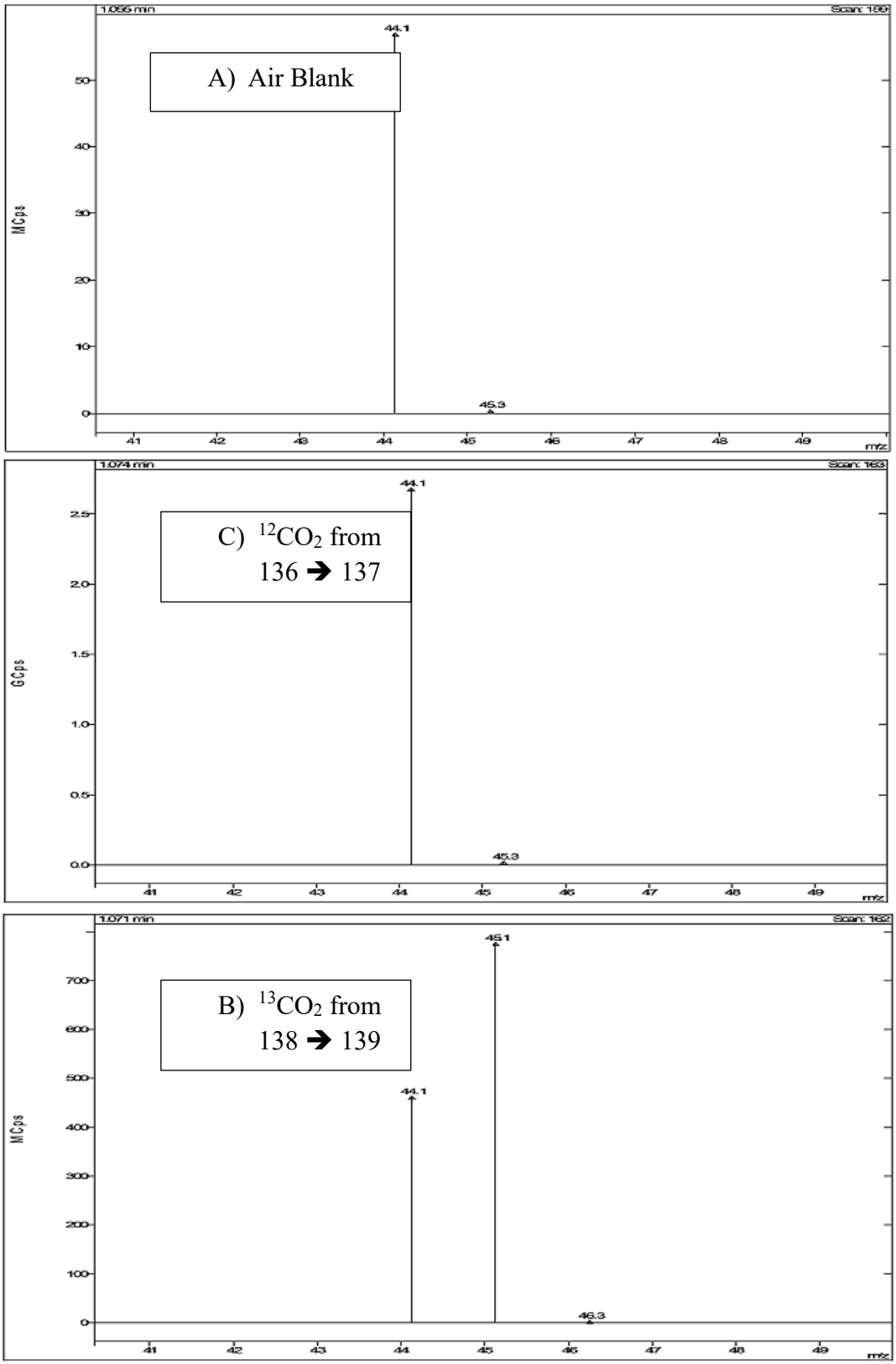
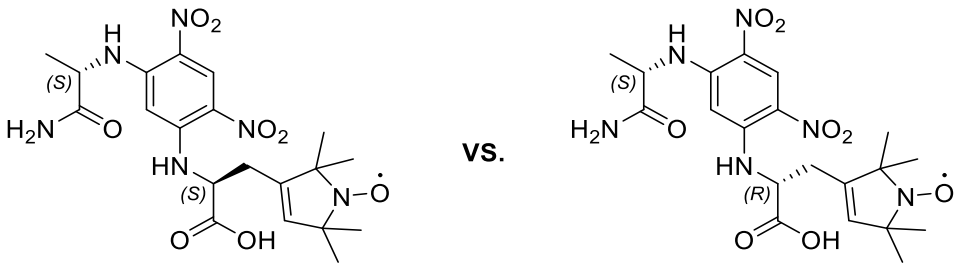


Figure 6.68 Showing the GCMS results from the analysis of the headspace gases. A) Air blank, B) $^{12}CO_2$ from Substrate 136➔137, C) $^{13}CO_2$ from substrate 138➔139.

6.5.1 Marfey's Assay



The Marfey's assay was used to determine the ratio between L and D forms of the amino acid synthesized. This was achieved by the following protocol. Spin labelled amino acid **(183)** (2.5 mg, 0.011 mmol) was added to a vial, followed by 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (15.0 mg, 0.055 mmol) dissolved in 2 mL of acetone. To this solution Et_3N (15.3 µL, 0.11 mmol) was added, and the reaction mixture was stirred for 30 min, at 40 ^oC, protected from light. The reaction mixture was then acidified at room temperature with 1 mL of 2 M HCl and dried under reduced pressure. The residue was dissolved in 10 mL acetone. Then analyzed by RP-HPLC-ESI-MS. RP–HPLC–HiRes-MS was performed using an Agilent 1200 SL HPLC System with a Kinetex C8 reverse phase analytical column (2.1 x 50 mm), 100 Å pore size, 1.7 µm particle size (Phenomenex, Torrance, CA, USA), thermostated at 40 °C followed by mass spectrometric detection. The buffer gradient system was composed of 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B at a flow rate of 0.5 mL/min; 0–0.5 min, 2% B; 0.5–5 min, 40% B, 5–7 min, 95% B; 7–9 min, 2% B. Mass spectra were acquired in positive mode of ionization using an Agilent 6220 Accurate–Mass TOF HPLC/MS system (Santa Clara, CA, USA) equipped with a dual sprayer electrospray ionization source with the second sprayer providing a reference mass solution. Mass correction was performed for every

individual spectrum using peaks at m/z 121.0509 and 922.0098 from the reference solution. Mass spectrometric conditions were drying gas 10 L/min at 325 °C, nebulizer 35 psi, mass range 100 – 1100 Da, acquisition rate of ~1.03 spectra/sec, fragmentor 150 V, skimmer 65 V, capillary 3200 V, instrument state 4 GHz High Resolution. Data analysis was performed using the Agilent MassHunter Qualitative Analysis software package version B.07.00 SP1.

6.5.2 D-Amino Acid Oxidase Assay

Spin labelled amino acid **(183)** (10.0 mg, 0.044 mmol) was dissolved into 2 mL of Tris HCl Buffer (200 mM, pH 8.3 at 25 °C, previously saturated with oxygen) followed by 1 mL of catalyse enzyme solution (21000 Unit/mg/mL, in deionized water). To this solution, was added 1 mL of D-amino acid oxidase enzyme solution (≥1.5 Unit/mg/mL, in 3.6 M ammonium sulfate solution, pH 6.5 at 25 °C). The assay mixture was stirred gently for 14 hrs, and the reaction was then quenched with 1 M HCl to precipitate the enzyme out of solution. The solids were filtered off and the solution was then purified on preparative HPLC. The crude sample was injected on a Phenomenex Luna 5 µ C18(2), 100 Å, AXIA, (21.2 mm ID x 250 mm); prep scale column, flow rate of 20 mL/min (A= H2O, 1% TFA and B = ACN, 1% TFA); 0–3 min, 10% B; 3–4.5 min, 20% B, 4.5–11 min, 35% B; 11–12 min, 95% B; 12–16 min, 95%; 16–17 min, 10%; 17–22 min, 10%. Compound **(183)** eluted at 7.9 min as a single peak to yield the product after removal of the solvent as an off-white powder. The product was then treated with 2 ml of 10% ammonium hydroxide and allowed to stand at room temperature for 4 h. The ammonium hydroxide was then removed *in vacuo* and then 5

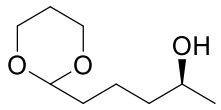
ml of water was added, frozen, then lyophilized to produce the final radical product as a brown-red solid, (8.3 mg, 92% recovery); IR (DCM, cast film) 3049, 2980, 2936, 1799, 1668, 1468, 1430 cm–1; CD (C, 57.23; H_2O), 22 ºC; $[\Theta]_{260}$ -77; $[\Theta]_{204}$ +2336; $[\Theta]_{195}$ -188; HRMS (ESI) calculated for $C_{11}H_{20}N_2O_3$ [M + H]+ 228.1468, found 228.1467.

The Marfey's assay was used to determine the ratio between L and D forms of the amino acid recovered. Spin labelled amino acid **(183)** (4.0 mg, 0.018 mmol) was added to a vial, followed by 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (24.0 mg, 0.088 mmol) dissolved in 2 mL of acetone. To this solution Et_3N (24.6 µL, 0.18 mmol) was added, and the reaction mixture was stirred for 30 min, at 40 ºC, protected from light. The reaction mixture was then acidified at room temperature with 1 mL of 2 M HCl and dried under reduced pressure. The residue was dissolved in 10 mL acetone. Then analyzed by RP-HPLC-ESI-MS. RP–HPLC–HiRes–MS was performed using an Agilent 1200 SL HPLC System with a Kinetex C8 reverse phase analytical column (2.1 x 50 mm), 100 Å pore size, 1.7 µm particle size (Phenomenex, Torrance, CA, USA), thermostated at 40 °C followed by mass spectrometric detection. The buffer gradient system was composed of 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B at a flow rate of 0.5 mL/min; 0–0.5 min, 2% B; 0.5–5 min, 40% B, 5–7 min, 95% B; 7–9 min, 2% B. Mass spectra were acquired in positive mode of ionization using an Agilent 6220 Accurate–Mass TOF HPLC/MS system (Santa Clara, CA, USA) equipped with a dual sprayer electrospray ionization source with the second sprayer providing a reference mass solution. Mass correction

was performed for every individual spectrum using peaks at m/z 121.0509 and 922.0098 from the reference solution. Mass spectrometric conditions were drying gas 10 L/min at 325 °C, nebulizer 35 psi, mass range 100 – 1100 Da, acquisition rate of ~1.03 spectra/sec, fragmentor 150 V, skimmer 65 V, capillary 3200 V, instrument state 4 GHz High Resolution. Data analysis was performed using the Agilent MassHunter Qualitative Analysis software package version B.07.00 SP1.

6.6 Synthesis and Characterization of Compounds

(*S*)–5–(1,3–Dioxan–2–yl)pentan–2–ol (40)

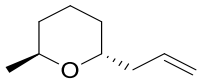


(40)

This known compound was synthesized following a literature preparation.[196] To a 100 mL flame dried round bottom flask the commercially available Grignard reagent **38** (2.2 g, 10 mmol) was added along with 20 mL dry THF and cooled to −30 °C using a Cryo Cooler. To this cold Grignard reagent solution, was added CuI (0.38 g, 2.0 mmol) stirred for 10 min, followed by the addition of (*S*)–propylene oxide **(39)** (1.2 g, 20 mmol). This mixture was stirred at −30 °C overnight, then quenched by addition of saturated NH_4Cl (10 mL) and stirred for 30 min. After stirring, the mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (40% EtOAc in Hex) affording the product (*S*)–5–(1,3–dioxan–2–yl)pentan–2–ol **(40)** as a colorless oil, R_f = 0.20 (60% EtOAc in Hex), (1.16 g, 67% yield). IR ($CHCl_3$, cast film): 3433, 2963, 2927, 1145 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.52 (t, *J* = 5.1 Hz, 1H), 4.09 (ddt, *J* = 10.4, 5.0, 1.4 Hz, 2H), 3.78–3.71 (m, 3H), 2.12–2.01 (m, 1H), 1.64–1.33 (m, 8H), 1.18 (d, *J* = 6.2 Hz, 2H); ^{13}C NMR (126

MHz, $CDCl_3$) δ 102.2, 67.9, 66.9, 39.1, 35.0, 25.9, 23.5, 20.2; $[\alpha]^{25}_D$ = + 6.40 (*c* = 1.0, $CHCl_3$); HRMS (EI) calcd for $C_9H_{17}O_3$ $[M - H]^-$ 173.1178, found 173.1176.

(2*R*,6*S*)–2–Allyl–6–methyltetrahydro–2H–pyran (41)

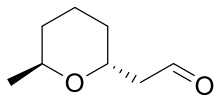


(41)

This known compound was synthesized following a literature preparation.[196] To a cooled (−40 ºC) solution of **40** (1.1 g, 6.3 mmol) and allyltrimethylsilane (3.0 mL, 19 mmol) in DCM (10 mL), TMSOTf (0.22 mL, 1.3 mmol) was added. The reaction mixture was then warmed to −20 °C and allowed to stir for 1 h. After quenching by addition of saturated $NaHCO_3$ solution, the resulting mixture was diluted with Et_2O (20 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified using column chromatography (10% EtOAc in Hex) affording the product (*2R,6S*)–2–allyl–6–methyltetrahydro–2H–pyran **(41)** as a colorless oil, R_f = 0.43 (10% EtOAc in Hex), (0.192 g, 22% yield). IR ($CHCl_3$, cast film): 2954, 2871, 1724 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.84–5.76 (m, 1H), 5.10–5.01 (m, 2H), 3.96–3.89 (m, 1H), 3.84–3.78 (m, 1H), 2.45–2.37 (m, 1H), 2.24–2.16 (m, 1H), 1.70–1.59 (m, 4H), 1.38–1.24 (m, 2H), 1.17 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.5, 116.4, 70.7, 67.0, 38.0, 31.6, 29.3, 19.7, 18.3; $[\alpha]^{25}_D$

= – 21.24 (*c* = 1.0, $CHCl_3$); HRMS *m/z* (relative intensity (%)): 99.0810 (100), 81.0685 (59), 55.0560 (39); calcd for $C_6H_{11}O$ $[M - \cdot C_3H_5]^+$ 99.0810, found 99.0809.

2–((2*R*,6*S*)–6–Methyltetrahydro–2*H*–pyran–2–yl)acetaldehyde (42)

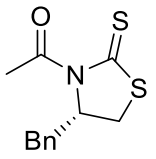


(42)

This known compound was synthesized following a modified procedure.[197] Compound **41** (0.18 g, 1.3 mmol) was dissolved in a mixture of 3:1 DCM:MeOH (20 mL) and the solution was then cooled to –78 ºC. Solid $NaHCO_3$ (1.0 g) was added, then ozone in a stream of oxygen was bubbled through until the solution turned blue (5 min). Ar was bubbled through for 2 min, then dimethyl sulphide (1.5 mL) was added and the mixture allowed to warm to room temperature and stirred for 16 h. The solvent and excess Me_2S were removed *in vacuo* and the residue was taken up with DCM (3 x 10 mL). The solution was then filtered and concentrated *in vacuo*. The residue was purified using column chromatography (50% Et_2O in EtOAc) affording the product 2–((2*R*,6*S*)–6–methyltetrahydro–2H–pyran–2–yl)acetaldehyde **(42)** as a colorless oil, R_f = 0.49 (50% Et_2O in Hex), (0.163 g, 89% yield). IR ($CHCl_3$, cast film): 2968, 2934, 1727 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.77 (dd, *J* = 3.2, 1.9 Hz, 1H), 4.41–4.35 (m, 1H), 3.94–3.91 (m, 1H), 2.76 (ddd, *J* = 16.0, 8.5, 3.0 Hz, 1H), 2.44 (ddd, *J* = 16.0, 5.3, 2.0 Hz,

1H), 1.77–1.58 (m, 4H), 1.40–1.28 (m, 2H), 1.18 (d, J = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 67.5, 66.4, 47.6, 31.1, 30.0, 19.4, 18.2; $[\alpha]^{25}_D = -22.54$ (c = 1.0, $CHCl_3$); HRMS (EI) calcd for $C_8H_{14}O_2$ $[M]^+$ 142.0994, found 142.0991.

(*S*)-1-(4-Benzyl-2-thioxothiazolidin-3-yl)ethan-1-one (43)

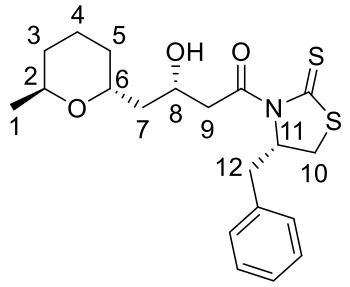


(43)

This compound was prepared following a modified protocol. In a flame dried round bottom flask (*S*)-4-benzylthiazolidine-2-thione (5.0 g, 26.1 mmol) was dissolved in 250 mL of dry THF at 0 °C and blanketed under Ar. While stirring the mixture, *n*–BuLi (2.5 M in Hex, 11.50 mL, 28.8 mmol) was slowly added and the reaction mixture was allowed to stir for 30 min. Acetyl chloride (2.95 mL, 31.4 mmol) was the slowly added and the reaction mixture was allowed to stir for another 60 min before quenching with saturated NH_4Cl (40 mL). The layers were separated, and the aqueous layer was extracted (3 x 40 mL), then the combined organic layer was washed with water (2 x 40 mL) then brine (2 x 40 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*S*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)ethan-1-one **(43)** as a yellow solid, R_f = 0.75 (50% EtOAc in Hex), (5.68 g, 86% yield). IR ($CHCl_3$, cast

film): 3086, 3066, 3025, 2989, 2954, 2862, 1701 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.33 (m, 2H), 7.30–7.26 (m, 3H), 5.38 (ddd, *J* = 10.8, 6.8, 3.9 Hz, 1H), 3.39 (dd, *J* = 11.4, 7.2 Hz, 1H), 3.22 (dd, *J* = 13.2, 3.7 Hz, 1H), 3.04 (dd, *J* = 13.2, 10.6 Hz, 1H), 2.89 (d, *J* = 11.6 Hz, 1H), 2.80 (s, Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 170.8, 136.6, 129.5, 129.0, 127.3, 68.3, 36.7, 31.9, 27.1; $[\alpha]^{25}_{D}$ = + 224 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{11}{}^{13}CH_{13}NNaOS_2$ $[M + Na]^+$ 274.0331, found 274.0331.

(*S*)–1–((*S*)–4–Benzyl–2–thioxothiazolidin–3–yl)–3–hydroxy–4–((*2R,6S*)–6–methyltetrahydro–2H–pyran–2–yl)butan–1–one (44)

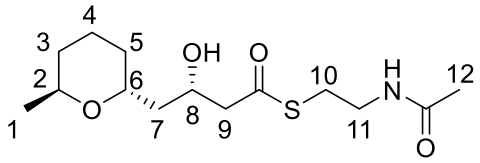


(44)

This new compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **43** (0.34 g, 1.4 mmol) in dry DCM (5 mL) was added $TiCl_4$ (1.0 M solution in DCM, 1.4 mL, 1.4 mmol) at 0 ºC under an Ar atmosphere. The reaction mixture was stirred for 5 min and then cooled to –78 °C. A solution of DIPEA (0.19 g, 1.5 mmol) was added. The reaction mixture was stirred at –78 °C for 2 h. A solution of

42 (0.25 g, 1.2 mmol) was added to the reaction mixture, which was then stirred for 30 min at –78 °C. The reaction mixture was quenched by the addition of saturated NH_4Cl (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (40% EtOAc in Hex) affording the product (*S*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxy–4–((*2R,6S*)–6–methyltetrahydro–2H–pyran–2–yl)butan–1–one (**44**) as a yellow oil, R_f = 0.37 (40% EtOAc in Hex), (37.0 mg, 76% yield). IR ($CHCl_3$, cast film): 3437, 2967, 2932, 1696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H), 7.31–7.27 (m, 3H), 5.40 (ddd, *J* = 10.8, 7.1, 3.7 Hz, 1H, H11), 4.42–4.40 (m, 1H, H8), 4.09–4.06 (m, 1H, H6), 4.02–3.94 (m, 1H, H2), 3.52 (dd, *J* = 17.4, 2.9 Hz, 1H, H9), 3.41 (ddd, *J* = 11.5, 7.2, 1.1 Hz, 1H, H10), 3.34 (dd, *J* = 17.5, 9.2 Hz, 1H, H9), 3.24 (dd, *J* = 15.4, 4.5 Hz, 1H, H12), 3.05 (dd, *J* = 13.2, 10.5 Hz, 1H, H12), 2.89 (d, *J* = 11.5 Hz, 1H, H10), 1.91–1.90 (m, 1H, H7), 1.74–1.52 (m, 5H, H3, 4, 5, 7), 1.44–1.29 (m, 2H, H3, 5), 1.22 (d, *J* = 6.6 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4, 172.8, 136.6, 129.5, 129.0, 127.3, 68.5, 67.8, 67.6, 65.6, 46.1, 39.6, 36.9, 32.1, 31.1, 30.7, 19.1, 18.4; $[\alpha]^{25}_D$ = + 74.43 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{20}H_{28}NO_3S_2$ $[M + H]^+$ 394.1505, found 394.1499.

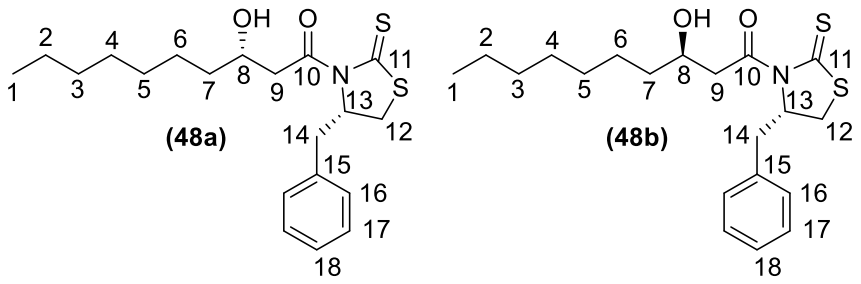
(*S*)–S–2–Acetamidoethyl 3–hydroxy–4–((*2R,6S*)–6–methyltetrahydro–2H–pyran–2–yl)butanethioate (46)



(46)

This compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **44** (0.37 g, 0.093 mmol) in 5 mL ACN was added K_2CO_3 (0.054 g, 0.32 mmol) and *HSNAC* (0.013 g, 0.11 mmol). The reaction mixture was stirred until the yellow color disappeared (5 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (100% EtOAc) affording the product (*S*)–S–2–acetamidoethyl 3–hydroxy–4–((*2R,6S*)–6–methyltetrahydro–2H–pyran–2–yl)butanethioate (**46**) as a clear oil, R_f = 0.12 (EtOAc), (12.0 mg, 44% yield). IR ($CHCl_3$, cast film): 3304, 3089, 2932, 1687, 1657 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.89 (s, 1H, NH), 4.31–4.29 (m, 1H, H8), 4.10–3.94 (m, 2H, H2, 6), 3.49–3.37 (m, 3H, H11, OH), 3.06–3.07 (m, 2H, H10), 2.84–2.69 (m, 2H, H9), 1.96 (s, 3H, H12), 1.84–1.83 (m, 1H, H7), 1.73–1.57 (m, 4H, H3, 5), 1.52–1.51 (m, 1H, H7), 1.42–1.30 (m, 2H, H4), 1.22 (d, J = 6.6 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.8, 170.4, 67.9, 67.5, 66.5, 51.2, 39.7, 39.4, 30.7, 30.7, 28.8, 23.2, 18.7, 18.3; $[\alpha]^{25}_D$ = – 6.82 (c = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{14}H_{26}NO_4S$ $[M + H]^+$ 304.1577, found 304.1575.

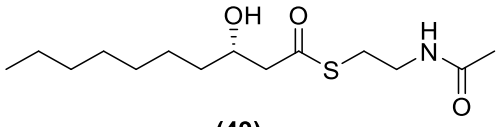
(*S*)–1–((*S*)–4–Benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydecan–1–one and (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydecan–1–one (48a/b)



These compounds were synthesized following a method adapted from the literature.[149] To a stirred solution of **43** (0.43 g, 1.7 mmol) in dry DCM (10 mL) was added $TiCl_4$ (1.0 M solution in DCM, 1.7 mL, 1.7 mmol) at –78 ºC under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.24 g, 1.9 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. Octanal **(47)** (0.20 g, 1.6 mmol) was added to the reaction mixture, which was then stirred for 30 min at –78 °C. The mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (15% EtOAc in Hex) affording two diastereomers; (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydecan–1–one **(48b) (anti)** and (*S*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydecan–1–one **(48a) (syn)** in a 1:2.2 ratio as yellow oils, R_f = 0.2 and 0.1 (15% EtOAc in Hex) respectively. **(48b) Anti** (0.117 g, 24% yield). IR ($CHCl_3$, cast film): 3444, 2926, 1692 cm^{-1}; ^1H NMR

(500 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 8.0, 6.9 Hz, 2H), 7.31–7.25 (m, 3H), 5.42 (ddd, *J* = 10.8, 7.1, 4.0 Hz, 1H, H13), 4.05 (td, *J* = 8.4, 7.5, 3.7 Hz, 1H, H8), 3.50 (dd, *J* = 17.4, 3.2 Hz, 1H, H9), 3.40 (ddd, *J* = 11.5, 7.2, 1.1 Hz, 1H, H12), 3.34 (dd, *J* = 17.5, 2.6 Hz, 1H, H9), 3.23 (dd, *J* = 13.2, 4.0 Hz, 1H, H14), 3.07 (s, 1H, OH), 3.05 (dd, *J* = 13.2, 10.5 Hz, 1H, H14), 2.91 (d, *J* = 11.5 Hz, 1H, H12), 1.65–1.23 (m, 12H, H2,3,4,5,6,7), 0.88 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.5, 173.9, 136.4, 129.5, 129.0, 127.3, 68.6, 68.3, 45.6, 36.9, 36.7, 32.1, 31.9, 29.6, 29.3, 25.5, 22.7, 14.2; $[\alpha]^{25}_D$ = + 137.15 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{20}H_{29}NNaO_2S_2$ $[M + Na]^+$ 402.1532, found 402.1535. **(48a) Syn** (0.263 g, 52% yield). IR ($CHCl_3$, cast film): 3458, 2927, 1691 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.32 (m, 2H), 7.31–7.26 (m, 3H), 5.40 (m, 1H, H13), 4.15 (dddd, *J* = 9.7, 7.3, 4.7, 2.4 Hz, 1H, H8), 3.65 (dd, *J* = 17.8, 2.4 Hz, 1H, H9), 3.41 (ddd, *J* = 11.6, 7.2, 1.0 Hz, 1H, H12), 3.23 (dd, *J* = 13.2, 4.0 Hz, 1H, H14), 3.13 (dd, *J* = 17.5, 9.5 Hz, 1H, H9), 3.10 (dd, *J* = 13.5, 10.5 Hz, 1H, H14), 2.90 (dd, *J* = 11.6, 0.7 Hz, 1H, H12), 2.75 (br s, 1H, OH), 1.66–1.21 (m, 12H, H2,3,4,5,6,7), 0.88 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4, 173.4, 136.5, 129.5, 129.0, 127.3, 68.4, 67.9, 46.0, 36.9, 36.4, 32.1, 31.9, 29.6, 29.3, 25.6, 22.7, 14.2; $[\alpha]^{25}_D$ = + 162.29 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{20}H_{29}NNaO_2S_2$ $[M + Na]^+$ 402.1532, found 402.1535.

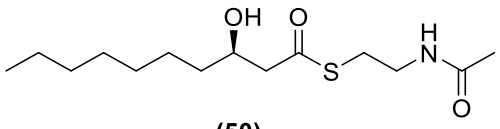
(*S*)–*S*–2–Acetamidoethyl 3–hydroxydecanethioate (49)



(49)

This known compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **48a** (0.26 g, 0.80 mmol) in 5 mL ACN was added K_2CO_3 (0.47 g, 2.82 mmol) and HSNAC **(45)** (0.12 g, 0.97 mmol). The reaction mixture was stirred until the yellow color disappeared (10 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*S*)–*S*–2–acetamidoethyl 3–hydroxydecanethioate (**49)** as a white solid, R_f = 0.24 (50% EtOAc in Hex), (147 mg, 63% yield), mp 64 – 66 ^oC. IR ($CHCl_3$, cast film): 3408, 3315, 3087, 2918, 2853, 1685, 1662 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.93 (s, 1H), 4.09–4.00 (m, 1H), 3.49–3.40 (m, 2H), 3.08–3.00 (m, 2H), 2.78–2.63 (m, 2H), 1.96 (s, 3H), 1.55–1.20 (m, 12H), 0.87 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.6, 170.5, 68.9, 51.1, 39.3, 36.8, 31.8, 29.5, 29.3, 28.9, 25.5, 23.2, 22.7, 14.1; $[\alpha]^{25}_D$ = + 18.40 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{14}H_{27}NNaO_3S$ $[M + Na]^+$ 312.1604, found 312.1606.

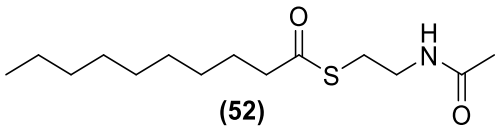
(*R*)–*S*–2–Acetamidoethyl 3–hydroxydecanethioate (50)



(50)

This known compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **48b** (0.100 g, 0.32 mmol) in 5 mL ACN was added K_2CO_3 (0.18 g, 1.10 mmol) and HSNAC (**45)** (0.05 g, 0.38 mmol). The reaction mixture was stirred until the yellow color disappeared (10 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*R*)–*S*–2–acetamidoethyl 3–hydroxydecanethioate (**50)** as a white solid, R_f = 0.24 (50% EtOAc in Hex), (73 mg, 81% yield), mp 64 – 66 ºC. IR ($CHCl_3$, cast film): 3407, 3313, 3087, 2918, 2853, 1685, 1662 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.03 (t, *J* = 5.8 Hz, 1H), 4.05–4.03 (m, 1H), 3.43 (m, 2H), 3.02 (m, 2H), 2.82 (s, 1H), 2.70 (m, 2H), 1.95 (s, 3H), 1.49–1.23 (m, 12H), 0.86 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.5, 170.6, , 68.8, 51.1, 39.3, 36.8, 31.8, 29.5, 29.2, 28.8, 25.5, 23.2, 22.6, 14.1; $[\alpha]^{25}_D$ = – 17.64 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{14}H_{27}NNaO_3S$ $[M + Na]^+$ 312.1604, found 312.1605.

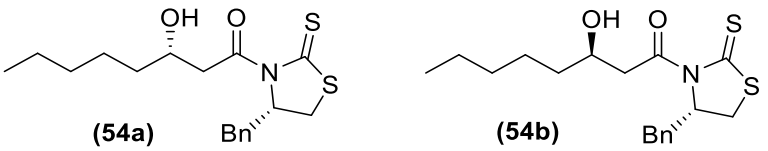
***S*–2–Acetamidoethyl decanethioate (52)**



(52)

This known compound was prepared following a modified literature protocol.[102] To a stirred solution of decanoic acid (**51**) (0.500 g, 2.90 mmol) in 25 mL dry DCM at 0 ºC, DCC (0.659 g, 3.19 mmol) was added, followed by DMAP (0.039 g, 0.32 mmol) and stirred for 10 min. HSNAC (**45**) (0.450 g, 3.77 mmol) was then added to the reaction mixture and allowed to stir for 1 h at 0°C, warmed up to room temperature and then allowed to continue stirring for 2 h. The reaction mixture was filtered to remove the precipitate, and the filtrate was washed twice with 1 M HCl and then with saturated $NaHCO_3$ solution and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified using column chromatography (30% EtOAc in DCM) affording the product *S*–2–acetamidoethyl decanethioate **(52)** as a white solid, R_f = 0.2 (50% EtOAc in Hex), (555 mg, 70% yield), mp 68–70 ºC. IR ($CHCl_3$, cast film): 3295, 3102, 2916, 2849, 1686, 1637 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.78 (s, 1H), 3.44 (q, *J* = 6.5, 2H), 3.03 (t, *J* = 6.5, Hz, 2H), 2.57 (t, *J* = 7.5 Hz, 2H), 1.96 (s, 3H), 1.66 (p, *J* = 7.5 Hz, 2H), 1.34–1.29 (m, 12H), 0.85 (t, *J* = 7.0, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.3, 170.2, 44.2, 39.9, 31.9, 29.4, 29.3, 29.3, 29.0, 28.5, 25.7, 23.3, 22.7, 14.2; HRMS (ESI) calcd for $C_{14}H_{27}NNaO_2S$ $[M + Na]^+$ 296.1655, found 296.1656.

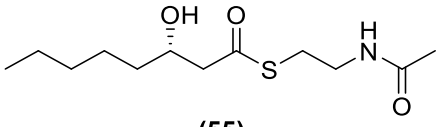
(*S*)–1–((*S*)–4–Benzyl–2–thioxothiazolidin–3–yl)–3–hydroxyoctan–1–one and (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxyoctan–1–one (54a/b)



These known compounds were synthesized following a modified literature protocol.[149] To a stirred solution of **43** (0.40 g, 1.6 mmol) in dry DCM (10 mL) was added $TiCl_4$ (1.0 M solution in DCM, 1.8 mL, 1.8 mmol) at –78 °C under Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.25 g, 1.9 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. Hexanal (**53**) (0.22 g, 2.2 mmol) was added to the reaction mixture, which was then stirred for 30 min at –78 °C. The reaction was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (10% EtOAc in Hex) affording two diastereomers; (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxyoctan–1–one (**54b) Anti** and (*S*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxyoctan–1–one (**54a) Syn** in a 1:2.9 ratio as yellow oils, R_f = 0.11 and 0.10 (10% EtOAc in Hex) respectively. **(54b) Anti** (0.109 g, 19% yield). IR ($CHCl_3$, cast film): 3440, 2928, 1692 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.39–7.33 (m, 2H), 7.30–7.24 (m, 3H), 5.42 (dddd, *J* = 10.3, 7.3, 4.0, 0.7 Hz, 1H), 4.06 (dddd, *J* = 9.1, 7.4, 4.6, 2.7 Hz, 1H), 3.46 (dd, *J* = 17.5, 10.5 Hz,

1H), 3.40 (ddd, *J* = 11.3, 8.0, 1.0 Hz, 1H), 3.31 (dd, *J* = 17.5, 2.5 Hz, 1H), 3.23 (dd, *J* = 13.3, 4.0 Hz, 1H), 3.08 (br s, 1H), 3.05 (dd, *J* = 13.5, 10.5 Hz, 1H), 2.91 (dd, *J* = 11.6, 0.7 Hz, 1H), 1.64–1.24 (m, 8H), 0.90 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.5, 173.9, 136.4, 129.5, 129.0, 127.3, 68.5, 68.3, 45.6, 36.9, 36.7, 32.1, 31.8, 25.2, 22.7, 14.1; $[\alpha]^{25}_D$ = + 126.21 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{25}NNaO_2S_2$ $[M + Na]^+$ 374.1219, found 374.1221. **(54a) Syn** (0.312 g, 56% yield); IR ($CHCl_3$, cast film): 3436, 2928, 1692 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.35 (m, 2H), 7.35–7.23 (m, 3H), 5.45–5.36 (m, 1H), 4.15 (dddd, *J* = 9.8, 7.3, 4.6, 2.4 Hz, 1H), 3.65 (dd, *J* = 17.7, 2.4 Hz, 1H), 3.41 (ddd, *J* = 11.5, 7.2, 1.0 Hz, 1H), 3.23 (dd, *J* = 13.2, 4.0 Hz, 1H), 3.13 (dd, *J* = 17.7, 9.4 Hz, 1H), 3.05 (dd, *J* = 13.2, 10.4 Hz, 1H), 2.90 (dd, *J* = 11.5, 0.7 Hz, 1H), 2.70 (s, 1H), 1.64–1.23 (m, 8H), 0.90 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4, 173.4, 136.5, 129.5, 129.0, 127.3, 68.4, 67.9, 46.0, 36.9, 36.4, 32.1, 31.8, 25.3, 22.6, 14.1; $[\alpha]^{25}_D$ = + 165.31 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{18}H_{25}NNaO_2S_2$ $[M + Na]^+$ 374.1219, found 374.1221.

(*S*)–*S*–2–Acetamidoethyl 3–hydroxyoctanethioate (55)

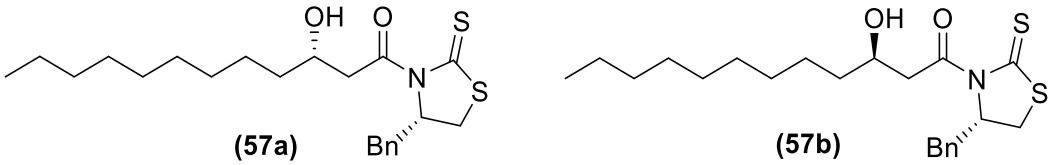


(55)

This known compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **54a** (0.20 g, 0.57 mmol) in 5 mL ACN was added

K_2CO_3 (0.33 g, 1.99 mmol) and HSNAC (**45)** (0.08 g, 0.68 mmol). The reaction mixture was stirred until the yellow color disappeared (10 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (70% EtOAc in Hex) affording the product (*S*)–*S*–2–acetamidoethyl 3–hydroxyoctanethioate (**55)** as an opaque oil, R_f = 0.08 (70% EtOAc in Hex), (110 mg, 74% yield). IR ($CHCl_3$, cast film): 3297, 3089, 2930, 2858, 1689, 1658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.89 (s, 1H), 4.05 (tq, *J* = 8.0, 4.1 Hz, 1H), 3.45 (m, 2H), 3.04 (m, 2H), 2.80–2.69 (m, 2H), 1.96 (s, 3H), 1.56–1.22 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.6, 170.5, 68.9, 51.1, 39.3, 36.8, 31.7, 28.9, 25.1, 23.3, 22.6, 14.0; $[\alpha]^{25}_D$ = + 12.16 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{12}H_{23}NNaO_3S$ $[M + Na]^+$ 284.1291, found 284.1292.

(*S*)–1–((*S*)–4–Benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydodecan–1–one and (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydodecan–1–one (57a/b)



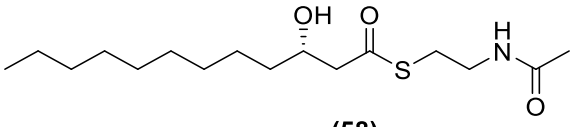
(57a) **(57b)**

These known compounds were synthesized following a method adapted from the literature.[149] To a stirred solution of **43** (0.40 g, 1.6 mmol) in dry DCM (10 mL) was added $TiCl_4$ (1.0 M solution in DCM, 1.8 mL, 1.8 mmol) at –78 ºC under Ar

atmosphere and stirred for 10 min. A solution of DIPEA (0.25 g, 1.9 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. Decanal (**56**) (0.35 g, 2.2 mmol) was added to the reaction mixture, which was then stirred for 30 min at –78 °C. The reaction was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (10% EtOAc in Hex) affording two diastereomers**:** (*R*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydodecan–1–one **(57b) Anti** and (*S*)–1–((*S*)–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxydodecan–1–one **(57a) Syn** in a 1:1.6 ratio as yellow oils, R_f = 0.11 and 0.05 (10% EtOAc in Hex) respectively. (**57b**) **Anti** (0.203 g, 31% yield). IR ($CHCl_3$, cast film): 3439, 2925, 1689 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.39–7.32 (m, 2H), 7.30–7.24 (m, 3H), 5.42 (dddd, *J* = 10.4, 7.3, 4.1, 0.7 Hz, 1H), 4.05 (tdd, *J* = 9.1, 4.5, 2.8 Hz, 1H), 3.46 (dd, *J* = 17.8, 9.5 Hz, 1H), 3.40 (ddd, *J* = 11.5, 7.0, 1.0 Hz, 1H), 3.30 (dd, *J* = 17.0, 2.5 Hz, 1H), 3.23 (dd, *J* = 13.2, 4.0 Hz, 1H), 3.08 (s, 1H), 3.05 (dd, *J* = 13.0, 10.5 Hz, 1H), 2.91 (dd, *J* = 11.6, 0.7 Hz, 1H), 1.64–1.22 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.5, 173.9, 136.4, 129.5, 129.0, 127.3, 68.6, 68.3, 45.6, 36.9, 36.7, 32.1, 31.9, 29.6, 29.6, 29.6, 29.4, 25.5, 22.7, 14.2; $[\alpha]^{25}_D$ = + 102.48 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{33}NNaO_2S_2$ $[M + Na]^+$ 430.1845, found 430.1852. **(57a) Syn** (0.315 g, 49% yield). IR ($CHCl_3$, cast film): 3459, 2925, 1693 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.32 (m, 2H), 7.32–7.24 (m, 3H), 5.44–5.36 (m, 1H), 4.19–4.10 (m, 1H), 3.65 (dd, *J* = 17.7, 2.4 Hz, 1H), 3.41 (ddd, *J* = 11.5,

7.2, 1.0 Hz, 1H), 3.23 (dd, *J* = 13.2, 4.0 Hz, 1H), 3.13 (dd, *J* = 17.8, 9.5 Hz, 1H), 3.05 (dd, *J* = 13.2, 10.4 Hz, 1H), 2.90 (dd, *J* = 11.6, 0.6 Hz, 1H), 2.76 (br s, 1H), 1.66–1.22 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4, 173.4, 136.4, 129.5, 129.0, 127.3, 68.4, 67.9, 45.9, 36.9, 36.4, 32.1, 31.9, 29.6, 29.6, 29.6, 29.3, 25.6, 22.7, 14.1; $[\alpha]^{25}_D$ = + 147.89 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{22}H_{33}NNaO_2S_2$ $[M + Na]^+$ 430.1845, found 430.1854.

(*S*)–*S*–2–Acetamidoethyl 3–hydroxydodecanethioate (58)

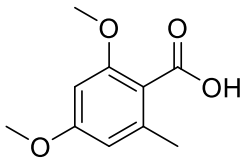


(58)

This known compound was synthesized following a method adapted from the literature.[149] To a stirred solution of **57a** (0.29 g, 0.72 mmol) in 5 mL ACN was added K_2CO_3 (0.42 g, 2.53 mmol) and HSNAC (**45)** (0.87 g, 0.68 mmol). The reaction mixture was stirred until the yellow color disappeared (10 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (70% EtOAc in Hex) affording the product (*S*)–*S*–2–acetamidoethyl 3–hydroxydodecanethioate (**58)** as a white solid, R_f = 0.13 (70% EtOAc in Hex), (189 mg, 82% yield), mp 66 – 68 ºC. IR ($CHCl_3$, cast film): 3398, 3311, 2918, 2849, 1682, 1666 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (s, 1H), 4.09–4.00 (m, 1H), 3.44 (m, 2H), 3.03 (m, 2H), 2.78–2.63 (m, 2H), 1.96 (s, 3H), 1.55–1.24 (m, 16H), 0.87 (t, *J* =

6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.5, 170.5, 68.9, 51.1, 39.3, 36.8, 31.9, 29.6, 29.5, 29.5, 29.3, 28.9, 25.5, 23.2, 22.7, 14.1; $[\alpha]^{25}_D$ = + 14.86 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{16}H_{31}NNaO_3S$ $[M + Na]^+$ 340.1917, found 340.1920.

2,4–Dimethoxy–6–methylbenzoic acid (60)

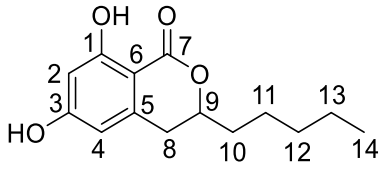


(60)

This known compound was prepared following a literature preparation.[198,199] To a solution of orsellinic acid **59** (2.5 g, 14.88 mmol) in acetone (80 mL), K_2CO_3 (12.67 g, 89.29 mmol) was added. MeI (5.6 mL, 89.29 mmol) was added and the reaction mixture was refluxed overnight. After the reaction was complete, the solvent and excess MeI was removed *in vacuo* and 100 mL water was added. The resulting solution was extracted with Et_2O (4 × 40 mL). The combined organic layers were washed with $NaHCO_3$ (40 mL) and brine (40 mL), then dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product as a pale-yellow oil. This crude sample was analyzed by LCMS and was found to contain the desired product. No further purification was done; this crude product was used for the next step. The crude product from the previous reaction was transferred to a 250 mL round bottom flask, containing 80 mL of 10%

KOH in EtOH and refluxed overnight. The mixture was allowed to cool then concentrated *in vacuo*. The resultant residue was acidified to pH 1 with 6 M HCl and extracted with EtOAc, washed with brine (40 mL), then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified using column chromatography (1% AcOH and 50% EtOAc in Hex) affording the product 2,4–dimethoxy–6–methylbenzoic acid **(60)** as a yellow–brown solid, R_f = 0.23 (50% EtOAc in Hex), (2.72 g, 93% yield), mp 142–144 °C. IR ($CHCl_3$, cast film): 2935, 2850, 2650, 1680, 1585 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.43 (d, J = 2.3 Hz, 1H), 6.38 (d, J = 2.3 Hz, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4, 162.3, 159.6, 144.1, 112.3, 109.1, 96.6, 56.5, 55.5, 22.6; HRMS (ESI) calcd for $C_{10}H_{11}O_4$ $[M - H]^-$ 196.0736, found 196.0736.

6,8–Dihydroxy–3–pentylisochroman–1–one (61)



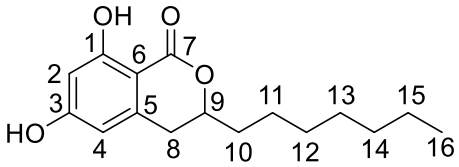
(61)

This new compound was prepared following a modified literature protocol.[200] In a flame dried 250 mL round bottom flask, 10 mL of dry THF was added and cooled to 0 ^oC over an ice bath and kept under Ar. To this cooled solvent, (*i*–Pr)$_2$NH (0.07 mL, 7.14 mmol) was added and stirred for 5 min, then 2.5 M *n*–BuLi (2.85 mL, 7.14 mmol)

was added dropwise. After 10 min of stirring, the reaction mixture was further cooled to –78 ºC. After 10 min, a solution of **60** (0.35 g, 1.78 mmol) dissolved in 5 mL dry THF, was slowly added and stirred for 30 min. Hexanal (0.88 mL, 7.14 mmol) was then added and stirred for 90 min maintaining –78 ºC. The reaction mixture was then allowed to warm to room temperature (15 min), and then quenched with 20 mL of water. The reaction mixture was acidified with 1 M HCl to pH 1 and then extracted 4 times with EtOAc. The combined organic phases were dried in anhydrous Na_2SO_4, filtered and then concentrated *in vacuo* to provide the crude protected product. This crude sample was analyzed by LCMS and was found to contain the desired methoxy–protected product. No further purification was done; this crude product was used for the next step. The crude product from the previous step was dissolved in 10 mL DCM, cooled to –78 ºC and BBr_3 (1 M, 11.3 mL, 11.30 mmol) was added dropwise. The reaction mixture was stirred for 1 h and then heated to reflux overnight. The reaction was allowed to cool, and 25 mL of water was added, and the layers were separated, and then extracted with 2 x 20 mL DCM. The combined organic layers were washed twice with 20 mL saturated $NaHCO_3$ then washed with 20 mL brine and dried over $MgSO_4$. The DCM was removed *in vacuo*, and the residue was purified using column chromatography (20% EtOAc in Hex) affording the product 6,8–dihydroxy–3–pentylisochroman–1–one **(61)** as a white solid, R_f = 0.16 (20% EtOAc in Hex), (223 mg, 47% yield), mp 86–88 ºC. IR ($CHCl_3$, cast film): 3246, 2951, 2925, 2851, 1626, 1584 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.32 (d, *J* = 2.2 Hz, 1H, H2), 6.21 (d, *J* = 2.3 Hz, 1H, H4), 5.97 (br s, 1H, OH), 4.57–4.47 (m, 1H, H9), 2.92– 2.81 (m, 2H, H8), 1.86 (dddd, *J* = 13.8, 10.3, 7.4, 5.1 Hz, 1H, H10), 1.70 (ddt, *J* = 13.9, 10.8, 5.5 Hz, 1H, H10),

1.61–1.53 (m, 1H, H11), 1.46 (m, 1H, H11), 1.33 (m, 4H, H12,13), 0.91 (t, J = 7.1 Hz, 3H, H14); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 164.4, 162.5, 141.9, 106.6, 102.1, 102.0, 79.3, 34.7, 33.2, 31.6, 24.6, 22.5, 14.0; HRMS (ESI) calcd for $C_{14}H_{17}O_4$ [M – H]$^-$ 249.1132, found 249.1131.

3–Heptyl–6,8–dihydroxyisochroman–1–one (62)

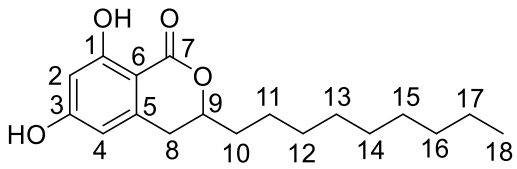


(62)

This new compound was prepared following a modified literature protocol.[200] In a flame dried 250 mL round bottom flask, 10 mL of dry THF was added and cooled to 0 ºC over an ice bath and kept under Ar. To this cooled solvent, (*i*–Pr)$_2$NH (0.14 mL, 10.19 mmol) was added and stirred for 5 min, then 2.5 M *n*–BuLi (4.08 mL, 10.19 mmol) was added dropwise. After 10 min of stirring, the reaction mixture was further cooled to –78 ºC. After 10 min, a solution of **60** (0.50 g, 2.55 mmol) dissolved in 5 mL dry THF, was slowly added and stirred for 30 min. Octanal (1.59 mL, 10.19 mmol) was then added and stirred for 90 min maintaining –78 ºC. The reaction mixture was then allowed to warm to room temperature (15 min) and then quenched with 20 mL of water. The reaction mixture was acidified with 1 M HCl to pH 1 and then extracted 4 times with EtOAc. The combined organic phases were dried in anhydrous Na_2SO_4, filtered

and then concentrated *in vacuo* to provide the crude protected product. This crude sample was analyzed by LCMS and was found to contain the desired methoxy–protected product. No further purification was done; this crude product was used for the next step. The crude product from the previous step was dissolved in 10 mL DCM and cooled to –78 ºC then BBr_3 (1 M, 15.3 mL, 15.30 mmol) was added dropwise. The reaction mixture was stirred for 1 h and then heated to reflux overnight. The reaction mixture was allowed to cool, and 25 mL of water was added. The layers were separated, and then extracted with 2 x 20 mL DCM. The combined organic layer was washed twice with 20 mL saturated $NaHCO_3$ then washed with 20 mL brine and dried over $MgSO_4$. The DCM was removed *in vacuo*, and the residue was purified using column chromatography (15% EtOAc in Hex) affording the product 3–heptyl–6,8–dihydroxyisochroman–1–one (**62**) as a white solid, R_f = 0.13 (15% EtOAc in Hex), (313 mg, 44% yield), mp 92–94 ºC. IR ($CHCl_3$, cast film): 3264, 2950, 2927, 2856, 1660, 1500 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.31 (d, *J* = 2.3 Hz, 1H, H2), 6.20 (d, *J* = 2.3 Hz, 1H, H4), 5.61 (s, 1H, OH), 4.51 (ddt, *J* = 11.1, 7.2, 4.7 Hz, 1H, H9), 2.93–2.78 (m, 2H, H8), 1.92–1.81 (m, 1H, H10), 1.75–1.64 (m, 1H, H10), 1.60–1.48 (m, 1H, H11), 1.45–1.42 (m, 1H, H11), 1.30–1.32 (m, 8H, H12,13,14,15), 0.89 (t, *J* = 6.8 Hz, 3H, H16); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.0, 164.5, 162.2, 141.9, 106.5, 102.2, 102.0, 79.3, 34.8, 33.2, 31.8, 29.4, 29.2, 24.9, 22.7, 14.1; HRMS (ESI) calcd for $C_{16}H_{21}O_4$ $[M - H]^-$ 277.1445, found 277.1444.

6,8–Dihydroxy–3–nonylisochroman–1–one (63)

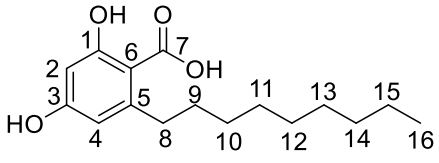


(63)

This new compound was prepared following a modified literature protocol.[200] In a flame dried 250 mL round bottom flask, 10 mL of dry THF was added and cooled to 0 ºC over an ice bath and kept under Ar. To this cooled solvent, (*i*–Pr)$_2$NH (0.07 mL, 7.14 mmol) was added and stirred for 5 min and then *n*–BuLi (2.5 M, 2.85 mL, 7.14 mmol) was added dropwise. After another 10 min of stirring, the reaction mixture was further cooled to –78 ºC. After 10 min, a solution of **60** (0.35 g, 1.78 mmol) dissolved in 5 mL dry THF, was slowly added and stirred for 30 min. Decanal (1.34 mL, 7.14 mmol) was then added and stirred for 90 min maintaining –78 ºC. The reaction mixture was then allowed to warm to room temperature (15 min), and then quenched with 20 mL of water. The reaction mixture was acidified with 1 M HCl to pH 1 and then extracted 4 times with EtOAc. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and then concentrated *in vacuo* to provide the crude protected product. This crude sample was analyzed by LCMS and was found to contain the desired methoxy–protected product. No further purification was done, and this crude product was used for the next step. The crude product from the previous step was dissolved in 10 mL DCM and cooled to –78 ºC the BBr_3 (1 M, 11.3 mL, 11.30 mmol) was added dropwise. The reaction mixture was stirred for 1 h and then heated to reflux overnight.

The reaction mixture was allowed to cool, and 25 mL of water was added, and the layers were separated, and then extracted with 2 x 20 mL DCM. The combined organic layer was washed twice with 20 mL saturated $NaHCO_3$ then washed with 20 mL brine and dried over $MgSO_4$. The DCM was removed *in vacuo*, and the residue was purified using column chromatography (20% EtOAc in Hex) affording the product 6,8–dihydroxy–3–nonylisochroman–1–one (**63**) as a white solid, R_f = 0.22 (20% EtOAc in Hex), (246 mg, 43% yield), mp 98–100 ºC. IR ($CHCl_3$, cast film): 3254, 2955, 2931, 2861, 1628, 1498 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.31 (d, *J* = 2.3 Hz, 1H, H2), 6.20 (d, *J* = 2.3 Hz, 1H, H4), 5.65 (br s, 1H, OH), 4.56–4.47 (m, 1H, H9), 2.92–2.81 (m, 2H, H8), 1.86 (dddd, *J* = 13.6, 10.2, 7.3, 5.1 Hz, 1H, H10), 1.75–1.64 (m, 1H, H10), 1.52 (ddd, *J* = 10.3, 7.8, 5.0 Hz, 1H, H11), 1.44 (tq, *J* = 13.3, 5.6, 4.5 Hz, 1H, H11), 1.38–1.25 (m, 12H, H12,13,14,15,16,17), 0.88 (t, *J* = 6.8 Hz, 3H, H18); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.0, 164.5, 162.2, 141.9, 106.5, 102.2, 102.0, 79.3, 34.8, 33.2, 31.9, 29.6, 29.5, 29.4, 29.3, 24.9, 22.7, 14.2; HRMS (ESI) calcd for $C_{18}H_{25}O_4$ $[M - H]^-$ 305.1758, found 305.1753.

2,4–Dihydroxy–6–nonylbenzoic acid (64)

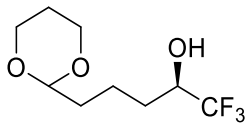


(64)

This known compound was prepared following a modified literature protocol.[200] In a flame dried 250 mL round bottom flask, 10 mL of dry THF was added and cooled to 0 ºC over an ice bath and kept under Ar. To this cooled solvent, (*i*–Pr)$_2$NH (0.07 mL, 7.14 mmol) was added and stirred for 5 min, then 2.5 M *n*–BuLi (2.85 mL, 7.14 mmol) was added dropwise. After 10 min of stirring, the reaction mixture was further cooled to –78 ºC. After another 10 min, a solution of **60** (0.35 g, 1.78 mmol) dissolved in 5 mL dry THF, was slowly added and stirred for 30 min. Octyl iodide (1.34 mL, 7.14 mmol) was then added and stirred for 30 min maintaining –78 ºC and then for 90 min at room temperature after which the reaction mixture was quenched with 20 mL of water. The reaction mixture was acidified with 1 M HCl to pH 1 and then extracted 4 times with EtOAc. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and then concentrated *in vacuo* to provide the crude protected product. This crude sample was analyzed by LCMS and was found to contain the desired methoxy–protected product. No further purification was done; this crude product was used as is, for the next step. The crude product from the previous step was dissolved in 10 mL DCM then cooled to –78 ºC and BBr_3 (1 M, 11.3 mL, 11.30 mmol) was added dropwise. The reaction mixture was stirred for 1 h, allowed to warm to room temperature and then heated to reflux overnight. The reaction mixture was allowed to cool, and 25 mL of water was added. The layers were separated, and then extracted with 2 x 20 mL DCM. The combined organic layers were washed twice with 20 mL saturated $NaHCO_3$ then washed with 20 mL brine and dried over $MgSO_4$. The DCM was removed *in vacuo*, and the crude product was purified by HPLC. Crude sample was dissolved in MeOH and injected on a Phenomenex Luna 5 µ C18(2), 100A, AXIA,

(21.2 mm ID x 250 mm); prep scale column, flow rate of 9 mL/min (A= H_2O, 1% TFA and B = ACN, 1% TFA); 0–5 min, 5% B; 15–20 min, 70% B, 38–43 min, 98% B; 45–50 min, 5% B. Compound **64** eluted at 32 min as a single symmetrical peak to yield the product 2,4–dihydroxy–6–nonylbenzoic acid (**64**) as a white solid after removal of the solvent (59 mg, 55% yield), mp 147–149 ºC. IR ($CHCl_3$, cast film): 3383, 2954, 2922, 2852, 2541, 1623, 1466 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.29 (s, 1H, H2), 6.27 (s, 1H, H4), 5.28 (br s, 1H, OH), 2.91 (t, *J* = 7.5 Hz, 2H, H8), 1.58–1.57 (m, 2H, H9), 1.36–1.27 (m, 14H, H10,11,12,13,14,15), 0.88 (t, *J* = 7.0 Hz, 3H, H16); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 166.5, 161.2, 150.5, 111.1, 103.7, 101.5, 36.7, 32.0, 31.8, 29.8, 29.6, 29.5, 29.4, 22.7, 14.1; HRMS (ESI) calcd for $C_{16}H_{23}O_4$ $[M - H]^-$ 279.1602, found 279.1608.

(*R*)-5-(1,3-Dioxan-2-yl)-1,1,1-trifluoropentan-2-ol (69)

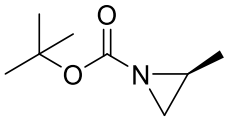


(69)

This compound was synthesized following a literature preparation.[196] To a 250 mL flame dried round bottom flask the commercially available Grignard reagent **38** (5.48 g, 50 mmol) was added along with 40 mL dry THF and cooled to −30 ºC using a Cryo Cooler. To this cold Grignard reagent solution, was added CuI (0.95 g, 5 mmol) stirred

for 10 min, followed by the addition of (*R*)–trifluoromethyl propylene oxide (**68**) (3.3 ml, 37.5 mmol). This mixture was stirred at −30 °C overnight, then quenched by addition of saturated NH_4Cl (10 mL) then stirred for 30 min. After stirring, the mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (15% EtOAc in Hex) affording the product (*R*)-5-(1,3-dioxan-2-yl)-1,1,1-trifluoropentan-2-ol **(69)** as a colorless oil, R_f = 0.32 (15% EtOAc in Hex), (7.2 g, quantitative conversion). IR ($CHCl_3$, cast film): 3408, 2961, 1280, 1167 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.53 (t, *J* = 4.5 Hz, 1H), 4.09 (dd, *J* = 11.0, 5.0 Hz, 2H), 3.86 –3.85 (m, 1H), 3.77–3.71 (m, 2H), 3.18 (br s, 1H), 2.09–2.01 (m, 1H), 1.71–1.49 (m, 6H), 1.34–1.31 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 125 (q), 102, 70, 67, 35, 30, 26,19; $[\alpha]^{25}_D$ = + 10.86 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_9H_{14}O_3F_3$ $[M – H]^+$ 227.0895, found 227.0894.

***tert*-Butyl (*S*)-2-methylaziridine-1-carboxylate (76)**

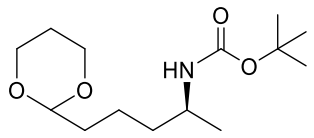


(76)

This compound was prepared by the following protocol. To a solution of compound **84** (4.1 g, 19.4 mmol) dissolved in 200 ml of dry DCM, TEA (13.5 ml, 96.99 mmol) was

added, followed by MsCl (1.80 ml, 23.3 mmol) was slowly added. The reaction mixture was allowed to stir for 2 h at 0 ºC. The reaction was quenched by addition of 20 ml of saturated NH_4Cl. DCM was added, and the layers were separated. The organic layer was washed with brine and dried with magnesium sulfate, then the solvent was removed *in vacuo* to provide a brown solid. The crude product was then dissolved into 50mL of dry THF and NaHMDS (29.1 ml, 29.1 mmol) was slowly added and the reaction was allowed to stir for 1 hour. The mixture was quenched with 10 % citric acid (20 ml), washed with water, sodium bicarbonate, water and finally brine. The organic layer was dried with magnesium sulfate, filtered and concentrated in vacuo to yield the product tert-butyl (*S*)-2-methylaziridine-1-carboxylate **(76),** R_f = 0.5 (20% Et_2O in Pentanes), (6.53 g, 93% yield). IR ($CHCl_3$, cast film): 3371, 2978, 2932, 1701, 1517 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.46–2.40 (m, 1H), 2.23 (d, *J* = 6.0 Hz, 1H), 1.87 (d, *J* = 4.0 Hz, 1H), 1.45 (s, 9H), 1.27 (d, *J* = 5.5 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162, 80.9, 33.6, 32.5, 28.4, 28.0, 27.9, 17.4; $[\alpha]^{25}_D$ = + 38.81 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{15}NNaO_2$ $[M + Na]^+$ 180.0995, found 180.0994.

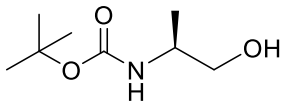
***tert*-Butyl (*S*)-(5-(1,3-dioxan-2-yl)pentan-2-yl)carbamate (77)**



(77)

This compound was synthesized following a literature preparation.[196] To a 250 mL flame dried round bottom flask the commercially available Grignard reagent **38** (8.4 g, 38.17 mmol) was added along with 40 mL dry THF and cooled to −20 ºC using a Cryo Cooler. To this cold Grignard reagent solution, was added CuBr.Me_2S (0.33 g, 1.59 mmol) stirred for 10 min, followed by the addition of compound **76** (5.0 g, 31.8 mmol). This mixture was stirred at −20 °C for 2 h, then quenched by addition of saturated NH_4Cl (10 mL) then stirred for 30 min. After stirring, the mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (10% EtOAc in Hex) affording the product tert-butyl (*S*)-(5-(1,3-dioxan-2-yl)pentan-2-yl)carbamate **(77)** as a waxy solid, R_f = 0.73 (50% EtOAc in Hex), (2.46 g, 28.3% yield). IR ($CHCl_3$, cast film): 3447, 2966, 2929, 2852, 2731, 1710, 1520 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.50 (t, *J* = 5.5 Hz, 1H), 4.32), 4.32 (br s, 1H), 4.09 (dd, *J* = 11.5, 6.5 Hz, 2H), 3.77–3.72 (m, 2H), 3.62 (br s, 1H), 2.11–2.03 (m, 1H), 1.61–1.57 (m, 2H), 1.46–1.37 (m, 2H), 1.34–1.31 (m, 2H), 1.10 (d, *J* = 6.5, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 102.3, 66.9, 37.1, 35.2, 35.1, 28.4, 25.8, 23.9, 21.2, 20.4; $[\alpha]^{25}_D$ = + 1.17 (*c* = 1.52, $CHCl_3$); HRMS (ESI) calcd for $C_{14}H_{28}NO_4$ $[M + H]^+$ 274.2013, found 274.2011.

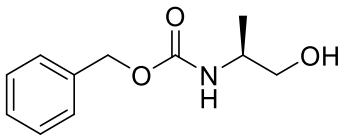
***tert*-Butyl (*S*)-(1-hydroxypropan-2-yl)carbamate (84)**



(84)

This compound was prepared by the following protocol. To a cold solution of Boc protected L-alanine (10.0 g, 52.85 mmol) in DME cooled by an ice-bath, methylmorpholine (5.9 g, 58.1 mmol) and isobutyl chloroformate (3.6 ml, 27.3 mmol) were added. After 5 minutes, the precipitated methylmorpholine hydrochloride was removed by filtration and washed with DME. The DME and the filtrate were combined in a large flask and cooled in an ice bath. $NABH_4$ (2.6 g, 68.2 mmol) in 25 mL of H_2O was slowly added (evolution of gas occurred), followed by another 250 ml of water. The solution was left to stir for 30 minutes, then the phases were separated, and the organic layer extracted with 200 mL of DCM, which was then washed with 200 mL of water, followed by 200 mL of brine. The organic layer was further dried with magnesium sulfate and concentrated *in vacuo* to provide the product tert-butyl (*S*)-(1-hydroxypropan-2-yl)carbamate **(84),** (8.33 g, 90% yield). IR ($CHCl_3$, cast film): 3336, 2977, 2933, 2877, 1689, 1527 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.60 (br s, 1H), 3.79–3.76 (m, 1H), 3.67–3.62 (m, 1H), 3.52–3.48 (m, 1H), 2.49 (br s, 1H), 1.45 (s, 9H), 1.14 (d, J = 8.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.5, 79.8, 67.4, 48.7, 28.53 (d), 17.4; $[\alpha]^{25}_D$ = – 10. 60 (c = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{17}O_3NNa$ [M + Na]$^+$ 198.1101, found 198.1099.

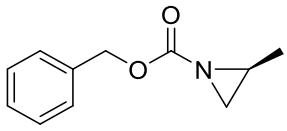
Benzyl (*S*)-(1-hydroxypropan-2-yl)carbamate (86)



(86)

This compound was prepared by the following protocol. To a cold solution of Cbz protected L-alanine (6.1 g, 27.28 mmol) in DME cooled by an ice-bath, *N*-methylmorpholine (3.0 g, 30.01 mmol) and isobutyl chloroformate (3.6 ml, 27.3 mmol) were added. After 5 minutes, the precipitated *N*-methylmorpholine hydrochloride was removed by filtration and washed with DME. The DME and the filtrate were combined in a large flask and cooled in an ice bath. $NABH_4$ (2.6 g, 68.2 mmol) in 25mL of H_2O was slowly added (evolution of gas occurred), followed by another 250 ml of water. The solution was left to stir for 30 minutes, then the phases were separated, and the organic layer extracted with 200mL of DCM, which was then washed with 200 mL of water, followed by 200mL of brine. The organic layer was further dried with magnesium sulfate and concentrated *in vacuo* to provide the product benzyl (*S*)-(1-hydroxypropan-2-yl)carbamate **(86),** (4.17 g, 73% yield). IR ($CHCl_3$, cast film): 3398, 3229, 2971, 2877, 1695, 1587, 1533 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.35 (m, 5H), 5.1 (s, 2H), 4.85 (br s, 1H), 3.85 (m, 1H), 3.68–3.67 (m, 1H), 3.55–3.54 (m, 1H), 2.18 (m, 1H), 1.18 (d, J = 5.0 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.4, 136.3, 128.5, 128.2, 128.1, 66.8, 49.0, 19.2; $[\alpha]^{25}_D$ = – 8.46 (c = 1.00, $CHCl_3$); HRMS (ESI) calcd for $C_{11}H_{15}NNaO_3$ $[M + Na]^+$ 232.0944, found 232.0950.

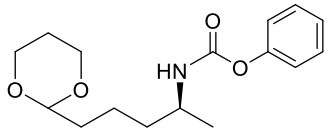
Benzyl (*S*)-2-methylaziridine-1-carboxylate (87)



(87)

This compound was prepared by the following protocol. To a solution of compound **86** (4.1 g, 19.4 mmol) dissolved in 200 ml of dry DCM, TEA (13.5 ml, 96.99 mmol) was added, followed by MsCl (1.80 ml, 23.3 mmol) was slowly added. The reaction mixture was allowed to stir for 2 h at 0 ºC. The reaction was quenched by addition of 20 ml of saturated NH_4Cl. DCM was added, and the layers were separated. The organic layer was washed with brine and dried with magnesium sulfate, then the solvent was removed *in vacuo* to provide a brown solid. The crude product was then dissolved into 50mL of dry THF and NaHMDS (29.1 ml, 29.1 mmol) was slowly added and the reaction was allowed to stir for 1 hour. The mixture was quenched with 10 % citric acid (20 ml), washed with water, sodium bicarbonate, water and finally brine. The organic layer was dried with magnesium sulfate, filtered and concentrated in vacuo to yield the product benzyl (S)-2-methylaziridine-1-carboxylate **(87),** (2.46 g, 66% yield). IR ($CHCl_3$, cast film): 3420, 3033, 2970, 1722, 1298 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.26 (m, 5H), 5.13 (s, 2H), 2.53–2.51 (m, 1H), 2.33 (d, J = 4.5, 1H), 1.96 (d, J = 3.0, 1H), 1.28 (d, J = 4.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.3, 135.9, 128.5, 128.2, 128.1, 68.0, 33.9, 32.7, 17.4; $[\alpha]^{25}_D$ = + 26.19 (c = 1.00, $CHCl_3$); HRMS (ESI) calcd for $C_{11}H_{13}NNaO_2$ $[M + Na]^+$ 214.0838, found 214.0837.

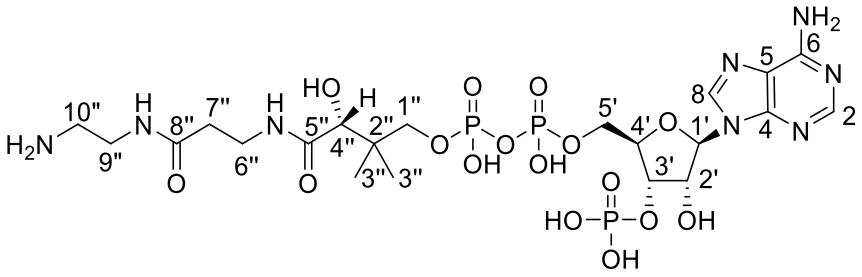
Phenyl (*S*)-(5-(1,3-dioxan-2-yl)pentan-2-yl)carbamate (88)



(88)

This compound was synthesized following a literature preparation.[196] To a 250 mL flame dried round bottom flask the commercially available Grignard reagent **38** (3.44 g, 15.69 mmol) was added along with 40 mL dry THF and cooled to −30 ºC using a Cryo Cooler. To this cold Grignard reagent solution, was added CuBr.Me_2S (0.10 g, 0.52 mmol) stirred for 10 min, followed by the addition of compound **87** (2.0 g, 10.46 mmol). This mixture was stirred at −30 °C for 2 h, then quenched by addition of saturated NH_4Cl (10 mL) then stirred for 30 min. After stirring, the mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (40% EtOAc in Hex) affording the product phenyl (*S*)-(5-(1,3-dioxan-2-yl)pentan-2-yl)carbamate **(88)** as a white solid, (1.59 g, 50% yield). IR ($CHCl_3$, cast film): 3301, 2958, 2556, 1686, 1546 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.3–7.29 (m, 5H), 5.08 (br s, 2H), 4.56–4.49 (m, 2H), 4.10–4.07 (m, 2H), 3.77–3.72 (m, 3H), 2.10–2.03 (m, 1H), 1.59–1.54 (m, 2H), 1.44–1.40 (2H), 1.35–1.31 (m, 2H), 1.14 (d, J = 5.0, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.4, 128.5, 128.0, 102.1, 66.9, 37.2, 35.4, 25.8, 20.4; $[\alpha]^{25}_D$ = + 2.94 (c = 1.00, $CHCl_3$); HRMS (ESI) calcd for $C_{17}H_{25}NNaO_4$ [M + Na]$^+$ 330.1676, found 330.1675.

Amino Coenzyme A (98)

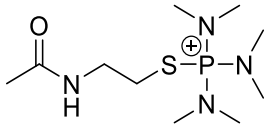


(98)

This known compound was prepared by the following method. To a screw cap glass vial, 2.0 mL of deionized water was added and purged with Ar, followed by the addition of Coenzyme A **(97)** (50.0 mg, 0.064 mmol). To this solution, boric acid (3.9 mg, 0.064 mmol), and ethylene diamine (43.0 μL, 0.64 mmol) were added, then the vial was capped under an Ar environment. The reaction mixture was then heated to 55° C for 48 h (progress of the reaction was followed by LCMS). Preparative HPLC was carried out on the crude reaction mixture using a reverse phase Grace Vydac 218TP1022 C18 column (22 mm ID× 250 mm); flow rate of 14 mL/min (A = 10 mM ammonium formate buffer, and B = 10 mM ammonium formate buffer in 95% ACN); 0–7 min, 0% B; 7–41 min, 0–4% B; 41–42 min, 4% B; 42–44 min, 4–95% B; 44–49 min, 95% B; 49–51 min, 95–0% B; 51–56 min, 0% B. Compound **98** eluted at 31.0 min as a single peak to yield the product **98** as a white powder after removal of the solvent (14.4 mg, 30% yield); ^{1}H NMR (700 MHz, D_2O) δ 8.58 (s, 1H, H8), 8.30 (s, 1H, H2), 6.20 (d, *J* = 6.7 Hz, 1H, H1'), 4.99–4.77 (m, 2H, H2', H3'), 4.61 (s, 1H, H4'), 4.27 (m, 2H, H5'), 4.03 (s, 1H, H4"), 3.81 (dd, *J* = 9.8, 4.9 Hz, 1H, H1"), 3.64 (dd, *J* = 9.8, 4.2 Hz, 1H, H1"),

3.53–3.48 (m, 4H, H6”, H9”), 3.16 (t, *J* = 5.8 Hz, 2H, H10”), 2.50 (t, *J* = 6.4 Hz, 2H, H7”), 0.89 (s, 3H, H3”), 0.84 (s, 3H, H3”). ^{13}C NMR (126 MHz, D_2O) δ 176.1 (C5”), 175.7(C8”), 153.8(C2), 150.4(C6), 148.5(C4), 140.9(C8), 119.4(C5), 87.5(C1’), 84.6(C4’), 75.4(C4”), 74.8(C2’), 74.7(C1”), 72.7(C1”), 66.5(C5’), 40.2(C10”), 39.3(C2”), 37.6(C9”), 36.5(C6”), 36.4(C7”), 21.5(C3”), 19.8(C3”). ^{31}P NMR (162 MHz, D_2O) δ 1.6, –4.2, –10.7. HRMS (ESI) Calcd for $C_{21}H_{36}N_8O_{16}P_3$ $[M - H]^-$ 749.1468, found 749.1454.

((2-Acetamidoethyl)thio)tris(dimethylamino)phosphonium (101)

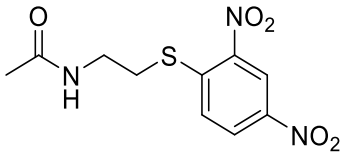


(101)

This compound was prepared by the following method. To a stirred solution of Ellman’s reagent (1.25 g, 3.14 mmol) in water (20 ml) was added K_2CO_3 (0.33 g, 2.0 mmol) and HSNAC **(45)** (0.25 g, 2.10 mmol). The reaction mixture was allowed to stir for another 2 hours at 35 ºC to form the SNAC-Ellman’s adduct **100**, then HMPT (0.66 ml, 4.20 mmol) was added. The reaction mixture was then stirred for another 2 h, and the product was cooled and purified by ion exchange chromatography to give the product ((2-acetamidoethyl)thio)tris(dimethylamino)phosphonium **(101)** as a red solid**,** (3.7 mg, 10%). IR ($CHCl_3$, cast film): 3393, 2929, 1675, 1468 cm^{-1}; ^{1}H NMR (500

MHz, $CDCl_3$) δ 3.54–3.50 (m, 2H), 3.14–3.08 (m, 2H), 2.92–2.84 (m, 18H), 1.98 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5, 39.0, 37.5, 29.4, 23.0; HRMS (ESI) calcd for $C_{10}H_{26}N_4O_3PS$ $[M]^+$ 281.1559, found 281.1558.

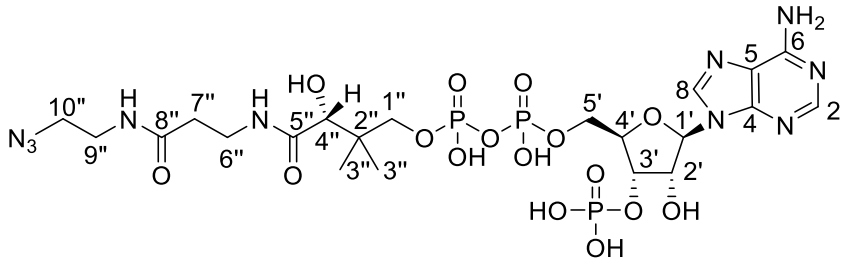
N-(2-((2,4-Dinitrophenyl)thio)ethyl)acetamide (103)



(103)

This compound was prepared by the following method. To a solution of Sanger's reagent (62.5 mg, 0.34 mmol) in 20 ml of water, was added K_2CO_3 (330 mg). To this solution, HSNAC (40 mg, 0.34 mmol) was added and stirred for 2 h. The water was removed and the product N-(2-((2,4-dinitrophenyl)thio)ethyl)acetamide **(103)** recrystalized from chloroform, (145.3 mg, quantitative conversion). IR ($CHCl_3$, cast film): 3411, 3292, 3080, 2930, 2849, 1593, 1520 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.08 (d, *J* = 2.5 Hz, 1H), 8.44 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.96 (d, *J* = 9 Hz, 1H), 5.89 (br s, 1H), 3.57 (q, *J* = 6.5 Hz, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.03 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 145.7, 144.4, 127.6, 127.6, 121.9, 38.3, 31.6, 23.3; HRMS (ESI) calcd for $C_{10}H_{11}N_3O_5SNa$ $[M + Na]^+$ 308.0312, found 308.0306.

Azido Coenzyme A (107)

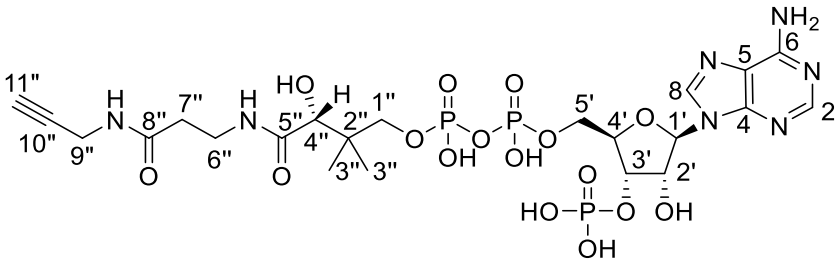


(107)

This compound was prepared by the following method. To a screw cap glass vial, 4.0 mL of deionized water was added and purged with Ar, followed by the addition of Coenzyme A **(97)** (50.0 mg, 0.064 mmol). To this solution, boric acid (3.9 mg, 0.064 mmol), and 2–azidoethylamine **(191)** (109.6 mg, 1.273 mmol) were added, then the vial was capped under an Ar environment. The reaction mixture was then heated to 55 ^{o}C for 48 h (progress of the reaction was followed by LCMS). Preparative HPLC was carried out on the crude reaction mixture using a reverse phase Grace Vydac 218TP1022 C18 column (22 mm ID × 250 mm); flow rate of 14 mL/min (A = 10 mM Ammonium Formate Buffer, and B = 10 mM Ammonium formate buffer in 95% ACN); 0–7 min, 0% B; 7–34 min, 0–13% B; 34–50 min, 13% B; 50–52 min, 13–95% B; 52–56 min, 95% B; 56–57 min, 95–0% B; 57–62 min, 0% B. Compound **107** eluted at 40.7 min as a single peak to yield the product as a white powder after removal of the solvent (14.0 mg, 28% yield); ^{1}H NMR (700 MHz, D_2O) δ 8.58 (s, 1H, H8), 8.32 (s, 1H, H2), 6.21 (d, *J* = 6.6 Hz, 1H, H1'), 4.93–4.86 (m, 2H, H2', H3'), 4.63 (s, 1H, H4'), 4.27 (m, 2H, H5'), 4.06 (s, 1H, H4"), 3.84–3.83 (m, 1H, H1"), 3.59–3.57 (m, 1H, H1"),

3.50 (t, J = 6.4 Hz, 2H, H6”), 3.45–3.36 (m, 2H, H9”), 2.71 (t, J = 6.6 Hz, 2H, H10”), 2.49 (t, J = 6.8 Hz, 2H, H7”), 0.93 (s, 3H, H3”), 0.82 (s, 3H, H3”). ^{13}C NMR (126 MHz, D_2O) δ 175.6(C5”), 175.1(C8”), 153.4(C2), 150.4(C6), 150.0(C4), 141.0(C8), 119.6(C5), 87.3(C1’), 84.5(C4’), 75.2(C4”), 74.7(C2’), 74.7(C2’), 72.8(C1”), 66.3(C5’), 39.6(C9”), 39.4(C2”), 36.4(C6”), 36.3(C7”), 31.1(C10”), 21.8(C3”), 19.1(C3”). ^{31}P NMR (162 MHz, D_2O) δ 0.1, –10.8, –11.4. HRMS (ESI) Calcd for $C_{21}H_{34}N_{10}O_{16}P_3$ $[M - H]^-$ 775.1373, found 775.1360.

Alkynyl Coenzyme A (108)

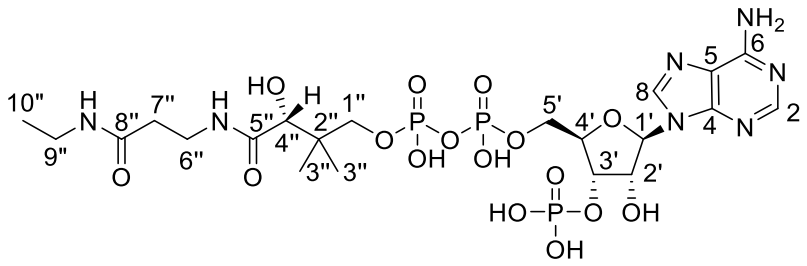


(108)

This compound was prepared by the following method. To a screw cap glass vial, 4.0 mL of deionized water was added and purged with Ar, followed by the addition of Coenzyme A **(97)** (50.0 mg, 0.064 mmol). To this solution, boric acid (3.9 mg, 0.064 mmol), and propargyl amine (81.0 µL, 1.27 mmol) were added, then the vial was capped under an Ar environment. The reaction mixture was then heated to 55 ºC for 48 h (progress of the reaction was followed by LCMS). Preparative HPLC was carried out

on the crude reaction mixture using a reverse phase semi–prep Grace Vydac 218TP510 C18 column (10 mm ID × 250 mm); flow rate of 5 mL/min (A = 10 mM Ammonium Formate Buffer, and B = 10 mM Ammonium formate buffer in 95% ACN); 0–7 min, 0% B; 7–37 min, 0–3% B; 37–40 min, 3% B; 40–41 min, 3–5% B; 41–44 min, 5% B; 44–46 min, 5–95% B; 46–49 min, 95% B; 49–51 min, 95–0% B; 51–56 min, 0% B. Compound **108** eluted at 29.2 min as a single peak to yield the product as a white powder after removal of the solvent (13.2 mg, 28% yield); ^{1}H NMR (700 MHz, D_2O) δ 8.58 (s, 1H, H8), 8.31 (s, 1H, H2), 6.21 (d, *J* = 6.4 Hz, 1H, H1), 4.92–4.85 (m, 2H, H2', H3'), 4.62 (s, 1H, H4'), 4.27 (m, 2H, H5'), 4.04 (s, 1H, H4"), 3.90 (s, 2H, H9"), 3.85–3.84 (m, 1H, H1"), 3.61–3.60 (m, 1H, H1"), 3.50 (t, *J* = 6.5 Hz, 2H, H6"), 2.61 (s, 1H, H11"), 2.49 (t, *J* = 6.5 Hz, 2H, H7"), 0.90 (s, 3H, H3"), 0.78 (s, 3H, H3"). ^{13}C NMR (126 MHz, D_2O) δ 175.0(C5"), 174.9(C8"), 152.9(C2), 150.7(C6), 149.9(C4), 139.9(C8), 118.3(C5), 86.4(C1'), 83.6(C4'), 79.5(C10"), 74.2(C4"), 73.8(C2'), 73.8(C3'), 71.9(C1"), 71.8(C11"), 65.4(C5'), 38.3(C2"), 35.5(C6"), 35.3(C7"), 28.8(C9"), 20.8(C3"), 18.2(C3"). ^{31}P NMR (162 MHz, D_2O) δ 1.4, –4.2, –11.2. HRMS (ESI) Calcd for $C_{22}H_{33}N_7O_{16}P_3$ $[M - H]^-$ 744.1202, found 744.1187.

Desulfurized Coenzyme A (110)

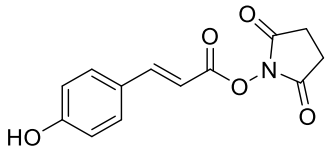


(110)

This compound was prepared by the following method. To a screw cap glass vial, 2.0 mL of deionized water was added and purged with Ar, followed by the addition of Coenzyme A **(97)** (50.0 mg, 0.064 mmol) and TCEP (91.3 mg, 0.318 mmol). To the reaction mixture, 2,2'–Azobis[2–(2–imidazolin–2–yl)propane]dihydrochloride **(109)** (3.20 mg, 0.013 mmol) was added and then the vial was capped under an Ar environment. The reaction mixture was then heated to 55 ^{o}C for 4 h (progress of the reaction was followed by LCMS). Preparative HPLC was carried out on the crude reaction mixture using a reverse phase Grace Vydac 218TP1022 C18 column (22 mm ID × 250 mm); flow rate of 14 mL/min (A = 10 mM Ammonium Formate Buffer, and B = 10 mM Ammonium formate buffer in 95% ACN); 0–7 min, 0% B; 7–23 min, 0–6% B; 23–30 min, 6% B; 30–35 min, 6–14% B; 35–44 min, 14% B; 44–46 min, 14–95% B; 46–49 min, 95% B; 49–51 min, 95–0% B; 51–56 min, 0% B. Compound **110** eluted at 42.5 min as a single peak to yield the product as a white powder after removal of the solvent (45.1 mg, 96% yield); ^{1}H NMR (700 MHz, D_2O) δ 8.73 (s, 1H, H8), 8.48 (s, 1H, H2), 6.27 (d, *J* = 6.1 Hz, 1H, H1'), 4.95–4.89 (m, 2H, H2', H3'), 4.65 (s, 1H, H4'), 4.30 (m, 2H, H5'), 4.07 (s, 1H, H4"), 3.90 (dd, *J* = 9.7, 4.0 Hz, 1H, H1"), 3.64 (dd, *J* = 10.0, 3.9 Hz, 1H, H1"), 3.51 (t, *J* = 6.6 Hz, 2H, H6"), 3.19 (q, *J* = 7.3 Hz, 2H,

H9”), 2.48 (t, J = 6.6 Hz, 2H, H7”), 1.11 (t, J = 7.3 Hz, 3H, H10”), 0.98 (s, 3H, H3”), 0.86 (s, 3H, H3”). ^{13}C NMR (126 MHz, D_2O) δ 175.7(C5”), 174.5(C8”), 153.8(C2), 151.0(C6), 149.0(C4), 143.2(C8), 119.6(C5), 88.4(C1’), 84.7(C4’), 75.1(C2’), 75.1(C4”), 75.0(C3’), 72.9(C1”), 66.1(C5’), 39.3(C2”), 36.5(C6”), 36.4(C7”), 35.5(C9”), 21.9(C3”), 19.2(C3”), 14.4(C). ^{31}P NMR (162 MHz, D_2O) δ –0.2, –11.1, –14.7. HRMS (ESI) Calcd for $C_{21}H_{35}N_7O_{16}P_3$ $[M - H]^-$ 734.1359, found 734.1361.

(*E*)–2,5–Dioxopyrrolidin–1–yl 3–(4–hydroxyphenyl)acrylate (111)

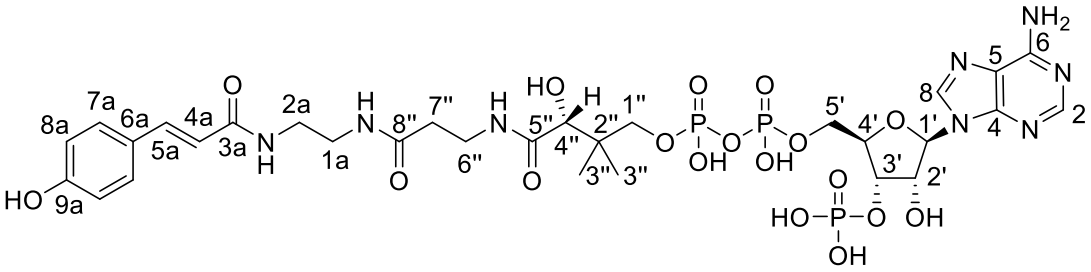


(111)

This known compound was prepared following a modified literature preparation.[201] In 10 mL of dry DMF, *p*–coumaric acid (10.0 mg, 0.086 mmol) was added followed by DIC (6.60 mg, 0.052 mmol) and NHS (9.80 mg, 0.061 mmol), then stirred for 2 h at room temperature. After 2 h the DMF was removed *in vacuo* and the solids were taken up by 20 mL EtOAc and then filtered. The filtrate was then extract with 20 mL of cold 1 M $NaHCO_3$. The EtOAc was then removed *in vacuo* and the product purified by recrystallization from EtOAc/Hex to yield (*E*)–2,5–dioxopyrrolidin–1–yl 3–(4–hydroxyphenyl)acrylate (**111**) as a white solid (13.2 mg, 58% yield). IR (Solid): 3270, 2939, 1781, 1759, 1710 cm^{-1}; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 7.87 (d, J = 15.5 Hz,

1H), 7.69 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 16.0 Hz, 2H), 2.90 (s, 4H). ^{13}C NMR (126 MHz, $(CD_3)_2CO$) δ 170.8, 163.5, 161.8, 150.6, 132.1, 126.5, 117.0, 109.6, 26.4. HRMS (ESI) Calcd for $C_{13}H_{10}NO_5$ $[M - H]^-$ 260.0564, found 260.0562.

***p*–Coumaroyl CoA Amide (112)**

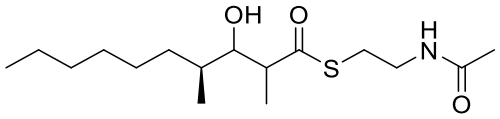


(112)

This molecular probe was prepared by the following method. To a screw cap reaction vial, phosphate buffer solution (2.0 mL, pH 7.4) was added followed by amino CoA **(98)** (6.31 mg, 0.008 mmol). To this reaction mixture 2–propenoic acid, 3–(4–hydroxyphenyl)–, 2,5–dioxo–1–pyrrolidinyl ester **(111)** (4.40 mg, 0.017 mmol) was added and the vessel was sealed under an Ar environment. The reaction mixture was stirred at room temperature for 14 h. Preparative HPLC was carried out on the crude reaction mixture using a reverse phase Grace Vydac 218TP1022 C18 column (22 mm ID × 250 mm); flow rate of 11 mL/min (A = H_2O, 0.5% formic acid and B = ACN, 0.5% formic acid); 0–6 min, 0% B; 6–53 min, 0–36% B; 53–54 min, 36% B; 54–59

min, 36–95% B; 59–64 min, 95% B; 64–65 min, 95–0% B; 65–70 min, 0% B. Compound **112** eluted at 35.4 min as a single peak to yield the product after removal of the solvent as a white powder (5.61 mg, 74% yield); ^{1}H NMR (500 MHz, D_2O) δ 8.49 (s, 1H, H8), 8.31 (s, 1H, H2), 7.35 (d, *J* = 8.0 Hz, 2H, H5a), 7.27 (d, *J* = 15.9 Hz, 1H, H7a), 6.79 (d, *J* = 8.3Hz, 2H, H4a), 6.30 (d, *J* = 16.1 Hz, 1H, H8a), 6.16 (d, *J* = 6.5 Hz, 1H, H1'), 4.99 – 4.80 (m, 2H, H2', H3'), 4.56 (s, 1H, H4'), 4.29 – 4.27 (m, 2H, H5'), 3.97 (s, 1H, H4"), 3.83 – 3.77 (m, 1H, H1"), 3.58 – 3.53 (m, 1H, H1"), 3.49 – 3.35 (m, 4H, H6", H1a), 3.30 (t, *J* = 5.9 Hz, 2H, H2a), 2.48 (t, *J* = 6.7 Hz, 2H, H7"), 0.85 (s, 3H, H3"), 0.77 (s, 3H, H3"). ^{13}C NMR (126 MHz, D_2O) δ 174.3(C8"), 172.3(C3a), 157.0(C9a), 152.7(C2), 150.2(C6), 149.0(C4), 144.5(C8), 130.5(C7a), 129.9(C5a), 127.6(C6a), 119.2(C5), 115.8(C8a), 115.2(C4a), 86.9(C1'), 85.9(C4'), 74.6(C5"), 74.3(C4"), 74.2(C2'), 74.2(C3'), 73.5(C1"), 65.8(C5'), 38.9(C2"), 38.8(C2a), 35.5(C6"), 35.4(C7"), 35.4(C1a), 20.7(C3"), 18.6(C3"). HRMS (ESI) Calcd for $C_{30}H_{42}N_8O_{18}P_3$ $[M - H]^-$ 895.1830, found 895.1844.

(4*S*)-*S*-2-Acetamidoethyl 3-hydroxy-4-methyldecanethioate (127)

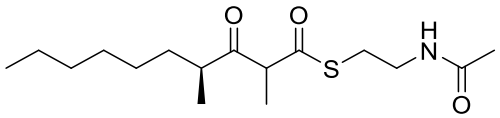


(127)

To a stirred solution of **(199)** (305 mg, 0.96 mmol) in 10 mL MeCN was added K_2CO_3 (556 mg, 3.4 mmol) and N-acetylcysteamine **(45)** (137 mg, 1.2 mmol). The reaction

mixture was stirred until the yellow color disappeared 40 min). The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (50 % EtOAc in Hex) affording the product (4*S*)-*S*-2-acetamidoethyl 3-hydroxy-4-methyldecanethioate (**127**) as a clear oil, R_f = 0.08 (50% EtOAc in Hex), (267 mg, 88% yield). IR ($CHCl_3$, cast): 3302, 2958, 2927, 2857, 1659, 1553 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.96 – 5.82 (m, 1H, NH), 3.98 (m, 1H, *J* = 8.9, 4.3, 3.5 Hz, H8, Major Diastereomer), 3.92 (ddd, 1H, *J* = 8.8, 5.5, 3.2 Hz, H8, Minor Diastereomer), 3.51 – 3.37 (m, 2H, H12), 3.10 – 2.97 (m, 2H, H11), 2.76 – 2.63 (m, 2H, H9), 1.96 (s, 3H, H14), 1.63 – 1.07 (m, 11H, H2-7), 0.90 – 0.87 (m, 6H, H1, H15); ^{13}C NMR (126 MHz, $CDCl_3$): Major diastereomer (anti): δ 200.5, 170.5, 72.0, 48.6, 39.4, 38.4, 32.8, 31.9, 29.6, 28.9, 27.2, 23.2, 22.7, 14.2, 14.1; Minor diastereomer (syn): δ 200.0, 170.5, 72.7, 47.7, 39.4, 38.4, 32.8, 32.2, 29.6, 28.9, 27.1, 23.2, 22.7, 15.0, 14.1; HRMS (ESI) Calcd for $C_{15}H_{29}NO_3SNa$ $[M + Na]^+$ 326.1760, found 326.1761.

(4*S*)-*S*-2-Acetamidoethyl 2,4-dimethyl-3-oxo-decanethioate (128)

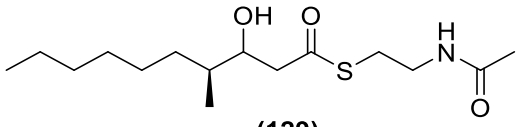


(128)

To a stirred solution of (**127)** (5.0 mg, 0.016 mmol) in 10 mL dry DCM at 0 ºC under argon, Dess-Martin periodinane (DMP) (13.4 mg, 0.032 mmol) in 5 ml dry DCM was

added and the reaction mixture was stirred for 5 min. The reaction was quenched by addition of 4 ml of 1:1 mixture of saturated $NaHCO_3$:$Na_2S_2O_3$ and stirred for 3 min. The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (10 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (1:1 Hexanes/EtOAc) affording the product (4*S*)-*S*-2-Acetamidoethyl 2,4-dimethyl-3-oxo-decanethioate (**128**) as a clear oil, R_f = 0.13 (50% EtOAc in Hex), (3.4 mg, 68% yield). (9:1 Keto:Enol); IR ($CHCl_3$, cast): 3288, 2958, 2931, 1722, 1661, 1549 cm-1; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.82 (br s, 1H, NH), 3.94 (m, 1H), 3.54 – 3.34 (m, 2H), 3.14 – 3.00 (m, 2H), 2.74 (m, 1H), 1.97 (s, 3H), 1.63 – 1.08 (m, 16H), 0.90 – 0.85 (m, 3H). ^{13}C NMR (126 MHz, CDCl3) δ (Mix of Keto and Enol): δ 208.8, 196.9, 196.7, 178.4, 170.4, 60.2, 59.9, 46.0, 45.8, 39.5, 33.2, 32.7, 31.7, 29.3, 28.9, 27.2, 27.2, 23.2, 22.6, 16.8, 16.4, 14.1, 14.0, 13.8; HRMS (ES) Calcd for $C_{16}H_{29}NO_3SNa$ ([M+Na]+) 338.1760, found 338.1761.

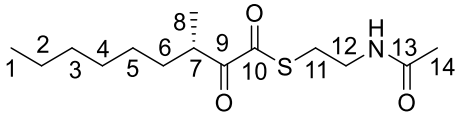
(*4S*)–*S*–2–Acetamidoethyl 3–hydroxy–4–methyldecanethioate (129)



(129)

This known compound was prepared following a literature protocol.[48] To a stirred solution of **198** (0.400 g, 1.31 mmol) in 10 mL ACN was added K_2CO_3 (0.760 g, 4.61 mmol) and HSNAC **(45)** (0.190 g, 1.58 mmol). The reaction mixture was stirred until the yellow color disappeared (40 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*4S*)–*S*–2–acetamidoethyl 3–hydroxy–4–methyldecanethioate **(129)** as a clear oil, R_f = 0.08 (50% EtOAc in Hex), (267 mg, 88% yield). IR ($CHCl_3$, cast film): 3302, 2958, 2927, 2857, 1659, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.96–5.82 (m, 1H), 3.98 (m, *J* = 8.9, 4.3, 3.5 Hz, 1H, Major Diastereomer), 3.92 (ddd, *J* = 8.8, 5.5, 3.2 Hz, 1H, Minor Diastereomer), 3.51–3.37 (m, 2H), 3.10–2.97 (m, 2H), 2.76–2.63 (m, 2H), 1.96 (s, 3H), 1.63–1.07 (m, 11H), 0.90–0.87 (m, 6H, H1); ^{13}C NMR (126 MHz, $CDCl_3$): Major diastereomer (anti): δ 200.5, 170.5, 72.0, 48.6, 39.4, 38.4, 32.8, 31.9, 29.6, 28.9, 27.2, 23.2, 22.7, 14.2, 14.1; Minor diastereomer (syn): δ 200.0, 170.5, 72.7, 47.7, 39.4, 38.4, 32.8, 32.2, 29.6, 28.9, 27.1, 23.2, 22.7, 15.0, 14.1; HRMS (ESI) calcd for $C_{15}H_{29}NO_3SNa$ $[M + Na]^+$ 326.1760, found 326.1761.

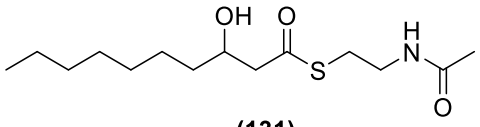
(*S*)–*S*–2–Acetamidoethyl 3–methyl–2–oxononanethioate (130)



(130)

This compound was prepared by the following method. To a stirred solution of **129** (0.10 g, 0.33 mmol) in 20 mL of DCM exposed to air, DMP (0.28 g, 0.66 mmol) was added and the reaction mixture was stirred for 2 h. The reaction mixture was quenched by addition of 10 mL of 1:1 mixture of saturated $NaHCO_3$:$Na_2S_2O_3$ and stirred for 5 min. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 x 20 mL), then brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*S*)–*S*–2–acetamidoethyl 3–methyl–2–oxononanethioate (**130**) as a yellow oil, R_f = 0.21 (50% EtOAc in Hex), (39 mg, 41% yield). $[\alpha]_D^{25}$ = 9.10 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$, cast film): 3288, 3079, 2957, 2930, 2858, 1720, 1676, 1552 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 5.81 (br s, 1H, NH), 3.46 (q, 2H, *J* = 6.3 Hz, H12), 3.28 (q, 1H, *J* = 7.0 Hz, H7), 3.11 (t, 2H, *J* = 6.3 Hz, H11), 1.97 (s, 3H, H14), 1.70–1.25 (m, 10H, H2,3,4,5,6) 1.12 (d, 3H, *J* = 7 Hz, H8), 0.86 (t, 3H, *J* = 7.0 Hz, H1); ^{13}C NMR (176 MHz, $CDCl_3$) δ 198.4 (C9), 191.6 (C10), 170.3 (C13), 39.6 (C7), 39.0 (C12), 32.3 (C6), 31.6 (C5), 29.2 (C4), 28.5 (C3), 27.1 (C11), 23.2 (C2), 22.6 (C14), 15.4 (C8), 14.1 (C1); HRMS (ESI) calcd for $C_{14}H_{26}NO_3S$ $[M + H]^+$ 288.1628, found 288.1628.

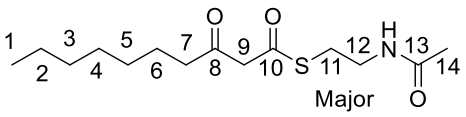
***S*–2–Acetamidoethyl 3–hydroxydecanethioate (131)**



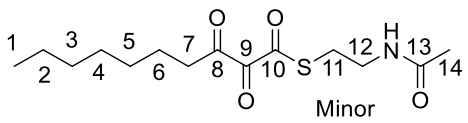
(131)

This known compound was prepared following a modified literature protocol.[39] To a round bottom flask containing 20 mL of dry ACN, was added **(200)** (3.35 g, 11.6 mmol) followed by K_2CO_3 (6.69 g, 40.5 mmol) at room temperature under an Ar atmosphere. Compound **(45)** (1.66 g, 11.9 mmol) was then added to the reaction mixture, and the mixture was stirred for 20 min. The solvent was then removed *in vacuo* and the residue was taken up in 20 mL EtOAc and 20 mL water. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (60% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 3–hydroxydecanethioate **(131)** as a yellow oil, R_f = 0.21 (EtOAc), (2.58 g, 77% yield). IR ($CHCl_3$ cast film): 3303, 3078, 2953, 2921, 2871, 2849, 1696, 1687, 1642 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.85 (s, 1H), 4.05 (s, 1H), 3.50–3.37 (m, 2H), 3.09–3.00 (m, 2H), 2.77–2.66 (m, 3H), 1.97 (s, 3H), 1.54–1.27 (m, 12H), 0.89–0.86 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.6, 170.5, 51.1, 51.1, 39.3, 36.8, 31.8, 29.5, 29.2, 28.9, 25.5, 23.3, 22.7, 14.1; HRMS (ESI) calcd for $C_{14}H_{27}NNaO_3S$ $[M + Na]^+$ 312.1604, found 312.1602.

***S*–2–Acetamidoethyl 3–oxodecanethioate (132) and *S*–2–acetamidoethyl 2,3–dioxodecanethioate (134)**



(132)

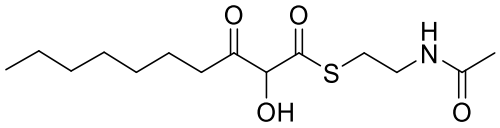


(134)

This compound was prepared by the following method. To a round bottom flask was added (**131)** (1.50 g, 5.19 mmol) followed by 20 mL of DCM. DMP (2.31 g, 5.45 mmol) was added and stirred for 30 min at room temperature, then quenched with 1:3 $Na_2S_2O_3$:$NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (3 x 10 mL), then the combined organic layers were washed with saturated $NaHCO_3$ (2 x 10 mL), dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 3–oxodecanethioate **(132)** and *S*–2–acetamidoethyl 2,3–dioxodecanethioate (**134**) as white solids, R_f = 0.11 (50% EtOAc in Hex), (1.14 g, 76% yield). IR ($CHCl_3$ cast film): 3281, 2949, 2923, 2856, 1717, 1687, 1637 cm^{-1}. Major; ^1H NMR (500 MHz, $CDCl_3$) δ 5.87 (br s, 1H, NH), 3.69 (s, 2H, H9), 3.46 (q, *J* = 6.5 Hz, 2H, H12), 3.09 (q, *J* = 6.0 Hz, 2H, H11), 2.52 (t, *J* = 7.5 Hz, 2H, H7), 1.97 (s, 3H, H14), 1.60–1.59 (m, 2H, H6), 1.28 (br s, 8H, H2,3,4,5), 0.88 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.4 (C8), 192.6 (C10), 170.5 (C13), 57.3 (C9), 43.6 (C7), 39.4 (C12), 31.8 (C5), 29.4 (C4), 29.1 (C6), 28.0 (C14), 26.4 (C3), 23.6 (C2), 14.2(C1); HRMS (ESI) calcd for $C_{13}H_{23}NNaO_3S$ $[M + Na]^+$ 310.1447, found 310.1444.

Minor; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (br s, 1H, NH), 3.51–3.46 (m, 2H, H12), 3.20–3.11 (m, 2H, H11), 2.58–2.54 (m, 2H, H7), 1.97 (s, 3H, H14), 1.60–1.59 (m, 10H, H6,5,4,3,2), 0.92 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.8 (C8), 190.3(C10), 184.7 (9), 170.5 (C13), 43.0 (C7), 39.4 (C12), 31.6 (C5), 29.1 (C4), 28.9 (C6), 27.9 (C14), 26.5 (C3), 23.3 (C2), 14.1(C1); HRMS (ESI) calcd for $C_{14}H_{24}NO_4S$ $[M + Na]^+$ 302.1426, found 302.1426.

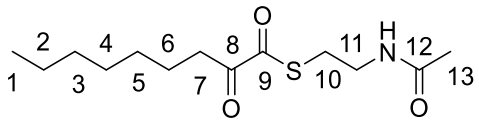
_S_–2–Acetamidoethyl 2–hydroxy–3–oxodecanethioate (133)



(133)

This new compound was prepared following a modified protocol.[202] To a flame dried vial containing 10 mL dry THF, **(205)** (10 mg, 0.02 mmol) was added followed by TBAF (1.0 M, 0.03 mL, 0.03 mmol) and stirred for 30 min. Due to the unstable nature of *S*–2–acetamidoethyl 2–hydroxy–3–oxodecanethioate no purification was possible, and this sample was used as is for the HiRes LCMS assay. HRMS (ESI) calcd for $C_{14}H_{26}NO_4S$ $[M + H]^+$ 304.1577, found 304.1584.

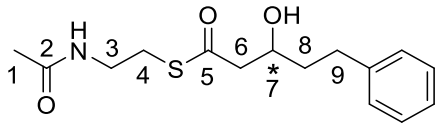
***S*–2–Acetamidoethyl 2–oxononanethioate (135)**



(135)

This new compound was prepared by the following method. To a round bottom flask was added **(131)** (0.400 g, 1.38 mmol) followed by 25 mL of DCM, open to air. DMP (2.93 g, 6.91 mmol) was added to the solution and stirred for 5 h. The reaction mixture was then quenched with 1:1 $Na_2S_2O_3$:$NaHCO_3$ (10 mL). The mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (3 x 10 mL), then dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 2–oxononanethioate **(135)** as a yellow oil, R_f = 0.14 (50% EtOAc in Hex), (279 mg, 74% yield). IR ($CHCl_3$, cast film): 3287, 3092, 2954, 2929, 2857, 1724, 1674 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.75 (s, 1H, NH), 3.49–3.45 (q, *J* = 12.5, 6.5 Hz, 2H, H11), 3.12–3.09 (t, *J* = 6.5 Hz, 2H, H10), 2.81–2.78 (t, *J* = 7.5 Hz, 2H, H7), 1.97 (s, 3H, H13), 1.66–1.59 (m, 2H, H6), 1.32–1.29 (m, 8H, H2,3,4,5), 0.89–0.86 (t, *J* = 6.5 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.3 (C8), 191.5 (C9), 170.4 (C12), 38.9 (C11), 36.5 (C7), 31.6 (C5), 29.0 (C10), 28.9 (C4), 28.5 (C3), 23.3 (C13), 23.1 (C6), 22.6 (C2), 14.1 (C1); HRMS (ESI) calcd for $C_{13}H_{23}NNaO_3S$ $[M + Na]^+$ 296.1291, found 296.1291.

***S*–2–Acetamidoethyl 3–hydroxy–5–phenyl[3–^{13}C]pentanethioate (136)**

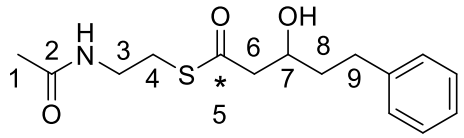


(136)

This new compound was prepared following a modified literature protocol.[39] To a flame dried round bottom flask containing 20 mL of dried acetonitrile (ACN) was added **(208)** (0.700 g, 2.36 mmol) followed by K_2CO_3 (1.17 g, 7.09 mmol) at room temperature under an Ar atmosphere. Compound **(45)** (0.340 g, 3.07 mmol) was added to the reaction mixture and stirred for 20 min under an Ar atmosphere. The solvent was removed *in vacuo*, and the product was taken up in 20 mL of EtOAc and 20 mL of water. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (65% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 3–hydroxy–5–phenyl[3–^{13}C]pentanethioate **(136)** as a pale yellow oil, R_f = 0.16 (EtOAc), (440 mg, 63% yield). IR ($CHCl_3$ cast film): 3297, 3085, 2929, 2862, 1687, 1656, 1550 cm^{-1}; ^{1}H NMR (500 MHz $CDCl_3$) δ 7.30–7.27 (m, 2H, ArH), 7.21– 7.17 (m, 3H, ArH), 5.73 (br s, 1H, NH), 4.24–4.20 (m, 0.5H, H7), 3.94–3.92 (m, 0.5H, H7), 3.47–3.42 (m, 2H, H3), 3.10–3.00 (m, 2H, H4), 2.84–2.67 (m, 5H, H6,9, OH), 1.96 (s, 3H, H1), 1.96–1.74 (m, 2H, H8); ^{13}C NMR (126 MHz $CDCl_3$) δ 199.4 (C5), 170.6 (C2), 141.6 (ArC), 128.48 (ArC), 128.45 (ArC), 126.0 (ArC), 68.2 (^{13}C, C7), 51.1 (d, C6), 39.2 (C9), 38.3 (d, C8), 31.8

(C3), 28.9 (C4), 23.2 (C1); HRMS (ESI) calcd for $C_{14}{}^{13}CH_{21}NNaO_3S$ $[M + Na]^+$ 319.1168, found 319.1162.

_S_–2–Acetamidoethyl 3–hydroxy–5–phenyl[1–^{13}C]pentanethioate (138)

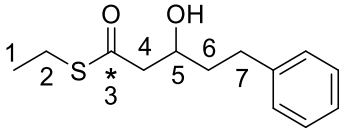


(138)

This new compound was synthesized following a modified literature protocol.[39] To a flame dried round bottom flask containing 20 mL of dried ACN was added compound **(210)** (0.600 g, 1.56 mmol) followed by K_2CO_3 (0.650 g, 4.67 mmol) at room temperature under an Ar atmosphere. Compound **(45)** (0.370 g, 3.11 mmol) was added to the reaction mixture while stirring for 20 min under Ar atmosphere. After stirring, the solvent was removed *in vacuo*, and the product was taken up in 20 mL of EtOAc and 20 mL of water. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 3–hydroxy–5–phenyl[1–^{13}C]pentanethioate **(138)** as a pale-yellow oil, $R_f = 0.19$ (EtOAc), (420 mg, 90% yield). IR ($CHCl_3$ cast film): 3294, 3085, 2927, 1652, 1550 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.29–7.26 (m, 2H, ArH), 7.21 – 7.16 (m, 3H, ArH), 5.79

(br s, 1H, NH), 4.05–4.03 (m, 1H, H7), 3.43–3.39 (m, 2H, H3), 3.03–3.00 (m, 2H, H4), 2.79–2.67 (m, 5H, H6,9,OH), 1.91 (s, 3H, H1), 1.80–1.73 (m, 2H, H8); ^{13}C NMR (126 MHz, CD_2Cl_2) δ 199.3 (C5), 170.5 (C2), 142.3 (ArC), 128.8 (ArC), 128.7 (ArC), 126.2 (ArC), 68.4 (C7), 51.8 (d, C6), 39.3 (C9), 38.8 (C8), 32.1 (C3), 29.4 (C4), 23.4 (C1); HRMS (ESI) calcd for $C_{14}{}^{13}CH_{21}NNaO_3S$ $[M + Na]^+$ 319.1168, found 319.1164.

_S_–Ethyl 3–hydroxy–5–phenyl[1–^{13}C]pentanethioate (140)

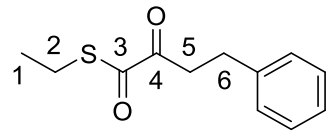


(140)

This new compound was synthesized following a modified literature protocol.[39] To a flame dried round bottom flask containing 20 mL of dried ACN was added compound **(210)** (1.50 g, 3.88 mmol) followed by K_2CO_3 (1.61 g, 11.7 mmol) at room temperature under Ar atmosphere. Ethanethiol (0.420 mL, 5.83 mmol) was added to the reaction mixture while stirring for 60 min under an Ar atmosphere. The solvent was removed *in vacuo*, and the product was taken up in 20 mL of EtOAc and 20 mL of water. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (20%

EtOAc in Hex) affording the product *S*–ethyl 3–hydroxy–5–phenyl[1–^{13}C]pentanethioate **(140)** as a pale yellow oil, R_f = 0.32 (EtOAc), (750 mg, 81% yield). IR ($CHCl_3$ cast film) 3438, 2930, 2872, 1643 cm^{-1}; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.30–7.27 (m, 2H, ArH), 7.21–7.18 (m, 3H, ArH), 4.09–4.04 (m, 1H, H5), 2.93–2.88 (m, 2H, H2), 2.84–2.67 (m, 5H, H7,4,OH), 1.87–1.71 (m, 2H, H6), 1.26 (t, *J* = 7.5 Hz, 3H, H1); ^{13}C NMR (126 MHz, CD_2Cl_2) δ 199.8 (^{13}C3), 141.8 (ArC), 128.6 (ArC), 128.5 (ArC), 126.1 (ArC), 68.1 (C5), 50.7 (d, C4), 38.2 (C6), 31.9 (C7), 23.6 (C2), 14.7 (C1); HRMS (ESI) calcd for $C_{12}{}^{13}CH_{18}NaO_2S$ $[M + Na]^+$ 262.0953, found 262.0950.

_S_–Ethyl 2–oxo–4–phenylbutanethioate (141)

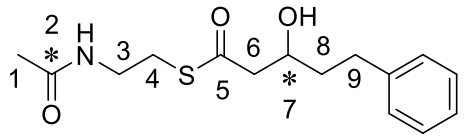


(141)

This compound was prepared by the following method. To a round bottom flask was added **(140)** (70.0 mg, 0.290 mmol) followed by 20 mL of DCM, and then DMP (0.683 mg, 1.61 mmol) was added and allowed to stir at room temperature for 6 h. The reaction miture was quenched with 1:1 $Na_2S_2O_3$/$NaHCO_3$ (5 mL). The mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (2 x 5 mL), then dried over brine and Na_2SO_4, filtered, and concentrated *in*

vacuo. The residue was purified using column chromatography (16.7% EtOAc in Hex) affording the product *S*–ethyl 2–oxo–4–phenylbutanethioate **(141)** as a yellow oil, R_f = 0.59 (15% EtOAC in Hex), (45.7 mg, 70% yield). IR ($CHCl_3$ cast film): 3086, 3063, 2956, 2926, 2853, 1723, 1667 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H, ArH), 7.22–7.20 (m, 3H, ArH), 3.15 (t, *J* = 7.5 Hz, 2H, H5), 2.97–2.91 (m, 4H, H6,2), 1.29 (t, *J* = 7.5 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8 (C4), 191.2 (C3), 140.1 (ArC), 128.6 (ArC), 128.4 (ArC), 126.4 (ArC), 38.2 (C5), 29.0 (C6), 23.1 (C2), 14.1 (C1); HRMS (ESI) calcd for $C_{12}H_{14}NaO_2S$ $[M + Na]^+$ 245.0607, found 245.0615.

[1–^{13}C]*S*–2–Acetamidoethyl 3–hydroxy–5–phenyl[3–^{13}C]pentanethioate (142)

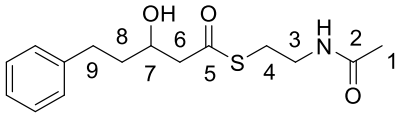


(142)

This new compound was synthesized following a method adapted from literature.[39] To a flame dried round bottom flask containing 20 mL of dried ACN was added compound **(208)** (0.15 g, 0.51 mmol) followed by K_2CO_3 (0.21 g, 1.5 mmol) at room temperature under Ar atmosphere. Compound **(212)** (0.09 g, 0.76 mmol) was added to the reaction mixture while stirring for 20 min under an Ar atmosphere. After stirring, the solvent was removed *in vacuo*, and the product was taken up in 20 mL of EtOAc and 20 mL of

water. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product [1–^{13}C]*S*–2–acetamidoethyl 3–hydroxy–5–phenyl[1–^{13}C]pentanethioate **(142)** as a pale-yellow oil, R_f = 0.15 (EtOAc), (139 mg, 92% yield). IR ($CHCl_3$ cast film): 3296, 3061, 2930, 1687, 1612 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H, ArH), 7.20–7.18 (m, 3H, ArH), 5.77 (br s, 1H, NH), 4.22 (m, 0.5H, H7), 3.93 (m, 0.5H, H7), 3.48–3.42 (m, 2H, H3), 3.07–3.00 (m, 2H, H4), 2.84–2.67 (m, 5H, H6,9,OH), 1.96 (d, *J* = 6.0Hz, 3H, H1), 1.86–1.74 (m, 2H, H8); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.4 (C5), 170.5 (C2), 141.6 (ArC), 128.5 (ArC), 128.4 (ArC), 126.0 (ArC), 68.0 (C7), 51.0 (d, C6), 39.3 (C9), 38.5 (d, C8), 32.6 (C3), 28.9 (C4), 23.5 (d, C1); HRMS (ESI) calcd for $C_{13}{}^{13}C_2H_{21}NNaO_3S$ $[M + Na]^+$ 320.1201, found 320.1202.

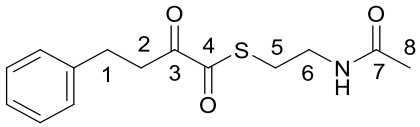
_S_–2–Acetamidoethyl 3–hydroxy–5–phenylpentanethioate (144)



(144)

This new compound was prepared following a modified literature protocol.[39] To a round bottom flask containing 20 mL of dry ACN, was added **(213)** (0.25 g, 0.85 mmol) followed by K_2CO_3 (0.420 g, 2.54 mmol) at room temperature, under Ar atmosphere. HSNAC **(45)** (0.130 g, 1.10 mmol) was then added to the reaction mixture, then the mixture was stirred for 20 min under an Ar atmosphere. The solvent was then removed *in vacuo* and the residue was taken up in 20 mL EtOAc and 20 mL water. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (65% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 3–hydroxy–5–phenylpentanethioate **(144)** as a yellow oil, R_f = 0.23 (EtOAc), (142.6 mg, 57% yield). IR ($CHCl_3$ cast film): 3289, 3084, 3061, 3025.19, 2936, 2864, 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30–7.27 (m, 2H, ArH), 7.21–7.18 (m, 3H, ArH), 5.90 (s, 1H, NH), 4.09–4.06 (m, 1H, H7), 3.48–3.42 (m, 2H, H3), 3.08–3.01 (m, 2H, H4), 2.84–2.67 (m, 4H, H6,9), 1.96 (s, 3H, H1), 1.86–1.75 (m, 2H, H8); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.5 (C5), 170.5 (C2), 141.6 (ArC), 128.5 (ArC), 128.5 (ArC), 126.1 (ArC), 68.1 (C7), 51.1 (C9), 39.3 (C3), 38.3 (C8), 31.8 (C6), 29.0 (C4), 23.3 (C1); HRMS (ESI) calcd for $C_{15}H_{21}NNaO_3S$ $[M + Na]^+$ 318.1134, found 318.1137.

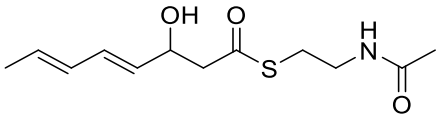
***S*–2–Acetamidoethyl 2–oxo–4–phenylbutanethioate (145)**



(145)

This new compound was prepared by the following method. To a round bottom flask containing 20 mL DCM was added **(144)** (138 mg, 0.470 mmol) followed by DMP (991 mg, 2.34 mmol) while stirring at room temperature. The reaction mixture was then allowed to stir for 6 h. The reaction mixture was quenched with 1:1 $Na_2S_2O_3$:$NaHCO_3$ (10 mL) and then extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 x 10 mL), dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (40% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 2–oxo–4–phenylbutanethioate **(145)** as a yellow oil, R_f = 0.09 (50% EtOAc in Hex), (81.9 mg, 63% yield). IR ($CHCl_3$ cast film): 3330, 3063, 2932, 1722, 1672 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H, ArH), 7.22–7.17 (m, 3H, ArH), 5.78 (s, 1H, NH), 3.47–3.43 (m, 2H, H6), 3.17–3.14 (m, 2H, H2), 3.11–3.08 (m, 2H, H5), 2.98–2.95 (m, 2H, H1), 1.98 (s, 3H, H8); ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.3 (C3), 191.3 (C4), 170.6 (C7), 141.9 (ArC), 131.8 (ArC), 128.1 (ArC), 126.6 (ArC), 39.0 (C6), 38.3 (C2), 29.1 (C1), 28.6 (C5), 23.3 (C8); HRMS (ESI) calcd for $C_{14}H_{17}NNaO_3S$ $[M + Na]^+$ 302.0821, found 302.0824.

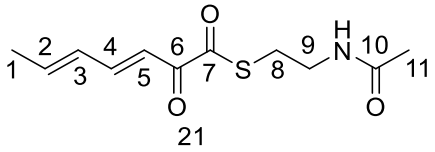
(*4E,6E*)–*S*–2–Acetamidoethyl 3–hydroxyocta–4,6–dienethioate (146)



(146)

This known compound was synthesized following a literature protocol.[48] To a stirred solution of **(214)** (0.60 g, 2.3 mmol) in 10 mL ACN was added K_2CO_3 (1.15 g, 7.00 mmol) and **(45)** (0.33 g, 2.8 mmol). The reaction mixture was stirred until the yellow color disappeared (40 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*4E,6E*)–*S*–2–acetamidoethyl 3–hydroxyocta–4,6–dienethioate **(146)** as a clear oil, R_f = 0.08 (50% EtOAc in Hex), (380 mg, 63% yield). IR ($CHCl_3$, cast film): 3298, 3090, 3019, 2932, 2878, 1658, 1551 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 6.23 (dd, *J* = 15.3, 10.4 Hz, 1H), 6.05–5.95 (m, 1H), 5.90 (br s, 1H), 5.73 (dq, *J* = 13.8, 6.7 Hz, 1H), 5.59–5.51 (m, 1H), 4.67–4.61 (m, 1H), 3.50 – 3.38 (m, 2H), 3.11–2.99 (m, 2H), 2.87–2.74 (m, 2H), 2.70 (br s, 1H), 1.95 (s, 3H), 1.77–1.73 (m, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 198.5, 170.5, 131.6, 131.1, 130.4, 130.4, 69.4, 51.0, 39.3, 28.9, 23.2, 18.2; HRMS (ESI) calcd for $C_{12}H_{19}NNaO_3S$ $[M + Na]^+$ 280.0978, found 280.0980.

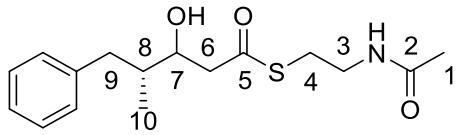
(*3E,5E*)–*S*–2–Acetamidoethyl 2–oxohepta–3,5–dienethioate (147)



(147)

This new compound was prepared by the following method. To a stirred solution of **(146)** (0.28 g, 1.1 mmol) in 20 mL of DCM exposed to air, DMP (0.91 g, 2.4 mmol) was added and the reaction mixture was stirred for 2 h. The reaction mixture was quenched by addition of 10 mL of 1:1 mixture of saturated $NaHCO_3$:$Na_2S_2O_3$ and stirred for 5 min. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 x 15 mL), then with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (5% EtOAc in Hex) affording the product (*3E,5E*)–*S*–2–acetamidoethyl 2–oxohepta–3,5–dienethioate **(147)** as a yellow oil, R_f = 0.41 (EtOAc), (171 mg, 66% yield). IR ($CHCl_3$, cast film): 3299, 3085, 2927, 2855, 1718, 1666, 1549 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.57 (dd, 1H, *J* = 15.5, 11.0 Hz, H5), 6.68 (d, 1H, *J* = 16.0 Hz, H4), 6.44–6.30 (m, 2H, H2,3), 5.77 (br s, 1H, NH), 3.48 (q, 2H, *J* = 6.5 Hz, H9), 3.12 (t, 2H, *J* = 6.5 Hz, H8), 1.97 (s, 3H, H11), 1.93 (d, 3H, *J* = 6.5 Hz, H1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.8 (C6), 183.0 (C7), 170.3 (C10), 150.0 (C5), 145.4 (C3), 131.0 (C2), 118.9 (C4), 39.0 (C9), 28.6 (C8), 23.3 (C11), 19.2 (C1); HRMS (ESI) calcd for $C_{11}H_{16}NO_3S$ $[M + H]^+$ 242.0845, found 242.0848.

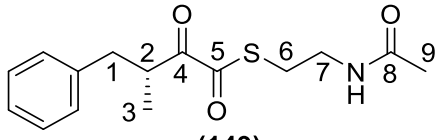
(*4R*)–*S*–2–Acetamidoethyl 3–hydroxy–4–methyl–5–phenylpentanethioate (148)



(148)

This new compound was synthesized following a modified literature protocol.[48] To a stirred solution of **(218)** (0.600 g, 1.94 mmol) in 20 mL ACN was added K_2CO_3 (0.960 g, 5.82 mmol) and HSNAC (0.300 g, 2.52 mmol). The reaction mixture was stirred until the yellow color disappeared 30 min. The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*4R*)–*S*–2–acetamidoethyl 3–hydroxy–4–methyl–5–phenylpentanethioate **(148)** as a pale-yellow oil, R_f = 0.06 (50% EtOAc in Hex), (420 mg, 70% yield). IR ($CHCl_3$, cast film): 3301, 3084, 2965, 2932, 1687, 1557 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.31–7.28 (m, 2H, ArH), 7.22–7.18 (m, 3H, ArH), 6.16 (br s, 1H, NH), 4.06–4.04 (m, 1H, H7), 3.47–3.43 (m, 2H, H3), 3.10–3.00 (m, 3H, H4,OH), 2.94–2.68 (m, 3H, H6,9), 2.47–2.37 (m, 1H, H9), 1.97 (s, 3H, H1), 1.87 – 1.82 (m, 1H, H8), 0.90 (d, *J* = 7.0 Hz, 3H, H10); ^{13}C NMR (176 MHz, $CDCl_3$) δ 199.7 (C5), 170.6 (C2), 140.6 (ArC), 129.2 (ArC), 128.3 (ArC), 125.9 (ArC), 70.8 (C7), 48.8 (C6), 40.6 (C8), 39.2 (C3), 38.6 (C9), 28.9 (C4), 23.1 (C1), 13.5 (C10); HRMS (ESI) calcd for $C_{16}H_{23}NNaO_3S$ $[M + Na]^+$ 332.1291, found 332.1286.

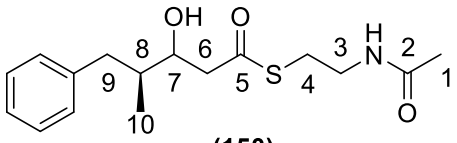
(*R*)–*S*–2–Acetamidoethyl 3–methyl–2–oxo–4–phenylbutanethioate (149)



(149)

This new compound was prepared by the following method. To a round bottom flask was added **(148)** (10.0 mg, 0.030 mmol) followed by 20 mL of DCM. DMP (68.5 mg, 0.160 mmol) was added then stirred for 4 h. The reaction was quenched with 1:1 $Na_2S_2O_3$:$NaHCO_3$ (10 mL). The mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (3 x 10 mL), then dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*R*)–*S*–2–acetamidoethyl 3–methyl–2–oxo–4–phenylbutanethioate **(149)** as a yellow oil, R_f = 0.11 (50% EtOAc in Hex), (5.71 mg, 60% yield). $[\alpha]_D^{25}$ = – 26.90 (*c* = 0.633, $CHCl_3$); IR ($CHCl_3$ cast film): 3411, 3295, 3085, 3064, 3028, 2972, 2934, 2876, 1719, 1676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.29–7.28 (m, 2H, ArH), 7.21–7.15 (m, 3H, ArH), 5.74 (s, 1H, NH), 3.65–3.60 (sextet, *J* = 5 Hz, 1H, H2), 3.44–3.40 (m, 2H, H7), 3.08–3.06 (m, 2H, H6), 3.04–3.01 (dd, *J* = 9.5, 5.5 Hz, 1H, H1a), 2.64–2.61 (dd, *J* = 9.5, 5 Hz, 1H, H1b), 1.96 (s, 3H, H9), 1.14–1.1 (d, *J* = 5Hz, 3H, H3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.7 (C4), 191.2 (C5), 171.0 (C8), 129.2 (ArC), 128.5 (ArC), 126.6 (ArC), 41.6 (C2), 38.9 (C7), 38.4 (C1), 31.6(C9), 28.2 (C6), 15.5 (C3); HRMS (ESI) calcd for $C_{15}H_{19}NNaO_3S$ $[M + Na]^+$ 316.0978, found 316.0977.

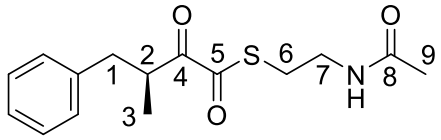
(*4S*)–*S*–2–Acetamidoethyl 3–hydroxy–4–methyl–5–phenylpentanethioate (150)



(150)

This new compound was prepared following a modified literature protocol.[48] To a stirred solution of **(222)** (0.900 g, 2.91 mmol) in 20 mL ACN was added K_2CO_3 (1.44 g, 8.73 mmol) and HSNAC (0.450 g, 3.78 mmol). The reaction mixture was stirred until the yellow color disappeared (30 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product (*4S*)–*S*–2–acetamidoethyl 3–hydroxy–4–methyl–5–phenylpentanethioate **(150)** as a pale-yellow oil, R_f = 0.06 (50% EtOAc in Hex), (790 mg, 88% yield). IR ($CHCl_3$, cast film): 3303, 3084, 2966, 2932, 1687, 1558 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H, ArH), 7.20–7.16 (m, 3H, ArH), 5.95 (br s, 1H, NH), 4.05–4.02 (m, 1H, H7), 3.46–3.42 (m, 2H, H3), 3.07–3.00 (m, 2H, H4), 2.87–2.67 (m, 4H, H6,9a,OH), 2.46–2.41 (m, 1H, H9b), 1.96 (s, 3H, H1), 1.83–1.81 (m, 1H, H8), 0.89 (d, *J* = 7.0 Hz, H10); ^{13}C NMR (176 MHz, $CDCl_3$) δ 199.9 (C5), 170.6 (C2), 140.6, 129.2 (ArC), 128.4 (ArC), 126.0 (ArC), 70.8 (C7), 48.8 (C6), 40.6 (C8), 39.5 (C3), 29.0 (C9), 28.9 (C3), 23.2 (C1), 13.6 (C10); HRMS (ESI) calcd for $C_{16}H_{23}NNaO_3S$ $[M + Na]^+$ 332.1291, found 332.1286.

(*S*)–*S*–2–Acetamidoethyl 3–methyl–2–oxo–4–phenylbutanethioate (151)

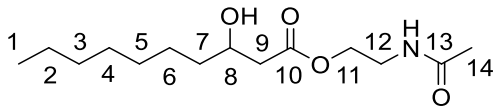


(151)

This new compound was prepared by the following method. To a round bottom flask was added **(150)** (10.0 mg, 0.030 mmol) followed by 20 mL of DCM. The DMP (68.5 mg, 0.160 mmol) was added then stirred for 3 h at room temperature. The reaction was then quenched with 1:1 $Na_2S_2O_3$:$NaHCO_3$ (10 mL). The mixture was extracted with Et_2O (3 x 10 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (3 x 10 mL), then dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (40% EtOAc in Hex) affording the product (*S*)–*S*–2–acetamidoethyl 3–methyl–2–oxo–4–phenylbutanethioate **(151)** as a yellow oil, R_f = 0.09 (40% EtOAc in Hex), (5.9 mg, 62% yield). $[\alpha]_D^{25}$ = + 28.09 (*c* = 0.603, $CHCl_3$); IR ($CHCl_3$ cast film) 3413, 3287, 3086, 3064, 3028, 2966, 2930, 2875, 2855, 1720, 1676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.29–7.24 (m, 2H, ArH), 7.22–7.14 (m, 3H, ArH), 5.70 (s, 1H, NH), 3.60 (m, 1H, H2), 3.46–3.41 (m, 2H, H7), 3.08–3.06 (m, 2H, H6), 3.04–3.02 (dd, *J* = 10.0, 5.0, 1H, H1a), 2.64–2.61 (dd, *J* = 9.5, 5.5 Hz, 1H, H1b), 1.98 (s, 3H, H9), 1.40–1.30 (dd, *J* = 5 Hz, 3H, H3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.7 (C4), 191.2 (C5), 170.4 (C8), 129.2 (ArC), 128.0 (ArC), 126.6 (ArC), 41.6 (C2), 38.9 (C7), 38.4 (C1), 28.6 (C6),

23.2 (C9), 15.5 (C3); HRMS (ESI) calcd for $C_{15}H_{19}NNaO_3S$ $[M + Na]^+$ 316.0978, found 316.0983.

2–Acetamidoethyl 3–hydroxydecanoate (152)

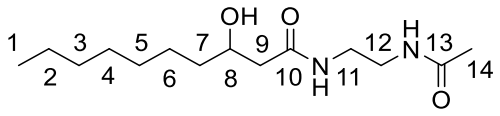


(152)

This new compound was prepared following a modified protocol. To a round bottom flask was added **(200)** (1.00 g, 3.45 mmol) followed by DMAP (0.210 g, 1.72 mmol) and 100 mL of dry DCM at room temperature. *N*–acetyl ethanolamine (1.43 g, 13.8 mmol) was added while stirring for 24 h. The layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (30–100% EtOAc in Hex) affording the product 2–acetamidoethyl 3–hydroxydecanoate **(152)** as a white solid, R_f = 0.19 (EtOAc), (870 mg, 92% yield). IR ($CHCl_3$, cast film): 3552, 3289, 3094, 2923, 2850, 1718, 1640 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.92 (br s, 1H, NH), 4.25–4.17 (m, 2H, H11), 4.02 (m, 1H, H8), 3.53 (q, *J* = 5.5 Hz, 2H, H12), 2.83 (d, *J* = 3.5 Hz, 1H, OH), 2.52 (dd, *J* = 16.0, 3.0 Hz, 1H, H9a), 2.41 (dd, *J* = 16.0, 9.5 Hz, 1H, H9b),1.98 (s, 3H, H14), 1.54–1.27 (m, 12H, H2,3,4,5,6,7), 0.88 (t, *J* = 7.5 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8 (C10),

170.5 (C13), 68.3 (C8), 63.5 (C11), 41.8 (C9), 38.7 (C12), 36.8 (C7), 31.8 (C6), 29.5 (C5), 29.3 (C4), 25.5 (C3), 23.3 (C14), 22.7 (C2), 14.1 (C1); HRMS (ESI) calcd for $C_{14}H_{27}NNaO_4$ $[M + Na]^+$ 296.1832, found 296.1829.

_N_–(2–Acetamidoethyl)–3–hydroxydecanamide (153)

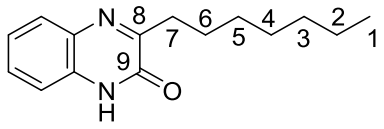


(153)

This new compound was prepared following a modified literature protocol.[39] To a round bottom flask was added **(200)** (1.00 g, 3.45 mmol) followed by 100 mL of dry DCM at room temperature, then *N*-acetyl ethylenediamine (1.40 g, 13.82 mmol) was added to the reaction mixture, and stirred for 60 min. The reaction mixture was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by recrystallization from Et_2O to yield the product *N*–(2–acetamidoethyl)–3–hydroxydecanamide **(153)** as a white solid R_f = 0.29 (acetone), (0.88 g, 93% yield). IR ($CHCl_3$ cast film): 3299, 3095, 2919, 2851, 1630, 1567 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.61, (br s, 1H, NH10), 6.19 (br s, 1H, NH13), 3.95 (m, 1H, H8), 3.60 (br s, 1H, OH), 3.43–3.35 (m, 2H, H11,12), 2.36 (dd, *J* = 15.0, 2.5 Hz, 1H, H9a), 2.25 (dd, *J*

= 15.0, 9.0 Hz, 1H, H9b), 1.99 (s, 3H, H14), 1.52–1.24 (m, 12H, H2,3,4,5,6,7), 0.88 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.6 (C10), 171.6 (C13), 68.8 (C8), 42.8 (C9), 40.2 (C11), 40.1 (C12), 37.1 (C7), 31.8 (C6), 29.5 (C5), 29.3 (C4), 25.5 (C3), 23.3 (C14), 22.7 (C2), 14.1 (C1); HRMS (ESI) calcd for $C_{14}H_{28}N_2NaO_3$ [M + Na]$^+$ 295.1992, found 295.1993.

3–Heptylquinoxalin–2(1*H*)–one (155)

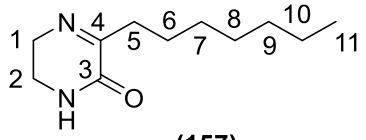


(155)

This new compound was prepared by the following method. To a vial containing 5 mL of dry DCM was added **(135)** (20.0 mg, 0.069 mmol), followed by **(154)** (14.9 mg, 0.138 mmol), then K_2CO_3 (28.7 mg, 0.207 mmol). The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was quenched by addition of 1 M HCl to pH 5. The layers were separated, and the aqueous layer was washed with DCM (2 x 5 mL). The organic layers were pooled and washed with water, brine and then dried over $MgSO_4$. The solvent was removed *in vacuo* to produce 3–heptylquinoxalin–2(1H)–one **(155)** as an orange/brown solid, R_f = 0.76 (80% EtOAc in Hex), (14.3 mg, 85% yield). IR ($CHCl_3$ cast film): 3011.52, 2955.84, 2914.99, 28849.26, 1667.68 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84–7.82 (d, *J* = 8 Hz, 1H,

ArH), 7.48–7.45 (t, *J* = 7 Hz, 1H, ArH), 7.34–7.31 (t, *J* = 7 Hz, 1H, ArH), 7.22–7.21 (d, *J* = 8 Hz, 1H, ArH), 2.97–2.94 (t, *J* = 8 Hz, 2H, H7), 1.85–1.79 (p, *J* = 15, 7.5 Hz, 2H, H6), 1.49–1.43 (m, 2H, H5), 1.42–1.35 (m, 2H, H4), 1.33–1.27, (m, 4H, H2,3), 0.90–0.84 (t, *J* = 7 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.3 (C9), 155.8 (C8), 132.8 (ArC), 130.8 (ArC), 129.6 (ArC), 128.9 (ArC), 124.1 (ArC), 115.1 (ArC), 33.7 (C7), 31.8 (C5), 29.6 (C4), 29.2 (C3), 26.9 (C2), 22.8 (C6), 14.1 (C1); HRMS (ESI) calcd for $C_{15}H_{19}N_2O$ $[M - H]^-$ 243.1503, found 243.1503.

3–Heptyl–5,6–dihydropyrazin–2(1*H*)–one (157)

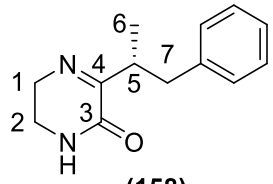


(157)

This new compound was prepared by the following method. To a vial containing 5 mL of dry DCM was added **(135)** (50.0 mg, 0.173 mmol), followed by **(156)** (20.8 mg, 0.346 mmol), then K_2CO_3 (71.6 mg, 0.518 mmol). The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was quenched by addition of 1 M HCl to pH 5. The layers were separated, and the aqueous layers was washed with DCM (2 x 5 mL). The organic layers were pooled and washed with water, brine and then dried over $MgSO_4$. The solvent was removed *in vacuo* to produce 3–heptyl–5,6–dihydropyrazin–2(1H)–one **(157)** as a yellow oil, R_f = 0.10 (50% EtOAc in Hex),

(30.2 mg, 89% yield). IR ($CHCl_3$ cast film): 3219, 3076, 2955, 2926, 2855, 1689, 1632 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 3.75 (t, *J* = 6.3 Hz, 2H, H1), 3.44–3.41 (m, 2H, H2), 2.59 (t, *J* = 7.7 Hz, 2H, H5), 1.60–1.55 (m, 2H, H6), 1.36–1.25 (m, 8H, H7,8,9,10), 0.86 (t, *J* = 13.3 Hz, 3H, H11); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7 (C3), 157.8 (C4), 47.8 (C1), 39.4 (C2), 33.9 (C5), 31.9 (C7), 29.9 (C8), 29.5 (C9), 26.4 (C6), 22.8 (C10), 14.2 (C11); HRMS (ESI) calcd for $C_{11}H_{21}N_2O$ $[M + H]^+$ 197.1648, found 197.1648.

(*R*)–3–(1–Phenylpropan–2–yl)–5,6–dihydropyrazin–2(1*H*)–one (158)

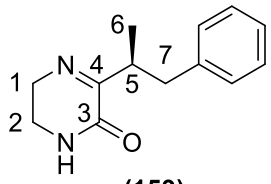


(158)

This new compound was prepared by the following method. To a vial containing 5 mL of dry DCM was added **(149)** (16.0 mg, 0.055 mmol), followed by **(156)** (6.57 mg, 0.109 mmol), then K_2CO_3 (22.6 mg, 0.164 mmol). The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was quenched by addition of 1 M HCl to pH 5. The layers were separated, and the aqueous layer was washed with DCM (2 x 5 mL). The organic layers were pooled and washed with water, brine and then dried over $MgSO_4$. The solvent was removed *in vacuo* to produce (*R*)–3–(1–phenylpropan–2–yl)–5,6–dihydropyrazin–2(1H)–one **(158)** as a yellow oil, R_f = 0.12

(50% EtOAc in Hex), (10.7 g, 91% yield). $[\alpha]_D^{25} = -4.62$ (*c* = 0.75, $CHCl_3$); IR ($CHCl_3$ cast film): 3226, 3062, 2967, 2930, 2871, 1686, 1632 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.17–7.15 (m, 2H, ArH), 7.10–7.06 (m, 3H, ArH), 6.20 (br s, 1H, NH), 3.67–3.63 (m, 2H, H1), 3.41–3.39 (m, 1H, H5), 3.27–3.24 (m, 2H, H2), 2.92 (dd, *J* = 13.3, 5.6 Hz, 1H, H7a), 2.51 (dd, *J* = 13.3, 8.4 Hz, 1H, H7b), 0.99 (d, *J* = 7.0 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5 (C3), 157.6 (C4), 140.5 (ArC), 129.4 (ArC), 128.3 (ArC), 126.1 (ArC), 47.8 (C1), 40.6 (C7), 39.1 (C2), 37.6 (C5), 17.7 (C6); HRMS (ESI) calcd for $C_{13}H_{17}N_2O$ $[M + H]^+$ 217.1335, found 217.1332.

(*S*)–3–(1–Phenylpropan–2–yl)–5,6–dihydropyrazin–2(1*H*)–one (159)

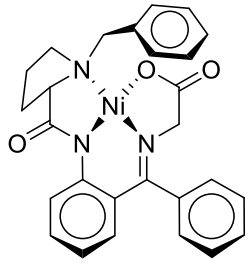


(159)

This new compound was prepared by the following method. To a vial containing 5 mL of dry DCM was added **(151)** (12.0 mg, 0.041 mmol), followed by **(156)** (4.93 mg, 0.082 mmol), then K_2CO_3 (17.0 mg, 0.123 mmol). The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was quenched by addition of 1 M HCl to pH 5. The layers were separated, and the aqueous layer was washed with DCM (2 x 5 mL). The organic layers were pooled and washed with water, brine and then dried over $MgSO_4$. The solvent was removed *in vacuo* to produce (*S*)–3–(1–

phenylpropan–2–yl)–5,6–dihydropyrazin–2(1H)–one **(159)** as a yellow oil, R_f 0.12 (50% EtOAc in Hex), (7.5 mg, 85% yield). $[\alpha]_D^{25}$ = + 2.32 (*c* = 0.75, $CHCl_3$); IR ($CHCl_3$ cast film): 3289, 3083, 3063, 2966, 2928, 1685, 1631 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 7.18–7.15 (m, 2H, ArH), 7.10–7.06 (m, 3H, ArH), 5.95 (br s, 1H, NH), 3.67–3.63 (m, 2H, H1), 3.41–3.39 (m, 1H, H5), 3.26–3.23 (m, 2H, H2), 2.92 (dd, *J* = 13.3, 5.6 Hz, 1H, H7a), 2.51 (dd, *J* = 13.3, 8.4 Hz, 1H, H7b), 0.99 (d, *J* = 7.0 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5 (C3), 157.4 (C4), 140.5 (ArC), 129.4 (ArC), 128.3 (ArC), 126.1 (ArC), 47.8 (C1), 40.6 (C7), 39.2 (C2), 37.6 (C5), 17.7 (C6); HRMS (ESI) calcd for $C_{13}H_{17}N_2O$ $[M + H]^+$ 217.1335, found 217.1332.

Glycine-Ni(II)-(*S*)–*N*–(2–benzoylphenyl)–1–benzylpyrrolidine–2–carboxamide (170)

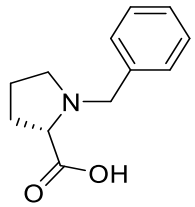


(170)

This compound was prepared by the following protocol.[194] To a round bottom flask compound **(173)** (2.54 g, 6.61 mmol) was dissolved in 35 mL of anhydrous MeOH, followed by glycine (2.48 g, 33.0 mmol) and $Ni(NO_3)_2 \cdot 6H_2O$ (3.84 g, 13.2 mmol). The mixture was heated to 45 °C, followed by the slow addition of freshly ground KOH (2.60 g, 46.3 mmol). The solution was left to stir for 2 h, and then the temperature was

raised to 50 °C and left overnight. The reaction mixture was quenched with glacial acetic acid (2.70 mL, 47.2 mmol). The mixture was rinsed with Milli Q H_2O and then concentrated *in vacuo*. The residue was purified using column chromatography (2.5% MeOH in EtOAc) affording the product **(170)** as a red solid, R_f = 0.23 (10% MeOH in EtOAc), (1.43 g, 44% yield). $[\alpha]_D^{26}$ = 1895.3 (*c* = 0.50, MeOH); IR (MeOH, cast film): 3442, 3057, 3030, 2956, 2821, 1675, 1639, 1590, 1545, 1494, 1471, 1495, 1456, 1441, 1407, 1363, 1336, 1310, 1260, 1166, 1129, 1073, 1029, 964, 927, 861, 811, 755, 725, 704, 657 cm^{-1}; ^1H (500 MHz, $CDCl_3$): δ 8.30 (dd, *J* = 8.5, 1.0 Hz, 1H), 8.07 (d, *J* = 7.0 Hz, 2H), 7.55–7.49 (m, 3H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.21 (td, *J* = 8.0, 2.0 Hz, 1H), 7.10 (d, *J* = 7.0 Hz, 1H), 7.00–6.87 (m, 1H, 1H), 6.80 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.70 (td, *J* = 7.0, 1.0 Hz, 1H), 4.49 (d, *J* = 12.5 Hz, 1H), 3.80 – 3.66 (m, 4H), 3.47 (dd, *J* = 10.5, 5.5 Hz, 1H), 3.40 – 3.30 (m, 1H), 2.61–2.55 (m, 1H), 2.47–2.38 (m, 1H), 2.19–2.12 (m, 1H), 2.11–2.03 (m, 1H); ^{13}C (126 MHz, $CDCl_3$): δ 181.4, 177.3, 171.7, 142.6, 134.7, 133.3, 133.2, 132.2, 131.8, 129.8, 129.6, 129.4, 129.1, 128.9, 126.3, 125.7, 125.2, 124.3, 120.9, 69.9, 63.1, 61.3, 57.5, 30.8, 23.7; HRMS (ESI) calculated for $C_{27}H_{26}N_3NiO_3$ $[M + H]^+$. 498.1322, found 498.1329.

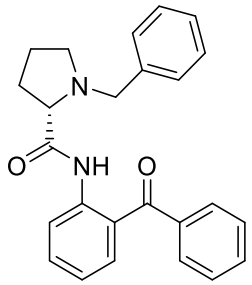
(*S*)–1–Benzylpyrrolidine–2–carboxylic acid (172)



(172)

This known compound was prepared by the following protocol.[194] To a 500 ml round bottom flask, L–proline **(171)** (5.00 g, 43.5 mmol) was dissolved in 35 mL of 2–propanol, followed by freshly ground KOH (8.52 g, 152 mmol). The mixture was heated to 40 °C and stirred until the solids disappeared. Benzyl chloride (7.5 mL, 65.1 mmol) was added dropwise over a period of 5 min, and the reaction mixture was allowed to stir for an additional 30 h maintaining 40 °C. The reaction mixture was quenched, by addition of HCl to pH 4, filtered through celite, and then concentrated *in vacuo*. The crude product was resuspended in acetone and then stored at –20 °C for 1 h. The solids were filtered off and the mother liquor was concentrated again *in vacuo*, resuspended in acetone, stored at –20 °C for 1 h and filtered. The product **(172)** was isolated as a brown solid (7.87 g, 88% yield). Due to the literature precedence of this procedure and the qualitative observations made throughout the reaction, the product (*S*)–1–benzylpyrrolidine–2–carboxylic acid **(172)** was used for the next step without characterization.[194]

(*S*)–*N*–(2–Benzoylphenyl)–1–benzylpyrrolidine–2–carboxamide (173)

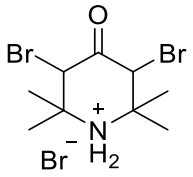


(173)

This known compound was prepared by the following protocol.[194] *N*–benzylproline hydrochloride salt **(172)** (63.0 g, 261 mmol) was dissolved in 300 mL of anhydrous DCM, then cooled to 0 °C. To this cooled solution, DMAP (38.5 g, 315 mmol) was added and the reaction mixture was allowed to stir for 15 min, after which the ice bath was removed. To the reaction mixture EDC·HCl (63.7 g, 332 mmol) was slowly added and the solution was stirred for 1 h. at room temperature. To this mixture, 2–aminobenzophenone (51.4 g, 261 mmol) dissolved in 100 mL of anhydrous DCM was added, followed by the addition of DMAP (12.7 g, 104 mmol). The reaction was then heated to 40 °C and stirred overnight. The reaction was quenched with 10% aqueous K_2CO_3 until the pH was ~8.5 (indicator paper). The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 100 mL). The collected organic phases were combined, washed with brine and dried with Na_2SO_4. The Na_2SO_4 was removed by filtration, the solution was then concentrated *in vacuo.* The residue was purified using column chromatography (15% EtOAc in Hex), then recrystallized from EtOH to

afford the product (*S*)–*N*–(2–benzoylphenyl)–1–benzylpyrrolidine–2–carboxamide (**173**) as a pale yellow solid, R_f = 0.42 (33.3% EtOAc in Hex), (47.7 g, 48% yield). $[\alpha]_D^{26}$ = – 111.1 (*c* = 0.50, DCM); IR (DCM cast film): 3261, 3062, 3029, 2967, 2873, 2808, 1962, 1812, 1690, 1645, 1598, 1578, 1508, 1447, 1375, 1354, 1318, 1290, 1265, 1211, 1180, 1157, 1120, 1076, 1047, 1028, 1000, 980, 938, 924, 894, 806, 753, 700, 670 cm^{-1}; ^1H (500 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.79–7.77 (m, 2H), 7.61 (tt, *J* = 7.5, 1.5 Hz, 1H), 7.55–7.48 (m, 4H,), 7.38 (d, *J* = 6.0 Hz, 1H), 7.32 (d, *J* = 7.0 Hz, 1H), 7.15–7.12 (m, 3H), 7.09 (td, *J* = 7.5, 1.0 Hz, 1H), 3.32 (dd, *J* = 10.0, 5.0 Hz, 1H), 3.22 (td, *J* = 6.5, 2.5 Hz, 1H), 2.41 (ddd, *J* = 9.5, 9.5, 6.5 Hz, 1H), 2.28–2.21 (m, 1H), 2.00–1.94 (m, 1H), 1.81–1.76 (m, 2H); ^{13}C (126 MHz, $CDCl_3$) δ 198.0, 174.6, 139.2, 138.6, 138.1, 133.4, 132.5, 132.5, 130.1, 129.1, 128.3, 128.2, 127.1, 125.3, 122.2, 121.5, 68.3, 59.9, 53.9, 31.0, 24.2; HRMS (ESI) calculated for $C_{25}H_{25}N_2O_2$ $[M + H]^+$ 385.1911, found 385.1907.

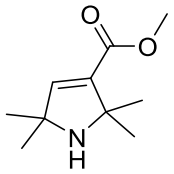
3,5-Dibromo-2,2,6,6-tetramethyl-4-oxopiperidin-1-ium bromide (175)



(175)

To a flame dried round bottom flask was added 2,2,6,6-tetramethyl-4-piperidinone **(174)** (40.0 g, 257 mmol), followed by 125 mL of glacial acetic acid at room temperature, giving a dark yellow solution. A solution of Br_2 (206 g, 1288 mmol) in 95 mL of glacial acetic acid was then added in a dropwise fashion, to the reaction mixture, and left to stir at room temperature for 16 hours. The precipitate that had formed was then filtered and washed using glacial acetic acid followed by diethyl ether, which gradually removed the yellow/orange colour to yield the product 3,5-Dibromo-2,2,6,6-tetramethyl-4-oxopiperidin-1-ium bromide (**175**) as a white powder (76.2 g, 95% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ H 9.41 (1H, br s), 5.49 (2H, s), 1.71 (6H, s), 1.36 (6H, s); ^{13}C NMR (150 MHz, DMSO-d_6) δ C 189.1, 64.7, 60.6, 28.2, 22.5.

Methyl 2,2,5,5–tetramethyl–2,5–dihydro–1H–pyrrole–3–carboxylate (177)

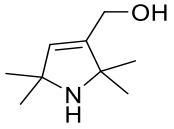


(177)

To a flame dried RBF under an atmosphere of Ar, NaOMe (52.9 g, 980 mmol) was suspended in 400 mL of anhydrous MeOH. This solution was cooled to 0 °C, followed by the addition of a suspension of 3,5–dibromopiperidin–4–one **(175)** (60.92 g, 195.9 mmol) in MeOH. The solution was brought up to room temperature and stirred for 16 h. The solution was concentrated *in vacuo*, suspended in 10% aqueous K_2CO_3, and

extracted with Et_2O (3 x 60 mL). The organic layers were combined, washed with brine (2 x 50 mL), dried over Na_2SO_4, and then concentrated *in vacuo* yielding methyl 2,2,5,5–tetramethyl–2,5–dihydro–1H–pyrrole–3–carboxylate (**177**) as an off yellow, nearly colorless oil. No purification was required (25.7 g, 72% yield). IR (neat): 3357, 2967, 2928, 2868, 1720, 1634, 1436, 1374, 1360, 1328, 1280, 1255, 1210, 1193, 1159, 1062, 1004, 928, 772, 704 cm^{-1}; ^1H (DMSO–d_6) δ 6.63 (s, 1H, C<u>H</u>CC(O)OCH_3), 3.65 (s, 3H, C(O)OC<u>H</u>$_3$), 1.27 (s, 6H, CC<u>H</u>$_3$), 1.17 (s, 6H, CC<u>H</u>$_3$); ^{13}C (DMSO–d_6) δ 163.9, 149.5, 138.1, 65.1, 63.0, 51.1, 30.0, 30.0; HRMS (ESI) calculated for $C_{10}H_{18}NO_2$ $[M + H]^+$184.1332, found 184.1332.

(2,2,5,5–Tetramethyl–2,5–dihydro–1H–pyrrol–3–yl)methanol (178)

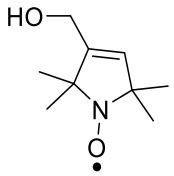


(178)

To a compound **(177)** (2.90 g, 12.8 mmol) under an atmosphere of Ar was added 19 mL of anhydrous toluene. The mixture was cooled to –40 °C and Red-Al (5.67g, 28.1 mmol) was added dropwise. The reaction mixture was brought up to room temperature and stirred for 45 min. The reaction mixture was quenched by adding 20% KOH at 0 °C. The crude product was extracted with Et_2O and then DCM, and the organic layers were pooled, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified using column chromatography (EtOAc) affording the product (2,2,5,5–tetramethyl–

2,5–dihydro–1H–pyrrol–3–yl)methanol **(178)**, R_f = 0.0 (EtOAc), (700 mg, 35% yield). IR (DCM cast film): 3299, 3176, 3055, 2960, 2922, 2869, 2728, 1467, 1443, 1425, 1380, 1364, 1277, 1236, 1194, 1156, 1108, 1035, 1013, 992, 962, 942, 896, 852, 836, 822, 763 cm^{-1}; ^1H (500 MHz, $CDCl_3$) δ 5.55 (t, *J* = 1.5 Hz, 1H), 4.21 (d, *J* = 2.0 Hz, 2H), 1.27 (s, 6H), 1.25 (s, 6H); ^{13}C (125 MHz, $CDCl_3$) δ 147.1, 131.9, 65.9, 63.4, 59.3, 30.9, 30.1; HRMS (ESI) calculated for $C_9H_{18}NO$ $[M + H]^+$ 156.1383, found 156.1382.

3-(Hydroxymethyl)-2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl (179)

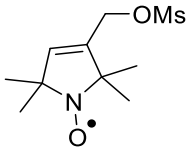


(179)

Compound **(178)** (700 mg, 4.51 mmol) was dissolved in 30 mL of anhydrous DCM under an atmosphere of Ar. The solution was cooled to 0 °C, followed by the addition of *m*CPBA (1.25 g, 7.21 mmol) as a suspension in 20 mL of DCM. The reaction was stirred for 24 h at 0 °C. The mixture was concentrated *in vacuo* and dissolved in Et_2O. The solution was then washed with saturated aqueous $NaHCO_3$ (3 x 20 mL), brine (3 x 20 mL) and dried over Na_2SO_4. The solution was filtered, concentrated *in vacuo*, and the residue was purified using column chromatography (10% acetone in Benzene) affording the product **(179)** as a yellow solid, R_f = 0.12 (10% acetone in benzene), (205 mg, 27% yield). Due to the paramagnetic properties of the molecule, NMR analysis

was not done. IR (DCM cast film): 3367, 2978, 2930, 2857, 2828, 2727, 1654, 1458, 1443, 1428, 1386, 1375, 1359, 1284, 1224, 1199, 1163, 1114, 1058, 1035, 953, 938, 830, 693 cm^{-1}; HRMS (EI) calculated for $C_9H_{16}O_2N$ $[M + H]^+$170.1181, found 170.1181.

1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydropyrrol-3-ylmethane sulfonate (180)

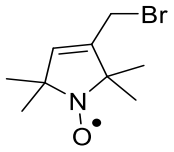


(180)

Compound **(179)** (2.31 g, 13.6 mmol) of was dissolved in anhydrous DCM under an atmosphere of Ar. To this solution, Et_3N (2.46 mL, 17.6 mmol) was added. The solution was stirred for 10 min at room temperature, and then put into an acetone/ice bath (–10 °C) followed by the addition of MsCl (1.16 mL, 14.9 mmol). The acetone/ice bath was removed, and the reaction was stirred for 3.5 hr. The solution was then diluted with DCM, washed with $NaHCO_3$ (3 x 10 mL) and brine (2 x 10 mL). The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and the residue was purified using column chromatography (5% acetone in Benzene) affording the product **(180)** as a yellow solid, R_f = 0.10 (5% acetone in benzene), (3.18 g, 95% yield). Due to paramagnetic properties of the molecule, NMR analysis was not done. IR (DCM cast film): 2978, 2933, 2871, 1725, 1655, 1468, 1428, 1357, 1285, 1226, 1176, 1100, 1056, 1036, 993, 969, 939,

920, 869, 835, 789, 752, 701, 670 cm^{-1}; HRMS (EI) calculated for $C_{10}H_{18}NO_4S$ [M^+] 248.0957, found 248.0951.

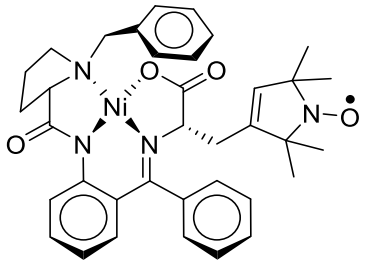
3-(Bromomethyl)-2,5-dihydro-2,2,5,5-tetramethyl-1H-pyrrol-1-yloxy (181)



(181)

To a solution of compound **(180)** (62.1 mg, 0.250 mmol) dissolved in anhydrous acetone, LiBr (50.2 mg, 0.578 mmol) was added. The solution was refluxed for 2 hr. The mixture was concentrated *in vacuo* and the residue was suspended in water. The aqueous solution was extracted with Et_2O (3 x 10 mL) and the organic fractions were dried over $MgSO_4$. The crude mixture was concentrated *in vacuo* and the residue was purified using column chromatography (2.5% acetone in Benzene) affording the product **(181)** as a yellow oil, R_f = 0.40 (5% acetone in benzene), (2.88 g, 96% yield). Due to paramagnetic properties of the molecule, NMR analysis was not done. IR (DCM cast film): 3398, 3055, 2977, 2930, 2866, 1733, 1640, 1467, 1429, 1359, 1321, 1285, 1233, 1209, 1161, 1101, 1057, 998, 955, 937, 898, 849, 816, 695, 673, 659 cm^{-1}. HRMS (EI) calculated for $C_9H_{15}NO^{81}Br$ [M^+] 234.0317, found 234.0311.

Spin labelled Ni-glycinate complex (182)

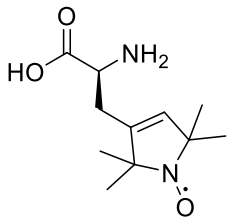


(182)

A solution of the Nickel-Schiff complex compound **(170)** (4.91 g, 9.85 mmol) of was added to a round bottom flask and dissolved in anhydrous DMF (100 mL). The solution was cooled to 0 °C, and then KOtBu (1.77 g, 15.7 mmol) was added slowly. The solution was stirred for 5 min and then compound **(181)** (2.61 g, 11.2 mmol) was added in anhydrous DMF (10 mL). The reaction mixture was left to stir for 45 min at 0 °C, and then brought up to room temperature and stirred for an additional 16 hr. The reaction was quenched using 10 mL of 5% aqueous acetic acid and the crude mixture was concentrated *in vacuo*. The solid was resuspended in water and extracted with DCM (3 x 20 mL). The organic layers were pooled, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified using column chromatography (2.5% acetone in Benzene) affording the product **(182)** as a red solid, R_f = 0.51 (10% MeOH in EtOAc), (5.26 g, 82% yield). Due to the paramagnetic properties of this molecule, NMR analysis was not done. IR (DCM, cast film) 3054, 2975, 2929, 2866, 1679, 1639, 1590, 1545, 1496, 1470, 1441, 1356, 1334, 1311, 1260,

1165 cm^{-1}; $[\alpha]_D^{26}$ = 1512.5; HRMS (ESI) calculated for $C_{36}H_{40}N_4NiO_4$ $[M + H]^+$ 650.2398, found 650.2407.

Spin labelled α-amino acid (183)

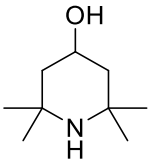


(183)

This compound was prepared by the following protocol adapted from the literature.[203] The Ni(II)-alkylated product **(182)** (1.3 g, 2.0 mmol) was dissolved in a methanol and DCM mixture (2:1, 3 mL/mmol) and added dropwise into a mixture of HCl (3 N, 4 mL) and methanol (4 mL) solution at 65 °C for 30 min. The methanol–water solution was evaporated, and the residue was re-dissolved in water (5 mL) and evaporated to remove the HCl. NH_4OH (5 mL), and then water (5 mL) was added and the mixture was concentrated *in vacuo* to dryness. The residue was then dissolved in water (5 mL) and $CHCl_3$ (5 mL). The organic phase was separated, and the water phase was washed with $CHCl_3$ (2×5 mL). The aqueous phase was evaporated to 10 mL and loaded on an ion-exchange column (DOWEX 50WX8, 100-200 mesh resin) which was pre-washed with water to neutral pH. The column was eluted by water to pH = 7 and then washed with ammonium hydroxide/water (4:1) until all the amino acid eluted. The aqueous

solution collected from the column was concentrated and the semi-pure spin labelled amino acid was collected after lyophilization. The crude sample was dissolved in water and injected on a Phenomenex Luna 5 μ C18(2), 100 Å, AXIA, (21.2 mm ID x 250 mm); prep scale column, flow rate of 20 mL/min (A= H_2O, 1% TFA and B = ACN, 1% TFA); 0–3 min, 10% B; 3–4.5 min, 20% B, 4.5–11 min, 35% B; 11–12 min, 95% B; 12–16 min, 95%; 16–17 min, 10%; 17–22 min, 10%. Compound (**183**) eluted at 7.9 min as a single peak to yield the product after removal of the solvent as an off-white powder. The product was then treated with 5 ml of 10% ammonium hydroxide and allowed to stand at room temperature for 4 h. The ammonium hydroxide was then removed *in vacuo* and then 5 ml of water was added, frozen, then lyophilized to produce the final radical product as a red solid, (315 mg, 69% yield). Due to the paramagnetic properties of the molecule, NMR analysis was not done. IR (DCM, cast film) 3049, 2980, 2936, 1799, 1668, 1468, 1430 cm^{-1}; CD (C, 57.23; H_2O), 22 ºC; $[\Theta]_{260}$ +137; $[\Theta]_{205}$ +2342; $[\Theta]_{195}$ +21; HRMS (ESI) calculated for $C_{11}H_{20}N_2O_3$ $[M + H]^+$ 228.1468, found 228.1467.

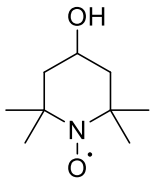
2,2,6,6–Tetramethylpiperidin–4–ol (185)



(185)

This known product was prepared following a literature protocol. To a solution of 2,2,6,6–tetramethyl–4–piperidone **(174)** (38.0 g, 244.8 mmol, 1.0 eq) in ethanol (250 mL), $NaBH_4$ (13.9 g, 367.2 mmol, 1.5 eq) was slowly added using a powder addition funnel. The reaction mixture was stirred at room temperature for 12 h, then quenched by addition of brine (10 mL). The reaction mixture was extracted with EtOAc (3 x 100 mL), and the combined organic phases were dried over K_2CO_3. The solvent was removed *in vacuo* affording the product **(185)** as an off white solid, $R_f = 0.74$ (EtOAc), (36.2 g, 94% yield). IR (solid) 3368, 3176, 2826, 2468 cm^{-1}; ^1H NMR (700 MHz, CD_3OD) δ 4.16–4.11 (m, 1H), 2.04 (dd, J = 14.0, 4.2 Hz, 2H), 1.51–148 (m, 14H); ^{13}C (176 MHz, CD_3OD) δ 62.4, 58.7, 48.6, 30.6, 25.9; HRMS (ESI) calculated for $C_9H_{20}NO$ $[M + H]^+$ 158.1539, found 158.1538.

4–Hydroxy–2,2,6,6–tetramethylpiperidine–1–oxyl (186)

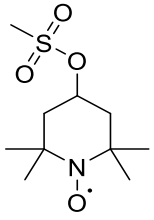


(186)

To a solution of **(185)** (35.0 g, 222.7 mmol) in degassed methanol (250 mL, degassed by purging with Ar) was added $Na_2WO_4 \cdot 2H_2O$ (11.0 g, 33.4 mmol). A solution of H_2O_2 (30% aq, 135.6 mL, 1336.3 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 48 h. The reaction mixture was then diluted with brine

(100 mL), the mixture was extracted with EtOAc (3 x 80 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give nitroxyl **(186)** as an orange solid (36.8 g, 96%) as a single spot by TLC (R_f=0.53, in EtOAc). Due to the paramagnetic properties of the molecule, NMR analysis was not done. IR (thin film) 3418, 2975, 2947, 1471, 1392 cm^{-1}; HRMS (ESI) calculated for $C_9H_{19}NO_2$ $[M + H]^+$ 173.1410, found 173.1406.

4–Methanesulfonyloxy–2,2,6,6–tetramethylpiperidine–1–oxyl (187)

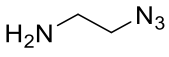


(187)

To a solution of (**186)** (36.5 mg, 211.9 mmol) in dry pyridine (250 mL) under Ar, was added MsCl (24.6 mL, 317.9 mmol) at 0 °C. The reaction mixture was warmed slowly to room temperature and stirred for 12 h. The reaction was quenched with sat. $NaHCO_3$ (100 mL) at 0 °C. The mixture was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (3 × 80 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (20 mL) and H_2O (20 mL) and dried over Na_2SO_4. The solvent was removed, and the residue was dried under high vacuum. The residue was purified using column

chromatography (30 % EtOAc in Hexanes) affording the product **(187)** as an orange-red solid, R_f = 0.75 (EtOAc), (51.4 g, 97% yield). Due to the paramagnetic properties of the molecule, NMR analysis was not done. IR (thin film) 2977, 2939, 1466, 1354, 1171 cm^{-1}; HRMS (ESI) calculated for $C_{10}H_{21}NO_4S$ $[M + H]^+$ 251.1186, found 251.1189.

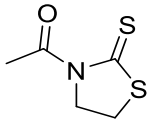
2–Azidoethanamine (191)



(191)

This known compound was prepared following a literature preparation.[204] To a solution of 2–bromoethylamine hydrobromide (700.0 mg 3.42 mmol) in 10 mL of water, sodium azide (666.3 mg, 10.25 mmol) was added and stirred at 75 ºC overnight. The reaction mixture was cooled to room temperature and extracted with Et_2O (3 x 50 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure over an ice bath to yield 2–azidoethanamine (**191**) as a clear oil (273 mg, 93% yield). IR (DCM, cast film): 3647, 2927, 2872, 2102, 1727 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.36 (t, *J* = 5.7 Hz, 1H), 2.88 (t, *J* = 5.7 Hz, 1H), 1.39 (br s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 54.8, 41.5. HRMS (ESI) Calcd for $C_2H_7N_4$ $[M + H]^+$ 87.0665, found 87.0670.

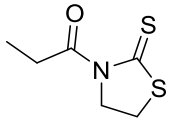
1–(2–Thioxothiazolidin–3–yl)ethenone (192)



(192)

This known compound was prepared using a modified protocol.[205] To a flame dried round bottom flask was added thiazolidine-2-thione (15.0 g, 126 mmol) along with 60 mL dry THF at –78 °C under an Ar atmosphere. *n*–BuLi (2.5 M, 55.4 mL, 138 mmol) was added to the solution and stirred for 30 min. The temperature was then reduced to 0 °C and stirred for a further 15 min. Acetic anhydride (15.5 mL, 164 mmol) was slowly added to the reaction mixture maintaining 0 °C and stirred for 2 h. The reaction mixture was quenched with saturated NH_4Cl (20 mL), and then extracted with Et_2O (3 x 10 mL). The combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (30% EtOAc in Hex) affording the product 1–(2–thioxothiazolidin–3–yl)ethanone **(192)** as a yellow oil, R_f = 0.39 (30% EtOAc in Hex), (18.2 g, 90% yield). IR ($CHCl_3$ cast film): 3004, 2942, 2890, 1696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.58 (t, J = 7.5 Hz, 2H), 3.288 (t, J = 7.5 Hz, 2H), 2.783 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 171.5, 55.7, 28.2, 27.0; HRMS (EI) calcd for $C_5H_7NOS_2$ $[M^{\cdot}]^+$ 160.9969, found 160.9968.

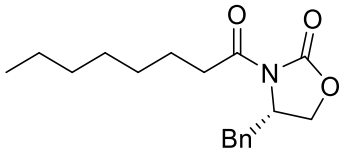
1-(2-thioxothiazolidin-3-yl)propan-1-one (193)



(193)

This known compound was prepared using a modified protocol.[205] To a flame dried round bottom flask was added thiazolidine-2-thione (4.55 g, 38.2 mmol) along with 60 mL dry THF at –78 °C under an Ar atmosphere. *n*–BuLi (2.4 M, 17.5 mL, 41.2 mmol) was added to the solution and stirred for 30 min. The temperature was then reduced to 0 °C and stirred for a further 15 min. Propionic anhydride (7.34 mL, 57.3 mmol) was slowly added to the reaction mixture maintaining 0 °C and stirred for 2 h. The reaction mixture was quenched with saturated NH_4Cl (20 mL), and then extracted with Et_2O (3 x 20 mL). The combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (30% EtOAc in Hex) affording the product 1-(2-thioxothiazolidin-3-yl)propan-1-one **(193)** as a yellow oil, R_f = 0.65 (50% EtOAc in Hex), (5.81 g, 87% yield). IR ($CHCl_3$, cast) 2978, 2938, 1701 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) δ 4.57 (t, 2H, *J* = 7.6 Hz, H2), δ 3.26 (t, 2H, *J* = 7.5 Hz, H1), 3.23 (q, 2H, *J* = 7.2 Hz, H3), 1.14 (dt, 3H, *J* = 15.3, 7.4 Hz, H4); ^{13}C NMR (175 MHz, $CDCl_3$) δ 201.5, 175.6, 56.0, 32.3, 28.3, 8.8. HRMS (ESI) Calcd for $C_6H_9NOS_2Na$ $[M + Na]^+$ 198.0018, found 198.0014.

(*S*)–4–Benzyl–3–octanoyloxazolidin–2–one (194)

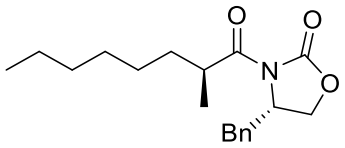


(194)

This known compound was synthesized following a literature protocol.[48] (*S*)-4-benzyloxazolidin-2-one (0.500 g, 2.82 mmol) was dissolved in 20 mL of dry THF and cooled to –78 °C using a dry ice/acetone bath. *n*–BuLi (2.14 M, 1.32 mL) was then added dropwise and the reaction mixture was maintained at –78 °C for 15 min. Octanoyl chloride (0.530 mL, 3.10 mmol) was added *via* syringe and the reaction mixture was allowed to warm over 16 h. The solution was quenched with H_2O (10 mL) and the bulk of the THF was removed *in vacuo*. The aqueous layer was then extracted with DCM (3 x 10 mL) and the organic layers were pooled, washed with brine and dried over Na_2SO_4. The crude product was concentrated *in vacuo* and the residue was purified using column chromatography (33.3% EtOAc in Hex) affording the product (*S*)–4–benzyl–3–octanoyloxazolidin–2–one **(194)** as a clear oil, R_f = 0.65 (33.3% EtOAc in Hex), (723 mg, 86% yield). $[\alpha]_D^{25}$ = + 43.87 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 2955, 2928, 2857, 1785, 1703 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.31 (m, 2H), 7.29–7.26 (m, 1H), 7.22–7.19 (m, 2H), 4.67 (ddt, *J* = 9.7, 7.5, 3.2 Hz, 1H), 4.22–4.13 (m, 2H), 3.30 (dd, *J* = 13.4, 3.3 Hz, 1H), 3.01–2.85 (m, 2H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.76–1.60 (m, 2H), 1.42–1.23 (m, 8H), 0.88 (t, *J* = 7.1, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 153.5, 135.4, 129.5, 129.0, 127.4, 66.2, 55.2, 38.0,

35.6, 31.7, 29.1, 29.1, 24.8, 22.7, 14.1; HRMS (ESI) Calcd for $C_{18}N_{26}NO_3$ $[M + H]^+$ 304.1907, found 304.1909.

(*S*)–4–Benzyl–3–((*S*)–2–methyloctanoyl)oxazolidin–2–one (195)

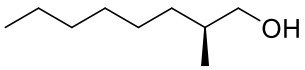


(195)

This known compound was synthesized following a literature protocol.[48] A solution of **(194)** (0.500 g, 1.65 mmol) in 10 mL of dry THF was blanketed with Ar and cooled to –78 °C. NaHMDS (1 M, 1.82 mL, 1.82 mmol) was added dropwise *via* syringe and the reaction mixture was stirred for 30 min at –78 °C before methyl iodide (0.31 mL, 4.9 mmol) was added. The reaction mixture was warmed to room temperature over 16 h and was quenched with saturated NH_4Cl. The THF was removed *in vacuo* and replaced with 10 mL of EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The organic layers were then pooled, washed with brine, and then dried over Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (14% EtOAc in Hex) affording the product (*S*)–4–benzyl–3–((*S*)–2–methyloctanoyl)oxazolidin–2–one **(195)** as a pale-yellow oil, R_f = 0.45 (14% EtOAc in Hex), (2.41 g, 92% yield). $[\alpha]_D^{25}$ = + 60.77 (*c* =

1.0, $CHCl_3$); IR ($CHCl_3$, cast): 2957, 2929, 2858, 1783, 1699, 1455, 1386, 1350 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 7.37–7.29 (m, 2H), 7.31–7.23 (m, 1H), 7.24–7.18 (m, 2H), 4.68 (ddt, *J* = 9.6, 7.4, 3.2 Hz, 1H), 4.23–4.13 (m, 2H), 3.71 (h, *J* = 6.8 Hz, 1H), 3.27 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.77–1.70 (m, 1H), 1.46–1.37 (m, 1H), 1.33–1.23 (m, 8H), 1.22 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 7.1 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.4, 153.1, 135.4, 129.5, 128.9, 127.3, 66.0, 55.4, 38.0, 37.8, 33.5, 31.7, 29.3, 27.3, 22.6, 17.4, 14.1; HRMS (ESI) Calcd for $C_{19}H_{27}NO_3Na$ [M + Na]$^+$ 340.1883, found 340.1886.

(*S*)–2–Methyloctan–1–ol (196)

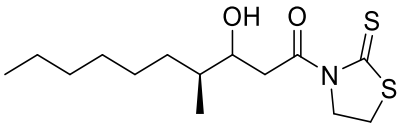


(196)

This known compound was synthesized following a literature protocol.[48] Absolute EtOH (1.06 mL, 18.2 mmol) and **(195)** (4.12 g, 15.1 mmol) were dissolved in dry Et_2O (120 mL) and cooled to 0 °C using an ice–water bath. $LiBH_4$ (2 M, 9.12 mL, 4.56 mmol) was added dropwise *via* syringe and the temperature was maintained at 0 °C for 1.5 h. The reaction mixture was quenched with 1 M NaOH (60 mL) and stirred until the solution was clear. The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 60 mL). The organic layers were pooled, washed with brine, and then dried over anhydrous Na_2SO_4. The organic solvent was removed *in vacuo* to yield a

crude colourless oil. The residue was purified using column chromatography (10% EtOAc in Hex) affording the product (*S*)–2–methyloctan–1–ol (**196**) as a clear oil, R_f = 0.20 (10% EtOAc in Hex), (2.41 g, 92% yield). $[\alpha]_D^{25}$ = – 11.14 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3330, 2957, 2925, 2873, 2857, 1466, 1379 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.49 (dd, *J* = 10.5, 5.8 Hz, 1H), 3.40 (dd, *J* = 10.5, 6.6 Hz, 1H), 1.64–1.55 (m, 1H), 1.42–1.32 (m, 1H), 1.32–1.20 (m, 8H), 1.13–1.03 (m, 1H) 0.90 (d, *J* = 6.7 Hz, 3H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 68.4, 35.8, 33.2, 31.9, 29.6, 27.0, 22.7, 16.6, 14.1; HRMS (ESI) Calcd for C_9H_{18} $[M – H_2O]^+$ 126.1409, found 126.1407.

(*4S*)–3–Hydroxy–4–methyl–1–(2–thioxothiazolidin–3–yl)decan–1–one (198)



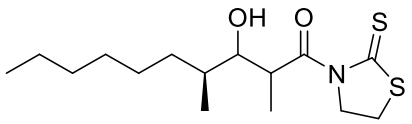
(198)

This known compound was prepared following literature protocol.[48] Oxalyl chloride (1.78 mL, 21.1 mmol) was cooled to –78 °C and a solution of DMSO (dried over 4 Å MS, 1.99 mL, 28.1 mmol) in 60 mL dry DCM was added dropwise. After 30 min at –78 °C, a solution of (**196)** (1.01 g, 7.01 mmol) in 5 mL of DCM was added dropwise and stirred at –78 °C for another 20 min. DIPEA, dried over 4 Å MS (13.34 mL, 105.3 mmol) was added dropwise and the temperature was maintained for 20 min, then

allowed to warm to –50 °C and held there for 60 min. The reaction mixture was quenched with 24 mL saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the combined organic layers were washed with 10 mL of saturated $NaHCO_3$, brine, and then dried on Na_2SO_4. The solvent was removed, and the crude aldehyde **(197)** was used immediately in the next steps. To a stirred solution of **(192)** (0.50 g, 3.1 mmol) in dry DCM (25 mL) was added $TiCl_4$ (1.0 M, 3.1 mL, 3.1 mmol) at –78 ºC under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.46 ml, 3.66 mmol) was added and the reaction mixture was stirred at –78 °C for 1 h. A solution of the freshly prepared crude aldehyde (400 mg, 2.81 mmol) in 5 mL of DCM was then added dropwise to the preformed enolate. After 1 h at –78 °C, the reaction mixture was quenched with 10 mL saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with DCM (3 x 10 mL). The organic layers were pooled, washed with brine and then dried on Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (25% EtOAc in Hex) affording the product (*4S*)–3–hydroxy–4–methyl–1–(2–thioxothiazolidin–3–yl)decan–1–one **(198)** as a yellow oil, R_f = 0.17 (25% EtOAc in Hex), (468 mg, 55% yield). IR ($CHCl_3$, cast film): 3518, 2956, 2926, 1697 cm^{-1}; Major diastereomer (anti): ^1H NMR (500 MHz, $CDCl_3$): δ 4.66–4.54 (m, 2H), 4.06–4.01 (m, 1H), 3.50–3.25 (m, 4H), 2.69 (br d, *J* = 4.0 Hz, 1H), 1.70–1.60 (m, 1H), 1.52–1.11 (m, 10H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.90–0.86 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 201.9, 174.8, 71.4, 55.8, 43.4, 38.2, 32.9, 31.9, 29.6, 28.4, 27.3, 22.7, 14.5, 14.2; Minor diastereomer (syn): ^1H NMR (500 MHz, $CDCl_3$): δ 4.66–4.54 (m, 2H), 3.98 (m, 1H), 3.50–3.25 (m, 2H), 2.82 (br d, *J* =

4.0 Hz, 1H), 1.70–1.60 (m, 1H), 1.52–1.11 (m, 10H), 0.93 (d, J = 6.8 Hz, 3H), 0.90–0.86 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 202.0, 174.9, 72.0, 55.8, 42.4, 38.3, 32.4, 31.9, 29.6 28.4, 27.2, 22.7, 15.1, 14.2; HRMS (ESI) Calcd for $C_{14}H_{25}NO_2S_2Na$ $[M + Na]^+$ 326.1219, found 326.1220.

(4*S*)-3-hydroxy-2,4-dimethyl-1-(2-thioxothiazolidin-3-yl)decan-1-one (199)

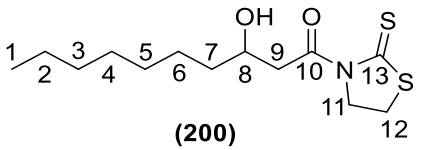


(199)

This known compound was prepared following a literature protocol.[48] To a stirred solution of **(193)** (225 mg, 1.28 mmol) in dry DCM (25 mL) was added $TiCl_4$ (1.0 M, 1.39 mL, 1.39 mmol) at –78 ºC under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.19 ml, 1.52 mmol) was added and the reaction mixture was stirred at –78 °C for 1 h. A solution of the freshly prepared crude aldehyde **(197)** (180 mg, 1.27 mmol) in 5 mL of DCM was then added dropwise to the preformed enolate. After 1 h at –78 °C, the reaction mixture was quenched with 10 mL saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with DCM (3 x 10 mL). The organic layers were pooled, washed with brine and then dried on Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (25% EtOAc in Hex) affording

the product (4*S*)-3-hydroxy-2,4-dimethyl-1-(2-thioxothiazolidin-3-yl)decan-1-one (**199**) as a yellow oil, R_f = 0.24 (25% EtOAc in Hex), (174 mg, 43% yield). (4:1 mix of diastereomers) IR ($CHCl_3$ cast): 3489, 2957, 2927, 2856, 1701, 1464 cm^{-1}; ^{1}H-NMR (500MHz, $CDCl_3$) Major diastereomer (syn): δ 4.80 (qd, 1H, *J* = 7.0, 2.7 Hz), 4.64 – 4.49 (m, 2H), 3.66 (dt, 1H, *J* = 8.7, 3.0 Hz), 3.35 – 3.22 (m, 2H), 2.68 (d, 1H, *J* = 3.6 Hz), 1.76 – 1.67 (m, 1H), 1.55 – 1.46 (m, 1H), 1.45 – 1.22 (m, 8H), 1.19 (app d, 1H, *J* = 7.0 Hz), 1.15 – 1.08 (m, 1H), 0.87 (t, 3H, *J* = 6.9 Hz), 0.84 (d, 3H, *J* = 6.8 Hz); Minor diastereomer (anti): δ 4.72 (dq, 1H, *J* = 8.4, 6.8 Hz), 4.64 – 4.49 (m, 2H), 3.73 (td, 1H, *J* = 8.2, 3.4 Hz), 3.35 – 3.22 (m, 2H), 2.35 (br d, 1H, *J* = 8.5 Hz), 1.65 – 1.55 (m, 1H), 1.55 – 1.46 (m, 1H), 1.45 – 1.22 (m, 8H), 1.17 (app d, 1H, *J* = 7.0 Hz), 1.15 – 1.08 (m, 1H), 0.88 (t, 3H, *J* = 6.9 Hz), 0.84 (d, 3H, *J* = 6.8 Hz); ^{13}C-NMR (125MHz, $CDCl_3$): Major diastereomer: δ 201.7, 179.7, 75.6, 56.5, 41.1, 36.0, 32.7, 32.0, 29.8, 28.2, 26.9, 22.7, 15.5, 14.2, 10.0; Minor diastereomer: δ 202.3, 179.3, 78.1, 56.4, 42.3, 35.0, 32.7, 31.9, 29.6, 28.5, 27.3, 22.7, 15.1, 14.1, 10.0; HRMS (ESI) Calcd for $C_{15}H_{27}NO_2S_2Na$ $[M + Na]^+$ 340.1375, found 340.1377.

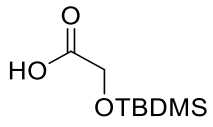
3–Hydroxy–1–(2–thioxothiazolidin–3–yl)decan–1–one (200)



(200)

This new compound was prepared following a modified literature protocol.[39] To a flame dried round bottom flask was added **192** (3.50 g, 21.7 mmol) along with 100 mL DCM at –78 °C under an Ar atmosphere. $TiCl_4$ (23.9 mL, 23.9 mmol) was added to the solution in the round bottom flask and the mixture was stirred for 10 min. DIPEA (3.58 mL, 28.2 mmol) was added and stirred for 1 h. Octanal **(47)** (3.72 mL, 23.88 mmol) was added to the reaction mixture and stirred for 1 h at –78 °C. The reaction mixture was allowed to warm to room temperature while stirred, then quenched with saturated NH_4Cl (20 mL). The reaction mixture was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (30% EtOAc in Hex) affording the product 3–hydroxy–1–(2–thioxothiazolidin–3–yl)decan–1–one **(200)** as a yellow oil, R_f = 0.42 (50% EtOAc in Hex), (4.44 g, 71% yield). IR ($CHCl_3$ cast film): 3509, 3423, 2927, 2855, 1697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.64–4.55 (m, 2H, H11), 4.09 (m, 1H, H8), 3.53–3.49 (dd, *J* = 18.0, 2.0 Hz, 1H, H9), 3.32–3.25 (m, 3H, H12,9), 2.88 (s, 1H, OH), 1.58–1.44 (m, 2H, H7), 1.36–1.28 (m, 10H, H2,3,4,5,6) 0.89–0.86 (t, *J* = 7.0 Hz, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.9 (C13), 174.3 (C10), 68.2 (C8), 55.7 (C11), 45.8 (C9), 36.6 (C7), 31.8 (C6), 29.5 (C5), 29.3 (C4), 28.4 (C12), 25.5 (C3), 22.7 (C2), 14.1 (C1); HRMS (EI) calcd for $C_{13}H_{23}NO_2S_2$ $[M^{\cdot}]^+$ 289.1170, found 289.1177.

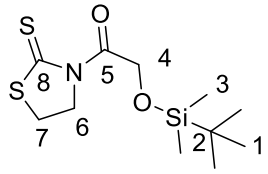
2–(*tert*–Butyldimethylsilyloxy)acetic acid (201)



(201)

This new compound was prepared by a modified method.[206] In 25 mL of dry DCM, methyl glycolate (1.20 g, 13.3 mmol) was dissolved, followed by imidazole (1.81 g, 26.6 mmol), then TBDMSCl (2.41 g, 16.0 mmol). The reaction mixture was stirred for 24 h at room temperature, then quenched by addition of 20 mL of saturated $NaHCO_3$. The mixture was extracted with DCM (3 x 20 mL), and the combined organic layers were dried over brine and $MgSO_4$, filtered, and concentrated *in vacuo*. This crude mixure was then dissolved in 30 mL of MeOH and 30 mL of 2N KOH, then stirred at room temperature for 24 h. The reaction mixture was acidified with 2N HCl to a pH of 2, then extracted with EtOAc (3 x 50 mL), washed with brine, and dried over Na_2SO_4, then concentrated *in vacuo*. This crude product 2–(*tert*–butyldimethylsilyloxy)acetic acid **(201)** was used as is in the next step.

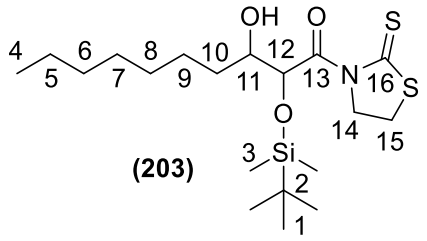
2–(*tert*–Butyldimethylsilyloxy)–1–(2–thioxothiazolidin–3–yl)ethanone (202)



(202)

This new compound was prepared by a modified method.[206] In 50 mL of dry DCM, compound **(201)** from the previous step was dissolved, followed by EDC (3.78 g, 19.7 mmol), then DMAP (0.320 g, 2.63 mmol), and thiazolidine-2-thione (1.72 g, 14.5 mmol) and stirred at 0 ºC for 30 min. The mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was partitioned between 40 mL Et_2O and 40 mL of 0.5 M HCl. The organic layer was washed with brine (2 x 20 mL) and dried over MgSO4, then concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product 2–(tert–butyldimethylsilyloxy)–1–(2–thioxothiazolidin–3–yl)ethanone **(202)** as a yellow solid, R_f = 0.85 (50% EtOAc in Hex), (2.42 g, 63% yield). IR ($CHCl_3$ cast film): 2952, 2929, 2856, 1714 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.12 (s, 2H, H4), 4.61 (t, *J* = 8.0 Hz, 2H, H6), 3.37 (t, *J* = 8.0 Hz, 2H, H7), 0.93 (s, 9H, H1), 0.12 (s, 6H, H3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.0 (C8), 173.7 (C5), 67.0 (C4), 55.8 (C6), 29.5 (C7), 25.8 (C1), 18.6 (C2), –5.3 (C3); HRMS (ESI) calcd for $C_{11}H_{21}NNaO_2S_2Si$ $[M + Na]^+$ 314.0675, found 314.0674.

2–(*tert*–Butyldimethylsilyloxy)–3–hydroxy–1–(2–thioxothiazolidin–3–yl)decan–1–one (203)

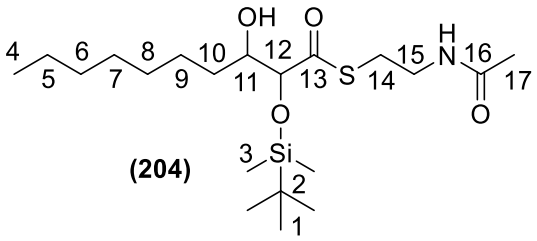


(203)

This new compound was synthesized following a modified protocol.[39] To a stirred solution of **(202)** (0.500 g, 1.72 mmol) in dry DCM (50 mL) was added $TiCl_4$ (1.0 M, 1.89 mL, 1.89 mmol) at –78 ºC under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.280 g, 2.23 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. Octanal **(47)** (0.240 g, 1.89 mmol) was added to the reaction mixture, which was then stirred for 1 h at –78 °C. The reaction mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product 2–(*tert*–butyldimethylsilyloxy)–3–hydroxy–1–(2–thioxothiazolidin–3–yl)decan–1–one **(203)** as a yellow oil, R_f = 0.74 (50% EtOAc in Hex), (510 mg, 71% yield). IR ($CHCl_3$, cast film): 3448, 2953, 2928, 2856, 1717 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.28 (s 1H, H12), 4.62 – 4.47 (m, 2H, H14), 3.97 (m, 1H H11), 3.50 – 3.42 (m, 1H, H15a), 3.25 –

3.19 (m, 1H, H15b), 1.56 (m, 1H, H10a), 1.55–1.24 (m, 11H, H10a,5,6,7,8,9), 0.95 (s, 9H, H1), 0.88 (t, J = 7.2 Hz, 3H, H4), 0.11 (s, 3H, H3a), 0.07 (s, 3H, H3b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9 (C13), 174.7 (C16), 75.7 (C12), 73.3 (C11), 57.1 (C14), 35.0 (C10), 31.9 (C9), 29.6 (C15), 29.4 (C8), 29.2 (C7), 26.0 (C6), 25.9 (C1), 22.8 (C5), 18.5 (C4), 14.2 (C2), –4.4 (C3a), –5.0 (C3b); HRMS (ESI) calcd for $C_{19}H_{37}NNaO_3S_2Si$ $[M + Na]^+$ 442.1876, found 442.1871.

_S_–2–Acetamidoethyl 2–(_tert_–butyldimethylsilyloxy)–3–hydroxydecanethioate (204)

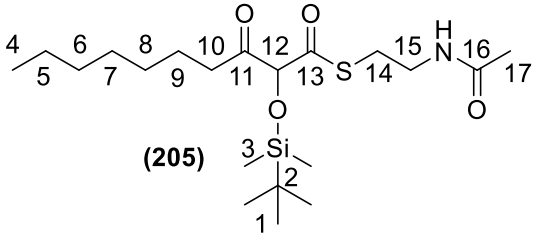


(204)

This new compound was synthesized following a method adapted from the literature.[39] To a stirred solution of **(203)** (0.450 g, 1.07 mmol) in 20 mL ACN, was added K_2CO_3 (0.260 g, 2.14 mmol) and **(45)** (0.440 g, 3.22 mmol). The reaction mixture was stirred until the yellow color disappeared (30 min). The solvent was removed *in vacuo* and the residue was purified using column chromatography (50% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 2–(*tert*–butyldimethylsilyloxy)–3–hydroxydecanethioate **(204)** as a clear oil, R_f = 0.22 (50% EtOAc in Hex), (320 mg, 70% yield). IR ($CHCl_3$,

cast film): 3287, 3080, 2954, 2929, 2856, 1685 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 5.75 (br s, 1H, NH), 4.12 (dd, *J* = 6.0, 3.5 Hz, 1H, H11), 4.01 (d, *J* = 7.0 Hz, 1H, H12), 3.45–3.43 (m, 2H, H14), 3.07–3.05 (m, 1H, H15), 2.96–2.94 (m, 1H, H15), 1.97 (s, 3H, H17), 1.78–1.70 (m, 1H, H10), 1.50–1.48 (m, 1H, H10), 1.48–1.27 (m, 10H, H5,6,7,8,9), 0.89 – 0.86 (m, 12H, H4,1), 0.6 (s, 3H, H3), 0.04 (s, 3H, H3); ^{13}C NMR (176 MHz, $CDCl_3$) δ 205.7 (C13), 170.1 (C16), 78.8 (C12), 73.0 (C11), 40.2 (C15), 39.3 (C10), 34.4 (C9), 31.7 (C8), 29.5 (C14), 29.2 (C7), 28.2 (C6), 25.8 (C5), 25.4 (C1), 22.7 (C17), 18.1 (C2), 14.1 (C4), –4.3 (C3a), –5.0 (C3b); HRMS (ESI) calcd for $C_{20}H_{41}NNaO_4SSi$ $[M + Na]^+$ 442.2418, found 442.2418.

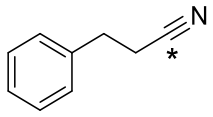
(205)



This new compound was prepared following a modified literature protocol.[48] To a round bottom flask was added **(204)** (0.20 g, 0.47 mmol) followed by 20 mL dry DCM. DMP (0.440 g, 1.03 mmol) was added and stirred for 3 h at room temperature, then quenched with 1:3 $Na_2S_2O_3$:$NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over brine and Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified using column chromatography (50% EtOAc in Hex) affording the product *S*–2–acetamidoethyl 2–

(*tert*–butyldimethylsilyloxy)–3–oxodecanethioate **(205)** as a white solid, R_f = 0.21 (50% EtOAc in Hex), (161.2 mg, 82% yield). IR ($CHCl_3$, cast film): 3289, 2956, 2929, 2857, 1733, 1658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (br s, 1H, NH), 4.83 (s, 1H, H12), 3.50–3.44 (m, 2H, H15), 3.14–3.11 (m, 2H, H14), 1.98 (s, 3H, H17), 1.75–1.26 (m, 12H, H5,6,7,8,9,10), 0.90–0.87 (m, 12H, H1,4), 0.5–0.4 (m, 6H, H3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.2 (C13), 191.9 (C11), 170.2 (C16), 74.1 (C12), 39.0 (C15). 33.7 (C10), 31.8 (C9), 29.3 (C8), 29.1 (C15), 28.5 (C1), 25.7 (C7), 25.2 (C17), 23.3 (C6), 22.6 (C5), 18.3 (C2), 14.1 (C4), –4.7 (C3a), –5.1 (C3b); HRMS (ESI) calcd for $C_{20}H_{39}NNaO_4SSi$ $[M + Na]^+$ 440.2261, found 440.2256.

3–Phenyl[1–^{13}C]propiononitrile (206)

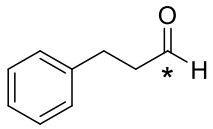


(206)

This known compound was prepared following a modified protocol.[207] In a round bottom flask containing 60 mL of acetone, compound (2-iodoethyl)benzene (1.87 mL, 12.9 mmol) was added, followed by ^{13}C labelled potassium cyanide (^{13}C, 99%) from Cambridge Isotope Laboratories (0.94 g, 14.2 mmol). The reaction mixture was then refluxed for 24 h and then cooled and quenched with saturated $NaHCO_3$ (20 mL). The crude reaction mixture was concentrated *in vacuo* then taken up in 20 mL of DCM and then washed with 20 mL of water. The layers were separated, and the aqueous layer

was extracted with DCM (3 x 15 mL). The combined organic layers were then washed with brine (2 x 15 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified using column chromatography (10% EtOAc in Hex) affording the product 3–Phenyl[1–^{13}C]propiononitrile **(206)** as a yellow oil, R_f = 0.62 (30% EtOAc in Hex), (1.45 g, 85% yield). IR ($CHCl_3$, cast film): 3064, 3030, 2934, 21.94, 1955, 1874, 1812, 1604, 1496 1455, 1425 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.36 (m, 2H), 7.32–7.26 (m, 3H), 2.99 (app q, 2H, *J* = 7 Hz), 2.67–2.63 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1, 128.9, 1.28.3, 129.3, 119.1(^{13}C), 31.6 (d), 19.4 (d); HRMS (EI) calcd for $C_8H_9{}^{13}CN$ [M^+] 132.0769, found 132.0767.

3–Phenyl[1–^{13}C]propanal (207)

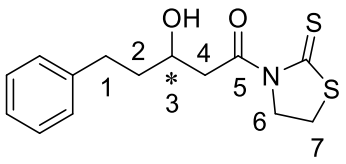


(207)

This known compound was prepared following a modified protocol.[208] To a flame dried round bottom flask containing 20 mL of dry DCM under Ar cooled to –78 ºC, the ^{13}C labelled compound **(206)** (1.00 g, 8.44 mmol), DiBAlH (1 M, 9.29 mL, 9.29 mmol) was added then stirred for 2 h. The reaction mixture was then allowed to warm to room temperature then stirred for 4 h, before adding 15 mL of saturated sodium potassium tartrate was added and allowed to stir for another 30 min. The reaction mixture was diluted with 20 mL of water and the layers were separated. The aqueous layer was

extracted with DCM (3 x 15 mL) and the combined organic layer was then washed with brine (2 x 15 mL), then dried over $MgSO_4$. The solvent was removed *in vacuo*, to yield the crude product 3–phenyl[1–^{13}C]propanal (**207**) which was used in the following reaction. Crude yield 1.07 g, 94% (R_f = 0.71, 30% EtOAc in Hex).

3–Hydroxy–5–phenyl–1–(2–thioxothiazolidin–3–yl)[3–^{13}C]pentan–1–one (208)

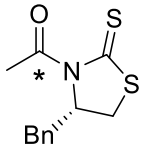


(208)

This new compound was synthesized following a method adapted from literature.[39] To a stirred solution of **(192)** (0.900 g, 5.58 mmol) in dry DCM (25 mL) was added $TiCl_4$ (1.0 M solution in DCM, 6.14 mL, 6.14 mmol) at –78 °C under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.850 g, 6.69 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. ^{13}C Labelled aldehyde **(207)** (0.830 g, 6.14 mmol) was added to the reaction mixture, which was then stirred for 1.5 h at –78 °C. The reaction mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was then removed *in vacuo* and the residue was purified using column chromatography (40% EtOAc in Hex) affording the

product 3–hydroxy–5–phenyl–1–(2–thioxothiazolidin–3–yl)[3–^{13}C]pentan–1–one **(208)** as a yellow oil, R_f = 0.32 (50% EtOAc in Hex), (1.06 g, 64% yield). IR ($CHCl_3$, cast film): 3162, 3023, 2941, 1694, 1495 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H, ArH), 7.22–7.18 (m, 3H, ArH), 4.60–4.56 (m, 2H, H6), 4.24–4.21 (m, 1H, H3), 3.53–3.50 (m, 1H, H1), 3.32–3.28 (m, 3H, H1,7), 2.85–2.70 (m, 2H, H4), 1.94–1.78 (m, 2H, H2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.7 (C8), 174.2 (C5), 141.9 (ArC), 128.5 (ArC), 128.1 (ArC), 126.0 (ArC), 67.4 (^{13}C, C3), 55.8 (C6), 46.1 (d, C4), 38.3 (d, C2), 31.9 (1), 28.5 (C7); HRMS (ESI) calcd for $C_{13}{}^{13}CH_{17}NNaO_2S_2$ $[M + Na]^+$ 319.0626, found 319.0626.

(*S*)–1–(4–Benzyl–2–thioxothiazolidin–3–yl)[1–^{13}C]ethanone (209)

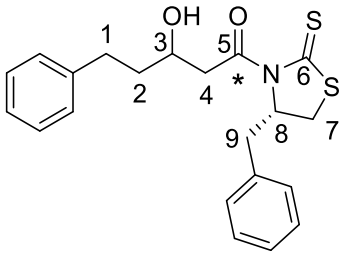


(209)

This known compound was prepared following a modified protocol.[39] In a flame dried round bottom flask compound (*S*)-4-benzylthiazolidine-2-thione (11.0 g, 57.5 mmol) was dissolved in 250 mL of dry THF at 0 ºC and blanketed under Ar. While stirring the mixture, *n*–BuLi (2.5 M in Hex, 25.3 mL, 63.3 mmol) was slowly added and the reaction mixture was allowed to stir for 30 min. The [1-^{13}C] acetyl chloride (4.49 mL, 63.3 mmol) was slowly added and the reaction mixture was allowed to stir for another 60 min before quenching with saturated NH_4Cl (40 mL). The layers were separated,

and the aqueous layer was extracted (3 x 40 mL), then the combined organic layer was washed with water (2 x 40 mL) then brine (2 x 40 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified using column chromatography (10% EtOAc in Hex) affording the product (*S*)–1–(4–benzyl–2–thioxothiazolidin–3–yl)[1–^{13}C]ethanone **(209)** as a yellow solid, R_f = 0.35 (35% EtOAc in Hex), (11.52 g, 79% yield). IR ($CHCl_3$, cast film): 3437, 2967, 2932, 1696 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.33 (m, 2H), 7.30–7.26 (m, 3H), 5.38 (ddd, *J* = 10.8, 7.0, 3.5 Hz, 1H), 3.38 (dd, *J* = 11.3, 9.5 Hz, 1H), 3.22 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.04 (dd, *J* = 13.5, 10.5 Hz, 1H), 2.89 (d, *J* = 11.5 Hz, 1H), 2.79 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 170.7, 136.5, 129.5, 129.0, 127.3, 68.2, 36.7, 31.8 (d), 27.07 (d); $[\alpha]^{25}_D$ = + 248.87 (*c* = 1.0, $CHCl_3$); HRMS (ESI) calcd for $C_{11}{}^{13}CH_{13}NNaOS_2$ $[M + Na]^+$ 275.0364, found 275.0367.

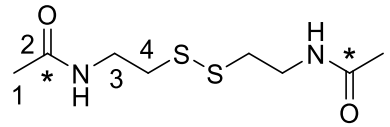
1–((*S*)–4–Benzyl–2–thioxothiazolidin–3–yl)–3–hydroxy–5–phenyl[1–^{13}C]pentan–1–one (210)



(210)

This new compound was synthesized following a modified literature protocol.[39] To a stirred solution of ^{13}C labelled compound **(209)** (3.20 g, 12.7 mmol) in dry DCM (100 mL) was added $TiCl_4$ (1.0 M solution in DCM, 13.9 mL, 13.9 mmol) at –78 ºC under Ar atmosphere and stirred for 10 min. A solution of DIPEA (1.97 g, 15.2 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. 3-Phenylpropanal (1.87 g, 14.0 mmol) was added to the reaction mixture, which was then stirred for 1.5 h at –78 °C. The reaction mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (30% EtOAc in Hex) affording the product 1–(*(S)*–4–benzyl–2–thioxothiazolidin–3–yl)–3–hydroxy–5–phenyl[1–^{13}C]pentan–1–one **(210)** as a yellow oil, R_f = 0.48 (30% EtOAc in Hex), (4.16 g, 85% yield. IR ($CHCl_3$, cast film): 3462, 3061, 3025, 2927, 1651, 1496 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.18 (m, 10H, ArH), 5.42–5.38 (m, 1H, H8), 4.16 (m, 1H, H3), 3.67 (dd, *J* = 19.0, 5.0 Hz, 1H, H4a), 3.42–3.28 (m, 1H, H7a), 3.23–3.13 (m, 2H, H9a,4b), 3.07–3.02 (m, 1H, H9b), 2.91–2.82 (m, 2H, H7b,1a), 2.77–2.71 (m, 1H, H1b), 1.94–1.80 (m, 2H, H2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4 (6), 173.7 (C5), 141.8 (ArC), 136.4 (ArC), 129.3 (ArC), 129.0 (ArC), 128.5 (ArC), 128.4 (ArC), 127.3 (ArC), 125.9 (ArC), 125.8 (ArC), 68.3 (C8), 67.6 (C3), 45.9 (d, C4), 37.9 (C2), 36.8 (C9), 31.8 (C7), 31.7 (C1); HRMS (EI) calcd for $C_{20}{}^{13}CH_{23}NO_2S_2$ [M^+] 386.1204, found 386.1203.

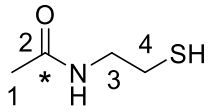
***N,N'*–(2,2'–Disulfanediylbis(ethane–2,1–diyl))[1,1'–$^{13}C_2$]diacetamide (211)**



(211)

This new compound was prepared by the following protocol. To a flame dried round bottom flask, containing 14 mL of pyridine cooled to 0 °C, 2,2'-disulfanediylbis(ethan-1-amine) (0.550 g, 2.45 mmol) was added. After stirring for 5 min, [1-^{13}C] acetyl chloride (1.00 g, 12.2 mmol) was added in a dropwise manner to the reaction mixture. The reaction mixture was quenched by addition of 10 mL of water and the 20 mL of DCM. The layers were separated, and the aqueous layer was extracted (3 x 20 mL) with DCM. The combined organic layers were then washed with brine (2 x 15 mL) then dried over $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified using column chromatography (gradient of EtOAc to acetone) affording the product *N,N'*–(2,2'–disulfanediylbis(ethane–2,1–diyl))[1,1'–$^{13}C_2$]diacetamide **(211)** as a white solid, R_f = 0.26 (acetone), (490 mg, 83% yield). IR ($CHCl_3$ cast film): 3292, 3057, 2972, 2864, 1607, 1540 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.29 (br s, 2H, NH), 3.59–3.55 (m, 4H, H3), 2.83 (t, 4H, *J* = 6.5 Hz, H4), 2.02 (d, *J* = 6 Hz, 3H,); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (C2), 38.6 (C3), 37.9 (C4), 23.2 (d, C1); HRMS (ESI) calcd for $C_6{}^{13}C_2H_{16}N_2NaO_2S_2$ $[M + Na]^+$ 261.0612, found 261.0614.

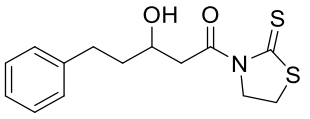
***N*–(2–Mercaptoethyl)[1–^{13}C]acetamide (212)**



(212)

This new compound was prepared by the following protocol. To a round bottom flask compound **(211)** (0.10 g, 0.42 mmol) was added, followed by 20 mL of water and then TCEP (0.24 g, 0.84 mmol) was added under Ar. The reaction mixture was stirred for 12 h. The reaction mixture was then extracted with DCM (3 x 20 mL) and the combined organic layers were washed with brine (2 x 20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified using column chromatography (acetone) affording the product *N*–(2–mercaptoethyl)[1–^{13}C]acetamide **(212)** as a clear oil, R_f = 0.88 (acetone), (94.7 mg, 94% yield). IR ($CHCl_3$, cast film): 3284, 3060, 2979, 2931, 2863, 1607, 1539 cm^{-1}; ^{1}H NMR (500 MHz $CDCl_3$) δ 6.05 (br s, 1H, NH), 3.43–3.39 (m, 2H, H3), 2.68–2.64 (m, 2H, H4), 1.99 (d, *J* = 6 Hz, 3H, H1), 1.35 (t, 1H, *J* = 8.5 Hz, SH); ^{13}C NMR (126 MHz $CDCl_3$) δ 170.1 (C2), 42.5 (C3), 24.7 (C4), 23.2 (d, C1)); HRMS (ESI) calcd for $C_3{}^{13}CH_9NNaOS$ $[M + Na]^+$ 143.0331, found 143.0331.

3–Hydroxy–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one (213)

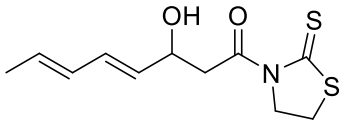


(213)

This known compound was synthesized following a method adapted from literature.[209] To a stirred solution of **(192)** (0.50 g, 3.1 mmol) in dry DCM (25 mL) was added $TiCl_4$ (1.0 M, 3.4 mL, 3.4 mmol) at –78 ºC under an Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.480 g, 3.72 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. 3–Phenyl propanal (0.46 g, 3.4 mmol) was added to the reaction mixture, which was then stirred for 1.5 h at –78 °C. The reaction mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (40% EtOAc in Hex) affording the product 3–hydroxy–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one **(213)** as a yellow oil, R_f = 0.32 (50% EtOAc in Hex), (600 mg, 66% yield). IR ($CHCl_3$, cast film): 3306, 3083, 3059, 3024, 2940, 1695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H), 7.21–7.18 (m, 3H), 4.61–4.57 (m, 2H), 4.13–4.11 (m, 1H), 3.52 (dd, 1H, *J* = 18.0, 2.5 Hz), 3.36 – 3.28 (m, 3H), 3.02 (d, 1H, *J* = 3.5 Hz), 2.84 – 2.70 (m, 2H), 1.92–1.80 (m, 2H); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 201.9, 174.1, 141.8, 128.5, 128.4, 125.9, 67.4, 55.7, 45.8, 38.1, 31.8, 28.4; HRMS (EI) calcd for $C_{14}H_{17}NO_2S_2$ $[M^{\cdot}]^+$ 295.0701, found 295.0700.

(*4E,6E*)–3–Hydroxy–1–(2–thioxothiazolidin–3–yl)octa–4,6–dien–1–one (214)

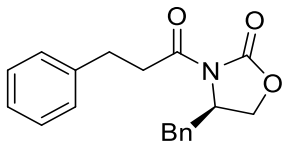


(214)

This known compound was synthesized following literature protocol.[48] To a stirred solution of **(192)** (0.80 g, 5.0 mmol) in dry DCM (25 mL) was added $TiCl_4$ (1.0 M solution in DCM, 5.5 mL, 5.5 mmol) at –78 ºC under Ar atmosphere and stirred for 10 min. A solution of DIPEA (0.83 g, 6.5 mmol) was added. The reaction mixture was stirred at –78 °C for 1 h. (2E,4E)-hexa-2,4-dienal (0.48 g, 5.0 mmol) was added to the reaction mixture, which was then stirred for 1 h at –78 °C. The reaction mixture was allowed to warm to room temperature and quenched by the addition of 10 mL saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using column chromatography (20% EtOAc in Hex) affording the product (*4E,6E*)–3–hydroxy–1–(2–thioxothiazolidin–3–yl)octa–4,6–dien–1–one **(214)** as a yellow oil, R_f = 0.39 (50% EtOAc in Hex), (828 mg, 65% yield). IR ($CHCl_3$, cast film): 3442, 3017, 2928, 1697

cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.25 (dd, *J* = 15.3, 10.4 Hz, 1H), 6.07–5.97 (m, 1H), 5.81–5.69 (m, 1H), 5.64–5.51 (m, 1H), 4.76–4.64 (m, 1H), 4.65–4.54 (m, 2H), 3.60–3.42 (m, 2H), 3.30 (m, 2H), 2.90 (br s, 1H), 2.84 (br s, 1H), 1.76 (m, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 201.8, 173.4, 131.3, 130.7, 130.7, 130.6, 127.6, 68.7, 55.7, 45.8, 28.4, 18.2; HRMS (ESI) calcd for $C_{11}H_{15}NNaO_2S_2$ $[M + Na]^+$ 280.0436, found 280.0437.

(*R*)–4–Benzyl–3–(3–phenylpropanoyl)oxazolidin–2–one (215)

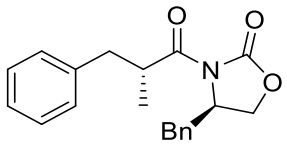


(215)

This known compound was prepared following a modified literature protocol.[210] (*R*)-4-Benzyloxazolidin-2-one (2.50 g, 14.1 mmol) was dissolved in 100 mL of dry THF and cooled to –78 °C using a dry ice/acetone bath. *n*–BuLi (6.21 mL, 15.5 mmol) was then added dropwise and the reaction mixture was maintained at –78 °C for 15 min. 3-Phenylpropanoyl chloride (2.31 mL, 15.5 mmol) was added *via* syringe and the reaction mixture was allowed to warm over 16 h. The solution was quenched with saturated NH_4Cl (10 mL) and the bulk of the THF was removed *in vacuo*. The aqueous layer was then extracted with DCM (3 x 20 mL) and the organic layer was pooled, washed with brine and dried over Na_2SO_4. The crude was concentrated, and the residue was purified

using column chromatography (20% EtOAc in Hex) affording the product (*R*)–4–benzyl–3–(3–phenylpropanoyl)oxazolidin–2–one **(215)** as a white solid, R_f = 0.44 (30% EtOAc in Hex), (4.01 g, 92% yield). $[\alpha]_D^{25} = -60.19$ (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3062, 3028, 2921, 1782, 1699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.29 (m, 7H), 7.25–7.19 (m, 3H), 4.71–4.68 (m, 1H), 4.22–4.17 (m, 2H), 3.3–3.25 (m, 3H), 3.08–3.04 (m, 2H), 2.79 (dd, *J* = 13.0, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 153.4, 140.5, 135.2, 129.5, 129.0, 128.6, 128.5, 127.4, 126.3, 66.2, 55.1, 37.9, 37.2, 30.3; HRMS (ESI) calcd for $C_{19}N_{19}NNaO_3$ $[M + Na]^+$ 332.1257, found 332.1257.

(*R*)–4–Benzyl–3–((*R*)–2–methyl–3–phenylpropanoyl)oxazolidin–2–one (216)

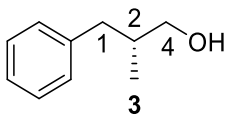


(216)

This known compound was prepared following a modified literature protocol.[211] A solution of **(215)** (3.90 g, 12.7 mmol) in 80 mL of dry THF was blanketed with Ar and cooled to –78 °C. NaHMDS (13.91 mL, 13.91 mmol) was added dropwise *via* syringe and the reaction mixture was stirred at –78 °C for 30 min before MeI (1.02 mL, 16.4 mmol) was added. The reaction mixture was allowed to warm to room temperature over 16 h and was quenched with saturated NH_4Cl. The THF was removed *in vacuo* and replaced with 20 mL of EtOAc. The layers were separated, and the aqueous layer was

extracted with EtOAc (3 x 20 mL). The organic layers were then pooled, washed with brine and dried over Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (5% EtOAc in Hex) affording the product (*R*)–4–benzyl–3–((*R*)–2–methyl–3–phenylpropanoyl)oxazolidin–2–one **(216)** as a pale-yellow oil, R_f = 0.50 (20% EtOAc in Hex), (3.31 g, 81% yield). $[\alpha]_D^{25}$ = –100.00 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3062, 3028, 2975, 2931, 1780, 1698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 7.35–7.32 (m, 2H), 7.29–7.26 (m, 3H), 7.24–7.18 (m, 5H), 4.54–4.51 (m, 1H), 4.15–4.07 (m, 2H), 3.96 (t, *J* = 8.5 Hz, 1H), 3.25 (dd, *J* = 13.5, 3.5 Hz, 1H), 3.04 (dd, *J* = 13.5, 8.0 Hz, 1H), 2.78–2.70 (m, 2H), 1.26 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.5, 153.0, 139.2, 135.3, 129.5, 129.2, 128.9, 128.3, 127.3, 126.4, 66.0, 55.4, 39.9, 39.5, 37.9, 17.1; HRMS (ESI) Calcd for $C_{20}H_{21}NO_3Na$ $[M + Na]^+$ 346.1414, found 346.1410.

(*R*)–2–Methyl–3–phenylpropan–1–ol (217)

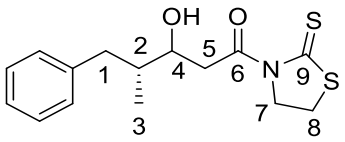


(217)

This new compound was prepared following a modified literature protocol.[48] In 100 mL of dry Et_2O compound **(216)** (2.70 g, 8.35 mmol) was dissolved and cooled to 0 °C

using an ice–water bath. $LiAlH_4$ (25.05 mL, 25.05 mmol) was added dropwise *via* syringe and the temperature was maintained at 0 °C for 1.5 h. The reaction mixture was quenched with 1M NaOH (60 mL) and stirred until the solution was clear. The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 60 mL). The organic layers were pooled, washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was removed *in vacuo* to yield a crude colourless oil. The residue was purified using column chromatography (20% EtOAc in Hex) affording the product (*R*)–2–methyl–3–phenylpropan–1–ol **(217)** as a pale-yellow oil, R_f = 0.66 (50% EtOAc in Hex), (1.08 g, 86% yield). $[\alpha]_D^{25}$ = + 11.62 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast): 3337, 2955, 2925, 2872, 1495, 1453 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.32–7.30 (m, 2H, ArH), 7.22–7.20 (m, 3H, ArH), 3.57–3.47 (m, 2H, H4), 2.79 (dd, *J* = 13.0, 6.0 Hz, 1H, H1), 2.45 (dd, *J* = 13.3, 8.0 Hz, 1H, H1), 1.99–1.95 (m, 1H, H2), 1.83 (br s, 1H, OH), 0.94 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.6 (ArC), 129.1 (ArC), 128.2 (ArC), 125.9 (ArC), 67.6 (C4), 39.7 (C1), 37.8 (C2), 16.4 (C3); HRMS (EI) Calcd for $C_{10}H_{14}O$ $[M^{\cdot}]^+$ 150.1045, found 150.1045.

(*4R*)–3–Hydroxy–4–methyl–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one (218)

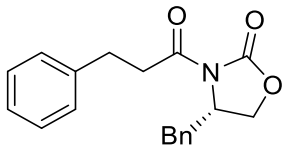


(218)

This new compound was prepared following a modified literature protocol.[48] Oxalyl chloride (1.01 mL, 12.0 mmol) in 50 mL of dry DCM was cooled to –78 °C and a solution of DMSO dried over 4 Å MS (1.13 mL, 16.0 mmol) in 20 mL dry DCM was added dropwise. After 30 min at –78 °C, a solution of **(217)** (0.600 g, 3.99 mmol) in 5 mL of DCM was added dropwise and stirred at –78 °C for another 20 min. DIPEA, dried over 4 Å MS (7.59 mL, 59.9 mmol), was added dropwise and the temperature was maintained for another 20 min and then warmed to –50 °C for 60 min. The reaction mixture was quenched with 10 mL of saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the organic was washed with 10 mL of saturated $NaHCO_3$ and brine before drying on Na_2SO_4. The solvent was removed, and the crude aldehyde was used immediately in the next step. A solution of the compound **(192)** (0.500 g, 3.10 mmol) in 50 mL of dry DCM was charged with $TiCl_4$ (3.41 mL, 3.41 mmol) at –78 °C. After 15 min at –78 °C, DIPEA (0.470 mL, 3.72 mmol) was added dropwise and a colour change was noted from bright orange–red to a deep red–brown. The reaction mixture was allowed to stir for 1 h then a solution of the crude aldehyde (0.460 g, 3.41 mmol) in 5 mL of dry DCM was added dropwise. After 1 h at –78 °C, the reaction mixture was quenched with 10 mL of saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with DCM (3 x 10 mL). The organic layers were pooled, washed with brine and dried on Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (30% EtOAc in Hex) affording the product *4R*)–3–hydroxy–4–methyl–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one

(218) as a yellow oil, R_f = 0.40 (50% EtOAc in Hex), (640 mg, 66% yield). IR ($CHCl_3$, cast film): 3529, 3024, 2963, 2931, 1694 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.29 – 7.26 (m, 2H, ArH), 7.20–7.17 (m, 3H, ArH), 4.62–4.56 (m, 2H, H7), 4.06–3.98 (m, 1H, H4), 3.59–3.27 (m, 4H, H5,8), 3.03–2.85 (m, 2H, H1,OH), 2.50–2.38 (m, 1H, H1), 1.99–1.87 (m, 1H, H2), 0.94–0.87 (m, 3H, H3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 201.9 (C9), 174.6 (C6), 140.8 (ArC), 129.3 (ArC), 128.3 (ArC), 125.9 (ArC), 71.5 (C4), 55.7 (C7), 43.6 (C5), 40.3 (C2), 39.6 (C1), 28.4 (C8), 14.0 (C3); HRMS (ESI) calcd for $C_{15}H_{19}NO_2S_2Na$ $[M + Na]^+$ 332.0749, found 332.0752.

(*S*)–4–Benzyl–3–(3–phenylpropanoyl)oxazolidin–2–one (219)

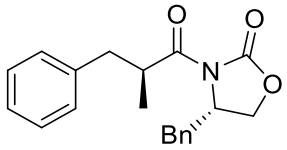


(219)

This known compound was prepared following a modified literature protocol.[212] (*S*)-4-Benzyloxazolidin-2-one (2.50 g, 14.1 mmol) was dissolved in 100 mL of dry THF and cooled to –78 °C using a dry ice/acetone bath. *n*–BuLi (2.5 M, 6.21 mL, 15.5 mmol) was then added dropwise and the reaction mixture was maintained at –78 °C for 15 min. Phenylpropanoyl chloride (2.31 mL, 15.5 mmol) was added *via* syringe and the reaction mixture was allowed to warm to room temperature over 16 h. The reaction

mixture was quenched with saturated NH_4Cl (10 mL) and the bulk of the THF was removed *in vacuo*. The aqueous layer was then extracted with DCM (3 x 20 mL) and the organic layers were pooled, washed with brine and dried over Na_2SO_4. The residue was purified using column chromatography (20% EtOAc in Hex) affording the product (*S*)–4–benzyl–3–(3–phenylpropanoyl)oxazolidin–2–one **(219)** as a white solid, R_f = 0.44 (30% EtOAc in Hex), (4.16 g, 95% yield). $[\alpha]_D^{25}$ = + 57.33 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3086, 3028, 2922, 2857, 1954, 1778, 1699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.34–7.26 (m, 7H), 7.23–7.17 (m, 3H), 4.69–4.64 (m, 1H), 4.20–4.14 (m, 2H), 3.34–3.22(m, 3H), 3.05–3.01 (m, 2H), 2.74 (dd, *J* = 13.5, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 153.4, 140.5, 135.2, 129.4, 129.0, 128.6, 128.5, 127.4, 126.3, 66.2, 55.1, 37.9, 37.1, 30.3; HRMS (ESI) calcd for $C_{19}N_{20}NO_3$ $[M + H]^+$ 310.1438, found 310.1434.

(*S*)–4–Benzyl–3–((*S*)–2–methyl–3–phenylpropanoyl)oxazolidin–2–one (220)

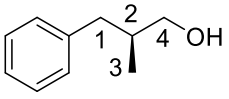


(220)

This known compound was prepared following a modified literature protocol.[213] To a solution of **(219)** (3.00 g, 9.72 mmol) in 80 mL of dry THF was blanketed with Ar and

cooled to –78 °C. NaHMDS (12.7 mL, 12.7 mmol) was added dropwise *via* syringe and the reaction mixture was stirred at –78 °C for 30 min before MeI (0.930 mL, 10.7 mmol) was added. The reaction mixture was allowed to warm to room temperature over 16 h and was quenched with saturated NH_4Cl. The THF was removed *in vacuo* and replaced with 20 mL of EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The organic layers were then pooled, washed with brine, and dried over Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (5% EtOAc in Hex) affording the product (*S*)–4–benzyl–3–((*S*)–2–methyl–3–phenylpropanoyl)oxazolidin–2–one **(220)** as a pale yellow oil, R_f = 0.50 (20% EtOAc in Hex), (2.52 g, 80% yield). $[\alpha]_D^{25}$ = 104.74 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3062, 3028, 2975, 2930, 1780, 1698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 7.34–7.31 (m, 2H), 7.29–7.22 (m, 3H), 7.24–7.18 (m, 5H), 4.55–4.50 (m, 1H), 4.15–4.07 (m, 2H), 3.96 (t, *J* = 8.5 Hz, 1H), 3.25 (dd, *J* = 13.5, 3.5 Hz, 1H), 3.04 (dd, *J* = 13.5, 8.0 Hz, 1H), 2.78–2.70 (m, 2H), 1.26 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.5, 153.0, 139.2, 135.3, 129.5, 129.2, 128.9, 128.3, 127.3, 126.4, 66.0, 55.4, 39.9, 39.5, 37.9, 17.1; HRMS (ESI) calcd for $C_{20}H_{21}NO_3Na$ $[M + Na]^+$ 346.1414, found 346.1417.

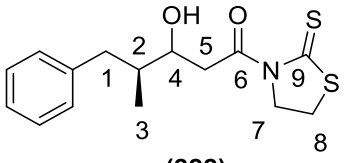
(*S*)–2–Methyl–3–phenylpropan–1–ol (221)



(221)

This new compound was prepared following a modified literature protocol.[48] In 100 mL of dry Et_2O compound **(220)** (2.40 g, 7.42 mmol) was dissolved and cooled to 0 °C using an ice–water bath. $LiAlH_4$ (1 M, 22.3 mL, 22.3 mmol) was added dropwise *via* syringe and the temperature was maintained at 0 °C for 1.5 h. The reaction mixture was quenched with 1 M NaOH (60 mL) and stirred until the solution was clear. The layers were separated, and the aqueous was extracted with Et_2O (3 x 60 mL). The organic layers were pooled, washed with brine, and dried over anhydrous Na_2SO_4. The organic solvent was removed *in vacuo* to yield the crude as a colourless oil. The residue was purified using column chromatography (20% EtOAc in Hex) affording the product (*S*)–2–methyl–3–phenylpropan–1–ol **(221)** as a pale-yellow oil, R_f = 0.66 (50% EtOAc in Hex), (1.06 g, 95% yield). $[\alpha]_D^{25}$ = – 10.92 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$, cast film): 3348, 2955, 2921, 2873, 1495, 1455 cm^{-1}; ^1H NMR (500 MHz, CDCl3) δ 7.29–7.26 (m, 2H, ArH), 7.21–7.16 (m, 3H, ArH), 3.53–3.43 (m, 2H, H4), 2.76 (dd, *J* = 13.5, 6.0 Hz, 1H, H1a), 2.41 (dd, *J* = 13.5, 8.0 Hz, 1H, H1b), 1.96–1.92 (m, 2H, H2,OH), 0.91 (d, *J* = 7.0 Hz, 3H, H3); ^{13}C NMR (125 MHz, CDCl3) δ 140.6 (ArC), 129.1 (ArC), 128.2 (ArC), 125.8 (ArC), 67.5 (C4), 39.7 (C1), 37.7 (C2), 16.4 (C3); HRMS (EI) Calcd for $C_{10}H_{14}O$ $[M^{\cdot}]^+$ 150.1045, found 150.1045.

(*4S*)–3–Hydroxy–4–methyl–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one (222)



(222)

This new compound was prepared following a modified literature protocol.[48] Oxalyl chloride (1.35 mL, 16.0 mmol) in 50 mL of dry DCM was cooled to –78 °C and a solution of DMSO dried over 4 Å MS (1.51 mL, 21.3 mmol) in 20 mL dry DCM was added dropwise. After 30 min at –78 °C, a solution of **(221)** (0.800 g, 5.32 mmol) in 5 mL of DCM was added dropwise and stirred at –78 °C for another 20 min. DIPEA, dried over 4 Å MS (10.12 mL, 78.88 mmol) was added dropwise and the temperature was maintained for another 20 min then warmed to –50 °C for 60 min. The reaction mixture was quenched with 10 mL of saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the organic layer was washed with 10 mL of saturated $NaHCO_3$ and brine before drying on Na_2SO_4. The solvent was removed, and the crude aldehyde was used immediately in the next step. A solution of the compound **(192)** (0.70 g, 4.34 mmol) in 50 mL of dry DCM was charged with $TiCl_4$ (4.78 mL, 4.78 mmol) at –78 °C. After 15 min at –78 °C, DIPEA (0.670 mL, 5.21 mmol) was added dropwise and a colour change was noted from bright orange–red to a deep red–brown. The reaction mixture was allowed to stir for 1 h then a solution of the crude aldehyde (0.64 g, 4.77 mmol) in 5 mL of dry DCM was added dropwise.

After 1 h at –78 °C, the reaction mixture was quenched with 10 mL of saturated NH_4Cl and allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with DCM (3 x 10 mL). The organic layers were pooled, washed with brine, and dried on Na_2SO_4. The reaction mixture was concentrated *in vacuo* and the residue was purified using column chromatography (30% EtOAc in Hex) affording the product (*4S*)–3–hydroxy–4–methyl–5–phenyl–1–(2–thioxothiazolidin–3–yl)pentan–1–one **(222)** as a yellow oil, R_f = 0.44 (50% EtOAc in Hex), (1.04 g, 77% yield). IR ($CHCl_3$, cast film): 3537, 3060, 2963, 2932, 1692 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.31–7.27 (m, 2H, ArH), 7.21–7.19 (m, 3H, ArH), 4.60–4.56 (m, 2H, H7), 4.09–4.06 (m, 1H, H4), 3.44–3.43 (m, 2H, H5), 3.30–3.26 (m, 2H, H8),2.90–2.86 (m, 1H, H1), 2.51 –2.46 (m, 1H, H1), 1.91 – 1.88 (m, 1H, H2), 0.94 (d, *J* = 6.5 Hz, 3H, H3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 201.9 (C9), 174.6 (C6), 140.8 (ArC), 129.2 (ArC), 128.2 (ArC), 125.8 (ArC), 71.5 (C4), 55.7 (C7), 43.6 (C5), 40.3 (C2), 39.5 (C1), 28.3 (C8), 14.1 (C3); HRMS (ESI) calcd for $C_{15}H_{19}NO_2S_2Na$ $[M + Na]^+$ 332.0749, found 332.0757.

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