Synthesis of Nitrogen Heterocyclic Compounds for Therapeutic Applications

by

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ABSTRACT

Reactive oxygen species (ROS) are a series of molecules, ions, and radicals derived from oxygen that possess remarkable reactivity. They act as signaling molecules when their concentration in cells is within a normal range. When the levels of ROS increase, reaching a concentration in which the antioxidants cannot readily quench them, oxidative stress will affect the cells. These excessive levels of ROS result in direct or indirect ROS-mediated damage of proteins, nucleic acids, and lipids. Excessive oxidative stress, particularly in chronic inflammation, has been linked with mutations and carcinogenesis. One of the main targets of ROS in severe oxidative stress is mitochondrial DNA (mtDNA). The synthesis of analogues of α-tocopherol is described as potential compounds with the ability to remediate defective mitochondria. An interesting possibility for eradicating cancer cells is to selectively target them with oxidative species while avoiding any deleterious effects on healthy cells. To accomplish this, analogues of the β-hydroxyhistidine moiety of the antitumor agent bleomycin (BLM) were synthesized.

The first part of this thesis focuses on the synthesis of simplified analogues of α-tocopherol. These analogues possess a bicyclic pyridinol as the antioxidant core and an alkyl group as the lipophilic chain to mimic α-tocopherol. Additionally, analogues with a completely oxidized pyridinol core were synthesized. Some of these analogues showed promising properties against ROS production and lipid peroxidation. The protection they conferred was shown to be tightly regulated by their concentration.

The second part of this thesis focuses on the synthesis of analogues of β-hydroxyhistidine. BLMs are glycopeptides that possess anticancer activity and have been used to treat testicular carcinomas, Hodgkin's lymphoma, and squamous cell carcinomas. The activity of BLM is based on the degradation of DNA, or possibly RNA, caused by a Fe(II)·BLM complex in the presence of O_2. The β-hydroxyhistidine moiety of BLM contributes to metal coordination via two ligands: the N-3 nitrogen atom of imidazole and possibly the nitrogen atom of the amide. A series of β-hydroxyhistidine analogues has successfully been synthesized.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

^{1}H	proton
^{13}C	carbon-13
°C	degrees Celsius
α	alpha
APCI	atmospheric-pressure chemical ionization
aq	aqueous
anh	anhydrous
β	beta
br s	broad singlet
BLM	bleomycin
Bn	benzyl
Bu_2OTf	dibutylboron triflate
d	doublet
dd	doublet of doublets
CAT	catalase
CNS	central nervous system
DBDMH	1,3-dibromo-5,5-dimethylhydantoin
DCF	dichlorodihydrofluorescein diacetate
DCFH	dichlorofluorescein
DIBAL-H	diisobutylaluminium hydride
DMAE	dimethylethanolamine
DEM	diethyl maleate
DMF	*N*,*N*-dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	*N*,*N*-dimethylsulfoxide
DNA	deoxyribonucleic acid
ds	double strand
dt	doublet of triplets
EI	electric ionization
ESI	electrospray ionization
EtOAc	ethyl acetate
FACS	fluorescence-activated cell sorting
FAB	fast atom bombardment
FCCP	carbonyl cyanide-4-(trifluoromethyl)phenylhydrazone
FmocOSu	*N*-(9-fluorenylmethoxy-carbonyloxy)succinate
FRDA	Friedreich's ataxia
g	gram(s)
GMP	guanosine monophosphate
GPx	glutathione peroxidase
GSH	glutathione
GSSG	glutathione disulfide
Hepes	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Hex	hexanes
HPLC	high performance liquid chromatography
h	hour(s)

J	coupling constant
JC-1	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide
m	multiplet
M	molarity
min	minutes
MAPK	mitogen-activated protein kinase
MALDI-TOF	matrix assisted laser desorption ionization – time of flight
MeOH	methanol
mL	milliliter(s)
μL	microliter(s)
mmol	millimole(s)
μmol	micromole(s)
mp	melting point
NADH	nicotinamide adenine dinucleotide
N	normality
nM	nanomolar
NMR	nuclear magnetic resonance spectroscopy
Q	ubiquinone
QH_2	ubiquinol
q	quadruplet
quint	quintuplet
RNA	ribonucleic acid
RCS	reactive chlorine species
ROS	reactive oxygen species
RNS	reactive nitrogen species
R_f	ratio of fronts
rt	room temperature
s	singlet
sat	saturated
SEM	standard error of the mean
SOD	superoxide dismutase
ss	single strand
$TBAF{\cdot}3H_2O$	tetra-*n*-butylammonium fluoride trihydrate
TEA	triethylamine
td	triplet of doublets
TBSCl	*tert*-butyldimethylsilyl chloride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
Tol	toluene
TPP^+	triphenylphosphonium
wt.	weight
UV	ultraviolet

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CHAPTER 1 - INTRODUCTION

1.1 General introduction

1.1.1 Redox homeostasis

Redox reactions are a class of reactions in which electrons are transferred from one molecule or atom to another. The reduction of one molecule is always coupled to the oxidation of another molecule. A molecule that gains electrons is termed an oxidizing agent while a molecule that loses electrons is called a reducing agent.[1] Redox reactions play a vital and complex role in the life of cells. They are necessary for proper metabolism, they are vital components of cofactors, and they are essential in assuring effective responses against endogenous and exogenous stimuli. The extensive list of oxidizing and reducing agents present in every organism interacts with each other forming a complex network. A balance between oxidizing and reducing agents is required for the proper functioning of cells. This balance is called redox homeostasis and it is an integral part of cells' environment and metabolism.[2]

Two types of antagonistic molecules serve as the main constituents of redox homeostasis: oxidants and antioxidants. Their interactions occur within a sophisticated and extensive redox network. Redox signaling is employed by a diverse range of organisms, including bacteria. Its goal is to induce protective mechanisms against oxidative stress and to reestablish the state of redox homeostasis after a stress phase.[3]

1.1.2 Oxidants

The normal oxidative metabolism observed in cells produces oxidants. The majority of these oxidants are known as reactive oxygen species (ROS).[4] Other

oxidants include cofactors, reactive nitrogen species (RNS), and reactive chlorine species (RCS). It must be noted that while ROS act mainly as oxidants on different organic substrates, some of them possess important functions as reductants. For example, superoxide ($O_2^{\bullet-}$) is an important reductant of metal ions, such as Fe^{3+}. By reducing Fe^{3+} ions, Fe^{2+} ions are regenerated. These Fe^{2+} ions are toxic and can generate hydroxyl radicals via the Fenton reaction in the presence of hydrogen peroxide.[5] Also, Fe^{2+} ions are able to reduce disulfide bonds.[6] Despite possessing a reductant nature, superoxide is considered a ROS due to its close relationship with other oxygen-containing substrates.

ROS possess different functions in organisms and their dysregulation has the ability to cause damage to cells. RNS are also important oxidants that are involved in cellular signaling processes. Additionally, their dysregulation can lead to cellular impairment.[7]

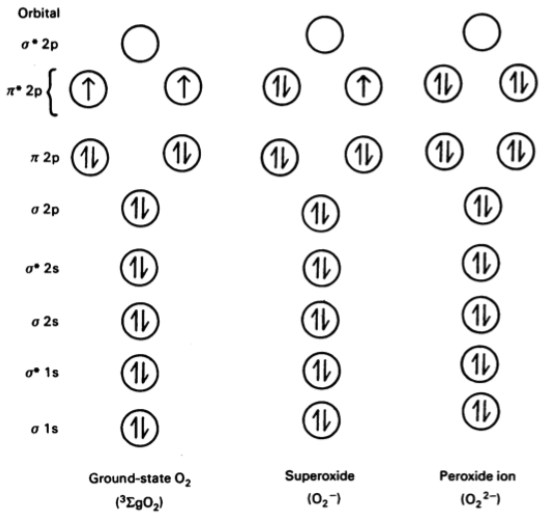


Figure 1.1. Molecular orbitals of selected ROS.[8]

ROS can be classified either as free radicals or non-radicals. Free radicals are a diverse species of independent existence that hold one or more unpaired electrons in their molecular or atomic orbitals (Figure 1.1).[9] Free radicals can be formed by the homolytic cleavage of a covalent bond, giving two radicals as products. The chemistry of these radicals consists of the transfer of only one electron. This is in contrast to the two electron processes observed in the majority of reactions of non-radicals.[10]

	Free radicals	Non-radicals
ROS (Reactive oxygen species)	Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, H_2O_2
	Hydroxyl, $HO^{\bullet}$	Hypochlorous acid, HClO
	Peroxyl, $ROO^{\bullet}$	Ozone, O_3
	Alkoxyl, $RO^{\bullet}$	Singlet oxygen, $^1O_2$
	Hydroperoxyl, $HOO^{\bullet}$	
RNS (Reactive nitrogen species)	Nitric oxide, $NO^{\bullet}$	Nitrosyl cation, NO^+
	Nitrogen dioxide, $NO_2^{\bullet}$	Nitrous acid, HNO_2
		Dinitrogen trioxide, N_2O_3
		Peroxynitrite, $ONOO^-$

Figure 1.2. Most common reactive species.[11]

The list of reactive species is extensive and diverse (Figure 1.2). The most common ROS in biological systems include singlet oxygen, hydrogen peroxide (H_2O_2), superoxide, and hydroxyl radical ($HO^{\bullet}$).[12] Additionally, nitric oxide ($NO^{\bullet}$) is an important RNS mediator used in numerous biological processes (Figure 1.3).[13]

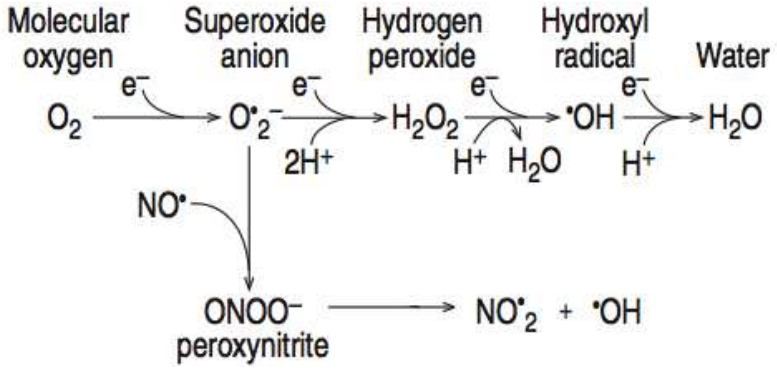


Figure 1.3. Chemical relationship between the major forms of ROS.[14]

Hydrogen peroxide is mainly produced in the mitochondria as a product of the enzyme superoxide dismutase reacting with superoxide.[15] The other product of this dismutation is molecular oxygen. Additionally, hydrogen peroxide is produced by the divalent reduction of molecular oxygen by several different oxidases, including uricase,[16] glucose oxidase,[17] and D-amino acid oxidase.[18] The toxicity of hydrogen peroxide is due to its reaction with Fe^{2+}, called the Fenton reaction (Figure 1.4). The product of this reaction is hydroxyl radical, which is responsible for the damage caused by hydrogen peroxide.[19]

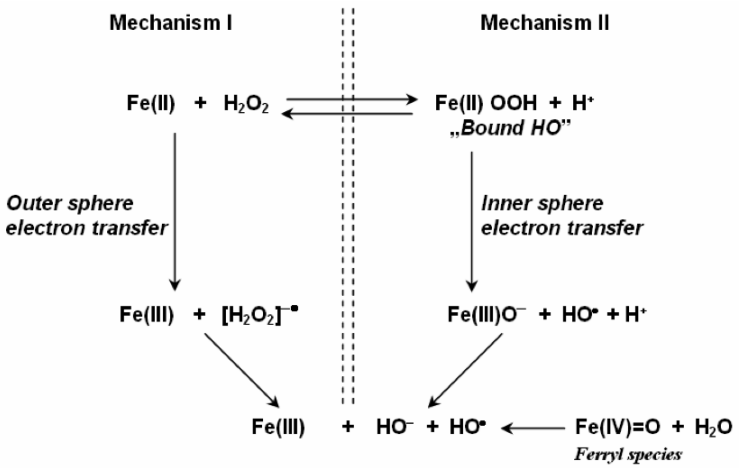


Figure 1.4. Fenton reaction and its two different mechanisms.[20]

Superoxide is obtained when molecular oxygen is reduced by one electron.[21] This radical is formed as a mitochondrial byproduct and it is produced by complexes I and III.[22] In complex I, superoxide is produced when one electron is transferred from the iron-sulfur cluster N1a to molecular oxygen. This is thermodynamically unfavorable because the reduction potential of cluster N1a ($E_{1/2}$ = –380 mV) coupled with the oxidation potential of NADH ($E_{1/2}$ = –320 mv) yields a negative reaction potential value.[23] This explains why superoxide production is very low compared to the normal processes of the electron transport chain. Additionally, the production of ROS in complex I is increased at higher ratios of NADH/NAD^+.[24] In complex III, ubiquinol (QH_2) is oxidized to produce ubisemiquinone ($Q^{\bullet-}$) and reduces cytochrome c_1. This ubisemiquinone transfers its electron to cytochrome *b* and reaches an ubiquinone (Q) from an ubiquinone pool, generating ubisemiquinone. This process occurs in order to generate a proton gradient from the matrix to the intermembrane space of the mitochondrion. This ubisemiquinone generated in the cytochrome *b* will

be reduced to ubiquinol by the oxidation of a second ubiquinol molecule (Figure 1.5).[25] However, on a significantly lesser scale, ubisemiquinone can donate one electron to molecular oxygen and produce superoxide.[26] The production of ROS by both complexes I and III may also be increased by the action of certain inhibitors.[27] Superoxide is also produced as a defensive mechanism during phagocytosis by neutrophils and macrophages.[28] The reactivity of superoxide is highlighted by its reaction with nitric oxide, in which peroxynitrite ($ONOO^-$) is produced. Peroxynitrite is a strong oxidizing molecule that decomposes to nitrogen dioxide ($NO_2^{\bullet}$) and hydroxyl radical, both of which are extremely reactive.[29] In addition, superoxide reacts with several enzymes by attacking their Fe-S clusters and releasing Fe^{3+} which is then followed by the Fenton reaction.[30]

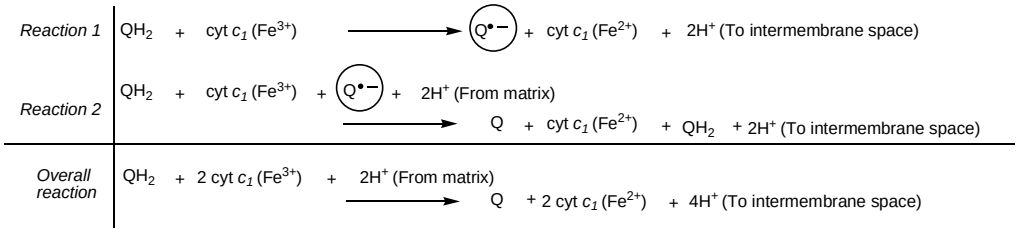


Figure 1.5. The cycle of ubiquinone (Q). Intermediate ubisemiquinone is encircled.[31]

As described earlier, hydroxyl radical is synthesized by the decomposition reaction of peroxynitrite. It is also the main product of the Fenton reaction (Figure 1.4).[32] Additionally, it is the decomposition product of the light excitation of hydrogen peroxide.[9] This radical is known as one of the most reactive chemical species, possessing second-order rate constants of more than $10^7\ M^{-1}\ s^{-1}$ when it

oxidizes other biological molecules.[33] It is responsible for the majority of damage caused to DNA that is attributed to ROS.[34]

Nitric oxide is produced by a diverse group of nitric oxide synthase (NOS) enzymes.[35] In this reaction, two molecules of L-arginine react with three molecules of NADH, forming two molecules of L-citrulline, two molecules of nitric oxide, and three molecules of NAD^+ (Figure 1.6).[36] Nitric oxide reacts with hydroxyl radical to produce peroxynitrite which is known as a powerful agent involved in nitration, nitrosation, and oxidation.[37]

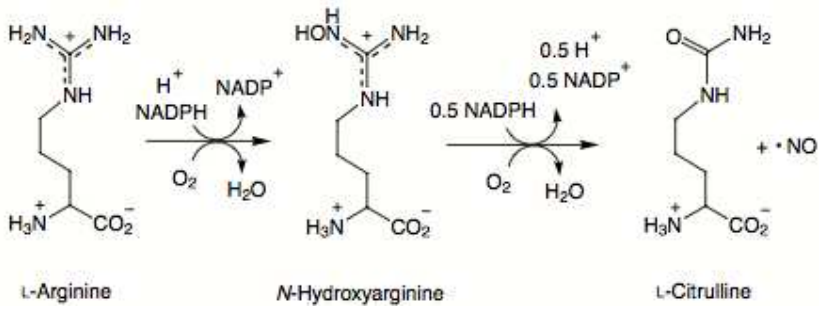


Figure 1.6. The nitric oxide synthase reactions. [38]

Even though these reactive species are generally recognized by their negative effects, they also have important roles in cellular signaling. Hydrogen peroxide has been linked to the activation of mitogen-activated protein kinase (MAPK) signaling pathways, inhibition of protein phosphatases, and activation of transcription factors.[39] The major part of these mechanisms involves the oxidation of cysteine residues by hydrogen peroxide.[40]

Superoxide activates protein kinases by promoting the phosphorylation of serine and threonine residues.[41] Additionally, superoxide is produced by neutrophils and mononuclear phagocytes to combat external pathogens.[42]

Hydroxyl radical also has an essential role in phagocytosis. Hydroxyl radicals are produced after superoxide releases Fe^{3+} from Fe-S clusters. These Fe^{3+} ions react with hydrogen peroxide forming hydroxyl radicals, which are the main oxidative forces against pathogens.[43]At the same time, the damage caused to tissue by hydroxyl radical initiates an inflammatory response.[44]

Nitric oxide has several diverse roles in cell signaling. Among these functions, nitric oxide and other vasodilators can activate soluble guanylyl cyclase in the endothelium to produce cyclic GMP (cGMP), leading to smooth muscle relaxation.[45] Additionally, nitric oxide can be added to the cysteine thiols of different proteins. This reaction is called nitrosylation, which can activate or inactivate proteins creating reversible and diverse signals.[46]

1.1.3 Antioxidants

Antioxidants compose the second type of species involved in redox homeostasis. These compounds are reducing agents that cells synthesize in order to delay, prevent, or remove oxidative damage. Their activity is crucial to afford redox homeostasis. A marked imbalance between antioxidants and oxidants will cause deleterious oxidative stress.[47]

Similar to oxidants, antioxidants exist in a variety of structural subtypes (Figure 1.7). These subtypes can be divided into two main groups: enzymatic and non-enzymatic antioxidants. The major enzymatic antioxidants are superoxide

dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). It must be noted that since superoxide is considered a reductant and not an oxidant, SOD must be referred to as an antireductant instead of as an antioxidant.[48] However, since superoxide dismutase is part of the protective mechanisms used against oxidative damage caused by ROS, sometimes it is discussed as an antioxidant. The major non-enzymatic antioxidants are ascorbate (vitamin C), tocopherol (vitamin E), glutathione (GSH), carotenoids (derived from vitamin A), and coenzyme Q_{10} (ubiquinone or Q).[49]

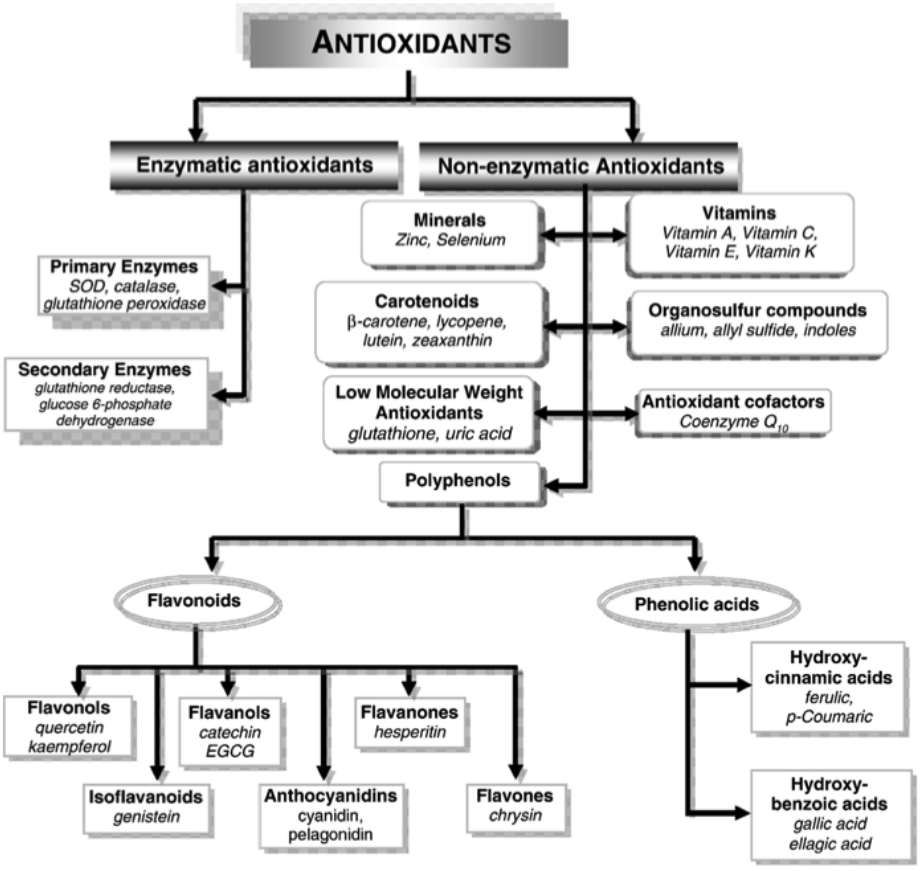


Figure 1.7. Classification of antioxidants.[50]

The enzymatic antioxidants quench different oxidants (or reductants in the case of superoxide dismutase) and their function is often complemented by other enzymes. Superoxide dismutase is responsible for converting superoxide into hydrogen peroxide (Figure 1.8). This reaction is normally coupled with other enzymes in order to remove the hydrogen peroxide formed.[51] These antioxidants possess one or several metal ions as active sites. The superoxide dismutase family is considered diverse since they possess Cu^{2+}, Zn^{3+}, Mn^{3+}, or Fe^{3+} at their catalytic center.[52]

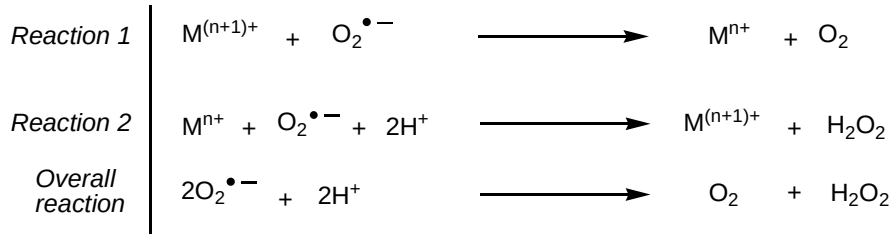


Figure 1.8. Mechanism of superoxide dismutase.[53]

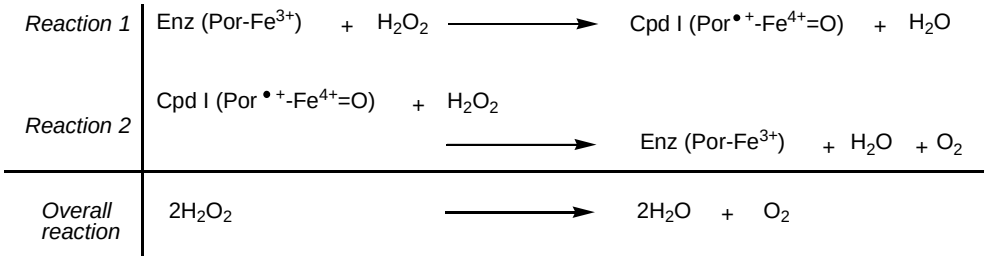


Figure 1.9. Mechanism of catalase.[54]

Catalase is responsible for reducing one molecule of hydrogen peroxide to water. In a secondary step, catalase oxidizes an additional molecule of hydrogen peroxide to molecular oxygen (Figure 1.9). The reactive site of catalase contains a

heme-iron cluster.[55] One of the main features of the catalase family is the formation of a high-valent iron intermediate after the first reaction. This intermediate is called Compound I (Cpd I).[56]

Glutathione peroxidase is responsible for reducing hydrogen peroxide to water. This enzyme has the same function as catalase, but it reduces hydrogen peroxide by a completely different mechanism. Additionally, glutathione peroxidase can transform lipid peroxides to their corresponding alcohols.[57] Glutathione peroxidase possesses a selenocysteine as its active site and utilizes glutathione (GSH) as a reducing agent.[58] Since the fate of this enzyme is exclusively tied to glutathione, more information is presented below regarding this non-enzymatic antioxidant.

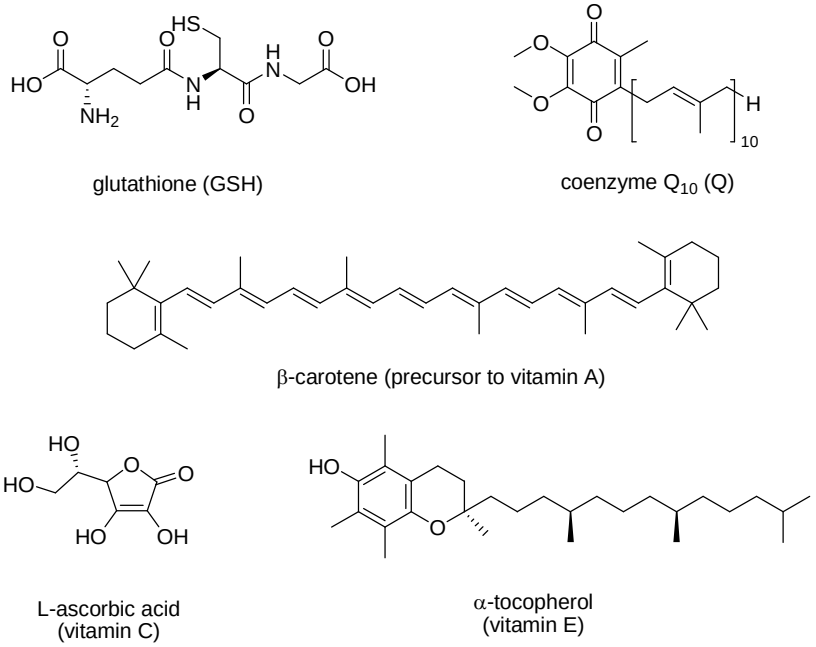


Figure 1.10. Structures of non-enzymatic antioxidants.

The non-enzymatic antioxidants can be divided into metabolic antioxidants and nutritionally derived antioxidants. Glutathione and ubiquinone are biologically important metabolic antioxidants while ascorbate (Vitamin C), tocopherol (Vitamin E), and the carotenoids are essential nutritionally derived antioxidants (Figure 1.10).[59]

Glutathione is composed of three amino acids: L-glutamate, L-cysteine, and glycine. In this molecule, glutamate is attached to cysteine through a gamma peptide bond while cysteine is attached to glycine by an alpha peptide bond.[60] Glutathione is capable of neutralizing hydrogen peroxide and lipid peroxides (Figure 1.11) as mentioned above. Glutathione can react with a wide array of oxidants. For example, glutathione can react in enzymatically catalyzed processes with other toxic species, such as formaldehyde and methylglyoxal, giving the relatively less toxic species formic acid and lactate, respectively.[61] Also, glutathione can react non-enzymatically with numerous oxidants. Glutathione has the ability to quench alkyl and peroxyl radicals. The coordination between superoxide dismutase and glutathione to quench alkyl radicals, peroxyl radicals, and superoxide is called a "free radical sink" (Figure 1.12).[62] Additionally, glutathione can react with superoxide generating glutathione thiyl radical ($GS^{\bullet}$) in order to protect other thiols. The final step of these reactions is the production of oxygen and peroxide by superoxide dismutase (Figure 1.12).[63]

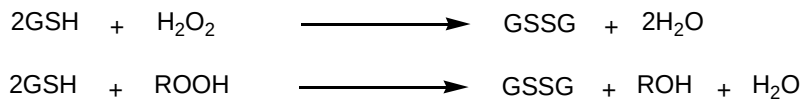


Figure 1.11. Reactions of glutathione with hydrogen peroxide and lipid peroxide.[64]

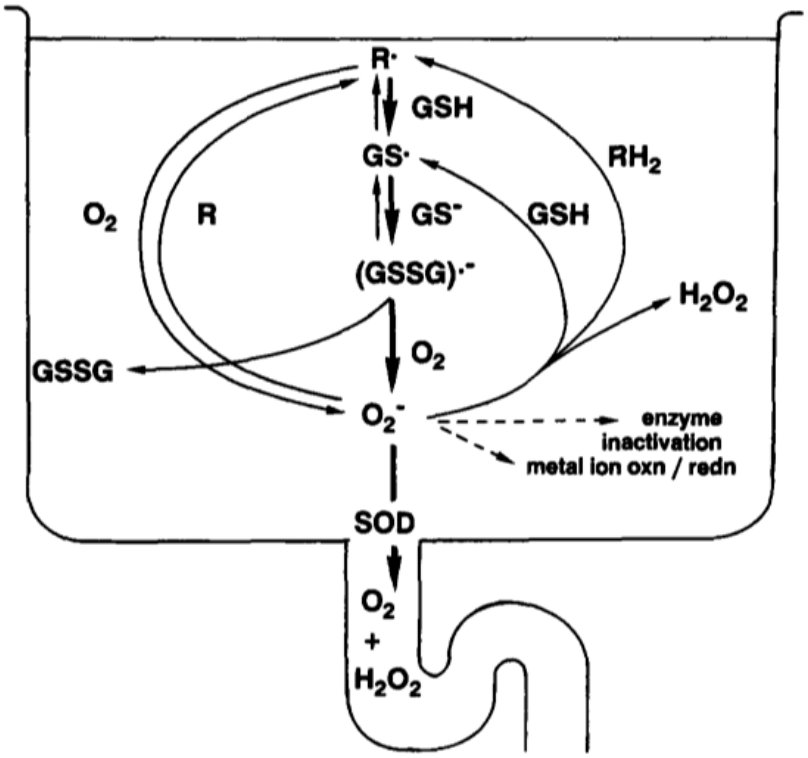


Figure 1.12. Molecular oxygen as radical sink for radicals generated from the reaction between glutathione and alkyl radicals.[65]

Coenzyme Q_{10} (ubiquinone or Q) is present in all cell membranes. The reduced form of coenzyme Q_{10} (ubiquinol or QH_2) is an important antioxidant that can neutralize peroxyl radicals (Figure 1.13).[66] It should be noted that the intermediate ubisemiquinone can undergo undesirable reactions such as the production of superoxide (Figure 1.5).[26] Additionally, ubiquinol can regenerate α-tocopherol from its oxidized form. Tocopherols are more efficient at quenching radicals than ubiquinol.[67]

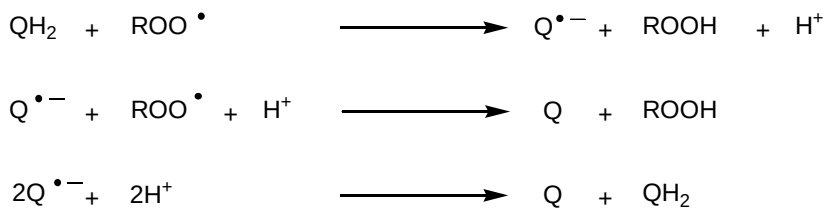


Figure 1.13. Reactions between peroxyl radicals and derivatives of ubiquinone (Q).[68]

Ascorbate (vitamin C) is an antioxidant that has the ability to react with a wide range of oxidants and radicals. It is present in significant quantities in the body and it can be regenerated.[69] Ascorbate interacts with several antioxidants, but its interaction with glutathione is vital in suppressing ROS (Figure 1.14). It is synthesized from D-gulonic acid in the majority of animals and from L-galactose in plants. Humans are among the few species that cannot synthesize this compound therefore it must be obtained in their diet.[70] Ascorbate can be regenerated by the dismutation of two molecules of ascorbate radical producing ascorbate and dehydroascorbate as products.[71] Additionally, ascorbate can be regenerated from dehydroascorbate by complex III of the electron transport chain (Figure 1.15). [72]

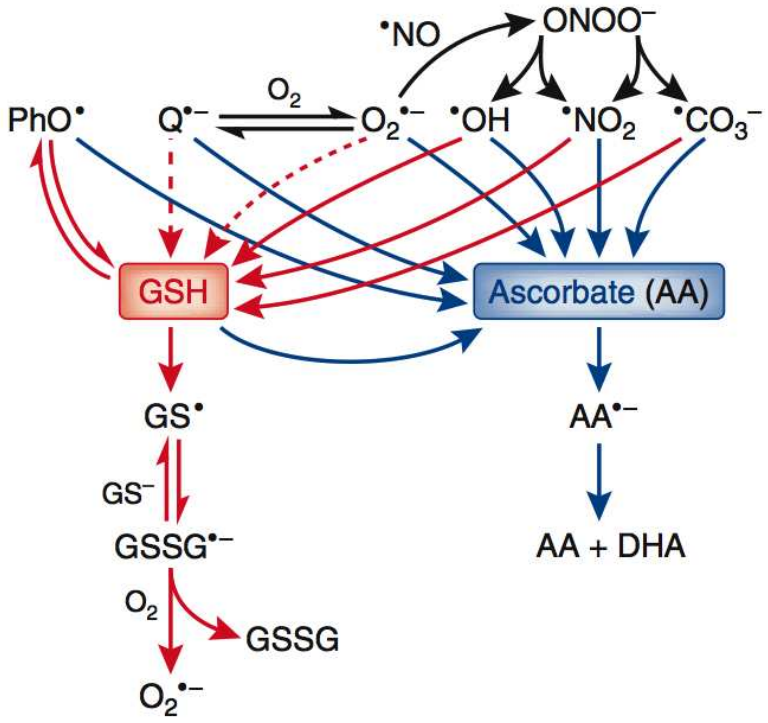
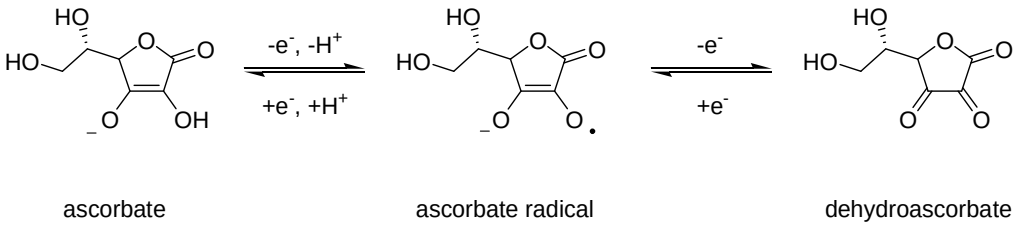


Figure 1.14. Major scavenging pathways by non-enzymatic oxidants. Dashed lines show less favorable reactions.[73]



ascorbate ascorbate radical dehydroascorbate

Figure 1.15. Oxidation products of ascorbate.[74,75]

The carotenoids are a family of natural pigments. More than a thousand have been identified in nature.[76] The carotenoids are biosynthesized from two geranylgeranyl moieties attached tail-to-tail producing a skeleton of forty carbons.[77] Their antioxidant properties are similar to those possessed by ubiquinol (Figure 1.12).

They are involved in the quenching of peroxyl radicals.[78] Additionally, carotenoids have the ability to regenerate tocopherol from the tocopheroxyl radical.[79]

Please note that vitamin E will be described in detail in section 2.1.

1.1.4 Balance between reactive species and antioxidants

As stated before, both oxidants and antioxidants possess properties that make them essential for proper cell signaling. At redox homeostasis, the concentration of ROS will be at low but measurable levels.[80] However, ROS concentrations can quickly change to address different situations. For example, during phagocytosis the cells involved enter into a state of oxidative stress. In this particular case, oxidative stress confers several advantages and becomes essential in order to combat external pathogens.[81]

When the presence of oxidants is at a significantly higher level than normal, severe oxidative stress can be observed (Figure 1.16). Severe oxidative stress results in direct or indirect ROS-mediated damage of proteins, nucleic acids, and lipids.[82] Severe oxidative stress has been implicated in carcinogenesis,[83] neurodegeneration,[84] aging,[85] and apoptosis.[86] The impact of oxidative stress in mitochondrial impairment and carcinogenesis will be reviewed in more detail below.

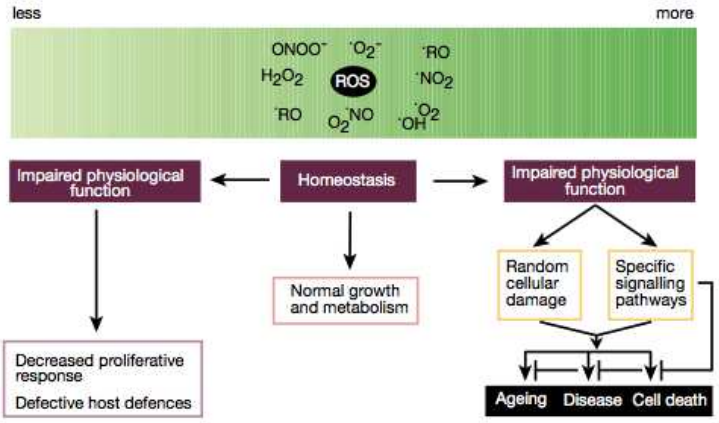


Figure 1.16. Consequences of dysregulated production of ROS.[87]

1.1.4.1 Oxidative stress and mitochondrial impairment

One of the main targets of ROS in severe oxidative stress is mitochondrial DNA (mtDNA). This is due to the close proximity between the mitochondrial DNA and the electron transport chain, which resides in the mitochondrial membrane.[88] Additionally, the absence of histones increases the susceptibility of mitochondrial DNA to the deleterious effects of ROS.[89] The net production of ROS and the accumulation of mitochondrial DNA mutations are important contributors to aging[90] and to several neurodegenerative such as Alzheimer's disease, Parkinson's disease, Friederich's ataxia (FRDA), hereditary spastic paraplegia, and amyotrophic lateral sclerosis, among others.[91] A second area of the human body with increased susceptibility to ROS is the central nervous system (CNS). The CNS is extremely sensitive to oxidative damage since it is the organ system that consumes the most oxygen in the body.[92]

In the majority of trials, nutritionally derived antioxidants have shown no effect in treating diseases caused by defective mitochondria.[93] However, drugs

derived from these antioxidants have the possibility of becoming pharmaceutical agents that may alleviate mitochondrial dysfunction. For example, the lipophilic cation triphenylphosphonium (TPP^+) accumulates in the mitochondria and has been attached to the core of several antioxidants generating MitoVit E and $MitoQ_{10}$.[94] MitoVit E and $MitoQ_{10}$, derived from vitamin E and ubiquinol respectively, target mitochondria selectively.[95] However, both compounds accumulate irreversibly in the mitochondria and eventually reach toxic concentrations. Another series of antioxidant analogues is derived from the modification of their core in order to increase its potency. For example, 5-pyrimidinols possess a core that is comparable in activity to the core of α-tocopherol, a component of vitamin E (Figure 1.18).[96] Chapter 2 will focus on the synthesis of modified core analogues of α-tocopherol.

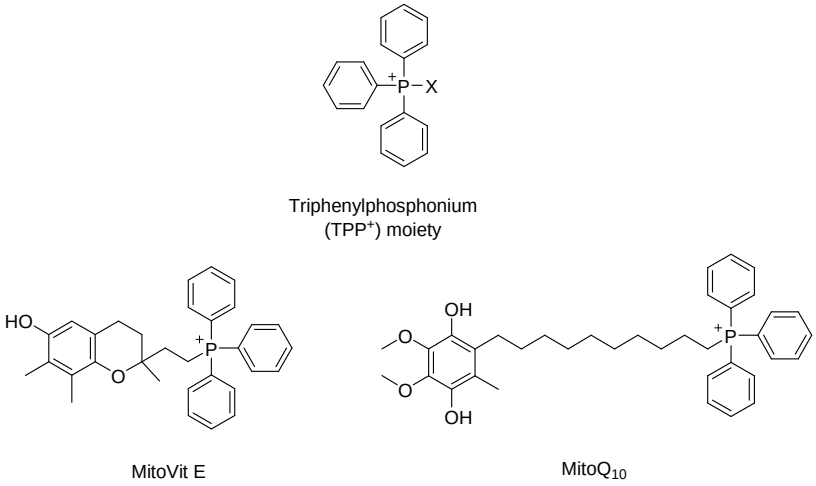
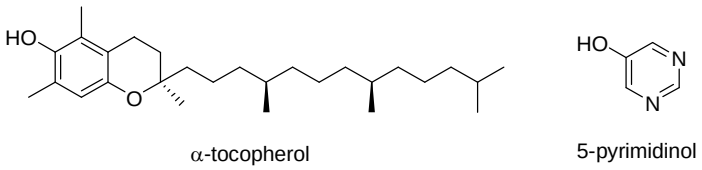


Figure 1.17. Structures of TPP^+ and antioxidant analogues possessing TPP^+ moiety.[94]



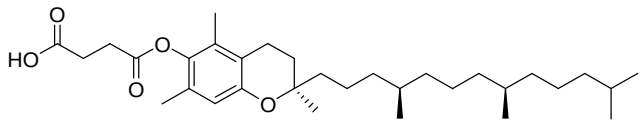
α-tocopherol 5-pyrimidinol

Figure 1.18. Structures of α-tocopherol and 5-pyrimidinol.[96]

1.1.4.2 Oxidative stress and carcinogenesis.

Excessive oxidative stress, particularly in chronic inflammation, has been linked with mutations and carcinogenesis.[97] Additionally, the organelle peroxisome and inflammatory cells such as neutrophils, eosinophils, and macrophages may be involved in carcinogenesis by producing high levels of ROS.[98] From ROS, hydroxyl radical is considered the major contributor of oxidative damage to DNA.[99] Hydroxyl radical is mainly produced inside the organism by the sources mentioned above and by the Fenton reaction.[100]

Nutritionally derived antioxidants have failed to provide any significant activity to prevent or to treat cancer.[101] However, some analogues of antioxidants have proven to be successful agents against cancer cells. One example is α-tocopheryl succinate (Figure 1.19), which is effective against several cancer cell lines. However, the anticancer activity of α-tocopheryl succinate occurs through pathways that do not involve ROS quenching.[102]



α-tocopheryl succinate

Figure 1.19. Structure of α-tocopheryl succinate.

Since the restoration of redox homeostasis in cancer cells by antioxidants does not seem to be possible, other mechanisms are being studied. An interesting possibility for eradicating cancer cells is to selectively target them with oxidative species while avoiding any adverse effects on healthy cells. Cancer cells carry higher levels of ROS in their mitochondria compared to healthy cells. Even in this situation, cancer cells are still susceptible to excessive levels of ROS.[103] The anticancer agent bleomycin has the ability to specifically target several tumor cell lines while having no effect on normal cell lines. Bleomycin is an anticancer agent that cleaves DNA and that is selective to cancer cells due to its disaccharide domain (Figure 1.20).[104] Analogues of bleomycin constitute an important target in order to increase the selectivity and potency against cancer cells. Chapter 3 will focus on the synthesis of analogues of β-hydroxyhistidine, which is one of the building blocks of bleomycin. These analogues will be used in the formation of new analogues of bleomycin after fully assembling the glycopeptide.

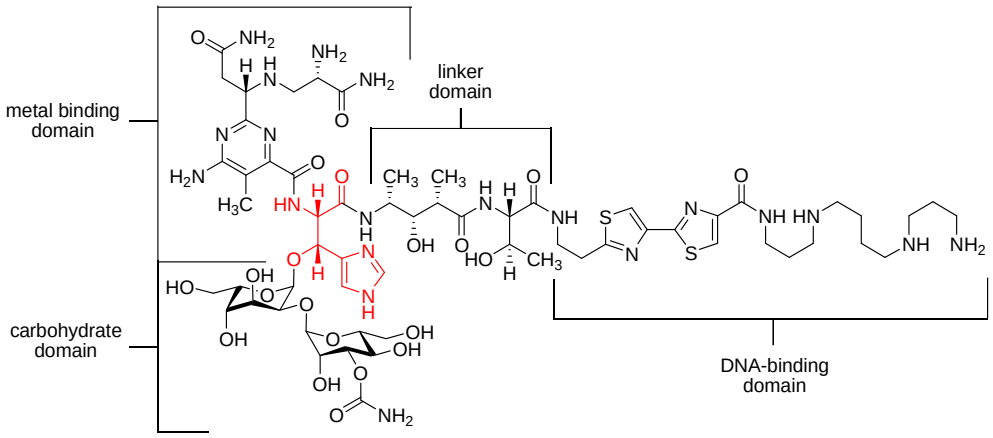


Figure 1.20. The different domains of BLM A_6. The β-hydroxyhistidine moiety is highlighted in red.

CHAPTER 2 - SYNTHESIS OF A NEW SERIES OF SIMPLIFIED α-TOCOPHEROL ANALOGUES

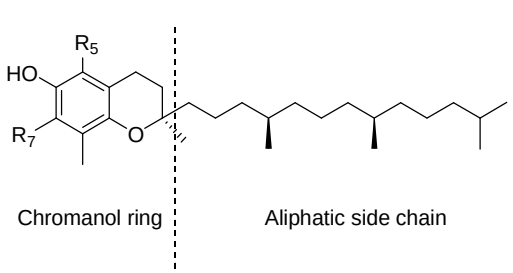
2.1 General introduction

The damage caused to mitochondria by excessive oxidative species impairs their function and also increases their susceptibility to further oxidative damage.[105] Defective mitochondria are considered one of the causes of disease progression in a wide range of diseases including Friedreich's ataxia, Alzheimer's disease, Parkinson's disease, hereditary spastic paraplegia, and Huntington's disease among others.[106]

Nutritionally derived antioxidants have failed to show any effect in the majority of clinical trials against diseases whose progression is caused by defective mitochondria. Experimental drugs structurally related to vitamin E and coenzyme Q are used to treat these mitochondrial dysfunctions.[107] Employing the knowledge obtained from the study of both antioxidants, more potent agents have been obtained.

Vitamin E is the common name of a group of eight molecules, each containing a chromanol ring and a lipophilic side chain.[108] Among this family, the most potent radical scavenger is α-tocopherol (Figure 2.1).[109] This compound reacts with lipid peroxyl radicals forming a stable tocopheroxyl radical. The stability of this radical is due to the resonance stabilization of the system. These tocopheroxyl radicals are quenched by reacting with other peroxyl radicals.[110] However, in the absence of another radical to terminate the radical propagation, the tocopheroxyl radical will act as an oxidant by reacting with fatty acids.[111] α-Tocopherol can be recycled in the body by ascorbic acid.[112] Both antioxidants in conjunction form the most efficient defense against lipid peroxidation in vivo.[113] Following the literature related to the structure and activity of α-tocopherol,[114] a thoughtful modification of its structure was

pursued. The synthesis of active analogues of α-tocopherol represents an interesting strategy to obtain potent and efficient antioxidants.[115] The activity of these new structures will be compared to α-tocopherol.



	R_5	R_7
α -T	CH_3	CH_3
β -T	H	CH_3
γ -T	CH_3	H
δ -T	H	H

Figure 2.1. Structures of components of vitamin E.

The Hecht laboratory has focused on the synthesis of diverse analogues of coenzyme CoQ_{10}.[116] Because of the potential of α-tocopherol analogues as promising antioxidants, research exploring these antioxidants has gained attention. 6-Amino-3-pyridinols possess cores that make them interesting antioxidants because biological assays have shown that they can quench peroxyl radicals better than α-tocopherol. Pyridinols **2.1** and **2.2** are 28 and 88 times more potent than α-tocopherol in quenching the peroxidation of methyl linoleate in benzene solution (Figure 2.2).[117]

For antioxidant **2.3**, it was proposed by Hecht and coworkers to synthesize an analogue possessing the same core as **2.1** and a lipophilic tail to improve its delivery to the mitochondrial membranes. This new antioxidant possessed a phytyl tail as a lipophilic group in position 4 of the pyridinol ring. Bicyclic antioxidant **2.3** was found to block peroxidation of the mitochondrial membranes and to protect cells against ROS more efficiently than α-tocopherol. However, the main drawback of **2.3** is that

its synthesis required sixteen steps. Additionally, several steps gave unexceptional yields and toxic reagents such as selenium dioxide (SeO_2) were used.[118]

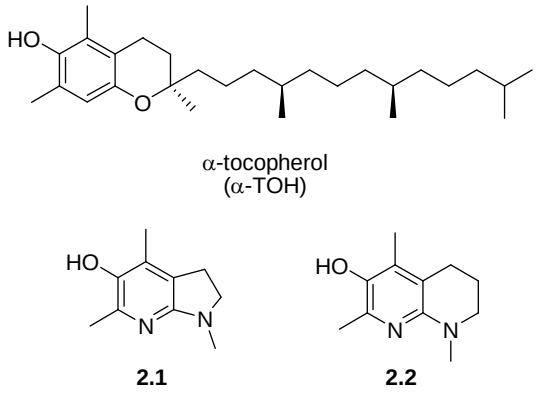


Figure 2.2. Structures of α-tocopherol and 6-amino-3-pyridinol cores **2.1** and **2.2**.

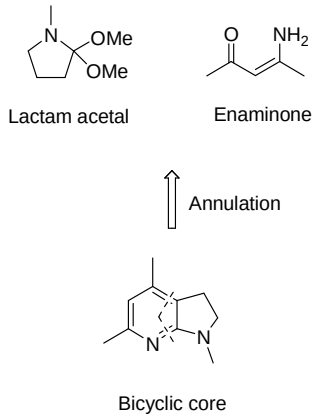


Figure 2.3. Retrosynthetic analysis of the bicyclic core by annulation.

In order to overcome these obstacles, Hecht and coworkers developed a novel method to synthesize bicyclic pyridinols **2.1** and **2.2** in a shorter and more manageable fashion. The main improvement consisted of the annulation of a lactam acetal with an enaminone (Figure 2.3).[119] With the efficient synthesis of the desired

cores, the lipophilic chain can be attached via regioselective metalation of the methyl group at the position 2 of the pyridine ring. Additionally, the phytyl tail was substituted with a linear alkyl chain. The role of the phytyl tail is to deliver the active core to the mitochondria efficiently therefore it can be replaced with an alkyl chain. Hecht and coworkers have studied the optimization of the length of the alkyl chain in ubiquinone analogues and discovered that the ten carbon chain was optimal.[120] Hecht and coworkers replaced the phytyl tail with alkyl substituents having five, ten and sixteen carbons (Figure 2.4). Among these analogues (**2.4**-**2.9**), only those possessing a ten carbon chain strongly decreased ROS levels, quenched lipid peroxidation, and maintained cell viability against induced oxidative stress. The analogues possessing five and sixteen carbons showed significantly less activity than **2.6** and **2.7**.[121]

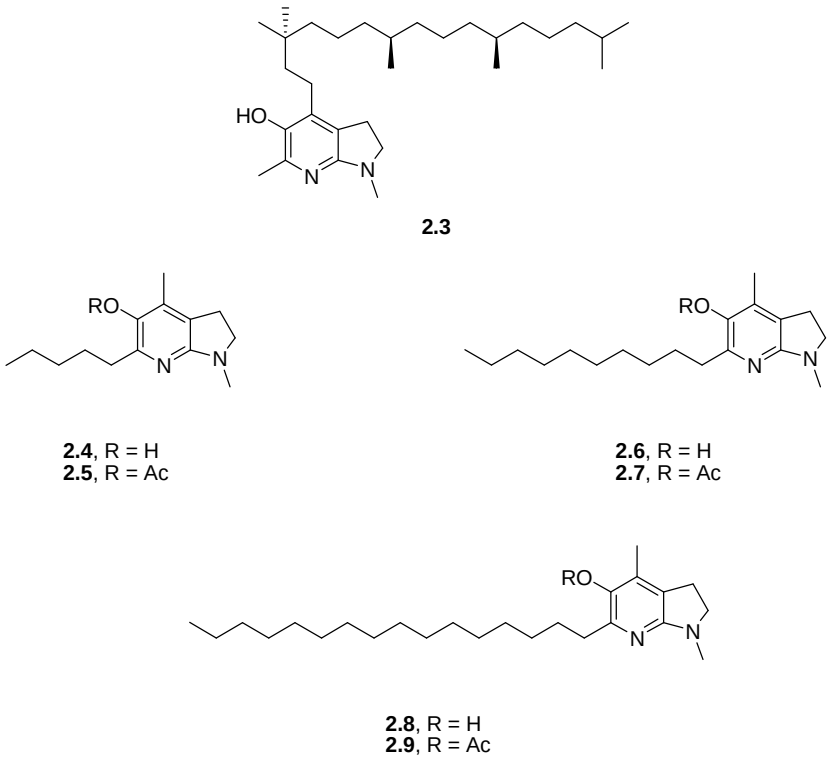
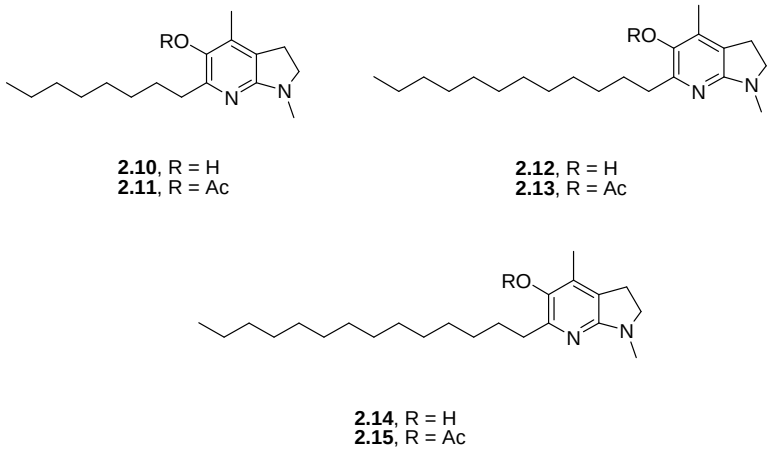


Figure 2.4. Structures of α-TOH analogues **2.3-2.9** synthesized by Hecht and coworkers.

The fact that only **2.6** and **2.7** had useful antioxidant properties while the analogues with shorter (**2.4** and **2.5**) and longer alkyl (**2.8** and **2.9**) chains showed significantly less activity, raised the question of the optimal length of the side chain for this series of analogues. To answer this question, additional analogues were synthesized (Figure 2.5).



2.10, R = H
2.11, R = Ac

2.12, R = H
2.13, R = Ac

2.14, R = H
2.15, R = Ac

Figure 2.5. Structures of new antioxidant analogues **2.10**-**2.15.**

To further elucidate properties of α-tocopherol analogues, a new core has been studied. Pyrrolopyridine **2.16**, the unsaturated form of **2.1**, offers a promising nucleus for an additional series of analogues (Figure 2.6). The extended planar structure and larger resonance of **2.16** compared with **2.1** could possibly contribute in the stabilization of the radical intermediate. Analogues **2.10**-**2.15** and **2.17**-**2.24** were tested for lipid peroxidation, mitochondrial membrane potential, cell viability, and ROS protection in cells depleted of glutathione. These results are presented below.

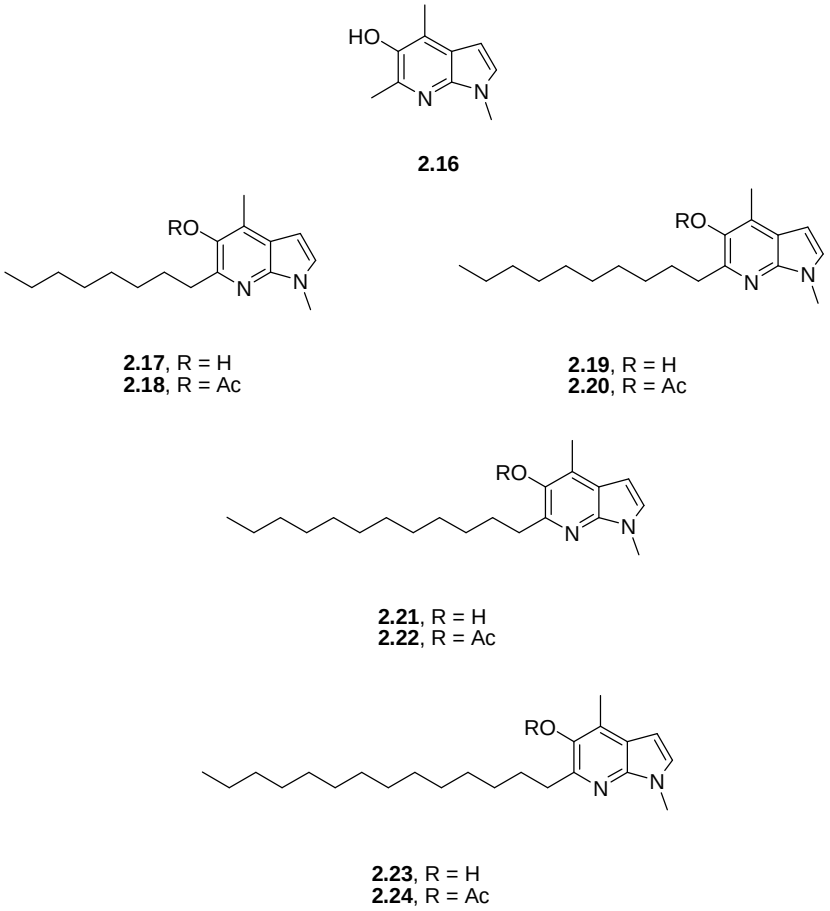


Figure 2.6. Structures of unsatured antioxidant **2.16** and antioxidant analogues **2.17-2.24.**

2.2 Results and discussion

2.2.1 Synthesis of bicyclic antioxidants

The synthesis of these analogues followed the retrosynthetic pathway that Hecht and coworkers used previously to obtain **2.4**-**2.9** (Figure 2.7).[121] The synthesis of these analogues used 1,4,6-trimethy-2,3-dehydro-1*H*-pyrrolo[2,3-*b*]pyridine (**2.26)** as a common intermediate.

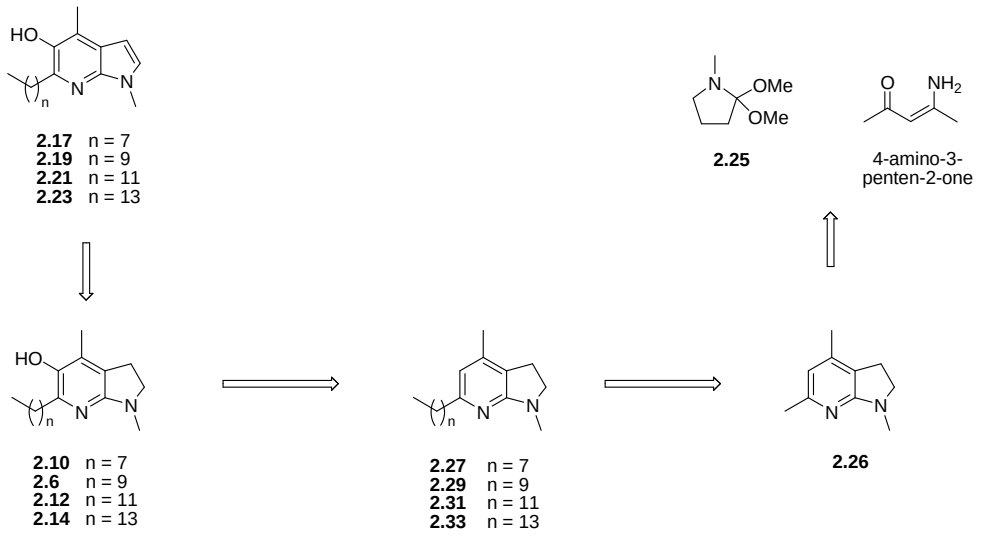
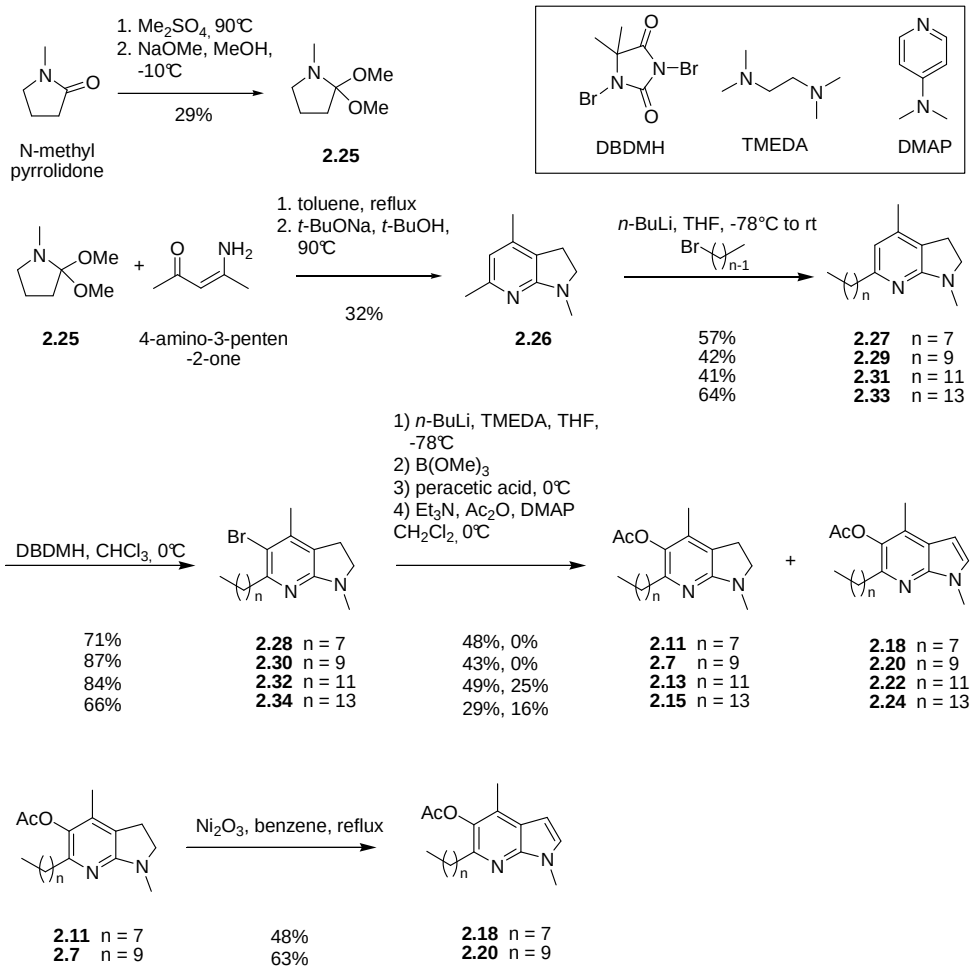


Figure 2.7. Retrosynthetic analysis of analogues **2.6**, **2.10**, **2.12**, **2.14**, **2.17**, **2.19**, **2.21**, and **2.23**.

Lactam acetal **2.25** was produced by reacting 1-methyl-2-pyrrolidone with dimethyl sulfate at 90 °C and subsequent treatment with sodium methoxide in methanol at −10 °C. Desired lactam acetal **2.25** was obtained in 29% yield after distillation and was stored in a desiccator after being purged with argon. The synthesis of bicyclic core **2.26** was the most important step in the scheme because it generates this complicated bicyclic core in one step. Lactam acetal **2.25** was cyclocondensed by treatment with 4-amino-3-penten-2-one in toluene at reflux followed by the addition of sodium *tert*-butoxide and *tert*-butanol at 90 °C to give bicyclic species **2.26** in 32% yield. Bicyclic core **2.26** was the main intermediate for all products (Scheme 2.1).



Scheme 2.1. Synthesis of pyridinol acetates **2.7**, **2.11**, **2.13**, **2.15**, **2.18**, **2.20**, **2.22**, and **2.24**.

The synthesis of pyridinol acetate **2.11**, containing an eight carbon side chain, continued with the alkylation of **2.26** using *n*-butyllithium and bromoheptane in tetrahydrofuran at −78 °C to afford bicyclic intermediate **2.27** in 57% yield. Bicyclic intermediate **2.27** was brominated using 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in chloroform at 0 °C to obtain bromide **2.28** in 71% yield. Bromide **2.28** was first treated with tetramethylethylenediamine (TMEDA) and *n*-butyllithium in

tetrahydrofuran at −78 °C. For the second step, trimethoxyborane was added at −78 °C; and for third step, peracetic acid was added at −78 °C to give the desired phenol. This phenol was acetylated using triethylamine, 4-dimethylaminopyridine and acetic anhydride to afford pyridinol acetate **2.11** in 48% yield. Oxidation of **2.11** with nickel peroxide gave oxidized pyridinol acetate **2.18** in 48% yield.

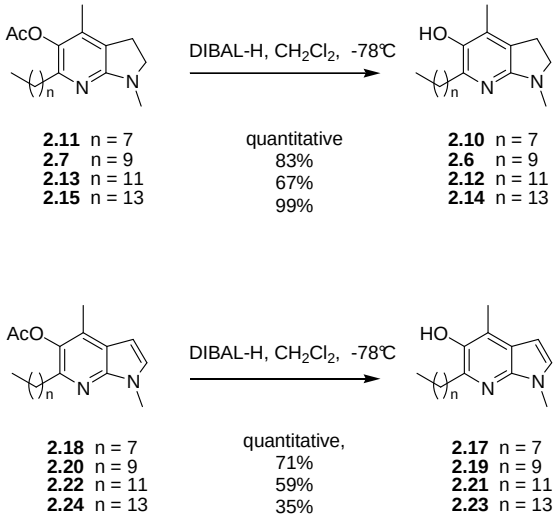
The synthesis of pyridinol acetate **2.7,** containing a ten carbon side chain, continued with the alkylation of **2.26** using *n*-butyllithium and bromononane in tetrahydrofuran at −78 °C to afford bicyclic intermediate **2.29** in 42% yield. Bicyclic intermediate **2.29** was brominated using 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in chloroform at 0 °C to obtain bromide **2.30** in 87% yield. Bromide **2.30** was first treated with tetramethylethylenediamine and *n*-butyllithium in tetrahydrofuran at −78 °C. For the second step, trimethoxyborane was added at −78 °C; and for third step, peracetic acid was added at −78 °C to give the desired phenol. This phenol was acetylated using triethylamine, 4-dimethylaminopyridine and acetic anhydride to afford pyridinol acetate **2.7** in 43% yield. Oxidation of **2.7** with nickel peroxide gave oxidized pyridinol acetate **2.20** in 63% yield.

The synthesis of pyridinol acetate **2.13,** containing a twelve carbon side chain, continued with the alkylation of **2.26** using *n*-butyllithium and bromoundecane in tetrahydrofuran at −78 °C to afford bicyclic intermediate **2.31** in 41% yield. Bicyclic intermediate **2.31** was brominated using 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in chloroform at 0 °C to obtain bromide **2.32** in 84% yield. Bromide **2.32** was first treated with tetramethylethylenediamine and *n*-butyllithium in tetrahydrofuran at −78 °C. For the second step, trimethoxyborane was added at −78

°C; and for third step, peracetic acid was added at −78 °C to give the desired phenol. This phenol was acetylated using triethylamine, 4-dimethylaminopyridine and acetic anhydride to afford pyridinol acetate **2.13** in 49% yield. Additionally, oxidized pyridinol acetate **2.22** was obtained as a byproduct in 25% yield.

The synthesis of pyridinol acetate **2.15,** containing a fourteen carbon side chain, continued with the alkylation of **2.26** using *n*-butyllithium and bromotridecane in tetrahydrofuran at −78 °C to afford bicyclic intermediate **2.33** in 64% yield. Bicyclic intermediate **2.33** was brominated using 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in chloroform at 0 °C to obtain bromide **2.34** in 66% yield. Bromide **2.34** was first treated with tetramethylethylenediamine and *n*-butyllithium in tetrahydrofuran at −78 °C. For the second step, trimethoxyborane was added at −78 °C; and for third step, peracetic acid was added at −78 °C to give the desired phenol. This phenol was acetylated using triethylamine, 4-dimethylaminopyridine and acetic anhydride to afford pyridinol acetate **2.15** in 29% yield. Additionally, oxidized pyridinol acetate **2.24** was obtained as a byproduct in 16% yield.

Free pyridinols were obtained from their respective pyridinol acetates by deacetylation using diisobutylaluminium hydride in dichloromethane at −78 °C. Then, pyridinols were converted to their triflate salts by treatment with a dilute solution of trifluoroacetic acetic acid (TFA) in water. The results of each final reaction can be observed in Scheme 3.2. The pyridinols and their respective acetates were tested in biological assays.



Scheme 2.2. Synthesis of free pyridinols **2.6**, **2.110**, **2.12**, **2.14**, **2.17**, **2.19**, **2.21**, and **2.23** from their respective acetates.

2.2.2 Biochemical and biological evaluation of synthesized analogues

2.2.2.1 Assessment of lipid peroxidation in cultured cells

Lipid peroxidation was determined in FRDA cells by monitoring the fluorescence of the peroxidation-sensitive dye C_{11}-BODIPY$^{581/591}$. Diethyl maleate (DEM) was used to deplete glutathione. The depletion of glutathione in FRDA cells increases the levels of ROS produced inside the cell and the mitochondria. Pretreatment of FRDA cells with phenolic compounds **2.14** (fourteen carbon chain, reduced core), **2.21** (twelve carbon chain, oxidized core), and **2.23** (twelve carbon chain, oxidized core) blocked lipid peroxidation even at 250 nM. Their acetates exhibited significantly smaller quenching activity, suggesting that the cleavage of the acetates' ester did not occur in a significant fashion.

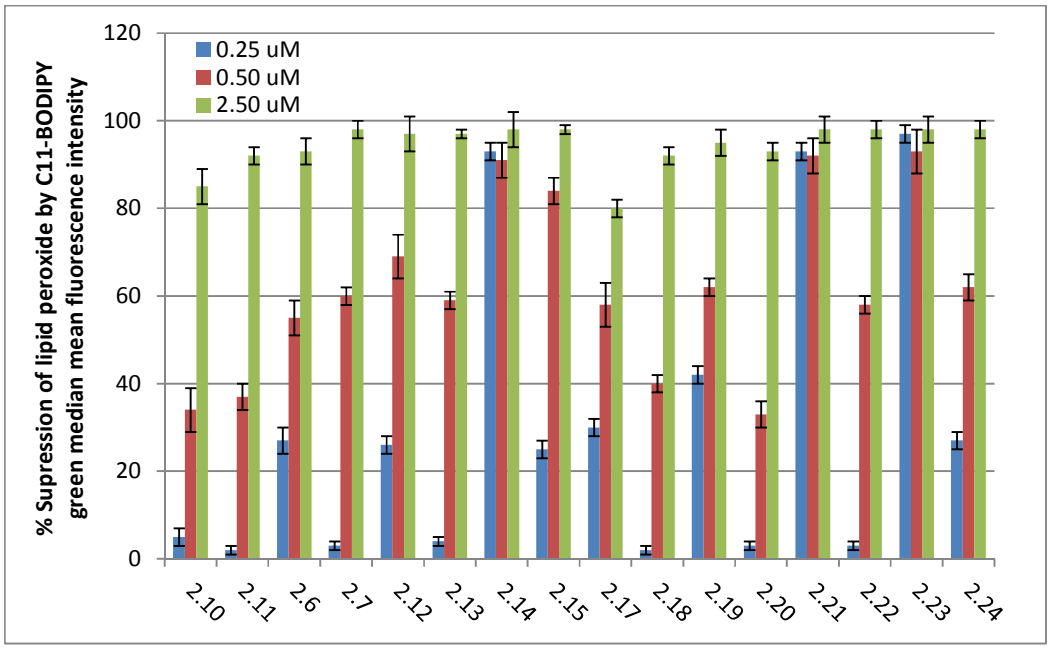


Figure 2.8. Lipid peroxidation in FRDA cells depleted of glutathione. Following pretreatment with the indicated compounds at 250 nM, 500 nM, and 2.50 μM concentration, the cells were treated with 5 mM of diethyl maleate (DEM) for 80 minutes to deplete glutathione. The cells were then treated with 500 nM C_{11}-BODIPY$^{581/591}$ in the dark at 37 °C for 30 minutes. The cells were washed twice in phosphate buffered saline and resuspended in Hanks' Balanced Salt Solution buffer before they were subjected to flow cytometry analysis using the FL1-H channel for C_{11}-BODIPY$^{581/591}$ – green (oxidized form). The figure shows a representative example of three independent experiments. In each analysis, 10,000 events were recorded. The bottom panel shows a bar graph of mean C_{11}-BODIPY$^{581/591}$ – green (oxidized form) fluorescence (a.u.) recorded by FACS and represents the percentage of the fluorescence means of the above flow cytogram profiles calculated using CellQuest software. Data are expressed as means ± SEM (n = 3). This experiment was performed by Dr. Omar Khdour.

2.2.2.2 Assessment of ROS production in cultured cells

Additionally, ROS quenching of these compounds was analyzed using FRDA cells depleted of glutathione. Diethyl maleate was used to deplete glutathione. Pretreatment of FRDA cells with phenolic compounds **2.14** (fourteen carbon chain, reduced core), **2.21** (twelve carbon chain, oxidized core), and **2.23** (fourteen carbon chain, oxidized core) quenched ROS even at 500 nM. Their respective acetates **2.15** (fourteen carbon chain, reduced core), **2.22** (twelve carbon chain, oxidized core), and **2.24** (fourteen carbon chain, oxidized core) were as effective as the phenolic compounds in the suppression of ROS. The most potent agent was **2.24**. It was the only compound able to quench ROS at 250 nM. From both studies, it can be observed that both twelve and fourteen carbon analogues were the most effective at protecting cells against ROS.

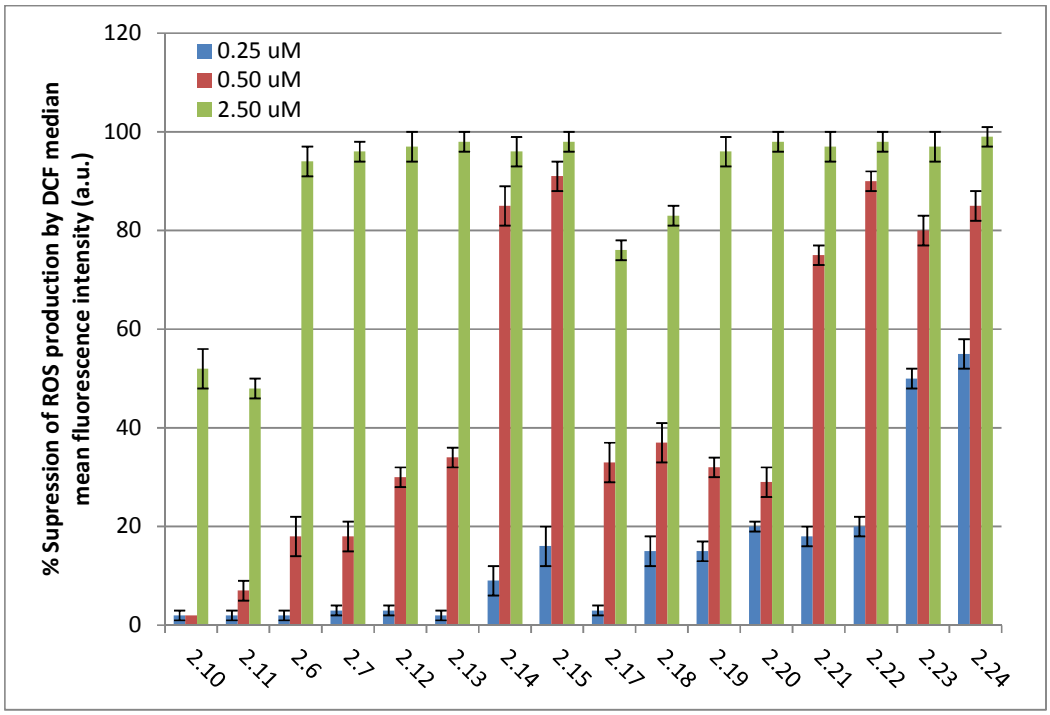


Figure 2.9. ROS production in FRDA cells depleted of glutathione. Following pretreatment with the indicated compounds (250 nM, 500 nM and 2.50 μM) for 16 h, the cells were treated with 5 mM of diethyl maleate (DEM) for 80 min to deplete glutathione. The cells were then washed in phosphate-buffered saline and suspended in phosphate-buffered saline containing 20 mM of glucose. Cells were loaded with 10 μM dichlorodihydrofluorescein diacetate (DCFH-DA) for 20 min and the green fluorescence (DCF) was measured by flow cytometry (C6 Accuri, BD Biosciences, San Jose, CA) using a 488 nm excitation laser and the FL1-H channel 530 ± 15 nm emission filter. The figure shows a bar graph of ROS % scavenging activity. Data are expressed as the mean ± SEM (n = 3). This experiment was performed by Dr. Omar Khdour.

2.2.2.3 Assessment of inhibition on the mitochondrial electron transport chain

The inhibitory effect of the synthesized compounds on bovine heart mitochondrial complexes I, II, and IV was studied. For each compound, higher concentrations correlated with a decrease in the activity of NADH oxidase. The acetates of the pyridinols that possessed oxidized cores showed an increase in NADH oxidase activity. This increase in activity correlated with an increase in length of the carbon side chain.

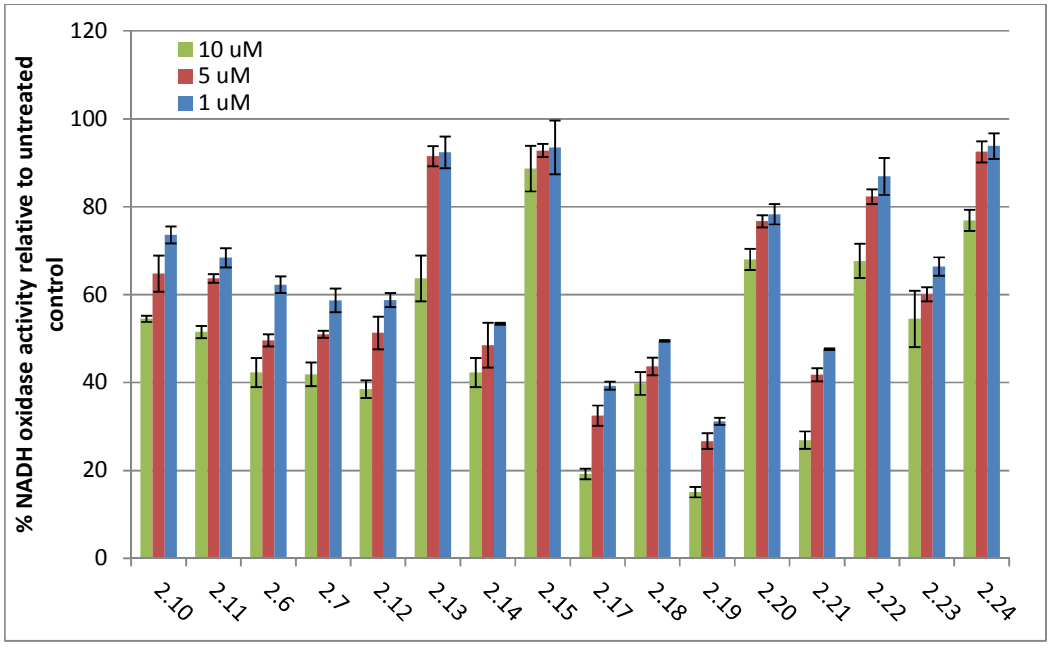


Figure 2.10. Inhibitory effects on bovine heart mitochondrial NADH oxidase activity. The inhibitory effects of the test compounds on bovine heart mitochondrial complexes I, III and IV were evaluated. The compounds were dissolved in *N,N*-dimethylsulfoxide (DMSO). Bovine heart submitochondrial particles were diluted to 0.5 mg/mL. NADH oxidase activity was determined in a reaction medium (2.5 mL total volume) containing 50 mM of Hepes, pH 7.5, and 5 mM of magnesium chloride.

The final mitochondrial protein concentration was 30 µg. After the pre-equilibration of submitochondrial particles with inhibitor for 5 min, the initial rates were calculated from the linear portion of the traces. The figure shows a bar graph of % NADH oxidase activity. Activity was expressed as the percentage of untreated cells. Data are expressed as the mean ± SEM (n = 3). This experiment was performed by Sriloy Dey.

2.2.2.4 Assessment of cell viability in cultured cells

The effect of the synthesized phenolic compounds on cell viability was assessed using FRDA cells depleted of glutathione. Diethyl maleate was used to deplete glutathione. Pretreatment with all compounds except **2.10** protected FRDA cells against oxidative stress at 0.5 µM. No compound offered protection at 0.1 µM.

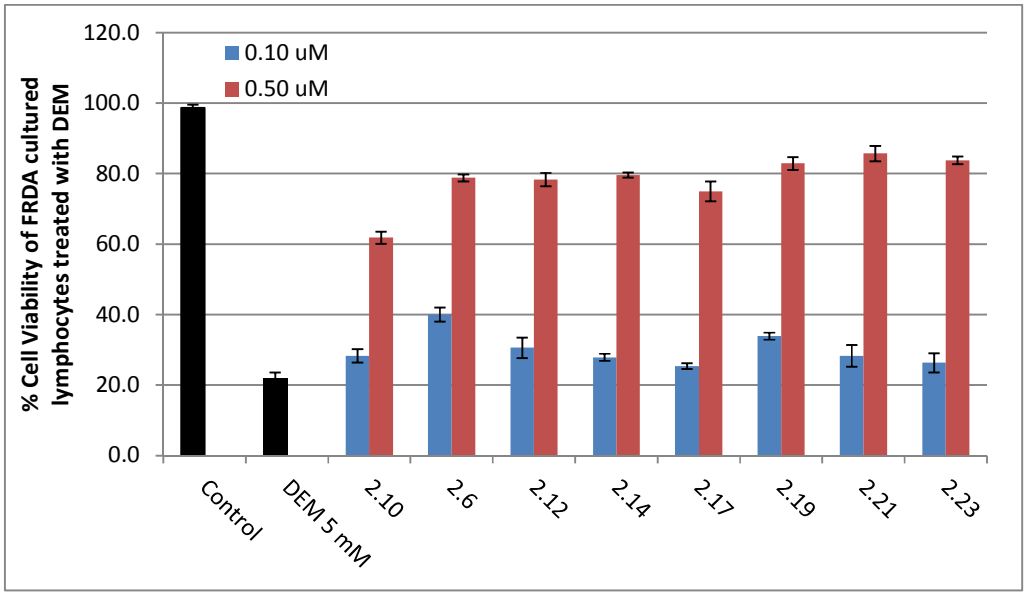


Figure 2.11. Cytoprotection of FRDA cells depleted of glutathione. Following pretreatment with the indicated compounds (0.10 µM and 0.50 µM) for 17 h, the cells were treated with 5 mM of diethyl maleate for 6 h to deplete glutathione and to induce

oxidative stress. Trypan blue was used to determine cell viability. Cell viability was expressed as the percentage compared to the untreated control. Data are expressed as means ± SEM. (n = 3). This experiment was performed by Basab Roy.

2.2.2.5 Assessment of maintenance of mitochondrial membrane potential ($\Delta\psi_m$)

The ability of these phenolic compounds to maintain mitochondrial membrane potential ($\Delta\psi_m$) was analyzed using FRDA cells depleted of glutathione. Diethyl maleate was used to deplete glutathione. Carbonyl cyanide-4-(trifluoromethyl) phenylhydrazone (FCCP) was used as a negative control, since FCCP depolarizes the mitochondrial membrane. JC-1 was used as a specific probe. JC-1 accumulates and attaches to healthy mitochondria exhibiting red fluorescence. Conversely, JC-1 fluoresces green when mitochondria lose potential and aggregates of JC-1 do not form. Pretreatment of FRDA cells with phenolic compounds **2.10** (eight carbon chain, reduced core) and **2.14** (fourteen carbon chain, reduced core) protected the mitochondria partially at 1 μM. Interestingly, no protection was provided by either compound at 5 μM. These data show the importance of the optimal concentration of these phenolic compounds to obtain protection of mitochondria (Figure 2.12).

The impact of these pyridinols in healthy FRDA cells (which means they were not treated with DEM) was also studied. The presence of compounds **2.10** (eight carbon chain, reduced core), **2.6** (ten carbon chain, reduced core), and **2.14** (fourteen carbon chain, reduced core) had no impact on mitochondrial potential at 1 μM. All these experiments were carried in absence of DEM. However, when the concentration was increased to 5 μM, all three compounds depolarized the mitochondria to different

degrees. The compound with the longest carbon chain **2.14** (fourteen carbon chain, reduced core) depolarized the mitochondria significantly, reaching levels comparable to FRDA cells treated with diethyl maleate. In contrast, the compound with the shortest chain **2.10** (eight carbon chain, reduced core) minimally depolarized the mitochondria (Figure 2.13).

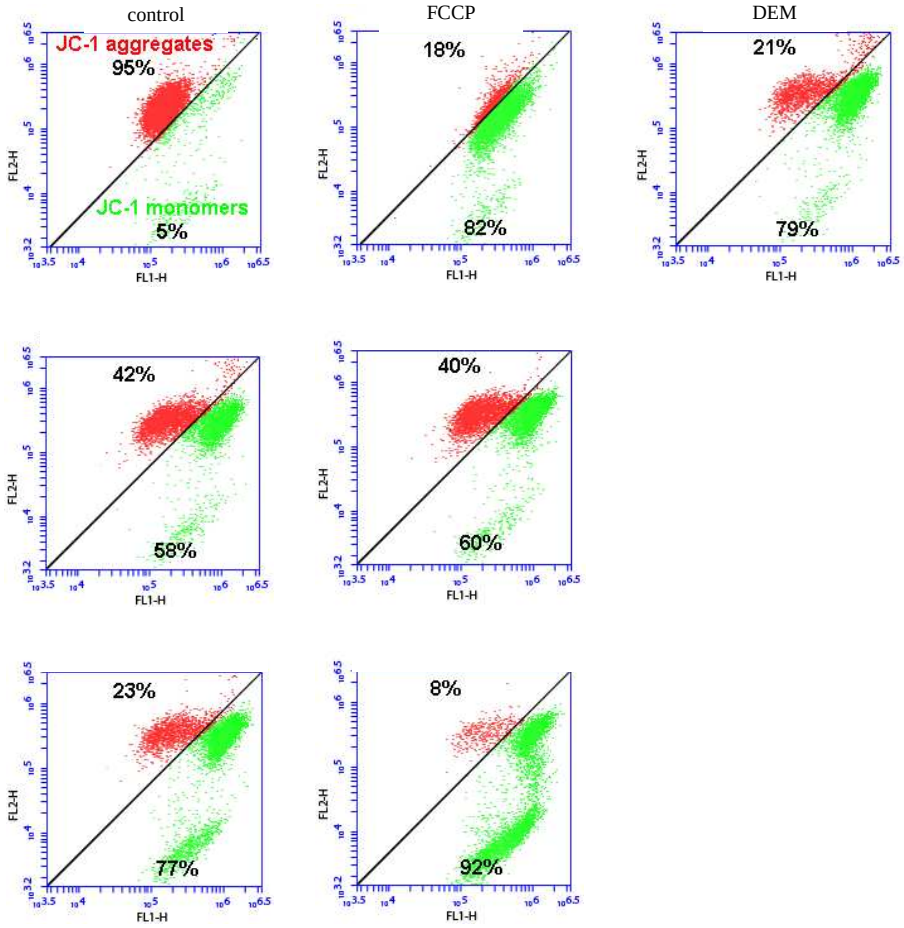


Figure 2.12. Maintenance of $\Delta\psi_m$ in FRDA cells depleted of glutathione. Representative flow cytometric two-dimensional color density dot plot analyses of $\Delta\psi_m$ in FRDA lymphocytes stained with JC-1. The percentage of cells with intact $\Delta\psi_m$ is indicated in the top left section of captions. In each analysis, 10,000 events

were recorded. Data are expressed as means ± SEM of three independent experiments run in duplicate. The bar graph shows the percentage of cells with intact $\Delta\psi_m$ calculated using CellQuest software. This experiment was performed by Dr. Omar Khdour.

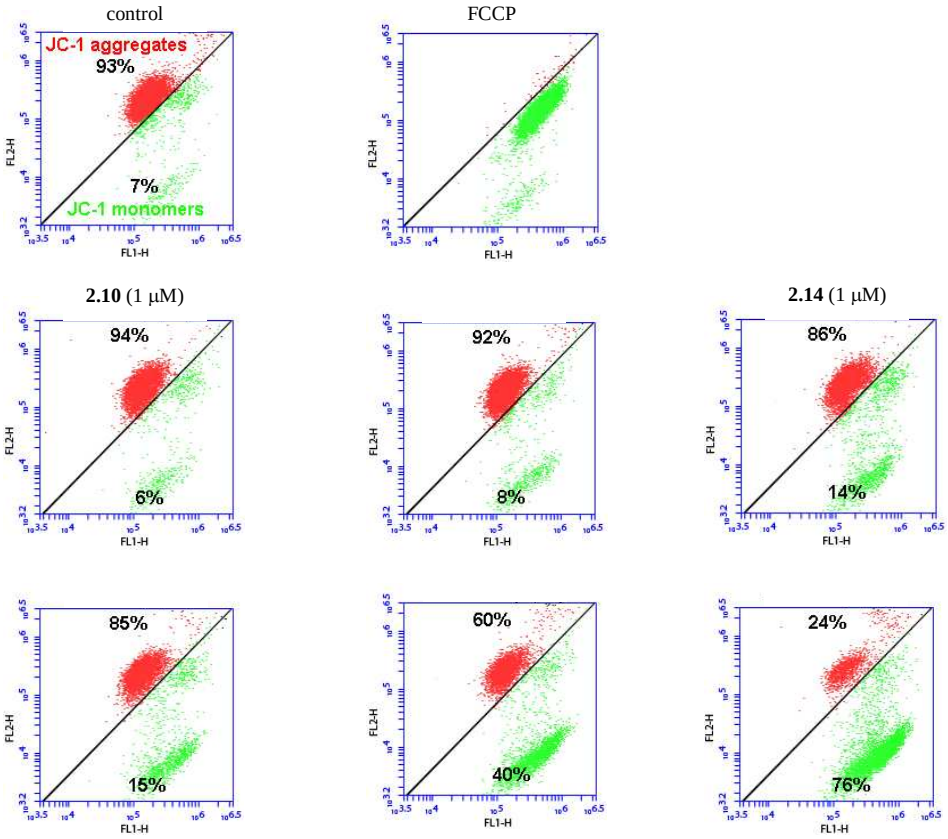


Figure 2.13. Maintenance of $\Delta\psi_m$ in FRDA cells. Representative flow cytometric two-dimensional color density dot plot analyses of $\Delta\psi_m$ in FRDA lymphocytes stained with JC-1. The percentage of cells with intact $\Delta\psi_m$ is indicated in the top left section of captions. In each analysis, 10,000 events were recorded. Data are expressed as means ± SEM of three independent experiments run in duplicate. The bar graph

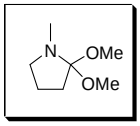
shows the percentage of cells with intact $\Delta\psi_m$ calculated using CellQuest software. This experiment was performed by Dr. Omar Khdour.

In conclusion, the best compounds to protect FRDA cells from lipid peroxidation and ROS are the pyridinols that possess twelve and fourteen carbon chain. However, it seems that cytoprotection is only afforded at low concentrations (250-500 nM). At higher concentrations (5 μM or higher), the data obtained from the mitochondrial membrane potential assay suggests that these compounds may be causing oxidative stress.

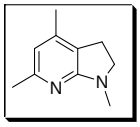
2.3 Experimental Section

General methods: Chemicals and solvents were of reagent grade and were used without further purification. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone under argon. Anhydrous dichloromethane (CH_2Cl_2) was distilled from calcium hydride under argon. Anhydrous diethyl ether (Et_2O) was distilled from sodium/benzophenone under argon. All reactions involving air or moisture sensitive reagents or intermediates were performed under an argon atmosphere. Flash chromatography was carried out using Silicycle 200-400 mesh silica gel. Analytical TLC was carried out using 0.25 mm EM silica gel 60 F250 plates that were visualized by UV irradiation (254 nm) or by staining with ceric ammonium molybdate stain. ^{1}H NMR and ^{13}C NMR spectra were obtained using 400 or 500 MHz Varian NMR spectrometers. Chemicals shifts were reported in parts per million (ppm, δ) referenced to the residual ^{1}H resonance of the solvent ($CDCl_3$, 7.26 ppm; CD_3CN, 1.94 ppm; C_6D_6, 7.16 ppm). ^{13}C spectra were referenced to the residual

^{13}C resonance of the solvent ($CDCl_3$, 77.0 ppm; CD_3CN, 1.32 ppm; C_6D_6, 128.06 ppm)). Splitting patterns were designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; quint, quintuplet; m, multiplet. High resolution mass spectra were obtained at the Arizona State University CLAS High Resolution Mass Spectrometry Laboratory.

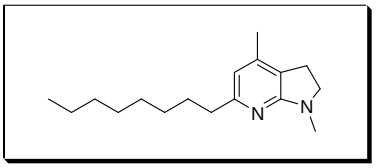


2,2-Dimethoxy-1-methylpyrrolidine (2.25).[121] A mixture containing 10.0 mL (10.3 g, 104 mmol) of 1-methyl-2-pyrrolidinone and 10.0 mL (13.3 g, 105 mmol) of dimethyl sulfate was stirred and heated at 90 °C for 1.5 h, then allowed to cool to room temperature. A solution containing 25.0 mL of 25% methanolic sodium methoxide and 72.0 mL of methanol was added at −10 °C under Ar over a period of 1 h. The precipitated solid was filtered and the solvent was concentrated under diminished pressure. The residue was dissolved in 100 mL of diethyl ether and stirred for 1 h, and then the precipitated solid was filtered. The solid was washed with two 10-mL portions of diethyl ether. After concentration of the combined filtrate under diminished pressure, the residue was distilled under diminished pressure to give **2.25** as a yellow liquid: yield 4.42 g (29%); ^1H NMR ($CDCl_3$) δ 1.50-1.61 (m, 2H), 1.71 (t, 2H, J = 7.8 Hz), 2.14 (s, 6H), 2.64 (t, 2H, J = 6.6 Hz), and 3.01 (s, 3H).



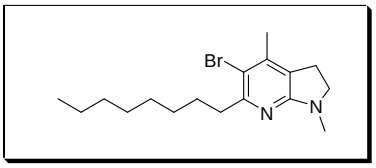
1,4,6-Trimethyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.26).[121] To a solution containing 1.20 g (12.1 mmol) of 4-amino-3-penten-2-one in 8 mL of toluene was

added 3.00 g (20.7 mmol) of **2.25**. The reaction mixture was heated at reflux and stirred for 2 h, then cooled to 90 °C and treated with 2.38 g (24.8 mmol) of sodium *tert*-butoxide and 2 mL of *tert*-butanol. The reaction mixture was stirred at 90 °C for another 16 h. The cooled reaction mixture was quenched by the addition of 10 mL of sat aq NH_4Cl. The reaction mixture was extracted with three 30-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (42 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **2.26** as a brown oil: yield 0.62 g (32%); silica gel TLC R_f 0.15 (1:4 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H), 2.20 (s, 3H), 2.65 (t, 2H, J = 8.4 Hz), 2.76 (s, 3H), 3.23 (t, 2H, J = 8.4 Hz), and 5.98 (s, 1H).



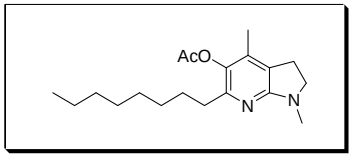
1,4-Dimethyl-6-octyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.27). To a solution containing 490 mg (2.71 mmol) of **2.26** in 7.2 mL of tetrahydrofuran was added 2.70 mL (4.34 mmol) of *n*-butyllithium (1.6 M in hexanes) followed by 450 μL (509 mg, 2.85 mmol) of 1-bromoheptane at –78 °C. The reaction mixture was warmed slowly to room temperature and stirred for 16 h. The reaction mixture was quenched by the addition of 25 mL of sat aq NH_4Cl at 0 °C. The reaction mixture was extracted with three 40-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (42 x 4 cm).

Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.27** as a yellow oil: yield 402 mg (57%); silica gel TLC R_f 0.36 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.79 (t, 3H, *J* = 7.2 Hz), 1.16-1.30 (m, 10H), 1.58 (quint, 2H, *J* = 8.0 Hz), 2.00 (s, 3H), 2.48 (t, 2H, *J* = 8.0 Hz), 2.72 (t, 2H, *J* = 8.4 Hz), 2.82 (s, 3H), 3.30 (t, 2H, *J* = 8.4 Hz), and 6.05 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.0, 22.7, 24.4, 29.4, 29.4, 29.6, 30.0, 32.0, 33.3, 38.1, 52.5, 112.5, 118.3, 141.2, 159.2, and 163.8; mass spectrum (EI), *m/z* 260.2247 $(M)^+$ ($C_{17}H_{28}N_2$ requires 260.2253).



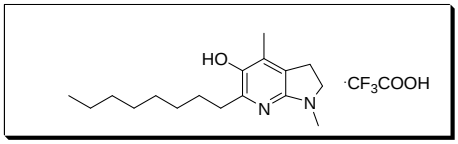
5-Bromo-1,4-dimethyl-6-octyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.28). To a solution containing 402 mg (1.54 mmol) of **2.27** in 6 mL of chloroform was added 220 mg (0.77 mmol) of 1,3-dibromo-5,5-dimethylhydantoin in five portions at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched by the addition of 8 mL of sat aq $NaHCO_3$. The reaction mixture was extracted with three 25-mL portions of chloroform. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:9 ethyl acetate–hexanes as eluant gave **2.28** as a yellow oil: yield 371 mg (71%); silica gel TLC R_f 0.65 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 6.0 Hz), 1.10-1.43 (m, 10H), 1.67 (quint, 2H, *J* = 8.0 Hz), 2.14 (s, 3H), 2.75-2.82 (m, 4H), 2.75-2.86 (s, 3H), and 3.37 (t, 2H, *J* = 8.4 Hz). ^{13}C NMR ($CDCl_3$) δ 14.1, 19.5, 22.7, 25.2,

28.7, 29.3, 29.5, 29.6, 31.9, 33.0, 38.1, 52.4, 110.9, 120.4, 141.1, 156.7, and 161.7; mass spectrum (APCI), *m/z* 339.1435 $(M+H)^+$ ($C_{17}H_{28}N_2Br$ requires 339.1436).



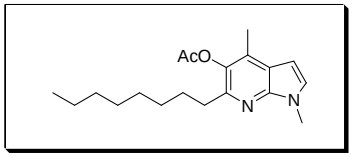
1,4-Dimethyl-6-octyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.11). To a solution containing 202 mg (0.60 mmol) of **2.28** in 5 mL of tetrahydrofuran was added 90.0 μL (69.7 mg, 0.60 mmol) of tetramethylethylenediamine at –78 °C followed by 0.75 mL (1.20 mmol) of *n*-butyllithium (1.6 M in hexanes). After 30 min, 147 μL (137 mg, 1.32 mmol) of trimethoxyborane were added and the resulting reaction mixture was stirred for another 1 h. To the reaction mixture was added dropwise 277 μL (100 mg, 314 mg total solution, and 1.32 mmol) of peracetic acid (32% wt.) and the solution was then warmed to 0 °C. The reaction mixture was diluted with 20 mL of water and extracted with three 25-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The resulting oil was dissolved in 10 mL of dichloromethane at 0 °C, followed by the addition of 485 μL (352 mg, 3.48 mmol) of triethylamine, 6.10 mg (0.05 mmol) of 4-dimethylaminopyridine and 164 μL (177 mg, 1.74 mmol) of acetic anhydride. The reaction mixture was stirred at room temperature for 1 h and quenched by the addition of 10 mL of sat aq NH_4Cl. The solution was extracted with three 30-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (46 x 3 cm). Step gradient elution with 1:9 →

1:4 ethyl acetate–hexanes as eluant gave **2.11** as a yellow oil: yield 92.0 mg (48%); silica gel TLC R_f 0.22 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, *J* = 7.2 Hz), 1.21-1.37 (m, 10H), 1.62 (quint, 2H, *J* = 7.2 Hz), 1.94 (s, 3H), 2.30 (s, 3H), 2.46 (t, 2H, *J* = 8.0 Hz), 2.83 (t, 2H, *J* = 8.4 Hz), 2.88 (s, 3H), and 3.42 (t, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 12.9, 14.1, 20.5, 22.6, 24.6, 28.7, 29.2, 29.5, 29.6, 31.9, 32.5, 33.3, 52.8, 120.1, 134.7, 136.5, 149.6, 161.2, and 169.9; mass spectrum (ESI), *m/z* 319.2390 $(M+H)^+$ ($C_{19}H_{31}N_2O_2$ requires 319.2386).



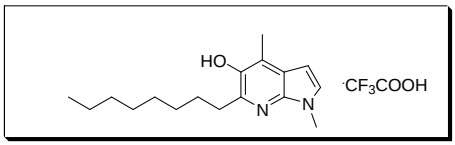
1,4-Dimethyl-6-octyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.10). To a solution containing 20.4 mg (64.1 μmol) of **2.11** in 1.0 mL of dichloromethane was added 200 μL (200 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at –78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.22 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq trifluoroacetic acid (TFA), frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1

methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 28 min, and were combined, frozen, and lyophilized to give **2.10** as a light yellow solid: yield 18.4 mg (quantitative); ^{1}H NMR (CD_3CN) δ 0.91 (t, 3H, *J* = 7.0 Hz), 1.28-1.40 (m, 10H), 1.57 (quint, 2H, *J* = 8.0 Hz), 2.15 (s, 3H), 2.72 (t, 2H, *J* = 8.0 Hz), 3.03 (t, 2H, *J* = 8.5 Hz), 3.08 (s, 3H), 3.77 (t, 2H, *J* = 8.5 Hz), and 7.73 (br s, 1H); ^{13}C NMR (CD_3CN) δ 13.0, 13.4, 22.4, 24.4, 27.3, 28.6, 28.7, 29.0, 29.0, 31.6, 32.4, 53.1, 126.3, 131.8, 140.3, 141.6, and 152.0; mass spectrum (EI), *m/z* 276.2207 $(M)^+$ ($C_{17}H_{28}N_2O_2$ requires 276.2202).



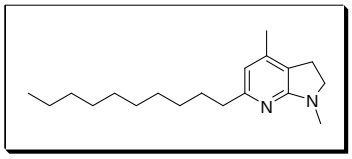
1,4-Dimethyl-6-octyl-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.18). To a solution containing 65.0 mg (0.20 mmol) of **2.11** in 5 mL of benzene was added 56.0 mg (0.61 mmol) of nickel peroxide. The reaction mixture was stirred at reflux for 18 h. The reaction mixture was filtered through a silica gel plug and washed with three 25-mL portions of benzene, followed by two 25-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (25 x 1.7 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.18** as a colorless oil: yield 50 mg (48%); silica gel TLC R_f 0.29 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, *J* = 7.2 Hz), 1.24-1.40 (m, 10H), 1.73 (quint, 2H, *J* = 7.6 Hz), 2.32 (s, 3H), 2.37

(s, 3H), 2.72 (t, 2H, *J* = 8.0 Hz), 3.83 (s, 3H), 6.38 (d, 1H, *J* = 3.6 Hz), and 7.07 (d, 1H, *J* = 3.6 Hz); ^{13}C NMR ($CDCl_3$) δ 12.8, 14.1, 20.6, 22.7, 28.9, 29.3, 29.5, 29.7, 31.3, 31.9, 33.2, 97.8, 119.4, 128.3, 131.0, 138.7, 144.9, 148.3, and 169.5; mass spectrum (FAB), *m/z* 317.2230 $(M+H)^+$ ($C_{19}H_{29}N_2O_2$ requires 317.2229).



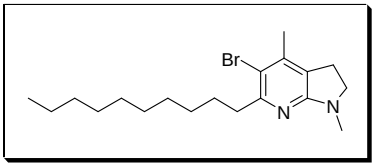
1,4-Dimethyl-6-octyl-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.17). To a solution containing 14.0 mg (44.2 μmol) of **2.18** in 1.0 mL of dichloromethane was added 140 μL (135 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.39 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 28.6 min, and were combined, frozen, and lyophilized to give **2.17** as a colorless

solid: yield 13.6 mg (quantitative); ^{1}H NMR (C_6D_6) δ 0.89 (t, 3H, *J* = 7.5 Hz), 1.21-1.48 (m, 10H), 2.00 (quint, 2H, *J* = 8.0 Hz), 2.18 (s, 3H), 2.20 (t, 2H, *J* = 8.0 Hz), 3.42 (s, 3H), 4.25 (br s, 1H), 6.29 (d, 1H, *J* = 3.5 Hz), and 6.60 (d, 1H, *J* = 2.5 Hz); ^{13}C NMR (C_6D_6) δ 12.0, 14.4, 23.1, 29.4, 29.8, 29.9, 30.1, 30.2, 30.9, 32.3, 33.6, 97.2, 120.1, 123.9, 143.2, 143.3, and 145.0; mass spectrum (EI), *m/z* 274.2043 $(M)^{+}$ ($C_{17}H_{26}N_2O$ requires 274.2045).

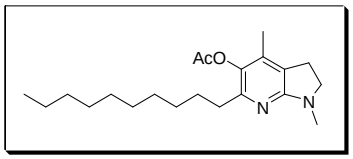


1,4-Dimethyl-6-decyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.29).[121] To a solution containing 600 mg (3.70 mmol) of **2.26** in 10 mL of tetrahydrofuran was added 3.70 mL (5.92 mmol) of *n*-butyllithium (1.6 M in hexanes) followed by 742 μL (805 mg, 3.89 mmol) of 1-bromononane at −78 °C. The reaction mixture was warmed slowly to room temperature and stirred for 16 h. The reaction mixture was quenched by the addition of 35 mL of sat aq NH_4Cl at 0 °C. The reaction mixture was extracted with three 60-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate−hexanes as eluant gave **2.29** as a yellow oil: yield 448 mg (42%); silica gel TLC R_f 0.44 (8:1:1 hexanes−ethyl acetate−methanol); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 7.2 Hz), 1.22-1.40 (m, 14H), 1.67 (quint, 2H, *J* = 8.0 Hz), 2.09 (s, 3H), 2.57 (t, 2H, *J* = 8.0 Hz), 2.82 (t, 2H, *J* = 8.0 Hz), 2.92 (s, 3H), 3.40 (t, 2H, *J* = 8.0 Hz), and 6.14 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 14.2, 18.1, 22.8, 24.5, 29.5, 29.6, 29.7, 29.7, 29.8, 30.0, 32.0, 33.3,

38.1, 52.6, 112.5, 118.3, 141.2, 159.2, and 163.8; mass spectrum (EI), *m/z* 288.2563 $(M)^+$ ($C_{19}H_{32}N_2$ requires 288.2566).

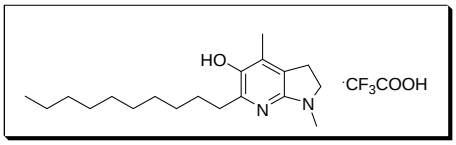


5-Bromo-1,4-dimethyl-6-decyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.30).[121] To a solution containing 100 mg (0.35 mmol) of **2.29** in 1.4 mL of chloroform was added 50.0 mg (0.18 mmol) of 1,3-dibromo-5,5-dimethylhydantoin in five portions at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched by the addition of 2 mL of sat aq $NaHCO_3$. The reaction mixture was extracted with three 15-mL portions of chloroform. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 2.5 cm). Step gradient elution with 1:9 ethyl acetate–hexanes as eluant gave **2.30** as a yellow oil: yield 112 mg (87%); silica gel TLC R_f 0.70 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.23-1.42 (m, 14H), 1.68 (quint, 2H, *J* = 7.6 Hz), 2.19 (s, 3H), 2.79 (t, 2H, *J* = 8.0 Hz), 2.84-2.90 (m, 5H), and 3.42 (t, 2H, *J* = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 14.3, 19.6, 22.8, 25.4, 28.8, 29.5, 29.7, 29.7, 29.8, 29.8, 32.1, 33.1, 38.3, 52.6, 111.1, 120.5, 141.2, 156.9, and 161.9; mass spectrum (FAB), *m/z* 367.1745 $(M+H)^+$ ($C_{19}H_{32}BrN_2$ requires 367.1749).



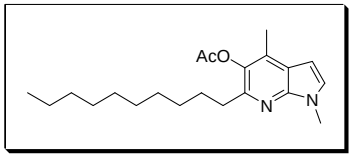
1,4-Dimethyl-6-decyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.7).[121] To a solution containing 293 mg (0.80 mmol) of **2.30** in 7 mL of tetrahydrofuran was added 113 μL (87.1 mg, 0.80 mmol) of tetramethylethylenediamine at −78 °C followed by 0.94 mL (1.50 mmol) of *n*-butyllithium (1.6 M in hexanes). After 30 min, 184 μL (171 mg, 1.65 mmol) of trimethoxyborane was added and the resulting reaction mixture was stirred for another 1 h. To the reaction mixture was added dropwise 346 μL (125 mg, 393 mg total solution, and 1.65 mmol) of peracetic acid (32% wt.) and the solution was then warmed to 0 °C. The reaction mixture was diluted with 25 mL of water and extracted with three 35-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The resulting oil was dissolved in 13 mL of dichloromethane at 0 °C, followed by the addition of 606 μL (440 mg, 4.36 mmol) of triethylamine, 8.50 mg (0.07 mmol) of 4-dimethylaminopyridine and 205 μL (221 mg, 2.18 mmol) of acetic anhydride. The reaction mixture was stirred at room temperature for 1 h and quenched by the addition of 12 mL of sat aq NH_4Cl. The solution was extracted with three 40-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.7** as a yellow oil: yield 118 mg (43%); silica gel TLC R_f 0.25 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J*

= 6.8 Hz), 1.22-1.37 (m, 14H), 1.62 (quint, 2H, *J* = 7.6 Hz), 1.94 (s, 3H), 2.30 (s, 3H), 2.46 (t, 2H, *J* = 8.0 Hz), 2.83 (t, 2H, *J* = 8.0 Hz), 2.88 (s, 3H), and 3.42 (t, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 12.9, 14.1, 20.5, 22.7, 24.6, 28.7, 29.3, 29.5, 29.5, 29.6, 29.6, 31.9, 32.5, 33.3, 52.8, 120.1, 134.7, 136.5, 149.6, 161.1, and 169.9; mass spectrum (EI), *m/z* 346.2624 $(M)^+$ ($C_{21}H_{34}N_2O_2$ requires 346.2620).



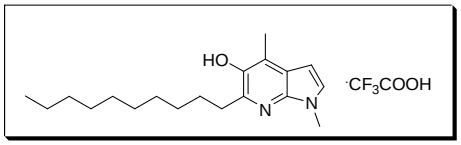
1,4-Dimethyl-6-decyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.6).[121] To a solution containing 21.1 mg (48.0 μmol) of **2.7** in 1.0 mL of dichloromethane was added 190 μL (19 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.28 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260

nm). Fractions containing the desired product eluted at 30 min, and were combined, frozen, and lyophilized to give **2.6** as a light yellow solid: yield 15.3 mg (83%); ^{1}H NMR (CD_3CN) δ 0.88 (t, 3H, *J* = 7.5 Hz), 1.21-1.35 (m, 14H), 1.53 (quint, 2H, *J* = 7.5 Hz), 2.12 (s, 3H), 2.68 (t, 2H, *J* = 8.0 Hz), 2.99 (t, 2H, *J* = 8.5 Hz), 3.05 (s, 3H), 3.73 (t, 2H, *J* = 8.5 Hz), and 5.57 (br s, 1H); ^{13}C NMR (CD_3CN) δ 13.0, 13.4, 22.4, 24.4, 27.3, 28.6, 29.0, 29.1, 29.1, 29.3, 29.3, 31.7, 32.4, 53.1, 126.2, 131.9, 140.3, 141.6, and 152.0; mass spectrum (APCI), *m/z* 305.2593 $(M+H)^+$ ($C_{19}H_{33}N_2O$ requires 305.2593).



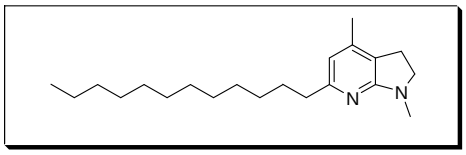
1,4-Dimethyl-6-decyl-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.20). To a solution containing 51.0 mg (0.15 mmol) of **2.7** in 4 mL of benzene was added 42.0 mg (0.46 mmol) of nickel peroxide. The reaction mixture was stirred at reflux for 18 h. The reaction mixture was filtered through a silica gel plug and washed with three 20-mL portions of benzene, followed by two 20-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (27 x 1.7 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.20** as a colorless oil: yield 35.0 mg (63%); silica gel TLC R_f 0.35 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.23-1.40 (m, 14H), 1.73 (quint, 2H, *J* = 7.6 Hz), 2.32 (s, 3H), 2.37 (s, 3H), 2.72 (t, 2H, *J* = 8.0 Hz), 3.83 (s, 3H), 6.37 (d, 1H, *J* = 3.6 Hz), and 7.07 (d, 1H, *J* = 3.6 Hz); ^{13}C NMR ($CDCl_3$) δ 12.8, 14.1, 20.6, 22.7, 28.9, 29.3, 29.5, 29.6,

29.6, 29.7, 31.3, 31.9, 33.3, 97.8, 119.4, 128.4, 131.0, 138.7, 144.9, 148.3, and 169.5; mass spectrum (EI), *m/z* 344.2459 $(M)^+$ ($C_{21}H_{32}N_2O_2$ requires 344.2464).

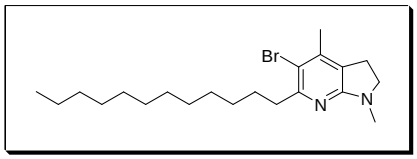


1,4-Dimethyl-6-decyl-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.19). To a solution containing 20.5 mg (59.5 μmol) of **2.20** in 1.0 mL of dichloromethane was added 180 μL (180 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.43 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 31 min, and were combined, frozen, and lyophilized to give **2.19** as a colorless solid: yield 12.8 mg (71%); ^{1}H NMR (C_6D_6) δ 0.91 (t, 3H, *J* = 7.2 Hz), 1.20-1.48 (m, 14H), 2.01 (quint, 2H, *J* = 7.6 Hz), 2.18 (s, 3H), 3.07 (t, 2H, *J* = 7.6 Hz), 3.42 (s, 3H), 4.13

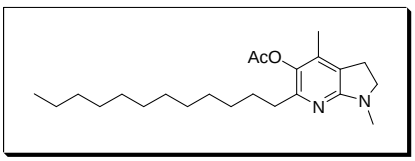
(s, 1H), 6.29 (d, 1H, *J* = 3.2 Hz), and 6.60 (d, 1H, *J* = 3.2 Hz); ^{13}C NMR (C_6D_6) δ 11.5, 14.0, 22.7, 29.0, 29.4, 29.7, 29.7, 29.8, 29.8, 29.9, 30.5, 31.9, 33.2, 96.8, 119.6, 123.4, 142.7, 142.9, and 144.6; mass spectrum (APCI), *m/z* 303.2435 $(M+H)^+$ ($C_{19}H_{31}N_2O$ requires 303.2436).



1,4-Dimethyl-6-dodecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.31). To a solution containing 300 mg (1.85 mmol) of **2.26** in 5 mL of tetrahydrofuran was added 1.75 mL (2.80 mmol) of *n*-butyllithium (1.6 M in hexanes) followed by 498 μL (524 mg, 2.23 mmol) of 1-bromoundecane at –78 °C. The reaction mixture was warmed slowly to room temperature and stirred for 16 h. The reaction mixture was quenched by the addition of 18 mL of sat aq NH_4Cl at 0 °C. The reaction mixture was extracted with three 30-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (28 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.31** as a yellow oil: yield 240 mg (41%); silica gel TLC R_f 0.49 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.20-1.38 (m, 18H), 1.66 (quint, 2H, *J* = 7.6 Hz), 2.08 (s, 3H), 2.56 (t, 2H, *J* = 8.0 Hz), 2.81 (t, 2H, *J* = 8.0 Hz), 2.91 (s, 3H), 3.39 (t, 2H, *J* = 8.4 Hz), and 6.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 18.0, 22.8, 24.5, 29.5, 29.5, 29.7, 29.7, 29.7, 29.8, 29.8, 30.0, 32.0, 33.3, 38.2, 52.5, 112.5, 118.3, 141.2, 159.3, and 163.8; mass spectrum (EI), *m/z* 316.2869 $(M)^+$ ($C_{21}H_{36}N_2$ requires 316.2879).



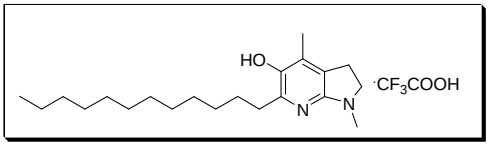
5-Bromo-1,4-dimethyl-6-dodecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.32). To a solution containing 240 mg (0.61 mmol) of **2.31** in 2.5 mL of chloroform was added 85.0 mg (0.31 mmol) of 1,3-dibromo-5,5-dimethylhydantoin in five portions at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched by the addition of 3.2 mL of sat aq $NaHCO_3$. The reaction mixture was extracted with three 15-mL portions of chloroform. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (28 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.32** as a yellow oil: yield 251 mg (84%); silica gel TLC R_f 0.74 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.25-1.40 (m, 18H), 1.67 (quint, 2H, *J* = 7.6 Hz), 2.17 (s, 3H), 2.78 (t, 2H, *J* = 8.0 Hz), 2.82-2.89 (m, 5H), and 3.40 (t, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.2, 19.6, 22.8, 25.3, 28.8, 29.5, 29.5, 29.7, 29.7, 29.8, 29.8, 29.8, 32.1, 33.1, 38.3, 52.5, 111.0, 120.4, 141.2, 156.9, and 161.9; mass spectrum (EI), *m/z* 394.1980 $(M)^+$ ($C_{21}H_{35}BrN_2$ requires 394.1984).



1,4-Dimethyl-6-dodecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.13). To a solution containing 258 mg (0.65 mmol) of **2.32** in 6.5 mL of

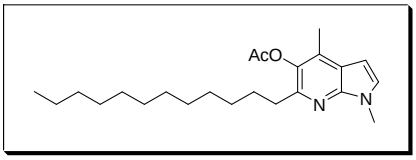
tetrahydrofuran was added 100 μL (75.8 mg, 0.65 mmol) of tetramethylethylenediamine at −78 °C followed by 815 μL (1.31 mmol) of *n*-butyllithium (1.6 M in hexanes). After 30 min, 155 μL (142 mg, 1.37 mmol) of trimethoxyborane was added and the resulting reaction mixture was stirred for another 1 h. To the reaction mixture was added dropwise 290 μL (104 mg, 326 mg total solution, 1.37 mmol) of peracetic acid (32% wt.) and the solution was then warmed to 0 °C. The reaction mixture was diluted with 25 mL of water and extracted with three 35-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The resulting oil was dissolved in 13 mL of dichloromethane at 0 °C, followed by the addition of 520 μL (376 mg, 3.72 mmol) of triethylamine, 6.40 mg (0.05 mmol) of 4-dimethylaminopyridine and 180 μL (193 mg, 1.89 mmol) of acetic anhydride. The reaction mixture was stirred at room temperature for 1 h and quenched by the addition of 12 mL of sat aq NH_4Cl. The solution was extracted with three 40-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.13** as a yellow oil: yield 120 mg (49%); silica gel TLC R_f 0.30 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.2 Hz), 1.25-1.37 (m, 18H), 1.62 (quint, 2H, J = 6.8 Hz), 1.94 (s, 3H), 2.30 (s, 3H), 2.46 (t, 2H, J = 8.0 Hz), 2.83 (t, 2H, J = 8.4 Hz), 2.88 (s, 3H), and 3.43 (t, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 12.9, 14.1, 20.5, 22.7, 24.6, 28.7, 29.3, 29.5, 29.5, 29.6, 29.6, 29.7, 29.7, 31.9, 32.5, 33.3, 52.8, 120.1, 134.7,

136.5, 149.6, 161.1, and 169.9; mass spectrum (EI), *m/z* 374.2939 $(M)^+$ ($C_{23}H_{38}N_2O_2$ requires 374.2933).

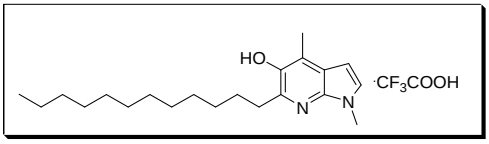


1,4-Dimethyl-6-dodecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.12). To a solution containing 14.2 mg (37.9 μmol) of **2.13** in 1.0 mL of dichloromethane was added 120 μL (120 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.34 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 32 min, and were combined, frozen, and lyophilized to give **2.12** as a light yellow solid: yield 8.4 mg (67%); ^{1}H NMR (CD_3CN) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.21-1.45 (m, 18 H), 1.54 (m, 2H), 2.12 (s,

3H), 2.68 (t, 2H, *J* = 8.8 Hz), 2.99 (t, 2H, *J* = 8.4 Hz), 3.05 (s, 3H), 3.73 (t, 2H, *J* = 8.4 Hz), and 5.05 (br s, 1H); ^{13}C NMR (CD_3CN) δ 13.0, 13.4, 22.3, 24.4, 27.2, 28.6, 29.0, 29.1, 29.3, 29.3, 29.3, 29.4, 29.9, 31.6, 32.4, 53.1, 126.2, 131.9, 140.3, 141.5, and 152.0; mass spectrum (APCI), *m/z* 333.2907 $(M+H)^+$ ($C_{21}H_{37}N_2O$ requires 333.2906).

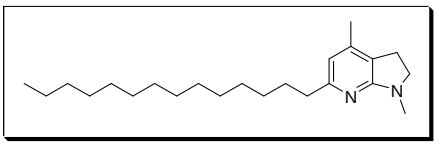


1,4-Dimethyl-6-dodecyl-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.22). The chromatographic procedure described previously to obtain to **2.13** also gave **2.22** as colorless solid: yield 58 mg (25%); silica gel TLC R_f 0.40 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.23-1.40 (m, 18H), 1.73 (m, 2H), 2.32 (s, 3H), 2.37 (s, 3H), 2.72 (t, 2H, *J* = 8.0 Hz), 3.83 (s, 3H), 6.37 (d, 1H, *J* = 3.2 Hz), and 7.07 (d, 1H, *J* = 3.2 Hz). ^{13}C NMR ($CDCl_3$) δ 12.8, 14.1, 14.2, 20.6, 22.7, 28.9, 29.3, 29.5, 29.6, 29.6, 29.7, 31.2, 31.9, 33.3, 60.3, 97.8, 119.4, 128.3, 131.0, 138.7, 144.9, 148.3, and 169.5; mass spectrum (ESI), *m/z* 372.2773 $(M)^+$ ($C_{23}H_{36}N_2O_2$ requires 372.2777).



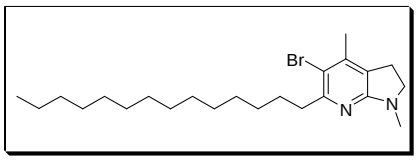
1,4-Dimethyl-6-dodecyl-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.21). To a solution containing 19.0 mg (59.5 μmol) of **2.22** in 0.8 mL of dichloromethane was added 160 μL (160 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction

mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.47 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 32.5 min, and were combined, frozen, and lyophilized to give **2.21** as a colorless solid: yield 9.9 mg (59%); ^{1}H NMR (C_6D_6) δ 0.92 (t, 3H, *J* = 6.8 Hz), 1.28-1.55 (m, 18H), 2.02 (quint, 2H, *J* = 8.0 Hz), 2.17 (s, 3H), 3.06 (t, 2H, *J* = 7.6 Hz), 3.42 (s, 3H), 4.03 (s, 1H), 6.30 (d, 1H, *J* = 3.6 Hz), and 6.60 (d, 1H, *J* = 3.6 Hz); ^{13}C NMR (C_6D_6) δ 11.5, 14.0, 22.7, 29.0, 29.4, 29.5, 29.7, 29.7, 29.7, 29.8, 29.8, 29.8, 30.5, 31.9, 33.2, 96.8, 119.6, 123.3, 142.8, 142.9, and 144.6; mass spectrum (APCI), *m/z* 331.2741 $(M+H)^+$ ($C_{21}H_{35}N_2O$ requires 331.2749).



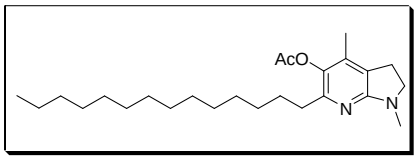
1,4-Dimethyl-6-tetradecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.33). To a solution containing 300 mg (1.85 mmol) of **2.26** in 5 mL of tetrahydrofuran was added 1.75 mL (2.80 mmol) of *n*-butyllithium (1.6 M in hexanes) followed by 498 μL

(524 mg, 2.23 mmol) of 1-bromotridecane at −78 °C. The reaction mixture was warmed slowly to room temperature and stirred for 16 h. The reaction mixture was quenched by the addition of 18 mL of sat aq NH_4Cl at 0 °C. The reaction mixture was extracted with three 30-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (28 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.33** as a yellow oil: yield 240 mg (64%); silica gel TLC R_f 0.53 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.24-1.38 (m, 22H), 1.67 (quint, 2H, J = 7.6 Hz), 2.06 (s, 3H), 2.55 (t, 2H, J = 7.6 Hz), 2.79 (m, 2H), 2.89 (s, 3H), 3.36 (m, 2H), and 6.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 17.9, 22.6, 24.4, 29.4, 29.5, 29.5, 29.6, 29.7, 29.7, 29.7, 29.8, 29.9, 31.9, 33.1, 33.2, 38.1, 52.9, 112.4, 118.1, 140.9, 159.1, and 163.7; mass spectrum (EI), *m/z* 344.3196 $(M)^+$ ($C_{23}H_{40}N_2$ requires 344.3192).



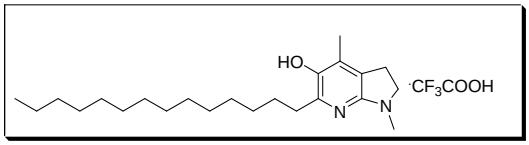
5-Bromo-1,4-dimethyl-6-dodecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridine (2.34). To a solution containing 240 mg (0.61 mmol) of **2.33** in 2.5 mL of chloroform was added 85.0 mg (0.31 mmol) of 1,3-dibromo-5,5-dimethylhydantoin in five portions at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched by the addition of 3.2 mL of sat aq $NaHCO_3$. The reaction mixture was extracted with three 15-mL portions of chloroform. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under

diminished pressure. The residue was purified via flash chromatography on a silica gel column (28 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.34** as a yellow oil: yield 251 mg (66%); silica gel TLC R_f 0.76 (8:1:1 hexanes–ethyl acetate–methanol); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.25-1.46 (m, 22H), 1.67 (quint, 2H, *J* = 8.0 Hz), 2.19 (s, 3H), 2.80 (t, 2H, *J* = 8.4 Hz), 2.84-2.90 (m, 5H), and 3.42 (t, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.3, 19.6, 22.8, 23.1, 23.9, 25.4, 28.8, 29.5, 29.7, 29.8, 29.8, 30.5, 31.4, 32.1, 33.1, 38.3, 38.9, 52.6, 111.1, 120.5, 141.3, 156.9, and 161.9; mass spectrum (EI), *m/z* 422.2307 $(M)^+$ ($C_{23}H_{39}BrN_2$ requires 422.2297).



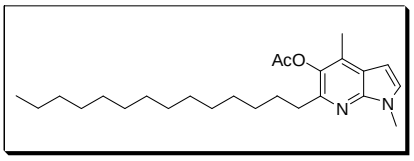
1,4-Dimethyl-6-tetradecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.15). To a solution containing 490 mg (1.16 mmol) of **2.34** in 11.5 mL of tetrahydrofuran was added 175 μL (134 mg, 1.16 mmol) of tetramethylethylenediamine at –78 °C followed by 1.44 mL (2.31 mmol) of *n*-butyllithium (1.6 M in hexanes). After 30 min, 270 μL (252 mg, 2.43 mmol) of trimethoxyborane was added and the resulting mixture was stirred for another 1 h. To the reaction mixture was added dropwise 511 μL (185 mg, 577 mg total solution, and 2.43 mmol) of peracetic acid (32% wt.) and the solution was then warmed to 0 °C. The reaction mixture was diluted with 50 mL of water and extracted with three 70-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The resulting oil was dissolved in 23 mL of dichloromethane at 0 °C, followed by the addition of

920 μL (667 mg, 6.60 mmol) of triethylamine, 11.0 mg (0.09 mmol) of 4-dimethylaminopyridine and 317 μL (343 mg, 3.36 mmol) of acetic anhydride. The reaction mixture was stirred at room temperature for 1 h and quenched by the addition of 25 mL of sat aq NH_4Cl. The solution was extracted with three 80-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified via flash chromatography on a silica gel column (43 x 2.5 cm). Step gradient elution with 1:9 → 1:4 ethyl acetate–hexanes as eluant gave **2.15** as a yellow oil: yield 140 mg (29%); silica gel TLC R_f 0.36 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.20-1.38 (m, 22H), 1.62 (quint, 2H, *J* = 7.6 Hz), 1.94 (s, 3H), 2.29 (s, 3H), 2.46 (t, 2H, *J* = 8.0 Hz), 2.83 (t, 2H, *J* = 8.4 Hz), 2.88 (s, 3H), and 3.42 (t, 2H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.0, 14.3, 20.6, 22.8, 24.8, 28.7, 28.7, 28.8, 28.8, 28.8, 29.5, 29.6, 29.7, 29.8, 29.8, 32.0, 32.6, 33.4, 52.9, 120.2, 134.8, 136.7, 149.8, 161.3, and 170.0; mass spectrum (FAB), *m/z* 403.3326 $(M+H)^+$ ($C_{25}H_{43}N_2O_2$ requires 403.3325).



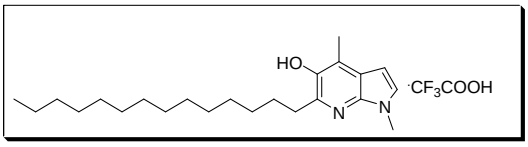
1,4-Dimethyl-6-tetradecyl-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.14). To a solution containing 20.0 mg (49.7 μmol) of **2.15** in 0.8 mL of dichloromethane was added 150 μL (150 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at –78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium

tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.41 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 34 min, and were combined, frozen, and lyophilized to give **2.14** as a light yellow solid: yield 17.7 mg (99%); ^{1}H NMR (CD_3CN) δ 0.92 (t, 3H, *J* = 7.5 Hz), 1.28-1.40 (m, 22H), 1.58 (quint, 2H, *J* = 7.0 Hz), 2.16 (s, 3H), 2.72 (t, 2H, *J* = 8.5 Hz), 3.03 (t, 2H, *J* = 8.5 Hz), 3.09 (s, 3H), 3.77 (t, 2H, *J* = 8.5 Hz), and 4.77 (br s, 1H); ^{13}C NMR (CD_3CN) δ 13.0, 13.4, 22.4, 24.4, 27.3, 28.6, 29.0, 29.1, 29.1, 29.2, 29.3, 29.3, 29.4, 29.4, 29.4, 31.7, 32.4, 53.2, 126.2, 131.9, 140.3, 141.5, and 152.0; mass spectrum (ESI), *m/z* 361.3216 $(M+H)^+$ ($C_{23}H_{41}N_2O$ requires 361.3219).



1,4-Dimethyl-6-tetradecyl-1*H*-pyrrolo[2,3-b]pyridin-5-yl Acetate (2.24). The chromatographic procedure described previously to obtain to **2.15** also gave **2.24** as

colorless solid: yield 80 mg (16%); silica gel TLC R_f 0.49 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.2 Hz), 1.23-1.40 (m, 22H), 1.73 (quint, 2H, J = 7.6 Hz), 2.32 (s, 3H), 2.37 (s, 3H), 2.71 (t, 2H, J = 8.0 Hz), 3.83 (s, 3H), 6.38 (d, 1H, J = 3.6 Hz), and 7.07 (d, 1H, J = 3.6 Hz). ^{13}C NMR ($CDCl_3$) δ 12.8, 14.1, 20.6, 22.7, 28.9, 29.3, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.8, 31.3, 31.9, 33.3, 97.8, 119.4, 128.3, 131.0, 138.7, 144.9, 148.3, and 169.5; mass spectrum (FAB), *m/z* 401.3157 $(M+H)^+$ ($C_{25}H_{41}N_2O_2$ requires 401.3168).



1,4-Dimethyl-6-tetradecyl-1*H*-pyrrolo[2,3-b]pyridin-5-ol and its Trifluoroacetic Acid Salt (2.23). To a solution containing 20.0 mg (49.7 μmol) of **2.24** in 0.8 mL of dichloromethane was added 150 μL (150 μmol) of diisobutylaluminium hydride (1.0 M in toluene) at −78 °C. The reaction mixture was stirred at the same temperature for 1 h before 2 mL of sat aq sodium potassium tartrate was added slowly. The reaction mixture was warmed slowly to room temperature over a period of 30 min. The solution was extracted with three 5-mL portions of ethyl acetate. The combined organic layer was washed with brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give the crude product as a yellow oil: silica gel TLC R_f 0.55 (1:9 methanol–hexanes). The residue was then dissolved in 1 mL of acetonitrile and 1% aq TFA, frozen and lyophilized. The crude product was purified on a Luna C_8 reversed phase semi-preparative (250 x 10 mm) HPLC column using gradients of methanol and 1% aq TFA. Linear gradients were employed using 1:4 methanol–1% aq TFA → 4:1 methanol–1% aq TFA over a period of 20 min, and

then 4:1 methanol–1% aq TFA → methanol over a period of 40 min, at a flow rate of 3.5 mL/min (monitoring at 260 nm). Fractions containing the desired product eluted at 35 min, and were combined, frozen, and lyophilized to give **2.23** as a colorless solid: yield 6.3 mg (35%); ^{1}H NMR (C_6D_6) δ 0.92 (t, 3H, *J* = 7.0 Hz), 1.28-1.54 (m, 22H), 2.02 (quint, 2H, *J* = 7.5 Hz), 2.16 (s, 3H), 3.06 (t, 2H, *J* = 7.0 Hz), 3.42 (s, 3H), 3.87 (br s, 1H), 6.29 (d, 1H, *J* = 3.5 Hz), and 6.60 (d, 1H, *J* = 3.0 Hz); ^{13}C NMR (C_6D_6) δ 11.9, 14.4, 23.2, 29.3, 29.9, 29.9, 30.0, 30.0, 30.2, 30.2, 30.2, 30.3, 30.3, 30.4, 30.9, 32.4, 33.6, 97.2, 120.0, 123.6, 143.2, 143.3, and 144.9; mass spectrum (FAB), *m/z* 359.3073 $(M+H)^+$ ($C_{23}H_{39}N_2O$ requires 359.3062).

CHAPTER 3 - SYNTHESIS OF A NEW SERIES OF β-HYDROXYHISTIDINE ANALOGUES OF BLEOMYCIN

3.1 General introduction

Umezawa and coworkers isolated the bleomycins (BLMs) in 1966 from *Streptomyces verticillus*.[122] These glycopeptides possess anticancer activity and have been used against testicular carcinomas,[123] Hodgkin's lymphoma,[124] and squamous cell carcinomas.[125] The activity of BLM is based on the degradation of DNA, or possibly RNA,[126] caused by a Fe(II)·BLM complex in the presence of O_2.[127] The active complex Fe(II)·BLM·O_2 degrades DNA by the abstraction of the 4′-hydrogen from a deoxyribose sugar of DNA.[128] This hydrogen abstraction triggers a reaction cascade that can lead to either of two different mechanisms (Figure 3.1): strand scission of DNA in presence of oxygen or an alkali-labile lesion via β-elimination.[129] The strand scission of DNA produces either single strand (ss) or double strand (ds) cleavage. Research suggests that the double strand cleavage seems to be responsible for the cytotoxicity of BLM.[130] Additionally, Fe(II)·BLM is reported to cleave transfer RNA (tRNA),[131] tRNA precursors,[132] ribosomal RNA (rRNA),[133] messenger RNA (mRNA),[134] and to inhibit protein synthesis.[135]

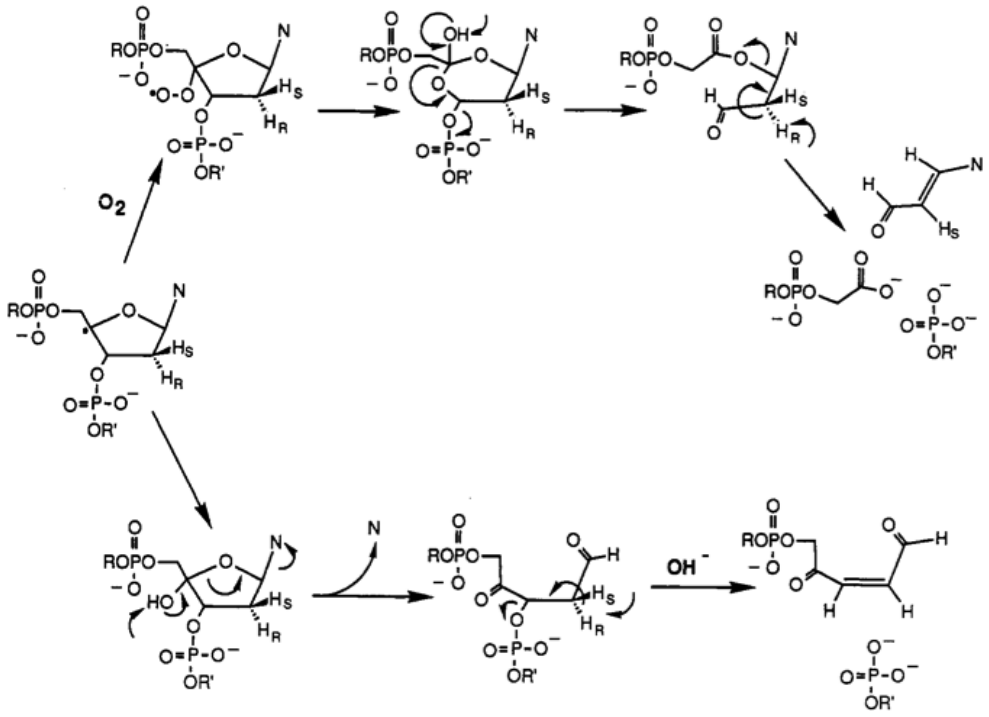


Figure 3.1. Proposed mechanism of DNA degradation by Fe(II)·BLM·O_2. The top pathway leads to strand scission of DNA while the bottom pathway produces the alkali-labile lesion.[136]

BLM is composed of four different functional domains (Figure 3.2). Each domain has a unique contribution to the selectivity and potency of BLM. Among these domains, the metal binding domain is crucial because of its clear role in metal coordination and in causing the oxidative damage to DNA by the abstraction of 4'-hydrogen atoms from DNA.[137] The structure of metallobleomycin was obtained by the analysis of the stable complex Fe(II)·BLM·CO[138] which mimics the structure of the unstable and "active" Fe(II)·BLM·O_2 complex. Subsequent research has been able to obtain a stable complex between Fe^{2+}, deglycoBLM (BLM without its disaccharide moiety), and carbon monoxide.[139] The study of these complexes has provided

information regarding the possible ligands that could coordinate with Fe^{2+} which has allowed different structures of Fe(II)·BLM to be suggested. A proposed structure of Fe(II)·BLM is presented below (Figure 3.3). In addition, complexes with other metals such as cobalt,[140] zinc,[141] and copper,[142] have been reported. The β-hydroxyhistidine moiety has been the least studied group of the metal binding domain. Its complete function and structural requirements for metal coordination are not yet fully understood (Figure 3.4).

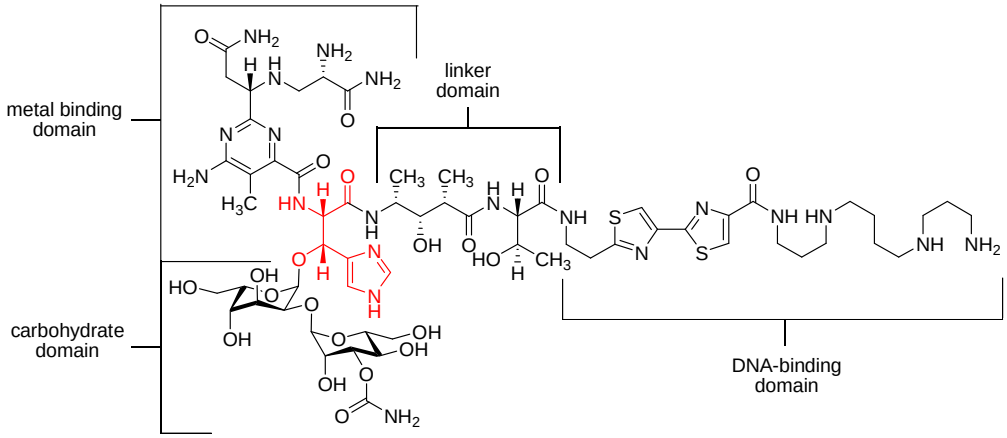


Figure 3.2. The different domains of BLM A_6. The β-hydroxyhistidine moiety is highlighted in red.

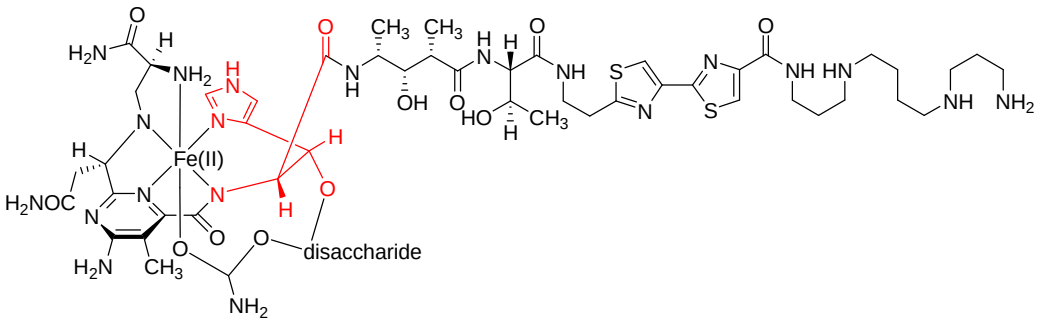


Figure 3.3. Proposed coordination between BLM and Fe(II). The β-hydroxyhistidine moiety is highlighted in red. Adapted from reference.[143]

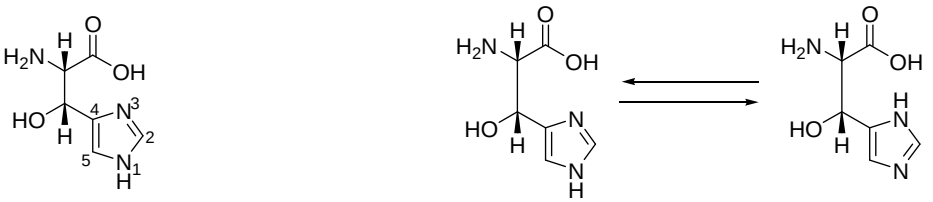


Figure 3.4. Nomenclature and tautomerization of the imidazole moiety in β-hydroxyhistidine.

The β-hydroxyhistidine moiety seems to contribute to metal coordination with two ligands: the N-3 nitrogen atom of imidazole and the nitrogen atom of the amide.[144] The contribution of the nitrogen atom of the amide is not universally accepted. It is possible that the protonation of the amide impacts the ability of this nitrogen to act as a ligand. At pH 6.4, Oppenheimer and coworkers demonstrated protonation of the amide showing that it does not interact with Fe(II).[138] Conversely, Bermel and coworkers showed that at pH 7.0 the deprotonated amide acted as an additional ligand for Fe(II).[145] To study the manner in which the amide nitrogen of β-

hydroxyhistidine coordinates with Fe(II), Boger and coworkers synthesized deshydroxy deglycoBLM A_2 (**3.1**), its N-methyl amide analogue (**3.2**), and its ester analogue (**3.3**) (Figure 3.5). Of these three analogues, **3.1** possessed a normal amide bond similar to the parent molecule, **3.2** was methylated and only coordinated with iron using π shell electrons, and **3.3** was unable to coordinate with Fe(II). Cleavage assays of supercoiled DNA showed that only **3.1** had activity comparable to deglycoBLM A_2, while **3.2** and **3.3** had no activity. These findings suggest that the amide nitrogen of β-hydroxyhistidine coordinates with Fe(II) using electrons from its σ shell. This nitrogen is only able to use its σ shell electrons for coordination in its deprotonated form.[146]

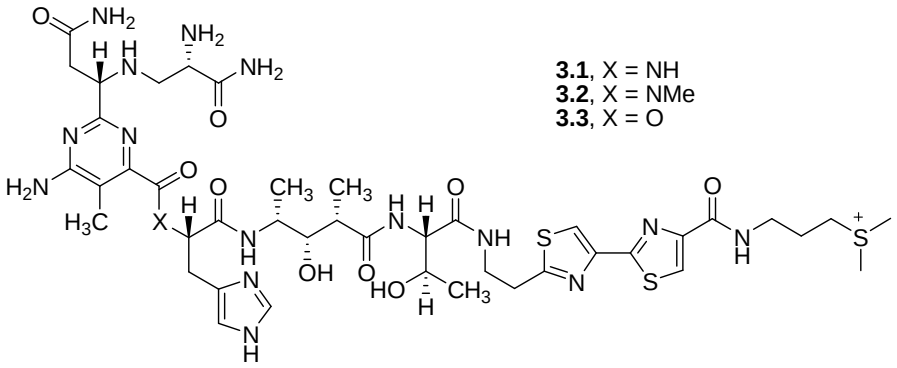


Figure 3.5 Structures of analogues synthesized by Boger and coworkers which were used to study the functionality of the nitrogen atom of the amide of β-hydroxyhistidine.[146]

In order to determine which tautomer (Figure 3.4) is crucial for complexation, Boger and coworkers studied the coordination of the N-3 nitrogen atom of imidazole with Fe(II). Three analogues were synthesized for this study: an analogue in which the

imidazole moiety was absent (**3.4**), an analogue in which oxazole was used instead of imidazole (**3.5**), and an analogue in which imidazole was replaced by pyrrole (**3.6**) (Figure 3.6). The N-3 nitrogen of oxazole **3.4** can only use its π shell electrons which suggests that it has the ability to mimic the N^1-H tautomer (Figure 3.4). Conversely, the N-3 nitrogen of pyrrole **3.5** coordinates mainly using σ electrons, similar to the N^3-H tautomer (Figure 3.4). Cleavage assays of supercoiled DNA showed that oxazole **3.5** had comparable activity with deglycoBLM A_2, while **3.4** and **3.6** had no activity. These findings indicate that the N-3 nitrogen of the N^1-H tautomer (Figure 3.4) of imidazole coordinates with Fe(II). This suggests that this nitrogen mainly uses electrons from its π shell for coordination.[147]

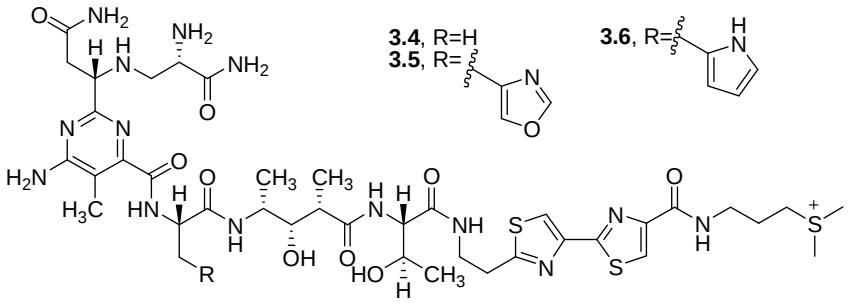


Figure 3.6. Structures of analogues synthesized by Boger and coworkers which were used to study the functionality of the nitrogen atom at position 3 of the imidazole of β-hydroxyhistidine.[147]

In the Hecht laboratory, much progress has been made in elucidating the functionality of β-hydroxyhistidine. In a study involving the synthesis of 108 different analogues of deglycoBLM by solid-phase synthesis, four different building blocks

utilizing the histidine moiety were addressed. All of the chosen compounds were commercially available. Thienylalanine (**3.7**), tryptophan (**3.8**), methionine (**3.9**), and histidine (Figure 3.7) were selected as β-hydroxyhistidine analogues.[148] The analogues **3.7**, **3.8**, and **3.9** showed a significant decrease in overall activity and much lower cleavage selectivity than those containing histidine.[149]

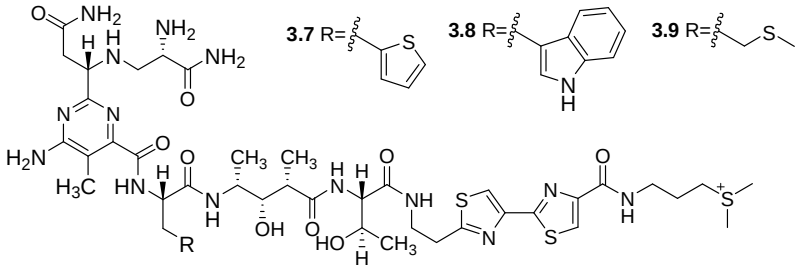


Figure 3.7. BLM analogues synthesized by Hecht and coworkers for the BLM library study.[148]

In order to complement these findings, more extensive research has been done in the Hecht laboratory to study which moieties would not only be able to mimic histidine but would also be able to show higher potency and selectivity. Hecht and coworkers synthesized a new series of analogues **3.11**-**3.18** (Figure 3.8) including the natural β-hydroxyhistidine moiety (**3.10**) for comparison.[150] The synthesized imidazole derived analogues were the 2-methyl (**3.11**), the 2-isobutyl (**3.12**), and the 5-methyl (**3.13**) imidazoles. These analogues have alkyl groups in positions 2 and 5 of the imidazole ring in order to increase their electron donating ability. It was expected that a greater electron donating character of the ring would increase the activity of the Fe(II)·BLM complex. However, this addition could also cause steric hindrance.

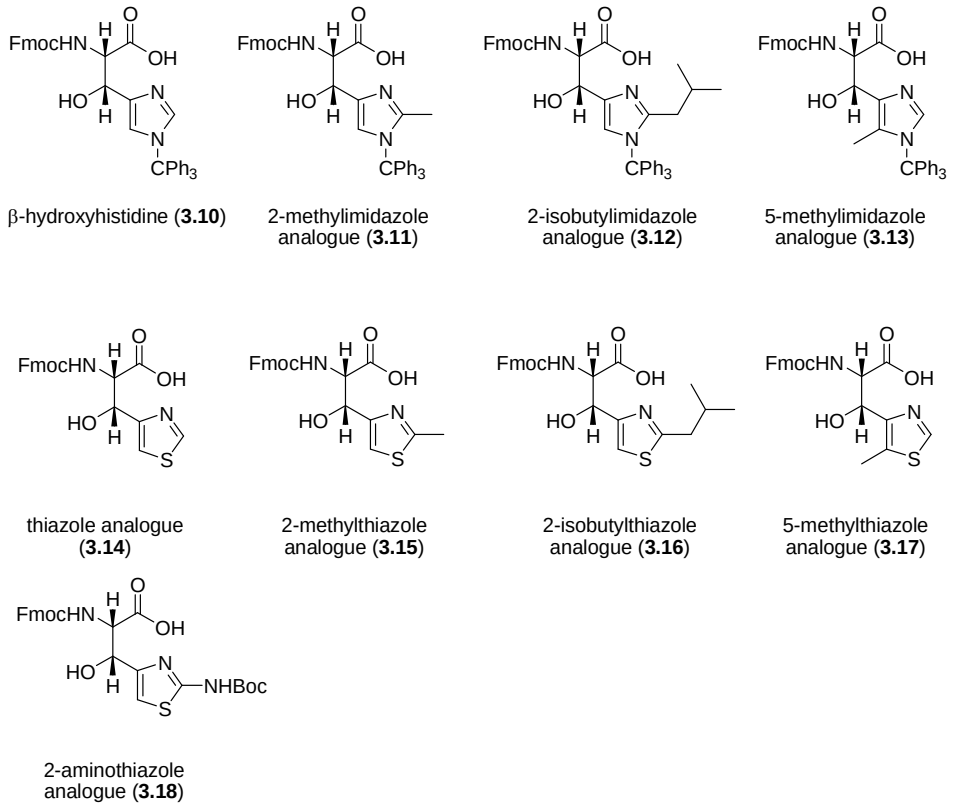
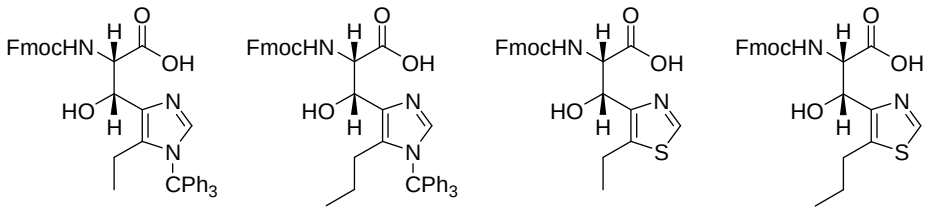


Figure 3.8. β-Hydroxyhistidine analogues synthesized by Hecht and coworkers.[150]

A series of thiazole analogues was synthesized in order to study the high electron density of the system compared with the imidazole ring. The thiazole analogues obtained had similar features when compared to the imidazole analogues: unmodified (**3.14**), 2-methyl (**3.15**), 2-isobutyl (**3.16**), 5-methyl (**3.17**), and 2-amino (**3.18**) thiazole analogues. Compared to imidazole, thiazole possesses a higher electron density, therefore, it may be able to better stabilize the metal complex. From these analogues, only **3.10**, **3.12**, **3.14**, **3.16** have been incorporated into deglycoBLM A_6 and tested in DNA cleavage assays. All the deglycoBLMs showed similar single

strand cleavage efficiency as deglycoBLM A_6, making them promising candidates for further characterization assays.[150]

The goal of this project was to prepare at least ten analogues of β-hydroxyhistidine that could be employed in creating a massive combinatorial BLM library in which 10^5 different BLMs can be obtained. This extensive library will hopefully provide interesting BLMs in terms of efficiency and potency. To accomplish this, four additional analogues (**3.19**-**3.22**) were synthesized (Figure 3.7). New schemes were developed utilizing intermediates analogous to those described in Elban's procedure.[150] These analogues were proposed in order to study the impact of increased steric hindrance in the position 5 of imidazole and thiazole. Previously, only methyl was used in this position. These larger alkyl groups will be used to determine their effect on DNA cleavage. Additionally, analogues **3.11**, **3.13**-**3.18** were synthesized for incorporation in BLMs.

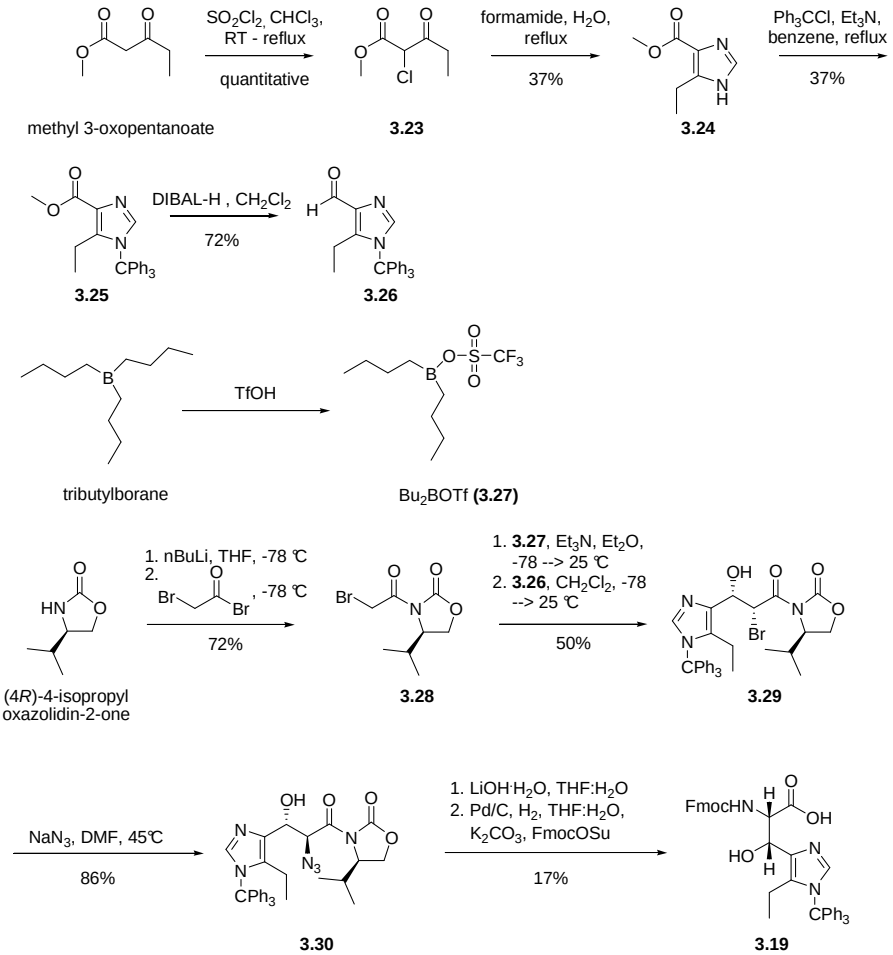


5-ethylimidazole analogue (**3.19**) | 5-propylimidazole analogue (**3.20**) | 5-ethylthiazole analogue (**3.21**) | 5-propylthiazole analogue (**3.22**)

Figure 3.9. β-Hydroxyhistidine analogues synthesized in this thesis.

3.2 Results and discussion

3.2.1 Synthesis of β-hydroxyhistidine analogues of bleomycin



Scheme 3.1. Synthesis of 5-ethylimidazole analogue (**3.19**).

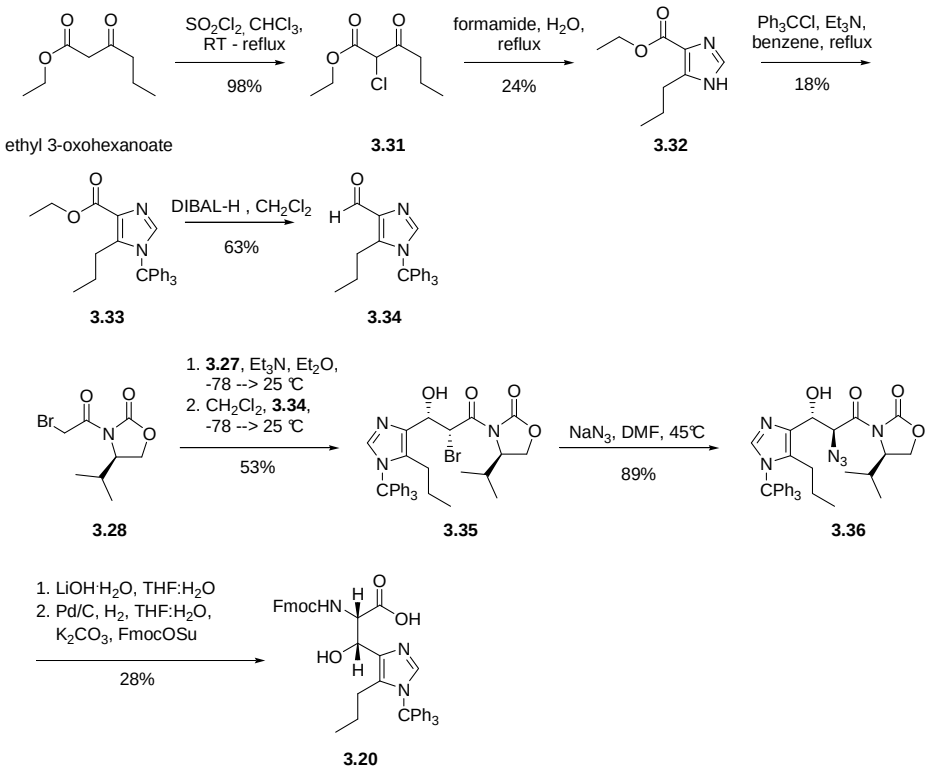
The aldehyde intermediate of the 5-ethylimidazole analogue (**3.19**) was synthesized using methyl 3-oxopentanoate as starting material (Scheme 3.1). Methyl 3-oxopentanoate was chlorinated using sulfuryl chloride in chloroform at reflux to give chlorinated intermediate **3.23** in quantitative yield.[151] Compound **3.23** was

condensed with an excess of formamide in the presence of two equivalents of water to obtain imidazole ester **3.24** in 37% yield. From this point, Elban's procedure was followed. Imidazole ester **3.24** was protected with trityl chloride using triethylamine in benzene at reflux to give protected imidazole **3.25** in 37% yield. Selective reduction with diisobutylaluminium hydride at −78 °C gave aldehyde intermediate **3.26** in 72% yield.

Dibutylboron triflate (**3.27**) was prepared by the reaction between tributylborane and trifluoromethanesulfonic acid at room temperature. After distilling the reaction mixture under diminished pressure, **3.27** was obtained as a light brown transparent liquid. (*4R*)-4-Isopropyloxazolidin-2-one was functionalized by deprotonation with *n*-butyllithium and coupling with bromoacetyl bromide in tetrahydrofuran at −78 °C to give functionalized chiral auxiliary **3.28** in 72% yield. A selective aldol condensation between **3.28** and **3.26** in the presence of dibutylboron triflate (**3.27**) at −78 °C gave bromide **3.29** in 50% yield after oxidative workup. Bromide **3.29** was converted to azide **3.30** in 86% yield by a S_N2 reaction with sodium azide in *N*,*N*-dimethylformamide at 45 °C. Finally, the chiral auxiliary of azide **3.30** was cleaved with lithium hydroxide in tetrahydrofuran. In addition to this, the azide was reduced with palladium on carbon, giving a free amine which was protected in-situ with *N*-(9-fluorenylmethoxy-carbonyloxy)succinate to give 5-ethylimidazole analogue **3.19** as colorless solid in 17% yield.

The aldehyde intermediate of the 5-propylimidazole analogue (**3.20**) (Scheme 3.2) was synthesized using ethyl 3-oxohexanoate as starting material. Ethyl 3-oxohexanoate was chlorinated using sulfuryl chloride in chloroform at reflux to give chlorinated intermediate **3.31** in 98% yield.[151] Compound **3.31** was condensed with an

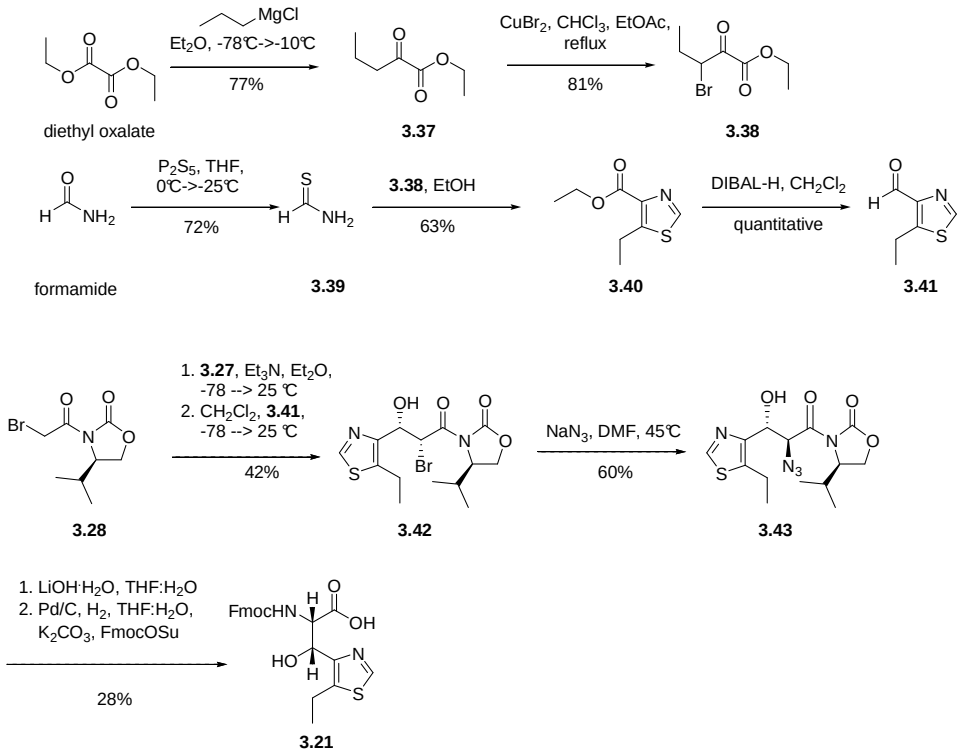
excess of formamide in the presence of two equivalents of water to obtain imidazole ester **3.32** in 24% yield. Imidazole ester **3.32** was protected with trityl chloride using triethylamine in benzene at reflux to give protected imidazole **3.33** in 18% yield. Selective reduction with diisobutylaluminium hydride at −78 °C gave aldehyde intermediate **3.34** in 63% yield.



Scheme 3.2. Synthesis of 5-propylimidazole analogue (**3.20**).

A selective aldol condensation between **3.28** and **3.34** in the presence of dibutylboron triflate (**3.27**) at −78 °C gave bromide **3.35** in 53% yield after oxidative workup. Bromide **3.35** was converted to azide **3.36** in 89% yield by a S_N2 reaction with sodium azide in *N*,*N*-dimethylformamide at 45 °C. Finally, the chiral auxiliary of

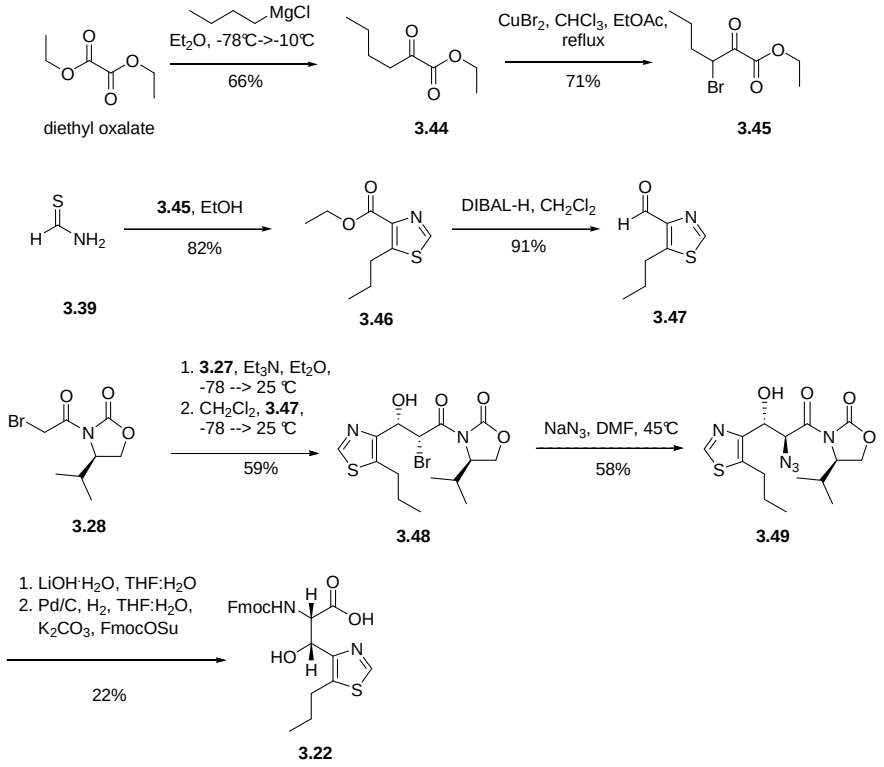
azide **3.36** was cleaved with lithium hydroxide in tetrahydrofuran. In addition to this, the azide was reduced with palladium on carbon, giving a free amine which was protected in-situ with *N*-(9-fluorenylmethoxy-carbonyloxy)succinate to give 5-propylimidazole analogue **3.20** as colorless solid in 28% yield.



Scheme 3.3. Synthesis of 5-ethylthiazole analogue (**3.21**).

The procedure followed for the synthesis of the 5-ethylthiazole analogue (**3.21**) was the same as that used by Elban, but using a different Grignard reagent in the first reaction. Diethyl oxalate was treated with *n*-propylmagnesium chloride in diethyl ether at −78 °C to give α-keto ester **3.37** in 77% yield. Compound **3.37** was brominated with $CuBr_2$ in a chloroform and ethyl acetate mixture at reflux to give β-

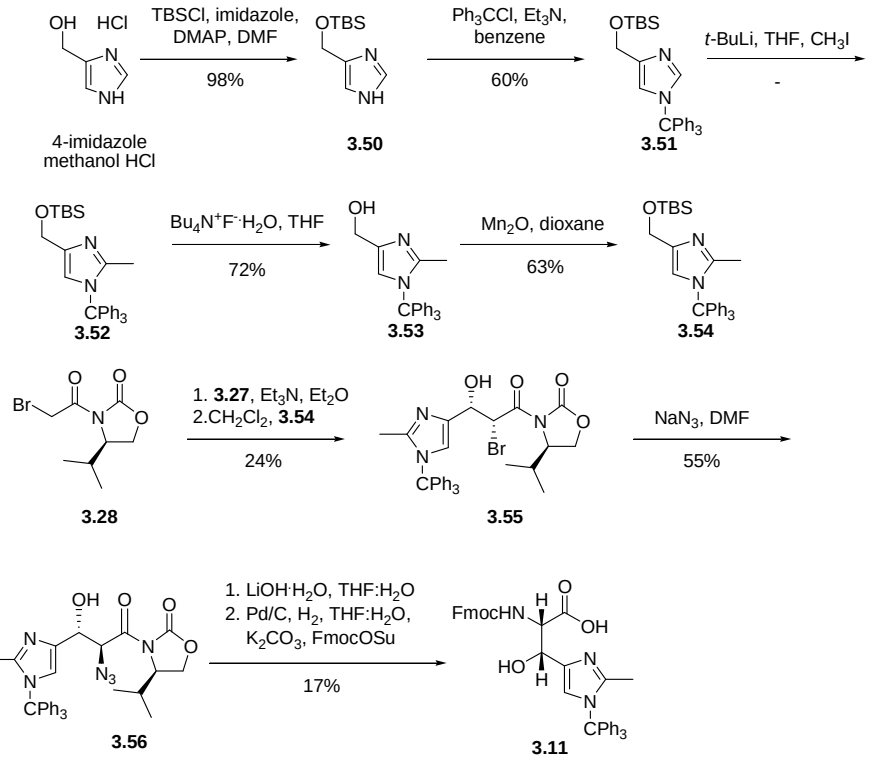
bromo ester **3.38** in 81% yield. Thioformamide **3.39** was obtained by the thionization of formamide with phosphorus pentasulfide in tetrahydrofuran and was used without further purification. β-Bromo ester **3.38** was condensed with crude thioformamide **3.39** in dry ethanol to give imidazole ester **3.40** in 63% yield. Selective reduction of ester **3.40** with diisobutylaluminium hydride in dichloromethane at −78 °C gave imidazole carboxaldehyde **3.41** in quantitative yield.



Scheme 3.4. Synthesis of 5-propylthiazole analogue (**3.22**).

A selective aldol condensation between **3.28** and **3.41** in the presence of dibutylboron triflate (**3.27**) at −78 °C gave bromide **3.42** in 42% yield after oxidative workup. Bromide **3.42** was converted to azide **3.43** in 60% yield by a S_N2 reaction

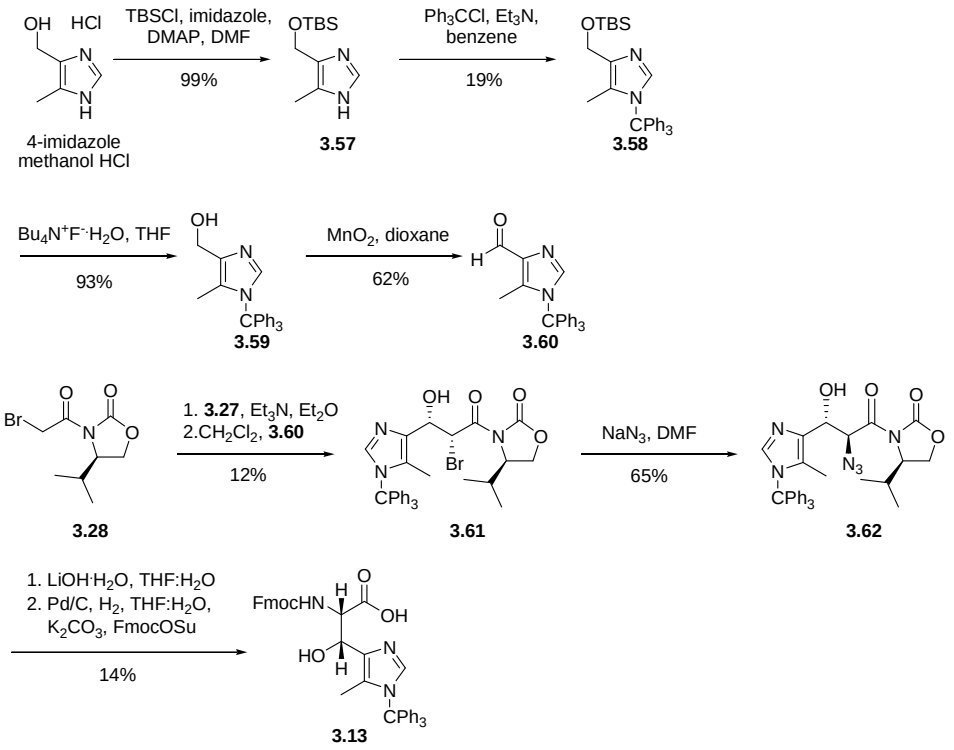
with sodium azide in *N*,*N*-dimethylformamide at 45 °C. Finally, the chiral auxiliary of azide **3.43** was cleaved with lithium hydroxide in tetrahydrofuran. In addition to this, the azide was reduced with palladium on carbon, giving a free amine which was protected in-situ with *N*-(9-fluorenylmethoxy-carbonyloxy)succinate to give 5-ethylthiazole analogue **3.21** as colorless solid in 28% yield.



Scheme 3.5. Synthesis of 2-methylimidazole analogue (**3.11**).

The synthesis of the 5-ethylthiazole analogue (**3.22**) utilized the same reactions as the previous analogue excluding the reagent used in the first reaction. Diethyl oxalate was treated with *n*-butylmagnesium chloride in diethyl ether at −78 °C to give α-keto ester **3.44** in 66% yield. Compound **3.44** was brominated with $CuBr_2$ in

a chloroform and ethyl acetate mixture at reflux to give β-bromo ester **3.45** in 71% yield. β-Bromo ester **3.45** was condensed with crude thioformamide **3.39** in dry ethanol to give imidazole ester **3.46** in 82% yield. Selective reduction of ester **3.46** with diisobutylaluminium hydride in dichloromethane at −78 °C gave imidazole carboxaldehyde **3.47** in 91% yield.

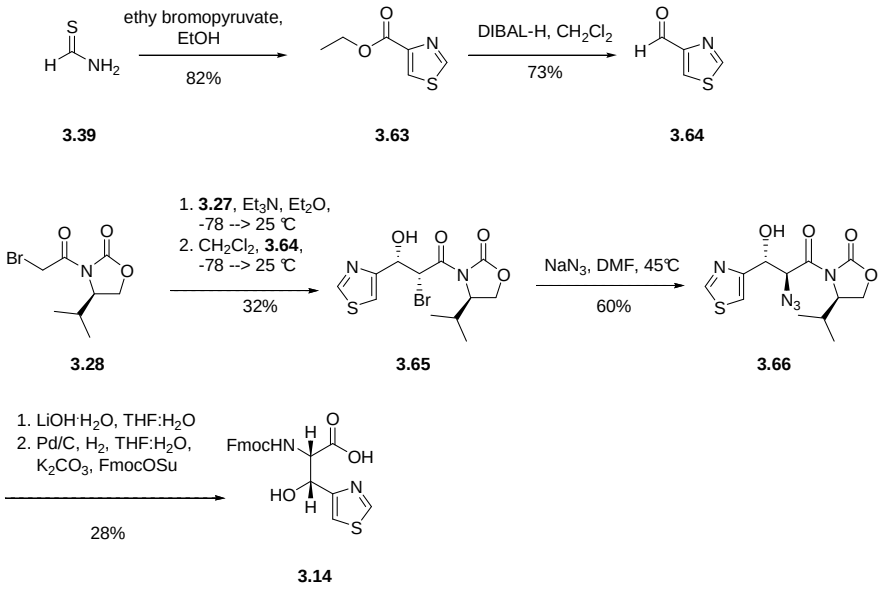


Scheme 3.6. Synthesis of 5-methylimidazole analogue (**3.13**).

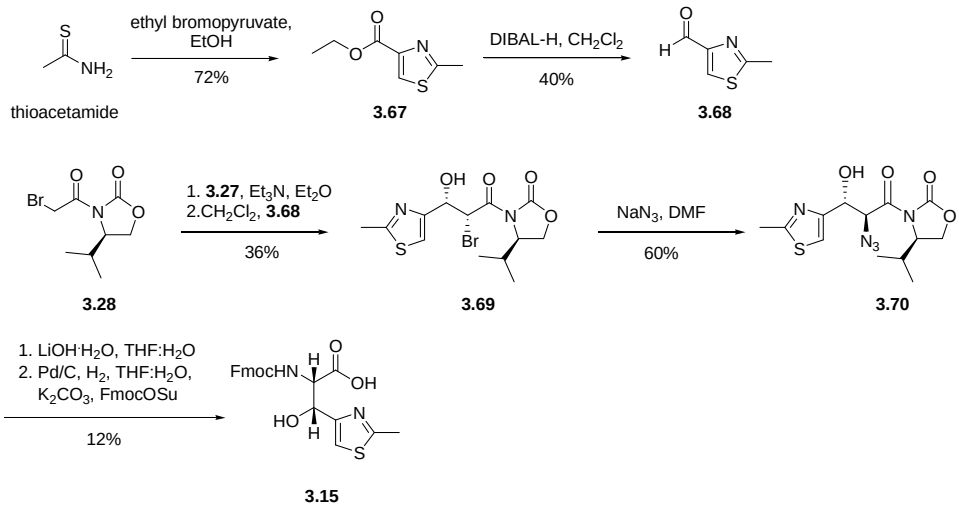
A selective aldol condensation between **3.28** and **3.47** in the presence of dibutylboron triflate (**3.27**) at −78 °C gave bromide **3.48** in 59% yield after oxidative workup. Bromide **3.48** was converted to azide **3.49** in 58% yield by a S_N2 reaction with sodium azide in *N*,*N*-dimethylformamide at 45 °C. Finally, the chiral auxiliary of

azide **3.49** was cleaved with lithium hydroxide in tetrahydrofuran. In addition to this, the azide was reduced with palladium on carbon, giving a free amine which was protected in-situ with *N*-(9-fluorenylmethoxy-carbonyloxy)succinate to give 5-propylthiazole analogue **3.22** as colorless solid in 22% yield.

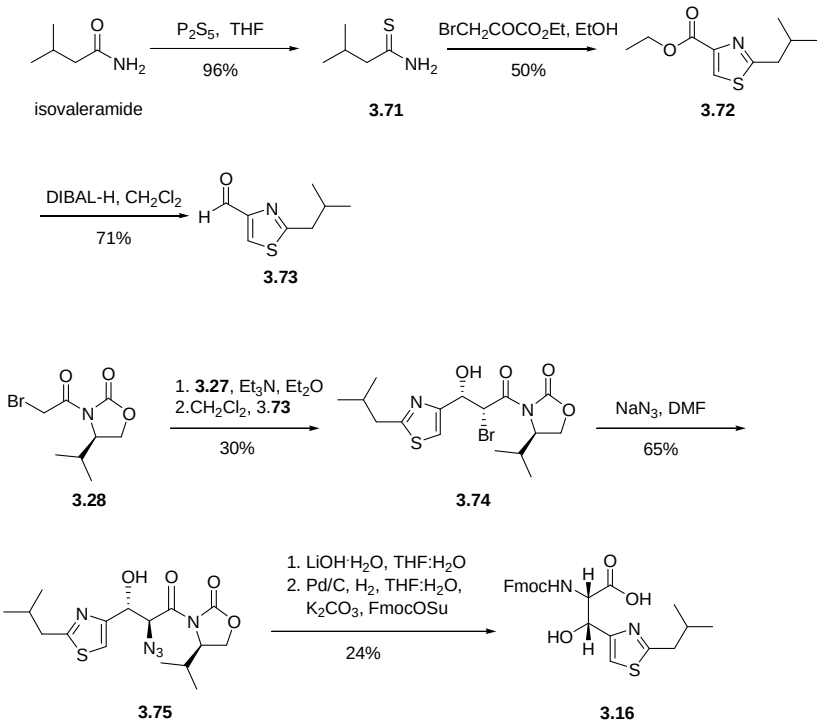
Additionally, analogues **3.11**, **3.13**-**3.18** were synthesized to be incorporated in bleomycins (Schemes 3.5-3.11).



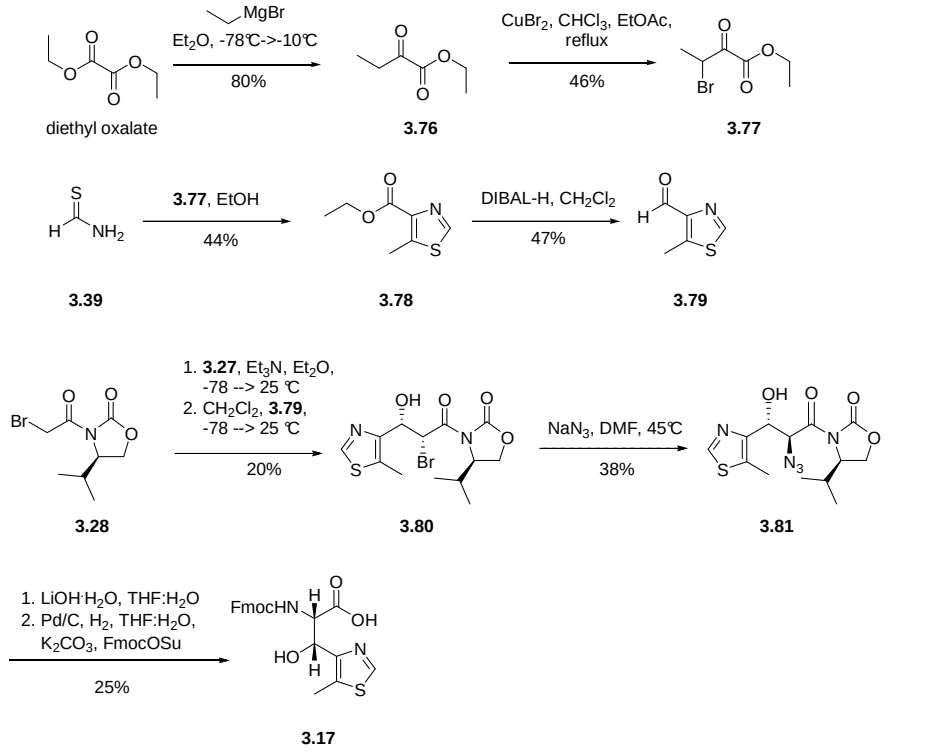
Scheme 3.7. Synthesis of thiazole analogue (**3.14**).



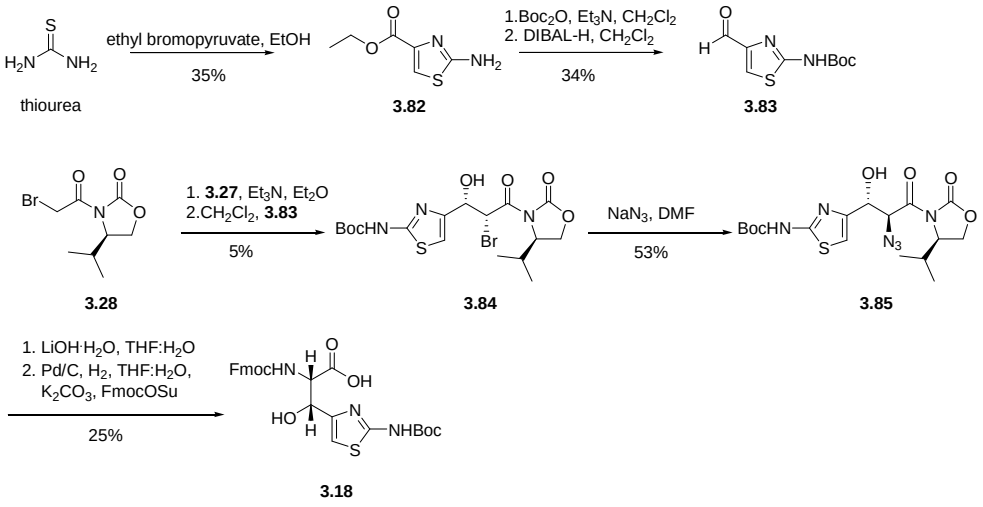
Scheme 3.8. Synthesis of 2-methylthiazole analogue (**3.15**).



Scheme 3.9. Synthesis of 2-isobutylthiazole analogue (**3.16**).



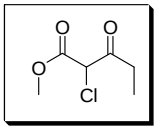
Scheme 3.10. Synthesis of 5-methylthiazole analogue (**3.17**).



Scheme 3.11. Synthesis of 5-aminothiazole analogue (**3.18**).

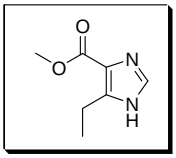
3.3 Experimental Section

General methods: Chemicals and solvents were of reagent grade and were used without further purification. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone under argon. Anhydrous diethyl ether (Et_2O) was distilled from sodium/benzophenone under argon. Anhydrous dichloromethane (CH_2Cl_2) was distilled from calcium hydride under argon. All reactions involving air or moisture sensitive reagents or intermediates were performed under an argon atmosphere. Flash chromatography was carried out using Silicycle 200-400 mesh silica gel. Analytical TLC was carried out using 0.25 mm EM silica gel 60 F250 plates that were visualized by UV irradiation (254 nm) or by staining with ceric ammonium molybdate stain. ^1H NMR and ^{13}C NMR spectra were obtained using 400 or 500 MHz Varian NMR spectrometers. Chemicals shifts were reported in parts per million (ppm, δ) referenced to the residual ^1H resonance of the solvent ($CDCl_3$, 7.26 ppm). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, 77.0 ppm). Splitting patterns were designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. High resolution mass spectra were obtained at the Michigan State University Mass Spectrometry Facility or at the Arizona State University CLAS High Resolution Mass Spectrometry Laboratory.



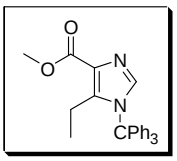
Methyl 2-chloro-3-oxo-pentanoate (3.23). To a solution containing 10.4 g (79.7 mmol) of 3-oxopentanoate in 50 mL of chloroform at 0 °C was added 6.80 mL (11.3 g, 83.9 mmol) of sulfuryl chloride. The reaction mixture was stirred for 30 min at

room temperature, and then at reflux for 2 h. The cooled reaction mixture was diluted with 150 mL of ethyl acetate, and then washed successively with 50 mL of water, 50 mL of sat aq $NaHCO_3$, 50 mL of water and 50 mL of brine. The solution was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to yield a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:6 ethyl acetate–hexanes as eluant gave **3.23** as a yellow oil: yield 13.0 g (quantitative); silica gel TLC R_f 0.33 (1:4 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7.2 Hz), 2.62 (q, 2H, J = 7.6 Hz), 3.71 (s, 3H), and 4.77 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 7.4, 32.4, 53.5, 60.4, 165.5, and 199.4; mass spectrum (EI), *m/z* 164.0235 $(M)^+$ ($C_6H_9O_3Cl$ requires 164.0240).

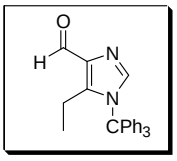


5-Ethyl-1*H*-imidazole-4-carboxylic Acid Methyl Ester (3.24). To a solution containing 13.0 g (79.0 mmol) of **3.23** in 32 mL (36.3 g, 806 mmol) of formamide was added 2.85 mL (2.85 g, 158 mmol) of distilled water. The reaction mixture was heated to 145 °C and stirred for 4 h. The cooled reaction mixture was diluted with 200 mL of chloroform, and then washed successively with 40 mL of water, 40 mL of sat aq $NaHCO_3$, 40 mL of water and 40 mL of brine. The solution was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to yield a colorless solid. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:9 methanol–chloroform as eluant gave **3.24** as a colorless solid: yield 4.50 g (37%); silica gel TLC R_f 0.37 (1:9 methanol–chloroform); ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.5 Hz), 3.03 (q, 2H, J = 7.5 Hz), 3.85 (s, 3H), 7.67 (s, 1H),

and 12.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 19.5, 51.3, 124.3, 135.3, 142.7, and 163.6; mass spectrum (APCI), *m/z* 155.0824 $(M+H)^+$ ($C_7H_{11}N_2O_2$ requires 155.0821).

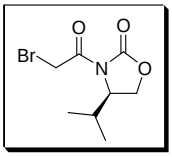


5-Ethyl-1-trityl-1*H*-imidazole-4-carboxylic Acid Methyl Ester (3.25). To a solution containing 4.57 g (29.6 mmol) of **3.24** in 220 mL of benzene was added 3.30 mL (4.54 g, 32.6 mmol) of triethylamine and 9.09 g (32.6 mmol) of trityl chloride. The reaction mixture was stirred at reflux for 4 h at which time the reaction mixture was washed with three 50-mL portions of water and dried over anh $MgSO_4$. Excess solvent was removed under diminished pressure to give a crude colorless solid. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.25** as a yellow solid: yield 4.39 g (37%); silica gel TLC R_f 0.43 (1:3 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.06 (t, 3H, *J* = 6.8 Hz), 2.48 (q, 2H, *J* = 7.2 Hz), 3.84 (s, 3H), 7.10 (m, 6H), 7.27 (m, 9H), and 7.32 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.1, 21.6, 51.4, 75.5, 128.0, 128.0, 128.0 128.2, 128.2, 128.2, 128.2, 128.2, 128.2, 129.9, 129.9, 129.9, 129.9, 129.9, 129.9, 130.7, 137.3, 141.3, 141.3, 141.3, 144.2, and 163.8; mass spectrum (APCI), *m/z* 397.1907 $(M+H)^+$ ($C_{26}H_{25}N_2O_2$ requires 397.1916).



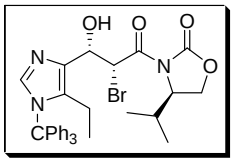
5-Ethyl-1-trityl-1*H*-imidazole-4-carbaldehyde (3.26). To a solution containing 4.39 g (11.7 mmol) of **3.25** in 85 mL of dichloromethane at −78 °C was added 23.6 mL

(5.03 g, 35.4 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 75 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.26** as a colorless solid: yield 2.91 g (72%); silica gel TLC R_f 0.54 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.13 (t, 3H, J = 7.6 Hz), 2.44 (q, 2H, J = 7.2 Hz), 7.10 (m, 6H), 7.28 (m, 9H), 7.36 (s, 1H), and 9.94 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.1, 21.4, 75.5, 128.2, 128.2, 128.2, 128.3, 128.3, 128.3, 128.3, 128.3, 128.3, 129.8, 129.8, 129.8, 129.8, 129.8, 129.8, 138.3, 139.2, 141.2, 141.2, 141.2, 143.9, and 187.5; mass spectrum (APCI), *m/z* 367.1800 $(M+H)^+$ ($C_{25}H_{23}N_2O$ requires 367.1810).



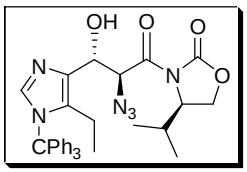
3-(2-Bromoacetyl)-(4*R*)-4-isopropyloxazolidin-2-one (3.28).[150] To a solution containing 2.50 g (19.4 mmol) of (4*R*)-4-isopropyloxazolidin-2-one in 125 mL of tetrahydrofuran at −78 °C was added 12.1 mL (19.4 mmol) of *n*-butyllithium (1.6 M in hexanes) over a period of 30 min. This solution was treated with 1.70 mL (3.94 g, 19.5 mmol) of bromoacetyl bromide. The cloudy solution was stirred for an additional 1.5 h. The reaction was quenched with 25 mL of sat aq NH_4Cl and 25 mL of sat aq

$NaHCO_3$. The solution was permitted to equilibrate at room temperature for 1 h. The organic and aqueous phases were separated, and the aqueous phase was extracted with three 50-mL portions of ethyl ether. The combined organic phase was washed with three 25-mL portions of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to yield a brown solid. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.28** as a yellow oil: yield 3.49 g (72%); silica gel TLC R_f 0.85 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (dd, 6H, *J* = 9.5 and 6.5 Hz), 2.40 (m, 1H), 4.24 (dd, 1H, *J* = 9.5 and 3.0 Hz), 4.33 (t, 1H, *J* = 8.5 Hz), 4.39 (dd, 1H, *J* = 12.5 and 1.0 Hz), 4.44 (m, 1H), and 4.56 (dd, 1H, *J* = 12.5 and 1.0 Hz).

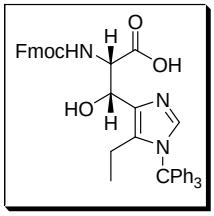


3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-ethyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.29). To a solution containing 1.08 g (4.32 mmol) of **3.28** in 20 mL of diethyl ether at −78 °C was added 1.15 mL (1.30 g, 4.75 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.90 mL (0.66 mg, 6.48 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.44 g (3.93 mmol) of **3.26** in 8 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The

reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.29** as colorless foam: yield 1.23 g (50%); silica gel TLC R_f 0.28 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.22 (t, 3H, J = 7.6 Hz), 0.89 (d, 6H, J = 6.8 Hz), 2.28 (m, 1H), 2.38 (m, 2H), 3.59 (br s, 1H), 4.18 (m, 2H), 4.30 (m, 1H), 5.10 (d, 1H, J = 8.4 Hz), 6.30 (d, 1H, J = 8.4 Hz), 7.06 (m, 6H), 7.26 (m, 9H), and 7.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.5, 14.8, 17.9, 28.0, 49.7, 58.3, 63.2, 68.3, 74.8, 127.8, 127.8, 127.8, 128.0, 128.0, 128.0, 128.0, 128.0, 128.0, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 133.7, 136.8, 137.8, 141.9, 141.9, 141.9, 152.6, 168.3, and 187.8. *Note: material decomposes rapidly, must be used immediately.*

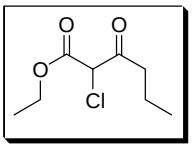


3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-ethyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.30). To a solution containing 1.23 g (2.20 mmol) of **3.29** in 40 mL of *N*,*N*-dimethylformamide was added 0.65 g (10.0 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.30** as a colorless foam: yield 1.00 g (86%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.24 (t, 3H, *J* = 7.2 Hz), 0.89 (t, 6H, *J* = 6.8 Hz), 2.28 (m, 2H), 2.50 (m, 1H), 3.84 (d, 1H, *J* = 9.2 Hz), 4.32 (m, 2H), 4.54 (m, 1H), 4.94 (d, 1H, *J* = 8.8 Hz), 5.86 (d, 1H, *J* = 8.4 Hz), 7.18 (m, 6H), 7.32 (m, 9H), and 7.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.9, 14.7, 17.9, 28.2, 58.9, 62.3, 63.6, 68.4, 74.9, 127.8, 127.8, 127.8, 128.1, 128.1, 128.1, 128.1, 128.1, 128.1, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 134.0, 137.6, 138.0, 141.8, 141.8, 141.8, 153.9, 169.8, and 187.8; mass spectrum (APCI), *m/z* 597.2712 $(M+H)^+$ ($C_{33}H_{35}N_6O_4$ requires 597.2720).

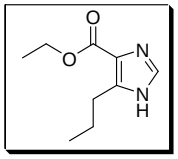


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-ethyl-1-trityl-1*H*-imidazol-4-yl)propionic Acid (3.19). To a solution containing 1.00 g (1.73 mmol) of **3.30** in 90 mL of 4:1 tetrahydrofuran–water was added 0.36 g (8.64 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 90 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.47 g (3.46 mmol) of K_2CO_3 and 0.87 g (2.59 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.19** as a yellow solid: yield 156 mg (17%); silica gel TLC R_f 0.22 (90:8:2 chloroform–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 0.24 (t, 3H, J = 6.8 Hz), 2.29

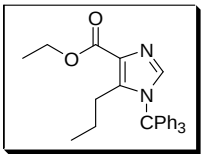
(m, 2H), 4.21 (m, 2H), 4.33 (m, 1H), 4.50 (m, 1H), 5.14 (m, 1H), 6.81 (br s, 1H), 7.18 (m, 7H), 7.28 (m, 14H), 7.63 (m, 2H), 7.78 (m, 2H), and 12.20 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.5, 19.9, 21.6, 47.2, 60.1, 67.1, 119.9, 125.4, 125.5, 125.6, 125.6, 127.1, 127.1, 127.2, 127.2, 127.7, 127.7, 127.7, 128.3, 128.3, 128.3, 128.3, 128.3, 128.3, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 134.6, 138.0, 138.0, 138.0, 140.4, 140.4, 141.2, 141.3, 143.9, 144.2, 156.1, and 172.6; mass spectrum (APCI), *m/z* 664.2822 $(M+H)^+$ ($C_{42}H_{38}N_3O_5$ requires 664.2811).



Ethyl 2-chloro-3-oxo-hexanoate (3.31). To a solution containing 19.8 g (125 mmol) of 3-oxohexanoate in 80 mL of chloroform at 0 °C was added 13.6 mL (22.8 g, 125 mmol) of sulfuryl chloride. The reaction mixture was stirred for 30 min at room temperature, and then at reflux for 2 h. The cooled reaction mixture was diluted with 300 mL of ethyl acetate, and then washed successively with 100 mL of water, 50 mL of sat aq $NaHCO_3$, 50 mL of water and 50 mL of brine. The solution was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to yield a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:6 ethyl acetate–hexanes as eluant gave **3.31** as a yellow oil: yield 23.2 g (98%); silica gel TLC R_f 0.35 (1:4 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, *J* = 7.2 Hz), 1.28 (t, 3H, *J* = 7.2 Hz), 1.63 (m, 2H), 2.64 (q, 2H, *J* = 7.2 Hz), 4.26 (q, 2H, *J* = 7.2 Hz), and 4.74 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.3, 13.9, 16.9, 40.7, 60.9, 63.0, 165.0, and 198.8; mass spectrum (EI), *m/z* 192.0550 $(M)^+$ ($C_8H_{13}O_3Cl$ requires 192.0553).

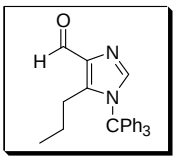


5-Propyl-1*H*-imidazole-4-carboxylic Acid Ethyl Ester (3.32). To a solution containing 29.1 g (151 mmol) of **3.31** in 60.0 mL (68.1 g, 1.51 mol) of formamide was added 5.44 mL (5.44 g, 302 mmol) of distilled water. The reaction mixture was heated to 145 °C and stirred for 4 h. The cooled reaction mixture was diluted with 300 mL of chloroform, and then washed successively with 100 mL of water, 50 mL of sat aq $NaHCO_3$, 50 mL of water and 50 mL of brine. The solution was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to yield a colorless solid. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:9 methanol–chloroform as eluant gave **3.32** as a colorless solid: yield 6.63 g (24%); silica gel TLC R_f 0.41 (1:9 methanol–chloroform); ^{1}H NMR ($CDCl_3$) δ 0.94 (m, 3H), 1.34 (t, 3H, J = 7.2 Hz), 1.69 (m, 2H), 2.95 (t, 2H, J = 8.0 Hz), 4.34 (q, 2H, J = 7.2 Hz), 7.70 (s, 1H), and 9.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 14.3, 22.7, 28.1, 60.3, 124.2, 135.3, 142.9, and 162.9; mass spectrum (APCI), *m/z* 183.1130 $(M+H)^+$ ($C_9H_{15}N_2O_2$ requires 183.1134).



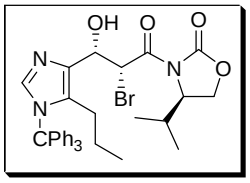
5-Propyl-1-trityl-1*H*-imidazole-4-carboxylic Acid Ethyl Ester (3.33). To a solution containing 6.63 g (36.4 mmol) of **3.32** in 270 mL of benzene was added 5.60 mL (4.05 g, 40.0 mmol) of triethylamine and 11.2 g (40.0 mmol) of trityl chloride. The reaction mixture was stirred at reflux for 4 h at which time the reaction mixture was

washed with three 50-mL portions of water and dried over anh $MgSO_4$. Excess solvent was removed under diminished pressure to give a crude colorless solid. The residue was purified by flash chromatography on a silica gel column (43 x 6 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.33** as a yellow solid: yield 2.67 g (18%); silica gel TLC R_f 0.47 (1:3 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.31 (m, 2H), 0.37 (t, 3H, J = 6.4 Hz), 1.36 (t, 3H, J = 7.2 Hz), 2.38 (m, 2H), 4.32 (q, 2H, J = 7.2 Hz), 7.13 (m, 6H), and 7.31 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.6, 20.7, 30.5, 60.2, 75.5, 128.0, 128.0, 128.0 128.2, 128.2, 128.2, 128.2, 128.2, 128.2, 129.9, 129.9, 129.9, 129.9, 129.9, 129.9, 130.7, 137.3, 141.3, 141.3, 141.4, 142.9, and 163.8; mass spectrum (APCI), *m/z* 425.2227 $(M+H)^+$ ($C_{28}H_{29}N_2O_2$ requires 425.2229).



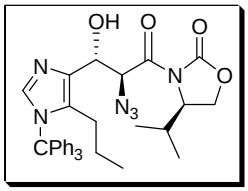
5-Propyl-1-trityl-1*H*-imidazole-4-carbaldehyde (3.34). To a solution containing 2.67 g (6.50 mmol) of **3.33** in 49 mL of dichloromethane at −78 °C was added 20.8 mL (2.96 g, 20.8 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 75 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.34** as a colorless

solid: yield 1.57 g (63%); silica gel TLC R_f 0.59 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.31 (m, 5H), 2.36 (m, 2H), 7.12 (m, 6H), 7.32 (m, 9H), 7.37 (s, 1H), and 9.95 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4, 20.7, 30.2, 75.6, 128.2, 128.2, 128.2, 128.3, 128.3, 128.3, 128.3, 128.3, 128.3, 129.9, 129.9, 129.9, 129.9, 129.9, 129.9, 138.3, 139.2, 141.2, 141.2, 141.2, 142.8, and 187.4; mass spectrum (APCI), *m/z* 381.1957 $(M+H)^+$ ($C_{26}H_{25}N_2O$ requires 381.1967).



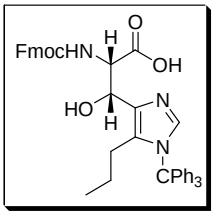
3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-propyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.35). To a solution containing 0.97 g (3.88 mmol) of **3.28** in 20 mL of diethyl ether at −78 °C was added 1.04 mL (1.17 g, 4.27 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.81 mL (0.59 mg, 5.82 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.34 g (3.52 mmol) of **3.34** in 8 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was

concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.35** as colorless foam: yield 1.23 g (53%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.38 (m, 5H), 0.95 (d, 6H, *J* = 7.2 Hz), 2.07 (m, 1H), 2.28 (m, 1H), 2.45 (m, 1H), 3.59 (br s, 1H), 4.20 (m, 2H), 4.36 (m, 1H), 5.12 (d, 1H, *J* = 8.8 Hz), 6.35 (d, 1H, *J* = 9.2 Hz), 7.12 (m, 6H), 7.23 (m, 1H), and 7.31 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.8, 17.9, 22.0, 28.0, 49.7, 58.3, 63.1, 68.3, 74.8, 127.8, 127.8, 127.8, 128.0, 128.0, 128.0, 128.0, 128.0, 128.0, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 132.7, 136.9, 137.8, 141.9, 141.9, 141.9, 152.6, 168.2, and 187.8. *Note: material decomposes rapidly, must be used immediately.*



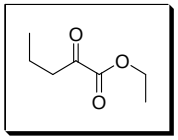
3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-propyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.36). To a solution containing 1.29 g (2.05 mmol) of **3.35** in 40 mL of *N*,*N*-dimethylformamide was added 0.66 g (10.2 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl

acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.36** as a colorless foam: yield 1.08 g (89%); silica gel TLC R_f 0.48 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.36 (t, 3H, *J* = 5.6 Hz), 0.89 (t, 6H, *J* = 5.2 Hz), 1.22 (t, 2H, *J* = 5.2 Hz), 2.18 (m, 2H), 2.44 (m, 1H), 3.91 (br s, 1H), 4.24 (m, 1H), 4.31 (m, 1H), 4.50 (m, 1H), 4.88 (m, 1H), 5.82 (d, 1H, *J* = 7.2 Hz), 7.13 (m, 6H), 7.27 (m, 9H), and 7.31 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.8, 17.9, 22.0, 28.2, 58.8, 62.3, 63.6, 68.3, 74.8, 127.8, 127.8, 127.8, 128.0, 128.0, 128.0, 128.0, 128.0, 128.0, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 132.8, 137.6, 138.1, 141.8, 141.8, 141.8, 153.8, 168.2, and 187.8; mass spectrum (ESI), *m/z* 615.2684 $(M+Na)^+$ ($C_{34}H_{36}N_6O_4Na$ requires 615.2696).

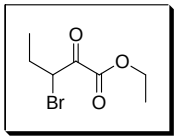


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-propyl-1-trityl-1*H*-imidazol-4-yl)propionic Acid (3.20). To a solution containing 1.08 g (1.82 mmol) of **3.36** in 90 mL of 4:1 tetrahydrofuran–water was added 0.38 g (9.12 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over

anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 90 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.50 g (3.65 mmol) of K_2CO_3 and 0.92 g (2.74 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.20** as a yellow solid: yield 0.35 g (28%); silica gel TLC R_f 0.22 (90:8:2 chloroform–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 0.37 (m, 5H), 2.18 (m, 2H), 4.23 (m, 3H), 4.58 (m, 1H), 5.08 (m, 1H), 6.20 (br s, 1H), 7.18 (m, 7H), 7.28 (m, 14H), 7.62 (m, 2H), 7.78 (d, 2H, J = 7.2 Hz), and 12.72 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4, 17.9, 22.0, 28.2, 47.1, 58.3, 68.6, 119.9, 125.4, 125.6, 125.6, 125.6, 127.1, 127.1, 127.2, 127.2, 127.8, 127.8, 127.8, 128.3, 128.3, 128.3, 128.3, 128.3, 128.3, 130.0, 130.0, 130.0, 130.0, 130.0, 130.0, 134.6, 141.3, 141.3, 141.3, 141.6, 141.6, 142.0, 142.0, 143.9, 144.2, 156.1, and 172.6; mass spectrum (ESI), *m/z* 678.2980 $(M+H)^+$ ($C_{43}H_{40}N_3O_5$ requires 678.2968).

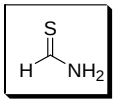


2-Oxopentanoic Acid Ethyl Ester (3.37). To a solution containing 15.0 mL (16.1 g, 110 mmol) of diethyl oxalate in 70 mL of diethyl ether at −78 °C was added 60.7 mL (12.5 g, 122 mmol) of *n*-propylmagnesium chloride (2.0 M in diethyl ether). The reaction mixture was stirred for 15 min and then allowed to warm to −10 °C for 2 h. The reaction mixture was quenched with 50 mL sat aq NH_4Cl and extracted with three 100-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.37** as a colorless oil: yield 12.2 g (77%); silica gel TLC R_f 0.72 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, *J* = 7.2 Hz), 1.37 (t, 3H, *J* = 6.8 Hz), 1.66 (m, 2H), 2.81 (t, 2H, *J* = 6.8 Hz), and 4.31 (q, 2H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.2, 13.7, 16.3, 40.9, 62.0, 161.1, and 194.3; mass spectrum (EI), *m/z* 144.0780 $(M)^+$ ($C_7H_{12}O_3$ requires 144.0787).

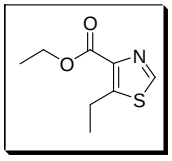


3-Bromo-2-oxopentanoic Acid Ethyl Ester (3.38). To a solution containing 12.2 g (84.9 mmol) of **3.37** in 300 mL chloroform was added a solution containing 56.9 g (255 mmol) of $CuBr_2$ in 600 mL of ethyl acetate. The reaction mixture was stirred at reflux for 18 h, cooled, filtered through a silica pad of silica gel, and washed with three 100-mL portions of ethyl acetate. The excess solvent was removed under

diminished pressure. The residue was purified by flash chromatography on a silica gel column (44 x 7 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.38** as a yellow oil: yield 15.4 g (81%); silica gel TLC R_f 0.64 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H, J = 7.2 Hz), 1.41 (t, 3H, J = 6.8 Hz), 2.14 (m, 2H), 4.40 (q, 2H, J = 7.2 Hz), and 5.03 (dd, 1H, J = 8.0 and 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 11.6, 13.8, 25.3, 50.0, 62.8, 160.4, and 185.7; mass spectrum (EI), *m/z* 221.9895 $(M)^+$ ($C_7H_{11}O_3Br$ requires 221.9892).

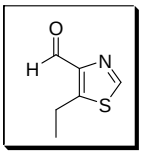


Thioformamide (3.39).[150] To a solution containing 5.00 g (111 mmol) of formamide in 34 mL of tetrahydrofuran at 0 °C was added 4.94 g (22.2 mmol) of P_2S_5. The reaction mixture was stirred while warming to room temperature for 3 h. The reaction mixture was filtered through a course glass frit and was washed with 50 mL of diethyl ether. The organic phase was concentrated under diminished pressure. The reaction crude was used without any further purification for the next reaction. Diminished pressure gave **3.39** as a yellow oil: yield 4.88 g (72%).



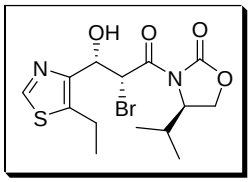
5-Ethylthiazole-4-carboxylic Acid Ethyl Ester (3.40). To a solution containing 15.0 mL (17.0 g, 278 mmol) of **3.39** in 82 mL of ethanol was added 15.4 g (70.0 mmol) of **3.38**. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 150 mL of water and extracted with three 250-mL portions of ethyl acetate. The

combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (42 x 7 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.40** as a colorless oil: yield 8.11 g (63%); silica gel TLC R_f 0.39 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.6 Hz), 1.42 (t, 3H, *J* = 7.6 Hz), 3.28 (q, 2H, *J* = 7.2 Hz), 4.40 (q, 2H, *J* = 7.2 Hz), and 8.71 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 15.5, 20.6, 60.5, 140.7, 149.2, 161.7, and 185.2; mass spectrum (EI), *m/z* 185.0514 $(M)^+$ ($C_8H_{11}NO_2S$ requires 185.0510).



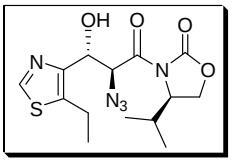
5-Ethylthiazole-4-carbaldehyde (3.41). To a solution containing 8.11 g (43.8 mmol) of **3.40** in 300 mL of dichloromethane at −78 °C was added 140 mL (19.9 g, 140 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.41** as a colorless solid: yield 6.18 g (quantitative); silica gel TLC R_f 0.49 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, *J* = 5.6 Hz), 3.09 (q, 2H, *J* = 6.4 Hz), 8.49 (s, 1H), and 9.99 (s,

1H); ^{13}C NMR ($CDCl_3$) δ 15.7, 20.2, 148.4, 150.0, 152.7, and 186.1; mass spectrum (EI), *m/z* 141.0242 $(M)^+$ (C_6H_7NOS requires 141.0248).



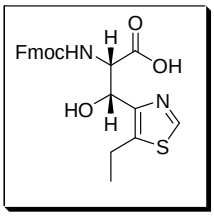
3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-ethylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.42). To a solution containing 2.50 g (10.0 mmol) of **3.28** in 50 mL of diethyl ether at −78 °C was added 2.67 mL (3.01 g, 11.0 mmol) of freshly prepared **3.27**, followed immediately by the addition of 2.09 mL (1.52 g, 15.0 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.41 g (10.0 mmol) of **3.41** in 25 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and

concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.42** as colorless foam: yield 1.65 g (42%); silica gel TLC R_f 0.28 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.93 (dd, 6H, *J* = 12.5 and 4.5 Hz), 1.33 (t, 3H, *J* = 7.5 Hz), 2.36 (m, 1H), 3.01 (m, 2H), 3.55 (br s, 1H), 4.22 (m, 2H), 4.32 (m, 1H), 5.33 (d, 1H, *J* = 7.5 Hz), 6.32 (d, 1H, *J* = 8.0 Hz), and 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.6, 14.7, 17.7, 28.0, 49.3, 58.4, 63.4, 68.6, 77.2, 139.9, 148.3, 149.9, 152.6, and 168.0. *Note: material decomposes rapidly, must be used immediately.*



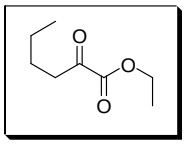
3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-ethylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.43). To a solution containing 1.65 g (4.23 mmol) of **3.42** in 53 mL of *N*,*N*-dimethylformamide was added 1.37 g (21.1 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.43** as a colorless foam: yield 0.90 g (60%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, *J*

= 7.2 Hz), 1.36 (t, 3H, *J* = 7.6 Hz), 2.49 (m, 1H), 2.96 (q, 2H, *J* = 7.6 Hz), 3.60 (d, 1H, *J* = 6.4 Hz), 4.31 (m, 1H), 4.38 (m, 1H), 4.55 (m, 1H), 5.09 (t, 1H, *J* = 8.8 Hz), 5.69 (d, 1H, *J* = 8.8 Hz), and 8.66 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.6, 16.6, 17.8, 19.4, 28.2, 59.0, 62.1, 63.8, 68.8, 139.9, 149.0, 150.6, 154.0, and 169.7; mass spectrum (FAB), *m/z* 354.1236 $(M+H)^+$ ($C_{14}H_{20}N_5O_4S$ requires 354.1236).



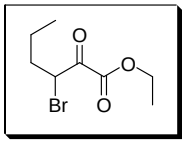
2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-ethylthiazol-4-yl)propionic Acid (3.21). To a solution containing 0.90 g (2.55 mmol) of **3.42** in 44 mL of 4:1 tetrahydrofuran–water was added 0.53 g (12.7 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 53 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.70 g (5.09 mmol) of K_2CO_3 and 1.29 g (3.82 mmol) of FmocOSu. This solution was added to ~200 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was

coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.21** as a light yellow solid: yield 0.35 g (28%); silica gel TLC R_f 0.22 (90:8:2 chloroform–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 1.26 (m, 3H), 2.87 (m, 2H), 4.21 (m, 1H), 4.37 (m, 4H), 4.68 (m, 1H), 5.32 (s, 1H), 6.62 (d, 1H, *J* = 7.2 Hz), 7.28 (m, 2H), 7.37 (m, 2H), 7.60 (t, 2H, *J* = 7.6 Hz), 7.73 (d, 2H, *J* = 7.2 Hz), and 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.5, 19.5, 47.1, 58.4, 67.4, 68.7, 119.9, 125.2, 125.3, 127.1, 127.1, 127.7, 127.7, 139.9, 141.2, 141.2, 143.8, 143.8, 148.1, 150.9, 156.4, 160.8, and 171.7; mass spectrum (FAB), *m/z* 439.1323 $(M+H)^+$ ($C_{23}H_{23}N_2O_5S$ requires 439.1328).

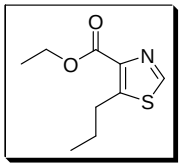


2-Oxohexanoic Acid Ethyl Ester (3.44). To a solution containing 15.0 mL (16.1 g, 110 mmol) of diethyl oxalate in 70 mL of diethyl ether at –78 °C was added 60.7 mL (14.3 g, 122 mmol) of *n*-butylmagnesium chloride (2.0 M in diethyl ether). The reaction mixture was stirred for 15 min and then allowed to warm to –10 °C for 2 h. The reaction mixture was quenched with 50 mL sat aq NH_4Cl and extracted with three 100-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.44** as a colorless oil: yield 11.5 g (66%); silica gel TLC R_f 0.78 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, *J* = 7.6 Hz), 1.38 (m, 5H), 1.63 (quint, 2H, *J* = 7.6 Hz), 2.85 (t, 2H, *J* =

7.6 Hz), and 4.31 (q, 2H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.2, 13.7, 14.3, 16.3, 40.9, 62.0, 161.1, and 194.3; mass spectrum (EI), *m/z* 158.0940 $(M)^+$ ($C_8H_{14}O_3$ requires 158.0943).

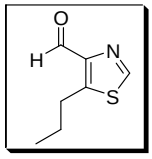


3-Bromo-2-oxohexanoic Acid Ethyl Ester (3.45). To a solution containing 11.5 g (72.9 mmol) of **3.44** in 300 mL chloroform was added a solution containing 48.8 g (219 mmol) of $CuBr_2$ in 600 mL of ethyl acetate. The reaction mixture was stirred at reflux for 18 h, cooled, filtered through a silica pad of silica gel, and washed with three 100-mL portions of ethyl acetate. The excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.45** as a yellow oil: yield 12.3 g (71%); silica gel TLC R_f 0.66 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, J = 7.2 Hz), 1.45 (m, 1H), 1.57 (m, 1H), 2.00 (m, 3H), 4.10 (q, 2H, J = 7.2 Hz), 4.38 (q, 2H, J = 7.2 Hz), and 5.07 (dd, 1H, J = 8.0 and 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.1, 13.6, 20.1, 33.6, 48.1, 62.6, 160.2, and 185.5; mass spectrum (FAB), *m/z* 237.0175 $(M+H)^+$ ($C_8H_{14}O_3Br$ requires 237.0126).



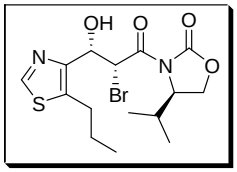
5-Propylthiazole-4-carboxylic Acid Ethyl Ester (3.46). To a solution containing 15.0 mL (17.0 g, 278 mmol) of **3.39** in 60 mL of ethanol was added 12.3 g (51.8 mmol) of **3.45**. The reaction mixture was stirred at reflux for 3 h at which time solid

$NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 150 mL of water and extracted with three 250-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.46** as a yellow oi: yield 8.47 g (82%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, *J* = 7.6 Hz), 1.43 (t, 3H, *J* = 7.6 Hz), 1.74 (m, 2H), 3.26 (t, 2H, *J* = 8.0 Hz), 4.42 (q, 2H, *J* = 7.2 Hz), and 8.64 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.6, 14.2, 24.8, 29.0, 60.9, 141.3, 149.2, 150.3, and 162.1; mass spectrum (EI), *m/z* 199.0676 $(M)^+$ ($C_9H_{13}NO_2S$ requires 199.0667).



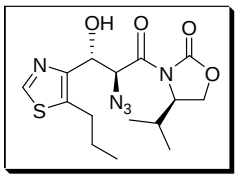
5-Propylthiazole-4-carbaldehyde (3.47). To a solution containing 8.32 g (41.8 mmol) of **3.46** in 300 mL of dichloromethane at −78 °C was added 134 mL (19.0 g, 134 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm).

Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.47** as a colorless solid: yield 5.90 g (91%); silica gel TLC R_f 0.60 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, J = 8.0 Hz), 1.54 (m, 2H), 3.06 (t, 2H, J = 7.6 Hz), 8.52 (s, 1H), and 10.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.5, 24.7, 28.2, 148.7, 150.3, 150.9, 186.0; mass spectrum (FAB), *m/z* 156.0483 $(M+H)^+$ ($C_7H_{10}NOS$ requires 156.0483).



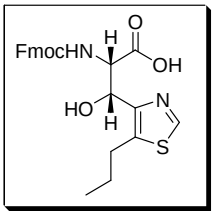
3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-propylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.48). To a solution containing 2.50 g (10.0 mmol) of **3.28** in 50 mL of diethyl ether at −78 °C was added 2.67 mL (3.01 g, 11.0 mmol) of freshly prepared **3.27**, followed immediately by the addition of 2.09 mL (1.52 g, 15.0 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.55 g (10.0 mmol) of **3.47** in 25 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10

mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.48** as colorless foam: yield 2.41 g (59%); silica gel TLC R_f 0.36 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.93 (dd, 6H, *J* = 12.5 and 4.5 Hz), 1.01 (t, 3H, *J* = 7.2 Hz), 1.70 (q, 2H, *J* = 7.2 Hz), 2.35 (m, 1H), 2.93 (m, 2H), 3.44 (br s, 1H), 4.22 (m, 2H), 4.30 (m, 1H), 5.31 (m, 1H), 6.30 (d, 1H, *J* = 7.6 Hz), and 8.55 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 14.7, 17.6, 25.1, 27.8, 27.9, 49.2, 58.0, 62.3, 68.5, 138.0, 148.7, 149.9, 152.5, and 167.9. *Note: material decomposes rapidly, must be used immediately.*



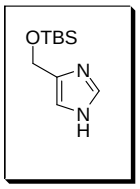
3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-propylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.49). To a solution containing 2.41 g (5.95 mmol) of **3.48** in 75 mL of *N*,*N*-dimethylformamide was added 1.93 g (29.7 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~60 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash

chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.49** as a colorless foam: yield 1.26 g (58%); silica gel TLC R_f 0.52 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.98 (d, 6H, *J* = 7.2 Hz), 1.03 (t, 3H, *J* = 6.0 Hz), 1.74 (m, 2H), 2.51 (m, 1H), 2.90 (t, 2H, *J* = 6.0 Hz), 3.40 (d, 1H, *J* = 8.0 Hz), 4.30 (m, 1H), 4.37 (m, 1H), 4.55 (m, 1H), 5.06 (t, 1H, *J* = 7.2 Hz), 5.70 (d, 1H, *J* = 6.8 Hz), and 8.66 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 14.7, 17.9, 25.4, 27.8, 28.3, 59.1, 62.1, 63.8, 69.0, 138.0, 149.5, 150.8, 154.0, and 169.8; mass spectrum (FAB), *m/z* 368.1403$(M+H)^+$ ($C_{15}H_{22}N_5O_4S$ requires 368.1393).



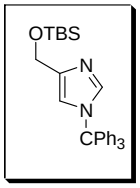
2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-propylthiazol-4-yl)propionic Acid (3.22). To a solution containing 1.26 g (3.43 mmol) of **3.49** in 60 mL of 4:1 tetrahydrofuran–water was added 0.72 g (17.1 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 71 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.95 g (6.86 mmol) of K_2CO_3 and 1.74 g (5.14 mmol) of FmocOSu. This solution was added to ~200 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then

filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.22** as a light yellow solid: yield 0.31 g (22%); silica gel TLC R_f 0.28 (90:8:2 chloroform–methanol–acetic acid). ^{1}H NMR ($CDCl_3$) δ 1.26 (m, 3H), 1.65 (m, 2H), 2.81 (t, 2H, *J* = 7.2 Hz), 4.19 (t, 2H, *J* = 7.2 Hz), 4.35 (m, 3H), 4.75 (m, 1H), 5.32 (s, 1H), 6.66 (d, 1H, *J* = 8.0 Hz), 7.28 (m, 2H), 7.37 (m, 2H), 7.60 (t, 2H, *J* = 7.6 Hz), 7.72 (d, 2H, *J* = 7.6 Hz); and 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 17.9, 25.1, 27.7, 58.4, 67.4, 68.7, 119.9, 125.2, 125.3, 127.1, 127.1, 127.7, 127.7, 138.1, 141.2, 141.2, 143.7, 143.9, 148.8, 151.1, 156.4, 161.6, and 171.9; mass spectrum (FAB), *m/z* 453.1486 $(M+H)^+$ ($C_{24}H_{25}N_2O_5S$ requires 453.1484).

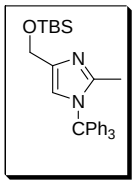


4-(*tert*-Butyl-dimethylsilanyloxymethyl)-1*H*-imidazole (3.50).[150] To a solution containing 1.00 g (51.0 mmol) of 3-imidazolemethanol hydrochloride in 25 mL of *N*,*N*-dimethylformamide was added 1.52 g (22.3 mmol) of imidazole, 1.23 g (8.18 mmol) of *tert*-butyldimethylsilyl chloride, and a catalytic amount of 4-dimethylaminopyridine. The reaction mixture was stirred for 16 h. The reaction mixture was quenched with 25 mL of water and extracted with three 150-mL portions

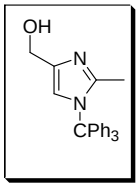
of ethyl acetate. The combined organic phase was washed with three 15-mL portions of brine, dried over anh $MgSO_4$, filtered, and excess solvent was removed under diminished pressure to give **3.50** as a yellow oil: yield 1.59 g (98%); silica gel TLC R_f 0.65 (10:1:0.1 dichloromethane–methanol–ammonium hydroxide); ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 6H), 0.95 (s, 9H), 4.78 (s, 2H), 6.99 (s, 1H) and 7.62 (s, 1H).



4-(*tert*-Butyldimethylsilanyloxymethyl)-1-trityl-1*H*-imidazole (3.51).[150] To a solution containing 1.55 g (7.30 mmol) of **3.50** in 60 mL of benzene was added 1.12 mL (0.80 g, 8.02 mmol) of triethylamine and 2.38 g (8.02 mmol) of trityl chloride. The reaction mixture was stirred at reflux for 1.5 h. The cooled reaction mixture was washed with three 25-mL portions of water, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a crude yellow oil. The residue was purified by flash chromatography on a silica gel column (34 x 5 cm). Step gradient elution with 1:3 → 1:1 ethyl acetate–hexanes as eluant gave **3.51** as a yellow solid: yield 2.20 g (60%); silica gel TLC R_f 0.38 (1:4 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 6H), 0.88 (s, 9H), 4.75 (s, 2H), 6.78 (s, 1H), 7.18 (m, 6H), 7.37 (m, 9H), and 7.51 (s, 1H).

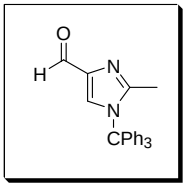


4-(*tert*-Butyldimethylsilanyloxymethyl)-2-methyl-1-trityl-1*H*-imidazole (3.52).[150] To a solution containing 2.20 g (4.83 mmol) of **3.51** in 60 mL of tetrahydrofuran at −78 °C was added 5.70 mL (9.68 mmol) of *tert*-butyllithium (1.7 M in pentane) over 5 min. The resulting red solution was stirred at −78 °C for 2 h at which time 0.60 mL (1.37 g, 9.68 mmol) of methyl iodide was added during 5 min. The resulting brown solution was allowed to warm to room temperature and was stirred for 18 h. The reaction mixture was quenched with 25 mL of sat aq NH_4Cl and extracted with three 25-mL portions of chloroform. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a dark solid. The solid was recrystallized from 1:4 ethyl acetate–hexanes. The remaining solvent was removed under diminished pressure to give a brown residue. The residue was purified by flash chromatography on a silica gel column (38 x 5 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.52** as a brown solid: yield 1.38 g (61%); silica gel TLC R_f 0.47 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H), 0.84 (s, 9H), 1.61 (s, 3H), 4.66 (s, 2H), 6.59 (s, 1H), 7.13 (m, 6H), and 7.31 (m, 9H).



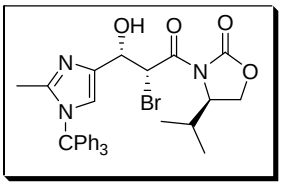
(2-Methyl-1-trityl-1*H*-imidazol-4-yl)methanol (3.53).[150] To a solution containing 61.38 g (2.94 mmol) of **3.52** in 40 mL of tetrahydrofuran was added 1.02 g (3.24

mmol) of TBAF·$3H_2O$. The reaction mixture was stirred at room temperature for 48 h. The resulting milky white solution was diluted with 30 mL of water and extracted with three 50-mL portions of chloroform. The combined organic phase was washed with three 15-mL portions of sat aq $NaHCO_3$ and 10 mL of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid which was recrystallized from ethanol. The remaining solvent was removed under diminished pressure to give a colorless oil. The residue was purified by flash chromatography on a silica gel column (39 x 4 cm). Elution with 1:4 methanol–dichloromethane as eluant gave **3.53** as a colorless solid: yield 0.75 g (72%); silica gel TLC R_f 0.66 (1:4 methanol–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.68 (s, 3H), 4.56 (s, 2H), 6.70 (s, 1H), 7.17 (m, 6H), and 7.31 (m, 9H).



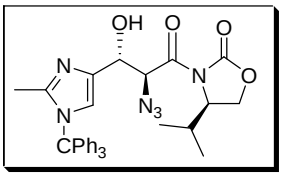
2-Methyl-1-trityl-1*H*-imidazole-4-carbaldehyde (3.54).[150] To a solution containing 0.75 g (2.11 mmol) of **3.53** in 40 mL of dioxane was added 0.90 g (10.5 mmol) of activated MnO_2. The reaction mixture was stirred at reflux for 18 h. The reaction mixture was filtered through a Celite pad and washed with three 60-mL portions of hot dioxane. Excess solvent was removed under diminished pressure to give a colorless solid. The residue was purified by flash chromatography on a silica gel column (30 x 4 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.54** as a colorless solid: yield 0.47 g (63%); silica gel TLC R_f 0.58 (1:2 ethyl acetate–hexanes);

^{1}H NMR ($CDCl_3$) δ 1.74 (s, 3H), 7.19 (m, 6H), 7.40 (m, 9H), 7.56 (s, 1H), and 9.84 (s, 1H).



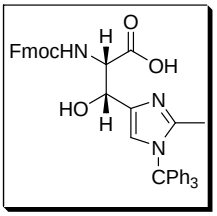
3-[2-*R*-Bromo-3-*R*-hydroxy-3-(2-methyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.55).[150] To a solution containing 0.53 g (2.12 mmol) of **3.28** in 20 mL of diethyl ether at −78 °C was added 0.56 mL (0.64 g, 2.33 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.33 mL (0.24 g, 2.33 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 0.75 g (2.12 mmol) of **3.54** in 8 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether−dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether−dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and

concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (41 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.55** as colorless oil: yield 0.31 g (24%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.97 (d, 6H, J = 6.9 Hz), 1.61 (s, 3H), 2.43 (m, 1H), 4.32 (m, 2H), 4.44 (quint, 1H, J = 4.2 Hz), 5.16 (d, 1H, J = 5.7 Hz), 6.04 (d, 1H, J = 6.0 Hz), 6.82 (s, 1H), 7.14 (m, 6H), and 7.34 (m, 9H). *Note: material decomposes rapidly, must be used immediately.*



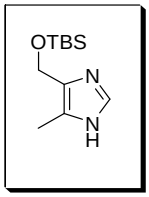
3-[2-*S*-Azido-3-*R*-hydroxy-3-(2-methyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.56).[150] To a solution containing 250 mg (0.41 mmol) of **3.55** in 20 mL of *N*,*N*-dimethylformamide was added 134 mg (2.08 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~15 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (40 x 2.5 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.56** as a colorless oil: yield 129 mg (55%); silica gel TLC R_f 0.51 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.90 (dd, 6H, J = 6.9 and 1.2 Hz), 1.63 (s, 3H), 2.31 (m, 1H), 4.31 (m, 2H), 4.51 (quint,

1H, *J* = 3.9 Hz), 4.93 (d, 1H, *J* = 7.8 Hz), 5.60 (d, 1H, *J* = 8.4 Hz), 6.81 (s, 1H), 7.16 (m, 6H), and 7.32 (m, 9H).

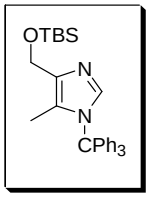


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(2-methyl-1-trityl-1*H*-imidazol-4-yl)propionic Acid (3.11).[150] To a solution containing 141 mg (0.25 mmol) of **3.56** in 20 mL of 4:1 tetrahydrofuran–water was added 52 mg (1.25 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 30 mL of 9:1 tetrahydrofuran–water. To this solution was added 70 mg (0.50 mmol) of K_2CO_3 and 126 mg (0.37 mmol) of FmocOSu. This solution was added to ~30 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (42 x 4 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.11** as a

colorless solid: yield 27 mg (17%); silica gel TLC R_f 0.70 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H), 4.19 (m, 3H), 4.32 (m, 1H), 4.71 (m, 1H), 6.62 (s, 1H), 7.05 (d, 6H, *J* = 7.0 Hz), 7.35 (m, 13H), 7.68 (t, 2H, *J* = 9.0 Hz), and 7.88 (d, 1H, *J* = 7.0 Hz).

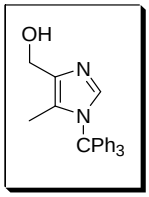


4-(*tert*-Butyldimethylsilanyloxymethyl)-5-methyl-1*H*-imidazole (3.57).[150] To a solution containing 3.00 g (20.2 mmol) of 4-hydroxymethyl-5-methylimidazole in 75 mL of *N*,*N*-dimethylformamide was added4.12 g (60.6 mmol) of imidazole, 3.35 g (22.2 mmol) of *tert*-butyldimethylsilyl chloride, and a catalytic amount of 4-dimethylaminopyridine. The reaction mixture was stirred for 16 h at room temperature. The reaction mixture was quenched with 25 mL of water and extracted with three 100-mL portions of ethyl acetate. The combined organic phase was washed with three 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give **3.57** as a colorless foam: yield 4.53 (99%); silica gel TLC R_f 0.1 (1:1 hexanes–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H), 0.85 (s, 9H), 2.22 (s, 3H), 4.63 (s, 2H), and 7.47 (s, 1H).



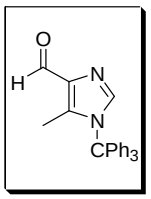
4-(*tert*-Butyldimethylsilanyloxymethyl)-5-methyl-1-trityl-1*H*-imidazole (3.58).[150] To a solution containing 4.20 g (18.6 mmol) of **3.57** in 250 mL of benzene was added

2.86 mL (2.06 g, 20.4 mmol) of triethylamine and 5.70 g (20.4 mmol) of trityl chloride. The reaction mixture was stirred at reflux for 4 h. The reaction mixture was washed with three 25-mL portions of water and dried over anh $MgSO_4$. Excess solvent was removed under diminished pressure to give a crude colorless solid. The residue was purified by flash chromatography on a silica gel column (35 x 7 cm). Step gradient elution with 1:3 → 1:1 ethyl acetate–hexanes as eluant gave **3.58** as a colorless solid: yield 1.69 g (19%); silica gel TLC R_f 0.77 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.86 (s, 9H), 1.46 (s, 3H), 4.03 (s, 2H), 7.12 (m, 6H), 7.22 (s, 1H), and 7.28 (m, 9H).

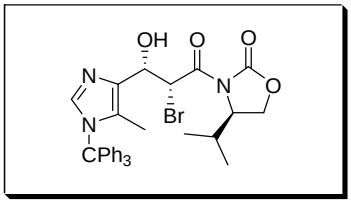


(5-Methyl-1-trityl-1*H*-imidazol-4-yl)methanol (3.59).[150] To a solution containing 1.00 g (2.13 mmol) of **3.58** in 50 mL of tetrahydrofuran was added 0.74 g (2.34 mmol) of TBAF·$3H_2O$. The reaction mixture was stirred at room temperature for 18 h and the resulting milky white solution was diluted with 30 mL of water and was extracted with three 50-mL portions of ethyl acetate. The combined organic phase was washed with three 20-mL portions of sat aq $NaHCO_3$ and 10 mL of brine, dried over anh $MgSO_4$, filtered, and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (33 x 4 cm). Elution with 1:4 methanol–dichloromethane as eluant gave **3.59** as a colorless solid: yield 2.93 g (93%); silica gel TLC *Rf* 0.47 (1:4

methanol–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 4.53 (s, 2H), 7.13 (m, 6H), and 7.31 (m, 10H).

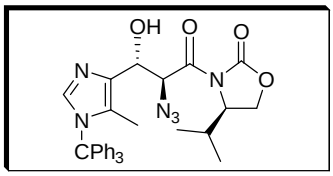


5-Methyl-1-trityl-1H-imidazole-4-carbaldehyde (3.60).[150] To a solution containing 0.70 g (3.09 mmol) of **3.59** in 50 mL of dioxane was added 0.90 g (9.88 mmol) of activated MnO_2. The reaction mixture was stirred at reflux for 16 h. The reaction mixture was filtered through a Celite pad and washed with three 80-mL portions of hot dioxane. Excess solvent was removed under diminished pressure to give a colorless solid. The residue was purified by flash chromatography on a silica gel column (37 x 4 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.60** as a colorless solid: yield 3.33 g (62%); silica gel TLC *Rf* 0.44 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 7.27 (m, 6H), 7.45 (m, 9H), 7.60 (s, 1H), and 10.14 (s, 1H).

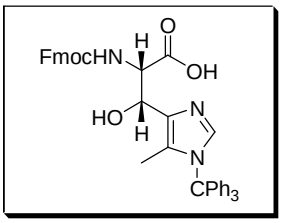


3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.61).[150] To a solution containing 0.43 g (1.72 mmol) of **3.28** in 50 mL of diethyl ether at −78 °C was added 0.50 mL (0.52 g, 1.90 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.26 mL (0.19 mg, 1.90 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and

then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 0.63 g (1.72 mmol) of **3.60** in 8 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (40 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.61** as colorless foam: yield 0.13 g (12%); silica gel TLC R_f 0.32 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.94 (dd, 6H, J = 6.5 and 2.4 Hz), 1.55 (s, 3H), 2.42 (m, 1H), 4.31 (m, 3H), 5.13 (d, 1H, J = 8.4 Hz), 6.21 (d, 1H, J = 8.4 Hz), 7.14 (m, 6H), 7.31 (m, 9H), and 7.39 (s, 1H). *Note: material decomposes rapidly, must be used immediately.*

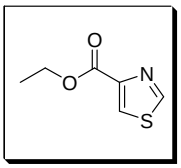


3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.62).[150] To a solution containing 0.14 g (0.22 mmol) of **3.61** in 50 mL of *N*,*N*-dimethylformamide was added 0.08 g (1.07 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a colorless oil. The residue was purified via flash chromatography on a silica gel column (37 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.62** as a colorless foam: yield 0.09 g (65%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 6H), 1.53 (s, 3H), 2.46 (m, 1H), 4.25 (m, 1H), 4.35 (t, 1H, *J* = 9.3 Hz), 4.54 (m, 1H), 4.87 (d, 1H, *J* = 7.8 Hz) 5.77 (d, 1H, *J* = 9.0 Hz), 7.18 (m, 6H), 7.33 (m, 9H), and 7.36 (s, 1H).

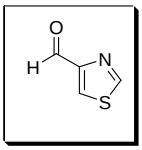


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-methy-1-trityl-1*H*-imidazol-4-yl)propionic Acid (3.13).[150] To a solution containing 0.33 g (0.58 mmol) of **3.62** in 30 mL of 4:1 tetrahydrofuran–water was added 0.35 g (2.11 mmol) of

$LiOH \cdot H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 30 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.18 g (1.10 mmol) of K_2CO_3 and 0.28 g (0.94 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (42 x 5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.13** as a colorless solid: yield 86 mg (14%); silica gel TLC R_f 0.68 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 1.57 (s, 3H), 4.18 (m, 3H), 4.36 (m, 1H), 5.20 (m, 1H), 7.09 (m, 7H), 7.31 (m, 13H), 7.59 (m, 2H), and 7.72 (m, 2H).

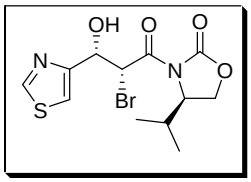


Thiazole-4-carboxylic Acid Ethyl Ester (3.63).[150] To a solution containing 22.1 mL (25.1 g, 410 mmol) of **3.39** in 120 mL of ethanol was added 14.3 mL (12.9 g, 103 mmol) of 3-bromo-2-oxopropionic acid ethyl ester. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 150 mL of water and extracted with three 250-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.63** as a yellow solid: yield 13.2 g (82%); silica gel TLC R_f 0.70 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3H, J = 7.2 Hz), 4.38 (q, 2H, J = 7.2 Hz), 8.21 (s, 1H), and 8.82 (s, 1H).



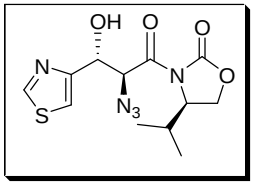
Thiazole-4-carbaldehyde (3.64).[150] To a solution containing 6.00 g (38.2 mmol) of **3.63** in 300 mL of dichloromethane at −78 °C was added 122 mL (17.3 g, 122 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at

room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.64** as a yellow solid: yield 3.15 g (73%); silica gel TLC R_f 0.51 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1H), 8.81 (s, 1H), and 9.92 (s, 1H).



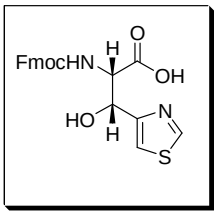
3-(2-*R*-Bromo-3-*R*-hydroxy-3-thiazol-4-ylpropionyl)-4-*R*-isopropyloxazolidin-2-one (3.65).[150] To a solution containing 2.20 g (10.0 mmol) of **3.28** in 100 mL of diethyl ether at −78 °C was added 2.35 mL (2.65 g, 9.68 mmol) of freshly prepared **3.27**, followed immediately by the addition of 1.84 mL (1.34 g, 13.2 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.00 g (10.0 mmol) of **3.64** in 15 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under

diminished pressure, and the resulting residue was dissolved in 10 mL of and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 24 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.65** as colorless oil: yield 1.03 g (32%); silica gel TLC R_f 0.28 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H, *J* = 15.0 and 5.4 Hz), 2.36 (m, 1H), 4.24 (m, 2H), 4.47 (m, 1H), 5.36 (dd, 1H, *J* = 3.0 and 1.2 Hz), 6.16 (d, 1H, *J* = 3.9 Hz), 7.84 (dd, 1H, *J* = 1.2 and 0.9 Hz), and 8.75 (d, 1H, J = 2.1 Hz).



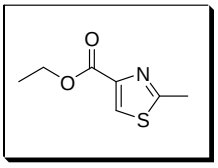
3-(2-*S*-Azido-3-*R*-hydroxy-3-thiazol-4-ylpropionyl))-4-*R*-isopropyloxazolidin-2-one (3.66).[150] To a solution containing 1.03 g (2.84 mmol) of **3.65** in 40 mL of *N*,*N*-dimethylformamide was added 0.92 g (14.2 mmol) of sodium azide. The reaction mixture was warmed to 45 ℃ and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.66** as a colorless oil: yield 0.55 g (60%); silica gel TLC R_f 0.45 (1:1 ethyl

acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (dd, 6H, *J* = 7.2 and 2.7 Hz), 2.37 (m, 1H), 4.27 (m, 2H), 4.54 (m, 1H), 5.31 (d, 1H, *J* = 7.5 Hz), 5.65 (d, 1H, *J* = 7.5 Hz), 7.54 (d, 1H, *J* = 2.1 Hz), and 8.84 (d, 1H, *J* = 2.1 Hz).

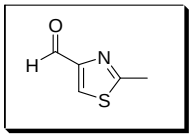


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-thiazol-4-ylpropionic Acid (3.14).[150] To a solution containing 0.55 g (1.69 mmol) of **3.66** in 20 mL of 4:1 tetrahydrofuran–water was added 0.35 g (8.45 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 20 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.47 g (3.38 mmol) of K_2CO_3 and 0.85 g (2.54 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Elution with

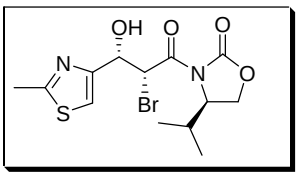
90:8:2 chloroform–methanol–acetic acid as eluant gave **3.14** as a colorless solid: yield 0.18 g (28%); silica gel TLC R_f 0.22 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR (DMSO-d_6) δ 4.19 (m, 3H), 4.57 (m, 1H), 5.06 (d, 1H, J = 5.5 Hz), 7.32 (t, 2H, J = 7.5 Hz), 7.42 (t, 2H, J = 7.5 Hz), 7.51 (s, 1H), 7.67 (d, 2H, J = 7.5 Hz), 7.88 (d, 2H, J = 7.5 Hz), and 9.05 (s, 1H).



2-Methylthiazole-4-carboxylic Acid Ethyl Ester (3.67).[150] To a solution containing 500 mg (6.65 mmol) of thioacetamide in 5 mL of ethanol was added 0.82 mL (1.23 g, 6.63 mmol) of 3-bromo-2-oxopropionic acid ethyl ester. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 100 mL of water and extracted with three 150-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.67** as a colorless solid: yield 0.73 g (72%); silica gel TLC R_f 0.51 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.2 Hz), 2.71 (s, 3H), 4.34 (q, 2H, J = 7.2 Hz), and 7.98 (s, 1H).

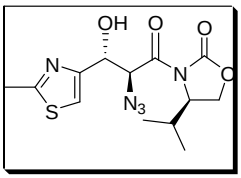


2-Methylthiazole-4-carbaldehyde (3.68).[150] To a solution containing 0.73 g (4.26 mmol) of **3.67** in 50 mL of dichloromethane at −78 °C was added 13.2 mL (1.90 g, 13.2 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 100-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 1:1 ethyl acetate–hexanes as eluant gave **3.68** as a colorless solid: yield 0.26 g (40%); silica gel TLC R_f 0.53 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.63 (s, 3H), 7.95 (s, 1H), and 9.81 (s, 1H).

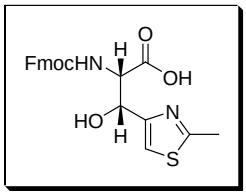


3-[2-*R*-Bromo-3-*R*-hydroxy-3-(2-metylthiazol-4-yl)propionyl]-4-*R*-isopropyloxazolidin-2-one (3.69).[150] To a solution containing 420 mg (1.68 mmol) of **3.28** in 20 mL of diethyl ether at −78 °C was added 0.45 mL (0.51 g, 1.85 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.26 mL (0.19 g, 1.85 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then

allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 0.21 g (1.68 mmol) of **3.68** in 8 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether−dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether−dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (41 x 4 cm). Step gradient elution with 1:4 ethyl acetate−hexanes → 100% ethyl acetate as eluant gave **3.69** as colorless oil: yield 0.23 g (36%); silica gel TLC R_f 0.37 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 6H, J = 7.0 Hz), 2.35 (m, 1H), 2.64 (s, 3H), 4.21 (d, 1H, J = 9.0 Hz), 4.29 (t, 1H, J = 9.0 Hz), 4.46 (m, 1H), 5.25 (s, 1H), 6.06 (s, 1H), and 7.21 (s, 1H).

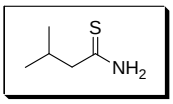


3-[2-*S*-Azido-3-*R*-hydroxy-3-(2-methylthiazol-4-ylpropionyl)]-4-*R*-isopropyloxazolidin-2-one (3.70).[150] To a solution containing 0.30 g (0.61 mmol) of **3.69** in 20 mL of *N*,*N*-dimethylformamide was added 0.20 g (3.05 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (42 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.70** as a colorless foam: yield 124 mg (60%); silica gel TLC R_f 0.54 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (dd, 6H, *J* = 6.6 and 3.9 Hz), 2.43 (m, 1H), 2.68 (s, 1H), 4.25 (m, 2H), 4.51 (m, 1H), 5.10 (t, 1H, *J* = 8.1 Hz), 5.56 (d, 1H, *J* = 7.8 Hz), and 7.23 (s, 1H).



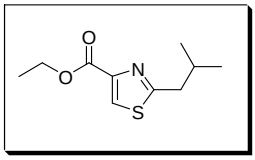
2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(2-methylthiazol-4-yl)propionic Acid (3.15).[150] To a solution containing 0.23 g (0.68 mmol) of **3.70** in 5 mL of 4:1 tetrahydrofuran–water was added 0.14 g (3.39 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until

pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 10 mL of 9:1 tetrahydrofuran–water. To this solution was added 0.19 g (1.36 mmol) of K_2CO_3 and 0.34 g (1.01 mmol) of FmocOSu. This solution was added to ~40 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.15** as a light yellow solid: yield 34 mg (12%); silica gel TLC R_f 0.60 (1:9 methanol–dichloromethane); ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 4.21 (m, 3H), 4.56 (t, 1H, J = 6.0 Hz) 4.90 (d, 1H, J = 4.5 Hz), 7.24 (s, 1H), 7.31 (m, 5H), 7.68 (d, 2H, J = 7.5 Hz), and 7.89 (d, 1H, J = 7.5 Hz).

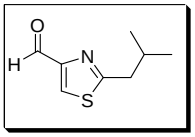


Thioisovaleramide (3.71).[150] To a solution containing 2.00 g (19.8 mmol) of isovaleramide in 50 mL tetrahydrofuran at 0 °C was added 0.88 g (3.95 mmol) of P_2S_5. The reaction mixture was stirred while warming to room temperature for 3 h. The reaction mixture was filtered through a course glass frit and was washed with 50

mL of diethyl ether. The organic phase was concentrated under diminished pressure. The reaction crude was used without any further purification for the next reaction. Diminished pressure gave **3.71** as a yellow oil: yield 2.22 g (96%).

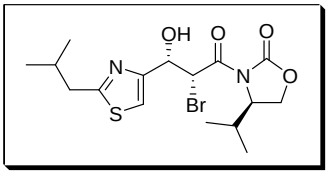


2-Isobutylthiazole-4-carboxylic Acid Ethyl Ester (3.72).[150] To a solution containing 122 mg (1.05 mmol) of **3.71** in 50 mL of ethanol was added 0.14 mL (0.22 g, 1.14 mmol) of 3-bromo-2-oxopropionic acid ethyl ester. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 15 mL of water and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 5 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.72** as a yellow oil: yield 127 mg (50%); silica gel TLC R_f 0.80 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 6H, J = 6.6 Hz), 1.35 (t, 3H, J = 7.2 Hz), 2.08 (m, 1H), 2.87 (d, 2H, J = 7.2 Hz), 4.33 (q, 2H, J = 7.1 Hz), and 8.01 (s, 1H).



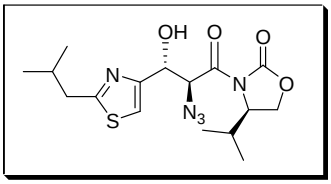
2-Isobutylthiazole-4-carbaldehyde (3.73).[150] To a solution containing 127 mg (0.60 mmol) of **3.72** in 20 mL of dichloromethane at −78 °C was added 1.85 mL (266 mg,

1.85 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 1.5 mL of methanol followed by 15 mL of 1:1 sat aq sodium potassium tartrate−pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 10-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 2.5 cm). Elution with 1:1 ethyl acetate−hexanes as eluant gave **3.73** as a brown oil: yield 71 mg (71%); silica gel TLC R_f 0.84 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 6H, J = 6.6 Hz), 2.05 (quint, 1H, J = 6.6 Hz), 2.81 (d, 2H, J = 7.2 Hz), 8.01 (s, 1H), and 9.89 (s, 1H).



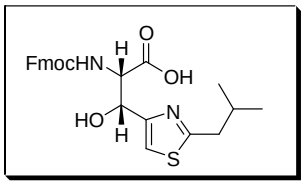
3-[2-*R*-Bromo-3-*R*-hydroxy-3-(2-isobutylthiazol-4-yl)propionyl]-4-*R*-isopropyloxazolidin-2-one (3.74).[150] To a solution containing 84 mg (0.34 mmol) of **3.28** in 4 mL of diethyl ether at −78 °C was added 90 μL (100 mg, 0.37 mmol) of freshly prepared **3.27**, followed immediately by the addition of 51 μL (37 mg, 0.37 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 57 mg (0.34 mmol) of **3.73** in 1.5 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The

reaction mixture was then diluted with 15 mL of 2:1 diethyl ether–dichloromethane and washed with two 5-mL portions of sat aq $NaHSO_4$ and 2 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 3 mL of methanol. To this solution was added 1 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 10-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 5-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (41 x 2.5 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.74** as colorless foam: yield 42 mg (30%); silica gel TLC R_f 0.59 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 12H), 1.94 (m, 1H), 2.33 (m, 1H), 2.74 (d, 2H, J = 7.2 Hz), 4.21 (m, 2H), 4.40 (m, 1H), 5.23 (d, 1H, J = 4.8 Hz), 6.07 (d, 1H, J = 4.8 Hz), and 7.20 (s, 1H).



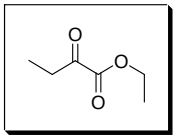
3-[2-*S*-Azido-3-*R*-hydroxy-3-(2-isobutylthiazol-4-ylpropionyl)]-4-*R*-isopropyloxazolidin-2-one (3.75).[150] To a solution containing 42 mg (0.10 mmol) of **3.74** in 5 mL of *N*,*N*-dimethylformamide was added 32 mg (0.50 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time

it was poured into ~4 g of ice and extracted with three 10-mL portions of ethyl acetate. The combined organic phase was washed with two 10-mL portions of water and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (42 x 2 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.75** as a colorless oil: yield 25 mg (65%); silica gel TLC R_f 0.75 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.85 (m, 12H), 1.99 (m, 1H), 2.37 (m, 1H), 2.75 (d, 2H, J = 7.2 Hz), 4.22 (m, 2H), 4.44 (m, 1H), 5.09 (m, 1H), 5.49 (d, 1H, J = 7.5 Hz), and 7.19 (s, 1H).



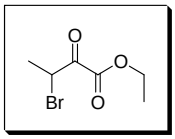
2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(2-isobutylthiazol-4-yl)propionic Acid (3.16).[150] To a solution containing 350 mg (0.91 mmol) of **3.75** in 20 mL of 4:1 tetrahydrofuran–water was added 192 mg (4.60 mmol) of $LiOH{\cdot}H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 20 mL of 9:1 tetrahydrofuran–water. To this solution was added 253 mg (1.83 mmol) of K_2CO_3 and 460 mg (1.38 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction

vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.16** as a colorless solid: yield 100 mg (24%); silica gel TLC R_f 0.44 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR (DMSO-d_6) δ 0.87 (d, 6H, J = 6.6 Hz), 1.93 (m, 1H), 2.77 (d, 2H, J = 7.2 Hz), 4.20 (m, 3H), 4.44 (m, 1H,) 4.90 (d, 1H, J = 5.7 Hz), 7.31 (m, 5H), 7.31 (m, 5H), 7.65 (d, 2H, J = 7.2 Hz), and 7.87 (d, 1H, J = 7.5 Hz).

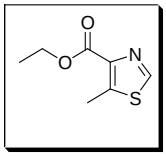


2-Oxobutanoic Acid Ethyl Ester (3.76).[150] To a solution containing 1.00 mL (1.08 g, 6.82 mmol) of diethyl oxalate in 20 mL of diethyl ether at −78 °C was added 2.27 mL (0.89 g, 6.82 mmol) of ethylmagnesium bromide (3.0 M in diethyl ether). The reaction mixture was stirred for 15 min and then allowed to warm to −10 °C for 2 h. The reaction mixture was quenched with 10 mL sat aq NH_4Cl and extracted with three 50-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered and excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.76** as a colorless oil: yield

0.72 g (80%); silica gel TLC R_f 0.89 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H, *J* = 7.2 Hz), 1.28 (t, 3H, *J* = 6.8 Hz), 2.77 (q, 2H, *J* = 7.5 Hz), and 4.22 (q, 2H, *J* = 6.9 Hz).

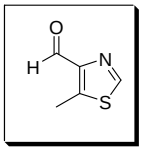


3-Bromo-2-oxobutanoic Acid Ethyl Ester (3.77).[150] To a solution containing 720 mg (5.53 mmol) of **3.76** in 30 mL chloroform was added a solution containing 2.38 g (16.6 mmol) of $CuBr_2$ in 60 mL of ethyl acetate. The reaction mixture was stirred at reflux for 18 h, cooled, filtered through a silica pad of silica gel, and washed with three 50-mL portions of ethyl acetate. The excess solvent was removed under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 1:3 ethyl acetate–hexanes as eluant gave **3.77** as a yellow oil: yield 537 mg (46%); silica gel TLC R_f 0.82 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, *J* = 6.9 Hz), 1.71 (d, 3H, *J* = 6.9 Hz), 4.28 (q, 2H, *J* = 7.2 Hz), and 5.09 (q, 1H, *J* = 6.9 Hz).

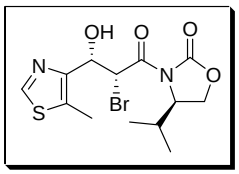


5-Methylthiazole-4-carboxylic Acid Ethyl Ester (3.78).[150] To a solution containing 0.76 g (12.4 mmol) of **3.39** in 40 mL of ethanol was added 0.52 g (2.48 mmol) of **3.77**. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 15 mL of water and extracted with three 50-mL portions of ethyl acetate. The

combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.78** as a colorless solid: yield 469 mg (44%); silica gel TLC R_f 0.33 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, J = 7.0 Hz), 2.36 (s, 3H), 3.97 (q, 2H, J = 7.0 Hz), and 8.23 (s, 1H).

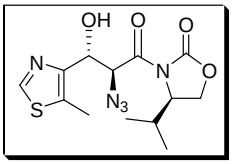


5-Methylthiazole-4-carbaldehyde (3.79).[150] To a solution containing 2.11 g (12.3 mmol) of **3.78** in 300 mL of dichloromethane at −78 °C was added 38 mL (5.51 g, 38.6 mmol) of diisobutylaluminium hydride (1.0 M in toluene) over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate–pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 150-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 2.5 cm). Elution with 1:2 ethyl acetate–hexanes as eluant gave **3.79** as a colorless solid: yield 0.74 g (47%); silica gel TLC R_f 0.61 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H), 8.47 (s, 1H), and 9.97 (s, 1H).

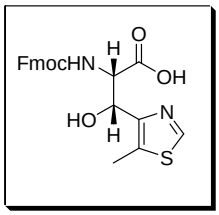


3-[2-*R*-Bromo-3-*R*-hydroxy-3-(5-methylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.80).[150] To a solution containing 2.00 g (8.15 mmol) of **3.28** in 50 mL of diethyl ether at −78 °C was added 2.18 mL (2.50 g, 8.98 mmol) of freshly prepared **3.27**, followed immediately by the addition of 1.25 mL (0.90 g, 8.98 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a solution containing 1.00 g (5.91 mmol) of **3.79** in 20 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4

ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.80** as colorless oil: yield 640 mg (20%); silica gel TLC R_f 0.54 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 6H), 2.27 (m, 1H), 2.51 (s, 3H), 4.18 (m, 3H), 5.31 (d, 1H, *J* = 8.1 Hz), 6.17 (d, 1H, *J* = 8.1 Hz), and 8.60 (s, 1H). *Note: material decomposes rapidly, must be used immediately.*

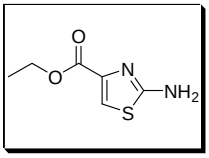


3-[2-*S*-Azido-3-*R*-hydroxy-3-(5-methylthiazol-4-yl)propionyl)]-4-*R*-isopropyloxazolidin-2-one (3.81).[150] To a solution containing 640 mg (1.70 mmol) of **3.80** in 53 mL of *N*,*N*-dimethylformamide was added 550 mg (8.50 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~45 g of ice and extracted with three 50-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (43 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.81** as a colorless oil: yield 218 mg (38%); silica gel TLC R_f 0.65 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 1H, *J* = 6.3 Hz), 2.43 (m, 1H), 2.52 (s, 3H), 4.24 (m, 1H), 4.34 (t, 1H, *J* = 9.0 Hz), 4.51 (m, 1H), 5.05 (t, 1H, *J* = 8.7 Hz), 5.61 (d, 1H, *J* = 9.0 Hz), and 8.59 (s, 1H)

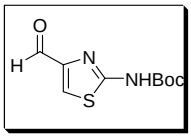


2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-3-(5-methylthiazol-4-yl)propionic Acid (3.17).[150] To a solution containing 218 mg (0.64 mmol) of **3.81** in 10 mL of 4:1 tetrahydrofuran–water was added 134 mg (3.21 mmol) of $LiOH·H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 15 mL of 9:1 tetrahydrofuran–water. To this solution was added 177 mg (1.28 mmol) of K_2CO_3 and 325 mg (1.97 mmol) of FmocOSu. This solution was added to ~50 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 4 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.17** as a colorless solid: yield 68 mg (25%); silica gel TLC R_f 0.76 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 4.19 (m,

2H), 4.48 (m, 1H), 4.68 (m, 1H), 5.36 (s, 1H), 6.90 (br s, 1H), 7.17 (m, 4H), 7.59 (t, 1H, J = 8.4 Hz), 7.73 (t, 1H, J = 7.5 Hz), and 8.57 (s, 1H).

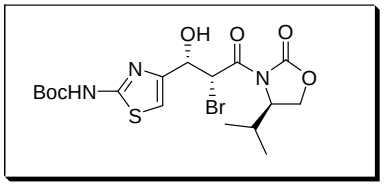


2-Aminothiazole-4-carboxylic Acid Ethyl Ester (3.82).[150] To a solution containing 17.9 g (225 mmol) of thiourea in 200 mL of ethanol was added 28.3 mL (44.0 g, 225 mmol) of 3-bromo-2-oxopropionic acid ethyl ester. The reaction mixture was stirred at reflux for 3 h at which time solid $NaHCO_3$ was added carefully until no bubbling was observed. The reaction mixture was poured into 150 mL of water and extracted with three 250-mL portions of ethyl acetate. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to afford a crude residue. The residue was purified by flash chromatography on a silica gel column (43 x 7 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.82** as a colorless solid: yield 13.7 g (35%); silica gel TLC R_f 0.51 (10% methanol in 1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.2 Hz), 4.32 (q, 2H, J = 7.2 Hz), 3.85 (br, 2H), and 7.39 (s, 1H).



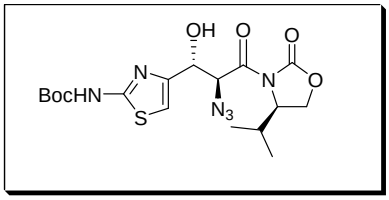
(4-Formylthiazol-2-yl)carbamic Acid *tert*-Butyl Ester (3.83).[150] To a solution containing 13.70 g (79.0 mmol) of **3.82** in 100 mL of dichloromethane at –78 °C was added 19.10 g (86.9 mmol) of di-*tert*-butyl dicarbonate. The reaction was stirred for 2 h at room temperature. Quenched with 50 mL of brine, and extracted with three 100-

mL portions of dichloromethane. The combined organic phase was dried over anh $MgSO_4$, filtered, and concentrated at diminished pressure. The residue was taken up in 60 mL of dichloromethane, it was cooled at −78 °C and 80.0 mL (7.09 g, 80.0 mmol) of diisobutylaluminium hydride (1.0 M in toluene) were added over a period of 30 min. The reaction mixture was stirred at −78 °C for 4 h at which time the reaction was quenched by the addition of 15 mL of methanol followed by 150 mL of 1:1 sat aq sodium potassium tartrate−pH 7 buffer. The reaction mixture was stirred vigorously at room temperature for 16 h, extracted with three 100-mL portions of dichloromethane, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (43 x 6 cm). Elution with 1:1 ethyl acetate−hexanes as eluant gave **3.83** as a colorless solid: yield 6.20 g (34%); silica gel TLC R_f 0.63 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 7.80 (s, 1H), and 9.89 (s, 1H).



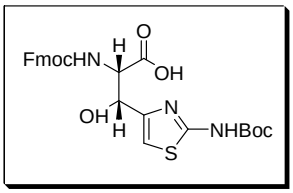
{4-[2-*R*-Bromo-1-*R*-hydroxy-3-(4-*R*-isopropyl-2-oxooxazolidin-3-yl)-3-oxopropyl]thiazol-2-yl}carbamic Acid *tert*-Butyl Ester (3.84).[150] To a solution containing 1.45 g (5.80 mmol) of **3.28** in 40 mL of diethyl ether at −78 °C was added 1.55 mL (1.75 g, 6.40 mmol) of freshly prepared **3.27**, followed immediately by the addition of 0.90 mL (0.64 g, 6.40 mmol) of triethylamine. The reaction mixture was stirred for 0.5 h at −78 °C and then allowed to warm to room temperature and stirred for an additional 2 h. The resulting dark maroon solution was cooled to −78 °C, and a

solution containing 0.68 g (11.0 mmol) of **3.83** in 20 mL of dichloromethane was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was then diluted with 60 mL of 2:1 diethyl ether–dichloromethane and washed with two 45-mL portions of sat aq $NaHSO_4$ and 25 mL of brine. The organic phase was concentrated under diminished pressure to give a brown oily residue which was dissolved in 20 mL of methanol. To this solution was added 6 mL of 30% aq H_2O_2 and the reaction was stirred for 1 h. The milky solution was concentrated under diminished pressure, and the resulting residue was dissolved in 10 mL of water and extracted with three 25-mL portions of 2:1 diethyl ether–dichloromethane. The combined organic phase was washed with two 10-mL portions of sat aq $NaHCO_3$ and 5 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (41 x 4 cm). Step gradient elution with 1:4 ethyl acetate–hexanes → 100% ethyl acetate as eluant gave **3.84** as colorless foam: yield 145 mg (5%); silica gel TLC R_f 0.23 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 6H, J = 6.9 Hz), 1.50 (s, 9H), 2.29 (m, 1H), 4.19 (m, 2H), 4.35 (m, 1H), 4.66 (br s, 1H), 5.21 (m, 1H), 6.01 (m, 1H), and 6.90 (s, 1H).



{4-[2-*S*-Azido-1-*R*-hydroxy-3-(4-*R*-isopropyl-2-oxooxazolidin-3-yl)-3-oxopropyl]thiazol-2-yl}carbamic Acid *tert*-Butyl Ester (3.85).[150] To a solution containing 145 mg (0.30 mmol) of **3.84** in 10 mL of *N*,*N*-dimethylformamide was

added 100 mg (1.52 mmol) of sodium azide. The reaction mixture was warmed to 45 °C and stirred for 1.25 h at which time it was poured into ~8 g of ice and extracted with three 25-mL portions of ethyl acetate. The combined organic phase was washed with two 25-mL portions of water and 10 mL of brine, dried over anh $MgSO_4$, filtered and concentrated under diminished pressure to give a brown oil. The residue was purified via flash chromatography on a silica gel column (42 x 4 cm). Step gradient elution with 1:4 → 1:1 ethyl acetate–hexanes as eluant gave **3.85** as a colorless oil: yield 71 mg (53%); silica gel TLC R_f 0.51 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81 (dd, 6H, *J* = 16.8 and 7.2 Hz), 1.50 (s, 9H), 2.33 (m, 1H), 4.15 (dd, 6H, *J* = 9.0 and 3.0 Hz), 4.27 (t, 1H, *J* = 9.0 Hz), 4.44 (m, 1H), 4.58 (br s, 1H), 4.95 (m, 1H), 5.55 (d, 1H, *J* = 9.0 Hz), and 6.92 (s, 1H).



3-(2-*tert*-Butoxycarbonylaminothiazol-4-yl)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxypropionic Acid (3.18).[150] To a solution containing 71 mg (0.15 mmol) of **3.85** in 20 mL of 4:1 tetrahydrofuran–water was added 31 mg (0.74 mmol) of $LiOH{\cdot}H_2O$. The reaction was stirred for 30 min at room temperature and quenched with 1 N HCl until pH 2.5 was reached. The reaction mixture was extracted with three 25-mL portions of ethyl acetate and two 25-mL portions of chloroform. The combined organic phase was washed with two 25-mL portions of brine, dried over anh $MgSO_4$, filtered, and concentrated under diminished pressure to give a colorless solid. The residue was dissolved in 20 mL of 9:1

tetrahydrofuran–water. To this solution was added 41 mg (0.30 mmol) of K_2CO_3 and 75 mg (0.22 mmol) of FmocOSu. This solution was added to ~100 mg of 10% Pd/C under an Ar atmosphere. The reaction vessel was purged with H_2 several times and stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and washed thoroughly with two 100-mL portions of 90:8:2 chloroform–methanol–acetic acid followed by 50 mL of toluene. Excess solvent was removed under diminished pressure and the resulting residue was coevaporated with several portions of toluene to give a yellow oil. The residue was purified by flash chromatography on a silica gel column (43 x 2.5 cm). Elution with 90:8:2 chloroform–methanol–acetic acid as eluant gave **3.18** as a colorless solid: yield 21 mg (25%); silica gel TLC R_f 0.68 (88:10:2 dichloromethane–methanol–acetic acid); ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 4.19 (m, 1H), 4.36 (m, 1H), 4.60 (m, 1H,) 4.86 (m, 1H), 5.42 (br, 1H), 5.73 (m, 1H), 6.66 (s, 1H), 7.28 (m, 4H), 7.54 (d, 1H, J = 7.2 Hz), and 7.74 (d, 1H, J = 7.5 Hz).

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