THE CHEMICAL AND BIOLOGICAL APPLICATIONS OF THAM

INVESTIGATIONS INTO THE CHEMOSELECTIVE MODIFICATION OF THAM DIRECTED TOWARDS BIOLOGICAL APPLICATIONS

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy

McMaster University DOCTOR OF PHILOSOPHY (2012) Hamilton, Ontario (Chemistry)

TITLE: Investigations into the chemoselective modification of THAM directed towards biological applications. AUTHOR: Janice Calzavara, B.Sc. (The University of Western Ontario) SUPERVISOR: Professor James McNulty PAGES: xix, 256

Abstract:

Tris(hydroxymethyl)aminomethane (THAM) was a readily-available and financially economical amino-triol that was viewed as having a large untapped potential as a starting material. The full chemoselective functionalization and differentiation of the amino group and the three primary alcohol residues present in THAM was extensively investigated. The development of this methodology allowed for the rapid assembly of a differentiated core that lead to existing and new potential drug scaffolds, both based on symmetrical and non-symmetrical molecules.

The discovery of a novel oxidative fragmentation and rearrangement process was made leading to the synthesis of differentiated oxazolidinone rings. This process allowed for the creation of novel chemical library situated around THAM-based oxazolidinones, as well as THAM-based 1,3-dioxanes.

THAM was also used as a starting material for sphingosine analogs, including sphingosine 1-phosphate (S1P) and anticancer S1K inhibitors. Selective functionalization of the amine and one alcohol within an oxazolidinone ring allowed access to a new family of Linezolid-type oxazolidinones as well. Additionally, various triazole-based compounds were prepared, including oxazolidinone triazoles, which allowed access to a new family of potential antifungal agents based on the lead compound Fluconazole.

A total synthesis of the immunosuppressant molecule FTY720 was also reported, employing double Wittig-olefination protocol, from THAM. This synthesis avoided certain pitfalls that were present in previously documented literature methods. Along the pathway to FTY720, many intermediates and analogs were synthesized and tested for biological activity alongside the novel oxazolidinone compounds, resulting in interesting lead compounds for various biological applications. A UV-active FTY720 scaffold was also synthesized for potential future *in vivo* tracking of the immunosuppressant and its metabolites.

Acknowledgements:

First, my countless thanks to my mentor Dr. James McNulty for the constant support and guidance he brought to my work days. Only because of him can I appreciate and respect organic chemistry as highly as I do. His vast knowledge of chemistry and science in general made each day a true learning experience that few people will ever be blessed with, and for this

I will be forever grateful.

To my committee members, Drs. Fred Capretta and Paul Harrison, thank you for helping me complete this project with your exceptional insight and wonderful ideas. I would also like to acknowledge Dr. Steven Kornic for his technical assistance on the instruments, as well as for the moral support during my Ph.D. Your services did not, and could not, go unnoticed.

Thank you to my parents: My father Larry for his sense of humor and for shaping me into who I am today, and my mother Jacqueline for her encouragement throughout my Ph.D. and for instilling in me the importance of an education. Throughout my life, you offered me your unwavering belief that I could do anything, and as a result I am doing everything I've ever dreamed of. To my sister Kathryn, we are sisters by chance, but friends by choice.

My husband and best friend Adam Guzzo is deserving of a fantastic level of thanks not only for marrying me, but also for always backing me and supporting me no matter what. Your all-around awesomeness helped me get through my undergraduate

degree and my doctorate, and with any luck, you will continue to inspire me throughout the rest of my life. All my love and thanks.

My lab members, both past and present, deserve special mention. It was with their help that the lab could function at all and I will miss their less-than-silent tolerance for Swern reaction after Swern reaction. Specifically, my thanks to David McLeod for your help in lab when reactions didn't go quite as planned, or when anything didn't go as planned, even though it was often your fault. I was extremely fortunate to work within such a talented lab filled with amazingly brilliant people, so I would like to extend my thanks to those individuals.

To my friends throughout my life, again I must offer my thanks. Two friends that require special mention are Lisa Walter and Nicole Rice: Lisa, for being by my side through every step of grad school, and Nicole, for the most amazing wedding cake toppers that have ever existed. To my many friends who have come and gone over the years: there aren't enough pages allotted to mention you all, but thank you nevertheless.

To the Department of Chemistry at McMaster, without your financial support I would not have been able to spend the past five years here completing this thesis, so thank you. Thanks also to the office staff, who assisted me with innumerable questions and concerns over these years.

Finally, thanks must be bestowed upon Dr. Farnsworth, Michael Burkett, Robert Heinlein, and Dexter Holland for raising my spirits for as long as I care to recall.

Thank you to you all, both listed and unmentioned. To quote the late Douglas Adams, "So long, and thanks for all the fish".

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Abbreviations:

^{3}H = tritium

Abs. = absorbance

Ac = acetate

AcO = acetoxy

Ac_2O = acetic anhydride

AIBN = azobisisobutyronitrile

APD = 3-amino-1,2-propanediol

Boc = *tert*-butoxycarbonyl

Boc_2O = di-*tert*-butyl dicarbonate

BPO = benzoyl peroxide

Bn = benzyl

Bz = benzoyl

Bu = butyl

^{n}Bu = 1-butyl

^{t}Bu = *tert*-butyl

Calc'd = calculated

CD_3CN = deuterated acetonitrile

CDase = ceramidase

$CDCl_3$ = deuterated chloroform

CI = chemical ionization

Conc. = concentration

CS = ceramide synthase

D_2O = deuterium oxide

dba = dibenzylideneacetone

DCC = *N*,*N*'-dicyclohexylcarbodiimide

DCM = dichloromethane

DHP = 2,3-dihydropyran

DIBAL = DIBAL-H = diisobutylaluminium hydride

Dioxane = 1,4-dioxane

DMAP = 4-dimethylaminopyridine

DMF = dimethylformamide

DMP = Dess–Martin periodinane

DMSO = dimethyl sulfoxide

DMSO-d_6 = deuterated dimethyl sulfoxide

ε = molar extinction coefficient

EI: electron ionization

eq = equivalent(s)

ES+/ESI+: Electrospray Mass Spectrometry (positive mode)

Et = ethyl

Et_2O = diethyl ether

EtOAc = ethyl acetate

eV = electron volt

FDA = Food & Drug Administration, USA.

Fingolimod^{TM} = FTY720

FTY720 = Fingolimod^{TM}

FTY720–P = phosphate derivative of FTY720

g = gram

h = hours

HPLC = high pressure liquid chromatography

Hz = hertz

^{i}Pr = *iso*-propyl

L = liter

LiHMDS = lithium bis(trimethylsilyl)amide

MAO-A = monoamine oxidase A

MDR = multiple drug resistant

Me = methyl

MeCN = acetonitrile

MeOD = MeOD-d_4 = deuterated methanol

Mic = microwave

mins = minutes

MOM = methoxymethyl

MP = melting point

MRSA = methicillin-resistant *Staphylococcus aureus*

Ms = mesylate

MS = multiple sclerosis

MW = microwave

NaHMDS = sodium bis(trimethylsilyl)amide

NBS = *N*-bromosuccinimide

NK = natural killer

nm = nanometer

OTf = triflate = trifluoromethanesulfonate

Pd/C = palladium on carbon

Ph = phenyl

PLP = pyridoxal phosphate

p.p.m. = parts per million

Pr = propyl

PTSA = *p*-toluenesulfonic acid monohydrate

r.b.f. = rbf = round bottom flask

R_f = retention factor

rt = room temperature

S.W. Ex = Excitation Slit Width

S.W. Em = Emission Slit Width

S1P = sphingosine 1-phosphate

S1P P = S1P phosphatase

SAR = structure-activity relationship

Sat'd = saturated

SERM = selective estrogen receptor modulator

Spec. Act. = Specific Activity

SphK = sphingosine kinase

SPT = serine palmitoyltransferase

TDACH = trans-diaminocyclohexane

TEA = triethylamine

Tf = triflyl = trifluoromethanesulfonyl

THAM = TRIS = tris(hydroxymethyl)aminomethane

THF = tetrahydrofuran

THP = tetrahydropyranyl ether

TLC = thin layer chromatography (silica-based)

TMEDA = *N*,*N*-tetramethyl ethylenediamine

Tol = toluene

Tritium = ^{3}H

Ts = Tos = tosylate = 4-methylbenzenesulfonate

UV = ultraviolet light

w/*w* = weight by weight

Z-Pro-OH = Cbz-proline = Z-L-proline

Introduction:

Background and Overview of THAM:

Tris(hydroxymethyl)aminomethane (THAM, Figure 1) is a densely functionalized amino triol that is readily available in high purity on a commercial scale and is relatively inexpensive. Also known as TRIS, although THAM is mainly used as a component within aqueous buffers, it has also been used for the following: in the synthesis of medications such as FTY720, Antrimycin D_v, and (+)-lactacystin; as a starting material for antihistamines; a central building block for ligands used for coordination in inorganic polymers; a central core unit for dendrimer construction; a component in inorganic catalysis; as a direct treatment for acidosis in acute lung injury; and as a starting material in the synthesis of 5-hydroxy-1,3-dioxanes.[1–12] THAM's versatility for many different types of synthetic uses stems from its scaffold that is highly functionalized with a primary amine and three primary alcohol functional groups.

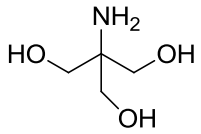


Figure 1 – Structure of THAM

While this dense functionalization is potentially useful, we considered that the actual utility of THAM as a building block would be greatly enhanced if methods for the functionalization and rapid differentiation of these individual functional groups could be

developed. We envisioned that these differentially protected THAM derivatives would allow access to a range of useful intermediates. As an example, amine protection followed by differentiation of the three hydroxyl groups of the triol would open a branch point for the synthesis of linear, non-symmetrical building blocks. The chemoselective differentiation via a protective cyclization of the molecule involving only one of the three hydroxyl residues and the amino group could prove highly strategic, allowing the synthesis of 4,4'-substituted oxazolidinone targets (Figure 2). A substituted oxazolidinone ring would hold the benefit of protecting an alcohol and the amine concurrently, thus removing their reactivity for chemistry to be performed on the remaining two primary alcohol branches; these alcohols, either one or both, would require subsequent chemoselective differentiation. Other possibilities include differentiation of the functional groups, followed by enantiomeric resolution, to open access to chiral asymmetric building blocks from THAM.

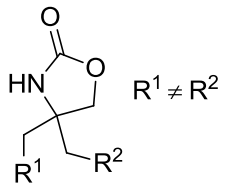


Figure 2 – 4,4'-substituted 2-oxazolidinone ring

Overall, the differentiation of the reactive primary amine and hydroxyl groups would be the key to unlocking the potential of THAM as a core building block for the synthesis of a wide range of synthons. We felt that this area was significantly

underdeveloped, and investigation into methods to achieve differential functionalization would allow rapid access to useful, densely functionalized synthons. The core objective and challenge in this Thesis is the development of synthetic methodology that will allow the rapid, selective differentiation of all three alcohols of THAM, while controlling or protecting the reactivity of the amine, in order to open access to synthetically useful building blocks. Once achieved, the synthesis of biologically active compounds, probes, and derivatives could be prepared with full control via the selective manipulation of the side-chains.

Oxazolidinone Ring Synthesis:

The synthesis of 2-oxazolidinone rings has been researched extensively, with the majority of syntheses based around reactions with 2-aminoethanol derivatives (Figure 3). Often these amino-ethanol precursors are derived from a reduced amino acid as these have an inherent, pre-set stereocenter and are readily commercially available. An important difference that must be taken into account is that THAM has four nucleophilic atoms (one amine and three alcohols); it is important to consider any syntheses used must be controllable and only react with the amine and one alcohol, not with all three alcohols. Finally, placement of the THAM scaffold was important, so as a result some syntheses were not appropriate as they would form the oxazolidinone ring directly from the reagents used, and not from THAM itself. This would place the THAM scaffold directly off the N-atom, rather than incorporate it within the oxazolidinone ring.[13]

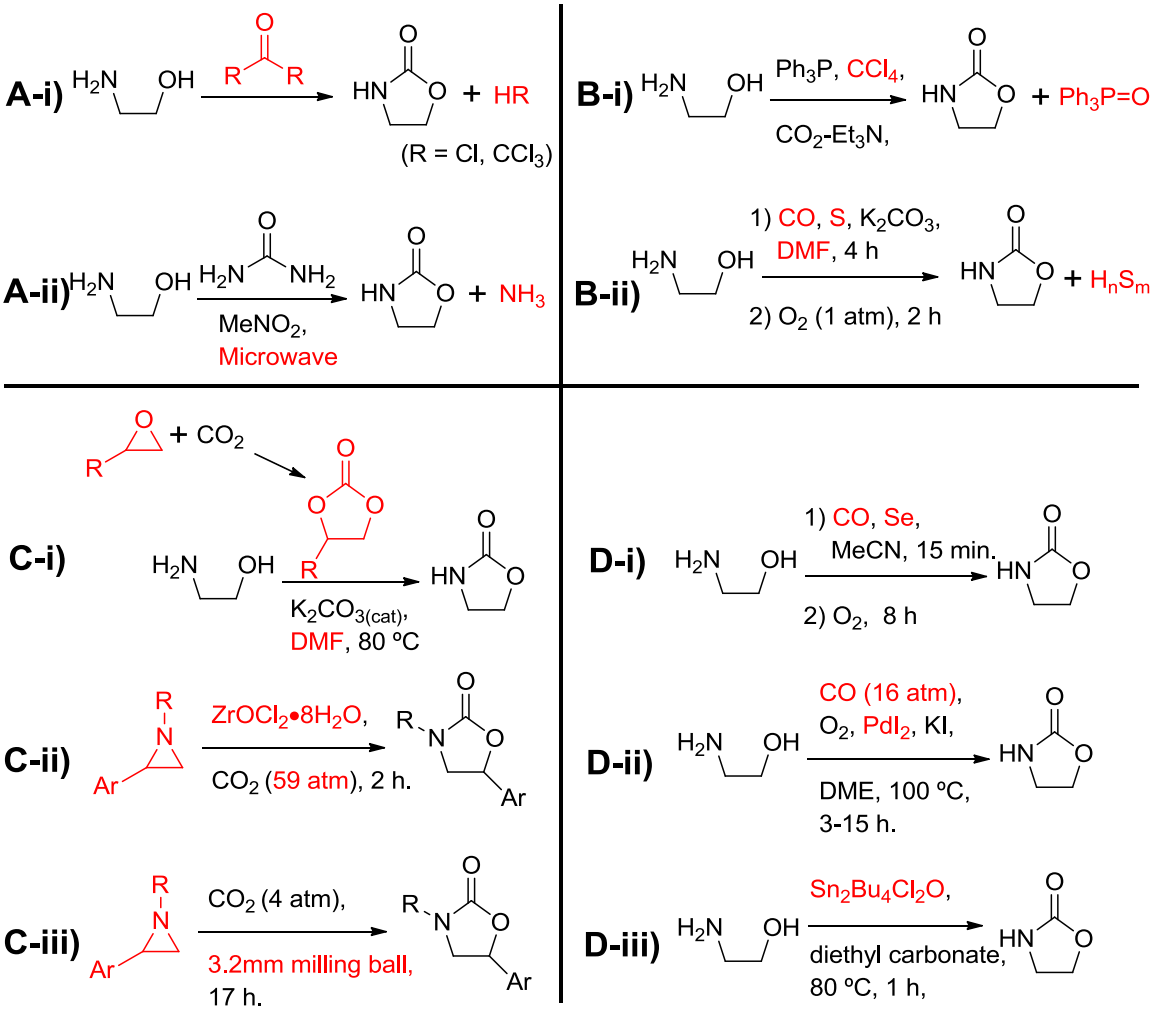


Figure 3 – Synthesis of Oxazolidinone Rings

Most notable amongst these syntheses is the reaction of the *N*- and *O*- terminus of the amino-alcohol with a carbonyl bearing two labile groups, such as chlorides, chloroform anions, or alkoxides (Figure 3: **A-i**).[14–17] This method has the drawback of not only producing a large amount of non-commercially useful by-product (non-atom economic), but in select cases using toxic and dangerous reagents, such as phosgene gas.

Additionally, careful stoichiometric control will be needed to prevent the remaining alcohols from reacting with the electrophilic reagent. The formation of an oxazolidinone ring is possible directly through a reaction with ethyl chloroformate, but similar drawbacks occur as HCl is produced in an equimolar amount, which must be scrubbed from the reaction using a base.[18] As well, other nucleophilic reactive groups cannot be present when using the methods previously mentioned. Within the same class of reactions to form an oxazolidinone ring was microwaving an amino-alcohol with urea and a catalytic amount of nitromethane (**A-ii**).[19] One of the main drawbacks of this method was the necessity of a chemical microwave. As well, the precursor amine/alcohol must be capable of tolerating microwave irradiation, as the nitromethane acts within the reaction to create localized hot-spots, which could damage more sensitive molecules.

An alternate route to oxazolidinone rings can be found using inorganic reagents, such as triphenylphosphine or sulfur (Figure 3: **B-i**/**B-ii**).[20,21] While useful, issues with selective functionalization of just one alcohol may be a concern with THAM. Additionally, for the **B-i** example, there are some major concerns: carbon tetrachloride is needed in equimolar amounts, which can be difficult to obtain due to its environmental issues; and triphenylphosphine oxide is produced in equimolar amounts, which often requires silica column chromatography to remove from the product mixture.[20]

Recently, it was found that using a cyclic carbonate in the presence of K_2CO_3 could transfer the carbonyl to an amino-alcohol (Figure 3: **C-i**).[22] However, this method not only required DMF as the solvent, but also an equimolar amount of carbonate, which is synthesized through an epoxide. Bypassing the need for an amino alcohol, an *N*-Boc

aziridine rearrangement directly to the oxazolidinone has been published in the literature (Figure 3: **C-ii**/**C-iii**).[23,24] However, the reagents required for this rearrangement can be difficult to obtain, such as a 3.2mm steel milling ball (**C-iii**), the zirconium catalyst (**C-ii**), or even the aziridine itself.[24-26] Additionally, the synthesis of an aziridine from the THAM scaffold may not be possible, due to the remaining nucleophilic alcohols.

A carbon monoxide/oxygen mixture (neat, or in the presence of a metal catalyst) can also produce oxazolidinones from 2-aminoethanols (Figure 3: **D-i**/**D-ii**).[27–31] However, this method often requires high pressure and produces toxic by-products, or leaves residual metal catalyst, which may be difficult to remove to a sufficient degree to obtain FDA approval if used in pharmaceutical formulations. A more recent addition to the literature uses diethylcarbonate as the carbonyl source, but this requires high heat (120-125 ºC), which could damage other functional groups that may be present in the molecule.[32]

Ideally, a novel method for oxazolidinone formation could be developed that makes it possible to form the oxazolidinone ring from the THAM derivative during any step within the synthetic route. Because THAM had a highly-functionalized core, the method chosen had to be compatible with the remaining alcohols within the molecule. Therefore, an oxazolidinone ring that was formed from non-reactive oxygen atoms and a nitrogen atom was preferred, rather than directly from an amino-alcohol. Forming the oxazolidinone ring from an intermediate would also hopefully eliminate side products caused when the remaining alcohols react in an undesirable manner with the reagent. As well, an intermediate would be easier to carry through the synthetic route than an amino-

alcohol, further prompting the need for a new oxazolidinone formation method. If possible, the method chosen would avoid the use of heavy-metals in the synthesis of the oxazolidinone ring, thus making the resulting product amenable to use *in vivo*.

THAM-based Potential Targets and Analogs:

Initially envisioned for this project were applications for differentiated derivatives that could be made available from THAM which might find use in diverse areas of medicinal chemistry, either directly or as structural analogs of known biologically-active compounds. Examples (Figure 4) of these active small molecules include: anti-tumor and anti-angiogenesis agents related to sphingosine/S1P[33]; immunosuppressants related to FTY720[34]; antibacterial compounds, such as Linezolid[35]; antifungal agents resembling Fluconazole and Voriconazole[36,37]; antiparasitic lead drugs; and aromatase inhibitors. All of these biologically active molecules mentioned, with the exception of Fluconazole and FTY720, require the complete differentiation of all three alcohols of THAM. With the case of Linezolid, the requisite of total differentiation of the alcohol groups goes one step further in also requiring the inclusion of the amine and an alcohol within an oxazolidinone ring. Because of the vast number of applications present for these drugs, eventual testing against a plethora of biological assays for all THAM derivatives would allow for a greater utilization of synthesized compounds.

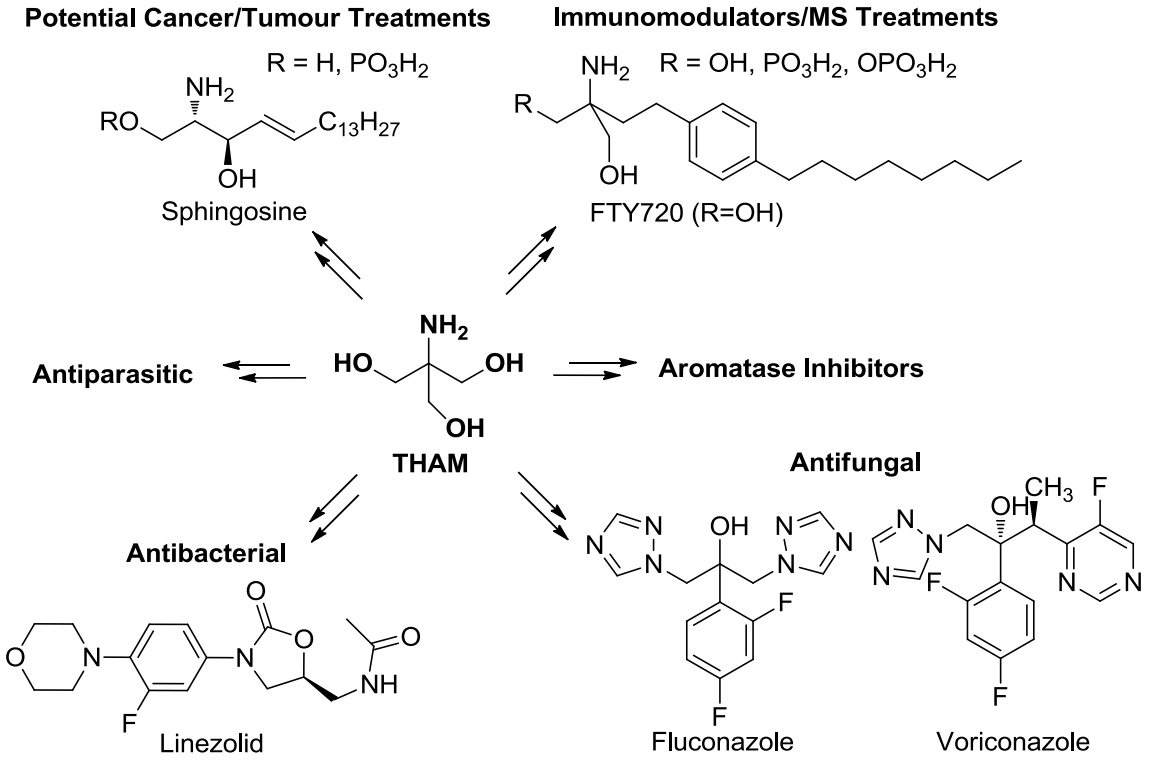


Figure 4 – Targets for THAM derivatives

Sphingosine/S1P:

One of the possible applications for this project is the synthesis of sphingosine analogs using THAM as the backbone starting material (Figure 5, R = H, R' = H). Sphingosine is a type of sphingolipid found in eukaryotic organisms as well as a select few prokaryotic organisms and viruses.[38,39] Sphingolipids are one of many types of lipids found in cells. Sphingolipids were discovered by Johann Thudichum and first reported in 1884, but their purpose within the cell was not determined for years, which is what led Thudichum to name them after the elusive sphinx.[38,39] It was not until a century later, in

1986 that it was discovered that the sphingolipids are bioactive compounds, not just backbones for membranes. Different sphingolipids, such as sphingosine, are made by altering the amine and head alcohol.

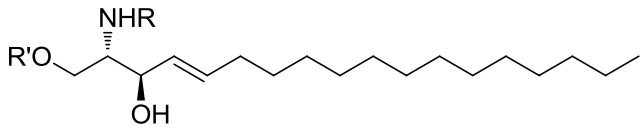


Figure 5 – Sphingolipid backbone

Sphingosine can be biotransformed into sphingosine 1-phosphate (S1P), an -*O*-phosphorylated version of sphingosine, through sphingosine kinase (SphK) using ATP as the phosphate and energy source for the reaction (Figure 6).[33] This conversion is of great biological importance as S1P can act as a cell messenger to induce angiogenesis, which is the growth of new blood vessels.[33] The cell-proliferation resulting from S1P is not limited to blood vessels, as it can also occur in other types of bodily fluids and tissues.[33,40] Within the S1P receptors there are 5 subtypes, $S1P_{1\text{-}5}$ and each of these subtypes can lead to different biological outcomes further downstream.[41,42] Additionally, there are two isoforms of SphK: SphK1and SphK2.[41,43] SphK1 is the isoform of SphK that is responsible for cell growth and proliferation, whereas SphK2 is thought to suppress cellular growth to lead to apoptosis.[41] This process of phosphorylation can be reversed through S1P phosphatase (S1P P) to return to the native sphingosine.[33,44]

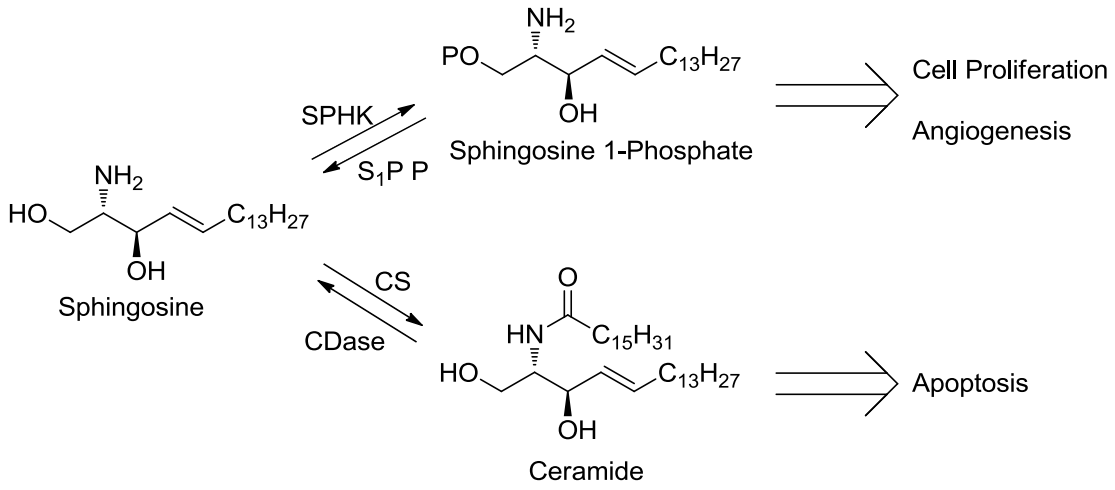


Figure 6 – Reaction Pathways of Sphingosine

Sphingosine is biosynthesized from ceramide, which is an *N*-acylated version of sphingosine. Ceramide acts in an opposite fashion to S1P, and is known to induce apoptosis in cells (Figure 6, see also scheme 1).[33] Ceramide in humans is biosynthesized from Palmitoyl-Coenzyme A (Palmitoyl-CoA) and the amino acid L-serine through serine palmitoyltransferase in the endoplastic reticulum.[33,45–48] In one process, a series of biotransformations via enzymes (3-ketoshinganine reductase, dihydroceramide synthase, and dihydroceramide desaturase) occur to biosynthesize sphingomeylin (Figure 7), which makes up about 30% of the myelin sheath in humans.[33,44,45,47]

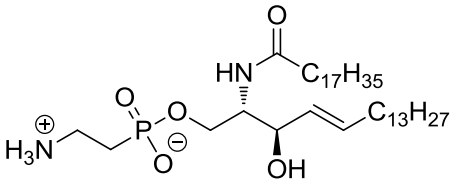


Figure 7 – Sphingomyelin[33]

Ceramide can also be biotransformed into sphingosine by loss of its acyl group through ceramidase (CDase), with the reverse occurring through the ceramide synthase (CS, Figure 6).[33,48] Overall, sphingosine is the intermediate between two competing reactions, the formation of S1P and ceramide; if these pathways are tailored preferentially towards one route, e.g. conversion to ceramide or inhibition of SphK, the treatment can be used in tumour therapy with the hopes of inducing apoptosis exclusively to the tumour cells or to limit cell growth and proliferation respectively. This potential control over the metabolic pathway of sphingosine creates a significant amount of interest and active research in this area as the applications for cancer treatment in the future are widespread and essential.[41,42]

Immunomodulators:

Myriocin:

Myriocin, also commonly known as (+)-myriocin, ISP-,1 and thermozymocidin, is an immunosuppressant isolated from the fungus *Isaria sinclairii* (Figure 8).[49,50] Myriocin was first reported as an antifungal agent by Vézina, *et al.* in 1972. As a result, this biological activity and structural complexity of meriocin makes it an interesting target to be researched.[49,51] Myriocin and its stereoisomers have been previously synthesized in no fewer than 15 different ways, with most syntheses revolving around the construction of the polar head followed by the addition of the non-polar tail. [52–66] In one specific synthesis by Jones *et al.*, the polar head is synthesized through an oxazolidinone, which is

easily accessible from THAM.[63] As such, the structure of myriocin possesses the basic THAM backbone (Figure 8, outlined in red), suggesting that some THAM derivatives synthesized within this thesis may have activity as immunosuppressive or antifungal agents.

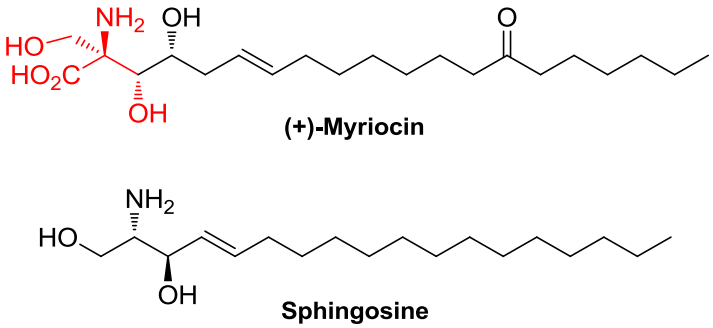
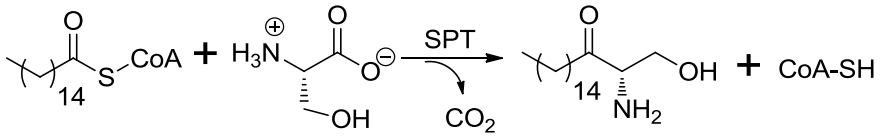


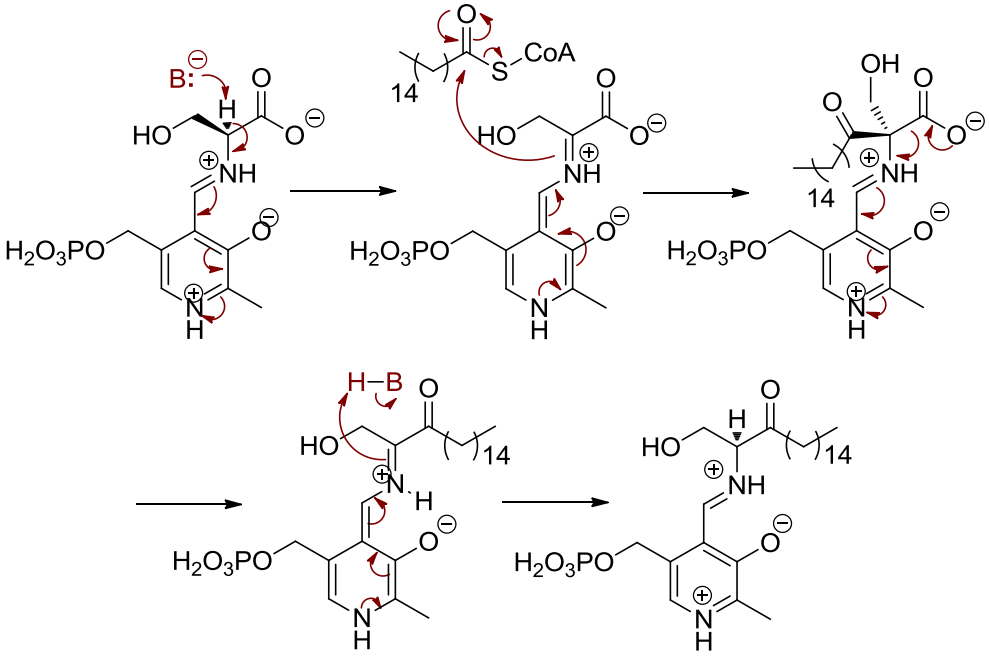
Figure 8 – Myriocin & Sphingosine[49]

Ceramide (Figure 6) is biosynthesized in the endoplasmic reticulum, where palmitoyl CoA and the amino acid L-serine are combined by serine palmitoyltransferase (SPT), using pyridoxal phosphate (PLP) as a co-factor, to give 3KDS.[33,47] Myriocin shares structural similarities to sphingosine (Figure 8), with respect to its polar head and non-polar tail.[47,49,50] Therefore, it is not surprising that myriocin can inhibit the SPT enzyme involved in the *de novo* synthesis of ceramide, preventing the formation of 3-keto-dihydrosphingosine (3KDS), also known as 3-oxosphingosine (Scheme 1). [33,49]



Scheme 1 – Biosynthesis of 3-keto-dihydrosphingosine

The mechanism behind this inhibition is straight forward: generally when L-serine combines with PLP, the proton adjacent to the amine is lost prior to decarboxylation (Scheme 2).[47] However, myriocin can react in lieu of L-serine on PLP to give the quaternary intermediate, which cannot undergo decarboxylation due to the lack of an acidic proton α to the carbonyl (Figure 9).[50]



Scheme 2 – Mechanism of SPT on L-serine[47]

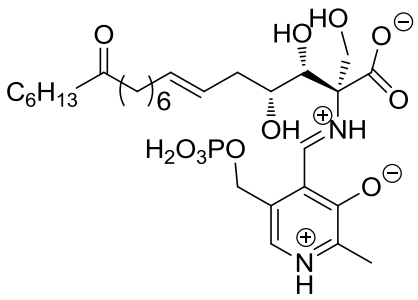


Figure 9 – Proposed binding of Myriocin with PLP[50]

FTY720:

FTY720, also known as Fingolimod and commercially marketed under the trademarked name Gilenya, is an alkyl amino diol that has shown activity as an immunosuppressant drug for the survival of transplanted tissues, for example kidney, liver, and heart tissues (Figure 10).[34,67,68] FTY720 was first synthesized in 1995 as an analog of myriocin, but to possess a simplified structure that could be more easily prepared.[69,70] A unique aspect of FTY720 was that it has the benefit of not hindering the body's natural production of lymphocytes.[34,67,68] FTY720 has also undergone clinical trials as a Multiple Sclerosis (MS) treatment, as FTY720 can help prevent the body's immune system response of attack against the myelin sheath around neurons.[71,72] More recently, it has been reported that FTY720 may be useful in the treatment of NK-leukemia. Promising results showed apoptosis with both human and rat NK-leukemic cells after treatment with FTY720 at μM concentrations.[73] While FTY720 is useful as an immunosuppressant, its main application within this project is to synthesize lead

compounds to act as S1K inhibitors or S1P mimics, potentially altering S1P production and/or S1P receptor modification.

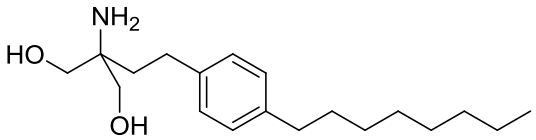
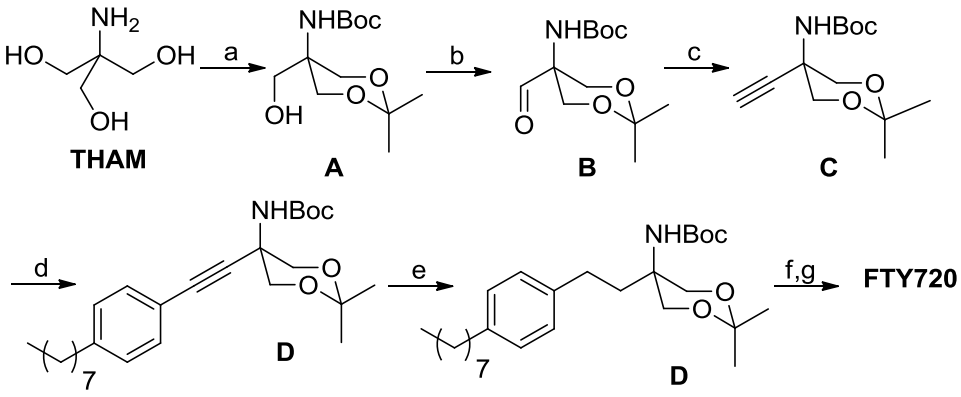


Figure 10 – FTY720[34]

There have been multiple different syntheses of FTY720 already reported in the literature[3,4,69,74–80], as well as syntheses of FTY720 analogs[81–88]. Most of these syntheses have revolved around the addition of the polar head group to the non-polar tail in a divergent-style synthesis. While some syntheses revolve around a malonate-derivative attacking an iodo-functionalized tail[76,89], there have been syntheses from THAM[3,4]. The most cited synthesis starting from THAM was published by Kim, *et al*. in 2006[3] (Scheme 3). Superficially, the synthesis reported by Kim, *et al*. may seem like an ideal synthesis, but there are a few problems that are inherent to the route taken; for example, a homogeneous metal catalyst is required for a cross-coupling reaction in one of the final steps, which could be an issue later with respect to purification for medications intended for human consumption. Additionally, while the overall yield may seem high (64%), this relies on an 85% yield for both the dual protection (87%) and subsequent Swern oxidation (98%) on the neopentyl alcohol of the THAM derivative **A**. This step was cited by a previous group[9], but it was not repeated within the publication and the yield is fairly high for a Swern oxidation on a such a hindered alcohol. Both protection and oxidation steps

rely on recrystallization as the sole purification method, further casting doubt on the reliability of an 87% and 98% yield, as recrystallization can often result in a lower yield. Also, not discussed within the paper is the procurement of the 4-octyl-1-iodobenzene reagent required for the synthesis, which would not be trivial to synthesize. The method employed by Kim *et al*. could potentially be improved by eliminating the Sonogashira cross-coupling reaction between the alkyne-THAM derivative and the iodophenyl species and substituting in its place an aqueous Wittig reaction between an aldehyde-THAM derivative and trialkyl phosphonium salt. This use of the aqueous Wittig would be an improvement in comparison for a few reasons, with the first being that the iodophenyl species required for the 2006 synthesis was not commercially available. Another major drawback to this reported method is the Sonogashira reaction requires degassed DMF-triethylamine as the solvent (instead of water), as well as a solution of KF for quenching, both of which may be difficult to handle if the synthesis is scaled up for commercial purposes.



a) Boc_2O, $(MeO)_2CMe_2$, $TsCl_{(cat)}$, DMF, rt, 3 h; b) 1) $(COCl)_2$, DMSO, DCM, -78 °C, 40 mins. 2) **A**, -78 °C to -20°C, 55 mins. 3) Et_3N, -20 °C to rt., 2 h. 85% over both steps; (c) $MeCOCH_2P(O)(OMe)_2$, TsN_3, K_2CO_3, MeCN–MeOH (1:1), rt, 5 h, 84%; (d) 4-octyl-1-iodobenzene, $Pd(PPh_3)_4$, CuI, DMF–Et_3N (4:1), rt, 3 h, 94%; (e) H_2, Pd (10% on C), benzene, rt, 5 h, 99%; (f) TFA–CH_2Cl_2–H_2O (2:2:1), rt, 12 h, 96%; (g) anhyd HCl, THF, rt, 3 h, 100%

Scheme 3 – Synthetic route for the synthesis of FTY720 as developed by Kim et al.[3]

From the diol FTY270, modification of one of the alcohols to a phosphate ester can be performed with the intent of synthesizing an S1P derivative and the activity monitored (Figure 11).[67,90] While FTY720 can bind to S1K and therefore inhibits the formation of S1P without being phosphorylated, after phosphorylation the inhibition activity increases dramatically.[84] Phosphorylation of FTY720 can be carried out synthetically through the reaction of protected FTY720 with phosphorylating agents. However, the formation of FTY720-P can also occur *in vivo* naturally via SphK2, the isoform of SphK mentioned previously that is pro-apoptotic.[42,91] Phosphorylated FTY720 binds to S1P receptors, acting as a full agonist with $S1P_1$, and partial agonist with $S1P_{3,4,5}$.[34,42,49,67,92] This biological activity seen on sphingosine receptors and enzymes

stems from the similar backbone that phosphorylated-FTY720 (FTY720-P, e.g. phosphate, phosphonate, vinyl phosphonate) derivatives possess with respect to S1P, with the simple reversal of the alkyl group from the adjacent carbon being the difference between S1P and FTY720-P (Figure 12).[49,67,83,93] Because FTY720 exhibits a high degree of similarity to sphingosine (a phenyl ring in lieu of an alkene), but requires a significantly less complex synthesis, investigation into FTY720/FTY720-P derivatives would be prudent provided that any such derivatives can be achieved in a regioselective manner.

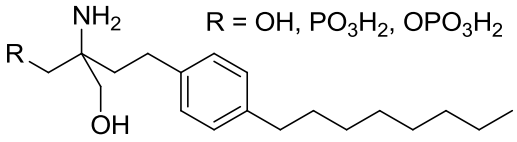
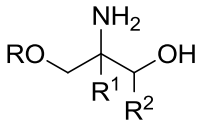


Figure 11 – Phosphorylated FTY720[34,49]

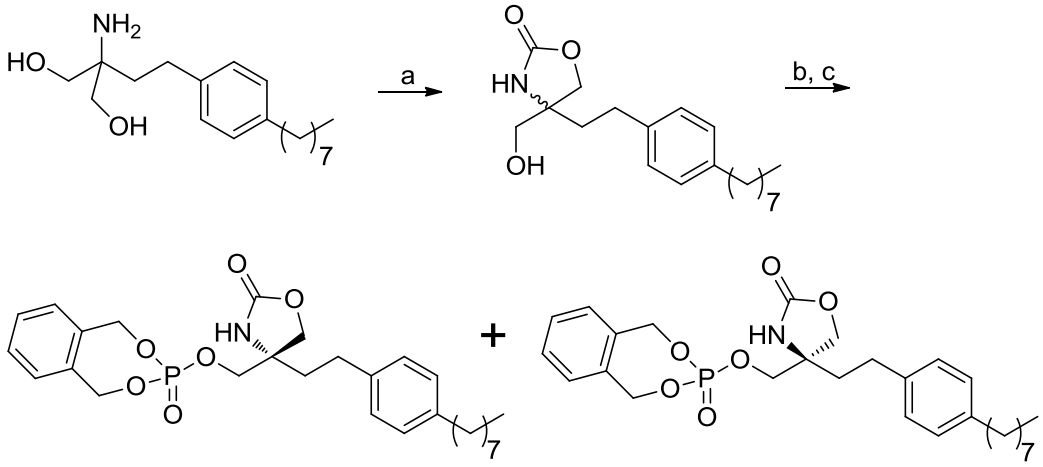


Sphingosine: R^1 = H, R^2 = Alkyl
FTY720-P: R^1 = Alkyl, R^2 = H

Figure 12 – Diagram of Sphingosine vs. FTY720 [49]

Separation of FTY720-P analogs has been reported in the literature using a variety of different methods.[67,75,78,83,84,86,93,94] Generally the separation is performed to resolve the *R* and *S* enantiomers of FTY720-P as the *S* enantiomer is the significantly more

biologically active enantiomer.[67,84,95] One method for separation is conversion of FTY720 to an oxazolidinone ring, followed by separation of the two resulting enantiomers through a chiral HPLC column. This conversion has previously been facilitated through reaction of benzyl chloroformate with FTY720; this synthetic route requires the removal of all protecting groups from the FTY720 precursor to render FTY720, followed by conversion of the newly-formed FTY720 to an oxazolidinone ring (Scheme 4).[67] This pathway could be expedited if there were some way to convert an FTY720 precursor directly to the oxazolidinone ring, as opposed to the current method of removing all protecting groups to render FTY720, followed by conversion of the amine and alcohol of FTY720 to an oxazolidinone ring.



a) Benzyl chloroformate, NaOH, rt, 48 h, 70%; b) 3-(diethylamino)-1,5-dihydro-2,4,3-benzodioxaphosphepintriphenyl phosphite, tetrazole, DCM/THF, rt, 18 h; then H_2O_2, rt, 90 min, 80%; c) Chiralpak® AS Baseline Separation

Scheme 4 – Reported resolution of FTY720 oxazolidinone derivative[67]

Antibacterial Agents:

Linezolid:

Linezolid (Figure 13) is an antimicrobial agent that is a part of the novel class of oxazolidinone-based antibiotics. Since its introduction, Linezolid has been found to be useful for treatment for multiple drug resistant (MDR) strains of tuberculosis.[68,96] Linezolid is used specifically against gram-positive antibiotic resistant bacteria. Currently, the sole FDA-approved oral treatment for methicillin-resistant *Staphylococcus aureus* (MRSA) skin infections is Linezolid, further illustrating the importance this medication has.[97,98] Linezolid is of interest to this project as it holds a similar backbone to THAM, with respect to its functionalized oxazolidinone ring.[99] As mentioned previously, if the amine and an alcohol of THAM are connected through a carbonyl, this will have formed an oxazolidinone ring with two methylene alcohol groups at the 4-position that are accessible for derivatization. The development of new scaffolds based on linezolid is necessary in order to keep one-step ahead of bacteria inevitably expressing resistance towards the Linezolid core. We envisioned that starting from a differentiated alcohol-containing oxazolidinone, addition of a bulky group similar to the phenyl-morpholine group can be added to closer resemble Linezolid. DuPont researchers have shown that the morpholine is not as important as the phenyl group, but that substitution is required on the *N*-terminus of the oxazolidinone ring.[35,99] It has also been shown that the *S*-configuration of Linezolid is the biologically-active isomer, however, dual substitution at the 4 site has not been fully investigated.[92,99] In the case of THAM-based oxazolidinones, this synthesis

can theoretically be directed to either enantiomer through asymmetric derivatization on the two primary alcohols.

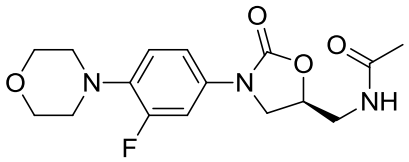


Figure 13 – Linezolid[35,99]

An analog of Linezolid that is of specific interest to this project is one containing a triazole ring in place of an acetate group the amide site (Figure 14).[100] Different substituents have been added to the triazole ring to alter the activity of the molecule, offering further options of differentiation in the compound and lengthening the duration that oxazolidinone-based antibiotics can be used prior to resistance being observed.[101] These findings suggest that it could be advantageous to introduce a triazole ring to specific THAM derivatives with the intent of increasing biological activity in any new THAM-based oxazolidinone analogs.

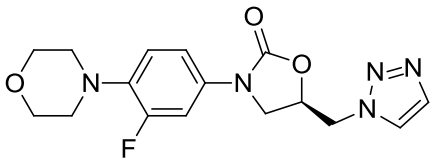


Figure 14 – Linezolid with triazole ring modification[100]

Torezolid, Radezolid, & Ranbezolid:

In an effort to fight MDR bacteria that have already developed resistance to Linezolid, alternate drug leads are being researched that are within the same oxazolidinone class.[102,103] Two examples of these novel medications are Torezolid (prodrug TR-701, active compound TR-700) and Radezolid (Figure 15), both of which share a striking resemblance to Linezolid.[104–107]

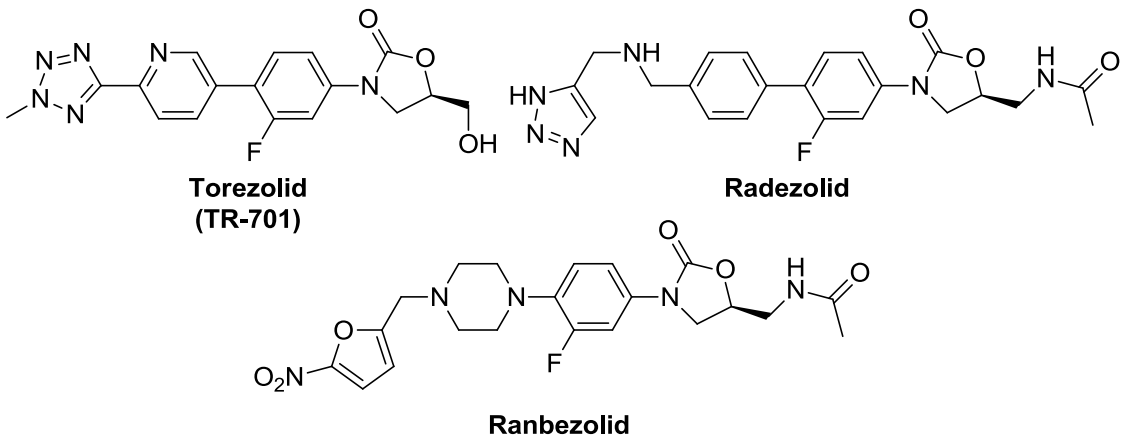


Figure 15 – Examples of oxazolidinone class of antibiotics

Ranbezolid (Figure 15) is an oxazolidinone-based antibacterial treatment that is structurally similar to Linezolid and others within the oxazolidinone antibiotic category. The feature that makes Ranbezolid of exceptional interest is that it can also act as an monoamine oxidase A (MAO-A) inhibitor.[108,109] MAO-A is responsible for the deamination of biogenic amines, oxidizing them to aldehydes.[110] MAO-A inhibitors have been known for many years to be biologically active as anti-depression agents.[111] This finding suggests that future biological testing for THAM-based oxazolidinone derivatives

as MAO-A inhibitors would be prudent, thus potentially opening an avenue into the financially lucrative area of anti-depression medications.

Antifungal Agents:

Fluconazole:

Fluconazole is a di-triazole compound that is currently used as an anti-fungal agent that has the versatility to be administered orally or intravenously (Figure 16).[36,37,112] The nitrogen atoms in the triazole ring act to inhibit the fungal cytochrome P450 enzyme that is accountable for the 14-α -demethylation of lanosterol by binding to the iron in the enzyme, though the success of this drug lies in the fact that fluconazole has less of an affinity for mammalian P450 enzymes than it does for fungal P450 enzymes.[113,114]

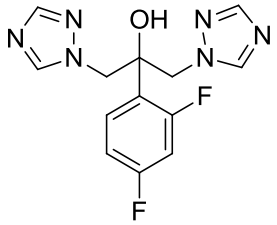


Figure 16 - Fluconazole[112]

Some resistance to Fluconazole has already emerged, prompting the need for new sister medications to be developed and tested; this resulted in the discovery of Voriconazole, which was marketed as an alternative to fluconazole in 2002 (Figure 17).[115]

Voriconazole is structurally similar to fluconazole in that it retains one of the triazole rings, but the other triazole ring has been substituted with a mono-fluorinated pyrimidine ring. This design preserves the placement of nitrogen atoms in the molecule for binding to the enzyme, while being significantly structurally unique that biological resistance present for fluconazole is not transferrable. This class of antifungal agents will ideally be mimicked by THAM analogs, but with a sufficient level of structural difference to avoid issues with previously-formed drug resistance or intellectual property (IP) infringement.

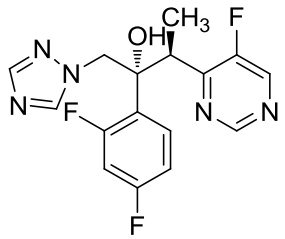


Figure 17 – Voriconazol[115]

Antiparasitic Agents:

Toxoplasmosis:

Toxoplasma gondii is a parasite that is responsible for toxoplasmosis, a disease most often contracted by humans from raw/undercooked meat or from the fecal matter of a feline.[116–118] For the majority of healthy adults, the parasite does not interfere with one's health or cause any medical issues, with the infected host often being asymptomatic and therefore, treatment is typically not recommended. However, the parasite can cause

detrimental effects to a fetus or an to an immune-compromised (e.g. HIV-infected) individual if left untreated.[116,118] The current general treatment for non-pregnant individuals that was first suggested in 1953 is a cocktail of pyrimethamine with sulfadiazine, with more recent regimens involving the addition of leucovorin (folinic acid). Alternate drugs may be used for more sensitive cases, such as in the case of pregnant women or HIV-affected individuals (figure 18).[118–120] Not unexpectedly, resistance to these prescribed drugs has formed, prompting the need for new medications for MDR strains of toxoplasmosis.[121] Specifically, a non-sulfa-drug-based approach would be ideal as resistance to and allergies against sulfa-drugs has been occurring since their induction into medicine in the 1930's.[121-123] As such, new treatments are being researched to eradicate the parasite from one's system for those who have contracted a more resistant strain or who cannot take sulfa drugs.

A predominant theme in these treatments is the use of heterocycles as main components. A THAM-based 1,3-dioxane or oxazolidinone may also be biologically active against *toxoplasma gondii*, and if so, the backbone will be of such a different scaffold that previously-formed resistance or existing patents will likely not be a concern.

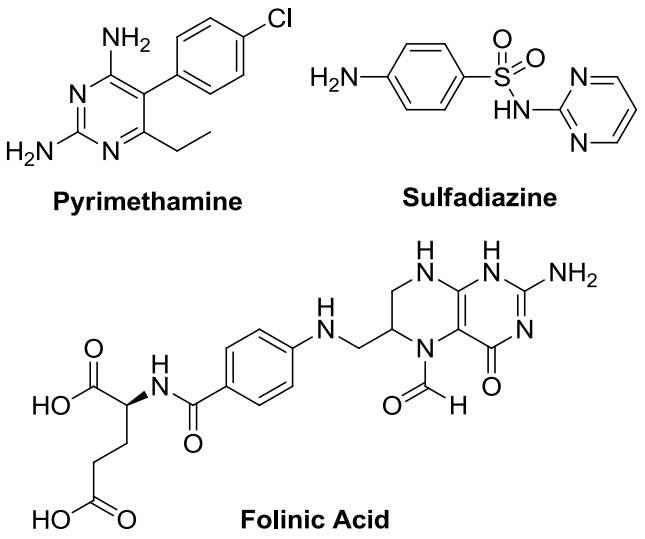


Figure 18– Toxoplasmosis treatments

Aromatase Inhibitors:

Previous work performed by fellow group members within the McNulty group has focused around synthesizing targets for aromatase, or cytochrome P450-19 (CPY 19A1), which is responsible for aromatizing estrogens *in vivo*.[124-126] While estrogens are incredibly important hormones to maintain a normal physiological lifestyle, they can also be detrimental in the case of estrogen-positive breast cancers.[127] These cancers are hormone-dependant and rely on estrogens to thrive, so one treatment method is to prevent the estrogens from binding to the tumor site. Several drugs have been developed to prevent estrogens from binding to receptors, such as Tamoxifen (Figure 19), which is a Selective Estrogen Receptor Modulator (SERM).[128] Resistance to Tamoxifen can also form, prompting the need for alternate treatment options.[127] One alternate option would

be to prevent the biosynthesis of estrogens all together, which is the purpose of aromatase (CPY 19A1) inhibitors.[129] Aromatase is the enzyme that catalyzes the conversion of testosterone to estradiol, aromatizing the A ring from an α-β unsaturated ketone to a phenol ring.[124] If this process is disrupted, the cancers will be starved for estrogen. One of the goals of this project was to synthesize a novel oxazolidinone ring compound that would ideally be similar to the compounds previously synthesized in the lab (Figure 19), but be faster/easier to synthesize and have a higher activity for inhibiting CPY 19A1.

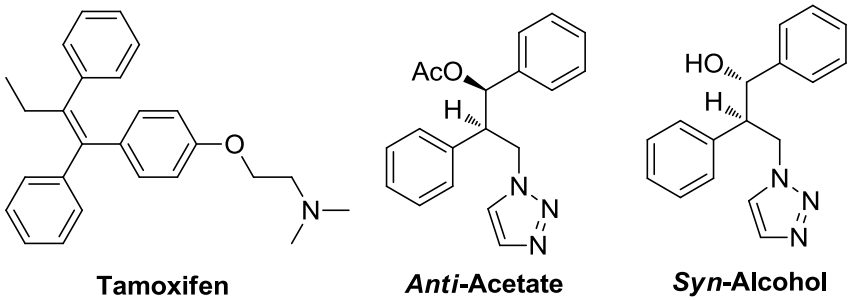


Figure 19 – Tamoxifen & CYP 19A1 inhibitors developed within the McNulty Group[126,127]

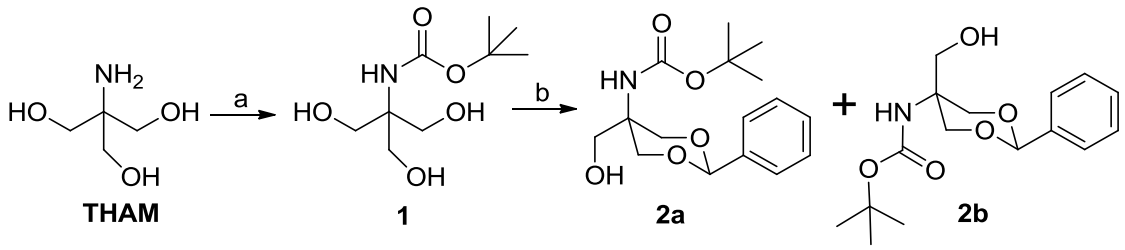
To conclude the introduction, THAM can readily be envisioned as a potentially valuable starting material towards the synthesis of a broad range of biologically active compounds in diverse therapeutic areas. These possibilities would be enhanced through the development of synthetic methodologies that allow rapid chemoselective differentiation on the dense amino-triol core inherent to THAM. Such processes should allow the introduction of structural diversity in terms of functional groups that can be added allowing us to realize the full potential of THAM. One of the primary drives of this Thesis, therefore, was directed towards the development of methodology allowing

selective differentiation of the alcohol residues with concurrent handling of the nucleophilic amino group present in THAM. Secondly, further manipulation though elaboration of the modified core in a selective manner to yield privileged core synthons that resemble known drug leads (such as oxazolidinones, sphingomimetics, etc.) would be investigated. Once differentiation of the alcohols and *N*-protection has been obtained, investigations as to the type of functional groups that can be incorporated within the THAM derivatives will be investigated to develop their potential. Thirdly, as an extension of this Thesis, various biological activities of all of these compounds would be explored in order to develop new hit compounds such as antimicrobial, anticancer and potential imaging agents.

Results and Discussion:

Fragmentation of THAM Derivative:

As most of the targets mentioned in this thesis require the chemoselective differentiation and control of all functional groups, with emphasis on the differentiation of the three alcohols, choice of the protecting groups was recognized as crucial. To facilitate the selective differentiation of the alcohols, cyclic diol protecting groups that would engage two alcohols simultaneously were the first type considered. In order to preferentially react with the alcohols the amine of THAM must first be sequestered by a protecting group. Therefore, the first protection to THAM carried out was protection of the amine, initially attempted using ethyl acetate with catalytic DMAP in DMF to afford an *N*-acetate derivative of THAM, but this reaction failed. Ramping up the reactivity of the electrophile, acetic anhydride with the same conditions, catalytic DMAP in DMF, was successful in protecting the amine; however, it was postulated that removal of an acetate group from the hindered amine in later synthetic steps may be difficult, so acetic anhydride was substituted for Boc anhydride. The addition of an *N*-Boc group was therefore carried out using Boc anhydride in the absence of a base to give **1** (Scheme 5). Addition of TEA as a base for this reaction was tried, but it did not improve the yields of **1** and actively hindered the next synthetic protection step, so it was excluded in future repetitions of this reaction.



(a) Boc_2O, DMF, 24 h; b) $PhCH(OMe)_2$, $PTSA_{(cat)}$, 24 h, 81% over both steps.

Scheme 5 – Protection of THAM

Literature suggested that a same-pot reaction to protect two alcohols on *N*-Boc THAM (**1**) was possible with either an acetonide or a benzylidene acetal.[9130] For the purposes of this project we decided on the benzylidene acetal, as it can be removed entirely with acid to render a diol, or reduced later to an alcohol and *O*-benzyl protecting group, allowing for further differentiation of the alcohols. In the same pot directly following addition of the Boc group, two of the alcohols were protected with a benzylidene protecting group using benzaldehyde dimethyl acetal and catalytic PTSA, leaving one alcohol free to give the Boc/Bz protected THAM as a mixture of diastereomers in an 81% yield over both steps (**2a**/**2b**, Scheme 5). The benefit of this synthetic route was that both of these protection steps can be performed sequentially in one pot with recrystallization for purification, lowering the cost and time required to perform the synthesis. This dual-protection reaction can also be scaled up easily and performed on the gram scale; as well the product can be stored for many years (>4) in the presence of air and ambient light with no special precautions taken, making **2a**/**2b** an ideal starting material.

Initially there were issues in synthesizing **2** as the reaction stopped after the formation of **1** and would not proceed to the acetal. It was believed that the formation of the acetal was not occurring because too much water was present in the reaction, which would prevent acetal formation and drive acetal degradation to the alcohols. This water was believed to be from the PTSA as the sample being used was monohydrate, and therefore 'wet'. To test this hypothesis different acids were employed as the catalyst for the acetal formation (PTSA and PPTS, from 5-15 mol%), but switching the acid used did not rectify the issue. The next possibility was the reaction was too dilute for acetal formation. As with traditional acetal formation, the concentration of the reaction was very important during this second synthetic step as anything too dilute (less than 0.5 mmol/mL) resulted in only formation of the *N*-Boc protected THAM triol **1**. Once the concentration issue had been resolved, it was found that 10 mol% of PTSA·H_2O was the most effective choice and that the added water had no noticeable detrimental effect on the reaction.

As mentioned, this reaction affords two diastereomers of **2**, referred to herein as the *cis* and *trans* isomers, in varying ratios, generally between 1:1 and 1:1.5 (Scheme 5). The isomers **2a** (*cis*) and **2b** (*trans*) can be resolved by TLC and eluted separately using silica column chromatography if desired. However, since the next step for differentiating the alcohols was a radical fragmentation that proceeded with a sufficiently high yield, separation of the isomers was generally not performed, with the exception for the purposes of characterization. While alcohol **2** had been reported in the literature in 1993 by Schmit, *et al.*, no mention was made that it was synthesized as two diastereomers. The

reporting of only one isomer is not surprising as superficially at a quick glance there wouldn't seem to be two isomers possible as the molecule possesses a plane of symmetry.[130] As a result, most spectra and physical characterization that has been carried out previously was done on a mixture of the two isomers with no mention that it was not a pure compound. More recently, in 2011 the same compound **2** was reported in *Tetrahedron* by Chiu, *et al*. having a full experimental with both the ^{1}H and ^{13}C NMR's, but only one isomer was reported.[88] Interestingly, the reported yield for said reaction was so low (65%), it is conceivable that the minor isomer was synthesized, but was disposed of accidently during the purification step after confirmation of the sole isomer was obtained from the previous literature. The oversight that **2** is synthesized as a pair of diastereomers (**2a**/**2b**) we were able to rectify in later publications.[131]

The addition of an alkene to the THAM backbone was envisioned with the intention of synthesizing a sphingosine analog. To achieve this type of scaffold the literature procedure set out by Hanessian was applied, wherein treatment of **2** with NBS in the presence of a radical initiator should afford the primary bromide **4** (Figure 20, mechanism Scheme 6). A bromide on the THAM scaffold would be useful as it could be reacted with triphenylphosphine to form a phosphonium salt, which could then be used in a Wittig reaction with a long-chain aldehyde to give the basic backbone of sphingosine. Protection of the remaining alcohol would likely have been required as treatment with a phosphonium salt version of **4** would very likely strip the benzoate group from the other alcohol. [132,133]

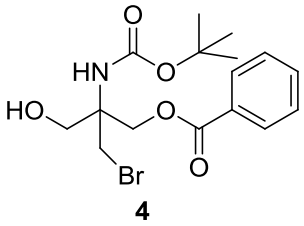
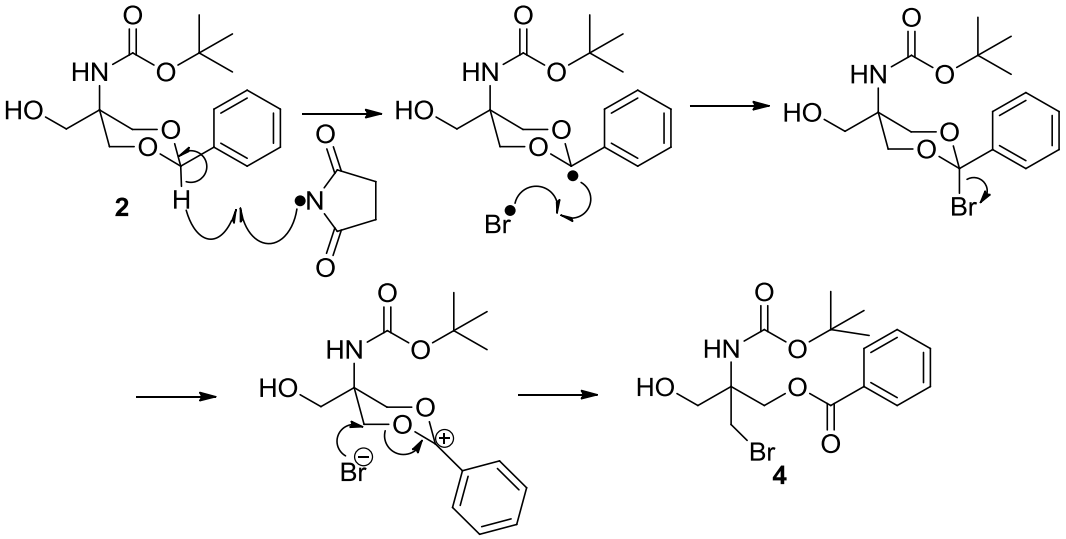
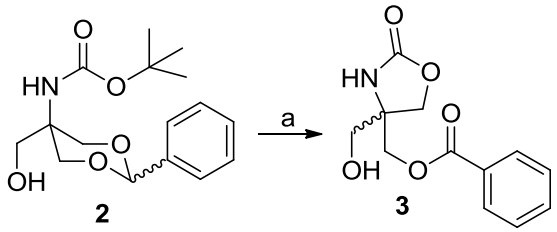


*Figure 20 – Expected product (**4**) of fragmentation*



Scheme 6– Expected radical fragmentation mechanism[133]

The radical fragmentation of the benzylidene group of alcohol **2** with *N*-bromosuccinimide (NBS) with a catalytic amount of BPO as an initiator in chlorobenzene at 70 ºC was performed and unexpectedly gave compound **3** as the sole isolated product with a 77% yield (Scheme 7). Obtaining only oxazolidinone **3** was unforeseen and unexpected as product **4** was the only expected product from this radical fragmentation reaction.[132,133]



a) NBS, $BPO_{(cat)}$ or $AIBN_{(cat)}$, PhCl, 70 ºC, 2 h, 77%

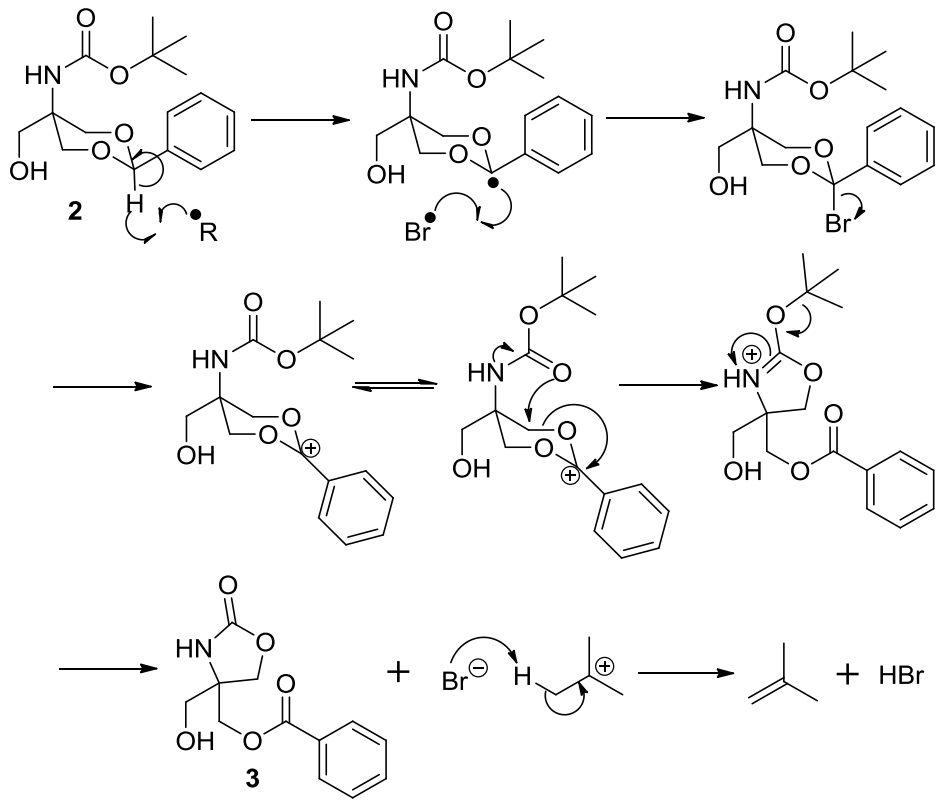
*Scheme 7 – Radical fragmentation of **2***

The conditions of the reaction were altered in several ways with the intent of producing product **4**. The initiator was changed to AIBN, which is purchased in an anhydrous state, as opposed to BPO which is sold as a 75% mixture with water, but this method also gave oxazolidinone **3** as the only isolated product. As the presence of an acid (HBr) is evidence of the continuation of the fragmentation, barium carbonate was added as a base to test whether this reaction is initiated in acidic conditions, but this had no effect as the pH remained below 2 during the reaction (tested by litmus paper).[132] The pH may not have been affected by the addition of base due to the low solubility of barium carbonate in chlorobenzene.

The structure of oxazolidinone **3** was partly verified through mass spectrometry as the molecular ion peak for **3** was lower than what is expected for **4**, and the spectra did not possess the characteristic bromine isotope peaks that would have also been found on **4**, but both of these findings could have resulted from **4** fragmenting within the mass spectrometer. The structure of compound **3** was further verified through ^{1}H NMR analysis, first through the lack of *N*-Boc protons at approximately 1.45 ppm. Other discrepancies were found downfield around 4.3 ppm, where large chemical shift

differences were seen between the two methylene protons on the oxazolidinone ring. This chemical shift suggested their conformation was fixed, for example, within a ring; that chemical shift difference would be absent, or less pronounced, if the protons were on a free-rotating chain. The ^{13}C NMR spectrum further confirmed the structure of compound **3**, with the *N*-Boc carbons absent, as well as the C-O peaks being shifted downfield to approximately 63 ppm.

The mechanism for this radical fragmentation was proposed to proceed from the carbocation intermediate rather than from **4**. This proposed mechanism was felt to be more correct due to the reactive nature of the semi-stabilized carbocation, and the fact that intramolecular reactions (carbonyl attacking the carbocation, Scheme 8) are generally faster than intermolecular reactions (bromine attacking the carbocation, Scheme 7). This theory was supported by previous research into intramolecular rearrangements via *N*-boc substrates formed by the reaction of iodine isocyanates and an alcohol, with subsequent treatment with an alkene as well as thermal treatment of β-iodocarbamates. Both of these reactions formed an oxazolidinone ring as the product via the carbonyl oxygen attacking the electrophilic carbon, followed by loss of the *tert*-butyl group.[134,135]



*Scheme 8 – Proposed mechanism for fragmentation of **2***

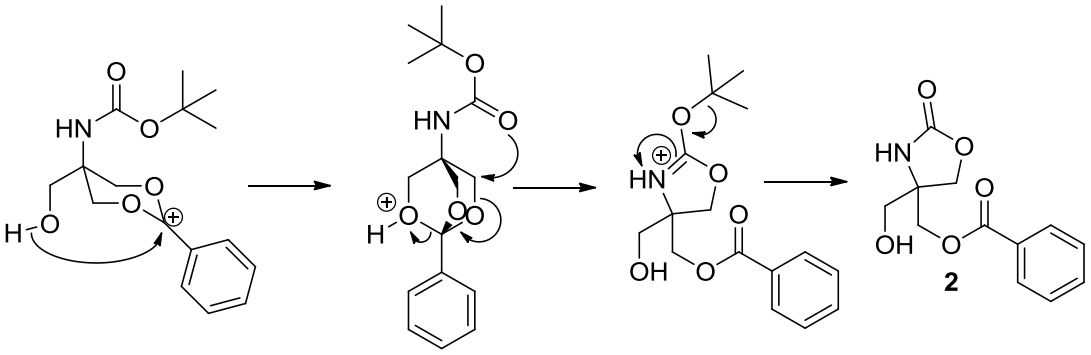
Although the differentiated intermediate **4** was not obtained, the novel oxidative radical fragmentation provided oxazolidinone **3** in three overall steps from THAM. Compound **3** contains all the elements desired for this project, including the protected amine and three differentiated alcohols. As described in the introduction, other medications, such as the antibiotic linezolid (Figure 13), muscle relaxant metaxalone[136,137], or migraine medication zolmitriptan[138], all possess a 2-oxazolidinone ring at their core, opening up the option of synthesizing analogs of these examples. Compound **3** was beneficial synthetically as it possesses only one reactive functional group, as opposed to having two as found on **4**, therefore possibly preventing side-

reactions in later synthetic steps. The non-reactive functional groups that were present on **3**, benzoyl and carbamate, are chemically orthogonal to one another, so selective removal of each is possible if desired. As a result, the robust scaffold found within oxazolidinone **3** will lend itself well to a plethora of reaction conditions, opening the synthetic pathway towards a multitude of different reactions and products.

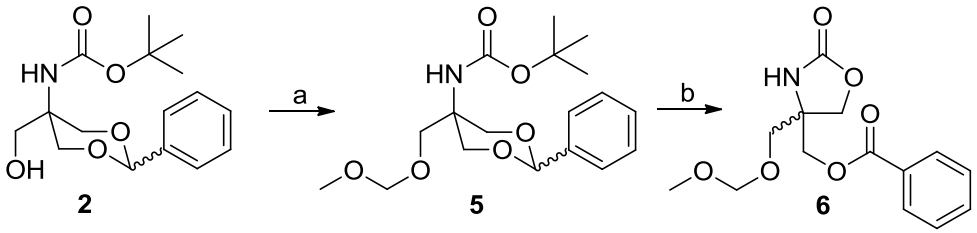
There were further benefits from this synthesis of oxazolidinone **3**, in that the product can be purified through recrystallization with a sufficient level of purity and a similar yield (76% with recrystallization vs. 77% with column chromatography), eliminating the need for purification via silica column chromatography. Separation of the *cis*/*trans* isomers seen with compound **2** was no longer a concern, simplifying any NMR spectra obtained for future compounds. Additionally, if previous methods cited in the literature were used to form an oxazolidinone ring, such as a carbonyl with two labile groups, differentiation of the remaining two alcohols would be very difficult. The unprotected product formed from using literature methods, 4,4-dimethoxymethyl-2-oxazolidinone, would also be of such high polarity that handling and purification may have been a serious concern.

At this stage it was unknown whether the free alcohol on **2** was involved in the fragmentation by creating a bicyclic orthoformate intermediate (Scheme 9). With the intent of probing the mechanism of this novel rearrangement, the alcohol of **2** was protected using MOM-Cl to afford **5** as a mixture of *cis*/*trans* diastereomers (Scheme 10). When the NBS fragmentation was performed on **5**, **6** was the isolated product when either catalytic BPO or AIBN was used as the initiator (Scheme 10). The formation of

oxazolidinone **6** was verified using the same methods as compound **3**, such as the loss of the *N*-Boc peaks on the proton and carbon NMR spectra, as well as the lower mass peak in the mass spectrum. This formation of the oxazolidinone ring suggests that this fragmentation was not limited to alcohol groups on the neighbouring branch. Although the reaction proceeded to the oxazolidinone, the yield was appreciably lower than the parent alcohol, which suggests that the alcohol may be playing another role in the reaction beyond stabilizing an intermediate.



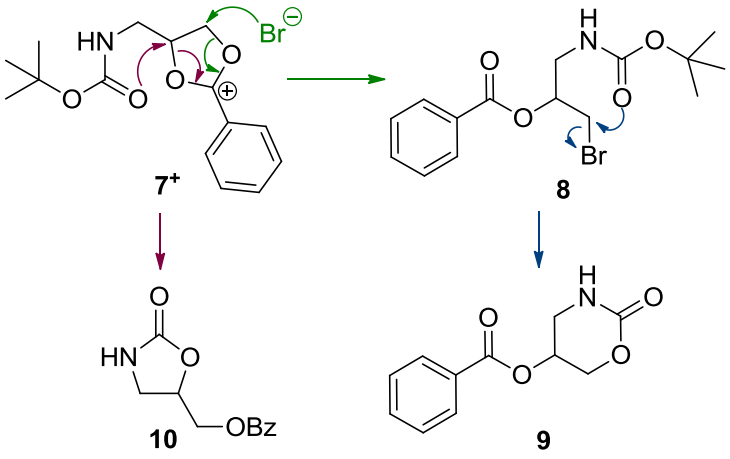
Scheme 9 – Possible alcohol involvement in radical fragmentation



(a) MOM-Cl, DIPEA, DCM, rt, 3 h, 72%; b) NBS, BPO or AIBN $_{(cat)}$, PhCl, 70 ºC, 1.5 h, 47%.

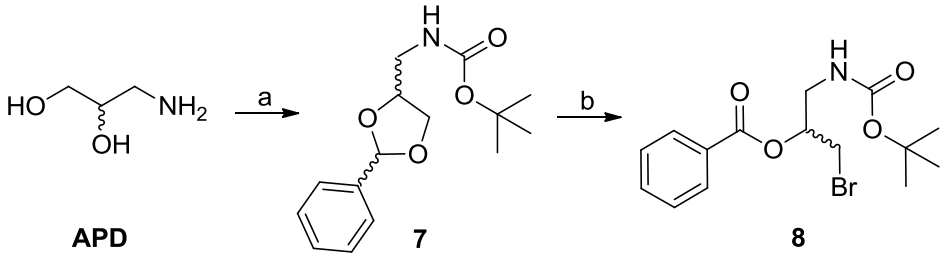
Scheme 10– Synthesis and fragmentation of MOM-protected Boc/Bz THAM

The mechanism of the fragmentation of Boc/Bz THAM (**2**) was further investigated using the substrate 3-amino-1,2-propanediol (APD), which would give a 5-member ring acetal and 5-member ring intermediate, as opposed to the previous example which had both a 6-member ring acetal and 6-member ring intermediate. Following the same protocol of the radical fragmentation of **2**, three distinct products, **8**, **9**, or **10**, were possible through the Boc/Bz protected APD compound **7** (Scheme 11). The first possible product would be formed with no rearrangement occurring, which would give the conventional product of the primary bromide **8** (in green, Scheme 11). The second and third possible mechanisms revolved around which route an intramolecular rearrangement would occur, whether directly from $\mathbf{7}^{+}$ (carbocation of **7**) or from **8** (Scheme 11). If the rearrangement were to proceed directly from the carbocation intermediate of **7** then oxazolidinone **10** would form, as was the case with alcohol **2** (in purple). The second alternative would be that an intramolecular rearrangement would occur from the primary bromide **8** to give carbamate **9** (in blue). The difference between these two mechanistic routes was how the rearrangement proceeds, whether it is via the conventional-product primary bromine intermediate **8** to give carbamate **9** (in blue), or through the carbocation intermediate to give oxazolidinone **10** (in purple). This experiment was designed to help determine if the primary bromide **4** was produced as an intermediate, or whether the reaction proceeded straight to the oxazolidinone **2**.



Scheme 11 – Possible APD radical fragmentation products

To synthesize the probe for this mechanism, the amine and two alcohols on APD were protected under the same conditions as Boc/Bz THAM (**2**) using Boc anhydride and benzaldehyde dimethyl acetal to afford **7** (Scheme 12). The Boc/Bz-protected compound **7** was then fragmented using the same conditions as previously employed on alcohol **2**: NBS, catalytic BPO, in PhCl at 70 ºC for several hours. The only product isolated from this reaction was the primary bromide **8** (Scheme 12), with no evidence of **9** or **10** by TLC or silica column chromatography. It was postulated that further heating of **8** might promote rearrangement after sufficient time, as perhaps the reaction simply required more time and energy to advance to the oxazolidinone ring. However, despite extensive heating of **8** no rearrangement was observed, as visualized by TLC.



a) 1) Boc_2O, DMF, rt, 7 h. 2) $PhCH(OMe)_2$, $PTSA_{(cat)}$, 16 h, 46%; b) NBS, $BPO_{(cat)}$, PhCl, 70 ºC, 5 h, 48%.

*Scheme 12 – Protection and radical fragmentation of APD to give **8***

The sterics within the mechanism were likely the cause for the 5-member ring analog not proceeding through the rearrangement. In the previous case of alcohol **2**, the carbocation would place the phenyl group in an sp^2 hybridized geometry, neither axial nor equatorial. As a result, the *N*-Boc and alcohol groups would set the configuration of the intermediate $\mathbf{2}^+$ (Figure 21). The bulkier *N*-Boc group of $\mathbf{2}^+$ would occupy the equatorial position, and therefore the alcohol was set in the axial position. Because this alcohol could weakly hydrogen-bond with the two oxygen atoms within the dioxane ring, this would further cement the ring in this configuration. However, because the electrons of the oxygens in the ring were stabilizing the adjacent benzylidene carbocation, the hydrogen-bond with the alcohol would be less pronounced than usual. Although weaker, because the less hydrogen-bonding MOM-protected analog **5** gave a lower yield than the stronger hydrogen-bonding alcohol **2**, the hydrogen-bonding may be considered a contributor to setting the configuration of the ring. As a result of this configuration, the bulky ^{t}butyl component of the *N*-Boc group of $\mathbf{2}^+$ would orient itself away from the molecule, pushing the carbonyl of the carbamate directly under the 6-member ring, adjacent to the

electrophilic carbons. However, in the case of the 5-membered analog **7**$^{+}$, the ^{t}butyl group would orient itself away from the ring, moving the carbonyl away from both of the electrophilic carbons (Figure 21). This distance between the nucleophilic carbonyl and electrophilic carbons slowed down the cyclization, thus giving time for the bromide to act as the nucleophile in the reaction. As a result, only **8** was isolated from the 5-member ring analog, rather than either a 5- or 6-member ring. This probing of the reaction suggested that the rearrangement from **2** to **3** proceeds via the mechanism proposed, in that the carbonyl attacks the carbon β to the carbocation.

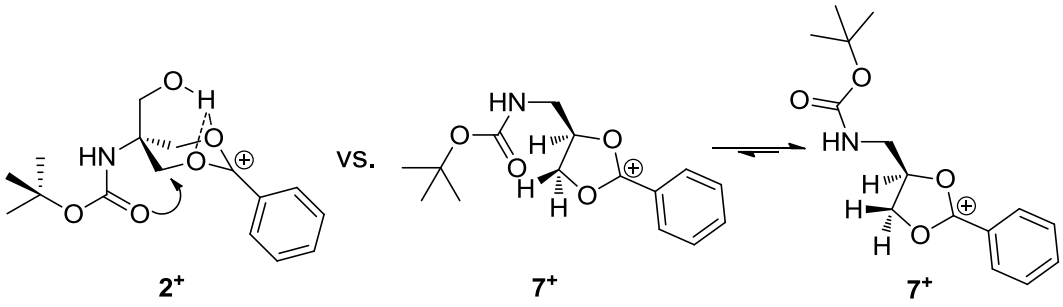


Figure 21 – Conformational Analysis of Novel Rearrangement

Oxazolidinone Library:

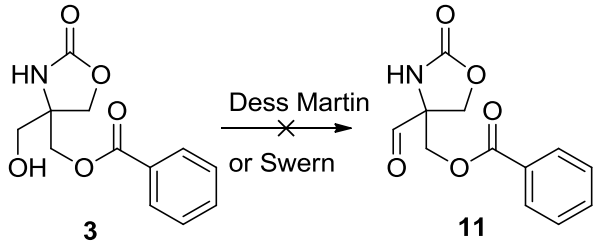
We next desired to take advantage of the fully-differentiated THAM derivative **3** as a compact reactive synthon that could be used towards oxazolidinone analogs, such as those found in Linezolid (Figure 13). The substituents on Linezolid are located on the carbon adjacent to the oxygen rather than the nitrogen, but this alteration may make a more cost-effective analog of Linezolid with potential activity. Alternatively, Sphingosine and FTY720 analogs could also be synthesized from oxazolidinone **3**, because one of the main objectives for this project, differentiation of the alcohols, has been achieved.

Sphingosine & FTY720 both possess a similar backbone of polar groups on one end of the molecule and a long, alkyl section on the other (Figure 12). To obtain this type of scaffold alcohol **2** or oxazolidinone **3** must have an alkyl chain added to one of its branches without the use of a heteroatom spacer. One method of adding an alkyl tail is to perform a Wittig reaction on alcohol **2** or oxazolidinone **3**, which would involve either transformation of the THAM derivative to the phosphonium salt or oxidizing the alcohol on the THAM derivative to the aldehyde.

To expand the oxazolidinone library it was thought that an alcohol to aldehyde conversion on oxazolidinone **3** would provide a more general synthetic approach within this project as a phosphonium salt has limited synthetic use beyond a Wittig reaction. Therefore, both a Dess-Martin oxidation and a Swern oxidation were attempted on oxazolidinone **3** to hopefully obtain aldehyde **11** (Scheme 13). The Dess-Martin oxidation route was quickly discounted as an option due to difficulties in forming and obtaining product **11**, resulting in no isolated product. The cost of the Dess-Martin reagent (DMP),

as well as difficulties in scaling the reaction up to the gram scale at a later time, also contributed to this method being abandoned.

The Dess-Martin oxidation may have failed due to sterics, as both the reagent and the neopentyl alcohol being oxidized are bulky. To circumvent this concern, a Swern oxidation was then attempted to synthesize aldehyde **11**. Several attempts to form the aldehyde through the Swern oxidation protocol were performed (Scheme 13), but the solubility of oxazolidinone **3** in DCM was too low. Dissolving **3** in a 1:1 DCM:DMSO solvent mixture prior to addition to the Swern reagent solved the solubility difficulties that were encountered. Despite the increase in polarity of the solvent, compound **3** precipitated from the solution when it reached the less-polar dimethylchlorosulfonium chloride solution. Attempts to solubilize **3** by warming the reaction mixture to -20 ºC also failed (note: if the reaction was warmed beyond this temperature the dimethylchlorosulfonium chloride intermediate required for the reaction decomposed).



3 **11**

*Scheme 13 – Failed oxidation of oxazolidinone **3***

As aldehyde **11** was not a viable pathway for the expansion the oxazolidinone library, there were a few alternate synthetic routes utilizing alcohol **3** that were explored.

One such route is conversion of the alcohol of **3** to a leaving group to facilitate substitution reactions. The mesylate-protected version **12** was prepared using MsCl in base, with verification through ^{1}H NMR and mass spectroscopy (Figure 22). However, mesylate **7** was found to be too unstable to be a viable intermediate for this project as it degrades too quickly to be reliably synthesized, scaled up, stored, or retained in a sufficient quantity. Regardless of the amount of MsCl and DIPEA added, the reaction maintains a large amount of starting material as shown by TLC, even with low temperatures experienced during the synthesis (-78 ºC). Experiments that involved the addition of a nucleophile prior to work up of the mesylation reaction to avoid degradation of **12** during purification also failed, with the usual resulting products being the starting material **3** and a mesylate-protected version of the nucleophile employed.

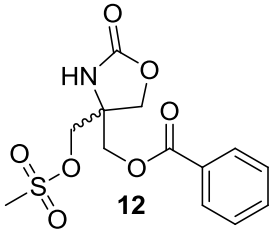
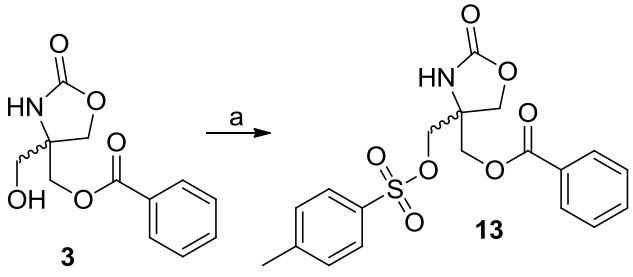


*Figure 22 – Mesylate-protected THAM-based oxazolidinone **12***

To obtain a stable electrophilic-branched oxazolidinone intermediate, the mesylate was substituted for a tosylate group. While mesylates are generally regarded to be less reactive than tosylates, it was thought that the additional bulk from the aromatic ring, as opposed to the methyl group, combined with being adjacent to a neopentyl alcohol, would

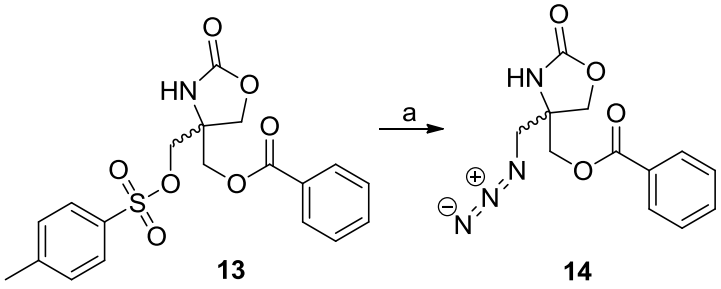
decrease the accessibility to the reactive carbon, thus increasing the stability of the compound to a sufficient level so as to allow for purification, handling, and storage. Addition of a tosylate group to **3** was performed using tosyl chloride, with lutidine as the base, and resulted in a good and reproducible yield of 87% (**13**, Scheme 14). Additionally, the tosylate **13** was stable enough for purification, handling, storage, and characterization, with no visible degradation of the product, even when stored as a solid at room temperature in the presence of air or light for several years. The number of equivalents of tosylate is pertinent to the reaction outcome (3 equivalents for mono-addition) since it was possible to add a tosyl group to the nitrogen of the oxazolidinone ring in addition to the alcohol if too many equivalents of tosyl chloride are used; tosylating the nitrogen must be avoided as removal of an *N*-tosyl generally requires very harsh conditions (e.g. refluxing in acid).[139] The addition of the tosylate group on the alcohol gives the flexibility to substitute the previous alcohol with a variety of different compounds to build a more comprehensive library of analogs.



a) Ts-Cl, lutidine, DCM, 15.5 h, 87%.

*Scheme 14 – Synthesis of tosyl-oxazolidinone **13***

To test the use of a nucleophile on such a sterically hindered electrophilic carbon as found on tosylate **13**, use of a less bulky nucleophile, such as an azide, was performed first. Sodium azide was employed successfully as a nucleophile to afford azide **14** from tosylate **13** (Scheme 15). This S_N2 substitution reaction gave excellent yields and purification was obtained through a simple DCM wash of the reaction mixture following removal of the solvent. The azide product proved to also be stable at room temperature in solution with exposure to indoor lighting for at least several days as well as in solid form in atmospheric air for many months without any noticeable degradation as visualized through the ^{1}H NMR spectrum. As azides can be reduced to an amine or reacted with an alkyne or cyanide to produce a triazole or tetrazole ring respectively, the addition of an azide to the THAM scaffold broadened the scope of compounds that could be derived from this synthetic pathway.



a) NaN_3, DMF, 80 ºC, 5 h, 95%.

*Scheme 15 – Nucleophilic substitution of **13** to afford azide **14***

Further confirmation of the structure of azide **14** was provided through single-crystal X-Ray crystallographic analysis (Figures 23a & 23b: same molecule at different

angles, with carbon in grey, hydrogen in white, oxygen in red, and nitrogen in blue). X-Ray crystallography further definitively proved our earlier analysis and conclusion that an oxazolidinone ring was formed during the radical fragmentation and subsequent rearrangement of alcohol **2**.

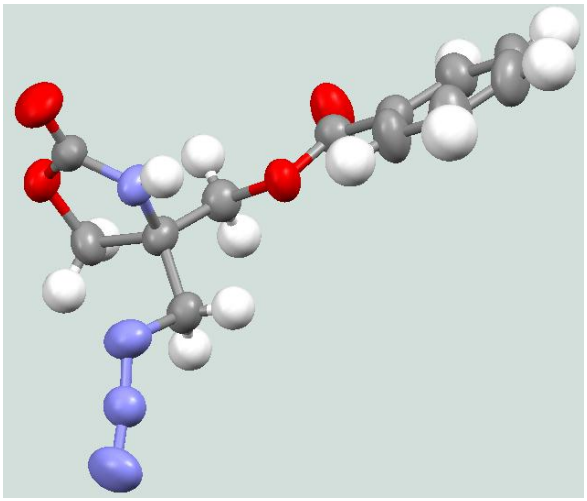


*Figure 23a – X-Ray crystal structure of azide **14***

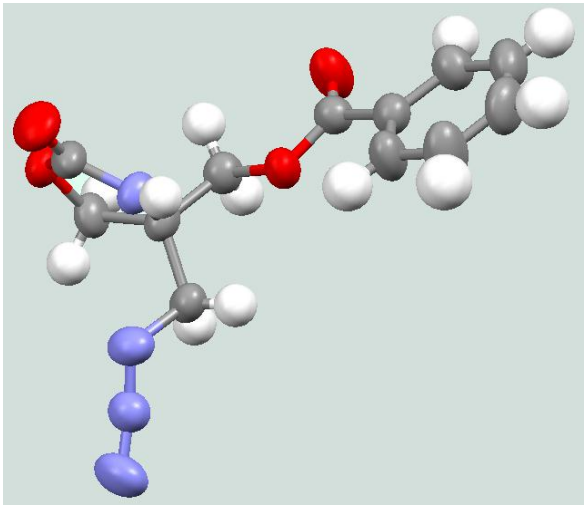
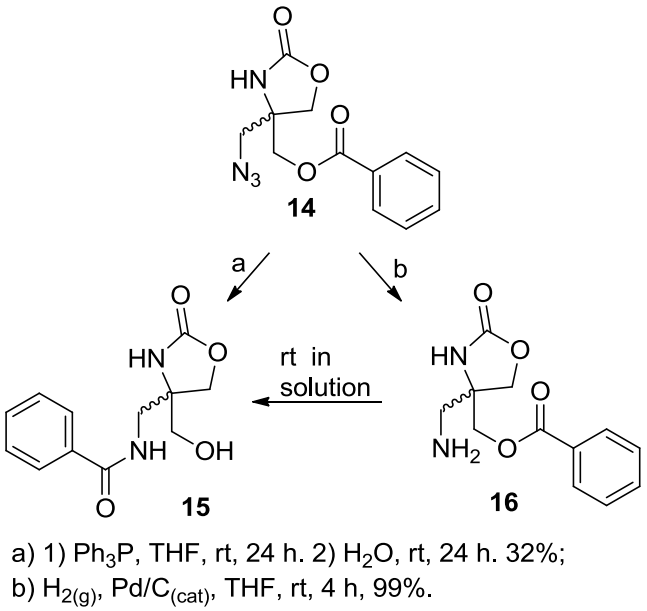


*Figure 23b – X-Ray crystal structure of azide **14***

Conversion of the azide to a more useful functional group, such as an amine, was desired to allow for the addition of electrophilic groups to the molecule. The reduction of the azide of **14** to the primary amine was performed under two different sets of conditions. The first method was through a Staudinger reaction, reacting triphenylphosphine with azide **14** for 24 hours, followed by treatment with water for an additional 24 hours (Scheme 16); these conditions gave primarily the *N*-benzoyl migrated product (**15**) as expected, formed via an acyl transfer when the amine reacted with the carbonyl of the benzoyl group in an intramolecular reaction, leaving a primary alcohol and a benzoyl-protected amine. The Staudinger reaction gave only trace amounts of the *O*-benzoyl product (**16**), likely as a result of the length of time required for the reaction. The Staudinger reaction had a few major drawbacks, mainly that the yield was barely over 30%, the reaction had to be performed over the course of two days, and column chromatography was required for purification. Due to the time and yield disadvantages the Staudinger reaction had with this substrate, this method was abandoned for the reduction of the azide functional group using hydrogen over catalytic palladium on carbon (Scheme 16). One of the most compelling benefits of this hydrogenation that is the reaction gave a quantitative yield with clean conversion to a single product (**16**), without significant amounts of side products. Another convincing improvement is this reduction uses filteration as the sole method of purification to remove the heterogeneous catalyst as opposed to silica column chromatography, which was required for the Staudinger reaction. Conversion of the amine/*O*-benzoyl (**16**) product to the alcohol/*N*-benzoyl product (**15**) occurs when the amine **16** is left in solution for an appreciable amount of

time. Thus, this acyl migration can be avoided if short reaction times are employed for the hydrogenation.

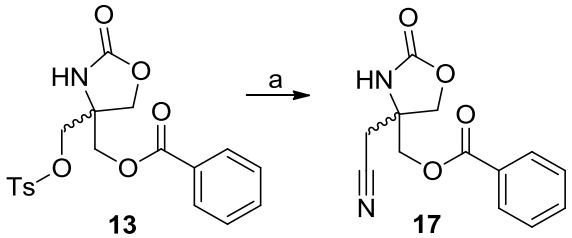


*Scheme 16 – Reduction of azide **14** to give **15** or **16***

Slow transfer of the benzoyl group from the oxygen (**16**) to the nitrogen (**15**) was observed when the amine was stirred in solution at room temperature in solvent over time (Scheme 16), as visualized by both TLC and ^{1}H NMR. This benzoyl migration has only been shown to occur when the product is in solution and there is no evidence of the migration occurring when **16** was stored in its solid form. This optional migration of the benzoyl group from oxygen to nitrogen gives the flexibility to run the reaction and store the stock product **16** under agreeable conditions without the need to finalize which precursor, **15** or **16**, will be desired in the future. The retention of the benzoyl group as a

protecting group on nitrogen also saves the synthetic step of protecting the amine, as well as retaining the option of solid-phase synthesis, with the benzoyl group as the linker to the solid support.

To expand the oxazolidinone library, substitution of the tosyl group was further investigated using various other 'arrow' nucleophiles, such as a nitrile. A cyano-oxazolidinone product could open the pathway to the synthesis of rings on the side chain, such as reaction of the nitrile with an azide to produce a tetrazole ring. Similar to the azide, cyanide was introduced to the oxazolidinone ring through substitution at the *O*-tosyl carbon using potassium cyanide to give **17** in 76% yield (Scheme 17). This reaction proceeds fairly cleanly with a similar work up to the azide, in that the solvent is removed under reduced pressure and the resulting residue is washed with DCM to afford the product with no further purification required or carried out.



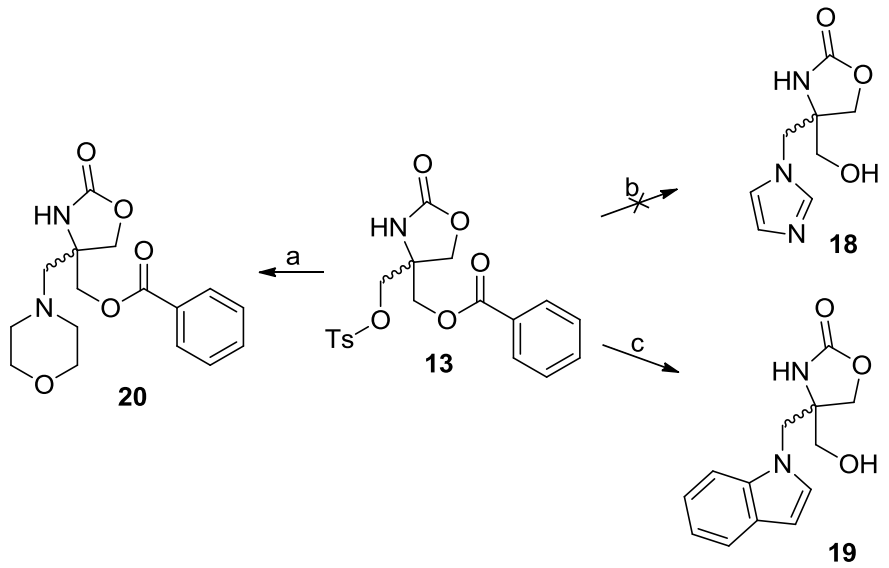
a) KCN, DMF, rt, 4 h, 76%.

*Scheme 17 – Synthesis of nitrile **17***

Reduction of the nitrile was attempted to obtain an analog of **16**, but with an additional methylene spacer between the amine and oxazolidinone ring. Nitrile **17** was treated with gaseous H_2 at one atmosphere of pressure over Pd/C, but this reaction failed

to produce any amine and only starting nitrile was isolated. An addition reaction using a Grignard reagent was also unsuccessful, likely due to the complexity of the molecule negatively affecting the chemoselectivity (e.g. the carbonyl of the benzoyl group or, given a sufficient length of time, the carbonyl of the oxazolidinone).

Primary and secondary amines, such as 1-dodecylamine, imidazole, and indole, were used as nucleophiles on **13** in an attempt to create the corresponding oxazolidinone. The substitution reaction between tosylate **13** and 1-dodecylamine failed to produce any amine-substituted product with or without the loss of the *O*-benzoyl group. The reaction between **13** and imidazole was attempted several times, however, it also failed to produce any product either, with only trace amounts of product formed (**18**, Scheme 18). Indole adds more readily than imidazole to make **19**, but the yield was very low (Scheme 18). Interestingly, unlike the reaction with sodium azide or potassium cyanide, the *O*-benzoate group was unavoidably removed by the nucleophile during the reaction as proved by the aromatic protons in the ^{1}H NMR associated with the benzoate group being absent.



a) Morpholine, DIPEA, THF, 70 ºC, 20 h, 49%; b) LiHMDS, imidazole, dioxane, 0 º to 70 °C, no rxn; c) NaH, indole, DMF, 0 ºC to rt, 21 h, 32%.

*Scheme 18 – Substitution of tosyl-oxazolidinone **13** with various amines*

Morpholine as a substrate was also envisioned for the substitution reaction due to its involvement in the backbone of Linezolid. Morpholine also proved to be a suitable nucleophile when DIPEA was used as the base (**9**, Scheme 17). When a base with a lower pKa was used, for instance lutidine, the reaction was unsuccessful. The use of DIPEA as a base had the added advantage of preventing the reaction from becoming acidic after the generation of *p*-toluenesulfonic acid. As opposed to earlier results, despite the large excess of morpholine used in the reaction (~20 eq.), the *O*-benzoate group remained on **20**.

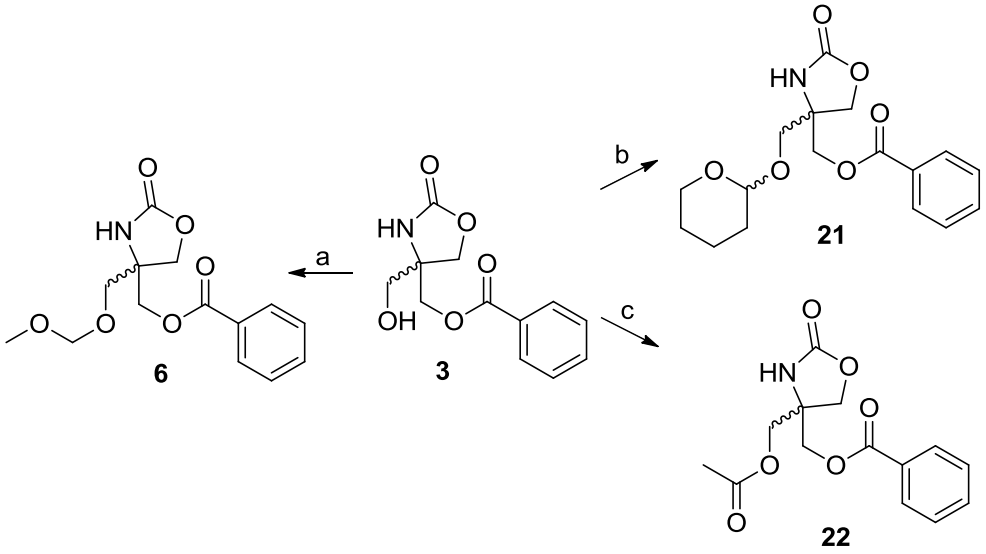
Although addition of further heterocylic rings and groups to THAM-based oxazolidinones would be useful for expanding the oxazolidinone library, it was abandoned as it was not advancing the project in a sufficiently-novel direction, especially

without further SAR data. We showed that tosylate **13** can be made in only four synthetic steps, with a relatively good yield. We have also proved that simple N_3^- and ^-CN substitution can occur, as well as reactions with amines such as morpholine and indole. Theoretically a library of compounds could be synthesized using this type of reaction, but to further the project we began looking at different types of reactions for the THAM scaffold.

Similar to substitution reactions, addition reactions to THAM-based oxazolidinone **3** were attempted to verify the scope of reaction types tolerated by this novel scaffold, as well as with the intention of expanding the oxazolidinone library. Addition reactions, mostly based around protecting groups, have been performed on oxazolidinone **3** with varying levels of success. Those protecting groups, such as MOM (**6**) and THP (**21**), were added without issue (Scheme 19); however, purification posed a problem as for no explicable reason both the MOM-oxazolidinone **6** (when synthesized from oxazolidinone **3**, as opposed to the synthesis that fragments alcohol **5**) and the THP-oxazolidinone **21** were solvated and very difficult to obtain as solids that were devoid of water. This solvation may have been the cause of issues in future water-sensitive amide aryl-coupling reactions that were later attempted with MOM-oxazolidinone **6** THP-oxazolidinone **21**. THP-oxazolidinone **21** was synthesized cleanly, but formation of **21** occurred with an equal ratio of both diastereomers, as was confirmed by ^{1}H NMR. The R_f values of the diastereomers were identical, and the compound was observed as a single spot as visualized on the TLC plate, making separation via traditional silica chromatography impossible for us. As a pure compound could not be isolated from the

mixture of isomers from THP-oxazolidinone **21**, that synthetic route was abandoned for a protecting group devoid of stereocenters.

Acetoxy-oxazolidinone **22** was synthesized from acetic anhydride and oxazolidinone **3** with relative ease, but later proved to be of no synthetic use for the *N*-aryl coupling or any other synthetic plan (scheme 19). Interestingly, acetoxy **22** was also formed by heating tosyl-oxazolidinone **13** in the presence of acetic acid, but this method was more time consuming than using acetic anhydride, and it gave a lower yield, so was never used to synthetize reasonable quantities of **22**.

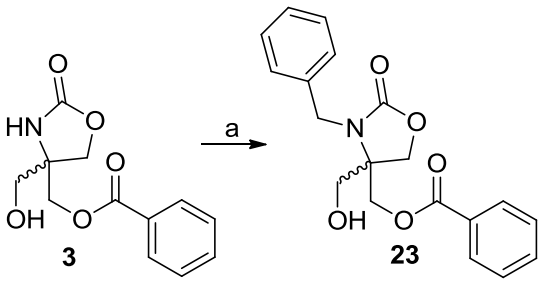


a) MOM-Cl, TEA, DCM, rt, overnight 65%; b) DHP, $PTSA_{(cat)}$, DCM, rt, 21 h, 68%;
c) Ac_2O, DMF, rt, 48 h, 89%.

*Scheme 19- Addition of MOM (**6**), THP (**21**) and acetoxy (**22**) to oxazolidinone **3***

Benzyl bromide was used with the intention of protecting the free alcohol with a benzyl group, capping the reactive alcohol with a chemically non-orthogonal protecting

group that could be removed with hydrogenation without affecting the remaining *O*-benzoyl group or oxazolidinone ring. The reaction was carried out with benzyl bromide in the presence of LiHMDS or NaH as the base in the reaction with the intent of first deprotonating the free alcohol. These reaction conditions resulted in the formation of the *N*-benzyl-protected product **23** in lieu of the intended *O*-benzoyl product (Scheme 20). Proof for the formation of **23** was found in the proton NMR spectrum, where the methylene protons adjacent to the alcohol remained at approximately 3.5 ppm, the N-H proton signal around 5-6 ppm was lost, and the methylene protons on the benzyl group were around 4.4 ppm, which is more upfield than had they been found adjacent to an oxygen. This unintended side-product **23** was formed when using either 1 or 2 equivalents of benzyl bromide/base for the reaction. There was trace amounts of both the amide and alcohol reacting with the benzyl bromide, but not to a sufficient degree to be of any practical synthetic value.



a) 1) LiHMDS, DMF, 0 ºC, 30 mins; 2) BnBr, 0 ºC to rt, 1.5 h, 41%.

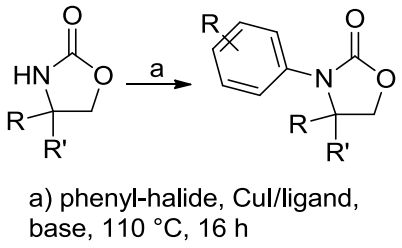
*Scheme 20 – N-benzylation of **3** to afford **23***

Due to the pKa of the oxazolidinone NH being lower than that of the alcohol (12 and 15.5 respectively), the reactivity of the alcohol may not be sufficient to obtain only the *O*-benzyl product when a base is first used to deprotonate the acidic hydrogens. The alcohol might be preferentially reacted with benzyl bromide in the presence of the oxazolidinone ring if prior addition of a base was avoided. The alcohol of **3** should be more nucleophilic than the nitrogen within the oxazolidinone ring as the lone pairs on the nitrogen are involved in resonance with the carbamate group. Though this synthetic route may have been optimized towards the formation of only the *O*-benzyl product, it was abandoned for more reliable methods.

More than the dual benzyl-protected product or *O*-benzylated product, the *N*-benzylated **23** can be considered beneficial to this project as it gives an additional avenue of attaching oxazolidinone **3** to a polymer support for solid phase synthesis, while maintaining a free reactive alcohol for derivatization. The synthetic method described in Scheme 20 would introduce alcohol **3** to the solid polymer support after the fragmentation step, thus preventing issues that may arise during the radical reaction and rearrangement. Additionally, the *N*-protected oxazolidinone **23** is structurally similar to an arylated oxazolidinone such as that type found in the structural backbone of the oxazolidinone-class of antibiotics (e.g. Linezolid, Figure 13); therefore this route could hypothetically be used to create a Linezolid analog from oxazolidinone **3**.

Linezolid-type Analogs:

As Linezolid and its derivatives (Figure 13, 14, & 15) have an aromatic ring bonded directly to the nitrogen of the oxazolidinone ring, it was hypothesized that the addition of an aromatic ring to a THAM derivative might increase the likelihood of having antibacterial activity for that class of compounds. The literature published within the last ten years cited aryl coupling to the nitrogen of the oxazolidinone ring in high yields through the use of affordable copper catalysts and ligands (Scheme 21).[140–142] Palladium can also be used as a catalyst or co-catalyst with copper for the aryl coupling to nitrogen with well-documented results.[143,144] The starting compounds within these papers illustrated that the novel coupling methods tolerate functional groups stemming off the oxazolidinone ring, but these groups are often situated at the methylene carbon adjacent to the oxygen (5-substituted position). In very few cases were there free hydroxyl groups present, in which case a low yield was reported (20%), and the hydroxyl was situated adjacent to the oxygen rather than the nitrogen atom.[142] Therefore it was believed that the previously synthesized THAM-based oxazolidinones were prime candidates for an *N*-arylation reaction, opening the possibility of antibacterial activity.



Scheme 21 – N-Aryl coupling of oxazolidinone

Different THAM-based oxazolidinones were used as starting materials in this reaction, such as the free alcohol **3** or a derivative with a protected alcohol, such as tosylate **13**, MOM ether **6**, or acetoxy **22**. The *N*-aryl coupling was carried out under a plethora of conditions, mostly altering the aromatic-halide used, the type and number of metals for the catalyst, the ligand/co-ligand for the catalyst(s), the protecting group on the alcohol of the oxazolidinone **3**, the solvent, the base, and source of heat (see Table 1). The duration the reaction was run remained fairly constant at approximately 16 hours of heating/stirring (overnight). Bromobenzene was the most common aromatic halide utilized for this reaction, however iodobenzene for the aryl source was also used, as well as 4-iodoacetophenone, with the intention that increasing the reactivity of the phenyl-halide may drive the reaction to completion. Potassium carbonate was the most commonly used base, but caesium carbonate was tried with no discernible difference. The solvents used were either toluene or 1,4-dioxane (dioxane) at 1 M concentration (with respect to the oxazolidinone) due to their high boiling points; dioxane was slightly favoured due to literature articles reporting its usefulness. As well, dioxane has increased polarity over toluene, assisting in solubilizing the oxazolidinones. While dioxane did respond more favourably to microwave radiation/heating than did toluene, the downside to the use of either solvent was that conventional heating in an oil bath was often required as even 1,4-dioxane is not considered a generally good solvent for microwave heating.

Some aryl-coupling reactions were successful, which are illustrated by being shaded grey in Table 1. Not all products were fully isolated, and as a result the yield of the successful reactions was not always reflective of their success.

Table 1 – Conditions for the *N*-aryl Coupling of Oxazolidinones & Halogenated Aromatics

	Ph-	**St. Mat.**	**Cu Cat.**	**Cu Lig.**	**Pd Cat.**	**Lig.**	**Solvent**	**Base**	**Yield**
1	Tol-I	-OH (**3**)	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
2	Tol-I	-Tosyl (**13**)	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
3	Ph-Br	-OH (**3**)	CuI (5%)	TDACH (11%)	-	-	Dioxane	K_2CO_3 (2eq)	-
4	2-IA	-OH (**3**)	CuI (1%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
5	2-IA	-MOM (**6**)	CuI (2.5%)	TDACH (10%)	-	-	Dioxane	K_3PO_4 (1eq)	-
6	4-AP	-OH (**3**)	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
7	4-AP	-Tosyl (**13**)	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
8	Ph-Br	Oxazoli-dinone	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	44%
9	4-AP	Oxazoli-dinone	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	47%
10	Ph-Br	-Ac (**22**)	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
11	Ph-Br	Oxazoli-dinone	-	-	$Pd_2(dba)_3$ (0.2%)	**27** (4%)	Toluene	Cs_2CO_3 (1.4eq)	-
12	Ph-Br	Oxazoli-dinone	-	-	$Pd_2(dba)_3$ (2.5%)	**28** (5%)	Toluene	Cs_2CO_3 (1.4eq)	-
13	Ph-Br	Oxazoli-dinone	CuOTf (5%)	-	$Pd_2(dba)_3$ (0.25%)	**28** (5%)	Toluene	Cs_2CO_3 (1.4eq)	<5%
14	Ph-Br	Oxazoli-dinone	CuOTf (2.5%)	-	$Pd_2(dba)_3$ (2.5%)	**28** (7.5%)	Dioxane	Cs_2CO_3 (1.4eq)	-
15	Ph-Br	Oxazoli-dinone	CuOTf (2.5%)	-	$Pd_2(dba)_3$ (2.5%)	**28** (7.5%)	^{s}BuOH	Cs_2CO_3 (1.4eq)	-
16	Ph-Br	Oxazoli-dinone	CuOTf (2.5%)	-	$Pd_2(dba)_3$ (2.5%)	**28** (7.5%)	DMF	Cs_2CO_3 (1.4eq)	-
17	Ph-Br	Oxazoli-dinone	Cu_2O (5%)	1,10-P.A. (5%)	$Pd_2(dba)_3$ (2.5%)	**28** (5%)	Dioxane	Cs_2CO_3 (1.4eq)	<5%
18	Ph-Br	Oxazoli-dinone	Cu_2O (5%)	1,10-P.A. (5%)	$Pd_2(dba)_3$ (2.5%)	**28** (5%)	^{s}BuOH	Cs_2CO_3 (1.4eq)	14%

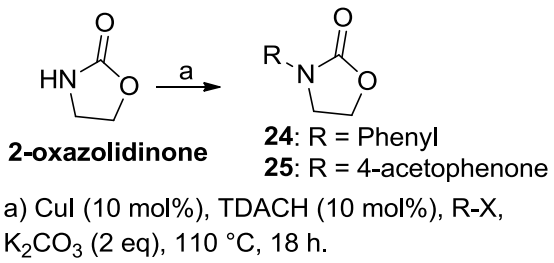
	Ph-	St. Mat.	Cu Cat.	Cu Ligand	Pd Catalyst	Lig.	Solvent	Base	Yield
19	Ph-Br	Oxazoli-dinone	Cu_2O (5%)	1,10-P.A. (5%)	$Pd_2(dba)_3$ (2.5%)	**28** (5%)	DMF	Cs_2CO_3 (1.4eq)	<5%
20	Ph-Br	Oxazoli-dinone	Cu_2O (5%)	1,10-P.A. (5%)	$Pd_2(dba)_3$ (2.5%)	-	^{s}BuOH	Cs_2CO_3 (1.4eq)	N.I.
21	Ph-Br	Oxazoli-dinone	Cu_2O (5%)	1,10-P.A. (5%)	$Pd_2(dba)_3$ (2.5%)	-	DMF	Cs_2CO_3 (1.4eq)	N.I.
22	Ph-Br	-MOM (**6**)	CuI (20%)	TDACH (20%)	-	-	Dioxane	K_2CO_3 (2eq)	-
23	Ph-Br	Oxazoli-dinone	CuI (10%)	TMEDA (10%)	-	-	Dioxane	K_2CO_3 (2eq)	-
24	Ph-Br	Oxazoli-dinone	CuI (10%)	TMEDA	-	-	TMEDA	-	5.5%
25	Ph-Br	Oxazoli-dinone	CuI (10%)	TDACH (10%)	-	-	Dioxane	K_2CO_3 (2eq)	30%
26	Ph-Br	-MOM (**6**)	CuI (20%)	TDACH (20%)	-	-	Dioxane	K_2CO_3 (2eq)	-
27	Ph-Br	-MOM (**6**)	CuI (120%)	TDACH (20%)	-	-	Dioxane	K_2CO_3 (2eq)	-
28	Ph-Br	Oxazoli-dinone	CuI (10%)	1,10-P.A. (10%)	-	-	Dioxane	K_2CO_3 (2eq)	40%
29	Ph-Br	-MOM (**6**)	CuI (20%)	1,10-P.A. (20%)	-	-	Dioxane	K_2CO_3 (2eq)	-
30	Ph-Br	Oxazoli-dinone	CuI (10%)	1,10-P.A. (10%)	$Pd_2(dba)_3$ (1.5%)	-	Dioxane	K_2CO_3 (2eq)	10%
31	Ph-Br	Oxazoli-dinone	CuI (10%)	1,10-P.A. (10%)	$Pd_2(dba)_3$ (1.5%)	**28** (3%)	Dioxane	K_2CO_3 (2eq)	<5%
32	Ph-Br	-OH (**3**)	CuI (20%)	1,10-P.A. (20%)	-	-	Dioxane	K_2CO_3 (2eq)	-
33	Ph-Br	-MOM (**6**)	CuI (50%)	1,10-P.A. (50%)	-	-	Dioxane	K_2CO_3 (2eq)	-
34	Ph-Br	-MOM (**6**)	Cu_2O (10%)	1,10-P.A. (10%)	$Pd_2(dba)_3$ (5%)	**28** (10%)	^{s}BuOH	Cs_2CO_3 (2eq)	-
35	Ph-Br	$-N_3$ (**14**)	CuI (110%)	TDACH (110%)	-	-	Dioxane	Cs_2CO_3 (2eq)	-
36	Ph-Br	-MOM (**6**)	CuI (196%)	TDACH (194%)	-	-	Dioxane	K_2CO_3 (2eq)	36%
37	Ph-Br	-MOM (**6**)	CuI (250%)	TDACH (250%)	-	-	Dioxane	K_2CO_3 (2eq)	62%

Table 1: All reactions run at 110 ºC, except entries 1 and 2 which were run at 80 ºC, N.I. = Not isolated, any yields <5% were determined by TLC, Lig. = ligand, T = temperature, Tol-I = 4-iodotoluene, 2-IA = 2-iodoanisole, 4-AP = 4-acetophenone, Ph-Br – bromobenzene, TDACH = 1,2-trans-diaminocyclohexane (racemic), $Pd_2(dba)_3$ = tris[dibenzylideneacetone]dipalladium(0), CuOTf = copper (I) triflate·benzene, 1,10-P.A. = 1,10-phenanthroline, TMEDA = *N*,*N*-tetramethyl ethylenediamine, **27** & **28** see Figure 24, 1% = 1 mol %, solvent concentration 1 M with respect to oxazolidinone unless otherwise specified, reaction run overnight (~16 h) unless otherwise specified, successful reactions in grey.

The free alcohol on the 4 position of the oxazolidinone ring found on compound **3** was likely interfering with the reaction/catalyst in some form and preventing product formation. As a result, any THAM oxazolidinone derivative that might be converted to the alcohol over the course of the reaction was likely to fail as well. Acetoxy **22** was eliminated as a potential starting material quickly as it was not stable in the presence of base at the elevated temperatures imperative for this reaction to occur without degrading to the alcohol. Similar issues with stability were seen with the tosyl derivative **13** being heated in base overnight, which likely accounts for the failure of the tosyl derivative to work. MOM-derivative **6** was also tried as the oxazolidinone source; however, obtaining a sufficiently dry sample was very difficult and this water might have resulted in the failed coupling in this reaction.

Despite a multitude of failed reactions it could still not be determined if the reaction was failing as a result of the oxazolidinone source or another facet of the conditions used. To rectify this lack of understanding, a trial reaction was run using unsubstituted 2-oxazolidinone; this reaction was successful using either bromobenzene or 4-iodoacetophenone as the phenyl source, with CuI and 1,2-*trans*-diaminocyclohexane as

the catalyst and ligand respectively (Scheme 22). 2-Oxazolidinone was also treated with bromobenzene and CuI at the catalyst, with tetramethylethylenediamine (TMEDA) acting as the ligand, base, and solvent; this variation of the reaction was successful, but resulted in a very low yield (5.5%). Novel phosphine ligands synthesized within the McNulty group were also used as a co-ligand (Figure 24), but experiments showed that they were not needed for the reaction to proceed, so they were abandoned.[145]



Scheme 22 – N-Arylation of 2-oxazolidinone

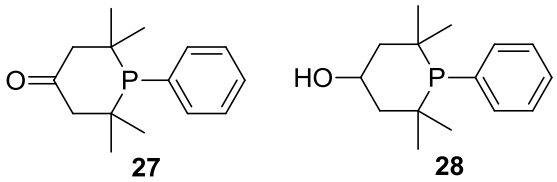
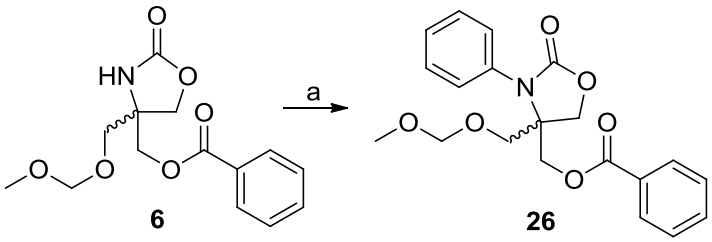


Figure 24 – Novel phosphine ligands developed by the McNulty group[145]

When the optimized conditions were used on a THAM derivative the reaction initially failed. Alterations to the optimized conditions continued with excess catalyst and ligand being the key to running this reaction on a THAM-based oxazolidinone. When 196 mol% catalyst and ligand were used on MOM-oxazolidinone **6** the *N*-arylation proceeded

with a 36% yield to afford **26**. If the catalyst/ligand loading was increased to 250 mol% the yield rises to 62% (Scheme 23). This increase was likely due to the first equivalent of copper being sequestered by the MOM group, therefore the more excess copper that was added to the mixture the more copper that would be available for use as a catalyst. As successful as these conditions were for the MOM-compound **6**, they failed when they were applied to tosylate **13** or azide **14**.



a) CuI, TDACH, PhBr, K_2CO_3, Dioxane, 110 ºC, 4 days, 62%

*Scheme 23 – N-Arylation of Mom-oxazolidinone **6***

Due to the requirements for the *N*-arylation reaction to proceed, there were limits to the scope and usefulness of this reaction. Should an *N*-arylated product have been desired, it would have required protection of the free alcohol, followed by *N*-arylation, and then deprotection of the alcohol, which would be a lengthy synthesis. This avenue may be revisited in the future for a more target-based synthesis, but was wrapped up to investigate more promising avenues of the project.

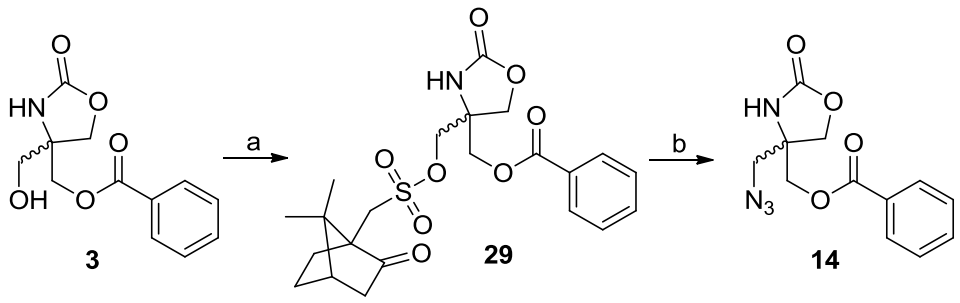
Chiral Resolution of Fragmented Protected THAM:

All oxazolidinone compounds synthesized within this project have been racemic mixtures of the compounds. We wanted to explore different routes to resolve the enantiomers, allowing us access to enantiomerically pure samples. Ideally, the first compound to be separated into its separate enantiomers would be **3** as once **3-*R*** and **3-*S*** were isolated, the synthesis could then be scaled up so that later derivatives (e.g. tosylate **13** and azide **14**) were synthesized directly from the enantiomerically pure sample of **3**, reducing the need to optimize the resolution on more than one compound.

There are several different ways to resolve the *R* and *S* enantiomers of the THAM-based oxazolidinone series. The first method would be HPLC separation of the compounds on a chiral column. The drawback to this method is the cost associated with running a preparative-scale HPLC separation; this method would only be a possibility if alternate methods failed. Although resolution of enantiomers is not possible by silica column chromatography, separation of diastereomers by said method is feasible. Therefore, the separation of THAM-based oxazolidinone enantiomers can also be done by adding a set chiral entity to the oxazolidinones to transform the mixture of enantiomers into a mixture of diastereomers. From there it is theoretically possible to separate the mixture of diastereomers through silica column chromatography, followed by the removal of the recently-added chiral entity to give the separated enantiomers.

The first chiral resolution tool used was a camphor sulfonyl derivative. This camphor sulfonate had a similar backbone to a tosylate, and therefore could act in a similar manner as the previously used tosyl leaving group. This similarity would allow

the synthesis of later derivatives in the oxazolidinone series directly without the need to remove the camphor adduct and then add a tosyl group to continue the synthetic pathway. Oxazolidinone **3** was treated with (*R*)-(-)-10-camphorsulfonyl chloride in DCM with base (TEA, pyridine, or lutidine) to afford **29** as a mixture of diastereomers (Scheme 24). While two distinct sets of peaks were visible in the ^{1}H NMR spectrum corresponding to the two diastereomers (*R*,*S* & *R*,*R*), only one spot was visible by TLC.



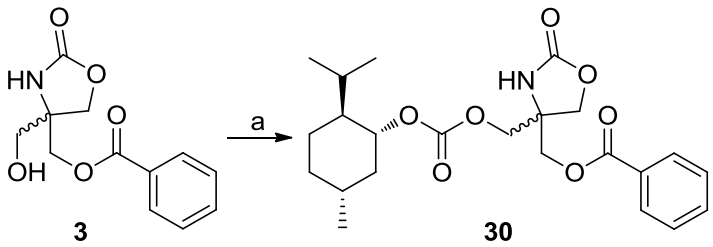
a) (1R)-(-)-10-camphorsulfonyl chloride, lutidine, rt, 22h, 39%; b) NaN_3, DMF, 70 ºC, overnight.

*Scheme 24- Synthesis of camphor-THAM derivative **29***

Effort was made to recrystallize the mixture of **29** into its separate diastereomers, however, this was also not feasible. To circumvent the need to separate the two diastereomers prior to use, it was hoped that one diastereomer of **29** would be more reactive and therefore react preferentially over the other in a substitution reaction, allowing for separation of the diastereomers to be avoided in lieu of separation of two different products. To test this hypothesis **29** was treated with 0.5eq of NaN_3 at 0 °C for 30 minutes, yet no product was observed by TLC and only starting material could be

visualized. The temperature was raised to room temperature for another 30 minutes, but there was still no reaction as visualized by TLC. Starting at 30 ºC and increasing to 70 ºC in 10 ºC increments the reaction was allowed to continue, but no evidence of product formation could be found. Eventually excess NaN_3 was added and the mixture was heated at 70 ºC, which afforded the same azide THAM derivative **14** (Scheme 14) as was originally synthesized from tosylate **13** (Scheme 24). As this was a test reaction the yield was not reflective of the success of the reaction, but as there was total spot-to-spot conversion by TLC analysis this reaction is thought to behave in the same fashion as tosylate **13**. Although this finding suggests that the camphor derivative **65** can be used in place of the tosylate **13** to carry out future derivatizations, selectively reacting one diastereomer in lieu of the other may not be possible.

The next chiral derivative tried was a menthol derivative. (1*R*)-(-)-menthyl chloroformate was added to **3** with TEA in DCM to yield the derivative **30** (Scheme 25). Unfortunately, separation quickly became an issue as menthol elutes at a similar R_f as **30**, making it very difficult to get pure samples of **30**, or even visualize how many spots/compounds are present. Effort was invested to purify the starting material (1*R*)-(-)-menthyl chloroformate by vacuum distillation, but this only produced a viscous, yellow oil that appeared to be less pure (by TLC and the products themselves after subsequent reaction with **3**) than the starting, non-distilled material. As it was impossible to obtain a pure sample of **30**, no spectra were obtained or yield calculated. It is doubtful that a sufficiently pure sample of (1*R*)-(-)-menthyl chloroformate will ever be obtained as to make this method a viable and attractive option.



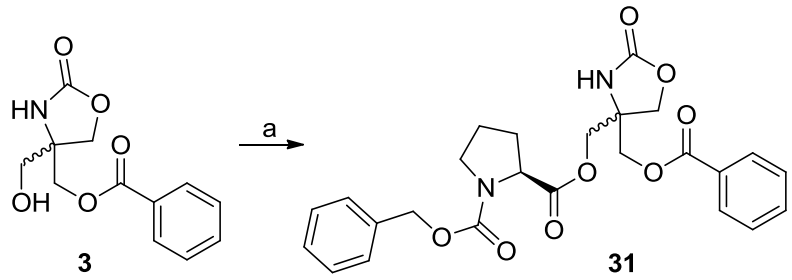
a) (-)-menthyl chloroformate, base, DCM, rt, overnight.

*Scheme 25- Synthesis of menthol-THAM derivative **30***

As the more polar a product is the more strongly it adsorbs to silica, thereby enhancing any differences in the R_f values between different structures, it was thought that a more polar derivative added to the THAM-based oxazolidinone may be easier to separate by silica column chromatography. The final derivative undertaken in an attempt to resolve the THAM oxazolidinones was Cbz-protected proline for a few reasons: the Cbz-proline derivative has a higher polarity than either the menthyl or camphor derivatives previously formed, and it possesses a much larger group than the menthyl entity, suggesting separation may be easier; Cbz-proline was readily available and had flexibility in its functionality; and the proline-oxazolidinone product would possess the chiral center closer to the oxazolidinone product than the camphor product **29** did, also suggesting separation to be more likely.

The first attempt to couple Cbz-proline to alcohol **3** was through a diethyl chlorophosphate-mediated esterification, but this reaction failed to produce any product and showed only starting alcohol in the ^{1}H NMR spectrum.[146] Oxazolidinone **31** was eventually isolated after a coupling of **3** to the protected amino acid through a Steglich esterification using DCC and catalytic DMAP in acetonitrile or DMF (Scheme 26).[147]

However, as was the issue previously encountered with the camphor and menthol derivatives, the ^{1}H NMR of **31** shows two diastereomers, while the TLC visualizes only one spot. In the future the Cbz group may be removed from **31**, further increasing the polarity, improving the chances of **31-*R*** and **31-*S*** having sufficiently different R_f values to be separated via a silica column.



a) Z-Pro-OH, DCC, $DMAP_{(cat)}$, MeCN or DMF, rt., overnight, 48%.

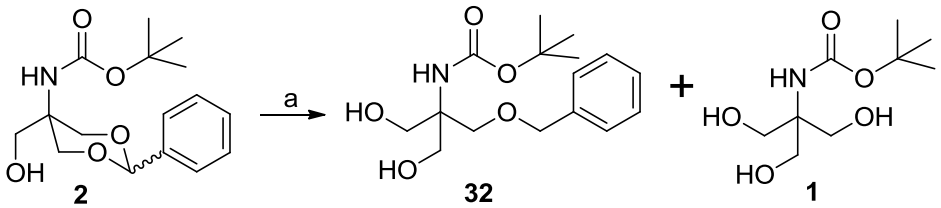
*Scheme 26 – Synthesis of L-proline-THAM derivative **31***

While these methods mentioned were unsuccessful, preparative-scale HPLC separation is still an option should the separate enantiomers of the oxazolidinone series be desired.

Symmetrization of THAM:

Deprotection of the alcohols of Boc/benzylidene acetal **2** and oxazolidinone/benzoate **3** were both envisioned for this project with the intent of creating symmetrical THAM analogs as this type of scaffold could lead to a symmetrical target molecule, such as fluconazole (Figure 16). This methodology of opening up the benzylidene diol-protecting group to a free alcohol and protected alcohol could also be useful in later THAM-based sphingosine or FTY720 derivatives as a method to differentiate the alcohols after manipulation of the alternate free alcohol has been completed.

Theoretically, the free alcohol on **2** could be used 'as-is' for any transformations prior to the diol being symmetrically functionalized, but as a test reaction the benzylidene acetal on **2** was reductively opened to an *O*-benzyl and hydroxyl derivative using DIBAL (**32**, Scheme 27).[148] The reaction proceeded to give diol **32** in a moderate yield (56%), in addition to giving triol **1** as a major side-product. A shorter reaction time (approximately 12 h) may increase the yield to an acceptable level and lessen the unwanted formation of triol **1**.



*Scheme 27 – Reduction of Boc/Bz THAM (**2**) using DIBAL*

Despite potential obstacles, this reaction did produce product **32** reliably and it was conclusively proved that the benzylidene acetal could be opened up in the presence of the *N*-Boc and hydroxyl groups. This reduction renders a free alcohol in the product, unlike the fragmentation, which renders a benzoate group; therefore if one desires an alcohol (or if an alcohol is already present, a diol) in the product this method would remove the deprotection step required if the fragmentation route is adopted. Additionally, the product formed from this reaction possesses two protecting groups, Boc & benzyl, both of which can be subsequently manipulated in a chemoselective manner.

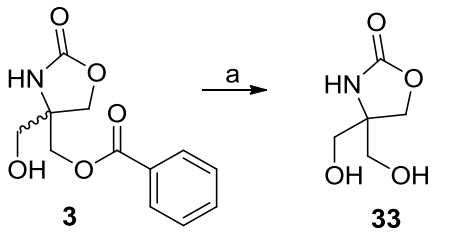
This reduction also allows the potential for differentiating the alcohols in situations where the novel fragmentation mentioned previously may not be a viable option. Additionally, retaining the original aromatic ring from the benzaldehyde dimethyl acetal, this synthetic route could be amenable to solid-phase synthesis; the novel fragmentation would require removal of the benzoate group to make available a free alcohol, which would liberate it from a solid phase synthesis had it been attached to one via the phenyl ring.

Functional groups within future products that are not compatible with DIBAL would be a potential issue with this synthetic pathway. It may also not be possible to remove the *O*-benzyl group from the future substrate (e.g. derivatives containing hydrogenation-sensitive functional groups), and therefore it may be necessary to go through a deprotected oxazolidinone rather than **32**.

While a DIBAL reduction of a benzylidene-containing product is an option, it means that the resulting compound will not contain an oxazolidinone ring during any

point of the synthesis, which would be disadvantageous for the purposes of biological testing and expanding the oxazolidinone-based chemical library. To rectify this issue, it may be more prudent to work directly from oxazolidinone **3** to obtain a diol/symmetrical molecule.

Removal of the benzoate group of oxazolidinone **3** to give diol **33** was performed using sodium hydroxide in a water/methanol solvent mixture to create symmetry within the molecule (Scheme 28). The reaction itself was successful in that the benzoate group was cleaved as proved by the loss of the aromatic peaks in the proton NMR. There were issues with the purification of **33** as after recrystallization the product was left solvated and could not be sufficiently dried. As a result, this reaction gave a 99% mass balance, whereas a 100% yield of **33** would have given a 58% mass balance. It is believed that the additional mass was due to water being complexed with **33**. This solvation limited the type of reactions that could be run in the future, such as by preventing the addition of tosyl groups to **33**. Attempts were made to dry diol **33**(desiccator under vacuum charged with sodium hydroxide as the desiccant), however, it failed to produce sufficiently dry product as would be needed in future synthetic steps.



3 **33**

a) NaOH (0.3 M), H_2O/MeOH, rt, 2 h.

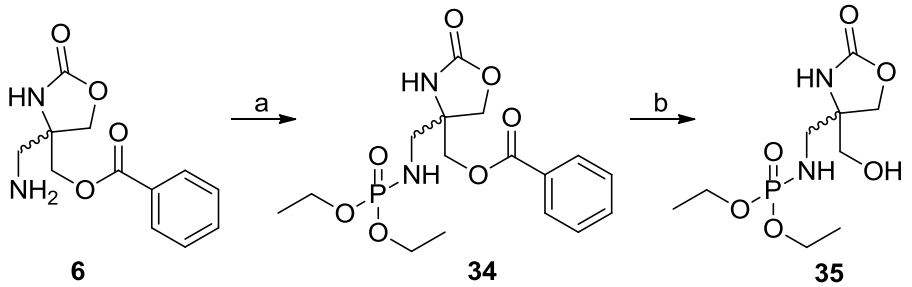
*Scheme 28 – Removal of benzoate from **3** to give diol **33***

Problems with purification of this deprotection reaction were more complex than the product simply being solvated; purification became a major issue as the diol product is so high in polarity that silica column chromatography would be difficult. Another option that had been tested was washing the product with solvent (with either water to remove the diol, or ether to remove just the benzoic acid), but this has failed to render a sufficiently pure product, and again solvation of the product would be a serious concern.

Even after acquisition of diol **33**, other problems were still present. One potential obstacle in following the oxazolidinone-diol route was that the diol may be too reactive: during the reaction of attaching leaving groups to the alcohols, only one alcohol would be converted to a leaving group/electrophile, while the other would remain as an alcohol/nucleophile. This mono-leaving group/mono-alcohol situation may cause an intramolecular cyclization between the two groups. Due to the difficulties presented with removal of the benzoate group of **3**, this synthetic avenue was abandoned; if a symmetrical molecule is desired it will have to be obtained via diol **32**.

Sphingosine Derivatives:

One of the main goals of this project was the synthesis of a THAM derivative with a phosphate group as it could resemble S1P or FTY720-P, and thus may inhibit S1K as a result. To achieve this scaffold, the amine derivative of THAM-based oxazolidinone **6** was reacted with a chlorophosphate derivative to give the mono-phosphoramide product **34** (Scheme 29). Ethyl chlorophosphate was the reactant of choice for this reaction as it was readily available and could be converted to the dimethyl phosphate or phosphoric acid derivative directly. Phosphoramide **34** can be used as a substitute for a phosphate ester for products such as THAM-based sphingosine analogs. Additionally, the phosphoramide product was stable at room temperature and can offer more stability for future synthetic steps than a phosphate ester, such as those found on S1P. Removal of the ethyl chains from the phosphoramide would be required to be a true S1P mimic, but this would most likely be achieved in the future from the dimethyl chlorophosphate, rather than diethyl chlorophosphate.



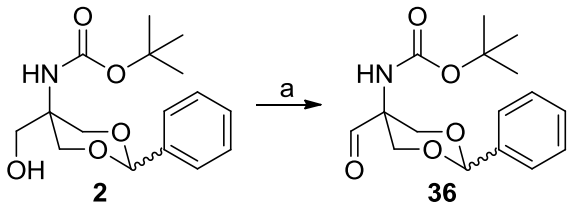
a) diethyl chlorophosphate, lutidine, DCM, 0 ºC to rt, 2 h, 51%; b) NaOH/H_2O (0.125 M), 1 h, 92%.

*Scheme 29 – Conversion of **6** to phosphoramides **34** & **35***

After addition of the phosphoramide, selective removal of the benzoate group of **34** was performed under basic aqueous conditions (~0.125M $NaOH/H_2O$) to render a free hydroxyl group in very high yields (**35**, Scheme 29). Despite treatment of **34** under such basic conditions, there was no evidence that the phosphoramide or oxazolidinone ring were affected in any manner. If desired, the free hydroxyl of **35** can be converted into a good leaving group, as was shown previously with the addition of a tosyl group (Scheme 13) and a second substitution performed to introduce further functionality. An alternate option would be oxidation of the alcohol to an aldehyde (if the previous solubility issues surrounding the oxazolidinone ring can be resolved). If the aldehyde version of **35** can be synthesized it could be used in a Wittig-type reaction to add the long-chain alkene required for Sphingosine.

Another route towards the synthesis of sphingosine analogs was through a Wittig reaction with an aldehyde version of **2**, thereby attaching an alkyl tail to the THAM scaffold via an alkene, giving the basic backbone of a long lipophilic tail with a polar head. Unlike the problematic issues encountered with oxidizing oxazolidinone **3** (Scheme 14), solubility of alcohol **2** was not a concern. As a result, the oxidation of the alcohol of **2** via a Swern reaction to the aldehyde proceeded with good yields (79%) to compound **36**, with a column performed for purification (Scheme 30). As with alcohol **2**, aldehyde **36** is a mixture of two *cis*/*trans* isomers; unfortunately, unlike the precursor alcohol **2**, the *cis*/*trans* isomers of aldehyde **36** could not be separated using silica column chromatography. Consequently, if separate isomers of **36** were desired for biological testing of it or later derivatives, separation would have to occur on alcohol **2**, prior to later

synthetic steps. However, **36** was reported as separate isomers by the McNulty group in the literature, with the spectral data reported for each isomer, further rectifying the oversight committed by previous research groups that reported **36** as a single isomer.[88,130,131]



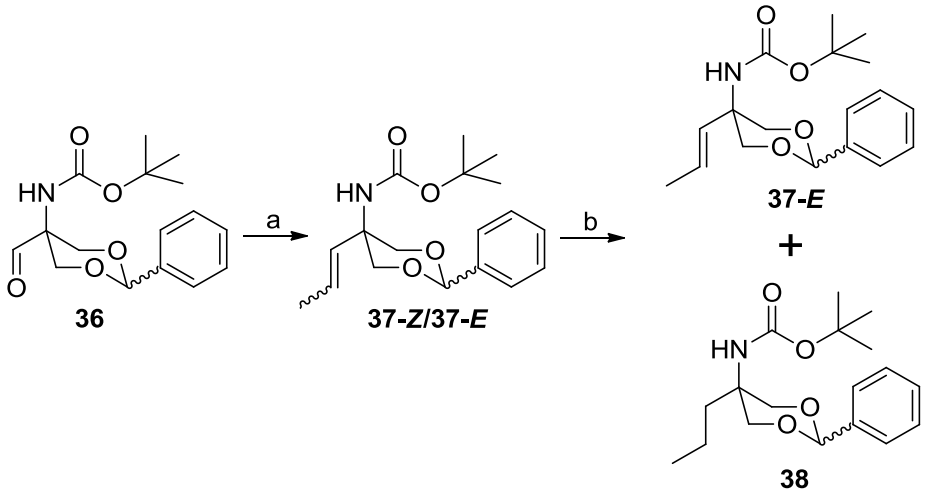
2 **36**

a) 1) $(COCl)_2$, DMSO, DCM, -78 °C, 40 mins; 2) **2**, -78 °C to -20°C, 55 mins; 3) Et_3N, -20 °C to rt., 2 h, 79%.

*Scheme 30 – Oxidation of alcohol **2** to aldehyde **36***

Because the aldehyde was adjacent to a tertiary carbon, the resulting neopentyl aldehyde **36** was stable at room temperature in the presence of atmospheric air for long periods of time (in excess of a year). The stability of the aldehyde was because the enol cannot be formed through tautomerization as the carbonyl was adjacent to a tertiary carbon, thus preventing any unwanted aldol addition reactions. This reaction was also amenable to larger batches as it was scaled up to the gram scale with no effect on the yield. The yield for the Swern oxidation (as well as the original *N*-Boc/benzylidene dual-protection to make **2**) was also consistent with literature values reported in 2011, unlike previous articles that reported unbelievably high yields (~95-98%) for the same or a similar compound.[88,130]

Since aldehyde **36** was synthesized to act as a precursor to a sphingosine/S1P-type analog, addition of an alkyl tail was the next synthetic step towards this target. As a proof-of-concept experiment, aldehyde **36** was first utilized in a Wittig reaction using triphenylethylphosphonium bromide to give a propylene tail on the THAM scaffold as a mixture of four isomers (*E*/*Z* & *cis*/*trans*, **37-*E*/37-*Z***, Scheme 31). While sphingosine had an alkene in its alkyl backbone, reduction of the double bond to the alkane through simple hydrogenation with hydrogen over Pd was attempted to verify what types of analogs with this scaffold would be possible (**38**, Scheme 31). This removal of the alkene functional group was verified through ^{1}H NMR by the disappearance of the sp^{2}-carbon protons around 5.7 ppm, as well as through ESI^{+} mass spec with a mass peak at 322.2 m/z. Interestingly, only the double bonds with a *Z* configuration were hydrogenated (both *cis*/*trans* chair isomers), while the *E* alkene (**37-*E***) remained after hydrogenation. This was verified through visualization of the alkene double bond protons at 5.9 ppm on the ^{1}H NMR spectrum, as well as a small peak in the ESI^{+} at 2 lower than the molecular ion peak, signifying the lack of two protons. This retention of the *E* isomer may have been due to the *E* isomer having difficulty getting physically close enough to the surface of the platinum due to steric hindrance, and therefore it was unable to react with the hydrogen on the surface of the platinum at the same rate as the *Z* isomer.

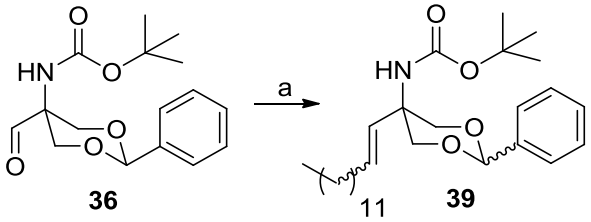


a) 1) $Ph_3PEt^+Br^-$, NaHMDS, THF, -78 ºC to 0 ºC, 30 mins; 2) **36**, -78 ºC to rt., 21 h, 78%; b) $H_{2(g)}$, Pd/C (10%), THF, rt, 3 h.

*Scheme 31 – Wittig reaction with **36** to give alkene **37-Z/E** and alkane **38***

To expand this concept of adding an alkyl tail to the THAM scaffold to better fit the goals of this project, a more sphingosine-like analog than propene needed to be synthesized. In lieu of the three-carbon chain, a fourteen-carbon chain was added to the THAM backbone through the addition of a tridecane chain. Therefore, a Wittig reaction was performed under the same conditions as the propene analog **37** (Scheme 31), but with triphenyltridecylphosphonium bromide as the alkylating agent, to afford the C_{14} analog **39** as a mixture of isomers (Scheme 32). A hydrogenation of this product was never performed as the alkane version did not match any analogs, as well as it failed to advance my project a significant amount. Removal of all protecting groups was also avoided as the sample contained both the *E* and *Z* isomers of the alkene, and therefore biological testing as a sphingosine analog would not have been accurate. Removal of the double bond prior to removal of the protecting groups would not have been useful as such a compound has

already been reported in the literature. However, this reaction did illustrate that different groups could be introduced to the THAM-scaffold via the aldehyde **36** through a Wittig reaction.



36 **39**

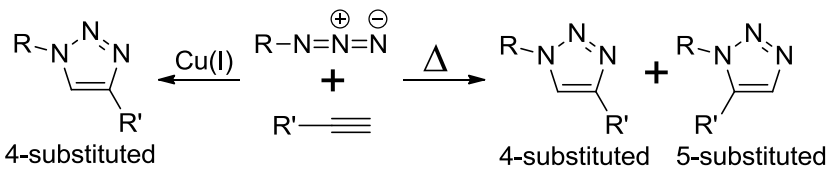
a) 1) Ph_3P-$C_{13}H_{25}^{+}$ Br^{-}, NaHMDS, THF, -78 ºC to 0 ºC, 30 mins. 2) **36**, -78 ºC to rt, 20 h.

*Scheme 32 – Wittig with aldehyde **36** to give **39***

FTY720 Analogs:

One main avenue envisioned for this project was the synthesis of FTY720 analogs. FTY720 is fairly structurally similar to sphingosine, but with a phenyl ring in the alkyl tail backbone in lieu of an alkene and with a slight change in the position of the alcohol groups. To steer away from a direct synthesis of FTY720, it was thought that a triazole ring in lieu of the phenyl ring may retain activity while creating a sufficiently-new compound. Addition of a triazole ring to the THAM-scaffold that had been previously synthesized could be achieved via a substitution reaction with a long-chain alkyl-bearing triazole ring, or more easily, through a Huisgen cycloaddition reaction.[149]

Colloquially known as the 'click' reaction between an azide and an alkyne, the Huisgen cycloaddition is a heterocyclic ring-forming reaction between an alkyne and azide to give a 1,2,3-triazole ring. This 'click' reaction was first performed using heat to drive the reaction forward. Unfortunately, with just heat to catalyze the reaction, both the 4- and the 5-substituted isomers of the triazole ring are usually formed (Scheme 33).[149,150] In 2002, Sharpless, *et al*. reported using copper (I) as a catalyst for the Huisgen cycloaddition reaction and produced only one regioisomer of the product (4-substituted, Scheme 33).[151]



Scheme 33 - Huisgen Cycloaddition Reaction

Triazole rings have been reportedly used as components within antibiotic Linezolid derivatives with some success.[101,152,153] Additionally, reports of triazole-containing FTY720 derivatives have been published in the literature, but with significantly less frequency than Linezolid analogs.[154] Therefore, it was believed that substitution of a triazole ring in lieu of a phenyl ring on FTY720 would be a logical step for this project as a triazole ring is approximately the same physical size and electronically behaves in a similar fashion to a phenyl ring (Figure 25). Furthermore, by placing the triazole ring one carbon closer to the THAM backbone, it would further avoid conflicts with existing patents.

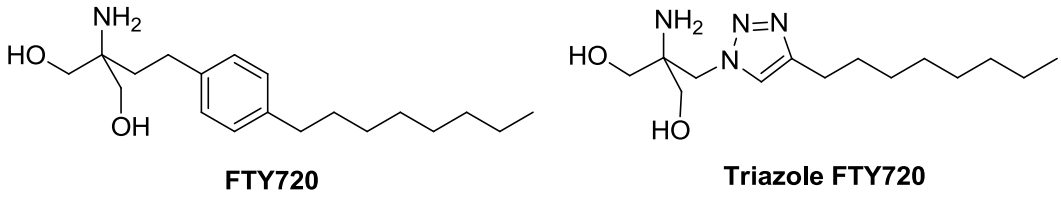
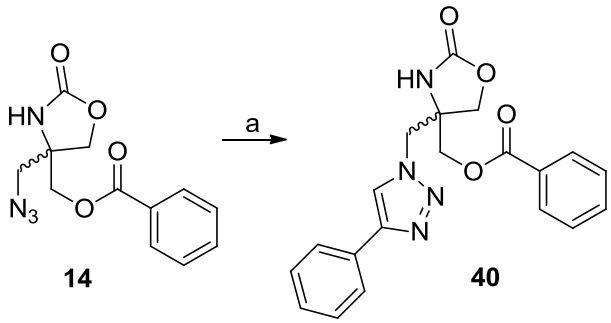


Figure 25 – Triazole FTY720 analog

Addition of the triazole ring onto the existing oxazolidinone scaffold can be achieved using an alkyne with azide **14** through a Huisgen cycloaddition reaction using a copper (I) catalyst to ensure the reaction proceeds regioselectively and renders a single regioisomer of the product. Prior to addition of a long-tailed alkyne, a test reaction was conceived to use phenyl acetylene as the alkyne as it was readily available. Azide **14** was therefore reacted with phenyl acetylene in the presence of copper (I) iodide and copper

(II) acetate (5 mol% each) as the catalysts in a model reaction to produce **40** with relatively good yields (Scheme 34).

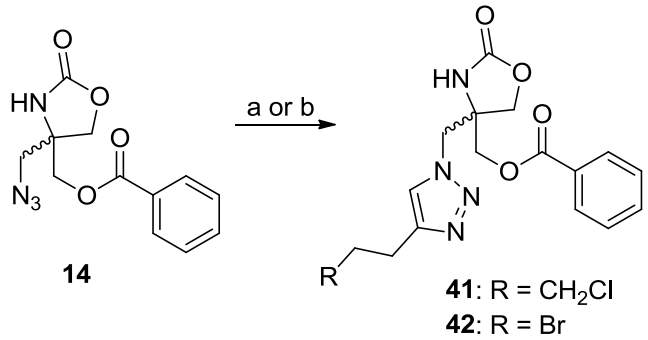


a) phenyl acetylene, $CuI_{(cat)}$, $Cu(OAc)_{2(cat)}$, THF, 40 °C, 21 h, 71%.

*Scheme 34 – 'Click' reaction on azide of **14** to give triazole **40***

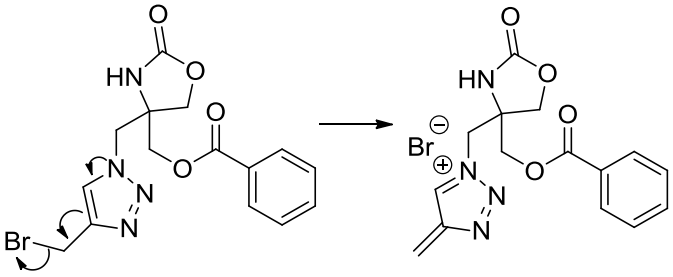
The ^{1}H NMR spectrum shows a singlet at approximately 7.9 ppm that integrated to one proton (with respect to the *O*-benzoate protons), signifying the likely presence of only one regioisomer, thought to be **40** from the regioisomer reported in the literature. Had both regioisomers of **40** been produced then the peak at 7.9 ppm would have integrated to less than one proton and there would very likely have been another singlet also integrating for less than one proton, around the same area of the spectrum.[155] This triazole derivative models certain antibiotics that possess a triazole ring and cyclic centre group, but with significant enough alterations that would make this an interesting lead compound that would likely not infringe on intellectual property rights. Triazole **40** may also mimic the aromatase inhibitors previously synthesized in the McNulty group, furthering the possibilities for this type of scaffold.[126]

Since the triazole-FTY720 analog desired possessed a long alkyl chain, it was believed that the use of a shorter-chain alkyne with a good leaving group on the terminal end of the chain would allow derivatization of the molecule without the need to procure a different alkyne for each individual reaction. 3-bromo-1-propyne was initially used as the alkyne reagent for this 'click' reaction, but the resulting product produced was unstable, and thus never isolated. However, when 5-chloro-1-pentyne was used as the alkyne in lieu of 3-bromo-1-propyne, the afforded product **41** was sufficiently stable to survive purification through silica column chromatography with reasonable isolated yields (Scheme 35). As was the case with **40**, it was thought that product **41** existed as a single regioisomer due to the presence of only one peak in the ^{13}C NMR at 147 ppm (representing the carbon at the 4 position of the triazole ring), with the belief that substitution occurred at the 4 position based on literature precedent.[151]



*Scheme 35 – "Click" Reaction Synthesizing **41** & **42***

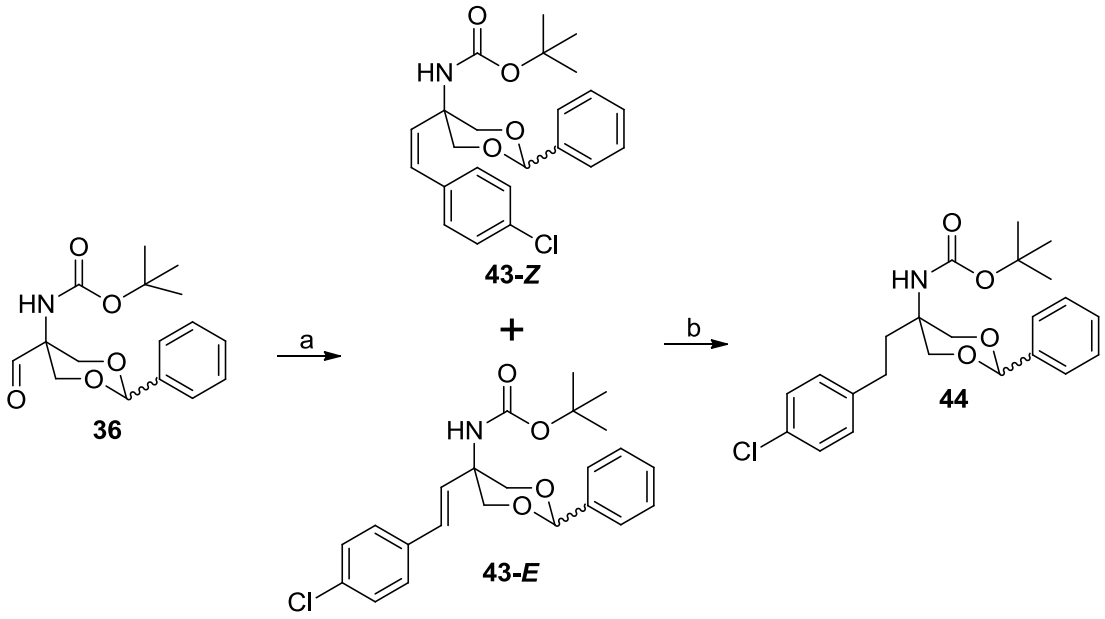
In an attempt to add an alkyl chain directly to the triazole-THAM backbone, triazole **41** was reacted with the Gilman organo-cuprate reagent Me_2CuLi, but no visible reaction occurred despite heating the reaction or adding additional Gilman reagent. It was suspected that the chlorine was not a sufficiently capable leaving group for the Gilman reaction to proceed, so bromo-derivative **42** was synthesized using the same protocol using 4-bromo-1-butyne as the alkyne. By using the bromo-butyl alkyne for the 'click' reaction it was hoped that the stability of the product would be increased as the detrimental resonance structure involved when the allylic bromide propyl version was used would be eliminated (Scheme 36). Although triazole **42** was synthesized and purified successfully as a single regioisomer using the same synthetic method mentioned earlier (Scheme 35), **42** has not been reacted with a Gilman reagent as of yet, but this may be done in the future as part of a continuation of the project.



Scheme 36 – Propyl product of 'click' reaction

Moving away from alkyne/azide cycloaddition reactions, another synthetic pathway for THAM derivatives that was investigated was the addition of an aromatic ring to the backbone. This aromatic ring would be in lieu of the alkene in the alkyl-chain tail

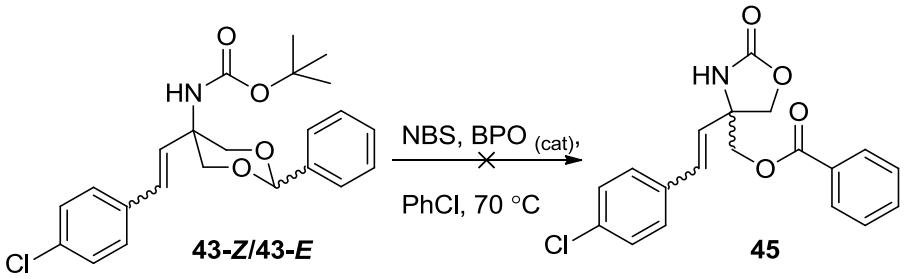
that was synthesized previously (Schemes 30 & 31), which steered the synthesis to more FTY720-type analogs as opposed to sphingosine/S1P analogs. As a proof of concept an aromatic-halide was thought to be a good choice as it would allow for later modular additions to the backbone, further expanding the chemical library. A 4-chloro substituted phenyl ring was added to the aldehyde **36** through a traditional Wittig reaction using triphenyl(4-chlorophenyl)phosphonium bromide with NaHMDS to give both the *cis*/*trans* isomers **43-*Z*/43-*E*** in very high yields (Scheme 37). This less-reactive chlorine analog was synthesized prior to the more-reactive bromine version as the 4-chlorophenyl phosphonium salt was previously made in the lab and ready for use. 4-chlorophenyl THAM derivative **43** was synthesized as both the *E* and *Z* isomers, which were separable by silica chromatography. Interestingly, **43-*Z*** slowly isomerized to **43-*E*** when dissolved in solution, so both isomers of the olefin can be isolated together and later isomerized to a single product (*E* isomer) if desired, or they can be purified separately and stored in their solid form.



a) 1) Ph_3P-(p-C_6H_4Cl)$^+$ Br^-, NaHMDS, THF, 0 ºC, 30 mins; 2) **36**, -78 ºC to rt, 15 h, 95% (as a mixture of *E*/*Z* isomers); b) $H_{2(g)}$, $Pd/C_{(cat)}$, THF, rt, overnight, 99.6%.

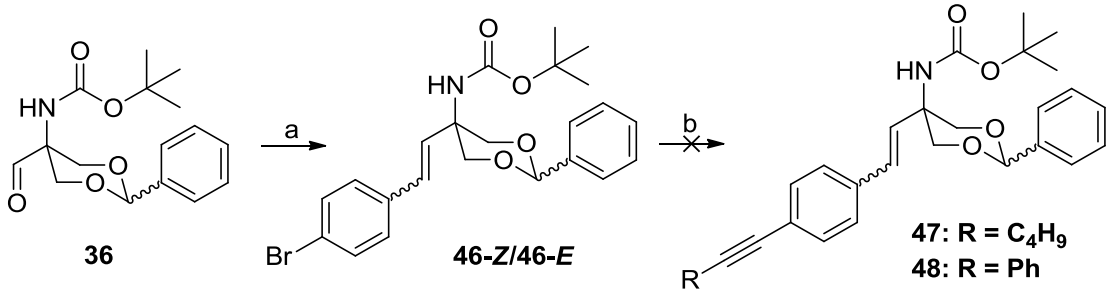
*Scheme 37 – Synthesis of **43** & **44** from aldehyde **36***

To ensure the double bond could be reduced to the alkane for the later steps in an FTY720 synthesis, **43** was hydrogenated using the classic conditions previously used in this project of hydrogen over carbon-supported palladium. Unlike the propyl analog **37**, both the *Z* and *E* isomers of **43** underwent hydrogenation (**44**, Scheme 37). Radical fragmentation of **43** to **45**, as was done previously with alcohol **2** to give oxazolidinone **3** (Scheme 6), was not successful as only starting material remained, as tested by TLC (Scheme 38). Unfortunately, the 4-chlorophenyl THAM-derivative lacks a sufficient level of reactivity to do many types of coupling reactions, so a similar 4-bromophenyl derivative was synthesized.



*Scheme 38 – Radical fragmentation of **43***

Triethyl(4-bromophenyl)phosphonium bromide was used in a Wittig reaction under similar reaction conditions as the 4-chlorophenyl derivative **43** to give the 4-bromophenyl-THAM derivative **46**, again as a mixture of four isomers with a marginally lower yield (*E*/*Z* & *cis*/*trans*, Scheme 39). Cross-coupling reactions to add functional groups to the phenyl ring of **46** via the bromine have thus far been unsuccessful. A Sonogashira coupling using a palladium (II) catalyst with copper (I) iodide has yet to form any product as tested by TLC and ^{1}H NMR, using either 1-hexyne or phenylacetylene (**47** & **48** respectively, Scheme 39).



a) 1) $Et_3P(p\text{-}C_6H_4Br)^+ Br^-$, NaHMDS, THF, 0 ºC, 2 h; 2) **36**, -78 ºC to rt, 20 h, 72% (as a mixture of *E*/*Z* isomers); b) $Pd(PPh_3)_{2(cat)}$, $CuI_{(cat)}$, R-C≡C-H, Et_3N, THF, rt, overnight.

*Scheme 39 – Synthesis of **46** and subsequent cross-coupling reactions*

Further investigations into troubleshooting the Sonogoashira coupling reaction for attaching the alkyl chain directly to the THAM scaffold for an FTY720 analog or novel FTY720 synthesis were abandoned. It may have been a possibility to attach the alkyl tail to the phenyl ring prior to its use in a Wittig reaction with the aldehyde-THAM derivative, but a previous synthesis of FTY720 reported the use of a Sonogashira coupling for the main attachment of the alkyl chain to the polar-head, thus making investigations down this avenue feckless.[3] To steer the synthesis in a more novel direction, one that would require milder reaction conditions, as well as avoided homogeneous metals for catalysts; thus we considered using a Wittig reaction for the joining of the polar head and non-polar tail.

FTY720 Total Synthesis:

As THAM possessed a significant resemblance to the polar head of FTY720, we envisioned developing a novel synthetic route to this multi-purpose medication. FTY720 can be separated into its base elements retrosynthetically (Scheme 40), which are a polar head (**36**) and a non-polar tail (**50**). These two groups, **36** & **50**, within the convergent synthesis can be joined through the use of an aqueous Wittig reaction that has been previously reported by the McNulty group, to create **49,** which is only 2 synthetic steps away from FTY720.[156]

Traditionally, a Wittig reaction was carried out under anhydrous conditions lest the phosphorus ylide act as a base and simply deprotonate the water (or any acidic proton within the reaction).[157] In addition, a traditional Wittig reaction generally favours the *Z* configuration of an alkene if an unstabilized phosphorane substrate was being used.[157] Purification for a traditional Wittig reaction also generally requires silica column chromatography due to the triphenylphosphine oxide side-product that was formed in an equimolar amount to the product. A further drawback of this anhydrous method was the requirement of a stronger base, such as an alkyl/aryl lithium, LDA, or a bis(trimethylsilyl)amide-based base: these bases are more difficult to handle and can be dangerous when a reaction is scaled up. As a consequence of using such a strong base, it is not uncommon to require the use of protecting groups on either the phosphorus salt or aldehyde as any acidic protons will be removed after addition of the base. Even if cost was not a concern, removal of an acidic proton elsewhere in the reaction could be

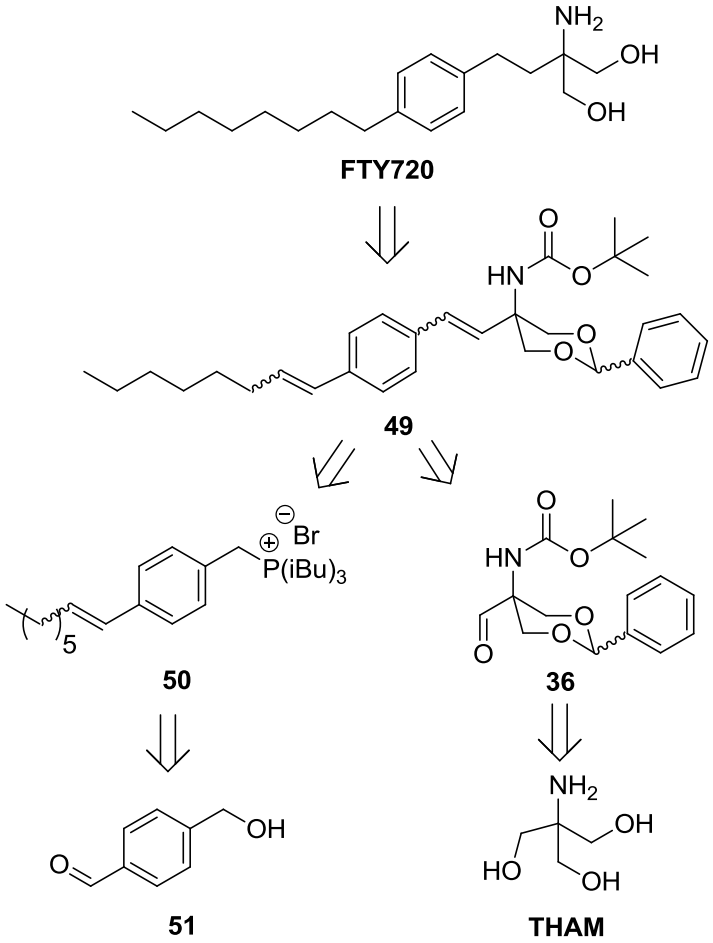
problematic; the deprotonation could potentially cause an intramolecular reaction with the newly-formed anion and another functional group that is present.

Research has been conducted into obtaining a higher proportion of the *E* alkene in lieu of the *Z* alkene from a Wittig reaction. The Schlosser modification is one method in which an additional equivalent of base is added to the reaction mixture after formation of the oxaphosphetane intermediate.[158] However, the drawback to this Wittig modification is that the reaction must still be performed under anhydrous conditions, in much the same way as the parent Wittig reaction. Another method is through a Peterson olefination or a Julia olefination, but these too would require anhydrous conditions.[159,160]

Recently the McNulty group has developed a method to perform the Wittig reaction under aqueous conditions using a milder base than traditional methods and which favors the *E* configuration of the alkene.[156,161162] The reaction is run in water and can be performed without the use of protecting groups on acidic protons, such as the N-H proton found on a carbamate or the O-H proton found on an alcohol.[163] Purification is also simplified in some cases as the resulting alkene can be filtered off from the water as the by-products (alkyl phosphonium salt, alkyl phosphine oxide, as well as base) are all water-soluble.

Continuing the retrosynthetic analysis, the polar head **36** has been synthesized previously from alcohol **2** via a Swern oxidation to render the aldehyde as a mixture of two isomers (*cis*/*trans*, Scheme 30).[130] Separation of these isomers is not required as the removal of the benzylidene protecting group in the final step to render FTY720 will remove this stereocenter. The phosphonium salt **50** can be synthesized from 4-

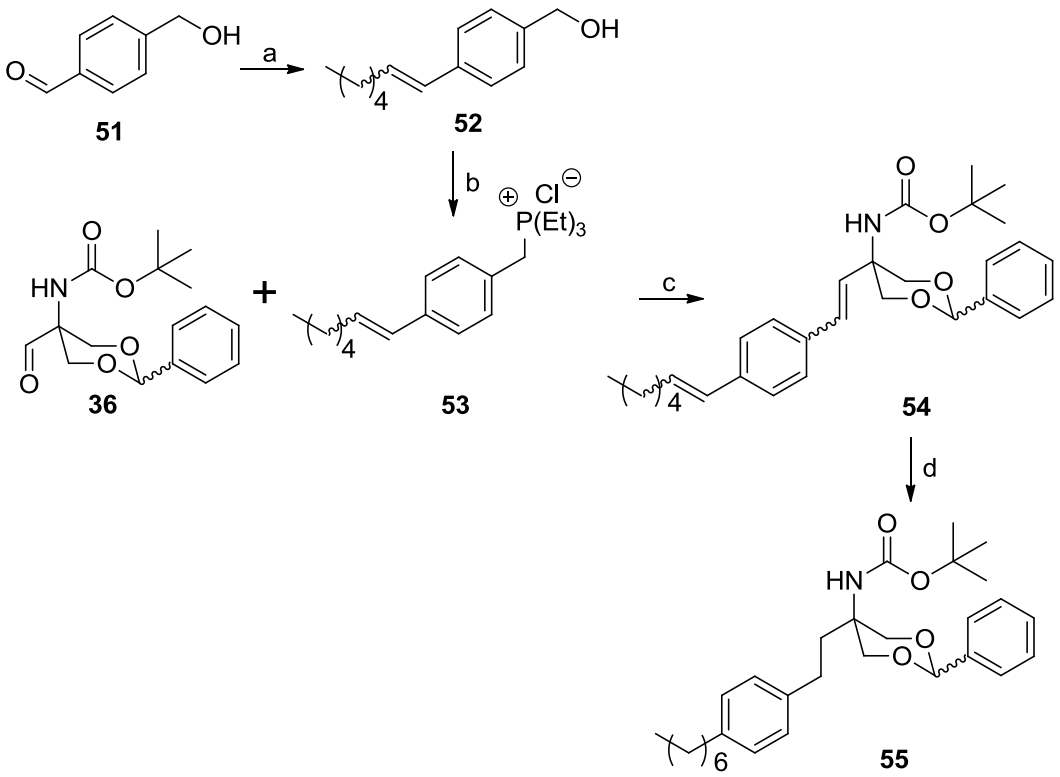
methylhydroxybenzaldehyde (**51**) through treatment with a trialkylphosphine hydrobromide salt (e.g. triisobutylphosphine hydrobromide). As the creation of FTY720 using this synthesis is modular, synthesis of analogs of FTY720 can be accommodated using this method. This research has been published by the McNulty group with myself as first author in *Tetrahedron Letters* (**2011**).[131]



Scheme 40 – Retrosynthesis of FTY720

The first step for this total synthesis was a literature procedure of a mono-reduction of terephthaldehyde using sodium borohydride in ethanol, which can be performed on the gram scale, to afford 4-(hydroxymethyl)benzaldehyde (**51**).[164] As there was a delay in the acquisition of 1-bromoheptane, 1-bromohexane was substituted for a trial reaction. Triphenylhexylphosphonium bromide was synthesized from 1-bromohexane and triphenylphoshine under traditional literature conditions (toluene, $NaI_{(cat)}$, reflux) to give triphenylhexylphosphonium bromide. Linker-aldehyde **51** was then reacted with triphenylhexylphosphonium bromide in a traditional Wittig reaction to afford **52** as primarily (>95%) the expected *E* isomer with good yields (Scheme 41).

Traditionally, the phosphonium salt **53** would have been synthesized from the benzyl bromide precursor. However, recent work performed by Dr. Das of the McNulty group showed that general benzyl phosphonium salts can be made directly from the alcohol without the need to go through the bromide intermediate. [156,161] Therefore, as a result the phosphonium salt **53** can be made from the benzyl alcohol derivative or the benzyl bromide precursor. As the alcohol precursor **52** was easier and faster to synthesize (required one fewer synthetic step), was safer to handle (benzyl bromides are often lachrymators, skin irritants, and toxic), and degraded less during storage, it was chosen as the precursor to for **53**.



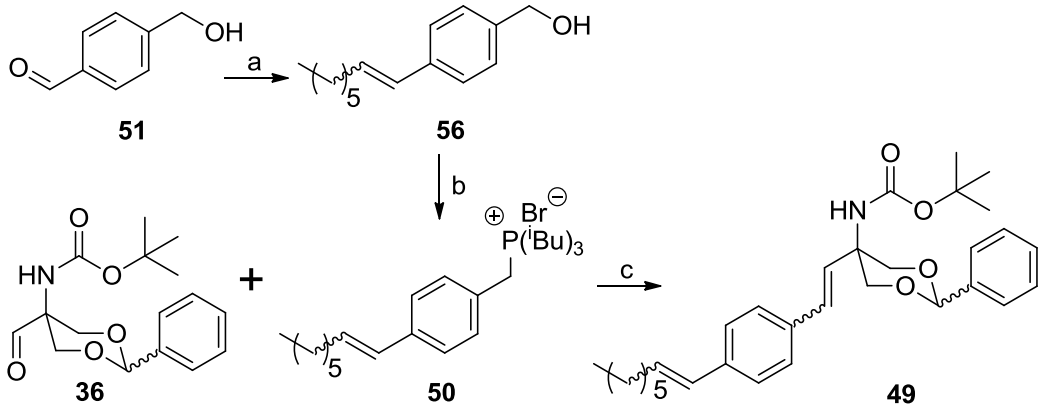
a) 1) $Ph_3P\text{-}C_6H_{13}^+Br^-$, NaHMDS, 0 °C, THF, 1.5 h; 2) **51**, -78 °C to rt, 19.5 h, 87%; b) $(Et)_3PH^+\ Cl^-$, 110 °C, 18 h, ($-H_2O$), 94%; c) K_2CO_3, H_2O, MW, 70 ºC, 30 mins, 37%; d) $H_{2(g)}$, Pd/C (10%), THF, rt, 42 h, 97%.

*Scheme 41 – Synthesis of **53**, **54**, & **55***

Benzyl alcohol **52** was then converted to the phosphonium salt **53** via an S_N2 reaction by heating the alcohol neat with triisobutylphosphine hydrobromide (Scheme 41). After monitoring the reaction by ^{31}P NMR, purification was obtained via drying over Na_2SO_4 to remove the water by-product. These phosphonium salts can be purified further through an aqueous workup, but for this trial synthetic route the salt was used without further purification. The trialkyl phosphonium salt **53** was reacted with aldehyde **36** under aqueous Wittig conditions using K_2CO_3 as the base, to afford **54** as a mixture of isomers

(Scheme 41). The alkene was hydrogenated under typical conditions of Pd/C and $H_{2(g)}$ to afford **55** as a mixture of two isomers (*cis*/*trans*, Scheme 41).

After the simple proof-of-concept was performed with the heptyl-chain derivative and deemed successful, the target-based synthesis of FTY720 began. Alcohol **51** was reacted in a traditional Wittig reaction with triphenylheptylphosphonium bromide (synthesized from triphenylphosphine and 1-bromoheptane) and NaHMDS to give **56** as a 90:10 mixture of *Z*/*E* isomers (Scheme 42), similar to **52**. The ratio of the olefinic isomers did not need to be controlled during formation, nor did they need to be separated during purification as the alkene would be reduced in a later step to render one isomer of the alkane.



a) 1) $Ph_3P\text{-}C_7H_{15}^+$ Br^-, NaHMDS, 0 ºC, THF, 1.5 h; 2) **51**, -78 ºC to rt, 19.5 h, 85%; b) $(^iBu)_3PH^+$ Br^-, 110 °C, overnight, ($-H_2O$), 99.7%; c) K_2CO_3, H_2O, MW, 70 ºC, 30 mins, 76%.

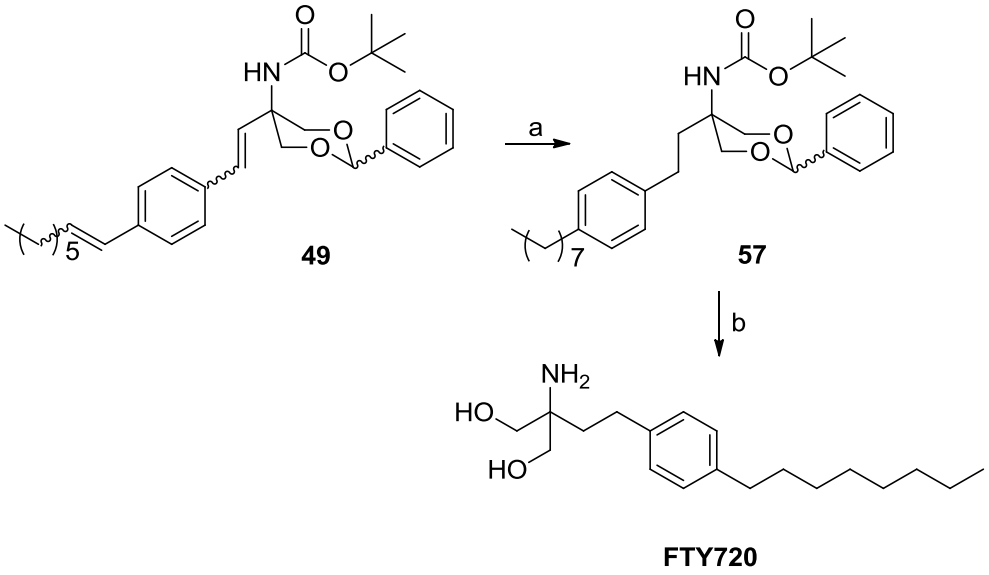
*Scheme 42 – Formation of FTY720 precursors **49** & **50***

The benzyl alcohol derivative **56** was reacted with triisobutylphosphine hydrobromide neat with traditional heating via an S_N2 mechanism to afford the phosphonium salt **50** in near quantitative yields, and this was used as-is with no additional purification beyond removal of the water over Na_2SO_4 (Scheme 42).

Utilizing the same aqueous Wittig reaction that has been previously developed by the McNulty group[156], the non-polar tail segment **50** was attached to the polar head group **36** (Scheme 42). In this example, the aqueous Wittig was performed under basic aqueous conditions (K_2CO_3 in H_2O), joining **36** and **50** together with microwave heating at 70 °C for approximately 30 minutes to afford **49** as a mixture of isomers. Although the Wittig reaction was also performed using traditional heating methods, the yield suffered slightly (64% vs. 76% for microwave heating) and the reaction itself took longer, making traditional thermal heating a less desirable method as opposed to microwave heating. The spectra obtained for **49** were complicated due to the large number of isomers present (*E*/*Z*, *E*/*Z*, *cis*/*trans*, for a total of up to 2^3 or 8 unique isomers), therefore total assignment of the ^1H & ^{13}C NMR spectra were not performed. Separation of the isomers was also not carried out as the separation would be problematic due to the similarity of R_f values, as well as acquisition of pure samples of each isomer is not a requirement for the synthesis as the isomers are to be removed in the final steps towards achiral FTY720.

The alkene groups of **49** were hydrogenated to alkanes using $H_{2(g)}$ over Pd/C to afford **57** as a mixture of two isomers (*cis*/*trans*, Scheme 43). While the hydrogenation was a seemingly simple and straightforward synthetic step which has been successful throughout this project, issues quickly arose with this reduction of **57** possibly due to

phosphorous contaminants remaining from the previous Wittig step poisoning the palladium catalyst. Once these set-backs were resolved the reduction proceeded smoothly to the hydrogenated analog **57**. This specific reduction had the added benefit that it can be monitored almost entirely by UV light by illuminating the flask at 354 nm. The starting material (**49**) fluoresced under long-wave UV light whereas the product (**57**) is UV-inactive at this wavelength, therefore the reduction was easily monitored as once the reaction ceased to glow with exposure to 354 nm light the reaction was judged to be complete.



a) $H_{2(g)}$, $Pd/C_{(cat)}$, THF, rt, overnight; b) HCl, DCM:MeOH, rt, overnight, 92% over both steps.

*Scheme 43 – Hydrogenation and deprotection of **49** to afford FTY720*

Addition of acid to remove both Boc and benzylidene protecting groups in the same step as the hydrogenation failed, generating a varying mixture of products. This unsuccessful deprotection was likely caused by the protecting groups of **49** being cleaved prior to the hydrogenation reducing the double bonds, and the subsequent intermediates then precipitating out of solution due to solubility constraints and not being physically available to react with the H_2/Pd. Increasing the polarity of the solvent to better tolerate a diol and amine, thus performing the hydrogenation in a methanol-based solvent system, also failed as the Pd/C catalyst aggregated into small pockets/beads within the reaction mixture, preventing the active sites on the catalyst from making sufficient contact with the reagents. As a result the hydrogenation was performed as a separate reaction from the removal of the protecting groups.

The final step of the synthesis is removal of both the Boc and Bz protecting groups on **57**, which was achieved cleanly through the use of $HCl_{(aq)}$ in a MeOH:DCM solvent mix to afford FTY720 as the hydrochloride salt, as determined by ^{1}H NMR (Scheme 43). To verify the formation of FTY720, a sample of FTY720•HCl was worked up using NaOH, followed by silica column chromatography using 10:1 DCM:MeOH doped with 1% NH_4OH to afford the free base. The spectra corresponding to the free base was in good agreement with the literature (see experimental for ^{1}H & ^{13}C NMR spectrums)[3]. The overall yield of this novel synthesis of FTY720 from THAM was 39%.

This novel double-Wittig based FTY720 synthesis has some distinguishing benefits over previous syntheses that have been reported. If the synthesis of FTY720 analogs using this approach was desired, the modular nature of this synthetic pathway

makes it amenable to derivatization: an alternate alkyl bromide in place of 1-bromoheptane can be used to render different chain lengths or to attach functional groups, or a different linker alcohol-aldehyde can be used in lieu of **51** if anything more complex than a benzene ring is desired. The amine/diol-protected version **57** would also have benefits previously not seen in all FTY720 syntheses, such as selective removal of only one protecting group, either the Boc or benzylidene group, or DIBAL reduction of the benzylidene group to an *O*-benzyl and alcohol, either of which could allow for future manipulation of FTY720 analogs. As a result, this novel FTY720 synthesis was not only succinct, it was also highly modular to allow for the synthesis of analogs, as well as amenable to the concept of scaling up to allow for its potential use on a more industrial scale.

Fragmented FTY720 Analogs:

As mentioned previously within this thesis, chiral FTY720-P derivatives have received a lot of interest in the literature lately, with separation of its two enantiomers being performed in a variety of ways. Specifically pertinent to this project, an alcohol and the amine could have been incorporated within an oxazolidinone ring for a double protection, then following phosphorylation of the remaining alcohol the two enantiomers can be separated.[67] An expedited synthesis of the oxazolidinone phosphate would have involved straight conversion of an FTY720 precursor to an oxazolidinone ring. Creation of this oxazolidinone ring thus could have been accomplished by having used the radical fragmentation previously researched by the McNulty group (Scheme 6). A potential drawback to this method of oxazolidinone ring formation was that there was more than one easily-abstracted hydrogen present in this specific substrate. While the benzylic hydrogen on **57** (blue, Figure 26) was the most readily abstracted, there were four (two sets of two geminal hydrogens each) benzylic hydrogens (purple and green, Figure 26) that could have also reacted NBS. As a result, there were 2^5, or 32, possible products that could have resulted from this reaction.

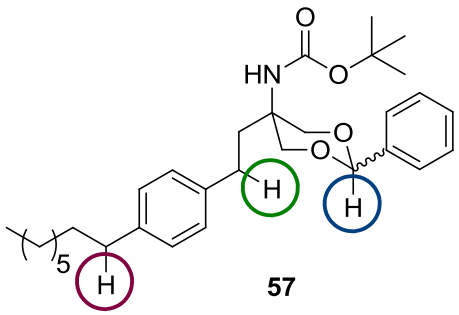
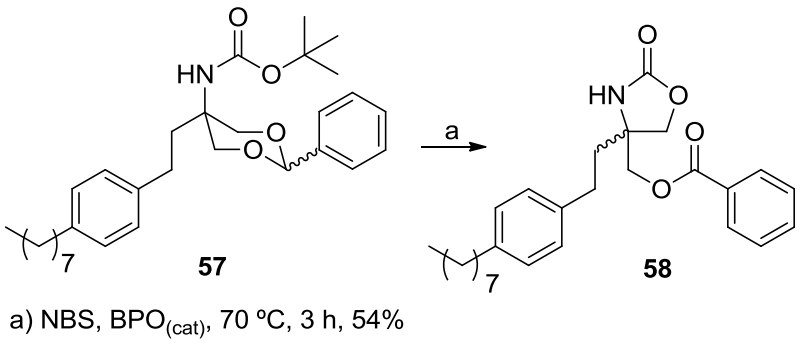


*Figure 26 – Abstractable hydrogens on **57***

Precursor **57** was treated with 1.8eq of NBS and 0.12 eq of BPO in chlorobenzene at 70 °C for several hours, similar to the fragmentation conditions that were applied to alcohol **2** (Scheme 44). After a TLC verified the presence of starting material present, an additional 1 eq of NBS and 0.09 eq of BPO were added to carry the reaction to completion. After workup, the only isolated product was oxazolidinone **58**. While the yield was not ideal, the previous method in use had only a 70% yield, and one must take into account the deprotection steps required between the FTY720 precursor which hover around 92-96%[3,131], which made the actual yield of the oxazolidinone formation reaction as low as 64%. This novel method of creating an FTY720 oxazolidinone has not been discussed within the literature, and after the requisite optimization, this process may be able to replace the methods currently used.



a) NBS, $BPO_{(cat)}$, 70 ºC, 3 h, 54%

*Scheme 44 – Synthesis of oxazolidinone FTY720 derivative **58***

FTY720 UV-Active Analogs:

Since this project began, FTY720 has passed phase III clinical trials and has since been marketed as a medication for the treatment of MS. Although much knowledge has been amassed on the mode of action of this drug from the X-ray crystal structure of bound S1P receptors, more information can be extracted if FTY720 could be traced *in vivo* through its course of action, as well as any derivatives that are biosynthesized in the process.[165] One method to obtain this information would be radiolabelling of FTY720 or FTY720-P with a radioactive isotope of an element, such as ^{3}H (tritium), ^{14}C, or ^{33}P.[90,92,166] However, radiolabelling FTY720 poses a concern over the medical safety as the use of tritiated-derivatives on humans or animals requires receiving ethics approval, which may prove problematic. Mice may be used in lieu of humans for the tritiated experiments, but ideally an experiment would be designed to track the drug pathways in humans.[92,167]

While there are known health concerns with the tritiated FTY720 analogs, the issues with bringing this idea to fruition run deeper. Synthesis of the tritiated analogs would be difficult as now the radioactive analogs not only have a half-life, but require expensive tritium and complex shipping methods. Additionally, the ^{3}H-labelled precursor required for the synthesis may not be commercially available. Even after acquisition of the tritiated starting materials, synthesis of the radioactive analogs is far from trivial as it requires highly-trained chemists and expensive starting materials. Storage, handling, cleaning, and general usage of radioactive compounds further requires more paperwork and facilities to meet standards, complicating the entire endeavour. Assuming all other

hurdles are overcome, once the tritiated material is used *in vivo*, expensive scintillation counters are required to measure the radiation emitted by the sample.[168] Because of all these drawbacks, a more practical approach for tracking FTY720 would be the synthesis of UV-active FTY720 analogs that could be monitored by UV light instead of radiation emission. Unlike ^{3}H-FTY720, a different structure would be required as FTY720 is not inherently UV-active at long wavelengths. Therefore, research into viable UV-active analogs is of interest to our group.

There were a number of possibilities for structures of the UV-active FTY720 analogs. The first possibility lay in the deprotected FTY720 precursor **49**, previously synthesized in the McNulty lab, which had already been shown to exhibit UV absorption (**59**, Figure 27).[131] The second and third possibilities would have been to have the analogs synthesized with a stilbene backbone, mimicking the long-tail alkyl portion of FTY720. An unsaturated propyl tail attached to the rear end of the tail would have been ideal, such as **60** (seen in the overlay in red on Figure 27, with black being FTY720). A simplified backbone possessing only a stilbene (**61**) might have potentially been as, or more, active than **60**, but it required fewer synthetic steps for its synthesis and thus was an attractive option. Previous stilbenes synthesized by the McNulty lab have been shown to be highly UV active, thus examples such as **60** and **61** should have retained their UV activity.[163]

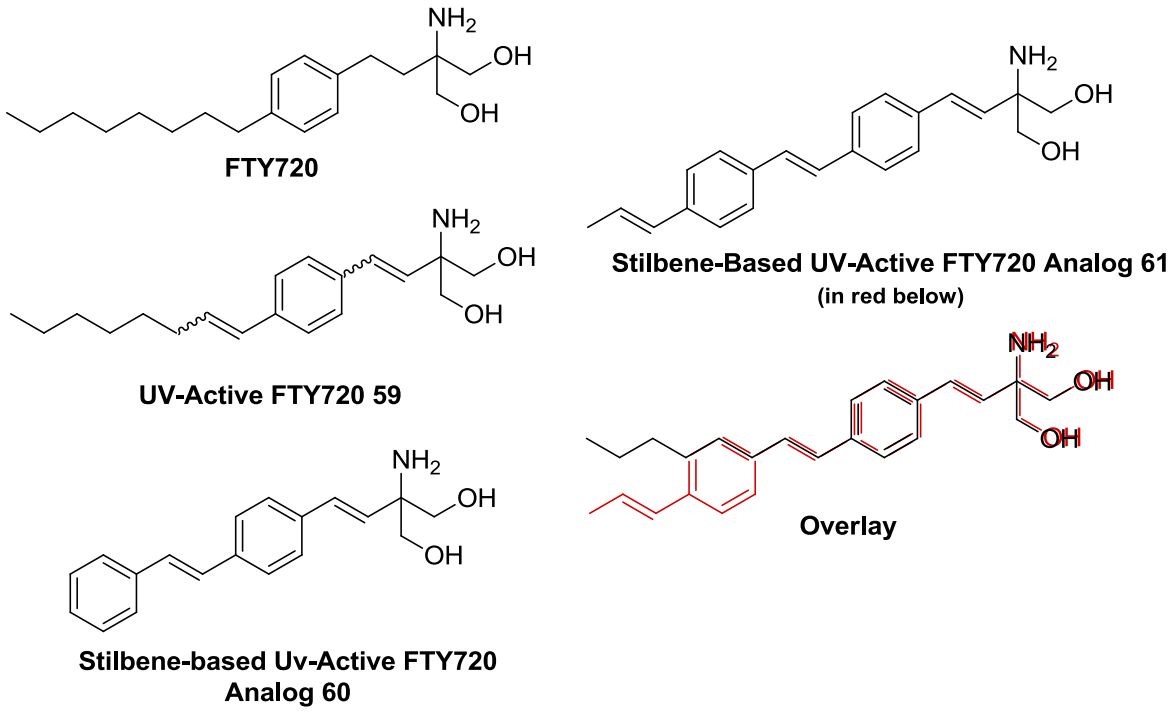
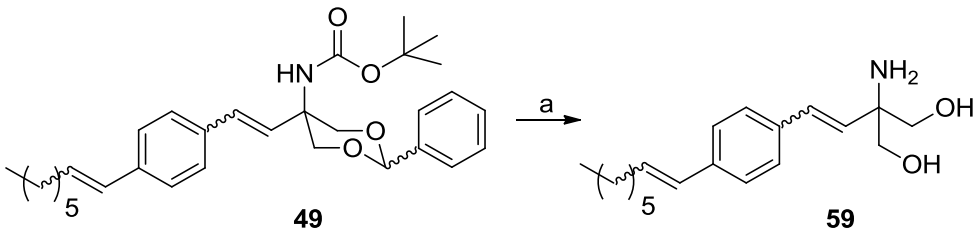


Figure 27– UV-active FTY720 Analogs

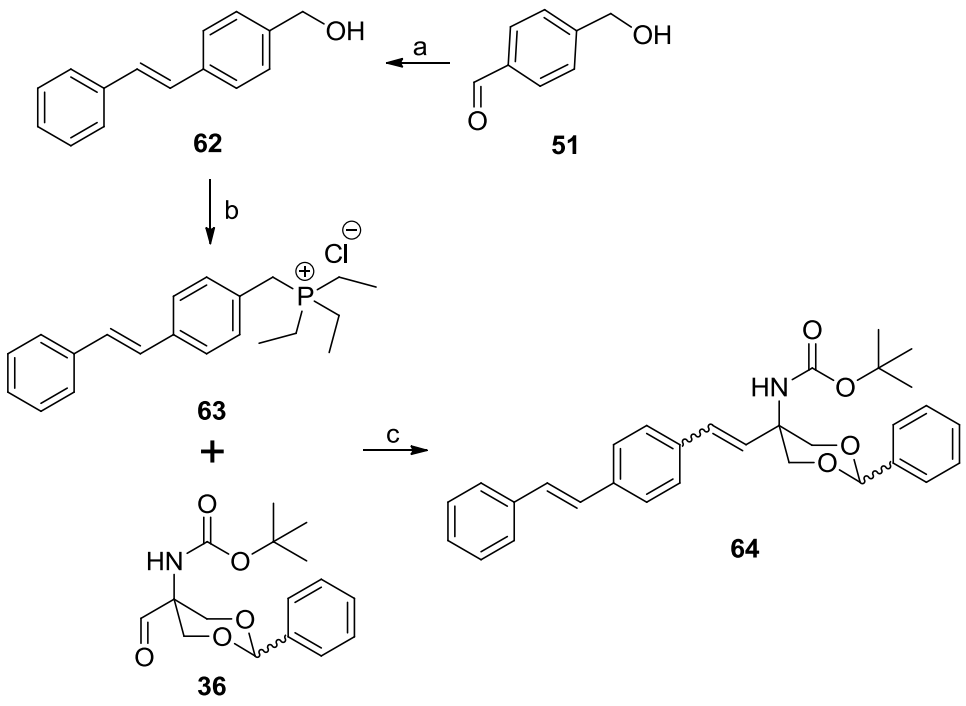
Synthesis of UV-active FTY720 analog **59** involves a theoretically straight-forward deprotection of the precursor **49** using H_2SO_4 in MeOH/DCM (Scheme 45). However, **49** failed to tolerate the treatment of acid well, resulting in the poor yield of 18% for the 'simple' removal of protecting groups. **59** also bore grease-like properties, causing streaking during silica column chromatography purification, which also affected the yield negatively. Due to all the drawbacks, this avenue was not developed further and our interests were diverted towards a stilbene-based analog.



a) H_2SO_4 (30% in MeOH), MeOH:DCM (1:1), rt, 5.5 h, 18%.

*Scheme 45 – Synthesis of UV-active FTY720 analog **59***

FTY720 derivatives which had a stilbene backbone in lieu of a long-chain alkyl group were investigated next. To facilitate this, methyl-hydroxy stilbene **62** was synthesized as per the literature from 4-hydroxymethyl benzaldehyde (**51**) and triethylbenzylphosphine hydrobromide using an aqueous Wittig reaction (Scheme 46).[163] The double-bond geometry of **62** was >95% *E*, reducing the number of isomers for later compounds, as compared to previous FTY720 precursors (e.g. **52** & **56**). Care needed to be taken with the handling and storage of these stilbene samples as the alkene could have isomerized from *E* to *Z* upon exposure to light.[169,170]



a) Et_3P-HCl, K_2CO_3, H_2O, 100ºC, 12 h, 84%; b) Et_3P-HCl, 110 ºC, 27 h, 79-97%;
c) K_2CO_3, H_2O, MW, 70 ºC, 35 mins, 75%.

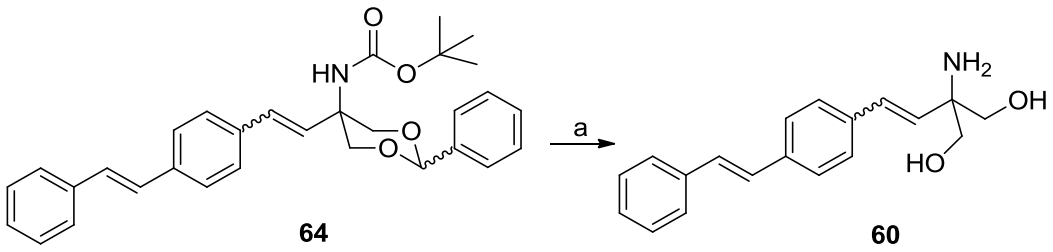
*Scheme 46 – Synthesis of stilbene FTY720 analog precursor **64***

Conversion of the alcohol of **62** to a phosphonium salt via triethylphosphine hydrochloride afforded trialkyl phosphonium salt **63** in good yields (Scheme 46). Purification of phosphonium salt **63** was carried out through sequential aqueous extractions, with removal of the alcohol impurity **62** through an EtOAc wash, followed by procurement of the product **63** via multiple DCM washes. However, as the structure of phosphonium salt **63** resembled a surfactant, the result was some loss of product through emulsification of the aqueous and organic layers during the work-up/purification. While it was manageable for this analog through the addition of solid NaCl during the water/DCM

washes, this emulsification problem became a more severe complication in later derivatives.

An aqueous Wittig reaction was used to fuse the phosphonium salt **63** and previously synthesized aldehyde **36** to yield **64** as a mixture of two major isomers (Scheme 46). Although separation of the spots was visible by TLC, the R_f values were too similar to allow for separate elution by silica column chromatography. During purification, one of the major isomers of **64** crystallized from the solution; however, these crystals were too fine to obtain an accurate melting point as of yet and only enough product was obtained for biological testing purposes.

Product **64** was treated under similar conditions to FTY720, HCl in MeOH/DCM with a 10% NaOH workup to avoid forming the salt, to give **60** (Scheme 47). The yield (61%) was lower than that of FTY720 (92%), likely due to the same issues that were encountered with the deprotection of FTY720 precursor **49**. In an attempt to potentially avoid these complications, treatment with TMS-Cl, followed by a basic workup, was used to remove the Boc and Benzylidene protecting groups, without the use of a harsh acid. This method was tested on **64**, and while initial TLC results seemed promising, upon further purification using silica column chromatography no discernible product could be isolated.

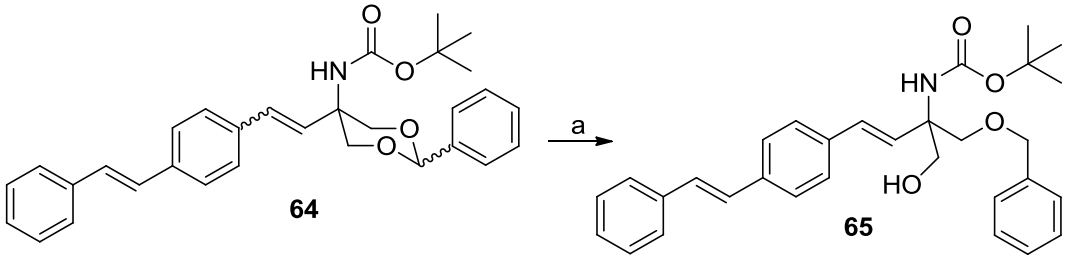


64 **60**

a) 1) HCl (37% in H_2O), DCM:MeOH, rt, 17 h; 2) NaOH (10% in H_2O), 61%.

*Scheme 47 – Synthesis of stilbene FTY720 analog **60***

Alternately, the benzylidene group of **64** can be opened to an alcohol and *O*-benzyl group using DIBAL with the intent of differentiating the alcohols. On FTY720-P, one alcohol is capped with a moderately polar phosphate functional group, which may be mimicked by an *O*-benzyl protecting group. The benzylidene reduction was carried out with excess DIBAL to afford **65** (Scheme 48). This reaction, while generally trivial, was more complicated than normal as there were many coordination sites for the aluminum, resulting in requiring an excess of DIBAL and a less than ideal yield.



64 **65**

a) DIBAL, DCM, 0 ºC to rt, 18 h, 43%.

*Scheme 48 – DIBAL reduction of **64** to **65***

The absorbance regions for diol **60** and *O*-benzyl **65** were scanned first (Tables 2 & 3 respectively, Figure 28). Both samples were diluted in methanol, to the concentration of 4.06×10^{-5} M for diol **60**, and 8.24×10^{-6} M for *O*-benzyl **65**. Both **60** and **65** had two similar λ_{max} values, but varying molar extinction coefficient (ε) values (Tables 2 & 3). Diol **60** absorbed at 205.6 nm and 327.6 nm, with ε values of 10,830 at 327.6 nm and 4,603 at 231.1 nm. The *O*-benzyl analog **65** absorbed at the similar wavelengths of 207.0 nm and 326.9 nm, with ε values of 45,648 at 326.9 nm and 17,604 at 232.0 nm. Therefore, the *O*-benzyl analog **65** was more capable of absorbing light than diol **60**.

Table 2 – Absorbance Data for Diol **60**

nm	Abs.	L (cm)	Conc. [mol/L]	**ε**
231.1	0.187	1.0	4.063×10^{-05}	**4603**
327.6	0.440	1.0	4.063×10^{-05}	**10830**

Table 3 – Absorbance Data for *O*-Benzyl **65**

nm	Abs.	L (cm)	Conc. [mol/L]	**ε**
232.0	0.145	1.0	8.237×10^{-06}	**17604**
326.9	0.376	1.0	8.237×10^{-06}	**45648**

Abs. = Absorbance, L = length of cell, ε = molar extinction coefficient

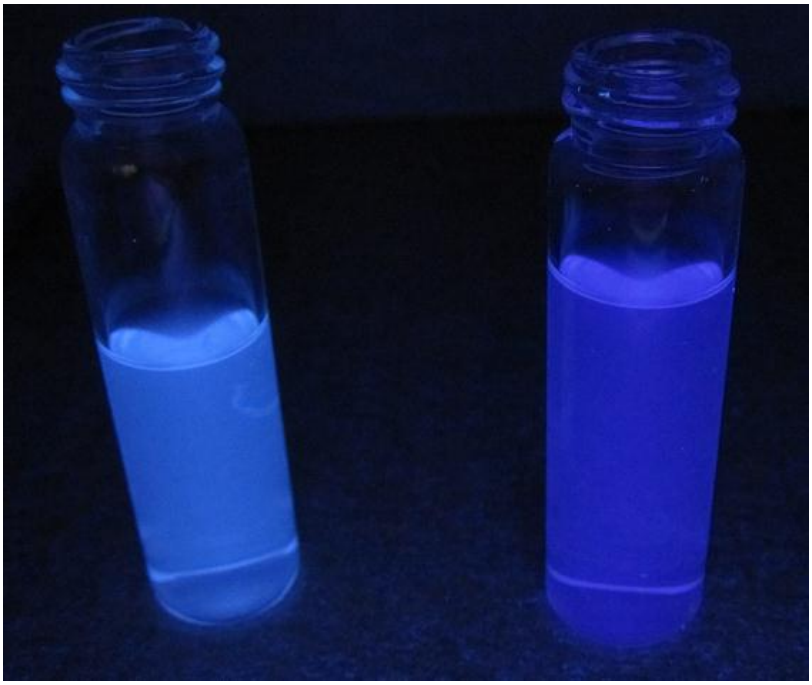


*Figure 28 – Diol **60** (left) and O-Benzyl **65** (right) irradiated at 365 nm*

Compounds **60** and **65** both fluoresced at two wavelengths, approximately 230 nm and approximately 327 nm (Tables 4 & 5). At both excitation wavelengths there were two fluorescent regions, generally around 370 nm, and another at 750 nm. However, the peaks found at approximately 700 nm were indicative of second order diffraction; therefore, these peaks are likely artifacts of the instrument, and not directly caused by the sample.

While both compounds fluoresced, *O*-benzyl **65** was more fluorescent than diol **60**. At the higher excitation wavelength 327 nm, both **60** and **65** emitted around 377 nm, but *O*-benzyl **65** emitted with a higher intensity. However, both of these compounds proved to be viable candidates for future fluorescence testing, with respect to their ability to absorb and emit light.

Table 4 – Fluorescence Data for Diol **60**

Excitation (nm)	S.W. Ex (nm)	S.W. Em (nm)	λ_{max} (nm)	Intensity
231.1	2.5	2.5	**377**	286
	10	5	**629**	116
	10	5	**653**	132
	10	5	**717**	253
	10	5	**750**	246
327.6	2.5	2.5	**379**	255
	5	5	**718**	307
	5	5	**753**	346

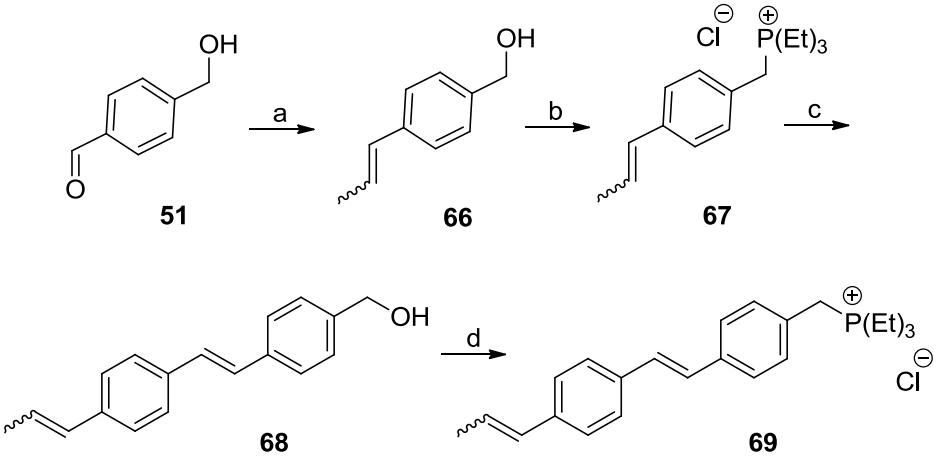
Table 5 – Fluorescence Data for *O*-Benzyl **65**

Excitation (nm)	S.W. Ex (nm)	S.W. Em (nm)	λ_{max} (nm)	Intensity
232.0	5	2.5	**359**	317
	5	2.5	**377**	379
	10	5	**622**	127
	10	5	**655**	136
	10	5	**717**	347
	10	5	**754**	319
326.9	2.5	2.5	**361**	261
	2.5	2.5	**378**	356
	5	5	**718**	464
	5	5	**754**	481

S.W. Ex = Excitation Slit Width, S.W. Em = Emission Slit Width

The synthesis for the alkyl-chain analog of **60** began by adding a propene tail to the linker aldehyde **51** using a traditional anhydrous Wittig reaction, giving **66** with an *E*/*Z* ratio of 1.75:1 (Scheme 49). As a repercussion of this far from optimal *E*:*Z* ratio, the NMR spectra were very complex and difficult to assign. Alcohol **66** was converted into a trialkyl phosphonium salt using triethylphosphine hydrobromide under similar conditions

as used in previous analogs (**67**, Scheme 49). The trialkyl phosphonium salt **67** was reacted with linker aldehyde **51** in an aqueous Wittig reaction to give hydroxymethyl stilbene **68**, again as a mixture of isomers (Scheme 49).



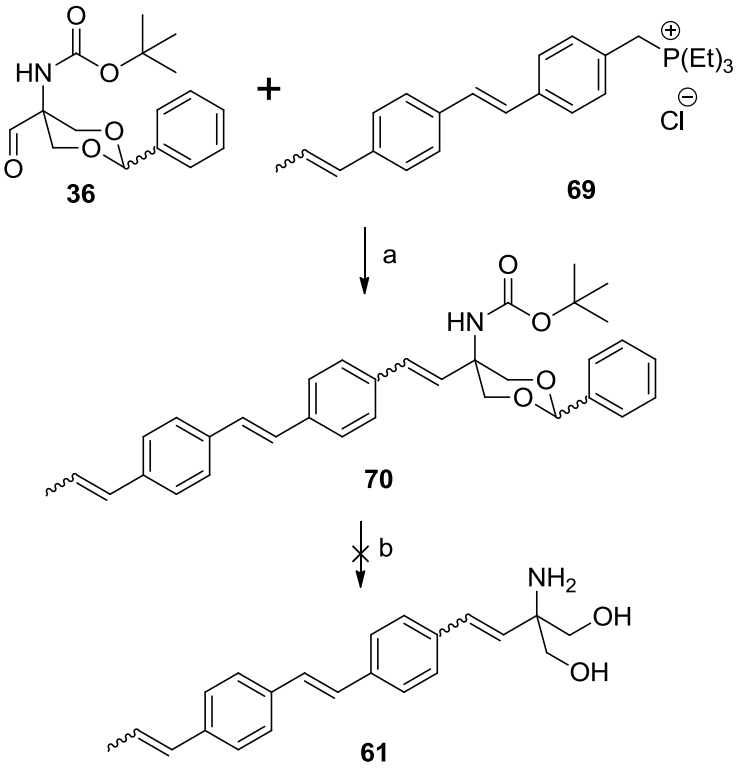
a) 1) Et_3P-Et^+ Br^-, ^nBuLi, THF, 0 ºC, 30 mins; 2) **51**, THF, - 78ºC, 6.5 h, 85%; b) Et_3P-HCl, 110 ºC, 17 h, 77%; c) **51**, K_2CO_3, H_2O, MW, 30 mins, 100 ºC, 99%; d) Et_3P-HCl, traditional 110 ºC for 17 h, MW 100-120 ºC for 3.25 h, 79%.

*Scheme 49 – Synthesis of phosphonium Salt **69***

Stilbene **68**, as with the previous stilbenes, was converted to the triethyl phosphonium salt **69** using triphenylphosphine hydrochloride (Scheme 49). As expected, purification of **69** was very difficult and problematic due to the surfactant-like nature of the salt. The general workup followed for this type of reaction was dissolving the crude mixture in water, extracting the alcohol/organic impurities with EtOAc (to be discarded), followed by multiple extractions of the product with copious rounds of DCM. This specific derivative though was more surfactant-like than usual (the polar side being the

phosphorus salt, and the stilbene tail acting as the non-polar region), causing the issue of emulsification of the aqueous and organic layers. Addition of brine during the EtOAc wash was of marginal help separating the layers, even after addition of less polar solvents such as Et_2O and hexanes. Solid NaCl was more useful for separating the layers until the DCM wash portion of the procedure, where emulsification again became an issue. These issues caused the yield for this reaction to suffer.

Stilbene-THAM derivative **70** was synthesized in an aqueous Wittig reaction using THAM aldehyde **36** and stilbene phosphonium salt **69** (Scheme 50). Purification of stilbene-FTY720 analog **70** was more difficult than similar derivative **64** as the complication was likely caused by the longer, more lipophilic tail. Analysis of the NMR spectra was also more of a predicament than was found with **64** as **70** has double the number of isomers due to the propene tail. As was seen previously during the experiment, an emulsification of the layers during the workup of the Wittig reaction occurred, negatively affecting the yields. Solid NaCl was once again employed to potentially avoid this hindrance, but with limited success.



a) K_2CO_3, H_2O, MW, 100 ºC, 20 mins, 46%; b) H_2SO_4, MeOH, DCM, no rxn.

*Scheme 50 – Synthesis of propene-stilbene FTY720 analog **61***

An attempt was made to remove the Boc and Bz protecting groups of **70** under treatment with acid, conditions similar to those used for FTY720 and stilbene-FTY720 previously (Scheme 50). While the reaction seemed promising by TLC, purification of **61** via silica column chromatography proved to be impossible as the product streaked heavily through the column (as traced by following the bands of the column by UV light and monitoring the fractions collected by TLC), and no discernible product could be isolated. An LH20 sephadex column was then utilized with the intent of exploiting the size of the molecule to separate the mixture as opposed to the traditional adsorption properties used

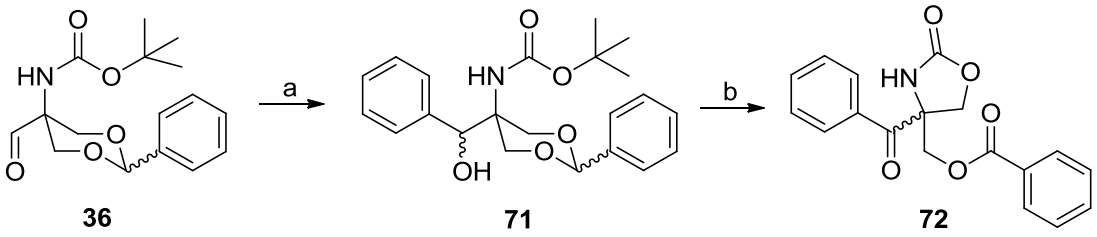
for silica column chromatography. This procedure ultimately failed as well, resulting in not being able to isolate **61**, and therefore not perform any biological testing on it.

While the propyl derivative **61** was never successfully isolated, compounds **60** and **65** were a good starting point to investigate the biological activities of UV-active FTY720 analogs. Should **60** or **65** have appreciable biological activity more effort can be invested into synthesizing **61**, but the propyl tail may not be relevant and by not including its addition two synthetic steps (synthesis of linker aldehyde **51** and benzyl alcohol **66**) can be avoided.

Aromatase Inhibitors:

As mentioned previously, work performed by fellow group members within the McNulty group has focused around synthesizing targets for selective inhibition of aromatase (CYP450 19A1).[126] Recently published were the two compounds *syn*-alcohol and *anti*-acetate with K_i values of 0.05μM and 0.06μM respectively (Figure 19). It was thought that a THAM derivative might mimic the basic backbone of several aryl rings surrounding a polar group and therefore possess aromatase inhibition properties.

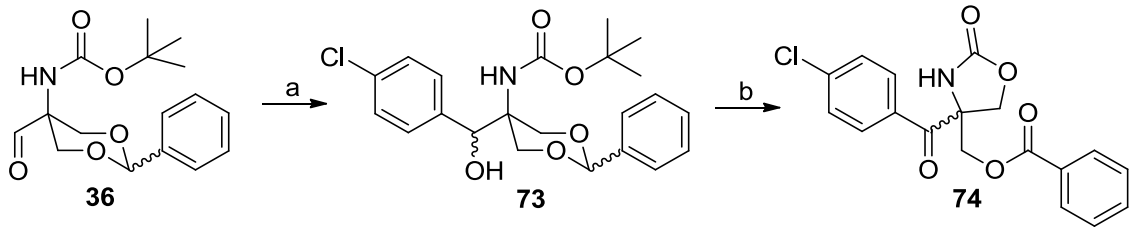
The previously synthesized aldehyde derivative of THAM (**36**) was reacted with the Grignard reagent phenylmagnesium iodide in Et_2O to afford **71** as a mixture of four isomers (*R*/*S* & *cis*/*trans*, Scheme 51). Fortunately, there was no evidence of the Grignard reagent reacting with the carbonyl of the *N*-Boc group, suggesting that an oxazolidinone ring may also be compatible with a Grignard reagent as it has similar electronic properties to a Boc group. The alcohol **71** can be treated with NBS under fragmentation conditions to yield **72**, where both the oxazolidinone ring rearrangement occurs, as well as oxidation of the benzyl alcohol to the benzyl ketone (Scheme 51). While maintaining the alcohol from **72** would have better resembled the aromatase inhibitors recently published, biological testing of **72** may be promising as activity was found with the *anti*-acetate derivative, suggesting that an alcohol is not a requirement for biological activity.



a) PhMgI, Et_2O, 0 ºC, rt, 1.75 h, 82%; b) NBS, $BPO_{(cat)}$, PhCl, 70 ºC, 2 h, 65%.

*Scheme 51 – Synthesis of potential aromatase inhibitors **71** & **72***

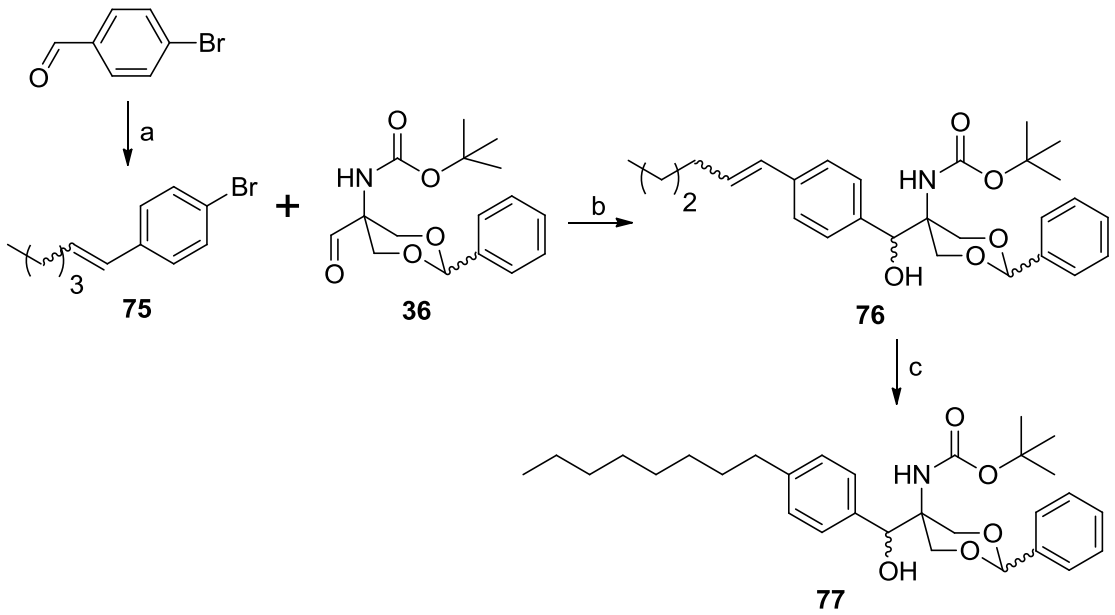
To allow for modular derivatization of analogs of **71** or **72**, a functionalized phenyl group could be used in place of iodobenzene in the Grignard reaction. The first type of functionalized phenyl group attempted was 4,4'-dibromobiphenyl, but due to solubility issues with 4,4'-dibromobiphenyl in THF or Et_2O, this substrate was abandoned. 4-Chlorobromobenzene was subsequently attempted for the synthesis of the Grignard reagent, which when reacted with aldehyde **36** afforded **73** (Scheme 52). The chloro derivative **73** was fragmented in the same fashion as **71**, in part to simplify the ^1H NMR of 74 to ensure its construction (Scheme 52). The radical fragmentation gave **74**, with retention of the chlorine atom. If this reaction were to be carried out in the future, 1,4-dibromobenzene would be preferred over 4-chlorobromobenzene as the bromo derivative of **73** or **74** would react more prolifically in substitution reactions.



a) 4-ClPhMgI, THF, 0 ºC, 2 h, rt, 49%; b) NBS, $BPO_{(cat)}$, PhCl, 70 ºC, 6 h, 78%

*Scheme 52 – Synthesis of chloro-derivatives **73** & **74***

An alkyl-chain aryl derivative version of **71** was synthesized to investigate the scope of possibilities on the THAM scaffold. 4-(oct-1-enyl)-bromobenzene (**75**) was synthesized from triphenylheptylphosphonium bromide and 4-bromobenzaldehyde as per literature procedure (*Z*:*E* ratio 9:1, Scheme 53).[171,172] This alkyl-phenyl halide was converted to the Grignard reagent using Mg^0 in THF. The newly-formed Grignard reagent was reacted with aldehyde **39** to give **76** as an inseparable mixture of 8 isomers (*E*/*Z*, *R*/*S*, *cis*/*trans*, Scheme 53).



a) Ph_3P-$C_7H_{15}^+$ Br^-, NaHMDS, THF, -78 ºC to rt, 2 h, 69%; b) 1) **75**, Mg^0, THF, 60 ºC, 1 h; 2) **36**, THF, 0 ºC to rt, 2 h, 45%; c) $H_{2(g)}$, Pd/C (10%), THF, rt, 21 h, 77%.

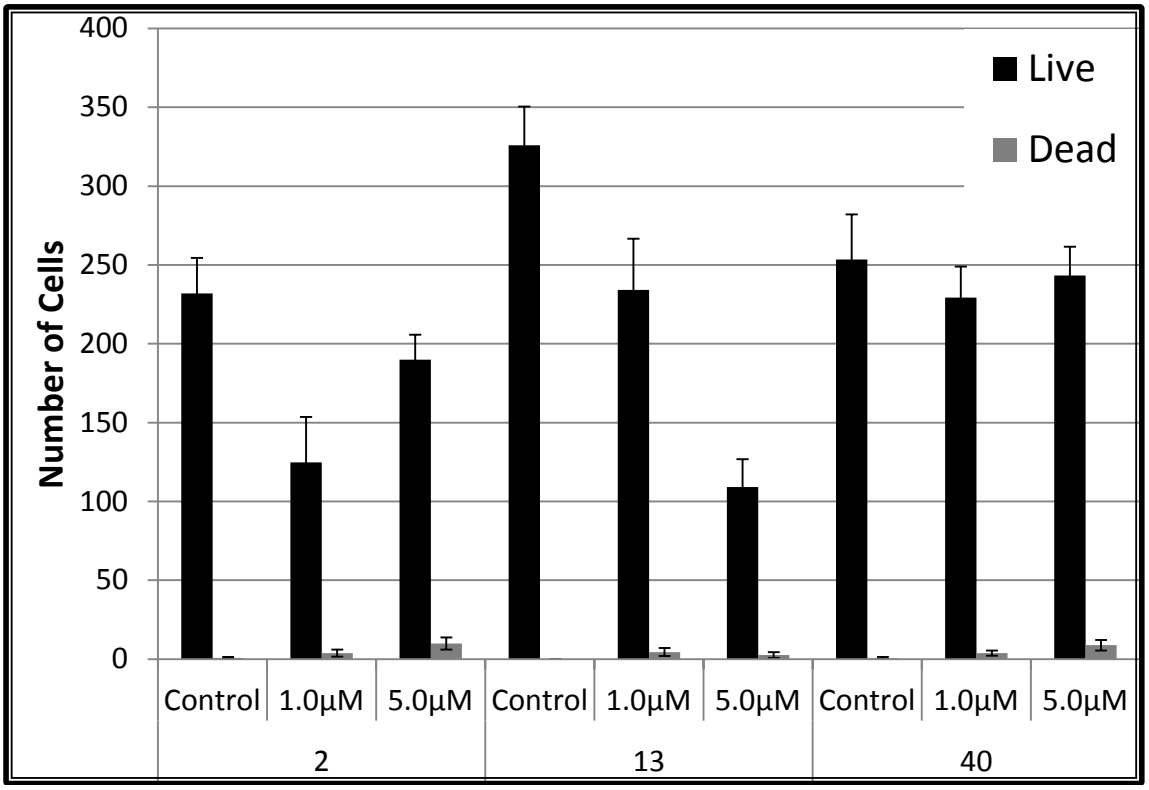
*Scheme 53 – Synthesis of aryl-alkyl derivatives **76** & **77***

The alkene of product **76** was reduced to an alkane so that the compound would closer mimic FTY720 derivatives, as well as elicit a simplified NMR spectrum for the purposes of full characterization. Pd/C with $H_{2(g)}$ in THF gave **77** as a product of two *cis*/*trans* isomers as expected (Scheme 53). Separation of the two *cis*/*trans* isomers or removal of the Boc/Bz protecting groups has yet to be performed, but these tasks may be carried out in the future to enlarge the FTY720 and sphingosine chemical library, depending on preliminary biological testing.

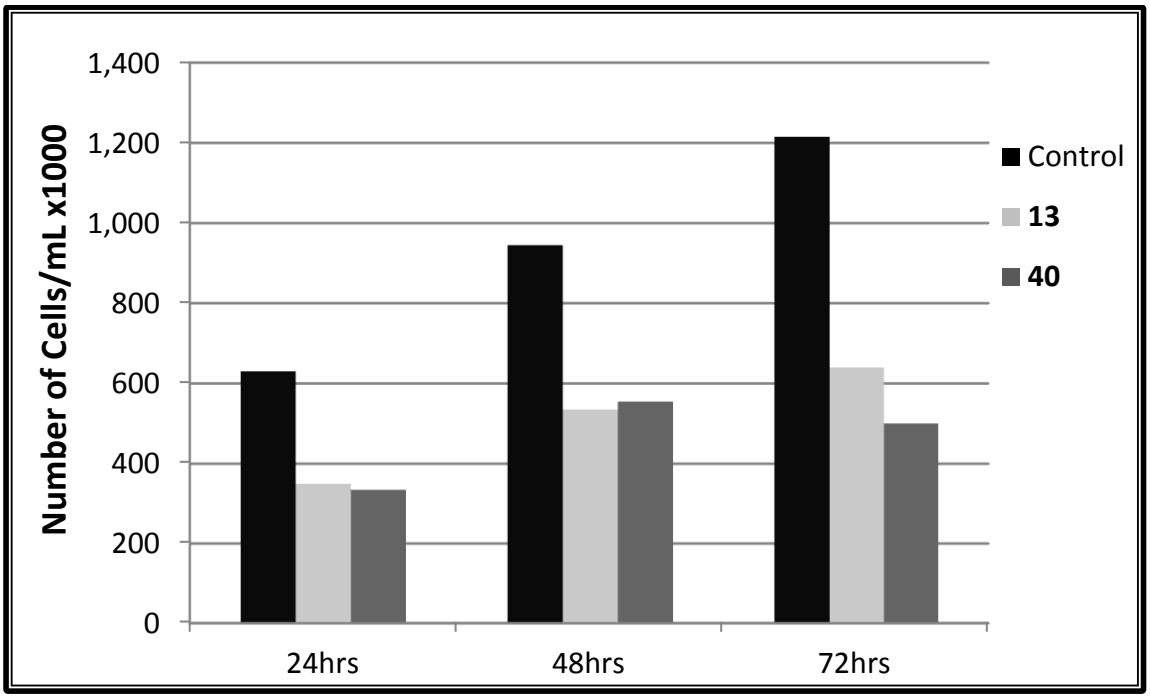
Biological Testing:

Anti-Melanoma and Anti-MCF-7 Breast Cancer Cells:

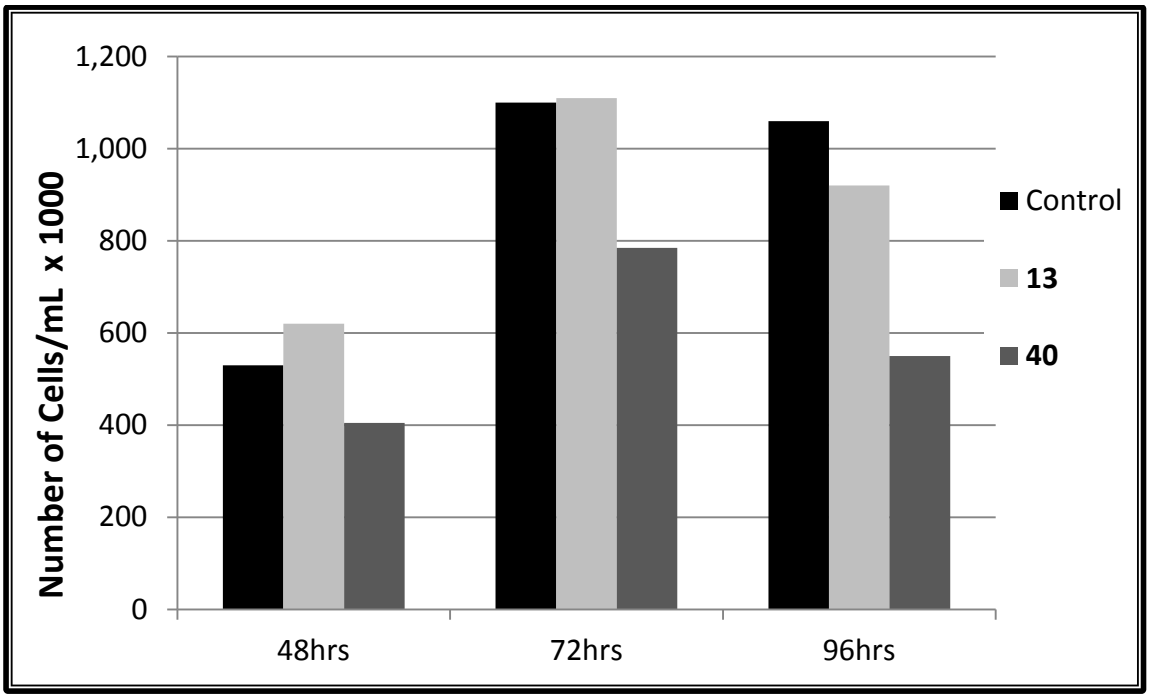
Compounds **3**, **13**, and **40** were tested against MCF-7 breast cancer cells, melanoma cells, and NHF cells at The University of Windsor by Ms. Pamela Ovadje, a senior thesis student working in the laboratory of Dr. Siyaram Pandey. Tested against melanoma cells, alcohol **3**, tosylate **13**, and triazole **40**, all showed activity, however, tosylate **13** showed the greatest decrease of live cells with respect to a control (Graph 1). The conspicuous lack of dead cells signifies that compound **13** inhibits the growth of new cells rather than inducing apoptosis in currently living cells.



*Graph 1- Dose-dependent effects of compounds **3**, **13**, and **40** on MCF-7 cells*



*Graph 2 – Time effects of compounds **13** & **40** on MCF-7 cells*



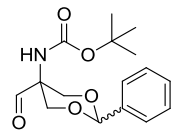
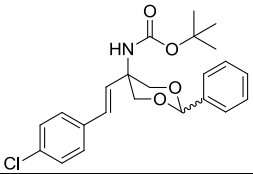
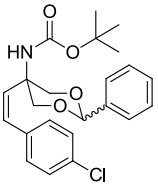
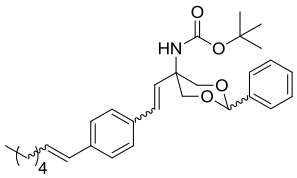
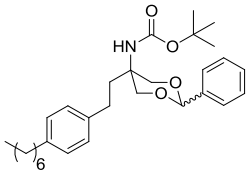
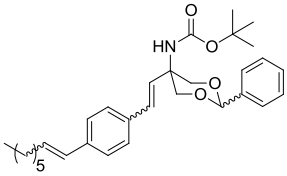
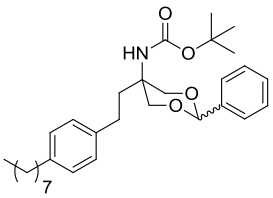
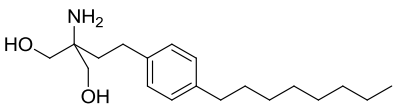
*Graph 3 – Time effects of compounds **13** & **40** on NHF cells*

Compounds **3**, **13**, and **40** were also tested against MCF-7 cells (Graph 2). Triazole **40** showed more retardation of MCF-7 cell growth; however, **13** affected NHF, or normal human cells, less (Graph 3), making it a better lead drug candidate.

Anti-Parasite Activity:

Biological testing of THAM-derivatives was performed by Dr. Brando at Johns Hopkins University, specifically against toxoplasma-infected cells and uninfected human cells (Table 6). The McNulty library of compounds was tested, some of which are derived from THAM, and the compound's suitability for being a lead drug candidate was calculated by taking the toxicity data against toxoplasma-infected cells (% Toxo. Inhib.) and subtracting the cytotoxicity to healthy cells (% Cytotox.) to generate a Specific Activity (Spec. Act.) value (Table 6, out of 100, closer to 100 the more suitable the candidate).

Table 6 – Biological Data for *Toxoplasma gondii* inhibitors

Structure	Cmpd	% Toxo. Inhib.	% Cytotox.	Spec. Act.	[Conc] (μM)
	**36**	96	2	94	65
	**43-(*E*)**	95	3	92	21
	**43-(*Z*)**	74	18	76	24
	**54**	95	4	91	27
	**55**	89	0	89	27
	**49**	93	10	83	30
	**57**	13	0	13	35
	**FTY 720**	96	91	5	39

Aldehyde **36** showed the largest amount of toxicity to the parasite, without compromising healthy cells. It is unknown at this time which isomer of **36** was more active, *cis* or *trans*, or even whether they were equally active. Separation of the *cis* and *trans* isomers of **36** may be performed in the future depending on IC_{50} values, but as both isomers had the same R_f value, they may have to be synthesized from a isomerically-pure sample of alcohol **2**. While having the highest level of toxicity to the parasite, the concentration of aldehyde **36** tested was two to three times higher than other compounds listed, thus casting some uncertainty onto its suitability as a lead compound. Before conclusive deductions can be drawn, and IC_{50} value would be needed of **36**, preferably in its pure *cis* or *trans* form.

The data also suggested interesting findings with respect to the SAR's. The first general theme was that only benzylidene analogs had appreciable activity, with the oxazolidinone series not presenting any significant anti-toxoplasmosis data. With the benzylidene series, the most active compounds possessed both the benzylidene and *N*-Boc protecting groups, as well as a phenyl entity on the final quadrant of THAM. The compound which showed the next highest Specific Activity after aldehyde **36** was **43**-(*E*). Activity dropped, while cytotoxicity increased, when comparing the *Z* and *E* isomers of **43**, with the more stable *E* isomer being the better drug candidate as it showed lower toxicity against healthy cells.

With FTY720 derivatives **49** and **54**, the activity against toxoplasma-infected cells stayed fairly constant, while there was a marked increase in cytotoxicity towards uninfected cells in **49**, which differs from **54** by the addition on a single carbon atom on

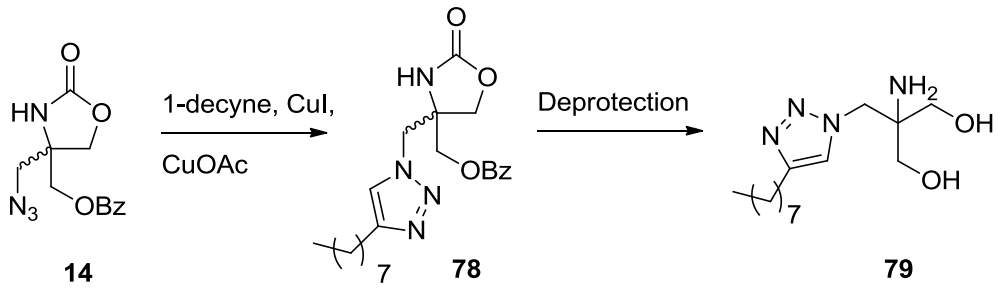
the terminal end of the alkyl chain. Reduction of the conjugated double bonds of **54** to give the alkyl version **55** also altered the toxoplasmosis inhibition, as well as lowering the cytotoxicity marginally. In comparison, when **49** had the double bonds replaced by saturated units (**57**), the activity against the toxoplasma-infected cells dropped significantly from 93% to 13%. This finding strongly suggested the requirement of a more rigid backbone or conjugated system to maintain biological activity against the toxoplasma-infected cells. Interestingly, FTY720 showed strong activity against toxoplasma-infected cells; fortunately it showed nearly the same activity against healthy, uninfected cells, marking the need to have the amine and oxygen atoms capped in some fashion to maintain selective toxicity.

Should further investigations into SAR be performed for this target, IC_{50} values would be needed, as well as separation of the *cis*/*trans* isomers to ensure accurate activity values were obtained.

Future Directions:

Triazole FTY720 Analogs:

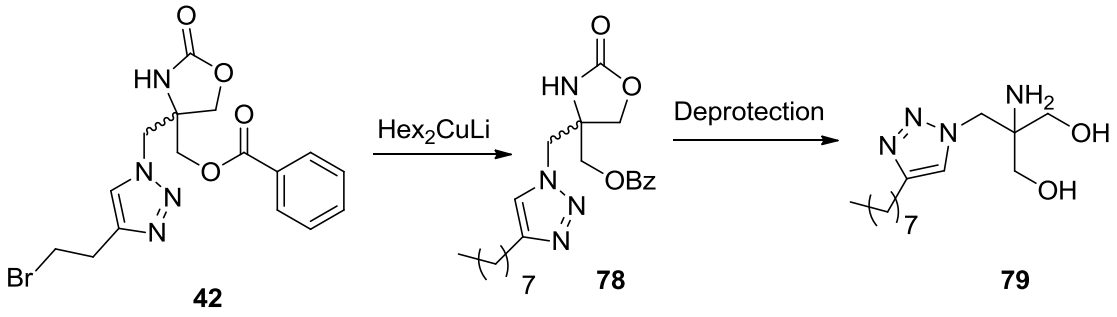
One of the avenues this project explored, but did not exhaust, is triazole-based FTY720 analogs. This facet of the project can be approached from either an end-target viewpoint or library viewpoint. If only the end-target **79** is desired, the synthesis can be carried out starting from 1-decyne in a Huisgen cycloaddition 'click' reaction with azide **14**, followed by total deprotection of the protecting groups to afford **79** (Scheme 54). Because the exact alkyne desired in the final product would be utilized in the reaction, this synthetic pathway is not amenable to creating a library as for each new analog that would be desired, the specific alkyne must be purchased and used.



*Scheme 54 – Synthesis of triazole-FTY720 analogs **78** & **79***

If a library of triazole-FTY720 compounds was desired, then the previously-synthesized bromo derivative **42** could be reacted with a Gilman reagent to afford **78**, followed by removal of the benzoate and oxazolidinone ring to render **79** (Scheme 55). The Gilman reagent should be a sufficiently soft nucleophile to preferentially react with

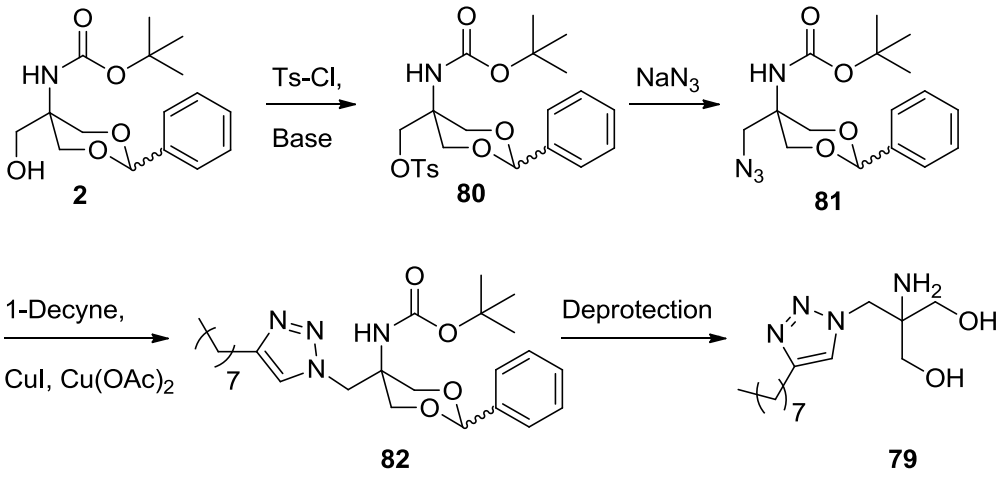
the alkyl bromide functional group and avoid reacting with the benzoate group or oxazolidinone ring. Even should a reaction occur between the protecting groups and the Gilman reagent, this would be acceptable as the protecting groups would be removed in the next synthetic step regardless. This synthetic method can use a variety of different Gilman reagents, making this pathway amenable to the synthesis of a triazole-FTY720 analog library. Should the synthesis of the Gilman alkyl cuprate reagent or Gilman reaction itself continue to be problematic, a Kumada coupling reaction could be run with a Grignard reagent.[173] While the benzoate group will almost certainly react and be removed, as was mentioned earlier this would not be an issue as the protecting group would have been removed via base in the next synthetic step. The oxazolidinone ring ought not to react with the Grignard reagent as the *N*-Boc group on **71** was preserved when in the presence of excess Grignard reagent.



*Scheme 55 – Synthesis of triazole-FTY720 analogs from triazole **42***

Depending on the difficulty of opening the oxazolidinone ring, the other option would be to begin the synthesis prior to the fragmentation through tosylation of alcohol **2**

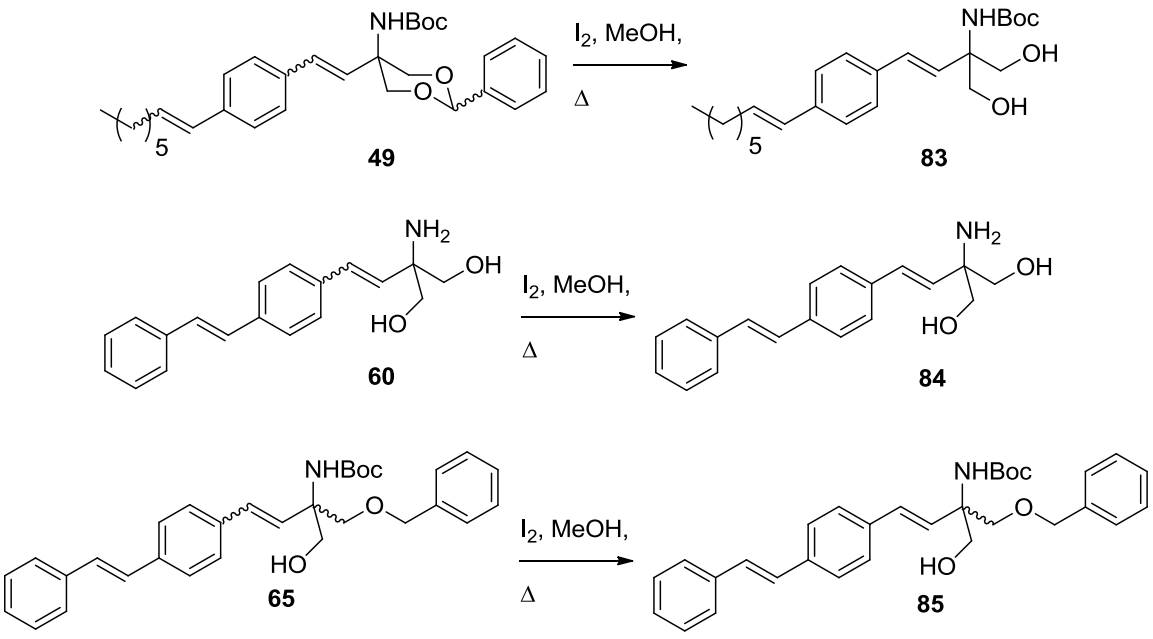
to **80**, followed by conversion to the azide using NaN_3 (**81**, Scheme 56). The 'click' reaction can then be performed on azide **81** to give triazole **82**, which then followed by the removal of the benzylidene and Boc groups will afford **79** (Scheme 56). The drawback to this synthetic route is that while the protecting groups are easier to remove, the oxazolidinone derivative is never prepared, and this compound may be interesting from a biological-testing viewpoint. Theoretically, **82** could be fragmented in the same way **57** was fragmented to **58** as it is likely the benzylic proton is more likely to be abstracted than the proton adjacent to the triazole ring (Scheme 56). If possible, this fragmentation would re-open the pathway for oxazolidinone rings and further expand our chemical library.



*Scheme 56 - Synthesis of triazole-FTY720 analogs from alcohol **2***

UV-Active FTY720 Derivatives:

Depending on the activity found with the UV-active FTY720 analogs, it may be prudent to isomerize the double bonds within the alkyl tail so that the stereochemistry is set and only one isomer is present in the sample (Scheme 57). This isomerization has been attempted before using I_2 with heat (~80 ºC) in methanol or a methanol:DCM mix, with the understanding that the deprotection of the benzylidene group in the same step may be unavoidable (**83**). However, these conditions failed to produce any isomerization of the double bond or removal of the benzylidene group. If optimization of this reaction was completed then this approach could be applied to any of the UV-active FTY720 derivatives, including the stilbene analogs (**84** & **85**, Scheme 27).



Scheme 57 – Future isomerization of UV-active FTY720 analogs

Conclusions:

It has been shown that there are many avenues that can be taken starting from the readily available and affordable compound THAM. Methodology within this project has included a novel fragmentation that opened a new pathway into the synthesis of oxazolidinones. The reaction was probed to determine the mechanism of the rearrangement, as well as to explore the scope of the reaction. This new rearrangement approach facilitated the synthesis of oxazolidinone rings for one of the main arteries of this project, the oxazolidinone library; this approach will continue to be tested for a plethora of biological applications over the coming years, as it has already shown promise with anti-parasitic activity with respect to toxoplasmosis.

Another synthetic route to FTY720 via THAM has also been successfully explored. This novel pathway had added advantages over previous reported procedures: the precursor is UV active, which simplified the monitoring of the reaction; no homogeneous metal catalysts were used, which lowered the cost of the synthesis, as well as made the conditions for the reactions milder; the yields were more reproducible; and an oxazolidinone ring was synthesized via an intermediate, which saved two synthetic steps should enantiomeric resolution via the oxazolidinone be desired. Depending on the biological activity inherent to the UV-active analogs, later testing and development could be investigated.

In conclusion, it has been shown that THAM was as versatile a starting material as had been hypothesized. The novel analogs since synthesized will ideally continue to show biological activity with a wider variety of applications. As well, the novel methods

towards known compounds will hopefully substitute the currently-used methods due to their advantages over the previously-known methods. Ideally the unfinished avenues within the project will continue to be researched, hopefully leading to novel lead compounds for a multitude of purposes in the future.

Experimental:

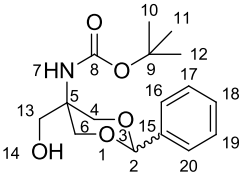
General:

'Flame-dried' implies the flask drying was performed under high vacuum with a Meker-Fisher burner unless otherwise stated. All chemicals were used, as-is, from supplier, with exceptions being: DCM, triethylamine (TEA), *N*,*N*-diisopropylethylamine (DIPEA), lutidine, and tetramethyl ethylenediamine (TMEDA) were distilled over CaH_2; THF, toluene, and 1,4-dioxane were distilled over Na^0 in the presence of benzophenone indicator; benzaldehyde dimethyl acetal and acetic anhydride were distilled over Na_2SO_4; chlorobenzene and bromobenzene were distilled over $CaCl_2$; morpholine were distilled over $MgSO_4$, dry DMF was from a solvent system, and N_2 gas was dried over Drierite© (97% $CaSO_4$, 3% $CoCl_2$). Triisobutylphosphine and triethylphosphine was obtained from Cytec Canada Inc. and converted to their HBr or HCl salts using a literature protocol.[156]

^1H, ^{13}C, and ^{31}P NMR spectra were aquired on a 600 MHz Bruker NMR spectrometer with chemical shift values given in parts per million (ppm) and IRs were run on a Nicolet 510 FT-IR spectrometer with chemical shift values given in cm^{-1} unless otherwise stated. Mass spectra were run on a Micromass Quattro Ultima spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV and HRMS (EI) were performed with a Micromass Q-TOF Ultima spectrometer. UV absorbance was measured on a Cary 50 UV-visible spectrophotometer, and fluorescence was measured on a Cary Eclipse fluorescence spectrophotometer. TLC's were run using Macherey-Nagel aluminum- or plastic-backed plates. Melting points were obtained on an Electronic Research Associates Inc. melting point apparatus and have been corrected against an external calibrant.

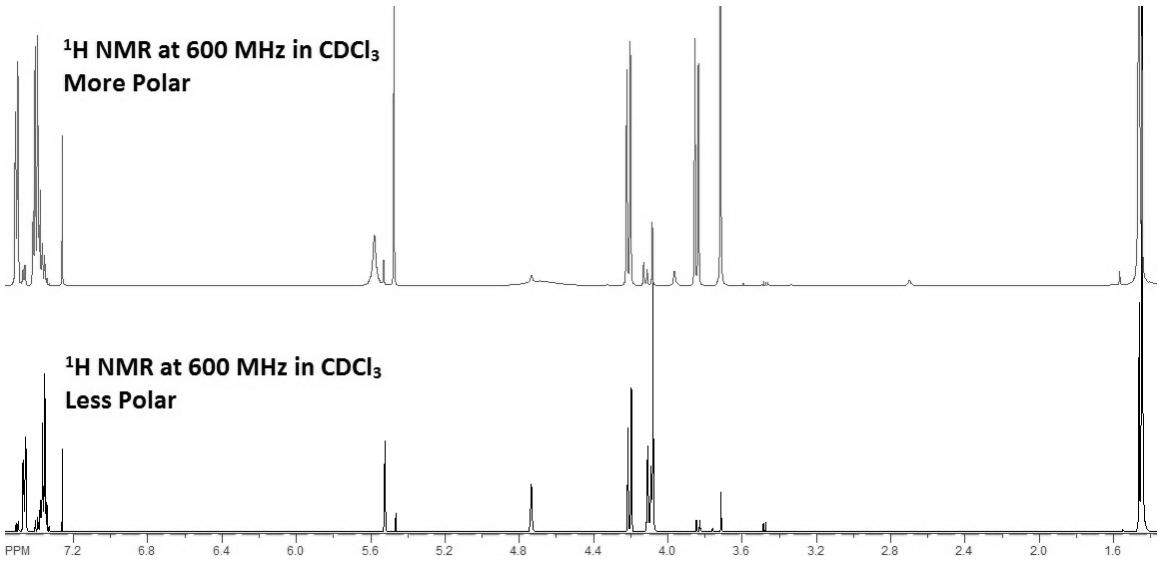
Synthesis of Compounds:

5-hydroxymethyl-5-(tert-butoxycarbonylamino)-propyl-1,3-benzylidene acetal (**2**):[130]

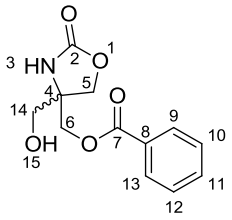


Tris(hydroxymethyl)aminomethane (THAM, 3.210 g, 26.50 mmol, 1.0 eq) and Boc_2O (6.6523 g, 30.48 mmol, 1.15 eq) were dissolved in dry DMF (24 mL) and stirred 24 h at rt. Benzaldehyde dimethyl acetal (5.1 g, 5.0 mL, 33 mmol, 1.2 eq) was added with catalytic *para*-toluenesulfonic acid monohydrate (PTSA, 0.192 g, 1.01 mmol, 0.06 eq) and stirred an additional 24 h. The mixture was diluted with Et_2O (60 mL) and extracted with sat'd $NaHCO_3$ (60 mL) mixed with additional water (20 mL). The aqueous layer was further diluted with water (30 mL) and washed with Et_2O (60 mL × 3), followed by a final dilution with water (20 mL) and final wash with Et_2O (100 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure. The product was recrystallized from Et_2O/hexane to give white crystals as a mixture of two separable (on silica-gel) isomers in a 60:40 ratio (6.666 g, 21.55 mmol, 81.3%). Isomer #1 (less polar): IR (4000-625ν cm^{-1}, NaCl): 3424, 3322, 3036, 2977, 2927, 2867, 1686, 1541, 1500, 1454, 1391, 1368, 1316, 1286, 1250, 1172, 1104, 1084, 1057, 989, 747, 699. ^{1}H NMR (600 MHz, $CDCl_3$): δ1.46 (9H, s, H-10/11/12), 3.71 (2H, s, H-13), 3.84 (2H, d, *J* = 11.6 Hz, H-4/6), 4.21 (2H, d, *J* = 11.6 Hz, H-4/6), 5.48 (1H, s, H-2), 5.8 (broad, 1H, s, NH-7), 7.40 (3H, m, H-17/18/19), 7.51 (2H, dd *J* = 1.5Hz, 8.1 Hz, H-16/20). ^{13}C NMR ($CDCl_3$, 150 MHz): δ28.4 (C-10/11/12), 53.7 (C-5), 65.0 (C-13), 72.0 (C-4/6), 80.8 (C-9),

102.1 (C-2), 126.1 (C-17/19), 128.5 (C-16/20), 129.4 (C-18), 137.6 (C-15), 156.9 (C-8). Isomer #2 (more polar): IR (4000-625ν cm^{-1}, NaCl): 3258, 3070, 2986, 2950, 2929, 2871, 2856, 1682, 1557, 1499, 1456, 1393, 1368, 1319, 1284, 1249, 1214, 1175, 1115, 1050, 1025, 984, 964, 941, 907, 871, 759, 697. ^{1}H NMR (600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 4.08 (2H, s, H-13), 4.10 (2H, s, *J* = 11.1 Hz, H-4/6), 4.20 (2H, d, *J* = 11.1 Hz, H-4/6), 4.74 (1H, s, NH-7), 5.52 (1H, s, H-2), 7.36 (3H, m, H-17/18/19), 7.46 (2H, dd, *J* = 2.4Hz, 8.4 Hz, H-16/20). ^{13}C NMR ($CDCl_3$, 150 MHz): δ28.4 (C-10/11/12), 52.0 (C-5), 63.3 (C-13), 69.4 (C-4/6), 72.0 (C-9), 101.7 (C-2), 126.3 (C-17/19), 128.5 (C-18/20), 129.2 (C-18), 137.6 (C-15), 155.4 (C-8). MS (mixture of isomers, ESI^+, TOF): Calc'd. for [$C_{16}H_{24}NO_5^+$]: 310.1654; found 310.1658.



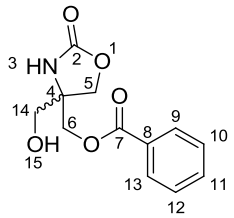
Fragmentation of alcohol **2** with BPO (**3**):[174]



To a flame-dried flask fitted with a condenser under argon, **2** (0.0502 g, 0.162 mmol, 1.0 eq), NBS (0.0309 g, 0.174 mmol, 1.05 eq), BPO (75% BPO in H_2O, 0.0020 g, 0.0062 mmol, 0.04 eq), and chlorobenzene (3 mL) were added and the mixture was heated at 70 ºC for 110 mins. A second portion of NBS (0.008 g, 0.05 mmol, 0.3 eq) and BPO (0.001 g, 0.003 mmol, 0.02 eq) were added and the solution was heated at 70 ºC for an additional 25 mins. The mixture was then cooled to room temperature and sat'd $NaHCO_3$ (2 mL) was added, with subsequent removal of the organic layer. The aqueous layer was washed with DCM (5 mL ×3), the organic layers combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (75:25 hexanes:ethyl acetate) to render white crystals (0.0313 g, 0.124 mmol, 77%). From recrystallization: To a flame-dried 2-neck 250 mL r.b.f. fitted with a condensor under N_2 was added **2** (1.36 g, 4.40 mmol, 1.0 eq), NBS (1.4069 g, 7.9048 mmol, 1.8 eq), BPO (75% in H_2O, 0.2481 g, 0.7682 mmol, 0.17 eq), and chlorobenzene (46 mL). The mixture was heated at 70 ºC for 4h, cooled to rt, and an additional 10 mL of chlorobenzene was added with a second portion of NBS (0.3939 g, 2.214 mmol, 0.5 eq) and BPO (0.0071 g, 0.22 mmol, 0.05 eq). After additional heating at 70 °C for 3h the mixture was cooled to rt, followed by an extraction with DCM (80 mL) and sat'd $NaHCO_3$ (100 mL). The aqueous layer was washed with DCM (80 mL × 4), the

organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The mixture was purified via recrystallization with EtOAc/hexanes, with a hot filtration performed after 100 mL of EtOAc was added to render a white solid (0.8367 g, 3.330 mmol, 76%) . IR (4000-625ν cm^{-1}, NaCl): 3248 (OH), 2923 (C-H), 2853 (C-H), 1712 (C=O), 1466, 1446, 1430, 1407, 1314, 1270 (C-O), 1178, 1114 (C-O), 1054 (C-O), 990, 961, 935, 926, 767, 708. ^{1}H NMR ($CDCl_3$, 600 MHz): δ2.58 (1H, s, OH-15), 3.69 (1H, dd, *J* = 5.2, 11.4 Hz, H-14), 3.73 (1H, dd, *J* = 5.2, 11.4 Hz, H-14) 4.33 (2H, dd, *J* = 9.0Hz, 16.8 Hz, H-6), 4.42 (1H, d, *J* = 11.6 Hz, H-5), 4.54 (1H, d, *J* = 11.6 Hz, H-5), 7.48 (2H, t, *J* = 7.8 Hz, H-10/12), 7.61 (1H, t, *J* = 7.7 Hz, H-11), 8.02 (2H, d, *J* = 7.7 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): 60.5 (C-4), 63.5 (C-14), 64.6 (C-5), 68.9 (C-6), 128.2 (C-10/12), 129.4 (C-9/13), 133.4 (C-11), 138.2 (C-8), 157.9 (C-2), 166.2 (C-8). MS (EI, TOF): Calc'd for [$C_{12}H_{14}NO_5^+$] 252.0872; found 252.0936. MP (corrected): 129.8 – 131.1 ºC.

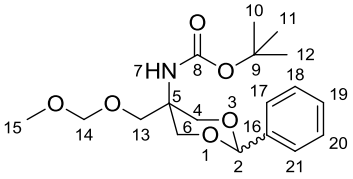
Fragmentation of **2** with AIBN (**3**):[175]



In a flame-dried flask fitted with a condenser under argon alcohol **2** (0.03 g, 0.0 mmol, 1 eq), NBS (0.019 g, 0.10 mmol, 1.0 eq), AIBN (0.0016g, 0.010mmol, 0.1eq), and chlorobenzene (2.5 mL) were added. The mixture was heated at 70ºC for 50 mins, then cooled to room temperature, with subsequent removal of the solvent under reduced

pressure. Purification was obtained through silica column chromatography (75:25 hexanes:ethyl acetate) to render white crystals (0.0177 g, 0.0704 mmol, 71.5%). IR (4000-625ν cm^{-1}, NaCl): 3261 (OH), 2958 (C-H), 2926 (C-H), 2855 (C-H), 1172, 1599, 1466, 1446, 1428, 407, 1389, 1313, 1268 (C-O), 1176 (C=O), 1112 (C-OH), 1052, 1021, 988, 960, 925, 805, 766, 743, 707,686. ^{1}H NMR ($CDCl_3$, 600 MHz): δ3.68 (1H, dd, *J* = 4.8, 12.8 Hz, H-14), 3.73 (1H, dd, J = 4.8, 12.8 Hz, H-14), 4.32 (1H, d, *J* = 16.1 Hz, H-5), 4.34 (1H, d, *J* = 16.1 Hz, H-5), 4.41 (1H, d, *J* = 11.6 Hz, H-6), 4.54 (1H, d, *J* = 11.6 Hz, H-6), 7.47 (2H, t, *J* = 7.6, 7.6 Hz, H-10/12), 7.62 (1H, t, *J* = 7.5, 7.6 Hz, H-11), 8.02 (2H, d, *J* = 7.5 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): 61.2 (C-4), 64.2 (C-14), 65.3 (C-5), 69.5 (C-6), 128.8 (C-10/12), 130.0 (C-9/13), 134.0 (C-11), 141.9 (C-8), 158.7 (C-2), 166.8 (C-7).

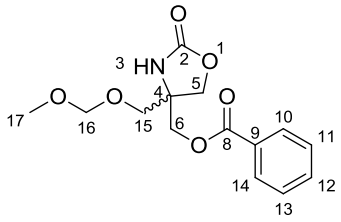
MOM-protection of oxazolidinone **3** (**5**):[174]



To a flame-dried 2-neck rbf alcohol **2** (0.2 g, 0.65 mmol, 1 eq) was dissolved in dry DCM (2 mL) with MOM-Cl (0.15 mL, 0.16 g, 2.0 mmol, 3.1 eq) and diisopropylethylamine (0.68 mL, 0.50g, 3.9 mmol, 6.0 eq). The mixture was stirred for 70 mins, then another 1.5 mL of DCM was added to replace any evaporated solvent. The mixture was stirred for an additional 130 mins, with subsequent removal of solvent under reduced pressure. Purification was obtained through column chromatography (15:85 ethyl acetate:hexanes)

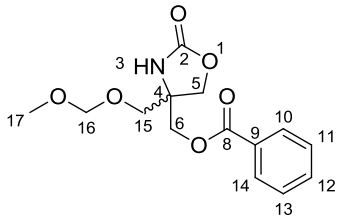
to give white crystals (1:1.4 isomer ratio, 0.1643 g, 0.4649 mmol, 72%). IR (4000-625ν cm-1, NaCl): 3427, 3353, 2977, 2933, 2886, 1718, 1500, 1456, 1392, 1367, 1313, 1287, 1248, 1218, 1167, 1109, 1077, 1046, 977, 918, 870, 825, 747, 699. ^{1}H NMR ($CDCl_3$, 600 MHz): δ1.48 (9H, s, H-10/11/12), 3.36 (major, 3H, s, H-15), 3.40 (minor, 3H, s, H-15), 3.81 (major, 2H, s, H-13), 3.96 (major, 2H, d, 11.2 Hz, H-4/6), 4.01 (minor, 2H, s, H-13), 4.16 (minor, 2H, d, *J* = 11.3 Hz, H-4/6), 4.34 (major, 2H, d, *J* = 11.2 Hz, H-4/6), 4.42 (minor, 2H, s-broad, H-4/6), 4.61 (major, 2H, s, H-14), 4.70 (minor, 2H, s, H-14), 4.83 (minor, 1H, s-broad, NH-7), 5.13 (major, 1H, s, NH-7), 5.48 (major, 1H, s, H-2), 5.54 (minor, 1H, s, H-2), 7.38 (3H, m, H-18/19/20), 7.46 (minor, 2H, dd, *J* = 1.8, 7.9 Hz, H-17/21), 7.51 (2H, d, *J* = 6.6 Hz, H-17/21). ^{13}C NMR (major, $CDCl_3$, 150 MHz): δ28.5 (C-10/11/12), 52.3 (C-15), 55.5 (C-5), 69.1 (C-13), 70.9 (C-4/6), 96.9 (C-14), 101.8 (C-2), 126.2 (C-17/21), 128.5 (C-18/20), 129.3 (C-19), 137.9 (C-16), 155.2 (C-8). ^{13}C NMR (minor, $CDCl_3$, 150 MHz): δ28.5 (C-10/11/12), 51.1 (C-15), 55.6 (C-5), 66.9 (C-4/6), 68.0 (C-13), 97.0 (C-14), 97.0 (C-2), 126.3 (C-17/21), 128.5 (C-18/20), 129.2 (C-19), 137.9 (C-16), 154.7 (C-8). MS (ESI+, TOF): Calc'd for [$C_{16}H_{24}NO_5^+$]: 354.1917; found 354.1906.

Fragmentation of **5** with BPO (**6**):[174]



In a flame-dried 10 mL rbf fitted with a condenser under argon, **10** (0.045 g, 0.13 mmol, 1.0 eq), NBS (0.0238 g, 0.133 mmol, 1.05 eq), and BPO (75% in H_2O, 0.005 g, 0.03 mmol BPO, 0.2 eq) were dissolved in chlorobenzene (2.5 mL) and refluxed at 70 ºC for 1.25 h. The mixture was then cooled to rt and the solvent removed under reduced pressure. Purification was obtained through column chromatography (85:15 hexanes:ethyl acetate) to give white crystals (0.0061 g, 0.021 mmol, 16% - not fully isolated, yield not reflective). ^{1}H NMR ($CDCl_3$, 200 MHz): δ3.36 (3H, s, H-17), 3.70 (2H, s, H-15), 4.26 (1H, d, *J* = 9.0 Hz, H-6), 4.35 (1H, d, *J* = 11.5 Hz, H-5), 4.38 (1H, d, *J* = 9.0 Hz, H-6), 4.54 (1H, d, *J* = 11.5 Hz, H-5), 4.66 (2H, s, H-16), 7.46 (2H, t, *J* = 7.5, 7.6 Hz, H-11/13), 7.60 (1H, t, 7.5 Hz, H-12), 8.02 (2H, d, *J* = 7.6 Hz, H-10/14). ^{13}C NMR ($CDCl_3$, 150 MHz): δ55.9 (C-17), 60.0 (C-4), 65.6 (C-15), 69.8 (C-5), 69.9 (C-6), 97.1 (C-16), 128.8 (C-11/13), 129.2 (C-12), 129.9 (C-10/14), 133.8 (C-9), 158.4 (C-2), 166.2 (C-8). MS (EI, TOF): Cal'd for [$C_{14}H_{18}NO_6^+$]: 296.1134; found 296.1140.

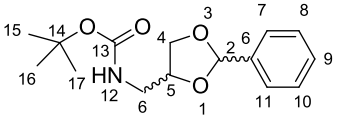
Synthesis of 4-methyldimethoxy-4-methylbenzoate-2-oxazolidinone from AIBN (**6**):[175]



In a flame-dried flask with condenser under argon **5** (0.041 g, 0.12 mmol, 1.0 eq), NBS (0.023 g, 0.13 mmol, 1.1 eq) and AIBN (0.0018 g, 0.011 mmol, 0.1 eq) were added. This was dissolved in chlorobenzene (2.5 mL) and refluxed at 70 ºC for 1.5 h. The mixture was then cooled to rt and diluted with dichloromethane (1 mL) and worked-up with of saturated sodium bicarbonate (2 mL). The aqueous layer was washed with DCM (5 mL × 3), and the organic layers were combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (85:15 hexanes:ethyl acetate, increasing to 75:25 hexanes:ethyl acetate) to render white crystals (0.0162g, 0.0549 mmol, 47%). IR (4000-625ν cm^{-1}, NaCl): 3428 (N-H), 2928 (C-H), 2888 (C-H), 2851 (C-H), 2824, 1719 (C=O ester), 1648 (C=O amine), 1603, 1581, 1497 (Ph), 1452 (Ph), 1392, 1366, 1314, 1274, 1251 (C-O ester), 1217, 1151 (C-O ether), 1111 (C-O ether), 1044 (C-N), 975, 919, 782, 749, 713, 700. ^{1}H NMR (600 MHz, $CDCl_3$) δ3.35 (3H, s, H-17), 3.67 (1H, d, *J* = 9.9 Hz, H-15), 3.70 (1H, d, *J* = 9.9 Hz, H-15), 4.27 (1H, d, *J* = 9.0 Hz, H-6), 4.36 (1H, d, *J* = 11.5 Hz, H-5), 4.36 (1H, d, *J* = 9.0 Hz, H-6), 4.52 (1H, d, *J* = 11.5 Hz, H-5), 4.65 (2H, s, H-16), 6.05 (1H, s, NH-3), 7.46 (2H, t, *J* = 7.5Hz, H-11/13) , 7.59 (1H, t, *J* = 7.5 Hz, H-12), 8.01 (2H, d, *J* = 7.9 Hz, H-10/14). ^{13}C NMR ($CDCl_3$, 150 MHz): 55.9 (C-17), 60.0 (C-4), 65.6 (C-5), 69.8

(C-6), 69.9 (C-15), 97.0 (C-16), 128.8 (C-11/13), 129.2 (C-9), 129.9 (C-10/14), 133.8 (C-12), 158.7 (C-2), 166.2 (C-8).

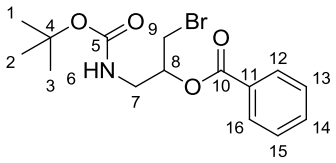
Protection of 3-Amino-1,2-propanediol (**7**):[9]



3-Amino-1,2-propanediol (0.1033 g, 1.134 mmol, 1.0 eq) was dissolved in a vial with DMF (0.6 mL) and added to a 10 mL flame-dried flask, rinsing the vial with a subsequent portion of DMF (0.4 mL), followed by addition to the flame-dried flask. Boc_2O (0.2652 g, 1.215 mmol, 1.08 eq) was then added to the solution and the mixture was stirred at rt for 7 h. Benzaldehyde dimethyl acetal (0.22 mL, 0.20 g, 1.3 mmol, 1.2 eq) and PTSA monohydrate (0.0125 g, 0.0657 mmol, 0.06 eq) was added and the reaction was stirred for 16 h. Sat'd $NaHCO_3$ (2 mL), H_2O (2 mL), and Et_2O (2 mL) were added and the mixture was extracted. The aqueous layer was washed with Et_2O (3 mL × 3), the organic layers were combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Silica column chromatography (100% hexanes gradually to 70:30 hexanes:ethyl acetate) yielded a white solid as an inseparable mixture of diastereomers in a 1:1.3 ratio (0.1441 g, 0.5159 mmol, 46%). IR (4000-625ν cm^{-1}, NaCl): 3316, 3001, 2947, 1873, 1667, 1520, 1479, 1406, 1088, 1071, 981, 914, 758. ^1H NMR (major isomer, 600MHz, $CDCl_3$): δ1.48 (9H, s, H-15/16/17), 3.25-3.35 (2H, m, H-6), 3.88 (1H, dd, *J* = 5.6, 8.2 Hz, H-4), 4.09 (1H, dd, *J* = 7.4, 8.1 Hz, H-4), 4.29-4.37 (1H, m, H-5), 4.8 (1H, broad, NH-12), 5.79 (1H, s, H-2), 7.39 (3H, m, H-8/9/10), 7.49 (2H, dd, *J* = 2.0, 7.5 Hz, H-7/11). ^1H

NMR (minor isomer, 600 MHz, $CDCl_3$): δ1.49 (9H, s, H-15/16/17), 3.41-3.54 (2H, m, H-6), 3.72 (1H, dd, *J* = 7.0, 8.2 Hz, H-4), 4.23 (1H, dd, *J* = 6.5, 8.3 Hz, H-4), 4.29-4.37 (1H, m, H-5), 4.9 (1H, broad, NH-12), 5.94 (1H, s, H-2), 7.39 (3H, m, H-8/9/10), 7.46 (2H, dd, *J* = 1.8, 7.6 Hz, H-7/11). ^{13}C NMR (mixture of isomers, $CDCl_3$, 150 MHz): δ28.0 (C-15/16/17), 28.4 (C-15/16/17), 42.4 (C-6), 43.0 (C-6), 67.8 (C-4), 67.9 (C-4), 75.6 (C-5), 75.9 (C-5), 79.6 (C-14), 79.6 (C-14), 103.5 (C-2), 104.3 (C-2), 126.4 (C-7/11), 126.6 (C-7/11), 128.4 (C-8/10), 128.5 (C-8/10), 129.3 (C-9), 129.5 (C-9), 137.2 (C-6), 137.9 (C-6), 156.2 (C-13). MS (ESI+): Calc'd. for $[C_{15}H_{21}NO_4Na^+]$: 302.1368; found 302.1362.

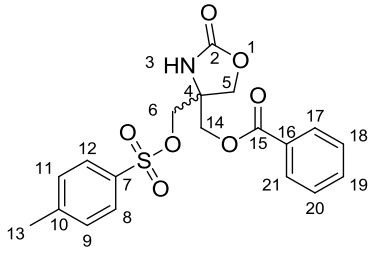
Fragmentation of **7** (**8**):[174]



7 (0.0491 g, 0.176 mmol, 1.0 eq), NBS (0.0528 g, 0.297 mmol, 1.76 eq), and BPO (75% in H_2O, 0.0094 g,0.029 mmol, 0.17 eq) were added to a flame-dried 10 mL rbf equipped with a condenser. Chlorobenzene (2.3 mL) was added and the mixture was heated at 70 ºC for 5 h. The solution was then cooled to rt and the solvent removed under reduced pressure. Purification was obtained by silica column chromatography (100 hexanes gradually increasing to 90:10 hexanes:ethyl acetate) to afford a white solid (0.0300 g, 0.0837 mmol, 48%). IR (4000-625ν cm^{-1}, NaCl): 3372, 2977, 1721, 1518, 1367, 1271, 1111, 1910, 711. ^1H NMR (600 MHz, $CDCl_3$): δ1.35 (9H, s, H-1/2/3), 3.46-3.54 (2H, m, H-7/9), 3.56 (1H, d, *J* = 5.2 Hz, H-9), 3.59 (1H, dd, *J* = 5.0, 11.1 Hz, H-7), 4.72 (1H, broad, NH-6), 5.23 (1H, q, *J* = 5.4 Hz, H-8), 7.39 (2H, t, *J* = 7.6 Hz, H-13/15), 7.52 (1H,

t, *J* = 7.5, 7.6 Hz, H-14), 8.00 (2H, d, *J* = 7.5 Hz, H-12/16). ^{13}C NMR (150 MHz, $CDCl_3$): δ28.4 (C-1/2/3), 31.5 (C-9), 42.6 (C-7), 72.4 (C-8), 80.1 (C-4), 128.6 (C-13/15), 129.7 (C-11), 130.0 (C-12/16), 133.6 (C-14), 156.0 (C-5), 165.9 (C-10). MS (ESI^+, TOF): Calc'd for [$C_{15}H_{20}BrNO_4Na^+$]: 380.0468, 382.0453; found 380.0480, 382.0511.

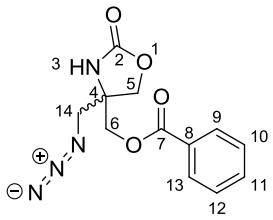
Synthesis of 4-methyltosyl-4-methylbenzoate-2-oxazolidinone **13**:



In a flame-dried 25 mL rbf charged with N_2 oxazolidinone **3** (0.3197 g, 1.272 mmol, 1.0 eq), tosyl chloride (0.7410 g, 3.886 mmol, 3.05 eq), and lutidine (3.0 mL) were added and stirred at rt for 20.5 h. The thick, dark-red mixture was diluted with DCM (1 mL) and stirred an additional 19.5 h. The solvent was then removed under reduced pressure and purification was obtained through silica column chromatography (80:20 hexanes:ethyl acetate) to afford a white, crystalline solid (0.4493 g, 1.108 mmol, 87%). IR (4000-625ν cm^{-1}, NaCl): 3343, 3066 (aromatic C-H), 3032, 2957, 2918, 1765 (C=O), 1725 (C=O carbamate), 1599 (C=C aromatic), 1585 (C=C aromatic), 1532, 1451 (C=C aromatic), 1401 (C=C aromatic), 1366, 1315, 1269 (C-O), 1212, 1191, 1177, 1113, 1097, 1071, 1050, 1027, 1019, 992, 937, 830, 814, 793, 764, 712, 686, 667. ^{1}H NMR (600 MHz, $CDCl_3$): δ2.37 (3H, s, H-13), 4.15 (2H, dd, *J* = 10.2, 16.3 Hz, H-6), 4.24 (1H, d, *J* = 9.4 Hz, H-14), 4.30 (1H, d, *J* = 11.6 Hz, H-5), 4.31 (1H, d, *J* = 9.4 Hz, H-14), 4.44 (1H, d, *J* =

9.4 Hz, H-5), 5.59 (1H, s, NH-3), 7.30 (2H, d, *J* = 8.1 Hz, H-9/11), 7.45 (2H, dd, *J* = 1.7, 7.4, 8.2 Hz, H-18/20), 7.61 (1H, dddd, *J* = 1.3, 1.3, 7.4, 7.4 Hz, H-19), 7.77 (2H, dd, *J* = 1.6, 8.2 Hz, H-8/12), 7.91 (2H, dd, *J* = 1.3, 8.2 Hz, H-17/21). ^{13}C NMR ($CDCl_3$, 150 MHz): δ21.8 (C-13), 59.2 (C-4), 64.9 (C-5), 69.1 (C-6), 69.3 (C-14), 128.1 (C-9/11), 128.8 (C-18/20), 129.9 (C-17/21), 130.3 (C-8/12), 131.8 (C-10), 134.0 (C-19), 146.0 (C-7), 157.7 (C-2), 165.8 (C-15). MS (CI): Calc'd for [$C_{19}H_{20}NO_7S^+$]: 406.0960; found 406.1000. Calc'd for [$C_{12}H_{12}NO_4^+$ = M^o-OTs^-]: 234.0766; found 234.0752. MP (corrected): 118.9-122.0 ºC.

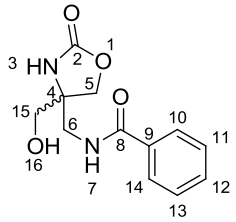
Synthesis of 4-methylazide-4-methylbenzoate-2-oxazolidinone **14**:



A flame-dry 2-neck rbf was loaded with tosylated oxazolidinone **13** (0.02010 g, 0.4958 mmol, 1.0 eq), sodium azide (0.1796 g, 0.2763 mmol, 5.5 eq), and DMF (1.4 mL), with subsequent heating of the solution at 80 ºC for 5 h. The solvent was removed under reduced pressure, followed by rinsing the resulting solid with DCM (3 mL × 4). The DCM washes were combined and the solvent removed under reduced pressure to afford a white solid (0.1505 g, 0.4724 mmol, 95%). IR (4000-625ν cm^{-1}, NaCl): 3312 (N-H/residual water), 3071, 2957, 2918, 2867, 2112, 1759 (C=O), 1725 (C=O), 1602, 1584, 1535, 1491, 1475, 1451, 1401, 1350, 1316, 1272, 1179, 1161, 1114, 1072, 1048, 1028, 1000, 961, 937, 806, 767, 712, 687, 668. ^{1}H NMR (600 MHz, $CDCl_3$): δ3.61 (1H, d, *J* =

12.4 Hz, H-14), 3.67 (1H, d, *J* = 12.4 Hz, H-14), 4.29 (1H, d, *J* = 9.2 Hz, H-6), 4.33 (1H, d, *J* = 11.4 Hz, H-5), 4.33 (1H, d, *J* = 9.2 Hz, H-6), 4.48 (1H, d, *J* = 11.4 Hz, H-5), 5.78 (1H, s, NH-3), 7.48 (2H, dd, *J* = 7.6, 7.8 Hz, H-10/12), 7.61 (1H, dd, *J* = 7.6 Hz, H-11), 8.02 (2H, d, *J* = 7.8 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): δ54.9 (C-14), 60.1 (C-4), 65.7 (C-5), 69.9 (C-6), 128.8 (C-10/12), 128.9 (C-8), 129.9 (C-9/13), 134.0 (C-11), 158.3 (C-2), 166.1 (C-7). MS. (ESI+, TOF): Calc'd for [$C_{12}H_{13}N_4O_4^+$]: 277.0937; found 277.0928. MP: decomposes >120 ºC.

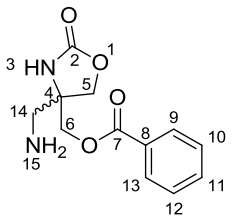
Synthesis of 4-methylhydroxy-4-methylbenzamide-2-oxazolidinone using Staudinger Reaction (**15**):[176]



Azide **14** (0.0164 g, 0.0594 mmol, 1.0 eq) was added to a microwave vial dissolved in DCM, with subsequent removal the DCM under reduced pressure. Triphenylphosphine (0.0231 g, 0.088 mmol, 1.5 eq) and THF (0.4 mL) were added to the vial, followed by stirring at rt for 24 h. H_2O (0.028 mL, 0.028 g, 1.56 mmol, 26 eq) was added and the mixture stirred for an additional 24 h at rt. Solvent was removed under reduce pressure and purified by silica column chromatorgraphy (hexanes:ethyl acetate 75:25) to give white crystals (0.0048 g, 0.019 mmol, 32%). IR (4000-625ν cm^{-1}, NaCl): 3313 (OH), 3287 (NH), 2920, 2851, 1801, 1743 (C=O), 1643 (C=O), 1602, 1578, 1540, 1490, 1437, 1402, 1309, 1272, 1177, 1158, 1119, 1048 (C-O), 965, 936, 803, 751, 712, 694. ^{1}H NMR

($CDCl_3$, 600 MHz): δ3.47 (1H, d, *J* = 6.5, 14.2 Hz, H-6), 3.56 (2H, dd, *J* = 11.0 Hz, H-15), 3.92 (1H, dd, *J* = 7.1, 14.2 Hz, H-6), 4.19 (1H, d, *J* = 9.2 Hz, H-5), 4.23 (1H, s, OH-16), 4.33 (1H, d, *J* = 9.2 Hz, H-5), 6.19 (1H, s, NH-3), 7.21 (1H, dd, *J* = 6.5, 7.1 Hz, NH-7), 7.46 (2H, t, *J* = 7.5 Hz, H-11/13), 7.55 (1H, tt, *J* = 1.0, 7.5, 8.2 Hz, H-12), 7.84 (2H, dd, *J* = 1.0, 8.2 Hz, H-10/14). ^{13}C NMR ($CDCl_3$, 150 MHz): δ44.0 (C-6), 62.2 (C-1), 64.4 (C-15), 71.5 (C-5), 127.4 (C-11/13), 129.0 (C-10/14), 132.6 (C-12), 132.8 (C-9), 159.4 (C-2), 169.9 (C-8).

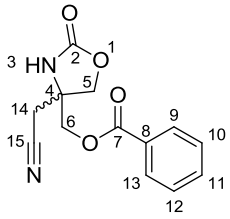
Synthesis of 4-methylamine-4-methylbenzoate-2-oxazolidinone (**16**):[175]



In a flamedried 2-neck 25 mL rbf under N_2 was added azide **14** (0.0152 g, 0.055 mmol, 1.0 eq) and Pd (10% on C, 0.0062 g, 0.0058 mmol, 0.1 eq), followed by THF (0.5 mL). The reaction vessel was flushed with copious amounts of $H_{2(g)}$, and the mixture stirred at rt for 4 h. The product mixture was filtered through celite, with washing of the residue with EtOAc (1.5 mL × 3). Solvent was removed under reduced pressure to afford white crystals (0.0137 g, 0.0547 mmol, 99.4%). IR (4000-625ν cm^{-1}, NaCl): 3262 (NH/NH_2/residual water), 2960, 2918, 1750 (C=O ester), 1711 (C=O carbamate), 1600 (C=C aromatic), 1451 (C=C aromatic), 1402 (C=C aromatic), 1374, 1314, 1274 (C-N), 1180, 1113, 1071, 1041, 947, 804, 769, 712. ^1H NMR ($CDCl_3$, 600 MHz): δ2.87 (1H, d, *J* = 13.4 Hz, H-14), 2.98 (1H, d, *J* = 13.4 Hz, H-14), 4.31 (1H, d, *J*= 8.9 Hz, H-6), 4.35

(1H, d, *J* = 8.9 Hz, H-6), 4.37 (1H, d, *J* = 11.4 Hz, H-5), 4.47 (1H, d, *J* = 11.4 Hz, H-5), 5.41 (1H, s, NH-3), 7.47 (2H, dd, *J* = 7.6, 8.0 Hz, H-10/12), 7.61 (1H, tt, *J* = 1.2, 7.5 Hz, H-11), 8.02 (2H, dd, *J* = 1.2, 8.0 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz):[177] δ45.7 (C-14), 61.3 (C-4), 66.1 (C-5), 70.4 (C-6), 128.8 (C-10/12), 129.2 (C-8), 129.9 (C-9/13), 133.6 (C-11), 159.2 (C-2), 166.3 (C-7). MS (ESI^+): Cal'd for [$C_{12}H_{15}N_2O_4^+$]: 251.1032; found 251.2.

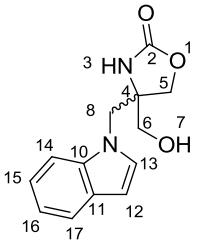
Synthesis of 4-methylamine-4-methylbenzoate-2-oxazolidinone (**17**):



To a flame-dried non-tapered microwave vial under N_2 was added tosylate **13** (0.0490 g, 0.121 mmol, 1.0 eq), KCN (0.0300 g, 0.461 mmol, 3.8 eq), DMF (0.3 mL), and stirred for 4 h at rt. Solvent was removed under reduced pressure, using toluene to aziotrope off any residual DMF. The solid was washed with copious amounts of DCM, filtered, and the DCM removed under reduced pressure to give the product as a white solid (0.0238 g, 0.915 mmol, 76%). IR (4000-625ν cm^{-1}, NaCl): 3334, 2923, 2249 (CN, small), 1755 (C=O), 1723 (C=O), 1403, 1269, 1112, 1044, 710. ^{1}H NMR (600 MHz, CD_3CN): δ2.92 (1H, d, *J* = 17.2 Hz, H-14), 2.98 (1H, d, *J* = 17.2 Hz, H-14), 4.33 (1H, d, *J* = 9.6 Hz, H-5), 4.34 (1H, d, *J* = 11.5 Hz, H-6), 4.38 (1H, d, *J* = 11.5 Hz, H-5), 4.43 (1H, d, *J* = 9.6 Hz, H-6), 6.39 (1H, s broad, NH-3), 7.53 (2H, dd, *J* = 7.5, 8.2 Hz, H-10/12), 7.66 (1H, tt, *J* = 1.2, 7.5 Hz, H-11), 8.06 (2H, dd, 1.2, 8.2 Hz, H-9/13). ^{13}C NMR (150 MHz, CD_3CN):

δ26.3 (C-14), 58.9 (C-4), 68.1 (C-5), 71.4 (C-6), 129.7 (C-10/12), 130.4 (C-6), 130.6 (C-9/13), 134.6 (C-11), 158.4 (C-2), 166.6 (C-7). MS (ESI+): Calc'd for [$C_{13}H_{13}N_2O_4^+$]: 261.0870; found 261.0850. MP (corrected): 131.0 – 132.2 ºC.

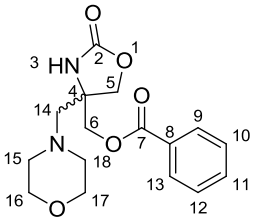
Conversion of Tosylate to Indole (**19**):



To a flame-dried tapered microwave vial was added sodium hydride (60% in mineral oil, 0.0304 g, 0.760 mmol, 5.1 eq), indole (0.0598 g, 0.510 mmol, 3.5 eq), and the mixture was cooled to 0 ºC, and DMF (0.25 mL) was added while stirring. After 20 mins additional DMF (0.2 mL) was added and the solution turned a dark brown. A final amount of DMF (0.2 mL) was added and stirred for 15 mins before tosyl oxazolidinone **13** (0.060g 0.148 mmol, 1.0 eq) was added in one portion. The solution was heated at 83 ºC for 18 h and then cooled to rt, with an additional 3 h of stirring. The solution was extracted with DCM (1 mL) in sat'd NH_4Cl (1 mL), and the aqueous layer washed with DCM (1 mL × 3). The organic layers were combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Purification was obtained through column chromatography (silica gel, 100 hexanes, slowly increasing the ratio to 50:50 hexanes:ethyl acetate, and eventually 100 ethyl acetate) to afford a white solid (0.0115 g, 0.0461 mmol, 31.6%). IR (4000-625ν cm^{-1}, NaCl): 3293 (O-H), 2924 (C-H), 1739 (C=O),

1461, 1313, 1049, 742. ^{1}H NMR (600 MHz, $CDCl_3$): δ3.54 (1H, d, J = 10.9 Hz, H-6), 3.57 (1H, d, *J* = 10.9 Hz, H-6), 4.22 (1H, d, *J* = 9.1 Hz, H-8), 4.26 (1H, d, *J* = 9.1 Hz, H-8), 4.32 (1H, d, *J* = 15.0 Hz, H-5), 4.36 (1H, d, *J* = 15.0 Hz, H-5), 5.82 (1H, s, NH-3), 6.55 (1H, d, *J* = 3.0Hz, H-12), 7.13 (2H, m, H-13/16), 7.23 (1H, dd, *J* = 7.3, 7.9 Hz, H-15), 7.40 (1H, d, *J* = 8.3 Hz, H-17), 7.62 (1H, d, *J* = 7.9 Hz, H-14). ^{13}C NMR (150 MHz, $CDCl_3$):[177] δ 49.1 (C-8), 63.1 (C-4), 64.1 (C-6), 70.1 (C-5), 103.2 (C-12), 109.2 (C-14), 120.2 (C-16/17), 121.4 (C-15), 122.6 (C-13), 128.6 (C-11), 137.0 (C-10), 159.1 (C-2). MS (ESI+): Cal'd for [$C_{13}H_{15}N_2O_3^+$]: 247.1083; found 247.1076.

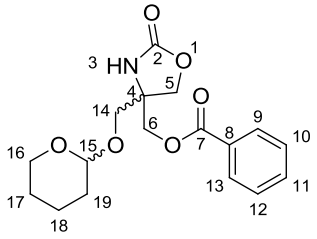
Synthesis of 4-methylmorpholine-4-methylbenzoate-2-oxazolidinone (**20**):



To a flame-dried flask, under N_2, was added tosylate **13** (0.015 g, 0.038 mmol, 1.0 eq), morpholine (0.07 mL, 0.070 g, 0.76 mmol, 21 eq), DIPEA (0.1 mL, 0.074 g, 0.57 mmol, 15 eq), and THF (0.15 mL). The mixture was heated at 80 ºC for 2 h, then additional morpholine (0.15 mL, 0.15 g, 1.73 mmol, 45 eq) was added. The mixture was heated at 70 ºC a further 18 h, followed by cooling to rt. Solvent was removed under reduced pressure and purified via silica column chromatography (70:30 Hexanes:Ethyl Acetate) to afford a yellow solid (0.0060 g, 0.019 mmol, 49%). IR (4000-625ν cm^{-1}, NaCl): 3278 (N-H), 3068, 2958, 2922, 2854, 2812, 2109, 1758 (C=O), 1723 (C=O), 1621, 1602, 1584, 1452, 1395, 1374, 1315, 1273 (C-O), 1178, 1116 (C-O), 1071, 1046 (C-N), 1028, 1013,

960, 937, 892, 865, 83, 768, 713, 687, 667. ^{1}H NMR (600 MHz, $CDCl_3$): δ2.60 (4H, dd, *J* = 4.4, 4.4 Hz, H-15/18), 2.62 (1H, d, *J* = 14.3 Hz, H-14), 2.67 (1H, d, *J* = 14.3 Hz, H-14), 3.68 (4H, t, *J* = 4.4 Hz, H-16/17), 4.24 (1H, d, *J* = 8.8 Hz, H-6), 4.31 (1H, d, *J* = 11.4 Hz, H-5), 4.36 (1H, d, *J* = 8.8 Hz, H-6), 4.47 (1H, d, *J*= 11.4 Hz, H-5), 5.49 (1H, s, NH-3), 7.47 (2H, dd, *J* = 7.5, 8.2 Hz, H-10/12), 7.59 (1H, tt, *J* = 1.2, 7.5 Hz, H-11), 8.01 (2H, dd, *J* = 1.2, 8.2 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): δ55.8 (C-15/18), 31.1 (C-4), 32.7 (C-14), 66.6 (C-1), 67.2 (C-16/17), 70.9 (C-6), 128.8 (C-10/12), 129.2 (C-8), 129.8 (C-9/13), 133.8 (C-11), 158.6 (C-2), 166.2 (C-7). MS (CI): Cal'd for [$C_{16}H_{21}N_2O_5^+$] 321.1450; found 321.1451.

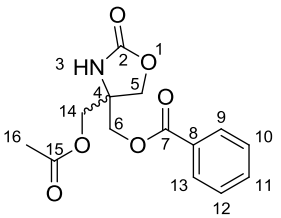
Synthesis of 4-methyl-O-THP-4-methylbenzoate-2-oxazolidinone (**21**):



To a flame-dried tapered microwave vial under N_2, oxazolidinone **3** (0.0488 g, 0.194 mmol, 1.0 eq), PTSA•H_2O (0.0012 g, 0.0063 mmol, 0.03 eq), DCM (0.3 mL), and DHP (0.030 mL, 0.030 g, 0.354 mmol, 1.8 eq) were added and the mixture was stirred at rt for 1.75 h. A subsequent portion of DHP (0.020 mL, 0.02 g, 0.236 mmol, 1.1 eq) was then added and the mixture was stirred an additional 19.5 h. The solvent was removed under reduced pressure and purification was obtained through silica column chromatography (silica gel, 100 hexanes, slowly increasing to 75:25 hexanes:ethyl acetate) to give a clear, colourless oil as a mixture of two inseparable isomers in a 50:50 distribution as indicated by ^{1}H NMR (0.0442 g, 0.132

mmol, 68%). IR (4000-625ν cm^{-1}, NaCl): 3278 (N-H/residual water), 2945 (C-H), 2871 (C-H), 1761 (C=O), 1723 (C=O), 1391 (C-O), 1272 (C-O), 1123 (C-O), 1071 (C-O), 1036(C-O), 713. ^{1}H NMR (mixture of diastereomers, integration set to 1H on 1 diastereomer = 1H, 600 MHz, $CDCl_3$): δ1.47-1.82 (15H, m, H-17/18/19, contaminated with H_2O), 2.70 (1H, impurity), 3.46-3.53 (3H, m, H-14/14/14), 3.56 (1H, dd, *J* = 2.1, 10.3 Hz, H-14), 3.76 (2H, ddt, *J* = 2.7, 9.4, 12.0 Hz, H-16), 3.88 (1H, d, *J* = 10.3 Hz, H-14), 3.93 (1H, dd, *J* = 0.7, 9.9 Hz, H-14), 4.24 (1H, dd, *J* = 1.1, 9.1 Hz, H-6), 4.28 (1H, d, *J* = 9.1 Hz, H-6), 4.32-4.38 (4H, m, H-5/5/6/6), 4.50 (1H, dd, *J* = 1.0, 11.5 Hz, H-5), 4.53 (1H, dd, *J* = 1.4, 11.5 Hz, H-5), 4.59-4.63 (2H, m, H-15), 6.08-3.36 (2H, broad-m, NH-2), 7.43 (2H, t, *J* = 7.7 Hz, H-10/12), 7.57 (1H, tt, *J* = 1.1, 7.4 Hz, H-11), 8.01 (2H, d, *J* = 7.4 Hz, H-9/13). ^{13}C NMR (mixture of diastereomers, $CDCl_3$, 150 MHz): δ19.2 (C-18), 19.3 (C-18), 25.2 (C-17), 25.3 (C-17), 30.3 (C-19), 30.4 (C-19), 60.0 (C-4), 62.6 (C-16), 62.6 (C-16), 65.8 (C-5), 65.9 (C-5), 69.4 (C-14), 69.8 (C-14), 69.8 (C-6), 69.9 (C-6), 99.3 (C-15), 99.7 (C-15), 128.7 (C-10/12), 129.3 (C-8), 129.8 (C-9/13), 133.6 (C-11), 158.8 (C-2), 158.9 (C-2), 166.2 (C-7). MS (ESI+): Calc'd for $[C_{17}H_{22}NO_6^+]$ 336.1447; found 336.1456.

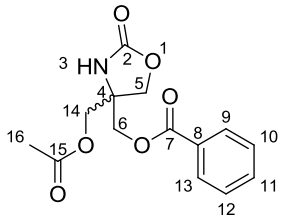
Acetate from Tosyl oxazolidinone (**22**):



Tosylate **13** (0.0253 g, 0.0624 mmol) was added to a tapered microwave vial with acetic acid (1 mL) and the vial was capped. The solution was microwaved at 200 ºC for 1 h,

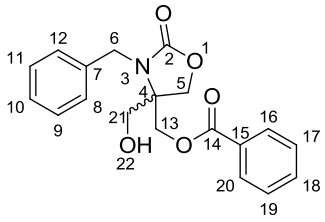
cooled, and microwaved a second time at 200 ºC for an additional 1 h. The solution was then cooled to rt and sat'd $NaHCO_3$ (2.5 mL) was added. The aqueous mixture was washed with DCM (1 mL × 3) and ethyl acetate (1 mL × 1). The organic layers were combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Purification was obtained through column chromatography twice (first column: silica gel, 100 hexanes, slowly increasing the ratio to 50:50 hexanes:ethyl acetate; second column: silica gel, 100 hexanes, slowly increasing the ratio to 60:40 hexanes:ethyl acetate) to produce a clear, colourless oil (0.0090 g, 0.031 mmol, 49.2%). IR (4000-625ν cm^{-1}, NaCl): 3322 (residual water/N-H), 2958 (C-H), 1764 (C=O), 1746 (C=O), 1726 (C=O), 1402 (C-O), 1272 (C-O), 1051, 712. ^{1}H NMR (600 MHz, $CDCl_3$): δ2.12 (3H, s, H-18), 4.17 (1H, d, *J* = 11.6Hz, H-14), 4.32 (1H, d, *J* = 9.2Hz, H-6), 4.34 (2H, d, *J* = 9.2, 11.6Hz, H-5/14 overlapping), 4.37 (1H, d, *J* = 9.2Hz, H-6), 4.49 (1H, d, *J* = 11.6Hz, H-5), 5.8 (1H, 2 peaks, NH-3), 7.46 (2H, t, *J* = 7.5, 8.2Hz, H-10/12), 7.60 (1H, tt, *J* = 1.1, 7.5Hz, H-11), 8.01 (2H, dd, *J* = 1.1, 8.2Hz, H-9/13). ^{13}C NMR (150 MHz, $CDCl_3$): δ20.7 (C-17), 59.5 (C-4), 65.1 (C-5), 65.5 (C-14), 69.5 (C-6), 128.8 (C-10/12), 128.9 (C-8), 129.9 (C-9/13), 133.9 (C-11), 158.2 (C-2), 166.1 (C-7), 170.5 (C-15). MS (ESI+): Calc'd for $[C_{14}H_{16}NO_6^+]$: 294.0977; found 294.0968.

Synthesis of 4-methyl-*O*-acetate-4-methylbenzoate-2-oxazolidinone (**22**):



To a flame-dried non-tapered microwave vial under N_2 were added oxazolidinone **3** (0.0386 g, 0.154 mmol, 1.0 eq), DMF (0.1 mL), acetic anhydride (0.15 mL, 0.162 g, 1.59 mmol, 10 eq), and the mixture was stirred for 48 h. The solvent was removed under reduced pressure and purification was obtained through silica column chromatography (100 hexanes, slowly increasing the ratio to 60:40 hexanes:ethyl acetate) to give a white solid (0.0404 g, 0.138 mmol, 89%). IR (4000-625ν cm^{-1}, NaCl): 3233 (residual water/N-H), 2959 (C-H), 1746 (C=O), 1725 (C=O), 1272 (C-O), 1114 (C-O), 1050 (C-O), 712. ^{1}H NMR (600 MHz, $CDCl_3$): δ2.12 (3H, s, H-16), 4.16 (1H, d, *J* = 11.7 Hz, H-14) , 4.32 (1H, d, *J* = 9.2 Hz, H-6), 4.34 (1H, d, *J* = 11.7 Hz, H-14) , 4.35 (1H, d, *J* = 11.6 Hz, H-5), 4.38 (1H, d, *J* = 9.2 Hz, H-6), 4.49 (1H, d, *J* = 11.6 Hz, H-5), 5.6-5.8 (1H over 2 peaks, NH-3), 7.47 (2H, t, *J* = 7.7 Hz, H-10/12), 7.61 (1H, t, *J* = 7.7 Hz, H-11), 8.01 (2H, d, *J* = 7.5 Hz, H-9/13). ^{13}C NMR (150 MHz, $CDCl_3$): δ20.7 (C-16), 59.5 (C-4), 65.1 (C-5), 65.5 (C-14), 69.5 (C-6), 128.8 (C-10/12), 128.9 (C-8), 129.9 (C-9/13), 133.9 (C-11), 158.2 (C-2), 166.1 (C-7), 170.5 (C-15). MS (ESI+): Calc'd for [$C_{14}H_{16}NO_6^+$] 294.0977; found 294.0983.

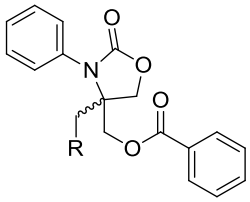
Synthesis of *N*-benzyl-4-methylhydroxy-4-methylbenzoate-2-oxazolidinone (**23**):



In a flame-dried tapered microwave vial under N_2 oxazolidinone **3** (0.030 g, 0.12 mmol, 1.0 eq) was added and cooled to 0 ºC. LiHMDS (1M in THF, 0.25 mL, 0.25 mmol, 2.1 eq) was added dropwise, followed by the addition of DMF (0.1 mL). The mixture was stirred at 0 ºC for 30 mins. Benzyl bromide (0.029 mL, 0.42 g, 0.24 mmol, 2.0eq) was then added. The reaction was then warmed to rt and allowed to stir for 1.5 h. The mixture was diluted with DCM (1 mL) and washed with NH_4Cl (1 mL). The aqueous layer was washed with DCM (1mL × 3) and the organic layers were combined. Ethanol (2 mL) was added to clarify the solution and the organic layer was then dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (silica gel, 100 hexanes, slowly increasing the ratio to 60:40 hexanes:ethyl acetate) to afford a white solid (0.0167 g, 0.0489 mmol, 41%). IR (4000-625ν cm^{-1}, NaCl): 3422 (O-H), 3064 (C-H), 3033 (C-H), 2924 (C-H), 1724 (C=O), 1602, 1585, 1451 (C=O), 1271 (C-O), 1069 (C-N), 710. ^{1}H NMR (600 MHz, $CDCl_3$): δ3.51 (1H, d, *J* = 12.5 Hz, H-21), 3.59 (1H, d, *J* = 12.5 Hz, H-21), 4.26 (1H, d, *J* = 12.0 Hz, H-5), 4.30 (1H, d, *J* = 8.9 Hz, H-13), 4.34 (1H, d, *J* = 8.9 Hz, H-13), 4.35 (1H, d, *J* = 15.7 Hz, H-6), 4.41 (1H, d, *J* = 12.0 Hz, H-5), 4.74 (1H, d, *J* = 15.7 Hz, H-6), 7.27-7.36 (3H, m, H-9/10/11), 7.39 (2H, d, *J* = 7.3 Hz, H-8/12), 7.39 (2H, t, *J* = 7.5, 8.2 Hz, H-17/19), 7.61 (1H, tt, *J* = 1.1, 7.5 Hz, H-18), 7.96 (1H, dd, *J* = 1.1, 8.2 Hz, H-16/20). ^{13}C NMR (150 MHz, $CDCl_3$): δ45.2 (C-6),

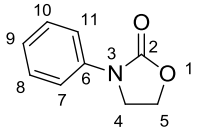
62.5 (C-5), 63.7 (C-21), 64.6 (C-4), 67.4 (C-13), 128.0 (C-8/12), 128.4 (C-10), 128.8 (C-17/19), 129.0 (C-15), 129.2 (C-9/11), 129.9 (C-16/20), 133.9 (C-18), 137.9 (C-7), 158.9 (C-2), 166.2 (C-14). MS (ESI+): Cal'd for [$C_{19}H_{20}NO_5^+$]: 342.1342; found 342.1349.

General Procedure for Aryl Coupling to THAM derivative:[141]



In a flame-dried tapered microwave vial under N_2 was added base (2 eq), THAM derivative (1 eq), CuI (cat.), and dioxane. The ligand (cat.) was then added, as well as the aromatic halide (1.2 eq). The microwave vial was capped and heated at 110 ºC overnight (~18 h). Formation of product was monitored by TLC.

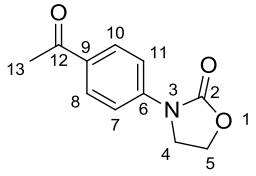
Arylation of 2-Oxazolidinone with Bromobenzene (**24**):[141]



In a flame-dried tapered microwave vial under N_2 was added 2-oxazolidinone (0.0999 g, 1.15 mmol, 1.0 eq), K_2CO_3 (0.317 g, 2.30 mmol, 2.0 eq), and CuI (0.0228 g, 0.120 mmol, 0.1 eq). Dioxane (1.0 mL) was added by syringe, followed by trans-diaminocyclohexane (0.014 mL, 0.0131 g, 0.115 mmol, 0.10 eq) by micropipette and PhBr (0.014 mL, 0.18 g, 1.71mmol, 1.5 eq) by microsyringe. The mixture was heated at 110 ºC with stirring for 7 h, then the temperature was lowered to 100 ºC for an additional 14 h of further stirring.

The mixture was then cooled to room temperature and purification was obtained through column chromatography (silica gel, 100 hexanes, slowly increasing the ratio to 60:40 hexanes:ethyl acetate) to afford a light brown solid (0.0825g, 0.0506 mmol, 44%). IR (4000-625ν cm^{-1}, NaCl): 1742 (C=O), 1129 (C-O), 1051, 752, 690. ^{1}H NMR (600 MHz, $CDCl_3$): δ4.07 (2H, dd, *J* = 7.8, 9.3 Hz, H-4), 4.49 (2H, dd, *J* = 7.8, 9.3 Hz, H-5), 7.14 (1H, tt, *J* = 0.8, 7.5 Hz, H-9), 7.38 (2H, tt, *J* = 7.5, 8.6 Hz, H-7/11), 7.55 (2H,.dd, *J* = 0.8, 8.6 Hz, H-8/10). ^{13}C NMR (150 MHz, $CDCl_3$): δ43.5 (C-4), 61.4 (C-5), 118.4 (C-7/11), 124.2 (C-9), 129.2 (C-8/10), 138.4 (C-6), 155.4 (C-2). MS (ESI+): Cal'd for $[C_9H_{10}NO_2^+]$: 164.0706; found 163.9.

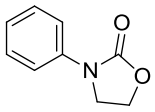
Arylation of 2-Oxazolidinone with 4-iodoacetophenone (**25**):[141,178]



In a flame-dried tapered microwave vial under N_2 2-oxazolidinone (0.0997 g, 1.15 mmol, 1.0 eq), K_2CO_3 (0.317 g, 2.30 mmol, 2.0 eq), CuI (0.022 g, 0.115 mmol, 0.10 eq), and 4-iodoacetephenone (0.272 g, 1.10 mmol, 0.95 eq) were added. Dioxane (1.0 mL) was added by syringe, followed by 1,2-*trans*-diaminocyclohexane (0.014 mL, 0.0131 g, 0.115 mmol, 0.10 eq) by micropipette. The mixture was heated at 110 ºC with stirring for 7 h, then the temperature was lowered to 100 ºC for a further 14 h of stirring. The mixture was then cooled to room temperature and purification was obtained by filtering the mixture through a celite plug and washing with warm DCM (15 mL). Purification was obtained

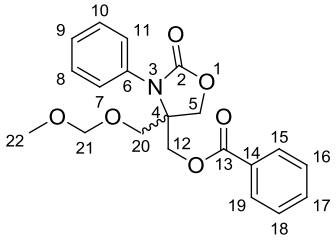
through recrystallization with an ethanol/diethyl ether mix to give a light brown solid (0.1100g, 0.536 mmol, 46.6%). IR (4000-625ν cm^{-1}, NaCl): 2973 (C-H), 2912 (C-H), 1747 (C=O), 1667 (C=O), 1275 (C-O), 1137 (C-O), 1049, 850, 785, 756. ^{1}H NMR (600 MHz, $CDCl_3$): δ2.59 (3H, s, H-13), 4.11 (2H, dd, *J* = 6.6, 7.8, 8.2, 9.4 Hz, H-4), 4.53 (2H, dd, *J* = 6.6, 7.8, 8.2, 9.4 Hz, H-5), 7.65 (2H, dt, *J* = 2.0, 9.0 Hz, H-7/11), 7.99 (2H, dt, *J* = 2.0, 9.0 Hz, H-8/10). MS (ESI+): Calc'd for $[C_{11}H_{12}NO_3^+]$: 206.0812; found 206.1.

Arylation of 2-Oxazolidinone with Bromobenzene and Novel Ligand **31** (**24**):[145]



In a flame-dried non-tapered microwave vial under N_2 was added $CsCO_3$ (0.1387 g, 0.453 mmol, 1.4 eq), 2-oxazolidinone (0.289 g, 0.332 mmol, 1.1 eq), Cu_2O (0.0019 g, 0.0133 mmol, 0.044 eq), 1,10-phenanthroline (0.0025 g, 0.0175 mmol, 0.057 eq), **31** (0.0039 g, 0.0156 mmol, 0.05 eq), and $Pd_2(dba)_3$ (0.0078 g, 0.0085 mmol, 0.028 eq). The vessel was evacuated under reduced pressure and charged with N_2, with repetition multiple times. N_2 was bled through ^{s}BuOH for 30 mins, followed by sonication while under argon for 3 mins. ^{s}BuOH (0.3 mL) and bromobenzene (0.032 mL, 0.0479 g, 0.3047 mmol, 1.0 eq) were added and the mixture was heated at 110 ºC overnight. The solvent was removed under reduced pressure with purification obtained through silica column chromatography (100% hexanes, slowly increasing percentage of ethyl acetate to give a white solid (0.0070 g, 14.1%). ^{1}H NMR in good agreement with literature.

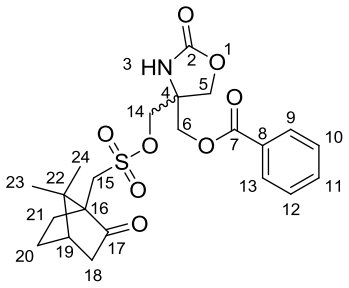
Synthesis of Arylated Mom-oxazolidinone **26**: [141]



To a flame-dried tapered microwave vial charged with N_2 was added CuI (0.0322 g, 0.169 mmol, 2.70 eq), dioxane (0.20 mL), then TDACH (0.020 mL, 0.019 g, 0.0169 mmol, 2.70 eq). The mixture was heated at 40 ºC for 5 mins, then cooled to rt. Bromobenzene (0.020 mL, 0.19 mmol, 0.030 g, 3.0 eq) was then added and the mixture was stirred for 20 mins at rt, followed by addition of MOM-protected oxazolidinone **6** (0.0162 g, 0.0625 mmol, 1.0 eq) and K_2CO_3 (0.0221 g, 0.160 mmol, 2.56 eq). The vial was subsequently capped and the mixture heated at 110 ºC for 24 h. The solution was diluted with dioxane (0.30 mL) and heated at 110 ºC for another 3 days. After cooling of the mixture the solvent was removed under reduced pressure and purification was obtained via silica column chromatography (100 hexanes, increasing to 70:30 hexanes:ethyl acetate) to afford a white solid (0.0144 g, 0.0388 mmol, 62%). ^{1}H NMR (600 MHz, $CDCl_3$): δ3.38 (3H, s, H-22), 3.60 (1H, d, *J* = 10.0 Hz, H-20), 3.70 (1H, d, *J* = 10.0 Hz, H-20), 4.22 (1H, d, *J* = 11.9 Hz, H-5), 4.48 (1H, d, *J* = 12.0 Hz, H-5), 4.49 (1H, d, *J* = 8.9 Hz, H-12), 4.57 (1H, d, *J* = 8.9 Hz, H-12), 4.66 (2H, dd, *J* = 6.7, 9.1 Hz, H-21), 7.26-7.28 (2H, m, H-8/10), 7.37 (1H, tt, *J* = 1.3, 6.2 Hz, H-9), 7.41 (2H, tt, *J* = 1.4, 7.0 Hz, H-7/11), 7.47 (2H, dt, *J* = 8.2 Hz, H-16/18), 7.61 (1H, tt, *J* = 1.3, 7.5 Hz, H-17), 7.99 (2H, dd, *J* = 1.2, 8.3 Hz, H-15-19). ^{13}C NMR (150 MHz, $CDCl_3$): δ56.1 (C-22), 64.5 (C-

4), 64.5 (C-20), 68.3 (C-12), 68.4 (C-5), 97.1 (C-21), 128.9 (C-16/18), 128.9 (C-7/11), 129.1 (C-9), 129.3 (C-17), 129.9 (C-8/10), 129.9 (C-15/19), 133.9 (C-14), 134.4 (C-6), 157.6 (C-2), 166.0 (C-13).

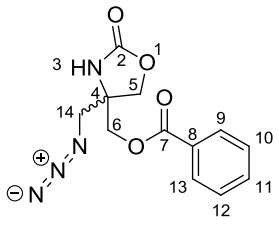
(1*R*)-(-)-10-Camphorsulfonyl oxazolidinone **29**:



In a tapered microwave vial charged with N_2 was added alcohol **3** (0.1028 g, 0.4091 mmol, 1.0 eq), (1*R*)-(-)-10-camphorsulfonyl chloride (0.2047 g, 0.8164 mmol, 2.0 eq), lutidine (0.20 mL, 0.19 g, 1.7 mmol, 4.2 eq), and DCM (0.3 mL). This was stirred for 22 h at rt, then the solvent was removed under reduced pressure. DCM (2 mL) and sat'd $NaHCO_3$ (2 mL) were added and the organic layer was removed. The aqueous layer was washed with DCM (2 mL × 2), the organic layers combined, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and purification was obtained through silica column chromatography (100 hexanes, eventually increasing to 50:50 hexanes:ethyl acetate) to give a white solid (0.0745 g, 0.160 mmol, 39%). IR (4000-625ν cm^{-1}, NaCl): 3297 (NH), 3068, 2963, 1771 (C=O), 1749 (C=O), 1716 (C=O), 1602, 1584, 1452, 1395, 1363, 1271, 1175, 1114, 1051, 998, 936, 830, 767, 714, 635. ^{1}H NMR (600 MHz, $CDCl_3$, as a mixture of 2 visable isomers in approximately a 50:50 ratio): δ0.84 (3H, s, H-16), 0.85 (3H, s, H-24), 1.05 (6H, s, H-23), 1.40-1.48 (2H, m, H-20/21), 1.64-

1.72 (2H, m, H-20/21), 1.94 (2H, t, *J* = 19.4 Hz, H-19), 2.00-2.06 (2H, m, H-20/21), 2.12 (2H, m, H-20/21), 2.36 (4H, d, *J* = 18.1 Hz, H-18), 3.05 (1H, d, *J* = 15.1 Hz, H-15), 3.07 (1H, d, *J* = 15.1 Hz, H-15), 3.60 (1H, d, *J* = 15.7 Hz, H-15), 3.61 (1H, d, *J* = 15.1 Hz, H-15), 4.32-4.42 (7H, m, H-5/14), 4.44 (1H, d, *J* = 10.4 Hz, H-5), 4.47 (1H, d, *J* = 10.4 Hz, H-5), 4.48-4.55 (3H, m, H-4/14), 6.3 (1H, s-broad, NH-3), 6.5 (1H, s-broad, NH-3), 7.45 (4H, t, *J* = 7.7 Hz, H-10/12), 7.59 (2H, t, *J* = 0.9, 7.4 Hz, H-11), 8.02 (4H, m, H-9/13). ^{13}C NMR (both isomers, 150 MHz, $CDCl_3$): δ19.6 (C-23/24), 19.7 (C-23/24), 25.1, 25.2, 27.0, 36.8, 42.7, 42.8, 42.8, 47.7, 47.9, 48.6 (C-15), 58.1 (C-16), 59.5 (C-4), 65.2 (C-5), 65.3 (C-5), 69.0 (C-14), 69.0 (C-14), 69.4 (C-6), 69.5 (C-6), 128.8 (C-10/12), 129.0 (C-8), 129.9 (C-9/13), 133.8 (C-11), 158.2 (C-2), 158.3 (C-2), 166.0 (C-7), 215.1 (C-17), 215.4 (C-17). MS (ESI^+, TOF): Calc'd for [$C_{22}H_{28}NO_8S^+$]: 466.1563; found: 466.1522.

Synthesis of 4-methylazide-4-methylbenzoate-2-oxazolidinone **14**from **29**:



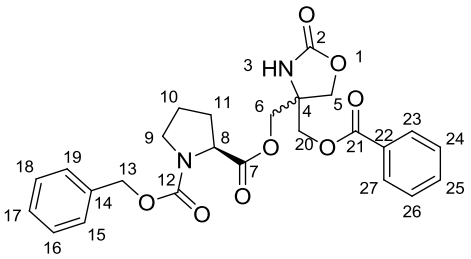
To a tapered microwave vial charged with N_2 was added Camphor THAM derivative **29** (0.0100 g, 0.0215 mmol, 1.0 eq), NaN_3 (0.0007 g, 0.0107 mmol, 0.5 eq), and DMF (0.15 mL). This was stirred at 0 ºC, 30 ºC, 40 ºC, 50 ºC, 60 ºC, and 70 ºC, with no visable change by TLC. Additional NaN_3 (0.0097 g, 0. 15 mmol, 7.0 eq) was added and the mixtured was stirred at 70 ºC overnight. The DMF was removed under reduced pressure, and further aziotroped off using DCM (2 mL × 2 additions). DCM was added to the

resulting slurry, the mixture dried over $MgSO_4$ with subsequent filtering, and the remaining solvent removed under reduced pressure to afford the azide **14**, as visualized by ^{1}H NMR (consistant with previous NMR).

(-)-menthyl fragmented Boc/Bz-protected THAM (**30**):

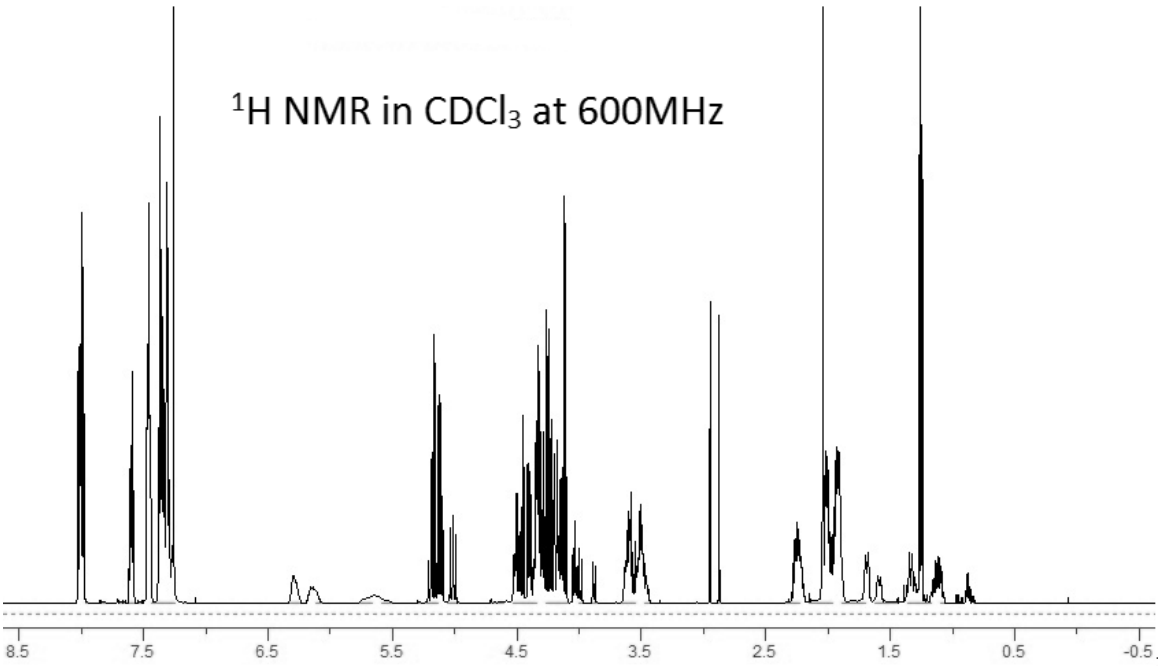
In a tapered microwave vial covered with tin foil and charged with N_2, alcohol **3** (0.0522 g, 0.208 mmol, 1.0 eq), DCM (0.2 mL), TEA (0.15 mL), lutidine (0.2 mL), and (1*R*)-(-)-menthyl chloroformate (0.20 mL, 0.204 g, 0.933mmol, 4.5 eq) were stirred together at rt overnight. The solvent was removed under reduced pressure and the separation via silica column chromatography was performed, however, total separation of the products was not possible.

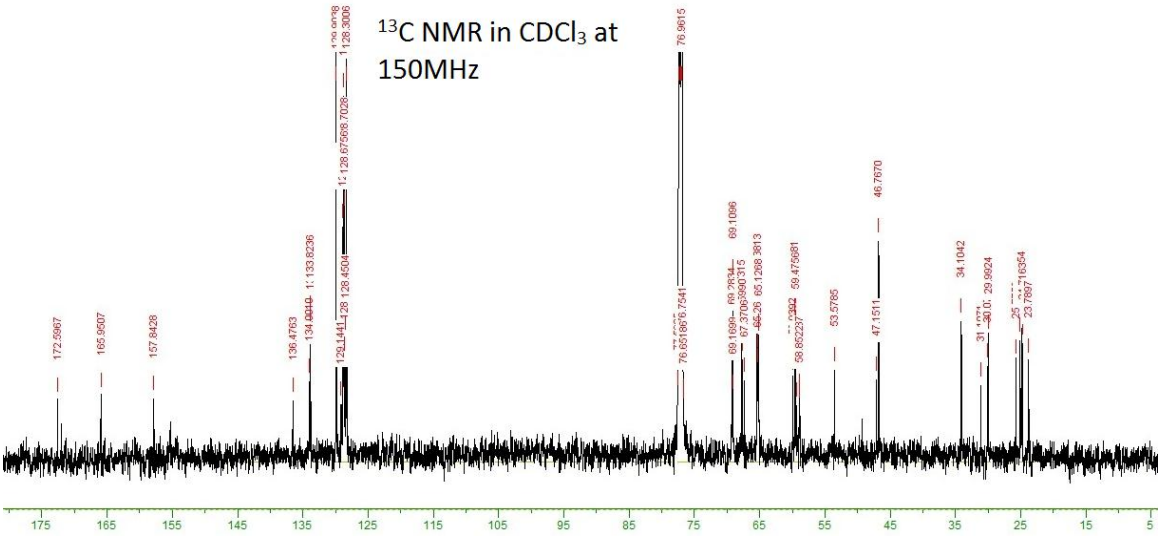
Synthesis of **67**:



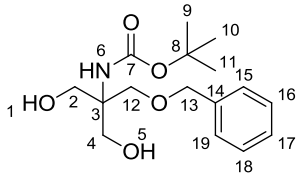
In two tapered microwave vials (V_1 & V_2) charged with N_2, alcohol **3** (V_1: 0.0213 g, 0.0848 mmol, 1.0 eq; V_2: 0.0215 g, 0.0856 mmol, 1.0 eq), Z-Pro-OH (V_1: 0.0261 g, 0.105 mmol, 1.2 eq; V_2: 0.0315 g, 0.126 mmol, 1.5 eq), DMAP (V_1: 0.0009 g, 0.007 mmol, 0.08 eq; V_2; small crystal), and DCC (V_1: 0.0275 g, 0.133 mmol, 1.5 eq; V_2: 0.0338 g, 0.164 mmol, 1.9 eq) were added. To the first vial was added MeCN (0.2 mL) and DMF

(0.2 mL) and to the second vial only DMF (0.2 mL) was added. Both were stirred overnight, mixed together, and their collectively the solvent removed under reduced pressure. Purification was obtained through silica column chromatography to produce a white solid as a mixture of 2 diastereomers (0.0393 g, 0.0815 mmol, 48% combined yield of both V_1 & V_2). IR (4000-625ν cm^{-1}, NaCl): 3320 (N-H), 3064, 3034, 2955, 2934, 2891, 2850, 1751 (C=O), 1730 (C=O), 1624, 1602, 1584, 1538, 1498, 1551, 1419, 1355, 1315, 1273, 1178, 1117, 1072, 1049, 1027, 936, 769, 736, 714, 700. ^{1}H NMR (600MHz, $CDCl_3$, as a mixture of 2 diastereomers): 7.27-7.40 (10H, m), 7.46 (4H, m), 7.60 (2H, m), 9.96-8.04 (4H, m). Due to complexity of spectrum, a diagram has been attached for both the ^{1}H NMR and the ^{13}C NMR. The ratio of isomers is 70:30 as calculated by the peaks at 5.01ppm on the ^{1}H NMR. MS (ESI+): Cal'd for $[C_{25}H_{27}N_2O_8^+]$: 483.1767; found 486.1754.





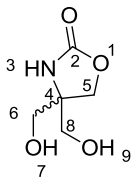
Reduction of Acetal **2** (**32**):[148,179]



In a flame-dried 2-neck rbf under N_2 alcohol **2** (0.1848 g, 0.5974 mmol, 1.0 eq) was added and cooled to -78 ºC. DIBAL (1M in toluene, 2.75 mL, 0.391 g, 2.75 mmol, 4.6 eq) was added slowly and the mixture was warmed to 0 ºC, stirring for 25 mins. The solution was then allowed to warm to rt, stirring for an additional 21 h. The mixture was then cooled to 0 ºC and MeOH (0.15 mL), sat'd NH_4Cl (0.7 mL), and DCM (4 mL) were added, followed by an additional 1h of stirring. The mixture was filter through a sintered glass funnel and rinsed with copious amounts of Et_2O and EtOAc. The solvent was removed under reduced pressure with purification obtained through silica column chromatography (100 hexanes, slowly increasing the ratio of ethyl acetate) to give a white

solid (0.1039g, 0.3337 mmol, 55.9%). IR (4000-625ν cm^{-1}, NaCl): 3414 (O-H), 2963 (C-H), 2924 (C-H), 2855 (C-H), 1688 (C=O), 1501, 1455, 1367 (C-O), 1259 (C-O), 1167 (C-O), 1052 (C-O), 1028, 801, 743, 698. ^{1}H NMR (600 MHz, $CDCl_3$): δ1.44 (9H, s, H-9/10/11), 3.55 (2H, d, *J* = 11.7 Hz, H-2/4), 3.61 (2H, s, H-12), 3.74 (2H, d, *J* = 11.7 Hz, H-2/4), 4.55 (2H, s, H-13), 5.51 (1H, s-broad, NH-6), 7.29-7.34 (3H, m, H-16/17/18), 7.35-7.38 (2H, m, H-15/19). MS (ESI+): Cal'd for [$C_{16}H_{26}NO_6^+$]: 312.1811; found 312.1085.

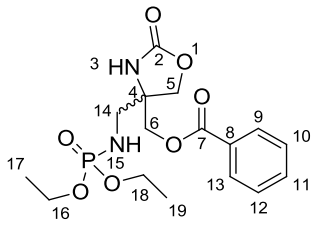
Synthesis of 4,4-methylhydroxy-2-oxazolidinone (**33**):



In a scintillation vial oxazolidinone **3** (0.0817 g, 0.325 mmol, 1.0 eq) was dissolved in an NaOH solution (~0.29 M, 0.115 g NaOH in 6 mL H_2O/MeOH 50:50 mix, 4.0 mL, 1.15 mmol, 3.6 eq) and stirred for 1 h at rt. The mixture was acidified with HCl (37% in water, 1:1 in H_2O). The solvent was removed under reduced pressure and the solid washed with EtOH (~20 mL). The ethanol was removed under reduced pressure and the resulting solid was washed with Et_2O (5 mL × 3) to remove benzoic acid. This rendered the product, which was a white solid (0.0806 g). The overall yield was indeterminable at this point due to the large quantity of water that remained in the sample. IR (4000-625ν cm^{-1}, NaCl): 3137 (O-H), 3046 (O-H), 2811 (C-H), 1746 (C=O), 1737 (C=O), 1404 (C-O), 1050 (C-O). ^{1}H NMR (600 MHz, D_2O): δ3.69 (2H, dd, *J* = 1.4, 11.9Hz, H-6/8), 3.74 (2H, dd, *J* =

1.4, 11.9Hz, H-6/8), 4.44 (2H, s, H-5). ^{13}C NMR (150MHz, D_2O): δ62.2 (C-6/8), 62.7 (C-4), 69.0 (C-5), 161.1 (C-2). MS (ESI+): Calc'd for [$C_5H_{10}NO_4^+$]: 148.0610; found 148.0571.

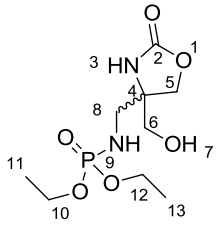
Synthesis of 4-methyl(diethylphosphoramide)-4-methylbenzoate-2-oxazolidinone (**34**):



In a flamedried, tapered microwave vial under N_2, amine **16** (0.0364 g, 0.146 mmol, 1.0 eq) was dissolved in DCM (0.5 mL) and cooled to 0 °C. Lutidine (0.02 mL, 0.0185 g, 0.173 mmol, 1.2 eq) was then added, followed by diethyl chlorophosphate (0.025 mL, 0.0297g, 0.168 mmol, 1.15 eq). The mixture was allowed to warm slowly to rt as it was stirred for 2 h. The solvent was removed under reduced pressure, with purification obtained through silica column chromatography (80:20 ethyl acetate:hexanes to 80:20 ethyl acetate:ethanol) to afford a white solid (0.0286 g, 0.0740 mmol, 51%). IR (4000-625ν cm^{-1}, NaCl): 3250 (N-H), 2983, 2930, 2907, 1762 (C=O), 1725 (C=O), 1602, 1584, 1538, 1451, 1395, 1316, 1273 (C-O ester), 1230 (P=O), 1178 (C-N), 1164 (C-N), 1114 (C-O ether), 1044 (P-O), 1029 (P-O), 970, 867, 802, 755, 713, 687, 666. ^{1}H NMR ($CDCl_3$, 600 MHz): δ1.23 (3H, t, *J* = 7.1 Hz, H-17), 1.30 (3H, t, *J* = 7.1 Hz, H-19), 3.10-3.20 (2H, m, H-14) 3.96-4.08 (4H, m, H-16/18), 4.23 (1H, d, *J*= 9.1 Hz, H-5), 4.30-4.40 (3H, m, H-6/15), 7.00-7.10 (1H, m, NH-3), 7.44 (2H, t, *J* = 7.3, 8.0 Hz, H-10/12), 7.57 (1H, tt, *J* = 1.2, 8.0 Hz, H-11), 7.98 (2H, dd, *J* = 1.2, 7.3 Hz, H-9/13). ^{13}C NMR: ($CDCl_3$,

150 MHz): δ16.2 (d, *J* = 7.5Hz, C-17), 16.3 (d, *J* = 7.5Hz, C-19), 45.9 (C-14), 60.8 (d, *J* = 5.9Hz, C-4), 63.1 (d, *J* = 5.9Hz, C-16), 63.2 (d, *J* = 5.9Hz, C-18), 65.8 (C-5), 70.1 (C-6), 128.7 (C-9/13), 129.2 (C-8), 129.8 (C-10/12), 133.7 (C-11), 158.9 (C-2), 166.2 (C-7). MS (CI): Cal'd for [$C_{16}H_{24}N_2O_7P^+$] 387.1321; found 387.1.

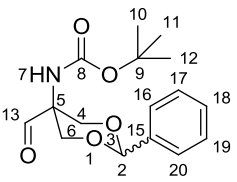
Synthesis of 4-methyl(diethylphosphoramide)-4-methylhydroxy-2-oxazolidinone (**35**):



In a small vial phosphoramide **34** (0.0136 g, 0.0352 mmol, 1.0 eq) was dissolved in a solution of aqueous sodium hydroxide (0.125M, 1 mL). This was stirred at room temperature for 1 h, followed by acidification using a dilute HCl solution in water (pH ≈ 2). DCM (2 mL) was added and the organic layer was extracted, which was then washed twice with water (1.5 mL × 2). The aqueous layer was kept, removing the solvent under reduced pressure with purification obtained through silica olumn chromatography (80:20 ethyl acetate:hexanes, to 80:20 ethyl acetate:ethanol) to afford a clear, colourless oil (0.0091 g, 0.32 mmol, 92%). IR (4000-625ν cm^{-1}, NaCl): 3274 (OH/NH), 2983, 2917, 2871, 2109, 1746, 1541 (C=C), 1444, 1395 (C-O), 1370, 1225 (P-N), 1164, 1144, 1099, 1033 (C-O), 969 (P-O), 867, 799, 768, 714. ^{1}H NMR: ($CDCl_3$, 600 MHz): δ1.34 (3H, t, *J* = 6.5 Hz, H-11), 1.33 (3H, t, *J* = 6.5 Hz, H-13), 3.05 (1H, ddd, *J* = 7.8, 11.3, 14.0 Hz, H-8), 3.21 (1H, ddd, *J* = 7.8, 11.3, 14.0 Hz, H-8), 3.62 (1H, d, *J* = 11.7 Hz, H-6), 3.69 (1H, d, *J* = 11.7 Hz, H-6), 3.94 (1H, dd, *J* = 7.8 Hz, NH-9), 4.07 (4H, dddd, *J* = 1.4, 6.5, 6.5,

14.5 Hz, H-10/12), 4.11 (1H, d, *J* = 9.0 Hz, H-5), 4.24 (1H, d, *J* = 9.0 Hz, H-5), 6.62 (1H, s, NH-3). ^{13}C NMR ($CDCl_3$, 150 MHz): 16.3 (*J* = 4.9Hz, C-11/13), 45.2 (C-4), 62.3 (*J* = 3.9Hz, C-8), 63.3 (*J* = 5.7Hz, C-10), 63.4 (*J* = 5.7Hz, C-12), 64.3 (C-6), 70.5 (C-5), 159.4 (C-2). ^{31}P NMR ($CDCl_3$, 243MHz, externally referenced to 85% H_3PO_4 in D_2O): δ10.0. MS (CI): Cal'd for [$C_9H_{20}N_2O_4P^+$]: 283.1059 [M + H^+], Actual: 283.1057.

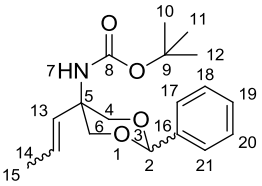
5-formyl-5-(tert-butoxycarbonylamino)-propyl-1,3-benzylidene acetal (**36**):[9,130]



Oxalyl chloride (0.30 mL, 0.44 g, 3.5 mmol, 2.1eq), was dissolved in DCM (9 mL) and cooled to -78 °C. DMSO (040 mL, 0.44 g, 5.6 mmol, 3.4 eq) was added to the mix slowly and the reaction was stirred for 40 mins. A solution of alcohol **2** (0.5045 g, 1.631 mmol, 1.0 eq) in DCM (2.5 mL) was added dropwise to the mixture over 3 mins and stirring was continued at -78 ºC for 55 mins. TEA (1.5 mL, 1.1 g, 11 mmol, 6.6 eq) was added to the reaction and the solution was slowly allowed to warm to rt, stirring over 2 h. The solution was quenched with 1M HCl (1 mL) and washed with sat'd $NaHCO_3$, followed by brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification was obtained through silica column chromatography (100:0 hexanes:ethyl acetate, gradually increasing to 80:20 hexanes:ethyl acetate) to render a white solid consisting of two inseparable isomers (0.3963 g, 1.289 mmol, 79%). IR (4000-625ν cm^{-1}, NaCl): 3341 (N-H), 2978, 2932, 2871, 1728 (C=O), 1700 (C=O), 1499, 1455, 1393, 1369, 1278, 1250, 1165, 1137, 1103, 1080, 1052, 989, 747, 699. ^{1}H NMR

(Major isomer, 600 MHz, $CDCl_3$): δ1.48 (9H, s, H-10/11/12), 4.14 (2H, d, *J* = 11.4 Hz, H-4/6), 4.28 (2H, d, *J* = 11.4 Hz, H-4/6), 5.5 (1H, s, H-2), 5.7 (1H, s, NH-7), 7.34-7.42 (3H, m, H-16/17/18), 7.50 (2H, dd, *J* = 1.5, 7.9 Hz, H-15/19), 9.59 (1H, s, H-13). ^{1}H NMR (Minor isomer, 600 MHz, $CDCl_3$): δ1.44 (9H, s, H-10/11/12), 4.34 (2H, d, *J* = 9.4 Hz, H-4/6), 4.52 (2H, d, *J* = 9.4 Hz, H-4/6), 5.25 (1H, s, NH-7), 5.70 (1H, s, H-2), 7.34-7.42 (3H, m, H-16/17/18), 7.48 (2H, dd, *J* = 1.5, 7.2 Hz, H-15/19), 10.20 (0.5H, s, H-13). ^{13}C NMR (Major isomer, 150 MHz, $CDCl_3$): δ28.4 (C-10/11/12), 60.6 (C-4), 69.7 (C-3/5), 81.4 (C-9), 101.8 (C-1), 126.3 (C-15/19), 128.5 (C-16/18), 129.5 (C-17), 137.2 (C-14), 155.9 (C-8), 198.5 (C-13). ^{13}C NMR (Minor isomer, 150 MHz, $CDCl_3$): δ28.4 (C-10/11/12), 56.3 (C-4), 69.0 (C-3/5), 80.9 (C-9), 101.7 (C-1), 126.0 (C-15/19), 128.6 (C-16/18), 129.5 (C-17), 137.0 (C-14), 154.4 (C-8), 200.5 (C-13). MS (ESI^+): Calc'd. for [$C_{16}H_{21}NO_5Li^+$] 314.1580; found 314.1579.

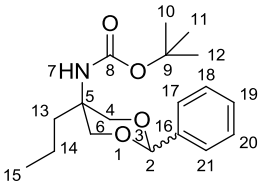
Synthesis of propene-derivative **37**:



In a flame-dried tapered microwave vial triphenylethylphosphonium bromide (0.1086 g, 0.2925 mmol, 3.8 eq) was dissolved in THF (0.4 mL) and the mixture was cooled to -78 ºC. NaHMDS (1M in THF, 0.30 mL, 0.30 mmol, 0.055 g, 3.9 eq) was added slowly, followed by warming of the mixture 0 ºC. The solution was stirred for 30 mins, followed by cooling to -78 °C. Aldehyde **36** (0.0237 g, 0.0771 mmol, 1.0 eq), which was dissolved

in THF (0.35 mL), was added slowly to the ylide and the whole mixture was allowed to slowly warm to rt. After stirring for 21 h the solvent was removed under reduced pressure and purification was obtained through silica column chromatography (100 hexanes, gradually increasing to 90:10 hexanes:ethyl acetate) to give a clear, colourless oil (0.0193 g, 0.0604 mmol, 78%). IR (4000-625ν cm^{-1}, NaCl): 3341 (N-H), 2976, 2930, 2857, 1717 (C=O), 1701 (C=O), 1497, 1455, 1391, 1366, 1244, 1165, 1111, 1018, 975, 747, 759, 698. ^{1}H NMR (*Z* isomers, 50:50 ratio of each, reference to combined H-17/21 = 2H, 600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 1.82 (3H, dt, *J* = 1.6, 7.2 Hz, H-15), 4.13 (2H, d, *J* = 10.7 Hz, H-4/6), 4.22 (2H, m, H-4/6), 4.49 (0.5H, s, NH-7), 4.63 (0.5H, s, NH-7), 5.16 (0.5H, d, *J* =11.8 Hz, H-13), 5.58 (0.5H, s, H-2), 5.59 (0.5H, s, H-2), 5.68 (0.5H, dddd, *J* = 7.2, 11.8 Hz, H-14), 5.73 (0.5H, dddd, *J* = 7.2, 11.7 Hz, H-14), 5.85 (0.5H, d, *J* = 11.7 Hz, H-13), 7.37 (3H, m, H-18/19/20), 7.48 (2H, dd, *J* = 1.4, 7.1 Hz, H-17/21). ^{1}H NMR (*E* isomer, reference H-17/21 = 2H, 600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 1.78 (3H, d, *J* = 5.9 Hz, H-15), 3.81 (2H, d, *J* = 11.4 Hz, H-4/6), 4.38 (2H, d, *J* = 11.4 Hz, H-4/6), 5.38 (1H, s, NH-7), 5.50 (1H, s, H-2), 5.86 (1H, dddd, *J* = 5.9, 16.1 Hz, H-14), 5.92 (1H, d, *J* = 16.1 Hz, H-13), 7.36 (3H, m, H-18/19/20), 7.50 (2H, dd, *J* =1.4, 7.1 Hz, H-17/21). ^{13}C NMR (mixture of isomers, 150 MHz, $CDCl_3$): δ14.4 (C-15), 18.3 (C-15), 28.48 (C-10/11/12), 28.53 (C-10/11/12), 51.0 (C-5), 51.4 (C-5), 52.5 (C-5), 72.5 (C-4/6), 101.1 (C-2), 101.3 (C-2), 101.8 (C-2), 125.9 (C-16), 126.2 (C-18/20), 126.4 (C-18/20), 128.5 (C-17/21), 129.1 (C-17/21), 129.3 (C-19), 131.2 (C-14), 131.8 (C-14), 137.7 (C-13), 137.8 (C-13), 137.9 (C-13). MS (ESI+): Calc'd for [$C_{18}H_{26}NO_4^+$]: 320.1856; found 320.2.

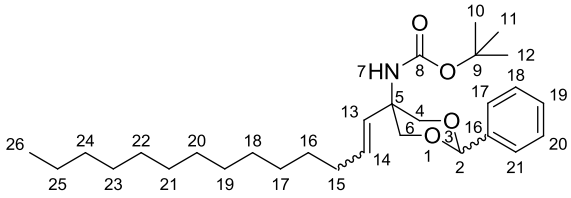
Hydrogenation of **37** to afford **38**:



Alkene **37** (0.0098 g, 0.030 mmol, 1.0 eq) was dissolved in THF (0.7 mL) and added to a flame-dried 10 mL rbf. Pd (10% *w*/*w* loading on C, 0.0034 g, 0.0032 mmol, 0.11 eq) and additional THF (0.5 mL) was then added and the flask was flushed with H_2 gas, topping up the THF to replace evaporated solvent. The reaction was stirred for 3 h at rt, followed by filtration through celite. The solvent was removed under reduced pressure and silica column chromatography was performed for purification (100 hexanes, gradually increasing to 60:30 hexanes:ethyl acetate) to give a clear, colourless oil (0.0062 g, 0.019 mmol, 64% based on full conversion). IR (4000-625ν cm^{-1}, NaCl): 3344 (N-H), 2964, 2931, 2871, 1717 (C=O), 1701 (C=O), 1498, 1391, 1366, 1245, 1166, 1110, 1076, 1029, 977, 946, 698. ^{1}H NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 2.01 (3H, dd, *J* = 1.1, 6.1 Hz, H-15), 4.16 (2H, beneath peaks, H-4/6), 4.25 (2H, beneath peaks, H-4/6), 4.48 (1H, s, NH-7), 5.58 (1H, s, H-2), 5.86 (1H, ddd, *J* = 6.2, 15.8 Hz, H-14), 5.92 (1H, dd, *J* = 1.1, 15.8 Hz, H-13), 7.32-7.41 (3H, m, H-18/19/20), 7.49 (2H, dd, *J* = 1.7, 8.2 Hz, H-17/21). ^{1}H NMR (Reduced major isomer, 600 MHz, $CDCl_3$): δ0.99 (3H, t, *J* = 7.4 Hz, H-15), 1.3 (2H, m, H-14), 1.45 (9H, s, H-10/11/12), 2.00 (2H, m, H-13), 4.09 (2H, d, *J* = 10.3 Hz, H-4/6), 4.13 (2H, d, *J* = 10.3 Hz, H-4/6), 5.52 (1H, s, H-2), 7.32-7.41 (3H, m, H-18/19/20), 7.48 (2H, d, *J* = 8.0 Hz, H-17/21). ^{1}H NMR (Reduced minor isomer, 600 MHz, $CDCl_3$): δ0.94 (3H, t, *J* = 7.3 Hz, H-15), 1.39 (2H, m, H-14),

1.45 (9H, s, H-10/11/12), 1.63 (2H, m, H-13), 3.66 (2H, d, *J* = 11.3 Hz, H-4/6), 4.29 (2H, d, *J* = 11.3 Hz, H-4/6), 5.07 (1H, s, NH-7), 5.44 (1H, s, H-2), 7.32-7.41 (3H, m, H-18/19/20), 7.48 (2H, d, *J* = 8.0 Hz, H-17/21). ^{13}C NMR (Mixture of isomers, α denotes alkene **37**, 150 MHz, $CDCl_3$): δ14.5 (C-15), 14.7 (C-15), 15.9 (C-14), 16.3 (C-14), 18.3 (C-13), 28.4 (C-10/11/12), 28.5 (C-10/11/12 $_\alpha$), 28.6 (C-10/11/12), 35.1 (C-15$_\alpha$), 50.9 (C-5), 51.4 (C-5), 51.9 (C-5), 69.8 (C-4/6), 71.9 (C-4/6), 73.5 (C-4/6), 80.0 (C-8), 101.3 (C-2), 101.7 (C-2), 102.0 (C-2), 126.1 (C-13$_\alpha$), 126.3 (C-14$_\alpha$), 126.3 (C-18/20), 126.4 (C-18/20), 126.5 (C-18/20), 128.5 (C-17/21), 128.5 (C-17/21), 128.6 (C-17/21), 129.1 (C-19), 129.2 (C-19), 131.8 (C-19), 137.8 (C-16), 138.0 (C-16), 138.0 (C-16). MS (ESI+): Calc'd for [$C_{18}H_{28}NO_4^+$]: 322.2013; found 322.2.

Synthesis of **39**:

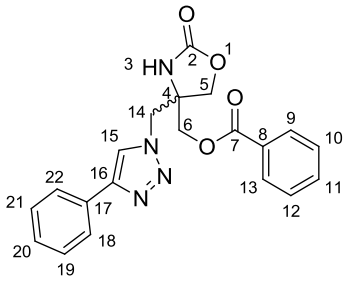


In a flame-dried tapered microwave vial triphenyltridecylphosphonium bromide (0.1070 g, 0.2036 mmol, 2.5 eq) and THF (0.5 mL) were added, followed by cooling of the mixture to 0 ºC. NaHMDS (1M in THF, 0.35 mL, 0.35 mmol, 0.064 g, 4.3 eq) was added slowly and the mixture was stirred for 30 mins. In a separate vial aldehyde **36** (0.0250 g, 0.0813 mmol, 1.0 eq) was dissolved in THF (0.4 mL). The phosphonium ylide was cooled to -78 ºC and the aldehyde solution was added to the mixture dropwise. The vial containing aldehyde **36** was then rinsed with THF (0.1 mL) and this portion of THF was

also added to the ylide solution. The solution was allowed to warm to rt slowly, followed by stirring for 20 h. The solvent was removed under reduced pressure and the crude product was purified through silica column chromatography (100 hexanes, gradually increasing to 90:10 hexanes:ethyl acetate) to give a clear, colourless oil (0.5187 g, 1.09 mmol, 134%). IR (4000-625ν cm^{-1}, NaCl): 3432 (N-H), 3340 (N-H), 2925, 2854, 1722 (C=O), 1701 (C=O), 1490, 1455, 1390, 1366, 1242, 1165, 1132, 1113, 1075, 1029, 979, 745, 721, 697. ^{1}H NMR (Major *Z* Alkene, 600 MHz, $CDCl_3$): δ0.88 (3H, t, *J* = 7.1 Hz, H-26), 1.26 (18H, m, H-16/17/18/19/20/21/22/23/24), 1.38 (2H, m, H-25), 1.45 (9H, s, H-10/11/12), 2.25 (2H, ddd, *J* = 1.4, 7.2 Hz, H-15), 3.79 (2H, d, *J* = 11.4 Hz, H-4/6), 4.38 (2H, d, *J* = 11.4 Hz, H-4/6), 5.14 (1H, d, *J* = 11.7 Hz, H-13), 5.34 (1H, s, NH-7), 5.49 (1H, s, H-2), 5.53-5.63 (1H, m, H-14), 7.32-7.42 (3H, m, H-18/19/20), 7.50 (2H, dd, *J* = 1.7, 8.2 Hz, H-17/21). ^{1}H NMR (Minor *Z* Alkene, 600 MHz, $CDCl_3$): δ0.88 (3H, t, *J* = 7.1 Hz, H-26), 1.26 (18H, m, H-16/17/18/19/20/21/22/23/24), 1.38 (2H, m, H-25), 1.44 (9H, s, H-10/11/12), 2.20 (2H, ddd, *J* = 1.5, 7.5 Hz, H-15), 4.16-4.26 (4H, m, H-4/6), 4.66 (1H, s, NH-7), 5.53-5.63 (2H, m, H-2/14), 5.80 (1H, d, *J* = 11.7 Hz, H-13), 7.32-7.42 (3H, m, H-18/19/20), 7.50 (2H, dd, *J* = 1.7, 8.5 Hz, H-17/21). ^{1}H NMR (Trace *E* Alkene, 600 MHz, $CDCl_3$, referenced to *Z*-10/11/12): δ2.10 (2H, dd, *J* = 7.1 Hz), 4.47 (0.15H, s), 5.8 (0.16H, ddd, *J* = 6.0, 16.1 Hz), 5.88 (0.16H, d, *J* = 16.1 Hz). ^{13}C NMR (α denotes major & β denotes minor, 150 MHz, $CDCl_3$): δ14.3 (C-26), 22.8 (C-25), 28.5 (C-10/11/12_α), 28.6 (C-10/11/12_β), 28.8 (C-24_α), 28.9 (C-24_β), 29.3 (C-16-23), 29.4 (C-16-23), 29.5 (C-16-23), 29.6 (C-16-23), 29.6 (C-16-23), 29.7 (C-16-23), 29.8 (C-16-23), 29.8 (C-16-23), 29.8 (C-16-23), 29.9 (C-16-23), 30.0 (C-16-23), 30.5 (C-16-23), 32.1 (C-15_α), 32.8 (C-15

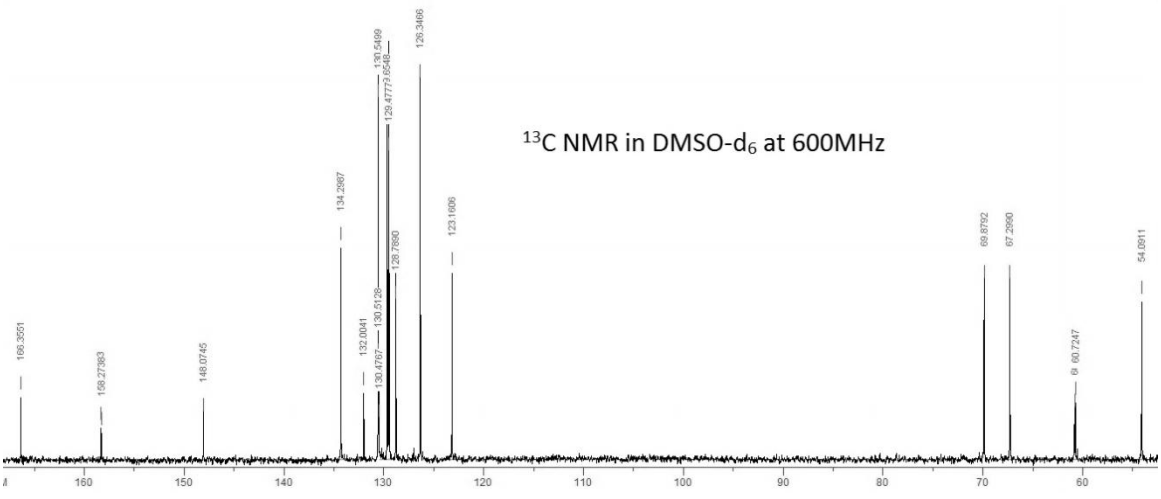
β), 51.1 (C-5 β), 52.3 (C-5 α), 72.3 (C-4/6 β), 72.7 (C-4/6 α), 79.5 (C-9), 101.0 (C-2 β), 101.7 (C-2 α), 126.2 (C-18/20 α), 126.5 (C-18/20 β), 128.5 (C-17/21 α), 128.5 (C-17/21 β), 129.1 (C-19 β), 129.1 (C-19 α), 129.3 (C-19 β), 130.0 (C-14 α), 130.5 (C-14 β), 134.1 (C-13 β), 135.4(C-13 α), 137.7 (C-16 β), 137.9 (C-16 α), 154.7 (C-8). MS (ESI+): Cal'd for $[C_{29}H_{48}NO_4^+]$: 474.3578; found 474.5.

Synthesis of triazole (**40**):[155]

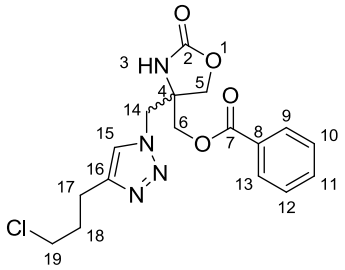


In a flame-dried tapered microwave vial under N_2 azide **14** (0.0091 g, 0.33 mmol, 1.0 eq), copper (I) iodide (0.0008 g, 0.0042 mmol, 0.13 eq), and copper acetate monohydrate (0.006 g, 0.003 mmol, 0.09 eq) were dissolved in dry THF (0.07 mL). Phenylacetylene (0.004 mL, 0.0037 g, 0.036 mmol, 1.1 eq) was added and heated at 50 °C for 5.5 h, then temperature was lowered and the mixture was heated at 40 °C for a further 18 h, with THF added throughout the reaction to counteract the evaporation of solvent. The mixture was cooled to room temperature and diluted with water (0.2 mL), then washed with DCM (0.2 mL, 3 times) and the organic layers combined and rinsed with saturated $NaHCO_3$. The organic layer solvent was removed under reduced pressure and purification was obtained through silica column chromatography (80:20 hexanes:ethyl acetate, slowly increasing the ratio to 50:50 hexanes:ethyl acetate) to give a white solid (0.0089g, 0.024 mmol, 71.3%). IR (4000-625ν

cm^{-1}, NaCl): 2920 (C-H), 2850, 2110, 1760 (C=O), 1722 (C=O), 1700 (C=O), 1700, 1406, 1318, 1266 (C-O), 1181, 1111, 1073, 1042, 1028, 909, 765, 713. ^{1}H NMR ($CDCl_3$, 600 MHz): δ4.32 (1H, d, *J* = 11.7 Hz, H-14), 4.44 (1H, d, *J* = 9.3 Hz, H-6), 4.45 (1H, d, *J* = 11.7 Hz, H-14), 4.53 (1H, d, *J* = 9.3 Hz, H-6), 4.65 (1H, d, *J* = 14.4 Hz, H-5), 4.69 (1H, d, *J* = 14.4 Hz, H-5), 6.5 (1H, s – broad, NH-3), 7.34 (1H, tt, *J* = 1.1, 7.5 Hz, H-20), 7.41 (2H, t, *J*= 7.5 Hz, H-19/21), 7.45 (2H, t, *J* = 7.5, 8.1 Hz, H-10/12), 7.60 (1H, tt, *J* = 1.2, 8.1 Hz, H-11), 7.78 (2H, dd, *J* = 1.1, 7.3 Hz, H-18/22), 7.87 (1H, s, H-15), 8.00 (2H, dd, *J* = 1.2, 8.1 Hz, H-9/13). ^{13}C NMR (150 MHz, DMSO-d_6): δ54.1 (C-4), 60.7 (C-14), 60.8 (C-14), 67.3 (C-5), 69.9 (C-6), 123.2 (C-20), 126.3 (C-18/22), 128.8 (C-11), 129.5 (C-19/21), 129.7 (C-10/12), 130.5 (C-17), 130.5 (C-9/13), 132.0 (C-8), 134.4 (C-15), 148.1 (C-16), 158.3 (C-2), 158.3 (C-2), 166.4 (C-7). MS (CI): Calculated: 379.1406 [M + H^+]. Actual: 379.1405.



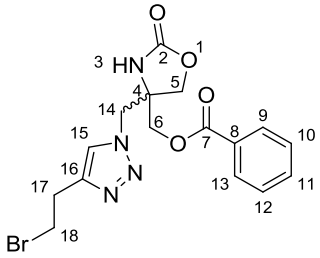
Synthesis of chloro-triazole **41**:[10]



In a flame-dried tapered microwave vial under N_2 azide **14** (0.030 g, 0.11 mmol, 1.0 eq), copper (I) iodide (0.0013 g, 0.0068 mmol, 0.06 eq), and copper (II) acetate monohydrate (0.0015 g, 0.0075 mmol, 0.079 eq) were dissolved in dry THF (0.2 mL). 5-chloro-1-pentyne (0.018 mL, 0.017 g, 0.17 mmol, 1.5 eq) was added and the mixture was heated at 50 °C for 18 h, with THF added throughout the reaction to counter the evaporation of solvent. The mixture was cooled to room temperature and the solvent removed under pressure and purification was obtained through column chromatography (silica gel, 80:20 hexanes:ethyl acetate, slowly increasing the ratio to 50:50 hexanes:ethyl acetate) to give a white solid (0.0292 g, 0.0771 mmol, 70%). %. IR (4000-625ν cm^{-1}, NaCl): 3341 (N-H), 3126, 3013, 2920, 1765, 1747 (C=O), 1715 (C=O), 1471, 1451, 1439, 1426, 1395, 1350, 1335, 1313, 1299, 1277 (C-O), 1264 (C-O), 1225, 1177, 1163, 1123, 1110, 1097, 1071, 1041 (C-N), 999, 951, 929, 910, 863, 826, 82, 766, 706 (C-Cl), 696, 651. ^{1}H NMR ($CDCl_3$, 600 MHz): δ2.13 (2H, tt, *J* = 6.3, 7.3 Hz, H-18), 2.87 (2H, t, *J* = 7.3 Hz, H-17), 3.54 (2H, t, *J* = 6.3 Hz, H-19), 4.29 (1H, d, *J* = 11.7 Hz, H-4), 4.41 (1H, d, *J* = 9.2 Hz, H-6), 4.41 (1H, d, *J* = 11.7 Hz, H-14), 4.51 (1H, d, 9.2 Hz, H-6), 4.60 (1H, d, *J* = 14.4 Hz, H-5), 4.66 (1H, d, *J* = 14.4 Hz, H-5), 6.76 (1H, s, NH-3), 7.46 (2H, dd, 7.2, 8.1 Hz, H-10/12), 7.47 (1H, s, H-15), 7.61 (1H, t, *J* = 7.2 Hz, H-11), 8.01 (2H, d, *J* = 8.1 Hz, H-

9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): 22.6 (C-18), 31.7 (C-17), 44.2 (C-19), 53.4 (C-14), 60.3 (C-4), 65.9 (C-5), 69.8 (C-6), 123.2 (C-15), 128.7 (C-8), 128.9 (C-10/12), 129.9 (C-9/13), 134.1 (C-11), 147.1 (C-16), 158.2 (C-2), 165.9 (C-7). MS (CI): Cal'd for [$C_{17}H_{20}ClN_4O_4^+$]: 379.11; found 379.1.

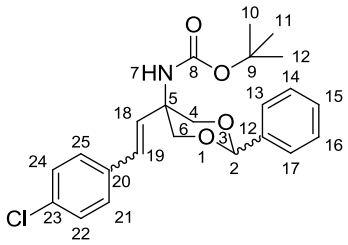
Synthesis of bromo-triazole **42**:[155]



In a flame-dried tapered microwave vial under N_2 azide **14** (0.0308 g, 0.115 mmol, 1.0 eq), copper (I) iodide (0.0018 g, 0.0094 mmol, 0.085 eq), and copper acetate monohydrate (0.0016 g, 0.0080 mmol, 0.07 eq) were dissolved in dry THF (0.2 mL). 4-bromo-1-butyne (0.010 mL, 0.014g, 0.11mmol, 1.0eq) was added and heated at 50 °C for 18 h, with THF added throughout the reaction due to the evaporation of solvent. The mixture was cooled to rt and the solvent removed under pressure and purification was obtained through column chromatography (silica gel, 80:20 hexanes:ethyl acetate, slowly increasing the ratio to 30:70 hexanes:ethyl acetate) to afford a white solid (0.0252 g, 0.616 mmol, 56%). IR (4000-625ν cm^{-1}, NaCl): 3263 (N-H), 3143, 3074, 3012, 2961 (N=N), 2919 (N=N), 1762 (C=O), 1725 (C=O), 1602 (C-N), 1584, 1554, 1451, 1403, 1316, 1270 (C-O), 1178, 1110, 1071, 1047, 1028, 1000, 929, 879, 805, 762, 712, 686, 667. ^{1}H NMR ($CDCl_3$, 600 MHz): δ3.26 (2H, t, *J* = 6.7 Hz, H-17), 3.61 (2H, t, *J* = 6.7

Hz, H-18), 4.29 (1H, d, *J* = 11.6 Hz, H-14), 4.40 (1H, d, *J* = 9.5 Hz, H-6), 4.41 (1H, d, *J* = 11.6 Hz, H-14), 4.54 (1H, d, *J* = 9.5 Hz, H-6), 4.63 (1H, d, *J* = 14.4 Hz, H-5), 4.68 (1H, d, *J* = 14.4 Hz, H-5), 6.89 (1H, s, NH-3), 7.46 (2H, dd, *J* = 7.5, 7.7 Hz, H-10/12), 7.61 (1H, t, *J* = 7.5 Hz, H-11), 7.61 (1H, s, H-15), 8.01 (2H, d, *J* = 7.7 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): 29.3 (C-18), 31.5 (C-17), 53.3 (C-14), 60.3 (C-4), 65.9 (C-5), 69.8 (C-6), 123.9 (C-15), 128.7 (C-8), 128.9 (C-10/12), 129.9 (C-9/13), 134.1 (C-11), 145.6 (C-16), 158.3 (C-2), 165.9 (C-7). MS (ESI+): Calc'd for [$C_{16}H_{18}BrN_4O_4^+$]: 409.0511; found 409.0528.

Synthesis of **43**:

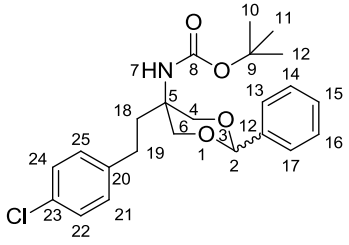


In a flame-dried 2-neck 10 mL rbf triethyl(4-chlorobenzyl)phosphonium bromide (0.1300 g, 0.4017 mmol, 4.5 eq) and THF (1 mL) were added and cooled to 0 ºC. NaHMDS (1M in THF, 0.45 mL, 0.45 mmol, 2.8 eq) was then added dropwise to the solution and the mixture was stirred for 30 mins, then cooled to -78 ºC. Aldehyde **36** (0.0487 g, 0.158 mmol, 1.0 eq) was dissolved in a separate vial with THF (0.6 mL) and then added dropwise to the phosphonium ylide. Additional THF (0.3 mL) was used to rinse the vial with the aldehyde, which was then added to the ylide mixture. The solution is left to warm to rt, stirring for 15 h. Purification was obtained through silica column

chromatography (100 hexanes, increasing gradually to 95:5 hexanes:ethyl acetate) to give a total of 4 isomers, two of which were separable *E*/Z isomers, as a white solid (0.0626 g, 0.151 mmol, 95%). IR (*Z* isomer, 4000-625ν cm^{-1}, NaCl): 3429 (N-H), 2976, 2928, 2860, 1717 (C=O), 1490, 1454, 1366, 1293, 1167, 1128, 1074, 1015, 1029, 980, 745, 698. ^{1}H NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ1.38 (9H, s, H-10/11/12), 3.74 (2H, d, *J* = 11.3 Hz, H-4/6), 4.31 (2H, d, *J* = 11.3 Hz, H-4/6), 5.27 (1H, broad s, NH-7), 5.39 (1H, s, H-2), 5.52 (1H, d, *J* = 12.6 Hz, H-18), 6.66 (1H, d, *J* = 12.6 Hz, H-19), 7.23 (2H, d, *J* = 8.4 Hz, H-21/25), 7.29 (2H, d, *J* = 8.4 Hz, H-22/24), 7.48-7.38 (2H, m, H-14/15/16), 7.44 (2H, dd, *J* = 1.7, 7.6 Hz, H-13/17). ^{13}C NMR (*Z* isomer, 150 MHz, $CDCl_3$): δ28.5 (C-10/11/12), 52.6 (C-5), 72.7 (C-4/6), 79.7 (C-9), 101.7 (C-2), 126.1 (C-14/16), 128.2 (C-13/17), 128.5 (C-22/24), 128.8 (C-15), 129.3 (C-19), 130.2 (C-21/25), 132.0 (C-18), 133.3 (C-23), 135.9 (C-20), 137.7 (C-12), 154.6 (C-8). MS (*Z* isomer, ESI+): Calc'd for [$C_{23}H_{27}ClNO_4^+$]: 416.1623, 418.1599, 417.1662; found 416.2, 418.2, 417.2. IR (*E* isomer, 4000-625ν cm^{-1}, NaCl): 3349 (N-H), 2976, 2928, 2860, 1705 (C=O), 1491, 1392, 1367, 1392, 1367, 1167, 979, 698. ^{1}H NMR (*E* isomer, 600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 3.92 (2H, d, *J* = 11.3 Hz, H-4/6), 4.37 (2H, d, *J* = 11.3 Hz, H-4/6), 5.37 (1H, broad s, NH-7), 5.53 (1H, s, H-2), 6.17 (1H, d, *J* = 16.5 Hz, H-18), 6.52 (1H, s, *J* = 16.5 Hz, H-19), 7.27-7.31 (4H, dd, *J* = 8.3 Hz, H-21/22/24/25), 7.37-7.43 (3H, m, H-19/20/21), 7.53 (2H, dd, *J* = 1.2, 8.3 Hz, H-13/17). ^{13}C NMR (*E* isomer, 150 MHz, $CDCl_3$): δ28.6 (C-10/11/12), 53.2 (C-5), 72.9 (C-4/6), 80.0 (C-9), 102.0 (C-2), 126.2 (C-14/16), 127.8 (C-13/17), 128.4 (C-12), 128.5 (C-22/24), 128.9 (C-21/25), 129.4 (C-19), 129.9 (C-18), 130.1 (C-15), 133.7 (C-23), 135.1 (C-20), 137.7 (C-12), 155.0 (C-8). MS

(*E* isomer, ESI+): Calc'd for [$C_{23}H_{27}ClNO_4^+$]: 416.1623, 418.1599, 417.1662; found 416.2, 418.2, 417.2.

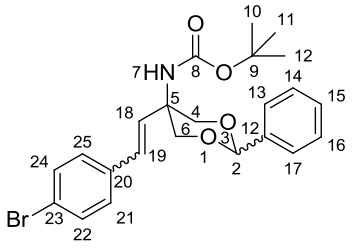
Hydrogenation of **43** to alkane **44**:



To a small sample vial was added alkene **43** (0.0172 g, 0.0141 mmol, 1.0 eq), and the vial was placed under reduced pressure and the atmosphere within replaced with N_2. THF (0.80 mL) was added, followed by Pd (10% *w*/*w* loading on C, 0.0043 g, 0.0040 mmol, 0.098 eq). The flask flushed with $H_{2(g)}$ and left to stir under a hydrogen atmosphere for 17.5 h The solution was filtered through a pipette packed with cotton, Na_2SO_4, and celite, with subsequent washings with copious amounts of THF. The solvent was removed under reduced pressure to afford a white solid as a mixture of 2 *cis*/*trans* isomers (0.0173 g, 0.0412 mmol, 99.6%). IR (4000-625ν cm^{-1}, NaCl): 3425 (N-H), 3359 (N-H), 2967, 2917, 2850, 1713 (C=O), 1493, 1455, 392, 1366, 1261, 1134, 1092, 1077, 1015, 807, 743, 699. ^{1}H NMR (Major isomer, 600 MHz, $CDCl_3$): δ1.48 (9H, s, H-10/11/12), 1.96 (2H, dt, *J* = 5.7, 8.4 Hz, H-19), 2.57 (2H, dt, *J* = 5.7, 8.4 Hz, H-18), 3.68 (2H, d, *J* = 11.1 Hz, H-4/6), 4.30 (2H, d, *J* = 11.1 Hz, H-4/6), 5.19 (1H, broad s, NH-7), 5.45 (1H, s, H-2), 7.13 (2H, d, *J* = 8.1 Hz, H-21/25), 7.28 (2H, d, *J* = 8.1 Hz, H-22/24), 7.34-7.43 (3H, m, H-14/15/16), 7.5 (2H, dd, *J* = 1.3, 7.6 Hz, H-13/17). ^{1}H NMR (Minor isomer, 600 MHz, $CDCl_3$): δ1.46

(9H, s, H-10/11/12), 2.38 (2H, dt, *J* = 5.3, 8.3 Hz, H-19), 2.66 (2H, dt, *J* = 5.3, 8.3 Hz, H-18), 4.02 (1H, broad s, NH-7), 4.22 (2H, d, *J* = 10.9 Hz, H-4/6), 4.43 (2H, within major H-4/6 peak, H-4/6), 5.53 (1H, s, H-2), 7.16 (2H, d, *J* = 8.4 Hz, H-21/25), 7.25 (2H, within major H-22/24 & solvent, H-22/24), 7.34-7.43 (3H, m, H-14/15/16), 7.47 (2H, d, *J* = 6.7 Hz, H-13/17). ^{13}C NMR (Major isomer, 150 MHz, $CDCl_3$): δ28.6 (C-10/11/12), 29.8 (C-19), 33.2 (C-18), 51.9 (C-5), 73.4 (C-4/6), 79.6 (C-9), 102.0 (C-2), 126.2 (C-14/16), 128.5 (C-13/17), 128.7 (C-21/25), 139.3 (C-15), 129.9 (C-22/24), 131.9 (C-23), 137.8 (C-12), 140.5 (C-20), 155.1 (C-8). ^{13}C NMR (Major isomer, 150 MHz, $CDCl_3$): δ28.5 (C-10/11/12), 29.1 (C-19), 34.0 (C-18), 50.8 (C-5), 72.2 (C-4/6), 79.6 (C-9), 101.9 (C-2), 126.3 (C-14/16), 128.5 (C-13/17), 126.7 (C-21/25), 129.2 (C-15), 129.8 (C-22/24), 131.8 (C-23), 137.7 (C-12), 140.5 (C-20), 155.1 (C-8). MS (ESI+): Cal'd for [$C_{23}H_{29}ClNO_4^+$]: 418.1785, 420.1756; found 418.3, 420.2.

Synthesis of **46**:

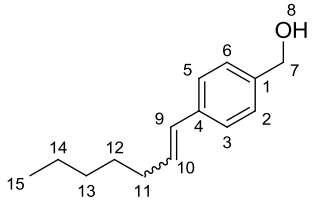


In a flame-dried tapered microwave vial triethyl-4-bromobenzylphosphomium bromide (0.2473 g, 0.6718 mmol, 2.3 eq) was dissolved in THF (1.6 mL) and cooled to 0 ºC. NaHMDS (1M in THF, 0.75 mL, 0.75 mmol, 0.14 g, 2.5 eq) is added slowly and stirred for 2 h. In a separate vial aldehyde **36** (0.0910 g, 0.293 mmol, 1.0 eq) was dissolved in

THF (0.8 mL). The phosphorus ylide was cooled to -78 ºC and the aldehyde solution was added dropwise. The vial previously containing aldehyde **36** was rinsed with THF (0.4 mL) and this was also added to the phosphorus ylide. The solution was allowed to warm to rt slowly, followed by stirring for 20 h. The solvent was removed under reduced pressure and the product was purified through silica column chromatography (100 hexanes, gradually increasing to 70:30 hexanes:ethyl acetate) to give a white solid, with *Z* being the predominant isomer (0.0975 g, 0.212 mmol, 72%). IR (4000-625ν cm^{-1}, NaCl): 3399 (N-H), 2977, 2917, 2866, 1700 (C=O), 1515, 1487, 1391, 1366, 1245, 1156, 1073, 1047, 1020, 1009, 987, 968, 855, 764, 698. ^{1}H NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ1.45 (9H, s, H-10/11/12), 4.01 (2H, d, *J* = 10.6 Hz, H-4/6), 4.29 (2H, d, *J* = 10.6 Hz, H-4/6), 5.37 (1H, s, NH-7), 5.59 (1H, s, H-2), 6.24 (1H, d, *J* = 12.5 Hz, H-18), 6.67 (1H, d, *J* = 12.5 Hz, H-19), 7.10 (2H, d, *J* = 8.2 Hz, H-22/24), 7.34-7.46 (5H, m, H-14/15/16/21/25), 7.48 (2H, dd, *J* = 1.4, 8.0 Hz, H-13/17). ^{1}H NMR (*E* isomer, 600 MHz, $CDCl_3$): δ1.33 (9H, s, H-10/11/12), 3.92 (2H, d, *J* = 11.4 Hz, H-4/6), 4.37 (2H, d, *J* = 11.4 Hz, H-4/6), 4.35 (1H, s, NH-7), 5.53 (1H, s, H-2), 6.19 (1H, d, *J* = 16.5 Hz, H-18), 6.51 (1H, d, *J* = 16.5 Hz, H-19), 7.24 (2H, d, *J* = 8.5 Hz, H-22/24), 7.34-7.46 (5H, m, H-14/15/16/21/25), 7.52 (2H, dd, *J* = 1.4, 7.9 Hz, H-13/17). ^{13}C NMR (*Z* isomer, 150 MHz, $CDCl_3$): δ28.4 (C-10/11/12), 53.6 (C-5), 73.2 (C-4/6), 80.0 (C-9), 101.3 (C-2), 121.1 (C-23), 126.4 (C-14/16), 128.1 (C-19), 128.6 (C-13/17), 129.2 (C-18), 130.4 (C-21/25), 131.3 (C-22/24), 133.0 (C-15), 136.5 (C-20), 137.4 (C-12), 155.0 (C-8). ^{13}C NMR (*E* isomer, 150 MHz, $CDCl_3$): δ28.5 (C-10/11/12), 53.2 (C-5), 72.9 (C-4/6), 80.0 (C-9), 101.9 (C-2), 121.8 (C-23), 126.2 (C-14/16), 128.0 (C-19), 128.5 (C-13/17), 129.4 (C-18), 129.9 (C-21/25),

131.8 (C-22/24), 133.0 (C-15), 135.5 (C-20), 137.7 (C-12), 153.8 (C-8). MS (ESI+): Calc'd for [$C_{23}H_{27}BrNO_4^+$]: 460.1123, 462.1103; found 460.2, 462.2.

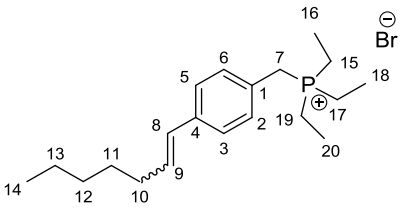
4-(hept-1-en-1-yl)benzyl alcohol (**52**):



In a 10 mL rbf under N_2, triphenylhexylphosphonium bromide (0.5949 g, 1.392 mmol, 2.06 eq) and THF (2.25 mL) were added. The mixture was cooled to 0 ºC and NaHMDS (1M in THF, 1.60 mL, 1.60 mmol, 2.4 eq) was added, stirring for 1.5 h. The phosphylide was then cooled to -78 ºC and 4-(hydroxymethyl)benzaldehyde (**51**, 0.0918 g, 0.674 mmol, 1.0 eq) in THF (0.70 mL) was added. The mixture was stirred 19.5 h, followed by addition of sat'd NH_4Cl (3 mL). The layers were separated and the aqueous layer was washed with Et_2O (3 mL × 3), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The product was obtained pure via silica column chromatography (100 hexanes, gradually increasing to 90:10 hexanes:ethyl acetae) to afford the product as a clear, faintly yellow oil as primarily (<95%) the (*Z*)-isomer (0.1174 g, 0.5751 mmol, 85%). ^{1}H NMR (600 MHz, $CDCl_3$): δ0.90 (3H, m, H-15), 1.29-1.37 (4H, m, H-12/13), 1.47 (2H, q, *J* = 7.2, 14.4 Hz, H-14), 2.33 (2H, dd, *J* = 7.3, 14.9 Hz, H-11), 4.64 (2H, s, H-7), 5.68 (1H, dt, *J* = 7.0, 11.8 Hz, H-10), 6.40 (1H, d, *J* = 12.1 Hz, H-9), 7.27 (2H, d, *J* = 8.0 Hz, H-2/6), 7.30 (2H, d, *J* = 8.0 Hz, H-3/5). ^{13}C DeptQ NMR (150 MHz, $CDCl_3$):

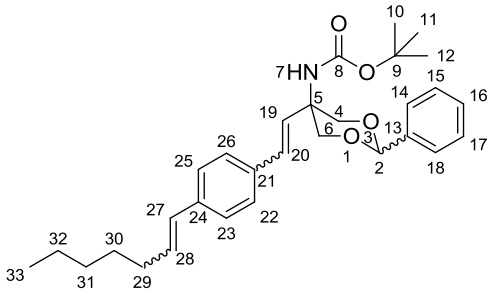
δ14.1 (C-15), 22.6 (C-14), 28.7 (C-12/13), 29.7 (C-12/13), 31.6 (C-11), 65.0 (C-7), 126.9 (C-3/5), 128.4 (C-9), 129.0 (C-2/6), 133.4 (C-10), 137.2 (C-4), 139.1 (C-1).

Formation of Phosphonium salt **53**:

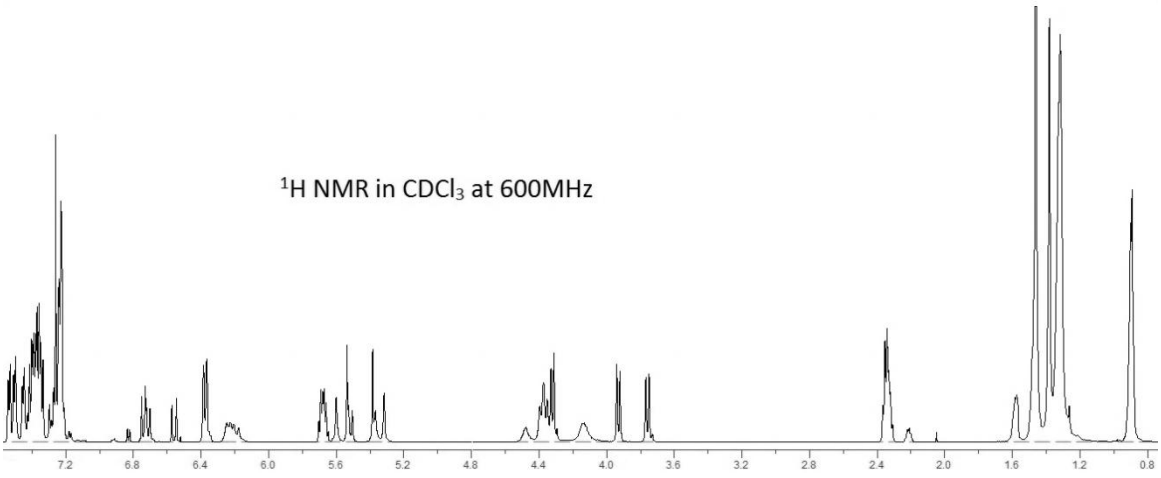


In a tapered microwave vial alcohol **52** (0.1032 g, 0.5055 mmol, 1.0 eq) was added to triethylphosphine hydrobromide (0.0.803 g, 0.519 mmol, 1.03 eq) and the mixture was heated at 110 ºC with stirring for 20 h. The mixture was dissolved with DCM, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford a white solid that was used without further purification (0.1628 g, 0.4775 mmol, 94%). ^{1}H NMR (minor contamination with starting alcohol, 600 MHz, $CDCl_3$): δ0.87 (4H, m, H-14), 1.18-1.26 (12H, m, H-16/18/20), 1.26-1.35 (6H, m, H-11/12), 1.40-1.48 (3H, m, H-13), 2.26 (2H, dd, *J* = 7.4, 14.8 Hz, H-10), 2.46-2.56 (8H, m, H-15/17/19), 4.21 (2H, d, *J* = 15.6 Hz, H-7), 5.69 (1H, dt, *J* = 7.3, 12.0, 14.1 Hz, H-9), 6.33 (1H, d, *J* = 11.7 Hz, H-8), 7.23-7.27 (2H, m, obscured by solvent peak, H-3/5), 7.41 (2H, dd, *J* = 2.1, 8.0 Hz, H-2/6). ^{13}C NMR (minor contamination with starting alcohol, 150 MHz, $CDCl_3$): δ6.1 (J_{CP} = 5.3 Hz, C-16/18/20), 12.1 (J_{CP} = 48.6 Hz, C-15/17/19), 14.1 (C-14), 22.6 (C-12), 26.0 (J_{CP} = 45.4 Hz, H-7), 28.7 (C-11), 29.6 (C-13), 31.6 (C-10), 127.0 (C-4), 127.8 (C-9), 129.9 (C-3/5), 130.1 (J_{CP} = 4.7 Hz, C-2/6), 134.6 (C-8), 138.2 (C-1). ^{31}P NMR (80 MHz, $CDCl_3$, externally referenced to 85% H_3PO_4 in D_2O): δ37.5.

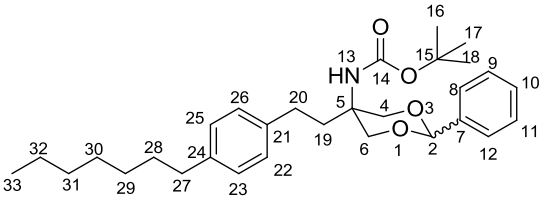
Synthesis of **54**:



To a flame-dried tapered microwave vial under N_2 was added phosphonium salt **53** (0.1351 g, 0.3806 mmol, 2.11 eq), aldehyde **36** (0.0554 g, 0.180 mmol, 1.0 eq), K_2CO_3 (0.1075 g, 0.7778 mmol, 4.3 eq), and H_2O (0.25 mL). The vial was capped and microwaved at 70 ºC for 30 mins. To the solution was added H_2O (1 mL) and the product was extracted with Et_2O (3 mL × 7), the organic layers combined, dried over Na_2SO_4, filterd, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (100 hexanes to 95:5 hexanes:ethyl acetate) to afford a white solid, however, alcohol **52** contaminated the mixture and began to elute with the product, so isolated yield not reflective (0.0315 g, 0.660 mmol, 37%). Due to complexity of the spectra, total ^{1}H NMR assignment was not attempted.

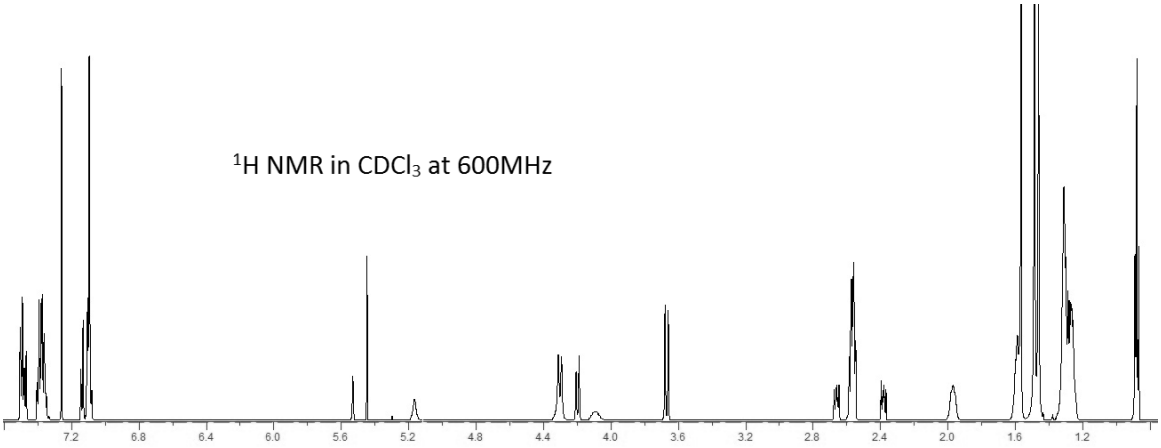


Formation of **55**:

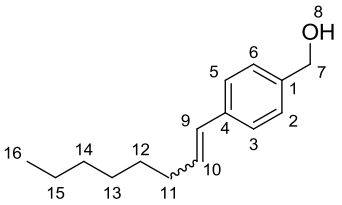


To a small sample vial **54** (0.0135 g, 0.0283 mmol, 1.0 eq) was added and the atmosphere was evacuated and replaced with N_2. THF (1.0 mL) and Pd (10% *w*/*w* loading on C, 0.0092 g, 0.086 mmol, 0.31 eq) were added and the vial was charged with $H_{2(g)}$, with addition of THF throughout to maintain a constant volume of solvent. The reaction was stirred for16 h at rt, followed by filtration through celite. The celite washed with copious amounts of Et_2O and the organic portions were combined and the solvent removed under reduced pressure. Further purification was obtained through a silica plug to afford **55** as a white solid (*cis*:*trans* 35:65, 0.0132 g, 0.0274 mmol, 97%). ^{1}H NMR not fully assigned due to complexity. ^{1}H NMR (major isomer, 600 MHz, $CDCl_3$): δ0.88 (3H, t, *J* = 6.8 Hz, H-33), 1.22-1.36 (8H, m, H-29/30/31/32), 1.49 (9H, s, H-16/17/18), 1.56-1.62 (2H, m, H-

28), 1.97 (2H, t, *J* = 7.0 Hz, H-19), 2.53-2.60 (4H, m, H-20/27), 3.67 (2H, d, *J* =10.7 Hz, H-4/6), 4.30 (2H, d, *J* = 4.29 Hz, H-4/6), 5.16 (1H, s, NH-13), 5.45 (1H, s, H-2), 7.07-7.12 (4H, m, H-22/23/25/26), 7.33-7.41 (3H, m, H-9/10/11), 7.50 (2H, d, *J* = 7.3 Hz, H-8/12). ^{1}H NMR (minor isomer, 600 MHz, $CDCl_3$): δ0.88 (3H, t, *J* = 6.8 Hz, H-33), 1.22-1.36 (8H, m, H-29/30/31/32), 1.46 (9H, s, H-16/17/18), 1.56-1.62 (2H, m, H-28), 2.36-2.31 (2H, m, H-19), 2.53-2.60 (2H, m, H-27), 2.64-2.68 (2H, m, H-20), 4.00-4.16 (2H, β, m, H-4/6), 4.20 (2H, d, *J* = 10.7 Hz, H-4/6), 5.53 (1H, s, H-2), 7.07-7.12 (2H, m, H-23/25), 7.14 (2H, d, *J* = 7.9 Hz, H-22/26), 7.33-7.41 (3H, m, H-9/10/11), 7.48 (2H, d, *J* = 7.6 Hz, H-8/12). Or: ^{1}H NMR (as a mixture of isomers with α = major and β = minor, 600 MHz, $CDCl_3$): δ0.88 (3H, αβ, t, *J* = 6.8 Hz, H-33), 1.22-1.36 (8H, αβ, m, H-29/30/31/32), 1.46 (9H, β, s, H-16/17/18), 1.49 (9H, α, s), 1.56-1.62 (2H, αβ, m, H-28), 1.97 (2H, α, t, *J* = 7.0 Hz, H-19), 2.36-2.31 (2H, β, m), H-19, 2.53-2.60 (α×4H & β×2H, m, H-20/27$_{αβ}$ & 27$_{β}$), 2.64 (2H, β, m, H-20), 3.67 (2H, α, d, *J* =10.7 Hz, H-4/6), 4.00-4.16 (2H, β, m, H-4/6), 4.20 (2H, β, d, *J* = 10.7 Hz, H-4/6), 4.30 (2H, α, d, *J* = 4.29 Hz, H-4/6), 5.16 (1H, α, s, NH-13), 5.45 (1H, α, s, H-2), 5.53 (1H, β, s, H-2), 7.07-7.12 (α×4H & β×2H, m, H-22/23/25/26$_{α}$ & H-22/26$_{β}$), 7.14 (2H, β, d, *J* = 7.9 Hz, H-23/25), 7.33-7.41 (3H, αβ, m, H – 9/10/11), 7.48 (2H, β, d, *J* = 7.6 Hz, H-8/12), 7.50 (2H, α, d, *J* = 7.3 Hz, H-8/12). ^{13}C NMR (mixture of isomers, 150MHz, $CDCl_3$): δ14.6 (C-33), 22.7, 28.4, 28.5, 28.6, 29.2, 29.3, 29.4, 31.7, 31.9, 34.3, 35.6, 51.9 (C-5), 72.0 (C-15), 73.4 (C-4/6), 101.8 (C-2), 101.9 (C-2), 126.1, 126.3, 128.2, 128.3, 128.4, 128.6, 129.2, 129.2, 137.8, 138.9, 139.1, 140.7, 156.3 (C-14).



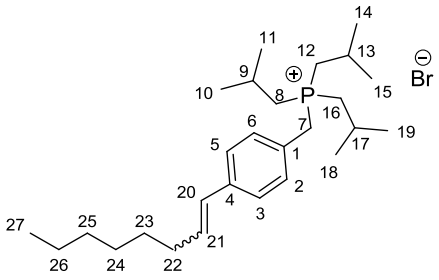
4-(oct-1-en-1-yl)benzyl alcohol (**56**):



Triphenylheptylphosphonium bromide (4.0588 g, 9.1957 mmol, 3.03 eq) was suspended in dry THF (9.0 mL) and the mixture was cooled to 0 °C while stirring. LiHMDS (1M in THF, 9.4 mL, 9.4 mmol, 3.1 eq) was added dropwise to the mixture and stirred for 1 h, followed by cooling to -78 °C. A solution of 4-(methoxymethyl)benzaldhyde[164] (**51**, 0.4133 g, 3.036mmol , 1.0 eq) in dry THF (5 mL) was then added dropwise to the phosphoranylide. The solution was allowed to warm to rt slowly and stirred for 20 h. The mixture was quenched with NH_4Cl (20 mL), separated, and the aqueous layer was washed with Et_2O (20 mL × 2, 10 mL × 1) and EtOAc (10 mL). The organic layers were combined and the solvent removed. Purification was obtained through column chromatography (100 hexanes to 90:10 hexanes:ethyl acetate) to give a faint yellow oil as

a mixture of (*Z*):(*E*) isomers in a 9:1 ratio (0.5780 g, 2.647 mmol, 87%). IR (4000-625ν cm^{-1}, NaCl): 3335 (broad OH), 3007, 2955, 2925, 2855, 1457, 1437, 1174, 1014, 722, 695. ^{1}H NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ0.88 (3H, t, *J* = 7.0 Hz, H-16), 1.23-1.39 (6H, m, H-12/13/14), 1.41-1.50 (2H, m, H-15), 2.33 (2H, dq, *J* = 1.8, 7.4 Hz, H-11), 4.67 (2H, s, H-7), 5.68 (1H, dt, *J* = 7.4, 11.7 Hz, H-10), 6.40 (1H, d, *J* = 11.7 Hz, H-9), 7.25 (2H, d, *J* = 8.1 Hz, H-2/6), 7.33 (2H, d, *J* = 8.1 Hz, H-3/5). ^{1}H NMR (*E* isomer, 600 MHz, $CDCl_3$): δ0.90 (3H, t, *J* = 6.9 Hz, H-16), 1.23-1.39 (6H, m, H-12/13/14), 1.41-1.50 (2H, m, H-15), 2.21 (2H, dq, *J* = 1.3, 7.2 Hz, H-11), 4.64 (2H, s, H-7), 6.23 (1H, dt, *J* = 6.9, 15.9 Hz, H-10), 6.37 (1H, d, *J* = 15.9 Hz, H-9), 7.26 (2H, d, *J* = 8.1 Hz, H-2/6), 7.32 (2H, d, *J* = 8.1 Hz, H-3/5). ^{13}C NMR (*Z* & *E* isomers, 150 MHz, $CDCl_3$): δ14.2, 14.2, 22.7, 28.8, 29.0, 29.2, 29.5, 30.1, 31.8, 31.9, 33.2, 65.3, 126.2, 127.0, 127.4, 128.4, 129.0, 129.4, 131.5, 133.6, 137.4, 137.6, 139.1, 139.5. MS (ESI^+, TOF): Cal'd. for $C_{15}H_{22}O$ [M + Ag^+] 325.0722; found 325.0720.

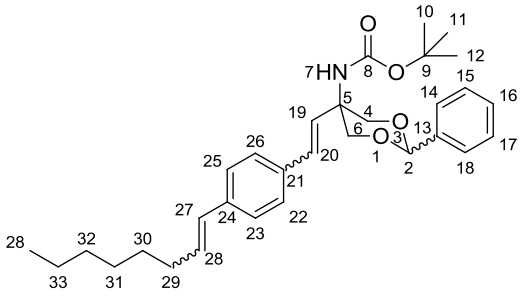
Formation of Phosphonium salt **50**:



4-(Oct-1-enyl)benzylalcohol (**56**, 0.3029 g, 1.387 mmol, 1.0 eq) and triisobutylphosphonium hydrobromide (0.3619 g, 1.501 mmol, 1.08 eq), were added to a microwave vial neat. The mixture was placed under reduced pressure and subsequently

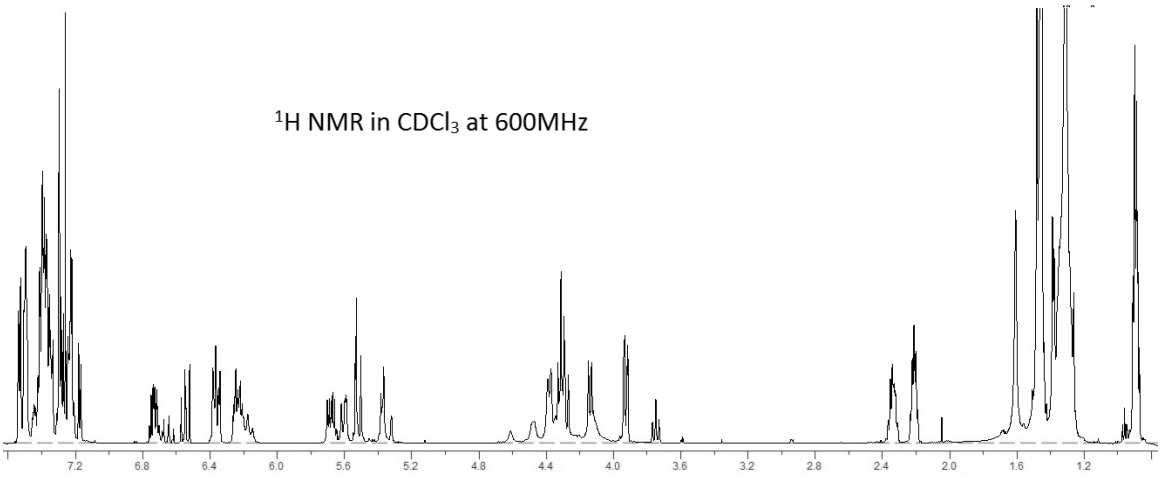
flushed with N_2. The mixture was stirred and heated at 110 °C overnight. The resulting mixture was cooled, diluted with DCM (5 mL), dried (Na_2SO_4) filtered, and the solvent removed under reduced pressure. The product was used without further purification ((*Z*):(*E*) 75:25 0.6701 g, 1.386 mmol, 99.7%). IR (4000-625ν cm^{-1}, NaCl): 2959, 2926, 2871, 1511, 1466, 1388, 1369, 1163, 1112, 964, 865. ^{1}H NMR (mixture of *Z* isomer (major) and *E* isomer (minor), 600 MHz, $CDCl_3$): δ0.87 (3H, t, *J* = 7.0 Hz, major, H-27), 0.89 (3H, t, *J* = 6.6 Hz, minor, H-27), 1.11 (18H, s, minor, H-10/11/14/15/18/19), 1.12 (18H, s, major, H-10/11/14/15/18/19), 1.20-1.37 (12H, m, major & minor, H-23/24/25), 1.40-1.49 (4H, m, major & minor, H-26), 2.10-2.18 (6H, m, major & minor, H-9/13/17), 2.20 (2H, dd, *J* = 6.9, 14.2 Hz, minor, H-22), 2.27 (2H, dd, *J* = 7.6, 14.9 Hz, major, H-22), 2.39 (12H, ddd, *J* = 6.4, 12.4, 12.7 Hz, major & minor, H-8/12/16), 4.26 (2H, d, *J* = 14.6 Hz, minor, H-7), 4.29 (2H, d, *J* = 14.6 Hz, major, H-7), 5.70 (1H, dt, *J* = 7.5, 11.8 Hz, major, H-21), 6.25 (1H, dt, *J* = 6.7, 15.5 Hz, minor, H-21), 6.33 (1H, d, *J* = 15.5 Hz, minor, H-20), 6.35 (1H, d, *J* = 11.8 Hz, major, H-20), 7.27 (2H, d, *J* = 8.1 Hz, minor, H-2/6), 7.32 (2H, d, *J* = 7.74 Hz, major, H-2/6), 7.38 (2H, dd, *J* = 2.1, 8.1 Hz, minor, H-3/5), 7.44 (2H, dd, *J* = 2.3, 8.1 Hz, major, H-3/5). ^{13}C NMR (mixture of isomers, 150 MHz, $CDCl_3$): δ14.2, 22.7, 23.9, 25.1, 25.1, 28.5, 28.8, 28.9, 28.9, 29.0, 29.1, 29.1, 29.2, 29.3, 29.4, 29.9, 31.8, 31.8, 33.2, 126.9, 127.8, 128.8, 129.8, 130.5, 130.5, 130.8, 130.9, 132.8, 134.6, 134.6, 138.2, 138.3. ^{31}P NMR (240 MHz, $CDCl_3$, externally referenced to 85% H_3PO_4 in D_2O): δ30.06, 30.19. MS (ESI^+, TOF): Calc'd. for [$C_{27}H_{48}P^+$] 403.3494; found 403.3499.

Formation of **49**:[156]

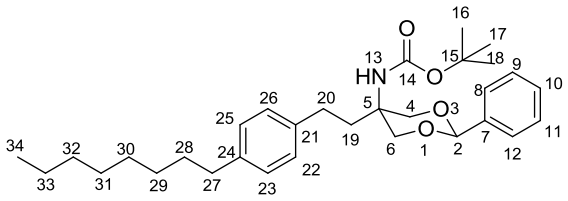


Microwave heating: phosphonium salt **50** (0.1419 g, 0.2934 mmol, 1.7 eq), LiOH (0.0187 g, 0.781 mmol, 4.5 eq), water (0.30 mL), and aldehyde **36** (0.0535 g, 0.174 mmol, 1.0 eq) were added to a tapered microwave vial. The microwave vial was flushed with N_2, capped, and heated in a microwave at 70 °C for 30 mins. The reaction was worked up with DCM (1 mL) and sat'd NH_4Cl (1 mL), and the aqueous layer was extracted with DCM (1 mL × 3), followed by EtOAc (1 mL). The organic layers were combined, dried (Na_2SO_4) filtered and the solvent removed under reduced pressure. Purification was obtained via silica column chromatography (100 hexanes to 90:10 hexanes:ethyl acetate) to give the mixture of isomers as a white amorphous solid (0.0648 g, 0.132 mmol, 76%). *Traditional heating:* phosphonium salt **50** (0.1259 g, 0.2604 mmol, 1.97 eq), K_2CO_3 (0.0727 g, 0.526 mmol, 3.98 eq), aldehyde **36** (0.0407 g, 0.132 mmol, 1.0 eq), and water (0.10 mL) were added to a tapered microwave vial. The vial was sealed with a septa and the mixture was stirred with heating at 70 ºC for 18 h. The mixture was diluted with water (1 mL) and extracted with Et_2O (2 mL × 5). The organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification via silica column chromatography (100 hexanes, increasing to 90:10 hexanes:ethyl acetate)

gave a mixture of isomers as a colourless amorphous mass (0.0416 g, 0.999 mmol, 64%). Due to the complexity of the spectra, full characterization has not been performed.



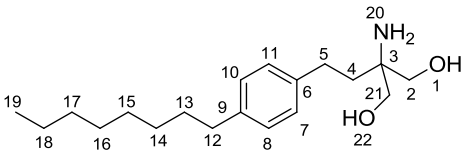
Formation of **57**:



A solution of **49** (0.0451 g, 0.0917 mmol, 1.0 eq) in THF (1 mL) and Pd (10% *w*/*w* loading on C, 0.0110 g, 0.0103 mmol, 0.11 eq) were added and the flask was flushed with H_2, with THF added throughout to maintain a volume of 1 mL. Additional Pd (10% *w*/*w* loading on C, 0.0134 g, 0.0126 mmol, 0.14 eq) was added after 18 h, with subsequent flushing of hydrogen gas. After a further 24 h of stirring the reaction was filtered through celite and washed with Et_2O, dried over $MgSO_4$, and the solvent removed under reduced pressure to give **57** as a white solid. Compound **60** was isolated cleanly as

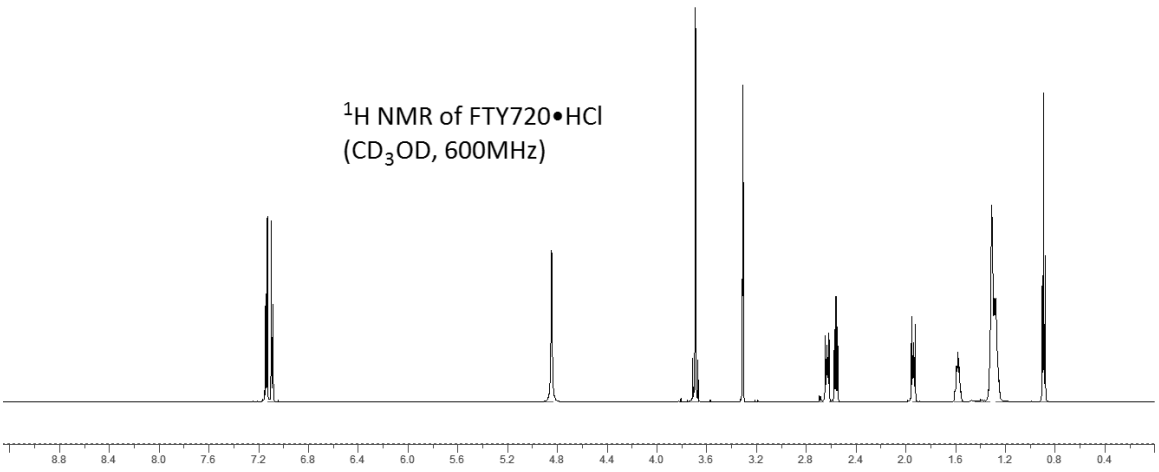
a 75:25 mixture of *cis/trans*-diasteromers and normally immediately deprotected (see 2.7). Compound **10** had: IR (4000-625ν cm^{-1}, NaCl): 3431, 3353, 2958, 2926, 2855, 1718, 1498, 1455, 1391, 1366, 1244, 1167, 1135, 1076, 1021, 975, 804, 745, 697. ^{1}H NMR (600 MHz, $CDCl_3$, mixture of isomers in 50:50 ratio): δ0.89 (7H, t, *J* = 7.0 Hz, H-35), 1.20-1.36 (28H, m, H-30/31/32/33/34), 1.47 (9H, s, H-16/17/18), 1.50 (9H, s, H-16/17/18), 1.60 (5H, m, H-29), 1.98 (2H, m, H-20), 2.39 (2H, dt, *J* = 5.3, 8.8 Hz, H-28), 2.53-2.60 (6H, m, H-20/28/H29), 2.67 (2H, dt, 5.6, 8.8, H-29), 3.67 (2H, d, *J* = 11.2 Hz, H-4/6), 4.21 (2H, d, *J* = 11.0 Hz, H-4/6), 4.31 (2H, d, *J* = 11.2 Hz, H-4/6), 4.34 (1H, s, NH-13), 5.17 (1H, s, NH-13), 5.45 (1H, s, H-2), 5.54 (1H, s, H-2), 7.11 (3H, m, H-23/24/26/27), 7.15 (2H, d, *J* = 8.0 Hz, H-23/27), 7.34-7.42 (6H, m, H-9/10/11), 7.49 (2H, dd, *J* = 1.7, 8.2 Hz, H-8/12), 7.51 (2H, dd, *J* = 1.7, 8.4 Hz, H-12). ^{13}C DEPTq NMR (150 MHz, $CDCl_3$, mixture of isomers in a 50:50 ratio): δ 14.2, 22.8, 28.5, 28.6, 28.7, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8, 31.7, 32.0, 34.4, 35.7, 50.9, 51.9, 72.0, 73.5, 101.9, 102.0, 126.2, 126.4, 128.3, 128.4, 128.5, 128.5, 128.6, 129.2, 129.3, 137.8, 137.9, 139.0, 139.1, 140.7, 140.7. MS (ESI^+, TOF): Calc'd. for [$C_{31}H_{45}NO_4Na^+$]: 518.3246; found 518.3224.

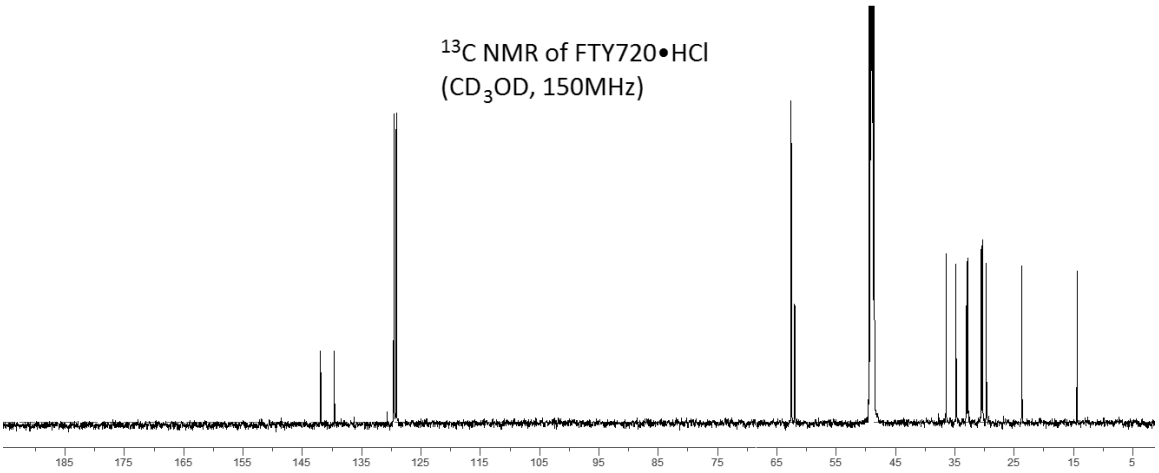
FTY720:

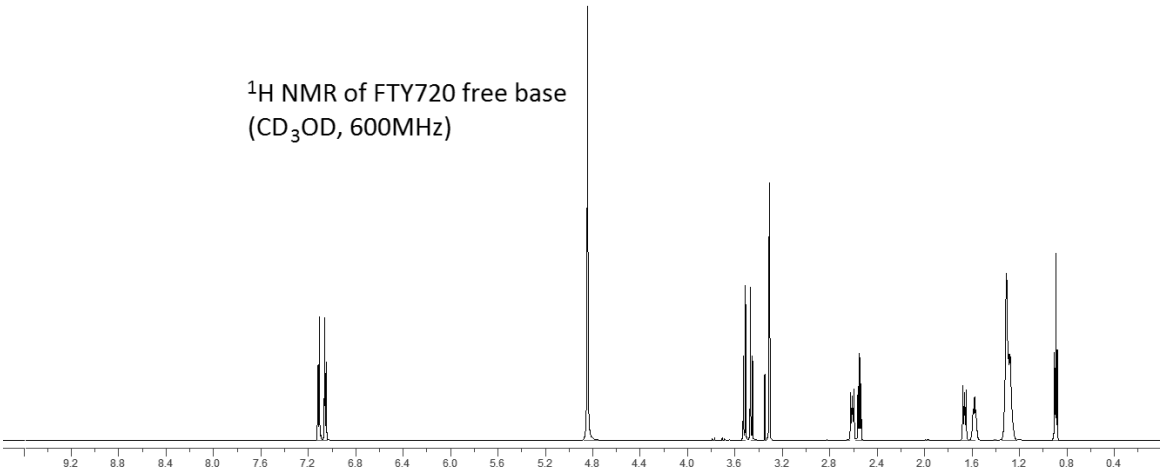


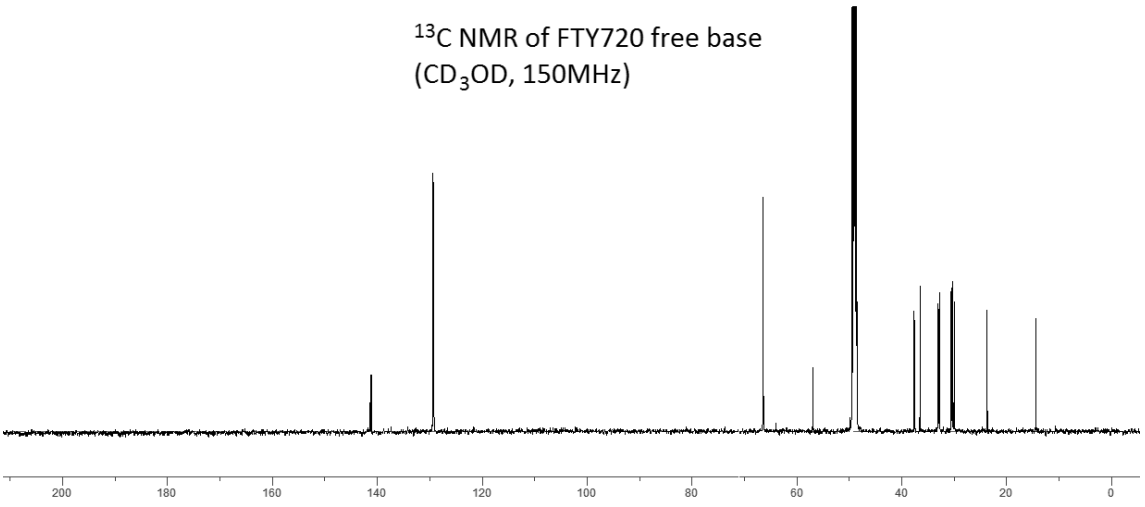
Compound **60** was dissolved in MeOH (0.6 mL) and DCM (0.4 mL) and HCl (37% in H_2O, 0.3 mL) was added. The mixture was stirred for 6 h, whereupon a second portion of MeOH (0.2 mL) and DCM (0.5 mL). Stirring was continued overnight (19 h) at which

time the reaction was neutralized through slow addition of sat'd $NaHCO_3$. The product was extracted with EtOAc (4 mL x 1, 2 mL x 4), the organic layers combined, dried (Na_2SO_4), filtered and solvent was removed under reduced pressure. The product was purified over silica to give FTY720-hydrochloride as a white solid (0.0259 g, 92% over both steps hydrogenation and deprotection steps). IR (4000-625ν cm^{-1}, NaCl): 3265, 3033, 2924, 2852, 1601, 1515, 1468, 1456, 1070, 1046. ^{1}H NMR (600 MHz, MeOD-d_4): δ0.89 (3H, t, *J* = 7.0 Hz, H-19), 1.22-1.35 (10H, m, H-14/15/16/17/18), 1.58 (2H, tt, *J* = 7.2, 7.3 Hz, H-13), 1.95 (2H, m, H-12), 2.56 (2H, t, *J* = 7.6 Hz, H-12), 2.64 (2H, m, H-5), 3.69 (1H, d, *J* = 11.6 Hz, H-2/3), 3.71 (1H, d, *J* = 11.6 Hz, H-2/3), 7.09 (2H, d, *J* = 8.0 Hz, H-8/10), 7.14 (2H, d, *J* = 8.0 Hz, H-7/11). ^{13}C NMR (150 MHz, MeOD-d_4): δ 14.4, 23.7, 29.7, 30.3, 30.4, 30.6, 32.8, 33.0, 34.8, 36.5, 62.0, 62.6, 129.2, 129.6, 139.6, 141.9. MS (ESI^+, TOF): Calcd for $C_{19}H_{33}NO_2$ [M + H^+] 308.2589; found: 308.2583. After passing through silica with 10:1 DCM:MeOH with 1% NH_4OH: ^{1}H NMR (600 MHz, MeOD-d_4): δ0.89 (3H, t, *J* = 7.0 Hz, H-19), 1.22-1.35 (10H, m, H-14/15/16/17/18), 1.58 (2H, m, H-13), 1.66 (2H, m, H-12), 2.55 (2H, t, *J* = 7.6 Hz, H-12), 2.61 (2H, m, H-5), 3.46 (1H, d, *J* = 10.9 Hz, H-2/3), 3.52 (1H, d, *J* = 10.9 Hz, H-2/3), 7.06 (2H, d, *J* = 7.9 Hz, H-8/10), 7.11 (2H, d, *J* = 7.9 Hz, H-7/11). ^{13}C NMR (150MHz, MeOD-d_4): δ14.4, 23.7, 30.0, 30.3, 30.4, 30.6, 32.8, 33.0, 36.5, 37.6, 57.0, 66.5, 129.2, 129.4, 141.1, 141.3.

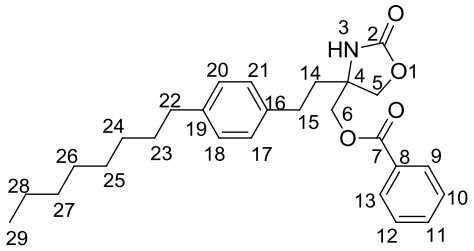

^{1}H NMR of FTY720•HCl
(CD_3OD, 600MHz)
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4


^{13}C NMR of FTY720•HCl
(CD_3OD, 150MHz)
185
175
165
155
145
135
125
115
105
95
85
75
65
55
45
35
25
15
5


^{1}H NMR of FTY720 free base
(CD_3OD, 600MHz)
9.2
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4

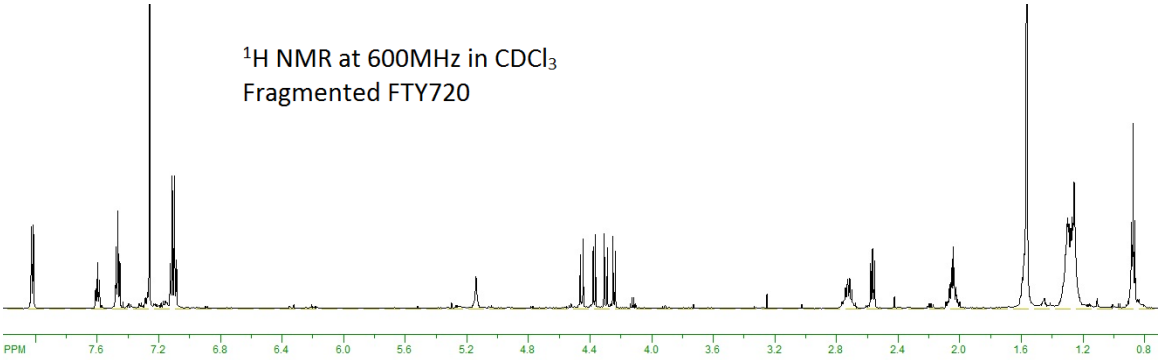

^{13}C NMR of FTY720 free base
(CD_3OD, 150MHz)
200
180
160
140
120
100
80
60
40
20
0

Synthesis of 4-(ethyl-(1-octyl-4-phenyl))-4-methylbenzoate-2-oxazolidinone (**58**):

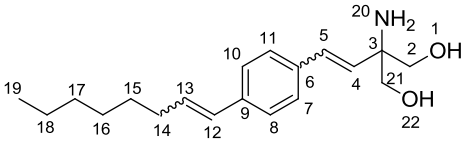


To a small vial was added *N*-Boc-benzylidene protected-FTY720 (**57**, 0.0197 g, 0.0397 mmol, 1.0 eq) and NBS (0.0082 g, 0.070 mmol, 1.8 eq), with subsequent charging of the vial with N_2. BPO (75% in H_2O, 0.0016 g, 0.0050 mmol, 0.12 eq) and chlorobenzene (0.4 mL) were then added, followed by heating at 70 ºC for 2 h. A second portion of NBS (0.0046 g, 0.039 mmol, 0.98 eq) and BPO (75% in H_2O, 0.0012 g, 0.037 mmol, 0.093 eq) were added with an additional 1 h of heating at 70 ºC. The mixture was cooled and diluted with sat'd $NaHCO_3$ (0.8 mL) and DCM (0.8 mL), then separated. The aqueous later was extracted with DCM (1 mL × 3), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purity was obtained via column chromatography (100 hexanes, slowly increasing to 90:10 hexanes:ethyl acetate) to yield a white powder (0.0093 g, 0.021 mmol, 54%). ^{1}H NMR (600 MHz, $CDCl_3$): δ0.81 (3H, t, *J* = 7.0 Hz, H-29), 1.15-1.28 (12H, m, H-23/24/25/26/27/28), 1.93-2.02 (2H, m, H-14), 2.50 (2H, t, *J* = 7.7 Hz, H-22), 2.60-2.70 (2H, m, H-15), 4.17 (1H, d, *J* = 9.0 Hz, H-6), 4.23 (1H, d, *J* = 11.6 Hz, H-5), 4.30 (2H, d, *J* = 9.0 Hz, H-6), 4.38 (2H, d, *J* = 11.6 Hz, H-5), 5.0 (1H, broad s, NH-3), 7.02 (2H, d, *J* = 8.1 Hz, H-18/20), 7.05 (2H, d, *J* = 8.1 Hz, H-17/21), 7.40 (2H, t, *J* = 7.8 Hz, H-10/12), 7.53 (1H, tt, *J* = 1.1, 7.4 Hz, H-11), 7.95 (2H, dd, *J* = 1.2, 8.0 Hz, H-9/13). ^{13}C NMR ($CDCl_3$, 150 MHz): δ14.3

(C-29), 22.8 (C-28), 29.4, 29.5, 29.5, 29.6, 31.7, 32.0, 35.7, 37.8 (C-14), 60.0 (C-4), 67.7 (C-5), 71.8 (C-6), 128.2, 128.8 (C10/12), 129.0, 129.2 (C-8), 129.9 (C-9/13), 133.8 (C-11), 137.2, 141.5, 158.4 (C-2), 166.3 (C-7). MS (ESI^+, TOF): Calc'd for [$C_{27}H_{35}NO_4$ + H^+]: 438.2644; found 438.2636.



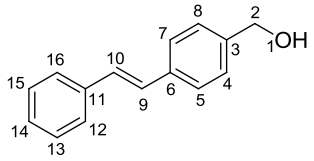
Synthesis of **59**:



To a small vial was added **49** (0.0371 g, 0.755 mmol), 30% H_2SO_4/MeOH (0.30 mL), MeOH (0.30 mL), and DCM (0.30 mL). The mixture was stirred at rt for 5.5 h, followed by neutralization with 10% NaOH, as tested by litmus paper. The solution was extracted with EtOAc (2 mL × 5), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained via column chromatography (100 hexanes, increasing to 100 ethyl acetate, then 100 DCM, finally 90:10 DCM:MeOH) to afford a clear oil as a mixture of isomers (0.0041 g, 0.014 mmol, 18%). ^{1}H NMR (600 MHz,

MeOD-d_4): δ0.83-0.94 (3H, m, H-19), 1.12-1.51 (8H, m, H-15/16/17/18), 2.18-2.26 (0.5H, m, H-14), 2.33 (1H, m, H-14), 3.46-3.50 (1H, m, H-2/21), 3.51 (1H, s, H-2/21), 3.60-3.68 (1H, m, H-2/21), 3.69 (1H, s, H-2/21), 5.62-5.70 (1H, m, H-13), 6.22-6.36 (0.5H, m, H-12), 6.39 (0.5H, t, *J* 11.1Hz, H-12), 6.63-6.80 (1H, m, H-5), 7.17-7.47 (4H, m, H-7/8/10/11). ^{13}C NMR (150 MHz, MeOD-d_4): δ14.4 (C-19), 23.7, 29.7, 30.1, 30.5, 31.0, 32.9, 32.9, 34.1, 55.1 (C-3), 61.6 (C-3), 66.4 (C-2/21), 67.0 (C-2/21), 126.7, 127.2, 127.3, 127.7, 129.4, 129.6, 129.7, 129.7, 130.0, 133.2, 134.2. MS (ESI+): Calc'd for [$C_{19}H_{31}NO_2H^+$]: 304.2277; found 304.2277.

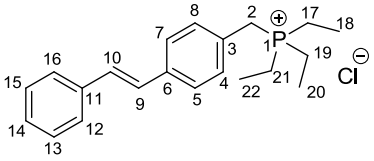
Synthesis of Stilbene **62**:[156]



To a non-tapered microwave vial was added 4-methoxymethylbenzaldehyde (0.1539 g, 1.130 mmol, 1.0 eq), triethylbenzylphosphonium chloride (0.6554 g, 2.266 mmol, 2.0 eq), K_2CO_3 (0.6390 g, 4.623 mmol, 4.1 eq), and H_2O (0.9 mL). The vial was capped and the solution was heated at 100 ºC for 22 h. The mixture was cooled to rt, diluted with H_2O (5 mL) and stirred for 2.5 h, followed by filtering to afford a white solid that was used without further purification (0.2002g, 0.9516mmol, 84%). IR (4000-625ν cm^{-1}, NaCl): 3308, 3080, 3023, 2923, 2864, 1684, 1599, 1447, 1418, 1267, 1215, 1112, 1073, 1043, 1001, 970, 964, 913, 841, 809, 777, 747, 690. ^{1}H NMR (600 MHz, $CDCl_3$): δ4.71 (2H, s, H-2), 7.11 (2H, s, H-9/10), 7.26 (1H, dt, *J* = 1.1, 7.4 Hz, H-14), 7.34-7.38 (4H, m, H-4/8/13/15), 7.52 (4H, d, *J* = 8.2 Hz, H-5/7/12/16). ^{13}C NMR (150 MHz, $CDCl_3$): δ65.3

(C-2), 126.7, 126.8, 127.5, 127.8 (C-9), 128.4 (C-10), 128.8 (C-4/8), 128.9 (C-14), 137.0 (C-6), 137.4 (C-11), 140.4 (C-3).

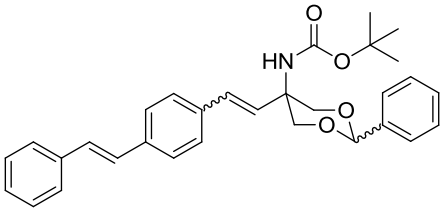
Synthesis of Salt **63**:[156]



To a microwave vial was added stilbene **62**[163] (0.1801 g, 0.8565 mmol, 1.0 eq) and triethylphosphine hydrochloride (0.1353 g, 0.8750 mmol, 1.02 eq). The vial was capped and the mixture heated at 110 ºC for 27 h. The mixture was cooled to rt, dissolved in DCM, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford a white solid as primarily (>98%) the *E* isomer that can generally be used without further purification (0.2871 g, 0.8277 mmol, 97%). For a purified salt: to a microwave vial was added stilbene **62**[163] (0.4954 g, 2.356 mmol, 1.0 eq) and triethylphosphine hydrochloride (0.3899 g, 2.522 mmol, 1.07 eq). The vial was capped and the mixture heated at 110 ºC for 17 h. The mixture was cooled to rt, dissolved in H_2O (35 mL) and extracted with EtOAc (10 mL × 4), with the organic layer subsequently discarded. The aqueous layer was then washed with DCM (15 mL × 10), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to give a white solid as primarily (>98%) the *E* isomer (0.6444 g, 1.858 mmol, 79%). IR (4000-625ν cm^{-1}, NaCl): 2975, 2920, 2884, 2320, 1700, 1512, 1449, 1414, 1270, 1250, 1165, 1107, 1049, 1023, 970, 830, 755, 724, 692. ^{1}H NMR (600 MHz, $CDCl_3$): δ1.24 (9H, dt, *J* = 7.7, 17.9 Hz, H-18/20/22), 2.52 (6H, ttd, *J* = 7.6, 15.4, 20.7 Hz, H-17/19/21), 4.26 (2H,

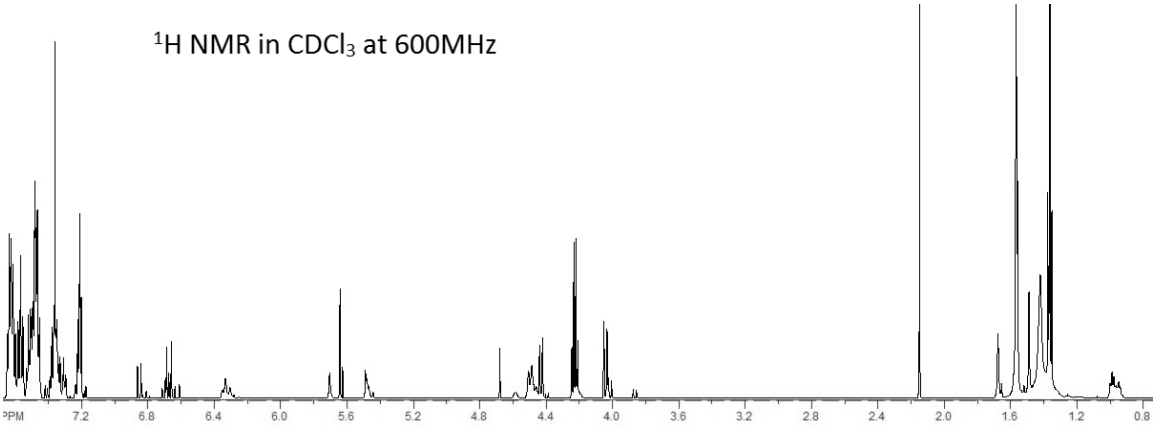
d, J = 15.5 Hz, H-2), 7.05 (1H, d, J = 16.6 Hz, H-9), 7.10 (1H, d, J = 16.6 Hz, H-10), 7.27 (1H, obscured by solvent peak, J = 7.2 Hz, H-14), 7.35 (2H, t, J = 7.6 Hz, H-4/8), 7.44 (2H, dd, J = 2.3, 8.4 Hz, H-13/15), 7.49 (4H, dt, J = 1.1, 7.6 Hz, H-5/7/12/16). ^{13}C NMR (150 MHz, $CDCl_3$): δ6.1 (J_{CP} =5.4Hz, C-18/20/22), 12.1 (J_{CP} = 48.2Hz, C-17,19,21), 26.0 (J_{CP} = 44.4Hz, C-2), 126.7, 127.5, 127.5, 127.5, 128.0, 128.8, 129.58, 130.6, 130.6 (J_{CP} = 4.6Hz, C-3), 136.9 (C-6), 137.6 (C-11). ^{31}P NMR (80 MHz, $CDCl_3$, externally referenced to 85% H_3PO_4 in D_2O): δ37.22. MS (ESI+): Calc'd for $C_{21}H_{28}P^+$: 311.1923; found 311.1922.

Synthesis of **64**:[161,162]

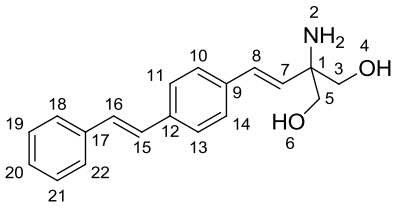


Aldehyde **36** (0.0264 g, 0.0859 mmol, 1.0 eq), phosphonium salt **63** (0.0451 g, 0.130 mmol, 1.5 eq), K_2CO_3 (0.0374 g, 0.271 mmol, 3.2 eq), and H_2O (0.11 mL) were added to a microwave vial. The vial was capped and the mixture was microwaved at 70 ºC for 35 mins. Sat'd NH_4Cl (0.6 mL) was added to the mixture and the solution was extracted with DCM (2 mL × 4), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained via silica column chromatography (100 hexanes, increasing to 90:10 hexanes:ethyl acetate) to afford **65**, a pale yellow solid, as a mixture of two major *cis*/*trans* isomers (0.0311 g, 0.643 mmol, 75%). Due to complexity of the spectra, full assignment has not been performed. IR

(4000-625ν cm^{-1}, NaCl): 3338, 2977, 2927, 2856, 1700, 1496, 1455, 1393, 1368, 1277, 1248, 1164, 1136, 1101, 1078, 1029, 986, 975, 915, 868, 747, 698. MS (ESI+): Calc'd for $C_{31}H_{34}NO_4$ [M + H^+] 484.2488; found 484.2495.

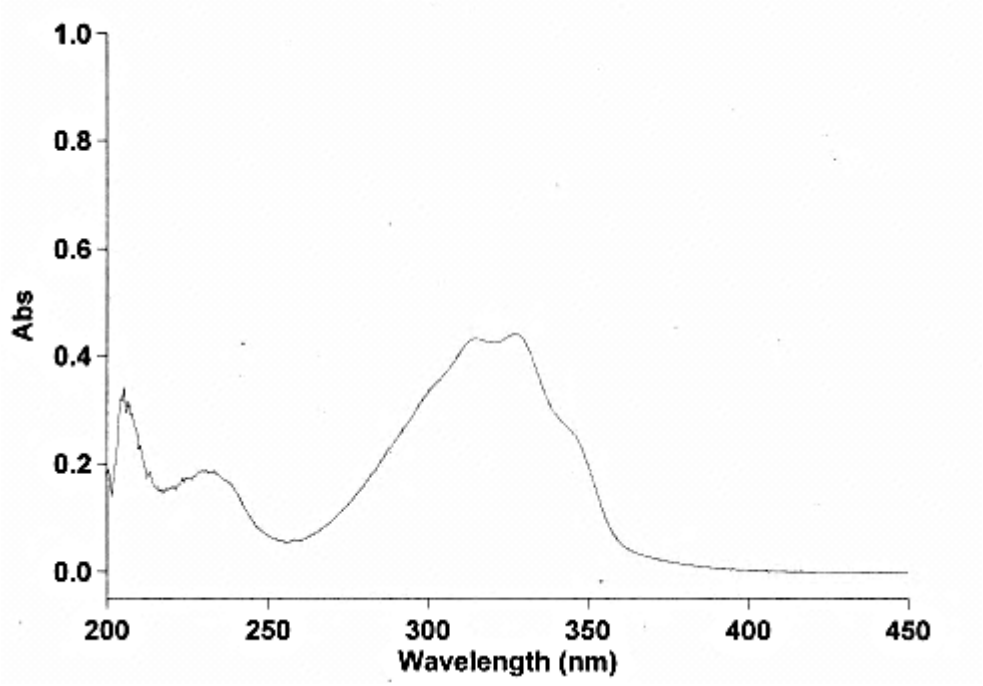


Synthesis of Stilbene FTY720 **60**:



To a small vial was added **64** (0.0123 g, 0.0254 mmol), DCM (0.20 mL), MeOH (0.20 mL), and HCl (37% in H_2O, 0.15 mL). The mixture was stirred at rt for 17 h, followed by addition of an NaOH solution (1% in H_2O, 2 mL) and more concentrated NaOH solution (10% in H_2O, 0.6 mL), until the reaction mixture was basic as tested by litmus paper. The now-basic solution was stirred for 15 mins to eliminate formation of the salt form of **60**. The mixture was washed with DCM (2 mL × 4) and EtOAc (2 mL × 4), the organic layers combined and diluted with DCM (4 mL) to resolubilize the particulate, dried over

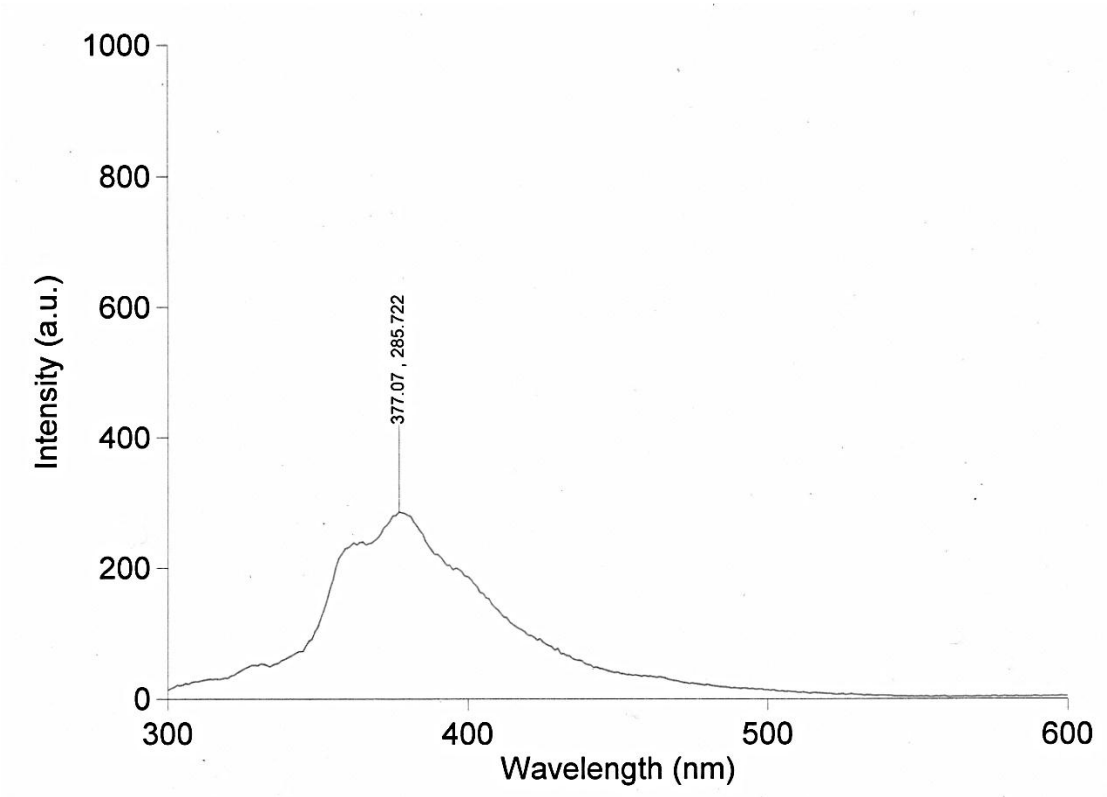
Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (100 hexanes, increasing to 100 DCM, then 90:10 DCM:MeOH) to afford a white solid as a mixture of two major and two minor isomers (0.0046 g, 0.016 mmol, 61%). ^{1}H NMR (two minor isomer peaks omittied, two major isomers of C-7/8 *E*:*Z* ratio 2:1, 600 MHz, MeOD-d_4): δ3.54 (*Z*, 4H, s, H-3/5), 3.67 (*E*, 2H, d, *J* = 11.2 Hz, H-3/5), 3.69 (s, OH-4/6), 3.71 (*E*, 2H, d, *J* = 11.2 Hz, H-3/5), 5.67 (*Z*, 1H, d, *J* = 12.8 Hz, H-7), 6.32 (*E*, 1H, d, *J* = 16.5 Hz, H-7), 6.71 (*E*, 1H, d, *J* = 16.5 Hz, H-8), 6.79 (*Z*, 1H, d, *J* = 12.8 Hz, H-8), 7.17 (*E*, 2H, d, *J* = 5.1 Hz), 7.18 (*Z*, 2H, d, *J* = 3.7 Hz), 7.22-7.26 (1H, m, H-20), 7.31-7.37 (2.7H, m), 7.45 (1.3H, d, *J* = 8.3 Hz), 7.51-7.57 (4H, m). ^{13}C NMR (mixture of 2 isomers, α-major and β-minor, 600 MHz, MeOD-d_4): δ60.0 (C-1_β), 61.2 (C-1_α), 67.0 (C-$3/5_\beta$), 67.4 (C-$3/5_\alpha$), 127.2, 127.3, 127.5, 127.5, 127.8, 127.8, 128.6, 128.7, 129.1, 129.2, 129.6, 129.7, 129.9, 130.0131.2, 131.7, 132.7, 134.5, 137.8, 137.8, 138.2, 138.8, 138.8. MS (ESI+): Calc'd for [$C_{19}H_{23}NO_2^+$]: 296.1650; found 296.1663. Absorbance and fluorescence samples diluted in MeOH.



λ_{max} = 205.6 & 327.6 nm

L = 1 cm, C = 4.063×10^{-5} M (0.0006 g in 0.0500 L of MeOH), $A_{327.06}$ = 0.440, $A_{231.1}$ = 0.187.

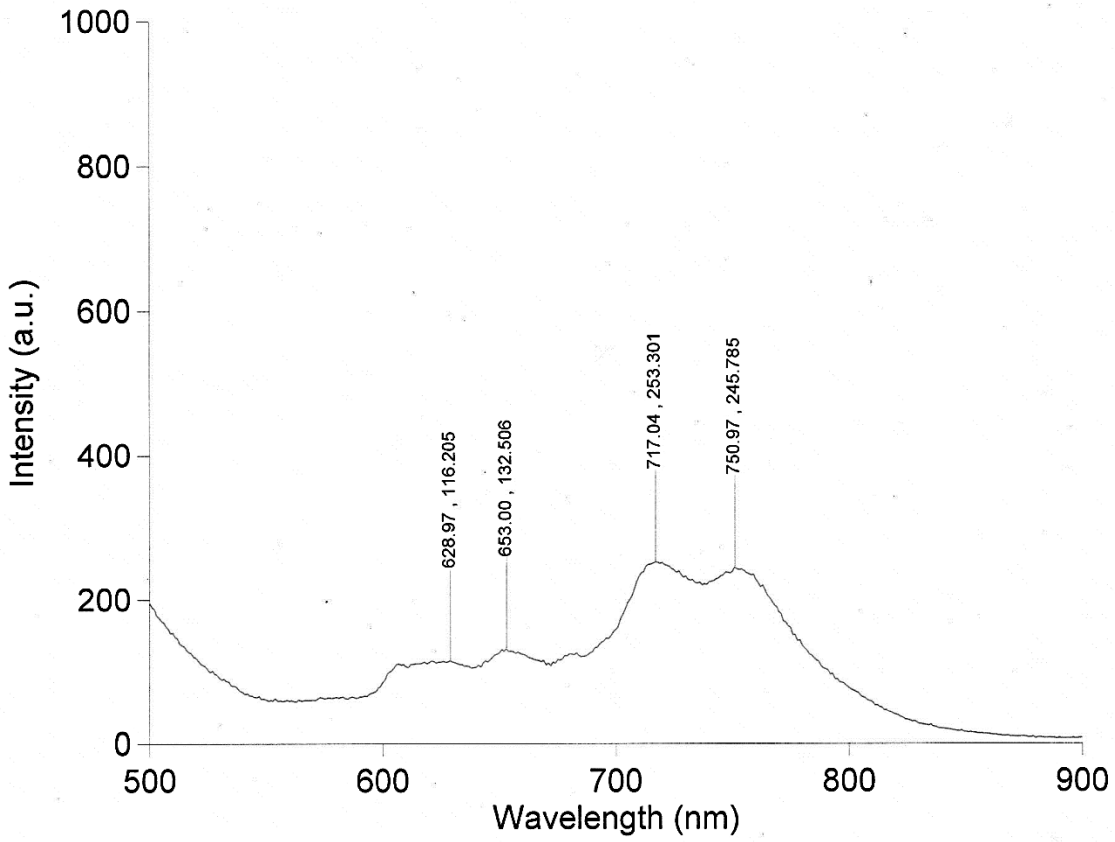
$\varepsilon_{231.1}$ = 4,603; $\varepsilon_{327.6}$ = 10,830.



Excitation: 231.1 nm

Excitation Slit Width: 5 nm

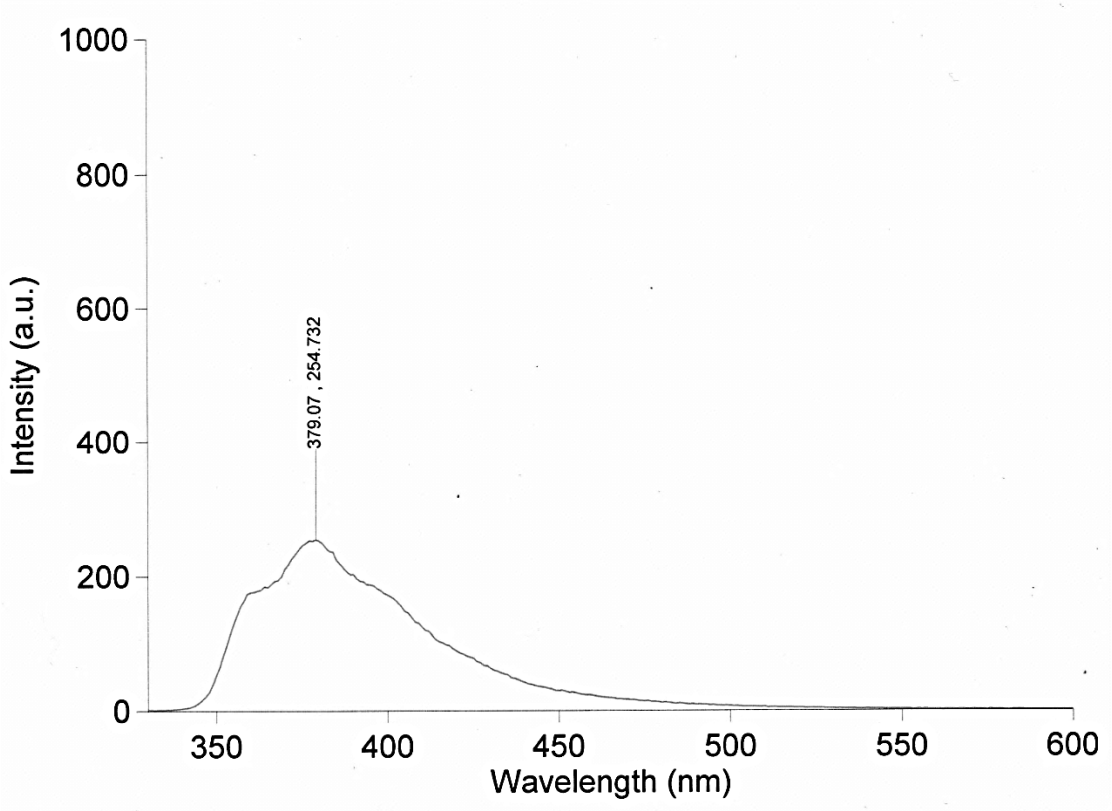
Emission Slit Width: 2.5 nm



Excitation: 231.1 nm

Excitation Slit Width: 10 nm

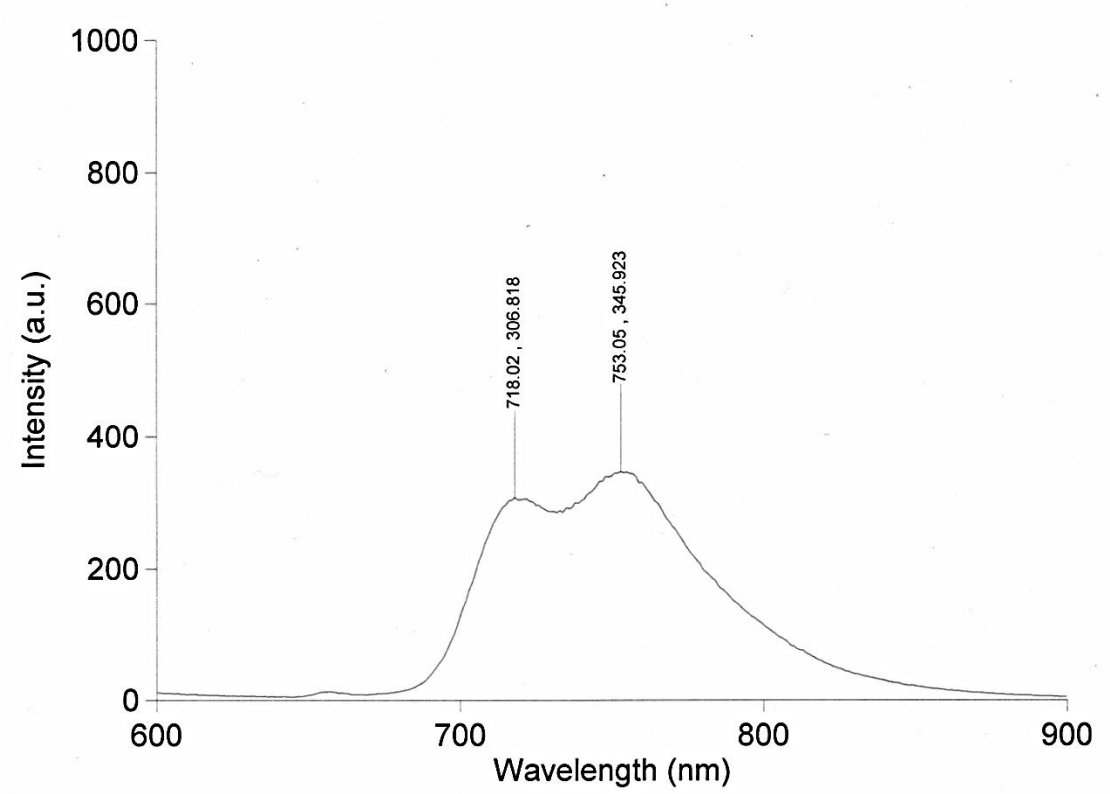
Emission Slit Width: 5 nm



Excitation: 327.6 nm

Excitation Slit Width: 2.5 nm

Emission Slit Width: 2.5 nm

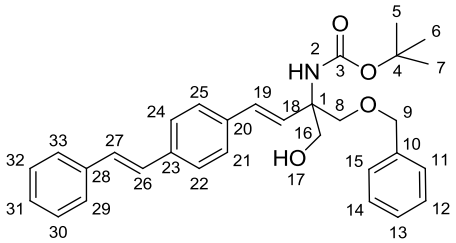


Excitation: 327.6 nm

Excitation Slit Width: 5 nm

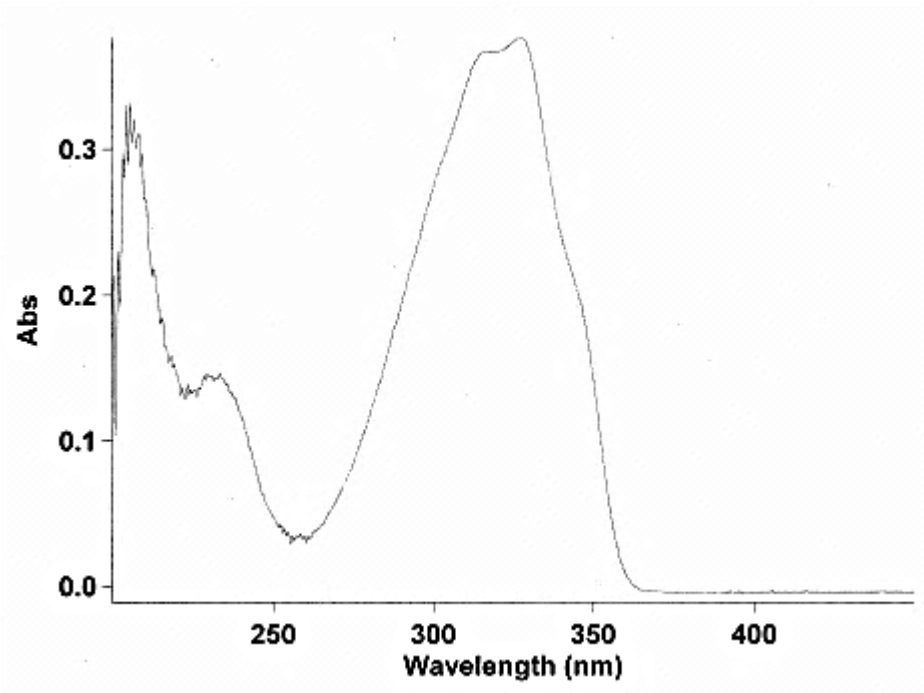
Emission Slit Width: 5 nm

Synthesis of Benzyl-stilbene **65**:



To a tapered microwave vial was added **64** (0.0178 g, 0.0368 mmol, 1.0 eq), and the vial was evacuated and refilled with N_2, with subsequent cooling to 0 ºC. **64** was dissolved in DCM (0.10 mL) and DIBAL (1M in DCM, 0.35 mL, 0.35mmol, 9.5eq) was added slowly. The mixture was allowed to warm to rt and was stirred 18.5 h. A 3.7% HCl solution (10% solution of 37% HCl in H_2O, 1 mL) was used to quench the reaction, followed by extraction using EtOAc (2 mL × 6). The organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford **65** as a white solid (0.0061 g, 0.016 mmol, 43%). ^{1}H NMR (two isomers of C-18/19, *E*:*Z* ratio 1.25:1, 600 MHz, $CDCl_3$): δ1.25 (*Z*, 9H, s, H-5/6/7), 1.47 (*E*, 9H, s, H-5/6/7), 3.54 (*Z*, 1H, d, *J* = 9.2 Hz, H-16), 3.68 (*E*, 1H, d, *J* = 9.4 Hz, H-16), 3.72 (*Z*, 1H, d, *J* = 9.3 Hz, H-16), 3.75 (*E*, 1H, d, *J* = 9.3 Hz, H-16), 3.77 (*E*, 1H, d, *J* = 11.6 Hz, H-8), 3.79 (*Z*, 1H, d, *J* = 11.8 Hz, H-8), 3.81 (*Z*, 1H, d, *J* = 11.8 Hz, H-8), 3.84 (*E*, 1H, d, *J* = 11.7 Hz, H-8), 4.54 (*Z*, 2H, dd, *J* = 12.0, 16.4 Hz, H-9), 4.58 (*E*, 2H, s, H-9), 5.16 (*Z*, 1H, s, NH-2), 5.40 (*Z*, 1H, s, NH-2), 5.62 (*Z*, 1H, d, *J* = 12.7 Hz, H-18), 6.24 (*E*, 1H, d, *J* = 16.2 Hz, H-18), 6.52 (*E*, 1H, d, *J* = 16.2 Hz, H-19), 6.70 (*Z*, 1H, d, *J* = 12.8 Hz, H-19), 7.06-7.13 (2H, m), 7.24-7.29 (1H, m), 7.29-7.42 (8H, m), 7.46 (*E*, 2H, d, *J* = 8.3 Hz), 7.49-7.54 (2H, m). ^{13}C NMR (mixture of isomers, 600 MHz, $CDCl_3$): δ28.3 (*Z*,C-5/6/7), 28.5 (*E*, C-5/6/7), 61.1

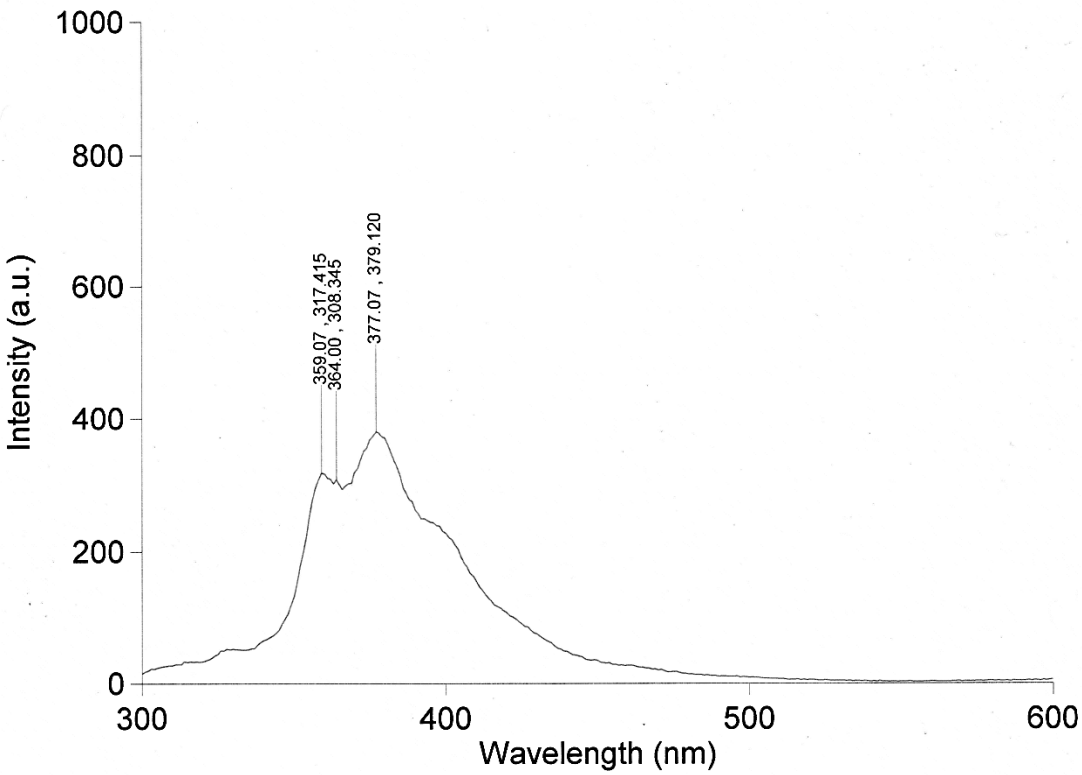
(C-1), 67.6 (*E*, C-16), 67.7 (*Z*, C-16), 73.5 (*Z*, C-8), 73.6 (*E*, C-8), 73.8 (C-9), 79.7 (*Z*, C-4), 80.2 (*E*, C-4), 126.1, 126.6, 126.8, 127.0, 127.8, 127.9, 128.1, 128.1, 128.4, 128.5, 128.7, 128.7, 128.8, 128.9, 128.9, 129.3, 130.3, 130.5, 131.9, 136.1, 136.6, 136.9, 137.5, 137.5, 137.7, 137.7, 155.5 (*E*, C-3), 156.1 (*Z*, C-3). MS (ESI+): Calc'd for $[C_{31}H_{36}NO_4^+]$ 486.2644; found 486.2639. Absorbance and fluorescence samples diluted in MeOH.



λ_{max} = 207.0 & 326.9 nm

L = 1 cm, C = 8.237 × 10^{-6} M (0.0004 g in 0.100L of MeOH), $A_{326.9}$ = 0.376, $A_{232.0}$ = 0.145.

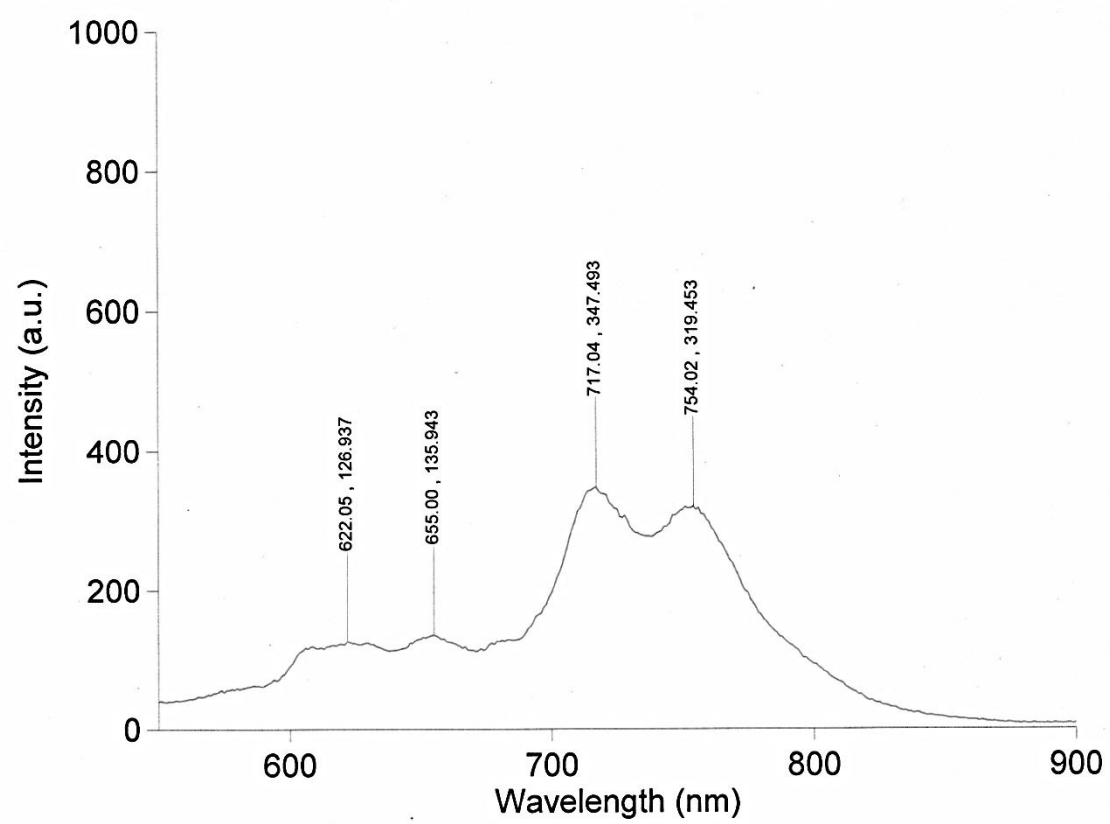
$\varepsilon_{232.0}$ = 17,604; $\varepsilon_{326.9}$ = 45,648.



Excitation: 232.0 nm

Excitation Slit Width: 5 nm

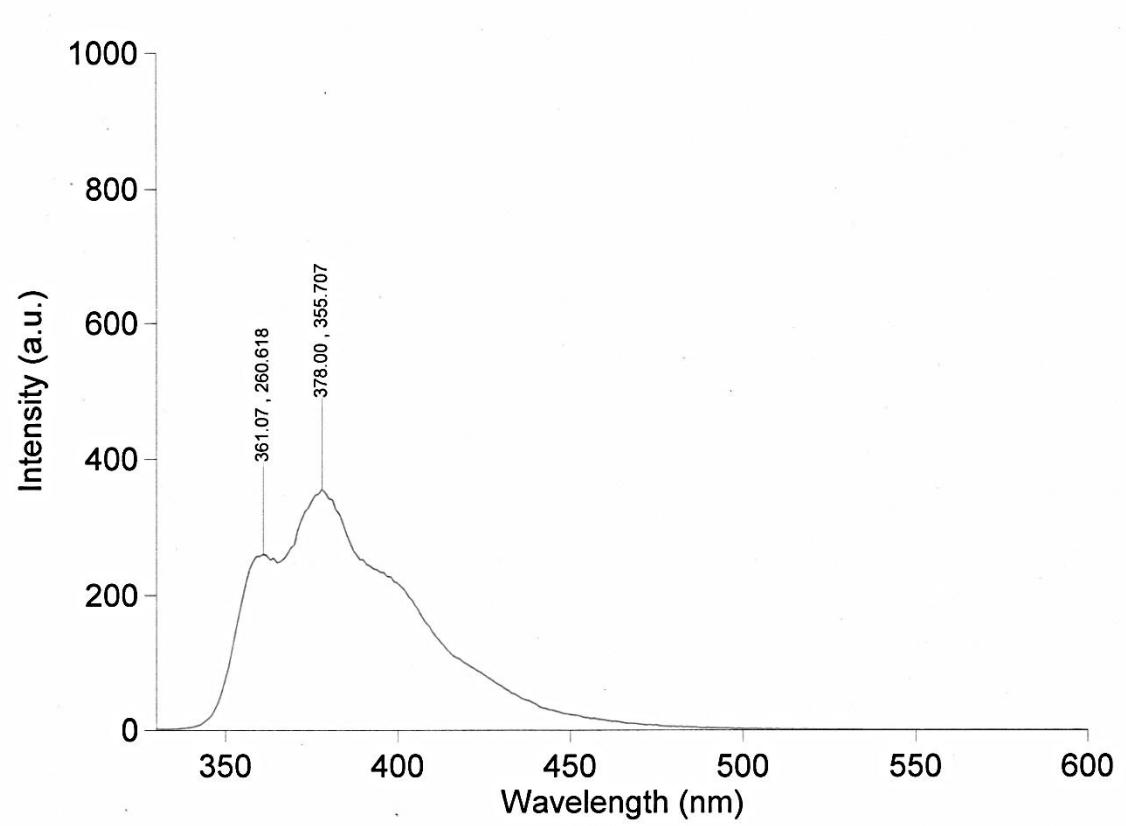
Emission Slit Width: 2.55 nm



Excitation: 232.0 nm

Excitation Slit Width: 10 nm

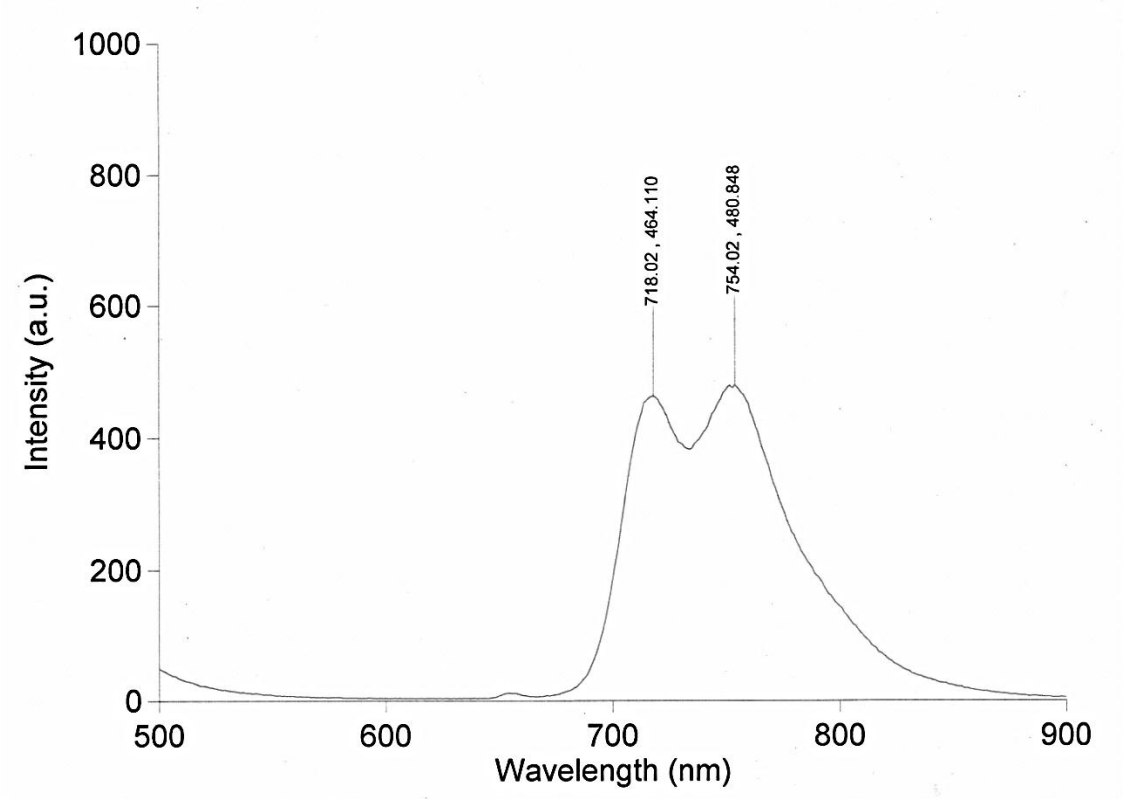
Emission Slit Width: 5 nm



Excitation: 326.9 nm

Excitation Slit Width: 2.5 nm

Emission Slit Width: 2.5 nm

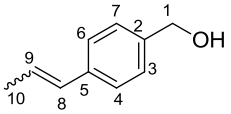


Excitation: 326.9 nm

Excitation Slit Width: 5 nm

Emission Slit Width: 5 nm

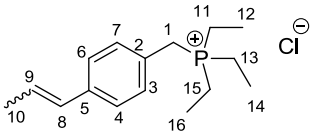
Synthesis of 4-(1-propene)-benzyl alcohol **66**:[180]



To a 50 mL 2-neck rbf was added triphenylethylphosphonium bromide (1.1028 g, 2.970 mmol, 1.36 eq) and the apparatus was flushed with N_2 for 30 mins. THF (3.0 mL) was then added and the mixture was cooled to 0 °C, followed by slow addition of ^{n}BuLi (2.5M

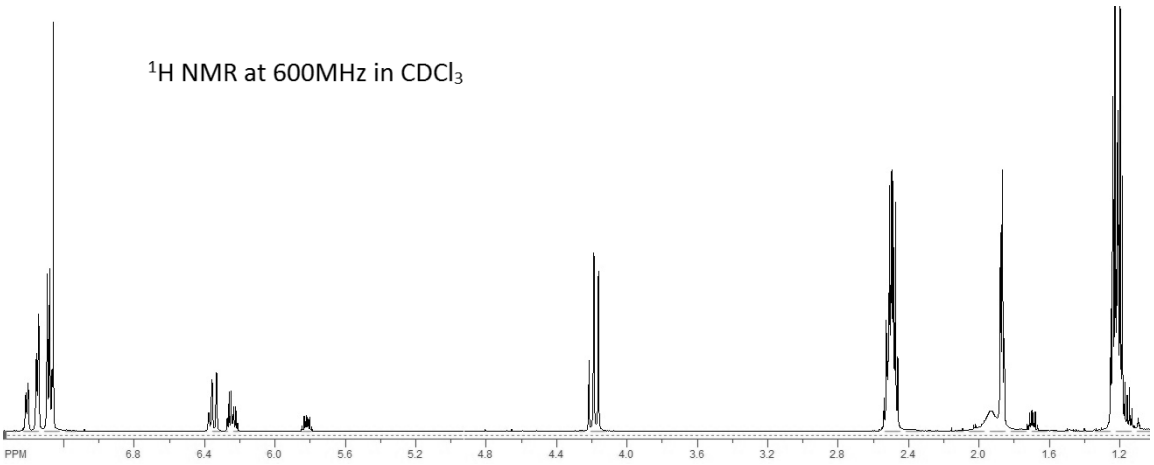
in hexanes, 2.0 mL, 5.0 mmol, 2.3 eq) which turned the reaction a deep red colour. The reaction was stirred for 20 mins and then cooled to -78 ºC, whereupon 4-hydroxymethyl benzaldehyde (0.2981 g, 2.191 mmol, 1.0 eq) in THF (1.0 mL) was added slowly, with additional THF (0.5 mL) used to rinse out the vial previously containing 4-hydroxymethyl benzaldehyde. The mixture was then allowed to warm to rt. with stirring. Following 6.5 h of stirring, sat'd NH_4Cl (15 mL) was added and the layers separated, setting aside the organic partition. The product extracted from the aqueous layer with Et_2O (15 mL × 3), then the organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained using silica column chromatography (100 hexanes, increasing gradually to 80:20 hexanes:ethyl acetate) to afford a clear, colourless oil as a mixture of isomers in approximately a 1.75:1 *E*:*Z* ratio (0.2839 g, 1.916 mmol, 87%). ^{1}H NMR (*E* isomer, major, 600 MHz, $CDCl_3$): δ1.88 (3H, dd, *J* = 1.7, 6.6Hz, H-10) , 4.66 (2H, s, H-1), 6.24 (1H, dd, *J* = 6.6, 13.2, 15.8Hz, H-9), 6.40 (1H, dd, *J* = 1.6, 15.8Hz, H-8), 7.27-7.35 (4H, m, H-3/4/6/7). ^{13}C NMR (*E* isomer, 600 MHz, $CDCl_3$): δ18.6 (C-10), 65.4 (C-1), 126.1 (C-9), 127.4 (C-4/6), 129.2 (C-3/7), 130.8 (C-8), 137.1 (C-5), 139.4 (C-2). ^{1}H NMR (*Z* isomer, minor, 600 MHz, $CDCl_3$): δ1.90 (3H, dd, *J* = 1.9, 7.2 Hz, H-10), 4.69 (2H, s, H-1), 5.80 (1H, ddd, *J* = 7.2, 11.5, 14.4 Hz, H-9), 6.43 (1H, dd, *J* = 1.7, 11.5 Hz, H-8), 7.27-7.35 (4H, m, H-3/4/6/7). ^{13}C NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ14.8 (C-10), 60.6 (C-1), 126.0 (C-9), 127.0 (C-4.6), 127.1 (C-3.7), 129.6 (C-8), 137.2 (C-5), 139.1 (C-2).

Synthesis of 4-(1-prop-1-ene)-benzyl triethylphosphine hydrobromide **67**:[156]

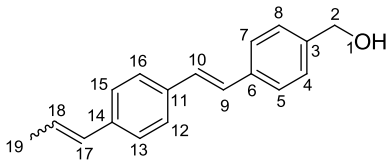


4-(1-prop-1-ene)-benzyl alcohol **66** (0.4170 g, 2.814 mmol, 1.0 eq) and triethylphosphine hydrochloride (0.4589 g, 2.968 mmol, 1.05 eq) were added to a tapered microwave vial, then the vial was capped and the mixture was heated at 110 ºC for 17 h. H_2O (5 mL) was added and the mixture was extracted with Et_2O (2mL × 1, 4 mL × 3) to remove the alcohol impurity, then these organic layers were discarded. The aqueous layer was then washed with DCM (10 mL × 11), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to give a pale yellow solid as a mixture of isomers (2.3:1 *E*:*Z*, 0.6146 g, 2.158 mmol, 77%). IR (4000-625ν cm^{-1}, NaCl): 3019, 2973, 2915, 2885, 1512, 1454, 1419, 1277, 1260, 1052, 967, 863, 810, 770, 753. ^{1}H NMR (mixture of isomers, 600 MHz, $CDCl_3$): δ1.17-1.26 (9H, m, H-12/14/16), 1.85-1.88 (3H, m, H-10), 2.45-2.55 (6H, m, H-11/13/15), 4.17 (*E*, 2H, d, *J* = 15.3 Hz, H-1), 4.20 (*Z*, 2H, d, *J* = 15.6Hz, H-1), 5.82 (*Z*, 1H, ddd, *J* = 7.4, 11.9, 14. 5Hz, H-9), 6.24 (*E*, 1H, ddd, *J* = 6.6, 13.2, 15.6 Hz, H-9), 6.35 (*E*, 1H, d, *J* = 15.3 Hz, H-8), 6.37 (*Z*, 1H, d, *J* = 10.5 Hz, H-8), 7.27 (*Z*, 2H, d, *J* = 8.3 Hz, H-3/7), 7.29 (*E*, 2H, d, *J* = 8.2 Hz, H-3/7), 7.35 (*E*, 2H, dd, *J* = 2.0, 8.2 Hz, H-4/6), 7.41 (*Z*, 2H, dd, *J* = 2.2, 8.2 Hz, H-4/6). ^{13}C NMR (mixture of isomers, 600 MHz, $CDCl_3$): δ6.1 (*J* = 5.5 Hz, C-12/14/16), 12.1 (*J* = 48.2 Hz, C-11/13/15), 14.8 (*Z*, C-10), 18.6 (*E*, C-10), 19.8 (*Z*, *J* = 66.2 Hz, C-1), 26.0 (*J* =45.5 Hz, C-1), 126.2 (*Z*, *J* = 9.0 Hz, C-2), 126.4 (*E*, *J* = 9.0 Hz, C-2), 126.9 (*J* = 3.3 Hz), 127.3, 128.1, 128.9, 130.0 (*J* = 2.7 Hz), 130.1, 130.1, 130.4, 130.4, 138.0 (*Z*, *J* = 3.3 Hz, C-5),

138.3 (*E*, *J* = 3.7 Hz, C-5). ^{31}P NMR (80 MHz, $CDCl_3$, externally referenced to 85% H_3PO_4): δ37.41 (major), 37.49 (minor). MS (ESI+): Calc'd for [$C_{16}H_{26}P^+$]: 249.1772; found 249.1767.



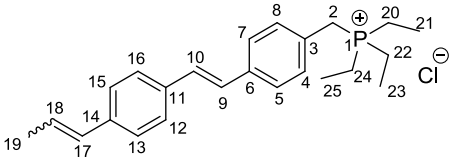
Synthesis of 1-hydroxymethyl stilbene **68**:[180]



To a non-tapered microwave vial was added 4-(1-prop-1-ene)-benzyl triethylphosphine hydrobromide (**67**, 0.5937 g, 2.085mmol, 1.4 eq), 4-hydroxymethyl benzaldehyde (**51**, 0.1983 g, 1.456 mmol, 1.0 eq), K_2CO_3 (0.5798 g, 4.296 mmol, 2.88 eq), and H_2O (0.83 mL). The vial was capped and microwaved at 100 ºC for 30 mins. H_2O (6 mL) was added, the mixture stirred for 1 h, then filtered to afford a yellow solid as a mixture of isomers with no further purification (5:1 *E*:*Z* of alkene C-17/18, 0.3612 g, 1.443 mmol, 99%). IR (4000-625ν cm^{-1}, NaCl): 3343, 3020, 2917, 2850, 1696, 1653, 1598, 1512,

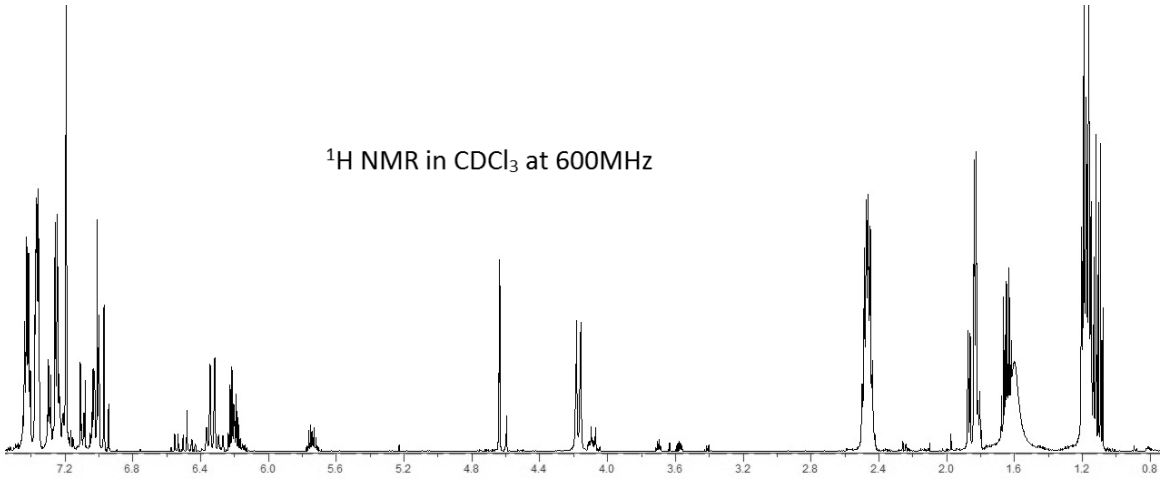
1456, 1419, 1375, 1271, 1210, 1167, 1110, 1044, 1015, 967, 883, 828, 778, 722, 695. ^{1}H NMR (mixture of 4 isomers, integration set methylene protons on C2 at 2H, 600 MHz, $CDCl_3$): δ1.86-1.96 (3H, m, H-19), 4.65-4.73 (2H, m, H-2), 5.80 (0.35H, ddd, *J* = 7.3, 11.8, 14.6 Hz, H-18$_{cis}$), (0.7H, ddd, *J* = 6.6, 13.2, 15.6 Hz, H-18$_{trans}$), 6.33-6.45 (1H, m, H-17), 6.56 (0.35H, d, *J* = 13.3 Hz, H-9/17$_{cis}$), 7.09 (1.7H, d, *J* = 15.0 Hz, H-9/17), 7.16-7.25 (1H, m), 7.247-7.39 (4H, m), 7.44 (1H, d, *J* = 8.3Hz), 7.5 (2H, dd, *J* = 7.1, 15.2 Hz, H-5/7). ^{13}C NMR (mixture of isomers, 600 MHz, $CDCl_3$): δ18.7 (C-19), 65.3 (C-2), 126.0, 126.3, 126.4, 126.8, 126.8, 127.2, 127.5, 127.8, 128.1, 128.7, 129.2, 129.4, 129.7, 130.9, 135.9, 137.6, 143.3. MS (ESI+): Calc'd for [$C_{18}H_{19}O^{+}$]: 250.1358; found 250.1358.

Synthesis of Phosphonium salt **69**:[156]

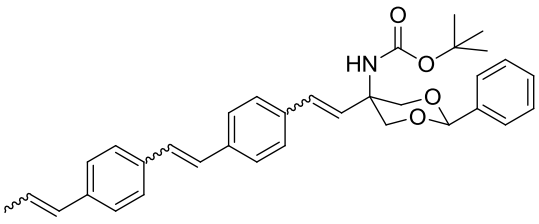


To a non-tapered microwave vial was added stilbene **68** (0.1960 g, 0.7830 mmol, 1.0 eq), triethylphosphine hydrochloride (0.1288 g, 0.8330 mmol, 1.06 eq), and dioxane (0.6 mL). The vial was sealed and heated at 110 ºC for 20 h. After cooling to rt, H_2O (10 mL) was added and the aqueous layer was washed with Et_2O (10 mL × 2) to remove the alcohol impurity. Those organic layers were discarded and the aqueous layer was then washed with DCM (10 mL × 10) with solid NaCl added to eliminate emulsification of the layers. The DCM-based organic layers were combined, dried over Na_2SO_4, filtered, and the

solvent removed under reduced pressure to afford a yellow solid (0.2425 g, 0.6873 mmol, 88%). Due to complexity the ^{1}H NMR was not fully characterized, see attached figure. IR (4000-625ν cm^{-1}, NaCl): 3021, 2925, 2851, 1696, 1684, 1513, 1456, 1418, 1244, 1161, 1107, 1047, 1012, 965, 925, 845, 785, 724. ^{31}P NMR (80 MHz, $CDCl_3$, externally referenced to 85% H_3PO_4): δ37.50. MS (ESI+): Calc'd for [$C_{24}H_{32}P^+$]: 351.2242; found 351.2254.

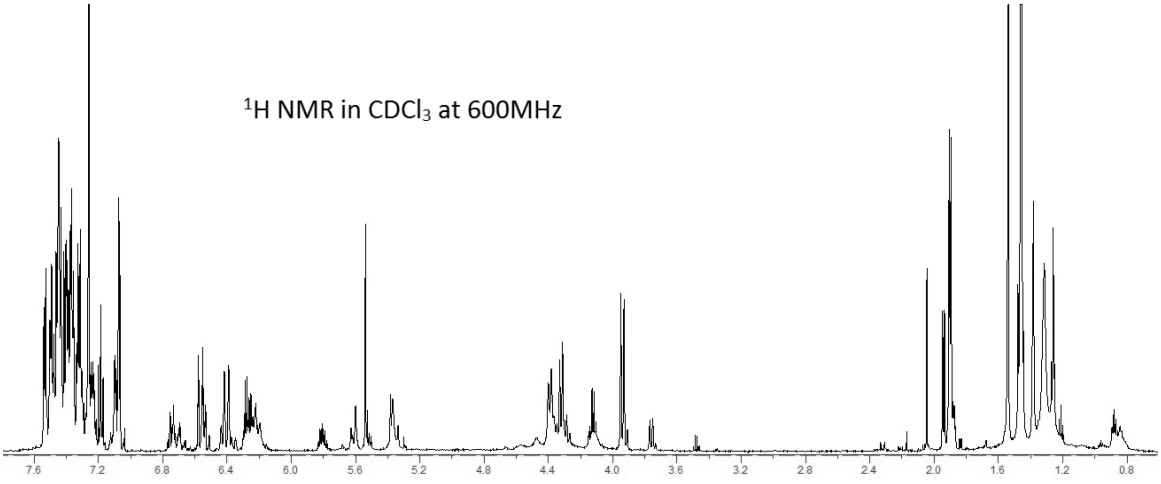


Synthesis of **70**:[161,162]

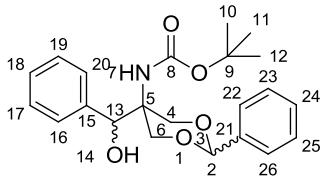


To a tapered microwave vial was added **69** (0.1497 g, 0.4259 mmol, 1.9 eq), aldehyde **36** (0.684 g, 0.222 mmol, 1.0 eq), K_2CO_3 (0.1210 g, 0.8755 mmol, 3.9 eq), and H_2O (0.3 mL). The vial was capped and microwave heated at 110 ºC for 20 mins, followed by

traditional heating at 110 ºC for 19 h. After cooling to rt, H_2O (6 mL) was added and the product was extracted with Et_2O (2 mL) and EtOAc (4 mL). Following the first separation solid NaCl was added to prevent the aqueous and organic layers from forming an emulsion. EtOAc (4 mL) was added and separated, followed by additional H_2O (5 mL) and Et_2O (10 mL). After waiting 15 mins the layers were extracted, with subsequent separations from Et_2O (10 mL × 2) washes occurring after 15 mins of waiting. Purification was obtained via silica column chromatography (100 hexanes, gradually increasing to 95:5 hexanes:ethyl acetate, and finally to 90:10 hexanes:ethyl acetate) to afford a yellow solid (0.0572 g, 0.1092 mmol, 49%). Due to the complexity of the ^1H NMR, total assignment was not performed.



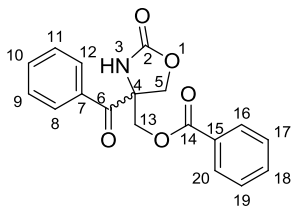
Grignard on aldehyde **36** to produce **71**:



To a 25 mL 2-neck rbf charged with N_2 and affixed a condensor, Mg^0 turnings (0.1205 g, 4.959 mmol, 6.5 eq), I_2 (0.0075 g, 0.0030 mmol, 0.04 eq), and Et_2O (2.5 mL) were added. Iodobenzene (0.15 mL, 0.27 g, 1.3 mmol, 1.7 eq) was added and heated at 45 ºC for 20 mins to initiate the reaction. The remaining iodobenzene (0.41 mL. 0.75 g, 3.7 mmol, 4.9 eq) was added over 20 minutes, and the solution was refluxed at 45 ºC for 1 h. This rendered 3 mL of stock grignard solution (~1.65M). In a separate flask charged with N_2, aldehyde **36** was dissolved in Et_2O (3 mL). The solution was cooled to 0 ºC and 2 mL (3.3 mmol, 4.3 eq) of the stock Grignard solution was added. The mixture was warmed to rt and allowed to stir for 1.75 h. Sat'd NH_4Cl (3 mL) was added and allowed to mix for 1h. The solution was separated and the aqueous layer was washed with Et_2O (4 mL × 3), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography (100 hexanes, increasing to 80:20 hexanes:ethyl acetate) to produce white, powder-like crystals as a mixture of isomers (0.2389 g, 0.6189 mmol, 82%). IR (4000-625ν cm^{-1}, NaCl): 3414 (O-H), 3331 (O-H), 3064, 3034, 2978, 2931, 2869, 1713 (C=O), 1683 (C=O), 1508, 1454, 1392, 1368, 1292, 1250, 1163, 1126, 1088, 1049, 1029, 982, 937, 916, 874, 787, 744, 699, 636. ^{1}H NMR (as a mixture of 2 *cis*/*trans* isomers in a 50:50 ratio, $CDCl_3$, 600 MHz): δ1.44 (9H, s, H-10/11/12), 1.50 (9H, s, H-10/11/12), 3.85 (1H, dd, *J* = 2.25, 11.4

Hz, H-4/6), 3.90 (1H, d, *J* = 11.4 Hz, H-4/6), 3.95 (1H, d, *J* = 12.2 Hz, H-4/6), 4.03 (1H, d, *J* = 11.7 Hz, H-4/6), 4.04 (1H, d, *J* =11.5 Hz, H-4/6), 4.11-4.16 (1H, m, H-4/6), 4.57 (1H, s, OH-13), 4.121368-4.75 (2H, m, H-4/6), 4.83 (1H, d, *J* = 10.3 Hz, OH-14), 5.12 (1H, s, NH-7), 5.44 (1H, s, H-2), 5.53 (1H, d, *J* = 5.7 Hz, H-13), 5.63 (1H, s, H-2), 6.31 (1H, d, *J* = 8.3 Hz, H-13), 7.22-7.52 (18H, m, H-16/17/18/19/20/23/24/25), 7.55 (2H, dd, *J* = 1.5, 8.2 Hz, H-22/26). ^{13}C NMR (as a mixture of 2 isomers in a 50:50 ratio, $CDCl_3$, 150 MHz): δ28.4 (C-10/11/12), 28.5 (C-10/11/12), 54.6 (C-5), 56.0 (C-5), 70.3 (C-13), 70.4 (C-13), 72.1 (C-4/6), 73.4 (C-4/6), 81.2 (C-9), 101.8 (C-2), 102.2 (C-2), 126.2 (C-18), 126.5 (C-23/25), 127.0 (C-23/25), 127.7 (C-17/19), 128.1 (C-17/19), 128.3 (C-16/20), 128.3 (C-16/20), 128.5 (C-22/26), 128.6 (C-22/26), 129.3 (C-24), 129.3 (C-24), 137.7 (C-21), 137.7 (C-21), 139.9 (C-15), 139.9 (C-15). MS (ESI+): Calc'd for [$C_{22}H_{28}NO_5^+$]: 386.1967; found 386.1960.

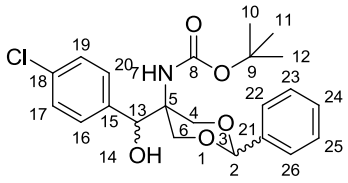
Synthesis of **72**:



To a 50 mL 2-neck rbf fitted with a condenser under N_2, **71** (0.1563 g, 0.4055 mmol, 1.0 eq), NBS (0.2197 g, 1.234 mmol, 3.0 eq), BPO (75% in H_2O, 0.0416 g, 0.113 mmol, 0.32 eq), and chlorobenzene (4 mL) are added. The mixture is heated at 70 °C for 2 h before being cooled to rt. Sat'd $NaHCO_3$ (4 mL) and DCM (2 mL) were added and the layers were separated, followed by the aqueous layer being washed with DCM (4 mL × 3). The

organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography to render a white solid as a mixture of two enantiomers (0.0854 g, 0.263 mmol, 65%). IR (4000-625ν cm^{-1}, NaCl): 3351 (N-H), 3065, 2923, 1764 (C=O), 1725 (C=O), 1686, 1598, 1582, 1450, 1395, 1270, 1179, 1110, 1050, 1027, 710, 689. ^{1}H NMR (600 MHz, $CDCl_3$): δ4.75 (1H, d, *J* = 11.7 Hz, H-5), 4.78 (1H, d, *J* = 11.7 Hz, H-5), 4.78 (1H, d, *J* = 11.9 Hz, H-13), 4.81 (1H, d, *J* = 11.9 Hz, H-13), 5.96 (1H, s – broad, NH-3), 7.45 (2H, t, *J* = 7.7 Hz, H-17/19), 7.54 (2H, t, *J* = 7.8 Hz, H-9.11), 7.59 (1H, tt, *J* = 1.2, 7.4 Hz, H-16), 7.66 (1H, tt, *J* = 1.1, 7.4 Hz, H-10), 7.85 (2H, dd, *J* = 1.3, 8.25 Hz, H-8/12), 7.96 (2H, dd, *J* = 1.3, 8.4 Hz, H-16/20). ^{13}C NMR (150 MHz, $CDCl_3$): δ68.0 (C-5), 68.3 (C-4), 69.2 (C-13), 128.7 (C-15), 128.8 (C-9/11), 129.0 (C-17/19), 129.5(C-8/12), 129.9 (16/20), 132.9 (C-7), 134.0 (C-18), 134.6 (C-10), 157.2 (C-2), 166.1 (C-14), 195.9 (C-6). MS (ESI+): Calc'd for [$C_{18}H_{16}NO_5^+$]: 326.1028; found 326.1016. MP (corrected): 137.1 - 138.7 ºC.

Synthesis of **73**:[181]

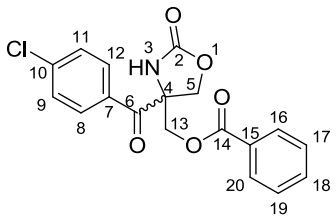


To a 10 mL rbf affixed with a condenser under N_2 was added Mg^o turnings (0.0354 g, 1.46 mmol, 2.4 eq), catalytic I_2 (0.0028 g, 0.011 mmol, 0.018 eq), and THF (0.6 mL). 4-chlorobromobenzene (0.2760 g, 1.442 mmol, 2.3 eq) was dissolved in THF (0.5 mL) and an aliquot (0.15 mL) of this mixture was added to the Mg^o solution and heated to 70 °C to

initiate the reaction. Once the reaction began, the remaining 4-chlorobromobenzene mixture was added over 30 mins. The mixture was heated for a further 60 mins before being cooled to 0 °C. **36** (0.1895 g, 0.6166 mmol, 1.0 eq) dissolved in THF (1 mL) was added over 2 mins, then the entire reaction was allowed to warm to rt while being stirred, with an additional 2 h of stirring. Sat'd NH_4Cl (3mL) was then added and stirred for 1h before separating. The aqueous layer was washed with Et_2O (2 mL × 3), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography to produce, as a mixture of isomers, a pale orange solid (50:50 ratio of isomers, 0.1275 g, 0.3036 mmol, 49%). IR (4000-625ν cm^{-1}, NaCl): 3409 (OH), 3326 (OH), 3067, 2979, 2931, 2868, 1714 (C=O), 1683 (C=O), 1597, 1507, 1491, 1456, 1393, 1368, 1291, 1250, 1200, 1162, 1126, 1091, 1048, 1015, 988, 941, 914, 876, 841, 743, 698. ^{1}H NMR (mixture of *cis*/*trans* isomers in a 1:1 ratio, 600 MHz, $CDCl_3$): δ1.44 (9H, s, H-10/11/12), 1.49 (9H, s, H-10/11/12), 3.80 (1H, dd, *J* = 2.8, 11.7 Hz, H-4/6), 3.87 (1H, d, *J* = 11.6 Hz, H-4/6), 3.95 (1H, d, *J* = 12.1 Hz, H-4/6), 3.98 (1H, d, *J* = 11.9 Hz, H-4/6), 4.00 (1H, d, *J* = 11.7 Hz), 4.06 (1H, d, *J* = 11.1 Hz, H-4/6), 4.36 (1H, s, OH-14), 4.48 (1H, s, NH-7), 4.66-4.72 (2H, m, H-4/6), 4.85 (1H, d, *J* = 9.3 Hz, OH-14), 5.12 (1H, s, NH-7), 5.44 (1H, s, H-2), 5.49 (1H, d, *J* = 5.6 Hz, H-13), 5.62 (1H, s, H-2), 6.38 (1H, d, *J* = 8.6 Hz, H-13), 7.19 (2H, d, *J* = 8.4 Hz), 7.30-7.44 (12H over 2 isomers, m), 7.46 (2H, dd, *J* = 1.9, 8.0 Hz, H-22/26), 7.54 (2H, dd, *J* = 1.5, 8.1 Hz, H-22/26). ^{13}C NMR (mixture of *cis*/*trans* isomers, 150 MHz, $CDCl_3$): δ28.4 (C-10/11/12), 28.4 (C-10/11/12), 54.6 (C-5), 55.9 (C-5), 70.1 (C-9), 70.2 (C-4/6), 70.4 (C-9), 72.1 (C-4/6), 76.3 (C-13), 81.5 (C-13), 101.9 (C-2), 102.3 (C-2),

126.1, 126.4, 128.3, 128.5, 128.5, 128.6, 129.0, 129.4, 129.4, 133.9, 133.9, 137.5, 138.5, 138.5. MS (ESI+): Calc'd for [$C_{22}H_{27}ClNO_5^+$]: 420.1578; found 420.1559.

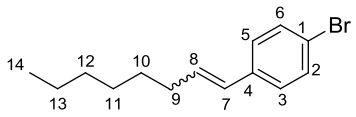
Synthesis of **74**:



To a tapered microwave vial was added **73** (0.0325 g, 0.0722 mmol, 1.0 eq), NBS (0.0560 g, 0.315 mmol, 4.4 eq), and catalytic BPO (75% in H_2O, 0.0118 g, 0.0365 mmol, .047 eq), with $N_{2(g)}$ flushed through the vessel for 20 mins. Chlorobenzene (0.6 mL) was then added and the mixture was heated at 70 °C for 6 h. The reaction was then cooled to rt and to it sat'd $NaHCO_3$ (1 mL) and DCM (0.5 mL) were added. The layers were separated and the aqueous portion was washed with DCM (1 mL × 3). The organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained through silica column chromatography to produce a clear, colourless, amorphous solid (0.0216 g, 0.0600 mmol, 78%). IR (4000-625ν cm^{-1}, NaCl): 3345 (NH), 3070, 2977, 2929, 1763 (C=O), 1725 (C=O), 1690 (C=O), 1588, 1488, 1451, 1397, 1369, 1271, 1178, 1110, 1095, 1071, 1051, 1027, 961, 843, 712. ^{1}H NMR (600 MHz, $CDCl_3$): δ4.67-4.80 (4H, m, H-5/13), 6.48 (1H, s-broad, NH-3), 7.43 (2H, t, *J* = 7.8 Hz, H-17/19), 7.49 (2H, d, *J* = 8.6 Hz, H-9/11), 7.58 (1H, dt, *J* = 0.7, 7.2 Hz, H-18), 7.81 (2H, d, *J* = 8.6 Hz, H-8/12), 7.94 (2H, dd, *J* = 0.7, 7.8 Hz, H-16/20). ^{13}C NMR (150 MHz, $CDCl_3$): δ67.8 (C-4), 68.2 (C-5), 69.2 (C-13), 128.7, 128.8 (C-9/11), 129.8 (C-17/19),

130.0 (C-16/20), 130.4 (C-8/12), 131.4 (C-7), 134.0 (C-18), 141.2 (C-10), 157.5 (C-2), 166.1 (C-14), 195.11 (C-6). MS (ESI+): Calc'd for [$C_{18}H_{15}ClNO_5^+$] 360.0639; found 360.0628.

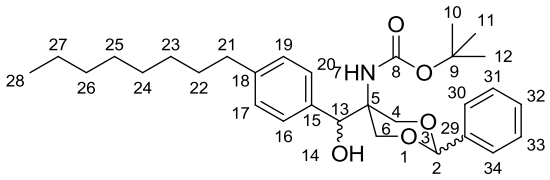
4-(1-oct-1-ene)-bromobenzene (**75**):[171,172]



Triphenylheptylphosphonium bromide (1.2772 g, 2.8937 mmol, 1.05 eq) was added to a 2-neck 25 mL rbf and suspended in THF (3.0 mL). The mixture was cooled to 0 ºC and NaHMDS (1M in THF, 3.24 mL, 3.24 mmol, 1.2 eq) was added slowly. The phosphorylide was stirred for 1 h, then cooled to -78 ºC. 4-bromo benzaldehyde (0.5100 g, 2.756 mmol, 1.0 eq) was dissolved in THF (1.0 mL) in a vial and was added to the mixture dropwise, with subsequent rinsing of the vial with THF (0.5 mL × 2). The solution was allowed to warm to rt, stirring for 2 h. NH_4Cl (10 mL) was added to quench the reaction, followed by extraction of the aqueous later with Et_2O (20 mL). The aqueous layer was diluted with H_2O (10 mL), followed by further rinsing with Et_2O (20 mL × 2). The organic layers were combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to give a clear oil as an approximate 0.1:1 mixture of *Z*/*E* isomers (0.5093 g, 1.906 mmol, 69%). ^{1}H NMR (*Z* isomer, 600 MHz, $CDCl_3$): δ0.87 (3H, t, *J* = 7.2 Hz, H-14),1.21-1.37 (6H, m, H-11/12/13), 1.44 (2H, quintet, *J* = 7.2, 14.5 Hz, H-10), 2.28 (2H, ddd, *J* = 1.9, 7.5, 15.0 Hz, H-9), 5.69 (1H, dt, *J* = 7.3, 11.7 Hz, H-8), 6.32 (1H, d, *J* = 11.7 Hz, H-7), 7.14 (2H, d, *J* = 8.5 Hz, H-2/6), 7.44 (*Z* isomer, 2H, dt, *J* = 2.5, 8.4

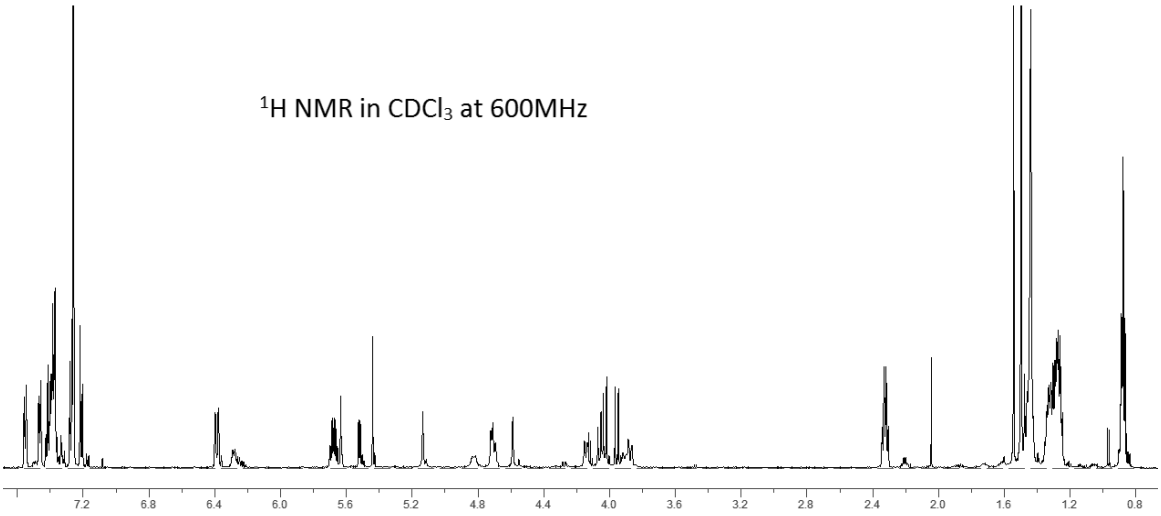
Hz, H-3/5). ^{1}H NMR (*E* isomer, 600 MHz, $CDCl_3$): δ0.87 (3H, t, *J* = 7.2 Hz, H-14),1.21-1.37 (6H, m, H-11/12/13), 1.44 (2H, quintet, *J* = 7.2, 14.5 Hz, H-10), 2.19 (2H, ddd, *J* = 1.1, 7.1, 14.2 Hz, H-9), 6.22 (1H, dt, *J* = 6.8, 15.8 Hz, H-8), 6.30 (1H, d, *J* = 16.0 Hz, H-7), 7.18 (1H, d, *J* = 7.3 Hz, H-2/6), 7.40 (2H, dt, *J* = 2.4, 8.6 Hz, H-3/5).

Synthesis of **76**:

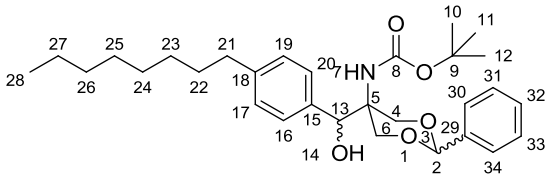


To a 25 mL 2-neck rbf fitted with a condenser under N_2 was added Mg^o turnings (0.0470 g, 1.95 mmol, 2.9 eq), catalytic I_2 (0.0019 g, 0.0075 mmol, 0.01 eq), and THF (1 mL). 4-(1-oct-1-ene)-bromobenzene (**75**, 0.4560 g, 1.707 mmol, 2.5eq), is combined with THF (0.2 mL), then a portion of this reagent (0.15 mL) was added to the mix and the mixture heated with a heating gun to reflux until initiation of the reaction. The reaction was then heated at 60 ºC and the remaining 4-(1-oct-1-ene)-bromobenzene solution was added over 30 mins. The mixture is heated an additional 30 mins, then cooled to 0 ºC. Aldehyde **36** (0.2062 g, 0.6709 mmol, 1.0 eq) in THF (1.3 mL) is added dropwise to the Grignard reagent and the resulting mixture is allowed to slowly warm to rt with stirring. After 2 h of stirring sat'd NH_4Cl (2 mL) is added and stirred an additional 2 h. The mixture is separated and the aqueous layer is washed with Et_2O (2 mL ×3), the organic layers combined, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. Purification was obtained via silica column chromatography to render a white solid as a

mixture as a mixture of isomers (*cis*:*trans* 2:3, (*Z*):(*E*) 9:1, 0.1510 g, 0.3047 mmol, 45%). IR (4000-625ν cm^{-1}, NaCl): 3276 (OH), 3072, 3010, 2955, 2928, 2855, 1678 (C=O), 1558, 1456, 1394, 1364, 1301, 1256, 1178, 1136, 1086, 1048, 1020, 994, 969, 764, 702. MS (ESI+): Calc'd for [$C_{30}H_{42}NO_5^+$]: 496.3063; found 496.3065.



Synthesis of **77**:



To a small vial was added **76** (0.0524 g, 0.106 mmol, 1.0 eq) and THF (0.9 mL), then subsequently flushed with N_2. Pd (10% *w*/*w* loading on C, 0.0080 g, 0.0075 mmol, 0.07 eq) was added, then the vessel was flushed with copious amounts of H_2, with addition of THF as was needed. The reaction was stirred for 21 h at rt and then filtered through celite.

The celite was rinsed with Et_2O, and the organic filtrate dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford **77** as a clear oil (*cis*:*trans* 45:55, 0.0405 g, 0.0813 mmol, 77%). IR (4000-625ν cm^{-1}, NaCl): 3417 (OH), 3345 (OH), 2927, 2855, 1717 (C=O), 1686 (C=O), 1509, 1456, 1391, 1367, 1291, 1249, 1163, 1126, 1102, 1086, 1048, 1020, 988, 783, 745, 697. ^{1}H NMR (as a mixture of isomers, 600 MHz, $CDCl_3$): δ0.89 (6H, t, *J* = 7.0 Hz, H-28), 1.22-1.37 (20H, m, H-23/24/25/26/27), 1.44 (9H, s, H-10/11/12), 1.51 (9H, s, H-10/11/12), 1.57-1.65 (4H, m, H-22), 2.61 (4H, t, *J* = 7.8 Hz, H-21), 3.86 (1H, dd, *J* = 2.3, 11.7 Hz, H-4/6), 3.89 (1H, d, *J* = 11.2 Hz, H-4/6), 3.94 (1H, d, *J* = 12.2 Hz, H-4/6), 4.01 (1H, d, *J* =11.7 Hz, H-4/6), 4.09 (1H, d, *J* = 11.5 Hz, H-4/6), 4.15 (1H, t, *J* = 10.9 Hz, H-4/6), 4.63-4.73 (3H, m, H-4/6/13), 4.79 (1H, d, *J* = 10.0 Hz, OH-14), 5.14 (1H, s, H-2), 5.43 (1H, s, H-2), 5.50 (1H, d, *J* = 5.7 Hz, H-13), 5.63 (1H, s, NH-7), 6.24 (1H, d, *J* = 7.3 Hz, OH-14), 7.14-7.18 (6H, d, H-16/20), 7.33 (2H, d, *J* = 8.0 Hz, H-16/20),7.35-7.44 (6H, m, H-31/32/33), 7.46 (2H, dd, *J* = 1.8, 7.9 Hz, H-30/34), 7.55 (2H, dd, *J* = 1.5, 8.2 Hz, H-30/34). ^{13}C NMR (as a mixture of isomers, 150 MHz, $CDCl_3$): δ14.2 (C-10/11/12), 22.8, 28.4, 28.5, 29.4, 29.5, 29.5, 29.6, 31.5, 31.6, 32.0, 35.8 (C-21), 35.8 (C-21), 54.5 (C-5), 56.0 (C-5), 70.4 (C-4/6), 72.0 (C-4/6), 73.2 (C-13), 76.8 (C-13), 80.6 (C-9), 81.1 (C-9), 101.8 (C-2), 102.1 (C-2), 126.2, 126.5, 126.8, 127.5, 128.3, 128.4, 128.5, 128.5, 129.3, 129.3, 137.0, 137.7, 137.8, 142.8, 142.9, 156.2 (C-8), 157.9 (C-8). MS (ESI+): Calc'd for [$C_{30}H_{44}NO_5^+$] 498.3220; found 498.3225.

Biological Testing for MCF-7 and Melanoma Cells:

Cancerous Cell Lines:

Human mammary gland adenocarcinoma (MCF-7) cell line was purchased from American Type Culture Collection (ATCC), Manassa, VA, USA. These cells were grown and maintained under conditions at 37 ^{o}C, 5% CO_2 and 95% humidity in an incubator. Culturing of cells was carried out using RPMI-1640 media enhanced with 10% fetal Bovine Serum (FBS) and 10 mg/mL gentamycin (Gibco, BRL, VWR, Canada).

Melanoma cells were obtained from American Type Culture Collection (ATCC), Manassa, VA, USA and were grown and maintained under the same conditions as the MCF-7 cells. Culturing was carried out using RPMI-1640 media supplemented with 10% FBS and 10 mg/mL gentamycin.

Non-cancerous Cell Lines:

For comparative studies using normal cells, Normal Human Fibroblasts (NHF) were obtained from Coriell Institute for Medical Research, USA. These cells were also subjected to the same conditions at 37 ^{o}C, 5% CO_2 and 95% humidity in an incubator. These cells were cultured in DMEM media supplemented with 10% FBS, 10 mg/mL gentamycin (Gibco, BRL, VWR, Canada) and 2mM glutamine.

Cell Treatment:

Melanoma and MCF-7 breast cancer cells were grown to approximately 60 to 70% confluence and were treated at 1.0 µM and 5.0 µM of each compound added directly

to the media over various time periods to determine which time period showed significant activity. NHF cells were treated at the same concentrations and time periods to test the safety of these compounds.

Cell Viability Assay:

An MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Sigma Chemical Company, Mississauga, Ontario, Canada) was performed by seeding onto 96-well plates and treating with sphingosine kinase inhibitors over a 72 h period. After treatment, 5mg/mL MTT dye was added directly into the medium in each well and plates were allowed to incubate for 4 h at 37 ^{o}C in the dark. The media was removed gently and replaced with MTT solvent (0.1M HCl in anhydrous isopropanol) and allowed to incubate in the dark at room temperature. The absorbance was then read at 590nm using a spectrophotometer.

References:

1. Darabantu, M.; Plé, G.; Maiereanu, C.; Silaghi-Dumitrescu, I.; Ramondenc, Y.; Mager, S. Synthesis and Stereochemistry of Some 1,3-Oxazolidine Systems Based on TRIS (α,α,α-trimethylolaminomethane) and Related Aminopolyols Skeleton. Part 2: 1-Aza-3,7-dioxabicyclo[3.3.0]octanes. *Tetrahedron* **2000**, *56*, 3799-3816.
2. Darabantu, M.; Plé, G.; Silaghi-Dumitrescu, I.; Maiereanu, C.; Turos, I.; Silberg, I. A.; Mager, S. Synthesis and Stereochemistry of Some 1,3-Oxazolidine Systems Based on TRIS (α,α,α-Trimethylolaminomethane) and Related Aminopolyols Skeleton. Part 1: (Di)spiro-1,3-oxazolidines. *Tetrahedron* **2000**, *56*, 3785-3798.
3. Kim, S.; Lee, H.; Lee, M.; Lee, T. Efficient Synthesis of the Immunosuppressive Agent FTY720. *Synthesis* **2006**, 753-755.
4. Balasubramaniam, S.; Annamalai, S.; Aidhen, I. Weinreb Amide Based New Synthetic Equivalents for Convenient Access to Immunosuppressive Agent FTY720 and Analogues. *Synlett* **2007**, 2841-2846.
5. Weibel, N.; Charbonnière, L.; Ziessel, R. Catalytic Etherification of *N*-protected Tris(hydroxymethyl)aminomethane for the Synthesis of Ligands With C_3 Symmetry. *Tetrahedron Lett.* **2006**, *47*, 1793-1796.
6. Han, J. W.; Hill; C. L. A Coordination Network That Catalyzes O_2-Based Oxidations. *J. Am. Chem. Soc.* **2007**, *129*, 15094-15095.
7. Atkinson, S, J.; Ellis, V. J.; Boyd, S. E.; Brown, C. L. Synthesis and Characterization of Peripherally Functionalized Dendritic Molecules. *New J. Chem.* **2007**, *31*, 155-162.

8. Han, J. W.; Hardcastle, K. I.; Hill, C. L. Redox-Active Coordination Polymers from Esterified Hexavanadate Units and Divalent Metal Cations. *Eur. J. Inorg. Chem.* **2006**, 2598-2603.

9. Ooi, H.; Ishibashi, N.; Iwabuchi, Y.; Ishihara, J.; Hatakeyama S. A Concise Route to (+)-Lactacystin. *J. Org. Chem.* **2004**, *69*, 7765-7768.

10. Forbes, D. C.; Ene, D. G.; Doyle, M. P. Stereoselective Synthesis of Substituted 5-hydroxy-1,3-dioxanes. *Synthesis* **1998**, *6*, 879-882.

11. Kallet, R. H.; Jasmer, R. M.; Luce, J. M.; Lin, L. H.; Marks, J. D. The Treatment of Acidosis in Acute Lung Injury with Tris-Hydroxymethyl Aminomethane (THAM). *Am. J. Respir. Crit. Care Med.* **2000**, *161*, 1149-1153.

12. Brinkman, G. L. The Use of THAM to Prevent Hyperventilation and Acidosis While Breathing Carbon Dioxide. *Am. J. Med. Sci.* **1960**, *239*, 728-731.

13. Osa, Y.; Hikima, Y.; Sato, Y.; Takino, K.; Ida, Y.; Hirono, S.; Nagase, H. Convenient Synthesis of Oxazolidinones by the Use of Halomethyloxirane, Primary Amine, and Carbonate Salt. *J. Org. Chem.* **2005**, *70*, 5737-5740.

14. Crowther, H.L.; McCombie, H. V. The Formation of Tetrahydro-oxazoles from α-hydroxy-β-anilino-αβ-diphenylethane and its Homologues. *J. Chem. Soc. Trans.* **1913**, *13*, 27-31.

15. Stien, D.; Anderson, G. T.; Chase, C. E.; Koh, Y.-H.; Weinreb, S. M. Total Synthesis of the Antitumor Marine Sponge Alkaloid Agelastatin A. *J. Am. Chem. Soc.* **1999**, *121*, 9574-9579.

16. Dyen, M. E.; Swern, D. 2-Oxazolidones. *Chem. Rev.* **1967**. *67*, 197-246.

17. Mercklé, L.; de Andrés-Gómez, A.; Dick, B.; Cox, R. J.; Godfrey, C. R. A. A Fragment-Based Approach to Understanding Inhibition of 1-deoxy-D-xylulose-5-phosphate Reductoisomerase. *ChemBioChem* **2005**, *6*, 1866-1874.

18. Cason, J.; Prout, F. S. The Synthesis of Tris-(hydroxymethyl)-methylnitramine and Other Compounds Related to Tris-(hydroxymethyl)-aminomethane. *J. Am. Chem. Soc.* **1949**, *71*, 1218-1221.

19. Bratulescu, G. An Excellent Procedure for the Synthesis of Oxazolidin-2-ones. *Synthesis* **2007**, 3111-3112.

20. Kubota, Y.; Kodaka, M.; Tomohiro, T.; Okuno, H. Formation of Cyclic Urethanes from Amino Alcohols and Carbon Dioxide Using Phosphorus (III) Reagents and Halogenoalkanes. *J. Chem. Soc. Perkin Trans.* **1993**, *30*, 5-6.

21. Applegath, F.; Franz, R. A. Preparation of Azoles. U.S. Patent 2,857,392, 1958; *Chem. Abstr.* **1959**, *53*, 5286.

22. Xiao, L.-F.; Xu, L.-W.; Xia, C.-G. A Method for the Synthesis of 2-Oxazolidinones and 2-Imidazolidinones from Five-Membered Cyclic Carbonates and β-aminoalcohols or 1,2-Diamines. *Green Chem.* **2007**, *9*, 369-372.

23. Phung, C.; Ulrich, R. M.; Ibrahim, M.; Tighe, N. T. G.; Lieberman D. L.; Pinhas, A. R. The Solvent-Free and Catalyst-Free Conversion of an Aziridine to An Oxazolidinone Using only Carbon Dioxide. *Green Chem.* **2011**, *13*, 3224-3229.

24. Wu, Y.; He, L.-N.; Du, Y.; Wang, J.-Q.; Miao, C.-X.; Li W. Zirconyl Chloride: an Efficient Recyclable Catalyst for Synthesis of 5-aryl-2-oxazolidinones from

Aziridines and CO2 Under Solvent-Free Conditions. *Tetrahedron* **2009**, *65*, 6204-6210.

25. Casarrubios, L.; Pérez, J. A.; Brookhart, M.; Templeton, J. L. Lewis Acid-Catalyzed Synthesis of Aziridines. *J. Org. Chem.* **1996**, *61*, 8358-8359.
26. Kilbas, B.; Balci, M. Palladium-Catalyzed Formation of Oxazolidinones from Biscarbamates: A Mechanistic Study. *Beilstein J. Org. Chem*. **2011**, *7*, 246-253.
27. Mizuno, T.; Takahashi, J.; Ogawa, A. Synthesis of 2-Oxazolidones by Sulfur-Assisted Thiocarboxylation with Carbon Monoxide and Oxidative Cyclization with Molecular Oxygen under Mild Conditions. *Tetrahedron* **2002**, *58*, 7805-7808.
28. Gabriele, B.; Mancuso, R.; Salerno, G.; Costa, M. An Improved Procedure for the Palladium-Catalyzed Oxidative Carbonylation of β-Amino Alcohols to Oxazolidin-2-ones. *J. Org. Chem*. **2003**, *68*, 601-604.
29. Li, P.; Yuan, X.; Wang, S.; Lu, S. A Novel Way to Chiral 2-Oxazolidinones: Selenium-Catalyzed Cyclocarbonylation of 2-Aminoethanols. *Tetrahedron* **2007**, *63*, 12419-12423.
30. Díaz, D. J.; Hylton, K.-G.; McElwee-White, L. Selective Catalytic Oxidative Carbonylation of Amino Alcohols to Ureas. *J. Org. Chem.* **2006**, *71*, 734-738.
31. Pulla, S.; Unnikrishnan, V.; Ramidi, P.; Sullivan, S. Z.; Ghosh, A.; Dallas, J. L.; Munshi, P. Interaction of Substrate and Catalyst During the Formation of Oxazolidinones from 2-Aminoalcohols and Diethyl Carbonate Using Recyclable 1,3-Dichlorodistannoxanes. *J. Mol. Catal. A: Chem.* **2011**, *338*, 33-43.

32. Munshi, P.; Padmanabhan, S.; Sullivan, S. Z.; Mustafa, T. A.; Brezden, A. M.; Ghosh, A. Particulate Science and Technology An Economically Viable Process for the Synthesis of a Chiral Oxazolidinone ((*S*)-4-Benzyl-2-oxazolidinone) from Amino Acid. *Synthesis* **2011**, 37-41.

33. Pyne, S.; Pyne, N. J. Sphingosine 1-Phosphate Signalling in Mammalian Cells. *Biochem. J.* **2000**, *349*, 385-402.

34. Suzuki, S. FTY720: Mechanisms of Action and its Effect on Organ Transplantation (Review). *Transplantation Proc.* **1999**, *31*, 2779-2782.

35. Brickner, S. J.; Barbachyn, M. R.; Hutchinson, D. K. Manninen, P. R. Linezolid (ZYVOX), the First Member of a Completely New Class of Antibacterial Agents for Treatment of Serious Gram-Positive Infections. *J. Med. Chem.* **2008**, *51*, 1981-1990.

36. Grant, S. M.; Clissold, S. P. Fluconazole. A Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in Superficial and Systemic Mycoses. *Drugs* **1990**, *39*, 877-916.

37. Goa, K. L.; Barradell, L. B. Fluconazole. An Update of its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Use in Major Superficial and Systemic Mycoses in Immunocompromised Patients. *Drugs* **1995**, *50*, 658-690.

38. Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry,* 4th ed.; W. H. Freeman, New York, NY, 2005.

39. Vance, D.; Vance, J., Eds. 5th *Biochemistry of Lipids, Lipoproteins and Membranes (5th Edn.)*; Elsevier, Hungary, 2008.

40. Liu, F.; Verin, A. D.; Wang, P.; Day, R.; Wersto, R. P.; Chrest, F. J.; English, D. K.; Garcia, J. G. Differential Regulation of Sphingosine-1-Phosphate- and VEGF-Induced Endothelial Cell Chemotaxis: Involvement of $G_{i\alpha 2}$-Linked Rho Kinase Activity. *Am. J. Respir. Cell Mol. Biol.* **2001**, *24*, 711-719.

41. Pyne, N. J.; Pyne, S. Sphingosine 1-Phosphate and Cancer. *Nat. Rev. Cancer* **2010**, *10*, 489-503.

42. Pyne, S.; Bittman, R.; Pyne, N. J. Sphingosine Kinase Inhibitors and Cancer: Seeking the Golden Sword of Hercules. *Cancer Res.* **2011**, *71*, 6576-6582.

43. Maceyka, M.; Sankala, H.; Hait, N. C.; Le Stunff, H.; Liu, H.; Toman, R.; Collier, C.; Chang, M.; Satin, L. S.; Merrill, Jr., A. H.; Milstien, S.; Spiengel, S. SphK1 and SphK2, Sphingosine Kinase Isoenzymes with Opposing Functions in Sphingolipid Metabolism. *J. Biol. Chem.* **2005**, *280*, 37118-37129.

44. Ogretmen, B.; Hannun, Y. A. Biologically Active Sphingolipids in Cancer Pathogenesis and Treatment. *Nat. Rev. Cancer* **2004**, *4*, 604-616.

45. Shayman, J. A. Sphingolipids. *Kidney Int.* **2000**, *58*, 11-26.

46. Radin, N. S. Killing Tumours by Ceramide-Induced Apoptosis: A Critique of Available Drugs. *Biochem. J.* **2003**, *371*, 243-256.

47. Hanada, K. Serine Palmitoyltransferase, a Key Enzyme of Sphingolipid Metabolism. *Biochim. Biophys. Acta.* **2003**, *1632*, 16-30.

48. French, K. J.; Schrecengost, R. S.; Lee, B. D.; Zhuang, Y.; Smith, S. N.; Eberly, J. L.; Yun, J. K.; Smith, C. D. Discovery and Evaluation of Inhibitors of Human Sphingosine Kinase. *Cancer Res.* **2003**, *63*, 5962-5969.

49. Miyake, Y.; Kozutsumi, Y.; Nakamura, S.; Fujita, T.; Kawasaki, T. Serine Palmitoyltransferase is the Primary Target of a Sphingosine-Like Immunosuppressant, ISP-1/Myriocin. *Biochem. Biophys. Res. Commun.* **1995**, *211*, 396-403.

50. Byun, H.-S.; Lu, X.; Bittman, R. Stereoselective Total Synthesis of Serine Palmitoyl-CoA Transferase Inhibitors. *Synthesis* **2006**, 2447-2474.

51. Kluepfel, D.; Bagli, J.; Baker, H.; Charest, M.-P.; Kudelski, A.; Sehgal, S. N.; Vézina, C. Myriocin, a New Antifungal Antibiotic from Myriococcum Albomyces. *J. Antibiot.* **1972**, *25*, 109-115.

52. Payette, D. R.; Just, G. A Total Synthesis of the Enantiomer of Anhydromyriocin (Anhydrothermozymocidin). *Can. J. Chem.* **1981**, *59*, 269-282.

53. Banfi, L.; Beretta, M. G.; Colombo, L.; Gennari, C.; Scolastico, C. Total Synthesis of (+)-Thermozymocidin (Myriocin) from D-Fructose. *J .Chem. Soc. Chem. Commun.* **1982**, 488-490.

54. Banfi, L.; Beretta, M. G.; Colombo, L.; Gennari, C.; Scolastico, C. 2-Benzoylamino-2-deoxy-2-hydroxymethyl-D-hexono-1,4-lactones: Synthesis from D-fructose and Utilization in the Total Synthesis of Thermozymocidin (Myriocin). *J. Chem. Soc., Perkin Trans. 1* **1983**, 1613-1619.

55. Deloisy, S.; Thang, T. T.; Olesker, A.; Lukacs, G. Synthesis of α-azido Aldehydes. Stereoselective Formal Access to the Immunosuppressant Myriocin. *Tetrahedron Lett.* **1994**, *35*, 4783-4786.

56. Yoshikawa, M.; Yokokawa, Y.; Okuno, Y.; Yagi, N.; Murakami, N. Synthesis of New Immunosuppressive Myriocin Analogs, 2-epi-myriocin, 14-deoxomyriocin, *Z*-14-deoxomyriocin, and Nor-deoxomyriocins: Their Structure-Activity Relationships. *Chem. Pharm. Bull.* **1994**, 42, 2662-2664.

57. Yoshikawa, M.; Yokokawa, Y.; Okuno, Y.; Murakami, N. Total Synthesis of a Novel Immunosuppressant, Myriocin (Thermozymocidin, ISP-I), and Z-Myriocin. *Chem. Pharm. Bull.* **1994**, *42*, 994-996.

58. Sano, S.; Kobayashi, Y.; Kondo, T.; Takebayashi, M.; Maruyama, S.; Fujita, T.; Nagao, Y. Asymmetric Total Synthesis of ISP-I (Myriocin, Thermozymocidin), a Potent Immunosuppressive Principle in the Isaria Sinclairii Metabolite. *Tetrahedron Lett.* **1995**, *36*, 2097-2100.

59. Hatakeyama, S.; Yoshida, M.; Esumi, T.; Iwabuchi, Y.; Irie, H.; Kawamoto, T.; Yamada, H.; Nishizawa, M. A Novel Enantioselective Synthesis of (+)-Myriocin Based on the Chemistry of 1-trimethylsilylbuta-2,3-dienes. *Tetrahedron Lett.* **1997**, *38*, 7887-7890.

60. Oishi, T.; Ando, K.; Chida, N. Stereoselective Total Synthesis of (+)-Myriocin from D-mannose. *Chem. Comm.* **2001**, 1932-1933.

61. Lee, K.-Y.; Oh, C.-Y.; Kim, Y.-H.; Joo, J.-E. Ham, W.-H. Total Synthesis of Myriocin. *Tetrahedron Lett.* **2002**, *43*, 9361-9363.

62. Inai, M.; Goto, T.; Furuta, T.; Wakimoto, T.; Kan, T. Stereocontrolled Total Synthesis of (−)-Myriocin. *Tetrahedron: Asymm.* **2008**, *19*, 2771-2773.

63. Jones, M. C.; Marsden, S. P. Total Synthesis of the Immunosuppressants Myriocin and 2-epi-myriocin. *Org. Lett.* **2008**, *10*, 4125-4128.

64. Yoshikawa, M.; Yokokawa, Y.; Okuno, Y.; Murakami, N. Total Syntheses of Myriocin and Z-Myriocin, Two Potent Immunosuppressants, from 2-deoxy-D-glucose. *Tetrahedron* **1995**, *51*, 6209-6228.

65. Rao, A. V. R.; Gurjar, M. K.; Devi, T. R.; Kumar, K. R. A Formal Synthesis of a Novel Immunosuppressant ISP-1: Stereocontrolled Pd(0) Catalyzed *cis*-hydroxyamination of Carbohydrate Derived Vinyl Epoxide. *Tetrahedron Lett.* **1993**, *34*, 1653-1656.

66. Gonda, J.; Martinková, M.; Raschmanová, J.; Balentová, E. Creation of Quarternary Stereocentres via [3,3]-Sigmatropic Rearrangement of Allylic Thiocyanates. A Synthetic Approach to (+)-Myriocin. *Tetrahedron: Asymm.* **2006**, *17*, 1875-1882.

67. Albert, R.; Hinterding, K.; Brinkmann, V.; Guerini, D.; Müller-Hartwieg, C.; Knecht, H.; Simeon, C.; Streiff, M.; Wagner, T.; Welzenbach, K.; Zécri, F.; Zollinger, M.; Cooke, N.; Francotte, E. Novel Immunomodulator FTY720 is Phosphorylated in Rats and Humans to Form a Single Stereoisomer. Identification, Chemical Proof, and Biological Characterization of the Biologically Active Species and its Enantiomer. *J. Med. Chem.* **2005**, *48*, 5373-5377.

68. Brinkmann, V.; Chen, S.; Feng, L.; Pinschewer, D.; Nikolova, Z.; Hof, R. FTY720 Alters Lymphocyte Homing and Protects Allografts Without Inducing General Immunosuppression. *Transplantation Proc.* **2001**, *33*, 530-531.

69. Adachi, K.; Kohara, T.; Nakao, N.; Arita, M.; Chiba, K.; Mishina, T.; Sasaki, S.; Fujita, T. Design, Synthesis, and Structure-Activity Relationships of 2-substituted-2-amino-1,3-propanediols: Discovery of a Novel Immunosuppressant, FTY720. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 853-856.

70. Fujita, T.; Yoneta, M.; Hirose, R.; Sasaki, S.; Inoue, K.; Kiuchi, M.; Hirase, S.; Adachi, K.; Arita, M.; Chiba, K. Simple Compounds, 2-alkyl-2-amino-1,3-propanediols Have Potent Immunosuppressive Activity. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 847-852.

71. Brinkmann, V. FTY720 (Fingolimod) in Multiple Sclerosis: Therapeutic Effects in the Immune and the Central Nervous System. *Br. J. Pharmacol.* **2009**, *158*, 1173-1182.

72. Haghikia, A.; Gold, R. The Impact of Fingolimod (FTY720) in Neuroimmunologic Diseases. *Am. J. Pathol.* **2010**, *176*, 2599-2601.

73. Liao, A.; Broeg, K.; Fox, T.; Tan, S.-F.; Watters, R.; Shah, M. V.; Zhang, L. Q.; Li, Y.; Ryland, L.; Yang, J.; Aliaga, C.; Dewey, A.; Rogers, A.; Loughran, K.; Hirsch, L.; Jarbadan, N. R.; Baab, K. T.; Liao, J.; Wang, H.-G.; Kester, M.; Desai, D.; Amin, S.; Loughran, Jr. T. P.; Liu, X. Therapeutic Efficacy of FTY720 in a Rat Model of NK-cell Leukemia. *Blood* **2011**, *118*, 2793-2800.

74. Kiuchi, M.; Adachi, K.; Kohara, T.; Minoguchi, M.; Hanano, T.; Aoki, Y.; Mishina, T.; Arita, M.; Nakao, N.; Ohtsuki, M.; Hoshino, Y.; Teshima, K.; Chiba, K.; Sasaki, S.; Fujita, T. Synthesis and Immunosuppressive Activity of 2-

Substituted 2-aminopropane-1,3-diols and 2-aminoethanols. *J. Med. Chem.* **2000**, *43*, 2946-2961.

75. Hale, J. J.; Yan, L.; Neway, W. E.; Hajdu, R.; Bergstrom, J. D.; Milligan, J. A.; Shei, G.-J.; Chrebet, G. L.; Thornton, R. A.; Card, D.; Rosenbach, M.; Rosen, H.; Mandala, S. Synthesis, Stereochemical Determination and Biochemical Characterization of the Enantiomeric Phosphate Esters of the Novel Immunosuppressive Agent FTY720. *Bioorg. Med. Chem.* **2004**, *12*, 4803-4807.

76. Seidel, G.; Laurich, D.; Fürstner, A. Iron-Catalyzed Cross-Coupling Reactions. A Scalable Synthesis of the Immunosuppressive Agent FTY720. *J. Org. Chem.* **2004**, *69*, 3950-3952.

77. Sugiyama, S.; Arai, S.; Kiriyama, M.; Ishii, K. A Convenient Synthesis of Immunosuppressive Agent FTY720 Using the Petasis Reaction. *Chem. Pharm. Bull.* **2005**, *53*, 100-102.

78. Lu, X.; Bittman, R. Enantioselective Synthesis of the Phosphate Esters of the Immunosuppressive Lipid FTY720. *Tetrahedron Lett.* **2006**, *47*, 825-827.

79. Durand, P.; Peralba, P.; Sierra, F.; Renaut, P. A New Efficient Synthesis of the Immunosuppressive Agent FTY-720. *Synthesis* **2000**, 505-506.

80. Kalita, B.; Barua, N.C.; Bezbarua, M.; Bez, G. Synthesis of 2-Nitroalcohols by Regioselective Ring Opening of Epoxides with $MgSO_4$/MeOH/$NaNO_2$ System: A Short Synthesis of Immunosuppressive Agent FTY-720. *Synlett* **2001**, 1411-1414.

81. Högenauer, K.; Hinterding, K.; Nussbaumer, P. S1P Receptor Mediated Activity of FTY720 Phosphate Mimics. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1485-1487.

82. Liu, K. K.-C.; Sakya, S. M.; O'Donnell, C. J.; Flick, A. C.; Li, J. Synthetic Approaches to the 2009 New Drugs. *Bioorg. Med. Chem.* **2011**, *19*, 1136-1154.

83. Valentine, W. J.; Kiss, G. N.; Liu, J.; E, S.; Gotoh, M.; Murakami-Murofushi, K.; Pham, T. C.; Baker, D. L.; Parrill, A. L.; Lu, X.; Sun, C.; Bittman, R.; Pyne, N. J.; Tigyi, G. (*S*)-FTY720-Vinylphosphonate, an Analogue of the Immunosuppressive Agent FTY720, is a Pan-Antagonist of Sphingosine 1-Phosphate GPCR Signaling and Inhibits Autotaxin Activity. *Cell. Signalling* **2010**, *22*, 1543-1553.

84. Tonelli, F.; Lim, K. G.; Loveridge, C.; Long, J.; Pitson, S. M.; Tigyi, G.; Bittman, R.; Pyne, S.; Pyne, N. J. FTY720 and (S)-FTY720 Vinylphosphonate Inhibit Sphingosine Kinase 1 and Promote its Proteasomal Degradation in Human Pulmonary Artery Smooth Muscle, Breast Cancer and Androgen-Independent Prostate Cancer Cells. *Cell. Signalling* **2010**, *22*, 1536-1542.

85. Camp, S. M.; Bittman, R.; Chiang, E. T.; Moreno-Vinasco, L.; Mirzapoiazova, T.; Sammani, S.; Lu, X.; Sun, C.; Harbeck, M.; Roe, M.; Natarajan, V.; Garcia, J. G. N.; Dudek, S. M. Synthetic Analogs of FTY720 [2-Amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol] Differentially Regulate Pulmonary Vascular Permeability in Vivo and in Vitro. *J. Pharmacol. Exp. Ther.* **2009**, *331*, 54-64.

86. Lu, X.; Sun, C.; Valentine, W. J.; Shuyu, E.; Liu, J.; Tigyi, G.; Bittman, R. Chiral Vinylphosphonate and Phosphonate Analogues of the Immunosuppressive Agent FTY720. *J. Org. Chem.* **2009**, *74*, 3192-3195.

87. Hinterding, K.; Cottens, S.; Albert, R.; Zecri, F.; Buehlmayer, P.; Spanka, C.; Brinkmann, V.; Nussbaumer, P.; Ettmayer, P.; Hoegenauer, K.; Gray, N.; Pan, S.

Synthesis of Chiral Analogues of FTY720 and its Phosphate. *Synthesis* **2003**, 1667-1670.

88. Ko, R. Y. Y.; Chu, J. C. K.; Chiu, P. Synthesis of Fluorinated Analogues of the Immunosuppressive Drug FTY720. *Tetrahedron* **2011**, *67*, 2542-2547.

89. Fujita, T.; Matsumoto, N.; Uchida, S.; Kohno, T.; Shimizu, T.; Hirose, R.; Yanada, K.; Kurio, W.; Watabe, K. Antibody Against a Novel, Myriocin (ISP-I)-Based Immunosuppressant, FTY720. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 337-339.

90. Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C.A.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. Alteration of Lymphocyte Trafficking by Sphingosine-1-Phosphate Receptor Agonists. *Science* **2002**, *296*, 346-349.

91. Zhu, R.; Snyder, A. H.; Kharel, Y.; Schaffter, L.; Sun, Q.; Kennedy, P. C.; Lynch, K. R.; Macdonald, T. L. Asymmetric Synthesis of Conformationally Constrained Fingolimod Analogues – Discovery of an Orally Active Sphingosine 1-Phosphate Receptor Type-1 Agonist and Receptor Type-3 Antagonist. *J. Med. Chem.* **2007**, *50*, 6428-6435.

92. Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors. *J. Biol. Chem.* **2002**, *277*, 21453-21457.

93. Kiuchi, M.; Adachi, K.; Tomatsu, A.; Chino, M.; Takeda, S.; Tanaka, Y.; Maeda, Y.; Sato, N.; Mitsutomi, N.; Sugahara, K.; Chiba, K. Asymmetric Synthesis and Biological Evaluation of the Enantiomeric Isomers of the Immunosuppressive FTY720-Phosphate. *Bioorg. Med. Chem.* **2005**, *13*, 425-432.

94. Takeda, S.; Chino, M.; Kiuchi, M.; Adachi, K. Direct Mono-Phosphorylation of 1,3-diols. A Synthesis of FTY720-Phosphate. *Tetrahedron Lett.* **2005**, *46*, 5169-5172.

95. Kiuchi, M.; Adachi, K.; Kohara, T.; Teshima, K.; Masubuchi, Y.; Mishina, T.; Fujita, T. Synthesis and Biological Evaluation of 2,2-disubstituted 2-aminoethanols: Analogues of FTY720. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 101-106.

96. Gutierrez-Lugo, M.-T.; Bewley, C. A. Natural Products, Small Molecules, and Genetics in Tuberculosis Drug Development. *J. Med. Chem.* **2008**, *51*, 2606-2612.

97. Leach, K. L.; Brickner, S. J.; Noe, M. C.; Miller, P. F. Linezolid , the First Oxazolidinone Antibacterial Agent. *Ann. N.Y. Acad. Sci.* **2011**, *1222*, 49-54.

98. Enright, M. C.; Robinson, D. A.; Randle, G.; Feil, E. J.; Grundmann, H.; Spratt, B. G. The Evolutionary History of Methicillin-Resistant Staphylococcus aureus (MRSA). *Proc. Natl. Acad. Sci.* **2002**, *99*, 7687-7692.

99. Barbachyn, M. R.; Ford, C. W. Oxazolidinone Structure-Activity Relationships Leading to Linezolid. *Angew. Chem. Int. Ed.* **2003**, *42*, 2010-2023.

100. Komine, T.; Kojima, A.; Asahina, Y.; Saito, T.; Takano, H.; Shibue, T.; Fukuda, Y. Synthesis and Structure-Activity Relationship Studies of Highly Potent Novel Oxazolidinone Antibacterials. *J. Med. Chem.* **2008**, *51*, 6558-6562.

101. Reck, F.; Zhou, F.; Girardot, M.; Kern, G.; Eyermann, C. J.; Hales, N. J.; Ramsay, R. R.; Gravestock, M. B. Identification of 4-substituted 1,2,3-triazoles as Novel Oxazolidinone Antibacterial Agents with Reduced Activity Against Monoamine Oxidase A. *J. Med. Chem.* **2005**, *48*, 499-506.

102. Auckland, C.; Teare, L.; Cooke, F.; Kaufmann, M. E.; Warner, M.; Jones, G.; Bamford, K.; Ayles, H.; Johnson, A. P. Linezolid-Resistant Enterococci: Report of the First Isolates in the United Kingdom. *J. Antimicrob. Chemother.* **2002**, *50*, 743-746.

103. Tsiodras, S.; Gold, H. S.; Sakoulas, G.; Eliopoulos, G. M.; Wennersten, C.; Venkataraman, L.; Moellering Jr., R. C,; Ferraro, M. J. Linezolid Resistance in a Clinical Isolate of Staphylococcus Aureus. *The Lancet* **2001**, *358*, 207-208.

104. Skripkin, E.; Mcconnell, T. S.; DeVito, J.; Lawrence, L.; Ippolito, J. A.; Duffy, E. M.; Sutcliffe, J.; Franceschi, F. Rχ -01, a New Family of Oxazolidinones That Overcome Ribosome-Based Linezolid Resistance. *Antimicrob. Agents Chemother.* **2008**, *52*, 3550-3557.

105. Schaadt, R.; Sweeney, D.; Shinabarger, D.; Zurenko, G. In Vitro Activity of TR-700, the Active Ingredient of the Antibacterial Prodrug TR-701, a Novel Oxazolidinone Antibacterial Agent. *Antimicrob. Agents Chemother.* **2009**, *53*, 3236-3239.

106. Shaw, K. J.; Poppe, S.; Schaadt, R.; Brown-Driver, V.; Finn, J.; Pillar, C. M.; Shinabarger, D.; Zurenko, G. In Vitro Activity of TR-700, the Antibacterial Moiety

of the Prodrug TR-701, against Linezolid-Resistant Strains. *Antimicrob. Agents Chemother.* **2008**, *52*, 4442-4447.

107. Jones, R. N.; Moet, G. J.; Sader, H. S.; Mendes, R. E.; Castanheira, M. TR-700 In Vitro Activity Against and Resistance Mutation Frequencies Among Gram-Positive Pathogens. *J. Antimicrob. Chemother.* **2009**, *63*, 716- 720.

108. Das, B.; Rudra, S.; Yadav, A.; Ray, A.; Rao, A. V. S. R.; Srinivas, A. S. S. V.; Soni, A.; Saini, S.; Shukla, S.; Pandya, M.; Bhateja, P.; Malhotra, S.; Mathur, T.; Arora, S. K.; Rattan, A.; Mehta, A. Synthesis and SAR of Novel Oxazolidinones: Discovery of Ranbezolid. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4261-4267.

109. Naruganahalli, K. S.; Shirumalla, R. K.; Bansal, V.; Gupta, J. B.; Das, B.; Ray, A. Ranbezolid, a Novel Oxazolidinone Antibacterial: In vivo Characterisation of Monoamine Oxidase Inhibitory Potential in Conscious Rats. *Eur. J. Pharmacol.* **2006**, *545*, 167-172.

110. Hotamisligil, G. S.; Breakefield, X. O. Human Monoamine Oxidase A Gene Determines Levels of Enzyme Activity. *Am. J. Hum. Genet.* **1991**, *49*, 383-392.

111. Finberg, J. P.; Youdim, M. B. Selective MAO A and B Inhibitors: Their Mechanism of Action and Pharmacology. *Neuropharmacol.* **1983**, *22*, 441-446.

112. Como, J. A.; Dismukes, W. E. Oral Azole Drugs as Systemic Antifungal Therapy. *N. Engl. J. Med.* **1994**, *330*, 263-272.

113. Kunze, K. L.; Wienkers, L. C.; Thummel, K. E.; Trager, W. F. Warfarin-Fluconazole I Inhibition of the Human Cytochrome P450-Dependent Metabolism

of Warfarin by Fluconazole: In Vitro Studies. *Drug Metab. Dispos.* **1996**, *24*, 414-421.

114. Ervine, C. M.; Matthew, D. E.; Brennan, B.; Houston, J. B. Comparison of Ketoconazole and Fluconazole as Cytochrome P450 Inhibitors. Use of Steady-State Infusion Approach to Achieve Plasma Concentration-Response Relationships. *Drug Metab. Dispos.* **1996**, *24*, 211-215.
115. Kofla, G.; Ruhnke, M. Voriconazole: Review of a Broad Spectrum Triazole Antifungal Agent. *Expert Opin. Pharmacother.* **2005**, *6*, 1215-1229.
116. Tenter, A. M.; Heckeroth, A. R.; Weiss, L. M. Toxoplasma gondii: From Animals to Humans. *Int. J. Parasitol.* **2000**, *30*, 1217-1258.
117. Alvarado-Esquivel, C.; Liesenfeld, O.; Estrada-Martínez, S.; Félix-Huerta, J. Toxoplasma Gondii Infection in Workers Occupationally Exposed to Raw Meat. *Emerg. Infec. Dis.* **2011**, *9*, 1371-1374.
118. Montoya, J. G.; Liesenfeld, O. Toxoplasmosis. *Lancet* **2004**, *363*, 1965-1976.
119. Eyles, D. E.; Coleman, N. Synergistic Effect of Sulfadiazine and Daraprim Against Experimental Toxoplasmosis in the Mouse. *J. Antimicrob. Chemother.* **1953**, *3*, 483-490.
120. Sheffield, H. G.; Melton, M. L. Effect of Pyrimethamine and Sulfadiazine on the Fine Structure and Multiplication of Toxoplasma Gondii in Cell Cultures. *J. Parasitol.* **1975**, *61*, 704-712.
121. Aspinall, T. V.; Joynson, D. H. M.; Guy, E.; Hyde, J. E.; Sims, P. F. G. The Molecular Basis of Sulfonamide Resistance in Toxoplasma Gondii and

Implications for the Clinical Management of Toxoplasmosis. *J. Infec. Dis.* **2002**, *185*, 1637-1643.

122. Drews, J. Drug Discovery: A Historical Perspective. *Science* **2000**, *287*, 1960-1964.

123. Kazanjian, P.; Armstrong, W.; Hossler, P. A.; Burman, W.; Richardson, J.; Lee, C.-H.; Crane, L.; Katz, J.; Meshnick, S. R. Pneumocystis Carinii Mutations Are Associated with Duration of Sulfa or Sulfone Prophylaxis Exposure in AIDS Patients. *J. Infec. Dis.* **2000**, *182*, 551-557.

124. Simpson, E. R.; Clyne, C.; Rubin, G.; Boon, W. C.; Robertson, K.; Britt, K.; Speed, C. Jones, M. Aromatase – A Brief Overview. *Annu. Rev. Physiol.* **2002**, *64*, 93-127.

125. Meinhardt, U.; Mullis, P. E. The Aromatase Cytochrome P-450 and its Clinical Impact. *Horm. Res.* **2002**, *57*, 145-152.

126. McNulty, J.; Nair, J. J.; Vurgun, N.; Difrancesco, B. R.; Brown, C. E.; Tsoi, B.; Crankshaw, D. J.; Holloway, A. C. Discovery of a Novel Class of Aldol-Derived 1,2,3-triazoles: Potent and Selective Inhibitors of Human Cytochrome P450 19A1 (Aromatase). *Bioorg. Med. Chem. Lett.* **2012**, *22*, 718-722.

127. Jordan, V. C. Tamoxifen (ICI46, 474) as a Targeted Therapy to Treat and Prevent Breast Cancer. *Br. J. Pharmacol.* **2006**, *147 (S1)*, S269-S276.

128. McDonnell, D. P.; Wardell, S. E. The Molecular Mechanisms Underlying the Pharmacological Actions of ER Modulators: Implications for New Drug Discovery in Breast Cancer. *Curr. Opin. Pharmacol.* **2010**, *10*, 620-628.

129. Chen, S.; Masri, S.; Wang, X.; Phung, S.; Yuan, Y.-C.; Wu, X. What Do We Know About the Mechanisms of Aromatase Inhibitor Resistance?. *J. Steroid Biochem. Mol. Biol.* **2006**, *102*, 232-240.

130. Schmidt, U.; Riedl, B. The Total Synthesis of Antrimycin D_v; III:[1] Construction of the Protected Hexapeptide and Deprotection. *Synthesis* **1993**, 815-818.

131. Calzavara, J.; McNulty, J. A Convergent Synthesis of the Immunosuppressant FTY720 Employing Aqueous Wittig Chemistry. *Tetrahedron Lett.* **2011**, *52*, 5672-5675.

132. Hanessian, S.; Plessas, N. R. The Reaction of O-Benzylidene Sugars with N-Bromosuccinimide. II. Scope and Synthetic Utility in the Methyl 4,6-O-Benzylidenehexopyranoside Series. *J.Org. Chem.* **1969**, *34*, 1035-1044.

133. Hanessian, S.; Plessas, N. R. The Reaction of O-Benzylidene Sugars with N-Bromosuccinimide. IV. Neighboring-Group Effects and Rearrangements. *J. Org. Chem.* **1969**, *34*, 1053-1058.

134. Foglia, T.A; Swern, D. 2-Oxazolidone Formation by Pyrolysis of β-Iodocarbamates. A Stereoselective Reaction. *J. Org. Chem.* **1969**, *34*, 1680-1684.

135. Heathcock, C.; Hassner, A. Formation of 2-Oxazolidones from β-iodourethanes. *Angew. Chem. Int. Ed.* **1963**, *2*, 1963-1963.

136. Diamond, S. Double-Blind Study of Metaxalone. Use as Skeletal-Muscle Relaxant. *J. Am. Med. Assoc.* **1975**, *18*, 433-440.

137. Vigroux, A.; Bergon, M.; Zedde, C. Cyclization-Activated Prodrugs: N-(Substituted 2-hydroxyphenyl and 2-hydroxypropyl)carbamates Based on Ring-

Opened Derivatives of Active Benzoxazolones and Oxazolidinones as Mutual Prodrugs of Acetaminophen. *J. Med. Chem.* **1995**, *38*, 3983-3994.

138. Perez, M.; Pauwels, P. J.; Fourrier, C.; Chopin, P.; Valentin, J.-P.; John, G. W.; Halazy, S. Dimerization of Sumatriptan as an Efficient Way to Design a Potent, Centrally and Orally Active 5-HT1B Agonist. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 675-680.

139. Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis, 4th Ed.* John Wiley & Sons, Inc., New Jersey, USA, 2007.

140. Ghosh, A.; Sieser, J. E.; Caron, S.; Couturier, M.; Dupont-Gaudet, K.; Girardin, M. Cu-Catalyzed N-Arylation of Oxazolidinones: an Efficient Synthesis of the κ-opioid receptor Agonist CJ-15161. *J. Org. Chem.* **2006**, *71*, 1258-1261.

141. Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. A General and Efficient Copper Catalyst for the Amidation of Aryl Halides and the N -Arylation of Nitrogen Heterocycles. *J. Am. Chem. Soc.* **2001**, *123*, 7727-7729.

142. Mallesham, B.; Rajesh, B. M.; Reddy, P. R.; Srinivas, D.; Trehan, S. Highly Efficient CuI-Catalyzed Coupling of Aryl Bromides with Oxazolidinones Using Buchwald's Protocol: A Short Route to Linezolid and Toloxatone. *Org. Lett.* **2003**, *5*, 963-965.

143. Cacchi, S.; Fabrizi, G.; Goggiamani, A.; Zappia, G. 3-Aryl-2-Oxazolidinones Through the Palladium-Catalyzed N-Arylation of 2-Oxazolidinones. *Org. Lett.* **2003**, *3*, 2539-2541.

144. Ghosh, A.; Sieser, J. E.; Riou, M.; Cai, W.; Rivera-Ruiz, L. Palladium-Catalyzed Synthesis of N-Aryloxazolidinones from Aryl Chlorides. *Org. Lett.* **2003**, *5*, 2207-2210.

145. Ullah, E.; McNulty, J.; Kennedy, C.; Robertson, A. One Step Entry to P,O- and P,N-Type Heterocyclic Tertiary Phosphine Ligands and Application in Suzuki-Miyaura Cross-Coupling Reactions. *Org. Biomol. Chem.* **2011**, *9*, 4421-4424.

146. McNulty, J.; Krishnamoorthy, V.; Robertson, A. Direct Formation of Esters and Amides from Carboxylic Acids Using Diethyl Chlorophosphate in Pyridine. *Tetrahedron Lett.* **2008**, *49*, 6344-6347.

147. Neises, B.; Steglich, W. Simple Method for the Esterification of Carboxylic Acids. *Angew. Chem. Int. Ed.* **1978**, *17*, 522-524.

148. Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K. A Facile Cleavage of Benzylidene Acetals with Diisobutylaluminum Hydride. *Chem. Lett.* **1983**, 1593-1596.

149. Huisgen, R. 1,3-Dipolar Cycloadditions. Past and Future. *Angew. Chem. Int. Ed.* **1963**, *2*, 565-596.

150. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

151. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper (I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.

152. Phillips, O. A.; Udo, E. E.; Samuel, S. M. Synthesis and Structure – Antibacterial Activity of Triazolyl Oxazolidinones Containing Long Chain Acyl Moiety. *Eur.J. Med. Chem.* **2008**, *43*, 1095-1104.

153. Ebner, D. C.; Culhane, J. C.; Winkelman, T. N.; Haustein, M. D.; Ditty, J. L.; Ippoliti, J. T. Synthesis of Novel Oxazolidinone Antimicrobial Agents. *Bioorg. Med. Chem.* **2008**, *16*, 2651-2656.

154. Li, Z.; Bittman, R. Synthesis and Spectral Properties of Cholesterol- and FTY720-Containing Boron Dipyrromethene Dyes. *J. Org. Chem.* **2007**, *72*, 8376-8382.

155. Kuijpers, B. H. M.; Dijkmans, G. C. T.; Groothuys, S.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; van Delft, F. L.; Rutjes, F. P. J. T. Copper(I)-Mediated Synthesis of Trisubstituted 1,2,3-Triazoles. *Synlett* **2005**, 3059-3062.

156. McNulty, J.; Das, P.; McLeod, D. Microwave-Assisted, Aqueous Wittig Reactions: Organic-Solvent- and Protecting-Group-Free Chemoselective Synthesis of Functionalized Alkenes. *Chem. Eur. J.* **2010**, *16*, 6756-6760.

157. Maryanoff, B. E.; Reitz, A. B. The Wittig Olefination Reaction and Modifications Involving Phosphoryl-Stabilized Carbanions. Stereochemistry, Mechanism, and Selected Synthetic Aspects. *Chem. Rev.* **1989**, *89*, 863-927.

158. Schlosser, M.; Christmann, K. F. Trans-Selective Olefin Syntheses. *Angew. Chem. Int. Ed.* **1966**, *5*, 126.

159. Peterson, D. J. Carbonyl Olefination Reaction Using Silyl-Substituted Organometallic Compounds. *J. Org. Chem.* **1968**, *33*, 780-784.

160. Julia, M.; Paris, J.-M. Syntheses a L'aide de Sulfones $V^{(+)}$ – Methode de Syntheses Generale de Doubles Liaisons. *Tetrahedron Lett.***1973**, *4*, 4833-4836.

161. McNulty, J.; Das, P. Highly Stereoselective and General Synthesis of (E)-Stilbenes and Alkenes by Means of an Aqueous Wittig Reaction. *Eur. J. Org. Chem.* **2009**, *2009*, 4031-4035.

162. McNulty, J.; Das, P. Aqueous Wittig Reactions of Semi-Stabilized Ylides. A Straightforward Synthesis of 1,3-dienes and 1,3,5-trienes. *Tetrahedron Lett.* **2009**, *50*, 5737-5740.

163. McNulty, J.; McLeod, D. An Iterative Approach Toward the Synthesis of Discrete Oligomeric p-Phenylene Vinylene Organic Dyes Employing Aqueous Wittig Chemistry. *Tetrahedron Lett.* **2011**, *52*, 5467-5470.

164. Loim, N. M.; Kelbyscheva, E. S. Synthesis of Dendrimers with Terminal Formyl Groups. *Russ. Chem. Bull., Int. Ed.* **2004**, *53*, 2080-2085.

165. Hanson, M. A.; Roth, C. B.; Jo, E.; Griffith, M. T.; Scott, .; Reinhart, G.; Desale, H.; Clemons, B.; Cahalan, S. M.; Schuerer, S. C.; Sanna, M. G.; Han, G. W.; Kuhn, P.; Rosen H.; Stevens, R. C. Crystal Structure of a Lipid G Protein-Coupled Receptor. *Science* **2012**, *335*, 851-855.

166. Foster, C. A.; Howard, L. M.; Schweitzer, A.; Persohn, E.; Hiestand, P. C.; Balatoni, B.; Reuschel, R.; Beerli, C.; Schwartz, M.; Billich A. Brain Penetration of the Oral Immunomodulatory Drug FTY720 and its Phosphorylation in the Central Nervous System During Experimental Autoimmune Encephalomyelitis:

Consequences for Mode of Action in Multiple Sclerosis. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 469-475.

167. Brinkmann, V.; Lynch, K. R. FTY720: Targeting G-Protein-Coupled Receptors for Sphingosine 1-Phosphate in Transplantation and Autoimmunity. *Curr. Op. Immunol.* **2002**, *14*, 569-575.

168. Guzzo, A. C.; Berger, R. G.; deCatanzaro, D. Excretion and Binding of Tritium-Labelled Oestradiol in Mice (Mus musculus): Implications for the Bruce Effect. *Reproduction* **2010**, *139*, 255-263.

169. Lewis, G. N.; Magel, T. T.; Lipkin, D. The Absorption and Re-emission of Light by Cis- and Trans-Stilbenes and the Efficiency of their Photochemical Isomerization. *J. Am. Chem. Soc.* **1940**, *62*, 2973-2980.

170. Orlandi, G.; Siebrand, W. Model for the Direct Photo-Isomerization of Stilbene. *Chem. Phys. Lett.* **1975**, *30*, 352-354.

171. Brown, J. H.; Marvel, C. S. Hexaalkylphenylethanes. IV. Preparation of Some Alkylbromobenzenes. *J. Am. Chem. Soc.* **1937**, *59*, 1176-1178.

172. Franksand, S.; Hartley, F. R. Preparation and Properties of Tertiary p-Alkylarylphosphines Containing Straight-Chain Alkyl Groups. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2233-2237.

173. Tamao, K.; Sumitani, K.; Kumada, M. Selective Carbon-Carbon Bond Formation by Cross-Coupling of Grignard Reagents with Organic Halides. Catalysis by Nickel-Phosphine Complexes. *J. Am. Chem. Soc.* **1972**, *94*, 4374-4376.

174. McNulty, J.; Wilson, J.; Rochon, A. C. Regiocontrol in the Oxidative Radical Fragmentation of Benzilidene Acetals and its Mechanistic Implications. *J. Org. Chem.* **2004**, *69*, 563-565.

175. McNulty, J.; Nair, J. J.; Griffin, C.; Pandey, S. Synthesis and Biological Evaluation of Fully Functionalized Seco-Pancratistatin Analogues. *J. Nat. Prod.* **2008**, *71*, 357-363.

176. Fernández, I.; Muñoz, L. Synthesis of Enantiomerically Pure (+)- and (−)-Protected 5-aminomethyl-1,3-oxazolidin-2-one Derivatives from Allylamine and Carbon Dioxide. *Tetrahedron: Asymm.* **2006**, *17*, 2548-2557.

177. Morales-Rios, M. S.; Espiñeira, J.; Joseph-Nathan, P. 13C NMR Spectroscopy of Indole Derivatives. *Mag. Reson.Chem.* **1987**, *25*, 377-395.

178. Mallesham, B.; Rajesh, B. M.; Reddy, P. R.; Srinivas, D.; Trehan, S. Highly Efficient CuI-Catalyzed Coupling of Aryl Bromides with Oxazolidinones Using Buchwald's Protocol: A Short Route to Linezolid and Toloxatone. *Org. Let.* **2003**, *5*, 963-965.

179. Balakumar, V.; Aravind, A.; Baskaran, S. A Highly Regio- and Chemoselective Reductive Cleavage of Benzylidene Acetals with EtAlCl2-Et3SiH. *Synlett* **2004**, 647-650.

180. Asao, N.; Shimada, T.; Yamamoto, Y. Chelation Control Through the Coordination of an Olefinic π-bond to Lewis Acid. *Tetrahedron Lett.* **2000**, *41*, 9533-9536.

181. Schiemenz, G. P. Diphenyl-p-bromophenylphosphine. *Org. Synth.* **1973**, *Coll. Vol. 5*, 496-499.