ADDRESSING ANTIBIOTIC RESISTANCE: THE DISCOVERY OF NOVEL KETOLIDE ANTIBIOTICS THROUGH STRUCTURE-BASED DESIGN AND IN SITU CLICK CHEMISTRY

A Dissertation

Submitted to the

Temple University Graduate Board

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

by

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December 2016

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ABSTRACT

Antibiotic resistance has become and will continue to be a major medical issue of the 21st century. If not addressed, the potential for a post-antibiotic era could become a reality, one that the world has not been familiar with since the early 1900's. Multidrug-resistant hospital-acquired bacterial infections already account for close to 2 million cases and 23,000 deaths in the United States, along with 20 billion dollars of additional medical spending each year. The CDC released a report in 2013 regarding the seriousness of antibiotic resistance and providing a snapshot of costs and mortality rates of the most serious antibiotic resistant bacteria, which includes 17 drug resistant bacteria, such as carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *Enterococcus* and *Staphylococcus aureus*, and multidrug-resistant *Acinetobacter* and *Pseudomonas aeruginosa*. The development of antibiotic resistance is part of bacteria's normal evolutionary process and thus impossible to completely stop. To ensure a future where resistant bacteria do not run rampant throughout society, there is a great need for new antibiotics and accordingly, methods to facilitate their discovery

Macrolides are a class of antibiotics that target the bacterial ribosome. Since their discovery in the 1950's medicinal chemistry has created semi-synthetic analogues of natural product macrolides to address poor pharmacokinetics and resistance. Modern X-Ray crystallography has allowed the chemist access to high resolution images of the bacterial ribosome bound to antibiotics including macrolides which has ushered in an era of structure-based design of novel antibiotics. These crystal structures suggest that the C-4 methyl group of third generation ketolide antibiotic telithromycin can sterically clash

with a mutated rRNA residue causing loss of binding and providing a structural basis for resistance.

The Andrade lab hypothesized that the replacement of this methyl group with hydrogen would alleviate the steric clash and allow the antibiotic to retain activity. To this end, the Andrade lab set out on a synthetic program to synthesize four desmethyl analogues of telithromycin by total synthesis that would directly test the steric clash hypothesis and also provide structure-activity relationships about these methyl groups which have not been assessed in the past. Following will contain highlights of the total synthesis of (–)-4,8,10-didesmethyl telithromycin, (–)-4,10-didesmethyl telithromycin, and (–)-4,8-desmethyl telithromycin and my journey toward the total synthesis of (–)-4-desmethyl telithromycin

Traditional combinatorial chemistry uses chemical synthesis to make all possible molecules from various fragments. These molecules then need to be purified, characterized, and tested against the biological target of interest. While high-throughput assay technologies (i.e., automation) has streamlined this process to some extent, the process remains expensive when considering the costs of labor, reagents, and solvent to synthesize, purify, and characterize all library members. Unlike traditional combinatorial chemistry, *in situ* click chemistry directly employs the macromolecular target to template and synthesize its own inhibitor. *In situ* click chemistry makes use of the Huisgen cycloaddition of alkyne and azides to form 1,2,3-triazoles, which normally reacts slowly at room temperature in the absence of a catalyst. If azide and alkyne pairs can come together in a target binding pocket the activation energy of the reaction can be lowered and products detected by LC-MS. Compounds found in this way generally show tighter binding than the individual fragments.

Described in the second part of this dissertation is the development of the first *in situ* click methodology targeting the bacterial ribosome. Using the triazole containing third generation ketolide solithromycin as a template we were able to successfully show that *in situ* click chemistry was able to predict the tightest binding compounds.

DEDICATION

This dissertation is dedicated to my wife, Sue,

whose encouragement and support has been instrumental

in my success

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Rodrigo B. Andrade for his constant support. His enthusiasm for synthetic organic chemistry and is unrelenting excitement and support of my projects provided motivation to go the extra mile to accomplish my goals. One of my favorite memories is listening to Rod's seminars given for the new batch of graduate students. Although I was already working on the projects being described, his ability to tell a story always left me with a renewed excitement for our projects. I want to thank Rod for his letters of support which have helped me secure a Co-Op position with Glaxo Smith Kline and a fulltime positon with my current employer Abzena.

I would like to thank Dr. Christian Schafmeister for serving as chair of my graduate committee. My experience in his first year physical organic chemistry class is still one of my favorite learning experiences I have had at Temple University. I would like to thank Dr. William M. Wuest for serving on my graduate committee. I appreciate all Bill has done to elevate the chemistry department since he joined the faculty. His active recruitment of top students, starting a mechanism club, and participating in the annual Swern cup have all had a positive impact on department. I am also grateful for the letters of support he provided for my GSK internship and current position at Abzena. I would like to thank Dr. Kevin C. Cannon for his willingness to serve as my external committee member. I would like to give a special thanks to Dr. Charles DeBrosse, for all the time he took to train me in various 2-D NMR methods and for his work on our various projects. I would like to thank Dr. Barry Cooperman for his suggestions during my work with *in situ* click chemistry, as they were instrumental in the success of the project.

I would like to give a special thanks to the original members of the macrolide project, Dr. Tapas Paul, Dr. Venkata Velvadapu, and Dr. Bharat Wagh. The training and advice I received from them in my first years were instrumental to my success as an organic chemist. I would like to thank Christiana Teijaro for her work on the MIC experiments and Samer Daher for help with compound characterization. I would like to thank the rest of the macrolide team, Miseon Lee and Xiao Jin for their continued work and dedication to the macrolide project. I would like to thank the rest of my former and current lab members, Gopal Sirasani Justin Kaplan, Senzhi Zhao, Praveen Kokkonda, Vijay Chatare, Chary Munagala, and Keaon Brown for all the great memories in and out of the lab. I would also like to thank each and every person I have had the pleasure to interact with during my time at Temple University.

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A	Adenine
Å	Angstrom
Ac	Acetyl
ACN	Acetonitrile
Ac_2O	Acetic anhydride
AcOH	Acetic acid
AgOTf	Silver Triflate
aq.	Aqueous
Ar	Aryl
$BF_3 \bullet Et_2O$	Boron trifluoromethane
Bn	Benzyl
BnOH	Benzyl alcohol
Bu_2BOTf	Dibutylboron triflate
Bu_3SnH	Tributyltin hydride
C	Cytosine
Calc'd	Calculated
CBr_4	Tetrabromomethane
CDI	Carbonyldiimidazole

$CeCl_3$	Cerium (III) chloride
CH_2Cl_2	dichloromethane
$Cl_3C_6H_2COCl$	2,4,6-trichlorobenzoyl chloride
$(COCl)_2$	oxalyl chloride
$CrCl_2$	Chromium (II) Chloride
CSA	Camphor sulphonic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbonyldiimidazole
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethylazodicarboxylate
DIBAL-H	Diisobutylaluminum hydride
DIPT	Diisopropyl tartrate
DMAP	*N*,*N*-4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess–Martin periodinane
DMSO	Dimethyl sulfoxide
dr	Diastereomeric ratio

DTBMP	2,6-di-*tert*-butyl-3-methyl pyridine
Equiv.	Equivalent
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
ESI	Electrospray ionization
FAB	Fast atomic bombardment
H_2	Hydrogen
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HF	Hydrofluoric acid
HG	Hoveyda–Grubbs
HRMS	High resolution mass spectrometry
I_2	Iodine
iPr_2NEt	*N*,*N*-diisopropylethylamine (Hünig's base)
IR	Infrared spectroscopy
K_d	Dissociation constant

KHMDS	Potassium bis(trimethylsilyl)amide
LDA	Lithium diisopropylamine
$LiAlH_4$	Lithium aluminum hydride
LiOH	Lithium hydroxide
Me	Methyl
Me_2S	Dimethyl sulfide
MeOH	Methanol
MeOTf	Methyl triflate
mg	Milligram
MHz	Mega hertz
mL	Milliliter(s)
mmol	Millimole(s)
MsCl	Methanesulfonyl chloride
MeI	Methyl iodide
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
n-BuLi	*n*-Butyl lithium
NaBH4	Sodium borohydride
NaH	Sodium hydride

NaOMe	Sodium methoxide
$NiCl_2$	Nickel (II) Chloride
NMR	Nuclear magnetic resonance
p-TsOH	*para*-Toluene sulfonic acid
Pd/C	Palladium on carbon
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium (0)
Ph	Phenyl
PhH	Benzene
PhMe	Toluene
Ph_3CCO_2H	Triphenylacetic acid
PivOH	Pivalic acid
PMBCl	*p*-Methoxybenzyl chloride
PPh_3	Triphenylphosphine
PPTS	Pyridinium *p*-toluene sulfonic acid
Pyr	Pyridine
rt	Room temperature
SAD	Sharpless Asymmetric Dihydroxylation
SAE	Sharpless Asymmetric Epoxidation

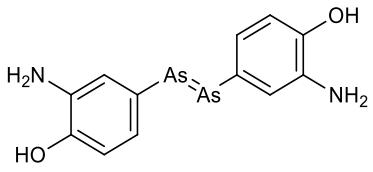
T	Thymine
TAS-F	Tris(dimethylamino)sulfonium difluoromethylsilicate
TBAF	*n*-tetrabutylammonium fluoride
TBSCl	*t*-Butyldimethylsilyl chloride
TBSOTf	*t*-Butyldimethylsilyl triflate
t-BuOK	Potassium *t*-butoxide
t-BuOOH	*t*-butylhydroperoxide
TESCl	Triethylsilyl chloride
TESOTf	Triethylsilyl trifluoromethanesulfonate
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
THF	Tetrahydrofuran
$Ti(OEt)_4$	Titanium (IV) ethoxide
TMSCl	Trimethylsilyl chloride
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
U	Uracil

CHAPTER 1: THIRD-GENERATION MACROLIDE ANTIBIOTICS AND THE DESMETHYL HYPOTHESIS TO ADDRESS ANTIBIOTIC RESISTANCE

1.1 Introduction

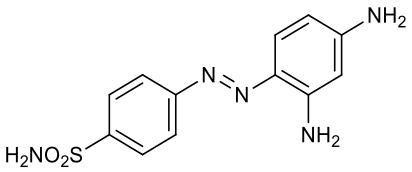
The beginning of our modern antibiotic era is generally associated with Paul Ehrlich and his work with organic dyes. The observation that synthetic dyes could stain certain microbes but not others led him to the idea that synthetic compounds could be used to specifically target disease in the human body, which he described as a "magic bullet". In 1904, Ehrlich began the first modern screening and compound optimization program where he and his co-workers synthesized hundreds of organoarsenic compounds to find a cure for syphilis. This massive undertaking resulted in the discovery of salvarsan (**1.1**) in 1909, which remained the main treatment for syphilis until the advent of penicillin in the 1940s (Figure 1.1).[1]

Figure 1.1. Structures of salvarsan, prontosil and sulfanilamide.



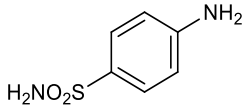
1.1

Salvarsan



1.2

Prontosil



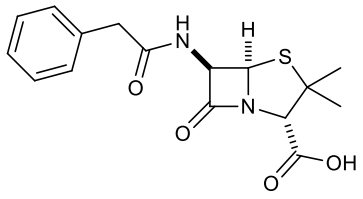
1.3

Sulfanilamide

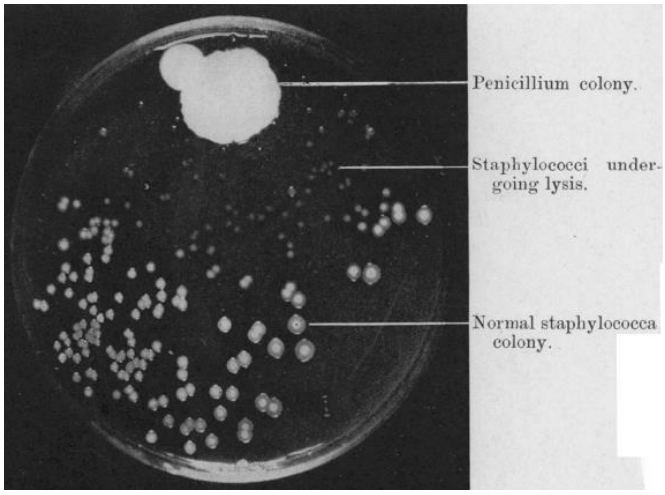
The progression of antibiotics continued with the discovery of sulfonamide or sulfa drugs, such as prontosil (**1.2**) by Gerhard Domagk, which ultimately led to a Nobel Prize in 1939 (Figure 1.1).[2] This antibiotic was marketed by Bayer and was found to effectively treat a wide range of bacterial infections, especially *Streptococci*.[2] Unfortunately for Bayer,

it was found that prontosil was not actually the active antibacterial agent, but instead was a metabolite thereof, sulfanilamide (**1.3**), which had been discovered and patented in the early 1900s (Figure 1.1). This spawned a wave of synthetic analogues of **1.3** which provided a major source of antibiotic treatment until penicillin in the 1940s.[3]

Figure 1.2. Structure of mold derived Penicillin G and Fleming's culture plate showing the effect of *Staphylococci* growth in the vicinity of a *Penicillium* colony.[4]



1.4
Penicillin G

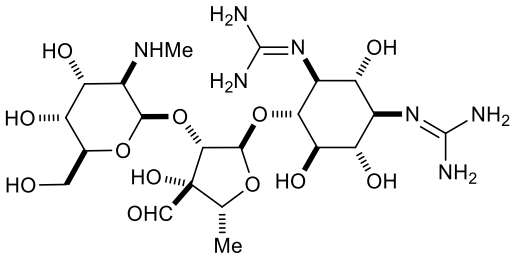


The next significant breakthrough in antibiotics came in 1928 when Sir Alexander Fleming discovered the effects of penicillin (**1.4**). He inadvertently discovered penicillin when he found that the growth of *Staphylococci* on a discarded petri dish was inhibited by a mold that had contaminated it (Figure 1.2).[4] Getting penicillin out of the laboratory setting and into the clinic turned out to be much more difficult than the initial discovery. Fleming worked until 1940 on methods to isolate and purify penicillin but was never successful. Fortunately, two scientists from Oxford, Howard Florey and Ernst Chain, published a paper describing a successful purification technique for penicillin that would

allow clinical testing and eventual mass production.[5] Penicillin would become one of the most successful antibiotics in history.

On the heels of penicillin's success came the discovery of streptomycin (**1.5**) in 1943 by Selman Waksman at Rutgers University, which was isolated from *Streptomyces griseus*, a soil bacterium from the phylum *Actinomycetes*.[6] Streptomycin was found to be active against Gram-negative bacteria, most importantly *Mycobacterium tuberculosis,* which causes tuberculosis. By 1946, streptomycin had passed through enough clinical trials to be put into general patient use, becoming the first real cure for tuberculosis. The success of both penicillin and streptomycin would turn the focus of antibiotic research from synthetic compounds to natural products. *Actinomycetes* would be found to have a wealth of antibiotic compounds and the collection and systematic screening of soil samples from across the globe would spawn the golden age of antibiotic research and development.[5-8]

Figure 1.3. Structure of streptomycin (**1.5**).



1.5
Streptomycin

1.2 Antibiotic Classes and Their Mechanism of Action

From the time of discovery of salvarsan, penicillin, and streptomycin until the 1960s, hundreds of novel antibiotics comprising of more than 20 distinct classes were

discovered. After the 1960s, only four truly unique classes of antibiotics were introduced with the majority of new antibiotics being derivatives of known compounds produced by medicinal chemistry efforts. Even though bacteria contain over 200 conserved essential proteins that could be potential targets for antibiotics, all clinically relevant antibiotics act on just four pathways that can be grouped as follows: (1) those affecting transcription, which targets RNA polymerase[9]; (2) DNA replication which targets DNA topoisomerase[10,11]; (3) cell wall synthesis[12,13], which targets peptidoglycan; and (4) protein synthesis, which targets the bacterial ribosome[14]. These four unique mechanisms are discussed below, along with antibiotic classes that operate accordingly.

1.2.1 Antibiotics Affecting Transcription by Targeting Bacterial RNA Polymerase

Bacterial DNA-dependent-RNA polymerases are made up of four subunits with a molecular mass of approximately 400 kDa. They are responsible for transcribing RNA from DNA in a process consisting of three steps: (1) DNA binding and RNA chain initiation; (2) RNA chain elongation; and, (3) termination.[9,15] These enzymes represent excellent antibacterial targets because they are highly conserved between different species of bacteria, and the enzyme and process for transcription differ from that of Eukaryotes.

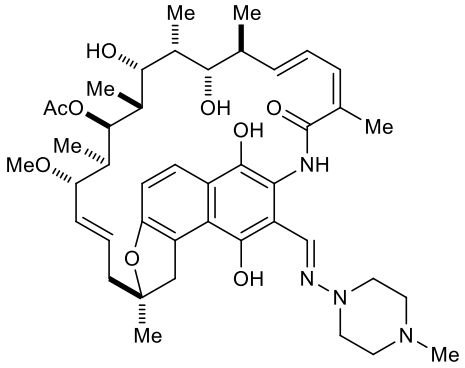
Rifamycins are a class of antibiotics isolated from soil bacterium *Steptomyces spectabilis* from the phylum *Actinomycetes*, which bind bacterial RNA polymerase and block the extension of the nascent RNA chain.[16] Rifamycins have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria.[16] They were first isolated in 1959, and the first clinically used rifamycin was rifampicin (**1.6**) (Figure 1.4), which came into use during the late 1960s and has long been the first order of treatment for

tuberculosis. Resistance generally develops rapidly and rifampicin is, therefore, paired with other antibiotics to reduce resistance.[17] Including rifampicin, there are currently four rifamycins approved for use in the U.S. rifabutin (**1.7**) was approved in 1992 for AIDS-related *Mycobacterium* infections. Rifapentine (**1.8**) was approved in 1998 for the treatment of tuberculosis. rifamixin (**1.9**) was approved in 2004 for the treatment of traveler's diarrhea.[17]

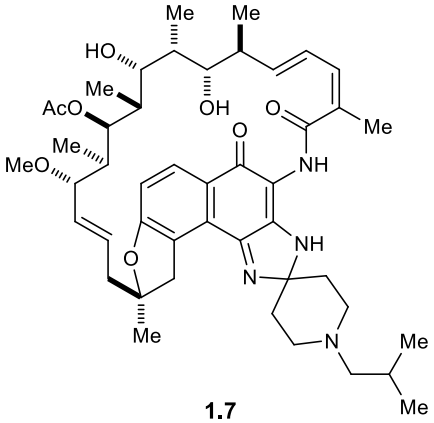
1.2.2 Antibiotics Targeting DNA Topoisomerase II and DNA Topoisomerase IV

DNA topoisomerases are essential enzymes used in the replication of DNA.[6] DNA gets wound tightly during the transcription and replication processes forming positive super coils. If positive supercoils are left to form without restriction the DNA strands will be under increasing torsion until the DNA and RNA polymerases cannot continue their progression down the DNA strand. Topoisomerases relieve this torsion by catalyzing changes in DNA topology by converting positive supercoils, left-handed in the same direction of twisting as the DNA helix, into negative supercoils, right-handed in the opposite direction of the twist in the DNA helix.[10] This is an ATP-driven process wherein both strands of the DNA are cut, passed through each other, and reconnected. This is an essential enzymatic process in the cell, without alleviating the strain associated with positive supercoils the DNA could not be unwound during the transcription process.[11] Like RNA polymerase, bacterial topoisomerases make excellent antibacterial targets as they are highly conserved amongst bacteria, yet completely different than topoisomerases found in eukaryotes.

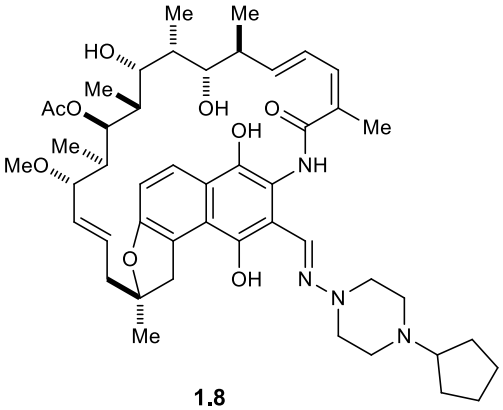
Figure 1.4. FDA approved bacterial RNA polymerase inhibitors.



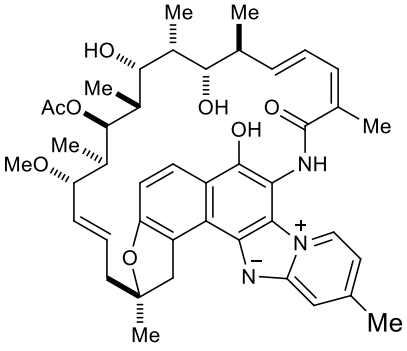
1.6
Rifampicin



1.7
Rifabutin



1.8
Rifapentine



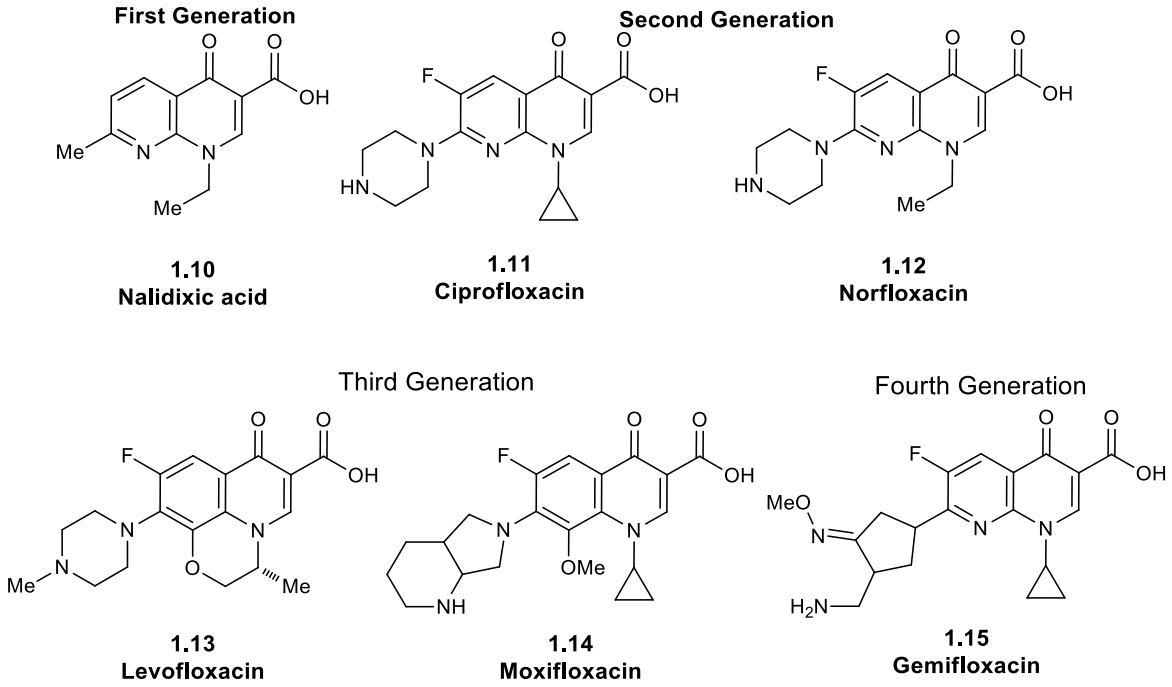
1.9
Rifamixin

Bacterial topoisomerases are targeted by two classes of antibiotics, the quinolones and aminocoumarins. The first quinolone antibiotics, nalidixic acid (**1.10**), was discovered in 1962.[6] It is a completely synthetic molecule that was found as an impurity in the synthesis of chloroquine.[18] It was found to have modest activity against Gram-negative bacteria and is still on the market today used to treat urinary tract infections. Medicinal chemistry has produced three subsequent generations of quinolones, known more commonly as fluoroquinolones due to the addition of the fluorine on the aromatic ring typically at the C-7 position (Figure 1.5). The second-generation consists of ciprofloxacin (**1.11**) and norfloxacin (**1.12**), the third-generation consists of levofloxacin (**1.13**) and moxifloxacin (**1.14**), and the fourth-generation consists of gemifloxacin (**1.15**).[19] These later generations of quinolones have a significant increase in potency against both Gram-positive and Gram-negative bacteria and are approved for a variety of indications. Both levofloxacin and ciprofloxacin have long been found on the list of top 100 most frequently prescribed drugs.[10,20]

The aminocoumarins are a class of natural product antibiotics produced by several *Streptomyces* species. They are characterized by a core 3-amino-4,7-dihydroxycoumarin moiety. The first aminocoumarin, novobiocin (**1.16**), was isolated in the 1950's and approved for clinical use in 1964.[8] It was used in the treatment of *Staphylococcus aureus* infections and to treat Lyme disease. Later, clorobiocin (**1.17**) and coumermycin A_1 (**1.18**) were discovered and found to have similar activity as **1.16** (Figure 1.6).[21] The three compounds have been found to act as competitive inhibitors of ATP.[22] A fourth compound, simocyclinone (**1.19**), was discovered later. Although it is part of the aminocoumarin family, it lacks the deoxy sugar, which is a key binding element for the ATP binding site.

Nonetheless, it was found to be approximately 50% more active than the previous three compounds affecting a novel binding site on topoisomerase.[23]

Figure 1.5. Four generations of quinolones.



1.2.3 Antibiotics Targeting Cell Wall Synthesis

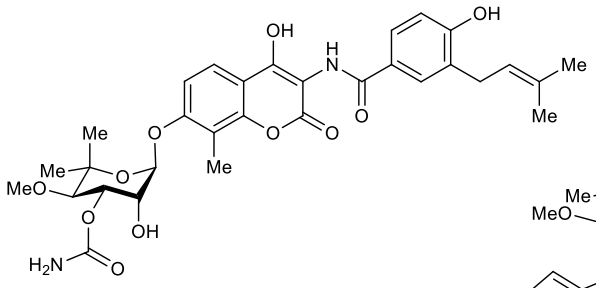
The bacterial peptidoglycan cell wall consist of repeating units of lipid II, which is a disaccharide (GlcNAc and MurNAc) pentapeptide monomer attached to a prenylated diphosphate anchor. The prenylated sidechain inserts itself into the lipid bilayer of the cell membrane. The lipid II monomers are connected together through glycosylation and different layers are cross-linked together through the amino acid sidechains. The prenylated sidechain is recycled back to the cytosol.[12] Antibiotics target this process through a number of different pathways.[6]

The penicillins were the first natural product antibiotics discovered with penicillin G (Figure 1.2) being the most active of the initial compounds isolated. They are broad spectrum antibiotics against Gram-positive bacteria, but are not very active against Gram-negative strains. The penicillins inhibit bacterial cell wall synthesis by targeting penicillin binding proteins, which are responsible for cross-linking the amino acid sidechains of the peptidoglycan layers.[24] The oral bioavailability of penicillin G was improved in the 1960's and 70's through synthesis resulting in the introduction of ampicillin (**1.20**) and amoxicillin (**1.21**).[25] The penicillins also suffered from resistance due to β-lactamases. Analogues, such as methicillin (**1.22**), were developed with improved stability toward these enzymes. Further development would spawn molecules, such as piperacillin (**1.23**), which increased activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa*.[6]

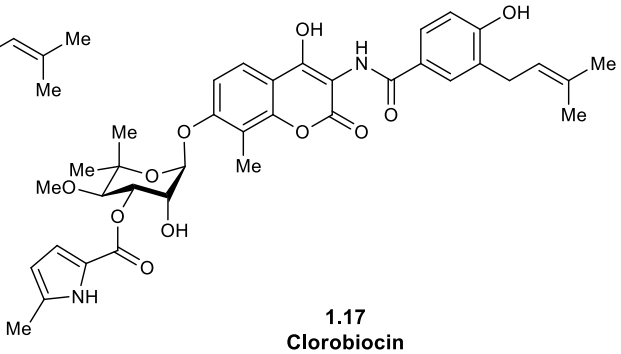
A related class of molecules, the cephalosporins, containing an unsaturation in the 6-membered ring were found to be much more stable against β-lactamases. Medicinal chemistry was able to produce many analogues including cephalexin[26] (**1.24**), which is still widely used today.[6]

Another class of molecules similar to penicillin are the carbapenems, such as thienamycin (**1.25**), are characterized by a 5-membered ring with an exocyclic sulfur compared to the 6-membered endocyclic sulfur containing ring in penicillins and cepholosporins.[27] These compounds are especially resistant to β-lactamases and research to discover novel carbapenems continues today.[6,28,29]

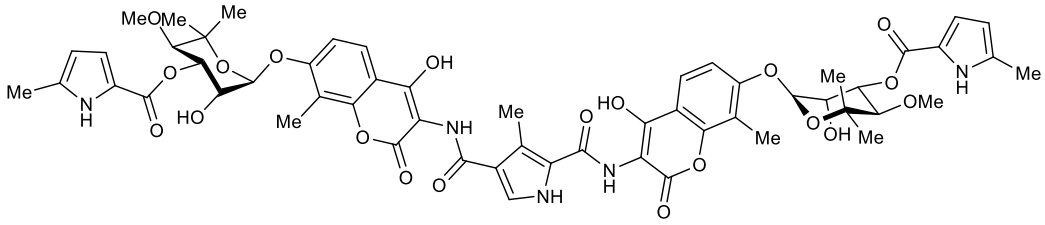
Figure 1.6. Aminocoumarin antibiotics.



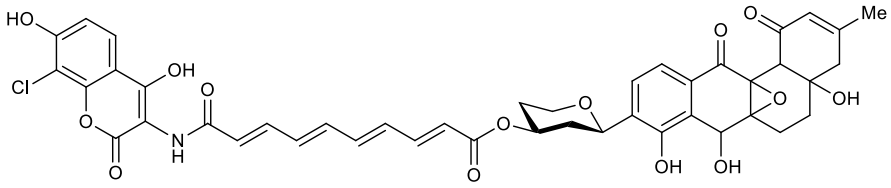
1.16
Novobiocin



1.17
Clorobiocin

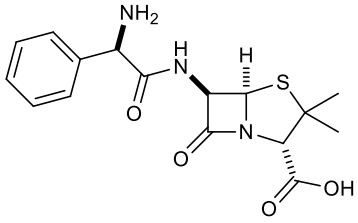


1.18
Coumermycin A_1

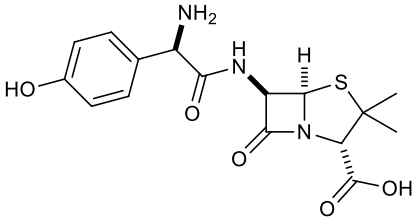


1.19
Simocyclinone

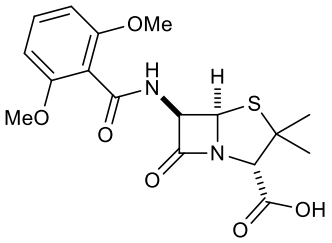
Figure 1.7. Representative penicillins, cephalosporins, and carbapenems.



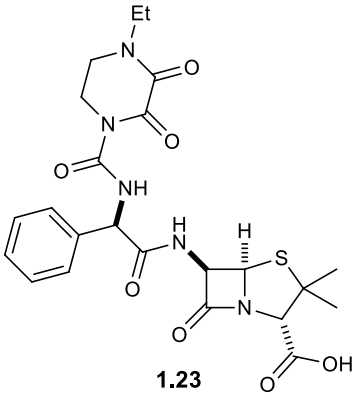
1.20
Ampicillin



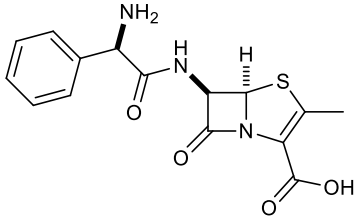
1.21
Amoxicillin



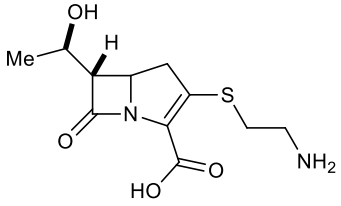
1.22
Methicillin



1.23
Pipericillin



1.24
Cephalexin



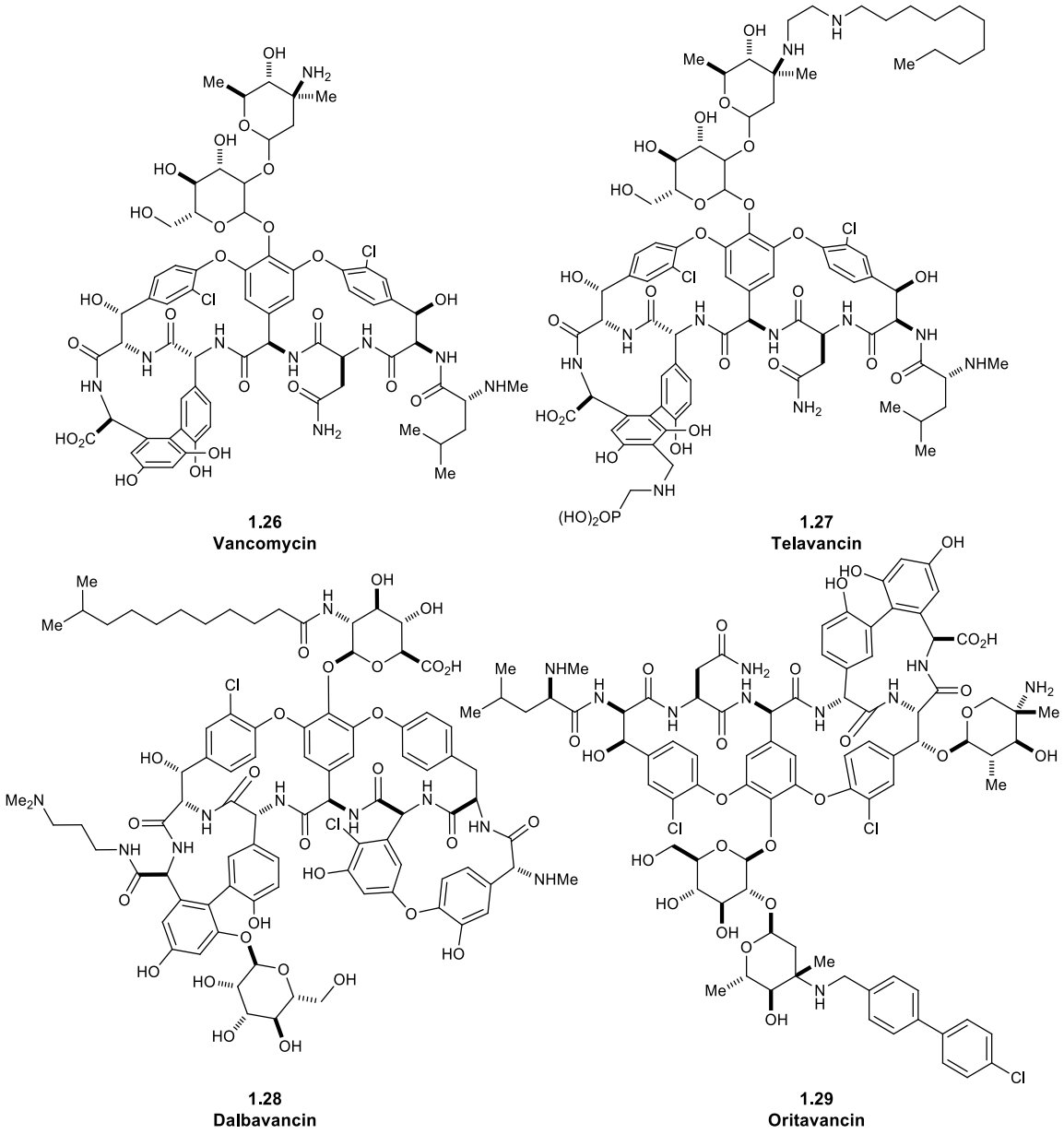
1.25
Thienamycin

Glycopetides are a class of sugar containing antibiotics that target the D-ala-D-ala portion of lipid II. With the terminus of the peptide chain blocked, enzymes are unable to bind and cross-link peptidoglycan layers effectively, destroying the cell wall and leading to eventual cell death.[12] The best known antibiotic in this class is vancomycin (**1.26**) (Figure 1.8), which was discovered in 1955 from an isolate of *Amycolatopsis orientalis*.[30,31] Vancomycin is restricted to Gram-positive bacteria due to its inability to cross the lipopolysaccharide containing outer membrane of Gram-negative bacteria. While it does have broad spectrum activity against most Gram-positive bacteria, its use is generally limited to serious infections, such as methicillin resistant *Staphylococcus aureus* (MRSA), which cannot be addressed with other antibiotics. For this reason, resistance has developed slowly to vancomycin, although increased reliance has seen an increase in the incidence of resistance.[32] Recent FDA approvals of lipophilic sidechain containing lipoglycopeptides telavancin (**1.27**), dalbavancin (**1.28**), and oritavancin (**1.29**) show superior activity to **1.26**[13] and will allow us to regain lost ground in the battle against resistance.

1.2.4 Antibiotics Targeting Protein Synthesis

The bacterial ribosome is a complex molecular machine responsible for the translation of mRNA into proteins.[33,34] On the molecular scale it is an enormous 2.5 MDa structure, consisting of a large 50S subunit and a small 30S subunit, which combine to make the 70S ribosome. The 50S subunit is made of 23S and 5S rRNA regions, as well as 34 proteins. The 30S subunit consists of the 16S rRNA and 20 proteins (Figure 1.9).[35] The ribosome is the target of over half of all antibiotics, which bind several different sites on the ribosome, acting on different phases of protein synthesis and is rightly described as a

Figure 1.8. Glycopeptide antibiotic vancomycin (**1.26**) and lipoglycopeptides telavancin (**1.27**), dalbavancin (**1.28**), and oritavancin (**1.29**).



1.26
Vancomycin

1.27
Telavancin

1.28
Dalbavancin

1.29
Oritavancin

target of targets. The functional centers most targeted by antibiotics are the tRNA–mRNA decoding regions of the 30S subunit, the peptidyl transferase center (PTC) and the peptide exit tunnel, both of which are located on the large 50S subunit.[14,36]

Figure 1.9. The 70S bacterial ribosome is composed of the small 30S and large 50S subunits, each consisting of rRNA and proteins.

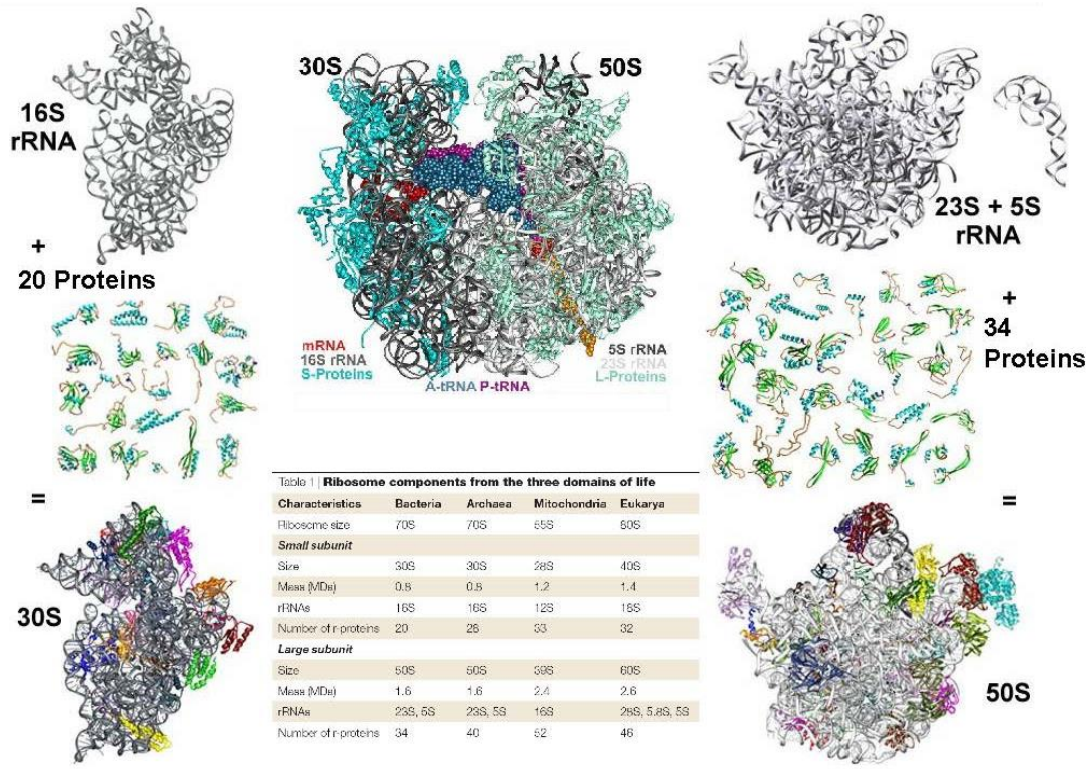


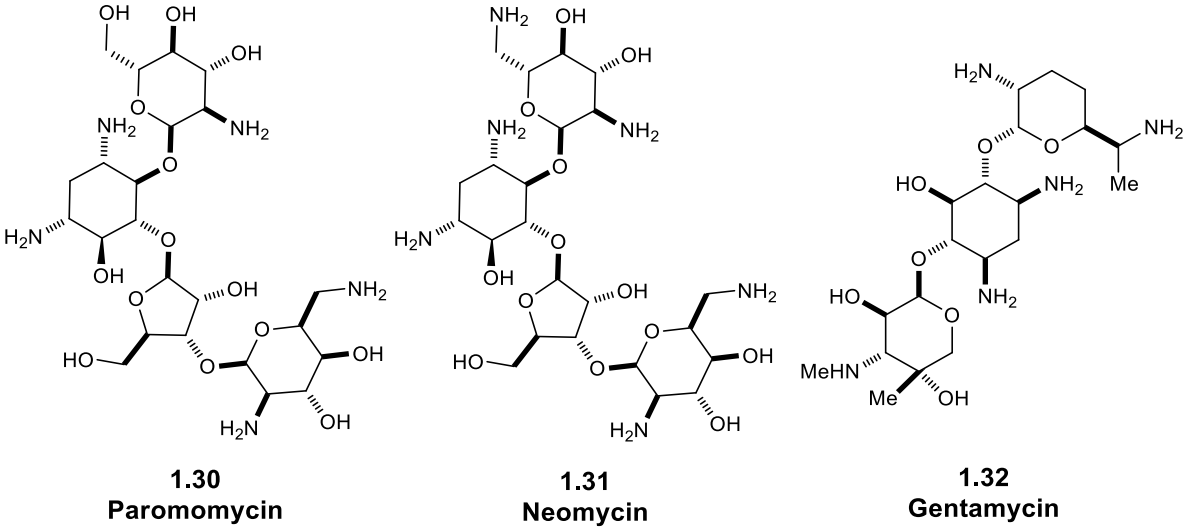
Table 1 | **Ribosome components from the three domains of life**

Characteristics	Bacteria	Archaea	Mitochondria	Eukarya
Ribosome size	70S	70S	55S	80S
Small subunit				
Size	30S	30S	28S	40S
Mass (MDa)	0.8	0.8	1.2	1.4
rRNAs	16S	16S	12S	18S
Number of r-proteins	20	26	33	32
Large subunit				
Size	50S	50S	39S	60S
Mass (MDa)	1.6	1.6	2.4	2.6
rRNAs	23S, 5S	23S, 5S	16S	28S, 5.8S, 5S
Number of r-proteins	34	40	52	46

The aminoglycosides are a family of bactericidal antibiotics that target the accuracy of translation. They bind the A-site causing the rRNA to adopt conformations, allowing non-cognate tRNA to bind and deliver the incorrect amino acid into the sequence.[37] The first aminoglycoside antibiotic was streptomycin[38] (**1.5**) (Figure 1.3). Its success led to the search for other natural product antibiotics, which led to the discovery of additional

aminoglycoside antibiotics: paromomycin (**1.30**), neomycin (**1.31**), and gentamycin (**1.32**) (Figure 1.10).[36] The aminoglycosides show potent activity against Gram-negative bacteria, especially *Enterobacter*, *Acinetobacter*, and *Pseudomonas* species. Due to toxicity and resistance, the aminoglycosides are used less often, although novel clinical uses are being developed, such as synergistic treatments with other antibiotics to combat Gram-positive infections, the use of inhaled aminoglycoside to treat lung infections suffered by cystic fibrosis patients, and coated onto surgical devices to avoid infection.[39]

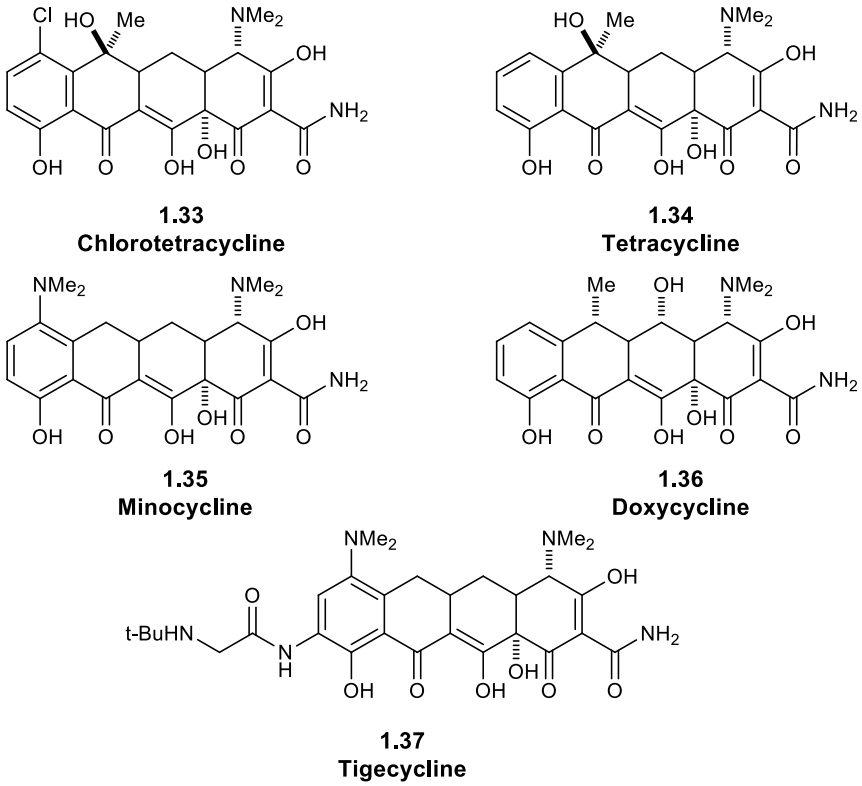
Figure 1.10. Common aminoglycoside antibiotics.



The tetracyclines are a class of antibiotics that exert their antibacterial properties by binding the 30S ribosomal subunit in the A-site tRNA binding pocket, thus preventing protein synthesis.[14,36,40] The first tetracycline, chlorotetracycline (**1.33**) (Figure 1.11), was first isolated in 1948 from *Steptomyces aureofaciens*. The structure of **1.33** was determined by Woodward in 1953, and as a result of chemical modifications, it was discovered that the removal of the chlorine atom of **1.33**, forming tetracycline (**1.34**), produced a more

potent antibiotic. Continued optimization efforts led to a new generation of compounds such as minocycline (**1.35**) and doxycycline (**1.36**). These compounds possessed better pharmacokinetic properties and increased potency against both Gram-positive and Gram-negative bacteria.[40] Resistance to tetracyclines through an efflux mechanism would lead to the development of a new generation of compounds known as glycylcyclines, most notably tigecycline (**1.37**), which showed similar activity to both tetracycline susceptible and resistant strains.[41]

Figure 1.11. Tetracycline antibiotics.

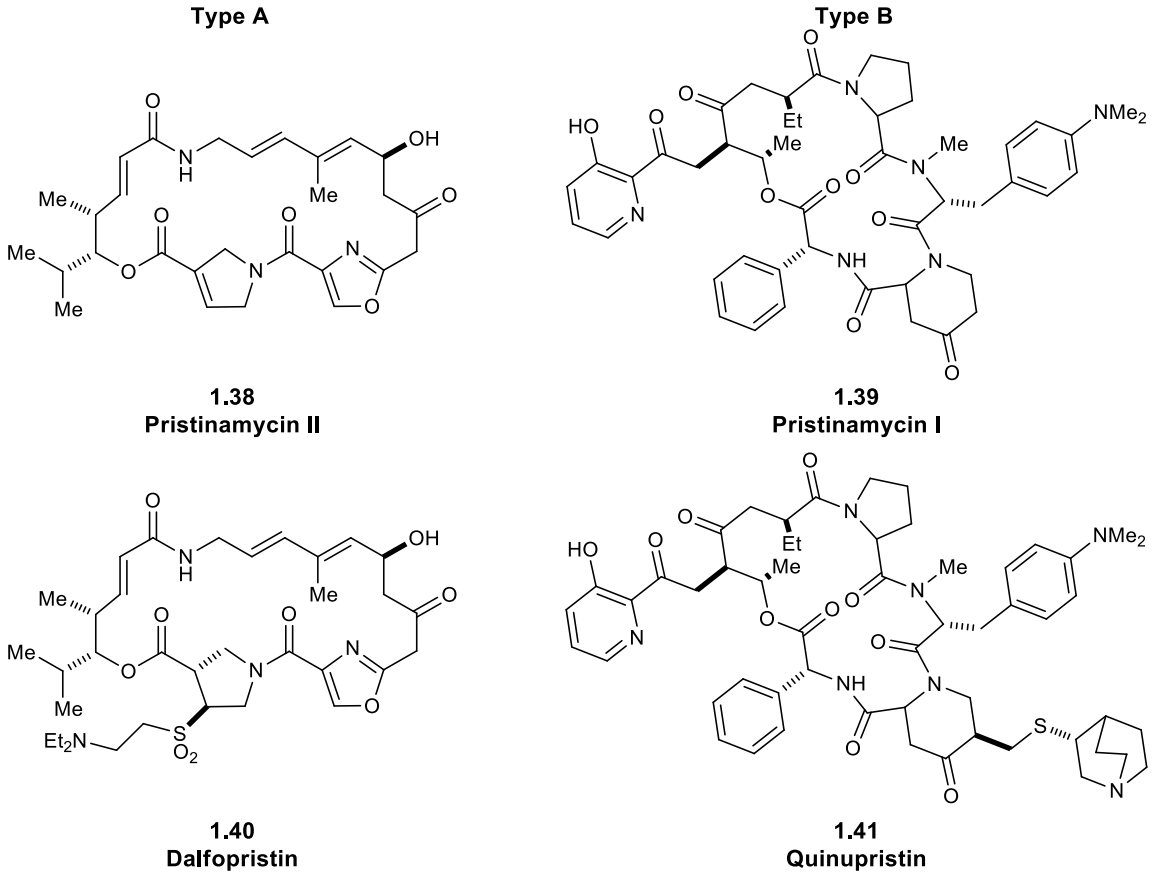


The streptogramins are a class of antibiotics first discovered in the 1950s during the golden age of antibiotic discovery. These natural product compounds are isolated from

a variety of *Streptomyces* species.[6] There are two subtypes of streptogramins, A and B, which are isolated together in a 70:30 mixture, respectively. While the two types of streptogramins are mildly bacteriostatic by themselves, when used in combination they become 100-fold more potent and show bacteriocidal activity.[42] The increased activity is attributed to their binding in adjacent locations. Type A streptogramins bind the PTC and interfere with tRNA binding in both the A-site and P-site, while type B streptogramins bind the peptide exit tunnel blocking the extension of the growing peptide chain.[14,36] The first streptogramins to find widespread use were the combination of pristinamycin II (**1.38**) and pristinamycin I (**1.39**), which were first put into use more than 50 years ago to treat a variety of Gram-positive bacteria, including some resistant strains, such as VRSA and MRSA (Figure 1.12). Poor aqueous solubility and increasing resistance would lead medicinal chemistry to derivatize pristinamycin, resulting in the discovery of Synercid, a combination of dalfopristin (**1.40**) and quinupristin (**1.41**).[43] The combination therapy was approved in 1999 for the treatment of vancomycin resistant *Enterococcus faecium* infections.[43]

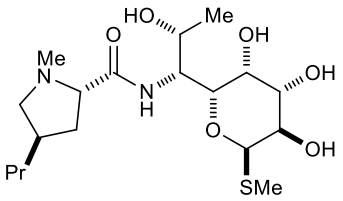
The lincosamides are another group of natural product antibiotics that share a mechanism of action with the streptogramins.[8] The first lincosamide antibiotic, lincomycin (**1.42**) (Figure 1.13), was isolated in 1962 from *Sreptomyces lincolnensis*.[44] A semi-synthetic derivative, clindamycin (**1.43**), was developed in in 1967. The lincosamides are generally active against only Gram-positive bacteria, especially aerobic bacteria and are used to treat skin infections. Lincomycin shows little activity against Gram-negative bacteria, while clindamycin does show some activity although resistance occurs rapidly. Lincosamides are also potent against some protozoan species.[45]

Figure 1.12. Clinically relevant streptogramins.

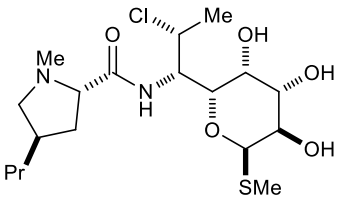


Oxazolidinone antibiotics are one of the few synthetically designed antibiotics in clinical use, along with quinolones. This class of compounds was first synthesized in 1978 and showed excellent activity against Gram-positive bacteria.[46] Due to toxicity issues, the class of compounds was not pursued again until the late 1980's when DuPont developed **1.44** and **1.45**, which contain the acetamidomethyl sidechain with (*R*)-stereochemistry, which was shown to be necessary for activity.[47] Upjohn would continue this work resulting in the 2000 FDA approval of linezolid (**1.46**) for several different Gram-positive infections.[43] The oxazolidinone antibiotics bind the A-site inhibiting accommodation of the tRNA and compete with the binding site of the lincosamides and chloramphenicols.[14,36]

Figure 1.13. Lincosamide antibiotics.

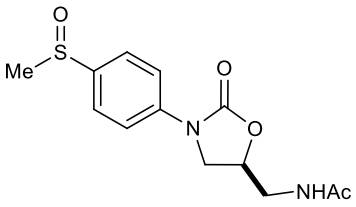


1.42
Lincomycin

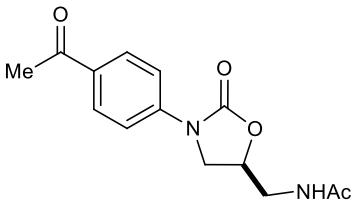


1.43
Clindamycin

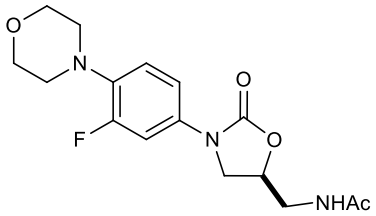
Figure 1.14. Oxizolidinone antibiotics.



1.44



1.45



1.46
Linezolid

1.3 The Discovery and Development of Macrolide Antibiotics

1.3.1 Macrolide Antibiotics and their Mechanism of Action

Macrolides, a term coined by R. B. Woodward in 1953 as an abbreviation for "macrolactone glycoside antibiotics[48] are polyketide-derived natural products consisting of a 12-16 membered lactone ring decorated with one or more carbohydrates. Macrolides are synthesized by polyketide synthases (PKS) and are produced in two stages. In the first stage, PKS sequentially stitches together propionyl CoA and methylmalonyl CoA subunits

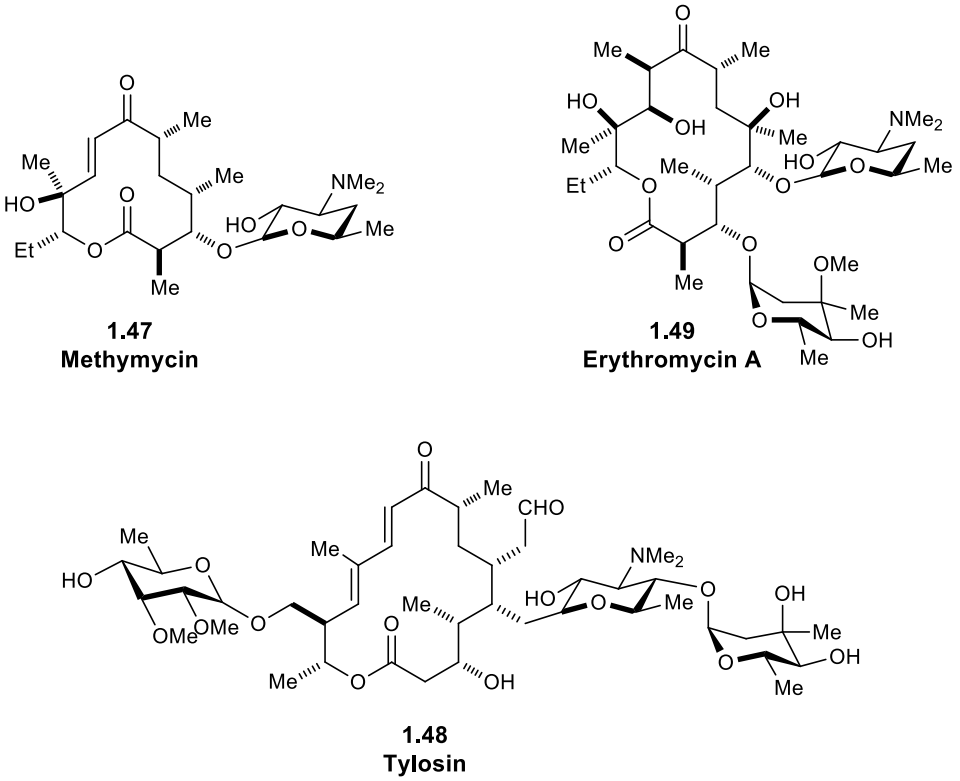
and cyclizes the resulting chain to provide the aglycone ring. The ring is then sequentially decorated with hydroxyl groups and carbohydrate moieties by the action of various hydroxylase and glycosyltransferase enzymes, respectively.[49] Macrolide antibiotics inhibit protein synthesis by reversibly binding to the bacterial ribosome. Specifically, they inhibit progression of the nascent peptide chain by binding to the peptidyl transferase center (PTC) of 23S rRNA located in the 50S subunit.[14,36] Unlike other inhibitors targeting the PTC, macrolide antibiotics bind slightly lower at the beginning of the peptide exit tunnel. It is generally accepted that macrolides do not interfere with the ribosomal active site machinery directly, but instead block the growing peptide as it begins to move into the exit tunnel and cause the ribosome to stall, releasing only a small peptide sequence instead of the full chain encoded by the mRNA.[50] Chemical footprinting studies have shown that macrolides mostly interact with RNA, not protein, specifically interacting with residues A2058, A2059, and G2505 in domain V and A752 of domain II.[50] Macrolides are commonly isolated from *Streptomyces* species of bacteria. Common representatives of this class are the 12-membered methymycin (**1.47**), 16-membered tylosin (**1.48**), and the most famous, clinically relevant, and commercially successful erythromycin A (**1.49**) (Figure 1.15).

1.3.2 Discovery of the Erythromycins

Erythromycin A (**1.49**), commonly referred to as erythromycin, was discovered in 1949 by McGuire et al. It was first isolated from the *Actinomycete Saccharopolyspora erythraea*, formerly *Streptomyces erythraea.*[7,8] It has been used clinically since 1952 and is an effective treatment for many Gram-positive upper and lower respiratory bacterial

infections, as well as skin and soft tissue infections. It has an excellent safety profile with the only major side effect being gastrointestinal discomfort.[51] Erythromycin (**1.49**) was the first macrolide antibiotic used clinically. [6]

Figure 1.15. Macrolide antibiotics methymycin (**1.47**), tylosin (**1.48**) and erythromycin A (**1.49**).

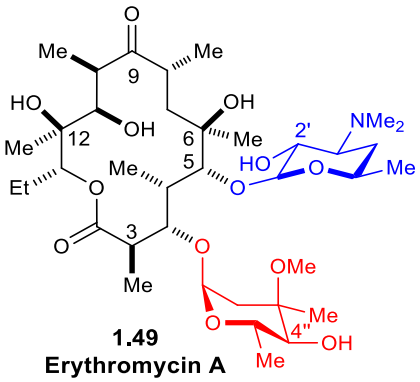


1.3.3 Structure of Erythromycin A

The structure of erythromycin A (**1.49**) was determined by chemical degradation studies in 1957.[52] The structure was confirmed and absolute stereochemistry was determined by X-ray crystallography in 1965.[53] **1.49** consists of a 14-membered lactone ring containing 10 stereocenters, as well as an aminosugar (desosamine), which is located

at the C-5 position and a deoxysugar (cladinose), which is located at the C-3 position (Figure 1.16).

Figure 1.16. Structure of erythromycin A with carbohydrate residues desosamine and cladinose.



1.49
Erythromycin A

1.3.4 Erythromycin A: A History of Structure–Activity Relationships

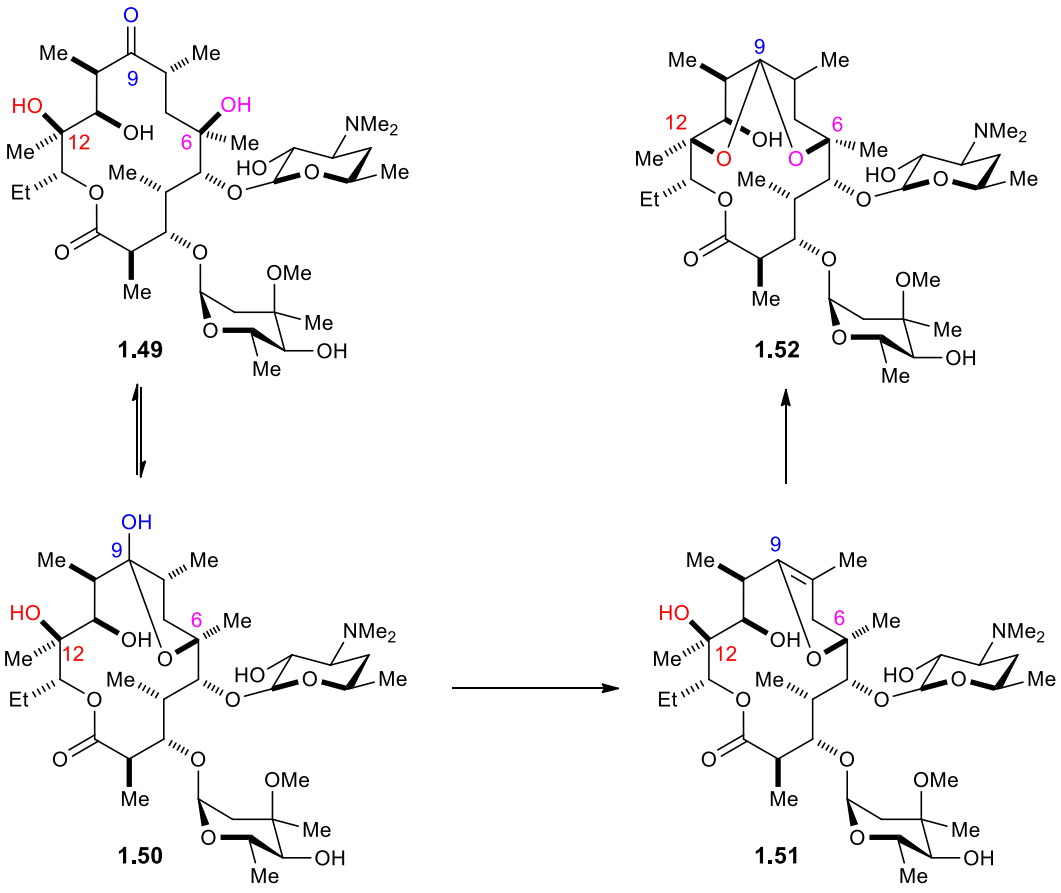
Extensive work has been done on the modification of **1.49** and the effects of those modifications on its antibacterial activity, giving us a wealth of structure–activity relationships (SAR) about this complex natural product. The presence of the desosamine sugar is essential for it to maintain antibacterial activity, specifically the presence and stereochemistry of the C-2' hydroxyl and the C-3' dimethylamine.[54] Modification of the cladinose sugar at C-3 showed that demethylation of the C-3'' methyl ether reduced activity. The presence or stereochemistry of the C-4'' was shown to be inconsequential. Transformation of the C-4'' hydroxyl to the ketone, oxime, or amino analogue did reduce activity compared to **1.49**. Studies starting in 1975 produced a number of C-3 decladinosyl derivatives including C-3 aromatic esters and the C-3 ketone analogue. All of these compounds were devoid of activity compared with erythromycin and it was thought that

the cladinose sugar was necessary for activity.[55,56] It was not until 1998 when it was found that the structure of C-3 keto-**1.49** reported was incorrect, as it would readily react with the unprotected C-6 hydroxyl forming the inactive hemiacetal. Protection of the C-6 hydroxyl as its methyl ether followed by oxidation of the C-3 hydroxyl allowed for the successful isolation of the C-3 ketone. Subsequent MIC analysis showed that this new compound was indeed active against both wild-type (WT) and resistant strains of bacteria paving the way for the development of the ketolides (*vide infra*).[57] Esterification of the C-11, C-4'', and C-2' hydroxyls resulted in less active compounds. Alkylation of the C-6 hydroxyl was shown to not affect activity. The C-9 keto was found to be replaceable with oxime, amino, or hydroxyl functionalities, although, in the case of the amino and hydroxyl functionality, the (*S*)-stereochemistry was found to be much more active.[58]

1.3.5 Erythromycin Acid Instability and Its Impact on Pharmacokinetics

Erythromycin (**1.49**) has poor oral bioavailability, and its major side effect is gastrointestinal discomfort. As part of the ongoing chemical modification and SAR studies with **1.49**, chemists at Abbott showed that under acidic conditions **1.49** converts to hemiketal **1.50,** then reacts further to form enol ether **1.51**, which can be isolated by crystallization (Scheme 1.1). Further treatment under acidic conditions forms the 6,9-9,12-spiroketal **1.52**, which is also isolable.[59] Both **1.51** and **1.52** are bereft of antibacterial activity. This finding would explain the poor bioavailability and side effects. Efforts to improve acid stability, such as different salt forms and enteric coated capsules stable at low pH, would provide limited improvement in bioavailability.

Scheme 1.1. Acid-mediated degradation of erythromycin (**1.49**) to hemiacetal **1.50**, enol ether **1.51**, and spiroketal **1.52**.



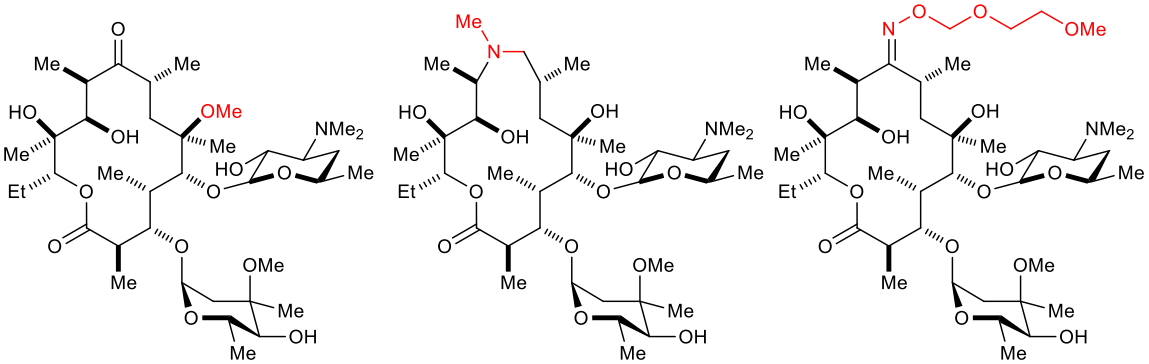
1.3.6 Development of Second-Generation Macrolides

With the structural cause of poor bioavailability known and a wealth of SAR data on chemically modified erythromycins, it wouldn't be long before chemists would synthesize a number of analogues that would increase acid stability. Medicinal chemists would concentrate on modifications to the C-6 hydroxyl, as well as the C-9 ketone, both of which had already been shown to be modifiable without loss of activity.[58,60] From this work would emerge clarithromycin[61] (**1.53**), azithromycin[62,63] (**1.54**) and roxithromycin[64] (**1.55**),

which would become some of the most commercially successful antibiotics in the post antibiotic golden age (Figure 1.17).

Clarithromycin (**1.53**) was first brought to market in Japan by Taisho Pharmaceuticals in 1991 and later gained FDA approval for use in the United States under Abbott.[6] **1.53** is the C-6 methyl ether analogue of **1.49**. The addition of the methyl group successfully blocks formation of the 6,9-acetal formation. Due to the removal of the acid instability, **1.53** shows excellent bioavailability compared to **1.49**, and has a decreased incidence of gastrointestinal problems. **1.53** shows slightly better *in vitro* activity when compared to **1.49**, while clinical efficacy is equivalent.[61]

Figure 1.17. Second-generation macrolide antibiotics clarithromycin (**1.53**), azithromycin (**1.54**), and roxithromyin (**1.54**).



1.53
Clarithromycin

1.54
Azithromycin

1.55
Roxithromycin

Azithromycin (**1.54**) was discovered by Pliva in the 1980's and was successfully brought to market in Europe in the late 1980's. It gained FDA approval in the United States in 1991 and was marketed by Pfizer.[63,65] **1.54** is a 15-membered macrolide analogue of **1.49**, commonly referred to an azalide, which is formed from the Beckman rearrangement

of **1.49** C-9 oxime. Removal of the electrophilic C-9 ketone removed the possibility of 6,9-hemiacetal formation. Like **1.53**, **1.54** has excellent bioavailability compared to **1.49**. **1.54** maintains similar activity against Gram-positive bacteria compared to **1.49**. Due to the addition of the basic nitrogen, **1.54** also shows exceptional activity against Gram-negative bacteria, such as *H. influenza*.[62]

Roxithromyin (**1.55**) was first developed by the French pharmaceutical company Roussel Uclaf, now part of Sanofi Aventis, in 1986.[66] It is a C-9 oxime ether derivative of **1.49** and much like **1.53** and **1.54** possesses stability under acidic conditions. **1.55** was shown to have an excellent bioavailability in mice (72%). It has a very similar clinical profile to **1.49**, but is characterized by a much higher half-life, plasma, and tissue concentrations. **1.55** has only a 4% incidence of gastrointestinal side effects.[64]

1.4 Development of Third-Generation Macrolides to Combat Antibiotic Resistance.

Antibiotic resistance has become and will continue to be a major medical issue of the 21st century. If not addressed, the potential for a post-antibiotic era could become a reality, one that the world has not been familiar with since the early 1900's. Multidrug-resistant hospital-acquired bacterial infections already account for close to 2 million cases and 23,000 deaths in the United States, along with 20 billion dollars of additional medical spending each year. The CDC released a report in 2013 regarding the seriousness of antibiotic resistance and providing a snapshot of costs and mortality rates of the most serious antibiotic resistant bacteria, which includes 17 drug resistant bacteria, such as carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *Enterococcus* and *Staphylococcus aureus*, and multidrug-resistant *Acinetobacter* and *Pseudomonas*

aeruginosa. The development of antibiotic resistance is part of bacteria's normal evolutionary process and thus impossible to completely stop. To ensure a future where resistant bacteria do not run rampant throughout society, there is a great need for new antibiotics and accordingly, methods to facilitate their discovery.

1.4.1 Resistance to Macrolide Antibiotics

Bacterial Resistance to macrolides has been known since erythromycin A was released in 1952.[67] While only a small percentage of bacteria showed resistance to macrolides in the 1950's, the continued reliance and overuse of macrolides over the last 60 years has caused a dramatic increase in resistance. The most common types of resistance to macrolide antibiotics are drug efflux, ribosomal modification, and ribosomal mutation.[68]

Efflux of macrolides out of the bacterial cell to reduce cellular concentrations of the antibiotic are encoded by either the *mef* of *msr* genes. *Mef* genes have been found in a variety of bacteria including *S. pyogenes* and *S. pneumoniae*. Studies have shown that the cladinose sugar of **1.49** is a recognition element for *mef* induced efflux. *Msr* genes have been found in *S. pneumoniae* but are most common in *S. aureus*. *Msr* encoded efflux pumps have been shown to efficiently remove first and second generation macrolides from the cell.[6]

Post translational methylation of bacterial ribosomes is accomplished by methyltransferase enzymes utilizing S-adenosylmethionine, which are encoded by *erm* genes. The *erm* genes are commonly found in *S. pneumoniae, S. pyognes*, and *S. aureus*.[69] The *erm* enzymes specifically target and mono- or dimethylate the N-6 exocyclic nitrogen of the adenine base at position 2058. (A2058). This modification causes resistance by

sterically blocking interactions of the 2'-hydroxyl and 3'-dimethylamine of erythromycin's desosamine sugar with A2058. Monomethylation provides only low levels of resistance while dimethylation confers high levels of resistance. *Erm* enzymes also confers resistance to lincosamides and streptogramin B antibiotics and are commonly referred to as Macrolide-Lincosamide-Streptogramin (MLS_B) resistance.[70]

Ribosomal RNA mutation is also a common form of MLS_B resistance. The most common mutation is the switch of A2058 to G2058 (A2058G). This mutation has a similar effect as *erm* methylation, causing the desosamine sugar of erythromycin to lose key binding interactions. Other common mutations are A2059G, A2058U, A2059U, and C2611T. rRNA mutations conferring resistance to macrolides are not as prevalent to *erm* resistance, but have, nonetheless, been found in *S. pneumoniae, S. aureus,* and *H. influenza*.[71]

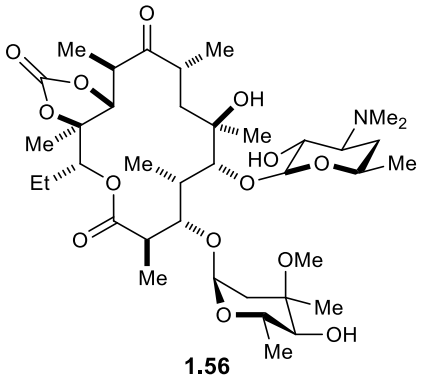
Mutations of ribosomal proteins L4 and L22 have been shown to give resistance to bacteria. These proteins are located near the macrolide binding site but do not make any direct binding interactions with the antibiotics. Instead, it is thought that these single amino acid mutations alter the peptide exit tunnel binding pocket sufficiently to eliminate binding interactions with the drug.[72]

1.4.2 Discovery of the Ketolides

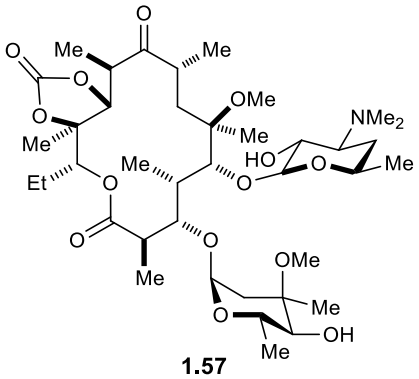
During the years after the discovery of erythromycin (*vide supra*), the extensive work to determine the SAR and the development of a generation of acid stable macrolides, led to many compounds that never reached fruition as drugs, but would, ultimately, have a role in the discovery of a new generation of macrolide antibiotics, called ketolides, that

maintained activity to resistant strains of bacteria. One such discovery was of the erythromycin 11,12-carbonate (**1.56**) and clarithromycin 11,12-carbonate (**1.57**), which were reported in 1983 (Figure 1.18). These compounds had increased acid stability and improved *in vivo* potency and half-life compared to erythromycin. Unfortunately, these compounds had increased hepatotoxicity compared to erythromycin and were not explored further.[54,58]

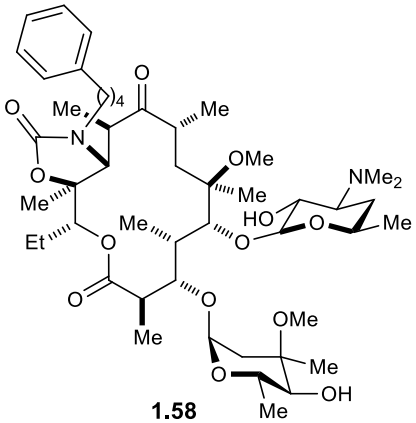
Figure 1.18. Structures of erythromycin 11,12-carbonate (**1.56**), clarithromycin 11, 12-carbonate (**1.57**), and A66321 (**1.58**)



1.56
Erythromycin 11, 12 carbonate



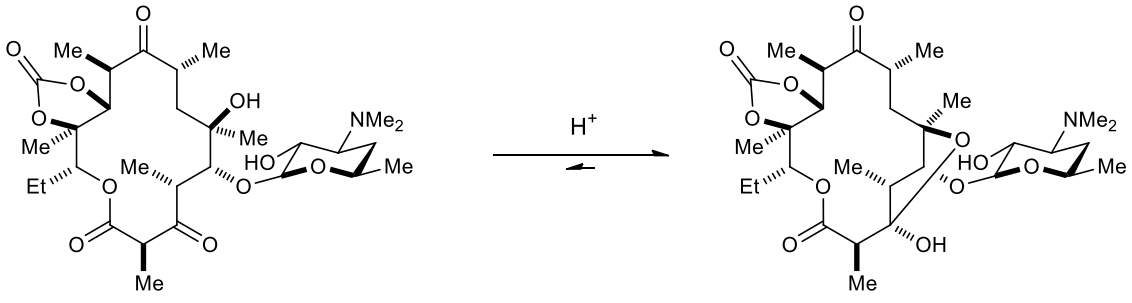
1.57
Clarithromycin 11, 12 carbonate



1.58
A66321

In 1989, Baker and co-workers out of Abbott Laboratories synthesized a series of clarithromycin analogues where the carbonate was replaced with a carbamate linked to a side chain in the hope that they could maintain the novel activity of the carbonate analogues, while eliminating the increase in hepatotoxicity. One compound to come out of this work was A66321 (**1.58**), containing a butylphenyl sidechain (Figure 1.18).[73] **1.58** was 8-fold more potent against *S. pyogenes* with an inducible MLS_B resistance and >32-fold more potent against *S. pyogenes* with a constitutive MLS_B resistance. Due to the initial reports suggesting that 3-keto erythromycin (**1.59**) was inactive,[56] the functionality was not explored again until 1998 when Agouridas and co-workers with Hoechst Marion Roussel (HMR) decided to explore the synthesis of this ketolide derivative.[57] They found that the structure of **1.59** was not the keto, but instead the hemiacetal (**1.60**), which is formed from the free C-6 hydroxyl under acidic conditions (Scheme 1.2).

Scheme 1.2. Conversion of 3-keto erythromycin (**1.59**) to 3,6-hemiketal erythromycin (**1.60**) under acidic conditions.



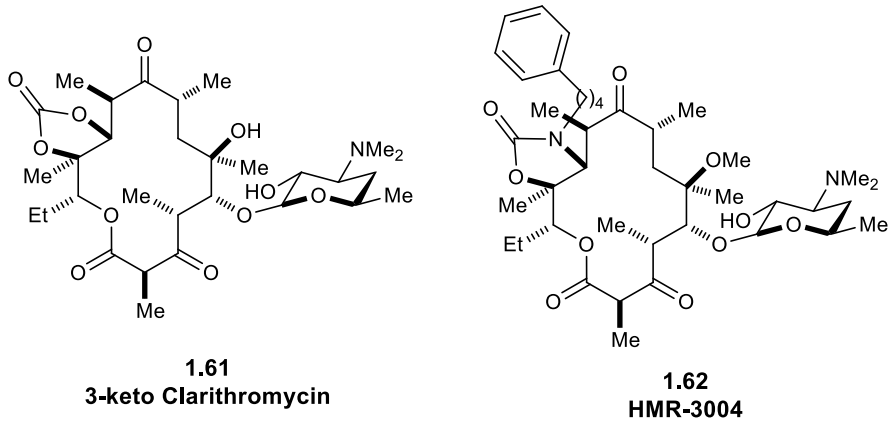
1.59
3-keto Erythromycin

1.60
3,6-hemiketal Erythromycin

Starting with clarithromycin (**1.53**), which features a 6-*O*-methyl ether as opposed to a free hydroxyl as in erythromycin (**1.49**), Agouridas and co-workers were able to

synthesize 3-keto-11,12-carbonate clarithromycin (**1.61**).[57] It was found that this compound was slightly active against both inducible and constitutive resistant strains and was explored further. The HMR team turned toward Baker's work at Abbott with **1.58** and synthesized a number of cyclic carbamate derivatives with various sidechains ultimately leading to HMR-3004 (**1.62**), which is shown in Figure 1.19.

Figure 1.19. Structures of novel ketolides 3-keto-11,12-carbonate clarithromycin (**1.61**) and HMR-3004 (**1.62**).



Further work by HMR would lead to the discovery of telithromycin (**1.63**), which showed excellent antibacterial activity against resistant strains and a good pharmacokinetic profile (Figure 1.20).[74] The new ketolide antibiotic was approved for use in Europe in 2001 and approved by the FDA in the U.S in 2004 for the treatment of acute bacterial sinusitis, chronic bronchitis, and community-acquired pneumonia (CAP).

While **1.63** was the first marketed ketolide antibiotic able to combat bacterial resistance, its release was marred in controversy due to fraudulent data reporting during the clinical trials by a single physician who attempted to cover up data that suggested **1.63**

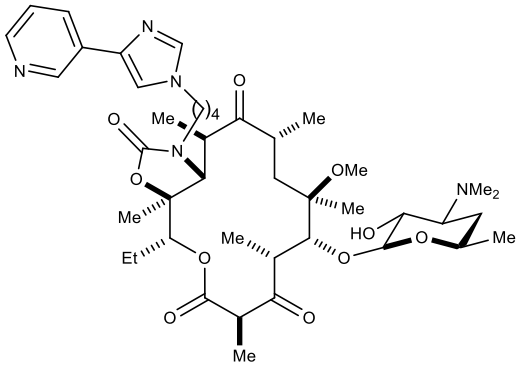
was more hepatotoxic than would be allowed for an antibiotic.[75] In 2007, the FDA issued a "black box warning" for telithromycin and removed acute bacterial sinusitis and chronic bronchitis from its approved indications. The next ketolide antibiotic to arrive would be Abbott's cethromycin (**1.64**), which underwent Phase III clinical trials until 2009. The FDA did not approve the drug, citing the need for more clinical data proving efficacy. Work with the DOD, Advanced Life Sciences has shown that **1.64** can be used to cure an inhaled lethal dose of anthrax with a 100% success rate in non-human primates. In response, the FDA classified **1.64** as an orphan drug for the treatment of anthrax exposure. Advanced Life Sciences is still awaiting approval for CAP and anthrax.[76] Solithromycin (**1.65**), which was discovered by Optimer Pharmaceuticals and further developed at Cempra Pharmaceuticals,[77] is similar to telithromycin (**1.63**). It possesses an aniline ring in place of pyridine, a triazole in place of imidazole, and contains a C-2 fluoro group. The FDA has accepted Cempra's NDA for solithromycin, backed by two separate Phase III clinical trials. The company hopes to gain approval for CAP before the end of 2016.

1.5 Ushering in an Era of Structure-Based Drug Design of Ribosome Targeting Antibiotics

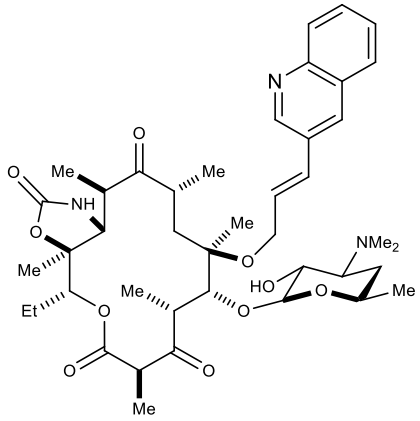
In 2009, Thomas A. Steitz, Ada E. Yonath, and Venatakatraman Ramakrishnan were jointly awarded the Nobel Prize in Chemistry for their contributions to solving the crystal structure of the bacterial ribosome and understanding its function. Steitz was responsible for solving the structure of the large 50S subunit of the archaebacterium *Haloarcula marismortui*,[78,79] while Yonath[80,81] and Ramakrishnan[82] solved the structures

of the small 30S subunit in eubacteria *Deinococcus radiodurans and Thermus thermophilus,* respectively.

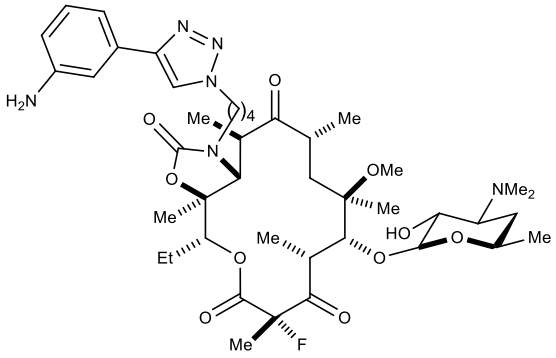
Figure 1.20. Structures of FDA approved ketolide antibiotic telithromycin (**1.63**) and ketolide antibiotics awaiting FDA approval, cethromycin (**1.64**) and solithromycin (**1.65**)



1.63
Telithromycin



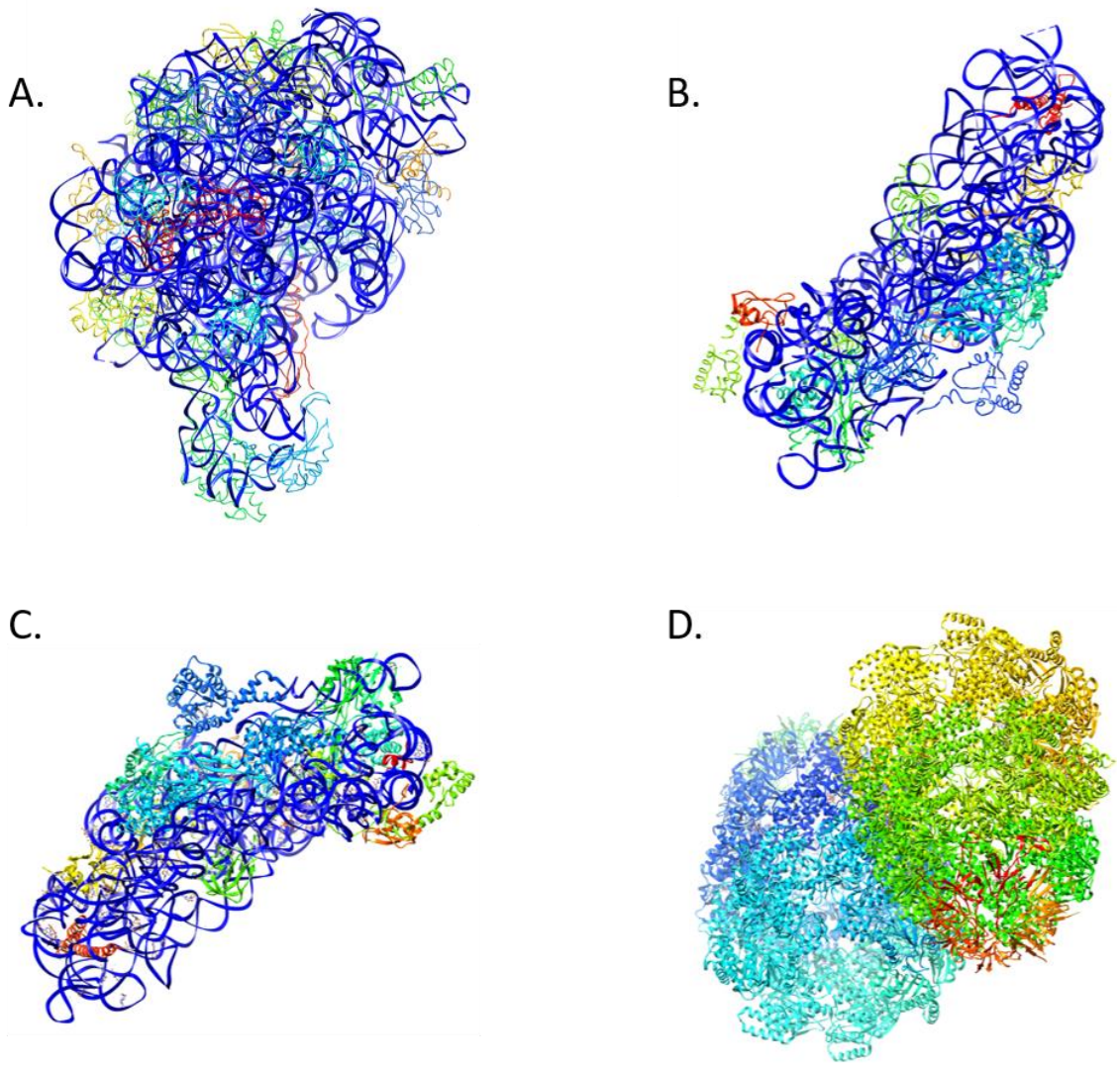
1.64
Cethromycin



1.65
Solithromycin

.

Figure 1.21. X-ray crystal structures of (A) 50S subunit from *H. marismortui* (Steitz et al., PDB = 1FFK; (B) 30S subunit from *T. thermophilus* (Yonath et al., PDB = 1FKA); (C) 30S subunit from *T. thermophilus* (Ramakrishnan et al., PDB = 1FJF); (D) 70S ribosome from *E. coli* (Cate et al. PDB = 4V4O).



While Steitz did report a low resolution structure of the 70S ribosome in 2000[83], Jamie H. Doudna-Cate, Harry F. Noller, and Marat Yusupov would solve the first high

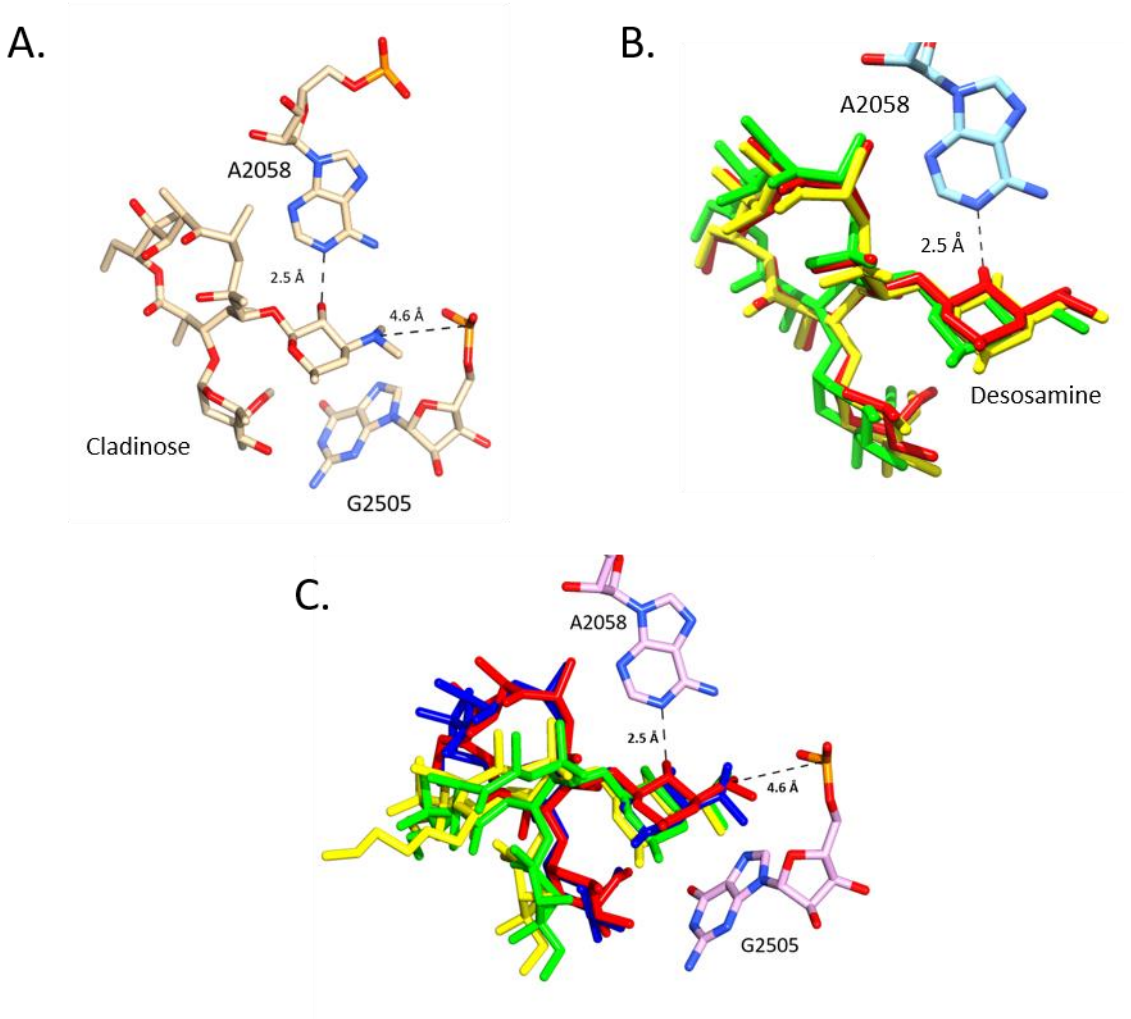
resolution crystal structure of the entire 70S bacterial ribosome (Figure 1.21).[84] All of these scientists would play a key role in the crystallization of antibiotics in the bacterial ribosome.

Shortly after these groups published the first high-resolution crystal structures of the bacterial ribosome, publications showing the crystal structures of the bacterial ribosome bound to antibiotics were reported.[78,80,85-89] Today, advances in technology have allowed the acquisition of crystal structures of seven different species of bacteria containing more than 47 different antibiotics.[14] These co-crystal structures would allow visualization of the exact nature of antibiotic binding, providing direct evidence for the wealth of biochemical experiments that have been used to determine the location of binding and which residues are involved. Biochemical footprinting assays have shown that residues A2058 and G2505 are directly involved in binding and SAR studies have shown the importance of the 2'-hydroxyl and 3'-dimethylamine moieties (*vide supra*). Examining the crystal structure of erythromycin bound to the *E. coli* ribosome shows us the exact nature of this important interaction (Figure 1.22A). The 2'-hydroxyl makes a hydrogen bond with the N1 nitrogen of A2058. Removal of this hydroxyl or change in stereochemistry greatly effects the ability of the macrolide to bind. The 3'-dimethylamine exists in its protonated form under physiological conditions and is in place to form an ionic interaction with the phosphate backbone of G2505. Comparing the binding mode of erythromycin A in three different bacterial species, *E. coli*, *D. radiodurans,* and *H. marismortui* shows a high degree of overlap of both the macrolactone core, desosamine, and cladinose structures (Figure 1.22B). This provides evidence that despite differences in sequence, the active sites of bacterial ribosomes are highly conserved among species. The structure of erythromycin

and second-generation macrolides azithromycin, clarithromycin, and roxithromycin, again shows the importance of the binding of desosamine to A2058 (Figure 1.22C). The macrolactone cores of the molecules adopt different conformations; azithromycin's macrolactone backbone maintains a binding mode identical to erythromycin, while the clarithromycin and roxithromyin ring adopts a slightly different binding orientation. Even with these differences in binding the desosamine ring of all four compounds remains highly conserved.

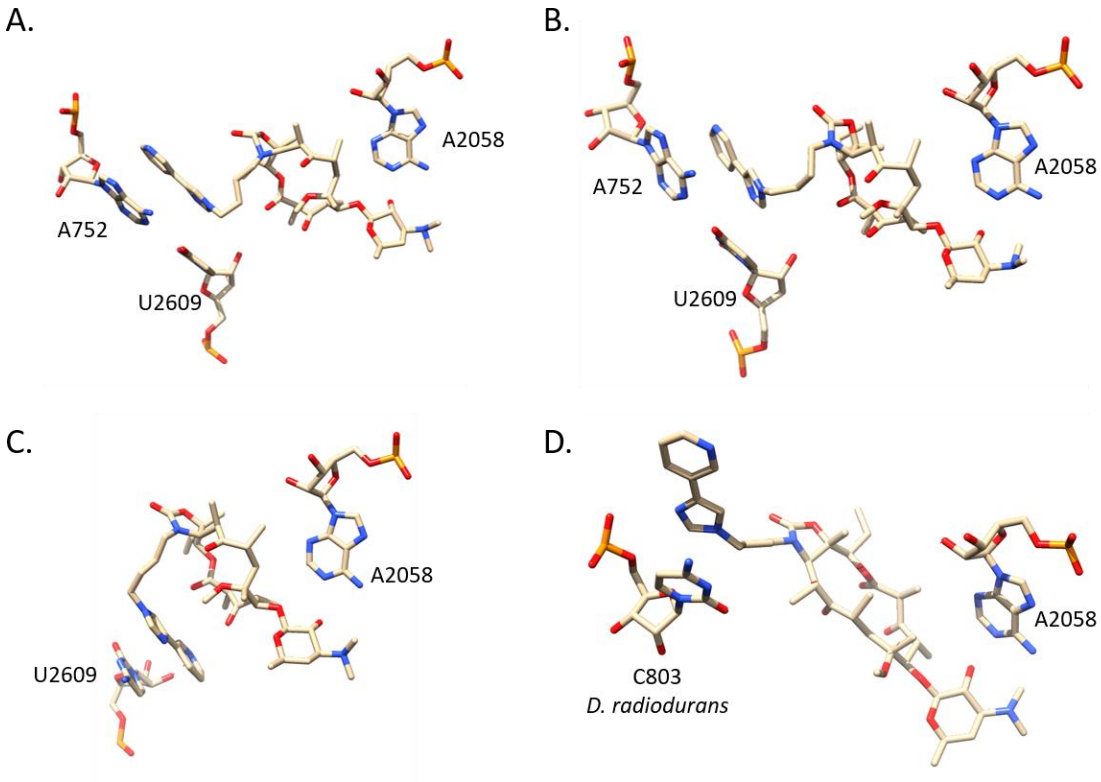
Crystal structures of telithromycin have also been reported for several bacterial species.[85,89-92] Unlike the desosamine moiety, the imidazole-pryidyl side chain of telithromycin does not show a conserved binding mode among all bacterial species. In *E.coli* and *T. thermophilus*, residue A752 in domain II and U2609 of domain IV come together to form a base pair (Figure 1.23A & B). These two aromatic bases form the binding region of the telithromycin sidechain where the pyridine ring π-stacks with A752 and the imidazole ring π-stacks with U2609. Biochemical experiments with clinically relevant bacteria have shown that this binding interaction most closely matches that of pathogenic bacteria and is seen as the best model system for design of novel macrolide and ketolide antibiotics.[90] It has been shown that the deletion of A752 or a U2609C mutation confers resistance to ketolide antibiotics but not to macrolides.[93] The binding orientation of the telithromycin sidechain in both *H. marismortui* (Figure 1.23C) and *D. radiodurans* (Figure 1.23D) supports this hypothesis. *H. marismortui* lacks the A752 residue while retaining the U2609 residue. The sidechain π-stacks with U2609 but adopts a different conformation

Figure 1.22. (A) Erythromycin A bound to 50S *E. coli* (PDB = 4V7U) (B) Overlap of Erythromycin A bound to *E. coli* (red, PDB = 4V7U), *D. radiodurans* (green, PDB = 1JZY), and *H. marismortui* (yellow, PDB = 1YI2). (C) Overlap of erythromycin A bound to *E. coli* (red, PDB = 47VU), Azithromycin bound to *T. thermophilus* (blue, PDB = 4V7Y), Clarithromycin bound to *D. radiodurans* (green, PDB = 1KO1), and roxithromyin bound to *D. radiodurans* (yellow, PDB = 1JZZ).



in the absence of the U2609-A752 base pair. *D. radiodurans* contains a cytosine residue in place of U2609. Again, the sidechain forms a π-stacking interaction with this base, but adopts a different conformation in the absence of A752.

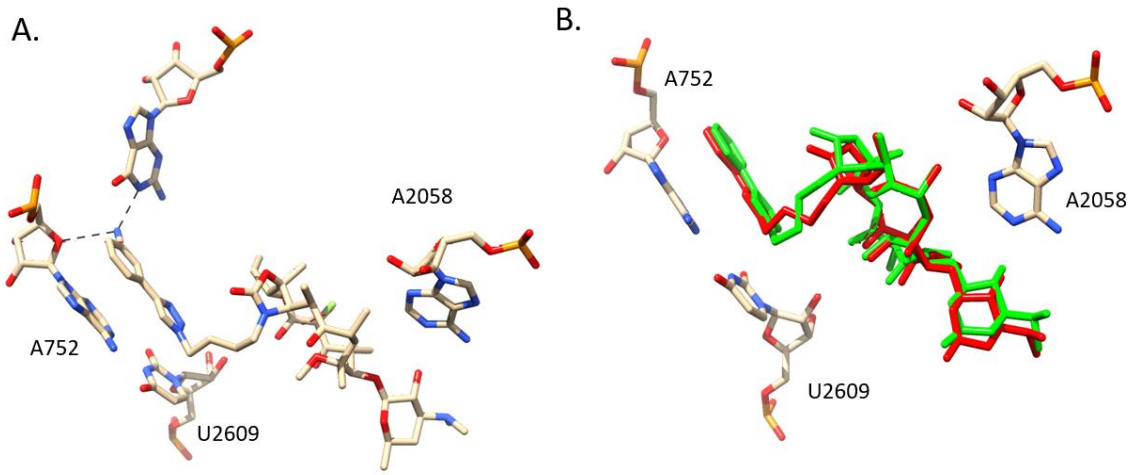
Figure 1.23. Telithromycin bound to bacterial ribosomes from (A) *E. coli* (PDB = 3OAT), (B) *T. thermophilus* (PDB = 3OI5), (C) *H. marismortui* (PDB = 1YIJ), and (D) *D. radiodurans* (PDB = 1P9X).



The structure of solithromycin bound to the *E. coli* ribosome has also been reported.[89] Compared to telithromycin, solithromycin contains an aniline in the place of pyridine, a 1,2,3-triazole in place of imidazole, and a C-2 fluoro moiety (Figure 1.20). The binding orientation of solithromycin correlates almost perfectly with that of telithromycin

(Figure 1.24B). Solithromycin is slightly more active than telithromycin against resistant strains of *S. pneumoniae* and *S. pyogenes*.[89] This increase in activity can be attributed to an additional H-bond interaction between the solithromycin aniline nitrogen and either N6 of G748 or the ribose sugar of A752, in addition to the C2-fluoro that forms a hydrophobic interaction with the aromatic heterocycle of C2611[89] (Figure 1.24A).

Figure 1.24. (A) Solithromycin bound to *E. coli* ribosome (PDB = 3ORB) (B) Solithromycin (red, PDB = 3ORB) and telithromycin (green, PDB = 3OAT) bound to *E. coli* ribosome.

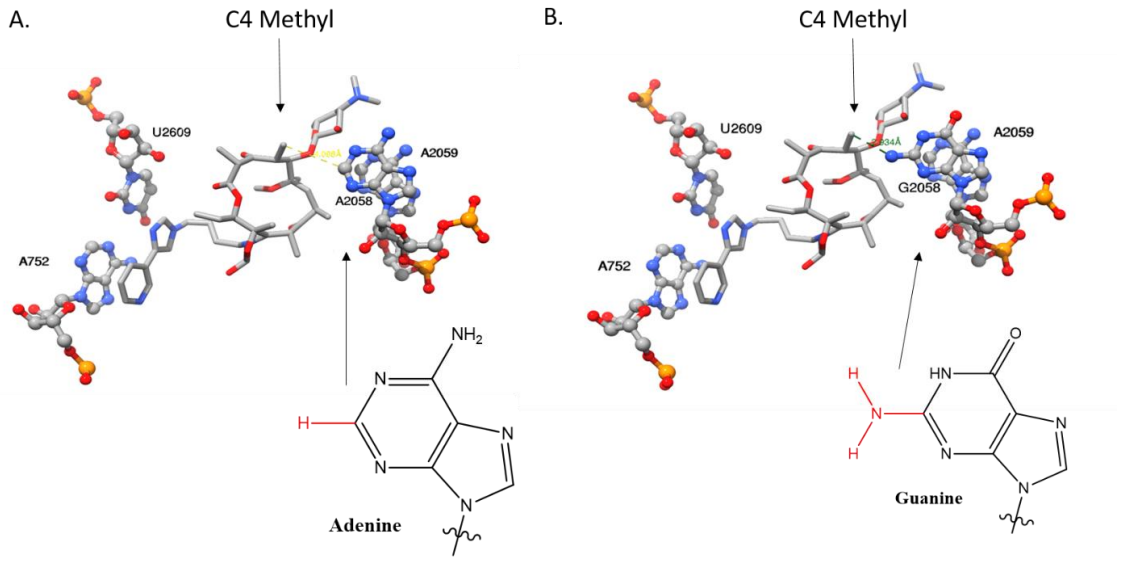


1.6 Desmethyl Telithromycin Analogues – Combating Bacterial Resistance

As previously mentioned, the A2058 residue is of the upmost importance for maintaining strong binding interactions with the desosamine sugar of macrolides. *H. marismortui* belongs to the domain Archaea, and as such naturally contains a G2058 residue, like Eukaryotes, in place of the A2058 residue that is present in bacteria. Mutation of bacterial rRNA (A2058G) confers resistance to macrolide antibiotics and is common in

S. pneumoniae, *S. aureus*, and *H. influenza*.[61,62] Steitz, in his work crystalizing macrolides and other antibiotics in ribosomes isolated from *H. marismortui*, had to first isolate G2058A mutants of the ribosomes.[78,85,86] After analysis of the high-resolution crystal structure of macrolides bound to the G2058A *H. marismortui* ribosomes, Steitz suggested that a steric clash between the C-4 methyl of macrolides and the exocyclic guanine nitrogen in A2058G bacterial mutants.[85] Examination of the crystal structure of telithromycin bound to *E.coli* (Figure 1.25A) shows there is a 4.1 Å distance between the C-4 methyl and residue A2058. In comparison, a computational mutation of adenine to guanine shows only a 2.9 Å distance from the exocyclic nitrogen of guanine to the C-4 methyl of telithromycin, which could sterically push the macrolactone away from the 2058 residue disrupting the binding interaction (Figure 1.25B).

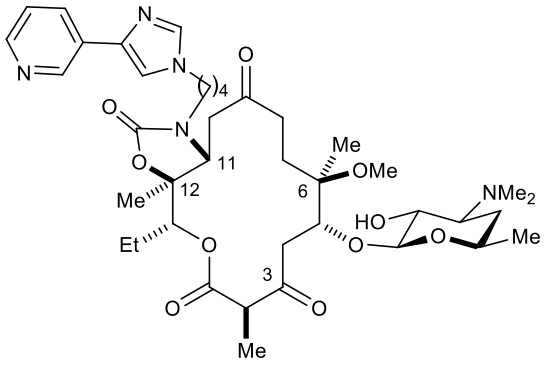
Figure 1.25. (A) Crystal structure of telithromycin bound to *E. coli* (PDB = 3OAT). (B) Computationally modified A2058G mutant of telithromycin bound to *E. coli*.



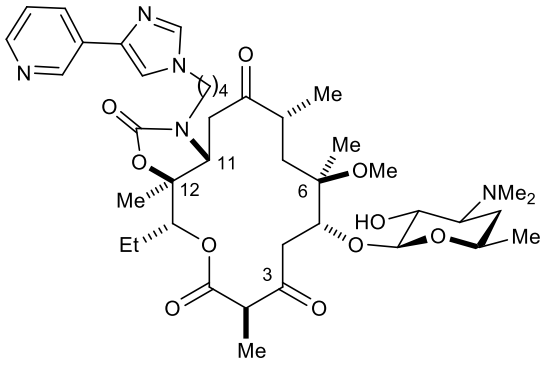
1.6.1 Design of Desmethyl Telithromycin Analogues

While the SAR and development of new generations of macrolides has been extensively explored by manipulating reactive functionalities (i.e. hydroxyls, carbonyls, amines) on both the macrolactone and carbohydrates, little to no modification of the hydrocarbon skeleton has been accomplished due to the need for multistep *de novo* synthesis, which has generally been avoided in the pharmaceutical industry.

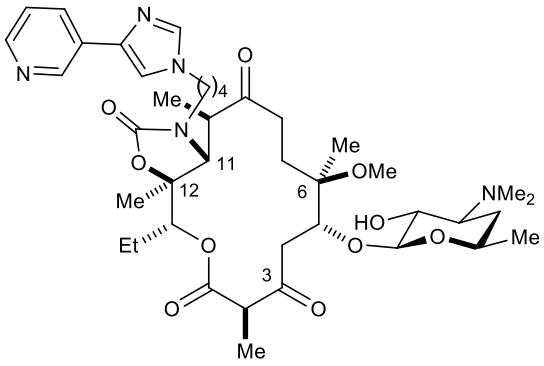
Figure 1.26. Structure of 4,8,10-tridesmethyl telithromycin (**1.66**), 4,10-didesmethyl telithromycin (**1.67**), 4,8-didesmethyl telithromycin (**1.68**), and 4-desmethyl telithromycin (**1.69**).



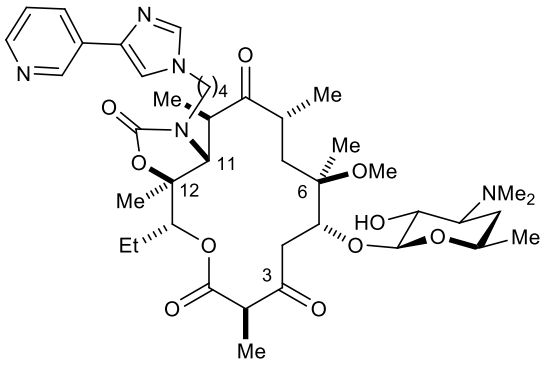
1.66
4,8,10-tridesmethyl telithromycin



1.67
4,10-didesmethyl telithromycin



1.68
4,8-didesmethyl telithromycin



1.69
4-desmethyl telithromycin

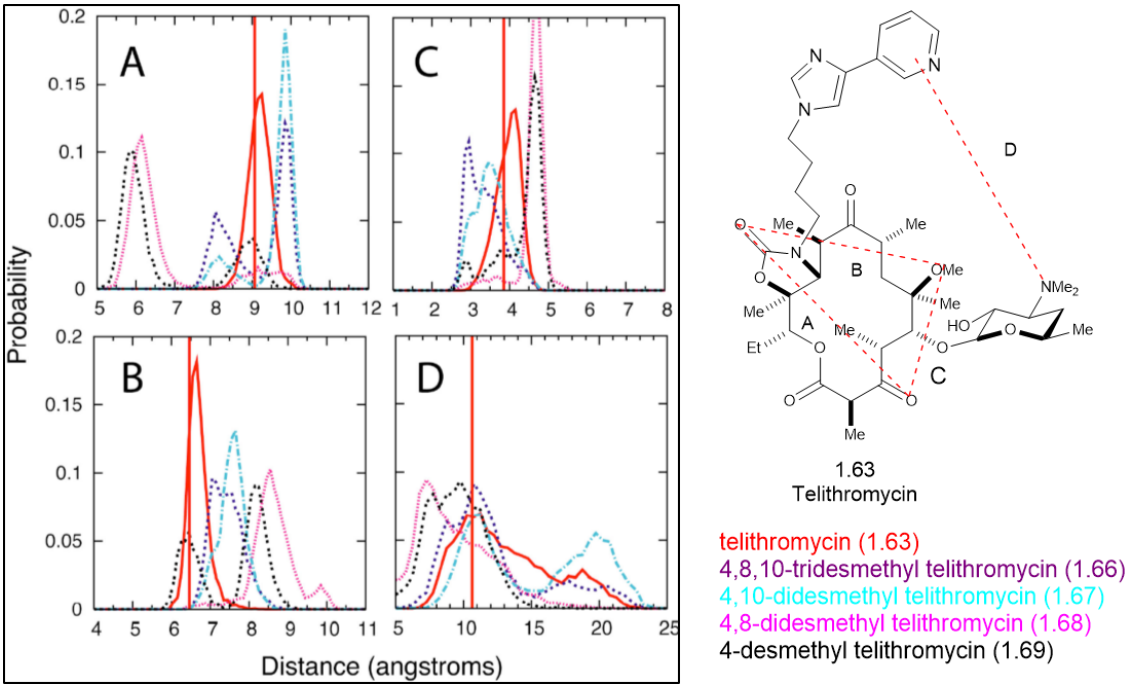
As part of a plan to show that 4-desmethyl telithromycin (**1.63**) could improve activity to A2058G mutants, the Andrade lab embarked on a plan to create four desmethyl analogues that would test the steric clash hypothesis and also provide some much needed SAR regarding the importance of the methyl groups decorating the macrolactone core. The plan entailed the total synthesis of 4,8,10-tridesmethyl telithromycin (**1.66**)[94,95], 4,10-didesmethyl telithromycin (**1.67**)[96], 4,8-didesmethyl telithromycin (**1.68**)[97], and 4-desmethyl telithromycin (**1.69**)[98] (Figure 1.26). Described below are the major challenges encountered during the synthesis of the first three desmethyl analogues **1.66**, **1.67**, and **1.68**. These syntheses have been thoroughly discussed in the Ph. D. theses of Dr. Venkata Velvadapu and Dr. Bharat Wagh.

1.6.2 Computer Aided Drug Design: Conformationally Sampled Pharmacophores (CSP)

A priori, the removal of methyl groups (i.e., desmethylation) will have some effect on the possible conformations the desmethyl analogues could achieve, which in turn could affect the binding of the compound to the ribosomal target. To test the consequence of desmethylation on the conformational flexibility, we entered into a collaboration with Dr. Alexander MacKerell–the Director of the Computer Aided Design Center at the University of Maryland, School of Pharmacy–who conducted a Conformationally Sampled Pharmacophore (CSP) analysis to test the conformational space of the four desmethyl analogues targeted for chemical synthesis. To this end, Hamiltonian Replica Exchange Molecular Dynamics simulations were used to sample the conformational space of **1.63**, **1.66**, **1.67**, **1.68**, and **1.69**. Next, probability distributions were plotted for the four distances

(A-D) shown in Figure 1.27. The data generated was compared to the crystal structure of **1.63** bound to the ribosome (solid red line). While the probability distributions of the four desmethyl telithromycin analogues sample a much greater chemical space than telithromycin, there is significant overlap to suggest that the desmethyl analogues could bind the ribosome.

Figure 1.27. Comparison of CSP probability distributions of **1.63**, **1.66**, **1.67**, **1.68**, **1.69**.



1.7 Highlights, Synthetic Challenges, and Solutions Toward the Synthesis of (–)-4,8,10-Tridesmthyl Telithromycin (1.66)

1.7.1 Retrosynthetic Analysis of Desmethyl Telithromycin Analogues

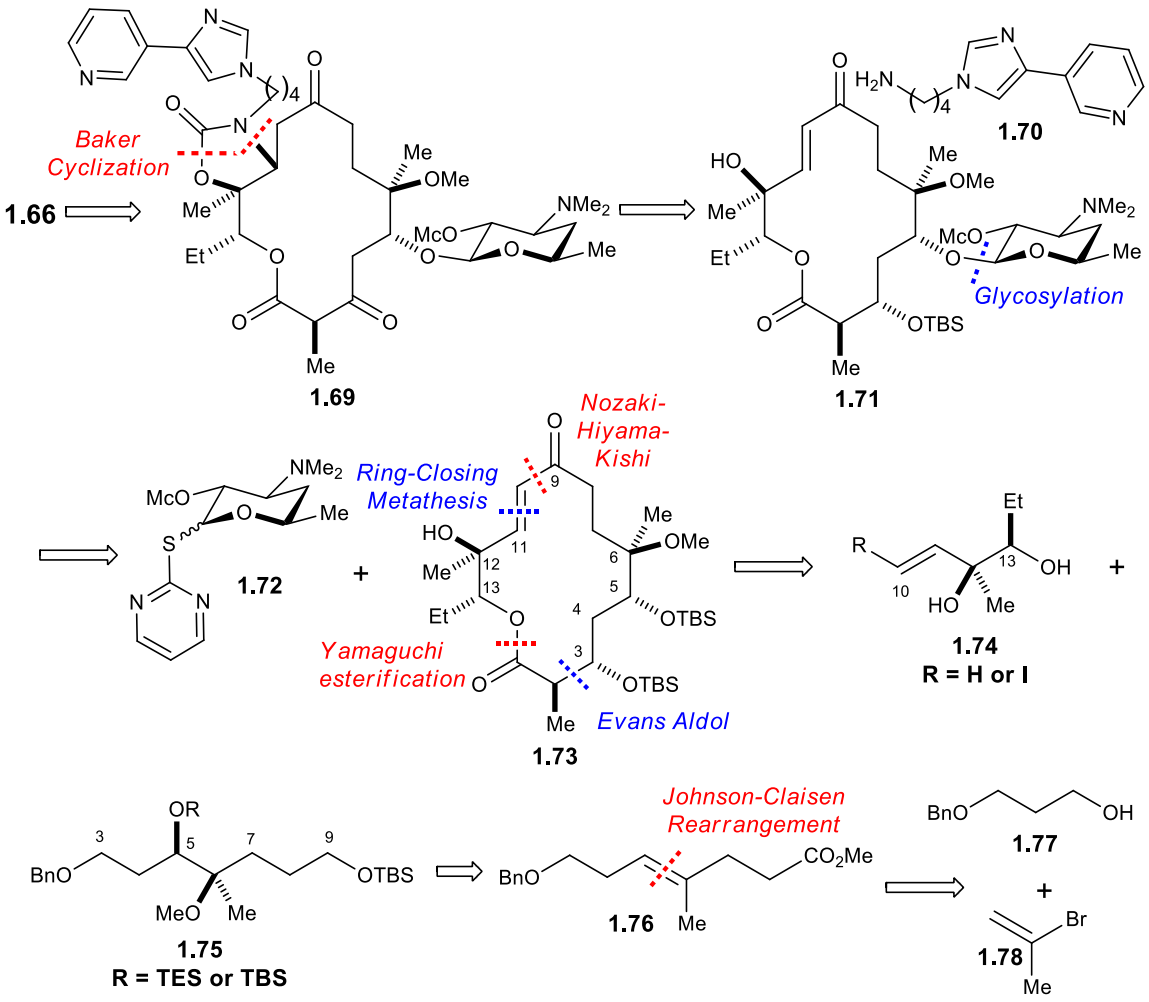
The retrosynthetic analysis of tridesmethyl analogue **1.66** begins with 2'-*O*-methylcarbonate-protected telithromycin (**1.69**) and represents the general plan that was

followed for all four analogues. Installation of the biaryl, butylamino sidechain **1.70** was chosen to be the last major synthetic operation in the synthesis being formed from glycosylated macroketolactone **1.71**. Stereoselective glycosylation would be accomplished utilizing the Woodward thiopyrimidine donor **1.72** and macrolactone **1.73**. The rationale for the 2'-O-methylcarbonate (Mc) protecting group was to ensure a highly stereoselective, 1,2-*trans* glycosylation event by means of well-established neighboring group participation.[99] The macrolactone was envisioned to be formed through reactions such as the Yamaguchi macrolactonization[100] to close the C-1–O-14 bond, Ring Closing Metathesis[101] (RCM) to access the C-10–C-11 double bond, or Nozaki-Hiyama-Kishi[102,103] (NHK) coupling to form the C-9–C-10 single bond. Macrolactone **1.73** would be formed by joining fragments **1.74** and **1.75**. The former would be derived from a kinetic resolution of racemic allylic alcohols using the Sharpless Asymmetric Epoxidation[104] (SAE) reaction to set both the C-12 and C-13 stereocenters. The latter would be prepared with the Sharpless Asymmetric Dihydroxylation[105] (SAD) reaction to set vicinal C-5 and C-6 stereocenters, in addition to the Johnson-Claisen rearrangement[106] to establish the C-3–C-9 framework.

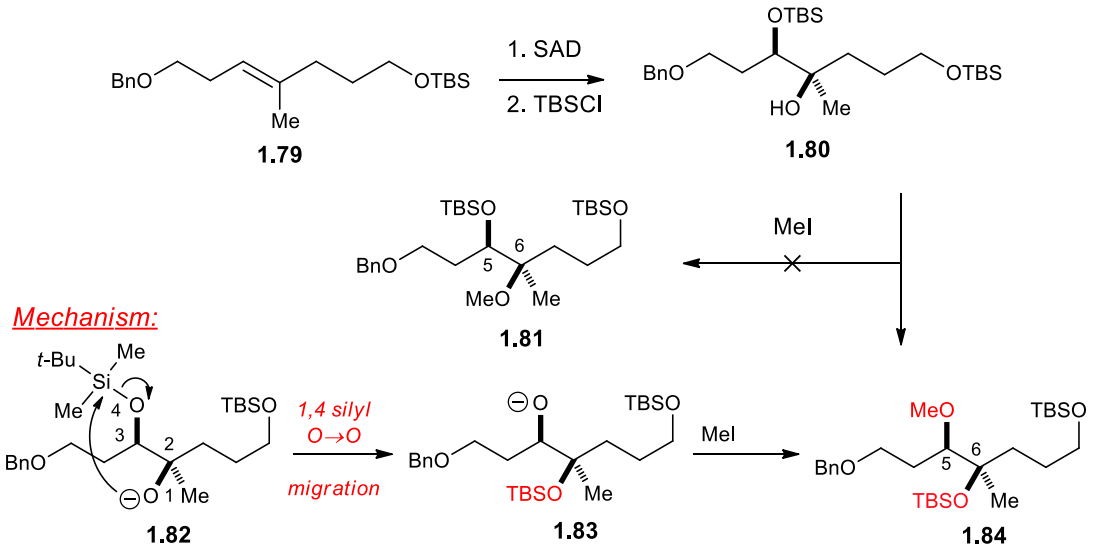
1.7.2 An Unexpected 1,4-Silyl Migration

The first major challenge in the synthesis of **1.66** came after SAD and TBS protection to form tertiary alcohol **1.80**. Methylation with MeI and NaH did not result in the expected tertiary methyl ether **1.81**. Alternatively, the alkoxide **1.82** attacked the silicon of the TBS ether transferring to the C-6 hydroxy. The resulting reactive intermediate **1.83** was quenched with MeI to form isolated product **1.84**.

Scheme. 1.3. Retrosynthetic Analysis of Desmethyl Telithromycin Analogues.

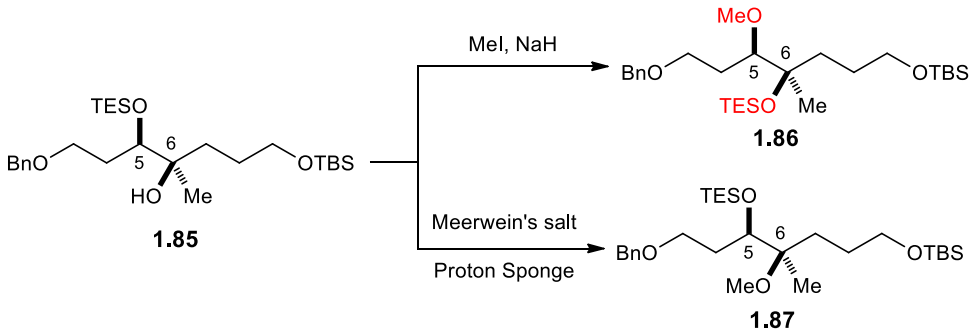


Scheme 1.4. 1,4-Silyl Migration to form undesired product **1.84**.



Ultimately, the problem was rectified through a swap of the C-5 protecting group to a triethylsilyl (TES) ether and particularly the use a more active alkylating agent, which would avoid the generation of the reactive alkoxide intermediate. As such, trimethyloxonium tetrafluoroborate (i.e., Meerwein's salt) with Proton Sponge were successfully employed on intermediate **1.85** to provide the desired product **1.87** over **1.86** (Scheme 1.5)**.**

Scheme 1.5. Avoidance of 1,4-Silyl Migration.



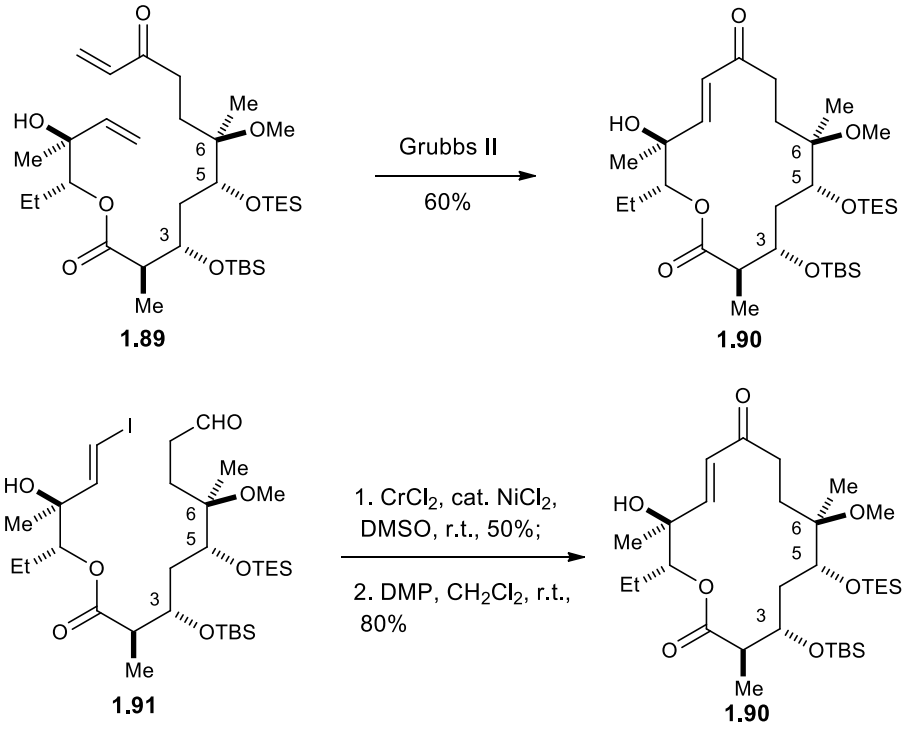
1.7.3 Successful Macrolactone Cyclization with NHK or RCM Tactics

One of the biggest challenges in the chemical synthesis of erythromycin and its congeners is a successful macrocyclization approach, which have provided many challenges for those familiar with the history of erythromycin's total synthesis (*vide infra*). Two separate cyclization methods, the NHK coupling and RCM reactions, were attempted. To the delight of the Andrade lab, both conditions provided a successful cyclization. Intermediate **1.89** was cyclized with Grubbs II by Dr. Venkata Velvadapu to give macrolactone **1.90** in 60% yield, whereas intermediate **1.91** was subjected to $CrCl_2$ with catalytic $NiCl_2$ to provide **1.90** in 40% yield by Drs. Tapas Paul and Bharat Wagh (Scheme 1.6). While the RCM approach resulted in a higher yield, it was advantageous to have an alternate cyclization method for future desmethyl analogues.

1.7.4 Deprotection of C-5 TES Ether

Initial attempts to deprotect the C-5 TES ether of **1.90** under acidic conditions with either *p*-TsOH or pyridinium *p*-toluenesulfonate (PPTS) resulted in the formation of hemiketal **1.91**. Use of the fluoride source *n*-tetrabutylammonium fluoride (TBAF) successfully removed the C-5 TES group; however, the C-3 TBS was also removed. To avoid the ketalization, the C-9 keto functionality was reduced under Luche conditions. The TES ether could then be safely removed with *p*-TsOH, followed by chemoselective TES protection of the allylic C-9 alcohol to give **1.92** while also setting the stage for glycosylation (Scheme 1.7).

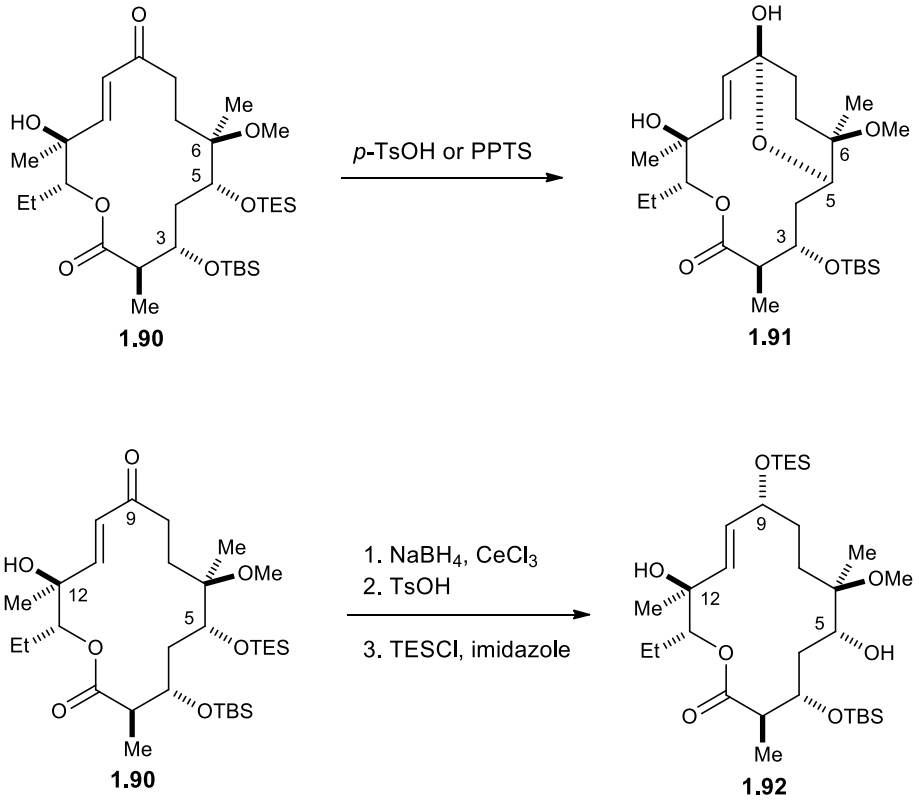
Scheme 1.6. Successful RCM and NHK cyclization reactions.



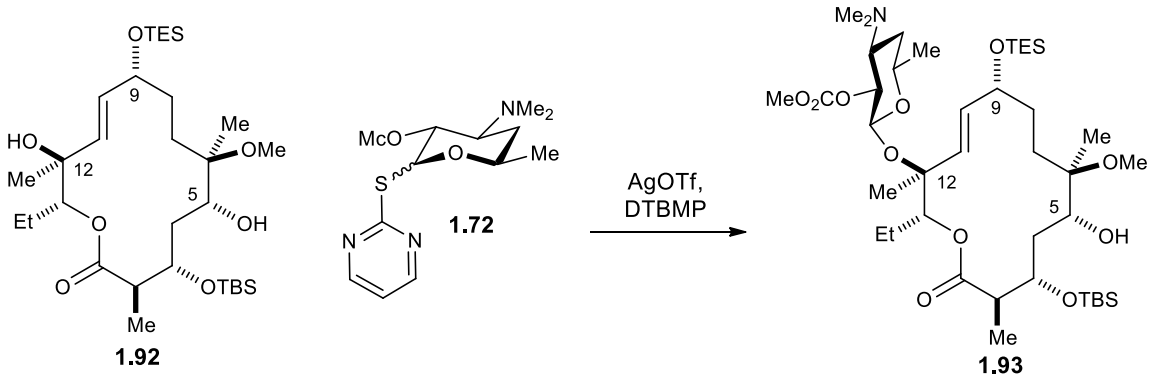
1.7.5 Route to Glycosylation

With **1.92** in hand, glycosylation was fully expected to react selectively at the secondary C-5 hydroxyl. Much to the dismay of the team, the reaction proceeded to form only one product which unfortunately was the C-12 glycosylated product **1.93**. The structure of **1.93** was rigorously established by 2D NMR experiments.[95] In the end, recourse to a fully protected acceptor would be needed. To this end, **1.90** was first reduced to the allylic alcohol then bis-TES protected at C-9 and C-12 positions with TESOTf. Removal of C-9 and C-5 TES groups with TsOH followed by reprotection of the allylic C-9 hydroxyl with TESCl gave glycosyl acceptor **1.94**. Glycosylation under standard conditions with **1.72** was successful. Bis-TES deprotection with TBAF followed by DMP oxidation provided **1.95**.

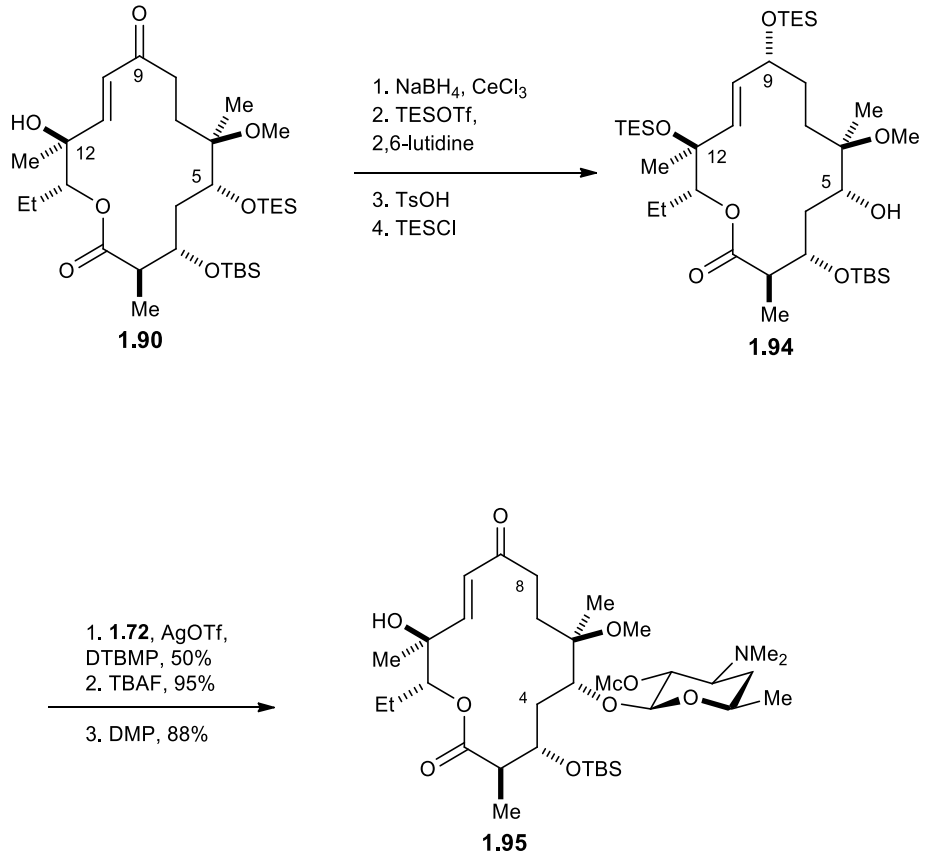
Scheme 1.7. Deprotection of the C-5 TES ether.



Scheme 1.8. Unwanted glycosylation of C-12 hydroxyl over C-5 hydroxyl.



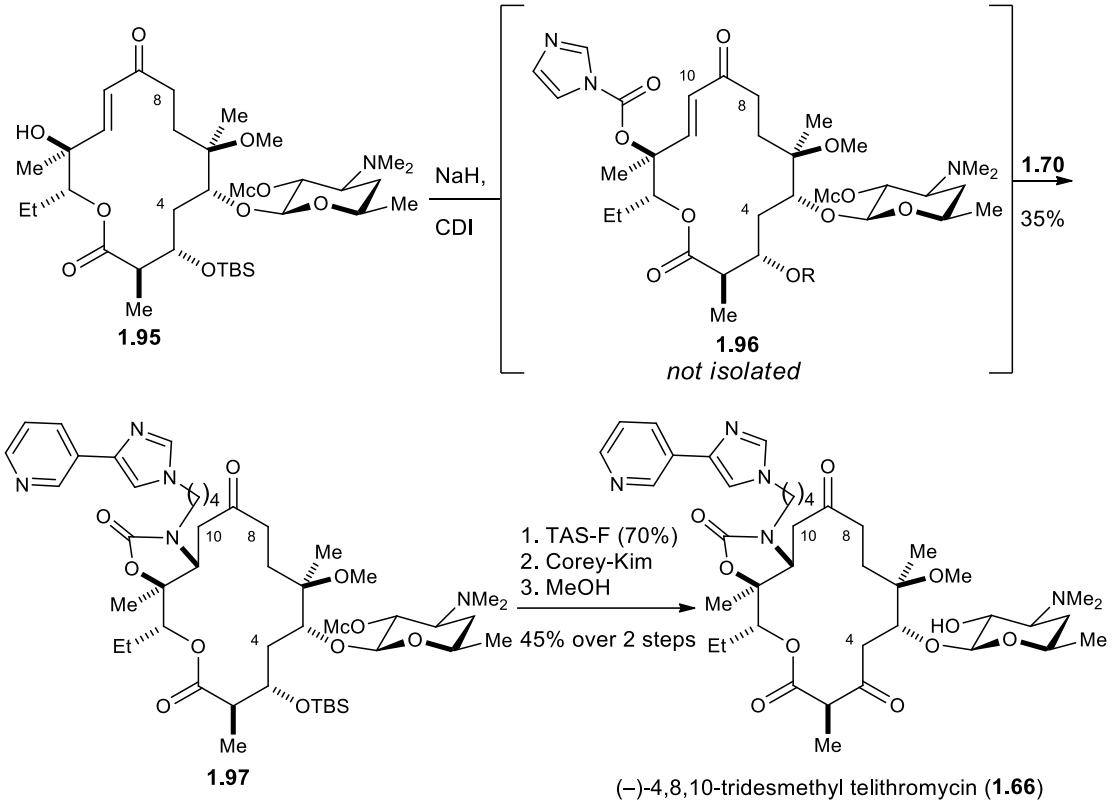
Scheme 1.9. Successful route to glycosylation.



1.7.6 Baker Cyclization and Successful Synthesis of (–)-4,8,10-Tridesmethyl Telithromycin

The end game to reach 4,8,10-tridesmethyl telithromycin (**1.66**) was accomplished without incident through the Baker cyclization to give **1.97** in 35% yield (Scheme 1.10). The three step sequence beginning with mild fluorine source tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F) to remove the C-3 TBS group in 70% yield[107], Corey-Kim oxidation to provide the C-3 ketone, and room temperature methanolysis of the 2'-methylcarbonate furnished 4,8,10-tridesmethyl telithromycin (**1.66**).[95]

Scheme 1.10. Endgame for 4,8,10-tridesmethyl telithromycin (**1.66**).



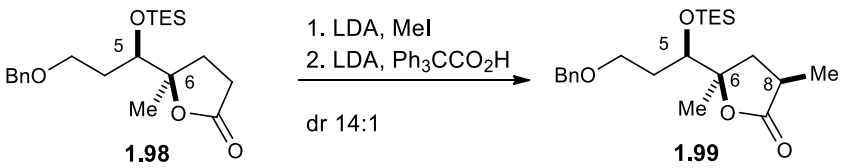
1.8. Highlights, Synthetic Challenges, and Solutions Toward the Synthesis of 4,10-Didesmthyl Telithromycin (1.67)

1.8.1. Stereoselective Installation of the C-8 Methyl Group

The first goal towards the total synthesis of 4,10-didesmethyl telithromycin (**1.67**) was to stereoselectively install the C-8 methyl group. This was accomplished in a two-step fashion by first alkylating the LDA-generated enolate of **1.98** with MeI. The resultant stereochemistry would be controlled by the bulky C-5 TES moiety and tertiary center at C-6 providing the unwanted diastereomer in greater yield. Re-enolization with LDA followed by kinetic protonation with PivOH quench gave the desired diastereomer **1.99** in a 6:1 dr

in 80% yield. Alternatively, use of the bulkier Ph_3CCO_2H in the kinetic protonation step furnished an even better dr of 14:1.

Scheme 1.11. Stereoselective Installation of C-8 Methyl.



1.8.2. Completion of the Total Synthesis of (–)-4,10-Didesmethyl Telithromycin (1.67)

The synthesis of 4,10-didesmethyl telithromycin (**1.67**) proceeded according to the procedures used in the synthesis of **1.66**. The RCM approach was successfully used to cyclize **1.100** to **1.101**. Hydroxyl protection conditions used earlier with **1.66** were employed again to allow regioselective glycosylation. The endgame of Baker cyclization, C-3 oxidation, and 2'-OH deprotection worked as described for **1.66** to yield 4,10-didesmethyl telithromycin (**1.67**) (Scheme 1.12).[96]

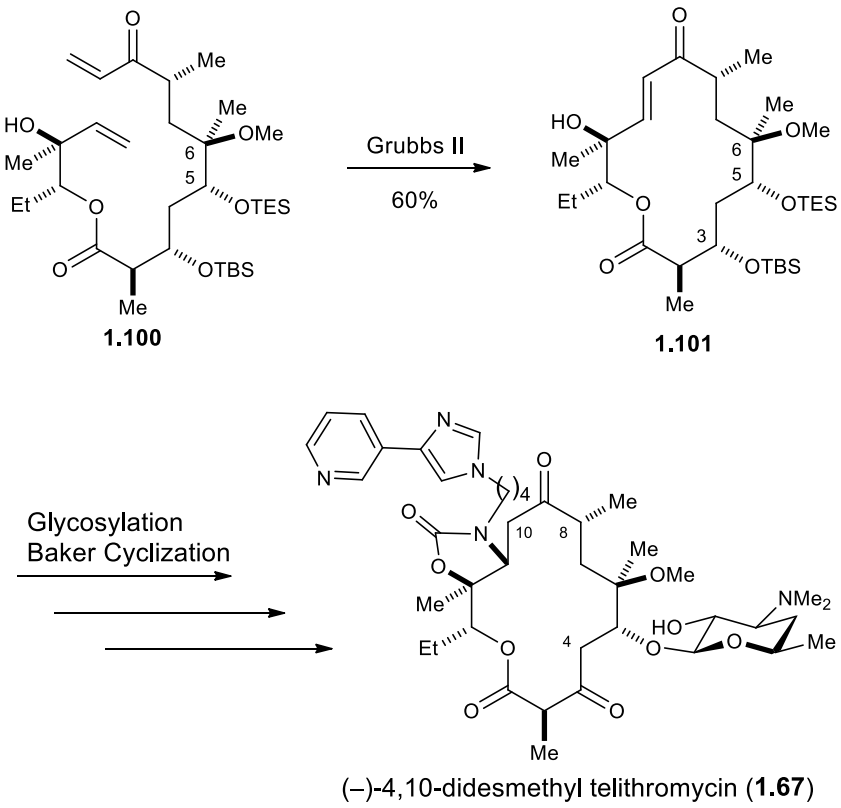
1.9 Highlights, Synthetic Challenges, and Solutions Toward the Synthesis of 4,8-Didesmthyl Telithromycin (1.68)

1.9.1 Cyclization Problems

The first challenge in the synthesis of 4,8-didesmethyl telithromycin (**1.68**) manifested in the macrocyclization key step. Attempts to utilize the RCM tactic on α,ω-diene **1.102** to access macrolactone **1.103** were unsuccessful, presumably due to the added bulk of the C-10 methyl. Recourse to the NHK tactic, which was initially developed in the synthesis of tridesmethyl analogue (**1.66**), successfully converted vinyl iodide **1.104** to

1.103 after DMP oxidation. Unfortunately, the synthesis of macrolactone **1.103** came at the expense of yield, delivering a painful 20% yield (Scheme 1.13).

Scheme 1.12. Endgame for 4,10-didesmethyl telithromycin.

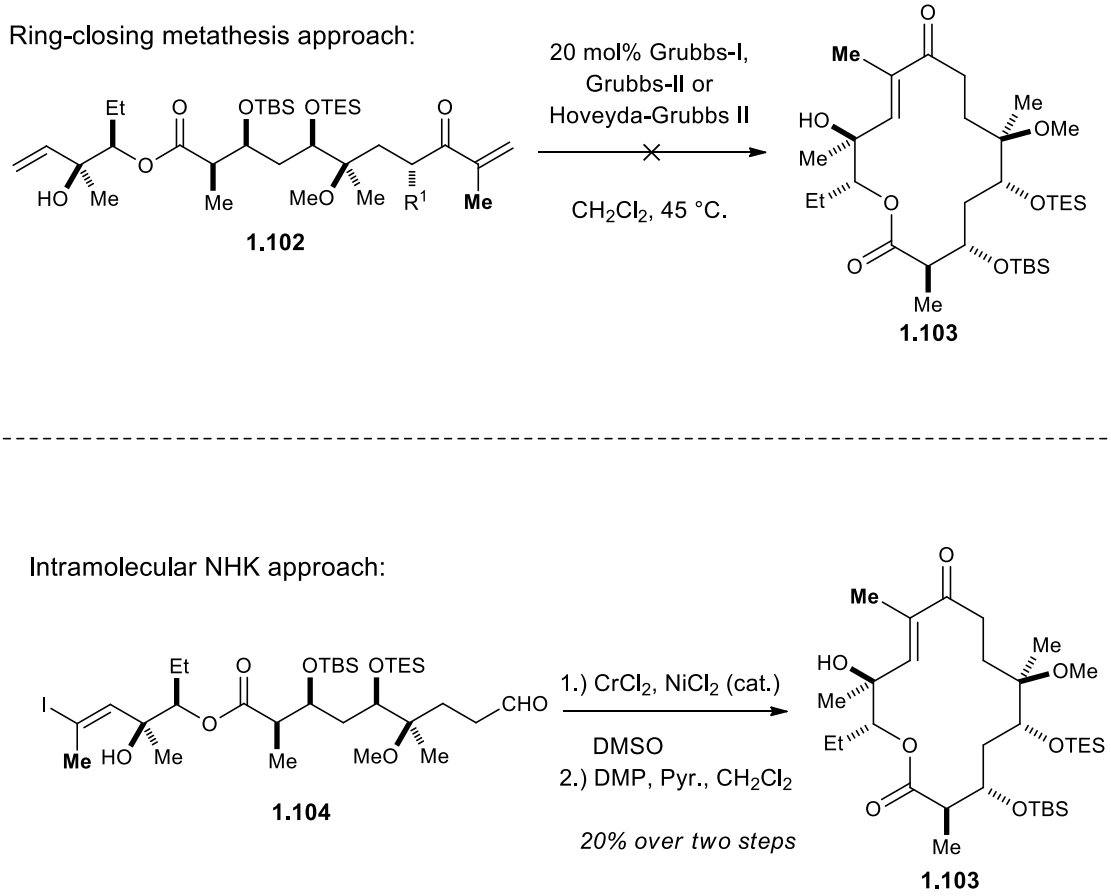


1.9.2 NHK Cyclization Post Glycosylation

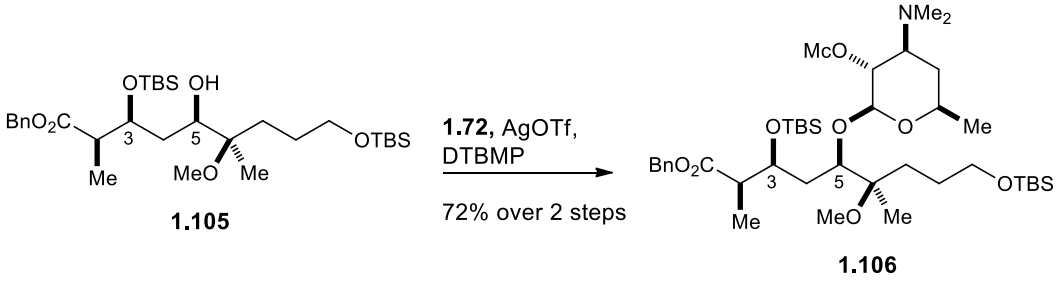
The low yield of the NHK cyclization event made the completion of **1.68** on scale a very difficult task. Fortunately, inspiration from Stephen Martin's synthesis of erythromycin B (i.e., 12-deoxyerythromycin A) would provide the solution.[108] Unlike Woodward's synthesis of erythromycin A, which installed the desosamine sugar *after* cyclization, Martin strategically chose to install desosamine *prior* to macrolactonization in a distinctly abiotic fashion that also benefited from increased convergency.[109] The current

glycosylation procedure called for a number of protection-deprotection steps to set the stage for glycosylation which would utilize too much material after a 20% yield NHK cyclization. If the glycosylation step could be performed prior to cyclization, then only the endgame consisting of Baker cyclization, C-3 oxidation, and 2'-carbonate deprotection would remain. This would ultimately serve to ameliorate the modest NHK yield help realize the synthesis of 4,8-didesmethyl telithromycin (**1.68**). To this end, **1.105** was successfully glycosylated with **1.72** furnishing **1.106** in 72% yield.

Scheme 1.13. Failed RCM attempt and Low Yielding NHK cyclization to **1.103**.



Scheme 1.14. Glycosylation of **1.105** to form key intermediate **1.106**.

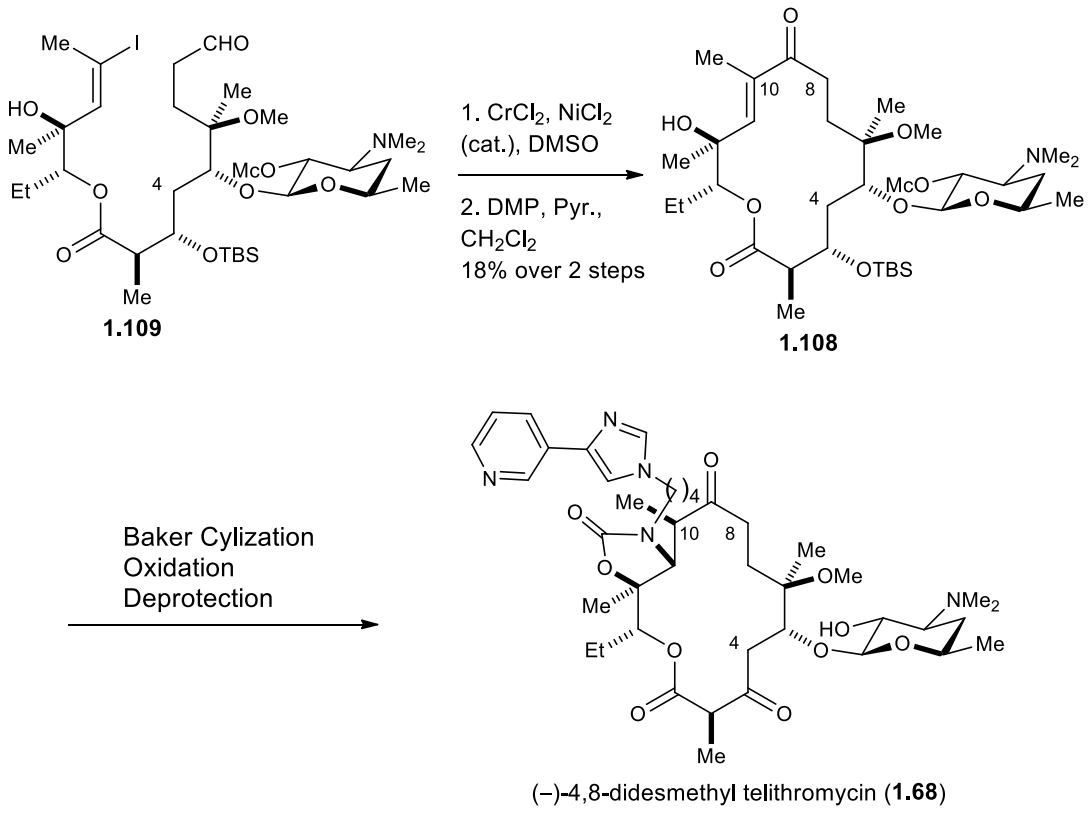


1.9.3 Intramolecular NHK Tactic in the Presence of Desosamine and Completion of (–)-4,8-Didesmethyl Telithromycin (1.68)

The intramolecular NHK reaction of vinyl iodide **1.109** in the presence of C-5 desosamine, followed by DMP oxidation of the diastereomeric allylic alcohols, proceeded in 18% yield over two steps (Scheme 1.15). The efficiency of this procedure was nearly identical to the C-5 TES ether **1.104** (20% yield). By employing the same endgame approach used for desmethyl analogues **1.66**, **1.67**, and **1.68**, 4,8-didesmethyl telithromycin (**1.68**) was completed without incident.[97]

At this stage, the completion of three of the desmethyl analogues was complete. The proceeding chapter will describe this total synthesis of (-)-4-desmethyl telithromycin and the challenges associated with it. While a comparison of biological evaluation results of the three previously described analogues could be placed here, it seems most appropriate to present all of the data at the end of the subsequent chapter.

Scheme 1.15. Endgame for 4,8-Didesmethyl Telithromycin (**1.68**).

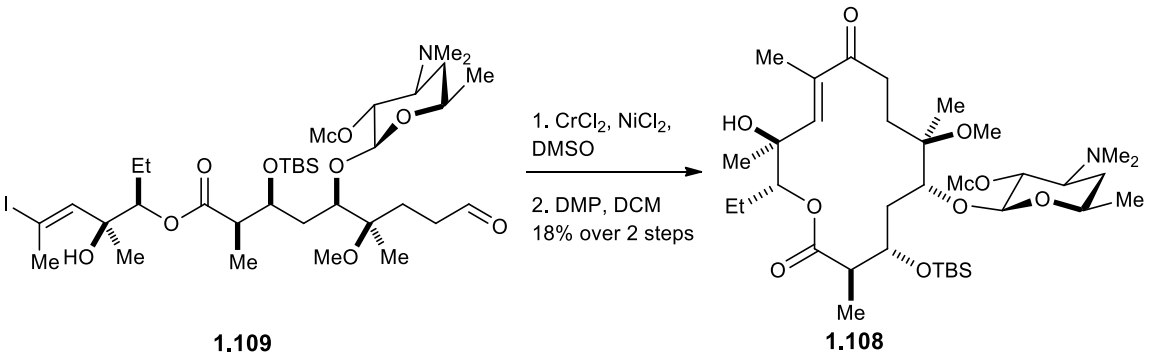


CHAPTER 2: TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF (–)-4-DESMETHYL TELITHROMYCIN

2.1 Introduction

With the successful completion of the total synthesis of 4,8,10-tridesmethyl telithromycin (**1.66**), 4,10-didesmethyl telithromycin (**1.67**), and 4,8-didesmethyl telithromycin (**1.68**) we felt confident that the final compound in the series, 4-desmethyl telithromycin (**1.69**), could be synthesized utilizing the knowledge gained to this point. The powerful Nozaki-Hiyama-Kishi (NHK) coupling used to cyclize the macrolactone of 4,8-didesmethyl telithromycin (**1.68**) could provide us with a viable route to complete the final analogue in the series (Scheme 2.1).[102,103]

Scheme 2.1. Successful NHK cyclization en route to (–)-4,8-didesmethyl telithromycin (**1.68**).

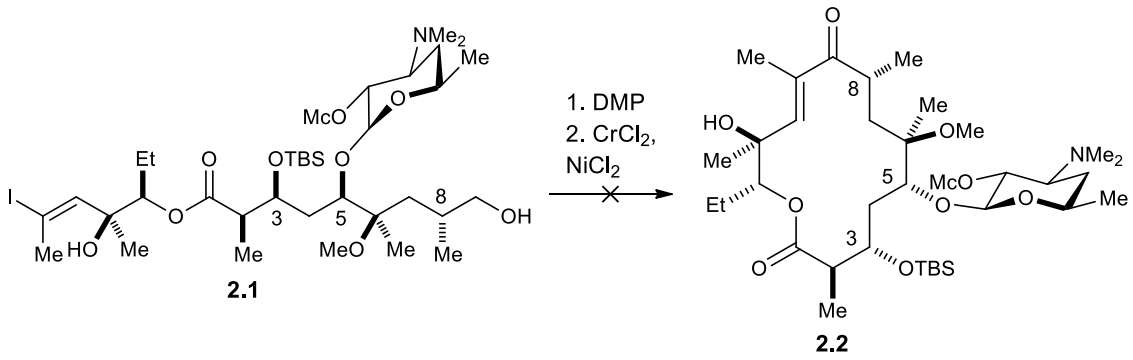


1.109 **1.108**

However, much like the failure of translating the RCM approach to cyclization for 4,8-didesmethyl telithromycin due to the additional C-10 methyl, 4-desmethyl telithromycin's additional methyl substituent at C-8 would severely limit the utility of the NHK cyclization as shown in Scheme 2.2 where NHK cyclization of macrolactone

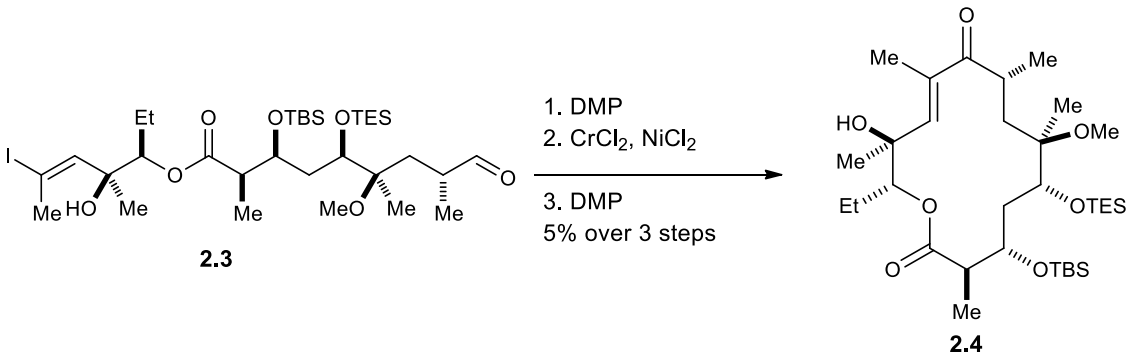
precursor **2.1** containing 2'-methyl carbonate protected desosamine at C-5 was unsuccessful (Scheme 2.2).

Scheme 2.2. Unsuccessful NHK cyclization of intermediate **2.1**.



Recourse to a C5-TES protected cyclization precursor **2.3** only provided 5% yield of the desired macrolactone **2.4** (Scheme 2.3). As with the previous analogues, a new route and method of cyclization would be needed.

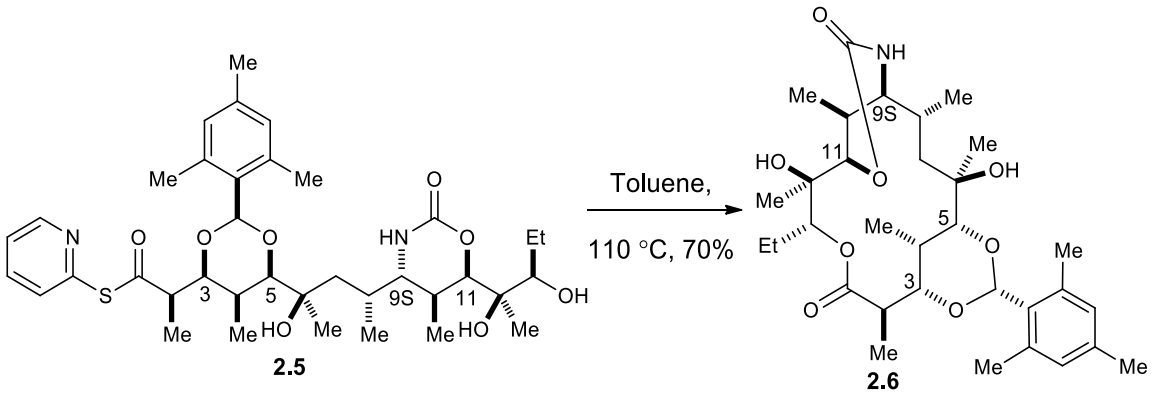
Scheme 2.3. Cyclization of C5-TES protected macrolactone precursor **2.3**.



While historically macrolactonization has been by far the most popular approach to the synthesis of erythronolides and erythromycins, starting with Corey's elegant syntheses

of erythronolide A[110] and B[111], it wasn't until Woodward's 1981 synthesis of erythromycin A that an intensive structure-reactivity study was performed on the macrolactonization step.[112] The cyclization was attempted on 15 different seco-acid substrates, and it was found that efficient macrolactonization would only occur when certain structural motifs were in place. The stereochemistry at C-9 must be the *S* configuration and cyclic protecting groups must be employed at the C-3/C-5 and C-9/C-11 positions. Woodward's macrolactonization was effected with a Corey-Nicolaou macrolactonization of seco-acid **2.5** to form macrolactone **2.6** in 70% yield (Scheme 2.4).

Scheme 2.4. Woodward's successful macrolactonization of seco-acid **2.5** bearing 9-(*S*) stereochemistry and cyclic protecting groups at C-3/C-5 and C-9/C-11.



Since completion of this synthesis, there have been many notable syntheses of erythromycin[108] congeners and its aglycone precursor, erythronolide[113-118], all of which have utilized macrolactonization and all which have followed the stringent preorganization requirements Woodward established (Figure 2.1).

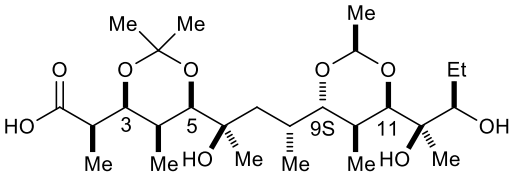
The observation can be made that while Woodward's macrolactonization used the Corey-Nicolaou methodology, all syntheses after Danishefsky's 1990 synthesis of 6-

deoxyerythronolide B utilized the Yamaguchi protocol, yet detailed structure-reactivity relationships were never re-explored. The persistent use of these stringent requirements for cyclization exhibits the colossal impact of Woodward's historical synthesis of erythromycin A. Fortunately, in 2011, Christina White explored these requirements and found that preorganization was not necessary.[119] Hydroxyl groups of a 6-deoxyerythronolide B seco-acid were protected as their methyl ethers and cyclization of **2.13** under Yamaguchi conditions resulted in **2.14** in 70% yield (Scheme 2.5). This encouraging study provided the inspiration for what would ultimately become the successful total synthesis of (−)-4-desmethyl telithromycin (**1.69**).

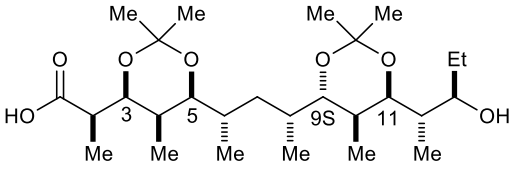
2.2 Retrosynthetic Analysis

Retrosynthetic analysis of **1.69** reveals late stage reactions to functionalize the macrolactone core (Scheme 2.6). The formation of the C-11–C-12 oxazolidinone ring bearing the butyl imidazole-pyridyl side chain would be derived from α, β-unsaturated ketone **2.15** and primary amine **1.70**, which was successful in the synthesis of previous desmethyl analogs. This one-pot carbamoylation/intramolecular aza-Michael reaction was first developed by Baker and co- workers[73] at Abbot Laboratories during their search for acid stable erythromycin A analogues, and late-stage execution of this methodology was eventually adopted by Hoechst Marion Roussel in their synthesis of telithromycin.[120] Next, stereoselective glycosylation of the C5 hydroxyl with Woodward's thiopyrimidine donor **1.72** results from suitably protected macrolactone **2.16**. Formation of the 14-membered lactone can be accomplished via intramolecular Yamaguchi macrolactonization of seco-acid **2.17**.

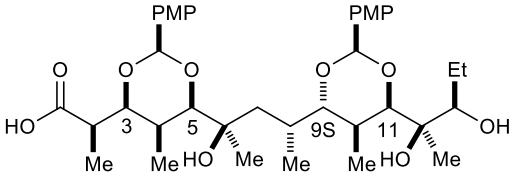
Figure 2.1. Preorganization of seco-acids of some notable Erythromycin and Erythronolide syntheses.



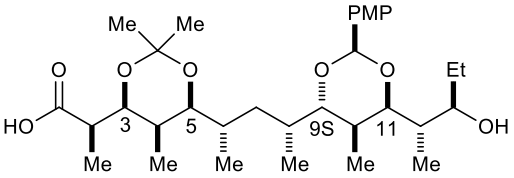
2.7
Stork et. al. 1987
(9*S*)-Dihydroerythronolide A



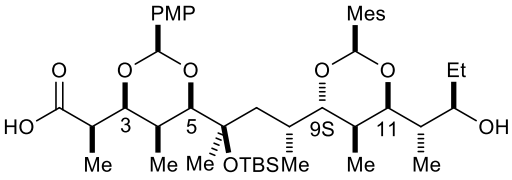
2.8
Danishefsky et. al. 1990
6-Deoxyerythronolide B



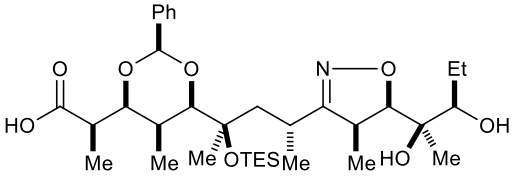
2.9
Hoffmann et. al. 1993
(9S)-Dihydroerythronolide A



2.10
Evans et. al. 1997
6-Deoxyerythronolide B

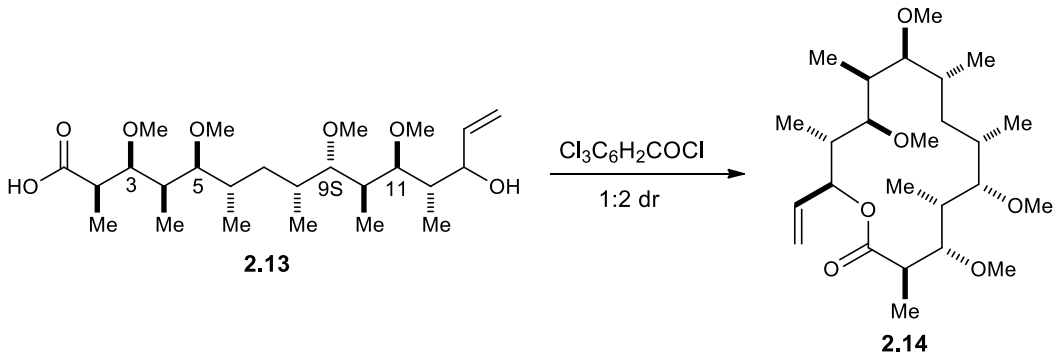


2.11
Martin et. al. 1997
Erythromycin B



2.12
Carreira et. al. 2005
Erythronolide A

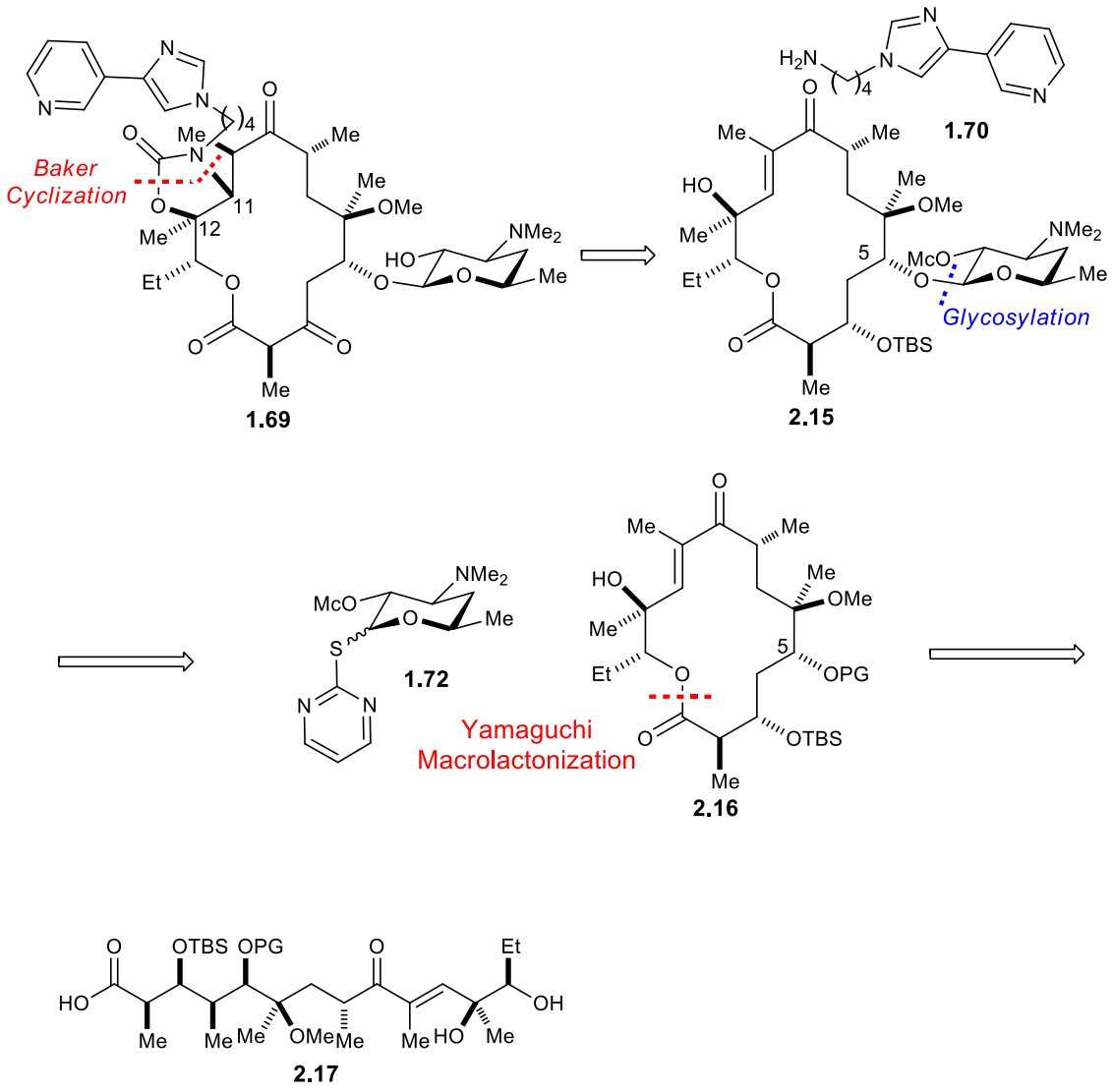
Scheme 2.5. White's successful macrolactonization of 6-deoxyerythronolide B seco-acid **2.13** containing no cyclic biasing elements.



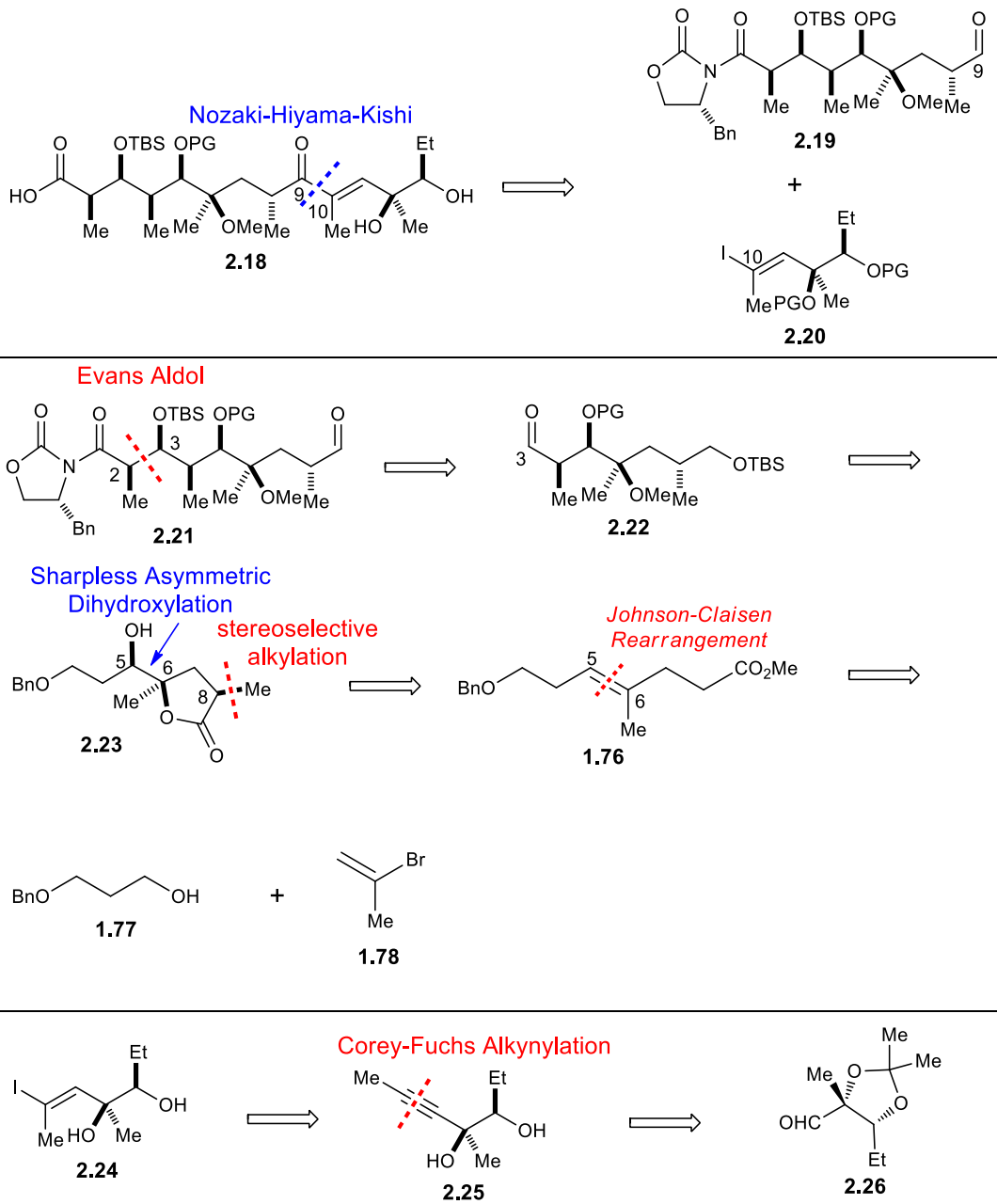
Seco-acid **2.18** can be formed by intermolecular Nozaki-Hiyama-Kishi coupling between aldehyde **2.19** and vinyl iodide **2.20**. Aldehyde **2.21** can be prepared by an Evans asymmetric propionate aldol with aldehyde **2.22**, which is prepared from lactone **2.23**.[121] Stereocenters at C-5 and C-6 can be set with a Sharpless asymmetric dihydroxylation of **1.76**, and the stereocenter at C-8 can be established with a substrate-controlled, diastereoselective alkylation. The C-5-C-6 double bond formation can be created from Johnson-Claisen Rearrangement of Grignard product formed from **1.77** and **1.78**. Vinyl iodide **2.24** can be synthesized from alkyne **2.25**, which can be synthesized from known aldehyde **2.26** by a Corey-Fuchs alkynylation (Scheme 2.7).

The coupling of aldehyde **2.27** and vinyl iodide **2.20** by a Nozaki-Hiyama-Kishi (NHK) reaction was a key transformation whose success was dependent on protecting groups used at C-12 and C-13 of **2.20** as well as the moieties located at C-5 of aldehyde **2.27**. The group implemented at C-5 also had major consequences for later stage chemistry

Scheme 2.6. Retrosynthetic analysis of (–)-4-desmethyl telithromycin (**1.69**).

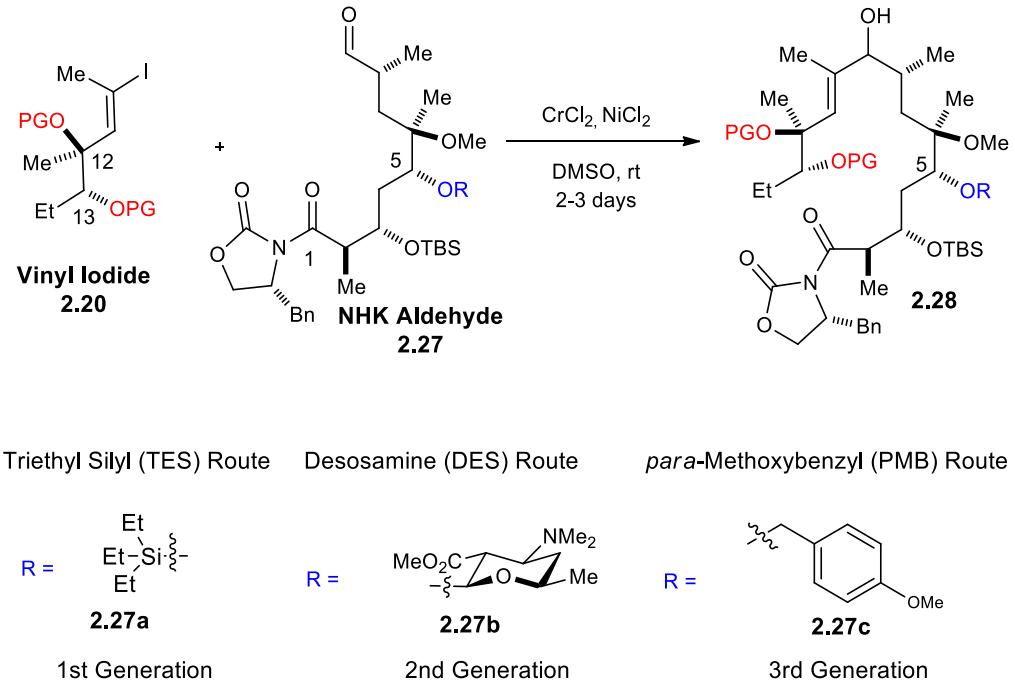


Scheme 2.7. Retrosynthetic analysis of seco-acid **2.18**.



performed after macrolactonization and as such several modifications to the synthesis were made and can be organized into three generations based on the group applied at C-5 (Scheme 2.8).

Scheme 2.8. Three major approaches toward the synthesis of 4-desmethyl telithromycin (**1.69**).



2.3 Current Study

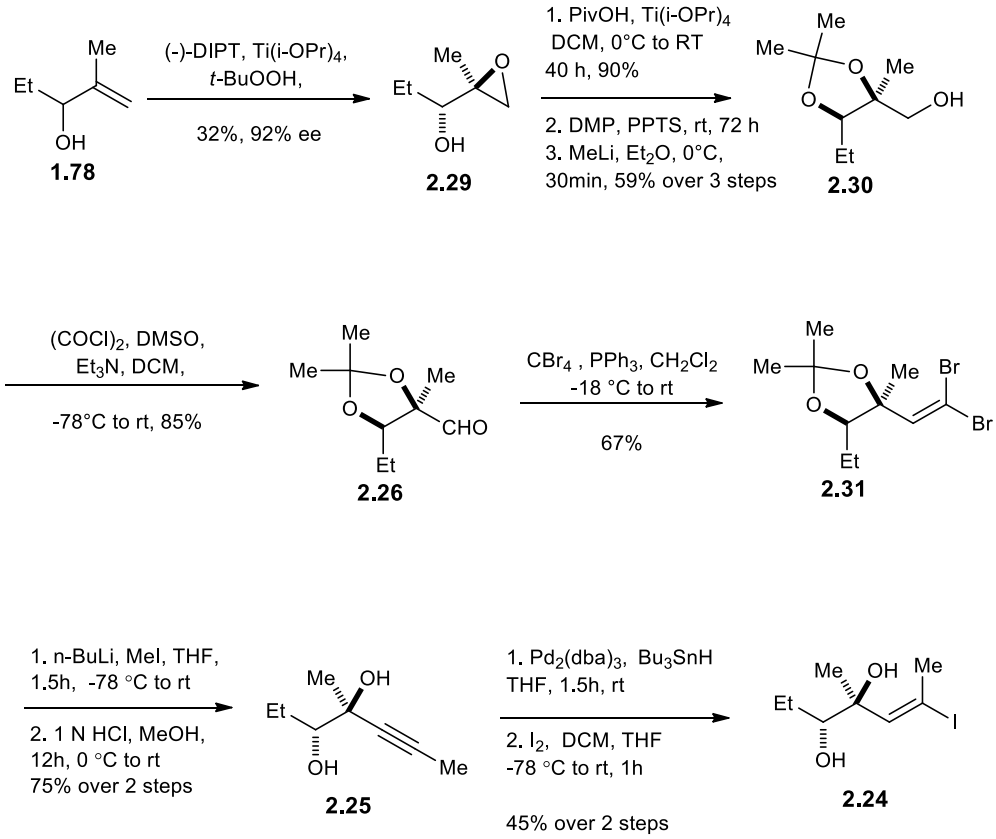
2.3.1 First-generation approach

2.3.1.1 Synthesis of vinyl iodide 2.24

The synthesis of vinyl iodide **2.24** (Scheme 2.9) begins with known racemic allylic alcohol **1.78** where a Sharpless asymmetric epoxidation provides a kinetic resolution resulting in a 32% yield of enantiopure epoxide **2.29**[122] Lewis acid-mediated epoxide

opening with pivalic acid, protection of the resultant diol as a dimethyl acetal, and cleavage of the ester with MeLi gave primary alcohol **2.30** in 63% over three steps. Swern oxidation furnished aldehyde **2.26** in 85% yield. Corey-Fuchs alkynylation provided a one carbon homologation to give dibromo olefin **2.31** in 67% yield.[123] Elimination of bromine with 2 equivalents of *n*-BuLi formed a lithium acetylide, which was subsequently trapped with methyl iodide. Acid-catalyzed hydrolysis of the acetonide afforded alkyne diol **2.25** in 75% over two steps. Palladium-catalyzed hydrostannylation furnished the requisite (*E*)-trisubstituted vinyl stannane, which underwent facile tin-iodide exchange to give vinyl iodide **2.24** in 45% yield over two steps.[124]

Scheme 2.9. Synthesis of vinyl iodide **2.24** for the NHK coupling reaction.



2.3.1.2 Synthesis of Aldehyde for NHK Coupling

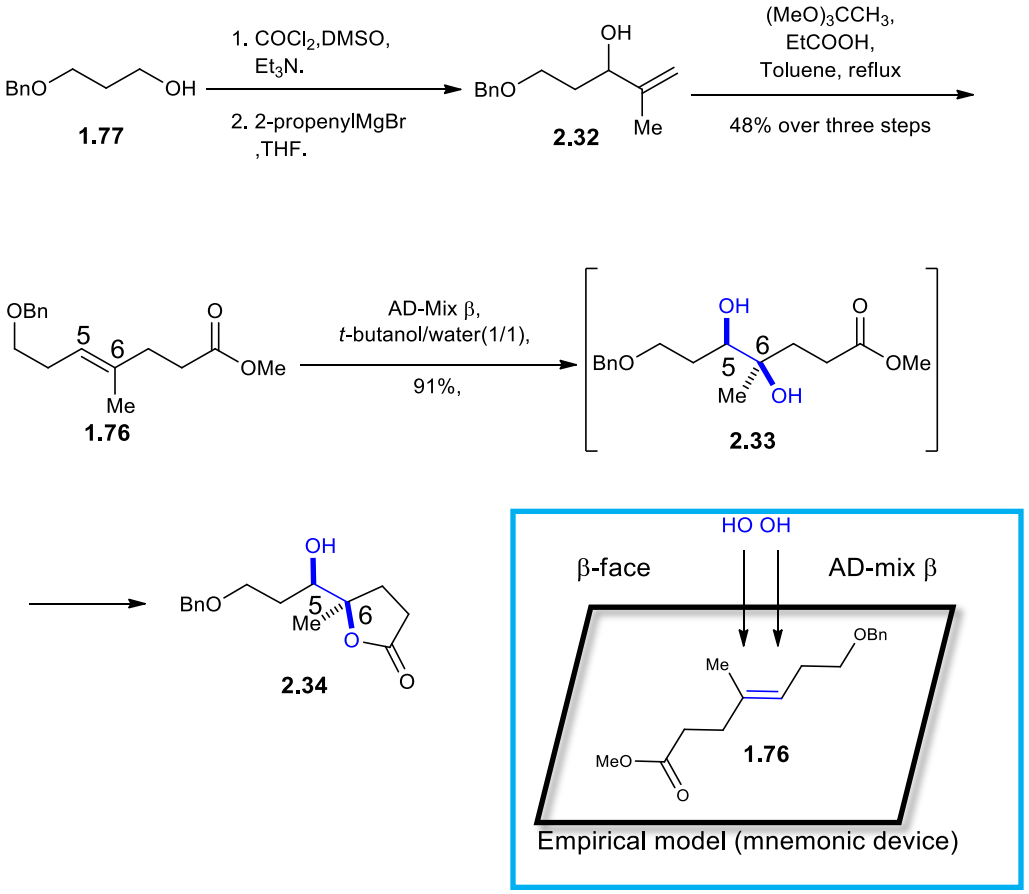
The synthesis of aldehyde **2.27a** starts with the synthesis of enantiopure lactone **2.34** (Scheme 2.10). To begin, Swern oxidation[125] of commercially available 3-benzyloxypropanol **1.77** followed by Grignard reaction[126] with 2-propenylmagnesium bromide (**1.78**) provided racemic allylic alcohol **2.32** which was taken to the next step without purification. Subjection to a Johnson-Claisen rearrangement[127] provided γ,δ-unsaturated ester **1.76** in 48% over three steps. The Sharpless Asymmetric Dihydroxylation (SAD) would then be used to set the stereochemistry at C-5 and C-6. Following the empirical model for the Sharpless Asymmetric Dihydroxylation[105], AD-Mix β was used to oxidize to *syn*-diol **2.33** which spontaneously cyclized to lactone **2.34** in 91% yield. Mosher analysis showed enantiomeric excess (ee) greater than 95%. The absolute stereochemistry was confirmed after subsequent steps (*vide infra*).

2.3.1.3 Synthesis of Alkylated Lactone and Determination of Absolute Stereochemistry of Alkylated Lactone 1.99

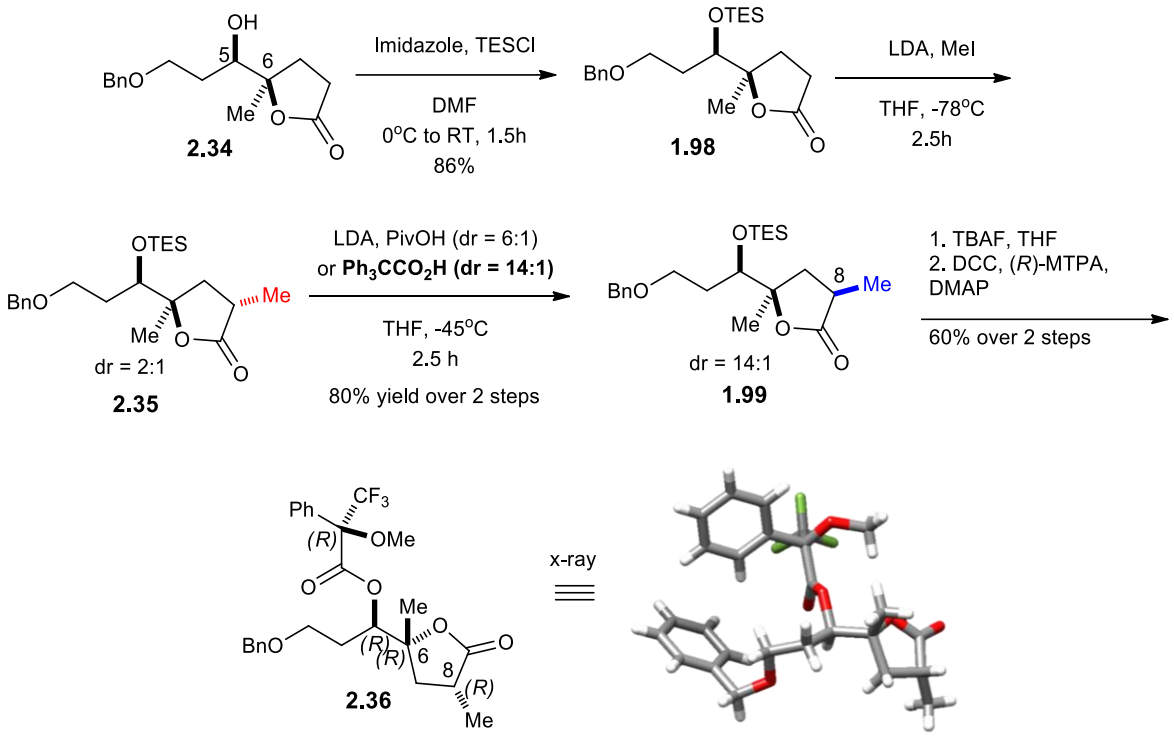
In order to methylate the C-8 carbon of lactone **2.34**, it was first protected as its triethyl silyl ether, which resulted in **1.98** in 86% yield (Scheme 2.11). Alkylation with LDA and trapping with MeI gave a 2:1 mixture of undesired diastereomer **2.35**. This can be rationalized by considering the transition state wherein methyl iodide approaches the less hindered enolate diastereoface (i.e., *syn* with respect C-6). Regeneration of the enolate and kinetic protonation with bulky pivalic acid successfully delivered the desired diastereomer **1.99** as a 6:1 mixture with the appropriate stereochemistry at C-8. Kinetic protonation was further optimized by utilizing the even bulkier triphenyl acetic acid, which

provided **1.99** as a 14:1 mixture of separable diastereomers. At this stage, the TES group was removed with tetra-*n*-butylammonium fluoride (TBAF) and the resultant alcohol coupled to (*R*)-methoxy(trifluoromethyl)phenylacetic acid ((*R*)-MTPA) with *N,N'*-dicyclohexylcarbodiimide (DCC) to give Mosher Ester **2.36**.[128] X-Ray crystallographic analysis of **2.36** confirmed both the absolute stereochemistry of the C-8 methyl as well as the C-5 and C-6 stereocenters derived from the Sharpless Asymmetric Dihydroxylation (*vide supra*).

Scheme 2.10. Synthesis of lactone **2.34** via Sharpless asymmetric dihydroxylation.



Scheme 2.11. Methylation of C-8 and determination of absolute stereochemistry by X-Ray crystallography.



2.3.1.4 Synthesis of NHK precursor 2.42

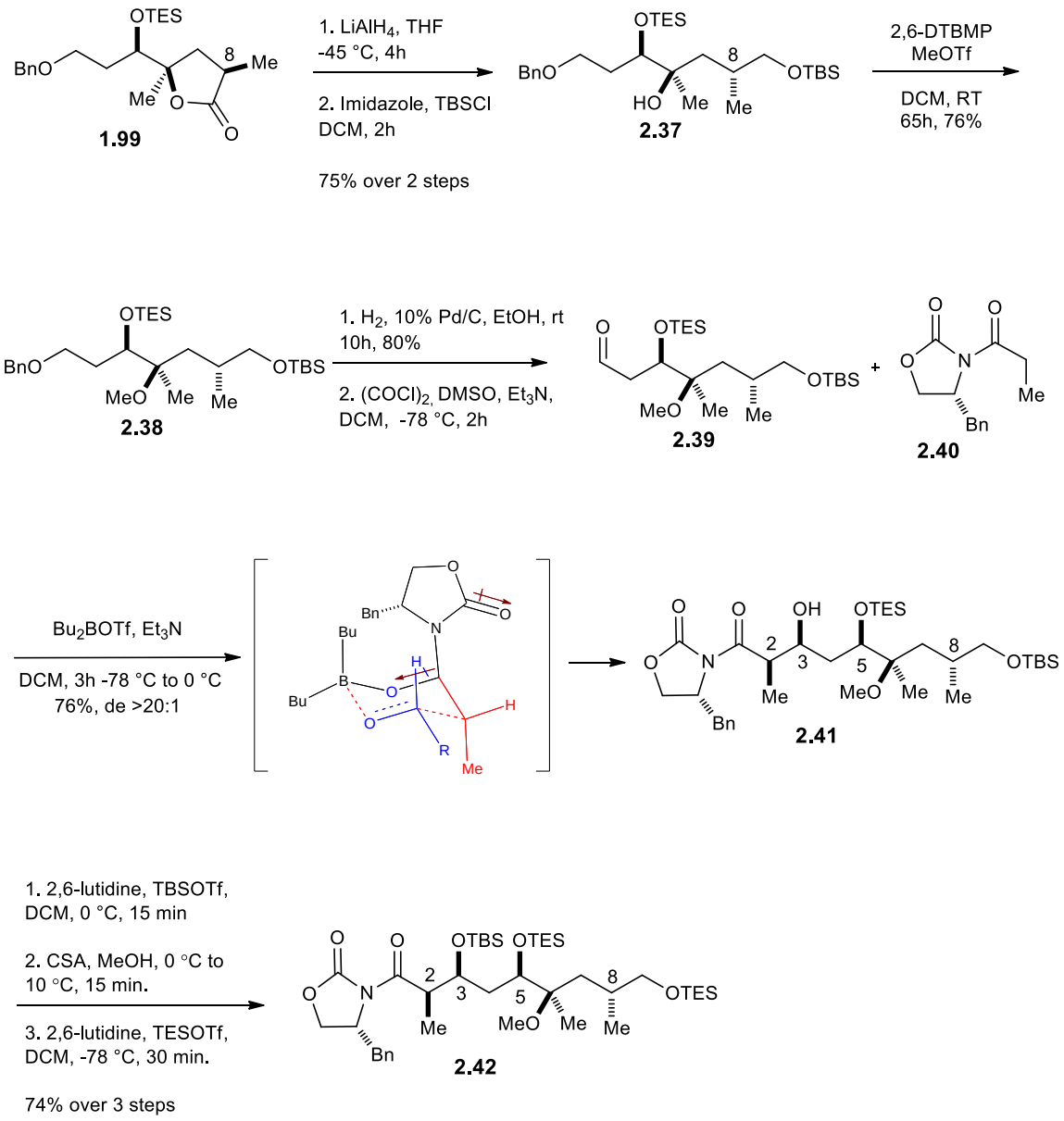
Lactone **1.99** was reduced with $LiAlH_4$, and the resultant primary alcohol was chemoselectively protected as its *tert*-butyldimethylsilyl (TBS) ether with TBSCl and imidazole to provide tertiary alcohol **2.37** in 75% yield over two steps. Utilizing methylation conditions optimized in the synthesis of 4,10-didesmethyltelithromycin (**1.67**), MeOTf and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) were utilized to obtain methyl ether **2.38** in 76% yield.[129] Hydrogenolysis of the benzyl ether with 10% Pd/C was accomplished in 80% yield. Swern oxidation[130] gave aldehyde **2.39** that was taken directly to the next step without purification. An Evans asymmetric aldol reaction[121] with (*R*)-4-benzyl-3-propionyl-2-oxazolidinone (**2.40**) set the configuration of the C-2 and C-3

carbons to furnish *syn* aldol **2.41** in 76% yield (dr > 20:1). Protection of the C-3 alcohol as its *tert*-butyldimethylsilyl ether using TBSOTf and 2,6-lutidine proceeded without incident. Protecting group manipulation involving the removal of the primary TBS and secondary TES ethers, followed by reprotection of both as TES ethers, gave **2.42** in 74% over three steps (Scheme 2.12).

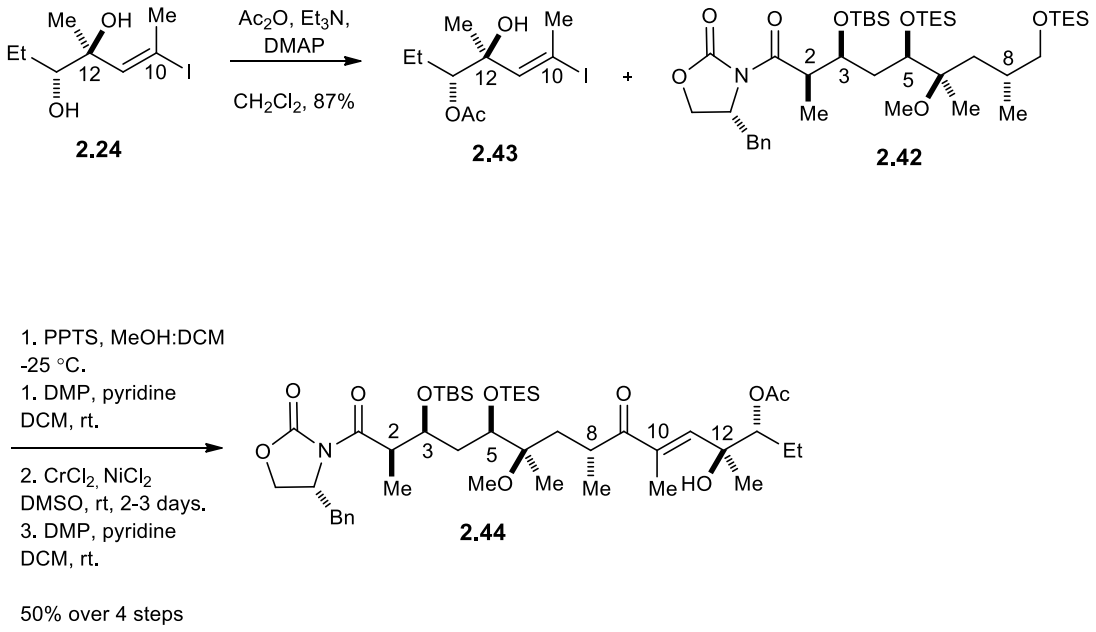
2.3.1.5 Nozaki-Hiyama-Kishi (NHK) Coupling

The Nozaki-Hiyama-Kishi (NHK) coupling to provide **2.44** was accomplished via a four-step sequence. The primary triethylsilyl ether on C-9 was cleaved chemoselectively in the presence of the secondary triethylsilyl ether on C-5 and the secondary *tert*-butyldimethylsilyl ether on C-6. The acquired alcohol was oxidized with Dess-Martin periodinane.[131] The resultant aldehyde then underwent NHK coupling with vinyl iodide **2.43**, which was synthesized from **2.24** in 87% yield, in the presence of an excess of $CrCl_2$ and catalytic $NiCl_2$ in degassed DMSO. DMP oxidation of the resultant diastereomeric mixture of allylic alcohols gave α, β-unsaturated ketone **2.44** in 50% yield over four steps. The acetate group of **2.43** played a crucial role in the successful coupling. Coupling attempts with the free diol and bis-acetate protected vinyl iodide resulted in no isolatable product. Failure of the NHK reaction with diol containing vinyl iodide was attributed to an intramolecular quenching of the chromium (III) nucleophile by the unprotected secondary alcohol. The failure of the bis-acetate protected diol was attributed to sterics or possibly reduced activity of the nucleophile by stabilization/deactivation of the nucleophile by coordination with the tertiary acetate (Scheme 2.13).[132]

Scheme 2.12. Synthesis of NHK precursor **2.48**.



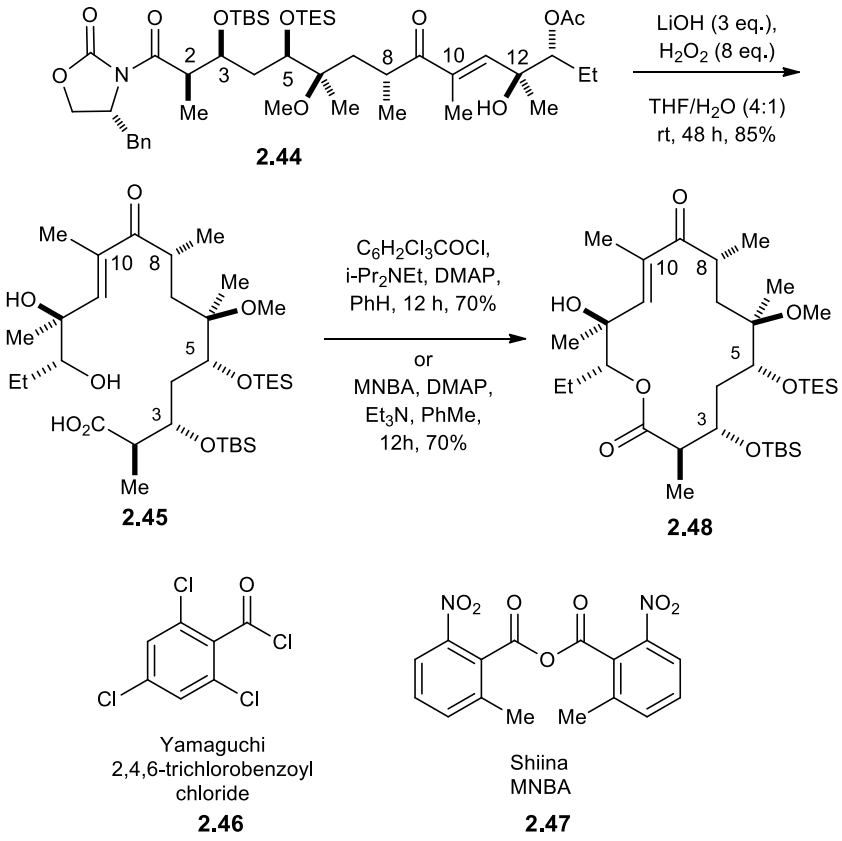
Scheme 2.13. Synthesis of NHK Product **2.44.**



2.3.1.6 Seco-acid formation and macrolactonization

Seco-acid formation (Scheme 2.14) was accomplished in a single step utilizing lithium hydroperoxide generated from hydrogen peroxide and lithium hydroxide in a 4:1.5 ratio to avoid epimerization. While we were confident that macrolactonization could be accomplished with our seco-acid (*vide supra*), two conditions were attempted. The Yamaguchi macrolactonization employed pre-generation of the mixed anhydride with 2,4,6-trichlorobenzoyl chloride (**2.46**) in dilute benzene (0.01 M) followed by addition of a large excess of DMAP (40 equiv).[119] The second condition used Shiina's catalyst (MNBA) (**2.47**) and utilized the slow addition of seco-acid to a dilute solution of DMAP in toluene (0.005 M).[133] Surprisingly, both conditions were successful yielding macroketolactone **2.48** in 70% yield. The synthesis was ultimately taken forward using the Yamaguchi macrolactonization due to an easier protocol.

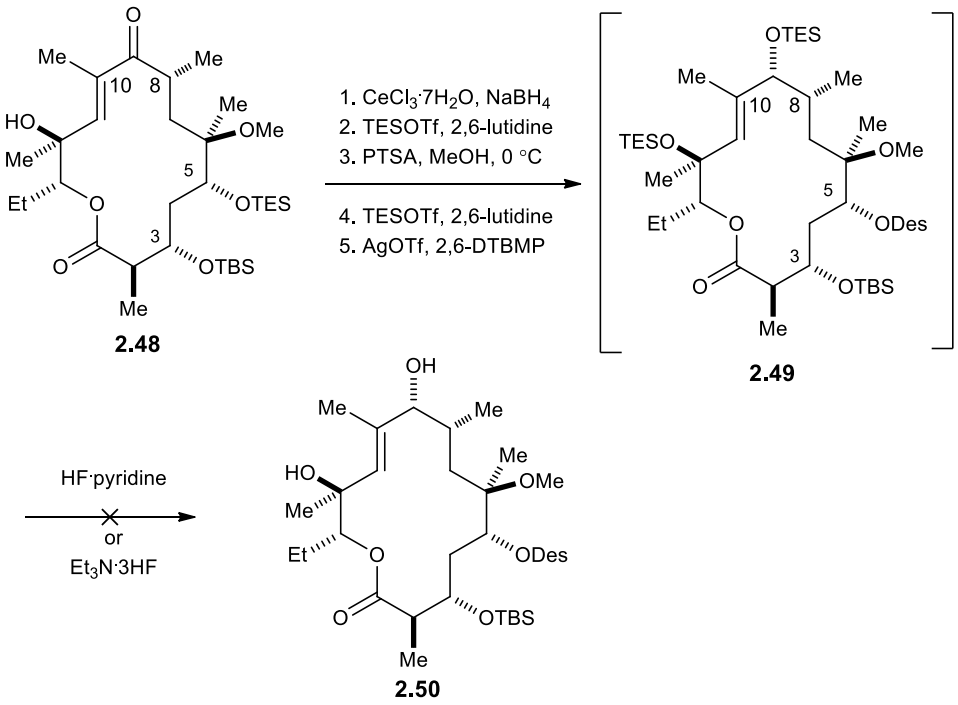
Scheme 2.14. Bis-deprotection of seco-acid **2.44** and macrolactonization to form macroketolactone **2.48**.



2.3.1.7. Failed Protection-Deprotection Scheme for Glycosylation

In order to successfully glycosylate macroketolactone **2.48**, we followed the protocol developed for the synthesis of 4,10-didesmethyl telithromycin (**1.68**), which is shown in Scheme 2.15.[129] Luche reduction provided a 2:1 mixture of separable diastereomers. TES protection was first attempted with TESCl; however, the additional methyl group at C-8 provided enough steric hindrance to prevent the reaction from going to completion. Recourse to TESOTf achieved bis-TES protection of the C-9 and C-12 hydroxyls. While glycosylation employing Woodward's thiopyrimidine donor **1.72** was able to provide bis-TES protected intermediate **2.56**, deprotection conditions with HF to give **2.50** were unsuccessful.

Scheme 2.15. Failed Protection-Deprotection Scheme for Glycosylation.



2.3.2. Second-Generation Approach: NHK Coupling with Desosamine Installed

Our next approach would remove the need for deprotection altogether by installing the desosamine prior to the Nozaki-Hiyama-Kishi Coupling. This strategy was employed first by Martin in his synthesis of erythromycin B[108], and later adopted by our group for the synthesis of 4,8-didesmethyl telithromycin.[97] With a methyl carbonate (Mc) protecting group for the C-2' hydroxyl of desosamine needed to ensure stereoselective β-glycosylation, we would require an orthogonal protecting group at C-1. Accordingly, we converted the Evans auxiliary to a benzyl ester with lithium benzyloxide and recruited three different protecting groups for vinyl iodide **2.24** that would not require saponification to remove. To this end, we began with aldol product **2.41** (Scheme 2.16). Protection of the

secondary alcohol as a TBS ether followed by transesterification of the Evans auxiliary with LiOBn provided **2.51** in 50% yield. Removal of the TES ether at C-5 and TBS ether at C-9 with CSA in MeOH was successful in the presence of the secondary TBS ether at C-3, giving **2.52** in 70% yield. Chemoselective protection of the primary C-9 alcohol and glycosylation with Woodward's thiopyrimidine donor **1.72** resulted in glycosylated product **2.53** in 74% over two steps. Three vinyl iodide coupling partners were synthesized from diol **2.24**. TES protected partner **2.55** was made in 75% yield utilizing TESCl. Diol protected partner **2.56** was prepared using 4-methoxybenzaldehyde dimethyl acetal in 70% yield. Coupling partner **2.57** was then fashioned in 95% from **2.62** by reductive cleavage using DIBAL-H.[134,135] After removal of the primary TBS ether with HF and oxidation to the aldehyde with Dess-Martin Periodinane, the coupling partners and aldehyde were subjected to Nozaki-Hiyama-Kishi conditions. To our disappointment, the reaction did not proceed with any of the protected vinyl iodides (**2.55-2.57**). Thus, a new protecting group scheme was needed.

2.3.3. Third-generation: C-5 *para*-methoxybenzyl (PMB) route.

To access our target, a new C-5 protecting group was needed. This group needed to be labile in the presence of silyl ethers and also had to withstand conditions used to deprotect a benzyl ether. We found inspiration from work done by Yonemitsu.[136] Many are familiar with the selective removal of *para*-methoxybenzyl (PMB) ethers with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of benzyl ethers, but Yonemitsu showed the dual orthogonality of these groups when he demonstrated that

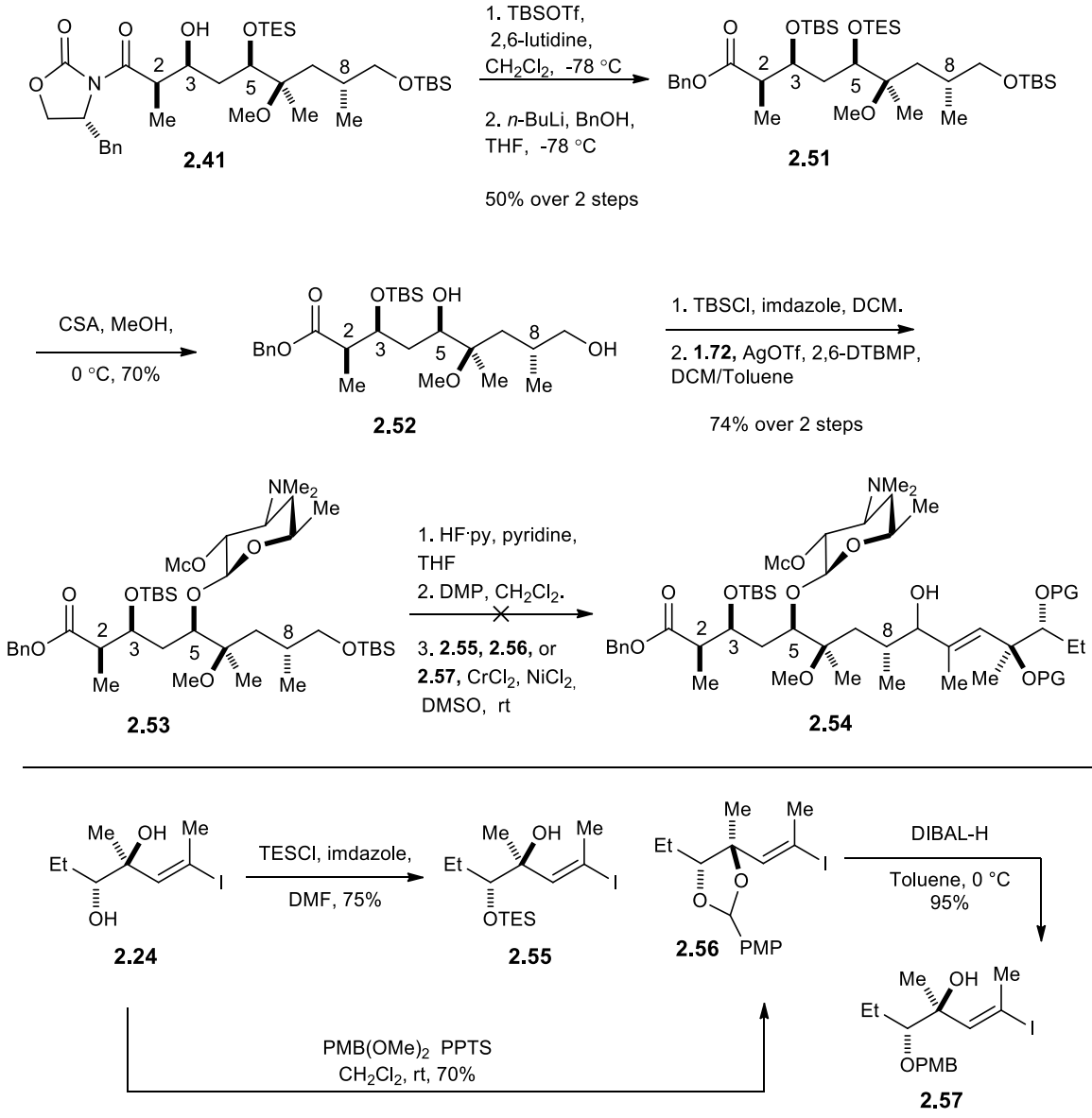
Raney-Ni allowed for the quantitative removal of benzyl ethers in the presence of the more electron rich PMB ethers (Scheme 2.17).

2.3.3.1 Routes to C-5 PMB protected Intermediates

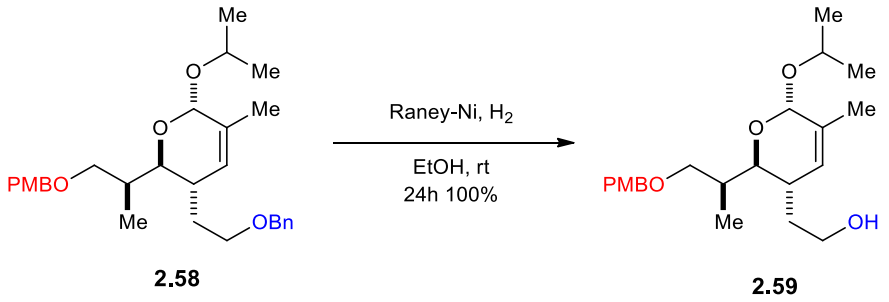
In order to test this deprotection method in our synthesis, the C-5 PMB protected intermediate **2.60** was synthesized from **2.38** by (1) bis-deprotection of both silyl ethers with camphor sulfonic acid (CSA), (2) chemoselective reprotection of the primary hydroxyl as its *tert*-butyldimethylsilyl (TBS) ether, and finally, protection of the C-5 secondary hydroxyl with the use of PMBCl to furnish **2.60** in 50% yield over three steps. Successful hydrogenolysis with Raney-Ni as described by Yonemitsu then provided primary alcohol **2.61** cleanly in 75% yield (Scheme 2.18)

The above route provided intermediate **2.61** in 19% yield over nine steps from lactone **1.98**. A more efficient route was developed in which the secondary hydroxyl of **2.34** was directly protected as its PMB ether in 65% yield to furnish **2.62** (Scheme 2.19). The alkylation and epimerization step provided only a 5:1 mixture of separable diastereomers leading to a 45% yield of **2.63** over two steps, compared to the 14:1 dr and 80% yield over two steps with TES installed on the C-5 hydroxyl. This result underscored the importance of the bulky TES group for high levels of diastereoselctivity in the kinetic protonation step. Fortunately, the unwanted diastereomer could be separated by flash column chromatography and recycled into future epimerization reactions. Although the dr and yield for these steps was lower, reductive opening of the lactone ring proceeded in 92% yield, chemoselective protection of the resultant primary alcohol as a TBS ether, and methylation of the tertiary alcohol with methyl triflate (72% over two steps) gave **2.60** in 19% over six steps, resulting in the same yield with three less steps.

Scheme 2.16. Attempted Nozaki-Hiyama-Kishi coupling with desosamine installed.

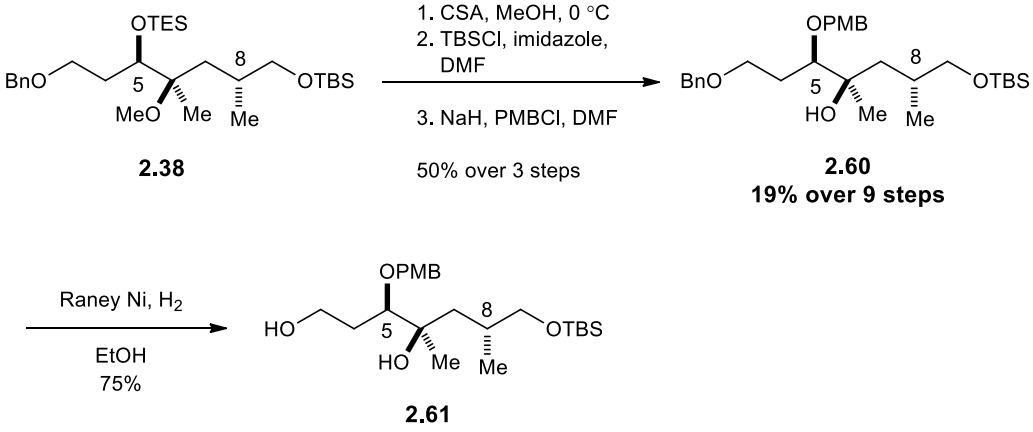


Scheme 2.17. Deprotection of a benzyl ether in the presence of a *para*-methoxybenzyl ether.



2.58 **2.59**

Scheme 2.18. Synthesis of **2.61** by chemoselective hydrogenation of benzyl ether **2.60**.



2.38 **2.60**
19% over 9 steps

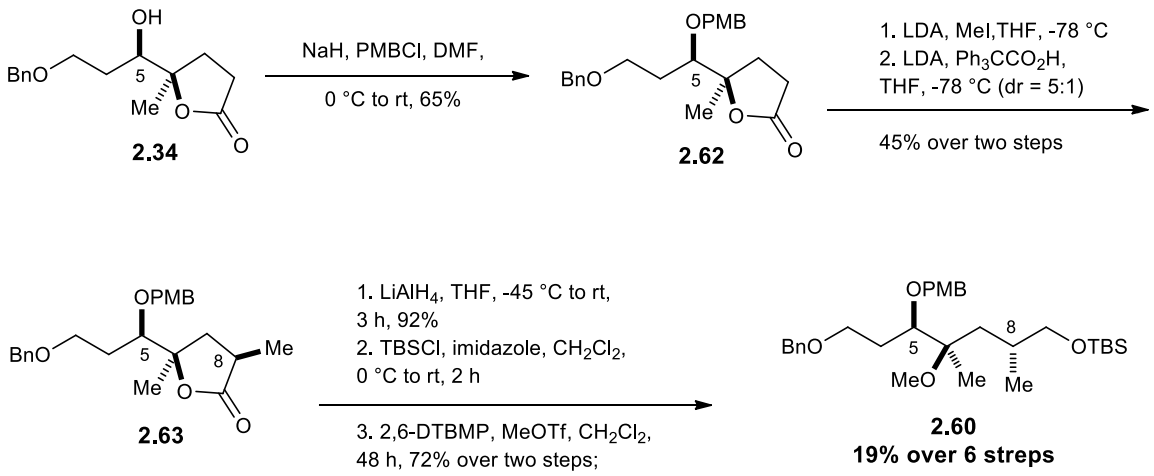
2.61

2.3.3.2 Synthesis of NHK Precursor

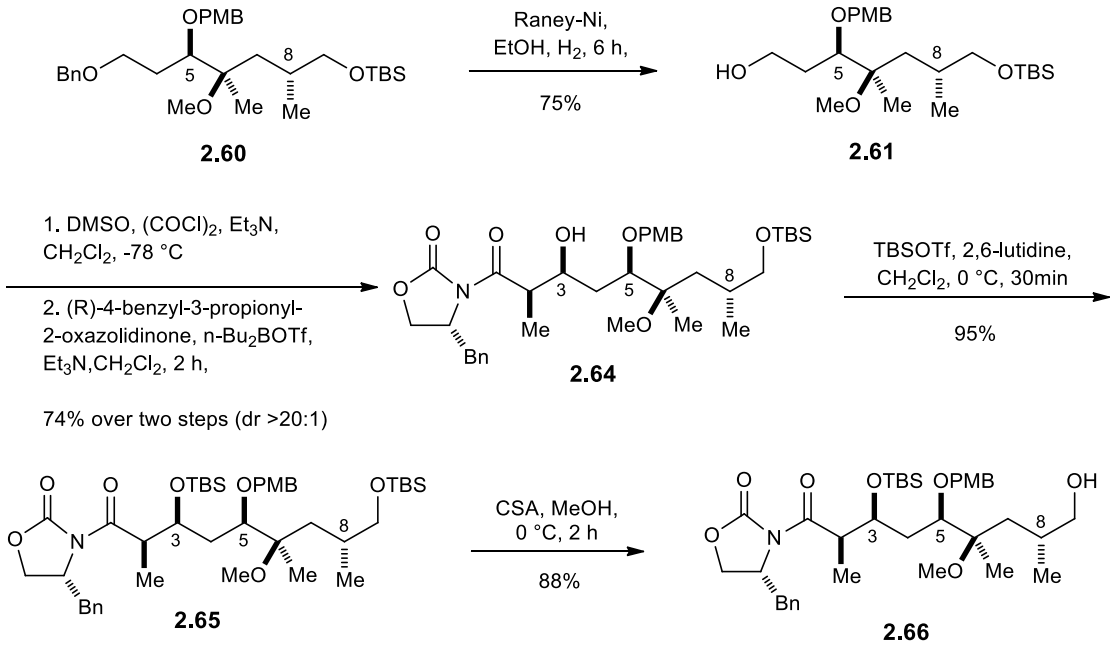
Hydrogenolysis of benzyl ether **2.60** was accomplished in 75% yield with Raney-Ni resulting in **2.61**. The aldehyde was generated via the Swern oxidation, which was then reacted under Evans asymmetric aldol conditions to give **2.64** in 74% yield over two steps with a dr > 20:1. The resultant secondary alcohol was protected as TBS ether **2.65** with TBSOTf and 2,6-lutidine in 95% yield. The primary TBS ether was then unmasked with

camphor sulphonic acid (CSA) providing **2.66** in 88% yield to set the stage for the Nozaki-Hiyama-Kishi coupling (Scheme 2.20).

Scheme 2.19. Alternate route to **2.61**.



Scheme 2.20. Synthesis of NHK precursor **2.67**.

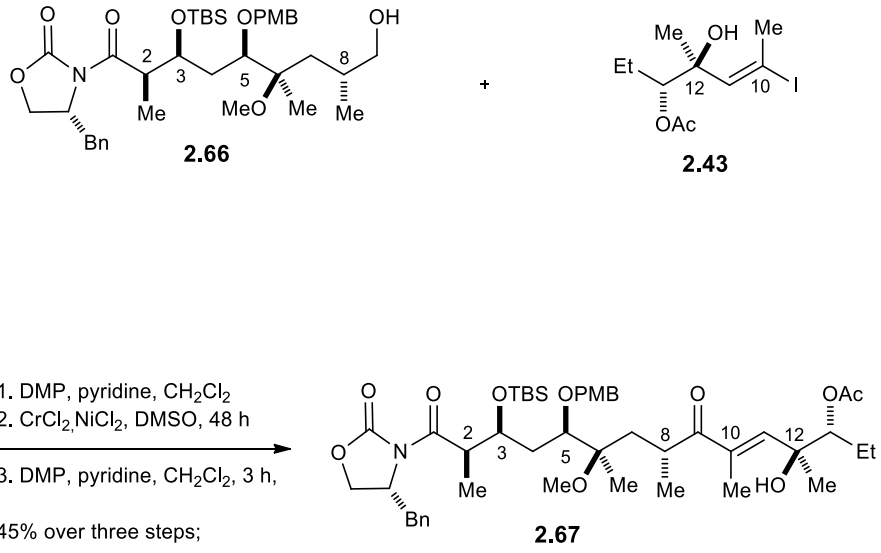


2.3.3.3 NHK Coupling and Yamaguchi Macrolactonization

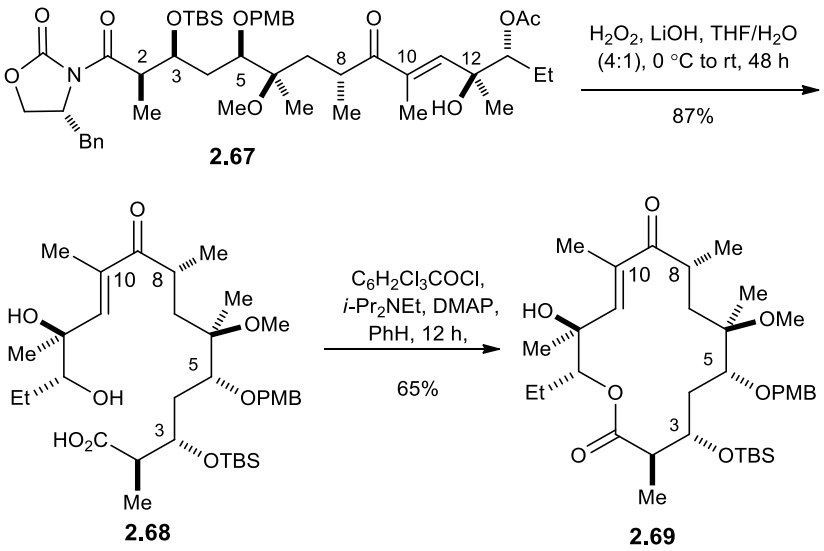
The crucial Nozaki-Hiyama-Kishi coupling was now attempted with the C-5 PMB group in place. The three step sequence began with oxidation of **2.66** to the aldehyde with Dess-Martin periodinane, followed by NHK coupling with **2.43**, and finally oxidation of the resultant allylic alcohol to enone **2.67** in 45% over three steps (Scheme 2.21).

Deprotection conditions developed previously employing lithium hydroperoxide gave seco-acid **2.68** in 87% yield. Yamaguchi macrolactonization then furnished macroketolactone **2.69** in 65% yield (Scheme 2.22). The stage was now set for glycosylation.

Scheme 2.21. Nozaki-Hiyama-Kishi Coupling.



Scheme 2.22. Yamaguchi Macrolactonization.



2.3.3.4 Glycosylation of macroketolactone C-5 hydroxyl acceptor 2.70

Luche reduction of the C-9 ketone of **2.69** provided a 4.6:1 mixture of separable diastereomers (Scheme 2.23). Although the stereochemistry would be inconsequential, the major isomer was isolated and taken forward to ease characterization of the complex intermediates. Protection of the C-9 and C-12 hydroxyls was deemed necessary due to the problems experienced with glycosylation of prior analogues.[95] The employment of a TES ether at C-9 made the protection and deprotection difficult. Instead, bis-TMS protection of the C-9 hydroxyl and the C-12 hydroxyl was employed.[95] DDQ was then used to oxidatively cleave the PMB group on the C-5 hydroxyl furnishing **2.70** in 76% over two steps. AgOTf mediated glycosylation with Woodward donor **7.72** then gave glycosylated macrolactone **2.71**. Selective deprotection of both TMS ethers in the presence of the C-3 TBS group with HF and oxidation of the C-9 hydroxyl with DMP supplied glycosylated macroketolactone **2.72** in 67% yield over two steps.

2.3.3.5 Baker Cyclization and Completion of (-)-4-Desmethyl Telithromycin

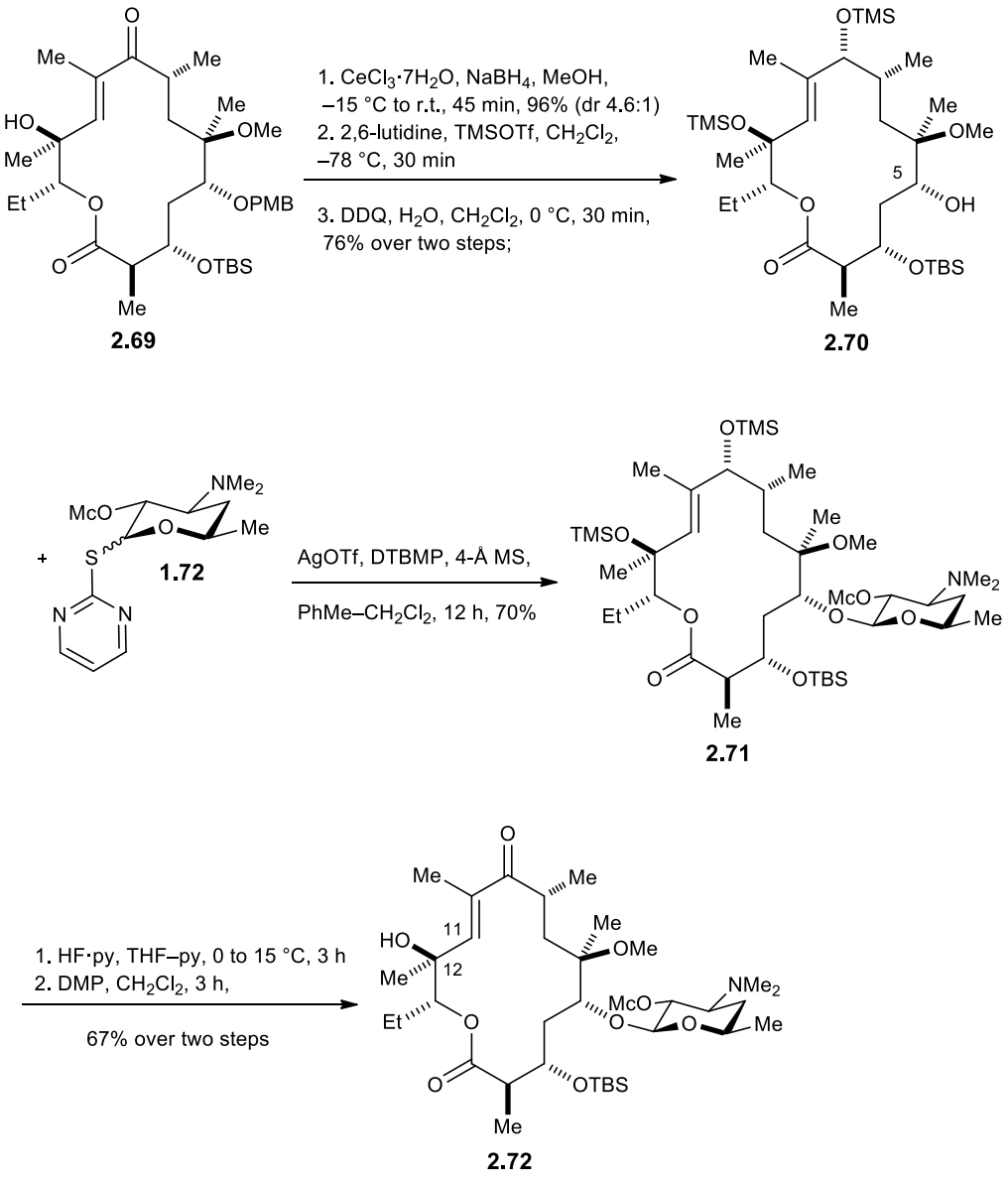
At this stage, the next step in the synthesis of 4-desmethyl telithromycin (**1.69**) was to install the pyridyl-imidazole side chain through the use of the Baker cyclization.[73] The C-12 tertiary hydroxyl of **2.72** was first activated with NaH and carbonyldiimidazole (CDI) to form an activated imidazole carbamate, which was then reacted with butyl amine **1.70** and underwent a sequential carbamoylation/intramolecular aza-Michael reaction to form oxazolidinone **2.73** in 61% yield over two steps (Scheme 2.24). With **2.73** in hand, all that was left to achieve the total synthesis was to deprotect the C-3 hydroxyl, oxidize to the ketone, and free the desosamine 2' hydroxyl. To this end, the TBS ether was removed using the mild TAS-F reagent[107] followed by Corey-Kim oxidation[137] to provide the C-3 ketone **2.74** in 53% yield over two steps. Methanolysis of the methyl carbonate at room temperature then gave (–)-4-desmethyl telithromycin **1.69** in 67% yield.

In summary, the synthesis of (–)-4-desmethyl telithromycin was accomplished utilizing a key intermolecular NHK coupling and Yamaguchi macrolactonization in 43 total steps with a longest linear sequence of 31 steps.

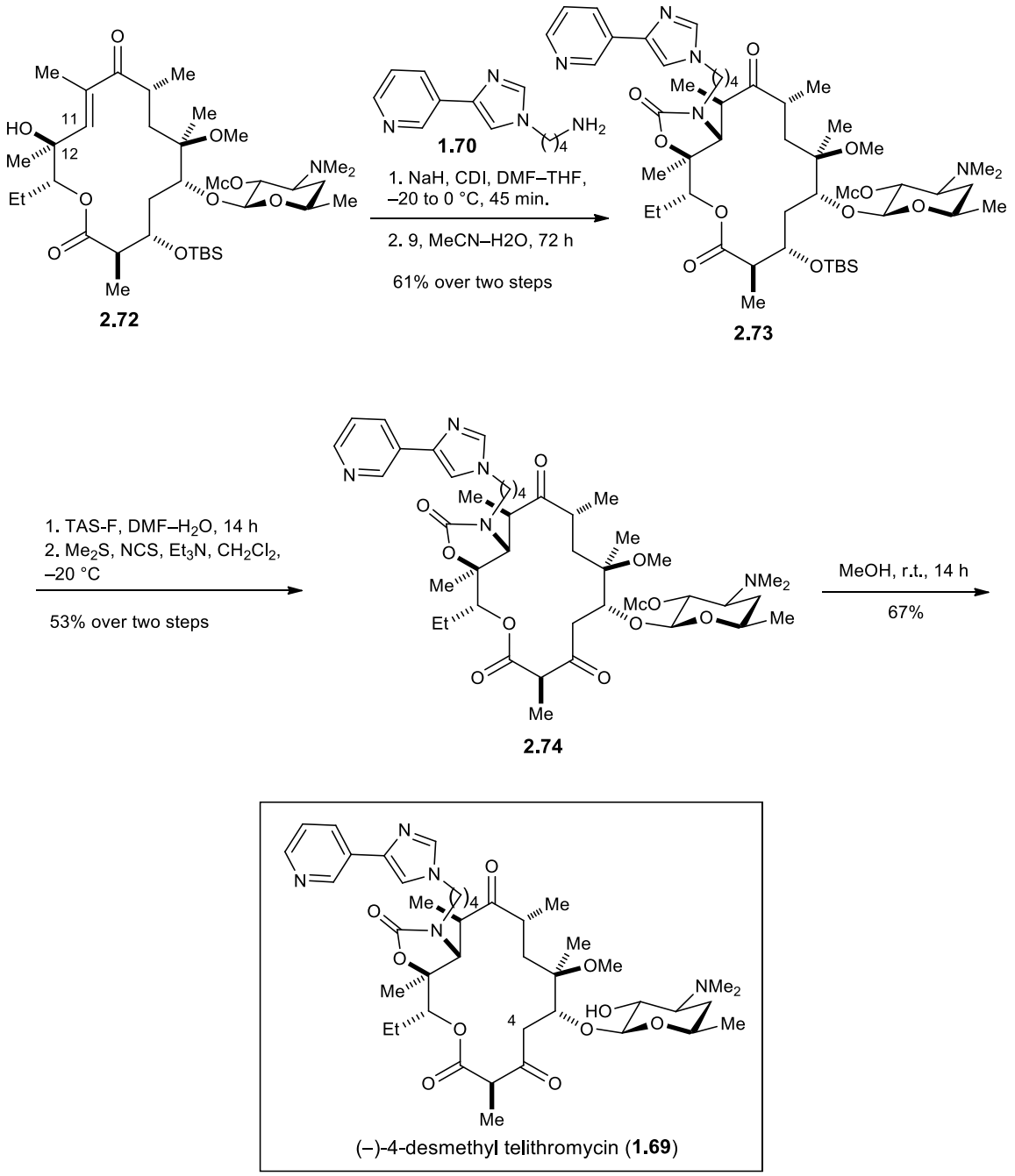
2.4 Minimum Inhibitory Concentration (MIC) Analysis

With (–)-4-desmethyl telithromycin (**1.69**) in hand, we could now directly test the desmethyl hypothesis. Minimum inhibitory concentrations (MIC's) were run against two *S. aureus* (entries 4-5) and three *E. coli* strains (entries 1–3) (Table 2.1). All desmethyl analogues including telithromycin were inactive against the two resistant *S. aureus* strains, which included UCN14 containing an A2058T mutation and ATCC33581 containing an *erm*A enzyme. One notable exception was the MIC of **1.66** against UCN14 which

Scheme 2.23. Glycosylation of macroketolactone C-5 hydroxyl acceptor **2.70**.



Scheme 2.24. Baker Cyclization and Completion of (–)-4-desmethyl telithromycin (**1.69**).



maintained activity compared to the other compounds. All compounds were also inactive against *E. coli* strain SQ171/2058G containing the A2058G mutation we wanted to test. All desmethyl analogues and telithromycin did however show activity against *E. coli* DK strains, which were engineered to lack the membrane transporter tolC, which effluxes macrolides such as erythromycin, telithromycin and desmethyl analogues. Notably, unlike any of the previous desmethyl analogues, (–)-4-desmethyl telithromycin (**1.69**) matched telithromycin's (**1.63**) MIC of 0.5 μg/mL against the DK wild type strain (entry 2). Unfortunately, (–)-4-desmethyl telithromycin (**1.69**) was 4-fold less potent than telithromycin against the DK A2058 mutant (entry 3).

Table 2.1. Minimum Inhibitory Concentration of (–)-4-desmethyl telithromycin (**1.69)** and comparison to telithromycin (**1.63)** and desmethyl analogues **1.66**, **1.67**, and **1.68**.

Entry	Strain	Bacteria	wt/mutant	1.66	1.67	1.68	1.69	TEL (1.63)
1	SQ171/2058G	*E. coli*	A2058G	>512	>256	>256	>256	>256
2	DK/pKK3535	*E. coli*	wt	32	8	4	0.5	0.5
3	DK/2058G	*E. coli*	A2058G	64	16	32	4	1
4	UCN14	*S. aureus*	A2058T	32	>256	>256	>256	>128
5	ATCC33591	*S.aureus*	*ermA*	>128	>128	>64	>128	>128

While we were unable to validate the desmethyl hypothesis, there was a noticeable structure-activity relationship in these data that may be useful for future antibiotic development. Against both the wild type and mutant DK strains, MIC values decrease as the number of backbone methyls increases. This clearly shows the importance of these methyl groups to provide (1) potential hydrophobic interactions with the side wall of the ribosomal exit tunnel. From X-ray structures it can be seen that all of the methyl

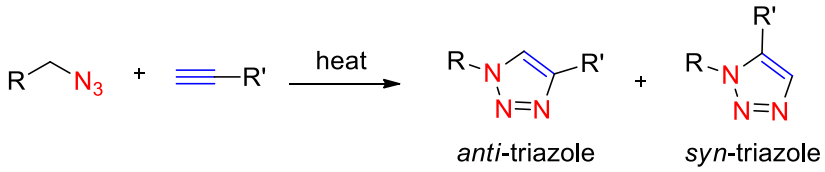
substituents are arranged on the side of the ring which faces the wall of peptide exit tunnel. We have clearly shown that elimination of these interactions has negative effects on binding. (2) Conformational rigidity of the 14-membered macrolactone allows for tighter binding. Both the in solution and bound structures of macrolides have been solved and surprisingly, in-solution, macrolides adopt a very rigid conformation for a 14-membered ring due to *syn*-pentane interactions of the various methyl substituents. This in-solution conformation aligns exactly with the macrolide bound to the ribosome. It has long been practice in the pharmaceutical industry to restrict degrees of freedom to enhance binding of small molecules used as drugs. While macrolide antibiotics seem vastly different in size and flexibility than the small planar molecules this approach is generally adopted for, it seems to hold true nonetheless. Further optimizing of ring rigidity in macrolides could lead to more potent antibiotics. From this work, it cannot be determined which of these factors is more important, but it is clear that total synthesis will be necessary to the future understanding of macrolide antibiotics and their interactions with the bacterial ribosome.

CHAPTER 3: THE DEVELOPMENT OF A RIBOSOME-TEMPLATED IN SITU CLICK METHODOLOGY FOR THE DISCOVERY OF ANTIBIOTICS TARGETING THE BACTERIAL RIBOSOME

3.1 Click Chemistry

The philosophy of click chemistry, as first imagined by Sharpless, was to design a set of powerful reactions and building blocks that can be used to build both simple and complex molecules. A stringent set of criteria was defined for these reactions. The reaction had to be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods, and be stereospecific.[138] Reactions that fit these criteria included 1,3-dipolar cycloadditions, ring-opening of strained heterocycles such as epoxides and aziridines, and "non-aldol" type carbonyl condensations to form oxime ethers and hydrazones.

Scheme 3.1. Huisgen thermal 1,3-dipolar cycloaddition.



Of these reaction types, Sharpless emphasized the importance that the 1,3-dipolar cycloadditions would have, particularly the Huisgen dipolar cycloaddition of azides and alkynes to form 1,2,3-triazoles, which was first described in 1971.[139] While Sharpless described this transformation as the "cream of the crop" for click reactions, it did have its limitations, most notably the high reaction temperatures needed to overcome the activation energy (25 kcal/mol for methyl azide and propyne)[140] and the mixtures of regioisomers (*anti*- and *syn*-triazoles) that were obtained with unsymmetrical alkynes (Scheme 3.1). The

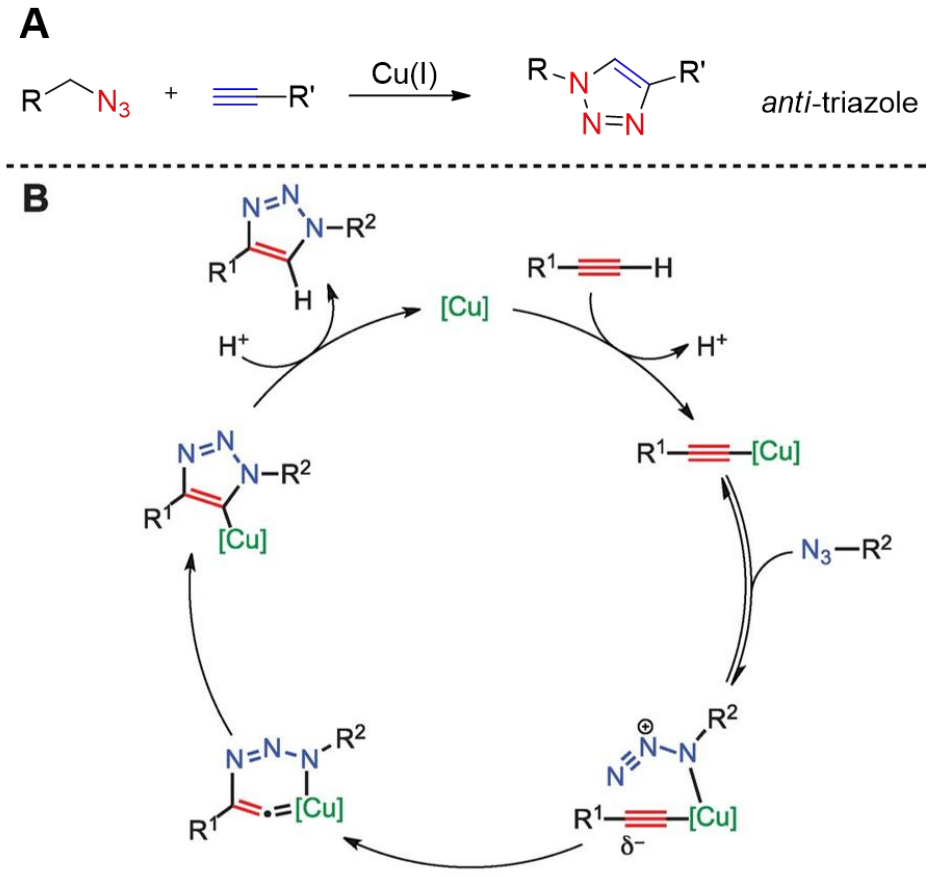
Huisgen cycloaddition would also have a huge impact in biology due to the limited reactivity of azides and alkynes with biological functionalities and compatibility with water. This would eventually lead Bertozzi and others to allow precise chemical modification of biomolecules *in vitro*.[141] The azide-alkyne cycloaddition has also found use by the Cravatt lab to discover new proteins involved in human disease using activity-based protein profiling.[142]

Sharpless's foresight into the importance of the Huisgen cycloaddition would be realized less than one year later when Sharpless and Fokin reported the Cu(I) catalyzed Huisgen cycloaddition of terminal alkynes and organic azides which formed exclusively the *anti*-triazole (Scheme 3.2A).[143] The "copper effect" was also independently reported by Meldal.[144] Not only did this reaction solve the regioselectivity problem of the thermal cycloaddition, but it now could be run at room temperature and in aqueous solvent. The selectivity for the *anti*-triazole was first explained mechanistically by the formation of a copper (I) acetylide and then the stepwise reaction with the azide proceeding through a six-membered copper containing intermediate shown in Scheme 3.2B. Density functional theory calculations showed this pathway to be thermodynamically more favorable compared to the direct cycloaddition mechanism.

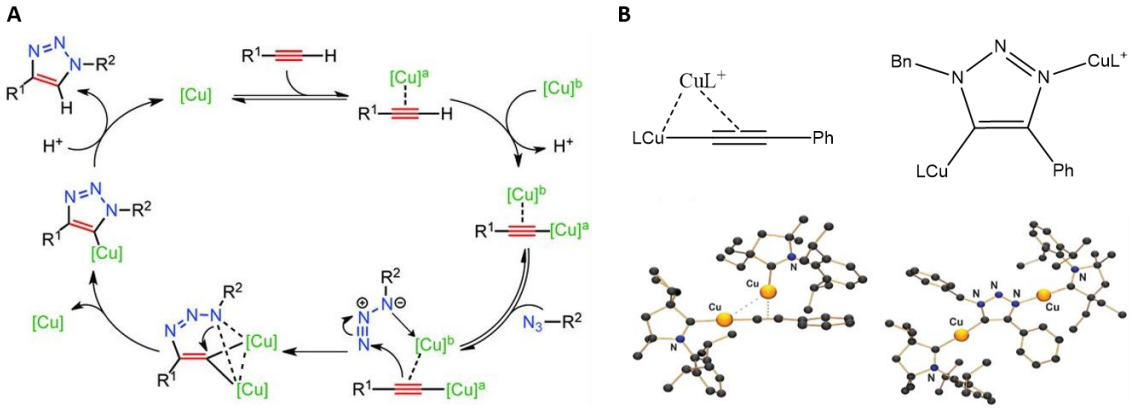
More recently, through the use of heat-flow reaction calorimetry, it was shown copper acetylides were not reactive toward azides unless additional copper catalyst is added, suggesting a mechanism involving two Cu centers. Also, crossover experiments utilizing isotopically enriched copper catalysts were carried out which showed the stepwise nature of the nitrogen-carbon bond forming events as well as the equivalence of the two copper atoms involved due to rapid interconversion.[145] With these data an updated

mechanism involving a π, σ-bis (copper) acetylide was proposed (Scheme 3.3A). Further evidence for a bis(copper) intermediate came in mid-2015 when Bertrand was able to isolate the postulated π,σ-bis(copper) acetylide with the use of strong σ-donating and π-accepting cyclic (alkyl)(amino) carbenes as ligands allowing the stabilization and isolation of this highly reactive intermediate once thought to be nonisolable.[146] Also isolated was a previously unimagined bis (copper) triazole (Scheme 3.3B).

Scheme 3.2. A) Copper (I) catalyzed azide alkyne cycloaddition. B) Original proposed mechanism.[143]

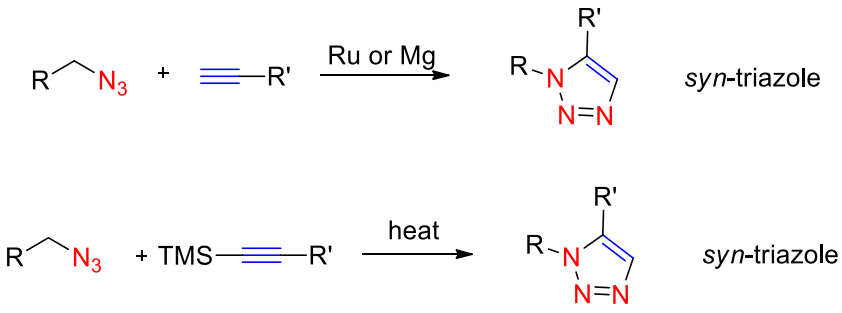


Scheme 3.3. A) Updated Bis(copper) Mechanism.[145] B) Isolated π,σ-Bis(copper) Acetylide and Bis(Copper) Triazole.[146]



While the majority of click chemistry applications utilize the *anti*-triazole forming copper catalyzed reaction, it is possible to selectively synthesize *syn*-triazoles, although the chemistry is not as robust and still in development (Scheme 3.4). Ruthenium-catalyzed *syn*-triazole formation has become a powerful method, but typically works well with only aromatic azides and alkynes and performs poorly with sterically bulky substituents.[147] Despite its difficulties, the Ru-catalyzed azide alkyne cycloaddition has great potential for future applications. Other methods for *syn*-triazole formation include the use of magnesium acetylides[148] and silyl protected acetylides.[149]

Scheme 3.4. Methods for the synthesis of *syn*-triazoles.



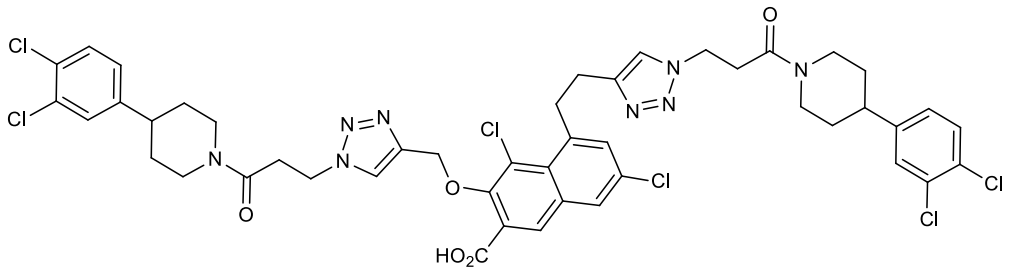
While the original definition of click chemistry was meant to encompass a broad set of reactions (*vide supra*), today it is synonymous with azide alkyne cycloadditions, especially the copper catalyzed version (CuAAC). Since its initial discovery in 2002, the applications of click chemistry have grown exponentially and have found a welcome place in drug development.

The 1,2,3-triazole formed from the azide alkyne click reaction are not just spectators. It is able to form hydrogen bonds and being aromatic can participate in π-stacking interactions. The 1,2,3-triazole is also considered as an isostere of an amide, having a similar dipole moment. This has allowed the targeting of pharmaceutically relevant enzymes with triazole containing compounds such as protein tyrosine phosphatase,[150] protein kinase inhibitors,[151] transferase inhibitors,[152] glycogen phosphorylase,[153] serine hydrolase,[154] cysteine and serine protease inhibitors,[155,156] and aspartic protease inhibitors[157] (Figure 3.1). More examples of pharmaceutically relevant enzymes targeted by molecules can be found in a recent review by Krzysztof Jozwiak.[158]

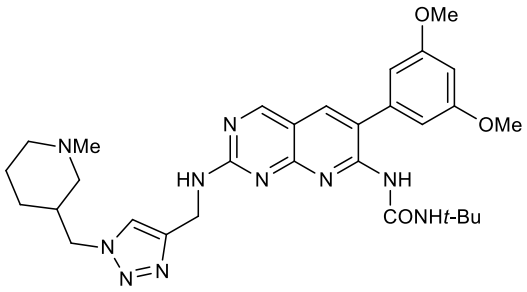
3.2 *In Situ* Click Chemistry

In situ click chemistry falls under the general category of kinetic target-guided synthesis because the cycloaddition reaction is irreversible. In contrast, dynamic combinatorial chemistry operates thermodynamically wherein fragments reversibly combine and shift the equilibrium when incubated with a target protein.[159] Although the Huisgen cycloaddition is extremely slow at room temperature due to the high activation energy, Mock and co-workers first showed that the rate of the reaction could be enhanced up to 10^5 when the azide and alkyne coupling partners were held in place near each other

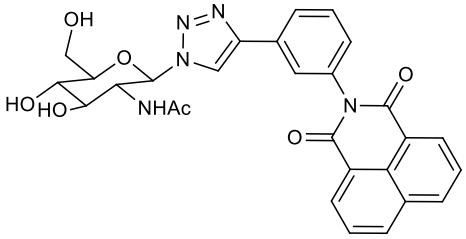
Figure 3.1. Some examples of enzyme inhibitors synthesized via CuAAC.



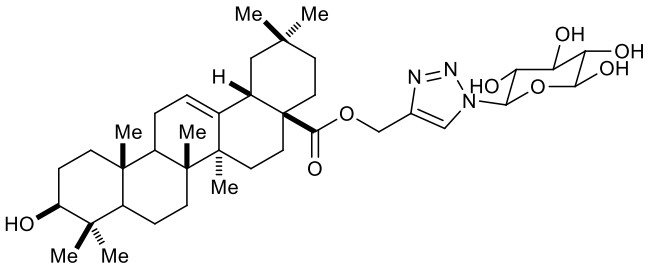
Mycobacterium protein tyrosine phosphotase B (mPTPB) inihitor (K_d = 160 nM)



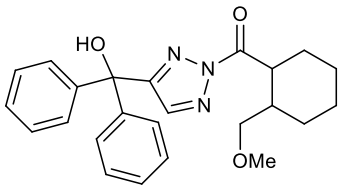
FGFR3 tyrosine kinase inhibitor



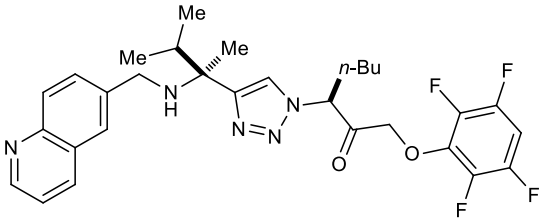
O-GlcNAcase Inhibitor (K_i = 185.6 μM)



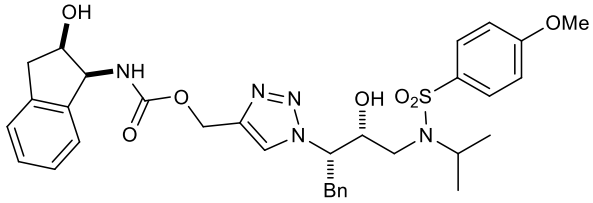
glycogen phosphorylase inhibitor (IC_{50} = 26 μM)



2,4-triazole
α,β-hydrolase-11 (ABHD11) (IC_{50} = 1 nM)



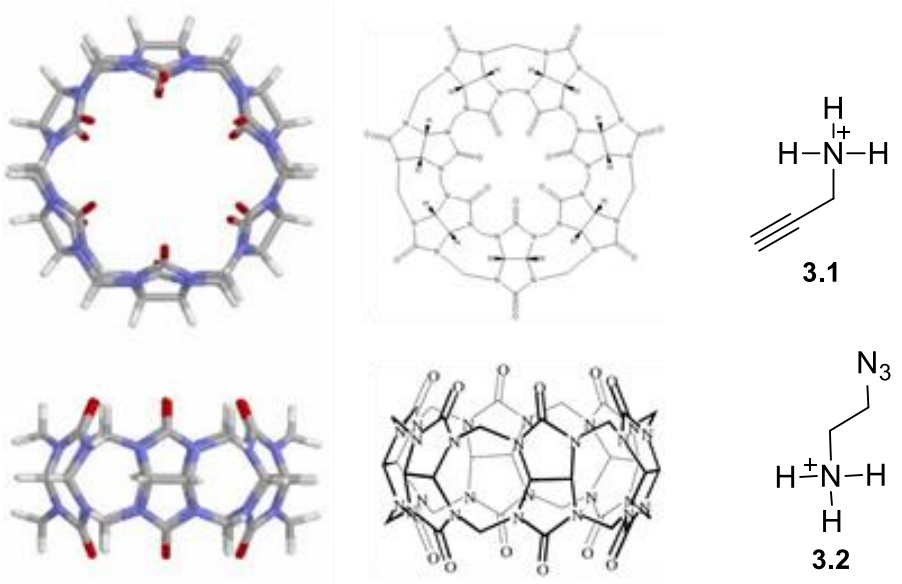
T. cruzi cysteine protease inhibitor (IC_{50} = 5.1 μM)



HIV-1 Protease inhibitor (K_i = 1.7 nM)

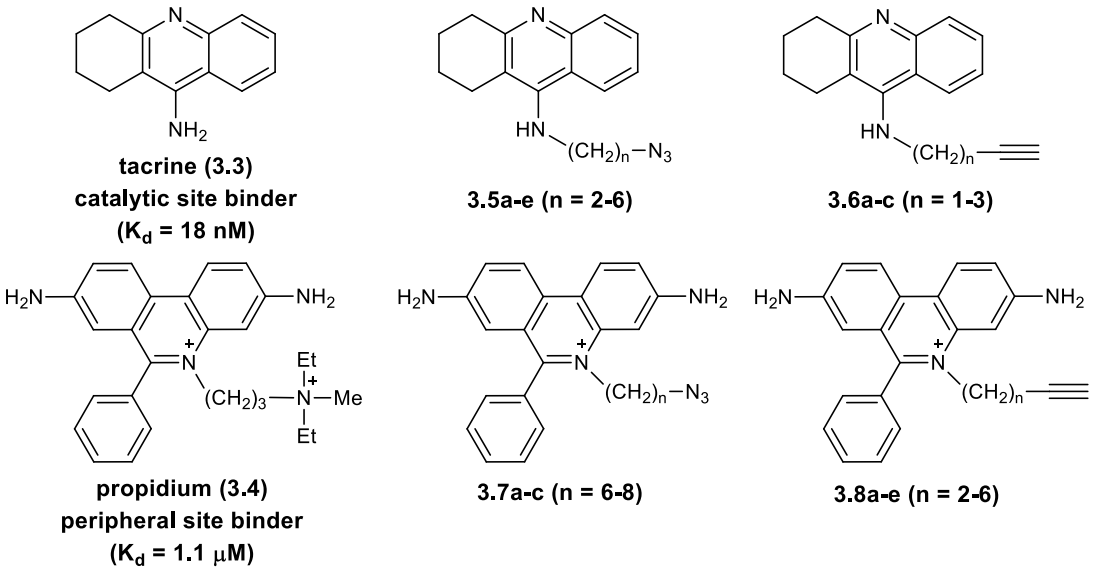
with the synthetic receptor cucurbituril, which contains a hollow core with the ability to bind ammonium ions (Figure 3.2). Ammonium cations **3.1** and **3.2** bind to cucurbituril simultaneously to each set of carbonyls with the alkyne and azide sidechains internalized thus allowing the enhanced rate of reaction of the cycloaddition.[160]

Figure 3.2. Structure of cucurbituril and azide and alkyne binding ammonium cations.



This work would inspire the first *in situ* click chemistry with an enzyme. Sharpless and co-workers chose acetylcholinesterase (AChE) as a target because of its importance in neurological function and because it contains two known binding sites; the catalytic site and a peripheral site. Compounds are known that bind both sites; tacrine (**3.3**) is a catalytic site binder possessing a dissociation constant (K_d) of 10 nM and propidium is a peripheral site binder containing a K_d of 1.1 μM (Figure 3.3).[159]

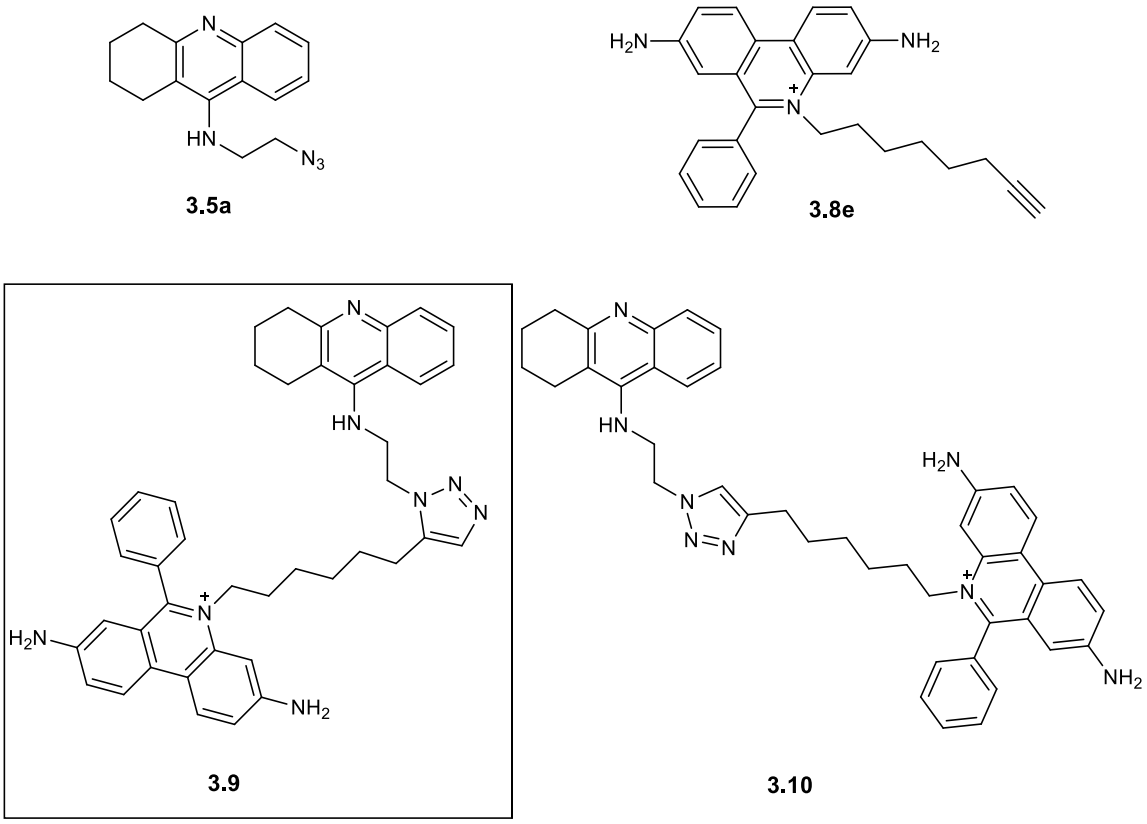
Figure 3.3. Tacrine (**3.3**) and propidium (**3.4**) with azide (**3.5a-e** and **3.7a-c**) and alkyne (**3.6a-c** and **3.8a-e**) derivatives for *in situ* study.



Using these two compounds, a small library of azides and alkynes were synthesized containing varying chain links. In all, eight compounds based on **3.3** (five azides **3.5a-e** and three alkynes **3.6a-c**) and eight compounds based on **3.4** (three azides **3.7a-c** and five alkynes **3.8a-e**) were synthesized. Each combination of **3.3** alkyne or azide with its corresponding **3.4** azide and alkyne were incubated separately with *Electrophorus* AChE at room temperature (Figure 3.4). These solutions were then analyzed by DIOS mass spectrometry, which was only able to detect the ion formed from one possible combination (i.e., **3.5a** and **3.8e** which combined to form **3.9** or **3.10**). HPLC of *in situ* and authentic samples followed by NMR experiments determined that *syn*-**3.9** was the compound formed in the *in situ* click experiment, which was shown to have 20-fold improvement in K_d against the target. Continued work on acetylcholinesterase showed that LC-MS was superior to DIOS-MS, providing nM limits of detection[161] and that *in situ* click could be performed

with mixtures of components instead of binary mixtures; in this case one azide and ten alkynes.[162] The ability to run multicomponent *in situ* click assays is of great significance as it allows for fast and efficient compound screening.

Figure 3.4. DIOS Mass spectrometry detection of **3.9** formed form **3.5a** and **3.8e**.



Building on these initial works with acetylcholine esterase, *in situ* click has been successful for the discovery of inhibitors of carbonic anhydrase,[163] HIV protease,[164] chitinase,[165,166] histone deacetylase,[167] mycobacterial transcriptional regulators,[168] and nictotinic acetylcholine receptors.[169] In addition, *in situ* click has found use for the development of protein-protein interaction modulators,[170] discovery of polyamide DNA binders,[171] and cell imaging.[172] Most recently, James Heath of Caltech has used *in situ* click

to discover in-cell inhibitors of botulinum neurotoxin[173] and found a selective inhibitor of a single point mutation of the Akt1 epitope.[174]

3.3 Current Study

3.3.1 Background

Solithromycin (**1.65**) is a 14-membered fluoroketolide based on the flagship ketolide telithromycin (Figure 3.5). Solithromycin was first developed by Optimer pharmaceuticals as OP-1068 and then developed by Cempra pharmaceuticals as CEM-101. It is currently in phase III clinical trials for the treatment of community-acquired pneumonia.[77] Solithromycin (**1.65**) possesses a fluorine at the C-2 position as well as a 1,2,3-triazole in place of the imidazole ring of telithromycin. In addition, it possesses an aniline in place of the pyridine, the latter of which is known to be the cause of hepatotoxicity in telithromycin.[75] **1.65** is known to bind the macrolide binding site of the 23S rRNA. Crystallographic data shows π-stacking interactions with U2609 and A752 as well as the potential hydrogen bond between the aniline nitrogen and the ribose sugar of A752 and/or G748 (Figure 3.6).

3.3.2 Proposed Ribosome-Templated *In Situ* Click Reaction to Make Solithromycin

We hypothesized that we could take advantage of these binding interactions to have the bacterial ribosome, specifically *E. coli* 70S ribosomes or 50S ribosomal subunits, template the formation of the 1,2,3-triazole ring of **1.65** from azide **3.11** and 3-ethynyl aniline (**3.12**) (Scheme 3.5). If successful, the bacterial ribosome would be validated as a target for the powerful drug discovery platform of *in situ* click chemistry. In order to test

this hypothesis, we would first need to synthesize **3.11**, which can be made from commercially available starting materials in nine steps.

Figure 3.5. Structure of fluoroketolide solithromycin (**1.65**) and ketolide telithromycin (**1.63**).

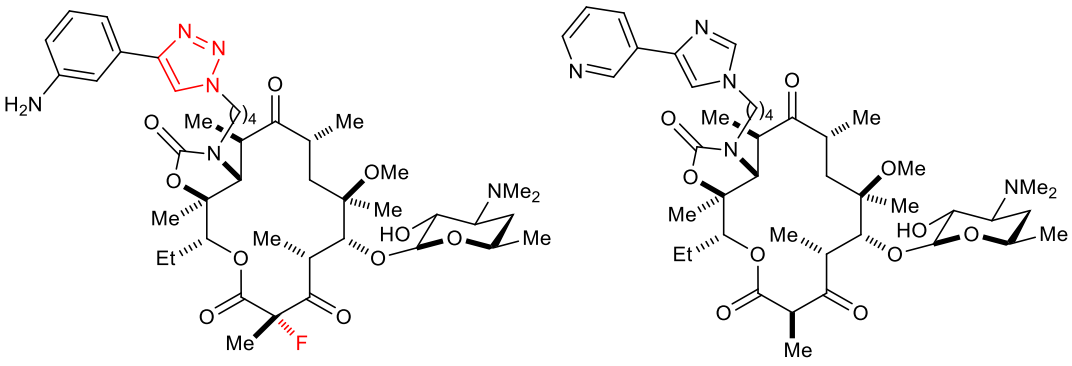
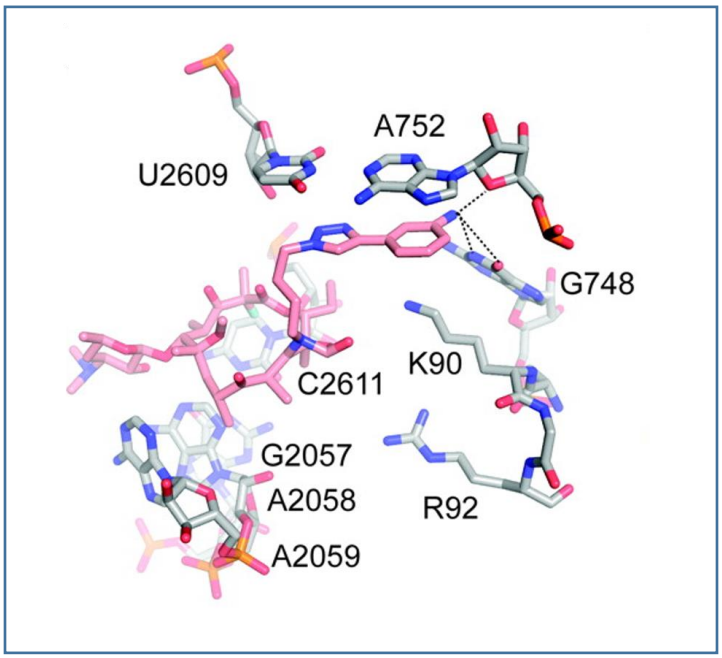
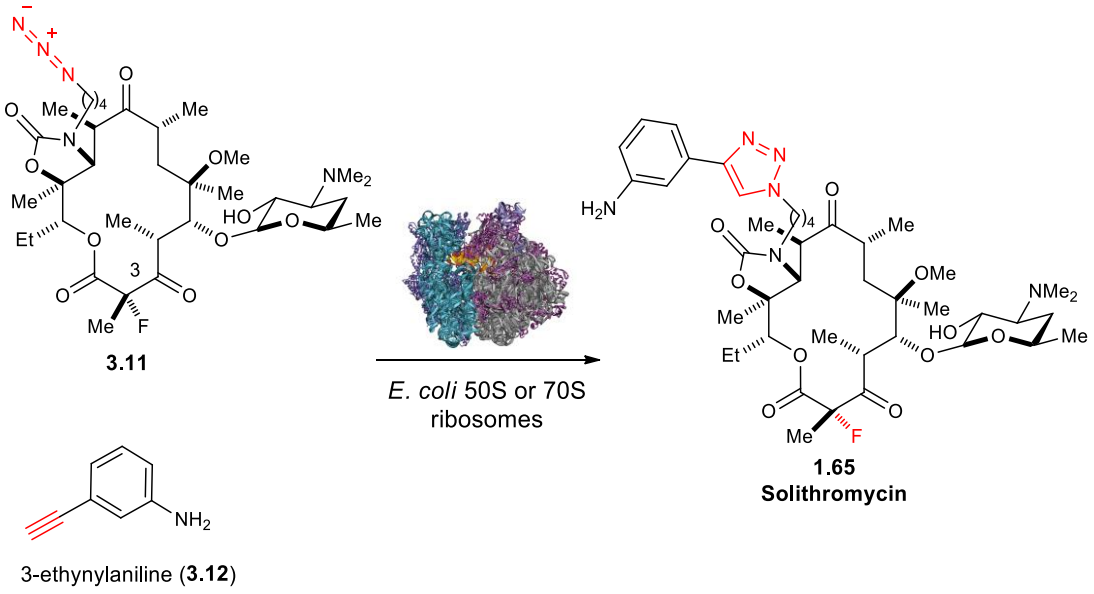


Figure 3.6. Crystal structure of solithromycin (**1.65**) bound to *E. coli* 70S ribosome showing key interactions with 23S rRNA residues.



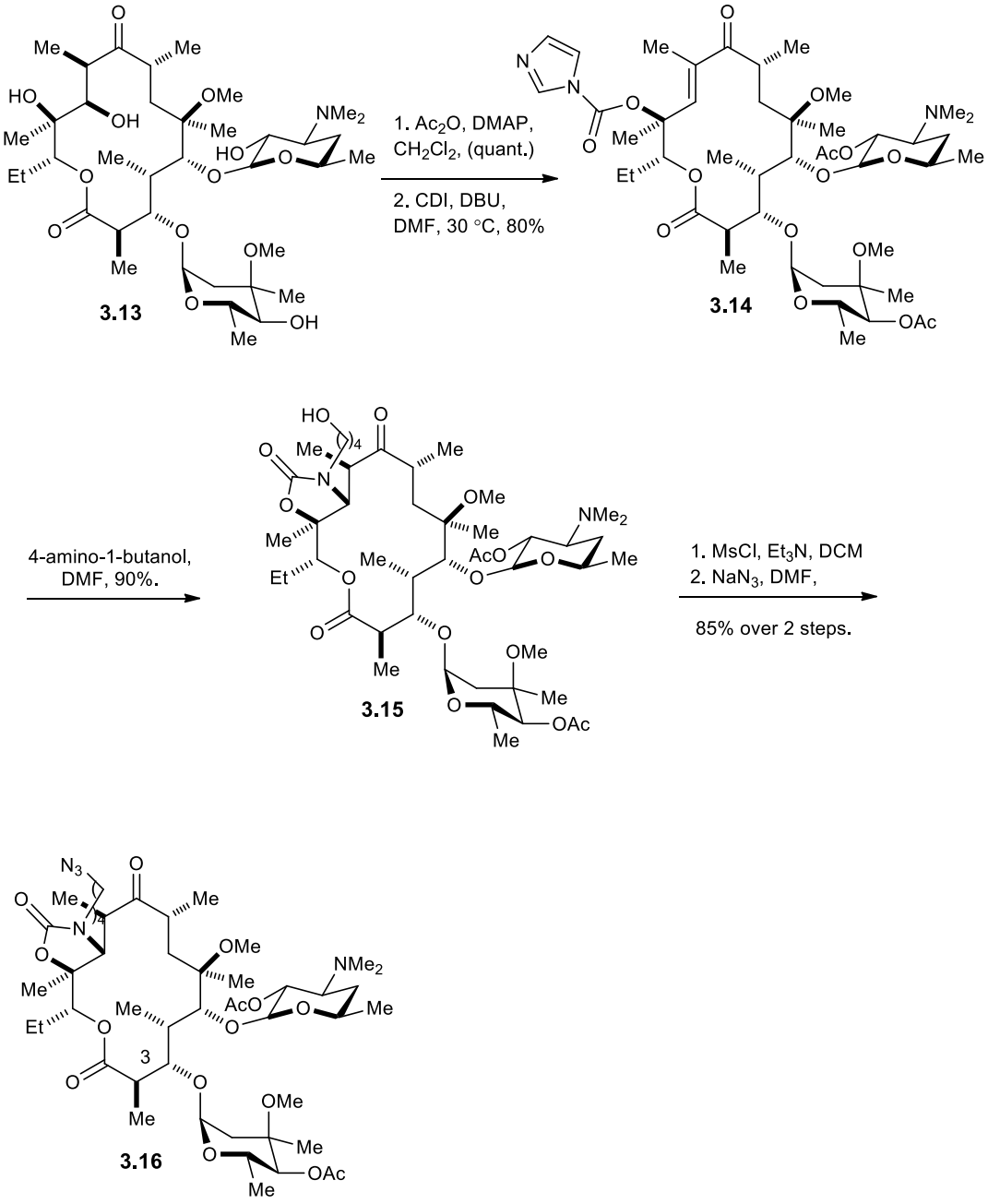
Scheme 3.5. Proposed Ribosome-Templated Azide-Alkyne Cycloaddition **3.11** and **3.12** to form **1.65**.



3.3.3 Synthesis of Azide 3.11

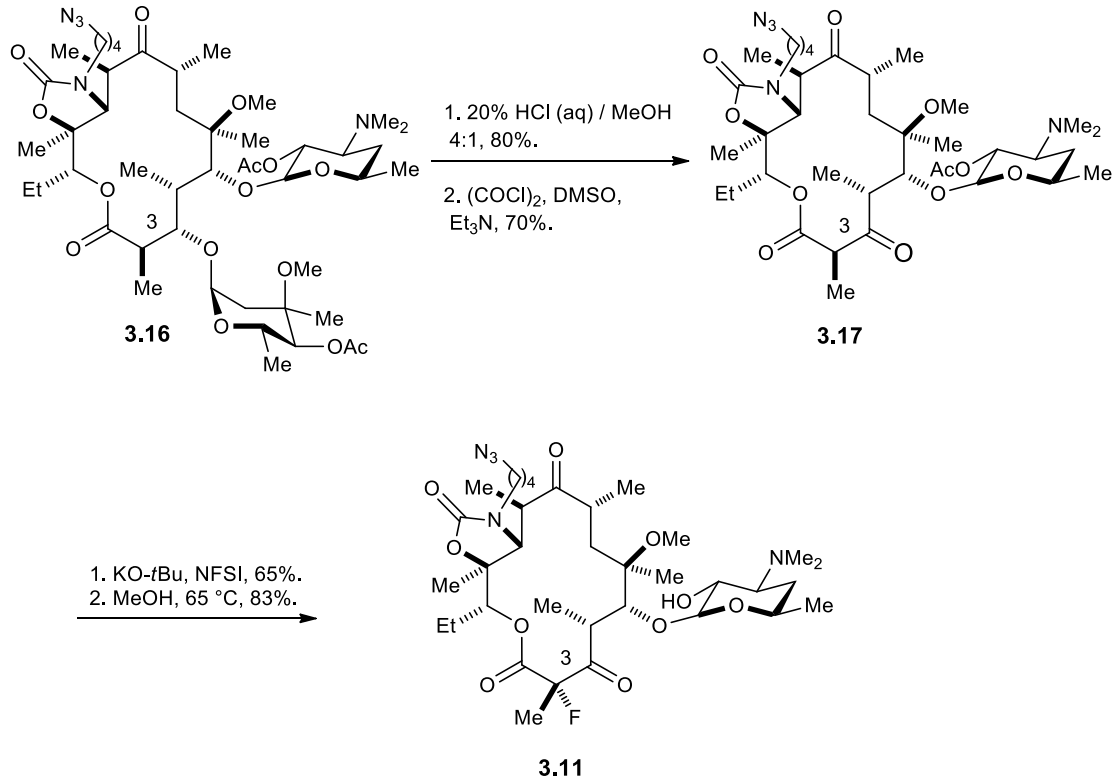
The synthesis of azide **3.11** was modified from that found in the literature.[175] The synthesis began with commercially available clarithromycin (**3.13**). The C-2' hydroxyl of desosamine and the C-4'' hydroxyl of cladinose were protected as acetates. Imidazole carbamate **3.14** was then formed in 80% yield by stirring at 30 °C in the presence of CDI and DBU. Baker cyclization of **3.14** with 4-amino-1 butanol provided **3.15** in 90% yield. The hydroxyl was then converted to the mesylate, which was found to be superior to the tosylate and was subsequently displaced with sodium azide to give azide intermediate **3.16** in 85% yield over two steps. It is worthwhile to note that these five steps were accomplished with no chromatography. Intermediates **3.14**, **3.15**, and **3.16** could be purified by crystallization by adding water to the reaction mixture (Scheme 3.6).[176]

Scheme 3.6. Synthesis of azide intermediate **3.16**.



Removal of the cladinose sugar was modified from the literature procedure by increasing the percentage of HCl from 10% to 20% to increase the reaction rate and the addition of methanol to enhance initial solubility of **3.16** leading to an 80% yield of hydrolyzed product. Swern oxidation of the resultant alcohol gave ketone **3.17** in 70% yield. Enolate formation with KO-*t*Bu and reaction with electrophilic fluorine source *N*-fluorobenzenesulfonimide (NFSI) gave fluorinated product in 65% yield. The conformation of the macrolactone provides exclusively the desired diastereomer.[177] Removal of the C2' *O*-acetate in refluxing methanol then provided azide **3.11** in 83% yield (Scheme 3.7).[176]

Scheme 3.7. Synthesis of azide **3.11**



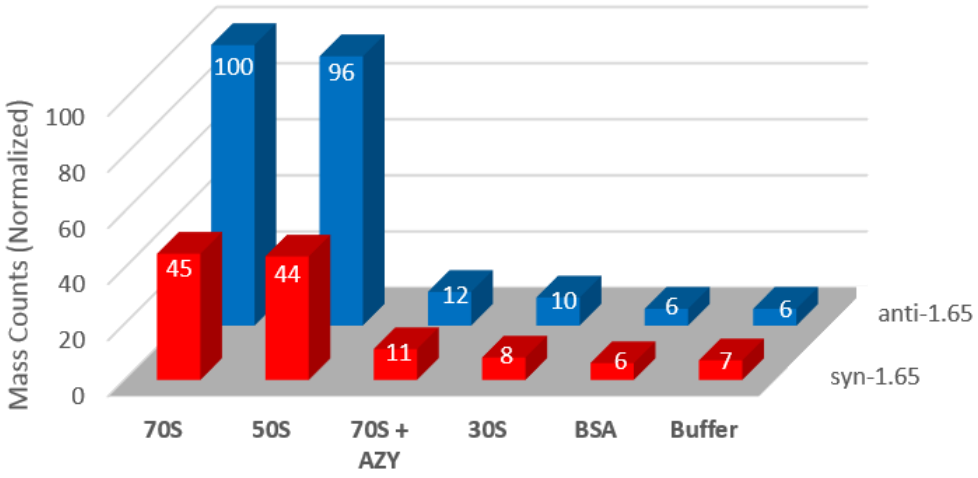
3.3.4 *In Situ* Click: Proof of Concept Experiments

With azide **3.11** in hand, we could now test our hypothesis that the bacterial ribosome is amenable to *in situ* click chemistry. Although the Huisgen 1,3-dipolar cycloaddition is very slow at room temperature, it can still produce detectable levels of 1,2,3-triazoles on the departmental Agilent 6520B Q-TOF LC-MS instrument. Therefore, the optimization of azide and alkyne concentrations was needed to ensure that the amount of triazole produced from this background reaction didn't exceed that produced by that templated by the ribosome. After carrying out various reaction conditions, it was found that incubating 70S or 50S *E.coli* ribosomes (5 μM), **3.11** (5 μM), and **3.12** (5 mM) in tris(hydroxymethyl)-aminomethane (Tris) buffer at room temperature for 24–48 hours showed >10-fold more *syn* and *anti*-**1.65** than when incubated in Tris buffer alone (Figure 3.7). Extracted ion chromatograms (EIC) were used to determine the location and quantity of *anti*- and *syn*-**1.65**. The retention time of a*nti*-**1.65** was determined by comparing to an authentic sample prepared by CuAAC and *syn*-**1.65** identified by comparing to a *syn*-*anti* mixture prepared through thermal cycloaddition.[176]

With a low nM dissociation constant, **3.11** acts like an anchor, fully saturating the macrolide binding site. **3.12** can only react with **3.11** if it can bind sufficiently long enough to facilitate the cycloaddition. Rigorous control experiments would show that the presence of the 50S ribosomal subunit, containing the macrolide binding site, was necessary for these results. Three important observations were made from these experiments. First, incubation in the presence of the 30S ribosomal subunit, with no macrolide binding site, or bovine serum albumin (BSA), which does not contain a macrolide binding site, showed approximately the same level of **1.65** as detected in Tris buffer alone. This clearly

demonstrates that an increased effective concentration or nonspecific protein or RNA interactions were not responsible for the increased presence of **1.65**. Secondly, addition to the *in situ* click reaction of known 2nd generation macrolide azithromycin (AZY, 25 μM), completely inhibited the formation of **1.65**, thus showing the cycloaddition does occur in the macrolide binding site. Thirdly, the ratio of *syn* and *anti*-**1.65** in all negative control experiments is approximately 1:1, yet the ratio of *syn* and *anti*-**1.65** is 1:2 in *in situ* click reactions with 70S or 50S *E. coli* ribosomes showing a definitive selectivity for *anti*-**1.65**.[176]

Figure 3.7. *In situ* click experiments with *E. coli* 70S ribosomes, 50S ribosomal subunits, 70S ribosomes with inhibitor azithromycin (AZY 25 μM) and negative controls (30S ribosomal subunits, BSA, or buffer only). Mass counts normalized to largest value.



3.3.5 Library Construction for Competition Assays

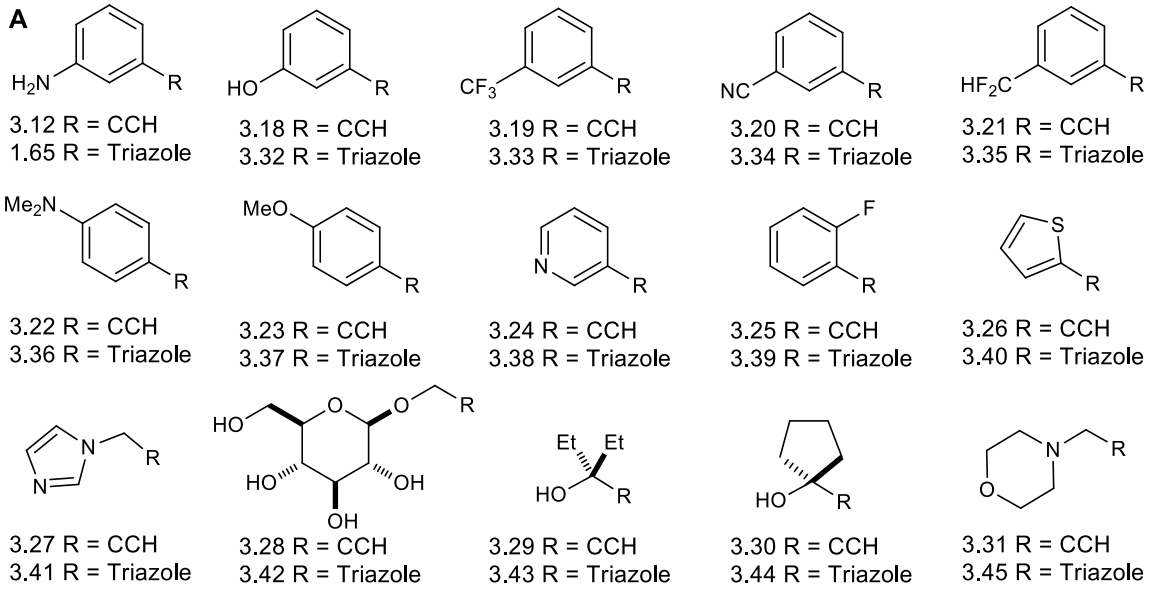
Having shown the bacterial ribosome to be a valid target for *in situ* click chemistry, we needed to show that the ribosome could template the tightest binding product from a pool of alkynes incubated with **3.11**. To this end, we selected a library of 15 structurally diverse alkynes, which included **3.12** (Table 1). The library consisted of aromatic alkynes with a *meta* substitution (**3.12, 3.18-3.21**), *para* substitution (**3.22-3.23**), an *ortho* substitution (**3.25**), and three heteroaromatic containing alkynes **(3.24, 3.26-3.27**). These alkynes have the potential to participate in π-stacking interactions with residues A752 and U2609 as well as other bases within the rRNA. They also have the ability to engage in hydrogen bonding with nearby residues. Nonaromatic alkynes were selected for their ability to function as hydrogen bond donors (**3.28**–**3.30**), hydrogen bond acceptors (**3.28**–**3.31**), and form electrostatic interactions with the negatively charged phosphate backbone of the rRNA (**3.31**).[176]

3.3.6 Determination of Dissociation Constants (K_d's)

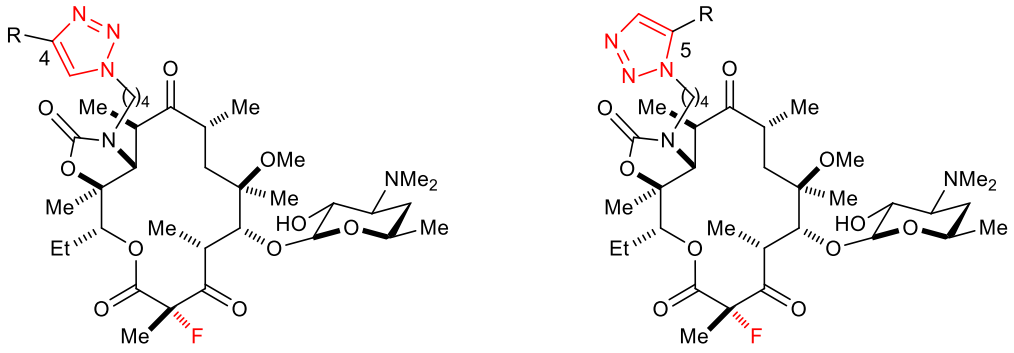
In order to compare results obtained from *in situ* click completion experiments, we first needed to experimentally determine the binding affinity of the clicked compounds. Because *anti*-**1.65** formed in twice the amount of *syn*-**1.65** in our initial proof of concept binary *in situ* click experiment, we chose to synthesize the *anti*-isomers of **1.65, 3.32-3.45** by copper (I) catalyzed click chemistry with standard copper sulfate, sodium ascorbate conditions to provide *anti*-**1.65**, **3.32-3.45** in 70-90% yield (Scheme 3.8).[176] Dissociation Constants (K_d) for *anti*-triazoles **1.65**, **3.32-3.45** as well as azide **3.11** were determined by a validated fluorescence polarization competition assay using a BODIPY-functionalized

Table 3.1. (A) Structure of alkyne fragments **3.12, 3.18-3.31** and triazoles products **1.65, 3.32-3.45** formed from cycloaddition with azide **3.11** (B) Possible regioisomeric *anti* - and *syn*-triazoles formed during i*n situ* click experiments.

A



B

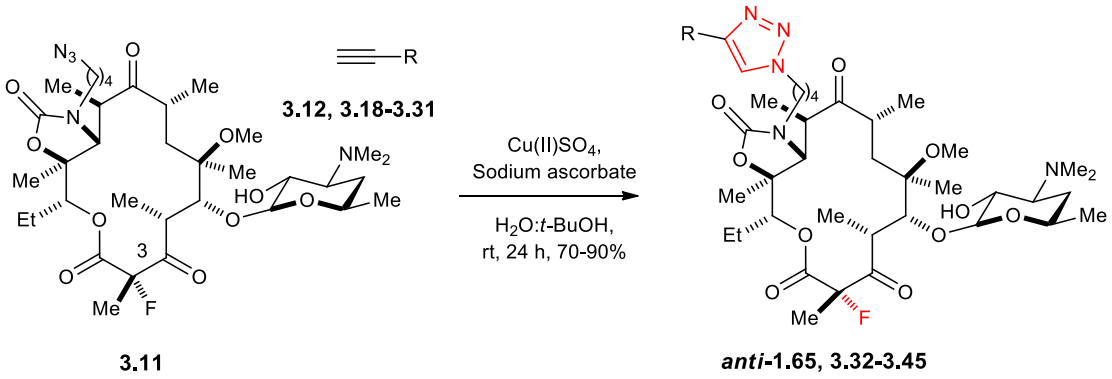


anti (1,4) isomer

syn (1,5) isomer

erythromycin.[178] Assays were run in triplicate and data derived from these experiments was fit to the Wang cubic equation to provide K_d's.[179] Gibbs free energy (ΔG) was determined from K_d with the equation (ΔG = -RTln K_d). These data are rank-ordered in **Table 3.2**. According to these data, there is only an 8-fold range in K_d for the compounds tested. 10 of the 15 compounds had a K_d better than azide **3.11** and five worse.[176]

Scheme 3.8. CuAAC of *anti*-**1.65, 3.32-3.45**.



3.3.7 Computational Work

To try and explain the very close range of K_d's, computational analysis utilizing site-identification by ligand competitive saturation (SILCS) was used to calculate the contributions the sidechains made to the overall binding affinity.[180-183] This method maps the functional group free energy pattern of molecules onto a grid and can quantitatively estimate the binding affinities as a ligand grid free energy (LGFE). The method allows for the estimation of the binding free energy contribution from different regions of the molecule. LGFE scores were calculated for (1) **1.65**, **3.32**–**3.45**, (2) the contribution of the side-chain alone, and (3) the contribution of the macrolactone and desosamine portion (Macro + DES). Interestingly, the predictive indices (PI) for total LGFE and the side-chain

were not predictive when compared to the experimental K_d data, but the LGFE for the Macro + DES portion did provide a satisfactory level of predictability with a PI of 0.37 (Table 3.2). This suggests that the binding is dominated by the macrolactone and desosamine portions of the molecule and that the sidechain portion, while adding to the total binding, can alter the structure of the rRNA to allow even tighter binding of the Macro + DES portion. This explanation seems reasonable knowing that rRNA is much more fluid in structure than protein[184] and computation has already shown that alteration of the A2058 residue has shown to alter the conformation of the U2609-A752 base pair.[185] This makes the application of *in situ* click chemistry to the bacterial ribosome even more powerful, as a traditional structure based design program would most likely fail using a static crystal structure of the ribosome to predict potential binding.[176]

3.3.8 Structure–Activity Relationships

Analysis of the K_d data gleaned several structure–activity relationships. *Meta*-substituted aromatic or heteroaromatic groups with the ability to participate in hydrogen bonding provided the greatest increase in binding affinity when compared to the azide precursor (e.g., **1.65, 3.32, 3.34-3.35, 3.38**). *Para*-substituted hydrogen bond acceptors **3.36** and **3.37** also received a boost in binding. Aromatic sidechains with no hydrogen bond donor/acceptors, such as trifluoromethyl **3.33** and monofluoro **3.39**, caused a decrease in K_d relative to the **3.11**. Nonaromatic sidechains, although containing the ability to hydrogen bond, also led to a decrease in binding (**e.g. 3.42-3.44**). This underscores the combined importance of π-stacking and hydrogen bonding with A752 to provide optimal binding. Five-membered heteroaromatic compounds **3.40** and **3.41,** containing a methylene spacer

between the triazole, also showed increased affinity. This type of sidechain is unrepresented in the literature and provides the opportunity to explore a novel chemo-type. Morpholine containing compound **3.45**, with the second best K_d, shows the importance of electrostatic interactions when targeting rRNA (i.e., the negatively charged phosphate backbone).[184]

Table 3.2. Rank-ordering of *anti*-triazoles **1.65**, **3.32-3.45** and azide **3.11** by dissociation constant (K_d) determined by fluorescence polarization competition assay with 70S *E. coli* ribosomes, along with experimentally determined Gibbs free energy values (ΔG) and calculated normalized LGFEs (kcal/mol) from SILCS analysis.

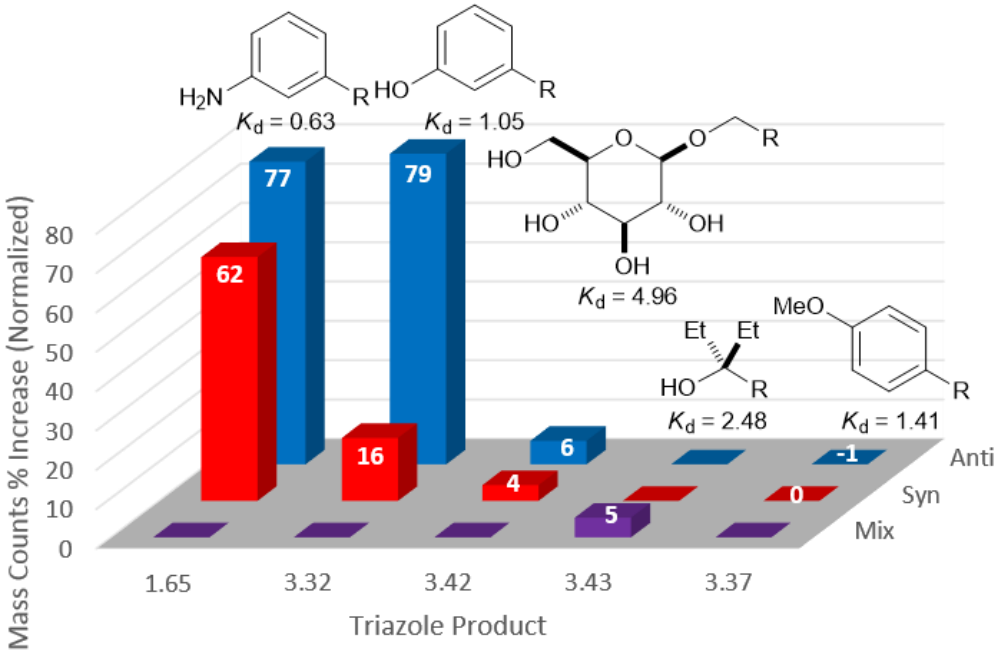
Compound (*anti*)	K_d (nM)	ΔG	total LGFE (kcal/mol)	side-chain LGFE (kcal/mol)	Macro+DES LGFE (kcal/mol)
1.65	0.63 ± 0.1	-12.54	-49.67	-16.20	-35.38
3.45	0.79 ± 0.12	-12.41	-51.83	-17.09	-35.3
3.32	1.05 ± 0.14	-12.24	-48.72	-14.01	-35.33
3.38	1.09 ± 0.12	-12.22	-48.70	-14.87	-35.57
3.36	1.26 ± 0.15	-12.13	-52.16	-17.19	-34.27
3.37	1.41 ± 0.15	-12.06	-50.83	-15.50	-34.86
3.41	1.53 ± 0.14	-12.02	-47.80	-14.27	-35.83
3.34	1.57 ± 0.15	-12.00	-50.82	-16.23	-35.24
3.35	1.74 ± 0.21	-11.94	-52.61	-17.81	-35.08
3.40	1.84 ± 0.16	-11.91	-47.93	-13.64	-34.7
Azide (3.11)	2.12 ± 0.39	-11.82	-38.85	-5.04	-35.85
3.44	2.43 ± 0.23	-11.74	-49.49	-15.15	-34.8
3.43	2.48 ± 0.25	-11.73	-48.96	-13.91	-35.37
3.39	2.52 ± 0.23	-11.72	-49.18	-15.39	-34.93
3.33	3.50 ± 0.51	-11.53	-52.86	-18.55	-34.91
3.42	4.96 ± 0.39	-11.32	-52.90	-18.10	-34.81
Predictive Indice	N/A	N/A	-0.22	-0.14	0.37

3.3.9 Five-Alkyne *In Situ* Click Competition Assays

With the K_d and computational data to guide us, we designed two *in situ* click experiments which would contain azide **3.11** and 5 alkynes in the presence of 50S *E. coli*

ribosomal subunits. These experiments would directly test whether the ribosome could selectively select triazole targets with a K_d lower than azide **3.11** over those with greater K_d values.

Figure 3.8. *In situ* click five-alkyne competition experiment with azide **3.11** (10 μM), alkynes **3.12, 3.18, 3.28, 3.29,** and **3.23** (2 mM each, 10 mM total) in the presence of 10 μM 50S *E. coli* ribosomal subunits (Anti = *anti*-triazole; Syn = *syn*-triazole; Mix = mixture of *syn*- and *anti*-triazoles).



The first 5-membered competition experiment (Figure 3.8) consisted of 10 μM azide **3.11** and 2 mM each of alkynes **3.12, 3.18, 3.28, 3.29**, and **3.23** incubated with 10 μM 50S *E. coli* ribosomal subunits at room temperature for 48 hours. **3.11** and ribosome concentrations were doubled from the initial binary experiment to insure formation of

triazoles products was sufficient to detect by LC-MS. Overall, alkyne concentration was doubled to 10 mM although each alkyne was present in only 2 mM. LC-MS analysis revealed that the formation of **1.65** and **3.32** dominated the experiment. *Anti*- and *syn*-**1.65** combined to have the highest amount of detected product followed by anti-**3.32**. With the two best K_d's of the 5 triazole products (*anti*-**1.65**; K_d = 0.63 and *anti*-**3.32**; K_d = 1.05), these data correlate very well with experimental binding affinity and again shows the importance of the *meta*-substituted aromatic sidechain with the ability to form hydrogen bonds. As expected, there was essentially no formation of **3.42** and **3.43**. Both of these compounds were found to have K_d's higher than azide **3.11**. Surprisingly, **3.37** was not detected in the *in situ* click reaction mixture even though its K_d is better than the **3.11**. This can be attributed competitive inhibition from the formation of **1.65** and **3.32**, which are two of the tightest binding triazole products produced from the set.[176]

The second five-membered *in situ* click experiment (Figure 3.9) contained 4 aromatic and 1 nonaromatic alkyne. **3.41** was detected in the greatest quantity, but unfortunately the *syn*- and *anti*- isomers were not resolvable by the HPLC method used. The next most abundant product was **3.38** which showed a 3:2 ration of *anti* to *syn*-triazole formation. Triazole **3.34** had a notable abundance and was also detected as a mixture of isomers. Out of the 5, these products had K_d improvement over azide **3.11**. Products **3.39** and **3.43**, with K_d values lower than azide **3.11**, were not detected in significant quantity.

3.3.10 Fifteen-Alkyne *In Situ* Click Competition Assay

Although the K_d values have a narrow range, the 5-membered *in situ* click experiments have demonstrated selectivity for the formation of triazoles with K_d's lower

than the azide starting material (**3.11**). To test the limits of this method, we designed an *in situ* click experiment utilizing all 15 alkynes (**3.12**, **3.18-3.31**). In order to improve solubility, the concentration of each alkyne was lowered to 1 mM from 2 mM, although the overall concentration of alkynes in solution increased to 15 mM from 10 mM. Alkyne solutions were also sonicated for 1–5 minutes and allowed to cool back to room temperature prior to addition of **3.11** or ribosomes. 70S *E. coli* ribosomes were used in place of 50S ribosomal subunits. The concentration of ribosomes was kept at 10 μM along with azide **3.11**.

Figure 3.9. *In Situ* Click Five Alkyne Competition Experiment with Azide **3.11** (10 μM), Alkynes **3.27, .3.24, 3.20, 3.25,** and **3.29** (2 mM each, 10 mM total) in the Presence of 10 μM 50S *E. coli* Ribosomal Subunits (Anti = *anti*-triazole; Syn = *syn*-triazole; mix = mixture of *syn*- and *anti*-triazoles).

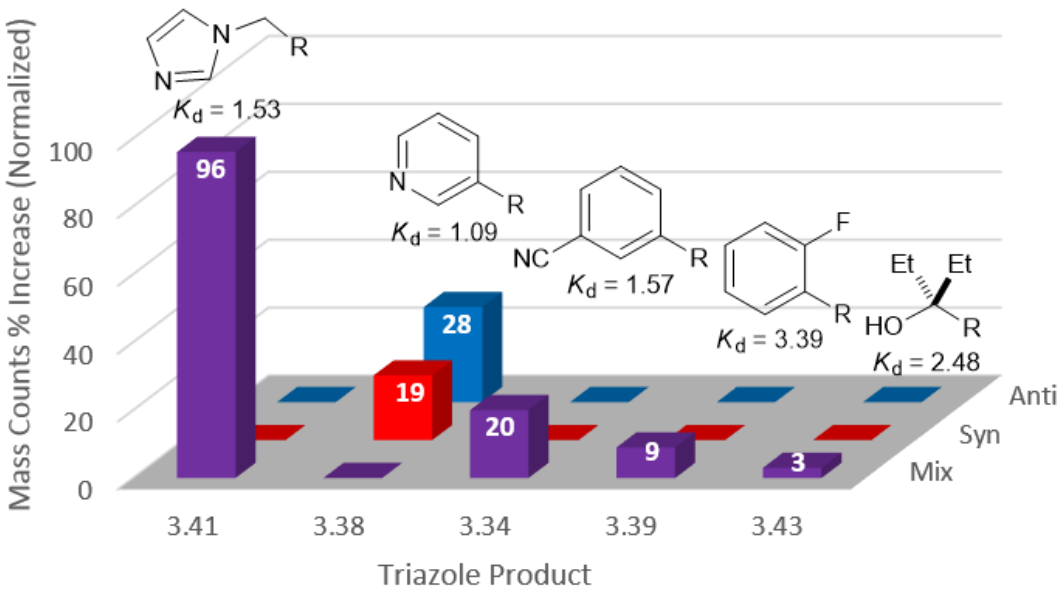
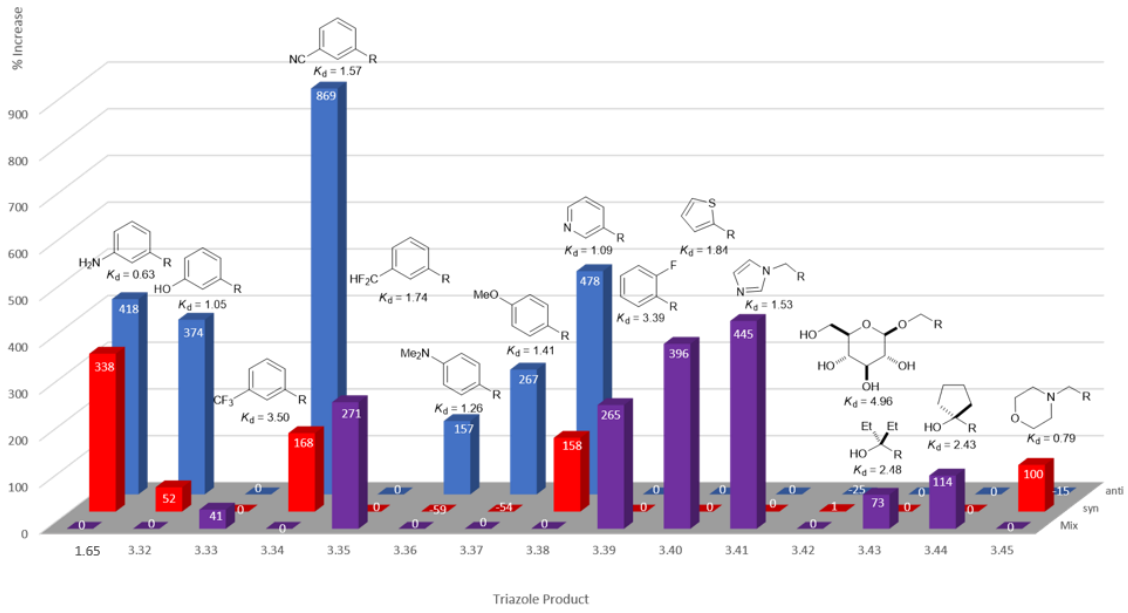


Figure 3.10. *In situ* click fifteen-alkyne competition experiment with azide **3.11** (10 μM), alkynes **1.65, 3.18**–**3.31** (1 mM each, 15 mM total) in the presence of 10 μM 70S *E. coli* ribosomes (anti = *anti*-triazole; syn = *syn*-triazole; mix = mixture of *syn*- and *anti*-triazoles).



The results of the fifteen-alkyne experiment are shown in Figure 3.10. Like the previous five-alkyne experiments, the fifteen-alkyne experiment was a good predictor of triazole products with K_d values better than azide **3.11**. **1.65, 3.32, 3.34, 3.35, 3.36, 3.37, 3.38, 3.40**, and **3.41** all showed significant formation in the assay. All have a K_d value lower than **3.11** and all are aromatic and contain the ability to hydrogen bond, thus showing the importance of this chemotype for increased binding affinity. Compounds **3.33, 3.42, 3.43**, and **3.44** showed little to no triazole formation in the assay. These compounds are

nonaromatic and all have K_d values worse than azide **3.11**. While these data were mostly consistent with the K_d data and previous five-alkyne experiments, there were a few anomalies. Fluorine containing triazole **3.39** binds less tightly than azide **3.11** but shows a mass count percent increase equal to some of the other tighter binding triazoles. Nitrile containing **3.34** had the highest detected mass count increase in the set. While the K_d value is better than **3.11**, the data is not consistent with the previous five alkyne experiment (Figure 3.4), where **3.34** was found in lower quantities than **3.41** and **3.38**. While still unexplained, the best hypothesis for the discrepancy is that the output is sensitive to the reaction conditions. There were three main differences between the five-alkyne and fifteen-alkyne experiments: (1) The fifteen-alkyne experiment represents a much more complex reaction mixture with 17 individual components and the possible formation of 30 triazole products; (2) The overall concentration of alkynes was less in the 5 alkyne experiment and the individual concentration was greater; (3) 70S ribosomes were used in the fifteen-alkyne experiment, while 50S ribosomal subunits were used in the 5 alkyne experiments. Although this didn't change the result in the binary experiment (Figure 3.2), this could have affected the outcome. Experiments were not run under both conditions due to the availability of 50S and 70S ribosomes. Another discrepancy was that morpholine containing triazole **3.45** was not detected in significant amounts even though it was found to have very high binding affinity (K_d = 0.8 nM). The enhanced binding of **3.45** is most likely due to an electrostatic interaction with a nearby phosphate. Ionic interactions like this are indiscriminate, meaning that they can add a great deal of binding affinity to a molecule but are not specific for the binding site. Without the macrolactone to tether it in place, alkyne **3.31** will interact indiscriminately with the rRNA phosphate backbone, thus effectively sequestering it from

the binding site. With the sea of negative charge in the ribosome, fragments containing protonated amines with no other binding elements will most likely not be compatible with *in situ* click chemistry. That said, more experiments need to be carried out to determine whether this is in fact the case.

3.3.11 Mechanism of Action and Minimum Inhibitory Concentration (MIC's)

The mechanism of action of all *anti*-triazole products **1.65, 3.32-3.45** and azide **3.11** were tested using a cell-free *in vitro* protein synthesis assay. Compounds were assayed at 1 μM and with low nM K_d's were expected to show inhibition of 70 ± 10%. As expected, all of the compounds inhibited protein synthesis, although the average range of inhibition was 48 ± 16%. Sequestration by assay components could limit the effective concentration of ligand. Results are shown in Table 3.3.

Minimum Inhibitory Concentrations (MIC) were determined against *Escherichia coli* (5 strains), *Staphylococcus aureus* (5 strains), and *Streptococcus pneumoniae* (6 strains). Full results are shown in Chapter 4. Representative strains where triazole compounds showed good activity are shown in Table 3.3.

Analysis of these data and comparison to the fifteen-alkyne *in situ* click experiment shows that *in situ* click was a good predictor of both tighter binders and MIC's. Out of the ten compounds found to have better K_d's than azide **3.11**, *in situ* click successfully predicted nine of these compounds (**1.65, 3.32, 3.38, 3.36, 3.37, 3.41, 3.34, 3.35**, and **3.40**). Comparing the eight novel compounds to solithromycin (**1.65**), two showed MIC values better than **1.65** in more than one assay (**3.32** and **3.40**). Seven of eight compounds showed an MIC value matching **1.65** in one or more MIC assay. Of the five compounds with a

higher K_d (i.e., weaker binder) than azide **3.11**, four compounds (i.e., **3.44, 3.43, 3.33,** and **3.42**) showed no significant triazole formation in the *in situ* click fifteen-alkyne experiment. In addition, the same four analogues showed MIC values worse than **1.65**.

Table 3.3. Dissociation constants (K_d); cell-free protein synthesis inhibition results; MIC analysis against *E. coli* DK pkk3535 and DK 2058G and S. *pneumonia* ATCC 49619 and 655 mefA (μg/mL); mass counts of *anti*- or mix triazoles from fifteen-alkyne i*n situ* click experiment (Values are normalized to solithromycin 1.65 as 100% for easier comparison).

Compound (*anti*)	K_d (nM)	% Inhibition	MIC *E. coli* DK pkk3535	MIC *E. coli* DK 2058G	MIC *S. pneumonia* ATCC 49619	MIC *S. pneumonia* 655 mefA	mass counts (*anti*-triazole)
1.65	0.63 ± 0.1	47 ± 11	2	2	0.004-0.008	0.25-0.5	100
3.32	1.05 ± 0.14	46 ± 3	2	1	≤ 0.002	0.5	89
3.38	1.09 ± 0.12	57 ± 10	2	2	0.016	0.5	114
3.36	1.26 ± 0.15	33 ± 4	4	4	0.008	1	50
3.37	1.41 ± 0.15	37 ± 5	2	2	0.002-0.008	1	64
3.41	1.53 ± 0.14	49 ± 12	2	2	0.032	4	106 (mix)
3.34	1.57 ± 0.15	58 ± 10	4	4	0.016	1	208
3.35	1.74 ± 0.21	42 ± 10	2	4	0.004	1	65 (mix)
3.40	1.84 ± 0.16	47 ± 12	1	1	0.004	0.5	95 (mix)
3.39	2.52 ± 0.23	47 ± 8	2	2	≤ 0.002	0.5	63 (mix)
Azide (3.11)	2.12 ± 0.39	55 ± 4	2	2	2	0.25	N/A
3.45	0.79 ± 0.12	64 ± 14	8	8	8	4	-4
3.44	2.43 ± 0.23	43 ± 2	4	4	0.016	2	27 (mix)
3.43	2.48 ± 0.25	47 ± 15	4	4	4	2	17 (mix)
3.33	3.50 ± 0.51	36 ± 9	4	4	4	2	10 (mix)
3.42	4.96 ± 0.39	32 ± 5	>32	>32	>32	>4	-6

There were two discrepancies found when comparing the fifteen-alkyne *in situ* click reaction to experimentally determined K_d's. 2-Flurobenzene-functionalized **3.39** showed a significant amount of triazole formation in the *in situ* click assay although it had a K_d worse than azide **3.11** whereas compound **3.45** had a very good K_d but showed no appreciable triazole formation in the *in situ* click assay. In both cases, the *in situ* click assays predicted correctly when compared to MIC's. **3.39** matched the MIC of **1.65** in 3 of 4 assays and was better in the 4th. **3.45** showed MIC values 4-fold to 200-fold worse than **1.65**. These data

shows that *in situ* click is a very accurate predictor of bacterial growth inhibition. It is most likely a coincidence that *in situ* click accurately predicted bacterial growth inhibition even when K_d values disagree due to the many complex variables that can attribute to the result, but future experiments should be able to provide an answer to this interesting result.

3.3.12 Mammalian Cell Toxicity Assay

While macrolide antibiotics have been used safely in humans since erythromycin A in the 1950's, these novel click derived compounds represent novel compounds that have not been tested in human and as such would need to be screened to determine if they are toxic to human cells. Utilizing a collaboration with the Moulder Center for Drug Discovery Research, potential cytotoxicity of human dermal fibroblasts (GM05659, Coriell Institute, Camden, NJ)[186] was measured using a commercial luciferase coupled ATP quantitation assay (CellTiter-Glo, Promega).[187] The assay was run on **3.11, 1.65, 3.32, 3.37, 3.40**, and **3.41**. The change in ATP content is measured by luminescence to determine the number of metabolically competent cells. The assays showed that all compounds tested had no effect on human fibroblasts until low micromolar concentration. The compounds, as expected, had great selectivity for bacteriostatic activity over mammalian cytotoxicity.[176]

3.4 Conclusion

In summary, we have developed the first ribosome templated *in situ* click assay with 70S *E. coli* ribosomes and 50S ribosomal subunits. Known triazole containing fluoroketolide solithromycin (**1.65**) was used to establish proof-of-concept. The *in situ* click method was shown to accurately predict compounds that bind better than azide

precursor **3.11** and have lower MIC's equal to solithromycin (**1.65**) when competition assays containing both five and fifteen alkynes were used. This assay can eliminate the laborious need to synthesize countless numbers of potential drug candidates, saving substantial resources.

The success of this initial study paves the way for the further advancement of the method. The Andrade lab is currently working on the synthesis of various macrolactone cores with diverse azide linkers. This will allow for exploration of novel chemical space near the macrolide binding pocket. The isolation of resistant *E. coli* ribosomes containing A2058 *N,N*-dimethylated nucleosides. Effective use of the *in situ* click method with resistant ribosomes would allow for the selective targeting of a particular resistance mechanism. Perhaps the most significant work currently under way is the translation of the *in situ* click method to an *in cellulo* click method using whole cell lysates. Isolation of active bacterial ribosomes is still a tedious process and isolation from many strains of bacteria, especially pathogenic bacteria, has yet to be accomplished. The use of a whole cell lysate to perform a kinetic target-guided screen would eliminate this difficulty and open the door to directly targeting any bacterial strain.

CHAPTER 4: EXPERIMENTAL SECTION

4.1 Chapter 2: Total Synthesis and Biological Evaluation of (–)-4-Desmethyl Telithromycin

4.1.1 General Methods

All reactions containing moisture or air sensitive reagents were performed in oven-dried glassware under nitrogen or argon. N,N-Dimethylformamide, tetrahydrofuran, toluene and dichloromethane were passed through two columns of neutral alumina prior to use. Anhydrous dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich and subjected to three cycles of freeze-pump-thaw before use. Pyridine, 2,6-lutidine, acetone, i-Pr_2NEt, and Et_3N were all distilled from CaH_2 prior to use. Molecular sieves (4Å) were activated by flame drying under vacuum prior to use. AgOTf was purchased from Sigma-Aldrich and azeotropically with dry toluene prior to use. Compounds **2.24**[97], **2.34**[129], **1.72**[112] and **1.70**[188] were prepared according to known literature procedures. All other reagents were purchased from commercial sources and used without further purification. All solvents for work-up procedures were used as received. Flash column chromatography was performed according to the procedure of Still using ICN Silitech 32-63 D 60Å silica gel with the indicated solvents. All HF reactions are performed in Nalgene containers. Thin layer chromatography was performed on Merck 60 F_{254} silica gel plates. Detection was performed using UV light, $KMnO_4$ stain, PMA stain and subsequent heating. 1H and 13C NMR spectra were recorded at the indicated field strength in $CDCl_3$ at RT. Chemical shifts are indicated in parts per million (ppm) downfield from tetramethylsilane (TMS, $\delta = 0.00$) and referenced to the $CDCl_3$. Splitting patterns are abbreviated as follows: s (singlet), d (doublet), bs (broad singlet), bd (broad doublet), t (triplet), q (quartet) and m (multiplet).

Dissociation constants (K_d) were determined by fluorescence polarization using a Tecan F200 plate reader and following procedure of Yan for determining K_d of macrolide antibiotics by competitive binding with a Bodipy labeled Erythromycin A.[178] Data was fit using Graphpad Prism using Wang's cubic derived equation for the direct determination of Kd for a competitive binding assay.[179]

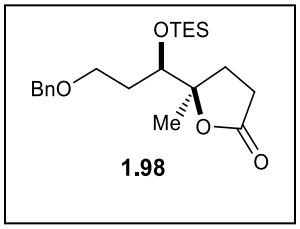
4.1.2 Computational Methods

Calculations are performed with the program CHARMM, version C35b6[189] and the CHARMM additive force field[189-193] including the protein nucleic acid,[194-197] carbohydrate,[129,190,198-201] and Geoff parameters and the TIP3P water model. Coordinates are obtained from the protein crystal database (PDB ID 3OAT), with hydrogens added using the HBUILd facility in CHARMM. All molecular dynamics (MD) simulations are performed using a stochastic boundary based approach that has been presented previously.[193] Briefly, the system is truncated to the region of interest around telithromycin by deleting residues outside of 40 Å of telithromycin's center of mass. Residues are considered within 40 Å if one atom is within the distance criterion. This truncation scheme reduces the number of atoms, making the MD simulations less computationally expensive. Then, three regions within the sphere are defined. Bases and residues containing one or more atoms within 28 Å comprise the dynamic region, those not in the dynamic region containing one or more atoms within 34 Å comprise the buffer region, and the remainder comprises the outer reservoir region. Atoms within the reservoir region are fixed for all calculations, while varying harmonic restraints are used on atoms within the buffer and dynamic regions as described below. Water is maintained within the sphere using a

spherical, quartic restraining potential as implemented in the MMFP module of CHARMM[202] using a 1 kcal/mol/Å force constant and offset parameter (P1) of 2.5 that was applied to the water oxygen atoms. Prior to dynamics, the entire system is first subjected to 250 steps of steepest descent (SD)[203] minimization with a harmonic restraint of 5 kcal/mol/Å on non-hydrogen atoms within the dynamic region and a mass-weighted harmonic restraint of 10 kcal/mol/Å on non-hydrogen atoms within the buffer region, followed by 250 steps of Adopted-Basis Newton Rhapson (ABNR)[189] using the same restraints. Equilibration consists of 400 ps (20 cycles) of Grand Canonical Monte Carlo/Molecular Dynamics (GCMC/MD) using the aforementioned restraints. GCMC/MD is implemented within the MC module in CHARMM [29] and has been described previously.[204] Following equilibration, the C4-desmethyl telithromycin and mutant/modified A2058 ribosomes are generated. Inactive water molecules from the GCMC/MD equilibration are deleted and patches are applied to telithromycin and A2058 in order to generate C4-desmethyl telithromycin with WT, A2058G, N6-monomethyl (MAD), and N6, N6'-dimethyl A2058 (DMAD). Atoms modified during the patch are subjected to minimization for 200 steps SD and 200 steps CG, and the entire system as allowed to relax for 50 steps SD and 50 steps CG. Parameters for the N6-mono and N6,N6'-dimethyl A2058 have been obtained by our lab previously[203]. Two monomethyl systems were studied due to the high energy barrier for the C6-N6 torsion, in which the methyl group in MAD1 is oriented toward telithromycin's desosamine sugar and away from it in MAD2. All systems were then subjected to 5 ns of Langevin dynamics[203] at 298 K with a friction coefficient of 5/ps and a 2 fs integration timestep using the "leapfrog" Verlet integrator.[205] SHAKE Nonbond lists are updated heuristically during dynamics with a

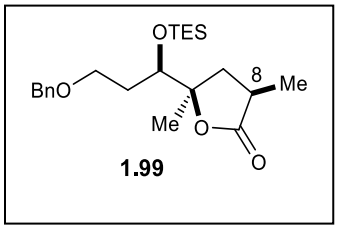
cutoff of 16 Å, the forces truncated at 12 Å and a switching function applied to the forces from 10 to 12 Å for both electrostatic and van der Waals energy terms. Interaction energies reported are calculated using the last 4 ns of the simulation, with the same non-bonded cutoffs as used during dynamics. Snapshots were written every 10 ps. The neutral group surrounding the C4 methyl [C3(=O)-C4(H2)-C5] is used so as not to calculate the interaction between species with non-integer charge.

4.1.3 Experimental Procedures and Characterization



1.98

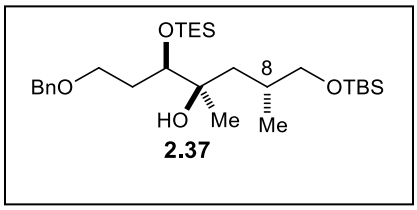
TES Lactone 1.98: Imidazole (3.32 g, 48375 mmol) and TESCl (6.3 g, 41.78 mmol) were added to a solution of 2.34 (9.2 g, 34.8 mmol) in DMF (250 mL) at 0 ºC. The solution was stirred for 1.5 h while warming to room temperature. Water (200 ml) was added and the mixture extracted with Et_2O (4 x 200 mL). The combined organic fractions were washed with water (100 mL), brine (100 mL), and filtered over sodium sulfate. The solvent was removed under reduced pressure and product purified by flash chromatography eluting with 0-30% EtOAc in hexanes to give 11.8 g (90%) of **1.98**. [α]23D +12.9° (c 1.05, CH_2Cl_2); IR (neat) 3521, 3030, 3064, 2953, 2874, 1771, 1496, 1454, 1416, 1385, 1240, 1205, 1113, 1041, 1006, 738 cm-1; 1H NMR (400 MHz) δ 7.32-7.20 (m, 5H), 4.44 (d, J = 12.0 Hz, 1H), 4.40 (d, J = 12.0 Hz, 1H), 3.77 (dd, J = 9.2, 2.4 Hz, 1H), 3.53 (m, 2H), 2.54-2.43 (m, 2H), 1.98-1.25 (m, 3H), 1.51-1.46 (m, 1H), 1.25 (s, 3H), 0.89 (t, J = 8.4 Hz, 9H), 0.65-0.50 (m, 6H); 13C NMR (100 MHz) δ 176.1, 138.1, 128.1 (2C), 127.5 (2C), 127.4, 88.6, 74.6, 72.8, 66.4, 32.7, 30.5, 28.8, 20.1 (3C), 4.8 (3C). HRMS (FAB) calc'd for $C_{21}H_{34}O_4Si$+Na= 401.2124, found 401.2126.



1.99

Methylated Lactone 1.99: *n*-BuLi (26.7 mL, 2.5 M in hexanes) was added dropwise to a solution of diisopropylamine (7.4 g, 72.8 mmol) in THF (300 mL) at -78 ºC. The solution was warmed to 0 ºC for 30 minutes then cooled back to -78 ºC. **1.98** (23 g, 60.7 mmol) in THF (100 mL) was added and the solution stirred for 2 h before addition of MeI (86g, 607 mmol) in THF (20 mL). After 30 minutes sat'd aq. NH_4Cl (100 mL) was added. The mixture was extracted with EtOAc (3 x 300 mL). The combined organic fractions were washed (brine), dried (sodium sulfate), and solvent removed under reduced pressure. After drying under high vacuum, the material was taken to the next step. To the freshly prepared LDA [THF (300 mL), diisopropylamine (8 g, 78.9 mmol), *n*-BuLi (29 mL, 2.5 M solution in hexanes)], was added the residue in THF (100 mL) at -78 ºC. The solution was stirred for 30 mins at -78 ºC and 1.5 h at -45 ºC. Triphenylacetic acid (17.5 g, 91.1 mmol) in THF (200 mL) was then cannulated into the reaction mixture at -78 ºC and allowed to warm to room temperature. After 2 h NH_4Cl (100 mL) was added. The mixture was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed (brine), dried (sodium sulfate) and filtered. The solvent was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with 0-30% EtOAc in hexanes to afford 14.3 g (60%) of **1.99** as a colorless oil. [α]23D +17.6° (c 1.06, CH_2Cl_2); IR (neat) 3088, 3041, 1772, 1494, 1360, 1103, 952, 698 cm-1; 1H NMR (400 MHz) δ 7.32-7.18 (m, 5H), 4.45 (d, J = 11.6 Hz, 1H), 4.39 (d, J = 11.6 Hz, 1H), 3.72 (dd, J = 9.6, 2.0 Hz, 1H), 3.52-3.48 (m, 2H), 2.71-2.66 (m, 1H), 2.00 (dd, J = 12.4, 3.6 Hz, 1H), 1.7-1.4 (m, 3H), 1.22 (s, 3H), 1.17 (d, J = 7.2 Hz, 3H), 0.88 (t, J = 8.6 Hz, 9H), 0.65-0.51 (m, 6H); 13C NMR (100 MHz) δ 178.5, 138.2, 128.3 (2C),

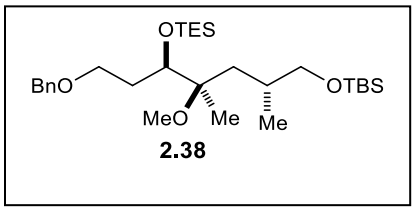
127.7 (2C), 127.6, 86.1, 75.1, 73.1, 66.6, 39.3, 34.8, 32.6, 18.9, 15.1, 6.9 (3C), 5.0 (3C). HRMS (FAB) calc'd for $C_{22}H_{36}O_4Si$+Na = 415.2281, found 415.2282.



2.37

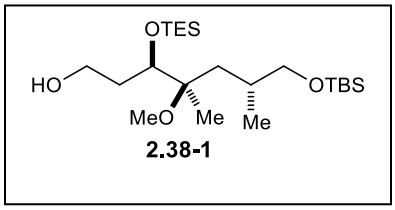
Tertiary Alcohol 2.37: To a suspension of $LiAlH_4$ (3 g, 80 mmol) in THF (300 mL) at -45 ºC was added lactone **1.99** (24 g, 61 mmol) dissolved in THF (100 mL) dropwise via cannula. After 3h, the reaction mixture was diluted with Et_2O (300 mL) and quenched by adding sat'd aq. sodium sulfate (200 mL) dropwise at 0 ºC. The aqueous layer was extracted with Et_2O (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, dried under high vacuum, and dissolved in DMF (300 mL). Imidazole (5.8 g, 85.5 mmol) and TBSCl (12.7 g, 73.3 mmol) were added, and the reaction mixture was stirred for 1 h. The reaction was quenched with water (100 mL) and extracted with CH_2Cl_2 (4 x 150 mL). The combined organic layers were washed with water (200 mL), brine (200 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with 0-30% EtOAc in hexanes to afford 23 g (75%) of **2.37** as a colorless oil. [α]23D +15.8° (c 3.3, CH_2Cl_2); IR (film) 3431, 3030, 2952, 2874, 2856, 1455, 1361, 1250, 1076, 1004, 938, 833,775, 696 cm-1; 1H NMR (400 MHz) δ 7.27-7.16 (m, 5H), 4.44 (d, J = 9.6 Hz, 1H), 4.40 (d, J = 10.0 Hz, 1H), 3.55-3.42 (m, 4H), 3.28 (dd, J = 6, 4.4 Hz, 1H), 1.95-1.84 (m, 2H), 1.58-1.42 (m, 2H), 1.33-1.26 (m, 1H), 1.05 (s, 3H), 0.87 (t, J = 8.6 Hz, 9H), 0.84 (s, 9H), 0.59-0.49 (m, 6H), -0.01 (s, 6H); 13C NMR (100 MHz) δ 138.5, 128.2 (2C), 127.6 (2C), 127.3, 77.1, 73.9, 72.8, 69.7, 67.9, 42.0, 33.0, 30.8, 25.8 (3C), 22.8, 19.4, 18.2, 7.0

(3C), 5.3 (3C), -5.5 (2C). HRMS (FAB) calc'd for $C_{28}H_{54}O_4Si_2$+Na= 533.3458, found 533.3439.



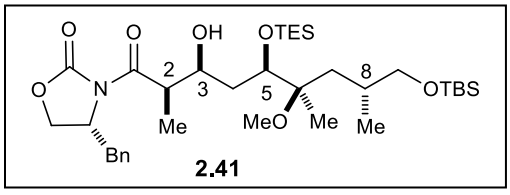
2.38

Methyl Ether 2.38: 2,6-DTBMP (92.0 g, 450 mmol) and MeOTf (44.3 g, 270 mmol) were added to a solution of **2.37** (23 g, 45 mmol) in CH_2Cl_2 (450 mL). After 48h, sat'd aq. $NaHCO_3$ (300 mL) was added and stirred for 10 mins followed by MeOH (300 mL) and stirred for 30 mins. The mixture was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic fractions were washed with brine (200 mL), dried (Na_2SO_4) and filtered. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography eluting with hexanes to recover 2,6-DTBMP the 0-30% EtOAc in hexanes to give 20 g (76%) of desired ether **2.38**. 5.2 g (76 %) of **3.7** as a colorless oil. [α]23D +11.0° (c 2.5, CH_2Cl_2); IR (film) 2950, 2929, 2874, 2855, 1461, 1360, 1249, 1093, 1072, 1006, 835, 775 cm-1; 1H NMR (400 MHz) δ 7.27-7.27 (m, 5H), 4.45 (d, J = 12.0 Hz, 1H), 4.41 (d, J = 12.0 Hz, 1H), (m, 1H), 3.83 (dd, J = 10, 1.6 Hz, 1H), 3.53-3.47 (m, 2H), 3.36-3.23 (m, 2H), 3.06 (s, 3H), 1.19 (m, 1H), 1.74 (bs, 1H), 1.05 (s, 3H), 0.88 (t, J = 8.6 Hz, 9H), 0.83 (s, 9H), 0.58-0.49 (m, 6H), -0.03 (s, 6H); 13C NMR (100 MHz) δ 138.7, 128.1 (2C), 127.5 (2C), 127.2, 79.5, 73.6, 72.7, 69.1, 67.8, 48.4, 35.7, 32.5, 31.0, 25.9 (3C), 18.3, 17.6, 7.06 (3C), 5.3 (3C), -5.4 (2C). HRMS (FAB) calc'd for $C_{28}H_{54}O_4Si_2$+Na= 547.3615, found 547.3596.



2.38-1

Alcohol 2.38-1. 10% Pd/C (576 mg, 0.5 mmol) was added to a solution of ether **2.38** (2.85 g, 5.4 mmol) in EtOH (54 mL) and stirred under an atmosphere of hydrogen. After 4 hours the hydrogen was removed and the reaction mixture was filtered through a Celite plug. The solvent was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with 0-40% EtOAc in hexanes to give 1.9 g (80%) of **2.38-1** as a colorless oil. [α]23D +6.0° (c 1.1, CH_2Cl_2); IR (film) 3349, 2929, 2875, 1642, 1376, 1251, 1166, 1062, 835, 775, 740, 666 cm-1; 1H NMR (400 MHz) δ 3.89 (dd, J = 8.8, 3.6 Hz, 1H), 3.78-3.63 (m, 2H), 3.31 (d, J = 6.4 Hz, 2H), 3.15 (s, 3H), 1.86-1.79 (m, 1H), 1.77-1.69 (m, 1H), 1.63-1.55 (m, 1H), 1.52 (dd, J = 15.2, 3.2 Hz, 1H), 1.34 (dd, J = 15.2, 8.4 Hz, 1H), 1.07 (s, 3H), 0.95 (t, J = 8.0 Hz, 9H), 0.95 (d, J = 6.4 Hz, 3H), 0.87 (s, 9H), 0.65-0.56 (m, 6H), 0.02 (s, 6H); 13C NMR (100 MHz) δ 79.6, 76.4, 69.1, 60.9, 48.7, 35.7, 34.9, 31.1, 25.9 (3C), 18.5, 17.7, 6.9 (3C), 5.2 (3C), -5.4 (2C). HRMS (FAB) calc'd for $C_{22}H_{50}O_4Si_2$+Na = 457.3145, found 457.3150.

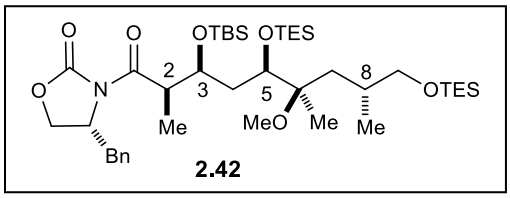


2.41

Aldol Product 2.41. DMSO (1.4 g, 18.4 mmol) was added dropwise to a solution of oxalyl chloride (1.1 g, 8.8 mmol) in CH_2Cl_2 (75 mL) at -78 ºC. After stirring for 10 min, alcohol **2.38-1** (3.2 g, 7.4 mmol) in CH_2Cl_2 (50 mL) was added and the reaction mixture was stirred at -78 ºC for 40 min. Triethylamine (1.85 g, 18.4 mmol) was then added dropwise by cannula, and the reaction mixture was slowly warmed to rt. Water (100 mL) was added and the organic layer was separated and washed with brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude

product was dissolved in Et_2O (100 mL), filtered through a plug of silica gel washing with ether (200 mL), concentrated under reduced pressure, and dried under high vacuum. The crude aldehyde (**2.39**) was use without further purification. Dibutylboron triflate (9.6 mL of a 1.0 M solution in CH_2Cl_2, 8.4 mmol) and triethylamine (1.1 g, 11 mmol) were added to a solution of (*R*)-4-benzyl-3-propionyl-2-oxazolidinone (**2.40**) (1.7 g, 7.6 mmol) in CH_2Cl_2 (20 mL) at 0 ºC. The solution was cooled down to -78 ºC and **2.39** in CH_2Cl_2 (10 mL) was cannulated into the reaction mixture. The solution was stirred for 20 min at -78 ºC. The solution was then warmed to 0 ºC and stirred an additional hour. The reaction was quenched by adding a pH 7 aq. phosphate buffer solution (0.2 M aq. sodium hydrogen phosphate/0.1 M aq. citric acid, 82:18, 12.8 mL) and methanol (38.5 mL). To this cloudy solution was added a solution of methanol and 30% hydrogen peroxide (2:1, 38.5 mL) and the resulting solution was stirred for 1 h at 0 ºC. The solution was concentrated and extracted with EtOAc (3 x 100 mL). The organic layer was washed with sat $NaHCO_3$ (100 mL), brine (100 mL), dried (Na_2SO_4) and filtered. The solvent was evaporated under reduced pressure and was purified by flash chromatography eluting with 0-20% EtOAc in hexanes to afford 3.88 g (80%) of **2.41.** [α]23D -35.8° (c 1.8, CH_2Cl_2); IR (film) 3526, 2950, 2875, 2855, 1780, 1696, 1456, 1381, 1288, 1207, 1237, 1094, 1005, 939, 935, 774, 736, 701 cm-1; 1H NMR (400 MHz) δ 7.38-7.24 (m, 5H), 4.76-4.76 (m, 1H), 4.24-4.15 (m, 3H), 3.99 (dd, J = 9.6, 6.0 Hz, 1H), 3.90-3.83 (m, 1H), 3.75 (bs, 1H), 3.37 (d, J = 6.0 Hz, 2H), 3.32 (dd, J = 13.2, 2.8 Hz, 1H), 3.18 (s, 3H), 2.81 (dd, J = 13.6, 9.6 Hz, 1H), 1.80-1.42 (m, 6H), 1.31 (d, J = 6.8 Hz, 3H), 1.11 (s, 3H), 1.02 (d, J = 6.4 Hz, 3H), 0.99 (t, J = 8.4 Hz, 9H), 0.93 (s, 9H) 0.72-0.66 (m, 6H), 0.08 (s, 6H); 13C NMR (100 MHz) δ 175.8, 158 152.9, 135.2, 129.3 (2C), 128.9 (2C), 127.1, 79.6, 76.8, 71.0, 69.0, 65.9, 55.3, 48.4,

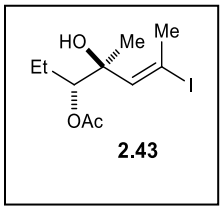
42.6, 37.6, 36.0, 35.4, 31.0, 25.8 (3C), 18.1 (2C), 17.2, 11.0, 6.8 (3C), 5.0 (3C), -5.5 (2C). HRMS (FAB) calc'd for C35H63NO7Si2 +Na = 688.4041, found 688.4028.



2.42

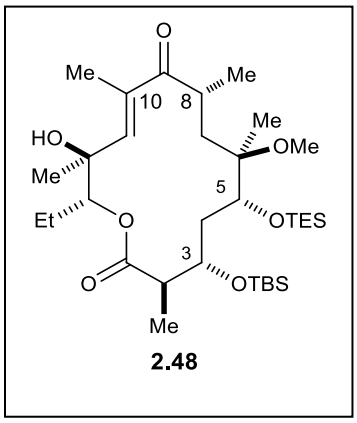
Aldol Product 2.42: To a stirred solution of aldol **2.41** (2.35 g, 3.53 mmol) in CH_2Cl_2 (20 mL) at 0 ºC was added 2,6-lutidine (0.67 g, 6.35 mmol) and TBSOTf (1.40 g, 5.30 mmol). The reaction mixture was stirred for 15 min at 0 ºC and quenched with sat'd aq. $NaHCO_3$ (10 mL). The organic layer was separated, washed with brine (10 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, and the crude product was azeotropically with toluene (10 mL). The crude product was dissolved in MeOH (70 mL) was added CSA (0.16 g, 0.70 mmol) at 0 ºC. After stirring for 2 h, the reaction was quenched with solid $NaHCO_3$ (0.30 g). The mixture was concentrated to remove MeOH. The residue was diluted with EtOAc (100 mL) and washed with saturated aq. $NaHCO_3$ (25 mL), dried (Na_2SO_4) and filtered. The solvent was evaporated under reduced pressure dried under high vacuum. The crude alcohol was dissolved in DMF (35 mL) and was added Imidazole (1.0 g, 14.12 mmol), DMAP (4 mg) and, TESCl (1.33 g, 8.83 mmol) at 0 ºC and stirred for 5 h at rt. The reaction was quenched with water (20 mL) and extracted with Et_2O (4 x 100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with EtOAc/hexanes (1/20) to afford 1.38 g (76%) of **2.42** as colorless oil. [α]23D -24.3° (c 1.15, CH_2Cl_2); IR (film) 2952, 2875, 1784, 1707, 1459, 1380, 1349, 1236, 1207, 1096, 1073, 1006, 971, 812, 775 cm-1; 1H NMR (400 MHz) δ

7.37-7.23 (m, 5H), 4.65-4.61 (m, 1H), 4.33-4.28 (m, 1H), 4.18-4.11 (m, 2H), 3.88 (dd, J = 6.8, 2.0 Hz, 1H), 3.78-3.75 (m, 1H), 3.40-3.30 (m, 3H), 3.15 (s, 3H), 2.78 (dd, J = 13.2, 9.2 Hz, 1H), 1.98-1.34 (m, 6H), 1.27 (d, J = 7.2 Hz, 3H), 1.07 (s, 3H), 1.00-0.95 (m, 24H), 0.93 (s, 9H) 0.69-0.59 (m, 14H), 0.13 (s, 3H), 0.09 (s, 3H); 13C NMR (100 MHz) δ 175.3, 152.9, 135.4, 129.4 (2C), 128.8 (2C), 127.2, 79.8, 73.6, 69.4, 68.9, 65.8, 55.5, 48.3, 42.0, 39.1, 37.6, 35.5, 31.3, 25.8 (3C), 18.2, 18.0, 17.0, 10.4, 7.1 (3C), 6.7 (3C), 5.3 (3C), 4.3 (3C), -4.2, -5.0; HRMS (FAB) calc'd for $C_{41}H_{77}NO_7Si_3$ +Na = 802.4906, found 802.4914.



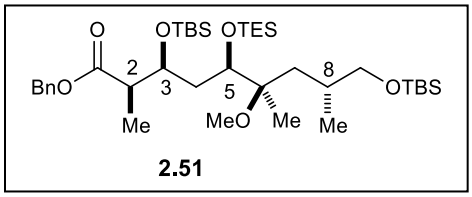
2.43

Vinyl Iodide OAc (2.43): Ac_2O (74.5 mg, 0,73 mmol) was added to a solution of vinyl iodide (**2.24**) (165 mg, 0.61 mmol), Et_3N (73.6 mg, 0.73 mmol) and DMAP (7.3 mg, 0.06 mmol) in DCM (6 mL) at 0 ˚C. The solution was stirred overnight while warming to RT. The solution was then diluted with sat'd aq. NH_4Cl (10 mL) and the aqueous fraction extracted with EtOAc (3 x 20 mL). The organic fraction was washed with brine (10 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 166 mg (87%) of vinyl iodide OAc) **(2.43)** as a white solid. $[\alpha]^{23}_D$ +74.6 (c 1.5, $CHCl_3$); IR (neat) 3499, 2971, 2932, 1713, 1373, 1247, 1189, 1049, 964, 844; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 – 6.02 (m, 1H), 4.78 (dd, *J* = 10.3, 3.0 Hz, 1H), 2.70 (d, *J* = 1.5 Hz, 3H), 2.12 (s, 3H), 1.76 – 1.52 (m, 2H), 1.28 (s, 3H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.16, 141.83, 99.94, 80.48, 77.88, 30.26, 26.29, 22.45, 20.95, 10.56; HRMS (ESI) calc'd for $C_{10}H_{17}IO_3$ + Na = 335.0120, found 335.0117.



2.48

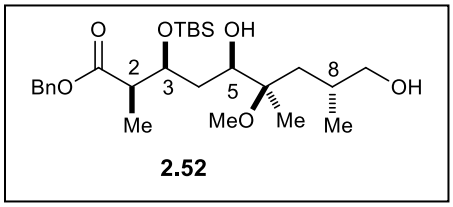
Macrolactone 2.48: A 10 mL flask was charged with **2.45** (10 mg), DIPEA (12 mg, 0.15 mmol), 2,4,6-trichlorbenzoyl chloride (15 mg, 0.08 mmol) and benzene (1.5 mL). The solution was stirred for 1 hour then an additional amount of DIPEA (12 mg, 0.15 mmol) and 2,4,6-trichlorbenzoyl chloride (30 mg, 0.15 mmol). After stirring for 10 hours, DMAP (74 mg) followed by benzene (1.5 mL) were added. After 45 minutes, sat'd aq. NH_4Cl was added and the mixture extracted with EtOAc (3 x 40 mL). The organic fractions were dried (Na_2SO_4) and the product purified by flash chromatography eluting with 0-30% EtOAc in hexanes to give 6.5 mg (65%) yield of desired lactone **2.48** IR (film) 3456, 2935, 2901, 1728, 1464, 1252, 1095, 1252, 1118, 1093, 1067 cm-1; 1H NMR (400 MHz) δ 5.66 (d, J = 1.4 Hz, 1H), 4.86 (dd, J = 10.4, 2.8 Hz, 1H), 4.08-4.04 (m, 1H), 3.84 (dd, J = 8.0, 4.4 Hz, 1H), 3.67 (d, J = 7.2 Hz, 1H), 3.20 (s, 3H), 2.55-2.52 (m, 1H), 2.27 (bs, 1H), 1.90 (d, J = 1.4 Hz, 1H), 1.80-1.52 (m, 5H), 1.38 (s, 3H), 1.20 (s, 3H), 1.16 (d, J = 7.2 Hz, 3H),), 1.06 (d, J = 6.8 Hz, 3H), 0.95 (t, J = 8.2 Hz, 9H), 0.91 (s, 9H), 0.86 (t, J = 7.2 Hz, 3H), 0.67-0.59 (m, 6H), 0.12 (s, 3H), 0.10 (s, 3H); 13C NMR (100 MHz) δ 208.0, 175.4, 142.6, 138.2, 80.4, 79.5, 74.2, 73.9, 70.8, 48.8, 48.6, 41.3, 35.8, 34.6, 31.9, 25.8 (3C), 22.7, 21.3, 21.1, 17.9, 16.7, 14.1, 12.8, 10.6, 7.2 (3C), 5.4 (3C), -3.7, -4.5; HRMS (FAB) calc'd for $C_{33}H_{64}O_7Si_2$+Na = 651.4088, found 651.4076.



2.51

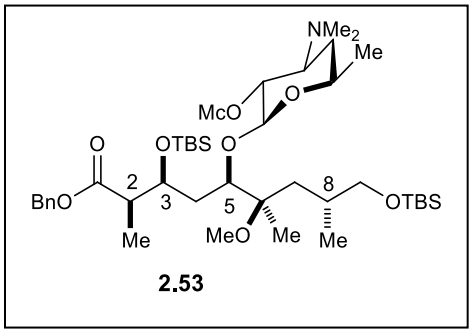
Benzyl Ester 2.51: To a stirred solution of aldol **2.41** (0.85 g, 1.28 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added 2,6-lutidine (0.24 g, 2.30 mmol) and

TBSOTf (0.50 g, 1.92 mmol). The reaction mixture was stirred for 15 min at 0 ºC and quenched with sat'd aq. $NaHCO_3$ (10 mL). The organic layer was separated, washed with brine (10 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure, azeotropically dried with toluene (3 mL). To BnOH (0.26 g, 1.99 mmol) in THF (6.5 mL) was added n-BuLi at 0 ºC and stirred for 30 mins. To this solution the residue dissolved in THF (6.5 mL) was cannulated at -78 ºC. The reaction mixture was allowed to warm slowly stirred for 16 hours at rt. The reaction was quenched by with NH_4Cl (10 mL). The aqueous layer was extracted with diethyl ether (2 x 20 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure and purified by flash chromatography eluting with 0-30% EtOAc in hexanes to afford 0.45 g (50%) of **2.51** as a colorless oil. [α]23D +19.8° (c 2.0, CH_2Cl_2); IR (film) 2928, 2877, 2856, 1736, 1462, 1413, 1387, 1361, 1251, 1171, 773, 735 cm-1; 1H NMR (400 MHz) δ 7.39-7.30 (m, 5H), 5.22 (d, J = 12.4 Hz, 1H), 4.96 (dd, J = 12.0 Hz, 1H), 4.51-4.46 (m, 1H), 3.58 (d, J = 10.0 Hz, 1H), 3.33 (dd, J = 9.6, 5.6 Hz, 1H), 3.22 (dd, J = 9.2, 7.2 Hz, 1H), 3.01 (s, 3H), 2.62-2.55 (m, 1H), 1.80-1.25 (m, 6H), 1.15 (d, J = 7.2 Hz, 3H), 1.02 (s, 3H), 0.94 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 8.0 Hz, 9H), 0.88 (s, 9H), 0.84 (s, 9H), 0.03-0.02 (m, 9H), -0.03 (s, 6H); 13C NMR (100 MHz) δ 174.8, 136.0, 128.7 (2C), 128.4 (2C), 128.1, 79.8, 73.7, 73.0, 69.2, 66.6, 48.4, 42.4, 37.3, 35.4, 29.6, 25.9 (3C), 25.7 (3C), 18.2, 18.0, 17.9, 16.9, 8.1, 7.1 (3C), 5.5 (3C), -3.1 (2C), -4.0, -5.5. HRMS (FAB) calc'd for $C_{38}H_{74}O_6Si_3$+Na = 733.4691, found 733.4689.



2.52

Diol 2.52: To a solution of benzyl ester **2.51** (0.38 g, 0.53 mmol) in MeOH (11 mL) was added CSA (0.025 g, 0.10 mmol) at 0 ºC. After 2 h, solid $NaHCO_3$ (100 mg) was added. The mixture was concentrated to remove MeOH. The residue was diluted with EtOAc (100 mL) and washed with sat'd aq. $NaHCO_3$ (25 mL), dried (Na_2SO_4) and filtered. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography eluting with 0-50% EtOAc in hexanes to provide 180 mg (70%) of **2.52.** [α]23D +7.1° (c 6.5, CH2Cl2); IR (film) 2952, 2930, 2876, 2856, 1731, 1461, 1413, 1375, 1251, 1093, 1005, 941, 836, 775, 737 cm-1; 1H NMR (400 MHz) δ 7.26-7.17 (m, 5H), 5.06 (dd, J = 12.4 Hz, 1H), 4.99 (d, J = 12.4 Hz, 1H), 3.55 (d, J = 10.0 Hz, 1H), 3.41 (dd, J = 10.4, 4.4 Hz, 1H), 3.19 (dd, J = 10.2, 3.6 Hz, 1H), 3.12 (s, 3H), 2.68-2.63 (m, 2H), 1.78-1.65 (m, 2H), 1.53-1.45 (m, 1H), 1.36 (d, J = 6.0 Hz, 3H), 1.01 (s, 3H), 1.05 (d, J = 6.0 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H), 0.77 (s, 9H), 0.00 (s, 3H), -0.04 (s, 3H); 13C NMR (100 MHz): δ 174.6, 135.9, 128.4 (2C), 128.2 (2C), 128.1, 79.1, 72.6, 72.2, 68.7, 66.2, 49.4, 44.7, 37.9, 35.1, 31.1, 25.7 (3C), 19.2, 18.4, 17.8, 11.7, -4.4, -4.7. HRMS (FAB) calc'd for $C_{26}H_{46}O_6Si$+Na = 505.2961, found 505.2966.

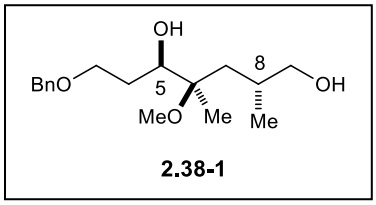


2.53

Benzyl Ester 2.53: To a solution of alcohol **2.52** (0.20 g, 0.41 mmol) in DMF (4 mL) at room temperature were added Imidazole (42 mg, 0.62 mmol) and TBSCl (75 mg, 0.49 mmol). After 16 h, the reaction H_2O (5 mL) was added. The mixture was diluted with EtOAc (10 mL), and the aqueous layer was extracted with EtOAc (2 x 5

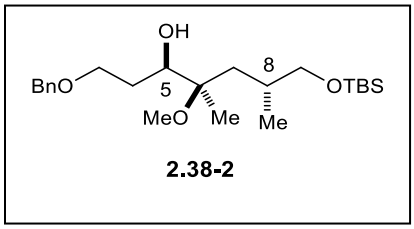
mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried (Na_2SO_4) and filtered. The solvent was concentrated under reduced pressure and azeotropically dried with toluene (5 mL), and the crude C-5 alcohol was taken to the next step. To a suspension of freshly activated 4 Å molecular sieves (4.5 g) and AgOTf (2.1 g, 8.1 mmol) in CH_2Cl_2 (7 mL) and toluene (7 mL) was added dropwise by cannula to a solution of C-5 alcohol (0.41 mmol), desosamine thiopyrimidine donor **1.72** (0.85 g, 2.46 mmol) and 2,6-di-tert-butyl-4-methylpyridine (0.505 g, 2.46 mmol) in CH_2Cl_2 (7 mL) at 0 ºC. The reaction flask was wrapped with aluminum foil, warmed to rt and stirred for an additional 20 h. The reaction was quenched with Et_3N (8.0 mL), filtered through Celite, and eluted with EtOAc (50 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (20 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 0-60% EtOAc in hexanes to afford 200 mg of **2.53** (55%). [α]23D -2.8° (c 0.5, CH_2Cl_2); IR (film) 3449, 3421, 2845, 2775, 1734, 1726, 1455, 1378, 1222, 1016, 1055, 889, 772 cm-1; 1H NMR (400 MHz) δ 7.26-7.20 (m, 5H), 5.08 (d, J = 12.8 Hz, 1H), 4.93 (d, J = 12.4 Hz, 1H), 4.39 (dd, J = 10.8, 7.6 Hz, 1H), 4.29 (d, J = 7.6 Hz, 1H), 3.61 (s, 3H), 3.51 (dd, J = 7.6, 2.8 Hz, 1H), 3.45 (dd, J = 9.6, 5.2 Hz, 1H), 3.17 (dd, J = 9.6, 7.6 Hz, 1H), 3.15-3.08 (m, 1H), 3.04 (s, 3H), 2.59-2.50 (m, 1H), 2.40 (dt, J = 12.0, 4.4 Hz, 1H), 2.14 (s, 6H), 1.94-1.87 (m, 1H), 1.73-1.66 (m, 1H), 1.54-1.41 (m, 3H), 1.22-1.11 (m, 2H), 1.07 (s, 3H), 1.03 (d, J = 6.0 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H), 0.78 (s, 9H), 0.75 (s, 9H), -0.05 (s, 3H), -0.07 (s, 6H), -0.09 (m, 3H); 13C NMR (100 MHz) δ 174.4, 155.3, 136.0, 128.4 (2C), 128.2 (2C), 128.0, 99.6, 78.6, 76.7, 75.1, 70.9, 68.8, 68.7, 66.2, 62.6, 54.4, 49.8, 43.4, 40.6 (2C), 36.4, 36.3, 31.0,

30.6, 25.9 (3C), 25.7 (3C), 20.9, 19.4, 18.3, 17.9, 8.6, -4.2, -5.2, -5.4 (2C). HRMS (FAB) calc'd for $C_{42}H_{77}NO_{10}Si_2$+Na = 834.4984, found 834.4880.



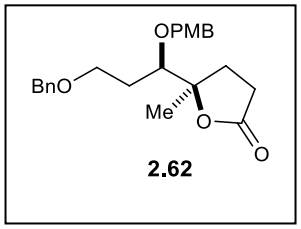
2.38-1

Diol 2.38-1: The methyl ether **2.38** (4.00 g, 10.16 mmol) was dissolved in MeOH (200 mL). To this solution was added *d,l*-10-camphorsulfonic acid (472 mg, 2.03 mmol). The resulting solution was stirred at 0 °C for 1 h. The reaction was terminated by addition of $NaHCO_3$ (853 mg, 10.15 mmol). The solution was concentrated under reduced pressure and the crude diol **2.38-1** was directly taken to the next step.



2.38-2

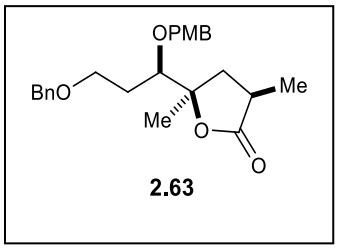
Alcohol 2.38-2: To a solution of **2.38-1**, prepared above, in CH_2Cl_2 (100 mL) at rt were added imidazole (1.24 g, 18.30 mmol) and TBSCl (2.30 g, 15.24 mmol). After stirring for 3 h at rt, the reaction was quenched by adding H_2O (50 mL) and stirred for 10 min. The aqueous layer was separated and extracted with additional CH_2Cl_2 (2 x 50 mL), the combined organic layers were then washed with brine (50 mL) and dried over Na_2SO_4. The solvent was concentrated under reduced pressure, and the crude alcohol **2.38-2** was azeotropically with toluene (4 x 20 mL) and dried for 4 h under high vacuum before taking it to the next step.



2.62

C5 PMB Ether 2.62: A Solution of **(R)-5-((R)-3-(benzyloxy)-1-hydroxypropyl)-5-methyldihydrofuran-2(3H)-one (2.34)** (1.1 g, 4.16 mmol) in DMF (5 mL) was cannulated into a

suspension of NaH (60%, 200 mg, 5 mmol) in DMF (15 mL) at 0 ˚C. The resulting mixture was stirred for 20 min at 0 ˚C before adding PMBCl (783 mg, 5 mmol). The solution was allowed to gradually warm to RT and after 4 hours cooled back to 0 ˚C and slowly quenched with water until the bubbling of H_2 ceased. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (3 x 40 mL). The combined organic fractions were then washed with H_2O (2 x 20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to afford 1.03 g (65%) **C5 PMB Ether (2.62)**. $[\alpha]^{23}_D$ +34.0 (c 1.5, $CHCl_3$); IR (neat) 3509, 2934, 2861, 1765, 1611, 1512, 1453, 1244, 1075, 1028, 941, 820, 737, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 7.20 (d, *J* = 8.7 , 2H), 6.84 (d, *J* = 8.7, 2H), 4.70 (d, *J* = 11.0 Hz, 1H), 4.50 – 4.40 (m, 3H), 3.62 (dt, *J* = 9.7, 4.8 Hz, 1H), 3.59 – 3.52 (m, 2H), 2.60 – 2.53 (m, 2H), 2.12 – 2.02 (m, 1H), 1.90 (ddd, *J* = 12.9, 8.9, 5.9 Hz, 1H), 1.86 – 1.77 (m, 1H), 1.65 (ddt, *J* = 14.2, 9.6, 4.3 Hz, 1H), 1.38 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.83, 159.30, 138.28, 130.50, 129.65 (2C), 128.41 (2C), 127.77 (2C), 127.69, 113.75 (2C), 89.44, 80.68, 73.88, 73.07, 66.45, 55.25, 31.29, 30.98, 28.79, 21.36; HRMS (ESI) calc'd for $C_{23}H_{28}O_5$ + H = 385.2015, found 385.2006.

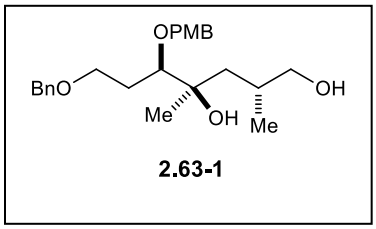


2.63

C8 Methylated Lactone (2.63): *n*-BuLi (1.0 mL, 2.41 M) was added drop wise to a stirring solution of diisoproplylamine (253 mg, 2.5 mmol) in THF (5 mL) at -78 ˚C and stirred for 10 min. The solution was warmed to 0 ˚C and stirred for an additional 20 min before cooling back to -78 ˚C. **C5 PMB Ether (2.62)** (503 mg, 1.31

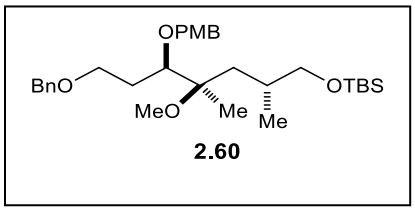
mmol) in THF (3 mL) was cannulated into the solution and stirred at -78 ˚C for 2 h. MeI (1.99 g, 14 mmol) in THF (3 mL) was cannulated into the solution and stirred for 1 h. Saturated NH_4Cl (25 mL) was added to the solution and was extracted with Et_2O (3 x 50 mL). The combined organic fractions were washed with brine (25 mL) and dried over Na_2SO_4. The dried solvent was removed under reduced pressure and dried under high vacuum before preceding to the next step. *n*-BuLi (1.0 mL, 2.41 M) was added drop wise to a stirring solution of diisoproplylamine (253 mg, 2.5 mmol) in THF (5 mL) at -78 ˚C and stirred for 10 min. The solution was warmed to 0 ˚C and stirred for an additional 20 min before cooling back to -78 ˚C. The product (*vide supra*) (510 mg, 1.31 mmol) in THF (3 mL) was cannulated into the solution and stirred for 30 min, then warmed to -45 ˚C for 1.5 h. The solution was cooled back to -78 ˚C and triphenylacetic acid (755 mg, 2.62 mmol) in THF (5 mL) was cannulated into the solution and stirred for 2 h while slowly warming to RT. Saturated NH_4Cl (25 mL) was added and the mixture was extracted with Et_2O (3 x 50 mL). The combined organic fractions were washed with brine (25 mL) and dried over Na_2SO_4. The dried solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 276 mg (54%) of **C8 Methylated Lactone (2.63)**. $[\alpha]^{23}_D$ +40.6 (c 1.5, $CHCl_3$); IR (neat) 2970, 2933, 2867, 1764, 1612, 1513, 1246, 1089, 1033, 821 ,699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.80 (d, *J* = 10.9 Hz, 1H), 4.50 – 4.38 (m, 3H), 3.78 (s, 3H), 3.62 (dd, *J* = 10.3, 2.0 Hz, 1H), 3.58 – 3.50 (m, 2H), 2.85 – 2.71 (m, 1H), 2.12 (dd, *J* = 12.5, 8.8 Hz, 1H), 1.79 – 1.50 (m, 3H), 1.37 (s, 3H), 1.27 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.00, 159.16, 138.24, 130.27, 129.76 (2C), 128.41 (2C), 127.79 (2C), 127.70, 113.71 (2C), 87.27, 81.05,

73.94, 73.10, 66.40, 55.24, 39.73, 34.23, 31.04, 19.61, 15.15; HRMS (ESI) calc'd for $C_{24}H_{30}O_5$ + Na = 421.1991, found 421.1982.



2.63-1

LAH Product 2.63-1: Methylated Lactone (**2.63**) (188 mg, 0.47 mmol) in THF (2 mL) was cannulated into a suspension of $LiAlH_4$ (23 mg, 0.61 mmol) in THF (3 mL) at -45 ˚C. The solution was stirred for 2 h, then allowed to slowly warm to RT over 1 hour. The mixture was diluted with Et_2O (10 mL) and cooled back to 0 ˚C. Saturated Na_2SO_4 (10 mL) was added slowly until all H_2 formation ceased. The mixture was extracted with Et_2O (3 x 20 mL). The combined organic fractions were washed with brine, filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-60% EtOAc in hexanes to afford 174 mg (92%) of **2.63-1** as a colorless oil. $[\alpha]^{23}_D$ +6.6 (c 1.5, $CHCl_3$); IR (neat) 3331, 2954, 2932, 2869, 1612, 1513, 1496, 1245, 1092, 1035, 821, 738, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 5H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 4.44 (m, 4H), 3.71 (s, 3H), 3.58 – 3.50 (m, 2H), 3.47 (dd, *J* = 10.9, 3.4 Hz, 1H), 3.31 (dd, *J* = 8.1, 3.6 Hz, 1H), 3.19 (dd, *J* = 10.9, 8.4 Hz, 1H), 1.97 – 1.83 (m, 2H), 1.66 (ddt, *J* = 14.5, 8.2, 4.9 Hz, 1H), 1.46 (dd, *J* = 14.5, 9.1 Hz, 1H), 1.36 (dd, *J* = 14.5, 3.1 Hz, 1H), 1.11 (s, 3H), 0.79 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.22, 137.92, 130.40, 129.41(2C), 128.40 (2C), 127.80 (2C), 127.72, 113.79 (2C), 83.69, 74.82, 74.07, 73.05, 69.12, 67.20, 55.22, 44.13, 31.47, 30.94, 21.72, 19.81; HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + H = 404.2484, found 404.2481.



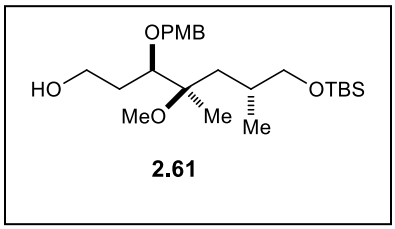
2.60

Benzyl Ether 2.60: Made from **2.38-2** or **2.63-1.**

From **2.38-2:** To a suspension of 60% dispersion of NaH (610 mg, 15.24 mmol) in DMF (75 mL) at 0 °C was added (cannulated) a solution of **2.38-2**, prepared above, in DMF (25 mL). The resulting mixture was stirred for 20 min before adding PMBCl (2.4 g, 15.24 mmol) and solution was gradually warmed to rt. After 3 h the reaction was cooled back down to 0 °C and slowly quenched with ice-cold water till the bubbling of H_2 ceased. This mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with fresh H_2O (2 x 50 mL) to rid of any DMF followed by brine (50 mL) and dried over Na_2SO_4. The dried solvent was concentrated under reduced pressure and purified by flash column chromatography eluting with 5% EtOAc in hexane to afford 2.70 g (50%) of the benzyl ether **2.60**.

From **2.63-1**: TBSCl (74 mg, 0.49 mmol) and imidazole (39 mg, 0.57 mmol) were added sequentially to a solution of LAH Product **(2.63-1)** (165 mg, 0.41 mmol) in DCM (4 mL) at 0 ˚C and stirred for 2 h while warming to rt. H_2O (4 mL) was added and the mixture extracted with DCM (3 x 8 mL). The organic fractions were washed with brine (4 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and dried under high vacuum. The crude product was dissolved in DCM (4 mL). 2,6-DTBMP (740 mg, 3.6 mmol) followed by MeOTf (361 mg, 2.2 mmol) were added and the solution stirred at rt for 48 h. Saturated $NaHCO_3$ (4 mL) was added and the mixture stirred for 15 minutes. MeOH (4 mL) was added and the mixture stirred for 30 min. The mixture was then extracted with Et_2O (3 x 10 mL). The combined organic fractions were washed with brine (5 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the

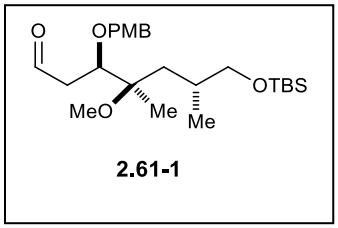
product purified by flash column chromatography eluting with hexanes to recover the 2,6-DTBMP and then 0-40% EtOAc in hexanes to give 157 mg (72%) of benzyl ether (**2.60**) as a colorless oil. $[\alpha]^{23}_{D}$ +15.8 (c 1.5, $CHCl_3$); IR (neat) 2963, 2929, 2855, 1514, 1463, 1248, 1098, 836, 775, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 5H), 7.24 – 7.18 (m, 2H), 6.87 – 6.81 (m, 2H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.55 – 4.40 (m, *J* = 10.9, 8.4 Hz, 3H), 3.79 (s, 3H), 3.65 (dd, *J* = 10.1, 2.3 Hz, 1H), 3.62 – 3.55 (m, 2H), 3.52 – 3.43 (m, 1H), 3.31 (dd, *J* = 9.7, 7.0 Hz, 1H), 3.23 (s, 3H), 1.98 (dddd, *J* = 15.7, 8.9, 6.9, 2.3 Hz, 1H), 1.85 (td, *J* = 13.0, 6.7 Hz, 1H), 1.68 – 1.52 (m, 2H), 1.36 – 1.25 (m, 1H), 1.20 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.94 – 0.88 (m, 9H), 0.04 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.93, 138.66, 131.50, 129.31 (2C), 128.29 (2C), 127.62 (2C), 127.42, 113.60 (2C), 80.48, 80.31, 74.42, 72.78, 69.02, 67.52, 55.23, 49.41, 36.89, 30.99, 30.02, 25.95 (3C), 19.55, 18.62, 18.34, -5.37, -5.39; HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + K = 569.3065, found 569.3047.



2.61

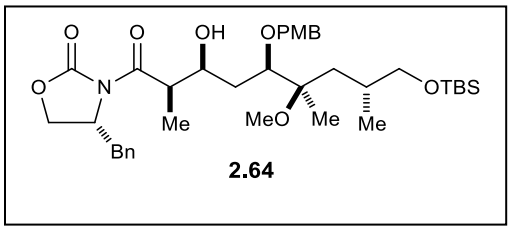
Aldol Alcohol **2.61:** Raney-Ni in H_2O was washed with EtOH and decanted. 8 spatulas full of Raney-Ni were then added to a solution of benzyl ether **(2.60)** (830 mg, 0.1.56 mmol) in EtOH (30 mL). The suspension was then placed under an atmosphere of H_2 and stirred approximately 6 h (Reaction times vary. TLC analysis is necessary to prevent over oxidation).The suspension was then filtered over Celite washing with EtOAc. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-20% EtOAc in hexanes to afford 520 mg (75%) of Aldol Alcohol **(2.61)** as a colorless oil. $[\alpha]^{23}_{D}$ +9.8 (c 1.5, $CHCl_3$); IR (neat) 3435, 2954, 2929, 2856, 1613, 1514, 1464, 1249, 1085, 836, 775 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.64 (dd, *J* = 75.8, 10.9 Hz, 2H), 3.80 (s, 3H), 3.77 – 3.70 (m, 1H), 3.70 – 3.63 (m, 2H), 3.40 – 3.30 (m, 2H), 3.24 (s, 3H), 2.55 (dd, *J* = 7.4, 4.0 Hz, 1H), 1.90 – 1.82 (m, 1H), 1.81 – 1.75 (m, 1H), 1.69 – 1.62 (m, 1H), 1.58 (dd, *J* = 14.9, 3.8 Hz, 1H), 1.34 (dd, *J* = 15.0, 7.8 Hz, 1H), 1.20 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.89 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.13, 131.01, 129.61 (2C), 113.77 (2C), 99.60, 82.60, 80.79, 74.12, 69.01, 60.93, 55.25, 49.19, 36.62, 32.91, 31.09, 25.93 (3C), 19.45, 18.29, -5.40 (2C); HRMS (ESI) calc'd for $C_{24}H_{34}O_5$ + K = 479.2595, found 479.2597.



2.61-1

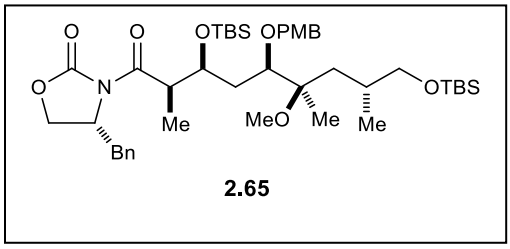
Aldol Aldehyde **2.61-1:** DMSO (2.8 g, 35.8 mmol) was added drop wise to a solution of oxalyl chloride (2.2 g, 17.2 mmol) in DCM (125 mL) at -78 ˚C. The solution was stirred for 20 min, then Aldol Alcohol **(2.61)** (6.3 g, 14.3 mmol) in DCM (20 mL) was cannulated into the solution and stirred for 45 min at -78 ˚C. Et_3N (3.6 g, 35.8 mmol) was then added and the solution allowed to warm to rt over 1 h. H_2O (70 mL) was added and the mixture extracted with DCM (3 x 145 mL). The combined organic fractions were washed with brine (70 mL) and removed under reduced pressure. The product was dissolved in Et_2O (100 mL) and passed through a plug of silica washing with Et_2O (3 x 150 mL). The solvent was removed under reduced pressure and azeotropically dried with toluene (3 x 50 mL). The product **2.61-1** was dried under high vacuum for 3 h before taking directly to the next step.



2.64

Aldol Product 2.64: Et_3N (2.2 g, 21.5 mmol) was added drop wise to a solution of (R)-4-benzyl-3-propionyl-2-oxazolidinone (**2.40**) (4g, 17.2 mmol) and Bu_2BOTf (18.6 mL, 1 M) in DCM (60 mL). The solution changes from red to yellow and is then cooled to -78 ˚C. Aldol Aldehyde (**2.61-1**) (6.3 g, 14.3 mmol) in DCM (10 mL) was cannulated in to the solution and stirred at -78 ˚C for 20 min and then at 0 ˚C for 1 h. Phosphate buffer (pH 7, 0.2 M Na_2HPO_4 (aq.):0.1M citric acid (aq.), 82:18, 50 mL) and MeOH (150 mL). The solution becomes cloudy and a solution of MeOH:30% H_2O_2 (2:1, 150 mL) was added and stirred at 0 ˚C for 1 h. The solution was then concentrated under reduced pressure and the remaining aqueous fraction was extracted with EtOAc (3 x 200 mL). The combined organic fractions were washed with sat'd $NaHCO_3$ (100 mL), brine (100 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash column chromatography eluting with 0-30% EtOAc in hexanes to give 7.1 g (74%) of Aldol Product (**2.64**) as a colorless oil. $[\alpha]^{23}_D$ +33.6 (c 1.5, $CHCl_3$); IR (neat) 3380, 2953, 2928, 2855, 1753, 1514, 1250, 1094, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 5H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.88 – 6.80 (m, 2H), 4.74 (d, *J* = 11.0 Hz, 1H), 4.65 (qd, *J* = 6.5, 3.1 Hz, 1H), 4.57 (d, *J* = 11.0 Hz, 1H), 4.22 – 4.11 (m, 2H), 4.11 – 4.03 (m, 1H), 3.79 (s, 3H), 3.74 (dd, *J* = 7.0, 3.8 Hz, 1H), 3.66 (dd, *J* = 8.8, 3.5 Hz, 1H), 3.42 (dd, *J* = 9.7, 6.2 Hz, 1H), 3.33 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.2 Hz, 1H), 3.23 (s, 3H), 2.76 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.87 – 1.61 (m, 4H), 1.58 (dd, *J* = 14.9, 4.0 Hz, 1H), 1.35 (dd, *J* = 14.9, 7.7 Hz, 1H), 1.25 (d, *J* = 7.0 Hz, 3H), 1.22 (s, 3H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.90 (s, *J* = 2.8 Hz, 9H), 0.04 (d, *J* = 1.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ

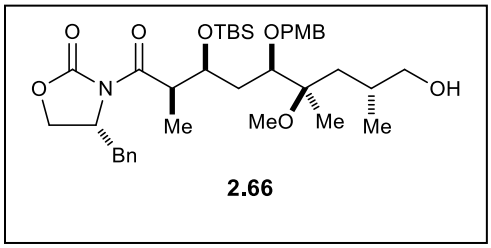
175.98, 159.01, 153.01, 135.29, 130.82, 129.58 (2C), 129.42 (2C), 128.91 (2C), 127.31, 113.66 (2C), 82.61, 80.78, 73.49, 70.50, 69.05, 66.00, 55.31, 55.22, 49.28, 42.56, 37.74, 36.61, 34.42, 31.11, 29.67, 25.95 (3C), 19.67, 18.39, 10.88, -5.37, -5.41; HRMS (ESI) calc'd for $C_{37}H_{57}NO_8Si$ + H = 672.3932, found 672.3903.



2.65

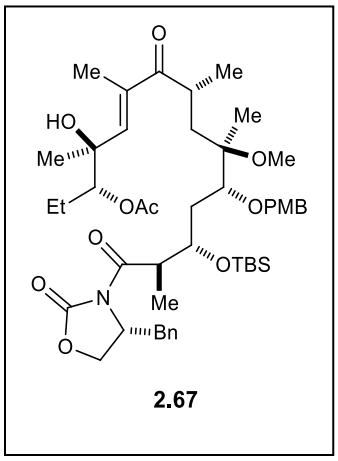
TBS Protected Aldol 2.65: 2,6-Lutidine (150 mg, 1.4 mmol) followed by TBSOTf (291 mg, 1.1 mmol) were added to a solution of **2.64** (486 mg, 0.7 mmol) in DCM (5 mL) at 0 °C and stirred for 30 min. Sat'd $NaHCO_3$ (5 mL) was added and the mixture extracted with DCM (3 x 10 mL). The combined organic fractions were washed with brine (5 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-10% EtOAc in hexanes to afford 560 mg (95%) of **2.65** as a colorless oil. $[\alpha]^{23}_D$ -22.9 (c 1.5, $CHCl_3$); IR (neat) 2953, 2928, 2856, 1780, 1707, 1514, 1463, 1386, 1248, 1094, 837, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.17 (m, 7H), 6.82 (d, *J* = 8.7 Hz, 1H), 4.66 (dd, *J* = 51.7, 11.4 Hz, 2H), 4.54 – 4.44 (m, 1H), 4.21 – 4.14 (m, 1H), 4.08 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.92 – 3.82 (m, 2H), 3.78 (s, 3H), 3.59 (d, *J* = 8.5 Hz, 1H), 3.47 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.33 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.27 (dd, *J* = 13.3, 3.0 Hz, 1H), 3.22 (s, 3H), 2.74 (dd, *J* = 13.3, 9.7 Hz, 1H), 2.08 – 1.95 (m, 1H), 1.81 (dd, *J* = 10.9, 6.6 Hz, 1H), 1.67 – 1.54 (m, 2H), 1.35 – 1.17 (m, 7H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 4.5 Hz, 18H), 0.05 – -0.08 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.02, 158.54, 152.98, 135.48, 132.20, 129.45 (2C), 128.89 (2C), 128.57 (2C), 127.26, 113.36 (2C), 80.46, 79.93, 73.24, 70.52, 69.05, 65.84, 55.78, 55.23, 49.44, 42.41, 37.50, 37.28,

36.74, 31.00, 25.96 (3C), 25.85 (3C), 19.78, 18.61, 18.34, 17.96, 10.83, -4.12, -5.27, -5.40 (2C); HRMS (ESI) calc'd for $C_{43}H_{71}NO_8Si_2$ + Na = 808.4616, found 808.4646.



2.66

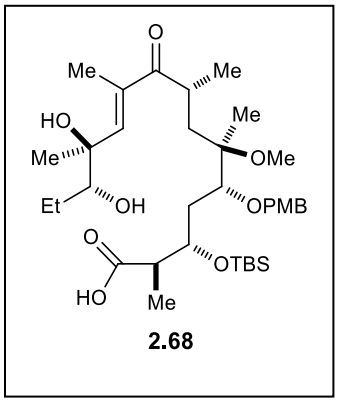
NHK Alcohol 2.66: CSA (33 mg, 0.14 mmol) was added to a solution of TBS Protected Aldol (**2.65**) (560 mg, 0.7 mmol) in MeOH (15 mL) at 0 ˚C and stirred for 2h. The MeOH was then removed under reduced pressure and the crude product dissolved in EtOAc (25 mL). The organic fraction was then washed with sat'd $NaHCO_3$ (10 mL), brine (10 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-40% EtOAc in hexanes to afford 425 mg (88%) **2.66** as a colorless oil. $[\alpha]^{23}_D$ -34.4 (c 1.5, $CHCl_3$); IR (neat) 3407, 3029, 2928, 2855, 1774, 1704, 1513, 1380, 1350, 1246, 1207, 1102, 1035, 835, 774, 733, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.22 (m, 7H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.68 (d, *J* = 3.0 Hz, 2H), 4.62 – 4.53 (m, 1H), 4.24 – 4.11 (m, 3H), 4.04 – 3.94 (m, 1H), 3.91 – 3.84 (m, 1H), 3.83 (s, 3H), 3.71 (d, *J* = 8.4 Hz, 1H), 3.63 (s, *J* = 9.1 Hz, 2H), 3.34 (s, *J* = 5.3 Hz, 3H), 3.33 – 3.29 (m, 1H), 2.81 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.17 (dd, *J* = 13.9, 9.4 Hz, 3H), 1.93 (s, 1H), 1.74 – 1.61 (m, 2H), 1.59 – 1.48 (m, 1H), 1.39 (s, 3H), 1.30 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.95 (s, *J* = 13.7 Hz, 9H), 0.05 (d, *J* = 38.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.69, 158.80, 153.16, 135.35, 131.44, 129.45 (2C), 128.91 (2C), 128.63 (2C), 127.32, 113.53 (2C), 79.93, 79.56, 73.73, 70.48, 68.64, 65.97, 55.79, 55.25, 49.95, 42.51, 39.18, 37.49, 37.02, 31.11, 25.83 (3C), 19.72, 19.29, 17.97, 10.47, -4.09, -5.28; HRMS (ESI) calc'd for $C_{37}H_{57}NO_8Si$ + H = 672.3932, found 672.3916.



2.67

NHK Enone 2.67: Dess-Martin periodinane (5 g, 11.9 mmol) was added to a solution of **2.66** (4 g, 6.0 mmol) and pyridine (2.4 g, 29.8 mmol) in DCM (60 mL). The solution was stirred at RT for 2 h. Sat'd aq. $NaHCO_3$ (50 mL), sat'd aq. Na_2SO_3 (50 mL) and H_2O (50 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 200 mL). The combined organic fractions were washed with brine (100 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure. The aldehyde (4 g, 6.0 mmol) was combined with vinyl iodide (**2.43**) (3.7 g, 12 mmol) and azeotropically dried with toluene (3 x 10 mL), dried under high vacuum, dissolved in DMSO (20 mL) and cannulated into suspension of $CrCl_2$ (2.9 g, 24 mmol) and $NiCl_2$ (29 mg, 0.2 mmol) in DMSO (4 mL). The suspension was stirred at RT for 48 h and then diluted with H_2O (50 mL). The mixture was extracted with EtOAc (5 x 100 mL) and the combined organic fractions were washed with brine (50 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure and dried under high vacuum. The crude NHK product was dissolved in DCM (60 mL) and pyridine (4.7 g, 59.5 mmol) followed by Dess-Martin periodinane (10 g, 23.8 mmol) were added and the solution stirred at RT for 3 h. Sat'd aq. $NaHCO_3$ (50 mL), sat'd aq. Na_2SO_3 (50 mL) and H_2O (50 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 200 mL). The combined organic fractions were washed with brine (50 mL) and filtered over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography eluting with 0-20% EtOAc in hexanes to give 5.4 g (45% over 3 steps) of **2.67** as a white foam. $[\alpha]^{23}_D$ -33.4 (c 1.5, $CHCl_3$); IR (neat) 2955, 2935, 1790, 1733, 1710,

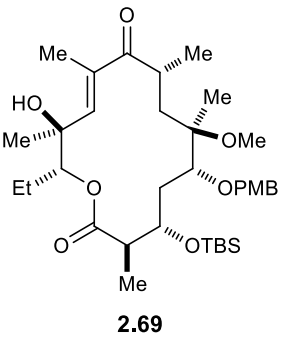
1514, 1463, 1375, 1247, 1098, 1043, 840, 776 cm^{-1}; 1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 7H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.50 (s, 1H), 5.00 (t, *J* = 6.6 Hz, 1H), 4.66 – 4.43 (m, 3H), 4.16 – 4.05 (m, 2H), 4.00 – 3.90 (m, 1H), 3.77 (s, 3H), 3.76 – 3.73 (m, 1H), 3.47 (d, *J* = 7.7 Hz, 1H), 3.44 – 3.33 (m, 1H), 3.26 (dd, *J* = 13.3, 3.0 Hz, 1H), 3.12 (s, 3H), 2.75 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.24 – 2.16 (m, 1H), 2.13 (s, *J* = 2.0 Hz, 3H), 2.02 (s, *J* = 1.1 Hz, 3H), 2.00 – 1.94 (m, 1H), 1.72 – 1.64 (m, 2H), 1.64 – 1.53 (m, 2H), 1.46 (dd, *J* = 14.4, 3.0 Hz, 1H), 1.36 (s, 3H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.20 (s, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.93 – 0.83 (m, 12H), -0.03 (d, *J* = 46.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.05, 175.25, 171.33, 159.09, 153.65, 140.78, 138.89, 135.78, 132.11, 129.84 (2C), 129.28 (2C), 128.96 (2C), 127.67, 113.86 (2C), 80.77, 80.49, 79.83, 77.59, 75.90, 73.82, 70.89, 66.35, 56.19, 55.63, 50.25, 42.95, 39.50, 37.86, 34.70, 26.23 (3C), 26.06, 23.01, 21.31, 20.80, 19.67, 18.35, 13.44, 10.97 (2C), -3.66, -4.95; HRMS (ESI) calc'd for $C_{47}H_{71}NO_{11}Si$ + Na = 876.4694, found 876.4694.



2.68

Secoacid 2.68: 30% H_2O_2 (219 mg, 6.5 mmol) followed by aq. LiOH (1.5 M, 2.42 mmol) were added to a solution of **2.67** (690 mg, 0.81 mmol) in THF:H_2O (4:1, 8 mL) at 0 °C. The solution was allowed to warm to RT stirring for 48 h. Sat'd aq. Na_2SO_4 (4 mL) and sat'd aq. NH_4Cl (4 mL) were added to the solution and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic fractions were washed with brine (10 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-5% MeOH in DCM to afford 458 mg (87%) of **2.68**

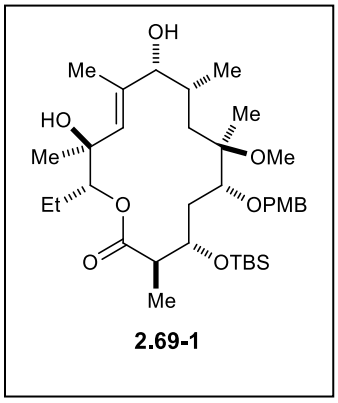
as a white foam. $[\alpha]^{23}_{D}$ +6.7 (c 1.5, $CHCl_3$); IR (neat) 3428, 2957, 2932, 2856, 1709, 1664, 1515, 1462, 1374, 1250, 1095, 1038, 837, 805, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.51 (d, *J* = 0.9 Hz, 1H), 4.54 (d, *J* = 1.1 Hz, 2H), 4.39 – 4.23 (m, 1H), 3.79 (s, 3H), 3.45 – 3.36 (m, 2H), 3.31 (dd, *J* = 9.5, 2.1 Hz, 1H), 3.13 (s, 3H), 2.64 (dd, *J* = 6.9, 3.5 Hz, 1H), 2.22 (dd, *J* = 14.3, 8.7 Hz, 1H), 2.01 (d, *J* = 0.9 Hz, 3H), 1.93 – 1.81 (m, 1H), 1.59 (ddd, *J* = 16.2, 9.5, 3.9 Hz, 2H), 1.46 – 1.39 (m, 2H), 1.38 (s, 3H), 1.20 (d, *J* = 6.1 Hz, 1H), 1.18 (s, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 0.86 (s, 9H), 0.03 (d, *J* = 14.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.88, 178.96, 159.11, 140.92, 138.04, 130.60, 129.31(2C), 113.73 (2C), 81.57, 80.01, 79.29, 76.24, 74.27, 70.78, 55.24, 50.14, 43.85, 39.23, 36.36, 34.56, 25.77 (3C), 24.95, 24.66, 20.39, 19.22, 17.92, 13.00, 11.09, 9.94, -4.14, -5.05; HRMS (ESI) calc'd for $C_{35}H_{60}NO_9Si$ + Na = 675.3904, found 675.3907.



2.69

Macrolactone 2.69: DIPEA (194 mg, 1.5 mmol) followed by 2,4,6-trichlorobenzoyl chloride (188 mg, 0.8 mmol) were added to an azeotropically dried (toluene 3 x 5 mL) solution of **2.68** (100 mg, 0.15 mmol) in benzene (15 mL) at RT. After 1 h, an additional amount of DIPEA (194 mg, 1.5 mmol) and 2,4,6-trichlorobenzoyl chloride (376 mg, 1.5 mmol) were added and the solution stirred for 12 h. DMAP (745 mg, 6.1 mmol) was added followed by benzene (15 mL) and stirred for 1 h. Sat'd aq. NH_4Cl (30 mL) was added and the mixture was extracted with EtOAc (5 x 100 mL). The combined organic fractions were washed with brine (100 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure.

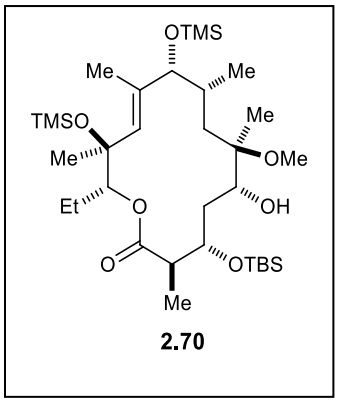
The product was purified by flash column chromatography to give 65 mg (65%) of **2.69** as a white foam. $[\alpha]^{23}_{D}$ +30.2 (c 1.5, $CHCl_3$); IR (neat) 2929, 2855, 1728, 1667, 1514, 1371, 1249, 1165, 1056, 835, 804, 776, 737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.2 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.53 (d, *J* = 1.0 Hz, 1H), 4.96 (dd, *J* = 10.7, 2.0 Hz, 1H), 4.66 (d, *J* = 5.5 Hz, 2H), 4.11 – 4.03 (m, 1H), 3.79 (s, *J* = 3.7 Hz, 3H), 3.70 (dd, *J* = 10.1, 4.0 Hz, 1H), 3.39 (dd, *J* = 11.9, 7.0 Hz, 1H), 3.19 (s, 3H), 2.68 (dd, *J* = 9.2, 7.3 Hz, 1H), 2.01 (d, *J* = 1.0 Hz, 3H), 1.97 – 1.41 (m, 6H), 1.28 (s, 3H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.13 (s, 3H), 1.11 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.88 (s, 9H), 0.02 (d, *J* = 21.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.40, 176.36, 158.78, 142.30, 138.82, 131.59, 129.05 (2C), 113.47 (2C), 81.13, 80.22, 79.27, 77.21, 73.94, 71.05, 55.25, 49.36, 47.79, 38.61, 37.22, 35.55, 25.96 (3C), 21.65, 21.32, 20.43, 18.69, 18.08, 17.14, 12.83, 10.65, -3.87, -4.94; HRMS (ESI) calc'd for $C_{35}H_{58}NO_9Si$ + Na = 657.3799, found 657.3816.



2.69-1

C9 Luche Alcohol 2.69-1: $CeCl_3$•7 H_2O (105 mg, 0.28 mmol) was added to a solution of **2.69** (76 mg, 0.12 mmol) in MeOH (2.4 mL) at RT and stirred for 30 min. The solution was cooled to -15 ˚C and $NaBH_4$ (9.8 mg, 0.26 mmol) was added. The solution was stirred at -15 ˚C for 15 min and allowed to warm to RT stirring for 30 min. The solution was then diluted with EtOAc (50 mL) and washed with 1M aq. HCl (10 mL), sat'd aq. $NaHCO_3$ (3 x 10 mL) and brine (10mL). The solution was filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-40% EtOAc in hexanes to afford 72 mg (96%) of **2.69-1** as a 4.6:1 mixture of separable

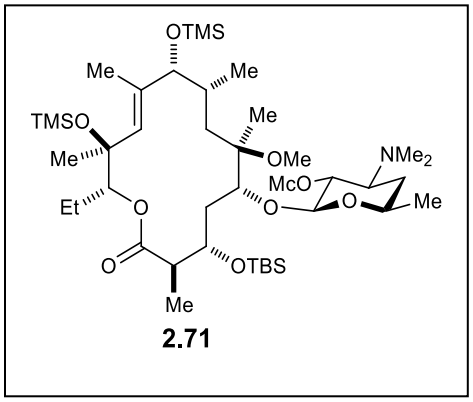
diastereomers. (The major isomer was taken forward separately for ease of characterization). $[\alpha]^{23}_{D}$ +14.3 (c 1.5, $CHCl_3$); IR (neat) 2956, 2932, 2856, 1729, 1514, 1463, 1370, 1249, 1171, 1061, 836, 775 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.81 (s, 1H), 4.88 (dd, *J* = 10.4, 2.9 Hz, 1H), 4.69 (d, *J* = 11.0 Hz, 1H), 4.43 (d, *J* = 11.0 Hz, 1H), 3.94 (s, 1H), 3.87 – 3.81 (m, 1H), 3.80 (s, *J* = 7.7 Hz, 3H), 3.59 (dd, *J* = 6.6, 4.4 Hz, 1H), 3.20 (s, 3H), 2.71 (dd, *J* = 8.7, 7.2 Hz, 1H), 2.12 – 2.05 (m, 1H), 1.95 – 1.86 (m, 1H), 1.83 (s, *J* = 0.7 Hz, 3H), 1.83 – 1.78 (m, 1H), 1.77 – 1.69 (m, 1H), 1.68 – 1.57 (m, 2H), 1.40 (s, 3H), 1.27 (s, 3H), 1.18 (d, *J* = 7.1 Hz, 3H), 1.15 (d, *J* = 7.1 Hz, 3H), 1.05 – 0.95 (m, 1H), 0.96 – 0.87 (m, 12H), 0.09 (d, *J* = 4.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.69, 158.74, 142.12, 131.61, 128.69 (2C), 125.62, 113.52 (2C), 83.10, 81.10, 80.73, 79.23, 74.25, 72.46, 71.52, 55.25, 50.90, 48.18, 39.63, 33.67, 30.03, 26.14 (3C), 24.65, 23.42, 21.02, 19.58, 18.26, 16.25, 15.58, 10.84, -3.34, -4.29; HRMS (ESI) calc'd for $C_{35}H_{60}NO_8Si$ + Na = 659.3955, found 659.3949.



2.70

Glycosylation Precursor 2.70: TMSOTf (33 mg, 0.15 mmol) was added to a solution of **2.69-1** (32 mg, 0.05 mmol) and 2,6-lutidine (21 mg, 0.2 mmol) in DCM (1 mL) at – 78 °C. The solution was stirred for 30 min and sat'd aq. $NaHCO_3$ (1 mL). The mixture was extracted with DCM (3 x 5 mL) and the combined organic fractions were washed with brine (1 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was dissolved in DCM:H_2O (8:1, 1.1 mL) and cooled to 0 °C. DDQ (23 mg, 0.1 mmol) was added and the solution stirred for 30 min. Sat'd aq. $NaHCO_3$ (1 mL) was added and the

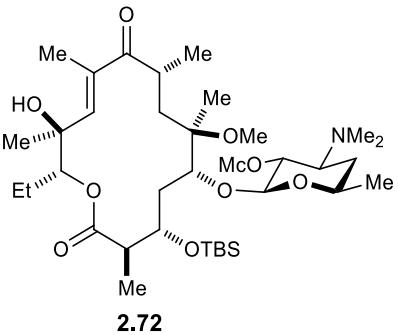
mixture extracted with DCM (3 x 10 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-10% EtOAc in hexanes to afford 25 mg (76%) of **2.70** as a white foam. $[\alpha]^{23}_D$ +53.2 (c 1.5, $CHCl_3$); IR (neat) 2957, 2856, 1731, 1250, 1096, 1065, 1047, 864, 838, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.41 (s, 1H), 4.82 (dd, *J* = 8.3, 3.6 Hz, 1H), 4.01 – 3.91 (m, 1H), 3.86 (d, *J* = 9.9 Hz, 1H), 3.78 (s, 1H), 3.14 (s, 3H), 2.56 (dd, *J* = 9.0, 7.2 Hz, 1H), 2.21 (s, 1H), 1.92 – 1.78 (m, 2H), 1.75 (s, 3H), 1.73 – 1.61 (m, 2H), 1.55 (ddd, *J* = 14.9, 10.2, 2.7 Hz, 1H), 1.47 – 1.40 (m, 1H), 1.39 (s, 3H), 1.35 – 1.26 (m, 1H), 1.24 (d, *J* = 7.3 Hz, 3H), 1.09 – 1.05 (m, 6H), 0.93 (t, *J* = 7.5 Hz, 3H), 0.89 (s, 9H), 0.14 (s, 3H), 0.10 (s, 12H), 0.08 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.37, 139.06, 128.80, 82.14, 81.43, 78.42, 76.54, 72.80, 72.00, 49.86, 49.41, 39.84, 31.97, 31.64, 25.91 (3C), 24.28, 23.56, 20.05, 19.52, 17.99, 17.00, 15.33, 11.57, 2.29 (3C), 0.39 (3C), -4.18, -4.71; HRMS (ESI) calc'd for $C_{33}H_{68}O_7Si_3$ + Na = 683.4171, found 683.4172.



2.71

Glycosylation Product 2.71: An azeotropically dried (toluene 3 x 3 mL) solution of **2.70** (45 mg, 0.07 mmol), desosamine thiopyrimidine donor **1.72** (134 mg, 0.41 mmol) and 2,6-DTBMP (84 mg, 0.41 mmol) in DCM (2 mL) was cannulated into a suspension of activated 4 Å molecular sieves and AgOTf (349 mg, 1.36 mmol) in 1:1 DCM:toluene (4 mL) at 0 °C. The mixture was allowed to warm to RT stirring for 12 h. Et_3N (3 mL) was added and stirred for 30 min before

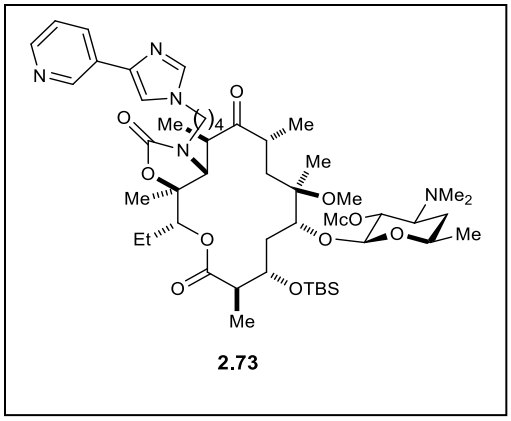
filtering over Celite and washing with EtOAc (25 mL). The organic fraction was then washed with sat'd aq. $NaHCO_3$ (3 x 5 mL) and brine (5 mL). The solution was filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-5% MeOH in DCM to give 40 mg (70%) of **2.71** as a colorless oil. $[\alpha]^{23}_D$ -12.9 (c 1.5, $CHCl_3$); IR (neat) 2956, 2857, 1756, 1733, 1261, 1163, 1095, 1073, 1052, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.48 (s, 1H), 5.00 (dd, *J* = 9.1, 2.6 Hz, 1H), 4.53 (dd, *J* = 10.5, 7.6 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 4.11 (s, 1H), 3.80 (d, *J* = 6.4 Hz, 1H), 3.74 (s, 4H), 3.52 (dd, *J* = 10.2, 5.4 Hz, 1H), 3.16 (s, 3H), 2.74 (s, 1H), 2.54 – 2.40 (m, 1H), 2.30 (s, 6H), 1.98 (dd, *J* = 15.1, 7.7 Hz, 1H), 1.92 – 1.76 (m, 2H), 1.74 (s, 3H), 1.44 (d, *J* = 14.0 Hz, 4H), 1.37 (s, 3H), 1.28 – 1.20 (m, 8H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.99 – 0.85 (m, 15H), 0.13 (s, 6H), 0.09 (d, *J* = 3.7 Hz, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.87, 155.13, 141.17, 130.60, 98.52, 81.65, 80.39, 78.65, 77.18, 75.87, 75.31, 71.86, 68.66, 62.64, 54.71, 50.57, 48.63, 40.71, 39.36, 34.16, 32.61, 31.24, 29.69, 26.39 (3C), 23.56, 22.75, 20.96, 20.14, 19.65, 18.43, 16.31, 15.45, 11.18, 2.37 (3C), 0.47 (3C), -2.95, -3.83; HRMS (ESI) calc'd for $C_{43}H_{85}NO_{11}Si_3$ + H = 876.5509, found 876.5514.



2.72

Enone with Desosamine 2.72: Pyridine (138 mg, 1.75 mmol) followed by 70% HF•pyridine (57 mg, 2.8 mmol) were added to a solution of **2.71** (30 mg, 0.03 mmol) in THF (1.2 mL) at 0 °C. The solution was allowed to warm to 15 °C stirring for 3 h. The solution was then cooled back to 0 °C and sat'd aq. $NaHCO_3$ was added drop wise until HF quenched. The mixture was then extracted with EtOAc (3 x

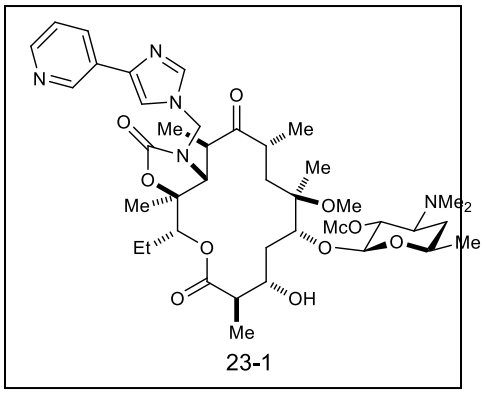
5 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was then dissolved in DCM (0.3 mL) and Dess-Martin periodinane (28 mg, 0.07 mmol) was added and the solution stirred at RT for 3 h. Sat'd aq. $NaHCO_3$ (1 mL), sat'd aq. Na_2SO_3 (1 mL) and H_2O (1 mL) were added to the reaction vessel and stirred for 30 min before extracting with EtOAc (3 x 10 mL). The combined organic fractions were washed with brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-5% MeOH in DCM to give 16 mg (67%) of **21** as a white foam. $[\alpha]^{23}_D$ +1.6 (c 1.5, $CHCl_3$); IR (neat) 1756, 1732, 1670, 1457, 1441, 1372, 1293, 1265, 1161, 1055, 995, 836, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.46 (d, *J* = 1.0 Hz, 1H), 4.99 (dd, *J* = 10.4, 2.1 Hz, 1H), 4.54 – 4.44 (m, 2H), 4.06 (s, 1H), 3.71 (s, 3H), 3.70 – 3.66 (m, 1H), 3.61 (s, 1H), 3.45 (d, *J* = 5.3 Hz, 1H), 3.22 (s, 3H), 2.82 – 2.69 (m, 1H), 2.46 – 2.31 (m, 1H), 2.28 (s, 6H), 2.00 (d, *J* = 0.8 Hz, 3H), 1.98 – 1.88 (m, 2H), 1.85 – 1.77 (m, 1H), 1.76 – 1.70 (m, 2H), 1.62 – 1.49 (m, 2H), 1.44 (s, 3H), 1.32 (dd, *J* = 23.9, 12.5 Hz, 1H), 1.27 – 1.20 (m, 7H), 1.10 (s, 3H), 1.06 (d, *J* = 6.7 Hz, 3H), 0.96 – 0.88 (m, 12H), 0.13 (d, *J* = 4.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.94, 176.04, 155.20, 141.83, 140.37, 98.54, 79.55, 78.11, 77.21, 76.31, 75.13, 73.58, 71.26, 68.60, 63.05, 54.55, 50.70, 48.05, 40.66 (2C), 38.07, 35.62, 30.60, 26.14 (3C), 23.43, 21.68, 21.09, 20.71, 20.21, 18.23, 16.91, 13.36, 10.77, -3.41, -4.24; HRMS (ESI) calc'd for $C_{37}H_{68}NO_7Si$ + H = 730.4562, found 730.4563.



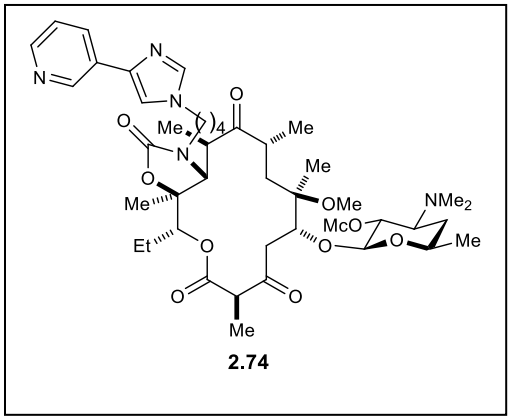
2.73

C11,12-oxazolidinone 2.73: 60% NaH (7.2 mg, 0.18 mmol) was added to a solution of **2.72** (31 mg, 0.04 mmol) and CDI (73 mg, 0.45 mmol) in 10:1 DMF:THF (0.35 mL) at -20 ˚C. The solution was stirred for 45 min while warming to 0 ˚C. Sat'd aq. $NaHCO_3$ (2 mL) was added dropwise and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic fractions were washed with NH_4OH (2 x 5 mL) and brine (5 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was dissolved in 9:1 MeCN:H_2O (1 mL). **1.70** (42 mg, 0.21 mmol) was added and the solution stirred at rt for 72 h. The solvent was then removed under reduced pressure and the product purified by flash column chromatography eluting with 0-5% MeOH in DCM to give 25 mg (61%) of **2.73** as yellow foam. $[\alpha]^{23}_D$ -8.8 (c 1.5, $CHCl_3$); IR (neat) 3117, 2929, 2853, 1751, 1457, 1264, 1166, 1061, 836, 776, 665 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.95 (d, *J* = 2.2 Hz, 1H), 8.44 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.08 – 8.02 (m, 1H), 7.56 (d, *J* = 1.1 Hz, 1H), 7.34 (d, *J* = 1.1 Hz, 1H), 7.31 – 7.26 (m, 1H), 4.94 (dd, *J* = 11.0, 1.9 Hz, 1H), 4.50 (dd, *J* = 10.7, 7.5 Hz, 1H), 4.35 (d, *J* = 7.5 Hz, 1H), 4.04 (t, *J* = 7.4 Hz, 2H), 3.76 (s, 3H), 3.74 – 3.58 (m, 4H), 3.52 (dd, *J* = 10.0, 5.4 Hz, 1H), 3.13 – 3.08 (m, 1H), 2.94 (s, 3H), 2.71 (td, *J* = 12.1, 4.3 Hz, 1H), 2.61 – 2.54 (m, 1H), 2.50 (dd, *J* = 9.4, 7.2 Hz, 1H), 2.27 (d, *J* = 9.2 Hz, 6H), 1.94 – 1.82 (m, 4H), 1.78 – 1.65 (m, 3H), 1.63 – 1.49 (m, 3H), 1.44 (d, *J* = 6.5 Hz, 3H), 1.30 (s, 3H), 1.22 (dd, *J* = 12.6, 6.5 Hz, 6H), 1.16 (t, *J* = 7.8 Hz, 3H), 1.12 (d, *J* = 7.1 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.94 (s, 9H), 0.80 (t, *J* = 7.3 Hz, 3H), 0.11 (d, *J* = 14.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.02, 176.58, 157.50, 155.23, 147.49, 146.21, 138.94, 137.81,

132.08, 130.27, 123.61, 115.59, 98.28, 82.76, 78.15, 77.64, 76.35, 75.13, 71.42, 68.84, 62.74, 59.96, 54.67, 50.76, 48.58, 46.86, 45.00, 42.49, 40.54 (3C), 39.96, 39.18, 38.31, 30.32, 28.64, 26.32 (3C), 24.21, 21.91, 20.94, 19.76, 18.84, 18.33, 16.20, 14.18, 13.78, 10.39, -2.93, -3.52; HRMS (ESI) calc'd for $C_{50}H_{81}N_5O_{12}Si$ + H = 972.5279, found 972.5738.

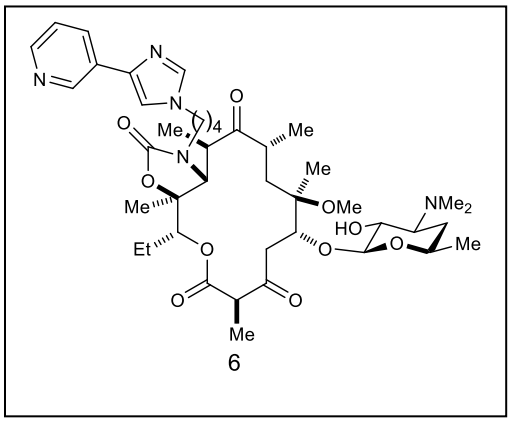


C3-OH 2.73-1: Tris(dimethylamino)sulfonium difluorotrimethylsilicate (36 mg, 0.13 mmol) in DMF (130 µL) was cannulated into a solution of **2.73** (25 mg, 0.03 mmol) in 65:1 DMF:H_2O (325 µL) at RT and stirred for 14 h. The solution was then diluted with EtOAc (10 mL) and washed with pH 7 phosphate buffer (2 x 2 mL) and brine (2 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. **2.73-1** was azeotropically dried with toluene (3 x 2 mL) and taken to the next step.



C3-Ketone 2.74: Me_2S (12.4 mg, 0.2 mmol) was added to a solution of NCS (16 mg, 0.12 mmol) in DCM (1 mL) at 0 ˚C and stirred for 5 min before cooling to -20 ˚C. **2.73-1** in DCM (1 mL) was cannulated into the solution and stirred for 1.5 h. Et_3N (29 mg, 0.29 mmol) was added and the solution allowed to warm to RT. Sat'd aq. $NaHCO_3$ (3 mL) was added and the mixture

extracted with DCM (3 x 10 mL). The combined organic fractions were washed with H_2O (3 mL) and brine (3 mL), filtered over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography eluting with 0-5% MeOH in DCM to give 12 mg (53%) of **2.74**. $[\alpha]^{23}_D$ +3.0 (c 0.97, $CHCl_3$); IR (neat) 2925, 1744, 1714, 1456, 1375, 1264, 1174, 1106, 1052, 1000, 734, 631 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.44 (d, *J* = 3.9 Hz, 1H), 8.12 – 8.03 (m, 1H), 7.54 (s, 1H), 7.33 (s, 1H), 7.31 – 7.27 (m, 1H), 4.95 (dd, *J* = 10.5, 2.3 Hz, 1H), 4.49 (dd, *J* = 10.5, 7.7 Hz, 1H), 4.37 (d, *J* = 8.1 Hz, 1H), 4.24 (d, *J* = 7.6 Hz, 1H), 4.00 (t, *J* = 7.4 Hz, 2H), 3.81 (s, 3H), 3.78 – 3.61 (m, 4H), 3.59 – 3.51 (m, 2H), 3.16 – 3.06 (m, 2H), 2.76 – 2.68 (m, 1H), 2.64 (s, 3H), 2.61 – 2.52 (m, 1H), 2.28 (s, 6H), 2.00 – 1.92 (m, 1H), 1.91 – 1.82 (m, 2H), 1.76 (dd, *J* = 12.3, 3.4 Hz, 1H), 1.67 – 1.52 (m, 6H), 1.51 (s, 3H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.31 (s, 3H), 1.26 (d, *J* = 6.2 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 215.67, 199.33, 169.71, 157.22, 155.14, 147.58, 146.48, 139.20, 137.78, 132.02, 130.33, 123.46, 115.47, 100.58, 82.28, 77.76, 77.50, 75.00, 69.48, 63.21, 60.34, 54.79, 53.32, 50.07, 46.84, 45.10, 43.41, 42.65, 40.58 (2C), 39.04, 38.69, 31.90, 30.45, 28.61, 24.39, 22.45, 20.95, 19.28, 18.53, 14.61, 14.09, 13.97, 10.54; HRMS (ESI) calc'd for $C_{44}H_{65}N_5O_{12}$ + H = 856.4708, found 856.4712.



6

(–)-4-desmethyl Telithromycin 1.69: 2.74 (12.1 mg, 0.014 mmol) in MeOH (2.8 mL) was stirred at RT for 10 h. The solvent was removed under reduced pressure and the product was purified by flash column chromatography eluting with 0-10% MeOH in DCM to give 7.5 mg (67%) of **1.69**. $[\alpha]^{23}_{D}$ -3.5 (c 0.23, $CHCl_3$); IR (neat) 3649, 2934, 2361, 1748, 1717, 1540, 1521, 1472, 1375, 1286, 1234, 1175, 1108, 1075, 668 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 1.7 Hz, 1H), 8.45 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.10 – 8.03 (m, 1H), 7.54 (d, *J* = 1.2 Hz, 1H), 7.34 (d, *J* = 1.2 Hz, 1H), 7.29 (dd, *J* = 8.2, 5.1 Hz, 1H), 4.96 (dd, *J* = 10.5, 2.3 Hz, 1H), 4.43 (d, *J* = 8.1 Hz, 1H), 4.17 (d, *J* = 7.4 Hz, 1H), 4.01 (t, *J* = 7.3 Hz, 2H), 3.82 – 3.50 (m, 5H), 3.28 (dd, *J* = 18.3, 9.2 Hz, 1H), 3.19 – 3.10 (m, 2H), 2.67 (s, 3H), 2.59 (dd, *J* = 12.5, 5.6 Hz, 1H), 2.54 – 2.39 (m, 2H), 2.27 (s, 6H), 2.02 – 1.79 (m, 5H), 1.76 – 1.59 (m, 5H), 1.50 (s, 3H), 1.33 (d, *J* = 6.3 Hz, 6H), 1.26 (d, *J* = 8.4 Hz, 3H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.02 (d, *J* = 6.9 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 215.93, 199.68, 169.78, 157.30, 147.58, 146.41, 139.08, 137.77, 131.99, 130.26, 123.49, 115.49, 103.38, 82.34, 78.27, 77.62, 69.84, 69.27, 65.87, 60.14, 53.25, 50.16, 46.84, 45.24, 44.37, 42.54, 40.22 (2C), 38.99, 38.83, 29.68, 28.63, 28.10, 24.31, 22.32, 21.22, 19.30, 18.54, 14.49, 14.13, 14.00, 10.56; HRMS (ESI) calc'd for $C_{42}H_{63}N_5O_{10}$ + H = 798.4653, found 798.4654.

4.2: Chapter 3: The Development of a Ribosome-Templated *In Situ* Click Methodology for the Discovery of Antibiotics Targeting the Bacterial Ribosome

4.2.1 General Methods

All reactions containing moisture or air sensitive reagents were performed in oven-dried glassware under nitrogen or argon. *N,N*-Dimethylformamide, tetrahydrofuran, toluene and dichloromethane were passed through two columns of neutral alumina prior to use. Water was double distilled prior for the *in situ* click reactions. Buffers were filtered prior to use. All solvents for work-up procedures were used as received. Alkynes **3.20** and **3.26** were prepared from the commercially available, TMS-protected alkyne variants by reaction with KOH in MeOH. Alkynes **3.27** and **3.31** were prepared by the reaction of imidazole and morpholine, respectively, with propargyl bromide. Alkyne **3.28** was prepared from the commercially available, peracetylated glucoside by deprotection with NaOMe in MeOH. Alkyne **3.21** was prepared in a three-step sequence starting with the Pd-catalyzed Sonogashira coupling of commercial *meta*-bromobenzaldehyde with TMS-acetylene, transformation of the aldehyde to the difloromethyl with commercial Deoxo-Fluor, and TMS deprotection with potassium carbonate in MeOH. All other reagents were purchased from commercial sources and used without further purification. Flash column chromatography was performed using an Isco Combiflash Rf 200 instrument with Isco Gold Silica Gel Columns with the indicated solvents. Thin layer chromatography was performed on Merck 60 F_{254} silica gel plates. Detection was performed using UV light, iodine, PMA stain and subsequent heating. ^{1}H and ^{13}C NMR spectra were recorded at the indicated field strength in $CDCl_3$ or MeOD at rt. Chemical shifts are indicated in parts per million (ppm) downfield from tetramethylsilane (TMS, $\delta = 0.00$) and referenced to the

$CDCl_3$. Splitting patterns are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), q (quartet) and m (multiplet). Optical rotations were measured on a Perkin-Elmer 341 Polarimeter at room temperature, using the sodium D line.

4.2.2 *In situ* Click Experiments

Procedure for the proof-of-concept ribosome-templated *in situ* Formation of solithromycin (1.65) from a binary Component Mixture of Azide 3.11 and 3-ethynylaniline (3.12).

***In situ* click:** Azide **3.11** [0.5 µL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 87 µL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 µL microcentrifuge tube. 50S subunits or 70S *E. coli* ribosomes [10 µL, 50 µM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to the solution and incubated at 37 °C for 30 minutes. 3-ethynylaniline **3.12** [2.5 µL, 200 mM in dimethyl sulfoxide (DMSO)] was added for a final volume of 100 µL and final concentrations of 5 µM azide **3.11**, 5 mM alkyne **3.12,** and 5 µM 50S subunits or 70S *E. coli* ribosomes. The solution was incubated at room temperature for 24 hours.

Buffer-only control: Azide **3.11** [0.5 µL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 97 µL of

aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 µL microcentrifuge tube and incubated at 37 °C for 30 minutes. 3-ethynylaniline **3.12** [2.5 µL, 200 mM in dimethyl sulfoxide (DMSO)] was added for final concentrations of 5 µM azide **3.11** and 5 mM alkyne **3.12**. The solution was incubated at room temperature for 24 hours.

BSA control: Azide **3.11** [0.5 µL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 97 µL of a solution of Bovine Serum Albumin [5 µM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] in a 300 µL microcentrifuge tube and incubated at 37 °C for 30 minutes. 3-ethynylaniline **3.12** [2.5 µL, 200 mM in dimethyl sulfoxide (DMSO)] was added for final concentrations of 5 µM azide **3.11** and 5 mM alkyne 3.12. The solution was incubated at room temperature for 24 hours.

30S *E. coli* subunit control: Azide **3.11** [0.5 µL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 87 µL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 µL microcentrifuge tube. 30S *E. coli* subunits [10 µL, 50 µM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to the solution and incubated at 37 °C for 30 minutes. 3-ethynylaniline **3.12** [2.5 µL, 200 mM in dimethyl sulfoxide (DMSO)] was added for a final volume of 100 µL and final concentrations of 5 µM azide **3.11**, 5 mM alkyne **3.12,** and 5

μM 50S subunits or 70S *E. coli* ribosomes. The solution was incubated at room temperature for 24 hours.

Azithromycin (AZY) inhibition experiment: Azide **3.11** [0.5 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 84.5 μL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 μL microcentrifuge tube. AZY [2.5 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] and 50S or 70S *E. coli* ribosomes [10 μL, 50 μM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] were added to the solution and incubated at 37 °C for 30 minutes. 3-ethynylaniline **3.12** [2.5 μL, 200 mM in dimethyl sulfoxide (DMSO)] was added for a final volume of 100 μL and final concentrations of 5 μM azide **3.11** and 5 mM alkyne **3.12** and 5 μM 50S or 70S *E. coli* ribosomes. The solution was incubated at room temperature for 24 hours.

LC-MS Analysis: 90 μL of the above solutions were injected on an Agilent 6520B Q-TOF LC-MS instrument utilizing an Agilent Poroshell 120 4.6 mm x 30 mm (2.1 um particle size) C8 reverse phase column with a flow rate of 1 mL per minute and a 10 minute gradient from 0% MeCN (0.1% HCO_2H)/100% water (0.1% HCO_2H)→100% MeCN (0.1% HCO_2H)/0% water (0.1% HCO_2H). Extracted ion chromatograms (EIC's) were used to locate & quantify amounts of **1.65.**

Table 4.1. LC-MS Analysis of *in situ* click experiments with *E. coli* 70S ribosomes, 50S subunits, 70S with inhibitor azithromycin (AZY, 25 μL) and negative controls (30S subunits, BSA, or buffer only). Mass counts (normalized) correspond to 1.65 (solithromycin) and *syn*-1.65 regioisomer ions.

	1.65 [counts]	***syn*-1.65 [counts]**	**1.65 (normalized)**	***syn*-1.65 (normalized)**
70S	1204889	540970	100	45
50S	1161665	534718	96	44
25 μM AZT	145321	131192	12	11
30S	116592	100953	10	8
BSA	73313	73634	6	6
Buffer	75504	81653	6	7

General Procedure for Five-Alkyne *In Situ Click* Competition Experiments

Azide **3.11** [1 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 74 μL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 μL microcentrifuge tube. 50S *E. coli* ribosomes [20 μL, 50 μM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] were added to the solution and incubated at 37 °C for 30 minutes. Five alkynes [1 μL each, 200 mM in dimethyl sulfoxide (DMSO)] were added for a final volume of 100 μL and final concentrations of 10 μM azide **3.11**, 2 mM each alkyne, and 10 μL 50S or 70S *E. coli* ribosomes. The solution was incubated at room temperature for 24 hours.

Buffer Control: Azide **3.11** [1 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 94 μL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 μL microcentrifuge tube and incubated at 37 °C for 30 minutes. Five Alkynes [1 μL each, 200 mM in dimethyl sulfoxide (DMSO)] were added for a final volume of 100 μL and final concentrations of 10 μL azide **3.11** and 2 mM each alkyne. The solution was incubated at room temperature for 24 hours.

LC-MS Analysis: 90 μL of the above solutions were injected on an Agilent 6520B Q-TOF LC-MS instrument utilizing an Agilent Poroshell 120 4.6 mm x 50 mm (2.1 um particle size) C8 reverse phase column with a flow rate of 1 mL per minute and a 10 minute gradient from 0% MeCN (0.1% HCO_2H)/100% water (0.1% HCO_2H) to 100% MeCN (0.1% HCO_2H)/0% water (0.1% HCO_2H). Extracted ion chromatograms (EIC) were used to locate & quantify amounts of clicked triazole products.

Table 4.2. LC-MS Analysis of *in situ* click experiment with azide **3.11** and alkynes **3.12**, **3.18**, **3.28**, **3.29**, and **3.23** with 50S *E. coli* ribosomal subunits

	Expt 1			Expt 2				
Cmpd#	50S [counts]	BUFFER [counts]	% INCREASE	50S [counts]	BUFFER [counts]	% INCREASE	average % INCREASE	standard error
anti-**3.42**	8183798	5204776	57	4523500	3667171	23	40	12
anti-**3.42**	3460609	2589691	34	2142196	1734690	23	29	4
anti-**3.32**	344598	45127	664	199476	33450	496	580	59
syn-**3.32**	91950	38066	142	48111	24115	100	121	15
3.43	25505	18505	38	15147	11347	33	36	2
anti-**3.37**	41568	46620	-11	26366	28255	-7	-9	1
syn-**3.37**	45157	45637	-1	41163	38932	6	2	2
anti-**1.65**	155901	18739	732	116629	23851	389	560	121
syn-**1.65**	376312	73916	409	220358	36426	505	457	34

Table 4.3. LC-MS Analysis of *in situ* click experiment with azide **3.11** and alkynes **3.27**, **3.24**, **3.20**, **3.25**, and **3.29** with 50S *E. coli* ribosomal subunits

	Expt 1			Expt 2				
	50S	BUFFER	%	50S	BUFFER	%	average %	standard
Cmpd#	[counts]	[counts]	INCREASE	[counts]	[counts]	INCREASE	INCREASE	error
3.34	951389	181150	425	997031	500926	99	262	115
3.41	372947	26902	1286	256826	20157	1174	1230	40
3.43	20670	13304	55	14304	10958	31	43	9
3.39	56089	34909	61	164476	60282	173	117	40
anti-**3.38**	169688	28874	488	133002	38919	242	365	87
syn-**3.38**	72822	17498	316	51502	18367	180	248	48

General Procedure for Fifteen-Alkyne *In Situ* Click Competition Experiments

***In situ* click:** Azide **2** [1 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 71.5 μL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 μL microcentrifuge tube. 70S *E. coli* ribosomes [20 μL, 50 μM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] were added to the solution and incubated at 37 °C for 30 minutes. 15 alkynes [0.5 μL each, 200 mM in dimethyl sulfoxide (DMSO)] were added for a final volume of 100 μL and final concentrations of 10 μL azide **2**, 1 mM each alkyne, and 10 μL 50S *E. coli* ribosomes. The solution was incubated at room temperature for 48 hours.

Buffer Control: Azide **2** [1 μL, 1 mM in aqueous buffer (20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol)] was added to 91.5 μL of a aqueous buffer [20 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$, 150 mM NH_4Cl, 6 mM 2-mercaptoethanol] in a 300 μL microcentrifuge tube and incubated at 37 °C for 30 minutes. 15 alkynes [0.5 μL each, 200 mM in dimethyl sulfoxide (DMSO)] were added for a final

volume of 100 µL and final concentrations of 10 µL azide **2** and 1 mM each alkyne. The solution was incubated at room temperature for 48 hours.

Table 4.4. LC-MS Analysis of *in situ* click experiments with azide **3.11** and alkynes **3.12**, **3.18-3.31** with 70S E. coli ribosomes.

	EXPT 1			EXPT 2			EXPT 3			EXPT 4			EXPT 5				
Cmpd #	70S [counts]	buffer [counts]	% increase	70S [counts]	buffer [counts]	% increase	70S [counts]	buffer [counts]	% increase	70S [counts]	buffer [counts]	% increase	70S [counts]	buffer [counts]	% increase	average % increase	standard error
anti-1.65	463037	76755	503	118233	25635	361	100750	29069	247	n.d.	n.d.	n.a.	106379	16103	561	418	71
syn-1.65	260046	89803	190	92108	26940	242	110991	21354	420	149603	47372	216	129513	17964	621	338	81
anti-3.32	334455	75062	346	93425	20104	365	84460	18048	368	138184	39584	249	190028	29501	544	374	48
syn-3.32	28030	40647	-31	25565	11720	118	15678	9327	68	mix	mix	n.d.	mix	mix	n.a.	52	44
3.33	3339059	2375207	41	270886	n.d.	n.a.	15335	n.d.	n.a.	109453	n.d.	n.a.	n.d.	n.d.	n.a.	41	n.a.
anti-3.34	1.1E+07	990922	972	675368	115034	487	351172	55474	533	616637	56470	992	690927	47342	1359	869	162
syn-3.34	653900	528261	24	133470	48857	173	63290	26766	136	118718	28097	323	59960	20996	186	168	54
3.35	1357140	507611	167	63912	11312	465	12867	n.d.	n.a.	33620	7103	373	28459	15851	80	271	89
anti-3.36	379646	159900	137	45533	n.d.	n.a.	13948	n.d.	n.d.	27115	n.d.	n.a.	n.d.	n.d.	n.a.	137	n.a.
syn-3.36	80121	196266	-59	48738	n.d.	n.a.	15483	n.d.	n.d.	30546	n.d.	n.a.	n.d.	n.d.	n.a.	-59	n.a.
anti-3.37	385567	104936	267	29101	n.d.	n.a.	n.d.	n.d.	n.a.	15994	n.d.	n.a.	17590	n.d.	n.a.	267	n.a.
syn-3.37	114707	250380	-54	32893	n.d.	n.a.	n.d.	n.d.	n.a.	15238	n.d.	n.a.	mix	mix	n.a.	-54	n.a.
anti-3.38	1281462	117355	992	82743	21437	286	64659	17056	279	88966	18384	384	135215	24693	448	478	132
syn-3.38	39670	42782	-7	27179	10132	168	28215	10169	177	27971	11252	149	46838	11623	303	158	49
3.39	979659	558900	75	76031	15347	395	13429	n.d.	n.a.	38958	9194	324	n.d.	n.d.	n.a.	265	97
3.40	449352	356756	160	88613	25438	248	25438	8785	190	56723	18186	212	28947	10644	172	396	191
3.41	530923	290417	83	231555	49310	370	196859	44047	347	247967	45560	444	449801	41600	981	445	147
anti-3.42	4451605	3266657	36	2720360	2681522	1	1939756	22219644	-91	2318501	2264299	-90	1854383	1590452	17	-25	27
syn-3.42	1754470	1424862	23	1219698	1087280	12	881023	1078854	-18	1005284	1080967	-7	704196	725805	-3	1	7
3.43	44877	25727	74	n.d.	n.d.	n.a.	10665	7223	48	n.d.	n.d.	n.a.	13955	7070	97	73	14
3.44	59862	28030	114	14881	n.d.	n.a.	11829	n.d.	n.a.	12630	n.d.	n.d.	27388	n.d.	n.a.	114	n.a.
anti-3.45	53813	36319	48	13183	n.d.	n.a.	12073	n.d.	n.a.	12117	n.d.	n.a.	24001	105578	-77	-15	63
syn-3.45	11514	10721	7	10266	n.d.	n.a.	6857	n.d.	n.a.	7092	n.d.	n.a.	15702	5371	192	100	93

4.3 Computational Methods

4.3.1 SILCS Sampling

Site-Identification by Ligand Competitive Saturation (SILCS) simulations were performed using a combined Grand Canonical-Monte Carlo/Molecular Dynamics (GCMC/MD) simulation[206] approach from which Grid Free Energy (GFE) Frag Maps[207] were obtained for MC-SILCS docking and scoring of the ligands based on ligand GFEs

(LGFE, see below).[208] Initial system preparation was performed using the program CHARMM[209] with the GCMC being performed using an in-house program and MD simulations being performed using GROMACS,[210] version 5.0 (developmental). The CHARMM additive force field was used including the CHARMM22 protein with the CMAP correction,[211-213] CHARMM36 nucleic acid,[214-217] CHARMM36 carbohydrate,[218-223] and CGenFF[224] (2b8) parameters and the TIP3P water model.[225] The simulation system involves a 40 Å radius spherical system, whose origin is the center of mass of telithromycin, extracted from the ribosomal 50S subunit crystal structure (PDB ID 3OAT)[226] as previously described.[227] Initial coordinates for the present study were obtained from a snapshot at 5 ns from a previous GCMC/MD simulation.[227] Non-hydrogen atoms in the outer region (from 34 to 40 Å) were restrained using a harmonic positional restraint of 10000 kJ/mol/nm^2 (24 kcal/mol/Å^2) and non-hydrogen atoms in the buffer region (from 28 to 34 Å) were restrained using a harmonic positional restraint of 2500 kJ/mol/nm^2 (6 kcal/mol/Å^2). Atoms in the inner region of 28 Å were not restrained. In all cases, ribosome positional restraints used the initial coordinates as reference.

For SILCS simulations, telithromycin and all crystal waters were removed. Water and the eight organic solutes (see below) were maintained within a 20 Å radius sphere using a spherical flat-bottomed potential of 4184 kJ/mol/nm^2 (10 kcal/mol/Å^2). The restraints were assigned to the following atoms of each solute: propane C2, benzene CG, methylammonium CE, acetate C1, methanol CB, formamide C, imidazole CG, and acetaldehyde C. Waters were subjected to a flat-bottom potential extending 45 Å from the center with the positional restraint applied to the oxygen.

The GCMC-SILCS procedure has been described in detail elsewhere,[206] such that the details of the general methodology will only briefly be addressed as they pertain to the simulations presented here. The macrolide binding pocket is primarily RNA and hence the charged nucleic acid backbone tends to favor the insertions of water, thereby limiting the insertions of hydrophobic fragments. Hence, an initial phase of 150,000 MC steps was performed in which all solutes except water were inserted; then, an additional "flooding" phase of 4,000,000 MC steps was performed for water only using an insertion radius of 45 Å. This radius was selected because it extends beyond the ribosome sphere and guarantees that all Mg^{2+} ions are solvated. The two phases were followed by a steepest decent (SD) minimization of 2000 steps using a force tolerance of 10 and maximum step size of 0.01 nm, in which positional restraints on the ribosome were removed, and 75 ps of molecular dynamics (MD) with a 1 fs time step to relax the system around the newly placed fragments.

GCMC/MD SILCS simulations were performed using 10 runs of 500 cycles each (a total of 500 ns cumulative MD simulation time) with each cycle consisting of GCMC, energy minimization, equilibration, and production. GCMC was performed for 150,000 steps using a radius of 20 Å. All fragments were given equal sampling time with a random assignment of the fragment insertion order. A cutoff of 15 Å beyond the sphere was used for calculation of the energies for each MC step. After each cycle of GCMC, relaxation of the system around the newly placed fragments and waters was accomplished with 500 steps of SD using a force tolerance of 10 and maximum step size of 0.01 nm. MD simulations were performed at 298 K using the LINCS[228] algorithm to constrain covalent bonds involving hydrogens and the leapfrog integrator (GROMACS integrator "md").

Nonbonded interactions were treated using grid neighbor searching employing a group cutoff-scheme with a cutoff of 10 Å. Neighbor lists were updated every 10 steps. A switching function was applied to the van der Waals energy terms from 5 to 8 Å, while the electrostatics were treated using Reaction-Field-Zero with a cutoff of 8 Å. Equilibration was accomplished using 50 ps of MD employing a 1 fs time step. Generation of the fragment maps was based on 100 ps production MD using a 2 fs time step, with coordinates saved every 2 ps. Coordinates from the last snapshot of the production phase were used to initiate a new cycle of GCMC. FragMaps were generated for the atom types: benzene carbons, propane carbons, acetaldehyde oxygen, formamide oxygen, formamide nitrogen, imidazole unprotonated nitrogen, imidazole protonated nitrogen, methanol oxygen, methylammonium nitrogen, and acetate oxygens following the protocol outlined in previous SILCS papers.[206-208,229-232] Notably, the generation of hydrogen bond donor maps (i.e. imidazole, methylammonium, formamide, methanol) is based on the non-hydrogen heteroatom directly bonded to the hydrogen.

4.3.2 MC-SILCS Sampling

Each ligand was generated in the Molecular Operating Environment (MOE, Chemical Computing Group, Inc., Quebec, Canada). The macrolactone ring coordinates were obtained from PDB ID 3OAT[226] with the aniline ring replaced by the R group of interest. For each ligand, an ensemble of conformations was generated using an in-house MC sampling algorithm in the field of the SILCS GFE FragMaps and exclusion map as described previously. Sampling of translational, rotational, and torsional degrees of freedom was performed in the presence flat-bottom potential of 10 Å radius on the center

of mass of the ligand. For the assembled macrolide analogs, the initial coordinates of the macrolactone and desosamine were based on the telithromycin crystal structure coordinates. Sampling was accomplished using 100 runs each consisting of two phases. In the first phase 100,000 MC steps are performed with translations allowed to vary by up to 1 Å, rotations by up to 180°, and torsions by up to 180° in each MC step. The second phase involves a simulated-annealing (SLOWCOOL) phase of 50,000 MC steps with translations allowed to vary by up to 0.2 Å, rotations by up to 9°, and torsions by up to 9° in each MC step with conformations saved every 1,000 steps. Only conformations from the second phase are collected for analysis giving a total of 5000 conformations for each ligand.

The calculation of LGFE scores has been described previously and is presented here to highlight details specific to this study. Unnormalized LGFE values were calculated by summing the GFE values for each classified atom in the congeners over each snapshot to calculate the Boltzmann average LGFE. Classified atoms are based on rules that assign atom types to a GFE FragMap type. Normalized LGFE scores were calculated by first dividing the Boltzmann average LGFE by the total number of classified atoms, which was then multiplied by the total number of non-hydrogen atoms to yield the normalized LGFE. Normalized LGFE values were also calculated for different regions of the molecules using subsets of ligand atoms: macrolactone+desosamine components and the side chains. The side chains are defined as the alkyl-R group moiety extending from N11 (including the 4 carbon linker), while the macrolactone+desosamine atoms correspond to the macrolactone and D-desosamine rings extending from C5. Predictive indices values were calculated according to Pearlman and Charifson[233] and rank from -1 (negative correlation) to +1 (positive correlation).

4.4 Dissociation Constant (K_d) Determination by Competition Binding of BODIPY Erythromycin A with Triazoles 1.65, Azide 3.11, 3.32-3.45 Utilizing Fluorescence Polarization (FP)[234]

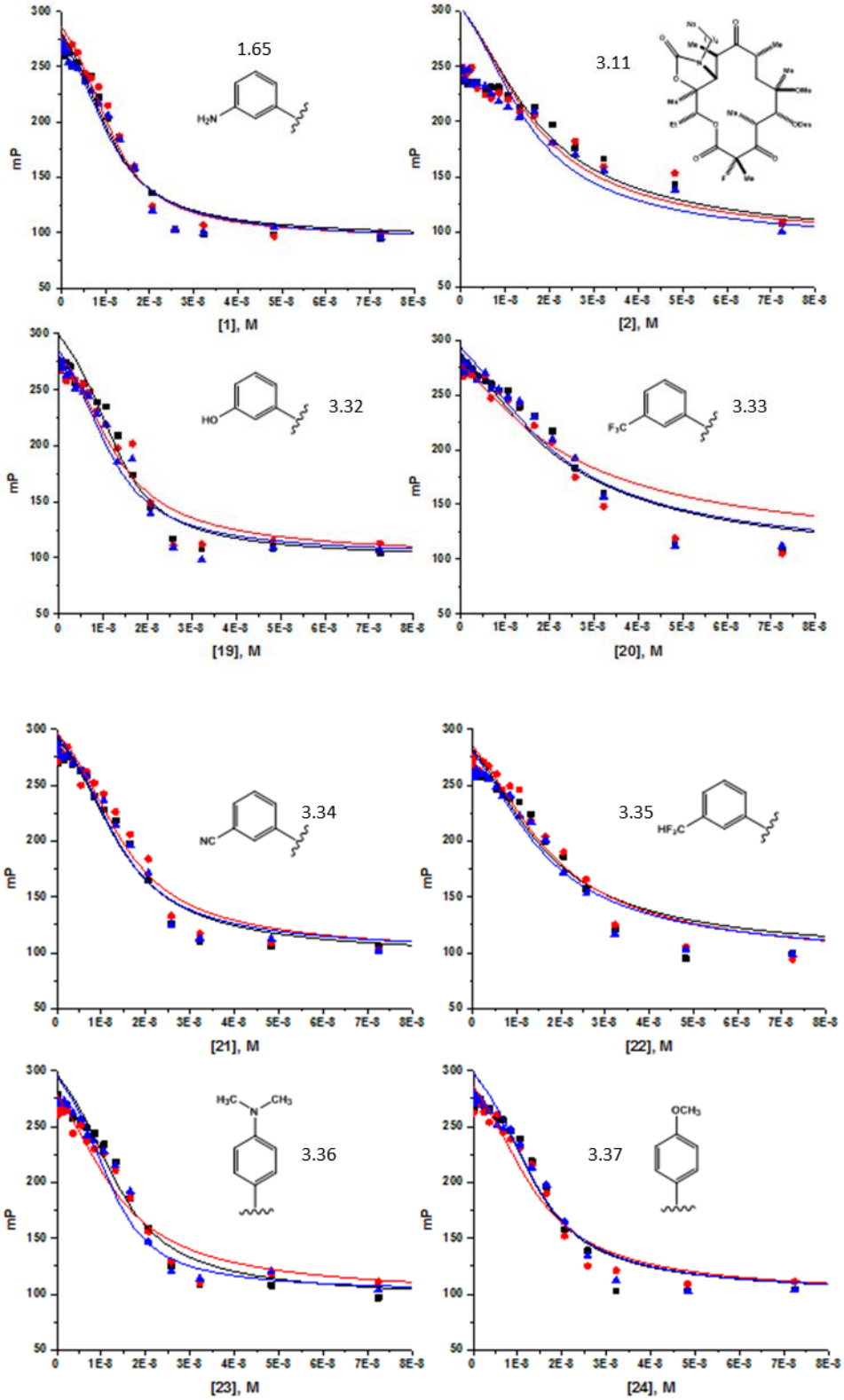
FP method:

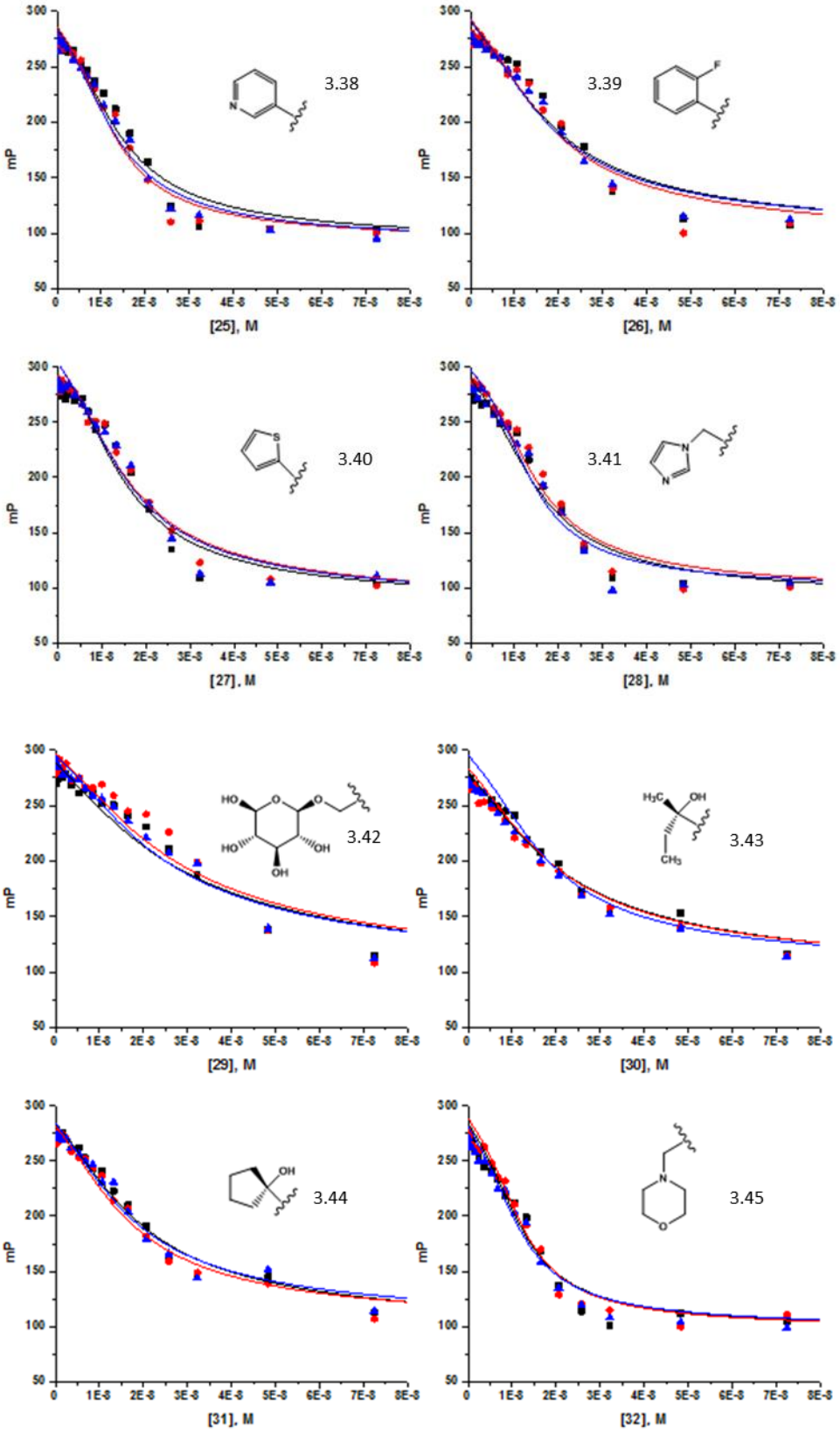
5.2 nM BODIPY-Erythromycin A was incubated with 37.8 nM 70S (12.9 nM active 70S as determined by binding assays) in buffer (20 mM HEPES pH 7.5, 50 mM NH_4Cl, 10 mM $MgCl_2$, 0.05% Tween 20) in a total volume of 96 µl in the wells of a 96-well plate for 30 min at room temp. 4 µl of 25X compound (1% DMSO final) or blank was added incubated at room temperature for one hour. The 96-well plate was then scanned on a Tecan F200 (485 nm excitation / 535 nm emission) to determine milliPolarization (mP).

***K*d fitting:** Data was plotted as [compound] vs. mP units. The data was fitted to Wang's cubic equation[235] in order to obtain the K_d of the competitive compound binding to 70S. Fitting parameters were Amax = 404, Amin = 82, Klig = 4.26e-9 (K_d of the BODIPY probe as determined by binding curves), [Lt] = 5.2 nM, [Rt] = 12.9 nM.

IC_{50} fitting: IC_{50} values were determined by extrapolating the compound concentration at which the mP signal was reduced to 50%.

Figure 4.1. Triplicate K_d fits of **1.65**, **3.11**, and **3.32-3.45**.

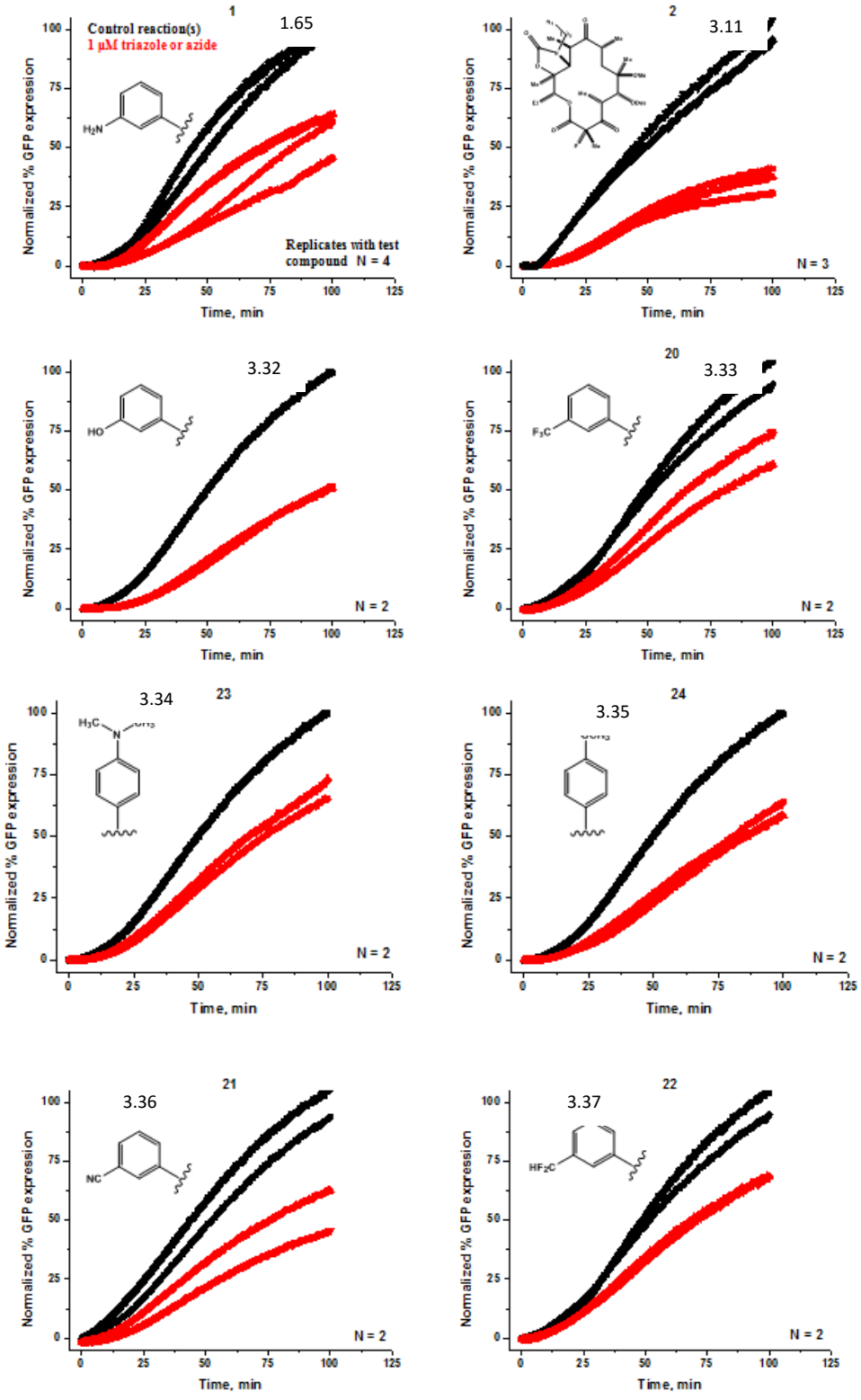


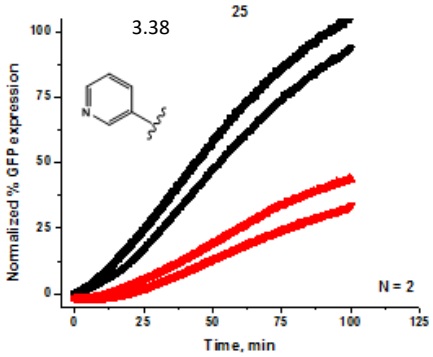

3.38
3.39
3.40
3.41
3.42
3.43
3.44
3.45
mP
[25], M
[26], M
[27], M
[28], M
[29], M
[30], M
[31], M
[32], M

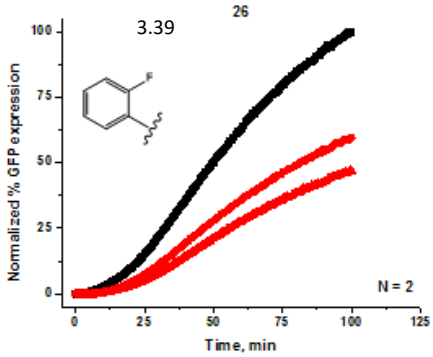
4.5 Cell-Free Translation Inhibition Method

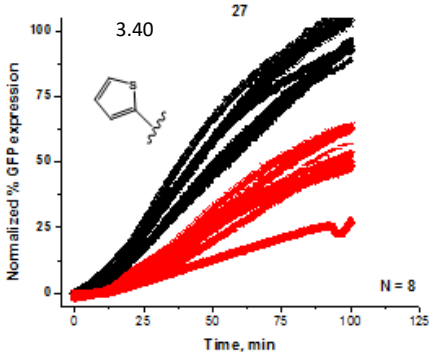
The ability of triazoles **1.65** and **3.32-3.45** and **azide 3.11** to inhibit protein synthesis was evaluated by monitoring the expression of a superfolder EmGFP in one of two cell-free protein expression kits: 5Prime's RTS 100 *E. coli* HY kit and Invitrogen's Expressway mini cell-free expression kit.[236] Reactions were performed as per the manufacturers' instructions on a 10 μL scale and with a final triazole concentration of 1 μM. The synthesis of EmGFP was monitored on a Tecan M1000 plate reader (excitation 486 nm, emission 535 nm) at 30 °C for 100 min. Translation inhibition was calculated from the percent reduction in the maximal slope of each curve relative to a DMSO only control. The ribosome concentration in the cell-free protein synthesis kits was determined as previously described.[236] Briefly, ribosomes were pelleted from the reconstituted reactions by ultracentrifugation in a Sorvall M120SE microultracentrifuge using a S120AT2 rotor at 110,000 rpm for 40 min. The 70S pellets were resuspended in buffer [50 mM Tris-HCl, pH 7.5, 30 mM NH_4Cl, 70 mM KCl, 15 mM $Mg(OAc)_2$ and 1 mM DTT]. The 70S concentration was determined by taking the absorbance at 260 nm assuming 1 AU = 26 pmol 70S. Inhibitor concentrations were determined based on the dry weight of the synthesized compounds.

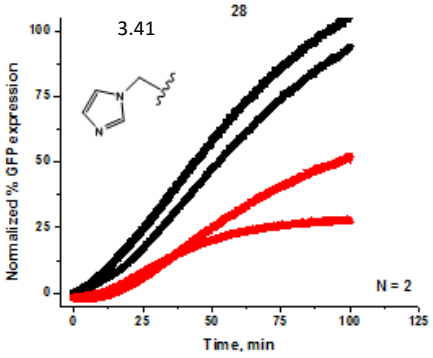
Figure 4.2. Cell-Free Translation Inhibition Results.

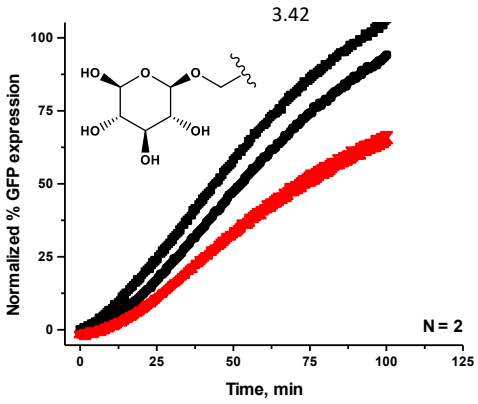


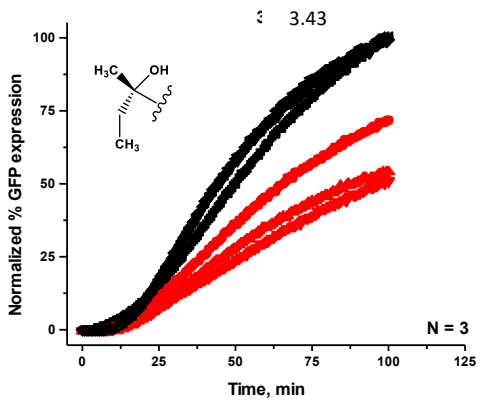

25
3.38
Normalized % GFP expression
Time, min
N = 2


26
3.39
Normalized % GFP expression
Time, min
N = 2


27
3.40
Normalized % GFP expression
Time, min
N = 8


28
3.41
Normalized % GFP expression
Time, min
N = 2


3.42
Normalized % GFP expression
Time, min
N = 2


3.43
Normalized % GFP expression
Time, min
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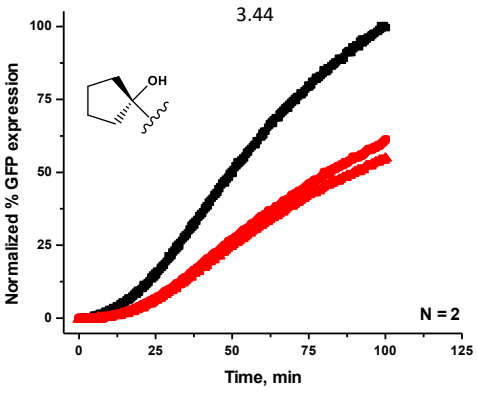
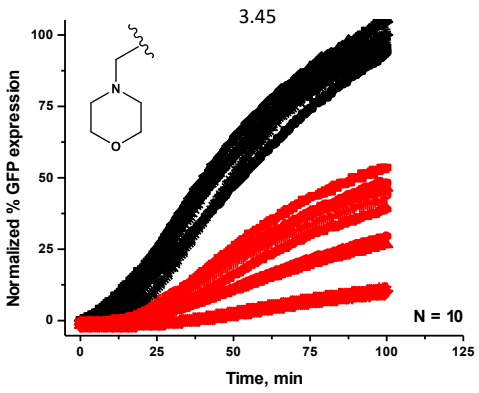

3.44
Normalized % GFP expression
Time, min
N = 2


3.45
Normalized % GFP expression
Time, min
N = 10

Compound	Number of replicates	Average % inhibition	Standard deviation
1.65	4	47.3	11.2
3.32	2	46.1	3.4
3.33	2	35.7	9.3
3.34	2	42.1	9.7
3.35	2	34.2	0.9
3.36	2	33.1	4.0
3.37	2	37.0	5.1
3.38	2	56.8	9.5
3.39	2	46.5	7.8
3.40	8	46.9	12.1
3.41	2	48.6	12.2
3.42	2	31.9	4.8
3.43	3	46.5	14.8
3.44	2	42.6	2.1
3.45	10	64.4	13.7

4.6 Minimum Inhibitory Concentration (MIC) Methods

***Escherichia coli*:** DK, DK (pkk3535), DK (2058G), SQ171, SQ171 (2058G)

E. coli strains were inoculated from freezer stock into Lysogeny Broth containing 50 µg/mL of Ampicillin (LB-Amp) and incubated at 37 °C overnight (No Ampicillin used with DK strain). The culture was then diluted 1:100 and grown to an OD_{600nm} = 0.6 (2-4 h). 75 µL of (LB-Amp) was added to wells in rows 1-11 of 96-well plates. 150 µL of a 64 µg/mL antibiotic solution for DK strains and 128 µg/mL for SQ strains in (LB-Amp) were then added to row 12. Serial dilutions were made from row 12 to 1 for a final volume of 75 µL in each well. The above prepared *E. coli* cultures were then diluted to OD_{600nm} = 0.004 with (LB-Amp) and 75 µL of the above prepared *E. coli* culture was then added to all wells. The last column of the 96-well plate was reserved for negative controls (LB-Amp

only) and positive controls (OD_{600nm} = 0.004 *E. coli* culture). 96-well plates were covered and incubated at 37 °C for 18 h. Plates were visually inspected to determine MIC.

***Staphylococcus aureus*:** UCN 14 (A2058U), UCN 17 (A2058G), UCN18 (A2059G), ATCC 29213 (wt), ATCC 33591 (MRSA)

S. aureus strains were inoculated from freezer stock into Brain Heart Infusion (BHI) Broth 37 °C overnight. The culture was then diluted 1:100 and grown to an OD_{600nm} = 0.6 (2-4 h). 75 µL of BHI Broth was added to wells in rows 1-11 of 96-well plates. 150 µL of a 256 µg/mL antibiotic solution in BHI Broth was then added to row 12. Serial dilutions were made from row 12 to 1 for a final volume of 75 µL in each well. The above-prepared *S. aureus* cultures were then diluted to OD_{600nm} = 0.004 with BHI Broth and 75 µL of the above-prepared *S. aureus* culture was then added to all wells. The last column of the 96-well plate was reserved for negative controls (BHI Broth only) and positive controls (OD_{600nm} = 0.004 *S. aureus* culture). 96-well plates were covered and incubated at 37 °C for 18 h. Plates were visually inspected to determine MIC.

***Streptococcus pneumoniae*:** ATCC 49619 (WT), 2196 (*erm* B), 655 (*mef* A), 1369 (*erm* B and *mef* A), 319 (A2059G), and 2634 (*mef* A and A2059G)

S. pneumoniae strains were plated from freezer stock on Tryptic Soy (TS) agar plates containing 5% sheep's blood and incubated at 37 °C in the presence of 5% CO_2 overnight. The *S. pneumoniae* was plated again from the above plates on new Tryptic Soy agar plates containing 5% sheep's blood and incubated at 37 °C in the presence of 5% CO_2 overnight. (Two growth cycles were needed to obtain a suitable amount of bacterial growth) Colonies

were then added to Mueller Hinton (MH) Broth until an OD_{600nm} = 0.1 was reached. 75 µL of MH broth was added to wells in rows 1-11 of 96-well plates. 150 µL of an 8 µg/mL antibiotic solution in MH broth was then added to row 12. Serial dilutions were made from row 12 to 1 for a final volume of 75 µL in each well. The above-prepared *S. pneumoniae* cultures were then diluted to OD_{600nm} = 0.001 with MH broth and 75 µL was added to all wells. The last column of the 96-well plate was reserved for negative controls (MH broth only) and positive controls (OD_{600nm} = 0.001 *S. pneumoniae* culture). 96-well plates were covered and incubated at 37 °C in the presence of 5% CO_2 for 20 h. Plates were visually inspected to determine MIC.

Table 4.5. MIC analysis of **1.65**, **3.11**, **3.32-3.45** against *Escherichia coli* DK, DK (pkk3535), DK (A2058G), SQ171, SQ171 (2058G).

MIC (µg/mL)	***Escherichia coli***				
Compound	***DK***	**DK pkk3535**	**DK 2058G**	**SQ 171**	**SQ171 A2058G**
SOL (1.65)	2	2	2	32	>64
Azide (3.11)	2	2	2	64	>64
3.32	2	2	1	32	>64
3.33	4	4	4	64	>64
3.34	8	4	4	>64	>64
3.35	4	2	4	64	>64
3.36	4	4	4	64	>64
3.37	2	2	2	64	>64
3.38	4	2	2	32	>64
3.39	2	2	2	32	>64
3.4	2	1	1	32	>64
3.41	2	2	2	64	>64
3.42	>32	>32	>32	>64	>64
3.43	8	4	4	>64	>64
3.44	4	4	4	>64	>64
3.45	8	8	8	>64	>64

Table 4.6. MIC analysis of **1**, **2**, **19-32** against *Staphylococcus aureus* UCN 14 (A2058U), UCN 17 (A2058G), UCN18 (A2059G), ATCC 29213 (wt), ATCC 33591 (MRSA).

MIC (µg/mL)	***Staphylococcus aureus***				
Compound	**ATCC**	**ATCC 33591**	**UCN14**	**UCN17**	**UCN18**
	29213 (WT)	**MRSA**	**A2058U**	**A2058G**	**A2059G**
SOL (1.65)	<0.0625	32-64	8	4	4
Azide (3.11)	< 0.0625	>64	>128	>128	>128
3.32	<0.0625	>128	16	8	8
3.33	<0.0625	32	32	32	32
3.34	0.5	>128	>128	128	128
3.35	<0.0625	128	32-64	16	8
3.36	<0.0625	128	128	32	32
3.37	<0.0625	>128	>128	32	32
3.38	<0.0625	>128	32-64	16	16
3.39	<0.0625	128	32	8	8
3.4	<0.0625	>128	32	8	8
3.41	0.25	>128	>128	128	128
3.42	128	>128	>128	>128	>128
3.43	0.25	>128	>128	>128	>128
3.44	0.125	>128	>128	128	>128
3.45	0.25	>128	128	>128	>128

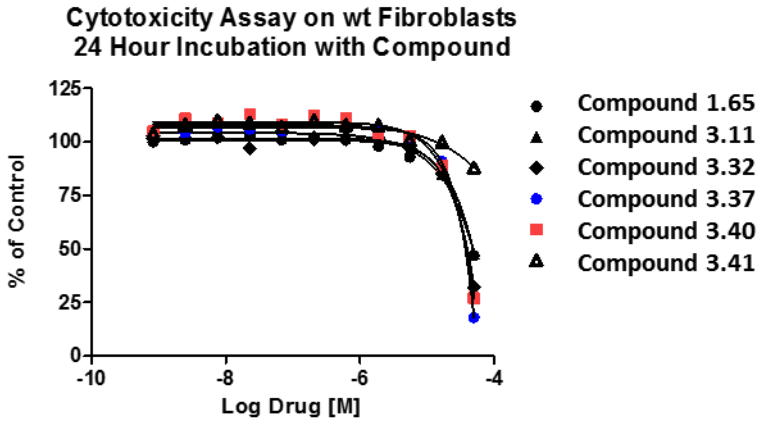
Table 4.7. MIC analysis of **1**, **2**, **19-32** against *Streptococcus pneumoniae* ATCC 49619 (WT), 2196 (*ermB*), 655 (*mefA*), 1369 (*ermB* and *mefA*), 319 (A2059G), and 2634 (*mefA* and A2059G).

MIC (µg/mL)	***Streptococcus pneumoniae***					
Compound	**ATCC 49619 (WT)**	**2196 *ermB***	**655 *mefA***	**1369 *ermB* & *mefA***	**319 A2059G**	**2634 *mefA* & A2059G**
SOL (1.65)	0.002-0.008	0.5-1	0.25-0.5	0.5	0.008	0.125-0.25
Azide (3.11)	≤ 0.002	>4	0.25	>4	1	1
3.32	≤ 0.002	2	0.5	4	0.016	0.25
3.33	0.016	>4	2	>4	0.5	1
3.34	0.016	>4	1	>4	0.25	0.5
3.35	0.004	4	1	>4	0.0625	0.5
3.36	0.008	4	1	>4	0.0625	0.5
3.37	0.002-0.008	4	1	>4	0.0625	0.25
3.38	0.016	1	0.5	>4	0.031	0.25
3.39	≤ 0.002	2	0.5	4	0.031	0.25
3.4	0.004	2	0.5	0.5	0.008	0.125
3.41	0.031	>4	4	>4	0.25	1
3.42	4	>4	>4	>4	>4	>4
3.43	0.031	4	2	>4	0.5	2
3.44	0.016	>4	2	>4	1	2
3.45	0.0625	>4	4	>4	2	4

4.7 Fibroblast Cytotoxicity Method

The potential cytotoxicity of the compounds on human fibroblasts cells was measured using a luciferase-coupled ATP quantitation assay (CellTiter-Glo viability assay, Promega). The change of intracellular ATP content indicates the number of metabolically competent cells. Human dermal fibroblasts (Coriell Institute GM05659) were seeded in 384-well white assay plates at 1000 cells/well in 30 µL complete growth media and grown overnight at 37 °C/5% CO_2. Dilutions were prepared from compound stocks, 50 mM in 100% DMSO and added to cells resulting in final concentrations of 50 µM to 0.88 nM, 0.1% DMSO. Cells were incubated at 37 °C/5% CO_2 for either 24 or 48 hours. Cell viability was measured after addition of 20 µL of CellTiter-Glo reagent (Promega) and luminescence read on an Envision plate reader. Percent viability was calculated as the ratio of luminescence intensity measured in compound treated cells relative to control 0.1 % DMSO treated cells.

Figure 4.3. Fibroblast viability assay results (24 and 48 hour incubation) for compounds **1.65, 3.11, 3.32, 3.37, 3.40, 3.41**.



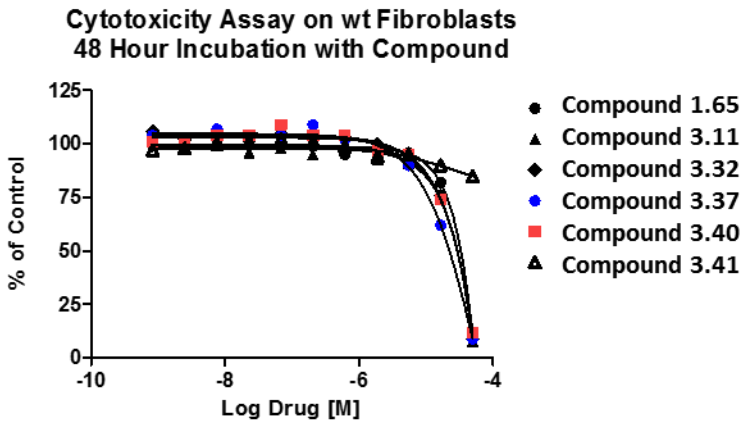
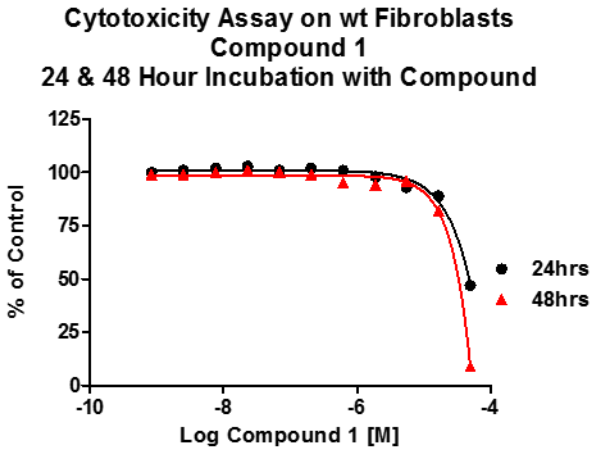


Figure 4.4. Individual fibroblast viability assay results (24 and 48 hours incubation).

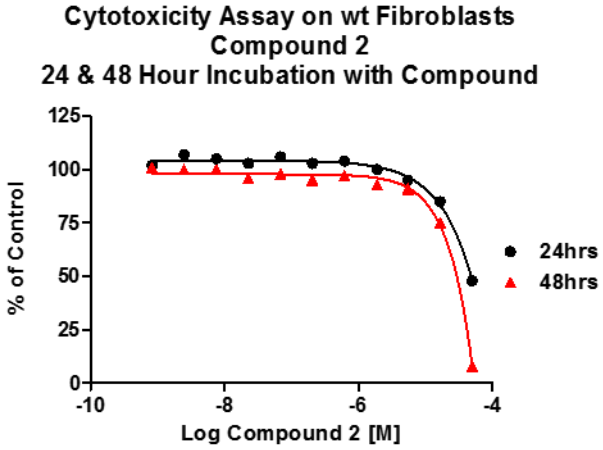
1.65

Compound 1		
[M]	24hr % CONT	48hr % CONT
5.00E-05	47	9
1.66E-05	89	82
5.50E-06	93	96
1.90E-06	98	94
6.17E-07	101	95
2.06E-07	102	99
6.90E-08	101	100
2.30E-08	103	101
7.60E-09	102	100
2.50E-09	101	99
8.33E-10	100	99



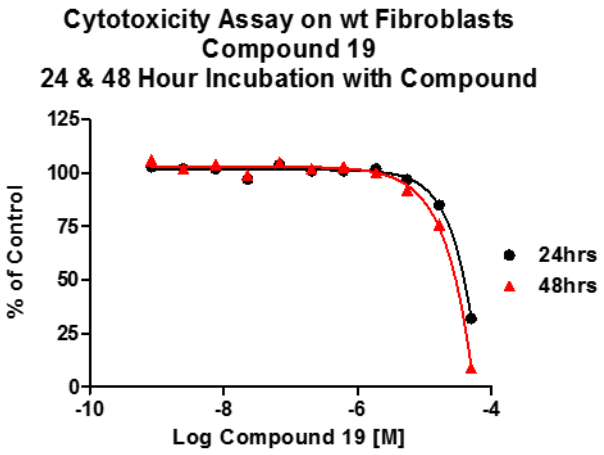
3.11

Compound 2		
[M]	24hr % CONT	48hr % CONT
5.00E-05	48	8
1.66E-05	85	75
5.50E-06	95	91
1.90E-06	100	93
6.17E-07	104	97
2.06E-07	103	95
6.90E-08	106	98
2.30E-08	103	96
7.60E-09	105	100
2.50E-09	107	100
8.33E-10	102	101



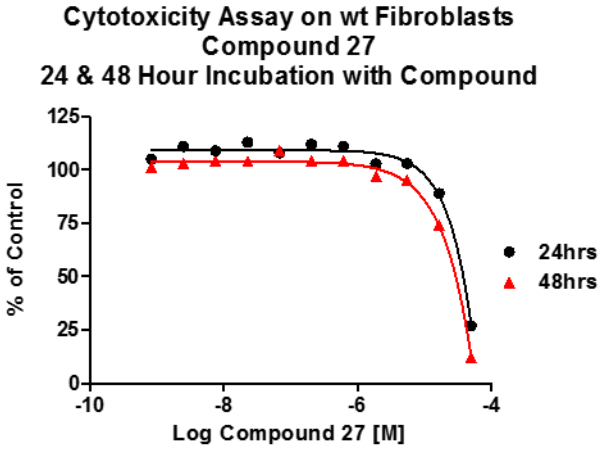
3.32

Compound 19		
[M]	24hr % CONT	48hr % CONT
5.00E-05	32	9
1.66E-05	85	76
5.50E-06	97	92
1.90E-06	102	100
6.17E-07	101	103
2.06E-07	101	102
6.90E-08	104	105
2.30E-08	97	99
7.60E-09	102	104
2.50E-09	102	102
8.33E-10	103	106



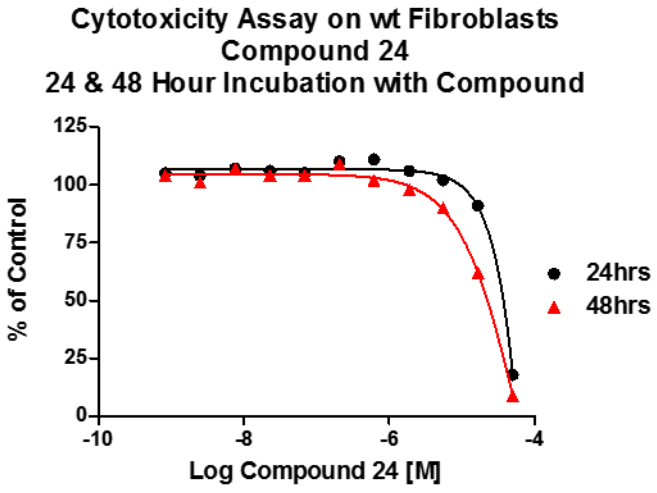
3.40

Compound 27		
[M]	24hr % CONT	48hr % CONT
5.00E-05	27	12
1.66E-05	89	74
5.50E-06	103	95
1.90E-06	103	97
6.17E-07	111	104
2.06E-07	112	104
6.90E-08	108	109
2.30E-08	113	104
7.60E-09	109	104
2.50E-09	111	103
8.33E-10	105	101



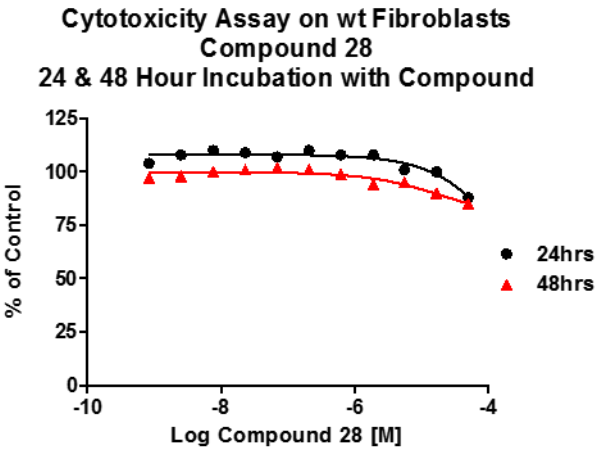
3.37

Compound 24		
[M]	24hr % CONT	48hr % CONT
5.00E-05	18	9
1.66E-05	91	62
5.50E-06	102	90
1.90E-06	106	98
6.17E-07	111	102
2.06E-07	110	109
6.90E-08	105	104
2.30E-08	106	104
7.60E-09	107	107
2.50E-09	104	101
8.33E-10	105	104

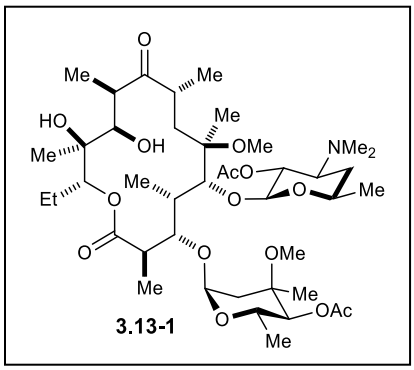


1.41

Compound 28		
[M]	24hr % CONT	48hr % CONT
5.00E-05	88	85
1.66E-05	100	90
5.50E-06	101	95
1.90E-06	108	94
6.17E-07	108	99
2.06E-07	110	101
6.90E-08	107	102
2.30E-08	109	101
7.60E-09	110	100
2.50E-09	108	98
8.33E-10	104	97

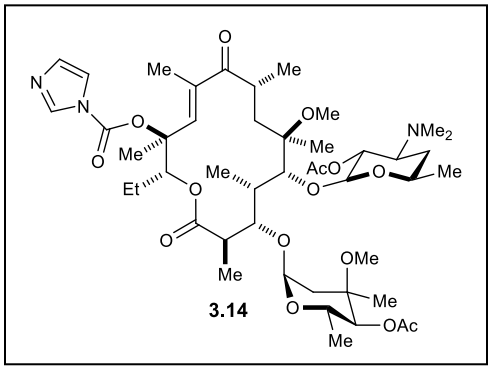


4.8 Experimental Procedures and Characterization



3.13-1: Ac_2O (3.42 g, 33.5 mmol) was added to a solution of Clarithromycin (5 g, 6.7mmol) and DMAP (205 mg, 1.67 mmol) in DCM (27 mL) under an inert atmosphere. After 18 hours stirring at room temperature TLC analysis showed complete consumption of the starting material. Sat. aq. NH_4Cl (15 mL) was added and the mixture was extracted with DCM (3 x 30 mL). The combined DCM fractions were washed with brine (10 mL), dried over sodium sulfate, and the solvent removed under reduced pressure to give 5.5 g (99%) of bis-acetate protected Clarithromycin **3.13-1** as a white foam. The product was sufficiently pure to carry on to the next step. $[\alpha]^{23}_D$ -151.6 (c 1.5, $CHCl_3$); IR (neat) 2974, 1740, 1457, 1373, 1235, 1171, 1047, 1010, 986; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.06 (dd, *J* = 11.1, 2.2 Hz, 1H), 4.98 (d, *J* = 5.0 Hz, 1H), 4.74 (dd, *J* = 10.4, 7.5 Hz, 1H), 4.67 (dd, *J* = 12.5, 8.6 Hz, 2H), 4.29 (dd, *J* = 9.7, 6.2 Hz, 1H), 3.97 (s, 1H), 3.78 – 3.69 (m, 3H), 3.59 (d, *J* = 6.4 Hz, 1H), 3.34 (s, *J* = 10.7 Hz, 3H), 3.19 (s, 1H), 3.00 (s, 3H), 2.99 – 2.94 (m, 1H), 2.87 (dd, *J* = 9.5, 7.3

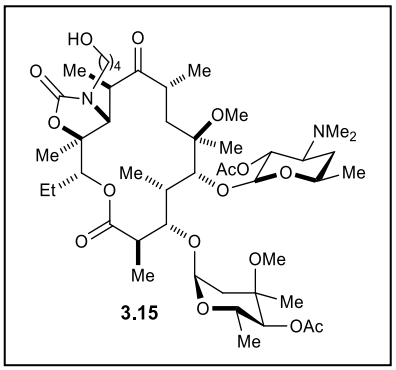
Hz, 1H), 2.72 (td, *J* = 12.3, 4.1 Hz, 1H), 2.60 – 2.50 (m, 1H), 2.40 (d, *J* = 15.2 Hz, 1H), 2.27 (s, 6H), 2.09 (s, 3H), 2.04 (s, 3H), 1.95 – 1.83 (m, 2H), 1.72 – 1.54 (m, 4H), 1.51 – 1.39 (m, 1H), 1.34 (s, 3H), 1.30 (d, *J* = 11.4 Hz, 1H), 1.20 (d, *J* = 6.1 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 1.12 (dt, *J* = 11.1, 4.7 Hz, 15H), 0.93 (d, *J* = 7.6 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.14, 175.51, 170.44, 169.98, 99.93, 95.75, 80.16, 78.56, 78.23, 77.67, 76.58, 74.18, 72.68, 71.95, 69.10, 67.23, 63.13, 63.10, 50.50, 49.31, 45.25, 44.83, 40.74 (2C), 38.62, 38.56, 37.21, 35.14, 31.15, 21.57 (2C), 21.10, 21.04, 20.89, 19.73, 18.33, 17.93, 16.07, 15.95, 12.34, 10.56, 9.01; HRMS (ESI) calc'd for $C_{42}H_{73}NO_{15}$ + H = 832.5058, found 832.5069.



3.14

3.14: Carbonyldiimidazole (5.4 g, 33.5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (2.04 g, 13.4 mmol), and **3.13-1** in DMF (110 mL) under an inert atmosphere were heated to 35 °C for 72 h. The solution was then cooled to 0 °C and water (110 mL) was added. The solid precipitate formed was then filtered off and washed with water (3 x 50 mL). The white solid was then dissolved in DCM and filtered over sodium sulfate to remove in residual water. The DCM was removed under reduced pressure to give 4.7g (77%) of **3.14** as a white solid. $[\alpha]^{23}_{D}$ -66.3 (c 0.79, $CHCl_3$); IR (neat) 2976, 2939, 2831, 1763, 1740, 1670, 1465, 1380, 1292, 1240, 1163, 1047, 1000, 752, 667; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.35 (s, 1H), 7.06 (s, 1H), 6.64 (s, 1H), 5.81 (dd, *J* = 10.4, 2.8 Hz, 1H), 4.96 (s, 1H), 4.75 – 4.64 (m, 2H), 4.60 (d, *J* = 7.5 Hz, 1H), 4.34 (dd, *J* = 9.5, 6.1 Hz, 1H), 3.88 – 3.76 (m, 1H), 3.70 – 3.60 (m, 1H), 3.58 (d, *J* = 6.8 Hz, 1H), 3.33 (s, *J* = 5.6 Hz, 3H), 3.27 – 3.18 (m, 1H), 3.13

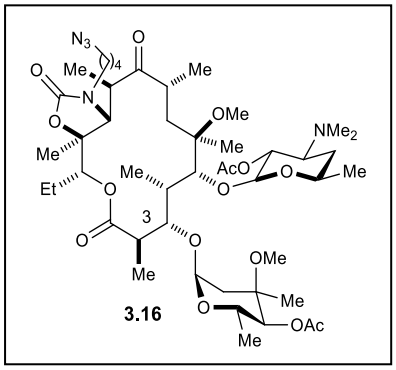
(s, 3H), 2.97 – 2.80 (m, 1H), 2.79 – 2.60 (m, 1H), 2.40 (d, *J* = 15.1 Hz, 1H), 2.26 (s, *J* = 9.6 Hz, 6H), 2.11 (s, 3H), 2.02 (s, *J* = 5.9 Hz, 3H), 1.93 – 1.87 (m, 1H), 1.84 (s, *J* = 5.0 Hz, 3H), 1.77 (s, *J* = 11.3 Hz, 3H), 1.74 – 1.59 (m, 4H), 1.41 – 1.30 (m, 3H), 1.25 (d, *J* = 5.3 Hz, 6H), 1.18 (d, *J* = 6.2 Hz, 6H), 1.14 (d, *J* = 5.0 Hz, 6H), 0.96 – 0.87 (m, *J* = 13.7, 6.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.61, 174.37, 170.50, 169.83, 145.76, 138.75, 137.82, 136.97, 130.82, 117.09, 100.46, 96.28, 84.43, 80.08, 78.51, 78.26, 77.21, 75.22, 72.65, 71.84, 67.56, 63.25, 63.03, 50.76, 49.42, 45.11, 40.68, 39.99, 39.25, 35.30, 30.82, 29.67, 22.33, 21.68, 21.49, 21.44, 21.06, 20.90, 19.91, 18.36, 18.13, 16.14, 13.32, 10.21, 9.26; HRMS (ESI) calc'd for $C_{46}H_{73}N_3O_{15}$ + H = 908.5114, found 908.5111.



3.15

3.15. A solution of **3.14** (4.7 g, 5.2 mmol) and 4-aminobutanol (2.3 g, 26 mmol) in DMF (17 mL) was heated to 35 °C for 48 h under an inert atmosphere. The solution was then cooled to 0 °C and water (17 mL) was added. The solid precipitate formed was then filtered off and washed with water (3 x 10 mL). The white solid was then dissolved in DCM and filtered over sodium sulfate to remove in residual water. The DCM was removed under reduced pressure to give 3.7g (80%) of **3.15** as a white solid. $[\alpha]^{23}_{D}$ -65.9 (c 1.2, $CHCl_3$); IR (neat) 2974, 2938, 1740, 1457, 1372, 1234, 1167, 1048, 1011, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.02 – 4.92 (m, 2H), 4.79 – 4.70 (m, 1H), 4.69 – 4.62 (m, 2H), 3.76 – 3.55 (m, 8H), 3.33 (s, *J* = 3.4 Hz, 3H), 3.14 (s, *J* = 5.8 Hz, 1H), 3.07 (q, *J* = 6.9 Hz, 1H), 3.01 (s, 3H), 2.90 (dd, *J* = 9.7, 7.2 Hz, 1H), 2.78 – 2.67 (m, 1H), 2.62 – 2.49 (m, 1H), 2.40 (d, *J* = 15.2 Hz, 1H), 2.27 (s, *J* = 6.1 Hz, 6H), 2.10 (s, 3H), 2.04 (s, 3H), 1.98 – 1.76 (m, 4H), 1.76

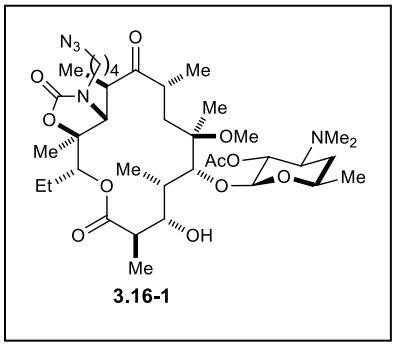
– 1.45 (m, 8H), 1.39 (s, *J* = 3.6 Hz, 3H), 1.34 (s, *J* = 8.9 Hz, 3H), 1.21 (d, *J* = 7.3 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 1.15 – 1.08 (m, 9H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 7.6 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.56, 176.20, 170.42, 169.96, 157.46, 99.96, 95.81, 82.58, 79.45, 78.64, 78.44, 77.14, 76.33, 72.63, 71.90, 67.32, 63.14, 63.07, 62.07, 60.08, 50.55, 49.32, 45.56, 45.02, 43.14, 40.72 (2C), 38.97, 38.36, 35.08, 31.08, 30.01, 29.68, 23.19, 21.99, 21.57, 21.53, 21.07, 20.88, 20.07, 18.83, 18.30, 15.97, 14.36, 14.17, 10.32, 8.98; HRMS (ESI) calc'd for $C_{47}H_{80}N_2O_{16}$ + H = 929.5581, found 929.5572.



3.16

Intermediate **3.16:** Et_3N (739 mg, 7.3 mmol) was added slowly to a solution of **3.15** and mesyl chloride (722 mg, 6.3 mmol) in DCM (26 mL) under an inert atmosphere at 0 °C. The solution was allowed to warm to room temperature stirring overnight for 18 hours. Water (13 mL) was added and the mixture extracted with DCM (3 x 30 mL). The combined organic fraction was washed with brine (10 mL), filtered over sodium sulfate, and the solvent removed under reduced pressure. The crude material was dissolved in DMF (125 mL) and NaN_3 (410 mg, 6.3 mmol) was added. The solution was heated at 80 °C under an inert atmosphere for 15 hours. The solution was cooled to 0 °C and water (125 mL) was added. The white precipitate was filtered and washed with water (3 x 50 mL). The white solid was then dissolved in DCM (50 mL) and filtered over sodium sulfate to remove residual water. The solvent was removed to give 4.6 g (77% over 2 steps) of **3.16** as a white solid. $[\alpha]^{23}_D$ -66.385.5 (c 0.99, $CHCl_3$); IR (neat) 2978, 2098, 1741, 1455, 1372, 1234, 1165, 1105, 1047,

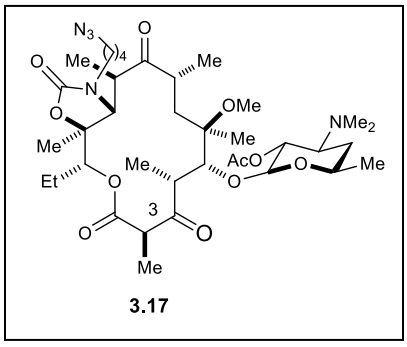
1010, 752, 666; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.02 – 4.88 (m, 2H), 4.80 – 4.71 (m, 1H), 4.71 – 4.61 (m, 2H), 4.36 – 4.24 (m, 1H), 3.76 – 3.66 (m, 3H), 3.65 – 3.52 (m, 4H), 3.34 (s, 3H), 3.32 – 3.28 (m, 1H), 3.05 (q, *J* = 6.8 Hz, 1H), 2.99 (s, 3H), 2.89 (dd, *J* = 9.5, 7.2 Hz, 1H), 2.80 – 2.65 (m, 1H), 2.61 – 2.48 (m, 1H), 2.40 (d, *J* = 15.2 Hz, 1H), 2.27 (s, 6H), 2.10 (s, *J* = 6.9 Hz, 3H), 2.04 (s, 3H), 1.98 – 1.44 (m, 12H), 1.38 (s, 3H), 1.34 (s, 3H), 1.21 (d, *J* = 7.1 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.15 – 1.10 (m, 9H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 7.6 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.14, 175.98, 170.41, 169.94, 157.37, 99.96, 95.80, 82.60, 79.50, 78.64, 78.44, 77.15, 76.09, 72.62, 71.87, 67.32, 63.13, 63.05, 60.18, 50.40, 49.32, 45.52, 45.00, 44.44, 42.79, 40.70 (2C), 38.89, 38.38, 38.33, 35.07, 31.08, 30.22, 24.66, 21.96, 21.56, 21.52, 21.06, 20.87, 20.02, 18.79, 18.32, 15.94, 14.33, 14.16, 10.33, 8.96; ; HRMS (ESI) calc'd for $C_{47}H_{79}N_5O_{15}$ + H = 954.5645, found 954.5630.



3.16-1

3.16-1: Intermediate **3.16** (3.2 g, 3.4 mmol) was added to a mixture of 20% HCl (40 mL) and MeOH (10 mL) in small portions allowing each portion to mix thoroughly before adding more. The resulting suspension was stirred at room temperature for 20 h. The solution was then basified to pH 10-12 with 2 N NaOH. This solution was extracted with EtOAc (3 x 100 mL). The combined organic fraction was washed with brine (100 mL), filtered over sodium sulfate, and solvent removed under reduced pressure. The product was purified by flash chromatography with a Combiflash instrument (MeOH/DCM(1 % NH_4OH) 0-10%) to give 2.0 g (80%) of cladinose removed product **3.16-1**. $[\alpha]^{23}_{D}$ -30.7 (c 2.0, $CHCl_3$); IR (neat)

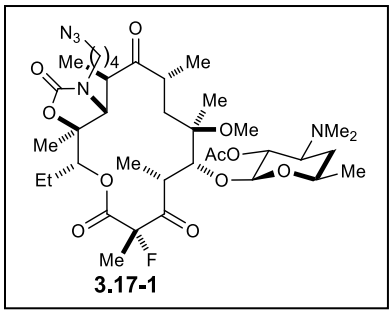
2972, 2096, 1735, 1456, 1375, 1235, 1164, 1055, 755, 667; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 – 4.96 (m, 1H), 4.78 – 4.70 (m, 1H), 4.58 (d, *J* = 7.6 Hz, 1H), 3.71 (d, *J* = 2.6 Hz, 1H), 3.65 (s, 1H), 3.62 – 3.54 (m, 2H), 3.50 – 3.43 (m, 1H), 3.39 (d, *J* = 10.5 Hz, 1H), 3.36 – 3.22 (m, 2H), 3.04 (q, *J* = 6.8 Hz, 1H), 2.92 (s, 3H), 2.74 – 2.63 (m, 2H), 2.58 – 2.45 (m, 1H), 2.23 (s, 6H), 2.16 (s, 1H), 2.04 (s, 3H), 2.00 (d, *J* = 7.3 Hz, 1H), 1.95 – 1.84 (m, 1H), 1.75 – 1.57 (m, 6H), 1.56 – 1.48 (m, 1H), 1.48 – 1.42 (m, 1H), 1.39 (s, 3H), 1.36 – 1.28 (m, 1H), 1.25 (s, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.20 (d, *J* = 6.1 Hz, 3H), 1.09 (d, *J* = 7.1 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 7.5 Hz, 3H), 0.80 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.75, 175.11, 169.90, 157.33, 99.80, 82.78, 80.43, 78.34, 77.37, 76.05, 71.37, 68.78, 63.11, 60.44, 50.95, 49.66, 45.71, 44.11, 42.98, 40.60 (2C), 38.78, 38.21, 35.79, 30.89, 26.31, 24.32, 22.11, 21.42, 21.06, 19.34, 18.81, 15.13, 14.26, 14.16, 10.20, 7.73; HRMS (ESI) calc'd for $C_{37}H_{63}N_5O_{11}$ + H = 754.45.97, found 754.4602.



3.17

3.17: Oxalylchloride (1.2 g, 9.6 mmol) and DCM (30 mL) were added to a flamed dried flask under an inert atmosphere. The solution was cooled to -78 °C and DMSO (1.5 g, 19.2 mmol) was added dropwise. The solution was allowed to stir for 45 minutes and then a solution of **3.16-1** (1.8 g, 2.4 mmol) in DCM (20 mL) was cannulated into the flask. This solution was then stirred for 1.5 hours at -78 °C. Triethylamine (1.9 g, 19.2 mmol) was added and the solution stirred for 30 minutes at -78 °C and then allowed to slowly warm to room temperature over 1.5 hours. Water (25 mL) was added to the solution and the mixture extracted with DCM (3 x 50 mL). The combined organic fraction was washed with

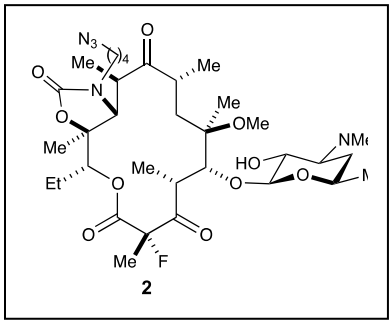
brine (50 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by flash chromatography on a Combiflash instrument (MeOH/DCM 0-10%) to give 1.3 g (70%) of C3-ketone **3.17**. $[\alpha]^{23}_{D}$ +17.5 (c 1.2, $CHCl_3$); IR (neat) 2979, 2100, 1744, 1455, 1376, 1215, 1163, 1061, 748, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (d, *J* = 8.7 Hz, 1H), 4.71 (dd, *J* = 10.4, 7.7 Hz, 1H), 4.33 (d, *J* = 7.6 Hz, 1H), 4.19 (d, *J* = 8.1 Hz, 1H), 3.79 (q, *J* = 6.7 Hz, 1H), 3.71 – 3.61 (m, 1H), 3.60 – 3.44 (m, 5H), 3.08 (q, *J* = 6.8 Hz, 1H), 3.04 – 2.93 (m, 1H), 2.69 – 2.62 (m, 1H), 2.62 (s, *J* = 13.0 Hz, 3H), 2.60 – 2.50 (m, 1H), 2.21 (s, *J* = 7.9 Hz, 6H), 2.03 (s, 3H), 1.98 – 1.87 (m, 1H), 1.82 – 1.58 (m, 7H), 1.57 – 1.48 (m, 2H), 1.45 (s, 3H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.29 (s, 3H), 1.22 (d, *J* = 6.0 Hz, 3H), 1.13 (t, *J* = 6.4 Hz, 6H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.00, 203.75, 169.70, 169.40, 157.10, 101.43, 82.03, 78.22, 78.05, 77.39, 71.45, 69.08, 63.30, 60.44, 51.15, 49.62, 46.95, 44.82, 44.30, 42.67, 40.54 (2C), 39.01, 38.93, 30.32, 29.99, 24.59, 22.24, 21.32, 20.89, 19.62, 18.29, 15.45, 14.66, 14.02, 13.84, 10.35; HRMS (ESI) calc'd for $C_{37}H_{61}N_5O_{11}$ + H = 752.4440, found 752.4438.



3.17-1

3.17-1: KO-*t*Bu (1.7 mL of 1 M in THF, 206.1 mg, 1.7 mmol) was added dropwise to a solution of **3.17** in THF (16 mL) at 0 °C under an inert atmosphere and stirred for 30 minutes. NFSI (410 mg, 1.3 mmol) was then added and the solution allowed to warm to room temperature stirring for 2 hours. Sat. aq. NH_4Cl (10 mL) was added and the mixture extracted with EtOAc (3 x 20 mL). The combined organic fractions were washed with brine (20 mL) and dried over

sodium sulfate. The solvent was removed under reduced pressure and the product purified by flash chromatography on a Combiflash instrument (MeOH/DCM 0-10%) to give 650 mg (65%) of **3.17-1**. $[\alpha]^{23}_{D}$ -3.2 (c 0.78, $CHCl_3$); IR (neat) 2980, 2100, 1744, 1372, 1214, 1108, 1062, 1001, 748, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (dd, *J* = 10.2, 2.1 Hz, 1H), 4.74 (dd, *J* = 10.6, 7.6 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.05 (d, *J* = 10.6 Hz, 1H), 3.72 – 3.42 (m, 4H), 3.42 (s, 1H), 3.34 – 3.21 (m, 2H), 3.09 (q, *J* = 7.0 Hz, 1H), 2.72 – 2.63 (m, 1H), 2.59 (d, *J* = 7.9 Hz, 1H), 2.56 (s, 3H), 2.25 (s, *J* = 9.9 Hz, 6H), 2.08 (s, 3H), 2.01 – 1.94 (m, 1H), 1.93 – 1.87 (m, 1H), 1.78 (d, *J* = 21.5 Hz, 3H), 1.73 – 1.56 (m, 8H), 1.49 (s, 3H), 1.32 (s, 3H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.20 – 1.14 (m, 6H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.54, 202.47 (d, *J* = 28.2 Hz), 169.77, 166.18 (d, *J* = 23.1 Hz), 157.05, 101.78, 97.88 (d, *J* = 205.8 Hz), 81.94, 79.58, 78.63, 78.58, 71.57, 69.22, 63.22, 60.88, 50.96, 49.14, 44.53, 43.01, 40.60 (2C), 39.28, 39.20, 30.44, 26.19, 25.16 (d, *J* = 22.3 Hz), 24.33, 22.41, 22.15, 21.39, 20.93, 19.67, 17.96, 14.69, 14.60, 13.73, 10.41; HRMS (ESI) calc'd for $C_{37}H_{60}FN_5O_{11}$ + H = 770.4346, found 770.4345.

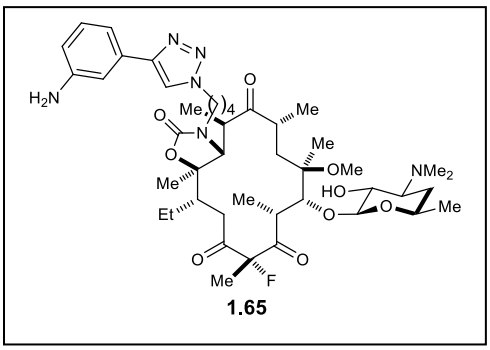


Azide 3.11. Intermediate **3.17-1** (650 mg, 0.85 mmol) was stirred in refluxing MeOH (26 mL) under an inert atmosphere for 3h. The solution was cooled to rt and the solvent removed under reduced pressure. The product was purified by flash chromatography on a Combiflash instrument (MeOH/DCM 0-10%) to give 520 mg (85%) of **3.11**. $[\alpha]^{23}_{D}$ +12.1 (c 1.3, $CHCl_3$); IR (neat) 2975, 2098, 1749, 1457, 1381, 1261, 1215, 1052, 1002, 749, 667; ^{1}H

NMR (500 MHz, $CDCl_3$) δ 4.86 (dd, *J* = 10.3, 2.0 Hz, 1H), 4.29 (d, *J* = 7.3 Hz, 1H), 4.08 – 4.04 (m, 1H), 3.66 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.60 – 3.47 (m, 3H), 3.41 (s, 1H), 3.33 – 3.22 (m, 2H), 3.17 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.09 (dd, *J* = 13.9, 6.9 Hz, 1H), 2.63 – 2.58 (m, 1H), 2.57 (s, 3H), 2.47 – 2.39 (m, 1H), 2.25 (s, 6H), 1.96 (ddd, *J* = 14.5, 7.5, 2.5 Hz, 1H), 1.87 (dd, *J* = 14.5, 2.7 Hz, 1H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.69 – 1.56 (m, 6H), 1.53 (d, *J* = 12.7 Hz, 1H), 1.48 (s, 3H), 1.34 (s, 3H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.22 (d, *J* = 6.1 Hz, 3H), 1.19 (s, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.93, [203.42, 203.14 (d, *J* = 28.3 Hz)], [166.78, 166.55 (d, *J* = 23.2 Hz)], 157.45, 104.57, [99.12, 97.07 (d, *J* = 206.1 Hz)], 82.35, 81.01, 78.91, 78.87, 70.70, 70.00, 66.15, 61.23, 51.33, 49.52, 44.98, 43.38, 41.13, 40.60 (2C), 39.92, 39.58, 28.45, 26.56, [25.72, 25.50 (d, *J* = 22.4 Hz)], 24.68, 22.50, 21.52, 20.10 18.28, 15.40, 15.08, 14.13, 10.77; HRMS (ESI) calc'd for $C_{35}H_{58}FN_5O_{10}$ + H = 728.4240, found 728.4231.

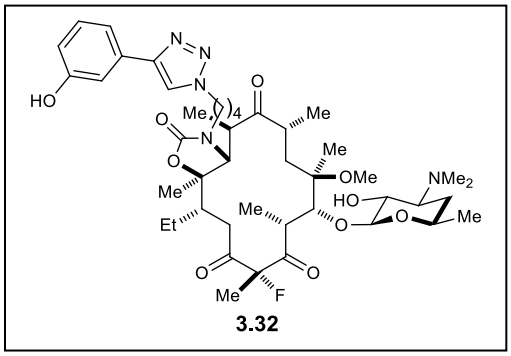
Experimental Procedure for Copper(I)-catalyzed Click Reactions:

Azide **2** (45 mg, 0.062 mmol, 1 eq.), $CuSO_4$ (1.24 µmol, 0.02 eq.), (+)-sodium L-ascorbate (6.2 µmol, 0.1 eq.), and alkyne **3.12, 3.18-3.31** (0.124 mmol, 2 eq.) in 1:1 water:*t*-BuOH (1.24 mL, 0.05 M) was stirred at rt for 24 hours. Water (2 mL) was added and the mixture extracted with EtOAc (3 x 5 mL). The combined organic fractions were washed with brine (5 mL), dried over sodium sulfate, and the solvent removed under reduced pressure. The product was purified by flash chromatography with a Combiflash instrument (MeOH/DCM (1% NH_4OH) 0-10%) to give triazoles **1.65, 3.32-3.45** (70-90% yield).



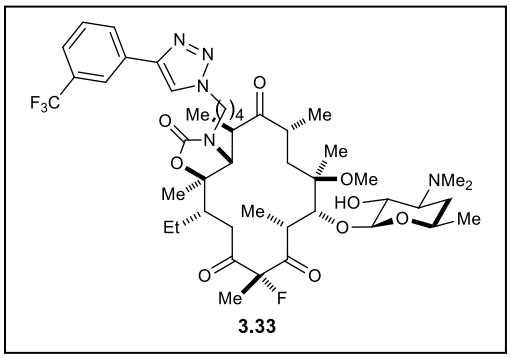
1.65

Solithromycin (1.65): $[\alpha]^{23}_D$ +12.6 (c 1.5, $CHCl_3$); IR (neat) 2980, 2360, 2341, 1750, 1457, 1374, 1261, 1162, 1109, 1078, 1051, 1003, 754, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.27 (d, *J* = 1.7 Hz, 1H), 7.20 – 7.14 (m, 2H), 6.66 – 6.61 (m, 1H), 4.87 (dd, *J* = 10.4, 2.0 Hz, 1H), 4.41 (td, *J* = 7.1, 1.4 Hz, 2H), 4.29 (d, *J* = 7.3 Hz, 1H), 4.03 (ddd, *J* = 18.4, 11.3, 3.7 Hz, 2H), 3.80 – 3.70 (m, 2H), 3.68 – 3.58 (m, 1H), 3.57 – 3.46 (m, 2H), 3.42 (s, 1H), 3.17 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 6.9 Hz, 1H), 2.65 – 2.56 (m, 1H), 2.54 (s, 3H), 2.48 – 2.41 (m, 1H), 2.26 (s, 6H), 2.02 – 1.92 (m, 3H), 1.87 (dd, *J* = 14.5, 2.7 Hz, 1H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.72 – 1.61 (m, 5H), 1.52 (d, *J* = 14.1 Hz, 1H), 1.49 (s, 3H), 1.33 (s, *J* = 8.6 Hz, 3H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.23 (d, *J* = 6.1 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.56, [202.97, 202.68 (d, *J* = 28.3 Hz)], [166.56, 166.33 (d, *J* = 23.2 Hz)], 157.20, 147.82, 146.82, 131.70, 129.63, 119.68, 116.13, 114.72, 112.35, 104.21, [98.79, 96.74 (d, *J* = 206.1 Hz)], 82.11, 80.70, 78.57, 78.52, 70.33, 69.62, 65.79, 61.03, 49.70, 49.20 (2C), 44.56, 42.75, 40.83, 40.22, 39.54, 39.18, 28.11, 27.58, [25.31, 25.09 (d, *J* = 22.5 Hz)], 24.25, 22.12, 21.14, 19.74, 17.88, 15.02, 14.69, 13.75, 10.45; HRMS (ESI) calc'd for $C_{43}H_{65}FN_6O_{10}$ + H = 845.4824, found 845.4817.



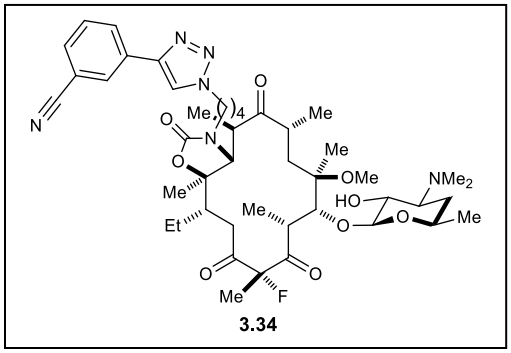
3.32

Triazole 3.32: $[\alpha]^{23}_{D}$ +12.7 (c 1.07, $CHCl_3$); IR (neat) 2972, 2978, 2361, 2341, 1750, 1559, 1489, 1260, 1163, 1078, 1052, 1003, 725, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.42 – 7.39 (m, 2H), 7.29 (t, *J* = 8.1 Hz, 1H), 6.88 – 6.84 (m, 1H), 4.88 (dd, *J* = 10.2, 2.1 Hz, 1H), 4.53 – 4.33 (m, 2H), 4.29 (d, *J* = 7.3 Hz, 1H), 4.02 (d, *J* = 9.5 Hz, 1H), 3.81 (dt, *J* = 13.8, 6.7 Hz, 1H), 3.68 – 3.60 (m, 1H), 3.60 – 3.48 (m, 3H), 3.43 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.11 (q, *J* = 6.9 Hz, 1H), 2.64 – 2.57 (m, *J* = 12.2, 6.9, 2.8 Hz, 1H), 2.51 (s, 3H), 2.50 – 2.41 (m, 1H), 2.27 (s, 6H), 2.03 – 1.92 (m, 3H), 1.88 (dd, *J* = 14.5, 2.7 Hz, 1H), 1.79 (d, *J* = 21.4 Hz, 3H), 1.72 – 1.59 (m, 5H), 1.54 – 1.40 (m, 4H), 1.33 – 1.28 (m, 6H), 1.22 (d, *J* = 6.1 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.98, [204.75, 204.46 (d, *J* = 28.5 Hz)], [166.98, 166.75 (d, *J* = 22.8 Hz)], 157.63, 156.90, 148.04, 132.44, 130.52, 120.13, 118.36, 115.57, 113.31, 104.59, [99.15, 97.10 (d, *J* = 206.3 Hz)], 82.52, 81.19, 79.14, 79.01, 70.69, 70.00, 66.17, 61.74, 50.29, 49.57, 44.91, 43.23, 41.38, 40.59 (2C), 39.96, 39.64, 28.53, 27.73, [25.71, 25.48 (d, *J* = 22.9 Hz)], 24.98, 22.51, 21.51, 20.07, 18.26, 15.49, 15.15, 14.17, 10.85; HRMS (ESI) calc'd for $C_{43}H_{64}FN_5O_{11}$ + H = 846.4664, found 846.4665.



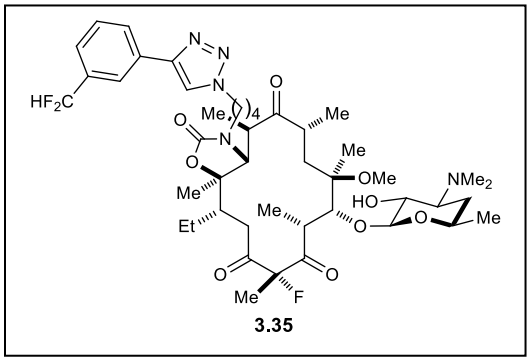
3.33

Triazole 3.33 $[\alpha]^{23}_D$ +8.5 (c 1.0, $CHCl_3$); IR (neat) 2972, 2361, 2341, 1751, 1489, 1323, 1261, 1165, 1126, 1072, 1052, 1003, 763, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 8.05 (m, 2H), 7.96 (s, 1H), 7.60 – 7.50 (m, 2H), 4.85 (dd, *J* = 10.4, 2.0 Hz, 1H), 4.45 (t, *J* = 7.4 Hz, 2H), 4.29 (d, *J* = 7.3 Hz, 1H), 4.05 (dd, *J* = 10.6, 1.3 Hz, 1H), 3.79 – 3.72 (m, 1H), 3.68 – 3.60 (m, 1H), 3.57 – 3.47 (m, 2H), 3.42 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 6.9 Hz, 1H), 2.64 – 2.57 (m, 1H), 2.52 (s, 3H), 2.48 – 2.41 (m, 1H), 2.26 (s, 6H), 2.02 – 1.93 (m, 3H), 1.87 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.74 (d, *J* = 21.4 Hz, 3H), 1.71 – 1.62 (m, 4H), 1.54 – 1.51 (m, 1H), 1.50 – 1.48 (m, 3H), 1.32 (s, 3H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.23 (d, *J* = 6.1 Hz, 3H), 1.22 – 1.19 (m, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 217.02, δ 202.99 (d, *J* = 28.1 Hz), 166.90 (d, *J* = 23.3 Hz), 157.59, 146.77, 132.03, 129.63, 129.31, 124.44 (q, *J* = 273.2 Hz), 124.88, 122.88, 120.69 (2C), 104.58, 98.15 (d, *J* = 206.2 Hz), 82.51, 81.01, 78.95, 78.89, 70.70, 69.99, 66.19, 61.27, 50.24, 49.56, 44.95, 43.05, 41.21, 40.60 (2C), 39.91, 39.55, 28.51, 27.98, 25.52 (d, *J* = 22.5 Hz), 24.57, 22.49, 21.51, 20.11, 18.26, 15.37, 15.05, 14.12, 10.82; HRMS (ESI) calc'd for $C_{44}H_{63}F_4N_5O_{11}$ + H = 898.4589, found 898.4640.

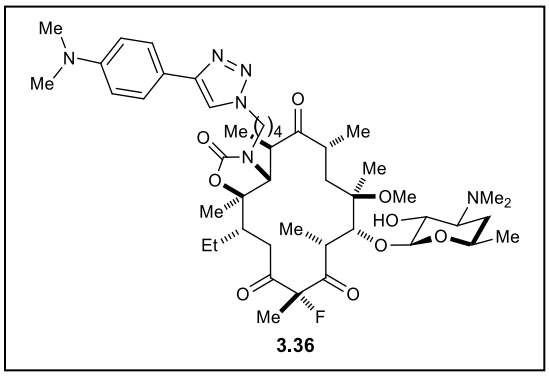


3.34

Triazole 3.34 $[\alpha]^{23}_{D}$ +8.7 (c 1.06, $CHCl_3$); IR (neat) 2972, 2941, 2360, 2341, 1751, 1457, 1262, 1161, 1109, 1078, 1052, 759, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.94 (s, 1H), 7.70 (s, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 4.88 (dd, *J* = 10.4, 2.1 Hz, 1H), 4.47 (t, *J* = 7.4 Hz, 2H), 4.32 (d, *J* = 7.3 Hz, 1H), 4.07 (dd, *J* = 10.7, 1.3 Hz, 1H), 3.82 – 3.73 (m, 1H), 3.70 – 3.61 (m, 1H), 3.54 (qd, *J* = 10.5, 4.9 Hz, 3H), 3.44 (s, 1H), 3.20 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.12 (q, *J* = 7.0 Hz, 1H), 2.67 – 2.59 (m, *J* = 12.0, 7.2 Hz, 1H), 2.55 (s, 3H), 2.51 – 2.42 (m, 1H), 2.29 (s, 6H), 2.05 – 1.95 (m, 3H), 1.93 – 1.86 (m, 1H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.73 – 1.61 (m, 5H), 1.58 – 1.52 (m, *J* = 14.5 Hz, 1H), 1.51 (s, 3H), 1.35 (s, 3H), 1.32 (d, *J* = 7.1 Hz, 3H), 1.25 (d, *J* = 6.1 Hz, 3H), 1.19 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.61, 202.66 (d, *J* = 28.2 Hz), 166.46 (d, *J* = 23.2 Hz), 157.22, 146.94, 133.09, 131.20, 128.95, 128.12, 126.14, 124.07, 120.11 (2C), 104.14, 97.75 (d, *J* = 206.0 Hz), 82.13, 80.64, 78.56, 78.48, 70.29, 69.55, 65.85, 60.92, 49.80, 49.18, 44.56, 42.67, 40.81, 40.23 (2C), 39.52, 39.17, 28.24, 27.59, 25.17 (d, *J* = 22.3 Hz), 24.18, 22.11, 21.13, 19.73, 17.87, 15.00, 14.66, 13.74, 10.45; HRMS (ESI) calc'd for $C_{44}H_{63}FN_6O_{10}$ + H = 855.4668, found 855.4652.

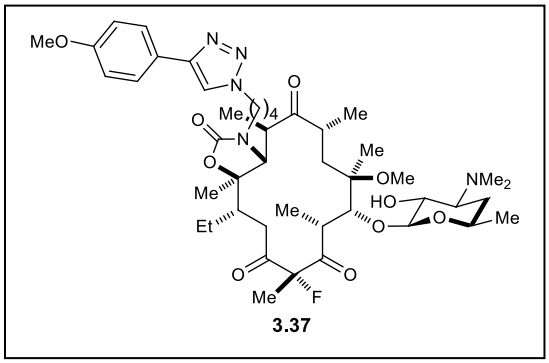


Triazole 3.35 $[\alpha]^{23}_{D}$ +17 (c 0.92, $CHCl_3$); IR (neat) 2978, 2943, 2356, 2341, 1750, 1710, 1455, 1251, 1167, 1052, 1003, 755, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 – 7.96 (m, *J* = 3.8, 2.6 Hz, 2H), 7.91 (s, 1H), 7.54 – 7.43 (m, 2H), 6.69 (t, *J* = 56.4 Hz, 1H), 4.87 (dd, *J* = 10.2, 2.3 Hz, 1H), 4.45 (t, *J* = 7.3 Hz, 2H), 4.30 (d, *J* = 7.3 Hz, 1H), 4.06 (dd, *J* = 10.6, 1.4 Hz, 1H), 3.80 – 3.71 (m, 1H), 3.69 – 3.61 (m, 1H), 3.57 – 3.48 (m, 922H), 3.44 (s, 1H), 3.19 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.11 (q, *J* = 7.0 Hz, 1H), 2.65 – 2.58 (m, 1H), 2.53 (s, 3H), 2.51 – 2.44 (m, 1H), 2.29 (s, 6H), 2.04 – 1.93 (m, 3H), 1.87 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.75 (d, *J* = 21.4 Hz, 3H), 1.72 – 1.61 (m, 4H), 1.57 – 1.51 (m, 1H), 1.50 (s, 3H), 1.33 (s, 3H), 1.31 (d, *J* = 7.1 Hz, 3H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.22 – 1.20 (m, 1H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.62, 202.68 (d, *J* = 28.2 Hz), 166.49 (d, *J* = 23.2 Hz), 157.21, 146.77, 134.89, 131.42, 129.20, 127.94, 124.83, 123.00, 120.13, 114.62 (t, *J* = 238.9 Hz), 104.20, 97.76 (d, *J* = 206.0 Hz), 82.12, 80.64, 78.56, 78.49, 77.25, 77.00, 76.75, 70.32, 69.63, 65.78, 60.91, 49.82, 49.18, 44.56, 42.68, 40.83, 40.22 (2C), 39.52, 39.17, 28.08, 27.59, 25.17 (d, *J* = 22.4 Hz), 24.20, 22.10, 21.14, 19.73, 17.88, 15.01, 14.67, 13.75, 10.45, -0.03, -18.36; HRMS (ESI) calc'd for $C_{44}H_{64}F_3N_5O_{11}$ + H = 880.4653, found 880.4654.



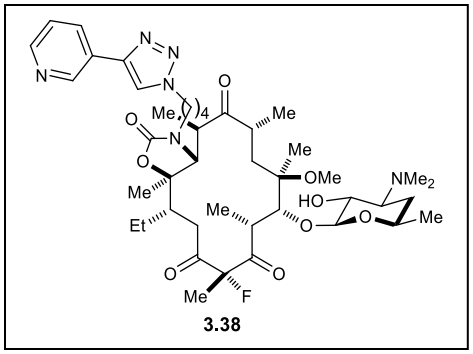
3.36

Triazole 3.36 $[\alpha]^{23}_{D}$ +9.8(c 0.61, $CHCl_3$); IR (neat) 2980, 2360, 2341, 1756, 1559, 1261, 1162, 1109, 1078, 1052, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 – 7.69 (m, 3H), 6.79 – 6.74 (m, 2H), 4.88 (dd, *J* = 10.4, 2.1 Hz, 1H), 4.40 (t, *J* = 7.5 Hz, 2H), 4.30 (d, *J* = 7.3 Hz, 1H), 4.06 (dd, *J* = 10.7, 1.2 Hz, 1H), 3.79 – 3.70 (m, 1H), 3.68 – 3.59 (m, 1H), 3.59 – 3.46 (m, 2H), 3.43 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 6.9 Hz, 1H), 2.98 (s, 6H), 2.66 – 2.59 (m, 1H), 2.56 (s, 3H), 2.49 – 2.40 (m, 1H), 2.26 (s, 6H), 2.02 – 1.92 (m, 3H), 1.88 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.78 (d, *J* = 21.4 Hz, 3H), 1.73 – 1.59 (m, 4H), 1.56 – 1.51 (m, 1H), 1.50 (s, 3H), 1.34 (s, 3H), 1.31 (d, *J* = 7.0 Hz, 3H), 1.23 (d, *J* = 6.1 Hz, 3H), 1.20 (s, *J* = 6.0 Hz, 1H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.52, 202.71 (d, *J* = 28.2 Hz), 166.43 (d, *J* = 23.3 Hz), 157.20, 150.30, 148.18, 126.68 (2C), 119.19, 118.19, 112.50 (2C), 104.19, 97.78 (d, *J* = 206.3 Hz), 82.09, 80.68, 78.57, 70.33, 69.60, 65.84, 60.95, 49.62, 49.24, 44.57, 42.78, 40.83, 40.51 (2C), 40.23 (2C), 39.55, 39.19, 34.10, 28.16, 27.66, 25.22 (d, *J* = 22.6 Hz), 24.22, 22.13, 21.15, 19.76, 17.89, 15.02, 14.69, 13.76, 10.47; HRMS (ESI) calc'd for $C_{45}H_{69}FN_6O_{10}$ + 2H = 437.2607, found 437.2605.



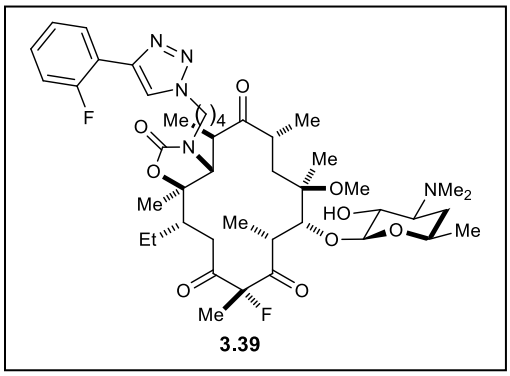
3.37

Triazole 3.37 $[\alpha]^{23}_{D}$ +5.6 (c 1.5, $CHCl_3$); IR (neat) 2972, 2940, 2360, 2341, 1755, 1709, 1457, 1379, 1250, 1162, 1108, 1078, 1051, 1003, 755, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.74 (m, 3H), 6.97 – 6.92 (m, 2H), 4.87 (dd, *J* = 10.4, 2.0 Hz, 1H), 4.41 (t, *J* = 7.4 Hz, 2H), 4.29 (t, *J* = 6.6 Hz, 1H), 4.07 – 4.04 (m, 1H), 3.83 (s, 3H), 3.79 – 3.70 (m, 1H), 3.67 – 3.59 (m, 1H), 3.58 – 3.47 (m, 2H), 3.43 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 6.9 Hz, 1H), 2.65 – 2.57 (m, 1H), 2.54 (s, 3H), 2.49 – 2.41 (m, 1H), 2.26 (s, 6H), 2.01 – 1.92 (m, 3H), 1.88 (dd, *J* = 14.5, 2.7 Hz, 1H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.73 – 1.60 (m, 4H), 1.55 – 1.51 (m, 1H), 1.49 (s, 3H), 1.33 (s, 3H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.25 (d, *J* = 1.7 Hz, 1H), 1.23 (d, *J* = 6.1 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.89 – 0.83 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.56, 202.65 (d, *J* = 28.2 Hz), 166.45 (d, *J* = 23.1 Hz), 159.41, 157.19, 147.56, 127.04 (2C), 123.54, 118.91, 114.12 (2C), 104.01, 97.76 (d, *J* = 206.0 Hz), 82.10, 80.63, 78.54, 70.22, 69.40, 65.92, 60.95, 55.28, 49.66, 49.20, 44.55, 42.73, 40.80, 40.25 (2C), 39.50, 39.17, 29.67, 28.48, 27.59, 25.19 (d, *J* = 22.3 Hz), 24.21, 22.11, 21.09, 19.73, 17.87, 15.00, 14.67, 13.74, 10.45; HRMS (ESI) calc'd for $C_{44}H_{66}FN_5O_{11}$ + 2H = 430.7450, found 430.7451.



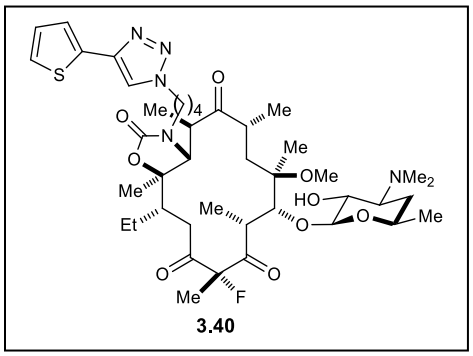
3.38

Triazole 3.38 $[\alpha]^{23}_{D}$ +9.0 (c 3.6, $CHCl_3$); IR (neat) 2972, 2940, 2360, 2341, 1752, 1708, 1457, 1379, 1261, 1109, 1078, 1051, 1003, 754, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.56 (s, 1H), 8.24 (dt, *J* = 7.9, 1.7 Hz, 1H), 7.96 (s, 1H), 7.37 (dd, *J* = 7.6, 5.0 Hz, 1H), 4.86 (dd, *J* = 10.5, 2.1 Hz, 1H), 4.52 – 4.41 (m, 2H), 4.30 (d, *J* = 7.3 Hz, 1H), 4.07 – 4.03 (m, 1H), 3.80 – 3.73 (m, 1H), 3.68 – 3.59 (m, 1H), 3.57 – 3.48 (m, 2H), 3.42 (s, 1H), 3.19 (dd, *J* = 10.1, 7.3 Hz, 1H), 3.10 (q, *J* = 7.0 Hz, 1H), 2.65 – 2.56 (m, 1H), 2.51 (s, 3H), 2.48 (d, *J* = 9.7 Hz, 1H), 2.29 (s, 6H), 2.03 – 1.93 (m, 3H), 1.87 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.75 (d, *J* = 21.4 Hz, 3H), 1.72 – 1.59 (m, 4H), 1.54 – 1.50 (m, 1H), 1.50 (s, 3H), 1.32 (s, 3H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.21 (d, *J* = 6.0 Hz, 1H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.62, 202.56 (d, *J* = 28.2 Hz), 166.49 (d, *J* = 23.4 Hz), 157.17, 148.99, 147.08, 144.62, 133.04, 123.66, 120.17, 104.06, 97.73 (d, *J* = 206.2 Hz), 82.11, 80.56, 78.52, 78.43, 70.24, 69.48, 65.80, 60.90, 49.86, 49.11, 44.53, 42.65, 40.77, 40.21 (2C), 39.46, 39.12, 28.27, 27.54, 25.16 (d, *J* = 22.2 Hz), 24.19, 22.07, 21.10, 19.69, 17.84, 14.94, 14.63, 13.72, 10.44; HRMS (ESI) calc'd for $C_{42}H_{63}FN_6O_{10}$ + H = 831.4647, found 831.4668.



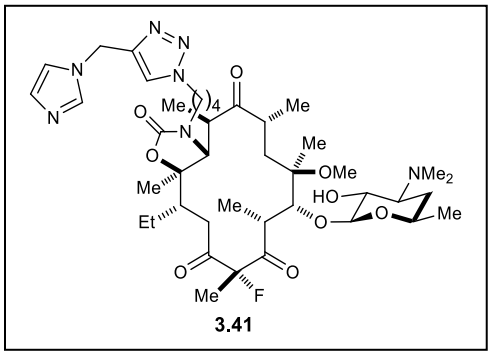
3.39

Triazole 3.39 $[\alpha]^{23}_{D}$ +7.1 (c 0.21, $CHCl_3$); IR (neat) 2972, 2940, 2360, 2341, 1754, 1709, 1457, 1379, 1260, 1233, 1162, 1108, 1077, 1051, 1003, 759, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 – 8.26 (m, 1H), 7.96 (t, *J* = 3.8 Hz, 1H), 7.31 – 7.21 (m, 2H), 7.14 – 7.07 (m, 1H), 4.86 (dd, *J* = 10.4, 2.0 Hz, 1H), 4.44 (t, *J* = 7.5 Hz, 2H), 4.30 (d, *J* = 7.3 Hz, 1H), 4.06 (dd, *J* = 10.7, 1.3 Hz, 1H), 3.79 – 3.69 (m, 1H), 3.68 – 3.59 (m, 1H), 3.57 – 3.47 (m, 2H), 3.42 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 7.0 Hz, 1H), 2.65 – 2.57 (m, 1H), 2.54 (s, 3H), 2.48 – 2.41 (m, 1H), 2.26 (s, 6H), 2.03 – 1.94 (m, 3H), 1.87 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.74 (d, *J* = 21.4 Hz, 3H), 1.71 – 1.60 (m, 4H), 1.55 – 1.51 (m, 1H), 1.49 (s, 3H), 1.33 (s, 3H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.20 (t, *J* = 3.0 Hz, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.51, 202.63 (d, *J* = 28.2 Hz), 166.34 (d, *J* = 23.4 Hz), 159.16 (d, *J* = 247.8 Hz), 157.15, 141.03 (d, *J* = 2.5 Hz), 129.00 (d, *J* = 8.3 Hz), 127.78 (d, *J* = 3.6 Hz), 124.44 (d, *J* = 3.0 Hz), 122.75 (d, *J* = 12.6 Hz), 118.72 (d, *J* = 13.1 Hz), 115.52 (d, *J* = 21.8 Hz), 104.04, 97.73 (d, *J* = 206.2 Hz), 82.04, 80.60, 78.50, 78.44, 70.23, 69.45, 65.83, 60.78, 49.76, 49.16, 44.50, 42.71, 40.77, 40.21(2C), 39.47, 39.12, 28.29, 27.67, 25.09 (d, *J* = 22.2 Hz), 24.12, 22.05, 21.08, 19.70, 17.84, 14.96, 14.62, 13.69, 10.34; HRMS (ESI) calc'd for $C_{43}H_{63}F_2N_5O_{10}$ + H = 848.4621, found 848.4615.



3.40

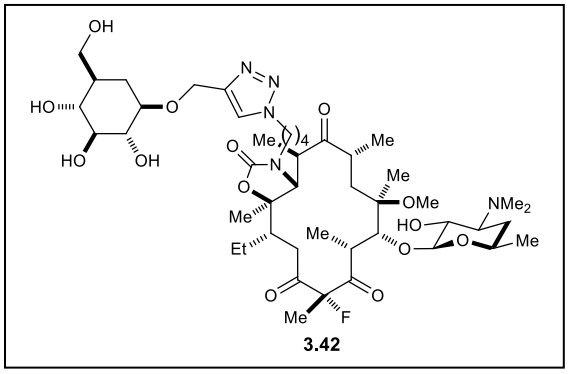
Triazole 3.40: $[\alpha]^{23}_{D}$ +2.0 (c 0.4, $CHCl_3$); IR (neat) 2971, 2360, 2341, 1750, 1653, 1464, 1260, 1078, 1052, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.39 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.28 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.07 (dd, *J* = 5.0, 3.6 Hz, 1H), 4.87 (dd, *J* = 10.3, 2.1 Hz, 1H), 4.42 (t, *J* = 7.4 Hz, 2H), 4.30 (d, *J* = 7.3 Hz, 1H), 4.06 (dd, *J* = 10.7, 1.3 Hz, 1H), 3.81 – 3.70 (m, 1H), 3.63 (dd, *J* = 14.9, 7.2 Hz, 1H), 3.58 – 3.48 (m, 2H), 3.43 (s, 1H), 3.19 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.10 (q, *J* = 7.1 Hz, 1H), 2.67 – 2.57 (m, 1H), 2.54 (s, 3H), 2.51 – 2.42 (m, 1H), 2.28 (s, 6H), 1.97 (ddt, *J* = 14.9, 9.6, 4.9 Hz, 3H), 1.88 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.64 (ddd, *J* = 14.4, 12.6, 9.5 Hz, 4H), 1.53 (s, 1H), 1.50 (s, 3H), 1.34 (s, 3H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 6.2 Hz, 3H), 1.21 (s, 1H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); HRMS (ESI) calc'd for $C_{41}H_{62}FN_5O_{10}S$ + H = 836.4279, found 836.4258.



3.41

Triazole 3.41: $[\alpha]^{23}_{D}$ +11.8 (c 0.69, $CHCl_3$); IR (neat) 2971, 2940, 2359, 1754, 1457, 1380, 1283, 1262, 1109, 1078, 1051, 1003, 763; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.42 (s, 1H), 7.05 (d, *J* = 11.2 Hz, 2H), 5.34 (q, *J* = 15.6 Hz, 2H), 4.79 (dd, *J* = 10.3, 2.1 Hz, 1H), 4.49 – 4.40 (m, 1H), 4.31 – 4.24 (m, 2H), 4.02 (dd, *J* = 10.7, 1.2 Hz, 1H), 3.76 – 3.68 (m, 1H), 3.63 – 3.49 (m, 3H), 3.35 (s, 1H), 3.18 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.07 (q, *J* = 7.0 Hz, 1H), 2.63 – 2.54 (m, 1H), 2.50 – 2.42 (m, 1H), 2.33 (s, 3H), 2.27 (s, 6H), 1.97 (ddd, *J* = 14.5, 7.5, 2.5 Hz, 1H), 1.92 – 1.83 (m, 3H), 1.79 (d, *J* = 21.3 Hz,

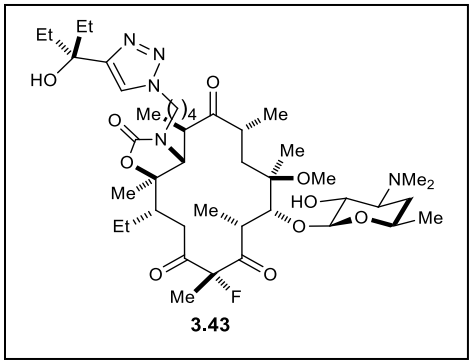
3H), 1.70 – 1.52 (m, 5H), 1.48 (s, 3H), 1.32 (s, 3H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.25 (d, *J* = 6.1 Hz, 3H), 1.21 (s, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.61, 202.87 (d, *J* = 28.4 Hz), 166.57 (d, *J* = 23.4 Hz), 157.14, 143.74, 137.21, 129.84, 122.19, 119.09, 104.25, 97.64 (d, *J* = 206.8 Hz), 82.11, 80.74, 78.57, 78.49, 70.31, 69.63, 65.83, 60.76, 50.06, 48.97, 44.55, 42.52, 42.40, 40.73, 40.24 (2C), 39.54, 39.14, 28.17, 27.53, 25.41 (d, *J* = 22.5 Hz), 24.33, 22.14, 21.17, 19.69, 17.83, 15.04, 14.65, 13.76, 10.56; HRMS (ESI) calc'd for $C_{41}H_{64}FN_7O_{10}$ + H = 834.4777, found 834.4755.



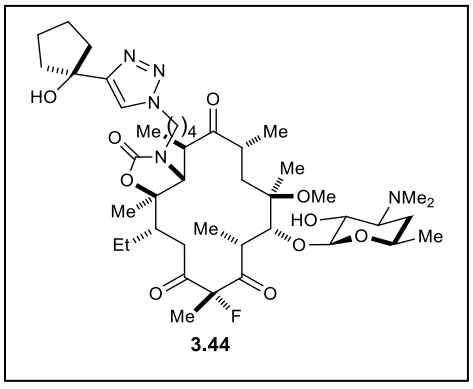
3.42

Triazole 3.42: $[\alpha]^{23}_D$ -2.5 (c 13.6, $CHCl_3$); IR (neat) 3384, 2971, 2939, 2360, 2340, 1750, 1457, 1262, 1161, 1106, 1078, 1051, 1003, 761, 668; ^{1}H NMR (400 MHz, MeOH) δ 8.01 (s, 1H), 5.04 (d, *J* = 12.5 Hz, 1H), 4.87 – 4.80 (m, 1H), 4.54 – 4.35 (m, 6H), 4.10 (d, *J* = 10.5 Hz, 1H), 3.88 (t, *J* = 10.4 Hz, 2H), 3.82 – 3.63 (m, 4H), 3.63 – 3.48 (m, 2H), 3.45 – 3.34 (m, 2H), 3.28 – 3.14 (m, 2H), 2.87 (s, 1H), 2.71 (s, 6H), 2.57 (s, 1H), 2.34 (s, 3H), 2.04 – 1.83 (m, 5H), 1.78 (d, *J* = 21.5 Hz, 3H), 1.74 – 1.57 (m, 3H), 1.54 (s, 3H), 1.51 – 1.39 (m, 2H), 1.37 – 1.25 (m, 9H), 1.21 (d, *J* = 6.7 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, MeOH) δ 218.03, 203.85 (d, *J* = 28.4 Hz), 167.80 (d, *J* = 23.2 Hz), 159.23, 145.77, 125.53, 104.24 (d, *J* = 99.4 Hz), 102.08, 99.27 (d, *J* = 205.4 Hz), 84.03, 81.20, 80.06, 79.75 (d, *J* = 8.5 Hz), 78.02 (d, *J* = 6.3 Hz), 76.20, 75.06, 74.86, 71.61, 70.81, 69.76, 66.43, 62.77 (dd, *J* = 47.5, 40.8 Hz), 56.52, 50.97, 49.96, 49.64,

49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 45.84, 43.85, 42.03, 40.52, 40.40, 40.08, 31.25, 30.73, 28.70, 25.55 (d, J = 25.9 Hz), 25.42, 23.34, 21.24, 20.42, 18.04, 15.50, 14.93, 14.06, 11.00; HRMS (ESI) calc'd for $C_{44}H_{72}FN_5O_{16}$ + H = 946.5036, found 946.5017.

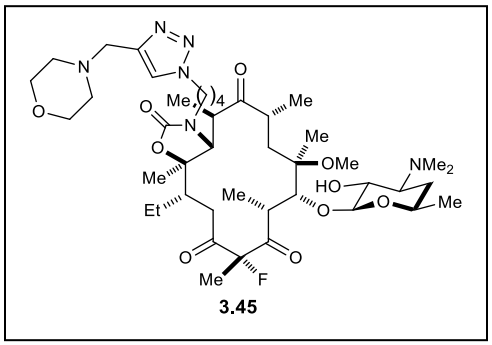


Triazole 3.43: $[\alpha]^{23}_D$ +14.4 (c 0.9, $CHCl_3$); IR (neat) 2971, 2939, 2880, 2360, 2341, 1751, 1457, 1375, 1261, 1161, 1109, 1078, 1052, 1003, 970, 753, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (s, 1H), 4.82 (dd, J = 10.4, 2.1 Hz, 1H), 4.45 – 4.37 (m, 1H), 4.36 – 4.27 (m, 2H), 4.07 – 4.02 (m, 1H), 3.74 – 3.66 (m, 1H), 3.61 – 3.46 (m, 3H), 3.38 (s, 1H), 3.19 (dd, J = 10.0, 7.4 Hz, 1H), 3.07 (q, J = 6.8 Hz, 1H), 2.64 – 2.57 (m, 1H), 2.52 – 2.47 (m, 1H), 2.45 (s, 3H), 2.28 (s, 6H), 1.99 – 1.82 (m, 8H), 1.77 (d, J = 21.4 Hz, 3H), 1.73 – 1.51 (m, 5H), 1.48 (s, 3H), 1.32 (s, 3H), 1.29 (d, J = 7.0 Hz, 3H), 1.24 (d, J = 5.9 Hz, 3H), 1.22 – 1.20 (m, 1H), 1.16 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H), 0.83 (t, J = 7.3 Hz, 6H); HRMS (ESI) calc'd for $C_{42}H_{70}FN_5O_{11}$ + H = 840.5134, found 840.5152.



Triazole 3.44: $[\alpha]^{23}_D$ +8.5 (c 1.77, $CHCl_3$); IR (neat) 2970, 2941, 2361, 2341, 1752, 1457, 1379, 1261, 1162, 1108, 1078, 1052, 1003, 755, 668; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (s, 1H), 4.79 (dd, J = 10.4, 2.1 Hz, 1H), 4.46 (dt, J = 12.8, 6.2 Hz, 1H), 4.31 – 4.20 (m, 2H), 4.02 (dd, J = 10.7, 1.2 Hz, 1H), 3.75

– 3.66 (m, 1H), 3.62 – 3.55 (m, 1H), 3.55 – 3.46 (m, 2H), 3.34 (s, *J* = 19.3 Hz, 1H), 3.16 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.06 (q, *J* = 6.9 Hz, 1H), 2.63 – 2.53 (m, 1H), 2.48 – 2.38 (m, 1H), 2.31 (s, 3H), 2.26 (s, *J* = 6.8 Hz, 6H), 2.23 – 2.13 (m, 3H), 2.11 – 2.03 (m, 1H), 2.03 – 1.91 (m, 4H), 1.91 – 1.83 (m, 4H), 1.77 (d, *J* = 21.4 Hz, 3H), 1.63 (ddt, *J* = 17.7, 14.6, 5.2 Hz, 4H), 1.52 – 1.42 (m, 1H), 1.47 (s, 3H), 1.30 (s, 3H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.24 (d, *J* = 6.1 Hz, 3H), 1.20 (s, 1H), 1.16 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.55, 202.98 (d, *J* = 28.4 Hz), 166.65 (d, *J* = 23.1 Hz), 157.05, 154.52, 120.06, 104.20, 97.54 (d, *J* = 206.7 Hz), 82.04, 80.57, 78.81, 78.54, 70.30, 69.63, 65.78, 60.76, 49.91, 48.92, 44.55, 42.64, 41.15 (2C), 40.70, 40.21(2C), 39.49, 39.10, 28.12, 27.61, 25.36 (d, *J* = 22.5 Hz), 24.60, 23.66 (2C), 22.07, 21.12, 19.64, 17.80, 14.95, 14.59, 13.74, 10.52; HRMS (ESI) calc'd for $C_{42}H_{68}FN_5O_{11}$ + H = 838.4977, found 838.4997.



3.45

Triazole 3.45: $[\alpha]^{23}_D$ +10.4 (c 1.44, $CHCl_3$); IR (neat) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (s, *J* = 8.5 Hz, 1H), 4.84 (dd, *J* = 10.3, 2.0 Hz, 1H), 4.43 – 4.31 (m, 2H), 4.29 (t, *J* = 7.0 Hz, 1H), 4.05 (dd, *J* = 10.6, 1.1 Hz, 1H), 3.72 – 3.69 (m, 4H), 3.68 (s, 2H), 3.62 – 3.57 (m, 1H), 3.57 – 3.48 (m, 3H), 3.40 (s, 1H), 3.17 (dd, *J* = 10.2, 7.3 Hz, 1H), 3.09 (q, *J* = 7.0 Hz, 1H), 2.64 – 2.57 (m, 1H), 2.51 (s, 4H), 2.48 (s, 3H), 2.46 – 2.41 (m, 1H), 2.26 (s, 6H), 2.01 – 1.83 (m, 4H), 1.77 (d, *J* = 21.3 Hz, 3H), 1.70 – 1.57 (m, 4H), 1.49 (s, 3H), 1.54 – 1.44 (m, 1H), 1.33 (s, 3H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 6.2 Hz, 3H), 1.20 (s, *J* = 4.3 Hz, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.88

(t, J = 7.4 Hz, 3H);^{13}C NMR (101 MHz, $CDCl_3$) δ 216.58, 202.74 (d, J = 28.2 Hz), 166.42 (d, J = 23.0 Hz), 157.17, 144.02, 122.68, 104.22, 97.73 (d, J = 206.3 Hz), 82.10, 80.64, 78.56, 78.45, 70.33, 69.64, 66.87 (2C), 65.79, 60.85, 53.66, 53.38 (2C), 49.71, 49.12, 44.57, 42.70, 40.79, 40.21(2C), 39.53, 39.15, 29.67, 27.62, 25.26 (d, J = 22.6 Hz), 24.23, 22.13, 21.16, 19.74, 17.87, 15.03, 14.66, 13.74, 10.48; HRMS (ESI) calc'd for $C_{42}H_{69}FN_6O_{11}$ + H = 853.5086, found 853.5105.

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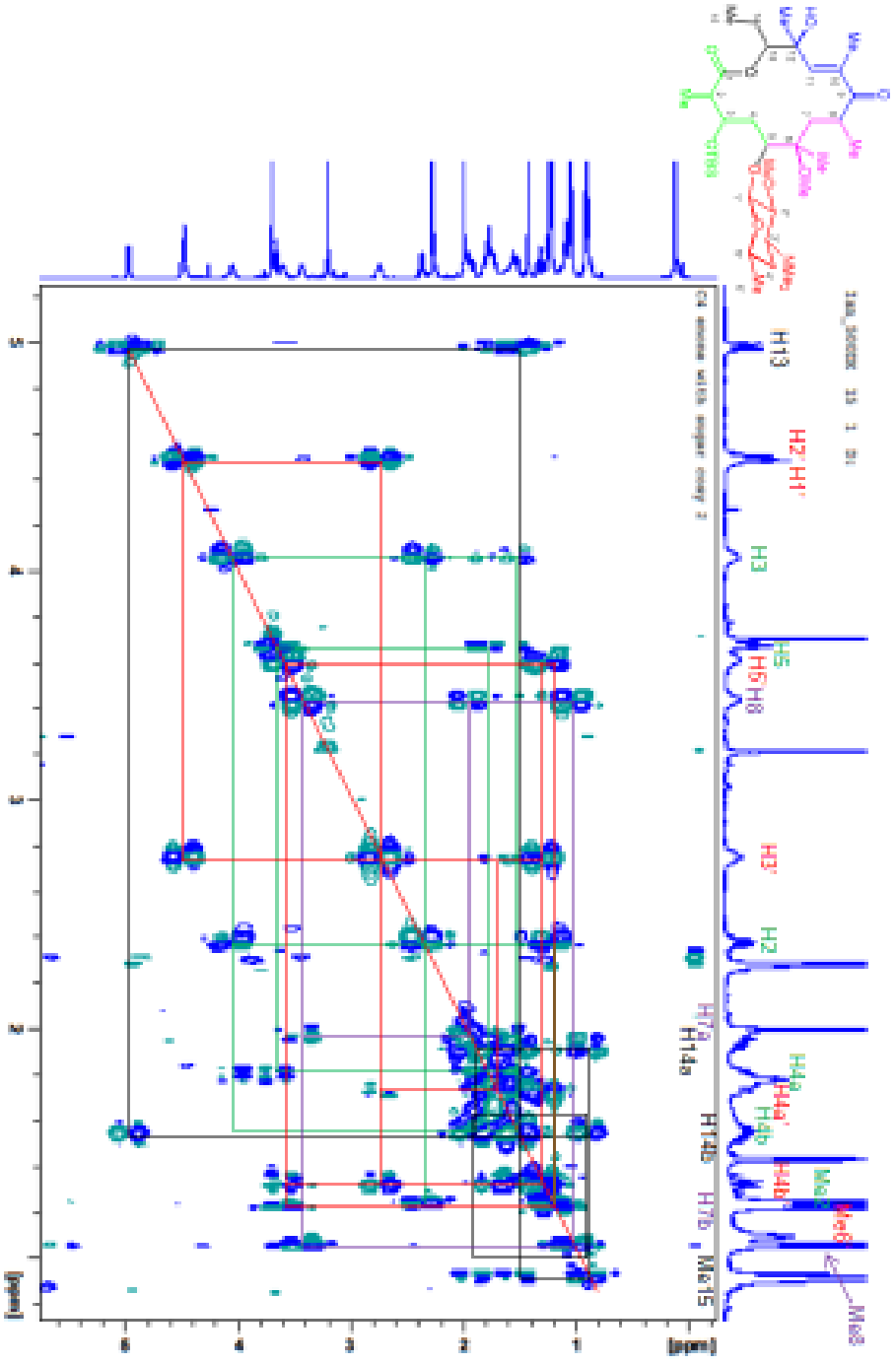
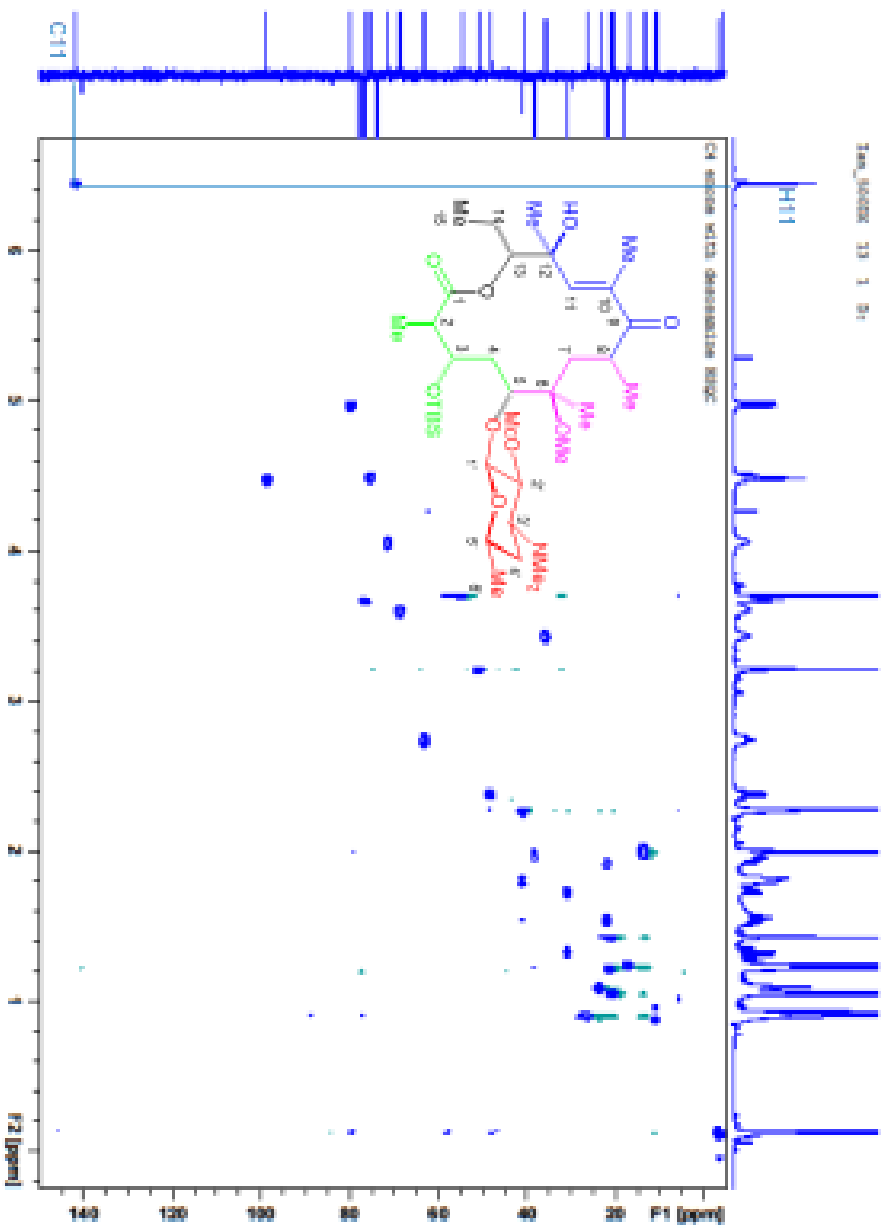
APPENDIX

A1. Structural Assignments for Macroketolactone 2.72

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H11
F2 [ppm]
F1 [ppm]
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120
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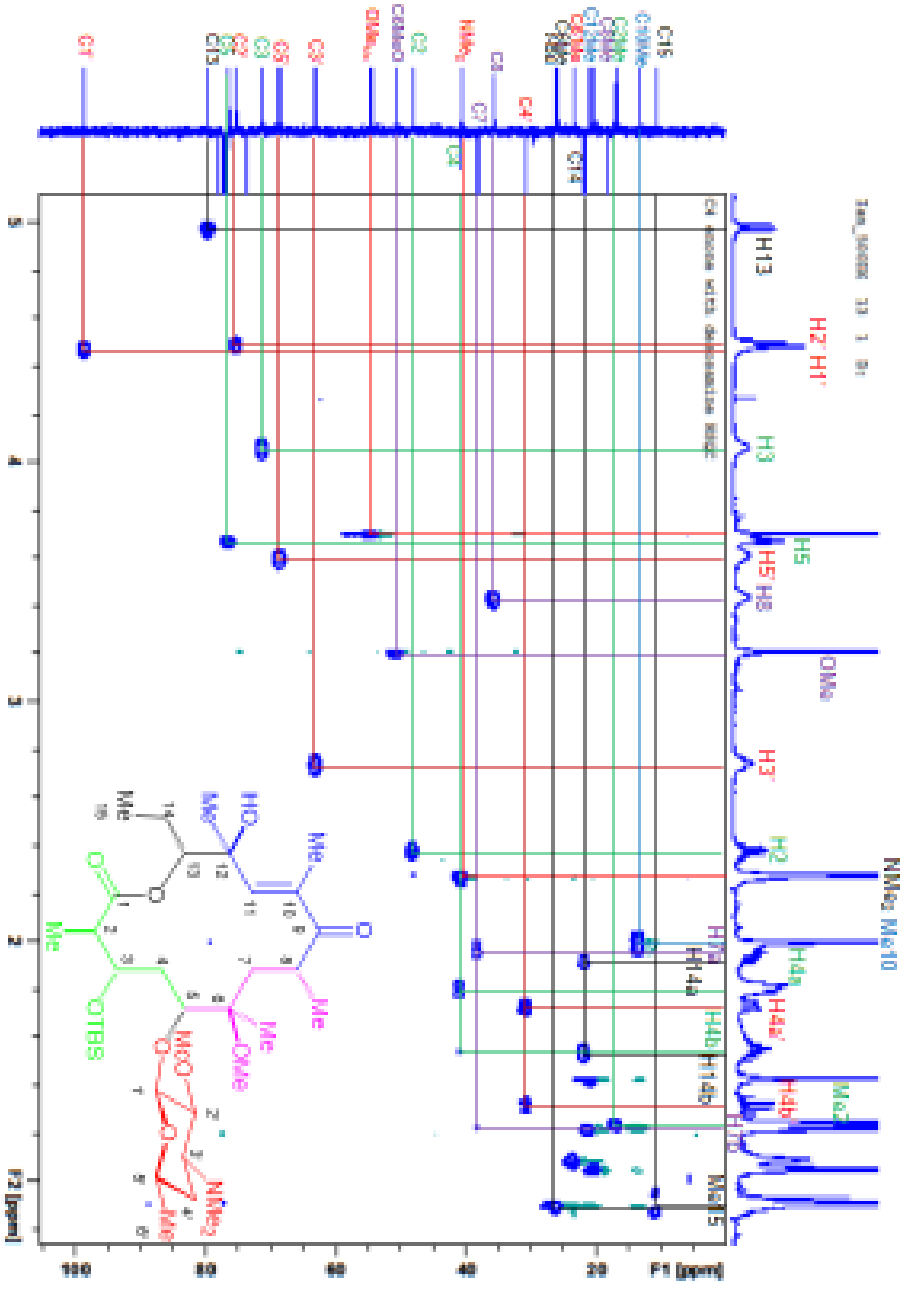
60
40
20
0
1
2
3
4
5
6
OTBS
OMe
NMe2
Me
HO


F1 [ppm]
100
80
60
40
20
H13
H2,H1'
H3
H5
H5,H8
OMe
H7
H2
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H4a'
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Me15
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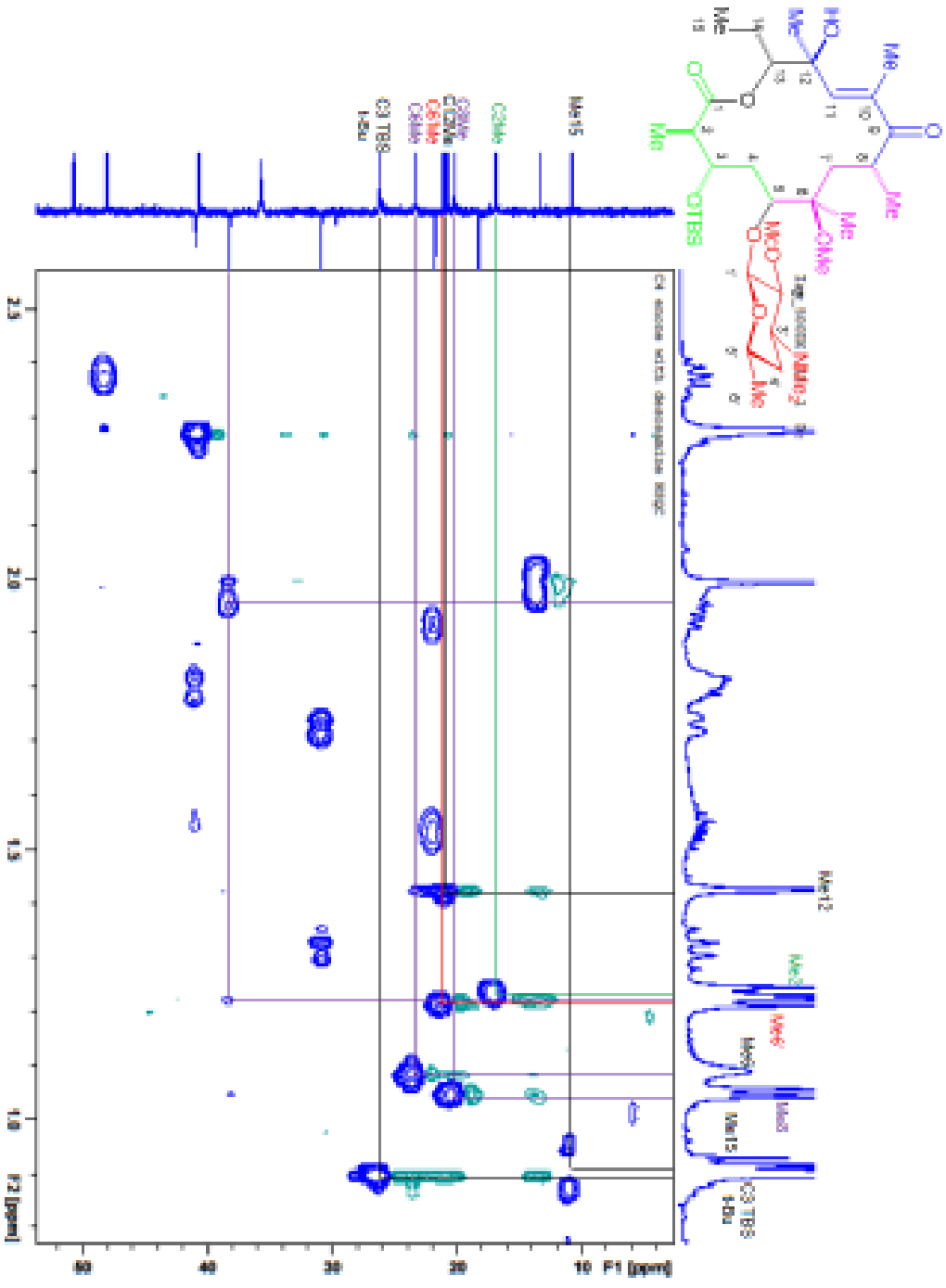
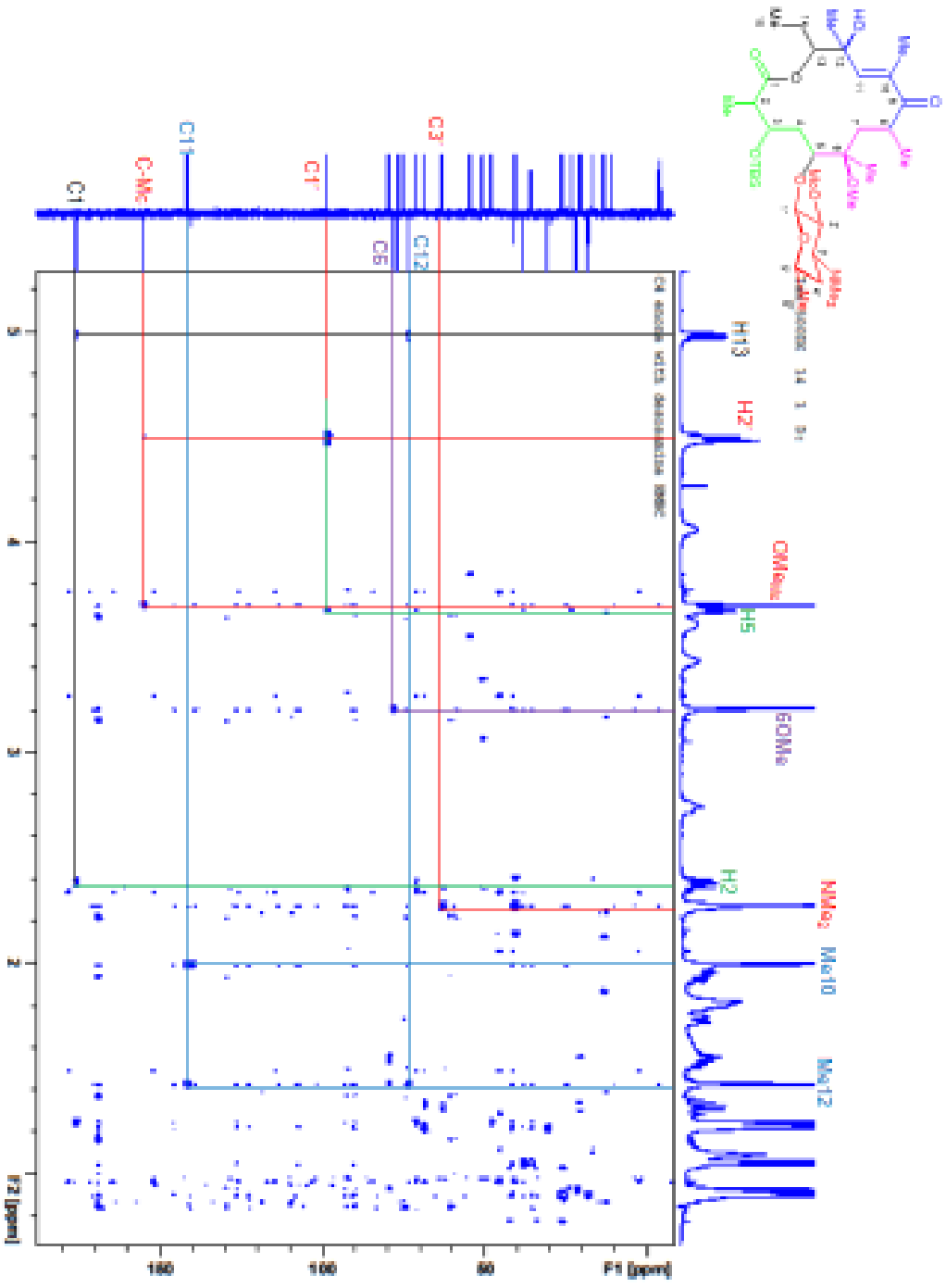
Me
HO
O

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C-Me
C11
C7
C3
C5
C12
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OMe
H5
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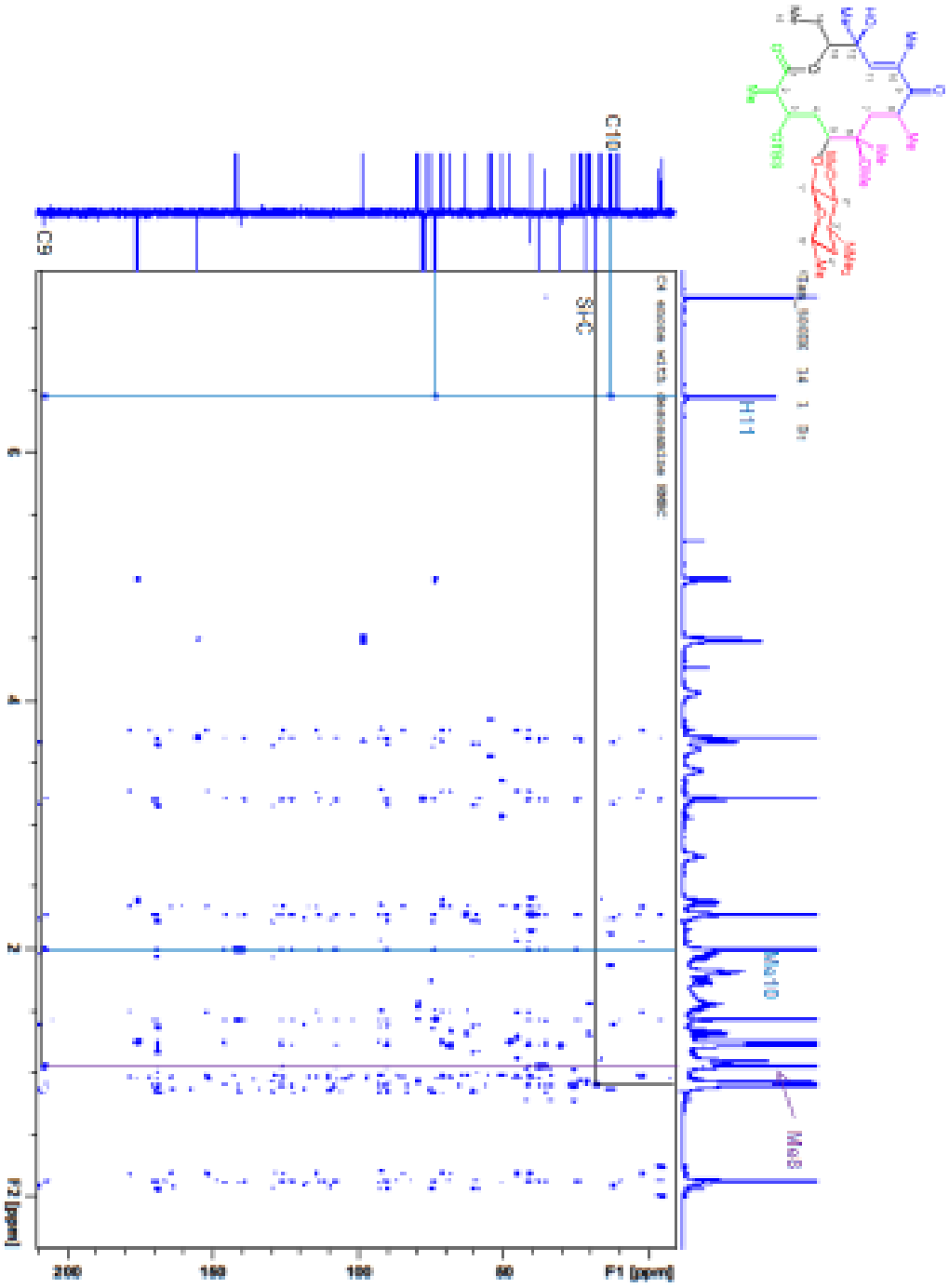
H2
NMe2
Me10
Me12
F2 [ppm]
100
F1 [ppm]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Me8
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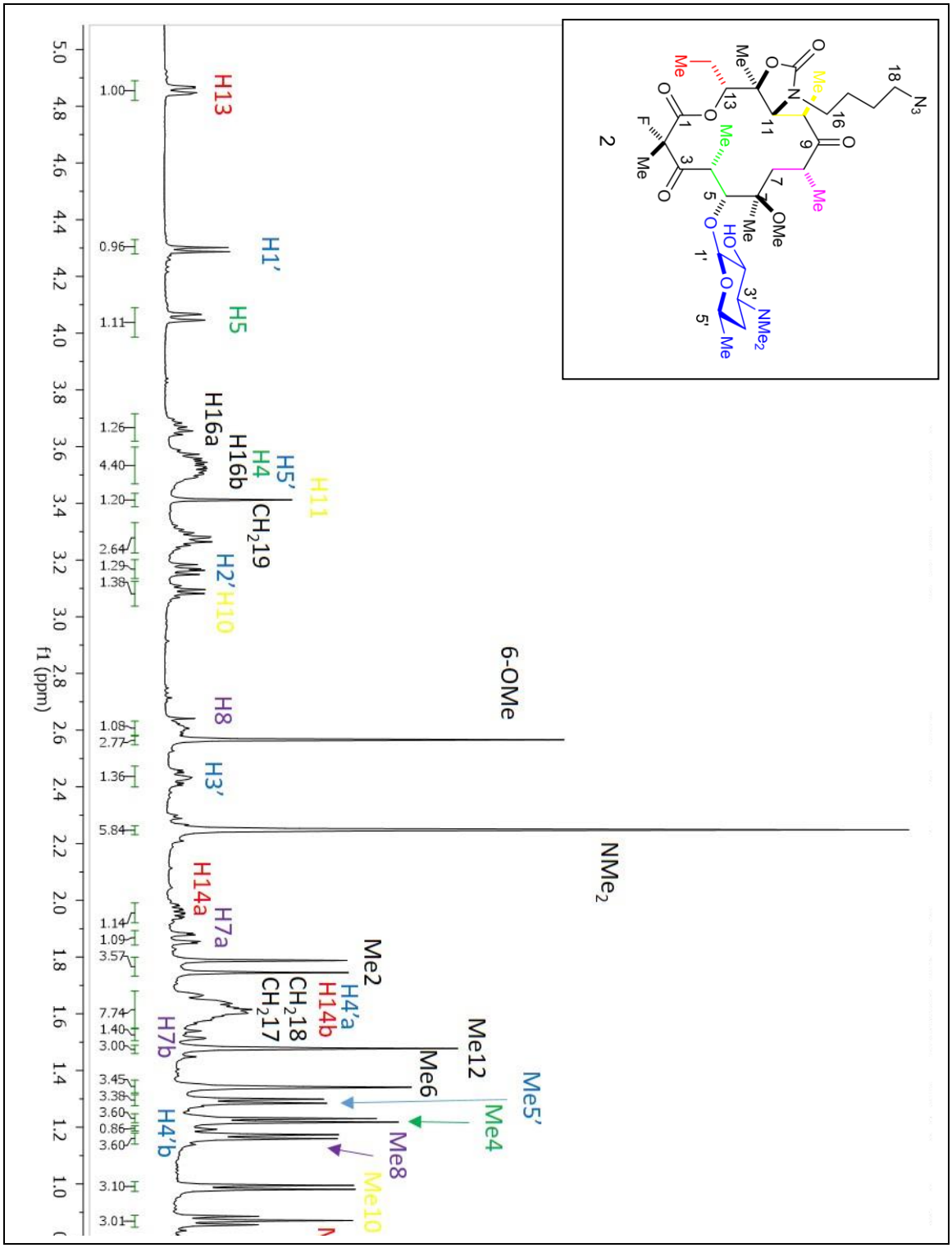
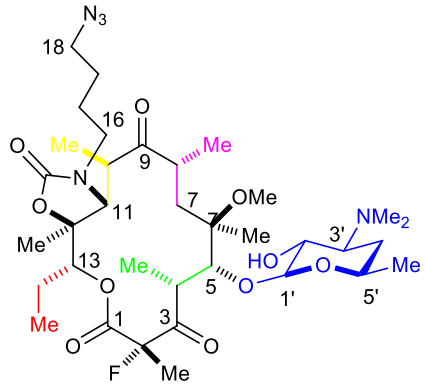
F1 [ppm]

A2. Structural Assignments for Azide 3.11

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18
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11
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OMe
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Me
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5'
Me
1
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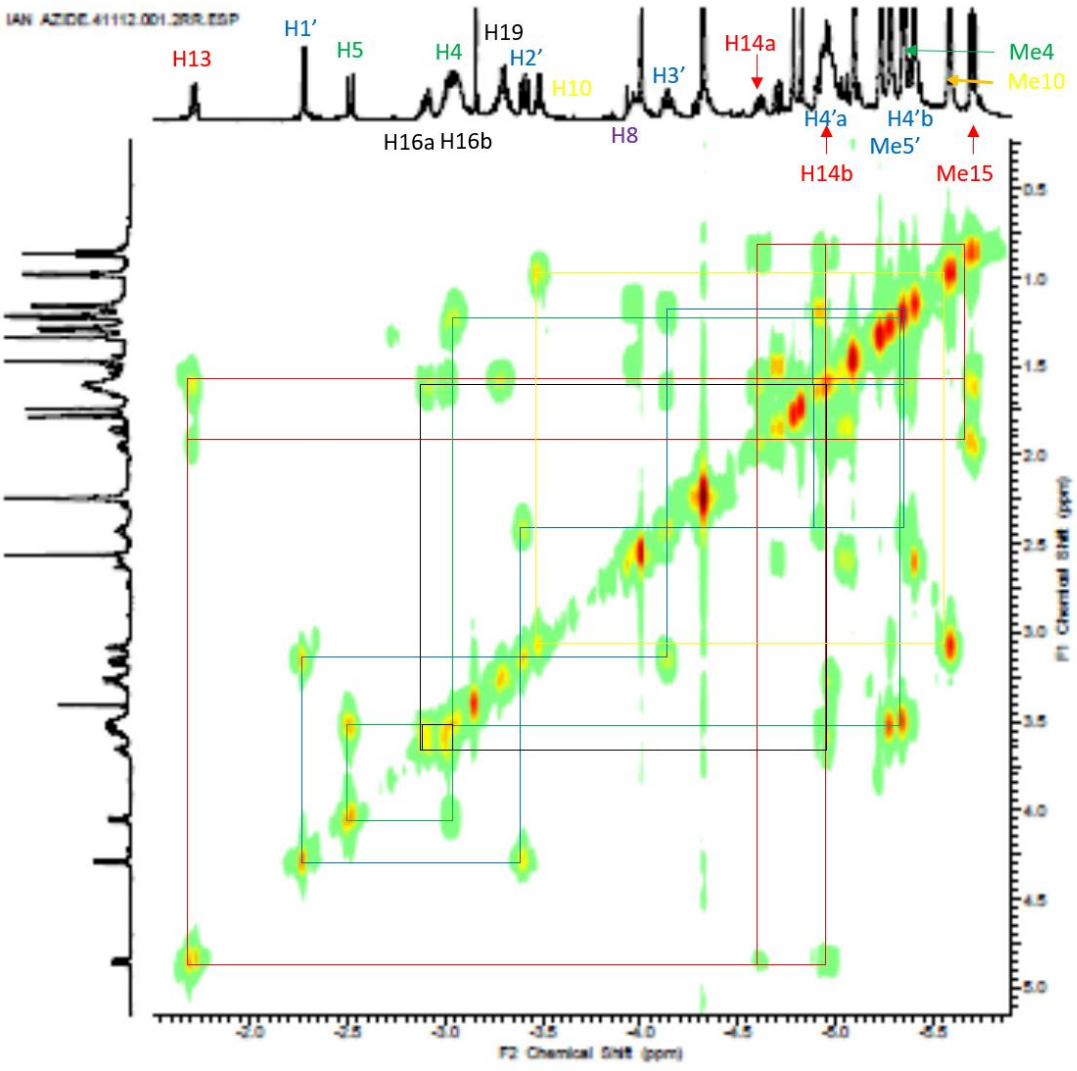
O
F
Me

2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H4
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H10
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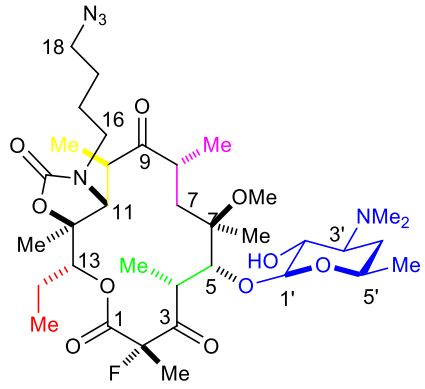
Me5'
H14b
Me15
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)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Me
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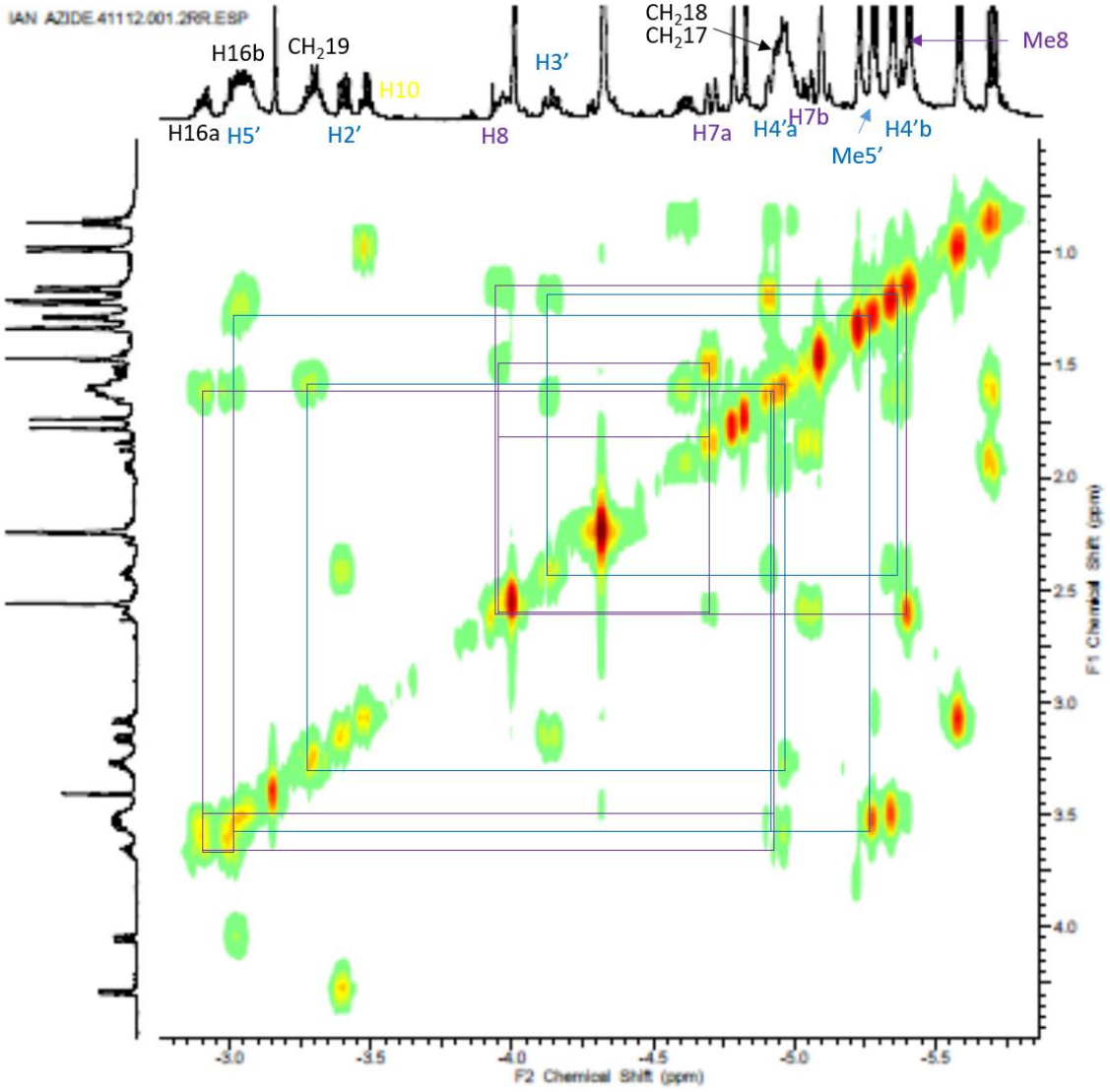
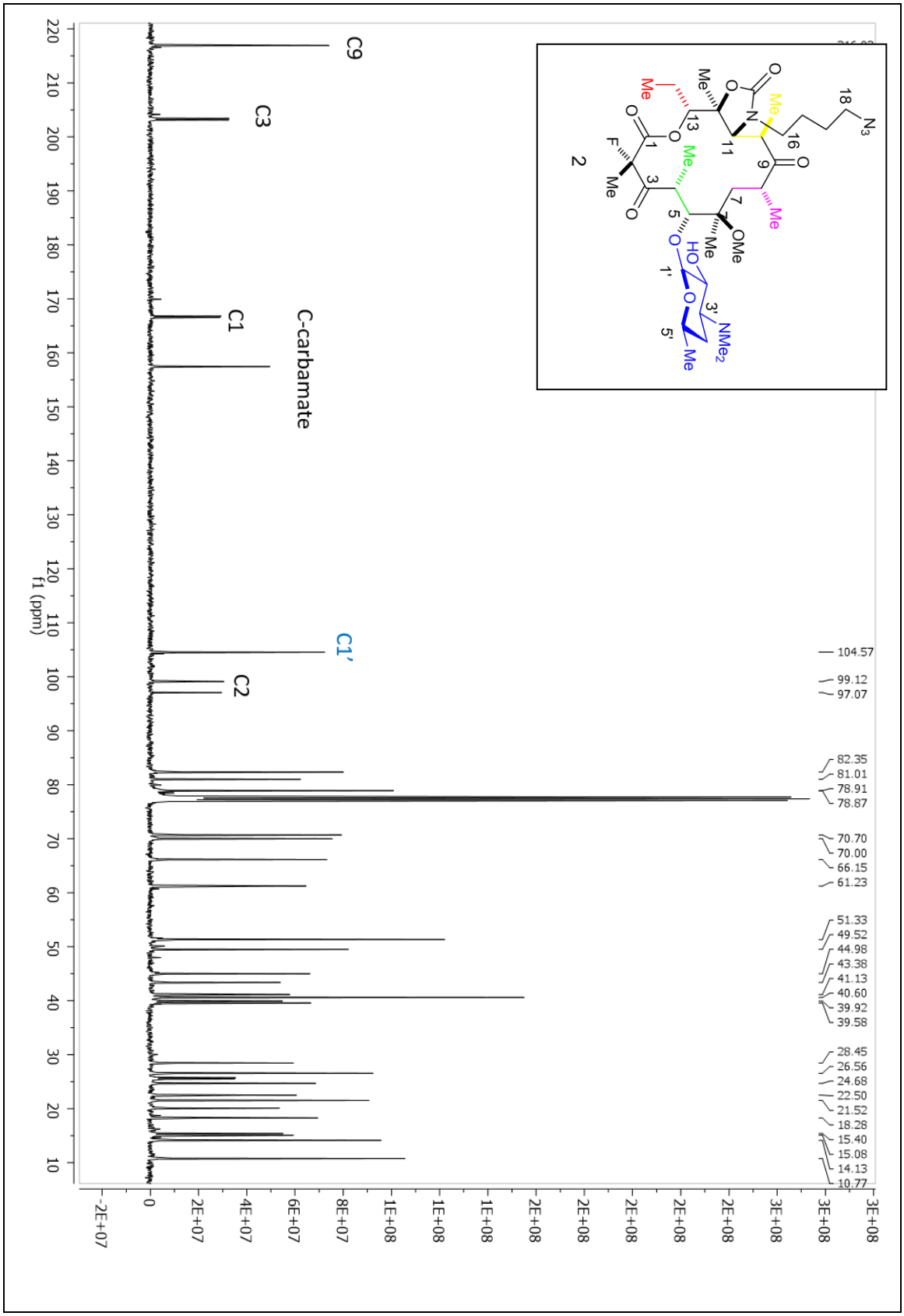
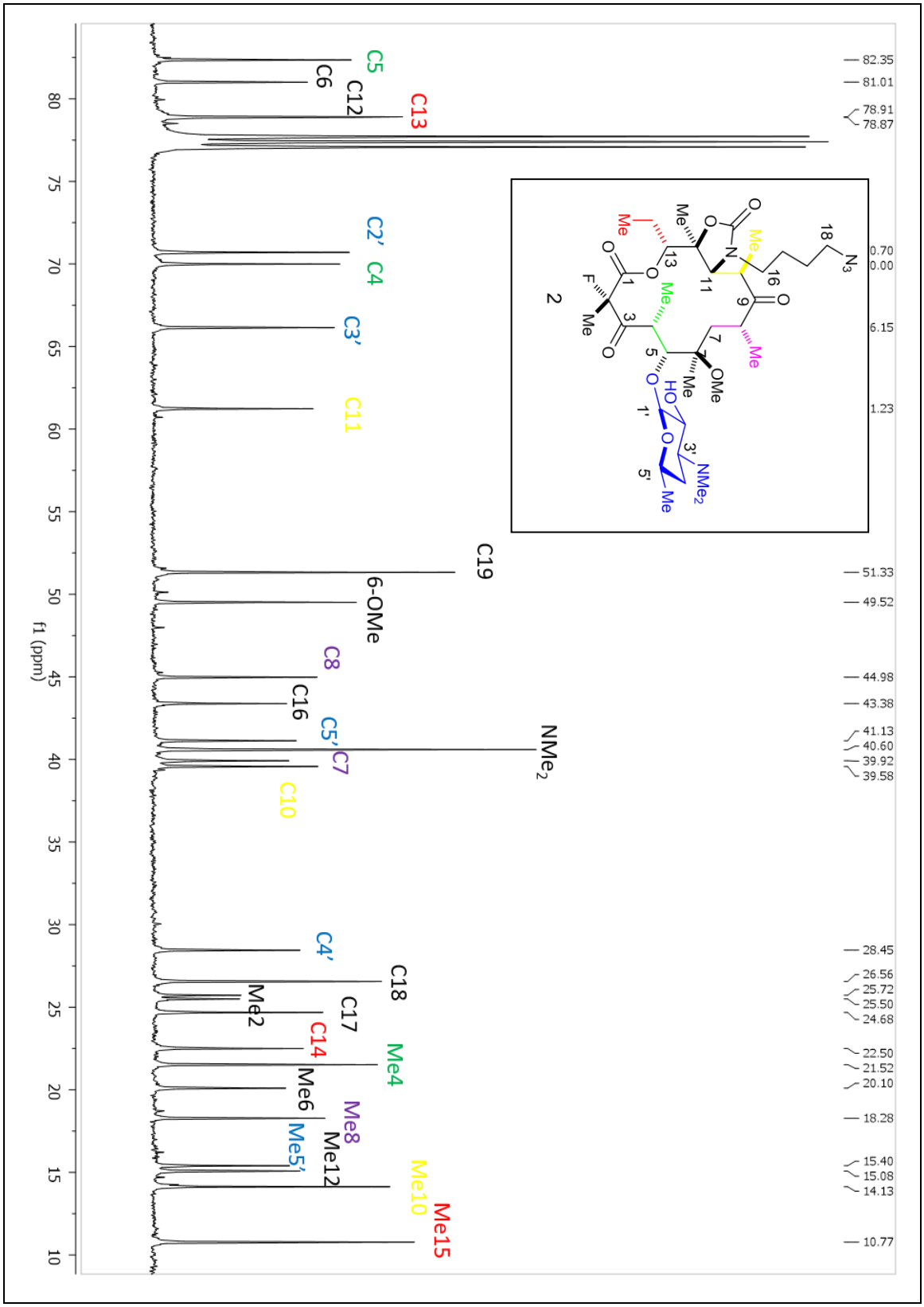
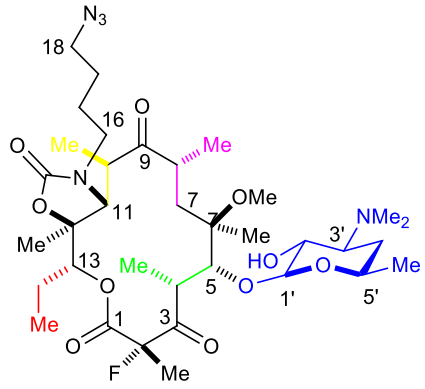
HO
O
Me
Me
O
5
O
1'
5'
1
3
O
O
F
Me

2

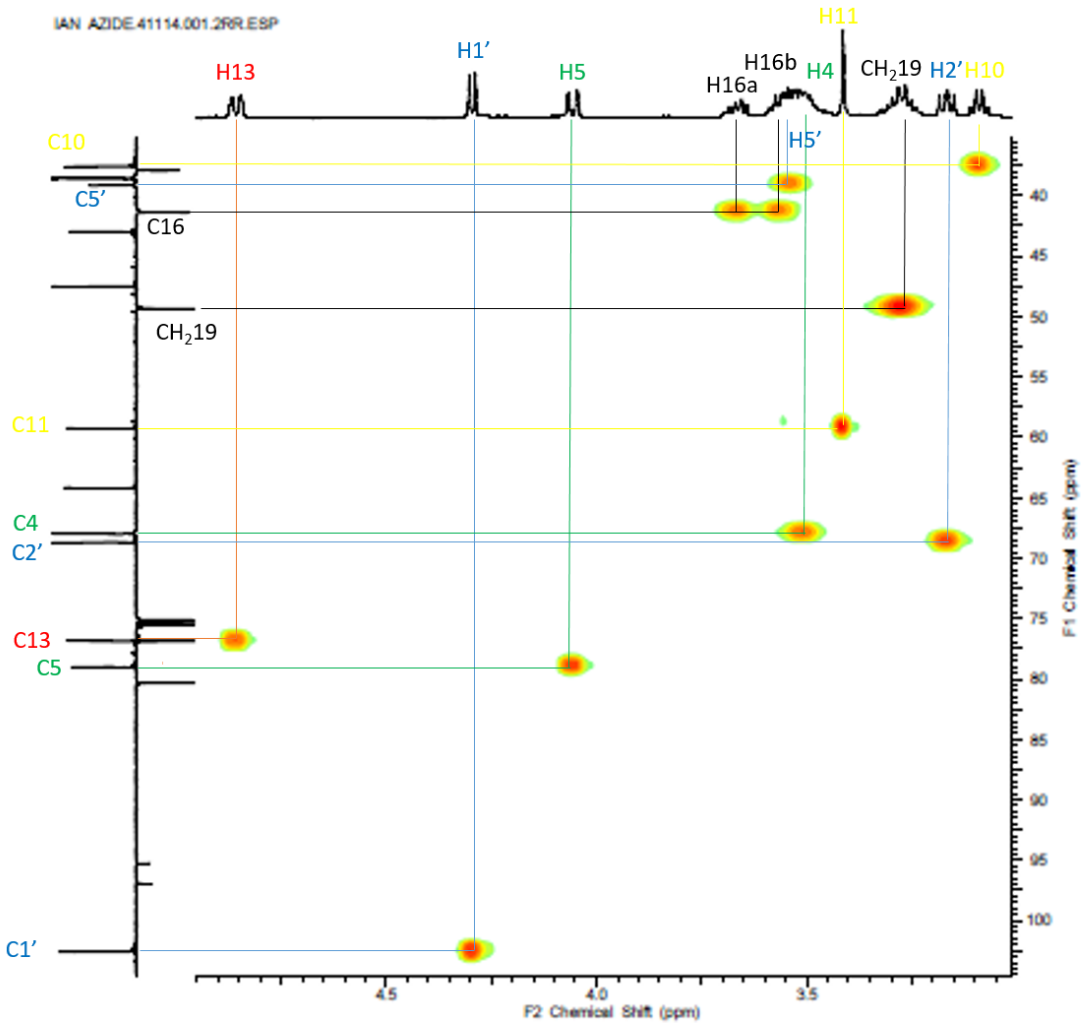

IAN AZIDE 41112.001.2RR.ESP
H16b
CH2 19
H10
H3'
CH2 18
CH2 17
Me8
H16a
H5'
H2'
H8
H7a
H4'a
H7b
H4'b
Me5'
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)

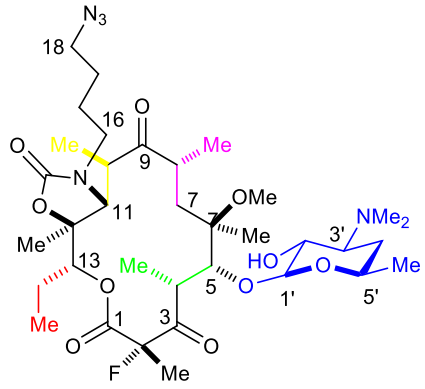

C9
C3
C1
C-carbamate
C1'
C2
f1 (ppm)
104.57
99.12
97.07
82.35
81.01
78.91
78.87
70.70
70.00
66.15
61.23
51.33
49.52
44.98
43.38
41.13
40.60
39.92
39.58
28.45
26.56
24.68
22.50
21.52
18.28
15.40
15.08
14.13
10.77


C5
C6
C12
C13
C2′
C4
C3′
C11
C19
6-OMe
C8
C16
C5′
C7
C10
NMe2
C4′
C18
C17
Me2
C14
Me4
Me6
Me8
Me5′
Me12
Me10
Me15
82.35
81.01
78.91
78.87
0.70
0.00
6.15
1.23
51.33
49.52
44.98
43.38
41.13
40.60
39.92
39.58
28.45
26.56
25.72
25.50
24.68
22.50
21.52
20.10
18.28
15.40
15.08
14.13
10.77
f1 (ppm)

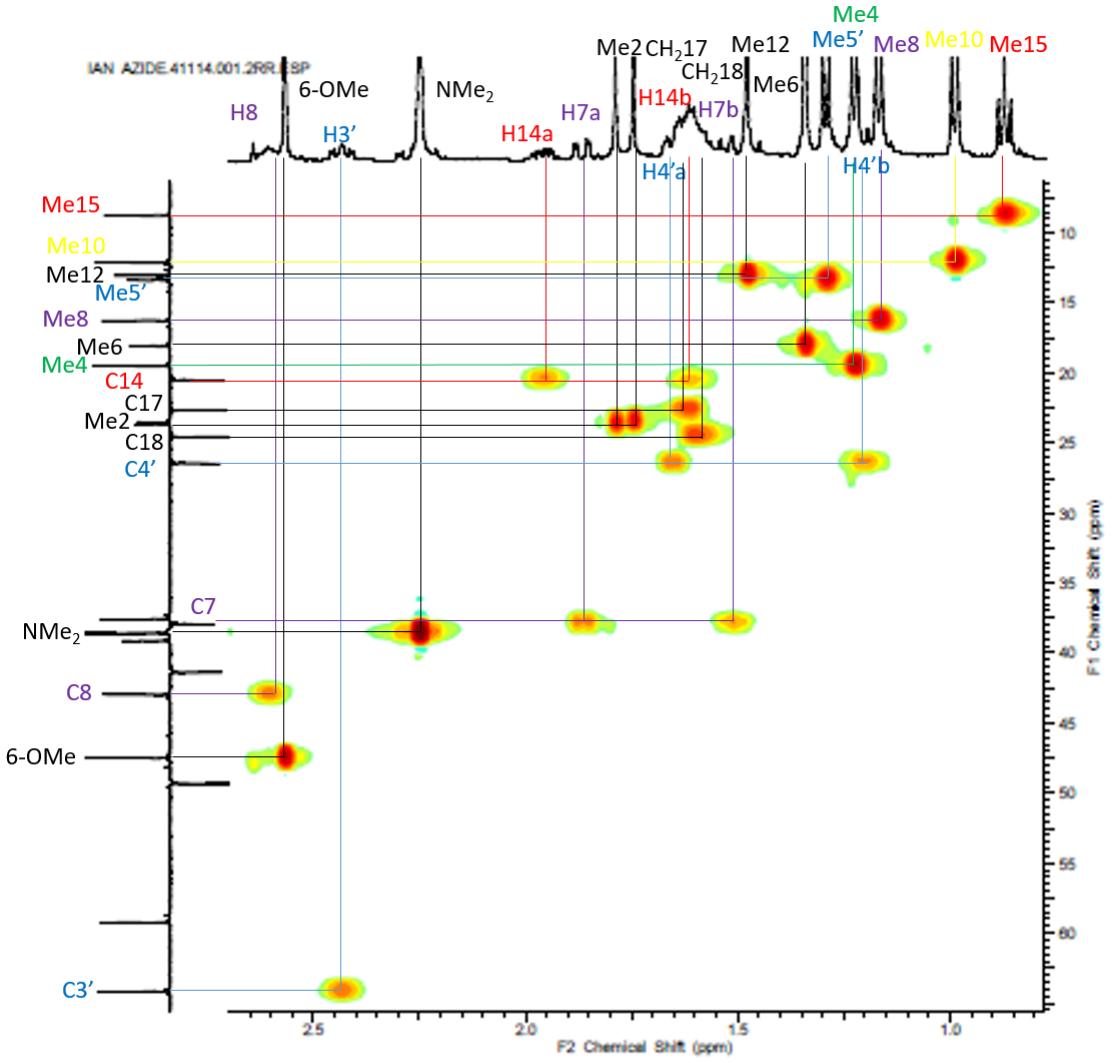

N3
18
16
O
Me
Me
9
O
N
O
11
7
OMe
Me
Me
13
Me
HO
O
3'
NMe2
Me
5
O
1'
5'
Me
1
3
O
O
F
Me

2


IAN AZIDE.41114.001.2RR.ESP
H11
H1'
H13
H5
H16b
H16a
H4
CH2 19
H2'
H10
H5'
C10
C5'
C16
CH2 19
C11
C4
C2'
C13
C5
C1'
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


N3
18
16
O
Me
Me
9
N
O
O
11
7
OMe
Me
Me
13
3'
NMe2
HO
O
Me
O
5
O
1'
5'
Me
1
3
O
O
F
Me

2


IAN AZIDE.41114.001.2RR.ESP
H8
6-OMe
H3'
NMe2
H14a
H7a
Me2
CH2 17
CH2 18
H14b
H7b
Me12
Me6
H4'a
Me4
Me5'
Me8
Me10
Me15
H4'b
Me15
Me10
Me12
Me5'
Me8
Me6
Me4
C14
C17
Me2
C18
C4'
C7
NMe2
C8
6-OMe
C3'
2.5
2.0
1.5
1.0
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)