The Use of Natural Product Substrates for the Synthesis of Libraries of Biologically Active, New Chemical Entities

Joshua Bryant Phillips

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THE USE OF NATURAL PRODUCT SUBSTRATES FOR THE SYNTHESIS OF
LIBRARIES OF BIOLOGICALLY ACTIVE, NEW CHEMICAL ENTITIES

by

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B.S. Chemistry, Northern Arizona University, 2002
B.S. Microbiology (health pre-professional), Northern Arizona University, 2002

Presented in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

Chemistry

The University of Montana

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THE USE OF NATURAL PRODUCT SUBSTRATES FOR THE SYNTHESIS OF LIBRARIES OF BIOLOGICALLY ACTIVE, NEW CHEMICAL ENTITIES

Advisor: Dr. Nigel D. Priestley
Chairperson: Dr. Bruce Bowler

ABSTRACT

Since Alexander Fleming first noted the killing of a bacterial culture by a mold, antibiotics have revolutionized medicine, being able to treat, and often cure life-threatening illnesses and making surgical procedures possible by eliminating the possibility of opportunistic infection. However, during the past 30 years many of the infections that were once easily cured by the proper antibiotic are no longer so due to a precipitous rise in multi-drug resistant organisms. This rise in multi-drug resistant organisms poses a grave threat to the medical advances that have been made in the past century and underscores the need for new antibiotics. We have developed two promising candidates for pharmaceutical applications, compounds 72 and 71, which are derived from nonactin, a biologically active natural product.

Nonactin 40, an ionophore macrotetrolide antibiotic that is produced by Streptomyces griseus ETH A7796, is an ideal candidate for the synthesis of new antimicrobial drugs. This secondary metabolite is composed of two units of (+)-nonactic acid 49 and two units of (–)-nonactic acid 50. Whereas nonactin does not possess a synthetically useful chemical ‘handle’, the nonactic acid subunits do. Through methanolysis of this structure and the separation of the two enantiomers, followed by a series of transformations, easily
diversifiable scaffolds have been synthesized, which allows for the relatively rapid synthesis of chemically diverse libraries.

From a small library of compounds that were synthesized, the compounds, 72 and 71 were found to show promising activities against vancomycin-resistant Enterococcus faecalis (VRE) and methicillin-resistant Staphylococcus aureus (MRSA). Compounds 88 and 90 were shown to be the active enantiomers. It was also shown by making the 1,4-substituted triazoles and the 1,5-substituted triazoles 91, 92 that only the 1,4-substituted triazoles gave the aforementioned activities. These results illustrate the vital importance of stereochemistry and regiochemistry.

To establish the importance of the nonactic acid moiety itself in the triazoloester compounds, analogues of these compounds were made by replacing the nonactic acid moiety with a cyclohexane moiety, specifically starting with both trans and cis 4-cyclohexanol-carboxylic acid. Neither the cis nor the trans analogues 131, 132, 142, 143, 151, 152, 160, 161 of either of the regioisomers of the compounds made to mimic 72 and 71 gave the activities of their nonactate-containing counterparts.

As an alternative to chemical synthesis we investigated biotransformation of nonactic acid analogs by Streptomyces griseus. While we were unable to generate new nonactin analogues we did discover an inhibitor of nonactin biosynthesis 186 and we were able to set limits on precursor directed biosynthesis in S. griseus.
The Missing Piece

In the fire the stone,
Turned in the pyre a bone,
Seasoned with the spice of ash.
In the hearth the cauldron,
Melted in the crucible this dust,
Out of the brazen kettle,
Arose a thing of brain, bone, and flesh.
And in the dark stumbled ‘round,
Until the morning light was found,
Though by all this amalgam had every part,
It fell at the door to dust for lack of a heart.

Joshua Bryant Phillips
Acknowledgements

It has been my distinct honor of working with some of the best professionals in the field and I would be truly without merit or consequence if I did not mention nor paid heed to the guidance, knowledge, and humor they have been kind and patient enough to bestow on me. First and foremost I would like to thank Dr. Nigel D. Priestley, my advisor, who has bore the brunt of my daily tribulations in organic chemistry and so eloquently provided much needed insight into its intricacy. I thank you for your generous support during this long and difficult endeavor.

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left me envious and pushed me to be ever greater. Rong Jian, a man with a wry and understated humor, who has seen fit to impart to me a wider view of the world. Of P. Whitney Swain III, who has taken pains in helping me with my research, and without who nothing would have been quite the same. And not in the least, Heather Ewing, a great friend, a skilled chemist, and truly a student I have felt proud to have had.

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<table>
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<th>Description</th>
</tr>
</thead>
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<tr>
<td>$^{13}$C NMR</td>
<td>13-carbon nuclear magnetic resonance spectrum</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>proton nuclear magnetic resonance spectrum</td>
</tr>
<tr>
<td>calcda.</td>
<td>calculated</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DCM</td>
<td>methylene chloride</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>DHP</td>
<td>3,4-dihydro-2H-pyran</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ESI-TOF-MS</td>
<td>electrospray ionization time of flight mass spectrometry</td>
</tr>
<tr>
<td>HMPA</td>
<td>hexamethylphosphoramide</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>50% of the Inhibitory concentration</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant / Hz</td>
</tr>
<tr>
<td>LC-MS (TOF)</td>
<td>time of flight liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MRSA</td>
<td>methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>pTsCl</td>
<td>para-toluene sulfonyl chloride</td>
</tr>
<tr>
<td>pTsOH-H$_2$O</td>
<td>para-toluene sulfonic acid monohydrate</td>
</tr>
<tr>
<td>py</td>
<td>pyridine</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature 22 °C</td>
</tr>
<tr>
<td>$R_f$</td>
<td>retention factor</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>SAR</td>
<td>structure activity relationship</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>TBDMSCI</td>
<td>tert-butyl dimethylsilyl chloride</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TOCSY-NMR</td>
<td>totally correlated spectrometry</td>
</tr>
<tr>
<td>VRE</td>
<td>vancomycin resistant <em>Enterococcus faecalis</em></td>
</tr>
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CHAPTER 1

INTRODUCTION AND BACKGROUND OF ANTIBIOTICS

1.1 Origin and history of antibiotics

1.1.A Definitions

An antibiotic is defined as a compound that kills or inhibits the growth of bacteria.[8, 9] Some antibiotics are bactericidal and others are bacteriostatic.[3, 8] Antibiotics include antibacterial agents such as penicillin, antifungals such as rifamycin, and antiprotozoal compounds such as tetracycline. They may be natural, synthetic, or semi-synthetic compounds which are derived from natural products and chemically modified. Chemical compounds that inhibit the growth or kill an organism are known collectively as antimicrobials.[9] Antimicrobials include antibacterials, antifungals, antiparasitics and antiprotozoal.[9] Compounds that kill microorganisms are bactericidal, while those that impede their growth are bacteriostatic agents.[8]

Before the isolation of antibiotics, infections had been treated orally and topically with a wide range of plants and molds in the Chinese, Greek, Egyptian, and Arab cultures.[10, 11] Modern medicine has since then isolated many of the naturally occurring antibiotic compounds that elicited the antibacterial, antifungal, or antiparasitic and antiprotozoal activity. Antibiotics were known originally as antibiosis, a term coined by French bacteriologist Vuillemin which meant ‘against life’ to describe the antibacterial nature of these compounds.[12] Antibiosis was first described in 1877 by Louis Pasteur and Robert Koch when they noted that the growth of Bacillus anthracis was inhibited by an
airborne strain of *bacillus*. In 1942 these compounds were renamed antibiotics (anti-‘against’, bios ‘life’) by the American microbiologist Selman Wakeman.[12, 14]

1.1B  Arsenicals and sulfonamides: development of the first synthetic antibiotics

Salvarsan 1a, 1b arguably, became the world’s first antibiotic compound used to treat a specific disease and was instrumental in ushering in the age of antibiotics. Paul Ehrlich, working with a team of scientists that included bacteriologist, Sahachiro Hata, and chemist Alfred Bertheim, set out to find a ‘magic bullet’, a compound that could affect only the target organism and not the host.[15] Ehrlich had noticed in his days as a medical student that certain dyes could be used to stain certain microorganisms which lead to this novel idea.[15] Putting this idea into practice, he lead a his team to find a compound that would kill *Treponema pallidum*, the parasite thought responsible for causing syphilis, and not affect the host.[15] Salvarsan was discovered in 1909 during the synthesis and testing of organoarsenic compounds by Ehrlich and Bertheim.[15]

![Salvarsan](image)

**Figure 1.1:** Structures of Salvarsan. Salvarsan consists of a mixture of cyclic arsenicals (1a and 1b).[1]

As described by Yarnell, A. et al., it was found that a single dose of the compound cured syphilis in rabbits. Salvarsan was placed on the market in 1910 for the treatment of syphilis and was used into the 1940’s, until the discovery and use of penicillin made
salvarsan obsolete by virtue of its superior bioavailability and lower toxicity. Despite difficulties in treatment using salvarsan, the pharmaceutical became the world’s first blockbuster drug.[15]

Cresol, an antiseptic, was the standard treatment for wounds inflicted in war.[2] Cresol, however, failed to prevent infection.[2] Due to the serious nature of the injuries and their frequency, the need for antibiotic research and development was spurred. It was said in 1914 by the Director General of the British Army Medical Service that “We have in this war gone straight back to all the septic infections of the Middle Ages.”[2, 16] Sulfonamides became the first antimicrobial drugs and the first of the synthetic antibiotics after salvarsan.[17]

This powerful new class of compounds was developed under the auspices of Heinrich Horlein of the Friedrich Bayer Company.[2] Heinrich Horlein was inspired by the work of Ehrlich and devised a plan similar to Ehrlich’s to develop synthetic antibacterial compounds.[2, 18] Domagk, who tested the newly synthesized compounds in vitro and in vivo using animals, was added to Horlein’s team in 1927.[2] The sulfonamide class of antibiotics would prove useful with the discovery of Prontosil 2 in 1932.

![Structure of Prontosil 2](image)

**Figure 1.2: Structure of Prontosil 2.**

Prontosil 2 is also called sulfonamidochrysoidine and was synthesized in 1932 by Mietzsch and Klarer.[2] Gerhard Domagk tested the compound against bacterial infections in mice and found the compound to be effective in the elimination of the infection,
specifically *Streptococcus hemolyticus*, a highly virulent Streptococcal strain.[2, 19] Prontosil 2 became the first medicine discovered that could treat a range of bacterial infections in vivo and the first commercially available antibacterial antibiotic.[19] Gerhard Domagk received the 1939 Nobel Prize for Medicine for his discovery. Prontosil 2 has activity against a wide range of Gram-positive cocci, with the exception of *Enterobacteria*. Sulfonamidochrysoidine was later found to be metabolized to sulfanilamide 4 by Jacques and Therese Trefouel at the Pasteur Institute in 1936. Sulfanilamide 4 had been discovered in 1909 by Paul Gelmo at the University of Vienna. Though its antimicrobial potential had not been discovered, sulfanilamide is cheaply produced and allows for ease of conjugation onto other molecules. Therefore, though Prontosil 2 failed to have exclusivity due to newer sulfonamides and the active component was a well-established compound, the discovery of Prontosil 2 ushered in the concept of ‘pro-drugs’ and the ‘sulfa drugs’ of which bactrim (sulfamethoxazole 3) would be a much later descendent.

![Figure 1.3: Structure of Sulfamethoxazole 3.](image)

It was also the first of the ‘wonder drugs’.

![Figure 1.4: Structure of Sulfanilamide 4.](image)

Sulfanilamide 4 was marketed in the United States, France, and Britain in the early part of 1937. By the Spring of 1937, any skepticism concerning chemotherapy had withered
away.[20] The biologically active sulfonamide compounds were useful antimicrobial drugs, a lead to the discovery of ‘bioactivation’, and were the first of the synthetic antibiotic pharmaceuticals. The sulfonamide itself was a compound widely used in dye manufacture, easy to conjugate to other compounds, and cheaply produced. Antibiotic sulfonamides are competitive inhibitors of the enzyme, dihydropteroate synthase, DHPS.[17] This inhibition of DHPS causes the inhibition of bacterial purine synthesis.[21-23] Sulfonamide spectrum of activity consists of Gram-positive cocci, such as Streptococci.[2]

1.1.C Beta-lactam antibiotics

As the discovery of the sulfonamide compounds became the first class of synthetic antimicrobials, the discovery of beta-lactam antibiotics marked the beginning of the naturally derived antibiotic compounds. Beta-lactam structures consist of a beta-lactam in a [3.2.0] bicyclic system.

Beta-lactam antibiotics act as bacteriocidal agents on Gram-positive bacteria and are divided into three categories based on structure: penams, penems, and cephems.[24, 25] Specifically, this class of antibiotics inhibits cell wall synthesis by targeting enzymes on the outer face of the cytoplasmic membrane, called peptidoglycan transpeptidases, which aid in the synthesis of the cell wall.[26] The beta-lactam antibiotics include several families of antibiotic compounds. These are the pencillins, monobactams, carbapenes, and cephalosporins. The first discovered compound in this class was the antibiotic compound, penicillin.
Figure 1.5: Penicillins, the structures of Penicillin F 5a and G 5b.[2]

Penicillin G 5b, or rather the effect, of the compound produced by a penicillian was discovered through serendipity by Alexander Fleming in 1928.[27] A petri dish which had been inoculated with *Staphylococcus* and accidently left uncovered in the basement of St. Mary’s Hospital in London became contaminated with a blue-green mold. It was observed that a halo of inhibited bacterial growth formed around the mold. Alexander Fleming concluded that the mold was producing a compound that lysed the *Staphylococcus* and, therefore killed them. This substance he named the ‘mould broth filtrate’ as penicillin from the name of the fungus.[28] A pure culture of the mold was grown and found to be *Penicillium notatum.*[2, 29] Fleming halted his research in 1931 after concluding that penicillin did not remain in the body long enough to be of value. Three years later he returned to it and attempted to enlist someone in deciphering its structure which was finally accomplished in the early 1940’s.[30]

After the end of WWII, the total synthesis of penicillin was completed by John C. Sheehan at MIT.[31] The synthetic method for the production of penicillin, however, did not allow for large scale production. Synthesis in drug production coupled with the production of natural products from fermentation became significantly important in the development of better antibiotics. As Fleming had observed, penicillin is not retained long in the human body. Its rapid renal clearance and the difficulties in large scale production did not allow penicillin to become a marketable drug. Within three to four hours after dosing,
approximately 80% of the compound was eliminated from the body through urination. As in early trials of the drugs, the patients urine was collected the compound extracted so that it could be reused. Infected patients who were given penicillin 5b had shown remarkable improvement, but due to the difficulties in the first attempts to isolate the drug from the filtrate of broth cultures of *Penicillium notatum* the patients would sicken and die when the last of the penicillin 5b was used.

Though penicillin 5b had been used successfully in the 1944 to treat soldiers, specifically amputees, the rapid loss of penicillin 5b posed a significant problem. An uricosuric agent called probenecid was found that could competitively inhibit the secretion of penicillin 5b via the organic acid transporter responsible for the elimination of penicillin 5b. The drugs were given together, thus establishing a synergistic strategy in drug development.

The application of two drugs was not an optimal solution and other penicillins 5b were sought after. The establishment of the structure of penicillin 5b and discovery of others led to the isolation of 6-APA, the pharmacophore of penicillin 5b, which in turn allowed for the semi-synthesis of penicillin 5b analogs. Among these analogs was ampicillin 6 which gave a broader spectrum of activity.

\[
\text{Figure 1.6: Structure of Ampicillin 6.}
\]

Penicillins 5a, 5b are wide spectrum antibiotics that are bacteriocidal against Gram-positive organisms. The mechanism of action of the penicillins 5b is the inhibition of cell walls synthesis which occurs by the inhibition of formation of peptidoglycan cross-linking in the cell wall. Cross-linking is inhibited by the binding and inactivation of the transpeptidases
by penicillin 5b or other beta-lactam, also known as the penicillin binding protein, PBP. The bacteria die via cytolysis caused by osmotic pressure.

1.1.D Cephalosporins

The Cephalosporins 7 are another class of beta-lactam antibiotics and are part of subgroup of beta-lactams called cephems.[32] Cephalosporins 7 are composed of a beta-lactam in a [4.2.0] bicyclic system.

![Figure 1.7: General Structure of Cephalosporins 7.](image)

Cephalosporin C was discovered by the Italian scientist Giuseppe Brotzu in 1948.[33] Cephalosporins were isolated from cultures of Cephalosporium acremonium in Sardinia.[33] Members of this antibiotic class are bactericidal and disrupt the synthesis of the peptidoglycan layer of bacterial cell walls in the same manner as the rest of the beta-lactam antibiotics.[32] Cephalosporins 7, however, show a greater resistance to beta-lactamases, otherwise known as penicillinases.[32] Several generations of cephalosporins 7 have allowed for an increase in their spectrum of activity. Cephalosporin structure 7 can be modified to alter the antibacterial activity or pharmacokinetic profile.[32] The acyl side chain at position 7 can be altered to change antibacterial activity and pharmacokinetics can be change by chemical modification to the 3-position.[32] The first generation of cephalosporins was active against Gram-positive bacteria, including penicillin resistant Staphylococci and Streptococci. Successive generations have shown activity against Gram-
negative bacteria, however, in doing so, the activity against Gram-positive bacteria has 
decreased. Activity against Gram-positive bacteria encapsulates the spectrum of activity of 
second and third generation cephalosporins. Third generation cephalosporins are able to 
penetrate the CNS which allows for their use against meningitis caused by *H. influenza*, 
*meningococci*, and *pneumococci*.[32] Some members of this class also have 
antipseudomonal activity. The fourth generation of cephalosporins shows broad spectrum 
activity being effective against Gram-positive bacteria as with first generation 
cephalosporins, but also have activity against meningitis, and against *Pseudomonas 
aeruginosa*.[32]

1.1.E Carbacephems

The carbacephems are beta-lactam antibiotics and are a subclass of the 
cephalosporins. Carbacephems, a member of the cephem class, are synthetically made and 
based upon the structure of cephalosporins. The basic structure of the carbacephems is 
altered from that of the cephalosporins and that of other cephems in that a carbon is 
substituted for the sulfur. A bacteriostatic effect is achieved by these synthetic structures by 
the inhibition of cell wall synthesis, which prevents cell division. However, unlike the other 
lactams, it does not lyse and kill the bacterium. An example of this class of antibiotics is 
Loracarbef 8, also referred to as Lorabid. This compound is more chemically stable than the 
widely used second generation cephalosporin, Cefaclor.
Figure 1.8: Structure of Loracarbef (Lorabid) 8, a carbacephem.

1.1.F Carbapenems

Figure 1.9: General Structure of the Carbapenems 9.

The carbapenems 9 are natural product analogues, based off the structure from Thienamycin 10.[34]

Figure 1.10: Structure of Thienamycin 10.

Carbapenems 9 are composed of an unsaturated five-membered carbon ring which is fused to one carbon and a nitrogen on the beta-lactam ring.[34] Thienamycin 10 is a natural product produced from Streptomyces cattelya and was the first described carbacephem.[34, 35] This compound, however, is chemically unstable and titers of this compound in fermentation are low. Carbapenems 9 are synthetically made via total synthesis. Carbapenems 9 have activity against Gram-positive and Gram-negative bacteria, having the broadest spectrum of activity in the beta-lactam antibiotic family.[34] They are also beta-lactamase resistant.[34]
Intracellular bacteria are not affected by carbapenems 9 due to the bioavailability of carbapenems.[36]

1.1.G Monobactams

The remaining class of beta-lactam antibiotics is the monobactams, which, unlike the other beta-lactams, have only a single four membered beta-lactam ring which is unattached to any other ring in the molecule.[37, 38] They are characterized by the presence of the 2-oxoazetidine-1-sulfonic acid moiety.[37] These compounds have been recently isolated from gram-negative bacteria.[38] Naturally occurring monobactams exhibit weak antibacterial activity, however, derivatives of these compounds are potent antibiotics.[37]

Figure 1.11: Structure of Aztreonam 11, a monobactam antibiotic.

Aztreonam 11 is synthetically produced.[37] Monobactams are only effective against Gram-negative bacterium, including *Pseudomonas aeruginosa*. [37, 38] Aztreonam 11 is also bactericidally effective against *E. coli, Klebsiella, Proteus, Citrobacter, Enterobacter*, and *Serratia* species.[39] Their mechanism of action is the inhibition of the muropeptide synthesis in the bacterial cell wall which blocks peptidoglycan cross-linking.

1.1.H Aminoglycosides

Aminoglycosides consist of multifunctional hydrophilic sugars which are derived from *Streptomyces* and *Micromonospora*. [40] They are protein synthesis inhibitors which
function by binding to the 30S ribosomal subunit.[41] A binding affinity for nucleic acids is also a characteristic of these antibiotics due to their polycationic nature. The majority of aminoglycosides are bactericidal.[40] Aminoglycosides are active against Gram-negative bacilli, including aerobic bacteria, such as Enterobacter, Pseudomonas, and Acinetobacter.[17]

Streptomycin 12 was first of the aminoglycosides antibiotics to be discovered.

Figure 1.12: Structure of Streptomycin 12, an aminoglycoside.

Streptomycin was isolated by Albert Schatz at Rutgers University in 1943 from Streptomyces griseus.[42] Streptomycin 12 is a bactericidal natural product and was the first antibiotic used to cure tuberculosis.[43] It is effective at inhibiting Gram-positive and Gram-negative bacteria and, therefore, classified as a broad spectrum antibiotic. This antibiotic inhibits the binding of formyl-methionyl-tRNA to the 30S subunit by binding to the 30S subunit of the bacterial ribosome, therefore, inhibiting protein synthesis.[44] Streptomycin 12 is also used to restrict the growth of fungi and algae.

1.1.1. Macrolides

Macrolides are members of a class of natural product antibiotics called polyketides. These antibiotics consist of a large macrocyclic lactone which is usually 14, 15, or 16-membered and two sugars, desosamine and cladinose.[45] Macrolides are protein synthesis
inhibitors and may have three different mechanisms of action.[45] They have been shown to inhibit ribosomal translocation, cause premature dislocation of peptidyl-tRNA from the ribosome, and inhibit the peptidyl of the peptidyl-tRNA from adding to the next amino acid in the sequence by inhibiting the peptidyltransferase.[45] Macrolides bind close to the peptidyl transferase center on the 50S ribosomal subunit.[45-48] In general, macrolides have a medium spectrum of activity, which is slightly greater than that of penicillin and are used for patients with a penicillin allergy.[49] Specifically, macrolides have activity against *Staphylococci*, beta-hemolytic *Streptococci*, *Pneumonococci*, *Enterococci*, *Chlamydia*, *Mycoplasma*, and *Mycobacteria*.[49] Commonly, they are used to treat respiratory infections. Erythromycin 13, azithromycin 15, clarithromycin 14, and telithromycin 16 are members of this class.

![Figure 1.13: Structure of Erythromycin 13, a macrolide antibiotic.](image)

Discovered by Eli Lilly in 1949 by J.M. McGuire, erythromycin 13 is a natural product macrolide antibiotic that was isolated from *Saccharopolyspora erythraea*. Erythromycin 13 contains a 14-membered ring and is composed of two sugars, cladinose and desosamine.[45, 50] As with other macrolides antibiotics, erythromycin 13 interferes with protein synthesis by binding to the 50s subunit of the bacterial 70s rRNA complex and causes interference with aminoacyl translocation.[45] This results in blockage of the entry way into
the large ribosomal subunit and, therefore, inhibits the production of useful proteins.[45, 51-53] Therefore the origin of antimicrobial activity is causing a lack of functional proteins necessary for life processes. Erythromycin 13 is not a particularly effective antibiotic and, therefore, not a first line drug.[49] It is quickly eliminated from the body by demethylation in the liver and is inactivated by gastric acid.[49] High doses are necessary and common side effects are diarrhea and nausea.[49] Due to the low acid tolerance, clarithromycin 14 was invented which is more acid stable.[49, 54] The C-6 hydroxyl group of erythromycin is substituted for a methoxy group which prevents the erythromycin 13 base from becoming the hemiketal intermediate via degradation. The gastrointestinal side-effects of erythromycin 13 are thought to originate from the production of the hemiketal intermediate, which induce stomach motility.[55]

![Figure 1.14: Structure of Clarithromycin 14.](image)

Azithromycin 15 is another member of the macrolides class of antibiotics, which is semisynthetic analogue of erythromycin 13.[49] The macrocycle ring is modified by the inclusion of a methyl amine and removal of the ketone at the 9a position of the aglycone ring giving a 15-membered ring in place of the 14-membered erythromycin macrocycle.[49, 56] This places azithromycin 15 into a subclass of macrolides antibiotics called azalides. Azithromycin 15 was discovered in 1980 by Pliva, a Croatian pharmaceutical company. By
binding to the 50S subunit of the bacteria ribosome, the translation of mRNA is inhibited.[56] This caused the inhibition of protein synthesis which prevents the growth of the bacteria.[56] Though azithromycin 15 shares a similar spectrum of activity with erythromycin 13, azithromycin is more effective against certain strains of Gram-negative species.[56] Azithromycin 15 is effective against *Staphylococcus aureus* and several species of *Streptococcus* including *pyogenes, agalactiae*, and *pneumonia*.[56] It is also effective against *Mycoplasma pneumonia, Chlamydia*, and *Haemophilus*.[56]

![Figure 1.15: Structure of Azithromycin 15.](image)

To treat respiratory tract infections caused by macrolide-resistant strains, ketolides were developed, such as telithromycin 16.[3, 49]

![Figure 1.16: Structure of Telithromycin 16, a ketolide antibiotic, related to macrolides antibiotics](image)

Ketolides are structurally related to macrolides, but differ in the removal of the L-cladinose sugar and the oxidation of the 3-hydroxy to 3-keto.[3, 49, 57] Ketolide antibiotics also
prevent protein synthesis via blocking of the ribosomal exit channel, but have activity against macrolide-resistant strains.[3]

1.1.J. Ansamycins

Ansamycins were discovered in 1959 by Sensi et al. These compounds are natural products, one that is produced from the Actinomycete, *Amycolatopsis mediterranei*.[58] This family of secondary metabolites contains several classes, each one a derivative with the primary structural characteristic of this unique family. The primary structural characteristic is the presence of an aromatic unit, bridged by an aliphatic chain.[59] Each structural derivative of this primary scaffolding engenders a new class. Examples of these are rifamycin and naphthomycin which contain a naphthalene ring or a naphthoquinone ring.[60] Another class includes the ansamycins, ansamitocin and geldanamycin 17 in which the primary aromatic moiety is composed of a benzoquinone ring or a benzene ring.[60]

![Image of Geldanamycin](image)

**Figure 1.17:** Structure of Geldanamycin 17, a benzoquinone ansamycin

Ansamycins have shown potency against a wide spectrum of Gram-positive and Gram-negative bacteria. Potent mycobacterial activity has been demonstrated by the rifamycin subclass of ansamycins and it is in wide spread use to combat fungal infections in immunocompromised patients, such as those with AIDS, as well as leprosy, and importantly
Lastly antiviral activity has also been demonstrated against bacteriophages and poxviruses. This gives ansamycins a wide spectrum of activity.

The rifamycin subclass of ansamycin antibiotics were discovered by Group Lepetit SpA, an Italian pharmaceutical company in Milan by Piero Sensi and Pinhas Margalith in 1957. Rifamycins were isolated from *Streptomyces mediterranei*, a soil bacterium. Over the years this species has been renamed several times and now includes the names *Nocardia mediterranei* and *Amycolatopsis rifamycinica*, which is perhaps the most recent nomenclature and was given in 2004.

![Figure 1.18: Structure of Rifamycin B 18.](image)

Rifamycin B 18 is able to inhibit DNA-dependent RNA synthesis via the binding to prokaryotic RNA polymerase.[62] By binding to the polymerase, its steric bulk physically blocks chain elongation of the oligonucleotide.[63] In HIV-compromised patients, tuberculosis, leprosy, and mycobacterium avium complex, as well as intestinal infections caused by *Escherichia coli* have all been treated with rifamycins.

1.1.K. Tetracyclines

Tetracyclines are a family of broad-spectrum, bacteriostatic antibiotics that were discovered in 1945 by Benjamin Minge Duggar.[64, 65] Structurally, the tetracycline antibiotics are uniquely defined for the presence of four hydrocarbon rings (A, B, C, and D)
and are derivatives of polycyclic naphthacene carboxamide.[4] These polyketide antibiotics, which are isolated from a Streptomyces species, comprise a very large group of antibiotics. The first of these antibiotics to be discovered was Aureomycin which was discovered in the late 1940s. Aureomycin, otherwise known as 7-chlorotetracycline 19, was isolated in the Lederle Laboratories from a soil-bacterium called Streptomyces aureofaciens.[4]

![Figure 1.19](image)

**Figure 1.19:** Structure of 7-Chlorotetracycline 19.[4]

Oxytetracycline was later isolated by A.C. Finlay et al. from Streptomyces rimosus. They are natural products and inhibit protein synthesis.[64, 65] Tetracyclines prevent the docking of amino-acylated tRNA to the ribosomal acceptor (A) site by binding to the 30S bacterial ribosome subunit.[4, 17] Tetracyclines are used in the treatment of skin infections such as acne and rosacea as well as anthrax, plague, and Legionnaires’ disease. In general tetracyclines are also employed as the primary class of antibiotics to treat infections such as urethritis, typhus, and spirochetal infections.

Tetracycline 20, the tetracycline antibiotic, was also discovered by Benjamin Duggar and described in 1948.

![Figure 1.20](image)

**Figure 1.20:** Structure of Tetracycline 20, a tetracycline antibiotic.[4]
It is used to treat a variety of Gram-positive and Gram-negative infections.[4] Certain protozoa are also treatable with tetracycline. Cholera was once widely treated with tetracycline. It is also used to treat common acne and rosacea. The discovery of this family of antibiotics was a precipitous event in that it lead to a cascade of chemically altered antibiotics and is noted as one of the most important discoveries in the field.

1.1.1. Tigecycline

Tigecycline \( \text{22} \) is a derivative of minocycline \( \text{21} \) and is related structurally to tetracyclines.[5, 66]

\[
\text{21. Minocycline}
\]

**Figure 1.21**: Structure of Minocycline \( \text{21} \).[4]

Tigecycline is a glycyldycline antibiotic and was developed by Wyeth in 2005.[5, 67, 68] Tigecycline \( \text{22} \) is a protein synthesis inhibitor and possesses a wide spectrum of activity.[3, 66, 69] It is bacteriostatic.[69] This antibiotic binds to the 30S ribosomal subunit and blocks aminoacyl-tRNA molecules entry into the A site of the 30S ribosomal subunit which prevents translation.[5, 69, 70] Activity has been documented against Gram-positive and negative bacteria including anaerobes, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, vancomycin-resistant *Enterococcus faecalis*, vancomycin-resistant *Enterococcus faecium*, and drug resistant strains *Actinetobacter baumannii*.[3, 5] This member of the new class of antibiotics, glycyldyclines, possesses a central four-ring carbocyclic skeleton and is therefore related to the tetracyclines.
Figure 1.22: Structure of Tigecycline 22, a glycyclycline.[5]

Tigecycline 22 is used for intravenous therapy for serious bacterial infections, among them, complicated skin, soft tissue, and intra-abdominal infections.[5, 70] It is mainly bacteriostatic; however, it has been shown to have bactericidal activity.

1.1.M. Glycopeptides

Glycopeptides are a class of antibiotics that consists of glycosylated cyclic or polycyclic nonribosomal peptides.[71] One of two core linear heptapeptide structures forms the backbone of these antibiotics.[71] This unique class inhibits peptidoglycan synthesis and therefore inhibits the construction of the cell wall. Interference with the construction of the bacterial cell wall is accomplished by two mechanisms. First, the glycopeptides inhibit transpeptidases which causes a lack of tensile strength in the cell wall resulting from a lack of cross-linked peptidoglycan necessary for the integrity of the organism.[71] Secondly, glycopepides prevent the growth of the peptidoglycan chain via the inhibition of transglycosylase.[71] Glycopeptides effectively target Gram-positive cocci.[71] Bacterial species that are susceptible to this class are *Staphylococci, Streptococci, Clostridia*, and *Enterococci.[71]* In *Enterococci*, these antibiotics are bacteriocidal, but are bacteriostatic in all almost all other species. Vancomycin 23 is a member of this class and has the distinction of being a last line of defense antibiotic, specifically for use against drug and multi-drug resistant species such as MRSA.[72]
Vancomycin 23, a natural glycopeptide antibiotic, was isolated by E.C. Kornfeld from *Amycolatopsis orientalis*, a soil bacterium found in Borneo.[73]

![Structure of Vancomycin](image)

**Figure 1.23**: Structure of Vancomycin 23, a glycopeptide antibiotic.

The antibiotic disrupts the synthesis of the cell wall in Gram-positive bacteria by inhibiting the assimilation of N-acetylglucosamine and N-acetylmuramic acid peptide subunits into the peptidoglycan matrix. Due to the side effects, such as ear and kidney damage as well as local pain and thrombophlebitis, and the prevention of the rise of vancomycin 23 resistant bacterial strains it is used as a drug of last resort.[73-76] The first use of the drug was the treatment of penicillin-resistant *Staphylococcus aureus* to combat beta-lactam resistant strains.[73, 74]

1.1.N. A lipopeptide antibiotic, daptomycin

Daptomycin 24 was discovered by Eli Lilly and marketed by Cubist in 2003 under the name Cubicin.[77-80] It is a novel lipopeptide antibiotic and a natural product.[81-88] Notably, it is the first natural product in 41 years to be categorized under a new structural class.[79, 80] Daptomycin 24 is produced by *Streptomyces roseosporus*.[81-88]
**Figure 1.24:** Structure of Daptomycin 24, a lipopeptide antibiotic

Daptomycin 24 consists of a 13-member amino acid cyclic lipopeptide with a decanoyl side-chain.[89] The mechanism of action of this novel compound is to bind to the bacterial cell membrane.[89] Its unique structure confers upon it this novel mechanism.[89] It is believed that the insertion of the lipophilic daptomycin tail into the bacterial cell membrane causes rapid membrane depolarization and potassium efflux which leads to the inhibition of DNA, RNA, and protein synthesis.[3, 89-91] This leads to cell death and, therefore, daptomycin 24 is bactericidal.[89-91] Daptomycin 24 has a very narrow spectrum of activity, only being active against Gram-positive organisms.[81-88] This cyclic lipopeptide has been shown to be effective against *Enterococci*, *Staphylococcus*, *Corynebacteria*, and *Streptococci*. The current main use of daptomycin 24 is to treat skin and skin structure infections.[92]

**1.1.O. Quinolones and fluoroquinolones**

Quinolones are synthetic broad spectrum antibiotics that were first synthesized in an attempt to make a synthetic form of chloroquine 26 which was used in World War II to treat malaria.[17] Chloroquine 26 was discovered in 1934 at Bayer by Hans Andersag. In the
process of this synthesis, nalidixic acid 25 was discovered and became the blueprint for the quinolone family of antibiotics.

Figure 1.25: Structure of Nalidixic Acid 25 and Chloroquine 26.[6]

Quinolones prevent DNA replication.[17, 93] Through porins in the outer membrane of bacterial cells, the quinolones diffuse through the plasma membrane and then selectively complex reversibly with DNA gyrase and topoisomerase IV.[94, 95] These enzymes are necessary for chromosomal decatenation, replication, and transcription. The bacteriostatic action is theorized to be caused by the slow reversible complex formation.[96] Higher concentrations of quinolones will have a bactericidal effect, depending upon the bacteriostatic or bactericidal effect of an individual quinolone, as some inhibit growth and are less effective in causing cell death.[96, 97] Quinolones also induced DNA lesions causing cell death by triggering the SOS response.[96, 98]

The fluoroquinolones 27 are a subclass of the quinolone antibiotics. The main difference between the two structures is the presence of a fluorine atom attached to the central ring system. This constituent is attached at either the 6 or 7 carbon atom position. Like the quinolones, the fluoroquinones 27 are synthetic. Fluroroquinolone antibiotics, as with the quinolones, originated from nalidixic acid, which was then employed in 1962 to treat urinary tract infections.[6, 99, 100]
The fluoroquinones’ bactericidal activity originates from its mechanism of action. The inhibition of the topoisomerase IV enzyme and DNA gyrase causes inference with DNA replication in bacteria.[6] Since human cells lack these topoisomerases, fluoroquinones are specific for bacterial strains and, due to the interference with DNA replication, they are also bactericidal.[6, 101, 102] Interference with DNA replication imbues fluoroquinones with a broad spectrum of activity. Effective bactericidal activity has been established against Gram-positive and Gram-negative bacteria.

Ciprofloxacin is a member of the fluoroquinolones, a second generation compound which was patented in 1983 by Bayer. Like the rest of the fluoroquinolones, ciprofloxacin is synthetic and inhibits DNA replication as well as transcription via the inhibition of DNA gyrase and topoisomerase IV.[103] Ciprofloxacin is used to treat urinary tract infections, bacterial diarrhea, gonorrhea, and community-acquired pneumonia.

**Figure 1.26:** General Structure of Fluoroquinolones 27.

**Figure 1.27:** Structure of Ciprofloxacin 28, a fluoroquinolone 27.[6]
1.1.P. Oxazolidinones

The oxazolidinone class of antibiotics were discovered in 1956.[104, 105]

![2-Oxazolidone](image)

**Figure 1.28:** The 2-Oxazolidone 29 Identifying Structure.

Members of this class are synthetic antibacterials, the first of which was cycloserine.

![Cycloserine](image)

**Figure 1.29:** Structure of Cycloserine 30.

This compound was developed as second-line drug in the treatment of tuberculosis.[106]

The mechanism behind the oxazolidinones antibacterial activity is the disruption of translation of mRNA into proteins in the ribosome.[107, 108] Oxazolidinones disrupts protein synthesis initiation complexes by interaction with the 23S ribosomal RNA on the 50S ribosomal subunit near the 30S ribosomal subunit in which the formation occurs.[3, 107, 109] Oxazolidinones are unique in this aspect in that unlike other protein synthesis inhibitors, they inhibit the initiation of protein synthesis, not the elongation process.[3, 110] It is theorized that these antibiotics prevent tRNA from entering the A site of the 50S ribosomal subunit by binding to the P site on the 50S ribosomal subunit, therefore causing the tRNA to dislodge from the ribosome completely.[111]

Oxazolidinones have been found to be effective against Gram-positive, specifically MRSA, and limited Gram-negative bacteria.[107, 110, 111] Like vancomycin 23, these
antibiotics are considered as last line defense against drug resistant organisms. Posizolid 31 and Linezolid 32 are members of this class.

**Figure 1.30:** Structure of Posizolid 31.

Linezolid 32 is a mostly bacteriostatic drug that was discovered in 1996 at Upjohn Laboratories.[111-113] This synthetic antibiotic was the first commercial available antibiotic and is used as a second line defense against multi-drug resistant Gram-positive strains, such as community-acquired pneumococcal pneumonia.[104, 105, 111, 114]

**Figure 1.31:** Structure of Linezolid 32.

The main mechanism of activity is the inhibition of protein synthesis. Linezolid 32 has been found to be effective against MRSA, VRE, and Streptococci strains.[110, 115] Surgical infections such as *S. epidermidis* can be treated with Linezolid 32.[111, 116, 117]

**1.1.Q. Polypeptide antibiotics**

The polypeptide antibiotics, such as Bacitracin 33, Gramicidin, and Polymyxin 34, mainly consist of repeated chains of amino acids. The compounds are used topically for treat of ear and eye infections.
Bacitracin 33 was first isolated by John T. Goorley from a *Bacillus subtilis* strain found in a compound fracture of the tibia in a female patient called Tracy I.[118, 119] Isolation gave a mixture of related cyclic polypeptides. Bacitracin 33 was isolated from this mixture in 1945.[118] Bacitracin 33 is a natural product antibiotic and is produced by strains of *Bacillus licheniformis*.[120]

![Structure of Bacitracin 33](image)

**Figure 1.32:** Structure of Bacitracin 33.

The natural product interferes the production of bacterial cell walls by preventing peptidoglycan subunits from being transported outside of the inner membrane.[121] The main mechanism of action occurs by inhibiting the dephosphorylation of the C55-isoprenyl pyrophosphate.[120, 121] This prevents the synthesis of the bacterial cell wall in Gram-positive organisms by inhibiting the regeneration of the lipid carrier which necessary for the cyclic synthesis of peptidoglycan.[120, 122, 123] Bacitracin 33 is used to treat topical infections when combined with other topical antibiotics such as polymyxin B 34. Eye and skin infections are treated with this polypeptide antibiotic and on small wounds to prevent infection.
Gramicidin was discovered by Rene Dubos in 1939 in the isolation of tyrothricin. Tyrothricin is composed of a mixture of linear pentadecapeptides, which are gramicidins A, B, and C.[124-126] It is also comprised of gramicidin S and tyrocidins, which are backbone-cyclized cationic decapetides.[124-126] This natural product mixture was isolated from Aneurinibacillus migulanus.[124] Collectively, they act to form a channel in the bacterial cell wall which results in the destruction of the ion gradient via an increase in permeability.[124, 127-131] Gramicidin acts to form a beta-helix in the hydrophobic interior of the cellular lipid bilayer, which then dimerizes to span the entire width of the membrane.[124, 127-131] Monovalent cations are able to travel between the extracellular space and the cell’s cytoplasm without control.[124, 127-131] This bactericidal mixture is applied topically and is potently active against a broad spectrum of Gram-positive and Gram-negative organisms.[124] Gramicidin S is a particular potent antibiotic however it may only be used topically due to its hemolytic effects.[124] Gram-positive bacilli are not affected by Gramicidin; however Gram-negative Neisseria stains are susceptible to these collective compounds.

Polymyxins 34 are produced by Bacillus polymyxa, a Gram-positive bacteria.[132] A pentabasic decapetide with a cycloheptapeptide ring and a C9 or C10 fatty acid chain constitute this antibiotic class. These compounds are surfactant-like in that their hydrophobic substituents cause damage to the cell membrane which results in cell death. Polymyxins 34 have a narrow spectrum of activity, specifically against Gram-negative species.[133, 134] In Gram-negative bacterial species, disturbances caused by the binding by the cationic polypeptide polymyxins 34 to the anionic lipopolysaccharides molecules in the outer membrane increase the permeability of the membrane.[135, 136] It has been hypothesized
that polymyxin may act on more than one target since cell death has been observed to take place before an increase in membrane permeability occurs.[137] A member of this subclass is Polymyxin B 34.

Figure 1.33: Structure of Polymyxin B 34, a cyclic polypeptide.

1.1.R. Chloramphenicol

Chloramphenicol 35 is a natural product bacteriostatic antimicrobial compound. David Gottlieb isolated the compound from Streptomyces venezuelae and used it to treat infection beginning in 1949.[138] Chloramphenicol 35 is a broad spectrum antibiotic, able to effectively treat Gram-positive and Gram-negative bacterial infections.[139] The compound is a protein synthesis inhibitor via the inhibition of peptide bond formation. Chloramphenicol inhibits peptidyltransferase activity of the bacterial ribosome via binding to the 50S ribosomal subunit.[17]

Figure 1.34: Structure of Chloramphenicol 35.
1.1.S. Trimethoprim

Trimethoprim 36 is a synthetic bactericidal antibiotic. Trimethoprim 36 was developed by GlaxoSmithKline and has been used in the United Kingdom since 1969. Potent, selective, and a competitive inhibitor of bacterial dihydrofolate reductase, Trimethoprim 36 acts to affect methyl transfer reactions due to the depletion of tetrahydrofolate.[17] This interferes with thymine synthesis by inhibiting folic acid synthesis by the disruption of dihydrofolate reductase.[17] It is therefore classified as a nucleic acid inhibitor.[17]

Figure 1.35: Structure of Trimethoprim 36.

1.2 Rise of antibiotic resistant strains

1.2.A. Introduction to antimicrobial resistance

As early as when the first ‘sulfa drugs’ and penicillins were being administered to patients, scientists and physicians have noted this ability of microorganisms to develop resistance to antimicrobial compounds.[140] An example of penicillin resistance is *S. pneumonia*, whose resistance was first detected in 1967, and has become increasingly prevalent worldwide.[140] However, in recent years, the world has seen a precipitous rise in resistant strains.[141] Such resistant strains are resistant to one or more compounds that had previously been effective at treating and curing the infection.[141] In a 2004 report from the IDSA (Infectious Disease Society of America), approximately 2 million individuals in the United States alone will acquire a bacterial infection.[141] Out of these infected people,
approximately 90,000 will succumb to the infection.[141] Bacterial strains that are resistant to one or antimicrobials make up more than 70 percent of these 2 million infections.[141]

As the number of bacterial and other microorganism such as fungi and protozoa, become immune to antibiotics, antifungal, or antiprotozoal pharmaceuticals, the approval rate for new antimicrobials diminished. Particularly, though new antimicrobial drugs are being synthesized, few new mechanisms of action have been discovered.[142]

1.2.B. Acquisition of antimicrobial resistance

Antimicrobial resistance is acquired through three main factors: plasmid exchange, random mutation, and zoonotic transfer.

Plasmid exchange is the lateral gene transfer of DNA or RNA via plasmids.[143] Resistance genes may be transferred from one microorganism to another microorganism and does not involve the mechanism of heredity.[143] Due to this process, one strain in which a particular antibiotic is effective may become resistant to that antibiotic via the acquisition of resistance genes from another resistant strain. Therefore, any resistant strains that exist in the environment may pass along their resistance, producing drug and multi-drug resistant organisms.[3] Plasmid exchange is very common among bacteria and is one of the most important causes of increased drug resistance.

Horizontal gene transfer can occur through three main mechanisms.[144] These mechanisms are transformation, transduction, and conjugation.[144] Transformation is the genetic alteration of a cell from uptake and expression of foreign genetic material, which may be either RNA or DNA.[144, 145] Transduction is the process in which bacterial DNA is moved from one bacterium to another by a bacterial virus, a bacteriophage.[144, 145] The
last of these is bacterial conjugation in which through direct cell-to-cell contract, genetic material is transferred.[144, 145]

Random mutation is a randomly derived change to the nucleotide sequence of the genetic material of an organism.[144] On the small scale random mutations include point mutations, insertions, and deletions.[144] Point mutations are mutations in which only a single base pair is altered or changed.[144, 145] These mutations may be caused by two main processes. Errors during the replication of DNA may cause the incorrect insertion of a base in the polynucleotide chain during DNA replication.[144, 145] Secondly, DNA bases may be changed directly via contact with chemical compounds.[144, 145] Insertions are the process in which segments of additional genetic material are added into the DNA sequence.[144, 145] Insertions are often caused by transposable elements, which are stretches of DNA that may move from one site on the chromosome to another.[144, 145] Insertions also may occur, though infrequently, through mistakes in DNA or RNA replication or recombination, or through exposure to acridines. Deletions may occur during insertion of a new DNA or RNA sequence.[144, 145] Deletion is the removal of a stretch of polynucleotide in the DNA or RNA segment.[144, 145] Deletions may be caused by exposure to acridines, mistakes in DNA or RNA replication, and commonly occur as part of insertion mutations or transposition mutations.[144, 145]

On the large scale random mutations include amplifications, deletions, chromosomal translocations, interstitial deletions, and chromosomal inversions. Amplifications are the production of additional copies of a nucleotide sequence.[145] These extra sequences are found either in intrachromosomal or extrachromosomal DNA or RNA.[145] Chromosomal translocations occur when a segment of genetic material detaches from the chromosome and
Chromosomal inversions are the inversion of a segment of a chromosome into the same site.[144] Zoonotic transfer of bacterial resistance relates to zoonosis, which is the infection of a human being with a disease that can be transmitted from a non-human animal. Humans can contract zoonotic diseases through direct contact with wild or domestic animals. Such labors as pig farming and ranching can result in zoonotic transfer. Dairies, fairs, soil, and food and water contaminated with animal feces are also highly likely sources of zoonoses. Zoonoses included bacteria, viruses, fungi, parasites, and prions. Examples of zoonotic diseases are anthrax, brucellosis, bubonic plague, and toxoplasmosis. Animals treated for certain infections with antibacterials or antiviral drugs may develop resistance strains. These drug resistant strains can then infect humans, causing a disease that may be very difficult to treat. Zoonotic antibiotic resistant infections are also on the rise, due to the overuse of antibiotics in humans and the use of antibiotics in agriculture and veterinary medicine. Furthermore, the resistance genes in zoonotic bacterial strains may be able to cross into an unrelated bacterial strain through plasmid exchange.

1.2.C. The mechanisms of antimicrobial resistance

Antimicrobial resistance is obtained through four main processes. These processes are drug modification or inactivation, alteration of the target site, alteration of the metabolic pathway, and reduced drug accumulation in the infected host.[3, 142, 143]
1.2.D. Antimicrobials and resistance mechanisms

1.2.D.1. Beta-Lactam resistance

The primary cause of resistance to beta-lactam antibiotics is the catalytic function of beta-lactamases.[17, 146] Inactivation of this family of antibiotics is caused by the enzymatic hydrolysis of the beta-lactam ring.[146] Current theory holds that these beta-lactamases evolved from penicillin-binding proteins.[146-152] Beta-lactamases are categorized into four classes, A, B, C, and D.[17, 146, 153-155] Each of these classes represents a different catalytic mechanism by which the lactamases cleave the beta-lactam and, therefore, enable resistance to this family of antibiotics.[146] A series of enzymes named extended-spectrum beta-lactamases (ESBLs) are also problematic and over 250 variants have been detected.[17] Therefore, strains of Enterobacteria can become resistance to cefotaxime, ceftriaxone, and ceftazidime.[17] To make matters worse, other species can easily acquire these enzymes.[17]

1.2.D.2. Aminoglycoside resistance

The roots of aminoglycoside resistance lies in four general mechanisms.[156] Altered uptake, target modification, chemical modification, and antibiotic efflux all contribute to the growing resistance to aminoglycosides.[156]

Uptake of aminoglycosides requires energy in order for transportation across the cell membrane.[157] Mutations that can confer aminoglycoside resistance are, therefore, those which can alter the membrane potential.[156, 157]

Target modification of the point in which the aminoglycoside antibiotic acts occurs through two mechanisms which are the methylation of the 16S rRNA and point mutation of ribosomal proteins or ribosomal RNA.[107, 156]
Chemical modification is possibly the most important mechanism in conferring resistance to aminoglycosides.[17, 156] A series of enzymes that act to adenylate, acetylate, or phosphorylate antibiotics are responsible for this resistance.[156] These enzymes are \( O \)-phosphotransferases, \( N \)-acetyltransferases, and \( O \)-nucleotidyltransferases.[17, 156] The sheer number and diversity of these enzymes in addition to enzyme inhibitor transportation across the cellular membrane, provide enormous challenges for the development of inhibition of these enzymes.[156]

Antibiotic efflux is also of great important and mostly a problem in Gram-negative organisms.[156] This mechanism of resistance exists due to the presence of the tripartite resistance-nodulation-division (RND) family of efflux proteins.[156] In Gram-negative organisms, such as \textit{E. coli}, AcrAD, a TolC-associated aminoglycoside efflux pump is responsible for the export of the aminoglycoside from the cytoplasm and periplasm.[156]

1.2.D.3. Quinolone resistance

As previously stated in the mechanism of action by quinolones, the targets of action are DNA gyrase and topoisomerase IV.[107] Target modification of either of these targets can result in resistance.[107] Point mutation can achieve this effect through a change in a single amino acid.[107] Mutations in the amino terminal domains of GyrA or ParC, as well as in GyrB and ParE have been shown to cause quinolone resistance.[107, 158] Changes in topoisomerase IV has the most effect in Gram-positive organisms, whereas, a mutation to the DNA gyrase elicits resistance more in Gram-negative species.[107, 159] Resistance in quinolones, with the exception of Qnr, plasmid mediated, proteins, is entirely chromosomal.[17, 160-164] Qnr proteins have the ability to protect DNA gyrase from
quinolones and have reached a wide distribution a variety of bacterial genera and plasmid environments .[17, 162]

1.2.D.4. Rifamycin resistance

Rifamycin resistance occurs due to mutations in the \( rpoB \) gene, specifically those which encode amino acid changes in the beta-subunit of the RNA polymerase.[107, 165] The highly conserved regions in which the mutations take place in the \( rpoB \) gene are thought to be involved in the polymerase antitermination process.[107, 165, 166] Connection between these mutations and the resistance effects have yet to be fully made, though it is theorized that they reduced the RNA polymerase affinity for rifampin.[107] \( E. \) coli, \( S. \) aureus, \( Mycobacterium \) leprae, \( S. \) pneumonia, \( Neisseria \) meningitides, and \( M. \) tuberculosis have all been shown to acquire resistance to rifamycins, specifically rifampin, through mutations in similar regions of the \( rpoB \) gene.[107, 147, 167-172]

1.2.D.5. Oxazolidinone resistance

The origin of oxazolidinone resistant strains is still under investigation.[107] It is deemed unlikely that mutation is responsible as multiple genomic copies of rRNA genes are present in most human pathogens including \( staphylococci \) and \( enterococci \).[107] Linezolid, the only oxazolidinone antibiotic in clinical use, has met with resistance in strains of VRE.[107, 147, 173, 174] Selective pressure in general has been ruled the most likely mechanism in which oxazolidinone resistance has been acquired by the vancomycin resistant \( Enterococcus \) faecalis.[107, 147] There is also a rough correlation between clinical isolates of \( E. \) faecalis which have a G2576T mutation on the 23S rRNA genes and the level of oxazolidinone resistance.[147, 175] Linezoid is the only oxazolidinone approved for clinical
use and is used to combat hospital acquired VRE nosocomial infections.[147] Current large surveillance studies have reported that linezoid resistance is rare in Enterococci.[176-180]

1.2.D.6. Macrolide resistance

Macrolide resistance stems from posttranscriptional alteration of a specific base of rRNA that results in ribosomes with reduced drug affinity.[107, 181] Methylation of an adenine at position 2058 of 23S rRNA occurs through the action of erythromycin resistance methylase.[107] Dimethylation at this locus engenders a high-level resistance to all generations of macrolides antimicrobials.[107, 182] Telithromycin, the only clinically available ketolide, is relatively unaffected by the monomethylation of A2058, but dimethylation of the site results in resistance.[107, 183] Macrolide resistance in Gram-positive bacteria can result from efflux mediated by \textit{mef} genes.[147, 184]

1.2.D.7. Glycopeptide resistance

Vancomycin resistance is achieved through the substitution of D-lactate for the terminal D-alanine of the peptide side chain.[107, 185] Vancomycin resistance in MRSA strains has been attributed to several abnormalities in these strains.[107] Enhanced autolysis, increased production of PBP-2, and cytoplasmic cell wall precursor monomer UDP-N-acetylmuramyl-pentapeptide, and enhanced incorporation of \textit{N}-acetylglucosamine into the bacterial cell wall contribute to the glycopeptide resistance in methicillin resistant \textit{Staphylococcus aureus}.[107, 186] In addition, the theory of the ‘false target model’ has been put forth due to the increased numbers of uncrosslinked pentapeptide chains which retain their binding affinity for vancomycin and the increase in cell wall thickness.[107, 187, 188] Non-critical binding of vancomycin to these targets may act to protect the active site of the
transglycosylase enzymes near the cell membrane.[107, 188] Vancomycin resistance has become increasingly widespread.[147, 189] In 2003, it was reported that 28.5% of enterococci infections in hospitalized patients in the U.S. intensive care units were vancomycin resistant, according to the National Nosocomial Infections Surveillance System.[147, 190] In 2004, an independent study that the percentage of *E. faecium* isolates that were vancomycin resistant was 72%.[176] Contamination of some hospital rooms has become so extreme as to warrant four hour long cleaning procedures and the gastrointestinal systems of patients that acquire VRE may persist in this condition for years.[147, 191, 192]

1.2.D.8. Tetracycline resistance

TetM, TetO, and OtrA are tetracycline resistance proteins that protect ribosomes from tetracycline, through interaction with the bacterial ribosome.[107, 147, 193-197] Research into the function of TetM suggests that it acts catalytically with tetracycline in addition to elongation factor, EF-G.[107, 198, 199] TetM does not prevent the binding of tetracyclines, but does catalytically control the release of tetracycline from the ribosome as well as acting as a competitive inhibitor.[107, 200] Drug efflux is also a main cause of tetracycline resistance which takes place in enterococci.[17, 107, 147] Tigecycline has been developed which can treat most tetracycline resistant strains.[17, 201, 202] Inhibitors of Tet efflux proteins have also been a recent development in combating tetracycline resistance.[17, 203]

1.2.D.9. Trimethoprim resistance

Exogenous acquisition of drug-resistant dihydrofolate reductases on transposons or plasmids are the most common way a species will acquire trimethoprim resistance.[17, 107, 204-207] Family 2 dihydrofolate reductases are subdivided into three classes.[107, 207] All
classes in this family of DHFRs have exceedingly high levels of resistance to trimethoprim. These trimethoprim resistance DHFRs are encoded by genes on mobile genetic elements. These included plasmids and transposons which are able to easily spread among bacteria.

1.2.D.10. Lipopeptide resistance

Resistance to the lipopeptide, daptomycin, has been noted in a few cases since its employ into clinical use. Lipopeptides may disrupt cell integrity which results in cell depolarization. Due to a lack of pertinent information concerning the mechanism of action of daptomycin, no resistance mechanism has been put forth.

1.2.D.11. Chloramphenicol resistance

Chloramphenicol acetyltransferases inactivate chloramphenicol. These enzymes are encoded on by genes on conjugative and non-conjugative resistance plasmids. They may also be present on the enterococci chromosome. Energy-driven efflux of chloramphenicol has also been documented.

1.2.D.12. Fluoroquinolone resistance

One mechanism or a combination of both mechanisms, infer fluoroquinolone resistance in Gram-negative species. Drug efflux and mutation are responsible for fluoroquinolone resistance, particularly in Enterococcus. Mutations in the genes that code for the topoisomerase target proteins impart resistance as do mutations in parC which encode for the A subunit of DNA gyrase, and subunits of gyrA and topoisomerase IV. Further mutations in the emeA gene and efrB gene that encode for an
enterococcus efflux pump and an ABC efflux pump respectively cause an increase in uptake
and export of the fluoroquinolone antibiotics.[147, 222-224]

1.3 Modern drug discovery methods

1.3.A. Introduction

Drug discovery consists of several lines of investigation including natural product
screening, synthesis, combinatorial chemistry, combinatorial biosynthesis, and the use of
synthesis, specifically combinatorial chemistry in conjunction with natural product screening.

Natural product screening is the isolation and characterization of potentially
pharmaceutically active biological molecules.[225] These molecules are derived from an
enumerable number of sources, which include bacteria, fungi, molds, plants, and marine
organisms. Synthesis is the artificial construction of both novel molecules and biological
molecule mimics using chemical techniques. Combinatorial chemistry is the use of synthesis
to create large libraries of compounds.[226] Combinatorial libraries mostly consist of small,
stereochemically poor products. Combinatorial biosynthesis is the use of knowledge of the
biosynthetic pathway, such as a polyketide biosynthesis pathway for the synthesis of a
macrolide like erythromycin, to alter it chemically in vivo.[227] These alterations involve
the addition and/or insertion of plasmid DNA or RNA into the producing strain of bacteria.
Such genetic alterations are commonly achieved with the introduction of a plasmid carrying
the desired segment. Combinatorial chemistry in conjunction with natural product screening
can be used to synthesize complex and difficult to synthesize natural products, allowing for
the efficient production of large libraries of compounds.[228] Combinatorial chemistry does
not involve genetic change, simply the use of a natural product as a scaffold which can be
elaborated using organic synthetic techniques to introduce foreign moieties for ease of
chemical modification. The introduction of easily modifiable chemical moieties is key to the production of large and quickly synthesized natural product analogue libraries.

1.3.B. Natural product screening

Compounds produced in nature were among the first substances used to combat infection.[11] These substances were derived from flora and fauna. This pharmacopeia of folklore medicines has been used as a standard medicinal arsenal for centuries.[11] Modern chemical techniques isolate the active component or components of various biological entities such as plants and molds. Thus, natural product research continues to be an active area of drug discovery. Many of the antibiotics previously mentioned, such as the penicillins and macrolides, are secondary metabolites of mold and bacteria respectively.

In the past 20 years, a major shift has occurred away from natural products and toward combinatorial libraries due to the advent of high throughput screening (HTS). [228] High throughput screening allows for the screening of large libraries of compounds that can be provided through combinatorial synthesis.[228] The value of natural products has not been in question; the efficiency in investigation of natural products has.

Natural products are compounds that are often low in sulfur and halogens and often contain such chemical groups as carboxylic acids, alcohols, esters, amides, and ethers. Natural products on average contain a fairly high number of ring structures. These structures are secondary metabolites (i.e. products from metabolic pathways that are necessary for the sustainment of life for an organism), and are often stereochemically rich. High numbers of chiral centers in conjunction with hydrogen bond donors allow for complex three dimension structures that can interact with target enzymes within organisms.[228] Some natural
products, such as those produced by microorganisms, can be synthesized in large quantities via culture and subsequent fermentation.

1.3.C. Synthesis

Synthesis is the mechanism by which molecules can be made artificially. Synthesis includes natural product synthesis, combinatorial synthesis, target oriented synthesis, and diversity oriented synthesis. Natural product synthesis, also called total synthesis, includes all synthetic steps necessary to re-create a natural product. Examples of this are the total synthesis of Taxol 37 and platencin 38.[229, 230]

![Taxol Structure](image1)

**Figure 1.36:** Structure of Taxol 37.

Taxol 39 is a diterpene which produced by plants of the genus *Taxus* and isolated from the bark of *T. brevifolia*. Taxol is used as a treatment of ovarian and breast cancers.[231]

![Platencin Structure](image2)

**Figure 1.37:** Structure of Platencin 38.

Platencin 38 is a potent inhibitor of bacterial fatty acid biosynthesis (Fab) and displays a broad-spectrum of activity against drug resistant pathogens such as methicillin-, macrolide-, and linezolid-resistant *S. aureus*, vancomycin intermediate *S. aureus*, vancomycin-resistant enterococci, and *Streptococcus pneumoniae*. [232] Target oriented synthesis is the synthesis of a particular compound designed to bind to specific receptor sites that have been
characterized in advance. Diversity-oriented synthesis is the synthesis of many compounds, varying in chemical groups, topography, stereochemistry, and regiochemistry. The aim of diversity-oriented synthesis is the construction of a variety of structures to test against a variety of targets in order to establish activity.

1.3.D. Combinatorial synthesis

Combinatorial synthesis is used to construct large libraries of compounds that have a low molecular weight, are stereochemically poor, uncomplicated and have a diversity of functional groups.[233] Combinatorial chemistry can exploit solid supports for ease of purification or can be free in solution.

1.3.E. Combinatorial biosynthesis

One significant problem in natural products research is the lack of selective chemical ‘handles’, or in other words, functional groups that provide for ease of structural diversification of the natural product. The lack of chemical ‘handles’ limits the synthesis of derivatives and analogues, which may be necessary in order to produce a product that has greater bioavailability, acid stability, activity, limits toxicity, and resists quick elimination from the body. Combinatorial biosynthesis can provide a way around this dilemma.

Combinatorial biosynthesis is the combinatorial construction of secondary metabolite biosynthesis gene clusters with the goal of generating new “non-natural” natural products. By using genetic recombinatorial techniques, changes can be made to the order in which the end product is assembled and, therefore, produce a novel natural product in vivo or a derivative of an already existing entity.[228, 234]
1.3.F. Natural products in combinatorial synthesis

Natural products are often stereochemically rich, topographically complex, contain ring structures, and can be produced through culture and fermentation processes, which eliminates the need for complex, time-consuming syntheses of similar compounds.[228] However, natural product isolation lacks the chemical diversity, large library synthesis, and high-throughput screening available in combinatorial synthesis.[228] A combination of these methods involves creating natural product-like compounds. After extraction of the desired natural product from fermentation, this product may be subjected to synthetic modification to create easily modifiable chemical moieties which can be exploited through combinatorial chemistry.[228] Once the natural product-like scaffold has been synthesized, thousands to millions of compounds can be synthesized using simple chemical techniques. Increases in the number of different moieties can be used to synthesize ever greater numbers of natural-product-like molecules that combine the chemical diversity of combinatorial compounds with the high rate of biological activity of natural products.[233] Not only is this harnessing of potentially vast libraries of biologically produced complex entities the latest evolution of combinatorial and natural product synthesis, it provides a strong and compelling strategy in modern drug discovery.[228]

1.4 Summary

Highly efficient combinatorial synthesis combined with scaffolds derived from nonactin, a natural product, was used to make triazoloester libraries of potentially antimicrobial compounds. The triazoloester library was assayed for antimicrobial activity. The impact of stereochemistry and structure on the biological activity of compounds exhibiting antimicrobial activity was studied as well as the necessity of the natural product
component of the antimicrobial active compounds for biological activity. Though many compounds can be synthesized from a single scaffold, the development of more than one scaffold from a single natural product is advantageous in the search for antibiotic compounds. Therefore, other scaffolds were synthesized from nonactin and libraries of compounds produced from these scaffolds. Finally, the synthesis of analogues of nonactin was attempted in order to develop a library from this ionophore antibiotic.
CHAPTER 2

METHYL NONACTATE AS A NATURAL PRODUCT SCAFFOLD FOR COMBINATORIAL SYNTHESIS

2.1 Why is methyl nonactate a good scaffold?

![Methyl Nonactate](image)

Figure 2.1: Structure of methyl (±)-nonactate 45, post-primary scaffold for the triazoloester nonactic acid analogue libraries.

Methyl nonactate 45 and, therefore, nonactic acid 49, 50 are highly useable and valuable structures for drug discovery.[235] Methyl nonactate 45 is a stereochemically rich structure (Figure 2.1). Gram quantities of highly pure enantiomers of methyl nonactate 46, 48 can be produced through the addition of racemic methyl nonactate to a fermentation of *Rhodococcus* and can then be used to synthesize enantiomerically pure primary scaffolds of the triazoloester nonactic acid analogue libraries (Scheme 2.1).[7]

![Enantiomers](image)

Figure 2.2: Enantiomers of nonactic acid and methyl nonactate.
Chemical ‘handles’ available on this structure make methyl nonactate 45 a compound from which many analogue scaffolds can be made and from these scaffolds, large derivative libraries.

\[
\begin{align*}
\text{46} & \quad \leftrightarrow \quad \text{48} \\
\text{(+)-triazoloesters} & \\
\text{(-)-triazoloesters}
\end{align*}
\]

**Scheme 2.1:** Overall scheme of triazoloester library synthesis from 46 and 48.

The secondary alcohol was conveniently available for esterification, which led to ester scaffolds, from which further modification could be initiated on the carboxylic acid (Scheme 2.1). The carboxylic acid was reduced to a primary alcohol, which was substituted with azide after tosylation. This allowed for the formation of substituted triazoles using terminal alkynes using the Huisgen’s 1,3-dipolar cycloaddition reaction under Sharpless conditions, which regioselectively formed 1,4-triazoles (Scheme 2.1).[236, 237]

The stereochemically rich structure 40 is available on the gram scale, which is made by *Streptomyces griseus* and extracted using standard procedures. Synthesis of the plus and minus nonactic acid 49, 50 is complicated, uses many steps, and results in low yields of the final products, resulting in only milligrams for use in scaffold synthesis.[238] Biosynthesis using fermentation cultures to produce nonactin 40 (Figure 2.3) followed by purification via crystallization, methanolysis, chromatographic separation, and stereoisomer separation via *Rhodococcus* (Scheme 2.2) is cost and time efficient as well as productive, producing
material 49, 50 in gram scale quantities.[7] Gram scale quantities are essential not only for the production of libraries from nonactic acid and nonactic acid analogue scaffolds, but for the mass scale production of commercial pharmaceutical compounds that may come from these libraries. High production of these compounds at lower cost and with less time will help make potential drugs more affordable and, therefore, commercially attractive.

2.2 How do we make methyl nonactate?

![Chemical Structure](image)

40, nonactin

### Figure 2.3: Naturally occurring macrotetrrolides 39, most importantly nonactin 40.

The parent compound of a group of ionophore (Na⁺ and K⁺) antibiotics called macrotetrrolides 39 (Figure 2.3) is nonactin 40, which is produced by *Streptomyces griseus* ETH A7796.[239] Acetate, propionate, and succinate are known to be units in the polyketide biosynthesis pathway that assemble to form nonactin 40.[239, 240] The nonactin 40 structure is a cyclic tetraester ionophore which is composed of four monomeric subunits, two plus enantiomers of nonactic acid 49 and two minus enantiomers of nonactic acid 50 (Figure 2.3).[238, 241, 242] The plus and minus subunits 49,50 are alternately joined to form a 32-
membered macrocycle.[238, 241] Though each subunit is chiral, the $S_d$ symmetry of the nonactin macrotetrolide 40 makes nonactin achiral, a meso compound.[238, 241]

Nonactin 40 has been shown to have both antibiotic and antitumor activity.[241, 243] It possesses antitumor activity against mammalian cell lines in vitro and in mice, against Crocker sarcoma 180.[240] Nonactin 40 has also been shown to be an inhibitor of the 170-kDa P-glycoprotein-mediated efflux of 4-$O’$-tetrahydropyrylidoxorubicin in multidrug-resistant erythroleukemia K562 cells at subtoxic concentrations.[240] Highly purified nonactin 40 is also commercially important for use in ammonium-sensitive electrodes.[235]

Fermentation of *Streptomyces griseus* yields nonactin 40, which is isolated from the fermentation by standard natural product extraction and isolation procedures.[235] The methods in Dinges J.M. et al. detail the extraction of the secondary metabolite from cell paste with acetone. After evaporation, the concentrated residue of the macrotetrolide mixture is then fractionated on a silica gel column and crystallized using standard methods to give nonactin crystals. The nonactin crystals are then subsequently recrystallized several times until a high purity is reached. A typical fermentation yields 5-10 g of nonactin 40 per liter.[235]

Methanolysis of the nonactin tetramer gives two plus and two minus nonactate subunits 49,50.[235] Methanolysis is carried out by refluxing nonactin 40 in 5% $\text{H}_2\text{SO}_4$ / MeOH for 12 hours. The nonactate subunits 49, 50 provide a powerful scaffold.
**Scheme 2.2:** The *Rhodococcus erythropolis* enzymatic separation of methyl (±)-nonactate 45 into 46 and 48.[7]

Perhaps the most important aspect to the nonactate scaffold 45 and its production from *Streptomyces griseus* also presents one of the most important problems. The stereochemistry provided by the natural product subunit is set biologically instead of chemically, but a racemic mix of the plus and minus enantiomers 45 is given by the methanolysis of nonactin 40. In order to meet the requirements of the study of stereochemically pure natural product libraries and the requirements of drug testing, the enantiomers were separated from each other. Enantiomers, however, have identical chemical properties, and, therefore, identical polarities which do not allow for their separation via chromatography on silica gel. A common, but costly way to separate out enantiomers is the use of Mosher’s ester to create diastereomers, which can be separated by chromatography on silica gel due to their distinct and different chemical properties. Mosher’s ester, however, is commercially very costly to buy. This cost limits the amount of material that can be separated. Therefore, in order to separate out the plus and minus nonactic acid stereoisomers 49, 50 another method was developed that would allow separation and purification on a gram scale and avoid expensive reagents. Racemic methyl nonactate 45 in shake flask cultures of *Rhodococcus erythropolis* allows for an efficient resolution (Scheme 2.2).[7] *Rhodococcus erythropolis* selectively oxidizes the secondary alcohol of the methyl (–)-nonactate 48.
making a ketone under aerobic conditions. The differences in polarity between the alcohol and the ketone functional groups allow for separation of the enantiomers on a silica gel column. The oxidized minus methyl nonactate 47 can then be reduced under anaerobic conditions by *Rhodococcus erythropolis* to give methyl (−)-nonactate 48. Therefore, both enantiomers of methyl nonactate can be separated from each other in excellent yields and with enantiomeric purity.[7] *Rhodococcus* fermentation is exceptionally cost effective and the separation method can be applied to grams of the racemic starting material.

### 2.3 Conclusion

Methyl nonactate is a versatile scaffold for the synthesis of libraries. It is a stereochemically complex molecule with readily modifiable alcohol and ester groups. It is derived from fermentation and methanolysis as opposed to a lengthy and expensive synthesis. The ability to separate out the enantiomers with inexpensive *Rhodococcus* fermentation makes methyl nonactate ideal for use in drug discovery.
CHAPTER 3

SYNTHESIS AND EVALUATION OF A COMBINATORIAL LIBRARY BASED UPON METHYL NONACTATE

3.1 Introduction

The primary library was created via the synthesis of an analogue to nonactic acid, which was developed as an azidoalcohol nonactic acid analogue scaffold 55. Through a short series of steps, all of which had good to excellent yields, the azidoalcohol 55 was synthesized. The azide and the secondary alcohol are easily modified by well-known synthetic techniques to produce triazoloesters (Scheme 3.1).

![Scheme 3.1](image)

**Scheme 3.1:** Overall scheme for the synthesis of the racemic triazoloester library from 45.

To build the triazoloester library, the secondary alcohol of the azidoalcohol nonactic acid analogue scaffold 55, which was synthesized from 45, was first esterified with a series of acyl chlorides, followed by the formation of a substituted triazole from the primary azide (Scheme 3.2).
3.2 Generation of scaffold material

Scheme 3.2: Synthesis the azidoalcohol (±)-nonactic acid analogue scaffold 55.

The azidoalcohol (±)-nonactic acid analogue scaffold 55 was synthesized with a 36.2% overall yield from methyl (±)-nonactate 45 (Scheme 3.2). The secondary alcohol of 45 was then THP protected using DHP in dichloromethane. The THP protection of the secondary alcohol went smoothly and quickly. The protection produced diastereomers which were purified and collected as a whole with minimal separation. The primary alcohol 52 from the reduction of the ester was purified by flash chromatography and dried to produce the protected nonactate alcohol. This primary alcohol 52 was then activated with pTsCl and pyridine into order to allow for the substitution of the alcohol for an azide. The protection of the secondary alcohol was necessary in order to assure that only the primary alcohol would be tosylated and replaced by azide. Though it has been shown that reduction of nonactate to a diol can be easily done, the following tosylation would depend upon a selective activation of a primary alcohol which was not a good strategy. The latter approach has been shown to
be slower, and does not produce high yields. Protection of the secondary alcohol is therefore necessary and uses an inexpensive and efficient method. The tosylation of the primary alcohol 53 was then followed by the azide substitution with sodium azide in DMF at 80 °C which quickly afforded the protected azide 54. The last remaining step was the straightforward removal of the THP protecting group by hydrochloric acid in methanol to give 55. The reaction was almost immediate but was given time to ensure good yields. With the removal of the THP group, the purification of the target scaffold produced was very simple and done by chromatography on silica gel. Each step is high yielding and efficient.

3.3 Diversification of scaffold 55 to generate a combinatorial library

The azidoalcohol nonactic acid analogue scaffold 55 was used as the primary scaffold for the synthesis of a library of triazoloester (±)-nonactic acid analogue compounds. The secondary alcohol and the primary azide provide chemical handles for derivatization. Esterification of the secondary alcohol allows for derivatization of one side of the scaffold (Scheme 3.3). Huisgen 1,3-dipolar cycloaddition under Sharpless conditions to the primary azide provides derivatization of the other side of the scaffold via the synthesis of substituted triazoles in a regiospecific manner (Scheme 3.4). In the formation of the triazoloester library the secondary alcohols were esterified forming azidoester nonactic acid analogues 56. The azidoester nonactic acid analogues then underwent the Huisgen’s 1,3-dipolar cycloadditions to produce the triazoloester nonactic acid analogue derivatives 62. For the initial prototype library we chose five terminal alkynes and five acyl halides to give twenty-five possible library members. The identity of the appended structures is shown in Table 3.1. Out of the twenty-five possible structures, ten of these compounds were chosen for synthesis.
Table 3.1: Chart of triazoloester compounds and numbering scheme.

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Cl</th>
<th>MeO</th>
<th>ClPh</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>55</td>
<td>57</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>60</td>
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<td></td>
<td>64</td>
<td>66</td>
<td>67</td>
<td></td>
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<tr>
<td></td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheme 3.3: Synthesis of the azidoester (±)-nonactic acid analogue scaffolds 56.

These reactions were carried out at room temperature and easily finished within three hours. The percent yields of these reactions were generally 70-90% (Figure 3.1).
Figure 3.1: Overall yields of azidoester nonactic acid analogue scaffolds from 45.

Scheme: 3.4: Synthesis of the racemic triazoloester nonactic acid analogue library 62.

The azidoester scaffold 56 was used to make each of the final derivatives compounds, the triazoloesters (Scheme 3.4). This second reaction performed was the 1,3-Huisgen dipolar cycloaddition in which to each azidoester 56 a monosubstituted alkyne was added, followed by 0.3 M copper sulfate in solution and a 1.1 M ascorbic acid solution. These conditions are commonly known as Sharpless conditions which act to control the regiospecificity of the reaction and improve the reaction rate. The percent yield of product 62 of these reactions generally exceeded 90% (Figure 3.2).
3.4 Biological evaluation of initial library

The 72, 71, and 67 compounds were shown by assay, which was performed by Adrienne Smith, Dr. Erin Bolstad, and Dr. Brooke Martin, to be the most active members of the 25 membered of the triazoloester (±)-nonactic acid analogue library (Figure 3.2). These compounds were tested against *B. subtilis*, *B. anthracis*, *S. aureus*, *S. aureus* (MRSA), and *Ent. faecalis* (VRE).
Table 3.2: Inactivities of azidoester compounds.

Alamar-blue assays on the azidoesters revealed that they have no antibacterial activity in *B. subtilis* or *S. aureus* (Table 3.2).[244] Since no azidoester showed any significant activity as did the original scaffold, only potential for the triazoloesters remained.

Table 3.3: MCF10 IC$_{50}$ (µM) and IC$_{99}$ (µM) values for azidoester compounds.

Cytotoxicity assays against the MCF10 breast cell line showed that none of the azidoesters had any significant cytotoxicity (Table 3.3).[245]
### Table 3.4: MIC (µM) values of racemic triazoloester compound library.

Alamar Blue Assay of the ten triazoloester compounds synthesized from the azidoalcohol scaffold 55 showed antibacterial activity for three compounds. 72 showed activity against *B. subtilis* at 94 µM and against *S. aureus* at 1500 µM. 67 had effective antibacterial activity against *B. subtilis* at 62 µM and against *S. aureus* at 1000 µM. The lead compound, 71, showed antibacterial activity against *B. subtilis* at 375 µM and against *S. aureus* at 750 µM (Table 3.4).
Table 3.5: IC$_{50}$ (µM) and IC$_{99}$ (µM) values for the triazoloester compound library.

The degree of cytotoxicity for the triazoloester compounds differed greatly. The bactericidal compounds 72 and 71, showed the highest IC$_{99}$ values for cytotoxicity. As the ratio of bactericidal activity compared to cytotoxicity was the greatest for 72 and 71, these became the lead compounds (Table 3.5). These compounds became the focus of SAR studies in order to increase the bactericidal potency of the compounds and decrease their cytotoxicity.

As shown in Figure 3.3, B. subtilis exposed to varying concentrations of the 72 compound with a DMSO carrier resulted in an effect similar to plasmolysis.[244] The SEM images of B. subtilis exposed to concentrations of 72 show a ‘shriveling’ of the plasma membrane with discontinuities present at the cell poles (Figure 3.3). Osmotic perturbations were ruled out by measuring MIC values over a wide range of NaCl concentrations. It was found that the MIC values were independent of ionic strength.
Figure 3.3: SEM images of *B. subtilis* after treatment with 72.

3.5 Evaluation of scaffold stereochemistry upon antibacterial activity

One of the most important aspects of the developmental plan in the projects synthetics schemes is stereochemistry. Stereochemistry played a prominent role in the projects for several reasons, the first being that for the development of any pharmaceutical both enantiomers must be tested to know the biological activities of each stereoisomer. Thalidomide was an anti-nausea medication given to pregnant women to alleviate morning sickness.[246] One enantiomer of thalidomide was found to be responsible for the anti-nausea effect, the other enantiomer proved to be a powerful teratogen that caused severe developmental defects.[246] The cellular component that a drug interacts with: efflux pumps, enzymes, etc, determines its activity or inactivity. One of the major goals of this research is not only the discovery of new powerful antibiotics, but antibiotics that will be selective. Therefore, in order to discover which if not both enantiomers of the racemic 72 and 71 triazoloester compounds is responsible for their antibacterial behavior, 81 and 79 were synthesized using an azidoalcohol (−)-nonactic acid analogue scaffold 77 which was synthesized from (−)-nonactic acid 48. The (−)-nonactate was separated out of the racemic stock according to the aforementioned *Rhodococcus* procedure.[7]
Scheme: 3.5: Synthesis of the azidoalcohol (−)-nonactic acid analogue scaffold 77.

The scaffold 77 was derived from (−)-nonactic acid. The secondary alcohol of the methyl (−)-nonactate starting material was THP protected using DHP and a catalytic amount of pTsOH at room temperature in methylene chloride for 5 hours. The eastern ester portion of the molecule was then reduced to primary alcohol 74 using lithium aluminum hydride in THF at room temperature for 12 hours and tosylated with pTsCl in pyridine at room temperature for 5 hours to give 75. The tosylate 75 then served as a leaving group for the introduction of azide via sodium azide in DMF at 100 °C for 3 hours. Deprotection of the secondary alcohol was achieved by stirring at room temperature with 6 M HCl in methanol and water at room temperature for 3 hours. This resulted in the nonactic acid analogue scaffold 77 derived from (−)-nonactic acid. The yields of this synthesis scheme matched well with those of the synthesis scheme for the racemic compound.
Scheme 3.6: Synthesis of the 71 triazoloester nonactic acid analogue 79.

From the azidoalcohol nonactic acid analogue scaffold 77 the triazolo-ester nonactic acid analogue 79 was synthesized (Scheme 3.6). As with the racemic scaffold, the secondary alcohol was esterified using methyl 4-chloro-4-oxobutyrate in pyridine at room temperature for 3 hours. The azide of the resultant azidoester 78 was then reacted with N-(3-ethynyl-phenyl)-acetamide in a Huisgen 1,3-dipolar cycloaddition reaction with Sharpless conditions. The reaction was carried out in a shaker at 37 °C for 12 hours. Initial addition of the components turned the mixture a yellow-orange color, clouded with undissolved solid. After 12 hours, the solid disappeared from the mixture but an insoluble residue clung to the glass of the vial. However, both reactions were high yielding and the products were easy to purify by chromatography on silica gel.
Scheme 3.7: Synthesis of the triazoloester (–)-nonactic acid analogue 81.

To synthesize the minus 72 triazoloester nonactic acid analogue 81, the azidoalcohol 77 derived from (–)-nonactic acid was esterified with benzoyl chloride in pyridine at room temperature for 3 hours (Scheme 3.7). Dipolar cycloaddition under Sharpless conditions was used to synthesize the triazole 81 by the addition of 2-ethynyl-pyridine to the azidoester 80 in 5:1 ethanol in water (Scheme 3.7). The solution turned a dark olive green with solid suspensions; the solution was then shaken at 37 °C for 12 hours. As with the 72 solution, a thin solid residue clung to the glass of the vial after rinsing with ethyl acetate. The benzylation was high yielding but purification was troublesome as the product spot coincided closely with leftover benzoyl chloride on separation followed on a thin layer aluminum-backed silica-gel chromatography plate. The triazole formation reaction was high yielding and purification was simple using chromatography on silica gel which gave pure 81.
Scheme 3.8: Synthesis of the azidoalcohol (+)-nonactic acid analogue scaffold 86.

Compound 86, derived from methyl (+)-nonactate 46, was generated using methods similar to those employed in generating 77. The secondary alcohol of methyl (+)-nonactate 46 was THP protected 82 with DHP and pTsOH at room temperature for 5 hours. The product of the reaction was reduced by lithium aluminum hydride in THF at room temperature for 12 hours to a primary alcohol 83. The yield over these two steps was 67%. The easily purified product 83 was then tosylation to give 84 with a 73.4% yield, which then underwent nucleophilic substitution with sodium azide in DMF at 80 °C for 5 hours to give the THP-protected primary azide 85, a process that gave a yield of 80%. In the final step, the secondary alcohol was deprotected with 6 M HCl in water and methanol at room temperature for 12 hours, giving a final 67% yield of the plus azido-alcohol nonactic acid analogue scaffold 86.
Scheme 3.9: Synthesis of the 72-triazoloester (+)-nonactic analogue 88.

Analogue 88 was synthesized by an almost identical method to that of 81. Benzoyl chloride was added to 86 with pyridine at room temperature for 3 hours giving a 77.5% yield of the ester analogue 87. Compound 87 was then used in a second reaction after purification with 2-ethynyl-pyridine, 1.1 M ascorbic acid in water, 0.3 M copper sulfate in water, 5:1 ethanol in water solution, which was shaken at 37 °C for 12 hours. This gave a product 88 yield of 74.4% after chromatography of silica gel.

Scheme 3.10: Synthesis of the 71-triazoloester (+)-nonactic acid analogue 90.
The plus 71 triazoloester nonactic acid analogue 90 was synthesized by methods almost identical to that of the minus stereoisomer 79. Methyl 4-chloro-4-oxobutyrate in pyridine was added to the plus azidoalcohol nonactic acid analogue 86 at room temperature for 3 hours giving a 66% yield after chromatography on silica gel. N-(3-ethynyl-phenyl)-acetamide was then added in a 5:1 ethanol to water solution to the resulting azidoester 89 followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was then shaken at 37 °C for 12 hours, giving an 81% yield of 90 after purification.

![Chemical structures of compounds 72 and 71]

<table>
<thead>
<tr>
<th>Organism</th>
<th>88</th>
<th>81</th>
<th>72</th>
<th>90</th>
<th>79</th>
<th>71</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus</td>
<td>(+)</td>
<td>(−)</td>
<td>Racemic (+)</td>
<td>(−)</td>
<td>Racemic</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>Inact.</td>
<td>1500</td>
<td>187</td>
<td>Inact.</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>B. subtilis</td>
<td>47</td>
<td>Inact.</td>
<td>94</td>
<td>187</td>
<td>Inact.</td>
<td>375</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>750</td>
<td>Inact.</td>
<td>1500</td>
<td>375</td>
<td>Inact.</td>
<td>750</td>
</tr>
<tr>
<td>S. aureus</td>
<td>750</td>
<td>Inact.</td>
<td>1500</td>
<td>750</td>
<td>Inact.</td>
<td>750</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>62</td>
<td>Inact.</td>
<td>Nd</td>
<td>125</td>
<td>Inact.</td>
<td>nd</td>
</tr>
</tbody>
</table>

Table 3.6: MIC (µM) Values of 72 and 71.

Compounds 72 and 71 both showed significant antibacterial activity against a range of organisms. Table 3.6 highlights the importance of stereochemistry in drug design. The racemic compounds 72 and 71, showed activity as did the plus enantiomer. The minus enantiomers 81 and 79 showed no activity. This assayed proved that it is the plus enantiomer of 72 and 71 that are antibacterial.
<table>
<thead>
<tr>
<th>Strain</th>
<th>88</th>
<th>81</th>
<th>90</th>
<th>79</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. anthracis</em></td>
<td>750</td>
<td>Inactive</td>
<td>375</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>1500</td>
<td>Inactive</td>
<td>187</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>47</td>
<td>Inactive</td>
<td>187</td>
<td>2000</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>500</td>
<td>Inactive</td>
<td>250</td>
<td>nd</td>
</tr>
<tr>
<td><em>E. faecalis van</em></td>
<td>62</td>
<td>Inactive</td>
<td>125</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>750</td>
<td>Inactive</td>
<td>750</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>S. aureus</em> MRSA</td>
<td>125</td>
<td>Inactive</td>
<td>250</td>
<td>750</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>250</td>
<td>Inactive</td>
<td>250</td>
<td>nd</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>64</td>
<td>Inactive</td>
<td>125</td>
<td>nd</td>
</tr>
</tbody>
</table>

Table 3.7: MIC ($\mu$M) Values for 88, 81, 90, and 79.

Compounds 88, 81, 90, and 79 were tested against a wide range of pathogens (Table 3.7). Significant antibacterial activity was shown against *B. subtilis*, *E. faecalis van*, *S. aureus* MRSA, *S. epidermidis*, and *S. pyogenes* for compound 88. The minus enantiomer 81 of 88 was found to be completely inactive against all the pathogens it was tested against. Compound 90 was found to have antibacterial activity against *B. cereus*, *B. subtilis*, *E. faecalis*, *E. faecalis van*, *S. aureus* MRSA, *S. epidermidis*, and *S. pyogenes*.

Modern drug discovery dictates that both enantiomers of a racemic mix must be tested for activity in order to determine which enantiomer gives rise to the biological action and, in the course of testing, determine the safety of each enantiomer. The *Rhodococcus* fermentation (Scheme 2.1) has allowed for the resolution of nonactic acid. Once the enantiomers had been separated, the pure enantiomers of 71 and 72 were synthesized and tested for activity. The assay showed very low activity for 81 and 79. The plus enantiomers, however, showed a marked increase in potency compared to that of the 50/50 mixture. This increase was double the activity of its racemic counterparts (Table 3.6).

In summary, this library was generated using a biologically active natural product, nonactin 40, from which the methyl nonactate scaffold 45 was generated. This scaffold was
then used to generate the azidoalcohol nonactate scaffold 55 which was used in turn to synthesize a library of compounds, in which members of which were shown to have antimicrobial activity. Pure plus and minus enantiomers of the azidoalcohol nonactate scaffold 86, 77 were synthesized and the enantiomerically pure antimicrobial compounds generated from each enantiomer. Assays revealed the plus enantiomer was responsible for the activity.

Though not entirely conclusive, the activity of only the plus enantiomer points to the specificity of the target site for these two compounds. It may also be noteworthy that out of only ten of the triazoloester nonactic acid analogue derivatives synthesized (Figure 3.2) two were found to have good activity.

### 3.6 Regiochemistry

Regiospecificity is also a major consideration for drug development just as stereospecificity is. Interactions between a potential drug and its binding site depend greatly on the molecular weight of the drug, its polarity, how lipophilic or hydrophilic it is, surface topology, weak or strong interactions and therefore binding with the drug target in order for the drug to fit in the right ‘pocket’. The stereospecificity of the nonactic acid analogue libraries can be controlled by the separation of enantiomers, and the organic synthesis tools used to make analogues with respect to making epimers. Regiospecificity in these analogues can also be controlled by the single site of regiodiversity. This site is the triazole made from the azide portion on the eastern side of the azido-esters such as 60 and 61. Using Sharpless conditions, the 1,4-triazole regioisomer dominates over the 1,5-triazole regioisomer in such a way by virtue of the reaction mechanism. The binding to the copper ligand to the terminal end of the alkyne allows for the reaction to proceed only one way by generating a copper
acetylide species. This gives the 1,4-triazole as the exclusive product. Under thermal conditions however the reaction favors the formation of the 1,5-triazole even with Sharpless conditions. In order to determine if regiospecificity is important to the triazoloester nonactic acid analogue libraries, both regioisomers were synthesized.

**Scheme 3.11:** Synthesis of the 1,5-triazoloester (+)-nonactic acid analogue 91.

Starting from a racemic amount of the azidoalcohol nonactic acid analogue scaffold 55, the secondary alcohol was esterified with benzoyl chloride in pyridine at room temperature for 3 hours (Scheme 3.11). The product 61 was purified by chromatography on silica gel and used directly in the dipolar cycloaddition (Scheme 3.11).

In the attempt to form the 1,5-triazole, the ester was added to a 5:1 ethanol to water solution and 1.1 M ascorbic acid in water was added, as was 0.3 M copper sulfate in water, along with 2-ethynyl-pyridine. The reaction was heated around 100 °C for 12 hours. The yield of the final product 91 of the reaction was 69%.

The IR, MS, and NMR data support the conclusion that the product of this reaction is a triazolo-ester nonactic acid analogue compound. The only spectral differences in 91 were changes in the aromatic region of the 1H NMR and those of the single proton attached to the
triazole. It was thought that the chemical shift of the triazole proton was shifted relative to that of the 1,4-triazole. However, it remained inconclusive by virtue of reliance on chemical shift data. Therefore, a 2D-NOE was run so that a through space correlation could be seen between the pyridine protons and the neighboring methyl group. Unfortunately no correlation was ever noted. A similar 2D-NOE was run on a sample of 72 but no correlation was noted between the 2-position methyl group and the triazolo proton. Since one of these interactions must exist, the logical conclusion is that the 2D-NOE was in some way faulty, possibly due to insufficient deoxygenation of the sample or because the positions of the protons relative to each other are too far away, resulting in little or no signal.

Scheme 3.12: Synthesis of the 1,5-triazoloester (+)-nonactic acid analogue 92.

The racemic analogue 55 was used to synthesize the racemic 1,5-triazole 92 (Scheme 3.11). The primary azide of the nonactic acid analogue 60 underwent dipolar cycloaddition reaction efficiently. The solution was stirred for 12 hours at 100 °C, giving a final yield of 92 after purification by chromatography on silica gel of 86.3%.

Compound 91 and 92 were analyzed with a 2D-NOE as well as 71 and 72. Neither of the aforementioned correlations was seen. Again, this is most likely due to either insufficient
deoxygenation of the sample or because the actual positions of the groups are too far apart to observe NOE.

No significant biological activity was noted for the putative 1,5-triazoloester (±)-nonactic acid analogue regioisomers 91 and 92.

3.7 Attempts to generate libraries from homononactic acid

Up till now, methyl nonactate 45 has been used as the primary scaffold in the synthesis of the major library. Methyl homononactate 93 is also a product of the methanolysis of the macrotetrolide mix which is separated out by chromatography on silica gel. In order to test the flexibility of the site of biological activity, the homologous azidoalcohol nonactic acid analogue scaffold 97 (Scheme 3.13) was made as well as their corresponding esters 98-102 (Figure 3.4). This particular synthetic scheme remains unfinished, the ultimate goal of which is to obtain the 72 and 71 homononactic acid analogue products 104, 103 (Figures 3.6 and 3.5). These would then be assayed for biological activity and compared with the biological data obtained for 72 and 71. This data would give a better idea as to the nature of the site which the compounds target.

Scheme 3.13: Synthesis of the azidoalcohol homononactic acid analogue scaffold 97.
Azidoalcohol homononactic acid analogue scaffold 97 was synthesized from methyl homononactate 93 (Scheme 3.13). As with the primary nonactic acid analogue scaffold 55, the secondary alcohol was THP-protected. Methyl homononactate 93 was added to methylene chloride, followed by DHP and pTsOH, and stirred for 5 hours at room temperature. During the reaction an increasing amount of polymeric product is produced in the reaction as a brown to black material that remains suspended in the reaction. Within the 5 hour time frame, the reaction remained fairly clear. Due to the diastereomers produced in the production of the THP protected product, the residue of the reaction was partially purified by chromatography on silica gel and used directly in the following reaction. The ester of the semi-pure THP protected product was then reduced to the primary alcohol by lithium aluminum hydride in THF and stirred at room temperature for 12 hours. This gave the product with a 73% yield of 94 after purification on silica gel. The primary alcohol 94 was then tosylated with pTsCl and pyridine and stirred in methylene chloride at room temperature for 12 hours giving a 78% yield after purification. The tosylated product 95 was then added to DMF with sodium azide at 100 °C for 24 hours which gave the azido product 96. The yield after chromatography on silica gel was 84.5%. The secondary alcohol was then deprotected with 6 M HCl in water and methanol at room temperature for 3 hours, resulting in a 70% yield 97 after purification by chromatography on silica gel.

Once the azidoalcohol homononactic acid analogue scaffold 97 had been synthesized, it was esterified and the 1,4-triazoles would then be synthesized to make the homologous 72 and 71-triazoloester nonactic acid analogues 104, 103. The synthesis of 104 and 103 remains incomplete.
Figure 3.4: Overall yields of azidoester homononactic acid analogue scaffold library 98, 99, 100, 101, 102.

Scheme 3.14: Synthesis of the 72-intermediate azidoester homononactic acid analogue 102.

The azidoalcohol homononactic acid analogue scaffold 97 was esterified with benzoyl chloride in pyridine at 37 °C for 12 hours (Scheme 3.14). The purification of the azidoester product 102 by chromatography on silica gel was difficult due to similar polarities. The final yield was 41%.

Scheme 3.15: Synthesis of the 71-intermediate azidoester homononactic acid analogue 101.
The azidoalcohol homononactic acid analogue scaffold 97 was esterified with methyl 4-chloro-4-oxobutyrate in pyridine at 37 ºC for 12 hours (Scheme 3.15). Unlike the 72-homononactic acid analogue azidoester 102, the 71-homononactic acid analogue azidoester 101 was simple to purify by chromatography on silica gel and gave a 66.1% yield.

![Figure 3.5: 71-triazoloester homononactic acid analogue 103.](image)

Due to a shortage of the compound, the triazoloesters 103,104 were never completed.

It remains unknown and part of a future synthesis to determine the utility of homononactic acid analogue libraries.

The azidoalcohol homononactic acid analogue scaffold 97 presented no biological activity against B. subtilis.

### 3.8 Conclusion

Triazoloester derivatives were synthesized from methyl nonactate, the product from the methanolysis of nonactin, and assayed for antibacterial activity and cytotoxicity. Out of the ten triazoloesters, two possessed significant antibacterial activity, 72 and 71. In time-kill
assays, it was found that they are bactericidal. SEM showed a ‘shriveling’ of B. subtilis in varying concentrations of 72, which may indicate the target of action of the triazolo-ester compounds to be the cytoskeleton. Compounds 88 and 90 were shown to be the active enantiomers. Compounds 81 and 79 had no antibacterial activity. Regioisomers of 72 and 71 were made. It was found that the 1,4-triazole regioisomer of 72 and 71 was the active regioisomer. The 1,5-triazole regioisomer of 72 and 71 showed no antibacterial activity. Homologs of 72 and 71 are as of yet incomplete.

Triazoloesters, by virtue of their bactericidal activity against S. aureus and E. faecalis as well as against other pathogens (Table 3.7) and their relatively low cytotoxicity have great potential for the development of new potent antibacterial compounds. Compounds 71 and 72 have shown great promise (Table 3.7) as antibacterial compounds and were later used as leads in SAR studies to produce even more potent compounds. To date, these compounds have undergone testing in animals and are proceeding to the next phase toward clinical studies.
SYNTHESIS OF THE BIS-TRIZAOLE NONACTIC ACID ANALOGUE LIBRARY

4.1 Synthesis of the bis-azide (±)-nonactic acid analogue scaffolds

The biological activity exerted by the (+)-72 and 71 nonactic acid analogue compounds 88, 90 has made the starting subunit, methyl nonactate 45 an attractive building block for making libraries of potentially active compounds. The azidoalcohol nonactic acid analogue scaffold 55 has lead to the triazoloester libraries which have shown even more promise through the continuation and expansion of the primary library (Figure 3.2). This primary scaffold 55, however, is not the only scaffold that can be developed from methyl nonactate 45. In this study, both the primary and secondary alcohols were transformed to azides to make an easy one step derivatization scaffold in which two triazoles could be made. Alkyne addition to this scaffold 115 produced the bis-triazole library 116 (Scheme 4.1).

Scheme 4.1: Overall scheme for the racemic bis-triazole library 116 from 45.

This downside to this scaffold is that both moieties on either end are the same. The advantage to this scaffold is that it greatly simplifies and shortens the scheme to make the scaffold, eliminates the esterification process that can be difficult, increases the overall yield, and allows for easy purification. The more scaffolds that can be made from methyl nonactate 45, the more attractive methyl nonactate 45 becomes for drug discovery.
Scheme 4.2: Synthesis of the bis-azide (±)-nonactic acid analogue scaffold 115.

The bis-azide (±)-nonactic acid analogue scaffold 115 was synthesized from methyl (±)-nonactate 45 (Scheme 4.2). The methyl (±)-nonactate 45 was reduced by lithium aluminum hydride in THF to give the diol 113 with a 99% yield. The diol 113 was then tosylated with pTsCl in pyridine. DMAP was added and the solution was stirred for 12 hours at room temperature giving a 39% yield of 114 after purification by column chromatography on silica gel. In the final step, sodium azide was added to the bis-tosylate product 114 in DMF at 80 °C for 12 hours, which resulted in an inversion of stereochemistry at C8 with an 80.5% yield after purification of the bis-azide nonactic acid analogue scaffold 115.

The bis-azide nonactic acid analogue scaffold 115 showed no biological activity.

4.2 Synthesis of the bis-triazole (±)-nonactic acid analogue library

Scheme 4.3: Synthesis of bis-triazole (±)-nonactic acid analogue library 116.

Libraries of bis-triazole (±)-nonactic acid analogue compounds 116 can be made through a dual Huisgen 1,3-dipolar cycloaddition on the bis-azide (±)-nonactic acid analogue
scaffold 115. In order to ensure the regiochemistry of the products, the 1,4-triazoles were made using Sharpless conditions at 37 ºC. Due to limited amounts of the material available, only five of the bis-triazole products (Figure 4.1) were synthesized.

![Chemical structures](image)

**Figure 4.1**: Overall yields for bis-triazole (±)-nonactic acid analogue library.

Each of the five compounds 117, 118, 119, 120, and 121, were synthesized by the addition of the bis-azide scaffold 115 to a 5:1 ethanol in water solution. To each of the five solutions prepared one of the previously used alkynes (Table 3.1) were added in excess, followed by 0.3 M copper sulfate in water and 1.1 M ascorbic acid in water. The solution was shaken at 37 ºC for 12 hours. Each compound was purified by chromatography on silica gel.
The yield of the bis-triazole product 117, 24.9%, was the lowest out of the five bis-triazoles synthesized possibly due to the steric hindrance caused by the tert-butyl groups. 118, 119, and 120 were obtained with a 56%, 61%, and a 63.4% yield respectively. A 99% yield of 121 was obtained. This may be due to electron donation into the ring and, therefore, into the alkyne.

4.3 Conclusions

The bis-triazole nonactic acid analogue library (Figure 4.1) was synthesized for two main purposes. The first of these purposes was to demonstrate that not one, but many different scaffolds could be synthesized from a single natural-product derivative. More than one scaffold allows for the possibility of parallel syntheses, even syntheses that use the addition of similar chemical moieties. The second purpose in their construction, with the success of the first scaffold in mind, and the results that showed the importance of the nonactic acid moiety, was to synthesize more potentially active compounds. Though these compounds did not show any significant useful biological activity, 118 did show a curious cytotoxic activity against normal mammalian cells over that of cancer cells. This preliminary finding has not yet been replicated or explored.
CHAPTER 5

CYCLOHEXANE ANALOGUE LIBRARY OF 72 AND 71

5.1 Introduction

The importance of the natural product component to the antimicrobial activity exhibited by 72 and 71 was determined by the replacement of the nonactic acid analogue scaffold with that of a cyclohexane chemically similar moiety (Figure 5.1a).

![Figure 5.1a](image)

**Figure 5.1a:** Comparison of 72 and 71 to cyclohexane analogues 131 and 132.

The cyclohexane moiety would approximate the spacing and stereochemistry of the natural product component of 72 and 71. The reasoning behind this replacement is not only to determine if the natural product component is necessary for biological activity, but if replacement of this moiety is possible using an inexpensive commercially available compound.
Figure 5.1b: 72 and 71 Comparison to the cyclohexane analogue library.

The cyclohexane analogue libraries of the 72 and 71 were synthesized to illustrate not just the importance of incorporating natural products, but the importance of the nonactic acid subunit itself (Figure 5.1). Cis and trans cyclohexanes were used to mimic the stereochemistry of the azidoalcohol nonactic acid analogue scaffolds 77, 86 in the synthesis of the 72 and 71 compounds.
5.2 Synthesis of the *Cis*-4-azidomethyl-cyclohexanol scaffold

![Scheme 5.1](image)

**Scheme 5.1:** Synthesis of the *cis*-4-azidomethyl-cyclohexanol scaffold 128.

A series of synthetic steps were executed to synthesize the *cis*-4-azidomethyl-cyclohexanol scaffold 128 (Scheme 5.1). The starting material for the synthesis was *cis*-4-hydroxycyclohexane-1-carboxylic acid 122 which was added to 5% sulfuric acid in methanol and stirred at room temperature, then refluxed for 5 hours. The methyl ester 123 was purified by chromatography on silica gel to give an 84% yield. The secondary alcohol 123 was protected by a THP group. The impure product 124 was reduced by lithium aluminum hydride in THF at room temperature for 12 hours. The two step reaction gave a 90% yield after purification by chromatography on silica gel. This gave a THP-protected secondary alcohol and a primary alcohol 125. The primary alcohol was then tosylation with pTsCl in pyridine at room temperature for 12 hours. The product 126 was purified by chromatography on silica gel to give a 69% yield. The tosylate was then substituted with azide. The tosylate product 126 in DMF with sodium azide was warmed to 80 °C, and stirred for 5 hours which gave an 82% yield of the primary azide product 127 after purification by chromatography on
silica gel. The 4-azidomethyl-cyclohexanol 128 was obtained by the deprotection of the secondary alcohol with 6 M HCl in water and methanol at room temperature after 3 hours. The final reaction gave a 90.3% yield of 128. This scaffold was obtained without any changes in the cis structure of the starting material to give the cis-4-azidomethyl-cyclohexanol product 128.

In order to approximate the structures and chemical reactivity of 72 and 71, the methyl 4-chloro-4-oxobutyrate and benzoyl chlorides were used to synthesize the corresponding esters in respect to the 72 and 71 products (Scheme 5.2).

![Scheme 5.2: Synthesis of the cis-4-azidomethyl-ester-cyclohexane scaffolds 129, 130.](image)

128 was added to pyridine and methyl 4-chloro-4-oxobutyrate and stirred for 3 hours then purified by chromatography on silica gel which gave 129 at 55% yield. Esterification reactions with methyl 4-chloro-4-oxobutryate often gave a good deal of dark residue which was easily separated from the product. 130 was obtained with an 81% yield in a similar reaction with the exception of using benzoyl chloride in place of methyl 4-chloro-4-oxobutyrate. Excess benzoyl chloride proved to be difficult to remove via chromatography on silica gel as it often coincided with 130.
Once the esters were synthesized the 72 and 71-like cyclohexane analogues could be synthesized. Each ester 129 and 130 underwent a dipolar cycloaddition using Sharpless conditions, with \( N-(3\text{-ethynyl-phenyl})\text{-acetamide} \) and \( 2\text{-ethynyl-pyridine} \) respectively (Scheme 5.3). These gave the 72 and 71-like triazolo-ester cyclohexane analogues 131 and 132.

**Scheme 5.3:** Synthesis of the *cis*-4-triazolo-ester cyclohexane analogues 131, 132.

\( N-(3\text{-ethynyl-phenyl})\text{-acetamide} \) was added to 5:1 ethanol and water, followed by the 71-like azidoester 129. 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water were added and the solution was shaken at 37 °C for 12 hours. After purification by chromatography on silica gel, this gave a 69% yield of the *cis* 71-like triazoloester cyclohexane analogue 131.

2-Ethynyl-pyridine was added to the 72-like azidoester 130 in 5:1 ethanol and water, followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. After the solution was shaken for 12 hours at 37 °C, the *cis* 72-like triazoloester cyclohexane analogue 132 was purified by chromatography on silica gel with a 62% yield.
Neither of the 72 nor the cis 71-like triazoloester cyclohexane analogue compounds 131,132 showed activity against a range of Gram positive pathogens.

5.3 **Synthesis of the Trans-4-azidomethyl-cyclohexanol scaffold**

![Scheme 5.4](image)

**Scheme 5.4:** Synthesis of the *trans*-4-azidomethyl-cyclohexanol scaffold 139.

With same lack of inversion of stereochemistry in the production of 72 and 71, the *trans*-4-azidomethyl-cyclohexanol scaffold 139 was synthesized with a similar series of synthetic steps (Scheme 5.4). *Trans*-4-hydroxy-cyclohexane-1-carboxylic acid 133 was added to 5% sulfuric acid in methanol and refluxed for 5 hours to give 4-hydroxy-cyclohexanecarboxylic acid methyl ester 134. The product was purified by chromatography on silica gel and gave 90% yield. The secondary alcohol of the methyl ester was protected by a THP group. The methyl ester analogue was added to methylene chloride at room temperature, followed by DHP and pTsOH. The solution was stirred for 5 hours and the THP protected product 135 was purified by chromatography on silica gel to give a 73% yield. The methyl ester analogue 135 was reduced by lithium aluminum hydride in THF at room temperature for 5 hours, which gave the primary alcohol product 136 at an 86.8% yield after purification by chromatography on silica gel. The primary alcohol 136 was tosylated by
the addition of the product to pyridine, followed by pTsCl at room temperature for 5 hours. The tosylate product 137 was purified by chromatography on silica gel which resulted in a 69% yield. Sodium azide in DMF was added to the tosylate product and the solution was warmed to 80 °C for 5 hours which gave an 83.7% yield of 138 after purification by chromatography on silica gel. The trans-4-azidomethyl-cyclohexanol scaffold 139 was produced by the deprotection of the THP group on the secondary alcohol with 6 M HCl in water and methanol at room temperature for 5 hours. An 81.7% yield of 139 was achieved after purification by chromatography on silica gel.

**Scheme 5.5:** Synthesis of the trans-4-azidomethyl-ester cyclohexane analogue scaffolds 140, 141.

Methyl 4-chloro-4-oxobutryrate was added to the trans-4-azidomethyl-cyclohexanol scaffold 139 in pyridine at room temperature and stirred for 3 hours to give the trans-71-like azidoester cyclohexane analogue 140. After purification by chromatography on silica gel, a 53% yield was achieved (Scheme 5.5).

Benzoyl chloride was added to the trans-4-azidomethyl-cyclohexanol scaffold 139 in pyridine at room temperature for 3 hours. The product was purified by chromatography on
silica gel and gave a 71% yield of the trans-72-like azidoester cyclohexane analogue 141 (Scheme 5.5).

![Scheme 5.6: Synthesis of the trans-4-triazoloester cyclohexane analogues 142, 143.](image)

The trans-71-azidoester cyclohexane analogue 140 was added to N-(3-ethynyl-phenyl)-acetamide in 5:1 ethanol and water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was stirred at 37 °C for 12 hours which gave the trans-71-triazoloester cyclohexane analogue 142 with a 73% yield after purification by chromatography on silica gel (Scheme 5.6).

2-Ethynyl-pyridine was added to the trans-72-azidoester cyclohexane analogue 141 in 5:1 ethanol in water. 1.1 M Ascorbic acid in water was added, followed by 0.3 M copper sulfate in water at 37 °C for 12 hours. Purification by chromatography on silica gel gave the trans-72-triazoloester cyclohexane analogue 143 with a 79% yield (Scheme 5.6).

No biological activity was exerted by either the 72 or 71-like trans triazoloester analogues 142, 143.
5.4 Synthesis of the Cis-(4-azido-cyclohexyl)-methanol scaffold

In order to cover the range of structures possible with the cyclohexane scaffold, two other scaffolds were synthesized (Schemes 5.7 and 5.10). These scaffolds are the cis and trans cyclohexanes with the azide directly connected to the ring. These syntheses involved an inversion of stereochemistry as the alcohol is substituted with an azide.

Scheme 5.7: Synthesis of the cis-(4-azido-cyclohexyl)-methanol scaffold 148.

Cis-(4-Azido-cyclohexyl)-methanol scaffold 148 was synthesized from trans-4-hydroxy-cyclohexane-1-carboxylic acid 133 (Scheme 5.7). Trans-4-hydroxy-cyclohexane-1-carboxylic acid 133 was reduced by lithium aluminum hydride and purified by chromatography on silica gel gave a 21% yield of trans-4-hydroxymethyl-cyclohexanol 144. The trans diol analogue 144 was added to DMF at 0°C, followed by TBDMSI, and imidazole. The solution was allowed to warm to room temperature as the solution was stirred for 12 hours. This kinetic based reaction gave the silyl protection of the primary alcohol almost exclusively. The percent yield of 145 was 57% after chromatography on silica gel. The secondary alcohol was then tosylated by the addition of the protected diol to pyridine at room temperature, followed by pTsCl. The solution was stirred for 12 hours, and
the tosylate product 146 was partially purified by chromatography on silica gel. Bimolecular nucleophilic substitution occurred on the secondary tosylated alcohol by the addition of the tosylated product to DMF, followed by sodium azide. The solution was warmed to 80 °C and stirred for 12 hours. The cis azido product 147 was purified by chromatography on silica gel, giving a 54.4% yield over 2 steps. Deprotection of the primary alcohol with pTsOH in water and acetic acid at room temperature for 24 hours and purification by chromatography on silica gel gave the cis-(4-azido-cyclohexyl)-methanol scaffold 148 with a 43% yield.

![Chemical structure](image)

**Scheme 5.8:** Synthesis of the cis-4-azidoester cyclohexane analogue scaffolds 149, 150.

Methyl 4-chloro-4-oxobutrylate was added to the cis-(4-azido-cyclohexyl)-methanol scaffold 148 in pyridine and stirred at room temperature for 3 hours to give the cis-71-like azidoester cyclohexane analogue 149. Purification by chromatography on silica gel gave a 78.4% yield (Scheme 5.8).

Benzoyl chloride was added to the cis-(4-azido-cyclohexyl)-methanol scaffold 148 in pyridine and stirred at room temperature for 3 hours. The cis-72-like azidoester cyclohexane analogue 150 was purified by chromatography on silica gel, which gave an 83% yield (Scheme 5.8)
Scheme 5.9: Synthesis of the \textit{cis}-4-triazoloester cyclohexane analogues 151, 152.

\textit{Cis}-71-like azidoester cyclohexane analogue 149 was added to \(N\)-(3-ethynyl-phenyl)-acetamide in 5:1 ethanol and water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was shaken at 37 °C for 12 hours which gave an 80.7\% yield after purification by chromatography on silica gel of the \textit{cis}-71-like-triazoloester cyclohexane analogue 151 (Scheme 5.9).

\textit{Cis}-72-like azidoester cyclohexane analogue 150 was added to 2-ethynyl-pyridine in 5:1 ethanol and water. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water was added and the solution was shaken for 12 hours at 37 °C. The \textit{cis}-72-like-triazoloester cyclohexane analogue 152 was purified by chromatography on silica gel which gave an 80\% yield (Scheme 5.9).

Unexpectedly, 152 did show activity against \textit{B. subtilis}. The assayed showed that 152 had an IC\textsubscript{99} of 125 µM against \textit{B. subtilis}.
5.5 Synthesis of the Trans-(4-azido-cyclohexyl)-methanol scaffold

The final scaffold for the synthesis of the 72 and 71 triazoloester cyclohexane analogues was the trans-(4-azido-cyclohexyl)-methanol scaffold 157 (Scheme 5.10). As with the previous syntheses, the synthetic scheme involves an inversion of stereochemistry, therefore, the trans scaffold is synthesized from the cis stereoisomer.

Scheme 5.10: Synthesis of the Trans-(4-azido-cyclohexyl)-methanol scaffold 157.

Cis-4-hydroxy-cyclohexane-1-carboxylic acid 122 was added to THF at 0 °C, followed by lithium aluminum hydride. The solution was allowed to warm to room temperature over 12 hours and the cis diol product 153 was purified by chromatography on silica gel to give a 76% yield. The primary alcohol of the diol was then protected by a silyl group by the addition of the diol to DMF at 0 °C, followed by TBDMSCl and imidazole. The solution was warmed to room temperature over 12 hours. Purification by chromatography on silica gel gave a 52% yield of 154, due to the kinetic nature of the reaction. The silyl protected product 154 was then added to pyridine at room temperature, followed by pTsCl and stirred for 5 hours which gave the tosylate 155 with a 91.6% yield after purification by chromatography on silica gel. The tosylate 155 was then added to DMF.
with sodium azide and warmed to 80 °C for 5 hours which after purification by chromatography on silica gel gave a 64.5% yield of the azido product 156. The scaffold 157 was obtained after purification with a 69.2% yield after the deprotection of the primary alcohol with 6 M HCl in water and methanol at room temperature for 5 hours (Scheme 5.10).

**Scheme 5.11:** Synthesis of the trans-4-azidoester cyclohexane analogue scaffolds 158, 159.

Methyl 4-chloro-4-oxobutyrate was added to the trans-(4-azido-cyclohexyl)-methanol scaffold 157 in pyridine at room temperature and stirred for 3 hours, which gave a 61% yield of the trans-71-like azidoester cyclohexane analogue 158 (Scheme 5.11). The product was purified by chromatography on silica gel.

Benzoyl chloride was added to the trans-(4-azido-cyclohexyl)-methanol scaffold 157 in pyridine at room temperature and stirred for 3 hours (Scheme 5.11). Purification by chromatography on silica gel gave a 72.6% yield of the trans-72-like azidoester cyclohexane analogue 159.
**Scheme 5.12:** Synthesis of the trans-4-triazoloester cyclohexane analogues 160, 161.

*Trans*-71-like azidoester cyclohexane analogue 158 was added to 5:1 ethanol in water, followed by N-(3-ethynyl-phenyl)-acetamide. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the solution was shaken at 37 °C for 12 hours. Purification by chromatography on silica gel gave a 79.6% yield of the *trans*-71-like triazoloester cyclohexane analogue product 160 (Scheme 5.12).

*Trans*-72-like azidoester cyclohexane analogue 159 was added to 5:1 ethanol in water, followed by 2-ethynyl-pyridine, 1.1 M ascorbic acid in water, and 0.3 M copper sulfate in water. The solution was shaken at 37 °C for 12 hours and the product was purified by chromatography on silica to give the *trans*-72-like triazoloester cyclohexane analogue 161 (Scheme 5.12).

None of the compounds showed any significant biological activity.
5.6 Synthesis of alternate cyclohexane-based scaffolds

The four cyclohexane scaffolds synthesized and the 72 and 71-like products made, illustrated that the methyl nonactate analogue subunit is important for the biological activity of 72 and 71. More cyclohexane scaffolds were made on a large scale to illustrate this point. Both cis and trans versions of the azido carboxylic acid scaffolds 164, 167 were synthesized and sent to the appropriate groups for further synthesis and studies. The secondary alcohol underwent a bimolecular nucleophilic substitution reaction resulting in an inversion in stereochemistry.

Scheme 5.13: Synthesis of the cis-4-azido-cyclohexane-carboxylic acid scaffold 164.

The cis-4-azido-cyclohexane-carboxylic acid scaffold 164 was synthesized from the trans-4-hydroxy-cyclohexanecarboxylic acid methyl ester 134 (Scheme 5.13). The secondary alcohol was tosylated with pTsCl in pyridine at room temperature and stirred for 5 hours. The tosylate 162 was purified by chromatography on silica gel and gave a 78% yield. The tosylate 162 was substituted with sodium azide in DMF at 80 °C for 5 hours and the product was then purified by chromatography on silica gel. The reaction gave an 89% yield of 163. The methyl ester was saponified overnight with 2.5 M lithium hydroxide in a water,
methanol, and THF solution and followed by 1 M HCl in water. Purification of the product by chromatography on silica gel gave a 91.5% yield of 164.

Scheme 5.14: Synthesis of the trans-4-azido-cyclohexane-carboxylic acid scaffold 167.

The trans-4-azido-cyclohexane-carboxylic acid scaffold 167 was synthesized from the cis-4-hydroxy-cyclohexanecarboxylic acid methyl ester 123 (Scheme 5.14). The secondary alcohol was tosylated in pyridine with pTsCl at room temperature which was stirred for 5 hours and gave a 67.4% yield of the tosylate 165 after purification by chromatography on silica gel. Trans-4-azido-cyclohexane-carboxylic acid methyl ester 166 was made by the nucleophilic substitution of the tosylate 165 with sodium azide in DMF at 80 °C for 5 hours. Purification by chromatography on silica gel gave a 77.5% yield of the azide 166. Saponification of the methyl ester by 2.5 M lithium hydroxide in a water, methanol, and THF mixture followed by the addition of 1 M HCl in water gave a 91% yield of the trans-4-azido-cyclohexanecarboxylic acid 167 after purification by chromatography on silica gel.

Using the cis-(164) and trans-substituted (167) azidoacids, the medicinal chemistry group at Promiliad Biopharma was able to generate 246 derivatives of the general structure.
Figure 5.2: General structure of the triazoloamide cyclohexane analogues.

Of these compounds, the following three had more than marginal activity against Gram-positive pathogens (Table 5.1).

<table>
<thead>
<tr>
<th>Designation</th>
<th>Compound</th>
<th>B. subtilis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-C-12</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>18-C-5</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>18-G-3</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Table 5.1: MIC (µM) Values for cyclohexane analogues for B. subtilis and S. aureus.

5.7 Conclusions

The synthesis of the cyclohexane, cis and trans, with alternating alcohol and azide groups attached to the ring or a single methylene away from the ring was greatly important in establishing the essential nature of the nonactic acid analogue moiety for the activity of compounds described earlier (Chapter 3). Despite the activity of 152, the rest of the eight member library was inactive. It should also be noted that 152 is only bactericidal against B. subtilis, a model Gram-positive bacteria and not a human pathogen. No biological activity was shown against S. aureus, S. aureus (MRSA), or E. faecalis (VRE). Though the chemical moieties were similar, with similar spacing to that of the nonactic acid central moiety, no significant activity was measured in the trans or the cis forms. Though this does not in and of itself prove that the nonactic acid moiety is responsible for the biological activity seen in
and 71, it does suggest the importance of this chirally rich, natural product derived analogue to the activity of these compounds against these antibiotic resistant strains.
6.1 Introduction

Though nonactin is an antibiotic, it is a poor drug.\[247\] Synthesis of nonactin analogue structures may be beneficial in developing a nonactin derivative that is less hydrophobic and more soluble.\[247\] The development of nonactin analogues was attempted using the azidoalcohol scaffolds synthesized from methyl nonactate 55 and methyl homononactate 97 and the linker compounds: isophthaloyl chloride, terephthaloyl chloride, 1,3-diethynylbenzene, and 1,4-diethynylbenzene (Figure 6.1).

\[\text{Nonactin, 40}\]

\[\text{Nonactin Analogues}\]

**Figure 6.1:** Cartoon of Nonactin Structure vs. Nonactin Analogue Structure.

Two units of the azidoalcohol scaffold are coupled with either isophthaloyl chloride or terephthaloyl chloride via an esterification reaction producing a bis-ester compound 168, 169, 170. Two units of the azidoalcohol scaffold were coupled with either 1,3-
diethynylbenzene or 1,4-diethynylbenzene via the Huisgen 1,3-dipolar cycloaddition reaction under Sharpless conditions giving a bis-triazole product 171, 172, 173. The final step in these syntheses would be coupling the bis-ester compounds with 1,3-diethynylbenzene or 1,4-diethynylbenzene to make a bis-ester bis-triazole macrolide. Likewise, the bis-triazole dimers would be coupled with isophthaloyl chloride or terephthaloyl chloride to produce a bis-triazole bis-ester macrolide.

The first step in the synthesis of these macrolides was the synthesis of dimers in order to work out the coupling conditions necessary for their formation (Scheme 6.1 and 6.2).

Scheme 6.1: Overal synthesis of dimer compounds from terephthaloyl chloride and isophthaloyl chloride.

Terephthaloyl chloride and isophthaloyl chloride were used to synthesize bis-ester bis-triazole dimer-like compounds of 72 and 71 (Scheme 6.1). Azidoalcohol nonactic analogue scaffolds 55 and 97 were used in conjunction with the dichlorides, terephthaloyl
chloride and isophthaloyl chloride, to synthesize bis-ester bis-azide scaffolds which were then used to synthesize the dimer compounds (Scheme 6.1).

A second set of scaffolds were synthesized with two molecules of the azidoalcohol scaffolds, 55 and 97, in conjunction with 1,3-ethynyl benzene and 1,4-ethynyl benzene (Scheme 6.2). Under Sharpless conditions this gave bis-triazole bis-hydroxy dimer compounds. These hydroxyl groups were then esterified with methyl 4-chloro-4-oxobutyrate and benzoyl chloride to make dimer-like compounds of 72 and 71 (Scheme 6.2).
6.2 Synthesis of dimer-like derivatives of nonactic acid analogue azideester and triazoloalcohol scaffolds

Scheme 6.3: Synthesis of Terephthalic acid bis-{1-[5-(2-azido-1-methyl-ethyl)-tetrahydrofuran-2-ylmethyl] propyl} ester scaffold 168.

Terephthalic acid bis-{1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl}-propyl} ester 168 was synthesized by the addition of 97 to terephthaloyl chloride in pyridine at room temperature. The solution was stirred for 24 hours and the product was purified by chromatography on silica gel. This gave a 50.8% yield of the 1,4-bis-esterazido product 168 (Scheme 6.3).

Scheme 6.4: Synthesis of Terephthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydrofuran-2-yl]-1-methyl ethyl} ester scaffold 169.
Terephthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester 169 was synthesized by the addition of 55 to terephthaloyl chloride in pyridine at room temperature and stirred for 3 days. Purification of the 1,4-bis-esterazido product 169 by chromatography on silica gel gave a 26.7% yield (Scheme 6.4).

\[
\begin{align*}
\text{Scheme 6.4:} & \quad \text{Synthesis of Terephthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester scaffold 169.} \\
\end{align*}
\]

Isophthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester 170 was synthesized by the addition of isophthaloyl chloride to 55 in pyridine at room temperature and stirred for 3 days. The 1,3-bis-esterazido product 170 was obtained with a 25.3% yield after purification by chromatography on silica gel (Scheme 6.5).
Scheme 6.6: Synthesis of 1,4-bis-triazolo-bis-alcohol homononactic acid analogue scaffold 171.

The 1,4-bis-triazolo alcohol 171 was synthesized by the addition of 97 to 5:1 ethanol in water, followed by 1,4-diethynylbenzene. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the mixture was shaken at 37 °C for 3 days. An 85% yield of 171 of the product was obtained after purification by chromatography on silica gel (Scheme 6.6).
Scheme 6.7: Synthesis of the 1,4-bis-triazolo-bis-alcohol nonactic acid analogue scaffold 172.

The 1,4-bis-triazoloalcohol 172 was synthesized by the addition of 1,4-diethynylbenzene to 5:1 ethanol in water, followed by 55. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the mixture was shaken for 12 hours at 37 °C to give the product 172 after purification by chromatography on silica gel with an 88% yield (Scheme 6.7).
Scheme 6.8: Synthesis of 1,3-bis-triazolo-bis-alcohol homononactic acid analogue scaffold 173.

The 1,3-bis-triazolo-bis-alcohol 173 was synthesized by the addition of 1,3-diethynylbenzene to 5:1 ethanol in water followed by 97. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added. The solution was shaken for 12 hours at 37 °C and the product 173 was purified by chromatography on silica gel. This resulted in an 85.4% yield (Scheme 6.8).

None of the dimer-like scaffolds synthesized for this library had any significant antimicrobial activity.
6.3 Synthesis of dimer-like derivatives of 72 and 71

![Chemical Structure](image)

**Figure 6.2:** Terephthalic acid bis-[1-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-ylmethyl}-propyl] ester 174.

Dimer 174 was synthesized by the addition of 168 to 5:1 ethanol in water followed by N-(3-ethynyl-phenyl)-acetamide. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the solution was shaken at 37 °C for 12 hours. Compound 174 was purified by chromatography on silica gel and resulted in a 70% yield (Figure 6.2).

![Chemical Structure](image)

**Figure 6.3:** Terephthalic acid bis-(1-{5-[1-methyl]-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl-tetrahydro-furan-2-ylmethyl}-propyl) ester 175.

The 1,4-bis-ester-bis-triazole homomethyl nonactic acid analogue 175 was synthesized by the addition of 168 to 5:1 ethanol in water. 2-Ethynyl-pyridine was added with 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water and the solution was shaken for 12 hours at 37 °C and gave a 57.7% yield of 175 after purification by chromatography on silica gel (Figure 6.3).
Figure 6.4: Synthesis of Terephthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-\[1,2,3\]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester 176.

2-Ethynyl-pyridine was added to 169 in 5:1 ethanol in water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was stirred at room temperature for 12 hours. Purification by chromatography on silica gel gave 176 (Figure 6.4).

Figure 6.5: Synthesis of Isophthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-\[1,2,3\]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester 177.

The 1,3-bis-ester-bis-triazole nonactic acid analogue 177 was synthesized by the addition of 2-ethynyl-pyridine to 5:1 ethanol in water followed by 170. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added, and the solution was stirred at room temperature for 12 hours. Purification of 177 by chromatography on silica gel gave an 85% yield (Figure 6.5).
Figure 6.6: Synthesis of 1,4-bis-triazole-bis-ester homononactic acid analogue 178.

Methyl 4-chloro-4-oxobutyrate was added to 171 in pyridine at room temperature. The solution was stirred for 12 hours and the product was purified by chromatography on silica gel which gave a 33.7% yield of 178 (Figure 6.6).

Figure 6.7: Synthesis of 1,4-bis-triazole-bis-ester homononactic acid analogue 179.

Benzoyl chloride was added to 171 in pyridine at room temperature and the solution was stirred for 12 hours. The reaction gave a 60% yield of 179 after purification by chromatography on silica gel (Figure 6.7).

Figure 6.8: Synthesis of 1,3-bis-triazole-bis-ester homononactic acid analogue 180.

Compound 173 was added to pyridine, followed by methyl 4-chloro-4-oxobutyrate. The solution was stirred for 12 hours at room temperature and 180 was purified by chromatography on silica gel to give a 47.8% yield (Figure 6.8).
Figure 6.9: Synthesis of 1,3-bis-triazole-bis-ester homononactic acid analogue 181.

Compound 173 was added to pyridine, followed by benzoyl chloride and stirred for 12 hours at room temperature which gave a 65.4% yield of 181 after purification by chromatography on silica gel (Figure 6.9).

For this library of eight 72 and 71-like dimeric compounds, no significant antibacterial activity was observed in Gram-positive or Gram-negative bacteria. No antibacterial activity was reported for any of the scaffold precursors.

6.4 Conclusions

The dimeric compounds showed no antimicrobial activity in the Alamar blue assays. This may indicate the specific nature of the target site and the conformation of 72 and 71 in respect to their interaction in order to elicit the bactericidal activity. Due to the demands of the synthesis of the nonactate and homononactate scaffolds and the limited material available at the time of synthesis, no macrolides were synthesized.
CHAPTER 7

FEEDING STUDIES

7.1 Nonactin biosynthesis competitive inhibitor in *Streptomyces griseus*

Nonactin 40 is ineffective as a therapeutic due to its high hydrophobicity and insufficient solubility. Making libraries of this structure could yield more effective antibiotic and antitumor agents and solve the pharmacodynamic properties. The structure of nonactin 40, however, presents a serious challenge to any structural modification for the purpose of synthesizing analogues and derivatization.

Nonactin 40 is a macrotetrolide which is assembled in vivo by *Streptomyces griseus* ETH A7796 from nonactic acid subunits. The resulting structure provides no easily modifiable chemical groups for easy synthetic transformations. Total synthesis of the macrotetrolide in which a chemical handle could be introduced is impractical. Precursor-directed biosynthesis derivatives may be a straightforward way to generate nonactin derivatives (Scheme 7.1).
Scheme 7.1: Overall synthesis for nonactic acid analogues.

As the natural production of nonactin is high (5-10 g/L), precursor directed biosynthesis should give detectable levels of analogs. Additionally, *S. griseus* or genetically modified strains of *S. griseus* in which nonactic acid production has been blocked, can convert nonactic acid into nonactin 40. This observation suggests that nonactic acid analogues may be added to these fermentative cultures and incorporated into nonactin-like structures, therefore, resulting in a biosynthesis of nonactin analogues with readily available synthetic handles for post-fermentative generation of nonactin analogues via synthesis.

The nonactin biosynthesis inhibitor 186 was synthesized from furan 182 as an analogue of nonactic acid.[247] Initially, this compound was sought after as a methyl nonactic acid analogue subunit with a chemically reactive handle for a later modification by synthesis. If the nonactic acid analogue subunits were incorporated into nonactin analogue structures, the unreduced furan could be used in alkylation or epoxidation, followed by addition reactions to make derivatives of nonactin 40.
Scheme 7.2: Synthesis of the Nonactin Biosynthesis Inhibitor 186.

Furan 182 was added to THF, was cooled to 0 °C and n-butyl lithium was added. After 2 hours of stirring at 0 °C, propylene oxide was added and the solution warmed to room temperature over 12 hours. The 1-furan-2-yl-propan-2-ol product 183 was purified by distillation and gave a 65.6% yield. This secondary alcohol of this product was then acetylated by acetic anhydride with pyridine at room temperature for 2 hours. The acetic acid 2-furan-2-yl-1-methyl-ethyl ester product 184 was given at a 78.8% yield after purification by distillation. Alkylation of the furan ring with ethyl (dl)-2-iodopropionate produced the 2,5-substituted furan product 185. The purified acetylated compound 184 was added to DMSO, followed by Fe₂(SO₄)₃-H₂O and ethyl (dl)-2-iodopropionate.[248] Triethyl borane in THF was added and the solution was stirred for 45 minutes.[248] A second addition of triethyl borane in THF was added and was stirred for an additional 45 minutes.[248] The 2,5-substituted furan nonactic acid analogue 185 was produced with a 10.9% yield after purification by chromatography on silica gel. Saponification of the bis-ester compound by 2.5 M LiOH in water, methanol, and THF at room temperature overnight, followed by the addition of 1 M HCl until the solution had a pH of 2, gave 186 after purification by chromatography on silica gel with an 81% yield (Scheme 7.2).
Two fermentative cultures of *S. griseus* ETH A7796 were prepared from the same vegetative culture and grown under standard conditions for 48 hours. The unreduced nonactic acid analogue 186 (56 mg in 0.5 mL of ethanol) was added to one of the fermentative cultures after inoculation after 48 hours. A blank sample (0.5 mL of ethanol) was added to the second fermentative cultures as a control culture. The macrotetrolide mixture produced from the fermentative cultures which were allowed to grow for 96 hours, were isolated according to standard protocols and analyzed with HPLC and LC-MS (TOF).

The nonactin biosynthesis inhibitor 186 was tested against the aforementioned bacterial and fungal species, but it was shown to have no significant activity. The first addition of the compound 186 to the fermentative culture did not result in the production of nonactin analogues. A far lower production of macrotetrolides than in the control fermentative cultures was also noted. Though the study did not result in the desired product, the much lower production of nonactin 40 proved to be of interest.

A second feeding study showed that the addition of the compound 186 did not affect biomass production, which was shown from the amount of mycelia recovered from each culture. The approximate wet weight of biomass of the experimental and control cultures was determined and shown to be approximately equal.

Secondly the data obtained from the reverse-phase HPLC quantification of the macrotetrolides present in the experimental culture (i.e. compound 186 added), revealed that nonactin 40 production in the fermentative cultures was reduced by more than 90% if either 10 mM or 1 mM of 186 was added. A concentration of 0.01 mM of the compound resulted in no inhibition of nonactin 40 production and a concentration of 0.1 mM gave an
intermediate amount of nonactin 40 compared to that of the control culture. Despite the lower levels of inhibition, MS and LC-MS analysis of the fermentative cultures showed no production of nonactin analogues. An IC$_{50}$ value of about 100 µM was obtained for the nonactin biosynthesis inhibitor 186.[247]

It has also been noted that the amount of phenazines, which are produced alongside macrotetrolides, did not diminish and remained unaffected by any amount of the inhibitor 186. Nonactic acid subunit production was unaffected as well. This leads to the hypothesis that the inhibitor only inhibits the assembly of nonactin 40 itself from its nonactic acid subunits.[247]

7.2 Nonactic acid derivatives for feeding studies to *Streptomyces griseus*

Having demonstrated the inhibition of nonactin biosynthesis by 186 we decided to generate additional analogues of 186. Where 186 did not become incorporated, we hoped for better results with analogues of 186.

![Scheme 7.3: Synthesis of a nonactic acid derivative 191.](image-url)
Compound 187 was synthesized by the addition of ethylene oxide to an anion generated from furan and purified by distillation to give a 29.7% yield. The primary alcohol 187 was acetylated with the addition of the alkylation product 187 to pyridine in THF at room temperature followed by acetic anhydride and stirred for 2 hours. The product 188 was purified by distillation to give a 78.3% yield. The 2,5-dialkylated bis-ester furan 189 was synthesized from the acetylated product 188 which was added to DMSO at room temperature, followed by Fe$_2$(SO$_4$)$_3$-H$_2$O and ethyl (dl)-2-iodopropionate. Air was blown through the solution, followed by the addition of 1.0 M Et$_3$B / THF. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et$_3$B / THF and stirred for 2 hours. A 7.8% yield of the product 189 was purified by chromatography on silica gel. Compound 189 was reduced by 5% rhodium on carbon under 500 psi of hydrogen for 5 hours to give 190. Purification of the tetrahydrofuran product 190 by chromatography on silica gel gave a 61.5% yield. The bis-ester product 190 was saponified by 2.5 M LiOH / H$_2$O / MeOH / THF (1:2:3) over 24 hours and followed by 1.0 M HCl resulting in a 32.4% yield of the nonactic acid analogue 191 after purification by chromatography on silica gel.(Scheme 7.3)
Scheme 7.4: Synthesis of a nonactic acid derivative 194.

The nonactic acid analogue 194 was synthesized from furan (Scheme 7.4). Furan 182 was added to THF at 0 °C followed by n-butyl lithium and stirred for 1 hour. Ethylene oxide in THF was added at 0 °C and the solution was warmed to room temperature over 12 hours. 0.1 M HCl was added to the solution which was stirred for 10 minutes. The product 187 was purified by distillation to give a 29.7% yield and added to pyridine in THF, followed by acetic anhydride at room temperature and stirred for 2 hours to give 78.3% yield of the acetylated product 188 after distillation. The acetylated product 188 was alkylated to give the 2,5-dialkylated-bis-ester furan product 192 by the addition to DMSO at room temperature followed by Fe₂(SO₄)₃-3H₂O and ethyl-iodoacetate. Air was bubbled through the solution followed by 1.0 M Et₃B / THF. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et₃B / THF. The solution was stirred for 2 hours and gave a 14.5% yield of the 2,5-dialkylated furan product 192 after purification by chromatography on silica gel. The furan was reduced by 5% Rh/C under 500 psi of hydrogen in ethyl acetate at room
temperature and stirred for 24 hours. The product 193 was given after purification by chromatography on silica gel to give a 51.4% yield. The reduced furan bis-ester product 193 was saponified by 2.5 M LiOH / H₂O / MeOH / THF (1:2:3) and followed by 1.0 M HCl. Purification by chromatography on silica gel gave a 52.4% yield of the nonactic acid analogue 194 (Scheme 7.4).

Scheme 7.5: Synthesis of a nonactic acid derivative 197.

Furan 182 was added to THF at 0 °C and followed by n-butyl lithium and stirred for 2 hours. Propylene oxide was added to the solution and warmed to room temperature over 12 hours. 0.1 M HCl was added to the solution and the alkylated product 183 was given with 65.6% yield after purification by distillation. The secondary alcohol 183 was acetylated by acetic anhydride in THF with pyridine at room temperature for 2 hours. Distillation gave 78.8% of the acetylated product 184 which was added to DMSO, followed by Fe₂(SO₄)₃·H₂O and ethyl-iodoacetate at room temperature. 1.0 M Et₃B / THF was added and air was bubbled through the solution. After 45 minutes, a second addition of 1.0 M Et₃B / THF was
added and the solution was stirred for an additional 2 hours. The 2,5-dialkylated furan bis-ester 195 was purified by chromatography on silica gel giving a 16.5% yield. The product 195 was added to ethyl acetate at room temperature followed by 5% Rh/C. The solution was stirred for 24 hours under 500 psi of hydrogen. The reduced furan product 196 was produced with a 28.3% yield after purification by chromatography on silica gel. Saponification of the bis-ester with 2.5 M LiOH / H2O / MeOH / THF (1:2:3) for 24 hours followed by 1.0 M HCl and gave the nonactic acid analogue 197 after purification by chromatography on silica gel with a 57.5% yield. (Scheme 7.5)

Scheme 7.6: Synthesis of a nonactic acid derivative 200.

Homononactic acid analogue 200 was synthesized from furan 182 (Scheme 7.6). 1,2-Epoxy-butane was added to an anion generated from the furan and the solution was stirred for 12 hours at 0 °C and the alkylated furan product 198 was purified by distillation and gave a 51.4% yield. The secondary alcohol was acetylated with the addition of the alkylated furan 198 to THF, followed by acetic anhydride and pyridine at room temperature and the solution was stirred for 20 hours. The product 199 was distilled and gave an 88.8% yield. The acetylated product 199 was added to DMSO at room temperature, followed by Fe2(SO4)3 - H2O and ethyl (dl)-2-iodopropionate. Air was bubbled through the solution and 1.0 M Et3B /
THF was added. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et₃B / THF with stirring for 2 hours. The dialkylated product was purified by chromatography on silica gel and added to 2.5 M LiOH / H₂O / MeOH / THF (1:2:3) with stirring overnight. 1.0 M HCl / H₂O was added and the unreduced homononactic acid analogue 200 was purified by chromatography on silica gel to give a 5% yield over 2 steps.

No significant antibacterial activity was noted for any of the nonactic acid analogues 186, 191, 194, 197 or 200.

It was decided to not attempt feeding studies in wild type S. griseus, but to wait for the construction of a ΔnonS/ΔnonS’ mutant blocked in the early stages of nonactin biosynthesis. The mutant strain of S. griseus would be unable to produce nonactic acid, but still have the ability to make nonactin if nonactic acid were added into the fermentation. The advantage of using the mutant strain is that it prevents the naturally produced nonactic acid from competing with the nonactic acid analogues. This is especially important considering the low overall yields of the nonactic acid analogues. Construction of this mutant is still being sought.

7.3 Conclusion

The feeding study experiments intended to create analogues of nonactin in vivo in Streptomyces griseus fermentations were only partially successful. The synthesis of all compounds intended to be fed into the cultures were performed successfully, including compound 186. When compound 186 was added, the production of nonactin 40 was inhibited. Since the only difference between nonactic acid and the nonactin biosynthesis inhibitor 186 was the unreduced furan, it is very likely that the unreduced furan caused this inhibition. Though analogues of nonactin were not synthesized, the production of the
nonactin biosynthesis inhibitor 186 did show a limit to the modifications allowed to nonactic acid.
CHAPTER 8

SYNTHESIS OF CHEMICALLY DIVERSE MOIETIES FOR TRIAZOLO-ESTER DIVERSITY

8.1 Introduction

We have already described the synthesis of libraries based upon the nonactate scaffold and synthetic analogues thereof, such as cis and trans-cyclohexane derivatives. While these libraries have generally relied upon commercially available acyl halides and alkynes to diversify the scaffold there are some limitations to relying upon commercial sources. For example, while many hundreds of acyl halides can be purchased, very few alkynes are available and those that are have poor structural diversity. A significant component of the experimental work in this thesis involved the generation of additional chemical entities for library construction. The routes, strategies, and methods for the synthesis of useful additional building blocks, while tangential to the larger questions studied herein, need to be archived for future reference. This last chapter serves that purpose.

8.2 Synthesis of racemic phenyl substituted allyl and vinyl secondary alcohols

\[
\begin{align*}
\begin{array}{c}
\text{MgBr} \\
\text{Et}_2\text{O}, 0 \degree \text{C, 30 min}
\end{array}
\end{align*}
\]

\[\text{201} \rightarrow \text{202}\]

Scheme 8.1: General synthesis of racemic phenyl substituted allyl secondary alcohols 202.

The Grignard reaction with the allyl magnesium bromide produced five phenyl substituted allyl secondary alcohols 202. 4-Isopropylbenzaldehyde, p-tolualdehyde, 4-bromobenzaldehyde, 3-benzyloxybenzaldehyde, and 4-(diethyl-amino)-benzaldehyde were
added to allyl magnesium bromide at 0 °C and the solutions were stirred for 30 minutes (Scheme 8.1). The secondary alcohols 203-207 were purified by chromatography on silica gel (Figure 8.1).

![Chemical structures](image)

**Figure 8.1:** Racemic phenyl substituted allyl secondary alcohols library.

**Scheme 8.2:** General synthesis of racemic phenyl substituted vinyl secondary alcohols 209.

In the second set of precursors, phenyl substituted vinyl secondary alcohols 209 were synthesized, which were then used in Sharpless epoxidation reactions to generate the (S)-alcohols (Scheme 8.2 and 8.3). p-Tolualdehyde, 4-isopropylbenzaldehyde, 4-(diethylamino)benzaldehyde, 2,3-dimethoxybenzaldehyde, 3-benzyloxybenzaldehyde, 4-bromobenzaldehyde, 3-phenoxybenzaldehyde, 4-ethoxybenzaldehyde, 4-ethylbenzaldehyde, 2-naphthaldehyde, and 3,5-di-tert-butyl-4-hydroxybenzaldehyde were each added to a
solution of THF and vinyl magnesium bromide at 0 °C. The solution was stirred for 12 hours, and the products 210-220 (Figure 8.2) purified by chromatography on silica gel.

Figure 8.2: Racemic phenyl substituted vinyl secondary alcohols library.

Scheme 8.3: General synthesis of synthesis of (S)-phenyl substituted vinyl secondary alcohols 221.
Sharpless epoxidation was used as a kinetic resolution to generate the (S)-phenyl substituted vinyl secondary alcohols 221 from the racemic starting materials 209 (Scheme 8.3).[249] Each stereochemically pure product was synthesized by the addition of its racemic precursor to methylene chloride at -20 °C with molecular sieves, followed by diethyl (−)-tartrate and Ti(iPrO)₄.[249] The solution was stirred for 30 minutes, after which t-BuOOH in methylene chloride was added to the -20 °C solution.[249] The solution was allowed to warm to room temperature over a 24 hours period.[249] Ferrous sulfate and d-(−)-tartaric acid with water were added and the solution stirred for 30 minutes.[249] The alcohol products were purified from the epoxides by chromatography on silica gel. The five stereochemically pure compounds 222-226 are featured in Figure 8.3.

![Figure 8.3: (S)-phenyl substituted vinyl secondary alcohols.](image-url)
8.3 Synthesis of racemic phenyl substituted allyl and vinyl terminal alkyne ethers

Scheme 8.4: General synthesis of racemic phenyl substituted vinyl terminal alkyne ethers 227.

The racemic phenyl substituted vinyl terminal alkyne ethers 227 were synthesized by the addition of the corresponding racemic phenyl substituted vinyl alcohols 209 to THF at room temperature. Sodium hydride was added followed by HMPA and the solution was stirred for 1 hour, after which propargyl bromide was added. The solution was stirred for 12 hours and the racemic phenyl substituted vinyl terminal alkyne ethers 228-234 (Figure 8.4) were purified by chromatography on silica gel. (Scheme 8.4)

Figure 8.4: Racemic phenyl substituted vinyl terminal alkyne ethers.
Scheme 8.5: General synthesis of racemic phenyl substituted allyl terminal alkyne ethers 235.

Though only the vinyl alcohols could be used in the Sharpless epoxidations to separate out the stereoisomers, the allyl terminal alkyne ethers 235 allow for greater chain length for the formation of macrolides using the Grubbs metathesis. The racemic phenyl substituted allyl terminal alkyne ethers 236-240 (Figure 8.5) were synthesized via the addition of the corresponding phenyl substituted allyl alcohol 202 to THF at room temperature followed by sodium hydride and HMPA which was stirred for 1 hour. Propargyl bromide was added and the solution was stirred at room temperature for 24 hours (Scheme 8.5).

Figure 8.5: Racemic phenyl substituted allyl terminal alkyne ethers library.
8.4 Synthesis of N-(3-Ethynyl-phenyl)-acetamide derivatives

Scheme 8.6: Synthesis of N-(3-ethynyl-phenyl)-amides.

The 3-ethynylaniline alkyne \( 241 \) provides an excellent moiety for the formation of triazoles. The free amine provides an easily modifiable chemical point for the synthesis of amides and carbamates and the possible future synthesis of secondary amines. Modification of the amine is necessary as the primary amine interferes with triazole formation in the 1,3-dipolar cycloaddition under Sharpless conditions as it complexes to Cu.

The \( N \)-(3-ethynyl-phenyl)-amides \( 242 \) (Figure 8.6) were synthesized by the addition of 3-ethynylaniline \( 241 \) to pyridine at room temperature. Acetic anhydride or an acyl chloride was added to the solution followed by stirring for 12 hours (Scheme 8.6). The products \( 243 \text{-} 249 \) (Figure 8.6) were purified by chromatography on silica gel in high yields.

Figure 8.6: The \( N \)-(3-ethynyl-phenyl)-amides library.
Scheme 8.7: Synthesis of (3-ethynyl-phenyl)-carbamic acid allyl ester 250.

3-Ethynylaniline 241 was added to pyridine at room temperature followed by allyl chloroformate (Scheme 8.7). The solution was stirred for 12 hours and the (3-ethynyl-phenyl)-carbamic acid allyl ester product 250 was obtained with a 75% yield after purification by chromatography on silica gel.

Scheme 8.8: Synthesis of (3-ethynyl-phenyl)-carbamic acid isobutyl ester 251.

(3-Ethynyl-phenyl)-carbamic acid isobutyl ester 251 was synthesized by the addition of 3-ethynylaniline 241 to pyridine at room temperature. Isobutyl chloroformate was added and the solution stirred for 12 hours (Scheme 8.8). Purification by chromatography on silica gel of the product 251 gave a 96.8% yield.

8.5 Biological activity of chemical diverse moieties for triazolo-ester diversity

All precursors were assayed for biological activity. Among these chemically diverse groups, 248 had an MIC of 62 {eq}\mu{M} {/eq} against {it B. subtilis}.

8.6 Conclusions

No compound in these libraries showed any significant biological activity, with the exception of 248, nor did the acyl chlorides or the alkynes used in the previous library. This
lack of activity highlights the necessity of the nonactic acid subunit to the active compounds. However, the importance of chemical diversity has been shown through the synthesis of the triazolo-ester nonactic acid analogue library. The synthesis of these new moieties for the creation of expanded triazoloester libraries was largely successful and the compounds were produced for the most part in good to excellent yields and relatively inexpensively. The vinyl alcohols were readily separated from the vinyl epoxides via column chromatography.
Combinatorial libraries were synthesized from racemic, and plus and minus methyl nonactate 45, 46, 48 the products of methanolysis of nonactin 40. The racemic triazoloester library made from the azidoalcohol 55, revealed 2 active compounds from the 10 (Figure 3.2) that were tested for activity. These compounds, 72 and 71, showed activity against, among other species, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* (Table 3.2). The individual enantiomers of 72 and 71, 88, 90, 81, and 79 were synthesized. The plus enantiomers, 88 and 90, were shown to exhibit biological activity (Table 3.2). 81 and 79 gave no significant biological activity against any of the test organisms (Table 3.2). Regiochemistry of the triazole was also considered. It was found that the 1,4-regioisomer was the active isomer. The 1,5-triazoles 91 and 92 were not bactericidal. Research into the homononactate equivalent versions of 72 and 71, 104 and 103 was begun, but was not completed. None of the intermediate compounds showed any activity of note.

The necessity of the nonactic acid subunit for activity was examined by the synthesis of the *trans* and *cis* cyclohexane analogues of 72 and 71, 141, 140, 132, 131, 152, 151, 161, 160. The chemical moieties were essentially the same in these analogues. None of the eight analogues synthesized possessed activity against MRSA or VRE. This study suggests that the nonactic acid subunit itself is important for activity.

The dimer library of 72 and 71 compounds and bis-triazole library synthesized from the bis-azide nonactic acid analogue scaffold 115 were not bactericidal. Several series of
chemical moieties, including enantiomerically pure moieties were synthesized for future library purposes in an effort to expand the chemical diversity of the triazoloester libraries.

The last of the projects entailed the biosynthesis of nonactin analogues via the construction of nonactic acid analogues to be used in feeding studies. The biosynthesis of the nonactin analogues remains incomplete due to developmental delays of the ∆nonS/∆nonS’-double knock out of *Streptomyces griseus*. However it was discovered that nonactic acid with an unreduced furan ring 186 inhibited the production of nonactin 40, at an IC$_{50}$ of 100 µM.
10.1 General Methods

Reagents were obtained from various chemical companies (TCI, Sigma-Aldrich, Acros, and Fischer Scientific) and were used both with and without prior purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Dichloromethane was distilled from calcium hydride (CaH₂). Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were used as bought from the chemical supplier without further purification. Pyridine (Py), used as both a solvent and a base, was purified via distillation under vacuum from potassium hydroxide (KOH). Anhydrous diethyl ether was used as received from the chemical supplier. para-Toluenesulfonyl chloride (pTsCl) was purified via crystallization from benzene. 3,4-Dihydro-2H-pyran (DHP) was used without distillation as were acyl chlorides and acetylenes.

Aqueous reagents used deionized water. All reactions that did not involve aqueous reagents, other than para-toluenesulfonic acid monohydrate (pTsOH-H₂O), were run under anhydrous conditions in an argon atmosphere. A hydrogen (H₂) atmosphere was used in the Pd/C catalytic reduction reactions. In anhydrous reactions, all glassware was flame-dried and/or oven dried. Reactions involving shaking were performed in the innOva 4000 Incubator Shaker of New Brunswick Scientific and the New Brunswick Scientific Classic Series C24 Incubator Shaker. Thin-layer chromatography plates were aluminum-backed.
with silica gel 60 F_{254}. Silica gel (230-400 mesh) from Silicycle was used in all column chromatography purifications.

Characterization of synthesized compounds was performed on the following instruments: Varian Unity Plus 400 MHz spectrometer was used to obtain proton and carbon nuclear magnetic resonance (NMR) spectra. Infrared spectra (IR) were obtained using the Nicolet Nexus 670 FT-IR using the SMART MIRacle attachment. Mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained with a Micromass LCT electrospray ionization (ESI) spectrometer in conjunction with a Waters 2790 Separations Module. The Alamar-Blue Assay, performed by Adrienne E. Smith, Erin S. Bolstad, and Brooke Martin was used to obtain the IC_{50} and MIC values for each compound in bacterial and cytotoxicity assays.

10.2 Library of Compounds

51: 2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl]-propionic acid methyl ester 51. Racemic methyl nonactate 45 (0.624 g, 2.9 mmol) was added to CH_{2}Cl_{2} (15 mL) followed by DHP (1.3 mL, 14.4 mmol) and pTsOH-H_{2}O (6.0 mg, 0.03 mmol). The solution was stirred for 5 hours at room temperature. The solution was dried (MgSO_{4}) and evaporated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give a clear oil 51 (0.86 g, 99%). R_{f} = 0.44 (EtOAc-hexanes (1:3)); \textsuperscript{1}H NMR (400 MHz, CDCl_{3}) \delta 4.73 (m, 0.5H), 4.62 (m, 0.5H), 3.92 (m, 4H), 3.67 (m, 3H), 3.46 (m, 1H), 2.50 (sextet, J = 14.0, 7.1, 7.0 Hz, 1H), 1.96 (m, 2H), 1.65 (m, 10H), 1.23 (m, 2H), and 1.10 (m, 4H);
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 175.4, 99.8, 94.5, 80.4, 80.3, 77.3, 77.0, 76.4, 72.6, 68.0, 63.0, 62.0, 51.54, 51.51, 45.4, 45.4, 44.1, 43.8, 31.5, 31.3, 31.1, 31.0, 28.6, 28.5, 25.51, 25.46, 22.7, 20.2, 19.5, 19.4, 13.4, and 13.3; IR (ATR) 2941, 2873, 1739, 1456, 1436, 1375, 1352, 1260, 1200, 1163, 1118, 1063, 1021, 992, 942, 905, 869, and 813 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{16}\)H\(_{26}\)O\(_3\)Na\(^+\): 323.18; found 323.35.

\[
\begin{align*}
\text{O} & \text{O} \\
\text{THP} & \text{O} \\
\text{O} & \text{LiAlH}_4 \\
\text{THF, 0 °C to r.t., 5h} & \text{THF, 0 °C to r.t., 5h} \\
\text{2-(5}'\text{-}(\text{Tetrahydro-pyran-2-yloxy})-\text{propyl}']-\text{tetrahydro-furan-2-yl]}-\text{propan-1-ol} & \text{52}
\end{align*}
\]

51 (0.841 g, 2.8 mmol) was added to THF (30 mL) followed by LiAlH\(_4\) (0.425 g, 11.2 mmol) at 0 °C. The solution was allowed to warm to room temperature and stirred for 5 hours. Water (0.43 mL) was added to the solution, followed by 3.0 M NaOH/H\(_2\)O (0.85 mL) and then with water (0.43 mL). Sodium sulfate was then added. After 5 minutes the solution was filtered and the filtrate evaporated. The residue was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give a clear oil 52 (0.576 g, 75.5%). R\(_f\) = 0.46 (EtOAc-hexanes (1:1)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.62 (m, 0.5H), 4.57 (m, 0.5H), 4.07 (m, 1H), 3.86 (m, 2H), 3.52 (m, 4H), 1.92 (m, 2H), 1.60 (m, 11H), 1.19 (d, \(J = 6.3\) Hz, 2H), 1.06 (d, \(J = 6.0\) Hz, 2H), and 0.75 (t, \(J = 6.8\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 99.7, 95.3, 85.6, 85.3, 76.6, 76.3, 72.3, 68.6, 68.3, 68.0, 62.9, 62.5, 43.9, 43.6, 41.0, 40.8, 31.0, 30.98, 30.7, 30.4, 30.2, 25.32, 25.28, 22.5, 20.1, 19.8, 19.5, and 13.5; IR (ATR) 3467, 2939, 2874, 1454, 1374, 1352, 1261, 1201, 1116, 1075, 1021, 991, 943, 905, 869, and 811 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{15}\)H\(_{28}\)O\(_4\)Na\(^+\): 295.19; found 295.34.
53: Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydrofuranyl-2-yl}-propyl ester 53. 52 (0.576 g, 2.1 mmol) was added to pyridine (10 mL) followed by pTsCl (0.806 g, 4.2 mmol) at room temperature and the resultant solution was stirred for 5 hours. Water (50 mL) was added and the solution extracted with ethyl acetate (3 × 100 mL). The organic layer was then washed with a saturated solution of copper sulfate (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL). The organic layer was dried (Na₂SO₄) and evaporated. The residue was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give a clear oil 53 (0.718 g, 79.6%). R_f = 0.41 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.78 (m, 2H), 7.33 (m, 2H), 4.73 (m, 0.5H), 4.60 (m, 0.5), 4.13 (m, 1H), 3.87 (m, 4H), 3.50 (m, 2H), 2.44 (s, 3H), 1.88 (m, 4H), 1.55 (m, 9H), 1.20 (m, 2H), 1.07 (m, 1H), and 0.89 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 144.6, 144.5, 133.0, 129.7, 129.7, 127.9, 99.8, 94.6, 80.0, 79.6, 76.3, 76.2, 73.2, 73.1, 72.8, 72.5, 67.9, 65.2, 63.0, 62.0, 44.1, 43.8, 43.2, 38.8, 38.5, 31.4, 31.2, 31.1, 31.0, 30.6, 29.12, 29.08, 28.98, 25.5, 25.4, 23.4, 22.6, 21.6, 20.2, 19.5, 19.4, 13.4, and 13.3; IR (ATR) 2941, 2875, 1739, 1599, 1454, 1359, 1291, 1188, 1176, 1118, 1097, 1075, 1021, 992, 962, 940, 812, 792, 706, and 666 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₂H₃₄O₆SNa⁺: 449.20; found 449.40.

54: 2-{2-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethoxy}-tetrahydro-pyran 54. 53 (0.718 g, 1.7 mmol) was added to DMF (7 mL) followed by
NaN₃ (0.547 g, 8.4 mmol). The solution was warmed to 80 °C and stirred for 5 hours. The solution was cooled to room temperature and water (25 mL) was added. The solution was washed with diethyl ether (3 × 50 mL). The organic layers were collected, dried (MgSO₄), and evaporated. The residue was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give a clear oil 54 (0.404 g, 80.6%). R_f = 0.7 (EtOAc-hexanes (1:3)); ^1H NMR (400 MHz, CDCl₃) δ 4.74 (m, 0.5H), 4.64 (m, 0.5H), 3.93 (m, 3H), 3.49 (m, 3H), 3.25 (m, 1H), 1.95 (m, 2H), 1.61 (m, 10H), 1.23 (d, J = 6.3 Hz, 2H), 1.11 (d, J = 6.0 Hz, 2H), and 0.91 (d, J = 6.8 Hz, 3H); ^13C NMR (100 MHz, CDCl₃) δ 99.8, 94.4, 80.7, 80.6, 76.2, 76.0, 72.5, 67.9, 62.9, 61.9, 55.1, 44.1, 43.9, 39.49, 3.47, 31.5, 31.3, 31.1, 31.0, 29.2, 25.5, 25.4, 22.7, 20.1, 19.41, 19.39, and 14.4; IR (ATR) 2939, 2875, 2096, 1453, 1380, 1351, 1282, 1201, 1115, 1075, 1021, 992, 942, 905, 870, and 813 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₅H₂₇N₃O₃Na⁺: 320.20; found 320.37.

55: 1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-propan-2-ol 55. 54 (0.404 g, 1.4 mmol) was added to 6 M HCl/MeOH (10 mL) and stirred at room temperature for 3 hours. A saturated solution of sodium bicarbonate was added until no gas evolved. The solution was then washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (MgSO₄), and evaporated. The residue was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give a light yellow clear oil 55 (0.218 g, 75.4%). R_f = 0.54 (EtOAc-hexanes (1:1)); ^1H NMR (400 MHz, CDCl₃) δ 3.93 (m, 1H), 3.85 (m, 1H), 3.47 (m, 1H), 3.30 (m, 1H), 3.18 (s, 1H), 3.12 (m, 1H), 1.83 (m, 2H), 1.51 (m, 5H), 1.05 (d, J = 6.3 Hz, 3H), and 0.80 (d, J = 6.9 Hz, 3H);
$^{13}$C NMR (100 MHz, CDCl$_3$) δ 80.8, 76.3, 64.7, 54.7, 43.5, 38.9, 30.5, 28.8, 23.2, and 14.0; IR (ATR) 3404, 2966, 2934, 2095, 1460, 1380, 1282, 1067, and 937 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{19}$H$_{19}$N$_3$O$_2$Na$: 236.14$; found 236.28.

\[ \text{py, THF, r.t., 12 h} \]

57: Acetic acid 2-{5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl}-1-methyl-ethyl ester 57. 55 (35.0 mg, 0.2 mmol) was added to THF (1 mL) followed by pyridine (2 mL) and acetic anhydride (2 mL). The solution was stirred for 12 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 57 (32.3 mg, 76.9%). R$_f$ = 0.63 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) δ 4.99 (m, 1H), 3.84 (m, 1H), 3.54 (m, 1H), 3.46 (m, 1H), 3.24 (m, 1H), 2.01 (s, 3H), 1.94 (m, 1H), 1.72 (m, 2H), 1.52 (m, 2H), 1.24 (d, $J = 6.3$ Hz, 3H), and 0.93 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.6, 80.8, 76.0, 69.1, 55.0, 42.4, 39.4, 31.3, 29.2, 21.4, 20.6, and 14.4

\[ \text{py, } 37 \degree C, 5 \text{ h} \]

58: 2,2-Dimethyl-propionic acid 2-{5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl}-1-methyl-ethyl ester 58. 55 (35.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by trimethylacetyl chloride (0.2 mL, 1.6 mmol). The solution was then
shaken at 37 °C for 5 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 58 (32.0 mg, 65.7%). Rᶠ = 0.80 (EtOAc-hexanes (1:3)); \(^1\)H NMR (400 MHz, CDCl₃) δ 4.96 (m, 1H), 3.83 (m, 1H), 3.53 (m, 1H), 3.47 (m, 1H), 3.23 (m, 1H), 1.93 (m, 2H), 1.72 (m, 3H), 1.51 (m, 2H), 1.21 (d, J = 6.2 Hz, 3H), 1.18 (s, 9H), and 0.92 (d, J = 6.8 Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl₃) δ 177.9, 80.8, 76.1, 68.8, 55.1, 42.5, 39.4, 31.4, 29.2, 27.2, 27.1, 20.5, and 14.4

59: Pent-4-enoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester 59. 55 (35.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by 4-pentenoyl chloride (0.2 mL, 1.6 mmol). The solution was shaken at 37 °C for 5 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 59 (24.7 mg, 51.0%). Rᶠ = 0.73 (EtOAc-hexanes (1:3)); \(^1\)H NMR (400 MHz, CDCl₃) δ 5.81 (m, 1H), 5.02 (m, 2H), 3.84 (m, 1H), 3.55 (m, 1H), 3.47 (m, 1H), 3.24 (m, 1H), 2.37 (m, 4H), 1.95 (m, 2H), 1.72 (m, 3H), 1.53 (m, 3H), 1.24 (d, J = 6.4 Hz, 3H), and 0.93 (d, J = 6.8 Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl₃) δ 172.5, 136.7, 115.4, 80.8, 76.0, 69.1, 55.1, 42.5, 39.4, 33.8, 31.3, 29.2, 28.9, 20.7, and 14.4
60: **Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester 60.** 55 (0.119 g, 0.6 mmol) was added to pyridine (3 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.6 mmol). The solution was stirred at room temperature overnight. Chloroform (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give 60 (0.109 g, 63.5%). Rₚ = 0.43 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 5.03 (m, 1H), 3.84 (m, 1H), 3.69 (s, 3H), 3.55 (m, 1H), 3.46 (m, 1H), 3.24 (m, 1H), 2.61 (m, 4H), 1.95 (m, 2H), 1.72 (m, 3H), 1.52 (m, 2H), 1.25 (d, J = 6.2 Hz, 3H), and 0.93 (d, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 173.1, 172.8, 80.8, 76.0, 69.6, 55.1, 51.8, 42.4, 39.5, 31.3, 29.4, 29.2, 28.9, 20.6, and 14.4; IR (ATR) 3954, 2360, 2097, 1731, 1438, 1363, 1269, 1162, 1065, 997, and 846 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₅H₂₅N₃O₅Na⁺: 350.17; found 350.21.

61: **Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester 61.** 55 (0.119 g, 0.6 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (0.18 mL, 1.6 mmol). The solution was stirred at room temperature overnight. Chloroform (50 mL) was added and the solution was washed with
saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 61 (0.128 g, 77.6%). \( R_f = 0.75 \) (EtOAc-hexanes (1:3)); \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 8.03 (m, 2H), 7.55 (m, 1H), 7.43 (m, 2H), 5.28 (m, 1H), 3.94 (m, 1H), 3.54 (m, 1H), 3.47 (m, 1H), 3.21 (m, 1H), 1.95 (m, 3H), 1.83 (m, 1H), 1.72 (m, 1H), 1.54 (m, 2H), 1.38 (d, \( J = 6.2 \) Hz, 3H), and 0.92 (d, \( J = 6.8 \) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl₃) \( \delta \) 166.0, 132.7, 130.8, 129.5, 128.3, 80.8, 76.1, 69.8, 55.0, 42.6, 39.4, 31.4, 29.2, 20.8, and 14.4; IR (ATR) 2972, 2877, 2096, 1715, 1603, 1585, 1451, 1381, 1356, 1314, 1274, 1175, 1109, 1069, 1026, and 711 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{17}\)H\(_{23}\)N\(_3\)O\(_3\)H\(^+\): 318.18; found 318.23.

63: **Acetic acid** 2-{5-[2-(4-tert-butyl-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydrofuran-2-yl}-1-methyl-ethy ester 63. 57 (16.2 mg, 0.06 mmol) was added to 5:1 EtOH/H\(_2\)O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 79.0 µL, 0.6 mmol). Ascorbic acid/H\(_2\)O (1.1 M, 127.0 µL, 0.2 mmol) and CuSO\(_4\)-5H\(_2\)O/H\(_2\)O (0.3 M, 0.1 mL, 0.03 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na\(_2\)SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 63 (18.0 mg, 84.1%). \( R_f = 0.38 \) (EtOAc-
hexanes (1:1)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.35 (s, 1H), 5.10 (m, 1H), 4.52 (m, 1H), 4.26 (m, 1H), 3.85 (m, 1H), 3.40 (m, 1H), 2.71 (d, $J = 7.3$ Hz, 2H), 2.19 (m, 1H), 2.04 (s, 3H), 2.00 (m, 2H), 1.66 (m, 9H), 1.25 (d, $J = 6.3$ Hz, 3H), 1.23 (m, 4H), and 0.82 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.6, 122.1, 80.8, 76.0, 68.9, 53.2, 42.4, 40.3, 40.0, 32.4, 31.6, 31.3, 29.7, 29.5, 25.1, 21.4, 20.7, and 14.4

Acetic acid 2-\{5-\{2-(4-cyclopentylmethyl-\{1,2,3\}triazol-1-yl\}-1-methyl-ethyl\}-tetrahydro-furan-2-yl\}-1-methyl-ethyl ester 64. 57 (16.2 mg, 0.06 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 84.0 µL, 0.6 mmol). Ascorbic acid/H$_2$O (1.1 M, 127.0 µL, 0.2 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 0.1 mL, 0.03 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 x 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 64 (9.4 mg, 41.0%). $R_f = 0.37$ (EtOAc-hexanes (1:1)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.35 (s, 1H), 5.10 (m, 1H), 4.52 (m, 1H), 4.26 (m, 1H), 3.85 (m, 1H), 3.40 (m, 1H), 2.71 (d, $J = 7.3$ Hz, 2H), 2.19 (m, 1H), 2.04 (s, 3H), 2.00 (m, 2H), 1.66 (m, 9H), 1.25 (d, $J = 6.3$ Hz, 3H), 1.23 (m, 4H), and 0.82 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.6, 122.1, 80.8, 76.0, 68.9, 53.2, 42.4, 40.3, 40.0, 32.4, 31.6, 31.3, 29.7, 29.5, 25.1, 21.4, 20.7, and 14.4

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Pent-4-enoic acid \(2\)-{\(5\)-[\(2\)-(4-tert-butyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester} 65. 59 (22.3 mg, 0.08 mmol) was added to 5:1 EtOH/H\(_2\)O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 94.0 \(\mu\)L, 0.8 mmol). Ascorbic acid/H\(_2\)O (1.1 M, 151.0 \(\mu\)L, 0.2 mmol) and CuSO\(_4\)-5H\(_2\)O/H\(_2\)O (0.3 M, 126.0 \(\mu\)L, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 \(\times\) 50 mL). The organic phases were collected, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 65 (10.1 mg, 35.6%). \(R_f = 0.50\) (EtOAc-hexanes (1:1)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.31 (s, 1H), 5.81 (m, 1H), 5.06 (m, 3H), 4.52 (m, 1H), 4.22 (m, 1H), 3.85 (m, 1H), 3.43 (m, 1H), 2.38 (m, 4H), 1.97 (m, 3H), 1.80 (m, 1H), 1.70 (m, 1H), 1.54 (m, 1H), 1.35 (s, 9H), 1.26 (d, \(J = 6.3\) Hz, 3H), and 0.83 (d, \(J = 6.9\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 172.5, 136.7, 119.5, 115.4, 80.8, 76.0, 68.9, 53.1, 42.5, 40.3, 33.8, 31.3, 30.7, 30.4, 29.4, 28.9, 20.8, and 14.3
66: 2,2-Dimethyl-propionic acid 2-{5-[2-(4-cyclopentylmethyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester 66. 58 (27.6 mg, 0.09 mmol) was added to 5:1 EtOH/H2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 122.0 µL, 0.9 mmol). Ascorbic acid/H2O (1.1 M, 185.0 µL, 0.2 mmol) and CuSO4·5H2O/H2O (0.3 M, 155.0 µL, 0.05 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na2SO4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 66 (17.2 mg, 46.0%). Rf = 0.50 (EtOAc-hexanes (1:1)); 1H NMR (400 MHz, CDCl3) δ 7.35 (s, 1H), 5.07 (m, 1H), 4.51 (m, 1H), 4.26 (m, 1H), 3.83 (m, 1H), 3.38 (m, 1H), 2.71 (d, J = 7.4 Hz, 2H), 2.18 (m, 1H), 1.97 (m, 4H), 1.66 (m, 11H), 1.24 (d, J = 6.2 Hz, 3H), 1.18 (s, 9H), and 0.82 (d, J = 6.9 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 178.0, 147.5, 122.0, 80.7, 76.1, 68.5, 53.1, 42.5, 40.3, 40.0, 38.7, 32.4, 31.6, 31.4, 29.5, 27.1, 25.1, 20.6, and 14.3
67: *Pent-4-enoic acid 2-[5-2-(4-cyclopentylmethyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* 67. 59 (22.3 mg, 0.08 mmol) was added to 5:1 EtOH/H2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 100.0 µL, 0.8 mmol). Ascorbic acid/H2O (1.1 M, 151.0 µL, 0.2 mmol) and CuSO4-5H2O/H2O (0.3 M, 126.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na2SO4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 67 (11.0 mg, 36.0%). 1H NMR (400 MHz, CDCl3) δ 7.36 (s, 1H), 5.82 (m, 1H), 5.12 (m, 1H), 5.03 (m, 1H), 4.52 (m, 1H), 4.27 (m, 1H), 3.84 (m, 1H), 3.39 (m, 1H), 2.72 (d, J = 7.4 Hz, 2H), 2.38 (m, 4H), 2.19 (m, 1H), 1.97 (m, 1H), 1.65 (m, 9H), 1.26 (d, J = 6.3 Hz, 3H), 1.23 (m, 4H), and 0.82 (d, J = 6.9 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 172.5, 136.6, 122.1, 115.4, 80.7, 76.0, 68.8, 53.2, 42.5, 40.3, 40.0, 33.8, 32.4, 32.4, 31.6, 31.3, 29.7, 29.5, 29.0, 25.1, 20.8, and 14.4

68: *Succinic acid 2-(5-[2-[4-(4-fluoro-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester* 68. 60 (22.4 mg, 0.07 mmol)
was added to 5:1 EtOH/H₂O (1.5 mL) followed by 1-ethynyl-4-fluorobenzene (78.0 µL, 0.7 mmol). Ascorbic acid/H₂O (1.1 M, 137.0 µL, 0.2 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 114.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 68 (17.7 mg, 57.8%).

Rᵣ = 0.38 (EtOAc-hexanes (1:1)); ¹H NMR (400 MHz, CDCl₃) δ 7.88 (m, 3H), 7.11 (t, J = 8.7 Hz, 2H), 5.23 (m, 1H), 4.55 (m, 1H), 4.43 (m, 1H), 3.83 (m, 1H), 3.65 (s, 3H), 3.40 (m, 1H), 2.63 (m, 4H), 1.98 (m, 3H), 1.82 (m, 1H), 1.67 (m, 2H), 1.53 (m, 2H), 1.26 (d, J = 6.4 Hz, 3H), and 0.90 (d, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 171.8, 127.5, 127.4, 120.9, 115.7, 115.5, 80.4, 75.9, 69.1, 53.2, 51.8, 42.5, 40.4, 31.2, 29.7, 29.4, 28.9, 20.8, and 14.5

2,2-Dimethyl-propionic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester 69. 58 (27.6 mg, 0.09 mmol) was added to 5:1 EtOH/H₂O (1.5 mL) followed by N-(3-ethynyl-pyridine)-acetamide (147.0 mg, 0.9 mmol). Ascorbic acid/H₂O (1.1 M, 185.0 µL, 0.2 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 155.0 µL, 0.05 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic
phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give 69 (32.4 mg, 76.6%). Rₛ = 0.51 (100% EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.98 (s, 1H), 7.91 (s, 1H), 7.81 (s, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.34 (t, J = 7.9 Hz, 1H), 5.09 (m, 1H), 4.53 (m, 1H), 4.39 (m, 1H), 3.81 (m, 1H), 3.41 (m, 1H), 2.17 (s, 3H), 1.98 (m, 3H), 1.81 (m, 1H), 1.58 (m, 3H), 1.20 (m, 12H), and 0.89 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 178.3, 168.6, 138.6, 131.2, 129.5, 121.3, 119.5, 116.7, 80.7, 76.2, 68.7, 53.5, 42.5, 40.4, 38.8, 31.3, 29.8, 27.1, 24.6, 20.6, and 14.6

70: **Benzoic acid 2-(5-[[2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester** 70. 61 (31.7 mg, 0.01 mmol) was added to 5:1 EtOH/H₂O (1.5 mL) followed by N-(3-ethynyl-pyridine)-acetamide (159.0 µL, 1.0 mmol). Ascorbic acid/H₂O (1.1 M, 200.0 µL, 0.2 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 165.0 µL, 0.05 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give 70 (43.5 mg, 91.4%). Rₛ = 0.49 (100% EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.03 (m, 3H), 7.91 (m, 1H), 7.84 (s, 1H), 7.77 (m, 1H), 7.56 (m, 2H), 7.44 (m, 2H), 7.32 (m, 1H), 5.44 (m, 1H), 4.51 (m, 1H), 4.40 (m, 1H), 147
3.92 (m, 1H), 3.38 (m, 1H), 2.14 (s, 3H), 1.98 (m, 4H), 1.81 (m, 1H), 1.55 (m, 2H), 1.37 (d, $J = 6.3$ Hz, 3H), and 0.87 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.7, 166.3, 146.9, 138.7, 132.9, 131.2, 130.6, 129.49, 129.46, 128.4, 121.5, 121.3, 119.4, 116.7, 80.6, 76.0, 69.5, 53.4, 42.5, 40.3, 31.3, 29.8, 24.6, 20.9, and 14.6

71: Succinic acid 2-(5-[2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester 71. 60 (21.0 mg, 0.1 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by N-(3-ethynyl-phenyl)-acetamide (102.0 mg, 0.6 mmol). Ascorbic acid/H$_2$O (1.1 M, 128.0 µL, 0.1 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 106.0 µL, 0.03 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 71 (25.2 mg, 80.8%). $R_f = 0.38$ (100% EtOAc); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.01 (s, 1H), 7.91 (s, 1H), 7.77 (m, 2H), 7.65 (d, $J = 7.8$ Hz, 1H), 7.35 (t, $J = 7.8$ Hz, 1H), 5.18 (m, 1H), 4.52 (m, 1H), 4.40 (m, 1H), 3.82 (m, 1H), 3.64 (s, 3H), 3.38 (m, 1H), 2.63 (s, 4H), 2.17 (s, 3H), 1.97 (m, 3H), 1.81 (m, 1H), 1.66 (m, 1H), 1.51 (m, 2H), 1.24 (d, $J = 6.3$ Hz, 3H), and 0.89 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.8, 172.0, 168.6, 146.9, 138.6, 131.4, 129.5, 121.4, 121.2, 119.4, 116.6, 80.6, 75.9, 69.4, 53.3, 51.8, 42.4, 40.3, 31.2, 29.8, 29.5, 29.0, 24.5, 20.7, and 14.6; IR (ATR) 3311, 3130, 2974, 2099, 1730, 1693, 1618, 1592, 1569, 1534, 1488, 1438, 1406, 1369, 1316,
1222, 1163, 1045, 997, 884, 847, 792, 757, and 694 cm⁻¹; ESI-TOF-MS m/z calcd for C$_{25}$H$_{34}$N$_4$O$_6$H$: 487.26; found 487.27.

72: *Benzoic acid 1-methyl-2-[5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl]-ethyl ester* 72. 61 (10.18 mg, 0.03 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 2-ethynyl-pyridine (98+, 33.0 µL, 0.3 mmol). Ascorbic acid/H$_2$O (1.1 M, 64.0 µL, 0.07 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 53.0 µL, 0.02 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (2 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 72 (12.2 mg, 90.4%). R$_f$ = 0.19 (EtOAc-hexanes (1:1)); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.56 (m, 1H), 8.16 (m, 2H), 8.05 (m, 2H), 7.75 (m, 1H), 7.54 (m, 1H), 7.43 (m, 2H), 7.21 (m, 1H), 5.36 (m, 1H), 4.64 (m, 1H), 4.32 (m, 1H), 3.98 (m, 1H), 3.51 (m, 1H), 2.00 (m, 4H), 1.86 (m, 1H), 1.58 (m, 2H), 1.40 (d, J = 6.3 Hz, 3H), and 0.85 (d, J = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 166.0, 150.4, 149.3, 148.1, 136.8, 132.7, 130.7, 129.5, 128.3, 122.9, 122.6, 120.2, 80.7, 76.3, 69.6, 53.5, 42.6, 40.4, 31.4, 29.6, 20.8, and 14.3; IR (ATR) 3133, 3065, 2972, 2954, 2935, 2877, 2100, 1736, 1708, 1600, 1593, 1569, 1544, 1472, 1455, 1430, 1420, 1382, 1356, 1319, 1275, 1260, 1235, 1201, 1155, 1108, 1070,
1060, 1031, 844, 790, and 718 cm\(^{-1}\); ESI-TOF-MS \(m/z\) calcd for \(\text{C}_{24}\text{H}_{28}\text{N}_{4}\text{O}_{3}\text{H}^+\): 421.22; found 421.24.

73: \(2\{-5\{-2\{(\text{Tetrahydro-pyran-2-yloxy})\text{-propyl}\}\}\text{-tetrahydro-furan-2-yl}\}\text{-propionic acid methyl ester 73}\). Methyl \((--)\)-nonactate 48 (0.704 g, 3.3 mmol) was added to methylene chloride (13 mL) followed by DHP (1.5 mL, 16.3 mmol) and pTsOH-H\(_2\)O (6.0 mg, 0.03 mmol). The solution was stirred at room temperature for 5 hours. The solution was evaporated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 73 (0.824 g, 84.4%). \({^1}\text{H}\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.73 (m, 0.5H), 4.62 (m, 0.5H), 3.92 (m, 4H), 3.67 (m, 3H), 3.46 (m, 1H), 2.50 (m, 1H), 1.96 (m, 2H), 1.65 (m, 10H), 1.22 (m, 2H), and 1.09 (m, 4H); \({^{13}}\text{C}\) NMR (100 MHz, CDCl\(_3\)) \(\delta\) 175.41, 175.38, 99.8, 94.5, 80.4, 80.3, 77.3, 77.0, 76.65, 76.4, 72.6, 68.0, 63.0, 62.0, 51.54, 5.51, 45.44, 45.36, 44.1, 43.8, 31.5, 31.3, 31.1, 31.0, 28.6, 28.5, 25.5, 25.4, 22.6, 20.2, 19.5, 19.4, 13.4, and 13.3; IR (ATR) 2941, 2873, 1739, 1456, 1436, 1375, 1352, 1260, 1200, 1163, 1118, 1063, 1021, 992, 942, 905, 869, and 812 cm\(^{-1}\); ESI-TOF-MS \(m/z\) calcd for \(\text{C}_{16}\text{H}_{28}\text{O}_{5}\text{Na}^+\): 323.18; found 323.24.

74: \(2\{-5\{-2\{(\text{Tetrahydro-pyran-2-yloxy})\text{-propyl}\}\}\text{-tetrahydro-furan-2-yl}\}\text{-propan-1-ol 74}\). 73 (0.824 g, 2.8 mmol) was added to THF (30 mL) followed by (0.208 g, 5.5 mmol) of lithium aluminum hydride at room temperature and then the solution was stirred overnight. Water (5.5 mL) was added, followed by 3.0 M NaOH/H\(_2\)O (11 mL) and then water (5.5 mL). The solution was filtered and evaporated to a residue which was
purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 74 (0.708 g, 94.8%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.66 (m, 0.5H), 4.60 (m, 0.5H), 4.12 (m, 1H), 3.88 (m, 2H), 3.54 (m, 4H), 1.96 (m, 2H), 1.65 (m, 1H), 1.23 (m, 2H), 1.10 (m, 2H), and 0.78 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 99.9, 95.4, 85.9, 85.6, 76.7, 76.5, 72.5, 68.8, 68.5, 68.1, 63.1, 62.7, 44.0, 43.7, 41.1, 40.9, 31.12, 31.09, 30.73, 30.7, 30.5, 30.4, 25.41, 25.37, 22.6, 20.2, 19.9, 19.6, and 13.6; IR (ATR) 3467, 2939, 2874, 1454, 1374, 1352, 1261, 1201, 1175, 1075, 1021, 991, 943, 905, 869, 811 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{15}$H$_{28}$O$_4$Na$: 295.19$; found 295.24.

75: Toluene-4-sulfonic acid 2-[(5-([2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl)-propyl ester 75. 74 (0.708 g, 2.6 mmol) was added to pyridine (2.1 mL, 26.0 mmol) followed by pTsCl (0.992 g, 5.2 mmol) and then the solution was stirred at room temperature for 5 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 75 (0.961 g, 86.6%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.77 (m, 2H), 7.33 (m, 2H), 4.72 (m, 0.5H), 4.59 (m, 0.5H), 4.13 (m, 1H), 3.85 (m, 4H), 3.48 (m, 2H), 2.43 (s, 3H), 1.68 (m, 13H), 1.17 (m, 2H), 1.07 (m, 1H), and 0.89 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 144.51, 144.45, 133.0, 129.7, 127.9, 99.8, 94.6, 80.0, 79.62, 79.60, 76.7, 76.3, 76.2, 73.2, 73.1, 72.8, 72.5, 67.9, 65.2, 63.0, 62.0, 44.1, 43.8, 43.2, 38.8, 38.5, 31.4, 31.2, 31.1, 31.0, 30.6, 29.1, 29.07, 29.0, 25.5, 25.4, 25.37, 22.6, 21.6, 20.2, 19.5, 19.4, 13.41, and 13.39; IR (ATR) 2941, 2875, 1739, 1599, 1454, 1359, 151
1291, 1188, 1176, 1118, 1097, 1021, 992, 962, 940, 812, 792, and 666 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{22}\)H\(_{34}\)O\(_6\)SNa\(^+\): 449.20; found 449.26.

76: 2-{2-{5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl}-1-methyl-ethoxy}-tetrahydro-pyran 76. 75 (0.961 g, 2.3 mmol) was added to DMF (10 mL) followed by sodium azide (1.464 g, 22.5 mmol) and the solution was then warmed to 100 \(^\circ\)C and stirred for 3 hours. Once cooled, water (50 mL) was added and the solution was washed with diethyl ether (3 \(\times\) 50 mL). The organic phases were collected, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 76 (0.479 g, 71.6%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.75 (m, 0.5H), 4.66 (m, 0.5H), 3.94 (m, 3H), 3.50 (m, 3H), 3.26 (m, 1H), 1.95 (m, 2H), 1.64 (m, 10H), 1.25 (m, 2H), 1.12 (m, 2H), and 0.93 (m, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 99.8, 94.5, 80.70, 80.67, 76.3, 76.1, 72.6, 67.9, 62.9, 62.0, 55.1, 44.2, 43.9, 39.53, 39.51, 31.5, 31.4, 31.1, 31.0, 29.3, 25.5, 25.45, 22.7, 20.2, 19.5, and 14.4; IR (ATR) 2939, 2875, 2096, 1453, 1380, 1351, 1282, 1201, 1115, 1075, 1021, 992, 942, 905, 870, and 813 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{15}\)H\(_{27}\)N\(_3\)O\(_3\)Na\(^+\): 320.20; found 320.25.

77: 1-{5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl}-propan-2-ol 77. 76 (0.479 g, 1.6 mmol) was added to 6 M HCl/MeOH (5 mL) and the solution was stirred for 3 hours at room temperature. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 \(\times\) 50 mL). The organic
phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 77 (0.268 g, 78%). ¹H NMR (400 MHz, CDCl₃) δ 4.06 (m, 2H), 3.60 (m, 1H), 3.42 (m, 1H), 3.27 (m, 1H), 2.75 (s, 1H), 1.96 (m, 2H), 1.67 (m, 5H), 1.20 (d, J = 6.4 Hz, 3H), and 0.94 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 81.3, 76.8, 65.3, 55.1, 43.1, 39.2, 30.6, 29.2, 23.3, and 14.4; IR (ATR) 3404, 2966, 2934, 2095, 1460, 1380, 1282, 1067, and 937 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₀H₁₉N₃O₂H⁺: 214.16; found 214.20.

78: Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester 78. 77 (0.134 g, 0.6 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate (97%, 155.0 µL, 1.3 mmol) and the solution was then stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 78 (0.150 g, 73%). ¹H NMR (400 MHz, CDCl₃) δ 5.01 (m, 1H), 3.82 (m, 1H), 3.66 (s, 3H), 3.53 (m, 1H), 3.44 (m, 1H), 3.22 (m, 1H), 2.58 (m, 4H), 1.92 (m, 2H), 1.71 (m, 3H), 1.49 (m, 2H), 1.22 (d, J = 6.4 Hz, 3H), and 0.91 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 171.6, 80.8, 75.9, 69.6, 55.0, 51.7, 42.4, 39.4, 31.2, 29.4, 29.1, 28.9, 20.5, and 14.4; IR (ATR) 2973, 2879, 2097, 1732, 1438, 1363, 1268, 1162, 1065, 997, 883, and 846 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₅H₂₅N₅O₅Na⁺: 350.17; found 350.22.
79: Succinic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester 79. 78 (0.150 g, 0.5 mmol) was added to 5:1 EtOH/H₂O (6 mL) followed by N-(3-ethynyl-pyridine)-acetamide (0.729 g, 4.6 mmol). Ascorbic acid/H₂O (1.1 M, 0.9 mL, 1.0 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 0.8 mL, 0.2 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 79 (0.215 g, 96.5%). ¹H NMR (400 MHz, CDCl₃) δ 8.11 (s, 1H), 7.89 (s, 1H), 7.75 (m, 2H), 7.62 (d, J = 7.8 Hz, 1H), 7.33 (m, 1H), 5.16 (m, 1H), 4.51 (m, 1H), 4.38 (m, 1H), 3.81 (m, 1H), 3.62 (s, 3H), 3.38 (m, 1H), 2.61 (s, 4H), 2.15 (s, 3H), 1.95 (m, 3H), 1.79 (m, 1H), 1.64 (m, 1H), 1.50 (m, 2H), 1.22 (d, J = 6.4 Hz, 3H), and 0.86 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.8, 172.0, 168.7, 146.9, 138.7, 131.3, 129.5, 121.4, 121.2, 119.4, 116.6, 80.6, 75.9, 69.4, 53.3, 51.8, 42.3, 40.3, 31.2, 29.7, 29.5, 28.9, 24.5, 20.7, and 14.6; IR (ATR) 3311, 3130, 2974, 2100, 1730, 1693, 1618, 1592, 1569, 1534, 1488, 1438, 1406, 1369, 1316, 1222, 1163, 1045, 997, 884, 847, 792, 757, and 694 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₅H₃₄N₄O₆H⁺: 487.26; found 487.27.
**80:** Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester 80. 77 (0.134 g, 0.6 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (146.0 µL, 1.3 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 80 (0.193 g, 96.7%). ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, J = 7.0 Hz, 2H), 7.53 (m, 1H), 7.43 (t, J = 7.9 Hz, 2H), 5.28 (m, 1H), 3.93 (m, 1H), 3.53 (m, 1H), 3.46 (m, 1H), 3.20 (m, 1H), 1.94 (m, 3H), 1.82 (m, 1H), 1.71 (m, 1H), 1.54 (m, 2H), 1.38 (d, J = 6.4 Hz, 3H), and 0.92 (d, J = 6.7 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.9, 132.7, 130.5, 129.4, 128.2, 80.8, 76.1, 69.7, 55.0, 42.5, 39.4, 31.4, 29.2, 20.7, and 14.4; IR (ATR) 2972, 2877, 2096, 1715, 1602, 1585, 1451, 1381, 1356, 1314, 1274, 1175, 1109, 1069, 1026, 935, and 711 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₇H₂₃N₅O₃H⁺: 318.18; found 318.23.

**81:** Benzoic acid 1-methyl-2-[5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl]-ethyl ester 81. 80 (0.166 g, 0.5 mmol) was added to 5:1 EtOH/H₂O (6 mL) followed by 2-ethylpyridine (98%, 0.53 mL, 5.2 mmol).
Ascorbic acid/H₂O (1.1 M, 1.0 mL, 1.2 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 0.9 mL, 0.3 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 81 (0.147 g, 67%).

**1H NMR (400 MHz, CDCl₃) δ 8.53 (m, 1H), 8.13 (m, 2H), 8.03 (m, 2H), 7.72 (m, 1H), 7.51 (m, 1H), 7.40 (m, 2H), 7.17 (m, 1H), 5.34 (m, 1H), 4.61 (m, 1H), 4.30 (m, 1H), 3.96 (m, 1H), 3.48 (m, 1H), 1.98 (m, 4H), 1.84 (m, 1H), 1.55 (m, 2H), 1.37 (d, J = 6.4 Hz, 3H), and 0.82 (d, J = 6.9 Hz, 3H);**

**13C NMR (100 MHz, CDCl₃) δ 165.9, 150.4, 149.3, 148.1, 136.7, 132.7, 130.6, 129.4, 128.2, 122.8, 122.6, 120.1, 80.6, 76.2, 69.5, 53.4, 42.5, 40.4, 31.3, 29.5, 20.8, and 14.2; IR (ATR) 3133, 3065, 2972, 2954, 2910, 2877, 2100, 1736, 1708, 1599, 1593, 1569, 1544, 1472, 1455, 1430, 1420, 1382, 1356, 1319, 1275, 1260, 1235, 1201, 1155, 1108, 1070, 1031, 844, 790, and 718 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₄H₂₈N₄O₃H⁺: 421.22; found 421.24.

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**82:** 2-(5-(2-(Tetrahydro-pyan-2-yloxy)-propyl)-tetrahydro-furan-2-yl)-propionic acid methyl ester 82. Methyl (+)-nonactate 46 (567.0 mg, 2.6 mmol) was added to methylene chloride (10 mL) followed by DHP (1.2 mL, 13.1 mmol) and pTsOH-H₂O (50.0 mg, 0.3 mmol). The solution was stirred for 5 hours at room temperature. The solution was evaporated to a residue which was partially purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 82. The semi-pure material was used in the following reaction. **1H NMR (400 MHz, CDCl₃) δ 4.72 (m, 0.5H), 4.61 (m,
0.5H), 3.92 (m, 4H), 3.66 (m, 3H), 3.46 (m, 1H), 2.49 (m, 1H), 1.95 (m, 2H), 1.60 (m, 10H), 1.20 (m, 2H), and 1.08 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 175.39, 175.36, 99.8, 94.5, 80.34, 80.25, 77.3, 77.0, 76.7, 76.6, 76.4, 72.6, 68.0, 62.9, 62.0, 51.52, 51.48, 45.4, 45.3, 44.0, 43.8, 31.5, 31.3, 31.1, 31.0, 28.53, 28.51, 25.5, 25.4, 22.6, 20.1, 19.43, 19.38, 13.33, and 13.28; IR (ATR) 2942, 2873, 1739, 1456, 1437, 1376, 1353, 1261, 1200, 1164, 1121, 1075, 1021, 992, 904, 869, and 812 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{16}$H$_{28}$O$_5$Na+: 323.18; found 323.22.


Semi-pure material 82 was added to THF (20 mL) followed by lithium aluminum hydride (0.3 g, 7.9 mmol) at room temperature and the solution was then stirred for 12 hours. Water (0.3 mL) was added, followed by 3.0 M NaOH/H$_2$O (0.6 mL) and then water (0.3 mL). The solution was filtered and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 83 (0.477 g, 66.9%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.67 (m, 1H), 4.61 (m, 1H), 4.13 (m, 1H), 3.90 (m, 2H), 3.55 (m, 4H), 1.97 (m, 2H), 1.65 (m, 11H), 1.24 (m, 2H), 1.11 (m, 2H), and 0.79 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 99.9, 95.5, 85.9, 85.7, 76.8, 76.52, 72.5, 68.9, 68.6, 68.1, 63.1, 62.7, 44.0, 43.7, 41.1, 40.9, 31.1, 31.1, 30.74, 30.72, 30.6, 30.5, 25.4, 25.4, 22.6, 20.2, 20.0, 19.6, and 13.7; IR (ATR) 3464, 2939, 2874, 1454, 1374, 1352, 1261, 1201, 1116, 1075, 1021, 991, 943, 905, 869, and 811 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{15}$H$_{28}$O$_4$Na+: 295.19; found 295.24.
84: Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propyl ester 84. 83 (0.452 g, 1.7 mmol) was added to pyridine (2 mL) followed by pTsCl (0.633 g, 3.3 mmol). The solution was stirred for 12 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 84 (0.520 g, 73.4%). ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 4.73 (m, 0.5H), 4.60 (m, 0.5H), 4.13 (m, 1H), 3.89 (m, 4H), 3.50 (m, 2H), 2.44 (s, 3H), 1.72 (m, 13H), 1.18 (m, 2H), 1.07 (m, 1H), and 0.90 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 144.6, 129.74, 129.7, 127.9, 99.8, 94.6, 80.0, 79.7, 76.7, 76.3, 76.2, 73.2, 73.1, 72.8, 72.5, 67.9, 65.3, 63.0, 62.0, 44.1, 43.8, 43.2, 38.8, 38.5, 31.4, 31.3, 31.1, 31.0, 30.6, 29.12, 29.08, 29.0, 25.5, 25.4, 23.4, 22.6, 21.6, 20.2, 19.5, 19.4, 13.42, 13.4, and 13.3; IR (ATR) 2941, 1599, 1455, 1359, 1188, 1188, 1097, 1075, 1021, 991, 962, 940, 813, and 667 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₂H₃₄O₆Na⁺: 449.20; found 449.25.

85: 2-{2-{5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl}-1-methyl-ethoxy}-tetrahydro-pyran 85. 84 (494.0 mg, 1.2 mmol) was added to DMF (3.5 mL) followed by sodium azide (376.0 mg, 5.8 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Once cooled to room temperature, water (20 mL) was added and
the solution was washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 85 (0.275 g, 79.9%). $^1$H NMR (400 MHz, CDCl$_3$) δ 4.75 (m, 1H), 4.66 (m, 1H), 3.96 (m, 3H), 3.51 (m, 3H), 3.26 (m, 1H), 1.96 (m, 2H), 1.65 (m 10H), 1.25 (m, 2H), 1.13 (m, 2H), and 0.93 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 99.8, 94.5, 80.72, 80.7, 76.3, 76.1, 72.6, 68.0, 63.0, 62.0, 55.2, 44.2, 43.9, 39.5, 31.5, 31.4, 31.1, 31.0, 29.3, 25.5, 25.46, 22.7, 20.2, 19.5, and 14.4; IR (ATR) 2939, 2875, 2096, 1453, 1380, 1351, 1282, 1201, 1115, 1075, 1021, 992, 941, 905, 870, and 813 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{15}$H$_{27}$N$_3$O$_3$Na$: 320.20; found 320.25.

86: 1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-propan-2-ol 86. 85 (0.249 g, 0.9 mmol) was added to 6 M HCl/H$_2$O/MeOH (10 mL) and the solution was stirred for 12 hours at room temperature. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 86 (0.120 g, 67%). $^1$H NMR (400 MHz, CDCl$_3$) δ 4.07 (m, 2H), 3.61 (m, 1H), 3.43 (m, 1H), 3.27 (m, 1H), 2.73 (s, 1H), 1.96 (m, 2H), 1.66 (m, 5H), 1.21 (d, $J$ = 6.2 Hz, 3H), and 0.94 (d, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 81.3, 76.8, 65.3, 55.1, 43.0, 39.2, 30.7, 29.2, 23.3, and 14.4; IR (ATR) 3411, 2939, 2875, 2096, 1453, 1380, 1351, 1282, 1201, 1115, 1079, 1282, 1067, and 937 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{10}$H$_{19}$N$_3$O$_2$H$: 214.16; found 214.18.
87: Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester 87. 86 (46.0 mg, 0.2 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (50.0 µL, 0.4 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 87 (53.1 mg, 77.5%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (m, 2H), 7.55 (m, 1H), 7.43 (m, 2H), 5.28 (m, 1H), 3.94 (m, 1H), 3.54 (m, 1H), 3.47 (m, 1H), 3.21 (m, 1H), 1.95 (m, 3H), 1.83 (m, 1H), 1.72 (m, 1H), 1.55 (m, 2H), 1.39 (d, J = 6.4 Hz, 3H), and 0.93 (d, J = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.0, 132.7, 130.8, 129.5, 128.3, 80.8, 76.1, 69.8, 55.0, 42.6, 39.4, 31.4, 29.2, 20.8, and 14.4; IR (ATR) 2972, 2877, 2096, 1715, 1602, 1585, 1451, 1381, 1356, 1314, 1274, 1175, 1109, 1069, 1026, 935, and 711 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₇H₂₃N₅O₃⁺: 318.18; found 318.23.

88: Benzoic acid 1-methyl-2-[5-{1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl]-ethyl ester 88. 87 (32.8 mg, 0.1 mmol) was added to 5:1 EtOH/H₂O (2.5 mL) followed by 2-ethynylpyridine (98%, 21.0 µL, 0.2 mmol). Ascorbic acid/H₂O (1.1 M, 207.0 µL, 0.2 mmol) and CuSO₄·5H₂O/H₂O (0.3 M,
172.0 µL, 0.05 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 88 (32.3 mg, 74.4%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.55 (m, 1H), 8.14 (m, 2H), 8.04 (m, 2H), 7.74 (m, 1H), 7.52 (m, 1H), 7.41 (m, 2H), 7.19 (m, 1H), 5.34 (m, 1H), 4.62 (m, 1H), 4.30 (m, 1H), 3.97 (m, 1H), 3.49 (m, 1H), 1.99 (m, 4H), 1.85 (m, 1H), 1.57 (m, 2H), 1.38 (d, $J$ = 6.4 Hz, 3H), and 0.83 (d, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.0, 150.4, 149.3, 148.1, 136.8, 132.7, 130.7, 129.5, 128.3, 122.6, 120.2, 80.7, 76.3, 69.6, 53.5, 42.6, 40.4, 31.3, 29.5, 20.8, and 14.3; IR (ATR) 3133, 3065, 2971, 2954, 2934, 2854, 2099, 1708, 1600, 1593, 1569, 1544, 1472, 1454, 1430, 1420, 1382, 1356, 1319, 1275, 1260, 1235, 1201, 1155, 1141, 1108, 1070, 1069, 1039, 844, 789, and 718 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{24}$H$_{28}$N$_4$O$_3$H$: 421.22$; found 421.28.

\[
\begin{align*}
\text{OH} & \quad \text{methyl 4-chloro-4-oxobutyrate} \\
\text{N}_3 & \quad \text{py, r.t., 3 h} \\
\text{MeO} & \quad \text{methyl 4-chloro-4-oxobutyrate}
\end{align*}
\]

**89:** Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester 89. 86 (46.0 mg, 0.2 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate (53.0 µL, 0.4 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3)
to give 89 (46.7 mg, 66.1%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.02 (m, 1H), 3.83 (m, 1H), 3.68 (s, 3H), 3.54 (m, 1H), 3.46 (m, 1H), 3.23 (m, 1H), 2.60 (m, 4H), 1.94 (m, 2H), 1.72 (m, 3H), 1.50 (m, 2H), 1.24 (d, $J = 6.2$ Hz, 3H), and 0.93 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.8, 171.7, 80.8, 75.9, 69.6, 55.1, 51.8, 42.4, 39.4, 31.3, 29.4, 29.2, 29.0, 20.6, and 14.4; IR (ATR) 2973, 2879, 2097, 1732, 1438, 1363, 1268, 1162, 1065, 997, 883, and 846 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{15}$H$_{25}$N$_3$O$_5$Na$^+$: 350.17; found 350.21.

90: Succinic acid 2-(5-[2-[(4-(3-acetylamino-phenyl)-1,2,3]triazol-1-yl]-1-methyl-ethyl)-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester 90. 89 (26.7 mg, 0.08 mmol) was added to 5:1 EtOH/H$_2$O (2 mL) followed by N-(3-ethynyl-pyridine)-acetamide (26.0 mg, 0.2 mmol). Ascorbic acid/H$_2$O (1.1 M, 163.0 µL, 0.2 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 136.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 90 (32.1 mg, 80.9%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (s, 1H), 7.90 (s, 1H), 7.77 (m, 2H), 7.63 (m, 1H), 7.35 (m, 1H), 5.17 (m, 1H), 4.52 (m, 1H), 4.39 (m, 1H), 3.82 (m, 1H), 3.64 (s, 3H), 3.39 (m, 1H), 2.63 (s, 4H), 2.16 (s, 3H), 1.97 (m, 3H), 1.80 (m, 1H), 1.66 (m,1H), 1.51 (m, 2H), 1.23 (d, $J = 6.4$ Hz, 3H), and 0.88 (d, $J = 7.0$ Hz, 3H); $^{13}$C NMR (100
MHz, CDCl$_3$) δ 172.8, 172.0, 168.7, 146.9, 138.7, 131.3, 129.5, 121.4, 121.2, 119.4, 116.6, 80.6, 75.9, 69.4, 53.3, 51.8, 42.3, 40.3, 31.2, 29.7, 29.5, 29.0, 24.5, 20.7, and 14.6; IR (ATR) 3309, 3130, 2930, 1729, 1676, 1618, 1592, 1568, 1534, 1488, 1438, 1406, 1368, 1316, 1221, 1163, 1120, 1045, 997, 884, 847, 792, 694, and 666 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{25}$H$_{34}$N$_4$O$_6$: 487.26; found 487.32.

91: Benzoic acid 1-methyl-2-[5-{1-methyl-2-(5-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl]-ethyl ester 91. 55 (110.0 mg, 0.5 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (0.6 mL, 5.2 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution washed with saturated copper sulfate solution (2 × 20 mL) and saturated sodium bicarbonate solution (2 × 20 mL), dried (Na$_2$SO$_4$), concentrated to a residue and was partially purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 61. Semi-pure product 61 was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 2-ethynyl-pyridine (98+%, 0.53 mL, 5.2 mmol). Ascorbic acid/H$_2$O (1.1 M, 1.0 mL, 1.1 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3M, 0.9 mL, 0.3 mmol) were added and the solution was heated on a hot plate at 100 °C and stirred for 12 hours. Once the solution was cool, saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by
chromatography on silica gel in EtOAc-hexanes (4:1) to give 91 (0.150 g, 69%). R_f = 0.55 (100% EtOAc); \(^1\)H NMR (400 MHz, CDCl\(_3\) ) δ 8.56 (m, 1H), 8.17 (m, 2H), 8.05 (m, 2H), 7.76 (m, 1H), 7.54 (m, 1H), 7.42 (m, 2H), 7.21 (m, 1H), 5.35 (m, 1H), 4.63 (m, 1H), 4.32 (m, 1H), 3.98 (m, 1H), 3.50 (m, 1H), 2.01 (m, 4H), 1.86 (m, 1H), 1.58 (m, 2H), 1.39 (d, J = 6.4 Hz, 3H), and 0.84 (d, J = 6.8 Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\) ) δ 166.2, 150.6, 149.5, 137.1, 133.0, 131.0, 129.8, 128.5, 123.2, 122.9, 120.5, 81.0, 76.5, 69.8, 53.8, 42.8, 40.7, 31.6, 29.8, 21.1, and 14.5; IR (ATR) 3132, 2955, 2924, 2854, 2099, 1709, 1600, 1461, 1420, 1381, 1357, 1319, 1275, 1201, 1155, 1108, 1070, 1044, 1031, 844, 790, 749, and 718 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{24}\)H\(_{28}\)N\(_4\)O\(_3\)H\(^+\): 421.22; found 421.28.

\[\text{MeO} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N-(3-ethynyl-phenyl)acetamide} \]
\[\xrightarrow{1.1 \text{ M Ascorbic acid} / \text{H}_2\text{O}} \]
\[\text{MeO} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N-(3-ethynyl-phenyl)acetamide} \]

\[\text{\text{AcHN}} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N-(3-ethynyl-phenyl)acetamide} \]

92: Succinic acid 2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester 92. 60 (113.0 mg, 0.4 mmol) was added to 5:1 EtOH/H\(_2\)O (1.5 mL) followed by N-(3-ethynyl-pyridine)-acetamide (550.0 mg, 3.5 mmol). Ascorbic acid/H\(_2\)O (1.1 M, 0.7 mL, 0.8 mmol) and CuSO\(_4\)-5H\(_2\)O/H\(_2\)O (0.3 M, 0.6 mL, 0.2 mmol) were added and the solution was heat on a hot plate at 100 °C and stirred for 12 hours. Once the solution was cool, saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue, which were purified by chromatography on silica gel in EtOAc-hexanes (4:1) to give 92 (0.145 g, 86.3%). R_f = 0.38 (100% EtOAc); \(^1\)H
NMR (400 MHz, CDCl$_3$) $\delta$ 7.92 (s, 1H), 7.79 (m, 2H), 7.73 (s, 1H), 7.67 (d, $J = 7.8$ Hz, 1H), 7.43 (t, $J = 7.9$ Hz, 1H), 5.19 (m, 1H), 4.53 (m, 1H), 4.43 (m, 1H), 3.86 (m, 1H), 3.65 (s, 3H), 3.39 (m, 1H), 2.64 (s, 4H), 2.18 (s, 3H), 1.98 (m, 3H), 1.82 (m, 3H), 1.67 (m, 1H), 1.52 (m, 2H), 1.25 (d, $J = 6.4$ Hz, 3H), and 0.91 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.8, 172.0, 168.6, 146.9, 138.7, 131.4, 129.5, 121.4, 121.2, 119.43, 119.41, 116.6, 80.6, 75.9, 69.4, 53.3, 51.8, 42.4, 40.3, 31.2, 29.7, 29.5, 24.5, 20.7, and 14.6; IR (ATR) 3305, 3130, 2970, 2100, 1728, 1678, 1618, 1592, 1536, 1488, 1439, 1409, 1369, 1317, 1219, 1164, 1046, 997, 885, 847, 792, 754, and 693 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{25}$H$_{34}$N$_{4}$O$_6$H$^+$: 487.26; found 487.31.

![Conversion of HOH to OTHP](image)


Methyl homononactate 93 (0.534 g, 2.3 mmol) was added to methylene chloride (9.3 mL) followed by DHP (1.1 mL, 11.6 mmol) and pTsOH-H$_2$O (4.5 mg, 0.02 mmol). The solution was stirred for 5 hours at room temperature. The solution was evaporated to a residue and partially purified by column chromatography on silica gel in EtOAc-hexanes (1:19) to give the semi-pure intermediate product. The semi-pure material was added to THF (25 mL) followed by lithium aluminum hydride (0.353 g, 9.3 mmol). The reaction was stirred at room temperature overnight. Water (0.4 mL) was added followed by 3.0 M NaOH/H$_2$O (0.8 mL) and then water (0.4 mL). The solution was filtered and evaporated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 94 (0.484 g, 72.9%). $^1$H
NMR (400 MHz, CDCl$_3$) $\delta$ 4.60 (m, 1H), 4.11 (m, 1H), 3.90 (m, 2H), 3.58 (m, 3H), 3.45 (m, 1H), 2.12 (s, 1H), 1.96 (m, 2H), 1.78 (m, 1H), 1.58 (m, 12H), and 0.83 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 99.6, 97.2, 86.0, 85.7, 77.6, 77.2, 76.8, 74.2, 69.0, 68.7, 63.3, 41.3, 41.1, 41.0, 40.6, 31.4, 31.3, 31.1, 31.0, 30.8, 28.9, 26.5, 25.62, 25.59, 20.46, 20.42, 13.85, 13.82, 9.8, and 9.0; IR (ATR) 3461, 2940, 2876, 1463, 1380, 1352, 1201, 1115, 1076, 1022, 999, 902, 868, 810, and 754 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{16}$H$_{30}$O$_4$Na$: 309.20; found 309.11.

95: Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-butyl]-tetrahydro-furan-2-yl}-propyl ester 95. 94 (0.426 g, 1.5 mmol) was added to methylene chloride (3 mL) was added to pyridine (1.2 mL, 14.9 mmol) and pTsCl (0.567 g, 3.0 mmol). The solution was stirred overnight at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 x 20 mL) and then saturated ammonium chloride solution (2 x 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 95 (0.512 g, 78.2%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.76 (d, $J = 8.3$ Hz, 2H), 7.32 (d, $J = 8.1$ Hz, 2H), 4.62 (m, 1H), 4.12 (m, 1H), 3.91 (m, 3H), 3.54 (m, 3H), 2.42 (m, 4H), 1.70 (m, 14H), and 0.85 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 144.6, 144.5, 132.9, 129.7, 129.67, 127.8, 99.3, 96.1, 94.6, 80.0, 79.60, 79.55, 76.9, 76.8, 76.5, 76.1, 73.8, 73.2, 73.1, 72.8, 70.3, 62.9, 62.87, 62.4, 41.0, 40.9, 40.4, 38.8, 31.4, 31.3, 25.9, 25.5, 21.6, 20.1, 19.8, 13.4, 9.97, 9.52, and 8.6; IR (ATR) 2940, 2876, 1738, 1599, 1464, 1359, 1188, 1176, 1118, 1097, 1075,
1022, 1001, 963, 902, 812, 667, and 554 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{23}$H$_{36}$O$_6$SNa$^+$: 463.21; found 463.03.

96: 2-\{1-\{(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethoxy\}-propoxy\}-tetrahydro-pyran 96. 95 (0.450 g, 1.0 mmol) was added to DMF (10 mL) followed by sodium azide (0.332 g, 5.1 mmol). The solution was warmed to 100 °C and stirred for 24 hours. Once cooled to room temperature, water (25 mL) was added and the solution was extracted with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 96 (0.269 g, 84.5%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.67 (m, 1H), 4.02 (m, 1H), 3.89 (m, 2H), 3.79 (m, 1H), 3.70 (m, 1H), 3.50 (m, 3H), 3.25 (m, 1H), 1.95 (m, 2H), 1.80 (m, 1H), 1.68 (m, 2H), 1.53 (m, 8H), and 0.88 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 99.4, 96.1, 80.7, 80.6, 76.9, 76.4, 76.1, 73.8, 62.9, 62.4, 55.14, 55.12, 41.0, 40.6, 39.5, 31.6, 31.5, 31.1, 31.0, 29.3, 28.9, 25.9, 25.5, 20.1, 19.8, 14.4, 9.58, and 8.7; IR (ATR) 2941, 2876, 2096, 1462, 1381, 1351, 1282, 1201, 1115, 1075, 1022, 1002, 903, 869, and 812 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{16}$H$_{29}$N$_3$O$_3$Na$^+$: 334.21; found 334.10.

97: 1-\{5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl\}-butan-2-ol 97. 96 (0.215 g, 0.7 mmol) was added to 6 M HCl/H$_2$O/MeOH (5 mL) and the solution was stirred at room temperature for 3 hours. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The
organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 97 (0.103 g, 70%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.07 (m, 1H), 3.70 (m 1H), 3.57 (m, 1H), 3.40 (m, 1H), 3.23 (m, 1H), 2.81 (s, 1H), 1.93 (m, 2H), 1.58 (m, 7H), and 0.90 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 81.2, 76.9, 70.4, 55.0, 40.9, 39.1, 30.6, 30.1, 29.1, 14.3, and 9.97; IR (ATR) 3412, 2965, 2936, 2876, 2096, 1462, 1381, 1282, 1069, and 883 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{11}$H$_{21}$N$_3$O$_2$H$: 228.17; found 228.09.

98: Acetic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethy]l-propyl ester)

98. 97 (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by acetic anhydride (0.15 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give 98 (28.4 mg, 66.7%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.93 (m, 1H), 3.84 (m, 1H), 3.55 (m, 1H), 3.48 (m, 1H), 3.23 (m, 1H), 2.04 (m, 3H), 1.88 (m, 2H), 1.60 (m, 7H), and 0.92 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.7, 80.8, 76.2, 73.4, 55.1, 40.0, 39.4, 31.3, 29.2, 27.5, 21.3, 14.4, and 9.4
99: 2,2-Dimethyl-propionic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester 99. 97 (42.6 mg, 0.2 mmol) was added to pyridine (5 mL) followed by trimethylacetyl chloride (230.0 µL, 1.9 mmol). The solution was stirred for 24 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na2SO4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 99 (46.8 mg, 80.6%). 1H NMR (400 MHz, CDCl3) δ 4.89 (m, 1H), 3.81 (m, 1H), 3.54 (m, 1H), 3.48 (m, 1H), 3.22 (m, 1H), 1.93 (m, 2H), 1.60 (m, 7H), 1.18 (s, 9H), and 0.89 (m, 6H); 13C NMR (100 MHz, CDCl3) δ 178.0, 80.7, 76.3, 73.0, 55.1, 40.1, 39.4, 38.8, 31.4, 29.2, 27.4, 27.2, 14.4, and 9.2

100: Pent-4-enoic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester 100. 97 (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by 4-pentenoyl chloride (0.18 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na2SO4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 100 (32.4 mg, 66.1%).
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.81 (m, 1H), 5.00 (m, 2H), 3.83 (m, 1H), 3.54 (m, 1H), 3.48 (m, 1H), 3.23 (m, 1H), 2.38 (m, 4H), 1.93 (m, 2H), 1.60 (m, 7H), 1.25 (m, 1H), and 0.90 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.6, 136.8, 115.4, 80.8, 76.1, 73.4, 55.1, 39.4, 33.8, 31.3, 29.2, 28.9, 27.5, 14.4, and 9.4

101: Succinic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester methyl ester 101. 97 (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 101 (35.7 mg, 66.1%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.96 (m, 1H), 3.82 (m, 1H), 3.68 (s, 3H), 3.54 (m, 1H), 3.47 (m, 1H), 3.23 (m 1H), 2.62 (m, 4H), 1.94 (m, 2H), 1.60 (m, 7H), and 0.92 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.8, 171.8, 80.8, 80.5, 76.1, 73.9, 67.2, 61.8, 55.1, 51.8, 40.0, 39.8, 39.4, 38.1, 31.3, 31.1, 29.3, 29.2, 29.1, 28.94, 28.9, 28.6, 27.5, 27.2, 14.4, 13.4, 10.4, and 9.3

102: Benzoic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester 102. 97 (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by benzoyl chloride (0.18 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl
acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 102 (21.5 mg, 41.0%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (m, 2H), 7.55 (m, 1H), 7.44 (m, 2H), 5.21 (m, 1H), 3.92 (m, 1H), 3.53 (m, 1H), 3.47 (m, 1H), 3.19 (m, 1H), 1.92 (m, 4H), 1.74 (m, 3H), 1.57 (m, 2H), and 0.94 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 166.1, 132.7, 130.7, 129.5, 128.3, 80.8, 76.3, 74.0, 55.1, 40.1, 31.4, 29.2, 27.7, 14.4, and 9.4

113: 2-[5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-propan-1-ol 113. Methyl nonactate 45 (1.072 g, 5.0 mmol) was added to THF (50 mL) followed by lithium aluminum hydride (0.376 g, 9.9 mmol). The solution was refluxed for 3 hours. Once cooled to room temperature, water (0.4 mL) was added followed by 3.0 M NaOH/H₂O (0.8 mL) and then water (0.4 mL). The solution was filtered and evaporated to a residue to give 113 (0.933 g, 99%). ¹H NMR (400 MHz, CDCl₃) δ 4.08 (m, 1H), 3.97 (m, 1H), 3.60 (m, 3H), 3.03 (s, 1H), 1.96 (m, 2H), 1.62 (m, 6H), 1.18 (d, J = 6.2 Hz, 3H), and 0.82 (d, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 84.8, 76.9, 67.7, 65.1, 44.1, 40.9, 30.4, 30.1, 23.8, and 13.6; IR (ATR) 3367, 2964, 2932, 2877, 1461, 1414, 1374, 1234, 1036, 938, 825, and 753 cm⁻¹

114: Bis-tosylated nonactate diol 114. 113 (0.207 g, 1.1 mmol) was added to pyridine (10 mL) followed by DMAP (0.134 g, 1.1 mmol) and pTsCl (1.05 g, 5.5 mmol).
solution was stirred at room temperature overnight. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then saturated ammonium chloride solution (2 × 50 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give **114** (0.213 g, 39%).

![Chemical structure](image)

**Reagents and conditions**

1. DMF, 80 °C, 12 h

**115**: 2-(2-Azido-1-methyl-ethyl)-5-(2-azido-propyl)-tetrahydro-furan **115**. **114** (0.213 g, 0.4 mmol) was added to DMF (2 mL) followed by sodium azide (0.279 g, 4.3 mmol). The solution was warmed to 80 °C and stirred for 12 hours. Once the solution was cooled to room temperature, water (20 mL) was added and the solution was washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **115** (82.3 mg, 80.5%). R_f = 0.63 (EtOAc-hexanes (1:9)); ^1H NMR (400 MHz, CDCl₃) δ 3.90 (m, 1H), 3.58 (m, 2H), 3.45 (m, 1H), 3.28 (m, 1H), 1.98 (m, 2H), 1.78 (m, 2H), 1.53 (m, 3H), 1.29 (d, J = 6.5 Hz, 3H), and 0.94 (d, J = 6.7 Hz, 3H); ^13C NMR (100 MHz, CDCl₃) δ 81.0, 76.0, 55.4, 55.0, 42.0, 39.4, 31.2, 29.1, 19.2, and 14.4; IR (ATR) 2970, 2937, 2877, 2093, 1461, 1380, 1253, 1091, 1041, 885, and 649 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₀H₁₈N₆O₃Na⁺: 261.14; found 261.19.
117: 4-tert-butyl-1-(1-15-(1-(4-tert-butyl-1H-1,2,3-triazol-1-yl)propane-2-yl)tetrahydrofuran-2-yl)propan-2-yl)-IH-1,2,3-triazole 117. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 52.0 µL, 0.4 mmol). Ascorbic acid/H$_2$O (1.1 M, 168.0 µL, 0.2 mmol) and CuSO$_4$·5H$_2$O/H$_2$O (0.3 M, 140.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give 117 (4.2 mg, 24.9%).

118: 4-(cyclopentylmethyl)-1-(1-15-(1-(1-(4-(cyclopentylmethyl)-1H-1,2,3-triazol-1-yl)propane-2-yl)tetrahydrofuran-2-yl)propan-2-yl)-IH-1,2,3-triazole 118. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 55.0 µL, 0.4 mmol). Ascorbic acid/H$_2$O (1.1 M, 168.0 µL, 0.2 mmol) and CuSO$_4$·5H$_2$O/H$_2$O (0.3 M, 140.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic
layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 118 (10.7 mg, 56.0%).

119: 4-(4-fluorophenyl)-1-(1-(5-(1-(4-(fluorophenyl)-1H-1,2,3-triazol-1-yl)propane-2-yl)tetrahydrofuran-2-yl) propane-2-yl)-1H-1,2,3-triazole 119. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) followed by 1-ethynyl-4-fluorobenzene (48.0 µL, 0.4 mmol). Ascorbic acid/H$_2$O (1.1 M, 168.0 µL, 0.2 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 140.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 119 (12.3 mg, 61.2%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.79 (m, 5H), 7.70 (m, 1H), 7.08 (m, 4H), 4.87 (m, 1H), 4.43 (m, 1H), 4.14 (m, 1H), 3.91 (m,1H), 3.45 (m, 1H), 2.18 (m, 1H), 2.07 (m 2H), 1.97 (m, 2H), 1.65 (d, $J = 6.8$ Hz, 3H), 1.55 (m, 2H), and 0.83 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 163.8, 161.3, 127.4, 127.3, 127.2, 127.1, 115.91, 115.87, 115.69, 115.65, 81.5, 76.6, 55.7, 53.4, 43.4, 40.5, 31.3, 29.4, 21.4, and 14.5
120: 2-(1-(1-(5-(1-(4-(pyridine-2-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)tetrahydrofuran-2-yl)propan-2-yl)-1H-1,2,3-triazol-4-yl)pyridine 120. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H₂O (1.5 mL) followed by 2-ethynylpyridine (98%, 42.0 µL, 0.4 mmol). Ascorbic acid/H₂O (1.1 M, 168.0 µL, 0.2 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 140.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 120 (11.8 mg, 63.4%). ¹H NMR (400 MHz, CDCl₃) δ 8.58 (m, 1H), 8.52 (m, 1H), 8.28 (s, 1H), 8.15 (m, 2H), 7.78 (m, 1H), 7.72 (m, 1H), 7.23 (m, 1H), 7.16 (m, 1H), 4.92 (m, 1H), 4.47 (m, 1H), 4.22 (m, 1H), 3.88 (m, 1H), 3.48 (m, 1H), 2.30 (m, 2H), 2.00 (m, 4H), 1.66 (d, J = 6.9 Hz, 3H), 1.53 (m, 2H), and 0.81 (d, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 149.0, 137.2, 123.0, 122.8, 122.7, 120.4, 120.3, 120.2, 81.2, 76.3, 55.6, 53.4, 43.2, 40.2, 31.2, 29.3, 21.3, and 14.3
121: \( N-(3-\{1-\{2-\{5-\{4-\{3\text{-Acetylamino-phenyl}\}\text{-}\text{triazol-1-yl}\}\text{-}\text{1-methyl-ethyl}\}\text{-}\text{tetrahydro-furan-2-yl}\}\text{-}\text{1-methyl-ethyl}\}\text{-}1H\text{-}\text{triazol-4-yl}\}\text{-}\text{phenyl}\)\)-acetamide

121. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H\(_2\)O (1.5 mL) followed by \( N\)-(3-ethynyl-phenyl)-acetamide (67.0 mg, 0.4 mmol). Ascorbic acid/H\(_2\)O (1.1 M, 168.0 µL, 0.2 mmol) and CuSO\(_4\)-5H\(_2\)O/H\(_2\)O (0.3 M, 140.0 µL, 0.04 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and the solution was washed with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 121 (23.1 mg, 99.1%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.65 (s, 1H), 8.43 (s, 1H), 7.94 (s, 1H), 7.85 (s, 1H), 7.77 (s, 1H), 7.61 (m, 3H), 7.47 (m, 2H), 7.31 (m, 1H), 7.19 (m, 1H), 4.74 (m, 1H), 4.24 (m, 1H), 4.00 (m, 1H), 3.88 (m, 1H), 3.36 (m, 1H), 2.56 (m, 1H), 2.12 (m, 9H), 1.90 (m, 1H), 1.60 (d, \( J = 6.8 \text{ Hz} \), 3H), 1.49 (m, 2H), and 0.75 (d, \( J = 6.8 \text{ Hz} \), 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 169.3, 138.8, 130.8, 129.5, 129.47, 121.4, 121.1, 120.0, 119.6, 117.5, 116.8, 82.0, 56.4, 53.7, 43.1, 40.4, 31.4, 29.5, 24.5, 24.4, 21.6, and 14.6

123: 4-Hydroxy-cyclohexanecarboxylic acid methyl ester 123. Cis-4-hydroxycyclohexane-1-carboxylic acid 122 (5.0 g, 34.7 mmol) was added to 5% H\(_2\)SO\(_4\)/MeOH (100 mL)
and the solution was stirred at room temperature then refluxed for 5 hours. Saturated sodium bicarbonate solution was added until no more gas evolved and the solution was then washed with ethyl acetate (3 × 100 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 123 (4.611 g, 84%). Rₓ = 0.19 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 3.80 (m, 1H), 3.60 (s, 3H), 2.32 (m, 2H), 1.89 (m, 2H), and 1.58 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.7, 66.5, 51.4, 41.0, 31.8, and 23.5; IR (ATR) 3407, 2937, 2867, 1729, 1435, 1237, 1198, 1169, 1137, 1067, 1033, 964, 911, 893, 839, 821, and 755 cm⁻¹; ESI-TOF-MS m/z calcd for C₈H₁₄O₃H⁺: 159.10; found 159.06.

124: 4-(Tetrahydro-pyran-2-yloxy)-cyclohexanecarboxylic acid methyl ester 124. 123 (1.0 g, 6.3 mmol) was added to methylene chloride (20 mL) followed by DHP (2.9 mL, 63.2 mmol) and pTsOH-H₂O (12.0 mg, 0.1 mmol). The solution was stirred at room temperature for 12 hours then evaporated to give a residue which was partially purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give semi-pure 124. The semi-pure product was used in the following reduction reaction. ¹H NMR (400 MHz, CDCl₃) δ 4.61 (m, 1H), 3.82 (m, 1H), 3.75 (m, 1H), 3.41 (m, 1H), 2.31 (septet, J = 5.6, 3.906 Hz, 1H), and 1.66 (m, 15H); ¹³C NMR (100 MHz, CDCl₃) δ 175.7, 96.3, 70.2, 62.4, 51.3, 41.4, 31.1, 30.7, 28.2, 25.4, 24.0, 23.6, and 19.6; IR (ATR) 2941, 2868, 1733, 1441, 1341, 1234, 1200, 1160, 1134, 1118, 1076, 1030, 1020, and 996 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₃H₂₂O₄Na⁺: 265.14; found 265.01.
125: [4-(Tetrahydro-pyran-2-yloxy)-cyclohexyl]-methanol 125. 124 was added to THF (30 mL) followed by lithium aluminum hydride (0.720 g, 19.0 mmol) at room temperature and allowed to stir overnight. Water (0.8 mL) was added, followed by 3.0 M NaOH/H₂O (1.6 mL), followed by water (0.8 mL) and sodium sulfate. The solution was filtered and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 125 (1.219 g, 90% over 2 steps). R_f = 0.23 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 4.63 (m, 1H), 3.87 (m, 2H), 3.45 (d, J = 6.0 Hz, 3H), 1.81 (m, 4H), 1.66 (m, 1H), 1.50 (m, 9H), and 1.35 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 96.6, 70.8, 67.6, 62.7, 39.1, 31.3, 30.7, 28.2, 25.5, 24.0, 23.7, and 19.9; IR (ATR) 3360, 3103, 2925, 2858, 1442, 1350, 1200, 1120, 1076, 1019, and 997 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₂H₂₂O₃Na⁺: 237.15; found 237.09.

126: Toluene-4-sulfonic acid 4-(tetrahydro-pyran-2-yloxy)-cyclohexylmethyl ester 126. 125 (1.108, 5.2 mmol) was added to pyridine (20 mL), followed by pTsCl (1.971 g, 10.4 mmol) and the solution was stirred for 12 hours at room temperature. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 100 mL) and then washed with saturated ammonium chloride solution (2 × 100 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 126 (1.32 g, 69.1%). ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 4.59
(m, 1H), 3.85 (m, 2H), 3.84 (d, J = 7.0 Hz, 2H), 3.45 (m, 1H), 2.44 (s, 3H), 1.74 (m, 5H), and 1.39 (m, 10H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 144.6, 133.1, 129.8, 127.8, 96.8, 74.6, 70.2, 62.8, 36.1, 31.5, 31.3, 30.4, 28.0, 25.5, 23.6, 23.3, 22.9, 21.6, and 20.0; IR (ATR) 2936, 2861, 1599, 1443, 1359, 1175, 1126, 1098, 1076, 1021, 997, and 937 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{19}$H$_{28}$O$_5$SNa$^+$: 391.16; found 391.02.

![Chemical structure](image)

**127:** 2-(4-Azidomethyl-cyclohexyloxy)-tetrahydro-pyran 127. 126 (1.211 g, 3.3 mmol) was added to DMF (15 mL) followed by sodium azide (1.07 g, 16.4 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Once the solution had cooled to room temperature, water (50 mL) was added and the solution washed with diethyl ether (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 127 (0.646 g, 82.2%). $^1$H NMR (400 MHz, CDCl$_3$) δ 4.629 (m, 1H), 3.875 (m, 2H), 3.46 (m, 1H), 3.14 (d, J = 6.8 Hz, 2H), 1.82 (m, 3H), and 1.50 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 96.7, 70.2, 62.7, 57.1, 36.9, 31.3, 30.7, 28.2, 25.5, 25.0, 24.7, and 19.9; IR (ATR) 2929, 2860, 2094, 1443, 1348, 1275, 1200, 1121, 1076, 1092, 1021, and 997 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{12}$H$_{21}$N$_3$O$_2$Na$^+$: 262.15; found 262.09.

![Chemical structure](image)

**128:** 4-Azidomethyl-cyclohexanol 128. 127 (0.543 g, 2.3 mmol) was added to 6 M HCl/H$_2$O/MeOH (10 mL) and the solution was stirred at room temperature for 3 hours. Saturated sodium bicarbonate solution was added to the solution until no gas
evolved. The solution was washed with ethyl acetate (3 × 50 mL) and the organic phases collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 128 (0.318 g, 90.3%).

$^1$H NMR (400 MHz, CDCl$_3$) δ 3.98 (m, 1H), 3.15 (d, $J = 6.6$ Hz, 2H), 1.71 (m, 3H), and 1.51 (m, 7H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 66.1, 57.0, 36.7, 31.7, and 24.2; IR (ATR) 3332, 2928, 2860, 2092, 1446, 1359, 1260, 1126, 1068, 1034, and 975 cm$^{-1}$.

129: Succinic acid 4-azidomethyl-cyclohexyl ester methyl ester 129. 128 (0.208 g, 1.4 mmol) was added to pyridine (3 mL) followed by methyl 4-chloro-4-oxobutyrate (1.65 mL, 13.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) then with saturated ammonium chloride solution (2 × 20 mL). The solution was dried (Na$_2$SO$_4$) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 129 (0.199 g, 55%). $^1$H NMR (400 MHz, CDCl$_3$) δ 5.03 (m, 1H), 3.67 (s, 3H), 3.16 (d, $J = 6.4$ Hz, 2H), 2.61 (s, 4H), 1.87 (m, 2H), 1.55 (m, 5H), and 1.28 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.7, 171.5, 69.6, 57.2, 51.8, 36.8, 29.5, 29.0, 28.9, and 24.7; IR (ATR) 2940, 2860, 2096, 1728, 1438, 1363, 1264, 1159, 1118, 1036, and 983 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{12}$H$_{19}$N$_3$O$_4$H$^+$: 270.15; found 270.06.
130: Benzoic acid 4-azidomethyl-cyclohexyl ester 130. 128 (0.208 g, 1.4 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (1.55 mL, 13.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL). The solution was dried (Na₂SO₄) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 130 (0.283 g, 81%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (m, 1H), 7.56 (m, 1H), 7.45 (m, 2H), 5.29 (m, 1H), 3.23 (d, J = 6.2 Hz, 2H), 2.05 (m, 2H), 1.67 (m, 5H), and 1.47 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 165.7, 132.8, 130.8, 129.4, 128.3, 69.7, 57.3, 37.0, 29.2, and 25.0; IR (ATR) 2938, 2860, 2095, 1712, 1602, 1584, 1450, 1274, 1108, 1069, 1026, and 710 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₄H₁₇N₃O₂H⁺: 260.14; found 260.08.

131: Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-ylmethyl]-cyclohexyl ester methyl ester 131. 129 (0.199 g, 0.7 mmol) was added to 5:1 EtOH/H₂O (10 mL) followed by N-(3-ethynyl-phenyl)-acetamide (0.235 g, 1.5 mmol). Ascorbic acid/H₂O (1.1 M, 1.5 mL, 1.6 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 1.2 mL, 0.4 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate (50 mL) was added and the solution extracted with ethyl acetate.
(3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 131 (0.217 g, 69%). Rₛ = 0.37 (100% EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.00 (s, 2H), 7.71 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.30 (t, J = 7.9 Hz, 1H), 5.02 (s, 1H), 4.22 (d, J = 7.3 Hz, 2H), 3.68 (s, 3H), 2.63 (s, 4H), 2.16 (s, 3H), 1.98 (m, 1H), 1.87 (m, 2H), 1.48 (m, 4H), and 1.34 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 172.8, 171.5, 168.8, 147.2, 138.7, 131.2, 129.5, 121.3, 120.3, 119.4, 116.9, 69.3, 55.8, 51.8, 37.5, 29.4, 28.9, 28.8, 24.6, and 24.5; IR (ATR) 3351, 2949, 2865, 2633, 2539, 2431, 2362, 2265, 2098, 1736, 1713, 1687, 1641, 1615, 1566, 1532, 1480, 1450, 1374, 1351, 1304, 1282, 1247, 1220, 1172, 1049, 1007, and 991 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₂H₂₈N₄O₅H+: 429.21; found 429.04.

132: **Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-ylmethyl)-cyclohexyl ester** 132. 130 (0.283 g, 1.1 mmol) was added to 5:1 EtOH/H₂O (10 mL) followed by 2-ethylpyridine (0.225 µL, 2.2 mmol). Ascorbic acid/H₂O (1.1 M, 2.2 mL, 2.4 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 1.8 mL, 0.6 mmol) were added. The solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate (50 mL) was added and the solution extracted with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 132 (0.244 g, 62%). Rₛ = 0.51 (100% EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.55 (m, 1H), 8.17 (d, J = 7.9 Hz, 2H), 8.14 (s, 1H),
8.00 (d, J = 8.2 Hz, 2H), 7.75 (t, J = 7.8 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.40 (t, J = 7.3, 2H), 7.20 (t, J = 6.2 Hz, 1H), 5.26 (s, 1H), 7.20 (d, 2H), 2.05 (m, 3H), and 1.55 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$; IR (ATR) 2929, 2860, 2633, 2538, 2444, 2266, 2098, 1721, 1607, 1599, 1471, 1449, 1427, 1368, 1278, 1266, 1119, 1046, 793, and 710 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{21}$H$_{22}$N$_4$O$_2$H$: 363.18$; found 363.04.

134: 4-Hydroxy-cyclohexanecarboxylic acid methyl ester 134. Trans-4-hydroxy-cyclohexane-1-carboxylic acid 133 (5.0 g, 34.7 mmol) was added to 5% H$_2$SO$_4$/MeOH (100 mL) and the solution was stirred and refluxed for 5 hours. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was extracted with ethyl acetate (3 × 100 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 134 (4.94 g, 90%). $R_f$ = 0.14 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.61 (s, 3H), 3.53 (pentet, J = 6.6, 4.028 Hz, 1H), 2.335 (s, 1H), 2.20 (m, 1H), 1.95 (m, 4H), 1.43 (m, 2H), and 1.23 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 176.0, 69.5, 51.5, 42.0, 34.3, and 27.0; IR (ATR) 3374, 2938, 2861, 1731, 1453, 1405, 1367, 1280, 1238, 1193, 1171, 1064, 959, 898, and 754 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_8$H$_{14}$O$_3$H$: 159.10$; found 159.03.
135: 4-(Tetrahydro-pyran-2-yloxy)-cyclohexanecarboxylic acid methyl ester 135. 134 (1.0 g, 6.3 mmol) was added to methylene chloride (20 mL) followed by DHP (2.9 mL, 31.6 mmol) and pTsOH-H₂O (12.0 mg, 0.06 mmol). The solution was stirred at room temperature for 5 hours. The solution was evaporated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 135 (1.11 g, 73%).

\[ R_f = 0.57 \ (\text{EtOAc-hexanes (1:3)}); \]
\[ ^1H \text{ NMR (400 MHz, CDCl}_3\delta\ 4.66 \ (m, \ 1H), \ 3.85 \ (m, \ 1H), \ 3.61 \ (s, \ 3H), \ 3.54 \ (m, \ 1H), \ 3.44 \ (m, \ 1H), \ 2.21 \ (m, \ 1H), \ 1.99 \ (m, \ 4H), \ 1.78 \ (m, \ 1H), \ 1.65 \ (m, \ 1H), \ 1.40 \ (m, \ 7H), \text{ and } 1.18 \ (m, \ 1H); \]
\[ ^1^3C \text{ NMR (100 MHz, CDCl}_3\delta\ 175.9, \ 96.7, \ 73.8, \ 62.6, \ 51.4, \ 42.2, \ 32.5, \ 31.1, \ 30.7, \ 27.3, \ 27.0, \ 25.3, \text{ and } 19.8; \]
\[ \text{IR (ATR) 2939, 2864, 1734, 1453, 1354, 1309, 1248, 1172, 1134, 1118, 1063, 1022, \text{ and } 987 \ \text{cm}^{-1}; \text{ ESI-TOF-MS m/z calcd for C}_{13}H_{22}O_4Na^+: 265.14; \text{ found 265.01}. \]

136: [4-(Tetrahydro-pyran-2-yloxy)-cyclohexyl]-methanol 136. 135 (1.0 g, 4.1 mmol) was added to THF (30 mL) followed by lithium aluminum hydride (0.471 g, 12.4 mmol). The solution was stirred at room temperature for 5 hours. Water (0.5 mL) was added, followed by 3.0 M NaOH/H₂O (1.0 mL), and then water (0.5 mL) again. The mixture was filtered and evaporated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 136 (0.769 g, 86.8%).

\[ R_f = 0.14 \ (\text{EtOAc-hexanes (1:3)}); \]
\[ ^1H \text{ NMR (400 MHz, CDCl}_3\delta\ 4.68 \ (m, \ 1H), \ 3.87 \ (m, \ 1H), \ 3.48 \ (m, \ 2H), \ 3.37 \ (d, \ J = 6.4 \ Hz, \ 2H), \ 2.10 \ (s, \ 1H), \ 2.01 \ (m, \ 2H), \ 1.79 \ (m, \ 3H), \ 1.65 \ (m, \ 1H), \]
1.42 (m, 6H), 1.18 (m, 1H), and 0.94 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 96.6, 75.0, 67.7, 62.6, 39.6, 32.9, 31.1, 27.8, 27.5, 25.4, and 19.8; IR (ATR) 3386, 2930, 2859, 1737, 1452, 1353, 1261, 1200, 1116, 1076, 1056, 1023, 970, 902, 867, 809, and 753 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{12}$H$_{22}$O$_3$Na$^+$: 237.15; found 237.10.

**137:** *Toluene-4-sulfonyl acid 4-(tetrahydro-pyran-2-yloxy)-cyclohexylmethyl ester 137.*

136 (0.641 g, 3.0 mmol) was added to pyridine (10 mL) followed by p-toluenesulfonyl chloride (0.855 g, 4.5 mmol). The solution was stirred at room temperature for five hours. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 137 (0.764 g, 69.3 %). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.75 (d, $J$ = 8.4 Hz, 2H), 7.32 (d, $J$ = 7.9 Hz, 2H), 4.66 (m, 1H), 3.86 (m, 1H), 3.79 (d, $J$ = 6.5 Hz, 2H), 3.47 (m, 2H), 2.42 (s, 3H), 1.99 (m, 2H), 1.75 (m, 3H), 1.61 (m, 1H), 1.49 (m, 4H), 1.28 (m, 1H), 1.14 (m, 1H), and 0.94 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 144.6, 132.9, 129.7, 127.7, 96.7, 74.5, 74.3, 62.7, 36.4, 32.5, 31.1, 30.7, 27.3, 27.1, 25.3, 21.5, and 19.8; IR (ATR) 2938, 2866, 1597, 1452, 1351, 1297, 1189, 1178, 1023, 950, and 810 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{19}$H$_{26}$O$_5$SNa$^+$: 391.16; found 391.04.

138: *2-(4-Azidomethyl-cyclohexyloxy)-tetrahydro-pyran 138.* 137 (0.406 g, 1.1 mmol) was added to DMF (5 mL) followed by sodium azide (0.292 g, 4.5 mmol). The solution
was warmed to 80 °C and stirred for 5 hours. After cooling to room temperature, water (25 mL) was added and the solution washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 138 (0.221 g, 83.7%). ¹H NMR (400 MHz, CDCl₃) δ 4.69 (m, 1H), 3.88 (m, 1H), 3.50 (m, 2H), 3.11 (d, J = 6.9 Hz, 2H), 2.03 (m, 2H), 1.80 (m, 3H), 1.68 (m, 1H), 1.51 (m, 5H), 1.36 (m, 1H), 1.20 (m, 1H), and 1.02 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 96.7, 74.6, 62.7, 57.3, 37.3, 32.8, 31.1, 31.0, 28.8, 28.5, 25.4, and 19.9; IR (ATR) 2935, 2860, 2095, 1738, 1453, 1354, 1275, 1200, 1076, 1056, 1030, and 978 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₂H₂₁N₃O₂Na⁺: 262.15; found 262.09.

139: 4-Azidomethyl-cyclohexanol 139. 138 (0.162 g, 0.7 mmol) was added to 6 M HCl/H₂O/MeOH (5 mL) and the solution was stirred for 5 hours at room temperature. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried, and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 139 (85.8 mg, 81.7%). ¹H NMR (400 MHz, CDCl₃) δ 3.54 (m, 1H), 3.13 (d, J = 6.6 Hz, 2H), 1.99 (m, 2H), 1.86 (s, 1H), 1.80 (m, 2H), 1.51 (m, 1H), 1.25 (m, 2H), and 1.03 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 70.5, 57.2, 37.0, 34.7, and 28.5; IR (ATR) 3332, 2929, 2857, 2360, 2094, 1454, 1360, 1273, 1200, 1046, and 1014 cm⁻¹.
**140:** *Succinic acid 4-azidomethyl-cyclohexyl ester methyl ester 140.* 139 (83.5 mg, 0.5 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxobutyrate (0.65 mL, 5.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 140 (76.4 mg, 53%).

**1H NMR** (400 MHz, CDCl₃) δ 4.66 (septet, J = 6.7, 4.4 Hz, 1H), 3.67 (s, 3H), 3.13 (d, J = 6.7 Hz, 2H), 2.58 (m, 4H), 2.00 (m, 2H), 1.83 (m, 2H), 1.53 (m, 1H), 1.34 (m, 2H), and 1.08 (m, 2H); **13C NMR** (100 MHz, CDCl₃) δ 172.7, 171.6, 73.1, 57.0, 51.7, 36.9, 30.8, 29.4, 28.9, and 28.2; IR (ATR) 2944, 2864, 2097, 1730, 1438, 1355, 1269, 1159, 1023, and 1001 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₂H₁₉N₃O₄H⁺: 270.15; found 270.06.

**141:** *Benzoic acid 4-azidomethyl-cyclohexyl ester 141.* 139 (83.5 mg, 0.5 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.62 mL, 5.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in
EtOAc-hexanes (1:3) to give 141 (100.1 mg, 71%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.04 (m, 2H), 7.55 (m, 1H), 7.43 (m, 2H), 4.92 (m, 1H), 3.18 (d, $J = 6.6$ Hz, 2H), 2.16 (m, 2H), 1.90 (m, 2H), 1.56 (m, 3H), and 1.19 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.9, 132.7, 130.6, 129.5, 128.2, 73.3, 57.0, 37.0, 30.9, and 28.3; IR (ATR) 2939, 2863, 2095, 1712, 1601, 1584, 1451, 1315, 1271, 1176, 1112, 1070, 1025, and 991 cm$^{-1}$

142: Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-ylmethyl]-cyclohexyl ester methyl ester 142. 140 (76.4 mg, 0.3 mmol) was added to 5:1 EtOH/H$_2$O (10 mL) followed by N-(3-ethynyl-phenyl)-acetamide (90.0 mg, 0.6 mmol). Ascorbic acid/H$_2$O (1.1 M, 0.57 mL, 0.6 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 0.47 mL, 0.2 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (25 mL) was added and the solution extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to form a residue which was purified by chromatography on silica gel in 100% EtOAc to give 142 (89.0 mg, 73%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.33 (s, 1H), 7.99 (s, 1H), 7.68 (s, 1H), 7.53 (m, 2H), 7.30 (m, 1H), 4.63 (m, 1H), 4.15 (d, $J = 7.2$, 2H), 3.65 (s, 2H), 2.58 (m, 4H), 2.14 (s, 3H), 1.95 (m, 2H), 1.87 (m, 1H), 1.66 (m, 2H), 1.26 (m, 3H), and 1.08 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.7, 171.7, 168.9, 147.2, 138.8, 131.0, 129.4, 121.2, 120.4, 119.5, 116.9, 77.2, 72.8, 55.5, 51.7, 37.5, 30.5, 29.3, 28.8, 28.1, and 24.4; IR (ATR) 3527, 3352, 2939, 1728, 1678,
1618, 1592, 1568, 1532, 1486, 1439, 1367, 1308, 1274, 1217, 1163, 1023, and 999 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{22}$H$_{28}$N$_4$O$_5$H$: 429.21; found 429.05.

143: Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-ylmethyl)-cyclohexyl ester 143. 141 (100.1 mg, 0.4 mmol) was added to 5:1 EtOH/H$_2$O (10 mL) followed by 2-ethylpyridine (98+%, 78.0 µL, 0.8 mmol). Ascorbic acid/H$_2$O (1.1 M, 0.77 mL, 0.9 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 0.64 mL, 0.2 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (25 mL) was added and the solution extracted with ethyl acetate (3 × 50 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 143 (111.0 mg, 79%). R$_f$ = 0.45 (100% EtOAc); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.57 (d, $J$ = 4.8 Hz, 1H), 8.18 (d, $J$ = 7.9 Hz, 1H), 8.12 (s, 1H), 8.00 (d, $J$ = 7.2 Hz, 2H), 7.77 (m, 1H), 7.52 (m, 1H), 7.40 (m, 2H), 7.22 (m, 1H), 4.92 (m, 1H), 4.29 (d, $J$ = 7.2 Hz, 2H), 2.14 (m, 2H), 2.02 (m, 1H), 1.80 (m, 2H), 1.48 (m, 2H), and 1.25 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.9, 150.2, 149.3, 148.2, 136.9, 132.8, 130.5, 129.4, 128.2, 122.8, 122.3, 120.2, 72.9, 55.7, 37.7, 30.7, and 28.2; IR (ATR) 2946, 2866, 2099, 1705, 1604, 1452, 1284, and 1121 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{21}$H$_{22}$N$_4$O$_2$H$: 363.18; found 363.04.
144: **4-Hydroxymethyl-cyclohexanol 144.** *Trans*-4-hydroxy-cyclohexane-1-carboxylic acid 133 (4.01 g, 27.8 mmol) was added to THF (140 mL) followed by lithium aluminum hydride (3.163 g, 83.4 mmol). The solution was stirred for 12 hours at room temperature. Water (3.2 mL) was added, followed by 3.0 M NaOH/H₂O (6.4 mL), and then water (3.2 mL). The mixture was filtered and evaporated to a residue which was purified by chromatography on silica gel in MeOH-chloroform (1:19) to give 144 (0.745 g, 21%). R_f = 0.30 (100% EtOAc); ^1H NMR (400 MHz, CDCl₃) δ 3.57 (m, 1H), 3.46 (d, J = 6.2 Hz, 2H), 2.02 (m, 2H), 1.83 (m, 2H), 1.62 (s, 1H), 1.45 (m, 2H), 1.27 (m, 2H), and 1.02 (m, 2H); ^13C NMR (100 MHz, CDCl₃) δ 71.0, 68.0, 39.5, 35.0, and 27.5; IR (ATR) 3271, 2940, 2922, 2862, 1460, 1363, 1297, 1258, 1048, 1055, and 976 cm⁻¹

145: **4-(tert-Butyl-dimethyl-silanyloxy)methyl-cyclohexanol 145.** 144 (0.50 g, 3.9 mmol) was added to DMF (7 mL) at 0 °C followed by imidazole (0.288 g, 4.2 mmol). TBSCI (0.579 g, 3.9 mmol) was added and the solution was stirred and slowly allowed to return to room temperature for 12 hours. Water (25 mL) was added and the solution extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 145 (0.534 g, 57%). IR (ATR) 3346, 2928, 2898, 2856, 1471, 1453, 1388, 1360, 1255, 1111, 1077, 833, and 773 cm⁻¹
146: **Toluene-4-sulfonic acid 4-(tert-butyl-dimethyl-silanyloxymethyl)-cyclohexyl ester**

146. 145 (1.05 g, 4.3 mmol) was added to pyridine (4 mL) followed by pTsCl (1.64 g, 8.6 mmol) and the solution was stirred for 12 hours at room temperature. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na₂SO₄), and concentrated to a residue which was partially purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 146. The semi-pure product was used directly in the following reaction. ESI-TOF-MS m/z calcd for C₂₀H₃₄O₄SSiNa⁺: 421.18; found 420.99.

147: **(4-Azido-cyclohexymethoxy)-tert-butyl-dimethyl-silane**

147. 146 (1.72 g, 4.3 mmol) was added to DMF (13 mL) followed by (1.4 g, 21.5 mmol) sodium azide. The solution was stirred at 80 °C for 12 hours. Water (50 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 147 (0.631 g, 54.4% over 2 steps). Rₛ = 0.57 (EtOAc-hexanes (1:19)); IR (ATR) 2928, 2891, 2856, 2099, 1471, 1447, 1253, 1107, 1090, 1068, 835, and 774 cm⁻¹.
148: (4-Azido-cyclohexyl)-methanol 148. 147 (0.586 g, 2.2 mmol) was added to water (1 mL) followed by acetic acid (9.8 mL) and pTsOH-H$_2$O (62.0 mg, 0.3 mmol). The solution was stirred at room temperature for 24 hours. Saturated sodium bicarbonate solution was added until no gas evolved. The solution was extracted with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 148 (145 mg, 42.9%). IR (ATR) 2932, 2860, 2097, 1738, 1448, 1365, 1233, and 1029 cm$^{-1}$.

149: Succinic acid 4-azido-cyclohexylmethyl ester methyl ester 149. 148 (57.0 mg, 0.4 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxobutyrate (0.5 mL, 3.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL). The solution was dried (Na$_2$SO$_4$) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 149 (77.5 mg, 78.4%).

150: Benzoic acid 4-azido-cyclohexylmethyl ester 150. 148 (57.0 mg, 0.4 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.4 mL, 3.7 mmol). The
solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 150 (79.2 mg, 83.2 %). IR (ATR) 3062, 2931, 2858, 2100, 1784, 1725, 1599, 1451, 1209, 1172, 1036, 1013, 993, and 700 cm⁻¹.

151: Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-cyclohexylmethyl ester methyl ester 151. 149 (77.5 mg, 0.3 mmol) was added 5:1 EtOH/H₂O (7 mL) followed by N-(3-ethynyl-phenyl)-acetamide (92.0 mg, 0.6 mmol). Ascorbic acid/H₂O (1.1 M, 0.58 mL, 0.6 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 0.48 mL, 0.2 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate (20 mL) was added and the solution washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 151 (99.5 mg, 80.7%). Rₛ = 0.37 (100% EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.25 (s, 1H), 7.95 (s, 1H), 7.77 (s, 1H), 7.55 (d, J = 7.8 Hz, 2H), 7.30 (t, J = 7.9 Hz, 1H), 4.52 (pentet, J = 3.8, 3.7 Hz, 1H), 4.035 (d, J = 7.3 Hz, 2H), 3.66 (s, 3H), 2.62 (s, 4H), 2.16 (m, 2H), 2.14 (s, 3H), 1.97 (m, 3H), 1.68 (m, 2H), and 1.58 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 172.74, 172.2, 168.9, 146.9, 138.7, 131.2, 129.4, 121.2, 119.4, 118.5, 116.8, 77.2, 66.7, 57.7, 51.8, 33.3, 29.0, 28.8, 28.7, 24.7, and 24.4; IR
(ATR) 3301, 2949, 2861, 1729, 1690, 1678, 1592, 1532, 1482, 1440, 1367, 1160, 998, 791, and 751 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{22}\)H\(_{28}\)N\(_4\)O\(_5\)H\(^+\): 429.21; found 428.99.

\[
\begin{align*}
\text{2-ethynyl-pyridine} & \quad \stackrel{1.1 \text{ M Ascorbic acid} / \text{H}_2\text{O}}{\rightarrow} \\
& \quad \stackrel{0.3 \text{ M CuSO}_4-5\text{H}_2\text{O} / \text{H}_2\text{O}}{\rightarrow} \\
& \quad \stackrel{5:1 \text{EtOH} / \text{H}_2\text{O}}{\rightarrow} \\
& \quad \stackrel{37 \, ^\circ\text{C}, 12 \, \text{h}}{\rightarrow}
\end{align*}
\]

\[\text{152: Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-cyclohexylmethyl ester 152.} \]

(79.2 mg, 0.3 mmol) was added to 5:1 EtOH/H\(_2\)O (7 mL) followed by 2-ethynyl-pyridine (98\%, 63.0 µL, 0.6 mmol). Ascorbic acid/H\(_2\)O (1.1 M, 0.61 mL, 0.7 mmol) and CuSO\(_4\)-5H\(_2\)O/H\(_2\)O (0.3 M, 0.50 mL, 0.2 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate (20 mL) was added and the solution washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 152 (88.2 mg, 79.7%).\(R_f = 0.53\) (100% EtOAc); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.58 (m, 1H), 8.33 (s, 1H), 8.23 (d, \(J = 7.9\) Hz, 1H), 8.03 (m, 2H), 7.82 (m, 1H), 7.55 (m, 1H), 7.42 (m, 2H), and 7.26 (m, 1H), 4.67 (pentet, \(J = 3.8, 3.296\) Hz, 1H), 4.29 (d, \(J = 7.2\) Hz, 2H), 2.37 (m, 2H), 2.12 (m, 3H), 1.83 (m, 2H), and 1.72 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 166.4, 132.9, 130.1, 129.5, 128.4, 122.8, 120.3, 67.1, 57.7, 33.8, 28.8, and 24.8; IR (ATR) 2952, 2855, 1716, 1599, 1449, 1426, 1272, 1116, 786, and 706 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{21}\)H\(_{22}\)N\(_4\)O\(_2\)H\(^+\): 363.18; found 362.99.
153: 4-Hydroxymethyl-cyclohexanol 153. *Cis*-4-hydroxy-cyclohexane-1-carboxylic acid 122 (4.01 g, 27.8 mmol) was added to THF (140 mL) at 0 °C followed by lithium aluminum hydride (3.17 g, 83.5 mmol). The solution was stirred overnight and allowed to warm to room temperature. Water (3.2 mL) was added, followed by 3.0 M NaOH/H$_2$O (6.4 mL), and then water (3.2 mL). The solution was filtered and evaporated to a residue which was purified by chromatography on silica gel in MeOH-chloroform (1:19) to give 153 (2.76 g, 76%). \( R_f = 0.32 \) (100% EtOAc); IR (ATR) 3315, 2924, 2859, 1442, 1372, 1255, 1126, 1022, 976, and 931 cm$^{-1}$.

154: 4-(tert-Butyl-dimethyl-silyloxymethyl)-cyclohexanol 154. 153 (0.50 g, 3.9 mmol) was added to DMF (7 mL) at 0 °C followed by imidazole (0.288 g, 4.2 mmol) and TBSCl (0.58g, 3.9 mmol). The solution was allowed to slowly warm to room temperature while stirring overnight. Water (50 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 154 (485 mg, 52%). $^1$H NMR (400 MHz, CDCl$_3$) δ 3.98 (s, 1H), 3.43 (d, \( J = 6.1 \) Hz, 2H), 1.69 (m, 2H), 1.52 (m, 6H), 1.35 (m, 2H), 0.88 (s, 10H), and 0.03 (s, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 67.7, 67.0, 39.1, 32.0, 25.9, 23.4, 18.4, and 5.4; IR (ATR) 3333, 2927, 2886, 2855, 1471, 1253, 1108, 1078, 1032, 979, 940, 896, 833, and 771 cm$^{-1}$.
155: *Toluene-4-sulfonic acid 4-(tert-butyl-dimethyl-silanyloxy methyl)-cyclohexyl ester*

155. 154 (1.9 g, 7.8 mmol) was added to pyridine (7 mL) followed by pTsCl (2.964 g, 15.6 mmol). The solution was stirred for 5 hours at room temperature. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 X 50 mL) and then with saturated ammonium chloride solution (2 X 50 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 155 (2.84 g, 91.6%). R_f = 0.72 (EtOAc-hexanes (1:3)); IR (ATR) 2929, 2885, 2859, 1598, 1470, 1348, 1247, 1187, 1170, 1129, 1094, 904, 831, 815, 775, and 676 cm⁻¹

156: *(4-Azido-cyclohexylmethoxy)-tert-butyl-dimethyl-silane*

156. 155 (2.733 g, 6.9 mmol) in DMF (20 mL) followed by sodium azide (2.23 g, 34.3 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Once cool, water (50 mL) was added to the solution which was then washed with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was then purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 156 (1.192 g, 64.5%). R_f = 0.57 (EtOAc-hexanes (1:19)); ¹H NMR (400 MHz, CDCl₃) δ 3.40 (d, J = 6.2 Hz, 2H), 3.20 (m, 1H), 2.02 (m, 2H), 1.84 (m, 2H), 1.43 (m, 1H), 1.32 (m, 2H), 1.00 (m, 2H), 0.88 (s, 10H), and 0.03 (s, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 67.8, 60.4, 39.3, 31.2, 27.8, 25.9, 18.3, and -5.42; IR (ATR) 2930, 2857, 2090, 1471, 1389, 1360, 1253, 1109, 1074, 1024, 834, and 774 cm⁻¹
157: (4-Azido-cyclohexyl)-methanol 157. 156 (1.109 g, 4.1 mmol) was added to THF (2 mL) followed by 6 M HCl/H₂O/MeOH (10 mL). The solution was stirred at room temperature for five hours. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 157 (0.442 g, 69.2%). ¹H NMR (400 MHz, CDCl₃) δ 3.88 (d, J = 6.5 Hz, 2H), 3.23 (m, 1H), 2.04 (s, 1H), 2.03 (m, 2H), 1.85 (m, 2H), 1.62 (m, 1H), 1.33 (m, 2H), and 1.06 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 68.6, 59.9, 35.9, 30.9, 27.8, and 20.9; IR (ATR) 2936, 2860, 2089, 1739, 1453, 1365, 1237, 1038, and 989 cm⁻¹.

158: Succinic acid 4-azido-cyclohexylmethyl ester methyl ester 158. 157 (26.0 mg, 0.2 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 158 (27.5 mg, 61%). ¹H NMR (400 MHz, CDCl₃) δ 3.90 (d, J = 6.5 Hz, 2H), 3.67 (s, 3H), 3.22 (pentet, J = 7.5, 4.150 Hz, 1H), 2.61 (s, 4H), 2.02 (m, 2H), 1.83 (m, 2H), 1.61 (m, 1H), 1.32 (m,
2H), and 1.05 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.6, 172.1, 68.8, 59.8, 51.8, 35.9, 30.9, 29.0, 28.8, and 27.7; IR (ATR) 2935, 2860, 2091, 1732, 1438, 1361, 1158, and 993 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{12}$H$_{19}$N$_3$O$_4$H$: 270.15; found 270.04.

**159:** Benzoic acid 4-azido-cyclohexylmethyl ester 159. 157 (26.0 mg, 0.2 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.2 mL, 1.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 159 (31.5 mg, 72.6%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.04 (m, 2H), 7.56 (m, 1H), 7.44 (m, 2H), 4.15 (d, $J$ = 6.5 Hz, 2H), 3.27 (pentet, $J$ = 7.4, 4.150 Hz, 1H), 2.07 (m, 2H), 1.96 (m, 2H), 1.79 (m, 1H), 1.38 (m, 2H), and 1.17 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.4, 132.9, 130.1, 129.5, 128.3, 68.9, 59.9, 36.1, 31.0, and 27.9; IR (ATR) 2936, 2860, 2089, 1713, 1452, 1315, 1267, 1175, 1120, 1070, 1025, 984, and 956 cm$^{-1}$

**160:** Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-cyclohexylmethyl ester methyl ester 160. 158 (27.5 mg, 0.1 mmol) was added to 5:1 EtOH/H$_2$O (3 mL) followed by $N$-(3-ethynyl-phenyl)-acetamide (33.0 mg, 0.2 mmol). Ascorbic
acid/H₂O (1.1 M, 0.2 mL, 0.2 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 0.2 mL, 0.05 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 160 (34.8 mg, 79.6%). ¹H NMR (400 MHz, CDCl₃) δ 8.67 (s, 1H), 8.01 (s, 1H), 7.70 (s, 1H), 7.51 (m, 2H), 7.26 (m, 1H), 4.34 (m, 1H), 3.92 (d, J = 6.4 Hz, 2H), 3.65 (s, 3H), 2.62 (s, 4H), 2.18 (m, 2H), 2.10 (s, 3H), 1.89 (m, 2H), 1.70 (m, 3H), and 1.16 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 172.7, 172.1, 169.1, 146.8, 138.8, 131.1, 129.2, 121.0, 119.4, 117.8, 116.8, 68.6, 59.7, 51.7, 35.8, 32.3, 28.9, 28.7, 27.9, and 24.3; IR (ATR) 3283, 2941, 1731, 1671, 1617, 1592, 1568, 1533, 1403, 1439, 1370, 1160, and 996 cm⁻¹; ESI-TOF-MS m/z calcd for C₂₂H₂₈N₄O₅H⁺: 429.21; found 428.97.

161: Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-cyclohexylmethyl ester 161. 159 (31.5 mg, 0.1 mmol) was added to 5:1 EtOH/H₂O (3 mL) followed by 2-ethynylpyridine, 98% (25.0 µL, 0.3 mmol). Ascorbic acid/H₂O (1.1 M, 0.25 mL, 0.3 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 0.2 mL, 0.06 mmol) were added and the solution was shaken at 37 °C for 12 hours. Saturated sodium bicarbonate solution (20 mL) was added and washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by
chromatography on silica gel in 100% EtOAc to give 161 (32.8 mg, 74.5%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.54 (m, 1H), 8.16 (s, 1H), 8.15 (m, 1H), 8.03 (m, 2H), 7.73 (m, 1H), 7.54 (t, $J$ = 7.5 Hz, 1H), 7.42 (m, 2H), 7.18 (m, 1H), 4.51 (m, 1H), 4.19 (d, $J$ = 6.5 Hz, 2H), 2.33 (m, 2H), 2.06 (m, 2H), 1.88 (m, 3H), and 1.35 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.3, 150.3, 149.2, 147.8, 136.8, 132.9, 130.0, 129.4, 128.3, 122.7, 120.0, 119.6, 68.7, 59.8, 36.1, 32.1, and 28.2; IR (ATR) 3145, 2941, 2855, 2094, 1718, 1603, 1451, 1438, 1417, 1275, 1176, 1118, 1045, 789, and 702 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{21}$H$_{22}$N$_4$O$_2$H$: 363.18; found 363.01.

162: 4-(Toluene-4-sulfonyloxy)-cyclohexanecarboxylic acid methyl ester 162. 134 (0.50 g, 3.2 mmol) was added to pyridine (5 mL) followed by pTsCl (0.663 g, 3.5 mmol) was added and the solution stirred for 5 hours at room temperature. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (3 $\times$ 50 mL) and then with saturated ammonium chloride solution (3 $\times$ 50 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 162 (0.772 g, 78.2%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.75 (d, $J$ = 8.3 Hz, 2H), 7.30 (d, $J$ = 7.9 Hz, 2H), 4.37 (m, 1H), 3.60 (s, 3H), 2.40 (s, 3H), 2.23 (m, 1H), 1.94 (m, 4H), and 1.46 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 174.9, 144.5, 134.2, 129.7, 127.4, 80.2, 51.6, 40.9, 30.9, 26.2, and 21.5; IR (ATR) 3001, 2951, 2905, 2866, 2836, 1729, 1597, 1451, 1431, 1365, 1312, 1192, 1174, 1043, and 952 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{15}$H$_{20}$O$_3$SH$: 313.11; found 312.93.
163: 4-Azido-cyclohexanecarboxylic acid methyl ester 163. 162 (1.193 g, 3.8 mmol) was added to DMF (10 mL) followed by sodium azide (1.241 g, 19.1 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Water (50 mL) was added to the solution once it was cooled to room temperature and the solution was washed with diethyl ether (3 x 100 mL). The organic phases were collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 163 (0.624 g, 89.1%). ¹H NMR (400 MHz, CDCl₃) δ 3.63 (m, 1H), 3.62 (s, 3H), 2.34 (m, 1H), 1.83 (m, 2H), and 1.64 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.0, 57.3, 51.4, 40.8, 28.6, and 23.9; IR (ATR) 2950, 2868, 2095, 1730, 1435, 1341, 1253, 1230, 1198, 1168, 1141, 1032, and 905 cm⁻¹

164: 4-Azido-cyclohexanecarboxylic acid 164. 163 (0.524 g, 2.9 mmol) was added to 2.5 M LiOH/H₂O/MeOH/THF (1:2:3) (10 mL) and the solution was stirred at room temperature overnight. 1.0 M HCl/H₂O was added until the solution had a pH of 2. The solution was washed with ethyl acetate (5 x 100 mL) and the organic phases collected, dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 164 (0.443 g, 91.5%). R_f = 0.49 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 12.03 (s, 1H), 3.69 (m, 1H), 2.44 (m, 1H), 1.91 (m, 2H), and 1.73 (m, 6H); ¹³C NMR (100 MHz, CDCl₃)
\[ \delta 181.6, 57.4, 40.7, 28.6, \text{and} 23.8; \text{IR (ATR) 3024, 2934, 2098, 1788, 1709, 1461, 1444, 1415, 1369 1312, 1233, 1199, \text{and} 1026 \text{ cm}^{-1} \]

165: 4-(Toluene-4-sulfonyloxy)-cyclohexanecarboxylic acid methyl ester 165. 123 (1.0 g, 6.3 mmol) was added to pyridine (10 mL) followed by pTsCl (1.446 g, 7.6 mmol). The solution was stirred at room temperature for 5 hours. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 165 (1.330 g, 67.4%). R\( _f \) = 0.31 (EtOAc-hexanes (1:3)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.77 (d, \( J = 8.3 \) Hz, 2H), 7.32 (d, \( J = 7.9 \) Hz, 2H), 4.69 (m, 1H), 3.65 (s, 3H), 2.43 (s, 3H), 2.31 (m, 1H), 1.85 (m, 4H), 1.69 (m, 2H), and 1.53 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 175.0, 144.4, 134.5, 129.7, 127.5, 78.3, 51.6, 40.9, 29.7, 23.2, and 21.6; ESI-TOF-MS m/z calcd for C\(_{15}\)H\(_{20}\)O\(_5\)SNa\(^+\): 335.09; found 334.95.

166: 4-Azido-cyclohexanecarboxylic acid methyl ester 166. 165 (1.275 g, 4.1 mmol) was added to (20 mL) DMF followed by sodium azide (1.327 g, 20.4 mmol). The solution was warmed to 80 °C and stirred for 5 hours. After cooling to room temperature, water (50 mL) was added and the solution was washed with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na\(_2\)SO\(_4\)), and concentrated
to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 166 (0.58 g, 77.5%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.59 (s, 3H), 3.22 (m, 1H), 2.21 (m, 1H), 1.97 (m, 4H), 1.44 (m, 2H), and 1.28 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 175.1, 58.9, 51.4, 41.5, 30.4, and 26.9; IR (ATR) 2949, 2864, 2092, 1731, 1453, 1435, 1365, 1305, 1254, 1194, 1171, 1118, 1042, 1021, and 898 cm$^{-1}$

**167:** 4-Azido-cyclohexanecarboxylic acid 167. 166 (0.520 g, 2.8 mmol) was added to 2.5 M LiOH/H$_2$O/MeOH/THF (1:2:3) (10 mL) and the solution was stirred for 12 hours at room temperature. 1.0 M HCl/H$_2$O was added until the solution had a pH of 2 and the solution was washed with ethyl acetate (5 x 100 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 167 (0.437 g, 91%). $R_f$ = 0.44 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 11.82 (s, 1H), 3.30 (m, 1H), 2.31 (m, 1H), 2.07 (m, 4H), 1.53 (m, 2H), and 1.37 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 181.7, 59.0, 41.6, 30.5, and 26.8; IR (ATR) 3033, 2935, 2862, 2091, 1693, 1466, 1427, 1360, 1307, 1250, 1213, 1041, 1021, and 939 cm$^{-1}$

**168:** Terephthalic acid bis-{1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl} ester 168. 97 (0.168 g, 0.8 mmol) was added to pyridine (2 mL) followed by terephthaloyl chloride (75.2 mg, 0.4 mmol) at room temperature. The solution was stirred for 24 hours. Ethyl acetate (50 mL) was added and the solution was washed
consecutively with saturated copper sulfate solution (2 × 25 mL) then saturated ammonium chloride solution (2 × 25 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexamnes (1:9) to give 168 (0.110 g, 50.8%). $R_f = 0.85$ (EtOAc-hexamnes (1:3)). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.10 (s, 4H), 5.23 (m, 2H), 3.91 (m, 2H), 3.53 (m, 2H), 3.46 (dd, $J = 4.1$ Hz, 2H), 3.20 (dd, $J = 7.4$ Hz, 2H), 1.92 (m, 8H), 1.74 (m, 6H), 1.54 (m, 4H), and 0.94 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.4, 134.3, 129.5, 80.8, 76.2, 74.6, 55.1, 40.1, 39.4, 31.4, 29.2, 27.6, 14.4, and 9.4; IR (ATR) 2967, 2878, 2095, 1717, 1579, 1504, 1461, 1408, 1380, 1268, 1116, 1101, 1018, 967, 876, and 730 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{30}$H$_{44}$N$_6$O$_6$H$^+$: 585.34; found 585.41.

169: Terephthalic acid bis-[2-{5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester 169. 55 (0.100 g, 0.5 mmol) was added to pyridine (2 mL) followed by terephthaloyl chloride (0.048 g, 0.2 mmol) at room temperature. The solution was stirred for 3 days. Ethyl acetate (50 mL) was added followed by a saturated copper sulfate solution (2 × 50 mL). The organic layer was then extracted with saturated ammonium chloride solution (2 × 50 mL) and then dried (Na$_2$SO$_4$) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexamnes (1:9) to give 169 (34.8 mg, 26.7%). $R_f = 0.76$ (EtOAc-hexamnes (1:3)). IR (ATR) 2966, 2934, 2876, 2096, 1717, 1579, 1504, 1461, 1408, 1380, 1268, 1116, 1101, 1018, 967, 876, and 731 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{28}$H$_{40}$N$_6$O$_6$H$^+$: 557.31; found 557.27.
**170:** *Isophthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester 170.* 55 (0.100 g, 0.5 mmol) was added to pyridine (2 mL) followed by isophthaloyl chloride (0.048 g, 0.2 mmol) at room temperature. The solution was stirred for 3 days. Ethyl acetate (50 mL) was added followed by a saturated copper sulfate solution (2 × 50 mL). The organic layer was then extracted with saturated ammonium chloride solution (2 × 50 mL) and then dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 170 (33.0 mg, 25.3%). $R_f=0.76$ (EtOAc-hexanes (1:3)). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.3, 133.6, 131.1, 130.6, 128.5, 80.9, 76.0, 70.3, 55.1, 42.5, 39.4, 31.4, 29.2, 20.8, and 14.4; IR (ATR) 2970, 2877, 2096, 1720, 1609, 1461, 1365, 1302, 1285, 1235, 1137, 1092, 1073, 938, and 731 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{28}$H$_{40}$N$_6$O$_6$H$^+$: 557.31; found 557.37.

**171:** *1-{5-(2-{4-[1-{2-[5-(2-Hydroxy-butyl)-tetrahydro-furan-2-yl]-propyl]-1H-[1,2,3]triazol-4-yl]-phenyl}-{1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl]-butan-2-ol 171.* 97 (0.100 g, 0.5 mmol) was added to 5:1 EtOH/H$_2$O (3 mL) followed by 1,4-diethylbenzene (0.0264 g, 0.2 mmol). Ascorbic acid/H$_2$O (1.1 M, 0.84 mL, 0.9 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 0.7 mL, 0.2 mmol) was added and the mixture was shaken for 3 days at 37 °C. Ethyl acetate (50 mL) was added and
the solution extracted with saturated sodium bicarbonate solution (2 × 100 mL). The organic layer was dried (Na₂SO₄) and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 171 (0.1034 g, 85%). R_f = 0.67 (MeOH-chloroform (1:9)). ¹H NMR (400 MHz, CDCl₃) δ 7.90 (m, 4H), 7.84 (s, 2H), 4.59 (m, 2H), 4.35 (m, 2H), 4.16 (m, 2H), 3.79 (m, 2H), 3.55 (m, 3H), 2.30 (s, 1H), 2.06 (m, 7H), 1.62 (m, 11H), and 0.88 (m, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 130.3, 126.0, 120.5, 81.2, 77.1, 70.6, 53.5, 41.3, 40.2, 30.8, 30.4, 29.5, 14.4, and 10.0; IR (ATR) 3409, 3130, 2965, 2937, 2877, 2242, 1462, 1416, 1371, 1223, 1070, 1047, 975, 909, 849, 821, 729, 646 cm⁻¹; ESI-TOF-MS m/z calcd for C₃₂H₄₈N₆O₄H⁺: 581.38; found 581.45.

172: 1-{5-(2-{4-[4-(1-{2-[5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-propyl}-1H-[1,2,3]triazol-4-yl)-phenyl]-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl]-propan-2-ol 172. 55 (50.0 mg, 0.3 mmol) was added to 5:1 EtOH/H₂O (2 mL), followed by 1,4-diethynyl benzene (96%, 15.0 mg, 0.1 mmol). Ascorbic acid/H₂O (1.1 M, 0.45 mL, 0.5 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 0.37 mL, 0.1 mmol) was added and the solution was stirred for at 37 °C for 12 hours. Saturated sodium bicarbonate solution (50 mL) was added and the solution was extracted with ethyl acetate (3 × 25 mL). The organic phases were collected, dried (Na₂SO₄), and evaporated to a residue. The product was purified by chromatography on silica gel MeOH-chloroform (1:9) to give 172 (54.3 mg, 88.0%). R_f = 0.20 (100% EtOAc). ¹H
NMR (400 MHz, CDCl$_3$) $\delta$ 7.88 (s, 4H), 7.84 (s, 2H), 4.57 (m, 2H), 4.34 (m, 2H), 4.10 (m, 4H), 3.53 (m, 2H), 2.65 (s, 2H), 2.05 (m, 6H), 1.65 (m, 8H), 1.23 (d, $J = 6.2$ Hz, 6H), and 0.88 (d, $J = 6.8$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 147.2, 130.3, 126.0, 120.5, 81.1, 76.9, 65.4, 53.5, 43.5, 40.1, 30.8, 29.4, 23.6, and 14.3

173: 1-[5-(2-{4-[3-(1-{2-[5-(2-Hydroxy-butyl)-tetrahydro-furan-2-yl]-propyl}-1H]-[1,2,3]triazol-4-yl]-phenyl]-[1,2,3]triazol-1-yl]-1-methyl-ethyl-tetrahydro-furan-2-yl]-butan-2-ol 173. 97 (97.1 mg, 0.4 mmol) was added to 5:1 EtOH/H$_2$O (3 mL) followed by 1,3-dilethynylbenzene (27 µL, 0.2 mmol). Ascorbic acid/H$_2$O (1.1 M, 0.8 mL, 0.9 mmol) and CuSO$_4$-5H$_2$O/H$_2$O (0.3 M, 0.7 mL, 0.2 mmol) was added and the mixture was stirred at 37 °C for 12 hours. Ethyl acetate (50 mL) was added and the solution extracted with saturated sodium bicarbonate solution (100 mL), dried (Na$_2$SO$_4$), and then concentrated to a residue. The residue was purified by chromatography on silica gel in 100% EtOAc to give 173 (100.9 mg, 85.4%). $R_f = 0.61$ (MeOH-chloroform (1:9)). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.33 (s, 1H), 8.03 (s, 2H), 7.86 (m, 2H), 7.49 (m, 1H), 4.57 (m, 2H), 4.42 (m, 2H), 4.15 (m, 2H), 3.83 (m, 2H), 3.51 (m, 2H), 2.45 (s, 2H), 2.05 (m, 10H), 1.60 (m, 8H), and 0.87 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 129.4, 125.0, 81.1, 76.9, 70.4, 53.6, 41.3, 40.0, 30.8, 29.6, 14.6, 14.5, and 10.0; IR (ATR) 3401, 3130, 2964, 2935, 2876, 2239, 1619, 1461, 1384, 1226, 1045, 971, 908, 795, 730, and 695 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{32}$H$_{48}$N$_6$O$_4$H$: 581.38; found 581.33.
174. *Terephthalic acid bis-[1-(5-[2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-ylmethyl)-propyl] ester* 174. 168 (21.0 mg, 0.04 mmol) was added to 5:1 EtOH/H₂O (1.5 mL) and ethyl acetate (1 mL) followed by N-(3-ethynyl-phenyl)-acetamide (17.0 mg, 0.1 mmol). Ascorbic acid/H₂O (1.1 M, 72 µL, 0.1 mmol) and CuSO₄-5H₂O/H₂O (0.3 M, 60 µL, 0.02 mmol) was added and the mixture was shaken at 37 °C for 12 hours. Ethyl acetate (25 mL) was added to the solution and washed with saturated sodium bicarbonate solution (20 mL). The organic phase was dried (Na₂SO₄) and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 174 (22.7 mg, 70%). Rᵢ = 0.12 (100% EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 8.13 (m, 4H), 8.00 (s, 2H), 7.87 (m, 4H), 7.72 (d, J = 7.7 Hz, 2H), 7.58 (d, J = 7.7 Hz, 2H), 7.31 (t, J = 7.9 Hz, 2H), 5.39 (m, 2H), 4.45 (m, 4H), 3.88 (m, 2H), 3.35 (m, 2H), 2.14 (s, 6H), 1.87 (m, 14H), 1.54 (m, 3H), 1.25 (m, 1H), 0.95 (m, 6H), and 0.85 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 168.7, 165.6, 138.6, 134.4, 131.4, 129.6, 129.4, 121.44, 121.42, 119.5, 116.8, 80.5, 75.9, 74.4, 53.2, 40.2, 40.1, 31.3, 29.7, 27.9, 24.6, 14.5, and 9.5; IR (ATR) 3304, 3127, 2962, 2926, 2855, 2243, 1712, 1691, 1678, 1619, 1592, 1567, 1536, 1484, 1462, 1443, 1406, 1369, 1269, 1167, 1103, 1018, 909, 791, 729, and 693 cm⁻¹; ESI-TOF-MS m/z calcd for C₅₀H₆₂N₈O₈H⁺: 903.48; found 903.49.
**175:** Terephthalic acid bis-(1-[5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-ylmethyl]-propyl) ester 175. 168 (21.0 mg, 0.04 mmol) was added to 5:1 EtOH/H$_2$O (1.5 mL) and ethyl acetate (1 mL) followed by 2-ethynyl-pyridine, 98+% (11.0 µL, 0.1 mmol). Ascorbic acid/H$_2$O (1.1 M, 72 µL, 0.08 mmol) and CuSO$_4$-H$_2$O/H$_2$O (0.3 M, 60 µL, 0.02 mmol) was added and the mixture was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added and the solution was washed with saturated sodium bicarbonate solution (20 mL). The organic phase was dried (Na$_2$SO$_4$) and concentrated to a residue then purified by chromatography on silica gel in 100% EtOAc to give 175 (16.4 mg, 57.7%). $R_f = 0.29$ (100% EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.57 (m, 2H), 8.15 (m, 8H), 7.76 (m, 2H), 7.21 (m, 2H), 5.32 (m, 2H), 4.64 (m, 2H), 4.34 (m, 2H), 3.94 (m, 2H), 3.49 (m, 2H), 1.97 (m, 6H), 1.76 (m, 4H), 1.57 (m, 4H), 1.25 (m, 4H), 0.97 (t, $J = 7.4$ Hz, 6H), and 0.85 (d, $J = 6.9$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.4, 150.5, 149.3, 148.1, 136.8, 134.3, 129.5, 122.9, 122.7, 120.2, 80.7, 76.3, 74.4, 53.5, 40.4, 40.3, 31.4, 29.6, 27.8, 14.3, and 9.5; IR (ATR) 2968, 2934, 2978, 2243, 1713, 1604, 1572, 1462, 1421, 1384, 1269, 1204, 1118, 1102, 1040, 1018, 910, 785, and 729 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{44}$H$_{54}$N$_8$O$_6$Na$^+$: 813.41; found 813.48.
176: Terephthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester 176. 169 (6.5 mg, 0.01 mmol) was added to 5:1 EtOH/H₂O (1.0 mL) and ethyl acetate (1.0 mL) followed by 2-ethynyl-pyridine, 98+% (4.0 µL, 0.04 mmol). Ascorbic acid/H₂O (1.1 M, 23.0 µL, 0.03 mmol) and CuSO₄·5H₂O/H₂O (0.3 M, 20.0 µL, 0.006 mmol) was added and the mixture was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added and the solution washed with saturated sodium bicarbonate solution (20 mL), dried (Na₂SO₄), concentrated to a residue, and purified by chromatography on silica gel in 100% EtOAc to give 176. Rᵢ = 0.50 (MeOH-chloroform (1:9)). IR (ATR) 2955, 2916, 2869, 1738, 1722, 1604, 1461, 1378, 1270, 1230, 1211, 1086, 1086, 908, 786, and 732 cm⁻¹; ESI-TOF-MS m/z calcd for C₄₂H₇₀N₈O₆Na⁺: 785.38; found 785.45.

177: Isophthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester 177. 170 (6.0 mg, 0.01 mmol) was added to 5:1 EtOH/H₂O (1.0 mL) and ethyl acetate (1.0 mL) followed by 2-ethynyl-pyridine, 98+% (3.0 µL, 0.03 mmol). Ascorbic acid/H₂O (1.1 M, 22.0 µL, 0.03 mmol) and
CuSO$_4$-H$_2$O/H$_2$O (0.3 M, 18.0 µL, 0.005 mmol) was added and the mixture was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added and the solution washed with saturated sodium bicarbonate solution (20 mL). The organic phase was dried (Na$_2$SO$_4$), concentrated to a residue and purified by chromatography on silica gel in 100% EtOAc to give 177 (7.0 mg, 85%). IR (ATR) 2954, 2916, 2869, 2841, 1722, 1605, 1377, 1241, 1081, 972, 908, 789, and 734 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{42}$H$_{50}$N$_8$O$_6$Na$: 785.38; found 785.44.

178: Succinic acid 1-[5-[2-(4-[4-[1-(2-[5-[2-(3-methoxycarbonyl-propionyloxy)-butyl]-tetrahydro-furan-2-yl]-propyl)-1H-[1,2,3]triazol-4-yl]-phenyl]-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-ylmethyl]-propyl ester methyl ester 178. 171 (49.0 mg, 0.09 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate, 97% (43.0 µL, 0.4 mmol). The solution was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL), followed by saturated ammonium chloride solution (2 × 20 mL). The organic phase was dried (Na$_2$SO$_4$) and concentrated to a residue then purified by chromatography on silica gel in 100% EtOAc to give 178 (23.0 mg, 33.7%). $R_f = 0.71$ (100% EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.93 (m, 6H), 5.15 (m, 1H), 4.58 (m, 1H), 4.45 (m, 1H), 3.83 (m, 1H), 3.66
(m, 6H), 3.41 (m, 1H), 2.63 (m, 8H), 1.44 (m, 21H), 1.22 (m, 2H), and 0.88 (m, 12H); 
$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.7, 172.0, 130.4, 126.04, 126.0, 121.2, 80.4, 75.9, 73.5, 53.2, 51.8, 40.3, 40.2, 31.3, 29.7, 29.4, 29.0, 27.8, 14.5, and 9.4; IR (ATR) 2969, 2879, 2256, 1728, 1438, 1366, 1217, 1161, 1066, 1044, 996, 973, 914, 847, and 729 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{42}$H$_{60}$N$_6$O$_{10}$H$: 809.44; found 809.48.

![Chemical structure]

179: $1,1'(5,5',(1,1'-((4,4'-phenylene)bis(1H-1,2,3-triazole-4,1-diyl))bis(propane-2,1-diyl))bis(tetrahydrofuran-5,2-diyl))bis(butane-2,1-diyl)dibenzoate$ 179. 171 (49.0 mg, 0.09 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (40.0 µL, 0.4 mmol). The solution was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na$_2$SO$_4$), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 179 (40.0 mg, 60%). $R_f = 0.82$ (100% EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.98 (m, 10H), 7.50 (m, 6H), 5.42 (m, 1H), 4.53 (m, 4H), 3.93 (m, 1H), 3.38 (m, 1H), 1.81 (m, 18H), 1.25 (m, 1H), 0.98 (t, $J = 7.4$ Hz, 6H), and 0.90 (d, $J = 6.8$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.3, 132.9, 130.5, 130.3, 130.1, 129.5, 128.4, 126.0, 121.3, 80.4, 75.9, 73.5, 53.2, 40.35, 40.27, 31.3, 29.8, 28.0, 14.5, and 9.6; IR (ATR) 2970, 2878, 2249, 1711, 1602, 1584,
1451, 1366, 1314, 1273, 1229, 1176, 1111, 1069, 1026, 973, 909, 804, 730, and 710 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{46}H_{56}N_6O_{10}\)H\(^+\): 789.43; found 789.45.

**180:** Succinic acid 1-{5-[2-(4-{3-[1-(2-{5-[2-(3-methoxycarbonyl-propionyloxy)-butyl]-tetrahydro-furan-2-yl]-propyl)-1H-[1,2,3]triazol-4-yl]-phenyl}-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-ylmethyl}-propyl ester methyl ester 180. 173 (36.0 mg, 0.06 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate, 97% (32.0 µL, 0.3 mmol). The solution was stirred at room temperature for 12 hours. Ethyl acetate (25 mL) was added to the solution and was washed with saturated copper sulfate solution (2 × 20 mL) and then washed with saturated ammonium chloride solution (2 × 20 mL), dried (Na\(_2\)SO\(_4\)), and concentrated to a residue which was purified by chromatography on silica gelin 100% EtOAc to give 180 (24.0 mg, 47.8%). \(R_f = 0.71\) (100% EtOAc). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.32 (m, 1H), 8.02 (s, 1H), 7.88 (m, 2H), 7.48 (m, 1H), 5.11 (m, 1H), 4.60 (m, 1H), 4.42 (m, 1H), 3.85 (m, 1H), 3.65 (s, 6H), 3.44 (m, 1H), 2.63 (s, 8H), 1.75 (m, 16H), 1.24 (m, 5H), and 0.88 (m, 15H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 172.7, 172.0, 147.2, 131.3, 125.2, 122.84, 122.78, 121.3, 80.5, 76.0, 73.5, 51.8, 51.3, 40.4, 40.2, 31.3, 29.7, 29.4, 28.9, 27.8, 14.4, and 9.4; IR (ATR) 2969, 2254, 1729, 1620, 1439, 1366, 1217, 1162, 1045, 997, 910, 846, 797, and 728 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{42}H_{60}N_6O_{10}\)H\(^+\): 809.44; found 809.48.
181: 1,1′-(5,5′-(1,1′-(4,4′-(1,3-phenylene)bis(1H-1,2,3-triazole-4,1-diyl))bis(propane-2,1-diyl))bis(tetrahydrofuran-5,2-diyl))bis(butane-2,1-diyl)dibenzoate 181. 173 (36.0 mg, 0.06 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (29.0 µL, 0.3 mmol). The solution was stirred for 12 hours at room temperature. Ethyl acetate (25 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na₂SO₄), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give 181 (32.0 mg, 65.4%). Rₕ = 0.82 (100% EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 8.35 (m, 1H), 8.05 (m, 6H), 7.88 (m, 2H), 7.55 (m, 2H), 7.43 (m, 5H), 5.38 (m, 2H), 4.56 (m, 2H), 4.41 (m, 2H), 3.93 (m, 2H), 3.40 (m, 2H), 1.96 (m, 9H), 1.75 (m, 2H), 1.56 (m, 3H), 1.42 (m, 2H), 1.25 (m, 2H), 0.97 (m, 6H), and 0.85 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 166.3, 132.9, 132.8, 131.3, 130.6, 130.0, 129.53, 129.49, 128.36, 128.27, 125.3, 80.5, 76.2, 73.6, 53.3, 40.31, 40.26, 31.3, 29.7, 27.9, 19.3, 14.4, and 9.5; IR (ATR) 2970, 2878, 2249, 1711, 1451, 1383, 1314, 1273, 1175, 1111, 1069, 1026, 909, 795, 730, and 710 cm⁻¹; ESI-TOF-MS m/z calcd for C₄₆H₅₆N₆O₆H⁺: 789.43; found 789.49.
183: *1-Furan-2-yl-propan-2-ol* 183. Furan 182 (5.0 mL, 68.8 mmol) was added to THF (70 mL), cooled to 0 °C. nBuLi in THF (2.37 M, 32.0 mL, 75.6 mmol) was added dropwise. The solution was stirred at 0 °C for 2 hours followed by the dropwise addition of propylene oxide (7.2 mL, 103.1 mmol). The solution was allowed to warm to room temperature overnight. After 12 hours, 0.1 M HCl/H₂O (25 mL) was added and the solution was stirred for 10 minutes. The solution was extracted with ethyl acetate (3 × 200 mL) and the organic layer was collected, dried (Na₂SO₄), and evaporated to an oil. The product was purified by distillation (48 °C, 15 mmHg) to give a clear liquid 183 (5.68 g, 65.6%). R₆ = 0.29 (EtOAc-hexanes (1:3)); ^1^H NMR (400 MHz, CDCl₃) δ 7.29 (m, 1H), 6.26 (m, 1H), 6.00 (m, 1H), 4.10 (m, 1H), 3.31 (s, 1H), 2.70 (m, 2H), and 1.17 (m, 3H); ^1^C NMR (100 MHz, CDCl₃) δ 152.7, 141.3, 110.1, 106.8, 66.6, 37.6, and 22.5; IR (ATR) 3415, 2971, 2933, 1717, 1507, 1376, 1080, 1008, and 941 cm⁻¹

184: *Acetic acid 2-furan-2-yl-1-methyl-ethyl ester* 184. 183 (5.7 g, 45.1 mmol) was added to THF (15 mL) followed by pyridine (18.4 mL, 225.4 mmol). Acetic anhydride (12.8 mL, 135.2 mmol) was added at room temperature and the solution was then stirred for 2 hours. Ethyl acetate (100 mL) was added to the solution, which was then washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated
ammonium chloride solution (2 × 50 mL). The organic layer was dried (Na₂SO₄) and concentrated to an oil. The product was purified by distillation (96 °C, 24 mmHg) to give slightly yellow oil 184 (5.97 g, 78.8%). Rₜ = 0.50 (EtOAc-hexanes (1:9); ¹H NMR (400 MHz, CDCl₃) δ 7.26 (s, 1H), 6.23 (s, 1H), 6.01 (s, 1H), 5.10 (sextet, J = 12.7, 6.4 Hz, 1H), 2.87 (dd, J = 15.0, 6.5 Hz, 1H), 2.78 (dd, J = 15.0, 6.4 Hz, 1H), 1.95 (s, 3H), and 1.19 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.1, 151.4, 141.2, 110.0, 106.7, 69.0, 34.1, 20.9, and 19.3; IR (ATR) 2982, 2937, 1736, 1597, 1507, 1456, 1372, 1237, 1147, 1061, 1009, 957, and 934 cm⁻¹.

185: 2-[5-(2-Acetoxy-propyl)-furan-2-yl]-propionic acid ethyl ester 185. 184 (30.6 g, 182.0 mmol) was added to DMSO (250 mL) followed by Fe₂(SO₄)₃-H₂O (72.8 g, 182.0 mmol) and ethyl-(dl)-2-iodopropionate (41.5 g, 182.0 mmol). Et₃B in THF (1.0 M, 182.0 mL, 182.0 mmol) was added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et₃B in THF (1.0 M, 182.0 mL, 182.0 mmol) was added and the solution was stirred for an additional 2 hours. Brine solution (200 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 185 (5.34 g, 10.9%). Rₜ = 0.56 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 6.01 (d, J = 3.3 Hz, 1H), 5.95 (d, J = 2.9 Hz, 1H), 5.06 (sextet, J = 12.8, 6.6, 6.2 Hz, 1H), 4.10 (q, J = 14.3, 7.3, 7.0, 2H), 3.69 (q, J = 7.3 Hz, 1H), 2.73 (dd, J = 15, 6.2 Hz, 1H) 2.73 (dd, J = 15, 6.2 Hz, 1H), 1.96 (s,
3H), 1.43 (d, J = 7.3 Hz, 3H), 1.21-1.17 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.5, 170.3, 152.1, 150.7, 107.6, 106.4, 69.2, 60.8, 39.4, 34.3, 21.1, 19.4, 15.6, and 14.0; IR (ATR) 2983, 2939, 1735, 1559, 1456, 1372, 1236, 1202, 1057, 1016, 954, and 786 cm$^{-1}$; ESI-TOF-HRMS m/z calcd for C$_{14}$H$_{20}$O$_5$H$^+$: 269.1398; found 269.1389.

186: 2-{5-(2-Hydroxy-propyl)-furan-2-yl}-propionic acid 186. 2.5 M LiOH/H$_2$O/MeOH/THF (1:2:3) (40 mL) was added to 185 (2.2 g, 8.2 mmol) and the solution was stirred at room temperature overnight. 1.0 M HCl/H$_2$O was added until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50 mL). The organic phase was dried (Na$_2$SO$_4$) and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give 186 (1.32 g, 81.0%). $R_f$ = 0.74 (EtOAc-MeOH-AcOH (95:4:1)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.40 (s, 2H), 6.05 (d, $J$ = 3.3 Hz, 1H), 5.98 (d, $J$ = 3.3 Hz, 1H), 4.02 (sextet, $J$ = 12.4, 6.2, 2H), 3.73 (q, $J$ = 7.3 Hz, 1H), 2.68 (d, $J$ = 6.2 Hz, 2H), 1.46 (d, $J$ = 7.3, 3H), and 1.15 (d, $J$ = 6.2 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 177.3, 151.9, 151.6, 107.6, 106.8, 66.8, 39.1, 37.4, 22.2, and 15.3; ESI-TOF-HRMS m/z calcd for C$_{10}$H$_{14}$O$_4$H$^+$: 199.0970; found 199.0971.
187:  

2-Furan-2-yl-ethanol 187. Furan 182 (20.0 mL, 275.0 mmol) was added to THF (150 mL) and cooled to 0 °C. nBuLi in THF (2.37 M, 128.0 mL, 302.5 mmol) was added dropwise to the solution. The solution was stirred for 1 hour and ethylene oxide (14.0 mL, 275.0 mmol) in THF (50 mL) was added dropwise into solution which was then stirred overnight and allowed to warm to room temperature. 0.1 M HCl/H₂O (100 mL) was added to the solution and was stirred for 10 minutes. The solution was extracted with ethyl acetate (3 × 200 mL) and the organic layers collected, dried (Na₂SO₄), and evaporated to an oil. The product was purified by distillation to give a clear oil 187 (9.16 g, 29.7%). R_f = 0.23 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.33 (m, 1H), 6.30 (m, 1H), 6.10 (m, 1H), 3.87 (t, J = 6.2 Hz, 2H), 2.89 (t, J = 6.2 Hz, 2H), and 1.25 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 141.5, 110.3, 106.5, 61.0, and 31.5; IR (ATR) 3402, 3078, 2929, 1717, 1600, 1507, 1364, 1217, and 1004 cm⁻¹

188:  

Acetic acid 2-furan-2-yl-ethyl ester 188. 187 (9.162 g, 81.7 mmol) was added to THF (27 mL) and pyridine (33.3 mL, 408.5 mmol) followed by acetic anhydride (23.2 mL, 245.1 mmol). The solution was stirred for 2 hours at 25 ºC. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium sulfate solution (2 × 50 mL). The organic
layer was dried (Na$_2$SO$_4$) and concentrated to an oil. The product was purified by distillation (85 °C, 10 mmHg) to give 188 (9.85 g, 78.3%). R$_f$ = 0.41 (EtOAc-hexanes 1:9); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.24 (m, 1H), 6.20 (m, 1H), 6.00 (m, 1H), 4.22 (m, 2H), 2.88 (m, 2H), and 1.96 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.6, 151.5, 141.2, 110.0, 106.0, 62.0, 27.4, and 20.4; IR (ATR) 2970, 1738, 1653, 1600, 1559, 1508, 1456, 1366, 1231, 1147, 1035, and 1009 cm$^{-1}$

189: 2-[(5-(2-Acetoxy-ethyl)-furan-2-yl)-propionic acid ethyl ester 189. 188 (30.0 g, 194.7 mmol) was added to DMSO (250 mL) followed by Fe$_2$(SO$_4$)$_3$·H$_2$O (78.0 g, 194.7 mmol) and ethyl-(dl)-2-iodopropionate (44.4 g, 194.7 mmol). Et$_3$B in THF (1.0 M, 195.0 mL, 194.7 mmol) was then added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et$_3$B in THF (1.0 M, 195.0 mL, 194.7 mmol) was added and the solution was stirred for an additional 2 hours. Brine solution (200 mL) was added and the solution was extracted with diethyl ether (3 x 100 mL). The organic layers were collected, dried (Na$_2$SO$_4$), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give a yellowish oil 189 (3.84 g, 7.8%). R$_f$ = 0.56 (EtOAc-hexanes 1:3); $^1$H NMR (400 MHz, CDCl$_3$) δ 6.03 (d, $J = 3.1$ Hz, 1H), 5.97 (d, $J = 3.2$ Hz, 1H), 4.24 (t, $J = 6.9$ Hz, 2H), 4.12 (q, $J = 7.2$, 7.1 Hz, 3H), 2.90 (t, $J = 6.9$ Hz, 2H), 2.00 (s, 3H), 1.45 (d, $J = 7.2$ Hz, 3H), and 1.21 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.6, 170.8, 152.2, 150.9, 106.9, 106.4, 62.2, 60.9, 39.4,
27.6, 20.8, 15.6, and 14.0; IR (ATR) 2984, 1735, 1653, 1559, 1456, 1367, 1231, 1036 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{13}\)H\(_{18}\)O\(_5\)Na\(^+\): 277.11; found 277.03.

\[\text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad 5\% \text{ Rh/C, H}_2, (500 \text{ psi}) \text{ethyl acetate, r.t., 5 h} \]

**190: 2-[5-(2-Acetoxyl-ethyl)-tetrahydro-furan-2-yl]-propionic acid ethyl ester 190.**  
(2.001 g, 7.9 mmol) was added to ethyl acetate (10 mL) in a hydrogenator followed by 5% Rh/C (0.6 g) and the solution was stirred at room temperature. The air was flushed out with argon, which was then flushed out with hydrogen. The pressure of the hydrogen atmosphere was raised to 500 psi and the solution was stirred for 24 hours. The pressure was released and the hydrogen was flushed out with argon. The solution was filtered through Celite and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give **190** (1.25 g, 61.5%). \(R_f = 0.50\) (EtOAc-hexanes (1:3)); \(^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) \(\delta 4.04 \text{ (m, 5H)}, 3.85 \text{ (m, 1H)}, 2.39 \text{ (m, 1H)}, 1.94 \text{ (s, 3H)}, 1.90 \text{ (m, 2H)}, 1.71 \text{ (m, 2H)}, 1.60 \text{ (m, 1H)}, 1.43 \text{ (m, 1H)}, \text{and 1.13 (m, 6H)}; \(^{13}\text{C} \) NMR (100 MHz, CDCl\(_3\)) \(\delta 174.3, 170.8, 80.0, 76.0, 61.7, 60.0, 45.0, 34.7, 30.8, 28.8, 20.7, 14.0, \text{and 13.8}; \) ESI-TOF-MS m/z calcd for C\(_{13}\)H\(_{22}\)O\(_3\)H\(^+\): 259.15; found 259.08.

\[\text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad 1. \quad 2.5 \text{ M LiOH} / \text{H}_2\text{O} / \text{MeOH} / \text{THF (1:2:3)} \]

**191: 2-[5-(2-Hydroxy-ethyl)-tetrahydro-furan-2-yl]-propionic acid 191.**  
2.5 M LiOH/H\(_2\)O/MeOH/THF (1:2:3) (25 mL) was added to **190** (1.2 g, 4.7 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H\(_2\)O was added until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 x 50
mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give **191** (0.283 g, 32.4%). R_f = 0.76 (EtOAc-MeOH-AcOH (95:4:1)); **¹H NMR** (400 MHz, CDCl₃) δ 7.65 (s, 1H), 4.13 (m, 2H), 3.98 (m, 2H), 2.51 (m, 1H), 2.00 (m, 4H), 1.66 (m, 3H), and 1.15 (m, 3H); **¹³C NMR** (100 MHz, CDCl₃) δ 179.3, 179.0, 171.2, 80.3, 79.8, 76.6, 76.5, 61.9, 45.1, 44.4, 34.8, 34.7, 30.9, 30.8, 28.7, 28.5, 20.9, and 13.3

**192**: [5-(2-Acetoxy-ethyl)-furan-2-yl]-acetic acid ethyl ester **192**. **188** (32.09 g, 208.3 mmol) was added to DMSO (300 mL) followed by Fe₂(SO₄)₃·H₂O (83.3 g, 208.3 mmol) and ethyl iodoacetate (45.0 mL, 208.3 mmol). Et₃B in THF (1.0 M, 208.5 mL, 208.3 mmol) was added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et₃B in THF (1.0 M, 208.5 mL, 208.3 mmol) was added and the solution was stirred for an additional 2 hours. Brine solution (200 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **192** (7.25 g, 14.5%). R_f = 0.63 (EtOAc-hexanes (1:3)); **¹H NMR** (400 MHz, CDCl₃) δ 6.02 (s, 1H), 5.92 (s, 1H), 4.17 (s, 2H), 4.06 (s, 2H), 4.06 (s, 2H), 3.52 (s, 2H), 2.83 (s, 2H), 1.93 (s, 3H), and 1.15 (s, 3H); **¹³C NMR** (100 MHz, CDCl₃) δ 170.5, 169.1, 151.0, 146.4, 108.3, 107.0, 61.9, 60.7, 33.8, 27.4, 20.5, and 13.8; IR (ATR)
2971, 1736, 1657, 1568, 1229, 1031, and 788 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{12}\)H\(_{16}\)O\(_5\)Na\(^+\): 263.09; found 263.01.

\[ \begin{align*}
\text{5% Rh/C, H\(_2\), (500 psi) ethyl acetate, r.t., 24 h} \\
\end{align*} \]

193: \([5-(2-\text{Acetoxy-ethyl})-\text{tetrahydro-furan-2-yl}]\text{-acetic acid ethyl ester 193. 192 (3.001 g, 12.5 mmol) was added to ethyl acetate (10 mL) in a hydrogenator followed by 5% Rh/C (0.7 g) and the solution was stirred at room temperature. The air was flushed out with argon, which was then flushed out with hydrogen. The pressure of the hydrogen atmosphere was raised to 500 psi and the solution was stirred for 24 hours. The pressure was released and the hydrogen was flushed out with argon. The solution was filtered through Celite and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 193 (1.57 g, 51.4%). R\(_f\) = 0.40 (EtOAc-hexanes (1:3)); \( \^{1} \)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 4.15 (m, 1H), 4.05 (m, 4H), 3.85 (m, 1H), 2.49 (dd, \( J \) = 15.8, 6.87, 6.776 Hz, 1H), 2.35 (dd, \( J \) = 15.9, 6.501, 6.409 Hz, 1H), 1.95 (m, 2H), 1.94 (s, 3H), 1.74 (m, 2H), 1.50 (m, 2H), and 1.17 (t, \( J = 7.2, 3H\)); \( ^{13} \)C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 170.9, 170.8, 76.2, 75.2, 61.7, 60.1, 40.9, 34.8, 30.7, 30.6, 20.7, and 14.0; ESI-TOF-MS m/z calcd for C\(_{12}\)H\(_{20}\)O\(_5\)H\(^+\): 245.14; found 245.07.

\[ \begin{align*}
\text{1. 2.5 M LiOH / H\(_2\)O / MeOH / THF (1:2:3)} \\
\text{2. 1.0 M HCl / H\(_2\)O} \\
\end{align*} \]

194: \([5-(2-\text{Hydroxy-ethyl})-\text{tetrahydro-furan-2-yl}]\text{-acetic acid 194. 2.5 M LiOH/H\(_2\)O/MeOH/THF (1:2:3) (25 mL) was added to 193 (1.454 g, 6.0 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H\(_2\)O was added} \])
until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50 mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give 194 (0.543 g, 52.4%). R_f = 0.70 (EtOAc-MeOH-AcOH (95:4:1)); ^1H NMR (400 MHz, CDCl₃) δ 8.03 (s, 1H), 4.20 (m, 1H), 4.09 (m, 2H), 3.93 (m, 1H), 2.55 (m, 1H), 2.45 (m, 1H), 2.00 (m, 3H), 1.78 (m, 2H), and 1.55 (m, 2H); ^13C NMR (100 MHz, CDCl₃) δ 175.7, 171.2, 76.5, 75.0, 61.8, 40.7, 34.7, 30.73, 30.68, and 20.8

195: [5-(2-Acetoxy-propyl)-furan-2-yl]-acetic acid ethyl ester 195. 184 (30.0 g, 178.4 mmol) was added to DMSO (250 mL) at room temperature. Fe₂(SO₄)₃-H₂O (71.35 g, 178.4 mmol) was added followed by ethyl iodoacetate (98%, 21.2 mL, 178.4 mmol). Et₃B in THF (1.0 M, 178.5 mL, 178.4 mmol) was added and the solution was stirred for 45 minutes as air was bubbled through the solution. After 45 minutes, Et₃B in THF (1.0 M, 178.5 mL, 178.4 mmol) was added and the solution was stirred for an additional 2 hours. Brine solution (200 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic layers were collected, dried (Na₂SO₄), and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give 195 (7.5 g, 16.5%). IR (ATR) 2982, 2936, 1735, 1653, 1559, 1457, 1372, 1237, 1031, and 955 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₃H₁₈O₅Na⁺: 277.11; found 277.03.
196: [5-(2-Acetoxy-propyl)-tetrahydro-furan-2-yl]-acetic acid ethyl ester 196. 195 (3.024 g, 11.9 mmol) was added to ethyl acetate (10 mL) followed by 5% Rh/C (0.7 g) and the solution stirred at room temperature. The air was flushed out with argon, which was then flushed out with hydrogen. The pressure of the hydrogen atmosphere was raised to 500 psi and the solution was stirred for 24 hours. The pressure was released and the hydrogen was flushed out with argon. The solution was filtered through Celite and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 196 (0.87 g, 28.3%). 1H NMR (400 MHz, CDCl₃) δ 4.91 (m, 1H), 4.11 (m, 1H), 4.04 (q, J = 7.2, 7.050 Hz, 2H), 3.78 (m, 1H), 2.48 (m, 1H), 2.34 (m, 1H), 1.91 (s, 3H), 1.90 (m, 2H), 1.65 (m, 1H), and 1.48 (m, 3H); 13C NMR (100 MHz, CDCl₃) δ 170.96, 170.94, 170.32, 170.29, 76.16, 76.13, 75.07, 75.01, 68.9, 68.5, 60.1, 42.3, 41.8, 40.99, 40.9, 31.0, 30.9, 30.6, 30.5, 21.11, 21.09, 20.4, and 13.9; ESI-TOF-MS m/z calcd for C₁₃H₂₂O₅Na⁺: 281.14; found 281.05.

197: [5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-acetic acid 197. 2.5 M LiOH/H₂O/MeOH/THF (1:2:3) (10 mL) was added to 196 (0.493 g, 1.9 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H₂O was added until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50 mL). The organic layers were collected, dried (Na₂SO₄), and concentrated to a
residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give 197 (0.206 g, 57.5%). R_f = 0.54 (EtOAc-MeOH-AcOH (95:4:1)); ^1H NMR (400 MHz, CDCl_3) δ 5.99 (s, 2H), 4.14 (m, 3H), 2.52 (m, 2H), 2.04 (m, 2H), 1.62 (m, 2H), and 1.16 (m, 3H); ^13C NMR (100 MHz, CDCl_3) δ 80.0, 77.1, 76.0, 75.5, 68.0, 65.1, 44.3, 43.0, 30.7, 30.5, 30.4, 31.6, and 23.0; ESI-TOF-MS m/z calcd for C_{9}H_{16}O_{4}H^+: 189.11; found 189.06.

198: 1-Furan-2-yl-butan-2-ol 198. Furan 182 (50.0 mL, 687.4 mmol) was added to THF (400 mL) and cooled to 0 ºC. nBuLi in hexanes (2.37 M, 320.0 mL, 756.2 mmol) was added dropwise and the solution was stirred at 0 ºC for 1 hour. 1,2-epoxybutane (66.0 mL, 756.2 mmol) was added dropwise into solution and the solution was allowed to warm to room temperature as it was stirred overnight. 0.1 M HCl/H_2O (200 mL) was added and the solution was stirred for 10 minutes. The solution was extracted with ethyl acetate (3 × 200 mL) and the organic layers collected, dried (Na_2SO_4), and evaporated to a residue. The product was purified by distillation under vacuum to give a clear oil 198 (49.57 g, 51.4%). ^1H NMR (400 MHz, CDCl_3) δ 7.28 (s, 1H), 6.25 (s, 1H), 6.05 (s, 1H), 3.75 (s, 1H), 2.73 (m, 2H), 2.36 (s, 1H), 1.46 (s, 2H), and 0.93 (s, 3H); ^13C NMR (100 MHz, CDCl_3) δ 152.9, 141.2, 110.1, 106.7, 71.5, 35.5, 29.3, and 9.7; IR (ATR) 3569, 3438, 3421, 3353, 3084, 2968, 2935, 2879, 1739, 1597, 1507, 1461, 1378, 1281, 1218, 1171, 1146, 1113, 1079, 1008, and 976 cm⁻¹
199: *Acetic acid 1-furan-2-ylmethyl-propyl ester* 199. 198 (30.0 g, 214.0 mmol) was added to THF (44 mL) followed by pyridine (88.0 mL, 1070.1 mmol). Acetic anhydride (61.0 mL, 642.0 mmol) was added and the solution was stirred at room temperature for 7 hours. Acetic anhydride (20.0 mL) was added followed by pyridine (20.0 mL) and the solution was stirred overnight to complete the reaction. Ethyl acetate (100 mL) was added to the solution which was then washed with saturated copper sulfate solution (2 × 100 mL) and then with saturated ammonium chloride solution (2 × 100 mL). The organic phase was then washed with water (100 mL), dried (MgSO$_4$), and evaporated to a residue. The product was purified by distillation (92°C, 9.6 mmHg) to give a clear liquid 199 (34.62 g, 88.8%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.24 (m, 1H), 6.21 (m, 1H), 5.99 (m, 1H), 4.98 (m, 1H), 2.81 (d, $J$ = 6.1 Hz, 2H), 1.95 (s, 3H), 1.54 (m, 2H), and 0.86 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.3, 151.5, 141.2, 110.0, 106.7, 73.4, 32.1, 26.3, 20.8, and 9.3; IR (ATR) 2971, 2937, 2883, 1735, 1596, 1507, 1458, 1373, 1236, 1148, 1079, 1011, 966, and 737 cm$^{-1}$.

200: *2-[5-(2-Hydroxy-butyl)-furan-2-yl]-propionic acid* 200. 199 (15.0 mL, 82.3 mmol) was added to DMSO (200 mL) followed by Fe$_2$(SO$_4$)$_3$·H$_2$O (33.0 g, 82.3 mmol) and DL-ethyl-2-iodopropionate (20.0 g, 82.3 mmol). Et$_3$B in THF (1.0 M, 82.5 mL, 82.3
mmol) was added and the solution was stirred for 45 minutes at room temperature while air was bubbled through the solution. After 45 minutes, Et$_3$B in THF (1.0 M, 82.5 mL, 82.3 mmol) was added and the solution was stirred for an additional 2 hours. Brine solution (200 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) which was then added to 2.5 M LiOH/H$_2$O/MeOH/THF (100 mL) and the solution stirred at room temperature overnight. 1.0 M HCl/H$_2$O was added until the solution had a pH of 2. The solution was then extracted with ethyl acetate (5 × 100 mL). The organic phases were collected, dried (Na$_2$SO$_4$), and evaporated to a residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give 200 (0.882 g, 5% over two steps). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.80 (s, 2H), 6.07 (d, $J = 3.2$ Hz, 1H), 6.01 (d, $J = 3.1$ Hz, 1H), 3.77 (m, 2H), 2.77 (m, 1H), 2.66 (m, 1H), 1.48 (d, $J = 7.2$ Hz, 4H), 1.19 (m, 1H), and 0.92 (t, $J = 7.3$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 177.4, 152.1, 151.6, 107.6, 106.8, 71.84, 71.81, 39.1, 35.4, 35.3, 29.15, 29.13, 15.4, 15.3, and 9.74; ESI-TOF-MS m/z calcd for C$_{11}$H$_{16}$O$_4$Na$: 235.09; found 235.03.

![203: 1-(4-Isopropyl-phenyl)-but-3-en-1-ol](image)

203: 1-(4-Isopropyl-phenyl)-but-3-en-1-ol 203. Allyl magnesium bromide in Et$_2$O (1.0 M, 40.5 mL, 40.5 mmol) was chilled to 0 ºC and 4-isopropylbenzaldehyde (5.11 mL, 33.8 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted
with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (\(\text{Na}_2\text{SO}_4\)) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 203. \(R_f = 0.65\) (EtOAc-hexanes (1:3)); \(^1\text{H} \text{NMR (400 MHz, CDCl}_3\)) \(\delta\) 7.24 (m, 4H), 5.81 (m, 1H), 5.12 (m, 2H), 4.68 (s, 1H), 2.91 (m, 1H), 2.50 (s, 2H), 2.25 (s, 1H), and 1.27 (m, 6H); \(^{13}\text{C} \text{NMR (100 MHz, CDCl}_3\)) \(\delta\) 148.4, 141.6, 135.0, 126.7, 126.1, 118.3, 73.5, 43.9, 34.1, and 24.3; IR (ATR) 3385, 3077, 2960, 2933, 2903, 2871, 1726, 1641, 1615, 1512, 1461, 1417, 1246, 1048, 999, 913, and 831 cm\(^{-1}\)

\[
\text{\begin{tikzpicture}
  \node at (0,0) {\text{O}};
  \node at (0.5,0) {\text{H}};
  \draw (-0.5,0) -- (0.5,0);
  \draw (0,-0.5) -- (0,0.5);
  \node at (1.5,0) {\text{MgBr}};
  \node at (2,0) {\text{Et}_2\text{O, 0 }^\circ\text{C, 30 min}};
  \draw (1,0) -- (2,0);
  \node at (2.5,0) {\text{O}};
  \node at (3,0) {\text{H}};
  \draw (2.5,0) -- (3,0);
  \draw (2.5,-0.5) -- (3,0);
  \draw (3,-0.5) -- (3,0);
  \draw (3,-0.5) -- (3,-1);
\end{tikzpicture}}
\]

204: \(1\text{-p-Tolyl-but-3-en-1-ol 204}\). Allyl magnesium bromide in Et\(_2\)O (1.0 M, 50.0 mL, 50.0 mmol) was chilled to 0 °C and p-tolualdehyde (97%, 4.93 mL, 41.6 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (\(\text{Na}_2\text{SO}_4\)) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 204. \(R_f = 0.54\) (EtOAc-hexanes (1:3)); \(^{13}\text{C} \text{NMR (100 MHz, CDCl}_3\)) \(\delta\) 140.9, 137.0, 134.6, 128.9, 125.7, 117.9, 73.1, 60.3, and 43.7; IR (ATR) 3369, 3076, 3007, 2978, 2923, 1903, 1641, 1514, 1432, 1306, 1247, 1179, 1108, 1045, 998, 914, 872, and 816 cm\(^{-1}\)
205: 1-(4-Bromo-phenyl)-but-3-en-1-ol 205. Allyl magnesium bromide in Et₂O (1.0 M, 32.43 mL, 32.4 mmol) was chilled to 0 °C and 4-bromobenzaldehyde (5.0 g, 27.0 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 205. Rₚ = 0.47 (EtOAc-hexanes (1:3)); $^{13}$C NMR (100 MHz, CDCl₃) δ 142.9, 133.9, 131.2, 127.5, 121.0, 118.4, 72.5, and 43.6; IR (ATR) 3428, 3077, 2980, 2934, 2904, 1724, 1641, 1593, 1488, 1403, 1374, 1244, 1098, 1045, 1010, 917, 871 and 824 cm⁻¹

206: 1-(3-Benzylxy-phenyl)-but-3-en-1-ol 206. Allyl magnesium bromide in Et₂O (1.0 M, 28.3 mL, 28.3 mmol) was chilled to 0 °C and 3-benzylxybenzaldehyde (5.0 g, 23.6 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 206. Rₚ = 0.47 (EtOAc-hexanes (1:3)); $^{13}$C NMR (100 MHz, CDCl₃) δ
159.1, 146.0, 137.2, 134.7, 129.7, 128.2, 127.8, 118.7, 118.5, 114.1, 112.6, 73.4, 70.2, and 44.0; IR (ATR) 3429, 3068, 3032, 2979, 2904, 1736, 1641, 1600, 1585, 1487, 1448, 1376, 1317, 1248, 1154, 1041, 1027, 994, 916, and 873 cm$^{-1}$

![Chemical structure](image)

**207:** 1-(4-Diethylamino-phenyl)-but-3-en-1-ol **207.** Allyl magnesium bromide in Et$_2$O (1.0 M, 33.9 mL, 33.9 mmol) was chilled to 0 °C and 4-(diethyl-amino)-benzaldehyde (99%, 5.0 g, 28.2 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **207.** $R_f = 0.46$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.19 (m, 2H), 6.66 (m, 2H), 5.83 (m, 1H), 5.13 (m, 2H), 4.60 (m, 1H), 3.35 (m, 4H), 2.51 (m, 2H), 2.14 (s, 1H), and 1.16 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 147.2, 135.1, 130.5, 127.0, 117.4, 111.5, 73.2, 44.2, 43.3, and 12.4; IR (ATR) 3414, 3075, 2973, 2932, 2899, 1739, 1640, 1613, 1567, 1520, 1467, 1448, 1397, 1374, 1355, 1265, 1196, 1153, 1045, 1011, 912, and 814 cm$^{-1}$

![Chemical structure](image)

**210:** 1-p-Tolyl-prop-2-en-1-ol **210.** Vinyl magnesium bromide in THF (1.0 M, 49.9 mL, 50.0 mmol) was chilled to 0 °C and p-tolualdehyde (97%, 5.0 g, 41.6 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution
(50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 210 (6.1 g, 98.9%). $R_f = 0.57$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.20 (m, 4H), 6.02 (m, 1H), 5.31 (m, 1H), 5.13 (m, 2H), 2.34 (s, 3H), and 2.02 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 140.3, 139.7, 137.2, 129.0, 126.2, 114.6, 74.9, and 21.0; IR (ATR) 3367, 3078, 3020, 2981, 2922, 2870, 1907, 1724, 1641, 1513, 1422, 1375, 1247, 1196, 1178, 1107, 1041, 1020, 988, 923, 848, 815, and 773 cm$^{-1}$

\[
\begin{align*}
\text{MgBr} & \rightarrow \text{HO} \\
\text{THF, 0 °C, 12 h} & \\
\end{align*}
\]

211: 1-(4-Isopropyl-phenyl)-prop-2-en-1-ol 211. Vinyl magnesium bromide in THF (1.0 M, 40.5 mL, 40.5 mmol) was chilled to 0 °C and 4-isopropylbenzaldehyde (5.0 g, 33.8 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with of brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 211 (5.31 g, 89.3%). $R_f = 0.55$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.25 (m, 4H), 6.04 (m, 1H), 5.34 (m, 1H), 5.17 (m, 2H), 2.90 (m, 1H), 2.04 (s, 1H), and 1.24 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 148.5, 140.2, 140.0, 126.6, 126.3, 114.8, 75.1, 33.8, and 24.0; IR (ATR) 3349, 3082, 3015, 2960,
2929, 2870, 1641, 1512, 1461, 1420, 1383, 1363, 1292, 1248, 1200, 1110, 1055, 1017, 988, 922, and 827 cm$^{-1}$

**212: 1-(4-Diethylamino-phenyl)-prop-2-en-1-ol 212.** Vinyl magnesium bromide in THF (1.0 M, 33.9 mL, 33.9 mmol) was chilled to 0 °C and 4-(diethyl-amino)-benzaldehyde (99%, 5.0 g, 28.2 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 212 (3.96 g, 68.4%). $R_f = 0.39$ (EtOAc-hexanes 1:3); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 (m, 2H), 6.66 (m, 2H), 6.06 (m, 1H), 5.33 (m, 1H), 5.11 (m, 2H), 3.34 (m, 4H), 2.03 (s, 1H), and 1.16 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 147.7, 140.9, 129.7, 128.0, 114.2, 112.0, 75.2, 44.6, and 12.8; IR (ATR) 3384, 3076, 2971, 2931, 2871, 1611, 1567, 1519, 1466, 1449, 1397, 1374, 1355, 1264, 1187, 1153, 1095, 1077, 988, 919, and 811 cm$^{-1}$

**213: 1-(2,3-Dimethoxy-phenyl)-prop-2-en-1-ol 213.** Vinyl magnesium bromide in THF (1.0 M, 36.11 mL, 36.1 mmol) was chilled to 0 °C and 2,3-dimethoxybenzaldehyde (5.0 g, 30.1 mmol) was added. The solution was stirred for 12 hours. Saturated
ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 213 (2.2 g, 37.6%). R_f = 0.32 (EtOAc-hexanes (1:3)); ^1H NMR (400 MHz, CDCl₃) δ 7.04 (t, J = 8.0 Hz, 1H), 6.91 (d, J = 7.8 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 6.08 (m, 1H), 5.42 (m, 1H), 5.31 (m, 1H), 5.16 (m, 1H), 3.85 (s, 6H), and 2.82 (s, 1H); ^13C NMR (100 MHz, CDCl₃) δ 152.5, 146.4, 140.2, 136.2, 124.2, 119.2, 114.4, 111.8, 71.1, 60.9, and 55.7; IR (ATR) 3427, 3083, 2939, 2905, 2835, 1737, 1640, 1586, 1479, 1430, 1264, 1219, 1170, 1065, 1003, 923, 872, 809, 787, and 748 cm⁻¹.

214: 1-(3-Benzylxy-phenyl)-prop-2-en-1-ol 214. Vinyl magnesium bromide in THF (1.0 M, 28.27 mL, 28.3 mmol) was chilled to 0 ºC and 3-benzyloxybenzaldehyde (5.0 g, 23.6 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:7) to give 214 (4.84 g, 85.5%). R_f = 0.37 (EtOAc-hexanes (1:3)); ^1H NMR (400 MHz, CDCl₃) δ 7.34 (m, 5H), 7.03 (m, 1H), 6.96 (m, 1H), 6.89 (m, 1H), 6.03 (m, 1H), 5.35 (m, 1H), 5.19 (m, 1H), 5.16 (m, 1H), 5.06 (m, 3H), and 2.00 (s, 1H); ^13C
NMR (100 MHz, CDCl$_3$) δ 159.0, 144.3, 140.0, 136.9, 129.6, 128.6, 128.0, 127.5, 118.8, 115.2, 114.1, 112.7, 75.2, and 70.0; IR (ATR) 3386, 3065, 3032, 2978, 2871, 1737, 1598, 1584, 1486, 1446, 1380, 1317, 1257, 1153, 1026, 991, 923, 878, 773, 735, and 696 cm$^{-1}$. 

**215:** 1-(4-Bromo-phenyl)-prop-2-en-1-ol 215. Vinyl magnesium bromide in THF (1.0 M, 32.5 mL, 32.4 mmol) was chilled to 0 ºC and 4-bromobenzaldehyde (5.0 g, 27.0 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 215 (2.5 g, 43.4%). $R_f = 0.41$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.43 (d, $J = 8.3$ Hz, 2H), 7.19 (d, $J = 8.4$ Hz, 2H), 5.94 (m, 1H), 5.27 (m, 1H), 5.15 (m, 1H), 5.07 (m, 1H), and 3.11 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 141.6, 139.8, 131.4, 128.0, 121.3, 115.4, and 74.4; IR (ATR) 3346, 3081, 2978, 2871, 1907, 1642, 1592, 1486, 1402, 1292, 1248, 1192, 1100, 1071, 1040, 1010, 988, 926, 843, and 816 cm$^{-1}$. 

**216:** 1-(3-Phenoxy-phenyl)-prop-2-en-1-ol 216. Vinyl magnesium bromide in THF (1.0 M, 30.3 mL, 30.3 mmol) was chilled to 0 ºC and 3-phenoxybenzaldehyde (5.0 g, 25.2
mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 216 (4.75 g, 83.2%). Rᵢ = 0.42 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.31 (m, 3H), 7.02 (m, 6H), 6.01 (m, 1H), 5.31 (m, 1H), 5.17 (m, 1H), 5.15 (m, 1H), and 2.72 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 157.3, 157.0, 144.8, 140.0, 129.6, 123.2, 121.0, 118.8, 117.8, 116.7, 115.2, and 74.8; IR (ATR) 3386, 3075, 2980, 2878, 1712, 1583, 1484, 1443, 1375, 1242, 1211, 1163, 1141, 1072, 1043, 1023, 990, 926, 821, 751, 700, and 692 cm⁻¹

217: 1-(4-Ethoxy-phenyl)-prop-2-en-1-ol 217. Vinyl magnesium bromide in THF (1.0 M, 39.9 mL, 39.9 mmol) was chilled to 0 °C and 4-ethoxybenzaldehyde (5.0 g, 33.3 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 217 (5.61 g, 94.5%). Rᵢ = 0.37 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.24 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 6.01 (m, 1H), 5.29 (m, 1H), 5.14 (m, 1H), 5.10 (m, 1H), 3.99 (q, J = 7.0 Hz, 2H), 2.47 (s, 1H), and 1.38
(t, J = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.4, 140.4, 134.7, 127.6, 114.5, 114.3, 74.7, 63.3, and 14.7; IR (ATR) 3388, 3077, 2980, 2930, 2879, 1893, 1725, 1641, 1611, 1585, 1510, 1478, 1393, 1302, 1241, 1173, 1115, 1045, 989, 921, and 826 cm$^{-1}$

218: 1-(4-Ethyl-phenyl)-prop-2-en-1-ol 218. Vinyl magnesium bromide in THF (1.0 M, 44.7 mL, 44.7 mmol) was chilled to 0 ºC and 4-ethylbenzaldehyde (5.0 g, 37.3 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 $\times$ 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 218 (5.53 g, 91.4%). $R_f$ = 0.50 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.29 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H), 6.05 (m, 1H), 5.34 (m, 1H), 5.19 (m, 1H), 5.16 (m, 1H), 2.67 (q, J = 7.6 Hz, 2H), 2.33 (s, 1H), and 1.26 (t, J = 7.6 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 143.7, 140.3, 139.9, 127.9, 126.3, 114.7, 75.0, 28.5, and 15.5; IR (ATR) 3369, 3083, 3014, 2965, 2931, 2873, 1908, 1641, 1614, 1512, 1455, 1418, 1282, 1249, 1197, 1178, 1110, 1018, 988, 923, and 826 cm$^{-1}$

219: 1-Naphthalen-2-yl-prop-2-en-1-ol 219. Vinyl magnesium bromide in THF (1.0 M, 38.5 mL, 38.4 mmol) was chilled to 0 ºC and 2-naphthaldehyde (3.0 g, 19.2 mmol)
was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexamnes (1:3) to give 219 (2.84 g, 82.2%). R_f = 0.42 (EtOAc-hexamnes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.82 (m, 4H), 7.48 (m, 3H), 6.11 (m, 1H), 5.38 (m, 1H), 5.31 (m, 1H), 5.22 (m, 1H) and 3.18 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 140.2, 140.0, 133.2, 132.8, 128.1, 127.9, 127.5, 126.0, 125.7, 124.8, 124.5, 115.0, and 75.1; IR (ATR) 3369, 3056, 3017, 2978, 2877, 1709, 1639, 1601, 1508, 1361, 1270, 1203, 1164, 1125, 1039, 988, 926, 856, 819, 780, 762, and 744 cm⁻¹

220: 2,6-Di-tert-butyl-4-(1-hydroxy-allyl)-phenol 220. Vinyl magnesium bromide in THF (1.0 M, 47.0 mL, 46.9 mmol) was chilled to 0 ºC and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (5.0 g, 21.4 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexamnes (1:4) to give 220 (3.5 g, 62.5%). R_f = 0.55 (EtOAc-hexamnes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.18 (s, 2H), 6.09 (m, 1H), 5.37 (m, 1H), 5.22 (s, 1H), 5.19 (m, 1H), 5.12 (m, 1H), 2.07 (s, 1H), and 1.45 (s,
18H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 153.5, 140.4, 135.9, 133.2, 123.2, 114.4, 75.7, 34.4, and 30.2; IR (ATR) 3636, 3542, 3430, 3076, 2956, 2910, 2871, 1643, 1592, 1433, 1391, 1362, 1317, 1234, 1205, 1155, 1119, 1032, 993, 927, and 885 cm$^{-1}$.

222: 1-(4-Ethyl-phenyl)-prop-2-en-1-ol 222. 218 (3.5 g, 21.5 mmol) was added to CH$_2$Cl$_2$ (5.4 mL), followed by 13 x 2µ sieves (1.2 g). Diethyl (-)-tartrate (0.67 g, 3.2 mmol) and Ti(iPrO)$_4$ (0.61 g, 2.2 mmol) were added and the solution was cooled to -20 °C. After 30 minutes, t-BuOOH in DCM (4.408 M, 2.92 mL, 12.9 mmol) was added and the reaction was stirred and allowed to warm to room temperature for 24 hours. Ferrous sulfate (9.0 g) was then added followed by d-(-)-tartaric acid (3.0 g) and water (100 mL). The solution was stirred for 30 minutes. The solution was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 222 (1.31 g, 37.4%). $R_f = 0.43$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.29 (d, $J = 8.2$ Hz, 2H), 7.20 (d, $J = 8.2$ Hz, 2H), 6.05 (m, 1H), 5.34 (m, 1H), 5.18 (m, 1H), 5.15 (m, 1H), 2.67 (q, $J = 7.6$ Hz, 2H), and 1.26 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 143.6, 140.3, 139.9, 127.9, 126.3, 114.6, 75.0, 28.4, and 15.5; IR (ATR) 3368, 3083, 3014, 2965, 2932, 2873, 1640, 1614, 1512, 1456, 1418, 1375, 1282, 1249, 1197, 1179, 1110, 1018, 988, 923, and 826 cm$^{-1}$. 

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223: 1-(2,3-Dimethoxy-phenyl)-prop-2-en-1-ol 223. 213 (1.0 g, 5.2 mmol) was added to CH₂Cl₂ (20.6 mL), followed by 13 x 2µ sieves (1.0 g). Diethyl (-)-tartrate (0.16 g, 0.8 mmol) and Ti(iPrO)₄ (0.15 g, 0.5 mmol) were added and the solution was cooled to -20 °C. After 30 minutes, t-BuOOH in DCM (4.408 M, 0.7 mL, 3.1 mmol) was added and the reaction was stirred and allowed to warm to room temperature for 24 hours. Ferrous sulfate (3.0 g) was then added followed by d-(-)-tartaric acid (1.0 g) and water (100 mL). The solution stirred for 30 minutes. The solution was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 223 (0.245 g, 24.5%). R₇ = 0.27 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.02 (t, J = 8.0 Hz, 1H), 6.91 (m, 1H), 6.83 (m, 1H), 6.07 (m, 1H), 5.43 (m, 1H), 5.30 (m, 1H), 5.15 (m, 1H), 3.83 (s, 6H), and 2.96 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 152.4, 146.2, 140.1, 136.2, 124.1, 119.1, 114.3, 111.7, 70.8, 60.8, and 55.6; IR (ATR) 3428, 3078, 2936, 2836, 1640, 1586, 1479, 1430, 1263, 1219, 1169, 1065, 1003, 922, 871, 809, 787, and 748 cm⁻¹

224: 1-(4-Isopropyl-phenyl)-prop-2-en-1-ol 224. 211 (3.0 g, 17.0 mmol) was added to CH₂Cl₂ (50 mL), followed by 13 x 2 µ sieves (3.0 g). Diethyl (-)-tartrate (0.53 g, 2.6 mmol) and Ti(iPrO)₄ (0.5 g, 1.7 mmol) were added and the solution was cooled to -20
°C. After 30 minutes, t-BuOOH in DCM (4.408 M, 2.3 mL, 10.2 mmol) was added and the reaction was stirred for 24 hours and allowed to warm to room temperature. Ferrous sulfate (9.0 g) was then added followed by d-(-)-tartaric acid (3.0 g) and water (100 mL). The solution stirred for 30 minutes. The solution was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried (Na2SO4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give **224** (0.855 g, 28.5%). Rf = 0.50 (EtOAc-hexanes 1:3); ¹H NMR (400 MHz, CDCl3) δ 7.31 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 6.05 (m, 1H); 5.35 (m, 1H), 5.19 (m, 1H), 5.15 (m, 1H), 2.93 (septet, J = 6.9 Hz, 1H), 2.66 (s, 1H), and 1.28 (d, J = 7.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl3) δ 148.2, 140.3, 140.0, 126.5, 126.3, 114.6, 75.0, 33.7, and 23.9; IR (ATR) 3369, 3082, 3014, 2960, 2928, 2870, 1641, 1512, 1460, 1419, 1383, 1363, 1292, 1201, 1180, 1109, 1055, 1017, 988, 922, and 827 cm⁻¹.

**225:** 1-(3-Benzylxoy-phenyl)-prop-2-en-1-ol **225**. **214** (1.84 g, 7.7 mmol) was added to CH₂Cl₂ (30 mL), followed by 13 x 2 μ sieves (2.0 g). Diethyl (−)-tartrate (0.20 mL, 1.2 mmol) and Ti(iPrO)₄ (0.23 mL, 0.8 mmol) were added and the solution was cooled to -20 °C. After 30 minutes, t-BuOOH in DCM (4.408 M, 1.04 mL, 4.6 mmol) was added and the reaction was stirred for 24 hours and allowed to warm to room temperature. Ferrous sulfate (6.0 g) was then added followed by d-(−)-tartaric acid (2.0 g) and water (100 mL). The solution stirred for 30 minutes. The solution was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried.
(Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give **225** (0.864 g, 47.0%). $R_f = 0.33$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.36 (m, 5H), 7.06 (m, 1H), 6.98 (m, 1H), 6.04 (m, 1H), 5.35 (m, 1H), 5.19 (m, 1H), 5.14 (m, 1H), 5.06 (m, 3H), and 2.92 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 158.8, 144.4, 140.1, 136.8, 129.4, 128.4, 127.8, 127.4, 118.8, 114.9, 113.8, 112.6, 74.9, and 69.8; IR (ATR) 3406, 3065, 3032, 2978, 2871, 1734, 1598, 1584, 1486, 1446, 1380, 1317, 1257, 1153, 1122, 1081, 992, 881, 774, 735, and 696 cm$^{-1}$

**226**: *1-(3-Phenoxy-phenyl)-prop-2-en-1-ol* **226**. **216** (3.0 g, 13.3 mmol) was added to CH$_2$Cl$_2$ (50 mL), followed by 13 x 2 $\mu$ sieves (3.0 g). Diethyl (–)-tartrate (0.41 g, 2.0 mmol) and Ti(iPrO)$_4$ (0.38 g, 1.3 mmol) were added and the solution was cooled to -20 ºC. After 30 minutes, t-BuOOH in DCM (4.408 M, 1.8 mL, 8.0 mmol) was added and the reaction stirred for 24 hours and allowed to warm to room temperature. Ferrous sulfate (9.0 g) was then added followed by d-(-)-tartaric acid (3.0 g) and water (100 mL). The solution was stirred for 30 minutes. The solution was extracted with ethyl acetate (3 x 10 mL) and the organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **226** (0.859 g, 28.6%). $R_f = 0.40$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.33 (m, 3H), 7.06 (m, 5H), 6.92 (m, 1H), 6.01 (m, 1H), 5.19 (m, 1H), 5.14 (m, 1H), and 2.47 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.3, 157.0, 144.7, 139.8, 129.74, 129.67, 123.2, 121.0, 118.8,
117.8, 116.7, 115.3, and 74.9; IR (ATR) 3348, 3069, 2876, 1864, 1583, 1484, 1443, 1242, 1211, 1163, 1140, 1072, 1023, 989, 926, 821, 780, 751, and 691 cm\(^{-1}\)

228: \textit{1-Methyl-4-(1-prop-2-ynyloxy-allyl)-benzene 228}. Sodium hydride in mineral oil (60\%, 0.272 g, 6.8 mmol) was added to dry THF (10 mL) followed by 210 (0.503 g, 3.4 mmol). HMPA (1.2 mL, 6.8 mmol) was added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80\%, 0.6 mL, 6.8 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 x 20 mL). The organic layer was separated, dried (Na\(_2\)SO\(_4\)), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 228 (0.351 g, 55.5\%). \(R_f = 0.77\) (EtOAc-hexanes (1:9)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.30 (d, \(J = 7.7\) Hz, 2H), 7.22 (d, \(J = 7.4\) Hz, 2H), 6.00 (m, 1H), 5.32 (m, 2H), 5.06 (d, \(J = 6.4\) Hz, 1H), 4.16 (m, 2H), 2.47 (m, 1H), and 2.40 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 137.9, 137.5, 136.8, 129.1, 127.0, 116.8, 80.9, 79.7, 74.2, 55.0, and 21.0; IR (ATR) 3292, 3023, 2921, 2856, 1738, 1642, 1612, 1513, 1442, 1414, 1365, 1304, 1259, 1217, 1180, 1108, 1068, 1021, 991, 926, 849, and 815 cm\(^{-1}\)

229: \textit{1,2-Dimethoxy-3-(1-prop-2-ynyloxy-allyl)-benzene 229}. Sodium hydride in mineral oil (60\%, 0.045 g, 1.1 mmol) was added to dry THF (10 mL) followed by 213 (0.110 g, 0.6 mmol). HMPA (0.2 mL, 1.1 mmol) was then added to the solution which was
stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.1 mL, 1.1 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na$_2$SO$_4$), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 229 (30.2 mg, 22.9%). $R_f = 0.34$ (EtOAc-hexanes (1:9)); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.07 (t, $J = 7.9$ Hz, 1H), 6.98 (d, $J = 7.9$ Hz, 1H), 6.85 (d, $J = 8.1$ Hz, 1H), 5.97 (m, 1H), 5.48 (d, $J = 6.5$ Hz, 1H), 5.30 (d, $J = 17.1$ Hz, 1H), 5.21 (d, $J = 10.3$ Hz, 1H), 4.12 (m, 2H), 3.85 (m, 6H), and 2.41 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 152.6, 146.9, 137.6, 133.8, 124.3, 119.2, 116.8, 111.7, 79.9, 75.2, 74.2, 60.9, 55.7, and 55.4; IR (ATR) 3287, 2970, 2918, 2849, 1738, 1587, 1480, 1431, 1365, 1264, 1219, 1170, 1060, 1005, and 927 cm$^{-1}$

230: $1$-Phenoxy-$3$-($1$-prop-$2$-ynyloxy-allyl)-benzene 230. Sodium hydride in mineral oil (60%, 0.177 g, 4.5 mmol) was added to dry THF (10 mL) followed by 216 (0.502 g, 2.2 mmol). HMPA (0.8 mL, 4.5 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.4 mL, 4.5 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na$_2$SO$_4$), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 230 (0.301 g, 51.4%). $R_f = 0.60$ (EtOAc-hexanes
(1:9)); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 7.38 (m, 2H), 7.17 (m, 2H), 7.08 (m, 2H), 6.99 (m, 1H), 5.98 (m, 1H), 5.36 (m, 2H), 5.09 (d, \( J = 6.9 \) Hz, 1H), 4.22 (m, 2H), and 2.49 (m, 1H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \( \delta \) 157.2, 156.9, 142.0, 137.3, 129.6, 129.7, 123.1, 121.7, 118.7, 118.0, 117.5, 117.4, 80.6, 79.5, 74.5, and 55.1; IR (ATR) 3293, 3065, 2856, 1738, 1583, 1484, 1443, 1244, 1211, 1163, 1067, 1023, 990, 930, 884, 835, 782, 753, and 692 cm\textsuperscript{-1}

\[ \begin{array}{c}
\text{Br} & \text{OH} \\
\text{1. NaH, HMPA, THF, r.t., 1 h} & \text{2. Propargyl bromide, r.t., 1 h}
\end{array} \]

231: \textit{1-Bromo-4-(1-prop-2-ynyloxy-allyl)-benzene 231}. Sodium hydride in mineral oil (60%, 0.158 g, 4.0 mmol) was added to dry THF (10 mL) followed by 215 (0.422 g, 2.0 mmol). HMPA (0.7 mL, 4.0 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.4 mL, 4.0 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 \( \times \) 20 mL). The organic layer was separated, dried (Na\textsubscript{2}SO\textsubscript{4}), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 231 (0.23 g, 46.3%). \( R_f = 0.71 \) (EtOAc-hexanes (1:9)); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 7.48 (d, \( J = 8.5 \) Hz, 2H), 7.24 (d, \( J = 8.3 \) Hz, 2H), 5.88 (m, 1H), 5.30 (m, 2H), 5.00 (d, \( J = 6.9 \) Hz, 1H), 4.14 (m, 2H), and 2.46 (m, 1H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \( \delta \) 139.0, 137.2, 131.5, 128.7, 121.7, 117.8, 80.3, 79.4, 74.6, and 55.2; IR (ATR) 3297, 3082, 2982, 2855, 1726, 1591, 1486, 1404, 1264, 1069, 1011, 990, 929, and 817 cm\textsuperscript{-1}
232: 1-Ethyl-4-(1-prop-2-ynyloxy-allyl)benzene 232. Sodium hydride in mineral oil (60%, 0.224 g, 5.6 mmol) was added to dry THF (10 mL) followed by 218 (0.454 g, 2.8 mmol). HMPA (1.0 mL, 5.6 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.5 mL, 5.6 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na2SO4), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 232 (0.447 g, 79.8%). Rf = 0.77 (EtOAc-hexanes (1:9)); 1H NMR (400 MHz, CDCl3) δ 7.36 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 6.025 (m, 1H), 5.34 (m, 2H), 5.09 (d, J = 6.7 Hz, 1H), 4.19 (m, 2H), 2.71 (q, J = 7.6, 2H), 2.485 (m, 1H), and 1.31 (t, J = 7.6 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 143.7, 137.8, 137.0, 127.8, 127.0, 116.7, 80.8, 79.7, 74.2, 54.9, 28.4, and 15.4; IR (ATR) 3292, 3012, 2966, 2933, 2873, 1727, 1669, 1612, 1513, 1442, 1417, 1360, 1266, 1068, 1020, 990, and 827 cm⁻¹

233: 2-(1-Prop-2-ynyloxy-allyl)naphthalene 233. Sodium hydride in mineral oil (60%, 0.217 g, 5.4 mmol) was added to dry THF (10 mL) followed by 219 (0.5 g, 2.7 mmol). HMPA (1.0 mL, 5.4 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.5 mL, 5.4
mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 23 (0.307 g, 50.9%). Rf = 0.63 (EtOAc-hexanes (1:9)); ¹H NMR (400 MHz, CDCl₃) δ 7.95 (m, 4H), 7.60 (m, 3H), 6.18 (m, 1H), 5.48 (m, 2H), 5.34 (d, J = 6.6 Hz, 1H), 4.31 (m, 2H), and 2.61 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 137.6, 137.1, 133.0, 132.9, 128.2, 127.8, 127.5, 126.0, 125.8, 124.7, 117.2, 81.0, 79.7, 77.2, 74.5, and 55.1; IR (ATR) 3294, 3056, 3017, 2983, 2854, 1738, 1601, 1509, 1359, 1067, 991, 928, 857, 820, and 746 cm⁻¹.

23: 1-Ethoxy-4-(1-prop-2-ynyloxy-allyl)-benzene 23. Sodium hydride in mineral oil (60%, 0.225 g, 5.6 mmol) was added to dry THF (10 mL) followed by 21 (0.501 g, 2.8 mmol). HMPA (1.0 mL, 5.6 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.5 mL, 5.6 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na₂SO₄), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 23 (0.256 g, 42.1%). Rf = 0.71 (EtOAc-hexanes (1:9)); ¹H NMR (400 MHz, CDCl₃) δ 7.28 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.965 (m, 1H), 5.27 (m, 2H), 4.99 (d, J = 6.6 Hz, 1H), 4.12 (m, 2H), 4.01 (q, J =
7.0 Hz, 2H), 2.46 (m, 1H), and 1.41 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 158.5, 137.9, 131.6, 128.2, 116.5, 114.2, 80.5, 79.7, 74.2, 63.1, 54.8, and 14.6; IR (ATR) 3289, 2981, 2901, 1738, 1610, 1585, 1511, 1478, 1442, 1393, 1303, 1240, 1174, 1115, 1067, 1047, 991, 923, and 826 cm$^{-1}$

**236:** 1-Benzyloxy-3-(1-prop-2-ynyloxy-but-3-enyl)-benzene 236. Sodium hydride in mineral oil (60%, 0.159 g, 4.0 mmol) was added to dry THF (10 mL) followed by 206 (0.504 g, 2.0 mmol). HMPA (0.7 mL, 4.0 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.4 mL, 4.0 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na$_2$SO$_4$), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 236 (0.339 g, 58.5%). $R_f = 0.68$ (EtOAc-hexanes (1:9)); $^1$H NMR (400 MHz, CDCl$_3$) δ 7.38 (m, 6H), 7.01 (m, 1H), 6.96 (m, 2H), 5.81 (m, 1H), 5.11 (m, 4H), 4.57 (m, 1H), 4.16 (dd, $J = 2.5$, 2.4 Hz, 1H), 3.90 (dd, $J = 2.4$ Hz, 1H), 2.66 (m, 1H), 2.49 (m, 1H), and 2.44 (t, $J = 2.4$ Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 158.9, 142.2, 136.8, 134.3, 129.4, 128.5, 127.9, 127.5, 119.6, 117.0, 114.3, 113.1, 80.2, 79.8, 74.2, 69.8, 55.5, and 42.0; IR (ATR) 3285, 3065, 3032, 2977, 2940, 2906, 2881, 1590, 1445, 1384, 1355, 1320, 1248, 1167, 1158, 1078, 1013, 992, and 924 cm$^{-1}$
237: *Diethyl-[4-(1-prop-2-ynyloxy-but-3-enyl)-phenyl]-amine* 237. Sodium hydride in mineral oil (60%, 0.186 g, 4.7 mmol) was added to dry THF (10 mL) followed by 207 (0.511 g, 2.3 mmol). HMPA (0.8 mL, 4.7 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.4 mL, 4.7 mmol) was added and the solution was stirred overnight. Saturated ammonium chloride solution (20 mL) was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated, dried (Na$_2$SO$_4$), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 237 (0.205 g, 34.2%). $R_f = 0.68$ (EtOAc-hexanes (1:9)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.18 (d, $J = 8.7$ Hz, 2H), 6.68 (d, $J = 8.8$ Hz, 2H), 5.83 (m, 1H), 5.08 (m, 2H), 4.46 (m, 1H), 4.11 (m, 1H), 3.88 (m, 1H), 3.38 (q, $J = 7.1$ Hz, 4H), 2.68 (m, 1H), 2.48 (m, 1H), 2.41 (m, 1H), and 1.19 (t, $J = 7.1$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 147.5, 135.1, 128.2, 126.4, 116.4, 111.3, 80.2, 80.0, 73.7, 54.8, 44.2, 41.8, and 12.5; IR (ATR) 3295, 3075, 2971, 2933, 2898, 1738, 1612, 1520, 1398, 1375, 1355, 1265, 1187, 1154, 1073, 1011, 914, and 814 cm$^{-1}$

238: 1-Bromo-4-(1-prop-2-ynyloxy-but-3-enyl)-benzene 238. Sodium hydride in mineral oil (60%, 0.180 g, 4.5 mmol) was added to dry THF (10 mL) followed by 205 (0.510
g, 2.3 mmol). HMPA (0.8 mL, 4.5 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.4 mL, 4.5 mmol) was added and the solution was stirred overnight. 20 mL of saturated ammonium chloride solution was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated and dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 238 (0.287 g, 48.2%). R₉ = 0.79 (EtOAc-hexanes (1:9)); ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 5.73 (m, 1H), 5.04 (m, 2H), 4.53 (m, 1H), 4.11 (dd, J = 2.4 Hz, 1H), 3.85 (dd, J = 2.4 Hz, 1H), 2.59 (m, 1H), and 2.41 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 139.5, 133.8, 131.5, 128.6, 121.6, 117.4, 79.5, 79.4, 74.4, 55.5, and 41.8; IR (ATR) 3298, 3078, 2978, 2903, 2855, 1738, 1642, 1592, 1485, 1442, 1408, 1344, 1229, 1070, 1010, 917, and 822 cm⁻¹

239: 1-Methyl-4-(1-prop-2-ynyloxy-but-3-enyl)-benzene 239. Sodium hydride in mineral oil (60%, 0.247 g, 6.2 mmol) was added to dry THF (10 mL) followed by 204 (0.500 g, 3.1 mmol). HMPA (1.1 mL, 6.2 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.6 mL, 6.2 mmol) was added and the solution was stirred overnight. 20 mL of saturated ammonium chloride solution was added and the solution was extracted with chloroform (3 × 20 mL). The organic layer was separated and dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel
in EtOAc-hexanes (1:19) to give 239 (72.2 mg, 11.7%). \( R_f = 0.82 \) (EtOAc-hexanes (1:9)); \(^1H\) NMR (400 MHz, CDCl\(_3\)) \( \delta 7.205 \) (m, 4H), 5.78 (m, 1H), 5.06 (m, 2H), 4.53 (m, 1H), 4.11 (dd, \( J = 2.5 \) Hz, 1H), 3.86 (dd, \( J = 2.4 \) Hz, 1H), 2.64 (m, 1H), 2.45 (m, 1H), 2.41 (t, \( J = 2.4 \) Hz, 1H), and 2.36 (s, 3H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \( \delta 137.6, 137.4, 134.6, 129.1, 126.9, 116.9, 80.1, 79.9, 74.0, 55.3, 42.0, \) and 21.1; IR (ATR) 3297, 3077, 3012, 2979, 2923, 2856, 1741, 1641, 1514, 1442, 1347, 1235, 1078, 1020, 996, 916, and 816 cm\(^{-1}\).

203: 1-Naphthyl-1-monothio-2-propyl benzene 203. Sodium hydride in mineral oil (60%, 0.214 g, 5.4 mmol) was added to dry THF (10 mL) followed by 203 (0.510 g, 2.7 mmol). HMPA (1.0 mL, 5.4 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide w/v in toluene (80%, 0.5 mL, 5.4 mmol) was added and the solution was stirred overnight. 20 mL of saturated ammonium chloride solution was added and the solution was extracted with chloroform (3 \( \times \) 20 mL). The organic layer was separated and dried (Na\(_2\)SO\(_4\)) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give 240 (0.359 g, 58.7%). \( R_f = 0.85 \) (EtOAc-hexanes (1:9)); \(^1H\) NMR (400 MHz, CDCl\(_3\)) \( \delta 7.25 \) (m, 4H), 5.82 (m, 1H), 5.08 (m, 2H), 4.56 (m, 1H), 4.12 (dd, \( J = 2.4 \) Hz, 1H), 3.87 (dd, \( J = 2.3 \) Hz, 1H), 2.93 (m, 1H), 2.65 (m, 1H), 2.47 (m, 1H), 2.39 (t, \( J = 2.4 \) Hz, 1H), and 1.28 (d, \( J = 7.0 \) Hz, 6H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)) \( \delta 148.3, 137.8, 134.6, 126.8, 126.3, 116.7, 80.0, 79.8, 74.0, 55.2,
42.0, 33.7, and 23.9; IR (ATR) 3302, 3078, 3014, 2961, 2904, 2870, 1738, 1642, 1510, 1461, 1422, 1364, 1298, 1229, 1078, 1018, 996, 916, and 831 cm\(^{-1}\)

\[
\begin{array}{c}
\text{Py, r.t., 12 h} \\
\text{N-H} \\
\text{O} \\
\text{NH}_2
\end{array}
\]

243: \(N\)-(3-Ethynyl-phenyl)-acetamide 243. \(N\)-Ethynylaniline 241 (2 mL, 19.1 mmol) was added to pyridine (20 mL). Acetic anhydride (5.4 mL, 57.4 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 \times 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 \times 10 mL) and then with saturated ammonium chloride solution (3 \times 10 mL). The organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give 243 (2.78 g, 91.3%). \(R_f = 0.46\) (EtOAc-hexanes (1:1)); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.23\) (s, 1H), 7.64 (s, 1H), 7.48 (m, 1H), 7.20 (m, 2H), 3.03 (s, 1H), and 2.13 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 169.1, 138.0, 128.9, 127.9, 123.5, 122.6, 120.6, 83.1, 77.4,\) and 24.3; IR (ATR) 3301, 3203, 1666, 1605, 1584, 1554, 1482, 1425, 1402, 1371, 1308, 1210, 1256, 1156, 1017, 880, and 786 cm\(^{-1}\); ESI-TOF-MS m/z calcd for C\(_{10}\)H\(_9\)NOH\(^+\): 160.08; found 160.05.

\[
\begin{array}{c}
\text{Py, r.t., 12 h} \\
\text{N-H} \\
\text{O} \\
\text{NH}_2
\end{array}
\]

244: \(N\)-(3-Ethynyl-phenyl)-2,2-dimethyl-propionamide 244. \(N\)-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Trimethylacetyl chloride (99%, 0.3 mL,
2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give **244** (0.149 g, 86.6%). $R_f = 0.78$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.64 (s, 1H), 7.53 (m, 2H), 7.21 (m, 2H), 3.04 (s, 1H), and 1.28 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 176.8, 138.0, 128.7, 127.7, 123.6, 122.5, 120.7, 83.1, 77.3, 39.5, and 27.4; IR (ATR) 3303, 3283, 2968, 2931, 2870, 1656, 1579, 1524, 1472, 1415, 1368, 1293, 866, 789, 692, 651, and 627 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{13}$H$_{15}$NOH$^+$: 202.12; found 201.99.

![Chemical structure of 244](image)

**245**: Pent-4-enoic acid (3-ethynyl-phenyl)-amide **245**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Pentenoyl chloride (98%, 0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give **245** (0.101 g, 59.4%). $R_f = 0.58$ (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$)
δ 7.925 (s, 1H), 7.635 (s, 1H), 7.52 (m, 1H), 7.22 (m, 2H), 5.83 (m, 1H), 5.05 (m, 2H), 3.04 (s, 1H), and 2.44 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 137.9, 136.6, 128.9, 127.9, 123.4, 122.6, 120.6, 115.9, 83.1, 77.4, 36.5, and 29.3; IR (ATR) 3284, 3079, 2978, 2918, 1660, 1602, 1584, 1536, 1482, 1425, 1406, 1367, 1294, 1237, 994, 920, 873, and 791 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₃H₁₃NOH⁺: 200.11; found 199.99.

246: N-(3-Ethynyl-phenyl)-succinamic acid methyl ester 246. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Methyl 4-chloro-4-oxobutyrate (97%, 0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 246 (0.139 g, 70.6%). Rᵣ = 0.19 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 7.64 (s, 1H), 7.52 (m, 1H), 7.22 (m, 2H), 3.71 (s, 3H), 3.05 (s, 1H), 2.75 (m, 2H), and 2.66 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 173.7, 169.8, 137.8, 128.9, 127.9, 123.1, 122.7, 120.2, 83.1, 77.4, 52.1, 32.0, and 29.1; IR (ATR) 3361, 3282, 3104, 3029, 3004, 2970, 2953, 1738, 1692, 1605, 1550, 1480, 1325, 1171, 884, 805, and 695 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₃H₁₃NO₃H⁺: 232.10; found 231.98.
247: *N-(3-Ethynyl-phenyl)-benzamide* 247. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Benzoyl chloride (0.1 mL, 1.0 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give 247 (0.130 g, 68.8%).

Rₛ = 0.58 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 8.00 (s, 1H), 7.83 (d, J = 7.2 Hz, 2H), 7.75 (s, 1H), 7.67 (m, 1H), 7.52 (m, 1H), 7.44 (m, 2H), 7.27 (m, 2H), and 3.06 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 165.9, 137.9, 134.6, 131.9, 129.0, 128.7, 128.2, 127.0, 123.6, 122.8, 120.8, 83.1, and 77.5; IR (ATR) 3278, 1645, 1612, 1579, 1547, 1424, 1296, 1253, and 798 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₅H₁₁NOH⁺: 222.09; found 221.99.

248: *Octanoic acid (3-ethynyl-phenyl)-amide* 248. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Octanoyl chloride (99%, 0.5 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10
mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 248 (0.197 g, 94.7%). R$_f$ = 0.69 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.20 (s, 1H), 7.66 (s, 1H), 7.54 (m, 1H), 7.20 (m, 2H), 3.02 (s, 1H), 2.33 (m, 2H), 1.65 (m, 2H), 1.25 (m, 8H), and 0.85 (m, 3H); IR (ATR) 3267, 2953, 2924, 2855, 1710, 1645, 1606, 1583, 1535, 1424, 1284, 1105, and 883 cm$^{-1}$; ESI-TOF-MS m/z calcd for C$_{16}$H$_{21}$NOH$^+$: 244.17; found 244.04.

249: N-(3-Ethynyl-phenyl)-propionamide 249. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Propionyl chloride (0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give 249 (0.139 g, 93.9%). R$_f$ = 0.39 (EtOAc-hexanes (1:3)); $^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.66 (s, 1H), 7.52 (m, 1H), 7.20 (m, 2H), 3.04 (s, 1H), 2.36 (q, J = 7.6, 7.508 Hz, 2H), and 1.19 (t, J = 7.6 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.8, 138.0, 128.7, 123.4, 122.5, 120.6, 83.1, 77.3, 30.5, and 9.6; IR (ATR) 3248, 3143, 3082, 2983,
250: (3-Ethynyl-phenyl)-carbamic acid allyl ester 250. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Allyl chloroformate (97%, 0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give 250 (0.129 g, 75.0%). Rf = 0.58 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.53 (s, 1H), 7.42 (m, 1H), 7.22 (m, 2H), 6.95 (s, 1H), 5.94 (m, 1H), 5.30 (m, 2H), 4.66 (m, 2H), and 3.06 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 153.2, 137.8, 132.2, 129.0, 127.1, 122.7, 122.0, 119.2, 118.3, 83.1, 77.4, and 65.9; IR (ATR) 3295, 3087, 2950, 1708, 1606, 1586, 1536, 1487, 1432, 1408, 1216, 1171, 1060, 996, 933, and 788 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₁H₁₁NOH⁺: 174.09; found 174.00.

251: (3-Ethynyl-phenyl)-carbamic acid isobutyl ester 251. 3-Ethynylaniline 241 (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Isobutyl chloroformate (98%, 0.4 mL, 2.6
mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 10 mL). The organic layer was separated and washed with saturated copper sulfate solution (3 × 10 mL) and then with saturated ammonium chloride solution (3 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give 251 (0.179 g, 96.8%). Rᵥ = 0.81 (EtOAc-hexanes (1:3)); ¹H NMR (400 MHz, CDCl₃) δ 7.52 (s, 1H), 7.42 (s, 1H), 7.23 (t, J = 7.9 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 6.87 (s, 1H), 3.95 (d, J = 6.6 Hz, 2H), 3.05 (s, 1H), 1.96 (nonet, J = 13.4, 6.7 Hz, 1H), and 0.95 (d, J = 6.7 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 153.6, 138.0, 128.9, 126.9, 122.7, 122.0, 119.1, 83.2, 77.3, 73.9, 71.4, 27.9, and 19.0; IR (ATR) 3298, 2963, 2875, 1702, 1606, 1586, 1536, 1488, 1471, 1431, 1410, 1285, 1268, 1217, 1172, 1062, 998, 978, 938, 886, 788, 770, 686, and 623 cm⁻¹; ESI-TOF-MS m/z calcd for C₁₃H₁₅NO₂H⁺: 218.12; found 218.02.
11.1 Spectra for (+)-1,4-Regioisomer, 72

72, Racemic 1,4-Regioisomer

Benzoic acid
1-methyl-2-[5-{1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^1$H NMR:
72, Racemic 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^{13}$C NMR:
72, Racemic 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

Infrared Spectrum:
11.2 Spectra for (−)-1,4-Regioisomer, 81

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)ethyl]-tetrahydro-furan-2-yl}ethyl ester

$^1$H NMR:
Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^{13}$C NMR:
81. Minus 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-y1-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

Infrared Spectrum:
81, Minus 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

Mass Spectrum:
11.3 Spectra for (+)-1,4-Regioisomer, 88

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^1$H NMR:
88, Plus 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^{13}$C NMR:
88. Plus 1,4-Regioisomer

Benzoic acid
1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl)-1,2,3triazol-1-yl]-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

Infrared Spectrum:
88. Plus 1,4-Regioisomer

Benzoic acid
1-methyl-2-[5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl]-ethyl ester

Mass Spectrum:
11.4 Spectra for (±)-1,4-Regioisomer, 71

Succinic acid
2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^1$H NMR:
Succinic acid
2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^{13}\text{C NMR}$:
Succinic acid
2-{5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl}-1-methyl-ethyl ester methyl ester

Infrared Spectrum:
11.5 Spectra for (-)-1,4-Regioisomer, 79

Succinic acid
2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^1$H NMR:
Succinic acid
2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^{13}$C NMR:
Succinic acid
2-{5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl}-1-methyl-ethyl ester methyl ester

Infrared Spectrum:
Succinic acid
2-{5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl}-1-methyl-ethyl ester methyl ester

Mass Spectrum:
11.6 Spectra for (+)-1,4-Regioisomer, 90

Succinic acid
2-\(\{5-\{2-\{4-(3\text{-acetylamino-phenyl})\{[1,2,3]\text{triazol-1-yl}\}\{1\text{-methyl-ethyl}\}\text{-tetrahydro-furan-2-yl}\}\{1\text{-methyl-ethyl}\}\text{ester methyl ester}}\)

\(^1\text{H NMR:}\)
Succinic acid
2-{5-[2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester methyl ester

^{13}C NMR:
90, Plus 1,4-Regioisomer

Succinic acid
2-{5-[2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl]-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester

Infrared Spectrum:
90, Plus 1,4-Regioisomer

Succinic acid
2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

Mass Spectrum:
11.7 Spectra for (±)-1,5-Regioisomer, 91

Benzoic acid 1-methyl-2-\{5-[1-methyl-2-(5-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl\}-ethyl ester

$^1H$ NMR:
91, Plus 1,5-Regioisomer

Benzoic acid 1-methyl-2-{5-[1-methyl-2-(5-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

$^{13}$C NMR:
Infrared Spectrum:
Benzoic acid 1-methyl-2-{5-[1-methyl-2-(5-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester

Mass Spectrum:
11.8 Spectra for (±)-1,5-Regioisomer, 92

Succinic acid
2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^1H$ NMR:
Succinic acid
2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

$^{13}$C NMR:
92, Plus 1,5-Regioisomer

Succinic acid
2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

Infrared Spectrum:
Succinic acid
2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester

Mass Spectrum:
11.9 Spectra for Nonactin Biosynthesis Competitive Inhibitor, 186

2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propionic acid

$^1$H NMR:
186. Nonactin Biosynthesis Inhibitor

2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propionic acid

$^{13}$C NMR:
186, Nonactin Biosynthesis Inhibitor

2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propionic acid

Infrared Spectrum:
2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propionic acid

Mass Spectrum:
CHAPTER 12

LITERATURE CITED


56. Retsema, J., Girard, A., Scheklly, W., Manousos, M., Anderson, M., Bright, G., Borovoy, R., Brennan, L., Mason, R., Spectrum and Mode of Action of Azithromycin (CP-62,993), a New 15-Membered-Ring Macrolide with Improved Potency against


70. Tygacil (tigecycline) clinical monograph, prescribing information., Wyeth Pharmaceuticals, Inc.: Philadelphia, PA.


215. Trieu-Cuot, P., de Cespedes, G., Bentorcha, F., Delbos, F., Gaspar, E., Horaud, T., 
Study of heterogeneity of chloramphenicol acetyltransferases (CAT) genes in 
streptococci and enterococci by polymerase chain reaction: characterization of a new 

216. Lynch, C., Courvalin, P., Nikaido, H., Active efflux of antimicrobial agents in wild- 

217. Tankovic, J., Mahjoubi, F., Courvalin, P., Duval, J., Leclerco, R., Development of 
fluoroquinolone resistance in Enterococcus faecalis and role of mutations in the DNA 

218. Eliopoulos, G.M., Activity of newer fluoroquinolones in vitro against Gram-positive 

219. Korten, V., Huang, W.M, Murray, B.E., Analysis by PCR and direct DNA sequencing 
of gyrA mutations associated with fluoroquinolone resistance in Enterococcus 

220. Kanmatsu, E., Deguchi, T., Yasuda, M., Kawamura, T., Nishino, Y., Kawada, Y., 
Alterations in the GyrA subunit of DNA gyrase and the ParC subunit of DNA 
topoisorame IV associated with quinolone resistance in Enterococcus faecalis. 

221. el Amin, N.A., Jalal, S., Wretlind, B., Alterations in GyrA and ParC associated with 
fluoroquinolone resistance in Enterococcus faecium. Antimicrob. Agents 


223. Oyamada, Y., Ito, H., Fujimoto, K., Combination of known and unknown mechanisms 
confers high-level resistance to fluoroquinolones in Enterococcus faecium. J. Med. 

are associated with fluoroquinolone resistance in Enterococcus faecalis. J. Med. 

225. newman, D.J., Cragg, G.M., Snader, K.M., Natural Products as Sources of New 


228. Ortholand, J., Ganesan, A., Natural products and combinatorial chemistry: back to 


367(630).


