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

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

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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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Of course, none of this would have been possible without the love and support of my family through these many years of education. My accomplishments, whatever they may be, either great or small, are also theirs in full measure. For my family, friends, and teachers, thank you for everything.

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LIST OF ABBREVIATIONS

^{13}C NMR	13-carbon nuclear magnetic resonance spectrum
^{1}H NMR	proton nuclear magnetic resonance spectrum
calcd.	calculated
d	doublet
DCM	methylene chloride
dd	doublet of doublets
δ	chemical shift
DHP	3,4-dihydro-2*H*-pyran
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
ee	enantiomeric excess
eq.	equivalent
ESI-TOF-MS	electrospray ionization time of flight mass spectrometry
HMPA	hexamethylphosphoramide
HPLC	high pressure liquid chromatography
IC_{50}	50% of the Inhibitory concentration
J	coupling constant / Hz
LC-MS (TOF)	time of flight liquid chromatography mass spectrometry
m	multiplet
m/z	mass to charge ratio
MIC	minimum inhibitory concentration
MRSA	methicillin resistant *Staphylococcus aureus*
MS	mass spectrometry
NMR	nuclear magnetic resonance
pTsCl	para-toluenesulfonyl chloride
pTsOH-H_2O	para-toluenesulfonic acid monohydrate
py	pyridine
r.t.	room temperature 22 ^{o}C
R_f	retention factor
s	singlet
SAR	structure activity relationship
SEM	scanning electron microscope
t	triplet
TBDMSCl	tert-butyl dimethylsilyl chloride
THF	tetrahydrofuran
TOCSY-NMR	totally correlated spectrometry
VRE	vancomycin resistant *Enterococcus faecalis*

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CHAPTER 1

INTRODUCTION AND BACKGROUND OF ANTIBIOTICS

1.1 Origin and history of antibiotics

1.1.A Definitions

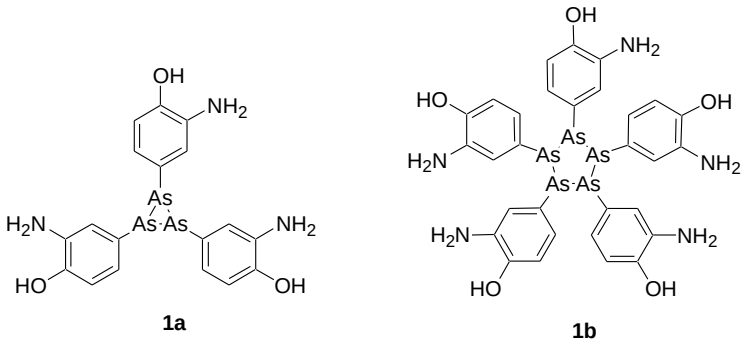
An antibiotic is defined as a compound that kills or inhibits the growth of bacteria.[8, 9] Some antibiotics are bactericidal and others are bacteriostatic.[3, 8] Antibiotics include antibacterial agents such as penicillin, antifungals such as rifamycin, and antiprotozoal compounds such as tetracycline. 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*.[13] In 1942 these compounds were renamed antibiotics (anti 'against', bios 'life') by the American microbiologist Selman Wakeman.[12, 14]

1.1.B 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 *Trepnema 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

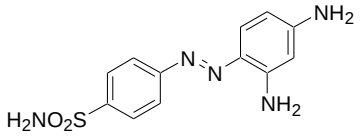
Figure 1.1: Structures of Salvarsan. Salvarsan consists of a mixture of cyclic arsenicals (**1a** and **1b)**.[1]

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.

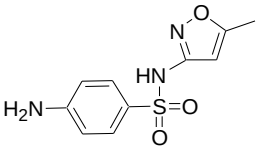


2, Prontosil

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,

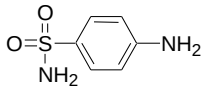
specifically *Streptococcus hemolyticus*, a highly virulent Streptococcal strain.[2, 19] Prontosil **2** became the first medicine discovered that could treat a range of bacterial infections in vivo and the first commercially available antibacterial antibiotic.[19] Gerhard Domagk received the 1939 Nobel 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.



3, sulfamethoxazole

Figure 1.3: Structure of Sulfamethoxazole **3**.

It was also the first of the 'wonder drugs'.



4, Sulfanilamide

Figure 1.4: Structure of Sulfanilamide **4.**

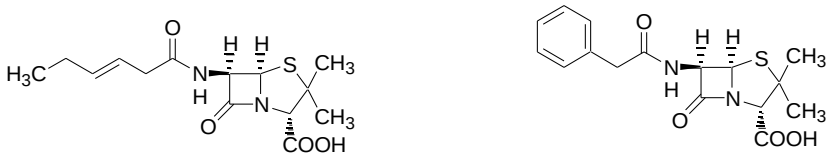
Sulfaniliamide **4** was marketed in the United States, France, and Britain in the early part of 1937. By the Spring of 1937, any skepticism concerning chemotherapy had withered

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.



5a, Penicillin F

5b, Penicillin G

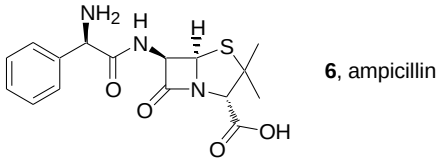
Figure 1.5: Penicillins, the structures of Penicillin F **5a** and G **5b**.[2]

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 urisosuric 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.



6, ampicillin

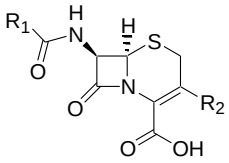
Figure 1.6: Structure of Ampicillin **6**.

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.



7, cephalosporin moiety

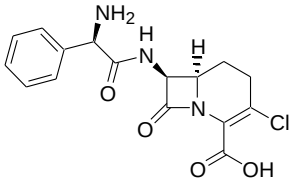
Figure 1.7: General Structure of Cephalosporins **7.**

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

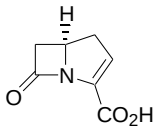
The carbacephems are beta-lactam antibiotics and are a subclass of the cephalosporins. Carbacephems, a member of the cephem class, are synthetically made and based upon the structure of cephalosporins. The basic structure of the carbacephems is altered from that of the cephalosporins and that of other 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.



8, Lorabid

Figure 1.8: Structure of Loracarbef (Lorabid) **8**, a carbacephem.

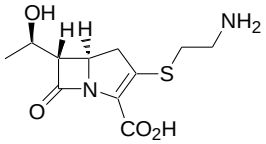
1.1.F Carbapenems



9, carbapenem moiety

Figure 1.9: General Structure of the Carbapenems **9**.

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



10, Thienamycin

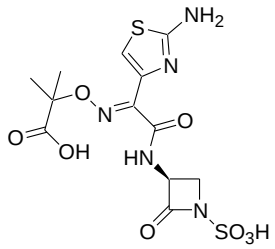
Figure 1.10: Structure of Thienamycin **10**.

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]



11, Aztreonam

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

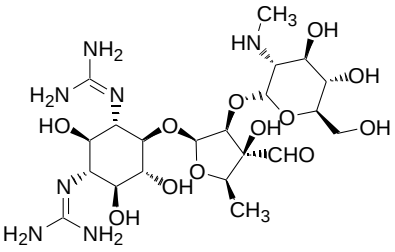
Aztreonam **11** is synthetically produced.[37] Monobactams are only effective against Gram-negative bacterium, including *Pseudomonas aeruginosa*.[37, 38] Aztreonam **11** is also bactericidally effective against *E. coli*, *Klebsiella*, *Proteus*, *Citrobacter*, *Enterobacter*, and *Serratia* species.[39] Their mechanism of action is the inhibition of the mucopeptide 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.



12, Streptomycin

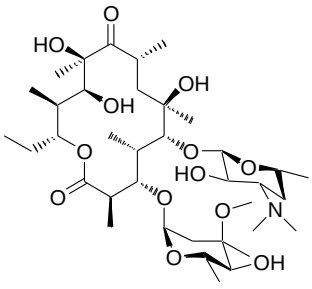
Figure 1.12: Structure of Streptomycin **12**, an aminoglycoside.

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.I. 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 *Staphylococc*i, beta-hemolytic *Streptococci*, *Pneumonococci*, *Enterococci*, *Chlamydia*, *Mycoplasm*, 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.

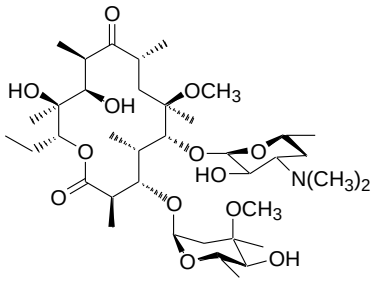


13, Erythromycin

Figure 1.13: Structure of Erythromycin **13**, a macrolide antibiotic.

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]

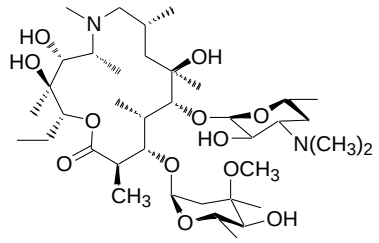


14, Clarithromycin

Figure 1.14: Structure of Clarithromycin **14**.

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

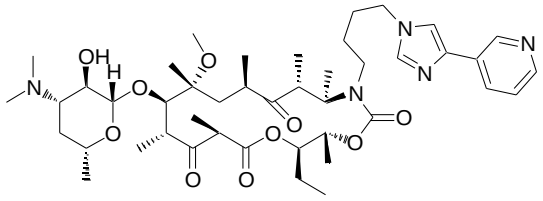
binding to the 50S subunit of the bacteria ribosome, the translation of mRNA is inhibited.[56] This caused the inhibition of protein synthesis which prevents the growth of the bacteria.[56] Though azithromycin **15** shares a similar spectrum of activity with erythromycin **13**, azithromycin is more effective against certain strains of Gram-negative species.[56] Azithromycin **15** is effective against *Staphylococcus aureus* and several species of *Streptococcus* including *pyrogenes*, *agalactiae*, and *pneumonia*.[56] It is also effective against *Mycoplasma pneumonia*, *Chlamydia*, and *Haemophilus*.[56]



15, Azithromycin

Figure 1.15: Structure of Azithromycin **15.**

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



16, Telithromycin

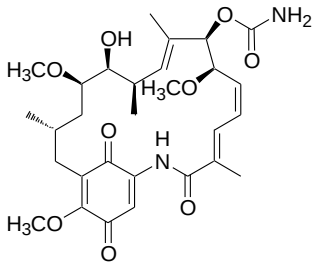
Figure 1.16: Structure of Telithromycin **16**, a ketolide antibiotic, related to macrolides antibiotics[3]

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]



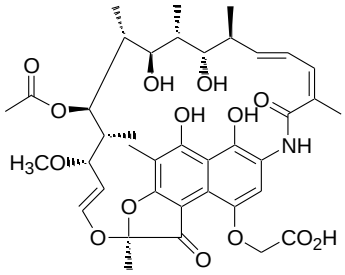
17, Geldanamycin

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

tuberculosis.[61] 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.



18, Rifamycin B

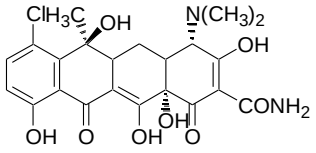
Figure 1.18: Structure of Rifamycin B **18**.

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]

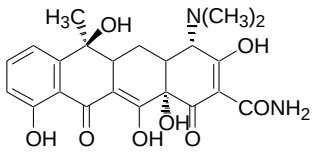


19, 7-Chlortetracycline

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.



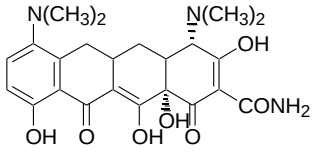
20, Tetracycline

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.L. Tigecycline

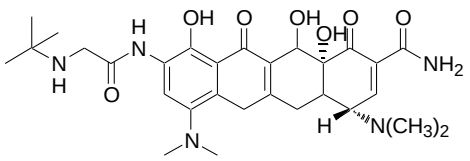
Tigecycline **22** is a derivative of minocycline **21** and is related structurally to tetracyclines.[5, 66]



21, Minocycline

Figure 1.21: Structure of Minocycline **21**.[4]

Tigecycline is a glycylcycline antibiotic and was developed by Wyeth in 2005.[5, 67, 68] Tigecycline **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, glycylcyclines, possesses a central four-ring carbocyclic skeleton and is therefore related to the tetracyclines.



22, Tigecycline

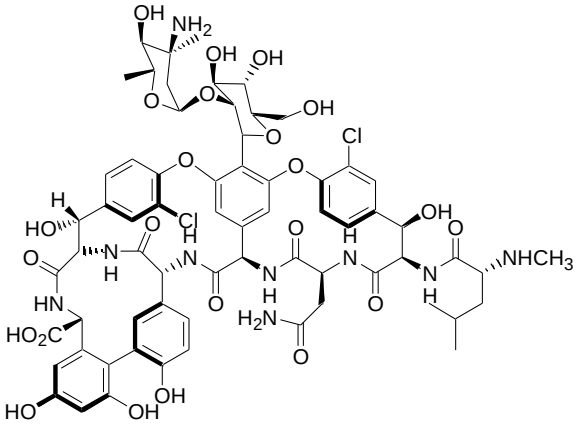
Figure 1.22: Structure of Tigecycline **22**, a glycylcycline.[5]

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]



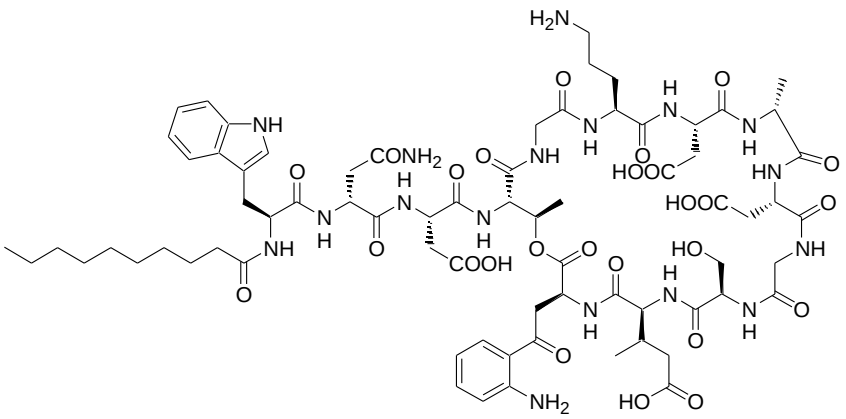
23, Vancomycin

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 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]



24, Daptomycin

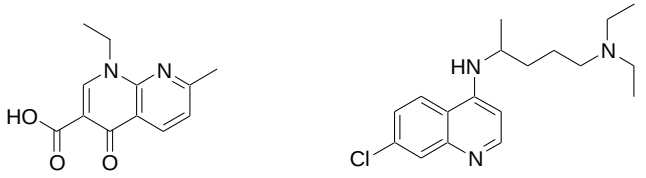
Figure 1.24: Structure of Daptomycin **24**, a lipopeptide antibiotic

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.

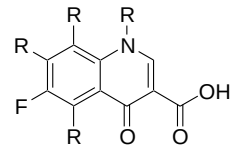


25, Nalidixic Acid **26**, Chloroquine

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]

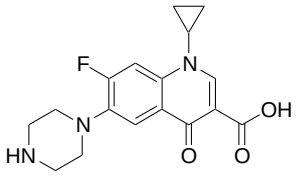


27, fluoroquine moiety

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

The fluoroquinones' **27** 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 **27** 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 **27** with a broad spectrum of activity. Effective bactericidal activity has been established against Gram-positive and Gram-negative bacteria.

Ciprofloxacin **28** is a member of the fluoroquinolones **27**, a second generation compound which was patented in 1983 by Bayer. Like the rest of the fluoroquinolones **27**, ciprofloxacin is synthetic and inhibits DNA replication as well as transcription via the inhibition of DNA gyrase and topoisomerase IV.[103] Ciprofloxacin **28** is used to treat urinary tract infections, bacterial diarrhea, gonorrhea, and community-acquired pneumonia.



28, Ciprofloxacin

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

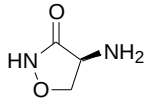
1.1.P. Oxazolidinones

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

29, 2-Oxazolidone

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

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



30, Cycloserine

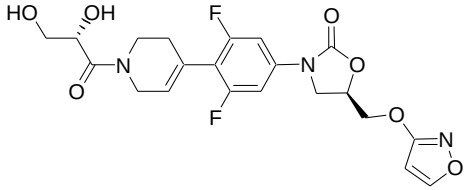
Figure 1.29: Structure of Cycloserine **30.**

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

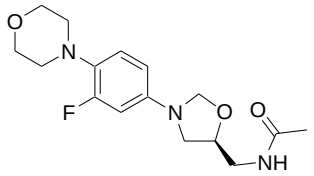
antibiotics are considered as last line defense against drug resistant organisms. Posizolid **31** and Linezolid **32** are members of this class.



31, Posizolid

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]



32, Linezolid

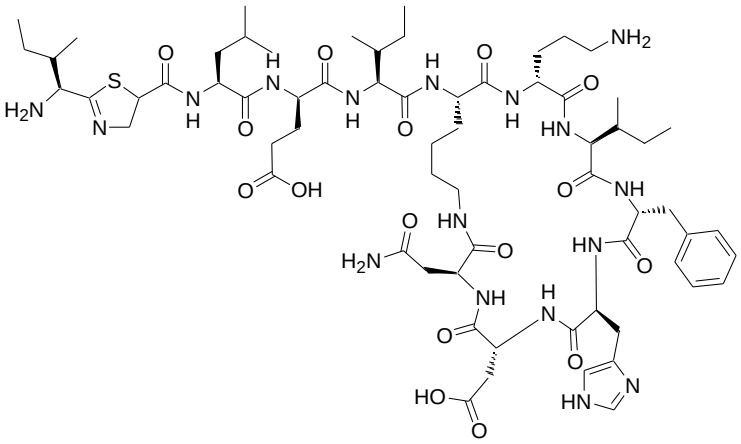
Figure 1.31: Structure of Linezolid **32.**

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]



33, Bacitracin

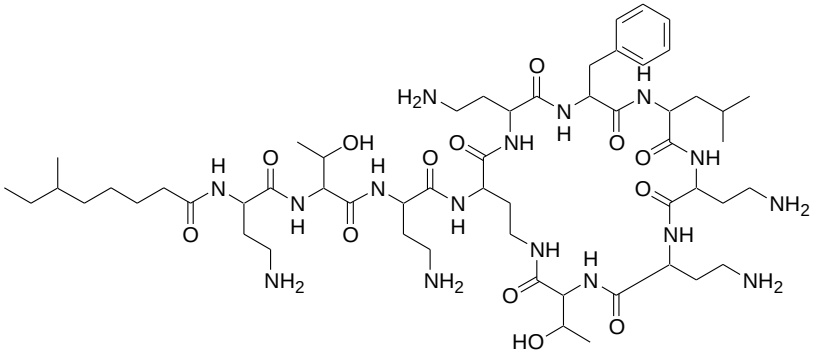
Figure 1.32: Structure of Bacitracin **33.**

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 C_{55}-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 decapeptides.[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 decapeptide 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**.

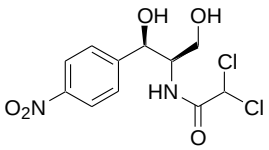


34, Polymyxin B

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]

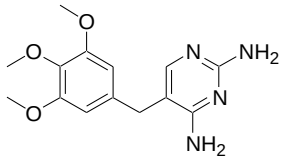


35, chloramphenicol

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]



36, Trimethoprim

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 intrachromaosomal or extrachromosomal DNA or RNA.[145] Chromosomal translocations occur when a segment of genetic material detaches from the chromosome and

attaches to another.[144] 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*-nucleotidyltranferases.[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 *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 *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 *N*-acetylglucosamine into the bacterial cell wall contribute to the glycopeptide resistance in methicillin resistant *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 placed 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 **38**.[107, 208, 209] These trimethoprim resistance DHFRs are encoded by genes on mobile genetic elements.[107, 207] These included plasmids and transposons which are able to easily spread among bacteria.[107, 207]

1.2.D.10. Lipopeptide resistance

Resistance to the lipopeptide, daptomycin, has been noted in a few cases since its employ into clinical use.[147, 210] Lipopeptides may disrupt cell integrity which results in cell depolarization.[147, 211] Due to a lack of pertinent information concerning the mechanism of action of daptomycin, no resistance mechanism has been put forth.[147]

1.2.D.11. Chloramphenicol resistance

Chloramphenicol acetyltransferases inactivate chloramphenicol.[17, 147, 212] These enzymes are encoded on by genes on conjugative and non-conjugative resistance plasmids.[147, 213-215] They may also be present on the enterococci chromosome.[147, 213-215] Energy-driven efflux of chloramphenicol has also been documented.[147, 216]

1.2.D.12. Fluoroquinolone resistance

One mechanism or a combination of both mechanisms, infer fluoroquinolone resistance in Gram-negative species.[147] Drug efflux and mutation are responsible for fluoroquinolone resistance, particularly in *Enterococcus*.[147, 216] 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.[147, 217-221] 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 pencillins and macrolides, are secondary metabolites of mold and bacteria respectively.

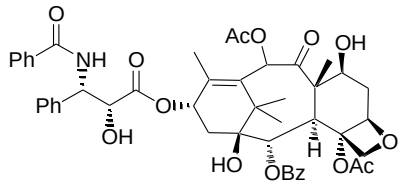
In the past 20 years, a major shift has occurred away from natural products and toward combinatorial libraries due to the advent of high throughput screening (HTS). [228] High throughput screening allows for the screening of large libraries of compounds that can be provided through combinatorial synthesis.[228] 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

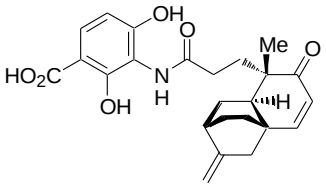
Synthesis is the mechanism by which molecules can be made artificially. Synthesis includes natural product synthesis, combinatorial synthesis, target oriented synthesis, and diversity oriented synthesis. Natural product synthesis, also called total synthesis, includes all synthetic steps necessary to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



37, Taxol

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]



38, platencin

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 pneumonia*.[232] Target oriented synthesis is the synthesis of a particular compound designed to bind 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

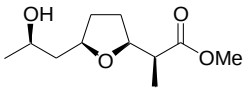
Highly efficient combinatorial synthesis combined with scaffolds derived from nonactin, a natural product, was used to make triazoloester libraries of potentially antimicrobial compounds. The triazoloester library was assayed for antimicrobial activity. The impact of stereochemistry and structure on 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

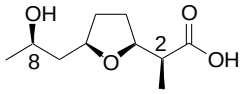
2.1 Why is methyl nonactate a good scaffold?



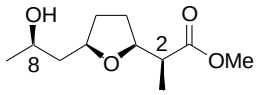
45, Methyl Nonactate

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

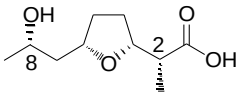
Methyl nonactate **45** and, therefore, nonactic acid **49**, **50** are highly useable and valuable structures for drug discovery.[235] Methyl nonactate **45** is a stereochemically rich structure (Figure 2.1). Gram quantities of highly pure enantiomers of methyl nonactate **46**, **48** can be produced 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]



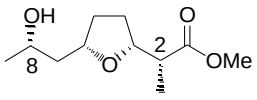
49, (+)-nonactic acid



46, methyl (+)-nonactate



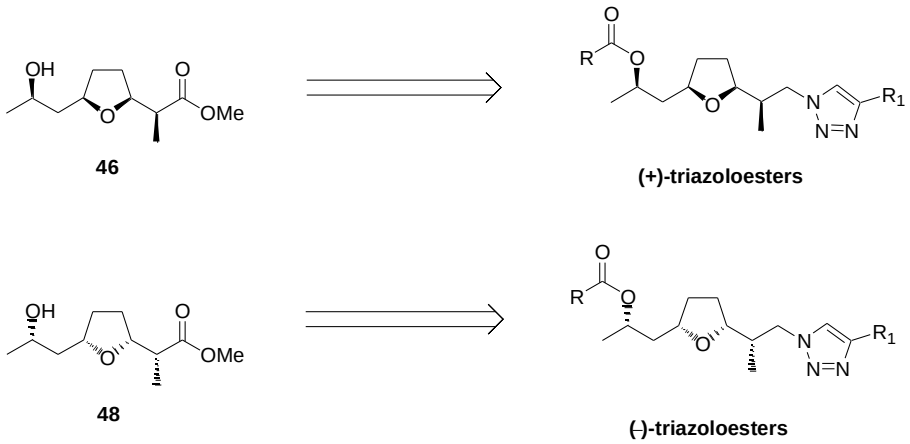
50, (-)-nonactic acid



48, methyl (-)-nonactate

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.



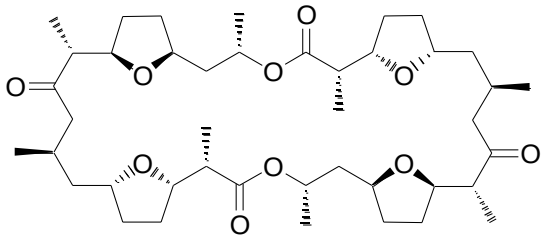
Scheme 2.1: Overall scheme of triazoloester library synthesis from **46** and **48**.

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]

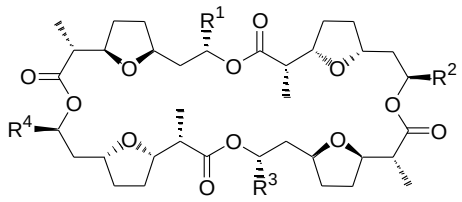
The stereochemically rich structure **40** is available on the gram scale, which is made by *Streptomyces griseus* and extracted using standard procedures. Synthesis of the plus and minus nonactic acid **49**, **50** is complicated, uses many steps, and results in low yields of the final products, resulting 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?



40, nonactin



39, macrotetrolides

	R^1	R^2	R^3	R^4
Nonactin (**40**)	Me	Me	Me	Me
Monactin (**41**)	Me	Me	Et	Me
Dinactin (**42**)	Et	Me	Et	Me
Trinactin (**43**)	Et	Et	Et	Me
Tetranactin (**44**)	Et	Et	Et	Et

Figure 2.3: Naturally occurring macrotetrolides **39**, most importantly nonactin **40**.

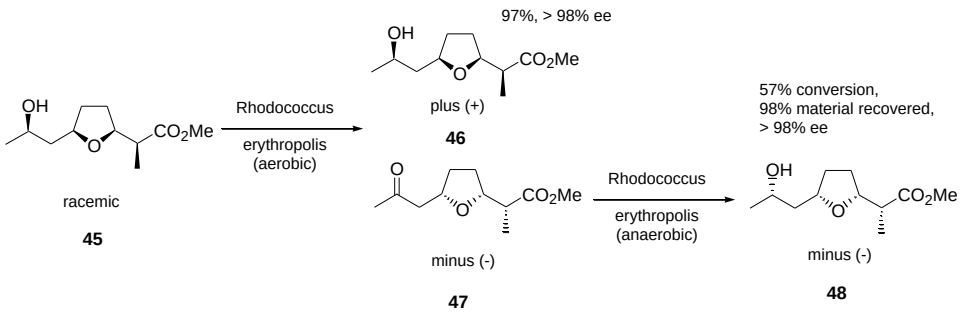
The parent compound of a group of ionophore (Na^+ and K^+) antibiotics called macrotetrolides **39** (Figure 2.3) is nonactin **40**, which is produced by *Streptomyces griseus* ETH A7796.[239] Acetate, propionate, and succinate are known to be units in the polyketide biosynthesis pathway that assemble 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_4 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*'-tetrahydropyranyldoxorubicin 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% H_2SO_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 erythropoli*s 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

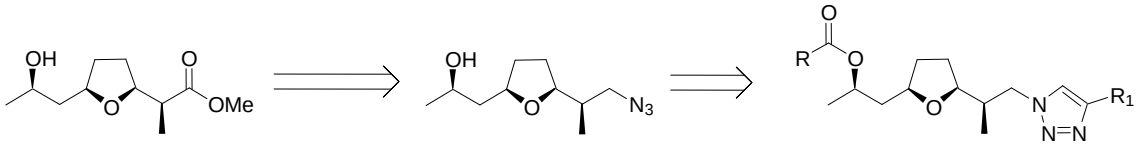
Methyl nonactate is a versatile scaffold for the synthesis of libraries. It is a stereochemically complex molecule with readily modifiable alcohol and ester groups. It is derived from fermentation and methanolysis as opposed to a lengthy and expensive synthesis. The ability to separate out the enantiomers 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).

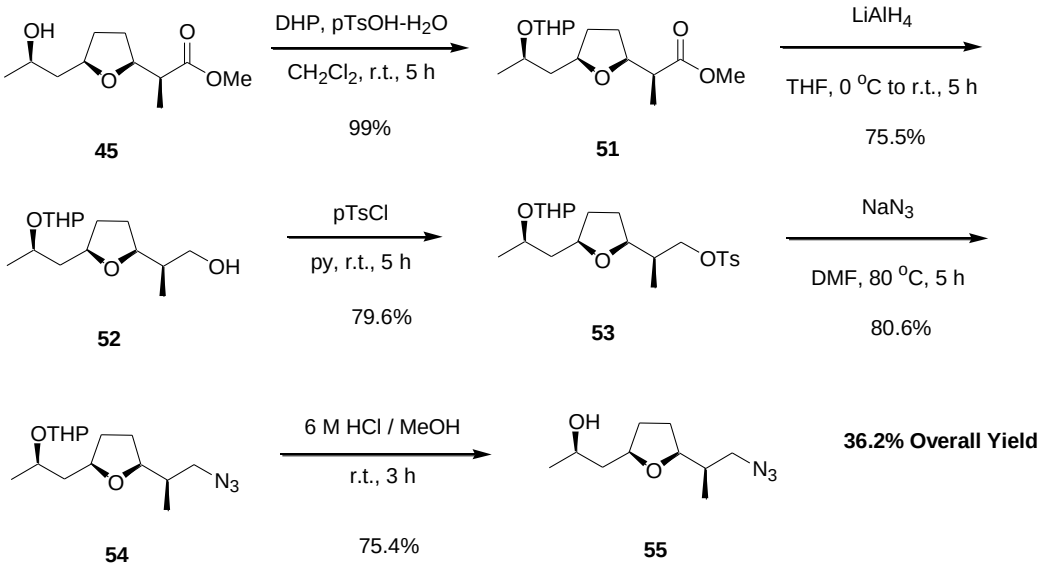


45 **55** **62,** racemic triazoloesters

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 ^{o}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.

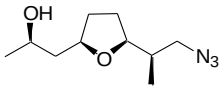
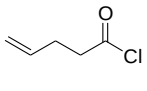
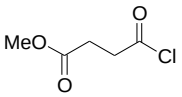
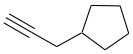
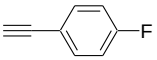
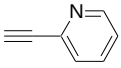
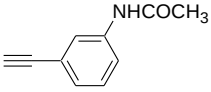
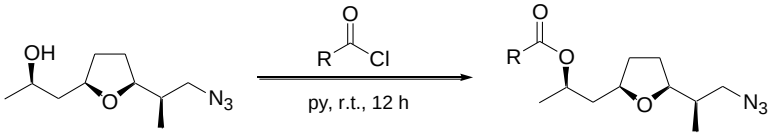
	**None**					
None	**55**	**57**	**58**	**59**	**60**	**61**
		**63**		**65**		
		**64**	**66**	**67**		
					**68**	
						**72**
			**69**		**71**	**70**

Table 3.1: Chart of triazoloester compounds and numbering scheme.



55 **56**

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).

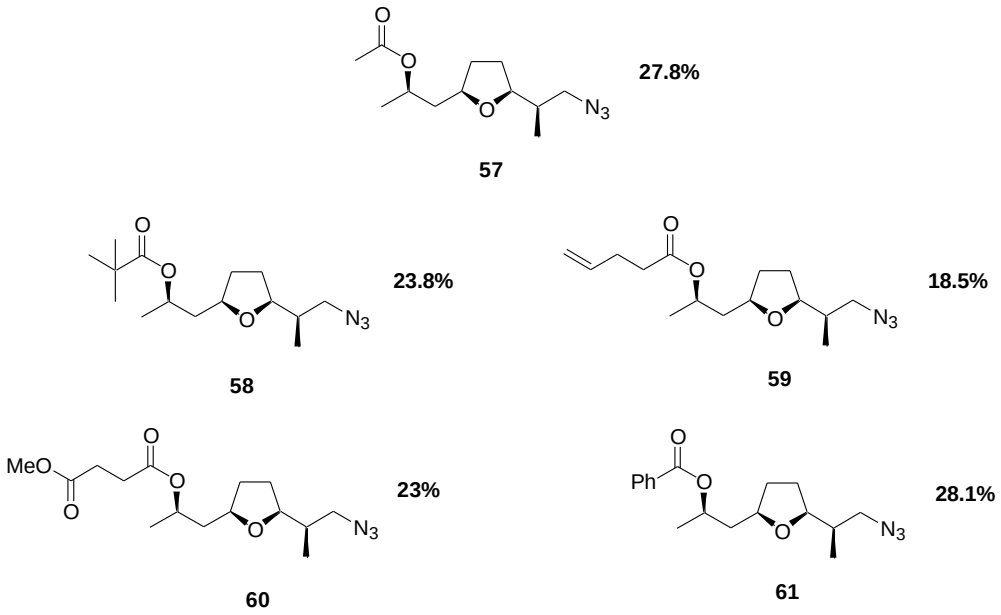
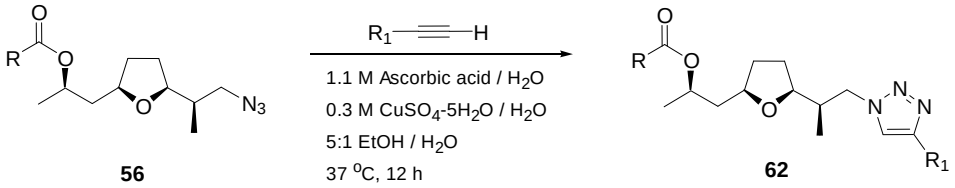


Figure 3.1: Overall yields of azidoester nonactic acid analogue scaffolds from **45**.



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).

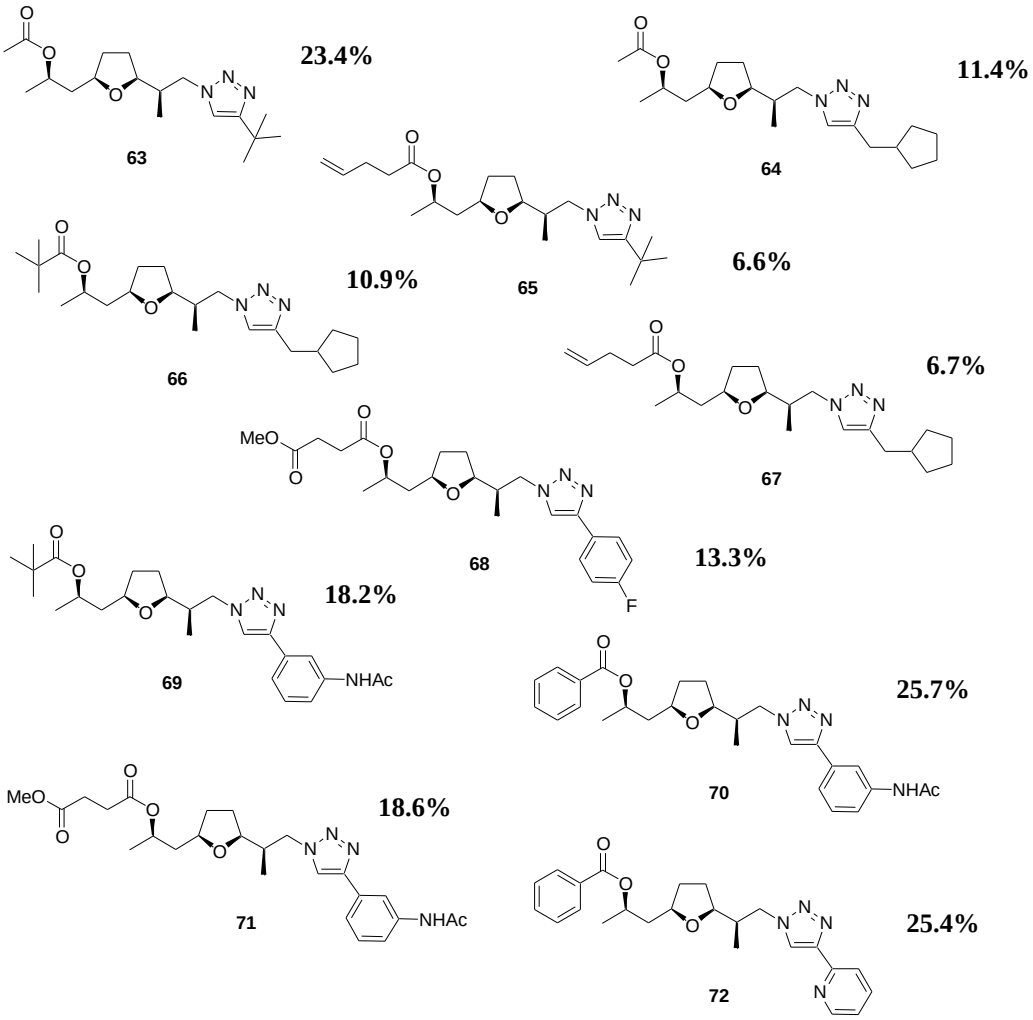
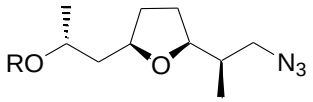


Figure 3.2: Overall yields of triazoloester (±)-nonactic acid analogue library from **45**.

3.4 Biological evaluation of initial library

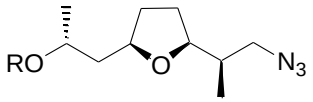
The **72**, **71,** and **67** compounds were shown by assay, which was performed by Adrienne Smith, Dr. Erin Bolstad, and Dr. Brooke Martin, to be the most active members of the 25 membered of the triazoloester (±)-nonactic acid analogue library (Figure 3.2). These compounds were tested against *B.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



R	Compound	*B. subtilis*	*S. aureus*
H	**55**	Inact.	Inact.
Ac	**57**	Inact.	Inact.
COtBu	**58**	Inact.	Inact.
COCH2CH2CHCH2	**59**	Inact.	Inact.
COCH2CH2CO2Me	**60**	Inact.	Inact.
Bz	**61**	Inact.	Inact.

Table 3.2: Inactivities of azidoester compounds.

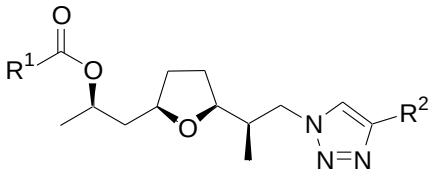
Alamar-blue assays on the azidoesters revealed that they have no antibacterial activity in *B. subtilis* or *S. aureus* (Table 3.2).[244] Since no azidoester showed any significant activity as did the original scaffold, only potential for the triazoloesters remained.



R	Compound	IC_{50} MCF10	IC_{99} (calc.)
H	**55**	791 ± 62	983 ± 62
OAc	**57**	685 ± 1125	720 ± 1126
COtBu	**58**	741 ± 18	926 ± 18
COCH2CH2CHCH2	**59**	704 ± 85	895 ± 86
COCH2CH2CO2Me	**60**	816 ± 56	1039 ± 56
Bz	**61**	769 ± 35	999 ± 35

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

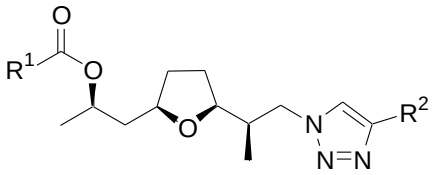
Cytotoxicity assays against the MCF10 breast cell line showed that none of the azidoesters had any significant cytotoxicity (Table 3.3).[245]



Compound	^{1}R	^{2}R	*B. subtilis*	*S. aureus*
63	Ac	tBu	Inact.	Inact.
66	tBu		Inact.	Inact.
65		tBu	Inact.	Inact.
68			Inact.	Inact.
72	Ph	2-pyridyl	94	1500
64	Ac		1125	Inact.
69	tBu	mPhNHAc	Inact.	Inact.
67			62	1000
71		mPhNHAc	375	750
70	Ph	mPhNHAc	Inact.	Inact.

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).



Compound	R1	R2	IC_{50} (MCF10)	IC_{99} (calc.)
63	Ac	tBu	716 ± 50	875 ± 50
66	tBu		114 ± 5.6	129 ± 5.7
65		tBu	407 ± 39	541 ± 40
68			767 ± 44	998 ± 44
72	Ph	2-pyridyl	291 ± 74	965 ± 109
64	Ac		481 ± 1.4	540 ± 1.4
69	tBu	mPhNHAc	140 ± 17	228 ± 18
67			247 ± 19	349 ± 19
71		mPhNHAc	758 ± 35	1017 ± 46
70	Ph	mPhNHAc	693 ± 83	1198 ± 88

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.

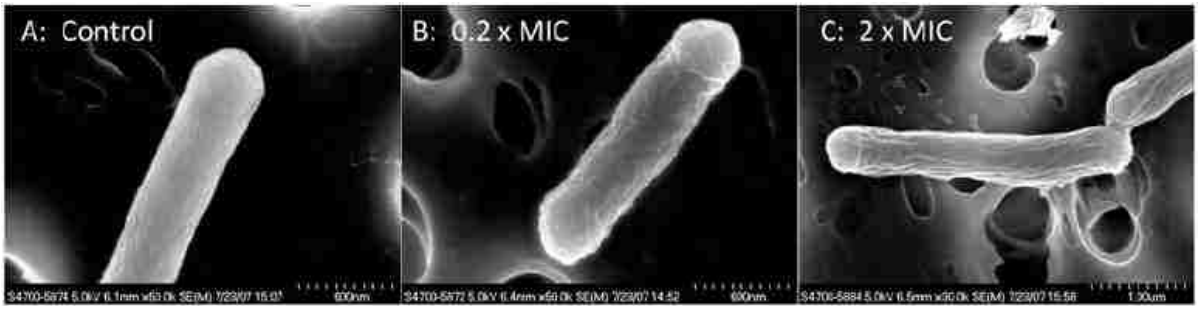
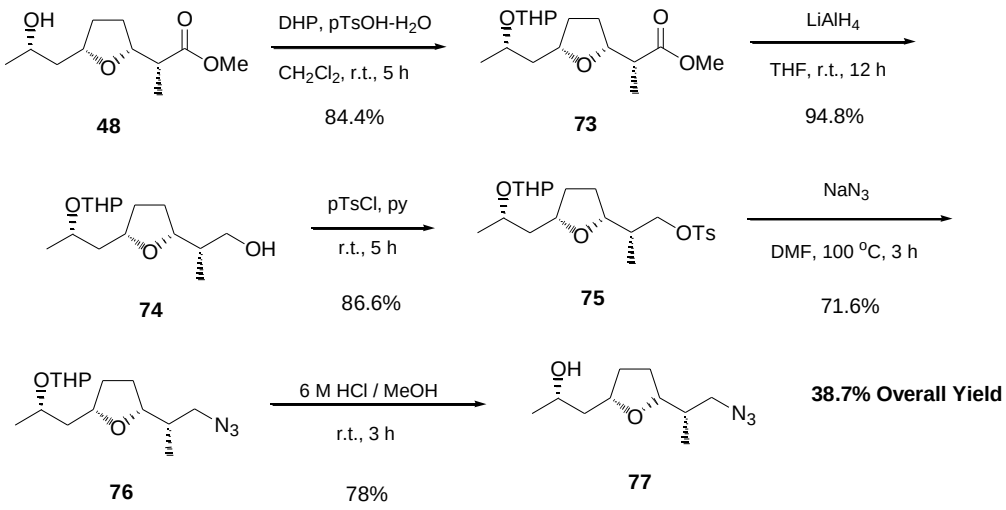


Figure 3.3: SEM images of *B. subtilis* after treatment with **72**.

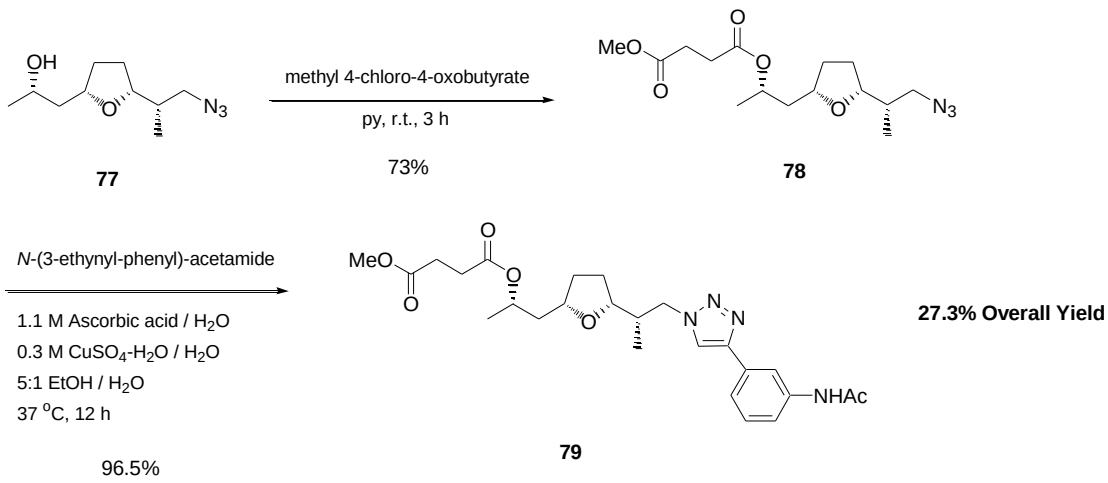
3.5 Evaluation of scaffold stereochemistry upon antibacterial activity

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 *Rhodoccocus* procedure.[7]



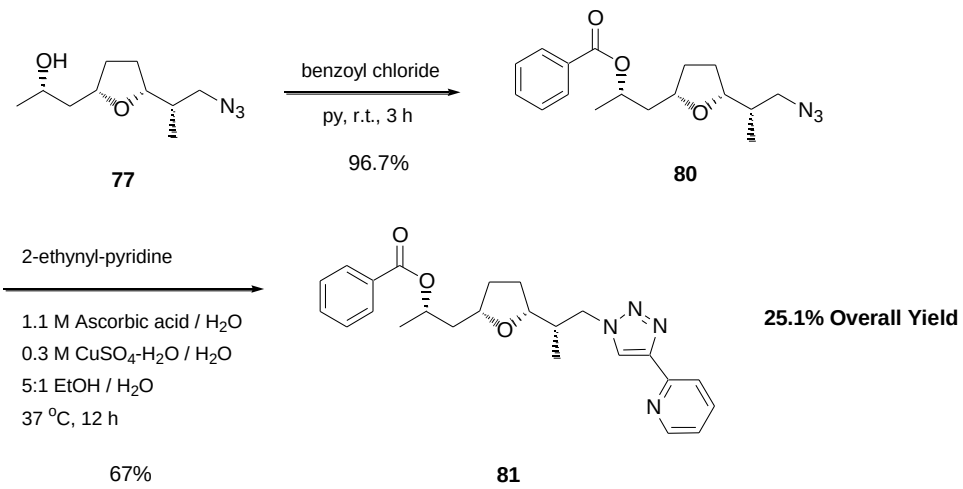
Scheme: 3.5: Synthesis of the azidoalcohol (–)-nonactic acid analogue scaffold **77**.

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.



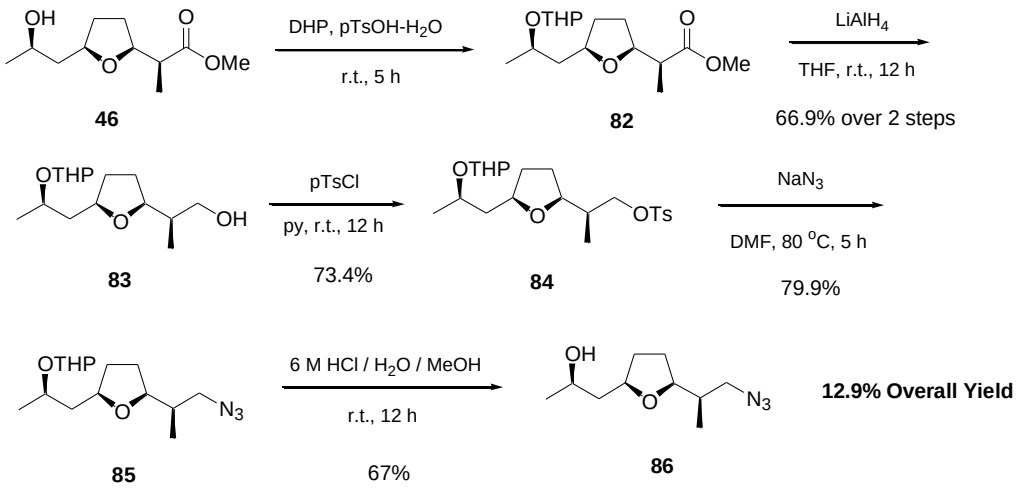
Scheme 3.6: Synthesis of the **71** triazoloester nonactic acid analogue **79**.

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.



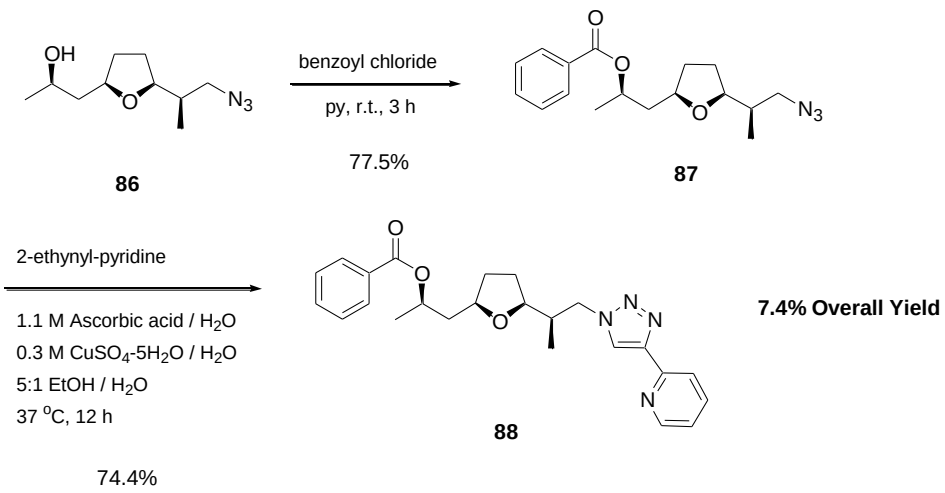
Scheme 3.7: Synthesis of the triazoloester (–)-nonactic acid analogue **81**.

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 benzoylation 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**.



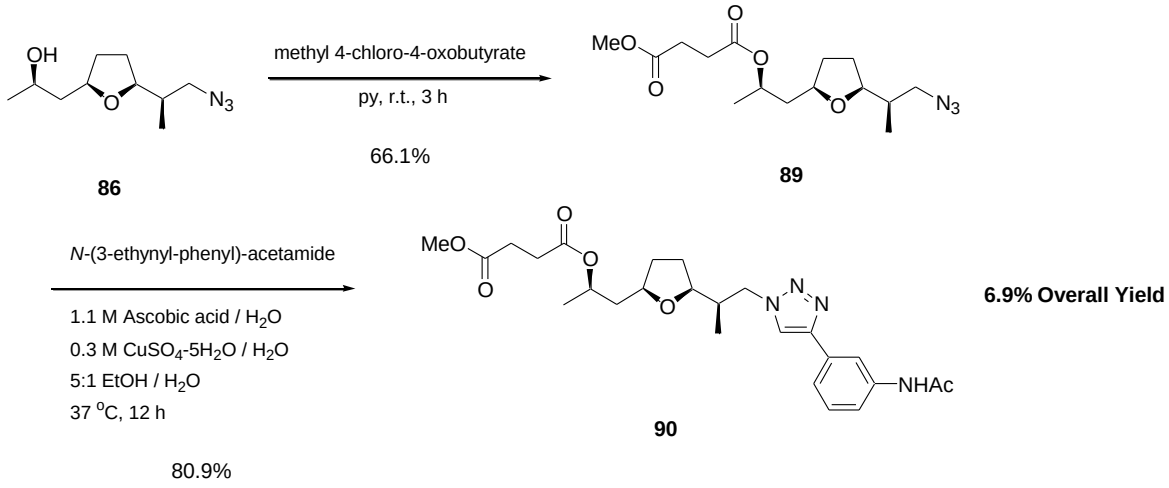
Scheme 3.8: Synthesis of the azidoalcohol (+)-nonactic acid analogue scaffold **86**.

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 tosylated 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**.



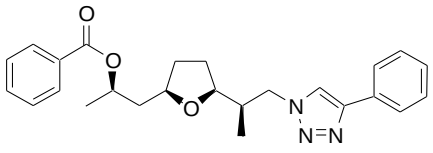
Scheme 3.9: Synthesis of the **72**- triazoloester (+)-nonactic analogue **88**.

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 ºC for 12 hours. This gave a product **88** yield of 74.4% after chromatography of silica gel.

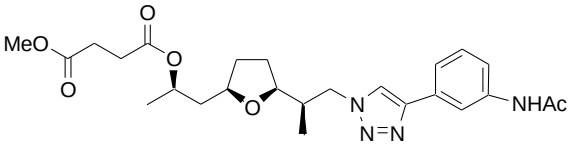


Scheme 3.10: Synthesis of the **71**-triazoloester (+)-nonactic acid analogue **90**.

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.



72



71

Organism	**88 (+)**	**81 (–)**	**72 Racemic**	**90 (+)**	**79 (–)**	**71 Racemic**
B. cereus	1500	Inact.	1500	187	Inact.	375
B. subtilis	47	Inact.	94	187	Inact.	375
B. anthracis	750	Inact.	1500	375	Inact.	750
S. aureus	750	Inact.	1500	750	Inact.	750
E. faecalis van^R	62	Inact.	Nd	125	Inact.	nd

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.

Strain	88	81	90	79
B. anthracis	750	Inactive	375	Inactive
B. cereus	1500	Inactive	187	Inactive
B. subtilis	47	Inactive	187	2000
E. faecalis	500	Inactive	250	nd
E. faecalis van^R	62	Inactive	125	Inactive
S. aureus	750	Inactive	750	Inactive
S. aureus MRSA	125	Inactive	250	750
S. epidermidis	250	Inactive	250	nd
S. pyogenes	64	Inactive	125	nd

Table 3.7: MIC (μM) 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 show against *B. subtilis*, *E. faecalis* van^R, *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^R, *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

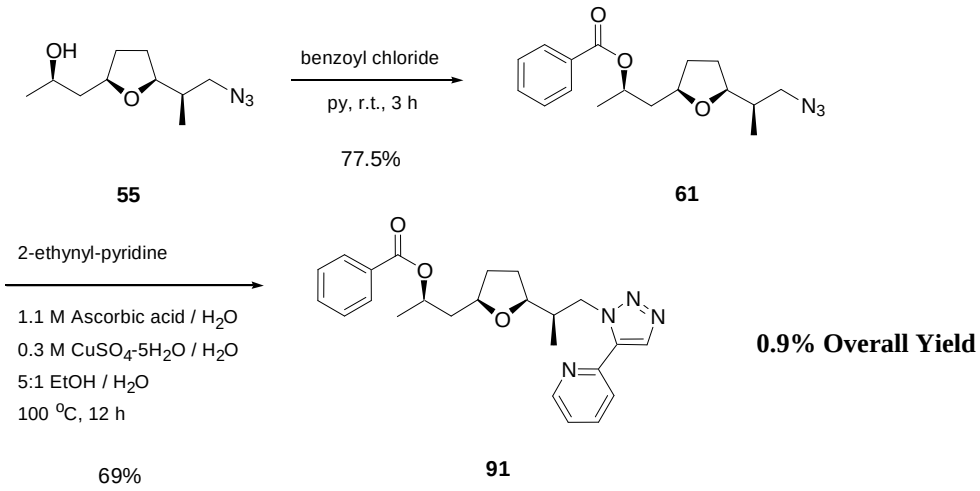
then used to generate the azidoalcohol nonactate scaffold **55** which was used in turn to synthesize a library of compounds, in which members of which were shown to have antimicrobial activity. Pure plus and minus enantiomers of the azidoalcohol nonactate scaffold **86**, **77** were synthesized and 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.



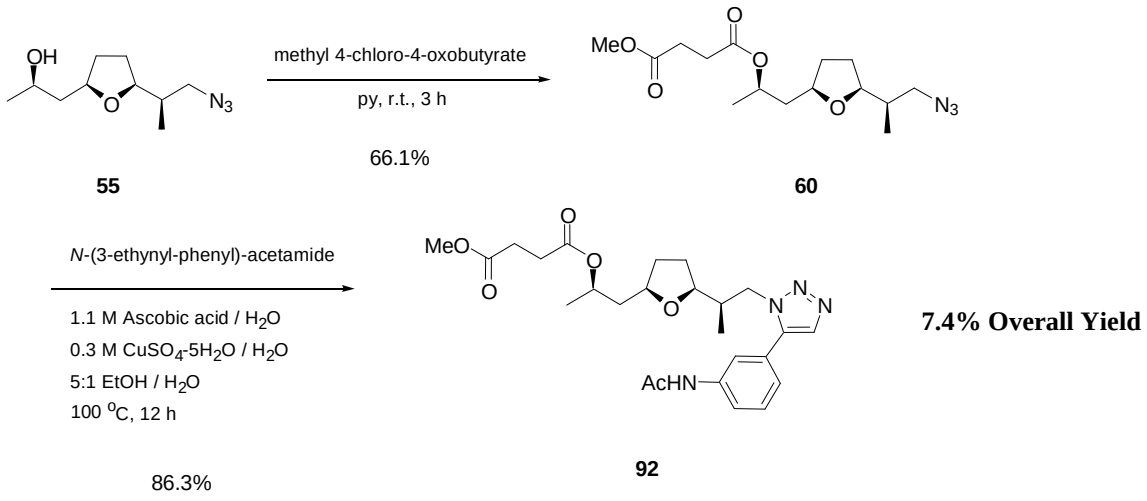
Scheme 3.11: Synthesis of the 1,5-triazoloester (+)-nonactic acid analogue **91**.

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%.

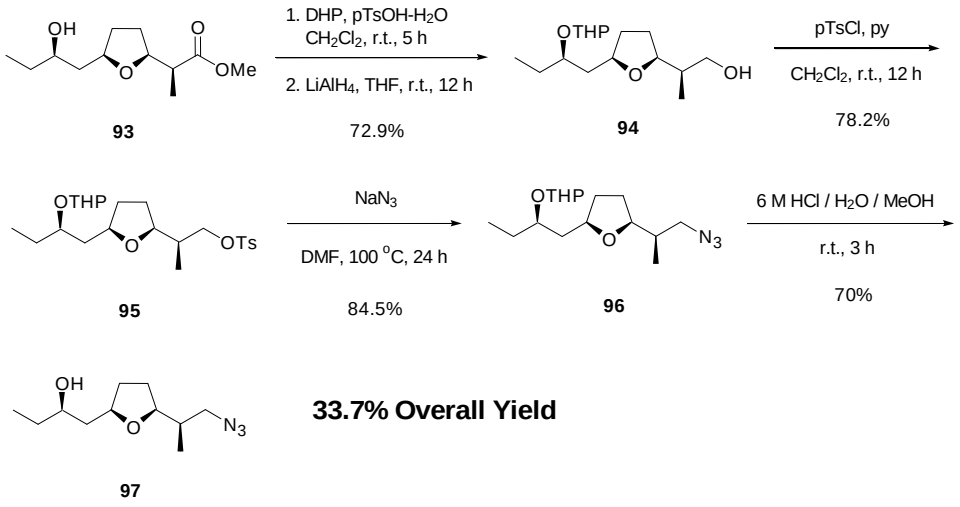
Compound **91** and **92** were analyzed with a 2D-NOE as well as **71** and **72**. Neither of the aforementioned correlations was seen. Again, this is most likely due to either insufficient

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 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.



93 **94** **95** **96** **97**

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.

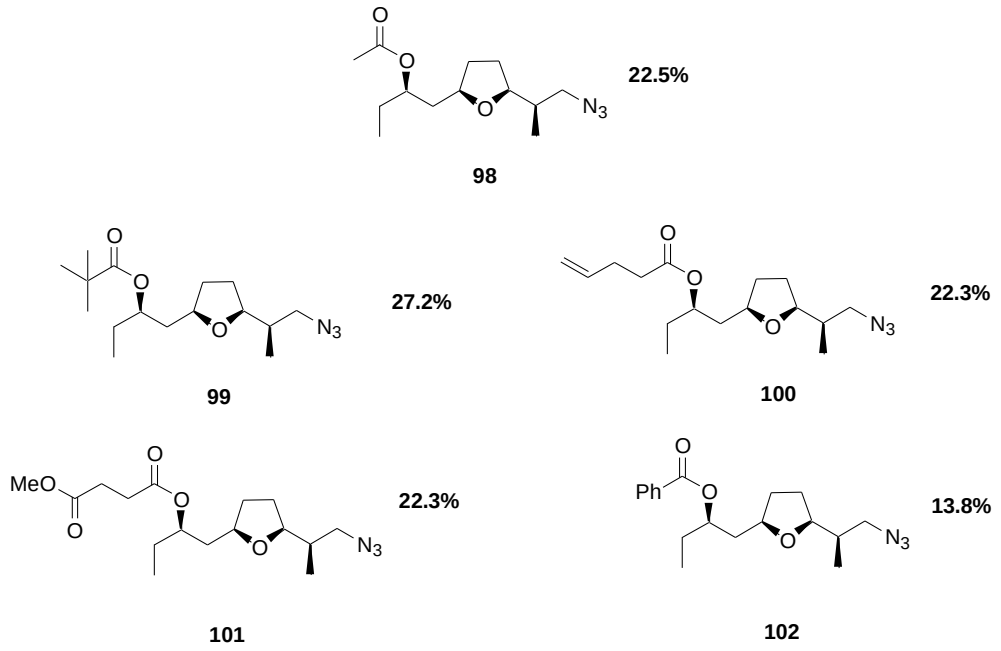
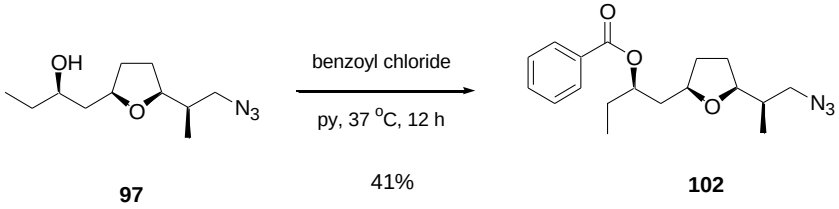
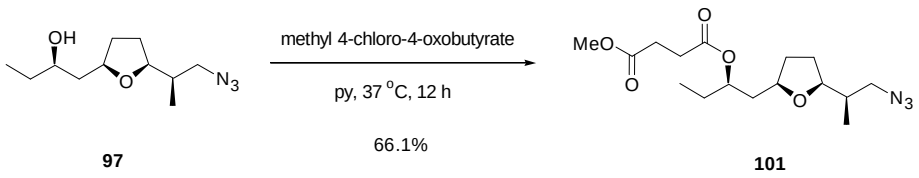


Figure 3.4: Overall yields of azidoester homononactic acid analogue scaffold library **98**, **99**, **100**, **101**, **102**.



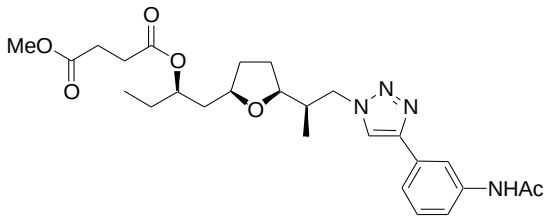
Scheme 3.14: Synthesis of the **72-**intermediate azidoester homononactic acid analogue **102**.

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%.



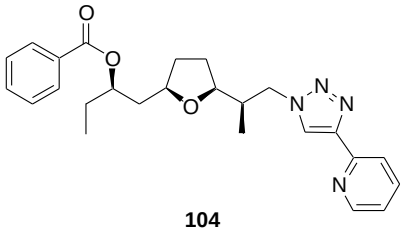
Scheme 3.15: Synthesis of the **71**-intermediate azidoester homononactic acid analogue **101**.

The azidoalcohol homononactic acid analogue scaffold **97** was esterified with methyl 4-chloro-4-oxobutryate 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.



103

Figure 3.5: 71-triazoloester homononactic acid analogue **103**.



104

Figure 3.6: 72-triazoloester homononactic acid analogue **104**.

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.

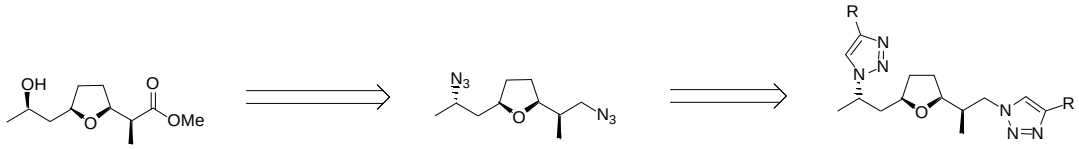
Triazoloesters, by virtue of their bactericidal activity against *S. aureus* and *E. faecalis* as well as against other pathogens (Table 3.7) and their relatively low cytotoxicity have great potential for the development of new potent antibacterial compounds. Compounds **71** and **72** have shown great 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.

CHAPTER 4

SYNTHESIS OF THE BIS-TRIZAZOLE NONACTIC ACID ANALOGUE LIBRARY

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

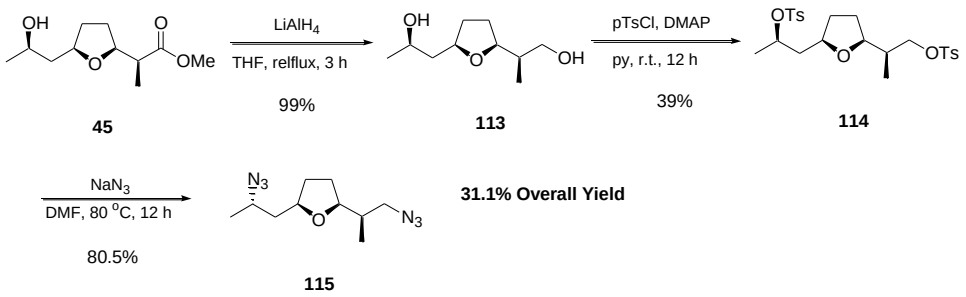
The biological activity exerted by the (+)-**72** and **71** nonactic acid analogue compounds **88**, **90** has made the starting subunit, methyl nonactate **45** an attractive building block for making libraries of potentially active compounds. The azidoalcohol nonactic acid analogue scaffold **55** 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).



45 **115** **116, bis-triazole library**

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.

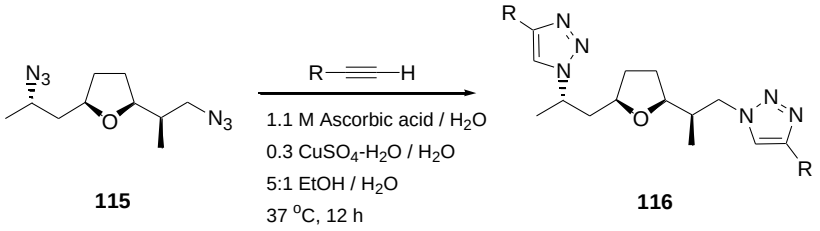


Scheme 4.2: Synthesis of the bis-azide (±)-nonactic acid analogue scaffold **115**.

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.

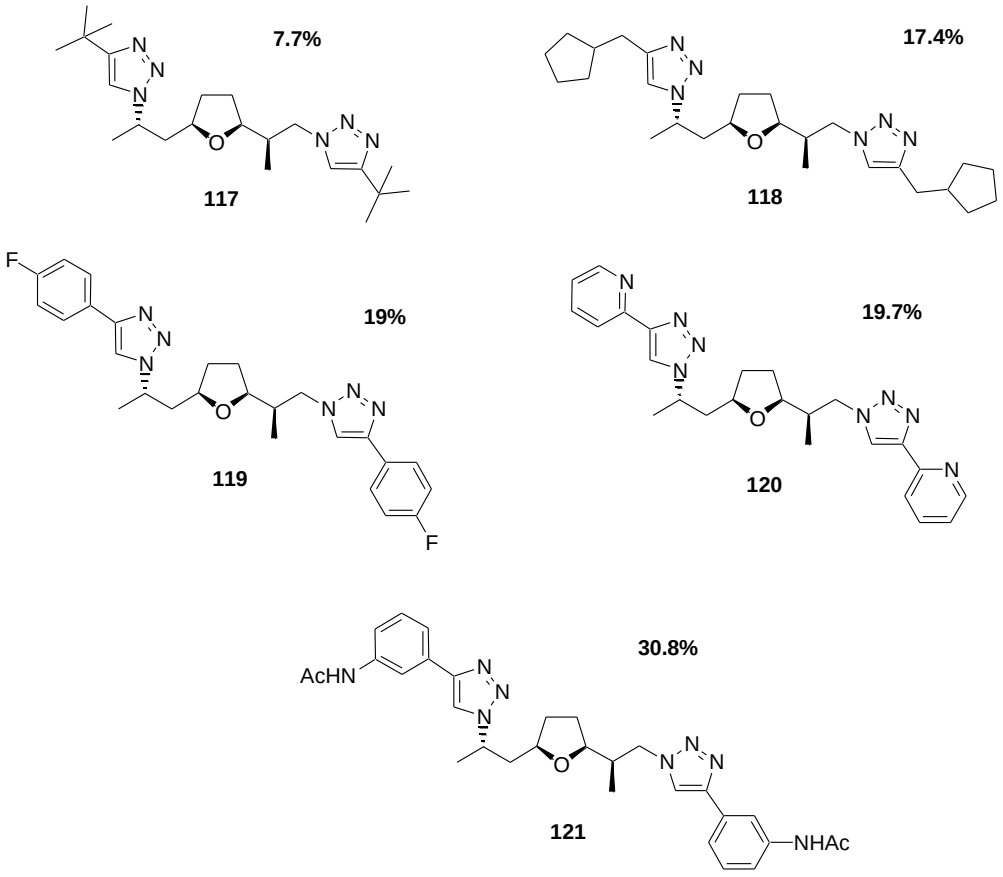


Figure 4.1: Overall yields for bis-triazole (±)-nonactic acid analogue library.

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).

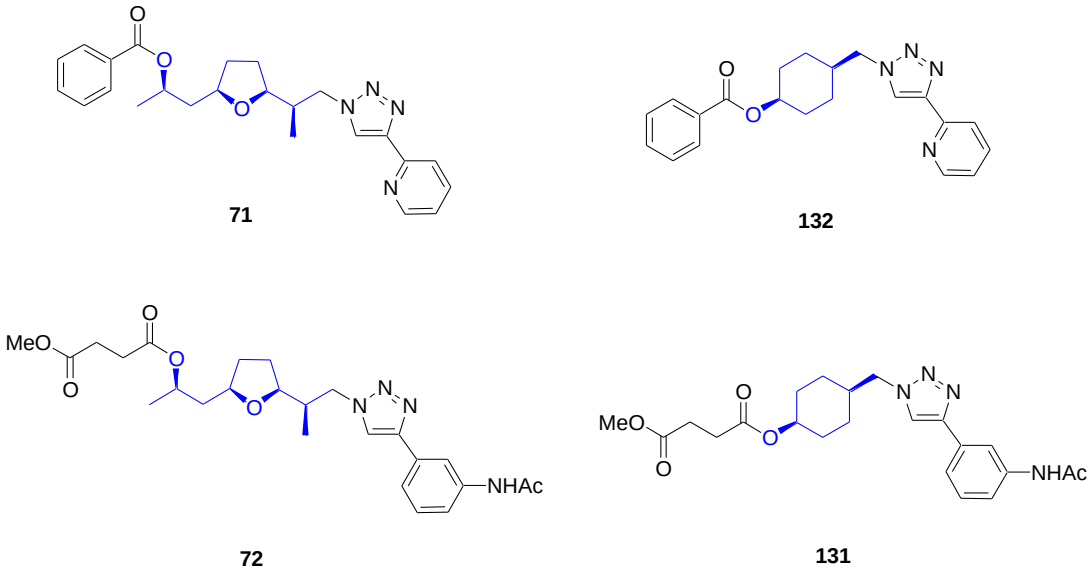


Figure 5.1a: Comparison of **72** and **71** to cyclohexane analogues **131** and **132**.

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.

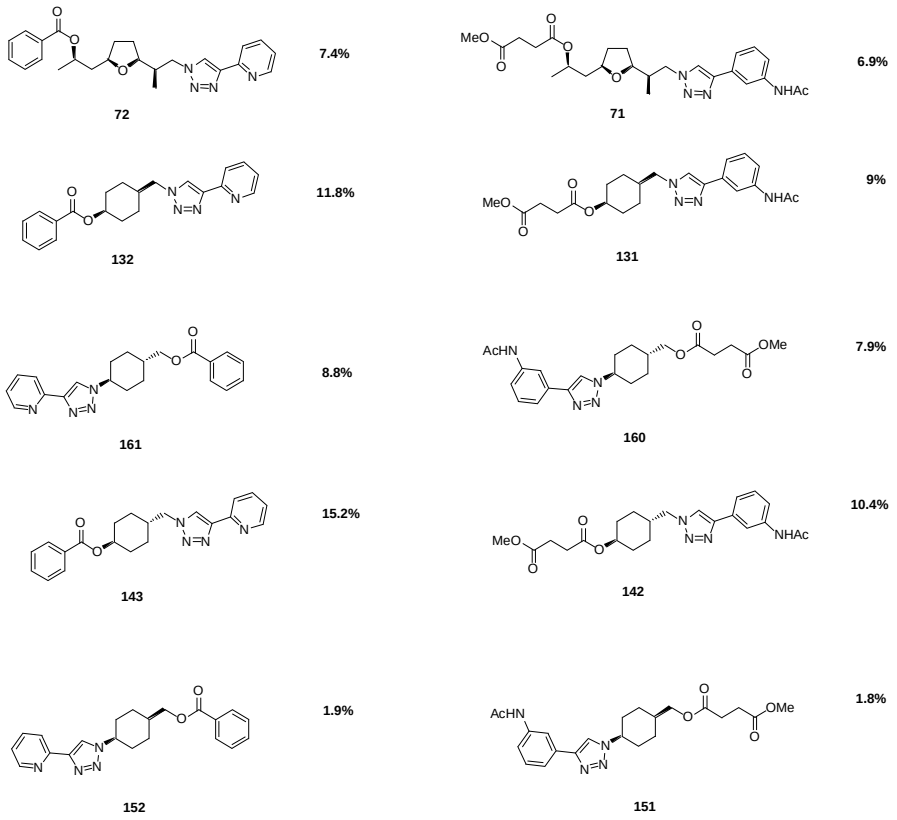
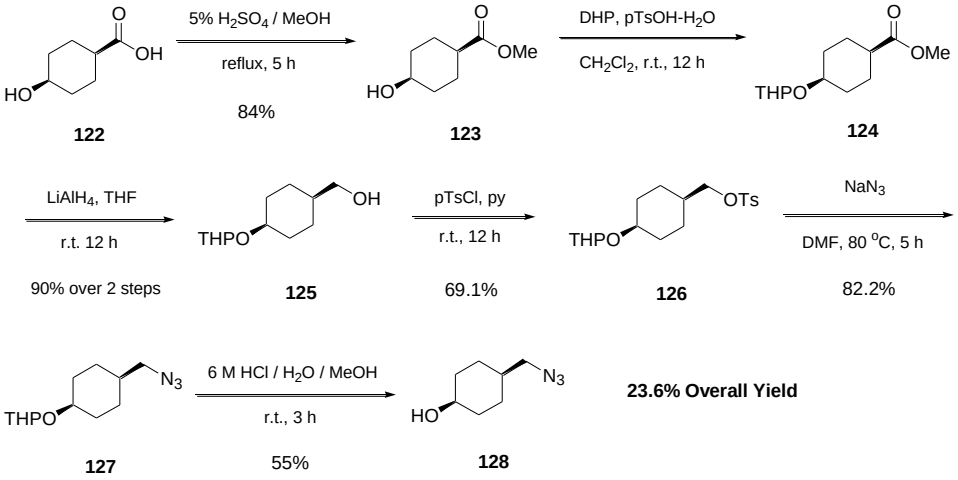


Figure 5.1b: **72** and **71** Comparison to the cyclohexane analogue library.

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

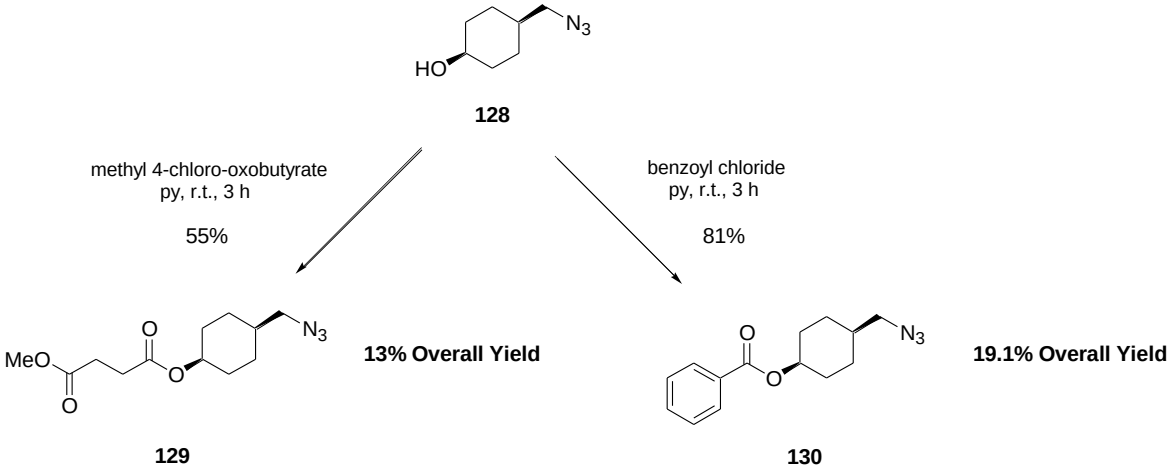


Scheme 5.1: Synthesis of the *cis*-4-azidomethyl-cyclohexanol scaffold **128**.

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 tosylated 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**.

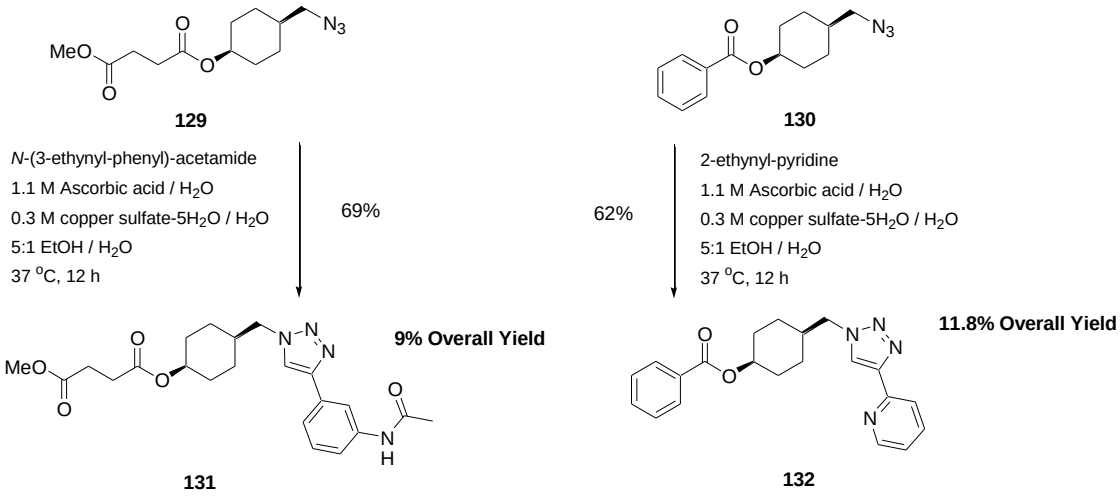
In order to approximate the structures and chemical reactivity of **72** and **71**, the methyl 4-chloro-4-oxobutyrate and benzoyl chlorides were used to synthesize the corresponding esters in respect to the **72** and **71** products (Scheme 5.2).



Scheme 5.2: Synthesis of the *cis*-4-azidomethyl-ester-cyclohexane scaffolds **129**, **130**.

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-ethynyl-phenyl)-acetamide and 2-ethynyl-pyridine respectively (Scheme 5.3). These gave the **72** and **71**-like triazolo-ester cyclohexane analogues **131** and **132**.



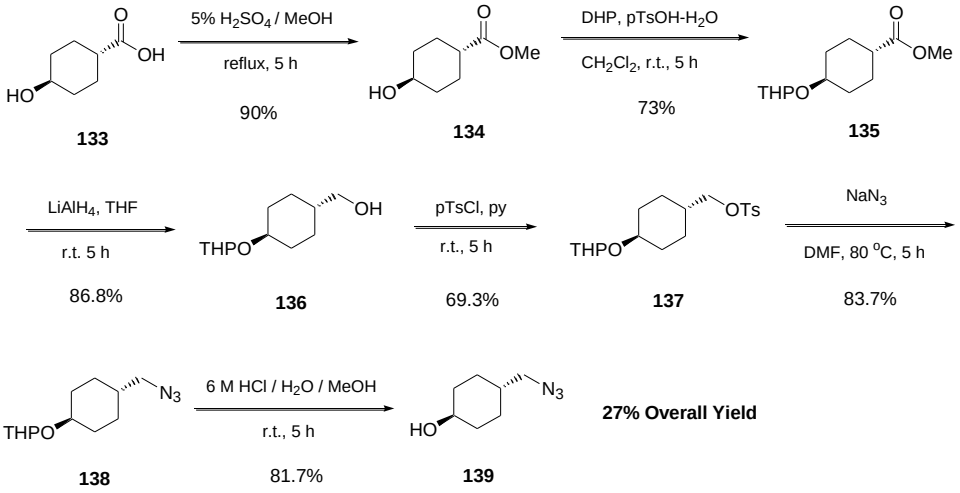
Scheme 5.3: Synthesis of the *cis*-4-triazolo-ester cyclohexane analogues **131**, **132**.

N-(3-Ethynyl-phenyl)-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.

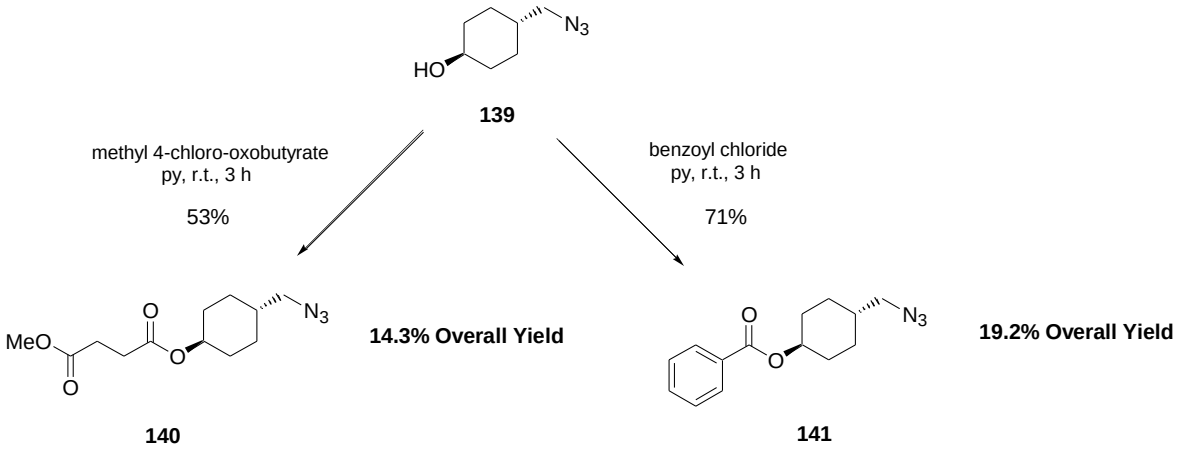
5.3 Synthesis of the *Trans*-4-azidomethyl-cyclohexanol scaffold



Scheme 5.4: 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.

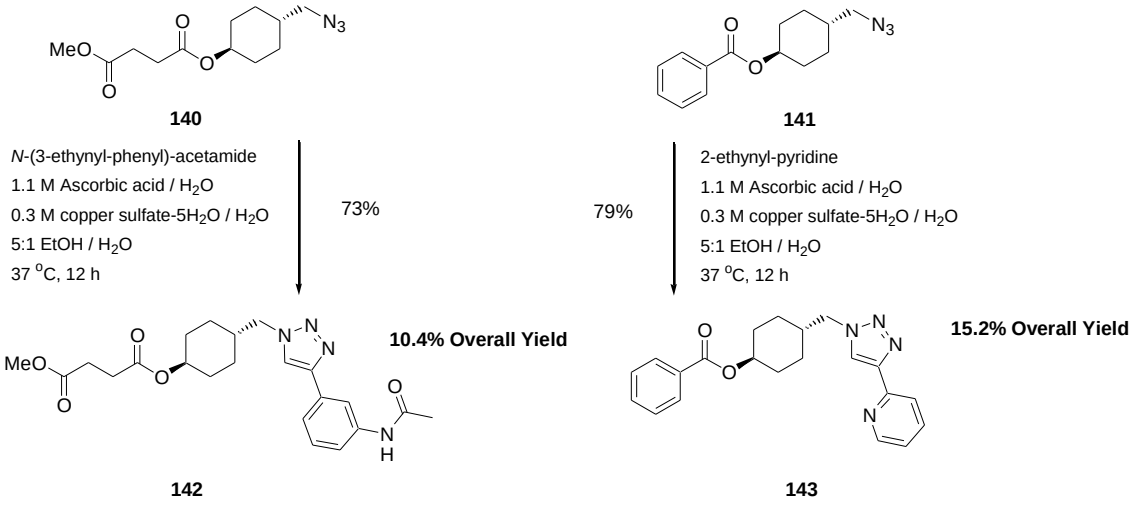


Scheme 5.5: Synthesis of the *trans*-4-azidomethyl-ester cyclohexane analogue scaffolds **140**, **141**.

Methyl 4-chloro-4-oxobutryate 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**.

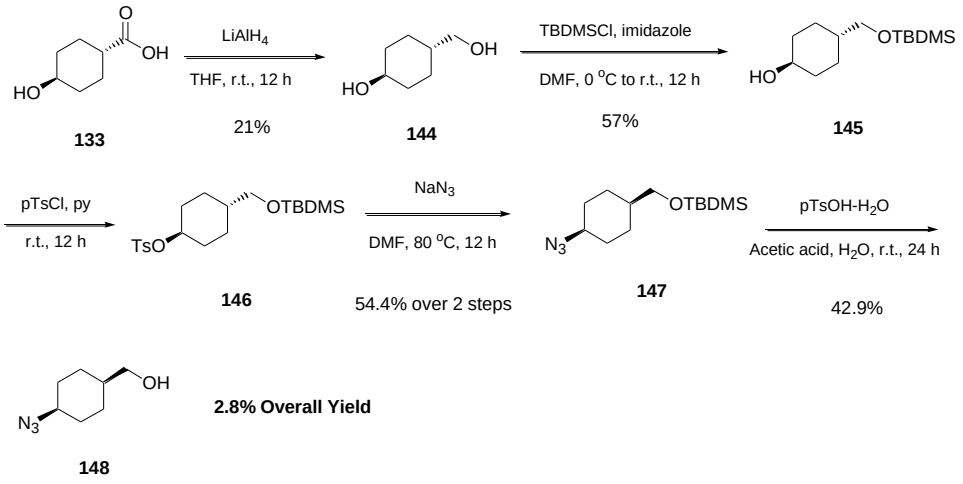
The *trans*-**71**-azidoester cyclohexane analogue **140** was added to *N*-(3-ethynyl-phenyl)-acetamide in 5:1 ethanol and water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was stirred at 37 °C for 12 hours which gave the *trans*-**71**-triazoloester cyclohexane 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

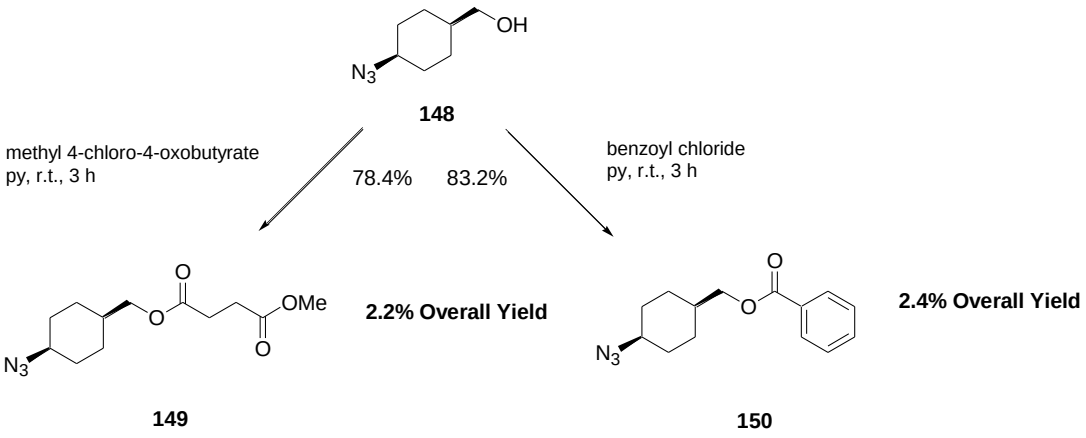
In order to cover the range of structures possible with the cyclohexane scaffold, two other scaffolds were synthesized (Schemes 5.7 and 5.10). These scaffolds are the *cis* and *trans* cyclohexanes with the azide directly connected to the ring. These syntheses involved an inversion of stereochemistry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

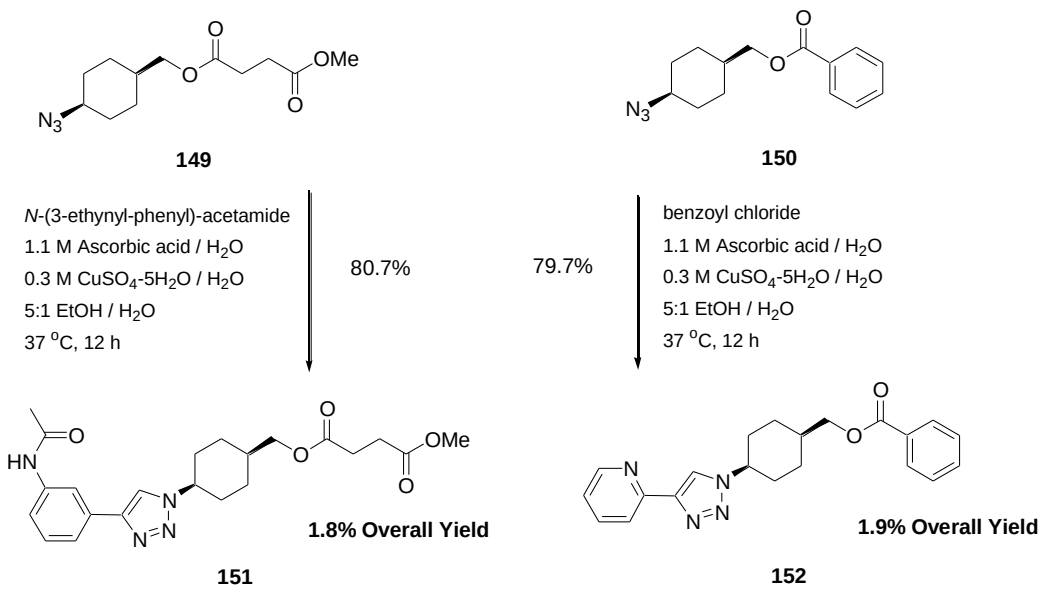
the tosylate product **146** was partially purified by chromatography on silica gel. Bimolecular nucleophilic substitution occurred on the secondary tosylated alcohol by the addition of the tosylated product to DMF, followed by sodium azide. The solution was warmed to 80 °C and stirred for 12 hours. 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.



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

Methyl 4-chloro-4-oxobutryate 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 *cis*-4-triazoloester cyclohexane analogues **151**, **152**.

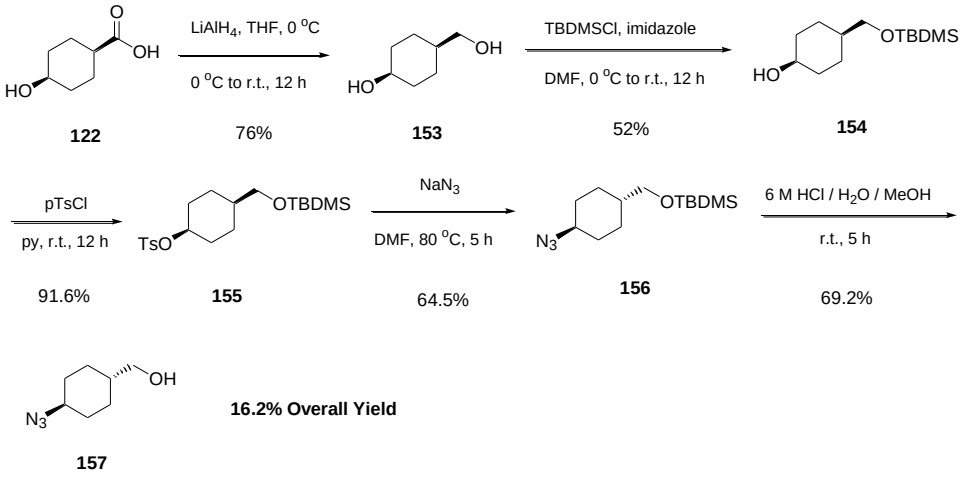
Cis-**71**-like azidoester cyclohexane analogue **149** was added to *N*-(3-ethynyl-phenyl)-acetamide in 5:1 ethanol and water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was shaken at 37 °C for 12 hours which gave an 80.7% yield after purification by chromatography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

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 *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 *B. subtilis*. The assayed showed that **152** had an IC_{99} of 125 μM against *B. subtilis*.

5.5 Synthesis of the *Trans*-(4-azido-cyclohexyl)-methanol scaffold

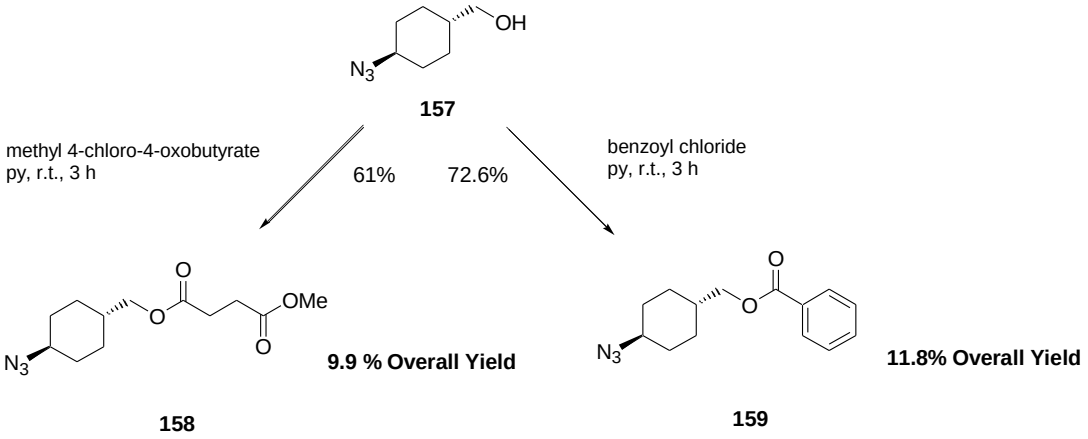
The final scaffold for the synthesis of the **72** and **71** triazoloester cyclohexane analogues was the *trans*-(4-azido-cyclohexyl)-methanol scaffold **157** (Scheme 5.10). As with the previous syntheses, the synthetic scheme involves an inversion of stereochemistry, therefore, the *trans* scaffold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

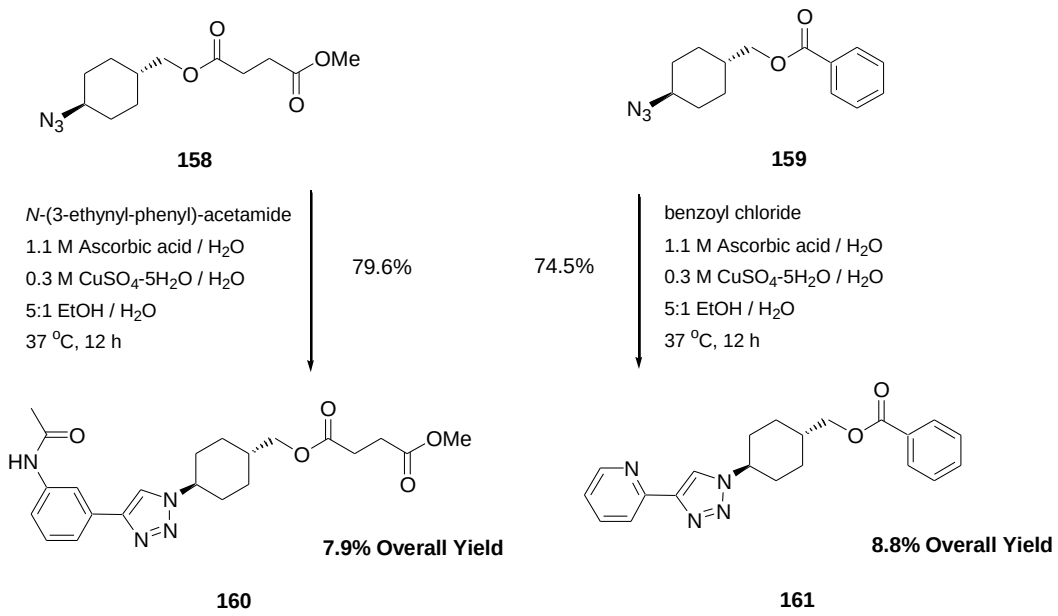
with sodium azide and warmed to 80 °C for 5 hours which after purification by chromatography on silica gel gave a 64.5% yield of the azido product **156**. The scaffold **157** was obtained after purification with a 69.2% yield after the deprotection of the primary alcohol with 6 M HCl in water and methanol 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**.

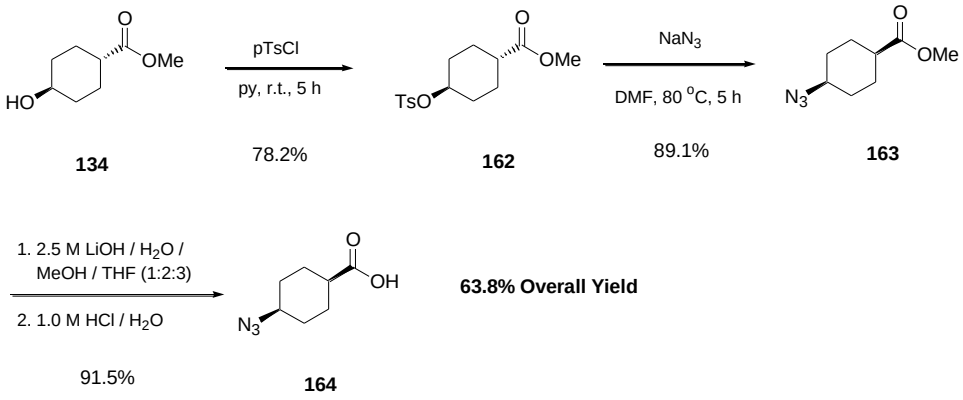
Trans-**71**-like azidoester cyclohexane analogue **158** was added to 5:1 ethanol in water, followed by *N*-(3-ethynyl-phenyl)-acetamide. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the solution was shaken at 37 °C for 12 hours. Purification by chromatography on silica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

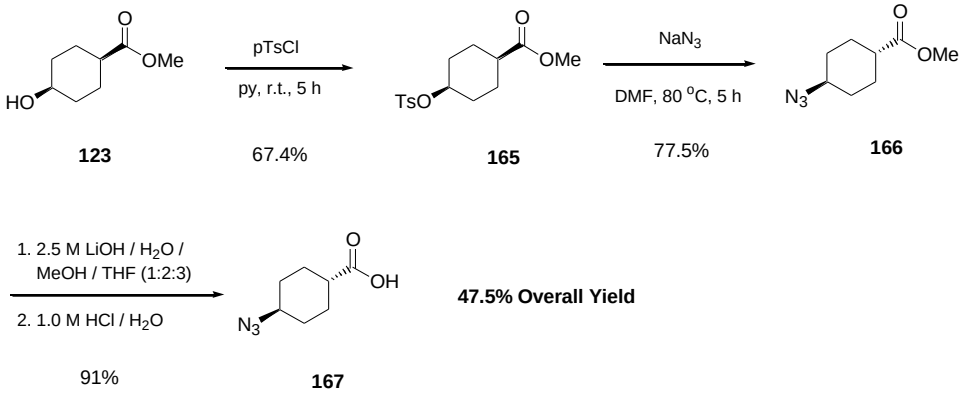
The four cyclohexane scaffolds synthesized and the **72** and **71**-like products made, illustrated that the methyl nonactate analogue subunit is important for the biological activity of **72** and **71**. More cyclohexane scaffolds were made on a large scale to illustrate this point. Both *cis* and *trans* versions of the azidocarboxylic 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**.



123 67.4% **165** 77.5% **166**

91% **167**

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.

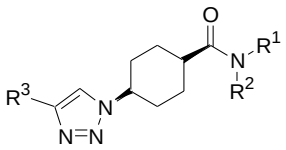


Figure 5.2: General structure of the triazoloamide cyclohexane analogues.

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

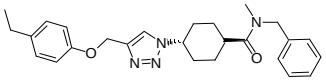
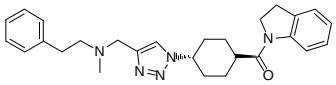
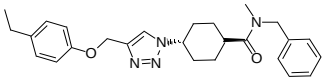
Designation	Compound	*B. subtilis*	*S. aureus*
18-C-12		250	250
18-C-5		250	250
18-G-3		250	250

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

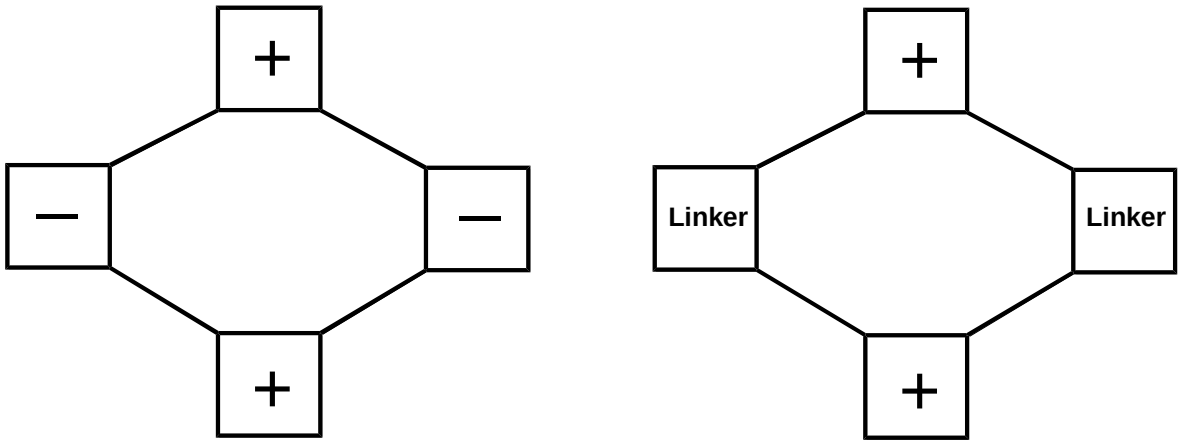
72 and **71**, it does suggest the importance of this chirally rich, natural product derived analogue to the activity of these compounds against these antibiotic resistant strains.

CHAPTER 6

OTHER NONACTIC ACID BASED STRUCTURES

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).



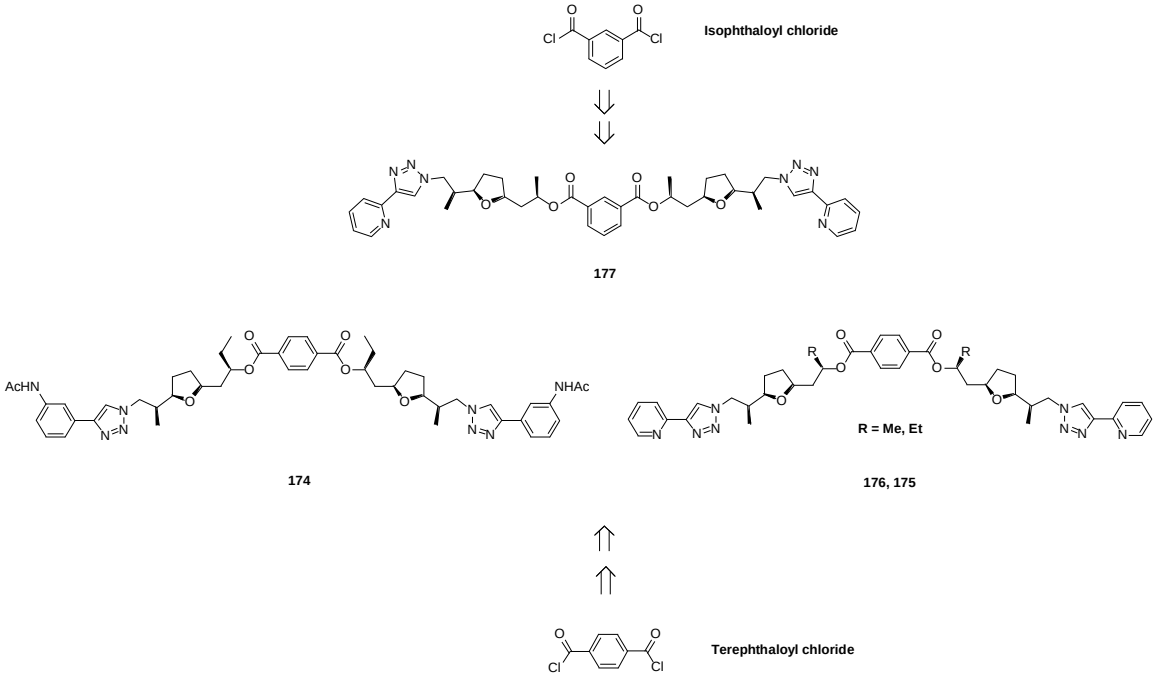
Nonactin, **40** Nonactin Analogues

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.

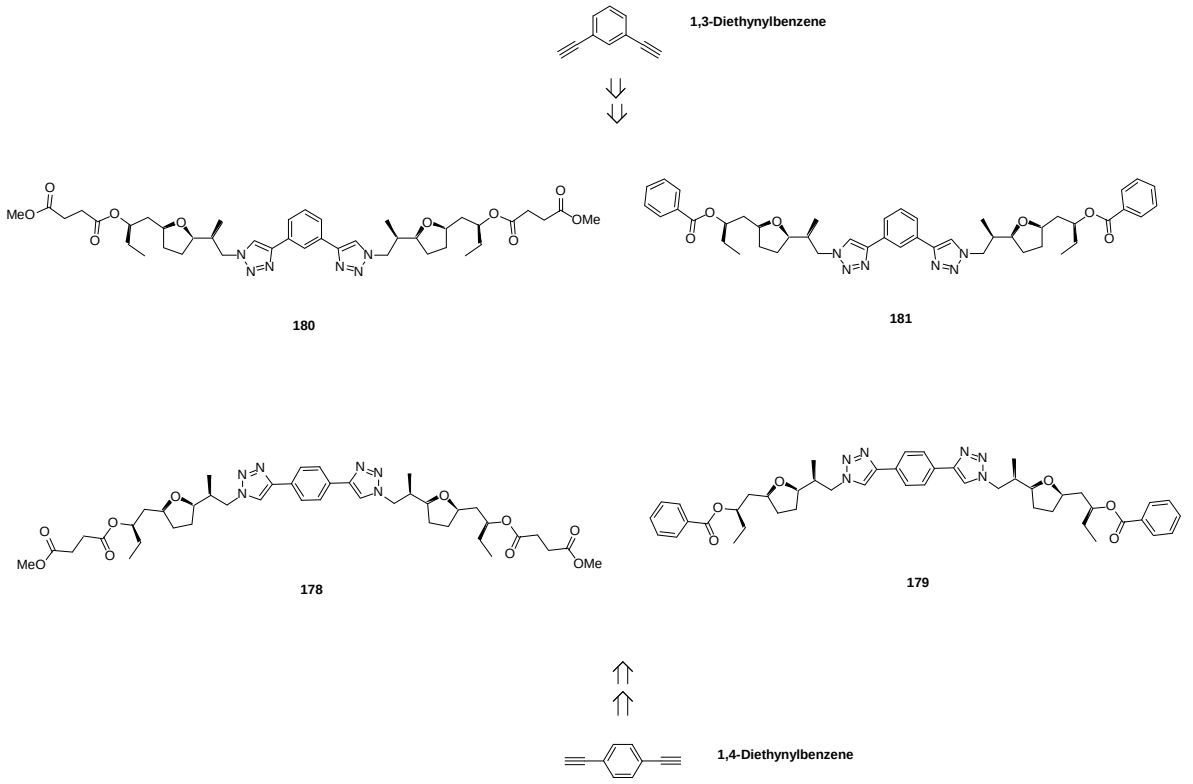
The first step in the synthesis of these macrolides was the synthesis of dimers in order to work out the coupling conditions necessary for their formation (Scheme 6.1 and 6.2).



Scheme 6.1: Overall 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

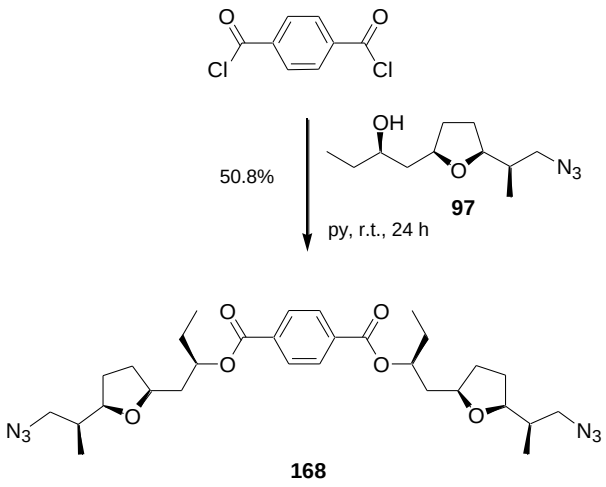
chloride and isophthaloyl chloride, to synthesize bis-ester bis-azide scaffolds which were then used to synthesize the dimer compounds (Scheme 6.1).



Scheme 6.2: Overall scheme of dimer compounds from 1,3-diethynylbenzene and 1,4-diethynylbenzene.

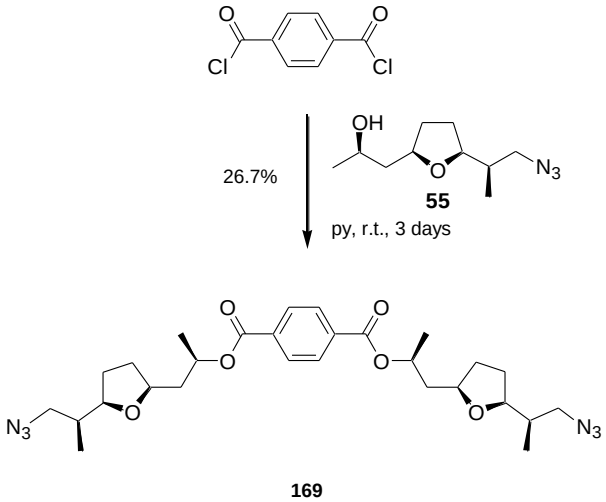
A second set of scaffolds were synthesized with two molecules of the azidoalcohol scaffolds, **55** and **97,** in conjunction with 1,3-ethynyl benzene and 1,4-ethynyl benzene (Scheme 6.2). Under Sharpless conditions this gave bis-triazole bis-hydroxy dimer compounds. These hydroxyl groups were then 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



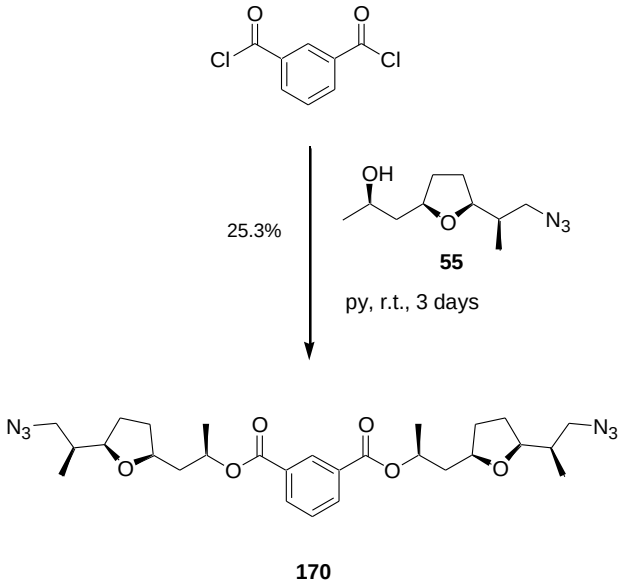
Scheme 6.3: Synthesis of Terephthalic acid bis-{1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl} ester scaffold **168**.

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).



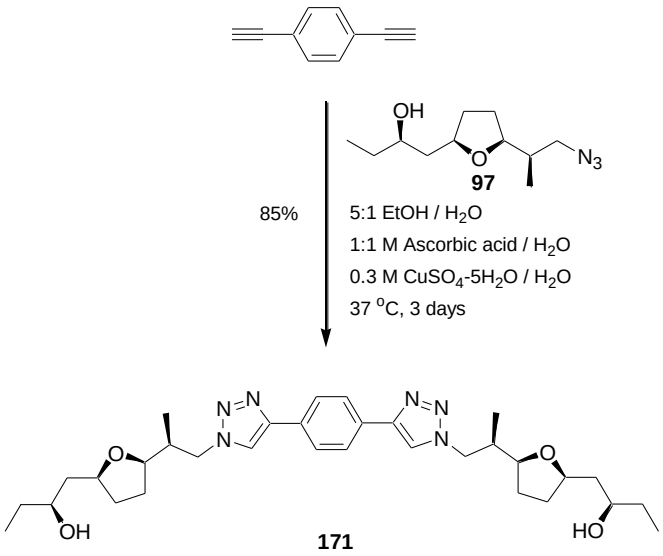
Scheme 6.4: Synthesis of Terephthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl ethyl} ester scaffold **169**.

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).



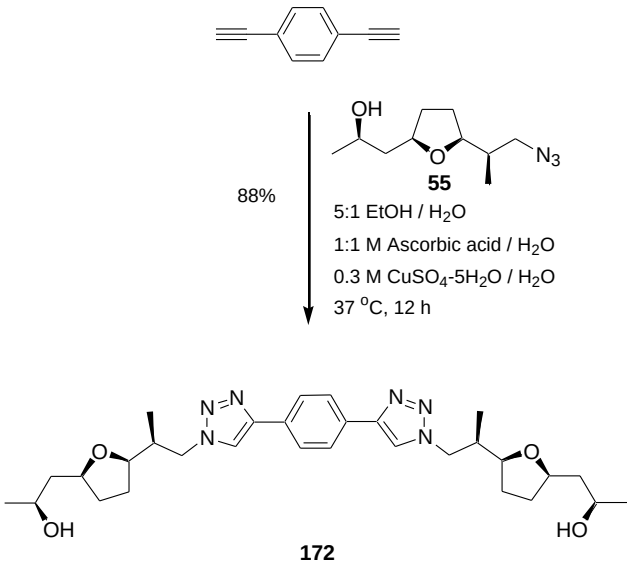
Scheme 6.5: Synthesis of Isophthalic acid bis-{2-[5-{2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester scaffold **170**.

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).



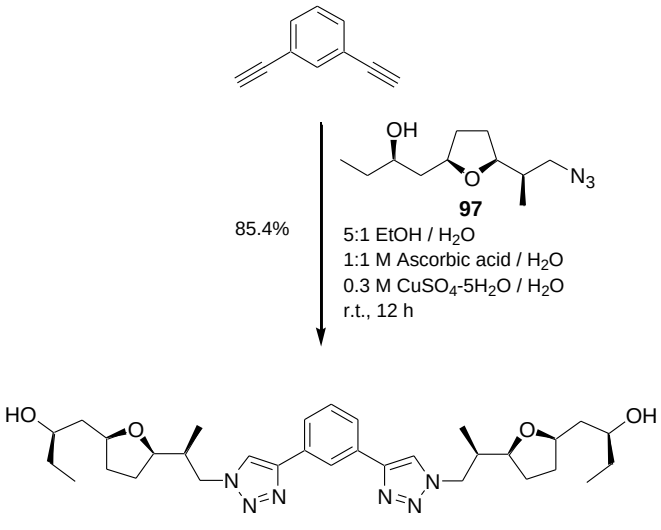
Scheme 6.6: Synthesis of 1,4-bis-triazolo-bis-alcohol homononactic acid analogue scaffold **171**.

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).



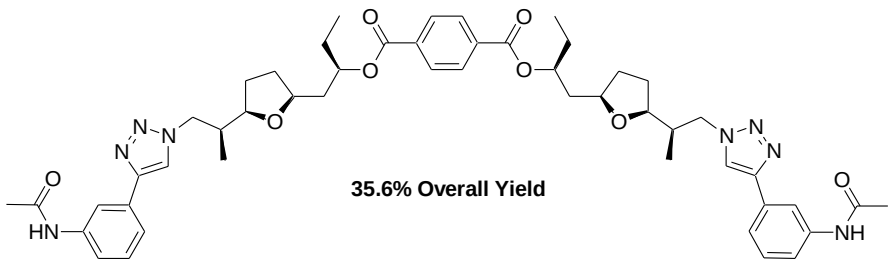
173

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 ºC 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.

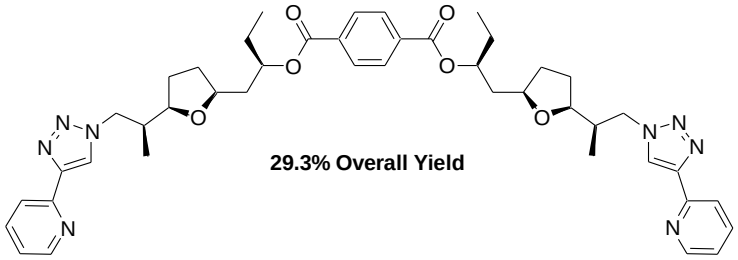
6.3 Synthesis of dimer-like derivatives of 72 and 71



174

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**.

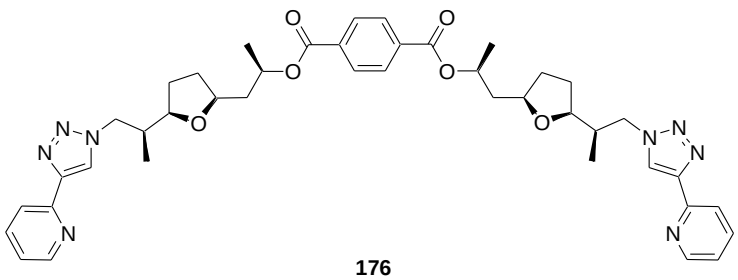
Dimer **174** was synthesized by the addition of **168** to 5:1 ethanol in water followed by *N*-(3-ethynyl-phenyl)-acetamide. 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added and the solution was shaken at 37 °C for 12 hours. Compound **174** was purified by chromatography on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



175

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**.

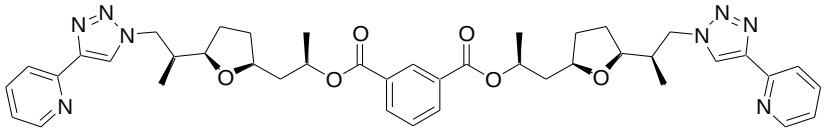
The 1,4-bis-ester-bis-triazole homomethyl nonactic acid analogue **175** was synthesized by the addition of **168** to 5:1 ethanol in water. 2-Ethynyl-pyridine was added with 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water and the solution was shaken for 12 hours at 37 °C and gave a 57.7% yield of **175** after purification by chromatography on silica gel (Figure 6.3).



176

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**.

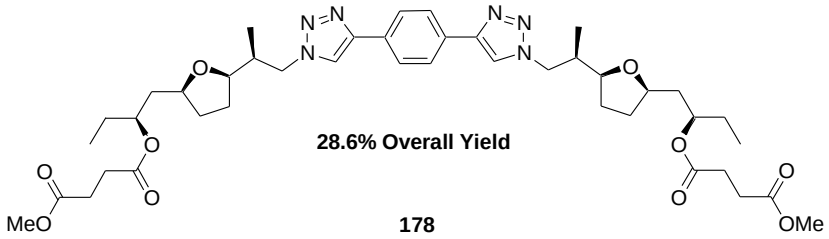
2-Ethynyl-pyridine was added to **169** in 5:1 ethanol in water followed by 1.1 M ascorbic acid in water and 0.3 M copper sulfate in water. The solution was stirred at room temperature for 12 hours. Purification by chromatography on silica gel gave **176** (Figure 6.4).



177 **21.5% Overall Yield**

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**.

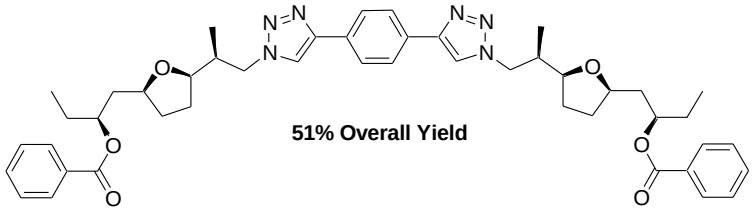
The 1,3-bis-ester-bis-triazole nonactic acid analogue **177** was synthesized by the addition of 2-ethynyl-pyridine to 5:1 ethanol in water followed by **170** . 1.1 M Ascorbic acid in water and 0.3 M copper sulfate in water were added, and the solution was stirred at room temperature for 12 hours. Purification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



178

Figure 6.6: Synthesis of 1,4-bis-triazole-bis-ester homononactic acid analogue **178**.

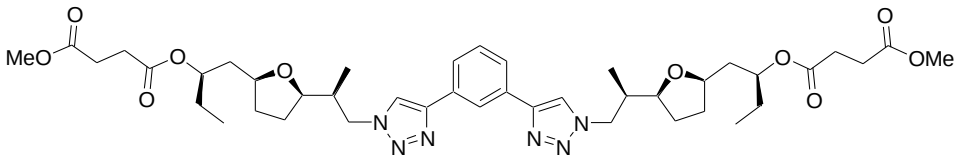
Methyl 4-chloro-4-oxobutyrate was added to **171** in pyridine at room temperature. The solution was stirred for 12 hours and the product was purified by chromatography on silica gel which gave a 33.7% yield of **178** (Figure 6.6).



179

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

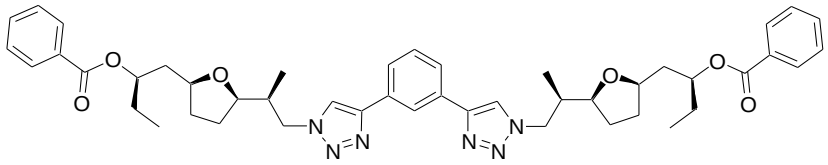
Benzoyl chloride was added to **171** in pyridine at room temperature and the solution was stirred for 12 hours. The reaction gave a 60% yield of **179** after purification by chromatography on silica gel (Figure 6.7).



180

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).



181

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.

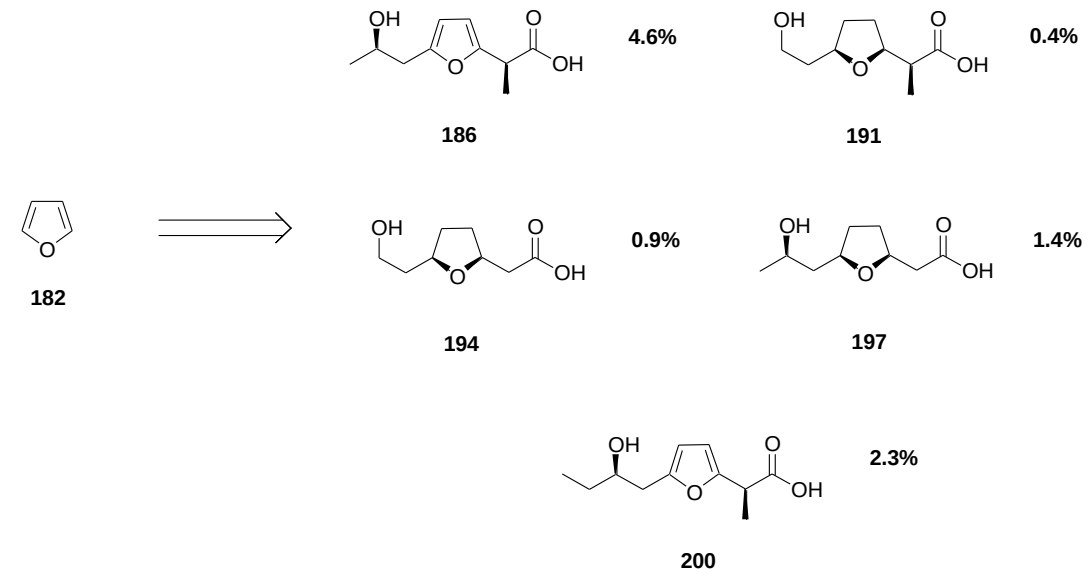
CHAPTER 7

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.

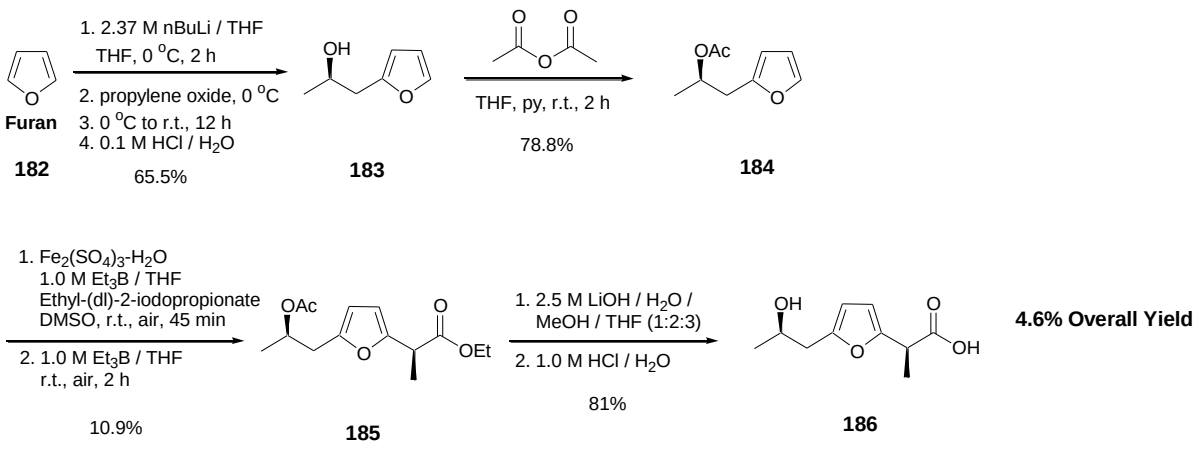
Nonactin **40** is a macrotetrolide which is assembled in vivo by *Streptomyces griseus* ETH A7796 from nonactic acid subunits. The resulting structure provides no easily modifiable chemical groups for easy synthetic transformations. Total synthesis of the macrotetrolide in which a chemical handle could 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 $Fe_2(SO_4)_3$-H_2O 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.[247] 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.[247] A blank sample (0.5 mL of ethanol) was added to the second fermentative cultures as a control culture.[247] 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).[247]

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.

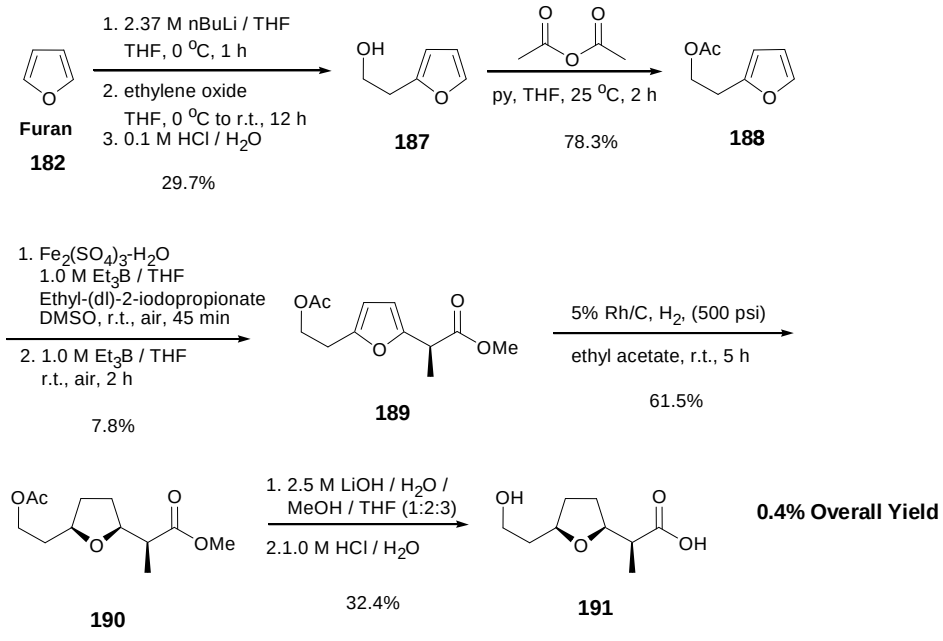
Secondly the data obtained from the reverse-phase HPLC quantification of the macrotetrolides present in the experimental culture (i.e. compound **186** added), revealed that nonactin **40** production in the fermentative cultures was reduced by more than 90% if either 10 mM or 1 mM of **186** was added. 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]

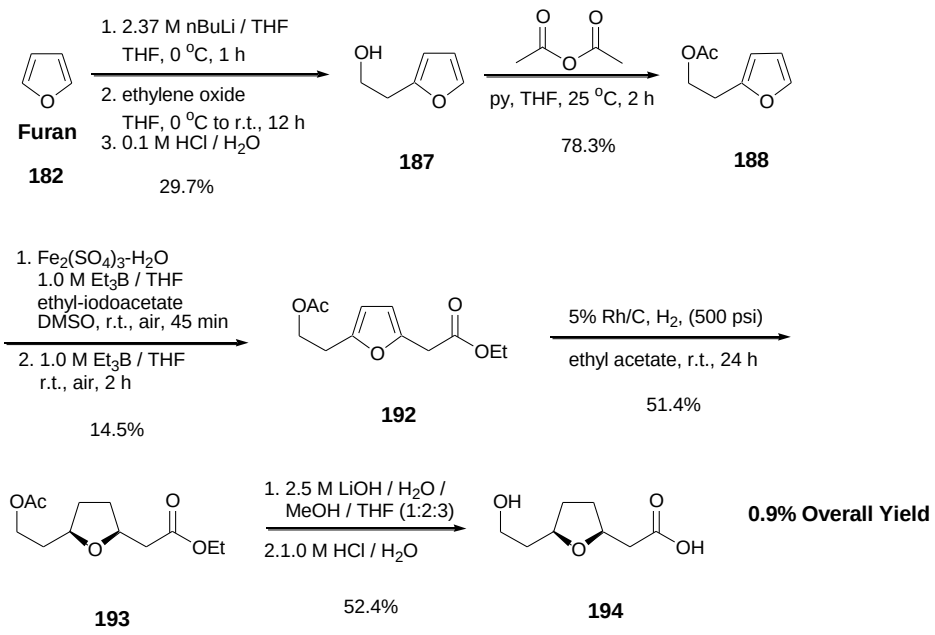
7.2 Nonactic acid derivatives for feeding studies to *Streptomyces griseus*

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**.

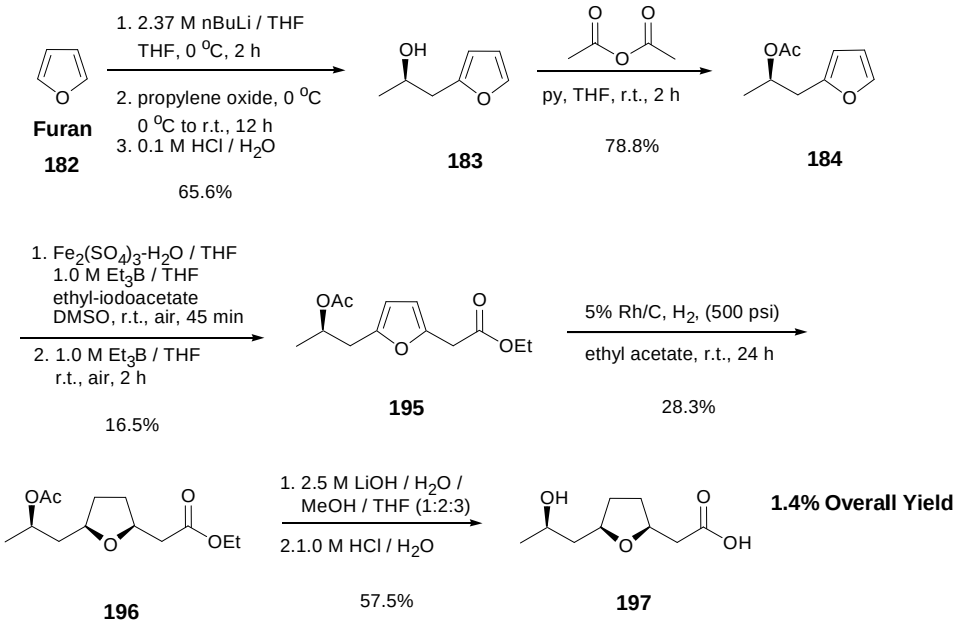
Compound **187** was synthesized by the addition of ethylene oxide to an anion generated from furan and purified by distillation to give a 29.7% yield. The primary alcohol **187** was acetylated with the addition of the alkylation product **187** to pyridine in THF at room temperature followed by acetic 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_2O and ethyl (dl)-2-iodopropionate. Air was blown through the solution, followed by the addition of 1.0 M Et_3B / THF. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et_3B / 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_2O / 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 $Fe_2(SO_4)_3$-H_2O and ethyl-iodoacetate. Air was bubbled through the solution followed by 1.0 M Et_3B / THF. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et_3B / 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

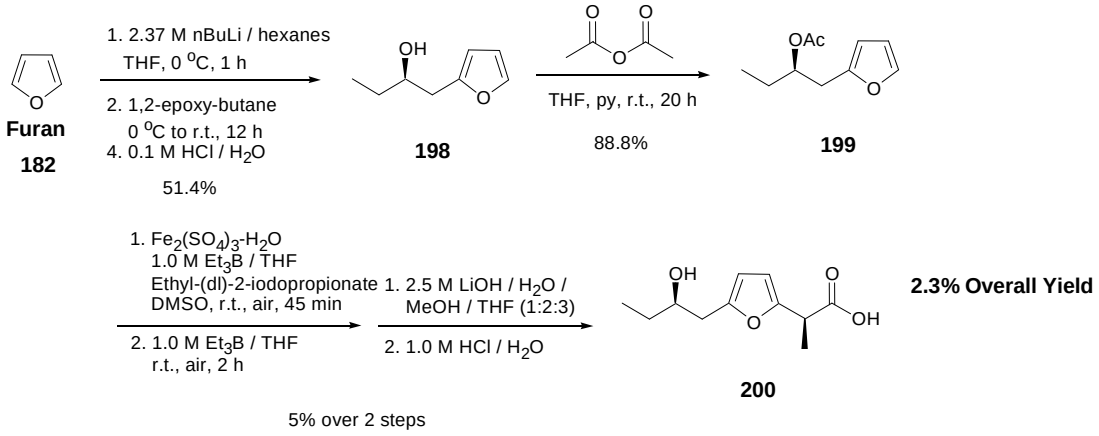
temperature and stirred for 24 hours. The product **193** was given after purification by chromatography on silica gel to give a 51.4% yield. The reduced furan bis-ester product **193** was saponified by 2.5 M LiOH / H_2O / MeOH / THF (1:2:3) and followed by 1.0 M HCl. Purification by chromatography 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 $Fe_2(SO_4)_3$-H_2O and ethyl-iodoacetate at room temperature. 1.0 M Et_3B / THF was added and air was bubbled through the solution. After 45 minutes, a second addition of 1.0 M Et_3B / 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 / H_2O / 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 $Fe_2(SO_4)_3$-H_2O and ethyl (dl)-2-iodopropionate. Air was bubbled through the solution and 1.0 M Et_3B /

THF was added. The solution was stirred for 45 minutes followed by a second addition of 1.0 M Et_3B / THF with stirring for 2 hours. The dialkylated product was purified by chromatography on silica gel and added to 2.5 M LiOH / H_2O / MeOH / THF (1:2:3) with stirring overnight. 1.0 M HCl / H_2O 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.

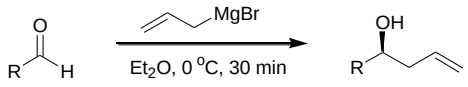
CHAPTER 8

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



201 **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).

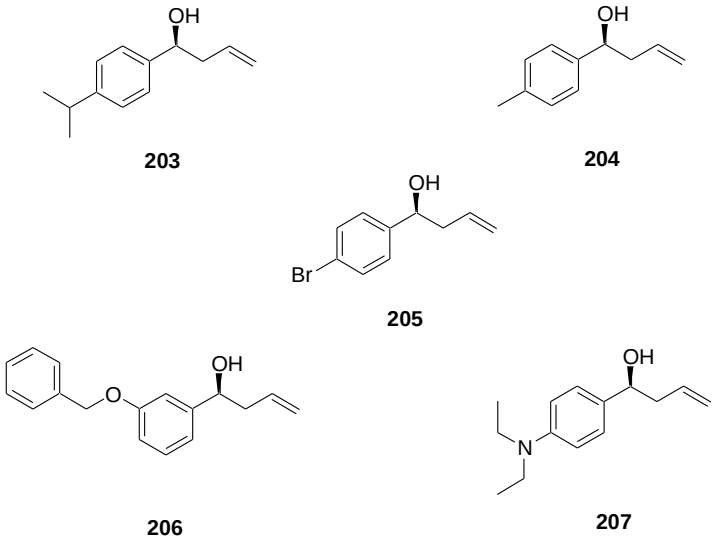
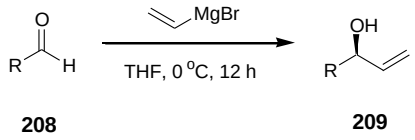


Figure 8.1: Racemic phenyl substituted allyl secondary alcohols library.



208 **209**

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-(diethyl-amino)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.

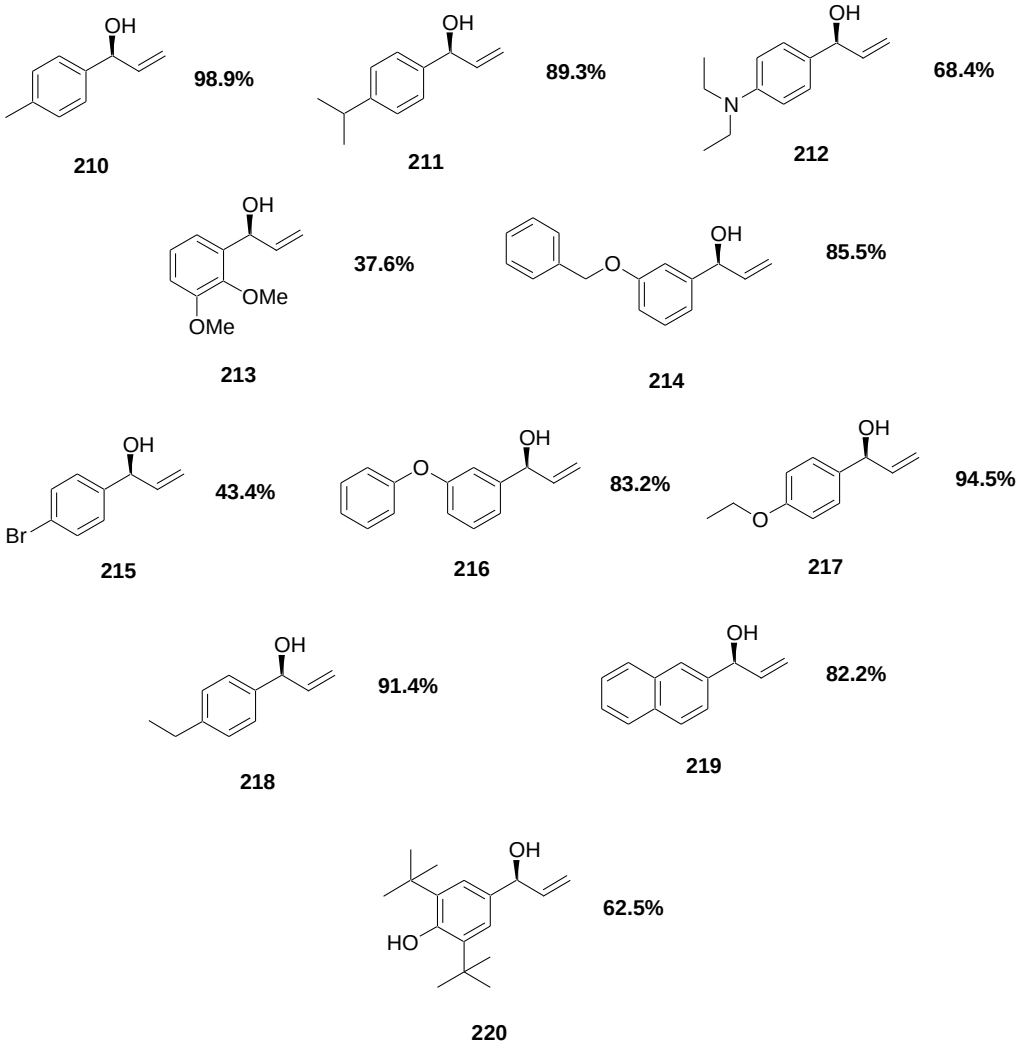
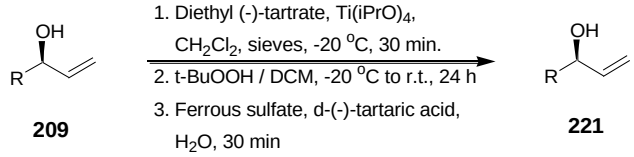


Figure 8.2: Racemic phenyl substituted vinyl secondary alcohols library.



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.

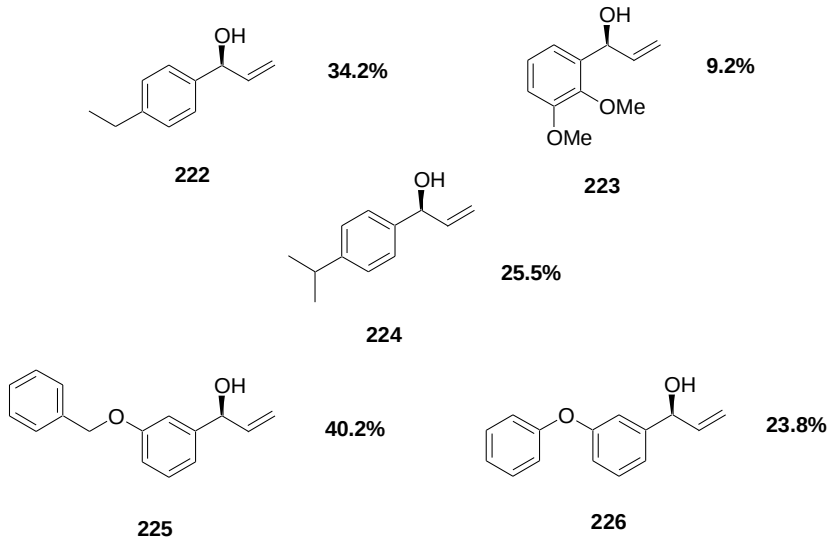
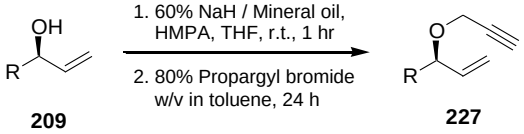


Figure 8.3: (S)-phenyl substituted vinyl secondary alcohols.

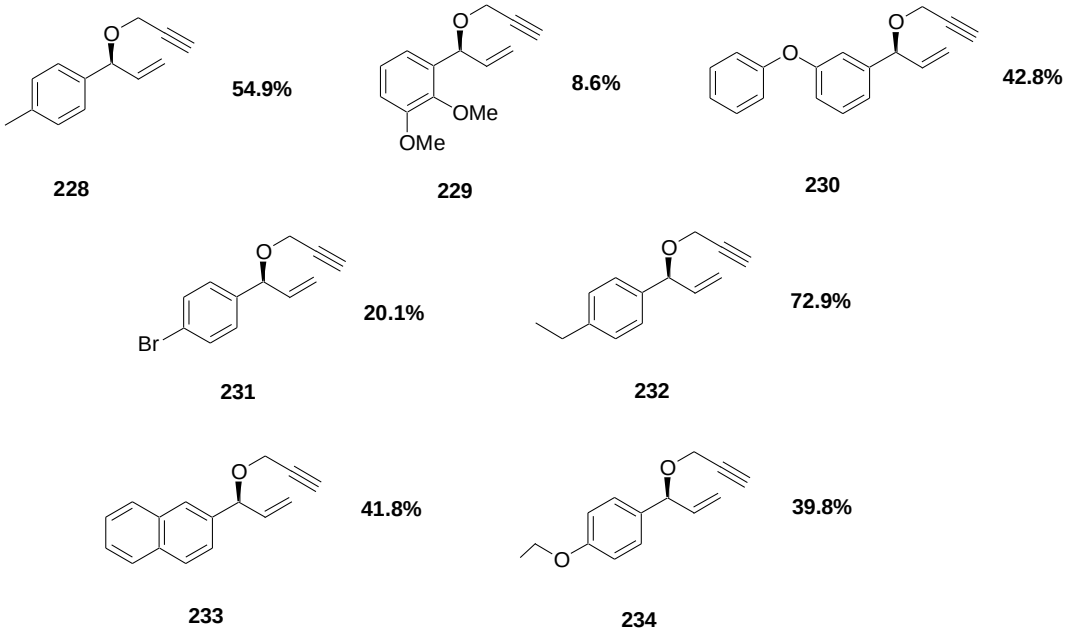
8.3 Synthesis of racemic phenyl substituted allyl and vinyl terminal alkyne ethers



209 **227**

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)

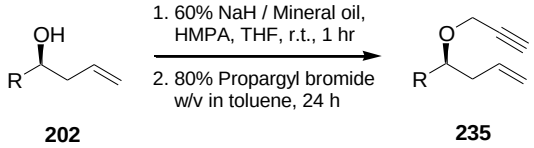


228 **229** **230**

231 **232**

233 **234**

Figure 8.4: Racemic phenyl substituted vinyl terminal alkyne ethers.



202 **235**

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).

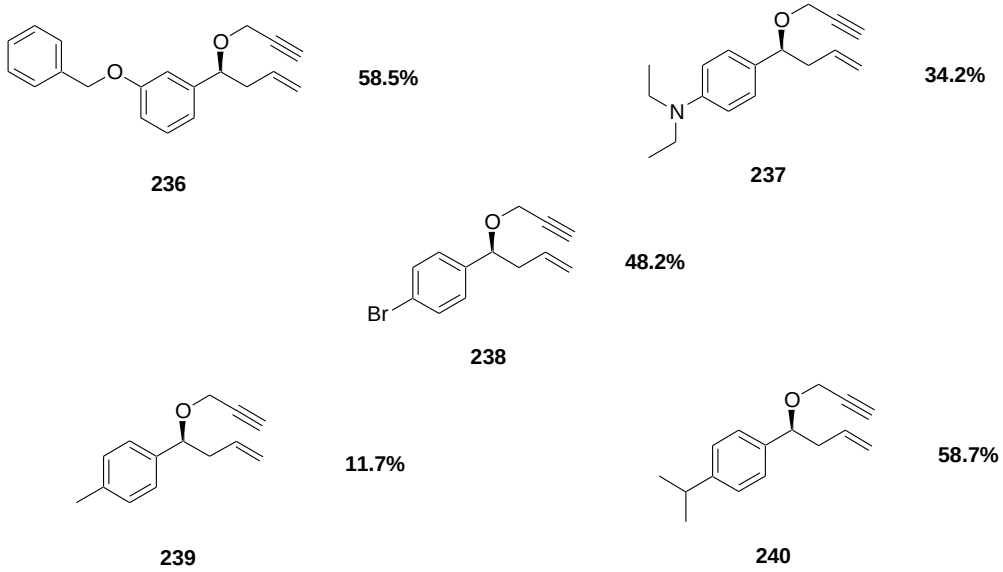
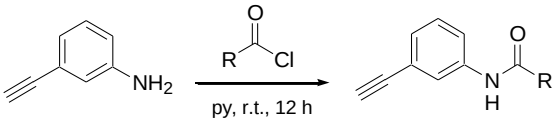


Figure 8.5: Racemic phenyl substituted allyl terminal alkyne ethers library.

8.4 Synthesis of *N*-(3-Ethynyl-phenyl)-acetamide derivatives

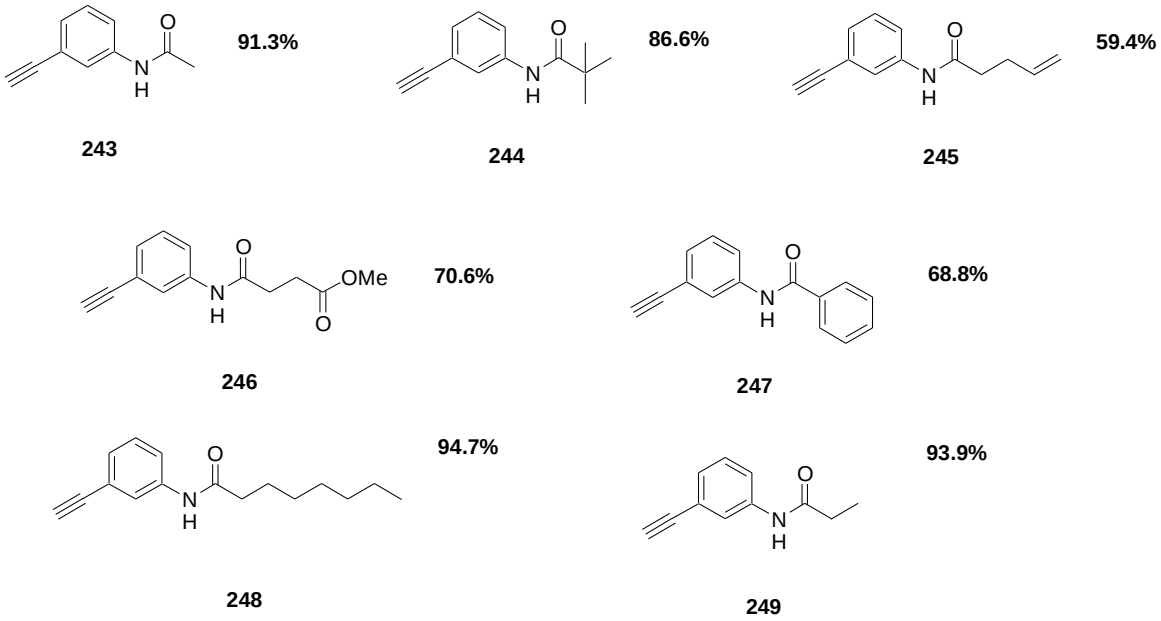


241 **242**

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 **243-249** (Figure 8.6) were purified by chromatography on silica gel in high yields.

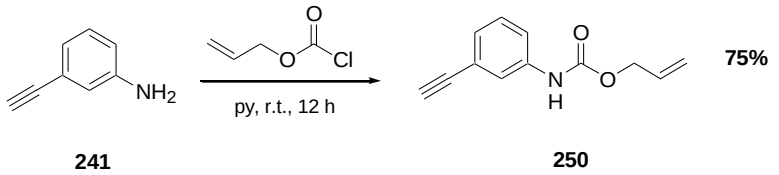


243 **244** **245**

246 **247**

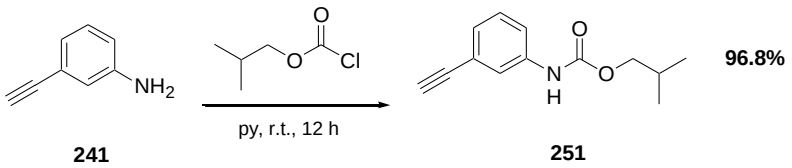
248 **249**

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 μM against *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.

CHAPTER 9

SUMMATION

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 Δ*nonS/*Δ*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.

CHAPTER 10

EXPERIMENTAL SECTION

10.1 General Methods

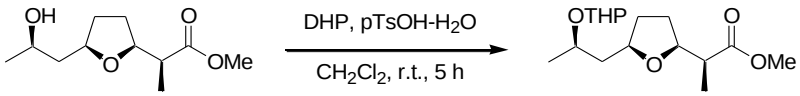
Reagents were obtained from various chemical companies (TCI, Sigma-Aldrich, Acros, and Fischer Scientific) and were used both with and without prior purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Dichloromethane was distilled from calcium hydride (CaH_2). Dimethylformamide (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-2*H*-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-H_2O), were run under anhydrous conditions in an argon atmosphere. A hydrogen (H_2) 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.

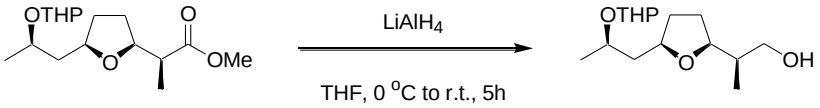
Characterization of synthesized compounds was performed on the following instruments: Varian Unity Plus 400 MHz spectrometer was used to obtain proton and carbon nuclear magnetic resonance (NMR) spectra. Infrared spectra (IR) were obtained using the Nicolet Nexus 670 FT-IR using the SMART MIRacle attachment. 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

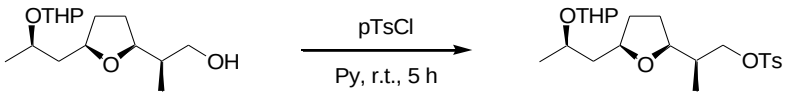


51: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propionic acid methyl ester* **51**. Racemic methyl nonactate **45** (0.624 g, 2.9 mmol) was added to CH_2Cl_2 (15 mL) followed by DHP (1.3 mL, 14.4 mmol) and pTsOH-H_2O (6.0 mg, 0.03 mmol). The solution was stirred for 5 hours at 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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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);

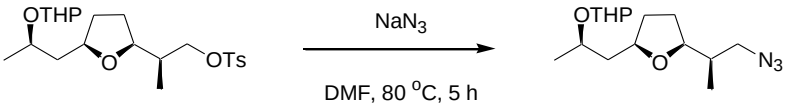
^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 99.8, 94.5, 80.4, 80.3, 77.3, 77.0, 76.4, 72.6, 68.0, 63.0, 62.0, 51.54, 51.51, 45.4, 45.4, 44.1, 43.8, 31.5, 31.3, 31.1, 31.0, 28.6, 28.5, 25.51, 25.46, 22.7, 20.2, 19.5, 19.4, 13.4, and 13.3; IR (ATR) 2941, 2873, 1739, 1456, 1436, 1375, 1352, 1260, 1200, 1163, 1118, 1063, 1021, 992, 942, 905, 869, and 813 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{16}H_{28}O_5Na^+$: 323.18; found 323.35.



52: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propan-1-ol* **52**. **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 ºC. 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 $NaOH/H_2O$ (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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{28}O_4Na^+$: 295.19; found 295.34.

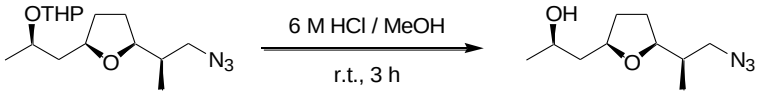


53: *Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propyl ester* **53**. **52** (0.576 g, 2.1 mmol) was added to pyridine (10 mL) followed by pTsCl (0.806 g, 4.2 mmol) at room temperature and the resultant solution was stirred for 5 hours. Water (50 mL) was 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 (Na_2SO_4) 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{22}H_{34}O_6SNa^+$: 449.20; found 449.40.



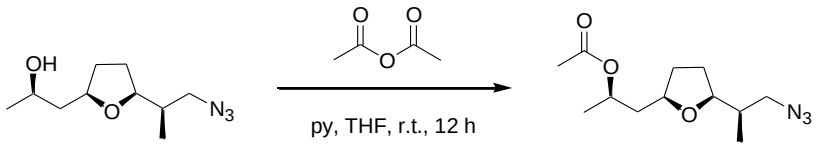
54: *2-{2-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethoxy}-tetrahydro-pyran* **54**. **53** (0.718 g, 1.7 mmol) was added to DMF (7 mL) followed by

NaN_3 (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 ($MgSO_4$), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{15}H_{27}N_3O_3Na^+$: 320.20; found 320.37.

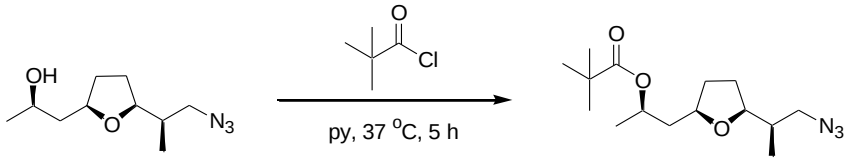


55: *1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-propan-2-ol* **55**. **54** (0.404 g, 1.4 mmol) was added to 6 M HCl/MeOH (10 mL) and stirred at room temperature for 3 hours. A saturated solution of sodium bicarbonate was added until no gas evolved. The solution was then washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried ($MgSO_4$), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{10}H_{19}N_3O_2Na^+$: 236.14; found 236.28.

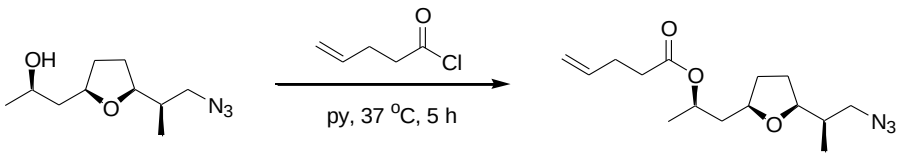


57: *Acetic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* **57**. **55** (35.0 mg, 0.2 mmol) was added to THF (1 mL) followed by pyridine (2 mL) and acetic anhydride (2 mL). The solution was stirred for 12 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_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

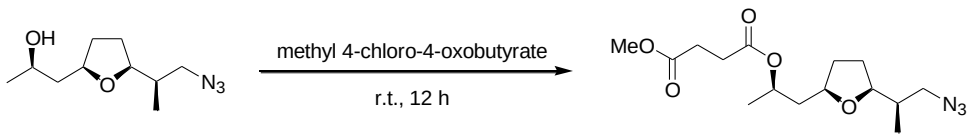


58: *2,2-Dimethyl-propionic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* **58**. **55** (35.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by trimethylacetyl chloride (0.2 mL, 1.6 mmol). The solution was then

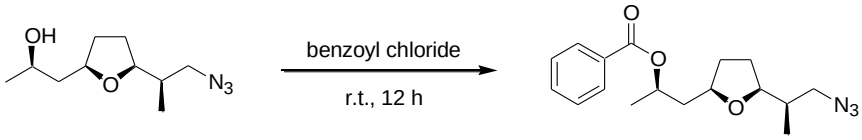
shaken at 37 °C for 5 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **58** (32.0 mg, 65.7%). R_f = 0.80 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) 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 (Na_2SO_4), 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%). R_f = 0.73 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) 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

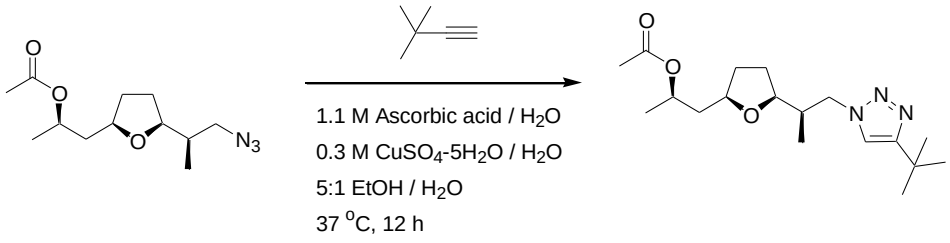


60: *Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester* **60**. **55** (0.119 g, 0.6 mmol) was added to pyridine (3 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.6 mmol). The solution was stirred at room temperature overnight. Chloroform (50 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 (Na_2SO_4), 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%). R_f = 0.43 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{15}H_{25}N_3O_5Na^+$: 350.17; found 350.21.



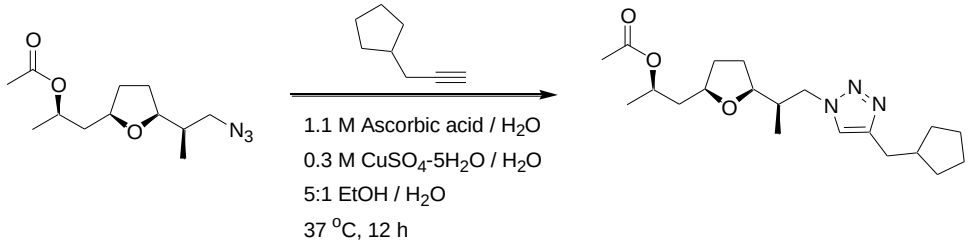
61: *Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* **61**. **55** (0.119 g, 0.6 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (0.18 mL, 1.6 mmol). The solution was stirred at room temperature overnight. Chloroform (50 mL) was added and the solution was washed with

saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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, $CDCl_3$) δ 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, $CDCl_3$) 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, 1603, 1585, 1451, 1381, 1356, 1314, 1274, 1175, 1109, 1069, 1026, and 711 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{17}H_{23}N_3O_3H^+$: 318.18; found 318.23.

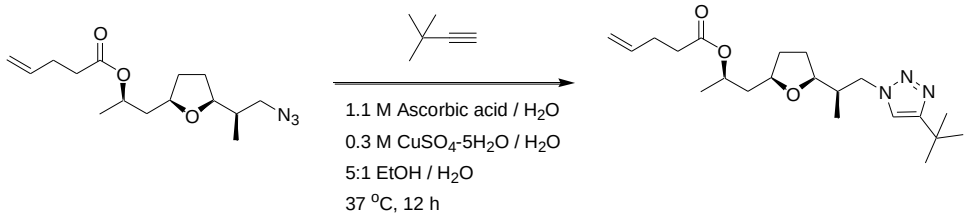


63: *Acetic acid 2-{5-[2-(4-tert-butyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester* **63**. **57** (16.2 mg, 0.06 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 79.0 µL, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 127.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_4), 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-

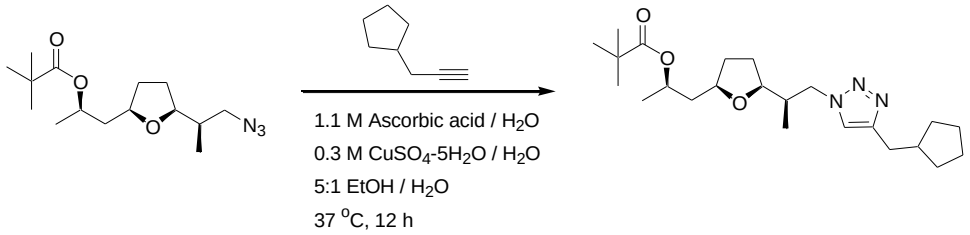
hexanes (1:1)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (s, 1H), 5.09 (m, 1H), 4.51 (m, 1H), 4.22 (m, 1H), 3.85 (m, 1H), 3.43 (m, 1H), 2.03 (s, 3H), 1.99 (m, 3H), 1.80 (m, 1H), 1.70 (m, 1H), 1.53 (m, 2H), 1.34 (s, 9H), 1.26 (d, J = 6.2 Hz, 3H), and 0.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 119.55, 119.52, 80.8, 76.0, 68.9, 53.1, 42.4, 40.3, 31.3, 30.7, 30.4, 29.4, 21.4, 20.7, and 14.3



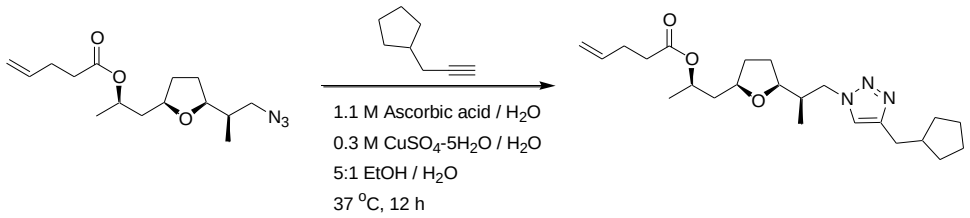
64: *Acetic acid 2-{5-[2-(4-cyclopentylmethyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester* **64**. **57** (16.2 mg, 0.06 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 84.0 µL, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 127.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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



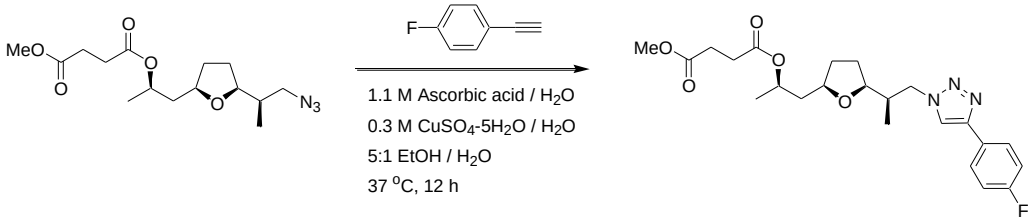
65: *Pent-4-enoic acid 2-{5-[2-(4-tert-butyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester* **65**. **59** (22.3 mg, 0.08 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 94.0 µL, 0.8 mmol). Ascorbic acid/H_2O (1.1 M, 151.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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/H_2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 122.0 µL, 0.9 mmol). Ascorbic acid/H_2O (1.1 M, 185.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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%). R_f = 0.50 (EtOAc-hexanes (1:1)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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

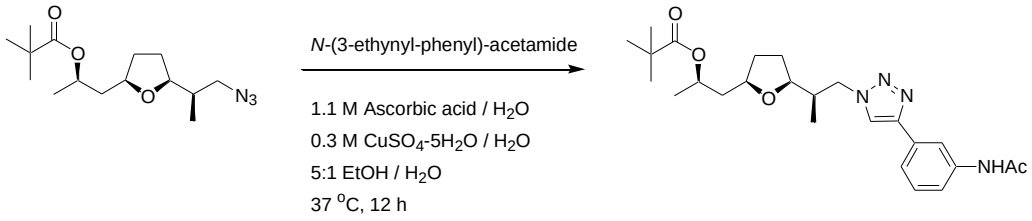


67: *Pent-4-enoic acid 2-{5-[2-(4-cyclopentylmethyl-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-yl}-1-methyl-ethyl ester* **67**. **59** (22.3 mg, 0.08 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 100.0 µL, 0.8 mmol). Ascorbic acid/H_2O (1.1 M, 151.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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



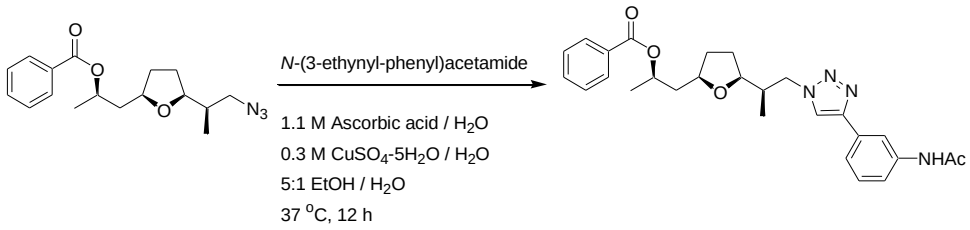
68: *Succinic acid 2-(5-{2-[4-(4-fluoro-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester* **68**. **60** (22.4 mg, 0.07 mmol)

was added to 5:1 EtOH/H_2O (1.5 mL) followed by 1-ethynyl-4-fluorobenzene (78.0 µL, 0.7 mmol). Ascorbic acid/H_2O (1.1 M, 137.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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%). R_f = 0.38 (EtOAc-hexanes (1:1)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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



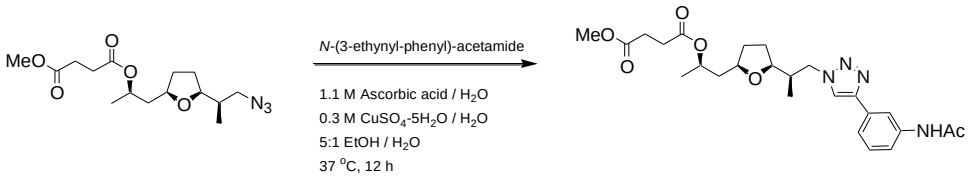
69: *2,2-Dimethyl-propionic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester* **69**. **58** (27.6 mg, 0.09 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by *N*-(3-ethynyl-pyridine)-acetamide (147.0 mg, 0.9 mmol). Ascorbic acid/H_2O (1.1 M, 185.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give **69** (32.4 mg, 76.6%). R_f = 0.51 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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



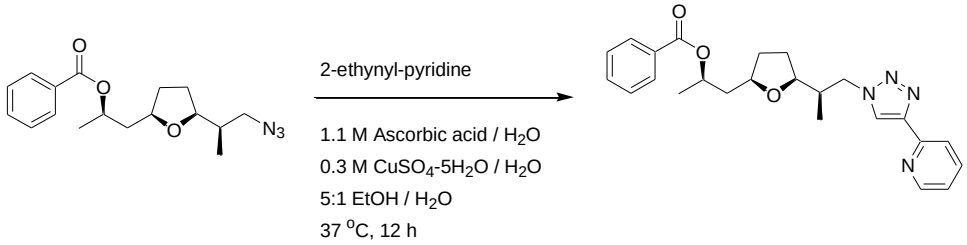
70: *Benzoic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester* **70**. **61** (31.7 mg, 0.01 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by *N*-(3-ethynyl-pyridine)-acetamide (159.0 µL, 1.0 mmol). Ascorbic acid/H_2O (1.1 M, 200.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give **70** (43.5 mg, 91.4%). R_f = 0.49 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) 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),

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, $CDCl_3$) δ 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



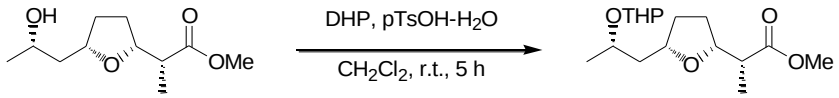
71: *Succinic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester* **71**. **60** (21.0 mg, 0.1 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (102.0 mg, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 128.0 µL, 0.1 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{25}H_{34}N_4O_6H^+$: 487.26; found 487.27.

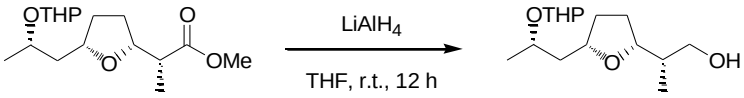


72: *Benzoic acid 1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester* **72**. **61** (10.18 mg, 0.03 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 2-ethynyl-pyridine (98+%, 33.0 µL, 0.3 mmol). Ascorbic acid/H_2O (1.1 M, 64.0 µL, 0.07 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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 $C_{24}H_{28}N_4O_3H^+$: 421.22; found 421.24.

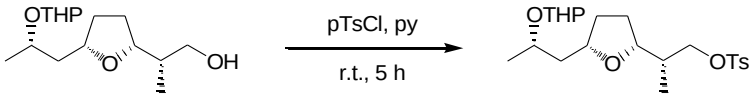


73: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propionic acid methyl ester* **73**. Methyl (–)–nonactate **48** (0.704 g, 3.3 mmol) was added to methylene chloride (13 mL) followed by DHP (1.5 mL, 16.3 mmol) and pTsOH-H_2O (6.0 mg, 0.03 mmol). The solution was stirred at room 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}H NMR (400 MHz, $CDCl_3$) δ 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}C NMR (100 MHz, $CDCl_3$) δ 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 $C_{16}H_{28}O_5Na^+$: 323.18; found 323.24.



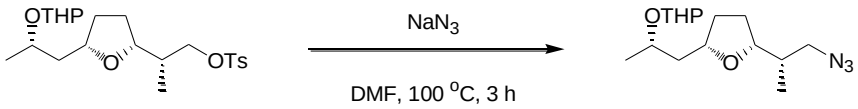
74: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propan-1-ol* **74**. **73** (0.824 g, 2.8 mmol) was added to THF (30 mL) followed by (0.208 g, 5.5 mmol) of lithium aluminum hydride at room temperature and then the solution was stirred overnight. Water (5.5 mL) was added, followed by 3.0 M NaOH/H_2O (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, $CDCl_3$) δ 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, 11H), 1.23 (m, 2H), 1.10 (m, 2H), and 0.78 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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, 1116, 1075, 1021, 991, 943, 905, 869, 811 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{15}H_{28}O_4Na^+$: 295.19; found 295.24.

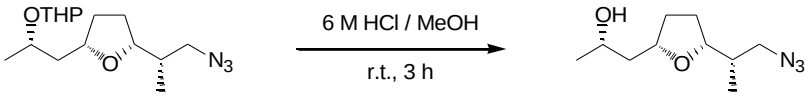


75: *Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propyl ester* **75**. **74** (0.708 g, 2.6 mmol) was added to pyridine (2.1 mL, 26.0 mmol) followed by pTsCl (0.992 g, 5.2 mmol) and then the solution was stirred at room temperature for 5 hours. Ethyl acetate (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 38.81, 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,

1291, 1188, 1176, 1118, 1097, 1075, 1021, 992, 962, 940, 812, 792, and 666 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{22}H_{34}O_6SNa^+$: 449.20; found 449.26.

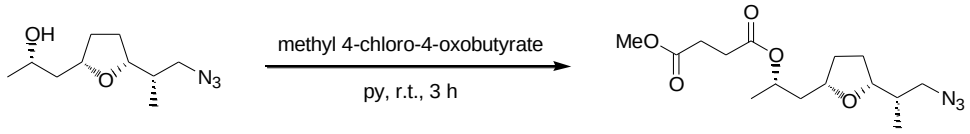


76: *2-{2-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethoxy}-tetrahydro-pyran* **76**. **75** (0.961 g, 2.3 mmol) was added to DMF (10 mL) followed by sodium azide (1.464 g, 22.5 mmol) and the solution was then warmed to 100 °C and stirred for 3 hours. Once cooled, water (50 mL) was added and the solution was washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{27}N_3O_3Na^+$: 320.20; found 320.25.

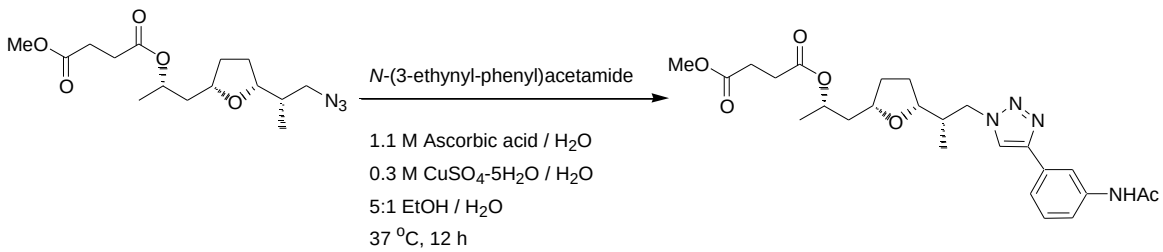


77: *1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-propan-2-ol* **77**. **76** (0.479 g, 1.6 mmol) was added to 6 M HCl/MeOH (5 mL) and the solution was stirred for 3 hours at room temperature. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The organic

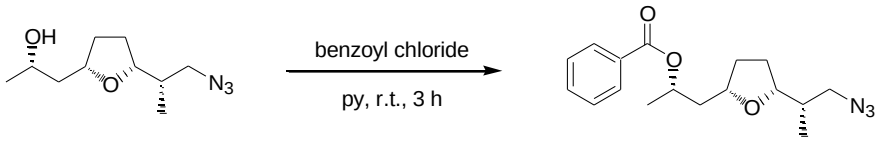
phases were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **77** (0.268 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.06 (m, 2H), 3.60 (m, 1H), 3.42 (m, 1H), 3.27 (m, 1H), 2.75 (s, 1H), 1.96 (m, 2H), 1.67 (m, 5H), 1.20 (d, J = 6.4 Hz, 3H), and 0.94 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{10}H_{19}N_3O_2H^+$: 214.16; found 214.20.



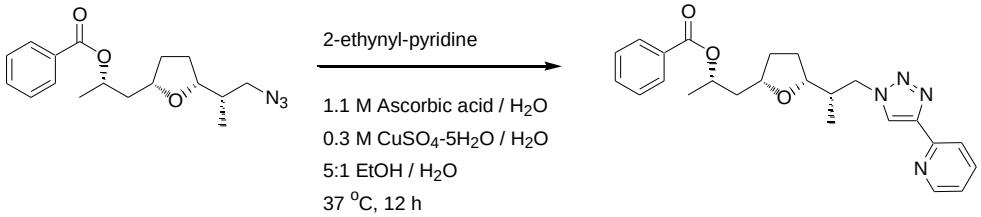
78: *Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester* **78**. **77** (0.134 g, 0.6 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate (97%, 155.0 µL, 1.3 mmol) and the solution was then stirred for 3 hours at room temperature. 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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{15}H_{25}N_3O_5Na^+$: 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/H_2O (6 mL) followed by *N*-(3-ethynyl-pyridine)-acetamide (0.729 g, 4.6 mmol). Ascorbic acid/H_2O (1.1 M, 0.9 mL, 1.0 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{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, 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 $C_{25}H_{34}N_4O_6H^+$: 487.26; found 487.27.

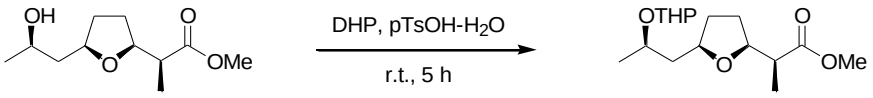


80: *Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* **80**. **77** (0.134 g, 0.6 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (146.0 µL, 1.3 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{17}H_{23}N_3O_3H^+$: 318.18; found 318.23.



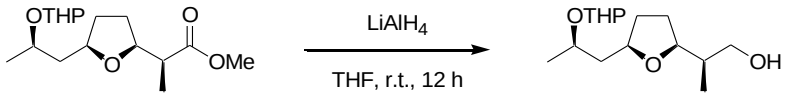
81: *Benzoic acid 1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester* **81**. **80** (0.166 g, 0.5 mmol) was added to 5:1 EtOH/H_2O (6 mL) followed by 2-ethynylpyridine (98%, 0.53 mL, 5.2 mmol).

Ascorbic acid/H_2O (1.1 M, 1.0 mL, 1.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{24}H_{28}N_4O_3H^+$: 421.22; found 421.24.

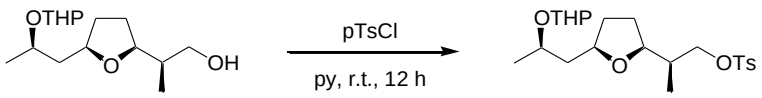


82: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propionic acid methyl ester* **82**. Methyl (+)-nonactate **46** (567.0 mg, 2.6 mmol) was added to methylene chloride (10 mL) followed by DHP (1.2 mL, 13.1 mmol) and pTsOH-H_2O (50.0 mg, 0.3 mmol). The solution was stirred for 5 hours 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. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.72 (m, 0.5H), 4.61 (m,

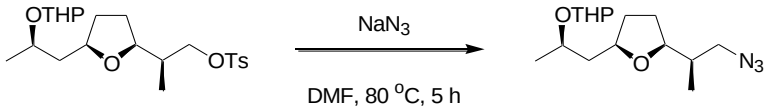
0.5H), 3.92 (m, 4H), 3.66 (m, 3H), 3.46 (m, 1H), 2.49 (m, 1H), 1.95 (m, 2H), 1.60 (m, 10H), 1.20 (m, 2H), and 1.08 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.39, 175.36, 99.8, 94.5, 80.34, 80.25, 77.3, 77.0, 76.7, 76.6, 76.4, 72.6, 68.0, 62.9, 62.0, 51.52, 51.48, 45.4, 45.3, 44.0, 43.8, 31.5, 31.3, 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 $C_{16}H_{28}O_5Na^+$: 323.18; found 323.22.



83: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propan-1-ol* **83**. 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 $NaOH/H_2O$ (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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{28}O_4Na^+$: 295.19; found 295.24.

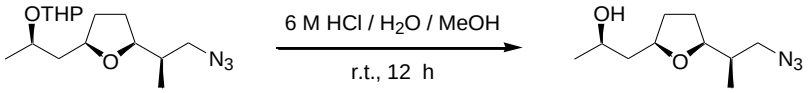


84: *Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-propyl]-tetrahydro-furan-2-yl}-propyl ester* **84**. **83** (0.452 g, 1.7 mmol) was added to pyridine (2 mL) followed by pTsCl (0.633 g, 3.3 mmol). The solution was stirred for 12 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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, 1176, 1097, 1075, 1021, 991, 962, 940, 813, and 667 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{22}H_{34}O_6SNa^+$: 449.20; found 449.25.

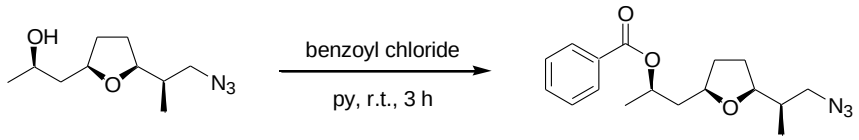


85: *2-{2-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethoxy}-tetrahydro-pyran* **85**. **84** (494.0 mg, 1.2 mmol) was added to DMF (3.5 mL) followed by sodium azide (376.0 mg, 5.8 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Once cooled to room temperature, water (20 mL) was added and

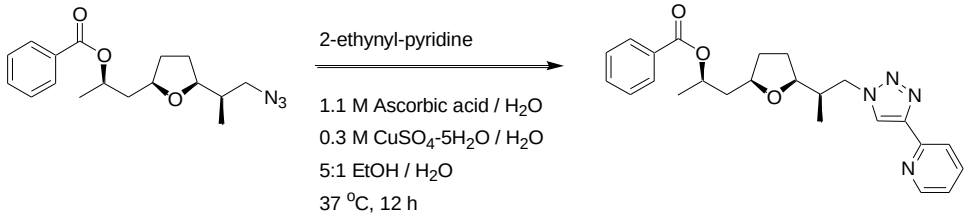
the solution was washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **85** (0.275 g, 79.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (m, 1H), 4.66 (m, 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 $C_{15}H_{27}N_3O_3Na^+$: 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_2O/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_2SO_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, 2967, 2934, 2878, 2095, 1460, 1379, 1282, 1067, and 937 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{10}H_{19}N_3O_2H^+$: 214.16; found 214.18.

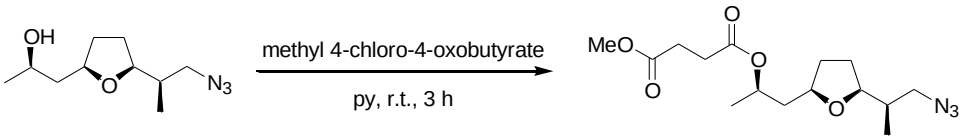


87: *Benzoic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester* **87**. **86** (46.0 mg, 0.2 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (50.0 µL, 0.4 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{17}H_{23}N_3O_3H^+$: 318.18; found 318.23.



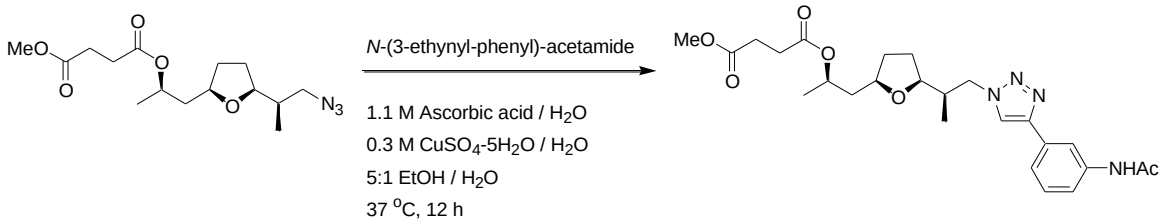
88: *Benzoic acid 1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester* **88**. **87** (32.8 mg, 0.1 mmol) was added to 5:1 EtOH/H_2O (2.5 mL) followed by 2-ethynylpyridine (98%, 21.0 µL, 0.2 mmol). Ascorbic acid/H_2O (1.1 M, 207.0 µL, 0.2 mmol) and $CuSO_4$-5H_2O/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 166.0, 150.4, 149.3, 148.1, 136.8, 132.7, 130.7, 129.5, 128.3, 122.8, 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 $C_{24}H_{28}N_4O_3H^+$: 421.22; found 421.28.



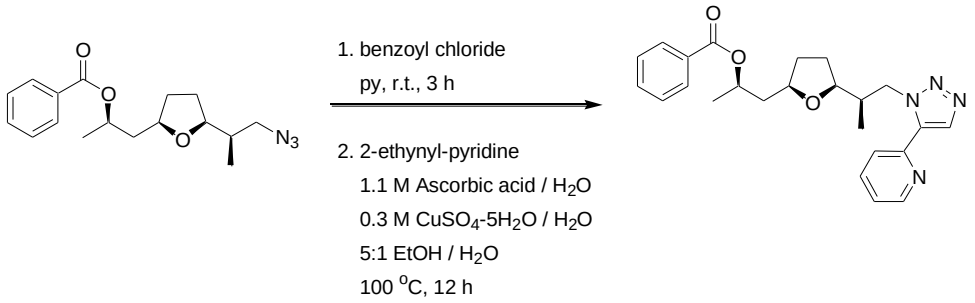
89: *Succinic acid 2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl ester methyl ester* **89**. **86** (46.0 mg, 0.2 mmol) was added to pyridine (1 mL) followed by methyl 4-chloro-4-oxobutyrate (53.0 µL, 0.4 mmol). The solution was stirred for 3 hours at room temperature. Ethyl acetate (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{25}N_3O_5Na^+$: 350.17; found 350.21.



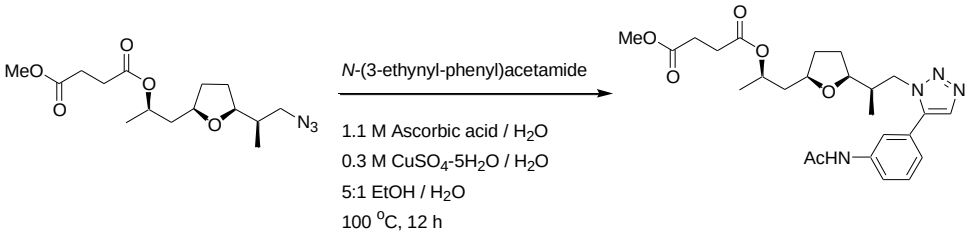
90: *Succinic acid 2-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester* **90**. **89** (26.7 mg, 0.08 mmol) was added to 5:1 EtOH/H_2O (2 mL) followed by *N*-(3-ethynyl-pyridine)-acetamide (26.0 mg, 0.2 mmol). Ascorbic acid/H_2O (1.1 M, 163.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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 $C_{25}H_{34}N_4O_6H^+$: 487.26; found 487.32.



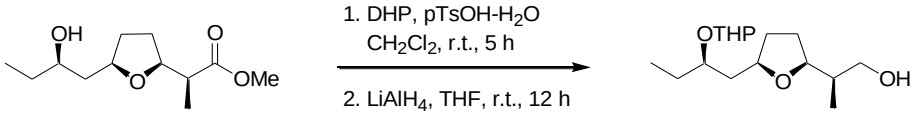
91: *Benzoic acid 1-methyl-2-{5-[1-methyl-2-(5-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl ester* **91**. **55** (110.0 mg, 0.5 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (0.6 mL, 5.2 mmol). The solution was stirred at room temperature for 3 hours. Ethyl 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_2SO_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_2O (1.5 mL) followed by 2-ethynyl-pyridine (98+%, 0.53 mL, 5.2 mmol). Ascorbic acid/H_2O (1.1 M, 1.0 mL, 1.1 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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 $C_{24}H_{28}N_4O_3H^+$: 421.22; found 421.28.



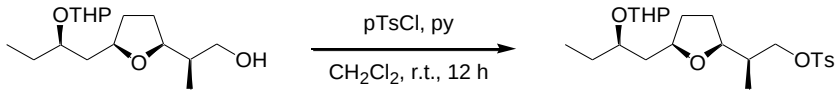
92: *Succinic acid 2-(5-{2-[5-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl ester methyl ester* **92**. **60** (113.0 mg, 0.4 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by *N*-(3-ethynyl-pyridine)-acetamide (550.0 mg, 3.5 mmol). Ascorbic acid/H_2O (1.1 M, 0.7 mL, 0.8 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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, 1H), 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, $CDCl_3$) δ 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, 29.0, 24.5, 20.7, and 14.6; IR (ATR) 3305, 3130, 2970, 2100, 1728, 1678, 1618, 1592, 1569, 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 $C_{25}H_{34}N_4O_6H^+$: 487.26; found 487.31.



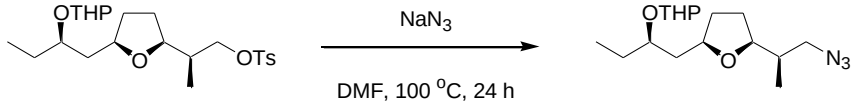
94: *2-{5-[2-(Tetrahydro-pyran-2-yloxy)-butyl]-tetrahydro-furan-2-yl}-propan-1-ol* **94**. Methyl homononactate **93** (0.534 g, 2.3 mmol) was added to methylene chloride (9.3 mL) followed by DHP (1.1 mL, 11.6 mmol) and pTsOH-H_2O (4.5 mg, 0.02 mmol). The solution was stirred for 5 hours at room temperature. 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_2O (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%). ^1H

NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{16}H_{30}O_4Na^+$: 309.20; found 309.11.

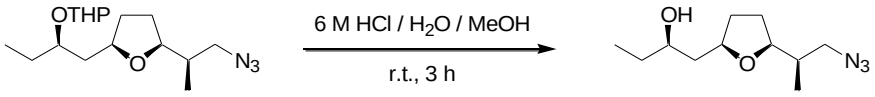


95: *Toluene-4-sulfonic acid 2-{5-[2-(tetrahydro-pyran-2-yloxy)-butyl]-tetrahydro-furan-2-yl}-propyl ester* **95**. **94** (0.426 g, 1.5 mmol) was added to methylene chloride (3 mL) was added to pyridine (1.2 mL, 14.9 mmol) and pTsCl (0.567 g, 3.0 mmol). The solution was stirred overnight at room temperature. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_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%). ^1H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{23}H_{36}O_6SNa^+$: 463.21; found 463.03.

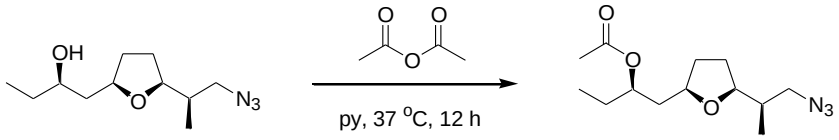


96: *2-{1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propoxy}-tetrahydro-pyran* **96**. **95** (0.450 g, 1.0 mmol) was added to DMF (10 mL) followed by sodium azide (0.332 g, 5.1 mmol). The solution was warmed to 100 °C and stirred for 24 hours. Once cooled to room temperature, water (25 mL) was added and the solution was extracted with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{16}H_{29}N_3O_3Na^+$: 334.21; found 334.10.

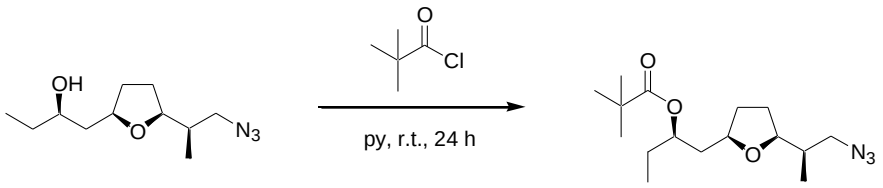


97: *1-[5-(2-Azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-butan-2-ol* **97**. **96** (0.215 g, 0.7 mmol) was added to 6 M HCl/H_2O/MeOH (5 mL) and the solution was stirred at room temperature for 3 hours. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The

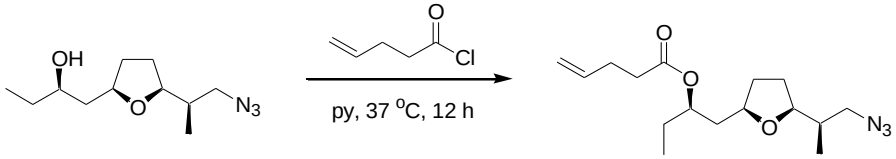
organic phases were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **97** (0.103 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 (m, 1H), 3.70 (m 1H), 3.57 (m, 1H), 3.40 (m, 1H), 3.23 (m, 1H), 2.81 (s, 1H), 1.93 (m, 2H), 1.58 (m, 7H), and 0.90 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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 $C_{11}H_{21}N_3O_2H^+$: 228.17; found 228.09.



98: *Acetic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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

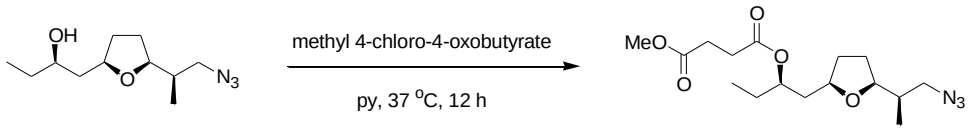


99: *2,2-Dimethyl-propionic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester* **99**. **97** (42.6 mg, 0.2 mmol) was added to pyridine (5 mL) followed by trimethylacetyl chloride (230.0 μL, 1.9 mmol). The solution was stirred for 24 hours at room temperature. Ethyl acetate (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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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

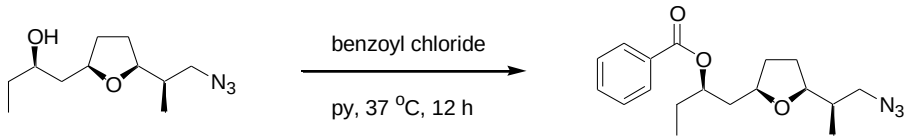


100: *Pent-4-enoic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester* **100**. **97** (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by 4-pentenoyl chloride (0.18 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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, $CDCl_3$) δ 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, $CDCl_3$) δ 172.6, 136.8, 115.4, 80.8, 76.1, 73.4, 55.1, 40.1, 39.4, 33.8, 31.3, 29.2, 28.9, 27.5, 14.4, and 9.4

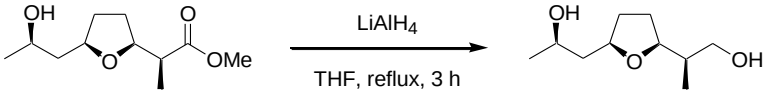


101: *Succinic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester methyl ester* **101**. **97** (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl acetate (50 mL) 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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

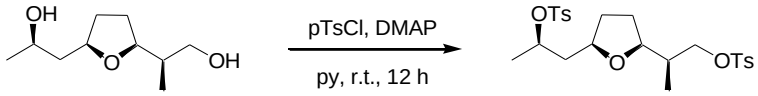


102: *Benzoic acid 1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl ester* **102**. **97** (36.0 mg, 0.2 mmol) was added to pyridine (2 mL) followed by benzoyl chloride (0.18 mL, 1.6 mmol). The solution was shaken at 37 °C for 12 hours. Ethyl

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 (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 132.7, 130.7, 129.5, 128.3, 80.8, 76.3, 74.0, 55.1, 40.1, 39.4, 31.4, 29.2, 27.7, 14.4, and 9.4

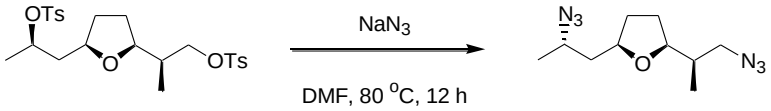


113: *2-[5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-propan-1-ol* **113**. Methyl nonactate **45** (1.072 g, 5.0 mmol) was added to THF (50 mL) followed by lithium aluminum hydride (0.376 g, 9.9 mmol). The solution was refluxed for 3 hours. Once cooled to room temperature, water (0.4 mL) was added followed by 3.0 M NaOH/H_2O (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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

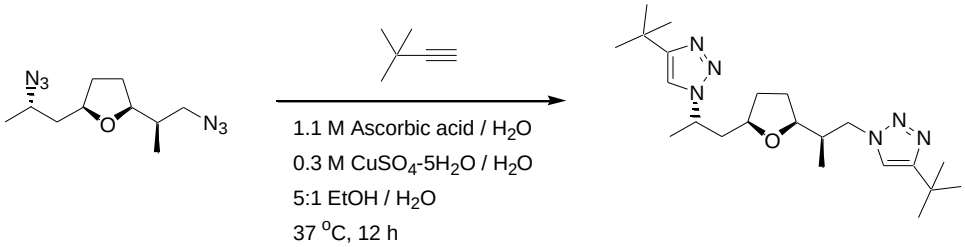


114: *Bis-tosylated nonactate diol* **114**. **113** (0.207 g, 1.1 mmol) was added to pyridine (10 mL) followed by DMAP (0.134 g, 1.1 mmol) and pTsCl (1.05 g, 5.5 mmol). The

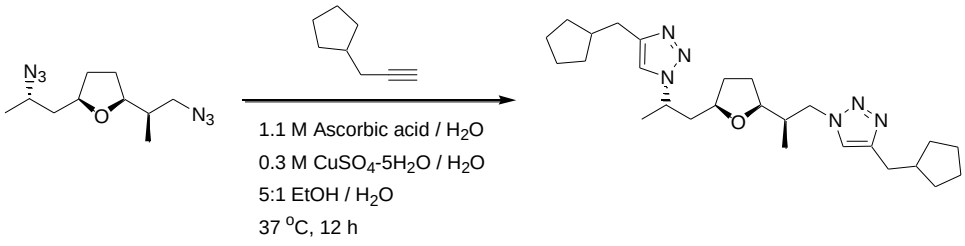
solution was stirred at room temperature overnight. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then saturated ammonium chloride solution (2 × 50 mL), dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give **114** (0.213 g, 39%).



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 (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{10}H_{18}N_6ONa^+$: 261.14; found 261.19.

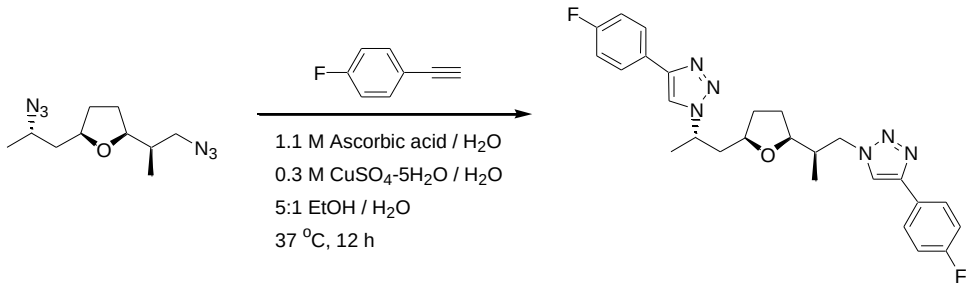


117: *4-tert-butyl-1-(1-15-(1-(4-tert-butyl-1H-1,2,3-triazol-1-yl)propane-2-yl)tetrahydrofran-2-yl)propan-2-yl)-1H-1,2,3-triazole* **117**. **115** (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3,3-dimethyl-1-butyne (98%, 52.0 µL, 0.4 mmol). Ascorbic acid/H_2O (1.1 M, 168.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel (100% EtOAc) to give **117** (4.2 mg, 24.9%).

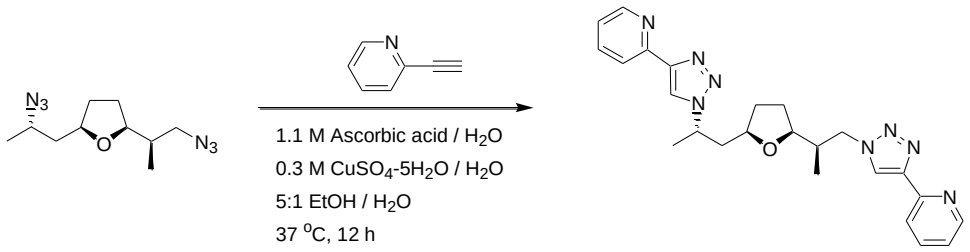


118: *4-(cyclopenylmethyl)-1-(1-15-(1-(1-(4-(cyclopentylmethyl)-1H-1,2,3-triazol-1-yl)propane-2-yl)tetrahydrofuran-2-yl)propan-2-yl)-1H-1,2,3-triazole* **118**. **115** (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 3-cyclopentyl-1-propyne (98%, 55.0 µL, 0.4 mmol). Ascorbic acid/H_2O (1.1 M, 168.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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

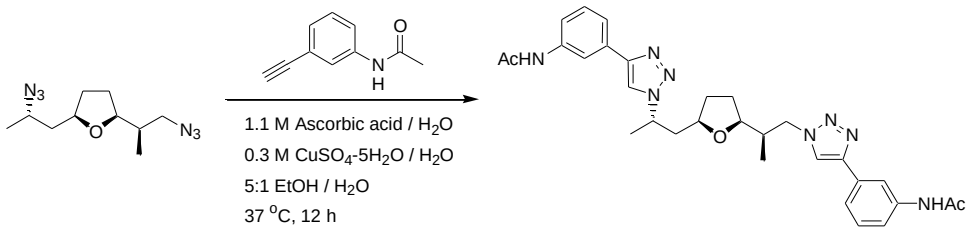
layers were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **118** (10.7 mg, 56.0%).



119: *4-(4-fluorophenyl)-1-(1-(5-(1-(4-(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_2O (1.5 mL) followed by 1-ethynyl-4-fluorobenzene (48.0 µL, 0.4 mmol). Ascorbic acid/H_2O (1.1 M, 168.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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

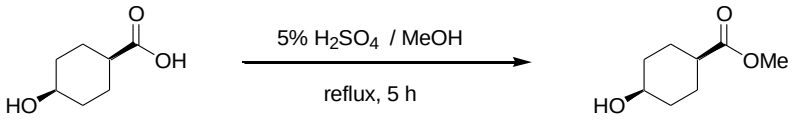


120: *2-(1-(1-(5-(1-(4-(pyridine-2-yl)-1H-1,2,3-triazol-1-yl)propan-2-yl)tetrahydrofuran-2-yl)propan-2-yl)-1H-1,2,3-triazol-4-yl)pyridine* **120**. **115** (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by 2-ethynylpyridine (98%, 42.0 µL, 0.4 mmol). Ascorbic acid/H_2O (1.1 M, 168.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **120** (11.8 mg, 63.4%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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



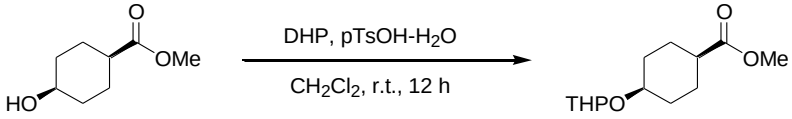
121: *N-(3-{1-[2-(5-{2-[4-(3-Acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-yl)-1-methyl-ethyl]-1H-[1,2,3]triazol-4-yl}-phenyl)-acetamide*

121. 115 (10.0 mg, 0.04 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (67.0 mg, 0.4 mmol). Ascorbic acid/H_2O (1.1 M, 168.0 µL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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 Hz, 3H), 1.49 (m, 2H), and 0.75 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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

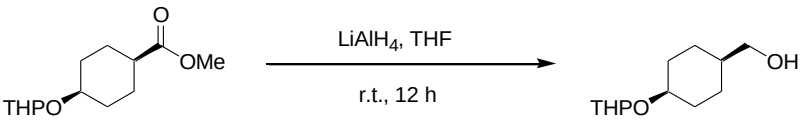


123: *4-Hydroxy-cyclohexanecarboxylic acid methyl este*r **123**. *Cis*-4-hydroxycyclohexane-1-carboxylic acid **122** (5.0 g, 34.7 mmol) was added to 5% H_2SO_4/MeOH (100 mL)

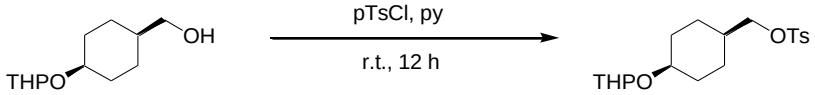
and the solution was stirred at room temperature then refluxed for 5 hours. Saturated sodium bicarbonate solution was added until no more gas evolved and the solution was then washed with ethyl acetate (3 × 100 mL). The organic phases were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:1) to give **123** (4.611 g, 84%). R_f = 0.19 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (m, 1H), 3.60 (s, 3H), 2.32 (m, 2H), 1.89 (m, 2H), and 1.58 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_8H_{14}O_3H^+$: 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-H_2O (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. ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{13}H_{22}O_4Na^+$: 265.14; found 265.01.

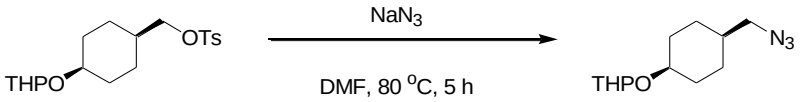


125: *[4-(Tetrahydro-pyran-2-yloxy)-cyclohexyl]-methanol* **125**. **124** was added to THF (30 mL) followed by lithium aluminum hydride (0.720 g, 19.0 mmol) at room temperature and allowed to stir overnight. Water (0.8 mL) was added, followed by 3.0 M $NaOH/H_2O$ (1.6 mL), followed by water (0.8 mL) 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{12}H_{22}O_3Na^+$: 237.15; found 237.09.

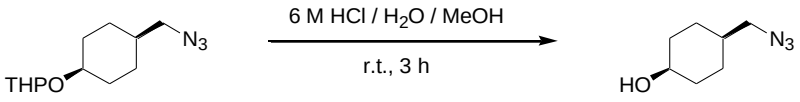


126: *Toluene-4-sulfonic acid 4-(tetrahydro-pyran-2-yloxy)-cyclohexylmethyl ester* **126**. **125** (1.108, 5.2 mmol) was added to pyridine (20 mL), followed by pTsCl (1.971 g, 10.4 mmol) and the solution was stirred for 12 hours at room temperature. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 100 mL) and then washed with saturated ammonium chloride solution (2 × 100 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{19}H_{28}O_5SNa^+$: 391.16; found 391.02.

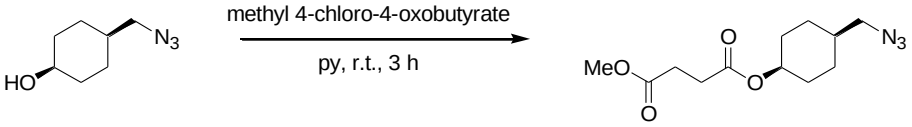


127: *2-(4-Azidomethyl-cyclohexyloxy)-tetrahydro-pyran* **127**. **126** (1.211 g, 3.3 mmol) was added to DMF (15 mL) followed by sodium azide (1.07 g, 16.4 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Once the solution had cooled to room temperature, water (50 mL) was added and the solution washed with diethyl ether (3 × 50 mL). The organic layers were collected, dried (Na_2SO_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 $C_{12}H_{21}N_3O_2Na^+$: 262.15; found 262.09.

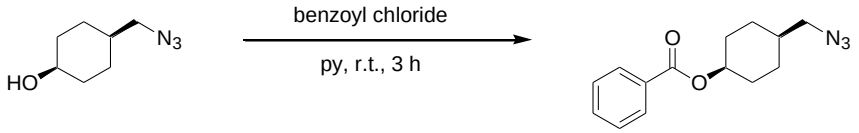


128: *4-Azidomethyl-cyclohexanol* **128**. **127** (0.543 g, 2.3 mmol) was added to 6 M HCl/H_2O/MeOH (10 mL) and the solution was stirred at room temperature for 3 hours. Saturated sodium bicarbonate solution was added to the solution until no gas

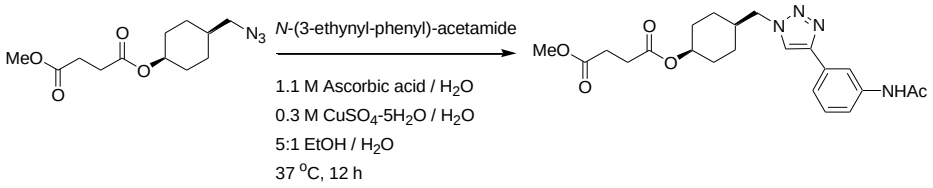
evolved. The solution was washed with ethyl acetate (3 × 50 mL) and the organic phases collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **128** (0.318 g, 90.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (m, 1H), 3.15 (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_2SO_4) and concentrated to a residue which as 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 $C_{12}H_{19}N_3O_4H^+$: 270.15; found 270.06.

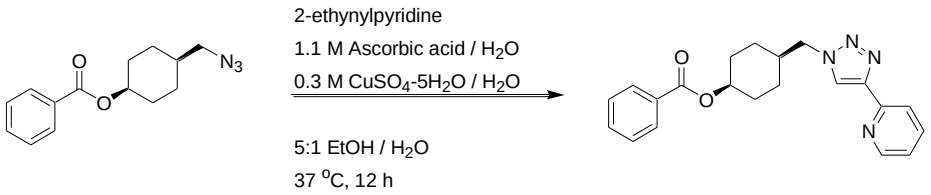


130: *Benzoic acid 4-azidomethyl-cyclohexyl ester* **130**. **128** (0.208 g, 1.4 mmol) was added to pyridine (3 mL) followed by benzoyl chloride (1.55 mL, 13.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL). The solution was dried (Na_2SO_4) 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{14}H_{17}N_3O_2H^+$: 260.14; found 260.08.



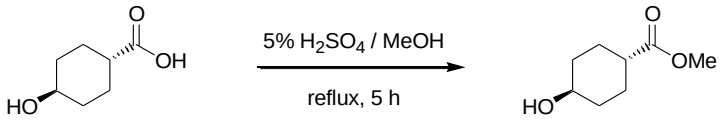
131: *Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-ylmethyl]-cyclohexyl ester methyl ester* **131**. **129** (0.199 g, 0.7 mmol) was added to 5:1 EtOH/H_2O (10 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (0.235 g, 1.5 mmol). Ascorbic acid/H_2O (1.1 M, 1.5 mL, 1.6 mmol) and $CuSO_4$-5H_2O/H_2O (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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **131** (0.217 g, 69%). R_f = 0.37 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{22}H_{28}N_4O_5H^+$: 429.21; found 429.04.

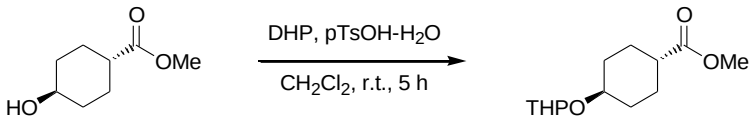


132: *Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-ylmethyl)-cyclohexyl ester* **132**. **130** (0.283 g, 1.1 mmol) was added to 5:1 EtOH/H_2O (10 mL) followed by 2-ethynyl-pyridine (0.225 µL, 2.2 mmol). Ascorbic acid/H_2O (1.1 M, 2.2 mL, 2.4 mmol) and $CuSO_4$-H_2O/H_2O (0.3 M, 1.8 mL, 0.6 mmol) 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 (N_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **132** (0.244 g, 62%). R_f = 0.51 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (m, 1H), 8.17 (d, J = 7.9 Hz, 2H), 8.14 (s, 1H),

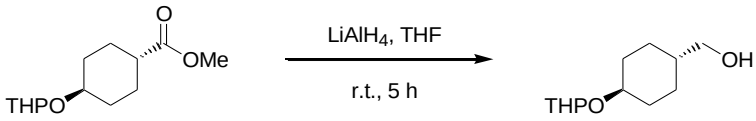
8.00 (d, *J* = 8.2 Hz, 2H), 7.75 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.3, 2H), 7.20 (t, *J* = 6.2 Hz, 1H), 5.26 (s, 1H), 7.20 (d, 2H), 2.05 (m, 3H), and 1.55 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ; IR (ATR) 2929, 2860, 2633, 2538, 2444, 2266, 2098, 1721, 1607, 1599, 1471, 1449, 1427, 1368, 1278, 1266, 1119, 1046, 793, and 710 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{21}H_{22}N_4O_2H^+$: 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_2SO_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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_8H_{14}O_3H^+$: 159.10 ; found 159.03.

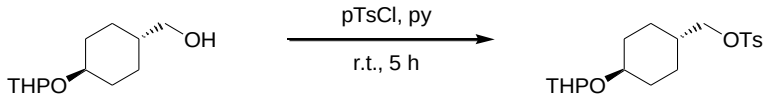


135: *4-(Tetrahydro-pyran-2-yloxy)-cyclohexanecarboxylic acid methyl ester* **135**. **134** (1.0 g, 6.3 mmol) was added to methylene chloride (20 mL) followed by DHP (2.9 mL, 31.6 mmol) and pTsOH-H_2O (12.0 mg, 0.06 mmol). The solution was stirred at room temperature for 5 hours. The solution was evaporated 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 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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), and 1.18 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 96.7, 73.8, 62.6, 51.4, 42.2, 32.5, 31.1, 30.7, 27.3, 27.0, 25.3, and 19.8; IR (ATR) 2939, 2864, 1734, 1453, 1354, 1309, 1248, 1172, 1134, 1118, 1063, 1022, and 987 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{13}H_{22}O_4Na^+$: 265.14; found 265.01.

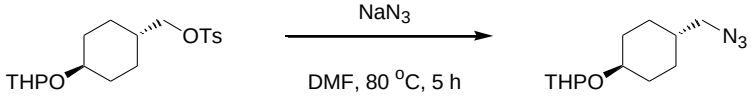


136: *[4-(Tetrahydro-pyran-2-yloxy)-cyclohexyl]-methanol* **136**. **135** (1.0 g, 4.1 mmol) was added to THF (30 mL) followed by lithium aluminum hydride (0.471 g, 12.4 mmol). The solution was stirred at room temperature for 5 hours. Water (0.5 mL) was added, followed by 3.0 M NaOH/H_2O (1.0 mL), and 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 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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 $C_{12}H_{22}O_3Na^+$: 237.15; found 237.10.

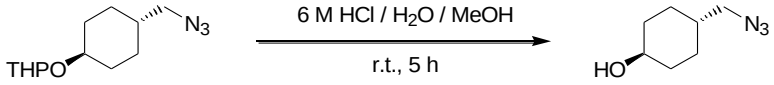


137: *Toluene-4-sulfonic acid 4-(tetrahydro-pyran-2-yloxy)-cyclohexylmethyl ester* **137**. **136** (0.641 g, 3.0 mmol) was added to pyridine (10 mL) followed by p-toluenesulfonyl chloride (0.855 g, 4.5 mmol). The solution was stirred at room temperature for five hours. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na_2SO_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, 1292, 1189, 1178, 1023, 950, and 810 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{19}H_{28}O_5SNa^+$: 391.16; found 391.04.

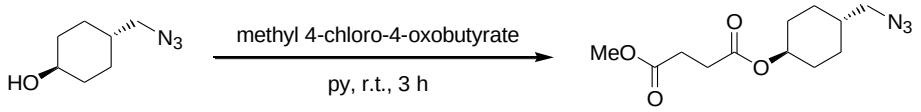


138: *2-(4-Azidomethyl-cyclohexyloxy)-tetrahydro-pyran* **138**. **137** (0.406 g, 1.1 mmol) was added to DMF (5 mL) followed by sodium azide (0.292 g, 4.5 mmol). The solution

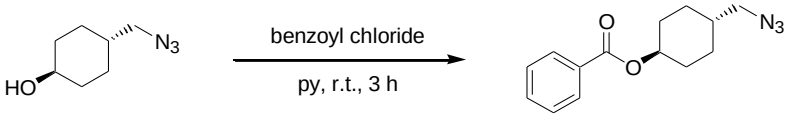
was warmed to 80 °C and stirred for 5 hours. After cooling to room temperature, water (25 mL) was added and the solution washed with diethyl ether (3 × 50 mL). The organic phases were collected, dried (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **138** (0.221g, 83.7%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{12}H_{21}N_3O_2Na^+$: 262.15; found 262.09.



139: *4-Azidomethyl-cyclohexanol* **139**. **138** (0.162 g, 0.7 mmol) was added to 6 M HCl/H_2O/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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

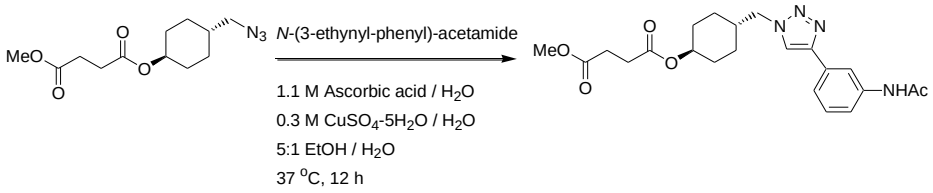


140: *Succinic acid 4-azidomethyl-cyclohexyl ester methyl ester* **140**. **139** (83.5 mg, 0.5 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxybutyrate (0.65 mL, 5.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{12}H_{19}N_3O_4H^+$: 270.15; found 270.06.



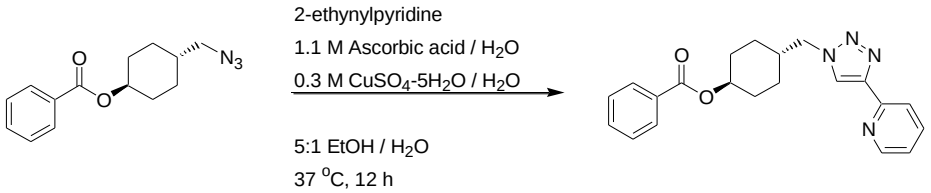
141: *Benzoic acid 4-azidomethyl-cyclohexyl ester* **141**. **139** (83.5 mg, 0.5 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.62 mL, 5.4 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

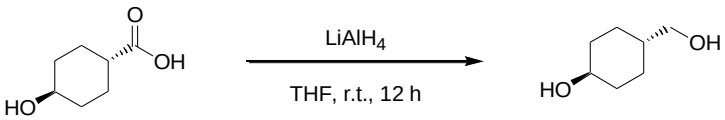


142: *Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-ylmethyl]-cyclohexyl ester methyl ester* **142**. **140** (76.4 mg, 0.3 mmol) was added to 5:1 EtOH/H_2O (10 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (90.0 mg, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 0.57 mL, 0.6 mmol) and $CuSO_4$-5H_2O/H_2O (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_2SO_4), and concentrated to 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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,

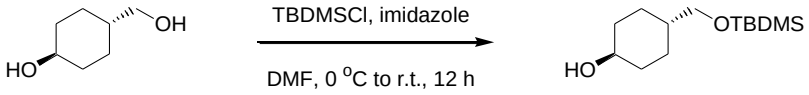
1618, 1592, 1568, 1532, 1486, 1439, 1367, 1308, 1274, 1217, 1163, 1023, and 999 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{22}H_{28}N_4O_5H^+$: 429.21; found 429.05.



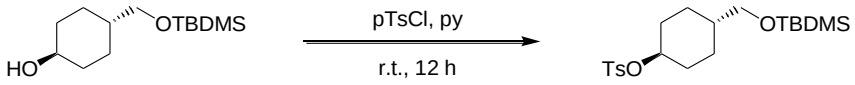
143: *Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-ylmethyl)-cyclohexyl ester* **143**. **141** (100.1 mg, 0.4 mmol) was added to 5:1 EtOH/H_2O (10 mL) followed by 2-ethynyl-pyridine (98+%, 78.0 µL, 0.8 mmol). Ascorbic acid/H_2O (1.1 M, 0.77 mL, 0.9 mmol) and $CuSO_4$-$5H_2O$/H_2O (0.3 M, 0.64 mL, 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_2SO_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 $C_{21}H_{22}N_4O_2H^+$: 363.18; found 363.04.



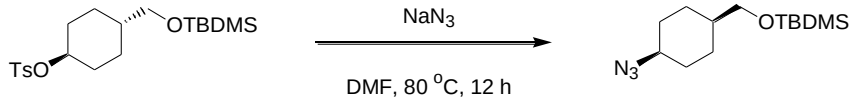
144: *4-Hydroxymethyl-cyclohexanol* **144**. *Trans*-4-hydroxy-cyclohexane-1-carboxylic acid **133** (4.01 g, 27.8 mmol) was added to THF (140 mL) followed by lithium aluminum hydride (3.163 g, 83.4 mmol). The solution was stirred for 12 hours at room temperature. Water (3.2 mL) was added, followed by 3.0 M $NaOH/H_2O$ (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, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



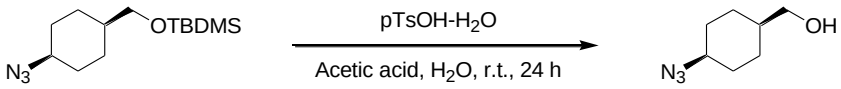
145: *4-(tert-Butyl-dimethyl-silanyloxymethyl)-cyclohexanol* **145**. **144** (0.50 g, 3.9 mmol) was added to DMF (7 mL) at 0 °C followed by imidazole (0.288 g, 4.2 mmol). TBSCl (0.579 g, 3.9 mmol) was added and the solution was stirred and slowly allowed to return to room temperature for 12 hours. Water (25 mL) was added and the solution extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na_2SO_4), 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}



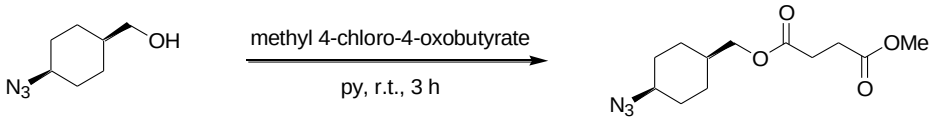
146: *Toluene-4-sulfonic acid 4-(tert-butyl-dimethyl-silanyloxymethyl)-cyclohexyl ester* **146**. **145** (1.05 g, 4.3 mmol) was added to pyridine (4 mL) followed by pTsCl (1.64 g, 8.6 mmol) and the solution was stirred for 12 hours at room temperature. Ethyl acetate (100 mL) was added and the solution washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na_2SO_4), 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 $C_{20}H_{34}O_4SSiNa^+$: 421.18; found 420.99.



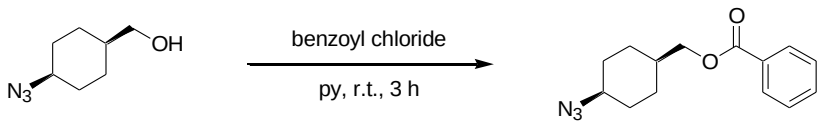
147: *(4-Azido-cyclohexylmethoxy)-tert-butyl-dimethyl-silane* **147**. **146 (**1.72 g, 4.3 mmol) was added to DMF (13 mL) followed by (1.4 g, 21.5 mmol) sodium azide. The solution was stirred at 80 °C for 12 hours. Water (50 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na_2SO_4), 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). R_f = 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_2O (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_2SO_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}

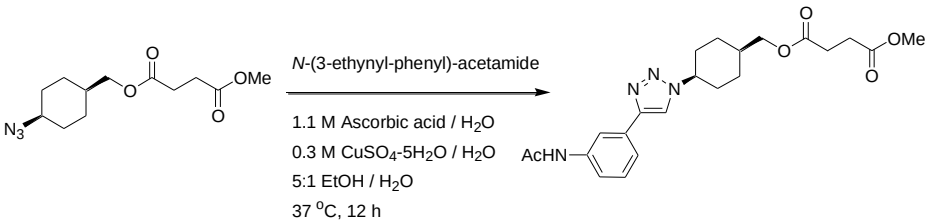


149: *Succinic acid 4-azido-cyclohexylmethyl ester methyl ester* **149**. **148** (57.0 mg, 0.4 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxobutyrate (0.5 mL, 3.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL). The solution was dried (Na_2SO_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 %).



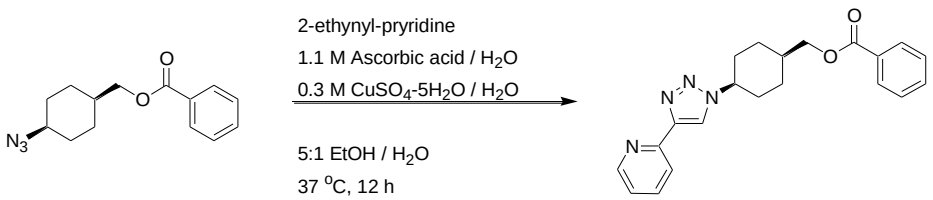
150: *Benzoic acid 4-azido-cyclohexylmethyl ester* **150**. **148** (57.0 mg, 0.4 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.4 mL, 3.7 mmol). The

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 (Na_2SO_4), 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}

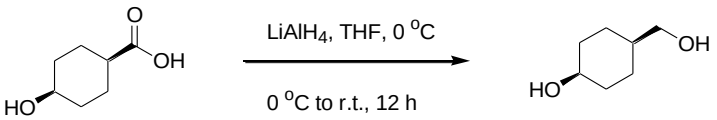


151: *Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-cyclohexylmethyl ester methyl ester* **151**. **149** (77.5 mg, 0.3 mmol) was added 5:1 EtOH/H_2O (7 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (92.0 mg, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 0.58 mL, 0.6 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **151** (99.5 mg, 80.7%). R_f = 0.37 (100% EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) 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) ; ^{13}C NMR (100 MHz, $CDCl_3$) 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

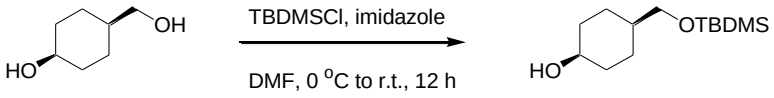
(ATR) 3301, 2949, 2861, 1729, 1690, 1678, 1618, 1592, 1567, 1532, 1482, 1440, 1367, 1160, 998, 791, and 751 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{22}H_{28}N_4O_5H^+$: 429.21; found 428.99.



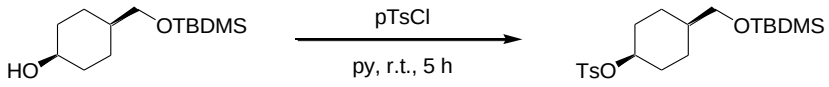
152: *Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-cyclohexylmethyl ester* **152**. **150** (79.2 mg, 0.3 mmol) was added to 5:1 EtOH/H_2O (7 mL) followed by 2-ethynyl-pyridine (98+%, 63.0 µL, 0.6 mmol). Ascorbic acid/H_2O (1.1 M, 0.61 mL, 0.7 mmol) and $CuSO_4$-$5H_2O$/H_2O (0.3 M, 0.50 mL, 0.2 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{21}H_{22}N_4O_2H^+$: 363.18; found 362.99.



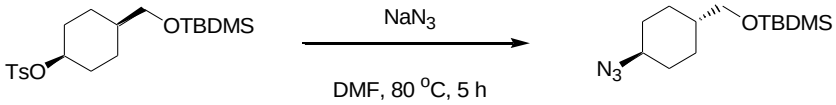
153: *4-Hydroxymethyl-cyclohexanol* **153**. *Cis*-4-hydroxy-cyclohexane-1-carboxylic acid **122** (4.01 g, 27.8 mmol) was added to THF (140 mL) at 0 °C followed by lithium aluminum hydride (3.17 g, 83.5 mmol). The solution was stirred overnight and allowed to warm to room temperature. Water (3.2 mL) was added, followed by 3.0 M $NaOH/H_2O$ (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}



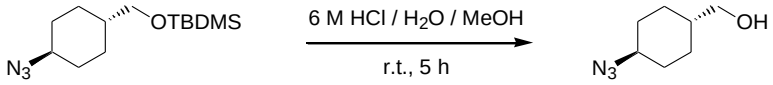
154: *4-(tert-Butyl-dimethyl-silanyloxymethyl)-cyclohexanol* **154**. **153** (0.50 g, 3.9 mmol) was added to DMF (7 mL) at 0 °C followed by imidazole (0.288 g, 4.2 mmol) and TBSCl (0.58g, 3.9 mmol). The solution was allowed to slowly warm to room temperature while stirring overnight. Water (50 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na_2SO_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%). ^1H 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}



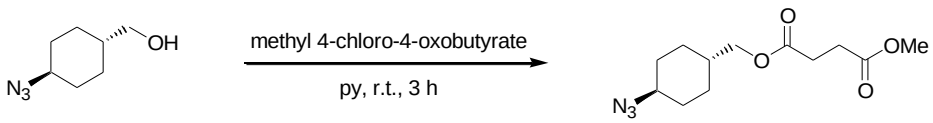
155: *Toluene-4-sulfonic acid 4-(tert-butyl-dimethyl-silanyloxymethyl)-cyclohexyl ester* **155**. **154** (1.9 g, 7.8 mmol) was added to pyridine (7 mL) followed by pTsCl (2.964 g, 15.6 mmol). The solution was stirred for 5 hours at room temperature. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 X 50 mL) and then with saturated ammonium chloride solution (2 X 50 mL), dried (Na_2SO_4), 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, 1092, 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 (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

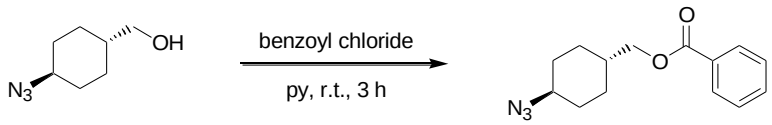


157: *(4-Azido-cyclohexyl)-methanol* **157**. **156** (1.109 g, 4.1 mmol) was added to THF (2 mL) followed by 6 M HCl/H_2O/MeOH (10 mL). The solution was stirred at room temperature for five hours. Saturated sodium bicarbonate solution was added until no gas evolved and the solution was washed with ethyl acetate (3 × 50 mL). The organic phases were collected, dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

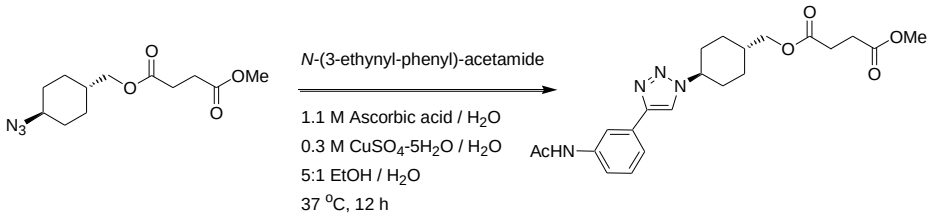


158: *Succinic acid 4-azido-cyclohexylmethyl ester methyl ester* **158**. **157** (26.0 mg, 0.2 mmol) was added to pyridine (5 mL) followed by methyl 4-chloro-4-oxobutyrate (0.2 mL, 1.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) δ 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 $C_{12}H_{19}N_3O_4H^+$: 270.15; found 270.04.

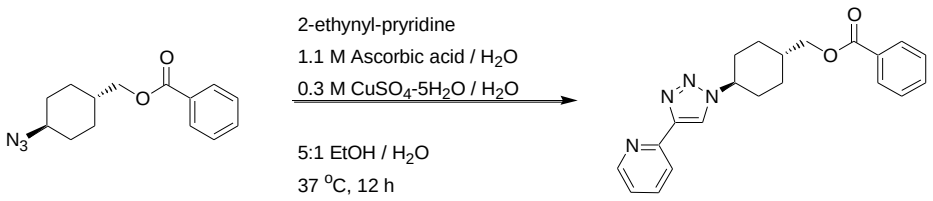


159: *Benzoic acid 4-azido-cyclohexylmethyl ester* **159**. **157** (26.0 mg, 0.2 mmol) was added to pyridine (5 mL) followed by benzoyl chloride (0.2 mL, 1.7 mmol). The solution was stirred at room temperature for 3 hours. Ethyl acetate (50 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 20 mL) and then with saturated ammonium chloride solution (2 × 20 mL), dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



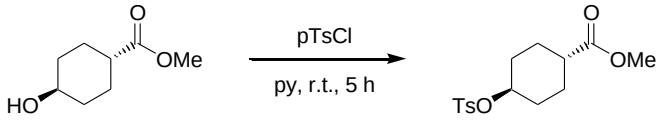
160: *Succinic acid 4-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-cyclohexylmethyl ester methyl ester* **160**. **158** (27.5 mg, 0.1 mmol) was added to 5:1 EtOH/H_2O (3 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (33.0 mg, 0.2 mmol). Ascorbic

acid/H_2O (1.1 M, 0.2 mL, 0.2 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **160** (34.8 mg, 79.6%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{22}H_{28}N_4O_5H^+$: 429.21; found 428.97.

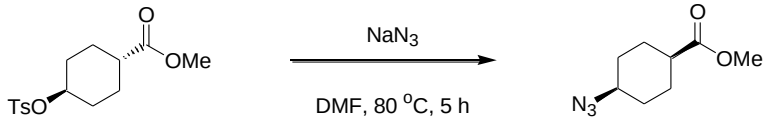


161: *Benzoic acid 4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-cyclohexylmethyl ester* **161**. **159** (31.5 mg, 0.1 mmol) was added to 5:1 EtOH/H_2O (3 mL) followed by 2-ethynyl-pyridine, 98% (25.0 µL, 0.3 mmol). Ascorbic acid/H_2O (1.1 M, 0.25 mL, 0.3 mmol) and $CuSO_4$-$5H_2O$/H_2O (0.3 M, 0.2 mL, 0.06 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 (Na_2SO_4), and concentrated to a residue which was purified by

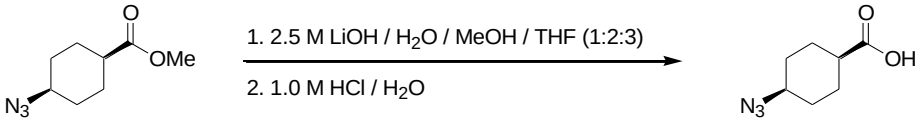
chromatography on silica gel in 100% EtOAc to give **161** (32.8 mg, 74.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (m, 1H), 8.16 (s, 1H), 8.15 (m, 1H), 8.03 (m, 2H), 7.73 (m, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.42 (m, 2H), 7.18 (m, 1H), 4.51 (m, 1H), 4.19 (d, J = 6.5 Hz, 2H), 2.33 (m, 2H), 2.06 (m, 2H), 1.88 (m, 3H), and 1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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 $C_{21}H_{22}N_4O_2H^+$: 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 × 50 mL) and then with saturated ammonium chloride solution (3 × 50 mL), dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{20}O_5SH^+$: 313.11; found 312.93.

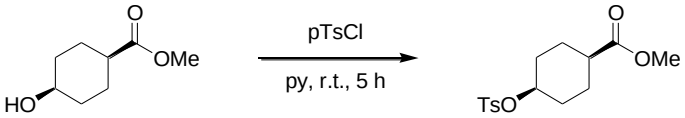


163: *4-Azido-cyclohexanecarboxylic acid methyl ester* **163**. **162** (1.193 g, 3.8 mmol) was added to DMF (10 mL) followed by sodium azide (1.241 g, 19.1 mmol). The solution was warmed to 80 °C and stirred for 5 hours. Water (50 mL) was added to the solution once it was cooled to room temperature and the solution was washed with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na_2SO_4), 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%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (m, 1H), 3.62 (s, 3H), 2.34 (m, 1H), 1.83 (m, 2H), and 1.64 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

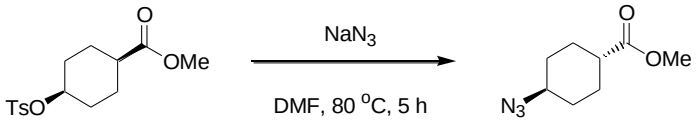


164: *4-Azido-cyclohexanecarboxylic acid* **164**. **163** (0.524 g, 2.9 mmol) was added to 2.5 M LiOH/H_2O/MeOH/THF (1:2:3) (10 mL) and the solution was stirred at room temperature overnight. 1.0 M HCl/H_2O was added until the solution had a pH of 2. The solution was washed with ethyl acetate (5 × 100 mL) and the organic phases collected, dried (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 3.69 (m, 1H), 2.44 (m, 1H), 1.91 (m, 2H), and 1.73 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 181.6, 57.4, 40.7, 28.6, and 23.8; IR (ATR) 3024, 2934, 2098, 1788, 1709, 1461, 1444, 1415, 1369 1312, 1233, 1199, and 1026 cm^{-1}

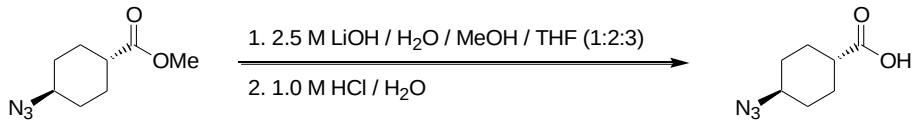


165: *4-(Toluene-4-sulfonyloxy)-cyclohexanecarboxylic acid methyl ester* **165**. **123** (1.0 g, 6.3 mmol) was added to pyridine (10 mL) followed by pTsCl (1.446 g, 7.6 mmol). The solution was stirred at room temperature for 5 hours. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium chloride solution (2 × 50 mL), dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{15}H_{20}O_5SNa^+$: 335.09; found 334.95.

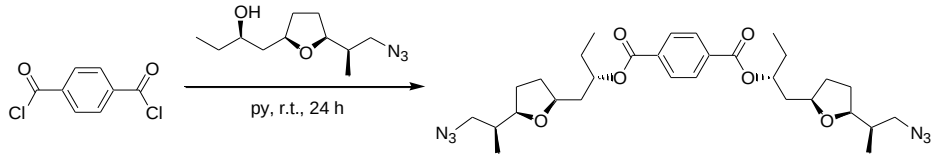


166: *4-Azido-cyclohexanecarboxylic acid methyl ester* **166**. **165** (1.275 g, 4.1 mmol) was added to (20 mL) DMF followed by sodium azide (1.327 g, 20.4 mmol). The solution was warmed to 80 °C and stirred for 5 hours. After cooling to room temperature, water (50 mL) was added and the solution was washed with diethyl ether (3 × 100 mL). The organic phases were collected, dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

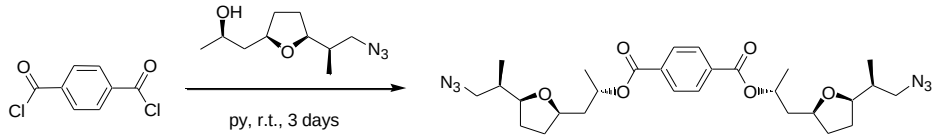


167: *4-Azido-cyclohexanecarboxylic acid* **167**. **166** (0.520 g, 2.8 mmol) was added to 2.5 M LiOH/H_2O/MeOH/THF (1:2:3) (10 mL) and the solution was stirred for 12 hours at room temperature. 1.0 M HCl/H_2O was added until the solution had a pH of 2 and the solution was washed with ethyl acetate (5 × 100 mL). The organic phases were collected, dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 181.7, 59.0, 41.6, 30.5, and 26.8; IR (ATR) 3033, 2935, 2862, 2091, 1693, 1446, 1427, 1360, 1307, 1250, 1213, 1041, 1021, and 939 cm^{-1}

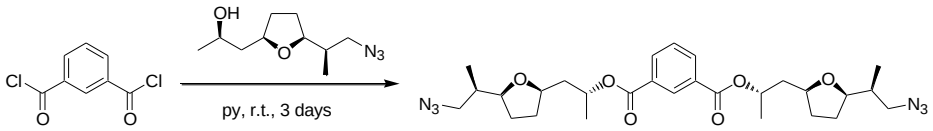


168: *Terephthalic acid bis-{1-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-ylmethyl]-propyl} ester* **168**. **97** (0.168 g, 0.8 mmol) was added to pyridine (2 mL) followed by terephthaloyl chloride (75.2 mg, 0.4 mmol) at room temperature. The solution was stirred for 24 hours. Ethyl acetate (50 mL) was added and the solution was washed

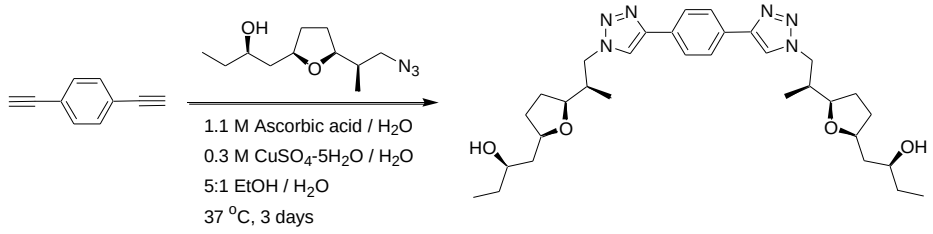
consecutively with saturated copper sulfate solution (2 × 25 mL) then saturated ammonium chloride solution (2 × 25 mL). The organic layer was dried (Na_2SO_4) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **168** (0.110 g, 50.8%). R_f = 0.85 (EtOAc-hexanes (1:3)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 1715, 1578, 1504, 1461, 1407, 1382, 1267, 1116, 1100, 1067, 1018, 945, 876, and 730 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{30}H_{44}N_6O_6H^+$: 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 terephthalolyl 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_2SO_4) and concentrated to a residue which was purified by chromatography on silica gel in EtOAc-hexanes (1:9) to give **169** (34.8 mg, 26.7%). R_f = 0.76 (EtOAc-hexanes (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 $C_{28}H_{40}N_6O_6H^+$: 557.31; found 557.27.

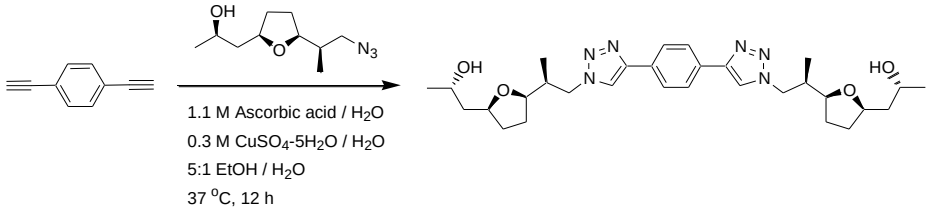


170: *Isophthalic acid bis-{2-[5-(2-azido-1-methyl-ethyl)-tetrahydro-furan-2-yl]-1-methyl-ethyl} ester* **170**. **55** (0.100 g, 0.5 mmol) was added to pyridine (2 mL) followed by isophthaloyl chloride (0.048 g, 0.2 mmol) at room temperature. The solution was stirred for 3 days. Ethyl acetate (50 mL) 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_2SO_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, $CDCl_3$) δ 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 $C_{28}H_{40}N_6O_6H^+$: 557.31; found 557.37.



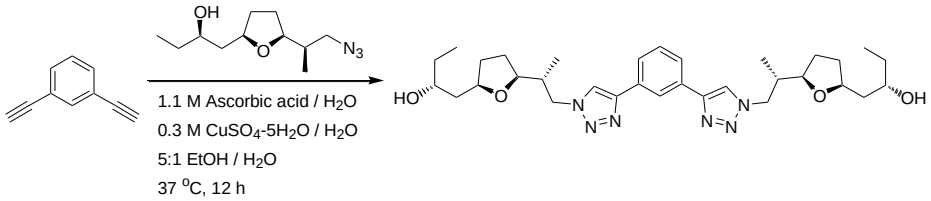
171: *1-[5-(2-{4-[4-(1-{2-[5-(2-Hydroxy-butyl)-tetrahydro-furan-2-yl]-propyl}-1H-[1,2,3]triazol-4-yl)-phenyl]-[1,2,3]triazol-1-yl}-1-methyl-ethyl)-tetrahydro-furan-2-yl]-butan-2-ol* **171**. **97** (0.100 g, 0.5 mmol) was added to 5:1 EtOH/H_2O (3 mL) followed by 1,4-diethylbenzene (0.0264 g, 0.2 mmol). Ascorbic acid/H_2O (1.1 M, 0.84 mL, 0.9 mmol) and $CuSO_4$-$5H_2O$/H_2O (0.3 M, 0.7 mL, 0.2 mmol) was added and the mixture was shaken for 3 days at 37 ^oC. Ethyl acetate (50 mL) was added and

the solution extracted with saturated sodium bicarbonate solution (2 × 100 mL). The organic layer was dried (Na_2SO_4) 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)). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{32}H_{48}N_6O_4H^+$: 581.38; found 581.45.

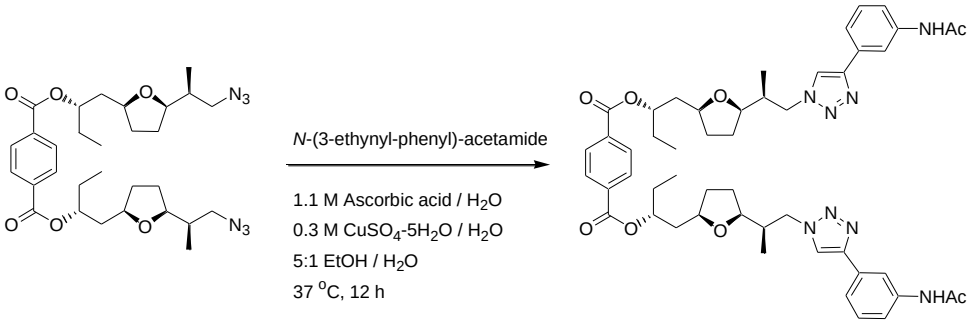


172: *1-[5-(2-{4-[4-(1-{2-[5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-propyl}-1H-[1,2,3]triazol-4-yl)-phenyl]-[1,2,3]triazol-1-yl}-1-methyl-ethyl)-tetrahydro-furan-2-yl]-propan-2-ol* **172**. **55** (50.0 mg, 0.3 mmol) was added to 5:1 EtOH/H_2O (2 mL), followed by 1,4-diethynyl benzene (96%, 15.0 mg, 0.1 mmol). Ascorbic acid/H_2O (1.1 M, 0.45 mL, 0.5 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), 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). ^{1}H

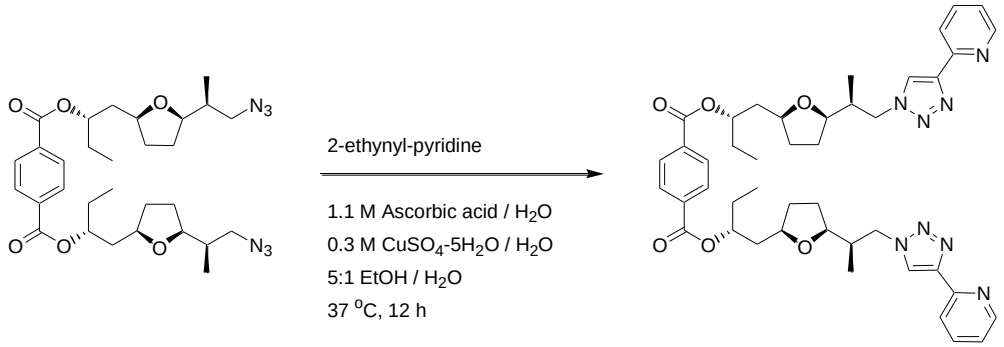
NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 4H), 7.84 (s, 2H), 4.57 (m, 2H), 4.34 (m, 2H), 4.10 (m, 4H), 3.53 (m, 2H), 2.65 (s, 2H), 2.05 (m, 6H), 1.65 (m, 8H), 1.23 (d, J = 6.2 Hz, 6H), and 0.88 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.2, 130.3, 126.0, 120.5, 81.1, 76.9, 65.4, 53.5, 43.5, 40.1, 30.8, 29.4, 23.6, and 14.3



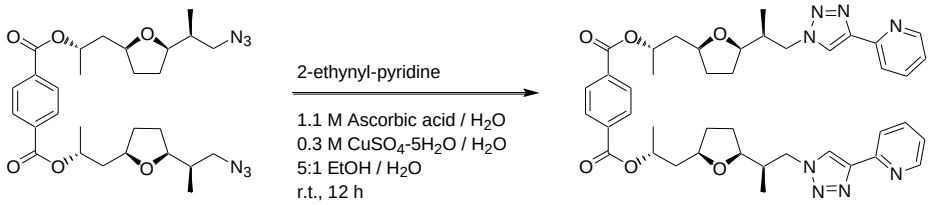
173: *1-[5-(2-{4-[3-(1-{2-[5-(2-Hydroxy-butyl)-tetrahydro-furan-2-yl]-propyl}-1H-[1,2,3]triazol-4-yl)-phenyl]-[1,2,3]triazol-1-yl}-1-methyl-ethyl)-tetrahydro-furan-2-yl]-butan-2-ol* **173**. **97** (97.1 mg, 0.4 mmol) was added to 5:1 EtOH/H_2O (3 mL) followed by 1,3-diethynylbenzene (27 µL, 0.2 mmol). Ascorbic acid/H_2O (1.1 M, 0.8 mL, 0.9 mmol) and $CuSO_4$-$5H_2O$/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{32}H_{48}N_6O_4H^+$: 581.38; found 581.33.



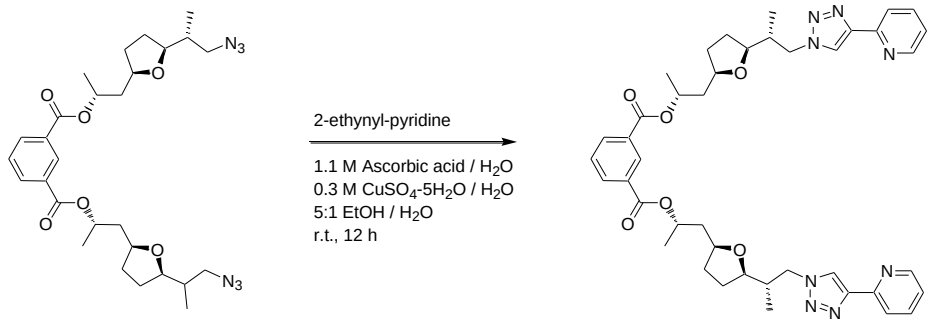
174: *Terephthalic acid bis-[1-(5-{2-[4-(3-acetylamino-phenyl)-[1,2,3]triazol-1-yl]-1-methyl-ethyl}-tetrahydro-furan-2-ylmethyl)-propyl] ester* **174**. **168** (21.0 mg, 0.04 mmol) was added to 5:1 EtOH/H_2O (1.5 mL) and ethyl acetate (1 mL) followed by *N*-(3-ethynyl-phenyl)-acetamide (17.0 mg, 0.1 mmol). Ascorbic acid/H_2O (1.1 M, 72 µL, 0.1 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4) and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **174** (22.7 mg, 70%). R_f = 0.12 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{50}H_{62}N_8O_8H^+$: 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_2O (1.5 mL) and ethyl acetate (1 mL) followed by 2-ethynyl-pyridine, 98+% (11.0 µL, 0.1 mmol). Ascorbic acid/H_2O (1.1 M, 72 µL, 0.08 mmol) and $CuSO_4$-H_2O/H_2O (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{44}H_{54}N_8O_6Na^+$: 813.41; found 813.48.

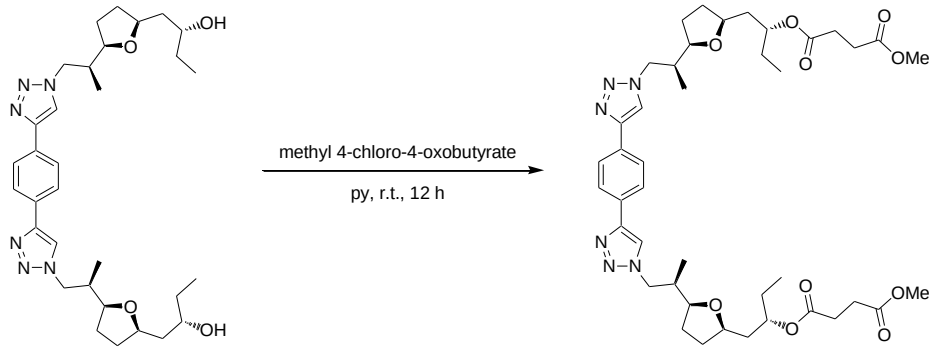


176: *Terephthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester* **176**. **169** (6.5 mg, 0.01 mmol) was added to 5:1 EtOH/H_2O (1.0 mL) and ethyl acetate (1.0 mL) followed by 2-ethynyl-pyridine, 98+% (4.0 µL, 0.04 mmol). Ascorbic acid/H_2O (1.1 M, 23.0 µL, 0.03 mmol) and $CuSO_4$-$5H_2O$/H_2O (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 (Na_2SO_4), concentrated to a residue, and purified by chromatography on silica gel in 100% EtOAc to give **176**. R_f = 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 $C_{42}H_{50}N_8O_6Na^+$: 785.38; found 785.45.



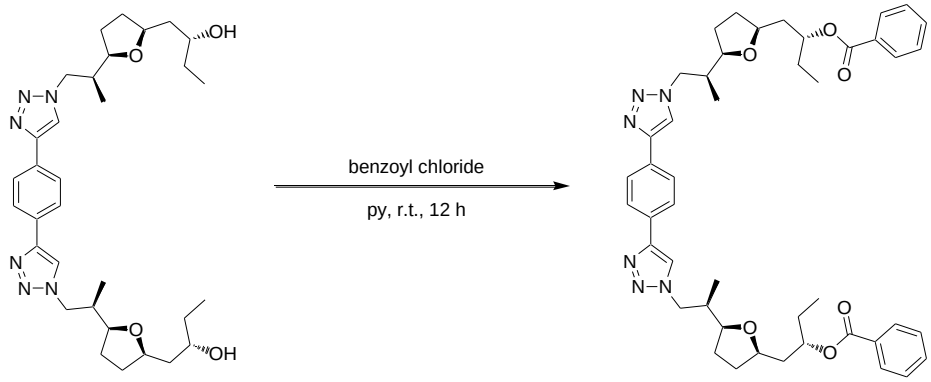
177: *Isophthalic acid bis-(1-methyl-2-{5-[1-methyl-2-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-ethyl]-tetrahydro-furan-2-yl}-ethyl) ester* **177**. **170** (6.0 mg, 0.01 mmol) was added to 5:1 EtOH/H_2O (1.0 mL) and ethyl acetate (1.0 mL) followed by 2-ethynyl-pyridine, 98+% (3.0 µL, 0.03 mmol). Ascorbic acid/H_2O (1.1 M, 22.0 µL, 0.03 mmol) and

$CuSO_4$-H_2O/H_2O (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_2SO_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, 1461, 1377, 1241, 1081, 972, 908, 789, and 734 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{42}H_{50}N_8O_6Na^+$: 785.38; found 785.44.



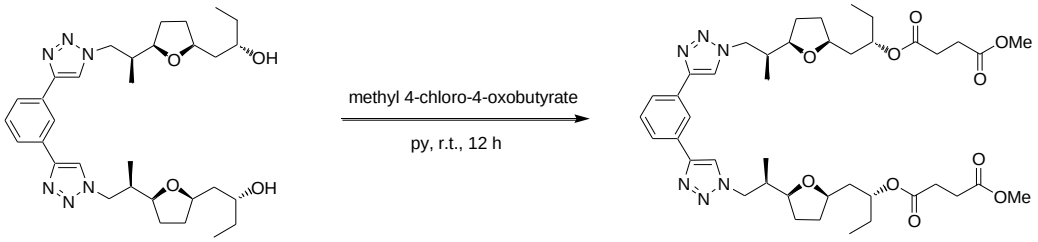
178: *Succinic acid 1-{5-[2-(4-{4-[1-(2-{5-[2-(3-methoxycarbonyl-propionyloxy)-butyl]-tetrahydro-furan-2-yl}-propyl)-1H-[1,2,3]triazol-4-yl]-phenyl}-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-ylmethyl}-propyl ester methyl ester* **178**. **171** (49.0 mg, 0.09 mmol) was added to pyridine (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{42}H_{60}N_6O_{10}H^+$: 809.44; found 809.48.

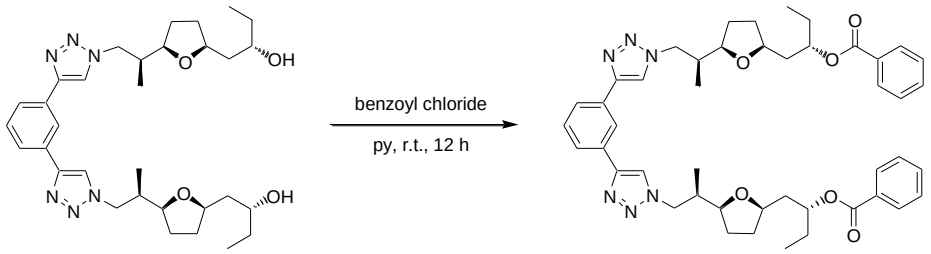


179: *1,1'-(5,5'-(1,1'-(4,4'-(1,4-phenylene)bis(1H-1,2,3-triazole-4,1-diyl))bis(propane-2,1-diyl))bis(tetrahydrofuran-5,2-diyl))bis(butane-2,1-diyl)dibenzoate* **179**. **171** (49.0 mg, 0.09 mmol) was added to pyridine (1 mL) followed by benzoyl chloride (40.0 µL, 0.4 mmol). The solution was stirred 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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,

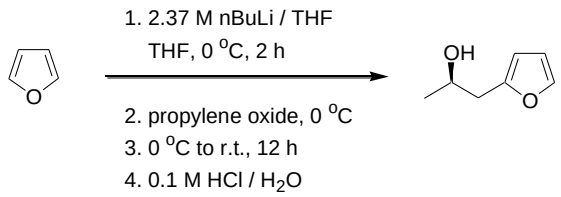
1451, 1366, 1314, 1273, 1229, 1176, 1111, 1069, 1026, 973, 909, 804, 730, and 710 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{46}H_{56}N_6O_6H^+$: 789.43; found 789.45.



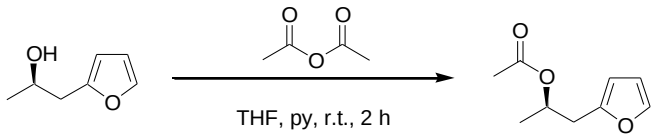
180: *Succinic acid 1-{5-[2-(4-{3-[1-(2-{5-[2-(3-methoxycarbonyl-propionyloxy)-butyl]-tetrahydro-furan-2-yl}-propyl)-1H-[1,2,3]triazol-4-yl]-phenyl}-[1,2,3]triazol-1-yl)-1-methyl-ethyl]-tetrahydro-furan-2-ylmethyl}-propyl ester methyl ester* **180**. **173** (36.0 mg, 0.06 mmol) was added to pyridine (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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 172.7, 172.0, 147.2, 131.3, 125.2, 122.84, 122.78, 121.3, 80.5, 76.0, 73.5, 53.3, 51.8, 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 (Na_2SO_4), and concentrated to a residue which was purified by chromatography on silica gel in 100% EtOAc to give **181** (32.0 mg, 65.4%). R_f = 0.82 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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, 1602, 1451, 1383, 1314, 1273, 1175, 1111, 1069, 1026, 909, 795, 730, and 710 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{46}H_{56}N_6O_6H^+$: 789.43; found 789.49.

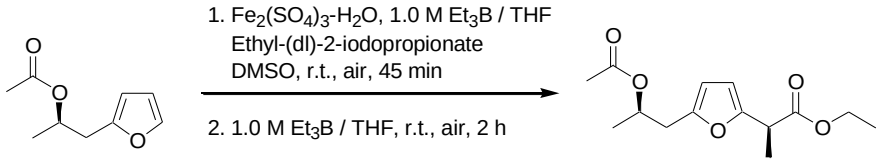


183: *1-Furan-2-yl-propan-2-ol* **183**. Furan **182** (5.0 mL, 68.8 mmol) was added to THF (70 mL), cooled to 0 ºC. nBuLi in THF (2.37 M, 32.0 mL, 75.6 mmol) was added dropwise. The solution was stirred at 0 ºC for 2 hours followed by the dropwise addition of propylene oxide (7.2 mL, 103.1 mmol). The solution was allowed to warm to room temperature overnight. After 12 hours, 0.1 M HCl/H_2O (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 (Na_2SO_4), and evaporated to an oil. The product was purified by distillation (48 ºC, 15 mmHg) to give a clear liquid **183** (5.68 g, 65.6%). R_f = 0.29 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



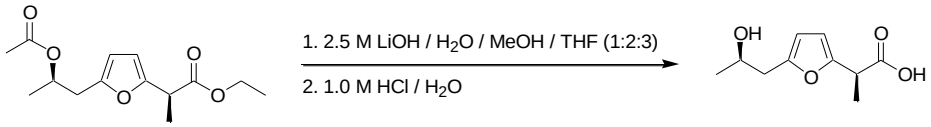
184: *Acetic acid 2-furan-2-yl-1-methyl-ethyl ester* **184**. **183** (5.7 g, 45.1 mmol) was added to THF (15 mL) followed by pyridine (18.4 mL, 225.4 mmol). Acetic anhydride (12.8 mL, 135.2 mmol) was added at room temperature and the solution was then stirred for 2 hours. Ethyl acetate (100 mL) was 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 (Na_2SO_4) and concentrated to an oil. The product was purified by distillation (96 ºC, 24 mmHg) to give slightly yellow oil **184** (5.97 g, 78.8%). R_f = 0.50 (EtOAc-hexanes (1:9); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

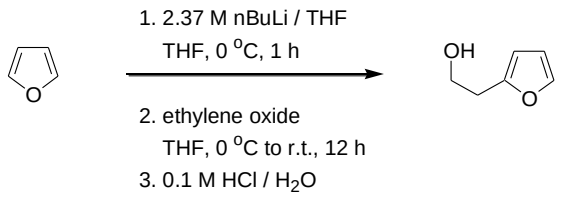


185: *2-[5-(2-Acetoxy-propyl)-furan-2-yl]-propionic acid ethyl ester* **185**. **184** (30.6 g, 182.0 mmol) was added to DMSO (250 mL) followed by $Fe_2(SO_4)_3$-H_2O (72.8 g, 182.0 mmol) and ethyl-(dl)-2-iodopropionate (41.5 g, 182.0 mmol). Et_3B in THF (1.0 M, 182.0 mL, 182.0 mmol) was added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et_3B 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 (Na_2SO_4), 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%). R_f = 0.56 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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,

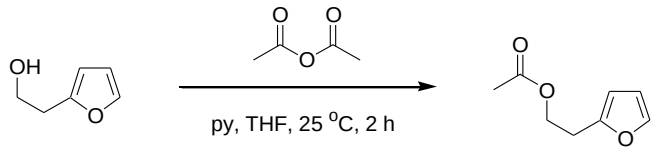
3H), 1.43 (d, J = 7.3 Hz, 3H), 1.21-1.17 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 170.3, 152.1, 150.7, 107.6, 106.4, 69.2, 60.8, 39.4, 34.3, 21.1, 19.4, 15.6, and 14.0; IR (ATR) 2983, 2939, 1735, 1559, 1456, 1372, 1236, 1202, 1057, 1016, 954, and 786 cm^{-1}; ESI-TOF-HRMS m/z calcd for $C_{14}H_{20}O_5H^+$: 269.1398; found 269.1389.



186: *2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propionic acid* **186**. 2.5 M $LiOH/H_2O$/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_2O 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{10}H_{14}O_4H^+$: 199.0970; found 199.0971.

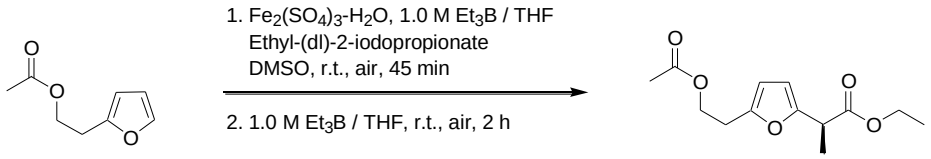


187: *2-Furan-2-yl-ethanol* **187**. Furan **182** (20.0 mL, 275.0 mmol) was added to THF (150 mL) and cooled to 0 ºC. nBuLi in THF (2.37 M, 128.0 mL, 302.5 mmol) was added dropwise to the solution. The solution was stirred for 1 hour and ethylene oxide (14.0 mL, 275.0 mmol) in THF (50 mL) was added dropwise into solution which was then stirred overnight and allowed to warm to room temperature. 0.1 M HCl/H_2O (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 (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



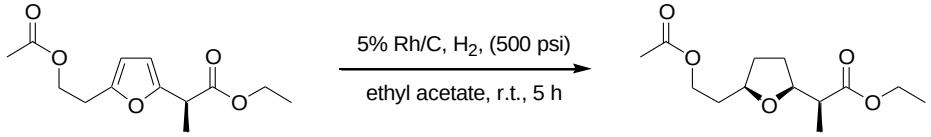
188: *Acetic acid 2-furan-2-yl-ethyl ester* **188**. **187** (9.162 g, 81.7 mmol) was added to THF (27 mL) and pyridine (33.3 mL, 408.5 mmol) followed by acetic anhydride (23.2 mL, 245.1 mmol). The solution was stirred for 2 hours at 25 ºC. Ethyl acetate (100 mL) was added and the solution was washed with saturated copper sulfate solution (2 × 50 mL) and then with saturated ammonium sulfate solution (2 × 50 mL). The organic

layer was dried (Na_2SO_4) and concentrated to an oil. The product was purified by distillation (85 ºC, 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}

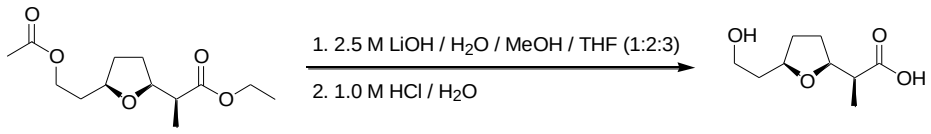


189: *2-[5-(2-Acetoxy-ethyl)-furan-2-yl]-propionic acid ethyl ester* **189**. **188** (30.0 g, 194.7 mmol) was added to DMSO (250 mL) followed by $Fe_2(SO_4)_3$-H_2O (78.0 g, 194.7 mmol) and ethyl-(dl)-2-iodopropionate (44.4 g, 194.7 mmol). Et_3B in THF (1.0 M, 195.0 mL, 194.7 mmol) was then added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et_3B 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 × 100 mL). The organic layers were collected, dried (Na_2SO_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 $C_{13}H_{18}O_5Na^+$: 277.11; found 277.03.

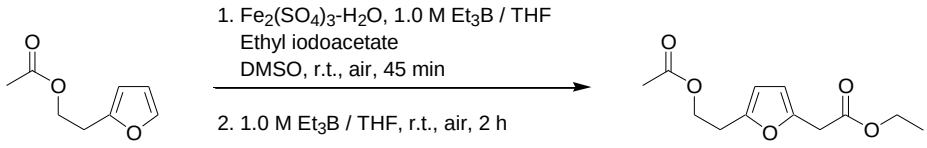


190: *2-[5-(2-Acetoxy-ethyl)-tetrahydro-furan-2-yl]-propionic acid ethyl ester* **190**. **189** (2.001 g, 7.9 mmol) was added to ethyl acetate (10 mL) in a hydrogenator followed by 5% Rh/C (0.6 g) and the solution was stirred at room temperature. The air was flushed out with argon, which was then flushed 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}H NMR (400 MHz, $CDCl_3$) δ 4.04 (m, 5H), 3.85 (m, 1H), 2.39 (m, 1H), 1.94 (s, 3H), 1.90 (m, 2H), 1.71 (m, 2H), 1.60 (m, 1H), 1.43 (m, 1H), and 1.13 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 170.8, 80.0, 76.0, 61.7, 60.0, 45.0, 34.7, 30.8, 28.8, 20.7, 14.0, and 13.8; ESI-TOF-MS m/z calcd for $C_{13}H_{22}O_5H^+$: 259.15; found 259.08.



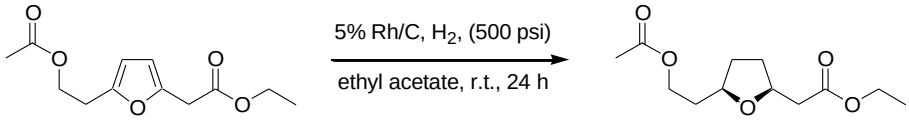
191: *2-[5-(2-Hydroxy-ethyl)-tetrahydro-furan-2-yl]-propionic acid* **191**. 2.5 M LiOH/H_2O/MeOH/THF (1:2:3) (25 mL) was added to **190** (1.2 g, 4.7 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H_2O was added until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50

mL). The organic layers were collected, dried (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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

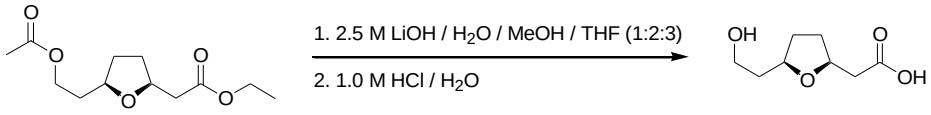


192: *[5-(2-Acetoxy-ethyl)-furan-2-yl]-acetic acid ethyl ester* **192**. **188** (32.09 g, 208.3 mmol) was added to DMSO (300 mL) followed by $Fe_2(SO_4)_3$-H_2O (83.3 g, 208.3 mmol) and ethyl iodoacetate (45.0 mL, 208.3 mmol). Et_3B in THF (1.0 M, 208.5 mL, 208.3 mmol) was added and the solution was stirred for 45 minutes while air was bubbled through the solution. After 45 minutes, Et_3B 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 (Na_2SO_4), 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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.92 (s, 1H), 4.17 (s, 2H), 4.06 (s, 2H), 3.52 (s, 2H), 2.83 (s, 2H), 1.93 (s, 3H), and 1.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 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, 1653, 1567, 1368, 1229, 1031, and 788 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{12}H_{16}O_5Na^+$: 263.09; found 263.01.

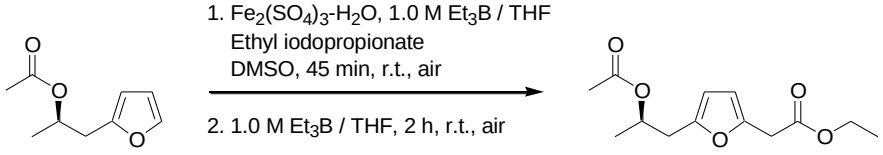


193: *[5-(2-Acetoxy-ethyl)-tetrahydro-furan-2-yl]-acetic acid ethyl ester* **193**. **192** (3.001 g, 12.5 mmol) was added to ethyl acetate (10 mL) in a hydrogenator followed by 5% Rh/C (0.7 g) and the solution was stirred at room temperature. The air was flushed out with argon, which was then flushed 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{12}H_{20}O_5H^+$: 245.14; found 245.07.

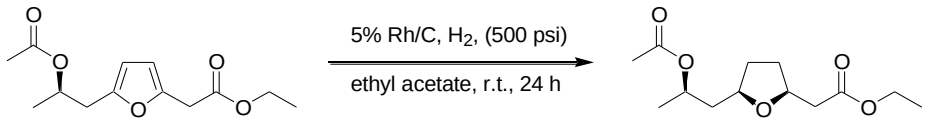


194: *[5-(2-Hydroxy-ethyl)-tetrahydro-furan-2-yl]-acetic acid* **194**. 2.5 M LiOH/H_2O/MeOH/THF (1:2:3) (25 mL) was added to **193** (1.454 g, 6.0 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H_2O was added

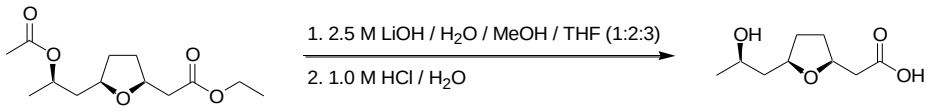
until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50 mL). The organic layers were collected, dried (Na_2SO_4), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give **194** (0.543 g, 52.4%). R_f = 0.70 (EtOAc-MeOH-AcOH (95:4:1)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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. $Fe_2(SO_4)_3$-H_2O (71.35 g, 178.4 mmol) was added followed by ethyl iodoacetate (98%, 21.2 mL, 178.4 mmol). Et_3B 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, Et_3B 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 (Na_2SO_4), 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 $C_{13}H_{18}O_5Na^+$: 277.11; found 277.03.

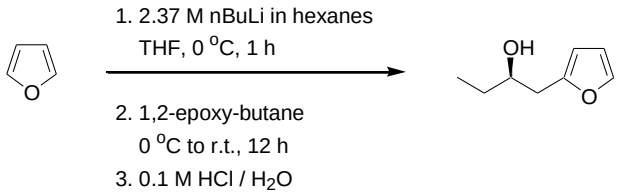


196: *[5-(2-Acetoxy-propyl)-tetrahydro-furan-2-yl]-acetic acid ethyl ester* **196**. **195** (3.024 g, 11.9 mmol) was added to ethyl acetate (10 mL) followed by 5% Rh/C (0.7 g) and the solution stirred at room temperature. The air was flushed out with argon, which was then flushed out with hydrogen. 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%). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{13}H_{22}O_5Na^+$: 281.14; found 281.05.

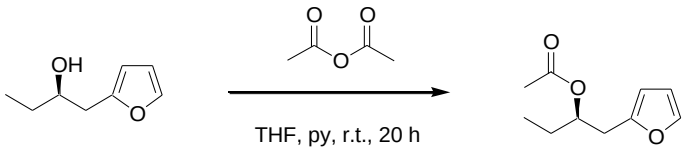


197: *[5-(2-Hydroxy-propyl)-tetrahydro-furan-2-yl]-acetic acid* **197**. 2.5 M $LiOH/H_2O/MeOH/THF$ (1:2:3) (10 mL) was added to **196** (0.493 g, 1.9 mmol) and the solution was stirred for 24 hours at room temperature. 1.0 M HCl/H_2O was added until the solution had a pH of 2. The solution was extracted with ethyl acetate (5 × 50 mL). The organic layers were collected, dried (Na_2SO_4), and concentrated to a

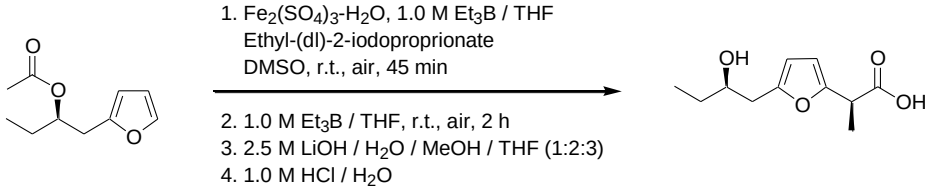
residue. The product was purified by chromatography on silica gel in EtOAc-MeOH-AcOH (194:5:1) to give **197** (0.206 g, 57.5%). R_f = 0.54 (EtOAc-MeOH-AcOH (95:4:1)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.99 (s, 2H), 4.14 (m, 3H), 2.52 (m, 2H), 2.04 (m, 2H), 1.62 (m, 2H), and 1.16 (m, 3H); ^{13}C NMR (100 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 $C_9H_{16}O_4H^+$: 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%). ^{1}H 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); ^{13}C 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}

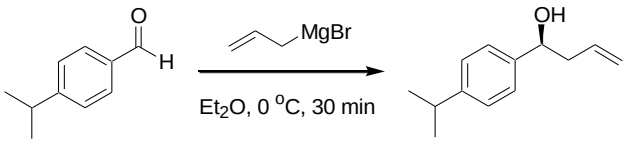


199: *Acetic acid 1-furan-2-ylmethyl-propyl ester* **199**. **198** (30.0 g, 214.0 mmol) was added to THF (44 mL) followed by pyridine (88.0 mL, 1070.1 mmol). Acetic anhydride (61.0 mL, 642.0 mmol) was added and the solution was stirred at room temperature for 7 hours. Acetic anhydride (20.0 mL) was 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 (92ºC, 9.6 mmHg) to give a clear liquid **199** (34.62 g, 88.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



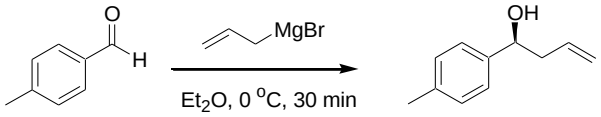
200: *2-[5-(2-Hydroxy-butyl)-furan-2-yl]-propionic acid* **200**. **199** (15.0 mL, 82.3 mmol) was added to DMSO (200 mL) followed by $Fe_2(SO_4)_3$-H_2O (33.0 g, 82.3 mmol) and DL-ethyl-2-iodopropionate (20.0 g, 82.3 mmol). Et_3B in THF (1.0 M, 82.5 mL, 82.3

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_3B 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_2SO_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_2O/MeOH/THF (100 mL) and the solution stirred at room temperature overnight. 1.0 M HCl/H_2O 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{11}H_{16}O_4Na^+$: 235.09; found 235.03.

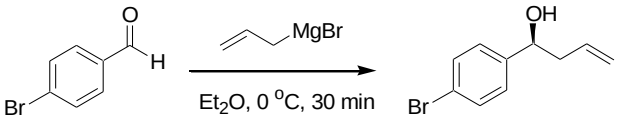


203: *1-(4-Isopropyl-phenyl)-but-3-en-1-ol* **203**. Allyl magnesium bromide in Et_2O (1.0 M, 40.5 mL, 40.5 mmol) was chilled to 0 ºC and 4-isopropylbenzaldehyde (5.11 mL, 33.8 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted

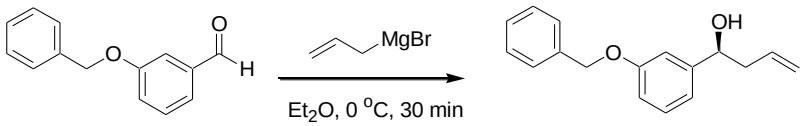
with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na_2SO_4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give **203**. R_f = 0.65 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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}C NMR (100 MHz, $CDCl_3$) δ 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}



204: *1-p-Tolyl-but-3-en-1-ol* **204**. Allyl magnesium bromide in Et_2O (1.0 M, 50.0 mL, 50.0 mmol) was chilled to 0 ºC 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 (Na_2SO_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}C NMR (100 MHz, $CDCl_3$) δ 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}

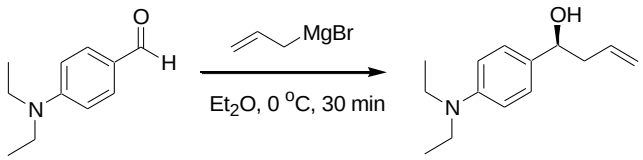


205: *1-(4-Bromo-phenyl)-but-3-en-1-ol* **205**. Allyl magnesium bromide in Et_2O (1.0 M, 32.43 mL, 32.4 mmol) was chilled to 0 ºC and 4-bromobenzaldehyde (5.0 g, 27.0 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution was 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_2SO_4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give **205**. R_f = 0.47 (EtOAc-hexanes (1:3)); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

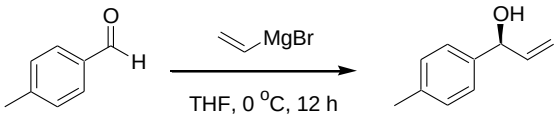


206: *1-(3-Benzyloxy-phenyl)-but-3-en-1-ol* **206**. Allyl magnesium bromide in Et_2O (1.0 M, 28.3 mL, 28.3 mmol) was chilled to 0 ºC and 3-benzyloxybenzaldehyde (5.0 g, 23.6 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added and the solution 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_2SO_4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **206**. R_f = 0.47 (EtOAc-hexanes (1:3)); ^{13}C NMR (100 MHz, $CDCl_3$) δ

159.1, 146.0, 137.2, 134.7, 129.7, 128.8, 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}

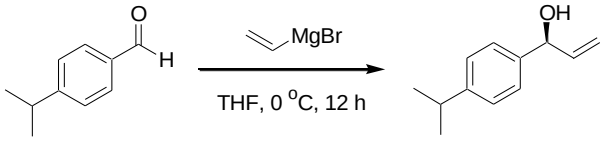


207: *1-(4-Diethylamino-phenyl)-but-3-en-1-ol* **207**. Allyl magnesium bromide in Et_2O (1.0 M, 33.9 mL, 33.9 mmol) was chilled to 0 °C and 4-(diethyl-amino)-benzaldehyde (99%, 5.0 g, 28.2 mmol) was added. The solution was stirred for 30 minutes. Saturated ammonium chloride solution (50 mL) was added 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_2SO_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)); ^1H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



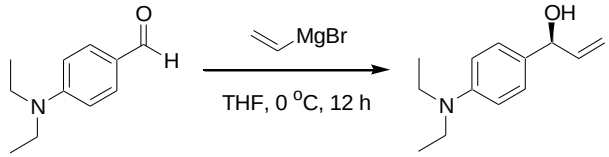
210: *1-p-Tolyl-prop-2-en-1-ol* **210**. Vinyl magnesium bromide in THF (1.0 M, 49.9 mL, 50.0 mmol) was chilled to 0 ºC and p-tolualdehyde (97%, 5.0 g, 41.6 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution

(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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

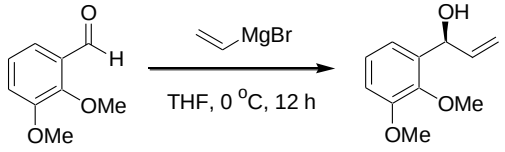


211: *1-(4-Isopropyl-phenyl)-prop-2-en-1-ol* **211**. Vinyl magnesium bromide in THF (1.0 M, 40.5 mL, 40.5 mmol) was chilled to 0 ºC and 4-isopropylbenzaldehyde (5.0 g, 33.8 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 1614, 1512, 1461, 1420, 1383, 1363, 1292, 1248, 1200, 1110, 1055, 1017, 988, 922, and 827 cm^{-1}

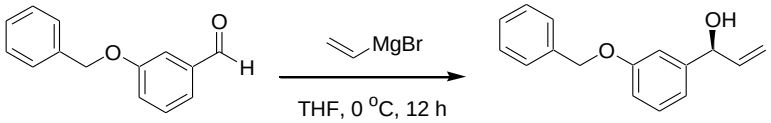


212: *1-(4-Diethylamino-phenyl)-prop-2-en-1-ol* **212**. Vinyl magnesium bromide in THF (1.0 M, 33.9 mL, 33.9 mmol) was chilled to 0 ºC and 4-(diethyl-amino)-benzaldehyde (99%, 5.0 g, 28.2 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and 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_2SO_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)); ^1H 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}



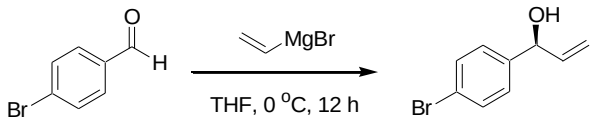
213: *1-(2,3-Dimethoxy-phenyl)-prop-2-en-1-ol* **213**. Vinyl magnesium bromide in THF (1.0 M, 36.11 mL, 36.1 mmol) was chilled to 0 ºC and 2,3-dimethoxybenzaldehyde (5.0 g, 30.1 mmol) was added. The solution was stirred for 12 hours. Saturated

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 (Na_2SO_4) 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

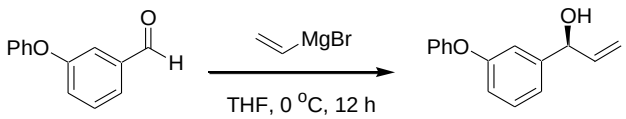


214: *1-(3-Benzyloxy-phenyl)-prop-2-en-1-ol* **214**. Vinyl magnesium bromide in THF (1.0 M, 28.27 mL, 28.3 mmol) was chilled to 0 ºC and 3-benzyloxybenzaldehyde (5.0 g, 23.6 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution 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 (Na_2SO_4) and concentrate 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)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C

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}

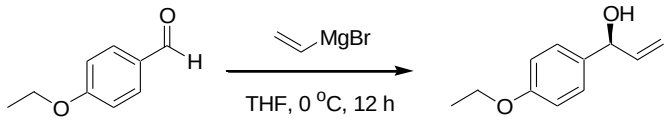


215: *1-(4-Bromo-phenyl)-prop-2-en-1-ol* **215**. Vinyl magnesium bromide in THF (1.0 M, 32.5 mL, 32.4 mmol) was chilled to 0 ºC and 4-bromobenzaldehyde (5.0 g, 27.0 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then 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_2SO_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, 2878, 1907, 1642, 1592, 1486, 1402, 1292, 1248, 1192, 1100, 1071, 1040, 1010, 988, 926, 843, and 816 cm^{-1}



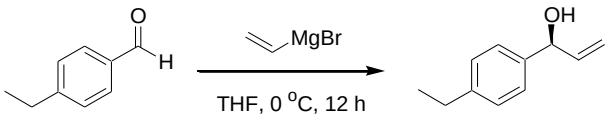
216: *1-(3-Phenoxy-phenyl)-prop-2-en-1-ol* **216**. Vinyl magnesium bromide in THF (1.0 M, 30.3 mL, 30.3 mmol) was chilled to 0 ºC and 3-phenoxybenzaldehyde (5.0 g, 25.2

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 (Na_2SO_4) 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%). R_f = 0.42 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

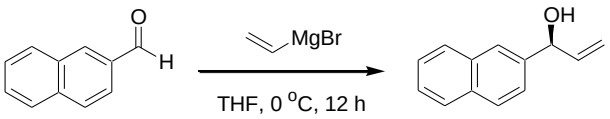


217: *1-(4-Ethoxy-phenyl)-prop-2-en-1-ol* **217**. Vinyl magnesium bromide in THF (1.0 M, 39.9 mL, 39.9 mmol) was chilled to 0 ºC and 4-ethoxybenzaldehyde (5.0 g, 33.3 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then 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 (Na_2SO_4) 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%). R_f = 0.37 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) δ 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}

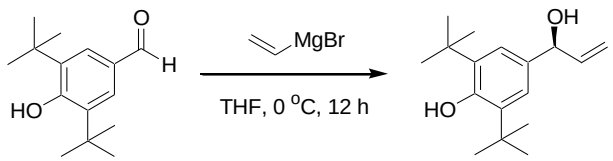


218: *1-(4-Ethyl-phenyl)-prop-2-en-1-ol* **218**. Vinyl magnesium bromide in THF (1.0 M, 44.7 mL, 44.7 mmol) was chilled to 0 ºC and 4-ethylbenzaldehyde (5.0 g, 37.3 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was added and the solution was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine solution (20 mL) and then water (20 mL). The solution was then dried (Na_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



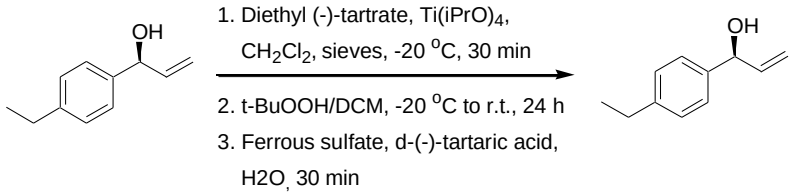
219: *1-Naphthalen-2-yl-prop-2-en-1-ol* **219**. Vinyl magnesium bromide in THF (1.0 M, 38.5 mL, 38.4 mmol) was chilled to 0 ºC and 2-naphthaldehyde (3.0 g, 19.2 mmol)

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 (Na_2SO_4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:3) to give **219** (2.84 g, 82.2%). R_f = 0.42 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

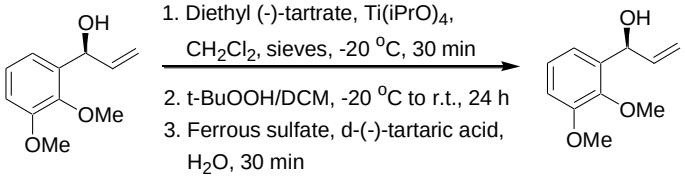


220: *2,6-Di-tert-butyl-4-(1-hydroxy-allyl)-phenol* **220**. Vinyl magnesium bromide in THF (1.0 M, 47.0 mL, 46.9 mmol) was chilled to 0 ºC and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (5.0 g, 21.4 mmol) was added. The solution was stirred for 12 hours. Saturated ammonium chloride solution (50 mL) was 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 (Na_2SO_4) and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:4) to give **220** (3.5 g, 62.5%). R_f = 0.55 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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,

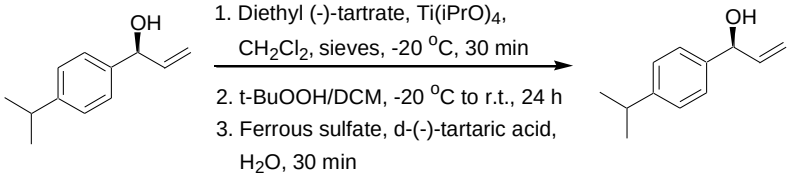
18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5, 140.4, 135.9, 133.2, 123.2, 114.4, 75.7, 34.4, and 30.2; IR (ATR) 3636, 3542, 3430, 3076, 3076, 2956, 2910, 2871, 1643, 1592, 1433, 1391, 1362, 1317, 1234, 1205, 1155, 1119, 1032, 993, 927, and 885 cm^{-1}



222: *1-(4-Ethyl-phenyl)-prop-2-en-1-ol* **222**. **218** (3.5 g, 21.5 mmol) was added to CH_2Cl_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 ºC. 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

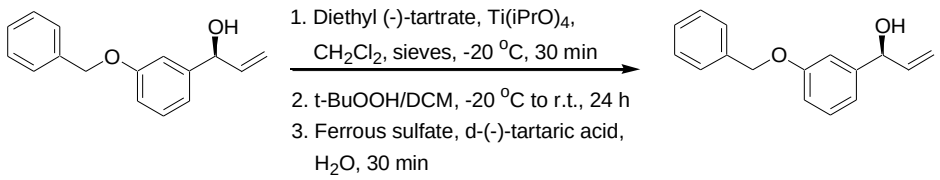


223: *1-(2,3-Dimethoxy-phenyl)-prop-2-en-1-ol* **223**. **213** (1.0 g, 5.2 mmol) was added to CH_2Cl_2 (20.6 mL), followed by 13 x 2µ sieves (1.0 g). Diethyl (–)-tartrate (0.16 g, 0.8 mmol) and $Ti(iPrO)_4$ (0.15 g, 0.5 mmol) were added and the solution was cooled to -20 ºC. After 30 minutes, t-BuOOH 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 (Na_2SO_4) 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%). R_f = 0.27 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



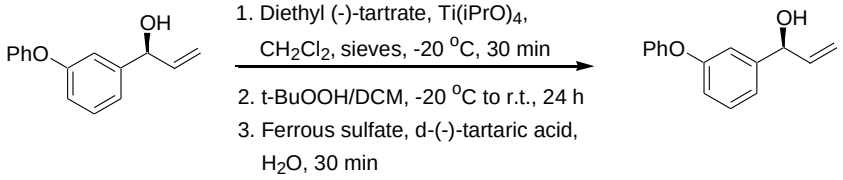
224: *1-(4-Isopropyl-phenyl)-prop-2-en-1-ol* **224**. **211** (3.0 g, 17.0 mmol) was added to CH_2Cl_2 (50 mL), followed by 13 x 2 µ sieves (3.0 g). Diethyl (–)-tartrate (0.53 g, 2.6 mmol) and $Ti(iPrO)_4$ (0.5 g, 1.7 mmol) were added and the solution was cooled to -20

ºC. 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 (Na_2SO_4) 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%). R_f = 0.50 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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, 1614, 1512, 1460, 1419, 1383, 1363, 1292, 1201, 1180, 1109, 1055, 1017, 988, 922, and 827 cm^{-1}



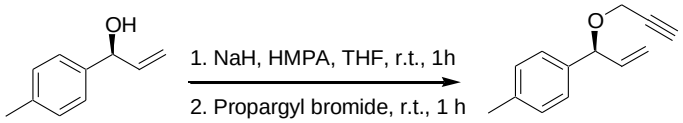
225: *1-(3-Benzyloxy-phenyl)-prop-2-en-1-ol* **225**. **214** (1.84 g, 7.7 mmol) was added to CH_2Cl_2 (30 mL), followed by 13 x 2 µ sieves (2.0 g). Diethyl (–)-tartrate (0.20 mL, 1.2 mmol) and Ti(iPrO)$_4$ (0.23 mL, 0.8 mmol) were added and the solution was cooled to -20 ºC. After 30 minutes, t-BuOOH 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_2SO_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, $CDCl_3$) δ 7.36 (m, 5H), 7.06 (m, 1H), 6.98 (m, 1H), 6.91 (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, $CDCl_3$) δ 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, 1026, 992, 881, 774, 735, and 696 cm^{-1}

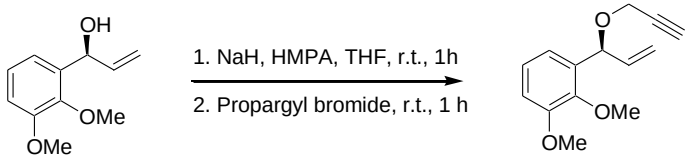


226: *1-(3-Phenoxy-phenyl)-prop-2-en-1-ol* **226**. **216** (3.0 g, 13.3 mmol) was added to CH_2Cl_2 (50 mL), followed by 13 x 2 µ sieves (3.0 g). Diethyl (–)-tartrate (0.41 g, 2.0 mmol) and $Ti(iPrO)_4$ (0.38 g, 1.3 mmol) were added and the solution was cooled to -20 ºC. After 30 minutes, t-BuOOH in 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 × 10 mL) and the organic layer was dried (Na_2SO_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, $CDCl_3$) δ 7.33 (m, 3H), 7.06 (m, 5H), 6.92 (m, 1H), 6.01 (m, 1H), 5.33 (m, 1H), 5.19 (m, 1H), 5.14 (m, 1H), and 2.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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, 2980, 2876, 1864, 1583, 1484, 1443, 1242, 1211, 1163, 1140, 1072, 1023, 989, 926, 821, 780, 751, and 691 cm^{-1}

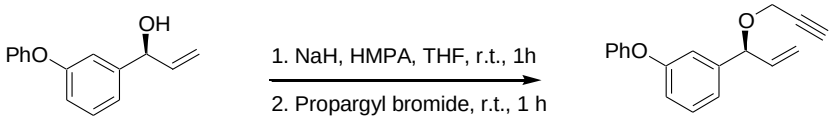


228: *1-Methyl-4-(1-prop-2-ynyloxy-allyl)-benzene* **228**. Sodium hydride in mineral oil (60%, 0.272 g, 6.8 mmol) was added to dry THF (10 mL) followed by **210** (0.503 g, 3.4 mmol). HMPA (1.2 mL, 6.8 mmol) was added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide 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 × 20 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (3:17) to give **228** (0.351g, 55.5%). R_f = 0.77 (EtOAc-hexanes (1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



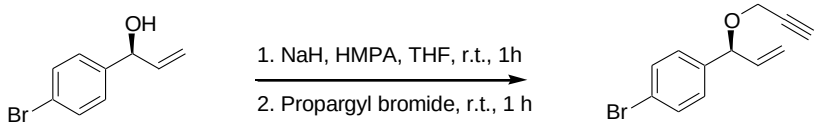
229: *1,2-Dimethoxy-3-(1-prop-2-ynyloxy-allyl)-benzene* **229**. Sodium hydride in mineral oil (60%, 0.045 g, 1.1 mmol) was added to dry THF (10 mL) followed by **213** (0.110 g, 0.6 mmol). HMPA (0.2 mL, 1.1 mmol) was then added to the solution which was

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_2SO_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}

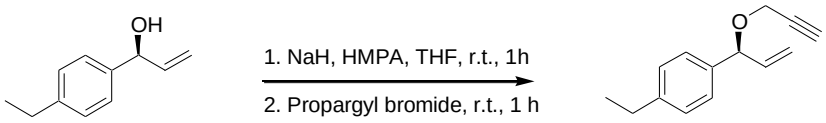


230: *1-Phenoxy-3-(1-prop-2-ynyloxy-allyl)-benzene* **230**. Sodium hydride in mineral oil (60%, 0.177 g, 4.5 mmol) was added to dry THF (10 mL) followed by **216** (0.502 g, 2.2 mmol). HMPA (0.8 mL, 4.5 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl 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_2SO_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

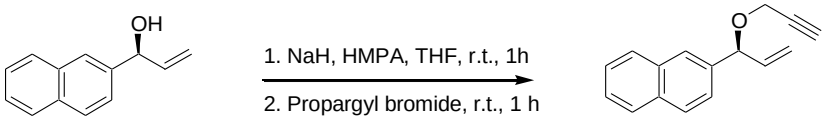
(1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 2H), 7.17 (m, 2H), 7.08 (m, 2H), 6.99 (m, 1H), 5.98 (m, 1H), 5.36 (m, 2H), 5.09 (d, J = 6.9 Hz, 1H), 4.22 (m, 2H), and 2.49 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 156.9, 142.0, 137.3, 129.6, 129.7, 123.1, 121.7, 118.7, 118.0, 117.5, 117.4, 80.6, 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^{-1}



231: *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 × 20 mL). The organic layer was separated, dried (Na_2SO_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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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^{-1}

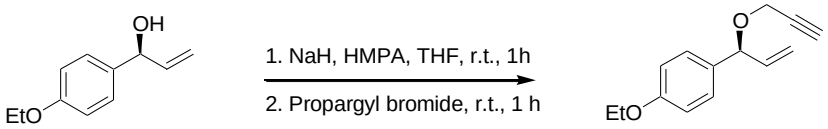


232: *1-Ethyl-4-(1-prop-2-ynyloxy-allyl)-benzene* **232**. Sodium hydride in mineral oil (60%, 0.224 g, 5.6 mmol) was added to dry THF (10 mL) followed by **218** (0.454 g, 2.8 mmol). HMPA (1.0 mL, 5.6 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide 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 (Na_2SO_4), 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%). R_f = 0.77 (EtOAc-hexanes (1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



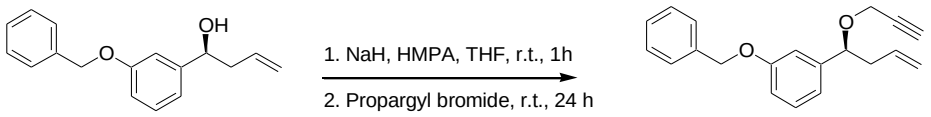
233: *2-(1-Prop-2-ynyloxy-allyl)-naphthalene* **233**. Sodium hydride in mineral oil (60%, 0.217 g, 5.4 mmol) was added to dry THF (10 mL) followed by **219** (0.5 g, 2.7 mmol). HMPA (1.0 mL, 5.4 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide 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 (Na_2SO_4), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give **233** (0.307 g, 50.9%). R_f = 0.63 (EtOAc-hexanes (1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}

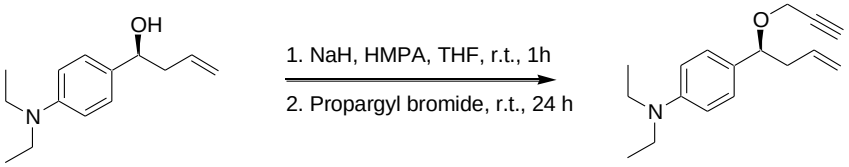


234: *1-Ethoxy-4-(1-prop-2-ynyloxy-allyl)-benzene* **234**. Sodium hydride in mineral oil (60%, 0.225 g, 5.6 mmol) was added to dry THF (10 mL) followed by **217** (0.501 g, 2.8 mmol). HMPA (1.0 mL, 5.6 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl bromide 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 (Na_2SO_4), and concentrated to a residue. The product was purified by chromatography on silica gel in EtOAc-hexanes (1:19) to give **234** (0.256 g, 42.1%). R_f = 0.71 (EtOAc-hexanes (1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) 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 =

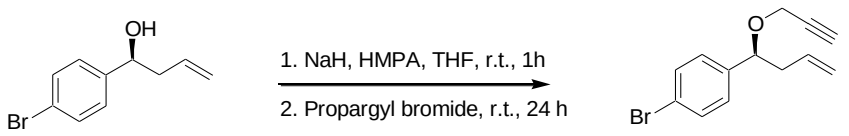
7.0 Hz, 2H), 2.46 (m, 1H), and 1.41 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 137.9, 131.6, 128.2, 116.5, 114.2, 80.5, 79.7, 74.2, 63.1, 54.8, and 14.6; IR (ATR) 3289, 2981, 2901, 1738, 1610, 1585, 1511, 1478, 1442, 1393, 1303, 1240, 1174, 1115, 1067, 1047, 991, 923, and 826 cm^{-1}



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_2SO_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}

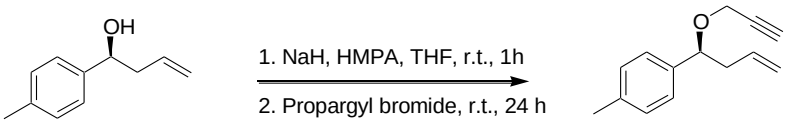


237: *Diethyl-[4-(1-prop-2-ynyloxy-but-3-enyl)-phenyl]-amine* **237**. Sodium hydride in mineral oil (60%, 0.186 g, 4.7 mmol) was added to dry THF (10 mL) followed by **207** (0.511 g, 2.3 mmol). HMPA (0.8 mL, 4.7 mmol) was then added to the solution which was stirred for 1 hour at room temperature. 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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}



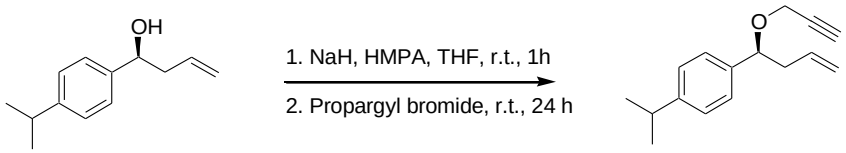
238: *1-Bromo-4-(1-prop-2-ynyloxy-but-3-enyl)-benzene* **238**. Sodium hydride in mineral oil (60%, 0.180 g, 4.5 mmol) was added to dry THF (10 mL) followed by **205** (0.510

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 (Na_2SO_4) 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%). R_f = 0.79 (EtOAc-hexanes (1:9)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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}



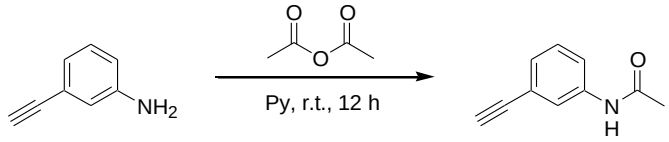
239: *1-Methyl-4-(1-prop-2-ynyloxy-but-3-enyl)-benzene* **239**. Sodium hydride in mineral oil (60%, 0.247 g, 6.2 mmol) was added to dry THF (10 mL) followed by **204** (0.500 g, 3.1 mmol). HMPA (1.1 mL, 6.2 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl 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 (Na_2SO_4) 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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

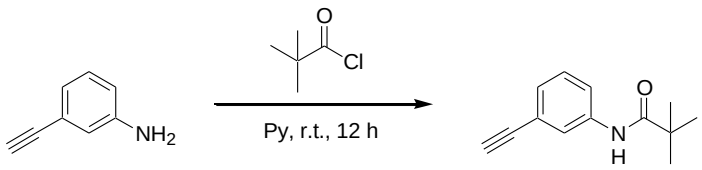


240: *1-Isopropyl-4-(1-prop-2-ynyloxy-but-3-enyl)-benzene* **240**. Sodium hydride in mineral oil (60%, 0.214 g, 5.4 mmol) was added to dry THF (10 mL) followed by **203** (0.510 g, 2.7 mmol). HMPA (1.0 mL, 5.4 mmol) was then added to the solution which was stirred for 1 hour at room temperature. Propargyl 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 × 20 mL). The organic layer was separated and dried (Na_2SO_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)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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}

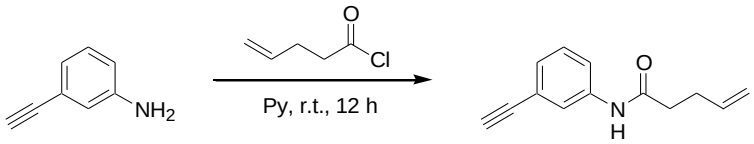


243: *N-(3-Ethynyl-phenyl)-acetamide* **243**. 3-Ethynylaniline **241** (2 mL, 19.1 mmol) was added to pyridine (20 mL). Acetic anhydride (5.4 mL, 57.4 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with ethyl acetate (3 × 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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{10}H_9NOH^+$: 160.08; found 160.05.



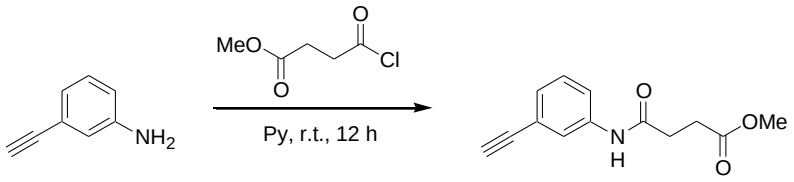
244: *N-(3-Ethynyl-phenyl)-2,2-dimethyl-propionamide* **244**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Trimethylacetyl chloride (99%, 0.3 mL,

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_2SO_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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 1186, 923, 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.

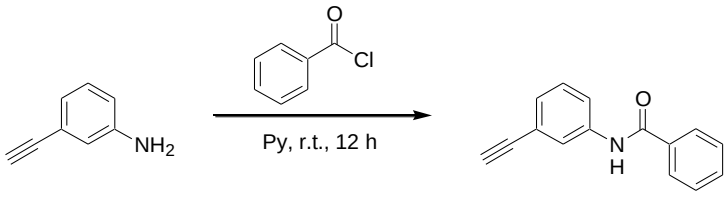


245: *Pent-4-enoic acid (3-ethynyl-phenyl)-amide* **245**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Pentenoyl chloride (98%, 0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then 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_2SO_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, $CDCl_3$)

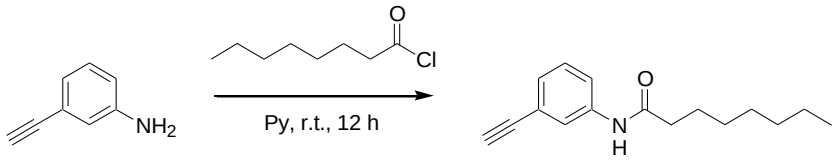
δ 7.925 (s, 1H), 7.635 (s, 1H), 7.52 (m, 1H), 7.22 (m, 2H), 5.83 (m, 1H), 5.05 (m, 2H), 3.04 (s, 1H), and 2.44 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 137.9, 136.6, 128.9, 127.9, 123.4, 122.6, 120.6, 115.9, 83.1, 77.4, 36.5, and 29.3; IR (ATR) 3284, 3079, 2978, 2918, 1660, 1602, 1584, 1536, 1482, 1425, 1406, 1367, 1294, 1237, 994, 920, 873, and 791 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{13}H_{13}NOH^+$: 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 (Na_2SO_4) 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%). R_f = 0.19 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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, 1727, 1738, 1692, 1605, 1550, 1480, 1325, 1171, 884, 805, and 695 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{13}H_{13}NO_3H^+$: 232.10; found 231.98.

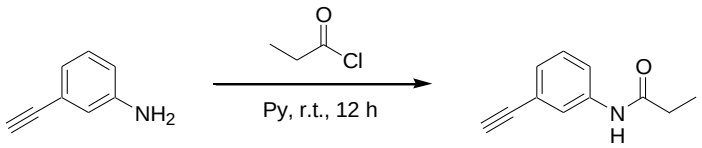


247: *N-(3-Ethynyl-phenyl)-benzamide* **247**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Benzoyl chloride (0.1 mL, 1.0 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted with 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 (Na_2SO_4) 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%). R_f = 0.58 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{15}H_{11}NOH^+$: 222.09; found 221.99.



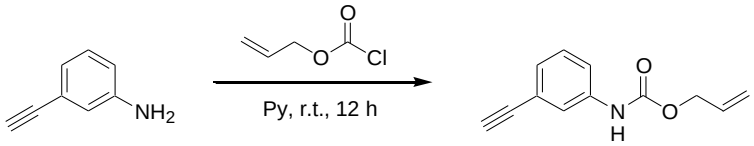
248: *Octanoic acid (3-ethynyl-phenyl)-amide* **248**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Octanoyl chloride (99%, 0.5 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted 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_2SO_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.

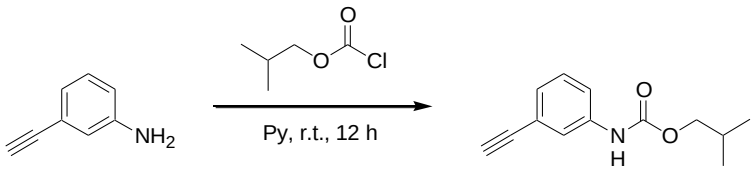


249: *N-(3-Ethynyl-phenyl)-propionamide* **249**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Propionyl chloride (0.3 mL, 2.6 mmol) was added and the solution was stirred for 12 hours at room temperature. Water (10 mL) was added to the solution which was then extracted 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_2SO_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, 128.8, 127.7, 123.4, 122.5, 120.6, 83.1, 77.3, 30.5, and 9.6; IR (ATR) 3248, 3143, 3082, 2983,

2971, 2938, 1650, 1607, 1579, 1547, 1476, 1462, 1417, 1378, 1282, 1221, 1074, 949, 888, and 788 cm^{-1}; ESI-TOF-MS m/z calcd for $C_{11}H_{11}NOH^+$: 174.09; found 174.00.



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 (Na_2SO_4) 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%). R_f = 0.58 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{12}H_{11}NO_2H^+$: 202.09; found 202.00.



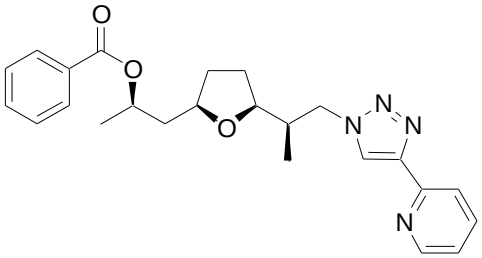
251: *(3-Ethynyl-phenyl)-carbamic acid isobutyl ester* **251**. 3-Ethynylaniline **241** (0.100 g, 0.9 mmol) was added to pyridine (3 mL). Isobutyl chloroformate (98%, 0.4 mL, 2.6

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 (Na_2SO_4) 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%). R_f = 0.81 (EtOAc-hexanes (1:3)); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{13}H_{15}NO_2H^+$: 218.12; found 218.02.

CHAPTER 11

APPENDIX

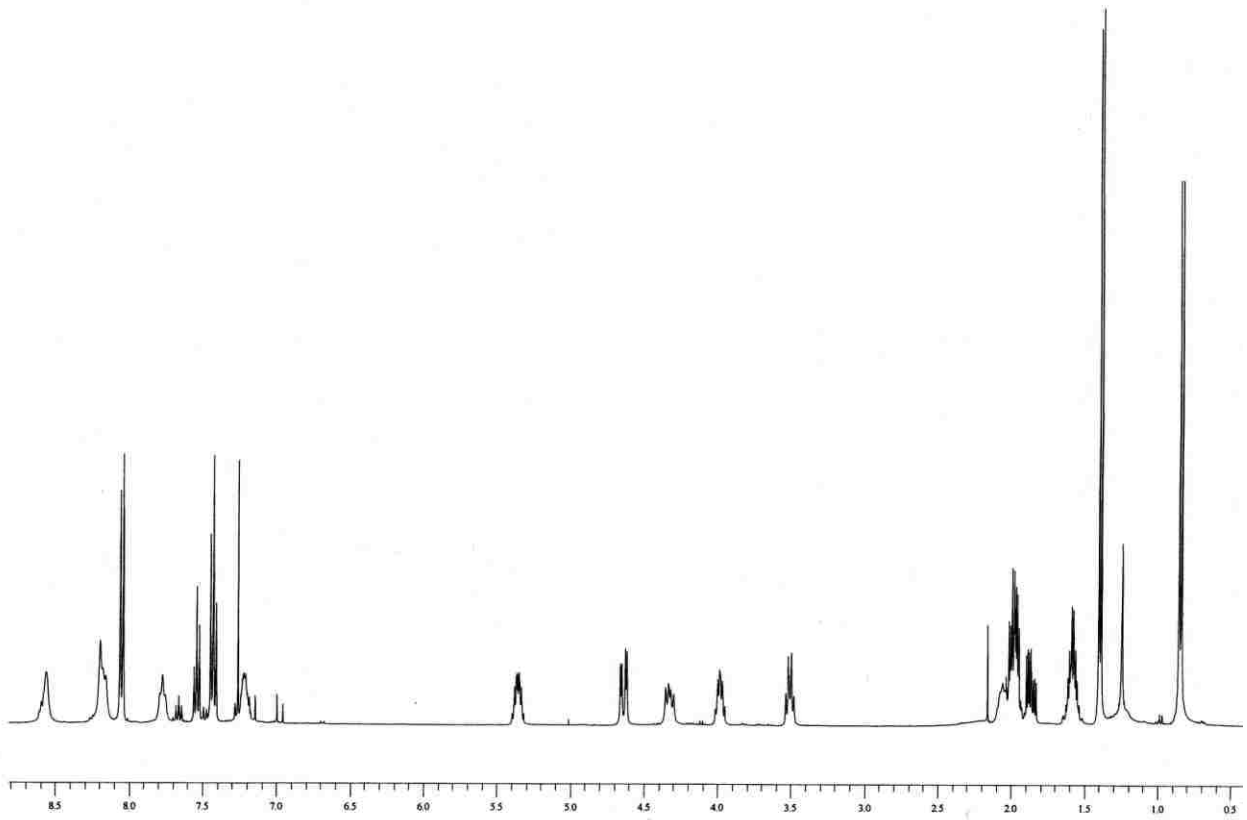
11.1 Spectra for (±)-1,4-Regioisomer, 72

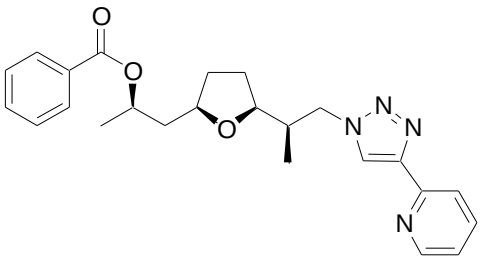


72, Racemic 1,4-Regioisomer

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:

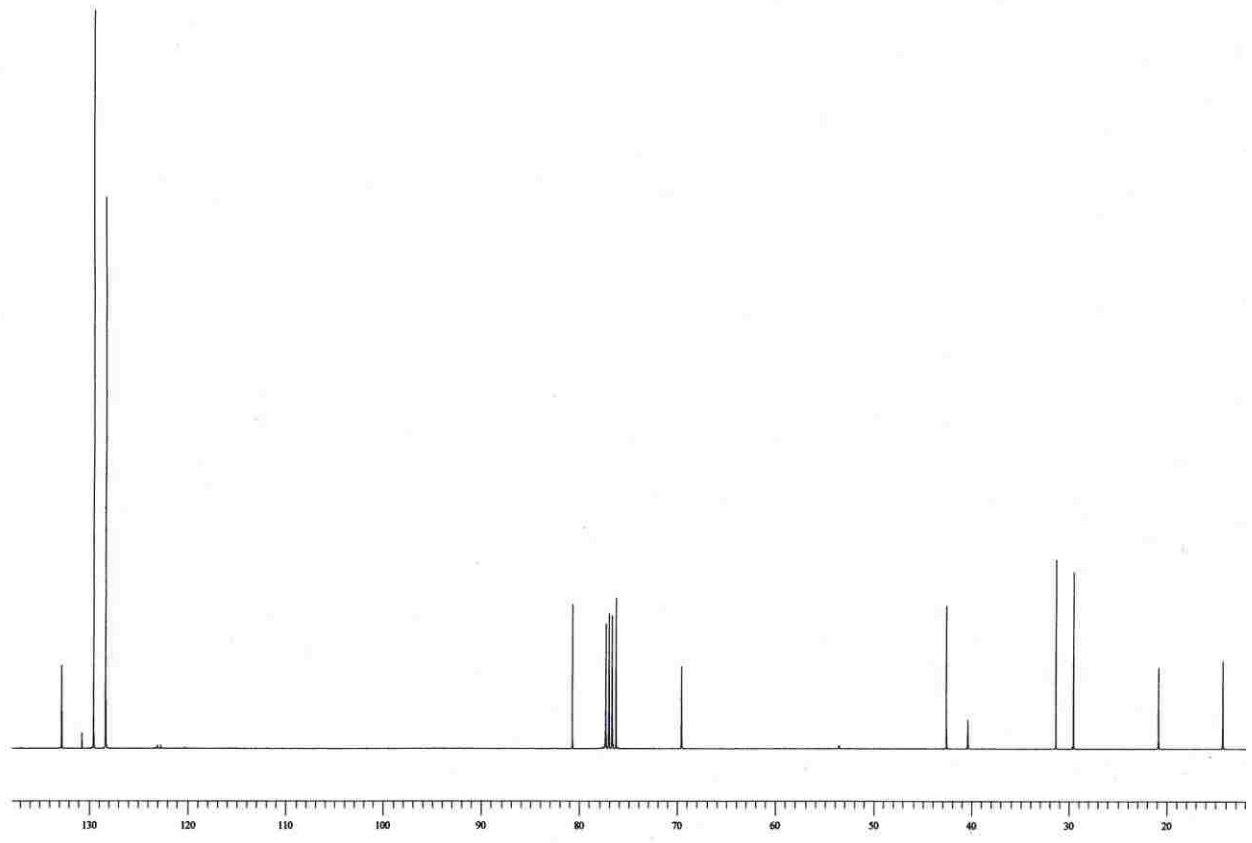


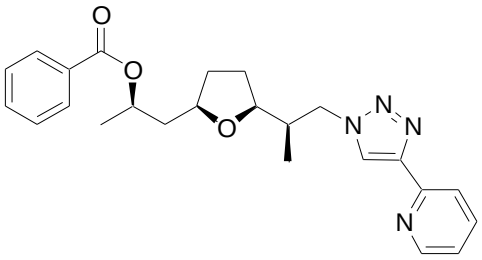


72, Racemic 1,4-Regioisomer

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:

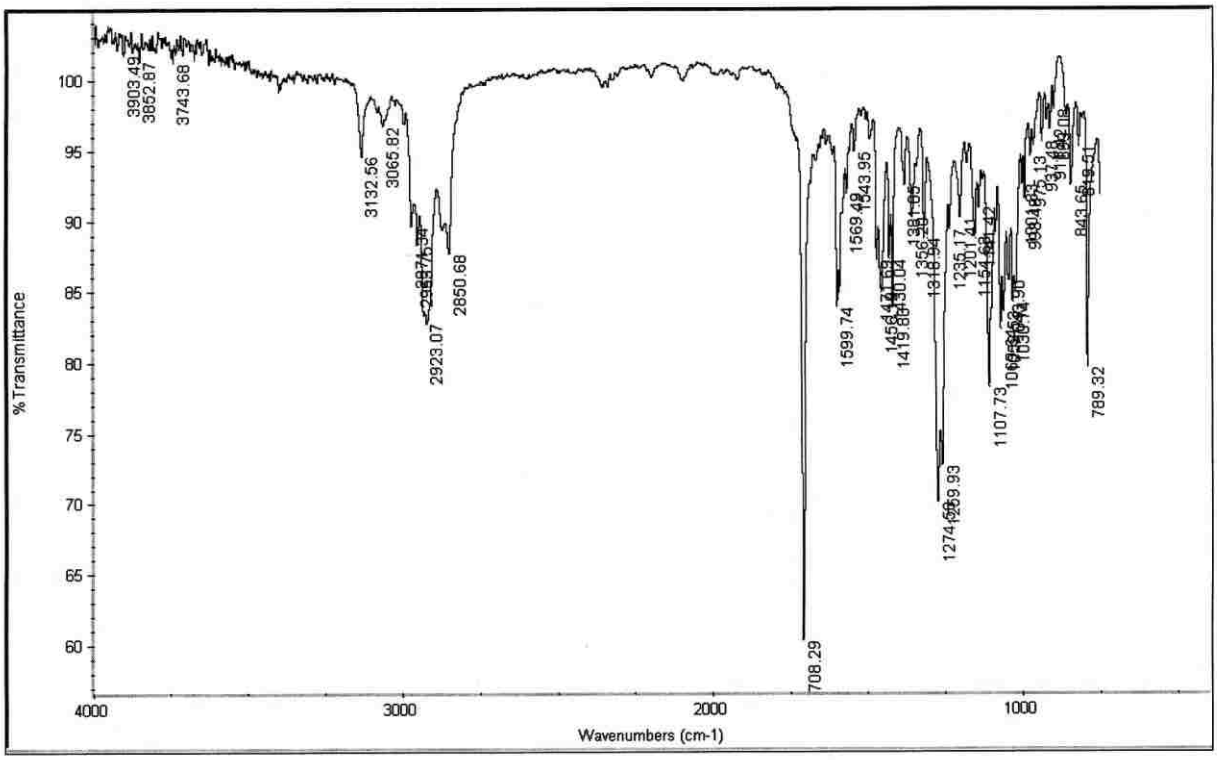




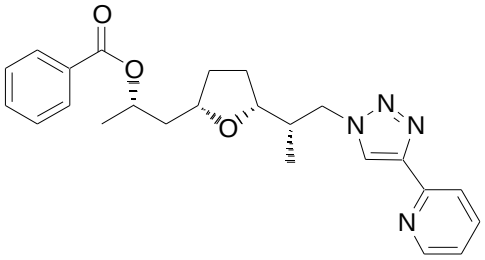
72, Racemic 1,4-Regioisomer

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:



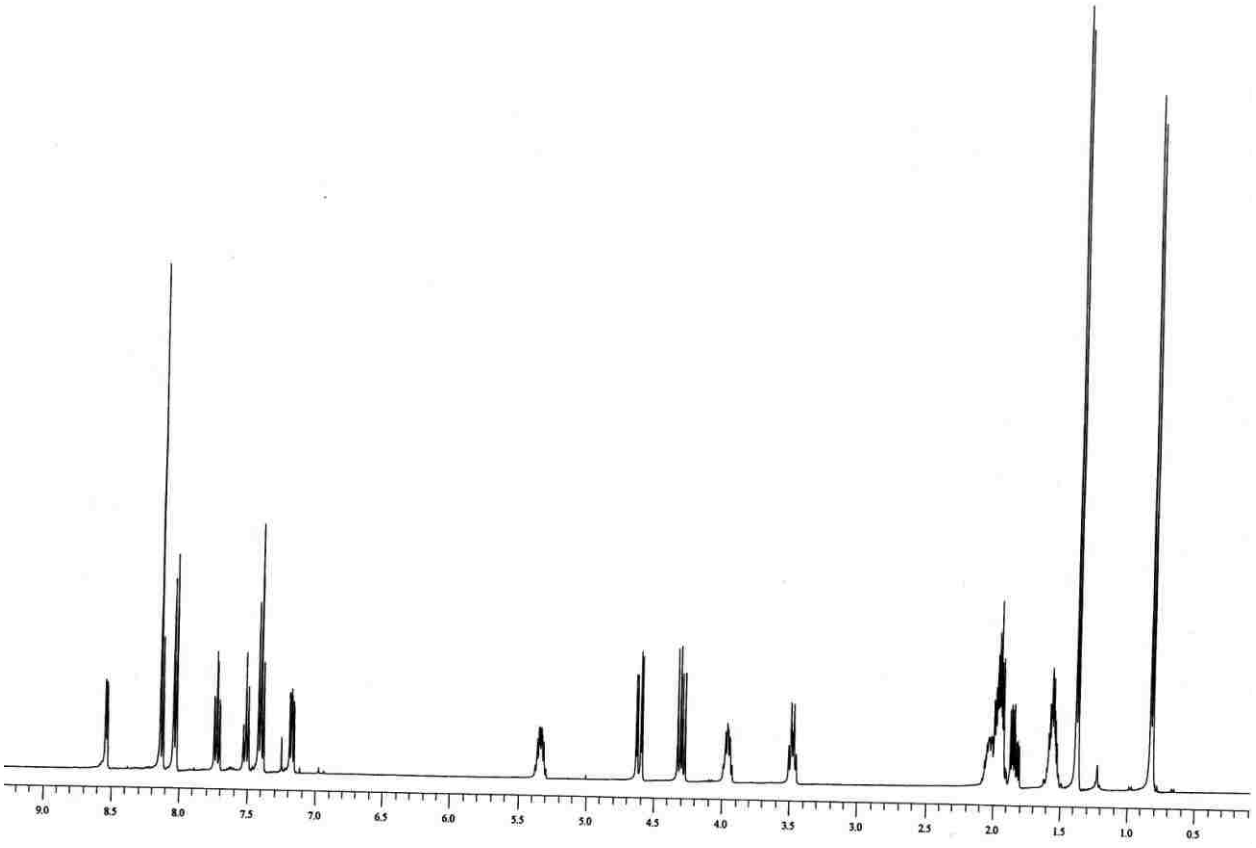
11.2 Spectra for (–)-1,4-Regioisomer, 81

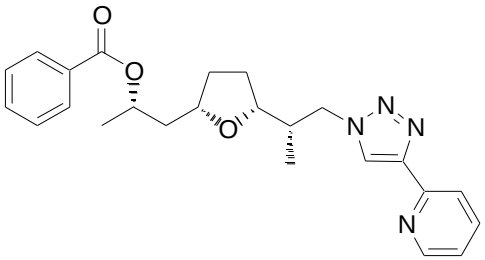


81, Minus 1,4-Regioisomer

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

^{1}H NMR:

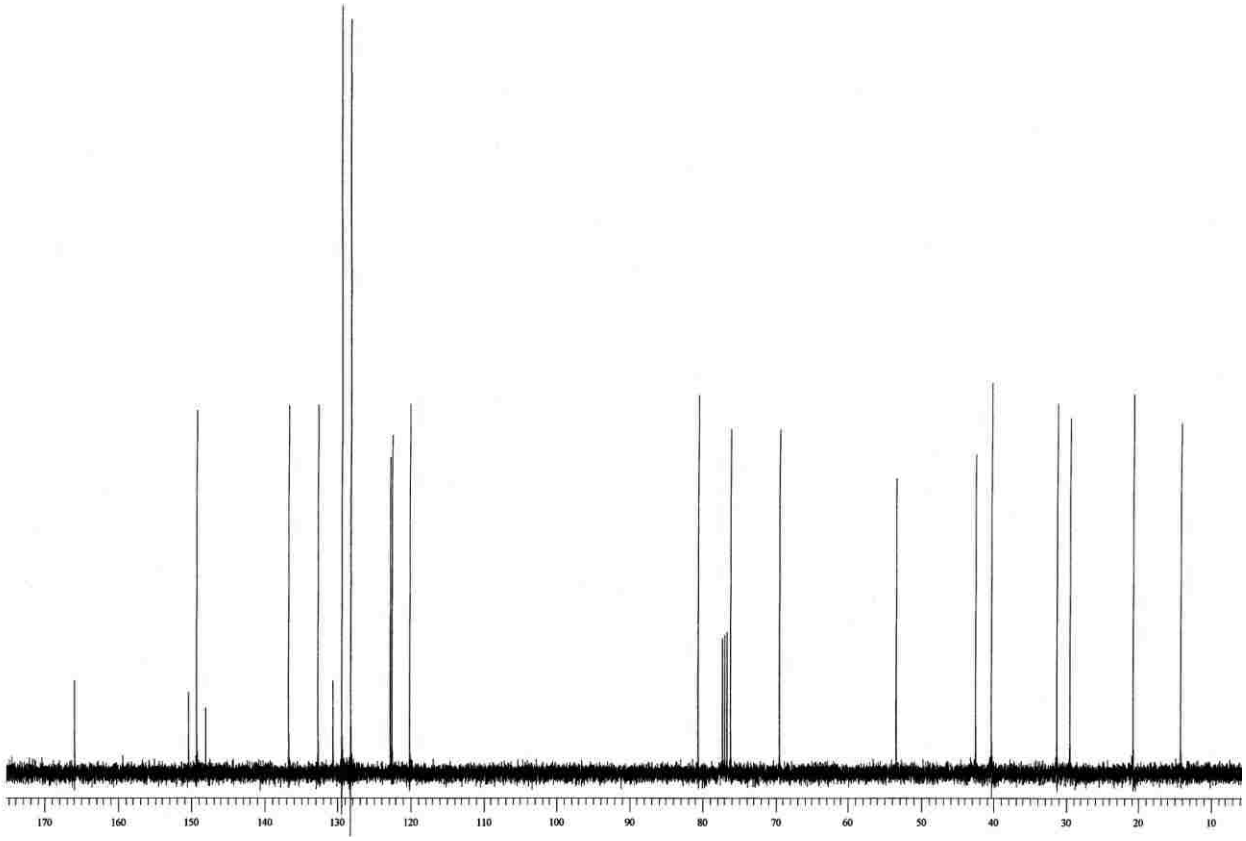


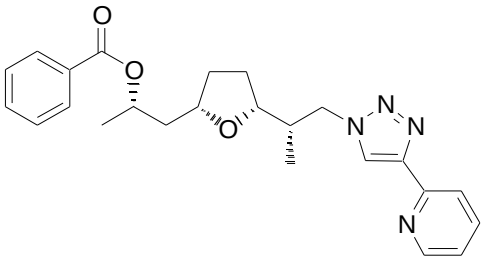


81, Minus 1,4-Regioisomer

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

^{13}C NMR:

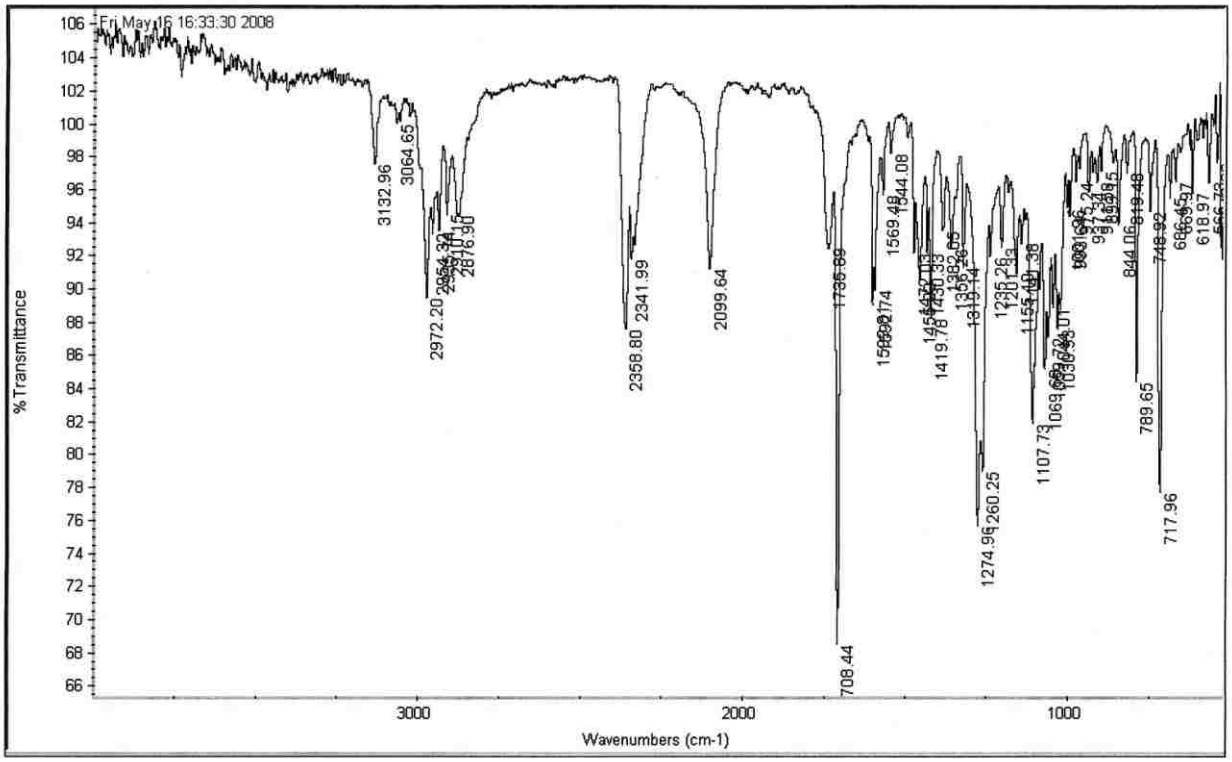


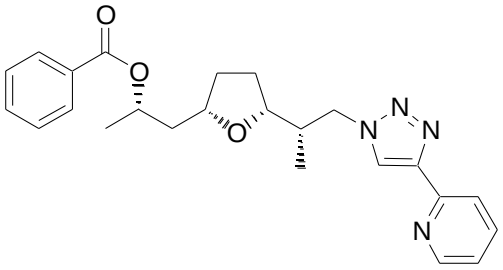


81, Minus 1,4-Regioisomer

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

Infrared Spectrum:

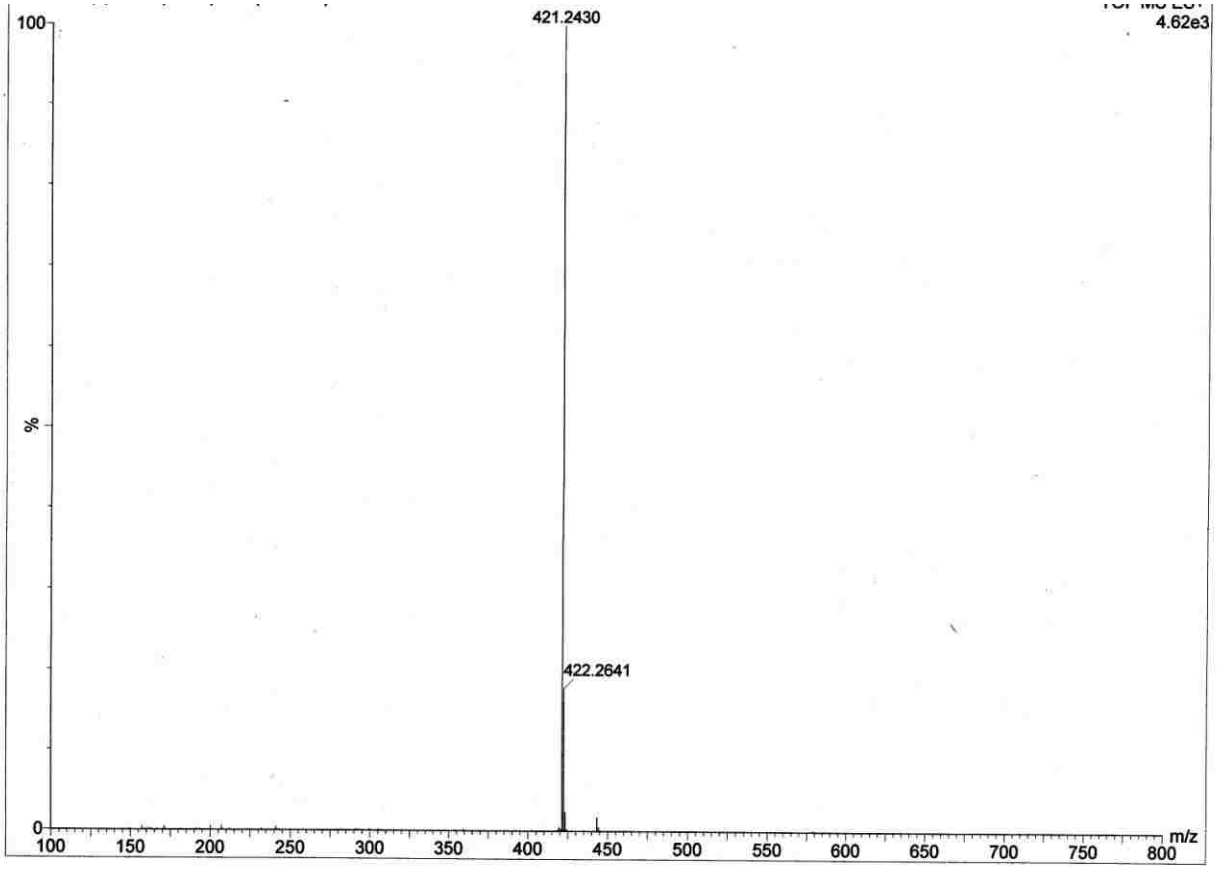




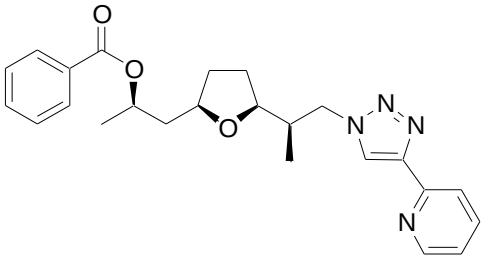
81, Minus 1,4-Regioisomer

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:



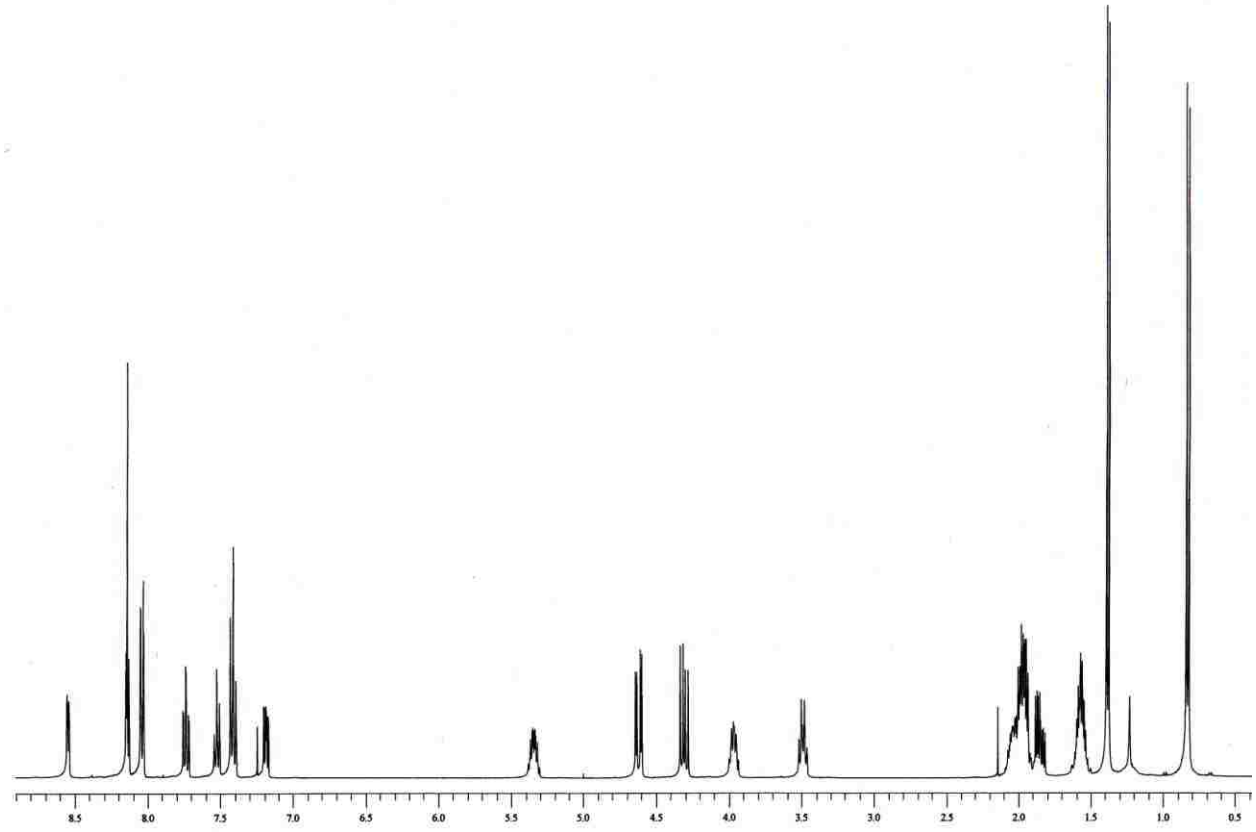
11.3 Spectra for (+)-1,4-Regioisomer, 88

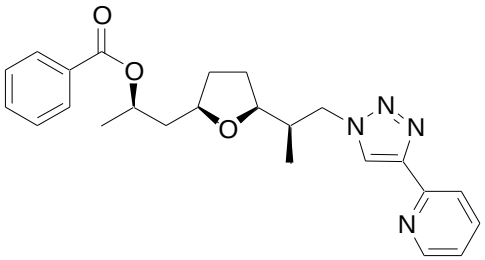


88, Plus 1,4-Regioisomer

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

^{1}H NMR:

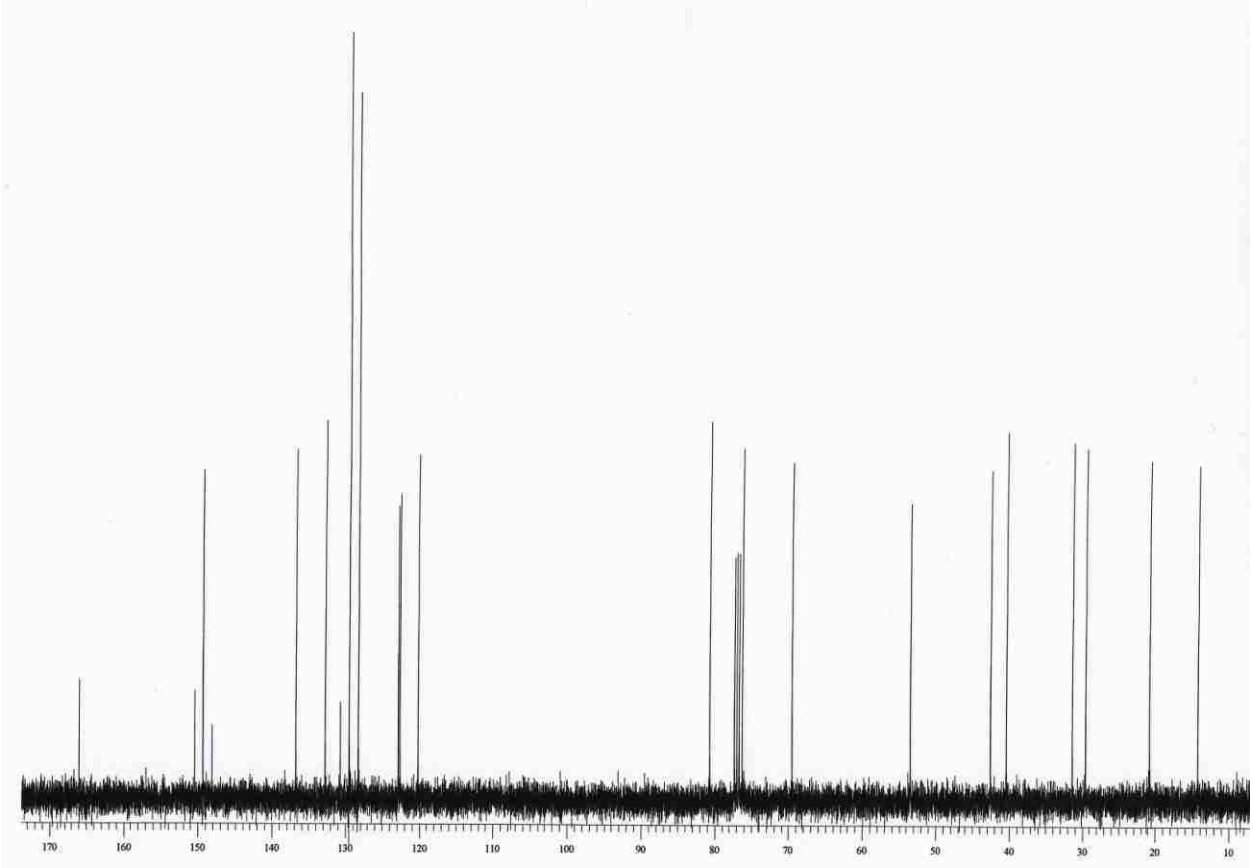


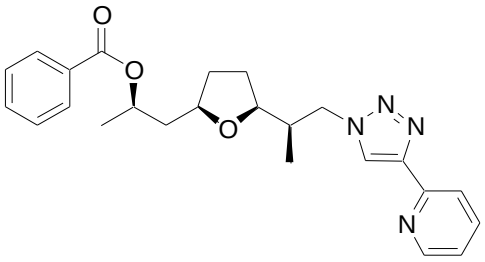


88, Plus 1,4-Regioisomer

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:

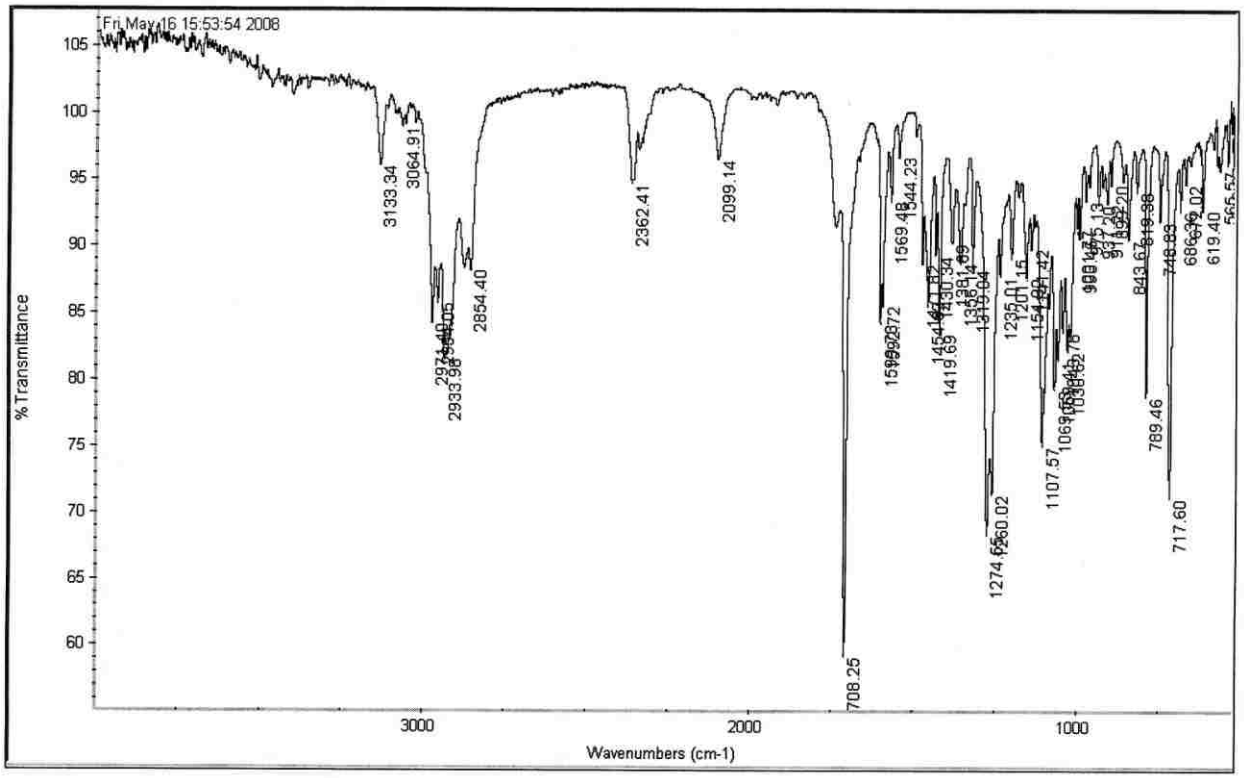


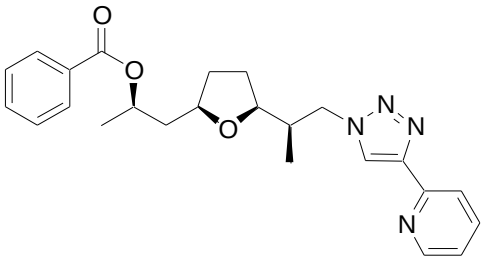


88, Plus 1,4-Regioisomer

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

Infrared Spectrum:

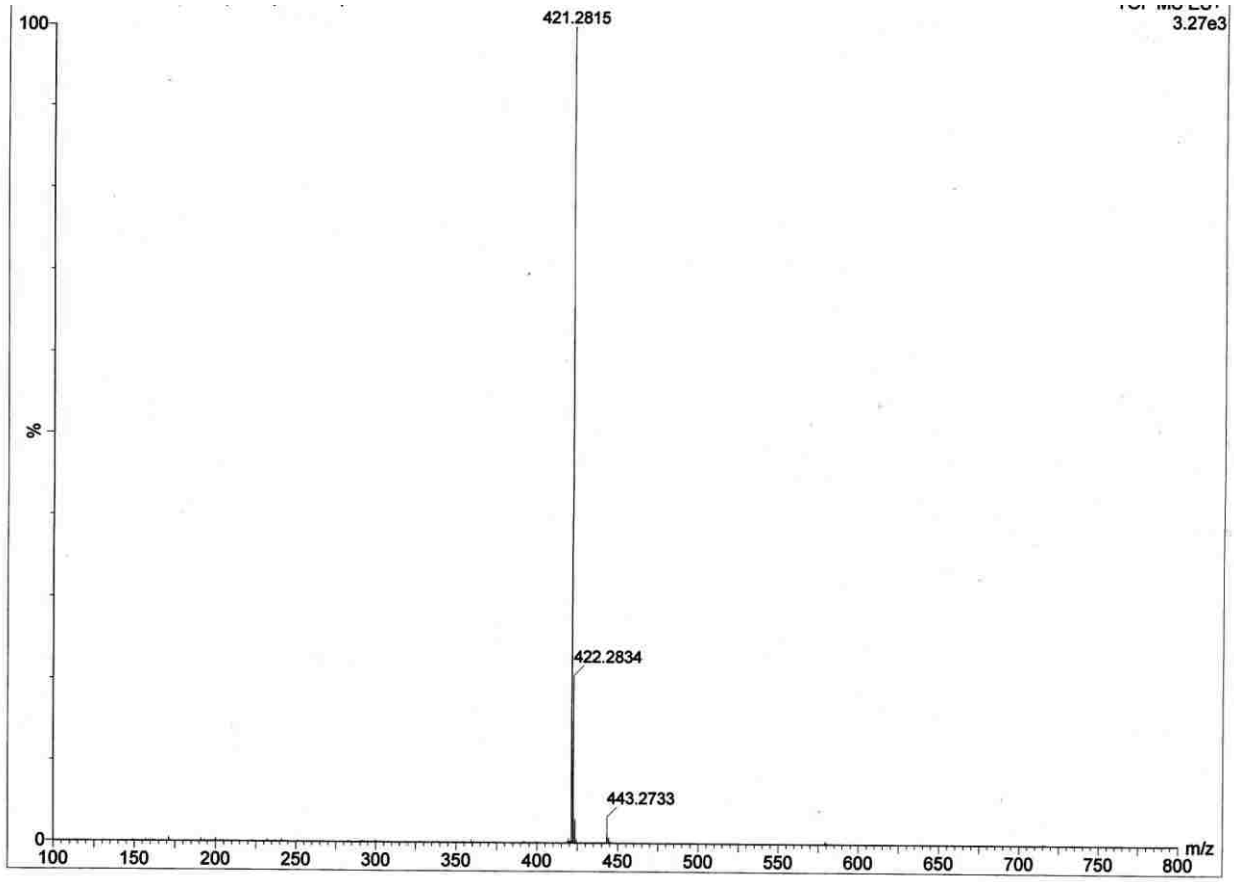




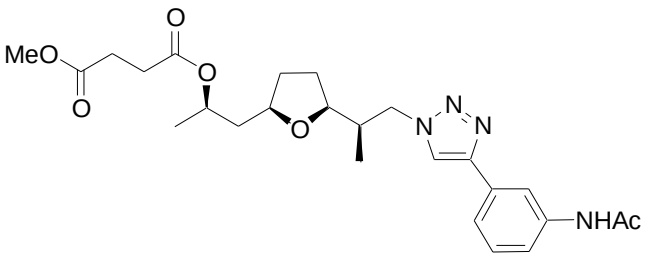
88, Plus 1,4-Regioisomer

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:



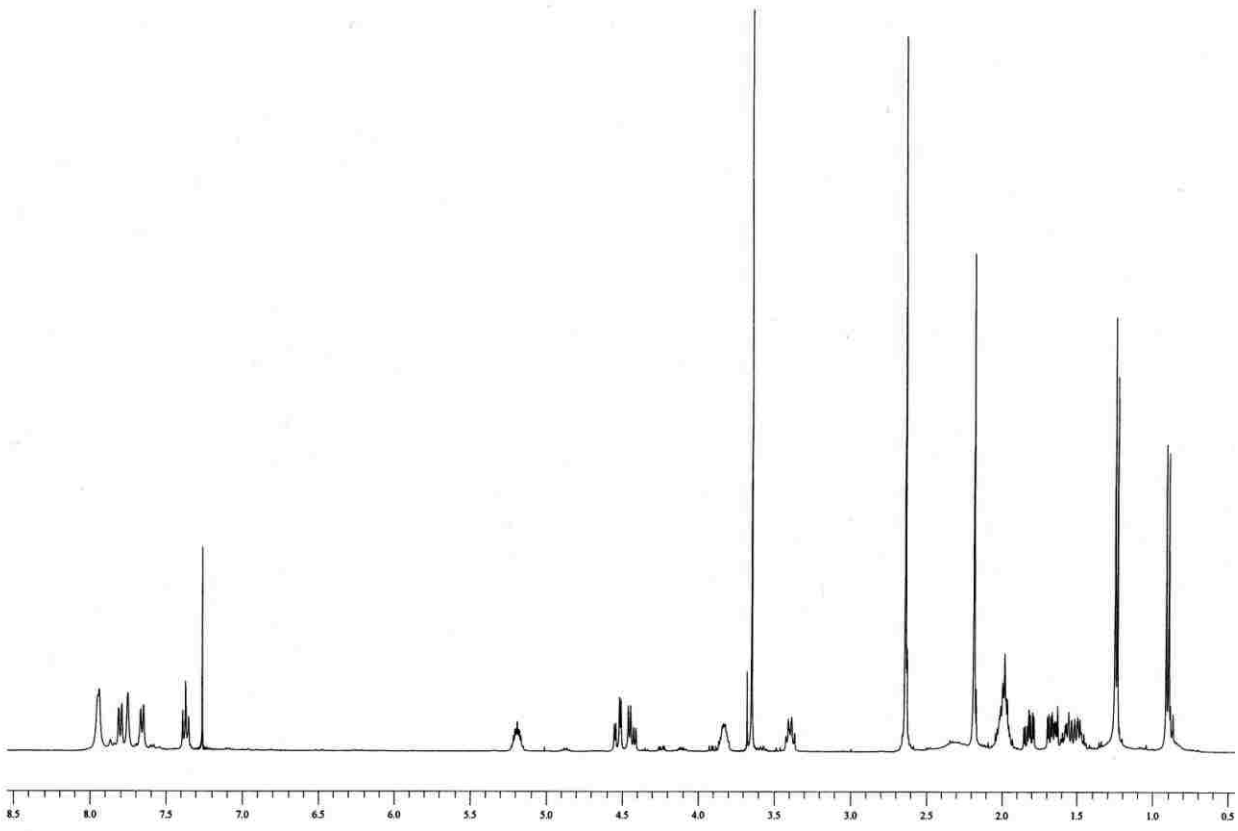
11.4 Spectra for (±)-1,4-Regioisomer, 71

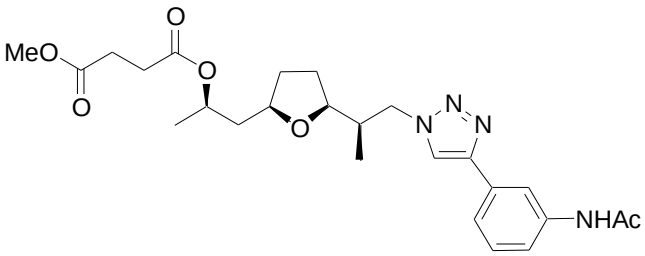


71, Racemic 1,4-Regioisomer

**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**

^{1}H NMR:

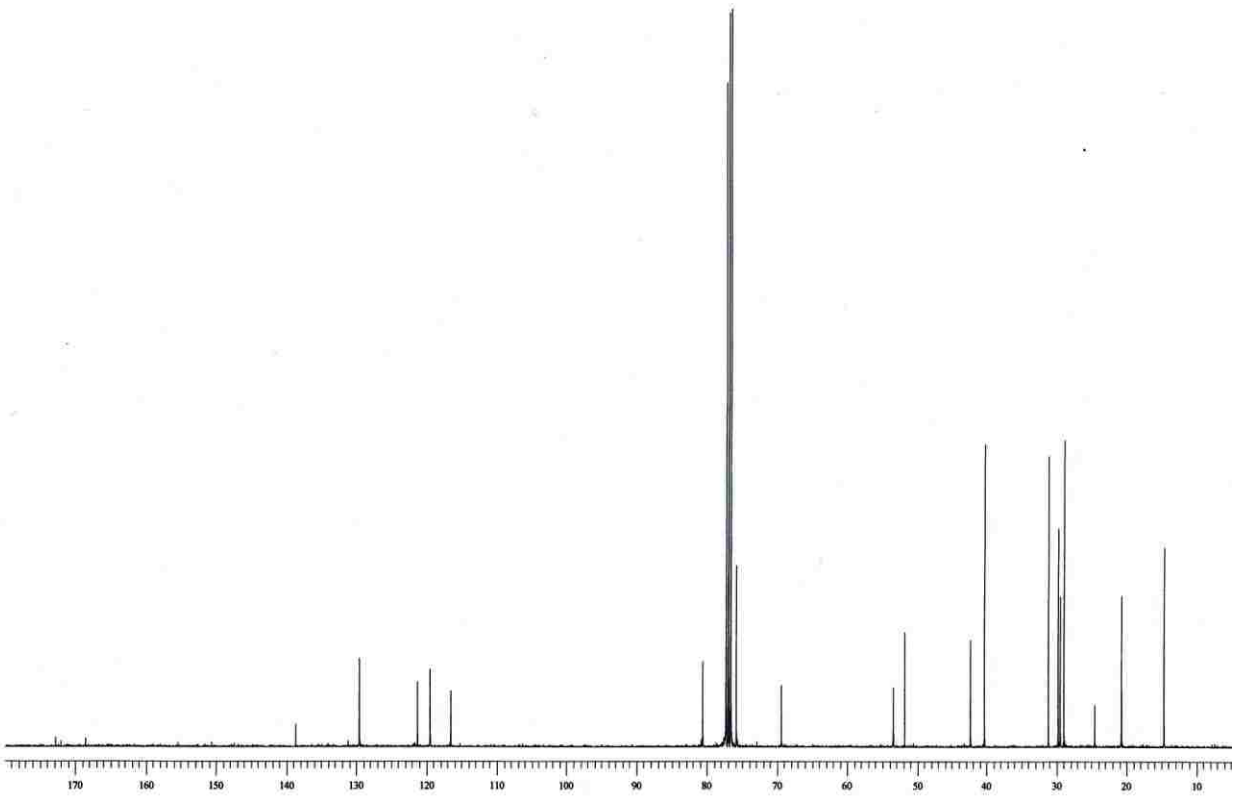


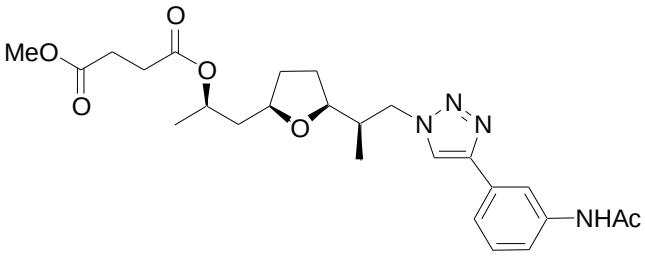


71, Racemic 1,4-Regioisomer

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

^{13}C NMR:

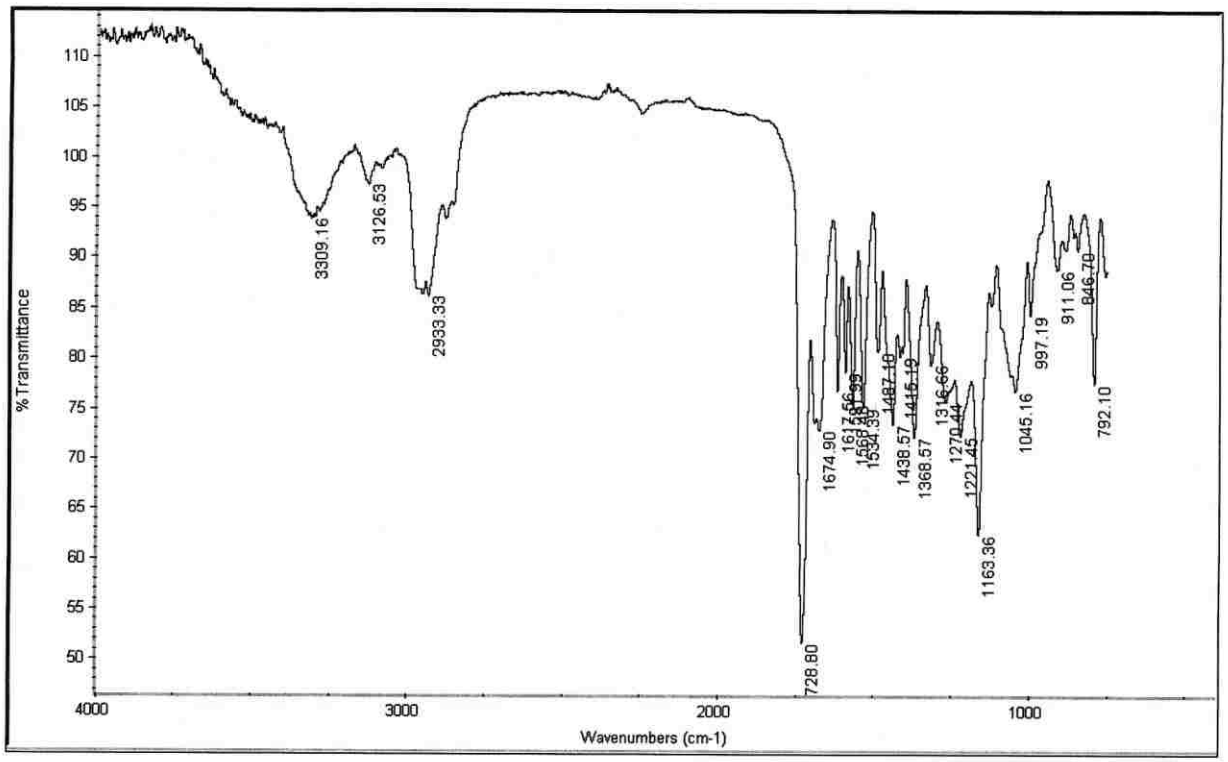




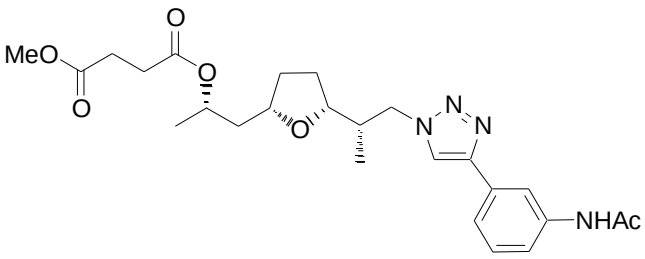
71, Racemic 1,4-Regioisomer

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:



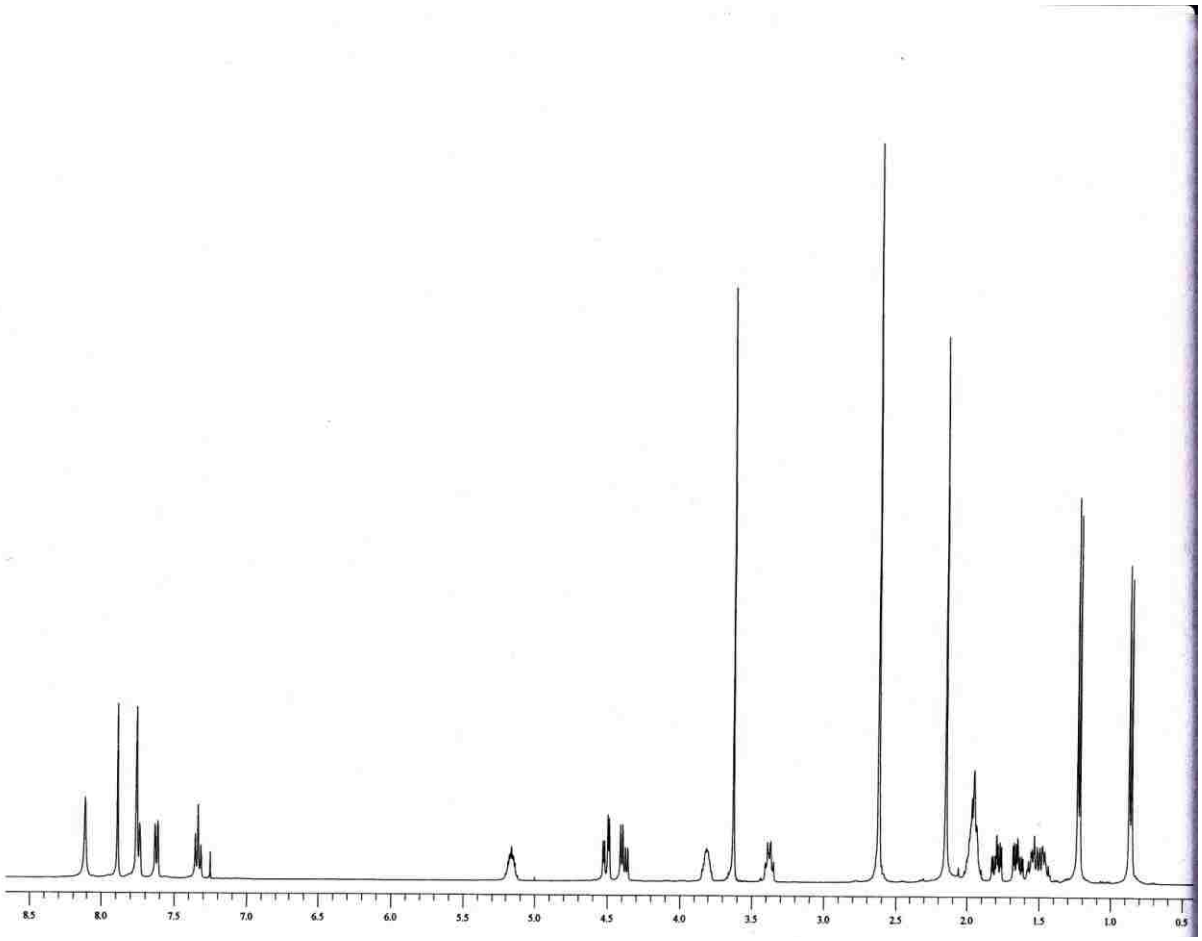
11.5 Spectra for (–)-1,4-Regioisomer, 79

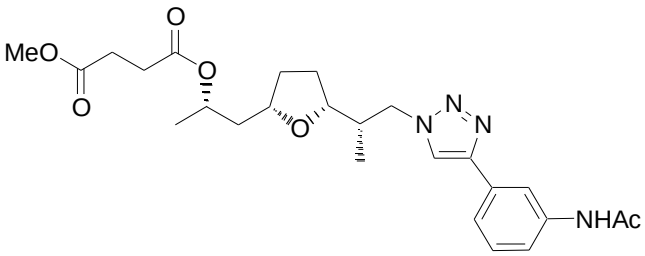


79, Minus 1,4-Regioisomer

**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**

^{1}H NMR:

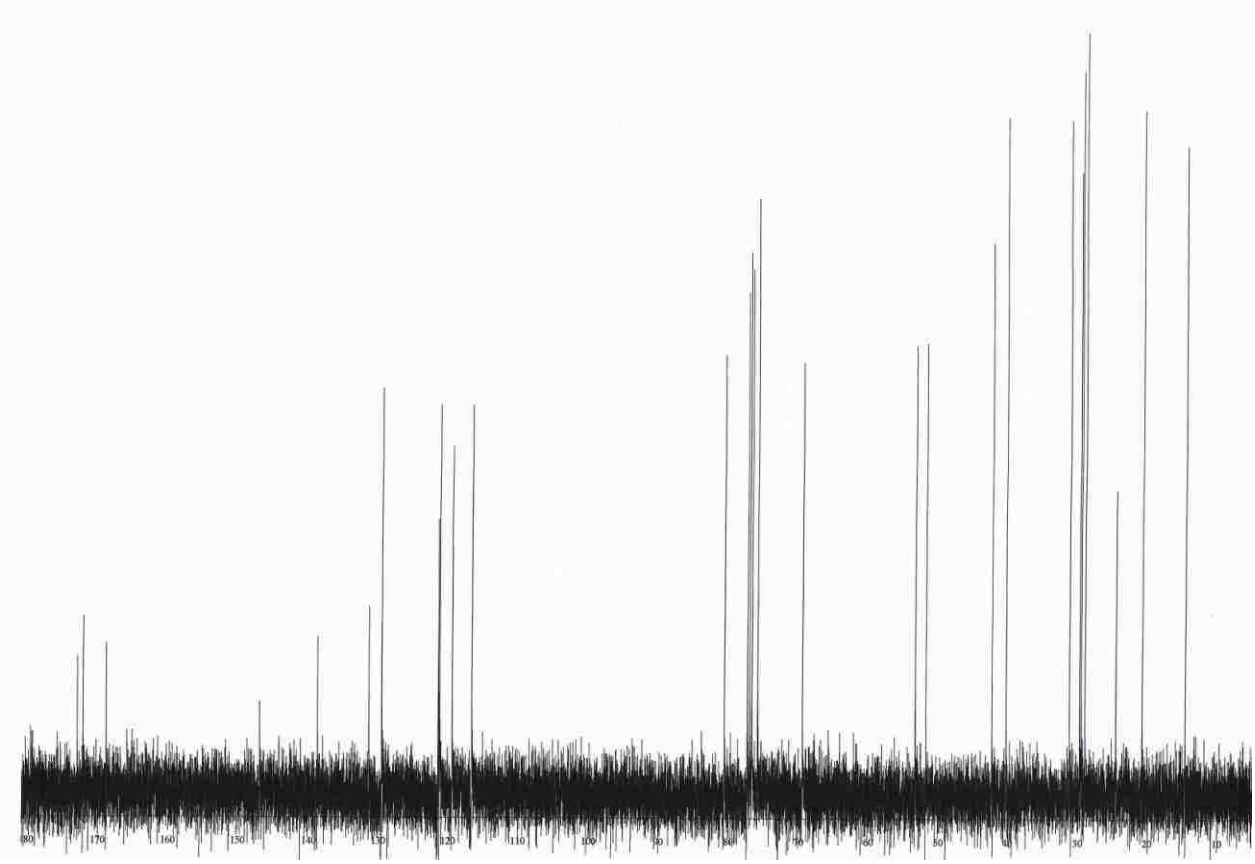


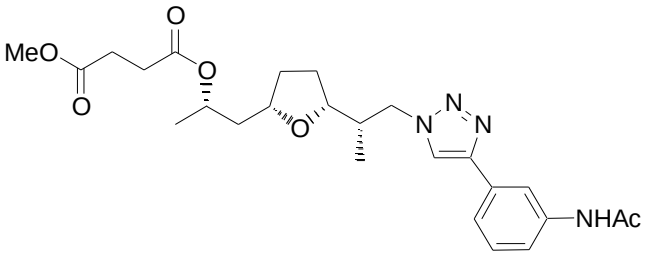


79, Minus 1,4-Regioisomer

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

^{13}C NMR:

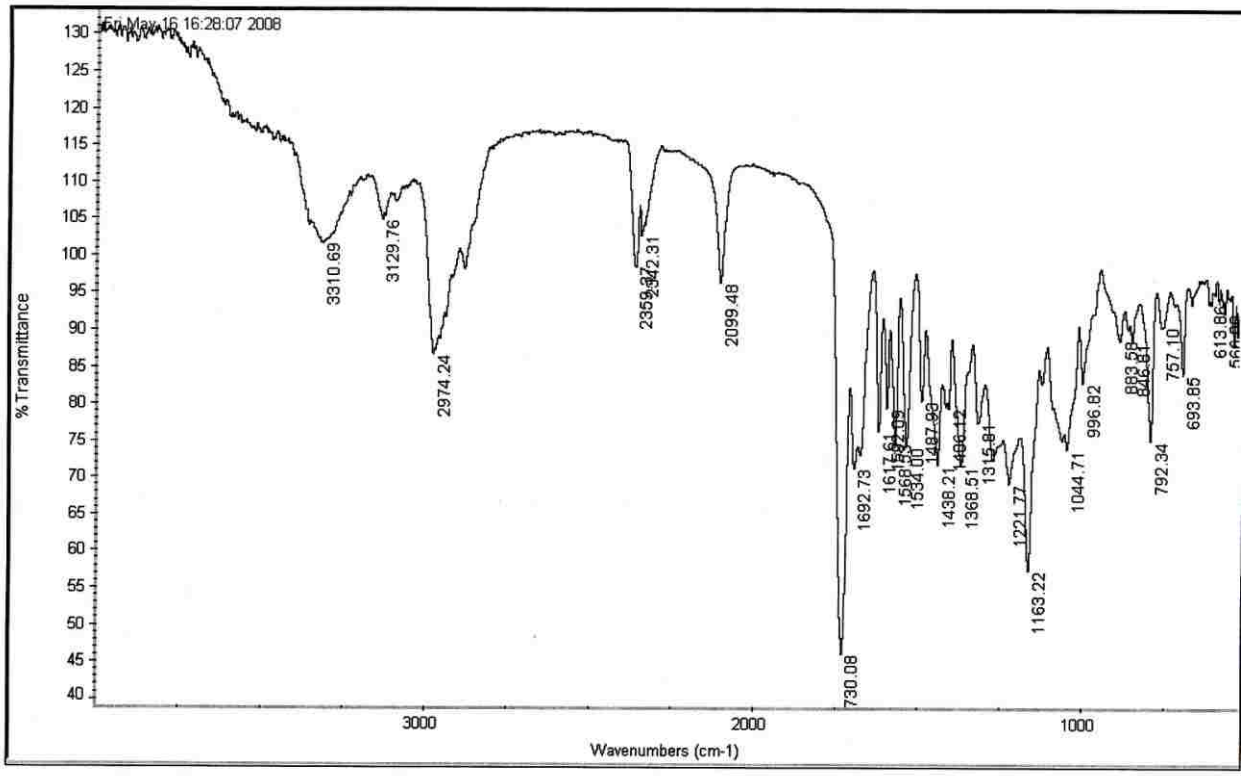


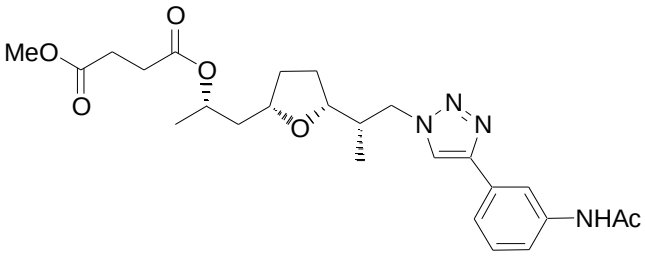


79, Minus 1,4-Regioisomer

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:

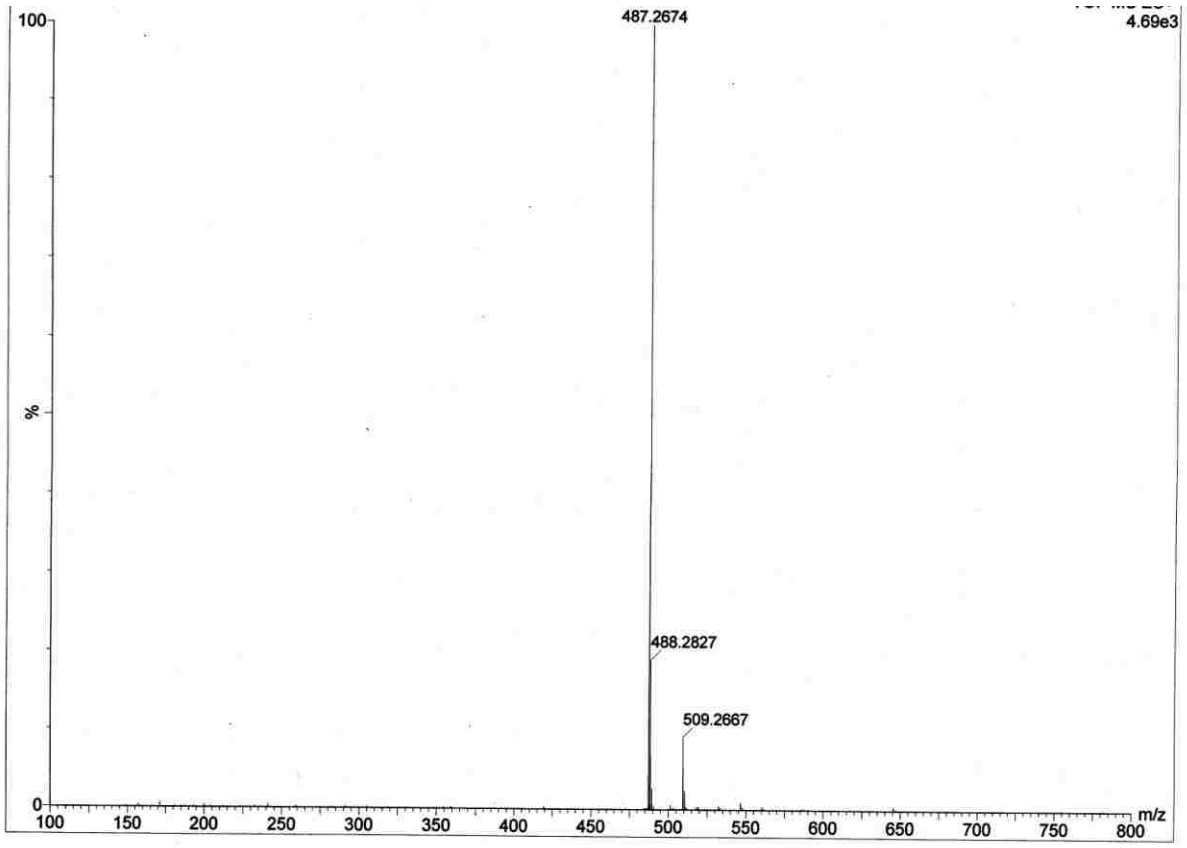




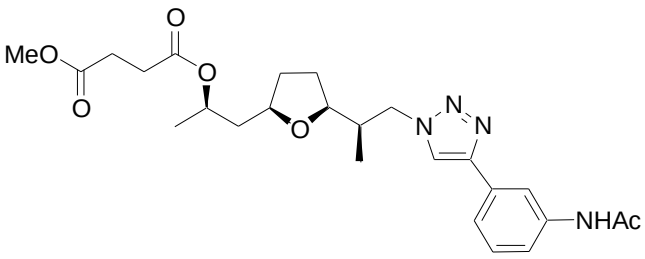
79, Minus 1,4-Regioisomer

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:



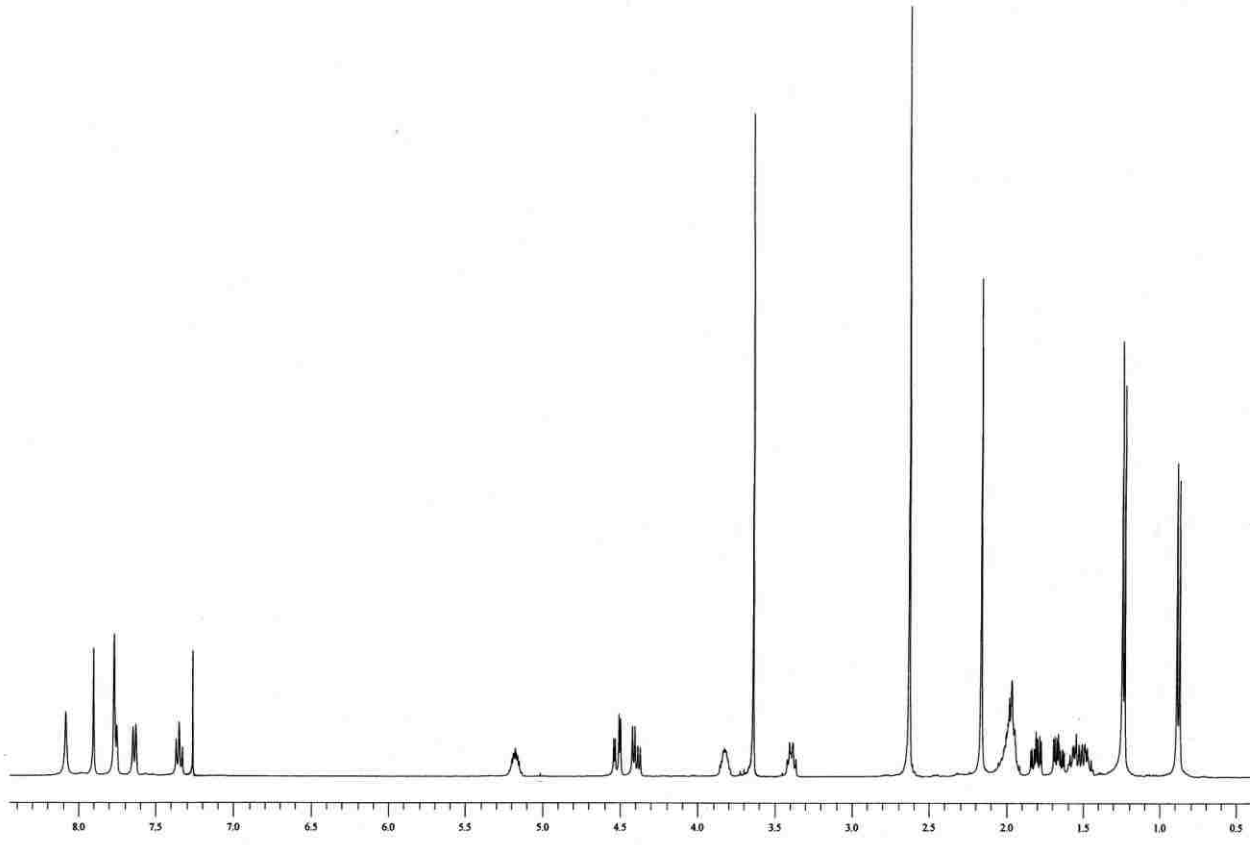
11.6 Spectra for (+)-1,4-Regioisomer, 90

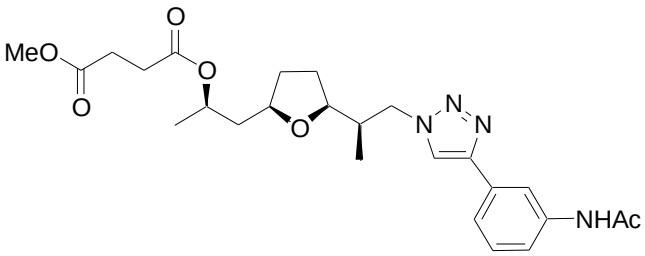


90, Plus 1,4-Regioisomer

**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**

^{1}H NMR:

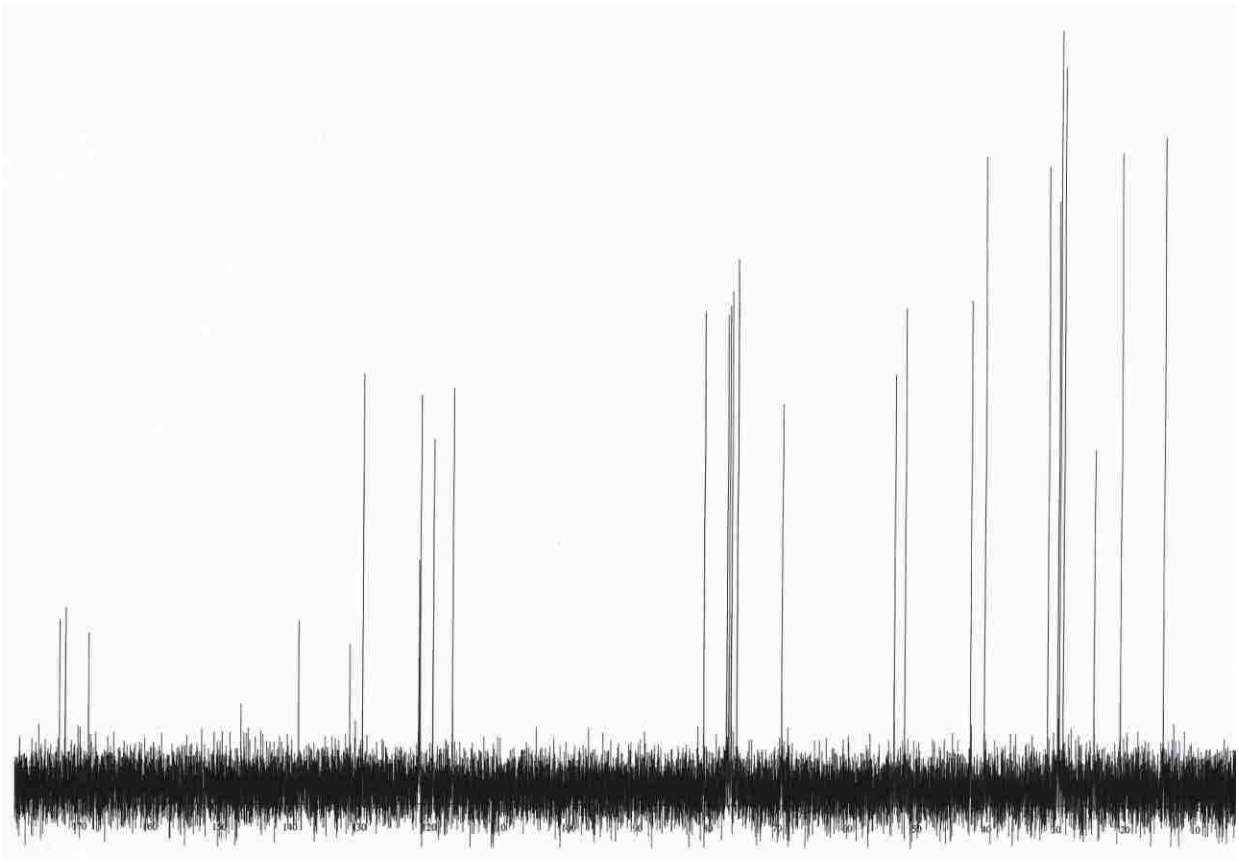


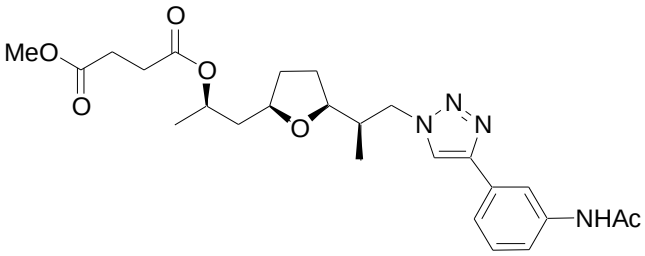


90, Plus 1,4-Regioisomer

**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**

^{13}C NMR:

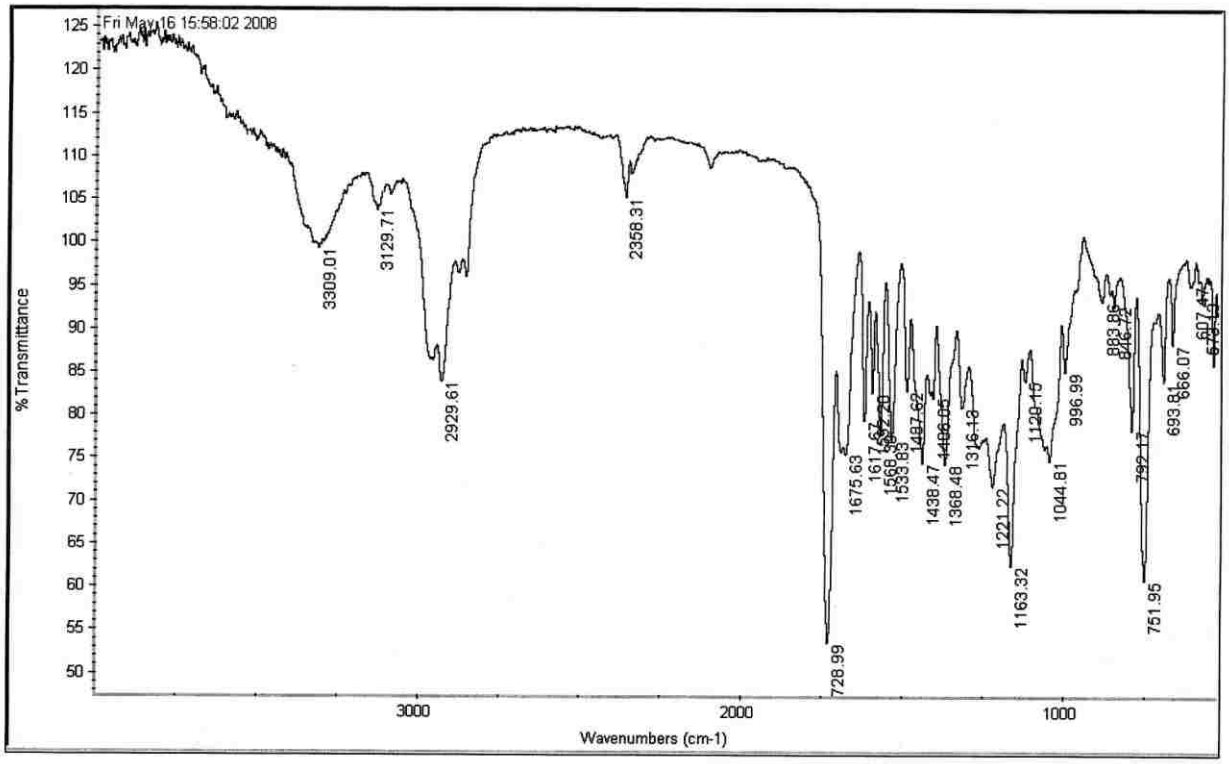


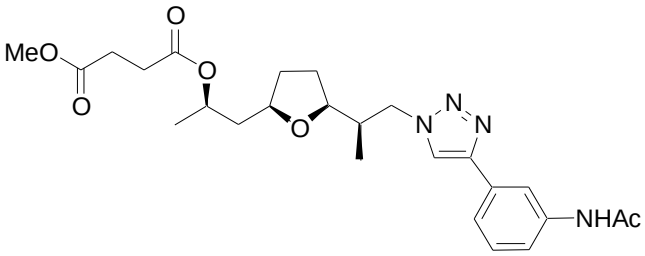


90, Plus 1,4-Regioisomer

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:

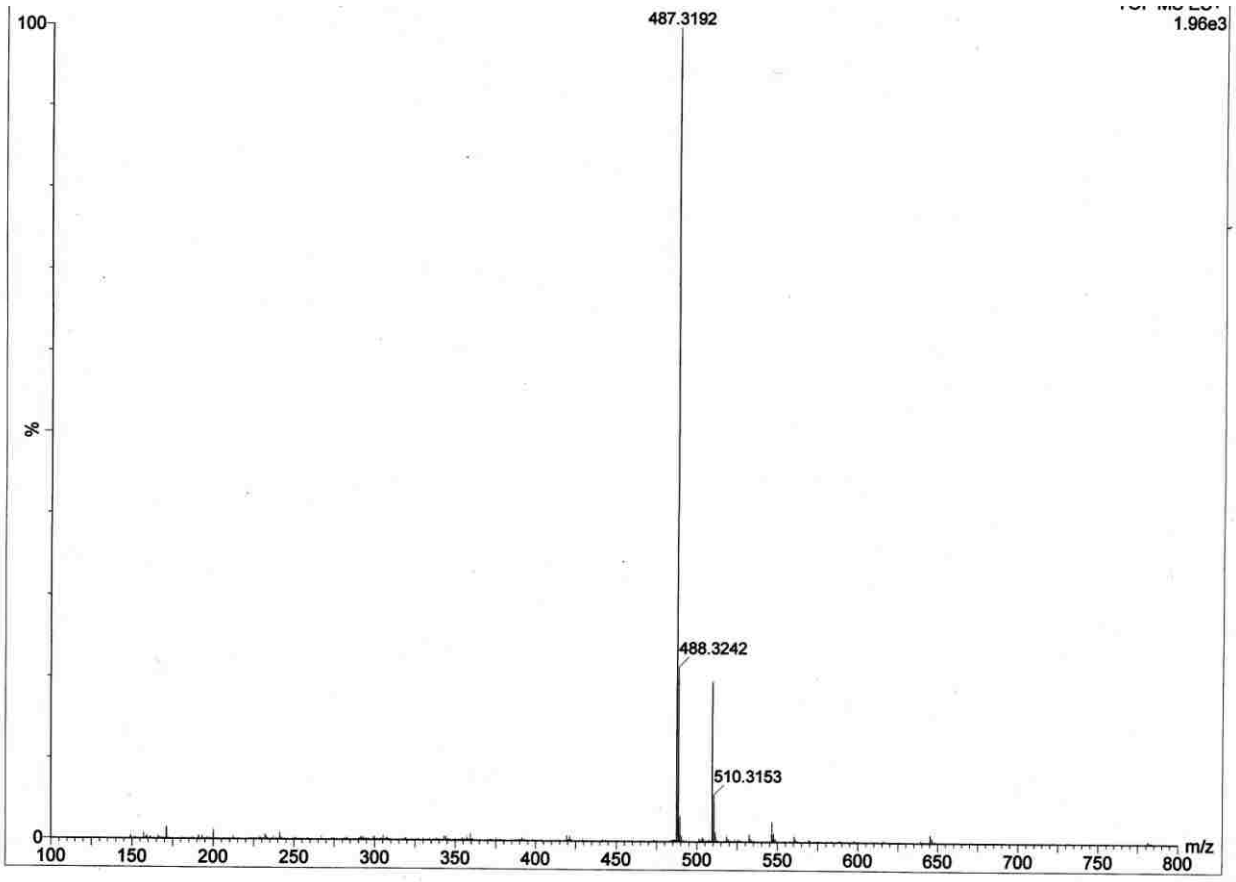




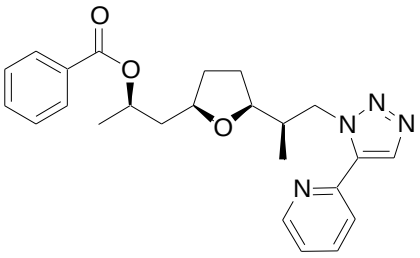
90, Plus 1,4-Regioisomer

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:



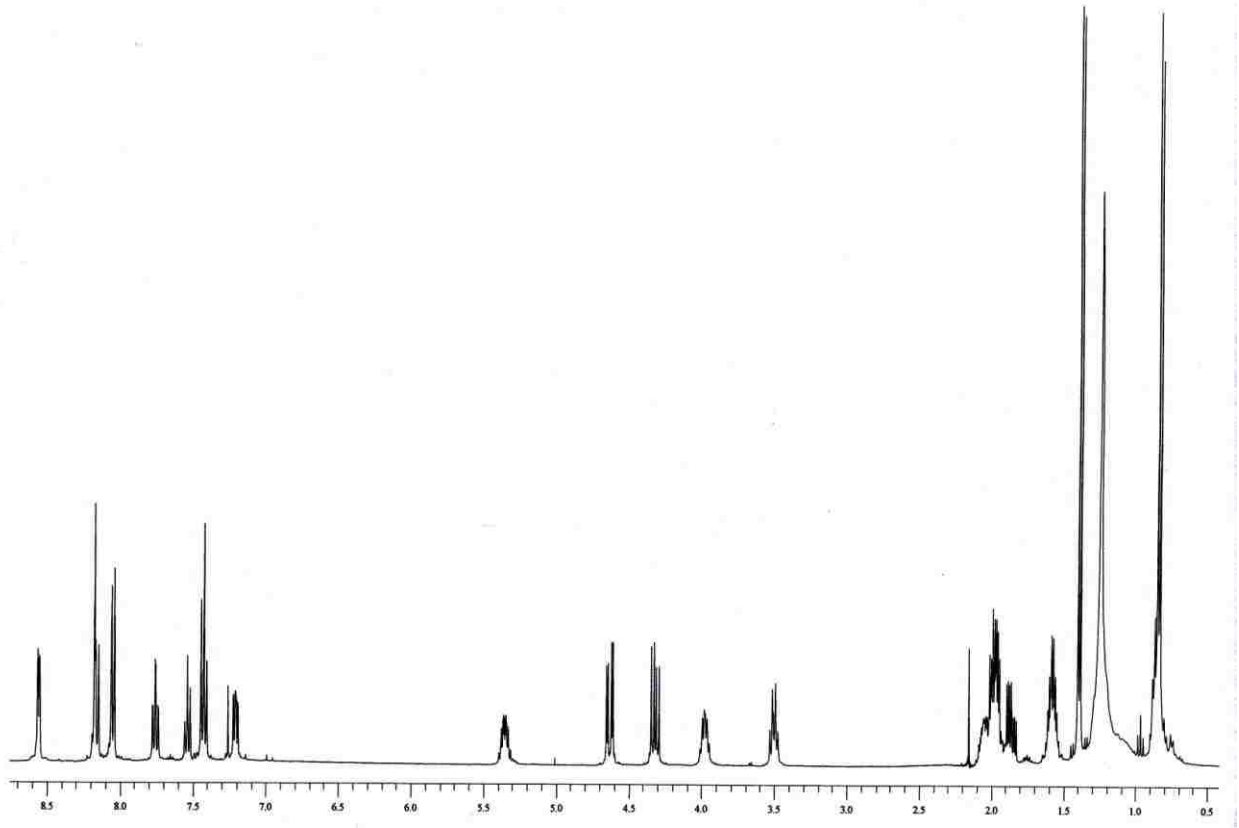
11.7 Spectra for (±)-1,5-Regioisomer, 91

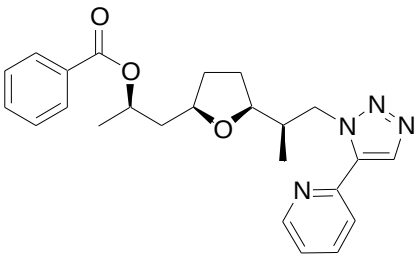


91, Plus 1,5-Regioisomer

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

^{1}H NMR:

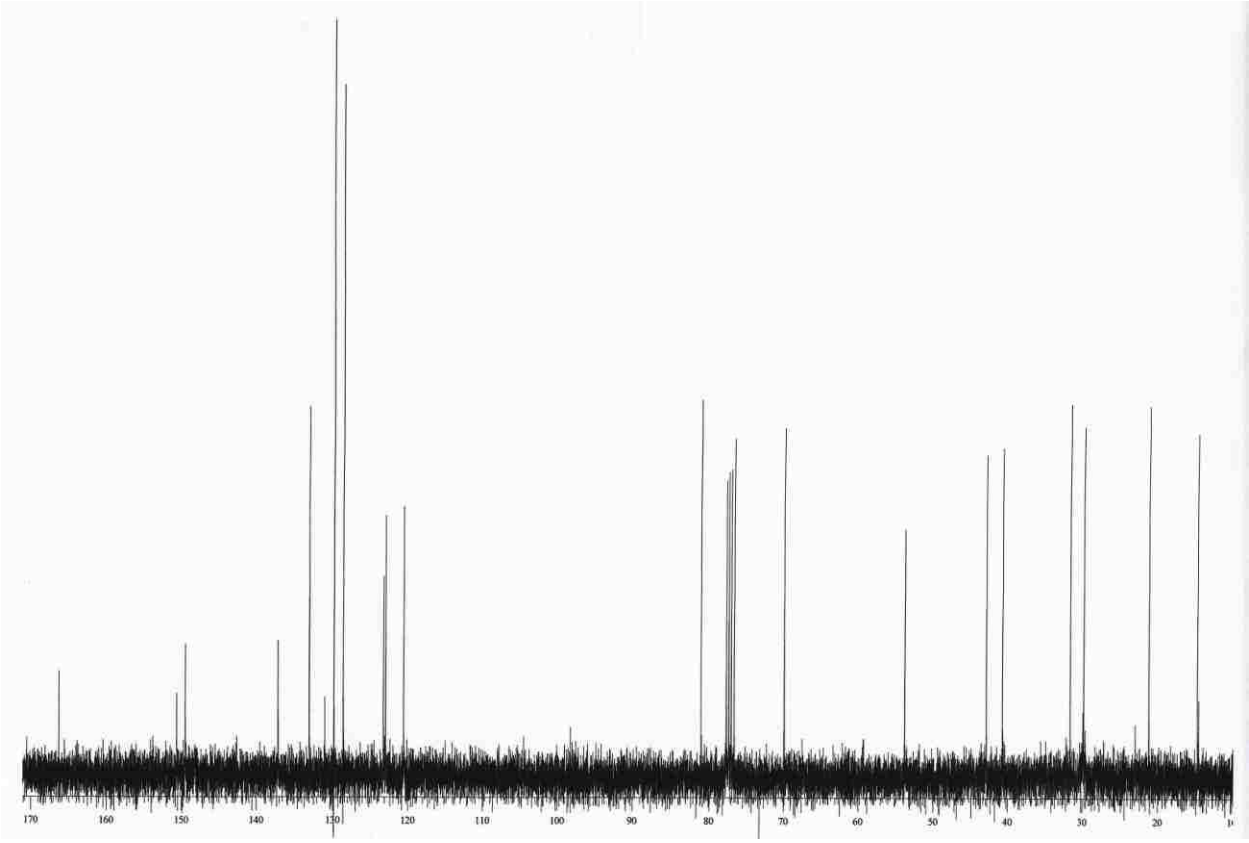


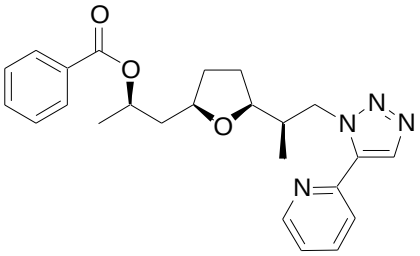


91, Plus 1,5-Regioisomer

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:

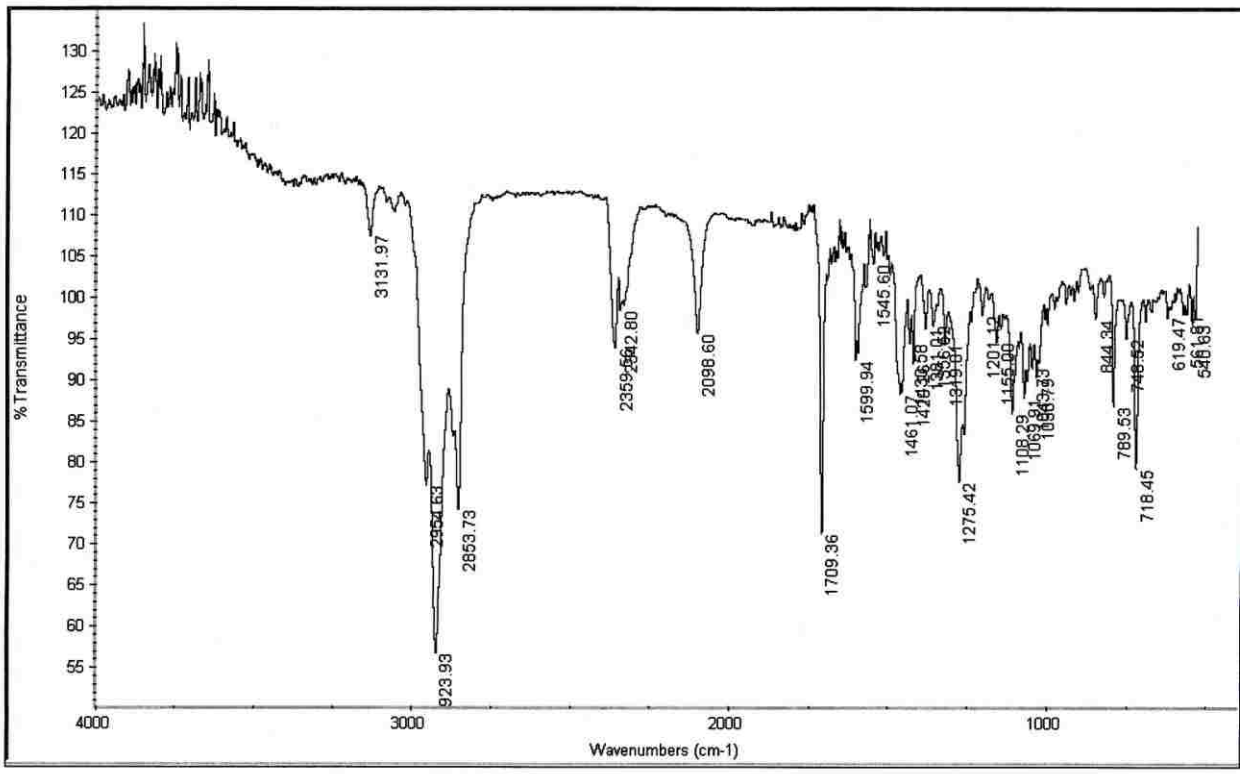


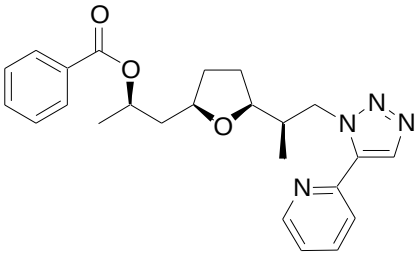


91, Plus 1,5-Regioisomer

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

Infrared Spectrum:

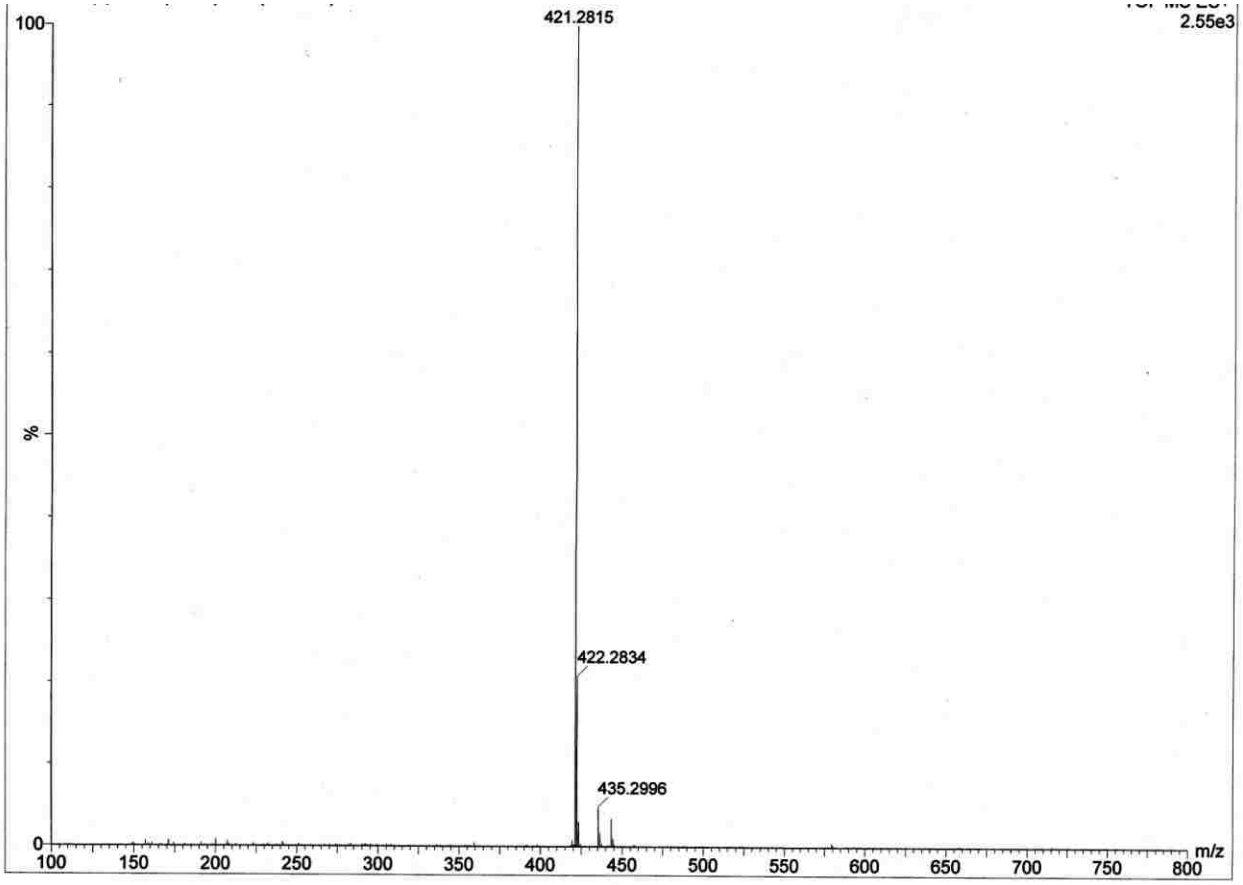




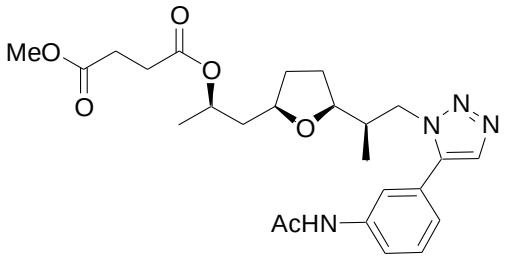
91, Plus 1,5-Regioisomer

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

Mass Spectrum:



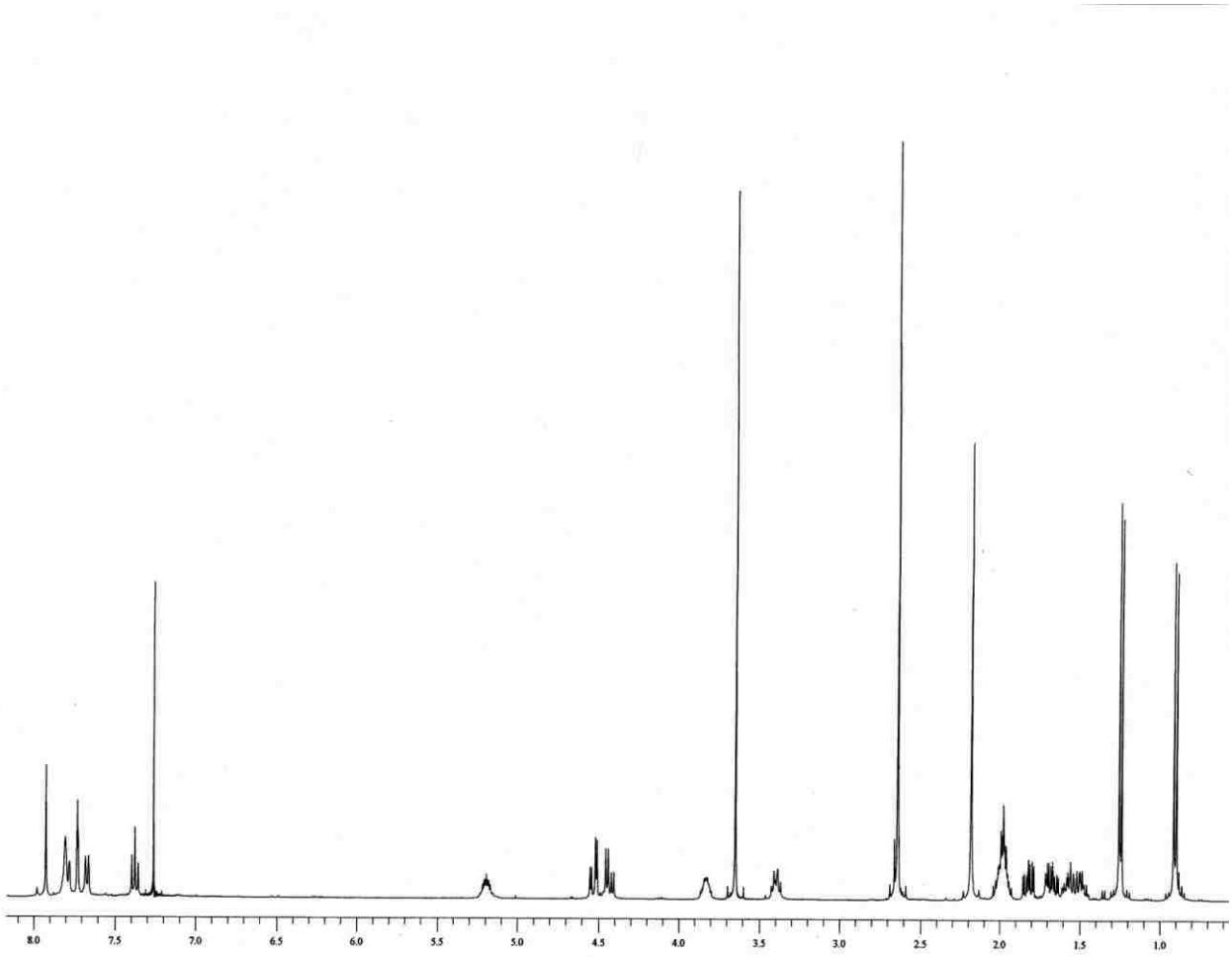
11.8 Spectra for (±)-1,5-Regioisomer, 92

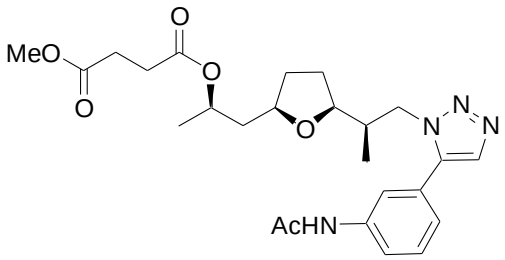


92, Plus 1,5-Regioisomer

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

^{1}H NMR:

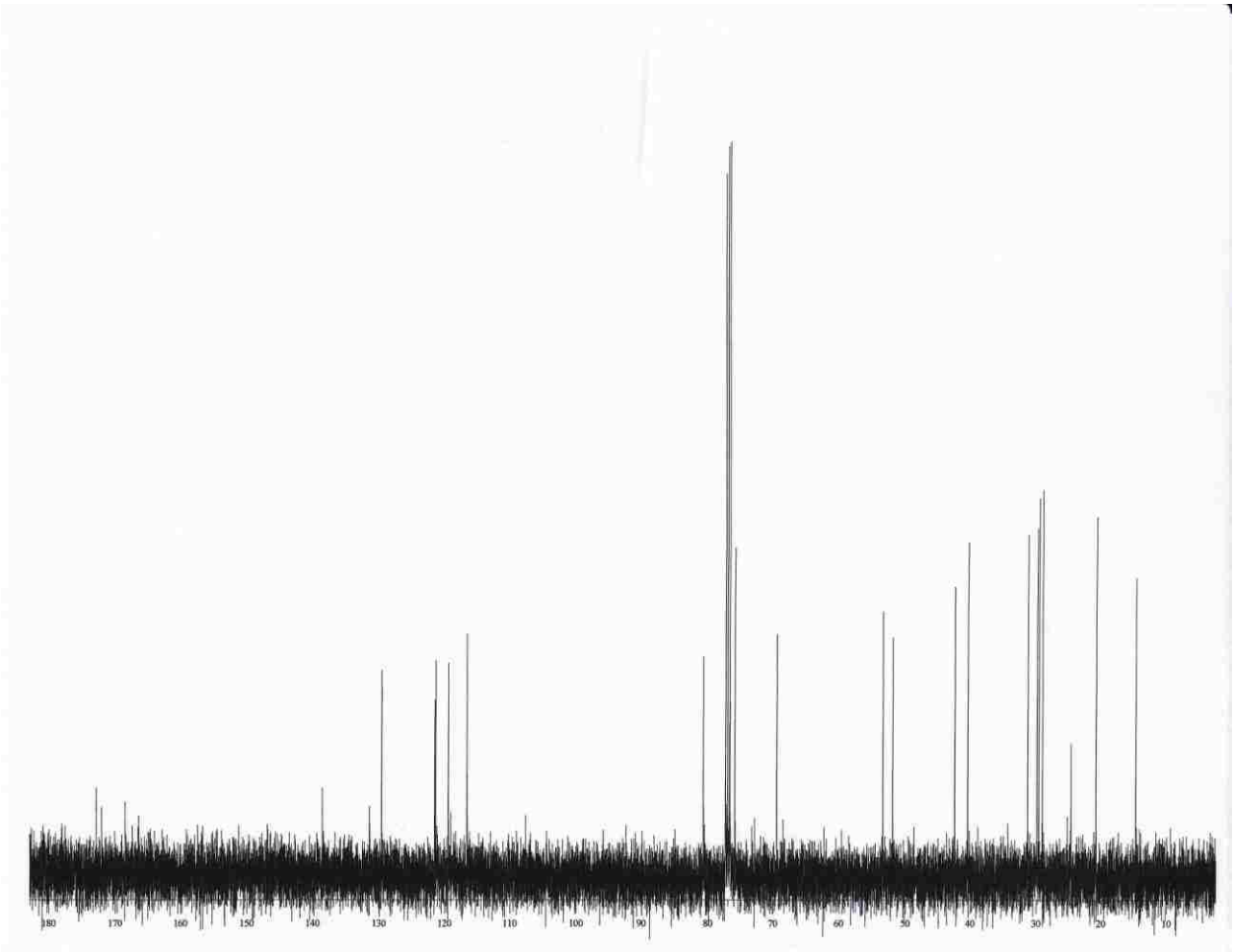


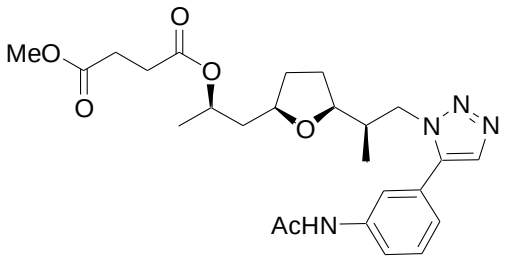


92, Plus 1,5-Regioisomer

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

^{13}C NMR:

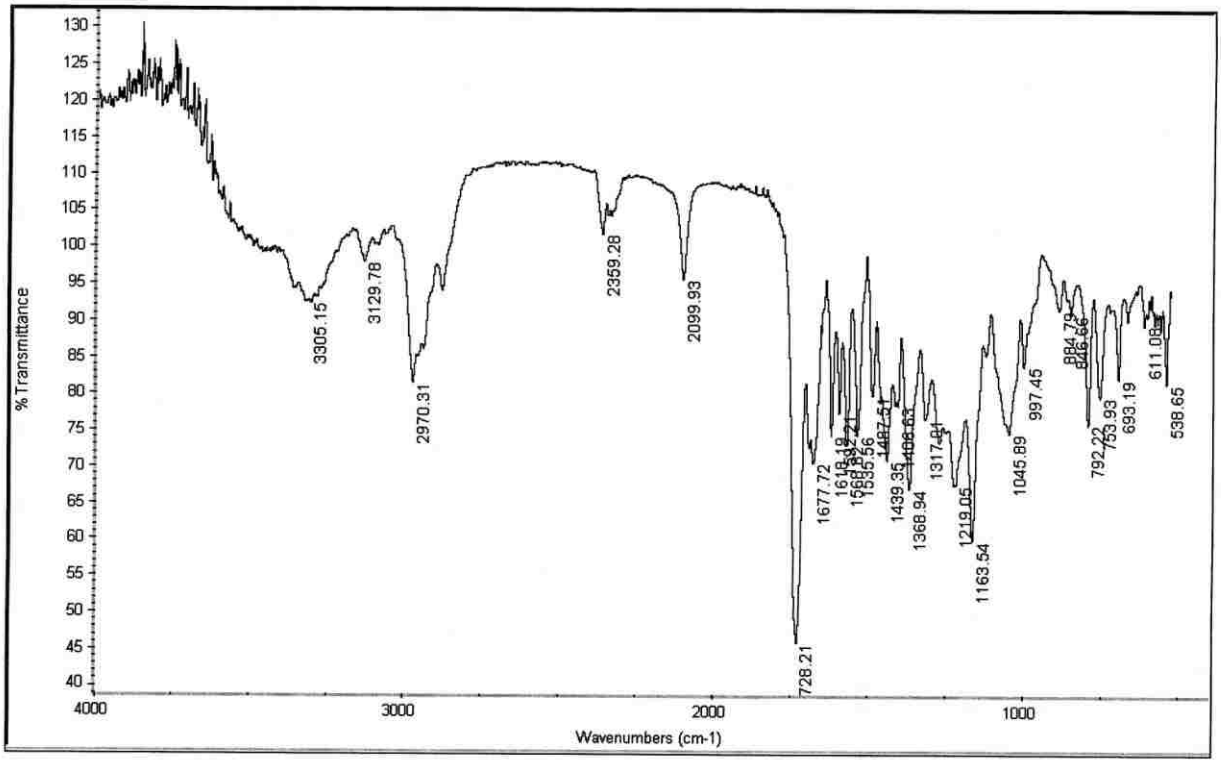


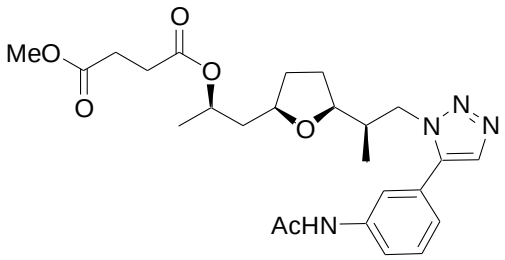


92, Plus 1,5-Regioisomer

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:

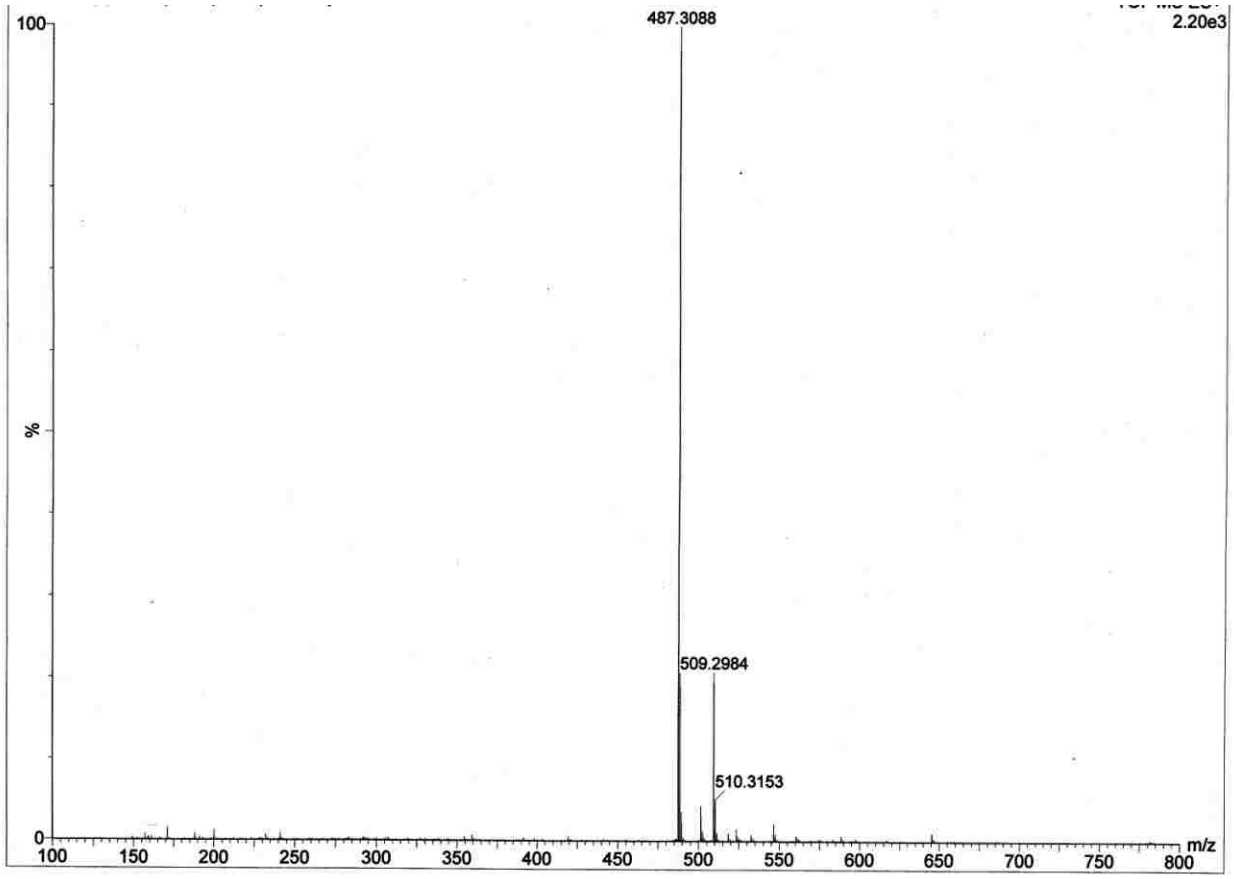




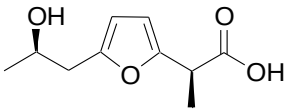
92, Plus 1,5-Regioisomer

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

Mass Spectrum:



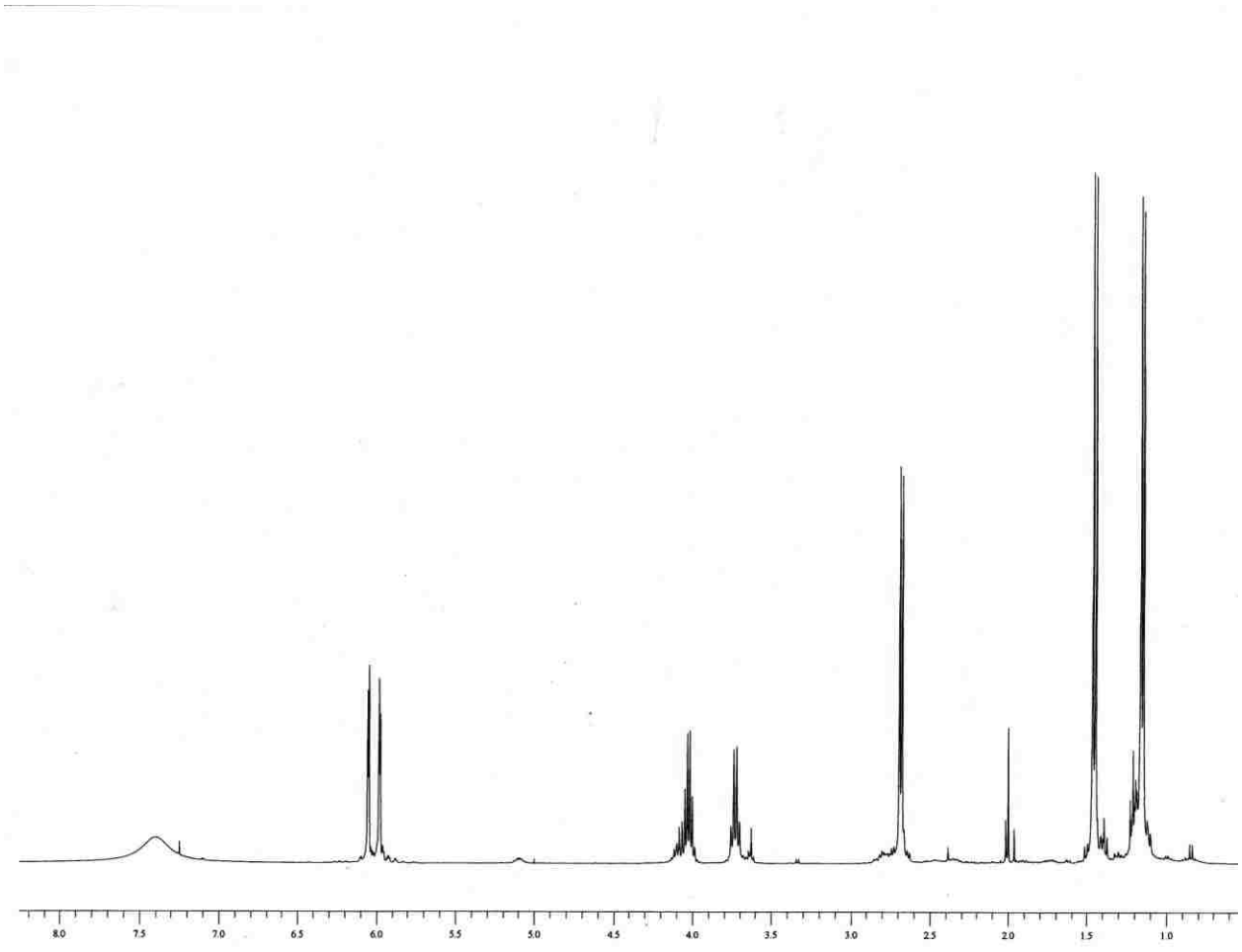
11.9 Spectra for Nonactin Biosynthesis Competitive Inhibitor, 186

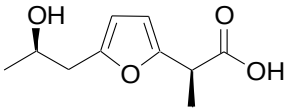


186, Nonactin Biosynthesis Inhibitor

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

^{1}H NMR:

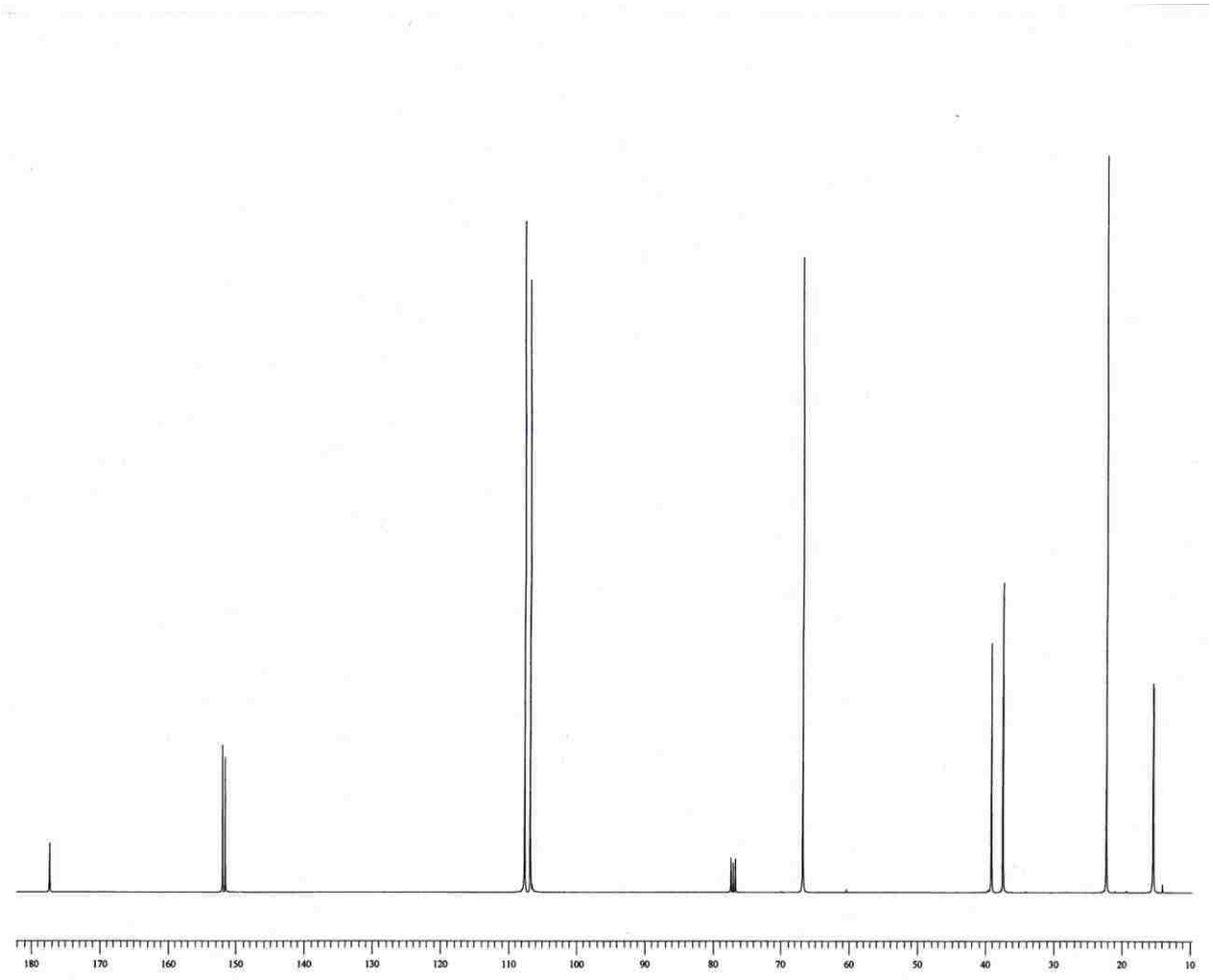


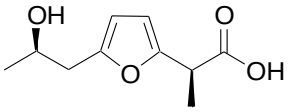


186, Nonactin Biosynthesis Inhibitor

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

^{13}C NMR:

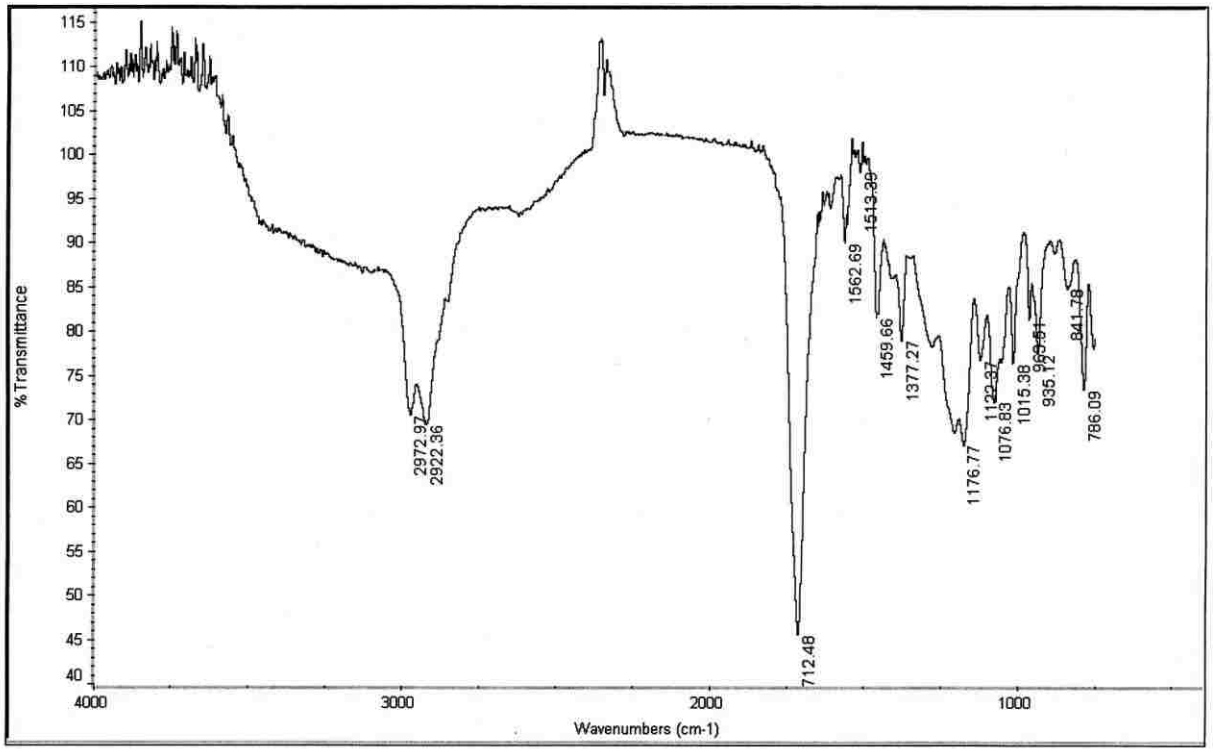


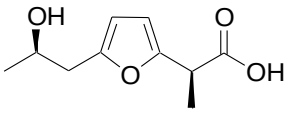


186, Nonactin Biosynthesis Inhibitor

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

Infrared Spectrum:

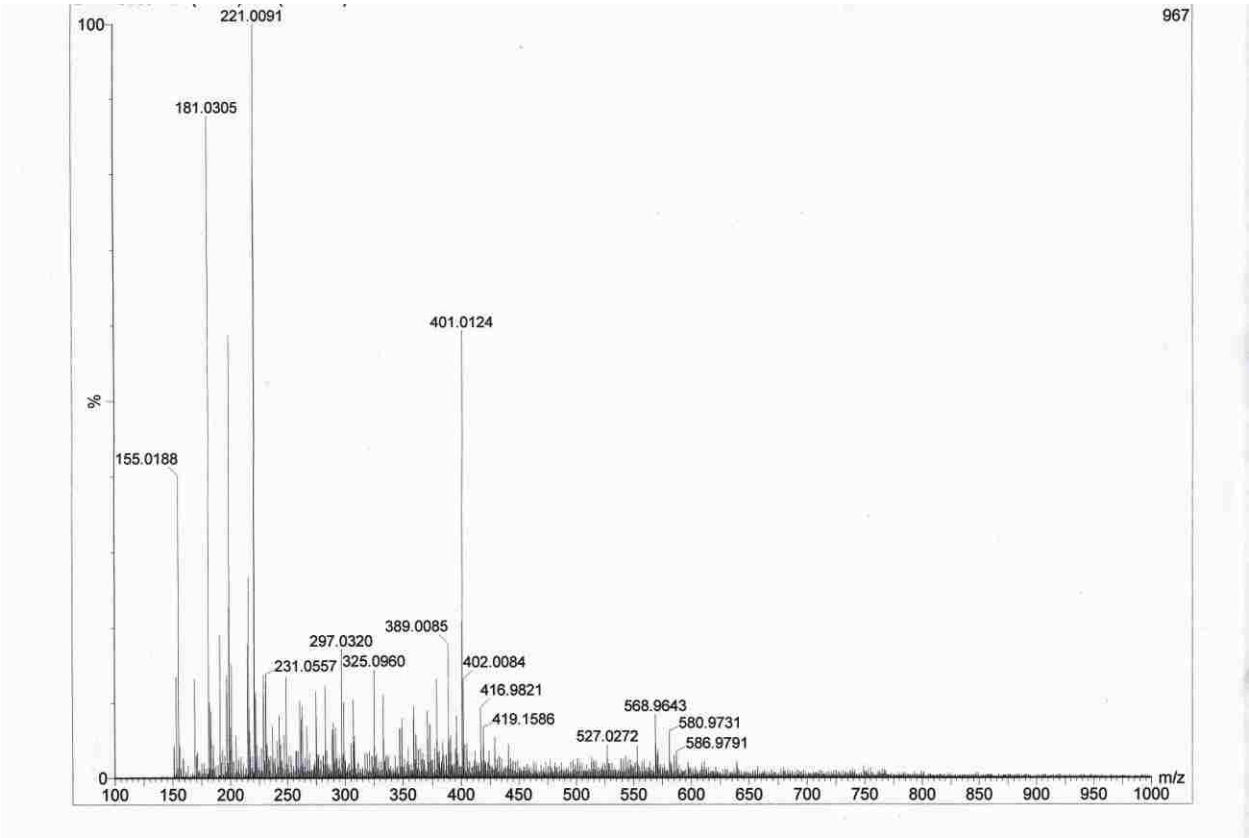




186, Nonactin Biosynthesis Inhibitor

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

Mass Spectrum:



CHAPTER 12

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