Stereoselective Synthesis of Pseudosaccharide Derivative and Bisphosphoramidate Core of EM2487

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STEREOSELECTIVE SYNTHESIS OF PSEUDOSACCHARIDE DERIVATIVE AND

BISPHOSPHORAMIDATE CORE OF EM2487

By

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A DISSERTATION

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HIV is among the leading causes of death worldwide resulting in over 1 million deaths annually according to the World Health Organization. Despite advances in the treatment of HIV, eradicating this pathogen remains a top priority in the medical and scientific communities. EM2487 is a natural product produced by Streptomyces sp. Mer-2487 that possesses promising activity against HIV. Specifically, EM2487 is a potent and selective inhibitor of HIV replication in both acutely and chronically infected cells. The observed HIV activity is hypothesized to be the result of targeting the Tat-TAR (trans-activation response) element which activates RNA polymerase II at the stage of transcriptional elongation. EM2487 has a unique structure that poses two important synthetic challenges: 1) The stereochemical relationships of the complex pseudosaccharide core, and 2) The synthesis of the rare bisphosphoramidate in EM2487. First, the stereochemical relationships of the complex pseudosaccharide core were not elucidated during the isolation due to the significant line broadening of the NMR spectra and must be
determined by total synthesis. Toward this goal, Achmatowicz reaction of furfuryl alcohol followed by rearrangement of the pyranone into a cyclopentenone provided the core pseudosaccharide in 30% yield over three steps. Bromination, Stille coupling, and phosphoramidite coupling produced the protected EM2487 pseudosaccharide. Finally, global deprotection using trifluoroacetic acid and boron trichloride provided an isomeric mixture of the target pseudosaccharide (trans and cis) in two steps overall yield of 30%. Chemical correlation experiments were used to determine the trans relationship between the hydroxyl groups in EM2487. In addition to approaches towards the pseudosaccharide, the synthesis of the rare bisphosphoramidate in EM2487 will be described. In addition to resolving the structure of EM2487, novel analogues with improved potency could be pursued by discovering an efficient synthesis scheme.
DEDICATION

To my parents, and my brother and sisters

For All the Prayer, Support, Encouragement, and Love They have always provided
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CHAPTER 1

1 INTRODUCTION TO HUMAN IMMUNODEFICIENCY VIRUS (HIV), AND ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) AND THE CHEMISTRY AND BIOLOGY OF EM2487

1.1 Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) is a lentivirus which is a subgroup of retrovirus. A retrovirus is a single-stranded, positive-sense RNA that can insert a copy of its RNA genome into the host cells.\textsuperscript{1,2} Human immunodeficiency virus is a sexually transmitted disease, and it was first clinically reported in 1981 by the Centers for Disease Control and Prevention (CDC). There are two types of HIV virus: HIV-1 which evolved from non-human primate immunodeficiency virus from central African chimpanzees, and HIV-2 from West African sooty mangabeys.\textsuperscript{1} A chronic infection with human immunodeficiency virus can cause Acquired ImmunoDeficiency Syndrome (AIDS).\textsuperscript{3} The HIV-1 virus can target CD4 cells which are called T-helper cells and these cells play an important role in the immune response, signaling other cells such as the cytotoxic T cell and the B cells to perform their functions. The resulting infection has a prolonged disease progression and it is characterized by a prolonged interval between the initial infection and the onset of serious symptoms as shown in Figure 1.1.\textsuperscript{4} Healthy people have CD4 cell count of 800 to 1200 cells per cubic millimeter (mm\textsuperscript{3}). After infection, the HIV-1 virus kills the CD4 cells, and decreases the number of cells inside the person’s body. If the CD4 cell count is <500, the infected person will get some symptoms such as cold sores, condyloma, and fungal infections. Once the CD4 cells count of <200 cells/mm\textsuperscript{3}, the person has progressed to the end stage of the disease and officially has AIDS.
1.1.1 Transmission of HIV

HIV can be transmitted in different ways. The most common way is through a sexual contact, but there are other pathways that humans can get the HIV virus. If a person has contact with infected blood, transmission of HIV could happen and transmission can also happen by contacting others bodily fluids from infected people such as breast milk, semen, and vaginal secretions. Mothers also can transfer the HIV virus to their child during pregnancy, during delivery, or through breast feeding. The HIV virus cannot be transmitted through day-to-day contact such as kissing, hugging, shaking hands, or sharing personal objects, food, or water. Importantly, patients who are taking the Antiretroviral Therapies (ART), do not transmit the HIV virus to their sexual partners.\(^2\)
1.1.2 Data and statistics about Human Immunodeficiency Virus

HIV is one of the most common diseases that cause death around the world. Many organizations such as the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) have collected data regarding the HIV virus. These organizations have also played an important role to inform people around the world about this disease. In 2019, the number of people living with this virus was 38 million. Most people living with the HIV virus live in Africa, especially in South Africa. In 2019, there were 1.7 million new infections and 690,000 deaths due to HIV. The number of people that were infected with HIV since the start of the epidemic in 1981 until the end of 2019 was 76 million and the number of people who have died from HIV since the start of the epidemic until the end of 2019 was 33 million as shown in Figure 1.2.5,6

![Adult HIV prevalence map](image)

Figure 1.2: Adult HIV prevalence. Reprinted with permission.6
1.1.3 The structural biology of HIV-1

During the last century, HIV-1 has emerged through many independent zoonotic transitions of simian immunodeficiency viruses. The HIV-1 virus is from the Retroviridae family, and its structure has two copies of positive-sense, single-stranded RNA. It also has envelope glycoproteins gp41 and gp120 as shown in Figure 1.3. These glycoproteins form the envelope spikes. The spikes shape contains trimers of noncovalently linked heterodimers. The surface glycoprotein is known as gp120, and the transmembrane glycoprotein is gp41.

Figure 1.3: The structure of HIV-1. Reprinted with permission.

Together this envelope spike plays an important role in the HIV-1 replication cycle. HIV-1 also has a protein capsid (CA). The capsid shape is a cone-shaped shell which covers the viral RNA genome. Other proteins are also found in the HIV-1 virus such as tat (Trans-Activator of Transcription)
which is essential in the transcription process. A variety of enzymes also play important roles in
the replication cycles such as reverse transcriptase, integrase, and protease. When the HIV-1
virus is outside the human bodies; it is harmless. Once the HIV-1 virus enters our bodies, it starts
to attack the human cell and replicate by using the reverse transcriptase enzyme which converts
the RNA to DNA.²

1.1.4 HIV-1 replication cycle

The HIV-1 replication cycle consists of 13 steps which is one of the most complex viral
replication cycles (Figure 1.4).⁸ The replication cycle components can come from the virus itself
or be co-opted from the host cell to facilitate the steps in the HIV-1 replication cycle.

Figure 1.4: The replication cycle of HIV-1. Reprinted with permission.⁸
The first step is the attachment between the virus and the host cell membranes. This interaction happens between the surface glycoprotein gp120 and the co-receptor of the CD4 cell which is called CC-chemokine receptor 5 (CCR5).\textsuperscript{10–15} After the attachment happens, conformational changes occur in the HIV-1 envelope. These changes lead to the insertion of the peptide of glycoprotein gp41 into the cell membrane, and this triggers fusion as shown in Figure 1.5.\textsuperscript{16}

![Diagram of HIV-1 attachment and fusion steps](image)

**Figure 1.5:** HIV-1 attachment and fusion steps. Reprinted with permission.\textsuperscript{16}

At the end of the fusion step, a six-helix hairpin structure forms, and then entry of the viral particle into the cell occurs. After the fusion step, the post-entry events happen. Partial uncoating of the viral capsid protein is the first step of the post-entry events. The capsid shell contains \textit{N}-terminal and \textit{C}-terminal domains connected by a flexible linker.\textsuperscript{17,18} These domains can assemble into ring structures containing five or six promoters.\textsuperscript{19,20} The ring that is formed can get together to create a fullerene-like cone that consists mainly of hexamers, and also possesses seven pentamers at the wide end and five at the narrow end.\textsuperscript{9,19} Because of this arrangement, it makes shape declinations. The uncoating may happen because of the high concentration of the pentameric forms that is expected at the narrow end of the cone as shown in Figure 1.6.\textsuperscript{8}
After the uncoating step, the viral particles (replication enzymes (RT), integrase (IN), and the viral genomic RNA) are released into the host cell allowing reverse transcription of viral RNA into linear DNA. Humans do not possess the replication enzyme, but the HIV-1 genome encodes this enzyme so that the reverse transcription could happen. The enzyme contains N-terminal RNA and DNA dependent DNA polymerase, and C-terminal RNase H which predigest the RNA component of the RNA-DNA hybrids. After completion of the reverse transcription step, import of the pre-integration complex (PIC) into the cell nucleus occurs. The integrase enzyme has two catalytic activities. First, it cuts the 3’-termini of the HIV DNA revealing the 3’-hydroxyls, then it
uses the 3’-hydroxyls to cut the chromosomal DNA. Then the PIC connects the viral DNA end to the host DNA 5’-phosphates. The distinction between the early and the late phase of HIV-1 replication is the integration step. After integration, the transcription step starts. This process requires elements both from the viral and the host cell. These elements are the viral transactivator protein (Tat), viral transactivation response (TAR), protein positive transcription elongation factor b (P-TEFb), which includes cyclin-dependent kinase 9 (CDK9), and cyclin T1.\textsuperscript{8} Tat activates RNA polymerase II by interacting with cyclin T1 and subsequently recruiting the viral transactivation response. Tat does not function without binding both ligands.\textsuperscript{21–25}

After replication of the viral RNA, nuclear export transfers the viral RNA from the nucleus to the cytoplasm of the host cells. This step requires HIV-1 Rev regulatory protein, Rev response element (RRE), and the host nuclear export factor CRM1.\textsuperscript{26,27} The mRNA that is exported into the cytoplasm serves as a template for protein production including the accessory viral proteins (Tat, Rev, Vpu, and Vif) and the retroviral structural proteins capsid, matrix (MA), and the nucleocapsid (NC). The retroviral structural proteins assemble to form the precursor polypeptide Gag and the HIV-1 Gag protein which now amass like a particle at the plasma membrane.\textsuperscript{28,29} After the assembly step, the particles are ready to bud from the cell through unbinding of the myristic acid and the conserved basic amino acid residues.\textsuperscript{30–34} After budding, the viral particles are released. The final step of the HIV-1 replication cycle is maturation which is mediated by the protease (PR) enzyme. Proteolysis of the precursor peptides Gag and Gag-pol by the protease enzyme produces mature infectious virions which have all the replication machinery.\textsuperscript{35}
1.2 Treatment of HIV infections: Highly active antiretroviral therapy

Many scientific and clinical studies over the last 40 years,\textsuperscript{36-40} have led to the development of highly active antiretroviral therapy (HAART) which is currently considered the standard of care to treat the HIV virus infections.\textsuperscript{41} Collectively, over 50 antiretroviral drugs have been approved for use in the HIV treatment.\textsuperscript{42} In this strategy, at least three drugs that belong to two classes of the antiretroviral therapy are used.\textsuperscript{2,8} The goal of the highly active antiretroviral therapy is first to restrain the HIV-associated morbidity and mortality.\textsuperscript{43} Another goal is to decrease the risk of the HIV transmission.\textsuperscript{44-46} There are six classes of highly active antiretroviral therapy that inhibit the HIV-1 replication cycle. HAART targets four different steps in the HIV-1 replication cycle. Namely, 1) entry, 2) reverse transcription, 3) integration and 4) maturation, which are all crucial steps in the HIV-1 replication cycle.

1.2.1 gp120 and the co-receptor CC-chemokine receptor 5 interaction inhibitors

The first step in the HIV-1 replication cycles is entry. The glycoprotein gp120 of the HIV-1 virus needs to interact with the surface receptor CD4 and the co-receptor CC-chemokine receptor 5 enabling viral entry. Scientists have studied the crystal structure of the HIV-1 envelope, using cryo-electron tomography\textsuperscript{47,48}, to design antibodies that neutralize the virus.\textsuperscript{49} When gp120 interacts with the CD4 cell, it leads to a conformational change in the gp120 of the HIV-1 envelope as shown in Figure 1.7.\textsuperscript{48}
Also, there is hydrophobic cavity in gp120 which is partially filled with the CD4 residue Phe43. Small molecules can bind in this cavity leading to antiviral activity. This discovery could open the way to find many useful small molecules that have affinity for the gp120. An example of this inhibitor is maraviroc.

1.2.2 Fusion inhibitors

Once gp120 of the HIV virus interacts with the CD4 cell, then the glycoprotein gp41 facilitates fusion of the virus into the cell. This fusion results from a cascade of conformational changes in the gp41 that leads to formation of a six-helix hairpin structure. Peptides have been developed that can disrupt the formation of the six-helix hairpin structure as shown in Figure 1.8. These peptides were synthesized and were derived from the N-terminal or C-terminal sequences of the gp41, and have antiviral activity.
Subsequently, small-molecule entry inhibitors have been developed and approved by the FDA. These include enfuvirtide, fostemsavir, maraviroc, ibalizumab, and leronoimab. The structures of fostemsavir (1-1) and maraviroc (1-2) are shown in Figure 1.9.42

Figure 1.8: Model shows two possible mechanisms for N-peptide inhibitory activity. Reprinted with permission.51

Figure 1.9: Structures of fostemsavir and maraviroc.
1.2.3 Nucleoside reverse transcriptase inhibitors

In the HIV-1 replication process, reverse transcription converts the viral genomic RNA into linear double-stranded DNA. HIV-1 RT has two active sites: 1) N-terminal RNA- and DNA-dependent DNA polymerase and 2) C-terminal RNase H. The nucleoside reverse transcriptase drugs inhibit DNA polymerization. NRTIs drugs mimic the natural dNTPs and would be incorporated by the reverse transcriptase (RT) enzyme into the viral DNA. Because these drugs lack the 3’-hydroxyl group, NRTIs act as chain terminators so that DNA synthesis cannot continue. Examples of the nucleoside reverse transcriptase inhibitors are lamivudine, abacavir, zidovudine, and others as shown in Figure 1.10.

![Figure 1.10: Structures of lamivudine, abacavir, and zidovudine.](image)

1.2.4 Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are drugs that bind at an allosteric site of the enzyme. NNRTIs change the conformation of the Tyr181, Tyr188 and the primer grip of the reverse transcriptase. An example of this type of drug is nevirapine which pi-stacks with the aromatic side chains of Tyr181 and Tyr188. Nevirapine drug resistance is common resulting in mutations that remove the aromatic side chains of RT. Subsequently, the more potent etravirine (TMC-125) and rilpivirine (TMC-278) were discovered. These latest drugs
have better resistance profiles because they have high affinity to the mutant reverse transcriptase. Another inhibitor of reverse transcriptase is the cellular restriction factor APOBEC3G. The HIV-1 protein Vif binds to APOBEC3G and inhibits HIV-1 infection.

1.2.5 Integrase inhibitors: integrase strand transfer inhibitor

The viral integrase enzyme inserts the viral DNA into the DNA of the host cell. Raltegravir is an integrase inhibitor that is approved for the treatment of HIV. The structure of Raltegravir is shown in Figure 1.11 and possesses two important moieties. The first moiety is the co-planar heteroatoms which are three oxygen atoms. The heteroatoms chelate the active site metal ions of the integrase enzyme and influence the position of the metal ions within the integrase active site. The other important moiety is the halogenated benzyl group that interacts with the terminal adenine ring.

![Structure of Raltegravir](image)

Figure 1.11: Structure of Raltegravir.

1.2.6 Protease inhibitors

In the final maturation step, protease converts the immature particles to the infectious virions. Scientists have studied the structure of the protease enzyme of HIV-1. The structure of the HIV protease consists of a homodimeric aspartyl protease. Inhibitors typically bind to
the active site of the protease and block the enzymatic activity as shown in Figure 1.12.\textsuperscript{82} The space that the inhibitors occupy is also called the inhibitor envelope.\textsuperscript{8} Several FDA approved drugs target protease including saquinavir, indinavir, ritonavir, fosamprenavir. Side effects with these inhibitors are common and include dyslipidemia, insulin-resistance, lipodystrophy/lipoatrophy, and cardiovascular and cerebrovascular diseases.\textsuperscript{84–87}

![Figure 1.12: The structure of the HIV-1 protease enzyme in complex with an inhibitor. Reprinted with permission.\textsuperscript{82}]

1.3 Tat-TAR interaction

Tat, which is the transcriptional transactivator from the HIV virus, is a small viral protein that consists of 86 to 102 amino acid residues.\textsuperscript{88} Tat plays an important role in the HIV-1 replication cycle which is a regulatory protein in the transcription step.\textsuperscript{23,24} Tat activates the RNA polymerase II at the level of transcription elongation by interacting with cyclin-dependent
serine/threonine kinases (CDK7 and CDK9). These two cyclins phosphorylate the C-terminal
domain of RNA polymerase II. Tat interacts with the CDK7 which phosphorylates serine 5 and
CDK9 which phosphorylates serine 2. When Tat interacts with CDK9 and Cyclin T1, which
are both the kinase component of the positive transcription elongation factor b (P-TEFb)
complex, it recruits the transactivation-response TAR which acts as a promoter element in the
transcribed 5’ end of the viral long terminal repeat. In general, the binding of P-TEFb to TAR
only happens in the presence of Tat. The results of the Tat/TAR interaction enhance the
HIV transcription elongation as shown in Figure 1.93 There are no approved drugs that target
the Tat/TAR interaction.
Scientists have started to study this concept which is the Tat/TAR interaction and have found that some molecules disrupt the Tat/TAR interaction. These molecules interfere with the binding of the Tat/TAR interaction. Examples of these molecules are dCA, Temacrazine, Celastrol, and 15d-PGJ₂, as shown in Figure 1.14.\textsuperscript{93,98}
1.4 EM2487, a novel anti-HIV-1 antibiotic

Due to prevalence of drug resistance caused by rapid HIV-1 mutation, there is a continuing need to discover new drugs that work by mechanisms different than HAART.\textsuperscript{2,36,61,63,64,75,83,99--102,37,40,53--58} One novel target would be the inhibition of the HIV-1 replication cycle at the transcriptional level. Takeuchi et al. have screened approximately 10,000 microorganism products. They found that the culture supernatant of \textit{Streptomyces} sp. Mer-2487 inhibits the HIV-1 Tat-induced gene expression.\textsuperscript{103} EM2487 is a novel Anti-HIV-1 antibiotic that is produced by \textit{Streptomyces} sp. Mer-2487. EM2487 inhibits the Tat-induced gene expression without affecting the basal or tumor necrosis factor-\(\alpha\)-induced transcription. This compound can inhibit the HIV-1 replication in both chronically and acutely infected cells. Scientists have studied the biological
properties of the EM2487, and they found that its IC$_{50}$ for acute HIV-1 infection was 0.27 µM in peripheral blood mononuclear cells (PBMCs).\textsuperscript{104} Importantly the 50% cytotoxic concentration for mock-infected PBMCs was 13.3 µM. EM2487 was also a selective inhibitor for human T-lymphotropic virus type I (HTLV-I) replication, and its IC$_{50}$ effective concentrations for HTLV-I p19 antigen production were 3.6 and 1.2 µM in MT-2 and MT-4 cells, respectively. Notably, EM2487 did not decrease the cell proliferation and viability at these concentrations. EM2487 showed 50% cytotoxic concentrations of 30.6 and 5.7 µM in MT-2 and MT-4 cells, respectively.\textsuperscript{105} EM2487 did not inhibit the early steps of the HIV-1 replication cycle, and instead selectively inhibits the viral mRNA synthesis.

1.4.1 Structure of EM2487

EM2487 has a unique structure compared to other nucleoside natural products.\textsuperscript{40,41,106–111} Nucleoside natural products are important compounds and can be used as antibacterial, antitumor, and antiviral agents.\textsuperscript{107,112,121,122,113–120} EM2487 has a nucleoside unit (uridine), long alkyl group, alkyl hydroxylamine, phosphate imide of glycinamide, and highly oxygenated cyclopentene. The alkyl hydroxylamine, phosphate imide of glycinamide, and the highly oxygenated cyclopentene substructures make EM2487 structurally unique. Also, EM2487 has an enol-ether linkage that is acid labile as shown in Figure 1.15.
Takeuchi et al. characterized and studied the physico-chemical properties of EM2487. They used NMR spectra, infrared spectra, UV, FAB mass spectra, and specific rotations to determine the structure of EM2487. They found that EM2487 has a white appearance, and has the formula $C_{32}H_{57}N_5O_{16}P_2$, High-Resolution Fast Atom Bombardment Mass Spectrometry (HRFABMS) $[m/z, (M-H)] (828.3216)$, and a lambda max at 262 nm.\textsuperscript{103} The IR and NMR data were also acquired in order to deduce the structure.\textsuperscript{103} Even though the scientists used 2D NMR (COSY, TOCSY, HMQC, and HMBC), they could not get more correlative information because of the significant line broadening of the NMR spectra. Notably, there are three stereocenters in the pseudosaccharide derivative of the EM2487 that are not defined.

### 1.4.2 Isolation of EM2487

Takeuchi et al. did a large-scale fermentation of the natural product EM2487 and took the fermentation broth (24 liter), and then they did the separation by centrifugation to get two layers the mycelia cake and the supernatant. The mycelia cake layer was extracted with methanol (20 liters), and the methanol layer was concentrated under vacuum. They mixed the concentrated solution with the supernatant and did a Diaion HP-20 column. The eluent for the column was 20%
methanol (6 liters), then they used 80% acetone (41 liters). The elution solution was concentrated under reduced pressure to get the crude EM2487 powder. Then the crude powder was injected into octadecylsilane (ODS) column using 35% acetonitrile in buffer (20 mM phosphate buffer pH 7.0). A Dianion HP-20 column was used to desalt the eluate and the solution was concentrated under reduced pressure. Finally, HPLC preparative (mobile phase: 35% acetonitrile/ 20 mM phosphate buffer, pH 7.0) was used to isolate the EM2487 (432 mg) by using a ODS column.103

1.4.3 Hydrolysis of EM2487

After isolation of EM2487, Takeuchi et al. performed routine characterization techniques but could not fully elucidate the structure due to the structural complexity and line broadening in the NMR spectra. However, the source of this line broadening was not indicated but could be due to the structure has many protons that are close to each other. To fully understand the structure, EM2487 (1-11) was hydrolyzed with 0.1 N HCl, and the solution was stirred at room temperature for 0.5 hr. The solution was neutralized with 1 N NaHCO3. The reaction mixture was subjected to a C18 column, washed with water, then eluted with methanol. The fraction that was eluted with water was purified with ODS-HPLC (MeOH/H2O 5:95) to get uridine 1-12. The uridine structure was confirmed when comparing to the approved data. The other fraction that was eluted from methanol of the C18 column was dried under nitrogen to get white powder 1-13. The stereochemistry of the pseudosaccharide in the compound 1-13 was not defined, and the structural determination was not completed. Compound 1-13 was also hydrolyzed with 1.0 N HCl and heated at 100 ºC for 1 hour. The reaction solution was cooled and neutralized with 1.0 N NaHCO3. The mixture was purified with a C18 column and washed with water to get the products 1-15 and 1-16. The other fraction that eluted from methanol was the product 1-14 as shown in
Scheme 1.1. The structure of these compounds (1-14, 1-15, and 1-16) was confirmed using IR, NMR, and mass spectra. The stereochemistry was not defined for the compound 1-15.103
Scheme 1.1: Hydrolysis of EM2487.
1.5 Muraymycins, nucleoside antibiotic natural products

Muraymycins are one of the most important nucleoside natural products. There are five types of muraymycins (A1, A5, B6, C4, and D2) as shown in Figure 1.16.

![Figure 1.16: Structures of muraymycins (A1, A5, B6, C4, and D2) and EM2487.](image)

Muraymycins are produced by *Streptomyces* and are found to inhibit the bacterial membrane protein translocase I. Scientists have started to synthesize the muraymycin derivatives and Spork et al. reported the synthesis of 5'-deoxy analogue of muraymycin which leads to the development of novel antibacterial muraymycin-derived structures. First, they synthesized the epicapreomycidine-containing a urea-dipeptide building block and this building block is very important in synthesizing the muraymycin natural products. For the synthesis of the nucleoside building block, they did the reaction of the protected uridine-5'-aldehyde 1-18 with the sulfur ylide 1-19 to get 1-20 in diastereoselectivity (d.r. > 95:5). They did a double inversion at the 6'-position and did the inversion first by treating the epoxide 1-20 with Bu₄NBr to get the bromohydrin 1-21, and second by treating the compound 1-22 with Bu₄NN₃. Following several
reactions, finally, compound **1-26** was treated with 1,4-cyclohexadiene **1-27** to provide the desired nucleoside building block **1-28**.

For the final stages of the total synthesis of muraymycin, they reacted the nucleoside building block **1-28** with the aldehyde **1-30** resulting in decomposition reactions. Based on the TLC investigations, they found that the starting material and the product were not stable under acidic reaction conditions. They used a different nucleoside building block, **1-29**\(^{128,129}\), and reacted with the aldehyde **1-30** to provide the product **1-31** in 74% yield. Following several reactions, the target compound **1-33** was provided as the bis-TFA salt as shown in Scheme 1.2.
Scheme 1.2: Synthesis the target 5'-deoxy analogue 1-33. DIC = N,N'-Diisopropylcarbodiimide, Lev = levulinyl, DMAP = 4-(dimethylamino)pyridine, TBDMS = tert-butyldimethylsilyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, HOBt = 1-Hydroxybenzotriazole, PyBOP = (benzotriazol-1-yl oxy)-trityrrolidinophosphonium hexafluorophosphate, DIPEA = N,N-diisopropylethylamine.

1.6 Thesis overview

EM2487 has a unique and complicated structure, and these properties increase the difficulty of its synthesis. There are two important cores in the EM2487 which are the cyclopentenone and the bisphosphoramidate. The cyclopentenone core is the degradation product of the EM2487 hydrolysis which has undefined stereochemistry, but the NMR data is published. The goal of this research is to find synthesis routes for synthesizing these two cores.
and determine the stereochemistry of the cyclopentenone core which leads to the synthesis of the EM2487 natural product. In Chapter 2 and Chapter 3, the relative stereochemistry of cyclopentenone derivative should be determined because the stereochemistry in the natural product EM2487 was not defined. Determination the stereochemistry of the cyclopentenone core is very an important because it will give an information about the reactivity of the natural product EM2487. A synthesis route with 8 steps for the cyclopentenone core is shown in Scheme 1.3.

Scheme 1.3: Stereoselective retrosynthetic route for the cyclopentenone core.

There are four possible isomers for the target cyclopentenone, either trans or cis and their enantiomers. By comparing the NMR data of the target cyclopentenone core that is synthesized with the published NMR data, a stereochemistry relationship could be defined. The synthesis is started with the commercially available furfuryl alcohol. By using the known approach of Caddick et al., pyrano-3-one was synthesized. Ring contraction of the pyrano-3-one was achieved using
the procedure in the literature\textsuperscript{131} to get the \textit{trans} stereochemistry. Bromination and Stille reactions were done. Then, a phosphoramidite coupling reagent at the secondary hydroxyl was reacted. Finally, a global deprotection using trifluoroacetic acid and BCl\textsubscript{3} was done to get the final target product cyclopentenone. Chemical correlation reactions were used to figure out the stereochemistry.

The other core, which is very rare in natural products, is the bisphosphoramidate. The P-N-P is also rare in organic synthesis. A synthetic route was designed for this core as shown in Scheme 1.4.

Scheme 1.4: Synthetic route for the bisphosphoramidate core.
CHAPTER 2

2 SYNTHESIS OF CYCLOPENTENONE DERIVATIVE OF THE DEGRADATION PRODUCT OF EM2487 STEREOSELECTIVITY

2.1 Introduction

Nucleoside natural products are very important compounds that have a variety of uses. Generally, nucleoside natural products have a nucleoside unit and other functional groups.\textsuperscript{106} In the EM2487, it has the nucleoside unit, but it also has the unique functional group which is the pseudosaccharide. The pseudosaccharide in the EM2487 is a highly oxygenated cyclopentene structure. When Takeuchi et al. did the hydrolysis for the EM2487, they got three substructures and one of them is the cyclopentenone core. The cyclopentenone core is a very important building block in organic chemistry which is useful in synthesizing many important natural products that have biological activities.\textsuperscript{130,131,140–145,132–139} Stephen Caddick et al. have studied the cyclopentenone core towards the synthesis of natural and unnatural dienediyynes such as Neocarzinostatin Chromophore A\textsuperscript{130,136,145–147} as shown in Figure 2.1.

![Figure 2.1: Synthesis approach to functionalized cyclopentenones.](image)

Neocarzinostatin Chromophore A
Takeuchi et al. published the NMR data of the cyclopentenone degradation product of the EM2487, but the stereochemistry was not defined.\textsuperscript{103} It is important to determine the stereochemistry of the cyclopentenone core by total synthesis because it will give us an information about the reactivity of the EM2487. There are four possible isomers for the target cyclopentenone, either \textit{trans} or \textit{cis} and their enantiomers. This chapter describes the synthesis of the cyclopentenone derivative with known stereochemistry. The synthesis started from the furfuryl alcohol and used the already published approach by Caddick et al.\textsuperscript{130} to get pyrano-3-one. The next step was the ring contraction for the pyrano-3-one to get the \textit{trans} stereochemistry product.\textsuperscript{131} Throughout 8 steps, the degradation product of the EM2487 was synthesized with defined stereochemistry. Unfortunately, in the final step, the two isomers \textit{cis} and \textit{trans} were produced. Chemical correlation reactions were used to determine which one was \textit{cis}, and which one was \textit{trans}. Comparing the NMR results to the published NMR data of the degradation product cyclopentenone of the EM2487, the stereochemistry was determined to be \textit{trans}.

2.2 Experimental

2.2.1 General information

All reactions were carried out using oven-dried glassware. Some of the reactions were carried under a nitrogen atmosphere as indicated in the methods below. All stirring was performed with an internal magnetic stirrer. All chemicals were purchased from commercial sources without further purification. Solvents were dried with activated 4 Å molecular sieves. Methylene chloride and THF were dried using the PureSolv Micro Purification system. Thin-layer chromatography (TLC) was performed on 250 \( \mu \)m silica gel 60 F-254 plates. The products were purified by flash column chromatography using silica gel F60 (230-400 mesh Silicycle 40-60 \( \mu \)m, Quebec City,
Quebec, Canada), and HPLC prep using a Gilson GX-271 instrument (3000 Parmenter Street Middleton, WI 53562, USA) at 210 and 254 nm with Trilution LC V3 software using Kinetex 5 µm EVO C18 100Å 21.2 x 150 mm. Analytical HPLC was also performed using Agilent Technologies instrument (Hewlett-Packard-Strasse 8 76337 Waldbronn, Germany) using Kinetex 5 µm EVO C18 100Å 4.6 x 150 mm. $^1$H, $^{13}$C, and $^{31}$P were recorded on a Varian INOVA 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), Varian Unity 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), and Bruker AVANCE NEO 500 (500 MHz, 125 MHz, 202 MHz). $^{31}$P NMR spectra were externally referenced to 85% H$_3$PO$_4$ at 0 ppm. Chemical shifts were reported in ppm and multiplicities were reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentet), h (hextet), hep (heptet), m (multiplet), and br (broad). Mass spectrometry analysis was performed by the School of Chemical Sciences Mass Spectrometry Laboratory at the University of Illinois.

2.2.2 Synthesis of 6-acetoxy-2,3-dihydro-6H-pyran-3-one (2-1)

![Chemical structure](image)

Compound 2-1 was prepared according to the literature.$^{130,131}$ In a round bottom flask, furfuryl alcohol (6 g, 61.2 mmol, 1 equiv.) and THF:H$_2$O (48mL:12mL) were cooled to 0 ºC. To the stirring solution, a finely ground mixture of NBS (16.3 g, 91.8 mmol, 1.5 equiv.) and sodium bicarbonate (10.3 g, 122.4 mmol, 2 equiv.) were added in portion wise. To this solution, acetic anhydride (11.6 mL, 122.4 mmol, 2 equiv.) was added, and the stirring was continued at room temperature for
24 h. Enough solid sodium bicarbonate and saturated sodium bicarbonate solution were added to the resulting orange solution to neutralize the solution. After that, the orange solution was extracted with ethyl acetate (5×100 mL), dried with MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (Rf = 0.51, 70:30% Hexanes: EtOAc) with a gradient of 90:10-80:20-70:30% (Hexanes: EtOAc) as an eluent to provide a yellow oil in 45% yield (4.3 g). The NMR data matched the previously published results.¹³⁰,¹³¹ ¹H NMR (400 MHz, CDCl₃) δ 6.97–6.88 (m, 1H), 6.49 (d, J = 3.6 Hz, 1H), 6.27 (d, J = 10.4 Hz, 1H), 4.51 (d, J = 17.0 Hz, 1H), 4.23 (d, J = 17.0 Hz, 1H), 2.14 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 193.4, 169.6, 142.3, 128.9, 86.7, 67.5, 21.0.

2.2.3 Synthesis of 6-t-butoxy-2,3-dihydro-6H-pyrano-3-one (2-2)

![Reaction Scheme]

Compound 2-2 was prepared according to the literature.¹³⁰,¹³¹ To a stirred solution of compound 2-1 (3.86 g, 24.7 mmol, 1 equiv.) in dried vial was added dry dichloroethane (25 mL) and tert-butanol (11.8 mL, 123.5, 5 equiv.) under nitrogen. To the resulting solution, a solution of tin (IV) chloride (1.24 mL of 1 M in dichloromethane, 1.24 mmol, 5 mmol%) was slowly added, and the stirring was continued at room temperature for 8 h. The reaction was quenched with saturated sodium bicarbonate solution (25 mL) and the solution was extracted with ethyl acetate (2×100 mL). The organic layer was washed with saturated sodium bicarbonate solution (50 mL) and
water (50 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified using silica gel column chromatography (R<sub>f</sub> = 0.76, 70:30% Hexanes: EtOAc) with 80:20% (Hexanes: EtOAc) as an eluent to provide a yellow oil in 94% yield (3.94 g). The NMR data matched the previously published results. 130,131 1H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.80 (dd, J = 10.3, 3.5 Hz, 1H), 6.09 (d, J = 10.3 Hz, 1H), 5.48 (d, J = 3.5 Hz, 1H), 4.53 (d, J = 16.9 Hz, 1H), 4.05 (d, J = 16.9 Hz, 1H), 1.31 (s, 9H). 13C NMR (101 MHz, CDCl<sub>3</sub>) δ 195.6, 146.3, 127.3, 88.0, 76.1, 66.2, 28.7.

2.2.4 Synthesis of trans-4-tert-butoxy-5-hydroxycyclopent-2-enone (±) (2-3)

![Chemical Structure](attachment:structure.png)

Compound 2-3 was prepared according to the literature. 131 In a dry vial, the compound 2-2 was added (2.75 g, 16.15 mmol, 1 equiv.) to tert-butanol (13.5 mL, 141.15 mmol, 1.2 mmol/1 mL). Then DABCO (0.27 g, 2.4 mmol, 0.15 equiv.) was added, and the solution was stirred at 50 °C for 24 h. The solution was cooled, and the reaction solution was quenched by adding an acetic acid/sodium acetate buffer solution at pH 5 (50 mL). The reaction mixture was extracted with dichloromethane (2×50 mL). The combined organic layers were dried using sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified using silica gel column chromatography (R<sub>f</sub> = 0.35, 70:30% Hexanes: EtOAc) with 70:30% (Hexanes: EtOAc) as an eluent to give a yellow solid in 75% yield (2.07 g). The NMR data matched the previously published
results.\textsuperscript{131} \textbf{1H NMR (400 MHz, CDCl\textsubscript{3})} \(\delta 7.34 (dd, J = 6.0, 2.0 \text{ Hz}, 1\text{H}), 6.25 (dd, J = 6.1, 1.5 \text{ Hz}, 1\text{H}), 4.59 (q, J = 2.1 \text{ Hz}, 1\text{H}), 4.09 (d, J = 2.5 \text{ Hz}, 1\text{H}), 1.32 (s, 9\text{H}). \textbf{13C NMR (101 MHz, CDCl\textsubscript{3})} \delta 205.1, 161.7, 131.5, 80.9, 76.5, 75.3, 28.4.

\textbf{2.2.5 Synthesis of trans and cis-2-bromo-4-tert-butoxy-5-hydroxycyclopent-2-enone (2-4 and 2-5)}

\begin{center}
\includegraphics[width=\textwidth]{synthesis_diagram}
\end{center}

In a dry vial, anhydrous CH\textsubscript{2}Cl\textsubscript{2} (3 mL) was added to the compound 2-3 (0.60 g, 3.5 mmol, 1 equiv.), and the solution was stirred at 0 \degree C under N\textsubscript{2}. Br\textsubscript{2} (0.19 mL, 3.7 mmol, 1.05 equiv.) in dry CH\textsubscript{2}Cl\textsubscript{2} (3 mL) was added dropwise for 10 min. to the previous solution. The reaction solution was stirred at 0 \degree C under N\textsubscript{2} for 1 h. Then a solution of Et\textsubscript{3}N (0.63 mL, 4.55 mmol, 1.3 equiv.) in dry CH\textsubscript{2}Cl\textsubscript{2} (3 mL) was added dropwise to the reaction mixture for 10 min. at 0 \degree C under N\textsubscript{2}, and the reaction allowed to stir at this temperature for 3 h. The reaction was extracted with CH\textsubscript{2}Cl\textsubscript{2} (2\times50 mL), and washed with H\textsubscript{2}O (25 mL), and brine (25 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography using a gradient eluent of 90:10-80:20-70:30\% (Hexanes: EtOAc) to give two compounds. One is a yellow solid (2-4) (\(R_f = 0.57\), 70:30\% Hexanes: EtOAc) in 75\% yield (0.65 g). \textbf{1H NMR (400 MHz, CDCl\textsubscript{3})} \(\delta 7.47 (dd, J = 2.3, 0.9 \text{ Hz}, 1\text{H}), 4.56 (t, J = 2.6 \text{ Hz}, 1\text{H}), 4.16 (d, J = 2.3 \text{ Hz}, 1\text{H}), 1.32 (s, 9\text{H}). \textbf{13C NMR (101 MHz, CDCl\textsubscript{3})} \delta 198.6, 159.6, 123.8,
79.5, 76.0, 75.7, 28.3. **HRMS (ESI)** m/z [M - tBuO]+ calcd. For C₅H₄O₂Br 174.9395, found 174.9397.

The other is brown solid (2-5) (0.03 g) (Rᵣ = 0.82, 70:30% Hexanes: EtOAc). **¹H NMR (400 MHz, CDCl₃)** δ 7.40 (d, J = 2.0 Hz, 1H), 4.48 – 4.45 (m, 1H), 4.10 (d, J = 3.0 Hz, 1H), 1.28 (s, 9H). **¹³C NMR (101 MHz, CDCl₃)** δ 196.5, 157.7, 124.6, 79.6, 75.9, 75.4, 28.6. **HRMS (ESI)** m/z [M - tBuO]+ calcd. For C₅H₄O₂Br 174.9395, found 174.9398.

### 2.2.6 Synthesis of trans-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-tert-butoxy-5-hydroxycyclopent-2-enone (2-6)

![Reaction Diagram]

In a dry vial, compound 2-4 (0.4 g, 1.61 mmol, 1 equiv.) was azeotropically dried (3 times) using extra dry toluene. Then, anhydrous 1,4-dioxane (14 mL), tetrakis(triphenylphosphine)palladium (0.19 g, 0.16 mmol, 0.1 equiv.), and Bu₃SnCH₂OTBS (1.05 g, 2.4 mmol, 1.5 equiv.) were added successively. The reaction mixture was heated to 100 °C overnight, then cooled to room temperature, concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (Rᵣ = 0.74, 70:30% Hexanes: EtOAc) using a gradient eluent of 90:10-80:20% (Hexanes: EtOAc) to give a yellow solid in 82% yield (0.42 g). **¹H NMR (400 MHz, CDCl₃)** δ 7.15 (d, J = 1.9 Hz, 1H), 4.54 – 4.51 (m, 1H), 4.40 – 4.34 (m, 2H), 4.11 (t, J = 2.4 Hz, 1H), 1.58 (s, 1H), 1.33 (s, 9H), 0.91 (s, 9H), 0.08 (d, J = 2.2 Hz, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 204.0, 154.2, 144.5, 81.2, 75.3, 74.9, 57.6, 28.3, 25.9, -5.5. **HRMS (ESI)** m/z [M + Na]+ calcd. for C₁₆H₃₀O₄NaSi 337.1811, found 337.1813.
2.2.7 Synthesis of \( \text{trans}-2-\left(\left(\text{tert-butylidimethylsilyl}\right)\text{oxy}\right)\text{methyl}-4\text{-tert-butoxy-5-}
\text{dibenzylphosphato-2-enone (2-7)} \)

\[
\text{OH} \quad \text{N} \quad \text{P} \quad \text{BnO} \quad \text{Bn} \quad \text{O} \quad \text{P} \quad \text{OBn}
\]

In a dry vial, compound (2-6) (0.45 g, 1.45 mmol, 1 equiv.) was azeotropically dried (3 times) using extra dry toluene. To this vial, dry CH\(_2\)Cl\(_2\) (14 mL) was added under N\(_2\), and then followed by 4,5-Dicyanoimidazole DCI (0.26 g, 2.2 mmol, 1.5 equiv.). The reaction mixture was cooled to 0 °C, then dibenzyl-N, N-diisopropylphosphoramidite (0.76 mL, 2.2 mmol, 1.5 equiv.) was added. The reaction mixture was stirred at 0 °C for 2 h, and then cooled to -78 °C. t-BuOOH (0.53 mL of 5-6M in decane, 2.9 mmol, 2 equiv.) was added slowly. Then the reaction was stirred at this temperature for 1 h. The reaction mixture was concentrated under vacuum. The reaction product was purified by silica gel column chromatography (R\(_f\) = 0.62, 70:30% Hexanes: EtOAc) using a gradient eluent of 90:10-80:20% (Hexanes: EtOAc) to give a yellow oil in 30% yield (0.25 g). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.36–7.27 (m, 10H), 7.09 (d, \(J = 2.0\) Hz, 1H), 5.23–5.03 (m, 4H), 4.80 (dd, \(J = 13.3, 2.6\) Hz, 1H), 4.70 (p, \(J = 2.3\) Hz, 1H), 4.38–4.31 (m, 2H), 2.11 (s, 1H), 1.22 (s, 9H), 0.89 (s, 9H), 0.05 (d, \(J = 1.8\) Hz, 6H). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 197.5, 153.3, 145.1, 128.5, 128.0, 127.9, 84.2, 75.3, 73.8, 69.6, 69.5, 57.6, 28.2, 25.9, 18.3, -5.4. \(^31\)P NMR (162 MHz, CDCl\(_3\)) \(\delta\) -1.9. HRMS (ESI) m/z [M + Na]\(^+\) calcd. for C\(_{30}\)H\(_{43}\)O\(_7\)NaPSi 597.2413, found 597.2416.
2.2.8 Synthesis of trans-2-(hydroxymethyl)-4-hydroxy-5-dibenzylphosphato-2-enone (2-8)

In a dry vial, dry CH₂Cl₂ (6.5 mL) was added to the compound 2-7 (0.197 g, 0.34 mmol, 1 equiv.). The reaction solution was cooled to -10 °C using NaCl+ ice. Then trifluoroacetic acid 99% (0.26 mL, 3.4 mmol, 10 equiv.) was added slowly to the reaction mixture. The stirring was continued at room temperature for 10 h. The reaction mixture was concentrated, then purified by silica gel column chromatography (Rf = 0.58, 90:10% dichloromethane: Methanol) using 90:10% dichloromethane: methanol as an eluent to give a yellow oil in 50% yield (0.070 g). ^1H NMR (400 MHz, CDCl₃) δ 7.38–7.28 (m, 10H), 7.26 (s, 1H), 5.11 (dd, J = 15.1, 8.3 Hz, 4H), 4.83–4.77 (m, 1H), 4.63 (dt, J = 7.8, 2.1 Hz, 1H), 4.34 (s, 2H). ^13C NMR (101 MHz, CDCl₃) δ 194.9, 153.3, 149.2, 144.6, 129.0, 128.2, 84.7, 74.4, 70.5, 70.3, 56.9. ^31P NMR (162 MHz, CDCl₃) δ -0.4. HRMS (ESI) m/z [M + H]⁺ calcd. for C₂₀H₂₂O₇P 405.1103, found 405.1103.

2.2.9 Synthesis of trans and cis-2-hydroxymethyl-4-hydroxy-5-dihydrogen phosphate-2-enone (2-9 and 2-10)

Peak at 1.5 min. HPLC: trans-2-9 ratio 60 : 40% Peak at 2.8 min. HPLC: cis-2-10
In a dry vial, dry CH$_2$Cl$_2$ (3 mL) was added to the compound 2-8 (0.12 g, 0.29 mmol, 1 equiv.). The reaction mixture was cooled to -10 °C, then BCl$_3$ (1.2 mL of 1M in dichloromethane, 1.2 mmol, 4 equiv.) was added slowly to the reaction solution. The reaction was stirred at room temperature overnight and quenched with (1 mL) anhydrous methanol. Then, the reaction mixture was concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/ min., mobile phase (isocratic) = (99.5:0.5) % (water-acetonitrile) in (0.1% TFA) to give two compounds with total 50% yield (0.033 g). One is separated at 1.5 min. retention time to get the compound trans-2-9 white solid (0.020 g). $^1$H NMR (500 MHz, D$_2$O) δ 7.40 (s, 1H), 4.89–4.87 (m, 1H), 4.62 (dd, $^3$J$_{PH}$ = 10.9, $J$ = 2.5 Hz, 1H), 4.30 (s, 2H). $^{13}$C NMR (126 MHz, D$_2$O) δ 201.5 (d, $^3$J$_{PC}$ = 6.4 Hz), 155.1, 143.8, 82.9 (d, $^2$J$_{PC}$ = 5.9 Hz), 73.7, 55.2. $^{31}$P NMR (202 MHz, D$_2$O) δ -0.05. HRMS (EI) m/z [M - H] calcd. for C$_6$H$_8$O$_7$P 223.0008, found 223.0004. The other compound is separated at 2.77 min. retention time to get compound cis-2-10 white solid (0.013 g). $^1$H NMR (500 MHz, D$_2$O) δ 7.51 (s, 1H), 4.94 (p, $J$ = 1.9 Hz, 1H), 4.67 (dd, $^3$J$_{PH}$ = 10.8, $J$ = 2.6 Hz, 1H), 4.24 (s, 2H), 3.42 (s). $^{13}$C NMR (126 MHz, D$_2$O) δ 201.8 (d, $^3$J$_{PC}$ = 6.5 Hz), 157.2, 140.7, 82.8 (d, $^2$J$_{PC}$ = 5.8 Hz), 73.9, 65.0, 58.1. $^{31}$P NMR (202 MHz, D$_2$O) δ -0.05. HRMS (EI) m/z [M - H] calcd. for C$_6$H$_8$O$_7$P 223.0008, found 223.0006.

2.2.10 Synthesis of trans-2-hydroxymethyl-4,5-dihydroxy-2-enone (2-11)

![Reaction Scheme](reaction_scheme.png)
In a dry vial, compound 2-6 (0.1 g, 0.32 mmol, 1 equiv.) was dissolved in dry CH₂Cl₂ (3 mL). The reaction solution was cooled to 0 °C, and then trifluoroacetic acid 99% (0.25 mL, 3.2 mmol, 10 equiv.) was added slowly. The reaction mixture was stirred at room temperature for 10 h, then concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/min., mobile phase (isocratic) = (99:1) % (water-acetonitrile) in (0.1%TFA) to get the trans-triol 2-11 yellow oil in 65% yield (0.03 g). ¹H NMR (500 MHz, CD₃OD) δ 7.25 (q, J = 1.8 Hz, 1H), 4.55 – 4.53 (m, 1H), 4.25 – 4.23 (m, 2H), 4.05 (d, J = 2.7 Hz, 1H), 3.35 (s, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 204.8, 154.9, 145.7, 82.9, 76.5, 56.8. HRMS (EI) m/z [M – H₂O]⁺ calcd. For C₆H₆O₃ 126.03170, found 126.03154.

2.2.11 Synthesis of trans-2-hydroxymethyl-4,5-dihydroxy-2-enone (2-12)

Compound 2-9 which was obtained at 1.5 min. retention time in HPLC (0.005 g, 0.02 mmol, 1 equiv.) was dissolved in 0.1 M citrate buffer pH = 5.6 (0.8 mL). To the reaction mixture, phosphatase acid from potato enzyme (lyophilized powder ≥ 3.0 units/ mg solid P1146-200 UN) (1 mg, 10 units) was added. The reaction mixture was incubated at 37 °C (stirring 100 rpm) overnight. The reaction mixture was concentrated, and the crude product was dissolved in methanol. The solution was concentrated again. The crude reaction was purified by HPLC prep
chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/min., mobile phase (isocratic) = (99:1) % (water-acetonitrile) in (0.1% TFA) to give the trans-triol 2-12 yellow oil in 50% yield (0.0014 g). \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\) 7.25 (s, 1H), 4.55–4.53 (m, 1H), 4.25–4.23 (m, 2H), 4.05 (d, \(J = 2.7\) Hz, 1H), 3.35 (s, 1H). \(^1\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 204.8, 154.9, 145.7, 82.9, 76.5, 56.8. HRMS (EI) m/z [M – H\(_2\)O]\(^+\) calcd. For C\(_6\)H\(_6\)O\(_3\) 126.03170, found 126.03154.

2.3 Results and Discussion

The synthesis for the target molecule which is the pseudosaccharide was designed to find the unknown stereochemistry for the two centers. This pseudosaccharide core is a result of the degradation of the EM2387 natural product as described in the literature. The NMR data and the mass spectrometry were provided\(^{103}\) but the stereochemistry was not identified. Through eight steps, the synthesis of the pseudosaccharide was achieved and the stereochemistry was identified for the pseudosaccharide derivative product from EM2487. Starting with a structure with known stereochemistry, this core could be synthesized and the NMR results of the structure with known stereochemistry compared with the one in the paper that was already published.

2.3.1 Synthesis of the trans-4,5-dioxygenated cyclopentenone

Furfuryl alcohol was used as starting material for the synthesis of the cyclopentenone as shown in

Scheme 2.1.
Scheme 2.1: Synthesis of the cyclopentenone 2-3 from furfuryl alcohol.

Achmatowicz reaction\textsuperscript{148} was used in the first reaction to get the acetoxy pyranone (2-1). Achmatowicz rearrangement is an oxidative ring expansion, and it is useful to get bioactive molecules. The mechanism for this rearrangement is shown in Figure 2.2.

The intermediate of this reaction is hydroxypyranone which has low stability in aqueous solutions at room temperature and decompose under basic conditions so that in this reaction, \textit{N}-bromosuccinimide was used in the presence of sodium bicarbonate and was directly followed by adding the acetic anhydride to obtain the more stable acetoxy pyranone (2-1). The yield for this reaction was 45\%, and the procedure was obtained from Caddick, S et al.\textsuperscript{130} Acetoxy pyranone
(2-1) was reacted with tert-butanol in the presence of SnCl$_4$ to obtain the butoxy pyranone (2-2) in 94% yield. The reaction was carried out under nitrogen because SnCl$_4$ is sensitive to moisture. Also, the reaction was run under a dry condition to get the high yield.$^{130}$ Then, compound butoxy pyranone (2-2) was reacted with 1,4-diazabicyclo[2.2.2]octane (DABCO) to get the trans-4,5-dioxygenated cyclopentenone derivative (2-3) in 75% yield. To obtain a high yield, it was important to run the reaction at 45-50 °C and the base (DABCO) equivalent was 0.15. If the temperature was increased higher than 50 °C and the base equivalent was also increased, the yield was lower because compound 2-3 would undergo decomposition or formation of a side product. The mechanism of this rearrangement was proposed by Nunes et al.$^{131}$ They proposed three different mechanisms: a 1,2-Wittig rearrangement including a biradical, cyclization to give epoxide followed by nucleophilic ring opening, or cyclization of the intermediate formed by electrocyclic ring opening of an enol as shown in Figure 2.3.
2.3.2 Synthesis of the trans and cis-2-bromo-4-tert-butoxy-5-hydroxycyclpent-2-enone

Bromo alkene compounds (2-4 and 2-5) were synthesized from the trans-4,5-dioxygenated cyclopentenone as shown in Scheme 2.2. In this reaction, the stoichiometry was important especially for the bromine amount. The equivalent amount of bromine was increased to 2 equivalents to get a higher yield, but the product was not obtained. The best conditions found were to use 1.05 equiv. of Br\textsubscript{2} to get the bromo alkene products in 75% yield. Interestingly, when the reaction was monitored by thin-layer chromatography (TLC), two spots were obtained. Fortunately, there was a big difference in R\textsubscript{f} values between the two spots.
Scheme 2.2: Synthesis of bromo alkene 2-4 and 2-5.

The two compounds were separated by flash column chromatography using a gradient eluent of 90:10-80:20-70:30% (Hexanes: EtOAc) to get the two products. One compound was a yellow solid (2-4), and the retention factor (R<sub>f</sub>) = 0.57, 70:30% Hexanes: EtOAc. The other compound was a brown solid (2-5), and the R<sub>f</sub> = 0.82, 70:30% Hexanes: EtOAc. After getting the NMR data, two isomers were observed, and the ratio is 96:4 for the trans and cis, respectively. It could be that there was an isomerization through a ring opening or through the formation enol/keto structures.

2.3.3 Stille coupling and phosphorylation reactions

The major product was used which is the trans (2-4) for the Stille coupling reaction to replace the bromine with CH<sub>2</sub>OTBS and for this reaction, Bu<sub>3</sub>SnCH<sub>2</sub>OTBS reagent was used with the tetrakis(triphenylphosphine)palladium catalyst as shown in Scheme 2.3.
Stille coupling is one of the best ways to make a C-C bond, and the catalytic cycle involves oxidative addition, transmetallation, and reductive elimination. In this reaction, a vinyl bromide underwent a Pd-catalyzed substitution reaction with a nucleophilic alkylmetal compound (Bu$_3$SnCH$_2$OTBS) to obtain the product (2-6) in 82% yield. The yield could be enhanced when the reaction was done at 100 °C and the starting material was azeotropically dried with extra dry toluene.

The phosphorylation reaction at the hydroxyl group of compound 2-6 is the next step. Phosphorylation of the hindered alcohol in 2-6 was challenging and many phosphorylating agents were tried including di-tert-butyl $N,N$-diisopropylphosphoramidite, and di(p-methoxybenzyl)-$N,N$-diisopropylphosphoramidites as well as a variety of polar and nonpolar aprotic solvents. Unfortunately, the phosphorylation coupling did not work even though different conditions were used, for example, dry acetonitrile and dry THF were used as solvents. In the literature, it has been found that the cycle of this kind of core which is the cyclopentenones tends to open especially when there is a good leaving group on the α-position to the carbonyl functional group and phosphorylation of alcohol alpha to the carbonyl primes the ring for a retro-Nazarov cyclization.$^{145,149}$ To solve this problem, it was thought that putting a stable phosphoramidite group in acidic conditions could get this reaction to work. Dibenzyl phosphoryl chloride, Et$_3$N and DMAP were used, but the reaction did not work. This may be due to the hydroxyl group being too sterically hindered to react with dibenzyl phosphoryl chloride. Finally, dibenzyl-$N,N$-diisopropyl phosphoramidite was used, and fortunately, the phosphate triester (2-7) was observed in a 30% yield as shown in Scheme 2.4.
2.3.4 Global deprotection using trifluoroacetic acid (TFA) and BCl₃

In order to complete the synthesis of this core, deprotection was performed using trifluoroacetic acid. The molar ratio of the trifluoracetic acid played an important role in the success of the reaction. Under the very acidic conditions, the product was found to be acid-labile and the deprotection had to be carefully controlled. It was found that the best condition was to use 10 equivalents of TFA reagent to get the compound (2-8) with 50% yield. The final step of this synthesis was the deprotection of the benzyl group using BCl₃ reagent to get the target product (trans-2-9) and (cis-2-10) as shown in Scheme 2.5.

![Scheme 2.4: Synthesis of the compound 2-7.](image)

Scheme 2.5: Synthesis of the target product cyclopentenone core (pseudosaccharide derivative).

When the NMR spectrum of the crude was checked, two products were provided with the ratio (60-40) % HPLC (1.5 min. and 2.8 min.). These two compounds could be separated using
HPLC chromatography with the condition (99.5-0.5) % (water-acetonitrile) in 0.1% TFA, wavelength 210 nm and 254 nm, run time 10 min. as shown in Figure 2.4.

![HPLC Chromatogram](image)

Figure 2.4: HPLC analysis for the target cyclopentenone core products. HPLC chromatography: Mobile Phase (isocratic) = 99.5% water: 0.5% acetonitrile with 0.1% TFA; Stationary phase: octyldecylsilane (C18); Detection: 210 nm (blue) and 254 nm (green); Run time: 10 minutes.

NMR (\(^{1}\text{H},^{13}\text{C},^{31}\text{P}\)), and mass spectroscopy were done for both products, and it was found that one compound which had a retention time of 1.5 min. matched the NMR of the degradation product in the literature.\(^{103}\) The other compound from the HPLC which had a retention of time 2.8 min. has a different NMR than the degradation product in the literature. It was thought that two compounds were obtained because using BCl\(_3\) as a deprotection reagent could form the enol form so that the enol could take the proton from both sides up or down as shown in Figure 2.5.

![Chemical Structures](image)

Figure 2.5: The isomerization process to give the \textit{trans-2-9} and \textit{cis-2-10}.
From known stereochemistry was started, which was *trans*, and it was thought that the target product was *trans*, and then compared the NMR results to the literature. Unfortunately, it did not work for us this hypothesis, so 2D NMR (NOESY) was done, but also the 2D NMR did not give us distinct information so that it could be said that one is *trans* and the other is *cis*. Also, J-coupling constants were checked for both compounds, but the numbers were too close (2.5 Hz and 2.6 Hz), so it was difficult to say which one was *trans* or *cis* as shown in Table 2.1.

Table 2.1: NMR results for the degradation product and the two compounds in HPLC.

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<tr>
<td></td>
<td>^H NMR in D_2O</td>
<td>^H NMR in D_2O</td>
</tr>
<tr>
<td></td>
<td>(literature)</td>
<td>rt = 1.5 min.</td>
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<td></td>
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<td>rt = 2.8 min.</td>
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<tr>
<td>7.40 (1H, br m)</td>
<td>7.40 (1H, app. s)</td>
<td>7.51 (1H, app. s)</td>
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<tr>
<td>4.88 (1H, br m)</td>
<td>4.87-4.89 (1H, m)</td>
<td>4.94 (1H, p, J = 1.9 Hz)</td>
</tr>
<tr>
<td>4.62 (1H, dd, J= 2.6 Hz, ^3J_{PH} = 10.7 Hz.)</td>
<td>4.62 (1H, dd, J= 2.5 Hz, ^3J_{PH} = 10.9 Hz.)</td>
<td>4.67 (1H, dd, J= 2.6 Hz, ^3J_{PH} = 10.8 Hz.)</td>
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<tr>
<td>4.30 (2H, br s)</td>
<td>4.30 (2H, s)</td>
<td>4.24 (2H, s)</td>
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</table>

Since the *trans* and *cis* configuration could not be definitively established due to isomerization in the final step, we started to think about another way which was using the chemical correlation reactions as shown in Scheme 2.6.
Scheme 2.6: Chemical correlation reactions to figure out the _trans_ stereochemistry.

Deprotection for compound 2-6 with trifluoroacetic acid was done to get the _trans-triol_ 2-11 with 65% yield. The presumed _trans_ derivative 2-9 (retention time = 1.5 min) was treated with acid phosphatase enzyme from potato and enzymatic deprotection was done to remove the phosphate group.\(^{150}\) _trans-triol_ 2-12 was obtained when the NMR results were compared.

From these results, it could be known that the HPLC separation product 2-9 which had a retention time of 1.5 min. has a _trans_ stereochemistry, and this product _trans-2-9_ matched NMR results of the cyclopentenone degradation product EM2487 in the literature.\(^{103}\) From these results, it could be confirmed that the unknown stereochemistry of the cyclopentenone derivative core in the natural product EM2487 is _trans_. The other compound _cis-2-10_ which had a retention time of 2.8 min. has a _cis_ stereochemistry.
2.4 Conclusions

Retrosynthesis steps were designed toward the synthesis of the cyclopentenone core which has unknown stereochemistry in the EM2487 natural product. Using an 8-step synthesis, the cyclopentenone target was synthesized. It was started from the Achmatowicz reaction to get known stereochemistry which is \textit{trans}. Some of the steps in the total synthesis were challenging. The phosphorylation of the hydroxyl group was difficult because of the cyclopentenone nature which tends to be opened under these conditions. We found that the dibenzyl-\textit{N,N}-di isopropyl phosphoramidite was the best reagent to do the reaction with the hydroxyl group because it was stable under the reaction conditions. The final step of the total synthesis was the deprotection with \(\text{BCl}_3\) reagent. This step gave two isomers which were separated using HPLC chromatography. The NMR was done for both isomers and one of them matched the cyclopentenone degradation product of EM2487 in the literature and the other did not match. It could not be known which one was \textit{trans} and which one was \textit{cis} from NMR results. Also, the J-coupling constants were very close for both, so it was difficult to distinguish between them. Using the 2D NMR NOESY was also not helpful. Finally, chemical correlation reactions were done, and it was found that the unknown stereochemistry of the cyclopentenone core of the degradation product of EM2487 in the literature was \textit{trans} and the other isomer was \textit{cis}. 

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CHAPTER 3

3 SYNTHESIS OF CIS-CYCLOPENTENONE DERIVATIVE OF EM2487

3.1 Introduction

To support our results that the unknown stereochemistry of cyclopentenone degradation product of EM2487 was trans, synthetic routes were designed to make the cis cyclopentenone derivative. The NMR results for both the trans and cis cyclopentenone degradation product of EM2487 will add more confidence to figure out the unknown stereochemistry. Starting from commercially available cyclopentenone derivative that has a known cis stereochemistry, the target cis cyclopentenone derivative (cis-2-10) could be prepared. Chemical correlation reactions could also be used to determine the unknown stereochemistry by comparing the NMR results of the trans-triol 2-11, and cis-triol 3-9 as shown in Scheme 3.1.

![Scheme 3.1: Synthetic route for the cis cyclopentenone derivative cis-2-10 and cis-triol 3-9.](image)

3-5  \[ \text{TBDPSO} \rightarrow \text{OH} \rightarrow \text{BnO} \rightarrow \text{OH} \rightarrow \text{Bn} \rightarrow \text{OH} \rightarrow \text{OH} \rightarrow \text{cis-2-10} \]

3-6  \[ \text{Trfluoro acetic acid} \rightarrow \text{THF} : H_2O \rightarrow \text{OH} \rightarrow \text{cis-triol 3-9} \rightarrow \text{trans-triol 2-11} \]
3.2 Experimental

3.2.1 General information

All reactions were carried out using oven-dried glassware. Some of the reactions were carried under a nitrogen atmosphere as indicated in the methods below. All stirring was performed with an internal magnetic stirrer. All chemicals were purchased from commercial sources without further purification. Solvents were dried with activated 4 Å molecular sieves. Methylene chloride and THF were dried using the Pure Solv Micro Purification system. Thin-layer chromatography (TLC) was performed on 250 μm silica gel 60 F-254 plates. The products were purified by flash column chromatography using silica gel F60 (230-400 mesh Silicycle 40-60 μm, Quebec City, Quebec, Canada), and HPLC prep using a Gilson GX-271 instrument (3000 Parmenter Street Middleton, WI 53562, United States of America) at 210 and 254 nm with Trilution LC V3 software using Kinetex 5 μm EVO C18 100Å 21.2 × 150 mm. Analytical HPLC was performed using Agilent Technologies instrument (Hewlett-Packard-Strasse 8 76337 Waldbronn, Germany) using Kinetex 5 μm EVO C18 100Å 4.6 × 150 mm. ¹H, ¹³C, and ³¹P were recorded on a Varian INOVA 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), Varian Unity 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), and Bruker AVANCE NEO 500 (500 MHz, 125 MHz, 202 MHz). ³¹P NMR spectra were externally referenced to 85% H₃PO₄ at (0 ppm). Chemical shifts were reported in ppm and multiplicities were reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentet), h (hextet), hep (heptet), m (multiplet), and br (broad). Mass spectrometry analysis was performed by the School of Chemical Sciences Mass Spectrometry Laboratory at the University of Illinois.
3.2.2 Synthesis of (3aR,6aR)-5-(hydroxymethyl)-2,2-dimethyl-3a,6a-dihydrocyclopenta[1,2-
  \textit{d}]1,3-dioxolan-4-one (3-2)

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\textbf{3-1}};
  \node (b) at (1,0) {\textbf{3-2}};
  \node (c) at (0,-0.5) {\textbf{Formaldehyde 37\%}};
  \node (d) at (0,-1) {\textbf{Imidazole}};
  \node (e) at (0,-1.5) {\textbf{THF : H$_2$O, 0 \degree C \ 50\%}};
  \node (f) at (1.5,0) {\textbf{}};
  \draw[->] (a) -- (b);
  \draw[->] (a) -- (c);
  \draw[->] (a) -- (d);
  \draw[->] (a) -- (e);
  \draw[->] (a) -- (f);
\end{tikzpicture}
\end{center}

The compound 3-2 was prepared according to the literature.\textsuperscript{134} Compound 3-1 (0.2 g, 1.29 mmol, 1 equiv.) was dissolved in 1:1 THF:H$_2$O (4 mL). To the reaction solution, aqueous formaldehyde 37\% (0.58 mL, 7.74 mmol, 6 equiv.) and imidazole (0.045 g, 0.66 mmol, 0.51 equiv.) were added. The reaction mixture was stirred at 0 \degree C for 5 h. Then the reaction mixture was extracted with ethyl acetate (3× 10 mL), and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography ($R_f = 0.33$, 50:50\% Hexanes: EtOAc) using 50:50\% Hexanes: EtOAc to get a yellow oil in 50\% yield (0.12 g). The NMR data matched the previously published results.\textsuperscript{134} \textbf{1H NMR (400 MHz, CDCl$_3$)} $\delta$ 7.41 (q, $J = 1.7$ Hz, 1H), 5.19 (dq, $J = 3.8$, 1.1 Hz, 1H), 4.47 (d, $J = 5.5$ Hz, 1H), 4.27 (s, 2H), 1.34 (s, 3H), 1.32 (s, 3H). \textbf{13C NMR (101 MHz, CDCl$_3$)} $\delta$ 202.7, 153.5, 146.4, 115.8, 77.9, 77.7, 57.1, 27.6, 26.2.
3.2.3 Synthesis of ((3aR,6aR)-2,2-dimethyl-4-oxo-3a,6a-dihydrocyclopental [1,2-d]1,3-dioxolan-5-yl)methyl benzoate (3-3)

The compound 3-3 was prepared according to the literature. In a dry vial, compound 3-2 (0.09 g, 0.49 mmol, 1 equiv.) was dissolved in dry CH₂Cl₂ (3 mL). The reaction solution was cooled to 0 ºC, and benzoyl chloride (0.09 mL, 0.74 mmol, 1.5 equiv.) and pyridine (0.06 mL, 0.74 mmol, 1.5 equiv.) were added. The reaction mixture was stirred at the same temperature for 3 h, and then at room temperature for 1 h. NH₄Cl (saturated solution, 1.8 mL) was added, and the reaction solution was extracted with CH₂Cl₂ (2 × 5 mL). The combined organic layers were washed with NH₄Cl (saturated solution, 1.8 mL), dried with sodium sulfate, and then concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography (Rf = 0.42, 70:30% Hexanes: EtOAc) using a gradient eluent of 90:10-80:20-70:30% (Hexanes: EtOAc) to get a yellow oil in 53% yield (0.075 g). ¹H NMR (400 MHz, CDCl₃) δ 8.06 (dd, J = 19.5, 8.4 Hz, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.50 – 7.40 (m, 3H), 5.26 – 5.21 (m, 1H), 5.08 – 4.96 (m, 2H), 4.55 (d, J = 5.5 Hz, 1H), 1.40 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 201.1, 166.1, 154.5, 142.3, 133.7, 130.4, 130.0, 129.6, 128.8, 115.8, 77.7, 77.3, 58.5, 27.7, 26.3.
3.2.4 Synthesis of compound 3-4

![Chemical structure](image)

Compound 3-3 (0.075 g, 0.26 mmol, 1 equiv.) was dissolved in 60% aqueous acetic acid (1.85 mL). The reaction solution was heated to 50 °C for 9 h. The reaction solution was concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography ($R_f = 0.35$, 20:80% Hexanes: EtOAc) using a gradient eluent of 60:40-50:50-40:60% (Hexanes: EtOAc) to get a white solid in 80% yield (0.052 g). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.03 – 7.93 (m, 2H), 7.63 – 7.56 (m, 1H), 7.51 – 7.42 (m, 3H), 4.93 (s, 2H), 4.68 (d, $J = 13.1$ Hz, 1H), 4.05 (s, 1H), 3.60 (s, 2H, OH).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 205.2, 171.0, 166.2, 155.9, 141.7, 133.5, 130.2, 129.8, 128.7, 71.9, 67.8, 58.5.

3.2.5 Synthesis of (3aS,6aS)-5-(hydroxymethyl)-2,2-dimethyl-3a,6a-dihydrocyclopenta[1,2-d]1,3-dioxolan-4-one (3-6)

![Chemical structure](image)

The compound 3-6 was prepared according to the literature. $^{134}$ Compound 3-5 (0.500 g, 3.24 mmol, 1 equiv.) was dissolved in 1:1 THF:H$_2$O (10.5 mL). To the reaction solution, aqueous formaldehyde 37% (1.6 mL, 19.44 mmol, 6 equiv.) and imidazole (0.100 g, 1.65 mmol, 0.51 equiv.)
were added. The reaction mixture was stirred at 0 °C for 5 h. Then the reaction mixture was extracted with ethyl acetate (3× 25 mL), and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography (Rf = 0.33, 50:50% Hexanes: EtOAc) using 50:50% Hexanes: EtOAc to obtain a yellow oil in 60% yield (0.35 g). The NMR data matched the previously published results.\(^{134}\)**1H NMR (400 MHz, CDCl\(_3\))** δ 7.44–7.41 (m, 1H), 5.21 (ddd, \(J = 5.2, 2.4, 1.2\) Hz, 1H), 4.50 (d, \(J = 5.4\) Hz, 1H), 4.33 (s, 2H), 1.37 (s, 3H), 1.36 (s, 3H).**\(^{13}C\) NMR (101 MHz, CDCl\(_3\)) δ 202.7, 153.4, 146.3, 115.8, 77.9, 77.6, 57.3, 27.6, 26.2.

### 3.2.6 Synthesis of compound 3-7

In a dry vial, compound 3-6 (0.34 g, 1.85 mmol, 1 equiv.) was dissolved in dry CH\(_2\)Cl\(_2\) (17 mL) under nitrogen. The solution was cooled to 0 °C. Then, tert-butyl(chloro)diphenylsilane (0.96 mL, 3.7 mmol, 2 equiv.) and imidazole (0.32 g, 4.63 mmol, 2.5 equiv.) were added successively to the reaction solution, and the stirring was continuous for 3 h at the same temperature. To the reaction mixture, brine (10 mL) was added, and the reaction solution was extracted with CH\(_2\)Cl\(_2\) (3 × 10 mL). The combined organic layers were washed with brine (sat. NaCl) (10 mL), and then dried using sodium sulfate. The solution was concentrated under reduced pressure. The crude reaction product was purified by silica gel column chromatography (Rf = 0.44, 80:20% Hexanes: EtOAc) using a gradient eluent of 95:5-90:10% (Hexanes: EtOAc) to get a colorless oil in 62% yield.
(0.49 g). \[^1H\text{NMR (400 MHz, CDCl}_3\]\ δ 7.75 – 7.60 (m, 4H), 7.58 – 7.55 (m, 1H), 7.47 – 7.33 (m, 6H), 5.27 – 5.19 (m, 1H), 4.50 (d, \(J = 5.6\) Hz, 1H), 4.43 – 4.40 (m, 2H), 1.42 (s, 3H), 1.40 (s, 3H), 1.09 (s, 9H). \[^{13}C\text{NMR (101 MHz, CDCl}_3\]\ δ 201.6, 152.8, 147.2, 135.6, 133.0, 132.9, 130.2, 128.1, 115.5, 78.2, 77.6, 59.0, 27.8, 27.0, 26.5, 19.5.

### 3.2.7 Synthesis of compound 3-8

![Chemical structure](image)

Compound 3-7 (0.1 g, 0.24 mmol, 1 equiv.) was dissolved in a mixture of THF (0.18 mL) and H\(_2\)O (0.13 mL). The reaction solution was cooled to 0 °C, and then trifluoroacetic acid (0.37 mL, 4.8 mmol, 20 equiv.) was added dropwise. The reaction mixture was stirred for 3 h at the same temperature. The solution was neutralized by addition of sodium bicarbonate at 0 °C. The reaction mixture was extracted with ethyl acetate and water. The organic layer was washed with brine, and dried with sodium sulfate. The solution was concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/ min., mobile phase = (50-70) % gradient of acetonitrile (water-acetonitrile) to give a colorless oil in 40% yield (0.036 g). \[^1H\text{NMR (400 MHz, CDCl}_3\]\ δ 7.67 – 7.61 (m, 4H), 7.59 (q, \(J = 2.0\) Hz, 1H), 7.40 (dd, \(J = 15.2, 6.6\) Hz, 6H), 4.86 – 4.79 (m, 1H), 4.43 (q, \(J = 16.6\) Hz, 2H), 4.12 (d, \(J = 5.5\) Hz, 1H), 1.08 (s, 9H). \[^{13}C\text{NMR (101 MHz, CDCl}_3\]\ δ 205.0, 153.9, 146.7, 135.6, 132.9, 130.1, 128.0, 72.2, 67.8, 58.8, 27.0, 19.4.
3.2.8 Synthesis of cis-2-hydroxymethyl-4,5-dihydroxy-2-enone (3-9)

Compound 3-6 (0.038 g, 0.21 mmol, 1 equiv.) was dissolved in 1:1 THF: H₂O (0.2 mL). The reaction solution was cooled to 0 ºC, and trifluoroacetic acid 99% (0.16 mL, 2.1 mmol, 10 equiv.) was added slowly. The reaction mixture was stirred at room temperature for 6 h and concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/min., mobile phase (isocratic) = (99:1) % (water-acetonitrile) in (0.1%TFA) to provide the cis-triol 3-9 yellow oil in 80% yield (0.024 g). ¹H NMR (500 MHz, CD₃OD) δ 7.43 (s, 1H), 4.75 (s, 1H), 4.26 (s, 2H), 4.11 (d, J = 5.4 Hz, 1H), 3.35 (s, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 207.6, 155.1, 147.1, 73.7, 68.9, 57.1. HRMS (EI) m/z [M – H₂O]+ calcd. For C₆H₆O₃ 126.03170, found 126.03133.

3.2.9 Synthesis of compound 3-10

Compound 3-10 was prepared according to the literature.¹⁵¹ Compound 3-1 (0.10 g, 0.65 mmol, 1 equiv.) was dissolved in dry MeOH (4 mL). To the reaction solution, CeCl₃ 7H₂O (0.24 g, 0.65 mmol, 1 equiv.) was added. The reaction solution was cooled to 0 ºC, and NaBH₄ (0.031 g, 0.85
mmol, 1.3 equiv.) was added. The reaction mixture was stirred for 1 h at this temperature. Then, the mixture was neutralized with 1 N HCl (1 mL). The reaction solution was extracted with diethyl ether (15 mL) and washed with brine. The organic layer was dried with sodium sulfate and concentrated under reduced pressure to give a colorless oil in 50% yield (0.05 g). The NMR data matched the previously published results. The product was used directly in the next step without further purification.

### 3.2.10 Synthesis of compound 3-11

![Chemical structure](image)

In a dry vial, Compound 3-10 (0.050 g, 0.3 mmol, 1 equiv.) was dissolved in dry CH$_2$Cl$_2$ (3 mL). The reaction solution was cooled to 0 °C, and then the benzoyl chloride (0.05 mL, 0.45 mmol, 1.5 equiv.) and pyridine (0.036 mL, 0.45 mmol, 1.5 equiv.) were added. The reaction mixture was stirred at the same temperature for 3 h, and at room temperature overnight. Then NH$_4$Cl (saturated solution, 1 mL) was added, and the reaction solution was extracted with CH$_2$Cl$_2$ (2 × 5 mL). The combined organic layers were washed with NH$_4$Cl (saturated solution, 1 mL), dried with sodium sulfate, and then concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography ($R_f = 0.74$, 70:30% Hexanes: EtOAc) using 90:10% Hexanes:EtOAc to give a colorless oil in 50% yield (0.039 g). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.19 – 8.05 (m, 2H), 7.72 – 7.64 (m, 2H), 7.59 – 7.40 (m, 1H), 6.17 (dt, $J = 5.9$, 1.7 Hz, 1H), 6.05 – 5.99 (m, 1H), 5.60 (dt, $J = 5.6$, 1.8 Hz, 1H), 5.09 (d, $J = 1.9$ Hz, 1H), 5.04 (t, $J = 5.7$ Hz, 1H), 1.37 (s, 3H), 1.35 (s,


3.2.11 Synthesis of compound 3-12

Compound 3-11 (0.039 g, 0.15 mmol, 1 equiv.) was dissolved in a mixture of THF (0.1 mL) and H₂O (0.1 mL). The reaction solution was cooled to 0 °C, and trifluoroacetic acid (0.23 mL, 3 mmol, 20 equiv.) was added dropwise. The reaction mixture was stirred overnight at room temperature. The reaction solution was concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography (Rf = 0.41, 50:50% Hexanes: EtOAc) using 50:50% Hexanes: EtOAc to give a colorless oil in 40% yield (0.013 g). ^1H NMR (400 MHz, CDCl₃) δ 8.16 – 7.99 (m, 3H), 7.67 – 7.35 (m, 2H), 6.27 (d, J = 5.8 Hz, 1H), 6.23 – 6.09 (m, 1H), 5.69 (d, J = 7.1 Hz, 1H), 4.58 (d, J = 5.3 Hz, 1H), 4.47 (t, J = 5.5 Hz, 1H). ^13C NMR (101 MHz, CDCl₃) δ 149.2, 137.8, 133.5, 132.1, 129.9, 128.6, 76.5, 73.9, 71.1.

3.2.12 Synthesis of compound 3-13

Compound 3-13 was prepared according to the procedure of preparing 2-7. In a dry vial, compound 3-8 (0.014 g, 0.036 mmol, 1 equiv.) was azeotropically dried (3 times) using extra dry
toluene. To this vial, anhydrous CH$_3$CN (0.3 mL) was added under N$_2$, followed by 4,5-dicyanoimidazole (DCI) (0.006 g, 0.054 mmol, 1.5 equiv.). The reaction mixture was cooled to 0 ºC and dibenzyl-N,N-diisopropylphosphoramidite (0.018 mL, 0.054 mmol, 1.5 equiv.) was added. The reaction mixture was stirred at 0 ºC for 2 h and cooled to -78 ºC. t-BuOOH (0.013 mL of 5-6 M in decane, 0.072 mmol, 2 equiv.) was added slowly and the reaction was stirred at this temperature for 1 h. The reaction mixture was concentrated under a vacuum. The crude product was purified by silica gel column chromatography (70:30% Hexanes: EtOAc). (Yield= 0%).

### 3.2.13 Synthesis of compound 3-14

![Chemical Structure](image)

In a dry vial, compound 3-12 (0.007 g, 0.027 mmol, 1 equiv.) was dissolved in dry CH$_2$Cl$_2$ (0.20 mL). Activated manganese (IV) oxide (0.020 g, 0.27 mmol, 10 equiv.) was added to the reaction solution and the mixture was vigorously stirred at room temperature overnight. The mixture was filtered through a pad of Celite and washed with methylene chloride. The filtrates were concentrated in vacuo, and the crude was purified by silica gel column chromatography (50:50% Hexanes: EtOAc). (Yield= 0%).

### 3.3 Results and Discussion

Synthetic route steps were designed to prepare the cis cyclopentenone derivative that has the same chemical structure to the cyclopentenone degradation product of EM2487. Fortunately, cyclopentenone derivatives (3-1) and (3-5) which have known cis stereochemistry
were commercially available. Starting from these cyclopentenone derivatives, the cis cyclopentenone derivative target *(cis-2-10)* and *(cis-triol 3-9)* could be synthesized as shown in Scheme 3.2.

Scheme 3.2: Synthesis of cis cyclopentenone phosphate *cis*-2-10 and cis cyclopentenone triol *cis*-triol 3-9.

### 3.3.1 Baylis–Hillman reactions for synthesizing compounds 3-2 and 3-6

Baylis-Hillman reactions are the reactions between the alpha position of an activated alkene and a carbon electrophile such as aldehydes and ketones in the presence of a nucleophilic catalyst. The suitable catalyst for this reaction is a tertiary amine such as DABCO, quinuclidine, etc. For the formation of compounds 3-2 and 3-6, starting materials 3-1 and 3-5, which are both commercially available, were used as activated alkenes. The electrophile that was used was formaldehyde 37%, and the nucleophilic catalyst was imidazole. Also, for this reaction, a mixture of 1:1 THF: H₂O was used as solvent. Sanzhong Luo et al. found that the presence of a protic
solvent such as water could increase the yield and the reaction rate. The yields for the formation of compounds (3-2) and (3-6) were 50% and 60%, respectively, as shown in Scheme 3.3.

Scheme 3.3: Synthesizing 3-2 and 3-6 compounds using Baylis-Hillman reaction.

### 3.3.2 Synthesizing compound 3-15 through protection/ deprotection and phosphorylation

After preparing compounds 3-2 and 3-6, the primary alcohol should be protected. It was important to use benzoyl and tert-butyldiphenylsilyl rather than tert-butyldimethylsilyl ether (OTBS) because these protecting group (BzO and OTBDPS) are more stable to acetic acid 60% and trifluoroacetic acid than OTBS.

For compound 3-2, benzoyl chloride was used to protect the alcohol functional group in the presence of pyridine as a base and compound 3-3 was obtained in 53% yield as shown in Scheme 3.4. After protecting the primary alcohol, deprotection of dioxol in benzoyl protected cis cyclopentenone (3-3) was achieved by using acetic acid 60% to obtain the diol 3-4 in 80% yield.
Scheme 3.4: Synthesizing the benzoyl protected cis cyclopentenone phosphate 3-15.

After getting compound (3-4), the next step was the phosphorylation reaction at the hydroxyl group alpha to the ketone functional group. Compound (3-4) was reacted with dibenzyl-N,N-diisopropyl phosphoramidite, but unfortunately there none of the desired product was observed. In the NMR of the crude compound, most of the peaks appeared around 5 ppm and it was difficult to analyze the compound. It seems that there was a hydrogen bonding between the hydroxyl groups in the cis cyclopentenone, and the OH group was not nucleophilic enough.

3.3.3 Synthesizing compound 3-13 through protection/ deprotection and phosphorylation

For compound (3-6), tert-butyl(chloro)diphenylsilane (TBDPSCI) was used to protect the primary alcohol in the presence of imidazole as a base to get the compound (3-7) in 62% yield. Compound 3-7 was deprotected by using trifluoroacetic acid (TFA) to obtain (3-8) in 40% yield. A different protecting group was installed which was tert-butylidiphenylsilyl to get the compound 3-8. With this protecting group, it was thought that there would be no interference in the NMR peaks, and the NMR pattern would be different compared to the previous reaction of the
compound 3-4 when most of the peaks appeared around 5 ppm. In the case of using tert-butyldiphenylsilyl as protecting group, it was thought that the problem could be solved, and the NMR would be easier to analyze and figure out what the problem was in the reaction. When compound 3-8 was reacted with dibenzyl-\(N,N\)-diisopropyl phosphoramidite, as shown in Scheme 3.5, also most of the peaks in the NMR spectrum appeared around 5 ppm. The same issue happened for both reactions of the compounds (3-4) and (3-8) with dibenzyl-\(N,N\)-diisopropyl phosphoramidite and neither reactions gave the desired product.

![Scheme 3.5: Synthesizing the tert-butyldiphenylsilyl protected cis cyclopentenone phosphate 3-13.](image)

Even though two different protecting groups were used, the phosphorylation reactions with diols (3-4) and (3-8) did not work. It seems that the hydroxyl group was not nucleophilic enough to react with dibenzyl-\(N,N\)-diisopropyl phosphoramidite, and this might be due to the hydrogen bonding.
3.3.4 Stereospecific hydride reduction (Luche reduction)

After the failure of the previous route reactions, different route reactions were designed to get the desired product which was the cis cyclopentenone derivative. This route was chosen because there was no diol intermediate so that the nucleophilicity of the hydroxyl could be increased and the reaction with dibenzyl-N,N-diisopropyl phosphoramidite would work.

Compound (3-1) was reduced using Luche conditions\textsuperscript{151} (CeCl\textsubscript{3}·7H\textsubscript{2}O and NaBH\textsubscript{4}) to get compound (3-10) in 50\% yield as shown in Scheme 3.6. The product was not stable when it was purified by silica gel column chromatography, so the product was used directly in the next step without further purification.

![Scheme 3.6: Luche reduction to prepare compound 3-10.](image)

Luche reduction is a stereospecific hydride reduction for α,β-unsaturated ketones so it reduces the ketone in the presence of other functional groups such as alkene and aldehyde. To do the 1,2-addition on the carbonyl groups, hard nucleophiles should be used. The ketone is a hard Lewis base, so it needs a hard Lewis acid according to HSAB theory (hard and soft (Lewis) acids and bases). The hardness of the borohydride is increased by replacing hydride groups with alkoxide groups. Furthermore, cerium (III) chloride (CeCl\textsubscript{3}) activates methanol, and this Lewis acid catalyst coordinates to the ketone which increases the electrophilicity of the carbonyl group.
3.3.5 Synthesizing compound 3-12 through protection/deprotection

After the reduction reaction to get the alcohol functional group, the next reaction was to protect the hydroxyl by using benzoyl chloride in the presence of pyridine as a base. The protection was necessary here because eventually the diol intermediate would not form. The reaction product was (3-11) in 50% yield. After protection of the hydroxyl group, compound 3-11 was reacted with trifluoroacetic acid to deprotect the diol to obtain (3-12) in 40% yield as shown in Scheme 3.7.

Scheme 3.7: Preparation of compound 3-12.

3.3.6 Selective oxidation of allylic alcohol using MnO₂

Selective oxidation of allylic alcohol (3-12) to ketone was done to get the compound 3-14. There were two hydroxyl groups in the compound (3-12), therefore a selective oxidation agent should be used. Manganese (IV) oxide (MnO₂) is a very weak oxidant, and it oxidizes allylic or benzylic alcohols in the presence of other hydroxyl groups. Compound (3-12) was reacted with activated manganese (IV) oxide following the procedure of Won Jun Choi et al.¹⁵³ to get the compound (3-14) as shown in Scheme 3.8. Unfortunately, there was no product, and the reaction was repeated many times, but always the starting material (3-12) is what was obtained.
Scheme 3.8: Selective oxidation of allylic alcohol 3-12 to ketone 3-14.

Unfortunately, this route reaction also failed to prepare the target product, which was the cis cyclopentenone phosphate derivative (cis-2-10), so it started to think about an alternative way which was the chemical correlation reaction.

3.3.7 Chemical correlation reaction cis stereochemistry

Since the cis cyclopentenone phosphate could not be made by the previous routes, it was decided to make the triols. Chemical correlation reactions were already done to figure out the trans stereochemistry as described in Chapter 2 and shown in Scheme 3.9. It was proved that the HPLC separation product (2-9) which had a retention time of 1.5 min. has a trans stereochemistry, and this product (trans-2-9) matched NMR results of the cyclopentenone degradation product of EM2487 in the literature.
Scheme 3.9: Chemical correlation reactions to prepare \textit{trans-triol} 2-11 and \textit{trans-triol} 2-12.

To support these results and to be more confident, compound (3-6) which has the \textit{cis} stereochemistry was reacted with trifluoroacetic acid to obtain (\textit{cis-triol} 3-9) in 80\% yield as shown in Scheme 3.10. When the NMR spectrum was obtained for compound (\textit{cis-triol} 3-9), it had different NMR results compared to compounds (\textit{trans-triol} 2-11) and (\textit{trans-triol} 2-12) as shown in

Table 3.1.

Scheme 3.10: Preparation of compound \textit{cis-triol} 3-9.
Table 3.1: NMR results for chemical correlation reactions.

<table>
<thead>
<tr>
<th></th>
<th>(^1\text{H NMR in CD}_3\text{OD})</th>
<th>(^1\text{H NMR in CD}_3\text{OD})</th>
<th>(^1\text{H NMR in CD}_3\text{OD})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{trans-triol 2-12})</td>
<td>(\text{trans-triol 2-11})</td>
<td>(\text{cis-triol 3-9})</td>
</tr>
<tr>
<td>7.25 (1H, s)</td>
<td>7.25 (1H, q, (J = 1.8) Hz)</td>
<td>7.43 (1H, s)</td>
<td></td>
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<tr>
<td>4.55-4.53 (1H, m)</td>
<td>4.55-4.53 (1H, m)</td>
<td>4.75 (1H, s)</td>
<td></td>
</tr>
<tr>
<td>4.25-4.23 (2H, m)</td>
<td>4.25-4.23 (2H, m)</td>
<td>4.26 (2H, s)</td>
<td></td>
</tr>
<tr>
<td>4.05 (1H, (d, J = 2.7) Hz)</td>
<td>4.05 (1H, (d, J = 2.7) Hz)</td>
<td>4.11 (1H, (d, J = 5.4) Hz)</td>
<td></td>
</tr>
</tbody>
</table>

These results support that the unknown stereochemistry of the cyclopentenone derivative core in the natural product EM2487 is \(\text{trans}\). The other compound \((\text{cis-2-10})\) having a retention time of 2.8 min. has a \(\text{cis}\) stereochemistry.

### 3.4 Conclusion

Two synthetic routes were designed to prepare the target product, which was the \(\text{cis}\) cyclopentenone derivative. The first route did not work because of the phosphorylation reactions, and the second route also did not work because oxidation of allylic alcohol \((3-12)\) to ketone \((3-14)\) did not work. To support the NMR results for Chapter 2, a chemical correlation reaction was done for the \(\text{cis}\) stereochemistry. The NMR results were compared to the NMR results for the chemical correlation reactions for \(\text{trans}\) stereochemistry in Chapter 2. These results support that the HPLC separation product \((2-9)\) has a \(\text{trans}\) stereochemistry, and this product \((\text{trans-2-9})\) matched the NMR results of the cyclopentenone degradation product of EM2487 in the literature. The other compound \((\text{cis-2-10})\) has a \(\text{cis}\) stereochemistry. Finally, it was found that the unknown stereochemistry of the cyclopentenone core of the degradation product of EM2487 in the literature was \(\text{trans}\).
CHAPTER 4

SYNTHESIS OF BISPHOSPHORAMIDATE CORE

4.1 Introduction

The second core functional group that needed to be synthesized on route to EM2487 was the bisphosphoramidate. The synthesis of this compound will lead to the synthesis of the natural product EM2487 and EM2487 has the bisphosphoramidate part in the structure of phosphate imide of glycinamide. The bisphosphoramidate is rare in natural products and furthermore the P-N-P link is found in few organic compounds. Examples of compounds that have the bisphosphoramidate functional group are shown in Figure 4.1 and include EM2487 and two closely related analogues, 1100-50 and K-563. 1100-50 was isolated from Streptomyces lavendulae SANK 64297 and K-563 was isolated from actinomycete Streptomyces sp. These compounds are biologically active.

Figure 4.1: Structures of natural products 1100-50, K-563, EM2487 and bisphosphoramidate 4-18.
The synthesis described in this chapter was conducted to prepare a bisphosphoramidate similar to the motif found in EM2487. The target of this synthesis was to prepare the compound 4-12.

4.2 Experimental

4.2.1 General information

All reactions were carried out using oven-dried glassware. Some of the reactions were carried under a nitrogen atmosphere. All stirring was performed with an internal magnetic stirrer. All chemicals were purchased from commercial sources without further purification. Solvents were dried with activated 4 Å molecular sieves. Methylene chloride and THF were dried using the pure solv micro purification system. Thin-layer chromatography (TLC) was performed on 250 µm silica gel 60 F-254 plates. The products were purified by flash column chromatography using silica gel F60 (230-400 mesh Silicycle 40-60 µm, Quebec City, Quebec, Canada), and HPLC prep using a Gilson GX-271 instrument (3000 Parmenter Street Middleton, WI 53562, USA) at 210 and 254 nm with Trilution LC V3 software using Kinetex 5 µm EVO C18 100Å 21.2 × 150 mm. Analytical HPLC was also performed using Agilent Technologies instrument (Hewlett-Packard-Strasse 8 76337 Waldbronn, Germany) using Kinetex 5 µm EVO C18 100Å 4.6 × 150 mm. $^1$H, $^{13}$C, and $^{31}$P were recorded on a Varian INOVA 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), Varian Unity 400 FT-NMR (400 MHz, 101 MHz, 162 MHz), and Bruker AVANCE NEO 500 (500 MHz, 125 MHz, 202 MHz). $^{31}$P NMR spectra were externally referenced to 85% H$_3$PO$_4$ at (0 ppm). Chemical shifts were reported in ppm and multiplicities were reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentet), h (hextet), hep (heptet), m (multiplet), and br
(broad). Mass spectrometry analysis was performed by the School of Chemical Sciences Mass Spectrometry Laboratory at the University of Illinois.

### 4.2.2 Synthesis of N-(2-Ethoxyethyl)-P,P',P'-tetrabenzylimidodiphosphate (4-4)

![Chemical Structure](image)

This compound was prepared according to the literature.\(^{161}\) In a dry vial, POCl\(_3\) (0.19 mL, 2.1 mmol, 2.1 equiv.) and Et\(_3\)N (0.31 mL, 2.2 mmol, 2.2 equiv.) were added to dry THF (3.2 mL), and this solution was cooled to -78 °C. In another dry vial, 2-ethoxyethylamine (0.089 g, 1 mmol, 1 equiv.) was dissolved in dry THF (3.2 mL) and this solution was added dropwise over 4 h to the previous reaction solution at -78 °C with stirring. The reaction mixture was stirred at room temperature for 30 min. The reaction solution was filtered through Celite, and the filtrate was cooled to -78 °C. In another dry vial, sodium benzylate was prepared from benzyl alcohol (0.42 mL, 4.1 mmol, 4.1 equiv.) and sodium hydride (60% in oil) which was washed 3 times with hexanes (0.164 g, 4.1 mmol, 4.1 equiv.) in dry THF (17.5 mL). The sodium benzylate solution was added to the filtrate at -78 °C. The reaction mixture was stirred for 1 h at -78 °C and then 1 h at room temperature. Aqueous NH\(_4\)Cl was added, and the solution was extracted with ethyl acetate (3× 50 mL), and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography (\(R_f = 0.34, 40:60\%\) Hexanes: EtOAc) using a gradient eluent of 70:30-40:60\% (Hexanes: EtOAc) to give a yellow oil in 10% yield (0.061 g). The NMR data matched the previously published results.\(^{161}\)
\( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.39 – 7.27 (m, 20H), 5.12 – 5.00 (m, 8H), 3.66 – 3.51 (m, 4H), 3.42 (q, \( J = 8.2 \) Hz, 2H), 1.11 (t, \( J = 7.0 \) Hz, 3H). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 136.0 (t, \( J = 3.9 \) Hz), 128.6, 128.4, 128.0, 69.2, 69.1, 66.4, 46.7, 15.2. \( ^{31}P \) NMR (202 MHz, CDCl\(_3\)) \( \delta \) 4.0.

### 4.2.3 Synthesis of 2-((tert-Butyldiphenylsilyl)oxy)ethan-1-amine (4-6)

![Chemical Reaction](https://via.placeholder.com/150)

This compound was prepared according to the literature.\(^{162}\) In a dry vial, ethanolamine (0.12 mL, 2 mmol, 1 equiv.) and imidazole (0.299 g, 4.4 mmol, 2.2 equiv.) were dissolved in anhydrous CH\(_3\)CN (10 mL) and the reaction solution was cooled to 0 \(^{\circ}\)C. tert-butyldiphenylsilyl chloride (TBDPSCl) (0.57 mL, 2.2 mmol, 1.1 equiv.) was added to the reaction solution at 0 \(^{\circ}\)C dropwise. The reaction mixture was stirred for 3.5 h at 0 \(^{\circ}\)C and then quenched with a saturated aqueous sodium bicarbonate solution. The reaction mixture was diluted with water and extracted with CH\(_2\)Cl\(_2\) (3× 25 mL) and the combined organic layers were washed with brine, dried with sodium sulfate, and concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 20 min., Flow rate = 20 mL/min., mobile phase = 1% to 100% gradient of acetonitrile (water-acetonitrile) to give a colorless oil in 90% yield (0.531 g). The NMR data matched the previously published results.\(^{162}\)\(^{1}H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.67 (dd, \( J = 7.8, 1.6 \) Hz, 4H), 7.44 – 7.35 (m, 6H), 3.88 (t, \( J = 5.2 \) Hz, 2H), 3.11 (t, \( J = 5.2 \) Hz, 2H), 2.17 (s, 2H, NH\(_2\)), 1.07 (s, 9H). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 135.7, 132.6, 130.1, 128.0, 60.2, 41.8, 27.0, 19.3.
4.2.4 Synthesis of \( N-(2-((\text{tert-Butyldiphenylsilyl})\text{oxy})\text{ethyl})-P,P',P'-\text{tetrabenzylimidodiphosphate} \) (4-7)

Compound 4-7 was attempted by adapting the procedure from the literature.\textsuperscript{161} In a dry vial, POCl\(_3\) (0.09 mL, 0.95 mmol, 2.1 equiv.) and Et\(_3\)N (0.14 mL, 0.99 mmol, 2.2 equiv.) were added to dry THF (1.5 mL), and this solution was cooled to \(-78^\circ\text{C}\). In another dry vial, compound (4-6) (0.135 g, 0.45 mmol, 1 equiv.) was dissolved in dry THF (1.5 mL) and this solution was added dropwise over 4 h to the previous reaction solution at \(-78^\circ\text{C}\) with stirring. The reaction mixture was stirred at room temperature for 30 min. Then, the reaction solution was filtered through Celite, and the filtrate was cooled to \(-78^\circ\text{C}\). In another dry vial, sodium benzylate was prepared from benzyl alcohol (0.19 mL, 1.84 mmol, 4.1 equiv.) and sodium hydride (60\% in oil) which was washed 3 times with hexanes (0.07 g, 1.84 mmol, 4.1 equiv.) in dry THF (8 mL). The sodium benzylate solution was added to the filtrate at \(-78^\circ\text{C}\). The reaction mixture was stirred for 1 h at \(-78^\circ\text{C}\) and then 1 h at room temperature. Aqueous NH\(_4\)Cl was added, and the solution was extracted with ethyl acetate (3\times 25 mL), and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by silica gel column chromatography using a gradient eluent of 70:30 to 40:60\% (Hexanes: EtOAc). (Yield = 0\%).
4.2.5 Synthesis of compound 4-14

\[
\text{TBDPSO-}\text{NH}_2 + \text{POCl}_3 \xrightarrow{\text{Et}_3\text{N, CH}_2\text{Cl}_2, 0 \degree\text{C to r.t., 3.5 h}} \text{TBDPSO-}\text{PO(O)NH-PO(O)O-TBDPS}
\]

In a dry vial, compound (4-6) (0.195 g, 0.65 mmol, 1 equiv.) and Et₃N (0.10 mL, 0.78 mmol, 1.2 equiv.) were dissolved in dry CH₂Cl₂ (5 mL) and the reaction solution was stirred and cooled to 0 °C. Diethyl chlorophosphatate (0.097 mL, 0.65 mmol, 1 equiv.) was added dropwise to the reaction solution at 0 °C. Then the reaction mixture was stirred for 3.5 h at room temperature. The reaction mixture was extracted with a saturated aqueous solution of NaHCO₃ and CH₂Cl₂ (3 × 25 mL), and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/min., mobile phase (isocratic) = (40%:60%) (water-acetonitrile) to get a colorless oil in 41% yield (0.115 g).

\(^1\text{H NMR}\) (500 MHz, CDCl₃) \(\delta\) 7.65 (dd, \(J = 8.0, 1.5\) Hz, 4H), 7.45 – 7.35 (m, 6H), 4.10 – 3.97 (m, 4H), 3.69 (t, \(J = 5.1\) Hz, 2H), 3.04 (t, \(J = 10.1, 5.3\) Hz, 2H), 1.29 (t, \(J = 7.1\) Hz, 6H), 1.05 (s, 9H).

\(^{13}\text{C NMR}\) (126 MHz, CDCl₃) \(\delta\) 135.6, 133.4, 129.9, 127.9, 64.3 (d, \(J = 6.4\) Hz), 62.4 (d, \(J = 5.3\) Hz), 43.6, 27.0, 19.3, 16.3 (d, \(J = 7.0\) Hz).

\(^{31}\text{P NMR}\) (202 MHz, CDCl₃) \(\delta\) 9.1.

4.2.6 Synthesis of compound 4-15

\[
\text{TBDPSO-}\text{PO(O)NH-PO(O)-} + \text{POCl}_3 \xrightarrow{\text{LDA, THF, -78 °C to r.t., Overnight}} \text{TBDPSO-}\text{PO(O)NPO(O)-PO(O)-PO(O)-TBDPS}
\]
In a dry vial, diisopropylamine (0.030 mL, 0.214 mmol, 1.2 equiv.) was dissolved in dry THF (0.5 mL) and the reaction solution was stirred and cooled to -78 ºC. A solution of n-butyllithium (0.133 mL, 0.214 mmol, 1.2 equiv., 1.6 M in hexanes) was added at -78 ºC and the reaction solution was stirred for 30 min. Then, compound (4-14) (0.078 g, 0.179 mmol, 1 equiv.) dissolved in dry THF (0.2 mL) was added to the reaction solution at -78 ºC and the reaction solution was stirred for 15 min. To the reaction solution, diethyl chlorophosphate (0.031 mL, 0.214 mmol, 1.2 equiv.) dissolved in dry THF (0.2 mL) was added at -78 ºC. The reaction mixture was stirred at this temperature for 5 h and then the reaction was allowed to warm slowly to room temperature and was stirred overnight. The reaction mixture was concentrated under reduced pressure and extracted with water and CH₂Cl₂ (3× 25 mL). The combined organic layers were washed with 0.5 M HCl, then with a saturated aqueous solution of NaHCO₃. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 20 min., Flow rate = 20 mL/ min., mobile phase (isocratic) = (40%:60%) (water-acetonitrile) to get a colorless oil in 25% yield (0.025 g).

**¹H NMR (500 MHz, CDCl₃)** δ 7.66 (ddd, J = 15.8, 7.9, 1.5 Hz, 4H), 7.45 – 7.35 (m, 6H), 4.14 – 3.98 (m, 8H), 3.82 (t, J = 7.5 Hz, 2H), 3.54 (tt, J = 14.2, 7.5 Hz, 2H), 1.26 (t, J = 7.1 Hz, 12H), 1.04 (s, 9H).

**¹³C NMR (126 MHz, CDCl₃)** δ 135.7, 133.7, 129.7, 127.8, 63.5 (t, J = 2.8 Hz), 62.8, 48.4, 26.9, 19.3, 16.2 (t, J = 3.6 Hz).

**³¹P NMR (202 MHz, CDCl₃)** δ 3.7.
4.2.7 Synthesis of compound 4-16

In a dry vial, compound (4-15) (0.025 g, 0.044 mmol, 1 equiv.) was dissolved in dry CH$_2$Cl$_2$ (0.5 mL) and the reaction solution was stirred and cooled to -40 °C. Iodotrimethylsilane (0.0065 mL, 0.044 mmol, 1 equiv.) was added at -40 °C and the reaction solution was stirred at this temperature for 1 h and for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was redissolved in THF (2 mL) and H$_2$O (0.5 mL). The resulting solution was stirred for 1.5 h at room temperature, and then concentrated under reduced pressure. The crude reaction was purified by HPLC prep chromatography using a C18 column (wavelength = 210 nm, run time = 10 min., Flow rate = 20 mL/ min., mobile phase (isocratic) = (40:60) % (water-acetonitrile). (Yield= 0%).

4.3 Results and discussion

The synthetic route was designed to prepare the bisphosphoramidate functional group which is found in the EM2487 and some natural products.

4.3.1 Synthesis of compound 4-4 using POCl$_3$

The bisphosphoramidate functional group is rare in natural products. Searching in the literature, it was found a few examples of synthesizing the P-N-P link in organic compounds. Vincent et al.\textsuperscript{161} made the tetrabenzyalted 4-4 using two equivalents of POCl$_3$ in one-pot method. However, this example was the only bisphosphoramidate compound that was prepared by this
method. The proposed mechanism for making the bisphosphoramidate 4-4 is shown in Figure 4.2. The amine 4-3 was reacted with two equivalents of POCl₃ to make an intermediate product, and then the sodium benzylate (4 equivalents) which was prepared in situ was added to the intermediate to make the bisphosphoramidate 4-4.

![Chemical structures](image)

Figure 4.2: The proposed mechanism for making the bisphosphoramidate 4-4.

The synthesis of bisphosphoramidate 4-4 was repeated and a 10% yield was obtained after purification. The reported yield was 26%. Although the yield is low, it makes so many bonds that it is still synthetically viable. Based on the similarity of 4-4 to the target bisphosphoramidate a route was devised that would be useful for the synthesis of EM2487 using other primary amines including glycineamide, glycine methyl ester, 2-aminoacetonitrile, and 2-((tert-butyldiphenylsilyl)oxy) ethan-1-amine. Unfortunately, all these starting materials did not work towards the synthesis of the target bisphosphoramidate as shown in Scheme 4.1.
Scheme 4.1: Synthesis of bisphosphoramidate 4-4, 4-7, 4-9, 4-11, and 4-13.

It seems that there was interference between the starting materials (glycinamide, glycine methyl ester, and 2-aminoacetonitrile) and the reagents that were used in the reaction, especially POCl₃. Another reason is that these starting materials were not nucleophilic enough to react with POCl₃ and make the bisphosphoramidate. The amine could react with the first equivalent of POCl₃ to make phosphoramidate but it was difficult for the lone pair on the nitrogen to react with another equivalent of POCl₃ and made the bisphosphoramidate. This can be rationalized by the fact that the lone pair now is in tautomerism with the (P=O) functional group. NH-P=O is like an amide functional group so the lone pair on nitrogen is not nucleophilic enough. Also, other challenges were faced with each example of these starting materials. There was a problem with the solubility of glycinamide and 2-aminoacetonitrile in the solvent (THF). Another issue was with the polymerization of the glycine methyl ester when the free base of glycine methyl ester was tried to be prepared.
Surprisingly, even though the starting material (4-6) was protected with (OTBDPS), there was also no product. It was thought that using (OTBDPS) as a protecting group would give more stability to the starting material and would be more reactive to making the bisphosphoramidate. There is not a lot in the literature about the P-N-P, so it was difficult to understand the behavior of the P-N-P link in organic chemistry.

Saady et al. have studied this type of molecule that has a P-N-P link. They did many different reactions to prepare the P-N-P link and they found that phosphoramides have a poor reactivity and relative fragility of the phosphorus benzyl esters. They proposed some mechanisms for what happened to their reactions as shown in Figure 4.3. When they tried to increase the temperature to improve the reactivity, decomposition of their compounds happened. For this reason, a different route was designed to prepare the target bisphosphoramidate.

Figure 4.3: Proposed mechanisms of phosphoramides reactions.
4.3.2 Preparation of phosphoramidate 4-14

Since the previous route did not work to give the target bisphosphoramidate compound, a different route was designed as shown in Scheme 4.2. In this route, the phosphoramidate was first prepared, and then in a separate reaction, the bisphosphoramidate functional group (P-N-P link) was made.

Scheme 4.2: Synthesis of bisphosphoramidate core 4-18.

A phosphoramidate 4-14 was first prepared. Ethanolamine 4-5 was reacted with tert-butylidiphenylsilyl chloride (TBDPSCI) in the presence of imidazole as a base to get the (4-6) in 90% yield. Then, compound 4-6 was reacted with diethyl chlorophosphate in the presence of Et$_3$N as a base to get the phosphoramidate (4-14) in 41% yield as shown in Scheme 4.3.
Diethyl chlorophosphate was used instead of dibenzyl chlorophosphate because it was thought that diethyl chlorophosphate was more stable towards the synthesis of the target bisphosphoramidate compound as the phosphorus benzyl esters have relative fragility.

**4.3.3 Synthesis of bisphosphoramidate 4-16**

Phosphoramidate 4-14 was reacted with another mole of diethyl chlorophosphate to get the bisphosphoramidate 4-15 in 25% yield. Generally, phosphoramidate cannot react directly with diethyl chlorophosphate because the lone pair on the nitrogen is not sufficiently nucleophilic. For this reason, lithium diisopropylamide (LDA) was used as a base to produce the more nucleophilic anionic nitrogen. To prepare LDA, diisopropylamine was reacted with n-butyllithium. This reaction is sensitive to moisture and temperature because n-Butyllithium is a strong base and a strong nucleophile at the same time, so it was important to do the reaction at -78 °C and the vials that were used should be dried very well. LDA is a suitable base in this reaction because it is a non-nucleophilic and strong base.

Deprotection of the ethyl group of the phosphoric ester 4-15 was performed using Iodotrimethylsilane, but unfortunately, the desired product was not obtained as shown in Scheme 4.4. Iodotrimethylsilane was used because it has selective deprotection for the ethyl
group. Even though the reaction was done at -40 °C, there was no product. It seems that the bisphosphoramidate got decomposed when it was reacted with Iodotrimethylsilane and it was thought that the P-N-P bonds were broken.

Scheme 4.4: Synthesis of bisphosphoramidate 4-16.

4.4 Conclusion

The synthetic route was designed to prepare the target bisphosphoramidate compound which is a very important core towards the synthesis of the natural product EM2487. Bisphosphoramidates are rare in natural products. Compound 4-4 was successfully prepared so this inspired us to prepare the compound 4-7 in a one-pot reaction. The reaction was unsuccessful to prepare compound 4-7. Bisphosphoramidate 4-15 was synthesized in 25% yield but the target bisphosphoramidate could not be prepared.
EM2487 is a natural product produced by *Streptomyces* sp. Mer-2487 that possesses promising activity against HIV. Specifically, EM2487 is a potent and selective inhibitor of HIV replication in both acutely and chronically infected cells. The synthesis of the two important cores of EM2487 which are the cyclopentenone derivative and bisphosphoramidate functional group were achieved. Synthetic route was designed toward the synthesis of the cyclopentenone derivative as shown in Scheme 5.1. Furfuryl alcohol was transformed into butoxy pyranone \((2-2)\) in two steps. The butoxy pyranone was reacted with 1,4-diazabicyclo[2.2.2]octane to get the compound \(2-3\) in 75% yield. The compound \(2-3\) was reacted with bromine then the product of this reaction was reacted with \(\text{Bu}_3\text{SnCH}_2\text{OTBS}\) to get the compound \(2-6\) in 82% yield. The next step was challenging which was the phosphorylation reaction at the hydroxyl group. Many phosphorylation reagents were tried but there was no product. Dibenzy1-\(N,N\)-diisopropyl phosphoramidite was the only reagent that worked in this reaction to get the compound \(2-7\) in 30% yield. Finally, global deprotection was performed using trifluoroacetic acid and then using \(\text{BCl}_3\) to get both isomers (\textit{trans-2-9} and \textit{cis-2-10}).
Scheme 5.1: Total synthesis of the cyclopentenone derivative.

After comparing the NMR results for both isomers (trans-2-9 and cis-2-10) with the NMR results of the cyclopentenone degradation product EM2487 in the literature, it was found that the undefined stereochemistry of the cyclopentenone degradation product EM2487 in the literature was trans as shown in Figure 5.1. The final alcohol in the pseudosaccharide core is still unknown and there are still four possible isomers.

Figure 5.1: Determination of the relative stereochemistry of the cyclopentenone derivative core.
After synthesis the cyclopentenone derivative, it was found that isomerization occurred to obtain both isomers (trans-2-9 and cis-2-10). This is an important information towards the synthesis of the natural product EM2487. Takeuchi et al.\textsuperscript{103} reported the hydrolysis of EM2487 with 1.0 N HCl to get three compounds 1-14, 1-15 and 1-16 as shown in Figure 5.2 and one of these compounds was the cyclopentenone derivative 1-15. It should be noticed that the isomerization also could happen when they did the hydrolysis of EM2487.

![Figure 5.2: Compounds resulting in hydrolysis of EM2487.](image)

For the future work, the synthesis of the two important cores (cyclopentenone derivative and bisphosphoramidate functional group) will lead us towards the synthesis of the natural product EM2487. The next step is to determine the third undefined stereochemistry of the pseudosaccharide derivative in EM2487. Sharpless epoxidation and reductive ring opening reactions should be used to stereoselective install the third hydroxyl as shown in Scheme 5.2. After obtaining compounds 5-3 and 5-4, NMR analysis will be done to determine the third alcohol stereochemistry of the pseudosaccharide derivative in EM2487. J-coupling constants, NOESY and COSY will be done, and compare the results with natural products EM2487 to tentatively assign the stereochemical relationship of the two alcohols.
The bisphosphoramidate is rare in natural products and furthermore the P-N-P link is found in few organic compounds. For the bisphosphoramidate functional group, deprotection of one of the ethyl groups will be achieved to get the desired product as shown in Scheme 5.3. We tried to deprotect the ethyl group with iodotrimethylsilane but we could not get the desired product so we will try to use different reagents to do the deprotection.

Scheme 5.3: Synthesis of compound 4-16.

After synthesis of these compounds 5-3, 5-4 and 4-16, other reactions will be done towards the synthesis the EM2487. Once the final stereochemistry of the natural product has been established, retrosynthesis analysis will be designed to prepare the natural product EM2487 as shown in Scheme 5.4.
Scheme 5.4: Retrosynthesis analysis of the natural product EM2487.

To prepare the EM2487, we will have 4 compounds (protected pseudosaccharide derivative, bisphosphoramidate, aldehyde component, and the protected uridine). We will do the Ullmann coupling between the protected pseudosaccharide derivative and the protected uridine. Then, we will do the phosphorylation reaction between the product of the Ullmann coupling and the bisphosphoramidate compound. The final product will be reacted with the alkyl hydroxyl amine compound which will be prepared from two fragments (the aldehyde and the hydroxylamine). After obtaining the final product, structural analysis will be conducted using NMR spectroscopy and mass spectrometry and the results compared with the isolation data to confirm the product. Finally, the purported biological activity of the natural compound will be evaluated as well as all synthetic intermediates.
REFERENCES


APPENDICES

APPENDIX A: NMR SPECTRA FOR CHAPTER 2

Figure A.1 $^1$H NMR spectrum of compound 2-1

Figure A.2 $^{13}$C NMR spectrum of compound 2-1
Figure A.3 $^1$H NMR spectrum of compound 2-2

Figure A.4 $^{13}$C NMR spectrum of compound 2-2
Figure A.5 $^1$H NMR spectrum of compound 2-3

Figure A.6 $^{13}$C NMR spectrum of compound 2-3
Figure A.7 $^1$H NMR spectrum of compound 2-4

Figure A.8 $^{13}$C NMR spectrum of compound 2-4
Figure A.9 $^1$H NMR spectrum of compound 2-5

Figure A.10 $^{13}$C NMR spectrum of compound 2-5
Figure A.11 $^1$H NMR spectrum of compound 2-6

Figure A.12 $^{13}$C NMR spectrum of compound 2-6
Figure A.13 $^1$H NMR spectrum of compound 2-7

Figure A.14 $^{13}$C NMR spectrum of compound 2-7
Figure A.15 $^{31}$P NMR spectrum of compound 2-7

Figure A.16 $^1$H NMR spectrum of compound 2-8
Figure A.17 $^{13}$C NMR spectrum of compound 2-8

Figure A.18 $^{31}$P NMR spectrum of compound 2-8
Figure A.19 $^1$H NMR spectrum of compound trans-2-9

Figure A.20 $^{13}$C NMR spectrum of compound trans-2-9
Figure A.21 $^{31}$P NMR spectrum of compound *trans*-2-9

Figure A.22 $^1$H NMR spectrum of compound *cis*-2-10
Figure A.23 $^{13}$C NMR spectrum of compound \textit{cis-2-10}

Figure A.24 $^{31}$P NMR spectrum of compound \textit{cis-2-10}
Figure A.25 $^1$H NMR spectrum of compound *trans-triol 2-11*

Figure A.26 $^{13}$C NMR spectrum of compound *trans-triol 2-11*
Figure A.27 $^1$H NMR spectrum of compound *trans-triol 2-12*

Figure A.28 $^{13}$C NMR spectrum of compound *trans-triol 2-12*
APPENDIX B: NMR SPECTRA FOR CHAPTER 3

Figure B.1 $^1$H NMR spectrum of compound 3-2

Figure B.2 $^{13}$C NMR spectrum of compound 3-2
Figure B.3 $^1$H NMR spectrum of compound 3-3

Figure B.4 $^{13}$C NMR spectrum of compound 3-3
Figure B.5 $^1\text{H}$ NMR spectrum of compound 3-4

Figure B.6 $^{13}\text{C}$ NMR spectrum of compound 3-4
Figure B.7 $^1$H NMR spectrum of compound 3-6

Figure B.8 $^{13}$C NMR spectrum of compound 3-6
Figure B.9 $^1$H NMR spectrum of compound 3-7

Figure B.10 $^{13}$C NMR spectrum of compound 3-7
Figure B.11 $^1$H NMR spectrum of compound 3-8

Figure B.12 $^{13}$C NMR spectrum of compound 3-8
Figure B.13 $^1$H NMR spectrum of compound cis-triol 3-9

Figure B.14 $^{13}$C NMR spectrum of compound cis-triol 3-9
Figure B.15 $^1$H NMR spectrum of compound 3-11

Figure B.16 $^{13}$C NMR spectrum of compound 3-11
Figure B.17 $^1$H NMR spectrum of compound 3-12

Figure B.18 $^{13}$C NMR spectrum of compound 3-12
APPENDIX C: NMR SPECTRA FOR CHAPTER 4

Figure C.1 $^1$H NMR spectrum of compound 4-4

Figure C.2 $^{13}$C NMR spectrum of compound 4-4
Figure C.3 $^{31}$P NMR spectrum of compound 4-4

Figure C.4 $^1$H NMR spectrum of compound 4-6
Figure C.5 $^{13}$C NMR spectrum of compound 4-6

Figure C.6 $^1$H NMR spectrum of compound 4-14
Figure C.7 $^{13}$C NMR spectrum of compound 4-14

Figure C.8 $^{31}$P NMR spectrum of compound 4-14
Figure C.9 $^1$H NMR spectrum of compound 4-15

Figure C.10 $^{13}$C NMR spectrum of compound 4-15
Figure C.11 $^{31}$P NMR spectrum of compound 4-15
BIOGRAPHY OF THE AUTHOR

Saad Zuhair Mohammed Ali was born in Mosul, Iraq on July 31, 1982. He attended the University of Mosul and graduated in 2004 with B.Sc. in Chemistry. He attended the University of Mosul and graduated in 2011 with M.Sc. in Organic Chemistry. In 2006, He got a job at the state company for drug industries and medical appliances in Mosul, Ninawa, Iraq, and worked in the quality control laboratory department. He came to the United States in 2015 to study the English as a second language (ESL) program at the University of Kentucky. He attended the University of Maine in January 2017 to pursue a doctoral degree in organic chemistry. Saad has worked as a Teaching Assistant and a Research Assistant at the University of Maine. Saad is a Candidate for the Doctor of Philosophy degree in Chemistry from the University of Maine in August 2022.