ALLENE-BASED APPROACH TO THE SYNTHESIS OF DE NOVO ERYTHROMYCINOIDS

by

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ABSTRACT OF THE DISSERTATION

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By HIYUN KIM

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Dr. Lawrence J. Williams Ph.D
We prepared an advanced synthetic module (bis[allene] macrolactone at center) equipped with two allenes embedded in a macrolactone scaffold. The plan was to effect heterogeneous derivatization of the allenes, in tandem or separately. In addition to diversity, this approach is maximally concise and economic, especially in terms of steps. Moreover, immediate derivatives of the macrocyclic bis[allene] can be taken into further steps, thus providing canonical build-up of erythromycin analogs.

To date, our bis[allene] macrolactone has been converted to over 30 novel macrolides. These *de novo* analogs serve to validate the strategy and lay the ground work for further work. Taken together, the allene-based reactions/transformations employed in this study, such as DMDO oxidation/nucleophile addition, allene osmylation/electrophile addition, bromination, allene oxide rearrangement, spirodiepoxide rearrangement, benzylic migration/elimination, mono- and bis-oxidation of bis[allene], chelation-controlled reduction and oxime formation, demonstrate that 8 of the 11 modifiable carbons in this antibiotic can be modified. It is especially noteworthy that each congener was made in less than three steps from the bis[allene] macrolactone.
Acknowledgements

“The mind of man plans his way, But the LORD directs his steps (Proverbs 16:9)"

I would like to note my sincere appreciation to my advisor Prof. Lawrence J. Williams. He has been an excellent teacher, researcher, and writer who possesses “naturally-born” scientific insight and care for the students at the same time. He gave me the second opportunity to pursue a Ph.D. degree at Rutgers. From the first day in his group, everything was new. However, I quickly realized that the novelty which I observed was the extension of creativity, enthusiasm, scientific contribution, and pride in his research. I worked on several very challenging projects, including quite a few different interdisciplinary projects, which were scientifically and experimentally challenging, but fully rewarding at the end. In his group, I have had my eyes opened to science.

The structural assignment of complex compounds reported in this thesis would have been nearly impossible without Dr. Novruz G. Akhmedov. I would like to express my sincere gratitude to Dr. Akhmedov for his continuous scientific and technical guidance on issues of complex NMR analysis.

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Lastly, I would like to thank my husband Ozgur Celik, our parents Jin-Seop Kim, Ryoung-Ja Kim, Yasar Celik, and Naciye Celik, and our families. They have always supported and encouraged me in my studies.
Dedication

To My Lord, Jesus Christ, and Orphans in North Korea
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
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<tr>
<td>Å</td>
<td>Angstroms</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>Ag₂O</td>
<td>silver (I) oxide</td>
</tr>
<tr>
<td>Akt</td>
<td>protein kinase B</td>
</tr>
<tr>
<td>AZM</td>
<td>azithromycin</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Bu₂BOTf</td>
<td>dibutylboron trifluoromethanesulfonate</td>
</tr>
<tr>
<td>CAM</td>
<td>clarithromycin</td>
</tr>
<tr>
<td>ClTi(O⁻Pr)₃</td>
<td>chlorotriisopropoxytitanium (IV)</td>
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<td>DABCO</td>
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<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-1,4-benzoquinone</td>
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<td>DIAD</td>
<td>diisopropyl azodicarboxylate</td>
</tr>
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<td>DIC</td>
<td>N,N'-dicycloisopropylcarbodiimide</td>
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<tr>
<td>DMAP</td>
<td>4-(N,N-dimethylamino)pyridine</td>
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<tr>
<td>DMDO</td>
<td>dimethylidioxirane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<td>DTT</td>
<td>dithiothreitol</td>
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xviii
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>eGFP</td>
<td>enhanced green fluorescence protein</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>epidermal growth factor receptor variant III</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalents</td>
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<tr>
<td>Et</td>
<td>ethyl</td>
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<tr>
<td>Et$_2$O</td>
<td>diethyl ether</td>
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<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
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<tr>
<td>FCC</td>
<td>flash column chromatography</td>
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<td>GBM</td>
<td>glioblastoma multifome</td>
</tr>
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<td>HFL</td>
<td>heterofunctional linkers</td>
</tr>
<tr>
<td>HIV</td>
<td>human imminodeficiency virus</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple-bond correlation spectroscopy</td>
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<tr>
<td>i-Bu</td>
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<td>i-Pr$_2$NEt</td>
<td>diisopropylethylamine</td>
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<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>LiCCH-EDA</td>
<td>lithium acetylide-ethylenediamine</td>
</tr>
<tr>
<td>m-</td>
<td>meta-</td>
</tr>
<tr>
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<td>molecular sieves</td>
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<tr>
<td>Me</td>
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<td>MLS$_B$</td>
<td>macrolide-lincosamide-streptogramin B</td>
</tr>
<tr>
<td>MsCl</td>
<td>methanesulfonyl chloride</td>
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</table>
mTOR  mammalian target of Rapamycin
NBS  $N$-bromosuccinimide
NCS  $N$-chlorosuccinimide
NMO  $N$-methylmorpholine $N$-oxide
nOe  nuclear overhauser effect
$\sigma$-  ortho-
OsO$_4$  osmium (VIII) tetroxide
$p$-  para-
PBS  phosphate buffered saline
PEG  polyethylene glycol
Ph  phenyl
PhH  benzene
PhMe  toluene
PI13K  phosphatidylinositol 3-kinase
PKS  polyketide synthase
PTEN  protein tyrosine phosphatase
pyr  pyridine
QD  quantum dot
RGD  arginine-glycine-aspartate peptide
RISC  RNA-induced silencing complex
rt  room temperature
RuO$_4$  ruthenium (VIII) tetroxide
SAR  structure activity relationship
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>SDE</td>
<td>spirodiepoxide</td>
</tr>
<tr>
<td>siRNA</td>
<td>small interfering RNA</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-butyldiphenylsilyl</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>triethyl amine</td>
</tr>
<tr>
<td>TESCl</td>
<td>triethylsilyl chloride</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TOPO</td>
<td>tri-octylphosphine oxide</td>
</tr>
<tr>
<td>wt%</td>
<td>weight percent</td>
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Chapter 1

Erythromycin and Its Derivatives

1.1. Introduction

Erythromycin 1.1, an archetypal 14-membered macrolide, originally exhibited unique and superior biological properties such as its activity, safety, and mode of action in inhibiting bacterial protein synthesis. Recently, Rubin et al. reported other biological functions such as the selective uptake by macrophages, extracellular kinase activity, and anti-asthmatic functions.

Figure 1.1-1: Erythromycin

![Erythromycin structure](image)

Even though its profuse utility for clinical use in human medicine since 1950, it showed several disadvantages, such as weak activity against gram-negative bacteria, induction of macrolide resistance, a bitter taste, and a tendency to produce gastrointestinal cramp as a consequence of the instability in the acidic medium of the stomach. This acid-instability caused a low and inconsistent bioavailability. These were
the initial major problems which directed the tremendous synthetic efforts to modify erythromycin.\textsuperscript{1,3,4}

\textbf{1.2. \textit{Erythromycin Derivatives}}

It had been recognized that the degradation of erythromycin proceeds via interaction of the 6- and 12-hydroxy, 9-carbonyl and 8-hydrogen groups through intramolecular ketalization (Scheme 1.2-1).\textsuperscript{5} The resulting compound mimics the activity of motilin which causes stomach cramps.\textsuperscript{4} Consequently, significant research was devoted to the modification of these specific positions. The 2\textsuperscript{nd} generation macrolides, clarithromycin \textsuperscript{1.3}, azithromycin \textsuperscript{1.4}, roxithromycin \textsuperscript{1.5}, flurithromycin \textsuperscript{1.6} and dirithromycin \textsuperscript{1.7} were developed in the 1980s, and eventually commercialized in the 1990s (Figure 1.2-1).

\textbf{Scheme 1.2-1: Acid Induced Transformation of Erythromycin}
Figure 1.2-1: Commercialized Erythromycin Derivatives

All these drugs, however, present several drawbacks. They are inactive against macrolide, lincosamide, type B streptogramin (MLS\textsubscript{B})-resistant streptococci and \textit{Streptococcus pneumoniae} and, with exception of azithromicin, weakly active against \textit{Haemophilus influenzae}.\textsuperscript{4} Furthermore, the resistance of \textit{S. pneumonia} to erythromycin has increased significantly in recent years.\textsuperscript{6}
A 2005 report indicated that ~30% of the *S. pneumoniae* strains in the United States were resistant to macrolides (erythromycin, azithromycin and clarithromycin). Although resistance among *S. pneumoniae* to single agents is problematic, more troublesome is the emergence and dissemination of multidrug-resistant (MDR) strains: MDR phenotypes cross several drugs, including macrolides, β-lactams, sulphonamides and tetracyclines. This emerging drug resistance clearly suggested an exigency for the 3rd generation macrolide and this quest resulted in the development of ketolides, which exhibit increased affinity to the ribosome as well as apparent lower sensitivity to known mechanisms of resistance. These 14-membered ring drugs are characterized by the keto group at the C3 position of the lactone ring, rather than the cladinose sugar found in the macrolides. Among many ketolides, two compounds were commercialized, telithromycin 1.8 and cethromycin 1.9. Both feature an 11,12-carbamate group (Figure 1.2-1). In addition, telithromycin has an extended alkyl-aryl extending from the 11,12-carbamate group, whereas cethromycin has a quinolylallyl side chain at the C6 position of the lactone ring.

1.3. **Binding Site and Modes of Action**

The details of the molecular interactions of the various classes of macrolides with the ribosome were determined from X-ray crystallographic structures of the archaeal (*H. marismortui*) and bacterial (*D. radiocurans*) large ribosomal subunits in the laboratories of Tom Steitz 7, 8 and Ada Yonath 9, 10 respectively. The X-ray crystallographic structures revealed that the macrolide binding site is located on the large ribosomal subunit inside the nascent peptide exit tunnel near the peptidyl transferase center. Basically, this
binding blocks the exit tunnel of the ribosome to prevent the departure of the nascent polypeptide, thereby halting protein synthesis. A number of nucleotide residues in domain V of 23S rRNA are involved in bonding with the macrolide molecule. Important contacts are found between the C5 mono- or disaccharide side chains of 14-, 15-, and 16-membered ring macrolides and rRNA. The desosamine sugar of erythromycin and other related 14-membered ring macrolides forms hydrogen bonding with the nitrogen bases of the nucleotide residues A2058, A2059 (E. coli numbering). The nucleotide, occupying position 2057, may also be involved in hydrogen bonding with the C5 desosamine of macrolide, or it may be establish hydrophobic interactions with the lactone ring. In addition, the desosamine sugar can potentially interact with the backbone phosphate oxygen of G2505. Hence, certain resistant microorganisms with mutational changes in components of this subunit of the ribosome fail to bind the drug.

Ketolides displayed increased binding to the ribosome as compared to the macrolides of previous generations. Their binding site overlaps the macrolide-binding site of domain V of 23S rRNA. In addition, ketolides bind to domain II. For example, telithromycin\textsuperscript{11} binds to a specific adenine (A752) on domain II of 23S rRNA via the alkyl-aryl extension from the 11,12-carbamate bridge, whereas the quinolallyl side chain at the C6 position in cethromycin\textsuperscript{12} makes a contact with U790 of domain II of 23S rRNA.

Binding affinity is affected not only by hydrogen bonding, but also by the hydrophobic interactions of the lactone ring with the ribosomal subunit. Steitz\textsuperscript{13} illustrated that the side of the lactone ring facing the lumen of the tunnel is hydrophilic, including two axially oriented hydroxyl (C6 & C11) and two axially oriented carbonyl
oxygen (C1 & C9). In contrast, the face of the lactone ring that contacts the tunnel wall is hydrophobic. It contains no hydrogen bond donors or acceptors, only consisting of three methyl groups (C4, C8 & C12) that reside in the hydrophobic pocket formed by the base of A2100 (A2059Ec), the hydrophobic C2 edge of A2099 (A2058Ec), and the base and sugar of G2546 (C2611Ec).

Figure 1.3-1: Macrolides in Hydrophobic Pocket (adapted with permission from ref.14)

Regardless of the structures, the nucleotide at position 2058Ec was shown to play a major role in the binding of macrolides to the ribosome. As a consequence, it also plays a main role in the resistance to macrolides, often acquired by steric hindrance caused by its methylation or A-to-G mutation.15,16
1.4. Previous SAR Studies

The combination of low structural processability and high structural complexity of the erythromycins pose serious obstacles to addressing the problem of bacterial resistance. The known structure/activity profile (Figure 1.4-1) for erythromycin represents a tremendous effort.\textsuperscript{17-19} Briefly, a priori study has shown that: (a) portions of the glycans, especially desosamine, are critical and both the hydrophilic character of the β-face and hydrophobic character of the α-face dictate binding (erythromycin);\textsuperscript{20} (b) C9 amine or oxime/ether functionality improves pharmacokinetic profiles (I);\textsuperscript{17, 19} (c) C9-C11 or C11-C12 heteroannulation can improve binding and appears to provide opportunities to overcome MLS\textsubscript{B} resistance (II, III);\textsuperscript{21, 22} (d) ring formation between C6 and C9 can be beneficial,\textsuperscript{1} leading to interesting bioactivities such as gastrointestinal motor stimulant albeit with non-antibiotic function (IV);\textsuperscript{23} (e) retention of the C6 and C12 heteroatom connectivity is desirable and ether formation at C6 may improve antibiotic activity and suppress other activity (I, V);\textsuperscript{24, 25} (f) C3 ketone derivatives can improve efflux resistance (VI);\textsuperscript{22, 26} and (g) alterations to the hydrophobic face of the macrocycle (e.g. at C4, C8, and C10, VII and VIII) may overcome resistance.\textsuperscript{14, 27}
Hence, modification on the C3-C6 and C9-C12 regions offer opportunities to improve drug properties and avoid bacterial resistance.\textsuperscript{14} We therefore aimed to directly access diverse modifiable erythromycinoid variants to thereby expedite lead optimization studies.

Conventional medicinal chemistry approaches rely on erythromycin as a starting material (See Figure 1.1-2). However, total synthesis represents the only means by which to gain unrestricted access to this structure space. Indeed, \textit{de novo} synthesis of the erythromycins has a rich history, and the knowledge gained from efforts to synthesize these targets is considerable.\textsuperscript{29}
1.5. **Total Synthetic Approach to Erythromycin Derivatives**

In 2011, Andrade et al. reported the total synthesis of 4,8,10-tridesmethyl telithromycin. As described in Section 1.2, telithromycin is a 3rd generation semisynthetic drug derived from erythromycin and characterized by the 11,12-carbamate side chain and 3-keto group. Their desmethylation strategy is grounded in structural data by Steitz and co-workers. Bacterial strains resistant to macrolide, lincosamide, type B streptogramin, and ketolide (MLS$_B$K) antibiotics commonly possess either an N-methyl transferase that methylates the exocyclic N6 amine in A2058Ec in 23S rRNA or a mutated 23S rRNA, often A2058G. Steitz and coworkers postulated the cause of resistance might due to a steric clash of the amino group of guanine 2058 with the C4 methyl of the drug.

**Scheme 1.5-1 Andrades’ Synthesis of 4,8,10-Tridesmethyl Telithromycin**

The synthesis commenced with the known aldehyde 1.10 for the construction of C11-C13 unit. Sharpless asymmetric dihydroxylation established the requisite stereochemistry at C5 and C6 at step 6. Construction of C2 and C3 bond was
accomplished via an Evans aldol reaction at step 10. After ring-closing methathesis (RCM) at step 19, the glycosylation (step 24), the elaboration with C11,C12-carbamate (step 28) and the oxidation at C3 (step 30) were applied. From macrocyclic structure 1.11, it took additional 12 steps to finish the synthesis. The synthesis was very lengthy (42 steps overall, 31 steps in the longest linear sequence) though 1.12 exhibited antibacterial activity.

Andrades’ synthesis demonstrated the advantage of total synthesis over semi-synthesis, namely the ability to modify the carbon skeleton selectively. However, the access to the structural/biological space of erythromycin analogs is far from the reality with single target-oriented synthesis.

1.6. Conclusion

Despite the development of new synthetic methods and strategies toward the erythromycin family, structural complexity and low structural processability are serious obstacles to addressing the problem of bacterial resistance through semi- and/or total synthesis. It is clear that comprehensive knowledge of structure/function space is essential prerequisite for the development of better drugs. Hence, the development of a de novo strategy that grants efficient access to diverse structures is imperative in order to conduct a comprehensive SAR study for erythromycin-based antibiotics.

This dissertation focuses on our advances to the efficient generation of erythromycinoids.

Chapter 2: Strategy for the Synthesis of De Novo Erythromycinoids

Chapter 3: Synthesis of the Bis[Allene] Macrolactone
Chapter 4: Synthesis of Erythromycinoids

Chapter 5: Allene Osmylation and Multicomponent Coupling

Additionally, a multidisciplinary collaborative project with Prof. Ki-Bum Lee (Department of Chemistry & Chemical Biology, Rutgers) is described in Chapter 6: The Ligand Design and Synthesis for SiRNA Delivery.
1.7. References


Chapter 2

Strategy for the Synthesis of De Novo Erythromycinoids: Bis[Allene] Macrolactone

2.1. Introduction

Ideally, unrestricted access to diverse erythromycin analogs, including skeletal and stereochemical modifications, would require a minimal number of manipulations and no dramatic change in the synthetic route. Considering the model study and our extensive experience with and knowledge of allene transformations, we designated the fully functionalized bis[allene] macrolactone as a modular starting point for the construction of new analog scaffolds. Herein, I disclose our bis[allene] macrolactone strategy for the synthesis of de novo erythromycinoids.

2.2. Efficient Access to Diverse in Erythromycin Congeners

Our aim is to enable access to the diverse erythromycinoid structure space through efficient synthesis. The synthetic strategy considers: a) the structural space contains skeletal and stereochemical diversity; b) the versatile synthetic module leads to new sets of expandable intermediates, which ultimately constitute sets of targeted lead structure; and c) synthetic maneuvers should be minimal: the routes should be short.
2.3. Synthetic Strategy: Bis[Allene] Macrolactone

To achieve the aim, we developed a bis[allene] macrolactone strategy, which is characterized by the following key points:

- Total synthetic approach
- Late-stage functionalization: allene oxidation
- Macrocyclic stereocontrol
- Convergent synthesis of bis[allene] macrolactone
- Divergent synthesis of erythromycinoids

The most common method for erythromycin analog synthesis has been the derivatization of erythromycin itself. This semi-synthetic method has its own benefit from bountiful supply of erythromycin. However, the lack of synthetic flexibility not only requires lengthy synthetic manipulations of erythromycin but also limits the accessibility of potentially useful chemical and biological space.
Total synthesis might be a very lengthy way to generate analogs, but in the context of diversity-oriented synthesis,\textsuperscript{1,2} it provides an excellent means of modification, especially on the carbon backbone, which is intractable when using the semi-synthetic method. Late-stage introduction of heterofunctionality\textsuperscript{3} is not considered an ideal strategy for target-oriented synthesis due to the risk of failure at a late stage. However, when it is combined with the versatile synthetic module, it can be greatly appreciated in terms of efficiency and diversity.

2.4. \textit{Allene Oxidation: Spirodiepoxide (SDE) Chemistry}

As a result of their inherent chirality and high reactivity in such diverse transformations as addition, cyclization/cycloaddition, cycloisomerization, and cross-coupling reactions,\textsuperscript{4,5} functionalized allenes have been employed in recent years as highly versatile precursors to natural products and other target molecules. Despite their demonstrated utility, allenes are still considered to be an “under-developed” functional group. Prof. Williams’ group has studied the synthesis\textsuperscript{6} and reactivity\textsuperscript{7} of allenes, developing many novel transformations to access diverse motifs (See Figure 2.4.-1).

Crandall’s pioneering work\textsuperscript{8-16} demonstrated that upon DMDO oxidation of allenes, the corresponding spirodiepoxides (SDEs) formed and various nucleophiles could be added. However, the synthetic utility of allenes in complex molecule synthesis was realized through the work in the Williams group.
Various heteronucleophiles and carbon nucleophiles as well as ambiphilic reagents such as benzamide, thiobenzamide, benamidine, azide, intramolecular oxygen, and organometallic carbon\textsuperscript{17} are compatible with SDE chemistry and result in a complex motif such as a vicinal triad composed of hydroxyl, ketone, and syn-substituted nucleophiles,\textsuperscript{18} haloketones, $\alpha$'-hydroxy enone,\textsuperscript{19} heterocycles (oxazoles, thiazoles, and imidazoles),\textsuperscript{20} dihydrofuranone,\textsuperscript{7} butenolides, diendiols, diyndiol, $\gamma$-lactones, spiroketalts,\textsuperscript{21} and pyrans\textsuperscript{22} with high regio- and stereoselectivity in a single step. The utility of SDEs was extended in synthetic studies of complex natural products such as epoxomicin,\textsuperscript{18} psymberin,\textsuperscript{22} pectenotoxin 4,\textsuperscript{23} and erythromycin.\textsuperscript{24, 25}
2.5. *Allene Oxidation: Osmylation*

We have recently developed a method of allene osmylation. Osmium tetroxide (OsO₄) readily oxidized simple and complex linear allenes and the hydrolysis of the intermediate osmate ester gave α-hydroxy ketones both under catalytic and stoichiometric conditions (*See* Chapter 5 for details). When an electrophile is present upon the oxidation, it not only accelerated the hydrolysis of osmate ester 2.18 but also formed a multi-component adduct, giving α-hydroxy-α′-electrophile ketones (2.16 → 2.19). For now, this OsO₄-catalyzed multicoupling reaction of allenes is not fully appreciated in complex settings nor has the scope of electrophiles been fully explored. However, studies are ongoing. So far we have found compatible electrophiles are N-bromosuccinimide (NBS), N-chlorosuccinimide (NCS), Selectfluor, Eshenmoser’s salt (dimethylmethylidene ammonium chloride), and chloramines-T (N-chloro tosylamide).

**Scheme 2.5-1: Allene Oxidation**
When applied on chiral allenes, the stereochemical outcome of the reaction complements our allene spirodiepoxidation/nucleophile capture methodology (2.16 → 2.22). Therefore, when applied on the macrocyclic bis[allene], it provides access to stereoisomers, and their unique SAR profiles, without invoking a new asymmetric route. Furthermore, the stereoselectivity is enhanced due the macrocyclic stereocontrol. The use of protecting groups was minimized via late-stage macrocyclic stereocontrol; furthermore, highly stereoselective reactions were achieved due to the conformational preferences of the macrolide intermediates.

2.6. **Model Study: Bis[allene] Macrolactone**

Dr. Ghosh and co-workers in Prof. Williams’ group demonstrated the macrocyclic bis[allene] strategy on a simplified racemic macrocyclic bis[allene] (Figure 2.6-1). The study demonstrated that: (a) heterogeneous derivatization of the bis[allene] by stepwise oxidation; (b) successful addition of an organocuprate (MeCuCNLi) to the SDE, which represented the feasibility of SDE chemistry for polyketide-containing natural product synthesis; (c) complete macrocyclic stereocontrol; and (d) compatibility of SDE/nucleophile addition chemistry in a macrocyclic platform.
2.7. *Convergent Synthesis of Bis[Allene] Macrolactone*

Single-flask allene preparation, a method recently developed in Prof. Williams’ group, helped us envision the synthesis of both allene (C4 to C6, C10 to C12) moieties in 2.1 from propargyl alcohols. The convergent, recursive alkynylation sequence (*step 6* and *step 8*), coordinated allene installation (*step 9*), and lactone formation (*step 11*) produce bis[allene] macrolactone (2.1).
Figure 2.7-1: Convergent & Recursive Assembly of a Macrocyclic Bis[Allene]  
(adapted with permission from ref. 25)

The synthesis of three building blocks, an alkynol (I), an alkynal (II), and an aldehyde (III), and subsequent coupling of these for the synthesis of (2.1) will be discussed in detail in the following chapter.

2.8. Conclusion

The bis[allene] macrolactone strategy is designed to confer the efficiency and diversity on the structure/function space of erythromycinoids. Diversity will be achieved by late-stage oxidation of the bis[allene], which will undergo selective independent or concurrent modification. We have shown that allene oxidation, depending on the oxidant, can enable multi-component coupling with various nucleophiles and electrophiles in a highly stereoselective manner. This coordinated synthetic maneuver will provide the stereochemical and skeletal diversities, which can easily be expanded into functional group and appendage diversities. The route is designed so as to enable modification on the carbon skeleton where precedent methodologies cannot access.
Hence, the unexplored structure/function space of erythromycin antibiotics can be realistically reached.

In the following chapter, I discuss the synthesis of the bis[allene] macrolactone.
2.9. References


Chapter 3

Synthesis of the Bis[Allene] Macrolactone

3.1. Introduction

We have focused on developing a single short route for the chemical synthesis of diverse erythromycinoids. The bis[allene] macrolactone was envisioned as a versatile synthetic module. The first generation of the synthesis was carried out by our former colleague, Dr. Partha Ghosh.¹ Kai Liu and I have since made considerable improvements, especially in terms of reproducibility. This chapter focuses on the experimental details of the synthetic route for the bis[allene] macrolactone.

3.2. Synthesis of Coupling Units

Three readily available fragments (I, II, and III) were prepared and combined to provide the bis[allene] macrolactone IV (Figure 3.2-1). For the sake of convenience, they are referred to here as the C5 alkyne, C6 alkyne, and C4 aldehyde for I, II, and III, respectively. Two versions of the bis[allene] macrolactone were prepared, one with benzylic ethers and the other with meta-fluorobenzyl ethers, masking the hydroxyl groups at C3 and C9.
The synthesis of C6 alkyne 3.5 commenced with an Evans asymmetric aldol reaction (Scheme 3.2-1). The addition of the enolate, derived from oxazolidinone 3.1, to commercially available dimethoxyacetaldehyde afforded the expected aldol product 3.2 as a single isomer (90%). 1,1-dimethoxyacetaldehyde is commercially available as a solution in H₂O. The anhydrous form of the aldehyde was obtained by extraction with dichloromethane (DCM) and drying over 4Å molecular sieves. Subsequent benzyl etherification was accomplished using benzyl bromide, Ag₂O, and 4Å molecular sieves in anhydrous DCM 3.3 (95%). Though the reaction was done cleanly, it was quite slow (2 days). Our former colleague Dr. Ghosh reported that when using conventional NaH and benzyl bromide, the benzylation gave several by-products. He also noted that this is the first example of an Ag₂O mediated benzylation of an Evans aldol product.
Scheme 3.2-1: Synthesis of the C6 Alkyne Coupling Partner

The hydride reduction\(^4\) provided the desired alcohol 3.4 (97%). It was observed that the yield was reproducible when the temperature was kept at 0 °C, rather than increasing the temperature from 0 °C to room temperature. The tosylation was done within 1 hour when employing 1,4-diazabicyclo[2.2.2]octane (DABCO) as the base.\(^5\) Dr. Ghosh reported that the tosylation took about 12 hours with pyridine. The primary tosylate was not purified; rather, the crude material was subjected to lithium acetylide to give alkyne 3.5 (80% over two steps).\(^6\) Upon quenching with saturated aqueous solution of NH\(_4\)Cl, the side-product 3.6 formed considerably and was very difficult to separate from 3.5. Side-product formation was prevented by cooling to 0 °C before quenching.
The antipode of 3.3 was prepared from ent-3.1 using identical procedures (Scheme 3.2-2). For the coupling with alkyne 3.5, ent-3.3 was hydrolyzed under acidic conditions. Dr. Ghosh reported that the hydrolysis took 12 hours in 80% aqueous acetic acid, but when trifluoroacetic acid was added into the cleavage cocktail, the hydrolysis went remarkably faster (3.5 hours). It was found that purification of 3.7 by flash column chromatography (FCC) was not necessary for the subsequent alkynylation step. Notably, significant amount of 3.7 decomposed on silica gel.
Propargyl alcohol 3.11 was prepared from commercially available bis-TMS acetylene 3.8, adopting a procedure developed by our former colleague, Dr. Stephen D. Lotesta (Scheme 3.2-3).⁷ AlCl₃-promoted Friedel Crafts-type acylation gave the alkynone 3.9 (95%), which was followed by a Noyori asymmetric reduction of the ketone. The propargyl alcohol was obtained in 90% yield with >95% ee (Mosher ester analysis).⁸ The alcohol was protected as the tert-butyldimethylsilyl (TBS) ether, then the trimethylsilyl (TMS) group was cleaved in the same pot (72% yield over two steps).

### 3.3. Optimization of the 1st Coupling (Step 6): C1 to C9

Two coupling units, aldehyde 3.7 and alkyne 3.5, were chosen for a chelation-controlled addition to generate propargyl lactone 3.13 (Scheme 3.3-1). Dr. Ghosh reported that the chelation-controlled addition of the zinc alkynylide of alkyne 3.5 to aldehyde 3.7, followed by spontaneous in situ lactonization gave 3.13 in a modest 64% yield as a 8:1 mixture of diastereomers (entry 1, Table 3.3-1). Using the meta-fluorobenzyl ether, he also reported 3.16 in a 35 % yield as a 6:1 mixture of diastereomers (entry 4, Table 3.3-1). However, my colleague Kai Liu and I realized that the reaction yield is not reproducible using Dr. Ghosh’s condition for both benzylic and meta-fluorobenzylic ethers.
Scheme 3.3-1: The 1st Coupling: Chelation-Controlled Alkynylation

In his thesis\(^1\), he argued that the propargylation competes with a side reaction in which the starting aldehyde 3.7 undergoes rearrangement to 3.18, promoted by the auxiliary anion 3.17. The amount of anion 3.17 increases as the propargylation proceeds. Consequently, the relative rate of the side reaction increase, relative to the rate of formation of the desired product.\(^9\) Minimizing this side reaction by adjusting temperature proved difficult as the propargylation only takes place within a narrow temperature range. The reaction is sluggish below -10 °C, but proceeds rapidly at 0 °C.

Table 3.3-1: Optimization of 1st Coupling Reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equivalents</th>
<th>Addition temp. (°C)</th>
<th>Addition Rate</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkyne</td>
<td>Aldehyde</td>
<td>Base (metal)</td>
<td>ZnBr(_2)</td>
</tr>
<tr>
<td>1(^a) (P=Bn)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-40 → -10</td>
</tr>
<tr>
<td>2 (P=Bn)</td>
<td>2.3</td>
<td>1</td>
<td>2.3</td>
<td>-40 → -10</td>
</tr>
<tr>
<td>3 (P=Bn)</td>
<td>2.3</td>
<td>1</td>
<td>2.3 (3.3)</td>
<td>-40 → -10</td>
</tr>
<tr>
<td>4(^a) (P=mFBn)</td>
<td>1.3</td>
<td>1</td>
<td>1.3</td>
<td>-78</td>
</tr>
<tr>
<td></td>
<td>5 ((P=mFBn))</td>
<td>6 ((P=mFBn))</td>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-----</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Reaction done by Dr. Ghosh and reported in his Ph.D thesis and not reproducible.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Indicated when the equivalence of metal is different from that of base.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This reaction was optimized by altering several variables such as the equivalents of alkyne and base, the equivalents of chelation metal, the addition temperature of aldehyde, the chelation metal, and the rate of aldehyde addition (Table 3.3-1).

For the benzylic ether, the reaction yield and selectivity were reproducible when using a large excess of alkyne (entry 2), and the diastereoselectivity was better when the equivalents of chelation metal was the sum of the equivalents of alkyne and aldehyde (entry 3). To avoid the side reaction, the temperature was kept at 10 °C for 8 hours after the addition of aldehyde.

After thorough optimization, reproducible conditions were found and the reaction was scaled up to a maximum of 3.5 g (C4 aldehyde). The highest obtained yield of 3.13 was 63% (entry 3). Based on recovered alkyne 3.5, however, the yield is an excellent 95%.

Optimization studies using the meta-fluorobenzylic ether resulted in a highest yield of 35%. From all the data that was collected, it was concluded that zinc may not be an effective metal in the case of the meta-fluorobenzylic ether. The electron-withdrawing nature of the meta-fluorobenzyl group may be reducing the ability of the aldehyde oxygen to chelate with the metal. Therefore, we explored alternative metals. Using diethylaluminum chloride as a Lewis acid, we achieved a higher combined yield of
the product, but the selectivity was poor. The undesired diastereomer was preferred under these conditions, giving a 2:1 ratio of undesired/desired diastereomers.

Chelation-controlled addition of zinc alkynilides to \(\alpha\)-alkoxy aldehydes is known to proceed with high syn selectivity.\(^\text{10}\) Although the expected stereochemistry was confirmed later by X-ray crystallography data of erythromycinoids (See Chapter 4), it was not clear at this moment if the desired diastereoselection was achieved. The syn selectivity in the major diastereomer of 3.13 was rationalized a number of ways such that: 

\(\text{a})\) the coupling constant of the propargylic proton in \(^1\text{H} \text{NMR}\) appeared (1H, td, 2.0 Hz, 6.5 Hz) in the major isomer and (1H, td, 2.0 Hz, 5.0 Hz) in the minor isomer. Although this difference is small, the trend is the same as that for similar substrates reported in the literature;\(^\text{11}\) 

\(\text{b})\) Carreira’s asymmetric alkynylation gave the desired product 3.13 as the sole product.\(^\text{12}\) When (-)-N-methylephedrine 3.20 was used, the reaction yield was 30% with complete diastereoselection. Jiang et al. reported the modified ligand 3.19 for the Carreira reaction.\(^\text{13}\)

**Scheme 3.3-2: The 1st Coupling: Carreira Asymmetric Alkynylation**

![Scheme 3.3-2](image-url)
3.19 was synthesized in two steps according to Jiang et al. (Scheme 3.3-3).\textsuperscript{14} For both protecting groups, it gave complete diastereoselection with moderate yields, but a large excess of alkyne was required (4 equivalents). Since among the three coupling units the synthesis of alkyne 3.5 is the longest linear sequence, it makes ZnBr\textsubscript{2}-mediated chelation-controlled alkyynylation the most practical approach to pushing material forward.

**Scheme 3.3-3: Synthesis of Jiang’s Modified Carreira Ligand\textsuperscript{14}**

\[ \text{Scheme 3.3-3: Synthesis of Jiang’s Modified Carreira Ligand} \]

3.4.  **Optimization of the 2nd Coupling: C1 to C14**

When propargyl lactone 3.13 was subjected to the cleavage cocktail (HOAc:TFA:H\textsubscript{2}O = 4:1:1), less than 50\% was converted to the desired aldehyde 3.23 in 3.5 hours. This was in sharp contrast to the conversion of ent-3.3 → 3.7 in 90\% yield under the same conditions (Scheme 3.4-1).

**Scheme 3.4-1: Hydrolysis of the Acetal (Step 7)**
Nonetheless, subjecting the acetal 3.13 to a 4:1:1 mixture of HOAc, TFA and H₂O afforded the aldehyde 3.23 in 90% yield after 12 hours of stirring at room temperature. The aldehyde 3.23 turned out to be somewhat unstable to silica gel chromatography. Therefore, it was quickly advanced to the next reaction without chromatography.

**Scheme 3.4-2: The Second Coupling (Step 8)**

Non-chelation controlled additions of titanium triisopropoxy alkynilides to α-alkoxy aldehydes are known to give products with high *anti* selectivity. The alkynylide derived from 3.11 was combined with 3.23 in the presence of titanium triisopropoxide chloride. These addition products were also readily separable and the major product 3.26 was taken forward (89%, dr 6:1). The stereochemistry was postulated at this stage of the synthesis by the comparison of a major product from chelation controlled zinc alkynilide, using ZnBr₂ instead of Ti(OiPr)₃Cl and Carreira asymmetric alkynylation. Using ZnBr₂ as the chelation metal, Dr. Ghosh reported that the product 3.27 was obtained in 83% yield, which strongly supported the structural assignment. Carreira’s
asymmetric alkynylation with the modified ligand 3.19 gave the desired product with complete diastereoselection in 63% yield.

### 3.5. Synthesis of the Bis[allene] Seco-acid

A single-flask conversion of the hydroxyl unit of 3.26 to bis[allene] 3.28 was accomplished by converting 3.26 to the mesylate followed by addition of excess lower order methyl cyanocuprate (Scheme 3.5-1). Deprotection of the TBS group under acidic conditions gave the bis[allene] seco-acid 3.29 in 92% yield. It was found that crude 3.28 could also be converted to 3.29 without flash column chromatography (83% over two steps).

**Scheme 3.5-1: Synthesis of the Bis[Allene] Seco-Acid (Step 10)**

When the temperature was kept below -20 °C after the addition of lower order methyl cyanocuprate to *in situ* mesylate, the major product was mono[allene] 3.27. Although the single-flask conversion to the bis[allene] is very convenient, mono[allene]
3.27 is a useful intermediate that offers the flexibility to generate heterogeneous functionalities on C6 and C12.

3.6. **Macrolactonization**

Yamaguchi macrolactonization of seco-acids 3.29 and 3.30 provided bis[allene] macrolactones 4.1 and 4.2 in 64% and 75% yield, respectively. It is well preceded that the addition of sp² centers on the periphery of the ring decreases the transannular ring strain, thereby it facilitates the ring closure. The major byproduct of these lactonizations is the dimer 3.31 derived from the seco-acids. Four methyl groups on allenes as doublet of doublet in 1H NMR confirm the dimer formation.

**Scheme 3.6-1: Synthesis of Bis[Allene] Macrolactone (Step 11)**
3.7. Conclusion: Flexibility of the Synthetic Route

The bis[allene]macrolactone was prepared in a longest linear sequence of 11 steps (overall yield ~ 18%) by two consecutive alkynations (steps 6 & 8), single-flask bis[allene] synthesis (step 9) and macrocyclization (step 11). Considering the relatively late-stage elaboration of the carbon backbone (C6, C12, and C13) and adjustable methodologies, the flexibility of the current synthetic route can be easily envisioned for incorporation of hetero-functionalities at C6, C12, and C13 (V, Figure 3.7-1).

Figure 3.7-1: Flexibility of the Synthetic Route

In Chapter 4, I will discuss the synthesis of erythromycinoids (IV → VI).
3.8. References


Chapter 4

The Synthesis of De Novo Erythromycinoids

4.1. Introduction

To date, bis[allene] macrolactone 4.1 has been converted to over 30 novel macrolides (Figure 4.1-1). These de novo analogs serve to validate the strategy and lay the foundation for further work. Taken together, the allene-based transformations employed in this study, such as DMDO oxidation/nucleophile addition, allene osmylation/electrophile addition, halohydrin formation, allene oxide rearrangement, spirodiepoxide (SDE) rearrangement, benzylic migration/elimination, mono- and bis-oxidation of bis[allene], chelation-controlled reduction, and oxime formation, demonstrate that 9 of 11 carbons in ring core of erythromycin can be modified. It is especially noteworthy that each congener was made in less than three steps from a bis[allene] macrolactone of type 4.1. The congeners contain a variety of functional groups including enone, epoxy ketone, furanone, α'-halo ketone (fluoro-, chloro-, bromo-), vinyl bromide, and furan. These variations of the macrolactone core skeleton are novel, particularly in comparison to conventional analogs, inaccessible from the natural product, and, most importantly, represent further opportunities to create new derivatives. Herein is presented the detailed reaction conditions, mechanistic rationales, and synthetic significance of each compound synthesized to date.
Figure 4.1-1: Synthesis of Erythromycinoids

4.2. DMDO Oxidation and Nucleophile Addition

A recent disclosure\(^1\) from our group demonstrated that lithium halide salts are better nucleophiles than tetraalkylammonium salts for addition reactions to SDEs. The former reacts rapidly and reliably to give haloketones in excellent yield upon addition to SDEs, derived from simple trisubstituted allenes.

The model macrolactone, \(\text{2.24}\), was treated with DMDO (3 equiv.). Scheme 4.2-1 outlines the epoxidation/bromide addition reactions of model macrolactone \(\text{2.24}\). Addition of LiBr to crude SDE \(\text{4.3}\) gave \(\alpha\)-bromo ketone \(\text{4.4}\). X-ray crystallographic
analysis (Figure 4.2-1) confirmed the bromide addition at C4 which is anti to the hydroxy at C6 as expected.\(^2\)

However, the addition of large excess LiBr (10 equiv.) to the SDE, without removing excess DMDO, resulted in bromine addition to the central carbon (C11) of the unreacted allene \((4.5 \rightarrow 4.6)\). Bromine isotope effect (\(^79\)Br and \(^81\)Br) in HRMS confirmed the presence of two bromines ([M+]:[M+2]:[M+4] = 51:100:47) in \textbf{4.6}.

\textbf{Scheme 4.2-1: Nucleophile Addition to SDE}

\[^1\text{H}\]\ NMR spectrum of \textbf{4.6} revealed the presence of a single vinyl proton resonance (6.03 ppm, CDCl\(_3\)). The vinyl proton resonance was coupled to both methylene protons of C9 (2.37 ppm and 2.31 ppm, CDCl\(_3\)) as determined by HMBC spectroscopy. A strong nOe was observed between the vinyl proton and the methyl protons at C12, confirming Z-vinyl bromide/\(\beta\)-C12 alcohol in \textbf{4.6}. 
The same strategy was applied to fully functionalized bis[allene] macrolactone 4.1. Treatment of 4.1 with DMDO (6 equiv.) in CDCl$_3$ gave bis[SDE] 4.7 (Scheme 4.2-2). Similar to the model study, one allene underwent oxidation to SDE below 0 °C while the other allene persisted. After 15 minutes at room temperature, the unreacted allene was completely oxidized to give the second SDE. However, at room temperature, significant cleavage of the benzyl ether was observed (~ 25% by $^1$H NMR). Use of CHCl$_3$ as solvent instead of CDCl$_3$ resulted in the recovery of more benzyl cleaved product.

Scheme 4.2-2: Dr. Ghosh’s Attempt to Add Nucleophile to Bis[SDE]$^4$
Due to the instability of SDEs, LiBr was added at -15 °C to bis[SDE]s (4.1 → 4.8, Scheme 4.2-3). Albeit in low yield (10%), we isolated the first nucleophile adduct 4.9 to the corresponding SDEs from fully functionalized macrocyclic bis[allene] 4.1. Remarkably, the reaction installed two ketones, two halides, and four stereocenters in a single flask transformation. The well-known versatility of haloketones also gives more importance on the utility of SDE chemistry.

Scheme 4.2-3: Nucleophile Addition to SDE

Crandall et al. reported the formation of α,α'-dihydroxy ketones upon the
oxidation of acyclic trisubstituted allenes by DMDO in aqueous THF.\textsuperscript{6} Under the same conditions, we have also observed hydroxyl addition to SDEs 4.12, however the tertiary C6 alkoxide subsequently added into the C10 SDE terminus 4.11. It was not a surprising outcome when considering the reactivity of the hydroxyl at C6 to form 6,9-hemiketal in erythromycin.\textsuperscript{7} Excess DMDO was not removed in either reaction in light of the instability of the macrocyclic SDE and its tendency to give complex mixtures upon DMDO removal at room temperature.

As per the previous observation of Dr. Partha Ghosh,\textsuperscript{4} there was benzylic ether cleavage. This type of oxidative cleavage is known and presumed to proceed via insertion of oxygen from DMDO into the benzylic C-H bond to give a hemiacetal which collapses into the alcohol and benzaldehyde.\textsuperscript{8} HRMS confirmed the cleavage of one benzylic group and nOe between the benzylic protons and H3 confirmed the location of cleavage is at C3 (See Figure 4.2-2).

Figure 4.2-2: Key nOe Analysis for Benzylic Ether Location in 4.12

4.3. DMDO Oxidation and Rearrangement

Scheme 4.3-1 shows products derived from epoxidation of 4.1 and 4.2 by DMDO. Upon the exposure of 4.1 to DMDO in methanol, the reaction smoothly delivered $\alpha$-
benzyloxyenone 4.13. However, epoxidation with DMDO in chloroform, followed by treatment with Lewis acid,\textsuperscript{1} delivered dihydrofuranone 4.16. Remarkably, lower order lithium methyl-cyanocuprate efficiently promoted this reaction giving 4.16 in 64% yield.

Benzyllic migration product 4.13 is presumably due to hydrogen-bond donation\textsuperscript{9,10} by methanol to the highly reactive allene oxide intermediate leading to opening of the strained heterocycle faster than a 2nd epoxidation event.

**Scheme 4.3-1: DMDO Oxidation and Rearrangement**
When the less electron-rich meta-fluorobenzyl group is used for the protection of the C3 and C9 hydroxyls (4.2), I observed the same pattern of migration from C3 → C6 as shown in 4.13 giving 4.15. This suggests that it might be a good methodology for the installation of methoxy group at C6 (4.14), which is essential to avoid 8,9-anhydro-6,9-hemi-ketal formation from erythromycin. Crandall et al. reported the addition of alcohols (e.g., n-PrOH, i-PrOH) to SDEs in the presence of base (i.e., K₂CO₃, NaH), however no methanol addition product was observed in reaction of 4.1 and 4.2.

Upon the oxidation of allene 4.1 using DMDO, SDE formation occurred readily at the C4-C6 allene at low temperature (−20 °C). The nearby electron withdrawing ester functionality retarded oxidation at the C10 to C12 allene. When 6 equivalents of DMDO were used, the mixture of products was obtained including mono-oxidized product 4.16, bis-oxidized product 4.17, containing dihydrofuranone and 10-desmethyl α-hydroxy ketone functionalities, and epoxy ketone 4.18.

Interestingly in the cases where benzylic migration occurred (4.13 and 4.18), the stereochemistry at C6 is retained. However, when the benzylic ether is lost (4.16 and 4.17), an inversion of configuration at C6 is observed. We postulate that formation of 4.13, 4.16, 4.17, and 4.18 are closely related and involve either allene oxide opening (4.19 → 4.20) or SDE opening (4.22 → 4.23) at C6 and subsequent capture of the C3 benzyloxy group (Scheme 4.3-2). The retained stereochemistry at C6 in 4.13 may reflect the comparatively high stability of oxyallyl zwitterions 4.20, which could explain benzyloxy capture with overall retention of configuration. In the case of 4.16, two factors such as the comparatively low stability of a cation derived from SDE and the proximity of the C3 benzyl ether to C6 could lead to 4.23 directly with inversion of
configuration at C6. This mechanistic framework is also consistent with the reaction conditions used for these transformations though further studies are needed to evaluate the hypothesis.

Scheme 4.3-2: Mechanistic Rationale for 4.13, 4.16, 4.17, and 4.18
Analogous to 4.20, subsequent bis-oxidized product 4.17 may involve the formation of oxyallyl zwitterion 4.25 and subsequent water addition from the more accessible top face accounts for retention of configuration at C12 (Scheme 4.3-2). The instability of SDEs in the presence of Lewis acid could form a transient carbocation 4.26. If the carbocation was captured by C3 benzyloxy group and concurrent (or subsequent) second epoxide opening 4.27 could lead to oxonium 4.28. The cyclic oxonium ion can then be opened up by closure of the oxygen anion onto C3 furnishing benzyloxy migrated epoxy ketone 4.18.

4.4. **Structural Analysis of 4.13 and 4.18**

Analysis of the spectral evidence (³H NMR, ¹³C NMR IR) presented below established the structure of compounds 4.13 and 4.18.

The NMR-spectra of 4.13 revealed the presence of olefin protons (6.73 and 7.05 ppm, CDCl₃). A trans configuration was established by the large vicinal couplings constants (ca. 15.7 Hz) observed (Figure 4.4-1). A coupling constant of 1.1 Hz corresponding to allylic coupling of H4 with H2 was observed as well as vicinal coupling of H2 with H3 (8.2 Hz). This firmly established the structure of this portion of the molecule.

The NMR-spectra of 4.18 revealed the presence of epoxide protons (3.37 and 3.97 ppm, CDCl₃) as well as the corresponding ¹³C NMR signals (59.4 and 52.4 ppm, CDCl₃). The epoxide protons were split by vicinal couplings (ca. 1.8 Hz) and the magnitude of these couplings established the epoxide geometry as trans, which was expected as per our mechanistic rationale.
Benzylic migration from C3 to C6 is unambiguously shown by nOes observed between the benzylic protons and the C6 methyl protons in 4.13 and 4.18. Extensive 2D nOe analysis revealed that the stereochemistry at C6 of 4.13 and 4.18 is retained in comparison to 4.1 (See Figure 4.4-1).

Observation of a signal at 1653 cm$^{-1}$ in the infrared spectrum, characteristic of the carbonyl at enones, confirmed the presence of an enone moiety in 4.13.

**Figure 4.4-1: Key nOe Analysis of 4.13 and 4.18**

4.5. **Structural Analysis of 4.16 and 4.17**

Due to their structural complexity, it is very hard to elucidate the structural assignment of 4.16 and 4.17 unambiguously. For compounds 4.16 & 4.17, the initial concern was whether the newly formed ring was dihydrofuranone or oxetanone. It was initially considered to be an oxetanone (4.29, Figure 4.5-1) because the $^{13}$C NMR signal at 217 ppm was in close agreement with literature values for other compounds containing oxetanone functionality ($\delta \approx 215$ ppm). Additionally, previous studies on model macrocyclic bis[allene] in which oxetanone was formed encouraged us to favor structure 4.29.$^3$ However, FTIR analysis showed a carbonyl absorption frequency at 1765 cm$^{-1}$,
which is consistent with furanone.\textsuperscript{11} Literature precedent for oxetanone containing molecules showed that an IR signal at 1802-1800 cm\textsuperscript{-1} corresponded to the oxetanone carbonyl.\textsuperscript{12}

Figure 4.5-1: Structural Analysis of 4.17

To further verify ring size and, by extension, the location of the hydroxyl (C4 in 4.17, C3 in 4.29), the material was acylated using acetic anhydride (Scheme 4.5-1). Upon acylation, the H4 proton in 4.30 shifted 1 ppm downfield in the 1H NMR spectrum, but the H3 proton did not show the shift. The same acylation strategy was used for mono-oxidized compound and returned similar data confirming the structure of 4.32.
Scheme 4.5-1: Acylation of 4.13 & 4.14 for Structural Proof of Furanone

Strong nOe between the C6 methyl protons and the H3 proton revealed that the stereochemistry at C6 of 4.16 and 4.17 is reversed in comparison to 4.1 (Figure 4.5-2).

Figure 4.5-2: Key nOe Analysis of 4.16 and 4.17

Mono-oxidation products such as 4.13, 4.16, and 4.18 provide the unique opportunities to enhance the heterogeneity among the erythromycinoids. The reactive
secondary hydroxyl in 4.16 was protected either with TES or benzylic ether for further derivatization of the second allene.

**Scheme: 4.5-2: Hydroxyl Protection in 4.16**

4.6. **De Novo Glycosylation Strategy**

The first reported oxime formation at C9 in the erythromycin macrolide family lead to invention of 2nd and 3rd generation erythromycins. When 4.16 was treated with hydroxylamine, oxime formation at C5 proceeded smoothly to give 4.35 in 78% yield (Scheme 4.6-1). This clean oxime formation suggested the possibility of adding the C5 sugar as a nucleophile (4.16 → 4.36) in contrast to the conventional methodologies.
For example, in Martin’s synthesis of erythromycin B, a sugar bearing a pyrimidyl thioglycoside at the anomeric position can be added via the intermediate oxocarbenium ion (Section 4.6-2).\textsuperscript{14} Woodward and Toshima employed similar methods to glycosylate erythromycin A.\textsuperscript{15,16} These strategies require the 2'-OH on the desosamine sugar to be masked. An alternative method for glycosylation with desosamine is to prepare the corresponding acetimidate as in the synthesis of Methymycin by Kang et al.\textsuperscript{17} Though the reaction proceeded regio- and stereoselectively to afford the desired C5-glycosylated β-glycoside as the sole anomer, the reaction requires rigorously anhydrous conditions and low yields are not uncommon.
In contrast, the aminooxydesosamine hydrochloride salt will react in aqueous conditions. The aminooxydesosamine hydrochloride salt\(^{18}\) was prepared in 4 steps (Scheme 4.6-3) by my colleague, Libing Yu, and the hypothesis was tested on simple ketones and macrolide \(4.50\) (Scheme 4.6-4). The desosamine was isolated from the degradation of erythromycin. Benzoyl protection of the 2'-OH gave \(4.42\). Mitsunobo reaction of the anomeric alcohol with \(N\)-hydroxyphthalimide provided \(4.43\) as a 1:1 mixture of \(\alpha:\beta\) anomers. Oxyphthalimide \(4.43\) was then reduced by hydrazine followed by the removal of benzoylether to give hydrochloride salt \(4.44\) in a single flask transformation.
Scheme 4.6-3: Modified Synthesis of Desosamine Hydrochloric Salt

Yu coupled both α and β glycosidic aminohydroxyl desosamine salts with simple ketones (i.e., acetone and menthone) to obtain oxime in 99% yield in all cases (Scheme 4.6-4). When macrolactone 4.50 was treated with 4.44β, $^{13}$C NMR data showed the appearance of the characteristic oxime signal ($\delta = 168$ ppm in 4.51) and the absence of the ketone signal of 4.50 ($\delta = 212$ ppm). The stereochemistry of the glycoside bond was confirmed by $^1$H NMR coupling constant analysis ($J_{1',2'} = 8.0$ Hz).
Scheme 4.6-4: Addition of Hydroxyl Amine Desosamine

4.7. OsO₄ Oxidation/Addition/Rearrangement/Application

According to the outcome of osmylation on linear allenes (See Chapter 5 for more detail), we hypothesized that the osmylation of macrocyclic bis[allene] 4.1 in the absence of an electrophile would give 4,10-desmethyl erythromycinoid 4.52 (Scheme 4.7-1). Desmethyl erythromycinoid 4.52 was targeted because Steitz and coworkers demonstrated the cause of resistance might be due to a steric clash of the amino group of guanine 2058 of the large ribosomal subunit with C4 methyl of the drug.¹⁹
Upon oxidation of 4.1 with 2 equivalents of OsO₄, Kai Liu isolated (9S)-dihydro-6,9-anhydro derivative 4.53 (46%). Initially, we thought that the bis-oxidized product was 4.52. However, long-range coupling between the proton at C9 and the methyl carbon at C6 suggested the formation of 6,9-furan. Furthermore, the tertiary hydroxyl at C12 was protected with a TES group and the product was recrystallized to unambiguously determine the structure to be 4.54 by X-ray crystallographic analysis (Figure 4.7-1). It is well precedented that 6,9-linked analogs, so called “motilide”, display biological activity as motilin acceptors, thus promoting gastrointestinal prokinetic activities.²⁰,²¹

Scheme 4.7-1: Desmethyl Erythromycinoids
In considering the different reactivity of the two allenes in macrocyclic bis[allene] 4.1, it was realized that we could oxidize them in a stepwise manner. Thereby, the 2\textsuperscript{nd} allene can be modified heterogeneously. In an attempt to affect stepwise oxidation, macrocyclic bis[allene] 4.1 was treated with 1 equivalent of OsO\textsubscript{4} to give mono-oxidized 4-desmethyl macrolide 4.50 (85\%). Addition of a 2\textsuperscript{nd} equivalent of OsO\textsubscript{4} oxidized the second allene to 4.53 (50\%).

Although the precise structures of the intermediates are a matter of speculation, the osmium adducts formed and were subsequently hydrolyzed (i.e., 4.55 $\rightarrow$ 4.50, 4.56 $\rightarrow$ 4.57, Scheme 4.7-2). Considering that these intermediates are also enolates,\textsuperscript{22} unlike simple alkene-derived osmate esters, $\beta$-elimination (4.56 $\rightarrow$ 4.57) and subsequent intramolecular conjugate addition (4.57 $\rightarrow$ 4.53) is reasonable and suggestive of other possible transformations. Interestingly, the C3 benzyloxy group is retained whereas the C9 group is eliminated, a phenomenon most likely traceable to the orientation of this group relative to the osmate ester.
C9-C12 $\alpha$-hydroxyenone 4.59 was isolated when hydroxyl ketone 4.50 was protected with a TES group (4.51 $\rightarrow$ 4.58), followed by a second osmylation (4.58 $\rightarrow$ 4.59, Scheme 4.7-3). $\alpha$-Hydroxyenone 4.59 adopts a trans configuration, as confirmed by vicinal coupling constants ($^3J_{H9, H10} = 15.5$ Hz). Considering the synthetic versatility of enones, this functionality in 4.59 and 4.13 provides enormous opportunity to derivatize C3, C4, C6, C9, C10 and C11 to include diverse functional arrays.
4.8. **Allene Halohydration**

Ionic addition to simple acyclic allenes is known, but has never been applied in asymmetric synthesis. We hypothesized that an NBS addition/hydrolysis reaction will be highly stereoselective due to macrocyclic stereocontrol in allene 4.50. Herein, I described the combination of allene osmylation and electrophilic bromination (Scheme 4.8-1).

**Scheme 4.8-1: Electrophilic Bromination**

Following osmylation of 4.1, the resultant ketoalcohol 4.50 was treated with NBS in acetonitrile. The C10-C11 Z-vinyl bromide/β-C12 hydroxyl 4.60 was isolated in excellent yield.
Although there are 2 possible faces of approach for the electrophilic bromination ion to the allene of 4.50 (4.61 and 4.63, Scheme 4.8-2), approach of the nucleophile is expected to occur from outside of the macrocycle. This would lead to Z-bromide 4.60 at a significantly faster rate than 4.62.

**Scheme 4.8-2: Mechanistic Postulation for 4.60**

The NMR-spectra revealed the presence of a single olefin proton (6.14 ppm in 4.60). The olefin proton was split by vicinal coupling ($J_{H10, H9} = 8.4$ Hz in 4.60). The transannular nOe of H10 with H13 and H3 unambiguously set Z-vinylbromide configuration of 4.60 (See Figure 4.8-1). The absence of an nOe for H10 with the C12 methyl group set the stereochemistry of the C12 hydroxyl as β as well as the nOe between the C12 methyl with the C14 methylene protons in 4.60.
Figure 4.8-1: Key nOe Analysis of 4.60

4.9. **Alternative to DMDO Oxidation**

Oxymercuration/demercuration\(^{25, 26}\) of allenes has been well studied, however it has never been applied in the context of macrocyclic allene systems. The stereochemical outcome of product formation in oxymercuration/demercuration is analogous to that of halohydration. Upon exposure of the allene to mercuric sulfate, the formation of the purported mercurium bridge inside the ring would allow for facile attack by a nucleophile from outside the macrocycle (\(2.24 \rightarrow 4.63\)) in comparison to formation of the mercurium bridge outside the ring and attack of the nucleophile from inside the macrocycle (\(2.24 \rightarrow 4.66\)). The former would lead to *trans* allylic alcohol 4.64. However, the demercuration step (reflux at 70 °C in sulfuric acid and water) lead to the elimination product 4.65.
Oxidative cleavage$^8,27,28$ of benzylic ethers and arenes is known and accounts for the instability of SDEs prepared from macrocycle 4.1 upon reaching ambient temperature. In contrast to DMDO oxidation, exposure of arene-containing allenes to $m$CPBA seems to give SDEs.$^{29}$ Hence, bis[allene] macrolactone 4.1 was oxidized with $m$CPBA (6 equiv.) in methanol. Instead of the methanol addition adduct, 4.68 was isolated containing a C2 olefin forms. This product was similar to 4.13, though differed by the position of the olefin.
A *trans* configuration of the olefin at C2-C3 in 4.68 was confirmed by nOe analysis. The C3 proton shows an nOe with H4β only, however in contrast, the C2-methyl protons shows an nOe with H4α only (Figure 4.9-1). In the *cis* olefin configuration, an nOe between H4α and the C2-methyl protons is unlikely. The mechanism is analogous to the formation of 4.13. The highly reactive allene oxide intermediate 4.69 opens up before the second epoxidation event due to hydrogen bond donation. The nearby benzyloxy added to the carbocation of intermediate 4.70 followed by removal of a proton by base promoted by the benzyloxonium ion in 4.71 (Scheme 4.9-3).

**Figure 4.9-1: Key nOe Analysis of 4.68**
**Scheme 4.9-3: Mechanistic Rationale for 4.68**

![Mechanism Diagram]

**4.10. Chelation-Controlled Reduction**

Zn(BH$_4$)$_2$ reduction of optically active $\alpha,\beta$-epoxy ketones$^{30}$ and $\alpha$-hydroxy ketones$^{31}$ in linear systems was reported to afford optically active *erythro* isomers with high selectivity. However, for macrocyclic systems, the stereochemical outcome is different from what is expected in linear systems. It depends on the topographical bias of the macrocycle. Contrary to the stereochemical prediction for linear systems, Paterson showed *threo* reduction at C5 by Zn(BH$_4$)$_2$ (4.72 → 4.73, Scheme 4.10-1).$^{32}$

**Scheme 4.10-1: Zn(BH$_4$)$_2$ Reduction on $\alpha$-Hydroxy Ketone by Paterson$^{32}$**

![Reduction Diagram]
Scheme 4.10-2: Zn(BH₄)₂ Reduction

Upon reduction with Zn(BH₄)₂, the macrolactones with epoxy ketone 4.18, keto alcohol with vinyl bromide 4.60, and keto alcohol 4.50 show the complete diastereoselection with moderate to good yield (Scheme 4.10-2). The careful and extensive NMR analysis established the structure of the reduction products.

In case of 4.18, the hydride attack would be more accessible from the opposite side of the epoxide 4.77, which account for α-hydroxy at C5 in 4.74. The stereochemistry was confirmed by nOe between H5 and benzylic protons at C6 in 4.74.
For keto alcohols 4.60 and 4.50, hydride attack on the ketone at C5 from the top face was predicted to be unlikely due to steric hindrance. 4.75 showed a very strong transannular nOe among H10, H3, and H13 and this led us to assume that the benzylic protons at C3 were positioned at the top face of the compound. The same benzylic protons showed an nOe with H5. This confirmed hydride attack at C5 from the top face as shown 4.78. However Zn(BH$_4$)$_2$ reduction of 4.50 gave β-hydroxy formation at C5 as a single diastereomer (4.76). The nOe between the C6-methyl and C5-α-hydroxy confirmed the assigned stereochemistry in 4.76.
4.11. *Benzyl Ether Cleavage by DDQ*

As shown in this chapter, the presence of benzylic ether resulted in a very interesting rearrangement due to its reactivity under the employed oxidation conditions. However, more stable protecting groups such as silyl ethers were also advised.

**Scheme 4.11-1: Benzyl Ether Cleavage by DDQ**

Instead of incorporating the different protecting group at the beginning of the synthesis, if we can manipulate the protecting groups at a later stage of the synthesis, this will ease the synthetic maneuver. Often times, benzylic ethers are cleaved by palladium catalyzed hydrogenolysis, however due to allene reactivity, we affected the cleavage with DDQ. The cleavage occurred stepwise leading to cleavage of the C9 benzyl ether before the C3 benzyl ether.

**Figure 4.11-1: Key nOe Analysis for Benzylic Ether Location in 4.80**
Computational analysis performed at the MM2 level suggests that the ground state of the bis[allene] macrolactone 4.1 populates a favorable conformation in which the benzylic ether at C9 is more open in space whereas the benzylic ether at C3 is tucked into the ring. This conformation is also consistent with nOe analysis.

**Figure 4.11-2: Ground State Minimum Energy Conformation of 4.1**

![Image of ground state minimum energy conformation of 4.1]

**4.12. Conclusion**

In summary, the four sites of unsaturation in a bis[allene] macrolactone were transformed with apparently complete selectively. The observed order of reactivity is C5-C6 > C4-C5 > C11-C12 and then C10-C11. In all of the reactions, the products were isolated as single stereoisomers. The observed stereochemical outcomes reflect the cooperative effects of intrinsic allene stereoselectivity, macrocyclic stereocontrol, and (for the intramolecular transformations) proximity of the reacting partners. Bis[allene] macrolactone has proven to be a processable intermediate and all compounds were
prepared from this species in 3 steps or less. 9 out of 11 modifiable carbons were synthetically manipulated and the new functional moieties included enone, epoxy ketone, furanone, \(\alpha\)'-halo ketone (fluoro-, chloro-, bromo-), halohydrin, and furan. Most importantly, this new class of erythromycinoids would allow us to systematically vary the point of interest on the macrolactone carbon backbone to shed light on the complete survey of structure/function space and the mechanisms by which these compounds work.

Taken all together, the bis[allene] macrolactone is a structurally processable intermediate that integrates the routes to targets that intersect with underexplored erythromycinoid structure space. As part of our program to develop \textit{de novo} strategies for the synthesis of erythromycinoids, we have been interested in improving methods to oxidize an allene to synthesize erythromycinoids. Allene osmylation and multicomponent coupling is discussed in more detail in chapter 5.
4.13. References


Chapter 5

Allene Osmylation and Multi-component Coupling

5.1. Introduction

A mechanistic rationale that reconciles earlier methodological shortcomings of allene osmylation is advanced and tested. These insights provide the basis for new efficient methods of catalytic allene osmylation and stereoselective multi-component allene hydroxylation/electrophile capture reactions. These methods constitute a powerful complement to allene spirodiepoxidation/nucleophile capture methods. Simple and complex substrates are used to demonstrate the generality of these new methods.

5.2. History of Allene Osmylation

In contrast to the extensive studies of alkene osmylation in the literature, we have found few studies on allene osmylation. These reports detail allene osmylation as sluggish and low yielding reactions with significant byproduct formation that is extremely limited in substrate scope. Fleming reported the asymmetric dihydroxylation of mono-substituted aryl allenes of type 5.1 using AD-mix (Scheme 5.2-1). The reaction gave chiral $\alpha$-hydroxy ketones with complete regioselectivity and a high degree of stereoselectivity, however the yields were only moderate (45 – 63%) with the exception of the para-methoxy substituted allene (79%). In the case of non-aryl 1,1-disubstituted allenes of type 5.3, the yield and enentioselectivity were poor. Crabbe disclosed the
stoichiometric osmium tetroxide (OsO₄)-mediated oxidation of steroid 5.7 gave dihydroxy-keto steroid 5.8 (Scheme 5.2-2).³

Scheme 5.2-1: Asymmetric Dihydroxylation on Allene by Fleming

Previous allene osmylations showed that oxidation occurs at the more electron-rich terminus. However Cazes demonstrated a steric effect, that led unexpectedly to unique α-hydroxyketone 5.10 from N-Boc aminomalonate 5.9 while osmylation of homolog 5.11 resulted in reversed regioselectivity giving regiumers 5.12 and 5.13 in a
OsO₄ was also shown by Agosta to oxidize allenic ketones to cyclic hemiketals (5.14 → 5.15, 5.16 → 5.17, Scheme 5.2-4).

**Scheme 5.2-3: Effect of Sterics on Regioselectivity**

(Cazes, 1996)

**Scheme 5.2-4: Osmylation on Allenic Ketones**

(Agosta, 1994)

5.14

5.15

5.16

5.17

5.3. **Mechanistic Rationale and Stereochemical Outcome**

The Sharpless group has reported their findings on “ligand-accelerated catalyst” systems in which the addition of a specific ligand increases the reaction rate of an already existing catalytic transformation. The nature of the ligands bound to a metal center or complex always affects the selectivity and rate of the organic transformations catalyzed by such a species. If the reaction rate is increased upon ligand binding, the stereoselective pathway can dominate over the nonselective one.

Along similar lines, we hypothesized that the addition of an electrophile to an allene osmylation reaction would accelerate decomposition of the relatively stable osmate.
enol ester intermediate (5.20 → 5.23, Figure 5.3-1), thereby increasing reaction rate and selectivity. Allenes are oxidized at the most electron-rich terminus and subsequent decomposition of the osmate ester captures the electrophile syn-facial to the smaller substituent (R<sub>s</sub>, 5.20). This provides regio- and stereoselective allene hydroxylation/electrophile addition anti-adduct. The stereochemical outcome of the reaction is complementary to allene spirodiepoxidation/nucleophile addition syn-adduct 5.24.

Figure 5.3-1: Allene Oxidation

In the DMDO oxidation of acyclic allenes, the first epoxidation will also occur at the most electron-rich terminus with excellent stereoselectivity (> 20:1), favoring the less sterically hindered side (H vs. R<sub>1</sub> on 5.21). The second epoxidation is less selective, but still favors the smaller substituent (R<sub>s</sub>). Nucleophile addition then occurs at the least
sterically hindered SDE term on the face of the larger substituent ($R_L$), providing the syn-addition product (5.24).

5.4. **Allene osmylation and multi-component coupling**

These hypotheses were tested on achiral tri-substituted allene 5.25 as a model system (Table 5.4-1). All reactions were carried out under catalytic conditions (0.1 equiv. OsO₄) with a specific stoichiometric oxidant. The typical experimental procedure is as follows: to the allene in a $t$-BuOH:pH 7.4 phosphate buffer (1:1) solution was added an electrophile (Selectfluor, NCS, or NBS at room temperature). The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.1 equiv.) was added slowly followed by stoichiometric oxidant. After the complete disappearance of allene by TLC observation, the reaction mixture was quenched with a saturated sodium sulfite solution. Purification by flash column chromatography on silica gel following aqueous workup afforded the corresponding $\alpha$-hydroxy-$\alpha'$-halo ketone. Under anhydrous conditions (entry 5), a solution of allene in dichloromethane (DCM) was added to a solution of OsO₄ (0.1 equiv.), NMO (1.2 equiv.), acetic acid (4 equiv.) and phenylboronic acid (1.2 equiv.) in DCM. The workup and purification process was identical to that of the above aqueous conditions and afforded the corresponding $\alpha$-hydroxy ketone.

In all cases, the reaction proceeds with complete regioselectivity. As expected, allene 5.25 is oxidized at the more substituted (electron-rich) terminus and decomposition of the osmate enol ester captures the electrophile at the less substituted terminus. In the presence of a halide electrophile, the reaction provides an $\alpha$-hydroxy-$\alpha'$-halo ketone and the rate of the reaction increases (cf. entries 1, 9, 12, and 13) with NMO as the stoichiometric oxidant in a $t$-BuOH:pH 7.4 phosphate buffer (1:1) solution.
Table 5.4-1: Catalytic Allene Oxidation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Stoichiometric oxidant (equiv.)</th>
<th>Additive (equiv)</th>
<th>E</th>
<th>Solvent</th>
<th>Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NMO (2)</td>
<td>-</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>48 hr</td>
<td>trace</td>
</tr>
<tr>
<td>2</td>
<td>AD-mix (1.4g/mmol)</td>
<td>MeSO₂NH₂(0.2)</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>48 hr</td>
<td>56 %</td>
</tr>
<tr>
<td>3</td>
<td>K₃Fe(CN)₆ (3)</td>
<td>K₂CO₃(0.2)</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>12 hr</td>
<td>65 %</td>
</tr>
<tr>
<td>4</td>
<td>NMO (2)</td>
<td>DABCO (0.1)</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>12 hr</td>
<td>68 %</td>
</tr>
<tr>
<td>5</td>
<td>NMO (1.2)</td>
<td>AcOH w/ PhB(OH)₂</td>
<td>-</td>
<td>DCM</td>
<td>2 hr</td>
<td>65 %</td>
</tr>
<tr>
<td>6</td>
<td>NMO (2)</td>
<td>AcOH</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>4 hr</td>
<td>70 %</td>
</tr>
<tr>
<td>7</td>
<td>NMO (2)</td>
<td>NCS</td>
<td>-</td>
<td>t-BuOH: H₂O (1:1)</td>
<td>12 hr</td>
<td>10 %</td>
</tr>
<tr>
<td>8</td>
<td>NMO (2)</td>
<td>NCS</td>
<td>-</td>
<td>acetone: H₂O (1:1)</td>
<td>3 hr</td>
<td>34 %</td>
</tr>
<tr>
<td>9</td>
<td>NMO (2)</td>
<td>NCS</td>
<td>-</td>
<td>t-BuOH: buffer (1:1)</td>
<td>24 hr</td>
<td>67 %</td>
</tr>
<tr>
<td>10</td>
<td>NMO (2)</td>
<td>NCS</td>
<td>-</td>
<td>acetone: buffer (1:1)</td>
<td>24 hr</td>
<td>68 %</td>
</tr>
<tr>
<td>11</td>
<td>NMO (2)</td>
<td>NBS</td>
<td>-</td>
<td>acetone: buffer (1:1)</td>
<td>2 d</td>
<td>31 %</td>
</tr>
<tr>
<td>12</td>
<td>NMO (2)</td>
<td>NBS</td>
<td>-</td>
<td>t-BuOH: buffer (1:1)</td>
<td>5 hr</td>
<td>68 %</td>
</tr>
<tr>
<td>13</td>
<td>NMO (2)</td>
<td>Selectfluor</td>
<td>-</td>
<td>t-BuOH: buffer (1:1)</td>
<td>12 hr</td>
<td>40 %</td>
</tr>
</tbody>
</table>

AD-mix with methanesulfonamide also accelerates the rate of the reaction (entry 2). Sharpless et al.⁸ demonstrated that hydrolysis of the osmium (VI) glycolate product can be accelerated considerably by methanesulfonamide.⁴ Potassium cyanoferrate (K₃Fe(CN)₆) in the presence of K₂CO₃, was also evaluated and found to react smoothly in the osmylation of 5.25 (entry 3). Sharpless argued that using K₃Fe(CN)₆, instead of NMO, eliminates the second catalytic cycle because there is no oxidant other than OsO₄.
in the organic layer due to biphasic conditions with K$_3$Fe(CN)$_6$. Thereby, the reported ee under K$_3$Fe(CN)$_6$/K$_2$CO$_3$ is better than ones using NMO. The tertiary amine, DABCO, is also capable of accelerating the reaction (entry 4).

The reaction can be conducted in organic solvent alone (entry 5). For the dihydroxylation of olefins, aqueous conditions are usually employed to hydrolyze the osmate ester. However, this not only makes it difficult to isolate water soluble polyol derivatives but many electrophiles also react fast with water. Iwasawa, Kato, and Narasaka reported that the use of phenylboronic acid as a diol captor prevents over-oxidation and also enables the reaction to be performed in organic solvent alone.

Table 5.4-2: Allene Oxidation

<table>
<thead>
<tr>
<th>allene</th>
<th>electrophile</th>
<th>solvent</th>
<th>products</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Bu=$\equiv$H n-Bu</td>
<td>Cl</td>
<td>t-BuOH: H$_2$O</td>
<td>5.30 (10%)</td>
</tr>
<tr>
<td>5.25</td>
<td>Na</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DCM</td>
<td>5.31 (83%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 5.25 | Cl | CH$_2$ | N$_2$Me |
| | | | Me |

Other than halide electrophiles, we also tried C-N and C-C bond forming reactions. The addition of Chloramine-T was performed under stoichiometric conditions (1.1 equiv. OsO$_4$) to give 5.30 (10% yield, Table 5.4-2). The majority of the product was chlorine addition 5.28 (50% yield). The addition of Eshenmoser salt (dimethylmethyleneammonium chloride) was carried out under catalytic conditions.
with NMO as the co-oxidant in anhydrous DCM giving 5.31 in good yield (63% yield). When DCC or DIC were added, heterocycles 5.32 and 5.33 were isolated in good yield (Scheme 5.4-1).

Scheme 5.4-1: DCC and DIC Addition

5.5. **Chiral allene: osmylation and multi-component coupling**

The osmylation reaction was also performed on chiral di- and tri-substituted allenes (Table 5.5-1). Optically active allenes 5.34 and 5.35 were prepared according to known procedures,\(^{10,11}\) however the racemate of 5.36 was used. Three electrophilic halide sources (Selectfluor, NCS, and NBS) were tested for both the catalytic and stoichiometric conditions. The reaction conditions for the catalytic and stoichiometric processes were the same as described in section 5.4.
Table 5.5-1: Chiral Allene Osmylation and Halide Addition

<table>
<thead>
<tr>
<th>entry&lt;sup&gt;A&lt;/sup&gt;</th>
<th>allene</th>
<th>product&lt;sup&gt;B&lt;/sup&gt;</th>
<th>selectivity&lt;sup&gt;C&lt;/sup&gt;</th>
<th>yield&lt;sup&gt;D&lt;/sup&gt;(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="5.34" /></td>
<td><img src="image" alt="5.37" /></td>
<td>&gt; 20:1</td>
<td>5.9 : 1</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="5.34" /></td>
<td><img src="image" alt="5.38" /></td>
<td>&gt; 20:1</td>
<td>5.3 : 1</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="5.35" /></td>
<td><img src="image" alt="5.39" /></td>
<td>(&gt; 20:1)</td>
<td>(&gt; 20:1)</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="5.35" /></td>
<td><img src="image" alt="5.40" /></td>
<td>(&gt; 20:1)</td>
<td>(&gt; 20:1)</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="5.35" /></td>
<td><img src="image" alt="5.41" /></td>
<td>(&gt; 20:1)</td>
<td>3.2 : 1</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="5.35" /></td>
<td><img src="image" alt="5.41" /></td>
<td>(&gt; 20:1)</td>
<td>(1.5:1)</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="5.36" /></td>
<td><img src="image" alt="5.42" /></td>
<td>(&gt; 20:1)</td>
<td>(7: 1)</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="5.36" /></td>
<td><img src="image" alt="5.42" /></td>
<td>(&gt; 20:1)</td>
<td>(2: 1)</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="5.36" /></td>
<td><img src="image" alt="5.43" /></td>
<td>(&gt; 20:1)</td>
<td>(1.7: 1)</td>
</tr>
</tbody>
</table>

A) stoichiometric reactions employed 1.1 equiv. of OsO₄ w/ electrophile (2 or 5 equiv.) at rt.
catalytic reactions employed 0.1 equiv of OsO₄ w/ NMO (2 equiv.) & electrophile
(2 or 5 equiv.) at rt.  B) only anti product is shown.  C) black = selectivity in stoichiometric condition,
blue = selectivity in catalytic condition.  D) combined yield of anti and syn products.

In all cases, we observed complete regioselectivity. In tri-substituted allenes
(Entries 1, 2, 5, 6, and 7), the osmate enol ester formed at the more substituted terminus as
expected, leading to carbon-halide bond formation at the less substituted terminus. In
1,3-disubstituted allenes (entries 3 and 4), the steric effect, induced by gem-dimethyl substituents, overrides the electronic effect of the electron withdrawing TBDPS group. The site of fluorine addition (5.35 → 5.39) was confirmed by observation of H4 geminal splitting by fluorine which appeared as a doublet with a characteristic strong splitting constant (Figure 5.5-1, \( J = 47.2 \ \text{Hz} \)) while the regioselectivity of 5.40 was confirmed by TOCSY and vicinal splitting of H2, which appeared as a triplet of doublets (Figure 5.5-1).

**Figure 5.5-1: Regiochemistry of 5.39 and 5.40**

The stereoselectivity is particularly good with bulky Selectfluor in comparison to NCS and NBS. It is likely that the asymmetric bias is enhanced due to the bulkiness of SelectFlour.\(^\text{12}\) In all cases, the stoichiometric reaction gave better selectivity than catalytic reactions. For the addition of bromine, NBS had to be pre-solvated in \( t-\)BuOH:buffer mixture, then added to the mixture of OsO\(_4\), NMO, and allene slowly over at least 1 hour. If these requirements are not met, the allene will react with NBS faster than osmylation can occur.
When the substrate without the para-methoxy benzyl (PMB) group 5.44 is oxidized in the presence of electrophiles, the osmylation reaction gave halo-substituted furanose (Scheme 5.5-1). Unlike the osmylation on the substrate with PMB group 5.35, I found the mixture of two diastereomers for fluorine adducts (Scheme 5.5-1). Upon the cleavage of PMB group in 5.39 by DDQ (Scheme 5.5-2), the reaction generated the same products 5.45 and 5.46 in the same ratio (1:1.8). This experiment indicated that the diastereoselectivity on fluorine addition is >20:1.

Scheme 5.5-2: In-Situ Cleavage and Cyclization of 5.39
5.6. **Cyclic Allene Osmylation: Model Study**

Macrocyclic bis[allene] 2.24 and compounds of this type provide a unique platform for diversity oriented synthesis due to the reactivity of allenes in various chemical transformations, the heterogeneous manipulation of bis[allene] and enhanced macrocyclic stereocontrol.\textsuperscript{13, 14} Since acyclic allenes show complete regioselectivity and moderate stereoselectivity, the successful application of allene osmylation/electrophile addition methodology for cyclic bis[allene]s of type 2.24 will further prove the utility of the methodology.
All reactions (Scheme 5.6-1) were carried out under stoichiometric conditions (2.2 equiv. OsO₄). The typical experimental procedure is as follows: to the allene in a t-BuOH:water (1:1) solution was added an electrophile (SelectFlour, NCS) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution was added dropwise. After the complete disappearance of allene by TLC observation, the reaction mixture was quenched with a saturated sodium sulfite solution.
In the absence of an electrophile, bis[keto] alcohol 5.49 was isolated as expected. Both NCS and Selectfluor proved to be the compatible electrophiles for macrocyclic bis[allene] 2.24 under the above reaction conditions giving the desired products (2.24 → 5.50, 2.24 → 5.51) in moderate yields. Under the reaction conditions, the conversion from mono-oxidized products of types 5.52 and 5.53 to bis-oxidized product 5.51 is easily monitored by TLC over 1 or 2 hours. Interestingly, the oxidation rate of the two allenes of the macyclic lactone (2.24) did not show much difference, which could be confirmed by the isolation of a 1:1 mixture of 5.52 and 5.53. This observation is contrary to the bis[allene] macrolactone oxidation of 2.24 by DMDO (See Chapter 4).

HMBC spectroscopy shows that the central allenic carbon resonances (C11 in 5.52, C5 in 5.53) was coupled to methyl protons at C12 in 5.52 and C6 in 5.53 while the tertiary methyl proton resonances at C6 in 5.52 and C12 in 5.53 was coupled carbonyl carbon resonances (C5 in 5.52, C11 in 5.53).

Figure 5.6-1: HMBC Confirmation of Two Central Allenic carbons

The bis[allene] hydroxylation/electrophile addition showed complete diastereoselection in the products. Kai Liu successfully recrystallized chlorine addition product 5.50 and was therefore able to establish the stereochemistry at C4 and C10 unambiguously (Figure 5.6-1).
Catalytic conditions with NMO as co-oxidant were also tried for fluorine and bromine additions (Scheme 5.6-2). With Selectfluor, bis-fluorine addition product \textbf{5.51} was isolated in 29\% yield. Since NBS is able to react with allenes independently, NBS was premixed in \textit{t}-BuOH:water and added over 10 hours into the mixture of allene, OsO$_4$, and NMO in \textit{t}-BuOH:water. However, the isolated halohydrin (\textbf{5.54}) showed NBS addition to the central carbon.
Scheme 5.6-2: Catalytic Osmylation on Macrocyclic Bis[Allene] 2.24

Stoichiometric conditions with OsO₄ in the presence of Chloramine-T led to the formation of polycyclic ether 5.55. The detailed mechanism for the formation of 5.55 was not clear, but one can presume that the over-oxidation product must be involved as an intermediate.

Scheme 5.6-3: Polycyclic Ether Formation

Long-range correlations through oxygens between the C6 methyl protons and C10 carbon (C6-CH₃ ↔ C10) as well as the C4 protons and C10 carbon (C4-H ↔ C10) confirmed the formation of cyclic ethers. The position of C10 was confirmed by long-range correlation between the C13 protons and C10 carbon (C13-H ↔ C10).
5.7. Cyclic Allene Osmylation

In chapter 4, we showed that treatment of macrocyclic bis[allene] 4.1 with OsO$_4$ can give 6,9-bicyclomacrolide 4.53 or C9-C12 hydroxyenone 4.59 if the osmylation was done stepwise and the hydroxyl group at C6 was masked before subsequent oxidation.

Model macrolactone of type 2.24 demonstrated that hydrolysis of the osmate ester in the presence of an electrophile will lead to the electrophile-captured adduct even in bis[allene] macocyclic platform. We can easily envision the synthetic utility of halogenated macrolide. Hence, Kai Liu reacted mono-osmylated adduct 4.50 with OsO$_4$ (1.1 equiv.) in t-BuOH:H$_2$O. NCS in t-BuOH and H$_2$O solution was slowly added to the
osmate enol ester in solution for 3 hours. The product showed chlorine addition 5.56 at C10 (Scheme 5.7-2). In the analogous way, NBS in t-BuOH and H2O solution was added to the osmate enol ester from bis[allene] macrolactone 4.1. Due to the reactivity of NBS with allenes, the complex mixture of products was observed on TLC and they were very hard to separate from flash column chromatography. After exhaustive chromatography, we could isolate mono bromine adduct 5.57 adduct in 10% yield.

Scheme 5.7-2: Chlorine Addition to Macrolactone

Since chlorine and bromine atoms possess similar Van der Waals radii to a methyl group (Cl = 1.80 Å, Br = 1.95 Å, Me = 2.00 Å), they could be possible candidates for methyl group bioisostere in erythromycin analogs.15 Especially, the chlorine atom is viewed not only to be isosteric but also isolipophilic with the methyl group and its replacement showed the alternation in drug metabolism.15
In modern medicinal chemistry, fluorinated compounds are synthesized on a routine basis.\textsuperscript{16} Owing to its chemical stability and small size, fluorine is often regarded as a hydrogen bioisostere and has become an important tool in modern drug discovery to fine-tune biological effects and for studies of binding affinity in protein-ligand complexes.\textsuperscript{17}

**Scheme 5.7-3: Fluorine and Bromine Addition to Macrolactone**

To incorporate fluorine, bis[allene] macrolactone \textit{4.1} was treated with OsO\textsubscript{4} followed by a solution of Selectfluor in \textit{t}-BuOH:H\textsubscript{2}O (1:1) (Scheme 5.7-3). This reaction was not optimized. The isolated products (\textit{5.58} and \textit{5.59}) showed fluorine addition. The syn addition of fluorine at C10 was confirmed by observation of a strong nOe between H10 and the C8 methyl group. Contrary to this, fluorine addition at C4 showed anti addition as expected.

**Figure 5.7-1: Coupling Constants and nOe Analysis of Cyclic Ether Formation**
Although the precise structures of the intermediates are a matter of speculation, the osmate enol ester intermediate 5.60 formed and subsequent decomposition of the osmate enol ester captured the electrophile at C4 syn-facial to the smaller substituent (methyl vs. osmate ester at C6). However, the osmylation at the C10-C12 allene resulted in β-elimination (5.62 → 5.63) and subsequent intramolecular conjugate addition formed enolate (5.63 → 5.64). Considering the relative ground state minimum energies, z-enolate is preferred and the steric in z-enolate preferred the top face access of electrophile. The same mechanistic rationale could be applied for the stereochemistry of chlorine and bromine adducts (5.56 and 5.57).
5.8. Conclusion

We discovered that a) the catalytic amount of OsO₄ with co-oxidants can be utilized to oxidize the chiral allenes with high regio- and stereoselective manner; b) the presence of electrophiles not only accelerates the rate of the reaction but also provides the multicomponent allene hydroxylation/electrophile capture adduct; c) when applied on the cyclic allene system, the reaction showed the complete stereoselection; d) the compatible electrophiles are Selectfluor, NCS, NBS, Chloramine-T, and Eshenmoser’s salt; e) taken together these advances have led to one general procedure which is applicable to a wide range of allenic substrates.
5.9. References


Chapter 6

The Ligand Design and Synthesis for siRNA Delivery

6.1. Introduction

Nanoscience refers to the study of manipulating matter on an atomic and molecular scale of approximately 1~1000 nm and their applications in various fields such as solar cells, electronic devices, drug/gene delivery, regenerative medicine, tissue engineering, and biological diagnosis.\(^1\)\(^2\) This field has been rapidly growing in the past two decades, and its biological application has been especially widely demonstrated as a proof-of-principle. However, a practical application to clinical medicine is required to solve many key problems concerning cancer biology for therapy as well as regenerative medicine using stem cells.

In this study, I focused on the design and synthesis of target specific quantum dot-based platforms to induce the apoptosis of brain cancer cells and to show their capacity to be extended to in vivo systems for the further clinical purpose of using them as theragnostics which combines therapy and diagnosis.\(^3\) This work in chapter 6 was all done in collaboration with Prof. Ki-Bum Lee’s group at Rutgers.
6.2. Quantum Dots (QDs)

QDs have been regarded as a representative nanosystem because it shows novel quantum physical properties such as quantum confinement effect and quantum size effect beyond classical mechanics.\textsuperscript{4,5} According to their sizes, QDs emit photons with different wavelengths due to different band gap energies between the conduction and valence bands: smaller sizes emit in the short wavelength range and larger size emits longer wavelength photons, which means the emission wavelength is readily tunable.

Figure 6.2-1: UV-Vis Absorption Spectra and TEM Image of Quantum Dots
(adapted with permission from ref. \textsuperscript{3})

Moreover, they have broad absorption ranges and narrow emission peaks, which enable multicolor detection with a single light source. Most of all, high quantum yields
and extinction coefficients generate about fifty times higher brightness than conventional organic dyes. Their enhanced photostability makes them ideal candidates for the substitution of organic dyes in the fields of single molecule biophysics and cell biology. In addition, QDs are especially suitable in biological applications since they provide high surface area which is ideal for a delivery agent and high quantum yield makes them the ideal tool for tracking agent.

However, despite of its advantages as described above, several drawbacks exist and need to be addressed for practical application, especially on cell biology. Its toxicity to cells is a major issue. Often times, the coating of quantum dots is done with mercaptoacetic acid, which is cytotoxic.\(^6\) Even though the coating of the quantum dots is compromised, the metallic core (i.e., cadmium or selenium ions) or the dissolution of the core can be toxic. Quantum dot metabolism and degradation within the body is still unknown and several studies have shown the accumulation of the quantums dots in the kidney, spleen, and liver.\(^7\) In addition, the bioconjugation with different molecules will increase the size of the dots making delivery into cells more difficult.

### 6.3. **Glioblastoma Multiforme (GBM) and the Mutant Epidermal Growth Factors Receptor (EGFRvIII)**

Glioblastoma multiforme (GBM) originating from glial cells is known to be the most malignant, invasive, fast-progressive, and difficult-to-treat brain tumor with just 10-12 months of the mean survival rate after cancer treatment.\(^8-10\) Therefore, it is necessary to develop novel early diagnostic tools and therapeutic strategies and nanotechnology can be a promising tool.
About 40% of glioblastoma show overexpression and amplification of the epidermal growth factor receptor (EGFR) gene locus and about half of these tumors express a mutant receptor (EGFRvIII) which lacks amino acids from 6 to 273 in the extracellular domain and is always activated without ligand binding.\textsuperscript{11}

We targeted EGFRvIII, since it is known that knockdown of this gene is one of the most effective ways to down-regulate the PI3K/Akt signaling pathway.\textsuperscript{12, 13} The PI3K/Akt signaling pathway plays a critical role in cell proliferation and apoptosis. Therefore, several drug molecules were developed to inhibit the function of the proteins in this pathway (i.e., Erlotinib/Gefinib for EGFR, and Rapamycin for mTOR). EGFR forms a dimeric complex upon the ligand binding on the extracellular domain. The kinase domain in cytoplasm region of each monomer is close together and subsequently phosphorylates the tyrosine residue on the long and flexible tail. This complex sends the proliferation triggering signal to following proteins in the downstream such as Akt, mTOR, and S6.

6.4. \textit{siRNA}

RNA interference (RNAi) is a system within living cells that is responsible for controlling which genes are active and how active they are. Two types of small RNA molecules; microRNA (miRNA) and small interfering RNA (siRNA), are central to RNA interference pathway. RNAs are the direct products of genes. These small RNAs can bind to target messenger RNA (mRNA) and either increase or decrease their activity. This usually results in translational repression or target degradation and gene silencing. RNAi-based approach can sequence-specifically inhibit the expression of targeted
oncogenes. Therefore, it enables modulation of signaling pathways and regulates the behavior of malignant tumor cells.\textsuperscript{14, 15}

**Figure 6.4-1: Mechanism of RNA interference\textsuperscript{16}**

However, in order to harness the full potential of this approach, the prime requirements are to deliver the siRNA molecules with high selectivity and efficiency into tumor cells and to monitor both siRNA delivery and the resulting knock-down effects at the single cell level.

Herein, we describe the synthesis and target-specific delivery of multifunctional siRNA-QD constructs for selectively inhibiting the expression of EGFRvIII in target
human U87 glioblastoma cells, and subsequently monitor the resulting down-regulated signaling pathway with high efficiency.

6.5. **Design and Synthesis of the multifunctional QD platform for delivery and tracking of siRNA**

We synthesized two types of siRNA-QD conjugates, one for siRNA delivery and the other for siRNA tracking (Figure 6.5-1). My former colleague Dr. Jongjin Jung prepared CdSe/CdS/ZnS core-shell QDs\textsuperscript{17, 18} with a diameter of 7 nm and coated with either trioctylphosphine oxide (TOPO) or hexadecylamine (HDA). In order to make the QD constructs water-soluble and suitable for conjugating with siRNA, we replaced these hydrophobic ligands with a dihydriophosphate acid (DHLA) derivatized with an amine-terminated polyethylene glycol (PEG) spacers.
We hypothesized that since the dithiol moiety would provide strong coordination to the QD surface and increase stability in aqueous media, the PEG space would increase water solubility and reduce nonspecific binding, and the amine group would enable conjugation to the siRNA element.

Two heterofunctional linkers (HFL) were synthesized and evaluated for siRNA conjugation. The linker **HFL1** was designed to release siRNA upon entering the cell by cleavage of the disulfide linkage, through enzymatic reduction or ligand exchange (e.g. glutathione). The second linker **HFL2** was designed to be more robust, thereby enabling evaluation of cellular uptake and localization of the siRNA construct within the cellular compartments.
The final design component was the combination of three biofunctional molecules, siRNA, thiol-modified RGD (Arginine-Glycine-Aspartic Acid) peptide and thiol-modified HIV-Tat derived peptide.

Since brain tumor cells (U87 and U87-EGFRvIII) overexpress the integrin receptor protein $\alpha_v\beta_3$, they strongly bind to the RGD binding domain.$^{21}$ Thereby, RGD-functionalized siRNA-QDs selectively accumulate in brain tumor cells in vitro, and can be tracked by fluorescence microscopy. In addition, the HIV-Tat peptide promotes efficient transfection of siRNA-QDs in cells when it is directly attached to the QD surface.$^{22}$

6.6. Transfection efficiency & target-specific delivery of siRNA-QDs

Prior to targeting EGFRvIII, the transfection efficiency was evaluated with EGFP (Enhanced Green Fluorescent Protein). Three different siRNA-QD constructs were prepared as follows.

- Construct modified with the RGD peptide only (siRNA-QD-RDG)
- Construct with HIV-Tat peptide only (siRNA-QD-HIV-Tat)
- Construct with both HIV-Tat and RGD peptide (siRNA-QD-RGD-HIV-Tat)

Among three, siRNA-QD-RGD-HIV-Tat, (the ratio of siRNA/RGD/HIV-Tat being 1:1:10 per QD) showed the maximum internalization within U87-EGFP cells. Therefore, this optimal condition was used for subsequent experiments.
The U87-EGFP cell line was then treated with siRNA-QD, modified with HIV-Tat and RGD, then simultaneously imaged by fluorescent microscopy.

**Figure 6.6-1: Transfection and Knockdown Effect by siRNA-QD Conjugates**

(adapted with permission from ref. 3)

(A) Control U87 cells without siRNA-QDs; (B) U87 cells with siRNA-QDs: A1, B1 = phase-contrast image, A2, B2 = fluorescence image. Scale bar: 100 μm.

In comparison to control U87-EGFP cells without siRNA-QD conjugates (A1 and A2, Figure 6.6-1), U87-EGFP cells with siRNA-QD conjugates (B1 and B2, Figure 6.6-1) clearly indicate the significant internalization of siRNA-QDs into the cells, which were easily distinguishable from the control cells owing to the bright fluorescence (red dots) of the QDs. Yellow arrows (B1 and B2, Figure 6.6-1) also indicate knockdown of EGFP in U87 cells as expected.
6.7. **Target-specific delivery of siRNA-QDs**

To further demonstrate the target-specific delivery of the siRNA-QDs, we incubated the siRNA-QDs, modified with HIV-Tat and RGD peptides, in co-cultures of the U87-EGFP cell line with PC-12 cell lines which are less-tumorigenic and have less integrin receptors. We hypothesized that the malignant U87 cells have higher cellular uptake of siRNA-QDs than the less tumorigenic PC-12 because the presence of RGD peptide on the surface of siRNA-QD led to specific binding with integrin receptors which over-expressed in the malignant U87 cells. As expected, the U87-EGFP cells showed selective accumulation of the QDs demonstrating the target-specific delivery of the siRNA-QDs (Figure 6.7-1).

**Figure 6.7-1: Co-cultures of U87-EGFP cells and PC-12 (adapted with permission from ref. 3)**

![Image showing EGFP cells (yellow arrow) and PC12 (blue arrow), Scale bar: 100 μm](image)

6.8. **Knockdown of EGFRvIII as a chemotherapeutic target by siRNA-QDs**

The successful demonstration of the selective manipulation of the U87-EGFP cell line led us to the examination of siRNA-QD constructs with EGFRvIII. U87-EGFRvIII cells were genetically modified to overexpress EGFRvIII, which only expressed within
cancer cells. This cell type was incubated with our siRNA-QDs, modified with Tat and RGD peptides. The cells were simultaneously imaged for the internalization of siRNA-QDs using fluorescence microscopy. Relative to the control (U87-EGFRvIII without siRNA-QDs), significant cell death was observed in the wells loaded with siRNA-QDs against EGFRvIII after 48 hours (A3, Figure 6.8-1). After 48 hours, the cells have clearly shrunk and appear to have collapsed (yellow arrow, A3, Figure 6.8-1).

**Figure 6.8-1: Knockdown of EGFRvIII in U87-EGFRvIII** (adapted with permission from ref. ³)

Western immunoblotting (Figure 6.8-2) showed the considerable decrease in the expression of EGFRvIII, and down-regulation of phosphor-Akt and phospho-S6 relative to control.
Taken all together, these results demonstrate the specificity of the siRNA against EGFRvIII, the inherent noncytotoxicity of the QDs, and the facile evaluation and manipulation of cancer cell proliferation with multifunctional QD constructs.

6.9. Conclusion

The small interfering RNA (siRNA) enables manipulation of key oncogenes that modulate signaling pathways and thereby regulate the behavior of malignant tumor cells. In collaboration with Prof. Lee’s group at Rutgers, we demonstrated as follows; (a) the synthesis and target-specific delivery of multifunctional siRNA-QD constructs; (b) the selective inhibition of the expression of epidermal growth factor receptor variant III
(EGFRvIII) in target human U87 glioblastoma cells; (c) the subsequent down-regulated signaling pathway with high efficiency was monitored.
6.10. References


Chapter 7

Experimental

7.1. General

All the commercial available starting materials, reagents and solvents were purchased from the suppliers (Aldrich–Sigma, Acros, Strem, TCI America, Fischer Scientific and Ochem.) and used without purification unless otherwise stated. Anhydrous solvent such as tetrahydrofurn (THF), diethyl ether (Et$_2$O), dichloromethane (DCM), toluene (PhMe) were purchased from Sigma–Aldrich, and then passed over a solvent purification system containing alumina based columns and dried by activated 4 Å molecular sieves for 1 hour before use. Anhydrous THF was further distilled from calcium hydride before use. All the glassware used for anhydrous reaction was flame–dried under vacuum. All the reactions were conducted under the atmosphere of high purity argon gas. The progress of all reactions were monitored by silica gel thin layer chromatography (TLC, Dynamic Absorbents Inc.), visualized under UV and/or stained using different stains such as anisaldehyde, vanillin, cerium ammonium molybdate, KMnO$_4$. Crude products were purified by flash column chromatography (FCC) on 120–400 mesh silica gel if necessary. Proton nuclear magnetic resonance spectra ($^1$H NMR) were obtained from either a Varian–300 instrument (300 MHz), Varian–400 instrument (400 MHz), Varian–500 instrument (500 MHz) or Inova–600 instrument (600 MHz). Chemical shifts are reported in ppm with tetramethylsilane (TMS) being the internal standard. Data is reported as follows: chemical shift (multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet, coupling constants in Hz and integration).
Carbon nuclear magnetic resonance spectra (\(^{13}\)C NMR) were obtained from either a Varian–400 instrument (100 MHz), Varian–500 instrument (125 MHz) or Inova–600 instrument (150 MHz). Chemical shifts are reported in ppm with tetramethylsilane (TMS) being the internal standard. Optical rotations were recorded at 25 °C using the sodium D line (589 nm), using Perkin–Elmer 241 polarimeter. Mass spectra were recorded on a Finnigan LCQ–DUO mass spectrometer (ESIMS) or Finnigan high resolution mass spectrometer (HRMS). Infrared (FTIR) spectra were recorded on ATI Mattson Genesis Series FT–Infrared spectrophotometer.
7.2. Chapter 3

The 4(S)–benzyl N–propionyl oxazolidinone 3.1 (20.3 g, 87.0 mmol) was dissolved in anhydrous DCM (607 mL). To this solution was added di–nbutylboron triflate (96.0 mL, 96.0 mmol) and TEA (10.6 g, 104 mmol) dropwise at −78 °C. The reaction mixture was then warmed to 0 °C and stirred for 1 hour at 0 °C, and then cooled back to −78 °C. Anhydrous DCM solution of 1.1–dimethoxy acetaldehyde (87.0 mL, 148 mmol, 0.18 g/mL) was added to the reaction mixture slowly at −78 °C, then the resulting solution was slowly warmed to 0 °C over 1 hour and stirred for 1 hour at 0 °C. The reaction was quenched with 100 mL solution of methanol and phosphate buffer (pH = 7.4) in 1:3 ratio at 0 °C, followed by the addition of 100 mL solution of 30% H₂O₂ and methanol (1:2 ratio). The reaction was stirred for 10 minutes at 0 °C, diluted with 200 mL DCM, washed with cold water (2 x 100 mL). Organic layer was separated and dried over anhydrous Na₂SO₄. The solvent was removed in vacuo. Silica gel column chromatography using 40% EtOAc in hexane gave aldol product 3.2 as white solid (26.4 g, 78.3 mmol, 90% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.35 – 7.25 (m, 3H), 7.21 (d, J = 7.0 Hz, 2H), 4.72 – 4.65 (m, 1H), 4.33 (d, J = 6.0 Hz, 1H), 4.23 – 4.15 (m, 2H), 4.05 – 3.96 (m, 1H), 3.42 (s, 3H), 3.38 (s, 3H), 3.26 (dd, J = 13.5, 3.5 Hz, 1H), 2.78 (dd, J = 13.5, 10 Hz, 1H), 2.68 – 2.60 (bs, 1H), 1.32 (d, J = 7.0 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 176.1, 153.2, 135.4, 129.6 (2), 129.1 (2), 127.5, 104.9, 71.4, 66.3, 55.4, 54.9, 54.4, 39.2, 38.1, 12.8; m/z
(ESIMS) calculated for $[\text{C}_{17}\text{H}_{23}\text{NO}_6\text{Na}]^+$: 360.2, found: 360.2; $[\alpha]^{25}_D = 51.5$ ($c = 0.01$, CHCl$_3$); IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 3485, 2937, 1778, 1696, 1386.

Aldol product 3.2 (17.6 g, 52.2 mmol), 4 Å powdered molecular sieve (20.0 g) and Ag$_2$O (33.0 g, 142 mmol) were mixed in anhydrous DCM (332 mL). Stirred for 10 minutes at room temperature, and then BnBr (24.4 g, 143 mmol) was added. The reaction mixture was then stirred thoroughly for 2 days under argon in the darkness at room temperature, and then filtered over a short column of celite. The solid residue was rinsed with DCM (3 x 100 mL). The solvent was removed in vacuo, silica gel column chromatography using 20% EtOAc in hexane gave compound 3.3 as colorless oil (20.3 g, 47.5 mmol, 91% yield).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.39 – 7.23 (m, 8H), 7.19 (d, $J = 7.0$ Hz, 2H), 4.81 (d, $J = 11.5$ Hz, 1H), 4.64 (d, $J = 11.5$ Hz, 1H), 4.59 – 4.53 (m, 1H), 4.34 (d, $J = 6.0$ Hz, 1H), 4.14 – 4.03 (m, 3H), 3.84 (dd, $J = 7.5$, 6.5 Hz, 1H), 3.43 (s, 3H), 3.34 (s, 3H), 3.24 (dd, $J = 13.5$, 3.5 Hz, 1H), 2.75 (dd, $J = 13.5$, 9.5 Hz, 1H), 1.31 (d, $J = 7.0$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 175.2, 153.3, 138.6, 135.5, 129.6 (2), 129.0 (2), 128.4 (2), 128.2 (2), 127.8, 127.4, 107.0, 79.8, 74.4, 66.1, 55.5, 55.4, 39.5, 38.1, 13.8; $m/z$ (ESIMS) calculated for $[\text{C}_{24}\text{H}_{29}\text{NO}_6\text{Na}]^+$: 450.2, found: 450.2; $[\alpha]^{25}_D = 22.0$ ($c = 0.01$, CHCl$_3$); IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 2934, 1778, 1698, 1383, 1107.
Aldol product 3.2 (8.25 g, 24.5 mmol), Ag₂O (17.0 g, 73.4 mmol) and 4 Å powdered molecular sieve (12.0 g) were mixed together and to that dry DCM (156 mL) was added. After stirring for 10 minutes at room temperature, 3-flurobenzyl bromide (6.00 mL, 48.9 mmol) was added to the reaction. The reaction was then stirred thoroughly for 5 days under nitrogen at room temperature. The reaction was then filtered through celite and the solid residue was rinsed with DCM (3 x 100 mL). Evaporation of the organic filtrate gave the crude product, which upon further purification by silica gel column chromatography using 20% EtOAc in hexane gave 3.32 as colorless oil (10.3 g, 23.1 mmol, 94% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.35 – 7.27 (m, 4H), 7.23 – 7.19 (m, 2H), 7.14 – 7.08 (m, 2H), 6.99 – 6.93 (m, 1H), 4.80 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 11.5 Hz, 1H), 4.64 – 4.60 (m, 1H), 4.35 (d, J = 6.4 Hz, 1H), 4.15 – 4.08 (m, 2H), 4.10 (d, J = 7.1 Hz, 1H), 3.82 (dd, J = 7.6, 6.1 Hz, 1H), 3.43 (s, 3H), 3.35 (s, 3H), 3.42 (dd, J = 13.5, 3.3 Hz, 1H), 2.76 (dd, J = 13.5, 2.8 Hz, 1H), 1.30 (d, J = 6.9 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 175.0, 163.8, 153.2, 141.1, 135.3, 129.8, 129.4, 128.9, 127.3, 123.2, 114.7, 114.4, 114.3, 106.7, 79.9, 73.4, 66.0, 55.3, 55.2, 55.0, 39.3, 38.0, 15.3.
Compound **3.3** (17.1 g, 40.0 mmol) was dissolved in Et₂O (370 mL), and then 11.0 mL methanol was added. The reaction mixture was cooled to 0 °C and then added a solution of LiBH₄ (35.2 mL, 77.4 mmol, 2.20 M solution in THF) slowly. Stirred at 0 °C for 2 hours, and then quenched with aqueous NH₄Cl (50 mL), extracted with EtOAc (3 x 200 mL). Organic layer combined, dried over anhydrous Na₂SO₄. The solvent was removed in vacuo. Silica gel column chromatography using 15% EtOAc in hexane gave **3.4** as colorless oil (9.90 g, 38.9 mmol, 97% yield).

**¹H NMR** (500 MHz, CDCl₃) δ 7.38 – 7.32 (m, 4H), 7.30 – 7.25 (m, 2H), 4.82 (d, J = 11.5 Hz, 1H), 4.58 (d, J = 11.5 Hz, 1H), 4.39 (d, J = 6.5 Hz, 1H), 3.59 (dd, J = 6.0, 3.5 Hz, 1H), 3.59 – 3.46 (m, 2H), 3.49 (s, 3H), 3.41 (s, 3H), 2.02 – 1.96 (m, 1H), 1.90 (bs, 1H), 0.94 (d, J = 7.0 Hz, 3H); **¹³C NMR** (125 MHz, CDCl₃) δ 138.9, 128.5 (2), 128.2 (2), 127.8, 106.5, 79.6, 74.1, 65.8, 56.2, 54.6, 36.9, 11.4; m/z (ESIMS) calculated for [C₁₄H₂₂O₄Na]⁺: 277.2, found: 277.2; IR ν_max(neat)/cm⁻¹ 3431, 2934, 1454, 1071.

Primary alcohol **3.4** (6.00 g, 23.7 mmol) was dissolved in anhydrous DCM (184 mL), and then added diazabicyclo[2.2.2]octane (DABCO) (3.66 g, 32.6 mmol). The reaction mixture was cooled down to 0 °C and tosyl chloride (4.97 g, 26.1 mmol) was added. The reaction mixture was warmed up to room temperature and stirred for 1 hour. The reaction was then diluted with 150 mL of DCM, washed with aqueous NH₄Cl (3 x 50 mL), water (50 mL). Organic layer combined and dried over anhydrous Na₂SO₄, and then...
concentrated down to dryness to give the crude tosylate, which could be used for the next step immediately without further purification.

The crude tosylate was azeotroped in toluene, and then dissolved in anhydrous DMSO (50 mL). To this, lithium acetylde-ethylenediamine (4.37 g, 47.4 mmol) solution in 100 mL DMSO was added at 0 °C slowly for 10 minutes. The reaction was stirred for 4 hours at room temperature. Upon the complete consumption of the tosylate, the reacton mixture was cooled down to 0 °C, and then quenched carefully with aqueous NH₄Cl (50 mL). The organic layer was extracted with EtOAc (300 mL), washed with water (3 x 100 mL), and then dried over anhydrous Na₂SO₄. The solvent was removed in vacuo. Silica gel column chromatography using 5% EtOAc in hexane gave 3.5 as colorless oil (4.90 g, 18.7 mmol, 80% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.30 (m, 4H), 7.28 – 7.22 (m, 1H), 4.68 (d, J = 11.5 Hz, 1H), 4.56 (d, J = 11.5 Hz, 1H), 4.36 (d, J = 7.0 Hz, 1H), 3.65 (dd, J = 7.0, 3.0 Hz, 1H), 3.47 (s, 3H), 3.38 (s, 3H), 2.23 – 2.12 (m, 2H), 2.10 – 2.00 (m, 1H), 1.96 (t, J = 7.5 Hz, 1H), 0.98 (d, J = 7.0 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 139.3, 128.4 (2), 127.9 (2), 127.6, 106.4, 83.7, 80.3, 74.8, 69.6, 56.0, 53.8, 34.4, 23.5, 14.0; m/z (ESIMS) calculated for [C₁₆H₂₂O₃Na⁺]: 285.1, found: 285.2; IR νmax(neat)/cm⁻¹ 3295, 2935, 2116, 1096.
The alkyne 3.14 was prepared from 3.32 in 3 steps using same procedure used for the synthesis of alkyne 3.5.

$^1$H NMR (500 MHz, CDCl$_3$) δ (7.34 – 7.23 (1H, m), 7.14 – 7.03 (2H, m), 6.97 – 6.91 (1H, m), 4.86 (1H, d, $J = 12.0$ Hz), 4.56 (1H, d, $J = 12.0$ Hz), 4.37 (1H, d, $J = 7.0$ Hz), 3.66 (1H, dd, $J = 7.0$, 2.5 Hz), 3.47 (3H, s), 3.39 (3H, s), 2.32 – 2.12 (2H, m), 2.10 – 2.00 (1H, m), 1.97 (1H, t, $J = 2.5$ Hz), 0.99 (3H, d, $J = 7.0$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 164.0, 162.1, 142.0 (d, $J = 69$ Hz), 129.9 (d, $J = 66$ Hz), 123.1 (d, $J = 29$ Hz), 114.6, 114.4, 114.4, 114.2, 106.3, 83.5, 80.4, 73.9 (d, $J = 7.5$ Hz), 69.7, 56.0, 53.8, 34.4, 23.4, 14.0; m/z (ESIMS) calculated for [C$_{16}$H$_{21}$FO$_3$Na]$^+$: 303.2, found: 303.2; IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 3306, 2936, 2117, 1789, 1592.

![Diagram of reactions](image)

Compound ent-3.2 and ent-3.3 could be synthesized by using the same procedure used for the synthesis of compound 3.2 and 3.3, respectively. The values for the observed optical rotation ([α]$^D_{25}$) are –50.0 ($c = 0.01$, CHCl$_3$) for the compound ent-3.2 and –21.0 ($c = 0.01$, CHCl$_3$) for the compound ent-3.3.

![Diagram of compound 3.7](image)

Benzyl protected aldol product ent-3.3 (3.90 g, 9.22 mmol) was dissolved in 46 mL of water: acetic acid: trifluoroacetic acid = 1:4:1 mixed solution at room temperature
for 3 hrs 30 minutes. The acidic solution was azeotroped with toluene (5 x 20 mL) and resulting crude product was taken into the next step immediately without further purification. It could also be further purified by silica gel column chromatography using 15% EtOAc in hexane gave \textit{3.7} as colorless viscous oil (3.16 g, 8.30 mmol, 90% yield).

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 9.81 (s, 1H), 7.38 – 7.22 (m, 8H), 7.17 (d, \(J = 7.0\) Hz, 2H), 4.75 (d, \(J = 12.5\) Hz, 1H), 4.64 – 4.54 (m, 1H), 4.59 (d, \(J = 12.5\) Hz, 1H), 4.32 – 4.24 (m, 1H), 4.16 – 4.06 (m, 2H), 3.92 (d, \(J = 6.0\) Hz, 1H), 3.20 (dd, \(J = 13.5, 3.5\) Hz, 1H), 2.76 (dd, \(J = 13.5, 10\) Hz, 1H), 1.33 (d, \(J = 7.0\) Hz, 3H); \textsuperscript{13}C NMR (125MHz, CDCl\textsubscript{3}) \(\delta\) 202.0, 173.8, 153.1, 137.2, 135.1, 129.6 (2), 129.1 (2), 128.7 (2), 128.4, 128.3 (2), 127.5, 83.3, 73.1, 66.4, 55.4, 41.5, 37.8, 13.4; \(m/z\) (ESIMS) calculated for [C\textsubscript{22}H\textsubscript{23}NO\textsubscript{5}Na]\textsuperscript{+}: 404.2, found: 404.2; IR \(\nu_{\text{max}}\) (neat)/cm\textsuperscript{-1} 1778, 1730, 1693, 1390, 1212.

3-flurobenzyl protected aldol product \textit{ent-3.32} (1.23 g, 2.77 mmol) was dissolved in water: acetic acid: trifluoroacetic acid = 1:4:1 mixed solution (13.7 mL) and stirred for 2 hours at room temperature. The mixture was azeotroped with toluene (3 x 50 mL) and resulting crude product upon further purification by silica gel column chromatography using 15% EtOAc in hexane gave \textit{3.15} as colorless viscous oil (1.05 g, 2.63 mmol, 95% yield).

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 9.82 (s, 1H), 7.34 – 7.24 (m, 4H), 7.19 – 6.95 (m, 4H), 4.73 (d, \(J = 12.5\) Hz, 1H), 4.71 – 4.63 (m, 2H), 4.57 (d, \(J = 12.5\) Hz, 1H), 4.34 – 4.26 (m, 1H), 4.16 – 4.13 (m, 1H), 4.12 (quartet, \(J = 7.0\), 1H), 3.92 (dd, \(J = 6.5, 1.2\) Hz,
Alkyne 3.5 (3.44 g, 13.1 mmol) was dissolve in Et₂O (130 mL) then cooled to –78 °C. To that mixture was added n–BuLi (8.2 mL, 13 mmol) slowly. The reaction mixture was stirred at –78 °C for 1 hour and added a solution of ZnBr₂ (4.13 g, 18.4 mmol) in Et₂O (35.9 mL). The reaction flask was moved to 0 °C bath. At 10 °C, the solution of aldehyde 3.7 (2.00 g, 5.24 mmol) in Et₂O (12.5 mL) was added dropwise over 1 hour via syringe pump. The reaction was stirred overnight. The reaction was quenched with aqueous NH₄Cl (50 mL) at 0 °C, and then extracted with EtOAc (200 mL). The organic layer was washed with water (2 x 50 mL), and then dried over Na₂SO₄. The organic layer was concentrated down to dryness to give the crude product (8:1 ratio by ¹H NMR). Silica gel column chromatography using 10% EtOAc in hexane gave major isomer 3.13 as colorless oil (1.53 g, 3.29 mmol, 63% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.24 (m, 10H), 5.12 (td, J = 2.0 Hz, 6.5 Hz, 1H), 4.82 (d, J =11.5 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.53 (d, J = 11.5 Hz, 1H), 4.51 (d, J = 11.5 Hz, 1H), 3.88 (dd, J = 9.5, 6.5 Hz, 1H), 3.57 (dd, J = 10.0, 7.0 Hz, 1H), 3.47 (s, 3H), 3.37 (s, 3H), 2.86 – 2.76 (m, 1H), 2.38 – 2.20 (m, 2H), 2.10 – 2.00 (m, 1H), 1.25 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 176.0, 139.1, 137.1, 128.8 (2), 128.5 (2), 128.4, 128.1 (2), 127.9 (2), 127.7, 106.4, 90.5, 81.0, 80.5, 74.7, 73.9, 72.4, 70.5, 56.1, 54.1, 39.4, 34.4, 24.0, 14.0, 12.7; m/z (ESIMS)
calculated for [C_{28}H_{34}O_{6}Na]^+: 489.3, found: 489.2; IR ν_{max}(neat)/cm^{-1} 2935, 2238, 1786, 1454.

Alkyne 3.14 (54.9 mg, 196 μmol) was dissolve in EtO (2 mL) then cooled to –78 °C. To that mixture was added n–BuLi (78 μL, 0.20 mmol) slowly. The reaction mixture was stirred at –78 °C for 1 hour and added a solution of ZnBr₂ (44.1 mg, 196 μmol) in EtO (0.4 mL). The reaction flask was moved to 0 °C bath. At 10 °C, the solution of aldehyde 3.15 (60.2 mg, 151 μmol) in EtO (0.4 mL) was added dropwise over 1 hour via syringe pump. The reaction was stirred overnight. The reaction was quenched with aqueous NH₄Cl (50 mL) at 0 °C, and then extracted with EtOAc (20 mL). The organic layer was washed with water (2 x 5 mL), and then dried over Na₂SO₄. The organic layer was concentrated down to dryness to give the crude product (8:1 ratio by ¹H NMR). Silica gel column chromatography using 10% EtOAc in hexane gave major isomer 3.16 as colorless oil (26.6 mg, 52.9 μmol, 35% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.40 – 7.25 (m, 3H), 7.09 – 6.93 (m, 5H), 4.88 – 4.82 (m, 2H), 4.75 (d, J = 12.0 Hz, 1H), 4.64 (d, J = 12.0 Hz, 1H), 4.54 (d, J = 11.7 Hz, 1H), 4.36 (d, J = 6.8 Hz, 1H), 3.89 (dd, J = 7.1, 5.6 Hz, 1H), 3.54 (dd, J = 7.0, 2.9 Hz, 1H), 3.48 (s, 3H), 3.39 (s, 3H), 2.68 (quartet, J = 7.3 Hz, 1H), 2.37 – 2.19 (m, 2H), 2.13 – 2.01 (m, 1H), 1.36 (d, J = 7.3 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H).
Acetal 3.13 (1.90 g, 4.10 mmol) was dissolved in 42.5 mL mixed solution of acetic acid, trifluoroacetic acid and water (4:1:1) at room temperature and stirred for 12 hours. The solvent was azeotroped with toluene (5 x 100 mL) to give a crude product which could be used for the next step without further purification. It could also be further purified by silica gel column chromatography using 12% EtOAc in hexane gave 3.23 as colorless viscous oil (1.50 g, 3.57 mmol, 90% yield).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.65 (s, 1H), 7.44 – 7.25 (m, 10H), 5.11 (d, $J$ = 6.0 Hz, 1H), 4.67 (d, $J$ = 13.0 Hz, 2H), 4.54 (d, $J$ = 11.5 Hz, 1H), 4.46 (d, $J$ = 12.0 Hz, 1H), 3.95 – 3.85 (m, 2H), 2.85 – 2.75 (m, 1H), 2.48 – 2.26 (m, 2H), 2.26 – 2.16 (m, 1H), 1.27 (d, $J$ = 7.0 Hz, 3H), 1.01 (d, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 204.3, 175.9, 137.6, 137.1, 128.8 (2), 128.7 (2), 128.5, 128.3, 128.2 (2), 128.0 (2), 89.2, 85.1, 81.0, 74.9, 73.4, 72.4, 70.4, 39.4, 35.2, 23.1, 14.5, 12.7; $m/z$ (ESIMS) calculated for [C$_{26}$H$_{28}$O$_5$Na]$^+$: 443.2, found: 443.2; IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 2935, 2240, 1786, 1730, 1455.

Acetal 3.16 (36.3 mg, 72.0 µmol) was dissolved in 0.75 mL mixed solution of acetic acid, trifluoroacetic acid and water (4:1:1) at room temperature and stirred for 12 hours. The solvent was azeotroped with toluene (5 x 100 mL) to give a crude product which could be used for the next step without further purification. It could also be further
purified by silica gel column chromatography using 12% EtOAc in hexane gave 3.32 as colorless viscous oil (31.3 mg, 69.0 μmol, 95% yield).

\(^1\text{H NMR (500 MHz, CDCl}_3\) \(\delta\) 9.67 (s, 1H), 7.44 – 6.92 (m, 8H), 5.21–5.11 (m, 1H), 4.71 (d, \(J = 12.0\) Hz, 2H), 4.68 (d, \(J = 11.7\) Hz, 1H), 4.64 (d, \(J = 11.7\) Hz, 1H), 4.42 (d, \(J = 12.0\) Hz, 1H), 3.96 – 3.84 (m, 2H), 2.90 – 2.78 (m, 1H), 2.50 – 2.19 (m, 2H), 1.29 (d, \(J = 7.3\) Hz, 3H), 1.01 (d, \(J = 6.7\) Hz, 3H).

A solution of alkyne 3.11 (1.38 g, 7.00 mmol) in THF (33 mL) was cooled to –78 °C. To that was added MeLi (3.70 mL, 5.87 mmol) slowly. The reaction mixture was stirred for 30 minutes and to that mixture was added the hexane solution of chlorotriisopropoxytitanium (IV) (5.87 mL, 5.87 mmol). The reaction was stirred for 1 hour and to that a solution of aldehyde 3.23 (1.50 g, 3.70 mmol) in THF (15 mL) was added slowly at –78 °C. The reaction was then warmed slowly to –30 °C over 1 hour. The reaction was quenched with aqueous NH\(_4\)Cl, extracted with Et\(_2\)O. The organic layer was dried over anhydrous Na\(_2\)SO\(_4\). Evaporation of the organic filtrate gave the crude product (6:1 ratio by \(^1\text{H NMR}) which upon further purification by silica gel column chromatography using 10% EtOAc in hexane gave major isomer of 3.26 as colorless oil (2.00 g, 3.23 mmol, 89% combined yield for both diastereomers).

\(^1\text{H NMR (300 MHz, CDCl}_3\) \(\delta\) 7.16 – 7.44 (m, 10H), 5.11 (td, \(J = 2.1\) Hz, 6.0 Hz, 1H), 4.76 (d, \(J = 12.0\) Hz, 1H), 4.72 (d, \(J = 12.0\) Hz, 1H), 4.58 (d, \(J = 12.0\) Hz, 1H), 4.53 (d, \(J = 12.0\) Hz, 1H), 4.43 – 4.54 (m, 1H), 4.32 (t, \(J = 6.6\) Hz, 1H), 3.87 (dd, \(J = 9.6, 6.6\) Hz, 1H).
Hz, 1H), 3.58 (dd, J = 5.4, 5.4 Hz, 1H), 2.88 – 2.76 (m, 1H), 2.46 – 2.13 (m, 1H), 1.75 – 1.60 (m, 1H), 1.24 (d, J = 7.2 Hz, 3H), 1.06 (d, J = 6.9 Hz, 3H), 0.87 (s, 9H), 0.08 (s, 3H), 0.10 (s, 3H); 13C NMR (125 MHz, CDCl3) δ 175.9, 138.4, 137.0, 128.8, 128.6, 128.5, 128.0, 127.9, 127.9, 89.7, 88.4, 83.5, 82.6, 80.9, 77.4, 77.2, 76.9, 74.6, 74.3, 72.3, 70.4, 64.4, 63.5, 39.4, 34.6, 31.9, 26.0, 23.8, 18.4, 14.9, 12.6, 9.8, 0.2, –4.2, –4.8; m/z (ESIMS) calculated for [C37H50O6SiNa]+: 641.3, found: 641.3.

A solution of alkyne 3.11 (26.8 mg, 135 μmol) in THF (640 μL) was cooled to –78 °C. To that was added MeLi (75 μL, 0.12 mmol) slowly. The reaction mixture was stirred for 30 minutes and to that mixture was added the hexane solution of chlorotriisopropoxyltitanium (IV) (120 μL, 120 μmol). The reaction was stirred for 1 hour and to that a solution of aldehyde 3.32 (36.3 mg, 80.0 μmol) in THF (320 μL) was added slowly at –78 °C. The reaction was then warmed slowly to –30 °C over 1 hour. The reaction was quenched with aqueous NH4Cl, extracted with Et2O. The organic layer was dried over anhydrous Na2SO4. Evaporation of the organic filtrate gave the crude product (6:1 ratio by 1H NMR) which upon further purification by silica gel column chromatography using 10% EtOAc in hexane gave major isomer of 3.33 as colorless oil (46.9 mg, 72.0 mmol, 90% combined yield for both diastereomers).

1H NMR (300 MHz, CDCl3) δ 7.37 – 7.24 (m, 3H), 7.15 – 6.93 (m, 3H), 5.18 (td, J = 2.1 Hz, 6.2 Hz, 1H), 4.78 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.58 (d, J = 5.3 Hz, 1H), 4.54 (d, J = 5.3 Hz, 1H), 4.50 (dd, J = 11.7, 5.6 Hz, 1H), 4.32 (td, J = 6.5,
1.5 Hz, 1H), 3.90 (dd, J = 9.4, 6.2 Hz, 1H), 3.58 (dd, J = 5.6, 4.4 Hz, 1H), 2.89 – 2.79 (m, 1H), 2.43 – 2.16 (m, 4H), 1.71 – 1.61 (m, 2H), 1.30 (d, J = 7.3 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H) 0.89 (s, 9H), 0.14 (s, 3H), 0.07 (s, 3H).

To a solution of 3.26 (0.25 g, 0.40 mmol) in 8 mL of Et₂O was added TEA (60.7 mg, 0.600 mmol) and methanesulfonyl chloride (68.7 mg, 0.606 mmol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (0.21 g, 2.4 mmol) and MeLi (1.5 mL, 2.4 mmol) in 12 mL of Et₂O at –35 °C. The temperature was kept below –20 °C for 1 hour. The reaction was then quenched with aqueous NH₄Cl (5 mL), and then extracted in Et₂O (3 x 10 mL). The organic layer was washed with water (10 mL) and dried over anhydrous MgSO₄. After removing the solvent under reduced pressure, the crude material was subject to silica gel column chromatography giving 3.27 (0.12g, 0.020 mmol) in 50% yield.

Data of 3.27: ¹H NMR (500 MHz, CDCl₃) δ 7.89 – 7.26 (m, 10H), 5.17 – 5.12 (m, 1H), 5.00 – 4.93 (m, 1H), 4.72 (d, J = 11.7 Hz, 1H), 4.68 (d, J = 12.2 Hz, 1H), 4.64 (d, J = 11.7 Hz, 1H), 4.30 (d, J = 12.0 Hz, 1H), 4.01 (t, J = 6.1 Hz, 1H), 3.89 (t, J = 6.4 Hz, 1H), 3.71 (dd, J = 8.8, 5.4 Hz, 1H), 2.86 – 2.80 (m, 1H), 2.68 – 2.62 (m, 1H), 2.19 (dd, J = 9.5, 2.0 Hz, 1H), 1.73 (d, J = 2.9 Hz, 3H), 1.64 (d, J = 3.0 Hz, 1H), 1.63 – 1.37 (m, 2H), 1.27
(d, J = 7.1 Hz, 3H), 1.10 (d, J = 6.6 Hz, 3H), 0.91 (s, 9H), 0.88 (t, J = 7.3 Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 202.5, 175.7, 138.5, 136.9, 128.5 (2), 128.2 (2), 127.8 (4), 127.5, 127.4, 102.7, 90.3, 89.4, 80.8, 80.7, 75.3, 73.4, 72.0, 70.3, 69.9, 39.1, 38.2, 29.2 (3), 25.8, 18.2, 15.1, 13.8, 12.4, 10.0, –4.6, –5.0.

To a solution of 3.26 (1.24 g, 2.00 mmol) in 40 mL of Et\(_2\)O was added TEA (0.30 g, 3.0 mmol) and methanesulfonyl chloride (0.34 g, 3.0 mmol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (1.07 g, 12.0 mmol) and MeLi (7.49 mL, 12.0 mmol) in 60 mL of Et\(_2\)O at –35 °C. The reaction mixture was then warmed to room temperature and stirred for 1h. The reaction was then quenched with aqueous NH\(_4\)Cl (50 mL) and extracted in Et\(_2\)O (3 x 100 mL). The organic layer was washed with water (100 mL), and then dried over anhydrous MgSO\(_4\). Evaporation of solvent gave the crude product 3.28 (1.27 g) and it is subjected to the next step without silica gel column chromatography.

\(^{1}\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.36 – 7.26 (m, 10H), 5.05 – 4.90 (m, 2H), 4.70 (d, J = 11.5 Hz, 1H), 4.69 (d, J = 11.5 Hz, 1H), 4.43 (d, J = 11.5 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.03 (dd, J = 8.0 Hz, 6.0 Hz, 1H), 4.00 (t, J = 6.0 Hz, 1H), 3.66 (dd, J = 9.5, 5.5 Hz, 1H), 2.84 – 2.72 (m, 1H), 2.42 – 2.32 (m, 1H), 1.95 – 1.85 (m, 1H), 1.72 (d, J = 3.0 Hz, 3H), 1.65 (d, J = 3.0 Hz, 3H), 1.60 – 1.50 (m, 2H), 1.24 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.88 (s, 9H), 0.85 (t, J = 7.5 Hz, 3H), 0.02 (s, 3H), 0.00 (s, 3H); \(^{13}\)C
NMR (125 MHz, CDCl$_3$) $\delta$ 203.9, 202.9, 177.0, 138.9, 137.8, 128.6 (2), 128.5 (2), 128.0 (4), 127.8, 127.6, 102.5, 100.0, 89.7, 87.7, 82.2, 79.8, 75.8, 70.7, 70.2, 37.7, 36.7, 29.5, 26.1 (3), 19.0, 18.4, 15.7, 13.8, 10.3, $-4.3$, $-4.8$; $m/z$ (ESIMS) calculated for [C$_{39}$H$_{56}$O$_5$SiNa$^+$]: 655.4, found: 655.4; $[\alpha]^{25}_D = 47$ (c = 0.01, CHCl$_3$); IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 2929, 1965, 1710, 1456, 1066.

To a solution of 3.33 (10.5 mg, 16.0 $\mu$mol) in 320 $\mu$L of Et$_2$O was added TEA (4.9 mg, 48 $\mu$mol) and methanesulfonyl chloride (5.5 mg, 48 $\mu$mol) respectively at 5 °C. The reaction mixture was warmed to room temperature and stirred for 1 hr 20 minutes at room temperature. The mesylate solution was added a solution of methyl cyanocuprate, prepared from CuCN (14.4 mg, 160 $\mu$mol) and MeLi (100 $\mu$L, 160 $\mu$mol) in 800 $\mu$L of Et$_2$O at −35 °C. The reaction mixture was then warmed to room temperature and stirred for 1h. The reaction was then quenched with aqueous NH$_4$Cl (5 mL) and extracted in Et$_2$O (3 x 10 mL). The organic layer was washed with water (10 mL), and then dried over anhydrous MgSO$_4$. Evaporation of solvent gave the crude product is subjected to silica gel column chromatography, giving 3.35 (9.2 mg, 14 $\mu$mol) in 86% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.34 – 7.23 (m, 3H), 7.12 – 6.89 (m, 5H), 6.13 – 6.00 (m, 1H), 5.63 – 5.52 (m, 1H), 5.63 – 5.52 (m, 1H), 4.64 (d, $J = 12.3$ Hz, 1H), 4.36 (d, $J = 12.6$ Hz, 1H), 3.99 (t, $J = 6.2$ Hz, 1H), 3.73 (dd, $J = 8.5$ Hz, 5.3 Hz, 1H), 2.59 – 2.49 (m, 1H), 2.25 – 2.15 (m, 2H), 1.96 – 1.86 (m, 2H), 1.70 (d, $J = 2.6$ Hz, 3H), 1.65 –
1.49 (m, 2H), 1.29 (d, $J = 6.8$ Hz, 3H), 1.08 (d, $J = 6.8$ Hz, 3H), 0.92 (s, 9H), 0.79 (t, $J = 7.5$ Hz, 3H), 0.02 (s, 3H), 0.00 (s, 3H).

The crude Bis[allene] 3.28 was dissolved in 60 mL of acetic acid, water, and trifluoroacetic acid mixture (4:1:0.25 ratio) and stirred for 1 hour at rt. The solvent was azeotroped with toluene (3 x 50 mL) and resulting crude product upon further purification by silica gel column chromatography using 30% EtOAc in hexane gave 3.29 as colorless oil (860 mg, 1.66 mmol, 83% yield over 2 steps, 3.26 → 3.29).

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.36 – 7.27 (m, 10H), 5.18 – 5.10 (m, 1H), 5.08 – 5.02 (m, 1H), 4.70 (d, $J = 11.5$ Hz, 1H), 4.68 (d, $J = 12.0$ Hz, 1H), 4.45 (d, $J = 11.5$ Hz, 1H), 4.41 (d, $J = 12.0$ Hz, 1H), 4.04 (t, $J = 6.4$, 1H), 3.99 (t, $J = 6.8$ Hz, 1H), 3.69 (dd, $J = 8.5$, 5 Hz, 1H), 2.84 – 2.76 (m, 1H), 2.38 – 2.32 (m, 1H), 1.94 – 1.84 (m, 1H), 1.75 (d, $J = 3$ Hz, 3H), 1.67 (d, $J = 2.5$ Hz, 3H), 1.66 – 1.50 (m, 2H), 1.23 (d, $J = 7$ Hz, 3H), 0.98 (d, $J = 6.5$ Hz, 3H), 0.90 (t, $J = 7$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 203.6, 201.7, 175.7, 138.9, 137.7, 128.7 (2), 128.5 (2), 128.1, 128.1 (2), 127.8 (2), 127.7, 103.4, 100.6, 92.5, 88.0, 82.1, 79.3, 74.2, 70.7, 70.4, 44.4, 37.6, 37.0, 27.9, 19.4, 15.4, 15.1, 13.3, 9.8; m/z (ESIMS) calculated for [C$_{33}$H$_{42}$O$_5$Na]$^+$: 541.3, found: 541.3; [α]$^{25}_D$ = 45.0 (c = 0.01, CHCl$_3$); IR $\nu$$_{max}$(neat)/cm$^{-1}$ 3388, 2933, 1965, 1710, 1454.
The seco acid 3.29 (195 mg, 0.380 mmol) was azeotroped with toluene (3 x 10 mL) and then dissolved in 20 mL toluene. TEA (0.19 mg, 1.9 mmol) and 2,4,6-trichlorobenzoyl chloride (0.46 g, 1.9 mmol) was added to this solution slowly at room temperature, and then stirred for 6 hours at room temperature. The resulting active ester was then added dropwise over 2 hours to a solution of 4–(N, N–dimethylamino)pyridine (DMAP) (0.46 g, 3.5 mmol) in toluene (163 mL) at 80 °C. The mixture was then cooled back to room temperature and quenched with aqueous NH₄Cl (100 mL). The organic layer was washed with water (2 x 100 mL) and dried over Na₂SO₄. The organic layer was concentrated down to dryness under reduced pressure. Silica gel column chromatography using 5% EtOAc in hexanes gave the bis[allene] macrolactone 4.1 (121 mg, 0.240 mmol, 64% yield) and 3.31 (19 mg, 19 µmol, 5% yield) as a colorless oil.

For detailed NMR analysis of 4.1, see page 204; m/z (ESIMS) calculated for [C₃₃H₄₀O₄Na]⁺: 523.3, found: 523.3; [α]²⁵D = 50.0 (c = 0.01, CHCl₃); IR νmax(neat)/cm⁻¹ 2969, 2934, 1960, 1731, 1454, 1369, 1248, 1065.

Data of 3.31; ¹H NMR (500 MHz, CDCl₃) δ 7.36 – 7.16 (m, 20H), 5.12 – 5.02 (m, 4H), 4.98 – 4.90 (m, 2H), 4.65 – 4.58 (m, 4H), 4.37 – 4.28 (m, 4H), 3.97 – 3.94 (t, J = 7.8 Hz, 2H), 3.71 – 3.67 (m, 2H), 2.73 – 2.60 (m, 3H), 2.36 – 2.28 (m, 2H), 1.95 – 1.78 (m, 5H), 1.74 – 1.60 (m, 12H), 1.24 – 1.23 (m, 8H), 1.03 – 0.97 (m, 6H), 0.89 – 0.81 (m, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 203.6, 202.6, 173.3, 138.9, 138.6, 128.2, 128.1, 127.5,
m/z (ESIMS) calculated for [C_{66}H_{80}O_{8}Na]^+: 1023.3, found: 1023.3; IR ν_{max}(neat)/cm^{-1} 2922, 1968, 1735, 1453, 1377, 1068.

The seco acid 3.30 (280 mg, 0.510 mmol) was azeotroped with toluene (3 x 10 mL) and then dissolved in 15 mL toluene. TEA (260 mg, 2.52 mmol) and 2,4,6-trichlorobenzoyl chloride (617 mg, 2.52 mmol) was added to this solution slowly at room temperature, and then stirred for 6 hours at room temperature. The resulting active ester was then added dropwise over 2 hours to a solution of 4–(N, N–dimethylamino)pyridine (DMAP) (610 mg, 5.00 mmol) in toluene (250 mL) at 80 °C. The mixture was then cooled back to room temperature and quenched with aqueous NH_{4}Cl (100 mL). The organic layer was washed with water (2 x 100 mL) and dried over Na_{2}SO_{4}. The organic layer was concentrated down to dryness under reduced pressure. Silica gel column chromatography using 5% EtOAc in hexanes gave the bis[allene] macrolactone 4.2 (202 mg, 0.380 mmol, 75% yield) as a colorless oil.

^{1}H NMR (500 MHz, CDCl_{3}) δ 7.29 – 7.22 (2H, m), 7.11 – 7.01 (4H, m), 6.97 – 6.89 (2H, m), 5.38 – 5.30 (1H, m), 5.28 (1H, t, J = 6.5 Hz), 5.16 – 5.08 (1H, m), 4.64 (1H, d, J = 12.5 Hz), 4.52 (1H, d, J = 12.5 Hz), 4.48 (1H, d, J = 12.5 Hz), 4.31 (1H, d, J =
12.0 Hz), 3.96 (1H, dd, J = 8.0, 4.0 Hz), 3.77 (1H, dd, J = 7.0, 7.0 Hz), 2.86 – 2.76 (1H, m), 2.20 – 2.14 (1H, m), 2.04 – 1.94 (1H, m), 1.71 (3H, d, J = 3.0 Hz), 1.70 (3H, d, J = 3.0 Hz), 1.72 – 1.62 (2H, m), 1.26 (3H, d, J = 7.0 Hz), 1.05 (3H, d, J = 7.0 Hz), 0.90 (3H, t, J = 7.5 Hz); 13C NMR (125 MHz, CDCl$_3$) δ 203.8, 201.3, 173.8, 164.1 (d, J = 84 Hz), 162.1 (d, J = 89 Hz), 142.1(d, J = 75 Hz), 141.6 (d, J = 69 Hz), 129.9 (d, J = 46 Hz), 129.8 (d, J = 79 Hz), 123.1 (d, J = 27 Hz), 122.9 (d, J = 27 Hz), 114.6 – 114.3 (4C, m), 102.6, 99.3, 91.6, 90.5, 81.7, 77.9, 75.8, 70.0 (d, J = 17 Hz), 68.1 (d, J = 19 Hz), 44.9, 37.7, 35.9, 25.1, 20.5, 17.5, 15.3, 14.0, 9.9; [α]$^{25}_D$ = –3.0 (c = 0.01, CHCl$_3$); m/z (ESIMS) calculated for [C$_{33}$H$_{38}$F$_2$O$_4$Na]$^+$: 559.3, found: 559.3; IR $\nu_{\text{max}}$(neat)/cm$^{-1}$ 2971, 2935, 1968, 1732, 1617, 1592, 1174.
To a solution of bis[allene] macrolactone 2.24 (26.6 mg, 115 μmol) in CHCl₃ (1 mL) was added a solution of DMDO in CDCl₃ (1.1 mL, 0.30 mmol) dropwise at −40 °C. The reaction was stirred under nitrogen and let it to warm to −5 °C over 1 hr 30 minutes. After the consumption of 2.24, excess DMDO was removed under reduced pressure and the crude mixture was azeotroped with toluene. To the crude mixture was added a solution of LiBr (39.8 mg, 0.450 mmol) in THF (1 mL) via syringe pump for 1 hour at −5 °C. After the consumption of SDE, the reaction mixture was diluted with water and then extracted with DCM. The combined organic phase was dried over anhydrous Na₂SO₄. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave 4.4 (24.2 mg, 69.9 μmol, 61%) of as white solid. The product was recrystallized in 20% EtOAc in hexane.

For detailed X–ray crystallography, see page 214; ¹H NMR (300 MHz, CDCl₃) δ 5.27 – 5.17 (m, 1H), 4.94 (dd, J = 12.4, 2.9 Hz, 1H), 4.70 (t, J = 7.0 Hz, 1H), 4.11 (dd, J = 12.5, 1.8 Hz, 1H), 3.72 (s, 1H), 2.69 – 2.54 (m, 1H), 2.52 – 2.30 (m, 3H), 2.06 – 1.97 (m, 2H), 1.73 (d, J = 2.93 Hz, 3H), 1.59 – 1.57 (m, 2H), 1.55 (s, 3H), 1.39 – 1.24 (m, 1H), 0.96 – 0.80 (m, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 208.9, 203.2, 171.7, 95.8, 92.0, 79.1, 65.8, 41.7, 37.9, 31.8, 29.7, 28.4, 26.8, 22.7, 17.6; m/z (HRMS) calculated for
To a solution of bis[allene] macrolactone 2.24 (47.0 mg, 202 µmol) in CDCl$_3$ (1 mL) was added a solution of DMDO in CDCl$_3$ (2.0 mL, 0.60 mmol) dropwise at –40 °C. The reaction was stirred under nitrogen and let to warm to –5 °C over 1 hr 30 minutes. To the reaction mixture was added LiBr (52.8 mg, 0.610 mmol) at –5 °C. TLC showed the unreacted SDE. To the reaction mixture was added LiBr (123 mg, 1.42 mmol). After the complete consumption of SDE, the reaction was then diluted with water and extracted with DCM. The combined organic phase was dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent and silica gel column chromatography using 20% EtOAc in hexanes gave 4.6 as colorless oil (50.0 mg, 113 µmol, 56% yield).

For detailed NMR analysis, see page 222; m/z (HRMS) calculated for [C$_{15}$H$_{21}$Br$_2$O$_5$H]$^+$: 441.9, found: 422.9, 424.9, 426.9 [C$_{15}$H$_{21}$Br$_2$O$_5$H–H$_2$O]; IR $v_{\text{max}}$ (neat)/cm$^{-1}$ 3495, 2920, 2860, 1740, 1721, 1454, 1372, 1241, 1143, 1002.
To a solution of bis[allene] macrolactone 4.1 (15.0 mg, 30.0 μmol) in CDCl₃ (1 mL) was added a solution of DMDO in CDCl₃ (0.4 mL, 0.2 mmol) dropwise at −40 °C. The reaction was stirred under nitrogen and let to warm to −15 °C over 30 minutes. To the reaction mixture was added LiBr (53.2 mg, 0.610 mmol) at −15 °C. After the complete consumption of SDE, the reaction was diluted with water and extracted with DCM. The combined organic phase was dried over anhydrous Na₂SO₄. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave 4.9 as colorless oil (2.0 mg, 3.1 μmol, 11% yield).

For detailed NMR analysis, see page 235; ¹H NMR (600 MHz, CDCl₃) δ 7.42 – 7.33 (m, 10H), 6.00 (d, J = 8.51 Hz, 1H), 5.17 (dd, J = 2.64, 10.56 Hz, 2H), 4.55 (d, J = 7.92 Hz, 1H), 3.86 (d, J = 9.39 Hz, 1H), 3.83 (dd, J = 3.82, 9.69 Hz, 1H), 3.17 (t, J = 6.45 Hz, 1H), 1.50 (s, 3H), 1.36 (d, J = 7.34, 3H), 1.21 (s, 3H), 1.05 (d, J = 6.75, 3H), 0.99 – 0.91 (m, 3H); IR v_max (neat)/cm⁻¹ 3435, 2955, 2922, 2851, 1726, 1460, 1377, 1161, 1072.
The solution of freshly prepared DMDO in CHCl₃ (0.5 mL, 0.2 M, 0.1 mmol) was added to bis[allene] macrolactone 4.1 (18.0 mg, 36.0 µmol), dissolved in THF/water (1:1, total 1 mL) dropwise at 5 °C. Upon the consumption of allene, the reaction mixture was removed under reduced pressure. The crude was chromatographed with 20% EtOAc in hexane through silica gel and the product was obtained in 4.12 (1.3 mg, 2.6 µmol, 7%).

For detailed NMR analysis, see page 237; m/z (HRMS) calculated for [C₂₆H₃₆O₉H]⁺: 493.3, found 493.3.

To a solution of bis[allene] macrolactone 4.1 (12.0 mg, 23.9 µmol) in methanol (3 mL) was added a solution of DMDO (0.38 mL, 0.14 mmol) dropwise at –50 °C. The reaction was stirred under nitrogen and let to warm to –15 °C over 1 hr 30 minutes. Evaporation of solvent and silica gel column chromatography using 5% EtOAc in hexanes gave 4.13 (10.0 mg, 19.4 µmol, 80%) as colorless oil.

For detailed NMR analysis, see page 247; m/z (HRMS) calculated for [C₃₃H₄₀O₅Na]⁺: 539.3, found: 539.3; [α]D = 3.3° (c = 0.005, CHCl₃); IR νmax (neat)/cm⁻¹ 3065, 2919, 2849, 1966, 1723, 1653, 1455, 1376, 1175, 1066.
To a solution of bis[allene] macrolactone \textbf{4.2} (32.9 mg, 65.5 \textmu mol) in methanol (2 mL) was added a solution of DMDO (1.15 mL, 180 \textmu mol) dropwise at \(-20^\circ\text{C}\). The temperature was kept at \(-20^\circ\text{C}\) for 5 hours. Evaporation of solvent and silica gel column chromatography using 5\% EtOAc in hexanes gave \textbf{4.15} (10.0 mg, 18.1 \textmu mol, 30\%) of as colorless oil.

For detailed NMR analysis, see page 272; \textit{m/z} (HRMS) calculated for [C_{33}H_{38}F_{2}O_{5}Na]^+: 575.3, found: 575.3; [\alpha]_{25}^{\text{D}} = -85.9^\circ$ (c = 0.005, CHCl$_3$); IR $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3066, 2972, 2933, 2876, 1964, 1738, 1696, 1624, 1450, 1379, 1177, 1086.

To a solution of macrolactone \textbf{4.1} (17.7 mg, 35.4 \textmu mol) in CDCl$_3$ (0.5 mL) was added a solution of DMDO (0.56 mL, 0.21 mmol, 0.38 M in CDCl$_3$) dropwise at \(-40^\circ\text{C}\), warmed up to \(-15^\circ\text{C}\) over 30 minutes, then lower order methyl cyanocuprate (MeCuCNLi, 0.71 mmol) was added, prepared by addition of MeLi (0.44 mL, 0.71 mmol) to a slurry of CuCN (63.3 mg, 0.710 mmol) in 2–methyl THF (6 mL) at \(-78^\circ\text{C}\)
and then warming to −15 °C. The mixture was warmed to −2 °C over 1 hr 30 minutes, quenched with saturated aqueous solution of NH₄OH and NH₄Cl (1:4 ratio) and then extracted with Et₂O. The combined organic layer was dried over anhydrous Na₂SO₄, filtered and then concentrated under reduced pressure to give the crude product, which was purified by silica gel column chromatography using 15% EtOAc in hexane to afford 4.16 (10.0 mg, 22.6 µmol, 64% yield) as a colorless oil.

For detailed NMR analysis, see page 287; m/z (HRMS) calculated for [C₂₆H₃₄O₆Na]⁺: 465.2, found: 465.5; [α]²⁵D = 5.9° (c = 0.005, CHCl₃); IR νmax (neat)/cm⁻¹ 3434, 2968, 2925, 1959, 1764, 1725, 1452, 1370, 1155.

To the neat bis[allene] macrolactone 4.1 (25.3 mg, 50.6 µmol) was added a solution of DMDO (800 µL, 303 µmol, 0.380 M in CDCl₃) dropwise at −40 °C. The temperature was increased to −15 °C over 1 hr. In another flask, lower order methyl cyanocuprate was prepared by addition of MeLi (632 µL, 1.01 mmol) to a slurry of CuCN (91.0 mg, 1.01 mmol) in Et₂O (10 mL) at −78 °C and then warming to −15 °C. SDE solution was added to the cuprate solution at a −15 °C. After the complete consumption of SDE, the reaction was then quenched with water and extracted with Et₂O. The combined organic phase was dried over anhydrous Na₂SO₄. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave a
mixture of 4.16 (3.3 mg, 7.5 μmol, 15% yield), 4.17 (7.3 mg, 15 μmol, 30% yield) and 4.18 (5.4 mg, 10 μmol, 20% yield) as colorless oil.

Data of 4.17: For detailed NMR analysis, see page 311; m/z (HRMS) calculated for [C_{26}H_{36}O_{8}H]^+: 477.5, found: 477.2; IR ν_{max} (neat)/cm^{-1} 3427, 3063, 2968, 2934, 2878, 1764, 1734, 1454, 1375, 1164, 1068.

Data of 4.18: For detailed NMR analysis, see page 329; m/z (HRMS) calculated for [C_{33}H_{40}O_{6}Na]^+: 555.3, found: 555.3; [α]_{25}^D = 11.1° (c = 0.005, CHCl_{3}); IR ν_{max} (neat)/cm^{-1} 3436, 3063, 3031, 2969, 2933, 2876, 1966, 1729, 1454, 1376, 1270, 1179, 1065.

![Diagram](4.30)

To a solution of alcohol 4.17 (7.4 mg, 16 μmol) in acetone (500 μl) was added grinded K_{2}CO_{3} (4.3 mg, 31 μmol) and stirred for 30 minutes at room temperature. To the mixture was added acetic anhydride (6 μl, 0.07 mmol) at room temperature and stirred for overnight. The reaction mixture was diluted in DCM and the organic layer was washed with water. The combined organic phase was dried over anhydrous Na_{2}SO_{4}. Evaporation of solvent and silica gel column chromatography using 7% EtOAc in hexanes gave 4.30 (5.6 mg, 11 μmol, 70% yield) as colorless oil.

For detailed NMR analysis of 4.30, see page 345; m/z (HRMS) found: 541.2, calculated for [C_{28}H_{38}O_{9}Na]^+: 541.2.
To a solution of alcohol 4.16 (3.1 mg, 7.0 μmol) in acetone (500 μl) was added grinded K₂CO₃ (9.6 mg, 70 μmol) and stirred for 30 minutes at room temperature. To the mixture was added acetic anhydride (6 μl, 0.07 mmol) at room temperature and stirred for overnight. The reaction mixture was diluted in DCM and the organic layer was washed with water. The combined organic phase was dried over anhydrous Na₂SO₄. Evaporation of solvent and silica gel column chromatography using 7% EtOAc in hexanes gave 4.32 (1.7 mg, 3.5 μmol, 50% yield) as colorless oil.

For detailed NMR analysis of 4.32, see page 347; m/z (HRMS) calculated for [C₂₈H₃₆O₇Na]⁺: 507.2, found: 507.2.

To the solution of 4.16 (6.2 mg, 14 μmol) in 1 mL of anhydrous DCM was added imidazole (2.9 mg, 40 μmol) and DMAP (0.5 mg, 4 μmol). After stirring for 5 minutes at room temperature, triethylsilyl chloride (12.6 mg, 83.6 μmol) was added to the mixture at room temperature. Upon the completion of the reaction, the mixture was quenched with
aqueous NH₄Cl and the organic layer was extracted with DCM, dried over Na₂SO₄. The crude was chromatographed with 3% EtOAc in hexane through silica gel, giving 4.33 (7.2 mg, 13 µmol, 92% yield).

For detailed NMR analysis of 4.33, see page 353; m/z (HRMS) calculated for [C₃₂H₄₈O₆SiH]+: 557.3, found: 557.3; IR ν max (neat)/cm⁻¹ 3439, 2957, 2931, 2987, 2897, 2858, 1723, 1471, 1376, 1195, 1103, 837.

To a stirred solution of macrolactone 4.16 (10.0 mg, 22.6 µmol) in toluene (155 µL) was added benzyl trichloroacetimidate (8.0 mg, 32 µmol) and lanthanium triflate (1.2 mg, 2.2 µmol). Then the mixture was stirred at room temperature for 30 minutes. The reaction mixture was concentrated under vacuum and the residue was subjected to purification by silica gel column chromatography yielding 4.34 (8.4 mg, 16 µmol, 70%).

For detailed NMR analysis, see page 367; m/z (HRMS) calculated for [C₃₃H₄₆O₆Na]+: 557.3, found: 555.3; IR ν max (neat)/cm⁻¹ 3400, 2965, 2923, 2877, 2851, 1900, 1759, 1725, 1453, 1374, 1186, 1079.
To a solution of 4.16 (3.4 mg, 7.7 µmol) in ethanol (1 mL) was added hydroxylamine hydrochloride (16.0 mg, 0.230 mmol). The reaction mixture was stirred for 2hrs, then KOH (12.9 mg, 0.230 mmol) was added at room temperature. The reaction was diluted with water and extracted with Et$_2$O. The combined organic phase was dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent and silica gel column chromatography using 15% EtOAc in hexanes gave 4.35 (2.7 mg, 6.0 µmol, 78% yield) as colorless oil.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.35 – 7.31 (m, 5H), 5.83 (d, $J = 6.60$ Hz, 1H), 5.57 (dd, $J = 8.80$, 5.58 Hz, 1H), 5.07 – 5.03 (m, 1H), 4.61 (d, $J = 11.74$ Hz, 1H), 4.34 (d, $J = 11.99$ Hz, 1H), 3.93 (dd, $J = 6.36$ Hz, 4.40, 1H), 3.79(dd, $J = 9.05$ Hz, 1.46, 1H), 3.09 (s, 1H), 2.98 – 2.91(m, 1H), 2.03 – 1.96 (m, 1H), 1.76 (d, $J = 2.69$ Hz, 3H), 1.73 – 1.60 (m, 4H), 1.31(d, $J = 8.56$ Hz, 3H), 0.93 (d, $J = 7.33$ Hz, 3H), 0.91 (s, 3H), 0.90 – 0.84 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 206.3, 174.3, 167.1, 139.2, 128.2, 127.5, 127.2, 98.6, 90.4, 83.2, 82.3, 76.7, 75.4, 70.5, 67.8, 42.5, 39.9, 34.1, 24.2, 22.7, 14.8, 14.0, 13.6, 10.1; m/z (HRMS) calculated for [C$_{26}$H$_{35}$NO$_6$Na]$^+$: 480.2, found: 480.2; [$\alpha$]$^{25}_D = 6.3^\circ$ (c = 0.005, CHCl$_3$); IR $\nu$(neat)/cm$^{-1}$ 3309, 2917, 2849, 1959, 1729, 1709, 1458, 1375, 1156, 1070.
The macrolactone 4.1 (26.0 mg, 52.0 µmol) was dissolved in the mixture of t–BuOH and water (1:1 ratio, total 2 mL). To the solution was added 4% aqueous OsO₄ solution (495 µL, 62.2 µmol) at room temperature, and then stirred for 45 minutes. After the complete consumption of 4.1, the reaction was quenched by 20 mL saturated Na₂SO₃ solution, and then extracted with Et₂O (2 x 20 mL). The solvent was removed in vacuo. Silica gel column chromatography using 10% EtOAc in hexane gave the macrolactone 4.50 as light yellowish oil (23.0 mg, 43.0 µmol, 83% yield).

¹H NMR (500 MHz, CDCl₃) δ 7.48 – 7.17 (m, 8H), 5.15 (t, J = 6.0 Hz, 2H), 4.94 (dd, J = 6.8, 3.1 Hz, 1H), 4.73 (dd, J = 13.4, 8.4 Hz, 1H), 4.58 (dd, J = 21.0, 11.6 Hz, 2H), 4.35 (d, J = 12.2 Hz, 1H), 4.15 (dd, J = 8.7, 6.9, 4.0 Hz, 1H), 3.83 (s, 1H), 3.44 (dd, J = 8.0, 6.9 Hz, 1H), 2.95 (qd, J = 17.8, 5.4 Hz, 2H), 2.58 (dt, J = 15.5, 6.8 Hz, 1H), 1.90 (dd, J = 15.0, 3.1 Hz, 1H), 1.79 (d, J = 2.9 Hz, 2H), 1.76 – 1.64 (m, 2H), 1.57 – 1.46 (m, 2H), 1.42 – 1.28 (m, 4H), 1.28 – 1.15 (m, 2H), 1.07 (t, J = 13.0 Hz, 2H), 0.99 – 0.69 (m, 6H); ¹³C NMR (126 MHz, CDCl₃) δ 216.5, 208.4, 178.2, 143.0, 142.8, 133.2, 132.9, 132.5, 132.3, 132.3, 132.1, 131.6, 104.1, 96.1, 86.7, 83.3, 81.9, 81.6, 81.4, 80.8, 80.4, 78.2, 74.4, 50.0, 47.3, 47.0, 38.6, 34.3, 31.8, 29.6, 23.0, 19.9, 18.1, 14.0; m/z (HRMS) calculated for [C₃₃H₄₂O₆Na]⁺: 557.3, found: 557.3; IR νmax(neat)/cm⁻¹ 3475, 3065, 2930, 2919, 2872, 2849, 1963, 1727, 1453, 1375, 1172, 1069.
To a stirred solution of macrolactone 4.50 (7.8 mg, 15 µmol) in 1 mL of acetonitrile was added N–Bromosuccinimide (34.0 mg, 191 µmol) at room temperature. Starting material was consumed right away. The reaction mixture was quenched with 1 mL of saturated aqueous solution of Na₂S₂O₃, and then extracted with Et₂O. The solvent was removed in vacuo, and the residue was subjected to purification by silica gel column chromatography (14% EtOAc in hexane) yielding 4.60 (9.1 mg, 15 µmol, 99% yield).

For detailed NMR analysis, see page 372; m/z (ESIMS) calculated for [C₃₃H₄₃BrO₇Na]⁺: 653.3, 655.3, found: 653.2, 655.2; [α]_{D}^{25} = 7.6° (c = 0.005, CHCl₃); IR \ ν_{max} (neat)/cm⁻¹ 3442, 3062, 2956, 2922, 2850, 1728, 1711, 1454, 1376, 1165, 1070.

To a solution of bis[allene] macrolactone 2.24 (36.6 mg, 158 µmol) in THF (2 mL) was added sulfuric acid (15.5 mg, 0.160 mmol), water (13 µL, 0.71 mmol), mercuric sulfate (1.9 mg, 6.3 µmol) sequentially. The reaction mixture was heated under reflux at 70 °C for 5 hours. After the complete consumption of bis[allene] macrolactone, the reaction was cooled down to room temperature and diluted with water. The organic
phase was extracted with EtOAc and dried over anhydrous Na₂SO₄. The solvent was removed *in vacuo*, and silica gel column chromatography using 20% EtOAc in hexanes gave 4.65 (15.9 mg, 63.6 μmol, 40%) as colorless oil.

For detailed NMR analysis, see page 393; *m/z* (HRMS) calculated for [C₁₅H₂₂O₃Na]⁺: 273.1, found: 273.1.

\[ \text{To a solution of bis[allene] macrolactone 4.1 (15.0 mg, 30.0 μmol) in 2–methyl THF (3 mL) was added Na₂HPO₄ (51.0 mg, 0.360 mmol) and } m\text{CPBA (31.0 mg, 0.180 mmol) sequentially at } -50 \degree \text{C. The temperature was slowly increased to 12 } \degree \text{C over 5 hr 30 minute. To the reaction mixture was methanol (4 mL) and stirred overnight. The reaction was quenched with aqueous NaHCO₃ and extracted with EtOAc. The combined organic phase was dried over anhydrous Na₂SO₄. The solvent was removed *in vacuo*, and silica gel column chromatography using 15% EtOAc in hexanes gave 4.68 as colorless oil (7.0 mg, 14 μmol, 45% yield).} \]

For detailed NMR analysis, see page 419; *m/z* (ESIMS) calculated for [C₃₃H₄₀O₅+Na]⁺: 539.6, found: 539.1.
To epoxy ketone 4.18 (1.7 mg, 3.2 µmol) in Et₂O (1 mL) was added Zn(BH₄)₂ (46 µl, 14 µmol, 0.30 M in Et₂O) dropwise at 0 °C, then Zn(BH₄)₂ (115 µl, 35.0 µmol, 0.300 M in Et₂O) twice at room temperature. After the complete consumption of 4.18, the reaction was quenched with aqueous NH₄Cl and extracted with Et₂O. The combined organic phase was dried over anhydrous Na₂SO₄. The solvent was removed in vacuo, and silica gel column chromatography using 15% EtOAc in hexanes gave 4.74 (1.4 mg, 2.6 µmol, 82% yield) as colorless oil.

For detailed NMR analysis, see page 442; m/z (HRMS) calculated for [C₃₃H₄₂O₆H]⁺: 535.3, found: 535.3.

To a stirred solution of macrolactone 4.60 (5.0 mg, 7.9 µmol) in 1 mL of anhydrous Et₂O was Zn(BH₄)₂ solution in Et₂O (91 µl, 12 µmol) at 0 °C. The mixture was stirred for 30 minutes at 0 °C. The reaction mixture was quenched with 1 mL of aqueous NH₄Cl, and then extracted with Et₂O. The crude was subjected to purification
by silica gel column chromatography (20% EtOAc in hexane) yielding 4.75 (4.9 mg, 7.9 μmol, 99% yield).

For detailed NMR analysis, see page 463; m/z (HRMS) calculated for [C33H45BrO7Na]+: 655.2, 657.2, found: 655.2, 657.2; [α]D25 = 5.8° (c = 0.005, CHCl3); IR νmax (neat)/cm⁻¹: 3433, 2925, 2851, 1729, 1454, 1375, 1164, 1068.

![Chemical structure](image)

To a solution of bis[allene] macrolactone 4.1 (21.5 mg, 43.0 μmol) in 1,2-dichloroethane (3.6 mL) and phosphate buffer (0.4 mL, pH = 7) was added DDQ (39.0 mg, 172 μmol) and stirred for 15 minutes at room temperature. Then, raise the temperature to 40 °C until the complete disappearance of 4.1. The aqueous NaHCO₃ (3 mL) was added and the resulting dark solution was stirred vigorously for 1 hr. The crude mixture was extracted with DCM and the excess solvent was removed under reduced pressure. The crude mixture was chromatographed, yielding 4.80 (10.5 mg, 25.6 μmol, 60%) as colorless oil.

For detailed NMR analysis, see page 500; m/z (HRMS) calculated for [C26H34O4Na]+: 433.2, found: 433.2; [α]D25 = 15.1° (c = 0.005, CHCl3); IR νmax (neat)/cm⁻¹: 3435, 2968, 2917, 2848, 1963, 1729, 1453, 1376, 1177, 1069.
7.4. Chapter 5

To the solution of allene (31.3 mg, 0.150 mmol) \textbf{5.25} in \textit{t}–BuOH (2 mL) and water (0.7 mL) was added Chloramine T–trihydrate (73.8 mg, 0.300 mmol) at room temperature. The mixture was stirred for 1 minute, and then 4% aqueous OsO$_4$ solution (1.29 mL, 165 \(\mu\)mol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding \textbf{5.30} (6.2 mg, 10% yield) and \textbf{5.28} (20.8 mg, 50% yield).

Data of \textbf{5.30}: $^1$H NMR (500 MHz, CDCl$_3$) \(\delta\) 7.79 – 7.68 (2H, m), 7.30 – 7.23 (2H, m), 5.31 (1H, d, \(J = 9.5\) Hz), 4.51 (1H, dd, \(J = 2.7, 9.5\) Hz), 2.39 (3H, s), 1.98 (3H, s), 1.91 – 1.82 (1H, m), 1.66 – 1.00 (14H, m), 0.99 – 0.80 (12H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) \(\delta\) 212.9, 143.4, 137.3, 129.6, 82.3, 55.6, 41.6, 38.1, 37.4, 22.5, 24.7, 24.4, 23.5, 22.9, 22.8, 20.8, 13.8, 13.8; IR \(\nu_{\text{max}}\) (neat)/cm$^{-1}$ 3516, 3282, 3028, 2956, 2931, 2870, 1712, 1598, 1466, 1332, 1159.

Data of \textbf{5.28}: $^1$H NMR (500 MHz, CDCl$_3$) \(\delta\) 4.79 (1H, dd, \(J = 10.3, 3.91\) Hz), 3.02 (1H, s), 1.92 – 1.03 (15H, m), 0.99 – 0.98 (3H, d, \(J = 6.60\) Hz), 0.95 – 0.91 (3H, d, \(J = 6.60\) Hz), 0.91 – 0.87 (6H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) \(\delta\) 208.8, 82.7, 54.2, 41.9, 38.6, 38.5, 25.6, 25.5, 24.7, 23.1, 22.9 (2), 21.1, 13.9, 13.8; IR \(\nu_{\text{max}}\) (neat) /cm$^{-1}$ 3513, 2958, 2932, 2872, 1720, 1467; \textit{m/z} (HRMS) calculated for [C$_{15}$H$_{29}$ClO$_2$H]$^+$: 277.2, found: 277.2.
To the solution of allene (30.0 mg, 0.140 mmol) **5.25** in anhydrous DCM (3 mL), dimethylmethylideneammonium chloride (70.0 mg, 0.700 mmol), and OsO₄ solution (3.0 mL, 0.15 mmol, 0.050 M in DCM), and NMO (20.0 mg, 0.150 mmol) were added at room temperature. The mixture was stirred for 1 hour, and then quenched by saturated solution of Na₂SO₃. The organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding **5.31** (27.3 mg, 63% yield).

Data of **5.31**: ¹H NMR (500 MHz, CDCl₃) 3.92 (1H, s), 3.50 – 3.38 (1H, m), 2.46 (2H, t, J = 7.6), 2.30 (6H, s), 1.76 – 1.00 (15H, m), 0.96 – 0.84 (12H, m); ¹³C NMR (125 MHz, CDCl₃) δ 219.7, 81.2, 64.4, 45.6, 42.3, 39.0, 38.7, 37.4, 26.3, 25.6, 25.5, 23.2, 23.1, 23.0, 22.7, 14.1, 13.9; m/z (HRMS) calculated for [C₁₈H₃₇NO₂H]⁺: 300.3, found: 300.3.

To the solution of allene (10.0 mg, 48.0 µmol) **5.25** in t-BuOH (900 µL) and water (150 µL) was added N,N’-dicyclohexylcarbodiimide (76.5 mg, 371 µmol) at room temperature. The mixture was stirred for 20 minutes, and then 4% aqueous OsO₄ solution (415 µL, 53.0 µmol) was added slowly. After the complete disappearance of
allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding 5.32 (16.4 mg, 79% yield).

For detailed NMR analysis, see page 528; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.24 (1H, t, $J = 7.8$ Hz), 4.21 – 4.30 (2H, tt, $J = 4.1$ Hz), 2.04 – 2.08 (1H, m), 1.87 – 1.91 (2H, m), 1.7 (1H, m), 0.96 (3H, s), 0.95 (3H, s), 1.15 – 2.20 (20H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 182.6, 166.8, 100.5, 94.6, 59.8, 59.2, 41.3, 39.8, 33.9, 32.2, 32.0, 31.9, 31.8, 29.5, 25.9, 25.8, 25.7, 25.6(2), 25.5(2), 23.1, 22.6, 22.5, 22.5, 13.9(2)

To the solution of allene (9.4 mg, 45 µmol) 5.25 in $t$-BuOH (900 µL) and water (150 µL) was added N,N’-diisopropylcarbodiimide (114 mg, 902 µmol) at room temperature. The mixture was stirred for 20 minutes, and then 4% aqueous OsO$_4$ solution (390 µL, 50.0 µmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in DCM. The crude was purified by silica gel column chromatography, yielding 5.33 (8.8 mg, 56% yield).

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.79 – 7.68 (2H, m), 7.30 – 7.23 (2H, m), 5.31 (1H, d, $J = 9.5$ Hz), 4.51 (1H, dd, $J = 2.7, 9.5$ Hz), 2.39 (3H, s), 1.98 (3H, s), 1.91 – 1.82 (1H, m), 1.66 – 1.00 (14H, m), 0.99 – 0.80 (12H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$
To the solution of allene (17.5 mg, 65.2 μmol) 5.34 in t-BuOH (1 mL), phosphate buffer (0.50 mL, pH= 7.4), and acetone (0.5 mL) was added Selectfluor (115 mg, 326 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.51 mL, 65 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of 5.37 (13.4 mg, 41.8 μmol, 64% combined yield for anti and syn in 5.9:1 ratio)

Data of 5.37 (anti): ¹H NMR (500 MHz, CDCl₃) δ 5.48 (1H, dddd, J = 2.20, 10.27, 51.35 Hz), 4.05 (1H, q, J = 6.11 Hz), 2.83 (1H, s), 1.96 – 1.88 (1H, m), 1.81 – 1.68 (2H, m), 1.35 (3H, s), 1.08 (3H, d, J = 6.36 Hz), 1.01 (3H, d, J = 6.85 Hz), 0.98 (6H, d, J = 6.26 Hz), 0.90 (9H, s), 0.86 (3H, s); ¹³C NMR (125 MHz, CDCl₃) δ 210.4 (J CF = 16.75), 92.2, 82.1, 72.4, 39.7, 25.8 (3), 25.7, 24.7, 23.3, 21.2, 18.6, 18.0, –4.3, –4.9; m/z (HRMS) calculated for [C₁₆H₃₃FO₃SiNa⁺]: 343.2, found: 343.2; [α]²⁵_D = –46.5° (c = 0.01, CHCl₃); IR v max (neat)/cm⁻¹ 3546, 2958, 2931, 2858, 1733, 1464, 1374, 1256, 1159, 1120, 1037, 982, 836.
To the solution of allene (17.5 mg, 65.2 μmol) 5.34 in t-BuOH (1 mL) and water (0.5 mL) was added NCS (43.5 mg, 326 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.51 mL, 65 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding 5.38 (17.1 mg, 50.9 μmol, 78% combined yield for anti and syn in 5.3:1 ratio).

Data of 5.38 (anti): ¹H NMR (500 MHz, CDCl₃) δ 5.02 (1H, d, J = 4.7, 10.2 Hz), 3.97 (1H, q, J = 6.3 Hz), 2.77 (1H, s), 1.90 – 1.82 (1H, m), 1.71 – 1.66 (2H, m), 1.42 (3H, s), 1.04 (3H, d, J = 6.3 Hz), 0.97 (3H, d, J = 6.7 Hz), 0.93 (3H, d, J = 6.3 Hz), 0.91 (3H, s), 0.90 (9H, s), 0.88 (3H, s); ¹³C NMR (125 MHz, CDCl₃) δ 208.2, 82.1, 73.3, 55.7, 40.9, 25.8 (3), 25.7, 24.8, 23.1, 21.1, 18.4, 18.0, −4.26, −4.81; m/z (HRMS) calculated for [C₁₀H₂₃ClO₃SiNa]⁺: 359.2, found: 359.2; [α]²⁵° = 24.8° (c = 0.01, CHCl₃); IR νmax (neat)/cm⁻¹ 3544, 2958, 2931, 2858, 1723, 1464, 1375, 1257, 1169, 1104, 1029, 1008, 837.

Stoichiometric condition is as follows; to the solution of allene (24.6 mg, 49.2 μmol) 5.35 in t-BuOH (1 mL), phosphate buffer (0.60 mL, pH = 7.4), and acetone (0.5
mL) was added Selectfluor (87.0 mg, 246 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.39 mL, 49 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding 5.39 (17.4 mg, 31.5 μmol, 64% yield, single diastereomer).

Catalytic condition is as follows; to the solution of allene (35.0 mg, 70.0 μmol) 5.35, and Selectfluor (124 mg, 349 μmol) in t-BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO₄ solution (55 μL, 7.0 μmol) was added slowly. Then, NMO (16.4 mg, 140 μmol) was added. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding 5.39 (23.8 mg, 43.1 μmol, 62% yield, single diastereomer).

Data of 5.39 (anti): ¹H NMR (500 MHz, CDCl₃) δ 7.67 – 7.63 (4H, m), 7.45 – 7.36 (6H, m), 7.21 – 7.18 (2H, m), 6.87 – 6.84 (2H, m), 5.16 (1H, d, J = 47.2 Hz), 4.49 – 4.46 (1H, m), 4.37 (2H, s), 3.96 (1H, ddd, J = 1.0, 3.4, 10.8 Hz), 3.89 (1H, ddd, J = 1.0, 4.4, 10.8 Hz), 3.80 (3H, s), 3.53 (1H, dd, J = 6.9 Hz), 3.19 (1H, dd, J = 9.3 Hz), 1.02 (9H, s), 1.00 (6H, s); ¹³C NMR (125 MHz, CDCl₃) δ 208.0, 207.8, 159.2, 135.7, 135.6, 132.9, 132.6, 129.8, 129.1, 127.7, 113.7, 97.0, 95.5, 76.5, 75.0, 72.8, 65.2, 55.2, 40.2, 40.1, 26.7, 20.9, 19.2; m/z (HRMS) calculated for [C₃₂H₄₁FO₅SiNa]⁺: 575.3, found: 575.3; [α]²⁵D = 18.4° (c = 0.01, CHCl₃); IR ν max (neat)/cm⁻¹ 3071, 3048, 2957, 2931, 2857, 1725, 1427, 1362, 1247, 1173, 1112, 1035, 1008, 736, 702.
Stoichiometric condition is as follows; to the solution of allene (24.6 mg, 49.2 μmol) 5.35 in t–BuOH (1 mL), phosphate buffer (0.60 mL, pH = 7.4), acetone (0.5 mL) was added NCS (32.8 mg, 246 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.39 mL, 49 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of 5.40 (19.2 mg, 33.8 μmol, 69% yield for anti and syn in 3.2:1 ratio).

Catalytic condition is as follows; to the solution of allene (18.2 mg, 36.4 μmol) 5.35 in t–BuOH (1 mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (0.5 mL) were added N–Methylmorpholine N–oxide (NMO) (8.5 mg, 73 μmol) and 4% aqueous OsO₄ solution (28 μL, 3.6 μmol). Then, NCS (24.3 mg, 182 μmol), dissolved in t–BuOH (0.5 mL), phosphate buffer (0.50 mL, pH = 7.4), and acetone (0.5 mL), was added dropwise over 3 hours via syringe pump at room temperature. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture of 5.40 (16.3 mg, 28.2 μmol, 77% yield for anti and syn in 1.5:1 ratio).
Data of 5.40 (anti): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.69 – 7.66 (4H, m), 7.44 – 7.36 (6H, m), 7.20 – 7.19 (2H, m), 6.87 – 6.83 (2H, m), 4.87 (1H, s), 4.39 (2H, s), 4.35 (1H, dt, $J =$ 1.7, 5.4 Hz), 3.92 (1H, dd, $J =$ 3.7, 10.8 Hz), 3.82 (1H, dd, $J =$ 5.4, 10.8 Hz), 3.79 (3H, s), 3.44 (1H, d, $J =$ 9.1 Hz), 3.32 (1H, d, $J =$ 9.3 Hz), 3.12 (1H, d, $J =$ 9.23 Hz), 1.09 (6H, s), 1.04 (9H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 206.0, 159.2, 135.7, 132.8, 132.6, 129.9, 129.8, 129.2, 127.8, 113.8, 77.9, 76.1, 73.0, 65.2, 62.4, 55.2, 26.7, 22.2, 20.8, 19.2; m/z (HRMS) calculated for [C$_{32}$H$_{41}$ClO$^5$SiNa]$^+$: 591.2, found: 591.2; $[\alpha]^{25}_D = 25.6^\circ$ (c = 0.01, CHCl$_3$); IR $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3071, 3049, 2955, 2927, 2855, 1728, 1427, 1364, 1248, 1173, 1112, 1036, 1008, 737, 702.

Stoichiometric condition is as follows; to the solution of allene (29.6 mg, 77.8 µmol) 5.44 in $t$–BuOH (1 mL), phosphate buffer (0.50 mL, pH = 7.4), acetone (0.5 mL) was added Selectfluor at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO$_4$ solution (0.61 mL, 78 µmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (14.2 mg, 32.9 µmol, 42% yield for 5.45 and 5.46 in 1: 2.6 ratio).

Catalytic condition is as follows; to the solution of allene (30.0 mg, 78.9 µmol) 5.44, Selectfluor (140 mg, 394 µmol), and NMO (18.5 mg, 158 µmol) in $t$–BuOH (1
mL), phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO₄ solution (62 µL, 7.9 µmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (19.3 mg, 44.7 µmol, 57% yield for 5.45 and 5.46 in 1: 1.8 ratio).

For detailed NMR analysis, see page 549; m/z (HRMS) calculated for [C₂₄H₃₃FO₄SiNa]⁺: 455.2; IR νmax (neat)/cm⁻¹ 3427, 3071, 3049, 2957, 2929, 2890, 2856, 1471, 1427, 1392, 1113, 907, 731, 702.

Stoichiometric condition is as follows; to the solution of allene (30.2 mg, 79.4 µmol) 5.44 in t–BuOH (1 mL), phosphate buffer (0.37 mL, pH = 7.4), and acetone (0.5 mL) was added NCS (53.0 mg, 397 µmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.62 mL, 79 µmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (17.4 mg, 38.8 µmol, 49% yield for 5.47 and 5.48 in 1: 1.1 ratio).

Catalytic condition is as follows; to the solution of allene (30.0 mg, 78.9 µmol) 5.44, NCS (52.6 mg, 394 µmol), and NMO (18.5 mg, 158 µmol) in t–BuOH (1 mL),
phosphate buffer (1.0 mL, pH = 7.4), and acetone (1 mL) was added 4% aqueous OsO₄ solution (62 μL, 7.9 μmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding the mixture (29.7 mg, 84% yield for 5.47 and 5.48 in 1:1.3 ratio).

For detailed NMR analysis, see page 556; m/z (HRMS) calculated for [C₂₄H₃₃ClO₄SiNa]⁺: 471.2, 473.2 found: 471.2, 473.2; IR ν_max (neat)/cm⁻¹ 3421, 3071, 3049, 2957, 2928, 2856, 1428, 1417, 1374, 1112, 1070, 916, 737, 702.

![Image](image_url)

5.49

To the solution of allene (9.4 mg, 40 μmol) 2.24 in t-BuOH (400 μL) and water (50 μL) was added 4% aqueous OsO₄ solution (0.64 mL, 81 μmol) slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding 5.49 (4.6 mg, 38% yield).

For detailed NMR analysis of 5.49, see page 573; m/z (HRMS) calculated for [C₁₅H₂₄O₆Na]⁺: 323.2, found: 323.2; IR ν_max (neat)/cm⁻¹ 3479, 2923, 2851, 1713, 1458, 1374, 1133.
To the solution of allene (10.5 mg, 45.0 μmol) 2.24 in t-BuOH (400 μL) and water (50 μL) was added NCS (30.2 mg, 226 μmol) at room temperature. The mixture was stirred for 20 minutes, then 4% aqueous OsO₄ solution (0.70 mL, 90 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding 5.50 (10.0 mg, 65% yield).

For detailed NMR analysis of 5.50, see page 585; m/z (HRMS) calculated for [C₁₅H₂₂Cl₂O₆H]⁺: 367.1, 369.1, 372.1, found: 367.1, 369.1, 372.1; IR ν max (neat)/cm⁻¹ 3433, 2920, 2850, 1739, 1462, 1375, 1241, 1157.

To the solution of allene (10.4 mg, 45.0 μmol) 2.24 in t-BuOH (2 mL) and water (1.3 mL) was added Selectfluor (109 mg, 448 μmol) at room temperature. The mixture was stirred for 7 minutes, then 4% aqueous OsO₄ solution (0.70 mL, 90 μmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na₂SO₃ and the organic layer was extracted in EtOAc.

![Chemical Structures](image)
The crude was purified by silica gel column chromatography, yielding 5.51 (6.3 mg, 42% yield) and the mixture of 5.52 and 5.53 (1.2 mg, 9% combined yield).

For detailed NMR analysis of 5.51, see page 603; *m/z* (HRMS) calculated for [C_{15}H_{22}F_{2}O_{6}Na]^+: 359.1, found: 359.1; IR $\nu_{\text{max}}$/cm$^{-1}$ 3479, 2927, 2853, 1727, 1455, 1372, 1162.

For detailed NMR analysis of 5.52 and 5.53, see page 618; *m/z* (HRMS) calculated for [C_{15}H_{21}FO_{4}Na]^+: 307.1, found: 307.1; IR $\nu_{\text{max}}$/cm$^{-1}$ 3492, 2929, 1723, 1461, 1371, 1155.

![5.54](image)

To a solution of bis[allene] macrolactone 2.24 (10.0 mg, 43.0 $\mu$mol) in t–BuOH and water mixture (1:1, total 2 mL) was added NMO (20.2 mg, 0.170 mmol) and 4% aqueous OsO$_4$ solution (135 $\mu$L, 17.0 $\mu$mol) sequentially. NBS (23.0 mg, 129 $\mu$mol) in t–BuOH (0.5 mL), phosphate buffer (0.5 mL, pH = 7) and acetone (0.5 mL) was added slowly over 10 hours. After the complete disappearance of SM, the reaction was quenched saturated solution of Na$_2$SO$_3$ and the organic layer was extracted with EtOAc. The organic phase was dried with Na$_2$SO$_4$ and the excess solvent was removed under reduced pressure. The crude material was chromatographed in silica gel, yielding 5.54 (6.0 mg, 47% yield).

For detailed NMR analysis, see page 631; IR $\nu_{\text{max}}$/cm$^{-1}$ 3459, 2923, 2850, 1735, 1712, 1459, 1370, 1163.
To the solution of allene (24.5 mg, 0.110 mmol) \textbf{2.24} in \textit{t}-BuOH (2 mL) and water (1.3 mL) was added Chloramine T–trihydrate (259 mg, 1.05 mmol) at room temperature. The mixture was stirred for 1 minute, then 4% aqueous OsO$_4$ solution (1.66 mL, 0.210 mmol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding \textbf{5.55} (2.3 mg, 7% yield).

For detailed NMR analysis, see page 638; \textit{m/z} (HRMS) calculated for [C$_{15}$H$_{20}$O$_7$Na]$^+$: 335.1, found: 335.1; IR $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3498, 2921, 2850, 1730, 1446, 1357.

To the solution of allene (20.7 mg, 41.0 $\mu$mol) \textbf{4.1} in \textit{t}-BuOH (1 mL), phosphate buffer (0.29 mL, pH = 7.4), and acetone (0.50 mL) was added 4% aqueous OsO$_4$ solution (0.71 mL, 91 $\mu$mol) slowly. To the mixture was added NBS (14.7 mg, 83.0 $\mu$mol), dissolved in \textit{t}-BuOH (0.5 mL), phosphate buffer (0.50 mL, pH = 7.4), and acetone (0.5 mL). After the complete disappearance of allene on TLC, the reaction mixture was
quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The organic phase was dried with Na$_2$SO$_4$ and the excess solvent was removed under reduced pressure. The crude was purified by silica gel column chromatography, yielding **5.57** (2.5 mg, 10% yield).

For detailed NMR analysis of **5.57**, see page 651; m/z (HRMS) calculated for [C$_{33}$H$_{41}$BrO$_6$Na]$^+$: 635.2, 637.2, found: 635.2, 637.2; IR $\nu$$_{\text{max}}$ (neat)/cm$^{-1}$ 3452, 3030, 2924, 2852, 1959, 1736, 1371, 1179, 1068, 697.

To the solution of allene (25.0 mg, 45.0 $\mu$mol) **4.1** in $t$-BuOH (1 mL), water (0.27 mL), and acetone (2 mL) was added Selectfluor (177 mg, 499 $\mu$mol) at room temperature. The mixture was stirred for 5 minutes, then 4% aqueous OsO$_4$ solution (1.18 mL, 90.0 $\mu$mol) was added slowly. After the complete disappearance of allene on TLC, the reaction mixture was quenched by saturated solution of Na$_2$SO$_3$ and the organic layer was extracted in EtOAc. The crude was purified by silica gel column chromatography, yielding **5.58** (2.6 mg, 10% yield) and **5.59** (5.0 mg, 21% yield).

For detailed NMR analysis of **5.58**, see page 653; m/z (HRMS) calculated for [C$_{26}$H$_{34}$F$_2$O$_7$Na]$^+$: 519.2, found: 519.2; [$\alpha$]$^2$$_{D}$ = 7.3° (c = 0.005, CHCl$_3$); IR $\nu$$_{\text{max}}$ (neat)/cm$^{-1}$ 3452, 2952, 2922, 2851, 1737, 1456,1377, 1174, 1068.
For detailed NMR analysis of 5.59, see page 671; $m/z$ (HRMS) calculated for $[C_{26}H_{35}FO_7Na]^+$: 501.2, found: 501.2; $\left[\alpha\right]_{D}^{25} = 1.3^\circ$ (c = 0.005, CHCl₃); IR $v_{\text{max}}$ (neat)/cm⁻¹ 3500, 2918, 2849, 1736, 1455, 1375, 1169, 1069, 1011.
7.5. Chapter 6

Synthetic Routes to DHLA–PEG–NH₂ and Heterofunctional Linkers:

(adapted with permission from ref.¹¹)

Synthesis of the Core–Shell Quantum Dots: CdSe/CdS/ZnS core–shell QDs were synthesized using well established protocols.¹ ² The QDs were then characterized
for size and its distribution using a transmission electron microscope (TEM); See Figure 6.2-1. The spectroscopic properties of the QDs were obtained using a fluorometer (FluoroMax–3, HORIBA Scientific).

\[
\text{H}_2\text{N}\left(\underset{n}{\text{O}}\right)\text{NH}_2
\]

Diamino–PEG (NH\(_2\)–PEG\(_8\)–NH\(_2\)) (6.1)

This procedure from the literature\(^3\) was modified. Neat PEG\(_8\) (20.0 g, 50.0 mmol, average MW 400 g/mol) was dried at 100 °C for 4 hours with stirring to remove all traces of water. The flask was cooled on an ice bath before thionyl chloride (10.5 mL, 145 mmol) was added dropwise. The solution was warmed to room temperature and stirred for 22 hours. After 22 hours, TLC indicated complete conversion to the bis–chloride; TLC (R\(_f\) 0.75, 90% DCM/MeOH, plate dried under vacuum prior to running) and ESI–MS (m/z 517.4 [M+H]\(^+\)). The crude product was diluted with DMF (20 mL) and the solvent removed under reduced pressure. After repeating this three times, the flask was left under high vacuum overnight to remove all traces of thionyl chloride. To the crude dark yellow product was added a solution of sodium azide (9.40 g, 145 mmol) in 250 mL of DMF. The resulting dark-orange solution was stirred for 16 hours at 85 °C, indicating complete conversion to the diazide by TLC (R\(_f\) 0.6, 90% DCM/MeOH) and ESI–MS (m/z 531.4 [M+H]\(^+\)). The solvent was removed under reduced pressure, and diluted with 200 mL of DCM. The precipitate was removed by vacuum filtration through Celite\(^\circledR\) and the solvent removed under high vacuum overnight to yield the intermediate diazide as brown oil. The sample was dissolved in 300 mL of THF, and triphenylphosphine (27.8 g, 106
mmol) was added to the mixture. The dark orange solution was stirred at room temperature for 24 hours, showing complete consumption of the diazide by ESI–MS and formation of the iminophosphorane intermediate by TLC (R_f 0.4, 90% DCM/MeOH). To the crude mixture, 4 mL of water was added and the dark brown mixture was stirred for 24 hours, showing complete reduction to the bis–amine 6.1 by TLC (R_f 0.1, 90% DCM/MeOH) and ESI–MS (m/z 457.4 [M+H]^+). THF was removed in vacuo, yielding a crude orange solid. 100 mL of water was added, and the precipitate was removed by vacuum filtration through a Büchner funnel. The filtrate was washed with toluene (3 x 50 mL). The water was removed in vacuo to give a dark orange oil. The crude product was purified by alumina column chromatography (DCM/MeOH, 95:5), giving 6.1 as a light yellow oil (19.8 g, 99%). Spectroscopic data matched those previously reported.^3^ Traces of triphenylphosphine oxide were detected by ^1^H NMR.

The previously reported protocols^3^, ^4^ for the synthesis of 6.1 required multiple steps; therefore, we applied a Mitsunobu reaction^5^ to acquire the bis–amine in a single step: PEG_8_ (10.0 g, 25.0 mmol), previously dried over 3Å molecular sieves, triphenylphosphine (13.1 g, 2.0 equiv.), and phthalimide (7.4 g, 2.0 equiv.) were mixed in 150 mL of DCM and cooled to 0 °C under nitrogen. To this solution was added 95% diisopropylazodicarboxylate (DIAD, 10.4 mL, 2.0 equiv.) dropwise. The temperature being kept at or below 20 °C. After the addition was complete, the reaction was allowed to warm to room temperature and was stirred for the additional 1 hour. To the mixture was then added 55% hydrazine (3.6 mL, 2.5 equiv.) and the reaction was refluxed overnight under nitrogen. It was then cooled to room temperature and filtered. The
filtrate was stirred with 15 g of Amberlyst® 15 cation exchange resin for 5 hours. The resin was then filtered and washed with DCM (3 x 50 mL). It was then added to a solution of 2.9 g of NaOH in 100 mL of anhydrous EtOH and mechanically stirred overnight. The resin was filtered and washed with 95% EtOH (3 x 50 mL). The filtrate was concentrated in vacuo to give 6.1 as an amber oil (9.2 g, 93%). Spectroscopic data matched those previously reported.3

![Lipoic Acid N–Hydroxysuccinimide–Ester (LA–NHS) (6.2)](image)

Lipoic Acid N–Hydroxysuccinimide–Ester (LA–NHS) (6.2)

The literature procedure3 was modified. To a solution of lipoic acid (5.00 g, 24.2 mmol) and N–hydroxysuccinimide (3.35 g, 29.1 mmol) in 150 mL of THF at 4 °C was added slowly a solution of dicyclohexylcarbodiimide (DCC, 6.00 g, 29.1 mmol) in 10 mL of THF. The mixture was warmed to room temperature and stirred for 8 hours, then showing complete conversion to the coupling product 6.2 by TLC (Rf 0.7, 90% DCM/MeOH). The precipitate was removed by vacuum filtration through Celite® and the solvent was evaporated in vacuo, giving a yellow solid. The crude product was redissolved in EtOAc and filtered again. This filtrate was concentrated in vacuo, giving pale–yellow oil. Recrystallization from a solution of hot ethyl acetate/hexane (1:1 v/v) afforded pure dithiane 6.2 a pale–yellow solid (3.47 g, 47%). The mother liquor was concentrated down and saved for future recrystallizations. Spectroscopic data matched those previously reported.3
This procedure from the literature\textsuperscript{3} was modified. To a yellow solution of 6.1 (13.0 g, 32.8 mmol) and sodium bicarbonate (2.75 g, 32.8 mmol) in DMF/water (100 mL, 50:50 v/v) at 4 °C was added dropwise a solution of recrystallized pure 6.2 (1.80 g, 5.90 mmol) in 10 mL of DMF over 1 hour, turning the reaction mixture a lighter yellow. The solution was warmed to room temperature and stirred for additional 16 hours, showing complete conversion to the amide 6.3 by TLC (R\textsubscript{f} 0.15, 90% DCM/MeOH) and ESI–MS (m/z 601.3 [M+H]\textsuperscript{+}, see page 686, adapted with permission from ref.\textsuperscript{11}). The crude product mixture was extracted with chloroform (3 x 30 mL). The combined organic extracts were washed with water (3 x 30 mL), dried over Na\textsubscript{2}SO\textsubscript{4} and filtered. After removing the solvent under reduced pressure, the crude product was purified by alumina column (DCM/MeOH, 95:5), yielding 6.3 as a yellow oil (1.93 g, 58\%). The remainder of the product was contaminated with traces of 6.1. This material was recycled along with excess 6.1 which recovered from the aqueous layer. Spectroscopic data matched those previously reported.\textsuperscript{3}

The published procedure\textsuperscript{3} for the synthesis of 6.3 required excessive extractions from the DMF/water layer; therefore, we developed the following procedure: 6.1 (7.8 g, 5.0 equiv.) was dissolved in 50 mL of DCM. To this solution was added a solution of 6.2 in 50 mL of DCM, dropwise with stirring, over 1 hour. After another 30 minutes, the solvent was removed \textit{in vacuo} and the residue was subject to silica gel chromatograph
(1% NH₄OH/10% MeOH/DCM). The compound 6.3 was obtained as an amber oil (2.6 g, 96% yield). Spectroscopic data matched those previously reported.³

![Dihydrolipoic Acid–PEG–Amine (DHLA–PEG₈–NH₂) (6.4)](image)

Dihydrolipoic Acid–PEG–Amine (DHLA–PEG₈–NH₂) (6.4)

This procedure from the literature³ was modified. To a yellow solution of 6.1 (0.20 g, 0.33 mmol) in 4:1 water/ethanol (3 mL) at 4 °C was slowly added 4 equiv. of sodium borohydride (50.0 mg, 1.33 mmol) over 30 minutes. The bubbling solution was stirred for 4 hours at 4 °C, showing only 67% conversion to the reduction product 6.4 by ESI–MS (m/z 603.3 [M+H]⁺, see page 688, adapted with permission from ref.¹¹). The product and starting material were not distinguishable due to overlapping TLC spots (Rₖ 0.15, 90% DCM/MeOH). The reaction mixture was left stirring overnight at room temperature. No further conversion was observed, so a total of 8 more equiv. of sodium borohydride was added over 48 hours, driving the reaction to completion in 72 hours. The crude reaction mixture was acidified to pH 2 with 1 M HCl, and extracted with chloroform (3 x 15 mL). The combined organic layer was dried over magnesium sulfate and filtered. The solvent was removed in vacuo to furnish the dithiol 6.4 as yellow oil (0.196 g, 98% yield). Spectroscopic data matched those previously reported.³ Traces of triphenylphosphine oxide were detected by ¹H NMR. A stability study was conducted on the dithiol. A 6 mg of 6.4 in CDCl₃ was kept under argon in a capped NMR tube for 48 hours, and no change was observed in the ¹H NMR spectrum. The sample was then
exposed to the atmosphere in an uncapped NMR tube for 48 hours, again showing no change by $^1$H NMR. This product appeared to be air–stable.

![Chemical Structure](image)

3–(2–Pyridyl)–dithiopropionic acid (PDP–OH) (6.5)

This procedure from the literature$^6$ was modified. Pyridyl disulfide (3.11 g, 14.1 mmol) was dissolved in 30 mL of anhydrous EtOH and cooled to $-10$ °C. Mercaptopropionic acid (MPA, 1.0 g, 9.4 mmol) in 20 mL of anhydrous EtOH was added dropwise over 30 minutes at $-10$ °C. The reaction mixture was allowed to warm to room temperature. After 24 hours, the solvent was removed *in vacuo* and the residue was chromatographed (15% EtOAc/hexane). The compound 6.5 was isolated as a pale yellow solid (1.3 g, 64%). Spectroscopic data matched those previously reported.$^6$

![Chemical Structure](image)

3–(2–Pyridyl)–dithiopropionic acid pentafluorophenyl ester (HFL1) (6.6)

This procedure was modified from the literature.$^7$ 6.5 (1.3 g, 6.0 mmol), pentafluorophenol (PFP, 1.1 g, 1 equiv.), and DCC (1.25 g, 1 equiv.) were dissolved in 30 mL of DCM and the reaction mixture was stirred at room temperature under nitrogen. After 48 hours, another 100 mg of both PFP and DCC were added to drive the coupling to completion within 3 hours. The solvent was removed *in vacuo*, and the residue was
purified by silica gel column chromatography (5% EtOAc/hexane). 6.6 was obtained as a yellow oil (1.88 g, 82% yield). Spectroscopic data matched those previously reported.\(^8\) Traces of DCC were detected by \(^1\)H NMR.

![3-Maleimidopropionic acid (MP–OH) (6.7)](image)

According to the literature,\(^9\) MP-OH was synthesized. 6.7 was obtained as a white solid (43% yield). Spectroscopic data matched those previously reported.\(^9\)

![3-Maleimidopropionic acid pentafluorophenyl ester (HFL2) (6.8)](image)

HFL2 was synthesized according to the literature.\(^7\) 6.8 was acquired as a white solid (74% yield). \(^1\)H–NMR (CDCl\(_3\)) \(\delta\): 6.72 (2H, s, maleimide), 3.95 (2H, \(-\text{CH}_2\alpha\)), 3.04 (2H, \(-\text{CH}_2\beta\)). Our spectrum agreed with that of the reported hexanoic analogue.\(^7\)

**Synthesis of Quantum Dot Conjugates with siRNA:** The siRNA molecules for eGFP and EGFRvIII containing 5’ thiol group were purchased from Dharmacon, Invitrogen. The siRNA for eGFP was designed as follows: sense sequence was 5’–thiol–GGCUACGUCCAGGAGCGCACC and antisense sequence was 5’–phosphate–
UGCGCUCCUGGACGUAGCCUU. For the knockdown of EGFRvIII, sense sequence was 5’–thiol–GAAAGGUAAUUAUGUGGUdGdTdT and antisense sequence was 5’–phosphate–CACCACAUAUUACCUUUCdTdT. QDs in the PBS were mixed with a 1000–fold excess of the cross linker, 6.6 and 6.8, for 1 hour. After removing the unreacted linkers by ultracentrifugation, siRNA molecules were coupled to the QDs by mixing them together for 1 hour. After the conjugation, free siRNA was removed using ultracentrifugation.

To quantify the ratio of siRNA molecules per QD, the siRNA linked to QDs via 6.6 (disulfide bond) were treated with DTT for 1 hour. After centrifugation, the supernatant was analyzed under UV at 260 nm to quantify the amount of siRNA detached from QDs. By determining the concentration of QDs in the same volume at the first absorption peak, the number of siRNA molecules per QD was estimated. For the conjugation of HIV–Tat(CYGRKKRRQRRR) and RGD(RGDC) to siRNA–QD, the same method as that used to conjugate siRNA to QDs was used to determine the ratio of 1:10:10 (siRNA:RGD:HIV–Tat).
7.6. References


Appendix: Spectral Data
MeO
MeO
mFBnO
Me
O
N O
O
Bn
### X-ray structure of 4.4

![X-ray structure diagram](image)

### Table 1. Crystal data and structure refinement for brlact.

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Theta range for data collection 2.11 to 30.55°.
Index ranges -11<=h<=11, -13<=k<=13, -14<=l<=14
Reflections collected 9028
Independent reflections 4557 [R(int) = 0.0119]
Completeness to theta = 30.55° 98.4 %
Absorption correction Semi-empirical from equivalents
Max. and min. transmission 0.746 and 0.646
Refinement method Full-matrix least-squares on F²
Data / restraints / parameters 4557 / 125 / 212
Goodness-of-fit on F² 1.001
Final R indices [I>2sigma(I)] R1 = 0.0246, wR2 = 0.0612
R indices (all data) R1 = 0.0264, wR2 = 0.0621
Largest diff. peak and hole 0.612 and -0.236 e.Å⁻³

Table 2. Atomic coordinates ( x 10⁴) and equivalent isotropic displacement parameters (Å² x 10³) for brlact. U(eq) is defined as one third of the trace of the orthogonalized Uᵢⱼ tensor.

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\]

Table 4. Anisotropic displacement parameters (Å² x 10³) for brlact. The anisotropic displacement factor exponent takes the form: 
\[-2\pi^2 [ h^2 a^* a^* U^{11} + ... + 2 h k a^* b^* U^{12} ] \]

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<td>H(7B2)</td>
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<td>4314</td>
<td>10037</td>
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Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å² x 10^3) for brlact.
Table 6. Torsion angles [°] for brlact.

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<th>Torsion Angle [°]</th>
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<td>71.25(13)</td>
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<td>C(15)-C(1)-C(2)-C(3)</td>
<td>132.43(11)</td>
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<td>Br(1)-C(1)-C(2)-C(3)</td>
<td>-109.33(10)</td>
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<td>C(3)-C(5)-C(6)-C(7B)</td>
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<td>C(6)-C(7A)-C(8A)-C(9A)</td>
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<td>C(5)-C(6)-C(7B)-C(8B)</td>
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<td>C(7A)-C(6)-C(7B)-C(8B)</td>
<td>29.2(6)</td>
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<td>C(6)-C(7B)-C(8B)-C(9B)</td>
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<td>C(7B)-C(8B)-C(9B)-C(10)</td>
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<td>C(8A)-C(9A)-C(10)-C(9B)</td>
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<tr>
<td>C(8A)-C(9A)-C(10)-C(11)</td>
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Table 7. Hydrogen bonds for brlact [Å and °].

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<tr>
<th>D-H...A</th>
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<th>d(H...A)</th>
<th>d(D...A)</th>
<th>&lt;(DHA)</th>
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<td>2.6364(14)</td>
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<td>0.82</td>
<td>2.83</td>
<td>3.4865(10)</td>
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Symmetry transformations used to generate equivalent atoms: #1 -x,-y,-z
$^{2}J = 17.6 \text{ Hz}$

$2J = 17.6 \text{ Hz}$

$2.5 \text{ Hz}$

$9.5 \text{ Hz}$
spin system isolation

[Chemical structure image]
Spin system isolation
1D TOCSY spectra
NOESY correlations

H3
H4
3-OCH₂Ph

5.25 5.00 4.75 4.50
4.025
4.050
4.075
12-CH₃
C₁₁--C=O
C₅--C=O
6-CH₃
gHMB correlations
there is no more long range correlation to 55 ppm! only one between H3 and 55 ppm.

\textbf{gHMBC} correlations C4 C10

There is no more long range correlation to 55 ppm!
<table>
<thead>
<tr>
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<th>7.48</th>
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<th>6.98</th>
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**Shifts (ppm)**

**1H NMR chemical shifts (ppm)**

**Coupling constants (Hz)**

**Notes:**
- The values represent chemical shifts and coupling constants for specific protons in the compound.
- The shifts are measured in parts per million (ppm) relative to a standard.
- The coupling constants provide information about the interactions between protons.

**Diagram:**
A structure diagram of the compound is shown, indicating the positions of the hydrogen atoms and their connectivity.

**Legend:**
- The diagram includes labels for atoms and functional groups, with arrows indicating bonds and interactions.
- Specific atoms and bonds are highlighted to aid in identifying the structure.
2-CH$_3$

13-CH$_3$CH$_3$

H$_4$

H$_3$

H$_2$

7-CH$_2$

H$_8$

8-CH$_3$

H$_9$

H$_10$

13-CH$_2$H$_12$

9 CH$_3$

O

1 CH$_3$

5 O

2 CH$_3$

7 OH

8 OH

4

5

6

7

8

9

10

11

12
NOESY expansions

- H3
- H4
- 2-CH3
- H3
- H4
- H2

Diagram showing NMR spectra with peaks at various chemical shifts.
H9 (triplet, 9.1 Hz)

8-CH3

NOESY expansion

H2

9 CH3

O

1

CH3

5

O

CH3

OBn

O

OBn

•

H

H3C

H3C

H7\beta

H7\alpha

H2

H9 (triplet, 9.1 Hz)
H4
6-CH3
NOESY expansion
9 CH3
O
1
CH3
5
O
CH3
OBn
O
OBn
•
H
H3C
H3C
H7||β
H7||α

6-CH₃
gHMBC
expansion

9

C3

C4

H3

H4

O

CH3

O

CH3

OBn

O

OBn

•

H

H3C

H3C

H7β

H7α

β

α
Assignments of CO groups

6.6
6.7
6.8
6.9
7.0
7.1
205.0 202.5 200.0
3.5
4.0
4.5
205.0 202.5 200.0 197.5
1.4
1.5
1.6
1.7
1.8
1.9
gHMBC

expansions

H4
H3
12-CH3 6-CH3
H3
H9
C11   C5 C11   C5 C11   C5
Coupling constant (J_HH)
2-CH₃

H₂

H₃

H₄

1D TOCSY subspectrum
1D TOCSY subspectrum
Differentiation of OCH$_2$Ph groups by NOESY
Aromatic compound with gHMBC correlations

- C2, C7, C8

NMR spectrum showing chemical shift values:

- 9-OCH2
- C2, C7
- C8

3.1, 3.2, 3.3, 3.4

CH3, CH3, CH3, O

H9

9-OCH2
0.93 (s, 1.6-C-H3, H4 = 7.67 Hz, 1.4-C-H3)
1.65 (t, J10 = 1.70 Hz, 1.4-CH2)
5.60 (d, JH13 = 6.3 Hz, J13 = 1.70 Hz, H13)
1.70 (s, H10)
3.83 (dd, JH8 = 2.6 Hz, J8 = 8.1 Hz, H8)
4.62 (d, J12 = 4.3 Hz, H12)
2.06 Hz, C11
90.7
20.6
C10
9.6
C9
68.3
C8
34.0
C7
42.9
C6
83.4
C5
21.9
C4
72.5
C3
7.97
C2
40.2
C1
171.0

shift (ppm)
13C NMR chemical shifts (ppm, 8)
coupling constant (Hz, 8)
1H NMR chemical shifts (ppm, 8)
Coupling constant (J<sub>HH</sub>)

NOESY
HMBC

The diagram depicts a molecular structure with various chemical functionalities and atom labels, including hydrogens (H), carbons (C), and methyl groups (CH₃). The HMBC (Heteronuclear Multiple Bond Correlation) spectrum is represented by red arrows indicating correlations between different atoms across bonds.
HMBC
$^1\text{H NMR spectrum in CDCl}_3$

$^{1}J_{AB} = 12.1\ \text{Hz}$
$J_{AB} = 14.9 \text{ Hz}$

$J = 1.9 \text{ Hz}$

$J_{AB} = 14.9 \text{ Hz}$

$H \text{ NMR spectrum in CDCl}_3$
$^{13}$C NMR spectrum in CDCl$_3$
$\text{13C NMR spectrum in CDCl}_3$
NOESY expansion

8-CH$_3$

2-CH$_3$

6-CH$_3$

13-CH$_3$

8-CH$_3$
NOESY expansion

H2
H9
H3
H4/H10
H13
H7
2-CH3            6-CH3                            
•
H
Me
9
CH3
13
O
1
CH3
3
O
6
H3C
O
OBn
O
OHMe H
H
H
H7
H7
NOESY expansion

H2
H3         H9
H4
H10
4-OH
H13
•
H
Me
9
CH3
13
O
1
CH3
3
O
6
H3C
O
OBn
O
OHMe H
H
H
H7
H7
gHMBC correlations

H10/H4

C12

C3

•

H

Me

9

CH3

13

O

1

CH3

3

O

6

H

3C

O

OBn

O

OH

Me

H

H

H7

H7
gHMBC correlations

9-OCH$_2$
gHMBC correlations

9-OCH₂

C9

9-OCH₂

1JCH

1JCH

gHMBC correlations
gHMBC correlations
gHMBC correlations

$H \cdot Me \cdot 9 \cdot CH_3 \cdot 1 \cdot O \cdot 13 \cdot CH_3 \cdot 3 \cdot O \cdot 6 \cdot H_3C \cdot O \cdot OBn \cdot O \cdot OHMe H \cdot H \cdot H \cdot H_7\alpha \cdot H_7\beta$
gHMBC correlations
C13-C6 C3-C9
8-CH3 13-CH2-CH3
6-CH3 2-CH3
12-CH3 8-CH3
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<th>1H NMR Chemical Shifts (δ/ppm)</th>
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</table>

**1H NMR chemical shifts (δ/ppm)**

- 4.55 (dd, J = 9.0 Hz, 2.4 Hz)
- 3.50 (d, J = 2.8 Hz)
- 3.29 (dp, J = 2.4 Hz, 1.7 Hz)
- 3.09 (d, J = 1.4 Hz)
- 2.73 (m, J = 1.4 Hz, 1.4 Hz)
- 2.17 (t, J = 1.4 Hz)
- 1.75 (dd, J = 9.0 Hz, 3.0 Hz)
- 1.65 (m, J = 1.4 Hz, 1.4 Hz)
- 1.53 (dd, J = 9.0 Hz, 1.4 Hz)
- 1.38 (dd, J = 9.0 Hz, 3.0 Hz)
- 1.21 (t, J = 1.4 Hz)
- 0.96 (d, J = 2.4 Hz, 9.0 Hz)
- 0.86 (d, J = 8.0 Hz, 9.0 Hz)
- 0.69 (q, J = 8.0 Hz)
- 0.67 (m, J = 8.0 Hz, 3.0 Hz)
- 0.65 (m, J = 8.0 Hz, 9.0 Hz)
- 0.44 (dd, J = 8.0 Hz, 3.0 Hz)
- 0.37 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.34 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.32 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.29 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.27 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.25 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.23 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.21 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.19 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.17 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.15 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.13 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.11 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.09 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.07 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.05 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.03 (dd, J = 9.0 Hz, 3.0 Hz)
- 0.01 (dd, J = 9.0 Hz, 3.0 Hz)

**Coupling constants (J/Hz)**

- 13C NMR chemical shifts (δ/ppm)
- 1.47 (s, 12-CH3)
- 1.44 (d, 7-CH2)
- 1.41 (d, 8-CH2)
- 1.32 (t, 9-CH2)
- 1.31 (t, 10-CH2)
- 1.30 (t, 11-CH2)
- 1.29 (t, 12-CH2)
- 1.22 (t, 9-CH2)
- 1.21 (t, 8-CH2)
- 1.20 (t, 7-CH2)
- 1.19 (t, 6-CH2)
- 1.18 (t, 5-CH2)
- 1.17 (t, 4-CH2)
- 1.16 (t, 3-CH2)
- 1.15 (t, 2-CH2)
- 1.14 (t, 1-CH2)
- 1.13 (t, 10-CH2)
- 1.12 (t, 9-CH2)
- 1.11 (t, 8-CH2)
- 1.10 (t, 7-CH2)
- 1.09 (t, 6-CH2)
- 1.08 (t, 5-CH2)
- 1.07 (t, 4-CH2)
- 1.06 (t, 3-CH2)
- 1.05 (t, 2-CH2)
- 1.04 (t, 1-CH2)
- 1.03 (t, 10-CH2)
- 1.02 (t, 9-CH2)
- 1.01 (t, 8-CH2)
- 1.00 (t, 7-CH2)
- 0.99 (t, 6-CH2)
- 0.98 (t, 5-CH2)
- 0.97 (t, 4-CH2)
- 0.96 (t, 3-CH2)
- 0.95 (t, 2-CH2)
- 0.94 (t, 1-CH2)
1d TOCSY

Coupling constant (J_HH)
Observed NOESY cross peaks between H3 and 2-CH$_3$/6-CH$_3$.
Spin system identification

[Diagram of a molecular structure with labeled atoms and protons]

2-CH₃
H₂
H₃
H₄
13C-H

Spin system identification
NOESY correlations
H2
H3
H9
H3
H4
9-CH3
O
1-CH3
5-CH3
O
Bn
O
Bn
•
H
H3C
H3C O
H7β
H7α
9-CH2
9-CH2
6-CH2
H4
NOESY correlations

9 CH₃

O

1

CH₃

5

O

CH₃

OBn

O

OBn

•

H

H₃C

H₃C
NOESY correlations
9 CH₃
O
1
CH₃
5
O
CH₃
OBn
O
OBn
•
H
H₃C
H₃C O
H₇β
H₇α
H₉
H₃
H₁₀ H₁₃
H₄
9-OCH₂
6-OCH₂
NOESY correlations

[Chemical structure diagram]
NOESY correlations

9 CH₃
O
1
CH₃
5
O
CH₃
OBn
O
OBn
•
H
H3C
H3C O
H7β
H7α
8-CH₃
2-CH₃
H4                  H9  H3         H2

6-OCH₂       1.20  1.15  1.10

5.0  4.5  4.0  3.5  3.0
gHMBC correlations

9 CH₃
O
1
CH₃
5
O
CH₃
OBn
O
OBn
•
H
H₃C
H₃C O
H₇β
H₇α
C₉               C₆
H₉
H₃
9-OCH₂
9-OCH₂
6-OCH₂ 9-OCH₂
C₂
1.02 (t, J = 7.1 Hz, H-14, H-14)
1.45 - 1.29 (m, J = 7.1 Hz, H-14)
3.96 (dd, J = 7.1 Hz, H-14, J = 2.1 Hz, H-13)
1.60 (s, J = 2.1 Hz, H-13)
3.07 (dd, J = 7.1 Hz, J = 3.2 Hz, H-13, J = 13.5 Hz, H-10a)
2.63 (dd, J = 3.2 Hz, J = 13.5 Hz, H-10a)
4.33 (d, J = 3.2 Hz, H-10a)
4.39 (d, J = 3.2 Hz, H-10a)
0.88 (dd, J = 3.2 Hz, H-8)
1.52 (m, H-8)
1.75 (dd, J = 3.2 Hz, J = 13.5 Hz, H-10a)
1.84 (dd, J = 3.2 Hz, J = 13.5 Hz, H-10a)
1.26 (s, J = 6.8 Hz, H-8)
2.02 (s, J = 6.8 Hz, H-8)
6.18 (d, J = 9.6 Hz, H-4)
4.18 (d, J = 9.6 Hz, H-4)
1.29 (dd, J = 7.3 Hz, H-3, J = 7.3 Hz, H-3)
1.20 (d, J = 7.3 Hz, H-3, J = 7.3 Hz, H-3)
3.29 (dd, J = 7.3 Hz, H-3, J = 7.3 Hz, H-3)

w/ coupling constant (J Hz)
1H NMR chemical shifts (δ ppm)
0.93 (6 H, CH₃, 2-H, 3-H, 4-H, 9-H, 10-H)
1.70 - 1.60 (6 H, CH₂, 1-H, 12-H, 13-H)
3.63 (4 H, CH₂, 6-H, 8-H)
1.77 (6 H, CH₃, 7-H, 8-H, 10-H)
5.08 - 5.05 (2 H, CH=CH₂)
4.61, 4.33 (2 H, CH=CH₂, 5-H, 11-H)
4.07 (2 H, CH₂, 1-H, 2-H)
0.88 (9 H, CH₃, 15-H)
1.70 (3 H, CH₃, 14-H)
1.85 (3 H, CH₃, 13-H)
1.17 (3 H, CH₃, 12-H)
2.13 (3 H, CH₃, 9-H)
6.31 (1 H, J=13=8, H3, H4)
4.13 (1 H, J=13=8, H3, H4)
1.21 (1 H, J=13=8, H3, H4)
3.00 (2 H, CH₂, 1-H, 2-H, 3-H, 4-H)

Coupling constant (J/Hz)

1H NMR chemical shifts (δ/ppm)
spin systems identification
HMBC correlations

- H
- Me
- 9
- CH₃
- O
- 1
- CH₃
- 3
- O
- 6
- H₃C
- O
- O
- Bn
- O
- O
- Me
- H
- H
- H
- H
- 7β
- H
- 7α
- CH₃O
$3J_{H4H3} = 7.95 \text{ Hz}$
1D TOCSY spectrum

\[
\begin{align*}
\text{SiCH}_2\text{CH}_3 & \\
\text{SiCH}_2\text{CH}_3 & \\
\end{align*}
\]
1D TOCSY spectra
1D TOCSY spectra

H2

H3

H4

2-CH3

Me

H

OBn

OTES

Me

H

H

H

H

H

H

H
$\text{C NMR spectrum}$

$\text{H}$

$\text{Me}$

$9$

$\text{CH}_3$

$13$

$14$

$\text{O}$

$1$

$\text{CH}_3$

$3$

$\text{O}$

$6$

$\text{H}_3\text{C}$

$\text{O}$

$\text{OBn}$

$\text{O}$

$\text{OTESMe}$

$\text{H}$

$\text{H}$

$\text{H}$

$\beta$

$\text{H}$

$\alpha$
13C NMR spectrum
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<th>Coupling Constants (Hz)</th>
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<td>1.14 (4-H, CH₃) H4 = 1.1 Hz, 1.4 CH₃</td>
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<tr>
<td>7.12</td>
<td>1.62 (m, 1.4 CH₂)</td>
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<td>5.66</td>
<td>(dd, J=1.3 Hz, 1.4 Hz) H3 = 5.6 Hz, 9.0 Hz, H13</td>
</tr>
<tr>
<td>7.77</td>
<td>(d, J=2.7 Hz, 1.2 CH₃) H3</td>
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<tr>
<td>5.07</td>
<td>(m, H10)</td>
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<tr>
<td>7.12, 4.73</td>
<td>6.43 (4-H, 4.9 Hz, 2.0 Hz, 2.0 Hz, 2.0 Hz, 2.0 Hz)</td>
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<td>3.33</td>
<td>(dd, J=8.0 Hz, 6.0 Hz)</td>
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<td>1.25</td>
<td>(d, J=8.0 Hz, 8.0 Hz)</td>
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<td>1.89</td>
<td>(dd, J=6.8 Hz, 6.8 Hz, 15.7 Hz, 15.7 Hz)</td>
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<tr>
<td>1.66</td>
<td>(m, H17)</td>
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<tr>
<td>4.00</td>
<td>(dd, J=4.4 Hz, 1.3 Hz, J=7.6 Hz, H3)</td>
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<tr>
<td>0.91</td>
<td>(d, J=7.6 Hz, 2.0 Hz, 2.0 Hz)</td>
</tr>
<tr>
<td>2.99</td>
<td>(dd, J=4.4 Hz, 4.4 Hz, 7.6 Hz, 7.6 Hz, 7.6 Hz)</td>
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<td>1.4C₃</td>
<td>(6-OH)</td>
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</table>

1H NMR chemical shifts (δ ppm)
Chloroform-d

•

H

Me

9

CH₃

13

14

O

1

CH₃

3

O

6

H₃C

O

OBn

O

OBnMe

H

H

H

H

7β

H

7α
Coupling constant (\( J_{HH} \))
1D NOESY

H9

9-OCH₂

1H NMR (control spectrum)

Selective decoupled 1H NMR spectrum
1D TOCSY
C NMR spectrum expansions

C9                                                                    C6   C13                                         C3                                   C3-OCH

C9-OCH

C12                                                               C4         C2     C7                                C8                                 6-CH

12-CH                                                             12-CH

13-CH

13CH2CH3

9

Me

13

Et O

1

Me

OBn

O

Me

OH

O

OBn

H

BrHO

Me

H4

H4

H7

H7
DPFGSENOE subspectrum

H10
H13

Et O
Me
OBn
O
Me
OH
O
OBn
H
BrHO
Me
H4β
H4α
H7β
H7α

H10
H13

DPEGSENOE
gHMBC expansions

C9 \rightarrow \ldots \rightarrow 6-CH_3, 2-CH_3

12-CH_3, 8-CH_3, 13-CH_2CH_3
Multiplicity patterns of protons

$J(H_4, H_5) = 15.94 \text{ Hz}$

$J(H_{10}, H_{11}) = 15.63 \text{ Hz}$

$J(H_4, 3-\text{CH}_2) = 5.3 \text{ Hz}$

$J(H_4, H_7) = 0.6 \text{ Hz}$
1D TOCSY experimental subspectrum of H7 (δ 5.35)
1D TOCSY experimental subspectrum of H7 (δ 5.35)

- 6-CH$_3$
- 8-CH$_2$
- 9-CH$_2$

mix = 10 ms
mix = 250 ms
1D TOCSY experimental subspectrum of H7 (δ 5.35)
mix = 10 ms
mix = 250 ms

6-CH₃
8-CH₂
9-CH₂
H10
H11
H7

1D TOCSY experimental subspectrum of H7 (δ 5.35)
1D TOCSY experimental subspectrum of H5 (δ 6.39)
1D TOCSY experimental subspectrum of H5 ($\delta$ 6.39)
Resolution enhanced 1H NMR spectrum
Expansions of the 1H NMR spectrum
$^1$H COSY
H NMR spectrum in C₆D₆

3.47 doublet of doublets of quartets (ddq) 10.6 Hz

3.47 doublet 10.6 Hz

3.07 doublet of doublets of quartets 6.8 Hz

3.07 doublet 6.8 Hz

2.00 1H

2-CH₃

H₂C

H

H₃C

13C

O

2

O

C

H₃

BnO

7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

3.475 3.450 3.075 3.050
$^{1}H$ NMR spectrum in $D_{6}$
The image shows a 2D NMR spectrum with chemical shift values and assignments labeled. The spectrum includes peaks labeled with chemical shifts such as 7.22 ppm. A structural diagram of the compound is also present, with atoms and functionalities labeled, such as H3, H4, and CH3 groups. The peaks correspond to the chemical shifts observed in the NMR spectrum.
selective excitation of H3 at 7.22 ppm
Selective decoupled $^1$H NMR spectra
Selective decoupled 1H NMR spectrum
Selective decoupled 1H NMR spectrum

H4α

H4β

H3

H4α

H4β

H3

H4α

H4β

Selective decoupled 1H NMR
Selective decoupled $^1$H NMR spectrum
Selective decoupled $^1$H NMR spectrum of the compound.
selective excitation of H4
Selective decoupled $^1$H NMR spectrum

$H_{13}$

$H_{10}$

$\beta_{H7}$ $\alpha_{H7}$
$\beta_{H4}$ $\alpha_{H4}$
$H_{3C}$
$CH_3$
$O$
$CH_3$
$O$
$BnO$

4.5 4.0 3.5 3.0 5.0 5.5

$5.075$ $5.050$

$5.075$ $5.050$

Selective decoupled $^1$H NMR spectrum
Selective decoupled $^1$H NMR spectrum
<table>
<thead>
<tr>
<th>Shift (ppm)</th>
<th>1H NMR chemical shifts (ppm)</th>
<th>Coupling constants (J/Hz)</th>
</tr>
</thead>
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<td>11.5, 7.28 (m, 1H), Ph</td>
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<td>1.35</td>
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<td></td>
</tr>
<tr>
<td>1.34</td>
<td>0.8, 1H</td>
<td></td>
</tr>
<tr>
<td>1.33</td>
<td>0.6, 1H</td>
<td></td>
</tr>
<tr>
<td>1.32</td>
<td>0.4, 1H</td>
<td></td>
</tr>
<tr>
<td>1.31</td>
<td>0.2, 1H</td>
<td></td>
</tr>
<tr>
<td>1.30</td>
<td>0.0, 1H</td>
<td></td>
</tr>
<tr>
<td>1.29</td>
<td>1.8, 1H</td>
<td></td>
</tr>
<tr>
<td>1.28</td>
<td>1.6, 1H</td>
<td></td>
</tr>
<tr>
<td>1.27</td>
<td>1.4, 1H</td>
<td></td>
</tr>
<tr>
<td>1.26</td>
<td>1.2, 1H</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>1.0, 1H</td>
<td></td>
</tr>
<tr>
<td>1.24</td>
<td>0.8, 1H</td>
<td></td>
</tr>
<tr>
<td>1.23</td>
<td>0.6, 1H</td>
<td></td>
</tr>
<tr>
<td>1.22</td>
<td>0.4, 1H</td>
<td></td>
</tr>
<tr>
<td>1.21</td>
<td>0.2, 1H</td>
<td></td>
</tr>
<tr>
<td>1.20</td>
<td>0.0, 1H</td>
<td></td>
</tr>
<tr>
<td>1.19</td>
<td>1.8, 1H</td>
<td></td>
</tr>
<tr>
<td>1.18</td>
<td>1.6, 1H</td>
<td></td>
</tr>
<tr>
<td>1.17</td>
<td>1.4, 1H</td>
<td></td>
</tr>
<tr>
<td>1.16</td>
<td>1.2, 1H</td>
<td></td>
</tr>
<tr>
<td>1.15</td>
<td>1.0, 1H</td>
<td></td>
</tr>
<tr>
<td>1.14</td>
<td>0.8, 1H</td>
<td></td>
</tr>
<tr>
<td>1.13</td>
<td>0.6, 1H</td>
<td></td>
</tr>
<tr>
<td>1.12</td>
<td>0.4, 1H</td>
<td></td>
</tr>
<tr>
<td>1.11</td>
<td>0.2, 1H</td>
<td></td>
</tr>
<tr>
<td>1.10</td>
<td>0.0, 1H</td>
<td></td>
</tr>
</tbody>
</table>

Note: The table and diagram represent chemical shifts and coupling constants for a compound, likely obtained from a NMR spectroscopy experiment.
1d TOCSY
1D TOCSY subspectrum

H13

13-CH2

H13

CH3CH2

CH2CH2
1D TOCSY subspectrum

\begin{center}
\includegraphics[width=\textwidth]{1d_tocsys_spectrum.png}
\end{center}
1H NMR chemical shifts (δ ppm) & coupling constants (J/Hz)
Coupling constant (J_HH)
NOE
13-CH

2  1.73/1.85
 1.77  1.83
12-CH3
7-CH2
dd ... 3J = 6.7 Hz
t, 3J =7.4 Hz
13-CH2CH3
10 Me
Et O
1
Me
OBn
5
OH
Me
OH
O
OBn
H
Br
HO
Me
H4
H4
H7
H7
H3 (δ 4.36)

J(H4(α),H3) = 8.5 Hz

J(H4(β),H3) = 15.0 Hz

J(H4(α),H5) = 2.4 Hz

J(H4(β),H5) = 7.5 Hz

J(H4(α),H4(β)) = 15.0 Hz

J(H4(β),H4(α)) = 15.0 Hz

H5 (δ 3.48)

J(H4(α),H4(β)) = 15.0 Hz

J(H4(β),H3) = 3.0 Hz

J(H4(α),H3) = 8.5 Hz

J(H4(β),H3) = 2.4 Hz

J(H4(α),H4(β)) = 15.0 Hz

J(H4(β),H4(α)) = 15.0 Hz
\[ J(H_4^{\beta}, H_5) = 7.5 \, \text{Hz} \]
\[ J(H_4^{\alpha}, H_5) = 2.4 \, \text{Hz} \]

\[ J(H_7^{\alpha}, H_8) = 7.0 \, \text{Hz} \]
\[ J(H_7^{\beta}, H_7^{\alpha}) = 14.7 \, \text{Hz} \]
\[ J(H_7^{\beta}, H_8) = 6.1 \, \text{Hz} \]
\[ J(H_7^{\beta}, H_7^{\alpha}) = 14.7 \, \text{Hz} \]
Spin system identification

H13
CH3-CH2-13

10 Me
Et O
Me
OBn
5
OH
Me
OH

O
OBn
H
Br
HO
Me
H4β
H4α
H7β
H7α
Spin system identification

H10 ... H7
H7 8-CH3
10 Me
Et O
1 Me
OBn
5 OH
Me
OH
O
OBn
H Br
HO
Me
H4β
H4α
H7β
H7α
Spin system identification

2-CH$_3$

H$_3$                 
H$_5$                                        H$_4$
H$_2$

10 Me
Et O
1
Me
OBn
5
OH
Me
OH
O
OBn
H
Br
HO
Me
H$_4$$\beta$
H$_4$$\alpha$
H$_7$$\beta$
H$_7$$\alpha$
1D NOESY
NOESY expansion
H5 has to up in order to observe NOE with 3-OCH$_2$PH (methylene protons);
1.75 1.50 1.25 1.00
4.20
4.25
4.30
4.35
4.40
H3
H4α H4β
H7β
2-CH3
H7α
NOESY expansion
10 Me
Et O
5
OH
Me
OH
OBn
H
Br
HO
Me
H4β
H4α
H7β
H7α
gHMBC expansions
gHMBC expansions
$g$HMBC expansion

$13-$CH$_2$CH$_3$
6-CH$_3$ 2-CH$_3$  8-CH$_3$

gHMBC expansions

6-CH$_3$  2-CH$_3$                8-CH$_3$
C9                  
C3       C5 C6 C2          C7           C4 C8
gHMBC expansion
gHMBC expansion

C2  C7  C4  C8

H2  H8

2-CH3  8-CH3

Me  Et  O

OBn  OH  Me

H4β  H4α  H7β  H7α
\[ C_{13} \text{ NMR spectrum} \]
$^{13}$C NMR spectrum
$^{13}$C NMR spectrum


Coupling constant (J/Hz)

1H NMR chemical shifts (δ/ppm)

13C NMR chemical shifts
Selective decoupling of 2-CH$_3$
Long-range gHMBC correlations

\[ H \cdot C \cdot H \]

O

O

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H

H
confirming of C3 assignment
Selective irradiation of H₄

6-CH₃

H₃
Selective irradiation of H9

Quartet (J = 2.92 Hz) to 12-CH3
Selective decoupling of H8
$^{13}$C NMR spectrum in CDCl$_3$
2-CH₃
3-OCH₂
H₂
H₃
H₄
DPFGSENOE subspectra
3-OCH₂
H₂
H₃
$^{1}H$ NMR spectra of tert-butyl diisopropylamine (TBDI) in a solvent (CD$_3$OD).
1D TOCSY spectrum

Diagram showing chemical shifts and sub-spectra.
Cyclohexyl protons identification

N-CH

5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
APT
H8 / H10

C9

gHMBC

4.225 4.250 4.275
TBDDS-CI-Me$_2$
1D NOESY

J_{CH3,F} = 1.5 Hz

J_{CH3,F} = 4.4 Hz

J_{CH3,F} = 3.9 Hz

J_{CH3,F} = 0.6 Hz

$\delta = 3.19$

$\delta = 3.15$

$\delta = 3.18$

$\delta = 3.81$

$\delta = 3.93$

$\delta = 4.53$

$\delta = 3.79$

$\delta = 3.88$

$\delta = 4.38$

$\delta = 3.38$

$\delta = 3.80$

$\delta = 3.87$

$\delta = 3.86$

$\delta = 3.85$

$\delta = 3.84$

$\delta = 3.83$

$\delta = 3.82$

$\delta = 3.81$

$\delta = 3.80$

$\delta = 3.79$

$\delta = 3.78$

$\delta = 3.77$

$\delta = 3.76$

$\delta = 3.75$

$\delta = 3.74$

$\delta = 3.73$

$\delta = 3.72$

$\delta = 3.71$

$\delta = 3.70$

$\delta = 3.69$

$\delta = 3.68$

$\delta = 3.67$

$\delta = 3.66$

$\delta = 3.65$

$\delta = 3.64$

$\delta = 3.63$

$\delta = 3.62$

$\delta = 3.61$

$\delta = 3.60$

$\delta = 3.59$

$\delta = 3.58$

$\delta = 3.57$

$\delta = 3.56$

$\delta = 3.55$

$\delta = 3.54$

$\delta = 3.53$

$\delta = 3.52$

$\delta = 3.51$

$\delta = 3.50$

$\delta = 3.49$

$\delta = 3.48$

$\delta = 3.47$

$\delta = 3.46$

$\delta = 3.45$

$\delta = 3.44$

$\delta = 3.43$

$\delta = 3.42$

$\delta = 3.41$

$\delta = 3.40$

$\delta = 3.39$

$\delta = 3.38$

$\delta = 3.37$

$\delta = 3.36$

$\delta = 3.35$

$\delta = 3.34$

$\delta = 3.33$

$\delta = 3.32$

$\delta = 3.31$

$\delta = 3.30$

$\delta = 3.29$

$\delta = 3.28$

$\delta = 3.27$

$\delta = 3.26$

$\delta = 3.25$

$\delta = 3.24$

$\delta = 3.23$

$\delta = 3.22$

$\delta = 3.21$

$\delta = 3.20$

$\delta = 3.19$
\[ ^1J_{CF} = 189.0 \text{ Hz} \]

\[ ^1J_{CF} = 189.3 \text{ Hz} \]

\[ ^2J_{CF} = 25.6 \text{ Hz} \]

\[ ^2J_{CF} = 16.6 \text{ Hz} \]

\[ ^3J_{CF} = 3.5 \text{ Hz} \]

\[ ^4J_{CF} = 2.1 \text{ Hz} \]

\[ ^2J_{CF} = 18.3 \text{ Hz} \]

\[ ^2J_{CF} = 18.6 \text{ Hz} \]

\[ ^1H \quad \alpha \text{C-H}_3 \quad (\delta = 3\text{.}19) \]

\[ ^1H \quad \beta \text{C-H}_3 \quad (\delta = 3\text{.}28) \]

\[ ^1H \quad \text{TBDPSO} \quad (\delta = 3\text{.}38) \]

\[ ^1H \quad \text{TBDPSO} \quad (\delta = 3\text{.}38) \]

\[ ^1H \quad (\delta = 3\text{.}38) \]

\[ ^1H \quad (\delta = 1\text{.}12) \]

\[ ^1H \quad (\delta = 4\text{.}79) \]
$J_{CF} = 5.4 \text{ Hz}$

$J_{CF} = 15.2 \text{ Hz}$

$J_{CF} = 2.3 \text{ Hz}$

$J_{CF} = 12.0 \text{ Hz}$
Coupling constant (J\textsubscript{HH})

1d TOCSY
Magnetization transfer pathway (spin system identification)
Selective irradiation of H5.

H5

5-HOH

\[ \text{Selective irradiation of H5} \]
DPFGSENOE subspectra (selective excitation of CH$_3$'s)
gCOSY H5
5-OH
5
HO
4
6
OHTBDPSO
HB
HA
H
O
Cl
H
CH3
CH3
H
H
5.48
$^{13}C$ NMR spectra
$3J(H_9\alpha-H_8\alpha) = 3.63$
$3J(H_9\alpha-H_8\beta) = 9.78$
$3J(H_9\beta-H_8\alpha) = 6.64$
$3J(H_9\beta-H_8\beta) = 9.78$
$2J(H_8\alpha-H_8\beta) = 14.02$
$3J(H_8\alpha-H_7\alpha) = 3.27$
$3J(H_8\alpha-H_7\beta) = 12.65$
$3J(H_8\beta-H_7\alpha) = 13.12$
$3J(H_8\beta-H_7\beta) = 3.67$
$2J(H_7\alpha-H_7\beta) = 13.91$
$3J(H_4\alpha-H_3\beta) = 9.41$
$3J(H_{10}\alpha-H_9\alpha) = 2.27$
$3J(H_{10}\alpha-H_9\beta) = 12.30$
$2J(H_{10}\alpha-H_{10}\beta) = 18.65$
$3J(H_4\alpha-H_3\alpha) = 6.22$
$2J(H_4\alpha-H_4\beta) = 18.40$
$3J(H_3\alpha-H_2\alpha) = 5.45$

$1^H$ and $13C$ NMR Chemical shifts (δ/ppm) and Coupling constants (J/Hz)
$J_{AB} = 11.7 \text{ Hz}$
Spin systems identifications

1D TOCSY
1D TOCSY subspectra

selective excitation of $H_8^\alpha$ at 0.54 ppm

$H_8^\beta$

$H_{10}^\alpha$ $H_{10}^\beta$

$H_7^\alpha$

$H_8^\alpha$ $H_7^\beta$

$H_9^\alpha$ $H_9^\beta$
Selective decoupled $^1$H NMR spectrum
Selective decoupled $^1$H NMR spectrum

\[ H_8 \beta \]

\[ H_7 \alpha \]

\[ H_7 \alpha \]

\[ H_8 \beta \]

\[ H_7 \alpha \]
gHMB
\[ J(\text{H9}\alpha\rightarrow\text{H8}\alpha) = 3.79 \]
\[ J(\text{H9}\alpha\rightarrow\text{H8}\beta) = 3.76 \]
\[ J(\text{H9}\beta\rightarrow\text{H8}\alpha) = 11.98 \]
\[ J(\text{H9}\beta\rightarrow\text{H8}\beta) = 5.95 \]
\[ J(\text{H8}\alpha\rightarrow\text{H8}\beta) = 11.87 \]
\[ J(\text{H8}\alpha\rightarrow\text{H7}\alpha) = 5.80 \]
\[ J(\text{H8}\alpha\rightarrow\text{H7}\beta) = 12.04 \]
\[ J(\text{H8}\beta\rightarrow\text{H7}\alpha) = 12.43 \]
\[ J(\text{H8}\beta\rightarrow\text{H7}\beta) = 3.51 \]
\[ J(\text{H7}\alpha\rightarrow\text{H7}\beta) = 12.58 \]
\[ J(\text{H4}\alpha\rightarrow\text{H3}\beta) = 10.54 \]
\[ J(\text{H10}\alpha\rightarrow\text{H9}\alpha) = 3.76 \]
\[ J(\text{H10}\alpha\rightarrow\text{H9}\beta) = 11.37 \]
\[ J(\text{H9}\alpha\rightarrow\text{H9}\beta) = 12.11 \]
\[ J(\text{H4}\alpha\rightarrow\text{H3}\alpha) = 3.85 \]
\[ J(\text{H3}\alpha\rightarrow\text{H3}\beta) = 14.11 \]
\[ J(\text{H3}\alpha\rightarrow\text{H2}\alpha) = 5.45 \]
\[ J(\text{H3}\alpha\rightarrow\text{H2}\beta) = 10.92 \]
\[ J(\text{H2}\alpha\rightarrow\text{H2}\beta) = 16.53 \]
\[ J(\text{H2}\alpha\rightarrow\text{H3}\beta) = 4.07 \]
\[ J(\text{H2}\beta\rightarrow\text{H3}\beta) = 3.63 \]
$^1$H NMR spectrum in CDCl$_3$
H NMR spectrum expansion

H8 β
H7 β
H4
H10
H13
H3a
H3b
H2a
H2b
H3c
H6a
H6b
H7a
H7b
H8a
H8b
H9a
H9b
H10a
H13a
H13b

1H NMR spectr um - expansion
1D TOCSY subspectra

H10

H9α

6-OH H9β

H7β

H8β H8α

H4

10

1

O

O

O

O

OH

CH3

HO

Cl

H3C

Cl

H2α

H2β

H3α

H3β

H4α

H7α

H7β

H8α

H8β

H9α H9β

H10α

H13α

H13β

1D TOCSY
$^{13}$C NMR spectrum in CDCl$_3$
The image contains a gCOSY spectrum and a corresponding molecular structure. The spectrum shows cross-peaks indicating the connectivity of protons in the molecule. The structure is labeled with various hydrogens (H), oxygens (O), and chlorines (Cl). The labels include α and β for diastereotopic protons, and numbers for specific positions in the molecule. The gCOSY label indicates that this is a gradient correlation spectrometry experiment.
NOESY expansions

H8a

H7a

H9a

H8g

H9g

H7g

H6g

H5g

H4g

H3g

H2g

H1g

H3f

H4f

H5f

H6f

H7f

H8f

H9f

H10f

H11f

H12f

H13f

H14f

H15f

H16f

H17f

H18f

H19f

H20f

H21f

H22f

H23f

H24f

H25f

H26f

H27f

H28f

H29f

H30f

H31f

H32f

H33f

H34f

H35f

H36f

H37f

H38f

H39f

H40f

H41f

H42f

H43f

H44f

H45f

H46f

H47f

H48f

H49f

H50f

H51f

H52f

H53f

H54f

H55f

H56f

H57f

H58f

H59f

H60f

H61f

H62f

H63f

H64f

H65f

H66f

H67f

H68f

H69f

H70f

H71f

H72f

H73f

H74f

H75f

H76f

H77f

H78f

H79f

H80f

H81f

H82f

H83f

H84f

H85f

H86f

H87f

H88f

H89f

H90f

H91f

H92f

H93f

H94f

H95f

H96f

H97f

H98f

H99f

H100f
gHMBC long-range correlations
gHMBC long-range correlations
Long-range correlations
1D NOESY spectrum in Benzene-d$_6$

exchangeable HOH
H$_2$O in C$_6$D$_6$

$\alpha$ $\beta$ $\gamma$ $\delta$ $\epsilon$ $\zeta$ $\eta$ $\theta$ $\iota$ $\kappa$ $\lambda$ $\mu$ $\nu$ $\xi$ $\omicron$ $\pi$ $\rho$ $\sigma$ $\tau$ $\upsilon$ $\phi$ $\chi$ $\psi$ $\omega$ $\alpha$ $\beta$ $\gamma$ $\delta$ $\epsilon$ $\zeta$ $\eta$ $\theta$ $\iota$ $\kappa$ $\lambda$ $\mu$ $\nu$ $\xi$ $\omicron$ $\pi$ $\rho$ $\sigma$ $\tau$ $\upsilon$ $\phi$ $\chi$ $\psi$ $\omega$
$3J(H_3\alpha-F) = 8.83$
$3J(H_3\beta-H_4\alpha) = 3.25$
$3J(H_3\beta-F) = 37.82$
$2J(H_4\alpha-F) = 46.39$
$2J(H_7\alpha-H_7\beta) = 13.27$
$3J(H_7\alpha-H_8\alpha) = 4.79$
$3J(H_7\alpha-H_8\beta) = 12.41$
$3J(H_7\beta-H_8\alpha) = 12.67$
$3J(H_7\beta-H_8\beta) = 3.74$
$2J(H_8\alpha-H_8\beta) = 12.23$
$3J(H_2\alpha-H_3\alpha) = 5.42$
$3J(H_2\beta-H_3\beta) = 6.84$
$2J(H_3\alpha-H_3\beta) = 14.16$
$3J(H_3\alpha-H_4\beta) = 8.14$
$2J(H_2\alpha-H_2\beta) = 15.52$
$3J(H_2\alpha-H_3\beta) = 7.55$
$3J(H_2\beta-H_3\alpha) = 7.19$
$2J(H_3\alpha-H_3\beta) = 14.16$
$3J(H_2\alpha-H_3\beta) = 7.55$
$3J(H_2\beta-H_3\alpha) = 7.19$

$\text{H_13p} = 4.42$
$\text{H_13q} = 4.22$
$12-\text{OH} = 3.80$
$12-\text{CH}_3 = 1.46$
$12-\text{CH} = 2.88$
$\text{H_10a} = 5.22$
$\text{H_8b} = 1.64$
$\text{H_9a} = 6.08$
$\text{H_8a} = 1.46$
$\text{H_7b} = 3.08$
$\text{H_6a} = 2.08$
$\text{H_5a} = 1.46$

$\text{C_1} = 171.74$
$\text{C_2} = 28.16$
$\text{C_3} = 24.99$
$\text{C_4} = 90.91$
$\text{C_5} = 209.92$
$\text{C_6} = 80.11$
$\text{C_6-CH}_3 = 27.95$
$\text{C_7} = 40.43$
$\text{C_8} = 17.38$
$\text{C_9} = 31.71$
$\text{C_10} = 94.93$
$\text{C_11} = 209.36$
$\text{C_12} = 78.49$
$\text{C_12-CH}_3 = 21.08$
$\text{C_13} = 69.15$
$J_{H_{13} \alpha, F} = 3.8 \text{ Hz}$

$J_{H_{13} \beta, F} = 1.8 \text{ Hz}$
1D TOCSY subspectra—H10, 9-CH2, 8-CH2, and 7-CH2 spin system identification
1D TOCSY subspectra—H4, 3-CH3, and 2-CH2 spin system identification

1D TOCSY subspectra—H4, 3-CH3, and 2-CH2 spin system identification
1D TOCSY subspectra (C6D6)
2.0 1.5 1.0
O
O
O
O
OH
Me
HO
F
Me
H
H... 



1D TOCSY subspectra
(C6D6)
expansions

(C6D6)
1D TOCSY
subspectra
expressions
Carbon-flourine ($^{1}J_{CF}$, $n=1, 2, 3, 4$) coupling constants

$J = 17.8$ Hz

$J = 24.7$ Hz

$J = 184.1$ Hz

$J = 179.8$ Hz

$J = 1.1$ Hz

$J = 3.9$ Hz

$J = 7.5$ Hz

$\alpha$-

$\beta$-

$\delta$-

$\gamma$-
Carbon-fluorine ($^n\text{J}_{\text{CF}}$, $n=1, 2, 3, 4$) coupling constants.
$2J_{HF} = 48.1 \text{ Hz}$

$2J_{HF} = 48.2 \text{ Hz}$

$\frac{H_4}{H_{10}}$
Selective decoupling of $H_{10}$
5$J_{HH}$ couplings disappear

O
O
O
OH
CH$_3$
•
H
H$_3$C
F
H
H
H
H
H
H
H
H
H
H

Selective decoupling of $H_{10}$
gHMBC correlations

$C_{10}/C_{12}$

$C_4$
gHMBC correlations
mixture of 2 isomers
mixture of 2 isomers
mixture of 2 isomers
C NMR spectrum

Mixture of 2 isomers

12-CH3

6-CH3

C1 C11

C2 C12

C3 C9 / C8

C4 C13

C5 C10

C6

C7

C8

C13

C11

C4

C5

13C NMR spectrum
<table>
<thead>
<tr>
<th>Shift (δ ppm)</th>
<th>1H NMR Chemical Shifts (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.12 - 7.15</td>
<td>2.75 (1H, m, H9)</td>
</tr>
<tr>
<td>1.23 - 1.37</td>
<td>3.12 (s, 12-CH3)</td>
</tr>
<tr>
<td>2.33 - 2.50</td>
<td>4.36 (s, 12-CH3)</td>
</tr>
<tr>
<td>2.63 - 3.10</td>
<td>4.60 (SH)</td>
</tr>
<tr>
<td>3.12 - 3.25</td>
<td>5.20 (1H, m, H7)</td>
</tr>
<tr>
<td>3.43 - 3.50</td>
<td>6.70 (1H, m, H6)</td>
</tr>
<tr>
<td>4.10 - 4.20</td>
<td>7.80 (1H, m, H5)</td>
</tr>
<tr>
<td>6.20 - 7.50</td>
<td>8.10 (1H, m, H4)</td>
</tr>
</tbody>
</table>

13C NMR Chemical Shifts (δ ppm):

<table>
<thead>
<tr>
<th>Shift (δ ppm)</th>
<th>13C NMR Chemical Shifts (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>24.1</td>
<td></td>
</tr>
<tr>
<td>32.8</td>
<td></td>
</tr>
<tr>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>32.1</td>
<td></td>
</tr>
<tr>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>32.3</td>
<td></td>
</tr>
<tr>
<td>82.3</td>
<td></td>
</tr>
<tr>
<td>208.6</td>
<td></td>
</tr>
<tr>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>32.2</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td></td>
</tr>
</tbody>
</table>

with coupling constant (J/Hz) 6CD6
Long-range correlations in C\textsubscript{6}D\textsubscript{6}; \textit{J}_{\text{CH}} was set to 132 Hz.

GHMBC

12-CH\textsubscript{3}

6-CH\textsubscript{3}

H\textsubscript{13}

H\textsubscript{13}
Long-range correlations in C$_6$D$_6$; $^{1}J_{CH}$ was set to 132 Hz.
Long-range correlations in C₆D₆; J(CH) was set to 132 Hz.
Long-range correlations in C6D6; 1JCH was set to 132 Hz.
NOESY expansion

= 12-OH       H4                                           H13
6-CH3
12-CH3
Long-range correlations through oxygen in CDCl₃; $J_{^1}$CH was set to 145 Hz.
Spin system isolations
\[13^C\text{NMR spectrum in } C_6D_6\]

- C10
- C12
- C6
- C4
- C13
- C2
- 6-CH\text{3}
- 12-CH\text{3}
- C3
- C5
- C11
- C1
7.42 - 7.29 (m, 8H, Ph)
0.90 (t, J = 7.3 Hz, 1.4-C5 CH3)
2.18 - 2.11 (m, 1.4-C7 CH2)
5.31 (s, J = 7.3 Hz, 1.3-C6 H)
1.83 (t, J = 2.9 Hz, 1.2-C6 H)
5.25 - 5.27 (m, 2H)
4.76, 4.51 (d, J = 10.5 Hz, 9-OCCH2)
4.70, 4.58 (d, J = 10.5 Hz, 9-OCCH2)
4.38 (m, 1H)
1.50 (s, J = 6.9 Hz, 8-C5 CH3)
1.31 - 1.68 (m, 1H)
2.08 (dd, J = 6.1 Hz, 1.3-C6 H, H7d)
1.32 (JJ, J = 14.2 Hz, H7d)
1.49 (s, J = 6.6 Hz, H7a)
4.98 (q, J = 4.2 Hz, H4)
3.53 (dd, J = 8.6 Hz, 1.3-C6 H, 1.3-C6 H)
2.75 (q, J = 7.3 Hz, 2-C7 CH3)
2.76 (q, J = 6.6 Hz, 2-C7 H2)

Coupling constants (J/Hz)

1H NMR chemical shifts (6/ ppm)
1d TOCSY

NOESY
\[ \delta 5.18 - H_{10} \]

\[ J_{H10,H9} = 9.8 \text{ Hz} \]

\[ J_{H10,F} = 45.7 \text{ Hz} \]

\[ 3-OCH_2-Ph \]
1D TOCSY experimental subspectra (a-b); c- control 1H NMR spectrum

(a) 

(b) 

(c) 

1D TOCSY experimental subspectra (a-b); c- control 1H NMR spectrum
Selective decoupling of H2
NOESY cross peak between 8-CH$_3$ and H10
NOESY cross peaks between 8-CH\textsubscript{3} and others
NOESY cross peak between H8 and H9
'strong' NOE
NOESY cross peak between H3 and 2-CH3

H3
NOESY
Selective excitation of H10

$J_{HF} = 45.4 \text{ Hz}$

$J_{HH} = 9.2 \text{ Hz}$
<table>
<thead>
<tr>
<th>Selective decoupling of H3</th>
</tr>
</thead>
</table>

H10
H13
H9
H8
H4α
H4β
H7α
H7β
H2 / H4β

Selective decoupling of H3

<table>
<thead>
<tr>
<th>5.0 4.5 4.0 3.5 3.0 2.5 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.65 2.60 2.55 2.50</td>
</tr>
</tbody>
</table>

O
1
O
OBnO
CH3
HO
O
H3C
Et
O

Selective decoupling of H8
Selective decoupling of H9

H10
NOESY

8-CH₃

3

13-CH₂

H10

H13

H9

'Hweak' nOe

9

6

O

1

O

OBnO

CH₃

HO

O

H₃C

Et

O

CH₃

CH₃

H

F

H

H

H₇β

H₇α

H₄β

H₄α

H 3-OCH₂

H₂/H₄β

H2/H₄β

'Hstrong' nOe

3-OCH₂
triplet of CH$_3$ (CH$_3$CH$_2$)
C3 and C9 overlapped with solvent peaks.

- 12-CH$_3$
- 6-CH$_3$
- 8-CH$_3$

$\delta$ 76.9
$\delta$ 77.3

$J_{CF}$ ~ 13.6 Hz
selective irradiation of H3
at 3.82 ppm
quartet lines of H2
14.2 Hz (H4)$\beta$
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**PUBLICATIONS**


