Diversification of Functionalized [13]-Macrodilactones

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Macrocycles are currently an important area of investigation in drug design and development. Macrocycles can bind target biomolecules and affect various cellular processes due to their ability to modulate their conformations and their occupation of a unique region of chemical space. Our group has worked on the synthesis, structural properties, and biological activity of a class of [13]-macrodilactones in previous studies. Their key structural components include two ester groups, an alkene, and a hinge atom.

This dissertation takes advantage of the modular approach to the synthesis of [13]-macrodilactones and parlays it for the ability to prepare numerous analogs from a common intermediate. This late stage diversification was applied in two separate projects. In the first, a family of biaryl-decorated [13]-macrodilactones were synthesized via Suzuki couplings using a common C7 bromo-aryl macrocycle starting material. In the second project a C7-amine functionalized [13]-macrodilactone was prepared and acylated with a number of amino acids. These two families of C7-substituted-[13]-macrodilactones have also been evaluated via principal component analysis (PCA), antiproliferation, and antibacterial assays. Additionally, a minor project of an unsubstituted [13]-macrodilactam have been studied as a derivative from the two major projects.

The studies of functionalized [13]-macrodilactones indicated that the unique structural and physiochemical characteristics may allow them to exhibit biological activity. The information obtained can be linked to new bioactive molecules with designed properties.
APPROVAL PAGE

Doctor of Philosophy Dissertation

Diversification of Functionalized [13]-Macrodilactones

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Sections of this thesis have been published, or will be submitted for publication:

  
  *Contributions:* LJM: C3-amino [13]-macrodilactones synthesis.

  
  *Contributions:* BC: PCA; MC: early preliminary studies; MR and ZAM: cell proliferation assay.
Other work that was published but that is not included here:


  *Contributions*: CC: condition screen, hydroamination and SFC.
Chapter 1

Origin and relevance of [13]-macrodilactones in the landscape of macrocycles
1.1 Macrocycles as drugs and leads for drug development

1.1.1 Origins

Macrocycles are cyclic compounds that contain 12 or more atoms. Their history can be tracked back to 1926, when Ruzicka reported civetone\(^1\) and muscone\(^2\), which were 15- and 17-membered macrocyclic ketones, respectively. The existence of these two macrocycles offered the first opportunity to study the chemistry of those natural products with musky odor. The successful synthesis and characterization of these two large rings were a challenge to the theory that rings more than 8-membered were too unstable to preserve at that time.\(^3\) And this great discovery helped Ruzicka to win his Nobel Prize in 1939. Their unique character showed the importance of the macrocyclic compounds as well as inspired the research in this area.

About 20% of all known terrestrial and marine natural products own either small or large ring in structures.\(^4\) Macrocycles occupy unique volume in chemical space that lies in between small molecules and biologicals like antibodies. With significant structural pre-organization, they are also flexible in binding the target surface to large extent. Compared with small molecules, they are able to exhibit various functionalities to work as an active biomolecules.\(^5\) “Lipinski’s rule of 5”\(^6\) is a set of practical rules that can be applied to a compound to evaluate its potential to serve as an orally active drug. It states that a compound may disobey no more than one of these items: (1) no more than 5 H-bond donors; (2) no more than 10 H-bond acceptors; (3) the molecular weight (MW) can be no more than 500; (4) the calculated Log P (CLogP) is less than 5 (or MlogP > 4.15). Even some macrocycle don’t meet “Lipinski’s rule of 5”\(^6\) because of their higher molecular weight; nonetheless, they still possess “drug-like” properties like good solubility and
bioavailability. Therefore, macrocyclic compounds have drawn growing interest in the synthetic and medicinal chemistry fields due to their biological potency and their proven contributions to drug development. At present, there are more than 100 drugs on the market or in clinical development that contain macrocyclic scaffolds.\(^7\)

![Erythromycin](image1.png) ![Simeprevir](image2.png)

**Figure 1.1 Macro cyclic drugs — Erythromycin and Ulimorelin**

Macro cyclic drug candidates are generally organized into two major categories: natural products and de novo macrocycles.\(^8\) Figure 1.1 shows two macrocyclic drug examples, Erythromycin and Ulimorelin, each of which illustrates one of the two classes.

Erythromycin is a macrolide antibiotic used in clinical medicine against Gram positive bacterial infections for the past 50 years. It was isolated from the *Saccharopolyspora erythraea* in 1949 and first approved for clinical use in 1952.\(^9\) Erythromycin inhibits RNA-dependent protein synthesis and bacterial growth by reversibly binding near the peptide exit tunnel on the 50s ribosomal subunit of bacterial rRNA complex. Clarithromycin and Azithromycin are two semi-synthetic derivatives from Erythromycin that were developed to possess better tissue penetration and potency.\(^10\)

Simeprevir\(^11\) is a macrocyclic hepatitis C virus (HCV) NS3/4A protease inhibitor that used together with other inhibitors to treat HCV infections. It was initiated by the idea to convert a linear hexapeptide inhibitor into a potent macrocyclic tripeptide therapeutic
candidate. Through a systematic and structure-based approach, Simeprevir was discovered based on its excellent biological, PK, and safety pharmacology profile. It was approved by US FDA in 2013,\textsuperscript{12} which was the first HCV reagent that owned a macrocyclic ring.

The potential bioavailability of macrocycles can be attributed to their relative rigidity compared with linear analogs. With similar molecular weight, macrocycles have fewer rotatable bonds, which results in higher selectivity and target binding. Additionally, macrocyclic compounds can cover a broader surface area than linear ones while maintaining conformationally restricted. Thus, the reduced entropy of a macrocycle is less than flexible linear analog as well as the free binding enthalpy of it might be higher when binds to a target’s surface.\textsuperscript{13} These unique features that linear analogs don’t own gives them better target binding ability especially to those shallow-surfaced target.\textsuperscript{14}

\textbf{1.1.2 Targets}

A review by Whitty\textsuperscript{15} indicated that macrocycles tend to maximize their binding part to the protein surface area, forming networks interactions that can’t be attained by small molecules. Macrocyclic compounds can not only bind strongly to a large surface of target, but they also tend to possess favorable pharmaceutical properties \textit{in vivo}. They benefit from their unique structures that balance restriction and flexibility simultaneously to have interactions with multiple targets. The restricted flexibility enables macrocycle to improve permeability through cell membranes. There is also evidence that the intramolecular hydrogen bonds formed by macrocycles bestows better solubility and cell-penetrating ability.\textsuperscript{16} All these characteristics, as well as metabolic stability, differentiate macrocyclic compounds from small and linear molecules to play a key role in clinical use.
There are three major categories of de novo macrocycles application in drug discovery (Figure 1.2). The first main class is enzyme inhibitors and they are the major applications of de novo macrocycles. For example, in the protease field, a macrocycle is a good inhibitor for a lead compound formation because of their increased proteolytic stability and reduced polarity. The second class is working as agonists and antagonists of G protein-coupled receptors (GPCRs). GPCRs are the biggest class of pharmaceutical targets in the current market, and macrocyclic peptides have been recognized as a “privileged models” in GPCRs field. The third class is the employment in the disruption of protein-protein interactions (PPIs). Macrocycles can cover the surface area at PPI hot spots and perform both rigidity and flexibility. Moreover, macrocyclization can potentially increase the cellular penetration by decreasing peptidic leads polarity. Several examples support the suggestion that macrocycles contribute to the privileged structure that is required for the modulation of PPI. Additionally, there are some other applications of
macrocycles such as stabilizing DNA G-quadruplex, a secondary structure of DNA, to serve in anticancer activity.\textsuperscript{20}

1.1.3 PK/PD and Bioavailability

Macrocycles are compounds that possess properties to act as competitive candidates for pharmaceutical research. They can hit multiple targets and interact with variety of host biological processes. The pharmacokinetic properties of macrocycles, accompanied with pharmacodynamic activities are account for various application in clinical use.\textsuperscript{16}

Pharmacokinetics and pharmacodynamics (PK/PD) are two divisions of pharmacology, which is used to study the interactions between drug and body. According to the studies of macrolide class of macrocyclic drugs, it revealed that a minor modification of the structure can have a major influence to the absorption of the macrocycle. There was another study\textsuperscript{21} that carried out focusing the class of “de novo designed” macrocycles in clinical development suggested that more than 90\% of them were orally active. These researches indicate that it is able to improve the PK/PD properties, including oral bioavailability of macrocycles through structure-based design and approaches.

Conformational flexibility thus may account for poor membrane permeability and poor metabolic stability. Therefore, restrict the conformation and adjusting the H-bonding functionalities can affect the PK/PD properties. Besides, macrocyclization of linear molecules is also another important method that can lead to desired PK/PD properties.\textsuperscript{14}
1.2 Macrocyclic conformational analysis

1.2.1 Basics of macrocyclic conformations

In the past few years, conformational studies of macrocycles have attracted interest because those compounds occupy a unique area of chemical space.22 “Chemical space” is a concept related to the drug discovery in most cases. It embodies a set of structural and physical properties of a given compound, presenting them in the form of a geographical map.23 Also, the research on conformation of macrocycles can reveal the mechanisms and transformations in the macrocyclization.24,25 Theoretically, macrocycles are able to cover more surface area while keep conformation restriction compared to acyclic compounds with similar molecular weight. Therefore, apart from small acyclic compounds, this property enables macrocycles to aim efficiently to those targets which present shallow surfaces.14

Shape, which is intimately linked to a compound’s conformation, is a primary factor related to macrocycle’s chemical and biological properties.26 It is an inherent feature that governs the chemical reactivity and affinity for biomolecules of macrocycles. There are many factors that account for this complexity such as number of degrees of freedom, hydrogen bonds and hydrophobic interactions. Low barrier between hydrophobic conformations with polar groups in macrocycles may cause shape change when a macrocycle permeates cell membranes.27 Besides, a range of steric interactions as well as ring strain effects also account for alternative conformation.27–29 Making relatively small structural modifications to a macrocycle can induce local conformation changes and affect structural features, which affects the shape of the compounds. For example: (1) The introduction of stereocenters along the backbone can influence the overall shape.30
formation manner of macrocycles from acyclic form is determined by the absolute configuration of installed atoms at specific places (key atom). Schreiber\textsuperscript{31} reported an example that encode the stereochemistry of an element on the backbone before ring closure would produce distinct skeletons of the ring products. (2) The addition of exocyclic functionality can also change the ring conformation. Moody’s\textsuperscript{32} work about Geldanamycin and analogs demonstrated that the attachment of an exocyclic ring changed amide configuration on the ring and therefore induce a bioactive conformation. (3) Through the incorporation of heterocycles on the ring is able to reduce the degree of freedom as well as make the macrocycles flatter. The investigation of Fairlie and co-workers\textsuperscript{33} for 18-membered cyclic hexapeptides specified that only the compound with a D-Pro exhibited intramolecular hydrogen bonding in a hydrophilic environment, while the one without a heterocycle. (4) Modifying the oxidation state of a specific atom will play a role on the ring flexibility. Clardy and co-workers’ research on Tawicyclamide B indicated that the oxidation of the thiazoline to a thiazole on this cyclic peptide would stabilize the rotation of the adjacent carbonyl and contribute to its coplanar with the ring.\textsuperscript{34} (5) Attached alkyl groups on the macrocycle can stabilize one isomer of macrocycles. Moore and Paul\textsuperscript{35} proved that the installation of a methyl group on the key nitrogen atom of Apratoxin B would only give a trans-isomer. In contrast to Apratoxin B itself showed two conformers on \textsuperscript{1}HNMR spectrum. (6) Changing the ring size is able to govern trans/cis isomerization\textsuperscript{36} or rigidity of macrocycles.\textsuperscript{37} Doi\textsuperscript{36} synthesized Apratoxin A analogs to display that expanding the ring size resulted in higher ratio of cis to trans amide on the ring. Other factors include altering the unsaturated functionality/hybridization and hydrogen bond
donors/acceptors should also be considered to relate with conformation study. Additionally, the location of a modification is also a key when we analyze this subject.\textsuperscript{38}

As a result, the bioactivity and drug-discovery study will finally benefit from these conformational features. The “correct” conformation not only provides macrocycles good selectivity among PPIs with similar binding surfaces, metabolic stability in physiological conditions, but also promotes the oral absorption and membrane permeability.\textsuperscript{26,39–41}

1.2.2 Methods for characterizing macrocycle conformations

Several methods and techniques can be applied in the analysis of macrocycle conformation. Nuclear magnetic resonance (NMR) spectroscopy is the most common and routinely used one for interpretation. $^1$H NMR can give a lot of clues besides the basic information. Relative chemical shifts differentiate similar protons in different position of a macrocycle. According to the Karplus equation, dihedral angles of backbone, which is one of the elements of conformation, can be obtained by homonuclear vicinal ($^{3}J$) coupling constants. Variable temperature (VT) NMR and hydrogen/deuterium (H/D) exchange can help to figure the intramolecular hydrogen bonding. The interpretation of NOESY and ROESY can be used to determine effective compound models. Besides NMR, other methods also own different functions. Circular dichroism (CD)\textsuperscript{42} and infrared (IR) spectroscopy are good tools to provide the secondary structural characteristics of peptide fragments in a macrocycle. X-ray crystallography can give a detailed three-dimensional demonstration of a macrocycle in the solid phase. Structures for lossless ion manipulations (SLIM),\textsuperscript{43} high-performance liquid chromatography (HPLC) are used to differentiate conformational isomers among similar macrocycles.
In recent years, computational modeling methods are also involved to cooperate with the traditional techniques. The principle is to select a sampling compound to generate new models with varied shape to determine the stability of each one. One frequently used computational method is molecular mechanics (MM), which makes use of different equations and calculations to search for appropriate macrocycle conformation. Molecular dynamics (MD) simulations is another approach which is applied to study the local conformational space around a given macrocyclic ring. The Monte Carlo search can generate conformers, and offer an useful graphical method to present and analysis these conformers with various energy by collaborating with polar coordinate maps.

Therefore, understanding the factors that affect the shape of macrocycles as well as proper analysis methods and techniques will establish foundations to design macrocycles with expected functions.

1.3 Synthetic strategies for preparing macrocycles

1.3.1 Macrolactonization and macrolactamization

There are several approaches that have traditionally been employed in the synthesis of macrocycles. Lactonization and lactamization are usually the most standard ways to prepare macrocycles. These two are especially effective for peptidic macrocycles since various coupling reagents employed in the reactions have been developed peptide chemistry. Their widely utilization also benefited from the fact that they can provide various products, independent of any ring size and efficient in either solid or liquid phase. Baran’s group synthesized Kapakahines B, contained a twisted 16-membered ring, was an example of lactamization (Figure 1.3). Kapakahines B is the first member
that was isolated from the marine sponge *Cribrochalina olemda*, as well as showed modest anti-leukemia activity. The gram-scale, enantiospecific total synthesis of Kapakahines B offered an efficient access to this bioactive natural product by utilizing multiple starting amino acids for macrolactamization.

![Chemical structures](image)

**Figure 1.3** Structure of Kapakahines B and starting materials for its total synthesis\(^{48}\)

1.3.2 **Nucleophilic substitution and nucleophilic aromatic substitution**

Nucleophilic substitution (S\textsubscript{N}2) and nucleophilic aromatic substitution (S\textsubscript{N}Ar) are another major category of macrocyclizations. These two reactions enjoy the similar advantages as lactonization and lactamization; meanwhile have some disadvantages like inducing side reactions to provide dimers and oligomers. S\textsubscript{N}Ar can occur on resin, which make it widely employed for the construction of constrained macrocyclic peptidomimetics.\(^{49}\) A representative example of this macrocyclization type is the total synthesis of Vancomycin.\(^{50}\) It has a unique molecular skeleton, which consisted of several biaryl ether unit. S\textsubscript{N}Ar is a well-suited method to construct such moieties. Zhu\(^{51}\) also utilized S\textsubscript{N}Ar-based macrocyclization to get access to a class of bioactive macrocycles holding the same fragment. This macrocyclization is good for obtaining both natural and de novo products, thus allow it a common approach in drug design.
1.3.3 Organometallic chemistry utilized for macrocyclization

Another viable category of macrocyclization is transition metal catalyzed macrocyclization. Ring-closing metathesis (RCM) is one of the most widely used reaction in recent years. Grubbs\textsuperscript{52} has been working on optimizing the ruthenium catalyst for this reaction in order to improve the efficiency. Among them, the second-generation ruthenium catalyst is most popular choice. RCM is a versatile approach with mild condition that can tolerate broad substrate scope and various ring size condition. Another valuable approach is the “click reaction”, especially the copper catalyzed alkynes and azides cycloaddition (CuAAC).\textsuperscript{53} It has been mainly applied to peptidic macrocycles, including those derived from a β-turn mimic.\textsuperscript{54} Therefore, many bioactive macrocyclic rings can be produced by this methodology, though sometimes dimerization could be a problem. When an aromatic moiety will be produced in the macrocycle backbone, palladium-catalyzed Heck reaction\textsuperscript{55} and Suzuki-type coupling\textsuperscript{56} are good choice for building the target. Heck reaction has been utilized as an important tool in the synthetic route of HCV protease inhibitors.\textsuperscript{57}

The Diels–Alder reaction is fundamental method for the preparation of 6-membered ring. It is normally represented a reaction between a conjugated diene and a substituted alkene, known as dienophile. An improved modification will also make it possible to employing in macrocyclization.\textsuperscript{58} This alternative method, transannular Diels-Alder (TADA), was developed by employing malonate connectors (dimethyl malonate, malononitrile) to build a variety of macrocyclic trienes for ring closing.\textsuperscript{59}

1.3.4 Other valuable approaches

Other minor categories of macrocyclization are: 1) combinatorial chemistry, which is mainly for macrolide compounds\textsuperscript{60}; 2) multicomponent reactions (MCR) approach that
can combine several pieces of starting material to final product in a one-pot reaction\textsuperscript{61}; 3) photochemical reaction and etc. All these strategies of macrocyclization facilitate it an attractive and general methodology to prepare more macrocycles for pharmaceutical studies.

Selecting a well-suited macrocyclization type is a key step for ring closing in the synthetic strategy. Even though macrocycles can be closed by various ones, specific reactions should be selected cautiously to hit the desired product with high efficiency. There are two major items to check for determination: the presence of specific functional group on the backbone of the ring and intrinsic feature favoring the closure of the ring.

In addition to the traditional approach — macrocyclization, there are some novel methods like biosynthesis for macrocycle diversity and macrocycle-based library technologies. They are emerging in recent decades and developing rapidly to meet increasing requirement of pharmaceutically relevant macrocycles.

1.4 [13]-Macrodilactones: A family of de novo macrocycles in the Peczuh group

1.4.1 Fundamentals of the [13]-macrodilactone

Our group has worked on [13]-macrodilactones in previous studies.\textsuperscript{62–65} We put special emphasis on investigating the synthesis, structural properties, and biological activity of a class of [13]-macrodilactones. This class of [13]-macrodilactones, whose key structural components include two ester groups and an alkene, possesses three four-atom planar units and a “hinge” atom C3. Two ester units (C4-O5-C6-C7 and C2-O1-C13-C12) are connected to the alkene unit (C8-C9-C10-C11) by bonds between the α-carbon atom of the ester and the corresponding allylic carbon on the alkene. Meanwhile they are
linked to each other by a “hinge” atom, C3. The numbering of this macrocycle is started at an oxygen atom as position 1 and followed along the backbone to the oxygen from another ester unit as position 5 (Figure 1.4).

![Diagram](image)

**Figure 1.4** General representation of the unsubstituted [13]-macrolactone structure

The example of (E)-cyclooctene demonstrated in Figure 1.5 shows that, when the saturated segment is short, the ring is twisted and the alkene is pushed out of the plane. Then, the ring becomes chiral and planar chirality is introduced as the stereogenic element of the (E)-cyclooctene and decides the handedness of the ring. The determination of the planar chirality solely depends on the (E)-alkene unit. When viewed from out of plane to the alkene unit, pR defines a clockwise array, while pS defines a counterclockwise array.

![Diagram](image)

**Figure 1.5** Demonstration of twist about alkene in (E)-cyclooctene

Our primary method of conformational analysis was to use structures from X-ray crystallography obtained from the solid state of our representative [13]-membered macrocycles to make structural observations. It was found that the double bond of the alkene orients itself perpendicular to the mean plane of the macrocycle (Figure 1.6).
There is an inside and outside face of alkene, and two ester units are the other two edges of this triangle with one and the other pointing in and out of the plane. Therefore, the hinge atom C3 allows the four-atom planar units to twist relative to each other based on an axis that goes through the center of the ring, which enable the asymmetry of the ester arms around the alkene unit to determine the macrocycle’s chirality. This generates a stereogenic axis instead of stereogenic centers, manifested as planar chirality since, in the case of the unsubstituted [13]-macrodilactone, it is the only stereogenic element. Therefore, the crystal structure revealed the presence of both enantiomers in the unit cell for the compound (pR and pS as planar chiralities) referring to the planar chirality established by the ring. The determination of the planar chirality obeys the rule established by (E)-cyclooctene. We have defined the conformation of this macrocycles as “ribbon” shape, according to the alkene planar as the top edge of a triangle shape.

![Diagram](image)

**Figure 1.6** Structures of unsubstituted [13]-macrodilactone (1), (pR)-1 and (pS)-1 from X-ray crystallographic data

This class of [13]-macrodilactones has been used as a model in our group to investigate the factors that influence the shape and biological properties of macrocycles.
We intended to discover how the number, positioning, and absolute configuration of stereogenic centers on the backbone of the macrocycle effect the three-dimensional shape and bioavailability of the macrocycles.

Several years ago, we reported a small class of carbohydrate fused [13]-macrodilactones, which imitated natural products in their structure and functionality.\textsuperscript{68,69} The idea was initiated from increasing demand for synthetic unnatural products as potential therapeutic drugs.\textsuperscript{70} We have also done a highly diastereoselective DMDO epoxidation and transesterification followed to study the conformational control of [13]-macrodilactone (Figure 1.7). We found that the facial selectivity was determined by remote stereocenters instead of one adjacent to the alkene. We claimed that C2 and C4 were key stereocenters, while C3 was not critical to control the planar chirality and axis of the macrocycle. This outcome is useful to provide rule in choosing a key atom to initiate the conformational changes of macrocycles. Our concept of key atoms is that they are stereogenic centers and specific locations (edge of each unit) on the macrocyclic backbone. As an expansion of the study, a methyl group was installed at C2 of the unsubstituted [13]-macrodilactone.\textsuperscript{68} It turned out that a complete change in the facial presentation had accomplished, which confirmed the results of the previous project.

![Figure 1.7 DMDO epoxidation of carbohydrate-fused [13]-macrodilactone](image)

Later on, the project followed up to study how both key atoms and planar units collaborate to direct the overall shape.\textsuperscript{63} As discussed in section 1.2, there are several
parameters contributing to the conformation of a macrocycle. Based on our findings, the planar units like esters and alkenes play a role in the rigidification of [13]-macrodi lactones. By investigating the consequences of attaching substituents on C2 and C3 solely or together, we concluded that the configuration at key positions along the macrocycle affect its planar chirality. These key atoms can also work together to govern the overall shape. When C2 (equivalent to C4) was mono-substituted (4 in Figure 1.8), the macrocycle tended to occupy a ribbon-shape. When C7 (equivalent to C12) was mono-substituted, the macrocycle shows a similar ribbon-shape. However, when C8 (equivalent to C11) was mono-substituted, the crystal structure of macrocycles shows it can either have a ribbon-shape or an alternate shape according to the configuration of the C8 atom and the molecule planar chirality. All these cases will be discussed in more details in next section. The same ribbon-shape was observed when C3 was substituted with two methyl group on it simultaneously (6 in Figure 1.8). Based on the crystal structure study of compound 2 and 3, we inspected that the epoxide is essentially the same as corresponding alkene. We could apply the conformational information inspected from epoxide to alkene. Thus, the cup shape reflected by epoxide product indicated the same shape of the macrocycle 5, which attached two methyl group on C2. Furthermore, a more complicated case will be discussed in the di-substituted section when both C2 and C4 have been decorated a methyl group orienting different directions.
Figure 1.8 Demonstration of C2 or C3 substituted [13]-macrolactones

Additionally, it was demonstrated in the same study that reaction can only occur on the outside face of the alkene because the interior space of the macrocycle was limited for a molecule. Second, the reason that this macrocycle exists as a ribbon shape may attribute to the suspicion that this conformation has the lowest energy.

1.4.2 Mono-substituted [13]-macrolactones

In order to interpret the manner by which stereogenic centers at key positions along the [13]-macrolactone’s backbone contribute to its planar chirality, a class of mono-substituted [13]-macrolactones have been synthesized and characterized for the purpose.

It has been introduced in the section 1.4.1 that C2 or C4 is key atoms that dictates our macrocycle folds. Figure 1.9 shows the model of compound 4 and 7, which were two pairs of racemic mixtures produced by RCM from the diene precursor. By installing the simple group, methyl group into the macrocycle backbone (compound 4), it can influence the overall shape of the ring to show a ribbon-shape. The configuration of C2 gives rise
to the planar chirality of [13]-macrodi lactone. C2 with a $R$ configuration created a $pR$ planar chirality for the ring; while a C2 of $S$ configuration gave a $pS$ planar chirality.

A simple substituent such as methyl group can have an effect on the planar chirality. That makes it persuasive that the position of a substituent matters more than its complexity. And our interest was raised to explore the possibilities of different shapes that other key atoms could create. Therefore, a further research was conducted by installing a phenyl group on C7 position, which is $\alpha$ to the ester group, on the macrocycle backbone (7 in Figure 1.9).^{65}

![Figure 1.9 Structure of C2-methyl and C7-phenyl [13]-macrodilactones (4 and 7)](image)

Get compound 7 prepared enable us to compare the global structure with other [13]-macrodilactones such as compound 4; meanwhile study how it dictates the planar chirality in this case. Based on the X-ray structures we got, C7 with a $R$ configuration created a $pS$ planar chirality for the ring; while a C7 of $S$ configuration gave a $pR$ planar chirality. When the phenyl group takes the third priority, it has the same result as C2-substituted one. Which means $R$ point chirality gives $pR$; $S$ point chirality gives $pS$. DMDO epoxidation of rac-7 was proceeded. Theoretically, four products would be expected to
isolate. But it gave only one diastereomer from each starting material as a pair of enantiomers. And this molecule still adopted a ribbon-shape. This outcome draws the conclusion that mono-substituted [13]-macrodilatones on this C7-position could influence the ring shape.

Then, C8-substituted one have been made to do the same study.\textsuperscript{62} Surprisingly, compound 8 has two diastereomeric serious (all four isomers) existing simultaneously in the unit cell (Figure 1.10). \(R\) configuration of C8 determines both \(pR\) and \(pS\) of the macrocycle; \(S\) configuration of C8 has the same outcome. Furthermore, compound 8 possessed planar chirality of \(pR\) still adopted a ribbon-shape; while compound 8 with a planar chirality of \(pS\) adopted an alternative shape. We defined it as a heart-shape in this case.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure110.png}
\caption{Ribbon and heart shape of C8-\(p\text{BrPh} \ [13]\)-macrodilactones (8)}
\end{figure}

Planar chirality and point chirality both work together to define stereochemistry of macrodilactones. Point chirality is related to the configuration of stereogenic centers at key atoms on the macrocyclic backbone. The absolute configuration (\(R\) or \(S\)) of the key arom will then dictate the planar chirality because the “bulk” of the group attached to that stereogenic center wants to avoid steric interactions with the rest of the ring. These “trans-annular” interactions are akin to the force that drives groups to be equatorial on a ring.
Moreover, macrocycles synthesized from racemic starting materials were expected to be racemic. Each enantiomer of the starting material in the racemate would specify a planar chirality for the macrocycle, while the shape of the asymmetric macrocycle would be predicted by the absolute configuration of optically active starting material. We also speculated that the macrocycles obtained from achiral starting material will be either racemates with enantiomeric planar chiralities or show new shape that still symmetrical and achiral.

**1.4.3 Di-substituted [13]-macrodilactones**

The next step was to discover how the macrocycle would be when it was di-substituted on the specific atoms. We synthesized more [13]-macrodilactones to have a close look into the interplay of configurations and chiralities (Figure 1.1). Each example had two stereocenters established. Macrocycle 9 owns phenyl substituents at both C7 and C12; Macrocycle 10 owns methyl substituents at both C2 and C4; macrocycle 11 owns a methyl group at C2 and a phenyl group at C12; Macrocycle 12 owns a methyl group at C2 and a phenyl group at C7.

Then a three-dimensional conformation arose according to the number, positioning, and absolute configuration of stereocenters. The relationship of the di-substituted groups was described by the relative positions of the hydrogen atoms on the same stereocenter with the group. When the hydrogens at the two stereocenters point in the same direction, it’s a cis relationship, while it’s a trans relationship when hydrogens point in opposite directions.
Figure 1.11 Structure of di-substituted [13]-macrolactones (9-12)

Compared with the mono-substituted ones, the addition of the stereogenic element further rigidified the ring structure; meanwhile define the handedness of the planar. For macrocycle 9-11, the trans configuration led to a ribbon-shape of macrocycle; while the same shape was induced by the cis relationship of macrocycle 12. We indicated that it may due to the reason that these examples are akin to trans-1,2- and 1,4-disubstituted cyclohexanes and cis-1,3-di-substituted cyclohexane. An alternative shape was observed from the cis-configured macrocycle 9-11 and trans-configured macrocycle 12. For example, cis-configured macrocycle 10 gave a cup-like shape. Each of them has a five-atom alkene planar unit instead of the four atom unit that is in ribbon-shape of macrocycle. We also found that the functional group exhibit the pseudo-equatorial positions based on the stereocenter configuration. The key atoms collaborated constructively to accomplish a low-energy conformation. The substituted group attached on the macrocycle could either work together to reinforce the shape or against each other to alternate the shape.
It must be noted that those stereogenic centers are all placed at either end of the planar units. And that means when a stereocenter was created at the termini of a multi-atom planar unit, the entire macrocycle shape would change.

In conclusion, the key atoms (C2, C4, C7, C12) on the ring play an important role in the determination of its entire structure. These key atoms can either work together or against each other to restrict the freedom of macrocycles to appear in different shapes and topologies.

**1.4.4 A [15]-macrolactone to test the “rules”**

We have also prepared some [15]-macrolactatones (14 and 15 in Figure 1.12) to investigate if the rules for 13-membered ring discovered by our group are also effective in macrocycles of different size. We would like to investigate the interplay of planar units, a hinge atom and point chiralities in the case of [15]-macrolactatones. We were also curious to know if these fundamental findings still directly influence the global structure of an expanded ring. We believed that the success application of these rules to the rings with different size will be a strong support that new macrocyclic drug candidates can be designed with defined shapes.

![Chemical structures of 14 and 15](image)

**Figure 1.12 Structure of representative [15]-macrolactatones**

In this case, an expanded version of [13]-macrolactatones, [15]-macrolactatones, has been utilized to evaluate the versatility of the guidelines we
concluded from the 13-membered ring. The idea was initiated by the motivation that larger ring will reveal more surface for protein binding. To accomplish this goal, an additional alkene unit has been introduced in between the existing alkene and its allylic carbon to make this larger ring. Same as [13]-macrodilactones, we exploited that the interplay of planar units, a hinge atom and point chiralities still worked together to affect the planar chirality and control the global structure of [15]-macrodilactones. Moreover, C2 (14 in Figure 1.12) is a critical atom to lead the planar chirality.

Sugar-fused [15]-macrodilactone 15 (Figure 1.12) is an alternative version of the expanded ring. However, this type is a totally different case. It adopted a unique cup shape, rather than a ribbon shape like original [13]-macrodilactones. Besides C2, the exocyclic hydroxymethyl group in the sugar moiety was proved to be critical directing planar chirality as well; and thus change the overall shape. It also supported the opinion that the exocyclic fragment rigidified bond rotation, which would also guide the conformation of macrocycles. This case study also motivated us to build new macrocycles in desired shape that can interact with the selected protein surfaces.

1.5 Lipid linked [13]-macrodilactones

Since natural macrocycles show great potential in drug discoveries, we have been interested in evaluating activity and bioavailability of [13]-macrodilactones. Therefore, a class of lipid-linked [13]-macrodilactones were synthesized and tested for their antimigratory and antiproliferative activities.74 A moiety in the structures was an n-octyl decorated glycoside (Figure 1.13). The macrocycles were synthesized through
diacylation of functionalized diols and RCM. Their antimigratory, cytostatic and cytotoxic activity have been evaluated against cancer cells.

This study was inspired by simple natural product macrocycles like migrastatin and intended to modulate the identity and subsequent properties of the molecule’s hydrophobic portion while maintaining the planar chirality of macrocycle.

![Structures of lipid-linked [13]-macrodilactones](image)

**Figure 1.13** Structures of lipid-linked [13]-macrodilactones

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>MIC (μM)</th>
<th>IC₅₀ (μM)</th>
<th>Therapeutic index (MLC/MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>10</td>
<td>85.1</td>
<td>30</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>91.4</td>
<td>6</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
<td>38.1</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 1.1** Antiproliferative activities of lipid-linked compounds in BT-20 breast cancer cells

Compound 16 and 18 (Figure 1.13) were the most promising compounds as either showed the highest therapeutic index or exhibited antiproliferative activity against BT-20 breast cancer cells at ~30 μM, both contained [13]-macrodilactone (Table 1.1). On the other hand, they also displayed cytostatic or cytotoxic activity at 100 μM reduced the rate of cell proliferation. By further exploring the data of compound 16, it performed a wide range of concentration for its subtoxic antiproliferative activity. It implied that this specific compound might target some factors involved in cell cycle progression.

Observing this class of compounds, we found that all the compounds that show antiproliferative activities contain a glucosyl unit and an octyl chain. Unexpectedly, core
[13]-macrolactone was not necessary for activity. Anyhow, either compound 16 or 18, which are two most promising compounds, has this [13]-macrolactone structure. Regarding to this lipid-linked [13]-macrolactone project, we have demonstrated the application of a novel class of macrocycles to serve as antiproliferative agents.
1.6 Summary and Outlook

Macrocycles hold great potential as drug candidates and as tool compounds for chemical biology applications. Their bioactivity is due to the unique chemical space that macrocycles occupy as well as their special conformation that can both present flexibility and rigidity. The rigidification results in a certain shape that is associated with a specific macrocyclic backbone; additional features then allow for local flexibility. An understanding of all the factors that influence the shape of a macrocycle can be applied to the preparation of new compounds with designed properties.

13-membered macrocycles have received less attention than other rings because of their low abundance compared with the common macrocycles embedded even-numbered ring. although an exploration of their intrinsic properties could serve in the area of new pharmaceutical lead compounds. Our group is continuously making efforts to investigate the elements that can guide the shape of [13]-macrodilactones. A class of [13]-macrodilactones whose key structural components include two ester groups and an alkene was synthesized and characterized by our group. We have reported that the number, positioning, and absolute configuration of stereogenic centers on the backbone of the macrocycle affect its three-dimensional conformation. We found that C7 or C12 (α to the ester carbonyl), as well as the other stereogenic center, which is the edge of each unit influence the shape of the [13]-macrodilactones. When [13]-macrodilactone is mono-substituted, the point chirality guides the planar chirality and the shape of the macrocycle; when it is di-substituted, the substituted groups can either work together or against each other to affect the shape of the macrocycle. We have also demonstrated the application of our lipid-linked [13]-macrodilactones to work as a novel class of antiproliferative agents.
1.6.1 Objectives of the dissertation research

Having established that the C7 position is key to guiding shape in [13]-macrodilactones, two major projects have been carried utilizing late-stage diversification as the synthetic strategy. Each project aimed to evaluate the effects of systematically varying substituents at this position of the macrocycle. In the first project (Chapter 2), biaryl units containing heteroatoms were introduced at C7 to study whether the polarity of the hydrophobic macrocycles will increase their permeability while keep antiproliferative ability, related to the cytotoxicity of our earlier lipid-linked [13]-macrodilactones (i.e., 16-18). It turned out that C7-aryl class hold the similar potency as the lipid-linked class. This project also motivated the incorporation of the structural and physicochemical features into the design of related families of macrocycles. In the second project (Chapter 3), a group of [13]-macrodilactones containing a functionalized amine (amidation, urea formation) at the C7 atom were prepared in an attempt to imitate the antibiotic natural product Teixobactin. This class of compounds was designed to take advantage of the unique feature of Teixobactin structure in anion binding to work as promising drug candidate. Unexpectedly, they didn’t show competitive MIC activities as desired. But the access to the crystal structures of the important compounds gave us some inspiration for the further design. Another minor project on the synthesis of an unsubstituted [13]-macrodilactam (Chapter 4) is also originated here to explore the stability of this modified version of [13]-macrodilactone.

To sum up, through synthesis, characterization and analysis of these three class of [13]-membered macrocycle (C7-aryl [13]-macrodilactones, C7-amino [13]-macrodilactones and unsubstituted [13]-macrodilactam), an overall picture depicting story
of C7-substituted [13]-macrodilactones will be delivered. The synthesis strategies established for these compounds develop a method to expand the substrate scope of macrocycles by late-stage coupling reactions. The shape, together with biological potency of these macrocycles will pave the way to design novel [13]-macrodilactones decorated by promising substituents on critical atom for specific biological purpose. Derived research can be conducted to hit other key atoms on the ring, or add dynamic functional group on [13]-macrodilactam. Moreover, running reduction of the alkene unit of the ring as well as reductive amination of those existed ring can supplement the whole story of C7-substituted [13]-macrodilactones.
1.7 References


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(74) Magpusao, A. N.; Desmond, R. T.; Billings, K. J.; Fenteany, G.; Peczuh, M. W.
Chapter 2

Synthesis of a C7-aryl [13]-macrolactone and its physicochemical and antiproliferative properties
2.1 C7-aryl [13]-macrodilactones

In chapter 1, we discussed that macrocycles can hold potential as drug candidates and as tool compounds in chemical biology applications.\(^1,^2\) The reduced flexibility of macrocycles improves selectivity in binding to a biological target.\(^3\) This rigidification results in a certain shape that is associated with a specific macrocyclic backbone; the shape is largely dictated by the number of atoms in the ring, the hybridization of ring atoms, and stereogenic centers. Additional features then allow for local flexibility within a motif. By understanding the factors that influence the topology of a macrocycle, they can then be applied to the preparation of new compounds with designed properties.

Our group is therefore investigating the synthesis, structural and physical properties, and biological activity of a class of [13]-macrodilactones that contain an uncommon element of stereogenicity as a key structural feature.\(^4^8\) By evaluating the anti-migratory and anti-proliferative activity of our lipid-linked [13]-macrodilactones (Figure 2.1), we found that 16 and 18 are the most promising compounds.\(^9\) We became interested in having a way to modulate the identity and subsequent properties of the hydrophobic portion of the molecule while maintaining the planar chiral macrocycle. Therefore, we proposed a new class of biaryl-linked [13]-macrodilactones to study their principle component analysis (PCA), and anti-proliferation assays.
2.1.1 Retrosynthesis

Our intention was to prepare a [13]-macrodilactone containing a pendant group that was poised via a late-stage diversification. An aryl halide, attached at a position alpha to the ester carbonyl would enable us to implement such a plan. The key intermediate, C7-(p-bromophenyl)-[13]-macrodilactone 22 (Scheme 2.1), could be prepared by a strategy that leveraged our previous experience with the synthesis of these macrocycles.

We have established before that 1,3-propane diol and related 1,3-diols can be sequentially acylated with different pentenoic acids to give diacyl diesters for making [13]-macrodilactone.

Hence, the new designed retrosynthesis route was created (Scheme 2.1). It was intended to attach various aromatic ring via Suzuki coupling of C7-bromo-phenyl [13]-macrodilactone (22). Macrodi lactone 22 could be produced through ring closing metathesis from the precursor diene 23, which was revealed by acylation of p-bromo-phenyl acid 24 and mono-acyl alcohol 25. Alkylation and mono-acylation could be applied to acid 26 and 27 respectively for the supply of compound 24 and 25.
2.1.2 Synthesis of C7-(p-bromophenyl)-[13]-macrodilactone

In this work, our strategy made use of 2-(p-bromophenyl)-4-pentenoic acid 24 as the key acylating agent. Compound 24 was itself prepared from commercially available 4-bromophenyl acetic acid 26 via esterification to give ester 28 in 85% yield.\textsuperscript{11} Followed by an alkylation yielded the allylated methyl ester 29 (72% yield), hydrolysis gave the 2-p-bromophenyl-4-pentenoic acid 24 in 96% yield.\textsuperscript{12} The mono-acyl alcohol 25 was prepared by the acylation of 1,3-butanediol 30 and 4-pentenoic acid 27 using DCC and DMAP (Scheme 2.2).\textsuperscript{7} Substituted pentenoic acid 5 was then esterified using the mono-acyl alcohol 25 under the same action of DCC and DMAP to deliver diacyl-diene 23 in 50% yield (Scheme 2.3). RCM on diene 23 afforded C7-(p-bromophenyl)-[13]-macrodilactone 22 (84%).\textsuperscript{5}
2.1.3 Synthesis of C7-aryl [13]-macrodilactone

Having established access to 22, the stage was now set for its derivatization via Suzuki coupling reactions. Suzuki cross couplings were conducted to convert the pendant aryl bromide into a variety of biaryls attached to the [13]-macrodilactone scaffold. A common set of reaction conditions was identified that were able to deliver the product macrocycles with yields sufficient for their further characterization (Table 2.1).
Macrocycles containing biaryl moieties that were strictly hydrophobic (i.e. 21a, 21b), or that incorporated heteroatoms into the biaryl unit in one way or the other (i.e, 21c-21k) were prepared. Aryl units containing heteroatoms were introduced to increase the polarity of the hydrophobic macrocycles and potentially enable them to permeate through cell membranes via active uptake mechanisms.
Table 2.1 Suzuki reaction of [13]-macrolactone 22 with aryl boronic acids

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<th>product</th>
<th>yield (%)</th>
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<td>21a</td>
<td>77</td>
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<td><img src="Ar2.png" alt="image" /></td>
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<tr>
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<td><img src="Ar3.png" alt="image" /></td>
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<td>6</td>
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<td>31f</td>
<td>21f</td>
<td>56</td>
</tr>
<tr>
<td>7</td>
<td><img src="Ar7.png" alt="image" /></td>
<td>31g</td>
<td>21g</td>
<td>93</td>
</tr>
<tr>
<td>8</td>
<td><img src="Ar8.png" alt="image" /></td>
<td>31h</td>
<td>21h</td>
<td>98</td>
</tr>
<tr>
<td>9</td>
<td><img src="Ar9.png" alt="image" /></td>
<td>31i</td>
<td>21i</td>
<td>61</td>
</tr>
<tr>
<td>10</td>
<td><img src="Ar10.png" alt="image" /></td>
<td>31j</td>
<td>21j</td>
<td>73</td>
</tr>
<tr>
<td>11</td>
<td><img src="Ar11.png" alt="image" /></td>
<td>31k</td>
<td>21k</td>
<td>76</td>
</tr>
</tbody>
</table>
2.2 Antiproliferative assays

Cell viability assays were conducted using the ASZ cell line to evaluate the cytotoxicity of the new [13]-macrodilactones. The ASZ line is a mouse-derived, basal cell carcinoma cell line that is easy to manage in cell culture and replicates at an appreciable rate, making it a useful model to evaluate the compounds in the project. We had previously measured cell viability using BT-20 cells, a human breast cancer cell line, but opted here to use the ASZ cells for convenience; both cell lines are suitable for cell viability assays. The MTS/PMS assay used, quantifies cell viability in comparison to cells treated with DMSO by the reduction of tetrazolium salts which results in the formation of formazan dyes which can be quantified by absorbance at ~490 nm.\textsuperscript{13,14} Using MTS and PMS over other tetrazolium dyes such as MTT or XTT provides rapid color development, stable reagents in solution, and aqueously soluble formazan products.\textsuperscript{15}

**Table 2.2** GI\textsubscript{50} value for C7-aryl [13]-macrodilactones

<table>
<thead>
<tr>
<th>entry</th>
<th>product</th>
<th>GI\textsubscript{50}\textsuperscript{a} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21a</td>
<td>≥100</td>
</tr>
<tr>
<td>2</td>
<td>21b</td>
<td>N/A\textsuperscript{b}</td>
</tr>
<tr>
<td>3</td>
<td>21c</td>
<td>12.1±0.5</td>
</tr>
<tr>
<td>4</td>
<td>21d</td>
<td>≥75</td>
</tr>
<tr>
<td>5</td>
<td>21e</td>
<td>40.9±0.3</td>
</tr>
<tr>
<td>6</td>
<td>21f</td>
<td>≥75</td>
</tr>
<tr>
<td>7</td>
<td>21g</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td>21h</td>
<td>19.7±4.4</td>
</tr>
<tr>
<td>9</td>
<td>21i</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>21j</td>
<td>≥75</td>
</tr>
<tr>
<td>11</td>
<td>21k</td>
<td>≥100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}GI\textsubscript{50} values are the avg ± SEM of two separate experiments, run in triplicate.

\textsuperscript{b} N/A = insufficient data to determine GI\textsubscript{50}; data not available.
The effect on ASZ cell viability of compounds (21a-21k) was tested at concentrations ranging from 100 - 0.1 μM, to provide a dose response curve for the compounds. As shown in Table 2.2, several of the new [13]-macrodilactones possessed anti-proliferative activity. Potency in the assay was used to divide the compounds into four groups. Compounds 21c and 21h possessed the greatest antiproliferative activity, with GI50 values of 12.1 and 19.7 μM, respectively. Compound 21e showed intermediate antiproliferative activity, whereas compounds 21d, 21f, and 21j showed only slight changes in cell viability. The final cohort of compounds 21a and 21k showed no activity or change in cell viability in comparison to treatment with DMSO. Notably, the potency of compounds 21c and 21h was similar to that of the carbohydrate-fused, lipid-functionalized macrocycles that we had previously studied. Moreover, the fact that there was a range of activities amongst this family of compounds prompted us to revisit the physicochemical properties of the compounds.

2.3 Physicochemical characterization

We next endeavored to find out where, in chemical space, the new biaryl set of [13]-macrodilactones (biAr) resided. Principal component analysis (PCA) was performed on the biAr compounds in order to compare their properties to a diverse collection of natural products (NP), currently available drugs (drug), drug-like and natural product-like compounds, including macrocycles described by Tan and co-workers (other), and the carbohydrate fused [13]-macrodilactones we had investigated previously (carb). To that end, we adopted a method for PCA established by Tan and co-workers. Their collection of reference compounds was comprised of drugs, natural products, and drug-like library
members, in addition to some additional macrocycles to form a library of over 180 compounds used in the analysis. Once the entire library of compounds was assembled, twenty structural and physiochemical parameters were collected for them, and the data was then normalized so that it could be exported to a statistical program in order to calculate the principal components (PCs). The number of PCs obtained was equal to the number of parameters used so in this case there were twenty PCs; only the first three were used to generate the PCA plots (Figure 2.2) because together they expressed ≥75% of the variance present in the original data. As we set about analyzing the plots, we were cognizant that over-analysis of the PCA would add little additional value to the study.\textsuperscript{16}
Figure 2.2 Principal Component Analysis (PCA). A library of 208 compounds was broken into sets: natural products (NP), drugs, other, biaryl [13]-macrodi lactones 21a-21k (biAr), and [13]-Macrodi lactones 1, 4, and 7. A. PC1 vs PC2 B. PC1 vs PC3 C. PC3 vs PC2
Figure 2.3 Loading plots that correspond to the PCA plots in Figure 2.2 A. PC1 vs PC2 B. PC1 vs PC3 C. PC3 vs PC2
Inspection of the PCA plots led to observations on the general characteristics of the compound sets in the library. In the PC1 vs PC2 and PC3 vs PC2 plots (Figure 2.2A and 2.2C), compounds of the NP set were spread across a crescent-shaped space. In the PC1 vs PC3 plot (Figure 2.2C), NPs were distributed more widely. The shape defined by the set likely reflected the broad range of molecular weights, along with contributions from other parameters of NPs. The drug set was mostly localized in one octant of the chemical space and largely separate from the NP set, especially with respect to the PC1 vs PC2 and PC3 vs PC2 plots. Differences in how natural products and drugs occupy their respective chemical spaces may be attributable to the fact that natural products typically have more rotatable bonds and more stereogenic centers along with higher molecular weights. The “other” compounds set further enriched the chemical space; it shared some overlap with drugs as shown in the PC1 vs PC2 and PC3 vs PC2 plots, while also extending beyond it to occupy some unique space (i.e., upper right quadrants). Unlike drugs, the other set contains both biologically active and inactive compounds, which may reflect the fact that they have not been selected for by parameters related to bioavailability. Overall, the NP, drug, and other sets occupied relatively distinct chemical spaces in the PCA. The plots also contain simulated spots that represent virtual compounds composed from the average values of each defined set, and the NP average spot is distant from the average drug spot, and underscores the differences present between these two sets of compounds. The reasoning for this difference and the deeper meaning behind the location of the spots in chemical space can be determined by connecting the initial parameters to the principal components.
We gleaned information about which parameters contributed to the variance in the principal components from the loading plots (Figure 2.3) and the coefficients of each of the parameters in the PCA (See Appendix). Loading plots provided a useful qualitative view on how specific parameters affect the location of a compound on the PCA plots. Further, the coefficients from which the loading plots were built provided a quantitative understating of which parameters had the greatest effect on the PCA plots. Parameters such as H-bond acceptors (HBA), topological polar surface area (tPSA) and the number of oxygen atoms (O) had the greatest effect on PC1. For PC2, aromatic ring count (RingAr), hydrophobicity (ALOGPs), and aqueous solubility (ALOGpS) were key, and the number of nitrogen atoms (N) was a major component of PC3. Depending on the sign (+/-), these parameters could move in one direction or the other with respect to a given PC. Consideration of individual parameters within the PCs, as listed above, provided insight into molecular characteristics of the compound sets we defined. By considering the three PCs together it was determined that the following parameters are key to the entire PCA due their significance in more than one of the PCs: sp³-hybridized carbons (Fsp³), relative polar surface area (relPSA), ALOGPs, aqueous solubility (LogD and ALOGpS), and RingsAr (See table in the Appendix). This enables a better understanding of how the PCA plot as a whole 3D-space was affected by the parameters. Our evaluation of these parameters is comparable to that of Tan et al. who obtained similar results using the same base library to evaluate their compounds. The only major difference between our results and theirs is the importance RingsAr, which is in line with the fact that compounds like 21a-21k that we report here incorporate a biaryl moiety as a key structural feature.
Three simple [13]-macrolactones from our library – unfunctionalized macrocycle 1 and the C2-methyl and C7-phenyl macrocycles 4 and 7, respectively (Figure 2.4) – illustrate the practical effects of RingsAr and Fsp$^3$ by illustrating how these structures related to each other on the plots. This effect is most clearly seen in the PC1 vs PC2 and the PC3 vs PC2 plots (Figure 2.2A and 2.2C), which makes sense due to the fact that both parameters are more significant in PC2 and PC3. In the PC1 vs PC2 plot, for example, the phenyl substituted macrocycle 7 is located in the lower right quadrant underneath the other two compounds, which coincides with the downward influence of RingsAr (Figure 2.3A). Methyl substituted macrocycle 4 was to the left of the unfunctionalized ring 1, which is in agreement with the influence of Fsp$^3$. A similar arrangement is seen in PC3 vs PC2 with the only difference being that the methyl substituted ring is now to the right of the unfunctionalized ring, which agrees with the change in direction based on the influence of Fsp$^3$ (Figure 2.2C, Figure 2.3C).

![Diagram of macrocycles](image)

**Figure 2.4** [13]-Macrolactones 1, 4, and 7 described in the text and lipid-linked [13]-macrolactones 17-18, 33
By evaluating the distributions in the PCA plots of the new biaryl-functionalized [13]-macrodilactones (21a-21k, biAr) and their carbohydrate-fused counterparts (e.g., 17-18, 33 carb), valuable insights about the differences between them became apparent. Both sets contain the [13]-macrodilactone (i.e., 1) core in common. The synthetic approach used to access them is modular in nature and allows for broad functionalization. Consequently, differences arise from the details (i.e., diversification strategy or starting materials) in the preparation of each set. The biAr set relied on late-stage functionalization via Suzuki reactions, whereas the carb set used pyranose sugars to template the core of the [13]-macrodilactone. In general, the biAr [13]-macrodilactones (21a-21k) occupy chemical space that overlaps with compounds in the drug set of the library (Figure 2.5). These compounds also occupy a much smaller area of chemical space in comparison to the carb set. This is due to the factors discussed above as well as the influence from RingsAr. As the name suggests, all of the compounds in the biAr set contain a biaryl system due to the Suzuki coupling. Compounds in the carb set (e.g., 17-18, 33), on the other hand, tend to be more natural product-like, based on their distribution in the PCA plots. This is caused by to two main factors. First, these compounds have more heteroatoms, specifically oxygen atoms, from the sugar that has been incorporated into the macrocyclic structure. Second, the Fsp³ is higher due to both the carbohydrate ring and the alkyl chain in octyl glycosides.
Figure 2.5 PC1 vs PC3 plot that details location in chemical space of antiproliferative compounds from the carb set (compounds 17-18, 33) and biAr set (21c, 21h) to bioavailability

The chemical space that both sets cover is relatively broad, and the carb set occupies more overall PC space than does the biAr set. In the PC1 vs PC2 plot, for example (Figure 2.2A), most compounds in the biAr sets reside in the lower right part of the plot along with most drug compounds, while the carb set starts in the lower right and spreads out into the upper left quadrant, showing little separation between the sets. The clearest distinction between these two sets can be seen in the PC1 vs PC3 plot (Figure 2.5) where the biAr set is localized mostly in the upper left quadrant just above the x-axis while the carb set is in the upper right quadrant. In general, the biAr set tracks towards drugs and the carb set tracks towards NPs. The PCA, therefore, makes sense with respect to the overall nature of these two sets of [13]-macrodilactones. That is, appending heterobiaryl groups to the macrocycle makes their properties more drug-like, while a fused carbohydrate makes their properties more natural product-like. Both sets of derivatives maintain a common [13]-macrodilactone core but nonetheless occupy somewhat complementary areas of chemical space. Further, the fact that specific
compounds (i.e., 17-18, 33 and 21c, 21h) in each of the sets were antiproliferative suggests that activity can be maintained despite these slight variations in structure.

2.4 Solubility assay

Solubility is a useful physical parameter that helps to characterize compounds, and it can provide insight into their biological activity. We wanted to see how varying the aryl unit attached to macrocycles 21a-21k influenced their solubility. Solubilities were determined by experimentally measuring optical absorbance values across a range of concentrations to prepare standard curves for each compound. Methanol was used to generate standard curves because all the compounds were highly soluble in it, and it is also largely UV transparent. Experimentally, water or buffer was added to a solid macrocycle sample of known mass, then it was incubated with occasional agitation over 24 hours, followed by centrifugation and collection of the UV-Vis spectrum of the supernatant. The concentration of the experimental samples was then determined based on comparison to the standard curve.

Besides conducting aqueous solubility assay by optical absorbance values of UV-Vis, one simple method was also utilized to do a parallel set test for getting results. The solubility was determined by measuring the mass difference of the Eppendorf tube before and after 24h sample dissolving (See Appendix for details). Surprisingly, the results derived from these two methods had large difference for the solubility value. This may due to the large error induced by this imprecise mass-weighting method. Nevertheless, even though the value and its order of the compounds were not totally agreed, the data may be indicative to some extent. Such as compound 21e, 21g, 21j, their values showed
they are one of the most promising compounds for solubility. The values revealed from the calculated aqueous solubility (ALOGpS) also supported the same trend. In general, the solubilities of the new compounds range from slightly to sparingly soluble (Table 2.3).22

**Table 2.3** Experimental and calculated aqueous solubility

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sol (mg/mL) (UV-Vis)</th>
<th>Sol (mg/mL) (Mass)</th>
<th>ALOGpS</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>0.0013</td>
<td>0.13</td>
<td>-5.91</td>
</tr>
<tr>
<td>21b</td>
<td>0.0073</td>
<td>0.73</td>
<td>-6.65</td>
</tr>
<tr>
<td>21c</td>
<td>0.0051</td>
<td>0.33</td>
<td>-5.07</td>
</tr>
<tr>
<td>21d</td>
<td>0.00044</td>
<td>0.2</td>
<td>-5.21</td>
</tr>
<tr>
<td>21e</td>
<td>0.0210</td>
<td>0.72</td>
<td>-4.79</td>
</tr>
<tr>
<td>21f</td>
<td>0.0044</td>
<td>0.17</td>
<td>-4.92</td>
</tr>
<tr>
<td>21g</td>
<td>0.027</td>
<td>0.18</td>
<td>-3.92</td>
</tr>
<tr>
<td>21h</td>
<td>0.0078</td>
<td>0.3</td>
<td>-3.86</td>
</tr>
<tr>
<td>21i</td>
<td>0.0290</td>
<td>0.23</td>
<td>-6.06</td>
</tr>
<tr>
<td>21j</td>
<td>0.0110</td>
<td>0.2</td>
<td>-4.32</td>
</tr>
<tr>
<td>21k</td>
<td>0.0018</td>
<td>0.4</td>
<td>-5.85</td>
</tr>
</tbody>
</table>

Amongst all the compounds, solubilities of three were tested in buffer solutions (Figure 2.6). First, 21e, whose biaryl moiety includes an m-pyridyl ring, possessed the highest solubility (Table 2.3) in pure water. Inexplicably, a significant decrease in solubility of 21e was observed in the buffer systems. On the other hand, the solubility of 21c increased when going from water to buffer. It was most soluble in the pH 7.8 buffer, suggesting that its phenol functionality was contributing by being partially ionized at higher pH values. Last, 21h had the median solubility and therefore was considered representative of the group. Values for 21h were consistent across the range of pH values evaluated.
Figure 2.6 Solubility of compounds 21c, 21e, and 21h at pH 6.2, 7.0, and 7.8

Due to the significance of solubility as a physiochemical parameter determined by PCA, we opted to look at correlation plots that explicitly included these with experimental data. Since we had determined aqueous solubilities, we focused on solubility terms that directly reflected solubility (i.e., LogS, ALOGpS) rather than partition coefficients such as logP and logD for the correlations. The first correlation compared experimental solubility values to LogS and ALOGpS, as well as Swiss ADME solubility terms. While no significant correlation was observed, (R² correlation coefficients < 0.3; see Appendix for details.), the clustering of the data points demonstrated general agreement (precision) amongst the computational solubility calculations used. Overall, however, disagreement between the experimental and computed solubilities drew their accuracy into question. The second set of correlations sought to connect the GI₅₀ values from the cell proliferation assay and the solubility values. Compounds that did not show activity in the proliferation assay were removed from the plots in order to look for correlations. When they were included by assigning them artificially high GI₅₀ values, trendlines were skewed significantly. Once removed, plots for solubility values - both computational and experimental - were made. Once again there was poor correlation, indicating that there
was no true connection seen between GI\textsubscript{50} and solubility. The correlation plots partially suggest that the greater the lipophilicity of the compounds, the greater their cytotoxicity. Consequently, it can be determined that while solubility does appear to be an important factor in terms of chemical space for our compounds, it does not fully explain their activity.

Aqueous solubilities, while helpful, provide only a partial picture of solubility. To gain more perspective, we also looked at the water-octanol partition coefficients, LogP and LogD. These parameters take into account the concentration of a given compound across the solvent barrier and, by so doing, portray solubility in more of a druggable sense that can have practical applications. Values were determined computationally for our compounds and were compared to those of the drugs and natural products in the library used for PCA. From the library, a handful of compounds were found to have solubility parameters that were similar to ours (e.g., 21c/21d, Tricor/Cymbalta, Rapamycin), which can be seen in Table 3. A few of our compounds violate Lipinski’s rules, which call for a LogP (ALOGPs in Table 2.4) no greater than 5. For example, 21f has a LogP of 5.76.\textsuperscript{24} Both the drugs and natural products have rule breakers such as Abilify at 5.21 and MycobactinS at 5.78. This shows that the rules are a good guide but do not need to be strictly followed; this may be particularly true for macrocycles. Overall, while our [13]-macrodilactones share some similarity in terms of partition coefficients and solubility to small molecule drugs and biologically active natural products, our results show that these parameters may be necessary but insufficient for potent anti-proliferative activity.
Table 2.4  Computational solubility values for [13]-macrodilactones and select drug and natural product library members (ALOGPs is LogP and ALOGpS is LogS)

<table>
<thead>
<tr>
<th>[13]-macrodilactones</th>
<th>Drugs</th>
<th>Natural Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LogD</td>
<td>ALOGPs</td>
</tr>
<tr>
<td>21a</td>
<td>4.52</td>
<td>5.04</td>
</tr>
<tr>
<td>21b</td>
<td>6.06</td>
<td>5.76</td>
</tr>
<tr>
<td>21c</td>
<td>4.22</td>
<td>4.78</td>
</tr>
<tr>
<td>21d</td>
<td>4.63</td>
<td>5.09</td>
</tr>
<tr>
<td>21e</td>
<td>3.30</td>
<td>3.72</td>
</tr>
<tr>
<td>21f</td>
<td>3.30</td>
<td>3.75</td>
</tr>
<tr>
<td>21g</td>
<td>2.60</td>
<td>2.8</td>
</tr>
</tbody>
</table>

2.5 Esterase assay

We also conducted esterase assay to determine if this new class of compounds are substrate of Pig Liver Esterase (PLE). Esterases are hydrolases that can catalyze for ester bonds’ cleavage. Our hypothesis was that PLE should help with the hydrolysis of our C7-aryl [13]-macrodilactones owning two ester units, which can be figured by the value of UV-Vis absorbance of the macrocycle solution after PLE addition. p-Nitrophenyl acetate is a model substrate that normally used in esterase assay. The cleavage of ester bond of p-nitrophenyl acetate provides p-nitrophenol, which can be detected by UV-Vis absorbance at 405 nm. This guides us to run the reaction of PLE and our compounds in the buffer for this test (See Appendix for details). The control experiment was carried out in the pure buffer solution with the compound and DI water.

Table 2.5 and Figure 2.7 gives the absorbance and TLC demonstration of compound 21a. Our macrocycle should have an absorbance peak at 256 nm. Compound
21a was dissolved in MeOH to make the stock solution for original abs. Then after the reaction, the sample was extracted by DCM, and the DCM layer was made a 10-fold dilution to evaluate the absorbance. Theoretically, if there was no activity, based on the Beer’s law, the absorbance of stock solution to esterase one at 256 nm should have the same ratio with the compound concentration in stock solution to esterase one. However, it was observed that the absorbance was decreased more than 10 times of the esterase sample after 20 min agitation and there was a new peak at 230 nm appeared. The outcome indicated that compound 21a was hydrolyzed by PLE in the esterase assay. Additionally, the absorbance of the control test was higher than esterase one under the same concentration, which also implied PLE’s effect in the assay. The new peak at 230 nm is probably represented the acid cut from the macrocycle, whereas the higher value of the control test was objected to this assumption.

A TLC trial was utilized to track the component in the solution. After the agitation, the control solution still showed the spot of 21a, while a disappearance of 21a spot supported the observation made from UV-Vis.

In consequence, the esterase assay did give us some insights about the esterase activity of C7-aryl [13]-macrolactones. Future design of our macrocycle class can also derived from this preliminary results to take an improvement of the biological ability.
Table 2.5 UV-Vis absorbance of 21a before and after the reaction with PLE

<table>
<thead>
<tr>
<th>20 min agitation</th>
<th>conc. (μM)</th>
<th>abs. (256 nm)</th>
<th>abs. (230 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock solution</td>
<td>16.44</td>
<td>0.3688</td>
<td></td>
</tr>
<tr>
<td>Esterase</td>
<td>1.64 (if no activity)</td>
<td>0.01937</td>
<td>0.04378</td>
</tr>
<tr>
<td>Control</td>
<td>1.64 (if no activity)</td>
<td>0.03043</td>
<td>0.05625</td>
</tr>
</tbody>
</table>

Figure 2.7 TLC demonstration of 21a
2.6 Conclusion

The synthesis, physicochemical characterization, and antiproliferative activity of a new class of C7-substituted [13]-macrodilactones has been reported in this chapter. Biaryl analogs were prepared by Suzuki reactions conducted on a common intermediate that contained a bromophenyl unit at C7 position of the [13]-macrodilactone. Principal component analysis placed the new compounds in physicochemical context relative to a variety of pharmaceuticals and natural products. PCA allowed the new biaryl [13]-macrodilactones (21) to be placed in the context of natural products, drugs and other compounds, and the carbohydrate-fused [13]-macrodilactones (e.g., 17-18, 33) that were studied previously. Further, individual compounds in the set (i.e., 21c and 21h) showed anti-proliferative activity of similar potency to 17-18, 33. The modest antiproliferative effect of the [13]-macrodilactones reported here motivates the incorporation of their structural and physicochemical features into the design of related families of macrocycles.

Moreover, this investigation reported here suggests that, by determining which parameters are of key interest, PCA may help guide synthetic planning of sets of compounds. For example, incorporation of stereogenic centers may tilt a set of compounds toward greater natural product-like character. This work underscores the value of an approach toward the identification of bioactive compounds that places the evaluation of physicochemical parameters early in the search process. Alternatively, the addition of hetero-biaryl units may make a scaffold more drug-like. It is notable that the depsipeptide macrocycle teixobactin contains a polar ring connected to a pendant peptide containing hydrophobic amino acids. The teixobactin structure is loosely related to our [13]-macrodilactones and suggests additional motifs that can be explored.
2.7 Experimental

2.7.1 Methyl 4-bromophenylacetate 27

To a solution of 4-bromophenylacetic acid (1.00 g, 4.65 mmol) in methanol (50 mL) at 0 °C, was slowly added H₂SO₄ (5 mL). The mixture was heated under reflux for 3 hours and then allowed to cool to room temperature and diluted with ethyl acetate (350 mL). The solution was washed with water (3 x 60 mL), and the organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to give the methyl ester 27 in 90% yield as a pale-yellow oil. ¹H NMR (CDCl₃) 300 MHz: δ 7.43 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 3.67 (s, 3H), 3.56 (s, 2H); ¹³C NMR (CDCl₃) 75 MHz: δ 171.4, 133.0, 131.6, 131.0, 121.1, 52.1, 40.5.
2.7.2 Compound 29

In a flame dried 100 mL round bottomed flask, diisopropylamine (4.18 mmol, 0.59 mL) was dissolved in 20 mL dry THF. The solution was cooled to -78 °C under N\textsubscript{2} and n-butyllithium (2.7 mL, 4.39 mmol, 1.6 M in hexanes) was added dropwise over 10 min. After stirring for an additional 30 min, methyl 4-bromophenylacetate 27 (0.96 g, 4.18 mmol) in 3 mL THF was quickly added to the LDA solution. The reaction was maintained at -78 °C for 30 min, and then allyl bromide (0.39 mL, 4.56 mmol) was added dropwise to the reaction mixture. The reaction was stirred and allow to warm to room temperature over 2 hours. The solution was then diluted with ethyl acetate (40 mL) and washed with saturated NH\textsubscript{4}Cl solution (1 x 20 mL). The organic layer was dried over Na\textsubscript{2}SO\textsubscript{4} and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to give the alkylated ester 29 as a light-yellow oil in 72% yield.

R\textsubscript{f} 0.6 (10% EtOAc/Hex); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) 300 MHz: \(\delta\) 7.45 (d, \(J = 8.5\) Hz, 2H), 7.20 (d, \(J = 8.4\) Hz, 2H), 5.70 (dddd, \(J = 17.0, 10.2, 6.8, 6.8\) Hz, 1H), 5.18–4.93 (m, 2H), 3.66 (s, 3H), 3.63 (dd, \(J = 19.5, 9.7\) Hz, 1H), 2.81 (dddd, \(J = 14.3, 7.1, 7.1\) Hz, 1H), 2.50 (ddd, \(J = 14.0, 6.9, 6.9\) Hz, 1H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) 75 MHz: \(\delta\) 173.4, 137.4, 134.8, 131.7, 129.7, 121.3, 117.4, 52.1, 50.8, 37.4.
2.7.3 2-(p-Bromophenyl)-4-pentenoic acid 24

The alkylated ester 29 (0.81 g, 3.01 mmol) was dissolved in a mixture of water and methanol (3:1, 12 mL). Sodium hydroxide (0.16 g, 3.91 mmol) was then added as a solid in one portion and the mixture was stirred overnight at room temperature. After, the reaction was acidified by addition of 1M HCl to pH 1 and then extracted with ethyl acetate (3 x 20 mL). The combined extracts were dried over Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. Pure pentenoic acid 24 was obtained as a yellow oil in quantitative yield and was used without additional purification. $^1$H NMR (CDCl$_3$) 400 MHz: δ 11.74 (s,br, 1H), 7.47 (d, $J = 8.4$ Hz, 2H), 7.22 (d, $J = 8.5$ Hz, 2H), 5.71 (dddd, $J = 17.0$, 10.2, 6.8, 6.8 Hz, 1H), 5.15–5.02 (m, 2H), 3.64 (dd, $J = 14.6$, 6.9 Hz, 1H), 2.82 (ddd, $J = 14.5$, 7.1, 7.1 Hz, 1H), 2.53 (ddd, $J = 14.2$, 7.0, 7.0 Hz, 1H); $^{13}$C NMR (CDCl$_3$) 100 MHz: δ 179.6, 136.8, 134.5, 131.9, 129.9, 121.8, 117.8, 50.9, 37.0.
Dicyclohexylcarbodiimide (DCC) (1.34 g, 6.48 mmol) and N,N-dimethylaminopyridine (DMAP) (366.5 mg, 3.00 mmol) were dissolved in DCM (45 mL), and the solution was cooled to 0 °C on an ice bath. 4-Pentenoic acid (600.7 mg, 6.00 mmol) was added and the mixture was stirred at the same temperature for 30 min. Then, 1,3-propanediol (456.5 mg, 6.00 mmol) in DCM (5 mL) was added to the mixture which was stirred overnight at room temperature. The reaction mixture was then filtered through a short pad of Celite, the solvent was removed from the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography to give 25 as a colorless oil (455 mg, 48%). $R_f$ 0.17 (20% EtOAc/Hex); $^1$H NMR (CDCl$_3$) 300 MHz: $\delta$ 5.70 (dddd, $J$ = 16.6, 10.3, 6.2, 6.2 Hz, 1H), 5.01–4.83 (m, 2H), 4.10 (t, $J$ = 6.4 Hz, 2H), 3.56 (t, $J$ = 6.2 Hz, 2H), 3.18 (s, 1H), 2.37–2.17 (m, 4H), 1.75 (p, $J$ = 6.3 Hz, 2H); $^{13}$C NMR (CDCl$_3$) 75 MHz: $\delta$ 173.4, 136.4, 115.4, 61.3, 58.6, 33.3, 31.5, 28.6.
2.7.5 Compound 23

DCC (647.3 mg, 3.14 mmol) and DMAP (174.2 mg, 1.43 mmol) were dissolved in DCM (12 mL), and the solution was cooled to 0 °C. 2-(p-Bromophenyl)-4-pentenoic acid 24 (800.0 mg, 3.14 mmol) was added and the mixture was stirred at the same temperature for 30 min. Then, 3-hydroxypropyl 4-pentenoate 25 (450.6 mg, 2.85 mmol) in DCM (1 mL) was then added to the mixture and it was stirred at room temperature overnight. After, the mixture was filtered through a short pad of Celite and the solvent was removed from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography to give diene 23 as a colorless oil (563 mg, 50%). \( R_f \) 0.44 (10% EtOAc/Hex); \( ^1H \) NMR (CDCl\(_3\)) 300 MHz: \( \delta \) 7.41 (d, \( J = 8.4 \) Hz, 2H), 7.15 (d, \( J = 8.4 \) Hz, 2H), 5.83-5.59 (m, 2H), 5.10–4.91 (m, 4H), 4.11 (q, \( J = 6.3 \) Hz, 2H), 4.04 (t, \( J = 6.3 \) Hz, 2H), 3.58 (dd, \( J = 8.4, 7.1 \) Hz, 1H), 2.76 (dddd, \( J = 14.5, 7.4, 7.4 \) Hz, 1H), 2.46 (dddd, \( J = 13.9, 6.8, 6.8 \) Hz, 1H), 2.40–2.27 (m, 4H), 1.88 (p, \( J = 6.4 \) Hz, 2H); \( ^{13}C \) NMR (CDCl\(_3\)) 75 MHz: \( \delta \) 172.7, 137.4, 136.5, 134.7, 131.7, 129.6, 121.3, 117.4, 115.5, 61.4, 60.7, 50.8, 37.3, 33.4, 28.8, 27.9. TOF HRMS (DART): \( m/z \) calcd for C\(_{19}\)H\(_{24}\)O\(_4\)Br [M+H]+: 395.0858; found: 395.0845.
2.7.6 [13]-Macrodilactone 22\textsuperscript{5}

Grubbs' second-generation catalyst (60.2 mg, 0.071 mmol) was added as a solid in one portion to a solution of diene 23 (560 mg, 1.42 mmol) in toluene (200 mL). The mixture was heated to reflux overnight (18 h). The mixture was then allowed to cool to room temperature, and then the toluene was removed under reduced pressure and the crude product was purified by silica gel column chromatography to give [13]-macrodilactone 22 as a white solid (438 mg, 84%). mp 108.8–111.0 °C; R\textsubscript{f} 0.45 (20% EtOAc/Hex); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) 300 MHz: δ 7.45 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 5.66–5.44 (m, 2H), 4.59-4.40 (m, 2H), 4.03 (dt, J = 11.2, 4.2 Hz, 1H), 3.86 (dt, J = 11.2, 4.1 Hz, 1H), 3.57 (dd, J = 12.4, 2.9 Hz, 1H), 2.73 (ddd, J = 12.9, 12.9, 8.1 Hz, 1H), 2.52–2.22 (m, 5H), 2.10-1.99 (m, 2H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) 75 MHz: δ 173.7, 137.9, 131.8, 131.3, 129.5, 128.5, 121.5, 60.7, 60.2, 51.4, 37.8, 34.1, 28.6, 25.8. TOF HRMS (DART): m/z calcd for C\textsubscript{17}H\textsubscript{20}O\textsubscript{4}Br [M+H]+: 367.0545; found: 367.0545.
2.7.7 General procedure for Suzuki reactions on [13]-macrodilactone 22

The (hetero)aryl boronic acid (0.15 mmol), [Pd_2-(dba)_3] (1.3 mg, 0.0012 mmol), PCy_3 (0.9 mg, 0.0033 mmol) and the [13]-macrodilactone 22 (50 mg, 0.136 mmol) were added to a 5 mL vial equipped with a stir bar under nitrogen. The vial was sealed, evacuated and refilled with nitrogen for ten minutes. Dioxane (0.7 mL), and aqueous K_3PO_4 (0.5 mg, 0.23 mmol in 0.18 ml H_2O) were added by syringe. The vial was heated at 100 °C for 18 h with vigorous stirring. The mixture was allowed to cool to room temperature and then filtered through a pad of silica gel with washing by EtOAc. The filtrate was dried over anhydrous Na_2SO_4 and then concentrated. The residue was purified by column chromatography on silica gel using hexanes:ethyl acetate solutions as reported for each compound.
2.7.7.1 Compound 21a

This compound was prepared by using the general procedure for Suzuki reactions with compound 31a to give compound 21a as a white solid (38 mg, 77%). mp 126.1-127.0 °C; Rf 0.64 (15% EtOAc/Hex); \(^1\)H NMR (CDCl\(_3\)) 400 MHz: \(\delta\) 7.56 (td, \(J = 7.2, 6.5, 1.8\) Hz, 4H), 7.50–7.39 (m, 4H), 7.37–7.30 (m, 1H), 5.66–5.49 (m, 2H), 4.57 (ddd, \(J = 11.1, 8.2, 6.6\) Hz, 1H), 4.51–4.39 (m, 1H), 4.03 (dt, \(J = 11.2, 4.2\) Hz, 1H), 3.87 (dt, \(J = 11.2, 4.1\) Hz, 1H), 3.66 (dd, \(J = 12.4, 2.9\) Hz, 1H), 2.82 (ddd, \(J = 13.0, 13.0, 8.1\) Hz, 1H), 2.48–2.28 (m, 5H), 2.10–1.99 (m, 2H); \(^{13}\)C NMR (CDCl\(_3\)) 100 MHz: \(\delta\) 174.0, 173.8, 140.9, 140.5, 138.0, 131.0, 128.9, 128.2, 127.5, 127.4, 127.2, 60.6, 60.3, 51.7, 37.8, 34.2, 28.7, 25.9. TOF HRMS (DART): \(m/z\) calcd for C\(_{23}\)H\(_{24}\)O\(_4\) [M+H\(^+\)]: 365.1753; found: 365.1737.
2.7.7.2 Compound 21b

This compound was prepared by using the general procedure for Suzuki reactions with compound 31b to give compound 21b as a white solid (26 mg, 47%). mp 113.2-114.2 °C; Rf 0.74 (30% EtOAc/Hex); \(^1^H\) NMR (CDCl\(_3\)) 400 MHz: \(\delta\) 7.40 (d, \(J = 8.1\) Hz, 2H), 7.08 (d, \(J = 8.2\) Hz, 2H), 6.93 (s, 2H), 5.63–5.51 (m, 2H), 4.65–4.53 (m, 1H), 4.52–4.42 (m, 1H), 4.03 (dt, \(J = 11.1, 4.2\) Hz, 1H), 3.90 (dt, \(J = 11.1, 4.1\) Hz, 1H), 3.65 (dd, \(J = 12.5, 2.8\) Hz, 1H), 2.82 (ddd, \(J = 12.9, 12.9, 7.9\) Hz, 1H), 2.47–2.30 (m, 5H), 2.32 (s, 3H), 2.11–2.02 (m, 2H), 1.98 (s, 6H); \(^{13}\)C NMR (CDCl\(_3\)) 100 MHz: \(\delta\) 174.3, 173.8, 140.2, 138.7, 137.1, 136.7, 136.2, 130.9, 129.7, 129.0, 128.2, 127.7, 60.5, 60.3, 51.7, 37.9, 34.2, 28.7, 25.9, 21.1, 20.9. TOF HRMS (DART): \(m/z\) calcd for C\(_{26}\)H\(_{30}\)O\(_4\) [M+H]\(^+\): 407.2222; found: 407.2189.
2.7.7.3 Compound 21c

This compound was prepared by using the general procedure for Suzuki reactions with compound 31c to give compound 21c as a white solid (27 mg, 52%). mp 151.6-152.3 °C; Rf 0.25 (30% EtOAc/Hex); ^1H NMR (CDCl$_3$) 300 MHz: δ 7.51 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.32–7.24 (m, 1H), 7.15–7.04 (m, 2H), 6.84 (dd, J = 8.0, 2.5 Hz, 1H), 5.95–5.70 (br, 1H), 5.63–5.48 (m, 2H), 4.61–4.51 (m, 1H), 4.51–4.41 (m, 1H), 4.03 (dt, J = 11.2, 4.2 Hz, 1H), 3.87 (dt, J = 11.2, 4.1 Hz, 1H), 3.63 (dd, J = 12.5, 2.9 Hz, 1H), 2.79 (ddd, J = 12.9, 12.9, 7.8 Hz, 1H), 2.51–2.22 (m, 5H), 2.08–2.00 (m, 2H); ^13C NMR (CDCl$_3$) 75 MHz: δ 174.4, 174.2, 156.4, 142.5, 140.1, 138.0, 131.0, 130.1, 128.9, 128.2, 127.4, 119.5, 114.5, 114.1, 60.7, 60.4, 51.6, 37.8, 34.2, 28.7, 25.8. TOF HRMS (DART): m/z calcd for C$_{23}$H$_{24}$O$_5$ [M+H]+: 381.1702; found: 381.1680.
2.7.7.4 Compound 21d

This compound was prepared by using the general procedure for Suzuki reactions with compound 31d to give compound 21d as a white solid (34 mg, 61%). mp 117.2-117.9 °C; Rf 0.51 (30% EtOAc/Hex); ¹H NMR (CDCl₃) 300 MHz: δ 7.51 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.29–7.22 (m, 1H), 6.89 (d, J = 7.7 Hz, 1H), 6.87 (s, 1H), 6.73–6.70 (m, 1H), 5.61–5.45 (m, 2H), 4.57–4.48 (m, 1H), 4.46–4.37 (m, 1H), 3.98 (dt, J = 11.2, 4.2 Hz, 1H), 3.82 (dt, J = 11.2, 4.1 Hz, 1H), 3.61 (dd, J = 12.5, 2.8 Hz, 1H), 2.96 (s, 6H), 2.77 (ddd, J = 12.9, 12.9, 7.8 Hz, 1H), 2.43–2.25 (m, 5H), 2.04–1.96 (m, 2H); ¹³C NMR (CDCl₃) 75 MHz: δ 174.1, 173.8, 150.9, 142.0, 141.5, 137.8, 131.01, 129.6, 129.0, 128.0, 127.7, 116.0, 111.9, 111.7, 60.6, 60.3, 51.7, 40.9, 37.8, 34.2, 28.7, 25.9. TOF HRMS (DART): m/z calcd for C₂₅H₂₉NO₄ [M+H]+: 408.2175; found: 408.2155.
2.7.7.5 Compound 21e

This compound was prepared by using the general procedure for Suzuki reactions with compound 31e to give compound 21e as a white solid (41 mg, 83%). mp 132.6-133.5 °C; Rf 0.36 (50% EtOAc/Hex); ¹H NMR (CDCl₃) 400 MHz: δ 8.82 (s, 1H), 8.57 (s, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.50 (q, J = 8.0 Hz, 4H), 7.38–7.28 (m, 1H), 5.65–5.46 (m, 2H), 4.54 (dt, J = 10.7, 7.4 Hz, 1H), 4.44 (dt, J = 15.0, 7.8 Hz, 1H), 4.04–4.00 (m, 1H), 3.88–3.83 (m, 1H), 3.65 (dd, J = 12.4, 2.8 Hz, 1H), 2.79 (ddd, J = 13.0, 13.0, 8.1 Hz, 1H), 2.45–2.27 (m, 5H), 2.06–2.00 (m, 2H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.9, 173.7, 148.6, 148.4, 138.9, 137.1, 136.3, 134.3, 131.2, 128.7, 128.5, 127.5, 123.7, 60.7, 60.3, 51.6, 37.8, 34.1, 28.6, 25.9. TOF HRMS (DART): m/z calcd for C₂₂H₂₃NO₄ [M+H]: 366.1705; found: 366.1694.
2.7.7.6 Compound 21f

This compound was prepared by using the general procedure for Suzuki reactions with compound 31f to give compound 21f as a white solid (28 mg, 56%). mp 144.3–145.2 °C; Rf 0.61 (100% EtOAc); \(^1\)H NMR (CDCl\(_3\)) 300 MHz: \(\delta\) 8.64 (s, 2H), 7.59 (d, \(J = 8.3\) Hz, 2H), 7.53–7.43 (m, 4H), 5.64–5.48 (m, 2H), 4.55 (ddd, \(J = 11.0, 8.2, 6.5\) Hz, 1H), 4.45 (ddd, \(J = 11.2, 8.2, 6.5\) Hz, 1H), 4.02 (dt, \(J = 11.2, 4.2\) Hz, 1H), 3.86 (dt, \(J = 11.2, 4.1\) Hz, 1H), 3.66 (dd, \(J = 12.4, 2.9\) Hz, 1H), 2.80 (ddd, \(J = 13.0, 13.0, 7.9\) Hz, 1H), 2.48–2.24 (m, 5H), 2.08–2.00 (m, 2H); \(^{13}\)C NMR (CDCl\(_3\)) 75 MHz: \(\delta\) 173.8, 173.7, 150.4, 147.9, 140.0, 137.4, 131.3, 128.6, 127.4, 121.6, 60.7, 60.3, 51.7, 37.8, 34.1, 28.6, 25.9. TOF HRMS (DART): \(m/z\) calcd for C\(_{22}\)H\(_{23}\)NO\(_4\) [M+H]+: 366.1705; found: 366.1700.
2.7.7.7 Compound 21g

This compound was prepared by using the general procedure for Suzuki reactions with compound 31g to give compound 21g as a white solid (46 mg, 93%). mp 145.7-146.6 °C; Rf 0.68 (75% EtOAc/Hex); $^1$H NMR (CDCl$_3$) 300 MHz: δ 9.18 (s, 1H), 8.91 (s, 2H), 7.53 (s, 4H), 5.63–5.47 (m, 2H), 4.58–4.50 (m, 1H), 4.48–4.40 (m, 1H), 4.02 (dt, $J$ = 11.2, 4.2 Hz, 1H), 3.86 (dt, $J$ = 11.2, 4.1 Hz, 1H), 3.66 (dd, $J$ = 12.4, 2.9 Hz, 1H), 2.79 (ddd, $J$ = 12.9, 12.9, 8.0 Hz, 1H), 2.46–2.28 (m, 5H), 2.08–2.00 (m, 2H); $^{13}$C NMR (CDCl$_3$) 75 MHz: δ 173.7, 173.6, 157.6, 154.9, 140.0, 134.0, 133.5, 131.3, 128.9, 128.5, 127.3, 60.9, 60.3, 51.7, 37.8, 34.1, 28.6, 25.9. TOF HRMS (DART): m/z calcd for C$_{21}$H$_{22}$N$_2$O$_4$ [M+H]$^+$: 367.1658; found: 367.1663.
2.7.7.8 Compound 21h

This compound was prepared by using the general procedure for Suzuki reactions with compound 31h to give compound 21h as a white solid (57 mg, 98%). mp 114.3-114.7 °C; Rf 0.36 (30% EtOAc/Hex); ¹H NMR (CDCl₃) 400 MHz: δ 7.56 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 6.92 (s, 1H), 5.65–5.48 (m, 2H), 4.55 (ddd, J = 11.1, 8.5, 6.7 Hz, 1H), 4.45 (ddd, J = 11.2, 8.2, 6.5 Hz, 1H), 4.08 (s, 6H), 4.02 (dt, J = 11.2, 4.1 Hz, 1H), 3.86 (dt, J = 11.2, 4.1 Hz, 1H), 3.65 (dd, J = 12.4, 2.9 Hz, 1H), 2.79 (ddd, J = 13.0, 13.0, 8.3 Hz, 1H), 2.48–2.27 (m, 5H), 2.10–1.99 (m, 2H); ¹³C NMR (CDCl₃) 75 MHz: δ 173.7, 162.7, 159.5, 140.2, 133.7, 132.6, 131.2, 129.3, 128.6, 128.0, 119.1, 60.7, 60.2, 54.9, 54.6, 51.7, 37.7, 34.1, 28.6, 25.8. TOF HRMS (DART): m/z calcd for C₂₃H₂₆N₂O₄ [M+H]⁺: 427.1869; found: 427.1882.
2.7.7.9 Compound 21i

This compound was prepared by using the general procedure for Suzuki reactions with compound 31i to give compound 21i as a white solid (34 mg, 61%). mp 150.7-151.3 °C; Rf 0.58 (30% EtOAc/Hex); $^1$H NMR (CDCl$_3$) 400 MHz: δ 8.29 (s, 1H), 7.66 (d, $J = 8.2$ Hz, 2H), 7.48 (d, $J = 8.3$ Hz, 2H), 7.39 (dt, $J = 8.2$, 1.0 Hz, 1H), 7.27–7.23 (m, 2H), 7.17 (dd, $J = 7.3$, 1.0 Hz, 1H), 6.78–6.66 (m, 1H), 5.65-5.53 (m, 2H), 4.65–4.53 (m, 1H), 4.53–4.41 (m, 1H), 4.04 (dt, $J = 11.2$, 4.2 Hz, 1H), 3.89 (dt, $J = 11.2$, 4.1 Hz, 1H), 3.69 (dd, $J = 12.5$, 2.9 Hz, 1H), 2.85 (ddd, $J = 12.8$, 12.8, 7.9 Hz, 1H), 2.47–2.28 (m, 5H), 2.16–1.97 (m, 2H); $^{13}$C NMR (CDCl$_3$) 100 MHz: δ 174.2, 173.8, 140.5, 137.4, 136.4, 134.0, 130.9, 129.0, 129.0, 127.9, 126.2, 124.6, 122.3, 119.7, 110.4, 102.1, 60.6, 60.4, 51.7, 37.8, 34.2, 28.7, 25.9. TOF HRMS (DART): m/z calcd for C$_{25}$H$_{25}$NO$_4$ [M+H]+: 404.1862; found: 404.1832.
2.7.7.10 Compound 21j

This compound was prepared by using the general procedure for Suzuki reactions with compound 31j to give compound 21j as a white solid (38 mg, 73%). mp 124.8-125.8 °C; Rf 0.44 (30% EtOAc/Hex); \(^1\)H NMR (CDCl\(_3\)) 300 MHz: \(\delta\) 7.44 (d, \(J = 8.2\) Hz, 2H), 7.20 (d, \(J = 8.2\) Hz, 2H), 5.72–5.45 (m, 2H), 4.55 (ddd, \(J = 11.1, 8.3, 6.6\) Hz, 1H), 4.45 (ddd, \(J = 11.2, 8.3, 6.6\) Hz, 1H), 4.02 (dt, \(J = 11.2, 4.2\) Hz, 1H), 3.87 (dt, \(J = 11.2, 4.1\) Hz, 1H), 3.64 (dd, \(J = 12.4, 2.8\) Hz, 1H), 2.79 (ddd, \(J = 12.9, 12.9, 7.9\) Hz, 1H), 2.47–2.28 (m, 5H), 2.38 (s, 3H), 2.25 (s, 3H), 2.10–2.00 (m, 2H); \(^1\)C NMR (CDCl\(_3\)) 75 MHz: \(\delta\) 174.0, 173.7, 165.3, 158.8, 138.2, 131.2, 129.7, 129.4, 128.7, 128.2, 116.4, 60.7, 60.3, 51.7, 37.8, 34.2, 28.6, 25.9, 11.7, 11.0. TOF HRMS (DART): \(m/z\) calcd for C\(_{22}\)H\(_{25}\)NO\(_5\) [M+H]+: 384.1811; found: 384.1831.
2.7.7.11 Compound 21k

This compound was prepared by using the general procedure for Suzuki reactions with compound 31k to give compound 21k as a white solid (46 mg, 76%). mp 145.4-146.2 °C; Rf 0.36 (30% EtOAc/Hex); ¹H NMR (CDCl₃) 400 MHz: δ 7.80 (s, 1H), 7.59 (s, 1H), 7.47–7.16 (m, 9H), 5.61–5.49 (m, 2H), 5.33 (s, 2H), 4.58-4.51 (m, 1H), 4.47–4.40 (m, 1H), 4.04–4.00 (m, 1H), 3.86–3.81 (m, 1H), 3.59 (dd, J = 13.6, 2.8 Hz, 1H), 2.77 (ddd, J = 13.0, 13.0, 8.0 Hz, 1H), 2.45–2.26 (m, 5H), 2.11–1.94 (m, 2H); ¹³C NMR (CDCl₃) 75 MHz: δ 174.0, 173.7, 137.1, 137.1, 136.5, 131.9, 131.0, 129.0, 128.9, 128.2, 127.8, 126.3, 125.8, 123.2, 60.6, 60.3, 56.4, 51.6, 37.8, 34.2, 28.6, 25.9. TOF HRMS (DART): m/z calcd for C₂₇H₂₈NO₄ [M+H]+: 445.2127; found: 445.2128.
2.8 Appendix A: Cell proliferation assay\textsuperscript{14,15}

ASZ cells were plated onto 96-well tissue culture plates and allowed to attach and grow for 24 h. Test compounds dissolved in DMSO were added at varying concentrations to experimental cultures at 1% DMSO final concentration. Cultures were then incubated for another 72 h. Controls, treated only with DMSO, were incubated in parallel to the test wells. Then, an MTS/PMS (20:1) solution was added to each well and the mixture incubated for 3 hours at 37 °C. Following incubation, absorbance was measured by spectrophotometry at 490 nm using a Biotek Synergy H1 Hybrid Reader. Reported values are the mean of two separate runs (with errors shown), where each run was conducted in triplicate. Absorbance data was normalized using the DMSO control values after background subtraction of the media-only wells. The percentage cell viability values were analyzed in GraphPad Prism to provide a GI\textsubscript{50} value for cell viability through non-linear regression analysis. The GI\textsubscript{50} values are average ± standard error mean (SEM), calculated using GraphPad Prism. The graphs (Figure 2.8) are representative graphs of the data from two runs each performed in triplicate.
**Figure 2.8** GI$_{50}$ plots for C7-aryl [13]-macrolactones
2.9 Appendix B: Principle component analysis

The procedure for principal component analysis (PCA) used was the method used by Derek Tan and co-workers with minor modifications. Specifications on the process of defining Chemical Field Terms in Instant JChem as integers or decimal numbers can be found in Sec 2.9.1. This was a key step for obtaining any values that were not whole numbers and was especially true for values that were less than 1 such as Fsp, which would be returned as 0 if the specification was not made thus throwing off the entire calculation. In addition to this loading plots were generated using Excel. These plots were generated from PC1-PC3 coefficients of each parameter for each of the three PCA plots. Once the loading plots were made, the data labels were added. These plots were much clearer and easier to interpret than similar plots generated by R and allowed for facile determination of parameter importance.

2.9.1 Details on principal component analysis (PCA) computations

General Procedure Principal Component Analysis

The procedure that was used was the method used by Derek Tan and co-workers in their 2015 paper. The following notes are on changes that I made or issues that I encountered.

- **Integer vs decimal number in Instant JChem**
  If the “type” is integer the text will be black, if the “type” is decimal number the text will be blue.
  - Molecular weight (MW): mass() already done when importing the data
  - N atom count (N): atomCount("7")
  - O atom count (O): atomCount("8")
  - H-bond donor count (HBD): donorCount()
  - H-bond acceptor (HBA): acceptorCount()
  - Rotatable bond count (RotB): rotatableBondCount()
  - Stereocenter count (nStereo): chiralCenterCount()
  - Topological polar surface area (tPSA): PSA()
- Number of rings (Rings): `ringCount()`
- Aromatic ring count (RngAr): `aromaticRingCount()`
- Ring system count (RngSys): `ringSystemCount()`
- Size of largest ring (RngLg): `largestRingSize()`
- Fraction of sp\(^3\)-hybridized carbons (Fsp3): \(\frac{\text{count(filter('atno()==6 && connections()==4'))}}{\text{atomCount("6")}}\)
- \(n\)-Octanol/water partition coefficient at pH = 7.4 (LogD): `logd('7.4')`
- van der Waals surface area (VWSA): `vanDerWaalsSurfaceArea()`
- Relative polar surface area (relPSA): `PSA()/vanDerWaals SurfaceArea()`
Table 2.6 PCA parameter significance (For each PC the 3 most positive values are highlighted in green and the 3 most negative values are highlighted in red.)

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>-3.31E-01</td>
<td>-0.14623</td>
<td>0.056633</td>
</tr>
<tr>
<td>N</td>
<td>-1.87E-01</td>
<td>-0.11319</td>
<td>-0.38392</td>
</tr>
<tr>
<td>O</td>
<td>-3.33E-01</td>
<td>0.03623</td>
<td>0.104333</td>
</tr>
<tr>
<td>HBD</td>
<td>-3.08E-01</td>
<td>0.09795</td>
<td>-0.16235</td>
</tr>
<tr>
<td>HBA</td>
<td>-3.40E-01</td>
<td>0.008343</td>
<td>-0.05126</td>
</tr>
<tr>
<td>RotB</td>
<td>-2.51E-01</td>
<td>-0.11655</td>
<td>-0.06516</td>
</tr>
<tr>
<td>nStereo</td>
<td>-2.94E-01</td>
<td>0.071195</td>
<td>0.259479</td>
</tr>
<tr>
<td>tPSA</td>
<td>-3.39E-01</td>
<td>0.026628</td>
<td>-0.12329</td>
</tr>
<tr>
<td>Rings</td>
<td>-1.72E-01</td>
<td>-0.25477</td>
<td>0.036448</td>
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<tr>
<td>RingAr</td>
<td>-4.47E-05</td>
<td>-0.35217</td>
<td>-0.31076</td>
</tr>
<tr>
<td>RngSys</td>
<td>-1.12E-01</td>
<td>-0.31891</td>
<td>-0.12756</td>
</tr>
<tr>
<td>RngLg</td>
<td>-1.48E-01</td>
<td>-0.01306</td>
<td>0.232185</td>
</tr>
<tr>
<td>Fsp3</td>
<td>-1.20E-01</td>
<td>0.315609</td>
<td>0.307754</td>
</tr>
<tr>
<td>LogD</td>
<td>1.53E-01</td>
<td>-0.26597</td>
<td>0.350431</td>
</tr>
<tr>
<td>VWSA</td>
<td>-3.27E-01</td>
<td>-0.12806</td>
<td>0.123274</td>
</tr>
<tr>
<td>relPSA</td>
<td>-1.44E-01</td>
<td>0.263741</td>
<td>-0.31186</td>
</tr>
<tr>
<td>ALOGPs</td>
<td>5.55E-02</td>
<td>-0.39857</td>
<td>0.262294</td>
</tr>
<tr>
<td>ALOGpS</td>
<td>6.87E-02</td>
<td>0.414088</td>
<td>-0.15117</td>
</tr>
<tr>
<td>nStMW</td>
<td>-1.66E-01</td>
<td>0.243712</td>
<td>0.323667</td>
</tr>
<tr>
<td>RRSys</td>
<td>-7.85E-02</td>
<td>-0.01287</td>
<td>0.168213</td>
</tr>
</tbody>
</table>
Table 2.7 Computational and Experimental LogS values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>JChem LogS</th>
<th>VCCLabs ALOGpS</th>
<th>SwissADME LogS ESOL</th>
<th>SwissADME LogS Ali</th>
<th>SwissADME LogS SILICOS-IT</th>
<th>Experimental Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>-5.08</td>
<td>-5.91</td>
<td>-5.26</td>
<td>-5.54</td>
<td>-6.49</td>
<td>-5.43</td>
</tr>
<tr>
<td>21b</td>
<td>-6.53</td>
<td>-6.65</td>
<td>-6.18</td>
<td>-6.67</td>
<td>-7.61</td>
<td>-4.75</td>
</tr>
<tr>
<td>21c</td>
<td>-4.64</td>
<td>-5.07</td>
<td>-5.12</td>
<td>-5.59</td>
<td>-5.9</td>
<td>-4.87</td>
</tr>
<tr>
<td>21d</td>
<td>-4.84</td>
<td>-5.21</td>
<td>-5.51</td>
<td>-5.74</td>
<td>-6.56</td>
<td>-5.97</td>
</tr>
<tr>
<td>21e</td>
<td>-3.91</td>
<td>-4.79</td>
<td>-4.6</td>
<td>-4.7</td>
<td>-6.11</td>
<td>-4.24</td>
</tr>
<tr>
<td>21f</td>
<td>-3.91</td>
<td>-4.92</td>
<td>-4.6</td>
<td>-4.7</td>
<td>-6.11</td>
<td>-4.92</td>
</tr>
<tr>
<td>21g</td>
<td>-4.01</td>
<td>-3.92</td>
<td>-4.19</td>
<td>-4.3</td>
<td>-5.74</td>
<td>-4.13</td>
</tr>
<tr>
<td>21h</td>
<td>-4.78</td>
<td>-3.86</td>
<td>-4.55</td>
<td>-4.95</td>
<td>-5.95</td>
<td>-4.74</td>
</tr>
<tr>
<td>21i</td>
<td>-5.90</td>
<td>-6.06</td>
<td>-5.62</td>
<td>-6</td>
<td>-7.34</td>
<td>-4.15</td>
</tr>
<tr>
<td>21j</td>
<td>-3.68</td>
<td>-4.32</td>
<td>-4.86</td>
<td>-5.3</td>
<td>-6.09</td>
<td>-4.56</td>
</tr>
<tr>
<td>21k</td>
<td>-4.79</td>
<td>-5.85</td>
<td>-5.62</td>
<td>-5.81</td>
<td>-7.35</td>
<td>-5.38</td>
</tr>
</tbody>
</table>

Solubility in terms of LogS was calculated for all compounds using different programs in order to compare the computational values to the experimental results and determine if there was any correlation present. The plots shown below were made using the values from the Table shown above.
Figure 2.9 Computational and Experimental LogS Correlation Plots for compounds 21a-21k
Figure 2.10 LogS vs GI50 correlation plots for 21c-21f, 21h and 21j
2.10 Appendix C: Solubility assay

2.10.1 General procedure for solubility assay (by UV-Vis)

Approximately 2 mg each (The precise mass ±0.1 mg was noted.) of compounds 21a-21k were transferred into Eppendorf tubes. Then, 1 mL DI water was added to each tube to dissolve the compound. Each tube was rotated intermittently over 24 hr. Then, the tubes were spun on a benchtop microcentrifuge, and the supernatant was removed. A UV-Vis spectrum of the supernatant solution was taken and the absorbance at specified wavelengths were compared to standard curves for each compound. The standard curves were generated by making dilutions of each compound in methanol. The concentration of the solution was then calculated according to Beer’s Law and the corresponding extinction coefficient. The concentration of the solution based on this assay was then considered as the aqueous solubility of each compound. The solubilities of selected compounds 21c, 21e, and 21h were also evaluated at different pH values using phosphate buffer. See the Supporting Information for more details of the solubility assays.

2.10.1.1 Determination of extinction coefficients

First of all, all the compound (21a-21k) was weighed out for about 2 mg to prepare the stock solution. For every single one, 20 ml of methanol was used to dissolve each one. Then, 0.1, 0.25, 0.5, 0.75 and 1 ml was respectively extracted from 20 ml solution and diluted with methanol to make a 4 ml solution. Therefore, five different concentrations of solution for every compound had been prepared. The UV-Vis absorbance of all five had been achieved and the absorbance at 256 nm wavelength was used to make the standard curve. The slope of the standard curve would be the extinction coefficient at 256 nm for that specific compound. Based on the extinction coefficient we got and applied it
in beer’s law, the concentration of the solution was calculated and transferred to the solubility for per ml of water.
Standard Curve (21a-21c)

Figure 2.11 Standard dilution curves for compounds 21a-21c
Figure 2.12 Standard dilution curves for compounds 21d-21f
**Figure 2.13** Standard dilution curves for compounds 21g-21i
Figure 2.14 Standard dilution curves for compounds 21j-21k
**Table 2.8** Extinction coefficients for 21a-21k

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>22782</td>
</tr>
<tr>
<td>21b</td>
<td>3201</td>
</tr>
<tr>
<td>21c</td>
<td>18419</td>
</tr>
<tr>
<td>21d</td>
<td>24076</td>
</tr>
<tr>
<td>21e</td>
<td>12543</td>
</tr>
<tr>
<td>21f</td>
<td>28194</td>
</tr>
<tr>
<td>21g</td>
<td>23248</td>
</tr>
<tr>
<td>21h</td>
<td>11480</td>
</tr>
<tr>
<td>21i</td>
<td>6591</td>
</tr>
<tr>
<td>21j</td>
<td>5075</td>
</tr>
<tr>
<td>21k</td>
<td>17982</td>
</tr>
</tbody>
</table>

2.10.2 General procedure for solubility assay (by mass)

Weighed the empty Eppendorf first (M1). Each compound was weighed about 2 mg into Eppendorf tube. Took the mass of the total as well (M2). Then 1 ml DI water was added to each tube to dissolve the compound. Each tube was rotated intermittently for 24 hr. After 1 day, used pipette to extract all the solution from Eppendorf tube and left all the undissolved solid in the Eppendorf tube. Applied vacuum and dried the tubes in the desiccator overnight. Took the total weight of the tube and the solid in it again (M3). The mass of the solid which was dissolved in DI water can be obtained from M2-M3.
2.11 Appendix D: Esterase assay

2.11.1 General procedure

MeOH stock solution: Approximately 1.5 mg of compound was dissolved in 10 mL MeOH to prepare the MeOH stock solution. The MeOH stock solution was then diluted using MeOH in different proper concentrations for individual compound to measure UV-vis absorbance (S1). Another reference sample was prepared by adding 100 μL DCM and 1800 MeOH into the tube containing 100 μL MeOH stock solution (total 2 mL). Its absorbance was measured as well (S2).

Each esterase sample: 20 μL MeOH stock solution was transferred into Eppendorf tube. Then 9 μL esterase enzyme (4 units) and 171 μL PH 7 buffer was added to the tube (total 200 μL, Tube 1). Control was prepared by adding 9 μL DI water and 171 μL PH 7 buffer to the Eppendorf tube with 20 μL MeOH stock solution in it (total 200 μL, Tube 2). Both tubes were rotated for 20 min - 1h. Then, 200 μL DCM was added to each tube to do the tiny extraction. 100 μL solution from the bottom layer of Tube 1 was extracted to prepare a 2 mL solution in MeOH to measure UV-Vis absorbance (EB). Repeat this step for all the other layers from both tubes and get four UV-Vis for each compound. The UV of 100 μL solution from the top layer of Tube 1 was marked as TB. The UV of 100 μL solution from the top layer of Tube 2 was marked as WT. The UV of 100 μL solution from the bottom layer of Tube 2 was marked as WB. Six total UV was measured for each compound. Two were the original absorbance, while other four were the absorbance after the esterase assay.

At last, the solution left in the Eppendorf tube was used to do TLC to monitor the hydrolysis as well.
2.11.2 Demonstration of the general procedure

Stock solution (10 mL)
UV-Vis absorbance S1 & S2

Reaction:

Sample (Tube 1): or
9 µl esterase enzyme (4 units)
171 µL PH 7 buffer
20 µL stock solution in MeOH

Control (Tube 2):
9 µl DI water
171 µL PH 7 buffer
20 µL stock solution in MeOH

In Eppendorf tube
20 min – 1 hr agitation at r.t.

Isolation:
200 µL DCM extraction

Top layer (ET & WT)
1. Dilute 100 µL to 2 mL in DI dilute to measure UV absorbance

Bottom layer (EB & WB)
1. Dilute 100 µL to 2 mL in MeOH to measure UV absorbance
   2. TLC

ET: Esterase tube aqueous layer
WT: DI tube aqueous layer
EB: Esterase tube DCM layer
WB: DI tube DCM layer
S1: MeOH dilution of stock solution
S2: MeOH & DCM dilution of stock solution

\(^a\) PH 7 buffer (1X PBS: 135 mM [NaCl]; 2.7 mM [KCl]; 4.3 mM [Na\(_2\)HPO\(_4\)]; 1.4 mM [KH\(_2\)PO\(_4\)])

\(^b\) Concentration varied for each tested compound
2.11.3 UV-Vis absorbance of 21a, 21g, 21i

Table 2.9 UV-Vis absorbance of 21a

<table>
<thead>
<tr>
<th>20 min agitation</th>
<th>conc. (μM)</th>
<th>abs. (256 nm)</th>
<th>abs. (230 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>46.97</td>
<td>1.0106</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>16.44</td>
<td>0.3688</td>
<td></td>
</tr>
<tr>
<td>EB</td>
<td>1.64 (if no activity)</td>
<td>0.01937</td>
<td>0.04378</td>
</tr>
<tr>
<td>WB</td>
<td>1.64 (if no activity)</td>
<td>0.03043</td>
<td>0.05625</td>
</tr>
</tbody>
</table>

![Chemical structure of 21a with 30% EtOAc in Hex]

![Graphs for S1 and S2](chart.png)
Figure 2.15 Absorbance and TLC of 21a
Table 2.10 UV-Vis absorbance of 21g

<table>
<thead>
<tr>
<th>1 h agitation</th>
<th>conc. (μM)</th>
<th>abs. (256 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>35.42</td>
<td>0.8053</td>
</tr>
<tr>
<td>S2</td>
<td>70.85</td>
<td>1.5053</td>
</tr>
<tr>
<td>EB</td>
<td>7.08 (if no activity)</td>
<td>0.1336</td>
</tr>
<tr>
<td>WB</td>
<td>7.08 (if no activity)</td>
<td>0.1393</td>
</tr>
</tbody>
</table>

![Diagram of 21g with 25% EtOAc in Hex]

![Graphs of S1 and S2 absorbance spectra]
Figure 2.16 Absorbance and TLC of 21g
Table 2.11 UV-Vis absorbance of 21i

<table>
<thead>
<tr>
<th>30 min agitation</th>
<th>conc. (μM)</th>
<th>abs. (300 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>27.23</td>
<td>0.3236</td>
</tr>
<tr>
<td>S2</td>
<td>20.42</td>
<td>0.2257</td>
</tr>
<tr>
<td>EB</td>
<td>2.04 (if no activity)</td>
<td>0.05825</td>
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<tr>
<td>WB</td>
<td>2.04 (if no activity)</td>
<td>0.06465</td>
</tr>
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</table>

![Diagram](image-url)
**Figure 2.17** Absorbance and TLC of 21i
2.12 References


(8) Rutledge, K. M.; Hamlin, T. A.; Baldisseri, D. M.; Bickelhaupt, F. M.; Peczuh, M. W. Macrocycles All Aflutter: Substitution at an Allylic Center Reveals the


(30) Instant JChem was used for structure database management, search and prediction, Instant JChem 18.8.0, 2018, ChemAxon (http://www.chemaxon.com)
Chapter 3

Synthesis of a C7-amino [13]-macrolactone and its derivatization as amides and ureas
3.1 Peptide natural products

Proteins and peptides are both fundamental to the biological functions of cells.\textsuperscript{1} Peptides are normally compromised of 2-50 amino acids connected by amide bonds and are further classified into oligopeptides and polypeptides. Oligopeptide is peptide that contains less than twenty amino acids, while polypeptide contains up to fifty amino acids. Though proteins and peptides both contain the same building blocks, proteins have more than 50 amino acids and a more complex structure to present dynamic conformations as secondary, tertiary, and quaternary structures.

Peptides are the basis of all organisms, playing a role as hormones, growth factors, antibacterial agents, and neurotransmitters in all biology systems.\textsuperscript{2,3} The versatility of peptides arouses various research interest of chemists and biologists.\textsuperscript{4} Peptide natural products can be categorized into two different types: One group is the ribosomally synthesized and post-translationally modified peptides (RiPPs), and the other is nonribosomal peptides (NRPs).\textsuperscript{5} RiPPs can be found in prokaryotes, eukaryotes, and archaea. For most RiPPs, a leader peptide is typically attached to the N-terminus of the core peptide. Eukaryotic peptides are usually RiPPs and have the same feature for identification by many of the post-translational modification enzyme.\textsuperscript{6} However, except fungi, there is no NRPs existed in eukarya, neither in archaea.\textsuperscript{7,8} NRPs are found prokaryotes like bacteria and normally synthesized by multifunctional, modular enzyme complexes. Peptides are attracting more attention to explore their application in pharmaceutical area due to these biological related features.
3.1.1 Peptide Hormones: A case-study in pharmaceutical relevance and preparation methods

Research focused on peptide-based drugs continuously draws interest because of the chemical space that peptides occupy between small molecules and biologics. The chemical space of peptides mostly overlaps with the macrocyclic natural products and the US FDA approved drugs, which is benefit from the dynamic amino acids that constitute the peptides. Accordingly, peptides have good chance to be equipped with similar bioactive properties as drugs. The versatility of peptides is reflected by the multiple roles it played overall. They can serve as peptide hormones, antimicrobial peptides (AMP), antibacterial peptides, and growth factors. They can also help with cell adhesion, cell-penetrating, tumor-homing and cancer-killing. Their diversity in structures and biological abilities qualify peptides as candidates for pharmaceutical applications.

Table 3.1 Sources of some early peptides

<table>
<thead>
<tr>
<th>peptide</th>
<th>source</th>
<th>introduction to the clinic</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Isolated from canine and bovine pancreata</td>
<td>1920s</td>
<td>Native</td>
</tr>
<tr>
<td>Adrenocorticotropic hormone (ACTH)</td>
<td>Isolated from bovine and porcine pituitary glands</td>
<td>1950s</td>
<td>Native</td>
</tr>
<tr>
<td>Oxytocin &amp; Vasopressin</td>
<td>Synthetic</td>
<td>1962</td>
<td>Native</td>
</tr>
<tr>
<td>Leuprorelin</td>
<td>Synthetic analog</td>
<td>1984</td>
<td>Cyclic analog of somatostatin</td>
</tr>
<tr>
<td>Octreotide</td>
<td>Synthetic analog</td>
<td>1988</td>
<td>Nonapeptide analog of gonadorelin</td>
</tr>
</tbody>
</table>

The development of peptides for pharmaceutical studies can be tracked back to 1920s (Table 3.1), when peptides such as Insulin and Adrenocorticotropic hormone (ACTH) were first isolated from natural sources. Insulin is a peptide hormone that was...
isolated from canine and bovine pancreata for diabetics treatment. ACTH was separated from bovine and porcine pituitary glands for treatment of endocrine disorders. It is worth pointing out that the discovery the 51 amino acid (aa) hormone Insulin was an outstanding achievement in 1920s. Because it was the first commercially available peptide drug, which was issued in 1923. Moreover, it just took one year to apply on patients after its initial isolation. This representative peptide drug inspired the following research about structure and inherent properties of peptides.²

In the early years of peptide isolation, it was challenging to obtain a pure isolated product form whole animal tissue. These naturally isolated peptides provided drugs before the 1950s until the synthetic approaches started to be more wide spread. Chemical synthesis of peptides started to flourish in the 1960s, Oxytocin and Vasopressin entered clinical use at that time.¹³ They both play critical roles in lactation, labor, and water balance.¹⁴ Oxytocin was the first synthesized peptide hormone, since the synthesis of long peptide had a lot of difficulties with multi-step procedures and purification.¹⁵ This led to a new and extraordinary strategy, solid-phase peptide synthesis (SPPS) in the same period. It was first demonstrated on the synthesis of a model tetrapeptide, L-leucyl-L-alanyl-glycyl-L-valine, and then was widely applied for building peptides.¹⁶,¹⁷ The invention of SPPS optimized synthetic approach of peptide to overcome difficulties such as efficiently achievement of long and peptide complex. SPPS also enabled peptides to easily switch amino acids at any position, so that the synthesis of analogs was relatively simple.

In 1980s, the synthesis of natural peptide analogs such as Octreotide (Somatostatin analog) and Leuprolelin (Gonadorelin analog) has evolved.¹³ Octreotide is
is used to treat acromegaly and pituitary tumors. The synthesis of peptide analogs can take advantage of biological ability of the natural hormone, accompanied with improved selectivity and pharmacokinetic.  

In recent years, both industry and academia has concentrated on pursuing novel peptides as potential therapeutics. Annual global sales of peptide drugs in 2013 was ca. $28 billion and it continued to increase with an annual growth rate of 9-10%. The progressive technologies for peptide search, design, and synthesis, along with the growing demand for metabolic disease and cancer medicines may result in a higher growth rate of peptide drugs sale. The US FDA has approved over 60 peptide drugs by 2018, and more than 150 candidates are now in different stages of clinical trials.

3.1.2 Macrocyclic NRSRs as ligands in biology: features and challenges

Among the 70 macrocyclic drugs now in clinical use, more than half of them are peptides, which demonstrates the medicinal potential of macrocyclic peptides. Macrocyclic nonribosomally synthesized peptides (NRSRs) are a class of structurally novel cyclic peptides with interesting biological activity such as thermally stable and orally active. They also can show both the therapeutic potency of protein drugs and the synthetic access of small-molecule drugs. There is a large library for the building block selections as well as multiple synthetic strategies for these kinds of peptides. Besides, modern and advanced technique have provided a method for switching any piece of amino acids or peptide bonds to make diverse macrocyclic NPSRs with high efficiency.

NRSPs usually don’t satisfy Lipinski’s “rule of five” or Veber’s rules, which are used to evaluate if a molecule is drug-like and orally active. Lipinski’s “rule of five” indicates a drug-like molecule should have no more than five H-bond donors, no more
than ten H-bond acceptors, the molecular weight of it should be less than 500 and log P should not exceed 5. Veber’s rules states that an orally active molecule should have 10 or fewer rotatable bonds and its polar surface area should be less than 140 Å². However, the peptidic macrocycles can corporate with D- and N-methylated amino acids, and other specific amino acids to display their potency. This method will allow peptides to overcome the inherent biological weaknesses. For example, the cyclic structure and N-methylated amino acids in Cyclosporin A (CSA) together offer this NRSP some small molecule-like biological abilities including better permeability, gut stability and oral bioavailability.22 These unique properties of CSA allow it to be a model for studying. It was found that CSA is relatively flexible to present six different conformations in polar solvent. Experiments also demonstrated that CSA possessed a compact structure along with four intramolecular hydrogen bonds in nonpolar tetrachloromethane. This conformation may minimize the energy for the phase transition, which allow CSA to dissolve in a hydrophobic phase. Furthermore, CSA holds seven N-methylated amino acids in its structure, which is a key for the membrane permeability and decreasing the hydrogen bonding donor (HBD) character of CSA. This investigation inspired the modification of NRSPs to remove amide unit for better bioavailability.23

Improving selectivity and pharmacological ability could be a challenge to small molecules and antibodies. Macrocyclization with side chain functionalization and coupling is a valuable method for NRSPs to increase the potency over those molecules.24–26 The products from the reaction can also avoid common limitations of metabolic instability of peptides. Similar to synthetic macrocyclic rings, different kinds of macrocyclizations as described in Chapter 1 can be employed to obtain this peptide class with various
functional groups. Biological tools such as mRNA display, which is employed to discover a binding peptide for a desired target, can be utilized to screen multiple candidates varying in size, and quickly build a class of analogs with desired functions. NRSPs are safe to assemble with unnatural amino acids or non-peptidic pieces for the sake of enhanced potency and physicochemical properties. The various half-life extension tools developed for protein and peptide drugs are easily to apply on NRSPs to tailor the pharmacokinetic behavior as defined by the target product profile. These distinguishing construction features of NRSPs make them to be useful models in both lead discovery and lead optimization phases over natural product.

Thermodynamics consideration of these NRSPs is another topic in the research. Several factors are in charge of thermodynamics including the chemical nature of the Lipids, peptides, and carbohydrates involved, as well as the mechanism of the procedure. Electrostatic forces, hydrogen bond formation, and hydrophobic interactions all contribute to this problem. In general, natural peptides degrade rapidly since peptide bonds are subject to enzymatic hydrolysis by proteases and peptidases present in the intestine. A linear peptide is flexible allowing it to have a variety of inactive conformations. Therefore, the entropy affects the free Gibbs energy (ΔG) of binding and the dissociation constant (Kd) of the drug, which leads to low binding affinity. Cyclic peptides can avoid this issue by the preorganization of cyclic scaffold and amino acid side chains. It will result in the decreasing of polar surface area and tendency to intramolecular H-bond networks formation. Hence, macrocyclic peptides’ potency to serve as a drug candidate is maximized due to these features.
The bioavailability of NRSPs including orally active, permeability and stability, which can be improved by the inherent characters or modification of this peptide class have been discussed.\textsuperscript{29} It has high possibilities to hit these purposes according to the previous research. However, there is still shortcomings for this peptide type: an efficient way to resolve this problem is fast renal clearance. Even though several solutions have been proposed for this issue, its serum retention is still recognized as a big challenge. A little improvement of this problem will end in decreasing bioavailability, solubility and immunogenicity.\textsuperscript{23}

3.2 Teixobactin

The discovery of Teixobactin, a representative NRSP, was reported in 2015.\textsuperscript{30} Its novel method of isolation as well as its target and mechanism were all demonstrated in the initial report. Teixobactin (Figure 3.1) is an 11-mer peptidic natural product.\textsuperscript{30} It contains five unnatural amino acid residues, N-Me-\textit{D}-Phe\textsubscript{1}, \textit{D}-Gln\textsubscript{4}, \textit{D}-\textit{allo}-Ile\textsubscript{5}, \textit{D}-Thr\textsubscript{8}, as well as the rare \textit{L}-\textit{allo}-End\textsubscript{10}. The most notable amino acid, \textit{L}-\textit{allo}-End\textsubscript{10} contains a unique five-membered cyclic guanidine moiety and is hardly found in nature. Free enduracididine was only isolated from the seeds of the legume \textit{Lonchocarpus sericeus} with antibiotic properties.\textsuperscript{31,32} It can inhibit seedling germination of lettuce but it does not have effect on the inhibition of protein production in rat hepatoma cells. Overall, the structure of Teixobactin contains two main sections, which are its \textit{N}-terminal tail and the 13-membered depsipeptide macrocycle.\textsuperscript{33}
Despite great advances in the development of design principles and synthetic methods to invent new peptides, they can not replace Nature in terms of antibiotic development. It is critical to discover more natural products as promising peptide drugs and drug leads. The story of the discovery Teixobactin illustrates innovations in the isolation of natural products from bacteria. The producing $\beta$-proteobacteria, *Eleftheria terrae*, was cultivated in its natural environment to grow without the need to culture it in a lab. The iChip$^{34}$, a multichannel device, was used to isolate Teixobactin from soil. This multichannel device has semi-permeable membranes and two supporting side panels to separate the central plate from the environment. The iChip was used to dip into the soil to culture and capture Teixobactin through the small holes of the plate.$^{30}$ The nutrients and environment through the device makes unculturable organisms culturable in nature.

Teixobactin is found to be stable, has good potency in serum, and low toxicity. It is produced by a Gram-negative bacterium and has good activity against Gram-positive organisms. It is an excellent inhibitor of peptidoglycan biosynthesis, but it is unable to bind DNA, RNA or proteins. Rather, Teixobactin was considered to have the same action mechanism as vancomycin, which binds to Lipid II to play its inhibition role as a first member from a new class of antibiotics. The mechanism of its action will be further discussed in detail in the following section 3.2.2.$^{30}$

![Figure 3.1 Chemical structure of Teixobactin](image)
3.2.1 Analogs and structural considerations of Teixobactin

Peptide drugs are classified as native, heterologous, or (semi)-synthetic analogs, based on their relationship to the endogenous peptide.\textsuperscript{13} Although the first native peptides were obtained from the natural source, the majority of them on the market are now chemically synthesized or obtained through recombinant expression in another (heterologous) organism. The weaknesses of endogenous peptides including low metabolic stability, poor oral activity, and low membrane permeability. These shortcomings motivated scientists to make more analogs using strategies such as L to D amino acid replacements, cyclization, and incorporation of unnatural amino acid with improved pharmaceutical properties.\textsuperscript{35} In fact, most of the peptide drugs currently under study are analogs derived from native peptides through these 40 years.\textsuperscript{13} Those derived analogs can help identify mechanism of action of the original compound, and make more potent drugs. Therefore, a search for analogs of Teixobactin promises to provide more information and maximize the activity of Teixobactin.\textsuperscript{36} Teixobactin’s analogs can be prepared through standard solid phase peptide synthesis (SPPS). This approach offers a most effective way that can easily switch amino acids at any position to produce the Teixobactin’s analogs with simple purification as well as maintain their structural features.

![Teixobactin](image)

**Figure 3.2** Highlights of some modification sites of Teixobactin analogs
Therefore, various studies of Teixobactin analogs have been carried out to study its properties. Here show some major sites that have been modified for Teixobactin analogs (Figure 3.2). One was focused on analogs of the N-terminal N-Me-D-Phe₁ of Teixobactin. It turned out that changing this group to the N-acetyl derivative resulted in a total loss of antibacterial activity. Also, switching to N-Me-D-Tyr, where a hydroxyl group was consequently added to the aromatic ring led to more than 100 times reduction of antibacterial activity. Simple removal of the methyl group on the N-terminal nitrogen kept the similar activity as Teixobactin.³⁷

Another investigation directed at the replacement of rare L-allo-End₁₀ with other amino acids looked at effects on antibacterial potency. Lys₁₀ and Ala₁₀ analogs were studied to compare with the native L-allo-End₁₀ peptide. It was found that Lys₁₀ analog possessed similar MIC against S. salivarius yet poor MIC against Enterococcus. For Ala₁₀, moderate antimicrobial activity was observed against these targets. By substituting L- allo-End₁₀ with non-charged, non-polar or β-branched side chains were all tolerated well to perform antimicrobial activities.

The exploration into the configuration of amino acids demonstrated that the D-amino acids are critical for the activity. One report on switching the configuration of N-Me-D-Phe₁, D-Gln₄, D-allo-Ile₅ to corresponding L-amino acids.³⁸ A 64 times decrease of antibacterial activity against S. aureus occurred was observed ultimately. Another work also demonstrated the importance of D-Thr₈ for the antibacterial activity.³⁹ An exchanging by a L-Thr₈ led to a dramatically drop of antibiotic activity. The value of MIC was more than 32 μg/mL against the selected Gram-positive bacteria.
Besides moderating the amino acids on Teixobactin, some concentrated on the ring size were designed to host the Lipid II binding site better. However, MIC against \textit{S. aureus} implied a moderate to poor outcome for the alternation. It was concluded from these studies that 13-membered rings were a justified choice to bind pyrophosphate group of Lipid II and related cell wall precursors.\textsuperscript{40}

Several research groups\textsuperscript{41,42} have done structure-activity relationship study to monitor the tolerance by modifying each position with multiple substituents to get different analogs and evaluate their antimicrobial activities. Figure 3.3 demonstrates the results of each position in different colors. Combined with the findings above, the results exhibited that antimicrobial activity maintained when did different substitutions on position Ser\textsubscript{3}, D-Gln\textsubscript{4}, Ala\textsubscript{9}, \textit{L-allo}-End\textsubscript{10} and Ile\textsubscript{11} since these positions have moderate to high tolerance of modifications.\textsuperscript{42} Different analogs worked on the tail of Teixobactin revealed that most part of it was either sensitive to the substitution or had some specific requirement to maintain the bioactivity. Any methylation of position 1-7 on backbone would kill the antibiotics activity of this molecule. Hydroxyl group of Ser\textsubscript{7} is a key for hydrogen bonding, which can not be replaced. As the ring piece of Teixobactin, it was claimed that the 13-membered ring was the best size to balance the activity and binding. A lactam ring may be preferred over lactone ring for better biological potency in Teixobactin.
3.2.2 Teixobactin’s interaction with Lipid II

Teixobactin can inhibit peptidoglycan biosynthesis, which is important for maintaining the structural integrity of bacterial cells, and consequently cell survival. This is an area of bacterial biosynthesis that is successfully targeted by several clinically approved antibiotics. Teixobactin is the first member of a new antibiotic class whose uncultured producing method offers a promising source of new antibiotics different from many known antibiotics. Teixobactin plays a role by binding to the peptidoglycan precursor, Lipid II, instead of interfering with the activity of enzymes. Lipid II is embedded in the cell membrane and recognized as one of the most essential intermediates for the biosynthesis of peptidoglycan component of bacterial cell walls. Therefore, binding to Lipid II will finally lead to the death of the cell.

Teixobactin is produced by Gram-negative bacterium, *E. terrae*, and permeate the outer membrane. It then localizes on the outer leaflet (outside) of the Gram-positive bacterial cell membrane. It can access it there because without an outer membrane, Lipid II is accessible to bind on Gram-positive bacteria. Lipid II and Teixobactin form a stoichiometric complex to prevent peptidoglycan and cell wall teichoic acid (WTA)
synthesis and precursor recycling. Teixobactin binds to various targets besides Lipid II; it is also able to bind Lipid III, which is the precursor of WTA. Blocking the WTA producing may play a role in autolysins liberation, which accelerate the lytic and killing activity of Teixobactin.

The Nowick group utilized the hydrochloride salt of one Teixobactin’s analog, Ac-$\Delta_1$-Arg$_{10}$-Teixobactin, and its X-ray crystal structure to study its binding mode. Figure 3.4 shows the binding of Teixobactin with pyrophosphate group of Lipid II from different directions. They observed that Arg$_{10}$ created a binding cavity with Ser$_7$, Thr$_8$ and Ile$_{11}$, which are important in the antimicrobial activity of Teixobactin. They indicated that hydrogen bond between Ser$_7$ and Ala$_9$ is also critical to the activity. This research implied that the antimicrobial activity has the possibility to increase by strengthening the interaction between phosphate group and Teixobactin’s analogs. Molecular dynamics study for the binding modes confirmed that the pyrophosphate-MurNAc moiety of Lipid II is the minimal motif to guarantee a stable binding with Teixobactin. It also ensured the important role of the 13-membered macrocycle for the antimicrobial activity.

**Figure 3.4** X-ray crystallographic structure of Ac-$\Delta_1$-Arg$_{10}$-Teixobactin as the hydrochloride salt. (A) Top view. (B) Side view. (C) Rotated side view (Reproduced from Ref. 44 with permission from The Royal Society of Chemistry)

The example of Teixobactin raises a good chance to minimize resistance development meanwhile keep antimicrobial activity. Due to limited resources, soil
microorganism cultivates most antibiotics these days and this cannot be replaced by synthetic routes. The case to isolate Teixobactin provides an innovative way to discover new antibiotics in uncultured bacteria. Additionally, through the structure-activity relationship study of Teixobactin, it explains the important factors of its structure that is essential for the bioactivity. All these insights from Teixobactin allow further research for designed molecules with improved biological potency.

3.3 C7-amino substituted [13]-macrodilactones

The discovery and studies of Teixobactin inspired the design of derivatives based on our [13]-macrodilactone architecture since they both possess a 13-membered ring (Figure 3.5). One reason accounted for the idea is the ring with the amino acid side chains of Teixobactin can create a cavity for anion binding to play a role in its biological application.44 We were encouraged that the ring size was the same as well as the common ester unit on the ring backbone. Moreover, the C10 position of Teixobactin, by virtue of the fact it is an amino acid, is α to the carbonyl group (Figure 3.5), and is well tolerated for substitution of several amino acids according to previous research.36 Therefore, a new class of C7-amo [13]-macrodi lactones was proposed based on all these promising structure features. The core [13]-macrodilactones will be added various amino acids on the key atom, C7 in this project, to mimic the peptide on the [13]-membered ring of Teixobactin. We believed that this new class of macrocycles would have good chance to show similar antibacterial activity contributing by the binding pattern of the ring and the attached peptide bond.
3.3.1 Retrosynthesis

Our goal was to prepare a [13]-macrodilactone containing an amine handle for functionalization via peptide coupling reactions. C7-aryl [13]-macrodilactones has already established a methodology to introduce a bromophenyl group from very beginning and build biaryl system on C7 at the late stage Suzuki coupling. Therefore, a similar strategy to make new C7-amino [13]-macrodilactones was developed based on this previous approach. The amine group was introduced from the commercially available protected glycine onto the backbone of the macrocycle through several reactions.

Retrosynthetically, the target was designed to have the general structure of 36, depicted in Scheme 3.1. It was envisioned that it could be obtained through the peptide coupling of the corresponding amine 37 and variety of amino acids.46 The amine could be revealed by the deprotection of Boc-protected macrocycle 38, which is accessed from diene 39 processing a ring closure metathesis.47 Acylation of the mono-acylate propane diol 25 that our group has applied on numerous occasions, using acid 40 would provide diene 39.48 Similar with the C7-aryl class, acid 40 is prepared by alkylation of commercially available Boc-protected glycine ester and ally bromide and followed hydrolysis.49–51
Scheme 3.1 Retrosynthesis of C7-amino [13]-macrodimicinones

3.3.2 Synthesis of C7-Boc protected [13]-macrolactone

The synthetic route for C7-Boc protected [13]-macrolactone was derived from our previously established C7-aryl [13]-macrolactone scheme (Scheme 3.2). The procedure was started from alkylating the alpha position of the ester group in starting material N-(tert-Butoxycarbonyl)glycine methyl ester (41) applying allyl bromide with LDA (75%). Then the hydrolysis was performed on Boc-protected methyl ester 42 to give substituted pentenoic acid 40 in 90% yield. The key acylating agent 40 was esterified using mono-alcohol 25 with the aid of DCC and DMAP to give precursor diene 39 for RCM in 89% yield. The final RCM was carried out to afford this new macrocycle, C7-Boc protected [13]-macrolactone (38) in 70% yield.
Once the macrocycle 38 has been prepared, its crystal was grown for the X-ray crystal structure analysis (Figure 3.6). The obtained shape will give some information about the impact of the Boc-substituent on key atom (C7) to the macrocycle. A pair of enantiomers was obtained, and the Boc-protected group occupied equatorial position like the phenyl group on C7-substituted [13]-macrodilactone. The planar chirality of the C7-Boc amino [13]-macrodilactone is corresponding to the point chirality of C7 (7S to pS, 7R to pR). Furthermore, the shape of this set is also close to ribbon like our previous characterized macrocycles.

Figure 3.6 Crystal structure of C7-Boc protected [13]-macrodilactone 38
3.3.3 Deprotection

Having this important intermediate macrocycle 38 prepared, the deprotection was the next step to work for the final target. In order to look for the best condition for the deprotection product, several conditions were screened with a small-scale reaction (Table 3.2). The deprotection was tried starting from 0.14 mmol Boc-protected macrodilactone 38. Protected macrodilactone 38 was dissolved in TFA and DCM solution of 1:1 or 1:2 ratio. The conditions were also involved within or without H₂O. Different work-up procedures were also carried out for condition screen.

Firstly, 1:1 TFA : DCM with DI water was tried. The crude was diluted with EtOAc, washed with brine. The organic layer was dried over sodium sulfate and concentrated \textit{in vacuo} to provide the product. However, the $^1$HNMR spectrum still showed the existence of Boc group from the crude product.

After the failure of the first condition, the same condition was tried again since it worked well for some of our compound. And the work-up details were changed this time, the reaction mixture was neutralized with NaHCO₃ before the extraction. The following step was kept the same and there was no Boc group peak on the $^1$HNMR spectrum for this trial.

In order to evaluate if simplify the work-up would influence the product formation, the same condition was carried out a third time followed by a modified work-up for Boc-deprotection of [13]-macrodilactone. The reaction mixture was concentrated under high-vac directly for next step since the reaction was in a tiny-scale. Later on, the applied equivalent of TFA and DCM was adjusted as well as run the reaction without water to confirm the applicability of reducing the H₂O and/or acid ratio. Both were worked well.
Table 3.2 Conditions of deprotection for C7-Boc protected [13]-macrolactone 38

<table>
<thead>
<tr>
<th>entry</th>
<th>conditions</th>
<th>work-up</th>
<th>Boc group on NMR?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95% 1:1 TFA:DCM 5% H₂O</td>
<td>Extract with EtOAc Concentrate</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>95% 1:1 TFA:DCM 5% H₂O</td>
<td>Neutralize with NaHCO₃ Extract with EtOAc Concentrate</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>95% 1:1 TFA:DCM 5% H₂O</td>
<td>Concentrate</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>1:1 TFA:DCM</td>
<td>Concentrate</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>1:2 TFA:DCM</td>
<td>Concentrate</td>
<td>no</td>
</tr>
</tbody>
</table>

The solvent and excess acid should be fine to get rid of under high pressure and leave the product behind, which can be applied to the next coupling reaction as a one-pot reaction without further purification steps. Because the TFA salt will be obtained in the vial after the evaporation, which can be coupled with activated amino acid. Also, by applying base in the condition, the TFA salt will transfer to free amine for the acylation. This procedure was turned out to be a feasible method for the deprotected compound by observing the ¹H NMR of the crude product. Compound given via entry 3 showed the clearest ¹H NMR with appropriate splitting peaks. Additionally, this condition was connected to an easy work-up. As a result, it was determined that the deprotection step would follow condition 3.
3.3.4 Synthesis of C7-amino [13]-macrodilactones

Having established access to compound 37, the next challenge was its derivatization via peptide coupling. Peptide couplings would be carried out to acquire C7-amino [13]-macrodilactones from the pendant amine. Multiple coupling conditions were tried based on changing the parameters such as reagent, solvent, temperature and reaction time. Six different conditions \(^{46,47,52,53}\) were applied using Boc-L-phenylalanine (43) as the coupled amino acid to figure out the effective way for this step (Table 3.3). The acid was consistently pre-activated with the condensation reagent (DCC, EDC or HBTU) before addition of the amine in each condition.

Our original acylation condition with DCC, DMAP was employed at first. But there was no product given after the reaction. Another trial without DMAP under room temperature was another attempt, which was also unsuccessful. It turned out that using DCC as the reagent can not get the desired product even after purification. Then, DCC was replaced with EDC and DMAP to give a moderate yield. The yield decreased when utilizing the base like Et\(_3\)N as well as shorten the reaction time. HOBt was used to accelerate the reaction by activate the intermediate ester. The yield did increase under the overnight condition. As a result, both with DCC or EDC did not give product with high yield in the reaction.

Next, new coupling reagent, HBTU, was added with base DIEA to activate the protected amino acid in DCM for the reaction. This condition gave the highest yield with short reaction time under room temperature. Therefore, the last condition was determined to be the most efficient way among all those screened methods with good yield and good quality.
Table 3.3 Different conditions of peptide coupling using Boc-L-phenylalanine\textsuperscript{46,47,52,53}

<table>
<thead>
<tr>
<th>entry</th>
<th>condition</th>
<th>equiv.</th>
<th>solvent</th>
<th>T.</th>
<th>time</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCC DMAP</td>
<td>1.1</td>
<td>DCM</td>
<td>0°C</td>
<td>overnight</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DCC</td>
<td>1.5</td>
<td>DCM</td>
<td>r.t.</td>
<td>overnight</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
<td>EDC DMAP</td>
<td>2</td>
<td>DMF</td>
<td>r.t.</td>
<td>overnight</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>EDC Et\textsubscript{3}N</td>
<td>1.5</td>
<td>DCM</td>
<td>r.t.</td>
<td>1.5h</td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>EDC Et\textsubscript{3}N HOBt</td>
<td>1.1</td>
<td>CHCl\textsubscript{3}</td>
<td>0°C</td>
<td>overnight</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>HBTU DIEA</td>
<td>1</td>
<td>DCM</td>
<td>r.t.</td>
<td>3.5h</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Following the optimized condition, a set of C7-amino [13]-macrodilactones were prepared by HBTU and DIEA in reasonable to good yields by different amino acids (Table 3.4). Some were equipped with aromatic ring (Phe, Trp), while some were equipped with aliphatic portion (Pro). Some had acidic function (Glu), while some had basic function (Arg, His). These coupled amino acids contained different protecting groups to avoid any possible reaction the free amine of amino acid itself could take place. The C7 key atom were attached various amino acid with an amide bond to connect in between. Introducing diversified amino acid on C7 of our 13-membered macrocycle is also an approach to mimic the research of Teixobactin analogs. Those research was making the analogs by
substituting the rare allo-End₁₀ on 13-membered ring of Teixobactin with other amino acid for biological study (Figure 3.5).⁴³
Table 3.4 Peptide coupling of [13]-macrolactone 37 with amino acids or carboxylic acid\textsuperscript{46}

<table>
<thead>
<tr>
<th>entry</th>
<th>product</th>
<th>amino acid/carboxylic acid</th>
<th>R</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44a</td>
<td>Boc-Phe-OH</td>
<td></td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>44b</td>
<td>Boc-Pro-OH</td>
<td></td>
<td>25%</td>
</tr>
<tr>
<td>3</td>
<td>44c</td>
<td>Boc-Trp-OH</td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>4</td>
<td>44d</td>
<td>Boc-Arg(Tos)-OH</td>
<td></td>
<td>57%</td>
</tr>
<tr>
<td>5</td>
<td>44e</td>
<td>Boc-L-Orn(Boc)-OH</td>
<td></td>
<td>79%</td>
</tr>
<tr>
<td>6</td>
<td>44f</td>
<td>Boc-Glu(OtBu)-OH</td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>7</td>
<td>44g</td>
<td>Boc-His-OH</td>
<td></td>
<td>36%</td>
</tr>
<tr>
<td>8</td>
<td>44h</td>
<td>Z-(\beta)-Ala-OH</td>
<td></td>
<td>40%</td>
</tr>
<tr>
<td>9</td>
<td>44i</td>
<td>4-Biphenylcarboxylic acid</td>
<td></td>
<td>28%</td>
</tr>
</tbody>
</table>
The $^{13}$C NMR spectra of compounds 44a demonstrated doubling of some carbon peaks from the compound (Figure 3.7). The carbons in the aromatic range as well as the carbons that attached to the oxygen atom on the ring were doubled. Compared with other products, those carbons were always doubled. It was observed that those carbon were either from the functional group of amino acids or key atoms that on alkene and on the edge of the ester unit. It was assumed that the splitting was induced by the two diastereomers of the product, which was generated by the stereocenter of the [13]-macrodilactone and original protected amino acid. The stereocenter on the [13]-macrodilactone was introduced by the racemic starting material protected glycine. So, the amine going into the acylation is racemic. The amino acid that adding onto the ring, though, is homochiral since it was prepared from naturally occurring chiral L-amino acids. As a result, linking them together made two diastereomeric products. The spots of two products were almost overlap on TLC plate for some compounds, which made it difficult to observe and isolate even after column chromatography purification. That all accounted for the doubling of the carbon peak on the $^{13}$CNMR spectrum.

To test this hypothesis, Z-β-Ala-OH was used as a reagent without a stereocenter in it to observe how the carbon peak of the product would change on the $^{13}$CNMR spectrum (Figure 3.8). Also, Z-β-Ala-OH is structurally similar to L-phenylalanine, which is another reason to choose this amino acid for comparison. Consequently, the doublet at those specific range of compound 44a indeed disappeared in the $^{13}$CNMR of compound 44i, which supported our assumption. Overall, nine C7-amino [13]-macrodilactones were prepared via peptide coupling using different amino acids and carboxylic acid with macrodilactone 37.
Figure 3.7 $^{13}$C NMR demonstration of compound 44a

Figure 3.8 $^{13}$C NMR demonstration of compound 44i
There was always one problem along with succeeded getting the product. The side product, tetramethylurea (Figure 3.9), derived from HBTU was mixed with product after the work-up and recrystallization procedure. A peak around 2.8 ppm in $^1$HNMR spectrum indicated the existence of tetramethylurea with the C7-amino [13]-macrodilactone. For some products, even purification through a chromatography column did not help with the disappearance of the urea peak in NMR spectrum.

![Figure 3.9 Structure of tetramethylurea](image)

**Table 3.5** Preparation of C7-urea [13]-macrodilactone derivatives$^{54,55}$

<table>
<thead>
<tr>
<th>entry</th>
<th>product</th>
<th>isocyanate</th>
<th>Ar</th>
<th>condition</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46a</td>
<td>Phenyl</td>
<td><img src="image" alt="Phenyl" /></td>
<td>NE$_3$, DCM, overnight</td>
<td>34%</td>
</tr>
<tr>
<td>2</td>
<td>46b</td>
<td>4-Fluorophenyl</td>
<td><img src="image" alt="4-Fluorophenyl" /></td>
<td>toluene, overnight</td>
<td>24%</td>
</tr>
<tr>
<td>3</td>
<td>46c</td>
<td>4-Biphenyl</td>
<td><img src="image" alt="4-Biphenyl" /></td>
<td>hexane, overnight</td>
<td>39%</td>
</tr>
</tbody>
</table>

Another set of C7-amino [13]-macrodilatone urea derivatives was prepared via different reaction conditions from isocyanate (Table 3.5).$^{54,55}$ In order to obtain the desired products, different work-up procedures were developed for corresponding product. For compound 46a, the solid in reaction mixture was not the product, it had to washed, used
Celite to do a tiny scale filtration to get rid of, and then followed by a column. For compound 46b and 46c, the product was obtained as white powder after recrystallization by hexane and DCM.

For this type of compounds with urea in macrocycle structure, they were hard to dissolve in CDCl$_3$ when did NMR analysis, it turned out that CD$_3$CN and MeOD worked better to get good spectrum with clear splitting.

### 3.3.5 Final deprotection for C7-amino [13]-macrodilactone

Finally, some selected was processed with another deprotection to deliver free amine version of the macrocycle. The same with peptide coupling condition screen, phenylalanine one (44a) was picked to test the conditions. It turned out that either 0°C or involving H$_2$O with TFA in DCM was worked for this reaction. A new condition by utilizing EtOAc and HCl could also help this reaction. Finally, the condition with TFA was determined to follow since it was consistent with the previous deprotection. It would be sufficient to handle since several attempts had been made before and easy to run several parallel deprotection at one time.
Table 3.6 Final deprotection conditions for compound 44a

<table>
<thead>
<tr>
<th>entry</th>
<th>condition</th>
<th>equiv.</th>
<th>T.</th>
<th>product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFA, DCM, H₂O</td>
<td>1:1</td>
<td>5%</td>
<td>r.t.</td>
</tr>
<tr>
<td>2</td>
<td>TFA, DCM</td>
<td>1:1</td>
<td>r.t.</td>
<td>×</td>
</tr>
<tr>
<td>3</td>
<td>TFA, DCM</td>
<td>1:1</td>
<td>0°C</td>
<td>✓</td>
</tr>
<tr>
<td>4</td>
<td>EtOAc, HCl</td>
<td></td>
<td>r.t.</td>
<td>✓</td>
</tr>
</tbody>
</table>

Then, proline and tryptophan ones were both worked under the same condition. However, when this condition was applied to histidine and ornithine, it failed. Ornithine one has two equivalents of Boc group in the compound may account for the failure of cleavage. Also, one deprotection trial of Cbz protected β-alanine was conducted. Unfortunately, this condition didn’t give the product neither. Table 3.7 and 3.8 show the organized results.
Table 3.7 Deprotection of C7-amino [13]-macrolactones 44a, 44c, 44g

![Deprotection reaction](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>product</th>
<th>R</th>
<th>product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49a</td>
<td><img src="image" alt="R" /></td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>49b</td>
<td><img src="image" alt="R" /></td>
<td>✓</td>
</tr>
<tr>
<td>3</td>
<td>49c</td>
<td><img src="image" alt="R" /></td>
<td>✗</td>
</tr>
</tbody>
</table>

Table 3.8 Deprotection of C7-amino [13]-macrolactones 44b, 44e, 44h

![Deprotection reaction](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>product</th>
<th>R</th>
<th>R’</th>
<th>product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49d</td>
<td><img src="image" alt="R" /></td>
<td><img src="image" alt="R’" /></td>
<td>✓</td>
</tr>
<tr>
<td>2</td>
<td>49e</td>
<td><img src="image" alt="R" /></td>
<td><img src="image" alt="R’" /></td>
<td>✗</td>
</tr>
<tr>
<td>3</td>
<td>49f</td>
<td><img src="image" alt="R" /></td>
<td><img src="image" alt="R’" /></td>
<td>✗</td>
</tr>
</tbody>
</table>
3.3.6 MIC activity of C7-amino [13]-macrodilactones

With all these protected and deprotected final targets prepared, the MIC activity was evaluated toward them. Different kinds of bacteria and fungi have been used for the MIC assay. Unfortunately, just three of them showed some activities with modest value. C7-urea [13]-macrodilatone derivatives 46a and 46b have some activities against *S. aureus*. Compound 46b also has activity against *C. glabrata*. Deprotected compound 49b has some poor activity against *B. anthracis*.

**Table 3.9** MIC activities of 46a, 46b, 49b against selected bacteria and fungi

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>S. aureus (13709) MIC (μM)</th>
<th>C. glabrata MIC (μM)</th>
<th>B. anthracis MIC (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46a</td>
<td>125</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>46b</td>
<td>125</td>
<td>500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>49b</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>500</td>
</tr>
</tbody>
</table>

3.4 Di-substituted C7-amino [13]-macrodilactones

Our group identified that C2, C4, C7 and C12 of our macrodilactone are the stereogenic centers, which influence the shape and topology of the [13]-macrodilactones. Besides the mono-substituted ones, we also synthesized di-substituted at two of C2, C4, C7, C12 position to study the substitution pattern and the interplay of the configurations of two stereogenic centers. Through all four positions, C7 takes the priority for these two projects. Then it was decided to add a simple methyl group on C2 position of this C7-amino [13]-macrodilactone class. In this case, the numbering of C7 atom will be C12.
based on our nomenclature. The crystal structures of two mono- and di-substituted macrocycles will enable us to have a rough picture of these new compounds conformation.

The synthetic route (Scheme 3.3) mostly copied the mono-substituted one, in which only modification was replacing mono-alcohol 25 with methyl-substituted mono-alcohol 50 in acylation step to introduce an additional methyl group on C2 atom. The route to get acid 40 was described in the previous part. Similarly, the acylated reagent alcohol 50 was applied to esterify with acid 40 to deliver a new diene 51 for RCM in 90% yield. The final RCM was conducted to provide the new disubstituted macrocycle 52 in 73% yield for conformational study. Additionally, a deprotection was operated as well to afford the TFA salt of this macrocycle (53) for further biological study.

One thing to mention here was the isolation of compound 52. The TLC plate of the crude product was shown a separation of two diastereomeric series. One series owned the majority of the yield, while the other just had little amount. There should be a cis series and a trans series of the product. However, these two serious could not be completely isolated by column chromatography. As a result, the proton and carbon NMR of this compound showed this mixture set on the spectra.
Scheme 3.3 Synthesis of disubstituted C7-amino [13]-macrodiolactones

The di-substituted macrocycle 52 was selected to grow a fine crystal to get the X-ray crystal structure for the compound. The conformation of it would be therefore revealed through this analysis method, which provided us some brief information to study the impact of stereocenters and substituents on key atoms to the shape of the macrocycle. Figure 3.10 demonstrates the crystal structures of the cis set of macrocycle 52 (crystal of trans series has not been observed), decorated by a methyl group on C2 atom and a bulky substituted group on C12 atom simultaneously. It is found that this compound still bears a ribbon-like shape. This implies that the key atoms are still work together to guide the overall conformation. The planar chirality of this macrocycle is governed by the point chirality of C2 (2S gives pS, 2R gives pR). Interestingly, the Boc-amino group in this case occupies an axial position, which is the same as the amino acid chain side chain in Teixobactin. This preliminary result provides us some clues for the continuous investigation of ring shape and its application to the drug design.
The MIC activities have also been evaluated toward compound 52 and 53. However, none of them showed activities against selected bacteria and fungi.

**3.5 [13]-macrodilactones with peptide chain on hinge atom**

Our [13]-macrodilactone library show some unique features because of its interplay among planar units and key stereocenters. The study of C7-amino macrocycle has encouraged us to continue exploring the topology and biological activity when it has been attached a same group on the hinge atom instead of these key atoms. Theoretically, substitution on the hinge atom C3 would not change the planar chirality of the overall molecule. So, we are curious to check the outcome of this emerged idea. In this case, a new compound, C3-Boc protected [13]-macrodilactone has been developed for the purpose.
Scheme 3.4 Synthesis of C3-amino [13]-macrolactone

In scheme 3.4, diol 54 was diacylated by 2 equivalents of pentenoic acid 27 with EDC and DMAP to afford C3-substituted diene 55 in 48% yield. There was also some mono-acylated product initiated from this reaction. Accordingly, another acylation can be applied to the mono-acylated product with the same acid to produce the desired diene 55 as well.\(^4\) Then the RCM was conducted to form the designed macrocycle 56 (70% yield).\(^4\)

3.6 Conclusion

Teixobactin was an innovative natural product, which can inhibit peptidoglycan biosynthesis. Its structure contains a [13]-membered macrocycle with peptide chains attached onto it. This interesting discovery and the common ring size with our macrocycle invoke our curiosity to modify our [13]-macrolactone and develop amide derivatives on it for biological activity study.

The investigation of [13]-macrolactones guided us to find that C7, α to the ester carbonyl, is the key atom that guide the topology of this family of compounds. As a result, we reported a certain class of C7-amino [13]-macrolactone and its derivatization as amides and ureas in this section. Different amino acids were attached into C7 position of [13]-macrolactones via peptide coupling for conformational study and MIC study. A di-substituted and a C3-substituted [13]-macrolactone amide derivatives have also been
obtained for an extended investigation of this class. The identification of these new macrocycles provides us related information about macrocycle conformations and their physicochemical and biological properties. C7 is still the key atom that can guide the shape of C7-amino [13]-macrolactones to form their lowest energy conformation itself or with another key atom. The Boc-amino group in di-substituted one occupies an axial position, which is not a normal case in our [13]-macrolactones. The MIC activities of this class are poor against various bacteria and fungi.
3.7 Experimental

3.7.1 N-Boc-allylglycine methyl ester 42

Diisopropylamine (480 µL, 3.45 mmol, 0.717 g/mL) was dissolved in 7.5 mL dry THF in a dry round bottom flask. The solution was cooled to -78 °C under nitrogen and n-butyllithium (2.5 M in hexane, 1.44 mL, 3.6 mmol) was added dropwise to the cooled solution. After stirring for 30 min, a solution of N-(tert-butoxycarbonyl)glycine methyl ester (283.8 mg, 1.5 mmol) in THF (0.9 mL) was added rapidly to the round bottom flask via syringe. The mixture was maintained at -78 °C for 30 min, then allyl bromide (150 µL, 1.7 mmol) was added dropwise. The reaction was allowed to slowly warm to room temperature over 3 h. The solution was then diluted with ethyl acetate, transferred to a separatory funnel, and washed with saturated NH₄Cl solution (1 x 20 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The mixture was purified by silica gel column chromatography to give compound 42 as a colorless oil (258 mg, 75%). Rf 0.73 (30% EtOAc/Hex); ¹H NMR (CDCl₃) 400 MHz: δ 5.64 (dddd, J = 17.1, 10.3, 7.2, 7.2 Hz, 1H), 5.16-5.05 (m, 2H), 4.39 (dd, J = 13.4, 7.3 Hz, 1H), 3.68 (s, 3H), 2.47 (ddd, J = 20.2, 13.7, 5.7 Hz, 1H), 2.42 (ddd, J = 20.8, 14.1, 6.9 Hz, 1H), 1.37 (s, 9H); ¹³C NMR (CDCl₃) 100 MHz: δ 172.5, 155.2, 132.3, 118.9, 79.7, 52.9, 52.1, 36.7, 28.2.
3.7.2 *N*-Boc-allylglycine 40

Ester 42 (187 mg, 0.82 mmol) was dissolved in a 3:1 mixture of methanol and water (3.6 mL) to which was added 43 mg (1.06 mmol) of solid NaOH in one portion. The mixture was stirred overnight at rt. After, the reaction mixture was acidified by the addition of 1M HCl to pH of 1 (litmus paper). The solution was then extracted with EtOAc (3 x 20 mL) and the combined extracts were dried over Na₂SO₄. Solvent from the filtrate was removed under reduced pressure. The pure acid 40 was obtained without purification as a colorless oil (159 mg, 90%). ¹H NMR (CDCl₃) 400 MHz: δ 7.54 (s, br, 1H), 5.75 (dddd, J = 17.2, 9.7, 7.2, 7.2 Hz, 1H), 5.26-5.02 (m, 2H), 4.36 (dd, J = 12.7, 6.2 Hz, 1H), 2.71-2.32 (m, 2H), 1.46 (s, 9H); ¹³C NMR (CDCl₃) 100 MHz: δ 176.5, 155.5, 132.1, 119.4, 80.3, 52.8, 36.4, 28.3.
3.7.3 General procedure for acylation of \(N\)-Boc-allylglycine 40\(^{48}\)

Dicyclohexylcarbodiimide (DCC) (0.165 g, 0.8 mmol) and \(N,N\)-dimethylaminopyridine (DMAP) (0.045 mg, 0.37 mmol) were dissolved in DCM (3 mL), and the solution was cooled, under nitrogen, to 0 °C on an ice bath. The \(N\)-Boc-allylglycine 40 (0.172 g, 0.8 mmol) to be esterified was then added and the mixture was stirred at 0 °C for 30 min. There was precipitate initiated in the solution. A solution of corresponding mono-alcohol (0.73 mmol) in DCM (1 mL) was then added and the mixture was stirred, overnight, at rt. After, the mixture was filtered through a short pad of celite and solvent was removed from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/hexane solutions as reported for each compound.
3.7.3.1 Compound 39

This compound was prepared via the general acylation procedure acylation to give diene 39 as a light yellow oil (231 mg, 89 %). R<sub>f</sub> 0.59 (25 % EtOAc/Hex); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 400 MHz: δ 5.76 (dddd, J = 17.6, 10.4, 6.2, 6.2 Hz, 1H), 5.65 (dddd, J = 17.0, 9.7, 7.2, 7.2 Hz, 1H), 5.11–5.08 (m, 1H), 5.06 (s, br, 1H), 5.04–4.93 (m, 3H), 4.31 (dd, J = 13.6, 6.5 Hz, 1H), 4.16 (ddd, J = 6.2, 6.2, 3.8 Hz, 2H), 4.11 (t, J = 6.3 Hz, 2H), 2.46 (ddd, J = 13.4, 6.8, 6.8 Hz, 2H), 2.40–2.28 (m, 4H), 1.96–1.90 (m, 2H), 1.39 (s, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 100 MHz: δ 172.8, 172.0, 155.2, 136.5, 132.3, 119.1, 115.6, 79.9, 61.9, 60.7, 52.9, 36.8, 33.4, 28.8, 28.3, 28.0. TOF HRMS (ESI) m/z calcd for C<sub>18</sub>H<sub>29</sub>NO<sub>6</sub> [M+H]+: 356.2073; found: 356.2081.
3.7.3.2 Compound 51

This compound was prepared via the general acylation procedure to give diene 51 as a light yellow oil (242 mg, 90%). Rf 0.7 (30 % EtOAc/Hex); $^1$H NMR (CDCl$_3$) 400 MHz: δ 5.73 (ddddd, $J = 16.7, 10.4, 6.2, 6.2$ Hz, 1H), 5.62 (dddd, $J = 17.8, 9.4, 7.1, 7.1$ Hz, 1H), 5.08–5.04 (m, 1H), 5.03 (s, br, 1H), 5.01–4.90 (m, 3H), 4.31 (dd, $J = 13.0, 6.1$ Hz, 1H), 4.13–3.97 (m, 3H), 2.42 (ddd, $J = 13.3, 7.0, 7.0$ Hz, 2H), 2.34–2.24 (m, 4H), 1.92–1.76 (m, 2H), 1.35 (s, 9H), 1.21–1.16 (m, 3H); $^{13}$C NMR (CDCl$_3$) 100 MHz: δ 172.7, 171.5, 155.1, 136.5 (2), 132.3, 119.0, 115.5, 79.7, 69.1, 67.8, 67.6, 61.6, 60.6, 60.5, 53.0, 52.9, 36.7, 36.6, 34.8, 33.7, 33.4, 28.8, 28.7, 28.2, 20.0, 19.9.
3.7.3.3 Synthesis of compound 55

DCC (474.7 mg, 2.3 mmol) and DMAP (122.17 mg, 1.05 mmol) were dissolved in DCM (15 mL), and the solution was cooled to 0 °C. Pentenoic acid 27 (460 mg, 4.6 mmol) was added and the mixture was stirred at the same temperature for 30 min. Then, diol 54 (400 mg, 2.09 mmol) in DCM (5 mL) was added to the mixture and it was stirred at room temperature overnight. After, the mixture was filtered through a short pad of Celite and the solvent was removed from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography to give diene 55 as a white solid (357 mg, 48%). Rf 0.67 (30 % EtOAc/Hex); 1H NMR (CDCl3) 300 MHz: δ 5.77 (dddd, J = 16.7, 10.3, 6.3, 6.3 Hz, 2H), 5.05 (dd, J = 3.1, 1.5 Hz, 1H), 5.00–4.98 (m, 2H), 4.95 (dd, J = 2.7, 1.3 Hz, 1H), 4.84 (s, 1H), 4.28–3.92 (m, 4H), 2.43–2.29 (m, 8H), 1.41 (s, 9H); 13C NMR (CDCl3) 100 MHz: δ 172.7, 155.2, 136.5, 115.7, 80.0, 63.1, 48.5, 33.4, 28.8, 28.3. TOF HRMS (DART): m/z calcd for C18H29NO6[M+H]+: 356.2073; found: 356.2077.
3.7.4 General RCM procedure\textsuperscript{47}

Grubbs' second-generation catalyst (0.064 g, 0.075 mmol) was added as a solid in one portion to a solution of the diene (1.5 mmol) in toluene (200 mL). The concentration of diene in the RCM reactions was consistently maintained between 5-10 mM. The mixture was heated to reflux at 110 °C overnight (22 h). The mixture was then allowed to cool to r.t., solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/hexane solutions as reported to give each compound.
3.7.4.1 Compound 38

This compound was prepared by using the general RCM procedure to give compound 38 as a white solid (343 mg, 70%). mp 97.1-98.1 °C; Rf 0.36 (25 % EtOAc/Hex); \textsuperscript{1}H NMR (CDCl\textsubscript{3}) 400 MHz: δ 5.49 (ddd, J = 15.6, 6.2, 6.2 Hz, 1H), 5.37 (ddd, J = 14.8, 7.2, 7.2 Hz, 1H), 5.01 (d, J = 8.6 Hz, 1H), 4.27–4.24 (m, 1H), 4.22–4.08 (m, 4H), 2.52–2.47 (m, 1H), 2.43–2.22 (m, 5H), 2.09–1.95 (m, 2H), 1.41 (s, 9H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) 100 MHz: δ 173.5, 172.8, 155.1, 132.3, 125.3, 80.1, 61.6, 60.7, 54.0, 36.5, 33.5, 28.4, 28.1, 26.0. TOF HRMS (ESI): m/z calcd for C\textsubscript{16}H\textsubscript{26}NO\textsubscript{6} [M+H]+: 328.1760; found: 328.1766.
3.7.4.2 Compound 52

This compound was prepared by using the general procedure for RCM to give compound 52 as a white solid (373 mg, 73%). Rf 0.43 (25 % EtOAc/Hex); \(^1\)H NMR (CDCl\(_3\)) 300 MHz: \(\delta\) 5.55–5.43 (m, 1H), 5.42–5.29 (m, 1H) 5.18–4.81 (m, 2H), 4.47–4.29 (m, 1H), 4.24–4.07 (m, 1H), 4.05–3.88 (m, 1H), 2.70–2.61 (m, 1H), 2.44–2.18 (m, 5H), 2.12–1.95 (m, 1H), 1.94–1.76 (m, 1H), 1.43 (s, 5H), 1.40 (s, 4H), 1.29 (d, J = 8.4 Hz, 1.5H), 1.28 (d, J = 8.2 Hz, 1.5H); \(^{13}\)C NMR (CDCl\(_3\)) 75 MHz: \(\delta\) 173.7, 172.7, 155.4, 133.5, 132.3, 125.4 (2), 79.9, 69.4, 68.8, 60.6, 60.1, 54.8, 36.2, 34.6, 34.0, 33.8, 33.6, 33.4, 28.7, 28.5, 28.4, 28.3, 20.4, 20.3. TOF HRMS (ESI): \(m/z\) calcd for C\(_{17}\)H\(_{28}\)NO\(_6\) [M+H]+: 342.1917; found: 342.1885.
3.7.4.3 Compound 56

This compound was prepared by using the general procedure for RCM to give compound 56 as a white solid (353 mg, 72%). mp 139.6-140.5 °C; Rf 0.36 (25% EtOAc/Hex); $^1$H NMR (CDCl$_3$) 400 MHz: δ 5.39-5.38 (m, 2H), 4.84 (d, $J = 8.6$ Hz, 1H), 4.24–4.21 (m, 1H), 4.21–4.18 (m, 2H), 4.13–3.81 (m, 2H), 2.33–2.26 (m, 8H), 1.42 (s, 9H); $^{13}$C NMR (CDCl$_3$) 100 MHz: δ 173.2, 155.1, 130.0, 80.3, 62.8, 46.9, 34.2, 28.8, 28.4. TOF HRMS (DART): $m/z$ calcd for C$_{16}$H$_{25}$NO$_6$ [M+H]+: 328.1760; found: 328.1773.
3.7.5 General procedure for deprotection of N-Boc-amino-[13]-macrodilactones

The Boc-protected amino-[13]-macrodilactone (0.15 mmol) was dissolved in 200 µL DCM and 10 µL of DI water was added to the solution. Then 200 µL TFA was added dropwise and the reaction was stirred at r.t. for 2h. After, the solvent removed under reduced pressure, followed by drying under high vacuum overnight. Amino-[13]-macrodilactones were used without further purification.
3.7.5.1 Compound 37

This compound was prepared by using the general procedure for Boc deprotection to give compound 37 as a yellow oil. Rf 0.33 (5% CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 5.58 (ddd, J = 15.4, 6.4, 6.4 Hz, 1H), 5.39 (ddd, J = 15.1, 7.6, 7.6 Hz, 1H), 4.29–4.17 (m, 4H), 3.97 (dd, J = 10.0, 3.7 Hz, 1H), 2.74–2.68 (m, 1H), 2.52–2.44 (m, 1H), 2.43–2.30 (m, 4H), 2.12–1.98 (m, 2H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.8, 169.7, 133.8, 123.4, 63.8, 61.6, 53.3, 34.0, 33.5, 27.8, 26.2. TOF HRMS (ESI): m/z calcd for C₁₁H₁₈NO₄ [M+H]+: 228.1236; found: 228.1233.
3.7.5.2 Compound 53

This compound was prepared by using the general procedure for deprotection to give compound 53 as a yellow oil. Rf 0.6 (10 % CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 5.66 (ddd, J = 14.8, 6.5, 6.5 Hz, 1H), 5.48 (ddd, J = 14.6, 6.9, 6.9 Hz, 1H), 5.12–5.06 (m, 1H), 4.50 (dd, J = 9.4, 9.4 Hz, 1H), 4.13 (s, br, 1H), 3.97 (ddd, J = 8.3, 4.2, 4.2 Hz, 1H), 2.83–2.77 (m, 1H), 2.68–2.58 (m, 1H), 2.45–2.28 (m, 4H), 2.10–2.02 (m, 1H), 1.97–1.89 (m, 1H), 1.33 (d, J = 6.2 Hz, 3H); ¹³C NMR (CDCl₃) 100 MHz: δ 174.2, 167.1, 135.6, 123.2, 71.4, 60.3, 53.9, 33.8, 33.1, 32.6, 28.5, 19.6. TOF HRMS (ESI): m/z calcd for C₁₂H₂₀NO₄ [M+H]⁺: 242.1392; found: 242.1388.
3.7.6 General procedure for the acylation of C7-amino [13]-macrolactones

To a 0.25 M solution of the desired 0.18 mmol protected amino acid or carboxylic acid in 0.72 mL DCM, C7-amino [13]-macrolactone (0.041 g, 0.18 mmol), HBTU (0.068 g, 0.18 mmol), and DIEA (0.070 g, 0.54 mmol) were added. The reaction was left to stir for 3-5 hour. The solvent was removed under reduced pressure and the resulting residue was dissolved in EtOAc. The solution was washed with aqueous solution of citric acid (1 x 5 mL), saturated sodium bicarbonate (2 x 5 mL) and brine (1 x 5 mL). The organic layer was dried under sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/hexane or CH₃OH/DCM solutions as reported to give each compound.
3.7.6.1 Compound 44a

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44a as a white solid (60 mg, 70%). mp 132.3-134.3 °C; Rf 0.29 (25% EtOAc/Hex); ¹H NMR (CDCl₃) 400 MHz: δ 7.33-7.27 (m, 3H), 7.25-7.19 (m, 2H), 6.47 (d, J = 8.0 Hz, 0.5H), 6.30 (d, J = 7.5 Hz, 0.5H), 5.50-5.38 (m, 1H), 5.35-5.20 (m, 1H), 5.11 (s, br, 0.5H), 5.04 (s, br, 0.5H), 4.58-4.45 (m, 1H), 4.37 (s, br, 1H), 4.32-4.26 (m, 1H), 4.19-4.11 (m, 3H), 3.06 (dd, J = 7.0, 7.0 Hz, 2H), 2.52-2.45 (m, 1H), 2.42-2.36 (m, 2H), 2.35-2.28 (m, 3H), 2.11-1.95 (m, 2H), 1.41 (s, 5H), 1.40 (s, 4H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.5, 172.0, 171.7, 170.8, 136.9, 136.8, 132.6, 132.5, 129.6 (2), 128.9 (2), 127.2 (2), 125.4, 125.3, 80.4, 62.1, 62.0, 60.9, 60.7, 53.0, 52.5, 39.0, 38.6, 36.2, 35.9, 33.7, 28.5, 28.0(2), 26.3, 26.1. TOF HRMS (ESI): m/z calcd for C₂₅H₃₅N₂O₇ [M+H]+: 475.2444; found: 475.2441.
3.7.6.2 Compound 44b

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44b as a colorless oil (19 mg, 25%). Rf 0.49 (5 % CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 7.31 (s, br, 0.5H), 6.52 (s, br, 0.5H), 5.48 (ddd, J = 16.0, 5.6, 5.8 Hz, 1H), 5.42-5.31 (m, 1H), 4.54 (s, br, 1H), 4.42-4.07 (m, 5H), 3.58–3.27 (m, 2H), 2.56–2.43 (m, 1H), 2.33 (ddd, J = 11.0, 6.1, 6.1 Hz, 5H), 2.08–1.94 (m, 4H), 1.89-1.81 (m, 2H), 1.44 (s, 5H), 1.42 (s, 4H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.3, 172.2, 132.3, 125.4, 80.6, 64.4, 61.7, 60.8, 52.9, 47.2, 36.2, 33.6, 31.1, 28.4 (2), 28.3, 28.0, 27.9, 26.1, 25.9. TOF HRMS (ESI): m/z calcd for C₂₁H₃₉N₂O₇ [M+H]+: 425.2288; found: 425.2291.
3.7.6.3 Compound 44c

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44c as a white solid (74 mg, 80%). mp 75.5-77.0 °C; Rf 0.53 (5 % CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 8.55 (d, J = 14.2 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.34 (dd, J = 8.0, 3.2 Hz, 1H), 7.21–7.13 (m, 1H), 7.10 (dd, J = 7.5, 3.0 Hz, 1H), 7.07 (s, 1H), 6.44 (d, J = 7.8 Hz, 0.5H), 6.25 (d, J = 8.0 Hz, 0.5H), 5.42–5.14 (m, 2H), 4.45 (s, br, 2H), 4.23–4.15 (m, 1H), 4.14–4.06 (m, 2H), 4.03–3.96 (m, 1H), 3.29 (s, br, 1H), 3.19-3.11 (m, 1H), 2.33 (ddd, J = 14.7, 7.6, 3.5 Hz, 3H), 2.26–2.22 (m, 2H), 2.17-2.08 (m, 1H), 2.00–1.85 (m, 2H), 1.41 (s, 5H), 1.40 (s, 4H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.5, 171.6, 171.3, 155.5, 136.4, 132.4, 132.2, 127.6, 125.2, 125.0, 123.4, 123.2, 122.3 (2), 119.7 (2), 118.9, 111.3 (2), 80.2, 61.8, 60.7, 52.9, 52.5, 38.7, 36.0, 35.6, 33.5, 28.7, 28.4, 27.9, 25.9. TOF HRMS (ESI): m/z calcd for C₂₇H₃₆N₃O₇ [M+H]+: 514.2553; found: 514.2497.
3.7.6.4 Compound 44d

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44d as a white solid (65 mg, 57%). mp 82.0-84.0 °C; Rf 0.25 (5 % CH₃OH/DCM); ¹H NMR (CD₃OD) 400 MHz: δ 7.78 (d, J = 7.3 Hz, 2H), 7.35 (d, J = 7.9 Hz, 2H), 5.64-5.56 (m, 1H), 5.49 (ddd, J = 14.0, 6.9, 6.9 Hz, 1H), 4.36 (dd, J = 9.7, 4.3 Hz, 1H), 4.20 (ddd, J = 13.2, 13.2, 6.9 Hz, 2H), 4.20 (ddd, J = 11.7, 11.7, 5.6 Hz, 2H), 4.11 (s, br, 1H), 3.32–3.14 (m, 2H), 2.62–2.52 (m, 1H), 2.51-2.46 (m, 2H), 2.45 (s, 3H), 2.41-2.32 (m, 3H), 2.18-2.00 (m, 2H), 1.75 (s, br, 1H), 1.60 (s, br, 3H), 1.49 (s, 4H), 1.48 (s, 5H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.9, 172.4, 172.2, 157.1, 156.2, 142.4, 140.5, 132.6, 132.5, 129.4, 126.2, 125.1, 80.5, 80.4, 62.1 (2), 61.0 (2), 55.5, 53.2, 52.9, 40.8, 35.5, 33.6, 29.8, 28.4, 27.9, 27.8, 26.1, 25.4, 21.6. TOF HRMS (ESI): m/z calcd for C₂₉H₄₄N₅O₅S [M+H]+: 638.2860; found: 638.2832.
3.7.6.5 Compound 44e

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44e as a colorless oil (77 mg, 79%). Rf 0.71 (100% EtOAc); $^1$H NMR (CD$_3$OD+CDCl$_3$) 400 MHz: δ 5.63–5.50 (m, 1H), 5.53 (s, 0.5H), 5.49-5.36 (m, 1H), 5.44 (s, 0.5H), 4.50 (s, br, 1H), 4.38–4.30 (m, 1H), 4.24 (ddd, $J = 7.0$, 7.0, 4.5 Hz, 1H), 4.17 (ddd, $J = 11.2$, 11.2, 5.8 Hz, 2H), 4.10-4.03 (m, 1H), 3.09-3.00 (m, 2H), 2.56–2.46 (m, 1H), 2.44-2.38 (m, 2H), 2.37-2.26 (m, 3H), 2.10-2.02 (m, 2H), 1.72 (s, br, 1H), 1.62–1.48 (m, 3H), 1.44 (s, 4H), 1.43 (s, 6H), 1.42 (s, 8H); $^{13}$C NMR (CD$_3$OD+CDCl$_3$) 100 MHz: δ 174.0, 173.4, 172.5, 132.2 (2), 125.0, 61.7, 60.9, 53.0, 52.9, 39.5, 35.2, 35.0, 33.1, 29.4, 27.6, 27.5, 25.9, 25.7. TOF HRMS (ESI): $m/z$ calcd for C$_{26}$H$_{44}$N$_3$O$_9$ [M+H]+: 542.3078; found: 542.3001.
3.7.6.6 Compound 44f

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44f as a colorless oil (83 mg, 90%). R<sub>f</sub> 0.53 (5% CH<sub>3</sub>OH/DCM); <sup>1</sup>H NMR (CDCl<sub>3</sub>) 400 MHz: δ 6.83 (s, br, 1H), 5.53 (ddd, <i>J</i> = 15.2, 6.0, 6.0 Hz, 1H), 5.45–5.36 (m, 1H), 5.35–5.25 (m, 1H), 4.55 (ddd, <i>J</i> = 8.6, 3.8, 3.8 Hz, 1H), 4.32 (ddd, <i>J</i> = 17.0, 11.4, 5.0 Hz, 1H), 4.27-4.18 (m, 2H), 4.18-4.08 (m, 2H), 2.55 (ddd, <i>J</i> = 10.5, 10.5, 5.0 Hz, 1H), 2.41 (ddd, <i>J</i> = 6.7, 4.2, 4.2 Hz, 2H), 2.40-2.26 (m, 4H), 2.11-2.01 (m, 3H), 1.96-1.82 (m, 2H), 1.44 (s, 7H), 1.43 (s, 6H), 1.42 (s, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 100 MHz: δ 173.5, 171.9, 171.2, 155.9, 155.7, 132.6 (2), 125.3 (2), 62.0, 60.8 (2), 52.9, 52.6, 36.1, 33.6, 32.0 (2), 28.4, 28.2, 28.0 (2), 27.8 (2), 26.2, 26.1. TOF HRMS (ESI): <i>m/z</i> calcd for C<sub>25</sub>H<sub>41</sub>N<sub>2</sub>O<sub>9</sub> [M+H]+: 513.2812; found: 513.2787.
3.7.6.7 Compound 44g

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44g as a white solid (30 mg, 36%). mp 163.0-164.5 °C; Rf 0.51 (10 % CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 7.95 (s, br, 1H), 7.32 (s, br, 1H), 7.00 (s, 1H), 5.76 (d, J = 6.5 Hz, 0.5H), 5.67 (d, J = 7.7 Hz, 0.5H), 5.57-5.45 (m, 1H), 5.44-5.29 (m, 1H), 4.45 (s, br, 2H), 4.31-4.21 (m, 2H), 4.21-3.98 (m, 2H), 3.09 (d, J = 3.5 Hz, 2H), 2.60-2.46 (m, 1H), 2.40 (ddd, J = 7.4, 4.0, 4.0 Hz, 2H), 2.37-2.24 (m, 3H), 2.13-1.96 (m, 2H), 1.42 (s, 9H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.7 (2), 172.4, 171.5, 171.3, 155.9, 135.0, 134.7, 132.6, 125.1 (2), 119.2, 80.7, 62.4 (2), 61.0, 53.3, 53.2, 35.7, 33.7, 28.9, 28.7, 28.5, 28.4 (2), 28.1, 26.1. TOF HRMS (ESI): m/z calcd for C₂₂H₃₃N₄O₇ [M+H]+: 465.2350; found: 465.2380.
3.7.6.8 Compound 44h

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44h as a white solid (31 mg, 40%). mp 109.9-111.0 °C; Rf 0.57 (5 % CH₃OH/DCM); ¹H NMR (CDCl₃) 400 MHz: δ 7.53-7.28 (m, 5H), 6.14 (d, J = 7.0 Hz, 1H), 5.52 (ddd, J = 15.9, 6.1, 6.1 Hz, 1H), 5.44 (s, br, 1H) 5.38 (ddd, J = 15.3, 8.3, 6.4 Hz, 1H), 5.11 (s, 2H), 4.56 (ddd, J = 8.8, 8.8, 4.0 Hz, 1H), 4.35-4.28 (m, 1H), 4.23-4.08 (m, 3H), 3.48 (dd, J = 12.1, 6.0 Hz, 2H), 2.58-2.51 (m, 1H), 2.48-2.42 (m, 3H), 2.41-2.38 (m, 1H), 2.35-2.30 (m, 2H), 2.29-2.22 (m, 1H), 2.10-1.96 (m, 2H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.6, 172.2, 170.9, 162.2, 136.7, 132.6, 128.6, 128.3, 128.2, 125.3, 66.9, 62.0, 60.7, 52.8, 37.2, 36.2, 36.0, 33.6, 27.9, 26.2. TOF HRMS (ESI): m/z calcd for C₂₂H₂₉N₂O₇ [M+H]⁺: 433.1975; found: 433.1982.
3.7.6.9 Compound 44i

This compound was prepared by using the general procedure for acylation of compound 37 to give compound 44i as a white solid (21 mg, 28 %). mp 184.8-185.5 °C; Rf 0.8 (5 % CH₃OH/DCM); "H NMR (CDCl₃) 400 MHz: 7.93 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.49-7.44 (m, 2H), 7.39 (t, J = 7.3 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 5.60 (ddd, J = 15.6, 6.1, 6.1 Hz, 1H), 5.51 (ddd, J = 14.5, 6.6, 6.6 Hz, 1H), 4.91 (ddd, J = 8.5, 3.7, 3.7 Hz, 1H), 4.45 (ddd, J = 11.8, 7.6, 4.6 Hz, 1H), 4.33-4.19 (m, 2H), 4.15 (ddd, J = 11.3, 6.5, 4.7 Hz, 1H), 2.77-2.70 (m, 1H), 2.54-2.43 (m, 3H), 2.42-2.35 (m, 2H), 2.17-2.00 (m, 2H); "C NMR (CDCl₃) 100 MHz: δ 173.5, 172.3, 166.3, 144.8, 140.1, 132.6, 132.5, 129.1, 128.2, 127.8, 127.5, 127.4, 125.7, 61.9, 60.5, 53.2, 36.2, 33.5, 27.9, 26.1. TOF HRMS (ESI): m/z calcd for C₂₄H₂₆NO₅ [M+H]+: 408.1811; found: 408.1811.
3.7.7.1 Compound 46a$^{46,54}$

To a 0.12 M solution of phenyl isocyanate (10 μL, 0.1 mmol, 1.096 g/mL) in 0.9 mL DCM, compound 37 (34.1 mg, 0.15 mmol) were added, followed by triethylamine (30 μL, 0.225 mmol, 0.726 g/mL). The reaction was stirred overnight under nitrogen. The solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate. The solution was washed with aqueous solution of citric acid (1 x 5 mL), saturated sodium bicarbonate (2 x 5 mL) and brine (1 x 5 mL). The organic layer was dried under sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography to give compound 46a as a colorless oil (12 mg, 34%). \( R_f \) 0.45 (50 % EtOAc/Hex); \( \text{\textsuperscript{1}H NMR (CD}_3\text{OD)} \) 400 MHz: \( \delta \) 7.38 (d, \( J = 7.4 \) Hz, 2H), 7.28 (dd, \( J = 7.4, 7.4 \) Hz, 2H), 7.01 (dd, \( J = 7.4, 7.4 \) Hz, 1H), 5.60 (ddd, \( J = 12.1, 6.3, 6.3 \) Hz, 1H), 5.55–5.30 (m, 1H), 4.38 (dd, \( J = 9.1, 3.9 \) Hz, 1H), 4.27 (ddd, \( J = 7.1, 5.2, 1.9 \) Hz, 2H), 4.24–4.19 (m, 1H), 4.19–4.11 (m, 1H), 2.63–2.52 (m, 1H), 2.46 (ddd, \( J = 7.7, 4.0, 2.2 \) Hz, 2H), 2.42-2.32 (m, 3H), 2.17–2.07 (m, 2H); \( \text{\textsuperscript{13}C NMR (CDCl}_3 \) 100 MHz: \( \delta \) 173.9, 173.2, 155.2, 138.7, 132.4, 129.3, 129.2, 125.5, 123.7, 120.6, 120.5, 61.7, 60.8, 53.6, 36.3, 33.6, 28.1, 26.0. TOF HRMS (ESI): \( m/z \) calcd for \( \text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_5 \) [M+H]+: 347.1607; found: 347.1607.
3.7.7.2 Compound 46b\textsuperscript{54}

To a 0.2 M solution of 4-fluorophenyl isocyanate (20 \( \mu \)L, 0.15 mmol, 1.206 g/mL) in 0.75 mL toluene, a 0.2 M solution of compound 37 (34.1 mg, 0.15 mmol) in 0.75 mL toluene were added dropwise. The reaction was stirred overnight under nitrogen. The solvent was removed under reduced pressure to afford the crude product. Then, the crude product was recrystallized using DCM and hexane to give compound 46b as a white solid (13 mg, 24%). \textsuperscript{1}H NMR (CD\(_3\)OD) 400 MHz: \( \delta \) 7.36 (dd, \( J = 9.0, 4.8 \) Hz, 2H), 7.02 (dd, \( J = 8.7, 8.7 \) Hz, 2H), 5.61 (ddd, \( J = 12.5, 6.3, 6.3 \) Hz, 1H), 5.53–5.31 (m, 1H), 4.37 (dd, \( J = 9.1, 3.9 \) Hz, 1H), 4.27 (ddd, \( J = 6.6, 5.2, 2.0 \) Hz, 2H), 4.25–4.21 (m, 1H), 4.21–4.12 (m, 1H), 2.69–2.50 (m, 1H), 2.50–2.42 (m, 2H), 2.42–2.32 (m, 3H), 2.14–2.07 (m, 2H); \textsuperscript{13}C NMR (CDCl\(_3\)) 100 MHz: \( \delta \) 174.0, 172.8, 155.0, 134.5, 132.6, 125.5, 122.7, 122.6, 116.1, 115.9, 61.6, 60.6, 53.6, 36.2, 33.7, 28.1, 26.1. TOF HRMS (ESI): \( m/z \) calcd for C\(_{18}\)H\(_{22}\)FN\(_2\)O\(_5\) [M+H]+: 365.1513; found: 365.1509.
To a 0.24 M solution of 4-biphenylyl isocyanate (23.4 mg, 0.12 mmol) in 0.5 mL hexane, compound 37 (27.2 mg, 0.12 mmol) were added. The reaction was stirred overnight under nitrogen. The white precipitate was filtered out. The crude product was washed with a little hexane and recrystallized using DCM and hexane to give compound 46c as a white solid (20 mg, 39%). H NMR (CD$_3$OD) 400 MHz: δ 7.79–7.71 (m, 2H), 7.71–7.61 (m, 2H), 7.46 (dd, J = 7.6, 7.6 Hz, 2H), 7.41–7.28 (m, 3H), 5.68 (ddd, J = 15.1, 6.4, 6.4 Hz, 1H), 5.55–5.43 (m, 1H), 5.51 (s, 1H), 4.38–4.29 (m, 2H), 4.27–4.12 (m, 2H), 3.98 (dd, J = 10.6, 4.1 Hz, 1H), 2.78–2.59 (ddd, J = 10.5, 4.4, 4.4 Hz, 1H), 2.54–2.41 (m, 3H), 2.41–2.33 (m, 2H), 2.17–2.04 (m, 2H); C NMR (CD$_3$CN) 100 MHz: δ 173.2, 169.0, 139.9, 138.5, 134.0, 129.0, 128.7, 127.6 (2), 127.5, 126.8, 126.1, 125.9, 123.1, 121.5, 115.2, 63.9, 61.5, 53.2, 33.4, 33.0, 27.5, 26.1.
3.7.8.1 Compound 49a

This compound was prepared by using the general procedure for deprotection 3.7.5 on compound 44a to give compound 49a as a pale yellow oil. Rf 0.6 (10 % CH$_3$OH/DCM); $^1$H NMR (CD$_3$OD) 400 MHz: $\delta$ 7.41-7.36 (m, 2H), 7.35-7.26 (m, 3H), 5.62-5.53 (m, 0.5H), 5.52–5.40 (m, 1H), 5.32 (ddd, $J =$ 15.0, 7.4, 7.4 Hz, 0.5H), 4.39–4.24 (m, 2H), 4.23-4.10 (m, 4H), 3.25 (dd, $J =$ 7.7, 6.5 Hz, 0.5H), 3.14 (dd, $J =$ 7.6, 4.8 Hz, 1H), 3.04 (dd, $J =$ 14.2, 8.0 Hz, 0.5H), 2.53-2.47 (m, 1H), 2.45-2.40 (m, 2H), 2.39-2.29 (m, 2H), 2.23-2.12 (m, 1H), 2.10-2.04 (m, 2H); $^{13}$C NMR (CD$_3$CN) 100 MHz: $\delta$ 174.2, 172.5, 172.1, 168.5, 135.3, 135.2, 133.6, 133.4, 130.6, 129.8, 128.6, 125.6, 125.5, 63.0 (2), 61.7 (2), 55.3, 54.1, 54.0, 38.8, 38.0, 37.8, 36.1 (2), 34.1, 34.0, 28.7, 28.6, 26.7. TOF HRMS (ESI): $m/z$ calcd for C$_{20}$H$_{27}$N$_2$O$_5$ [M+H]+: 375.1920; found: 375.1914.
3.7.8.2 Compound 49d

This compound was prepared by using the general procedure for deprotection 3.7.5 on 44b to give compound 49d as a pale yellow oil. Rf 0.65 (100% EtOAc); $^1$H NMR (CD$_3$OD+CDCl$_3$) 400 MHz: δ 5.62-5.48 (m, 1H), 5.44-5.32 (m, 1H), 4.53–4.44 (m, 1H), 4.36 (s, br, 2H), 4.23 (ddd, J = 8.3, 8.3, 2.0 Hz, 1H), 4.20–4.10 (m, 2H), 3.36 (s, br, 1H), 3.33 (p, J = 1.7 Hz, 1H), 2.54 (ddd, J = 13.9, 13.9, 6.7 Hz, 1H), 2.50–2.39 (m, 2H), 2.38–2.33 (m, 2H), 2.32-2.28 (m, 1H), 2.23-1.91 (m, 5H), 1.89-1.79 (m, 1H); $^{13}$C NMR (CD$_3$OD+CDCl$_3$) 100 MHz: 173.9, 172.6, 168.0, 132.7, 132.6, 125.0, 124.8, 59.1, 58.0, 57.9, 52.3, 52.2, 46.0, 45.9, 34.3, 33.3, 31.0, 29.7, 29.7, 27.4, 27.3, 23.7, 23.6. TOF HRMS (ESI): m/z calcd for C$_{16}$H$_{25}$N$_2$O$_5$ [M+H]+: 325.1763; found: 325.1754.
3.7.8.3 Compound 49b

This compound was prepared by using the general procedure for deprotection 3.7.5 on 44c to give compound 49b as a light yellow oil. Rf 0.5 (100% EtOAc); $^1$H NMR (MeOD+CDCl$_3$) 400 MHz: $\delta$ 7.51 (dd, $J = 13.8$, 7.8 Hz, 1H), 7.35 (dd, $J = 8.2$, 3.4 Hz, 1H), 7.19-7.08 (m, 2H), 7.07-7.01 (m, 1H), 5.49-5.41 (m, 0.5H), 5.36-5.21 (m, 1H), 5.06 (ddd, $J = 14.7$, 7.0, 7.0 Hz, 0.5H), 4.38–4.24 (m, 1H), 4.18-4.01 (m, 5H), 3.31-27 (m, 1H), 3.26-3.21 (m, 1H), 2.52–2.36 (m, 2H), 2.35–2.25 (m, 2H), 2.24-2.15 (m, 1H), 2.14–2.05 (m, 1H), 2.02-1.94 (m, 1H), 1.87–1.77 (m, 1H); $^{13}$C NMR (CDCl$_3$) 100 MHz: $\delta$ 174.6, 171.9, 171.2, 136.5 (2), 132.8 (2), 126.8 (2), 124.9, 124.5, 124.3, 122.9, 122.8, 120.2 (2), 118.2, 111.9 (2), 106.8, 62.7, 62.5, 61.2, 61.1, 54.1 (2), 53.5 (2), 38.9, 35.4, 33.6 (2), 27.8, 27.6, 26.1, 25.9. TOF HRMS (ESI): m/z calcd for C$_{22}$H$_{26}$N$_3$O$_5$ [M+H]+$^+$: 414.2029; found: 414.2021.
3.8 Appendix E: Crystallographic Data for Compound 38

Low-temperature diffraction data (ω-scans) were collected on a Rigaku MicroMax-007HF diffractometer coupled to a Saturn994+ CCD detector with Cu Kα (λ = 1.54178 Å) for the structure of 007b-18091. The diffraction images were processed and scaled using Rigaku Oxford Diffraction software (CrysAlisPro; Rigaku OD: The Woodlands, TX, 2015). The structure was solved with SHELXT and was refined against F² on all data by full-matrix least squares with SHELXL (Sheldrick, G. M. Acta Cryst. 2008, A64, 112–122). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms to which they are linked (1.5 times for methyl groups). The only exception is H16, which was found in the difference map and freely refined. The full numbering scheme of compound 007b-18091 can be found in the full details of the X-ray structure determination (CIF), which is included as Supporting Information. CCDC number XXXXXX (007b-18091) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

![3.11](image.png)

**Figure 3.11** The complete numbering scheme of 007b-18091 with 50% thermal ellipsoid probability levels. The hydrogen atoms are shown as circles for clarity.
<table>
<thead>
<tr>
<th><strong>Table 3.10</strong> Crystal data and structure refinement for 007b-18091</th>
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<td><strong>Temperature</strong></td>
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<td><strong>Wavelength</strong></td>
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<td>c</td>
</tr>
<tr>
<td>Volume</td>
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<tr>
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<td><strong>Crystal color and habit</strong></td>
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<td><strong>Independent reflections</strong></td>
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<td><strong>Observed reflections (I &gt; 2sigma(I))</strong></td>
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<td><strong>Max. and min. transmission</strong></td>
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<tr>
<td><strong>Solution method</strong></td>
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<td><strong>Refinement method</strong></td>
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<td><strong>Goodness-of-fit on F²</strong></td>
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<td><strong>R indices (all data)</strong></td>
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<tr>
<td><strong>Extinction coefficient</strong></td>
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<tr>
<td><strong>Largest diff. peak and hole</strong></td>
</tr>
</tbody>
</table>
3.9 Appendix F: Crystallographic Data for Compound 52

Low-temperature diffraction data (ω-scans) were collected on a Rigaku MicroMax-007HF diffractometer coupled to a Dectris Pilatus3R detector with Mo Kα (λ = 0.71073 Å) for the structure of 007c-18056. The diffraction images were processed and scaled using Rigaku Oxford Diffraction software (CrysAlisPro; Rigaku OD: The Woodlands, TX, 2015). The structure was solved with SHELXT and was refined against F² on all data by full-matrix least squares with SHELXL (Sheldrick, G. M. Acta Cryst. 2008, A64, 112–122). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms to which they are linked (1.5 times for methyl groups). The only exception is H16, which was found in the difference map and freely refined. The full numbering scheme of compound 007c-18056 can be found in the full details of the X-ray structure determination (CIF), which is included as Supporting Information. CCDC number XXXXXX (007c-18056) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Figure 3.12 The complete numbering scheme of 007c-18056 with 50% thermal ellipsoid probability levels. The hydrogen atoms are shown as circles for clarity.
Table 3.11 Crystal data and structure refinement for 007c-18056

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<tr>
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<td>P2₁/n</td>
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<td>b = 8.1681(3) Å</td>
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<td></td>
<td>c = 20.9570(8) Å</td>
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<tr>
<td>Z</td>
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<td>Density (calculated)</td>
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<td>Observed reflections (I &gt; 2sigma(I))</td>
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<td>Max. and min. transmission</td>
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<tr>
<td>Refinement method</td>
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<td>R indices (all data)</td>
<td>R1 = 0.0758, wR2 = 0.1712</td>
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<tr>
<td>Largest diff. peak and hole</td>
<td>0.621 and -0.535 e.Å⁻³</td>
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3.10 References


Natural Products: Overview and Recommendations for a Universal Nomenclature.


Thank you for placing your order on Marketplace™.

**Order Summary:**
Order date: 10 Jul 2020  
Order number: 1047704  
No. of items: 1  
Order total: 0.00 USD

Sincerely,  
The CCC Marketplace Team


Chapter 4

Synthesis of an unsubstituted [13]-macrodilactam: A study on conditions and functional group effects on RCM
4.1 Macrolactams versus macrolactones

4.1.1 Biology

Our next step was to take a deep look at the ring of Teixobactin and move to modify our ring system itself after working on the substituents. Coincidently, the size of our ring is the same as the ring in Teixobactin and the backbone structures are similar to each other. Instead of changing the ring size, we were interested in switching the heteroatoms along the backbone since 13-membered ring was the best fit for the binding and activity based on the Teixobactin analogs studies. Moreover, the NH groups from the lactam are presumably H-bonding to the phosphates of Lipid II.

Another inducement for the design of the new ring was the stability concern. The esterase assays on the representative C7-aryl [13]-macrodilactones described in Chapter 2 were performed to determine whether they were in fact substrates. There was evidence that the ester unit of the compounds were cleaved using pig-liver esterase. Based on the preliminary results, we hypothesized that amides should be more stable metabolically in serum than esters. Furthermore, research on Teixobactin analogs also supported that the lactam ring worked better over lactone ring for the bioactivities.

Taking advantage that macrolactams have more H-bonding character and less susceptible to hydrolysis than macrolactones, a new derivative of the original [13]-macrodilactone was proposed (Figure 4.1). An unsubstituted [13]-macrodilactam, 68, was targeted for synthesis. The idea is that it can be utilized as a model and precursor for a whole class of new [13]-macrodilactams.
4.1.2 Bonding

Compared with oxygen on macrolactone, an NH group is preferred to bind anions by forming a hydrogen bond to the anion and thus lead to enhanced antibiotic activity. The 4-atom ester unit will be replaced by 4-atom amide unit. Since the ester bond is more flexible than the amide bond, the [13]-macrodilactam will be more rigid than [13]-macrodilactone (Figure 4.1). The structural units that make up macrolactone and macrolactam affect the overall shape, and therefore the conformation of the macrocycle. The greater rigidity of the new unsubstituted [13]-macrodilactam should still allow it to adopt the ribbon conformation, which is the same as unsubstituted [13]-macrodilactone. By the appropriate addition of functional groups, a new conformation, in the context of the amides NH units, may enable the macrocycle to exhibit potential pharmaceutical properties.

4.2 Previous [13]-macrolactam/lactones

Our group has synthesized and characterized some C2 and C3 substituted [13]-macrocycles before without the evaluation of bioactivity. Besides the substituted [13]-macrodilactones, we have already synthesized some other substituted [13]-macrodilactams. Figure 4.2 shows two [13]-macrodilactams that we have previously

![Unsubstituted [13]-macrodilactone and [13]-macrodilactam](image-url)
prepared. One is mono-substituted on C2, while another is di-substituted on C3. We didn't do deep exploration or do X-ray crystal structure analysis for this specific class.

![Figure 4.2](image)

**Figure 4.2** Previously synthesized substituted [13]-macrolactam

We intended to revisit this class of macrocycles, starting with the simple, unsubstituted one. In this case, the physicochemical and biological studies can be conducted systematically for this whole new class. Additionally, the impressive Teixobactin discovery and these two single examples make the new compounds more accessible and have some potential therapeutically properties. The synthetic route can be optimized and derived from the previous one, which also make the synthesis work more approachable.

### 4.3 Unsubstituted [13]-macrolactam

#### 4.3.1 Synthesis

We used a three-carbon diol to connect two acids for the RCM when synthesizing [13]-macrolactones. Therefore, a modified strategy can be established based on that to obtain the desired unsubstituted [13]-macrolactam by using diamine to connect two acids. At the very beginning, route 1 was proposed for getting diene 67 (Scheme 4.1), because it can be used as a general procedure for both unsubstituted and substituted macrocycle as it prepared the precursor stepwise and substituents on the ring can be introduced from individual acylation. For route 1, it can provide unsymmetrical
macrodilactams via acylation with various substituted acid or diamine stepwise. However, it consumes more steps in the overall scheme. Route 1 commenced with the mono-protection of commercially available 1,3-diaminopropane 63 (80%). Then, the first acylation was conducted to provide alkene 65 (70-80%). The second acylation proceeded after the deprotection by TFA. Conditions we previously used for acylation (DCC & DMAP, HBTU & DIEA) were employed to route 1 (Scheme 4.2). Nevertheless, this route to make the product is still ambiguous. Because the deprotection is hard to get success and the second acylation always gave low yield and inefficient purification.

Scheme 4.1 Two routes for the synthesis of diene 67

Consequently, Route 2 (diacylation) in Scheme 4.1 was designed for the diene 67 to try on the unsubstituted precursor first. This route presents a more direct and efficient method, which can provide the product all at one. However, this route will only give
symmetrical macrocycles since the acids should be the same. So, its efficiency and applicability will be limited. Assembling a substituted diamine that has attachment on the \( \alpha \) position to the amine with acids is the only way to obtain an unsymmetrical product in this route.

**Scheme 4.2 Different conditions and details of Route 1**
Then, different acylation reagents have been screened for this reaction. HBTU and DCC led to impure intermediate products. After several attempts by adjusting the reaction concentration and time, the EDC condition proved to be the most effective one that can give up to 70% yield (Table 4.1). According to all these conditions, a relatively high concentration of diamine (0.13 M) is good for isolating the pure product after column chromatography. Reaction time can be extended to 2 days to increase the yield.

<table>
<thead>
<tr>
<th>entry</th>
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<th>equiv.</th>
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<th>time</th>
<th>product</th>
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<td>2</td>
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<td>HBTU</td>
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<td></td>
<td>DIEA</td>
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<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Acid</td>
<td>2</td>
<td>2.5</td>
<td>0.25 M</td>
<td>overnight</td>
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<td></td>
<td>HBTU</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>DIEA</td>
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<tr>
<td>3</td>
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<td>2</td>
<td>2.16</td>
<td>0.07 M</td>
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<td></td>
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<td>DMAP</td>
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<td>DCC</td>
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<td>EDC</td>
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4.3.2 Ring closing metathesis (RCM)

RCM was the next step to achieve the final product. It took a lot efforts to reach the goal of getting the macrocycle product. Since we had done RCM to prepare [13]-
macrodilactones successfully already, those original conditions were used and expected to be successful. However, the lack of success prompted us to investigate the reaction more deeply.

Table 4.2 lists all the catalysts and solvents have been used for the RCM. It was assumed that DCM worked better for this class. The choice of catalysts and concentration is not that important for this specific reaction. Furthermore, the reaction has also been tried in pressure vessel with higher concentration, the result was far away from expectation. It was intended to run the reaction at a pressure that was different from the ambient pressure to investigate if it could accelerate the reaction. However, the ethylene gas was given off during the RCM, which could make this condition disfavor RCM. It may even push the reaction backwards, which didn’t help with the RCM in general.

The purification also presented technical challenges as only tiny amount of impure product in purple color was recovered with various eluent system help. Many spots appeared on TLC plates, regardless if EtOAc/Hex or CH$_3$OH/DCM solvent systems were used. The best way to obtain this macrocycle is still unclear. Even some soft crystal like needle has already grown by using EtOAc, the yield is super low. Further efforts should be put to work for this RCM like trying other catalyst (Schrock) and solvent (THF).
Table 4.2 Conditions of RCM for unsubstituted [13]-macrodilactam

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<td>toluene</td>
<td>3 mM</td>
<td>No product</td>
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<tr>
<td>3</td>
<td>Grubbs'' II</td>
<td>DCM</td>
<td>7 mM</td>
<td>25% with impure product</td>
</tr>
<tr>
<td>4</td>
<td>Grubbs'' II</td>
<td>DCM</td>
<td>18 mM (pressure vessel)</td>
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</tr>
<tr>
<td>5</td>
<td>Hoveyda</td>
<td>toluene</td>
<td>3 mM</td>
<td>Little conversion</td>
</tr>
<tr>
<td>6</td>
<td>Hoveyda</td>
<td>DCM</td>
<td>17 mM (2 days)</td>
<td>Some conversion</td>
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</table>

These preliminary experimental results gave some tips for the follow-up research. Dichloromethane is a better solvent than toluene for RCM of this specific macrocycle. Both catalysts can help with RCM while not efficient. Neither adjusting concentration nor increasing the pressure of this reaction improved the way to produce product. Although column purification was employed as well as recrystallization, an explicit procedure was still in urgent demand.

So, some study of the RCM and the factors that play a role in it will help with this requirement. Ring closing metathesis (RCM) reaction has been widely used in macrocycle synthesis. Catalyst is a major topic in the study of RCM. Ruthenium-based and molybdenum-based are two most common catalysts among them. The strategy our

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group established was applying Ru-2 (Figure 4.3), also a ruthenium-based catalyst, to the RCM reaction. The first well-defined ruthenium-based one was discovered in 1992 by Grubbs group.\(^5\) Then the Ru-0, which is effective in the RCM, was developed from the first one and prepared by reacting 3,3-diphenylcyclopropene with RuCl\(_2\) (PPh\(_3\))\(_4\) and substituting PPh\(_3\) with PCy\(_3\).\(^6,7\) Replacing one of the phosphine ligand of Ru-1 with a N-heterocyclic carbene (NHC) ligand will give a new complex, Ru-2, known as Grubbs’ II, to favor the dissociation of the phosphine from ruthenium and improve the reactivity of catalytic activity in RCM.\(^4\) Another representative ruthenium catalyst is Hoveyda-Grubbs catalyst (Ru-4), which was reported by Hoveyda group in 2000. Ru-4 possesses several advantages like high reactive, recyclable and stable in air and moisture to be applied in a large substrate scope.\(^8\)

![Figure 4.3 Commonly used Ruthenium catalysts for RCM](image)

Thermodynamic and kinetic factors are always potentially parameters that play an important role in the RCM. And speaking of thermodynamic factor, the noticeable unfavored entropy and enthalpy problem certainly bring difficulties to the macrocycle formation. The entropic concern of the RCM is raised from the increasing translational and rotational freedom it caused during the reaction. A conformational element such as preorganization can be introduced to solve this problem to some extent. Furthermore, the late stage functionalization of macrocycle is also a challenge because of their low-energy conformations and transannular interactions.\(^9\)
There are several ways can be introduced to promote the RCM, in which conformational pre-organization is common approach. It includes inducing conformational constraints by substituents, using tunable protecting groups, relieving ring strain by planar centers and introducing non-covalent interactions.\textsuperscript{10} In the following section, the Thorpe–Ingold effect of substituents will be discussed based on the experimental results.

Structural restriction of rotation is an important factor to reduce the entropic effect in RCM. Thorpe–Ingold effect is therefore introduced to explain the efficient RCM for ring formation in the presence of conformational constraints.\textsuperscript{11} Track back to the examples of our group (Figure 4.2), the successful synthesis of our C2 mono- and C3 di-substituted [13]-microdilactams is also a strong support for this argument. The conformational constraints can be a gem-dimethyl group, a ring or any elements initiating rigidity.\textsuperscript{4} Ring strain in the product is also an important factor that influence the RCM. It is easily to increase because transannular interaction between atoms is forced into proximity from opposite sides of the ring.\textsuperscript{12} It was found that the selectivity of RCM over acyclic dimers formation would benefit a lot from adjusting the number of methylenes on the diene chain.\textsuperscript{13,14}

Additionally, the enantioselectivity of the RCM products are affected by the factors like ring size and bulky substrate group (ie. NHBoc group) in several cases.\textsuperscript{4}

\textbf{4.3.3 Thorpe–Ingold effect}

Thorpe–Ingold effect among the factors mentioned above will be further discussed in detail. This effect, first raised by Thorpe and Ingold in 1915\textsuperscript{11}, is also known as gem-dimethyl effect (Figure 4.4). It was proposed that the repulsion of gem-dimethyl group in an open carbon chain induced an increase in angle $\alpha$ with a decrease in angle $\beta$. $\alpha$
represents the angle between to substituents on the atom, while $\beta$ represents the internal angle the main chain of the molecule forms. It resulted in the closer position of X and Y, which made it easier to perform a cyclization. Then in 1960, Allinger and Zalkow found that both entropy and enthalpy favored the cyclized product of substituted hexanes.\textsuperscript{15} One thing to be noted about this concept is that when “Thorpe-Ingold effect” is mentioned, it is more about the change of the angle in the reaction; while “\textit{gem}-dimethyl effect” is more about the overall acceleration of the reaction.\textsuperscript{16}

![Diagram of gem-dimethyl effect]

\textbf{Figure 4.4} Demonstration of \textit{gem}-dimethyl effect

Intramolecular reactions normally proceed easily than their intermolecular ones due to the favorable entropy change in the transition state. However, some intramolecular reactions are difficult because of the increased ring strain. In this case, dimethyl group was introduced on the chain to promote the reaction. Because the adjacent C-C bond can adopt as an anti-conformation to present a bend of the main chain for the accessible ring closure.\textsuperscript{16}

Unlike small ring, the research of the Thorpe–Ingold effect in the macrocycle like large-membered lactone revealed that the great influence may be diminished because there are several other factors immerged in the more complex structure. The entropy has less to do for Thorpe–Ingold effect since the rotational freedom of large ring is similar to that of the open-chain form.\textsuperscript{15} The substituted group is not limited to methyl group. There are several examples like difluoro group\textsuperscript{17}, tert-butyl group\textsuperscript{18} and \textit{etc.} show their ability to
restrict the substrate chain as well. In other words, other functional groups or ring on the chain can also promote the organic transformations like RCM.

Thorpe–Ingold effect has also been considered as an important element when process an inter/intramolecular cyclization in peptide chemistry. Both yield and selectivity of nitrogen-contained macrocyclization can be benefit from this effect.\textsuperscript{19} Also, there is evidence showing that the \textit{gem}-dimethyl group is able to help with the stability of natural products\textsuperscript{20} and even catalysts.\textsuperscript{21}

To sum up, Thorpe–Ingold effect is possible to explain the difficulty when running RCM of our unsubstituted diene precursor. Lack of the substituted groups on the backbone of the molecule resulted in a larger internal $\beta$ angle. And the molecule became flexible to increase the ring strain. All these maybe the factors that brought difficulties to the RCM reaction. Installing some \textit{gem}-alkyl group at a specific atom is worth trying for the future research of this class of compounds to accomplish a successful RCM. In our previous synthesis, the yield of RCM for compound 59 and 60 was 72\% and 46\%, respectively (Figure 4.2). Both yields are higher than the RCM of unsubstituted one (25\%), which is also a strong support for this proposed synthesis idea.

\textbf{4.4 Conclusion}

The design of new unsubstituted [13]-macrodilactams inspired by our [13]-macrodilactone and Teixobactin. The C7-amino [13]-macrodilactones class was inspired by the structurally similarity with Teixobactin. The [13]-macrodilactams can be regarded as an advanced version of it. It both benefit from the biology and bonding aspects to be a candidate with potential pharmaceutical properties.
The most basic and unsubstituted member of [13]-macrodilactam class have been successfully obtained and characterized. However, the route for the synthesis of this class still need to be improved especially the key RCM step. Thorpe–Ingold effect can be an aspect to considered for installment of the groups on the backbone to help with the reaction. Also, a derived project can be continued to introduce various substituted acids for the diversification of this [13]-macrodilactam class. Both symmetrical and unsymmetrical macrocycles can be prepared by the proposed scheme in this chapter. The future direction can be focus on the optimization of the scheme as well as the efficient derivatization of the compounds.
4.5 Experimental

4.5.1 1,3-Propane diamine di-(4-pentenamide) 67

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.621 g, 4.00 mmol) and N,N-dimethylaminopyridine (DMAP) (0.122 g, 1.00 mmol) were dissolved in 10 mL DCM, and the solution was cooled to 0 °C on an ice bath. 4-pentenoic acid (0.4 g, 4.00 mmol) was added and the mixture was stirred at the same temperature for 15 min. Then, 1,3-Diaminopropane (0.148 g, 2 mmol) in DCM was added to the mixture which was stirred for two days at room temperature. The reaction mixture was then filtered through a short pad of Celite, the solvent was removed from the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography to give diene 67 as a white solid (333 mg, 70 %). mp 129.1-130.6 °C; Rf 0.1 (100% EtOAc); ¹H NMR (CDCl₃) 400 MHz: δ 6.30 (s, br, 2H), 5.82 (dddd, J = 16.7, 10.2, 6.4, 6.4 Hz, 2H), 5.09 (dd, J = 3.2, 1.6 Hz, 1H), 5.05 (dd, J = 3.2, 1.6 Hz, 1H), 5.02 (dd, J = 2.8, 1.3 Hz, 1H), 4.99 (dd, J = 3.0, 1.4 Hz, 1H), 3.30–3.25 (m, 4H), 2.5–2.34 (m, 4H), 2.30 (dd, J = 8.0, 6.6, 1.2 Hz, 4H), 1.67–1.52 (m, 2H); ¹³C NMR (CDCl₃) 100 MHz: δ 173.2, 137.0, 115.7, 36.1, 35.7, 29.9, 29.8. TOF HRMS (ESI): m/z calcd for C₁₃H₂₃N₂O₂ [M+H]+: 239.1760; found: 239.1741.
4.5.2 [13]-Macrodilactam 68

Grubbs’ second-generation catalyst (0.022 g, 0.026 mmol) was added as a solid in one portion to a solution of the diene (0.124 g, 0.52 mmol) in DCM (75 mL) under nitrogen. The mixture was heated to reflux overnight (18 h), then the mixture was allowed to cool to room temperature. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to give [13]-macrodilactam 68 as a white solid (27 mg, 25%). mp 120.2-122.0 °C; Rf 0.21 (5% CH₃OH/DCM); ℏH NMR (CDCl₃) 400 MHz: δ 6.08 (s, br, 2H), 5.55 (t, J = 3.3 Hz, 2H), 3.38 (dd, J = 5.7, 5.7 Hz, 4H), 2.35–2.27 (m, 8H), 1.77–1.72 (m, 2H); ℏC NMR (CDCl₃) 100 MHz: δ 172.9, 130.7, 39.5, 37.0, 29.7, 28.4.
4.6 References


(18) Zhao, D. C.; Tidwell, T. T. A Stable and Persistent Bisketene: 2, 3-Bis (Trimethylsilyl)-1, 3-Butadiene-1, 4-Dione. *Journal of the American Chemical Society* **1992**, *114* (27), 10980–10981.


