Design and Synthesis of Natural and Unnatural Macrocycles

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Chemistry in the Graduate School
of Duke University

2019

ABSTRACT

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Abstract

Macrocycles have received growing appreciation for their potential in probing a broad biological space, and there are a significant number of bioactive macrocycles in nature. In addition, a macrocyclic scaffold provides interesting features, including high binding affinity and target selectivity due to the pre-organized conformation. Despite the therapeutic potential and valuable pharmacological characteristics of this class of scaffolds, they have been poorly exploited in the field of drug discovery because of synthetic challenges and incompatibility to the conventional Lipinski's rule of five. Therefore, efficient approaches for the generation of diverse and complex macrocycles are needed to develop novel macrocyclic agents.

In an attempt to construct a diverse set of macrocycles, we designed a natural product-like macrocycle library containing a tetrahydropyran core. Our strategy for the generation of the library is based on the tandem allylic oxidation/oxa-conjugate addition for the stereoselective synthesis of a 2,6-*cis*-tetrahydropyran ring. The incorporation of various building blocks and the utilization of diverse macrocyclization methods were carried out to rapidly increase skeletal diversity and complexity. Appendage diversification was performed to further modulate the physicochemical properties of the macrocycles. Cheminformatic analyses demonstrated that our macrocycle library covers

a distinct chemical space compared to drug-like space, while significantly overlapping with macrocyclic natural product space.

We also explored a modular strategy for the synthesis of a macrocycle from nature, forazoline A, a polyketide natural product. Forazoline A exhibits potent in vivo efficacy against the fungal pathogen *Candida albicans*. Our approach towards the synthesis of forazoline A relied on the construction of three major fragments with the desired stereochemistry and the assembly of those fragments. In the dissertation, the synthetic studies of cyclohexane, thiazolidine, and alkyl chain fragments were introduced. Construction of the highly functionalized cyclohexane moiety was efficiently accomplished utilizing Mukaiyama aldol, Bayer–Villeger reaction, and addition of lithium dimethyl cuprate. Synthesis of the thiazolidine fragment was investigated via oxa-conjugate addition and Pummerer-type rearrangements. Finally, the alkyl chain fragment was synthesized through epoxidation, regioselective ring opening, and β-ketoester formation.

Dedication

To my parents for their unconditional love and support

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List of Abbreviations

Å	angstrom
Ac	acetyl
AcOH	acetic acid
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Boc	*t*-butyloxycarbonyl
brsm	based on recovered starting material
bs	broad singlet
Bz	benzoyl
CDI	1,1'-carbonyldiimidazole
COSY	^{1}H-^{1}H correlation spectroscopy
CSA	camphorsulfonic acid
CuAAC	Cu(I)-mediated azide/alkyne cycloaddition
Cy	cyclohexyl
d	doublet
d	chemical shift (ppm)
DBU	1,8-diazobicyclo[5,4,0]undec-7-ene
DCC	*N*,*N*′-dicyclohexylcarbodiimide

dd	doublet of doublet
DDQ	2,3-dichloro-5,6-dicyano-p-benzoquinone
DET	diethyl tartrate
DFT	density functional theory
DIBAL	diisobutylaluminum hydride
DIC	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)-pyridine
DMF	*N*,*N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethylsulfoxide
dt	doublet of triplet
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	ethyl
FDA	the Food and Drug Administration
HATU	(1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HMQC	heteronuclear multiple quantum coherence
HRMS	high-resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration

LCMS	liquid chromatography–mass spectrometry
LiHMDS	lithium bis(trimethylsilyl)amide
m	multiplet
m-CPBA	3-chloroperbenzoic acid
Me	methyl
MNBA	2-methyl-6-nitrobenzoic anhydride
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NHC	*N*-heterocyclic carbene
NMR	nuclear magnetic resonance
PCA	principal component analysis
Ph	phenyl
PMB	*para*-methoxybenzyl
PMI	principal moments of inertia
PPIs	protein-protein interactions
Pr	propyl
q	quartet
s	singlet
t	triplet

TBAF	*tetra*-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	*para*-toluenesulphonyl

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1. Introduction

1.1 Macrocycles in Drug Discovery and Chemical Biology

The development of small molecules that can perturb various biological processes has long been an important goal in drug discovery and chemical biology.[1-2] Through synthetic and medicinal chemistry efforts, combined with a better understanding of biomolecule structures and functions, a significant number of bioactive small molecules has been developed and clinically validated.[3] By mimicking the interactions of receptors or enzymes with endogenous ligands or substrates, small molecules have been designed or modified. The resulting compounds have sufficient functionalities to tightly bind to hydrophobic pockets in the protein targets (Figure 1). However, such targets represent only a fraction of disease-relevant biomolecules, and small-molecule drugs are not highly effective to interact with targets presenting extended binding surfaces including protein–protein interactions (PPIs). This is partially due to the fact that the selection of the small molecules is often guided by the Lipinski's rule of five[4] to increase the probability of obtaining favorable physicochemical properties including oral bioavailability and cell permeability. In conjunction with this guideline, synthetic tractability for the generation of a large amount of compound collections is a crucial factor in pharmaceutical settings for screening therapeutic candidates. As a result, small molecule drugs typically represent simple and flat

structures with molecular weights less than 500 which are not sufficient sizes for addressing larger targets.

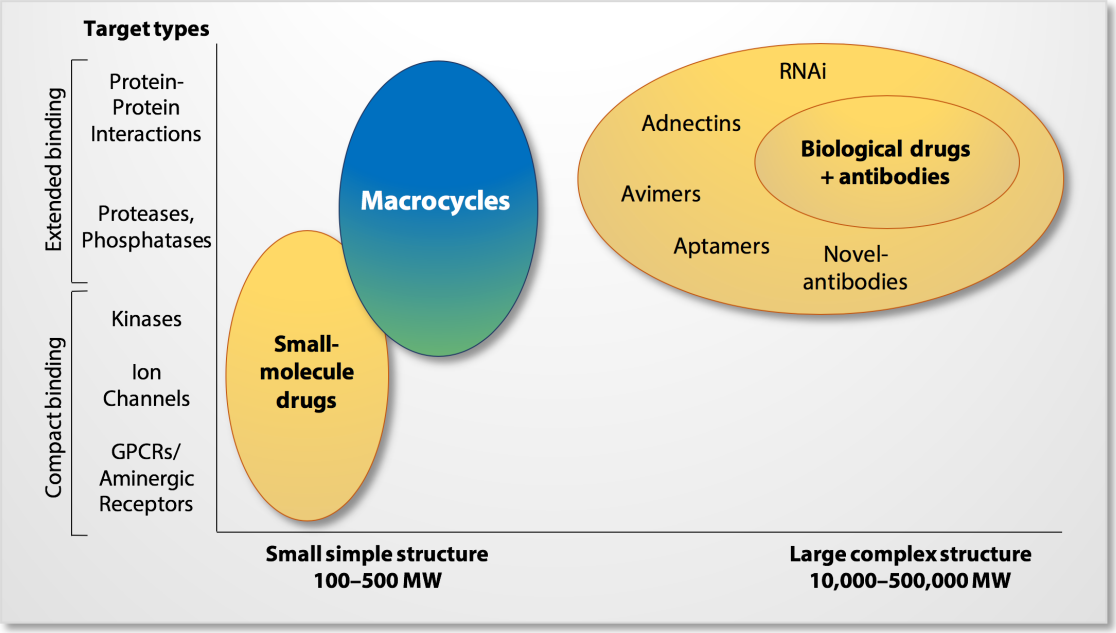


Figure 1. The potential of the macrocycle positioned between small-molecule drugs and biological drugs (Figure redrawn with the permission from ref.[5])

To overcome this challenge, researchers have developed biologics as a new tool for disrupting larger biomolecular interactions.[6] The utilization of monoclonal antibodies, artificial proteins, and semi-synthetic peptides have revolutionized this field, providing new therapeutic opportunities. Although they have shown high potency towards protein–protein interactions and other larger targets, there are significant limitations such as low cell permeability, high cost, and reduced patients compliance. Therefore, the need exists for the discovery of new chemotypes that have sufficient sizes

and functionalities to interact with larger targets but still maintain drug-like characters and favorable pharmacological properties. In this regard, macrocycles have a potential to be positioned between small-molecule drugs and biological drugs in chemical space.[5]

Macrocycles are an interesting class of small molecules which generally contains 14 or more atoms in ring systems. This cyclic scaffold provides structural pre-organization that can lead to an improved binding affinity and target selectivity by minimizing unnecessary conformational changes.[7-9] It is noteworthy that macrocycles are not rigid structures, rather balanced between structural pre-organization and sufficient flexibility to tightly bind to target surfaces.[7] This constrained flexibility of a macrocycle may contribute to enhanced cell permeation by formation of intramolecular hydrogen bonds.[10-11] However, due to this cyclic nature of scaffolds, the rationale design of macrocycles has been considered challenging using fragment-based, computational, or other conventional approaches for drug discovery.[12]

In order to guide the design of new macrocyclic compounds, Villar *et al.* analyzed the physicochemical properties of existing bioactive macrocycles (Figure 2).[13] They analyzed the chemical properties of the macrocycles from known macrocycle-protein crystal structures (MC test set) along with those of macrocycle drugs and all oral drugs that have been approved by the US Food and Drug Administration (FDA). The result showed that the macrocycle test set seems to resemble the subset of orally available drugs containing a macrocyclic scaffold. Notably, the data represented that

oral macrocycle drugs are not compatible with the conventional Lipinski's rule of five showing larger molecular weights, extended polar surface areas, and more rotatable bonds. They also analyzed the binding geometry of the macrocycles and quantified binding areas between macrocycle ligands and proteins, thus providing an initial clue for designing bioactive macrocycles.

Intrigued by favorable pharmacological properties and bioavailability, there have been endeavors to discover natural or unnatural macrocyclic compounds with potent biological activities outside the conventional metrics. The development of macrocyclic compounds in drug discovery will be briefly discussed in the following chapter.

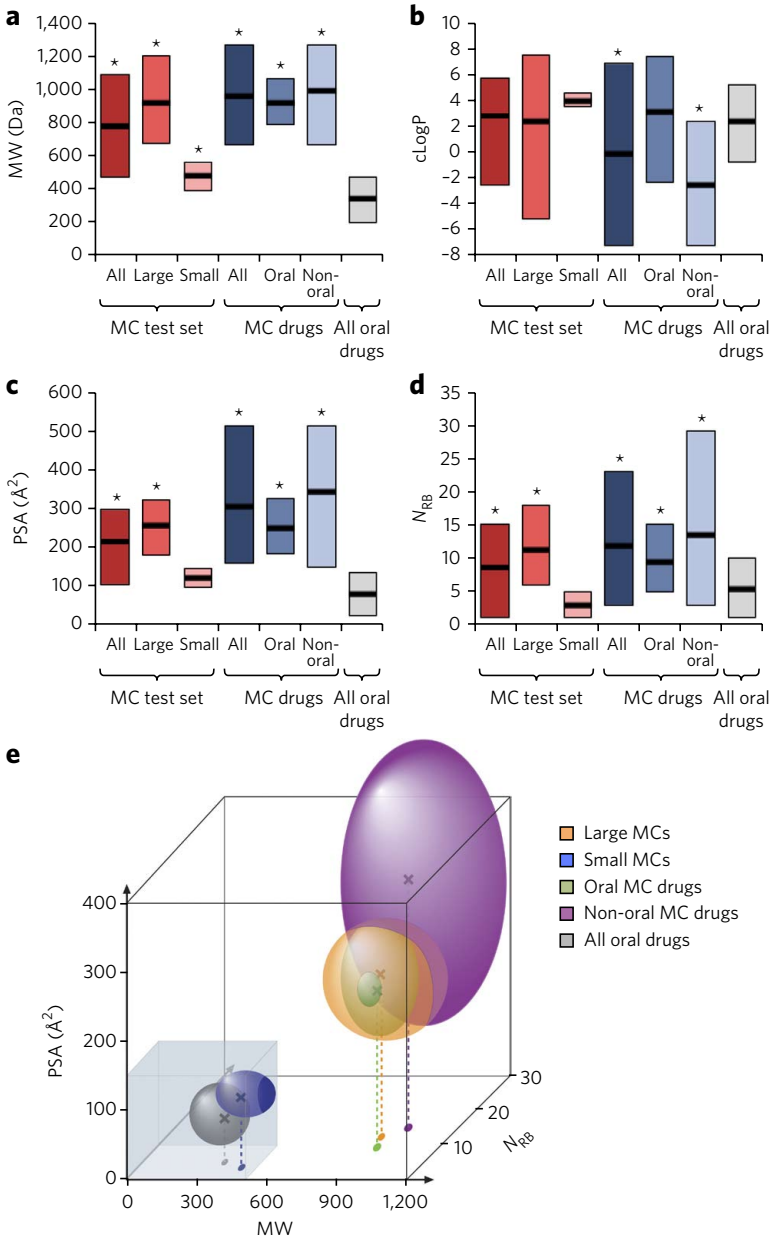


Figure 2. Analysis of physicochemical properties of macrocycles that have co-crystal structures with proteins, macrocyclic oral drugs, and all oral drugs; The macrocycle test set contains large macrocycles with MW >600 Da and small macrocycles with MW < 600 Da; The properties include molecular weight **(a)**, cLogP **(b)**, polar surface area **(c)**, and the number of rotatable bonds **(d)**. The distribution of the conventional oral drugs, macrocycle drugs, and the MC test set in chemical space were shown in **(e)** (Figure reprinted with permission from ref.[13])

1.1.1 Bioactive Macrocycles in Nature

Naturally occurring macrocycles have provided great opportunities to exhibit an impressive record of efficacy in modulating disease-relevant biopathways. In 2014, Giordanetto and Kihlberg reported that 68 macrocycle drugs have been approved for human use by FDA and 35 macrocyclic compounds are in clinical development.[14] Among the 68 macrocycle drugs in clinical use, 48 macrocycle drugs are natural products and 18 macrocycles are directly derived from natural products. The analysis showed that 34 compounds have been used to treat infectious diseases while 10 macrocycles are applied in oncology (Figure 3a). A large portion of the macrocycle drugs are macrolides and cyclic peptides, and it is noteworthy that the majority of the oral macrocycle drugs belong to the macrolide classes (Figure 3b).

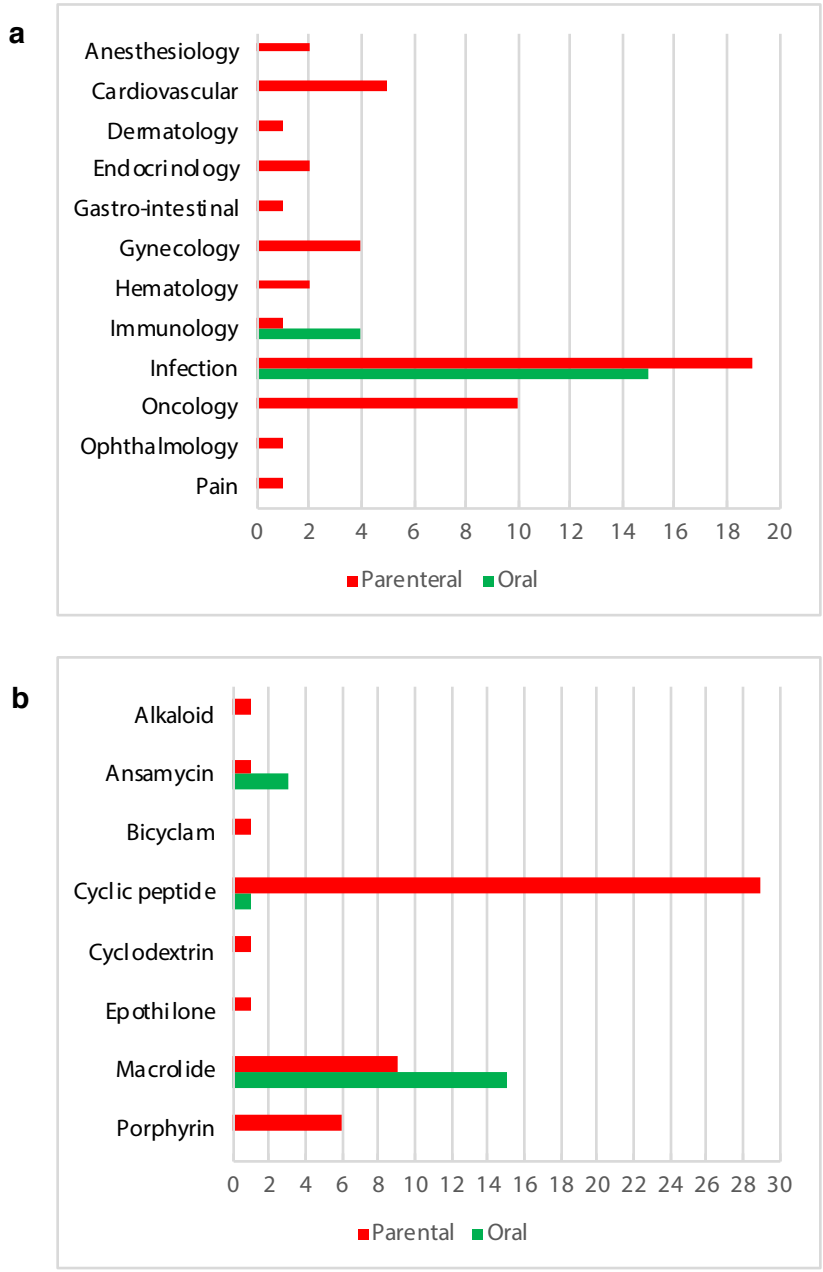


Figure 3. Distribution of oral (green bars, $N = 19$) and parenteral (red bars, $N = 49$) macrocyclic drugs across different therapeutic indications (**a**) and chemical classes (**b**) (Figure redrawn with permission from ref.[14] Copyright (2014) American Chemical Society.)

Figure 4 shows examples of non-peptidic macrocycle drugs identified and developed from natural sources. Macrolides for the treatment of infectious diseases include amphotericin B[15] and erythromycin[16] that are originally isolated from bacterial species. Rapamycin[17] and FK506[18] are immunosuppressants prescribed for patients who received organ transplantation. Not only these parent molecules, but a variety of analogs have been synthesized and investigated for the development of macrocycle drugs with improved properties.[19-20]

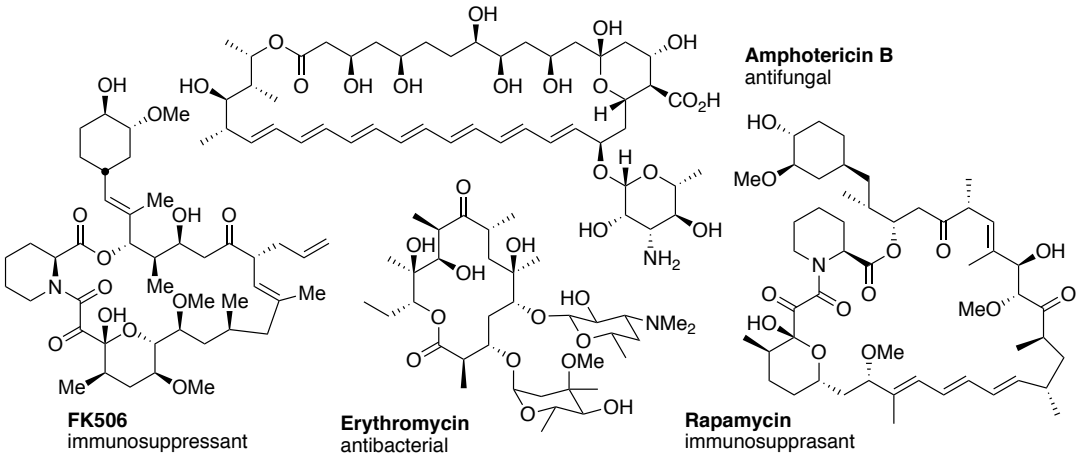


Figure 4. Examples of macrocyclic natural products approved by FDA

1.1.2 Synthetic Macrocycles with Therapeutic Applications

In conjunction with the discovery of bioactive macrocycles from nature, there has been growing efforts in the development of synthetic macrocycles for therapeutic uses (Figure 5). Eribulin is one example of synthetic macrocycles that are currently used in clinic. This antitubulin agent was developed and optimized by truncating the structure

of a marine natural product halichondrin B.[21-22] Temsirolimus is another drug developed by the exploitation of a macrocyclic natural product as a starting point. The late-stage modification of rapamycin yielded this mTOR kinases inhibitor which has been used for the treatment of advanced renal cell carcinoma.[23-24] In addition, high-throughput-screening (HTS) of macrocycle compound collections has identified hit compounds. TZP-101 was discovered from a 10,000-membered macrocycle library constructed using a proprietary solid phase parallel synthesis platform.[25] Screening of the macrocycle library against gastrointestinal targets by Tranzyme Pharma produced a clinical candidate, TZP-101, as a selective agonist of the ghrelin receptor.[26] This biologically active macrocycle PPI inhibitor shows the potential of macrocycles as powerful tools for targeting larger protein surfaces.

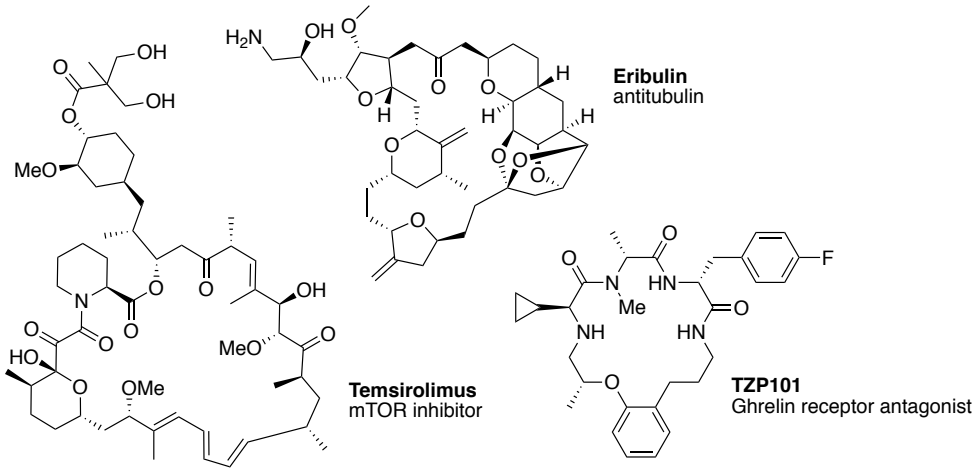


Figure 5. Examples of non-natural macrocycle drugs and a clinical candidate

Despite the beneficial characteristics and therapeutic potentials of macrocyclic scaffolds described above, this compound class has been underexplored and poorly exploited for the discovery of drugs and chemical probes. This is mainly due to the fact that the generation of complex macrocycles, especially non-peptidic macrocycles, needs extensive synthetic efforts. Structural complexity and stereochemical density of macrocyclic compounds often causes difficulties in analog synthesis, which further impede the design and use of these scaffolds. In regard to this matter, our strategies for the construction of complex natural and unnatural macrocycles will be introduced, using fragment assembly that allows the efficient modification on ring systems.

1.2 Goals of Dissertation

Considering the therapeutic potential of natural or unnatural macrocycle compounds with favorable properties, there has been growing needs for the discovery and development of a new class of macrocycles. In this regard, this dissertation will be focused on the synthesis of a natural product and natural-product-like compounds with a macrocyclic core. The goal of chapter 2 is to introduce a convergent synthetic strategy for the construction of a natural-product-like macrocycle library containing a high degree of chemical diversity. By adopting nature's design, tetrahydropyran-containing macrocycles have been generated via a tandem allylic oxidation/oxa-conjugation reaction. Cheminformatic analyses were utilized to demonstrate physicochemical and structural diversity of the designed macrocycles. Chapter 3 will focus on the synthetic studies towards the total synthesis of an antifungal natural polyketide, forazoline A. This chapter aims to describe the retrosynthetic analysis of the natural macrocycle and investigate the asymmetric synthesis of the three fragments consisting the polyketide backbone. Ultimately, the goal of the dissertation would be to provide valuable insights for designing and synthesizing macrocyclic small molecules through the studies on both natural and unnatural macrocycles.

2. Design and Synthesis of a Natural-Product-Like Macrocycle Library Containing Tetrahydropyran Moieties

(published in *Chem. Eur. J.* **2019**, *25*, 6500–6504)

2.1 Introduction

2.1.1 Limitations of Conventional Synthetic Libraries

Screening technologies of small-molecule libraries against various biological systems have served as crucial platforms for discovering new chemical modulators. Consequently, there have been extensive efforts for generating small-molecule libraries to identify potential drug candidates.[27-28] Although such endeavors have accomplished the development of lead compounds for new therapeutics, the majority of current small-molecule drugs address a relatively narrow range of protein families. In 2017, Overington and co-workers examined the distribution of the human protein families targeted by The Food and Drug Administration (FDA)-approved drugs.[29] The analysis revealed that rhodopsin-like G protein-coupled receptors (GPCRs), ion channels, kinases, and nuclear hormone receptors were dominant, as shown in Figure 6. Furthermore, Hopkins and Groom estimated that existing drug-like molecules can target only ~10–14% of the proteins encoded in the human genome and only a subset of these proteins are related to human diseases.[30] As a result, biological targets that are hard to

address using current drug-like molecules have been often regarded as intractable, which include protein–protein interactions and protein–nucleic acid interactions.[31]

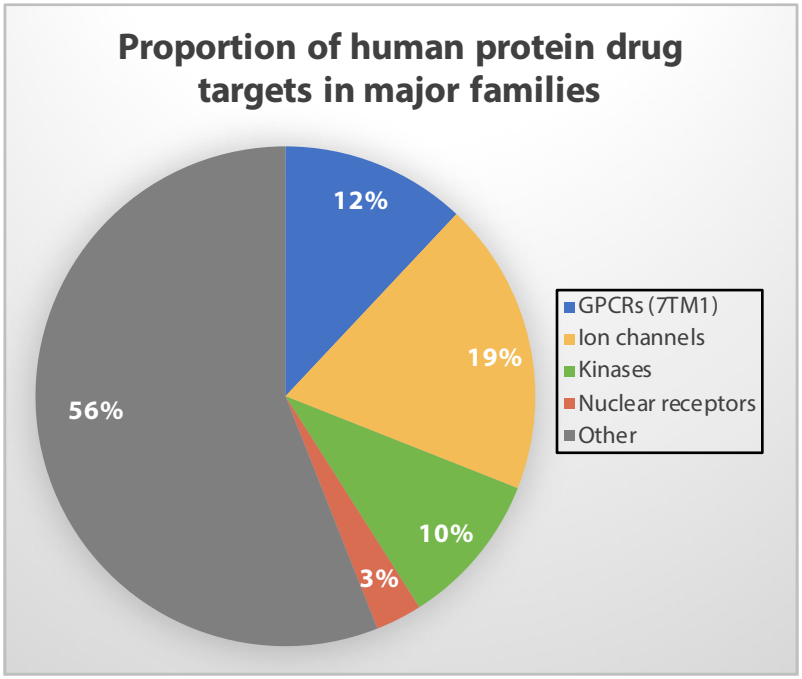


Figure 6. Proportion of human protein drug targets in major families (Figure reproduced with the permission from ref.[29])

It has been considered that one of the main reasons of target limitation is a low degree of structural/physicochemical diversity in conventional synthetic libraries.[32] It is notable that conventional drug-like molecules are often designed based on Lipinski's rule-of-five[4] which is derived from common features of existing drugs, to increase the likelihood of presenting favorable physicochemical properties. Although it has been utilized as a rapid and convenient tool for evaluating drug-likeness, the small molecules that fall within the rule-of-five share a high degree of structural similarity with one

another. Furthermore, there are exceptions, especially natural products, that are outside the rule-of-five chemical space but still exhibit favorable oral absorption.[33] Another factor contributing to a limited occupancy in chemical space is that traditional drug-like molecules have relatively simple scaffolds and low sp^3/sp^2 ratios due to their high synthetic accessibility (Figure 7).[34] Therefore, exploring a broader range of chemical space becomes an important aim in order to increase biological target diversity.

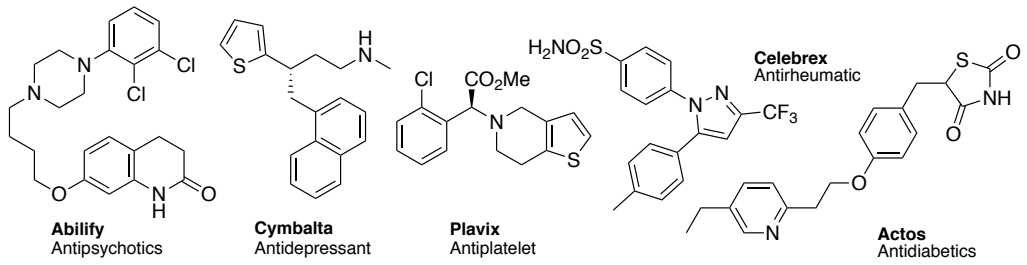


Figure 7. Examples of best-selling small molecule drugs

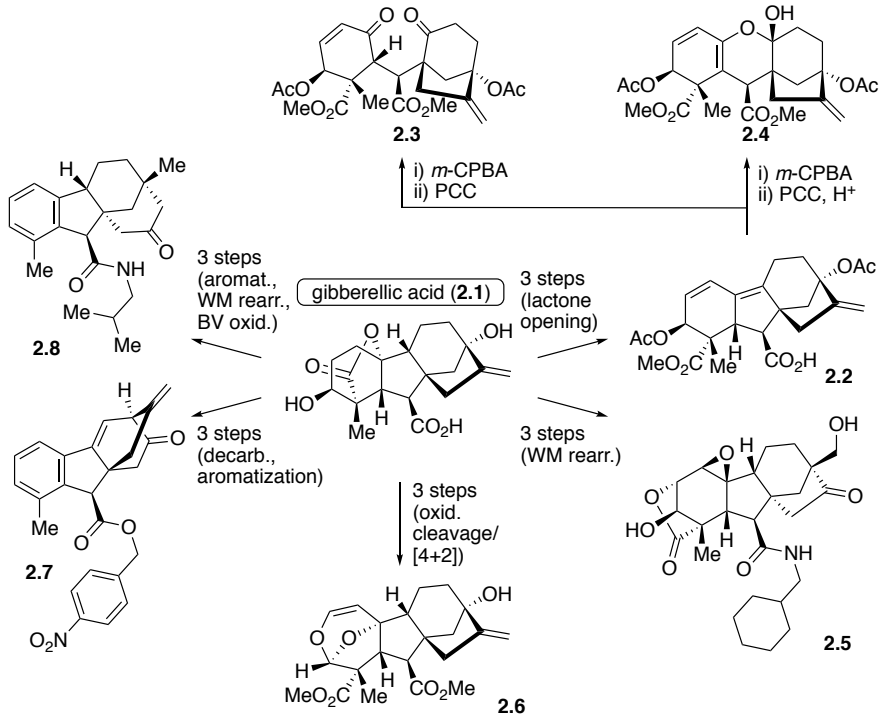
2.1.2 Natural-Product-Like Chemical Libraries

Natural-product-like small-molecule libraries have been generated to discover new lead compounds and chemical probes in the drug discovery field, since the natural product pool offers a high degree of skeletal diversity and structural complexity along with potent biological activities.[35-36] In order to provide natural-product-like compounds for screening, several strategies have been introduced.[37] The first strategy is the construction of compound collections by utilizing available natural products as starting points. Second, a divergent strategy can be adapted from biosynthetic design in nature

for the generation of complex and diverse molecules. Lastly, structurally simplified or truncated natural product scaffolds can be generated in order to provide a sufficient number of compounds for screening. Here, three examples of those strategies are described.

Hergenrother and co-workers reported a synthetic strategy for the creation of natural product-like compounds with high scaffold diversity using readily available natural products as common intermediates.[38] With combinations of ring-expansion, ring-fusion, and ring-rearrangement reactions, natural products were divergently transformed into complex compound collections. As an example, gibberellic acid **2.1** was utilized as a common intermediate for the synthesis of structurally diverse polycycles (Scheme 1). Gibberellic acid is a plant hormone isolated from *Gibberella fujikuroi*[39], containing a tetracyclic diterpene core with a fused lactone, two allylic alcohols, an exocyclic olefin and a carboxylic acid. These synthetic handles enabled the selective and independent functionalization of each ring of the core structure through a variety of reactions that distort the ring system. Lactone opening reaction of **2.1** followed by derivatizations on the carboxylic acid and alcohols produced tetracyclic triene **2.2**, which was further diversified into two scaffolds **2.3** and **2.4** using neutral or acidic oxidative conditions. Based-promoted lactone rearrangement of gibberellic acid **2.1** followed by amidation, epoxidation, and Wagner–Meerwein (WM) rearrangement furnished compound **2.5**. Cleavage of the lactone ring in **2.1** followed by methylation, oxidative

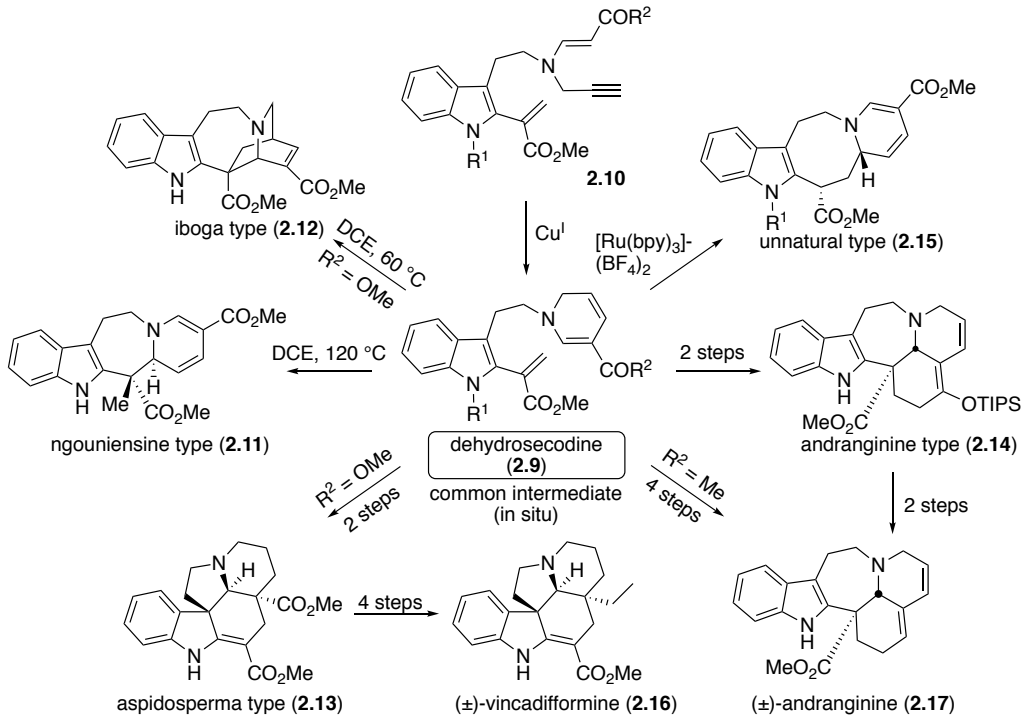
cleavage of the diol, and subsequent intramolecular [4+2] cycloaddition afforded acetal **2.6**. Two different sets of three-step reaction sequences efficiently built up the scaffolds **2.7** and **2.8**. The synthesized compound collections showed richer stereochemical content compared to that of commercial small-molecule libraries.



Scheme 1. Hergenrother's ring-distortion strategy to build natural product-derived diverse scaffolds from gibberellic acid[38]

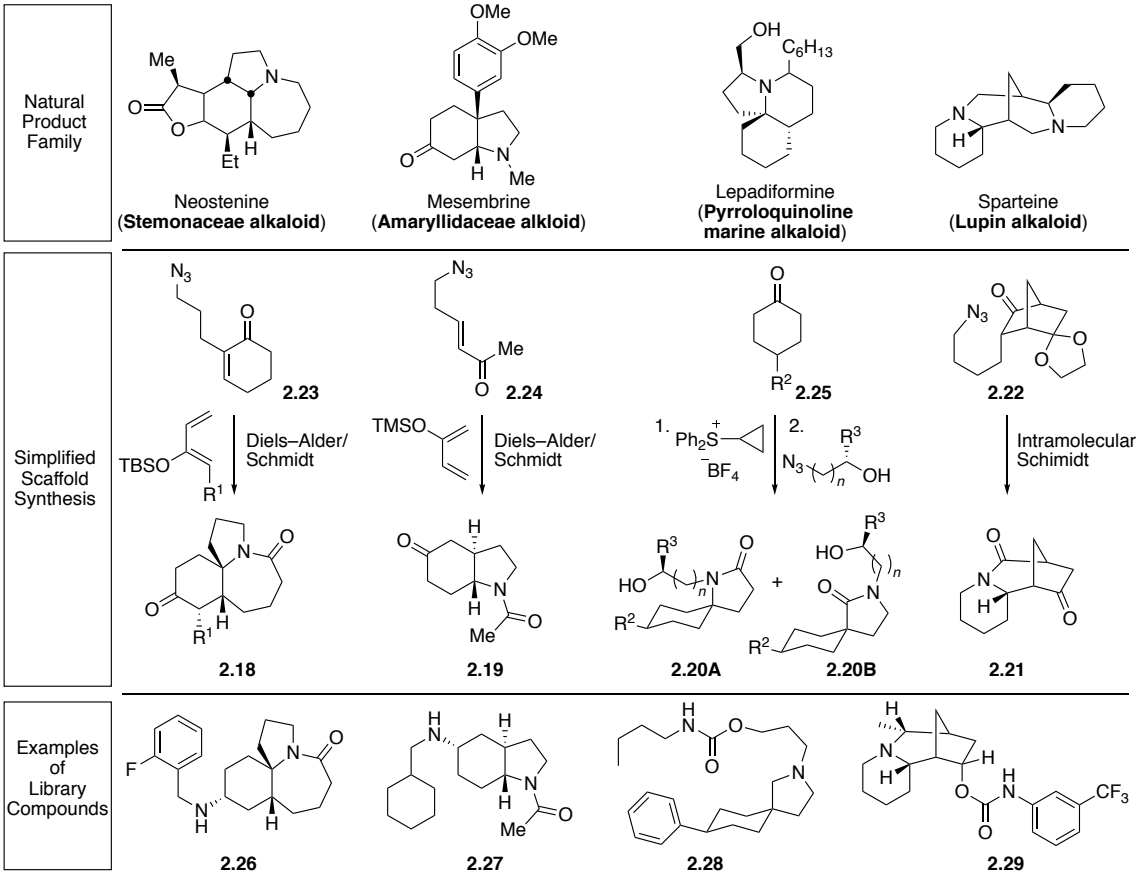
Oguri and co-workers designed the divergent synthesis of indole alkaloid-type compounds via a variety of intramolecular cyclizations of a polyunsaturated common intermediate **2.9** (Scheme 2).[40] This idea was inspired by biosynthetic assembly lines in nature, employing dehydrosecodine which has been proposed as an intermediate in the

biosynthesis of many indole alkaloids.[41] The synthesis began with the preparation of the common intermediate **2.9** in situ from a precursor enyne **2.10** through Cu(I)-catalyzed dihydropyridine formation. With the resulting intermediate **2.9**, different types of intramolecular cyclization were explored to provide four naturally occurring scaffolds (**2.11**–**2.14**) and non-natural type structure **2.15** in a divergent manner. Redox-mediated cyclizations produced either ngouniensine-type scaffold **2.11** or unnatural-type skeleton **2.15**. In addition, [4+2] cyclizations furnished iboga-type **2.12**, aspidosperma-type **2.13**, and andranginin-type scaffold **2.14**. The versatility of this synthetic approach enables the concise total syntheses of natural products (±)-vincadifformine **2.16** and (±)-andranginine **2.17** within 12 steps from tryptamine.



Scheme 2. Biosynthesis-inspired approach to furnish diverse alkaloidal scaffolds[40]

Aubé and co-workers reported the construction of a natural product-like compound library based on four families of biologically active alkaloids including Stemonaceae alkaloids, pyrroloquinoline marine alkaloids, lupin alkaloids, and Amaryllidaceae alkaloids.[42] In order to improve versatility and synthetic tractability, the original structures were simplified to produce the key scaffolds **2.18**–**2.21** while retaining attractive components (Scheme 3). Stemonaceae and Amaryllidaceae alkaloid scaffolds (**2.18** and **2.19**) were prepared using a Diels–Alder/Schmidt reaction. The scaffolds **2.20A** and **2.20B** were constructed through sulfur ylide-mediated spiroannulation followed by ring expansion using an azido alcohol. Lastly, azide **2.22** was converted into the tricyclic sparteine **2.21** via the intramolecular Schmidt reaction. After the construction of the key scaffolds **2.18**–**2.21**, derivatizations on amine or alcohol functionalities were performed to diversify the compound library (**2.26**–**2.29**). By balancing between the generation of natural product-like compounds and the improvement of the synthetic accessibility, 686 compounds were prepared that resemble natural alkaloids.



Scheme 3. Alkaloid-inspired compound library by the Aubé group[42]

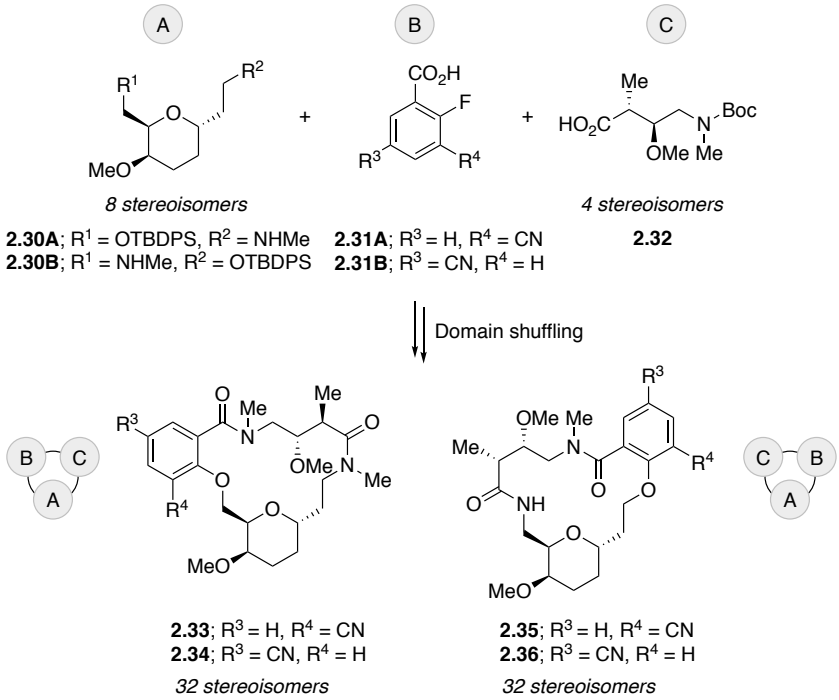
2.1.3 Macrocycles in Chemical Libraries

Macrocyclic drugs are another class of small molecules that are often positioned outside the conventional drug-like space but still exhibit potent biological activities and favorable physicochemical properties. With the recognition of therapeutic potential of macrocyclic scaffolds in the drug discovery field, there has been growing interest in generating macrocycle libraries using various technologies. Recent advances in the synthesis of non-peptidomimetic macrocycle libraries will be briefly reviewed, which include a fragment-based domain shuffling strategy, a ring-expansion approach, a diversity-oriented synthesis, and a DNA-templated synthesis.

2.1.3.1 Fragment-based Domain Shuffling Strategy

Marcaurelle and co-workers reported a modular approach for the synthesis of a macrocycle library via fragment-based domain shuffling.[43] This strategy was devised to rapidly construct structural/stereochemical analogs that allowed to obtain useful structure-activity relationship from the initial library members. Inspired by the numerous pyran-containing macrocycles in nature, they aimed to generate macrocycles with a pyran core. To achieve this, three fragments (**A**–**C**) were prepared which can be readily modified, interchanged, or shuffled (Scheme 4). The pyran-containing macrocycle scaffolds **2.34**–**2.36** were prepared through amide couplings and macrocyclization via nucleophilic aromatic substitution using the three fragments. The

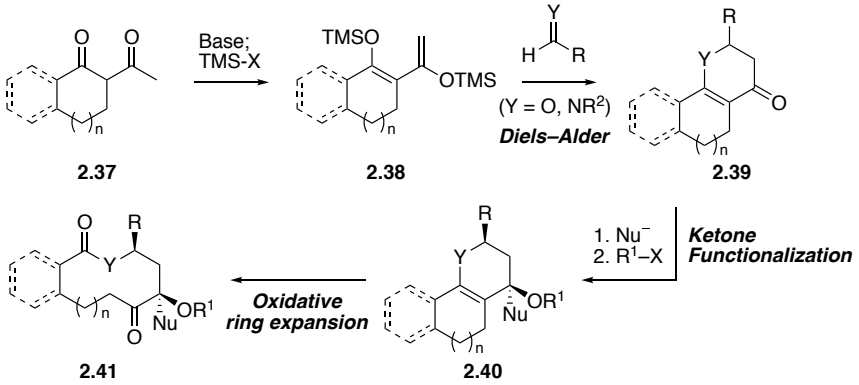
pyran fragment (**A**) has three stereogenic centers and 8 stereoisomers could be prepared. In addition, 4 diastereomers of the linear fragment (**B**) were prepared for the domain shuffling. Therefore, 32 combinations of stereoisomers for each macrocyclic scaffold can be produced. Furthermore, changing a position of a substituent on the aromatic fragment (**B**) and domain shuffling contributed to increasing the size of the library. This strategy features the synthesis of various stereoisomers using a modular approach and allows rapid access to derivatization for follow up medicinal chemistry efforts.



Scheme 4. Marcaurelle's fragment-based domain shuffling approach for the synthesis of pyran-based macrocycles[43]

2.1.3.2 Ring-Expansion Strategy

In 2012, the Tan group proposed a strategy for the construction of diverse macrocycles using a non-classical macrocyclization reaction.[44] The idea harnessed the synthesis of macrolactones and macrolactams via the oxidative ring expansion reaction (Scheme 5). The synthesis commenced with the conversion of the cyclic 1,3-diketone **2.37** into the corresponding 1,3-disiloxydienes **2.38**. Diels–Alder reaction of **2.38** with various dienophiles such as aldehydes or imines generated the polycyclic substrate **2.39**. The resulting ketone functionality in **2.39** can be further derivatized in a stereoselective way, which contributes to increased stereochemical diversity. Lastly, the oxidative ring expansion of the key substrate **2.40** with a bridging double bond was performed. After screening various reaction conditions, they found that a ruthenium(III)-based oxidant system is the most efficient method to prepare both macrolactones and macrolactams **2.41**. Using this strategy, 18 macrocyclic scaffolds of 10- to 12-membered lactones and lactams were generated within five steps and the subsequent appendage diversification was carried out to give additional 12 macrocycles.

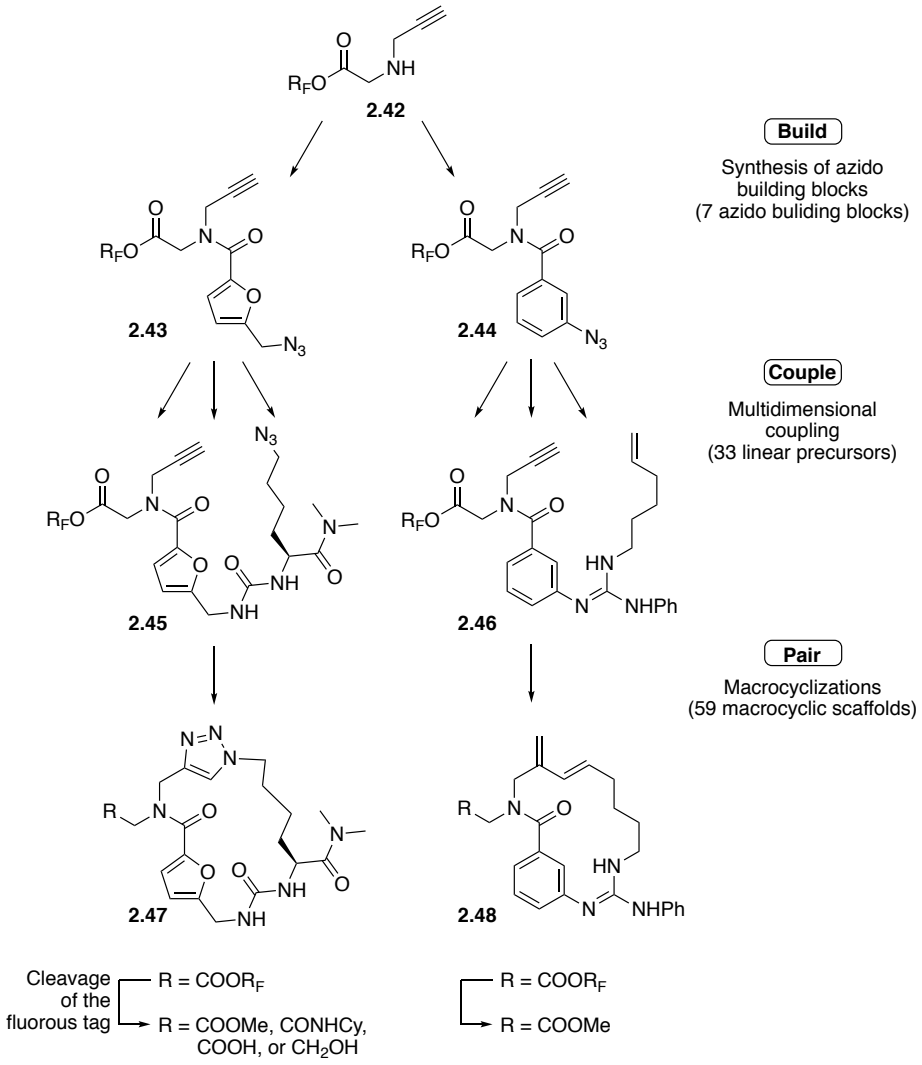


Scheme 5. Oxidative ring expansion strategy for the synthesis of the macrocycle library[44]

2.1.3.3 Diversity-Oriented Synthesis

Diversity-oriented synthesis (DOS) has been exploited for the efficient construction of diverse chemical libraries with high skeletal diversity.[45-47] Using the DOS concept, Spring and co-workers developed an advanced build/couple/pair algorithm to generate 59 distinct scaffolds (Scheme 6).[48] In the build phase, seven different azido building blocks (**2.43** and **2.44**) were prepared. These building blocks were coupled to a variety of electrophiles including acyl chlorides, aldehydes, and isocyanates to yield **2.45** and **2.46**. Through the multidimensional coupling phase, the azide functional groups in **2.43** and **2.44** were converted into various motifs such as urea and guanidine. Lastly, these linear substrates (**2.45** and **2.46**) were cyclized via azide/alkyne cycloaddition or enyne metathesis in the pair phase, leading to the formation of the macrocycles (**2.47** and **2.48**). It is notable that this approach took advantage of fluorous solid-phase extraction. Installation of a polyfluorocarbon tag to the building block **2.42** enabled the efficient

purification of products from the reagents after each reaction. At the last stage of the synthesis, the polyfluorocarbon tags were cleaved by transesterification, transamidation, ester hydrolysis, and ester reduction. As a result, the macrocycle library with a high degree of skeletal diversity was efficiently constructed using the DOS concept within five steps.



Scheme 6. Outline of the synthetic strategy used for the construction of Spring's diversity-oriented synthesis[48]

2.1.3.4 DNA-Encoded Libraries

DNA-encoded chemical libraries are a collection of synthetic molecules that are conjugated with short DNA strands. The technology was firstly introduced in 1994.[49] Over two decades, this method has been evolved to provide a variety of small molecules for in vitro selection in the medicinal chemistry field. Since DNA tags function as identification bar codes, this method allows the construction and screening of a massive-sized compound library. In 2018, Liu's group synthesized 256,000 macrocycles using DNA-encoded and DNA-templated library synthesis methodology (Figure 8).[50] Scaffold building block **D**, which was attached to the 5' end of the DNA template, was coupled to building block **A** linked to the corresponding 'anticodon' DNA. Under the same procedure, the building blocks **B** and **C** were incorporated to the scaffold building block **D**. After the three amidation coupling reactions, the oxidative cleavage of the diol with the treatment of sodium periodate furnished a glyoxyloyl group. Subsequent Wittig cyclization provided the macrocyclic scaffold. Along with the computational prediction for generating the compound collections with improved physicochemical properties, in vitro screening efforts against an insulin-degrading enzyme have identified a hit macrocycle with an IC_{50} value of 50 nM. Collectively, the DNA-templated strategy for the synthesis of macrocycles has proven to be a powerful tool for the discovery of bioactive small molecules.

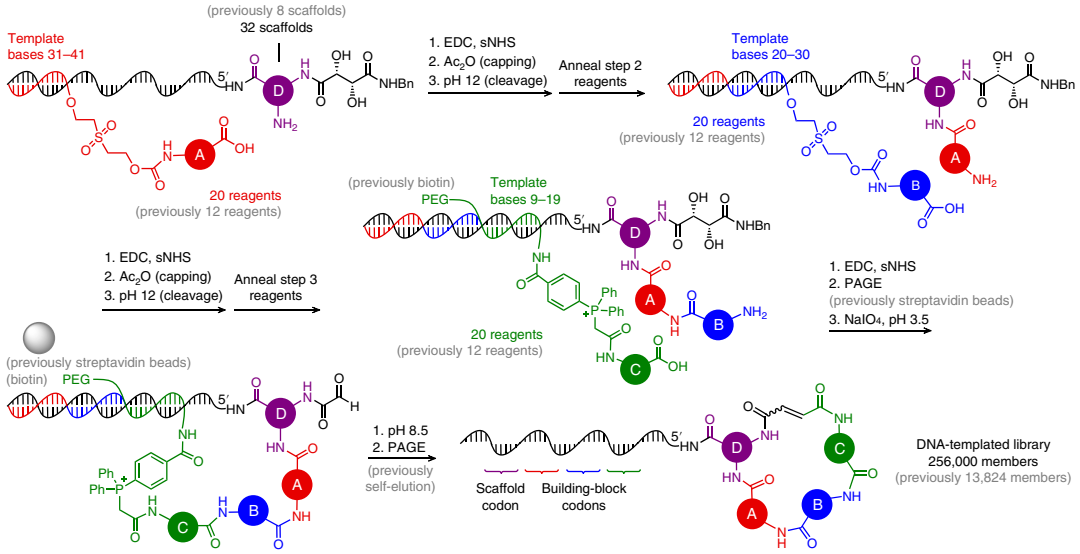


Figure 8. DNA-templated macrocycle library synthesis scheme (Figure reprinted with permission from ref.[50])

Although some progress has been made in the synthesis of macrocyclic libraries during the last decade, a new strategy is still needed to construct natural product-like macrocycles with a high degree of structural and stereochemical diversity. In this regard, a series of chemical properties should be considered including a high sp^3/sp^2 carbon ratio, high stereochemical density, various ring sizes, and diverse three-dimensional shapes.

2.1.4 Tetrahydropyran-Containing Natural Macrocycles with Biological Activities

Numerous natural and nature-inspired macrocycles have long been clinically and medicinally useful. The chemical diversity and complexity of macrocyclic natural products have provided inspiration for innovative drug design, especially in the context of biological relevancy. Among them, tetrahydrofuran (THF)/ tetrahydropyran (THP)-containing macrocycles are an interesting subgroup of natural products.[51] These privileged substructures can be found in polyketide natural products with a wide range of biological activity (Figure 9). For example, zampanolide has been demonstrated as a cytotoxic tubulin-assembly agent against multi-drug resistant cancer cell lines[52], and neopeltolide exhibits potent anticancer activity.[53] Dimeric versions of THP-containing macrolides have also shown unique effects on several therapeutic targets. Cocosolide is known to strongly inhibit IL-2 production and T-cell proliferation.[54] Inspired by the bioactive macrocycles with THP cores from nature, we embarked on the synthesis of a THP-containing macrocycle library with a high degree of chemical diversity that overlaps with natural macrocycles.

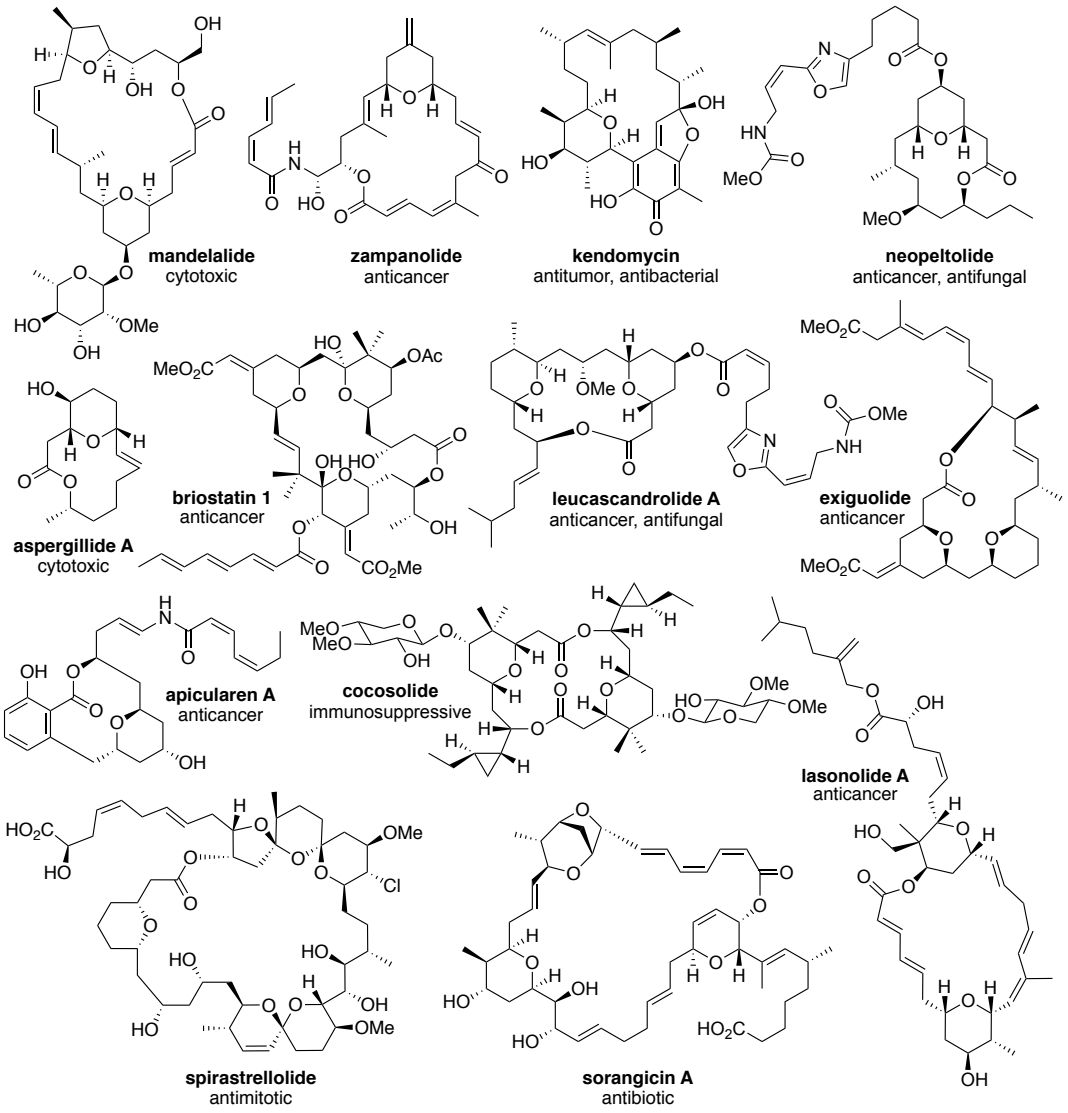


Figure 9. Examples of biologically active macrocycles containing tetrahydropyran moieties

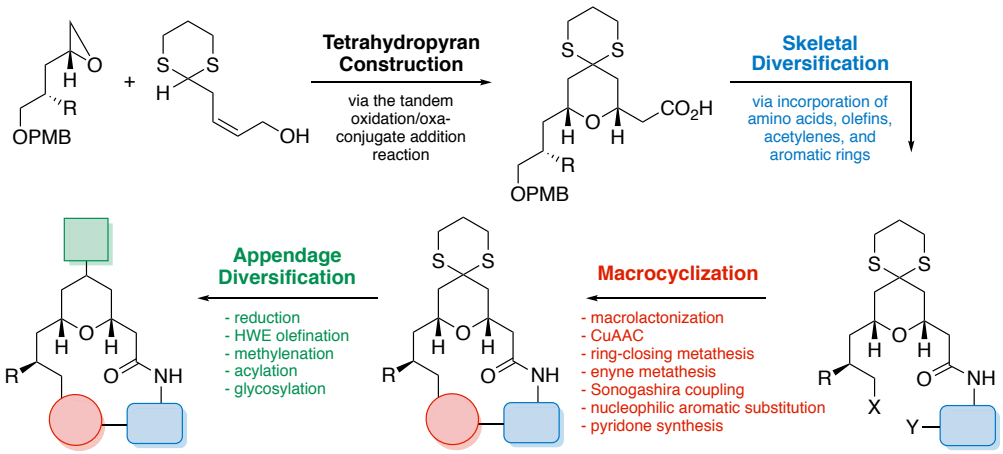
2.1.5 Goal of the Research

The goal of the research is to generate a new collection of macrocyclic compounds that can address a broader range of chemical space. Given the favorable features of natural-product-like scaffolds and macrocyclic cores discussed above, our synthetic approach involves a stereoselective THP ring synthesis along with three diversification methods. We envisioned that the goal would be achieved by a convergent strategy to rapidly increase the chemical diversity and structural complexity. Cheminformatic analyses including Principal Component Analysis (PCA) and Principal Moment of Inertia (PMI) would be performed to assess physicochemical/structural diversity of the generated macrocycle set by comparing with natural product and drug sets. Finally, biological potential of the macrocycle compounds would be evaluated. Thus, the construction of a library of tetrahydropyran (THP)-containing macrocyclic compounds would provide a new opportunity for exploring natural-product-like chemical space. The detailed synthetic approaches will be described in the following chapter.

2.2 Results and Discussion

2.2.1 Design a Tetrahydropyran-Containing Macrocycle Library

Our strategy for the synthesis of THP-containing macrocycles was illustrated in Scheme 7. The tetrahydropyran core would be constructed via the tandem allylic oxidation/oxa-conjugate addition[55-62] in a stereoselective manner to provide a common intermediate. With the resulting intermediate as a starting point, three diversification strategies were utilized to efficiently construct diverse macrocycle scaffolds. First, a variety of amine parts including amino acids, olefins, acetylenes, and aromatic rings would be incorporated to rapidly increase skeletal diversity and modify ring sizes. Second, seven different macrocyclization methods would be exploited to install various functionalities on a ring system. Finally, the appendage diversification would further modulate the physicochemical properties of macrocycles.

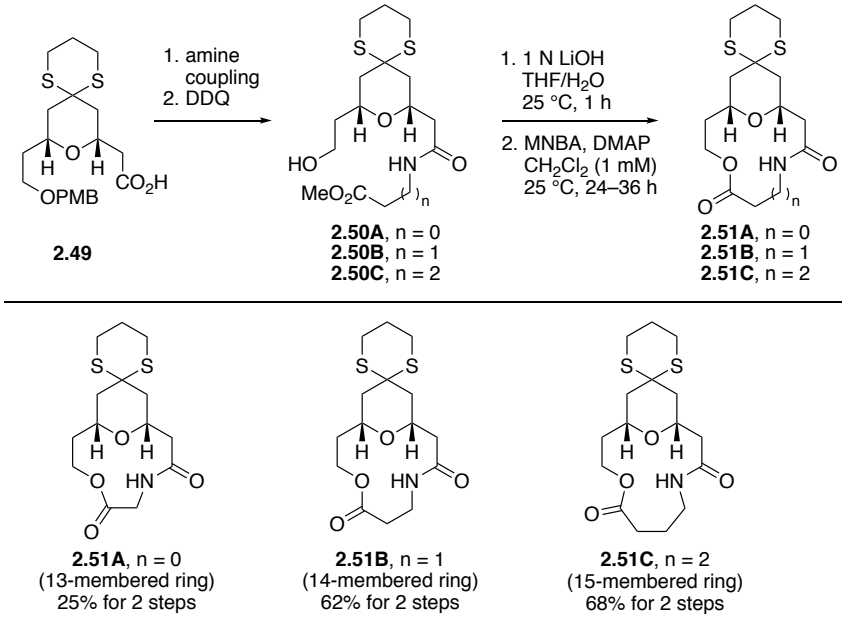


Scheme 7. Outline of our approach for the construction of a THP-containing natural-product-like macrocyclic library

2.2.2 Synthesis of Tetrahydropyran-Containing Macrocycles

2.2.2.1 Ring-size Optimization

Before designing individual structures of our macrocycle library, we initially tested macrocyclization reactions in order to find appropriate ring sizes for a THP-containing macrocycle (Scheme 8). Carboxylic acid **2.49** was derivatized with glycine methyl ester, β-alanine methyl ester, and methyl 4-aminobutanoate via HATU coupling and subsequent PMB deprotection using DDQ produced the substrates **2.50A–C**. Hydrolysis of the methyl esters **2.50A–C** under a basic condition generated the corresponding seco acids followed by Shiina macrolactonization[63] to give macrocycles **2.51A–C**. The 14- and 15-membered ring systems were smoothly constructed with the combination of MNBA and DMAP in moderate yields (62% and 68%, respectively), while the 13-membered macrocycle **2.51A** was obtained in a low yield (25%) possibly due to the increased ring strain. Based on this result, we decided to generate THP-containing macrocycles which contain more than or equal to 14 atoms in a ring system.

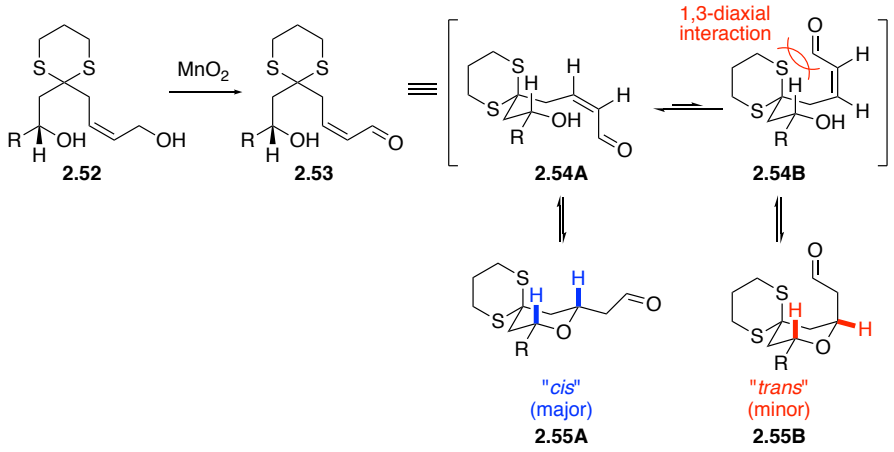


Scheme 8. Ring-size optimization for a THP-containing macrolactone

2.2.2.2 Construction of 2,6-*cis*-Tetrahydropyran Core

The stereoselective synthesis of 2,6-*cis*-tetrahydropyran using tandem allylic oxidation/oxa-conjugate addition was reported by the Hong group in 2009 (Scheme 9).[55] The synthesis began with allylic oxidation of diol **2.52** in the presence of MnO_2 to afford α,β-unsaturated aldehyde **2.53**. The intermediate **2.53** was subsequently cyclized to provide a tetrahydropyran ring with high stereoselectivity (dr >20:1). The rationale of the stereochemical outcome can be the energy difference between two possible transition states **2.54A** and **2.54B**. Since unfavorable 1,3-diaxial interaction of the α,β-unsaturated carbonyl group at C-6 and the dithiane group at C-4 in conformation **2.54B** is larger than that of the C-6 hydrogen atom and the dithiane group in **2.54A**, the oxa-conjugate

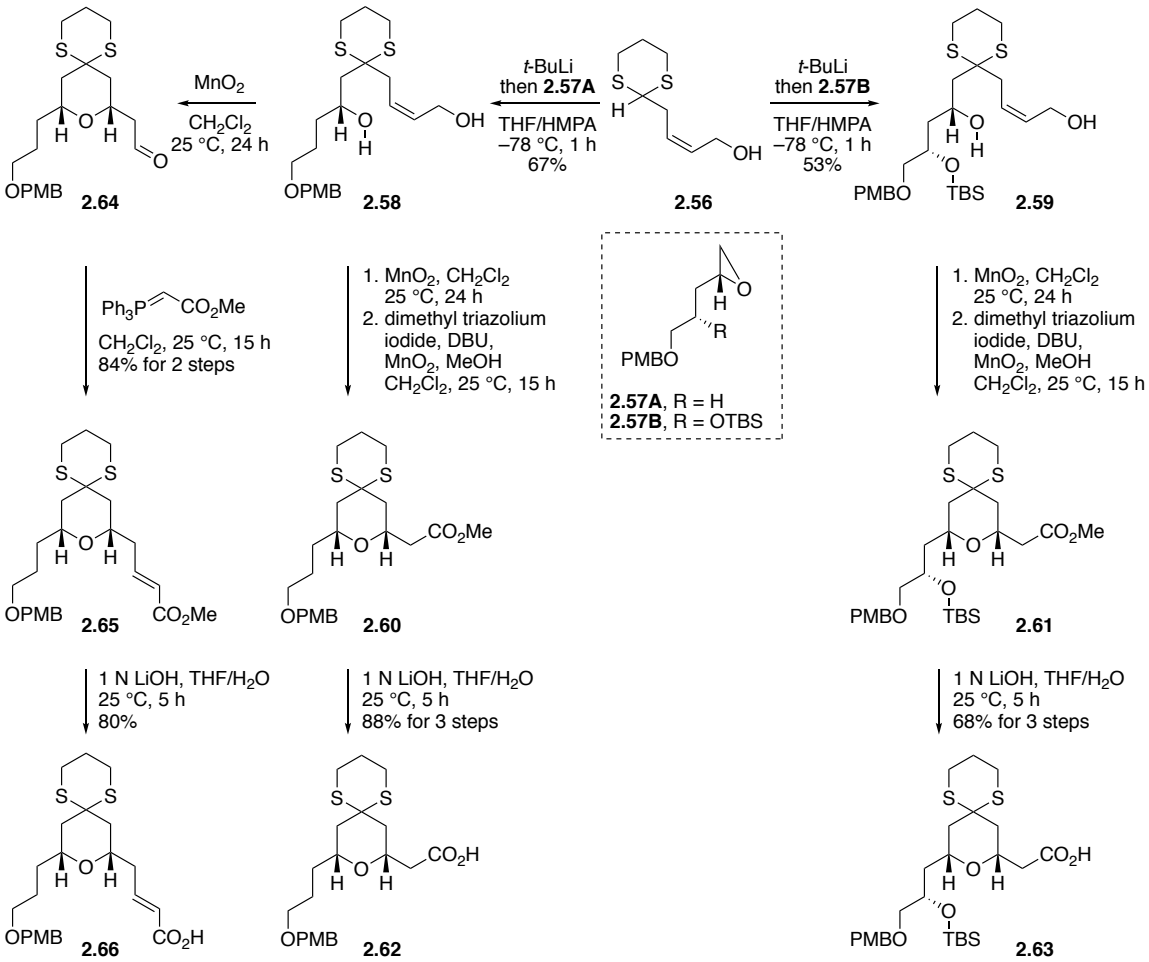
addition is favored to produce *cis* product **2.55A**. Furthermore, this synthetic method does not require harsh reaction conditions such as strong acids or transition-metal complexes to activate the conjugate acceptor. The intramolecular oxa-conjugate addition of the intermediate **2.53** proceeds under the mild reaction condition because the 1,3-dithiane group on C-4 position facilitates the reaction through the *gem*-disubstituent effect. By utilizing this synthetic method, various THP-containing natural products has been synthesized.[55, 57-58, 60-61] We decided to generate THP-containing macrocycles using this tandem allylic oxidation/oxa-conjugate addition due to the high efficiency and stereoselectivity of the reaction.



Scheme 9. Synthesis of a 2,6-*cis*-tetrahydropyran via a tandem allylic oxidation/oxa-conjugate addition

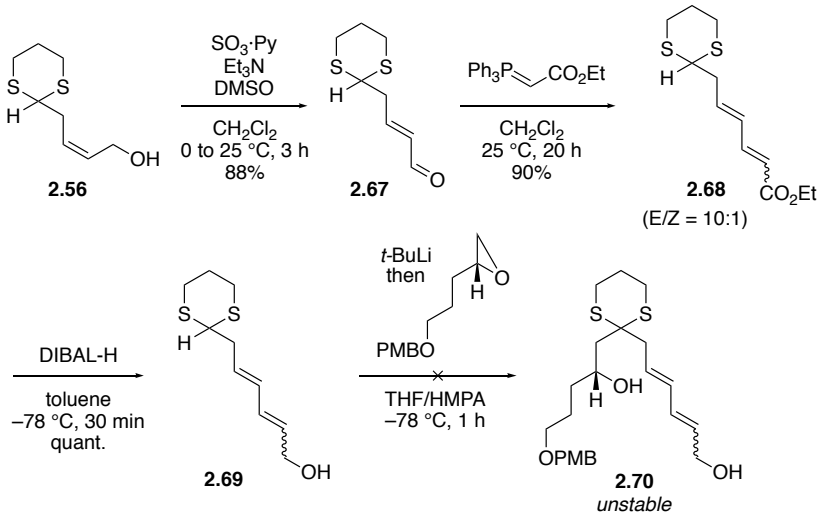
The synthesis of the THP-containing macrocycle library commenced with the preparation of allylic alcohols **2.58** and **2.59** as substrates for the tandem allylic oxidation/oxa-conjugate addition (Scheme 10). Chiral epoxide **2.57A** and **2.57B**[64-65] were

coupled to known dithiane **2.56**[55] to give allylic alcohols **2.58** and **2.59**. One-pot allylic oxidation and oxa-conjugate addition of **2.58** and **2.59** proceeded to generate the desired 2,6-*cis*-THP containing aldehydes with high stereoselectivity (dr >20:1), which were further converted into methyl esters **2.60** and **2.61** through *N*-heterocyclic carbene (NHC)-mediated esterification reaction using dimethyl triazolium iodide and MnO_2.[60] Hydrolysis of the methyl esters **2.60** and **2.61** by treatment with lithium hydroxide provided common intermediates **2.62** and **2.63**.



Scheme 10. Synthesis of the common intermediate **2.62**, **2.63**, and **2.66**

In a similar manner, a common intermediate **2.66** containing an additional double bond was prepared through the dithiane coupling followed by two-carbon elongation. After the tandem allylic oxidation/oxa-conjugation reaction of compound **2.58**, the resulting aldehyde **2.64** was treated with methyl (triphenylphosphoranylidene)acetate to furnish (*E*)-α,β-unsaturated methyl ester **2.65** in 84% yield for 2 steps. Basic hydrolysis with lithium hydroxide yielded the desired carboxylic acid **2.66**. This reaction sequence was more effective than the reverse sequence, which was the two-carbon homologation followed by the dithiane coupling, as shown in Scheme 11. The 1,3-dithiane **2.56** was converted into aldehyde **2.67** by Parikh–Doering oxidation. Wittig reaction of the aldehyde **2.67** provided the conjugated ethyl ester **2.68** in 90% yield as an isomeric mixture. DIBAL reduction of the ester **2.68** yielded the corresponding alcohol **2.69** which was subjected to the 1,3-dithiane coupling reaction. However, the resulting diene product **2.70** was unstable on silica gel purification, which resulted in very low yield.



Scheme 11. Attempt towards the synthesis of compound **2.70**

Using the tandem allylic oxidation/oxa-conjugate addition, the three common intermediates **2.62**, **2.63**, and **2.66** with a 2,6-*cis*-THP core were prepared. As these intermediates (**2.62**, **2.63**, and **2.66**) contain a primary alcohol and a carboxylic acid at the end of the structures, a variety of building blocks can be assembled to rapidly increase the chemical diversity of the macrocycle library. It is also notable that an additional hydroxyl group or double bond in compound **2.63** and **2.66** would contribute to further modulate log *P* value, polar surface area, and ring rigidity of final macrocycle structures.

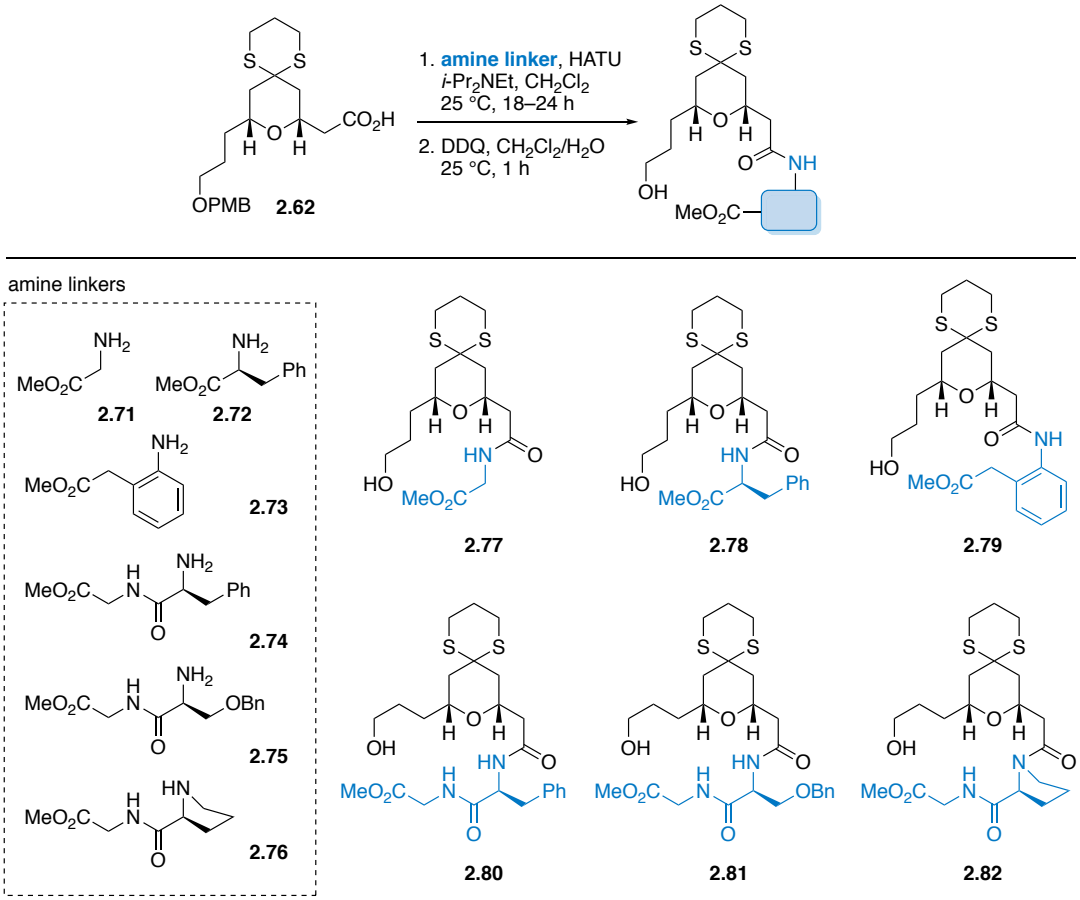
2.2.2.3 Building Blocks Assembly

With the common intermediates **2.62**, **2.63**, and **2.66** in hand, we carried out the skeletal diversification step by coupling various building blocks. In the meantime, the

resulting compounds would be linear substrates for macrocyclizations, which is the second diversification step. The synthesis of linear precursors for seven different macrocyclizations with high skeletal diversity would be described in the following chapter.

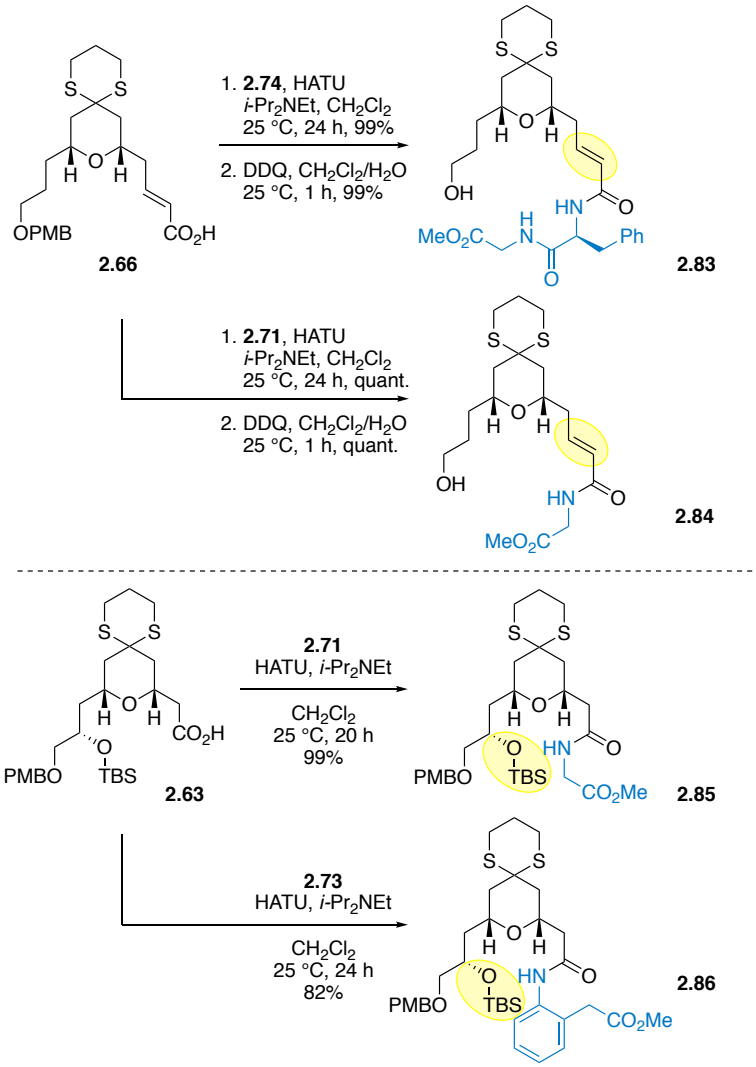
2.2.2.3.1 Synthesis of Linear Precursors for Macrolactonization

As a macrolactone is a common motif in polyketide natural products, we set out to generate diverse linear precursors for macrolactonizations as shown in Scheme 12. Glycine methyl ester (**2.71**) and phenylalanine methyl ester (**2.72**) were coupled to **2.62** via HATU coupling reaction followed by PMB deprotection using DDQ to furnish the linear precursors **2.77** and **2.78**. In order to incorporate a fused aromatic ring system to the final cyclic structure, methyl 2-(2-aminophenyl)acetate (**2.73**) was coupled to **2.62** as well. In a similar manner, dipeptides **2.74**–**2.76** were installed to **2.62** to prepare compounds **2.80**–**2.82**.



Scheme 12. Preparation of linear precursors for macrolactonization from **2.62**

Linear substrates **2.83**–**2.86** containing a double bond or a hydroxyl group were prepared using the same procedure with the common intermediates **2.66** and **2.63** (Scheme 13). The desired compounds **2.83**–**2.86** were obtained in excellent yields.

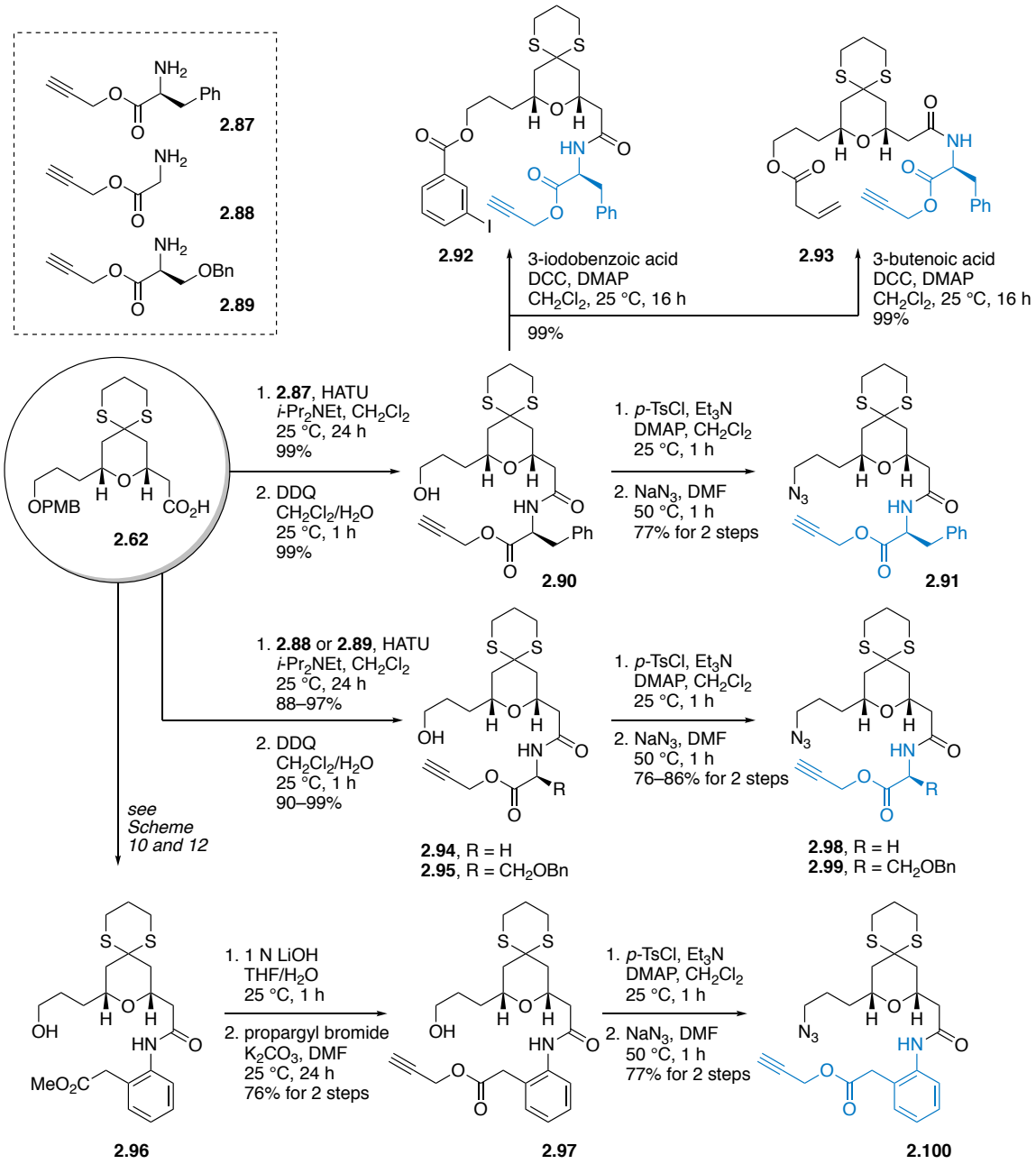


Scheme 13. Preparation of linear precursors for macrolactonization from **2.63** and **2.66**

2.2.2.3.2 Synthesis of Linear Precursors for Cu-catalyzed Azide/Alkyne Cycloaddition, Enyne Metathesis, and Sonogashira Cross-Coupling

Next, we generated the linear precursors for Cu(I)-mediated azide/alkyne cycloaddition (CuAAC)[66], enyne metathesis[67], and Sonogashira cross-coupling reaction[68-69] (Scheme 14). Carboxylic acid **2.62** was coupled to propargyl-containing amine **2.87**

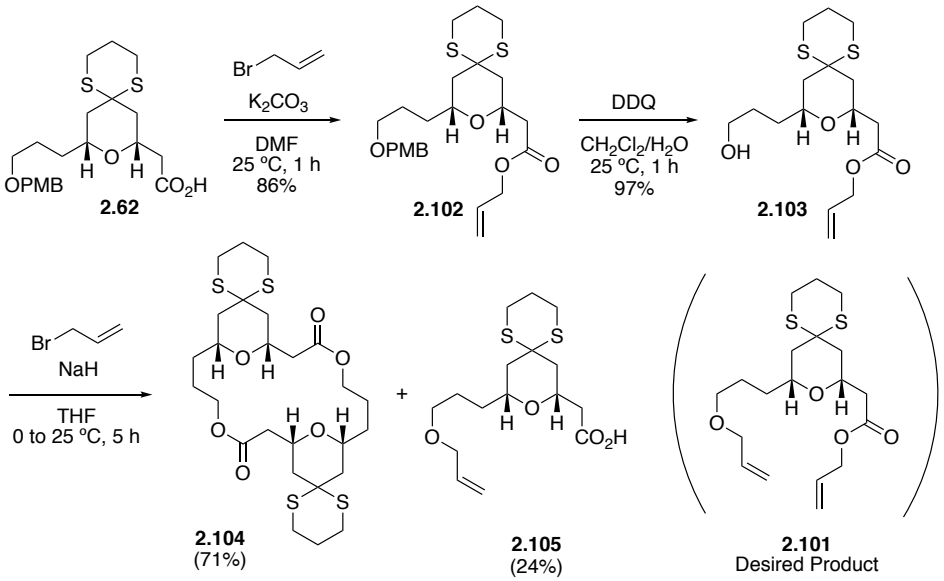
followed by PMB deprotection to give compound **2.90**. This compound can be further convergently diversified for the preparation of substrates of three different macrocyclization reactions. Tosylation of the primary alcohol in **2.90** followed by azidation yielded azide alkyne **2.91** as a substrate for CuAAC. Through DCC coupling of **2.90** with 3-iodobenzoic acid or 3-butenoic acid, the substrates **2.92** and **2.93** were prepared for Sonogashira cross-coupling reaction and enyne metathesis, respectively. Other azide alkynes (**2.98**–**2.100**) were also generated in a similar manner.



Scheme 14. Preparation of linear precursors for CuAAC, enyne metathesis, and Sonogashira cross-coupling reaction from **2.62**

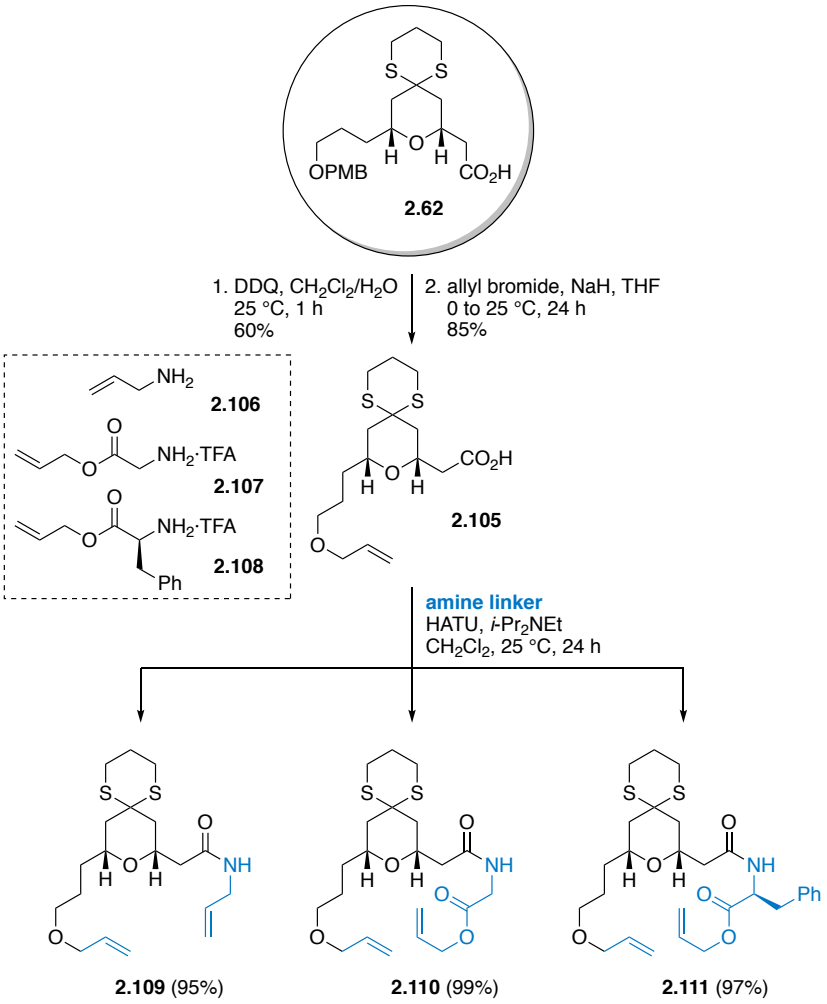
2.2.2.3.3 Synthesis of Linear Precursors for Ring-Closing Metathesis

Attempts for the synthesis of linear precursors of ring-closing metathesis were described.[70] Firstly, we designed a diene compound **2.101** which has two allyl groups attached to both the primary alcohol and carboxylic acid functional groups (Scheme 15). To achieve this, allylation of the carboxylic acid **2.62** using allyl bromide and potassium carbonate was performed. After PMB deprotection, the second allylation was attempted with the combination of sodium hydride and allyl bromide. However, the reaction gave a hydrolyzed product **2.105** (24%) and a cyclic dimer **2.104** (71%) instead of the desired product **2.101**. We speculated that the allyl ester functionality of **2.103** is quite reactive under the condition, thus being susceptible to either esterification with the primary alcohol in another molecule or hydrolysis by water upon aqueous workup. Based on the result, we decided to change the target structure changing an allyl ester to an allyl amide group.



Scheme 15. Attempt to the synthesis of diene **2.101**

The second attempt for the generation of diene compounds **2.109**–**2.111** is illustrated in Scheme 16. PMB deprotection of **2.62** followed by allylation on the primary alcohol yielded compound **2.105**. Three different amines **2.106**–**2.108** possessing allyl groups were coupled to the carboxylic acid **2.105** to furnish the desired diene substrates **2.109**–**2.111** in excellent yields (95–99%).

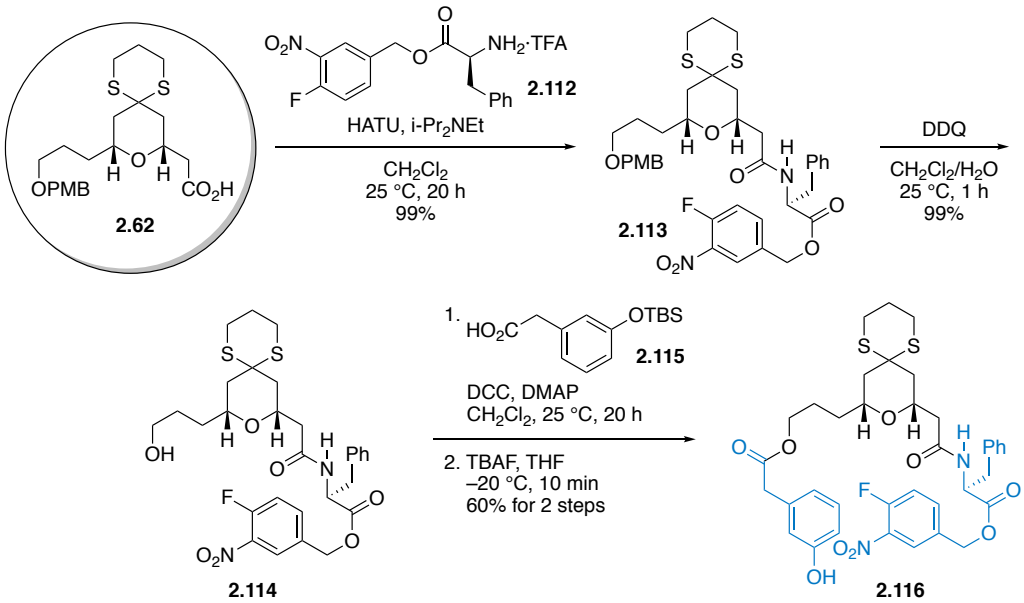


Scheme 16. Preparation of linear precursors for ring-closing metathesis from **2.62**

2.2.2.3.4 Synthesis of Linear Precursors for Nucleophilic Aromatic Substitution and Pyridone Synthesis

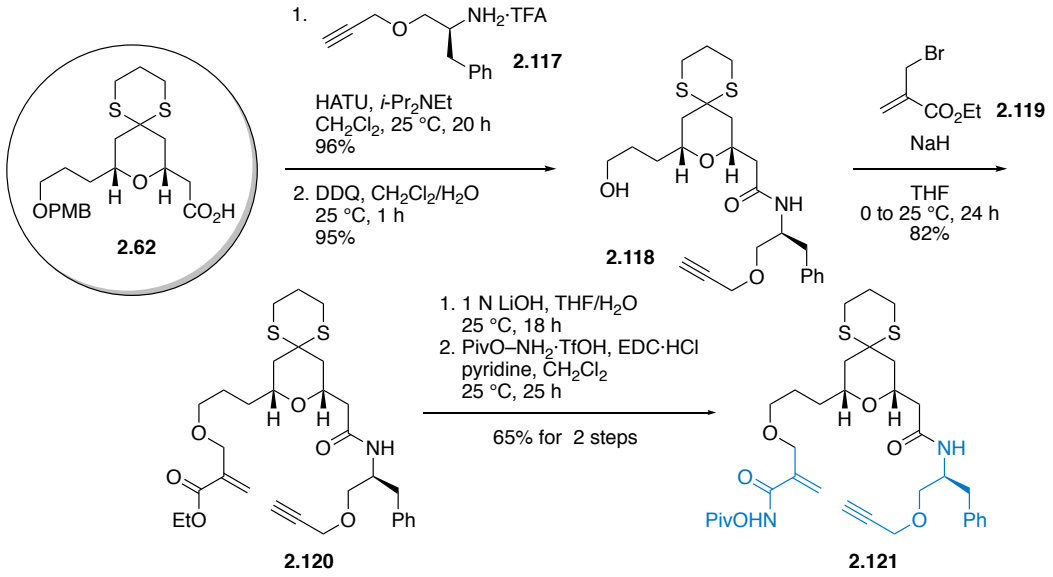
The preparation of substrates (**2.116** and **2.121**) for nucleophilic aromatic substitution[71] and pyridone formation[72] was performed using a similar procedure. For the synthesis of compound **2.116**, the common intermediate **2.62** was coupled to the amine linker **2.112** which was prepared from Boc-L-phenylalanine and 4-fluoro-3-

nitrobenzyl bromide (Scheme 17). The resulting **2.113** was subjected to PMB deprotection and further elaborated with *m*-hydroxyphenylacetic acid (**2.115**) to give **2.116** as a product.



Scheme 17. Preparation of linear precursors for nucleophilic aromatic substitution from **2.62**

HATU coupling of the carboxylic acid **2.62** with phenylalaninol ether **2.117**[73] followed by PMB deprotection provided alcohol **2.118** (Scheme 18). After the alkylation of the alcohol **2.118** with 2-bromomethylacrylate (**2.119**), the ethyl ester functionality in **2.120** was converted into pivaloyl hydroxamate through hydrolysis and an EDCI coupling reaction[72] to furnish the compound **2.121** in 65% yield for 2 steps.



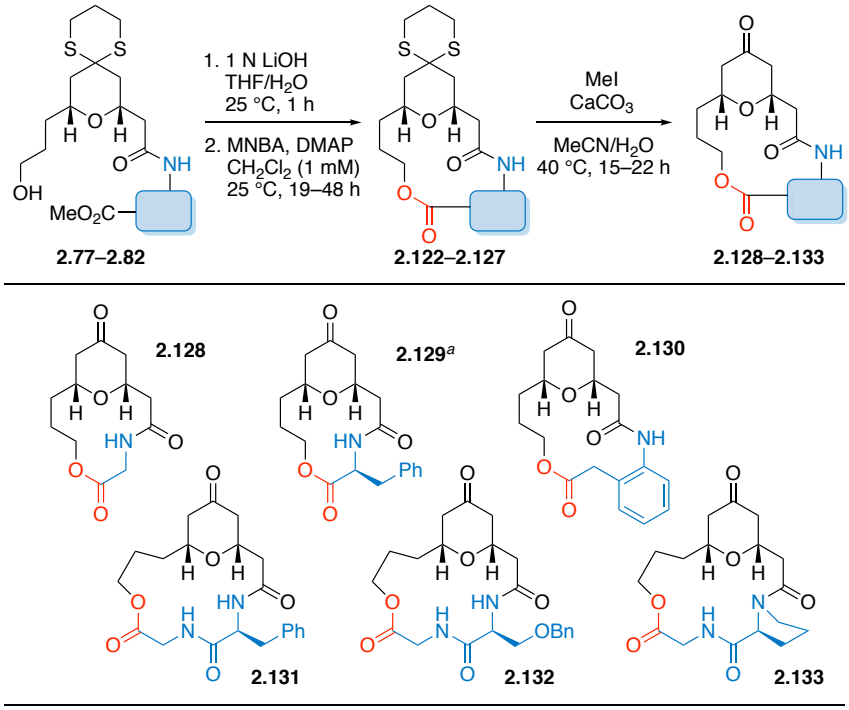
Scheme 18. Preparation of linear precursors for pyridine synthesis from **2.62**

2.2.2.4 Macrocyclizations

2.2.2.4.1 Macrolactonization

Having achieved the preparation of a variety of linear substrates, we set out to explore macrocyclization reactions in order to incorporate diverse functionalities into macrocyclic scaffolds. First, macrolactonization was carried out as shown in Scheme 19. Hydrolysis of the methyl esters **2.77**–**2.82** with lithium hydroxide provided the corresponding seco acids, which were treated with 2-methyl-6-nitrobenzoic anhydride[63] and converted to the desired macrolactones **2.122**–**2.127**. Harsher conditions should be used with elevated reaction temperature (80 °C) for the macrocyclization of the substrate **2.123**, possibly because the benzyl group right next to the carboxylic acid hindered the cyclization event. The cyclized products **2.122**–**2.127** were subjected to 1,3-dithiane

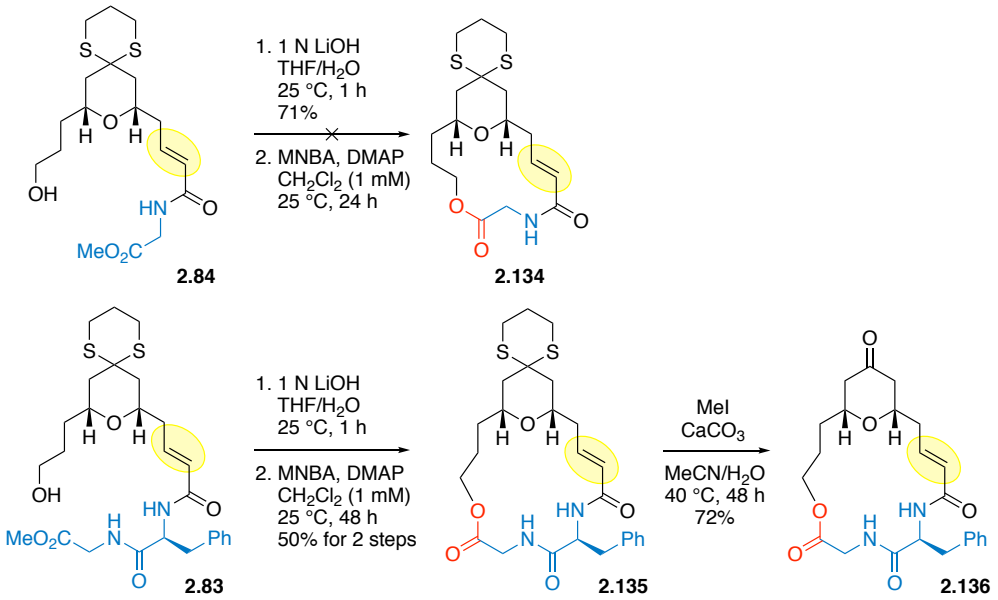
deprotection in the presence of methyl iodide and calcium carbonate to yield macrolactones **2.128–2.133**.



a: Modified Shiina condition was used (MNBA, DMAP, 4Å MS, toluene/THF, 80 °C, 21 h)

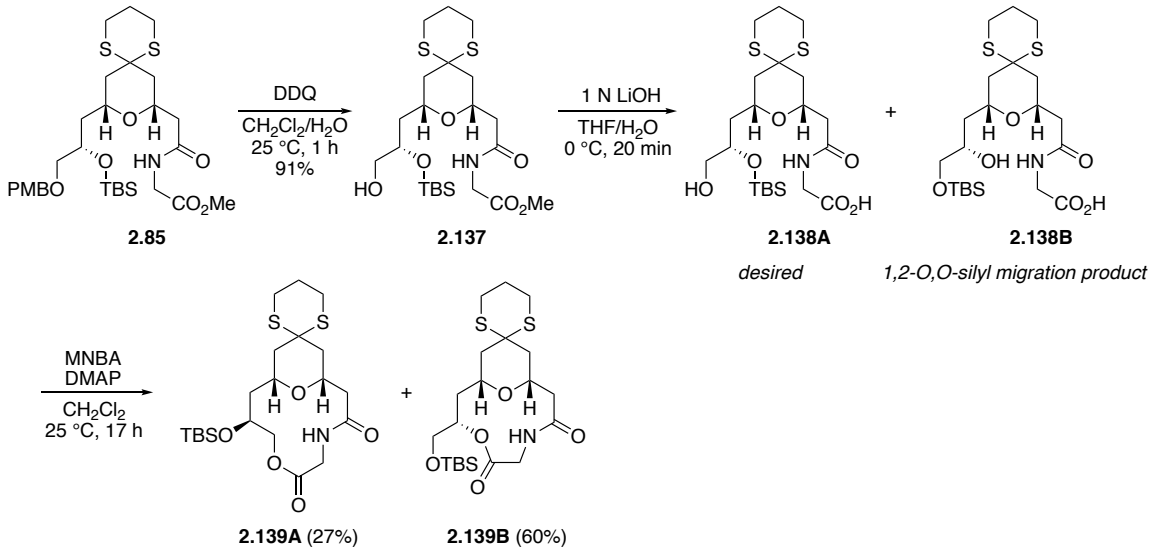
Scheme 19. Macrolactonization of **2.77–2.82** using the Shiina condition

Next, macrolactonizations of the unsaturated substrates **2.83** and **2.84** were attempted under the same Shiina condition (Scheme 20). Unfortunately, the linear precursor **2.84** was not cyclized likely due to the increased rigidity coming from the double bond in **2.84**. When the linker part was long enough to facilitate the cyclization, as in the case of the substrate **2.83**, the desired macrolactone **2.135** was obtained.



Scheme 20. Macrolactonization of **2.83** and **2.84** using Shiina condition

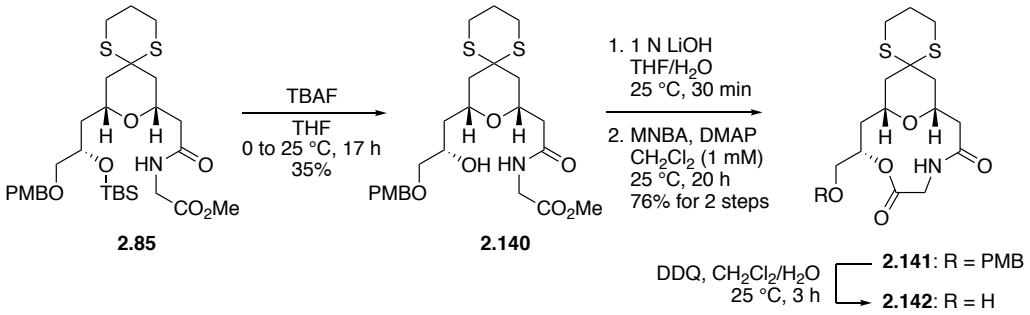
Lastly, macrolactonization reactions of the hydroxy substrates **2.85** and **2.86** were examined (Scheme 21–24). When the substrate **2.85** was subjected to PMB deprotection and basic hydrolysis as described above, an inseparable mixture of **2.138A** and **2.138B** was obtained (Scheme 21). MNBA-mediated cyclization of the mixture produced the 14-membered product **2.139A** (27%) and the 13-membered product **2.139B** (60%) which were separated and confirmed by ^{1}H NMR analysis. This unexpected outcome could be explained by 1,2-*O*,*O*-silyl migration from the secondary alcohol to the primary alcohol in **2.137** during the hydrolysis step.



Scheme 21. Silyl migration during the synthesis of the hydroxy macrolactone **2.139A**

To further confirm the structure of the 13-membered macrolactone **2.139B**, we synthesized 13-membered ring skeletons **2.141** and **2.142** via a different synthetic route as shown in Scheme 22. First, TBS group was removed using tetra-*n*-butyl ammonium fluoride to avoid the silyl migration. The resulting secondary alcohol **2.140** was hydrolyzed and subsequently cyclized to furnish the desired macrolactone **2.141**. In addition, PMB deprotection of **2.141** yielded the free alcohol **2.142**. Having the hydroxy macrolactones (**2.139A**, **2.139B**, **2.141**, and **2.142**) in hand, ^{1}H NMR spectra of the hydroxy macrolactones were compared to analyze the structures (Figure 10). We expected that the methine proton neighboring the secondary alcohol in the 13-membered macrolactones **2.139B**, **2.141**, and **2.142** would be more downfield shifted than that in the 14-membered macrolactone **2.139A**, since the former is linked to the

carbon directly connected to the ester oxygen. As we predicted, the methine protons from 13-membered rings (**2.139B**, **2.141**, and **2.142**) were shown between 4.9 and 5.1 ppm while the proton from 14-membered ring **2.139A** appeared at 4.1 ppm. Based on these results, we concluded that the obtained **2.139B** and **2.139A** were 13-membered and 14-membered lactone structures, respectively.



Scheme 22. Synthesis of the 13-membered hydroxy macrolactones **2.141** and **2.142**

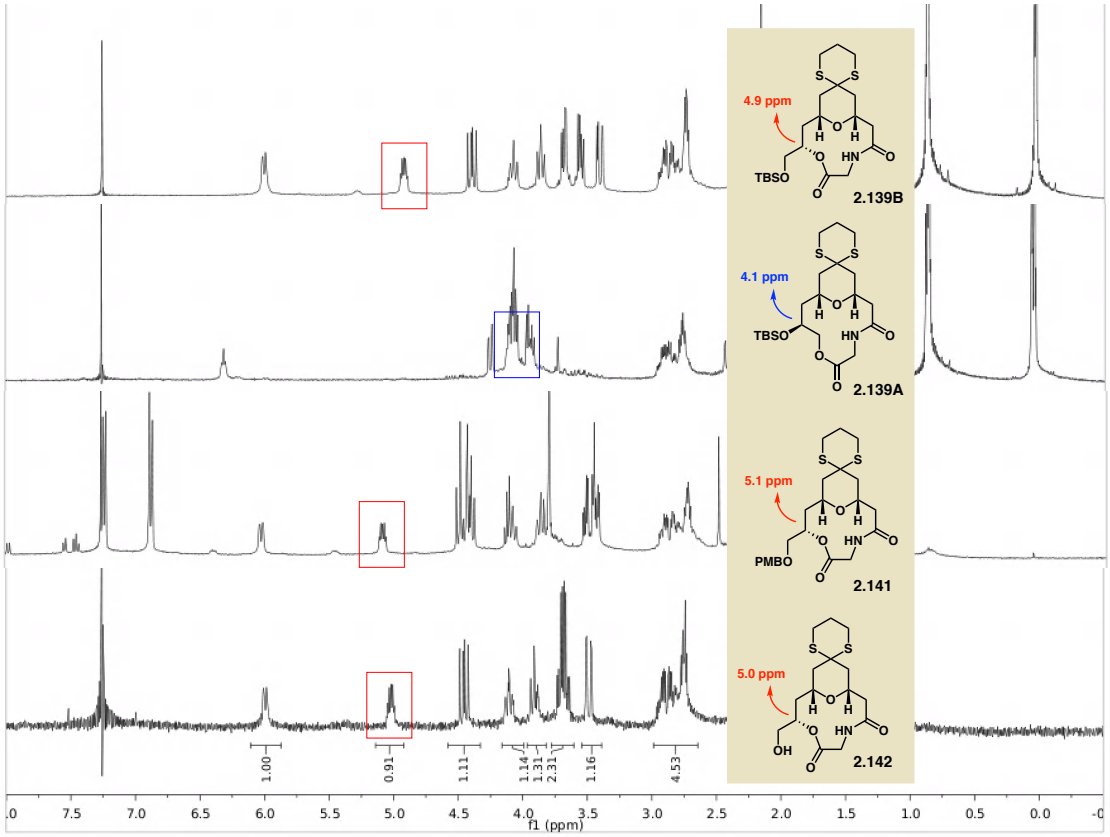
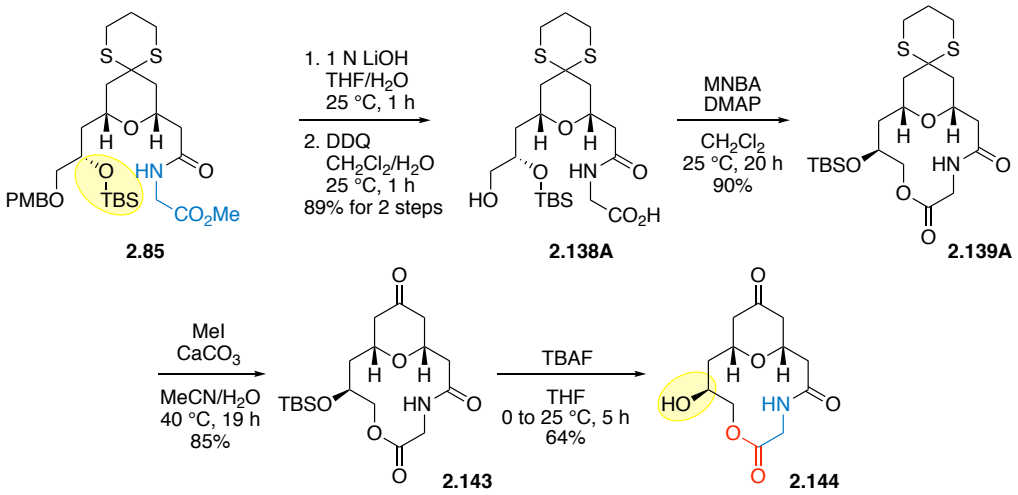


Figure 10. ^{1}H NMR spectra of hydroxy macrolactones (**2.139A**, **2.139B**, **2.141**, and **2.142**)

In order to synthesize the originally designed 14-membered hydroxy macrolactone **2.139A**, we devised a different route by changing the hydrolysis/PMB deprotection sequence (Scheme 23). In this scheme, basic hydrolysis was performed first and then PMB deprotection of the corresponding carboxylic acid was followed to prevent the silyl migration under the basic condition. The resulting seco acid **2.138A** was treated with MNBA and cyclized to provide the desired 14-membered macrocycle

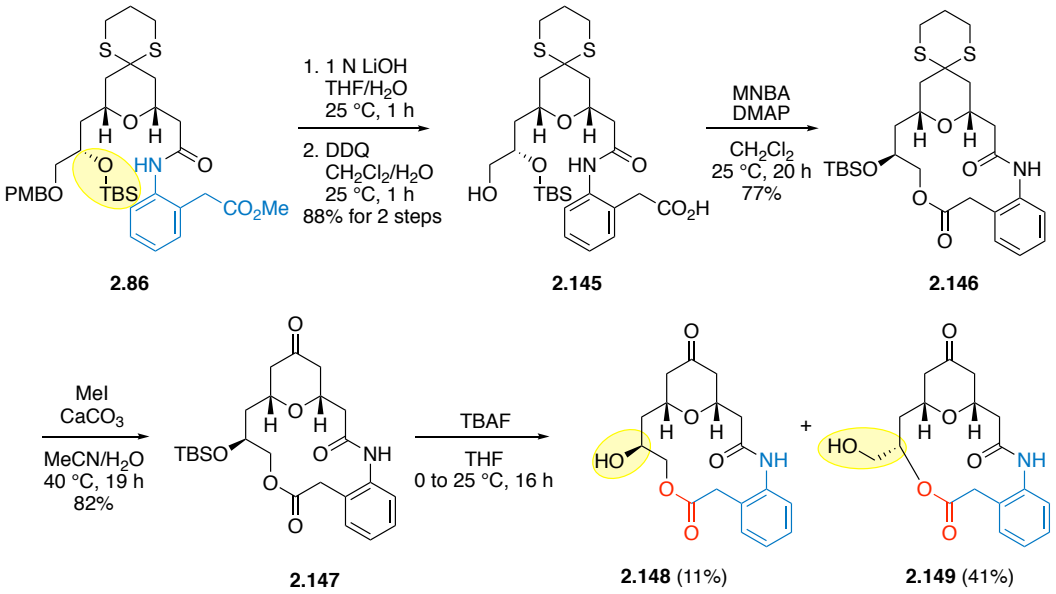
2.139A in 90% yield. 1,3-dithiane deprotection followed by desilylation generated the macrolactone **2.144** with an additional hydroxyl group.



Scheme 23. Synthesis of the hydroxy macrocycle **2.144**

Having secured a successful route to the preparation of a hydroxy macrolactone without the silyl migration, we embarked on the synthesis of another hydroxy analog **2.148** (Scheme 24). Deprotections of **2.86** and Shiina macrolactonization proceeded smoothly to yield the macrocyclic scaffold **2.146**, and the following 1,3-dithiane deprotection generated ketone **2.147**. When **2.147** was treated with tetra-*n*-butyl ammonium fluoride, the transesterification product **2.149** (41%) was obtained along with the desired product **2.148** (11%). Although we explored other desilylating reagents (HF·pyridine, $BF_3{\cdot}OEt_2$, AcCl/MeOH, TBAF/AcOH, and TFA), all reactions produced the mixture of **2.148** and **2.149** in which compound **2.149** was the major product. Taken

together, we generated 10 different macrolactone scaffolds with various ring sizes and functional groups utilizing MNBA-mediated cyclization.

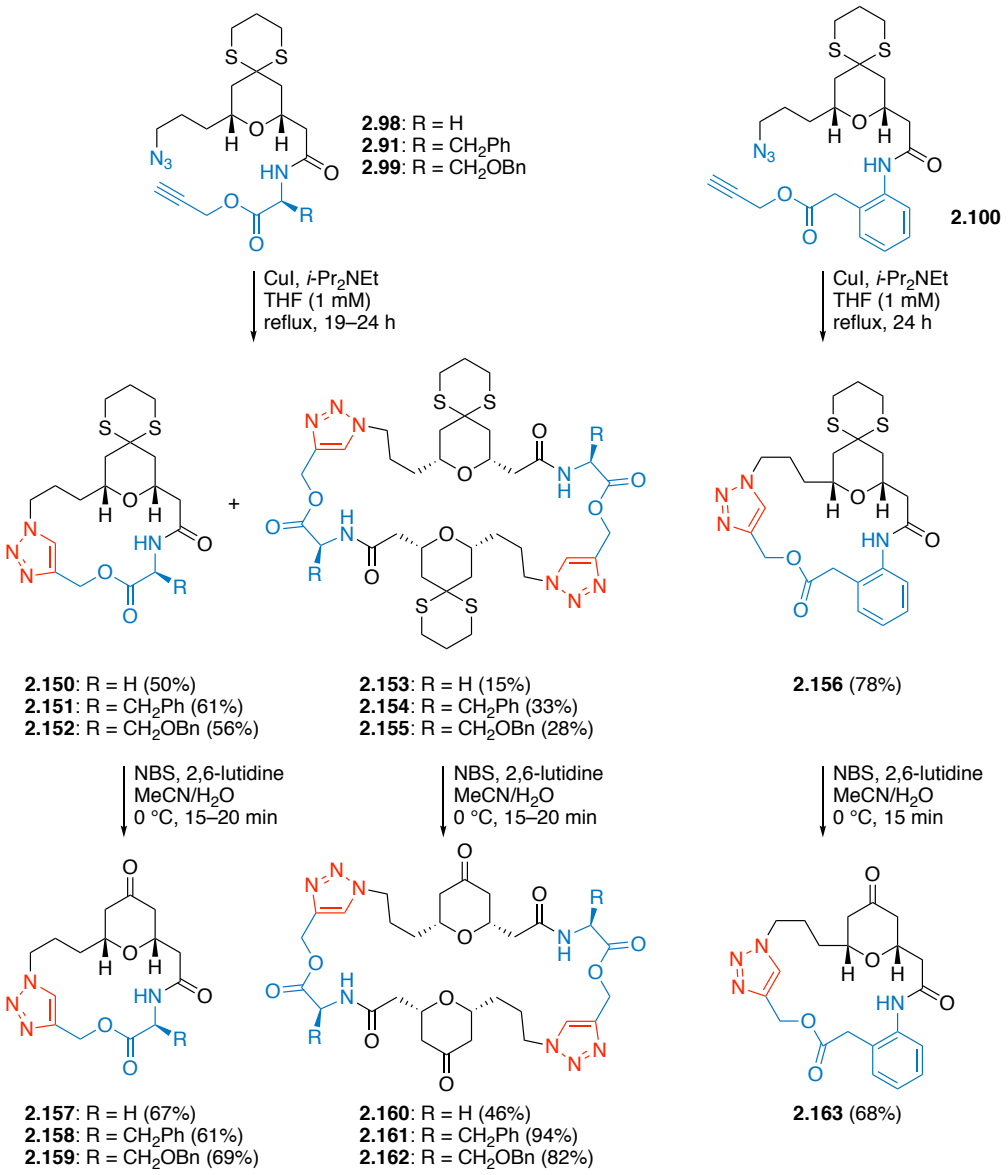


Scheme 24. Synthesis of hydroxy macrolactone **2.148** and **2.149**

2.2.2.4.2 Cu(I)-catalyzed Azide/Alkyne Cycloaddition

Our synthetic approach for Cu(I)-catalyzed azide/alkyne cycloaddition[66] of the azide alkynes (**2.91**, **2.98**–**2.100**) was illustrated in Scheme 25. In the presence of CuI and Hünig's base, the linear precursors (**2.91**, **2.98**, and **2.99**) were cyclized to produce the desired cyclic monomers (**2.150**–**2.152**) with cyclic dimers (**2.153**–**2.155**) which were confirmed by high-resolution mass spectrometry. Interestingly, a cyclic dimer of the aniline-containing substrate **2.100** was not obtained under the cycloaddition condition. With the triazole-fused macrocycles **2.150**–**2.156** in hand, deprotection of a 1,3-dithiane

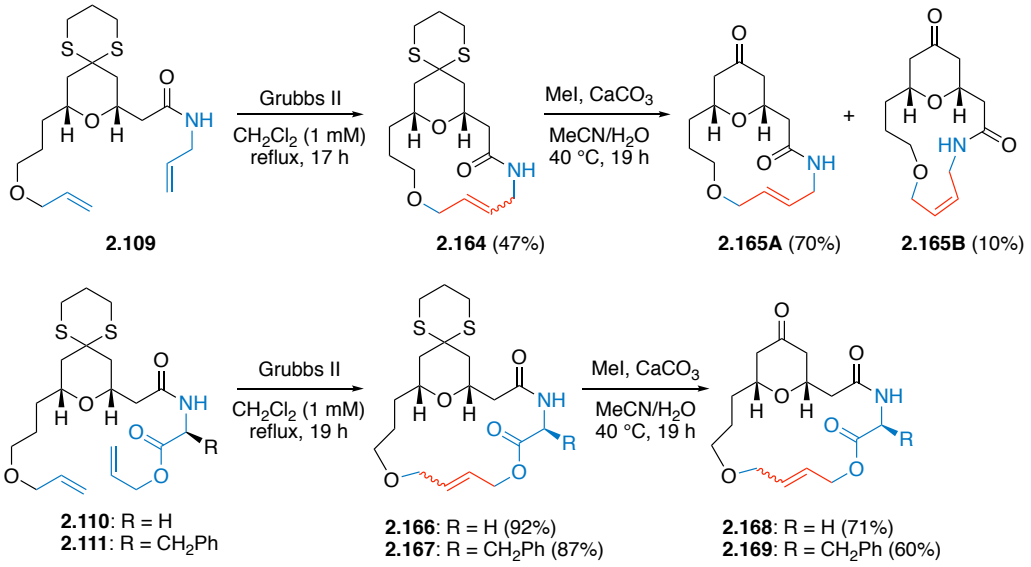
group was carried out. The typical MeI/$CaCO_3$ condition was not effective in this case, because a triazole moiety was also susceptible to methylation to produce a methyl triazolium salt. For this reason, the triazole-containing macrocycles **2.150–2.156** were treated with *N*-bromosuccinimide which successfully resulted in the formation of macrocycles **2.157–2.163**.



Scheme 25. Synthesis of the triazole-containing macrocycles **2.157**–**2.163** via Cu(I)-catalyzed cycloaddition.

2.2.2.4.3 Ring-Closing Metathesis

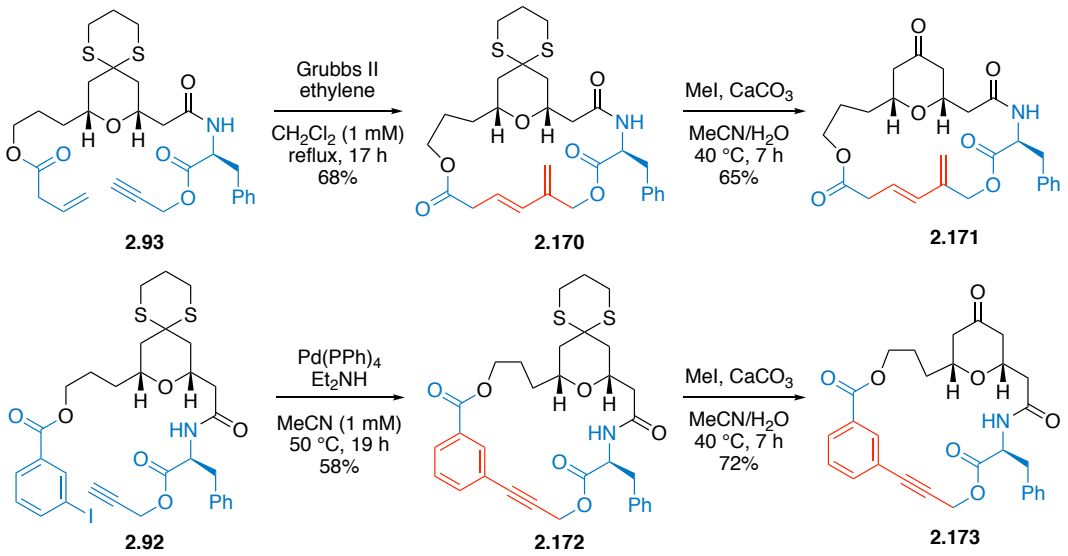
Ring-closing metathesis[70] of the linear substrates **2.109–2.111** was investigated (Scheme 26). The treatment of the diene **2.109** with Grubbs 2nd-generation catalyst under a refluxing condition yielded an inseparable mixture of *cis* and *trans* cyclized products. The mixture of two isomers smoothly underwent 1,3-dithiane deprotection to give the *trans* macrocycle **2.165A** (70%) and the *cis* macrocycle **2.165B** (10%). The stereochemistry was determined by analyzing coupling constants of the vicinal protons across a double bond, which were 15.6 Hz in the *trans* product **2.165A** and 11.5 Hz in the *cis* product **2.165B**. The double bond-containing macrocycles **2.168** and **2.169** were also prepared under the same procedure.



Scheme 26. Synthesis of the macrocycles (**2.165A**, **2.165B**, **2.168**, and **2.169**) through the ruthenium-catalyzed ring-closing metathesis

2.2.2.4.4 Enyne Metathesis and Sonogashira Cross-Coupling

Ring-closing enyne metathesis[67] and Sonogashira cross-coupling reaction[68-69] were exploited to generate the macrocycles **2.92** and **2.93** (Scheme 27). First, ring-closing metathesis of the enyne substrate **2.93** was studied. It was reported that the *endo*/*exo* selectivity of the ring closure reaction is significantly affected by ring size due to the differences in ring strain of the intermediates.[74] When a ring system contains more than 12 atoms, an *endo*-product is preferentially generated. Moreover, Hansen and Lee found that the *E*/*Z* selectivity of the 1,3-diene can be improved by performing the reaction under ethylene atmosphere.[74] According to this procedure, a mixture of our substrate **2.93** and the Grubbs 2nd-generation catalyst was refluxed under ethylene atmosphere leading to the formation of the desired enyne macrocycle **2.170**. The coupling constant of the protons on the internal olefin is 15.8 Hz (in CD_3OD), thus confirming the product is in a *trans* configuration. Next, intramolecular Sonogashira coupling reaction of substrate **2.92** was carried out. In the presence of tetrakis(triphenylphosphine)palladium, the reaction smoothly proceeded to furnish the aryl alkyne-containing macrocycle **2.172**. The resulting macrocycles **2.170** and **2.172** were subjected to dithiane deprotection to produce **2.171** and **2.173**.

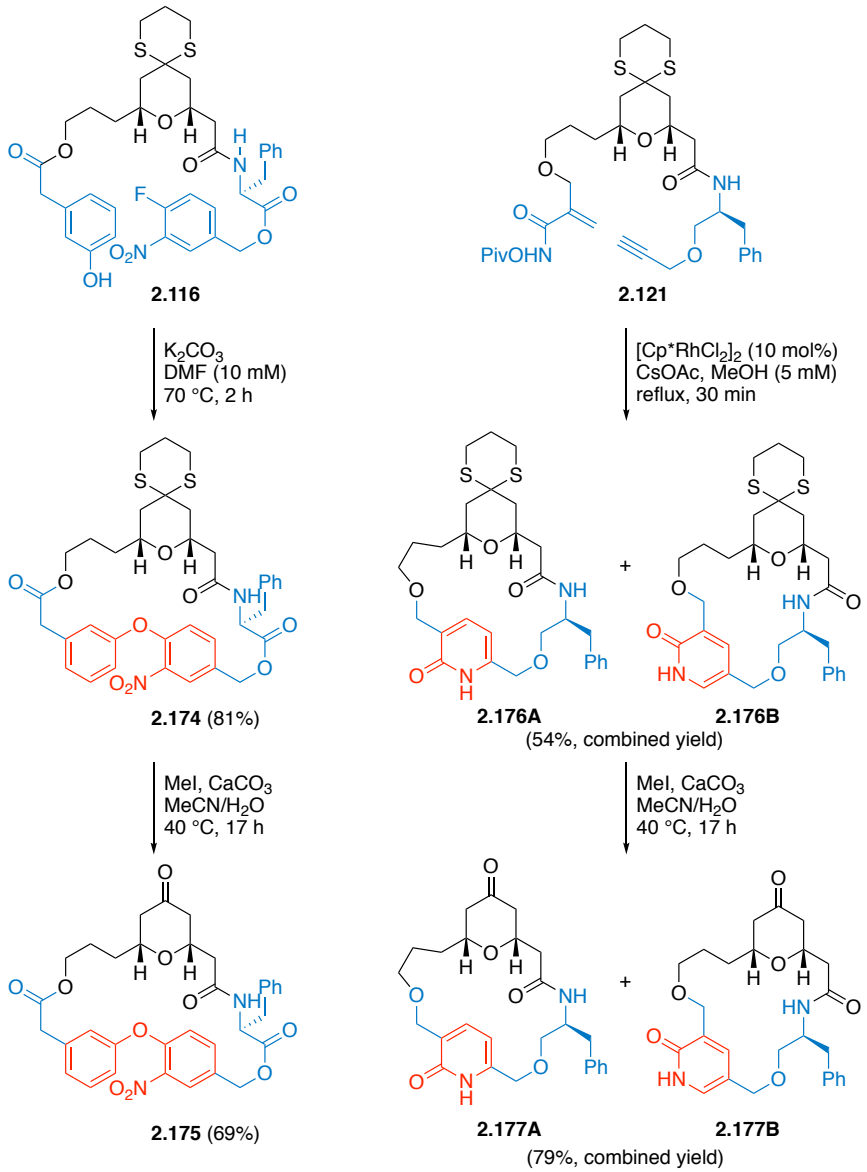


Scheme 27. Enyne metathesis and Sonogashira cross-coupling for the preparation of the macrocycle **2.171** and **2.173**

2.2.2.4.5 Nucleophilic Aromatic Substitution and Pyridone Synthesis

Nucleophilic aromatic substitution and pyridone synthesis were performed to incorporate a biaryl ether or a pyridone moiety into a macrocycle scaffold (Scheme 28). When substrate **2.116** was treated with potassium carbonate, the substitution reaction occurred on the activated aryl fluoride to generate the biaryl macrocycle **2.174** in 81% yield. Meanwhile, the installation of a pyridone ring in a macrocycle was carried out according to the reported procedure by Cossy and co-workers.[72] The linear acrylic hydroxamate **2.121** underwent a rhodium(III)-catalyzed C–H activation/heterocyclization to yield the cyclized products **2.176A** and **2.176B** as an inseparable mixture. Two regioisomers **2.176A** and **2.176B** were obtained possibly due to

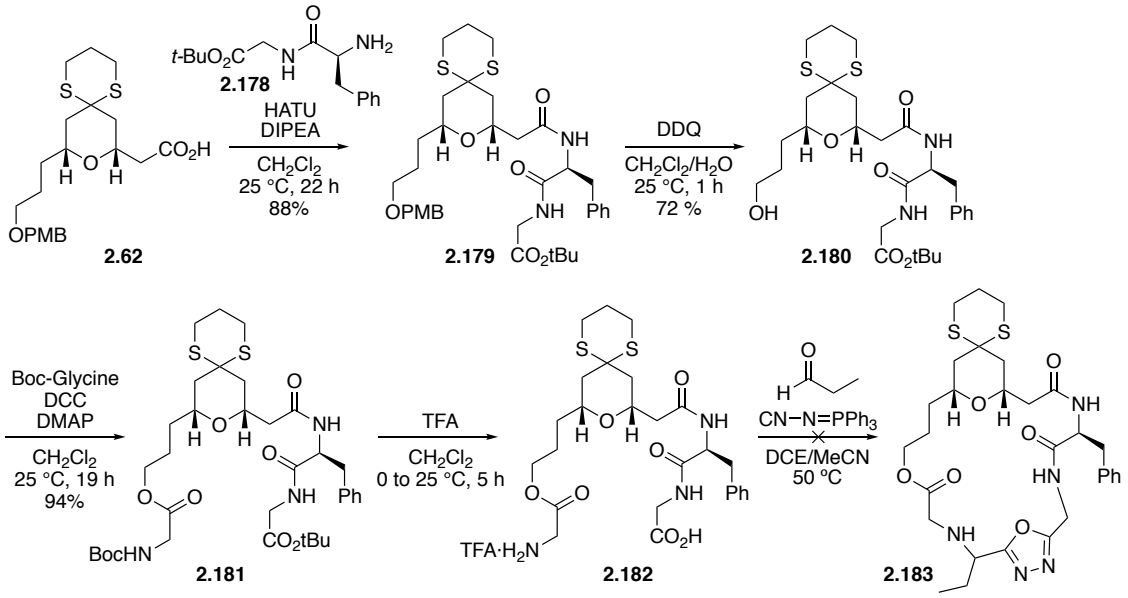
(1) the use of the terminal alkyne (not an internal alkyne) and (2) the sufficiently large ring size which provided a chance to have both conformations. With the cyclized products (**2.174**, **2.176A**, and **2.176B**) in hand, dithiane deprotection reactions were performed to give macrocycles (**2.175**, **2.177A**, and **2.177B**).



Scheme 28. Synthesis of macrocycle **2.175**, **2.177A**, and **2.177B** via nucleophilic aromatic substitution and ruthenium(III)-catalyzed pyridone synthesis

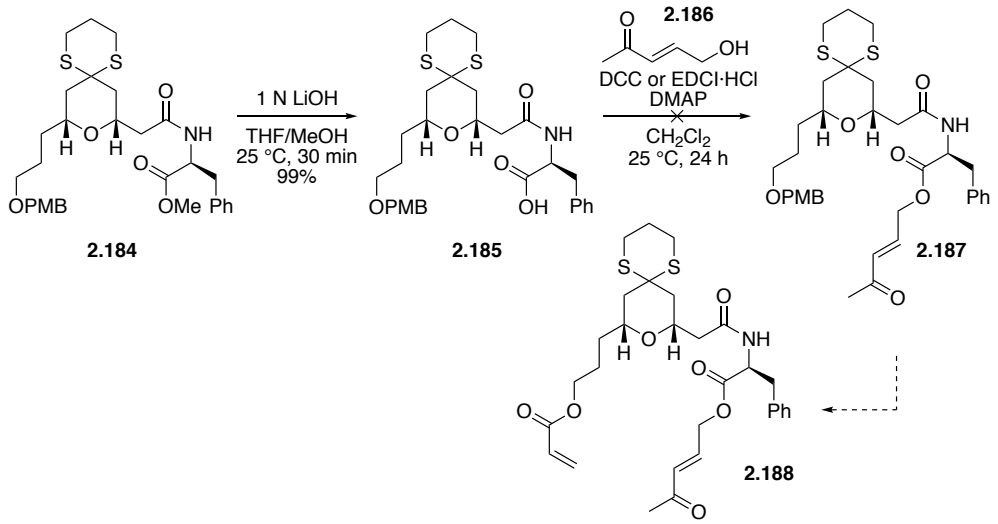
2.2.2.4.6 Other Attempts for Macrocyclization

Our attempts towards the installation of various heterocycles into a macrocycle scaffold are described here. We investigated the synthesis of a macrocycle containing a fused-oxadiazole system. In 2016, the Yudin group reported a macrocyclization reaction between a linear peptide, an aldehyde, and (*N*-isocyanimino)triphenylphosphorane.[75] In order to apply this method to our system, the substrate x was prepared which has amine and carboxylic acid handles (Scheme 29). The synthesis commenced with HATU coupling of **2.62** with dipeptide **2.178** to give **2.179** in 88% yield. PMB deprotection and DCC coupling with Boc-protected glycine followed by the treatment of trifluoroacetic acid generated substrate **2.182** for the oxadiazole formation. Unfortunately, this method was not applicable to our system, producing a complex mixture of unidentified compounds.



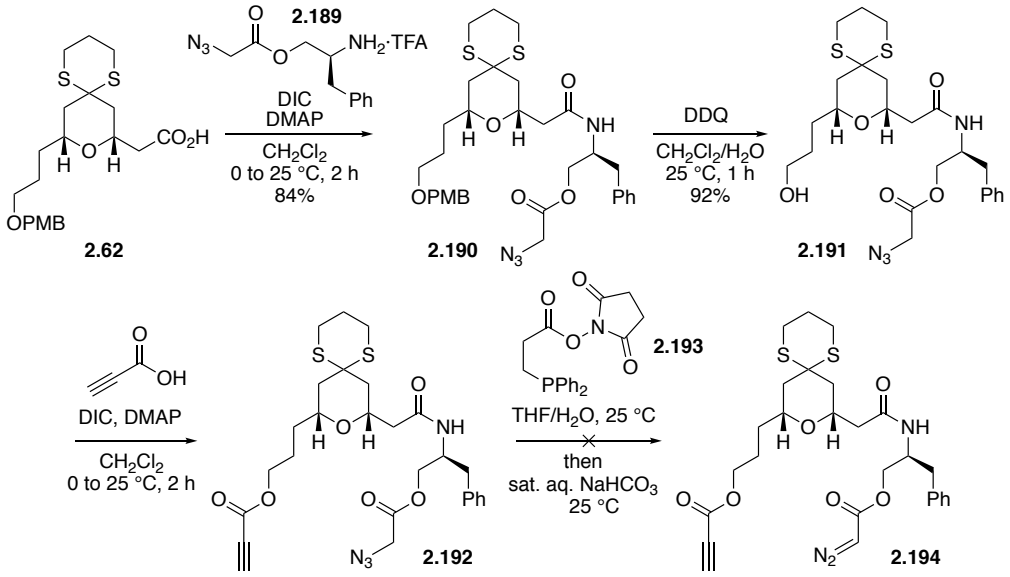
Scheme 29. Attempts towards the installation of an oxadiazole moiety

Next, the synthesis for a substrate of intramolecular Diels–Alder reaction[76-77] was attempted (Scheme 30). We designed substrate **2.188**, which has an electron donating group on the carboxylic acid side and a dienophile on the primary alcohol side. After hydrolysis of the methyl ester **2.184**, DCC or EDCI coupling reaction of the resulting carboxylic acid **2.185** with (*E*)-5-hydroxypent-3-en-2-one was explored. However, the desired product **2.187** was not obtained and the major product was highly unstable on silica gel purification.



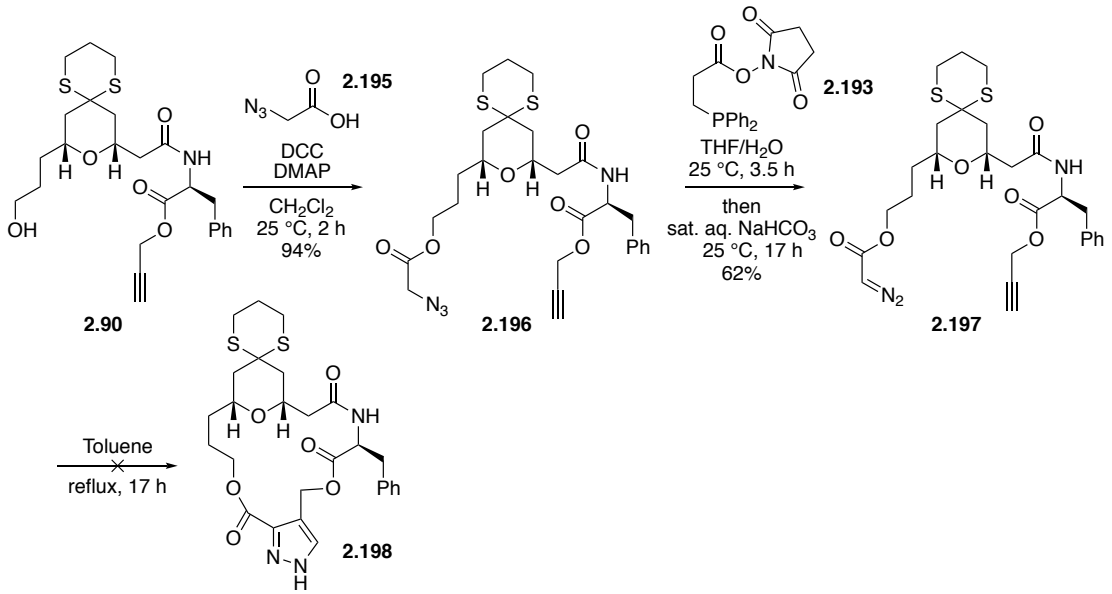
Scheme 30. Attempts to the synthesis of intramolecular Diels–Alder substrate **2.188**

We also tried the incorporation of a pyrazole moiety to a macrocycle via 1,3-dipolar cycloaddition of a diazoalkane with an alkyne. Initially, the substrate **2.194** was designed containing a diazo group on the carboxylic acid side and an alkyne on the other side (Scheme 31). HATU coupling with an amine, PMB deprotection, and DIC coupling with propionic acid were carried out to furnish the decorated azide alkyne **2.192** as a product. Conversion of the azide **2.192** into the diazo **2.193** was attempted according to Raine's procedure.[78] However, propionic ester was hydrolyzed during the reaction.



Scheme 31. Attempts to the synthesis of **2.194** for the pyrazole installation

To avoid the hydrolysis problem, we utilized the alkyne compound **2.90** as a starting material in which the triple bond was installed as a propargyl group (Scheme 32). DCC coupling of **2.90** with 2-azidoacetic acid (**2.195**) yielded the azide alkyne **2.196** in 94% yield. By adopting Raine's method, the azide group was successfully converted into the diazo group to furnish substrate **2.197**. However, the desired product **2.198** was not obtained possibly due to the lack of an activating group at the α-position of dipolarophile.

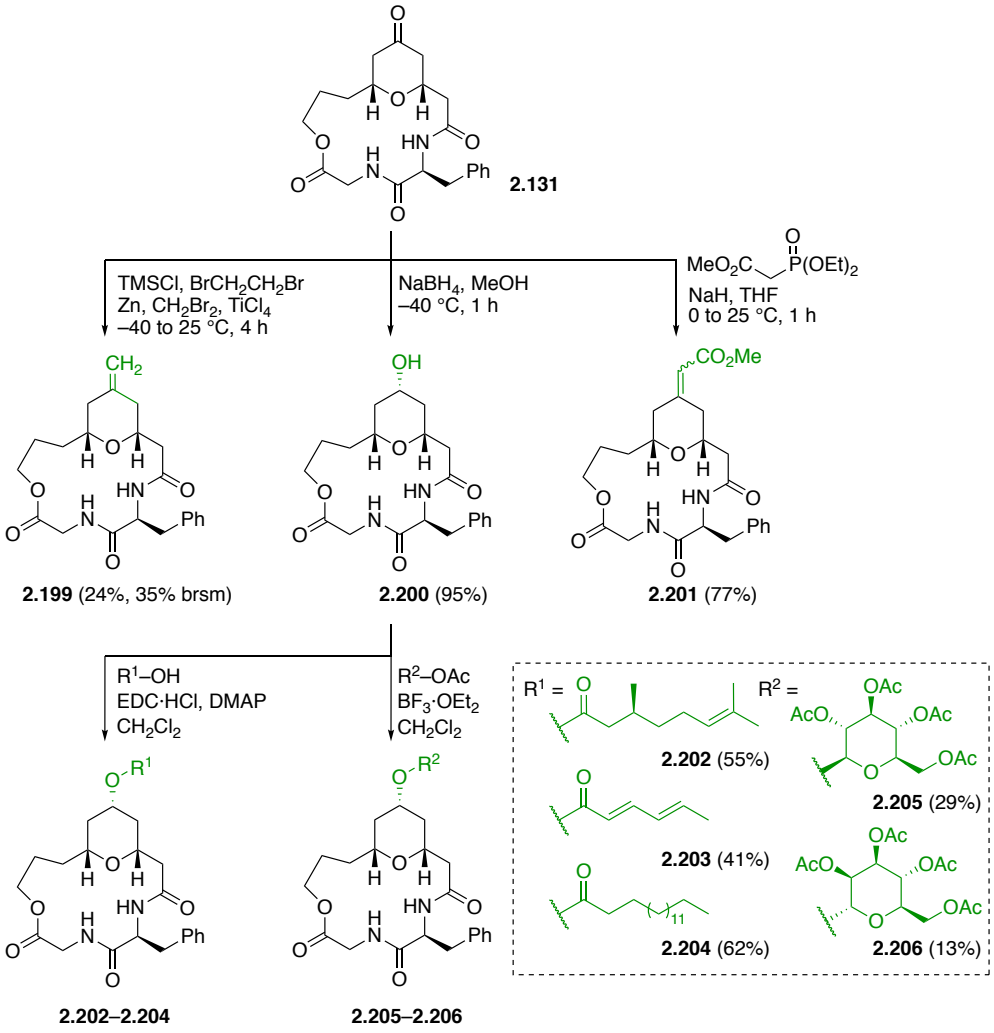


Scheme 32. Attempt towards the installation of a pyrazole moiety to a macrocycle

2.2.2.5 Appendage Diversifications

Having achieved the construction of THP-containing macrocyclic skeletons, we embarked on the final diversification step to further modulate the physicochemical properties of these macrocycles (Scheme 33). While the macrolactone **2.131** was chosen as an example for the appendage diversification step, this method can be applied to the other macrocyclic skeletons. The ketone functionality on the THP core of **2.131** was exploited as a synthetic handle to be converted into carbonyl, hydroxy, or olefinic functional group. The ketone **2.131** was stereoselectively reduced to alcohol **2.220** by $NaBH_4$ in 95% yield. In addition, the ketone was successfully converted to α,β-unsaturated ester **2.221** by a Horner–Wadsworth–Emmons reaction with methyl

diethylphosphonoacetate. Methylenation of the ketone **2.131** by Lombardo–Takai olefination[79-81] produced macrocycle **2.199** with a terminal olefin group (24%, 35% brsm). To improve the yield, Wittig-type olefination was also attempted with the combination of methyltriphenylphosphonium iodide and *n*-butyllithium. Unfortunately, this attempt resulted in a lower yield (10%) with inseparable triphenylphosphine oxide as a byproduct.



Scheme 33. Appendage diversification of the macrolactone **2.131**

To further expand the diversity of the macrocyclic library, we carried out acylation and glycosylation of the secondary alcohol **2.200**. This derivatization was devised since glycosylated or acylated macrolides are abundant in natural products and their physical properties and biological activities are significantly different from those of the parent compounds.[82] Acylation of **2.200** with citronellic acid, sorbic acid, and palmitic acid provided the decorated macrocycles **2.202**–**2.204**. Furthermore, glycosylation with glucose acetate and mannose acetate yielded the final compound **2.205** and **2.206**.

2.2.3 Cheminformatic Analyses

After the construction of our designed macrocycle library, we performed cheminformatic analyses including principal component analysis (PCA) and principal moment of inertia (PMI) to evaluate the chemical diversity of the library.[44] To achieve this, our 34 macrocycle library members were compared to 50 top-selling small-molecule drugs (Drug set), 75 diverse natural products (Natural Product Set), and 36 macrocyclic natural products (Macrocyclic NP Set) which are shown in Table 1.

Table 1. Compounds used in principal component analysis

Series	Compounds				
Drug Set (50 compounds)	Abilify	Coreg	Imitrex	Prevacid	Toprol
	Aciphex	Crestor	Januvia	Protonix	Tricor
	Actos	Cymbalta	Lamictal	Revlimid	Valtrex
	Adderall	Diovan	Levaquin	Risperdal	Wellbutrin
	Ambien	Effexor	Lexapro	Serevent	Xarelto
	Avandia	Eliquis	Lipitor	Seroquel	Zetia
	Benazepril	Flonase	Lyrica	Singulair	Zocor
	Celebrex	Fosamax	Nexium	Sovaldi	Zoloft
	Cialis	Gilenya	Norvasc	Tecfidera	Zyprexa
	Concerta	Gleevec	Plavix	Topamax	Zyrtec
Natural Product Set (75 compounds)	Actinonin	Cephamycin C	Estrone	Muscarine	Spongistatin 1
	Adrenaline	Cerebroside	FK228	Mycobactin S	SQ26180
	Adriamycin	Coformycin	FK506	Penicillin G	Staurosporine
	Amphotericin B	Colchicine	Forskolin	Phorbol MA	Streptogramin B
	Apoptolidin	Compactin	FTY720	Plaunotol	Streptomycin
	Arglabin	Cyclosporin A	Fumagillin	Prostaglandin f2a	Talaromycin B
	Artemisinin	Cytochalasin B	Geldanamycin	Pseudomonic acid A	Taxol
	Avermectin B1a	Dalfopristin	Ginkgolide B	Quinine	Telomestatin
	Bestatin	Daptomycin	Lactacystin	Radicicol	Thienamycin
	Bleomycin	Discodermolide	Lipstatin	Rapamycin	Trapoxin B
	Brefeldin A	Duocarmycin A	Longithorone A	Reserpine	Trichostatin
	Brevetoxin B	Echinocandin B	Midecamycin A1	Rifamycin B	Validamycin
	Caffeine	Epothilone A	Mizoribine	Salicylihalamide A	Vancomycin
	Calicheamicin G1	Ergotamine	Monensin	Scopolamine	Vincristine

	Calyculin A	Erythromycin A	Morphine	Spergualin	Zaragozic acid A
Macrocyclic NP Set (36 compounds)	2,4,6,8-Tetramethyl-3,4-dihydroxydec-8(9)-enolide	Cembrene A	Jasmine Ketolactone	Manzamine A	Pristinamycin IIB
	Apicularen A	Cladospolide D	Jatrophane 1	Methymycin	Sorangicin A
	Aspergillide A	Curvularin	Kendomycin	Neomethymycin	Xestospongin C
	Bacitracin A	Cyanolide A	Largazole	Neopeltolide	Zampanolide
	Batatoside L	Decarestrictine A1	Lasonolide A	Nostopeptolide A1	
	Bryostatin 1	Fluvirucin A1	Leucascandrolide A	Pinolidoxin	
	Budmunchiamine A	Hypothemycin	Lyngbouilloside	Pladienolide B	
	Caspofungin	Iriomoteolide 3a	Mandelalide A	Polymyxin B	
THP-MC Library Set (34 compounds)	**2.128**	**2.144**	**2.161**	**2.173**	**2.202**
	2.129	**2.148**	**2.162**	**2.175**	**2.203**
	2.130	**2.149**	**2.163**	**2.177A**	**2.204**
	2.131	**2.157**	**2.165A**	**2.177B**	**2.205**
	2.132	**2.158**	**2.168**	**2.199**	**2.206**
	2.133	**2.159**	**2.169**	**2.200**	**2.207**
	2.136	**2.160**	**2.171**	**2.201**	

PCA is a tool that uses a linear transformation to convert a set of correlated variables (in this case, molecular weights, polar surface area, number of stereogenic centers, etc) into a set of values of principal components.[83] PCA allows us to visualize multidimensional data into lower dimensional space. We examined 20 structural and physicochemical properties of 195 compounds (Table 2). Examination of the component loadings in PCA indicated that the main contributors of the PC1 axis are molecular weight, number of oxygens and hydrogen bonding acceptors, while the greatest contributors of the PC2 axis are hydrophobicity and calculated aqueous solubility. In PC1 vs. PC2 plot, the members of our macrocyclic library shift in a negative direction along the PC1 axis compared to the small-molecule drugs, covering a distinct region of the chemical space and considerably overlapping with macrocyclic natural product space (Figure 11a). In plots PC1 vs. PC3 (Figure 11b) and PC3 vs. PC2 (Figure 11c), the

macrocyclic library members are positioned between drug space and macrocyclic natural product space.

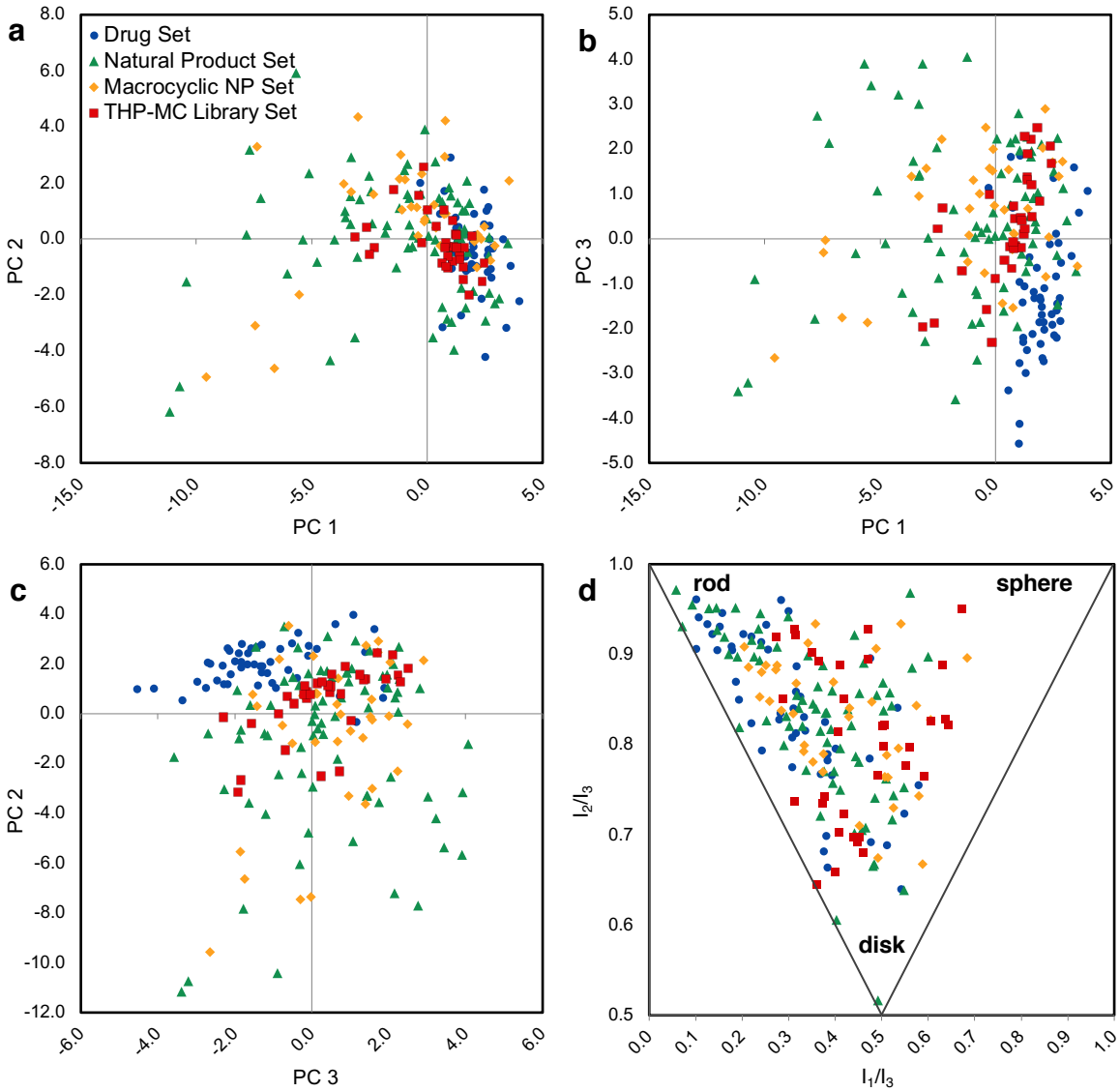


Figure 11. Principal component analysis (PCA) and principal moment of inertia (PMI) plots; (**a**) PCA plot of PC1 vs. PC2; (**b**) PCA plot of PC1 vs. PC3; (**c**) PCA plot of PC3 vs. PC2; (**d**) PMI plot illustrating the three-dimensional shape diversity of the compound sets

Table 2. Structural and physicochemical parameters used in PCA

Parameters	Description
MW	Molecular weight
N	Number of nitrogen
O	Number of oxygen
XlogP	Calculated *n*-octanol/water partition coefficient
HBD	Number of hydrogen bond donors
HBA	Number of hydrogen bond acceptors
RotB	Number of rotatable bonds
tPSA	Topological polar surface area
ALOGPs	Calculated *n*-octanol/water partition coefficient (alt)
ALOGpS	Calculated aqueous solubility
nStereo	Number of stereocenters
R	Number of *R* stereocenters
S	Number of *S* stereocenters
RSdelta	$R - S$
nStMW	nStereo ÷ MW (stereochemical density)
Rings	Number of rings
RngAr	Number of aromatic rings
RngSys	Number of ring systems
RngLg	Number of atoms in largest ring outline
RRsys	Rings ÷ RngSys (ring complexity)

Next, we carried out a PMI analysis to examine the molecular shape diversity of the test sets (Figure 11d). Principal moments of inertia calculations were performed using the Molecular Operating Environment (MOE, v2018.01) software package[84] by our collaborator, the Hargrove lab at Duke University. PMI analysis of three-dimensional shapes of compounds provides an intuitive description of molecular shape plotted on a three-sided triangular graph with three extremes (rod, disk, and sphere).[85] Low-energy conformations for each molecule in the compound sets were calculated and then plotted on a three-sided triangular map bounded by canonical shapes. The drug reference set lies along rod-like and disk-like axes. In contrast, our macrocyclic library showed

decreased rod-like character compared to the drug set, while extending more into sphere-like space and overlapping with natural products. The macrocyclic library possesses increased molecular shape diversity compared to small-molecule drugs.

2.2.4 Biological Evaluation

In order to assess the potential of the generated macrocycle library for chemical probe and therapeutic development, the library members were screened for inhibition of Huh7 cells, a hepatocyte-derived carcinoma cell line (Figure 12). The cell viability test was performed by our collaborator, the Derbyshire group at Duke University. Dimethyl sulfoxide and brefeldin A were used as a negative control and a positive control, respectively. When the 100 μM of each macrocycle was treated to the cancer cell line, the result showed that compound **2.173** exhibit the most potent inhibition (91%) among the THP-macrocycles, followed by **2.175** (33%), **2.148** (31%), and **2.128** (27%). These four macrocycle members were pointed on the PCA and PMI plots (figure 13). In addition to the cytotoxicity test, biological evaluation of the THP-containing macrocycle library in transcription factors, patient-derived xenograft tumors, and fungal infections is in progress.

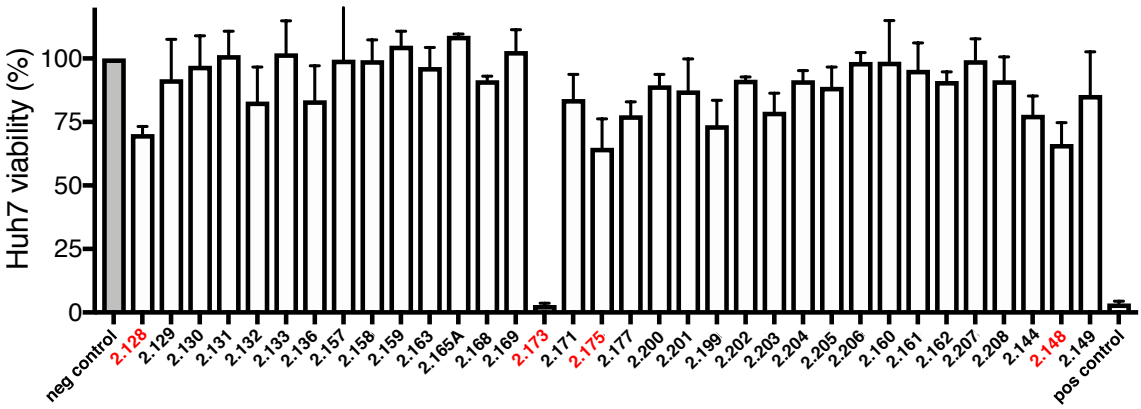
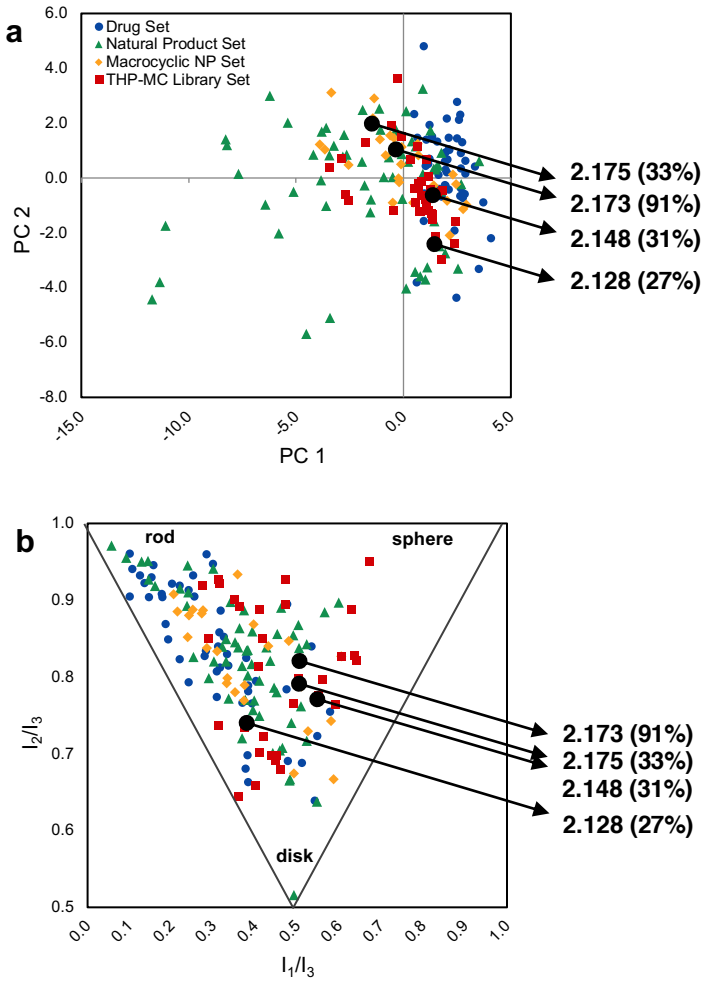


Figure 12. Cytotoxicity data of the macrocycle library in Huh7 cells (see Figure 13 for structure information)



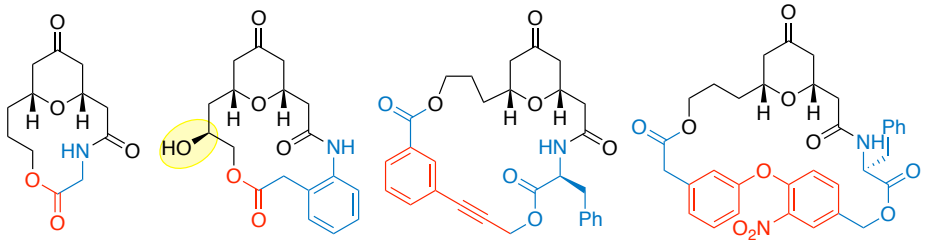


Figure 13. (**a**) The primary hits from the Huh7 cytotoxicity assay in PCA analysis; (**b**) The primary hits from the Huh7 cytotoxicity assay in PMA analysis

2.3 Conclusions

In summary, we introduced an approach for the generation of a structurally diverse and complex macrocycle library based on the tandem allylic oxidation/oxa-conjugate addition/oxidation reaction. For the skeletal diversity, we used the coupling of 1,3-dithianes to chiral epoxides followed by the tandem oxidation/oxa-conjugate addition reaction to introduce tetrahydropyrans to the macrocyclic scaffold. Expansion of the skeletal diversity of macrocycles was achieved by modifications in ring sizes (14- to 38- membered rings), scaffolds, and degree of saturation. A diverse set of macrocyclization reactions was successfully exploited to incorporate various functionalities into macrocyclic skeletons. The THPs in macrocycles were further elaborated with appendages such as acyl and glycosyl groups to expand the physicochemical properties of macrocycles. Cheminformatic analyses demonstrated that our macrocycle library covers a distinct chemical space compared to the conventional drug-like space, while significantly overlapping with macrocyclic natural product space. Since there exists a number of readily available chiral epoxides and functionalized 1,3-dithianes, we expect that our approach can be easily expanded to a larger size of macrocycle library with a high level of structural diversity. Moreover, it is fairly straightforward to incorporate structurally more complex tetrahydropyrans into macrocyclic backbones.[56-58, 61-62] This new set of macrocyclic compounds will provide

valuable insights for navigating a broader biological space that has been intractable using the conventional small molecules.[86]

2.4 Experimental Sections

2.4.1 General Methods

All reactions were conducted in oven-dried glassware under nitrogen. Unless otherwise stated all reagents were purchased from commercial suppliers and used without further purification. All solvents were American Chemical Society (ACS) grade or better and used without further purification except tetrahydrofuran (THF), which was freshly distilled from sodium/benzophenone each time before use. Analytical thin layer chromatography (TLC) was performed with glass backed silica gel (60 Å) plates with fluorescent indication (Whatman). Visualization was accomplished by UV irradiation at 254 nm, or by staining with *p*-anisaldehyde solution or potassium permanganate solution followed by heating. Flash column chromatography was performed by using silica gel (particle size 230−400 mesh, 60 Å). All 1H NMR and ^{13}C NMR spectra were recorded with a Varian 400 (400 MHz) and a Bruker 500 (500 MHz) spectrometer. All NMR δ values are given in parts per million (ppm) and are referenced to the residual isotopomer solvent signals ($CHCl_3$: δ = 7.26 ppm, CD_2HOD: δ = 3.31 ppm, $CD_3S(O)CHD_2$: δ = 2.50 ppm, CD_2HCN: δ =1.94 ppm) for ^1H NMR spectra, or the solvent signals for ^{13}C spectra. Coupling constants (*J*) are given in Hertz (Hz) and multiplicities are indicated using the conventional abbreviation (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of non-equivalent resonances, br = broad). Electrospray ionization (ESI) mass spectrometry (MS) was recorded with an Agilent 1100 series (LC/MSD trap)

spectrometer and performed to obtain the molecular masses of the compounds. Infrared (IR) absorption spectra were determined with a Thermo-Fisher (Nicolet 6700) spectrometer. Optical rotation values were measured with a Rudolph Research Analytical (A21102 API/1W) polarimeter.

2.4.2 General Procedures

Coupling of 1,3-Dithiane and Epoxide (GP1)

To a cooled (–78 °C) solution of an allylic alcohol (1.0 equiv) in THF/HMPA (10/1, 0.05 M) was added dropwise *t*-BuLi (1.7 M in pentane, 3.5 equiv). The resulting mixture was stirred for 5 min before epoxide (2.0 equiv) was added. After stirring for 1 h at –78 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

Allylic Oxidation, Oxa-conjugate Addition, Oxidative Esterification and Hydrolysis (GP2)

[Allylic oxidation/Oxa-conjugate addition/Oxidative esterification] To a solution of an allylic alcohol (1.0 equiv) in CH_2Cl_2 (0.1 M) was added MnO_2 (5.0 equiv), and the resulting mixture was stirred for 5 h at 25 °C. An addition of MnO_2 (5.0 equiv) was repeated three times every 3 h. When the starting material was completely consumed, a

mixture of dimethyltriazolium iodide (0.2 equiv), DBU (1.2 equiv, 0.1 M in CH_2Cl_2), MnO_2 (5.0 equiv) and MeOH (5.0 equiv) in CH_2Cl_2 was added in one portion. After stirred at the same temperature for 24 h, the reaction mixture was filtered through a pad of Celite and concentrated *in vacuo*. **[Hydrolysis]** The residue was dissolved in THF/MeOH/H_2O (2/1/1, 0.1 M) was added LiOH (1.0 M in H_2O, 3.0 equiv) at 25 °C. After stirred at the same temperature for 17–24 h, the reaction mixture was cooled to 0 °C, acidified with 1 N HCl (10 equiv), and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified as indicated.

HATU Amide Coupling Reaction (GP3)

To a solution of a carboxylic acid (1.0 equiv) in CH_2Cl_2 (0.1 M) were added amine (1.2 equiv), HATU (2.0 equiv), and *i*-Pr_2NEt (5.0 equiv) at 25 °C. After stirring for 18–24 h at 25 °C, the reaction mixture was concentrated *in vacuo*. The residue was purified as indicated.

PMB Deprotection (GP4)

To a solution of a PMB-protected alcohol (1.0 equiv) in CH_2Cl_2/H_2O (10/1, 0.1 M) was added DDQ (1.5 equiv) in one portion. After stirring the reaction mixture at 25 °C for 1

h, the reaction was quenched with saturated aqueous $NaHCO_3$ and diluted with excess H_2O. The resulting mixture was stirred vigorously for 1 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

Hydrolysis (GP5)

To a solution of an ester (1.0 equiv) in THF/H_2O (2/1, 0.1 M) was added LiOH (1.0 M in H_2O, 2.0 equiv) at 25 °C. After stirring at the same temperature for 1–18 h, the reaction mixture was cooled to 0 °C, acidified with 1 N HCl (6 equiv), and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified as indicated.

MNBA-Mediated Macrolactonization (GP6)

To a solution of 2-methyl-6-nitrobenzoic anhydride (MNBA, 1.2 equiv) in CH_2Cl_2 (0.002 M) was added DMAP (2.5 equiv). A solution of a seco-acid (1.0 equiv) in CH_2Cl_2 (0.003 M) was slowly added to the reaction mixture over 10 h via a dropping funnel at 25 °C. After stirring for additional 9–38 h, the reaction mixture was concentrated *in vacuo*. The residue was purified as indicated.

1,3-Dithiane Deprotection using MeI/$CaCO_3$ (GP7)

To a solution of a 1,3-dithiane-protected ketone (1.0 equiv) in MeCN/H_2O (3/1, 0.05 M) were added $CaCO_3$ (5.0 equiv) and MeI (40 equiv) in a sealed tube at 25 °C. The resulting suspension was slowly heated to 40 °C and stirred for 7–48 h. The reaction mixture was cooled to room temperature, and then diluted with CH_2Cl_2. The reaction mixture was filtered through a pad of Celite and concentrated *in vacuo*. The residue was purified as indicated.

TBS Deprotection (GP8)

To a cooled (0 °C) solution of a TBS-protected alcohol (1.0 equiv) in THF (0.03 M) was added TBAF (1.0 M in THF, 1.2 equiv) dropwise. After 5 min, the reaction mixture was allowed to warm to 25 °C and stirred for 5–16 h. The reaction was quenched by adding 1~2 drops of HOAc. The mixture was concentrated *in vacuo*, and the residue was purified as indicated.

Tosylation (GP9)

To a solution of an alcohol (1.0 equiv) in CH_2Cl_2 (0.5 M) were added *p*-toluenesulfonyl chloride (1.2 equiv), triethylamine (2.5 equiv), and DMAP (0.1 equiv) at 25 °C. After stirring at the same temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2 and H_2O. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2.

The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified as indicated.

Azidation (GP10)

To a solution of a tosylated alcohol (1.0 equiv) in CH_2Cl_2 (0.5 M) was added sodium azide (3.0 equiv) at 25 °C. The reaction mixture was slowly heated to 50 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and diluted with EtOAc and H_2O. The layers were separated, and the organic layer was washed with H_2O several times. The resulting organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified as indicated.

Cu(I)-catalyzed Azide/Alkyne Cycloaddition (GP11)

To a solution of a linear azide-alkyne (1.0 equiv) in dry THF (0.001 M) was added *i*-Pr_2NEt (3.0 equiv) at 25 °C. After bubbling N_2 through the solution for 15 min, CuI (2.0 equiv) was added and the reaction mixture was heated to reflux for 19–24 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified as indicated.

1,3-Dithiane Deprotection using *N*-bromosuccinimide (GP12)

To a cooled (0 °C) solution of a 1,3-dithiane-protected ketone (1.0 equiv) in MeCN/H_2O (4/1, 0.03 M) were added 2,6-lutidine (16 equiv) and *N*-bromosuccinimide (8.0 equiv). After stirring for 15 min at the same temperature, the reaction mixture was diluted with CH_2Cl_2, and the reaction was quenched by adding saturated aqueous $NaHCO_3$. The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$. The resulting organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified as indicated.

Ring-Closing Metathesis (GP13)

A diene (1.0 equiv) was dissolved in CH_2Cl_2 (0.001 M) at 25 °C. Grubbs 2nd-generation catalyst (10 mol%) was added, and the reaction mixture was heated to reflux. After refluxing for 17–19 h, the reaction mixture was cooled to 25 °C and dimethyl sulfoxide (~10 drops) was added to the solution. After 17 h, the reaction mixture was concentrated *in vacuo.* The residue was purified as indicated.

DCC Coupling (GP14)

To a solution of an alcohol (1.0 equiv) in CH_2Cl_2 (0.05 M) were added acid (1.1 equiv), *N*,*N*'-dicyclohexylcarbodiimide (1.2 equiv), and DMAP (0.1 equiv) at 25 °C. After stirring

for 16–20 h at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified as indicated.

2.4.3 The Members of the Tetrahydropyran-Containing Macrocyclic Library

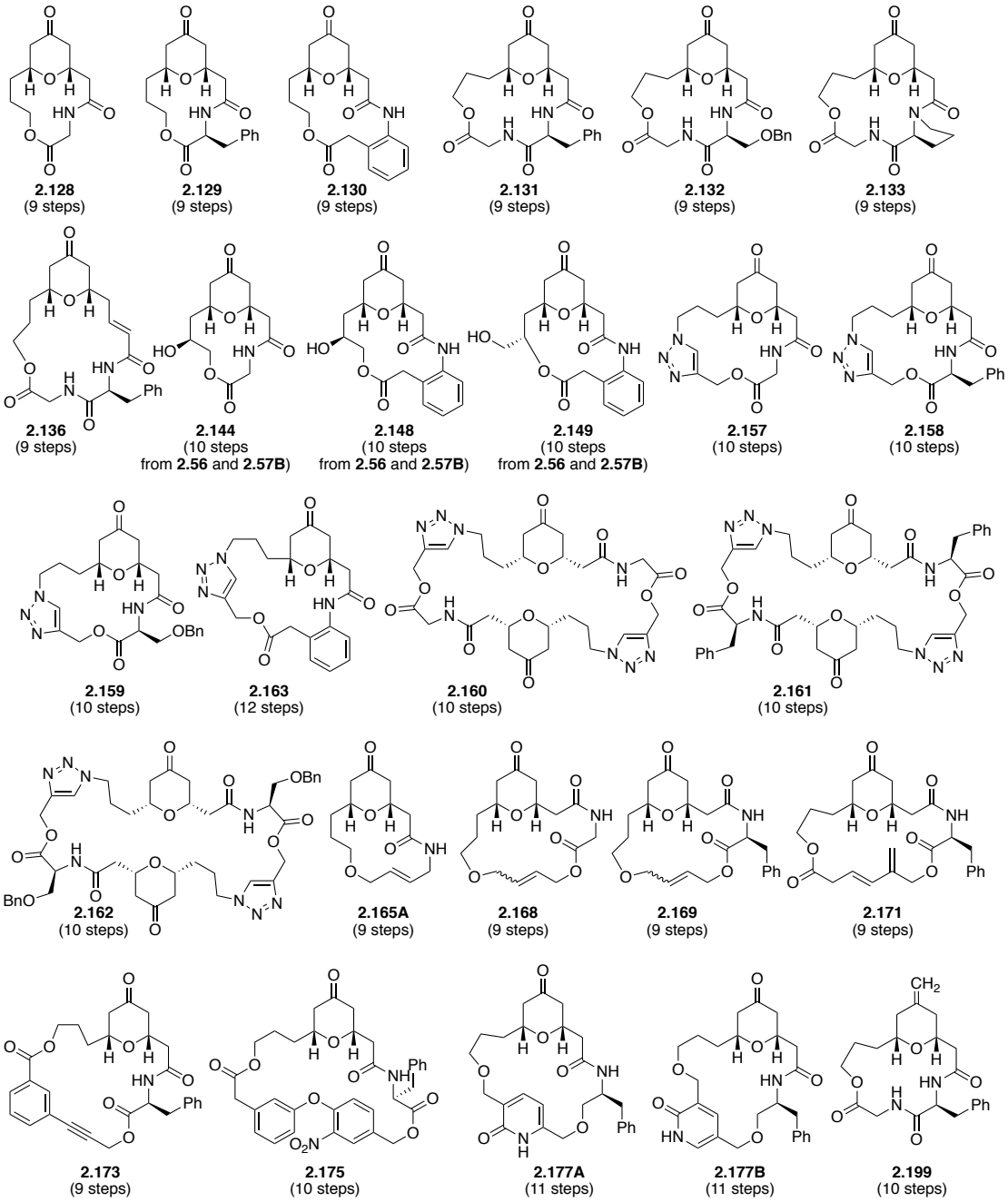


Figure 14. The members of the tetrahydropyran-containing macrocyclic library (continued on next page; the number of synthetic steps was counted from **2.56** and **2.57A** unless otherwise stated).

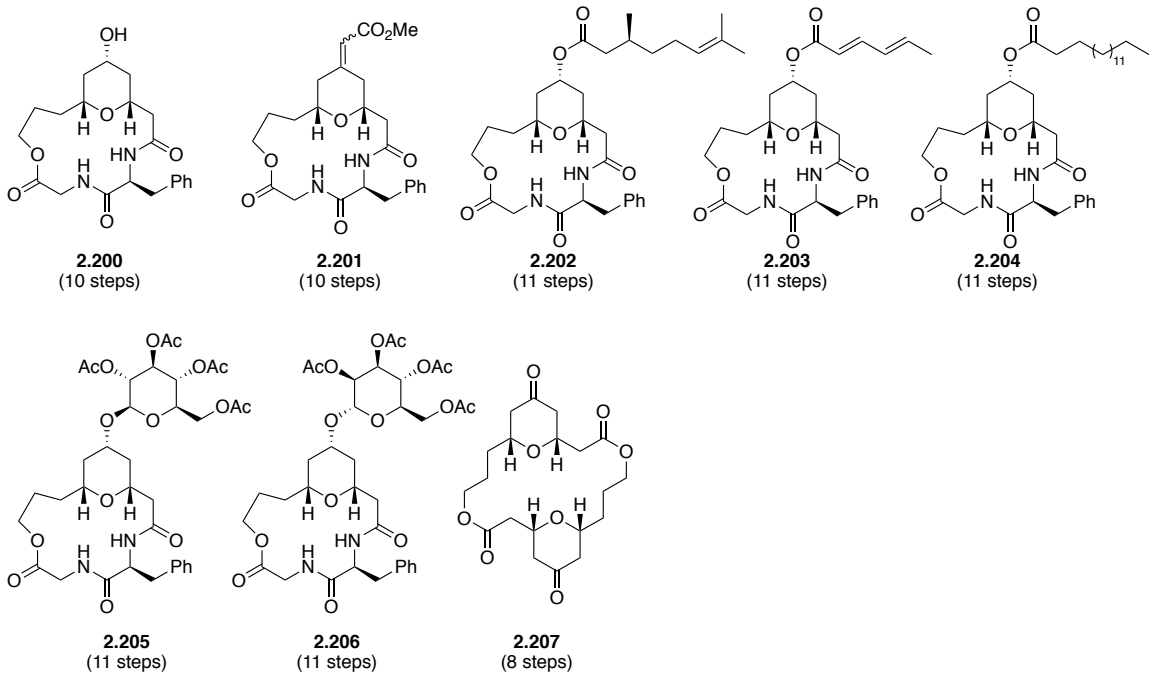
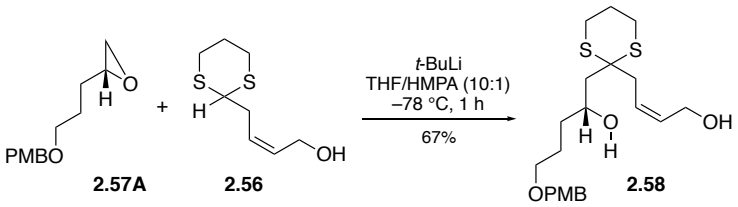
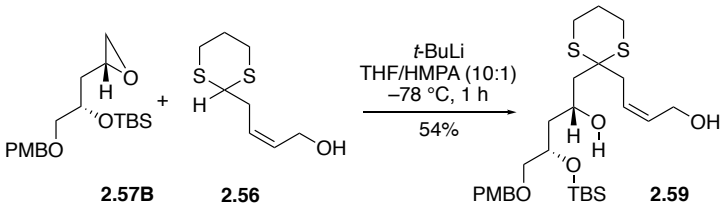


Figure 14 (continued). The members of the tetrahydropyran-containing macrocyclic library (the number of synthetic steps was counted from **2.56** and **2.57A** unless otherwise stated).

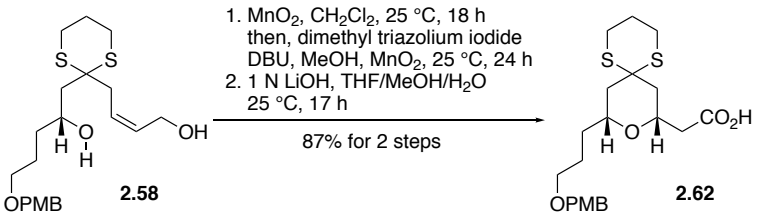
2.4.4 Synthetic Procedures and Analytical Data



Synthesized from allylic alcohol **2.56**[55] (2.31 g, 12.14 mmol) and epoxide **2.57A**[64] (5.40 g, 24.28 mmol) according to **GP1**. Purification by silica flash chromatography (hexanes/EtOAC, 1/1) afforded diol **2.58** (3.36 g, 67%) as colorless oil: $[\alpha]^{25}_{D}$ –11.2 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3400, 2932, 1512, 1440, 1246, 1032, 819 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.82 (dt, *J* = 11.0, 6.9 Hz, 1H), 5.70 (dt, *J* = 11.4, 7.4 Hz, 1H), 4.44 (s, 2H), 4.17–4.26 (m, 1H), 4.06–4.14 (m, 1H), 3.95–4.04 (m, 1H), 3.80 (s, 3 H), 3.63 (br s, 1H), 3.43–3.53 (m, 2H), 2.94 (br s, 1H), 2.71–2.91 (m, 5H) 2.66 (dd, *J* = 15.2, 6.8 Hz, 1H), 2.20 (dd, *J* = 15.1, 9.1 Hz, 1H), 1.91–2.07 (m, 3H), 1.67–1.77 (m, 2H), 1.52–1.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 131.9, 130.3, 129.3, 126.1, 113.7, 72.5, 69.9, 68.3, 58.1, 55.2, 51.9, 44.6, 37.0, 35.1, 26.2, 26.1, 25.7, 24.8; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{21}H_{32}O_4S_2$ 435.1634; found: 435.1641.

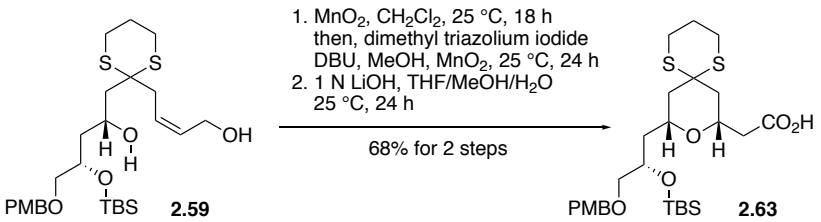


Synthesized from allyl alcohol **2.56**[55] (502 mg, 2.638 mmol) and epoxide **2.57B**[65] (1.86 g, 5.276 mmol) according to **GP1**. Purification by silica flash chromatography (hexanes/EtOAC, 2/1) afforded **2.59** (754 mg, 54%) as colorless oil; $[\alpha]^{25}_{D}$ –8.1 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 2930, 2855, 1514, 1248, 1100, 1034, 835, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.81 (dt, *J* = 11.5, 7.0 Hz, 1H), 5.71 (dt, *J* = 11.3, 7.2 Hz, 1H), 4.47 (s, 2H), 4.12– 4.25 (m, 2H), 3.98–4.10 (m, 2H), 3.81 (s, 3H), 3.61 (s, 1H), 3.47 (dd, *J* = 10.0, 4.6 Hz, 1H), 3.40 (dd, *J* = 9.9, 5.6 Hz, 1H), 2.76–2.96 (m, 5H), 2.72 (dd, *J* = 15.2, 6.7 Hz, 1H), 2.33 (t, *J* = 6.2 Hz, 1H), 2.17 (dd, *J* = 15.0, 8.4 Hz, 1H), 1.87–2.05 (m, 3H), 1.70–1.75 (m, 2H), 0.88 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2, 131.9, 130.1, 129.5, 126.5, 113.8, 74.1, 73.1, 70.3, 66.7, 58.2, 55.3, 52.1, 45.1, 43.2, 36.8, 26.3, 26.2, 25.9, 25.0, 18.1, −4.3, −4.7; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{46}O_5S_2Si$ 565.2448; found 565.2451.



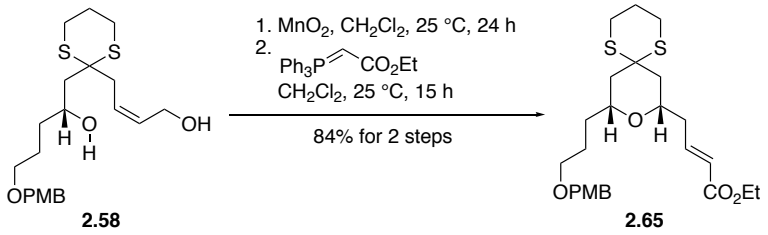
Synthesized from diol **2.58** (2.53 g, 6.132 mmol) according to **GP2**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded carboxylic acid **2.62** (2.28 g, 87% for 2 steps) as colorless oil: $[\alpha]^{25}_{D}$ –3.3 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 2937, 2937, 2360, 1712, 1513, 1247, 1090, 1035, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.27 (m, 2H),

6.87 (d, J = 7.9 Hz, 2H), 4.42 (s, 2H), 4.15–4.24 (m, 1H), 3.77–3.87 (m, 4H), 3.38–3.50 (m, 2H), 2.68–2.98 (m, 4H), 2.56 (dd, J = 15.7, 7.8 Hz, 1H), 2.48 (dd, J = 15.7, 4.9 Hz, 1H), 2.34 (d, J = 13.7 Hz, 1H), 2.16–2.22 (m, 1H), 1.94–2.04 (m, 2H), 1.50–1.78 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 159.1, 130.6, 129.4, 129.4, 113.9, 113.8, 72.7, 72.5, 69.8, 69.4, 55.3, 47.8, 43.1, 42.5, 40.5, 32.2, 26.0, 25.9, 25.8, 25.7; HRMS (m/z): $[M+Na]^+$ calcd for $C_{21}H_{30}N_5S_2$ 449.1438; found 449.1439.



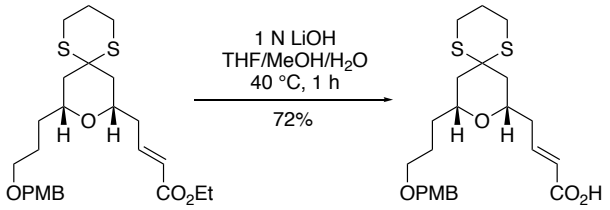
Synthesized from **2.59** (700 mg, 1.289 mmol) according to **GP2**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.63** (488 mg, 68% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ –2.5 (c 1.0, $CHCl_3$); IR (neat): ν_{max} = 2930, 2854, 2360, 1712, 1513, 1248, 1098, 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 4.44 (s, 2H), 4.16 (dddd, J = 11.6, 7.4, 5.4, 2.7 Hz, 1H), 3.92–4.02 (m, 2H), 3.79 (s, 3H), 3.28–3.50 (m, 2H), 2.66–2.97 (m, 4H), 2.53 (dd, J = 15.8, 7.7 Hz, 1H), 2.42 (dd, J = 15.7, 5.3 Hz, 1H), 2.34 (d, J = 13.6 Hz, 1H), 2.18 (d, J = 13.8 Hz, 1H), 1.92–2.05 (m, 2H), 1.49–1.78 (m, 4H), 0.87 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 159.2, 130.7, 129.4, 113.8, 74.1, 73.0, 69.8, 69.3, 68.7, 55.4, 47.8, 43.5, 42.4, 40.9, 40.6, 26.0, 25.9,

25.8, 18.2, −4.3, −4.6; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{44}O_6S_2Si$ 579.2241; found 579.2236.



[Allylic Oxidation/Oxa-conjugate Addition] To a solution of diol **2.58** (200 mg, 0.485 mmol) in CH_2Cl_2 (5 mL) was added MnO_2 (210 mg, 2.424 mmol), and the resulting mixture was stirred for 3 h at 25 °C. An addition of MnO_2 (210 mg, 2.424 mmol) was repeated three times every 3 h. When diol **2.58** was completely consumed, the reaction mixture was then filtered through a pad of Celite with CH_2Cl_2 to afford the crude aldehyde **2.64**, which was carried to the next step without further purification. **[Wittig Olefination]** To a solution of the crude aldehyde **2.64** in CH_2Cl_2 (10 mL) was added ethyl (triphenylphosphoranylidene)acetate (184 mg, 0.528 mmol) at 25 °C. The resulting mixture was stirred for 15 h, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 5/1) to afford (*E*)-enoate **2.65** (190 mg, 84% for 2 steps) as a major isomer: Colorless oil; $[\alpha]^{25}_D$ +4.7 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 2936, 2906, 2857, 1716, 1513, 1246, 1094, 1037, 819 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.24–7.28 (m, 2H), 6.96 (dt, *J* = 15.8, 7.2 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.87 (d, *J* = 15.7 Hz, 1H), 4.42 (s, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.82–3.90 (m, 1H), 3.80 (s, 3H), 3.69–3.77 (m,

1H), 3.39–3.51 (m, 2H), 2.85–2.91 (m, 2H), 2.72–2.77 (m, 2H), 2.36–2.45 (m, 1H), 2.27–2.35 (m, 1H), 2.16–2.25 (m, 2H), 1.70–1.82 (m, 1H), 1.50–1.69 (m, 3H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 159.1, 144.8, 130.7, 129.2, 123.4, 113.7, 72.6, 72.5, 71.2, 69.9, 60.2, 55.3, 48.0, 43.1, 42.9, 38.4, 32.4, 25.9, 25.8, 25.8, 14.3; HRMS (m/z): $[M+Na]^+$ calcd for $C_{25}H_{36}O_5S_2$ 503.1896; found 503.1896.

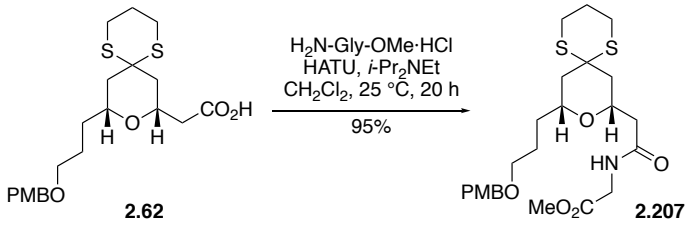


2.65 **2.66**

To a solution of ethyl ester **2.65** (161 mg, 0.345 mmol) in THF/MeOH/H_2O (2/1/1, total 3.5 mL) was added LiOH (0.69 mL, 1.0 M in H_2O, 0.690 mmol) at 25 °C. The reaction mixture was slowly heated to 40 °C and stirred for 1 h. The reaction mixture was cooled to 0 °C, acidified with 1 M citric acid (~2.5 mL), and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1) to afford carboxylic acid **2.66** (112 mg, 72%) as colorless oil: $[\alpha]^{25}_D$ +5.1 (c 0.95, $CHCl_3$); IR (neat): ν_{max} = 2939, 2906, 2860, 2359, 1695, 1651, 1513, 1247, 1091 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.28 (m, 2H), 7.07 (dt, J = 15.6, 7.2 Hz, 1H), 6.87 (d, J = 8.5 Hz, 2H), 5.90 (d, J = 15.7 Hz, 1H), 4.43 (s, 2H), 3.83–3.93 (m, 1H), 3.80 (s, 3H), 3.69–3.78 (m, 1H), 3.39–3.52

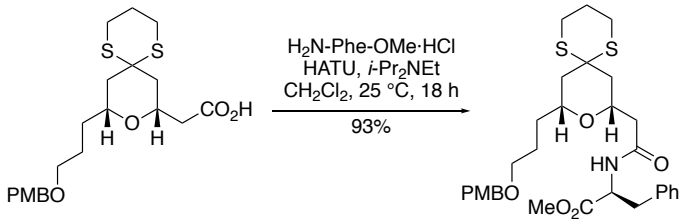
(m, 2H), 2.85–2.91 (m, 2H), 2.71–2.78 (m, 2H), 2.28–2.49 (m, 2H), 2.21 (d, J = 13.2 Hz, 2H), 1.95–2.04 (m, 2H), 1.50–1.80 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 159.2, 147.8, 130.7, 129.4, 122.8, 113.8, 72.7, 72.5, 71.2, 69.9, 55.4, 48.0, 43.2, 43.0, 38.5, 32.4, 26.0, 25.9, 25.8; HRMS (m/z): $[M+H]^+$ calcd for $C_{23}H_{32}O_5S_2$ 453.1764; found 453.1763.

Macrocycles from Macrolactonization



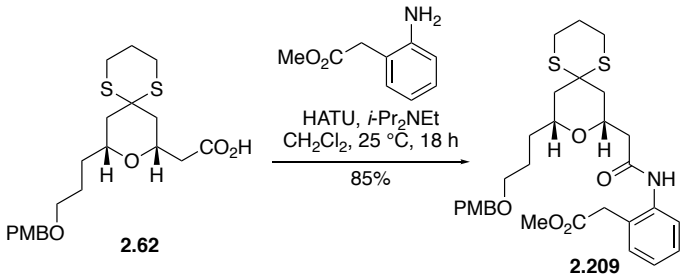
Synthesized from **2.62** (70 mg, 0.164 mmol) and glycine methyl ester hydrochloride (25 mg, 0.197 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 2/3) afforded **2.207** (77 mg, 95 %) as colorless oil: $[\alpha]^{25}_{D}$ –6.3 (c 1.0, $CHCl_3$); IR (neat): ν_{max} = 3319, 2946, 2907, 1748, 1660, 1512, 1245, 1207, 1033 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 8.6 Hz, 2H), 7.04 (t, J = 5.5 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 4.40 (s, 2H), 4.09–4.16 (m, 1H), 4.05 (dd, J = 18.4, 5.8 Hz, 1H), 3.91 (dd, J = 18.3, 5.0 Hz, 1H), 3.80–3.88 (m, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.38–3.48 (m, 2H), 2.92 (ddd, J = 14.3, 8.7, 3.4 Hz, 1H), 2.66–2.86 (m, 3H), 2.34–2.48 (m, 2H), 2.28 (dt, J = 13.7, 2.2 Hz, 1H), 2.16 (dt, J = 13.9, 2.1 Hz, 1H), 1.89–2.04 (m, 2H), 1.47–1.75 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.3, 159.2, 130.6, 129.3, 113.8, 72.6, 72.5, 69.9, 55.3, 52.3, 47.7, 43.1, 42.3, 42.3, 41.2,

32.5, 26.0, 25.9, 25.7, 25.7; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{24}H_{35}NO_6S_2$ 520.1798; found 520.1811.

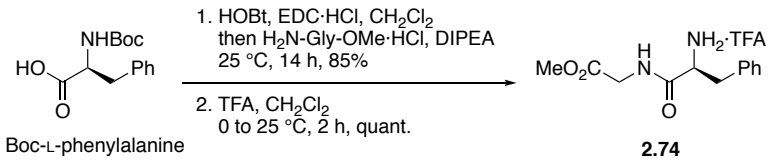


2.62 **2.208**

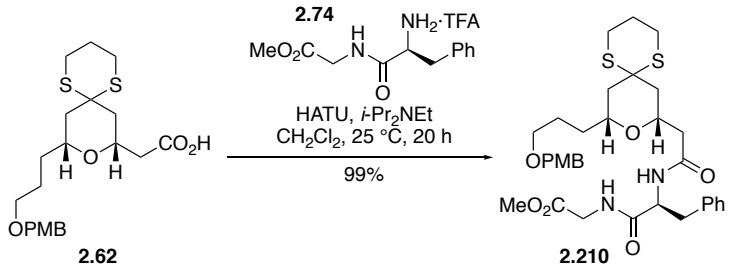
Synthesized from **2.62** (56 mg, 0.131 mmol) and L-phenylalanine methyl ester hydrochloride (31 mg, 0.144 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.208** (72 mg, 93%) as colorless oil: $[\alpha]^{25}_D$ +27.6 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3342, 2948, 2908, 1744, 1670, 1513, 1247, 1089, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.17–7.28 (m, 4H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 7.4 Hz, 2H), 4.92 (dt, *J* = 7.7, 5.5 Hz, 1H), 4.40 (s, 2H), 4.03–4.12 (m, 1H), 3.80 (s, 3H), 3.64–3.72 (m, 4H), 3.33–3.41 (m, 2H), 3.10 (t, *J* = 5.1 Hz, 2H), 2.89–2.98 (m, 1H), 2.70–2.86 (m, 3H), 2.33–2.47 (m, 2H), 2.05–2.32 (m, 2H), 1.90–2.07 (m, 2H), 1.28–1.70 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 170.3, 159.1, 136.1, 130.7, 129.4, 129.2, 128.5, 127.1, 113.8, 72.5, 69.8, 69.8, 55.3, 52.9, 52.2, 47.7, 42.8, 42.3, 42.1, 38.0, 32.2, 25.9, 25.9, 25.7, 25.6; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{31}H_{41}NO_6S_2$ 610.2268; found 610.2255.



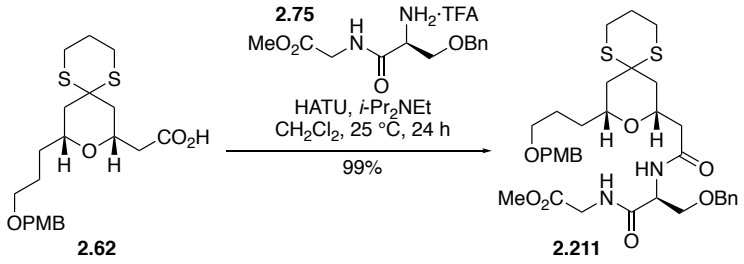
Synthesized from **2.62** (58 mg, 0.135 mmol) and methyl 2-(2-aminophenyl)acetate[87-88] (62 mg, 0.162 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.209** (66 mg, 85%) as colorless oil: $[\alpha]^{25}_{D}$ +1.6 (*c* 1.2, $CHCl_3$); IR (neat): ν_{max} = 3308, 2947, 2858, 1736, 1512, 1246, 1091, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 (br s, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.21 (m, 3H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 4.37 (s, 2H), 4.23–4.31 (m, 1H), 3.82–3.90 (m, 1H), 3.80 (s, 3H), 3.68 (s, 3H), 3.61 (s, 2H), 3.38–3.45 (m, 2H), 2.94 (ddd, *J* = 14.4, 8.7, 3.4 Hz, 1H), 2.75–2.87 (m, 2H), 2.71 (ddd, *J* = 14.5, 7.0, 3.6 Hz, 1H), 2.57 (d, *J* = 5.9 Hz, 2H), 2.41 (d, *J* = 13.4 Hz, 1H), 2.19 (d, *J* = 13.9 Hz, 1H), 1.93–2.08 (m, 2H), 1.53–1.75 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 169.2, 159.1, 136.3, 130.7, 130.6, 129.2, 128.3, 126.9, 125.7, 125.5, 113.8, 72.6, 72.5, 70.1, 69.8, 55.3, 52.5, 47.8, 43.8, 43.1, 42.5, 38.2, 32.4, 26.0, 25.9, 25.7, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{30}H_{39}NO_6S_2$ 574.2292; found 574.2297.



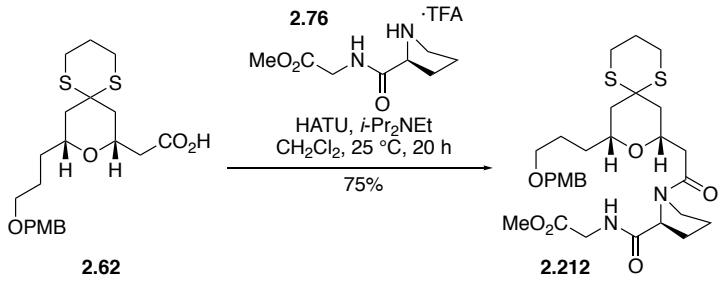
To a solution of Boc-L-phenylalanine (1.93 g, 7.27 mmol) in CH_2Cl_2 (73 mL) were added 1-hydroxybenzotriazole hydrate (1.18 g, 8.73 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 1.67 g, 8.73 mmol) at 25 °C. After stirring for 30 min at the same temperature, glycine methyl ester hydrochloride (959 mg, 7.64 mmol) and *i*-Pr_2Et (3.80 mL, 21.8 mmol) were added to the solution at 25 °C. After 14 h, the reaction mixture was diluted with CH_2Cl_2 and H_2O. The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$, 1 M citric acid, and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) to afford the product (2.08 g, 85%) as colorless oil. The product was dissolved in CH_2Cl_2 (60 mL) and the reaction was cooled to 0 °C. Trifluoroacetic acid (15 mL) was added dropwise, and the resulting mixture was stirred for 10 min at 0 °C and then allowed to warm to 25 °C. After stirring for 2 h at 25 °C, the reaction mixture was concentrated. The residue was co-evaporated with toluene several times to afford **2.74** (2.09 g, quant.) as colorless oil, which was solidified upon standing: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (br s, 1H), 7.16–7.34 (m, 5H), 4.39–4.57 (m, 1 H), 3.99 (dd, J = 18.0, 5.3 Hz, 1H), 3.74 (dd, J = 18.1, 5.1 Hz, 1H), 3.57 (s, 3H), 3.00–3.27 (m, 2H).



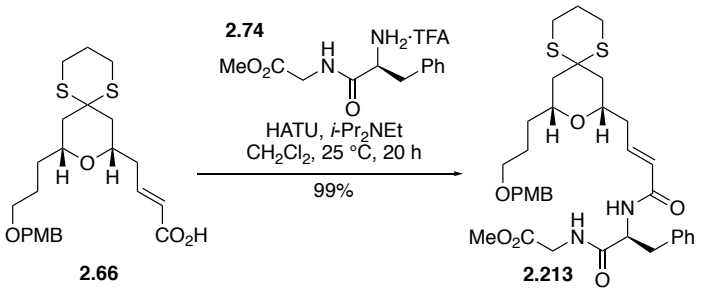
Synthesized from **2.62** (40 mg, 0.0983 mmol) and dipeptide **2.74** (27 mg, 0.113 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/3) afforded **2.210** (60 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –16.5 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3293, 2935, 2857, 1752, 1647, 1513, 1246, 1207, 1090 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.12–7.26 (m, 7H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.73 (t, *J* = 5.4 Hz, 1H), 4.74 (q, *J* = 7.0 Hz, 1H), 4.38 (s, 2H), 3.95–4.02 (m, 1 H), 3.92 (dd, *J* = 6.4, 5.4 Hz, 2H), 3.75 (s, 3H), 3.63–3.71 (m, 4H), 3.33–3.44 (m, 2H), 3.09 (dd, *J* = 14.0, 6.8 Hz, 1H), 2.99 (dd, *J* = 14.0, 7.1 Hz, 1H), 2.87 (ddd, *J* = 14.4, 8.1, 3.7 Hz, 1H), 2.64–2.84 (m, 3H), 2.35 (dd, *J* = 15.4, 8.4 Hz, 1H), 2.27 (dd, *J* = 15.4, 3.4 Hz, 1H), 2.19 (dt, *J* = 13.6, 2.1 Hz, 1H), 2.13 (dt, *J* = 13.6, 2.0 Hz, 1H) 1.86–2.02 (m, 2H), 1.41–1.69 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 171.0, 169.9, 159.1, 136.6, 130.6, 129.3, 129.2, 128.6, 126.9, 113.8, 72.6, 69.8, 69.7, 55.3, 53.9, 52.3, 47.6, 42.8, 42.5, 42.4, 41.2, 37.9, 32.3, 25.9, 25.8, 25.8; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{44}N_2O_7S_2$ 667.2482; found 667.2492.



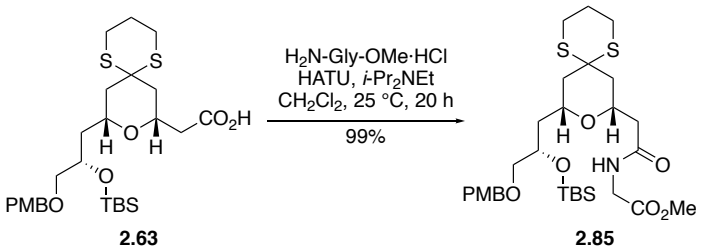
Synthesized from **2.62** (42 mg, 0.0985 mmol) and dipeptide **2.75**[89] (43 mg, 0.118 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/4) afforded **2.211** (66 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ +7.1 (*c* 0.45, $CHCl_3$); IR (neat): ν_{max} = 3300, 2949, 2906, 1753, 1657, 1513, 1247, 1097 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 7.23 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 7.1 Hz, 1H), 6.96 (t, *J* = 5.3 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.62 (td, *J* = 6.9, 4.1 Hz, 1H), 4.57 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 11.9 Hz, 1H), 4.38 (s, 2H), 4.10–4.19 (m, 1H), 4.02 (d, *J* = 5.3 Hz, 2H), 3.89 (dd, *J* = 9.3, 4.1 Hz, 1H), 3.74–3.83 (m, 4H), 3.72 (s, 3H), 3.55 (dd, *J* = 9.3, 6.9 Hz, 1H), 3.31–3.42 (m, 2H), 2.94 (ddd, *J* = 14.3, 8.6, 3.5 Hz, 1H), 2.67–2.87 (m, 3H), 2.37–2.48 (m, 2H), 2.30 (d, *J* = 14.0 Hz, 1H), 2.16 (d, *J* = 14.1 Hz, 1H), 1.89–2.08 (m, 2H), 1.44–1.72 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.3, 169.9, 159.2, 137.5, 130.7, 129.3, 128.6, 128.0, 127.9, 113.8, 73.5, 72.7, 72.6, 70.0, 69.9, 69.3, 55.4, 52.6, 52.4, 47.8, 43.0, 42.6, 42.5, 41.4, 32.3, 26.0, 25.9, 25.8, 25.8; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{34}H_{46}N_2O_8S_2$ 697.2588; found 697.2594.



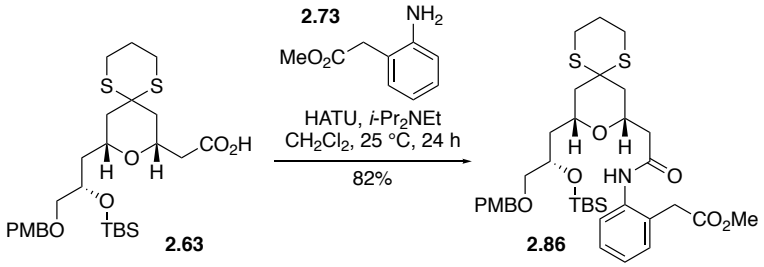
Synthesized from **2.62** (81 mg, 0.190 mmol) and dipeptide **2.76**[90] (81 mg, 0.285 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/4) afforded **2.212** (85 mg, 75%) as colorless oil: $[\alpha]^{25}_{D}$ –51.4 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 2945, 1751, 1629, 1513, 1439, 1246, 1091, 844 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (t, *J* = 5.5 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.60 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.38 (s, 2H), 4.25 (dt, *J* = 11.1, 5.9 Hz, 1H), 4.01 (dd, *J* = 18.2, 5.7 Hz, 1H), 3.93 (dd, *J* = 18.2, 5.3 Hz, 1H), 3.72–3.79 (m, 4H), 3.69 (s, 3H), 3.49–3.58 (m, 2H), 3.34–3.44 (m, 2H), 2.99 (ddd, *J* = 12.7, 8.9, 3.0 Hz, 1H), 2.73–2.88 (m, 2H), 2.67 (ddd, *J* = 14.5, 7.1, 3.3 Hz, 1H), 2.58 (dd, *J* = 14.7, 6.9 Hz, 1H), 2.50 (dt, *J* = 13.7, 2.0 Hz, 1H), 2.32–2.44 (m, 2H), 2.11 (d, *J* = 13.7 Hz, 1H), 1.78–2.05 (m, 5H), 1.37–1.72 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 171.0, 170.1, 159.1, 130.6, 129.2, 113.8, 72.5, 72.3, 72.3, 70.7, 69.7, 59.5, 55.3, 52.2, 48.0, 43.4, 42.6, 41.2, 40.7, 32.4, 27.5, 25.9, 25.8, 24.9; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{42}N_2O_7S_2$ 617.2326; found 617.2344.



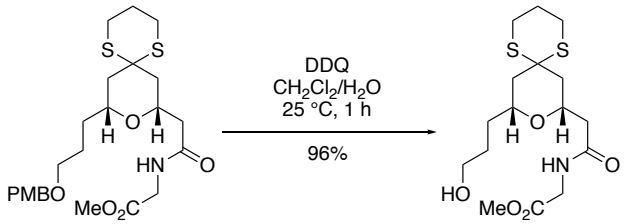
Synthesized from **2.66** (52 mg, 0.115 mmol) and dipeptide **2.74** (46 mg, 0.138 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/2) afforded **2.213** (79 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –6.2 (*c* 1.3, $CHCl_3$); IR (neat): ν_{max} = 3271, 2934, 2856, 2359, 1754, 1628, 1512, 1246, 1206, 1092, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.19–7.32 (7H), 6.79–6.92 (3H), 6.41 (br s, 1H), 6.16 (br s, 1H), 5.83 (d, *J* = 15.3 Hz, 1H), 4.77 (q, *J* = 7.4 Hz, 1H), 4.42 (s, 2H), 4.00 (dd, *J* = 18.2, 5.4 Hz, 1H), 3.91 (dd, *J* = 18.2, 5.2 Hz, 1H), 3.76–3.86 (m, 4H), 3.66–3.75 (m, 4H), 3.37–3.51 (m, 2H), 3.15 (dd, *J* = 13.9, 6.4 Hz, 1H), 3.08 (dd, *J* = 13.9, 7.5 Hz, 1H), 2.82–2.93 (m, 2H), 2.69–2.79 (m, 2H), 2.33–2.44 (m, 1H), 2.24–2.33 (m, 1H), 2.14–2.33 (m, 2H), 1.93–2.03 (m, 2H), 1.49–1.78 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 169.8, 165.6, 159.1, 141.3, 136.6, 130.7, 129.3, 129.3, 128.6, 127.0, 125.2, 113.8, 72.5, 71.3, 70.0, 55.3, 54.2, 52.4, 48.0, 43.1, 42.9, 41.2, 38.7, 38.3, 38.1, 32.4, 25.9, 25.8, 25.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{35}H_{46}N_2O_7S_2$ 671.2819; found 671.2828.



Synthesized from **2.63** (220 mg, 0.395 mmol) and glycine methyl ester hydrochloride (102 mg, 0.474 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.85** (245 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –5.0 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 2952, 2855, 1748, 1681, 1538, 1248, 1208, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.5 Hz, 2H), 7.04 (t, *J* = 5.3 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 2H), 4.39 (s, 1H), 3.91–4.11 (m, 4H), 3.79 (dd, *J* = 18.2, 4.9 Hz, 1H), 3.74 (s, 3H), 3.66 (s, 3H), 3.32–3.40 (m, 2H), 2.90 (ddd, *J* = 14.4, 8.7, 3.3 Hz, 1H), 2.58–2.83 (m, 3H), 2.31–2.43 (m, 2H), 2.25 (d, *J* = 13.6 Hz, 1H), 2.15 (d, *J* = 13.8 Hz, 1H), 1.87–2.01 (m, 2H), 1.77 (ddd, *J* = 13.2, 7.5, 5.2 Hz, 1H), 1.51–1.67 (m, 3H) 0.83 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.1, 159.1, 130.2, 129.3, 113.7, 74.2, 73.0, 69.6, 69.5, 68.3, 60.3, 55.2, 52.1, 47.6, 43.4, 42.1, 42.0, 41.1, 41.0, 25.9, 25.8, 25.7, 21.0, 18.1, 14.2, −4.4, −4.8; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{30}H_{49}NO_7S_2Si$ 650.2612; found 650.2610.

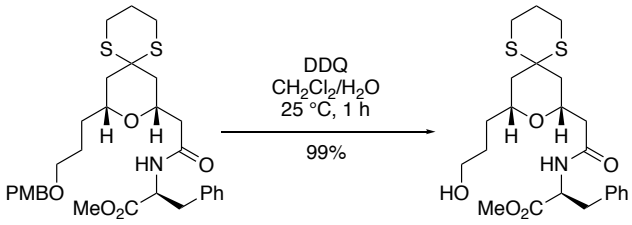


Synthesized from **2.63** (64 mg, 0.115 mmol) and methyl 2-(2-aminophenyl)acetate[87-88] (23 mg, 0.138 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 2/1) afforded **2.86** (66 mg, 82%) as colorless oil: $[\alpha]^{25}_{D}$ –1.1 (*c* 0.9, $CHCl_3$); IR (neat): ν_{max} = 3328, 2950, 2854, 1513, 1247, 1097, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.24–7.30 (m, 1H), 7.16–7.23 (m, 3H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.34 (s, 2H), 4.23–4.31 (m, 1H), 4.00–4.09 (m, 1H), 3.94 (p, *J* = 5.5 Hz, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 3.60 (s, 2H), 3.39 (dd, *J* = 9.7, 5.6 Hz, 1H), 3.34 (dd, *J* = 9.8, 5.1 Hz, 1H), 2.95 (ddd, *J* = 12.5, 8.7, 3.4 Hz, 1H), 2.76–2.87 (m, 2H), 2.71 (ddd, *J* = 14.5, 7.0, 3.6 Hz, 1H), 2.52–2.59 (m, 2H), 2.41 (d, *J* = 13.5 Hz, 1H), 2.23 (d, *J* = 13.8 Hz, 1H), 1.92–2.06 (m, 2H), 1.64–1.81 (m, 3H), 1.61 (dd, *J* = 13.7, 11.1 Hz, 1H), 0.85 (s, 9H), 0.02 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 169.2, 159.1, 136.4, 130.8, 130.6, 129.3, 128.4, 126.7, 125.7, 125.4, 113.8, 74.2, 73.0, 70.2, 69.8, 68.6, 55.3, 52.5, 47.9, 43.9, 43.5, 42.5, 42.4, 41.0, 38.4, 26.0, 25.8, 18.2, 18.2, −4.2, −4.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{36}H_{53}NO_7S_2Si$ 704.3106; found 704.3108.



2.207 **2.77**

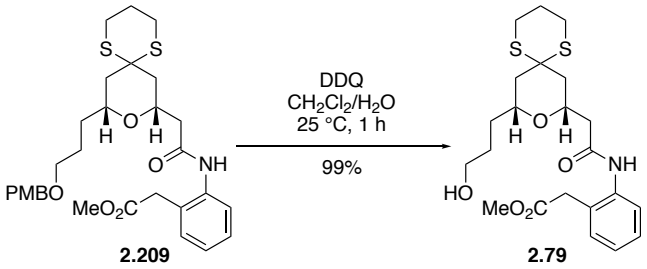
Synthesized from **2.207** (69 mg, 0.0139 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.77** (50 mg, 96%) as colorless oil: $[\alpha]^{25}_{D}$ –5.5 (*c* 0.83, $CHCl_3$); IR (neat): ν_{max} = 3344, 2946, 2359, 1749, 1655, 1542, 1437, 1210, 1058 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.03 (t, *J* = 5.0 Hz, 1H), 4.14 (dddd, *J* = 11.5, 7.6, 4.1, 2.0 Hz, 1H), 4.05 (dd, *J* = 18.4, 5.3 Hz, 1H), 3.98 (dd, *J* = 18.4, 5.1 Hz, 1H), 3.84 (dddd, *J* = 11.8, 6.4, 3.6, 1.5 Hz, 1H), 3.72 (s, 3H), 3.60 (td, *J* = 6.2, 1.2 Hz, 2H), 2.92 (ddd, *J* = 14.3, 8.9, 3.3 Hz, 1H), 2.65–2.85 (m, 3H), 2.35–2.46 (m, 3H), 2.29 (dt, *J* = 13.6, 2.1 Hz, 1H), 2.13 (dt, *J* = 13.8, 2.0 Hz, 1H), 1.87–2.06 (m, 2H), 1.50–1.71 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.6, 72.6, 70.1, 62.5, 52.4, 47.7, 43.2, 42.4, 42.3, 41.3, 32.0, 28.7, 26.0, 25.9, 25.7; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{16}H_{27}NO_5S_2$ 400.1223; found 400.1235.



2.208 **2.78**

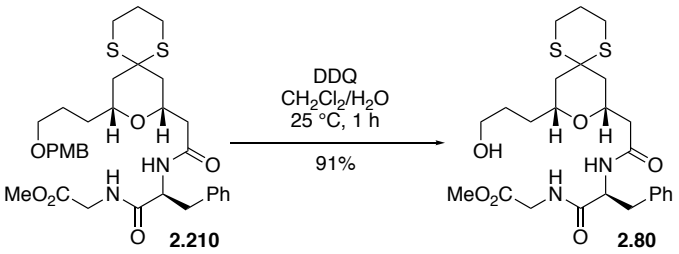
Synthesized from **2.208** (43 mg, 0.0728 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.78** (34 mg, 99%) as colorless oil:

$[\alpha]^{25}_{D}$ +46.2 (*c* 0.98, $CHCl_3$); IR (neat): ν_{max} = 3334, 2942, 2359, 1743, 1654, 1541, 1217, 1056 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.31 (m, 1H), 7.19–7.25 (m, 1H), 7.02–7.15 (m, 3H), 4.92 (dt, *J* = 8.0, 5.4 Hz, 1H), 4.08 (ddt, *J* = 11.0, 8.4, 2.7 Hz, 1H), 3.70 (s, 3H), 3.58–3.68 (m, 1H), 3.41–3.55 (m, 2H), 3.04–3.18 (m, 2H), 2.92 (ddd, *J* = 14.4, 9.0, 3.2 Hz, 1H), 2.68–2.87 (m, 3H), 2.42 (dd, *J* = 15.6, 8.3 Hz, 1H), 2.34 (dd, *J* = 15.6, 3.2 Hz, 1H), 2.25 (dt, *J* = 13.9, 2.1 Hz, 1H), 2.09 (dt, *J* = 13.8, 2.0 Hz, 1H), 1.88–2.06 (m, 2H), 1.63 (dd, *J* = 13.7, 11.3 Hz, 1H), 1.54 (dd, *J* = 13.8, 11.3 Hz, 1H), 1.22–1.50 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 170.3, 136.0, 129.5, 128.6, 127.1, 72.4, 69.9, 62.6, 52.9, 52.4, 47.6, 43.0, 42.3, 42.3, 38.0, 31.7, 28.5, 26.0, 25.9, 25.7; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{23}H_{33}NO_5S_2$ 490.1692; found 490.1694.

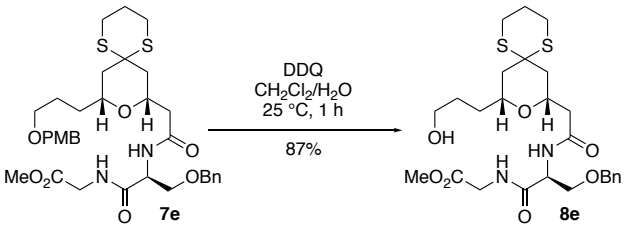


Synthesized from **2.209** (81 mg, 0.141 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.79** (63 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –2.1 (*c* 1.1, $CHCl_3$); IR (neat): ν_{max} = 3322, 2947, 1735, 1665, 1527, 1436, 1160, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 (br s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.29 (t, *J* = 7.9, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 4.29 (dtd, *J* = 11.7, 6.1, 1.9 Hz, 1H),

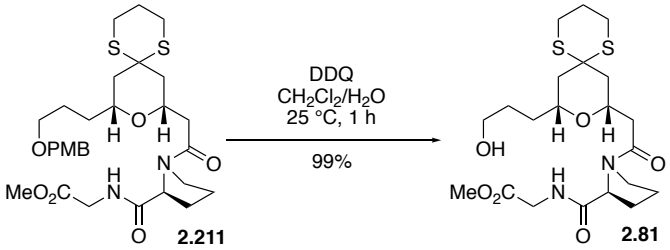
3.88 (dtt, J = 9.6, 4.9, 2.1 Hz, 1H), 3.70 (s, 2H), 3.61–3.66 (m, 2H), 3.54–3.60 (m, 2H), 2.95 (ddd, J = 14.5, 9.0, 3.3 Hz, 1H), 2.77–2.87 (m, 2H), 2.72 (ddd, J = 14.5, 7.0, 3.5 Hz, 1H), 2.51–2.62 (m, 2H), 2.41 (d, J = 13.7 Hz, 1H), 1.99–2.07 (m, 2H), 1.71 (dd, J = 13.7, 11.3 Hz, 1H), 1.50–1.67 (m, 5H); HRMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{31}NO_5S_2$ 454.1716; found 454.1724.



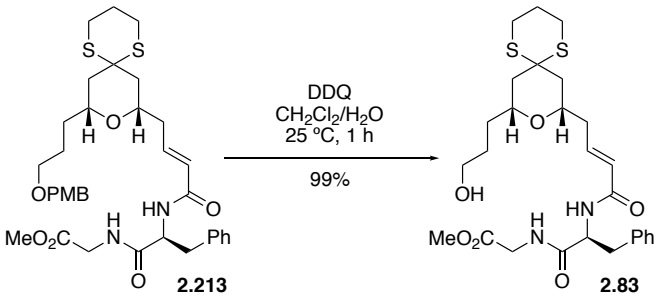
Synthesized from **2.210** (1.41 g, 2.187 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded **2.80** (1.05 g, 91%) as colorless oil: $[\alpha]^{25}_D$ –17.5 (c 1.0, $CHCl_3$); IR (neat): ν_{max} = 3306, 2946, 1751, 1648, 1529, 1438, 1209, 1053, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.18–7.33 (m, 5H), 6.65 (br s, 1H), 4.80 (dt, J = 8.2, 6.8 Hz, 1H), 3.98–4.08 (m, 2H), 3.89 (dd, J = 18.1, 5.0 Hz, 1H), 3.69–3.80 (m, 4H), 3.58 (td, J = 6.3, 2.3 Hz, 2H), 3.13 (dd, J = 13.8, 7.0 Hz, 1H), 3.07 (dd, J = 13.8, 6.6 Hz, 1H), 2.93 (ddd, J = 14.4, 8.6, 3.5 Hz, 1H), 2.73–2.87 (m, 3H), 2.70 (br s, 1H), 2.44 (dd, J = 15.8, 9.1 Hz, 1H), 2.33 (dd, J = 15.8, 2.7 Hz, 1H), 2.24 (dt, J = 13.6, 2.2 Hz, 1H), 2.14 (dt, J = 13.7, 2.1 Hz, 1H), 1.91–2.09 (m, 2H), 1.42–1.68 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 170.8, 169.9, 136.5, 129.4, 128.5, 126.9, 72.5, 69.7, 62.4, 53.8, 52.4, 47.5, 43.0, 42.6, 42.3, 41.1, 38.3, 31.8, 28.8, 25.9, 25.8, 25.7; HRMS (m/z): $[M+Na]^+$ calcd for $C_{25}H_{36}N_2O_6S_2$ 547.1907; found 547.1919.



Synthesized from **7e** (74 mg, 0.110 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded **8e** (53 mg, 87%) as colorless oil: $[\alpha]^{25}_{D}$ +14.0 (*c* 0.89, $CHCl_3$); IR (neat): ν_{max} = 3726, 2944, 2360, 1750, 1654, 1541, 1218, 1097, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28–7.37 (m, 5H), 7.05 (t, *J* = 5.5 Hz, 1H) 4.66 (td, *J* = 7.1, 4.1 Hz, 1H), 4.61 (d, *J* = 11.9 Hz, 1H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.15 (tdd, *J* = 8.5, 5.4, 2.6 Hz, 1H), 4.03 (dd, *J* = 5.4, 2.0 Hz, 2H), 3.91 (dd, *J* = 9.3, 4.2 Hz, 1H), 3.79–3.87 (m, 1H), 3.74 (s, 2H), 3.48–3.62 (m, 3 H), 2.95 (ddd, *J* = 14.5, 8.8, 3.4 Hz, 1H), 2.69–2.87 (m, 3H), 2.48 (dd, *J* = 15.5, 8.3 Hz, 1H), 2.41 (dd, *J* = 15.5, 3.4 Hz, 1H), 2.29 (dt, *J* = 13.7, 2.1 Hz, 1H), 2.14 (dt, *J* = 13.7, 2.1 Hz, 1H) 1.91–2.04 (m, 2 H), 1.48–1.71 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 170.0, 137.5, 128.5, 127.9, 73.5, 72.6, 69.9, 69.4, 62.5, 52.5, 52.5, 47.7, 43.1, 42.5, 41.4, 31.9, 28.8, 26.0, 25.9, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{38}N_2O_7S_2$ 555.2193; found 555.2193.

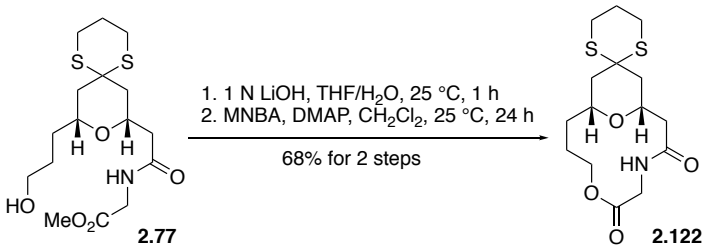


Synthesized from **2.211** (83 mg, 0.139 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded **2.81** (68 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –62.7 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3018, 2359, 1749, 1634, 1214, 744, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (t, *J* = 5.5 Hz, 1H), 4.59 (dd, *J* = 8.2, 2.3 Hz, 1H), 4.28 (dddd, *J* = 11.2, 7.3, 5.6, 1.9 Hz, 1H), 3.99 (dd, *J* = 18.2, 5.6 Hz, 1H), 3.93 (dd, *J* = 18.1, 5.4 Hz, 1H), 3.75–3.82 (m, 1H), 3.68 (s, 3H), 3.26–3.58 (m, 4H), 2.98 (ddd, *J* = 14.3, 9.2, 3.1 Hz, 1H), 2.64–2.85 (m, 3H), 2.57 (dd, *J* = 14.9, 7.1 Hz, 1H), 2.48 (d, *J* = 13.6 Hz, 1H), 2.41 (dd, *J* = 15.0, 5.5 Hz, 1H), 2.27–2.34 (m, 1H), 2.08 (d, *J* = 13.8 Hz, 1H), 1.81–2.03 (m, 5H), 1.39–1.67 (m, 6H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{34}N_2O_6S_2$ 475.1931; found 475.1936.



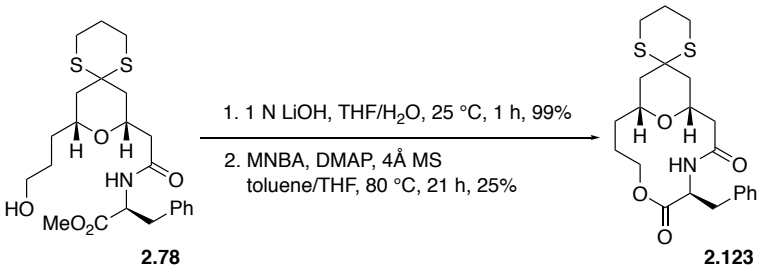
Synthesized from **2.213** (47 mg, 0.0686 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.83** (38 mg, 99%) as colorless oil:

$[\alpha]^{25}_{D}$ –16.3 (*c* 0.98, $CHCl_3$); IR (neat): ν_{max} = 3279, 2943, 2908, 1751, 1662, 1541, 1208, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.29 (m, 2H), 7.14–7.22 (m, 3H), 6.99 (t, *J* = 5.5 Hz, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.70 (dt, *J* = 15.5, 6.8 Hz, 1H), 5.88 (d, *J* = 15.5 Hz, 1H), 4.81 (dt, *J* = 8.1, 6.9 Hz, 1H), 3.98 (dd, *J* = 18.0, 5.6 Hz, 1H), 3.82–3.94 (m, 2H), 3.66–3.78 (m, 4H), 3.55 (t, *J* = 6.4 Hz, 2H), 3.14 (dd, *J* = 13.9, 6.8 Hz, 1H), 3.07 (dd, *J* = 14.0, 7.0 Hz, 1H), 2.82–2.92 (m, 2H), 2.69–2.77 (m, 2H), 2.24–2.38 (m, 2H), 2.12–2.23 (m, 2H), 1.92–2.02 (m, 2H), 1.47–1.72 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 170.0, 166.1, 140.5, 136.8, 129.4, 128.6, 126.9, 125.7, 72.9, 71.6, 62.5, 54.3, 52.4, 47.9, 43.3, 42.9, 41.3, 38.0, 32.2, 29.2, 26.0, 25.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{27}H_{38}N_2O_6S_2$ 551.2244; found 551.0042



[Hydrolysis] Synthesized from **2.77** (50 mg, 0.132 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^1H NMR (400 MHz, CD_3OD) δ 4.13–4.21 (m, 1H), 3.99 (d, *J* = 17.9 Hz, 1H), 3.76–3.88 (m, 2H), 3.51–3.57 (m, 2H), 2.97–3.06 (m, 1H), 2.79–2.93 (m, 2H), 2.68–2.76 (m, 1H), 2.53 (d, *J* = 14.1 Hz, 1H), 2.47 (dd, *J* = 14.1, 7.5 Hz, 1H), 2.36 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.17 (d, *J* = 14.0 Hz, 1H), 1.87–2.03 (m, 2H), 1.45–1.70 (m, 6H). **[Macrolactonization]** Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH,

40/1) afforded **2.122** as colorless oil (31 mg, 68% for 2 steps): $[\alpha]^{25}_{D}$ +30.4 (*c* 0.52, $CHCl_3$); IR (neat): ν_{max} = 3058, 3027, 2940, 1600, 1496, 1244, 754, 693 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.10 (br s, 1H), 4.49–4.56 (m, 1H), 4.36 (dd, *J* = 17.3, 8.8 Hz, 1H), 4.18 (t, *J* = 11.2 Hz, 1H), 3.86 (t, *J* = 11.1 Hz, 1H), 3.68–3.76 (m, 2H), 2.73–2.97 (m, 4H), 2.45 (dd, *J* = 12.9, 3.2 Hz, 1H), 2.33 (d, *J* = 13.5 Hz, 1H), 2.26 (dd, *J* = 12.6, 11.4 Hz, 1H), 1.88–2.05 (m, 2H), 1.46–1.77 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 169.1, 74.6, 71.7, 65.6, 48.0, 43.8, 43.4, 42.9, 42.4, 34.1, 27.4, 26.0, 26.0, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{15}H_{23}NO_4S_2$ 346.1141; found 346.1138.

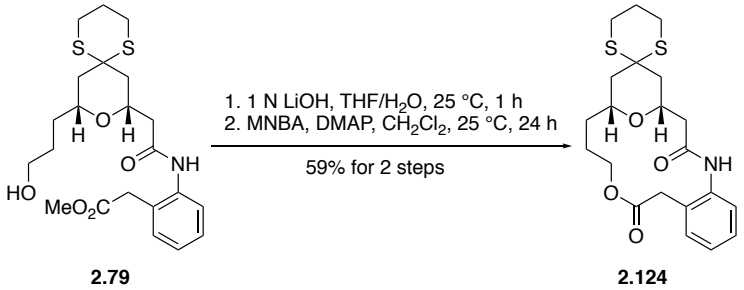


[Hydrolysis] To a solution of methyl ester **2.78** (61 mg, 0.130 mmol) in THF/H_2O (2/1, total 3 mL) was added LiOH (0.26 mL, 1.0 M in H_2O, 0.260 mmol) at 25 °C. After stirring at the same temperature for 1 h, the reaction mixture was cooled to 0 °C, acidified with 1 N HCl (~1 mL), and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/AcOH, 10/1/0.1) to afford seco-acid (59 mg,

99%) as colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.26–7.35 (m, 2H), 7.11–7.25 (m, 3H), 4.70 (dd, *J* = 7.7, 5.3 Hz, 1H), 4.10 (dddd, *J* = 11.2, 7.5, 5.4, 1.9 Hz, 1H), 3.67–3.76 (m, 1H), 3.41–3.55 (m, 2H), 3.19 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.94–3.08 (m, 2H), 2.77–2.90 (m, 2H), 2.72 (ddd, *J* = 14.6, 7.1, 3.2 Hz, 1H), 2.46 (d, *J* = 13.6 Hz, 1H), 2.41 (dd, *J* = 14.5, 7.7 Hz, 1H), 2.27–2.34 (m, 1H), 2.14 (d, *J* = 13.7 Hz, 1H), 1.86–2.04 (m, 2H), 1.37–1.62 (m, 6H).

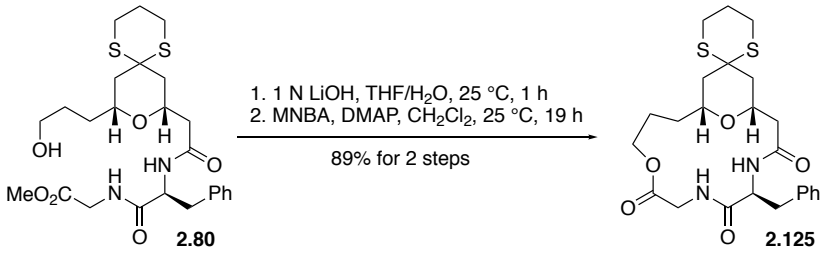
[Macrolactonization] To a solution of 2-methyl-6-nitrobenzoic anhydride (MNBA, 95 mg, 0.276 mmol) in toluene (30 mL) were added DMAP (67 mg, 0.551 mmol) and 4Å molecular sieves (500 wt%, 110 mg). After the resulting mixture was slowly heated to 80 °C, a solution of the seco-acid (22 mg, 0.0485 mmol) in toluene/THF (1/1, 25 mL) was added to a heated solution over 12 h via a syringe pump. The reaction was stirred for additional 9 h at 80 °C. The reaction mixture was cooled to 0 °C, and quenched by saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford macrolactone **2.123** (5.4 mg, 25%) as colorless oil: $[\alpha]^{25}_D$ +37.2 (*c* 0.33, $CHCl_3$); IR (neat): ν_{max} = 3296, 2938, 1730, 1660, 1534, 1222, 1013 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.33 (m, 2H), 7.14–7.25 (m, 3H), 5.98 (d, *J* = 9.8 Hz, 1H), 4.94 (ddd, *J* = 9.7, 7.1, 6.2 Hz, 1H), 4.71 (ddd, *J* = 11.2, 4.4, 2.9 Hz, 1H), 4.10–4.20 (m, 1H), 3.53–3.69 (m, 2H), 3.05–3.15 (m, 2H), 2.87–2.95 (m, 1H), 2.79–2.87 (m, 1H), 2.66–2.79 (m, 2H), 2.25–2.35 (m, 2H), 2.11–2.18 (m, 1H), 2.03–2.10 (m, 1H), 1.86–2.03 (m, 4H), 1.68–1.78

(m, 1H), 1.51–1.65 (m, 3H), 1.36–1.47 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 170.7, 136.5, 129.4, 128.7, 127.1, 75.0, 71.7, 66.0, 53.7, 47.9, 43.8, 43.8, 43.1, 37.5, 34.1, 27.7, 26.1, 26.0, 25.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{29}NO_4S_2$ 436.1611; found 436.1607.



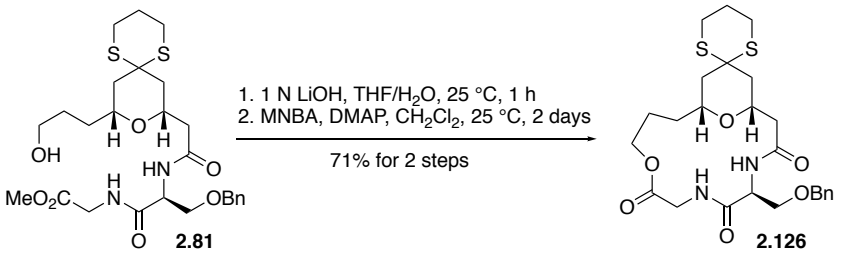
[Hydrolysis] Synthesized from **2.79** (40 mg, 0.0887 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.38 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.26–7.34 (m, 2H), 7.21 (td, *J* = 7.4, 1.4 Hz, 1H), 4.22–4.38 (m, 1H), 3.78–3.91 (m, 1H), 3.65 (s, 2H), 3.51–3.58 (m, 2H), 2.99 (ddd, *J* = 14.5, 8.6, 3.4 Hz, 1H), 2.82–2.92 (m, 2H), 2.78 (ddd, *J* = 14.4, 7.1, 3.5 Hz, 1H), 2.50–2.61 (m, 2H), 2.47 (d, *J* = 13.7 Hz, 1H), 2.24 (d, *J* = 13.7 Hz, 1H), 1.91–2.06 (m, 2H), 1.49–1.72 (m, 6H). **[Macrolactonization]** Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.124** (22 mg, 59% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ +63.2 (*c* 0.4, $CHCl_3$); IR (neat): ν_{max} = 3347, 2912, 2359, 1738, 1513, 1251, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (br s, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.29–7.36 (m, 2H), 7.17 (t, *J* = 7.4 Hz, 1H), 4.35 (t, *J* = 10.6 Hz, 1H), 3.98–4.16 (m, 3H), 3.76 (d, *J* = 13.0 Hz, 1H), 3.45 (d, *J* = 13.0 Hz, 1H), 2.93–3.01 (m, 1H), 2.73–2.91

(m, 4H), 2.49 (d, J = 17.2 Hz, 1H), 2.30 (d, J = 13.6 Hz, 1H), 2.19 (d, J = 13.9 Hz, 1H), 1.91–2.11 (m, 2H), 1.52–1.77 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.2, 135.5, 130.6, 128.3, 127.7, 127.6, 126.3, 70.5, 70.2, 66.7, 47.4, 44.3, 43.4, 42.9, 38.9, 35.0, 26.0, 26.0, 25.7, 23.0; HRMS (m/z): $[M+Na]^+$ calcd for $C_{21}H_{27}NO_4S_2$ 444.1274; found 444.1277.



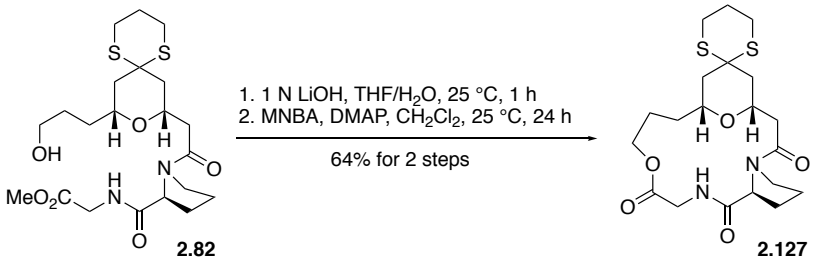
[Hydrolysis] Synthesized from **2.80** (584 mg, 1.113 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.20–7.35 (m, 5H), 4.74 (ddd, J = 8.2, 5.5, 2.6 Hz, 1H), 4.06– 4.15 (m, 1H), 3.92 (s, 2H), 3.73–3.81 (m, 1H), 3.50–3.62 (m, 2H), 3.22 (dd, J = 14.0, 5.6 Hz, 1H), 3.04 (ddd, J = 14.1, 9.1, 3,2 Hz, 1H), 2.98 (dd, J = 14.0, 8.4 Hz, 1H), 2.82–2.91 (m, 2H), 2.77 (ddd, J = 14.5, 7.1, 3.5 Hz, 1H, 2.38–2.47 (m, 2H), 2.33 (dd, J = 14.5, 4.9 Hz, 1H), 2.21 (dt, J = 13.7, 2.1 Hz, 1H), 1.89–2.09 (m, 2H), 1.43–1.69 (m, 6H). **[Macrolactonization]** Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) afforded **2.125** (488 mg, 89% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ +12.5 (c 1.0, $CHCl_3$); IR (neat): ν_{max} = 3306, 2929, 1732, 1652, 1531, 1278, 1104, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08–7.34 (m, 6H), 6.48 (d, J = 8.4 Hz, 1H), 4.93 (ddd, J = 9.9, 6.7, 3.6 Hz, 1H), 4.53 (dd, J = 18.0, 8.5 Hz, 1H), 4.01–4.12 (m, 2H), 3.85 (td, J = 10.7, 2.1 Hz, 1H),

3.55–3.66 (m, 2H), 3.51 (dd, J = 13.5, 3.7 Hz, 1H), 2.74–2.98 (m, 5H), 2.46 (dd, J = 16.5, 9.4 Hz, 1H), 2.38 (dd, J = 16.5, 3.2 Hz, 1H), 2.23 (d, J = 13.8 Hz, 1H), 1.91–2.08 (m, 3H), 1.44–1.73 (m, 4H), 1.22–1.35 (m, 1H), 0.99–1.12 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 168.2, 136.7, 129.9, 128.6, 127.0, 127.0, 70.6, 68.8, 64.7, 53.5, 47.5, 42.4, 42.1, 41.9, 40.7, 36.7, 30.0, 26.0, 25.9, 25.7, 21.4; HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{32}N_2O_5S_2$ 493.1825; found 493.1817.



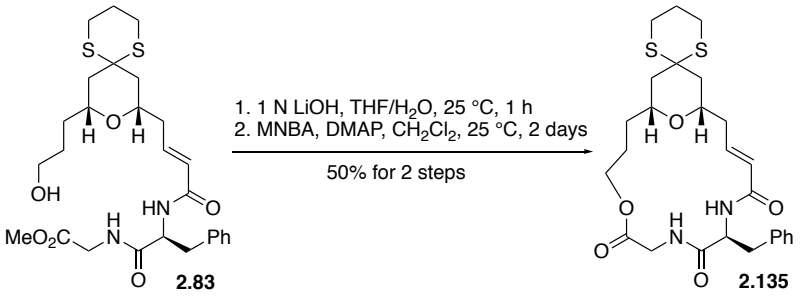
[Hydrolysis] Synthesized from **2.81** (42 mg, 0.0757 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.16–7.40 (m, 5H), 4.67 (t, J = 5.2 Hz, 1H), 4.49–4.61 (m, 2H), 4.13–4.22 (m, 1H), 3.91–3.97 (m, 2H), 3.75–3.82 (m, 2H), 3.72 (dd, J = 9.9, 5.0 Hz, 1H), 3.43–3.54 (m, 2H), 2.99 (ddd, J = 14.3, 8.9, 3.2 Hz, 1H), 2.80–2.90 (m, 2H), 2.73 (ddd, J = 14.5, 7.3, 3.4 Hz, 1H), 2.50 (dd, J = 14.4, 8.1 Hz, 1H), 2.44 (d, J = 13.9 Hz, 1H), 2.39 (dd, J = 14.4, 4.8 Hz, 2H), 2.18 (d, J = 13.7 Hz, 1H), 1.86–2.05 (m, 2H), 1.42–1.68 (m, 6H). **[Macrolactonization]** Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 60/1) afforded **2.126** (28 mg, 71% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ +29.7 (c 0.23, $CHCl_3$); IR (neat): ν_{max} = 3312, 2934, 1736, 1658, 1530,

1277, 1096 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 7.50–7.65 (m, 2H), 7.22-7.38 (m, 3H), 4.66 (ddd, J = 8.3, 5.8, 4.2 Hz, 1H), 4.46–4.57 (m, 2H), 3.97–4.18 (m, 4H), 3.77–3.87 (m, 3H), 3.62 (dd, J = 9.2, 5.8 Hz, 1H), 2.78–3.01 (m, 4H), 2.63 (dd, J = 14.5, 3.9 Hz, 1H), 2.24–2.34 (m, 2H), 2.19 (dt, J = 13.7, 2.2 Hz, 1H), 1.87–2.01 (m, 3H), 1.73–1.84 (m, 2H), 1.60 (dtd, J = 15.6, 7.7, 3.0 Hz, 1H), 1.40–1.53 (m, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 170.8, 170.5, 169.4, 139.4, 129.1, 128.5, 128.3, 73.6, 71.7, 70.5, 70.4, 65.3, 53.5, 48.7, 43.1, 42.8, 42.8, 42.3, 32.3, 26.7, 26.3, 26.1, 23.4; HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{34}N_2O_6S_2$ 523.1931; found 523.1934.



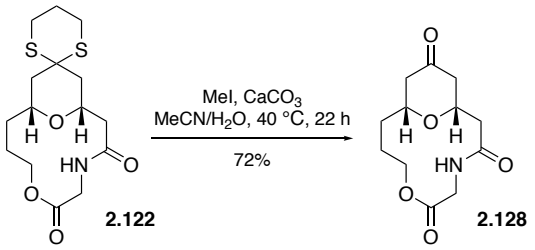
[Hydrolysis] Synthesized from **2.82** (42 mg, 0.0885 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^1H NMR (400 MHz, CD_3OD) δ 8.23 (t, J = 5.9 Hz, 1H), 4.47 (dd, J = 8.3, 3.5 Hz, 1H), 4.19–4.27 (m, 1H), 3.95 (dd, J = 17.7, 3.9 Hz, 1H), 3.88 (dd, J = 17.8, 4.5 Hz, 1H), 3.74–3.81 (m, 1H), 3.67–3.74 (m, 2H), 3.49–3.58 (m, 2H), 3.01 (ddd, J = 14.4, 9.0, 3.2 Hz, 1H), 2.81–2.92 (m, 2H), 2.73–2.80 (m, 1H), 2.68 (dd, J = 14.6, 8.4 Hz, 1H), 2.47–2.53 (m, 1H), 2.42 (dd, J = 14.6, 4.6 Hz, 1H), 2.15–2.23 (m, 2H), 1.87–2.12 (m, 5H), 1.42–1.68 (m, 6H). **[Macrolactonization]**

Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded rotameric mixture **2.126** (21 mg, 64% for 2 steps) as yellow oil: ^{1}H NMR (400 MHz, CD_3CN/D_2O = 10/1) δ 4.42–4.54 (m, 1H), 4.08–4.26 (m, 2H), 3.91–4.07 (m, 2H), 3.80–3.90 (m, 1H), 3.60–3.76 (m, 2H), 3.37–3.51 (m, 1H), 2.70–2.95 (m, 4H), 2.30–2.60 (m, 2H), 2.08–2.24 (m, 2H), 1.77–2.08 (m, 7H), 1.58–1.76 (m, 2H), 1.33–1.53 (m, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{20}H_{30}N_2O_5S_2$ 443.1669; found 443.1666.



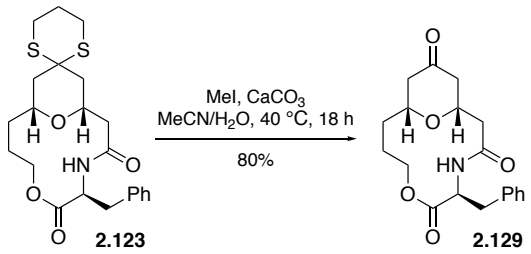
[Hydrolysis] Synthesized from **2.83** (38 mg, 0.0690 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, CD_3OD) δ 7.07–7.37 (m, 6H), 6.75 (dt, *J* = 15,2, 7.2 Hz, 1H), 5.99 (d, *J* = 15.4 Hz, 1H), 4.72 (dd, *J* = 9.1, 5.2 Hz, 1H), 3.79–3.91 (m, 3H), 3.71–3.78 (m, 1H), 3.50–3.57 (m, 2H), 3.22 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.86–2.96 (m, 3H), 2.74–2.83 (m, 2H), 2.21–2.38 (m, 4H), 1.90–2.03 (m, 2H), 1.43–1.70 (m, 6H). **[Macrolactonization]** Synthesized from the crude seco-acid according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded **2.135** (17 mg, 50% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ –66.4 (*c* 0.68, $CHCl_3$); IR

(neat): ν_{max} = 3293, 2904, 2359, 1733, 1660, 1288 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.29 (m, 2H), 7.16–7.23 (m, 3H), 7.00 (dd, *J* = 8.3, 4.2 Hz, 1H), 6.71 (ddd, *J* = 15.7, 7.4, 5.8 Hz, 1H), 6.10 (d, *J* = 8.7 Hz, 1H), 5.84 (d, *J* = 15.6 Hz, 1H), 4.75 (q, *J* = 7.8 Hz, 1H), 4.28 (dd, *J* = 17.3, 8.3 Hz, 1H), 3.91–4.11 (m, 2H), 3.81–3.90 (m, 1H), 3.59–3.68 (m, 1H), 3.51 (dd, *J* = 17.3, 4.2 Hz, 1H), 3.23 (dd, *J* = 14.0, 7.5 Hz, 1H), 3.03 (dd, *J* = 14.0, 7.5 Hz, 1H), 2.83–2.90 (m, 2H), 2.70–2.79 (m, 2H), 2.19–2.37 (m, 3H), 2.15 (d, *J* = 14.0 Hz, 1H), 1.94–2.04 (m, 2H), 1.59–1.82 (m, 3H), 1.54 (dd, *J* = 13.6, 11.0 Hz, 1H), 1.21–1.42 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 168.8, 166.6, 141.1, 136.9, 129.3, 128.7, 126.9, 125.5, 71.6, 71.4, 64.7, 54.4, 48.1, 43.3, 43.2, 42.1, 37.1, 36.3, 29.9, 26.0, 25.8, 24.5; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{34}N_2O_5S_2$ 519.1982; found 519.1978.

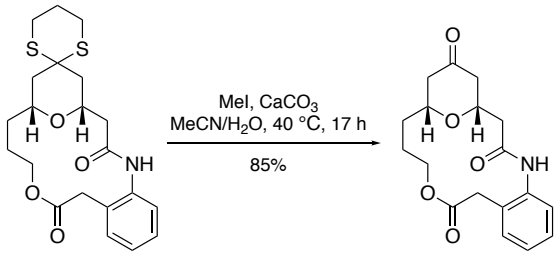


Synthesized from **2.122** (30 mg, 0.0868 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.128** (16 mg, 72%) as a white solid: $[\alpha]^{25}_D$ +58.4 (*c* 0.26, $CHCl_3$/MeOH = 60/1); IR (neat): ν_{max} = 3293, 2933, 1720, 1650, 1548, 1230, 996 cm^{-1}; ^1H NMR (400 MHz, CD_3CN/D_2O = 10/1) δ 4.17 (ddd, *J* = 11.3, 8.7, 1.7 Hz, 1H), 4.11 (ddd, *J* = 11.4, 6.3, 1.8 Hz, 1H), 3.93 (tt, *J* = 11.5, 3.2 Hz, 1H), 3.83 (s, 2H), 3.57–3.65 (m, 1H), 2.56 (dd, *J* = 13.3, 3.5 Hz, 1H), 2.37 (ddd, *J* = 14.9, 2.8, 1.4 Hz, 1H), 2.21–

2.33 (m, 4H), 1.73–1.84 (m, 1H), 1.53–1.72 (m, 3H); ^{13}C NMR (125 MHz, CD_3CN/D_2O = 10/1) δ 209.5, 174.2, 171.3, 77.9, 76.2, 66.1, 48.6, 47.4, 43.4, 43.1, 35.2, 27.2; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{12}H_{17}NO_5$ 256.1180; found 256.1178.

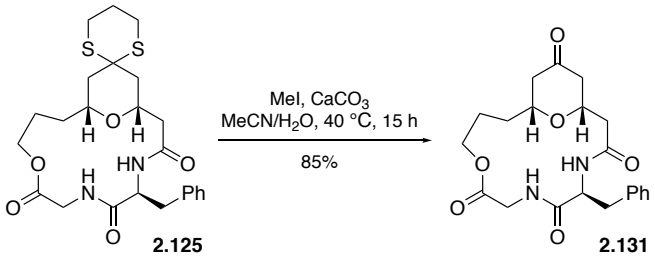


Synthesized from **2.123** (18 mg, 0.0397 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 50/1) afforded **2.129** (11 mg, 80%) as a white solid: $[\alpha]^{25}_D$ +85.7 (*c* 0.18, $CHCl_3$); IR (neat): ν_{max} = 3299, 2929, 1718, 1652, 1547, 1337, 1296, 1223, 995 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.36 (m, 2H), 7.25–7.29 (m, 1H), 7.16–7.23 (m, 2H), 5.90 (d, *J* = 9.8 Hz, 1H), 4.97 (ddd, *J* = 9.7, 7.1, 5.9 Hz, 1H), 4.74 (ddd, *J* = 11.6, 4.4, 2.4 Hz, 1H), 4.00 (tt, *J* = 11.4, 2.9 Hz, 1H), 3.63 (td, *J* = 11.2, 1.6 Hz, 1H), 3.49 (ddt, *J* = 11.5, 9.2, 2.0 Hz, 1H), 3.05–3.22 (m, 2H), 2.38–2.47 (m, 2H), 2.34 (dt, *J* = 14.7, 2.3 Hz, 1H), 2.14–2.29 (m, 3H), 1.91–2.02 (m, 1H), 1.76–1.87 (m, 1H), 1.52–1.66 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.5, 171.1, 170.7, 136.4, 129.4, 128.8, 127.2, 79.1, 75.1, 66.0, 53.7, 48.4, 48.3, 47.3, 44.5, 37.2, 34.8, 27.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{19}H_{23}NO_5$ 346.1649; found 346.1649.



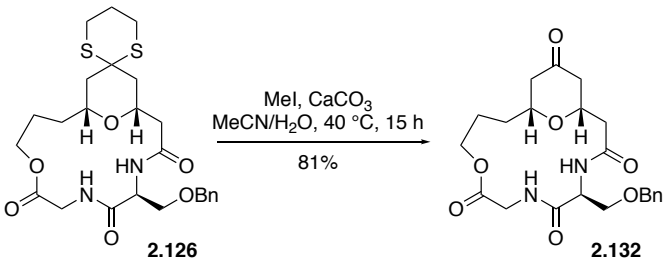
2.124 **2.130**

Synthesized from **2.124** (22 mg, 0.0522 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.130** (15 mg, 85%) as a white solid: $[\alpha]^{25}_{D}$ +52.0° (*c* 0.25, $CHCl_3$); IR (neat): ν_{max} = 3286, 2923, 1725, 1653, 1533, 1261, 1006, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.67 (dd, *J* = 8.1, 2.8 Hz, 1H), 7.28–7.37 (m, 2H), 7.18 (t, *J* = 7.5 Hz, 1H), 4.12–4.20 (m, 2H), 4.03 (t, *J* = 10.7 Hz, 1H), 3.94 (tt, *J* = 11.0, 2.7 Hz, 1H), 3.69 (d, *J* = 13.3 Hz, 1H), 3.47 (d, *J* = 13.3 Hz, 1H), 2.95 (dd, *J* = 17.0, 9.8 Hz, 1H), 2.58 (d, *J* = 17.0 Hz, 1H), 2.37–2.48 (m, 3H), 2.29 (dd, J = 14.7, 11.3 Hz, 1H), 1.99–2.10 (m, 1H), 1.83–1.94 (m, 1H), 1.65–1.83 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.0, 170.5, 169.1, 135.3, 130.7, 128.4, 127.7, 127.6, 126.6, 75.4, 74.1, 66.6, 48.4, 47.5, 43.9, 38.9, 35.7, 23.2; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{21}NO_5$ 332.1493; found 332.1492.



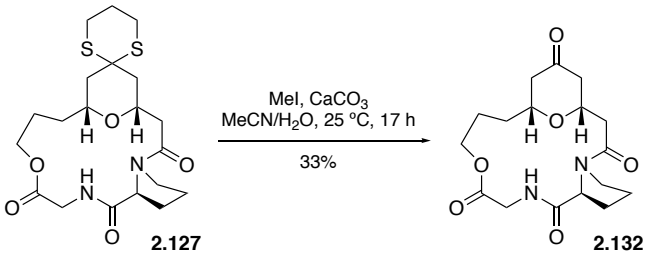
2.125 **2.131**

Synthesized from **2.125** (84 mg, 0.171 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.131** (59 mg, 85%) as a white solid: $[\alpha]^{25}_D$ –22.4 (*c* 0.33, CH_3CN/H_2O = 20/1); IR (neat): ν_{max} = 3283, 1727, 1640, 1552, 1245, 698 cm^{-1}; ^1H NMR (400 MHz, CD_3CN/D_2O = 10/1) δ 7.15–7.33 (m, 5H), 4.62 (dd, *J* = 8.2, 6.2 Hz, 1H), 4.06 (ddd, *J* = 10.5, 7.2, 3.0 Hz, 1H), 3.91–3.99 (m, 2H), 3.88 (d, *J* = 17.4 Hz, 1H), 3.77 (d, *J* = 17.4 Hz, 1H), 3.60–3.66 (m, 1H), 3.02 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.90 (dd, *J* = 13.8, 8.2 Hz, 1H), 2.41–2.56 (m, 2H), 2.13–2.32 (m, 4H), 1.75–1.87 (m, 1H), 1.62–1.74 (m, 1H), 1.51–1.62 (m, 1H), 1.40–1.50 (m, 1H); ^{13}C NMR (125 MHz, CD_3CN/D_2O = 10/1) δ 209.1, 172.4, 171.2, 170.2, 137.5, 129.6, 128.8, 127.0, 75.0, 73.7, 66.0, 66.0, 54.6, 47.0, 46.7, 42.1, 42.0, 37.6, 33.1, 22.5; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{26}N_2O_6$ 403.1864; found 403.1871.

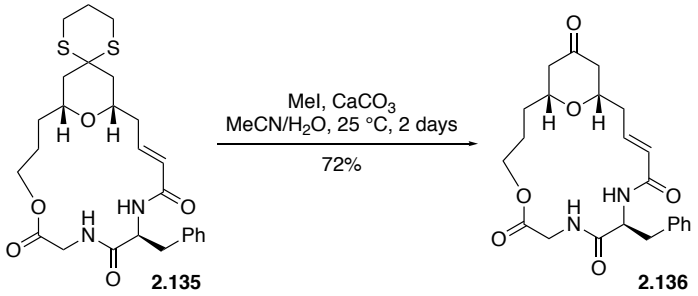


Synthesized from **2.126** (14 mg, 0.0268 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.132** (9.4 mg, 81%) as a white solid: $[\alpha]^{25}_D$ +12.1 (*c* 0.29, $CHCl_3$); IR (neat): ν_{max} = 3317, 2948, 2865, 1734, 1717, 1653, 1540, 1270, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.41 (m, 5H), 7.00 (d, *J* = 8.9 Hz, 1H),

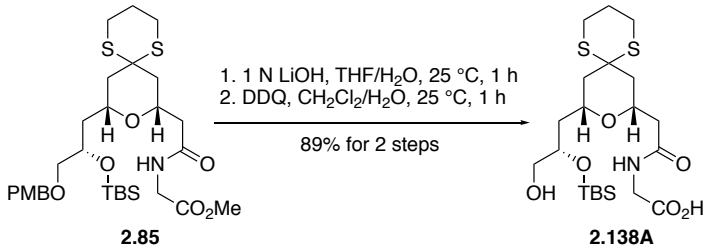
6.83 (dd, *J* = 6.2, 3.5 Hz, 1H), 4.74 (ddd, *J* = 9.0, 4.2, 2.4 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.37 (dd, *J* = 18.3, 6.7 Hz, 1H), 4.23 (ddd, *J* = 10.6, 7.4, 3.1 Hz, 1H), 4.14 (dd, *J* = 9.2, 2.5 Hz, 1H), 3.92–4.02 (m, 2H), 3.90 (dd, *J* = 18.2, 3.6 Hz, 1H), 3.62 (dd, *J* = 9.2, 4.3 Hz, 1H), 3.47–3.58 (m, 1H), 2.81 (dd, *J* = 14.6, 11.7 Hz, 1H), 2.57 (dd, *J* = 15.3, 4.0 Hz, 1H), 2.50 (dd, *J* = 15.3, 6.4 Hz, 1H), 2.36 (dt, *J* = 14.7, 2.0 Hz, 1H), 2.17–2.34 (m, 2H), 1.74–1.89 (m, 2H), 1.66 (dddd, *J* = 15.0, 9.0, 6.2, 2.8 Hz, 1H), 1.37–1.47 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.9, 169.7, 169.5, 167.9, 137.6, 128.6, 128.2, 128.0, 75.8, 73.9, 73.2, 69.5, 64.8, 53.0, 46.5, 42.5, 41.9, 31.4, 22.3; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{28}N_2O_7$ 433.1969; found 433.1965.



Synthesized from **2.127** (20 mg, 0.0452 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded rotameric mixture **2.132** (5.3 mg, 33%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.8–7.4 (m, 1H), 4.57–4.84 (m, 1H), 4.26–4.44 (m, 2H), 3.77–4.10 (m, 3H), 3.44–3.71 (m, 3H), 2.86–3.04 (m, 1H), 2.49–2.62 (m, 1H), 2.25–2.49 (m, 4H), 2.10–2.22 (m, 1H), 1.94–2.08 (m, 2H), 1.63–1.92 (m, 4H), 1.23–1.60 (m, 1H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{17}H_{24}N_2O_6$ 353.1707; found 353.1709.

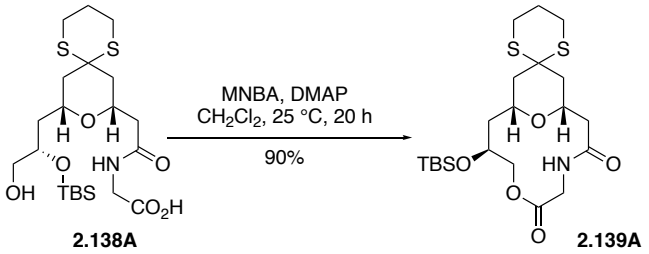


Synthesized from **2.135** (39 mg, 0.0752 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.136** (22 mg, 72%) as a white solid: $[\alpha]^{25}_{D}$ –64.7 (*c* 0.27, $CHCl_3$/MeOH = 17/2); IR (neat): ν_{max} = 3310, 2923, 1741, 1665, 1536, 1236, 702 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.30 (t, *J* = 6.0 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.13–7.30 (m, 5H), 6.64 (dt, *J* = 16.1, 5.6 Hz, 1H), 5.99 (d, *J* = 15.9 Hz, 1H), 4.65 (q, *J* = 7.6 Hz, 1H), 3.88 (dd, *J* = 10.7, 6.4 Hz, 1H), 3.80–3.86 (m, 1H), 3.72–3.79 (m, 1H), 3.48–3.58 (m, 2H), 3.00 (dd, *J* = 13.7, 7.5 Hz, 1H), 2.82 (dd, *J* = 13.7, 7.7 Hz, 1H), 2.40 (d, *J* = 17.2 Hz, 1H), 2.17–2.34 (m, 5H), 1.60–1.73 (m, 2H), 1.40–1.51 (m, 1H), 1.30–1.39 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 206.5, 171.2, 168.9, 164.6, 138.2, 137.9, 129.0, 128.1, 126.2, 125.9, 75.2, 74.2, 63.6, 47.4, 46.9, 41.8, 36.6, 36.2, 30.1, 24.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{23}H_{28}N_2O_6$ 429.2020; found 429.2013.



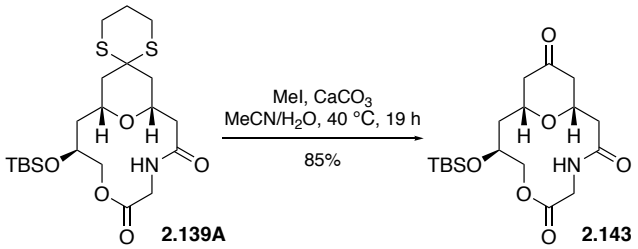
[Hydrolysis] To a solution of **2.85** (33 mg, 0.0526 mmol) in THF/H_2O (2/1, total 2 mL) was added LiOH (0.11 mL, 1.0 M in H_2O, 0.105 mmol) at 25 °C. After stirring at the same temperature for 1 h, the reaction mixture was cooled to 0 °C, acidified with 1 M citric acid (~1 mL), and diluted with CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford the crude carboxylic acid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.44 (s, 2H), 4.11 (ddd, *J* = 11.9, 7.3, 3.8 Hz, 1H), 3.93–4.07 (m, 3H), 3.85 (dd, *J* = 18.2, 5.0 Hz, 1H), 3.79 (s, 3H), 3.34–3.45 (m, 2H), 2.94 (ddd, *J* = 14.4, 8.9, 3.2 Hz, 1H), 2.74-2.85 (m, 2H), 2.70 (ddd, *J* = 14.6, 7.1, 3.8 Hz, 1H), 2.37–2.48 (m, 2H), 2.29 (d, *J* = 13.6 Hz, 1H), 2.17 (d, *J* = 13.8 Hz, 1H), 1.91–2.06 (m, 2H), 1.80 (ddd, *J* = 13.1, 7.5, 5.2 Hz, 1H), 1.53–1.71 (m, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). **[PMB deprotection]** The residue was dissolved in CH_2Cl_2/H_2O (10/1, total 2 mL) and DDQ (18 mg, 0.0788 mmol) was added in one portion at 25 °C. The reaction mixture was stirred at 25 °C for 1 h, and quenched with saturated aqueous $NaHCO_3$ (~1 mL). The resulting mixture was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/AcOH, 20/1/0.2) to afford seco-acid **2.138A** (23 mg, 89% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ +8.1 (*c*

0.4, $CHCl_3$); IR (neat): ν_{max} = 3347, 2929, 2856, 2341, 1734, 1653, 1541, 1252, 1101, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 4.14–4.26 (m, 1H), 3.92–4.13 (m, 4H), 3.60 (dd, *J* = 11.4, 5.2 Hz, 1H), 3.51 (dd, *J* = 11.6, 5.4 Hz, 1H), 2.96 (ddd, *J* = 14.3, 9.0, 3.2 Hz, 1H), 2.75–2.87 (m, 2H), 2.71 (ddd, *J* = 14.5, 7.1, 3.7 Hz, 1H), 2.51 (dd, *J* = 15.0, 8.1 Hz, 1H), 2.33–2.47 (m, 2H), 2.10 (d, *J* = 13.9 Hz, 1H), 1.91–2.07 (m, 2H), 1.78 (ddd, *J* = 12.5, 8.5, 3.2 Hz, 1H), 1.52–1.73 (m, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 171.3, 70.4, 69.4, 68.9, 65.9, 47.7, 43.7, 42.4, 42.2, 40.3, 26.0, 25.9, 25.8, 18.2, −4.5, −4.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{39}NO_6S_2Si$ 492.1904; found 492.1904.

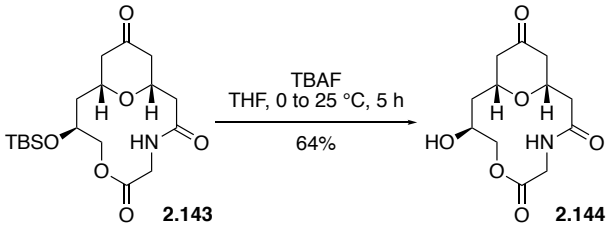


Synthesized from **2.138A** (22 mg, 0.0445 mmol) according to **GP6**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 60/1) afforded **2.139A** as a white solid (19 mg, 90%): $[\alpha]^{25}_D$ +19.7 (*c* 0.3, $CHCl_3$); IR (neat): ν_{max} = 2952, 2901, 2357, 1740, 1085, 839, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.29 (t, *J* = 6.4 Hz, 1H), 4.25 (dd, *J* = 11.3, 2.2 Hz, 1H), 4.04–4.16 (m, 4H), 3.92–4.00 (m, 2H), 2.71–3.00 (m, 4H), 2.43 (dd, *J* = 13.7, 3.3 Hz, 1H), 2.26–2.38 (m, 2H), 2.09 (d, *J* = 13.5 Hz, 1H), 1.96–2.06 (m, 2H), 1.90 (ddd, *J* = 14.7, 9.3, 2.9 Hz, 1H), 1.64–1.76 (m, 5H), 0.87 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (125 MHz,

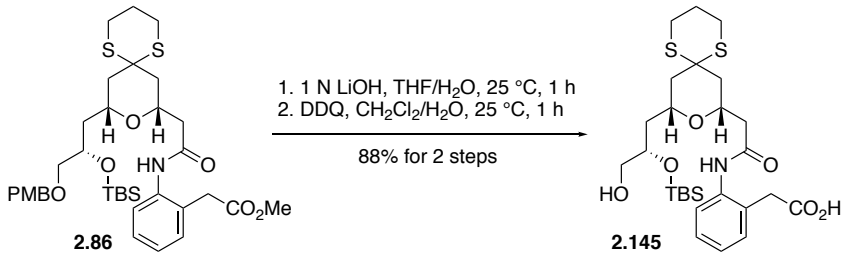
$CDCl_3$) δ 172.0, 169.0, 71.9, 68.7, 68.3, 67.2, 48.2, 44.2, 42.8, 42.6, 42.5, 41.9, 26.1, 25.8, 25.8, 18.1, −4.8, −4.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{37}NO_5S_2Si$ 476.1955; found 476.1958.



Synthesized from **2.139A** (27 mg, 0.0568 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.143** (19 mg, 85%) as a white solid: $[\alpha]^{25}_D$ +37.3 (*c* 0.18, $CHCl_3$); IR (neat): ν_{max} = 3255, 3075, 2955, 2858, 1738, 1716, 1654, 1252, 1097, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.21 (t, *J* = 6.2 Hz, 1H), 4.31 (d, *J* = 10.4 Hz, 1H), 3.88–4.11 (m, 6H), 2.54 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.41–2.49 (m, 2H), 2.27–2.40 (m, 3H), 2.04 (ddd, *J* = 14.8, 9.3, 2.9 Hz, 1H), 1.70–1.79 (m, 1H), 0.85 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.7, 171.3, 168.9, 75.4, 72.6, 68.1, 67.0, 48.4, 47.3, 43.4, 42.7, 42.6, 25.8, 18.1, −4.7, −4.9; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{31}NO_6Si$ 386.1993; found 386.1994.



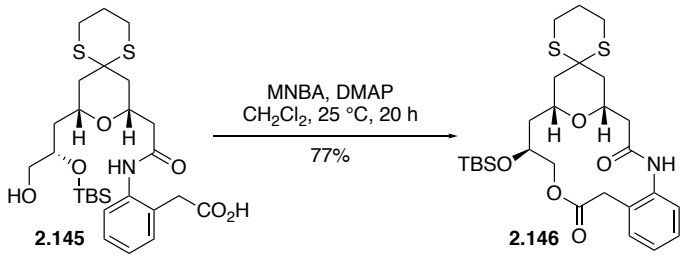
Synthesized from **2.143** (12 mg, 0.0311 mmol) according to **GP8**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.144** (5.4 mg, 25%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD=10/1) δ 4.18 (d, J = 11.3 Hz, 1H), 4.11 (dd, J = 11.6, 6.0 Hz, 1H), 3.97 (d, J = 17.1 Hz, 1H), 3.82–3.94 (m, 3H), 3.79 (d, J = 17.3 Hz, 1H), 2.52 (dd, J = 13.3, 3.6 Hz, 1H), 2.38 (d, J = 14.5 Hz, 1H), 2.18–2.34 (m, 4H), 1.91 (ddd, J = 14.0, 10.4, 3.0 Hz, 1H), 1.72 (ddd, J = 14.7, 9.5, 2.2 Hz, 1H); HRMS (m/z): $[M+H]^+$ calcd for $C_{12}H_{17}NO_6$ 272.1129; found 272.1127.



Compound **2.145** was prepared according to the same procedure as described for compound **2.138A**.

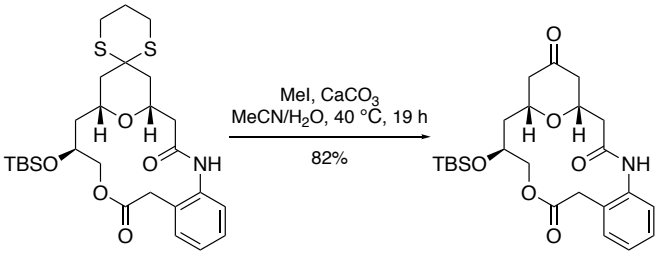
[Hydrolysis] Synthesized from **2.86** (56 mg, 0.0795 mmol) to afford the carboxylic acid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.56 (dd, J = 7.8, 1.3 Hz, 1H), 7.21–7.29 (m, 2H), 7.18 (d, J = 8.6 Hz, 2H), 7.14 (td, J = 7.5, 1.3 Hz, 1H), 6.82 (d, J = 8.6 Hz, 2H), 4.39 (s, 2H), 4.23–4.32 (m, 1H), 4.03–4.12 (m, 1H), 3.87–3.96 (m, 1H), 3.77 (s, 3H), 3.59 (d, J = 14.9 Hz, 1H), 3.52 (d, J = 15.1 Hz, 1H), 3.34–3.41 (m, 2H), 2.94 (ddd, J = 12.4, 9.0, 3.2 Hz, 1H), 2.74–2.86 (m, 2H), 2.69 (ddd, J = 14.5, 7.0, 3.5 Hz, 1H), 2.61 (dd, J = 15.6, 8.3 Hz, 1H), 2.54 (dd, J = 15.6, 3.5 Hz, 1H), 2.36 (d, J = 13.8 Hz, 1H), 2.21 (d, J = 13.7 Hz, 1H), 1.92–2.06 (m, 2H),

1.77–1.83 (m, 1H), 1.57–1.73 (m, 3 H), 0.83 (s, 9H), 0.01 (s, 3H), −0.05 (s, 3H). **[PMB deprotection]** Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.145** (40 mg, 88% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ –11.6 (*c* 0.5, $CHCl_3$); IR (neat): ν_{max} = 3331, 2950, 2856, 1710, 1525, 1252, 1101, 755 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.43 (d, *J* = 7.9 Hz, 1H), 7.25–7.36 (m, 2H), 7.21 (t, *J* = 7.5 Hz, 1H), 4.26–4.35 (m, 1H), 4.05 (dt, *J* = 11.3, 6.0 Hz, 1H), 3.89 (p, *J* = 5.4 Hz, 1H), 3.67 (d, *J* = 15.7 Hz, 1H), 3.62 (d, *J* = 15.7 Hz, 1H), 3.52 (dd, *J* = 11.4, 5.4 Hz, 1H), 3.48 (dd, *J* = 11.2, 5.0 Hz, 1H), 2.99 (ddd, *J* = 14.4, 8.5, 3.4 Hz, 1H), 2.82–2.91 (m, 2H), 2.77 (ddd, *J* = 14.5, 7.2, 3.6 Hz, 1H), 2.58 (dd, *J* = 13.6, 7.1 Hz, 1H), 2.53 (dd, *J* = 14.2, 5.0 Hz, 1H), 2.47 (d, *J* = 13.6 Hz, 1H), 2.27 (d, *J* = 13.7 Hz, 1H), 1.92–2.05 (m, 2H), 1.67–1.78 (m, 2H), 1.64 (dd, *J* = 13.6, 11.2 Hz, 1H), 1.55 (dd, *J* = 13.7, 11.2 Hz, 1H), 0.89 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{27}H_{43}NO_6S_2Si$ 570.2374; found 530.2372.



Synthesized from **2.145** (41 mg, 0.0719 mmol) according to **GP6**. Purification by silica flash chromatography (hexanes/EtOAc, 3/2) afforded **2.146** (31 mg, 77%) as colorless oil: $[\alpha]^{25}_D$ +39.4 (*c* 0.5, $CHCl_3$); IR (neat): ν_{max} = 3354, 2950, 2357, 1747, 1685, 1517, 1253, 1100

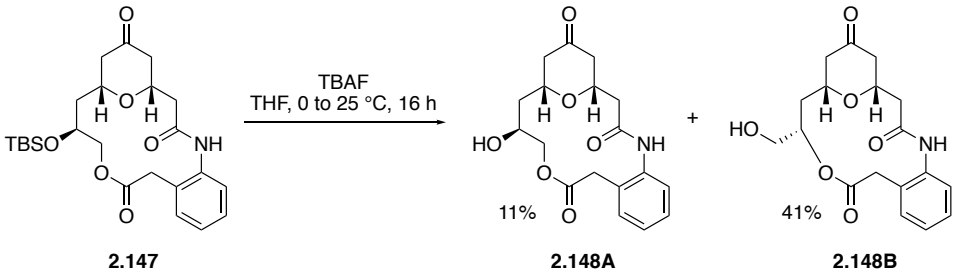
cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.27–7.37 (m, 2H), 7.16 (t, J = 7.5 Hz, 1H), 4.32 (t, J = 10.6 Hz, 1H), 4.01–4.16 (m, 3H), 3.91–4.00 (m, 1H), 3.72 (d, J = 13.4 Hz, 1H), 3.49 (d, J = 13.4 Hz, 1H), 2.72–3.00 (m, 5H), 2.46 (d, J = 17.0 Hz, 1H), 2.26 (d, J = 14.3 Hz, 1H), 2.21 (d, J = 15.0 Hz, 1H), 1.96–2.10 (m, 2H), 1.86–1.95 (m, 1H), 1.59–1.77 (m, 3H), 0.80 (s, 9H), −0.01 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 170.0, 135.4, 130.6, 128.4, 127.2, 126.3, 70.0, 69.7, 68.9, 66.4, 47.3, 44.2, 43.9, 43.4, 42.8, 38.7, 26.0, 26.0, 25.8, 25.7, 18.1, −4.7, −4.9; HRMS (m/z): $[M+H]^+$ calcd for $C_{27}H_{41}NO_5S_2Si$ 552.2268; found 552.2263.



2.146 **2.147**

Synthesized from **2.146** (40 mg, 0.0725 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.147** (25 mg, 82%) as a white solid: $[\alpha]^{25}_{D}$ +18.9 (c 0.33, $CHCl_3$); IR (neat): ν_{max} = 3365, 2955, 2857, 1723, 1683, 1517, 1253, 1093, 837 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.33 (td, J = 7.8, 1.3 Hz, 1H), 7.29 (d, J = 7.2 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 4.08–4.18 (m, 2H), 3.98–4.07 (m, 3H), 3.67 (d, J = 13.9 Hz, 1H), 3.53 (d, J = 13.9 Hz, 1H), 2.91 (dd, J = 16.9, 10.0 Hz, 1H), 2.56 (d, J = 16.9 Hz, 1H), 2.38–2.48 (m, 3H), 2.33 (dd, J = 14.6, 11.2 Hz, 1H), 2.06 (ddd,

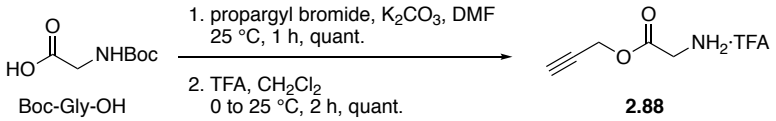
J = 14.4, 9.5, 3.7 Hz, 1H), 1.76 (ddd, J = 14.6, 8.7, 2.8 Hz, 1H), 0.81 (s, 9H), 0.00 (s, 3H), −0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.7, 170.1, 168.9, 135.3, 130.8, 128.5, 127.4, 127.2, 126.6, 73.9, 73.3, 69.3, 66.4, 48.5, 47.4, 44.2, 43.9, 38.7, 25.8, 18.1, −4.8, −4.9; HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{35}NO_6Si$ 462.2306; found 462.2304.



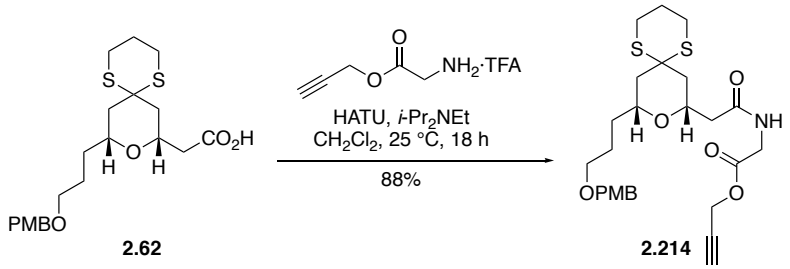
Synthesized from **2.147** (14 mg, 0.0303 mmol) according to **GP8**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 15/1) afforded **2.148A** (1.2 mg, 11%) and **2.148B** (4.3 mg, 41%) as a white solid; **2.148A**: White solid (11%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 7.7 Hz, 1H), 7.31 (d, J = 7.6 Hz, 1H), 7.18–7.24 (m, 1H), 4.12–4.27 (m, 3H), 4.01–4.10 (m, 2H), 3.71 (d, J = 13.7 Hz, 1H), 3.52 (d, J = 13.7 Hz, 1H), 2.92 (dd, J = 16.8, 10.0 Hz, 1H), 2.58 (d, J = 16.8 Hz, 1H), 2.33–2.51 (m, 4H), 2.13–2.22 (m, 1H), 1.78–1.87 (m, 1H); HRMS (m/z): $[M+H]^+$ calcd for $C_{18}H_{21}NO_6$ 348.1442; found 348.1444; **2.148B**: White solid (41%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.34 (td, J = 7.6, 1.8 Hz, 1H), 7.23–7.26 (m, 1H), 7.20 (t, J = 7.4 Hz, 1H), 5.08–5.16 (m, 1H), 4.05–4.17 (m, 1H), 3.90–4.00 (m, 1H), 3.77 (d, J = 16.4 Hz, 1H), 3.61–3.72 (m, 3H), 2.80 (dd, J = 16.3, 10.7 Hz, 1H), 2.57 (d, J = 15.7 Hz, 1H), 2.26–2.51 (m, 5H), 2.21

(dt, J = 15.5, 10.0 Hz, 1H); HRMS (m/z): $[M+H]^+$ calcd for $C_{18}H_{21}NO_6$ 348.1442; found 348.1437.

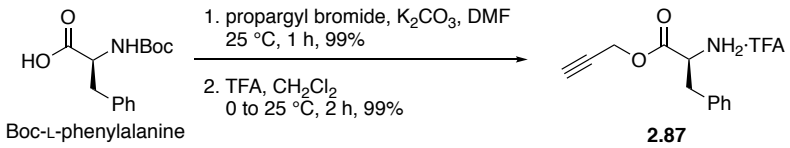
Macrocycles from Cu-catalyzed Azide/Alkyne Cycloaddition



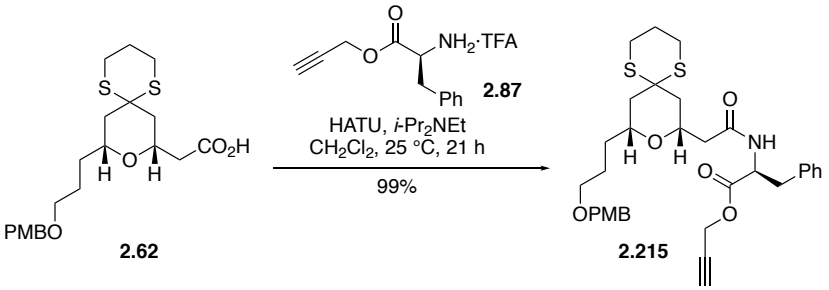
To a solution of Boc-Gly-OH (100 mg, 0.571 mmol) in DMF (3 mL) were added K_2CO_3 (236 mg, 1.713 mmol) and propargyl bromide (0.19 mL, 80 wt% in toluene, 1.713 mmol) at 25 °C. After stirring for 1 h at the same temperature, the reaction mixture was diluted with EtOAc and H_2O. The layers were separated, and the organic layer was washed with H_2O several times. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 5/1) to afford the product (122 mg, quant.) as a white solid. The product was dissolved in CH_2Cl_2 (6.0 mL) and the reaction was cooled to 0 °C. Trifluoroacetic acid (1.5 mL) was added dropwise, and the resulting mixture was stirred for 10 min at 0 °C and then allowed to warm to 25 °C. After stirring for 2 h at 25 °C, the reaction mixture was concentrated. The residue was co-evaporated toluene several times to afford glycine propargyl ester **2.88** (65 mg, quant.) as a white solid. ^{1}H NMR spectrum data is in accordance with the reported data.[91]



Synthesized from **2.62** (75 mg, 0.176 mmol) and glycine propargyl ester **S10a** (48 mg, 0.211 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.214** (81 mg, 88%) as colorless oil: $[\alpha]^{25}_{D}$ –5.0 (*c* 1.3, $CHCl_3$); IR (neat): ν_{max} = 2937, 2359, 1753, 1655, 1512, 1173, 1086, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.6 Hz, 2H), 7.06 (t, *J* = 5.4 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.69 (d, *J* = 2.3 Hz, 2H), 4.39 (s, 2H), 4.04–4.15 (m, 2H), 3.95 (dd, *J* = 18.4, 5.2 Hz, 1H), 3.81–3.88 (m, 1H), 3.77 (s, 3H), 3.37–3.49 (m, 2H), 2.92 (ddd, *J* = 14.4, 8.8, 3.5 Hz, 1H), 2.65–2.84 (m, 3H), 2.47 (t, *J* = 2.5 Hz, 1H), 2.43 (dd, *J* = 15.4, 7.9 Hz, 1H), 2.37 (dd, *J* = 15.5, 3.8 Hz, 1H), 2.28 (dt, *J* = 13.7, 2.1 Hz, 1H), 2.15 (dt, *J* = 13.9, 2.0 Hz, 1H), 1.89–2.04 (m, 2H), 1.48–1.72 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 169.1, 130.6, 129.3, 113.8, 75.5, 72.6, 72.4, 69.9, 69.8, 55.3, 52.6, 47.7, 43.1, 42.3, 42.2, 41.1, 32.5, 26.0, 25.8, 25.7, 25.6; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{26}H_{35}NO_6S_2$ 522.1979; found 544.1783.

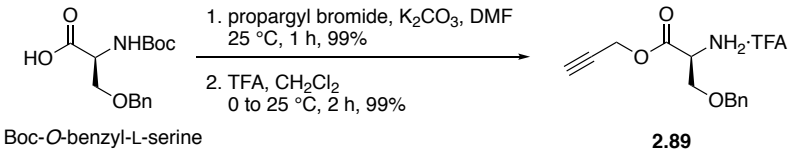


Synthesized from Boc-L-Phe-OH (118 mg, 0.445 mmol) according to the same procedure as described for **2.88**. Compound **2.87** (131 mg, 98% for 2 steps) was obtained as a white solid. **[Alkylation]** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.35 (m, 3H), 7.12–7.19 (m, 2H), 4.94 (d, J = 8.5 Hz, 1H), 4.77 (dd, J = 15.7, 2.5 Hz, 1H), 4.68 (dd, J = 15.6, 2.1 Hz, 1H), 4.56–4.67 (m, 1H), 3.15 (dd, J = 14.0, 5.7 Hz, 1H), 3.08 (dd, J = 14.0, 6.4 Hz, 1H), 2.51 (t, J = 2.5 Hz, 1H), 1.41 (s, 9H). **[Boc deprotection]** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.36 (m, 3H), 7.21–7.25 (m, 2H), 4.75 (dd, J = 15.6, 2.5 Hz, 1H), 4.66 (dd, J = 15.5, 2.5 Hz, 1H), 4.32 (t, J = 6.3 Hz, 1H), 3.33 (dd, J = 14.7, 5.8 Hz, 1H), 3.27 (dd, J = 14.5, 7.0 Hz, 1H), 2.53 (t, J = 2.4 Hz, 1H).

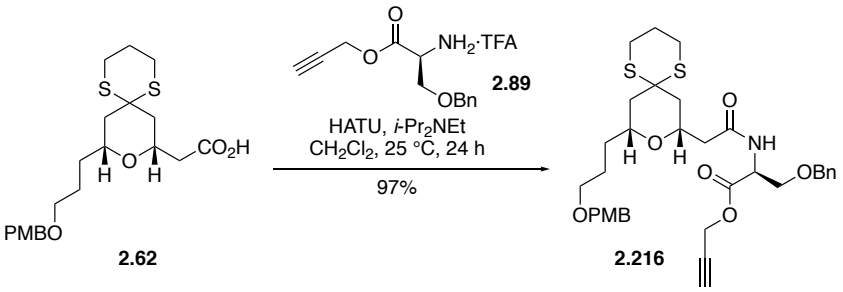


Synthesized from **6a** (85 mg, 0.200 mmol) and the amine part **S10b** (72 mg 0.240 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **13** (121 mg, 99%) as colorless oil: $[\alpha]^{25}_D$ +10.2 (c 1.6, $CHCl_3$); IR (neat): ν_{max} = 3285, 2937, 2908, 1748, 1666, 1512, 1246, 1173 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ⊚⊚⊚⊚⊚–7.25 (m, 5H), 7.08–7.13 (m, 2H), 7.06 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 4.94 (dt, J = 8.3, 5.7 Hz, 1H), 4.71 (dd, J = 15.5, 2.5 Hz, 1H), 4.64 (dd, J = 15.5, 2.5 Hz, 1H), 4.38 (s,

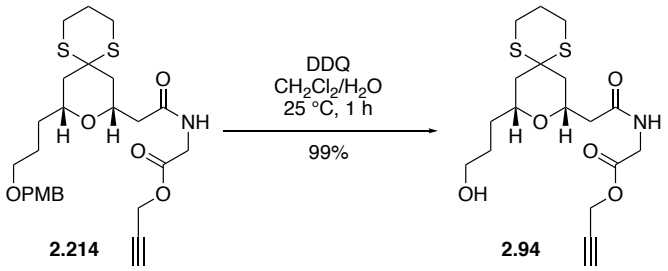
2H), 4.04 (dddd, J = 11.4, 7.6, 4.0, 2.1 Hz, 1H), 3.76 (s, 3H), 3.61–3.68 (m, 1H), 3.29–3.37 (m, 2H), 3.07–3.13 (m, 2H), 2.90 (ddd, J = 14.5, 8.7, 3.3 Hz, 1H), 2.66–2.83 (m, 3H), 2.48 (t, J = 2.5 Hz, 1H), 2.32–2.40 (m, 2H), 2.22 (dt, J = 13.8, 2.1 Hz, 1H), 2.11 (dt, J = 13.8, 2.0 Hz, 1H), 1.89–2.03 (m, 2H), 1.46–1.66 (m, 3H), 1.33–1.42 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.2, 159.1, 135.7, 130.6, 129.4, 129.2, 129.2, 128.5, 128.5, 127.1, 113.7, 75.5, 72.5, 72.4, 69.7, 69.7, 55.3, 52.6, 52.6, 47.6, 42.7, 42.2, 42.1, 37.8, 32.1, 25.9, 25.8, 25.7, 25.5; HRMS (m/z): $[M+H]^+$ calcd for $C_{33}H_{41}NO_6S_2$ 612.2448; found 612.2453.



Synthesized from Boc-*O*-benzyl-L-serine (118 mg, 0.400 mmol) according to the same procedure as described for **2.88**. Compound **2.89** (129 mg, 98% for 2 steps) was obtained as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.36 (m, 5H), 4.73 (dd, J = 15.7, 2.3 Hz, 1H), 4.68 (dd, J = 15.7, 2.3 Hz, 1H), 4.59 (d, J = 11.9 Hz, 1H), 4.46 (d, J = 11.9 Hz, 1H), 4.21–4.32 (m, 1H), 3.90 (br s, 2H), 2.95–3.02 (m, 1H), 2.86–2.93 (m, 1H), 2.50 (t, J = 2.4 Hz, 1H).

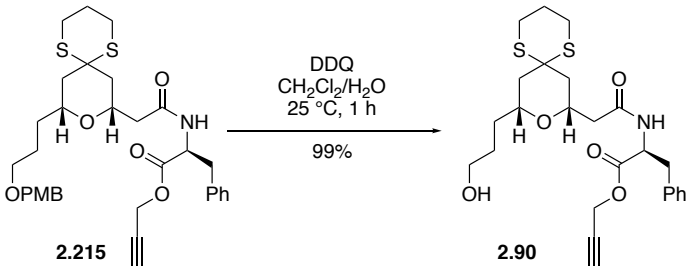


Synthesized from **2.62** (49 mg, 0.115 mmol) and the amine part **2.89** (46 mg 0.138 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.216** (72 mg, 97%) as colorless oil: $[\alpha]^{25}_{D}$ +5.7 (*c* 1.6, $CHCl_3$); IR (neat): ν_{max} = 3292, 2935, 2342, 1752, 1671, 1513, 1247 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.38 (m, 5H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.77–4.84 (m, 1H), 4.66–4.76 (m, 2H), 4.51 (d, *J* = 12.2 Hz, 1H), 4.44 (d, *J* = 12.2 Hz, 1H), 4.36 (s, 2H), 4.13 (dddd, *J* = 11.2, 9.4, 5.7, 3.2 Hz, 1H), 3.90 (dd, *J* = 9.5, 3.1 Hz, 1H), 3.79–3.85 (m, 1H), 3.77 (s, 3H), 3.65 (dd, *J* = 9.5, 3.2 Hz, 1H), 3.28–3.40 (m, 2H), 2.94 (ddd, *J* = 14.4, 8.8, 3.3 Hz, 1H), 2.74–2.86 (m, 2H), 2.70 (ddd, *J* = 15.2, 7.3, 4.0 Hz, 1H), 2.37–2.51 (m, 3H), 2.30 (d, *J* = 13.7 Hz, 1H), 2.14 (d, *J* = 13.7 Hz, 1H), 1.89–2.05 (m, 2H), 1.64–1.80 (m, 2H), 1.43–1.64 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 169.5, 159.1, 137.6, 130.7, 129.2, 128.4, 127.8, 127.6, 113.8, 77.3, 75.4, 73.2, 72.6, 72.5, 69.9, 69.8, 69.7, 55.3, 52.8, 52.5, 47.8, 42.9, 42.3, 42.0, 32.3, 26.0, 25.9, 25.8, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{34}H_{43}NO_7S_2$ 642.2554; found 642.2560.



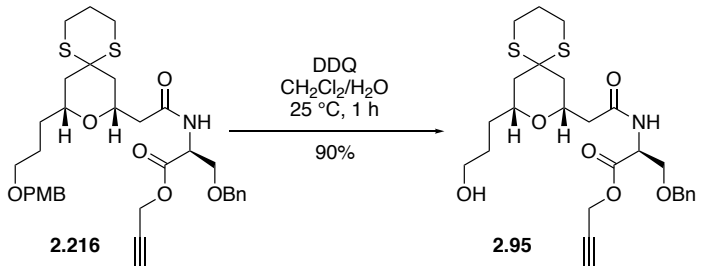
Synthesized from **2.214** (38 mg, 0.0728 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.94** (29 mg, 99%) as colorless oil:

$[\alpha]^{25}_{D}$ –5.4 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3287, 2940, 2359, 1756, 1654, 1184, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.01 (br s, 1H), 4.74 (d, *J* = 2.5 Hz, 2H), 4.14–4.22 (m, 1H), 4.01–4.14 (m, 2H), 3.83–3.92 (m, 1H), 3.57–3.70 (m, 2H), 2.95 (ddd, *J* = 14.4, 9.0, 3.3 Hz, 1H), 2.76–2.86 (m, 2H), 2.72 (ddd, *J* = 14.6, 7.0, 3.7 Hz, 1H), 2.51 (t, *J* = 2.5 Hz, 1H), 2.39–2.49 (m, 2H), 2.32 (dt, *J* = 13.7, 2.1 Hz, 1H), 2.13–2.18 (m, 1H), 1.89–2.07 (m, 2H), 1.52–1.74 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 169.4, 77.1, 75.7, 72.6, 70.1, 62.5, 52.8, 47.7, 43.2, 42.4, 42.3, 41.2, 32.1, 28.7, 26.0, 25.9, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{27}NO_5S_2$ 402.1403; found 402.1404.



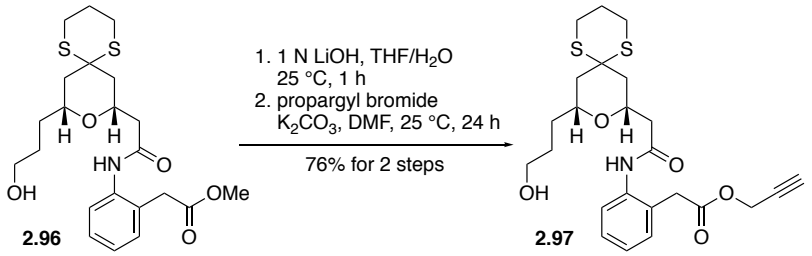
Synthesized from **2.215** (121 mg, 0.200 mmol) according to **GP4**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded **2.90** (98 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ +19.1 (*c* 1.4, $CHCl_3$); IR (neat): ν_{max} = 3292, 2939, 2909, 1247, 1652, 1529, 1176, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27–7.33 (m, 2H), 7.21–7.26 (m, 1H), 7.11–7.16 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 1H), 5.01 (dt, *J* = 8.2, 5.4 Hz, 1H), 4.79 (dd, *J* = 15.5, 2.5 Hz, 1H), 4.69 (dd, *J* = 15.5, 2.5 Hz, 1H), 4.05– 4.15 (m, 1H), 3.67 (dddd, *J* = 11.5, 6.9, 4.8, 2.0 Hz, 1H), 3.44–3.54 (m, 2H), 3.13–3.21 (m, 2H), 2.95 (ddd, *J* = 14.5, 9.0, 3.2 Hz, 1H), 2.78–2.86 (m,

2H), 2.75 (ddd, J = 14.5, 7.1, 4.0 Hz, 1H), 2.53 (t, J = 2.5 Hz, 1H), 2.45 (dd, J = 15.7, 8.4 Hz, 1H), 2.37 (dd, J = 15.7, 3.1 Hz, 1H), 2.27 (dt, J = 13.7, 2.2 Hz, 1H), 2.10 (dt, J = 13.8, 2.1 Hz, 1H), 1.92–2.06 (m, 2H), 1.65 (dd, J = 13.7, 11.3 Hz, 1H), 1.57 (dd, J = 13.8, 11.3 Hz, 1H), 1.25–1.51 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.3, 135.7, 129.6, 128.5, 127.1, 77.0, 75.7, 72.4, 69.8, 62.5, 52.7, 52.7, 47.6, 42.9, 42.2, 37.7, 31.7, 28.4, 25.9, 25.8, 25.7; HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{33}NO_5S_2$ 492.1873; found 492.1875.



Synthesized from **2.216** (94 mg, 0.146 mmol) according to **GP4**. Purification by silica flash chromatography (hexanes/EtOAc, 1/2) afforded **2.95** (69 mg, 90%) as colorless oil: $[\alpha]^{25}_D$ +12.1 (c 1.1, $CHCl_3$); IR (neat): ν_{max} = 3289, 2940, 2909, 1752, 1655, 1529, 1192, 1054, 744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.41 (m, 3H), 7.20–7.27 (m, 2H), 4.82 (dt, J = 8.6, 3.1 Hz, 1H), 4.72 (d, J = 2.5 Hz, 2H), 4.52 (d, J = 12.1 Hz, 1H), 4.47 (d, J = 12.1 Hz, 1H), 4.15 (dddd, J = 11.5, 6.9, 4.7, 2.1 Hz, 1H), 3.91 (dd, J = 9.5, 3.1 Hz, 1H), 3.77–3.86 (m, 1H), 3.68 (dd, J = 9.5, 3.2 Hz, 1H), 3.48 (t, J = 6.0 Hz, 2H), 2.94 (ddd, J = 14.4, 9.0, 3.3 Hz, 1H), 2.75–2.85 (m, 2H), 2.71 (ddd, J = 14.5, 6.9, 3.6 Hz, 1H), 2.47 (t, J = 2.5 Hz, 1H), 2.35–2.45 (m, 2H), 2.30 (dt, J = 13.8, 2.1 Hz, 1H), 2.11 (dt, J = 13.7, 2.1 Hz, 1H), 1.89–2.06 (m, 2H),

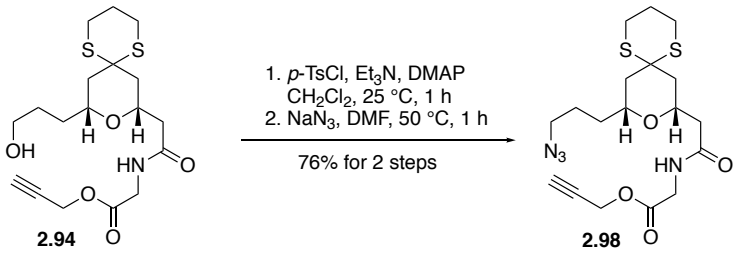
1.71 (dd, J = 13.8, 11.3 Hz, 1H), 1.41–1.67 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 169.7, 137.5, 128.5, 127.9, 127.7, 75.5, 73.3, 72.6, 69.9, 69.9, 62.5, 52.9, 52.5, 47.7, 43.1, 42.3, 42.0, 32.0, 28.7, 26.0, 25.9, 25.7; HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{35}NO_6S_2$ 522.1979; found 522.1981.



[Hydrolysis] Synthesized from **2.96** (40 mg, 0.0887 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification.

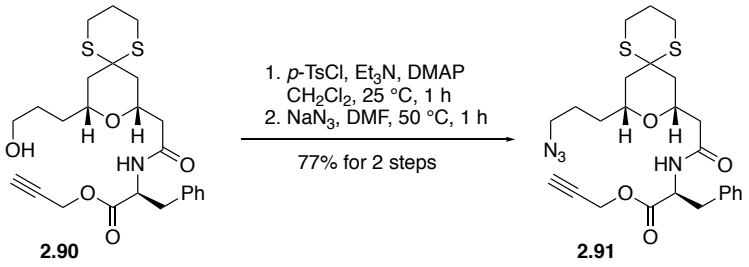
[Propargylation] To a solution of the crude residue in DMF (1 mL) were added K_2CO_3 (37 mg, 0.266 mmol) and propargyl bromide (0.03 mL, 80 wt% in toluene, 0.266 mmol). The reaction mixture was stirred at 25 °C for 24 h and diluted with EtOAc and excess H_2O. The layers were separated, and the organic layer was washed with H_2O several times. The resulting organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford alcohol **2.97** (32 mg, 76% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ –2.6 (c 0.4, $CHCl_3$); IR (neat): ν_{max} = 3290, 2939, 2360, 1741, 1663, 1522, 1145 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.32 (td, J = 7.7, 1.7 Hz, 1H), 7.24 (dd, J = 7.1, 1.4 Hz, 1H), 7.16 (td, J = 7.5, 1.2 Hz, 1H), 4.71 (d, J = 2.4 Hz, 1H), 4.24–4.36 (m, 1H), 3.86–

3.96 (m, 1H), 3.65–3.76 (m, 2H), 3.55–3.64 (m, 2H), 2.97 (ddd, *J* = 14.5, 8.9, 3.2 Hz, 1H), 2.78–2.90 (m, 2H), 2.74 (ddd, *J* = 14.6, 6.9, 3.5 Hz, 1H), 2.54–2.64 (m, 2H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.39–2.47 (m, 1H), 2.18 (dt, *J* = 13.7, 2.1 Hz, 1H), 1.91–2.10 (m, 2H), 1.73 (dd, *J* = 13.7, 11.3 Hz, 1H), 1.53–1.68 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ◎171.5, 169.3, 136.2, 130.9, 128.6, 126.9, 126.1, 125.9, 75.7, 72.7, 70.3, 62.6, 53.0, 47.8, 43.6, 43.2, 42.4, 38.1, 32.2, 28.7, 26.0, 26.0, 25.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{24}H_{31}NO_5S_2$ 478.1716; found 478.1714.



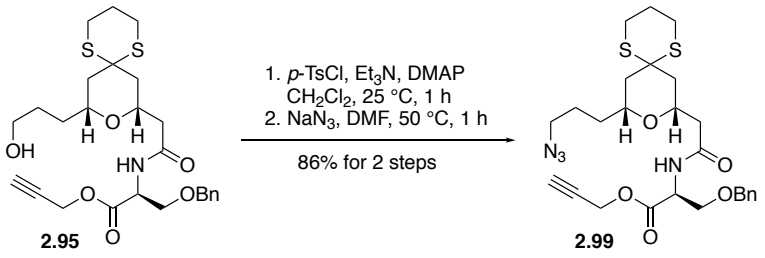
[Tosylation] Synthesized from **2.94** (57 mg, 0.142 mmol) according to **GP9**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.1, Hz, 2H), 6.83 (br s, 1H), 4.74 (d, *J* = 2.5 Hz, 1H), 4.03–4.16 (m, 5H), 3.72–3.84 (m, 1H), 2.94 (ddd, *J* = 14.4, 8.9, 3.4 Hz, 1H), 2.77–2.87 (m, 2H), 2.74 (dq, *J* = 10.9, 4.2, 3.7 Hz, 1H), 2.50 (t, *J* = 2.5 Hz, 1H), 2.45 (s, 3H), 2.39–2.44 (m, 2H), 2.30 (d, *J* = 13.5 Hz, 1H), 2.09 (dt, *J* = 13.7, 2.2 Hz, 1H), 1.93–2.04 (m, 2H), 1.75–1.87 (m, 2H), 1.65 (dd, *J* = 13.7, 11.3 Hz, 1H), 1.47–1.61 (m, 3H). **[Azidation]** Synthesized from the crude tosylate according to **GP10**. Purification by silica flash chromatography (hexanes/EtOAc, 2/1) afforded azide **2.98** (46 mg, 76% for 2 steps) as

colorless oil: $[\alpha]^{25}_{D}$ –5.1 (*c* 0.6, $CHCl_3$); IR (neat): ν_{max} = 3293, 2938, 2096, 1756, 1662, 1535, 1183 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.88 (t, *J* = 5.4 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 2H), 4.08–4.18 (m, 2H), 4.04 (dd, *J* = 18.6, 5.1 Hz, 1H), 3.79– 3.87 (m, 1H), 3.28 (t, *J* = 6.7 Hz, 2H), 2.93 (ddd, *J* = 14.4, 8.8, 3.3 Hz, 1H), 2.74–2.86 (m, 2H), 2.71 (ddd, *J* = 14.6, 7.0, 3.7 Hz, 1H), 2.50 (t, *J* = 2.5 Hz, 1H), 2.37–2.47 (m, 2H), 2.30 (dt, *J* = 13.8, 2.1 Hz, 1H), 2.13 (dt, *J* = 13.8, 2.0 Hz, 1H), 1.90–2.07 (m, 2H), 1.69–1.80 (m, 1H), 1.48–1.70 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 169.2, 77.1, 75.6, 72.2, 70.1, 52.8, 51.3, 47.6, 43.1, 42.3, 42.3, 41.2, 32.7, 26.0, 25.9, 25.7, 25.0; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{26}N_4O_4S_2$ 427.1468; found 427.1458.

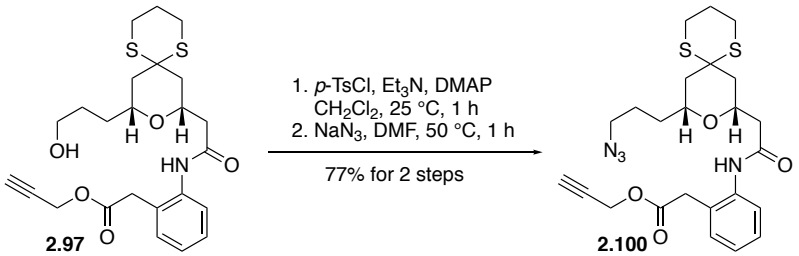


[Tosylation] Synthesized from **2.90** (42 mg, 0.0854 mmol) according to **GP9**. The crude reaction mixture was used for the next step without further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.18–7.25 (m, 3H), 7.07–7.12 (m, 2H), 6.94 (d, *J* = 8.2 Hz, 1H), 4.97 (dt, *J* = 8.2, 5.2 Hz, 1H), 4.76 (dd, *J* = 15.5, 2.5 Hz, 1H), 4.68 (dd, *J* = 15.5, 2.5 Hz, 1H), 4.00–4.09 (m, 1H), 3.82–3.94 (m, 2H), 3.52–3.60 (m, 1H), 3.11–3.16 (m, 2H), 2.92 (ddd, *J* = 14.5, 8.7, 3.3 Hz, 1H), 2.73–2.84 (m, 3H), 2.52 (t, *J* =

2.5 Hz, 1H), 2.44 (s, 3H), 2.32–2.39 (m, 2H), 2.23 (dt, *J* = 13.8, 2.1 Hz, 1H), 1.93–2.08 (m, 3H), 1.53–1.66 (m, 2H), 1.48 (dd, *J* = 13.8, 11.2 Hz, 1H), 1.22–1.36 (m, 3H). **[Azidation]** Synthesized from the crude tosylate according to **GP10**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.91** (34 mg, 77% for 2 steps) as colorless oil: $[\alpha]^{25}_{D}$ +19.5 (*c* 0.7, $CHCl_3$); IR (neat): ν_{max} = 3293, 2937, 2095, 1748, 1666, 1525, 1177 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.21–7.38 (m, 3H), 7.09–7.16 (m, 2H), 7.01 (d, *J* = 8.1 Hz, 1H), 4.99 (dt, *J* = 8.0, 5.2 Hz, 1H), 4.78 (dd, *J* = 15.6, 2.5 Hz, 1H), 4.70 (dd, *J* = 15.5, 2.4 Hz, 1H), 4.05–4.14 (m, 1H), 3.58–3.69 (m, 1H), 3.07–3.19 (m, 4H), 2.94 (ddd, *J* = 14.5, 9.0, 3.2 Hz, 1H), 2.70–2.85 (m, 3H), 2.53 (t, *J* = 2.4 Hz, 1H), 2.42 (dd, *J* = 15.8, 7.9 Hz, 1H), 2.37 (dd, *J* = 15.9, 3.5 Hz, 1H), 2.26 (dd, *J* = 13.8, 2.5 Hz, 1H), 2.09 (dt, *J* = 13.9, 2.1 Hz, 1H), 1.90–2.06 (m, 2H), 1.64 (dd, *J* = 13.7, 11.3 Hz, 1H), 1.48–1.60 (m, 2H), 1.23–1.39 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.2, 135.7, 129.7, 128.6, 127.2, 75.7, 72.1, 69.9, 52.8, 52.7, 51.4, 47.6, 42.9, 42.3, 42.2, 37.8, 32.6, 26.0, 25.9, 25.7, 24.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{32}N_4O_4S_2$ 517.1938; found 517.1936.

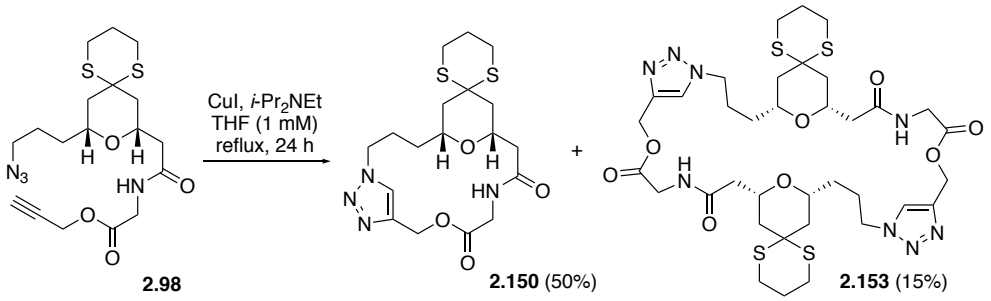


[Tosylation] Synthesized from **2.95** (68 mg, 0.130 mmol) according to **GP9**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.2 Hz), 7.17–7.30 (m, 7H), 6.44 (br s, 1H), 4.75 (dt, *J* = 8.6, 3.1 Hz, 1H), 4.63–4.72 (m, 2H), 4.38–4.48 (m, 2H), 4.02–4.10 (m, 1H), 3.81–3.92 (m, 2H), 3.64–3.70 (m, 1H), 2.84–2.91 (m, 1H), 2.67–2.81 (m, 3H), 2.44 (t, *J* = 2.3 Hz, 1H), 2.38 (s, 3H), 2.32–2.37 (m, 2H), 2.24 (d, *J* = 13.8 Hz), 1.87–2.04 (m, 3H), 1.61–1.76 (m, 2H), 1.45–1.58 (m, 2H), 1.25–1.41 (m, 2H). **[Azidation]** Synthesized from the crude tosylate according to **GP10**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.99** (62 mg, 86% for 2 steps) as colorless oil: $[\alpha]^{25}_{D}$ +9.1 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} = 3289, 2943, 2869, 2093, 1751, 1669, 1521, 1103 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.37 (m, 5H), 4.81 (dt, *J* = 8.4, 3.0 Hz, 1H), 4.69–4.79 (m, 2H), 4.45–4.55 (m, 2H), 4.10–4.18 (m, 1H), 3.93 (dd, *J* = 9.5, 3.0 Hz, 1H), 3.74–3.82 (m, 1H), 3.68 (dd, *J* = 9.5, 3.1 Hz, 1H), 3.04–3.16 (m, 2H), 2.95 (ddd, *J* = 14.5, 9.0, 3.3 Hz, 1H), 2.75–2.86 (m, 2H), 2.71 (ddd, *J* = 14.4, 7.0, 3.6 Hz, 1H), 2.47 (t, *J* = 2.5 Hz, 1H), 2.41–2.46 (m, 2H), 2.31 (dd, *J* = 13.8, 2.1 Hz, 1H), 2.10 (dd, *J* = 13.7, 2.1 Hz, 1H), 1.92–2.05 (m, 2H), 1.73 (dd, *J* = 13.9, 11.4 Hz, 1H), 1.64–1.71 (m, 1H), 1.60 (dd, *J* = 13.9, 11.4 Hz, 1H), 1.35–1.56 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 169.6, 137.6, 128.5, 127.9, 127.7, 77.3, 75.4, 73.4, 72.2, 70.0, 52.9, 52.5, 51.3, 47.7, 43.0, 42.3, 41.9, 32.6, 26.0, 25.9, 25.7, 24.9; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{34}N_4O_5S_2$ 547.2043; found 547.2044.



[Tosylation] Synthesized from **2.97** (34 mg, 0.0712 mmol) according to **GP9**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.73 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 1H), 7.27–2.37 (m, 3H), 7.23 (d, J = 7.5 Hz, 1H), 7.14 (t, J = 7.5 Hz, 1H), 4.68 (d, J = 2.4 Hz, 2H), 4.18–4.28 (m, 1H), 3.93–4.07 (m, 2H), 3.73–3.83 (m, 1H), 3.65 (s, 2H), 2.94 (ddd, J = 12.3, 8.5, 3.4 Hz, 1H), 2.76–2.87 (m, 2H), 2.72 (ddd, J = 14.7, 7.1, 3.8 Hz, 1H), 2.51–2.58 (m, 2H), 2.50 (t, J = 2.5 Hz, 1H), 2.43 (s, 3H), 2.39 (d, J = 13.8 Hz, 1H), 2.10 (d, J = 13.7 Hz, 1H), 1.93–2.07 (m, 2H), 1.73–1.83 (m, 1H), 1.63–1.73 (m, 2H), 1.42–1.60 (m, 3H). **[Azidation]** Synthesized from the crude tosylate according to **GP10**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.100** (28 mg, 28% for 2 steps) as colorless oil: $[\alpha]^{25}_D$ –2.9 (c 0.36, $CHCl_3$); IR (neat): ν_{max} = 3291, 2937, 1740, 1670, 1522, 1246, 1125, 754 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.31 (td, J = 7.8, 1.5 Hz, 1H), 7.24 (d, J = 7.4 Hz, 1H), 7.15 (t, J = 7.3 Hz, 1H), 4.71 (d, J = 2.5 Hz, 2H), 4.23–4.35 (m, 1H), 3.79–3.93 (m, 1H), 3.68 (s, 2H), 3.27–3.35 (m, 2H), 2.96 (ddd, J = 12.6, 8.9, 3.4 Hz, 1H), 2.78–2.91 (m, 2H), 2.74 (ddd, J = 14.6, 7.0, 3.6 Hz, 1H), 2.54–2.63 (m, 2H), 2.51 (t, J = 2.4 Hz, 1H), 2.42 (d, J = 13.5 Hz, 1H), 2.17 (d, J = 13.7 Hz, 1H), 1.93–2.10 (m, 2H), 1.48–1.78 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 169.3, 136.3, 130.9, 128.6, 126.6, 126.0, 125.8, 75.7, 72.3,

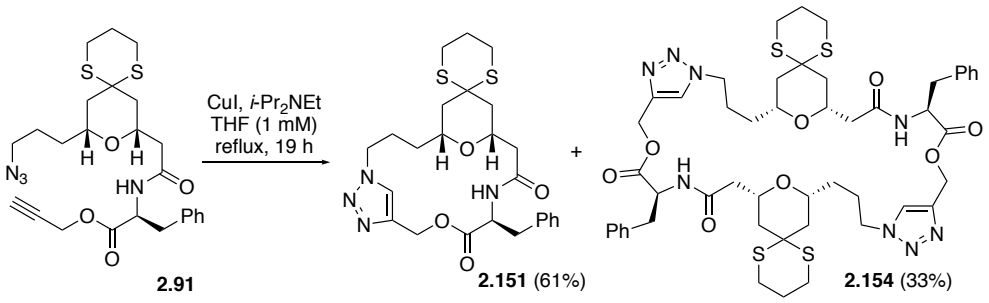
70.3, 53.0, 51.4, 47.8, 43.8, 43.2, 42.5, 38.2, 32.8, 26.1, 26.0, 25.8, 25.1; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{24}H_{30}N4O_4S_2$ 503.1781; found 503.1773.



Synthsized from **2.98** (20 mg, 0.0469 mmol) according to **GP11**. Purification by silica flash chromatography (silica gel, CH_2Cl_2/MeOH, 15/1) afforded cyclic monomer **2.150** (10 mg, 50%) and cyclic dimer **2.153** (3 mg, 15%).

Cyclic monomer **2.150**: Colorless oil; $[\alpha]^{25}_D$ +21.9 (*c* 0.23, $CHCl_3$); IR (neat): ν_{max} = 3302, 2944, 1741, 1662, 1540, 1215, 1086, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 6.35 (s, 1H), 5.38 (d, *J* = 12.8 Hz, 1H), 5.29 (d, *J* = 12.8 Hz, 1H), 4.38–4.49 (m, 2H), 4.34 (ddd, *J* = 16.7, 7.2, 1.2 Hz, 1H), 4.15–4.24 (m, 1H), 3.75 (dd, *J* = 16.9, 3.8 Hz, 1H), 3.65–3.73 (m, 2H), 2.92 (ddd, *J* = 12.1, 7.9, 3.8 Hz, 1H), 2.67–2.87 (m, 3H), 2.38 (d, *J* = 14.8 Hz, 1H), 2.29 (dd, *J* = 14.9, 9.5 Hz, 1H), 2.22 (d, *J* = 13.6 Hz, 1H), 2.12 (d, *J* = 13.7 Hz, 1H), 1.92–2.08 (m, 3H), 1.80–1.92 (m, 1H), 1.59–1.68 (m, 1H), 1.51–1.59 (m, 1H), 1.36 (ddt, *J* = 14.8, 10.1, 5.3 Hz, 1H), 1.10 (ddt, *J* = 14.9, 10.3, 5.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 169.5, 143.3, 124.2, 71.3, 70.0, 59.3, 49.4, 47.7, 42.6, 42.5, 42.3, 42.0, 31.0, 26.1, 26.0, 25.8, 24.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{26}N_4O_4S_2$ 427.1468; found 427.1476.

Cyclic dimer **2.153**: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 2H), 6.89 (t, J = 5.0 Hz, 2H), 5.24 (s, 4H), 4.33–4.52 (m, 4H), 4.14–4.24 (m, 2H), 4.06 (dd, J = 18.6, 5.1 Hz, 2H), 3.99 (dd, J = 18.6, 5.0 Hz, 2H), 3.80–3.90 (m, 2H), 2.93 (ddd, J = 12.0, 7.5, 3.6 Hz, 2H), 2.66–2.89 (m, 6H), 2.34–2.46 (m, 4H), 2.26 (d, J = 13.6 Hz, 2H), 2.09–2.21 (m, 4H), 1.90–2.08 (m, 6H), 1.49–1.70 (m, 6H); HRMS (m/z): $[M+H]^+$ calcd for $C_{36}H_{52}N_8O_8S_4$ 853.2864; found 853.2868.

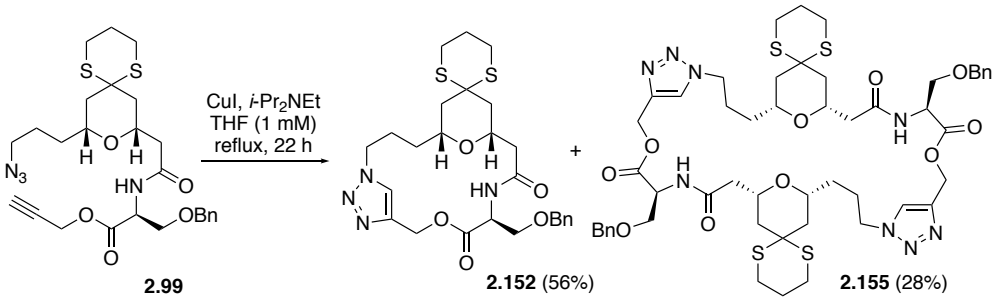


Synthesized from **2.91** (33 mg, 0.0639 mmol) according to **GP11**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 50/1) afforded cyclic monomer **2.151** (20 mg, 61%) and cyclic dimer **2.154** (11 mg, 33%).

Cyclic monomer **2.151**: Colorless oil; $[\alpha]^{25}_D$ +15.8 (c 0.4, $CHCl_3$); IR (neat): ν_{max} = 3306, 2934, 2359, 1740, 1666, 1522, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.26–7.32 (m, 2H), 7.19–7.24 (m, 1H), 7.11–7.17 (m, 2H), 6.28 (d, J = 8.6 Hz, 1H), 5.48 (d, J = 12.6 Hz, 1H), 5.13 (d, J = 12.6 Hz, 1H), 4.89 (ddd, J = 8.5, 6.8, 5.2 Hz, 1H), 4.41 (ddd, J = 14.2, 6.7, 4.3 Hz, 2H), 4.34 (ddd, J = 13.6, 9.4, 3.8 Hz, 2H), 3.98 (ddt, J = 10.6, 8.3, 2.4 Hz, 1H), 3.47–3.57 (m, 1H), 3.21 (dd, J = 13.7, 5.2 Hz, 1H), 3.04 (dd, J = 13.7, 6.8 Hz, 1H), 2.92 (ddd, J = 14.3,

8.4, 3.5 Hz, 1H), 2.71–2.86 (m, 3H), 2.37 (dd, *J* = 15.7, 8.4 Hz, 1H), 2.29 (dd, *J* = 15.7, 2.8 Hz, 1H), 2.16 (d, *J* = 13.9 Hz, 1H), 1.90–2.08 (m, 4H), 1.76–1.87 (m, 1H), 1.72 (dd, *J* = 13.8, 11.3 Hz, 1H), 1.53 (dd, *J* = 13.7, 11.5 Hz, 1H), 1.07 (ddt, *J* = 15.5, 10.7, 5.7 Hz, 1H), 0.90 (ddt, *J* = 14.8, 9.4, 4.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 169.8, 136.2, 129.6, 128.7, 127.1, 123.5, 70.8, 69.7, 59.2, 53.3, 49.4, 47.6, 42.3, 42.2, 41.6, 37.5, 30.7, 26.0, 26.0, 25.7, 24.0; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{32}N_4O_4S_2$ 517.1938; found 517.1933.

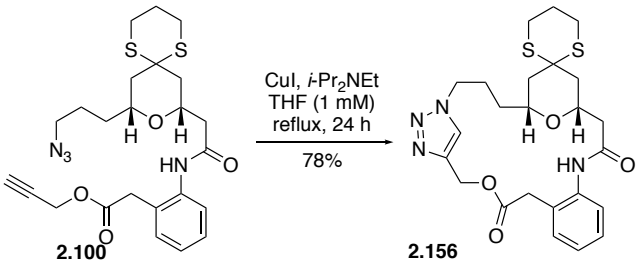
Cyclic dimer **2.154**: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 2H), 7.08–7.20 (m, 8H), 6.86–6.96 (m, 4H), 5.22–5.38 (m, 4H), 4.99 (ddd, *J* = 8.2, 5.6, 4.0 Hz, 2H), 4.02–4.26 (m, 6H), 3.51–3.63 (m, 2H), 3.15 (dd, *J* = 13.7, 4.0 Hz, 2H), 3.08 (dd, *J* = 13.7, 5.6 Hz, 2H), 2.93 (ddd, *J* = 14.4, 8.9, 3.3 Hz, 2H), 2.74–2.86 (m, 6H), 2.45 (dd, *J* = 16.0, 9.8 Hz, 2H), 2.31 (dd, *J* = 15.9, 1.8 Hz, 2H), 2.22 (d, *J* = 13.4 Hz, 2H), 1.94–2.12 (m, 6H), 1.71–1.84 (m, 4H), 1.60 (dd, *J* = 13.8, 11.3 Hz, 2H), 1.52 (dd, *J* = 13.7, 11.3 Hz, 2H), 1.18–1.32 (m, 4H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{50}H_{64}N_8O_8S_4$ 1033.3803; found 1033.3794.



Synthesized from **2.99** (41 mg, 0.0758 mmol) according to **GP11**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 50/1) afforded cyclic monomer **2.152** (23 mg, 56%) and cyclic dimer **2.155** (12 mg, 28%).

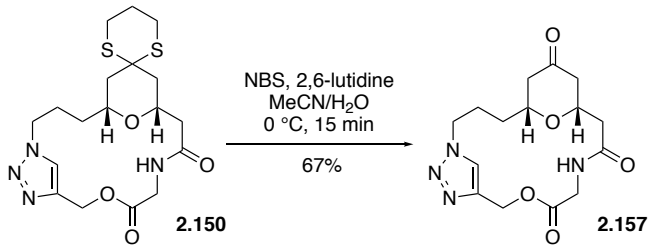
Cyclic monomer **2.152**: Colorless oil; $[\alpha]^{25}_{D}$ +14.5 (*c* 0.4, $CHCl_3$); IR (neat): ν_{max} = 3307, 2937, 1741, 1670, 1533, 1216, 1094, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.27–7.37 (m, 3H), 7.22–7.26 (m, 2H), 6.74 (d, *J* = 8.7 Hz, 1H), 5.45 (d, *J* = 12.8 Hz, 1H), 5.33 (d, *J* = 12.8 Hz, 1H), 4.78 (dt, *J* = 8.1, 3.7 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 11.6 Hz, 1H), 4.38 (ddd, *J* = 13.7, 6.9, 3.5 Hz, 1H), 4.30 (ddd, *J* = 13.7, 9.4, 3.3 Hz, 1H), 3.96–4.03 (m, 1H), 3.93 (dd, *J* = 9.3, 3.2 Hz, 1H), 3.67 (dd, *J* = 9.4, 4.3 Hz, 1H), 3.42–3.50 (m, 1H), 2.90 (ddd, *J* = 15.0, 7.6, 4.0 Hz, 1H), 2.83 (ddd, *J* = 14.3, 6.7, 4.0 Hz, 1H), 2.68–2.78 (m, 2H), 2.42 (dd, *J* = 15.5, 8.4 Hz, 1H), 2.33 (dd, *J* = 15.6, 2.7 Hz, 1H), 2.16 (d, *J* = 13.8 Hz, 1H), 1.93–2.08 (m, 4H), 1.80 (dd, *J* = 13.6, 11.3 Hz, 2H), 1.53 (dd, *J* = 13.7, 11.4 Hz, 1H), 1.05–1.25 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.1, 137.5, 128.6, 128.1, 128.0, 73.7, 71.1, 69.9, 69.5, 59.6, 52.6, 49.6, 47.7, 42.3, 42.1, 41.9, 31.2, 26.0, 26.0, 25.7, 23.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{34}N_4O_5S_2$ 547.2043; found 547.2052.

Cyclic dimer **2.155**: Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 2H), 7.42 (d, J = 8.6 Hz, 2H), 7.26–7.32 (m, 6H), 7.06–7.15 (m, 4H), 5.33 (d, J = 12.5 Hz, 2H), 5.24 (d, J = 12.6 Hz, 2H), 4.86 (dt, J = 8.8, 2.9 Hz, 2H), 4.34 (s, 2H), 4.06–4.19 (m, 4H), 3.84–3.95 (m, 4H), 3.73–3.81 (m, 2H), 3.65 (dd, J = 9.3, 3.0 Hz, 2H), 2.88–2.99 (m, 2H), 2.72–2.88 (m, 6H), 2.48 (dd, J = 15.9, 9.7 Hz, 2H), 2.34 (dd, J = 15.8, 1.8 Hz, 2H), 2.24 (d, J = 13.6 Hz, 2H), 1.94–2.13 (m, 8H), 1.64 (dd, J = 13.7, 11.3 Hz, 2H), 1.49–1.61 (m, 4H), 1.24–1.39 (m, 4H).

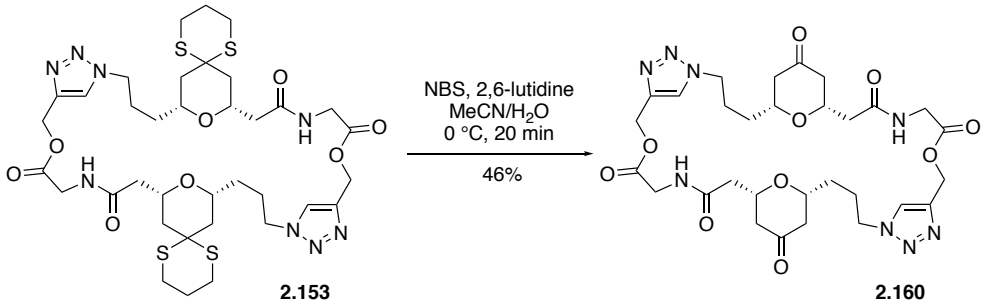


Synthesized from **2.100** (27 mg, 0.0537 mmol) according to **GP11**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) afforded cyclic monomer **2.156** (21 mg, 78%) as colorless oil: $[\alpha]^{25}_D$ –20.0 (c 0.37, $CHCl_3$); IR (neat): ν_{max} = 3318, 2938, 1732, 1683, 1527, 1452, 1243, 1145, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.96 (dd, J = 8.2, 1.3 Hz, 1H), 7.30 (td, J = 7.9, 1.5 Hz, 1H), 7.24 (dd, J = 7.8, 1.5 Hz, 1H), 7.12 (td, J = 7.5, 1.3 Hz, 1H), 7.02 (s, 1H), 5.50 (d, J = 13.0 Hz, 1H), 5.13 (d, J = 13.0 Hz, 1H), 4.18–4.37 (m, 3H), 3.62–3.73 (m, 3H), 2.92 (ddd, J = 14.2, 8.0, 3.7 Hz, 1H), 2.83 (ddd, J = 14.6, 7.0, 3.9 Hz, 1H), 2.71–2.80 (m, 2H), 2.55 (dd, J = 13.8, 3.1 Hz, 1H), 2.43 (dd, J = 13.8, 9.2 Hz, 1H), 2.32 (d, J = 13.6 Hz, 1H), 2.09 (d, J = 13.8 Hz, 1H), 1.94–2.05 (m, 2H), 1.83–1.93 (m, 2H), 1.68

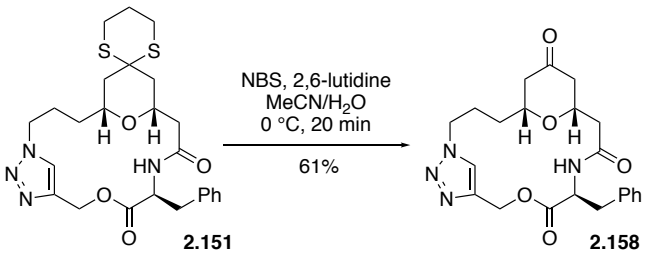
(dd, J = 13.7, 11.3 Hz, 1H), 1.55 (dd, J = 13.7, 11.3 Hz, 1H), 1.37–1.48 (m, 1H), 1.12–1.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 169.4, 143.4, 136.5, 131.6, 128.8, 125.9, 124.5, 123.5, 122.0, 72.1, 70.5, 59.6, 49.5, 47.7, 44.6, 43.3, 42.3, 39.7, 31.8, 26.4, 26.0, 26.0, 25.8; HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{30}N_4O_4S_2$ 503.1781; found 503.1784.



Synthesized from **2.150** (21 mg, 0.0492 mmol) according to **GP12**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) afforded ketone **2.157** (11 mg, 67%) as colorless oil, which was crystallized upon standing: $[\alpha]^{25}_D$ +39.0 (c 0.17, CH_3CN/H_2O = 20/1); IR (neat): ν_{max} = 3316, 2924, 2854, 1739, 1668, 1541, 1284, 1214, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 6.15 (br s, 1H), 5.40 (d, J = 12.7 Hz, 1H), 5.33 (d, J = 12.8 Hz, 1H), 4.40–4.51 (m, 2H), 4.37 (dd, J = 16.7, 7.4 Hz, 1H), 3.97–4.09 (m, 1H), 3.76 (dd, J = 16.7, 4.1 Hz, 1H), 3.44–3.57 (m, 1H), 2.46 (dd, J = 14.8, 2.4 Hz, 1H), 2.39 (d, J = 9.2 Hz, 1H), 2.30–2.37 (m, 3H), 2.23 (dd, J = 14.4, 11.7 Hz, 1H), 2.01–2.13 (m, 1H), 1.83–1.95 (m, 1H), 1.45–1.55 (m, 1H), 1.26–1.35 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.6, 169.7, 169.5, 143.5, 123.8, 75.6, 73.4, 59.4, 49.3, 46.8, 46.7, 42.7, 41.9, 31.7, 24.7; HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{20}N_4O_5$ 337.1507; found 337.1509.

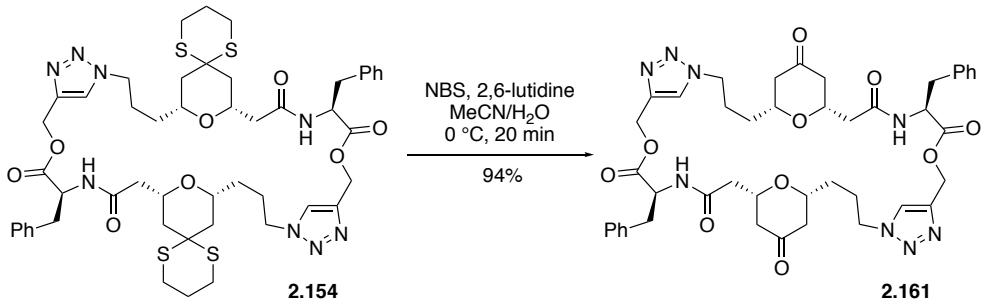


Synthesized from **2.153** (9.0 mg, 0.0106 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1/10 → CH_2Cl_2/MeOH, 10/1) afforded ketone **2.160** (3.3 mg, 46%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 2H), 6.68 (br s, 2H), 5.24 (s, 4H), 4.35–4.53 (m, 4H), 3.94–4.15 (m, 6H), 3.66–3.76 (m, 2H), 2.46–2.58 (m, 4H), 2.25–2.45 (m, 8H), 2.14–2.23 (m, 2H), 2.03–2.13 (m, 2H), 1.63–1.73 (m, 4H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{30}H_{40}N_8O_{10}$ 673.2940; found 673.2920.



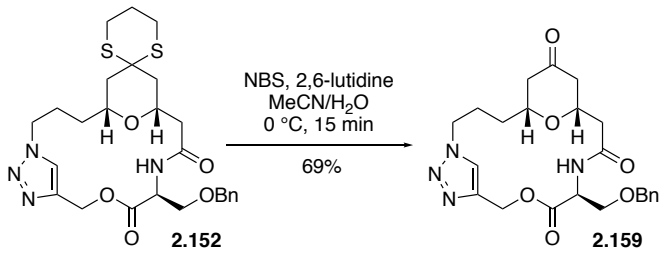
Synthesized from **2.151** (21 mg, 0.0406 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1/10 → CH_2Cl_2/MeOH, 10/1) afforded ketone **2.158** (11 mg, 61%) as a white solid: $[\alpha]^{25}_D$ –11.5 (*c* 0.18, $CHCl_3$); IR (neat): ν_{max} = 3027, 2951, 1719, 1374, 1264, 1176, 1154, 753 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 1H),

7.23–7.36 (m, 3H), 7.08–7.20 (m, 2H), 6.10 (d, *J* = 8.3 Hz, 1H), 5.41 (d, *J* = 12.7 Hz, 1H), 5.26 (d, *J* = 12.7 Hz, 1H), 4.84 (q, *J* = 7.1 Hz, 1H), 4.30–4.54 (m, 2H), 3.63–3.82 (m, 1H), 3.26 (ddt, *J* = 11.1, 7.0, 3.3 Hz, 1H), 3.16 (dd, *J* = 13.9, 6.4 Hz, 1H), 3.10 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.75 (dd, *J* = 14.2, 12.2 Hz, 1H), 2.32–2.47 (m, 2H), 2.22–2.30 (m, 2H), 2.16 (dd, *J* = 14.2, 11.7 Hz, 1H), 1.99–2.10 (m, 1H), 1.87–1.98 (m, 1H), 1.30–1.39 (m, 1H), 1.22–1.29 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.0, 171.5, 168.9, 143.8, 136.0, 129.4, 128.8, 127.3, 123.6, 75.5, 73.4, 59.2, 53.7, 49.6, 47.0, 46.6, 41.7, 37.8, 32.4, 25.0; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{26}N_4O_5$ 427.1976; found 427.1974.

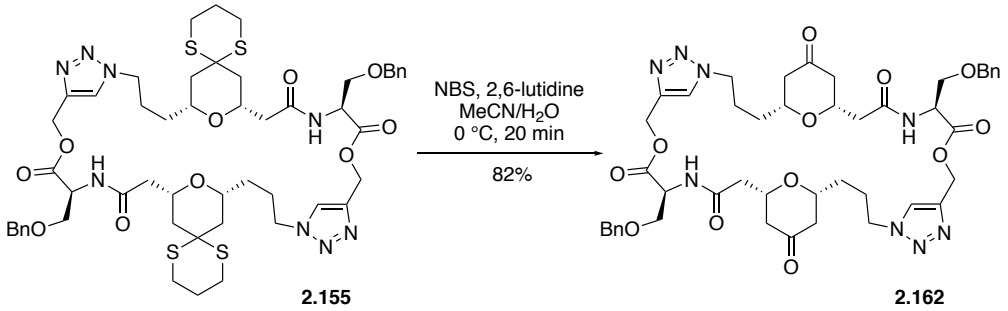


Synthesized from **2.154** (23 mg, 0.0223 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1:10 → CH_2Cl_2/MeOH, 10/1) afforded ketone **2.161** (18 mg, 94%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 2H), 7.06–7.22 (m, 6H), 6.85–6.99 (m, 4H), 5.37 (d, *J* = 12.4 Hz, 2H), 5.31 (d, *J* = 12.2 Hz, 2H), 4.85–5.03 (m, 2H), 4.28–4.39 (m, 2H), 4.12–4.23 (m, 2H), 3.80–3.92 (m, 2H), 3.35–3.47 (m, 2H), 3.05–3.18 (m, 4H), 2.55 (dd, *J* = 15.9, 9.2 Hz, 2H), 2.43 (d, *J* = 15.4 Hz, 2H), 2.15–2.40 (m, 8H),

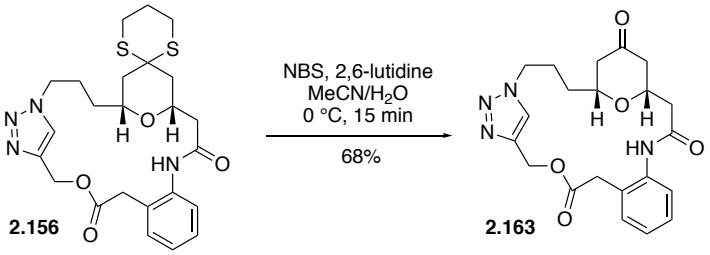
1.80–1.96 (m, 2H), 1.35–1.53 (m, 6H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{44}H_{52}N_8O_{10}$ 853.3880; found 853.3874.



Synthesized from **2.152** (43 mg, 0.0787 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1:10 → CH_2Cl_2/MeOH, 20/1) afforded ketone **2.159** (25mg, 69%) as a white solid: $[\alpha]^{25}_D$ –12.2 (*c* 0.41, $CHCl_3$); IR (neat): ν_{max} = 3302, 2953, 2868, 1740, 1717, 1667, 1535, 1213, 1094, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.27–7.37 (m, 3H), 7.20–7.26 (m, 2H), 6.56 (d, *J* = 8.5 Hz, 1H), 5.44 (d, *J* = 12.9 Hz, 1H), 5.31 (d, *J* = 13.1 Hz, 1H), 4.76 (dt, *J* = 7.7, 3.7 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.35–4.42 (m, 2H), 3.92 (ddd, *J* = 9.5, 3.6, 1.3 Hz, 1H), 3.65–3.75 (m, 2H), 3.10 (ddt, *J* = 10.8, 6.8, 3.2 Hz, 1H), 2.72–2.82 (m, 1H), 2.39–2.48 (m, 2H), 2.08–2.29 (m, 4H), 1.86–1.97 (m, 1H), 1.30–1.50 (m, 1H), 1.26–1.37 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.9, 170.3, 169.3, 143.8, 137.3, 128.6, 128.2, 128.1, 123.1, 75.5, 73.7, 73.6, 69.3, 59.6, 52.7, 49.8, 47.2, 46.7, 41.5, 32.7, 24.3; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{23}H_{28}N_4O_6$ 457.2082; found 457.2080.



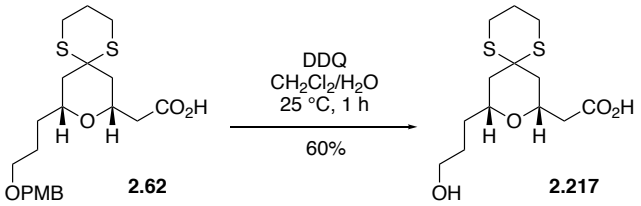
Synthesized from **2.155** (23 mg, 0.0210 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1:10 → CH_2Cl_2/MeOH, 10/1) afforded ketone **2.162** (16 mg, 82%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 2H), 7.25–7.33 (m, 6H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.05–7.14 (m, 4H), 5.33 (d, *J* = 12.5 Hz, 2H), 5.24 (d, *J* = 12.5 Hz, 2H), 4.77–4.88 (m, 2H), 4.27–4.40 (m, 4H), 4.15–4.25 (m, 2H), 3.87–4.04 (m, 6H), 3.62 (dd, *J* = 9.5, 3.0 Hz, 2H), 3.49–3.60 (m, 2H), 2.57 (dd, *J* = 15.7, 9.0 Hz, 2H), 2.46 (d, *J* = 15.2 Hz, 2H), 2.16–2.37 (m, 8H), 1.95–2.10 (m, 2H), 1.65–1.77 (m, 2H), 1.35–1.57 (m, 4H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{46}H_{56}N_8O_{12}$ 913.4091; found 913.4080.



Synthesized from **2.156** (24 mg, 0.0477 mmol) according to **GP12**. Purification by silica flash chromatography (hexanes/EtOAc, 1:10 → CH_2Cl_2/MeOH, 40/1) afforded ketone **2.163** (13 mg, 68%) as a white solid: $[\alpha]^{25}_D$ –31.7 (*c* 0.17, $CHCl_3$); IR (neat): ν_{max} = 3349,

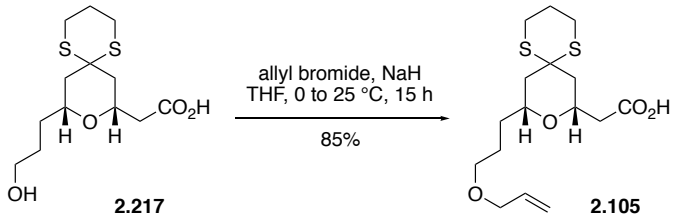
2956, 1721, 1563, 1452, 1251, 1150, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.26–7.29 (m, 1H), 7.13 (t, J = 7.5 Hz, 1H), 6.84 (s, 1H), 5.57 (d, J = 13.2 Hz, 1H), 5.13 (d, J = 13.1 Hz, 1H), 4.33 (ddd, J = 13.4, 9.4, 3.5 Hz, 1H), 4.21 (ddd, J = 14.0, 6.6, 3.4 Hz, 1H), 4.07–4.17 (m, 1H), 3.64–3.76 (m, 2H), 3.51–3.60 (m, 1H), 2.63 (dd, J = 13.8, 2.9 Hz, 1H), 2.54 (dd, J = 13.9, 9.4 Hz, 1H), 2.45 (d, J = 14.4 Hz, 1H), 2.30–2.40 (m, 2H), 2.23 (dd, J = 14.7, 11.3 Hz, 1H), 1.84–2.00 (m, 2H), 1.48–1.59 (m, 1H), 1.29–1.39 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.4, 171.5, 168.5, 143.7, 136.6, 131.8, 129.0, 125.8, 123.4, 122.7, 121.4, 76.4, 74.0, 60.0, 49.3, 47.9, 46.9, 45.1, 39.9, 32.3, 26.5; HRMS (m/z): $[M+H]^+$ calcd for $C_{21}H_{24}N_4O_5$ 413.1820; found 413.1813.

Macrocycles from Ring-Closing Metathesis



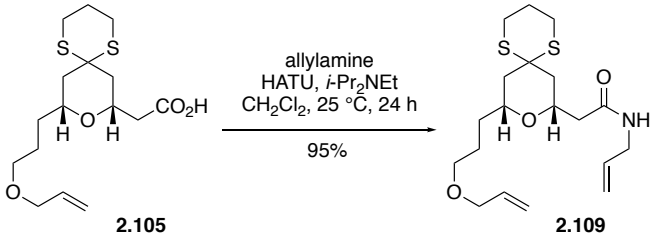
To a solution of **2.62** (186 mg, 0.435 mmol) in CH_2Cl_2/H_2O (10/1, total 4.4 mL) was added DDQ (148 mg, 0.653 mmol) in one portion. After stirring at 25 °C for 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (~1 mL). Na_2SO_4 was added to the reaction mixture. After stirring for 30 min, the reaction mixture was filtered, washed with MeOH, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/AcOH, 20/1/0.2) to afford alcohol **2.217** (80

mg, 60%) as colorless oil: $[\alpha]^{25}_{D}$ +8.1 (*c* 1.3, $CHCl_3$); IR (neat): ν_{max} = 3427, 2944, 2908, 2360, 1717, 1423, 1280 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 4.19 (ddd, *J* = 12.7, 7.5, 5.7 Hz, 1H), 3.78 (d, *J* = 11.9, 6.3 Hz, 1H), 3.49–3.62 (m, 2H), 2.74–3.00 (m, 4H), 2.38–2.49 (m, 3H), 2.22 (d, *J* = 13.9 Hz, 1H), 1.89–2.04 (m, 2H), 1.44–1.73 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.8, 73.8, 71.1, 62.8, 44.4, 43.7, 41.6, 33.0, 29.6, 27.1, 26.7, 26.5; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{13}H_{22}O_4S_2$ 307.1032; found 307.1038.



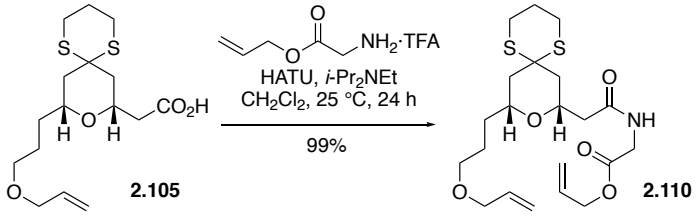
To a cooled (0 °C) solution of **2.217** (80 mg, 0.261 mmol) in THF (5 mL) was added NaH (31 mg, 60% dispersion in mineral oil, 0.783 mmol). After the reaction mixture was stirred for 20 min at the same temperature, allyl bromide was added. The reaction was allowed to warm to room temperature. After stirring for 15 h at 25 °C, the reaction mixture was diluted with CH_2Cl_2 and quenched by saturated aqueous NH_4Cl. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1) to afford **2.105** (77 mg, 85%) as colorless oil: $[\alpha]^{25}_{D}$ +5.0 (*c* 1.2, $CHCl_3$); IR (neat): ν_{max} = 2908, 2860, 1710, 1422, 1076, 924 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 5.87 (ddt, *J*

= 17.2, 10.4, 5.6 Hz, 1H), 5.23 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.14 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.18 (dddd, *J* = 11.2, 7.5, 5.4, 1.9 Hz, 1H), 3.93 (d, *J* = 5.6 Hz, 2H), 3.72–3.82 (m, 1H), 3.34–3.48 (m, 2H), 2.91 (ddd, *J* = 14.4, 8.0, 3.9 Hz, 1H), 2.82 (ddd, *J* = 14.5, 6.7, 3.9 Hz, 1H), 2.68–2.78 (m, 2H), 2.55 (dd, *J* = 15.7, 7.7 Hz, 1H), 2.44 (dd, *J* = 15.7, 5.4 Hz, 1H), 2.34 (dt, *J* = 13.7, 2.1 Hz, 1H), 2.16 (dt, *J* = 13.9, 2.1 Hz, 1H), 1.90–2.02 (m, 2H), 1.46–1.74 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 134.9, 116.9, 72.6, 71.7, 70.0, 69.3, 47.7, 43.0, 42.4, 40.5, 32.2, 25.9, 25.8, 25.7, 25.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{16}H_{27}O_4S_2$ 347.1345; found 347.1348.

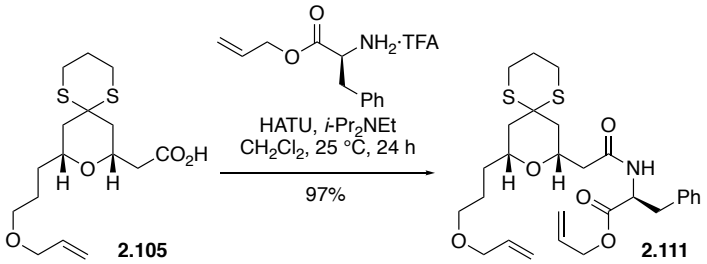


Synthesized from **2.105** (30 mg, 0.0866 mmol) and allylamine (13 μL, 0.173 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.109** (32 mg, 95%) as colorless oil: $[\alpha]^{25}_{D}$ –3.4 (*c* 0.4, $CHCl_3$); IR (neat): ν_{max} = 3300, 2939, 2857, 2359, 1643, 1541, 1093, 922 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (s, 1H), 5.75–5.95 (m, 2H), 5.24 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.13–5.20 (m, 2H), 5.10 (dd, *J* = 10.3, 1.2 Hz, 1H), 4.07–4.16 (m, 1H), 3.93 (d, *J* = 5.5 Hz, 2H), 3.78–3.90 (m, 3H), 3.35–3.47 (m, 2H), 2.93 (ddd, *J* = 12.3, 8.6, 3.3 Hz, 1H), 2.81 (ddd, *J* = 15.1, 7.5, 3.6 Hz, 1H), 2.67–2.78 (m, 2H), 2.41 (dd, *J* = 15.4, 8.5 Hz, 1H), 2.34 (dd, *J* = 15.6, 3.3 Hz, 1H), 2.28 (d, *J* = 13.7 Hz, 1H), 2.16 (d, *J* = 13.8 Hz, 1H), 1.90–2.06 (m, 2H), 1.48–1.74 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$)

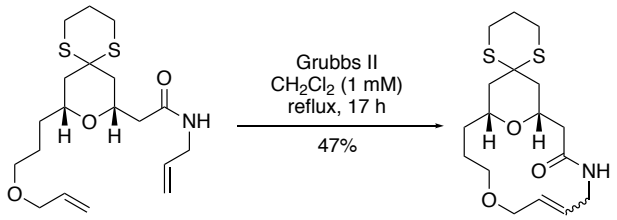
δ 170.7, 134.9, 134.4, 117.0, 116.3, 72.5, 71.9, 70.2, 70.0, 47.7, 43.3, 42.7, 41.8, 32.6, 26.0, 25.9, 25.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{19}H_{31}NO_3S_2$ 386.1818; found 386.1823.



Synthesized from **2.105** (20 mg, 0.0577 mmol) and glycine allyl ester[92] (15 mg, 0.0693 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.110** (25 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ –4.5 (*c* 0.4, $CHCl_3$); IR (neat): ν_{max} = 3225, 2936, 1749, 1660, 1534, 1423, 1379, 1191, 1090, 986 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.04 (t, *J* = 5.1 Hz, 1H), 5.81–5.98 (m, 2H), 5.28–5.36 (m, 1H), 5.21–5.28 (m, 2H), 5.15 (dd, *J* = 10.4, 1.2 Hz, 1H), 4.57–4.70 (m, 2H), 4.12–4.18 (m, 1H), 4.10 (dd, *J* = 18.4, 5.5 Hz, 1H), 3.99 (dd, *J* = 18.4, 5.2 Hz, 1H), 3.92–3.97 (m, 2H), 3.81–3.89 (m, 1H), 3.36–3.50 (m, 2H), 2.89–2.99 (m, 1H), 2.67–2.86 (m, 3H), 2.37–2.50 (m, 2H), 2.29 (d, *J* = 13.5 Hz, 1H), 2.17 (d, *J* = 13.8 Hz, 1H), 1.89–2.08 (m, 2H), 1.48–1.78 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 169.6, 135.0, 131.7, 119.0, 116.9, 72.5, 71.9, 70.2, 70.0, 66.0, 47.7, 43.2, 42.4, 42.3, 41.4, 32.5, 26.0, 25.9, 25.8, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{33}NO_5S_2$ 444.1873; found 444.1871.

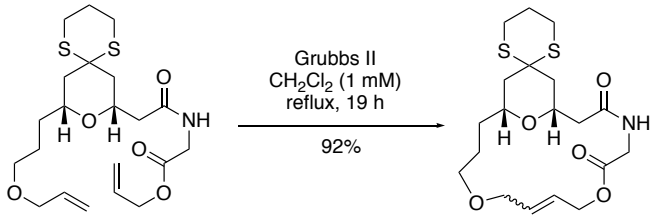


Synthesized from **2.105** (20 mg, 0.0577 mmol) and phenylalanine allyl ester[93] (21 mg, 0.0693 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 2/1) afforded **2.111** (25 mg, 97%) as colorless oil: $[\alpha]^{25}_{D}$ +17.5 (*c* 0.5, $CHCl_3$); IR (neat): ν_{max} = 3326, 2937, 2860, 2360, 1741, 1671, 1525, 1192, 1091, 929 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.33 (m, 2H), 7.18–7.24 (m, 1H), 7.11 (d, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 5.79–5.95 (m, 2H), 5.21–5.35 (m, 3H), 5.15 (d, *J* = 10.4 Hz, 1H), 4.94 (dt, *J* = 8.3, 5.9 Hz, 1H), 4.60 (d, *J* = 5.8 Hz, 2H), 4.02–4.12 (m, 1H), 3.93 (d, *J* = 5.4 Hz, 2H), 3.62–3.73 (m, 1H), 3.27–3.42 (m 2H), 3.05–3.18 (m, 2H), 2.93 (ddd, *J* = 14.6, 8.7, 3.0 Hz, 1H), 2.81 (ddd, *J* = 14.7, 7.1, 3.5 Hz, 1H), 2.68–2.78 (m, 2H), 2.32–2.45 (m, 2H), 2.25 (d, *J* = 13.7 Hz, 1H), 2.13 (d, *J* = 13.8 Hz, 1H), 1.90–2.07 (m, 2H), 1.65 (dd, *J* = 13.8, 11.4 Hz, 1H), 1.49–1.61 (m, 2H), 1.31–1.47 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.4, 136.1, 135.1, 131.6, 129.5, 128.6, 127.1, 119.1, 116.8, 72.6, 71.8, 70.2, 69.9, 66.0, 53.0, 47.7, 42.9, 42.4, 42.2, 38.1, 32.3, 26.0, 25.9, 25.8, 25.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{39}NO_5S_2$ 534.2342; found 534.2342.



2.109 **2.164**

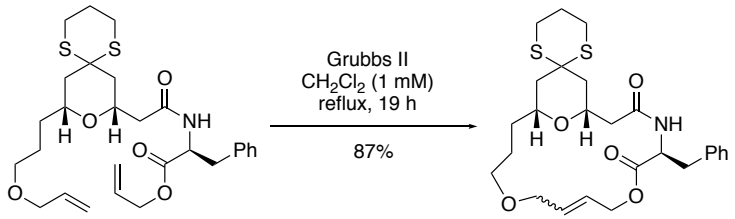
Synthesized from **2.109** (36 mg, 0.0934 mmol) according to **GP13**. Purification by silica flash chromatography (hexanes/EtOAc, 1/3) afforded an inseparable mixture of *E*/*Z* isomers (**2.164**, *E*/*Z* = 10/1, 16 mg, 47%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 6.19 (br s, 1H), 5.67–5.84 (m, 2H), 4.13–4.27 (m, 2H), 4.09 (dd, *J* = 13.3, 5.2 Hz, 1H), 3.95 (dd, *J* = 13.1, 6.1 Hz, 1H), 3.65–3.74 (m, 1H), 3.36–3.54 (m, 3H), 2.80–2.96 (m, 2H), 2.69–2.79 (m, 2H), 2.33 (dd, *J* = 14.3, 2.9 Hz, 1H), 2.21–2.30 (m, 2H), 2.07–2.15 (m, 1H), 1.94–2.04 (m, 2H), 1.65–1.74 (m, 2H), 1.61 (dd, *J* = 13.6, 11.3 Hz, 1H), 1.55 (dd, *J* = 13.7, 11.2 Hz, 1H), 1.43–1.52 (m, 1H), 1.34–1.43 (m, 1H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{17}H_{27}NO_3S_2$ 358.1505; found 358.1500.



2.110 **2.166**

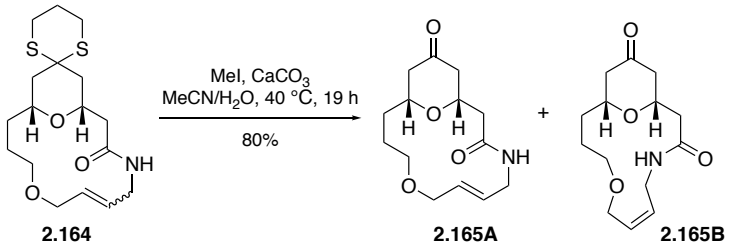
Synthesized from **2.110** (36 mg, 0.0812 mmol) according to **GP13**. Purification by silica flash chromatography (hexanes/EtOAc, 1/3) afforded an inseparable mixture of *E*/*Z* isomers (**2.166**, 31 mg, 92%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ

6.96 (br s, 1H), 5.79–5.97 (m, 2H), 4.61–4.73 (m, 2H), 4.31 (dd, *J* = 18.1, 6.4 Hz, 1H), 4.13–4.22 (m, 1H), 3.94–4.02 (m, 2H), 3.91 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.81–3.88 (m, 1H), 3.40–3.55 (m, 2H), 2.90–3.00 (m, 1H), 2.69–2.88 (m, 3H), 2.45 (dd, *J* = 15.4, 9.0 Hz, 1H), 2.38 (dd, *J* = 15.3, 2.7 Hz, 1H) 2.27 (dt, *J* = 13.6, 2.0 Hz, 1H), 2.20 (dt, *J* = 13.8, 1.9 Hz, 1H), 1.92–2.09 (m, 2H), 1.64–1.72 (m, 2H), 1.50–1.61 (m, 3H), 1.39–1.49 (m, 1H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{19}H_{29}NO_5S_2$ 416.1560; found 416.1557.



Synthesized from **2.111** (34 mg, 0.0637 mmol) according to **GP13**. Purification by silica flash chromatography (hexanes/EtOAc, 3/2) afforded an inseparable mixture of *E*/*Z* isomers (**2.167**, major:minor = 5:1, 28 mg, 87%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 7.20–7.35 (m, 4H), 7.08–7.17 (m, 2H), 5.62–5.78 (m, 2H), 5.07 (ddd, *J* = 8.7, 5.8, 4.2 Hz, 1H), 4.87 (dd, *J* = 11.6, 4.3 Hz, 1H), 4.28 (dd, *J* = 11.5, 4.4 Hz, 1H), 4.05–4.14 (m, 1H), 3.91–3.99 (m, 1H), 3.85 (dd, *J* = 13.7, 4.1 Hz, 1H), 3.55–3.66 (m, 1H), 3.42–3.50 (m, 1H), 3.26–3.34 (m, 1H), 3.23 (dd, *J* = 13.6, 4.3 Hz, 1H), 3.11 (dd, *J* = 13.6, 5.7 Hz, 1H), 2.96 (ddd, *J* = 14.3, 9.4, 3.1 Hz, 1H), 2.71–2.86 (m, 3H), 2.50 (dd, *J* = 16.1, 10.3 Hz, 1H), 2.31 (dd, *J* = 16.1, 1.6 Hz, 1H), 2.25 (dt, *J* = 13.7, 2.2 Hz, 1H), 1.95–2.11 (m, 3H), 1.62

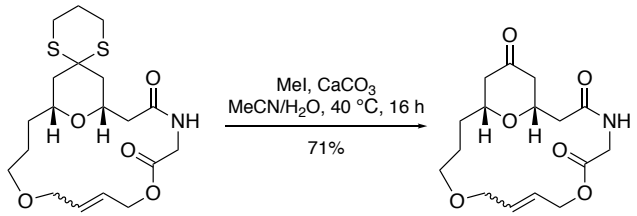
(dd, J = 13.7, 11.3 Hz, 1H), 1.51 (dd, J = 13.7, 11.4 Hz, 1H), 1.15–1.33 (m, 4H); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{35}NO_5S_2$ 506.2029; found 506.2030.



Synthesized from **2.164** (15 mg, 0.0419 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) afforded trans olefin **2.165A** (7.8 mg, 70%) with cis olefin **2.165B** (1.1 mg, 10%).

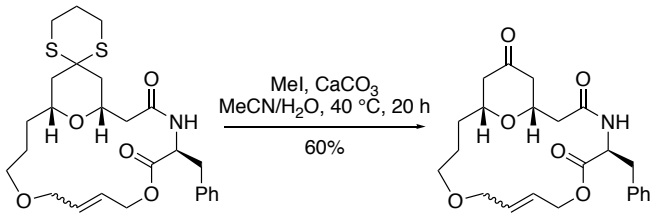
trans-alkene **2.165A**: White solid; $[\alpha]^{25}_D$ +117.1 (c 0.13, $CHCl_3$); IR (neat): ν_{max} = 3290, 2932, 2852, 1719, 1646, 1544, 1079 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.01 (br s, 1H), 5.83 (dt, J = 15.6, 6.0 Hz, 1H), 5.72 (dt, J = 15.6, 5.4 Hz, 1H), 4.32 (ddd, J = 14.9, 8.7, 5.7 Hz, 1H), 4.04–4.15 (m, 2H), 4.00 (dd, J = 13.4, 6.5 Hz, 1H), 3.49–3.60 (m, 2H), 3.34–3.45 (m, 2H), 2.45 (dd, J = 14.1, 2.6 Hz, 1H), 2.37–2.43 (m, 1H), 2.19–2.37 (m, 4H), 1.61–1.82 (m, 3H), 1.45–1.55 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.8, 169.8, 131.1, 128.7, 74.3, 71.6, 69.3, 48.4, 47.3, 44.1, 40.3, 32.1, 27.4; HRMS (m/z): $[M+H]^+$ calcd for $C_{14}H_{21}NO_4$ 326.1598; found 358.1602.

cis-alkene **2.165B**: ^1H NMR (400 MHz, $CDCl_3$) δ 6.98 (br s, 1H), 6.03–5.90 (m, 1H), 5.74 (dt, J = 11.5, 6.1 Hz, 1H), 4.24 (dt, J = 13.5, 8.1 Hz, 1H), 4.02–4.15 (m, 2H), 3.83–3.96 (m, 1H), 3.72–3.82 (m, 1H), 3.50–3.59 (m, 2H), 3.42–3.49 (m, 1H), 2.53 (dd, J = 15.6, 10.3 Hz, 1H), 2.18–2.42 (m, 6H), 1.92–2.04 (m, 1H), 1.67–1.78 (m, 2H).



2.166 **2.168**

Synthesized from **2.166** (27 mg, 0.0650 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) afforded an inseparable mixture of *E*/*Z* isomers (**2.168**, major:minor = 4:1, 15 mg, 71%): ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 6.69 (br s, 1H), 5.74–5.98 (m, 2H), 4.59–4.73 (m, 2H), 4.35 (dd, *J* = 18.1, 6.6 Hz, 1H), 3.93–4.07 (m, 3H), 3.89 (dd, *J* = 17.9, 4.0 Hz, 1H), 3.62–3.74 (m, 1H), 3.39–3.54 (m, 2H), 2.19–2.58 (m, 6H), 1.63–1.88 (m, 2H), 1.48–1.62 (m, 2H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{16}H_{23}NO_6$ 326.1598; found 326.1602.

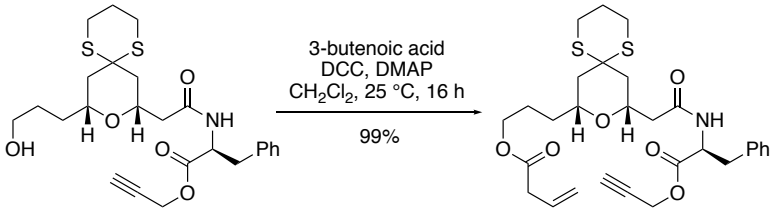


2.167 **2.169**

Synthesized from **2.167** (25 mg, 0.0494 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded an inseparable mixture of *E*/*Z* isomers (**2.169**, major:minor = 5:1, 12 mg, 60%): ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 7.19–7.30 (m, 3H), 7.07–7.13 (m, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 5.79 (dt, *J* = 15.9, 4.9 Hz, 1H), 5.73 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.03 (ddd, *J* = 8.4, 5.8, 4.4 Hz, 1H), 4.88 (dd, *J* =

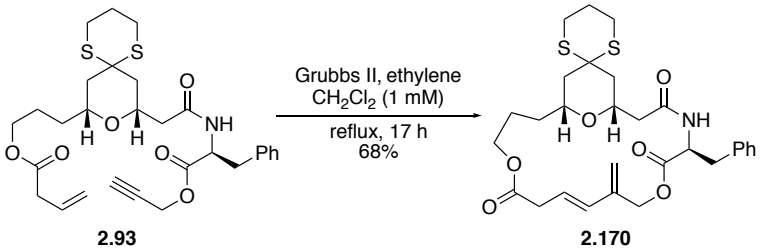
12.2, 5.4 Hz, 1H), 4.35 (dd, J = 12.2, 5.2 Hz, 1H), 3.99 (dd, J = 13.7, 4.3 Hz, 1H), 3.81–3.90 (m, 2H), 3.46–3.54 (m, 1H), 3.38–3.45 (m, 1H), 3.21–3.32 (m, 2H), 3.13 (dd, J = 13.7, 5.9 Hz, 1H), 2.59 (dd, J = 15.9, 9.8 Hz, 1H), 2.41 (dd, J = 15.9, 2.1 Hz, 1H), 2.30–2.36 (m, 3H), 2.19 (dd, J = 14.4, 11.5 Hz, 1H), 1.25–1.50 (m, 6H); HRMS (m/z): $[M+H]^+$ calcd for $C_{23}H_{29}NO_6$ 416.2068; found 410.2064.

Macrocycle from Enyne Metathesis



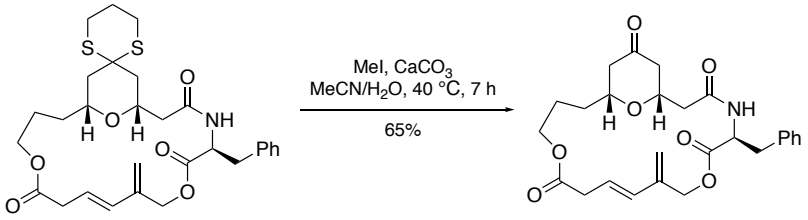
Synthesized from **2.90** (31 mg, 0.0631 mmol) according to **GP14**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.93** (35 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ +13.3 (c 0.6, $CHCl_3$); IR (neat): ν_{max} = 3291, 2931, 1735, 1665, 1530, 1174 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25–7.31 (m, 2H), 7.19–7.25 (m, 1H), 7.09–7.15 (m, 2H), 7.02 (d, J = 8.0 Hz, 1H), 5.84–5.96 (m, 1H), 5.15–5.20 (m, 1H), 5.11–5.15 (m, 1H), 4.98 (dt, J = 8.1, 5.5 Hz, 1H), 4.77 (dd, J = 15.6, 2.6 Hz, 1H), 4.68 (dd, J = 15.5, 2.7 Hz, 1H), 4.04–4.12 (m, 1H), 3.91–4.02 (m, 2H), 3.60–3.69 (m, 1H), 3.14 (d, J = 5.3 Hz, 2H), 3.06 (dd, J = 7.0, 1.4 Hz, 2H), 2.90–2.98 (m, 1H), 2.69–2.84 (m, 3H), 2.52 (t, J = 2.5 Hz, 1H), 2.33–2.46 (m, 2H), 2.26 (d, J = 13.6 Hz, 1H), 2.09 (d, J = 13.7 Hz, 1H), 1.89–2.03 (m, 2H), 1.50–1.68 (m, 3H), 1.28–1.40 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 170.9, 170.6, 136.0, 130.7, 129.9, 128.9,

127.5, 118.9, 75.9, 72.5, 70.2, 64.9, 53.0, 53.0, 47.9, 43.1, 42.5, 42.4, 39.5, 38.1, 34.2, 32.2, 26.2, 26.0, 25.3, 24.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{37}NO_6S_2$ 560.2135; found 560.2135.



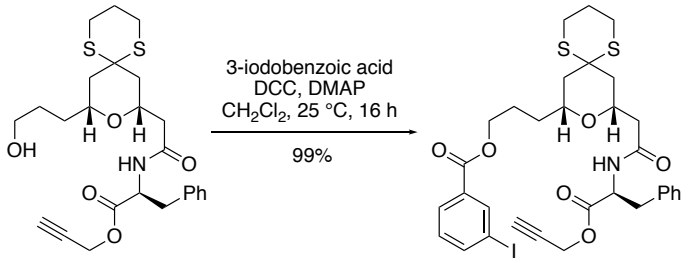
The linear precursor **2.93** (32 mg, 0.0572 mmol) was dissolved in dry CH_2Cl_2 (57 mL). Ethylene was bubbled through the solution for 10 min. Grubbs 2nd-generation catalyst (9.7 mg, 0.0114 mmol) was added and the reaction was heated to reflux under an ethylene atmosphere. After 17 h, the reaction mixture was cooled to 25 °C and dimethyl sulfoxide (~10 drops) was added to the solution. After stirring for 2 h at 25 °C, the reaction mixture was concentrated *in vacuo.* The residue was purified by silica flash chromatography (hexanes/EtOAc, 1/1) to afford **2.170** (22 mg, 68%) as colorless foam: $[\alpha]^{25}_D$ +55.1 (*c* 0.36, $CHCl_3$); IR (neat): ν_{max} = 3374, 2911, 1734, 1666, 1521, 1251, 1178, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.14–7.26 (m, 4H), 6.97–7.06 (m, 2H), 6.04–6.30 (m, 2H), 5.52 (d, *J* = 11.4 Hz, 1H), 5.40 (s, 1H), 5.36 (s, 1H), 5.00 (dt, *J* = 8.2, 5.2 Hz, 1H), 4.31 (d, *J* = 11.5 Hz, 1H), 4.12 (td, *J* = 9.8, 5.8 Hz, 1H), 3.96–4.04 (m, 1H), 3.73 (td, *J* = 9.9, 5.9 Hz, 1H), 3.52–3.60 (m, 1H), 3.21–3.29 (m, 1H), 3.03–3.17 (m, 3H), 2.90–2.98 (m, 1H), 2.49 (dd, *J* = 16.0, 9.7 Hz, 1H), 2.28 (d, *J* = 16.0 Hz, 1H), 2.22 (d, *J* = 13.7 Hz, 1H), 2.09 (d, *J* = 13.8 Hz, 1H), 1.93–2.06 (m, 2H), 1.50–1.67 (m, 3H), 1.36–1.46 (m, 1H), 1.14–1.23 (m, 1H), 0.88–1.02

(m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.6, 170.5, 140.6, 136.0, 131.6, 130.0, 128.6, 127.0, 124.9, 122.9, 72.0, 70.0, 64.3, 63.8, 52.6, 47.5, 43.4, 42.6, 42.4, 39.3, 37.7, 31.3, 26.0, 25.8, 24.5; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{37}NO_6S_2$ 560.2135; found 560.2139.

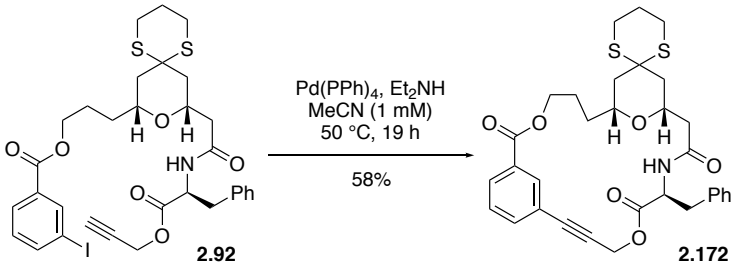


Synthesized from **2.170** (21 mg, 0.0375 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 10/1) afforded **2.171** (12 mg, 65%) as a white solid: $[\alpha]^{25}_D$ +44.8 (*c* 0.19, MeOH); IR (neat): ν_{max} = 3384, 2927, 1726, 1666, 1525, 1257, 1182, 752 cm^{-1}; ^{1}H NMR (400 MHz, CD_3CN/D_2O = 10/1) δ 7.13–7.31 (m, 3H), 7.00–7.12 (m, 2H), 6.22 (d, *J* = 16.1 Hz, 1H), 6.00 (ddd, *J* = 16.0, 8.1, 5.7 Hz, 1H), 5.33 (s, 1H), 5.32 (s, 1H), 5.15 (d, *J* = 11.7 Hz, 1H), 4.77 (t, *J* = 6.0 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.08 (ddd, *J* = 10.7, 7.4, 5.9 Hz, 1H), 3.76–3.90 (m, 2H), 3.41–3.54 (m, 1H), 3.14–3.21 (m, 1H), 3.10 (dd, *J* = 16.4, 8.1 Hz, 1H), 2.96–3.07 (m, 2H), 2.47 (dd, *J* = 16.0, 9.4 Hz, 1H), 2.33 (dd, *J* = 16.0, 2.4 Hz, 1H), 2.23–2.32 (m, 3H), 2.20 (dd, *J* = 14.8, 11.0 Hz, 1H), 1.50–1.67 (m, 2H), 1.31–1.42 (m, 1H), 1.19–1.30 (m, 1H); ^{13}C NMR (125 MHz, CD_3CN/D_2O = 10/1) δ 207.9, 172.2, 171.7, 171.4, 141.7, 137.2, 132.6, 130.6, 129.5, 127.9, 125.6, 122.7, 77.2, 74.2, 65.3, 65.1, 54.1, 47.6, 47.3, 42.8, 39.4, 38.1, 32.6, 25.2; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{31}NO_7$ 470.2173; found 470.2171.

Macrocycle from Sonogashira Cross-Coupling Reaction

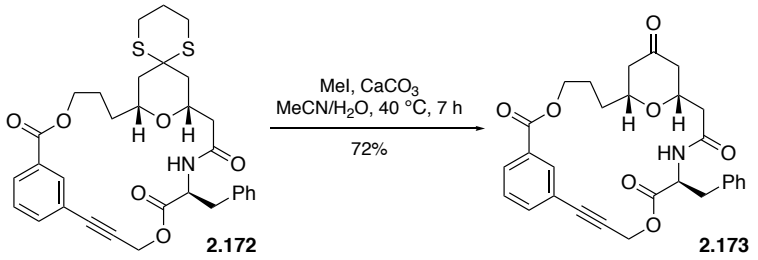


Synthesized from **2.90** (27 mg, 0.0541 mmol) and 3-iodobenzoic acid (15 mg, 0.0595 mmol) according to **GP14**. Purification by silica flash chromatography (hexanes/EtOAc, 4/3) afforded **2.92** (39 mg, 99%) as colorless oil: $[\alpha]^{25}_{D}$ +12.1 (*c* 0.7, $CHCl_3$); IR (neat): ν_{max} = 3296, 2933, 1748, 1666, 1256, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.97 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.87 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.21–7.30 (m, 3H), 7.17 (t, *J* = 7.9 Hz, 1H), 7.09–7.14 (m, 2H), 7.03 (d, *J* = 8.2 Hz, 1H), 4.99 (dt, *J* = 8.0, 5.3 Hz, 1H), 4.72 (dd, *J* = 15.6, 2.5 Hz, 1H), 4.65 (dd, *J* = 15.5, 2.5 Hz, 1H), 4.15–4.26 (m, 2H), 4.07–4.14 (m, 1H), 3.66–3.74 (m, 1H), 3.14 (d, *J* = 5.4 Hz, 2H), 2.94 (ddd, *J* = 14.4, 9.0, 3.3 Hz, 1H), 2.71–2.85 (m, 3H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.35–2.47 (m, 2H), 2.27 (d, *J* = 14.0 Hz, 1H), 2.12 (d, *J* = 13.9 Hz, 1H), 1.90–2.06 (m, 2H), 1.70–1.79 (m, 1H), 1.66 (dd, *J* = 13.8, 11.4 Hz, 1H), 1.57 (dd, *J* = 13.7, 11.2 Hz, 1H), 1.35–1.49 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.3, 165.1, 141.8, 138.4, 135.7, 132.3, 130.2, 129.7, 128.8, 128.6, 127.2, 93.9, 75.7, 72.3, 69.9, 65.4, 52.7, 52.7, 47.6, 42.9, 42.3, 42.2, 37.8, 32.0, 26.0, 25.8, 25.0, 24.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{32}H_{36}INO_6S_2$ 722.1102; found 722.1095.



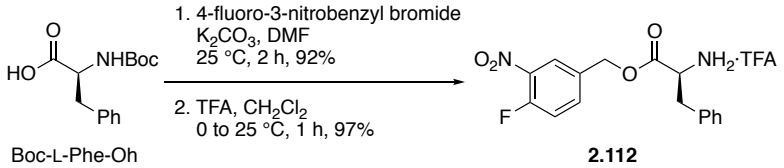
To a solution of **2.92** (19 mg, 0.0263 mmol) in MeCN (26 mL) were added tetrakis(triphenylphosphine)palladium (15 mg, 0.0132 mmol) and Et_2NH (5 μL, 0.0395 mmol) at 25 °C. The resulting mixture was heated to 50 °C and stirred for 17 h. The mixture was cooled to 25 °C and diluted with EtOAc and H_2O. The layers were separated, and the organic layer was washed with saturated aqueous NH_4Cl and brine. The resulting organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 1/1) to afford **2.172** (9.0 mg, 58%) as pale yellow oil: $[\alpha]^{25}_{D}$ +30.5 (*c* 0.32, $CHCl_3$); IR (neat): ν_{max} = 3354, 2947, 1751, 1720, 1667, 1298, 1231, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.81 (t, *J* = 1.6 Hz, 1H), 7.55 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.40–7.47 (m, 2H), 7.12–7.17 (m, 2H), 6.86–6.96 (m, 3H), 5.05–5.19 (m, 2H), 4.67 (d, *J* = 15.4 Hz, 1H), 4.56 (dt, *J* = 10.4, 3.8 Hz, 1H), 4.04–4.12 (m, 1H), 3.92–4.01 (m, 1H), 3.52–3.60 (m, 1H), 3.32 (dd, *J* = 13.9, 3.1 Hz, 1H), 3.15 (dd, *J* = 13.9, 6.0 Hz, 1H), 2.97 (ddd, *J* = 13.0, 9.6, 3.0 Hz, 1H), 2.69–2.87 (m, 3H), 2.45–2.52 (m, 2H), 2.28 (d, *J* = 13.8 Hz, 1H), 2.19 (d, *J* = 14.7 Hz, 1H), 2.02–2.12 (m, 1H), 1.90–2.01 (m, 1H), 1.77 (dd, *J* = 13.7, 11.4 Hz, 1H), 1.49–1.64 (m, 4H), 1.21–1.32 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.3, 165.5, 136.3, 135.7, 133.7, 130.6,

129.8, 129.8, 128.7, 128.6, 126.7, 122.5, 87.4, 85.2, 72.3, 70.0, 64.4, 54.3, 51.8, 47.6, 42.5, 42.2, 42.0, 37.5, 32.9, 26.1, 26.0, 25.8, 24.8; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{32}H_{35}NO_6S_2$ 594.1979; found 594.1980.

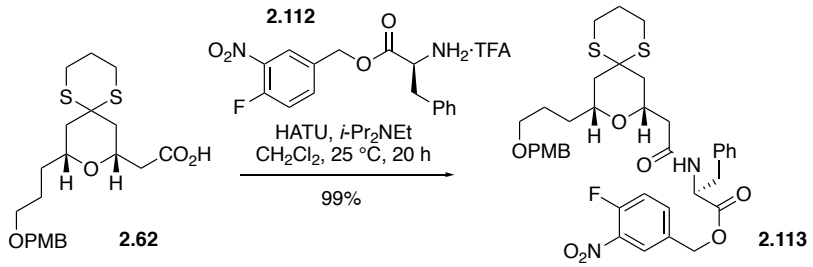


Synthesized from **2.172** (25 mg, 0.0421 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.173** (15 mg, 72%) as a white solid: $[\alpha]^{25}_D$ +37.0 (*c* 0.25, $CHCl_3$); IR (neat): ν_{max} = 3364, 2959, 1719, 1670, 1522, 1229, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 1H), 7.82 (s, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 7.10–7.18 (m, 2H), 6.85–6.97 (m, 3H), 5.10–5.18 (m, 2H), 4.70 (d, *J* = 15.4 Hz, 1H), 4.44 (ddd, *J* = 10.9, 5.8, 3.4 Hz, 1H), 4.11 (ddd, *J* = 11.0, 8.8, 3.0 Hz, 1H), 3.87 (ddt, *J* = 12.1, 6.0, 2.9 Hz, 1H), 3.38–3.46 (m, 1H), 3.35 (dd, *J* = 14.0, 3.2 Hz, 1H), 3.17 (dd, *J* = 14.0, 6.0 Hz, 1H), 2.68 (dd, *J* = 15.9, 2.7 Hz, 1H), 2.48–2.58 (m, 2H), 2.45 (d, *J* = 14.1 Hz, 1H), 2.35 (d, *J* = 14.4 Hz, 1H), 2.29 (dd, *J* = 14.1, 11.8 Hz, 1H), 1.58–1.78 (m, 2H), 1.36–1.56 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.6, 170.9, 169.2, 165.3, 136.2, 135.7, 133.9, 130.5, 129.9, 129.7, 128.8, 128.7, 126.8, 122.5, 87.4, 85.0, 73.6, 64.2, 54.4, 51.8, 46.9, 46.4, 42.4, 37.4, 33.7, 24.4; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{29}H_{29}NO_7$ 504.2017; found 504.2015.

Macrocycle from Nucleophilic Aromatic Substitution

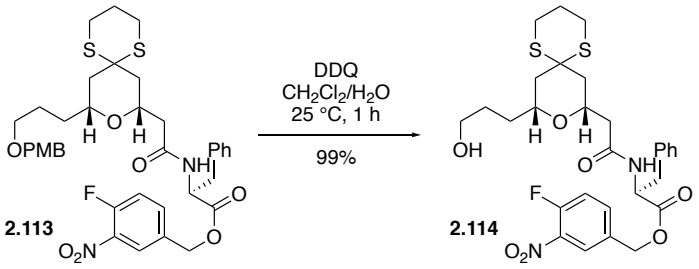


Synthesized from Boc-L-Phe-OH (106 mg, 0.400 mmol) and 4-fluoro-3-nitrobenzyl bromide (103 mg, 0.440 mmol) according to the same procedure as described for **2.88**. Compound **2.112** (149 mg, 89% for 2 steps) was obtained as pale yellow oil. **[Alkylation]** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 7.0, 2.3 Hz, 1H), 7.48 (ddd, J = 8.6, 4.2, 2.3 Hz, 1H), 7.18–7.32 (m, 4H), 7.05–7.14 (m, 2H), 5.10 (s, 3H), 4.97 (d, J = 8.1 Hz, 1H), 4.53–4.67 (m, 1H), 2.96–3.16 (m, 2H), 1.41 (s, 9H). **[Boc deprotection]** ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (dd, J = 7.1, 2.3 Hz, 1H), 7.64 (ddd, J = 8.6, 4.2, 2.3 Hz, 1H), 7.41 (dd, J = 10.9, 8.6 Hz, 1H), 723–7.35 (m, 3H), 7.14–7.23 (m, 2H), 5.25 (s, 2H), 4.39 (t, J = 7.1 Hz, 1H), 3.15–3.27 (m, 2H).



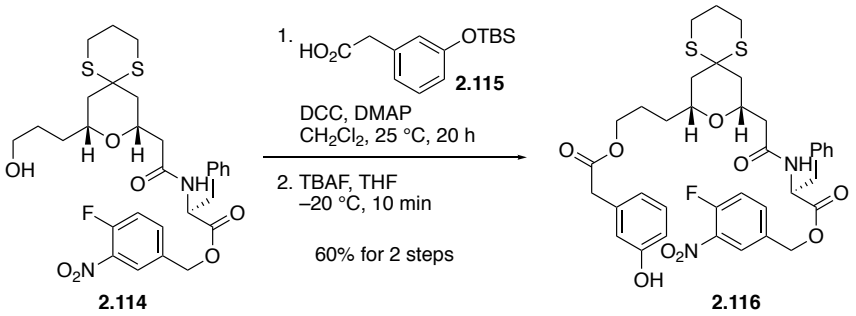
Synthesized from **2.62** (45 mg, 0.106 mmol) and amine part **2.112** (53 mg, 0.127 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 3/2) afforded **2.113** (77 mg, 99%) as yellow oil: $[\alpha]^{25}_D$ +3.6 (c 0.8, $CHCl_3$); IR (neat): ν_{max} = 3365,

2938, 1745, 1540, 1349, 1088 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 7.0, 2.3 Hz, 1H), 7.46 (ddd, J = 8.6, 4.2, 2.3 Hz, 1H), 7.17–7.25 (m, 6H), 7.10 (d, J = 8.0 Hz, 1H), 7.00–7.06 (m, 2H), 6.84 (d, J = 8.6 Hz, 2H), 5.07 (s, 2H), 4.91 (dt, J = 8.0, 6.3 Hz, 1H), 4.40 (s, 2H), 4.07 (dtd, J = 11.3, 5.7, 2.1 Hz, 1H), 3.77 (s, 3H), 3.66–3.75 (m, 1H), 3.32–3.42 (m, 2H), 3.01–3.12 (m, 2H), 2.91 (ddd, J = 14.4, 8.3, 3.5 Hz, 1H), 2.69–2.86 (m, 3H), 2.35–2.43 (m, 2H), 2.22 (dt, J = 13.6, 2.1 Hz, 1H), 2.14 (dt, J = 13.6, 2.0 Hz, 1H), 1.92–2.04 (m, 2H), 1.58–1.70 (m, 2H), 1.36–1.56 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 170.5, 159.1, 155.2 (d, $J_{C\text{-}F}$ = 266.7 Hz), 135.7, 135.4 (d, $J_{C\text{-}F}$ = 8.7 Hz), 132.4 (d, $J_{C\text{-}F}$ = 4.5 Hz), 130.6, 129.2, 128.6, 127.1, 126.0, 118.7 (d, $J_{C\text{-}F}$ = 21.1 Hz), 113.7, 72.6, 69.8, 69.7, 64.8, 55.3, 53.0, 47.6, 42.8, 42.2, 42.1, 38.1, 32.2, 25.9, 25.8, 25.7; HRMS (m/z): $[M+H]^+$ calcd for $C_{37}H_{43}FN_2O_8S_2$ 727.2518; found 727.2517.



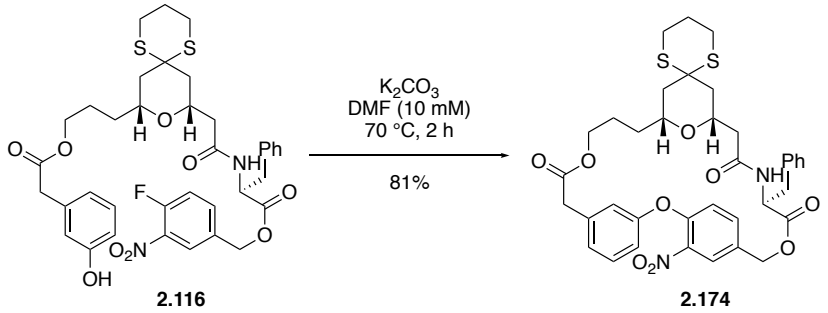
Synthesized from **2.113** (75 mg, 0.103 mmol) according to **GP4**. Purification by silica flash chromatography (hexanes/EtOAc, 1/2) afforded **2.114** (62 mg, 99%) as yellow oil: $[\alpha]^{25}_D$ +6.6 (c 0.7, $CHCl_3$); IR (neat): ν_{max} = 3358, 2940, 2360, 1744, 1653, 1540, 1350 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, J = 6.9, 2.3 Hz, 1H), 7.52 (ddd, J = 8.6, 4.2, 2.3 Hz, 1H),

7.26–7.31 (m, 1H), 7.19–7.26 (m, 3H), 7.09 (d, J = 8.0 Hz, 1H), 7.01–7.07 (m, 2H), 5.13 (s, 3H), 4.97 (dt, J = 8.1, 6.2 Hz, 1H), 4.08–4.16 (m, 1H), 3.70–3.78 (m, 1H), 3.55 (t, J = 5.7 Hz, 2H), 3.05–3.16 (m, 2H), 2.94 (ddd, J = 14.4, 8.7, 3.4 Hz, 1H), 2.71–2.86 (m, 3H), 2.37–2.48 (m, 2H), 2.26 (dt, J = 13.7, 2.2 Hz, 1H), 2.13 (dt, J = 13.8, 2.1 Hz, 1H), 1.93–2.08 (m, 2H), 1.63–1.70 (m, 1H), 1.52–1.60 (m, 2H), 1.38–1.48 (m, 3H); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{35}FN_2O_7S_2$ 607.1943; found 607.1941.



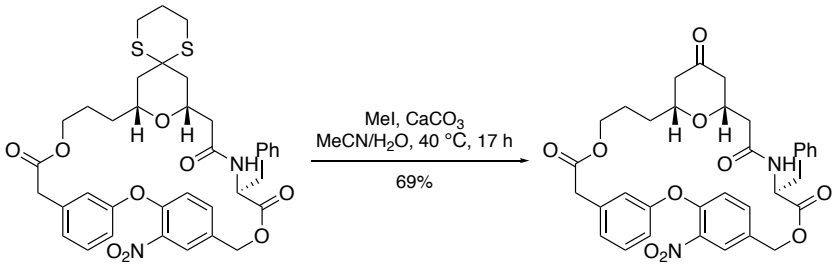
[DCC coupling] Synthesized from **2.114** (40 mg, 0.0660 mmol) and *m*-hydroxyphenylacetic acid (**2.115**)[94] (19 mg, 0.0725 mmol) according to **GP14**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, J = 6.9, 2.3 Hz, 1H), 7.50 (ddd, J = 8.6, 4.2, 2.3 Hz, 1H), 7.11–7.26 (m, 5H), 6.98–7.07 (m, 3H), 6.85 (d, J = 7.5 Hz, 1H), 6.77 (t, J = 2.1 Hz, 1H), 6.73 (dd, J = 8.0, 1.8 Hz, 1H), 5.11 (s, 3H), 4.93 (dt, J = 8.0, 6.1 Hz, 1H), 4.04–4.14 (m, 1H), 4.00 (t, J = 6.5 Hz, 2H), 3.64–3.73 (m, 1H), 3.53 (s, 2H), 3.12 (dd, J = 13.9, 5.7 Hz, 1H), 3.06 (dd, J = 13.8, 6.6 Hz, 1H), 2.88–2.97 (m, 1H), 2.69–2.86 (m, 3H), 2.34–2.46 (m, 2H), 2.25 (d, J = 13.5 Hz, 1H), 2.09 (d, J = 13.7 Hz, 1H), 1.91–2.03 (m, 2H), 1.58–1.76 (m, 3H), 1.51 (dd, J = 13.7, 11.2 Hz,

1H), 1.28–1.38 (m, 2H), 0.97 (s, 9H), 0.18 (s, 6H). **[TBS deprotection]** To a cooled (–20 °C) solution of the crude reaction mixture in THF (2 mL) was added TBAF (0.13 mL, 1.0 M in THF, 0.132 mmol) dropwise. After 10 min, the reaction mixture was allowed to warm to 0 °C and diluted with EtOAc and H_2O. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 2/3) to afford **2.116** (29 mg, 60% for 2 steps) as colorless oil: $[\alpha]^{25}_{D}$ +6.8 (*c* 0.49, $CHCl_3$); IR (neat): ν_{max} = 3338, 2940, 1734, 1650, 1540, 1349, 1278, 1178 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 6.8 Hz, 1H), 7.43–7.50 (m, 1H), 7.12–7.25 (m, 5H), 6.98–7.07 (m, 3H), 6.71–6.82 (m, 3H), 5.09 (s, 2H), 4.93 (q, *J* = 6.7 Hz, 1H), 4.07–4.16 (m, 1H), 3.94–4.07 (m, 2H), 3.66–3.75 (m, 1H), 3.54 (s, 2H), 3.12 (dd, *J* = 13.5, 6.1 Hz, 1H), 3.06 (dd, *J* = 13.8, 6.7 Hz, 1H), 2.88–2.98 (m, 1H), 2.68–2.86 (m, 3H), 2.38–2.46 (m, 2H), 2.26 (d, *J* = 13.5 Hz, 1H), 2.09 (d, *J* = 13.8 Hz, 1H) 1.89–2.05 (m, 2H), 1.57–1.69 (m, 2H), 1.43–1.55 (m, 2H), 1.28–1.42 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 171.5, 170.9, 156.5, 155.3 (d, $J_{C\text{-}F}$ = 266.3 Hz), 137.3, 135.6, 135.5, 132.3 (d, $J_{C\text{-}F}$ = 3.6 Hz), 129.9, 129.3, 128.7, 127.3, 126.1, 121.3, 118.8 (d, $J_{C\text{-}F}$ = 21.0 Hz), 116.2, 114.5, 72.3, 69.9, 65.1, 64.9, 53.2, 47.6, 42.9, 42.3, 42.2, 41.5, 38.1, 32.0, 26.0, 26.0, 25.8, 24.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{37}H_{41}FN_2O_9S_2$ 741.2310; found 741.2318.



To a solution of **2.116** (19 mg, 0.0256 mmol) in DMF (3 mL) was added K_2CO_3 (14 mg, 0.103 mmol) at 25 °C. The resulting mixture was slowly heated to 70 °C and stirred for 2 h. The reaction was cooled to 25 °C, and diluted with EtOAc and H_2O. The layers were separated, and the organic layer was washed with H_2O several times. The resulting organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 2/3) to afford **2.174** (21 mg, 81%) as colorless oil: $[\alpha]^{25}_{D}$ –0.92 (*c* 0.35, $CHCl_3$); IR (neat): ν_{max} = 3366, 2933, 1734, 1668, 1533, 1445, 1252, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 2.2 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.09–7.24 (m, 8H), 7.02–7.07 (m, 2H), 6.85 (t, *J* = 1.9 Hz), 6.80 (d, *J* = 8.6 Hz, 1H), 5.32 (d, *J* = 12.4 Hz, 1H), 5.13 (dt, *J* = 8.8, 5.2 Hz, 1H), 4.98 (d, *J* = 12.4 Hz, 1H), 3.97–4.07 (m, 2H), 3.84 (ddd, *J* = 10.8, 8.1, 5.0 Hz, 1H), 3.52–3.64 (m, 3H), 3.20 (dd, *J* = 13.8, 5.0 Hz, 1H), 3.06 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.78–2.91 (m, 2H), 2.69–2.77 (m, 2H), 2.53 (dd, *J* = 16.0, 2.9 Hz, 1H), 2.39 (dd, *J* = 16.0, 6.8 Hz, 1H), 2.16 (d, *J* = 13.6 Hz, 1H), 1.95–2.02 (m, 2H), 1.92 (d, *J* = 13.7 Hz, 1H), 1.71 (dd, *J* = 13.7, 11.5 Hz, 1H), 1.50–1.60 (m, 1H), 1.27–1.37 (m, 1H), 1.12–1.22 (m, 2H), 0.85–0.96 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.8, 170.2, 155.6, 150.9, 140.8, 136.4, 136.1, 134.6, 130.8, 130.8, 129.6, 128.6, 127.2, 126.5, 126.2,

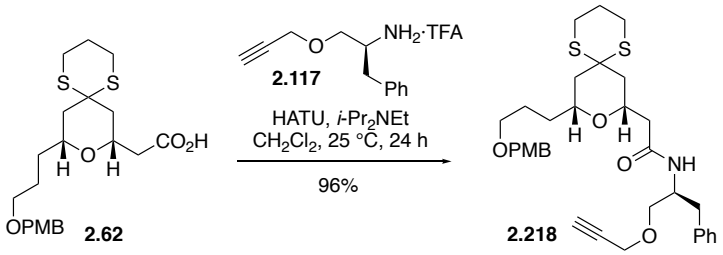
120.2, 119.5, 118.8, 72.5, 69.7, 65.6, 65.1, 52.6, 47.6, 42.4, 41.9, 41.8, 41.5, 38.2, 32.2, 26.0, 25.7, 24.7, 24.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{37}H_{40}N_2O_9S_2$ 721.2248; found 721.2234.



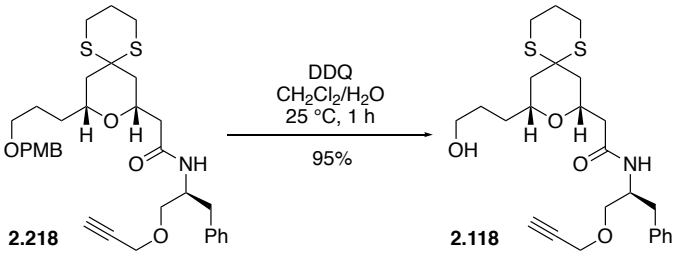
2.174 **2.175**

Synthesized from **2.174** (35 mg, 0.0486 mmol) according to **GP7**. Purification by silica flash chromatography (CH2Cl2/MeOH, 40/1) afforded **2.175** (21 mg, 69%) as a white solid: $[\alpha]^{25}_{D}$ +6.7 (*c* 0.3, $CHCl_3$); IR (neat): ν_{max} = 3373, 2958, 1727, 1669, 1532, 1250, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 2.1 Hz, 1H), 7.36–7.43 (m, 2H), 7.18–7.24 (m, 3H), 7.16 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 6.99–7.04 (m, 2H), 6.91–6.97 (m, 2H), 6.75–6.80 (m, 1H), 5.17 (d, *J* = 12.3 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 5.02 (dt, *J* = 8.1, 5.6 Hz, 1H), 3.90–4.01 (m, 2H), 3.78–3.86 (m, 1H), 3.56 (s, 3H), 3.37–3.46 (m, 1H), 3.19 (dd, *J* = 14.1, 5.4 Hz, 1H), 3.13 (dd, *J* = 14.1, 5.9 Hz, 1H), 2.48–2.58 (m, 2H), 2.39 (dd, *J* = 14.6, 11.1 Hz, 1H), 2.28–2.35 (m, 1H), 2.25 (d, *J* = 14.2 Hz, 1H), 2.09 (dd, *J* = 14.2, 11.8 Hz, 1H), 153–1.64 (m, 1H), 1.27–1.42 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.2, 171.2, 170.8, 169.4, 156.1, 150.7, 141.3, 136.2, 136.0, 134.9, 131.1, 130.7, 129.4, 128.6, 127.3, 126.3, 125.9, 121.0, 119.2, 118.3, 73.6, 66.0, 64.8, 52.8, 46.9, 46.5, 42.5, 41.6, 37.8, 32.5, 24.3; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{34}H_{34}N_2O_{10}$ 631.2286; found 631.2282.

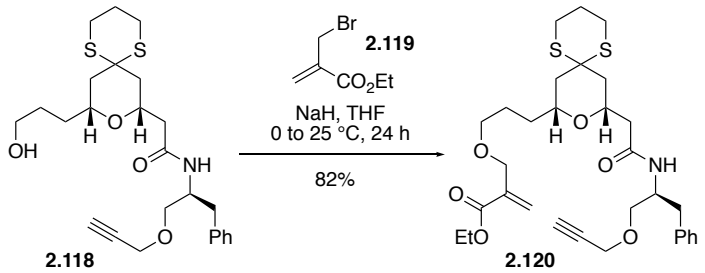
Macrocycle from Pyridone Synthesis



Synthesized from **2.62** (100 mg, 0.234 mmol) and phenylalaninol ether (**2.117**)[73] (81 mg, 0.281 mmol) according to **GP3**. Purification by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.218** (135 mg, 96%) as colorless oil: $[\alpha]^{25}_{D}$ –96.4 (*c* 2.4, $CHCl_3$); IR (neat): ν_{max} = 3285, 2934, 2858, 1658, 1512, 1246, 1089, 1033, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.31 (m, 2H), 7.15–7.26 (m, 5H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 4.42 (s, 2H), 4.28–4.36 (m, 1H), 4.14 (dd, *J* = 15.8, 2.4 Hz, 1H), 4.09 (dd, *J* = 15.8, 2.4 Hz, 1H), 4.01–4.07 (m, 1H), 3.68–3.89 (m, 4H), 3.34–3.52 (m, 4H), 2.93 (ddd, *J* = 14.3, 8.7, 3.4 Hz, 1H), 2.70–2.88 (m, 5H), 2.40 (t, *J* = 2.4 Hz, 1H), 2.30–2.39 (m, 2H), 2.26 (d, *J* = 13.7 Hz, 1H), 2.16 (d, *J* = 13.8 Hz, 1H), 1.87–2.10 (m, 3H), 1.70–1.79 (m, 1H), 1.60–1.68 (m, 1H), 1.48–1.60 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 159.1, 137.9, 130.6, 129.5, 129.2, 128.3, 126.4, 113.7, 79.5, 74.9, 72.5, 72.4, 69.9, 69.7, 69.6, 58.3, 55.2, 49.6, 47.6, 43.0, 42.6, 42.3, 37.4, 32.4, 25.9, 25.8, 25.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{33}H_{43}NO_5S_2$ 598.2655; found 598.2663.

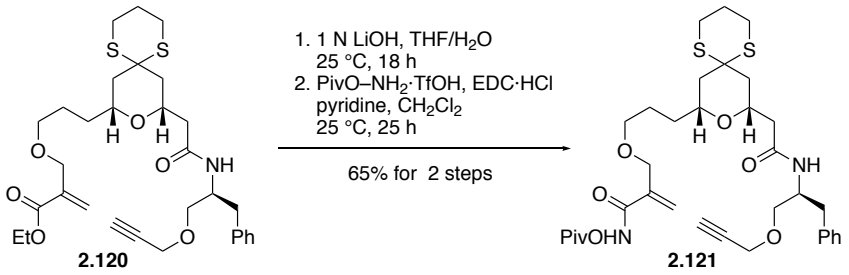


Synthesized from **2.218** (137 mg, 0.229 mmol) according to **GP4**. Purification by silica flash chromatography (hexanes/EtOAc, 1/2) afforded **2.118** (104 mg, 95%) as colorless oil: $[\alpha]^{25}_{D}$ –20.8 (*c* 1.7, $CHCl_3$); IR (neat): ν_{max} = 3288, 2940, 2907, 2865, 1647, 1537, 1090, 1066, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.37 (m, 2H), 7.15–7.26 (m, 3H), 6.78 (d, *J* = 8.8 Hz, 1H), 4.28–4.38 (m, 1H), 4.12–4.22 (m, 2H), 4.09 (dtd, *J* = 9.3, 5.3, 2.4 Hz, 1H), 3.82 (dtd, *J* = 11.8, 6.0, 1.9 Hz, 1H), 3.63 (t, *J* = 6.1 Hz, 2H), 3.47 (dd, *J* = 9.3, 3.9 Hz, 1H), 3.43 (dd, *J* = 9.4, 3.6 Hz, 1H), 2.94 (ddd, *J* = 14.3, 9.0, 3.2 Hz, 1H), 2.69–2.90 (m, 5H), 2.44 (t, *J* = 2.4 Hz, 1H), 2.32–2.43 (m, 2H), 2.29 (d, *J* = 13.8 Hz, 1H), 2.11–2.18 (m, 2H), 1.90–2.07 (m, 2H), 1.50–1.74 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 137.8, 129.4, 128.4, 126.4, 79.4, 74.9, 72.4, 70.0, 69.6, 62.5, 58.4, 49.7, 47.6, 43.0, 42.5, 42.2, 37.3, 32.0, 28.7, 25.9, 25.8, 25.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{25}H_{35}NO_4S_2$ 478.2080; found 478.2088.



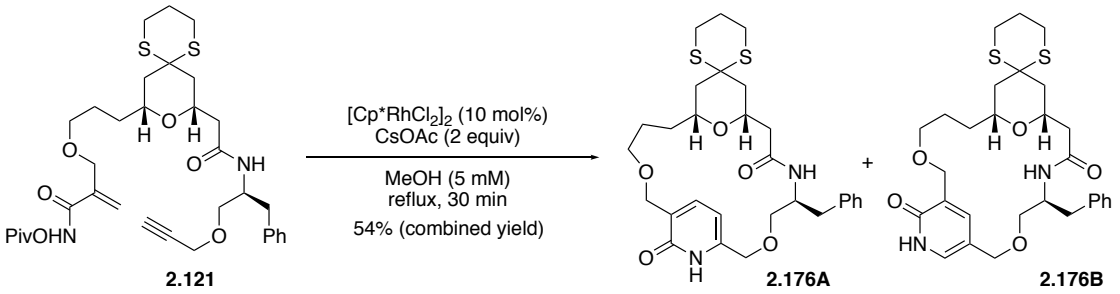
To a cooled (0 °C) solution of **2.118** (104 mg, 0.218 mmol) in THF (2.2 mL) was added NaH (17 mg, 60% dispersion in mineral oil, 0.435 mmol). After the reaction mixture was stirred for 30 min at the same temperature, ethyl 2-bromomethylacrylate (**2.119**) (0.15 mL, 1.09 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to warm to 25 °C and stirred for 20 h. The mixture was diluted with EtOAc and quenched with saturated aqueous NH_4Cl. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 3/2) to afford **2.120** (105 mg, 82%) as colorless oil: $[\alpha]^{25}_{D}$ –15.3 (*c* 1.7, $CHCl_3$); IR (neat): ν_{max} = 3288, 2907, 2864, 1713, 1666, 1530, 1273, 1103, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27–7.34 (m, 2H), 7.16–7.24 (m, 3H), 6.77 (d, *J* = 8.9 Hz, 1H), 6.26 (q, *J* = 1.5 Hz, 1H), 5.83 (q, *J* = 1.7 Hz, 1H), 4.28–4.36 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.16 (t, *J* = 1.6 Hz, 2H), 4.12–4.15 (m, 2H), 4.02–4.08 (m, 1H), 3.74–3.83 (m, 1H), 3.39–3.52 (m, 4H), 2.93 (ddd, *J* = 14.4, 8.8, 3.3 Hz, 1H), 2.70–2.86 (m, 5H), 2.42 (t, *J* = 2.4 Hz, 1H), 2.30–2.38 (m, 2H), 2.26 (dt, *J* = 13.8, 2.1 Hz, 1H), 2.15 (dt, *J* = 13.8, 2.1 Hz, 1H), 1.90–2.06 (m, 2H), 1.70–1.80 (m, 1H), 1.50–1.68 (m, 5H), 1.29 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

170.3, 166.0, 138.0, 137.6, 129.6, 128.5, 126.5, 125.4, 79.6, 75.0, 72.5, 70.8, 70.1, 69.7, 69.0, 60.8, 58.5, 49.8, 47.7, 43.2, 42.7, 42.3, 37.6, 32.5, 26.0, 25.9, 25.8, 25.8, 14.3; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{31}H_{43}NO_6S_2$ 590.2605; found 590.2593.

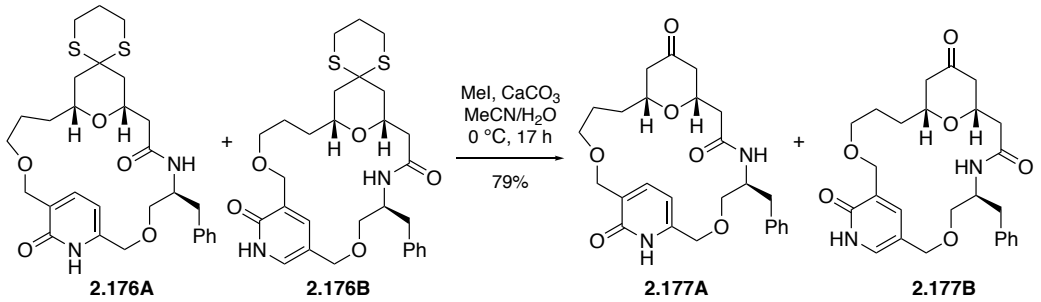


[Hydrolysis] Synthesized from **2.120** (50 mg, 0.0848 mmol) according to **GP5**. The crude reaction mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.32 (m, 5H), 6.79 (d, *J* = 8.8 Hz, 1H), 6.37 (d, *J* = 1.4 Hz, 1H), 5.91 (d, *J* = 1.6 Hz, 1H), 4.28–4.35 (m, 1H), 4.14–4.20 (m, 2H), 4.13 (t, *J* = 2.8 Hz, 2H), 4.03–4.08 (m, 1H), 3.75–3.83 (m, 1H), 3.46–3.54 (m, 2H), 3.45 (dd, *J* = 9.5, 3.8 Hz, 1H), 3.41 (dd, *J* = 9.3, 3.5 Hz, 1H), 2.65–2.97 (m, 6H), 2.41 (t, *J* = 2.3 Hz, 1H), 2.32–2.39 (m, 2H), 2.23–2.29 (m, 1H), 2.11–2.18 (m, 1H), 1.90–2.04 (m, 2H), 1.69–1.78 (m, 1H), 1.46–1.66 (m, 5H). **[EDC coupling]** To a solution of the crude residue in CH_2Cl_2 (2 mL) were added *O*-pivaloylhydroxylamine[95] (34 mg, 0.127 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 32 mg, 0.169 mmol), and pyridine (0.07 mL, 0.847 mmol) at 25 °C. After stirring for 25 h at the same temperature, the reaction mixture was diluted with EtOAc and neutralized by 1 M citric acid. The

layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 1/1) to afford **2.121** (36 mg, 65% for 2 steps) as colorless oil: $[\alpha]^{25}_{D}$ –10.3 (*c* 0.62, $CHCl_3$); IR (neat): ν_{max} = 2916, 1654, 1597, 1286, 1175, 1005, 809 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 7.26–7.30 (m, 2H), 7.15–7.23 (m, 3H), 6.72 (d, *J* = 8.8 Hz, 1H), 6.31 (d, *J* = 1.3 Hz, 1H), 5.62 (d, *J* = 1.4 Hz, 1H), 4.26–4.36 (m, 1H), 4.21 (s, 2H), 4.09–4.18 (m, 2H), 4.02–4.09 (m, 1H), 3.74–3.82 (m, 1H), 3.45–3.54 (m, 2H), 3.44 (dd, *J* = 9.4, 3.9 Hz, 1H), 3.40 (dd, *J* = 9.3, 3.6 Hz, 1H), 2.92 (ddd, *J* = 14.7, 8.8, 3.5 Hz, 1H), 2.67–2.87 (m, 5H), 2.42 (t, *J* = 2.3 Hz, 1H), 2.29–2.41 (m, 2H), 2.26 (d, *J* = 13.7 Hz, 1H), 2.15 (d, *J* = 13.8 Hz, 1H), 1.91–2.06 (m, 2H), 1.77 (dq, *J* = 13.4, 6.6 Hz, 1H), 1.57–1.69 (m, 2H), 1.41–1.57 (m, 3H), 1.31 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4, 170.2, 164.2, 138.0, 136.4, 129.6, 128.5, 127.1, 126.6, 79.6, 75.0, 72.4, 70.6, 70.4, 70.2, 69.7, 58.5, 49.8, 47.7, 43.1, 42.4, 38.5, 37.5, 32.2, 27.2, 26.0, 25.9, 25.8, 25.6; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{34}H_{48}N_2O_7S_2$ 661.2976; found 661.2983.

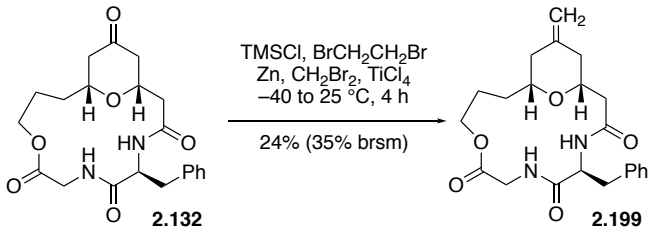


To a vigorously solution of $[Cp^*RhCl_2]_2$ (3.6 mg, 5.14 μmol, 10 mol %) and CsOAc (20 mg, 0.103 mmol) in refluxing MeOH (2.0 mL) was added a solution of hydroxamate **2.121** (33 mg, 0.0514 mmol) in MeOH (0.5 mL) dropwise over 30 min. Once the addition was complete, the reaction mixture was refluxed for additional 30 min, cooled to 25 °C, and then concentrated. The residue was dissolved in CH_2Cl_2 and the resulting solution was washed with a saturated aqueous $NaHCO_3$ and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 30/1) to afford an inseparable mixture of regioisomers (**2.176A** and **2.176B**, 15 mg, 54% combined yield) as orange oil. The structures of the isomeric pyridines were determined by NMR spectroscopic analysis.[72, 96]: ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 7.37 (d, J = 6.7 Hz, 1H), 7.27–7.33 (m, 2H), 7.13–7.22 (m, 3H), 6.97 (d, J = 8.5 Hz, 1H), 6.20 (d, J = 6.7 Hz, 1H), 4.52 (d, J = 12.4 Hz, 1H), 4.37 (d, J = 11.7 Hz, 1H), 4.32 (d, J = 11.9 Hz, 1H), 4.20–4.30 (m, 2H), 3.81–3.90 (m, 1H), 3.48–3.60 (m, 3H), 3.37 (d, J = 4.4 Hz), 2.62–2.99 (m, 6H), 2.12–2.27 (m, 3H), 1.80–2.08 (m, 4H), 1.52–1.72 (m, 3H), 1.37–1.49 (m, 1H), 1.22–1.34 (m, 1H); HRMS (m/z): $[M+H]^+$ calcd for $C_{29}H_{38}N_2O_5S_2$ 559.2295; found 559.2295.



Synthesized from regioisomeric mixture **2.176A** and **2.176B** (15 mg, 0.0268 mmol) according to **GP7**. Purification by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded an inseparable mixture of regioisomers (**2.177A** and **2.177B**, 9.7 mg, 77%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Major isomer) δ 7.49 (d, J = 8.9 Hz, 1H), 7.27–7.41 (m, 3H), 7.11–7.25 (m, 3H), 6.20 (d, J = 6.7 Hz, 1H), 4.56–4.67 (m, 2H), 4.28–4.38 (m, 1H), 4.18 (d, J = 12.7 Hz, 1H), 4.09 (d, J = 12.2 Hz, 1H), 3.82–3.93 (m, 1H), 3.56–3.66 (m, 2H), 3.26–3.46 (m, 3H), 2.86–3.00 (m, 2H), 2.50–2.57 (m, 1H), 2.27–2.33 (m, 1H), 2.12–2.24 (m, 2H), 1.89–2.01 (m, 2H), 1.67–1.81 (m, 2H), 1.40–1.55 (m, 2H); HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{32}N_2O_6$ 469.2333; found 469.2327.

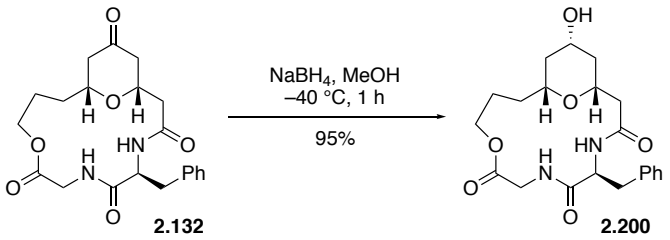
Macrocycles from Appendage Diversification



Activated zinc dust (285 mg, 4.37 mmol) was dried under high vacuum in a 20 mL vial fitted with a septum cap (in a 50 °C sand bath). Upon cooling, the vial was flushed with

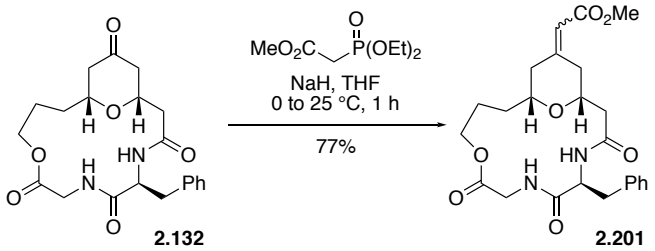
N_2. Anhydrous THF (7 mL), 1,2-dibromoethane (19 μL, 0.220 mmol), and TMSCl (5.6 μL, 0.044 mmol) were added to the vial. After stirring for 10 min, dibromomethane (0.10 mL, 1.42 mmol) was added. The mixture was cooled to –40 °C and $TiCl_4$ (1.01 mL, 1.0 M in CH_2Cl_2, 1.01 mmol) was added slowly, dropwise over 10 min. The resulting ash-blue suspension was warmed to 0 °C over 45 min then stirred at 0 °C for 2 days. An aliquot of the Lombardo reagent thus-obtained (452 μL, 0.0992 mmol, 4 eq) was added to ketone **2.132** (10 mg, 0.0248 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was allowed to warm to 25 °C and stirred for additional 4 h. The mixture was diluted with EtOAc and quenched with saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) to afford **33a** (2.4 mg, 24%, 35% brsm) as a white solid with the recovered starting material **2.199** (3.0 mg, 30%): $[\alpha]^{25}_{D}$ +26.4 (*c* 0.07, $CHCl_3$); IR (neat): ν_{max} = 3312, 2929, 1734, 1646, 1540, 1289 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09–7.33 (m, 6H), 6.46 (d, *J* = 7.2 Hz, 1H), 4.95 (ddd, *J* = 10.1, 6.7, 3.7 Hz, 1H), 4.78 (s, 2H), 4.55 (dd, *J* = 18.0, 8.5 Hz, 1H), 4.13 (dt, *J* = 11.0, 4.2 Hz, 1H), 3.80–3.91 (m, 1H), 3.54–3.68 (m, 2H), 3.51 (dd, *J* = 13.6, 3.6 Hz, 1H), 3.06–3.16 (m, 1H), 2.91 (dd, *J* = 13.5, 6.7 Hz, 1H), 2.51 (dd, *J* = 16.7, 9.6 Hz, 1H), 2.44 (dd, *J* = 16.9, 3.3 Hz, 1H), 2.15–2.23 (m, 1H), 1.91–2.06 (m, 3H), 1.67–1.77 (m, 1H), 1.47–1.64 (m, 2H), 1.32–1.40 (m, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 171.9, 170.4, 169.7, 145.6, 138.9, 130.5,

129.2, 127.4, 109.4, 77.5, 75.8, 65.5, 55.0, 43.1, 42.8, 40.9, 40.3, 38.4, 32.8, 23.3; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{28}N_2O_5$ 401.2071; found 401.2067.



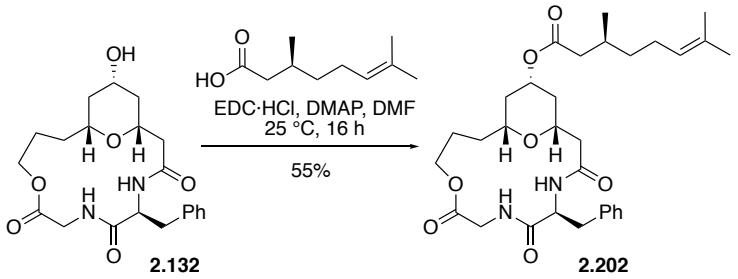
To a cooled (–40 °C) suspension of **2.132** (151 mg, 0.375 mmol) in MeOH (72 mL) was added sodium borohydride (21 mg, 0.563 mmol). After stirring for 1 h at the same temperature, the reaction mixture was slowly warm to 0 °C, quenched with saturated aqueous NH_4Cl, and concentrated. The residue was dissolved in CH_2Cl_2, and the resulting solution was washed with H_2O. The aqueous layer was extracted with CH_2Cl_2 several times. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford **2.200** (144 mg, 95%) as a white solid: $[\alpha]^{25}_{D}$ –12.0 (*c* 0.23, CH_3CN/MeOH = 1/1); IR (neat): ν_{max} = 3328, 2933, 1731, 1640, 1536, 1250, 999, 750 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.29 (t, *J* = 5.7 Hz, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.20–7.28 (m, 2H), 7.10–7.19 (m, 3H), 4.69 (d, *J* = 4.7 Hz, 1H), 4.56 (td, *J* = 8.2, 6.5 Hz, 1H), 3.85–3.96 (m, 2H), 3.76 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.65 (dd, *J* = 16.9, 5.8 Hz, 1H), 3.52–3.62 (m, 2H), 3.17–3.26 (m, 1H), 2.89 (dd, *J* = 13.5, 6.5 Hz, 1H), 2.77 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.39 (dd, *J* = 13.6, 3.5 Hz, 1H), 1.94 (dd, *J* = 13.7, 8.3 Hz, 1H), 1.53–1.75 (m, 4H), 1.32–1.43 (m, 2H), 1.15 (q, *J* = 11.4 Hz, 1H), 0.88 (q, *J* = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.5,

169.5, 169.3, 137.8, 129.0, 128.1, 126.2, 72.5, 72.1, 66.3, 64.6, 64.5, 53.5, 42.1, 41.9, 40.8, 40.7, 37.9, 32.2, 22.1; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{28}N_2O_6$ 405.2020; found 405.2022.



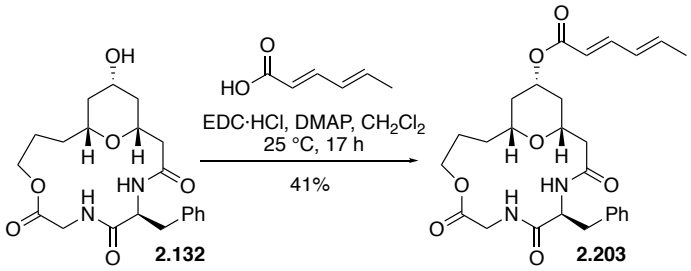
To a cooled (0 °C) suspension of NaH (5.4 mg, 60% dispersion in mineral oil, 0.134 mmol) in THF (1 mL) was added methyl diethylphosphonoacetate (19 μL, 0.134 mmol) dropwise. After 10 min, a suspension of **2.132** (18 mg, 0.0447 mmol) in THF (1 mL) was added dropwise at 0 °C. After stirring for 10 min at 0 °C, the reaction mixture was allowed to warm to 25 °C and stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl, and diluted with EtOAc and H_2O. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 80/1) to afford **2.201** (16 mg, 77%) as a white solid: $[\alpha]^{25}_D$ +1.3 (*c* 0.26, CH_3CN); IR (neat): ν_{max} = 3302, 2950, 1715, 1646, 1436, 1223, 1168, 1151, 752 cm^{-1}; ^1H NMR (400 MHz, CD_3CN/D_2O = 10/1) δ 7.14–7.32 (m, 5H), 5.70 (d, *J* = 2.0 Hz, 1H), 4.62 (t, *J* = 6.9 Hz, 1H), 4.05 (ddd, *J* = 10.3, 6.8, 3.0 Hz, 1H), 3.86–3.95 (m, 2H), 3.74 (dd, *J* = 17.4, 3.6 Hz, 1H), 3.61–3.69 (m, 4H), 3.30 (dddd, *J* = 22.9, 10.6, 5.6, 2.7 Hz, 1H), 3.00 (dd, *J* = 13.7, 6.3 Hz, 1H), 2.93 (dd, *J* = 13.8, 7.7 Hz, 1H), 2.45 (td, *J* = 14.9, 3.1 Hz, 1H),

2.09–2.28 (m, 3H), 1.97–2.06 (m, 1H), 1.68–1.84 (m, 1H), 1.55–1.67 (m, 1H), 1.45–1.54 (m, 1H), 1.34–1.44 (m, 1H); ^{13}C NMR (125 MHz, CD_3CN/D_2O = 10/1) δ 171.9, 171.3, 169.8, 167.2, 157.7, 137.2, 129.4, 128.4, 126.7, 114.2, 76.0, 74.7, 74.2, 65.7, 54.2, 50.9, 41.7, 41.3, 37.2, 35.0, 32.4, 22.0; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{24}H_{30}N_2O_7$ 459.2126; found 459.2121.



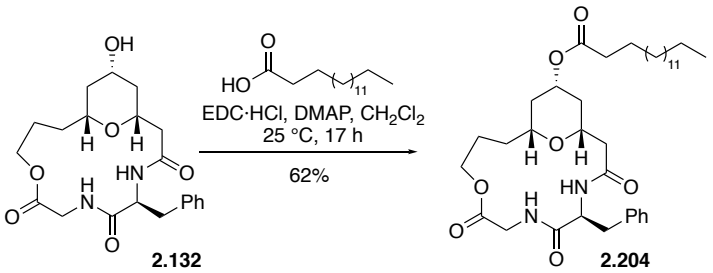
To a solution of **2.132** (16 mg, 0.0396 mmol) in DMF (1.3 mL) were added (*S*)-citronellic acid[97] (34 mg, 0.198 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 15 mg, 0.0791 mmol), and DMAP (1 mg) at 25 °C. After stirring for 16 h at the same temperature, the reaction mixture was diluted with EtOAc and H_2O. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.202** (12 mg, 55%) as a white solid: $[\alpha]^{25}_D$ +11.1 (*c* 0.2, $CHCl_3$); IR (neat): ν_{max} = 3300, 2924, 1730, 1637, 1541, 1296, 1224 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.36 (m, 3H), 7.14–7.21 (m, 2H), 7.04 (d, *J* = 9.5 Hz, 1H), 6.44 (d, *J* = 7.4 Hz, 1H), 5.07 (t, *J* = 7.0 Hz, 1H), 4.93 (ddd, *J* = 10.1, 7.0, 3.7 Hz, 1H), 4.80–4.89 (m, 1H), 4.54 (dd, *J* = 18.0, 8.4 Hz, 1H), 4.10 (ddd, *J* = 10.7, 5.0, 3.2 Hz, 1H), 3.87 (td, *J* = 10.6, 2.2 Hz, 1H),

3.62–3.68 (m, 1H), 3.60 (dd, *J* = 18.1, 2.5 Hz, 1H), 3.51 (dd, *J* = 13.6, 3.7 Hz, 1H), 3.14–3.23 (m, 1H), 2.91 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.49 (dd, *J* = 16.6, 9.3 Hz, 1H), 2.42 (dd, *J* = 16.6, 3.3 Hz, 1H), 2.28 (dd, *J* = 14.5, 5.9 Hz, 1H), 2.08 (dd, *J* = 14.6, 8.1 Hz, 1H), 1.88–2.02 (m, 4H), 1.75–1.82 (m, 1H), 1.64–1.72 (m, 4H), 1.49–1.62 (m, 4H), 1.18–1.39 (m, 5H), 1.07–1.17 (m, 1H), 0.93 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 170.2, 169.9, 168.0, 136.7, 131.6, 129.8, 128.6, 127.0, 124.2, 73.9, 71.2, 69.2, 64.6, 53.1, 42.3, 42.0, 41.9, 36.7, 36.6, 34.6, 30.1, 25.7, 25.4, 21.5, 19.6, 17.7; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{31}H_{44}N_2O_7$ 557.3221; found 557.3215.



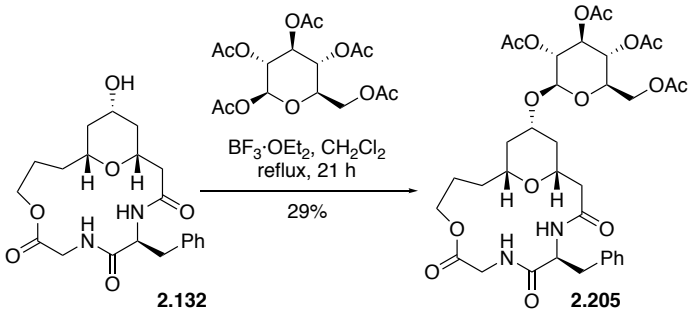
To a solution of **2.132** (7 mg, 0.0173 mmol) in CH_2Cl_2 (0.60 mL) were added sorbic acid (9.7 mg, 0.0865 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 17 mg, 0.0865 mmol), and DMAP (1 mg) at 25 °C. After stirring for 17 h at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 20/1) afforded **2.203** (3.5 mg, 55%) as a white solid: $[\alpha]^{25}_D$ +16.6 (*c* 0.1, $CHCl_3$); IR (neat) : ν_{max} = 3297, 2926, 1733, 1643, 1541, 1245, 1140, 1003 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.32 (m, 2H), 7.14–7.25 (m,

4H), 7.04 (d, *J* = 9.3 Hz, 1H), 6.39 (d, *J* = 8.5 Hz, 1H), 6.08–6.25 (m, 2H), 5.71 (d, *J* = 15.3 Hz, 1H), 4.85–5.01 (m, 2H), 4.55 (dd, *J* = 18.0, 8.5 Hz, 1H), 4.11 (ddd, *J* = 10.6, 4.6, 3.2 Hz, 1H), 3.86 (td, *J* = 10.5, 2.1 Hz, 1H), 3.64–3.73 (m, 1H), 3.61 (dd, *J* = 18.1, 2.5 Hz, 1H), 3.54 (dd, *J* = 13.6, 3.5 Hz, 1H), 3.15–3.27 (m, 1H), 2.91 (dd, *J* = 13.5, 6.8 Hz, 1H), 2.50 (dd, *J* = 16.6, 9.5 Hz, 1H), 2.43 (dd, *J* = 16.6, 3.1 Hz, 1H), 1.94–2.02 (m, 1H), 1.78–1.91 (m, 4H), 1.48–1.60 (m, 2H), 1.27–1.41 (m, 3H), 1.04–1.15 (m, 1H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{27}H_{34}N_2O_7$ 499.2439; found 499.2434.



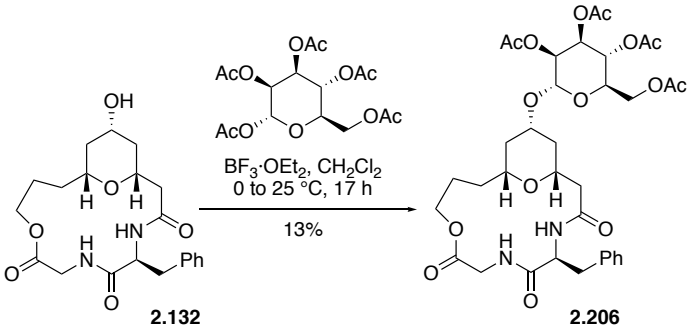
To a solution of **2.132** (7 mg, 0.0173 mmol) in CH_2Cl_2 (0.60 mL) were added palmitic acid (22 mg, 0.0865 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 17 mg, 0.0865 mmol), and DMAP (1 mg) at 25 °C. After stirring for 17 h at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexanes/EtOAc, 1/1) afforded **2.204** (6.9 mg, 62%) as a white solid: $[\alpha]^{25}_{D}$ +4.3 (*c* 0.18, $CHCl_3$); IR (neat): ν_{max} = 3296, 2921, 2851, 1731, 1637, 1542, 1224 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.33 (m, 2H), 7.21–7.25 (m, 1H), 7.13–7.20 (m, 2H), 7.03 (d, *J* = 9.5 Hz, 1H), 6.43–6.46 (m, 1H), 4.94 (ddd, *J* = 9.7, 6.8, 3.6 Hz,

1H), 4.80–4.90 (m, 1H), 4.55 (dd, *J* = 18.0, 8.3 Hz, 1H), 4.06–4.15 (m, 1H), 3.83–3.91 (m, 1H), 3.63–3.70 (m, 1H), 3.60 (d, *J* = 18.1 Hz, 1H), 3.53 (dd, *J* = 13.5, 3.5 Hz, 1H), 3.14–3.23 (m, 1H), 2.91 (dd, *J* = 13.5, 6.7 Hz, 1H), 2.50 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.42 (dd, *J* = 16.7, 2.6 Hz, 1H), 2.26 (t, *J* = 7.6 Hz, 1H), 1.91–1.98 (m, 1H), 1.75–1.82 (m, 1H), 1.50–1.60 (m, 2H), 1.05–1.40 (m, 30H), 0.88 (t, *J* = 6.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 170.2, 169.9, 168.0, 136.7, 129.8, 128.6, 127.0, 73.9, 71.2, 69.2, 64.6, 53.1, 42.3, 42.0, 36.7, 36.6, 34.6, 34.5, 31.9, 30.1, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 25.0, 22.7, 21.5, 14.1; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{37}H_{58}N_2O_7$ 643.4317; found 643.4302.



To a solution of **2.132** (5.0 mg, 0.0124 mmol) in CH_2Cl_2 (0.40 mL) were added β-D-glucose pentaacetate (7.2 mg, 0.0185 mmol) and boron trifluoride diethyl etherate (7.6 μL, 0.0618 mmol) at 25 °C in a sealed tube. The reaction was slowly heated and refluxed for 21 h. The reaction mixture was cooled to 25 °C, quenched by adding triethylamine (~3 drops), and concentrated. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 60/1) to afford **2.205** (2.6 mg, 29%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.33 (m, 2H), 7.21–7.25 (m, 1H), 7.14–7.19 (m, 2H), 7.09 (d, *J* = 9.5 Hz, 1H),

6.36 (d, J = 8.2 Hz, 1H), 5.20 (t, J = 9.0 Hz, 1H), 5.09 (t, J = 9.6 Hz, 1H), 4.86–4.98 (m, 2H), 4.62 (d, J = 8.1 Hz, 1H), 4.54 (dd, J = 18.0, 8.5 Hz, 1H), 4.26 (dd, J = 12.5, 4.6 Hz, 1H), 4.16 (dd, J = 12.1, 1.7 Hz, 1H), 4.05–4.12 (m, 1H), 3.83–3.92 (m, 1H), 3.72–3.81 (m, 1H), 3.66–3.72 (m, 1H), 3.55–3.64 (m, 2H), 3.52 (dd, J = 13.6, 2.9 Hz, 1H), 3.08–3.17 (m, 1H), 2.90 (dd, J = 13.4, 6.8 Hz, 1H), 2.50 (dd, J = 16.6, 9.2 Hz, 2H), 2.37–2.45 (m, 1H), 1.95–2.17 (m, 12H), 1.85–1.92 (m, 1H), 1.77–1.84 (m, 1H), 1.61–1.72 (m, 2H), 1.15–1.38 (m, 3H), 1.05–1.15 (m, 1H); HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{46}N_2O_{15}$ 735.2971; found 735.2957.



To a cooled (0 °C) solution of **2.132** (5.0 mg, 0.0124 mmol) in CH_2Cl_2 (0.40 mL) were added α-D-mannose pentaacetate (7.2 mg, 0.0185 mmol) and boron trifluoride diethyl etherate (7.6 μL, 0.0618 mmol). After stirring at the same temperature for 30 min, the reaction mixture was warmed to 25 °C and stirred for 17 h. The reaction mixture was quenched by adding triethylamine (~3 drops), and concentrated. The residue was purified by silica flash chromatography (CH_2Cl_2/MeOH, 40/1) to afford **2.206** (1.2 mg, 13%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.32 (m, 2H), 7.21–7.25 (m, 1H), 7.14–7.20 (m, 2H), 7.07 (d, J = 9.1 Hz, 1H), 6.37 (d, J = 6.8 Hz, 1H), 5.22–5.37 (m, 2H), 5.11–

5.19 (m, 1H), 4.95–5.03 (m, 1H), 4.87–4.95 (m, 1H), 4.55 (dd, *J* = 18.0, 8.4 Hz, 1H), 4.27 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.08–4.17 (m, 2H), 3.99–4.06 (m, 1H), 3.84–3.93 (m, 1H), 3.70–3.80 (m, 1H), 3.55–3.65 (m, 2H), 3.53 (dd, *J* = 13.8, 3.8 Hz, 1H), 3.04–3.15 (m, 1H), 2.91 (dd, *J* = 13.5, 6.9 Hz, 1H), 2.51 (dd, *J* = 16.5, 9.6 Hz, 1H), 2.42 (dd, *J* = 16.8, 2.5 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.75–1.82 (m, 1H), 1.63–1.74 (m, 2H), 1.54–1.61 (m, 1H), 1.31–1.44 (m, 2H), 1.20–1.28 (m, 1H), 1.07–1.17 (m, 1H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{35}H_{46}N_2O_{15}$ 735.2971; found 735.2948.

2.4.5 Cheminformatic Analyses

Principal component analysis (PCA) and principal moment of inertia (PMI) calculation were carried out to assess the chemical diversity of our macrocyclic library. Total 195 compounds (Table 1) were compared, which include the established reference sets[44, 98]:

- 40 top selling small molecule drugs in 2006 and 10 top selling small molecule drugs in 2016[99]
- 75 natural products[100]
- 36 macrocyclic natural products (Figure 16)
- 34 macrocyclic library set (Figure 14)

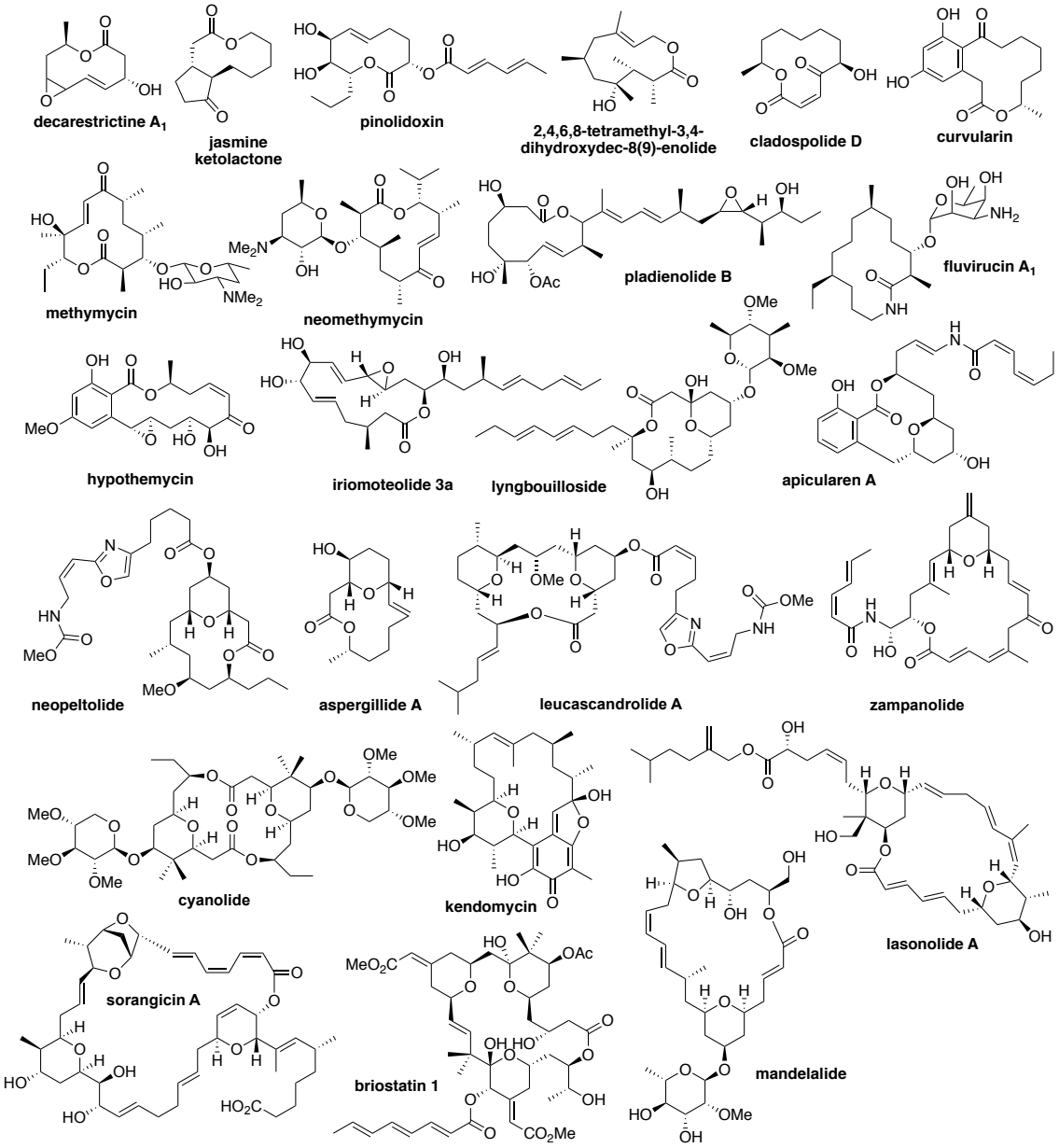


Figure 15. Macrocyclic natural product reference set used in the cheminformatic analysis

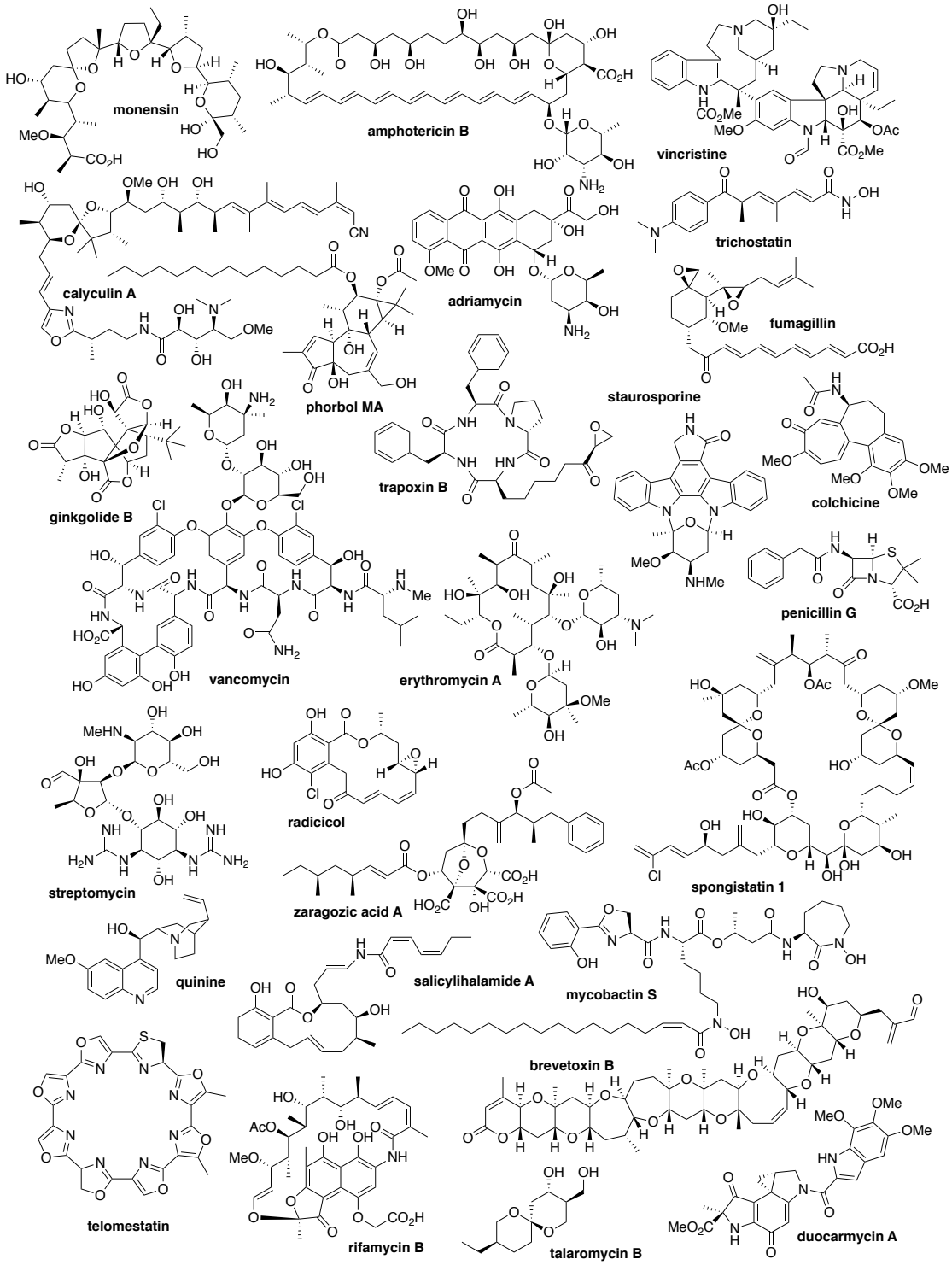


Figure 16. Natural product reference set used in the cheminformatic analysis (continued on nex page)

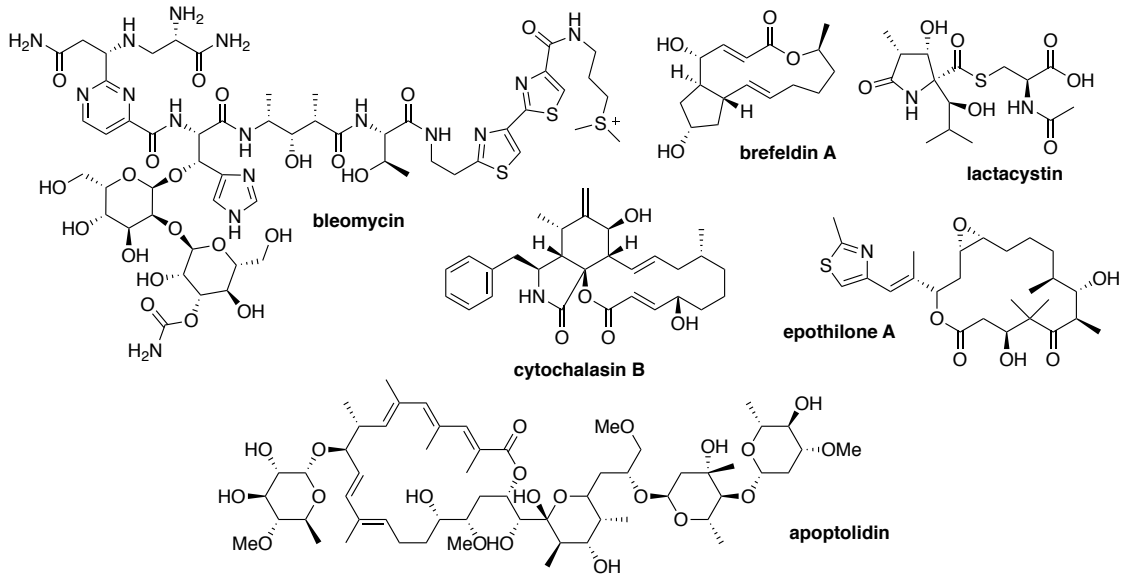


Figure 16 (continued). Natural product reference set used in the cheminformatic analysis

Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed using the procedure introduced by Tan[44, 98]. The plots shown in Figure 11 were generated from compound sets above, and the 20 structural and physicochemical properties were determined by free online cheminformatic tools (Molinspiration[101], VCC Lab[102]), ChemDraw and manual inspections (Table 2). The mean average values for each compound sets were calculated (Table 3).

Table 3. Average structural and physicochemical parameters by compound series († = nStMW × 1000 for clarity)

	Drug Set	Natural Product Set	Macrocyclic NP Set	THP-MC Library Set
MW	355	629	485	472
N	2.4	2.6	0.4	2.5
O	3.0	9.7	7.5	7.1
XlogP	2.5	1.5	3.4	1.1
HBD	1.6	4.9	2.2	1.5
HBA	5.6	10.8	7.9	9.6
RotB	6.2	9.7	5.3	3.1
tPSA	72	183	111	103
ALOGPs	2.5	2.1	3.1	1.3
ALOGpS	-3.7	-3.8	-3.8	-3.5
nStereo	1.3	9.1	7.5	3.6
R	0.6	4.1	3.7	2.5
S	0.7	5.0	3.9	1.2
RSdelta†	-0.1	-0.9	-0.2	1.3
nStMW	3.4	13.9	15.1	7.5
Rings	2.9	3.8	2.7	3.5
RngAr	2.0	1.0	0.2	1.3
RngSys	2.1	2.0	1.4	1.7
RngLg	8.1	15.8	16.8	20.9
RRsys	1.4	2.3	2.1	2.3

Table 4. Component loadings for PCA of the macrocyclic library with established reference sets (Top three contributing parameters are marked yellow)

	PC1	PC2	PC3
MW	-0.3349	0.0848	-0.0921
N	-0.1383	-0.1530	-0.4127
O	-0.3322	-0.0591	0.0543
XLogP	0.0394	0.5125	0.0670
HBD	-0.2633	-0.2188	-0.1581
HBA	-0.3236	-0.0895	-0.0798
RotB	-0.2075	0.0491	-0.2656
tPSA	-0.3163	-0.1632	-0.1409
nStereo	-0.3135	0.0114	0.2222
R	-0.2722	-0.0252	0.2935
S	-0.3076	0.0418	0.1305
nStMW	-0.1918	-0.0553	0.4031
RSdelta	0.1000	-0.0904	0.1774
Rings	-0.2065	0.1923	-0.0391
RngAr	-0.0030	0.1314	-0.4228
RngSys	-0.1549	0.0973	-0.2736
RngLg	-0.1987	0.1298	0.1798
RRSys	-0.0893	0.1361	0.2117
ALOGPs	-0.0168	0.5332	-0.0173
ALOGpS	0.1074	-0.4704	0.1140

Principal Moment of Inertia (PMI)

Merck molecular force field 94x (MMFF94x) and the generalized Born solvation model were used to generate stable conformers for each molecule. Principal moments of inertia calculations were performed using the Molecular Operating Environment (MOE, v2018.01) software package[103], and the conformational search was performed with the following settings:

Method	LowModeMD
Rejection Limit	150
RMS Gradient	0.005
Iteration Limit	10000
MM Iteration Limit	500
RMSD Limit	0.15
Energy Window	3
Conformation Limit	10000

Cytotoxicity Assay

Library members were screened for inhibition of Huh7 cells, a hepatocyte-derived carcinoma cell line. Compounds (100 μM) in triplicate were incubated with Huh7 (7,000 cells/well) cells in 384-well white microtiter plates for 5 days in a standard tissue culture incubator at 37 °C. The final concentration of DMSO was 1% and the assay volume was 30 μL. Brefeldin A (10 μM) was the positive control. Cell viability was assessed after addition of CellTiter-Glo (Promega) to each well and relative luminescence was determined with an EnVision (Perkin Elmer). Data were normalized to the DMSO control and plotted as the average ± the standard deviation. Significance was assessed by performing a two-way ANOVA, Dunnett's multiple comparison test (GraphPad Prism).

3. Synthetic Studies toward Forazoline A

3.1 Introduction

3.1.1 Clinical Needs in Antifungal Drug Development

Fungal infections have been a continuous threat to humans and medically important fungal diseases can be categorized into two types by tissue or organ levels, depending on primary colonization sites.[104] The first type includes mycoses of physical barriers of body surfaces such as superficial, cutaneous, subcutaneous, and mucosa. In healthy individuals, high body temperature and a powerful immune surveillance adequately resists the fungal challenges.[105] The second major group of fungal infections is an invasive fungal infection that can affect the central nervous system, bloodstream, or organs including kidney, lung, and liver. Immunocompromised patients, who have a reduced ability to fight off attack from pathogenic fungi, are particularly vulnerable to a large number of opportunistic invasive fungal infections.

Invasive fungal infection has become a major public health problem and is widely recognized as a leading cause of substantial morbidity and mortality in the healthcare environment. For individuals infected with human immunodeficiency virus (HIV), fungal infection can be life-threatening. It was estimated that there were more than 1 million cases of HIV-associated cryptococcal infection per year in the 2000s, and 600,000 of these cases led to death.[106] In addition, the advent of aggressive cancer chemotherapy and prescription of immunosuppressive medication for organ

transplantation have also contributed to the increased incidence of serious fungal infections.[107]

Despite the huge clinical need, therapeutic options for the treatment of invasive fungal infections are limited to only four major classes of drugs (polyenes, flucytosine, azoles and echinocandins) as shown in Figure 17. It is important to note that all four structural classes of antifungal agents were identified five decades ago. Polyenes are the oldest class of antifungals and first discovered in the 1950s, of which amphotericin B is the only treatment for systemic fungal infections (Figure 18). Amphotericin B has the most broad spectrum of antifungal action, used in combination with 5-flucytosine for the treatment of cryptococcal meningitis.[108] This discovery boosted the development of other antifungals such as azole-containing antifungals and echinocandins in the 1960s and 1970s. A small number of antifungal choices would not be a problem by itself, if they address invasive fungal infections with satisfactory outcomes. However, these fungal agents suffer from several limitations such as emerging drug resistance mechanism and high toxicity. Details of the four classes of antifungal drugs that are currently prescribed in clinic will be discuss in the next chapter.

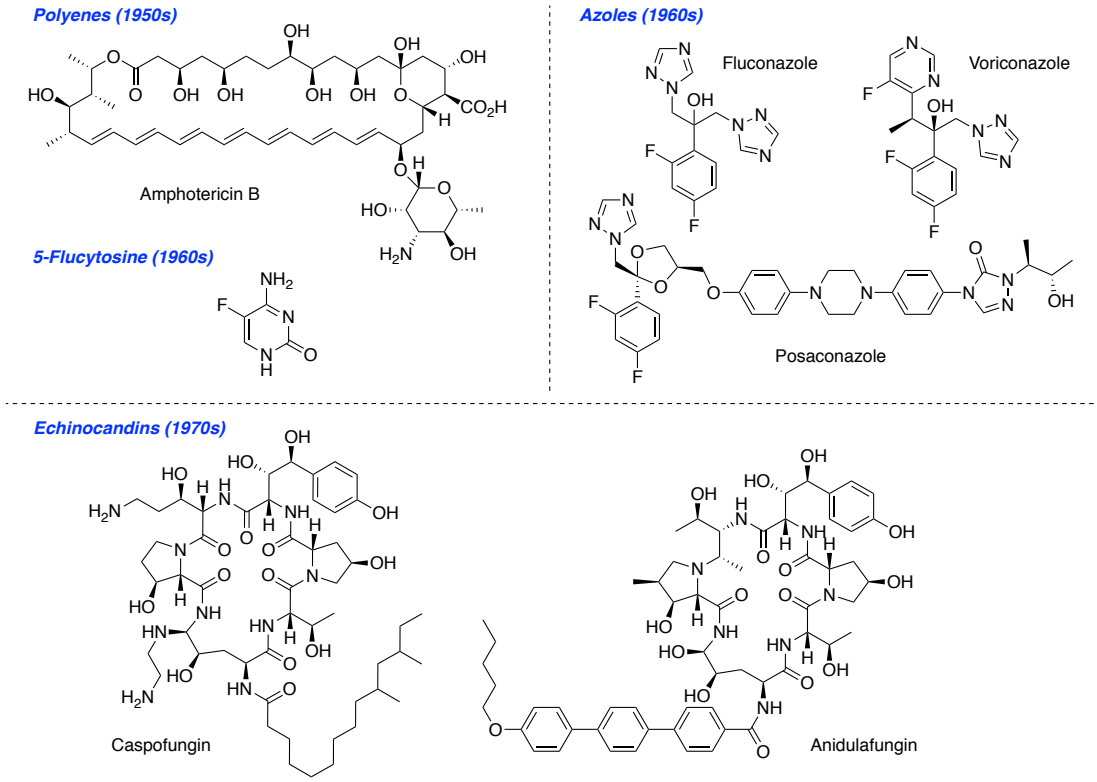


Figure 17. Current Antifungal Therapeutics

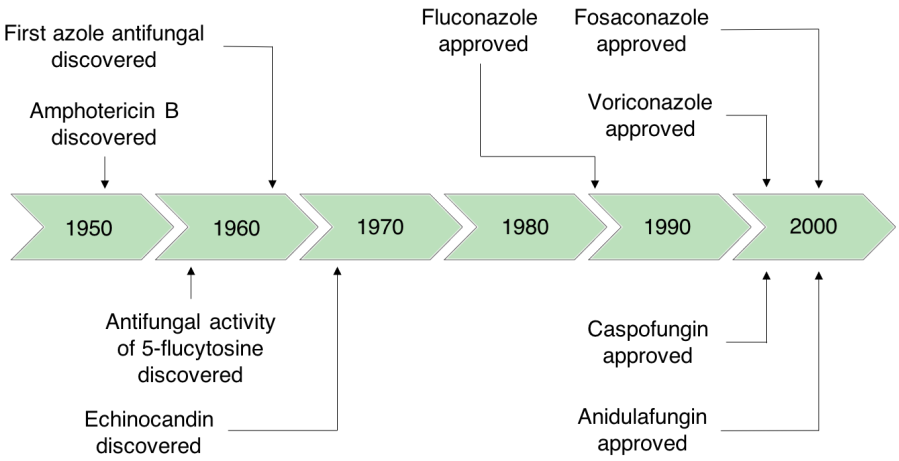


Figure 18. Timeline depicting key milestones of antifungal drug development (Figure redrawn with the permission from ref.[109])

3.1.2 Current Antifungal Therapeutics and Challenges

3.1.2.1 Polyenes

Amphotericin B is isolated from *Streptomyces* species and the most potent antifungal in clinical use today. This macrocyclic natural product possesses a number of hydroxyl groups and double bonds that are responsible for the mechanism of action.[110-111] Amphotericin B binds to ergosterol, which is a main component of fungal cell walls, and disrupts plasma membranes leading to leakage of vital cellular constituents and ultimately death of fungal cells.[112] However, amphotericin B can also interact with cholesterol (human membrane sterol) and this results in significant host toxicity including infusional toxicity, renal dysfunction and low blood potassium[113-114], which has been a primary drawback of amphotericin B. In addition, low solubility and bioavailability have been a major challenge. Since crystalline amphotericin B is insoluble in water at pH 7, the deoxycholate salt of amphotericin B (fungizone®) was developed in 1966 but it shows severe toxicity. To alleviate the toxicity problem, lipid formulations of amphotericin B such as Abelcet® and AmBisome® were developed and approved by FDA in the mid-1990s.[114-115]

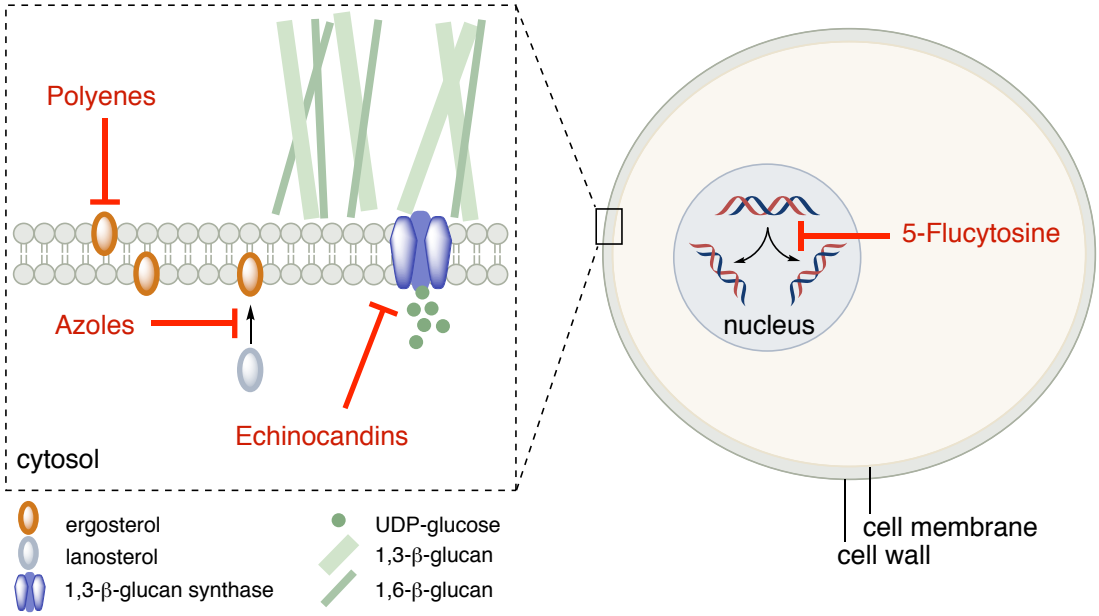


Figure 19. Antifungal targets (Figure adapted with permission from ref.[116])

3.1.2.2 Flucytosine

Flucytosine is a synthetic pyrimidine analog originally developed as an anticancer agent and later identified as an antifungal drug.[117-118] It is transformed into 5-fluorouracil by a cytosine deaminase, that is not present in humans, and this compound interferes with RNA and DNA metabolism by incorporation into RNA structures and inhibition of thymidylate synthase (Figure 20).[119] Flucytosine is generally used in adjunctive therapy with amphotericin B due to rapid development of resistance during monotherapy, caused by mutations of the enzymes.[120]

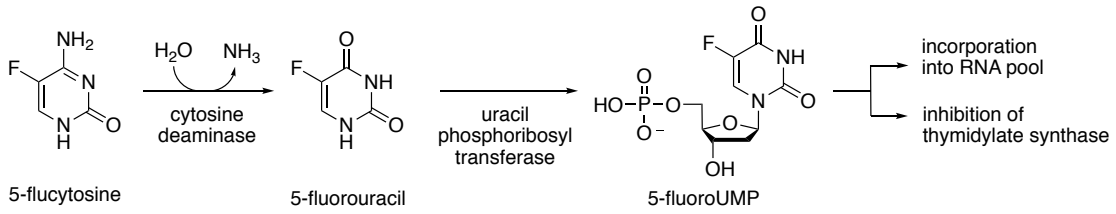


Figure 20. Mechanism of action of 5-flucytosine

3.1.2.3 Azoles

The first azole-based drug miconazole was introduced in the 1970s and the second and third generations of azole drugs such as fluconazole and voriconazole have been developed with an improved spectrum of antifungal activity, pharmacokinetics, safety, and formulations. Azoles are the most widely used antifungal agents for the treatment of invasive fungal infections[121], especially in resource-limited regions because amphotericin B and 5-flucytosine are quite expensive and not readily available. The mechanism of action of azoles involves the inhibition of the cytochrome P450-dependent enzyme 14-α-demethylase, which converts lanosterol into ergosterol, and this results in the decrease of ergosterol synthesis and the accumulation of 14-α-methylated precursors.[122-123] Since ergosterol has an important role in stimulating fungal cell growth, azoles in general exhibit fungistatic activity except for voriconazole which is fungicidal against *A. fumigatus*.[124] Major drawbacks of azoles might be due to a significant incidence of multiple resistance and the induction of drug–drug interactions due to their ability of cross-inhibition of P450-dependent enzymes.[125]

3.1.2.4 Echinocandins

Echinocandin B is the first prototype of echinocandins which is a fungal secondary metabolite isolated from *Aspergillus nidulans*.[125] Semisynthetic efforts have provided echinocandin analogs such as caspofungin, micafungin, and anidulafungin with favorable solubility and better pharmacokinetic properties for clinical settings. Echinocandins consist of a cyclic hexapeptide core with a lipid side chain that is responsible for antifungal activity.[126-128] These compounds are specific and non-competitive inhibitors against β-(1,3)-D-glucan synthase enzyme complex which is a glucosyltransferase enzyme involved in the formation of glucan polymers of fungal cell wall. Although this class of antifungals exhibits reduced efficacy against *Cryptococcus neoformans*[126-128], they show relatively broad-spectrum fungicidal activity with little host toxicity and reduced drug–drug interaction. As a result, echinocandins have become a first-line choice for the treatment of deadly nosocomial candidemia and invasive candidiasis.[129]

3.1.3 Natural Products as Antifungal Agents

With the unmet medical needs mentioned above, there have been efforts in discovery and development of new chemical entities which have low host toxicity, broad-spectrum activity, fungicidal activity, and new mechanisms of action different from existing antifungal drugs. In particular, natural products and their synthetic

derivatives have served as a great platform to develop therapeutics for infectious diseases[129], and it is noteworthy that two out of four classes of current antifungal drugs are derived from natural products (polyenes and echinocandins). Naturally produced small molecules have provided diverse and privileged scaffolds proven to interact with biological targets, along with target selectivity and intrinsic pharmacological properties. In this regard, many natural products with antifungal activity have been reported.[130-132]

As an example, sordarin was isolated from *Sordaria araneosa*[133] and its semi-synthetic derivatives were precilinically developed by Glaxo–Wellcome and Merck[134] (Figure 21). This class of antifungals is known to have a novel mode of action, which involves selective binding to the elongation factor 2 (EF-2) and ribosome complex.[135-136] By stabilizing the ribosome–elongation factor 2 complex, protein biosynthesis in fungi is inhibited. Although there have been synthetic efforts for the development of potent sordarin derivatives[137-138], in vivo efficacy of sordarins have not yet been sufficiently high to enter clinical development.

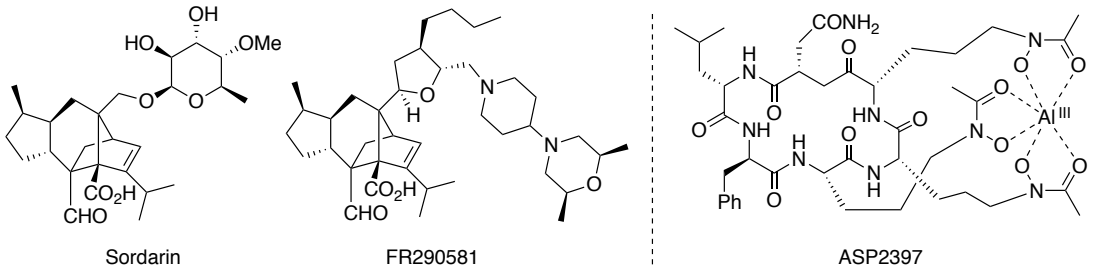


Figure 21. Structures of natural product-derived antifungals

Another example can be ASP2397, which is a hexapeptide metabolite of a fungal strain MF-347833 isolated from Malaysian leaf litter in 2017 by Astellas (Figure 21).[139] The molecular target of ASP2397 has not been clearly elucidated yet, but it is found that ASP2397 is taken up by the siderophore iron transporter 1 (Sit1). Since this transporter only exists in fungi, not mammalian cell, ASP2397 is believed to have low host toxicity. In particular, it shows high in vitro and in vivo efficacy toward azole-susceptible and azole-resistant strains of *Aspergillus* species.

3.1.4 Discovery, Identification, and Antifungal Activity of Forazoline A

Forazoline A is an antifungal natural product isolated from the strain WMMB-499, an *Actinomadura* sp. cultivated from the ascidian *Ecteinascidia turbinata* by Bugni's group in 2014 (Figure 22).[140] In parallel with disc diffusion assay, they analyzed 34 invertebrate-associated bacterial extracts using LCMS-based metabolomics that allows qualitative and quantitative measurement of metabolites in a complex sample.[141] LCMS profiling and principal component analysis (PCA) resulted in the identification of new antifungal natural products, forazoline A and B.[142-144] The relative configuration and connectivity were elucidated by a combination of extensive 1D and 2D NMR analyses, including ^{13}C–^{13}C gCOSY, ^{13}C–^{15}N HMQC, and DFT (density functional theory) calculations.

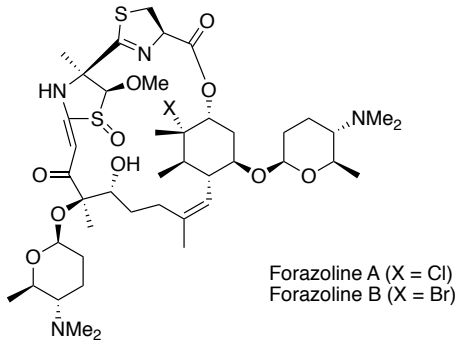


Figure 22. The structures of forazoline A and B

This study reveals that forazoline A exhibits promising antifungal activity against pathogenic *Candida albicans* strain *in vitro* with a minimum inhibitory concentration of 16 μg/mL, showing synergistic effect with amphotericin B. More importantly, this polyketide showed potent *in vivo* efficacy comparable to amphotericin B in an immunocompromised mouse model of disseminated candidiasis against *Candida albicans* K1. Efficacy in a fungal-infected mouse model was determined by measuring kidney fungal burden. When mice were treated with forazoline A at a range of concentrations, it was observed that 1 $\log_{10}$cfu/kidney (1.5 ± 0.12) reduction in organism burden compared to that of the control mice. Notably, there was no apparent host toxicity.

In addition, chemical genomic profiling was performed to investigate the mechanism of action of forazoline A. After screening forazoline A against over four thousand deletion mutant yeast strains, sensitivity or resistance to forazoline A was

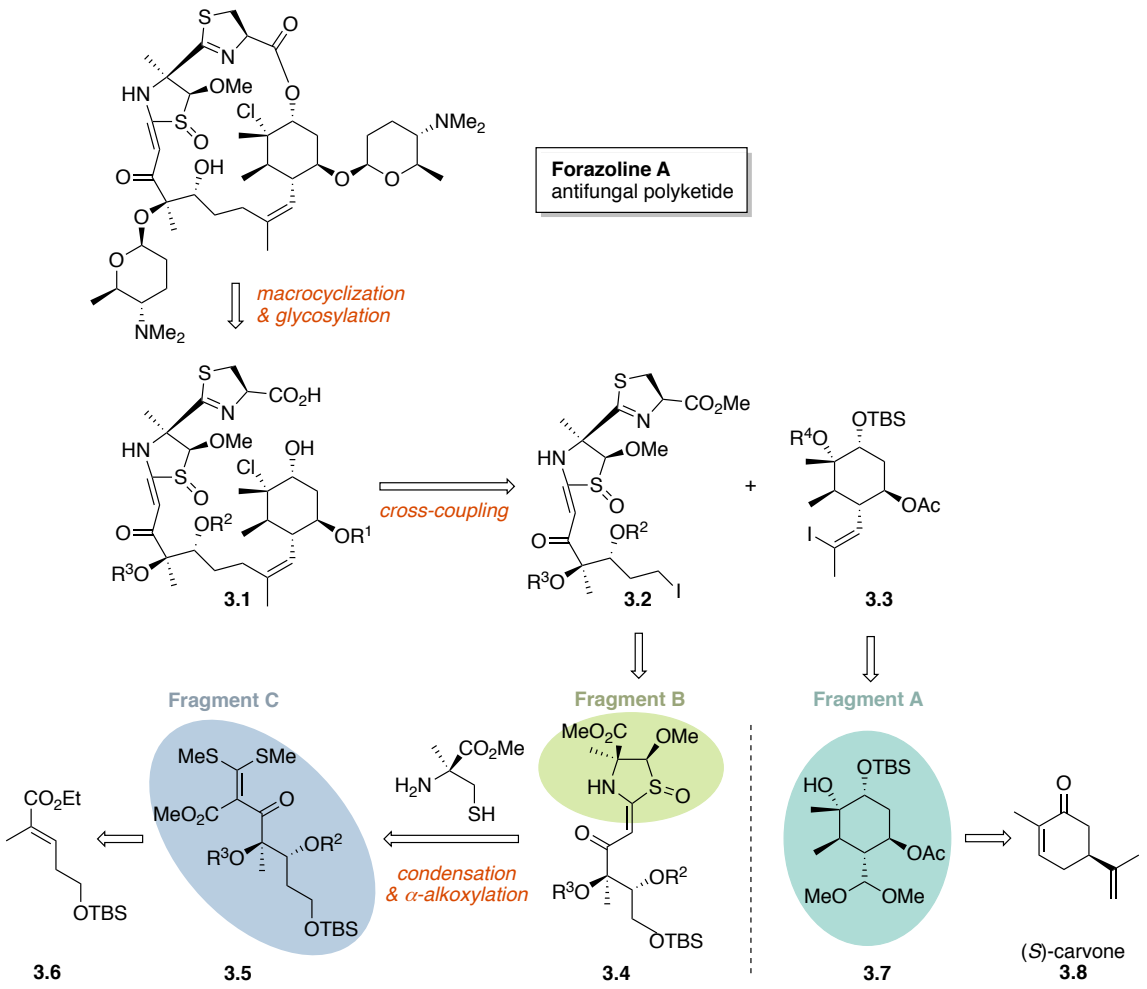
analyzed by quantification of DNA barcodes. Genomic profiling data suggested that forazoline A may have a unique mechanism of action different from caspofungin, fluconazole, or amphotericin B, because sensitive mutants to forazoline A with deletion of genes *LEM3* and *FPK1* were not most sensitive strains to the existing antifungals. Furthermore, mutants of *SDS3* and *DEP1* genes were most resistant to forazoline A, thus suggesting that forazoline A may be involved in the regulation of phospholipid biosynthesis and membrane integrity.

Along with the interesting antifungal activity of forazoline A, it has structurally intriguing features such as highly functionalized cyclohexane-1,3-diol with a tertiary chloride and an α-methoxy substituted thiazolidine. Therefore, we aim to explore the synthesis of forazoline A focusing on the construction of substructures of the molecule, which will be described in the following chapter.

3.2 Results and Discussion

3.2.1 Retrosynthetic Analysis

Our retrosynthetic analysis of forazoline A is depicted in Scheme 34. We envisioned that two identical glycosides, forosamines, could be simultaneously installed to the corresponding aglycon which would be generated via macrolactonization of the linear precursor **3.1**. Tri-substituted (*Z*)-alkene functionality in **3.1** would be generated through a Suzuki or Negishi cross-coupling reaction of key intermediate **3.2** and **3.3**. We anticipated that thiazolidine fragment **B** would be synthesized by condensation of alkyl chain **3.5** with cysteine in conjunction with α-alkoxylation, and fragment **3.5** would be derivatized from compound **3.6**[145] using asymmetric Sharpless epoxidation and dimethyl thioketene formation. Functionalized cyclohexane fragment **A** would be prepared from commercially available terpene (*S*)-carvone.



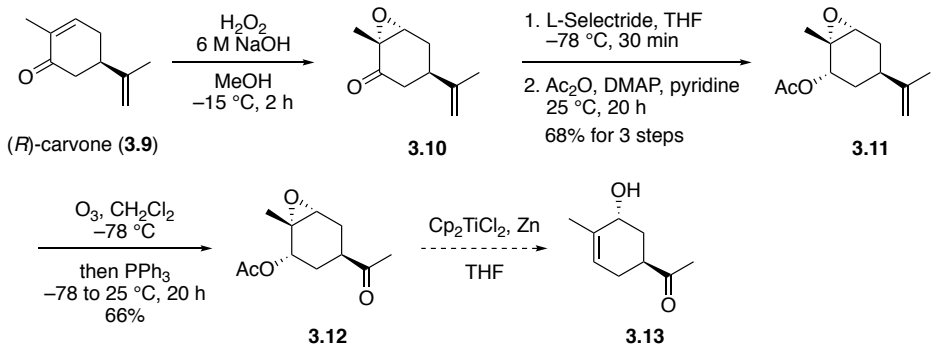
Scheme 34. Retrosynthetic analysis of forazoline A

Our goal of this chapter is the investigation towards the construction of the major fragments **A**–**C**. The detailed synthetic attempts for the enantioselective construction of cyclohexane, thiazolidine, and the alkyl chain backbones will be sequentially discussed. Ultimately, the synthetic strategy for the assembly of the three fragments will be outlined, which would accomplish the total synthesis of forazoline A.

3.2.2 Synthesis of a Functionalized Cyclohexane

3.2.2.1 Synthesis of the Cyclohexane Fragment via Titanium-Mediated Radical Reaction

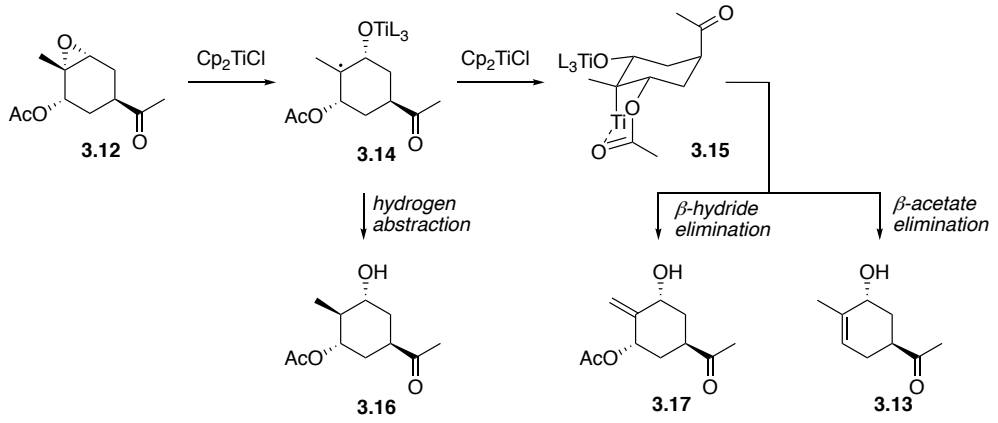
Our first attempt to the synthesis of the cyclohexane fragment A relied on a radical ring-opening reaction (Scheme 35).[146] The synthesis commenced with (*R*)-carvone (**3.9**) which was subjected to epoxide formation in the presence of hydrogen peroxide and sodium hydroxide. Subsequent ketone reduction using L-Selectride of **3.10** provided known secondary alcohol[147]. Acetyl protection of the alcohol followed by ozonolysis of the corresponding acetate afforded the substrate **3.12** for the titanium-mediated ring-opening reaction.



Scheme 35. Synthetic approach for **3.13** via the radical ring-opening reaction

We attempted ring-opening and isomerization of the tri-substituted epoxide **3.12** promoted by Cp_2TiCl to generate the known compound **3.13**[146]. According to the proposed mechanism of the reaction (Scheme 36), compound **3.12** is converted into alkoxytitanium radical **3.14** and further reduced to the alkoxy titanium carbanion **3.15** in

the presence of excess amount of Cp_2TiCl. In the course of the reaction, there are three possible products (**3.13**, **3.16**, and **3.17**) from the titanium species **3.14** and **3.15**. With the low concentration of Ti(III), the radical **3.14** abstracts hydrogen atom leading to the formation of the saturated alcohol **3.16**. In addition, alkoxy titanium carbanion **3.15** could undergo β-hydride elimination or β-acetate elimination to generate olefin **3.17** or **3.13**. We found that the desired β-acetate elimination product **3.13** was obtained with low yield and the other possible products **3.16** and **3.17** were also observed under the reaction condition. In order to generate the cyclohexane scaffold in a more efficient way, another synthetic route was devised.

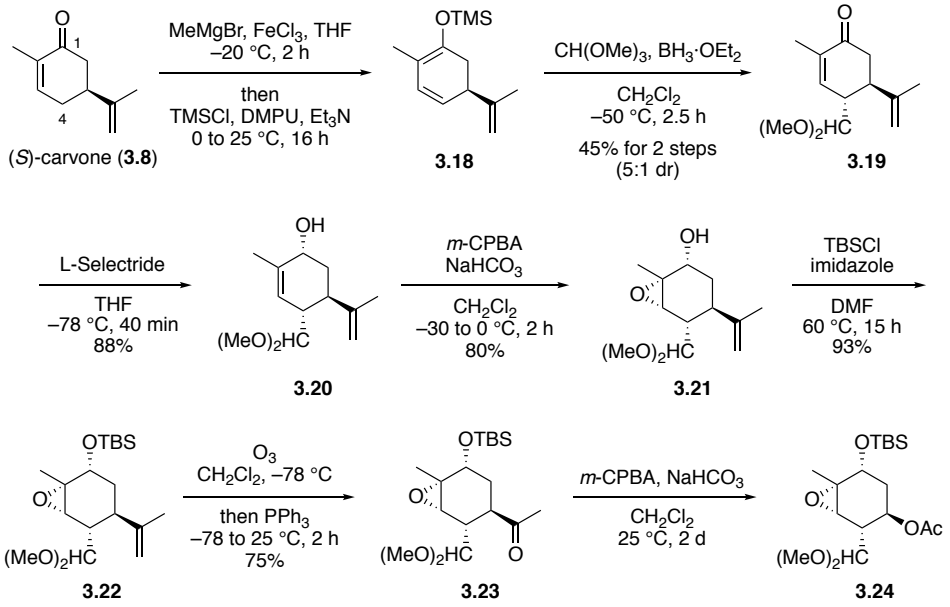


Scheme 36. Possible products from the Cp_2TiCl-promoted epoxide opening reaction[146]

3.2.2.2 Synthesis of the Cyclohexane Fragment via Mukaiyama Aldol and Baeyer–Villiger Oxidation Reaction

Our second approach was initiated with the preparation of cyclohexenone x by introducing a new stereocenter at C-4 position (Scheme 37). (*S*)-carvone (**3.8**) was treated

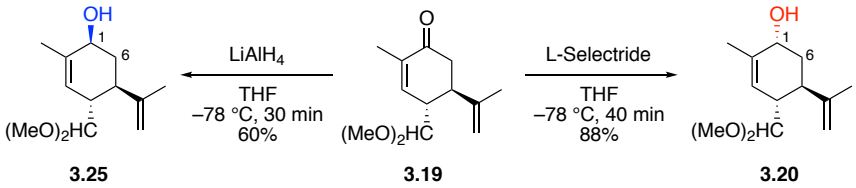
with methylmagnesium bromide in the presence of catalytic iron(III) chloride, providing a vinylogous enolate which was trapped by a *t*-butyl trimethylsilyl group. Subsequent Mukaiyama aldol reaction of **3.18** with trimethyl orthoformate provided compound **3.19**, and the NMR spectrum of **3.19** was in agreement with that of the reported enantiomer synthesized by Inoue's group.[148]



Scheme 37. Synthesis of **3.24** via Mukaiyama aldol and Baeyer–Villiger oxidation

Reduction of the resulting ketone **3.19** using L-Selectride was carried out in order to furnish axial alcohol **3.20**. To confirm the stereochemistry at C-1 position, the same substrate **3.19** was reacted with lithium aluminum hydride to afford equatorial alcohol **3.25** in which the stereochemical outcome was governed by torsional effect (Scheme 38). By comparing the proton splitting patterns in the NMR spectra, we confirmed that the

generated compound is the desired axial alcohol (Figure 23). The proton at C-1 position of the axial alcohol **3.20** appear as a triplet at 3.91 ppm with 3.2 Hz of coupling constant, while the proton (4.19 ppm) at C-1 position of the equatorial alcohol **3.25** shows a larger coupling constant due to the coupling with the proton at C-6 position in the anti configuration. Having the axial alcohol **3.20** in hand, we carried out hydroxy-directed epoxidation and TBS protection to yield cyclohexane **3.22**. At this point, stereochemistry induction from (*S*)-carvone at five stereogenic centers in fragment **A** was completed. Ozonolysis proceeded smoothly to give ketone **3.22** which was followed by Baeyer–Villiger oxidation.



Scheme 38. Ketone reductions to synthesize axial and equatorial alcohol **3.20** and **3.25**

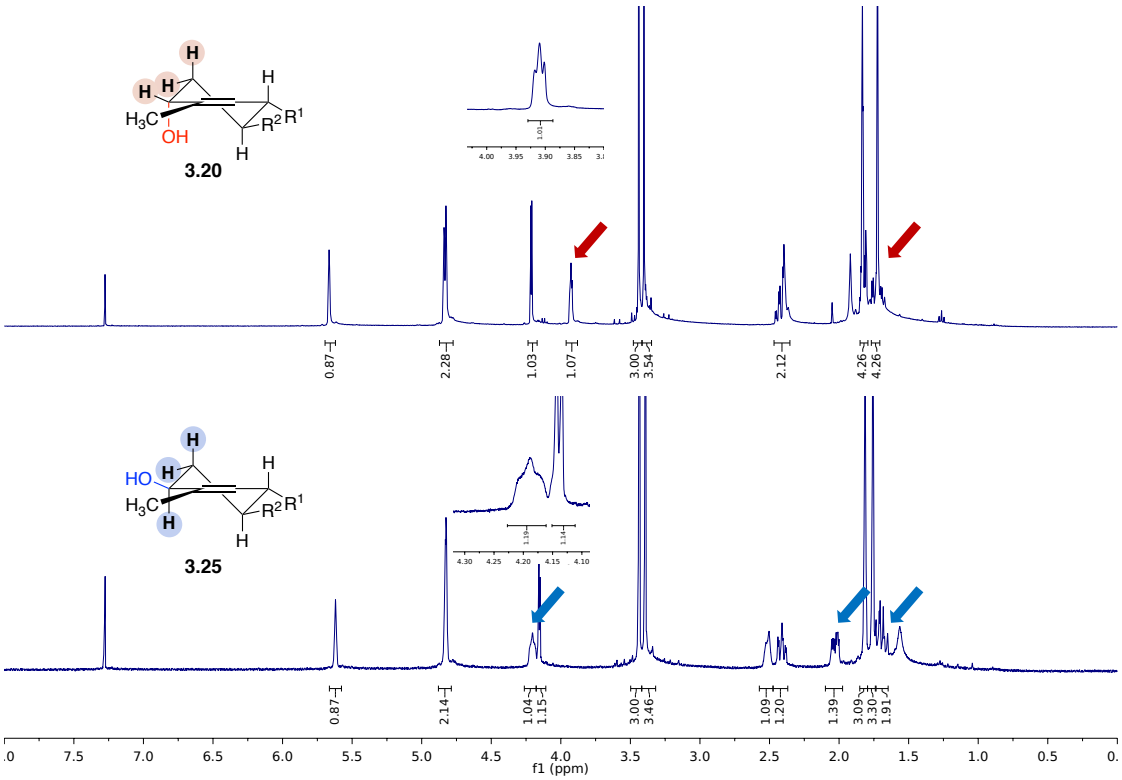
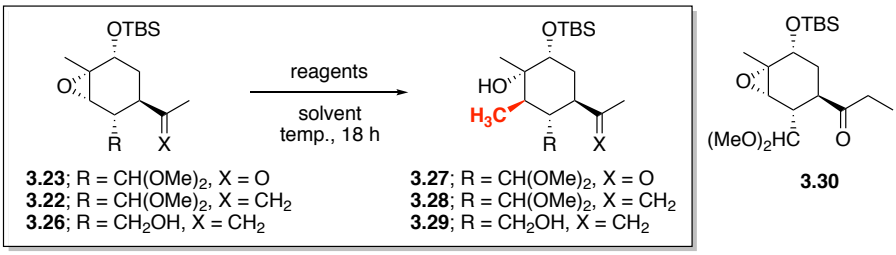


Figure 23. ^{1}H NMR spectra of the **3.20** and **3.25**

Our attempts for regioselective epoxide opening using organocuprate reagents is summarized in Table 3. Addition of lithium dimethylcuprate to cyclohexane epoxides has been used for the synthesis of highly substituted cyclohexanes.[149-150] As the regioselectivity of epoxide ring opening using Me_2CuLi is generally dictated by steric factors, we anticipated that the desired ring opening product in which the methyl group is inserted at sterically more accessible C-3 position would be obtained. The organocuprate was generated in situ using copper(I) iodide and methyl lithium and

treated with substrate **3.23** (entry 1). However, the reaction was sluggish, and the newly formed product was undesired compound **3.30** in which the methyl group was added at the end of the acetyl group. Exposure of compound **3.22** to MeCu(CN)Li or methylmagnesium bromide with $CuBr{\cdot}SMe_2$ led to no reaction or the formation of unidentified product (entry 2–3). To test whether steric bulkiness at adjacent C-4 position affects the epoxide ring opening reaction, substrate **3.26** was prepared from **3.22** by acetal deprotection and aldehyde reduction. When the resultant primary alcohol **3.26** was allowed to react with CuI and MeLi, the regioselective ring opening product **3.29** was obtained with 24% yield along with 60% of the recovered starting material (entry 4). We then examined the solvent effect and found that the use of diethyl ether instead of THF dramatically increased the reaction yield, even with the sterically hindered substrate **3.22** (entry 5).

Table 5. Optimization of epoxide ring opening reaction using methylcuprate



entry	substrate	Cu source	reagent	solvent	temperature	result
1	**3.23**	CuI	MeLi	THF	–78 to 25 °C	undesired (**3.30**, 18%) + **3.23** (69%)
2	**3.22**	CuCN	MeLi	THF	–78 to 25 °C	no reaction
3	**3.22**	$CuBr{\cdot}SMe_2$	MeMgBr	THF	–78 to 25 °C	unidentified + **3.22** (78%)
4	**3.26**	CuI	MeLi	THF	–25 to 25 °C	desired (**3.29**, 24%) + **3.26** (60%)
5	**3.22**	CuI	MeLi	Et_2O	–40 to 25 °C	desired (**3.28**, 97%)

In summary, the synthesis of an advanced precursor to the fragment **A** was successfully accomplished. The stereochemical information in the naturally occurring terpene **3.8** was successfully transferred by utilizing Mukaiyama aldol reaction, stereoselective ketone reduction and hydroxy-directed epoxidation, thus installing five stereocenters within a cyclohexane framework. This route enables rapid and efficient construction of the highly functionalized cyclohexane scaffold.

3.2.3 Attempts for the Synthesis of Methyl (2*S*,3*S*)-2-amino-3-mercapto-3-methoxy-2-methylpropanoate

Having achieved the synthesis of a precursor to fragment **A**, we turned our attention to the thiazolidine construction. A significant number of thiazolyl-containing natural products with macrocyclic cores have been reported especially in the area of thiopeptide antibiotics[151], but apratoxin sulfoxide is the only example of thiazoline natural products containing a sulfoxide moiety on the ring up to date (Figure 24). Furthermore, α-functionalized thiazolidine sulfoxides without a fused ring are extremely rare scaffolds.[152-154] In this regard, we aim to explore two different strategies for the synthesis of the α-alkoxy thiazolidine sulfoxide as shown in Scheme 39. First, oxa-conjugate addition reactions of α,β-unsaturated ester **3.31** were investigated. In addition, α-functionalizations were attempted using Pummerer-type reactions of thiazolidine sulfoxide substrates. Before discussing these attempts in detail, the studies for the synthesis of the thiazolidine backbone will be described.

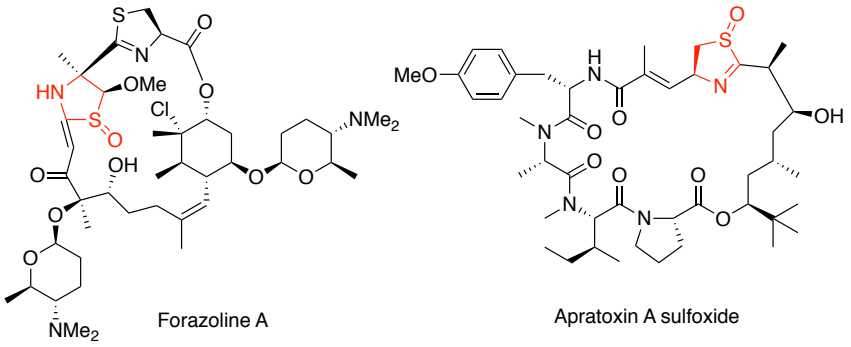
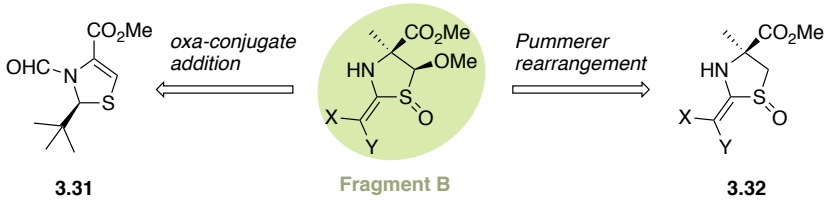


Figure 24. The structures of forazoline A and apratoxin A sulfoxide



Scheme 39. Two approaches toward the synthesis of a precursor to fragment **B**

3.2.3.1 Condensation Reactions

We explored condensation reactions of L-cysteine methylester with various condensation partners as a model study (Figure 25). Since condensation of cysteine with cyanides has been well studied for the synthesis of a thiazoline moiety[155-158], we primarily tested commercially available cyanides (**3.33**–**3.36**) containing ester, ketone, acetal, and alcohol functionalities. However, all the reactions with cyanides (**3.33**–**3.36**) resulted in the formation of a complex mixture of unidentified products under refluxing conditions.

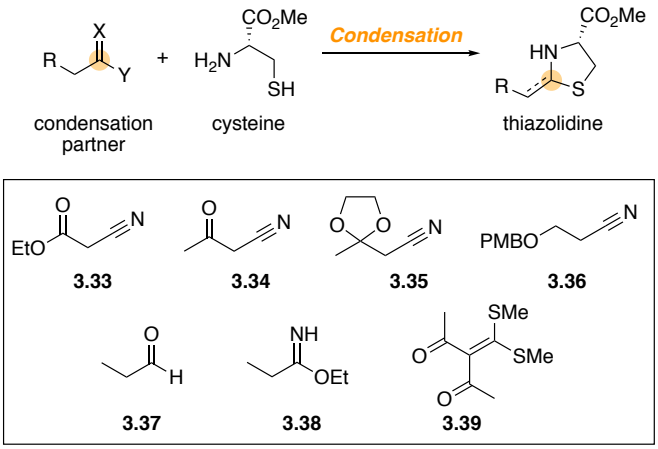
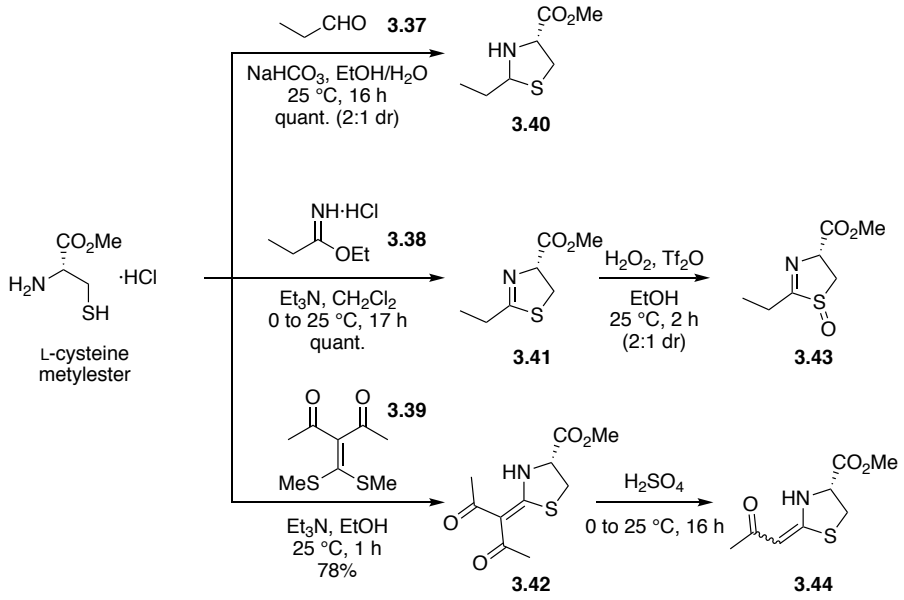


Figure 25. Model study for the construction of a thiazolidine scaffold

To generate the thiazolidine backbone under milder conditions, we examined more reactive condensation partners such as propionaldehyde (**3.37**), ethyl imidate **3.38**[159], and dimethyl thioketene **3.39**[160] (Scheme 40). All of the reactions proceeded smoothly at room temperature in the presence of mild bases such as sodium bicarbonate and triethylamine, to generate compound **3.40**[161], **3.41**[162], and **3.42** respectively. Next, we further tested the reactivity of compound **3.41** and **3.42** toward the installation of the sulfoxide and acid-promoted deacetylation.[163-164] Oxidation of the thioether functionality in thiazolidine **3.41** with H_2O_2/Tf_2O provided the corresponding sulfinyl derivative **3.43**. Treatment of sulfuric acid to β-diketone **3.42** furnished deacetylated product **3.44**, which possess the desired alkylidene thiazolidine scaffold.[163]

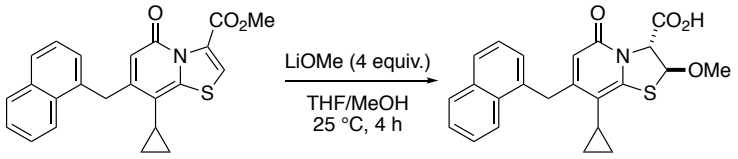


Scheme 40. Thiazolidine synthesis via various condensation reactions

Based on the model study, we decided to utilize the condensation of cysteine with dimethyl thioketene as a strategy for the construction of the ketene *N*,*S*-acetal moiety. In the following chapters, two approaches toward installation of α-methoxy thiazolidine will be discussed.

3.2.3.2 Oxa-Conjugate Addition

Our initial approach to the installation of an α-alkoxy group was based on oxa-conjugate addition. We prepared the thiazoline **3.47** according to the previously reported procedure for the enantiomer synthesis[165], and utilized this compound **3.47** as a substrate for oxa-conjugate addition study (Table 4). In 2017, Chorell *et al.* reported the synthesis of methoxy substituted compound **3.46** from α,β-unsaturated methyl ester **3.45** by the use of lithium methoxide in THF/MeOH solvent (Scheme 41).[166] This method was applied to our substrate **3.47** (entry 1).



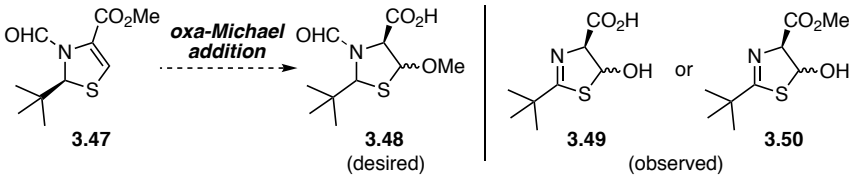
3.45 **3.46** (75%)

Scheme 41. Installation of a methoxy group to x reported by Chorell *et al.*[166]

We found that the methyl ester group was hydrolyzed by the use of sodium methoxide in THF/MeOH, which was consistent with our expectation. However,

aldehyde and *N*,*S*-acetal peaks were also not observed in the ^{1}H NMR spectrum. As similar types of oxidative deformylation have been reported, especially for the synthesis of quinolines[167-170], we assumed that the formyl group may not be stable under this condition. Furthermore, since the methoxy peak was not observed in the ^{1}H NMR of the product, we tentatively assigned the product structure as **3.49**. In order to obtain additional information about the structure of the product, compound **3.49** was exposed to Ag_2O and iodomethane which resulted in the formation of **3.50**.

Table 6. Attempts to oxa-conjugate addition of thiazolidine **3.47**



entry	conditions	temp / time	results
1	NaOMe (4 equiv), THF/MeOH	25 °C, 20 h	**3.49**
2	NaOMe (4 equiv), THF	25 °C, 20 h	no reaction
3	DBU (4 equiv), MeOH	25 °C, 2 d	**3.49** (slow)
4	Tf_2NH (0.1 equiv), MeOH	0 °C, 18 h	**3.50**
5	Tf_2NH (1 equiv), MeOH	0 °C, 1 h	**3.50**
6	TfOH (0.1 equiv), MeOH	0 °C, 3 h	**3.50**
7	P(*t*-Bu)$_3$, MeOH	25 °C, 20 h	no reaction
8	$Pd(MeCN)_2Cl_2$, MeOH, CH_2Cl_2	25 °C, 3 h	no reaction

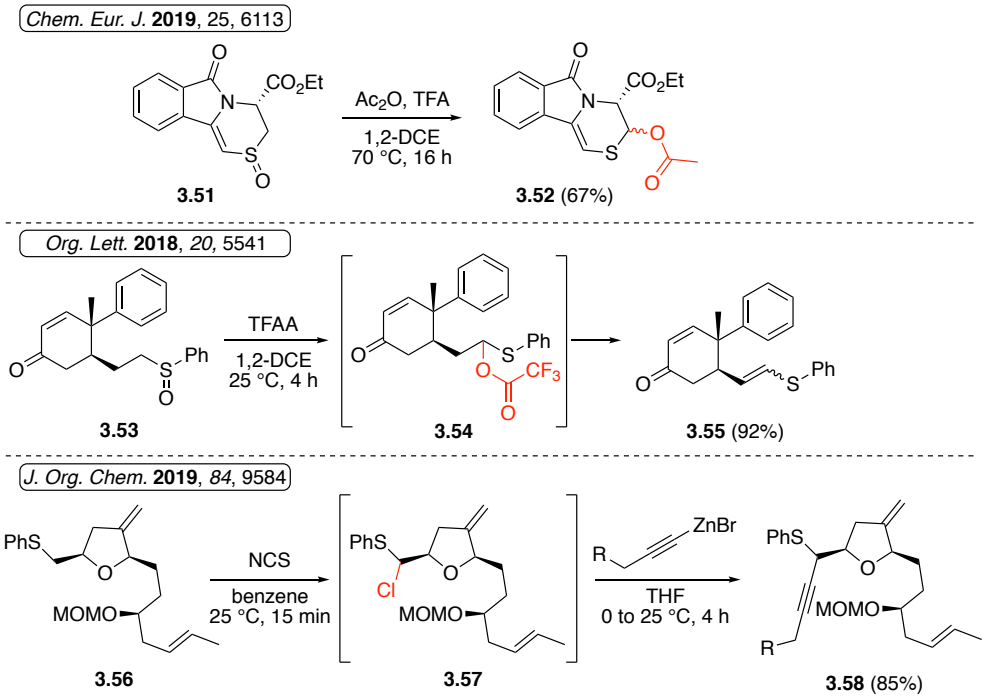
In the absence of methanol as a co-solvent, no reaction occurred possibly due to poor solubility of sodium methoxide (entry 2). When DBU was treated instead of

sodium methoxide to substrate **3.47**, the reaction was much slower than entry 1 generating the same product **3.49** (entry 3). Next, we investigated oxa-conjugate addition of **3.47** in acidic media. Substrate **3.47** was exposed to trifluoromethanesulfonimide affording compound **3.50** as a product which was slowly decomposed during the reaction (entry 4–5). The same result was obtained when substrate **3.47** was reacted with triflic acid (entry 6). Under these acidic conditions, hydrolysis of the methyl ester was not observed but still deformylation occurred. Although phosphine-catalyzed hydroalkoxylation[171] (entry 7) and a palladium-catalyzed reaction[172-173] (entry 8) were also explored, all attempts were not effective for the installation of an α-methoxy group to a thiazolidine ring.

3.2.3.3 Pummerer-type Rearrangement

Our second approach for the synthesis of an α-methoxy thiazolidine sulfoxide relied on a Pummerer-type rearrangement. The Pummerer rearrangement has been widely applied to construct α-functionalized sulfides.[174-175] Addition of a halogen, an acetoxy, or a trifluoroactoxy group using the Pummerer-type rearrangement has been reported and comprehensively studied and selected examples were shown in Scheme 42.[176-179] Pummerer reaction of a sulfoxide **3.51** using acetic anhydride resulted in the formation of **3.52** as a mixture of two diastereomers.[179] Functionalizations of the α-position of **3.53** and **3.56** were also reported to provide the trifluoroacetyl product **3.54**

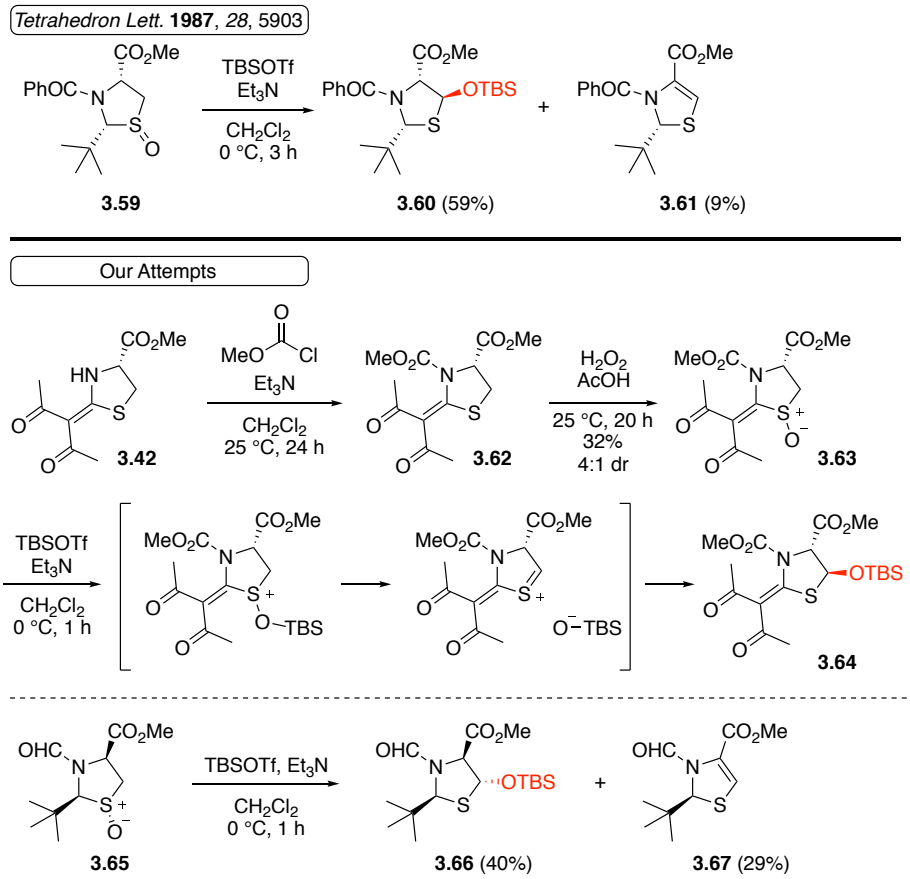
and α-chloro sulfide **3.57**, respectively.[177-178] Due to the high reactivity of these functional groups, **3.54** subsequently underwent an elimination reaction to give **3.55**, and **3.57** was subjected to a substitution reaction using an alkylzinc bromide as a nucleophile.



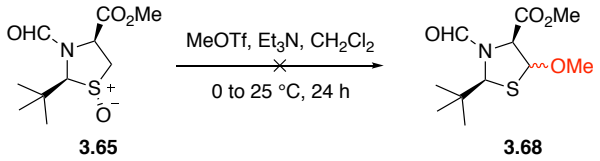
Scheme 42. Examples of a Pummerer-type rearrangement for α-functionalizations of a sulfide[177-179]

In 1987, Tokitoh *et al.* showed that a silyloxy group can be stereospecifically installed to the α-position of a sulfide using an optically active thiazolidine sulfoxide.[180] We decided to apply this method to our system (Scheme 43). To synthesize a substrate for this reaction, protection of amine in the ketene *N*,*S*-acetal **3.42** was carried out in the

presence of methyl chloroformate to give compound **3.62**, which was subjected to sulfide oxidation using hydrogen peroxide and acetic acid. When the resulting sulfoxide **3.63** was treated with *t*-butyldimethylsilyl trifluoromethanesulfonate and triethylamine, the corresponding α-silyloxy thiazolidine **3.64** was detected. Analysis of ^{1}H NMR suggested that the obtained product **3.64** is a single diastereomer due to the stereospecific nature of the reaction, but additional spectroscopic studies should be followed. The same reaction conditions were applied to substrate **3.65**[165], which led to the formation of the desired α-functionalized thiazolidine **3.66** along with the elimination product **3.67**. The observation of the elimination side product **3.67** was in accordance with the reported result, which is that mono-substitution at the *N*,*S*-acetal position resulted in the formation of a considerable amount of α,β-unsaturated sulfide.[180] Encouraged by the successful installation of α-siloxy functionality to the thiazolidines, substrate **3.65** was treated with methyl trifluoromethansulfonate but only the starting material was recovered (Scheme 44).

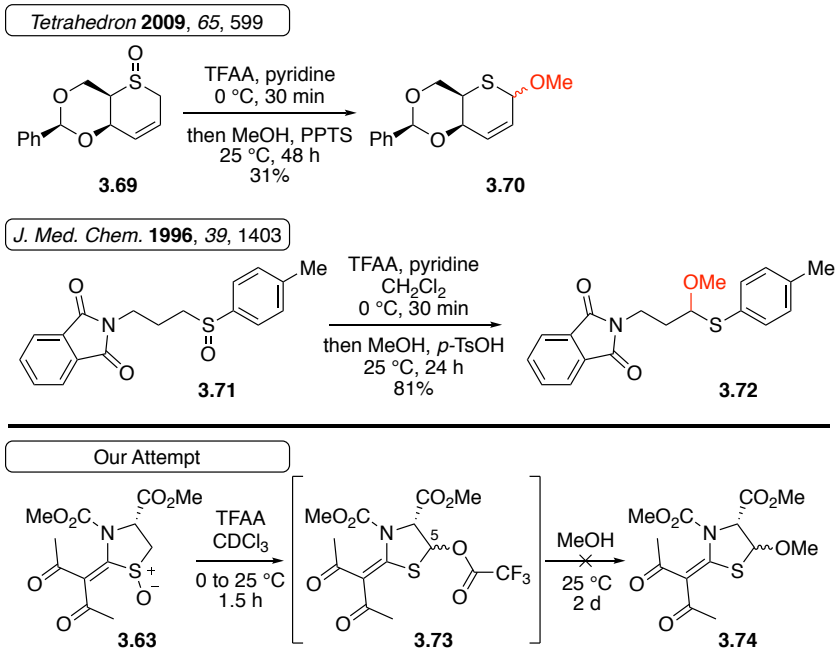


Scheme 43. Silicon Pummerer reaction[180] of the thiazolidine sulfoxide **3.63** and **3.65**



Scheme 44. Attempt to the synthesis of **3.68**

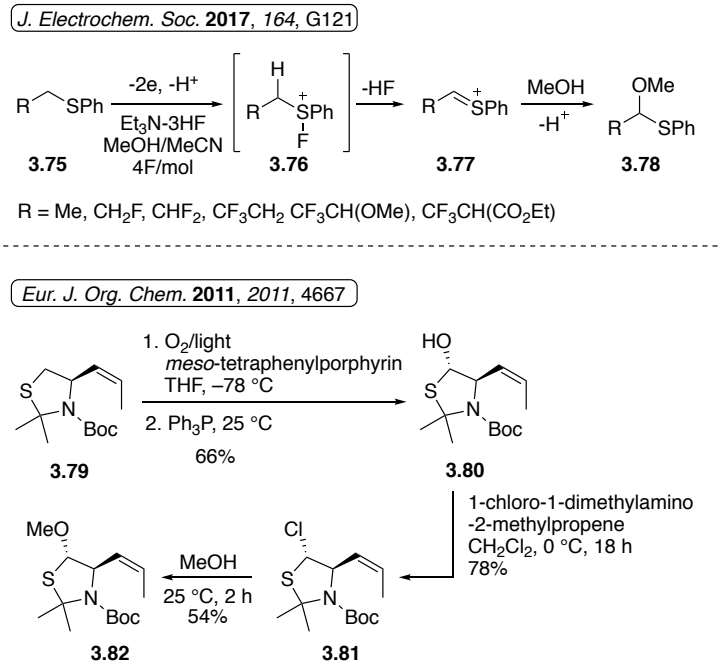
Thus, we turned our attention to the two-step sequence consisting of (1) activation of the α-position of the sulfide and (2) methoxy substitution. There are several reports about this type of transformation[181-182] and a typical procedure includes Pummerer rearrangement of a sulfoxide using trifluoroacetic anhydride and subsequent substitution of the trifluoroacetoxy group to a methoxy functionality (Scheme 45). We applied this procedure to the substrate **3.63** using $CDCl_3$ as a reaction solvent.[183] After exposure of the sulfoxide **3.63** to trifluoroacetic anhydride for 1.5 h, an intermediate was formed and detectable on TLC. The possible structure of the intermediate might be **3.73** due to the appearance of a downfield shifted doublet at 6.65 ppm in the ^{1}H NMR spectrum, which could correspond to the proton at C-5 position. However, the intermediate **3.73** did not react with methanol and it was highly unstable on silica chromatography. Although modified Pummerer-type reactions were attempted using *N*-chlorosuccinimide[184], benzoyl peroxide[185-186], or DDQ-induced oxidative coupling[187], these attempts led to either no reaction or the formation of an unstable intermediate.



Scheme 45. Attempt for the installation of the α-methoxy group to **3.63** according to the literatures[181-182]

In summary, two approaches towards the construction of the functionalized thiazolidine moiety were investigated via the oxa-conjugate addition and the Pummerer rearrangement. The alkylidene thiazolidine backbone was synthesized by the condensation of cysteine with dimethyl thioketene. Although the oxa-conjugate addition reaction of **3.47** with various conditions did not furnish the desired product, we confirmed the installation of the silyloxy group at the α-position of the thiazolidine sulfide via the stereospecific Pummerer rearrangement. In order to complete the synthesis of the α-methoxy thiazolidine scaffold, additional attempts can be undertaken (Scheme 46). One approach would be anodic methoxylation of sulfides using Et_3N-3HF

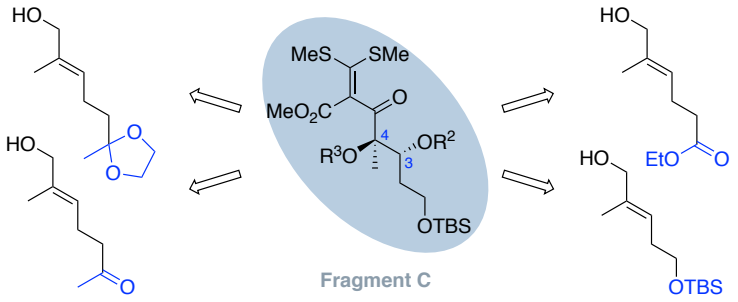
as a supporting salt reported by Isokawa *et al.* in 2017.[188] Fluorosulfonium ion **3.76** would be first formed by a fluoride ion, and the subsequent deprotonation and elimination reactions generate cation intermediate **3.77** which reacts with methanol to provide α-methoxylated product **3.78**. In addition, the α-functionalization could be performed via a singlet oxygen-mediated photooxidation.[189] Treatment with $^1O_2$ to **3.79** with a catalytic amount of sensitizing dye followed by in situ reduction of the corresponding peroxide provided hemiacetal **3.80**. Conversion of compound **3.80** to **3.81** proceeded with Ghosez's chlorenamine reagent[190], and the following solvolysis yielded α-methoxy thiazolidine **3.82**.



Scheme 46. Installation of a methoxy group to a sulfide substrate using a fluoride ion-mediated electrochemistry[188] or a singlet oxygen-mediated photooxidation[189]

3.2.4 Efforts towards the Synthesis of the Alkyl Chain Fragment

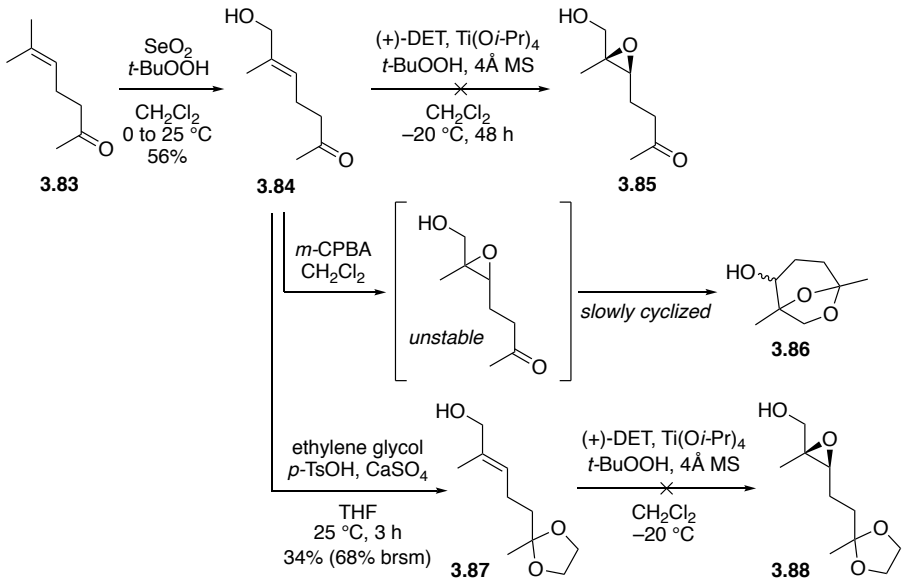
Synthetic studies for the alkyl chain fragment **C** were performed (Scheme 47). In this regard, we envisioned that the stereochemistry at C-3 and C-4 position could be readily installed utilizing Sharpless asymmetric epoxidation.[191] One end of the resulting alkyl chain would be further converted to β-ketoester for the condensation with (*S*)-2-methylcysteine, and the other end would be connected to the cyclohexane fragment **A**.



Scheme 47. Synthetic attempts to the synthesis of fragment **C** from various allylic alcohols

To test the feasibility of this route, known allyl alcohol **3.84** was prepared from 6-methyl-5-hepten-2-one **3.83** according to the reported procedure[192] and subjected to asymmetric Sharpless epoxidation (Scheme 48). However, the desired alcohol **3.85** was not detected during the reaction, and the same result was obtained with the treatment of *m*-chloroperbenzoic acid. After a literature search, we found that the desired structure **3.85** is quite unstable and slowly cyclizes to generate a 7-membered ring followed by epoxide opening to give compound **3.86**.[193-194] In order to prevent cyclization as a side

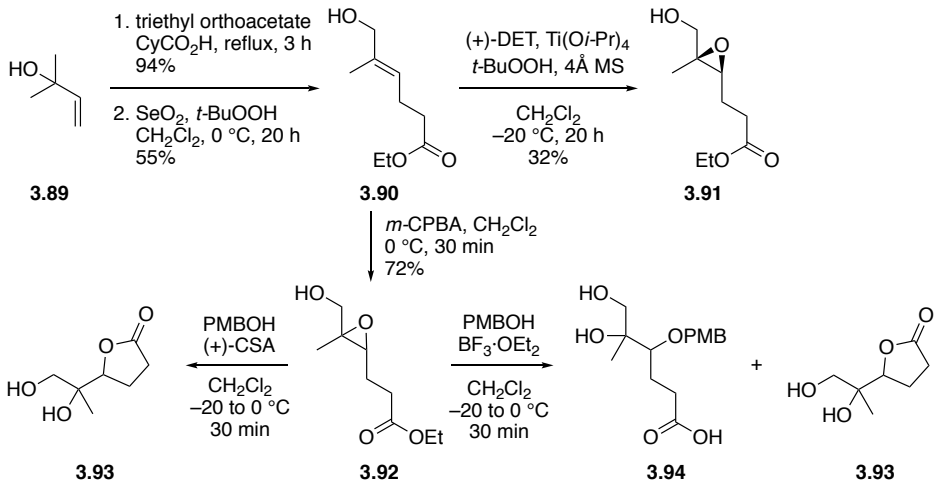
reaction, we decided to protect the ketone functionality using ethylene glycol to have the known allylic alcohol **3.87**[195] with an acetal group. Unfortunately, this substrate **3.87** was not reactive under the Sharpless asymmetric epoxidation condition and no reaction occurred.



Scheme 48. Synthetic study using the allylic alcohols **3.84** and **3.87**

Next, we examined the reactivities of allylic alcohol **3.90** containing an ethyl ester functionality which was prepared from 2-methyl-3-buten-2-ol (**3.89**).[196-197] Sharpless asymmetric epoxidation of the allylic alcohol **3.90** provided the desired epoxide product **3.91** (Scheme 49). To examine the feasibility of an acid-promoted regioselective ring-opening reaction, we prepared the racemic epoxide **3.92** as a substrate by the treatment of *m*-chloroperbenzoic acid to the allylic alcohol **3.90**. When the compound **3.92** was exposed to 4-methoxybenzyl alcohol in the presence of Lewis acid ($BF_3{\cdot}OEt_2$) or Brønsted

acid ((+)-camphorsulfonic acid), the desired regioselective ring-opening was observed but accompanying hydrolysis and cyclization to produce undesired product **3.93** in both cases.

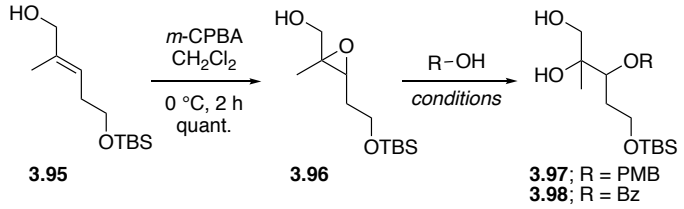


Scheme 49. Synthetic study using the ester-containing allylic alcohol **3.90**

Since the allylic alcohols with ketone, acetal, and ester (**3.84**, **3.87**, and **3.90**) were not effective for this synthetic route, we decided to prepare silyl-protected alcohol **3.95**. After obtaining the alcohol **3.95** according to the reported procedure[145], racemic epoxidation was carried out to furnish compound **3.96** which was subjected to a set of ring-opening conditions as summarized in Table 5. When substrate **3.96** was treated with 4-methoxybenzyl alcohol/$BF_3{\cdot}OEt_2$ combination, as the same condition discussed above, the reaction of compound **3.96** was much slower than that of the ester-containing epoxide **3.92** (entry 1). Most of the starting material remained even when the temperature was raised to 25 °C. ^{1}H NMR spectrum of the product showed that there

were no peaks corresponding to the TBS group, thus suggesting the TBS group is not stable enough under this condition. We utilized (+)-camphorsulfonic acid and titanium(IV) isopropoxide as an alternative acid promoter[198], but similar results were observed (entry 2–3). The reaction with higher temperatures led to complete consumption of the starting material **3.96**, but an unidentifiable product mixture was obtained (entry 4). Considering the low reactivity of 4-methoxybenzyl alcohol under the conditions, we decided to change the nucleophile to benzoic acid, which resulted in the formation of the desired ring-opening product **3.98** with moderate yield (entry 5).

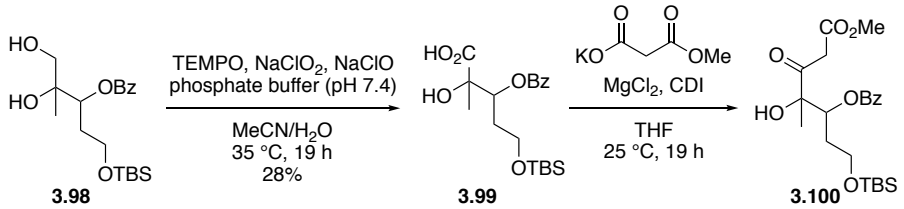
Table 7. Regioselective ring-opening reactions of the allylic alcohol **3.95**



entry	R	conditions	temp / time	results
1	PMB	$BF_3{\cdot}OEt_2$, CH_2Cl_2	–78 to 25 °C, 24 h	mostly starting material
2	PMB	(+)-CSA, CH_2Cl_2	0 to 25 °C, 3 h	mostly starting material
3	PMB	Ti(O*i*-Pr)$_4$, toluene	25 °C, 5 h	mostly starting material
4	PMB	Ti(O*i*-Pr)$_4$, toluene	60 °C, 2 h	unidentifiable mixture
5	Bz	Ti(O*i*-Pr)$_4$, toluene	25 °C, 2 h	desired (**3.98**, 45%)

With the epoxide ring-opening product **3.98** in hand, we performed TEMPO-catalyzed oxidation[199] of the diol **3.98** (Scheme 50). In the presence of $NaClO_2$ as the stoichiometric oxidant, the corresponding carboxylic acid **3.99** was produced which was

further subjected to β-ketoester synthesis by adopting a procedure reported by Masamune and co-workers.[200-201] The carboxylic acid **3.99** was activated by 1,1′-carbonyldiimidazole which was reacted with magnesium methylmalonate to furnish the desired β-ketoester product **3.100**.



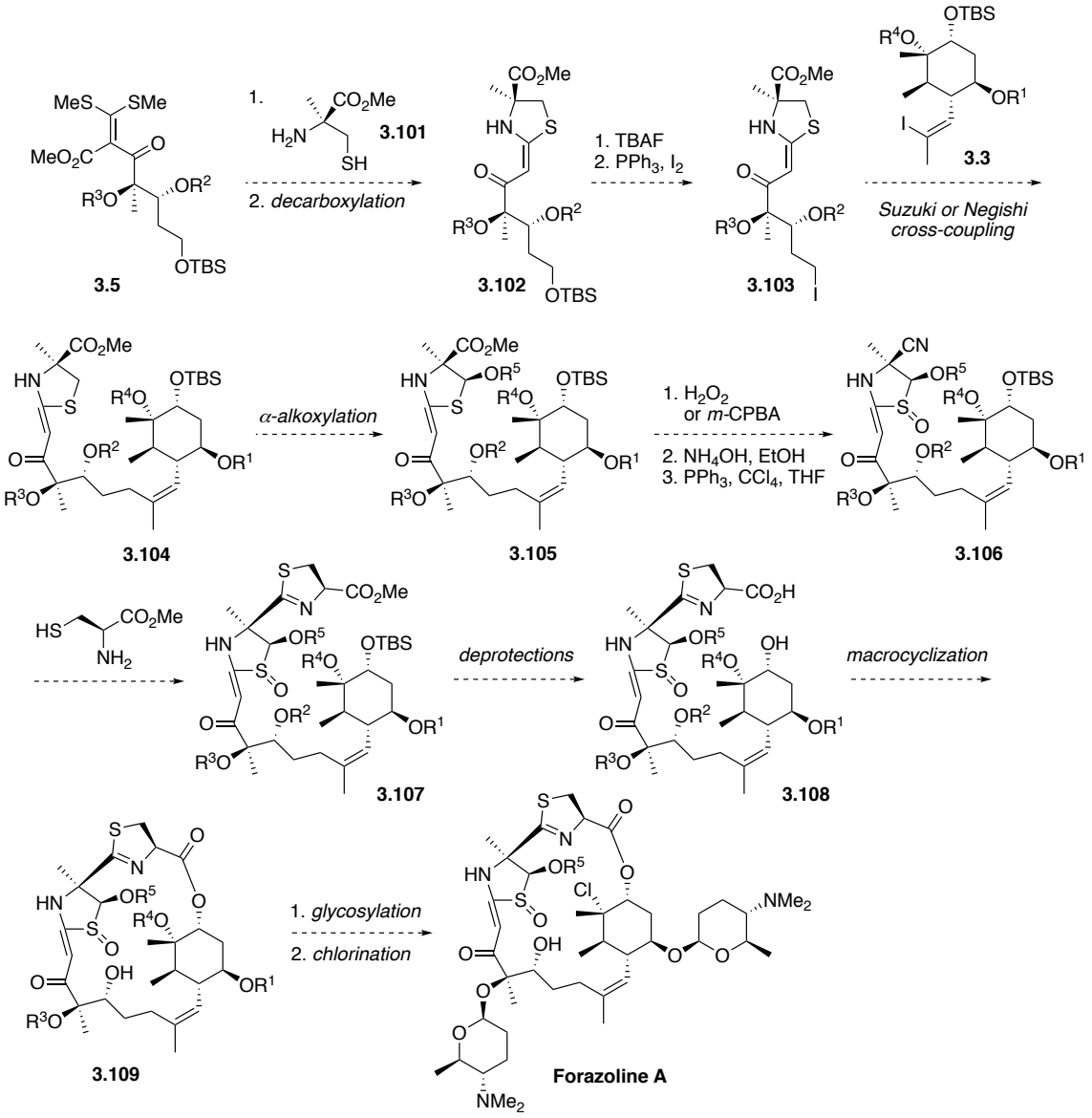
Scheme 50. Synthesis of the alkyl fragment **C** from alcohol **3.98**

In summary, the approach for the construction of alkyl fragment **C** scaffold was described which utilized Sharpless asymmetric epoxidation, acid-promoted epoxide opening, and dimethyl thioketene formation. After achieving enantioselective synthesis of the β-ketoester scaffold, the thioketene formation using CS_2/MeI followed by the condensation with 2-methylcysteine would be carried out to generate thiazolidine moiety.

3.2.5 Future Work towards Fragment Assembly

We have accomplished the synthesis of the functionalized cyclohexane **3.28** and the racemic construction of the alkyl chain part **3.100**, along with the investigation of the

synthesis of a α-methoxy thiazolidine moiety. Although we have not yet identified an effective strategy for the synthesis of the highly unusual and unprecedented α-methoxy thiazolidine sulfoxide, the assembly plan towards total synthesis of forazoline A using the three fragment **A**–**C** is outlined here (Scheme 51).



Scheme 51. Synthetic plan for the assembly of the fragment **A**–**C**

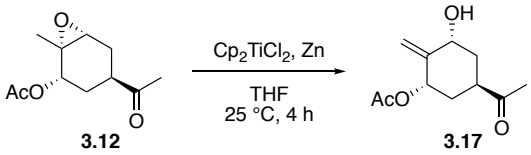
Fragment assembly would commence with condensation between dimethyl thioketene **3.5** and 2-methylcysteine methylester (**3.101**) followed by decarboxylation. The resulting ketene *N*,*S*-acetal **3.102** would be subjected to silyl deprotection and Appel reaction to prepare alkyl iodide **3.103**. We envision that a Suzuki or Negishi cross-coupling reaction of the iodide **3.103** with alkenyl iodide **3.3** would effectively generate the tri-substituted (*Z*)-alkene functionality, which would assemble the alkyl chain fragment **C** and the cyclohexane fragment **A**. This type of tri-substituted (*Z*)-alkene synthesis is well studied in literatures.[202-204] Next, oxidation of thioether **3.104** with H_2O_2 or *m*-CPBA would provide the corresponding sulfoxide, which could be possibly transformed into α-alkoxy thiazolidine. The ester **3.105** would be next converted to cyanide **3.106** followed by condensation with L-cysteine methylester to give **3.107**.[205] Subsequent hydrolysis and TBS deprotection would furnish substrate **3.108** which will be subjected to macrolactonization. Finally, glycosylation with two forosamines[206-207] and chlorination would finish the total synthesis of forazoline A.

3.3 Conclusion

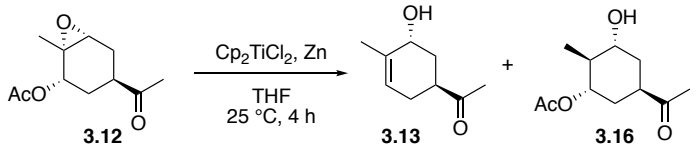
In conclusion, we have been investigating the synthesis of three major fragments **A**–**C** of forazoline A. Construction of the highly functionalized cyclohexane ring in fragment A was efficiently accomplished utilizing Mukaiyama aldol, Bayer–Villeger reaction, and addition of lithium dimethylcuprate. Among five stereocenters on the cyclohexane, four stereocenters were successfully transferred with the desired stereochemistry from the enantiomerically rich (*S*)-carvone. Synthetic studies towards fragment B were also carried out using oxa-conjugate addition and Pummerer-type reactions. After the construction of the ketene *N*,*S*-acetal moiety by the condensation of cysteine with dimethyl thioketene, attempts to oxa-conjugate addition of the thiazolidine **3.47** containing α,β-unsaturated ester were described. In addition, various Pummerer rearrangement conditions were investigated and we achieved the synthesis of the α-silyloxylated thiazolidine **3.64**. Finally, an advanced precursor to fragment **C** was synthesized in a racemic form to determine the synthetic feasibility of the reactions. We confirmed that epoxidation of allylic alcohol **3.95** followed by regioselective epoxide opening and β-ketoester formation furnished the desired **3.100**. Therefore, these initial studies will be a milestone towards the assembly of the three fragment **A**–**C** which would complete the total synthesis of forazoline A.

3.4 Experimental Sections

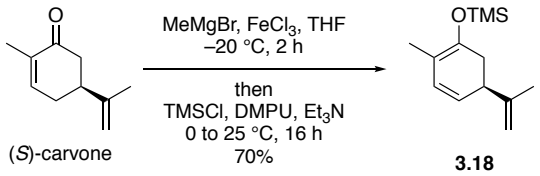
All reactions were conducted in oven-dried glassware under nitrogen. Unless otherwise stated all reagents were purchased from commercial suppliers and used without further purification. All solvents were American Chemical Society (ACS) grade or better and used without further purification except tetrahydrofuran (THF), which was freshly distilled from sodium/benzophenone each time before use. Analytical thin layer chromatography (TLC) was performed with glass backed silica gel (60 Å) plates with fluorescent indication (Whatman). Visualization was accomplished by UV irradiation at 254 nm, or by staining with *p*-anisaldehyde solution or potassium permanganate solution followed by heating. Flash column chromatography was performed by using silica gel (particle size 230−400 mesh, 60 Å). All ^{1}H NMR spectra were recorded with a Varian 400 (400 MHz) and a Bruker 500 (500 MHz) spectrometer. All NMR δ values are given in parts per million (ppm) and are referenced to the residual isotopomer solvent signals ($CHCl_3$: δ = 7.26 ppm, CD_2HOD: δ = 3.31 ppm) for ^{1}H NMR spectra. Coupling constants (*J*) are given in Hertz (Hz) and multiplicities are indicated using the conventional abbreviation (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of non-equivalent resonances, br = broad).



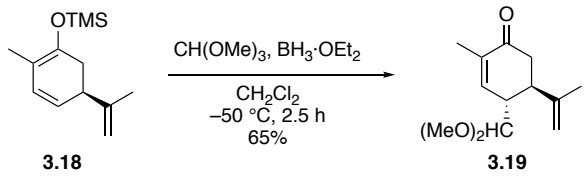
Titanocene dichloride Cp_2TiCl_2 (117 mg, 0.471 mmol) and powdered Zn (97 mg, 1.56 mmol) were placed in a round-bottomed flask under an argon atmosphere. Anhydrous fresh distilled and deoxygenated THF (0.6 mL) was added, and stirring was maintained for 1 h at 25 °C. To the reaction mixture, a solution of **3.12** (50 mg, 0.236 mmol) in fresh distilled and deoxygenated THF (2.4 mL) was added at 25 °C. After stirring for 4 h at 25 °C, the mixture was quenched with saturated aqueous NaH_2PO_4 (2 mL) and brine (2 mL) and stirred for 30 min. The reaction mixture was diluted with diethyl ether and then filtered through a pad of Celite. The filtrate was washed with brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 4/1) to afford **3.17** as colorless oil: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.48 (t, J = 4.1 Hz, 1H), 5.22 (s, 1H), 5.20 (s, 1H), 4.41 (q, J = 4.7 Hz, 1H), 3.11–3.20 (m, 1H), 2.22 (s, 3H), 2.05–2.14 (m, 6H), 1.85 (ddd, J = 14.1, 10.4, 3.6 Hz, 1H), 1.77 (ddd, J = 13.8, 10.6, 3.4 Hz, 1H).



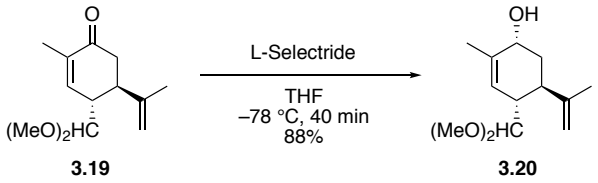
Titanocene dichloride Cp_2TiCl_2 (117 mg, 0.471 mmol) and powdered Zn (97 mg, 1.56 mmol) were placed in a round-bottomed flask under an argon atmosphere. Anhydrous fresh distilled and deoxygenated THF (0.6 mL) was added, and stirring was maintained for 1 h at 25 °C. The reaction mixture was added to a solution of **3.12** (50 mg, 0.236 mmol) in fresh distilled and deoxygenated THF (2.4 mL) at 25 °C. After stirring for 6 h at 25 °C, the mixture was quenched with saturated aqueous NaH_2PO_4 (2 mL) and brine (2 mL) and stirred for 30 min. The reaction mixture was diluted with diethyl ether and then filtered through a pad of Celite. The filtrate was washed with brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.13** and **3.16** as colorless oil; ^{1}H NMR spectrum data of **3.16** is in accordance with the reported data.[146]; **3.13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14–5.24 (m, 1H), 3.86–3.97 (m, 1H), 2.86–3.00 (m, 1H), 2.41 (d, J = 8.4 Hz, 1H), 2.19 (s, 3H), 2.07–2.16 (m, 6H), 1.60–1.68 (m, 1H), 1.47–1.54 (m, 1H), 1.08 (d, J = 7.1 Hz, 3H).



To a cooled (–20 °C) solution of $FeCl_3$ (24 mg, 0.146 mmol) in THF (12 mL) was added methylmagnesium bromide solution (3.0 M in diethyl ether, 4.88 mL, 14.645 mmol). To this mixture, a solution of (*S*)-carvone (1.0 g, 6.657 mmol) in THF (3.6 mL) was added over 2 h. After stirring 1.5 h at –20 °C, the mixture was allowed to warm to 0 °C. Trimethylsilyl chloride (1.17 mL, 9.187 mmol), DMPU (0.97 mL, 7.988 mmol), and triethylamine (1.21 mL, 8.654 mmol) were added sequentially at 0 °C. The resulting mixture was allowed to warm to 25 °C and stirred for 12 h. Saturated aqueous $NaHCO_3$ solution (10 mL) was added at 0 °C. The mixture was filtered through a pad of Celite and the filtrate was diluted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 50/1) to afford **3.18** (1.03 g, 70%) as colorless oil. The enantiomer of **3.18** is the known compound and ^{1}H NMR spectrum data is in accordance with the reported data.[148]

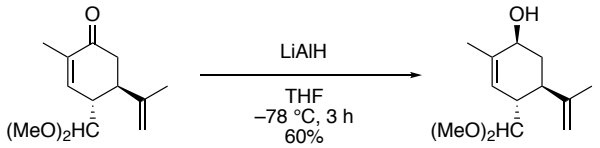


To a cooled (–50 °C) solution of **3.18** (1.03 g, 4.627 mmol) in CH_2Cl_2 (16 mL) were added trimethyl orthoformate (0.61 mL, 5.552 mmol) and boron trifluoride diethyl etherate (0.63 mL, 5.089 mmol). After stirring 2.5 h at –50 °C, saturated aqueous $NaHCO_3$ solution was added at 0 °C. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 20/1) to afford **3.19** (670 mg, 65%) as colorless oil. The enantiomer of **3.19** is the known compound and ^{1}H NMR spectrum data is in accordance with the reported data.[148]



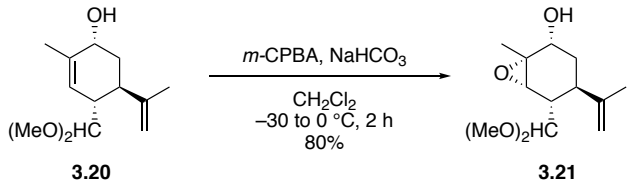
To a cooled (–78 °C) solution of **3.19** (2.80 g, 12.483 mmol) in THF (250 mL) was added L-Selectride (1.0 M in THF, 15.0 mL, 14.980 mmol) over 15 min and the resulting mixture was stirred for 40 min at –78 °C. 6 M NaOH (15 mL) and hydrogen peroxide solution (30 wt. % in H_2O, 15 mL) were added dropwise at 0 °C and the mixture was stirred for 1 h at 25 °C. The layers were separated, and the aqueous layer was extracted with EtOAc. The

combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford **3.20** (2.49 g, 88%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (s, 1H), 4.79–4.85 (m, 2H), 4.20 (d, J = 2.7 Hz, 1H), 3.92 (t, J = 3.2 Hz, 1H), 3.43 (s, 3H), 3.39 (s, 3H), 2.43 (dd, J = 10.3, 3.1 Hz, 1H), 2.34–2.40 (m, 1H), 1.82 (s, 3H), 1.74–1.81 (m, 2H), 1.71 (s, 3H).

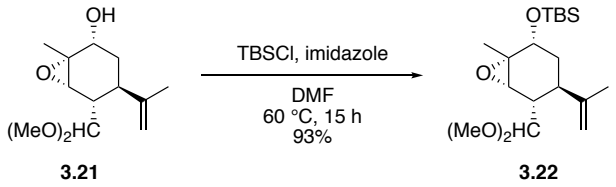


3.19 **3.25**

To a cooled (–78 °C) solution of **3.19** (25 mg, 0.111 mmol) in THF (1 mL) was added lithium aluminum hydride solution (2.0 M in THF, 0.07 mL, 0.1337 mmol) dropwise. After stirring 3 h at –78 °C, the reaction was quenched with water at 0 °C. The resulting mixture was diluted with EtOAc and water. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford **3.25** (15 mg, 60%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (s, 1H), 4.81 (s, 2H), 4.16–4.22 (m, 1H), 4.14 (d, J = 3.4 Hz, 6H), 3.42 (s, 3H), 3.38 (s, 3H), 2.47–2.53 (m, 1H), 2.36–2.43 (m, 1H), 2.01 (ddd, J = 12.2, 5.7, 2.8 Hz, 1H), 1.80 (s, 3H), 1.74 (s, 3H), 1.68 (td, J = 12.0, 9.0, 1H).

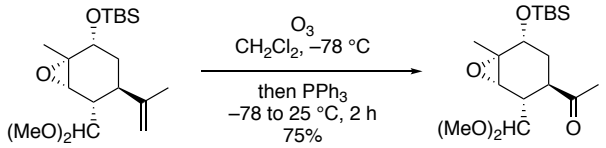


To a cooled (–30 °C) solution of **3.20** (2.29 g, 10.118 mmol) in CH_2Cl_2 (100 mL) were added sodium bicarbonate (1.28 g, 15.177 mmol) and *m*-chloroperbenzoic acid (≤77%, 2.72 g, 12.141 mmol). The reaction mixture was allowed to warm to 0 °C over 2 h. Saturated aqueous $Na_2S_2O_3$ solution and saturated aqueous $NaHCO_3$ solution were added at 0 °C. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (× 2), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.21** (1.96 g, 80%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82 (s, 1H), 4.68 (s, 1H), 4.39 (d, J = 6.2 Hz, 1H), 3.80 (dd, J = 6.2, 5.4 Hz, 1H), 3.48 (s, 3H), 3.37 (d, J = 3.2 Hz, 1H), 3.33 (s, 3H), 2.27–2.34 (m, 1H), 2.22 (td, J = 6.1, 3.3 Hz, 1H), 1.84 (br s, 1H), 1.71 (s, 3H), 1.66 (ddd, J = 13.6, 8.2, 5.2 Hz, 1H), 1.48 (ddd, J = 13.7, 6.4, 3.2 Hz, 1H), 1.41 (s, 3H).



To a solution of **3.21** (55 mg, 0.227 mmol) in DMF (0.3 mL) were added imidazole (31 mg, 0.454 mmol) and *tert*-butyldimethylsilyl chloride (51 mg, 0.341 mmol) at 25 °C. The

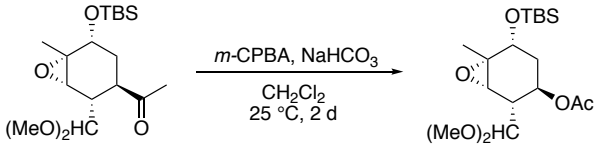
resulting mixture was heated to 60 °C and stirred at the same temperature for 12 h. The reaction mixture was cooled to 25 °C and then diluted with water and EtOAc. The layers were separated, and the organic layer was washed with water (× 3), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 10/1) to afford **3.22** (75 mg, 93%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (s, 1H), 4.60 (s, 1H), 4.51 (d, J = 8.6 Hz, 1H), 3.83 (dd, J = 10.2, 5.5 Hz, 1H), 3.49 (s, 3H), 3.25 (s, 3H), 3.17 (d, J = 4.8 Hz, 1H), 2.35 (dd, J = 8.6, 4.8 Hz, 1H), 2.26–2.32 (m, 1H), 1.75 (s, 3H), 1.53–1.62 (m, 2H), 1.30 (s, 3H), 0.90 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H).



3.22 **3.23**

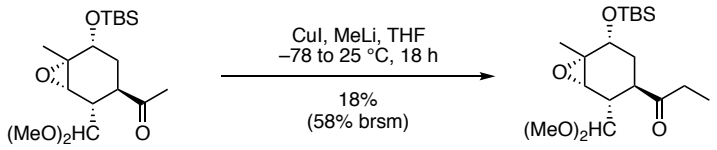
A solution of **3.22** (75 mg, 0.210 mmol) in CH_2Cl_2 (2 mL) was cooled to –78 °C. Ozone was bubbled through the solution until a blue color developed. After 5 min, the excess of ozone was removed with an O_2 purge, and triphenyl phosphine (110 mg, 0.421 mmol) was added. The solution was allowed to warm to 25 °C and stirred for 2 h. The solvent was removed under vacuum, and the residue was diluted with diethyl ether and brine. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 5/1) to afford **3.23** (56 mg, 75%) as colorless oil: ^{1}H NMR (400 MHz,

$CDCl_3$) δ 4.44 (d, *J* = 8.8 Hz, 1H), 3.76 (dd, *J* = 8.9, 5.6 Hz, 1H), 3.46 (s, 3H), 3.26 (s, 3H), 3.14 (d, *J* = 4.0 Hz, 1H), 2.74 (q, *J* = 4.6 Hz, 1H), 2.63 (dt, *J* = 8.8, 3.4 Hz, 1H), 2.16 (s, 3H), 1.69–1.82 (m, 2H), 1.31 (s, 3H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H).



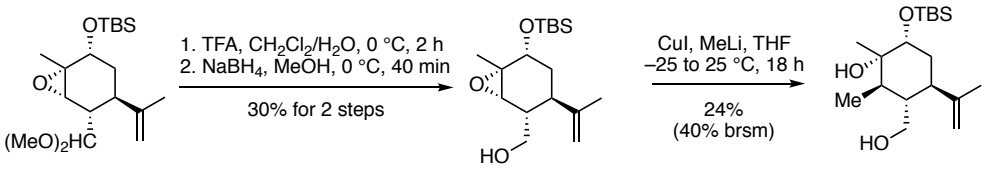
3.23 **3.24**

To a solution of **3.23** (25 mg, 0.070 mmol) in CH_2Cl_2 were added $NaHCO_3$ (9 mg, 0.105 mmol) and *m*-chloroperbenzoic acid (≤77%, 19 mg, 0.084 mmol). After stirring 2 days at 25 °C, saturated aqueous $Na_2S_2O_3$ solution was added. After stirring 20 min at 25 °C, the organic layer was washed with saturated aqueous $NaHCO_3$ solution (× 2). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 5/1) to afford **3.24** as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97–5.02 (m, 1H), 4.41 (d, *J* = 8.9 Hz, 1H), 4.11 (dd, *J* = 10.5, 5.1 Hz, 1H), 3.47 (s, 3H), 3.35 (s, 3H), 3.12 (d, *J* = 4.4 Hz, 1H), 2.23 (dd, *J* = 9.0, 4.4 Hz, 1H), 2.03 (s, 3H), 1.86 (dt, *J* = 13.9, 5.0 Hz, 1H), 1.61 (dd, *J* = 13.7, 10.7 Hz, 1H), 1.41 (s, 3H), 0.90 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H).



3.23 **3.30**

To a cooled (–15 °C) solution of copper iodide (35 mg, 0.18 mmol) in THF (0.36 mL) was added methyllithium solution (1.6 M in diethyl ether, 0.23 mL, 0.36 mmol) dropwise. After stirring 20 min at –15 °C, a solution of **3.23** (13 mg, 0.036 mmol) in THF (0.36 mL) was added dropwise at –78 °C. The resulting mixture was allowed to warm to 25 °C and stirred for 18 h. The reaction mixture was quenched with water and then filtered through a pad of Celite. The filtrate was diluted with EtOAc and water, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.30** as colorless oil: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.46 (d, J = 8.8 Hz, 1H), 3.81 (dd, J = 8.9, 5.2 Hz, 1H), 3.47 (s, 3H), 3.27 (s, 3H), 3.17 (d, J = 3.7 Hz, 1H), 2.72–2.81 (m, 1H), 2.59–2.64 (m, 1H), 2.40–2.55 (m, 2H), 1.76 (ddd, J = 14.6, 8.7, 4.5 Hz, 1H), 1.65–1.72 (m, 1H), 1.34 (s, 3H), 1.07 (t, J = 7.2 Hz, 3H), 0.92 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H).

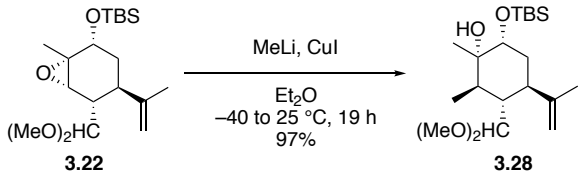


3.22 **3.26** **3.29**

[Deprotection/ Reduction] To a cooled (0 °C) solution of **3.22** (15 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (0.25 mL) dropwise. After stirring 2 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was dissolved in MeOH and the reaction mixture was cooled to 0 °C. Sodium borohydride was added, and the resulting suspension was stirred for 40 min at 0 °C. The reaction mixture was concentrated *in vacuo* and the residue was diluted with EtOAc and water. The organic layer was separated, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.26** (4 mg, 30%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.83 (s, 1H), 4.69 (s, 1H), 3.95 (t, J = 6.1 Hz, 1H), 3.79 (dd, J = 10.7, 7.2 Hz, 1H), 3.73 (dd, J = 10.8, 5.6 Hz, 1H), 3.21 (d, J = 3.4 Hz, 1H), 2.19–2.25 (m, 1H), 2.12–2.19 (m, 1H), 1.72 (s, 3H), 1.53–1.58 (m, 2H), 1.34 (s, 3H), 0.91 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H).

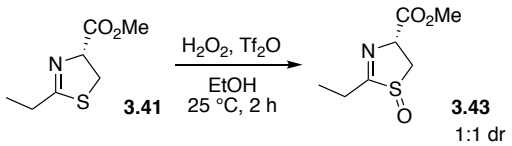
[Epoxide Opening] To a cooled (–20 °C) solution of copper iodide (24 mg, 0.128 mmol) in THF (0.3 mL) was added methyllithium solution (1.6 M in diethyl ether, 0.16 mL, 0.256 mmol) dropwise. After stirring 15 min at –20 °C, a solution of **3.26** (4 mg, 0.0128 mmol) in THF (0.3 mL) was added dropwise at –25 °C. After stirring 1 h at –25 °C, the

reaction mixture was allowed to warm to 25 °C and stirred for 17 h. The reaction mixture was quenched with water and then filtered through a pad of Celite. The filtrate was diluted with EtOAc and water, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.29** (1.0 mg, 24%, 40% brsm) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.83 (s, 1H), 4.79 (s, 1H), 3.67 (dd, J = 3.2, 2.3 Hz, 1H), 3.64 (dd, J = 11.3, 3.5 Hz, 1H), 3.60 (dd, J = 11.8, 2.4 Hz, 1H), 2.61 (td, J = 12.2, 3.7 Hz, 1H), 2.24–2.32 (br s, 1H), 1.85 (dd, J = 11.3, 6.9 Hz, 1H), 1.72 (dd, J = 12.8, 2.1 Hz, 1H), 1.66 (s, 3H), 1.59–1.65 (m, 2H), 1.05 (s, 3H), 1.03 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 0.10 (s, 6H).

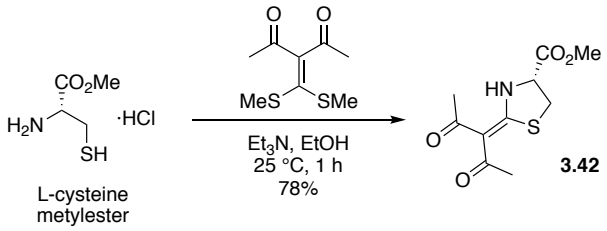


To a cooled (–20 °C) solution of copper iodide (80 mg, 0.421 mmol) in diethyl ether (0.42 mL) was added methyllithium solution (1.6 M in diethyl ether, 0.53 mL, 0.841 mmol) dropwise. After stirring 15 min at –20 °C, a solution of **3.22** (15 mg, 0.0421 mmol) in diethyl ether (0.42 mL) was added dropwise at –40 °C. After stirring 2 h at –40 °C, the reaction mixture was allowed to warm to 25 °C and stirred for 17 h. The reaction mixture was quenched with water and then filtered through a pad of Celite. The filtrate was diluted with EtOAc and water, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel,

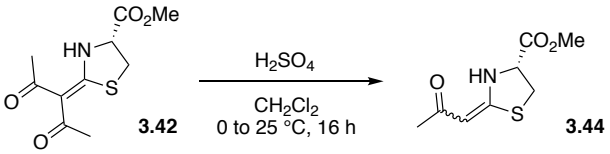
hexanes/EtOAc, 15/1) to afford **3.28** (15 mg, 97%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.77–4.80 (m, 1H), 4.75–4.77 (m, 1H), 4.19 (d, J = 0.9 Hz, 1H), 3.63 (d, J = 2.2 Hz, 1H), 3.45 (s, 3H), 3.34 (s, 3H), 2.52 (td, J = 12.2, 4.4 Hz, 1H), 2.17 (s, 1H), 2.01 (dd, J = 11.0, 6.8 Hz, 1H), 1.66 (s, 3H), 1.57–1.64 (m, 2H), 1.46 (t, J = 11.4 Hz, 1H), 1.05 (d, J = 6.8 Hz, 3H), 1.03 (s, 3H), 0.93 (s, 9H), 0.09 (s, 3H), 0.09 (s, 3H).



To a solution of **3.41** (31 mg, 0.179 mmol) in ethanol (0.9 mL) were added hydrogen peroxide (30 wt% in H_2O, 37 μL, 0.358 mmol) and triflic anhydride (89 μL, 0.089 mmol). After stirring 2 h at 25 °C, the reaction mixture was diluted with EtOAc and water. The layers were separated, and the aqueous layer was extracted with EtOAc (× 4). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to afford **3.43** as a crude product: ^{1}H NMR (400 MHz, CD_3OD) δ 4.92 (dd, J = 8.7, 3.8 Hz, 1H, diastereomer A), 4.77 (dd, J = 7.7, 5.2 Hz, 1H, diastereomer B), 4.10 (dd, J = 14.7, 3.8 Hz, 1H, diastereomer A), 3.96 (dd, J = 14.7, 8.7 Hz, 1H, diastereomer A), 3.73–3.79 (m, 6H), 3.68 (dd, J = 14.3, 5.2 Hz, 1H, diastereomer B), 3.49 (dd, J = 14.3, 7.8 Hz, 1H, diastereomer B), 2.21–2.35 (m, 4H), 1.09–1.19 (m, 6H).

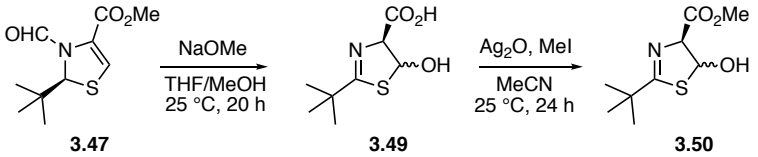


To a solution of diemthyl thioketene[160] (30 mg, 0.147 mmol) in ethanol (1.5 mL) were added cysteine methylester hydrochloric acid (25 mg, 0.147 mmol) and triethylamine (0.10 mL, 0.735 mmol). After stirring 1 h at 25 °C, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 40/1) to afford **3.42** (28 mg, 78%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.74–4.79 (m, 1H), 3.78 (s, 3H), 3.27–3.32 (m, 2H), 2.47 (s, 3H), 2.45 (s, 3H).

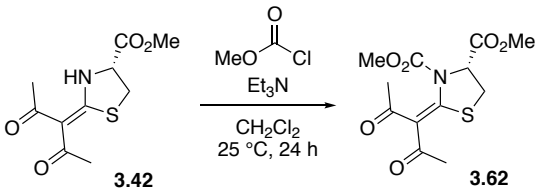


To a cooled (0 °C) solution of **3.42** (27 mg, 0.111 mmol) in CH_2Cl_2 (1 mL) was added concentrated sulfuric acid (0.03 mL, 0.444 mmol). After stirring 10 min at 0 °C, the reaction mixture was allowed to warm to 25 °C. After stirring 16 h at 25 °C, the reaction mixture was diluted with CH_2Cl_2 and neutralized by carefully adding saturated aqueous $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*

to afford **3.44** as a crude product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1H), 4.71 (dd, J = 7.3, 5.7 Hz, 1H), 3.80 (s, 3H), 3.43–3.49 (m, 2H), 2.06 (s, 3H).

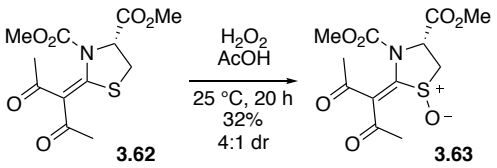


[Oxa-conjugate addition] To a solution of **3.47** (35 mg, 0.153 mmol) in THF/MeOH (1/1, 2.7 mL) was added sodium methoxide (33 mg, 0.61 mmol) at 25 °C. After stirring 20 h at 25 °C, the suspension was concentrated. **[Methylation]** The residue was dissolved in acetonitrile (2 mL) and Ag_2O (106 mg, 0.459 mmol) and MeI (0.10 mL, 1.53 mmol) were added at 25 °C. After stirring 24 h at 25 °C, the mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 1/1) to afford **3.50** as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (dd, J = 5.6, 4.5 Hz, 1H), 4.12 (d, J = 4.3 Hz, 1H), 4.11 (d, J = 5.9 Hz, 1H), 3.92 (s, 3H), 1.05 (s, 9H).

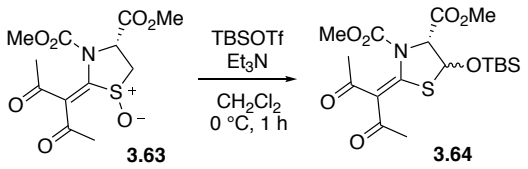


To a solution of **3.42** (40 mg, 0.164 mmol) in CH_2Cl_2 (2 mL) were added methyl chloroformate (19 μL, 0.250 mmol) and triethylamine (70 μL, 0.493 mmol). After stirring

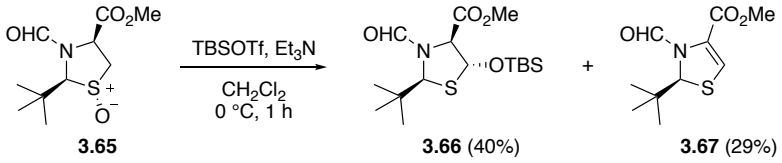
6 h at 25 °C, additional methyl chloroformate (19 μL, 0.250 mmol) and triethylamine (70 μL, 0.493 mmol) were added. After stirring 18 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc/CH_2Cl_2, 1/1/1) to afford **3.62** as yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.54 (d, J = 7.3 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.30 (dd, J = 11.7, 1.3 Hz, 1H), 3.24 (dd, J = 11.9, 7.5 Hz, 1H), 2.42 (s, 3H), 2.34 (s, 3H).



To a solution of **3.62** (22 mg, 0.073 mmol) in acetic acid (1 mL) was added hydrogen peroxide (30 wt% in H_2O, 22 μL, 0.219 mmol). After stirring 20 h at 25 °C, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 40/1) to afford **3.63** (7 mg, 32%) as colorless oil: Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (dd, J = 9.9, 6.2 Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.57 (dd, J = 13.9, 6.2 Hz, 1H), 2.79 (dd, J = 13.9, 9.9 Hz, 1H), 2.45 (s, 3H), 2.36 (s, 3H).

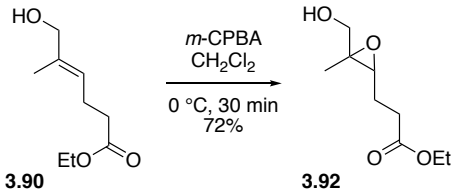


To a solution of **3.63** (7 mg, 0.022 mmol) in CH_2Cl_2 (0.5 mL) were added triethylamine (4 μL, 0.029 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (6 μL, 0.027 mmol) at 0 °C. After stirring 1 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 4/1) to afford **3.64** (less than 1 mg) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (s, 1H), 5.41 (s, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 0.83 (s, 9H), 0.15 (s, 3H), 0.11 (s, 3H).

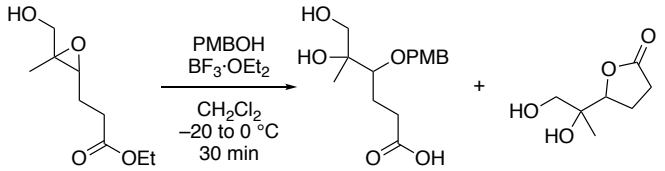


To a solution of **3.62** (60 mg, 0.243 mmol) in CH_2Cl_2 (2.4 mL) were added triethylamine (44 μL, 0.315 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (67 μL, 0.291 mmol) at 0 °C. After stirring 1 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 8/1) to afford **3.66** (35 mg, 40%) and

3.67 (16 mg, 29%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30–8.45 (m, 1H), 5.87–5.93 (m, 1H), 5.25–5.50 (m, 1H), 4.56–5.18 (m, 1H), 3.68–3.82 (m, 3H), 0.84–1.00 (m, 18H), 0.07–0.15 (m, 6H); B: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.07 (s, 1H), 5.82 (s, 1H), 3.82 (s, 3H), 0.95 (s, 9H).

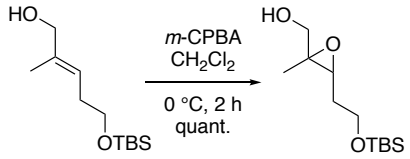


To a cooled (0 °C) solution of **3.90** (119 mg, 0.691 mmol) in CH_2Cl_2 (2.3 mL) was added *m*chloroperbenzoic acid (≤77%, 232 mg, 1.07 mmol). After stirring 30 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and quenched with saturated aqueous $NaHCO_3$ solution. The layers were separated, and the organic layer was washed with saturated aqueous $Na_2S_2O_3$ solution and saturated aqueous $NaHCO_3$ solution. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/2) to afford **3.92** (94 mg, 72%) as colorless oil.



3.92 **3.94** **3.93**

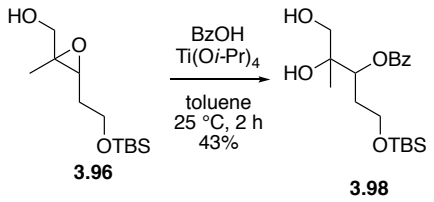
To a cooled (–20 °C) solution of **3.92** (12 mg, 0.064 mmol) in CH_2Cl_2 (0.6 mL) were added 4-methoxybenzyl alcohol (88 mg, 0.64 mmol) and boron trifluoride diethyl etherate (4 μL, 0.032 mmol). The reaction was allowed warm to 0 °C over 30 min. The reaction mixture was diluted with CH_2Cl_2 and water. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 40/1) to afford **3.94** (7 mg, 37%) and **3.93** as colorless oil; **3.94**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 4.54 (t, J = 7.4 Hz, 1H), 4.49 (d, J = 11.4 Hz, 1H), 4.44 (d, J = 11.5 Hz, 1H), 3.80 (s, 3H), 3.47 (d, J = 9.2 Hz, 1H), 3.33 (d, J = 9.2 Hz, 1H), 2.46–2.56 (m, 2H), 2.09–2.29 (m, 2H), 1.19 (s, 3H); **3.93**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.50 (t, J = 7.5 Hz, 1H), 3.65 (d, J = 11.1 Hz, 1H), 3.50 (d, J = 11.0 Hz, 1H) 2.48–2.62 (m, 2H), 2.13–2.35 (m, 2H), 1.23 (s, 3H).



3.95 **3.96**

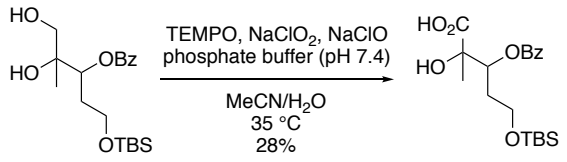
To a cooled (0 °C) solution of **3.95** (320 mg, 1.39 mmol) in CH_2Cl_2 (14 mL) was added *m*-chloroperbenzoic acid (≤77%, 370 mg, 1.67 mmol). After stirring 2 h at 0 °C, saturated

aqueous $Na_2S_2O_3$ solution and CH_2Cl_2 were added at 0 °C. The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ solution (× 2). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford **3.96** (342 mg, quant.) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75–3.82 (m, 2H), 3.70 (d, J = 12.2 Hz, 1H), 3.58 (d, J = 12.2 Hz, 1H), 3.18 (t, J = 6.2 Hz, 1H), 1.71–1.91 (m, 2H), 1.30 (s, 3H), 0.90 (s, 9H), 0.06 (s, 6H).



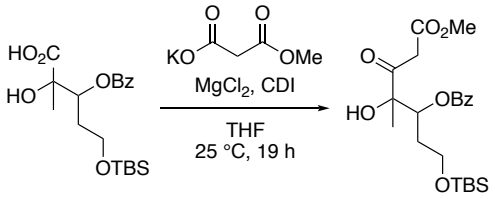
To a solution of **3.96** (200 mg, 0.812 mmol) in toluene (8 mL) were added benzoic acid (990 mg, 8.12 mmol) and titanium(IV) isopropoxide (0.24 mL, 0.82 mmol). After stirring 2 h at 25 °C, the reaction mixture was diluted with EtOAc and saturated aqueous $NaHCO_3$ solution. The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to afford **3.98** (130 mg, 43%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.4 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.7 Hz, 2H), 5.22 (dd, J = 8.5, 3.7 Hz, 1H), 3.67–3.80 (m, 2H), 3.48 (d, J = 12.0 Hz, 1H), 3.32 (d, J = 12.1 Hz,

1H), 2.23 (dddd, J = 14.8, 9.5, 5.9, 3.6 Hz, 1H), 1.93 (ddt, J = 14.5, 8.6, 4.4 Hz, 1H), 1.18 (s, 3H), 0.86 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H).



3.98 **3.99**

To a solution of **3.98** (130 mg, 0.353 mmol) in MeCN/H_2O (4/1, 5 mL) were added phosphate buffer solution (2 mL, 1.0 M, pH 7.4), TEMPO (28 mg, 0.176 mmol), and $NaClO_2$ (191 mg, 0.116 mmol). The resulting biphasic mixture was slowly heated to 35 °C and a solution of NaOCl in H_2O (0.4 mL 5% NaOCl in 1.2 mL H_2O) was added over 1 h. After stirring 18 h at 35 °C, the reaction mixture was cooled to 25 °C. The reaction mixture was diluted with CH_2Cl_2 and saturated aqueous NH_4Cl solution. The layers were separated, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo.* The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 20/1, 1% AcOH) to afford **3.99** (38 mg, 28%) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.5 Hz, 2H), 7.59 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.7 Hz, 2H), 5.58 (t, J = 5.3 Hz, 1H), 3.58–3.76 (m, 2H), 1.98–2.18 (m, 2H), 1.50 (s, 3H), 0.87 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H).



3.99 **3.100**

Magnesium chloride (3 mg, 0.031 mmol) and potassium monomethyl malonate (6 mg, 0.041 mmol) were suspended in THF (0.3 mL) and heated at 65 °C for 3 h. After the above reaction had proceeded for 1 h, **3.99** (6 mg, 0.016 mmol) was dissolved in THF (0.2 mL) and carbonyldiimidazole (4 mg, 0.024 mmol) was added at 25 °C. The reaction mixture was stirred at 25 °C for 2 h. The magnesium malonate suspension was cooled to 30 °C and added dropwise to the activated acid. A white precipitate formed rapidly during this addition so vigorous stirring was necessary to avoid clamping. The resulting suspension was stirred at 40 °C for 19 h then cooled to 0 °C and quenched with a saturated aqueous NH_4Cl solution. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 2/1) to afford **3.100** (0.2 mg) as colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.0 Hz, 2H), 7.59 (t, J = 6.8 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 5.56 (t, J = 5.3 Hz, 1H), 4.73 (s, 1H), 3.71 (s, 6H), 3.56–3.87 (m, 7H), 1.92–2.02 (m, 2H), 1.25 (s, 3H), 0.89 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H).

4. Conclusion

Macrocycles are positioned between conventional small molecules and gigantic biologics in chemical space. This class of scaffolds provides potential to interact with extended target surfaces while retaining favorable pharmacological properties. Given the growing need for generating diverse macrocycles for the development of new therapeutics and chemical probes, we investigated efficient strategies for the preparation of natural and unnatural macrocycles based on fragment assembly. First, our modular approach for the construction of a library of tetrahydropyran-containing macrocycles was introduced. By using three different diversification steps, natural product-like macrocycles were generated with a high level of physicochemical and structural diversity which were evaluated by principal component analysis and principal moment of inertia. Second, the synthetic study towards antifungal forazoline A was described. Based on the retrosynthetic analysis, we explored the stereoselective synthesis of three major fragments of the macrocyclic polyketide. We expect that our studies on the synthesis of diverse macrocycles would offer opportunities and insights for the development of new bioactive macrocycles.

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Biography

Hyunji Lee received a Bachelor of Science degree in pharmacy at Yeungnam University, South Korea in February of 2010. She earned her Master of Science degree in medicinal chemistry from the college of pharmacy at Seoul National University, South Korea in February of 2012. In the fall of 2014, she began her graduate career at Duke University and received her Doctor of Philosophy in organic chemistry in November of 2019.

Honors & Award

Kathleen Zielik Fellowship Award	2018–2019
C. R. Hauser Memorial Fellowship	2017–2018
National Research Foundation of Korea, BK 21 Research Scholarship	2010–2011
Yeungnam University, Scholarship for Academic Excellence	2008–2009

Publications and Patent

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