Stereoselective Synthesis of Sub-structures of Eribulin (C14-C21 and C22-C28) and Related Macrocyclic Compounds

A Thesis

Submitted for the Degree of

DOCTOR OF PHILOSOPHY

In Chemistry

By

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April 2016

DEDICATED TO MY FAMILY

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CERTIFICATE

This is to certify that the thesis entitled ***"Stereoselective Synthesis of Sub-structures of Eribulin (C14-C21 and C22-C28) and Related Macrocyclic Compounds"*** being submitted by ***Mr. Naveen Kumar Mallurwar*** to the University of Hyderabad for the award of ***Doctor of Philosophy in Chemistry*** has been carried out by him under my supervision and the same has not been submitted elsewhere for a degree. I am satisfied that the thesis has reached to the standard of fulfilling the requirements of the regulations relating to the nature of the degree.

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Supriya and their family for their valuable support in my life. I would like to thank my grandfather and grandmother ***Neelakant (late), Sakkubai*** for their moral support. My heart-full thanks to my wife ***Manasa*** for her love and the constant support, and for keeping me happy over the past few years and my little thanks to my nephew ***Shlok, Surya*** and my Niece ***Saanvi*** who has been a source of relief while I am away from the work.

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To all my friends, relatives and well wishers, thank you for your understanding and encouragement in my many moments of crisis. Your relationship makes my life a wonderful experience. I cannot list all the names here, but you are always on my mind.

I dedicate this thesis in memory of my father, ***Suryakanth*** (1952-2011) to my mother, ***Saraswathi***. My father was my best friend and a greatest role model. He was an amazing father, coach, mentor, and a friend. Dear father, wherever you are, you are gone but you will never be forgotten.

Dr. Reddy's Institute of Life Sciences ***Naveen Kumar Mallurwar***

April 2016

Synopsis

This thesis entitled, ***"Stereoselective Synthesis of Sub-structures of Eribulin*** *(C14-C21 and C22-C28)* ***and Related Macrocyclic Compounds"*** contain four chapters.

Chapter 1: Synthesis and Biological Evaluation of Cytoskeleton (Actin and Tubulin) Modulators and the Importance of Macrocyclic Natural Products and Synthetic Analogs

In this chapter, I explored the synthesis and biological evaluation of cytoskeleton (actin and tubulin) modulators. In tubulin class, I discussed the introduction, the structure of a microtuble, the importance of microtubules in mitosis (cell division process) and microtubules as a target for anticancer therapy by developing destabilizing and stabilizing agents. In the category of destabilizing agents, I explained the discovery and development of eribulin and the literature synthesis of its key fragments. In the category of stabilizing agents, I covered the chemistry, biology, and the synthesis of peloruside A, natural product. I have also explained the importance of actin, various natural products and their analogs targeting actin, and the importance of modulating its dynamics. In addition to this, I have also discussed the importance of protein-protein interactions as targets for drug discovery. Finally, I have also discussed the importance of macrocyclic compounds (natural products as well as synthetic compounds) in medicinal chemistry and their distinct structural features when compared to their equivalent acyclic compounds.

Chapter 2: Synthesis of C14-C21 Eribulin Fragment for Building A Diverse Set of Macrocycles *(submitted for publication).*

2.1: Literature Synthesis of C14-C21 Eribulin Fragment

This section covers the literature synthesis of C14-C21 fragment of eribulin, which is reported in the literature, during the past several years by various leading research groups.

2.2: Our Synthesis for the C14-C21 Eribulin Fragment

Eribulin **(F1.1)** is a non-taxane drug, and first-in-class microtubule dynamics inhibitor, which was approved by FDA in 2010 for use in patients who previously received at least two prior chemotherapeutic regiments for the metastatic breast cancer.

The birth of eribulin originated from the Kishi's group while working on the total synthesis of halichondrin and its various analogs while searching for better microtubule binding agents.

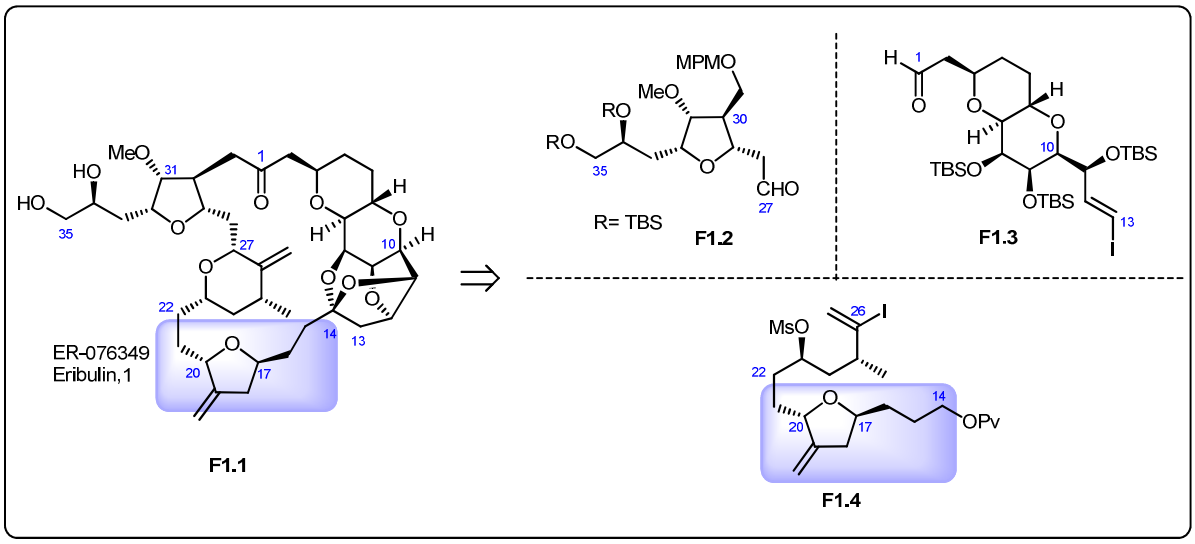


Figure 1: Three Key Fragments According to Kishi

With our continued interest in developing the practical synthesis approaches to various sub-structures of eribulin and other bioactive natural products, and their utilization in obtaining different sets of macrocyclic compounds, we focused our attention to the C14-C21 substituted tetrahydrofuran fragment of eribulin. This key fragment was further utilized in developing the modular synthesis for obtaining two different macrocyclic compounds having 17- and 18-membered rings. The key scaffold **F2.1** was utilized in developing our macrocyclic synthesis approach.

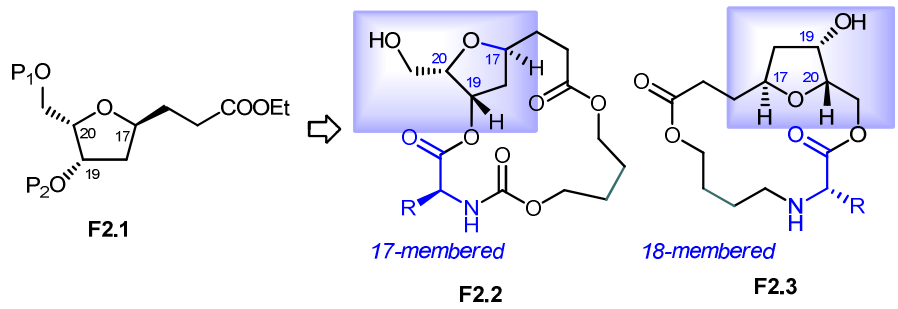


Figure 2: Two Planned Macrocyclic Targets having 17- and 18-Membered Rings (**F2.2** and **F2.3**) from the Substituted Tetrahydrofuran Moiety, **F2.1**

Our plan was to develop a practical and scalable, new methodology to the synthesis of C14-C21 fragment of eribulin. The retrosynthetic analysis of our target **F3.1** is shown in **Figure 3.** Compound **3.1** was obtained from carbon extension, oxidation and Wittig reaction on **F3.2**. The tetrahydrofuran ring formation could be achieved through our key, planned iodocyclization of **F3.3**. In our synthesis planning, we aimed for accessing the diol compound from a cheap chiral starting material *R,R*-tartaric acid, in a few simple transformations.

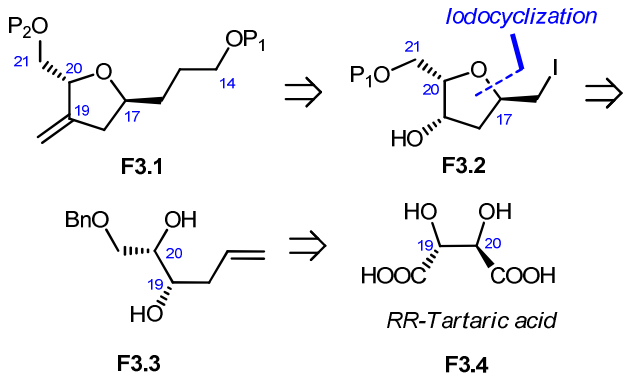
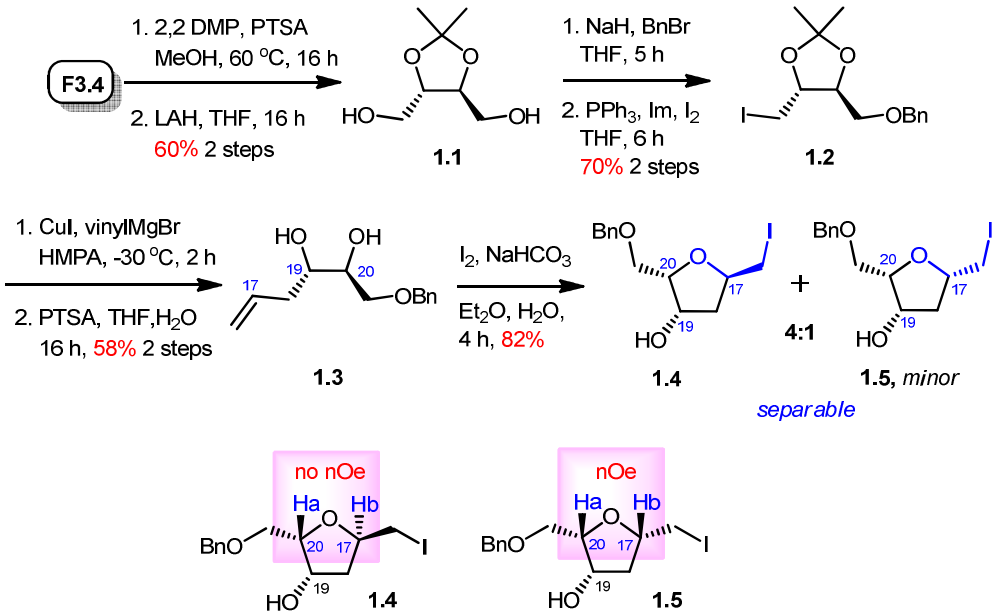


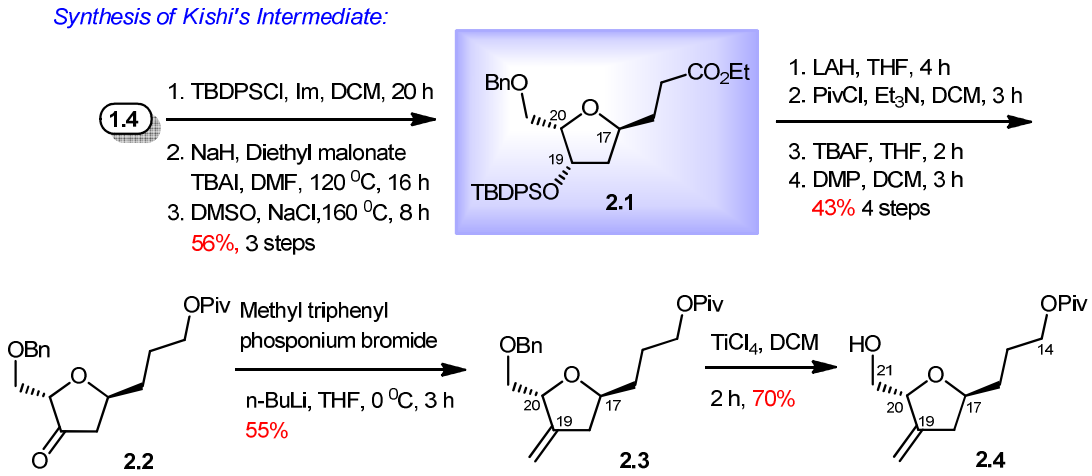
Figure 3: Retrosynthesis of C14-C21 Eribulin Fragment



Scheme 1: Iodocyclization Approach to the Synthesis of **1.4** and **1.5**.

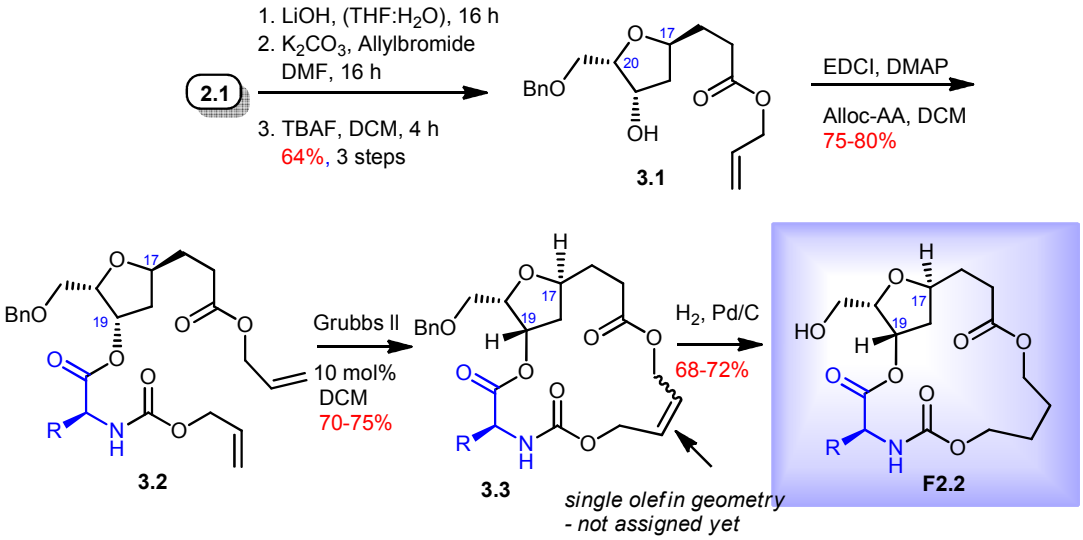
As shown in **Scheme 1**, *R,R*-tartaric acid (**F3.4**) was converted to **1.2** via **1.1** in simple steps. This upon treatment with vinyl MgBr/CuI followed by an acetonide removal gave compound **1.3.** Our key iodocyclization reaction with **1.3** produced two tetrahydrofuran derivatives, **1.4** and **1.5** as the major and minor products in a 4:1 ratio. These two diastereomers were easily separable, and the pure products were then thoroughly subjected to the structural analysis by 1D and 2D NMR studies

The remaining steps for completing the synthesis of the Kishi's tetrahydrofuran fragment are shown in **Scheme 2**. Compound **1.4** having a *trans*-2,5-disubstituted tetrahydrofuran moiety was converted to **2.1** in three steps, and this was then led to producing the keto derivative, **2.2**. Finally, the required product **2.4** was obtained from **2.2** in a series of steps that involved (i) Wittig reaction, and (ii) the -OBn deprotection under Lewis acidic conditions, and this approach completed our synthesis of the Kishi's fragment.

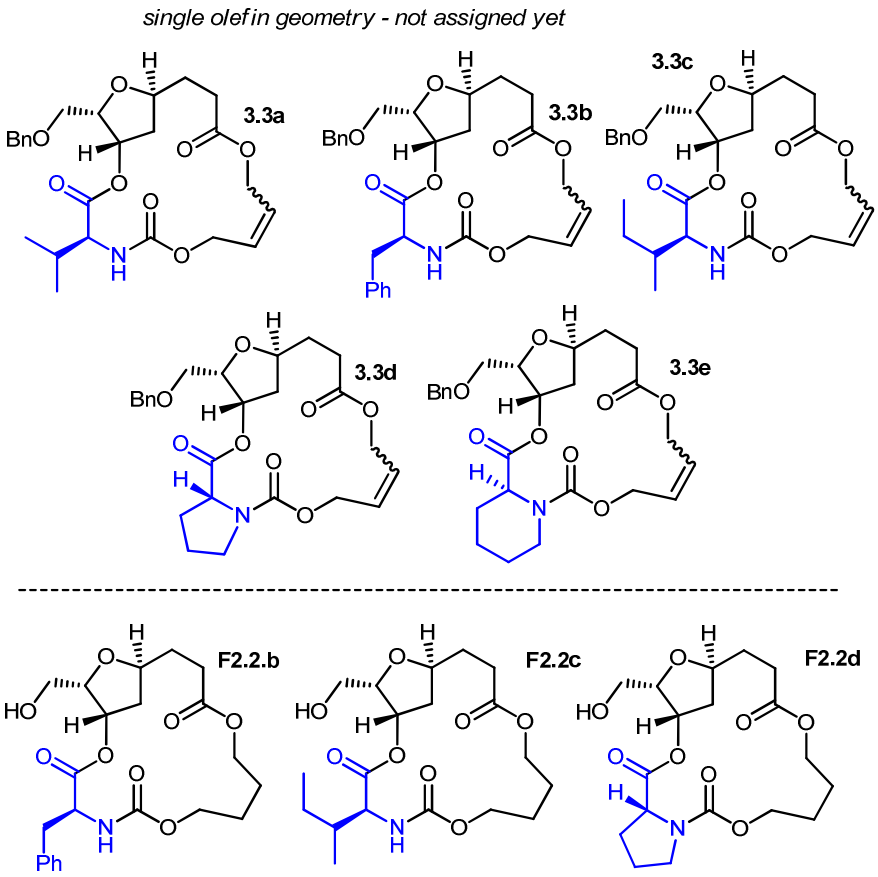


Scheme 2: Completion of the Synthesis of Kishi's Fragment.

Having a method for obtaining the sufficient amount of the key intermediate **2.1**, we then focused our attention to developing a modular approach to the synthesis of two different macrocyclic compounds, **F2.2(a-e)** and **F2.3(a-e)**. **Scheme 3** shows our approach to the synthesis of a macrocyclic compound **F2.2(a-e)**. Compound **2.1** was hydrolyzed for obtaining the free acid which was further allylated and subjected to the hydroxyl protection removal, giving **3.1**. It was then coupled with five different amino acids for obtaining a precursor **3.2** for our crucial Ring Closing Metathesis (RCM). The use of a second generation Grubbs' catalyst successfully produc-

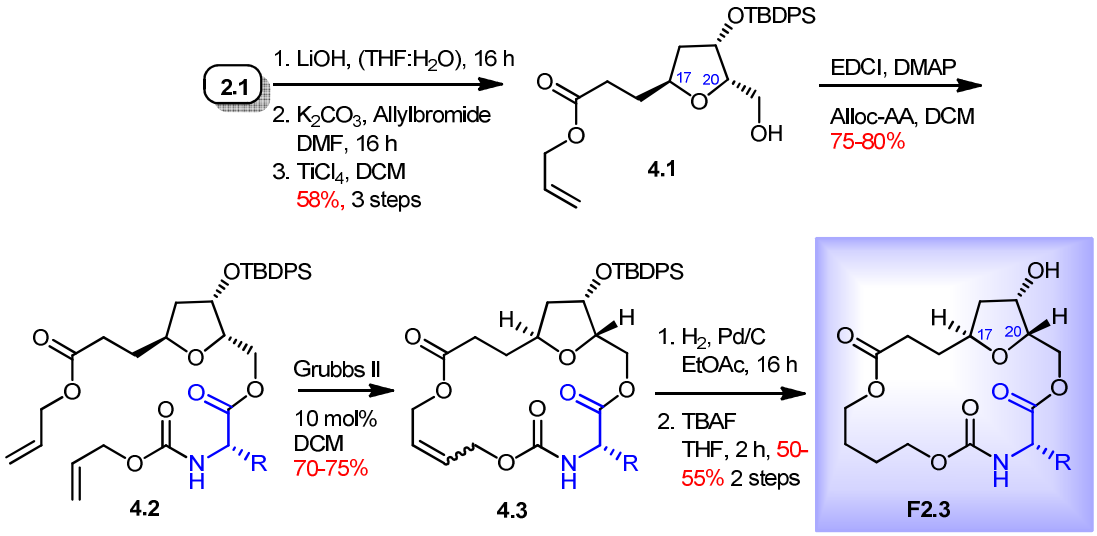


Scheme 3: 17-Membered Macrocyclic Compounds (**3.3(a-e)/F2.2(a-e)**) from *trans*-2,5-Disubstituted Tetrahydrofuran Fragment, **2.1**.

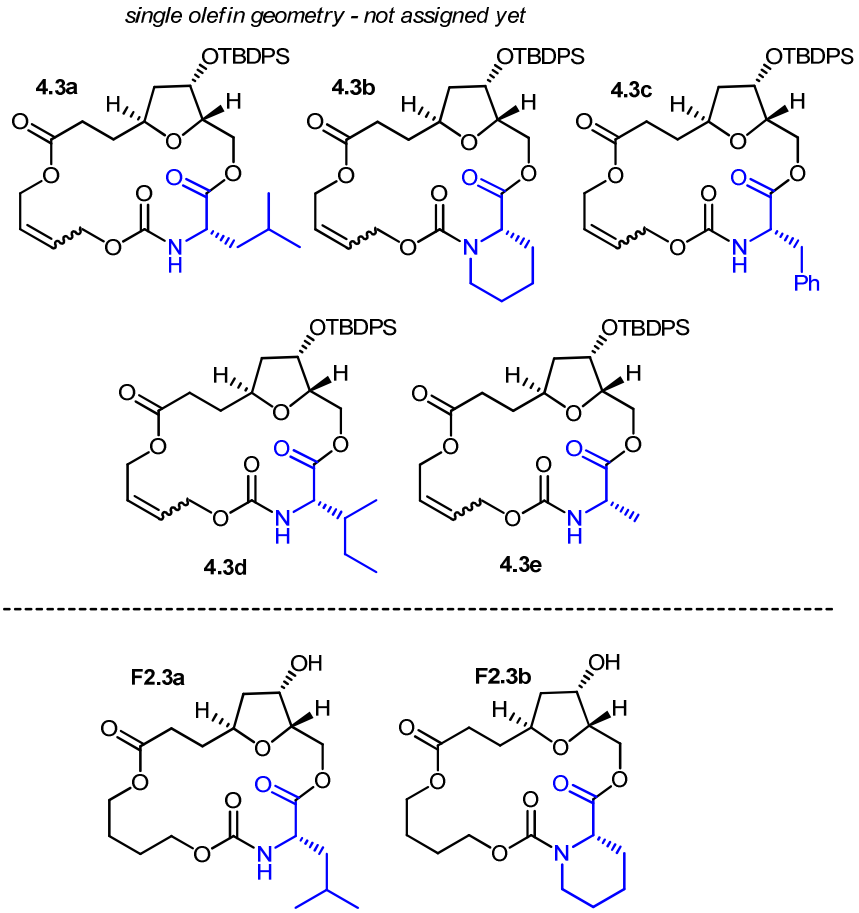


-ed the 17-membered ring macrocycle with a single olefin geometry, giving compound, **3.3**. The olefin geometry was not assigned due to overlapping signals in NMR. Five macrocyclic compounds were obtained by this approach, and this validated the feasibility of our ring formation, that is independent of an amino acid utilized in the synthesis. As a test case, in three examples, the hydrogenation conditions led to producing the de-protected compounds and the removal of the double bond, gave compound, **F2.2** (See **Scheme 3**).

In a similar manner, our synthesis approach for obtaining 18-membered macrocyclic compounds is shown in **Scheme 4**. All the products were thoroughly purified and well-characterized by MS and 1D and 2D NMR.



Scheme 4: 18-Membered Macrocyclic Compounds (**4.3(a-e)/F2.3(a-e)**) from *trans*-2,5-Disubstituted Tetrahydrofuran Fragment, **2.1**.



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Chapter 3: Synthesis of C22-C28 Eribulin Fragment for Building A Diverse Set of Macrocycles *(manuscript under preparation).*

3.1 Literature Synthesis of C22-C28 Eribulin Fragment

This section covers the literature synthesis of C22-C28 fragment of eribulin, reported over the years, by several leading research groups.

3.2 Our Synthesis of C22-C28 Eribulin Fragment

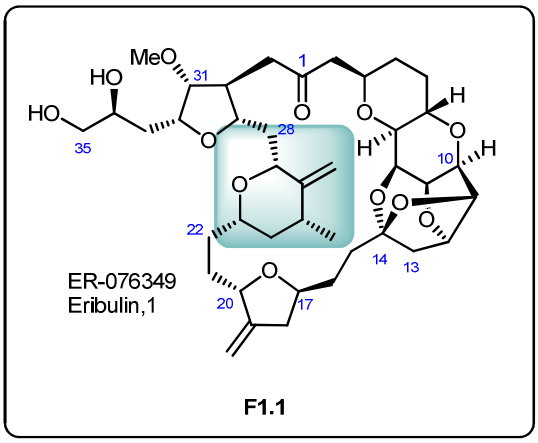


Figure 4: Structure of Eribulin

In this chapter, we focused our attention to the C22-C28 substituted tetrahydropyran fragment of eribulin, and further, the use of this fragment in developing a modular synthesis of various macrocyclic compounds having a 17 membered ring. The key scaffold **5.6** was utilized in developing this macrocyclic synthesis approach.

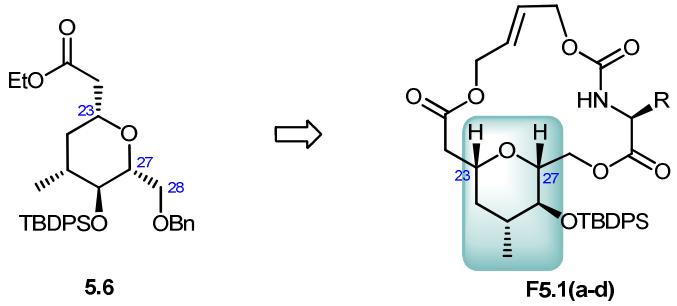


Figure 5: Macrocyclic Target having 17 Membered ring (**F5.1**) from the Substituted Tetrahydropyran Moiety **(5.6)**

The retrosynthetic analysis of our target **F6.1** is shown in **Figure 6.**

Compound **F6.1** could be obtained from **F6.2** by a Michael approach, protecting group removal, oxidation and terminal Wittig reaction. The α, β unsaturated ester **F6.2** was planned from the lactone derivative, **F6.3**. The lactone **F6.3** could be obtained from cyclization and methylation of compound **F6.4,** and this could be easily accessed from iso-ascorbic acid, **F6.5** as a cheap source of chiral strating material.

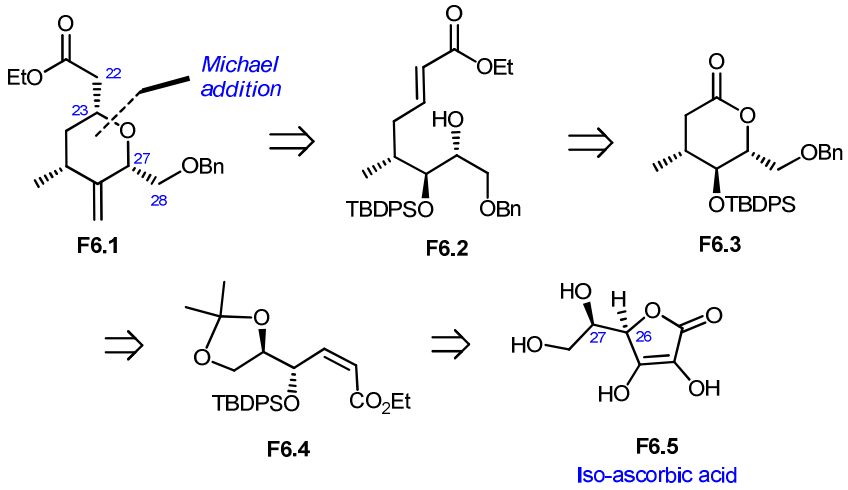
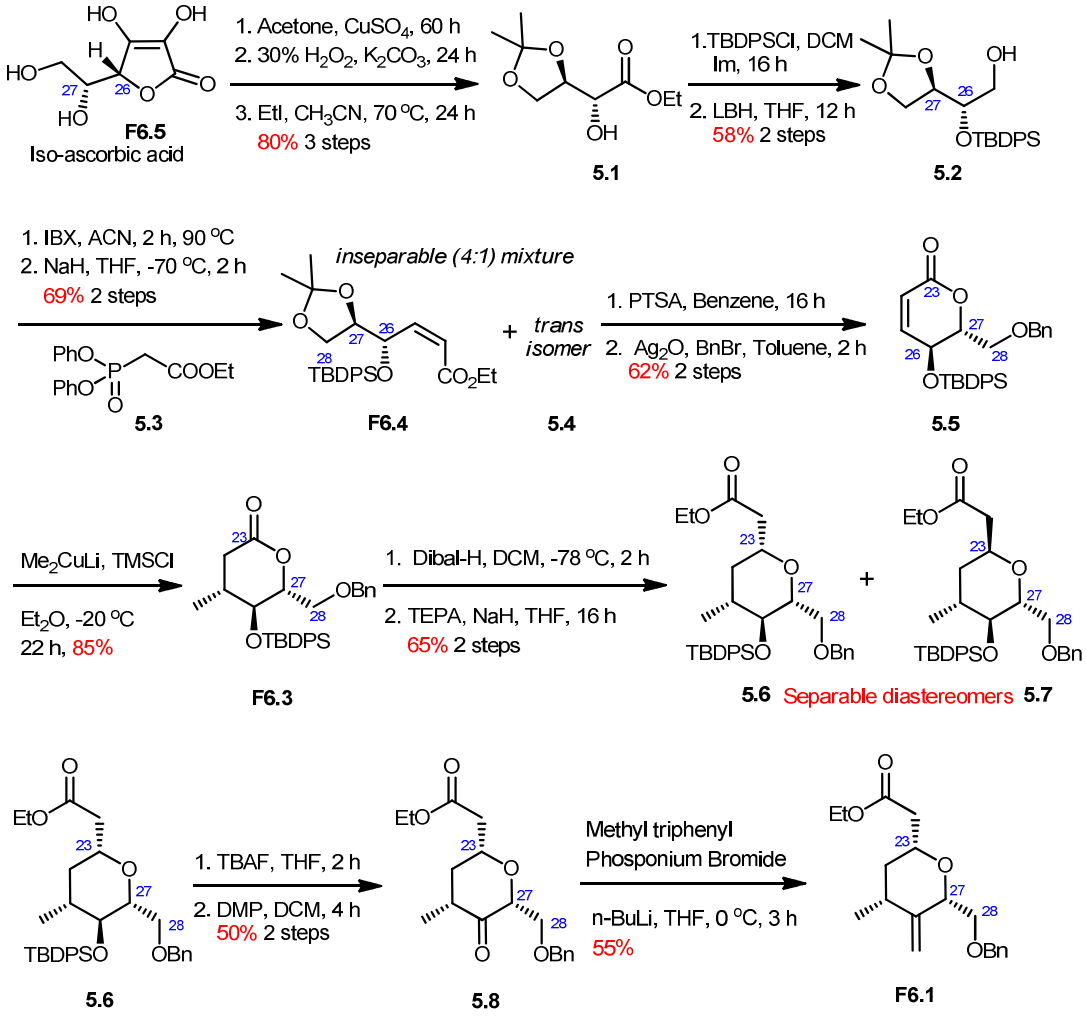


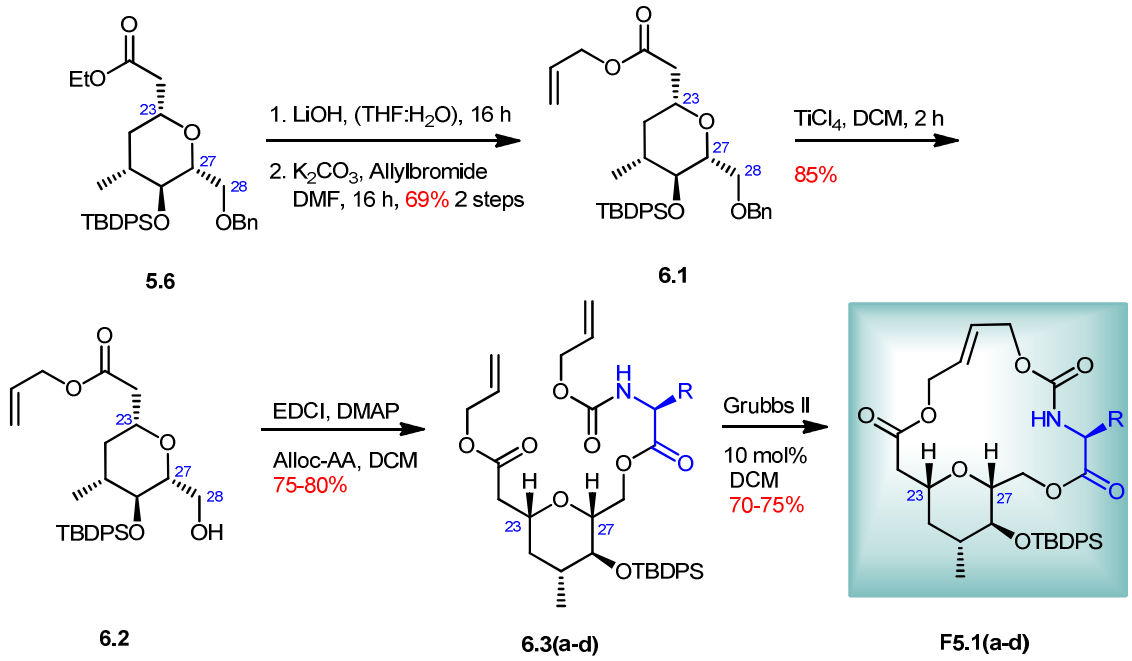
Figure 6: Retrosynthesis of C22-C28 Eribulin Fragment

Our synthesis was started with a chiral starting material, iso-ascorbic acid **F6.5** as shown in **Scheme 5**. It was to converted to **5.1** in three simple steps. This then lead to producing the corresponding alcohol **5.2**, which following oxidation was then subjected to *cis* Wittig reaction giving *cis*- (**F6.4**) and *trans*- (**5.4**) products as two separable geometrical isomers in a 4:1 ratio. Compound **F6.4** was then subjected for cyclization under PTSA condition giving the α,β lactone **5.5.** The stereoselective conjugate addition on **5.5**, produced compound **F6.3**. The reduction reaction followed by Horner-Wadsworth-Wittig reaction furnished two separable diastereomers **5.6** and **5.7.** Finally, the required product **F6.1** was obtained from **5.6** in a series of steps that involved (i) silyl group removal, (ii) oxidation and (iii) Wittig reaction.

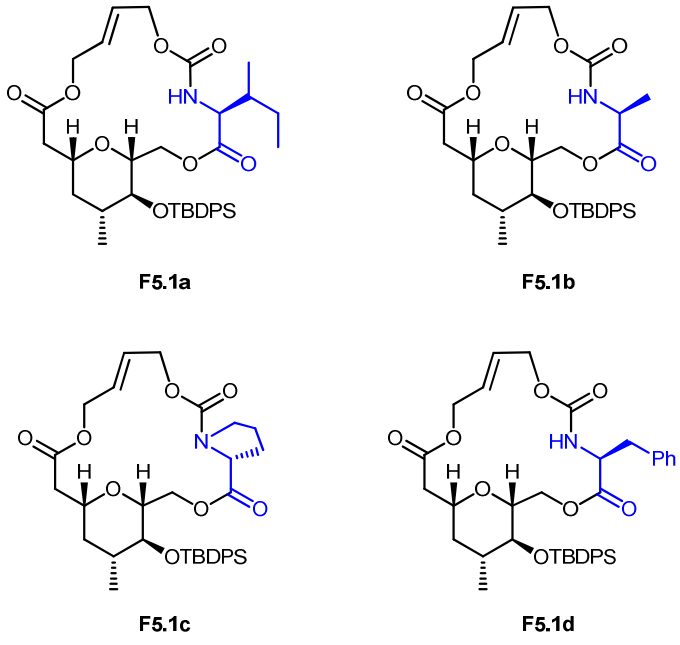


Scheme 5: Synthesis of C22- C28 Fragment of Eribulin.

With having a sufficient amount of the key intermediate **5.6** in hand, we then developed a modular approach to the synthesis of macrocyclic compounds, **F5.1**. **Scheme 6** shows our approach to the synthesis of a macrocyclic compound, **F5.1.**



Scheme 6: 17-Membered Macrocyclic Compounds (**F5.1**) from *cis*-2,5-Disubstituted Tetrahydropyran Fragment, **(5.6)**



The hydrolysis of **5.6** produced the free acid which was further allylated and the protecting group removal gave, **6.2.** It was then coupled with four different amino acids for obtaining a precursor **6.3** for the crucial Ring Closing Metathesis (RCM). The use of a second generation Grubbs'

catalyst successfully produced the 17-membered ring macrocycle with a single olefin geometry, **F5.1**. In one case, the olefin geometry was assigned by using extensive NMR studies. Four macrocyclic compounds were obtained by this approach and this further validated the feasibility of our ring formation that is independent of an amino acid utilized in the synthesis.

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Chapter 4: Synthesis of Isatin-based 13-Membered Macrocyclic Diversity

In nature, isatin is found in many plants such as *Calanthe discolor, Isatis tinctoria, and in Couroupita guianensis,* and it is a metabolic derivative of adrenaline, commonly found in humans.

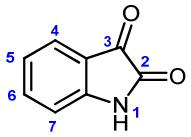


Figure 7: Structure of Isatin

Isatins have significant importance in medicinal chemistry and are used as a common starting material in the synthesis of a large variety of heterocyclic compounds which are biologically active. A variety of pharmacological actions are associated with isatin and its derivatives include several compounds having anticancer, antimicrobial, antiviral, anti-inflammatory, anticonvulsant, and analgesic activity.

Due to the distinct structural features of macrocyclic compounds, they have less conformational flexibility than an equivalent acyclic derivatives and so suffer a trivial entropic loss upon binding to a receptor. In contrast to the smaller cyclic systems, macrocycles are not rigid, and so, allowing them to potentially mould to a target surface for achieving an optimal binding. In addition to this, macrocyclic compounds also offer the possibility for binding across larger surfaces that are difficult to access with the traditional small molecules. From the chemistry point of view, macrocyclic compounds can offer various types of stereochemical complexity and the diverse functionality in a conformationally restricted manner.

With this objective, we were interested in developing a synthesis method for obtaining different types of 13-membered macrocyclic compounds using Isatin as the starting material and this plan is shown in **Scheme 7**. The introduction of an amino acid moiety in the macrocyclic ring would allow introducing a chiral diversity site for obtaining further analogs.

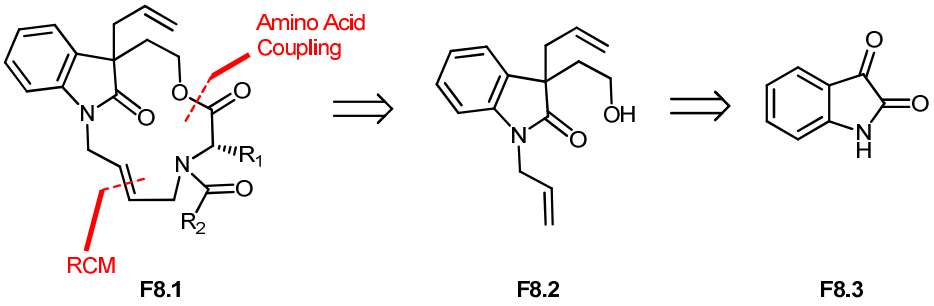
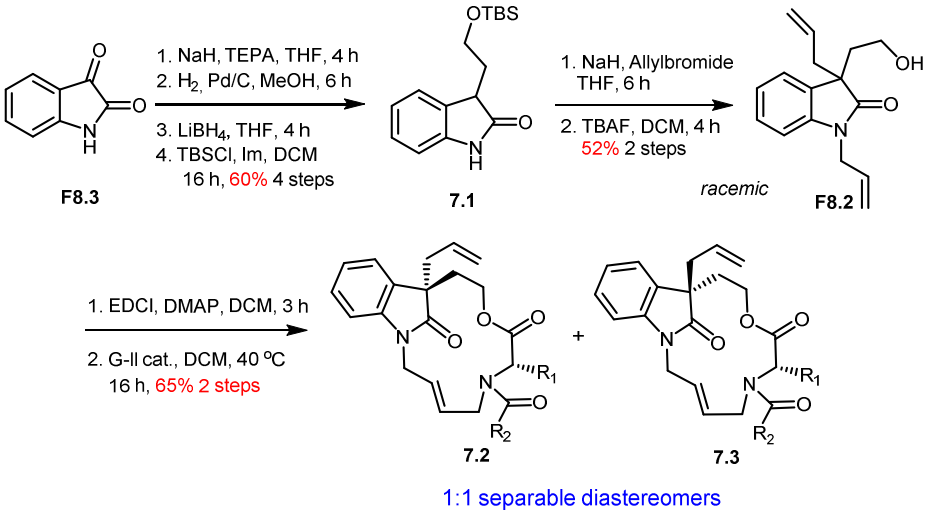


Figure 8: Retrosynthesis of Target Macrocycles.

The retrosynthetic analysis of our target **F8.1** is shown in **Figure 8.** Compound **F8.1** could be obtained from the amino acid building block coupling followed by ring closing metathesis of compound **F8.2**. This could be obtained from the commercially available isatin, **F8.3**

Our synthesis was started with Horner–Wadsworth-Emmons reaction of commercially available isatin, which gave the Wittig product. The reduction of the double bond with H_2, Pd/C gave us a racemic mixture, which was further followed by the reduction of an ester with lithium borohydride, protection of primary alcohol with silyl group furnished compound **7.1**. Allylation of compound **7.1** gave *bis*-allylated product which on deprotection with TBAF gave compound **F8.2**. It was then coupled with five different amino acids for obtaining a precursor for our crucial

ring closing metathesis approach. The use of the second generation Grubbs' catalyst produced the 13-membered ring macrocyles with the *trans* olefin geometry **7.2** & **7.3**



Scheme 7: Synthesis of Macrocyclic Compounds

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(2) Guo, Y.; Chen, F. *CA 104: 213068f* **1986**.

(3) Bergman, J.; Lindström, J.-O.; Tilstam, U. *Tetrahedron* **1985**, *41*, 2879.

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1. 14-Membered macrocyclic ring-derived toolbox: the identification of small molecule inhibitors of angiogenesis and early embryo development in zebrafish assay. Aeluri, M., Pramanik, C., Chetia, L., **Mallurwar, N.** K., Balasubramanian, S., Chandrasekar,G., Kitambi, SS, Arya, P. *Org. Lett.* **2015**, *15*(3), 436-439.
2. A Modular Approach to Building 17- and 18-Membered Macrocyclic Diversity from Eribulin C14-C21 Fragment, **Naveen Kumar Mallurwar**, Saidulu Konda, Mahender Khatravath, Pallavi Rao, Shivashankar Sripally, Javed Iqbal and Prabhat Arya, **2016**, *submitted for publication*.
3. Synthesis of C1-C11 Eribulin Fragment its Analogs for Building A Diverse Set of

Macrocycles, Mahender Khatravath, **Naveen Kumar Mallurwar**, Saidulu Konda, Pallavi Rao, Shivashankar Sripelly, Javed Iqbal and Prabhat Arya, **2016**, *accepted for publication in the journal, Synthesis.*

4. Stereoselective Synthesis of C27-C35 Eribulin Fragment and Its Utilization in Building Structurally Diverse Macrocycles, Saidulu Konda, Mahender Khatravath, **Naveen Kumar Mallurwar**, Pallavi Rao, Shivashankar Sripelly, Javed Iqbal and Prabhat Arya, **2016**, *accepted for publication* in a special issue of the journal, *Synthesis*, "Target Oriented Synthesis of Complex Molecules".
5. Stereoselective Synthesis of C22-C28 Eribulin Fragment for Building A Diverse set of 17-Membered Macrocyclic Compounds. *Manuscript under preparation, 2016*
6. Synthesis of Isatin based Diverse set of Macrocyclic Compounds. *Manuscript under preparation, 2016*

Seminars/Conferences Attended

2013 Presented a poster at DRILS, University of Hyderabad Campus, India

2014 Attended at the **X-JNOST** 2014 international conference, IIT, Madras, India

2015 Presented a poster at "Trend Setting Innovations in Chemical Sciences & Technology Applications in Pharma Industry" that was held at JNTUH, Hyderabad, India.

Abbreviations

Ac	:	Acetyl
Ag_2O	:	Silver oxide
Alloc-AA	:	N-Alloc amino acid
aq.	:	Aqueous
BnBr	:	Benzyl bromide
CH_3CN	:	Acetonitrile
$CDCl_3$	:	Deuterated chloroform
CuI	:	Copper Iodide
$CuSO_4$	:	Copper sulphate
C_6H_{12}	:	Benzene
DCM	:	Dichloromethane
DMF	:	Dimethylformamide
DMAP	:	4-Dimethylaminopyridine
DMSO	:	Dimethylsulphoxide
DMP	:	Dess martin periodinane
2,2 DMP	:	2,2 Dimethoxy propane
EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtI	:	Ethyl iodide
EtOAc	:	Ethyl acetate
Et_2O	:	Diethyl ether
Et_3N or TEA	:	Triethylamine
$EtO_2CCH_2PO(OEt)_2$	:	Triethyl phosphonoacetate
ES	:	Electro Spray
G-II	:	Grubbs 2^{nd} generation catalyst
H_2	:	Hydrogen
H_2O_2	:	Hydrogen peroxide
HMPA	:	Hexamethyl phosphoramide
2N HCl	:	2 Normal Hydrochloric acid
H	:	Hour
IBX	:	2-iodoxybenzoic acid
Im	:	Imidazole

I_2	:	Iodine
K_2CO_3	:	Potassium carbonate
KOH	:	Potassium hydroxide
$LiAlH_4$	:	Lithium aluminium hydride
$LiBH_4$	:	Lithium borohydride
LiOH	:	Lithium hydroxide
MeI	:	Methyl iodide
Ms	:	Mesyl
NH_4Cl	:	Ammonium chloride
$NaHCO_3$	:	Sodium bicarbonate
NaCl	:	Sodium chloride
Na_2SO_4	:	Sodium sulphate
NaH	:	Sodium hydride
$NaBH_4$	:	Sodium borohydride
NMR	:	Nuclear magnetic resonance
NaN_3	:	Sodium azide
Me	:	Methyl
MeOH	:	Methanol
OsO_4	:	Osmium tetroxide
*p*TSA	:	*para*-toluene sulfonic acid
PivCl	:	pivaloyl chloride
Pd/C	:	10% Palladium on Carbon
PPh_3	:	Triphenylphosphene
Rt	:	Room temperature
R_f	:	Retardation factor
TBDPSCl	:	tert-Butylchlorodiphenylsilane
TBAF	:	Tetra-n-butylammonium fluoride
TBAI	:	Tetrabutylammonium iodide
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TLC	:	Thin Layer Chromatography
TMSCl	:	Trimethylsilyl chloride
$TiCl_4$	:	Titanium tetrachloride
TsCl	:	*para*-toluenesulfonyl chloride

General Information

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Varian 400 MHz NMR spectrometer at the frequency indicated. Where indicated, the NMR peak assignments were made using COSY experiments. All chemical shifts are quoted on the δ-scale and were referenced to the residual solvent as an internal standard. Combinations of the following abbreviations are used to describe NMR spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Mass spectra and LC-MS were recorded using electron impact, chemical ionization or electrospray ionization techniques, on Agilent-6430 mass spectrometer. High-performance liquid chromatography was carried out on Agilent-1200 instrument using X-BRIDGE C-18 150×4.6mm 5µ column. Thin layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel $60F_{254}$ (Merck, 1.05554) and the spots were visualized with UV light at 254 nm or alternatively by staining with aqueous basic potassium permanganate or ceric ammonium molybdate or ninhydrin. Flash column chromatography was performed using silica gel (Merck, 60A, 230-400 Mesh). Commercially available reagents were used as supplied and some of them were distilled before use. All reactions were performed in oven dried glassware. DMF, DCM, MeOH and THF were dried immediately prior to use according to standard procedures: dimethylformamide, dichloromethane was distilled under N_2 from CaH_2, methanol was distilled under N_2 over Mg and tetrahydrofuran was distilled under N_2 over Na. All solvents were removed by evaporation under reduced pressure.

Chapter 1

Synthesis and Biological Evaluation of Cytoskeleton (Actin and Tubulin) Modulators and the Importance of Macrocyclic Natural Products and Synthetic Analogs

Synthesis and Biological Evaluation of Cytoskeleton (Actin and tubulin) Modulators and the Importance of Macrocyclic Natural Products and Synthetic Analogs

In this section, I am going to discuss about the synthesis and biological evaluation of cytoskeleton (actin and tubulin) modulators, an overview of protein-protein interactions (PPIs) and the importance of macrocyclic natural products and other related compounds.

1.1) Cytoskeleton

1.2) Tubulin (Microtubules)

1.3) Actin (Microfilaments)

1.4) Protein-Protein Interactions

1.5) Importance of Macrocycles

1.1 Introduction to Cytoskeleton

Cell is the basic structural and functional unit of all the living organisms, which is composed of many organelles surrounded by a membrane.[1] Cytoskeleton is a network of intracellular filaments which is present in the cytoplasm[1] of the cell and plays a major role in cell shape, division, and function of living organisms.[2] It is present both in prokaryotes and eukaryotes. In prokaryotes, it is formed not very distinct, while in eukaryotes, the cytoskeleton is made-up of complex structures and other accessory proteins necessary for the gene duplication and specialization.[2] The cytoskeleton in eukaryotes is highly motile (i.e. capable of motion) and functions according to the responses of the cell. It is dynamic[3] in nature as it dismantles and rearranges itself during the course of retaining the cell shape, structure, and also, allows the movement of cell organelles in the cytoplasm.[4] The functions of cytoskeleton include, the structural support to the cell, the cell motility, the circulation of materials, and the regulatory activities within the cell, etc.

Cytoskeleton is structurally made-up of microtubules, microfilaments and intermediate filaments, which differ in their diameter and the protein sub-units.[5] These filaments are attached to organelles in cytoplasm and plasma membrane with the help of various accessory proteins.[5]

- **Microfilaments**, also called as actin filaments, are the thin components.

- **Intermediate filaments** are fibers with diameters in a middle range.
- **Microtubules** are the thickest of the three components of the cytoskeleton.

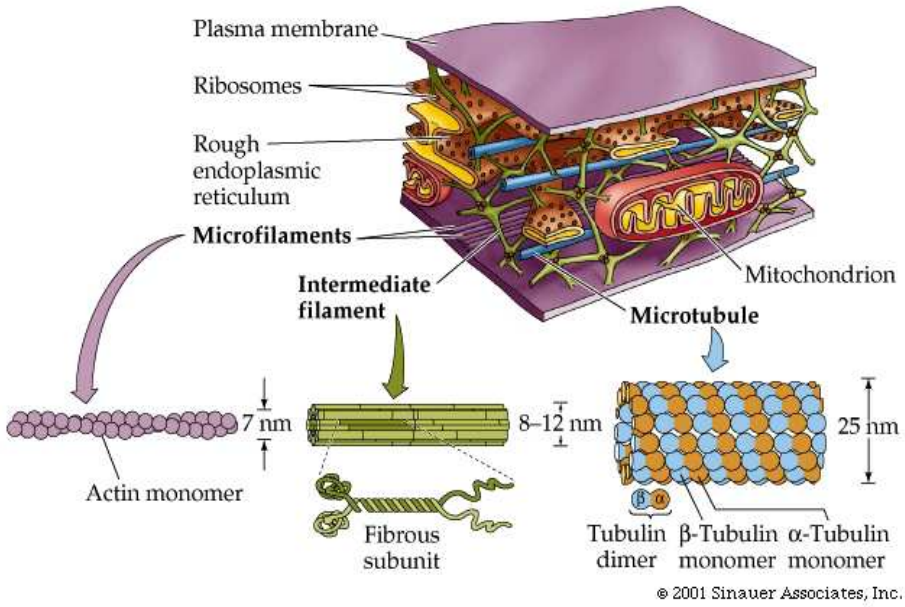


Figure 1: Eukaryotic Cytoskeletal Element Arrangements
(note: this figure is directly taken from Google net)

http://www.sinauer.com/catalog/biology/cell-and-molecular-biology/mechanics-of-motor-proteins-and-the-cytoskeleton.html#table_of_contents

1.2 Introduction to Tubulin

Tubulin is a small protein unit of microtubules, and was isolated in 1967, by Borisy and Taylor.[6] It is an important subunit of microtubules which play a key role in several cellular functions. The term tubulin was given by Mohri and co-workers[7] and the purification of the tubulin protein from brain was carried-out by Weisenberg and co-workers in 1968.[8] It was obtained from the sperm tails by Shelanski and Taylor.[8-9] Microtubules are present in all eukaryotic cytoskeleton and participate in several biological functions.[9]

Tubulin is a globular protein formed by two polypeptide units, α-tubulin and β-tubulin.[10] Both α- and β-tubulin have an approximate molecular weight of 50-55 kilo dalton.[3,11] The arrangement of amino acid in both the units is identical with a very minor changes. The third type of tubulin is known as γ-tubulin,[10] and it appears only at the centrosome. It plays a major role in the assembly of microtubules, during the process of the cell division. Apart from these, other types such as delta, zeta and

epsilon tubulins are also reported in the literature.[10] All these collectively are known as tubulin super family.

1.2.1 Microtubules

These are the principal components of cytoplasm present all over in the cytoplasm.[12] They are hollow, cylindrical structures present widely in cytoskeleton, and are formed by the heterodimers of tubulin. The continuous assembly and disassembly of microtubules is observed within the cell, which is similar to that of actin filaments.

Functions of microtubules:

- Cell shape determination and cell movements[11]
- Intracellular movement of organelles[11]
- Chromosome separation during mitosis i.e., cell division.

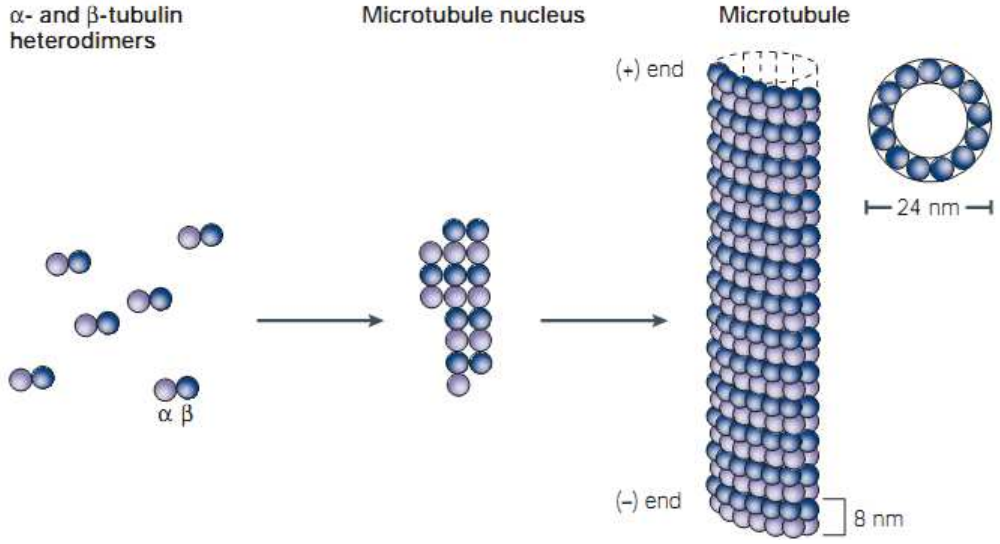


Figure 2: Polymerization of Microtubules

(note: this figure is taken from *Nat. Rev. Cancer* **2004**, *4*, 253-265)

1.2.2 Structure of Microtubules

Microtubules are structurally formed by the organization of protofilaments of tubulin around a hollow rod-like structure.[12] Protofilaments are formed by the polymerization of dimers of tubulin, in which, the distinct ends are termed as the head and tail. The protofilaments are also arranged in parallel, which give rise to a polar microtubule with positive (+ve) and negative (-ve) ends.[13] The growing intensity at the ends

depend upon the polarity which determines the movement of microtubules. The polarity is more at the plus end than the minus end.[13]

Both α- and β-tubulin are homologous to each other and bind to GTP (Guanosine Tri phosphate) nucleotide which is hydrolyzed by an enzyme, called as GTPase.[14] The GTP nucleotide - bound to α-tubulin is stable, which is consistent, while its binding to β-tubulin is labile and can alter easily.[14,15] As the nucleotide bound to α tubulin is stable, it is termed as the non-exchangeable site whereas the nucleotide of β tubulin is termed as exchangeable. This immediately converts itself to GDP with the help of an enzyme, known as, GTPase.[14] The lability of β tubulin is due to the presence of lysine at the end site which is highly hydrolyzable, and also, comparable to that of the presence of glutamic acid in α-tubulin E254.[16]

In the course of the formation of a protofilament, the heterodimers of tubulin are associated in such a way that the oppositely charged ends of the monomer units are attached to each other.[17] These protofilaments further bind laterally, eventually forming a microtubule. The surface of the microtubule appears as the 2-dimensional crystalline structure which grows as long as up to 24 nm.[18] Due to the distinct arrangement of monomers during the formation with charged ends, they end-up alternatively with an α and β tubulin units.[18] As the tubulins are known as polarized protein units, such an arrangement of polarized proteins is also called as *electret polymer***.**

1.2.3 Dynamic Instability of Microtubules

Microtubules are said to be highly dynamic polymer.[18] They are said to be highly dynamic as there is a co-existence of polymerization and depolymerization.[18] The phase in which the GTP subunits gets added to the end of the microtubule is commonly termed as polymerization.[18] Immediately after the polymerization, the bound subunits undergo the hydrolysis with the release of a phosphate molecule and GDP.[18] This GDP unit release from the microtubule at a faster rate which is nothing but the depolymerization phenomenon.[18] Thus, the dynamic behavior of microtubules is said to be a result of hydrolysis of GTP to GDP, which weakens the strength required for the binding of the monomer unit to the adjacent unit. Treadmilling[19] is the process of continuous loss of tubulin molecules attached to GDP from the minus end and the same numbers of tubulin molecules are added at the positive end. The loss

of molecules from the minus end is termed as "shrinkage" whereas the addition is called as "growth". Thus, alternate cycles of growth and shrinkage also lead to the dynamic instability of microtubules. As the tubulin molecules attached to the GDP are lost continuously, only the tubulin molecules that are attached to GTP are present in the cytoplasm of the cell, which can only get attached to the microtubule at the cap of GTP tubulins. This cap of GTP tubulins is present at the end of the microtubule whereas the GTP attached tubulin molecules gets binded for preventing the disassembly of the molecules.[19] Nevertheless, the β tubulins can hydrolyze at a faster rate than the attachment of new tubulins. If the rate of hydrolysis is faster than the attachment, the microtubules gets depolymerized, resulting in a phenomenon called, *catastrophe*.[3] The process of catastrophe can be terminated by *rescue*,[3,19] in which, a new cap of GTP tubulin is added to the tip. The half life of the microtubules within the cell is observed to be only few minutes due to their property of dynamic instability.[19]

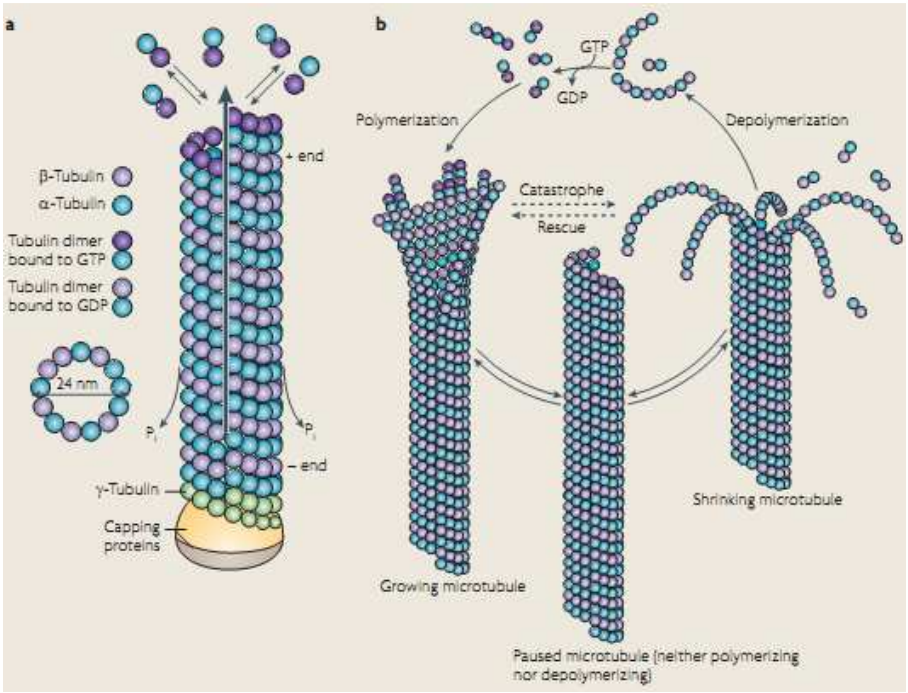


Figure 3: Microtubule Structure and Dynamic Instability

(note: this figure is taken from *Nat. Rev. Neurosci.* **2008**, *9*, 309-322)

1.2.4 Microtubules: Role in Cell Division

The process of cell division undergoes through various phases, such as Interphase, M-phase, G1 phase, S-phase and G2 phase. In Interphase,[20,21] the cell prepares for the

cell division. The nucleus and the nuclear envelope are separate and the chromatin appears as the thread-like structure. The cell spends maximum of its time in the Interphase. After this phase, it is the M-Phase, which is also called the actual dividing phase and it comprises of Prophase, Metaphase, Anaphase and Telophase.[21] The last is the cytokinesis, in which, the cell gets divided into two daughter cells.[21]

1.2.4a Organization of Microtubules at the Centrosome

During the process of cell division, the microtubules are attached at the centrosome.[17] The minus end of the microtubules is attached at the centrosome and the positive end is left freely.[17] Thus, the extension of the microtubules is nothing but the spindle fibers which plays a major role in the separation of chromosomes to daughter cells.[17] Therefore, the centrosome, here is, referred to, as the microtubule organizing center (MTOC) from which the assembly of microtubules gets initiated in forming mitotic spindles and play a major role in cell division.

Animal cells generally have a pair of centrioles at the centrosome (MTOC), perpendicular to each other, and are surrounded by a pericentriolar material.[17] The MTOC has two main functions, which include:

- ♣ organization of cilia and flagella in eukaryotic cells
- ♣ organization of mitotic and meiotic spindles

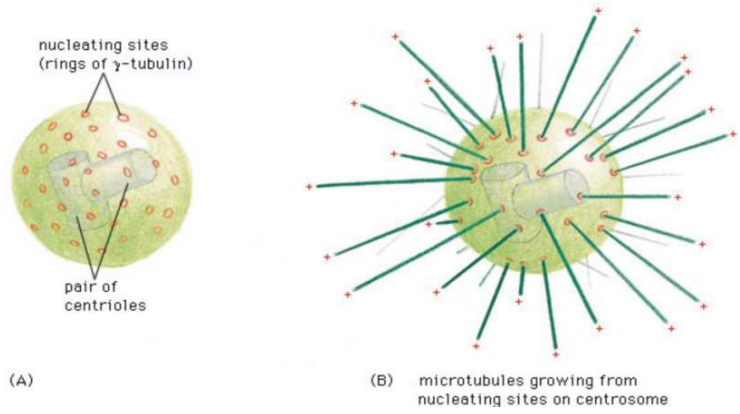


Figure 4: Representation of Centriole and Microtubules Emerging from Centrioles (note: this figure is directly taken from Google net)

During the mitosis as a proof of dynamic instability of microtubules, they rearrange completely to form mitotic spindles.[17] The microtubules arrangement of the interphase is disassembled and the tubulin units are further rearranged to form spindles.[17] This

formation of spindle fibers lead to the separation of daughter chromosomes and the formation of daughter cells.[17] The disassembly and reassembly of microtubules forms two microtubule organizing centers at the two opposite ends of the spindle fibers by the duplication of the centrosome.[21]

1.2.4b Phases of Cell Division

S.No	Phase		Explanation
1.	Interphase		Chromosomes are doubled in number; appear as thread-like chromatin initially but at the end, the actual as well as the sister chromosomes alters to sister chromatids (rod-like structures).
2.	M-Phase	Prophase	Beginning of cell division. Appearance of centrioles and the movement to opposite ends. Formation of spindle fibers.
		Metaphase	Attachment of chromatids to the spindle fibers.
		Anaphase	Splitting of sister chromatids and the movement of chromosomes to the opposite ends.
		Telophase	Formation of two daughter nuclei and the end of mitotic cell division.
3.	G_1-phase		Time gap between the mitosis and the synthesis phase
4.	S-Phase		Phase where the synthesis of DNA takes place.
5.	G_2-Phase		During this phase, the proteins required are prepared by the cell along with the cell growth in order to favor the cell division. Time gap between the replication of DNA and mitotic phase.

Table: Phases of Cell Division

The interphase of the cell division ends with the formation of mitotic spindles.[21] It is formed by the duplication of the centrioles and other components attached at the centrosome but they all are positioned at one end of the cell.[20] In the actual mitotic phase (M-Phase), the actual movement to the opposite ends then gets initiated. In the prophase, the centrosomes separate and move apart to the opposite poles.[20] During the separation of chromosomes, the chromatin gets condensed.[21] As discussed earlier, the

minus ends of the microtubules are anchored while the plus ends are left freely.[21] The plus end of the microtubule is the end which interacts with the chromosomes. The sister chromatids that are formed by the replication of single chromosome are joined by a common centromere, during the metaphase. The formed sister chromatids are then arranged on a vertical plane leading to the process of division which generally occurs at the anaphase. During this period, the plus ends of the microtubules are atta-

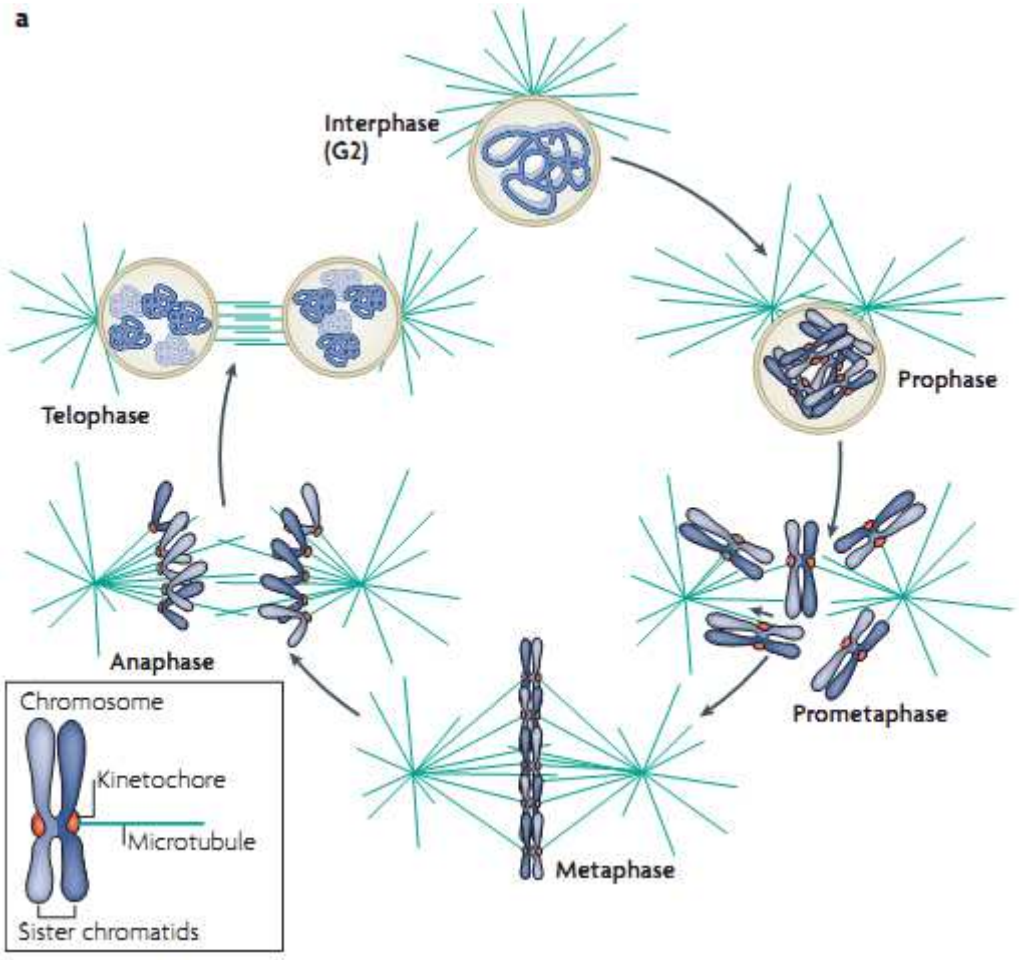


Figure 5: Phases in Mitosis (M-phase) Prophase, Metaphase, Anaphase and Telophase (note: this figure is taken from *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 33-46)

-ched to the centromere of the sister chromatids called as kinetochore.[20] The attached microtubules are called kinetochore microtubules which pull apart the sister chromatids, and thus, form a part of the cell division.[20] Apart from the kinetochore microtubules, astral and polar microtubules are also present, which does not attach to the centromere but strengthen the kinetochore microtubules by stabilizing them for helping in the cell division. As the sister chromatid are pulled apart, two spindles are

formed at the opposite ends and further undergo division into two daughter cells in Telophase followed by cytokinesis.[22]

The microtubules as discussed earlier are highly unstable, and therefore, stabilized by a type of special proteins, called as microtubule associated proteins (MAP's).[23] Although there are many types of proteins, MAP-1, MAP-2 and tau are the best identified from neuronal cells.[22] MAP-4 is identified from non-neuronal vertebrate cells.[21] Phosphorylation is the process associated with the activity of microtubule associated proteins (MAP's) and tau protein gained its importance as a major component in the characteristic lesions in the brains of Alzheimer's patients.[23] As discussed above, various types of proteins associated with microtubules help in the movement, hence termed as motor proteins. The two types of motor proteins, namely are, kinesins (towards + end) and dyenins (towards - end).[24] Both are called as the prototypes of microtubule motor proteins and move in opposite directions along the microtubules.[24]

1.2.5 Microtubules as Targets for Anti-cancer Therapy

As microtubules play a major role in the cell division process, they are considered to be the targets for anti-cancer therapy.[4] The mechanisms involve with microtubules as the targets for anti-cancer therapy include the blockade or altering anyone or more steps of cell division. This shows a direct effect on the multiplication of cells, which generally depends upon the dividing capacity of the cells.[4] The crucial involvement of microtubules in mitosis makes them a prime target for further developing anti-cancer agents. Microtubules play a major role in the movement and formation of mitotic spindles; suppressing these activities also inhibit the cell division.[4] Also, the inhibition of cell division leads to the destruction of microtubules.[4] In all the above cases, microtubules target mitotic division of cells. Hence, the drugs involving microtubules as targets are generally named as anti-mitotic drugs.[3] In addition, the microtubule targeted drugs can be effectively used in combination with other chemotherapeutic agents such as vinca alkaloids, taxanes etc. Hence, microtubules are considered as the targets for the study of cancer drugs.

Microtubule-targeted anti-mitotic drugs are mainly classified into two groups.[4] One class of anti-mitotic drugs demonstrate their activity by inhibiting the polymerization of tubulin in forming microtubules and they are called microtubule- destabilizing

agents or the polymerization inhibitors.[4] The second class of anti-mitotic drugs are termed as microtubule stabilizing agents or depolymerization inhibitors and these types compounds exhibit their activity by inhibiting the depolymerization of tubulin.

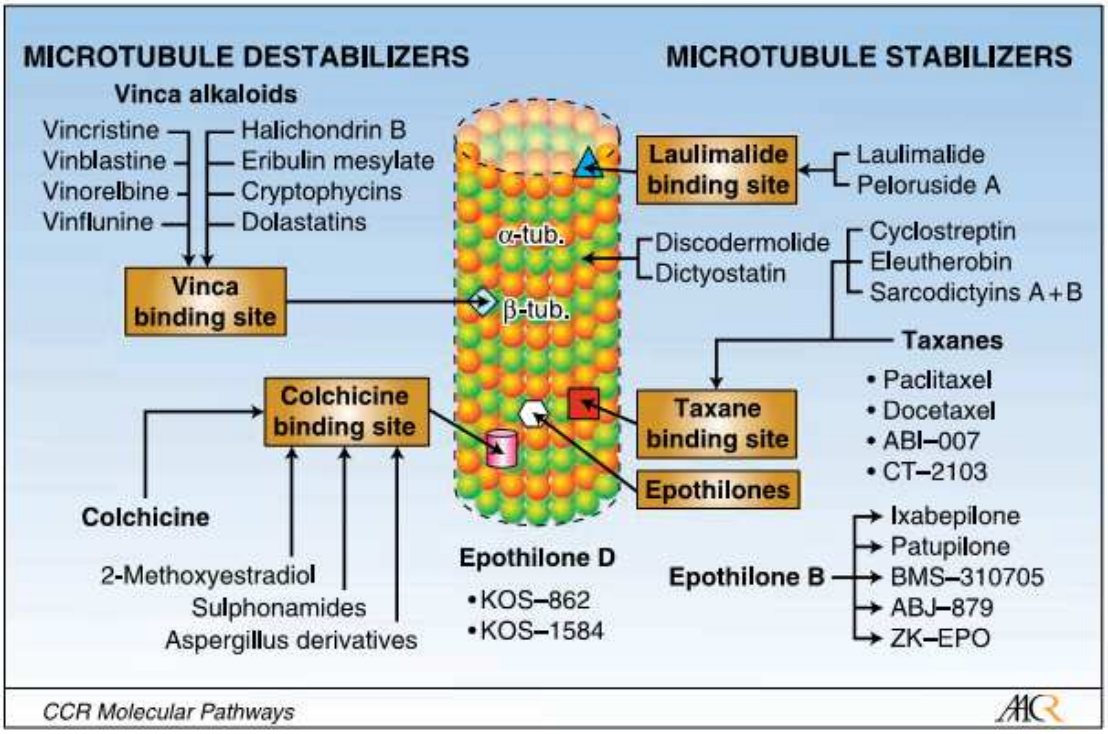


Figure 6: Microtubule Stabilizers and Destabilizers their Binding Sites on Tubulin (note: this figure is directly taken from *Clin. Cancer Res*. **2008**, *14*, 7167-7172)

1.2.6 Microtubule Destabilizing Agents

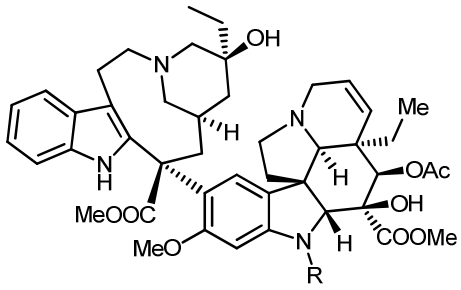
Microtubule destabilizers consist predominantly of drugs that act at the Colchicine-Vinca binding sites. Colchine and related analogs bind to the β-subunit of soluble tubulin and this leads to the formation of tubulin-colchine complex.[25] This complex undergo polymerization with normal tubulins in forming the microtubules.[25] The presence of this complex brings the conformational change which blocks the tubulin dimers from further addition. This process prevents the growth of the microtubules. As the tubulin-colchine complex slows down the addition of new dimmers, the microtubule depolymerizes due to the structural instability during the Metaphase of mitosis.[26] The vinca alkaloids bind to the β-subunit of tubulin dimers at a separate region, called the vinca-binding domain. The binding of vinca alkaloids to soluble tubulin is reversible and rapid, however, they induce the conformational change in tubulin in connection with tubulin self-association.[3] Vinca alkaloids binds to tubulin with a high affinity at the microtubule

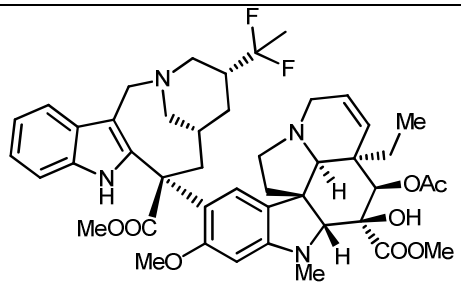
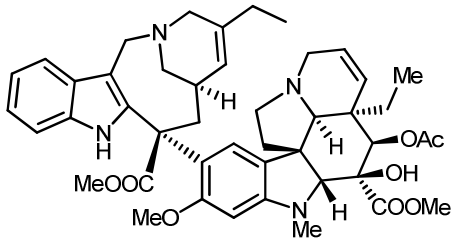
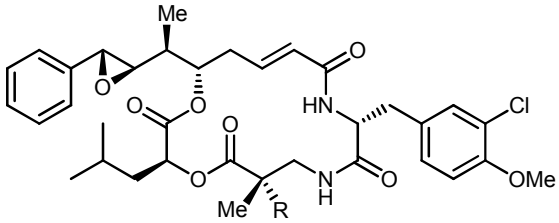
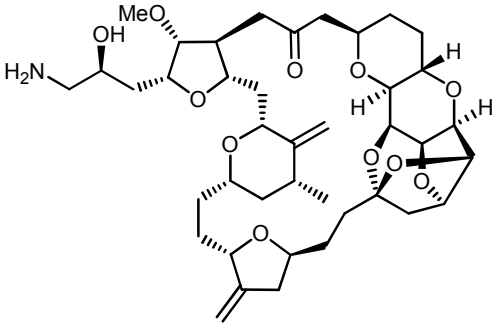
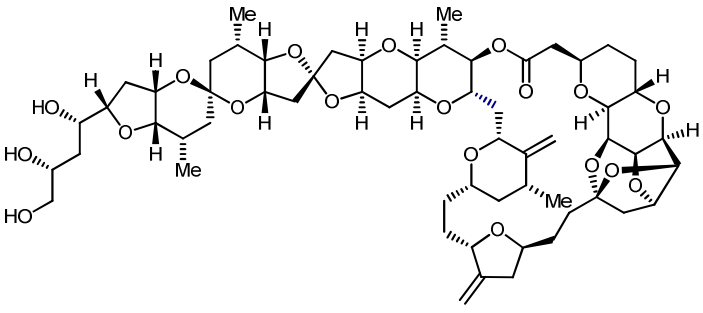
ends but with low affinity at the tubulin sites present along the sides of the microtubule cylinder.[3] The binding of these drugs at the high affinity sites results in the kinetic suppression of tubulin exchange even at a low drug concentration while their binding to the low affinity sites in relatively high drug concentration depolymerizes microtubules.[3]

1.2.7 Microtubule Stabilizing Agents

Microtubule stabilizers consist predominantly of drugs that act at the taxane-and laulimalide binding site. Paclitaxel (taxol) is the prototype (preliminary version) of the taxane family of antitumor drugs, and it is the first natural product shown to act as the microtubule stabilizer.[27] Taxol improves the microtubule polymerization by enhancing both the elongation and nucleation phases of the polymerization reaction, and it decreases the critical tubulin subunit concentration. The binding mechanism of taxol imitate that of the GTP nucleotide with some differences.[27] At one end of the tubulin dimer, the GTP binds and maintain the contact with the next dimer along with the protofilament. Whereas, taxol binds to one side of β-tubulin while keeping the contact with the next protofilament. Taxol binding sites are also located in the assembled tubulin while GTP binds to unassembled tubulin.[27] The hydrolysis of GTP intiates the disassembly of the microtubule system, while the activation of tubulin by taxol leads to permanent stabilization of the microtubule.[28]

Shown below are some of the tubulin destabilizing and stabilizing agents along with their binding domains and structures:

Domain	Name	Structure
	R=Me Vinblastin	
	R= CHO Vincristine	

Vinca domain	Vinflunine	
	Vinorelbine	
	Natural : Cryptophycin 1 R = H	
	Synthetic: Cryptophycin 52 R = Me	
	Eribulin mesylate (Halaven ^{TM})	
	Halichondrin B	

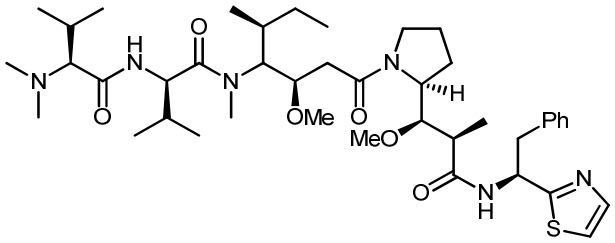
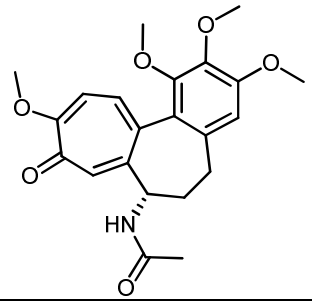
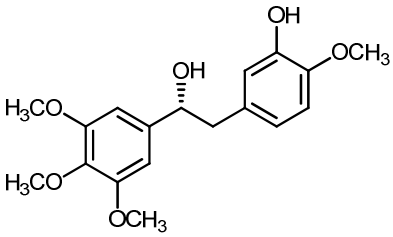
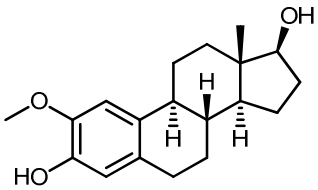
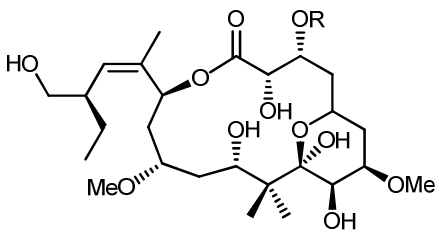
	Dolastatin 10	
Colchicine domain	Colchicine	
	Combretastatin	
	2-methoxy estradiol	

Figure 7: Tubulin Destabilizing Agents

laulimalide binding site	R=Me Peloruside A	
	R= H Peloruside B	

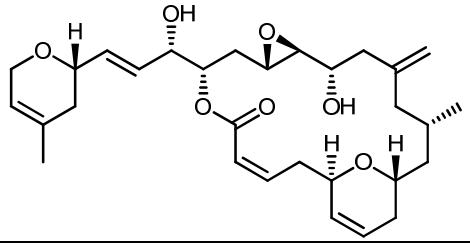
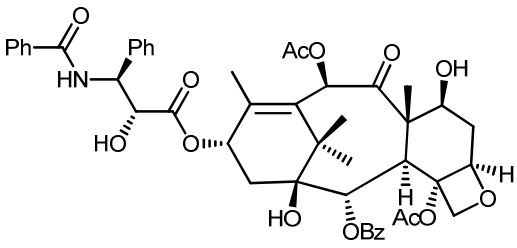
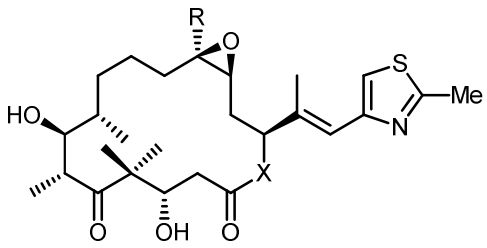
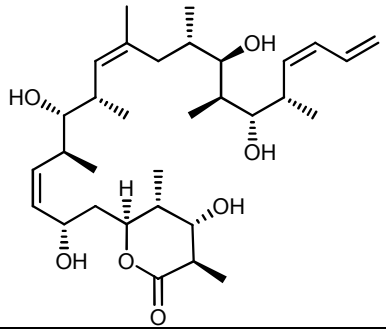
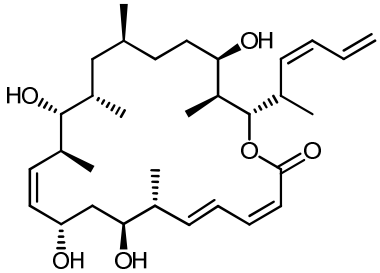
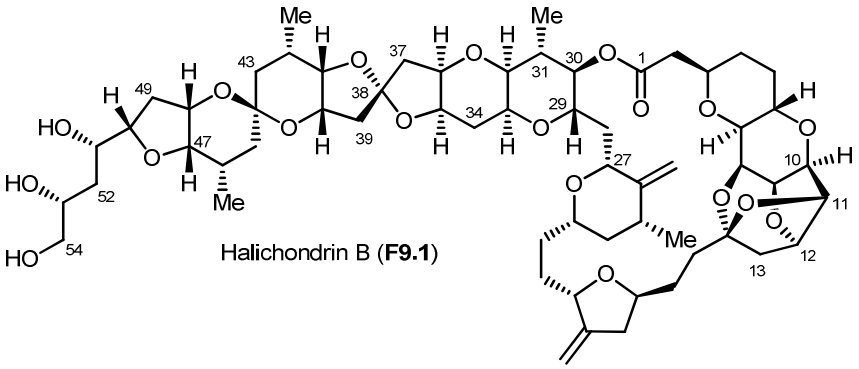
	(-)-Laulimalide	
Taxane binding site	Paclitaxel(taxol)	
	X = O R = H Epothilone A	
	X = O R = CH_3 Epothilone B	
	X = NH, R =CH3 Ixabepilone (BMS-247550)	
	Discodermolide	
	Dictyostatin	

Figure 8: Tubulin Stabilizing Agents

1.2.8 Synthesis of Microtubule Destabilizing Agents

Eribulin: Eribulin mesylate (Halaven®) (**Figure 7)** is a non-taxane microtubule dynamics destabilizer, and is approved for the treatment of late-stage metastatic breast cancer.[29] It is a macrocyclic ketone, non-peptidic, and fully synthetic drug that is derived from the truncated version of halichondrin B (**F9.1**).[30] Halichondrins are a class of polyether macrolides, first isolated from the marine sponge *Halichondria okadai* Kadota,[29] which is commonly located along the pacific coast of Japan. The potent *in vivo* activity of the crude extracts from *Halichondria okadai* Kadota, led the isolation and identification of nor-halichondrin A (**F9.2**) with the cytotoxicity, IC_{50} = 5 ng/mL vs. B16 melanoma as shown by the Uemura team[31] Later, Uemura and co-workers collected 600 kg of *H. Okadai*, and further identified seven halichondrins.[32]

Halichondrin family is having an unusual 2,6,9-trioxatricyclo [3.3.2.0] decane ring system, as well as a 22-membered macrolactone ring, two exocyclic olefins, and, an array of poly-oxygenated pyran and furan rings that define three major classes of halichondrins A, B and C.[32] The detailed isolation and structural elucidation of the halichondrin family is nicely reviewed by Phillips and co-workers in 2009.[31]



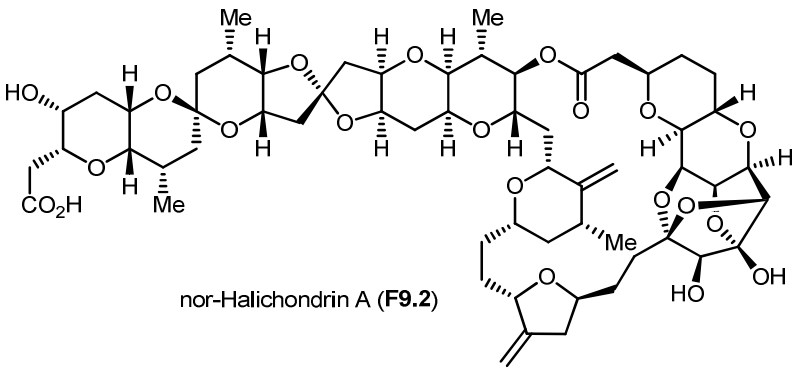
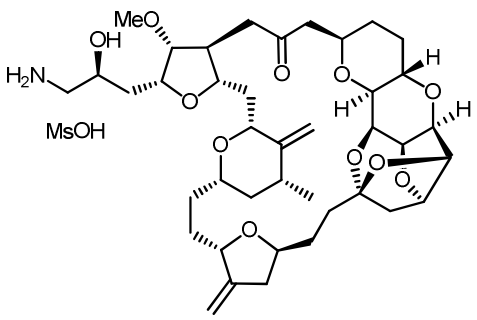


Figure 9: Halichondrin B and nor-Halichondrin A

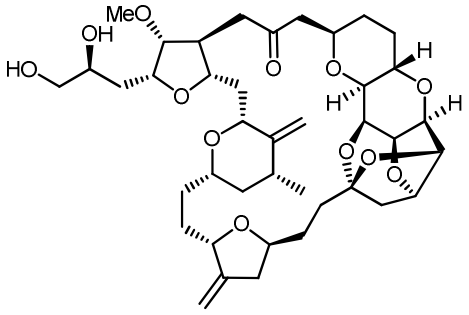
First, the total synthesis of halichondrin and norhalichondrin was achieved by Kishi and co-workers in 1992[33] Second, the total synthesis of norhalichondrin B was reported by Phillips and co-workers in 2009.[34] Some other groups also worked on the total synthesis of halichondrin family natural products (**Figure 10**).[35,36]

1.2.8a Discovery and Development of Eribulin

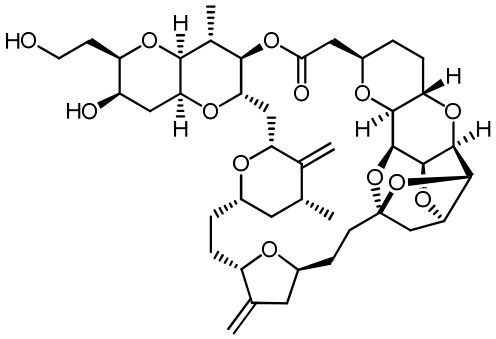
During the biological evaluation of halichondrin B and its intermediates, a macrocyclic macrolactone diol **F10.3** was discovered as a more potent compound than halichondrin B against DLD-1 human colon cancer cells.[37] Among these several derivatives, compounds having the ketone moiety were the most promising, and, thus, **F10.2** (ER-076349) and **F10.1** showed prominence in activity (E7389, previously ER-086526). Halaven **F10.1** is a non-taxane, first-in-class microtubule dynamics inhibitor. The FDA approved this compound for the metastatic breast cancer[38]



F10.1
Eribulin mesylate (Halaven ™)



F10.2 ER-076349



F10.3

Figure 10: Chemical Structures of Eribulin (**F10.1)**, ER-076349 (**F10.2)** and Diol (**F10.3)**

The novel mechanism of action of eribulin is different from other several classes of tubulin targeted agents (**Figure 11**).[39] These known compounds bind to an interdimer

interface or the tubulin subunit alone and, inhibits the microtubular growth phase of microtubular dynamics instability in interphase cells without causing any effect on shortening.[40,41] In addition to this, moreover, it also promotes the centromere spindle relaxation without affecting the rate of stretching.[40]

The preclinical studies of eribulin showed a broad spectrum of anti-tumor activity against a wide variety of human cancer types.[42] The phase II trials of eribulin in chemotherapy pre-treated advanced breast cancer patients showed a manageable tolerability profile with most common drug-related adverse effect

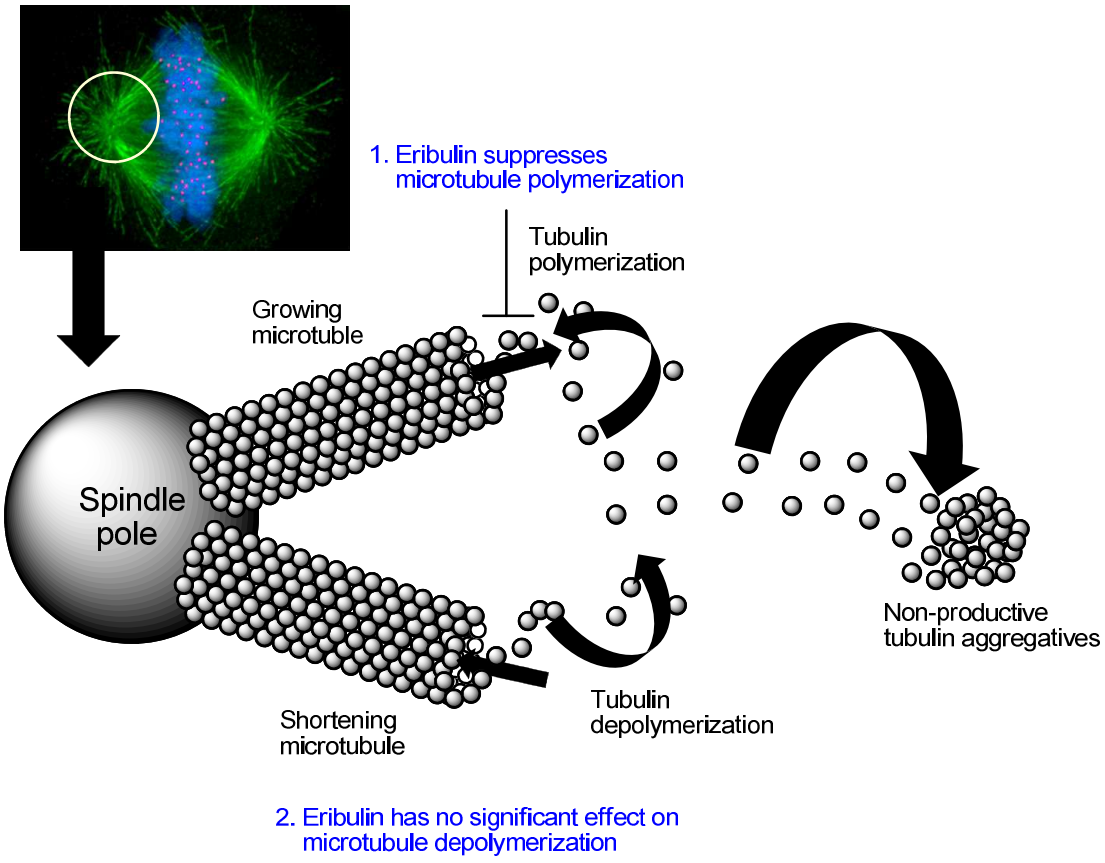


Figure 11: Eribulin Mechanism of Action[43]

(note: this figure is drawn from *Mol. Cancer Ther.* **2005**, *4*, 1086)

Peripheral neuropathy is a common toxicity associated with tubulin-targeted chemotherapeutic agents. The phase II study compared the incidence and severity of neuropathy associated with eribulin or ixabepilone in metastatic breast cancer was designed to detect a difference in neuropathy rate of 35% for eribulin versus 63% for ixabepilone.[44] These studies have shown the incidence of neuropathy (any grade) to

be 33.3 and 48.0% and peripheral neuropathy as 31.4 and 44.0% for eribulin and ixabepilone respectively even though these results were not significant. The phase III trial, 762 women with LABC (Locally Advanced Breast Cancer) or MBC(Metastatic Breast Cancer) were randomly allotted in 2:1 ratio to eribulin 1.4mg/m^2 over 2-5 min on days 1 and 8 of 21-days cycle (n=508). The treatment of the physicians choice (TPC) (n=254), result showed a significant increase in an overall survival for eribulin (13.1 months) compared with TPC (Treatment of Physician's Choice) (10.6 months).[45] Based on the result, the FDA approved eribulin mesylate as the third-line treatment for MBC (Metastatic Breast Cancer) refractory to anthracyclines and taxanes.

1.2.8b Key Fragments of Eribulin

The Eisai synthesis of ER-076349 and E7389 utilized most of the technology that was adopted from Kishi's approaches. ER-076349 was readily synthesized from three fragments, and, they are shown in **Figure 12**. Later, a practical, gram scale synthesis of eribulin was also reported by Yu and co-workers.[46]

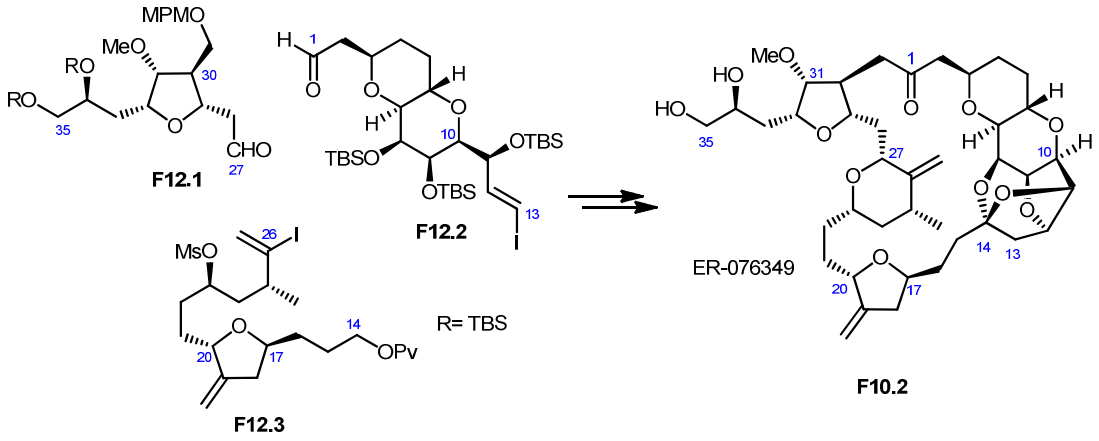


Figure 12: Key Fragments of ER-O76349

1.2.8c Synthesis of Eribulin Fragments

Fragment C14-C26: In this section, I am going to discuss the recent synthesis of C14-C26 fragment, reported by Chandrasekhar and co-workers.[47]

The retrosynthetic analysis of target **F13.1** is shown in **Figure 13.** Compound **F13.1** obtained from oxidation was followed by the Wittig reaction of **F13.2.** The tetrahydrofuran ring formation could be achieved through Sharpless asymmetric di-

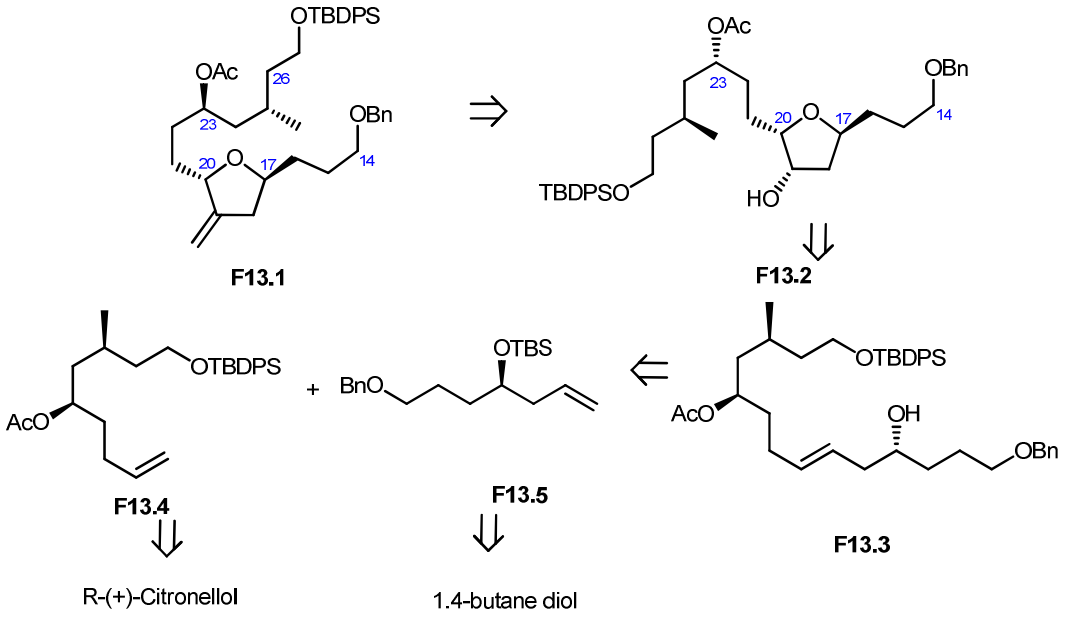
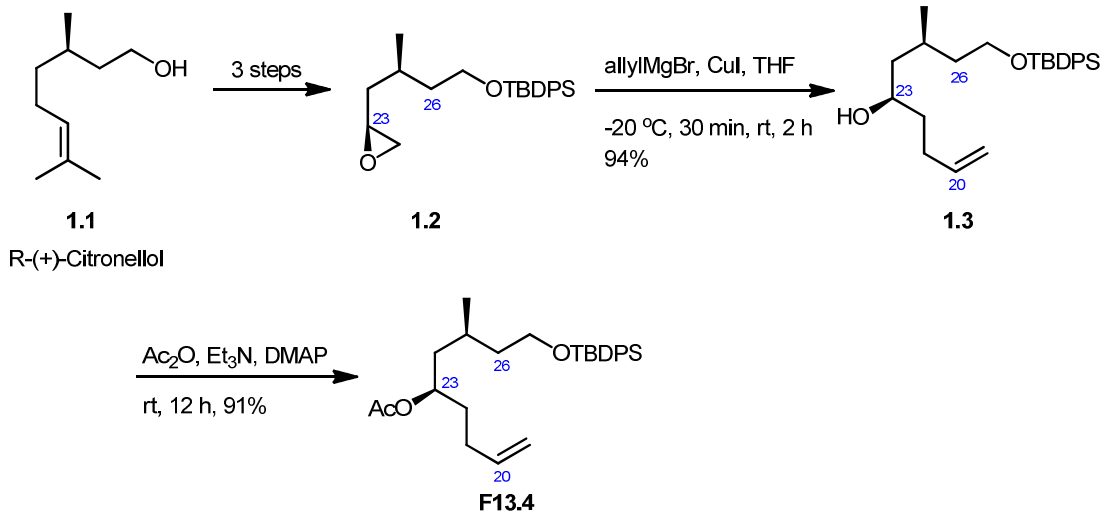


Figure 13: Retrosynthesis of C14-C26 Fragment

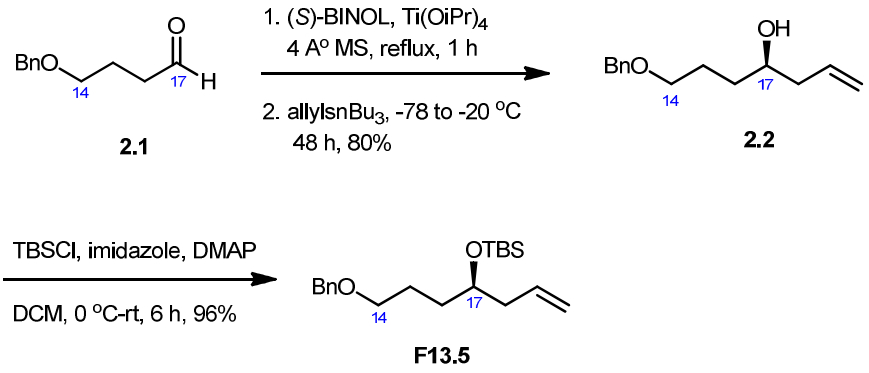
-hydroxylation followed by *in situ* SN^2-cyclization of alcohol **F13.3**. Compound **F13.3** could be furnished by the cross-metathesis reaction between two moieties, **F13.4** and **F13.5**, which were easily obtained from *R*-(+)-citronellol and 1,4-butane diol, respectively.



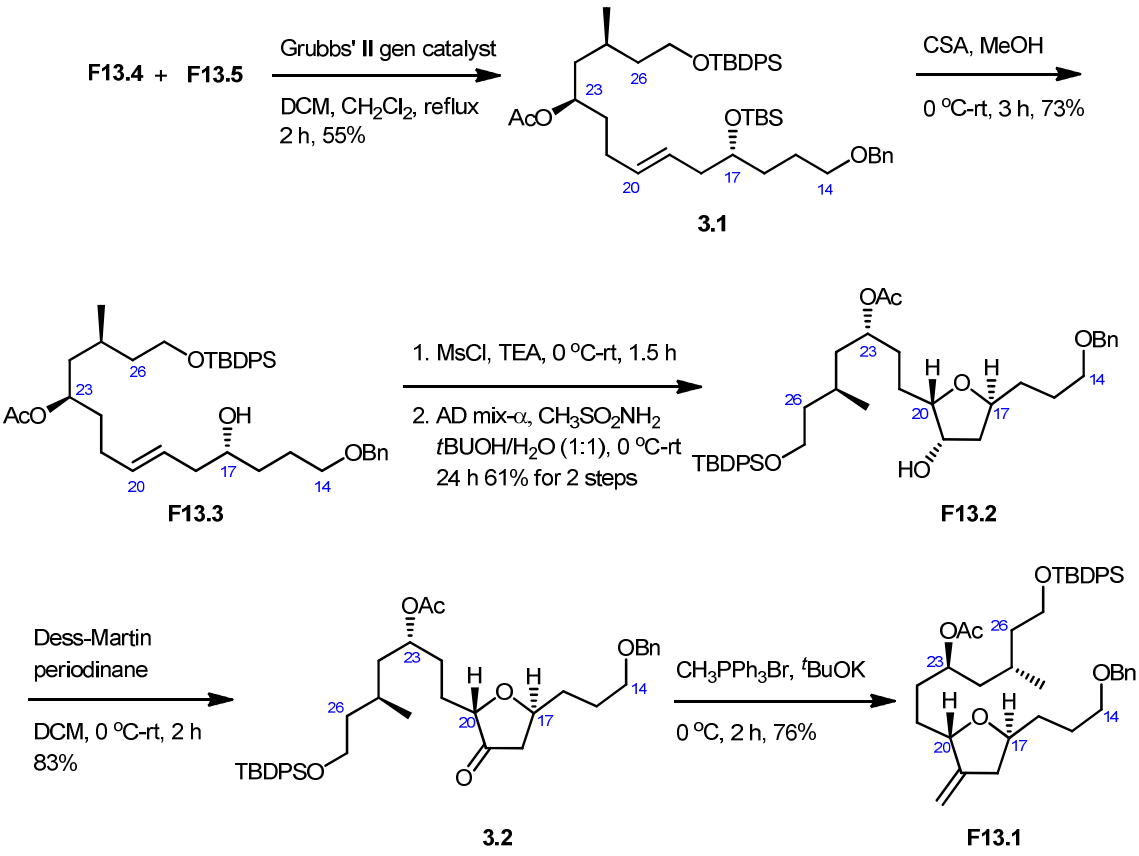
Scheme 1: Synthesis of Compound **F13.4**

A series of silyl group protection, ozonolysis of the olefinic bond on compound **1.1** yielded an aldehyde,[48] which was then converted to an epoxy silyl ether **1.2** using an organocatalytic reaction.[49,50,51] This upon treatment with allyl MgBr/CuI gave **1.3**.

Following the secondary alcohol protection, this approach furnished the synthesis of **F13.4.**



Scheme 2: Synthesis of Compound **F13.5**

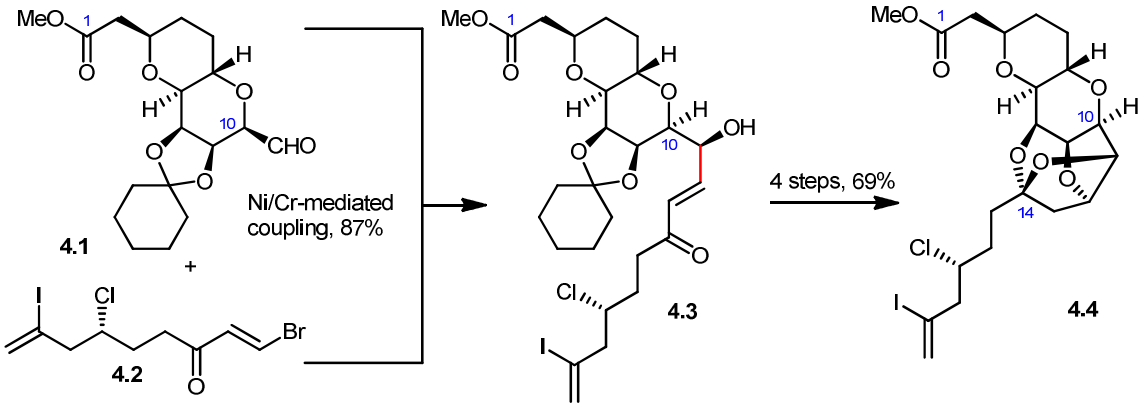


Scheme 3: Synthesis of C14-C26 Fragment

An asymmetric Keck allylation[52] of aldehyde, **2.1** (obtained from 1,4-butane diol)[53] gave homoallylic alcohol **2.2** (95% ee). This was further followed by the secondary alcohol protection with the silyl group, giving compound **F13.5.**

The differentially protected tetrol, compound **3.1** was then obtained by a cross-metathesis reaction between **F13.4** and **F13.5.** Selective deprotection of the secondary silyl group, finally, gave compound **F13.3.** The tetrahydrofuran ring formation could be achieved through Sharpless asymmetric dihydroxylation followed by *in situ* SN^2-cyclization[54,55] of alcohol, **F13.3.** Oxidation of compound **F13.2** gave the corresponding keto compound **3.2,** which upon Wittig reaction produced the target as the exomethylene product, **F13.1**

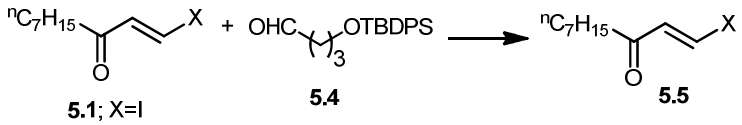
Fragment C1-C19: Here, I am explaining the recent synthetic strategy developed for the C1-C19 fragment of eribulin/halichondrin by the Kishi's group.[56]



Scheme 4: Synthesis of C1-C19 Fragment

The Cr-mediated coupling of organic triflates or halides with aldehydes is affiliated to a class of 1,2-carbonyl addition reactions.[57] Ni-or-Cr-Mediated coupling of alkenyl triflates or halides were originally reported by Hiyama, Takai, Nozaki and co-workers in 1983.[58] In this process, the active nucleophiles, $RCrX_2$, are generated from their corresponding triflates or halides *in situ*.

The researchers first studied the coupling efficiency of β-iodoenone (**5.1**) with an aldehyde (**5.4**) using 10mol% Cr catalyst prepared from sulfonamide **5.6**[58] and 1 mol% **5.7**. In addition to this, different types of catalysts,[59] β-bromoenones and aldehydes were also tested. Moreover, the computational experiments were performed to study the reactivity between β-bromoenones and vinyl iodides.[59,60] Finally, the coupling of **4.1** and vinylogous acyl anion of β-bromoenone **4.2** with Cr-catalyst (which was prepared from sulfonamide **5.6** and Ni-complex **6.3**) gave good results.

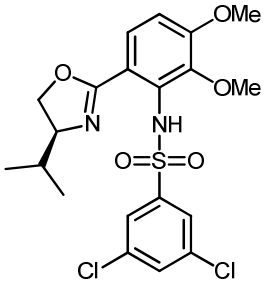


5.1; X=I
5.2; X=Br
5.3; X=Cl

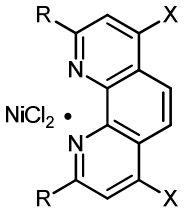
5.4

5.5

5.1-5.5 27% (condition A) and 36% (condition B)
5.2-5.5 57% (condition A) and 81% (condition B)
5.3-5.5 52% (condition A) and 48% (condition B)



5.6



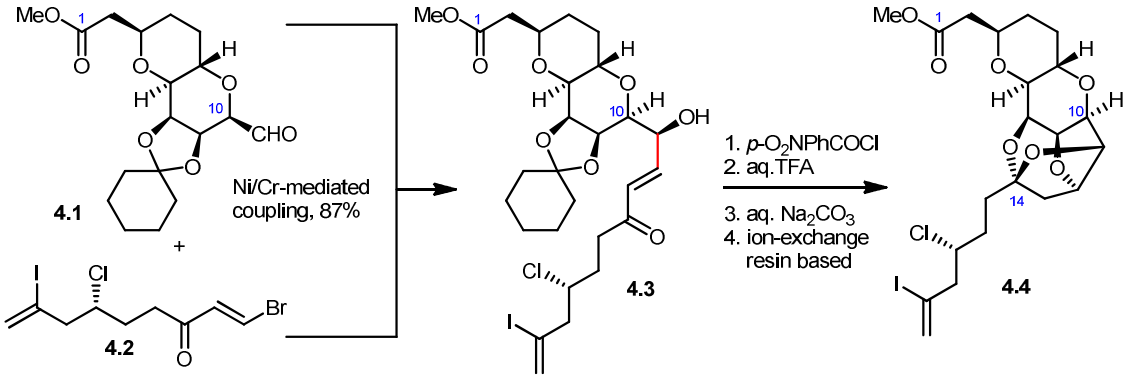
5.7: $(Me)_2Phen(H)_2 \bullet NiCl_2$
R=Me, X=H

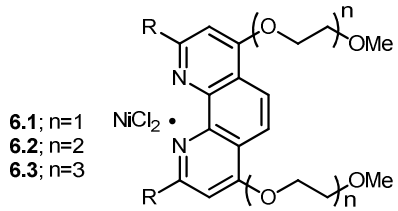
5.8: $(Me)_2Phen(OMe)_2 \bullet NiCl_2$
R=Me, X=OMe

A: 10 mol% Cr-complex prepared from sulfonamide **5.6** and 1 mol% nickel complex **5.7**

B: 10 mol% Cr-complex prepared from sulfonamide **5.6** and 0.05 mol% nickel complex **5.8**

Scheme 5: Optimized Conditions





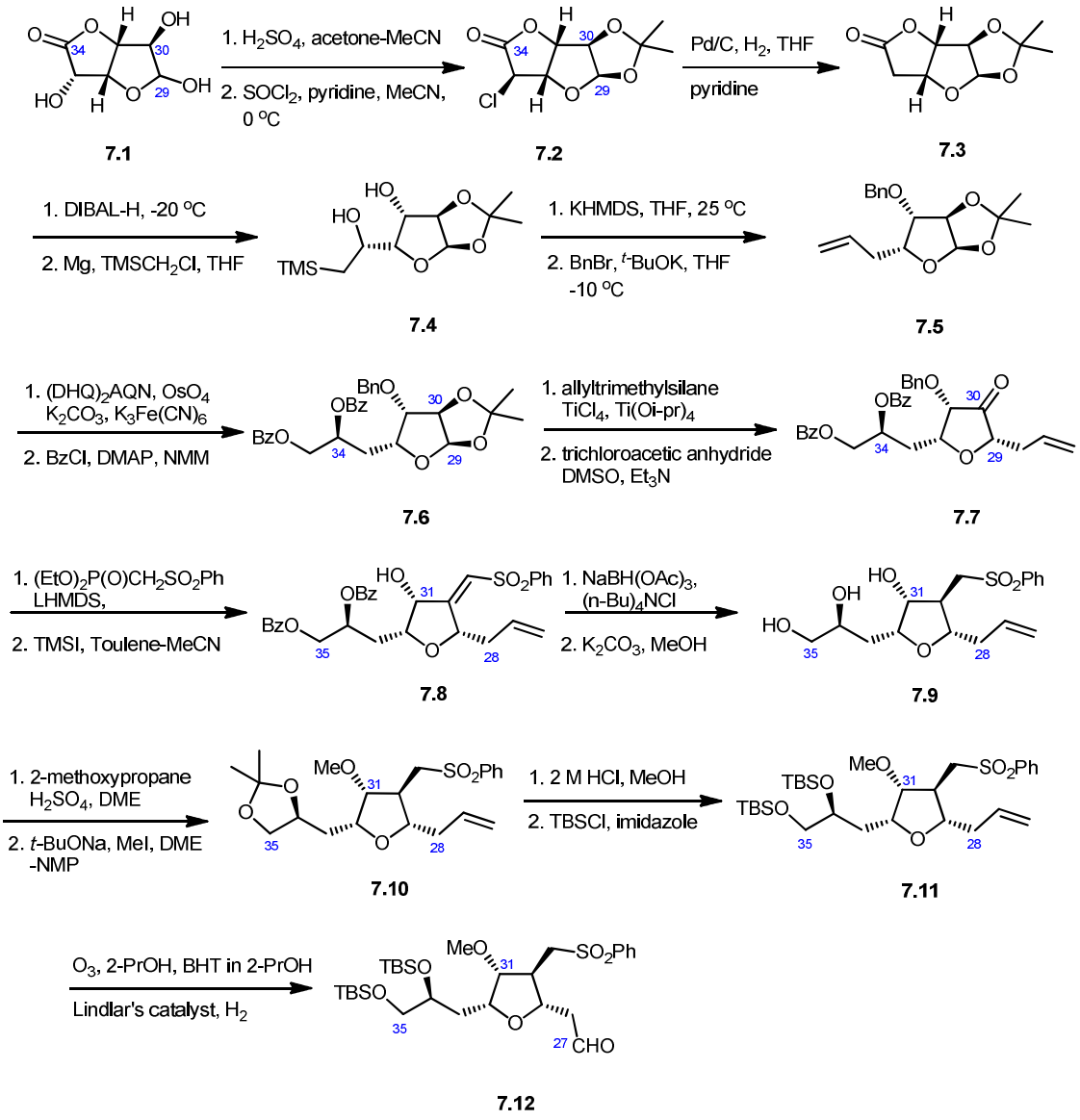
6.1; n=1
6.2; n=2
6.3; n=3

coupling conditions: 10 mol% Cr-catalyst prepared from sulfonamide **5.6**, 0.05 mol% Ni-complex **6.3**, Zr(Cp)2 (1.5 eq), LiCl (2 eq) and Mn (2 eq) in MeCN (0.4 M) at RT, 3h.

Scheme 6: Applied Reaction Conditions

The furan ring generation was carried-out by an acylation of allylic alcohol as the *p*-nitrobenzoate which was then followed by an aqueous TFA hydrolysis of the cyclohexilidene group, finally, giving the diol moiety. This was then treated with aqueous K_2CO_3 for the *p*-nitrobenzoate hydrolysis followed by an oxy-Michael addition of C9-hydroxyl group with an α, β-unsaturated ketone giving 1:2 mixture. It was then subjected to an ion exchange resin, thus completing the synthesis of the desired polycyclic compound, **4.4**[61,62]

Fragment C27-C35: In this section, I am going to discuss the recent synthesis of C27-C35 fragment from Chase and co-workers.[63]



Scheme 12: Synthesis of C27-C35 Fragment of Eribulin

Glucurono-3, 6-lactone acetonide,[64] **7.2** was obtained from **7.1**, which was then subjected to catalytic hydrogenation, giving deoxyacetonide **7.3.** DIBAL-H reduction and silyl protection of primary alcohol further produced **7.4**. Treatment of **7.4** with KHMDS induces the reductive elimination of trimethylsilanol followed by benzyl protection and this approach yielded the olefin **7.5.** The Sharpless asymmetric dihydroxylation[65,66] finally produced the chiral secondary alcohol diastereomeric ratio of 3:1 and this was then followed by the benzoyl protection giving the dibenzoate **7.6.** *C*-Glycosidation[67] of **7.6** with allyltrimethylsilane, followed by a modified Moffat oxidation, led the synthesis of compound, **7.7.** Horner-Wadsworth-Emmons reaction,[64] followed by the cleavage of the benzyl ether of compound **7.7** gave **7.8**. The hydroxy-directed conjugate reduction[68] of **7.8**, which upon treatment with base produced the corresponding triol**, 7.9.** The acetonide protection followed by methylation of compound **7.9** furnished the synthesis of **7.10.** Deprotection of acetonide in acidic medium followed by silylation produced **7.11.** Finally, the target compound was synthesized by ozonolysis of the terminal olefin of **7.11**, followed by the reductive workup.

1.2.9 Synthesis of Microtubule Stabilizing Agents

Peloruside A: Peloruside A was isolated by Northcote and co-workers in 2000 from the New Zealand marine sponge *Mycale hentscheli*,[69,70] and was identified as a tubulin-stabilizing agent by Miller and co-workers in 2002.[71] The *in vitro* studies with purified tubulin showed that peloruside directly promote the tubulin polymerization in the absence of microtubule-associated proteins.[72] Peloruside A binds to the laulimalide site with β-tubulin, which is different site to the one, tubulin binding to paclitaxel. It was further indicated that peloruside A and laulimalide have a different binding site from other microtubule-stabilizing agents.[72]

In the laulimalide-tubulin complex, the parts of the macrocycle and the side chain of laulimalide are deeply inserted into a pocket formed by residues Gln293, Phe296, Pro307, Arg308, Tyr312, Asn339, Val335, Tyr342, and Phe343 of β –tubulin **(Figure 14 b)**. The O27 atom of dihydropyran moiety in the side chain makes a water-mediated hydrogen bond with the side chain -OH group of Tyr312 and the main chain carbonyl of Phe296. The O20 atom in the side chain and the O9 atom of the

tetrahydropyran ring in the macrocycle make hydrogen bonds with the side chains of Asp297/Ser298 and Asn339 respectively.

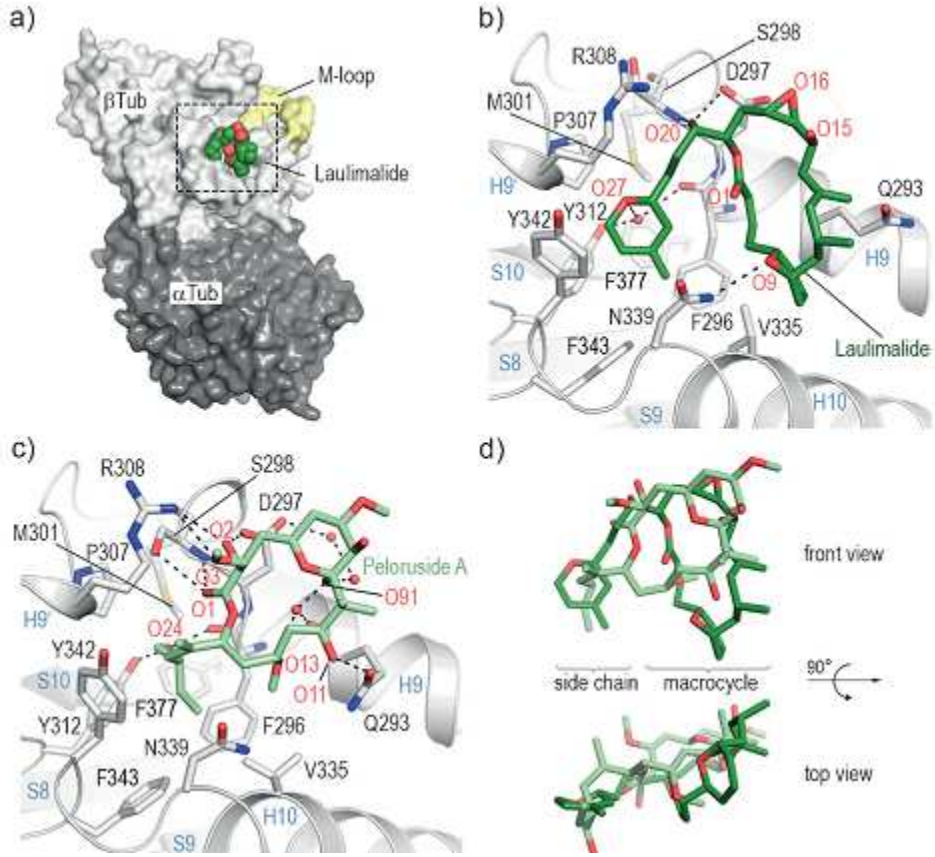


Figure 14: Complex Formed between laulimalide-Tubulin and the Peloruside A-tubulin (note: this figure is directly taken from *Angew. Chem. Int. Ed*. **2014**, *53*, 1621-1625)

In the peloruside A-tubulin complex, hydrogen bonds are formed between the O24, O11, O3, O2, and O1 atoms of peloruside and the side chains of Tyr312, Gln293, Arg308, Asp297/Arg308, Ser298 of β–tubulin **(Figure 14 c)** respectively.

Laulimalide-tubulin complex,[73] and peloruside A-tubulin complex,[73] both shows the macrocycles as well as the side chains of microtubule stabilizing agents superimpose well, further demonstrating a common binding mode **(Figure 14- d).**

1.2.9a Synthesis of Peloruside

Herein, I am going to discuss the recent synthesis of peloruside from Evans and co-workers.[74] The purpose of discussing this synthesis is to report a convergent approach that was developed by the researchers to this natural product.

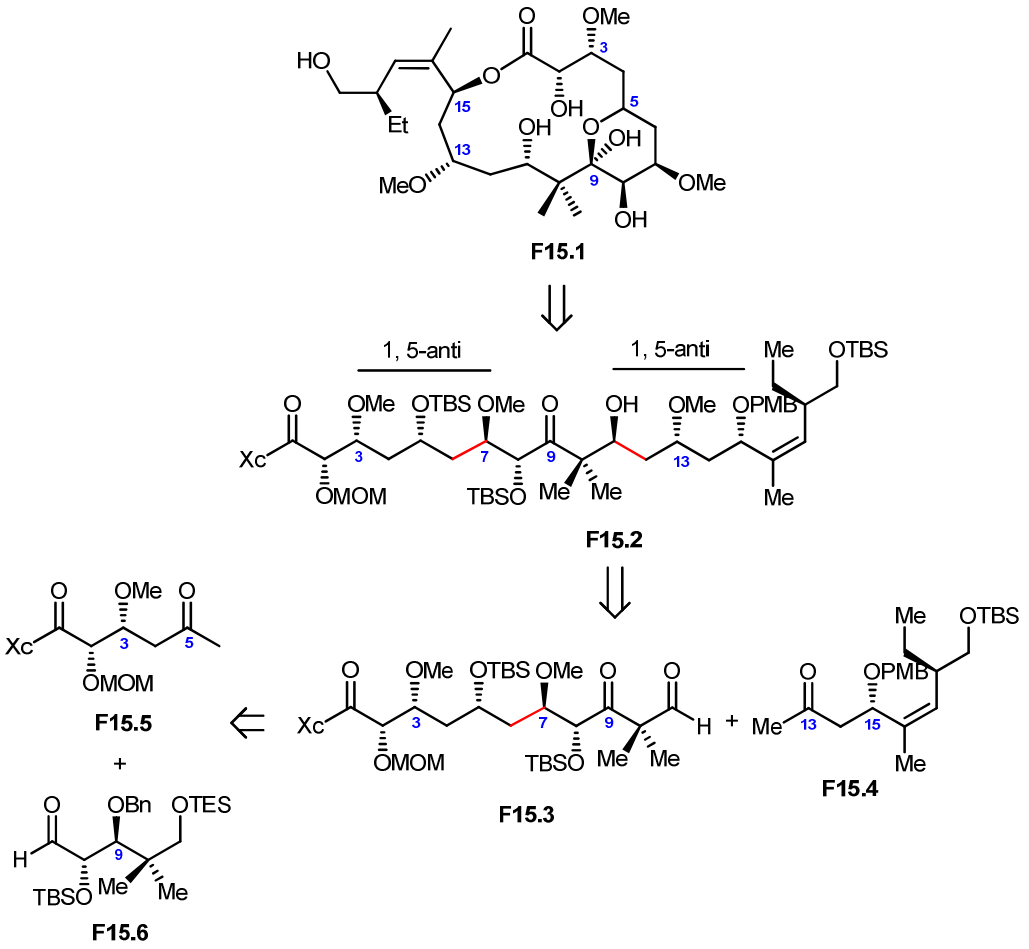
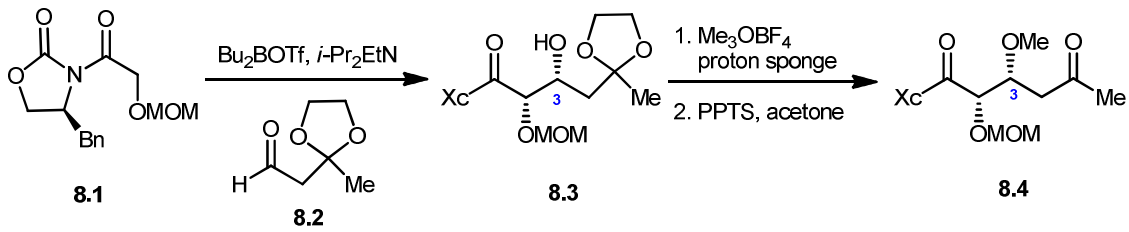


Figure 15: Retrosynthesis of Peloruside A

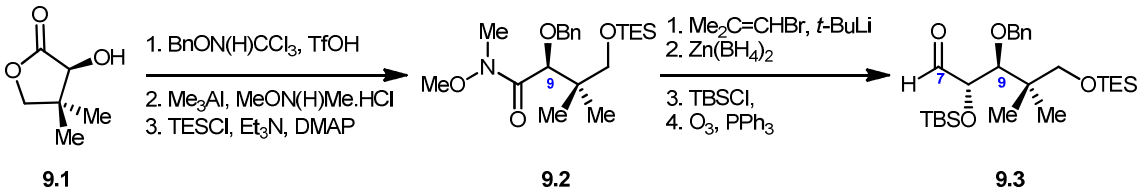
The disconnection of **F15.1** relies on the two highlighted aldol disconnections as it is illustrated in **Figure 15**. Target compound **F15.1** obtained from **F15.2,** which is obtained from aldol reaction of **F15.4** and **F15.3.** Compound **F15.3 was** obtained from aldol reaction between **F15.5** and **F15.6.**

Imide based aldol reaction between **8.1** and **8.2** established the C2-C3 *syn* stereochemistry with an excellent diastereoselection,[75] and this approach, gave **8.3**, which upon methylation followed by an acetal cleavage, produced synthon **8.4**

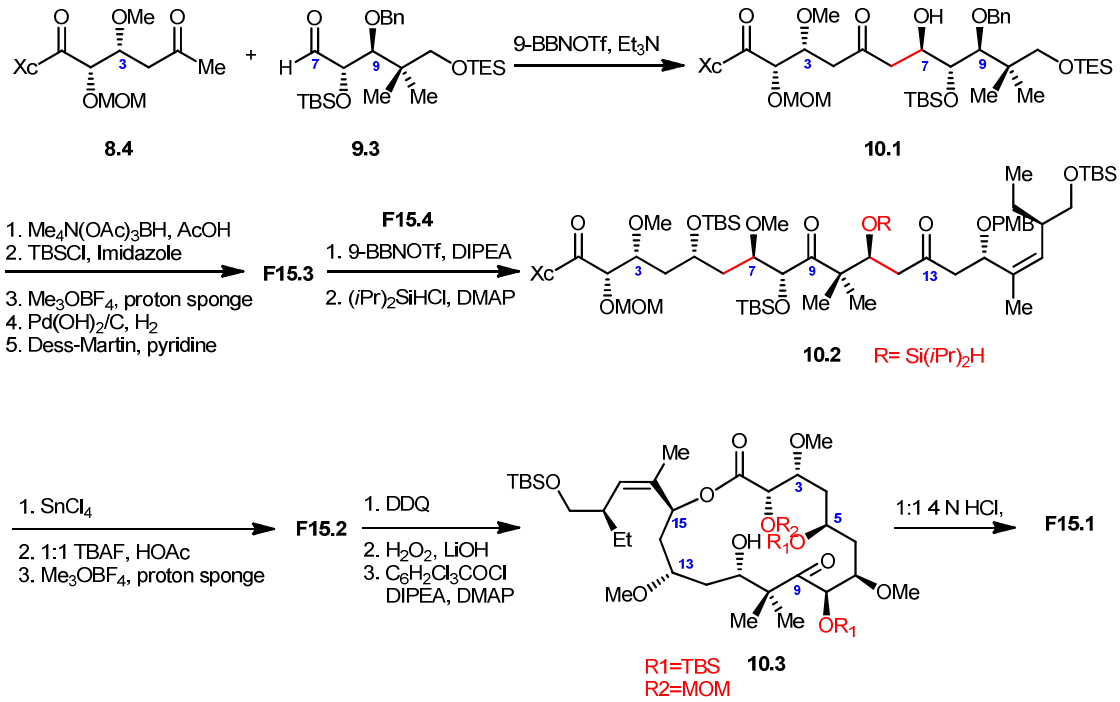


Scheme 8: Synthesis of Synthon **8.4**

Compound **9.2** was obtained from starting material (*S*)-pantolactone in three steps.



Scheme 9: Synthesis of Synthon **9.3**



Scheme 10: Completion of Synthesis

The synthon **9.3** was produced from **9.2** in four steps which involved Wittig, stereoselective borohydride reduction[76] (95:5 diastereoslectivity) of carbonyl group, the protection of alcohol with the silyl group and finally, the ozonolysis.

Compound **10.1** was obtained (dr = 98:02) by aldol reaction[77] between methyl ketone **8.4** and aldehyde **9.3.** A series of reaction on **10.1** which involved the reduction of C5 carbonyl with triacetoxyborohydride[78] (dr = 10:1), silyl group protection of less hindered C5 hydroxyl group, methylation of C7-hydroxyl group, removal of benzyl, triethylsilyl and oxidation, finally gave compound **F15.3.**

The aldol reaction of aldehyde **F15.3** with methyl ketone **F15.4** produced the diastereomers in 20:1 ratio which on protection with silyl group gave compound **10.2.** Chemo- and stereo-selective intramolecular *anti* reduction[79] of **10.2** with $SnCl_4$, gave the desired diastereomer with 40:1 selectivity. This upon subsequent C11-C13 silyl protecting group removal, selective methylation of C13 hydroxyl group, yielded **F15.2.** The -OPMB removal of **F15.2** followed by the hydrolysis and subsequent Yamaguchi macrocyclization[80] produced **10.3.** Finally, peloruside A **(F15.1)** was achieved through the protecting groups removal of **10.3.**

1.3 Introduction to Actin

The leading cytoskeletal protein of all eukaryotic cell is actin, and it participates in many essential cellular processes, including cell motility, cell division, cell signaling, muscle contraction, and cytokinesis.[81]

Actin was first isolated by Banga and Szent-Györgyi in 1942 from the rabbit's muscle cell, in which, it constitutes approximately 25-30% of total cell protein.[82] Actin have 3 isoforms, and they are: α-actin, β-actin and γ-actin. Within the cell, actin is existing in two forms. One is the monomeric called G-actin or globular actin, second a double stranded filamentous polymer called F-actin. The crystal structures of both G-actin (monomer) and F-actin (polymer) were determined in 1990 by Kabsch, Holmes and their colleagues.[83]

1.3.1 Microfilaments

These are solid rods about 7 nm in diameter and were named so because of their size and diameter when compared to other components of the cytoskeleton.[84] These filaments are made up of a protein called actin, which is present in muscle fibers and is involved in contraction.[85] Apart from the presence in muscles, it also forms a part of cytoskeleton. These protein units polymerizes into thin filaments, which are flexible in nature, called as actin filaments or microfilaments.[84]

Each G-actin (monomer) has binding sites that mediate head to tail interactions with two other G-actins. This addition of Mg^{+2}, K^+ or Na^+ to the G-actin monomer intiates the polymerization and forms F-actin (filaments),[81] in which, each monomer is rotated by 166^o. Therefore, F-actin threads have the appearance of a double-helical structure.[85] Polymerization of G-actin to F-actin, depolymerization of F-actin to G-

actin largely depends up on the critical concentration of G-actin.[85] Above the critical concentration of G-actin, the molecules tend to polymerize, and below the critical concentration, actin filaments depolymerizes.[86,87]

Due to the orientation of all actin monomers in the same direction, actin filaments (F-actin) have two distinct ends, which are called plus(+) and minus(-) ends differing in their polarity.[87] The plus end polymerizes 5-10 faster than the minus end. The minus and plus ends are important because proteins such as myosin move along the actin filament in only one direction. This process is important in muscle contraction.[87]

Actin strongly binds one adenosine nucleotide, and the polymerization process, from G-actin to F-actin also activates the ATPase.[81] The ATPase activity drives the actin filament treadmilling, in which, the polymerization at the one end and de-polymerization at the other end occurs at the same time. This biological process happens with the help of numerous accessory proteins which can further influence several aspects of actin filament dynamics.[86-87]

The majority of natural products targeting actin and modulating its dynamics are isolated from sponges, plants, marine and soil organisms, fungi, algae and bacteria. These bioactive natural products are classified into two types, depending on their effect on actin cytoskeleton[88] and these are: (i) microfilament stabilizing agents and (ii) microfilament destabilizing agents.

1.3.2 Actin Filament Stabilizers

Natural products that stabilize actin filaments or induce the actin polymerization are shown to be extremely useful as chemical tools to understanding the actin filament assembly and organization, as well as, the actin-mediated cellular functions.[89] The common chemical features of these actin filament stabilizers is the cyclic depsipeptide, which is a macrocyclic compound containing both amino acids and hydroxy acids joined by the peptide and ester bonds, respectively. Some of the natural products are chondramides,[90] doliculide,[91] jasplakinolide,[92] amphidinolide H,[93] seragamides,[94] hectochlorin,[95] phalloidin [96,97] and bisbromoamide[98]

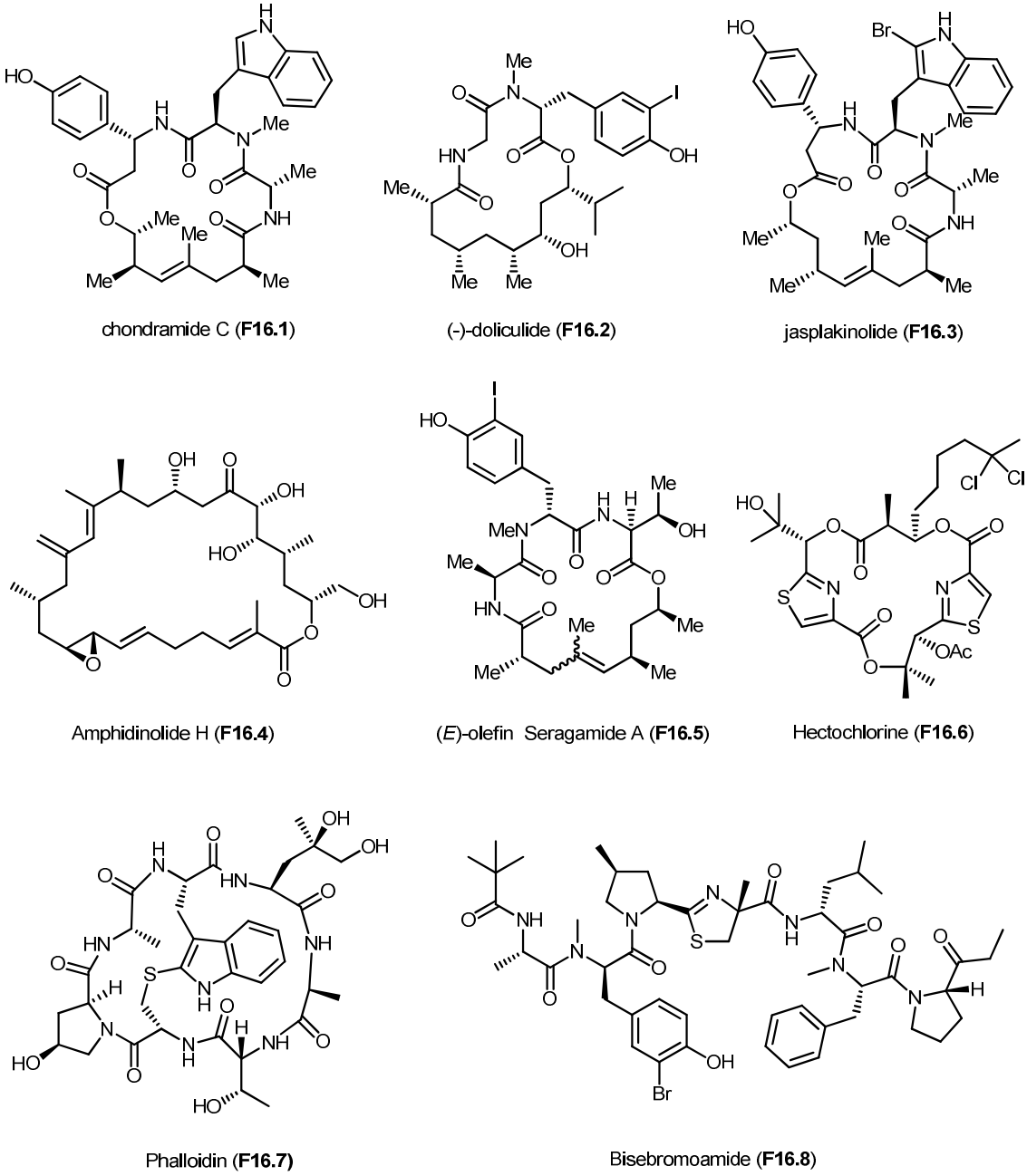


Figure 16: Actin Filament Stabilizers

1.3.2a Amphidinolide H

Amphidinolide H[99] is a 26-membered potent cytotoxic macrolide isolated from *Amphidinium sp*. dinoflagellates that lives off the coasts of Japan and the U.S.A. Virgin Islands. It covalently binds to actin sub-domain 4, and further, stabilizes the actin filament.[100] The structural features of an amphidinolide H are allylic epoxide and the vicinally located one-carbon branch. The absolute stereochemistry of amphidinolide -H was established on the basis of the X-ray diffraction analysis and

the synthesis of the degradation product. From the structure-activity relationship of amphidinolide-H-type macrolides, it was found that the presence of an allylic epoxide, an *S-cis*-diene moiety, and the ketone at C-20 were important for the cytotoxic activity.

Amphidinolide-H shows a potent cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells with the picogram order IC_{50} values.

In the following section, I described the Fuerstner's approach to the synthesis of amphidinolide H.

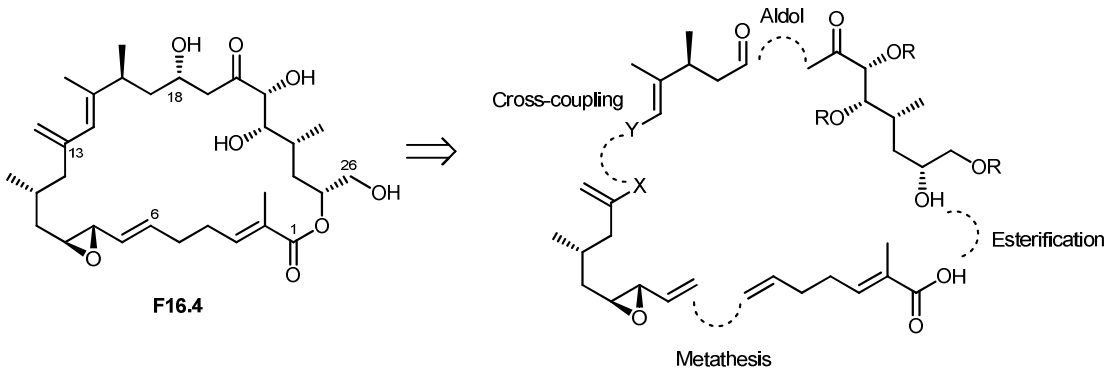
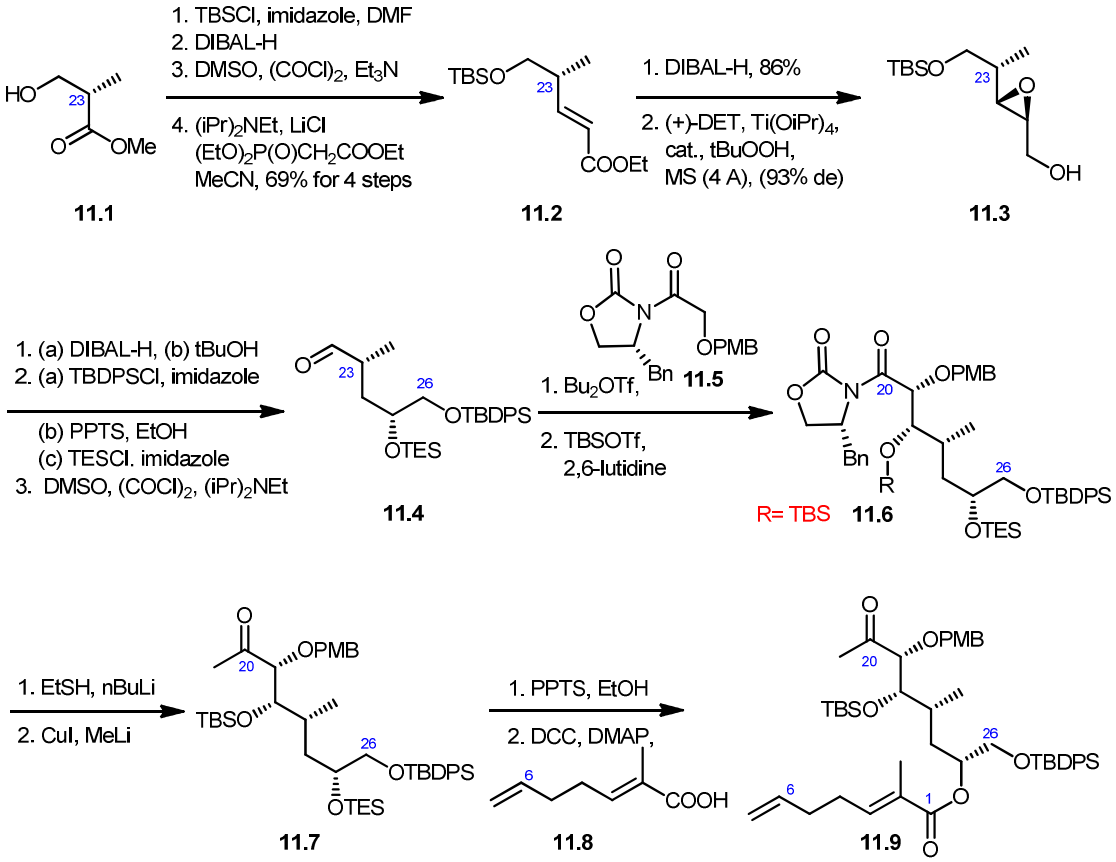


Figure 17: Retrosynthesis of Amphidinolide H

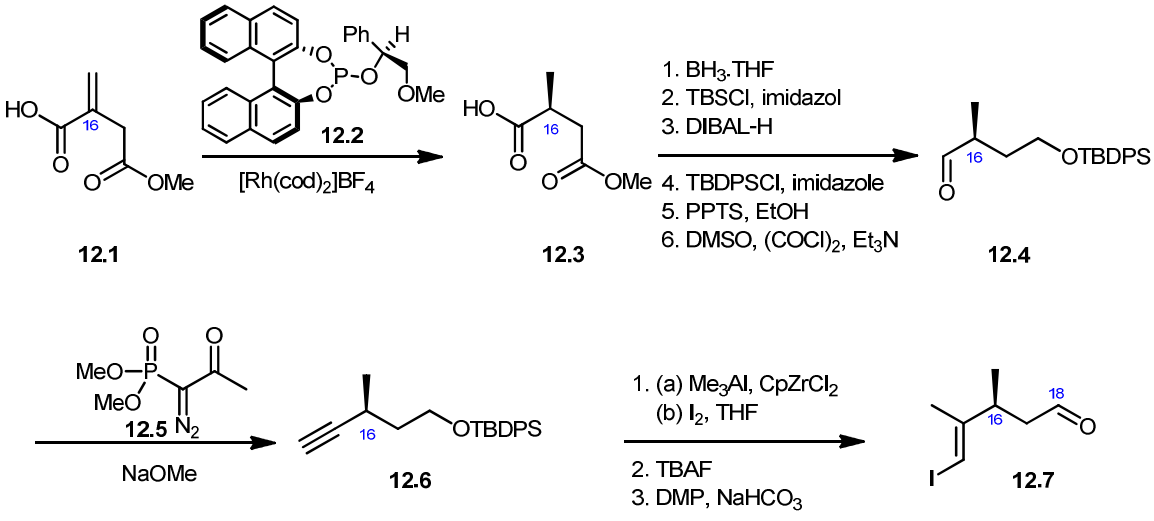
The retrosynthesis for amphidinolide H, it was disconnected into four building blocks which were to be combined by esterification, an aldol reaction, metal-catalyzed cross-coupling, and an olefin metathesis

The synthesis of the "south-eastern" part of **F16.4** began with the conversion of the Roche ester **11.1** into an enoate,[101] **11.2** followed by reduction and the Sharpless epoxidation. This approach afforded **11.3**; DIBAL-H cleanly opened the oxirane ring by a selective delivery of the hydride to the carbon atom distal to the alcohol (dr. >20:1) and the protecting group manipulations followed by Swern oxidation, led the synthesis of aldehyde **11.4**. The conversion of an aldehyde **11.4** to **11.6** was achieved by boron glycolate aldol reaction and the stereochemistry was controlled by the reliable Evans chiral auxiliary.[75] The hydroxyl group was then silylated with TBSOTf and 2,6-lutidine, and the resulting product **11.6** was converted into the thioester, which was reacted with Me_2CuLi, giving the methyl ketone,[102] **11.7**. Finally, the "south-eastern" part of **F16.4** was synthesized by the selective cleavage of the TES

moiety with PPTS followed by an esterification of the released alcohol with acid,[103] **11.8**, thus giving **11.9**

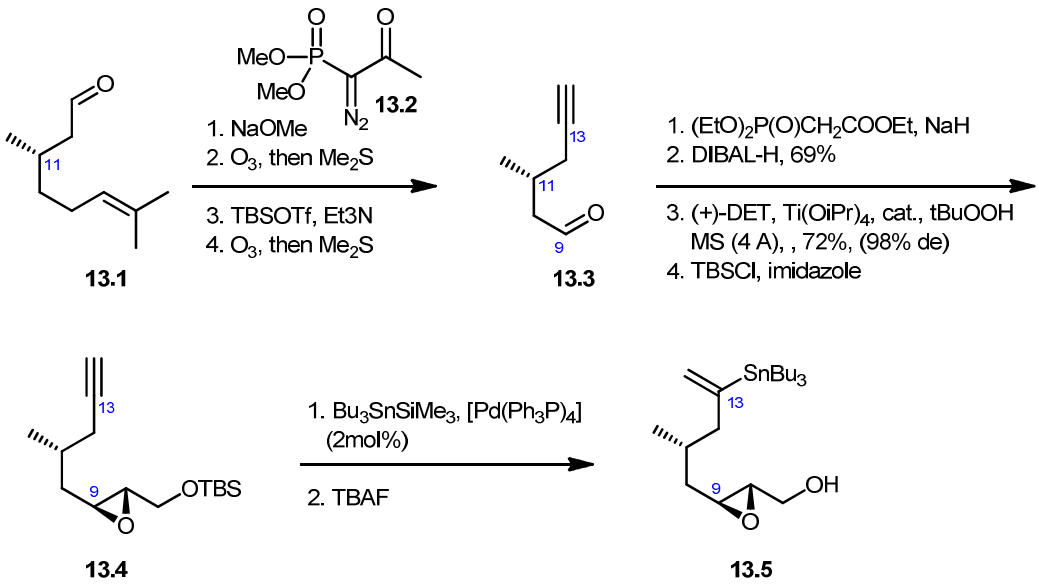


Scheme 11: Synthesis of South-Eastern part



Scheme 12: Synthesis of North-Western Section

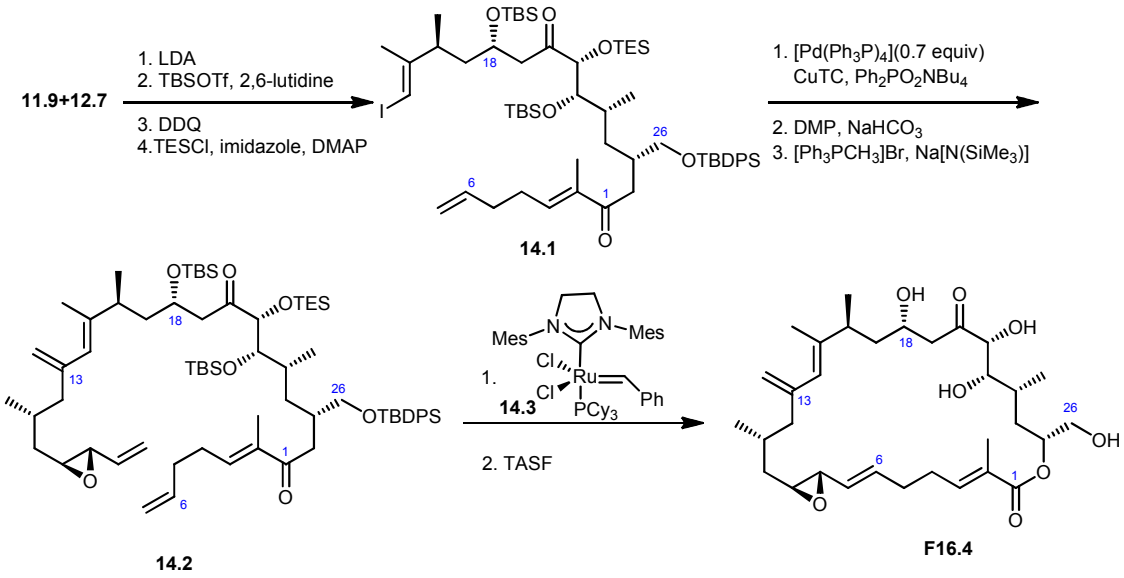
The synthesis of the "north-western" part of **F16.4** was started with an asymmetric hydrogenation of the itaconic acid monoester **12.1** by using a catalyst which was freshly prepared from $[Rh(cod)_2]BF_4$ and mono-dentate phosphite, **12.2**. The routine oxidation-state and the protecting-group manipulations of **12.3** afforded the corresponding aldehyde **12.4** which was then converted into an alkyne **12.6** using the Ohira–Bestmann reagent,[104] **12.5.** The treatment of **12.6** in a sequence of events that included zirconium-induced carbo-alumination, iodine-induced alkenyl iodide functionality,[105,106] the removal of TBDPS, and finally, the DMP oxidation, gave an aldehyde, **12.7.**



Scheme 13: Synthesis of South-Western part

The synthesis of the "south-western" part of **F16.4** was prepared from citronellal **13.1,** as shown in **Scheme 13**. Conversion of the carbonyl group into an alkyne subsequent chemo-selective ozonolysis of trisubstituted olefin gave aldehyde **13.3.** A four steps sequence which involved the silyl enol ether formation, selective ozonolysis, Horner-Emmons reaction, and a subsequent reduction followed by Sharpless epoxidation, give an epoxide **13.4**. Tributylstannane **13.5** was then prepared by a regioselective, palladium-catalyzed silylstannation of **13.4** with $Bu_3SnSiMe_3$[107] followed by a cleavage of the C-Si and O-Si bonds with TBAF.

The first sub-unit coupling was the connection of the lithium enolate of **11.9** with iodine containing an aldehyde **12.7**, followed by the protecting-group manipulations,



Scheme 14: Completion of Synthesis

and this approach, gave **14.1**. Steps towards the completion of the total synthesis were further carried-out by Stille reaction[108] of **13.5** and iodide **14.1**, and this was performed with a combination of $[Pd(PPh_3)_4]$, CuTC, and $Ph_2PO_2NBu_4$ in DMF. Followed by the conversion of an epoxy alcohol to vinyl oxirane, RCM and the removal of silyl protecting groups with TASF afforded, amphidinolide H, **F16.4**

1.3.3 Actin Filament Destabilizers

Natural products that bind to two distinct regions of an actin monomer, which are the ATP-biding cleft and the barbed end, results in destabilizing the actin filament or in inhibiting the filament assembly. Some of actin destabilizers are bistramide A,[109] mycalolide A,[110,111] sphinxolides/reidispongiolide A,[112] swinholide A,[113] aplyronines[114,115, 116]

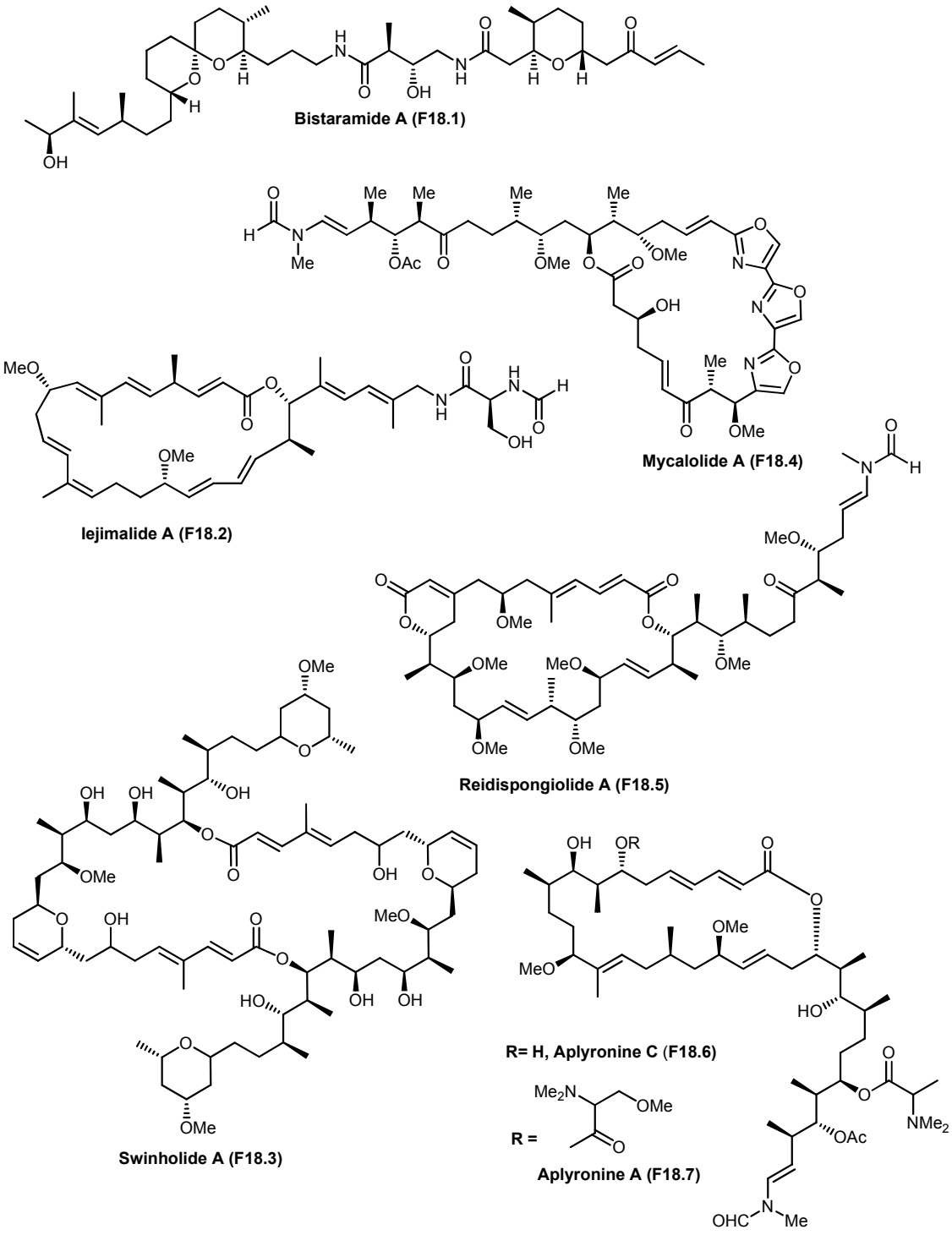


Figure 18: Actin Filament Destabilizers

1.3.3a Bistramide A

Bistramides are a unique family of dilactam polyethers isolated from the marine ascidian *Lissoclinum bistratum* that are responsible for severing actin filaments and a covalent sequestration of a monomeric actin in the cell.[117,118]

Bistramide A was isolated in 1988 by Verbist and co-workers.[119] Bistramide A blocks the sodium channels, and induces a highly selective activation of a single protein kinase C (PKC) isotype. Kozmin and co-workers reported the first total synthesis of bistramide A in 2004.

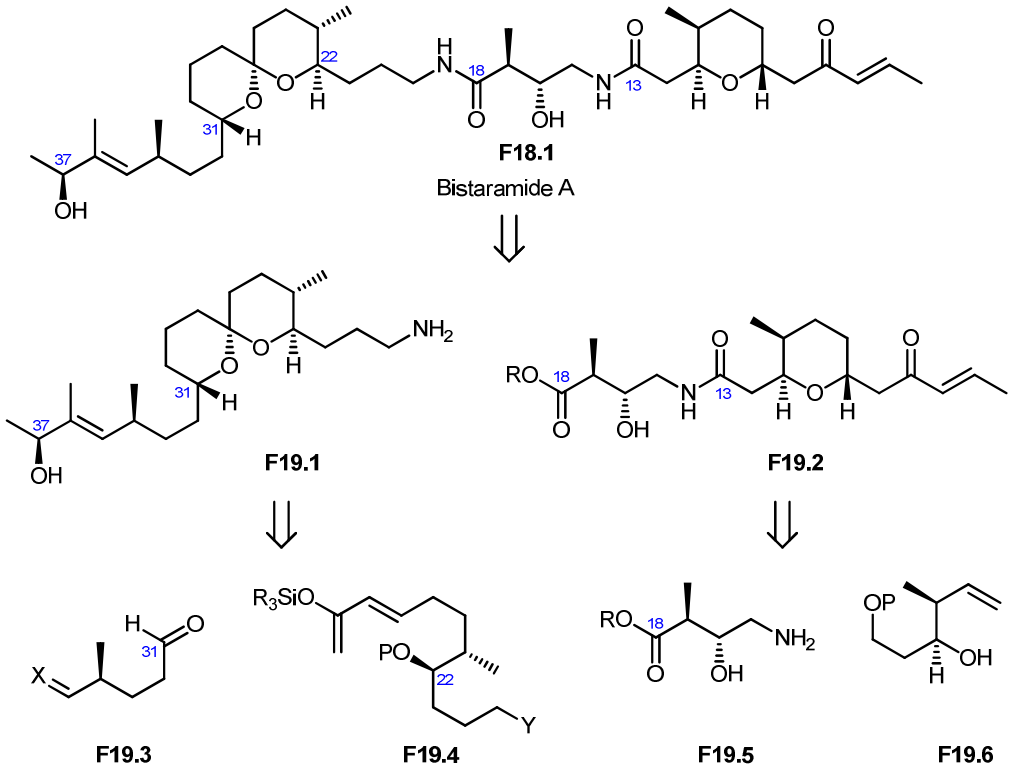
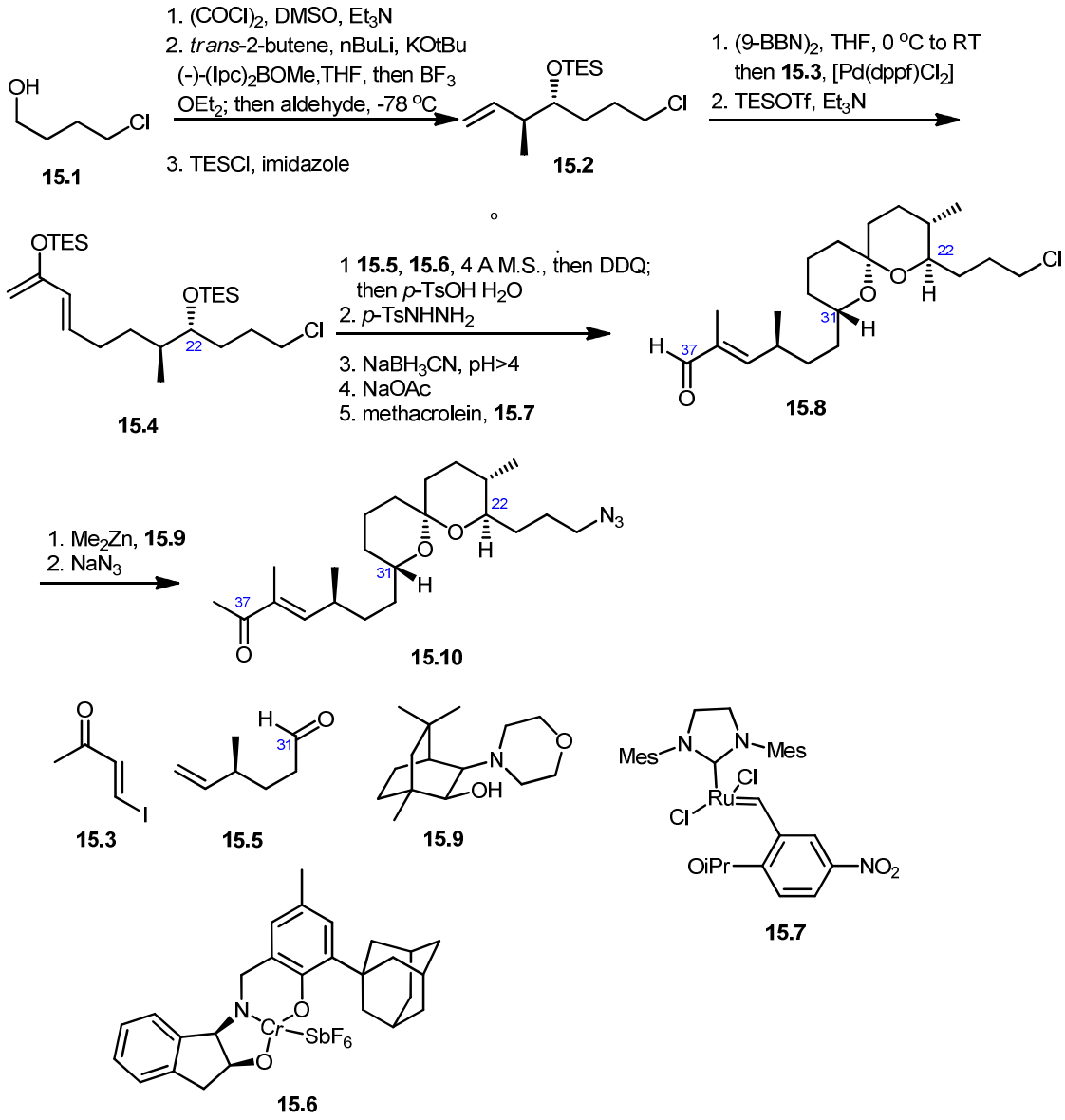


Figure 19: Retrosynthesis of Bistramide A

The retrosynthetic analysis is shown in **Figure 19**. It was started from an amide bond formation of the two fragments, **F19.1** and **F19.2**. The fragment **F19.1** was formed from the cycloaddition and oxidative cyclization between aldehyde **F19.3** and a diene compound, **F19.4**. The fragment **F19.2** was formed from amine **F19.5** and a tetrahydropyran-containing carboxylic acid derived from the alcohol, **F19.6**

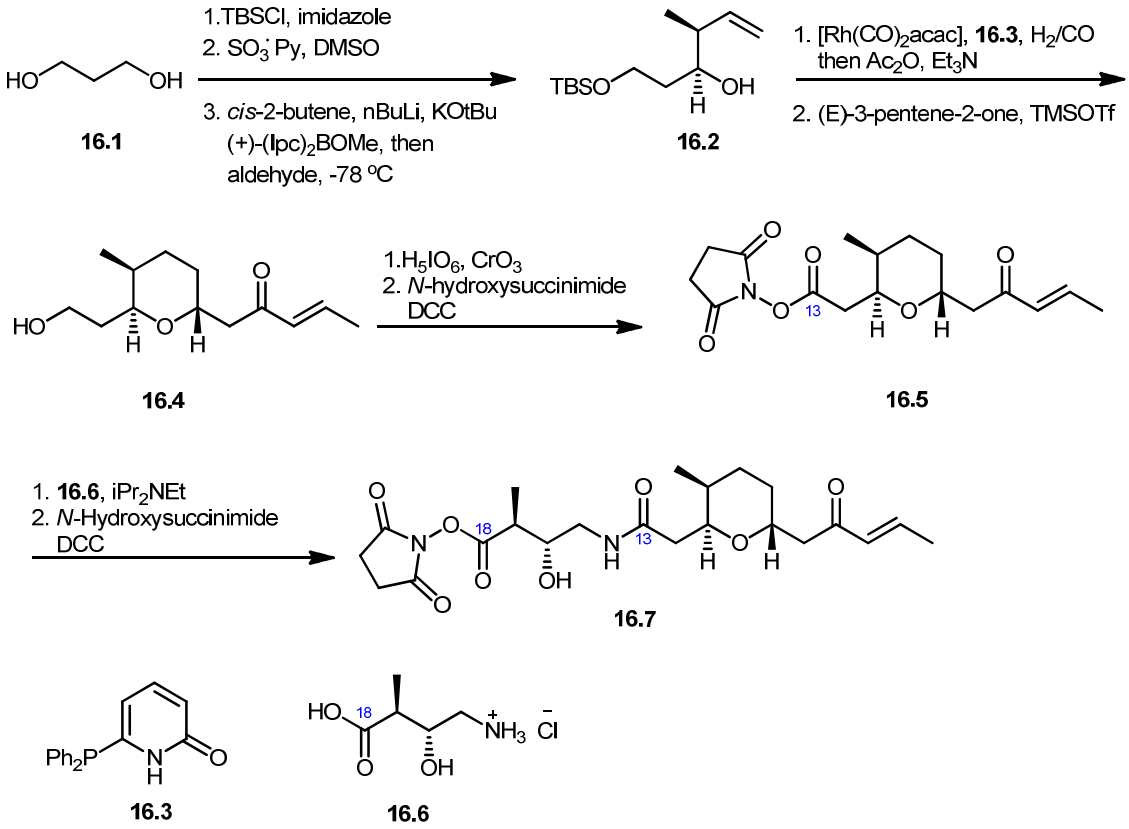
Synthesis of fragment **F19.1** was started with an oxidation, Brown crotylation, and silylation of 4-chloro-1-butanol, giving **15.2**. Suzuki coupling with **15.3** furnished the corresponding ketone, which was followed by conversion into the silyloxy diene **15.4**. Coupling of **15.4** with an aldehyde **15.5** in the presence of a catalyst **15.6** followed by DDQ and acid-mediated ring closing, finally gave, spiroacetal in 58% yield as a single stereoisomer. The deoxygenation of the carbonyl moiety by Wolff- Kishner re-



Scheme 15: Synthesis of **15.10**

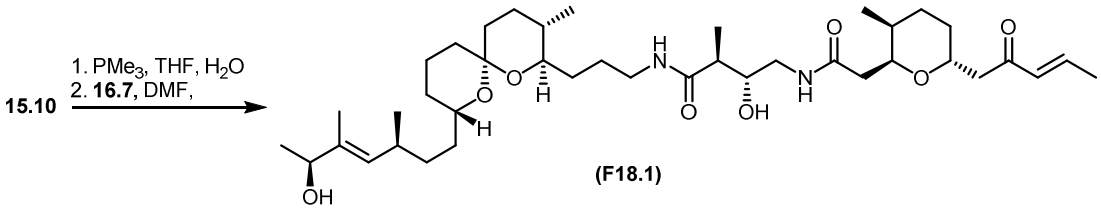
-duction followed by the cross-metathesis with methacrolein using Grela-Grubbs catalyst (**15.7**)**,** finally furnished the aldehyde, **15.8.** A diastereoselective addition of Me_2Zn in the presence of **15.9** followed by the chloride to an azide conversion completed the synthesis of **15.10.**

Synthesis of the right fragment was started with 1,3-propanediol as following: the conversion of propanediol into the homoallylic alcohol **16.2** proceeded through the selective monosilylation, oxidation, and an asymmetric crotylation. Hydroformylation of **16.2** through rhodium catalysis in the presence of Breit's exceptional DPP on ligand **16.3**, followed by conversion of the lactol moiety into an acetate *in situ* and the



Scheme 16: Synthesis of **16.7**

subsequent addition of (*E*)-3-pentene-2-one in the presence of TMSOTf and an acidic work up, gave **16.4**. Conversion of primary alcohol into acid followed by an active ester formation with *N*-hydroxysuccinamide and DCC furnished **16.5**. The coupling of **16.5** with **16.6** provided an amide derivative, which was further converted into an activated ester **16.7**.



The reduction of **15.10** with PMe_3 in aqueous THF, led the formation of a crude amine mixture combined with **16.7** to complete the synthesis of bistramide A.

1.4 Introduction to Protein-Protein Interactions

Protein-protein interactions (PPI) are extremely important for virtually most biological processes, for example DNA replication, translation, transcription,

secretion, splicing, signal transduction, cell cycle control, and intermediary metabolism.[120] Thus, targeting specific protein-protein interactions has many potential uses in the broad drug discovery arena.[121]

Many protein-protein interactions interfaces are biologically interesting targets for drug discovery, but the design of compounds that are able to interfere with the PPI surface is highly difficult when compared to design of small molecules for the smaller deep cavities of enzymes.[121,122] Unlike enzymes, protein-protein interaction have multiple smaller areas called as "hotspot" that contain the subset of residues which give the majority of the binding free energy.[123]

Functional genomics describes that there are 3,000–10,000 disease-transforming proteins but pharma industry has explored only a small portion yet, ~400 proteins to date, for medically helpful developments. Most of these protein targets belongs to few families, such as nuclear hormone receptors, enzymes, G protein-coupled receptors, ion channels and other targets.[124] Small-molecule drugs use the endogenous ligand-binding sites to control the activity of their target proteins, because these proteins normally function through the binding of small molecules, which are either substrates or cofactors. For two main reasons, PPIs are the main targets for small-molecules. First, the contact area between proteins generally involve a large number of hydrophobic and polar interactions distributed across a large interface; second, the protein-protein interfaces are generally offer fault surfaces.

In general, small molecules that affect protein-protein interactions function through three distinct mechanisms: (i) orthosteric inhibition; In this approach, there is a competition between orthosteric inhibitor and the partner protein for the same binding site on target protein. Orthosteric inhibitors bind to the target protein prior to the partner protein for the same binding site, thus directly inhibiting the formation of macromolecular complexes.

(ii) Allosteric inhibitors: In this approach, small-molecule ligands (allosteric inhibitors) bind to the target protein at the sites distinct from the partner protein binding site (used for macromolecular complex). The binding of an allosteric inhibitor on target protein changes the dynamic properties or static conformation of the target

protein(s) and with this, partner protein cannot form the macromolecular complex with the target protein.

(iii) The third category is interfacial binders: In this approach, ligands and proteins form a ternary complex. An interfacial inhibitor binds to a pocket at the macromolecular interface, the ligand and proteins which sometimes is only transiently formed in a transition state, and locks the complex into a non-productive arrangement.[125,126]

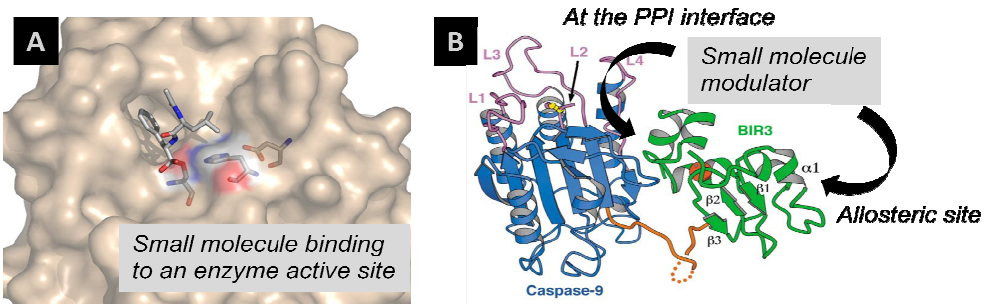


Figure 20: (A) Small Molecule Binding to an Enzyme Active Site; (B) Small Molecule Modulators of Protein-Protein Interactions via Interface or an Allosteric Site.[127] (note: this is figure is directly taken from a review article from our group, *Chem. Rev.* **2014**, *114*, 4640−4694)

1.5 Introduction to Macrocycles

Macrocycles are defined as cyclic compounds with the ring structure containing 12 or more atoms and the molecular weight 500-2,000 Da; often, do not possess the drug-like "rule of five" properties.[128] Macrocyclic natural products often display interesting and diverse biological activities. Currently, there are more than 100 approved macrocycle drugs from natural products, for example, anticancer, antifungal, antibiotic and immuno-suppressive activities as seen for epothilone B **(F21.1),**[129,130,131] amphotericin B **(F21.2),**[132,133] erythromycin **(F21.3)**[134] and rapamycin. **(F21.4)**[135] Macrocyclic drugs primarily are originated from two sources: natural products provide unique drugs, such as above mentioned compounds **(F21.1-F21.4)**; the second traditional source of macrocycles comes from peptides generated drugs from synthetic or natural sources, some of which also belong to the natural products family.

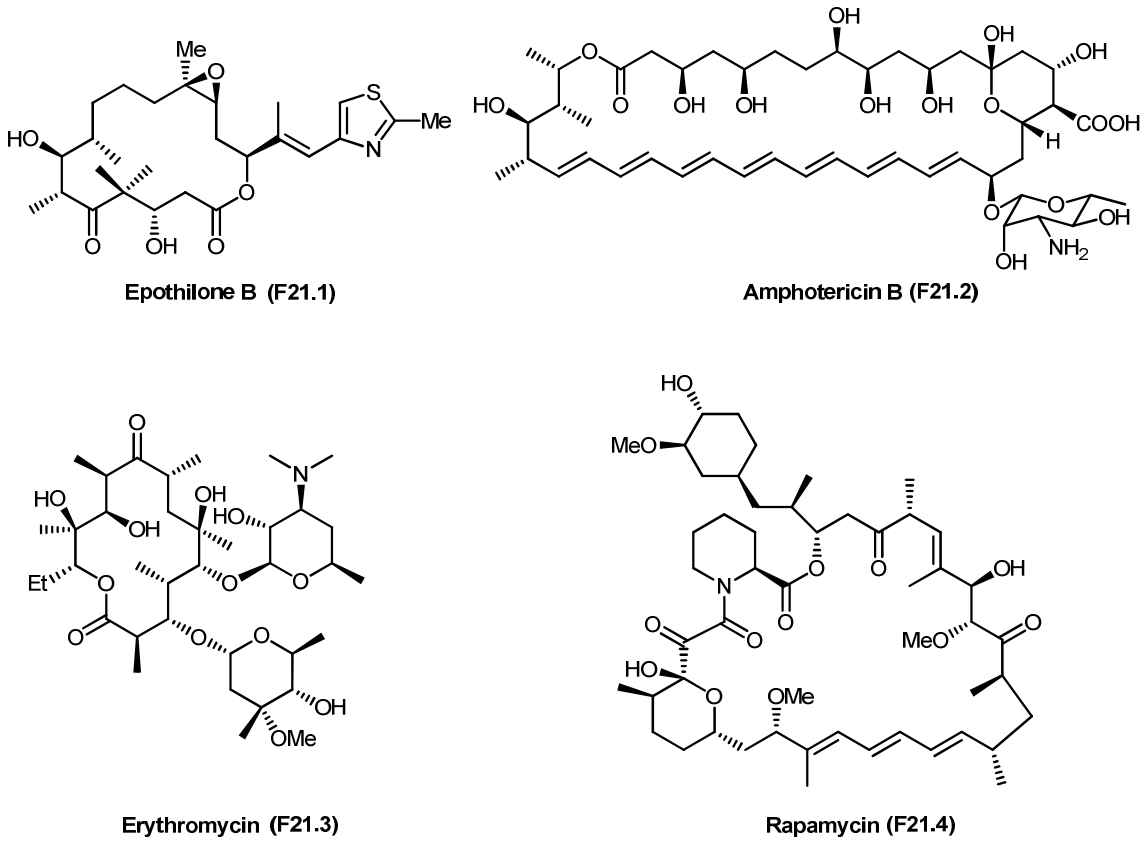


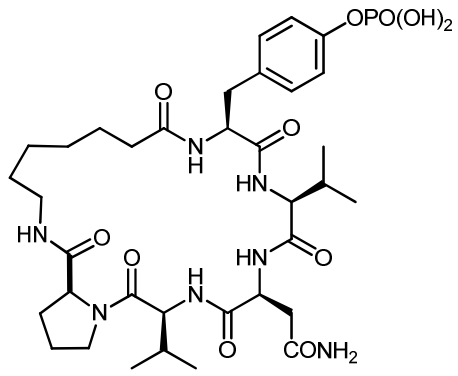
Figure 21: Structures of Naturally Occurring Macrocyclic Drugs: Epothilone B **(F21.1),** Amphotericin B **(F21.2)**, Erythromycin **(F21.3)**, and Rapamycin **(F21.4)**

1.5.1 Importance of Macrocycles

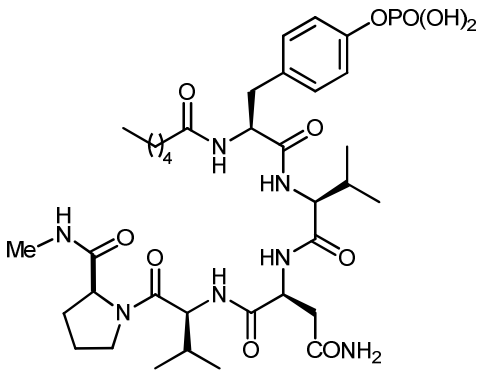
The primary objective in medicinal chemistry is the design of small molecules for selective binding to proteins with a high affinity. For this approach, one general strategy is increasing, such as ligand binding affinities involve pre-organizing the ligands into their biologically active conformations by introducing further conformational restrictions.[136]

Due to the distinct structural features of macrocycles, they have less conformational flexibility compared to their equivalent acyclic compounds. In this process, they suffer a trivial entropic loss upon binding to the receptor. In contrast to the smaller cyclic systems, macrocycles are not rigid and so allowing them to potentially mould to a target surface in achieving optimal binding. In addition to this, macrocycles also offer the possibility for binding across larger surfaces that are difficult to access with the traditional small molecules.[137] From the chemistry point of view, macrocycles can offer stereochemical complexity and diverse functionality in a conformationally

restricted manner. Shown below are some of macrocycles that have more potential than their equivalent linear analogs. More than that, macrocyclic compounds can demonstrate several favorable drug-like properties, which include an improved membrane penetration, good solubility, enhanced metabolic stability, good oral bioavailability, and an increased lipophilicity with desirable pharmacodynamic and pharmacokinetic properties.[138, 139,137b]

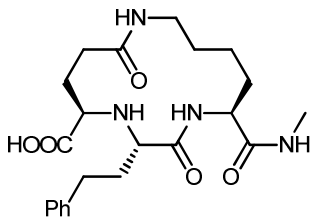


F22.1

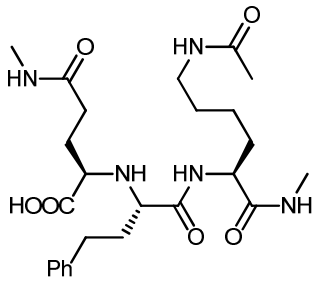


F22.2

Figure 22: Macrocycle **F22.1** is 2-fold Greater Affinity than it's Linear Acyclic Compound **F22.2**[136] for Stabilizing the Biologically Active Conformation of Grb2 SH2 Peptides.



F23.1



F23.2

Figure 23: Macrocycle **F23.1** is 17-fold More Affinity than it's Acyclic Compound **F23.2**[140] in the Inhibition of MMP-8 which Belongs to the Family of MMPs needed for the Maintenance of the Extracellular Matrix.

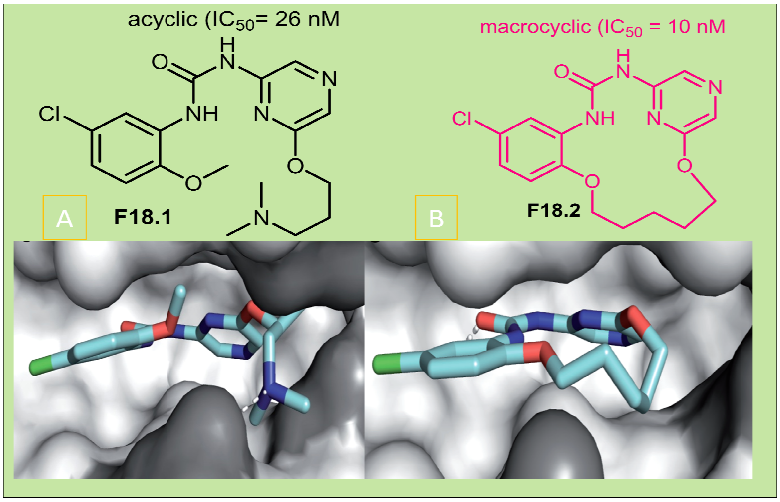


Figure 23: Acyclic and Macrocyclic CHK1 Inhibitors. X-ray Co-Crystal Structure of Inhibitors in CHK1: (A) Acyclic inhibitor (B) Macrocyclic inhibitor.[141] (note: this figure is taken from *Future Med. Chem*. 2012, 1412)

1.6 Conclusions

As microtubules play a major role in the cell division process, they are considered to be the targets for anti-cancer therapy. The mechanisms involved for the microtubules as targets for anti-cancer therapy include blockade or altering any one or more steps of cell division. This approach shows the direct effect on the multiplication of cells, which generally depends upon the dividing capacity of the cells. The crucial involvement of microtubules in mitosis could be combined adequately with the molecularly targeted drugs. With reference to upgrading the basic knowledge of these drugs, we require a deeper understanding of the interactions of the microtubule targeted drugs with mitotic-spindle poles or centrosome, as well as the relationship between the drug inducing the mitotic block and the cell death. In this chapter, I discussed some of the natural products and their analogs with the mechanisms involved for microtubule as targets for anti-cancer therapy. I have also explained the importance of actin, natural products and their analogs targeting actin and the importance of modulating its dynamics. In addition to this, I have also discussed the area of protein-protein interactions as the targets for drug discovery. Finally, I have also discussed the importance of naturally as well as synthetic macrocycles in medicinal chemistry and their distinct structural features when compared to their equivalent acyclic compounds.

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Chapter 2

Synthesis of C14-C21 Eribulin Fragment for Building A Diverse Set of Macrocycles

2.1. Introduction

Eribulin is an approved drug for the treatment of metastatic breast cancer,[1 2 3] and it resulted from an extensive synthesis efforts that were made over the years toward halichondrins.[4 5 6] In 1992, Kishi's group reported the synthesis of halichondrin B. During their marathon synthesis program, they were also working with National Cancer Institute (NCI), USA and Eisai Research Institute for *in vitro* and *in vivo* antitumor testing. In one of the studies, a macrocyclic macrolactone diol (the structure is not shown here) was shown to be as potent as halichondrin B against human colon cancer cells. Both, the diol derivative and halichondrin B, showed the blocking of a cell cycle progression at the G2/M phase, and this further, caused the microtubule destabilization. Through several structural analogs syntheses, eribulin emerged as the winner and as the first non-taxane, microtubule dynamics inhibitor-based drug. Compared to other anti-cancer drugs (i.e. taxanes, vinca alkaloids and epothilones etc), it seems to function through the tubulin binding site that was not established before.[7] It has been shown that eribulin binds to an inter-dimer face or β-tubulin subunit alone, and through this interaction, it inhibits the microtubular growth phase of the microtubular dynamics instability without causing any effect on the shortening of the microtubules.[8]

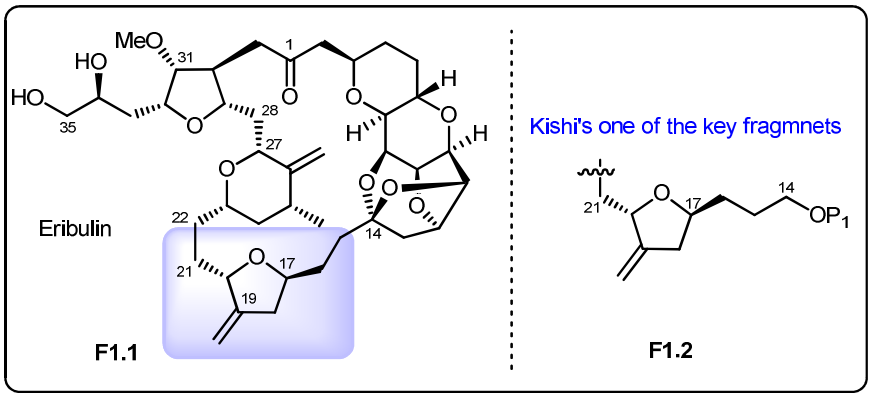


Figure 1: Eribulin (**F1.1**) and One of the Key Fragments of Eribulin **(F1.2)** Having 2,5 *trans*-Tetrahydrofuran Ring

2.2. Working Hypothesis

With our continued interest in developing practical synthesis approaches to various sub-structures of eribulin and other bioactive natural products,[9 10] and their further utilization in obtaining different sets of macrocyclic compounds, we focused our

attention to the C14-C21 substituted tetrahydrofuran fragment. This type of sub-structures along with the macrocyclic rings are also commonly found in several other bioactive natural products. For example, amphidinolide E (**F2.2**) and amphidinolide F (**F2.3**), both belong to the family of amphidinolides,[11, 12] and are also known as highly potent cytotoxic agents. In the case of amphidinolide E (**F2.2**), *cis*-2,5- disubstituted tetrahydrofuran is embedded in the functionalized 18-membered macrocyclic ring, whereas, two *trans*-2,5-disubstituted tetrahydrofuran moieties are a part of a densely functionalized 23-membered ring in amphidinolide F (**F2.3**). Another family of natural products that contain this moiety along with a macrocyclic ring is the haterumalides (**F2.1**), and they are known for their potent cytotoxicity. [13]

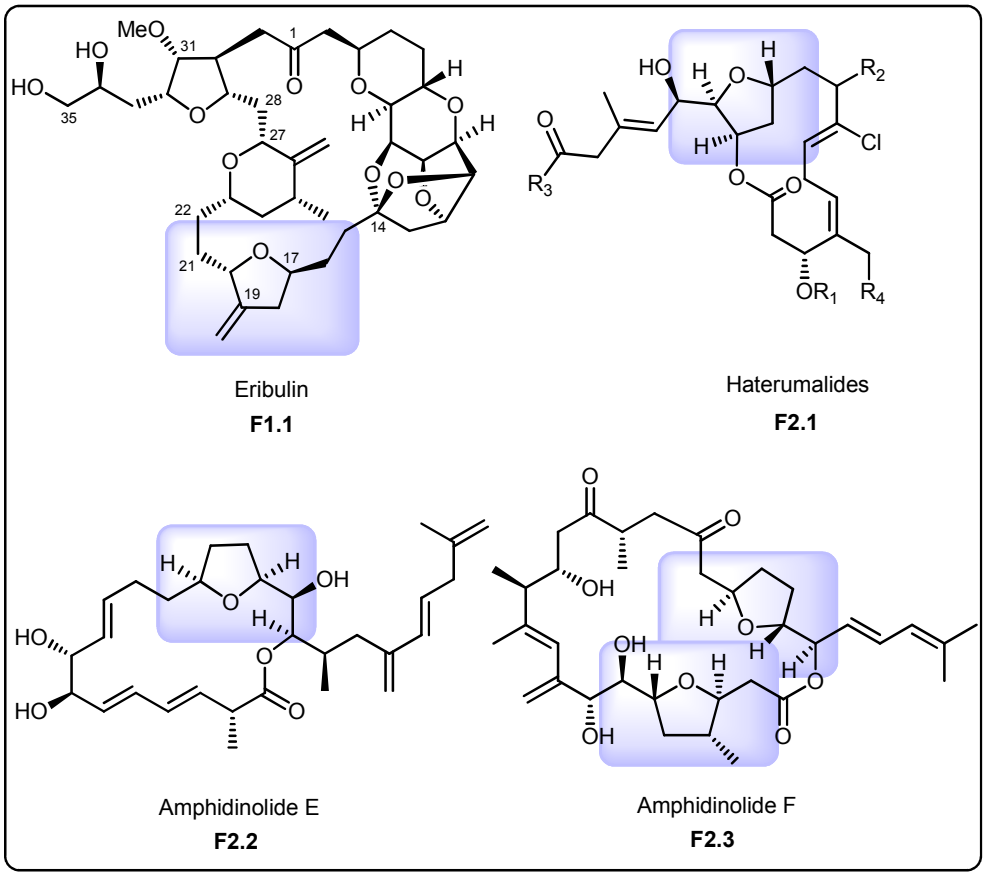


Figure 2: Eribulin and Other Bioactive Natural Products Containing the *cis*- or *trans*-2,5-Disubstituted Tetrahydrofuran Moiety Embedded in the Macrocyclic Rings.

Due to the presence of *trans*-2,5-disubstituted tetrahydrofuran moiety in eribulin and several other important bioactive natural products, we considered this as the privileged scaffold, and it can serve as a good starting point in building a chemical toolbox with a diverse set of macrocyclic compounds. With this goal in mind, we set

three objectives, and these were: (i) to develop a practical and scalable synthesis of *trans*-2,5-disubstituted tetrahydrofuran as the key scaffold **F3.1** (note: eribulin numbering is shown in **Figure 3**), (ii) to complete the synthesis of Kishi's eribulin fragment having this moiety, and (iii) to develop a modular synthesis for obtaining two different macrocyclic compounds with 17- and 18-membered rings (see, **F3.2** and **F3.3**). In our approach, the utilization of *trans*-2,5-disubstituted groups at C17 and C19 leads to a macrocycle with 17-membered ring whereas the use of *trans* C17 and C20 functional groups provides an 18-membered ring. The incorporation of an amino acid moiety in both macrocyclic rings allow introducing a chiral diversity site for obtaining further analogs. The long-term goal of this study is to obtain several different types of macrocyclic compounds having this privileged *trans*-2,5-disubstituted tetrahydrofuran moiety.

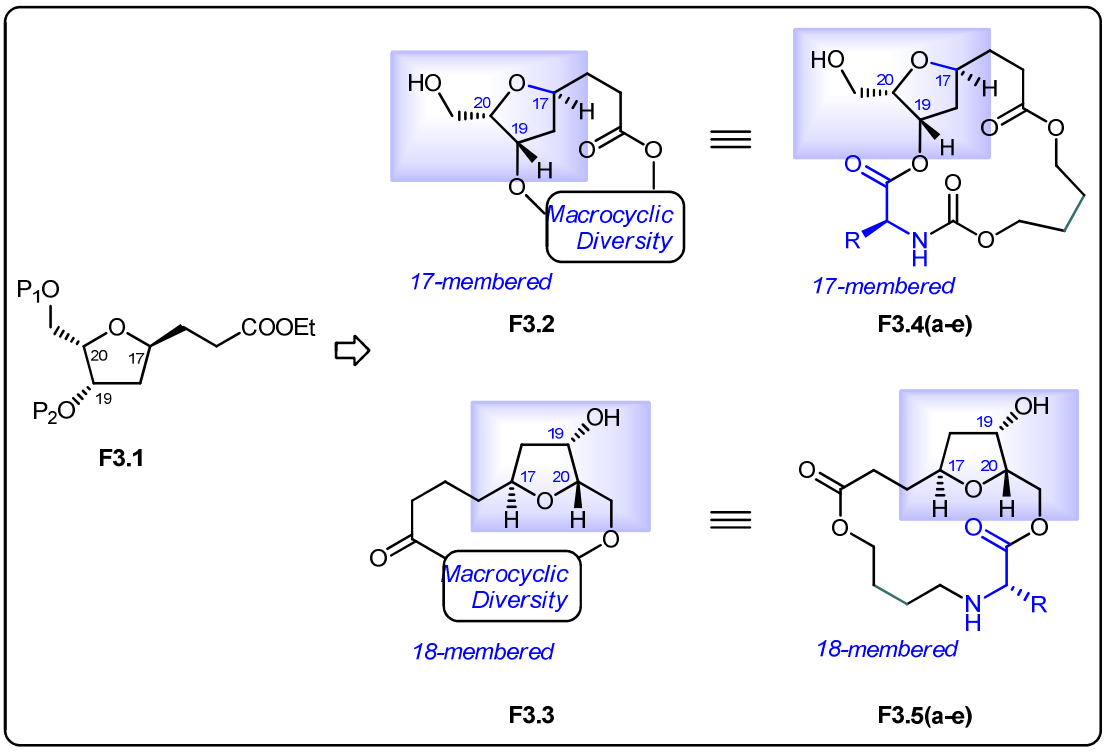


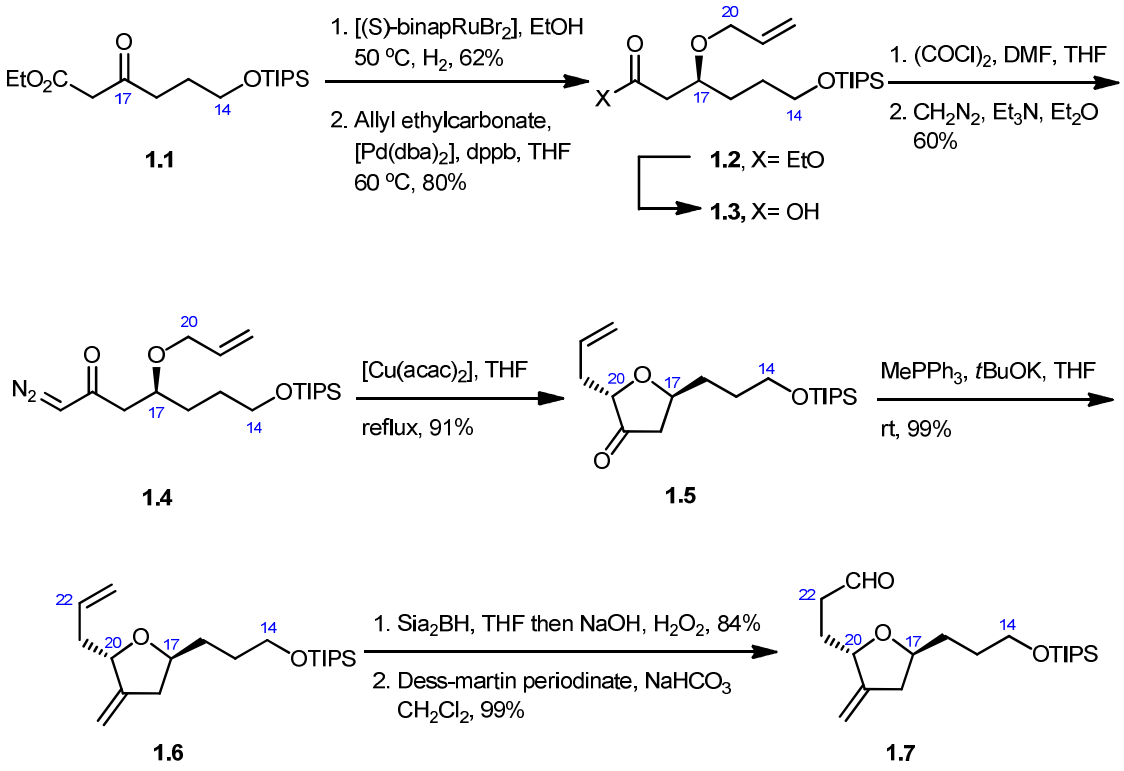
Figure 3: Two Planned Macrocyclic Targets having 17- and 18-Membered Rings (**F3.2** and **F3.3**) from the Substituted Tetrahydrofuran Moiety, **F3.1**

2.3 Literature Synthesis of C14-C21 Eribulin Fragment

In this section, I have covered some of the literature approaches to the synthesis of the C14-C21 eribulin fragment.

2.3.1 Phillips' Approach

In 2009, Phillips and co-workers synthesized the C14-C26 fragment of eribulin[14] and herein, I have highlighted the synthesis upto C22 carbon center only. The synthesis started with Noyori hydrogenation of β-ketoester **1.1** and the subsequent, Pd-mediated allylation, which furnished the synthesis of *O*-allyl ester **1.2.** The ester derivative was converted into diazoketone **1.4**; when treated with $Cu(acac)_2$ in THF under reflux, the rearrangement took place, yielding the 2,5-*anti*-tetrahydrofuran derivative.[15] A Wittig olefination of compound **1.5** produced the diene **1.6,** which was further hydroborated selectively with $SiaBH_2$ reagent. This was then followed by an oxidation of the alcohol with Dess-Martin periodinane, giving the corresponding aldehyde **1.7.**

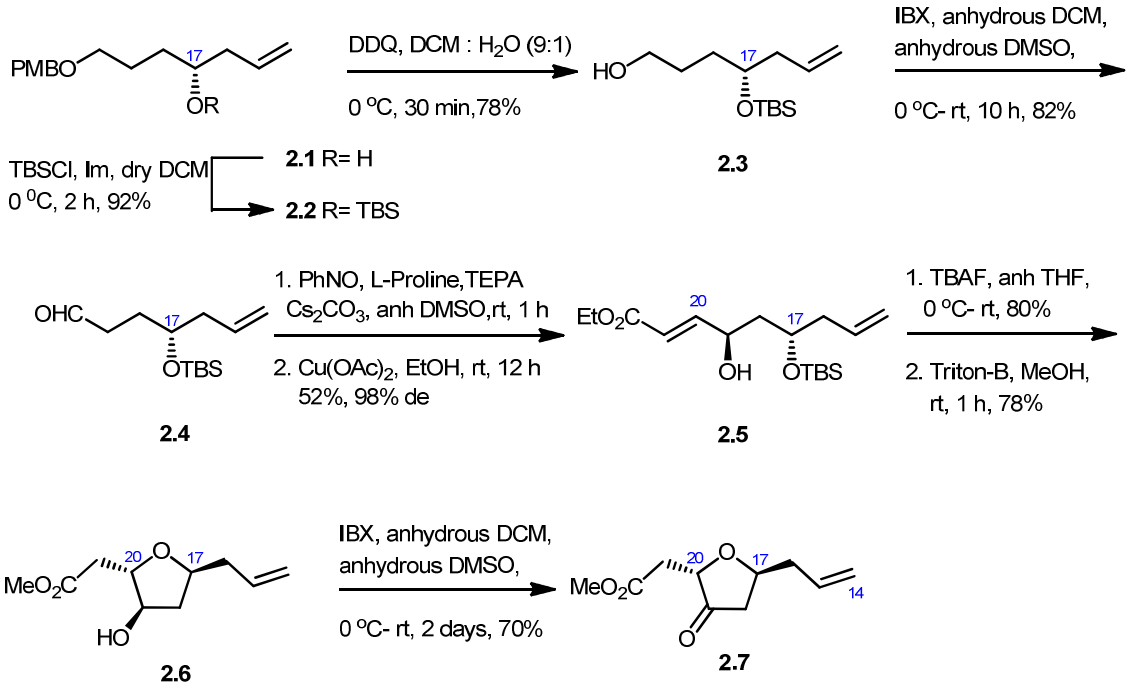


Scheme 1: The Synthesis of the C14-C22 Tetrahydrofuran Fragment

2.3.2 Sabitha's Approach

In 2012, Sabitha and co-workers synthesized the C14-C22 fragment of eribulin from a known homoallyl alcohol. This homoallyl alcohol[16] **2.1** was converted into **2.2** by a simple protection approach. After the deprotection of the PMB group, the resulting alcohol **2.3** was then oxidized to an aldehyde **2.4.** This compound upon treatment with nitrosobenzene in the presence of L-proline (40 mol%) in DMSO at rt followed by *in*

situ Horner–Wadsworth–Emmons olefination produced an aminoxyolefinic ester. The cleavage of the O–N bond in aminoxyolefinic ester using $Cu(OAc)_2$ gave the corresponding γ-hydroxy unsaturated ester **2.5** in 52% yield with 98% de. The absolute stereochemistry of the newly generated chiral center in compound **2.5** was confirmed at a later stage by NOE studies of the cyclic product, **2.6.** The silyl group removal followed by the cyclization of compound **2.5**, finally, gave the required *anti* 2,5 tetrahydrofuran **2.6**, where the *trans*-esterification occurred. The oxidation of alcohol **2.6** with IBX produced **2.7**



Scheme 2: The Synthesis of the C14-C22 Tetrahydrofuran Fragment

2.4 Our Synthesis for the C14-C21 Eribulin Fragment

2.4.1 Retrosynthesis of C14-C21 Eribulin Fragment.

Retrosynthetic analysis of our target **F4.1** is shown in **Figure 4.** Compound **F4.1** obtained from carbon extension, oxidation and Wittig reaction on **F4.2**. The tetrahydrofuran ring formation could be achieved through an iodocyclization of **F4.3**. This diol compound obtained from the cheap chiral starting material, *R,R*-tartaric acid, in a few simple transformations.

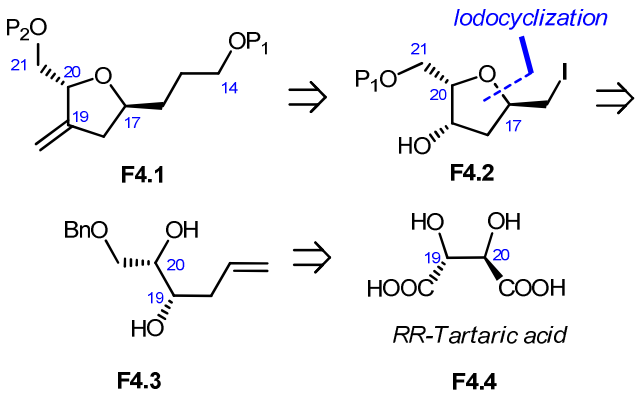
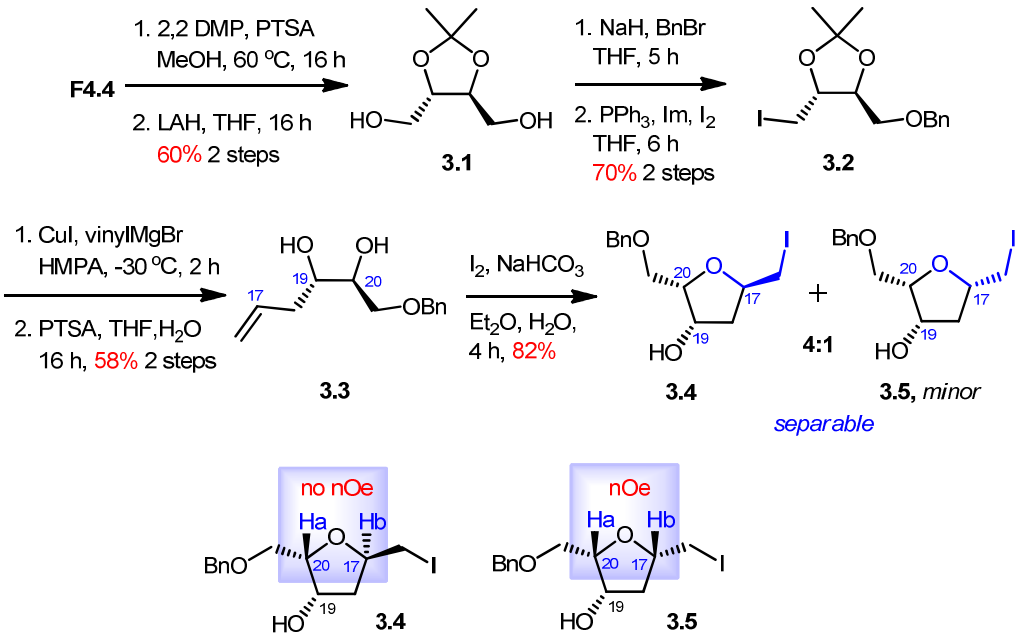


Figure 4: Retrosynthesis of C14-C21 Eribulin Fragment

2.4.2 Synthesis of C14-C21 Eribulin Fragment

Our synthesis started with a chiral starting material, *R,R*- tartaric acid **(F4.4)**, which was converted into **3.1** by using one pot esterification and an acetonide protection.[17] This was further followed by reduction with $LiAlH_4$; selective mono-protection of the diol afforded the mono *O*-benzyl ether,[18] and this was then converted to its corresponding iodo compound, **3.2** as a colorless oil. This upon treatment with vinyl MgBr/CuI followed by an acetonide removal gave **3.3.**[19] An iodocyclization reaction[20,21] with **3.3** produced *trans* and *cis* 2,5-disubstituted tetrahydrofuran derivatives, **3.4** and **3.5** as the major and minor products in a 4:1 ratio.



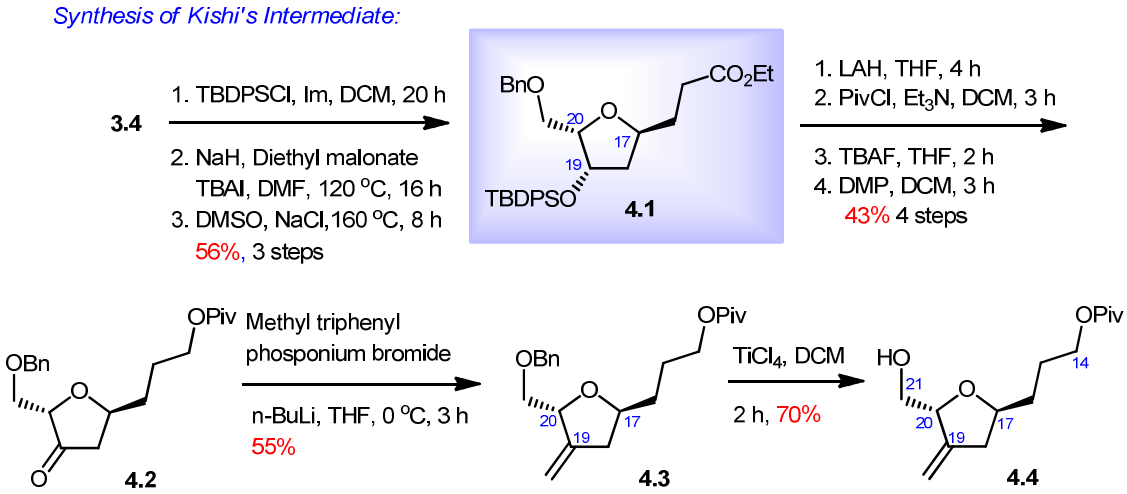
Scheme 3: Iodocyclization Approach to the Synthesis of **3.4** and **3.5**.

These two diastereomeric compounds were easily separable, and the pure products were then thoroughly subjected to the structural analysis by 1D and 2D NMR studies.

Structural Analysis

The details of the stereochemical assignments are provided as follows. Based on 2D nOe analysis, we assigned the stereochemistry of C17 carbon in compounds **3.4** and **3.5.** In compound **3.5**, the H_a proton showed nOe with H_b which indicated that it is having a 2,5 *cis*-isomer, whereas in compound **3.4**, there is no nOe between H_a and H_b, indicating this diastereomer as the 2,5 *trans*-isomer (see **Scheme 3**)

The remaining steps for completing the synthesis of the Kishi's tetrahydrofuran fragment are shown in **Scheme 4**.

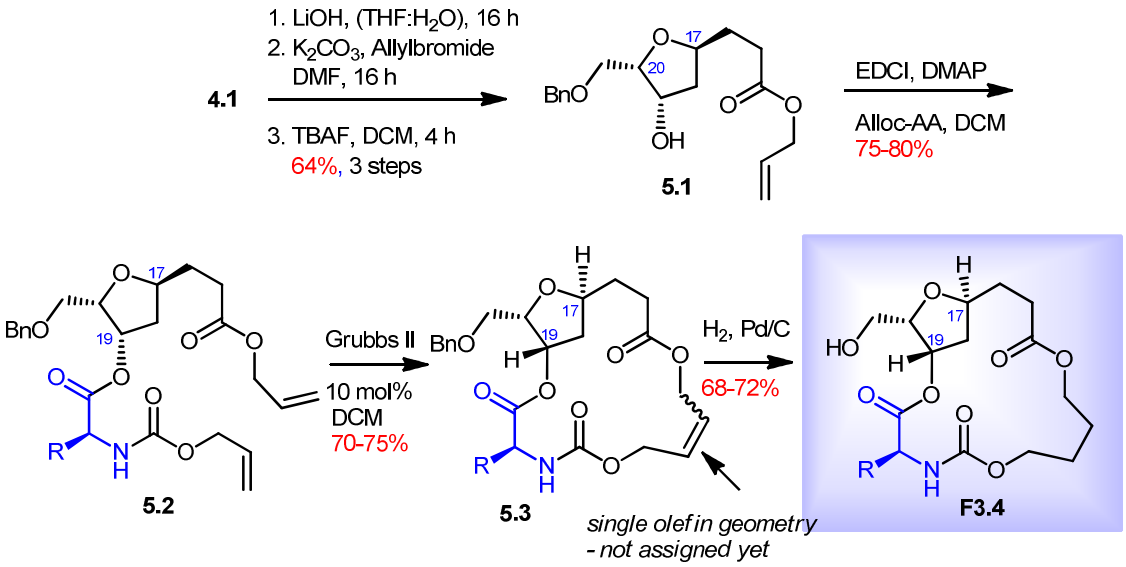


Scheme 4: Completion of the synthesis of Kishi's fragment

The protection followed by the substitution of iodide with the diethylmalonate and subsequent decorboxylation[22] of **3.4** gave **4.1** as a colorless liquid. A series of simple reactions steps, such as: (i) reduction, (ii) standard protection, (iii) the deprotection and functional group manipulation, and (iv) oxidation, finally, produced the keto compound, **4.2.** Finally, the required product **4.4** was obtained from **4.2** in a series of steps that involved (i) Wittig, and the OBn deprotection under Lewis acidic conditions, and this approach completed the synthesis of Kishi's fragment.[23]

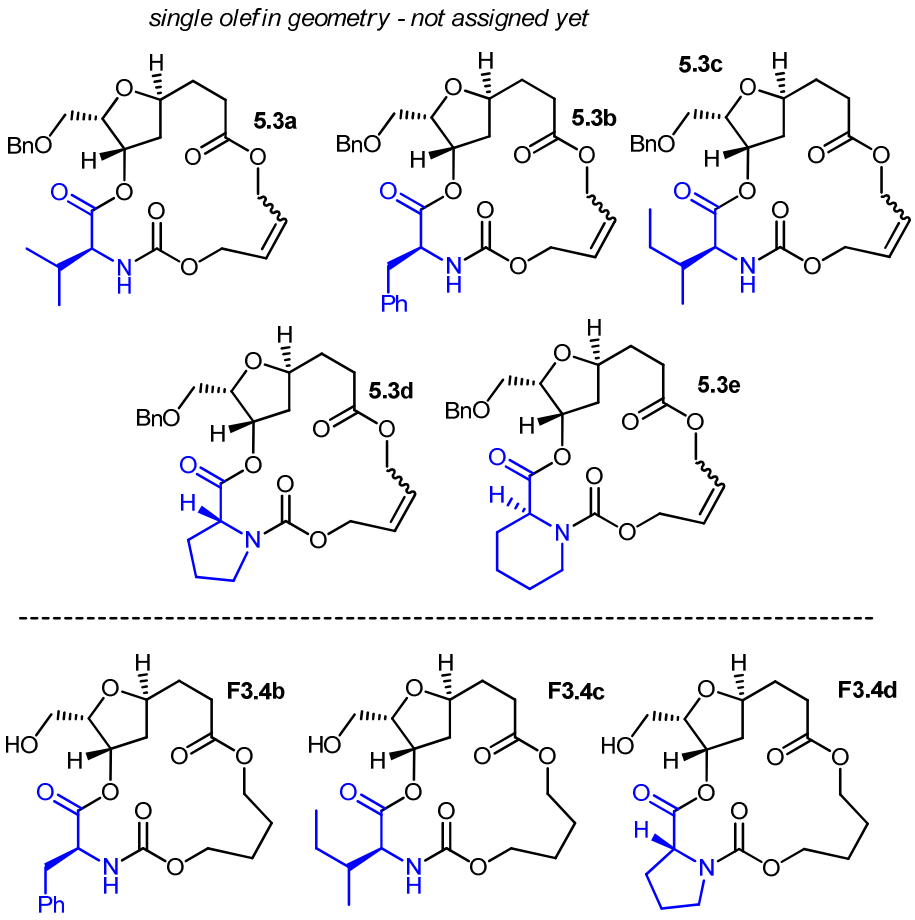
2.4.3 Synthesis of 17-Membered Macrocyclic Compounds

With this sufficient amount of the key intermediate **4.1,** we then developed a modular approach to the synthesis of two different macrocyclic compounds, **F3.4** and **F3.5**. **Scheme 5** shows our approach to the synthesis of a macrocyclic compound **F3.4.** The hydrolysis of **4.1** produced free acid which was then allylated, and following the protecting group removal, gave compound **5.1.** It was then coupled with five different amino acids for obtaining the precursor **5.2** for the crucial Ring Closing Metathesis (RCM). The use of the second generation Grubbs' catalyst successfully produced the 17-membered ring macrocycle with a single olefin geometry, **5.3**. The olefin geometry was not assigned due to overlapping signals in NMR. Five macrocyclic

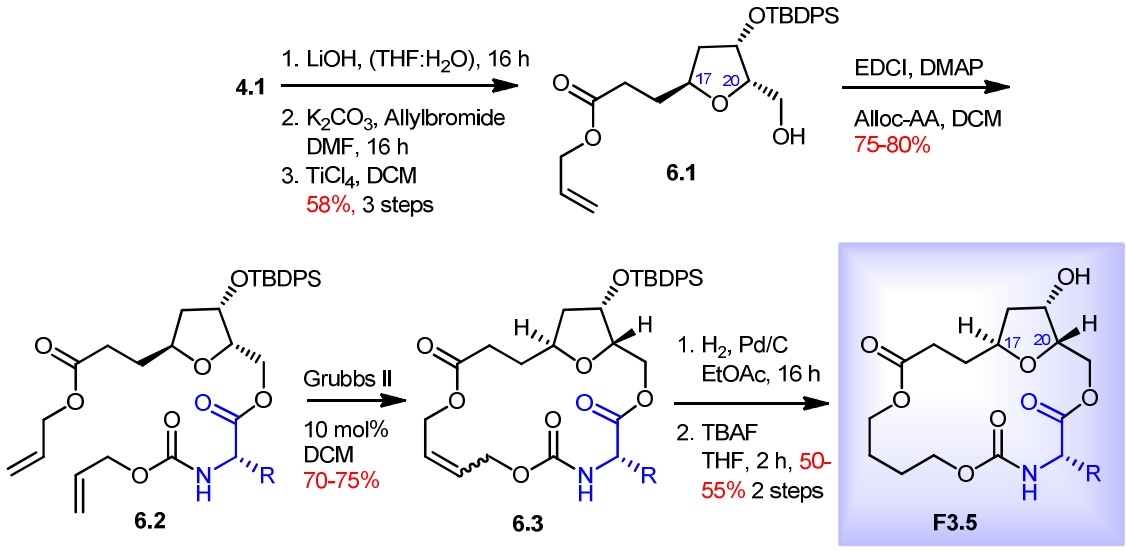


Scheme 5: 17-Membered Macrocyclic Compounds (**5.3/F3.4**) from *trans*-2,5-Disubstituted Tetrahydrofuran Fragment, **4.1**.

compounds were obtained by this approach and this further validated the feasibility of our ring formation that is independent of an amino acid utilized in the synthesis. As a test case, in three examples, the hydrogenation conditions led to producing the de-protected compounds, and the removal of the double bond, finally giving **F3.4**.



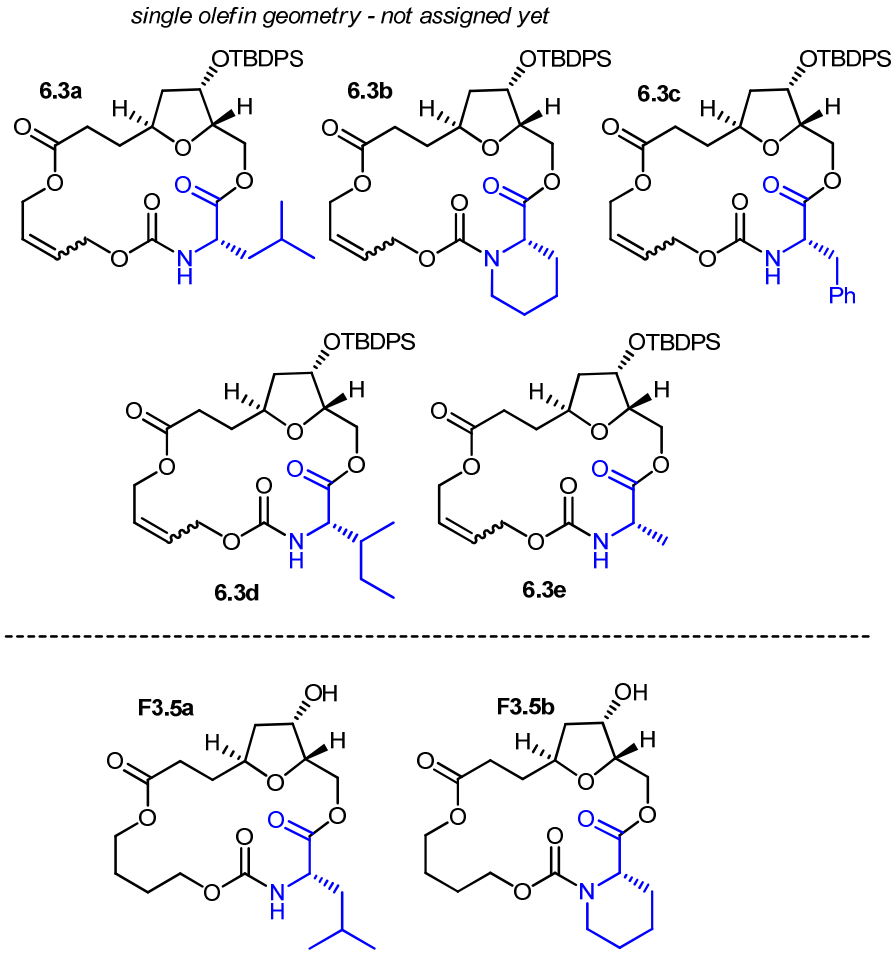
2.4.4 Synthesis of 18-Membered Macrocyclic Compounds



Scheme 6: 18-Membered Macrocyclic Compounds (**6.3/F3.5**) from *trans*-2,5-Disubstituted Tetrahydrofuran Fragment, **4.1**.

In a similar manner, our synthesis approach for obtaining 18-membered macrocyclic compounds is shown in **Scheme 6**. The hydrolysis of **4.1** produced free acid which

was then allylated and the protecting group removal, gave **6.1.** It was then coupled with five different amino acids for obtaining a precursor **6.2** for the crucial ring closing metathesis. The use of a second generation Grubbs' catalyst successfully produced the 18-membered ring macrocycle with a single olefin geometry, **6.3**. The olefin geometry was not assigned due to overlapping signals in NMR. All the products were thoroughly purified and well-characterized by MS and 1D and 2D NMR



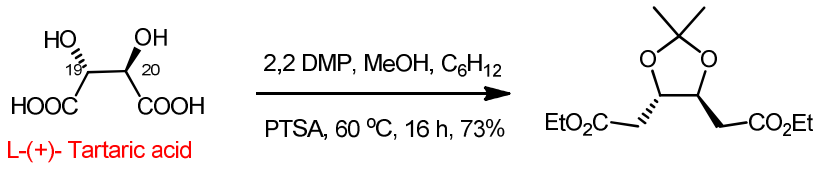
2.4.5 Conclusions

- To summarize, we succeeded in developing a practical and scalable synthesis of *trans*-2,5 substituted tetrahydrofuran moiety which is present in eribulin as well as in several other bioactive natural products.
- The privileged scaffold **4.1** was further utilized in obtaining two different types of i.e. 17- and 18-membered macrocyclic rings; moreover, it was also utilized in completing the synthesis of the Kishi's fragment.

- The incorporation of an amino acid moiety in both macrocyclic rings allow introducing a chiral diversity site for further obtaining several analogs having a variation in the chiral side chain present within the macrocyclic ring skeleton.
- The key step involved in our synthesis was an iodocyclization. An advantage of our approach is the use of a cheap chiral starting material *R,R*-tartaric acid.
- The biological evaluation of all the compounds generated from this program is ongoing in collaboration with Dr. Satish Kitambi, Karolinska Institute, Sweden and Dr. Subhadra Dravida, Trans Cell Biologics, Hyderabad in various patient-derived cancer cells / cancer stem cells to search for novel selective cancer cell killers, for cell migration, and in general, as the cytoskeleton modulators.

2.4.6 Experimental Section

2.4.6a Synthesis of Tetrahydrofuran 3.4



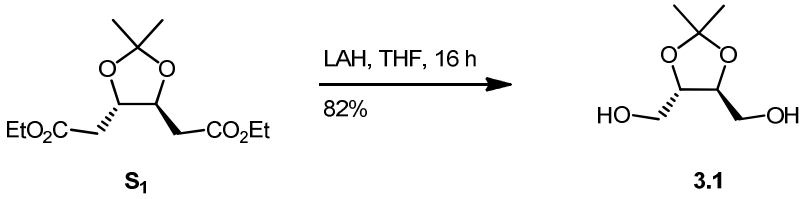
F4.4 **S_1**

Diethyl 2,2'-((4S, 5S)-2,2-dimethyl-1,3-dioxolane-4,5-diyl)diacetate (S_1):

To a solution of L-(+)-tartaric acid (11.5 g, 76 mmol) in a mixture of anhydrous MeOH (5 mL) and cyclohexane (6 mL) was added 2,2-dimethoxypropane (21mL, 17.5 mmol, 2.3 eq) and p-toluenesulfonic acid (157 mg, 0.012 eq) The reaction mixture was refluxed for 12 h, and then quenched with solid K_2CO_3 (115 mg, 1 mol%). The resulting reaction mixture was filtered through a celite pad. The solvent was concentrated under reduced pressure and purified by normal column chromatography (silica gel 60-120 mesh, 20% EtOAc in n-hexane, to afford diester **S_1** as a colourless oil (12 g) in 73% yield.

Molecular Formula: $C_{13}H_{22}O_6$

R_f (solvent system): 0.2 (20%, EtOAc/hexane).



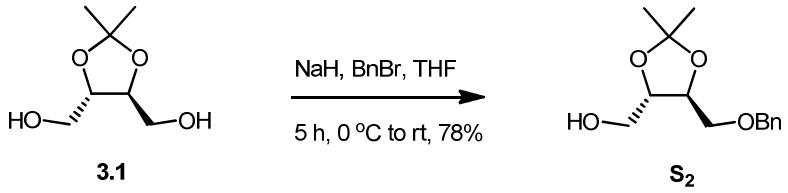
S1 **3.1**

((4S, 5S)-2,2-Dimethyl-1,3-dioxolane-4,5-diyl)dimethanol (3.1):

A suspension of $LiAlH_4$ (5.2 g, 13.7 mmol) in dry THF (70 mL) was cooled using ice bath and a solution of **S_1** (12 g, 55 mmol) in dry THF (15 mL) was added drop wise over 30 min. The reaction mixture was stirred for 16 h and completion of the reaction (TLC), it was cooled to 0 ^oC and quenched with 10% aq. NaOH (15 mL), water (10 mL) and EtOAc (50 mL). The white precipitate was filtered through a pad of silica gel and washed with a mixture of MeOH/EtOAc (1:3, 200 mL). The filtrate was concentrated to give the corresponding acetonide diol as colorless oil **3.1** (7.9 g, 82%).

Molecular Formula: $C_7H_{14}O_4$

R_f (solvent system): 0.2 (50%, EtOAc/hexane).



3.1 **S_2**

((4S, 5S)-5-((benzyloxy) methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (S_2):

To the solution of **3.1** (7.9 g, 48 mmol) in THF (20 mL) was added NaH (60%, 1.4 g, 58 mmol) at 0 ^oC, and stirred for 1 h to this benzyl bromide (58 mmol, 7 mL) was added and the reaction mixture was stirred for 5 h and was quenched with aqueous NH_4Cl. The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine. The resulting mixture was dried over Na_2SO_4 and concentrated under reduce pressure. Purification by column chromatography (silica gel 60-120 mesh, 25% EtOAc in n-hexane, TLC: R_f = 0.2) provided product **S_2** (7.5 g, 78%) as a colorless oil.

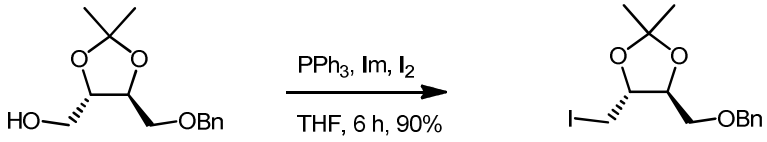
Molecular Formula: $C_{14}H_{20}O_4$

R_f (solvent system): 0.2 (25%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 253.2 (M+1).

1H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.38-7.25 (m, 5H), 4.58 (s, 2H), 4.09-4.01 (m, 1H), 3.98-3.91 (m, 1H), 3.76 (td, *J* = 11.32, 4.00 Hz, 1H), 3.67 (td, *J* = 13.22, 6.63

Hz, 2H), 3.56 (dd, *J* = 9.87, 5.67 Hz, 1H), 2.41 (d, *J* = 4.59 Hz, 1H), 1.42 (d, *J* = 2.17 Hz, 6H); **^{13}C NMR** (100MHz, $CDCl_3$) δ ppm : 137.6, 128.4, 127.8, 127.7, 109.3, 79.6, 76.6, 73.7, 70.4, 62.4, 27.0, 26.9.



S_2 **3.2**

(4S, 5R)-4-((Benzyloxy) methyl)-5-(iodomethyl)-2,2-dimethyl-1,3-dioxolane (3.2):

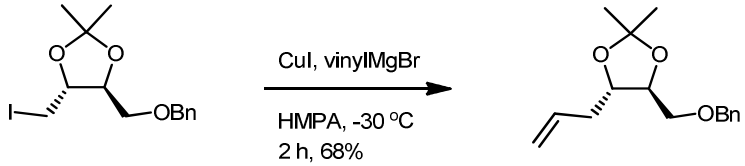
To a solution of the mono-Bn protected product (9.6 g, 38 mmol) in THF (100 mL) was added imidazole (9 g, 133 mmol), Ph_3P (15 g, 57 mmol) and iodine (14.5 g, 57 mmol) at 0 ^oC successively. The resulting mixture was warmed up to 23 °C over 2 h and stirred overnight and then quenched by 10% aqueous $Na_2S_2O_3$. The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine. The resulting mixture was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (silica gel 60-120 mesh, 5% EtOAc in n-hexane, TLC: R_f = 0.5) provided (12.4 g, 90%) iodide **3.2** as a colorless oil

Molecular Formula: $C_{14}H_{19}IO_3$

R_f (solvent system): 0.5 (5%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 363.0 (M+1).

1H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.38-7.28 (m, 5H), 4.59 (s, 2H), 4.00-3.92 (m, 1H), 3.89-3.83 (m, 1H), 3.68-3.62 (m, 2H), 3.35 (dd, *J* = 10.55, 5.14 Hz, 1H), 3.28 (dd, *J* = 10.54, 5.29 Hz, 1H), 1.47 (s, 3H), 1.42 (s, 3H); **^{13}C NMR** (100MHz, $CDCl_3$) δ ppm : 137.8, 128.4, 127.7, 109.8, 80.1, 77.6, 73.6, 70.5, 27.3, 27.3, 6.4.



3.2 **S_3**

(4S, 5S)-4-Allyl-5-((benzyloxy) methyl)-2, 2-dimethyl-1,3-dioxolane (S_3):

To a solution of thus obtained iodide (12.4 g, 34 mmol) in THF (40 mL) was added HMPA (30 mL) and CuI (4.5 g, 23.8 mmol) at 23 ^oC. The resulting mixture was cooled to -30 ^oC and vinylmagnesium bromide (136 mL, 1M in THF, 13.6 mmol) was added drop wise at that temperature over 1 h. The resulting mixture was stirred at -30

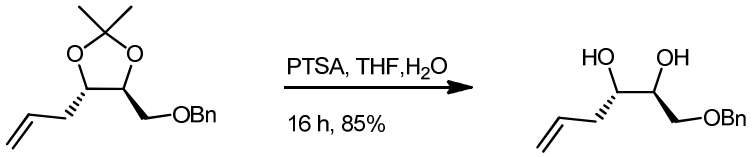
°C for 2 h and then warmed up to 10 °C and then quenched with aqueous NH_4Cl. The organic layer was separated and the aqueous layer was extracted with ethyl acetate, the combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (silica gel 60-120 mesh, 5% EtOAc in n-hexane, TLC: R_f = 0.5) provided product **S_3** (6.1 g, 68%)

Molecular Formula: $C_{16}H_{22}O_3$

R_f (solvent system): 0.5 (5%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 263.1 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.44-7.20 (m, 5H), 5.88-5.76 (m, 1H), 5.18-5.05 (m, 2H), 4.60 (S, 2H), 3.94-3.85 (m, 2H), 3.60-3.55 (m, 2H), 2.43-2.35 (m, 2H), 1.43 (S, 3H), 1.41 (S, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 138.0, 133.8, 128.4, 127.6, 127.6, 117.6, 108.8, 79.5, 77.3, 73.4, 70.4, 37.4, 27.3, 27.0.



S_3 **3.3**

(2S, 3S)-1-(Benzyloxy) hex-5-ene-2,3-diol (3.3):

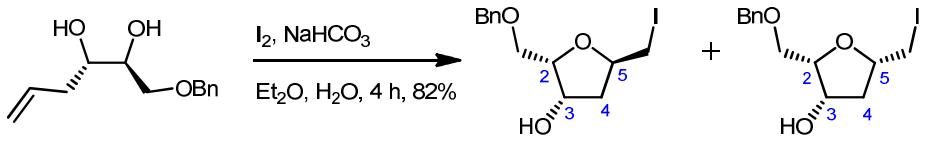
To the obtained olefin **S_3** (6.1 g, 23 mmol) in THF: H_2O (4:1) was added PTSA (6 g, 34 mmol) and the mixture was stirred at 23 °C for 16 h. The reaction was then quenched with Na_2CO_3 (11.4 g, 108 mmol) and extracted with ethyl acetate, dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (silica gel 60-120 mesh, 40% EtOAc in n-hexane, TLC: R_f = 0.4) provided product **3.3** (4.37 g, 85%);

Molecular Formula: $C_{13}H_{18}O_3$

R_f (solvent system): 0.4 (40%, EtOAc/hexane)

LRMS: (ES+) m/z = 223.1 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.39-7.28 (m, 5H), 5.84 (tdd, *J* = 17.23, 10.17, 7.12 Hz, 1H), 5.12 (ddd, *J* = 9.52, 7.15, 1.14 Hz, 2H), 4.61-4.51 (m, 2H), 3.76-3.67 (m, 2H), 3.65 (dd, *J* = 9.50, 3.32 Hz, 1H), 3.59 (dd, *J* = 9.50, 5.60 Hz, 1H), 2.62 (t, *J* = 6.64 Hz, 1H), 2.56 (t, *J* = 4.40 Hz, 1H), 2.33 (q, *J* = 6.34 Hz, 2H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 137.6, 134.4, 128.5, 127.9, 127.8, 117.9, 73.6, 72.6, 71.6, 71.5, 38.1.



3.3 **3.4** **3.5**

4:1 Separable diastereomers

$NaHCO_3$ (3.154 g, 38 mmol) and I_2 (5.9 g, 23 mmol) were added to a solution of **3.3** (4.3 g, 19 mmol) in Et_2O (400 mL) and H_2O (160 mL) at 0 ^oC. The mixture was stirred at 0 ^oC for 4 h and quenched with saturated $Na_2S_2O_3$ (100 mL). The aqueous layer was extracted with Et_2O (3 × 50 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated. Flash chromatography of the residue on silica gel (7:3 hexanes/DCM) gave 4.5 g (65%) of iodide (5R)-**3.4**, followed by 1.10 g (16%) of diastereomer (5S)-**3.5**. The stereochemistry of (5R)-**3.4** and (5S)-**3.5** was established by 2D & 1D NOESY studies.

Data for **(5R)-3.4 :**

Molecular Formula: $C_{13}H_{17}IO_3$

R_f (solvent system): 0.4 (30%, DCM/hexane)

LRMS: (ES+) *m/z* = 349.02 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.41-7.29 (m, 5H), 4.60 (S, 2H), 4.53 (dd, *J* = 7.6, 3.6 Hz 1H), 4.29 (dt, *J* = 14, 4.6 Hz, 1H), 4.19 (dd, *J* = 8.8, 5.2 Hz, 1H), 3.80 (d, *J* = 5.00 Hz, 2H), 3.32 (t, *J* = 5.48 Hz, 2H), 3.04 (d, *J* = 3.87 Hz, 1H), 2.22 (dd, *J* = 13.32, 5.82 Hz, 1H), 1.90-1.83 (m, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 137.3, 128.5, 128.0, 127.8, 80.9, 73.9, 73.9, 68.9, 42.2, 11.1.

Data for **(5S)-3.5 :**

Molecular Formula: $C_{13}H_{17}IO_3$

R_f (solvent system): 0.4 (30%, DCM/hexane)

LRMS: (ES+) *m/z* = 349.02 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.29-7.42 (m, 5H), 4.61 (s, 2H), 4.47 (dt, *J* = 9.6, 6.4 Hz, 1H), 4.11 (qd, *J* = 7.88, 5.47 Hz, 1H), 3.99 (dd, *J* = 9.34, 4.91 Hz, 1H), 3.81 (d, *J* = 5.02 Hz, 2H), 3.42-3.33 (m, 2H), 2.79 (d, *J* = 5.50 Hz, 1H), 2.38 (ddd, *J* = 13.96, 7.90, 6.19 Hz, 1H), 1.88 (ddd, *J* = 13.81, 5.22, 2.69 Hz, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 137.5, 128.5, 127.9, 127.8, 81.6, 77.9, 73.8, 73.0, 69.0, 40.8, 10.6.

2.4.6b Synthesis of Kishi's Fragment 4.4

3.4 **S_4**

(((2S,3S,5R)-2-((Benzyloxy)methyl)-5-(iodomethyl)tetrahydrofuran-3-yl)oxy) (tert-butyl)diphenylsilane (S_4):

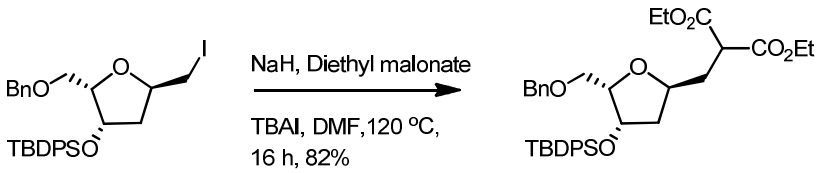
To a solution of **3.4** (4.5 g, 12 mmol) and imidazole (1.75 g, 25 mmol) in DCM (50 mL) was added tert-butylchlorodiphenylsilane (6.5 mL, 25 mmol) at 0 ^oC. After stirring overnight at room temperature, hexane (100 mL) and water (400 mL) were added. The organic layer was separated and the aqueous layer was extracted with hexane (50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel 60-120 mesh, 10% EtOAc in n-hexane, TLC: R_f = 0.4) to afford the title compound **S_4** (6 g, 94%) as a colorless oil.

Molecular Formula: $C_{29}H_{35}IO_3Si$

R_f (solvent system): 0.4 (10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 587.01 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64 (dd, *J* = 10.86, 4.00 Hz, 4H), 7.49-7.28 (m, 11H), 4.61 (d, *J* = 11.89 Hz, 1H), 4.50 (d, *J* = 11.81 Hz, 2H), 4.34-4.26 (m, 1H), 4.15 (dt, *J* = 5.73, 3.68 Hz, 1H), 3.72 (d, *J* = 5.80 Hz, 2H), 3.27 (dd, *J* = 9.96, 4.06 Hz, 1H), 3.18 (dd, *J* = 9.92, 7.06 Hz, 1H), 1.98 (ddd, *J* = 13.26, 5.86, 1.51 Hz, 1H), 1.55 (ddd, *J* = 13.50, 9.20, 4.63 Hz, 1H), 1.07 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 138.1, 135.8, 135.8, 133.9, 133.0, 129.9, 129.9, 128.3, 128.0, 127.8, 127.7, 127.6, 82.8, 76.9, 74.4, 73.5, 69.3, 42.0, 27.0, 19.3, 11.3.



S_4 **S_5**

Diethyl 2-(((2S, 4S, 5S)-5-((benzyloxy)methyl)-4-((terbutyldiphenylsilyl)oxy) tetrahydrofuran-2-yl)methyl)malonate (S_5):

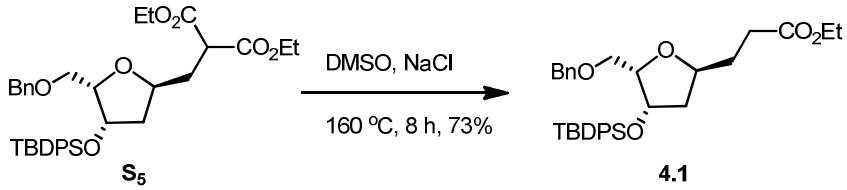
Sodium hydride (60% suspension in mineral oil, 344 mg, 14.3 mmol) was taken in 20 mL dry DMF, then diethyl malonate (2.44 g, 15.3 mmol) was added at 0 ^{o}C. After 0.5 h, compound **S_4** (6 g, 10.2 mmol) was added and the reaction mixture was refluxed for 12 h. After that time water was added at 0 ^{o}C and the reaction mixture was extracted with EtOAc, the organic layer was successively washed with brine and dried ($MgSO_4$). The organic extract was evaporated in vacuo and purified by silica gel chromatography (silica gel 60-120 mesh, 10% EtOAc in n-hexane, TLC: R_f = 0.4) to afford the title compound the diethyl malonate derivative **S_5** in 82% yield (5.2 g) as a clear liquid

Molecular Formula: $C_{36}H_{46}O_7Si$

R_f (solvent system): 0.4 (10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 619.3 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64-7.59 (m, 4H), 7.45-7.26 (m, 11H), 4.57 (d, *J* = 11.83 Hz, 1H), 4.51-4.45 (m, 2H), 4.32-4.24 (m, 1H), 4.23-4.08 (m, 4H), 3.99 (td, *J* = 6.66, 4.71 Hz, 1H), 3.74 (dd, *J* = 9.91, 4.86 Hz, 1H), 3.66 (dd, *J* = 9.88, 6.75 Hz, 1H), 3.51 (dd, *J* = 8.30, 6.25 Hz, 1H), 2.09-1.94 (m, 2H), 1.83 (ddd, *J* = 13.16, 5.97, 1.67 Hz, 1H), 1.42 (ddd, *J* = 13.43, 8.95, 4.85 Hz, 1H), 1.24 (td, *J* = 9.06, 7.13 Hz, 6H), 1.04 (d, *J* = 6.04 Hz, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 169.5, 169.3, 138.3, 135.8, 135.8, 133.9, 133.1, 129.8, 129.8, 128.3, 127.9, 127.7, 127.5, 81.3, 75.2, 74.3,73.4, 69.3, 62.0, 61.4, 61.3, 49.2, 41.1, 34.7, 26.9, 19.3, 14.1, 14.0.



S_5 **4.1**

Ethyl 3-((2S, 4S, 5S)-5-((benzyloxy)methyl)-4-((tert butyldiphenylsilyl)oxy) tetra hydrofuran-2-yl)propanoate (4.1):

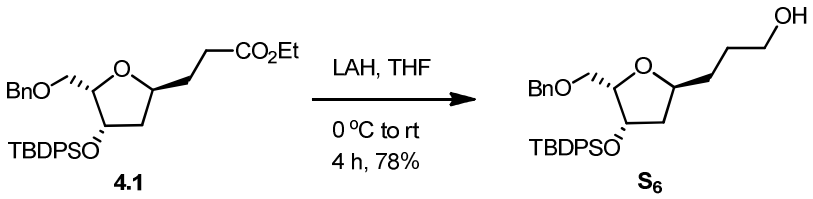
To a solution of the above compound (5.2 g, 8.4 mmol) in DMSO (20 mL) were added water (0.2 mL) and LiCl (2.8 g, 67.2 mmol). The mixture was stirred for 16 h at 160 ^{o}C. After cooling to room temperature, the mixture was poured into water, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with water and brine, and dried with Na_2SO_4. After concentration in vacuo, the residue was purified by column chromatography (silica gel 60-120 mesh, 10% EtOAc in n-hexane, TLC: R_f = 0.4) to afford 3.4 g (73%) of ester

Molecular Formula: $C_{33}H_{42}O_5Si$

R_f (solvent system): 0.4 (10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 547.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.63 (t, *J* = 6.61 Hz, 4H), 7.47-7.27 (m, 11H), 4.60 (d, *J* = 11.89 Hz, 1H), 4.49 (d, *J* = 12.08 Hz, 2H), 4.32-4.22 (m, 1H), 4.10 (q, *J* = 7.13 Hz, 2H), 4.01 (td, *J* = 6.91, 4.44 Hz, 1H), 3.71 (dq, *J* = 9.87, 5.85 Hz, 2H), 2.26-2.43 (m, 2H), 1.85-1.71 (m, 3H), 1.45-1.38 (m, 1H), 1.24 (dd, *J* = 14.14, 7.02 Hz, 3H), 1.05 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.5, 138.2, 135.8, 134.8, 134.0, 133.1, 129.8, 129.7, 129.6, 128.3, 127.9, 127.7, 127.6, 127.5, 81.3, 74.3, 73.4, 60.3, 40.9, 30.9, 26.9, 26.5, 19.3, 14.2.



4.1 **S_6**

-((2S,4S,5S)-5-((Benzyloxy)methyl)-4-((tert-butyldiphenylsilyl)oxy)tetrahydro furan-2-yl)propan-1-ol (S_6):

A suspension of $LiAlH_4$ (85 mg, 2.19 mmol) in dry THF (5 mL) was cooled using ice bath and a solution of **4.1** (1g, 1.83 mmol) in dry THF (10 mL) was added drop wise over 10 min. The reaction mixture was stirred for 3 h and completion of the reaction (TLC), it was cooled to 0 ^{o}C and quenched with 10% aq. NaOH (5 mL), water (5 mL) and EtOAc (15 mL). The white precipitate was filtered through a pad of silica gel and washed with EtOAc and dried with Na_2SO_4. After concentration in vacuo, the residue was purified by column chromatography (silica gel 60-120 mesh, 30% EtOAc in n-hexane, TLC: R_f = 0.4) to afford give compound **S_6** (720 mg, 78%).

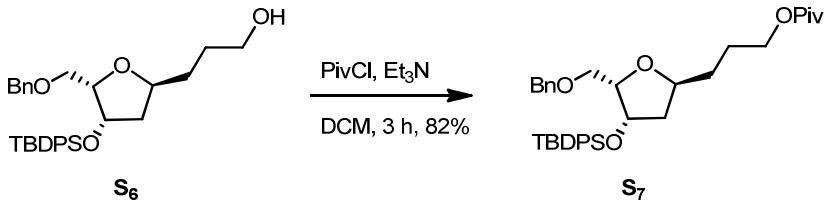
Molecular Formula: $C_{31}H_{40}O_4Si$

R_f (solvent system): 0.4 (30%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 505.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.63 (dd, *J* = 7.86, 6.83 Hz, 4H), 7.42 (td, *J* = 7.23, 6.31 Hz, 2H), 7.38-7.25 (m, 9H), 4.59 (d, *J* = 11.82 Hz, 1H), 4.52-4.46 (m, 2H), 4.29 (dq, *J* = 10.09, 5.33 Hz, 1H), 4.06 (td, *J* = 6.85, 4.66 Hz, 1H), 3.77-3.68 (m, 2H), 3.68-3.57 (m, 2H), 1.80 (ddd, *J* = 13.07, 5.53, 1.07 Hz, 1H), 1.70 (dd, *J* = 5.31, 3.37 Hz, 1H), 1.61 (dd, *J* = 13.20, 6.94 Hz, 2H), 1.57-1.49 (m, 2H), 1.46-1.36 (m, 1H), 1.05 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 138.2, 135.8, 135.8, 134.0,

133.1, 129.8, 129.8, 128.3, 128.0, 127.7, 127.7, 127.6, 81.5, 77.7, 74.2, 73.5, 69.4, 62.9, 41.4, 32.8, 29.7, 26.9, 19.3.



S_6 **S_7**

3-((2S,4S,5S)-5-((Benzyloxy)methyl)-4-((tert-butyldiphenylsilyl)oxy)tetrahydro furan-2-yl)propyl pivalate (S_7):

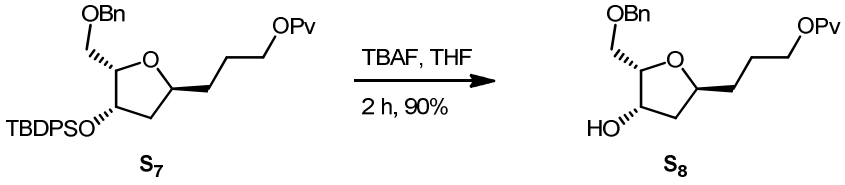
To a solution of **S_6** (720 mg, 1.42 mmol), DMAP (34 mg, 0.28 mmol) and triethylamine (0.8ml, 5.68 mmol) in CH_2Cl_2 (10 ml) was added PivCl (0.34 ml, 2.84 mmol) at 0 ^oC. The reaction mixture was warmed up to rt overnight (10h) before quenched with saturated aqueous $NaHCO_3$ solution, extracted with EtOAC (50 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column chromatography (silica gel 60-120 mesh, 5% EtOAc in n-hexane, TLC: R_f = 0.3) to afford **S_7** as a colorless oil (688 mg, 82%).

Molecular Formula: $C_{36}H_{48}O_5Si$

R_f (solvent system): 0.3 (5%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 589.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64 (t, *J* = 6.88 Hz, 4H), 7.43 (dd, *J* = 12.18, 7.18 Hz, 2H), 7.39-7.28 (m, 9H), 4.61 (d, *J* = 11.93 Hz, 1H), 4.50 (d, *J* = 11.99 Hz, 2H), 4.27 (td, *J* = 11.72, 5.89 Hz, 1H), 4.03 (dd, *J* = 10.78, 4.69 Hz, 3H), 3.77-3.66 (m, 2H), 1.89 (dd, *J* = 13.2, 9.2 Hz 1H), 1.69 (t, *J* = 9.34 Hz, 1H), 1.59 (dd, *J* = 10.05, 5.19 Hz, 2H), 1.47-1.34 (m, 2H), 1.17(s, 9H), 1.06 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 138.2, 135.8, 135.8, 134.0, 133.1, 129.7, 129.7, 128.3, 128.0, 127.6, 127.5, 81.3, 77.2, 74.3, 73.4, 69.4, 64.3, 41.1, 38.7, 32.2, 27.2, 26.9, 25.1, 19.3.



S_7 **S_8**

3-((2S,4S,5S)-5-((Benzyloxy)methyl)-4-hydroxytetrahydrofuran-2-yl)propyl pivalate (S_8):

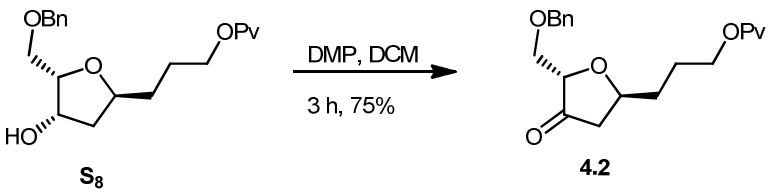
To a solution of **S_7** (688 mg, 1.17 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 2.34 mL, 2.34 mmol,) at 0 ^oC. The mixture was allowed to stand at room temperature for 1 h, then EtOAc (10 mL), H_2O (5 mL) and saturated aqueous NaCl (5 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2×20mL). The organic extracts were combined, dried (Na_2SO_4), concentrated under reduced pressure and purified by flash chromatography (silica gel 60-120 mesh, 30% EtOAc in n-hexane, TLC: R_f = 0.2) to give the title compound (360 mg, 90%) as a colour less oil.

Molecular Formula: $C_{20}H_{30}O_5$

R_f (solvent system): 0.2 (30%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 351.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.34 -7.25 (m, 5H), 4.57 (s, 2H), 4.48 (t, *J* = 3.95 Hz, 1H), 4.26 (qd, *J* = 11.70, 5.87 Hz, 1H), 4.07 (dd, *J* = 7.40, 5.41 Hz, 3H), 3.76 (d, *J* = 5.14 Hz, 2H), 2.12-2.05 (m, 1H), 1.81-1.61 (m, 5H), 1.58-1.48 (m, 1H), 1.19 (s, 9H).



S_8 **4.2**

3-((2S,5S)-5-((Benzyloxy)methyl)-4-oxotetrahydrofuran-2-yl)propyl pivalate (4.2):

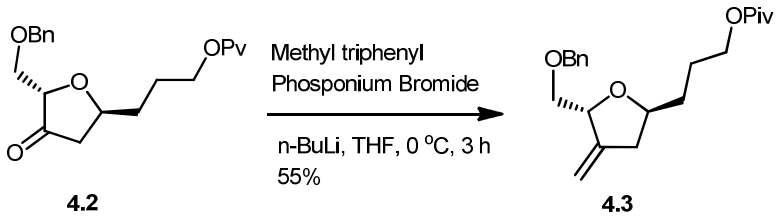
To a solution of alcohol **S_8** (360 mg, 1.02 mmol) in dry DCM (15 mL) added DMP (872 mg, 2.05 mmol) in one portion at 0 ^oC under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at 20 ^oC then completion of starting material by monitoring with TLC, poured a saturated solution of sodium thiosulfate ($Na_2S_2O_3$) (5 mL) and saturated $NaHCO_3$ solution (10 mL) allowed to stirred for 15 minutes. Two layers were separated and aqueous layer extracted with DCM, combined organic layers were washed with brine and dried over Na_2SO_4, concentrated under reduced pressure and purified by flash chromatography (silica gel 60-120 mesh, 10% EtOAc in n-hexane, TLC: R_f = 0.2) give **4.2** as colourless liquid (268 mg, 75%)

Molecular Formula: $C_{20}H_{28}O_5$

R_f (solvent system): 0.2 (10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 349.1(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.32-7.26 (m, 5H), 4.55-4.50 (m. 3H), 4.11-4.06 (m, 3H), 3.75 (dd, *J* = 10.4, 3.6 Hz, 1H), 3.70 (dd, *J* = 10.4, 3.6 Hz, 1H), 2.61 (dd, *J* = 18, 6.5 Hz, 1H), 2.19 (dd, *J* = 18, 8 Hz, 1H), 1.89-1.67 (m, 4H), 1.19(s, 9H).



4.2 **4.3**

3-((2S, 5R)-5-((Benzyloxy)methyl)-4-methylenetetrahydrofuran-2-yl)propyl pivalate (4.3):

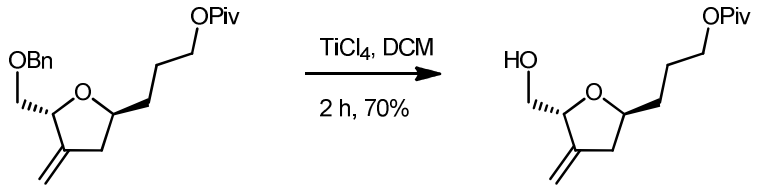
A suspension of methyltriphenylphosphonium bromide (1.6 g, 4.62 mmol) in dry THF (3 mL) was treated with n-BuLi (2.88 mL, 4.62 mmol, 1.6 M solution in hexane) under N_2 at 0 ^oC. The resulting yellow solution was allowed to stir at room temperature for 30 min, then cooled to -78 ^oC. A solution of ketone **4.2** (268 mg, 0.77 mmol) in dry THF (2.5mL) was added slowly and the reaction mixture was allowed to warm up to room temperature. The stirring was continued for 4 hr before it was quenched by saturated aqueous NH_4Cl. The aqueous layer was extracted with Et_2O three times, the combined organics were dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography (silica gel 60-120 mesh, 10% EtOAc in n-hexane, TLC: R_f = 0.3) gave pure **4.3** (146 mg, 55%)

Molecular Formula: $C_{21}H_{30}O_4$

R_f (solvent system): 0.3 (10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 347.2(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.37-7.25 (m, 5H), 5.04 (dd, *J* = 3.2, 2.07 Hz, 1H), 4.91 (dd, *J* = 4.18, 2.09 Hz, 1H), 4.60 (d, *J* = 4.53 Hz, 2H), 4.14-4.04 (m, 3H), 3.53-3.49 (m, 2H), 2.74-2.65 (m, 1H), 2.26 (dd, *J* = 15.50, 7.02 Hz, 1H), 1.82-1.62(m, 4H), 1.57-1.48 (m, 1H), 1.19 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 178.5, 148.6, 138.3, 128.3, 127.6, 127.5, 106.0, 79.3, 77.6, 73.3, 72.8, 64.2, 38.7, 31.5, 27.2, 25.2.



4.3 **4.4**

3-((2S,5R)-5-(Hydroxymethyl)-4-methylenetetrahydrofuran-2-yl)propylpivalate (4.4):

To a solution of **4.3** (146 mg, 0.42 mmol) in dry DCM (10 mL) added $TiCl_4$ (156 mg, 0.84 mmol) at 0 ^oC under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at then quenched with saturated NH_4Cl solution and two layers were separated and aqueous layer extracted with DCM, combined organic layers were washed with brine and dried over Na_2SO_4. Then organic layer was evaporated and obtained **4.4** as pure compound (75 mg, 70%).

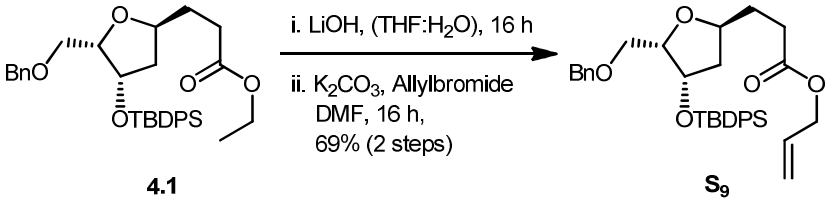
Molecular Formula: $C_{14}H_{24}O_4$

R_f (solvent system): 0.35(10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 257.1(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 5.08 (dd, *J* = 3.2, 2.07 Hz,, 1H), 4.93 (dd, *J* = 4.33, 2.17 Hz, 1H), 4.50 (s, 1H), 4.11 (td, *J* = 12.73, 4.30 Hz, 3H), 3.64-3.57 (m, 2H), 2.71 (dddd, *J* = 15.59, 6.06, 3.77, 1.92 Hz, 1H), 2.30 (ddd, *J* = 15.58, 5.18, 1.88 Hz, 1H), 2.0 (s, 1H), 1.81-1.74(m, 1H) 1.70-1.61 (m, 2H), 1.54-1.50 (m,1H), 1.20 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 178.6, 147.8, 106.2, 80.6, 77.5, 64.8, 64.1, 38.9, 38.7, 31.5, 27.2, 25.2.

2.4.6c Synthesis of 17-membered Macrocycles, 5.3/F3.4



4.1 **S_9**

Allyl 3-((2S, 4S, 5S)-5-((benzyloxy)methyl)-4-((tertbutyldiphenylsilyl) oxytetra hydrofuran-2-yl)propanoate (S_9) :

To a solution of compound **4.1** (600 mg, 1.12 mmol) in THF:H_2O mixture (4:1) added LiOH.H_2O (236 mg, 5.6 mmol) allowed to stirred for 24 h at room temperature then added 5% HCl solution (5 mL) and the compound extracted twice with EtOAc. The

organic phase was dried over Na_2SO_4, filtered and evaporated solvent afforded the carboxylic acid product as colourless oil which is subjected to Allylation reaction without further purification.

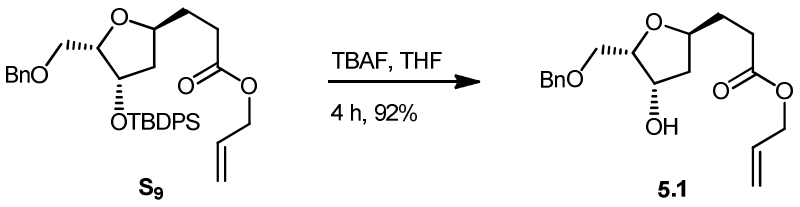
To the solution of above crude compound acid(1 eq) in dry DMF added K_2CO_3(4 eq), allylbromide (2 eq) at 0 ^oC then allowed stirred for 12 hours at room temperature under nitrogen atmosphere. Then reaction quenched with saturated NaCl and added cold water extracted twice with EtOAc. Combined organic layers were dried over Na_2SO_4, filtered and evaporated. Purification of crude compound by flash column chromatography over silica gel (10% EtOAc/hexane) afforded the compound **S_9** as light yellow oil (423 mg, 69% for two steps);

Molecular Formula: $C_{34}H_{42}O_5Si$

R_f (solvent system): 0.4(10%, EtOAc /hexane)

LRMS: (ES+) *m/z* = 559.2(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.73 (dd, *J* = 7.43, 1.44 Hz, 1H), 7.63 (t, *J* = 6.53 Hz, 4H), 7.43-7.32 (m, 10H), 5.90 (ddd, *J* = 16.22, 10.92, 5.71 Hz, 1H), 5.34-5.26 (m, 1H), 5.22 (d, *J* = 10.42 Hz, 1H), 4.63-4.47 (m, 5H), 4.28 (qd, *J* = 12.43, 6.18 Hz, 1H), 4.02 (td, *J* = 6.57, 4.38 Hz, 1H), 3.72 (dq, *J* = 9.74, 5.99 Hz, 2H), 2.49-2.32 (m, 2H), 1.80 (td, *J* = 15.17, 6.59 Hz, 3H), 1.41 (ddd, *J* = 13.61, 9.03, 4.74 Hz, 1H), 1.06 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.1, 138.2, 135.8, 135.7, 134.8, 134.0, 133.1, 132.2, 129.8, 129.7, 129.6, 128.3, 127.9, 127.7, 127.6, 127.5, 118.1, 81.3, 76.6, 74.3, 73.4, 69.4, 65.0, 40.9, 30.8, 30.8, 26.9, 26.5, 19.3.



S_9 **5.1**

Allyl-3-((2S,4S,5S)-5-((benzyloxy)methyl)-4-hydroxytetrahydrofuran-2-yl)prop anoate (5.1) :

To a solution of **S_9** (423 mg, 0.75 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 1.89 mL, 1.89 mmol,) at 0 ^oC. The mixture was allowed to stand at room temperature for 4 h, then EtOAc (20 mL), H_2O (10 mL) and saturated aqueous NaCl (5 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2×20 mL). The organic extracts were combined, dried ($MgSO_4$),

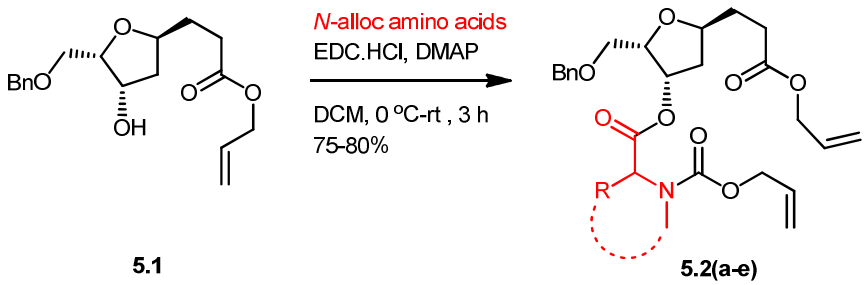
concentrated under reduced pressure purified by flash chromatography (silica gel 60-120 mesh, 30% EtOAc in n-hexane, TLC: R_f = 0.3) to give the title compound (223 mg, 92%) as a pale yellow oil.

Molecular Formula: $C_{18}H_{24}O_5$

R_f (solvent system): 0.3(30%, EtOAc /hexane)

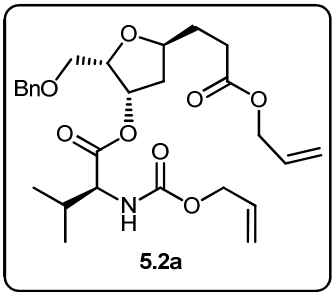
LRMS: (ES+) *m/z* = 321.1(M+1)

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm : 7.39-7.27 (m, 5H), 5.96-5.85 (m, 1H), 5.35-5.27 (m, 1H), 5.22 (dd, *J* = 10.43, 1.24 Hz, 1H), 4.56 (dd, *J* = 6.91, 5.59 Hz, 4H), 4.48 (d, *J* = 3.91 Hz, 1H), 4.26 (qd, *J* = 9.59, 6.16 Hz, 1H), 4.05 (dd, *J* = 9.11, 5.15 Hz, 1H), 3.75 (t, *J* = 4.99 Hz, 2H), 2.89 (d, *J* = 4.07 Hz, 1H), 2.55-2.35 (m, 2H), 2.08 (ddd, *J* = 13.15, 5.73, 1.14 Hz, 1H), 1.91-1.81 (m, 2H), 1.78-1.67 (m, 1H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm : 173.0, 137.5, 132.2, 128.5, 127.8, 118.1, 79.6, 73.8, 73.7, 69.0, 65.0, 41.3.



5.1 **5.2(a-e)**

To the solution of **5.1** (1 eq) in DCM solution added alloc amino acid building block (1 eq) and EDC.HCl (1.5 eq) at 0 ^{o}C under nitrogen atmosphere and allowed to stirred for 2 hours. Then added saturated $NaHCO_3$ solution to this reaction mixture extracted twice with EtOAc. Combined organic layers were washed with brine solution and dried anhydrous Na_2SO_4, evaporated the solvent, Purification of crude compound by flash chromatography over silica gel (40% EtOAc/hexane) afforded the compound **5.2(a-e)** as colourless oil.



5.2a

(S)-(2S,3S,5S)-5-(3-(allyloxy)-3-oxopropyl)-2-((benzyloxy)methyl) tetrahydro furan-3-yl 2-(((allyloxy)carbonyl)amino)-3-methylbutanoate (5.2a) :

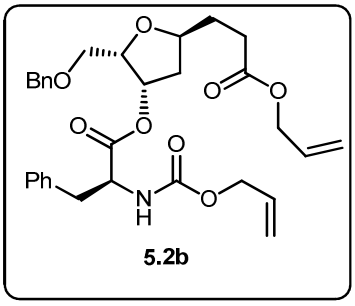
Molecular Formula: $C_{27}H_{37}NO_8$

R_f (solvent system): 0.5 (30%, EtOAc/hexane);

Yield: 78%

LRMS: (ES+) *m/z* = 504.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.36-7.27 (m, 5H), 5.90 (tdd, *J* = 16.36, 11.04, 5.70 Hz, 2H), 5.41 (d, *J* = 3.64 Hz, 1H), 5.29 (dd, *J* = 17.17, 1.08 Hz, 2H), 5.20 (t, *J* = 9.34 Hz, 3H), 4.56 (d, *J* = 5.64 Hz, 4H), 4.51 (d, *J* = 6.27 Hz, 2H), 4.26 (dd, *J* = 9.06, 4.31 Hz, 1H), 4.24-4.17 (m, 2H), 3.59-3.55 (m, 2H), 2.56-2.36 (m, 2H), 2.19-2.08 (m, 2H), 1.91-1.82 (m, 3H), 0.95 (d, *J* = 6.83 Hz, 3H), 0.81 (d, *J* = 6.82 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.8, 171.3, 156.1, 137.8, 132.5, 132.1, 128.3, 127.7, 127.6, 118.1, 117.9, 79.0, 77.0, 76.0, 73.4, 68.3, 65.8, 65.1, 59.1, 39.0, 31.0, 30.7, 30.7, 19.1, 17.0.



5.2b

(S)-(2S,3S,5S)-5-(3-(allyloxy)-3-oxopropyl)-2-((benzyloxy)methyl) tetrahydro furan-3-yl 2-(((allyloxy)carbonyl)amino)-3-phenylpropanoate (5.2b) :

Molecular Formula: $C_{31}H_{37}NO_8$

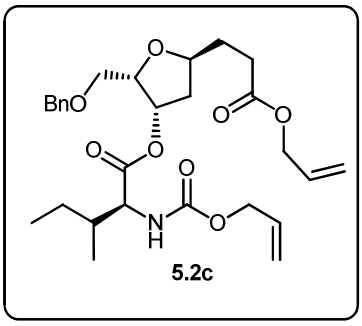
R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 78%

LRMS: (ES+) *m/z* = 552.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.34-7.25 (m, 8H), 7.10 (dd, *J* = 7.25, 1.99 Hz, 2H), 5.96-5.82 (m, 2H), 5.36 (t, *J* = 3.84 Hz, 1H), 5.34-5.26 (m, 2H), 5.26-5.18 (m, 3H), 4.59-4.48 (m, 7H), 4.19 (dt, *J* = 6.02, 3.86 Hz, 1H), 4.09 (qd, *J* = 9.53, 6.16 Hz, 1H), 3.51 (d, *J* = 5.99 Hz, 2H), 3.08 (dd, *J* = 13.94, 5.74 Hz, 1H), 2.98 (dd, *J* = 13.91, 6.48 Hz, 1H), 2.46 (tdd, *J* = 32.53, 16.39, 8.08 Hz, 2H), 2.10 (dd, *J* = 13.97, 5.87 Hz, 1H), 1.88-1.82 (m, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.8, 170.9, 155.4,

137.8, 135.4, 132.5, 132.2, 129.2, 128.7, 128.4, 127.7, 127.7, 127.2, 118.2, 117.9, 78.9, 76.9, 76.3, 73.4, 68.1, 65.8, 65.1, 54.8, 38.9, 38.1, 30.8, 30.7.



5.2c

(2S, 3R)-(2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-2-((benzyloxy)methyl) tetrahydro furan-3-yl 2-(((allyloxy)carbonyl)amino)-3-methylpentanoate (5.2c) :

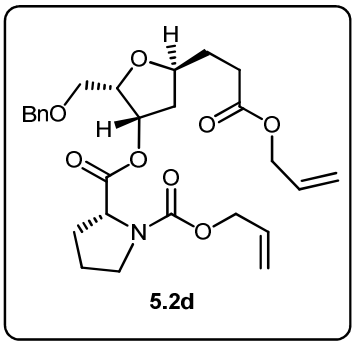
Molecular Formula: $C_{28}H_{39}NO_8$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 78%

LRMS: (ES+) *m/z* = 518.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 7.35-7.25 (m, 5H), 5.96-5.82 (m, 2H), 5.42 (td, *J* = 7.40, 3.77 Hz, 1H), 5.34-5.25 (m, 2H), 5.24-5.17 (m, 3H), 4.59-4.46 (m, 6H), 4.29 (dd, *J* = 8.86, 4.52 Hz, 1H), 4.20 (td, *J* = 9.39, 5.76 Hz, 2H), 3.57 (dd, *J* = 8.23, 3.13 Hz, 2H), 2.56-2.36 (m, 2H), 2.14 (td, *J* = 12.19, 6.08 Hz, 1H), 1.91-1.80 (m, 4H), 1.43-1.28 (m, 1H), 1.18-1.07 (m, 1H), 0.92 (t, *J* = 6.44 Hz, 3H), 0.85 (dd, *J* = 12.88, 5.47 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*): 172.8, 171.3, 155.9, 137.8, 132.5, 132.1, 128.3, 127.6, 127.6, 118.1, 117.8, 79.1, 76.9, 76.1, 73.4, 68.4, 65.8, 65.1, 58.7, 53.4, 39.0, 37.7, 30.7, 30.7, 24.5, 15.5, 11.5 .



5.2d

(R)-1-allyl 2-((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-2-((benzyloxy)methyl) tetrahydrofuran-3-yl) pyrrolidine-1,2-dicarboxylate (5.2d) :

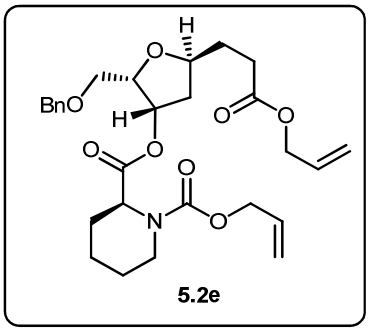
Molecular Formula: $C_{27}H_{35}NO_8$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 79%

LRMS: (ES+) *m/z* = 502.2 (M+1)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.36-7.26 (m, 5H), 5.97-5.79 (m, 2H), 5.45-5.39 (m, 1H), 5.32-5.12 (m, 4H), 4.62-4.47 (m, 6H), 4.33 (dt, *J* = 9.37, 9.10, 3.30 Hz, 1H), 4.23 (dd, *J* = 9.45, 5.62 Hz, 1H), 4.20-4.10 (m, 1H), 3.61 (d, *J* = 5.82 Hz, 1H), 3.57 (d, *J* = 5.86 Hz, 1H), 3.54-3.39 (m, 2H), 2.54-2.39 (m, 2H), 2.23-2.03 (m, 2H), 1.96-1.82 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 172.8, 172.8, 171.9, 171.8, 154.5, 153.9, 138.1, 137.9, 132.9, 132.6, 132.2, 128.3, 128.2, 127.8, 127.6, 127.6, 127.5, 118.1, 118.1, 117.4, 117.3, 79.3, 79.2, 76.9, 76.9, 75.7, 75.6, 73.4, 68.6, 68.5, 65.9, 65.8, 65.1, 65.0, 59.3, 58.8, 46.8, 46.3, 38.8, 38.7, 30.9, 30.7.



5.2e

(S)-1-allyl 2-((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-2-((benzyloxy)methyl) tetra hydrofuran-3-yl) piperidine-1,2-dicarboxylate (5.2e) :

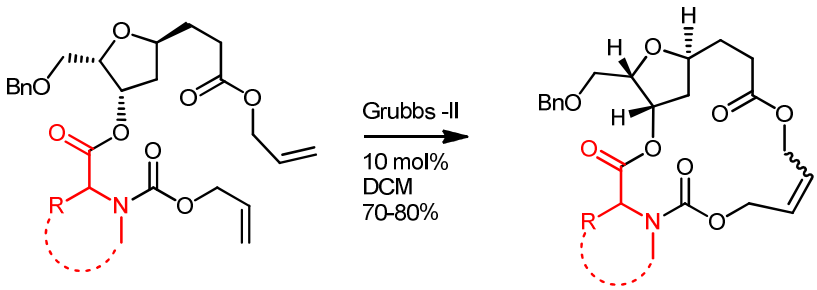
Molecular Formula: $C_{28}H_{37}NO_8$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

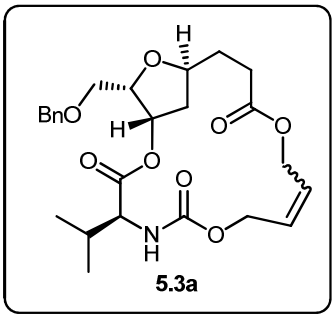
LRMS: (ES+) *m/z* = 516.2 (M+1).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.35-7.26 (m, 5H), 5.98-5.82 (m, 2H), 5.45 (s, 1H), 5.33-5.18 (m, 4H), 4.83 (dd, *J* = 45.98, 4.39 Hz, 1H), 4.61-4.48 (m, 6H), 4.27-4.14 (m, 2H), 4.12-3.95 (m, 1H), 3.60-3.50 (m, 2H), 3.05-2.82 (m, 1H), 2.57-2.37 (m, 2H), 2.20-2.06 (m, 2H), 1.87 (dd, *J* = 14.24, 6.92 Hz, 3H), 1.70-1.57 (m, 3H), 1.48-1.33 (m, 1H), 1.17 (dd, *J* = 22.82, 12.56 Hz, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 172.8, 170.8, 156.2, 137.9, 132.8, 132.1, 128.3, 127.7, 127.6, 127.6, 118.1, 117.4, 79.3, 79.2, 75.8, 75.7, 73.4, 68.5, 68.5, 66.2, 66.1, 65.1, 54.5, 54.4, 41.8, 41.7, 41.7, 39.0, 30.7, 30.6, 26.8, 26.7, 24.6, 24.4, 20.7, 20.5.



5.2(a-e) **5.3(a-e)**

To a solution of **5.2 (a-e)** (1eq) in dry DCM under nitrogen atmosphere added Grubbs' 2nd generation catalyst (10 mol%) and reaction mixture was allowed to stirred for 2 h at 40 ^{o}C. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **5.3(a-e).**



5.3a

Molecular Formula: $C_{25}H_{33}NO_8$

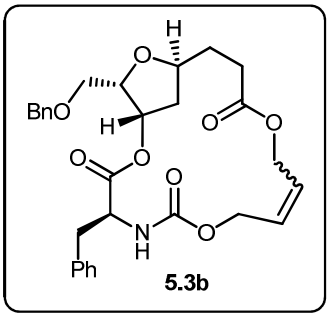
R_f (solvent system): 0.3 (40%, EtOAc/hexane)

Yield: 72%

LRMS: (ES+) *m/z* = 476.2 (M+1).

HRMS: (ESI) calcd. for $[C_{25}H_{33}NO_8 + H]^+$: 476.2284, found: 476.2284.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.37-7.22 (m, 5H), 5.76 (s, 2H), 5.62-5.30 (m, 1H), 4.85 (d, *J* = 3.76 Hz, 3H), 4.41 (d, *J* = 105.43 Hz, 4H), 4.26 (dd, *J* = 9.45, 5.96 Hz, 2H), 4.06-3.87 (m, 1H), 3.58 (d, *J* = 5.47 Hz, 2H), 2.81-2.52 (m, 1H), 2.43-2.33 (m, 1H), 2.32-2.18 (m, 1H), 2.01-1.89 (m, 1H), 1.88-1.65 (m, 3H), 0.96 (d, *J* = 6.80 Hz, 3H), 0.90 (d, *J* = 6.62 Hz, 3H).



5.3b

Molecular Formula: $C_{29}H_{33}NO_8$

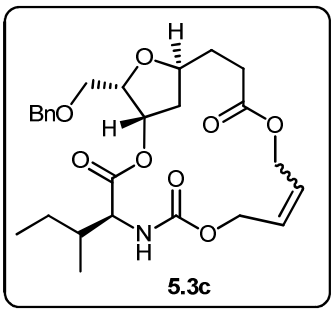
R_f (solvent system): 0.3 (40%, EtOAc/hexane)

Yield: 72%

LRMS: (ES+) *m/z* = 524.2 (M+1).

HRMS: (ESI) calcd. for $[C_{29}H_{33}NO_8 + H]^+$: 524.2284, found: 524.2284.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm : 7.36-7.23 (m, 8H), 7.17-7.08 (m, 2H), 5.65 (d, *J* = 51.59 Hz, 3H), 4.85 (d, *J* = 7.66 Hz, 3H), 4.51 (dd, *J* = 19.22, 4.98 Hz, 3H), 4.38-4.15 (m, 4H), 4.06-3.91 (m, 1H), 3.54 (s, 2H), 3.14 (d, *J* = 3.94 Hz, 1H), 3.02-2.80 (m, 1H), 2.80-2.51 (m, 1H), 2.40-2.32 (m, 1H), 1.94 (s, 1H), 1.74 (dd, *J* = 17.05, 12.06 Hz, 2H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm: 172.7, 170.4, 155.5, 136.3, 129.1, 128.9, 128.8, 128.3, 128.0, 127.7, 127.5, 127.2, 126.7, 80.2, 76.4, 75.7, 73.3, 68.4, 64.0, 62.7, 54.8, 39.4, 36.4, 31.4, 29.7.



5.3c

Molecular Formula: $C_{26}H_{35}NO_8$

R_f (solvent system): 0.3 (40%, EtOAc/hexane)

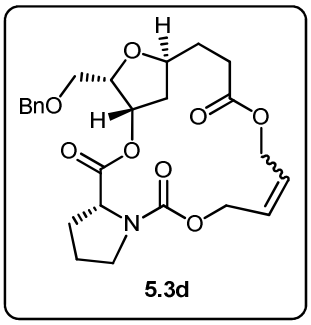
Yield: 69%

LRMS: (ES+) *m/z* = 490.2 (M+1)

HRMS: (ESI) calcd. for $[C_{26}H_{35}NO_8 + H]^+$: 490.2441, found: 490.2441.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.43-7.26 (m, 5H), 5.81 (dd, *J* = 8.27, 4.28 Hz, 2H), 5.64-5.33 (m, 1H), 5.29-5.07 (m, 1H), 5.05-4.91 (m, 1H), 4.84 (d, *J* = 8.62 Hz, 1H), 4.62-4.49 (m, 2H), 4.44-4.00 (m, 5H), 3.68-3.53 (m, 2H), 2.85-2.58 (m, 1H),

2.41 (ddd, *J* = 15.23, 7.46, 3.42 Hz, 1H), 2.21-2.09 (m, 1H), 2.04-1.87 (m, 1H), 1.83-1.60 (m, 3H), 1.48-1.34 (m, 1H), 1.19 (s, 1H), 1.02-0.87 (m, 6H).



5.3d

Molecular Formula: $C_{25}H_{31}NO_8$

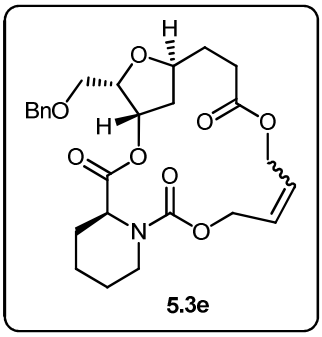
R_f (solvent system): 0.3 (40%, EtOAc/hexane)

Yield: 69%

LRMS: (ES+) *m/z* = 474.2 (M+1).

HRMS: (ESI) calcd. for $[C_{25}H_{31}NO_8+H]^+$: 474.2128, found: 474.2128.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.35-7.21 (m, 5H), 5.81-5.73 (m, 2H), 5.55 (t, *J* = 4.03 Hz, 0.6H), 5.35 (t, *J* = 4.09 Hz, 0.3H), 5.13-5.06 (m, 0.6H), 4.96 (dd, *J* = 12.76, 5.17 Hz, 0.3H), 4.83-4.78 (m, 0.5H), 4.62 (s, 0.4H), 4.58-4.42 (m, 3H), 4.38-4.28 (m, 2H), 4.19-4.06 (m, 2H), 3.56-3.45 (m, 2H), 3.41 (t, *J* = 6.60 Hz, 1H), 2.69-2.53 (m, 1H), 2.39-2.29 (m, 1H), 2.23-2.11 (m, 2H), 2.08-1.97 (m, 1H), 1.91-1.74 (m, 5H), 1.74-1.63 (m, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.2, 171.4, 154.3, 137.9, 128.3, 128.2, 127.7, 127.6, 126.7, 79.3, 76.3, 75.4, 73.4, 68.3, 64.3, 62.7, 60.1, 46.3, 39.7, 31.6, 31.2, 30.0, 24.5.



5.3e

Molecular Formula: $C_{26}H_{33}NO_8$

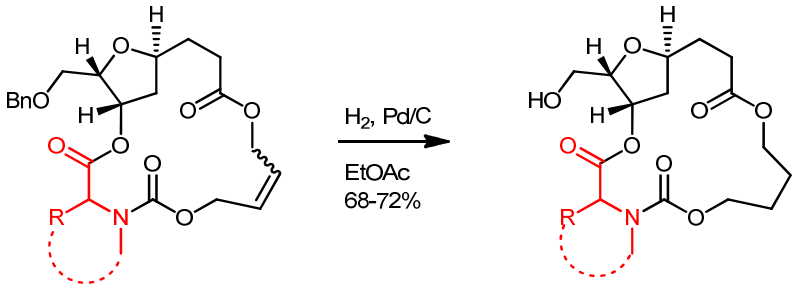
R_f (solvent system): 0.3 (40%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 488.2 (M+1).

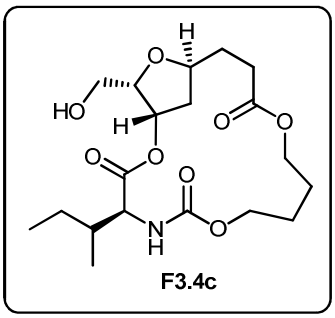
HRMS: (ESI) calcd. for $[C_{26}H_{33}NO_8+H]^+$: 488.2284, found: 488.2284.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.38-7.25 (m, 5H), 5.77 (d, *J* = 20.94 Hz, 2H), 5.40-5.26 (m, 1H), 4.97 (dd, *J* = 33.82, 13.51 Hz, 1H), 4.88-4.75 (m, 1H), 4.72-4.48 (m, 3H), 4.41-4.22 (m, 3H), 4.17-4.00 (m, 2H), 3.68-3.51 (m, 2H), 3.12 (dd, *J* = 18.30, 7.50 Hz, 0.6H), 2.61 (ddd, *J* = 36.43, 19.64, 11.29 Hz, 1.5H), 2.41-2.28 (m, 1H), 2.17 (dd, *J* = 13.47, 4.14 Hz, 1H), 1.95-2.05 (m, 2H), 1.85-1.55 (m, 6H), 1.48-1.38 (m, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.9, 171.2, 155.6, 137.8, 128.3, 128.2, 127.6, 127.3, 127.2, 79.3, 76.4, 75.8, 73.5, 68.3, 65.0, 62.8, 54.1, 41.6, 39.2, 31.1, 30.7, 26.6, 24.3, 19.8.



5.3 (a-e) **F3.4(a-e)**

To a solution of **5.3 (a-e)** (1eq) in ethyl acetate under hydrogen atmosphere added palladium charcoal (10 mol%) and reaction mixture was allowed to stirred for 16 h. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **F3.4 (a-e)**



F3.4c

(1S,4S,16S,18S)-4-((R)-sec-butyl)-18-(hydroxymethyl)-2,7,12,17-tetraoxa-5-azabicyclo[14.2.1]nonadecane-3,6,13-trione (F3.4c) :

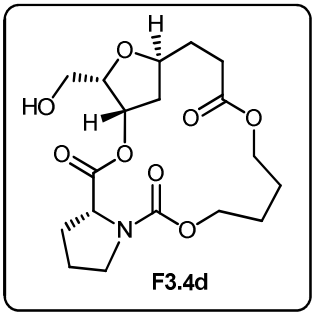
Molecular Formula: $C_{19}H_{31}NO_8$

R_f (solvent system): 0.3 (5%, MeOH/DCM)

Yield: 70%

LRMS: (ES+) *m/z* = 402.2 (M+1).

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm : 5.58-5.34 (m, 1H), 5.05-4.90 (m, 0.4H), 4.89-4.73 (m, 1.5H), 4.62-4.47 (m, 1H), 4.31-4.07 (m, 3H), 3.99-3.87 (m, 1H), 3.74-3.59 (m, 2H), 2.74-2.55 (m, 1H), 2.43-2.30 (m, 1H), 2.26-1.98 (m, 3H), 1.90-1.58 (m, 8H), 1.43 (s, 1H), 1.25-1.14 (m, 1H), 1.03-0.91 (m, 6H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm : 72.9, 172.0, 156.5, 81.5, 75.9, 64.4, 60.3, 59.0, 58.1, 53.4, 39.5, 36.2, 27.0, 26.0, 24.9, 22.6, 16.1, 14.1, 11.6.



Molecular Formula: $C_{18}H_{27}NO_8$

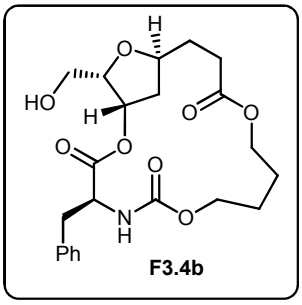
R$_f$ (solvent system): 0.3 (5%, MeOH/DCM)

Yield: 70%

LRMS: (ES+) *m/z* = 386.1 (M+1).

HRMS: (ESI) calcd. for $[C_{18}H_{27}NO_8 + Na]^+$: 408.1636, found: 408.1636.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm : 5.53 (t, *J* = 4.26 Hz, 0.7H), 5.36-5.30 (m, 1H), 4.69 (s, 0.3H), 4.50-4.42 (m, 1H), 4.37 (dd, *J* = 8.59, 4.23 Hz, 1H), 4.20 (dd, *J* = 7.60, 3.26 Hz, 1H), 4.16-4.07 (m, 1H), 3.99 (d, *J* = 2.42 Hz, 0.65H), 3.89-3.81 (m, 0.3H), 3.79-3.61 (m, 2H), 3.58-3.41 (m, 3H), 2.67-2.58 (m, 1H), 2.35 (ddd, *J* = 15.20, 8.00, 3.41 Hz, 1H), 2.30-2.19 (m, 2H), 1.82-1.92 (m, 9H), 1.66-1.55 (m, 2H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm : 172.8, 172.1, 154.6, 110.0, 80.8, 76.3, 75.6, 64.6, 63.8, 61.1, 60.0, 46.3, 39.9, 31.3, 27.1, 25.5, 24.5, 22.6, 14.1.



(1S,4S,16S,18S)-4-benzyl-18-(hydroxymethyl)-2,7,12,17-tetraoxa-5-azabicyclo [14.2.1]nonadecane-3,6,13-trione (F3.4b) :

Molecular Formula: $C_{22}H_{29}NO_8$

R_f (solvent system): 0.3 (5%, MeOH/DCM);

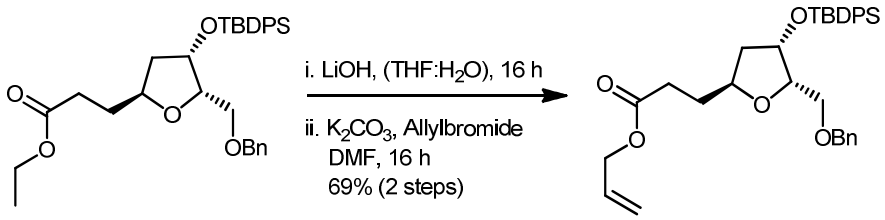
Yield: 70%;

LRMS: (ES+) *m/z* = 436.1 (M+1).

HRMS: (ESI) calcd. for $[C_{22}H_{29}NO_8 + Na]^+$: 458.1791, found: 458.1791.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.30 (td, *J* = 17.15, 5.37 Hz, 3H), 7.16 (d, *J* = 7.02 Hz, 2H), 5.46 (s, 0.6H), 5.27-5.21 (m, 0.2H), 5.16-5.05 (m, 0.2H), 4.98-4.89 (m, 0.6H), 4.56-4.23 (m, 3H), 4.11 (dd, *J* = 9.50, 5.94 Hz, 1H), 4.06-3.81 (m, 2H), 3.78-3.70 (m, 1H), 3.71-3.61 (m, 1H), 3.60-3.48 (m, 1H), 3.14 (s, 1H), 3.04-2.91 (m, 1H), 2.67-2.53 (m, 1H), 2.32 (d, *J* = 3.20 Hz, 2H), 2.11-1.94 (m, 1H), 1.90-1.79 (m, 1H), 1.78-1.65 (m, 3H), 1.59 (s, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.3, 171.7, 156.0, 136.1, 129.1, 128.7, 127.3, 81.3, 76.2, 64.5, 64.0, 60.4, 55.1, 39.4, 36.6, 31.4, 29.7, 26.9, 25.8.

2.4.6d Synthesis of 18-membered Macrocycles, 6.3/F3.5



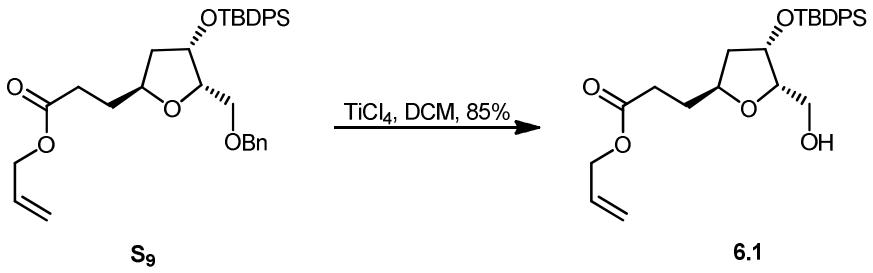
4.1 **S_9**

Allyl 3-((2S, 4S, 5S)-5-((benzyloxy)methyl)-4-((tert-butyldiphenylsilyl)oxy) tetra hydrofuran-2-yl)propanoate (S_9) :

To a solution of compound **4.1** (600 mg, 1.12 mmol) in THF:H_2O mixture (4:1) added LiOH.H_2O (236 mg, 5.6 mmol) allowed to stirred for 24 h at room temperature then added 5% HCl solution (5 mL) and the compound extracted twice with EtOAc. The organic phase was dried over Na_2SO_4, filtered and evaporated solvent afforded the carboxylic acid product as colourless oil which is subjected to allylation reaction without further purification.

To the solution of above crude compound acid(1 eq) in dry DMF added K_2CO_3(4 eq), allyl bromide (2 eq) at 0 ^oC then allowed stirred for 12 hours at room temperature under nitrogen atmosphere. Then reaction quenched with saturated NaCl and added cold water extracted twice with EtOAc. Combined organic layers were dried over Na_2SO_4, filtered and evaporated. Purification of crude compound by flash column

chromatography over silica gel (10% EtOAc/hexane) afforded the compound **S_9** as light yellow oil (423 mg, 69% for two steps)



S_9 **6.1**

Allyl 3-((2S, 4S, 5S)-4-((tert-butyldiphenylsilyl)oxy)-5-(hydroxymethyl)tetra hydrofuran-2-yl)propanoate (6.1) :

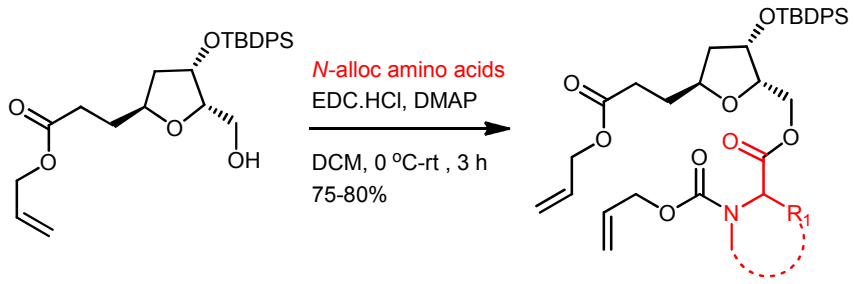
To a solution of **S_9** (423 mg, 0.75 mmol) in dry DCM (10 mL) added $TiCl_4$ (282 mg, 1.51 mmol) at 0 ^oC under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at then quenched with saturated NH_4Cl solution, and two layers were separated and aqueous layer extracted with DCM, combined organic layers were washed with brine and dried over Na_2SO_4. Concentrated under reduced pressure purified by flash chromatography (silica gel 60-120 mesh, 30% EtOAc in n-hexane, TLC: R_f = 0.4) give **6.1** as pure compound (301 mg, 85%);

Molecular Formula: $C_{27}H_{36}O_5Si$

R_f (solvent system): 0.4 (30%, EtOAc/hexane)

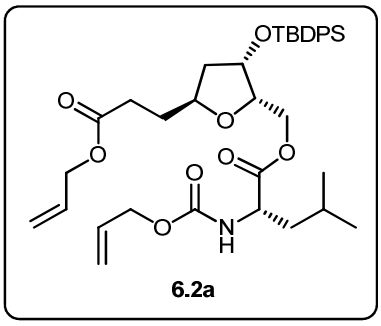
LRMS: (ES+) *m/z* = 469.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.67-7.61 (m, 4H), 7.48-7.36 (m, 6H), 5.94-5.84 (m, 1H), 5.33-5.19 (m, 2H), 4.54 (dt, *J* = 5.55, 1.2 Hz 3H), 4.26 (qd, *J* = 12.86, 6.27 Hz, 1H), 3.95-3.86 (m, 2H), 3.75 (d, *J* = 8.86 Hz, 1H), 2.48-2.29 (m, 2H), 2.17 (s, 1H), 1.89 (ddd, *J* = 13.12, 6.15, 2.69 Hz, 1H), 1.74 (dd, *J* = 14.31, 6.69 Hz, 2H), 1.53-1.43 (m, 1H), 1.08 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm: 173.1, 138.2, 135.8, 135.7, 134.7, 134.0, 133.1, 132.2, 129.8, 129.7, 128.3, 127.9, 127.7, 127.6, 127.5, 118.1, 81.3, 76.6, 74.3, 73.4, 69.4, 65.0, 40.9, 30.8, 26.9, 26.5, 19.3.



6.1 **6.2(a-e)**

To the solution of **6.1**(1 eq) in DCM solution added alloc amino acid building block (1 eq) and EDC.HCl (1.5 eq) at 0 ^{o}C under nitrogen atmosphere and allowed to stirred for 2 hours. Then added saturated $NaHCO_3$ solution to this reaction mixture extracted twice with EtOAc. Combined organic layers were washed with brine solution and dried anhydrous Na_2SO_4, evaporated the solvent, Purification of crude compound by flash chromatography over silica gel (40% EtOAc/hexane) afforded the compound **6.2(a-e)** as colourless oil.



6.2a

(S)-((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-3-((tert-butyldiphenylsilyl)oxy) tetrahydrofuran-2-yl)methyl 2-(((allyloxy)carbonyl)amino)-4-methylpentanoate (6.2a) :

Molecular Formula: $C_{37}H_{51}NO_8Si$

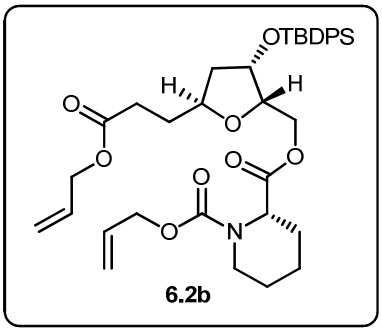
R_f (solvent system): 0.5 (40%, EtOAc/hexane)

Yield: 77%

LRMS: (ES+) *m/z* = 666.2(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.66-7.62 (m, 4H), 7.47-7.35 (m, 6H), 6.00-5.78 (m, 2H), 5.29 (ddd, *J* = 17.24, 3.55, 2.00 Hz, 2H), 5.19 (t, *J* = 11.66 Hz, 3H), 4.56 (dd, *J* = 7.55, 6.27 Hz, 4H), 4.50 (d, *J* = 11.24 Hz, 1H), 4.46-4.21 (m, 4H), 4.04-3.97 (m, 1H), 2.47-2.26 (m, 2H), 1.86 (ddd, *J* = 13.09, 5.98, 1.91 Hz, 1H), 1.78-1.69 (m, 3H), 1.69-1.58 (m, 1H), 1.55-1.37 (m, 2H), 1.07 (s, 9H), 0.93 (t, *J* = 5.99 Hz, 6H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.0, 172.9, 135.7, 133.7, 132.8, 132.2, 130.0,

129.9, 127.8, 127.7, 118.1, 117.6, 79.4, 74.2, 65.7, 65.0, 64.5, 52.5, 41.8, 40.8, 30.8, 30.7, 26.9, 24.6, 22.9, 21.7, 19.2.



6.2b

(S)-1-allyl 2-(((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-3-((tert-butyldiphenyl silyl)oxy)tetrahydrofuran-2-yl)methyl) piperidine-1,2-dicarboxylate (6.2b) :

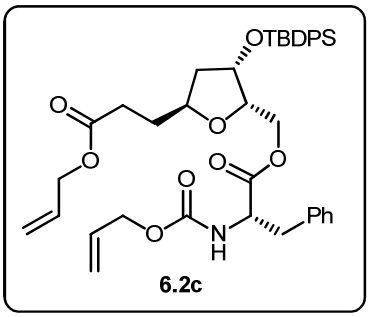
Molecular Formula: $C_{37}H_{49}NO_8Si$

R_f (solvent system): 0.5 (40%, EtOAc/hexane)

Yield: 73%

LRMS: (ES+) *m/z* = 664.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.66-7.61 (m, 4H), 7.48-7.35 (m, 6H), 5.89 (ddd, *J* = 16.15, 10.90, 5.70 Hz, 2H), 5.34-5.23 (m, 2H), 5.24-5.12 (m, 2H), 4.97 (d, *J* = 3.20 Hz, 0.5H), 4.87 (d, *J* = 3.20 Hz, 0.4H), 4.60 (dd, *J* = 9.80, 4.95 Hz, 2H), 4.55 (dd, *J* = 8.68, 7.35 Hz, 2H), 4.47 (d, *J* = 10.68 Hz, 2H), 4.39-4.29 (m, 1H), 4.24 (td, *J* = 7.80, 7.00 Hz, 1H), 4.07 (dd, *J* = 25.33, 9.26 Hz, 1H), 4.00 (dd, *J* = 7.87, 4.05 Hz, 1H), 3.06 (d, *J* = 33.77 Hz, 1H), 2.46-2.19 (m, 3H), 1.93-1.80 (m, 1H), 1.79-1.59 (m, 5H), 1.49-1.36 (m, 2H), 1.31 (dd, *J* = 17.67, 2.67 Hz, 1H), 1.07 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.9, 171.5, 156.2, 135.7, 135.7, 132.9, 132.8, 132.2, 129.9, 129.9, 127.8, 127.7, 118.1, 117.1, 79.4, 76.4, 74.2, 65.0, 40.8, 30.9, 30.7, 26.9, 19.2.



6.2c

(S)-((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-3-((tert-butyldiphenylsilyl)oxy) tetrahydrofuran-2-yl)methyl 2-(((allyloxy)carbonyl)amino)-3-phenylpropanoate (6.2c) :

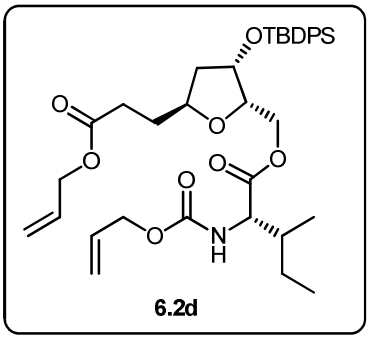
Molecular Formula: $C_{40}H_{49}NO_8Si$

R_f (solvent system): 0.5 (40%, EtOAc/hexane)

Yield: 73%

LRMS: (ES+) *m/z* = 700.3(M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72-7.62 (m, 4H), 7.52-7.36 (m, 6H), 7.32-7.21 (m, 3H), 7.16 (d, *J* = 7.6 Hz, 2H), 5.97-5.83 (m, 2H), 5.37-5.17 (m, 5H), 4.71 (dd, *J* = 13.94, 5.96 Hz, 1H), 4.59-4.53 (m, 4H), 4.48 (d, *J* = 10.59 Hz, 1H), 4.45-4.40 (m, 1H), 4.38 (dd, *J* = 11.53, 3.82 Hz, 1H), 4.30 (dt, *J* = 12.69, 6.16 Hz, 1H), 3.98 (td, *J* = 7.68, 3.98 Hz, 1H), 3.14 (dq, *J* = 13.94, 5.90 Hz, 2H), 2.50-2.30 (m, 2H), 1.93-1.85 (m, 1H), 1.82-1.74 (m, 2H), 1.49-1.40 (m, 1H), 1.09 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm: 172.9, 171.4, 155.4, 135.8, 135.7, 133.7, 132.8, 132.6, 132.2, 130.0, 130.0, 129.4, 128.5, 127.9, 127.7, 127.0, 118.2, 117.7, 79.4, 74.3, 65.7, 65.1, 65.0, 54.7, 40.9, 38.1, 30.8, 30.7, 26.9, 19.3.



6.2d

(2S, 3S)-((2S, 3S, 5S)-5-(3-(allyloxy)-3-oxopropyl)-3-((tert-butyldiphenylsilyl)oxy) tetrahydrofuran-2-yl)methyl 2-(((allyloxy)carbonyl)amino)-3-methylpentanoate (6.2d) :

Molecular Formula: $C_{37}H_{51}NO_8Si$

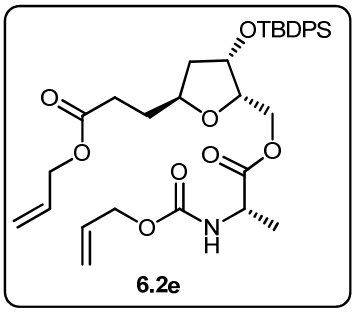
R_f (solvent system): 0.5 (40%, EtOAc/hexane)

Yield: 73%

LRMS: (ES+) *m/z* = 666.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64 (d, *J* = 7.79 Hz, 4H), 7.46-7.36 (m, 6H), 5.98-5.82 (m, 2H), 5.35-5.24 (m, 3H), 5.22 (t, *J* = 9.48 Hz, 2H), 4.55 (dd, *J* = 7.61, 6.46 Hz, 4H), 4.49 (s, 1H), 4.40 (ddd, *J* = 14.07, 11.46, 6.04 Hz, 3H), 4.30-4.21 (m, 1H), 4.00 (td, *J* = 7.81, 4.00 Hz, 1H), 2.46-2.26 (m, 2H), 1.94-1.82 (m, 2H), 1.77-1.67

(m, 2H), 1.47-1.38 (m, 2H), 1.23-1.13 (m, 1H), 1.07 (s, 9H), 0.95-0.89 (m, 6H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.9, 171.9, 155.8, 135.7, 135.7, 133.7, 132.8, 132.7, 132.2, 130.0, 129.9, 127.8, 127.7, 118.1, 117.7, 79.4, 76.6, 74.3, 65.7, 65.0, 64.6, 58.4, 40.8, 38.1, 30.8, 30.7, 26.9, 24.8, 19.2, 15.4, 11.6.



6.2e

allyl3-((2S,4S,5S)-5-((((S)-2-(((allyloxy)carbonyl)amino)propanoyl)oxy)methyl)-4-((tert-butyldiphenylsilyl)oxy)tetrahydrofuran-2-yl)propanoate (6.2e) :

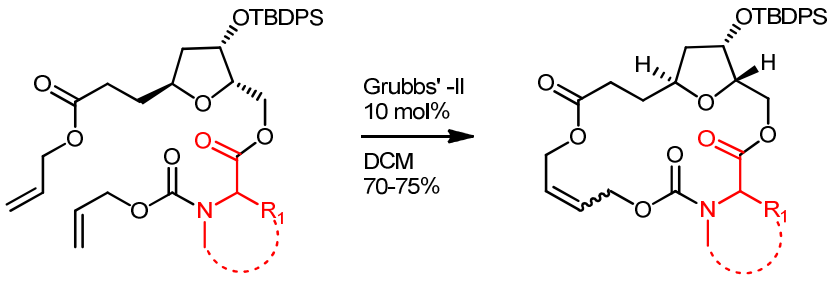
Molecular Formula: $C_{34}H_{45}NO_8Si$

R_f (solvent system): 0.5 (40%, EtOAc/hexane)

Yield: 70%

LRMS: (ES+) *m/z* = 624.2 (M+1)

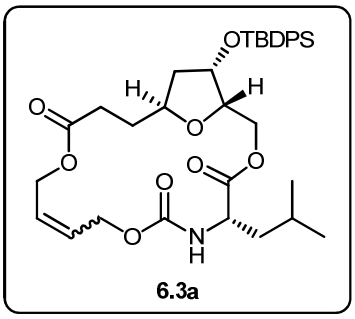
^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.66 (dd, *J* = 11.97, 5.46 Hz, 4H), 7.50-7.34 (m, 6H), 5.99-5.82 (m, 2H), 5.39 (d, *J* = 7.33 Hz, 1H), 5.35-5.18 (m, 4H), 4.56 (dd, *J* = 7.88, 6.53 Hz, 4H), 4.49 (dd, *J* = 5.33, 3.02 Hz, 1H), 4.41 (ddd, *J* = 21.57, 11.44, 4.10 Hz, 3H), 4.27 (dt, *J* = 12.61, 6.16 Hz, 1H), 4.05-3.98 (m, 1H), 2.47-2.28 (m, 2H), 1.87 (ddd, *J* = 13.03, 5.84, 1.87 Hz, 1H), 1.79-1.71 (m, 2H), 1.41 (d, *J* = 7.15 Hz, 4H), 1.07 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm: 172.9, 172.9, 155.4, 135.7, 135.7, 133.7, 132.8, 132.6, 132.2, 130.0, 129.9, 127.8, 127.7, 118.1, 117.7, 79.4, 76.7, 74.2, 65.7, 65.1, 64.7, 49.6, 40.8, 30.7, 30.7, 26.9, 19.3, 18.7.



6.2(a-e) **6.3 (a-e)**

To a solution of **6.2(a-e)** (1eq) in dry DCM under nitrogen atmosphere added Grubbs' 2nd generation catalyst (10 mol%) and reaction mixture was allowed to stirred for 2 h

at 40 ^{0}C. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **6.3(a-e)**



6.3a

Molecular Formula: $C_{35}H_{47}NO_8Si$

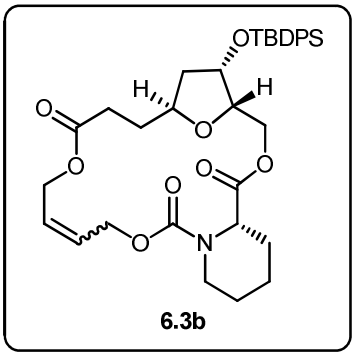
R_f (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 70%

LRMS: (ES+) *m/z* = 638.3 (M+1).

HRMS: (ESI) calcd. for $[C_{35}H_{47}NO_8Si + H]^+$: 638.3149, found: 638.3149.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.66-7.61 (m, 4H), 7.48-7.35 (m, 6H), 5.76 (s, 2H), 5.12-5.00 (m, 1H), 4.88-4.73 (m, 1H), 4.67-4.56 (m, 1H), 4.55-4.43 (m, 2H), 4.42-4.33 (m, 1H), 4.30-4.26 (m, 2H), 4.08-3.98 (m, 1H), 2.49-2.19 (m, 2H), 1.99-1.79 (m, 2H), 1.79-1.68 (m, 2H), 1.53 (td, *J* = 13.10, 6.62 Hz, 3H), 1.06 (s, 9H), 0.95 (d, *J* = 6.44 Hz, 7H), 0.86 (s, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.8, 135.7, 133.6, 132.9, 130.0, 129.9, 127.8, 127.7, 126.6, 77.2, 64.4, 63.2, 52.9, 41.1, 40.0, 30.2, 26.9, 24.7, 23.0, 21.4, 19.2.



6.3b

Molecular Formula: $C_{35}H_{45}NO_8Si$

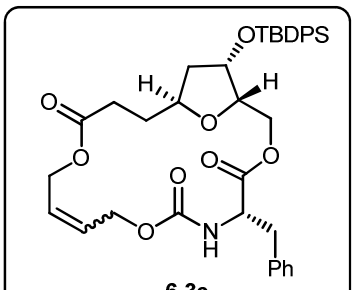
R_f (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 70%

LRMS: (ES+) *m/z* = 636.2 (M+1).

HRMS: (ESI) calcd. for $[C_{35}H_{45}NO_8Si + H]^+$: 636.2992, found: 636.2992.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.66-7.62 (m, 4H), 7.47-7.36 (m, 6H), 5.77 (t, *J* = 2.88 Hz, 2H), 4.88 (dd, *J* = 32.49, 8.85 Hz, 2H), 4.60 (s, 1H), 4.57-4.46 (m, 2H), 4.30 (s, 1H), 4.28-4.14 (m, 2H), 4.10 (dd, *J* = 16.34, 9.05 Hz, 2H), 3.15 (s, 1H), 2.42 (d, *J* = 12.24 Hz, 1H), 2.32-2.21 (m, 1H), 2.15 (s, 1H), 1.94-1.86 (m, 1H), 1.86-1.76 (m, 1H), 1.76-1.64 (m, 4H), 1.64-1.56 (m, 1H), 1.56-1.46 (m, 1H), 1.45-1.32 (m, 2H), 1.06 (s, 9H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm : 172.7, 172.0, 155.6, 135.7, 133.5, 132.9, 130.0, 129.9, 128.1, 127.8, 127.7, 126.0, 78.9, 75.3, 73.6, 64.4, 64.2, 63.3, 54.2, 41.7, 39.7, 30.6, 30.3, 26.9, 26.6, 24.5, 20.2, 19.1.



6.3c

Molecular Formula: $C_{38}H_{45}NO_8Si$

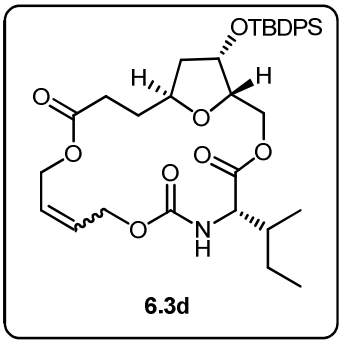
R$_f$ (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 71%

LRMS: (ES+) *m/z* = 672.2 (M+1).

HRMS: (ESI) calcd. for $[C_{38}H_{45}NO_8Si + H]^+$: 672.2992, found: 672.2992.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm : 7.68-7.61 (m, 4H), 7.49-7.36 (m, 6H), 7.34-7.23 (m, 3H), 7.17 (d, *J* = 6.42 Hz, 2H), 5.82-5.66 (m, 2H), 4.61 (s, 4H), 4.47 (ddd, *J* = 28.37, 14.03, 6.85 Hz, 3H), 4.38-4.24 (m, 2H), 4.04-3.93 (m, 1H), 3.25-2.81 (m, 2H), 2.52-2.18 (m, 2H), 1.99-1.76 (m, 3H), 1.53 (dd, *J* = 12.97, 6.52 Hz, 2H), 1.08 (s, 9H); **^{13}C NMR** (100 MHz, *CDCl$_3$*) δ ppm : 172.8, 171.1, 135.7, 133.5, 132.9, 130.0, 129.9, 129.2, 129.1, 128.7, 127.8, 127.7, 126.6, 73.7, 64.5, 63.3, 60.3, 53.4, 37.7, 29.7, 26.9, 21.0, 19.2, 14.2.



6.3d

Molecular Formula: $C_{35}H_{47}NO_8Si$

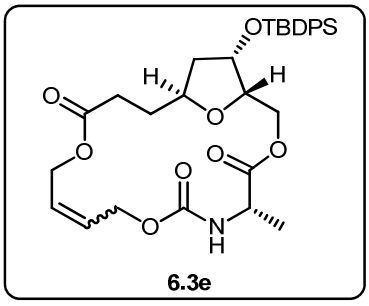
R_f (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 71%

LRMS: (ES+) *m/z* = 638.3 (M+1).

HRMS: (ESI) calcd. for $[C_{35}H_{47}NO_8Si + H]^+$: 638.3149, found: 638.3149.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.67-7.61 (m, 4H), 7.42 (qd, *J* = 14.32, 7.11 Hz, 6H), 5.76 (s, 2H), 5.23-5.03 (m, 1H), 4.91-4.76 (m, 1H), 4.69-4.41 (m, 3H), 4.26 (dd, *J* = 6.71, 4.27 Hz, 3H), 4.19-3.94 (m, 2H), 2.52-2.18 (m, 2H), 2.01-1.86 (m, 2H), 1.72 (s, 1H), 1.53 (dd, *J* = 13.03, 6.67 Hz, 2H), 1.20-1.13 (m, 1H), 1.07 (s, 9H), 0.96 (d, *J* = 6.44 Hz, 3H), 0.91 (dd, *J* = 13.96, 6.57 Hz, 5H), **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.7, 135.6, 133.5, 132.9, 130.0, 129.9, 127.8, 127.7, 59.0, 53.4, 31.9, 29.6, 29.6, 29.3, 26.8, 24.7, 24.7, 22.6, 19.1, 15.7, 14.1.



6.3e

Molecular Formula: $C_{32}H_{41}NO_8Si$

R_f (solvent system): 0.2 (60%, EtOAc/hexane)

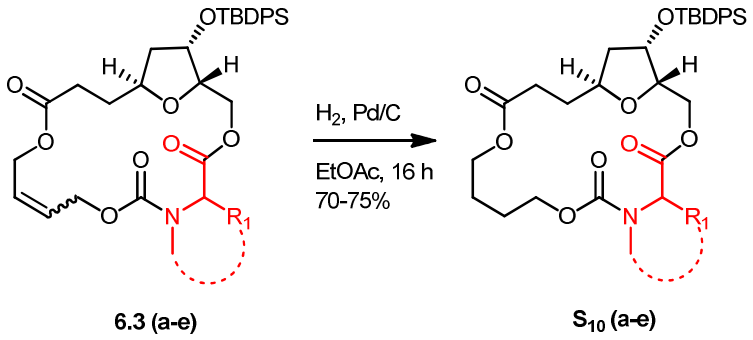
Yield: 71%

LRMS: (ES+) *m/z* = 596.2 (M+1).

HRMS: (ESI) calcd. for $[C_{32}H_{41}NO_8Si + H]^+$: 596.2679, found: 596.2679.

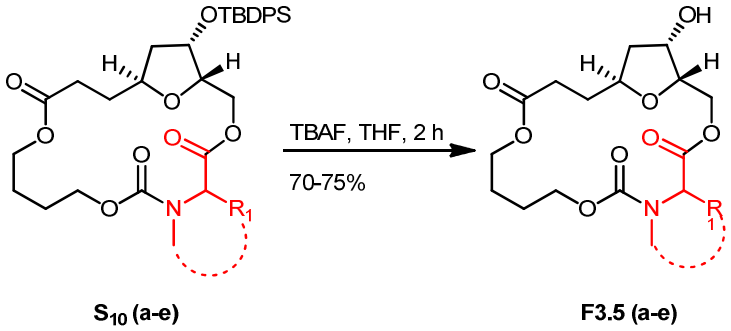
^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.63 (dd, *J* = 6.56, 1.41 Hz, 4H), 7.47-7.35 (m, 6H), 5.77 (s, 2H), 5.26-4.60 (m, 3H), 4.55-4.49 (m, 1H), 4.46 (dd, *J* = 11.82, 8.06 Hz, 1H), 4.41-4.13 (m, 4H), 4.07-3.98 (m, 1H), 2.51-2.17 (m, 2H), 1.98-1.76 (m, 3H), 1.53 (dd, *J* = 13.56, 6.72 Hz, 2H), 1.39 (s, 3H), 1.06 (s, 9H); **^{13}C NMR** (100 MHz,

$CDCl_3$) δ ppm : 172.9, 171.1, 152.9, 135.7**,** 133.6, 132.9, 130.0, 129.9, 127.8, 127.7, 126.6, 73.7, 64.4, 63.4, 60.4, 53.4, 50.0, 29.7, 26.9, 21.0, 19.2, 14.2.



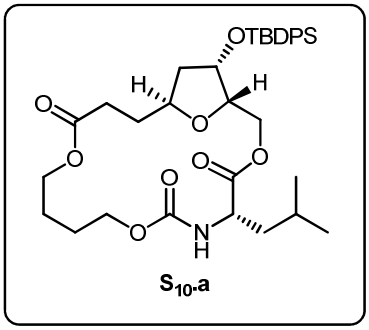
6.3 (a-e) **S_{10} (a-e)**

To a solution of **6.3 (a-e)** (1eq) in ethyl acetate under hydrogen atmosphere added palladium charcoal (10 mol%) and reaction mixture was allowed to stirred for 16 h. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **S_{10}(a-e).**



S_{10} (a-e) **F3.5 (a-e)**

To a solution of **S_{10} (a-e)** (1 eq) in THF (15 mL) was added TBAF (1.0 M in THF, 2 eq) at 0 ^{o}C. The mixture was allowed to stand at room temperature for 4 h, then EtOAc (20 ml), H_2O (10 mL) and saturated aqueous NaCl (5 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2×20 mL). The organic extracts were combined, dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography on silica gel (EtOAc) to give the title compound **F3.5(a-e).**



S_{10}.a

(1S,5S,17S,19S)-19-((tert-butyldiphenylsilyl)oxy)-5-isobutyl-3,8,13,20-tetraoxa-6-azabicyclo[15.2.1]icosane-4,7,14-trione (S_{10}.a) :

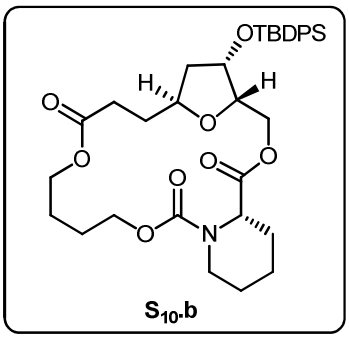
Molecular Formula: $C_{35}H_{49}NO_8Si$

R_f (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 71%

LRMS: (ES+) *m/z* = 640.3 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.69-7.58 (m, 4H), 7.49-7.34 (m, 6H), 5.12-4.98 (m, 0.6H), 4.75-4.64 (m, 0.3H), 4.42-4.53 (m, 2H), 4.43-4.33 (m, 1H), 4.34-4.17 (m, 3H), 4.10-4.02 (m, 1H), 3.97 (dd, *J* = 2.96, 0.76 Hz, 2H), 3.87-3.75 (m, 1H), 2.45-2.32 (m, 1H), 2.28 (d, *J* = 7.14 Hz, 1H), 1.85 (ddd, *J* = 12.92, 6.50, 3.27 Hz, 2H), 1.57-1.45 (m, 3H), 1.70 (s, 6H), 1.06 (s, 9H), 0.95 (d, *J* = 6.39 Hz, 6H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm: 173.5, 172.9, 156.3, 135.7, 133.6, 132.8, 130.0, 129.9, 127.9, 127.7, 79.2, 75.5, 74.2, 64.7, 64.0, 52.8, 41.3, 40.1, 30.0, 26.9, 25.4, 24.7, 23.0, 21.5, 19.2.



S_{10}.b

Molecular Formula: $C_{35}H_{47}NO_8Si$

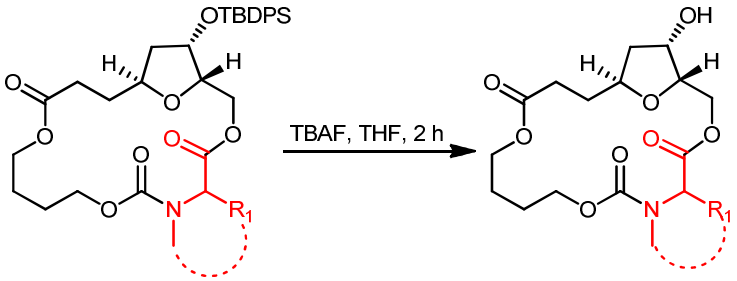
R_f (solvent system): 0.2 (60%, EtOAc/hexane)

Yield: 71%

LRMS: (ES+) *m/z* = 638.3 (M+1)

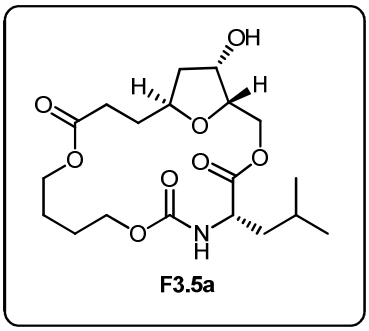
^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.63 (d, *J* = 7.65 Hz, 4H), 7.41 (ddd, *J* = 14.42, 8.27, 4.39 Hz, 6H), 4.86-4.78 (m, 1H), 4.60-4.46 (m, 2H), 4.36-4.18 (m, 2H), 4.12 (d,

J = 12.25 Hz, 2H), 4.05 (s, 2H), 3.95-3.85 (m, 1H), 3.12-3.01 (m, 1H), 2.46-2.33 (m, 1H), 2.32-2.22 (m, 1H), 2.22-2.11 (m, 1H), 1.92-1.78 (m, 2H), 1.68 (d, *J* = 4.37 Hz, 9H), 1.49 (s, 3H), 1.06 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.3, 171.9, 156.1, 135.7, 135.7, 133.6, 132.9, 130.0, 129.9, 127.8, 127.7, 79.3, 75.8, 74.0, 65.3, 64.8, 63.9, 54.3, 41.6, 40.1, 30.9, 30.8, 26.9, 26.7, 25.9, 25.5, 24.6, 20.4, 19.2.



S_{10} (a-e) **F3.5 (a-e)**

To a solution of **S_{10} (a-e)** (1 eq) in THF (15 mL) was added TBAF (1.0 M in THF, 2 eq) at 0 ^{o}C. The mixture was allowed to stand at room temperature for 4 h, then EtOAc (20 ml), H_2O (10 mL) and saturated aqueous NaCl (5 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2×20 mL). The organic extracts were combined, dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography on silica gel (EtOAc) to give the title compound **F3.5(a-e).**



F3.5a

(1S,5S,17S,19S)-19-hydroxy-5-isobutyl-3,8,13,20-tetraoxa-6-azabicyclo[15.2.1] icosane-4,7,14-trione (F3.5a) :

Molecular Formula: $C_{19}H_{31}NO_8$

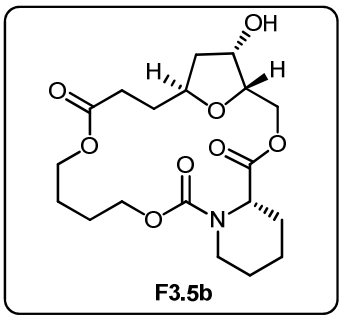
R_f (solvent system): 0.3 (5%, MeOH/DCM)

Yield: 70%

LRMS: (ES+) *m/z* = 402.2 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 5.49-5.30 (m, 0.7H), 5.09-4.97 (m, 0.2H), 4.85-4.62 (m, 1H), 4.53-4.42 (m, 1H), 4.39-4.18 (m, 3H), 4.18-4.03 (m, 2H), 4.02-3.78 (m,

2H), 3.76-3.56 (m, 2H), 2.72-2.59 (m, 1H), 2.44-2.31 (m, 1H), 2.12-1.98 (m, 4H), 1.72 (d, *J* = 2.54 Hz, 5H), 1.64-1.53 (m, 2H), 0.98-0.90 (m, 6H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.4, 172.7, 156.2, 81.4, 76.2, 64.1, 60.4, 53.2, 52.7, 39.8, 39.5, 31.4, 27.0, 26.0, 23.0, 22.9, 21.5, 21.3.



F3.5b

Molecular Formula: $C_{19}H_{29}NO_8$

R_f (solvent system): 0.3 (5%, MeOH/DCM)

Yield: 70%

LRMS: (ES+) *m/z* = 400.1 (M+1)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 4.98-4.79 (m, 1H), 4.76-4.46 (m, 1H), 4.33 (d, *J* = 6.48 Hz, 5H), 4.06 (d, *J* = 6.27 Hz, 6H), 2.93 (s, 1H), 2.48-2.20 (m, 3H), 2.03 (s, 2H), 1.97-1.78 (m, 6H), 1.49-1.37 (m, 2H), 1.27 (d, *J* = 1.52 Hz, 1H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 173.4, 172.7, 156.2, 81.4, 79.7, 76.2, 64.5, 64.1, 60.4, 53.2, 52.7, 39.7, 39.5, 31.4, 31.3, 27.0, 26.0, 24.7, 23.0, 22.9, 21.5.

2.4.7 References

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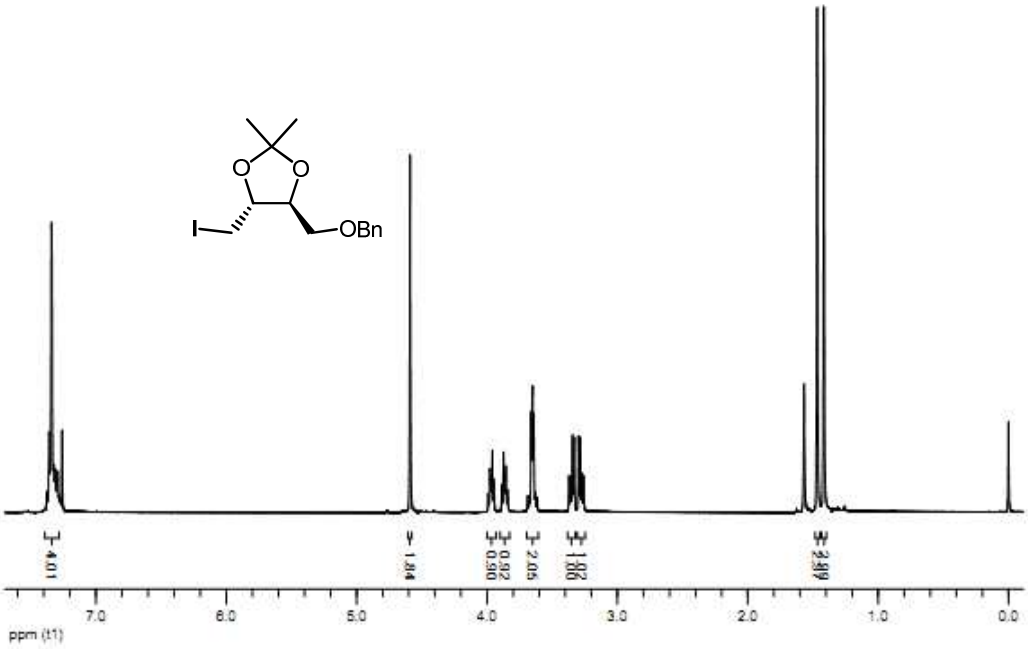
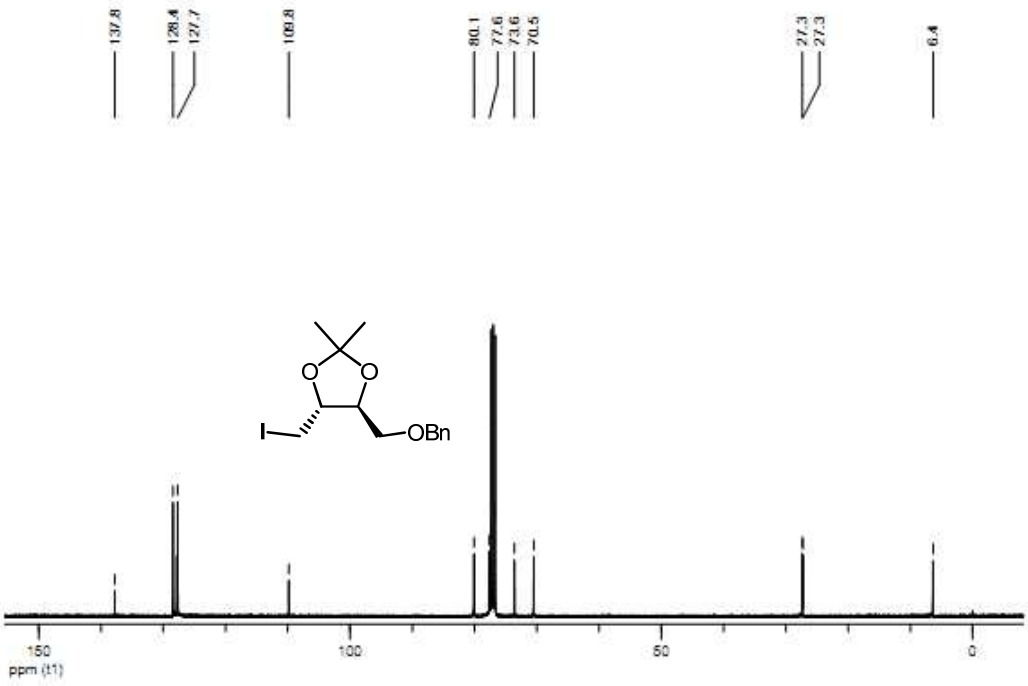
2.4.8 Spectral data

^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



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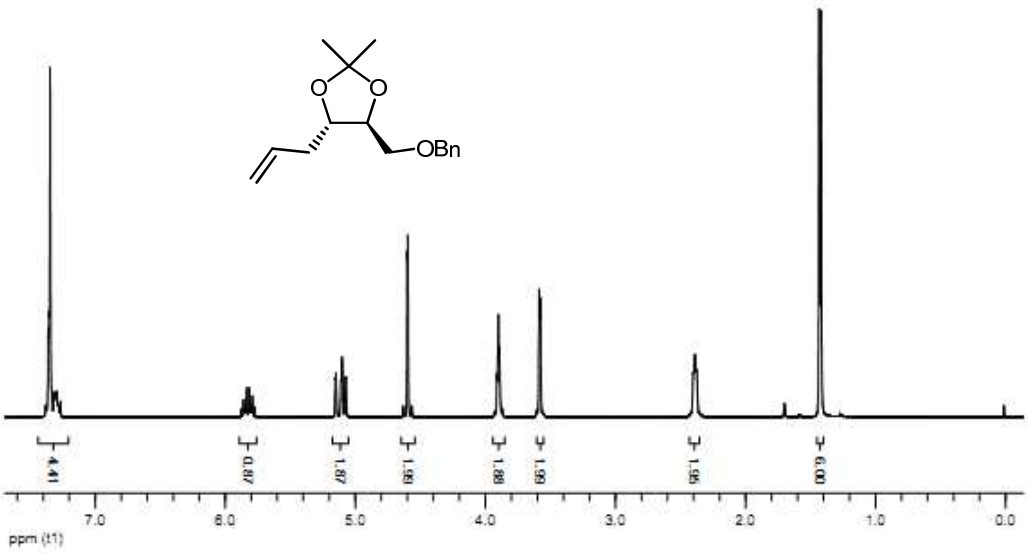

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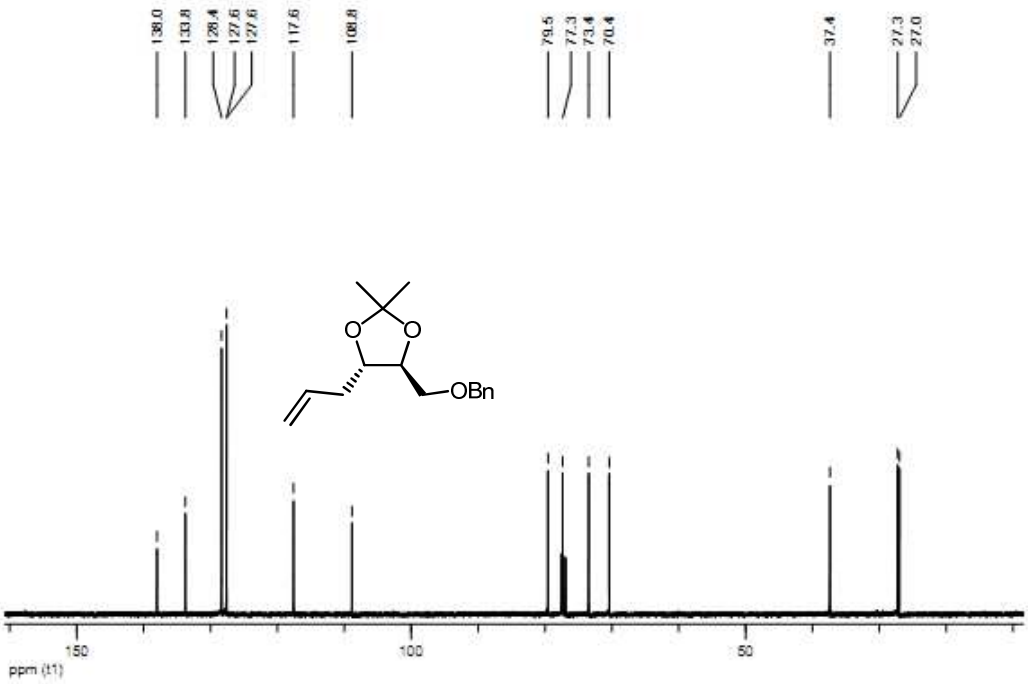

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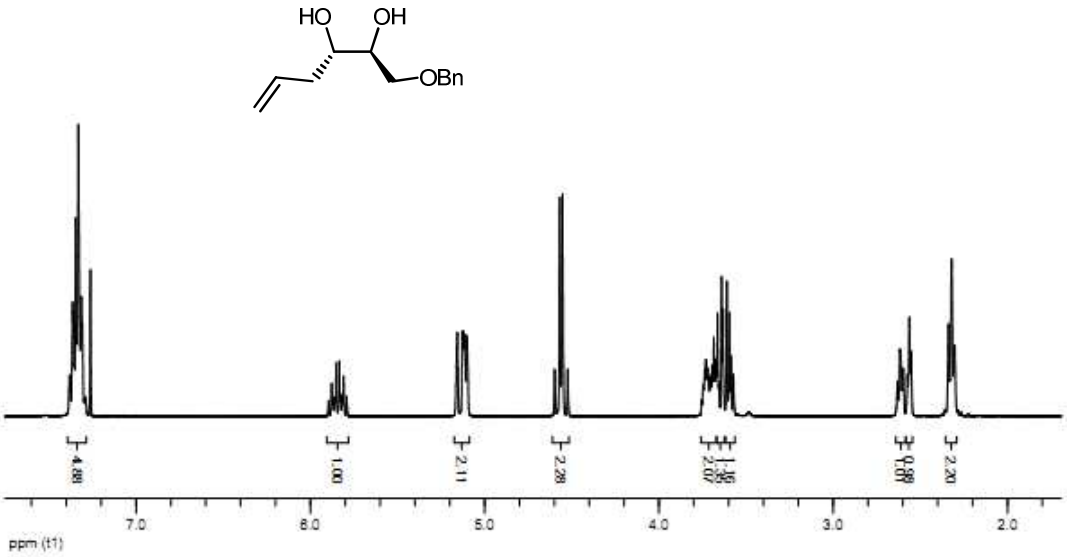

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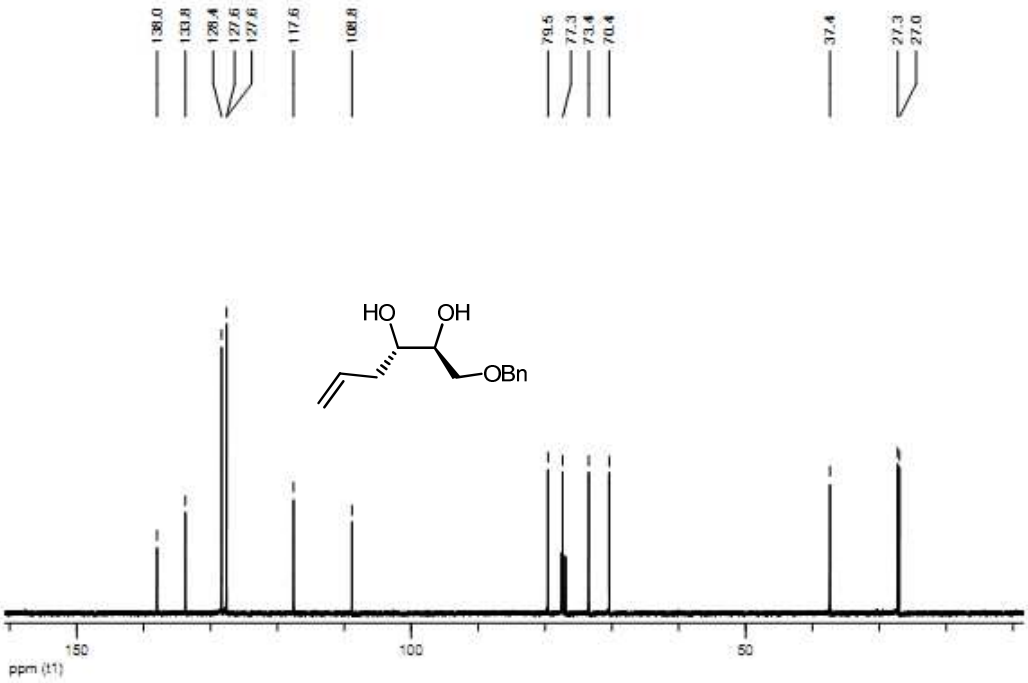

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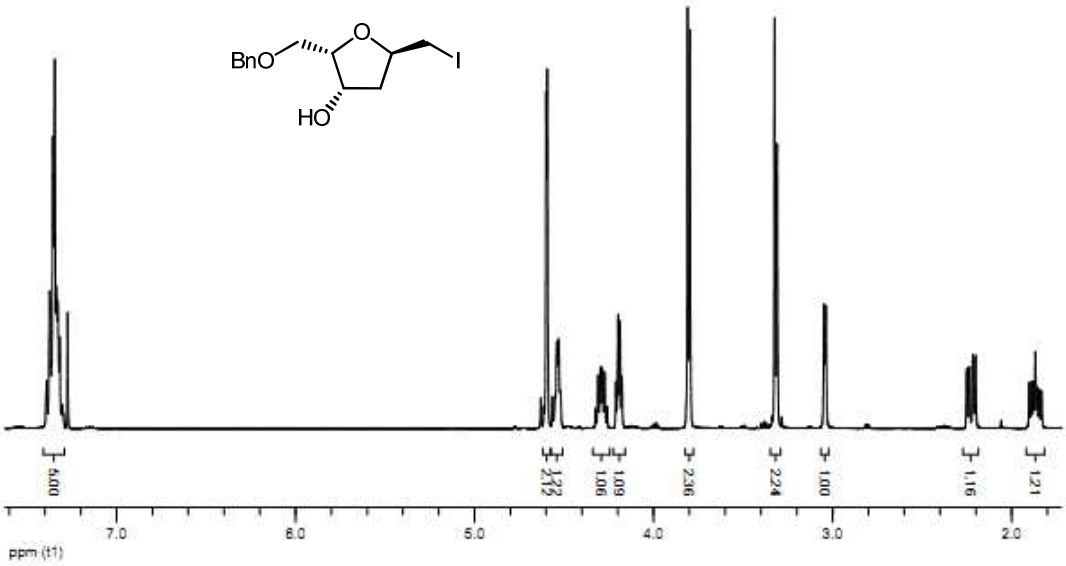

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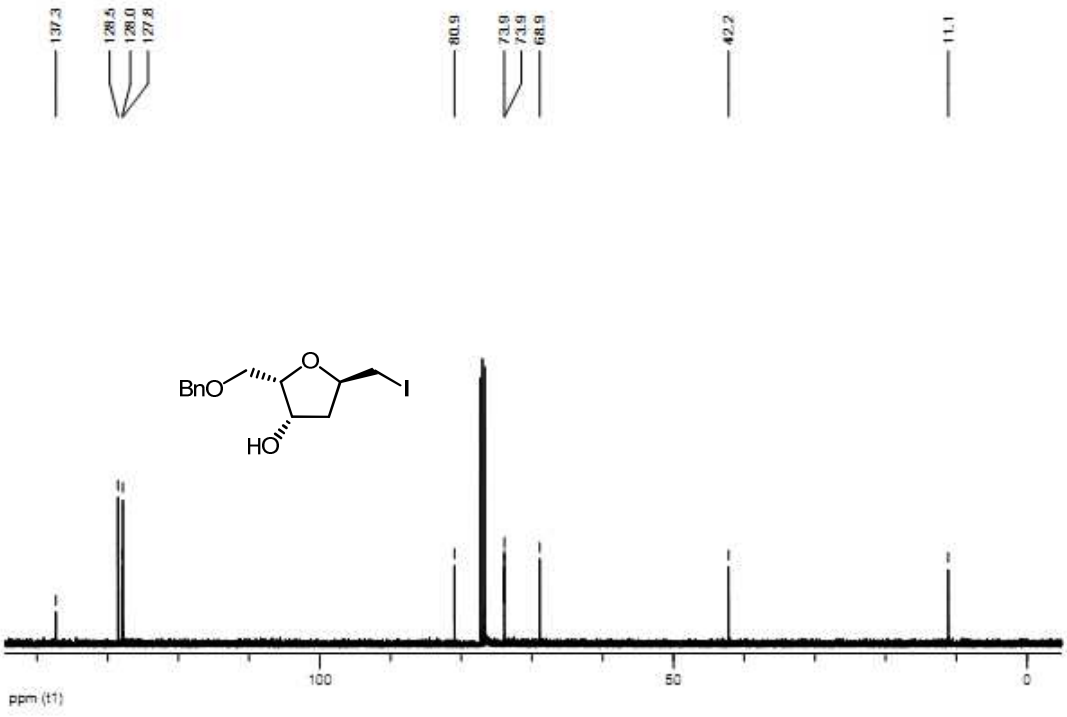
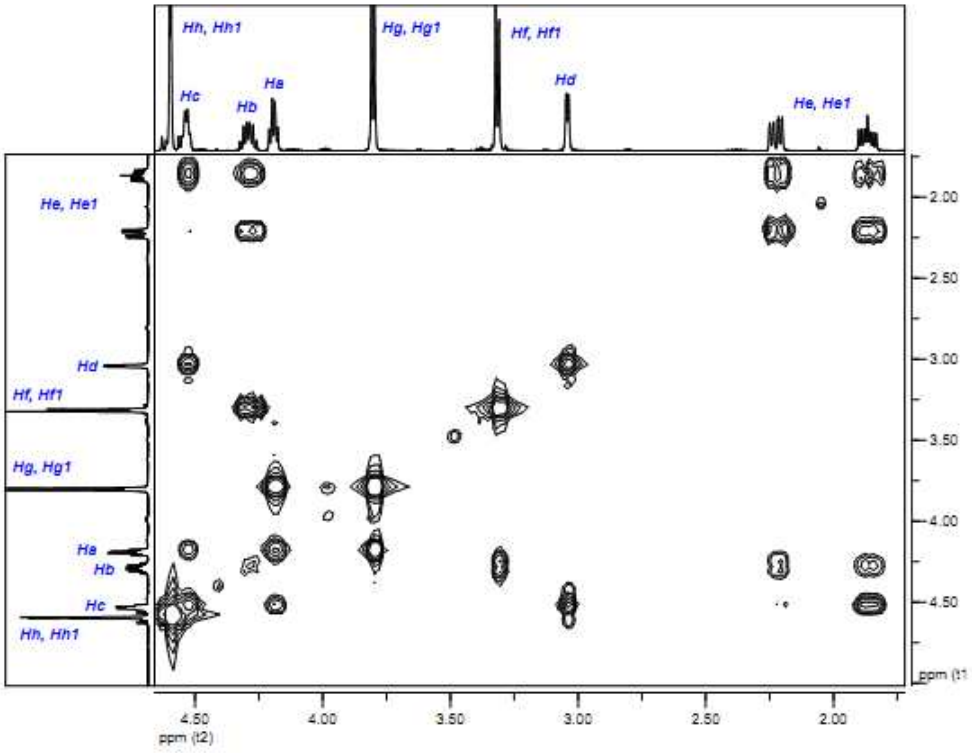
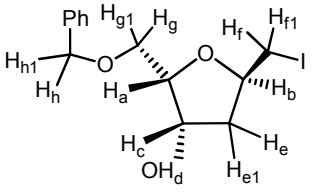
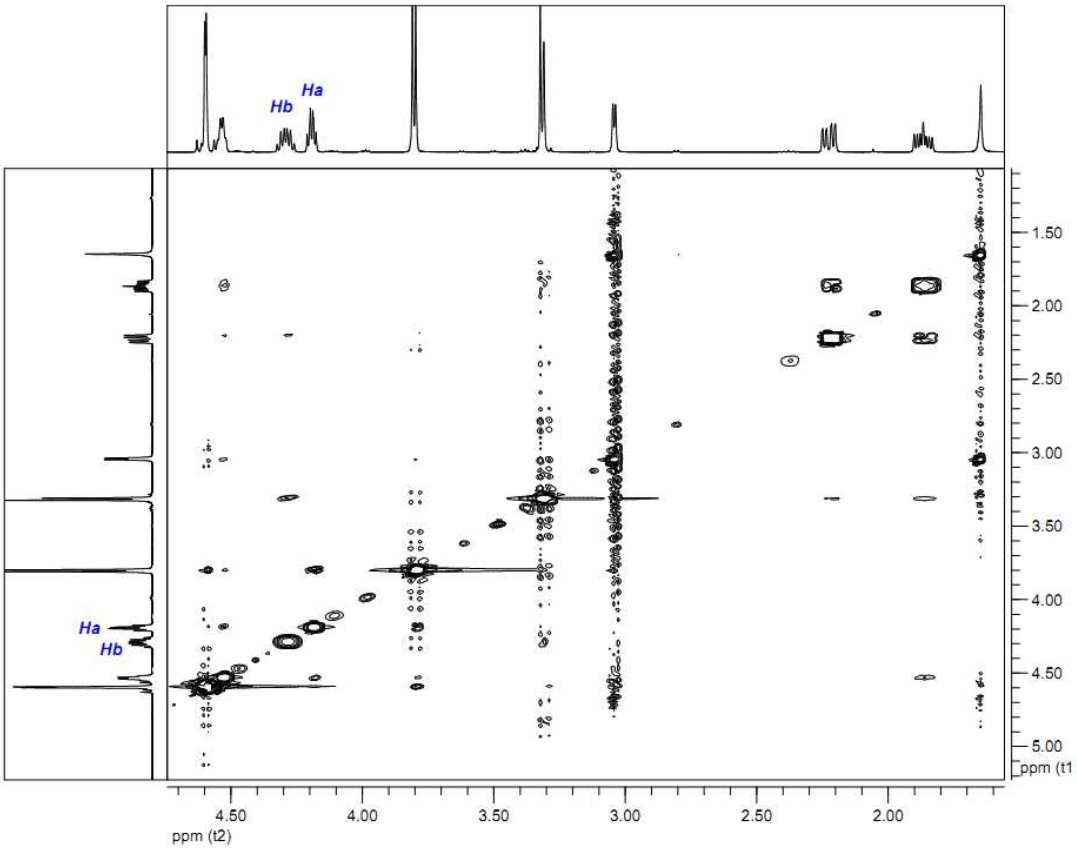


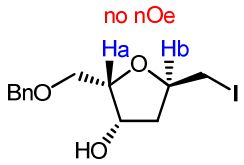
^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)



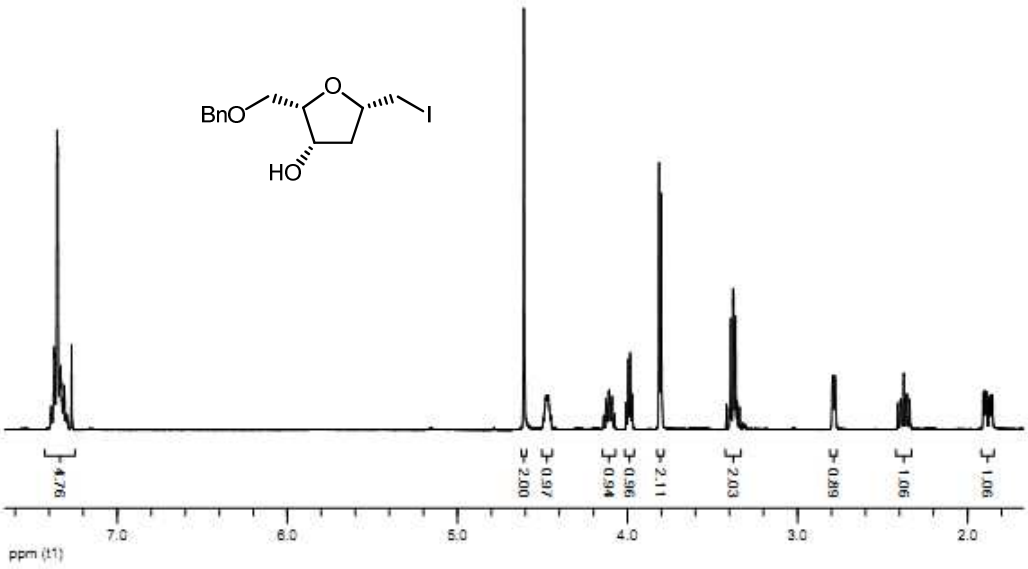


2D NOE (400 MHz, $CDCl_3$)

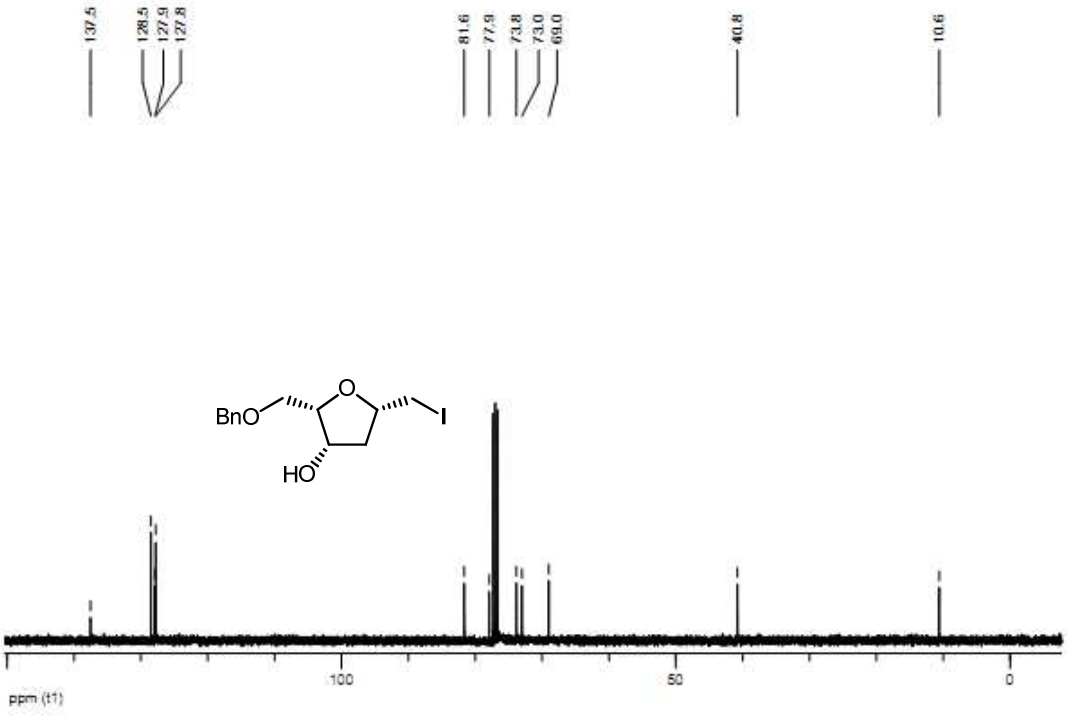




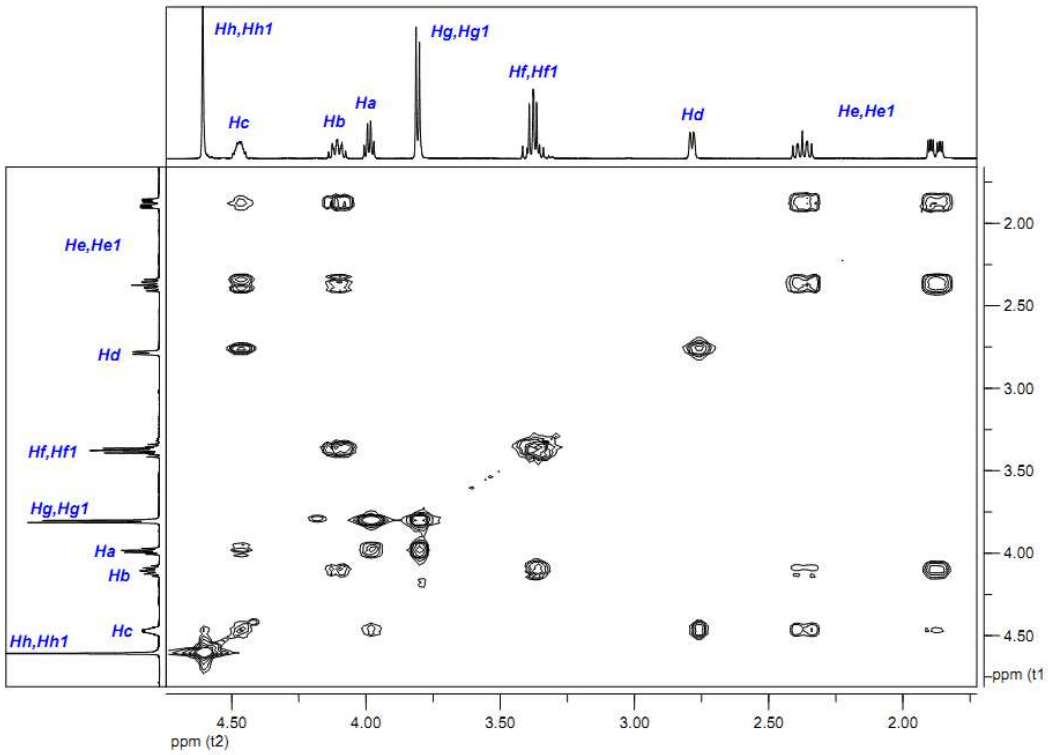
^{1}H NMR ($CDCl_3$, 400MHz)

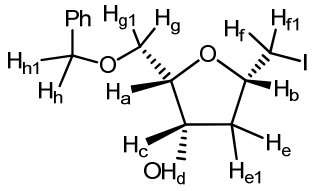


^{13}C NMR ($CDCl_3$, 100MHz)

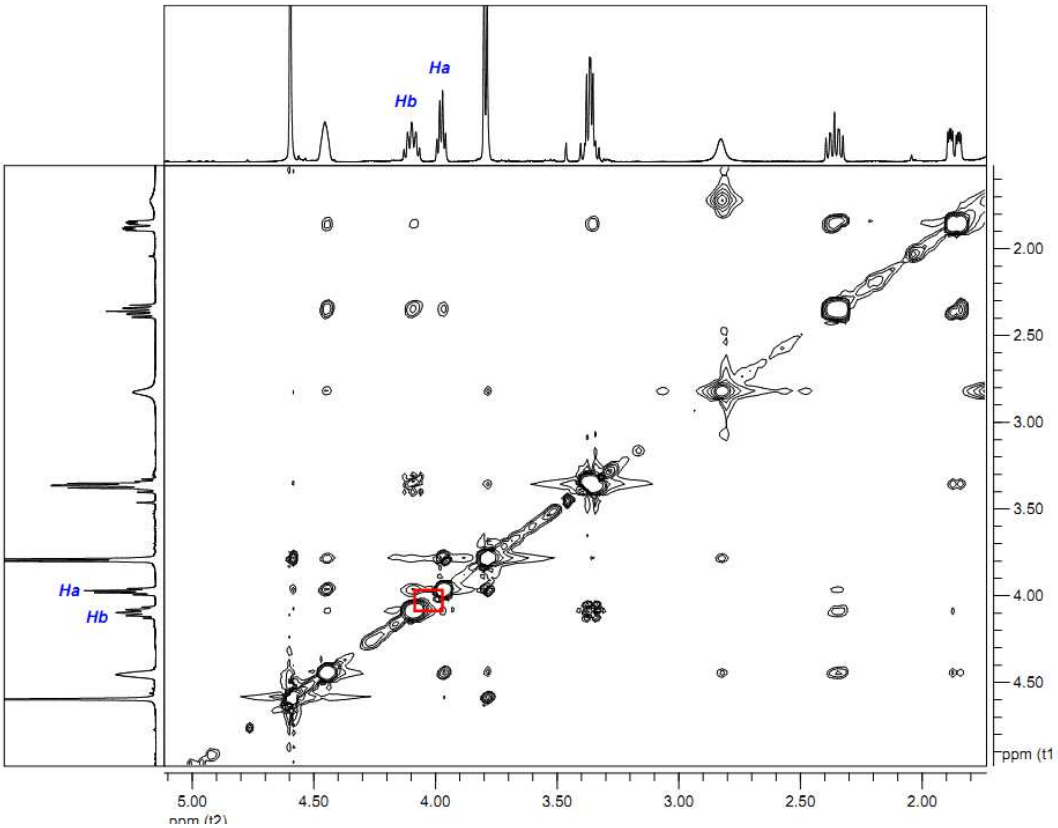


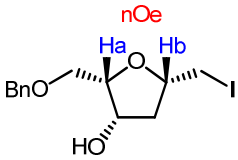
^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)



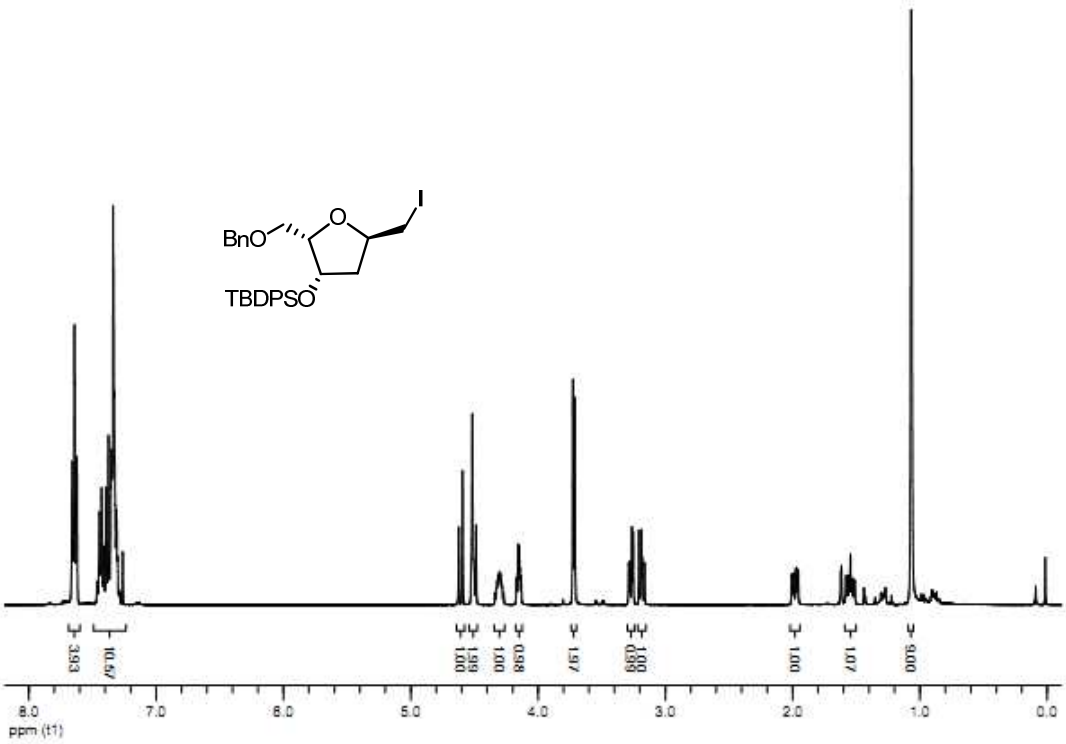


2D NOE (400 MHz, $CDCl_3$)

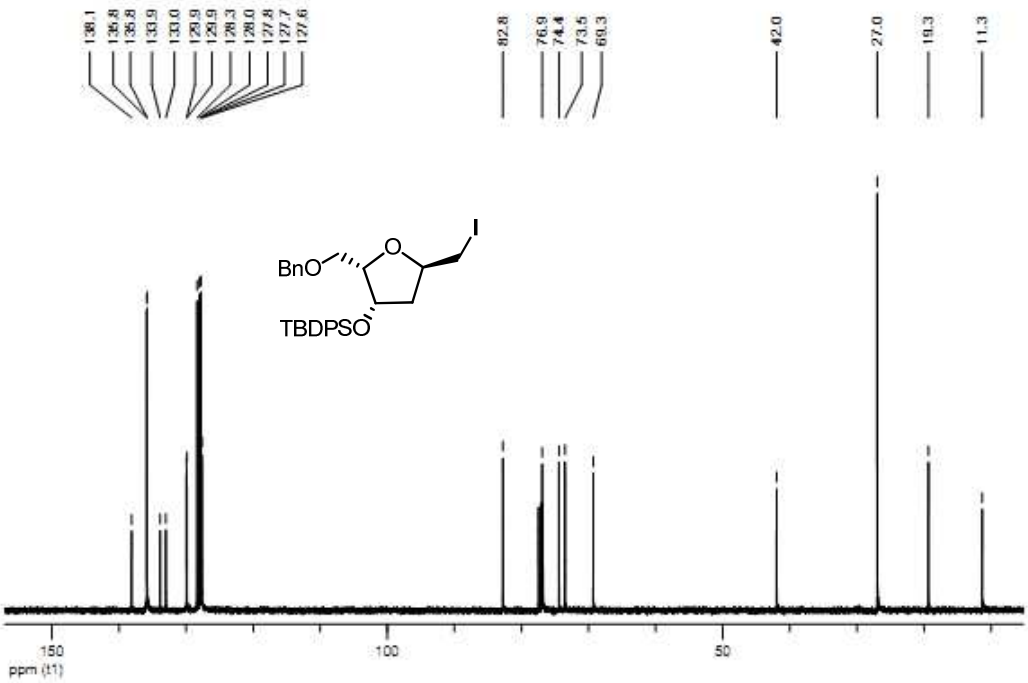




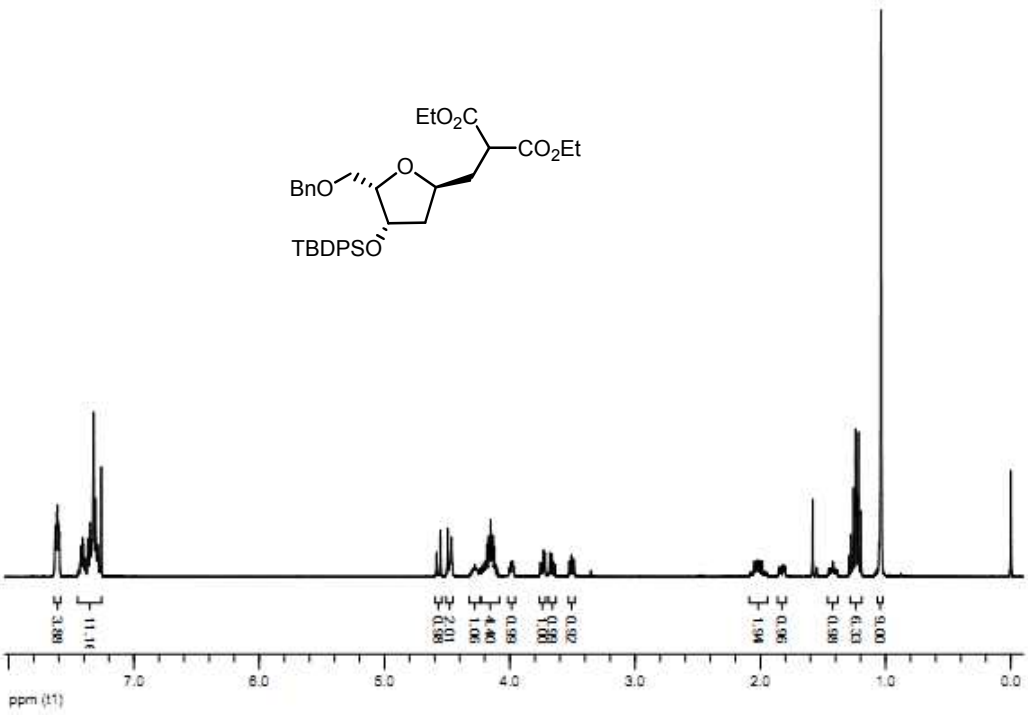
^{1}H NMR ($CDCl_3$, 400MHz)



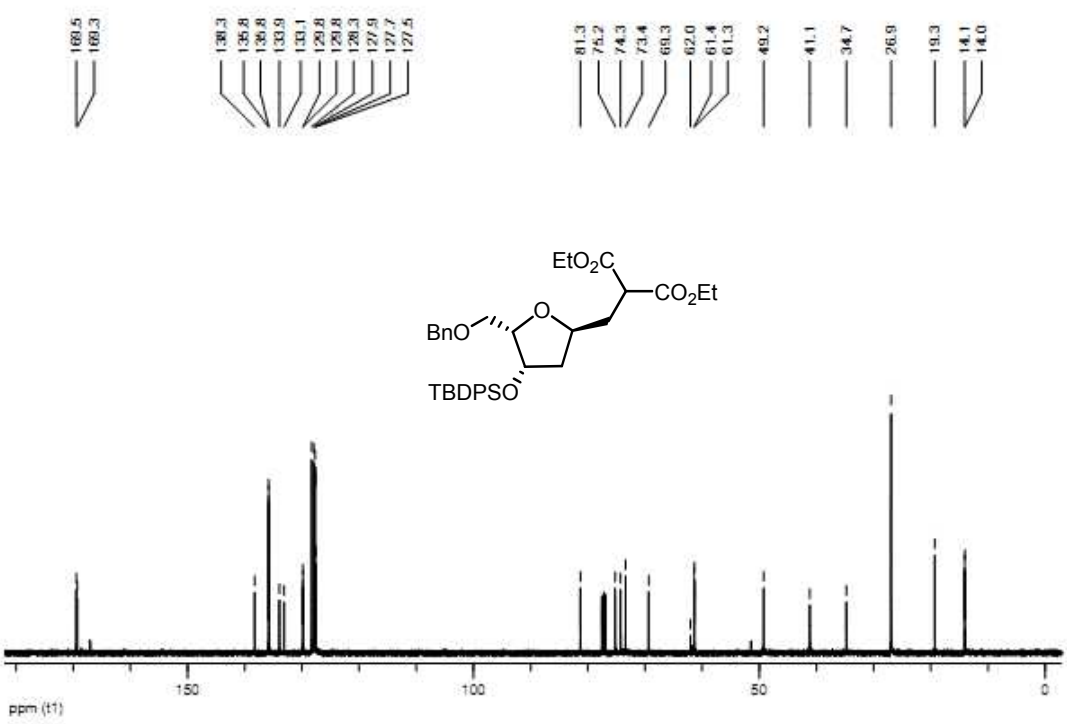
^{13}C NMR ($CDCl_3$, 100MHz)



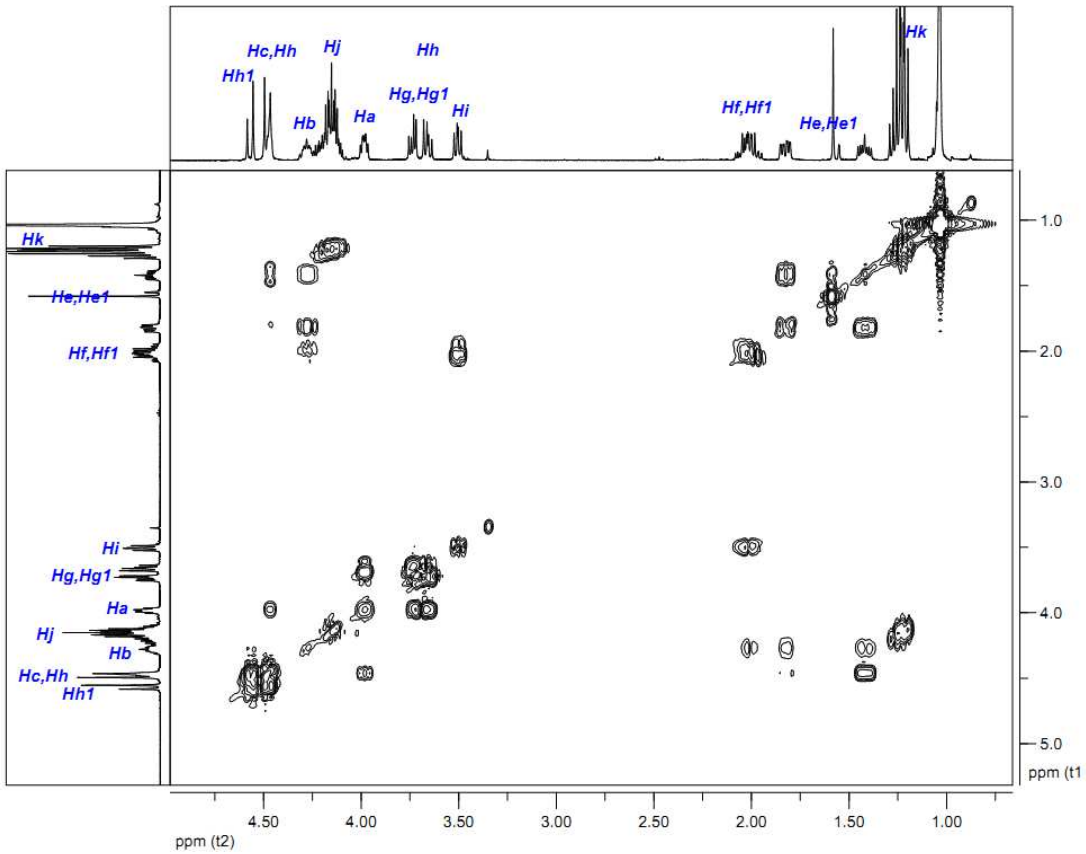
^{1}H NMR ($CDCl_3$, 400MHz)

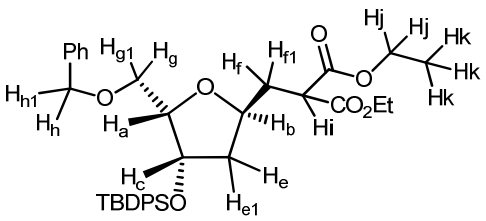


^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)





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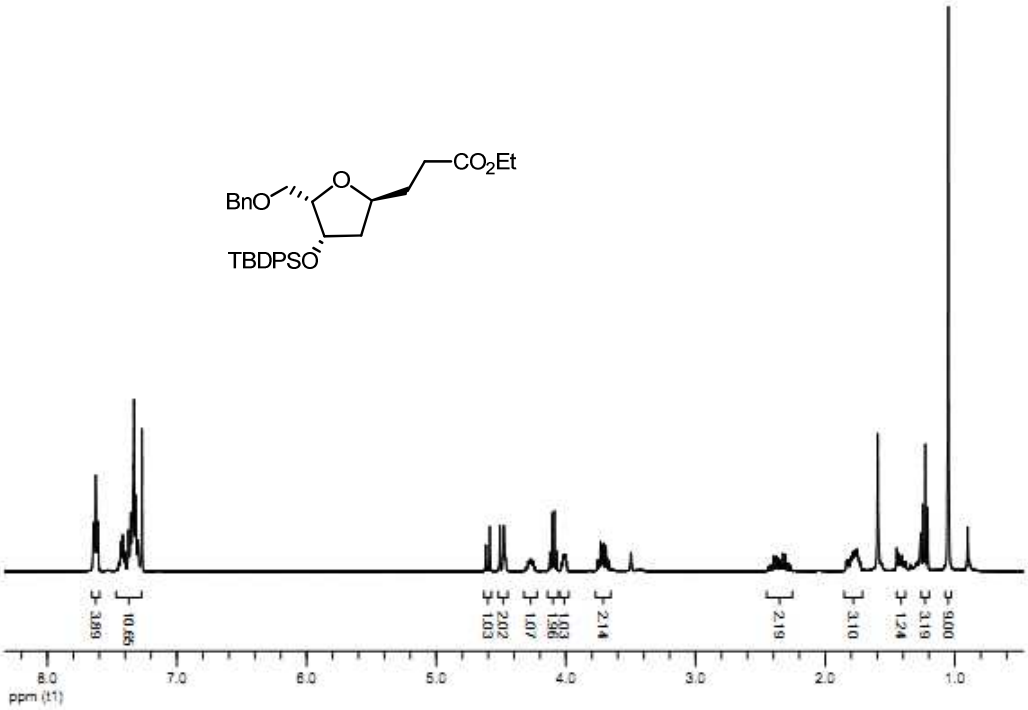
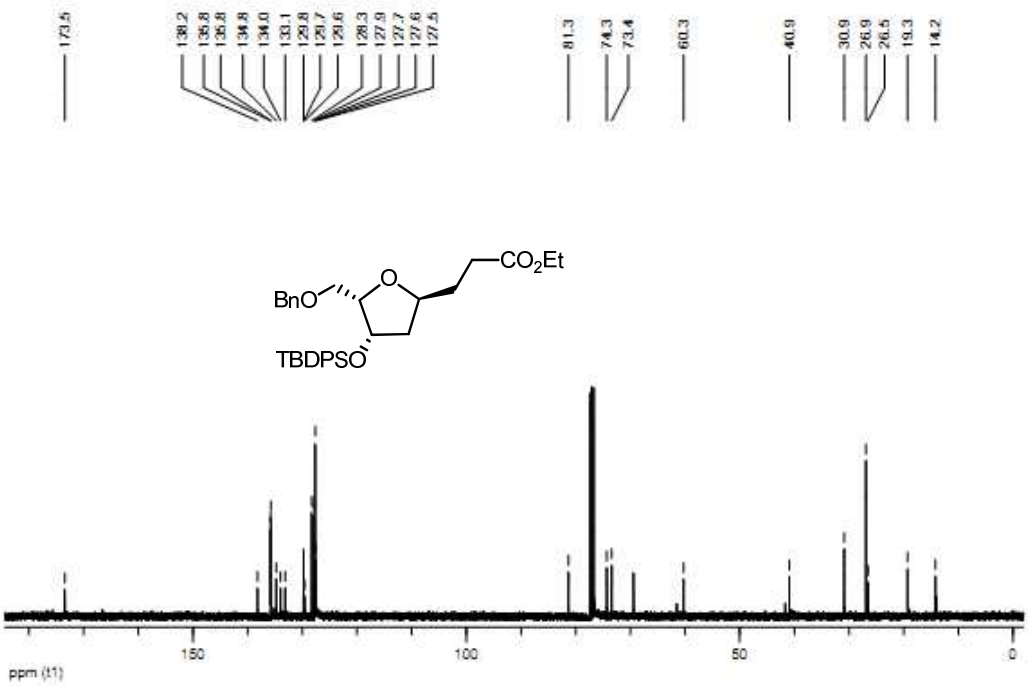
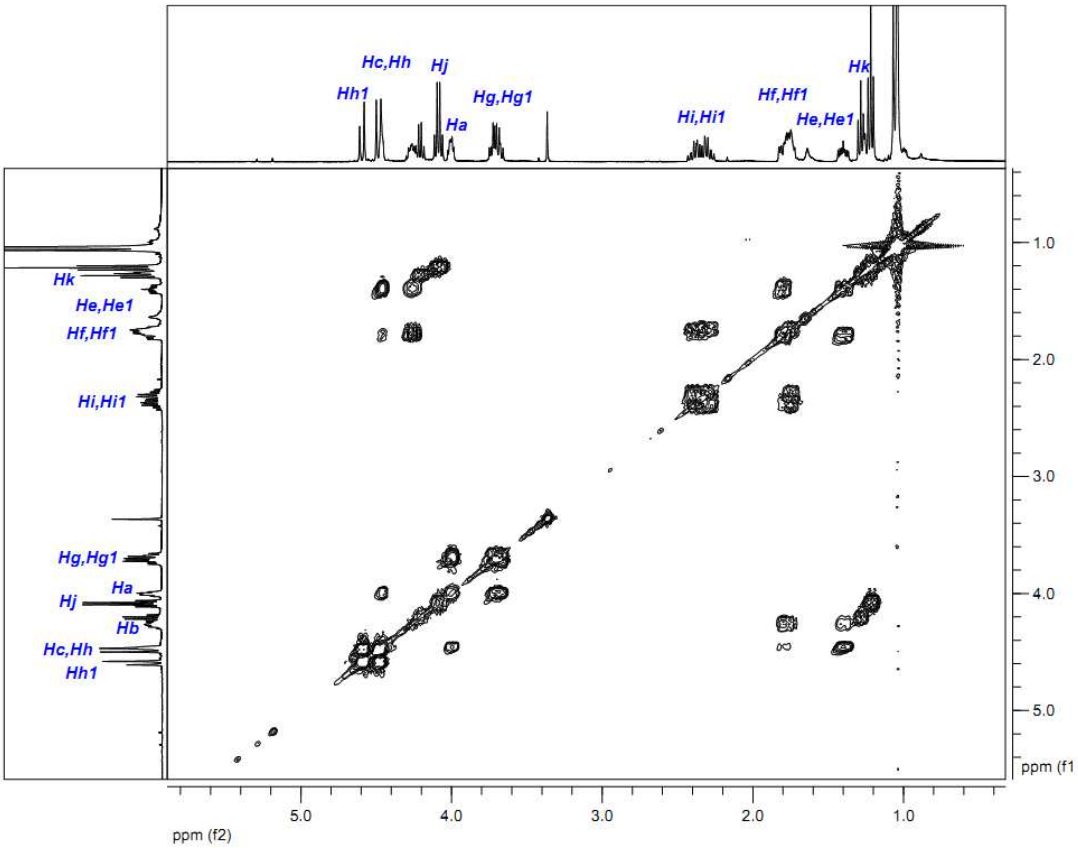
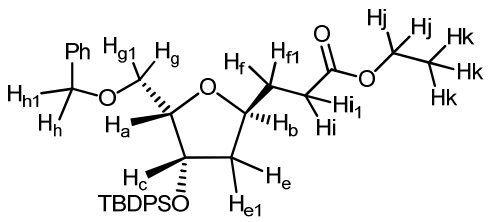


^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)





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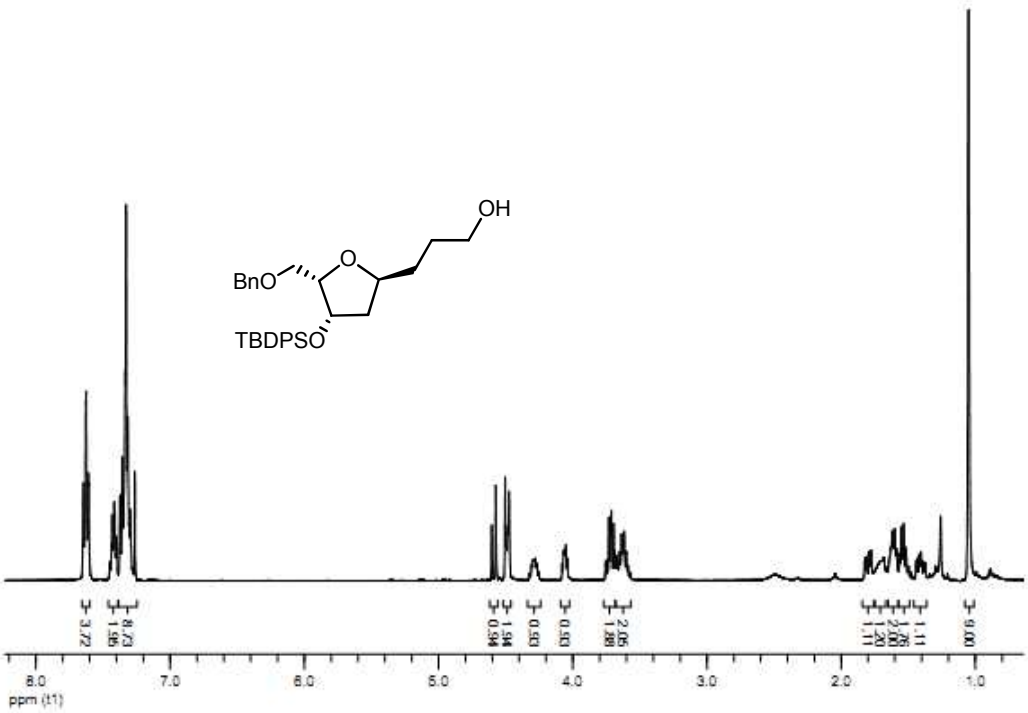
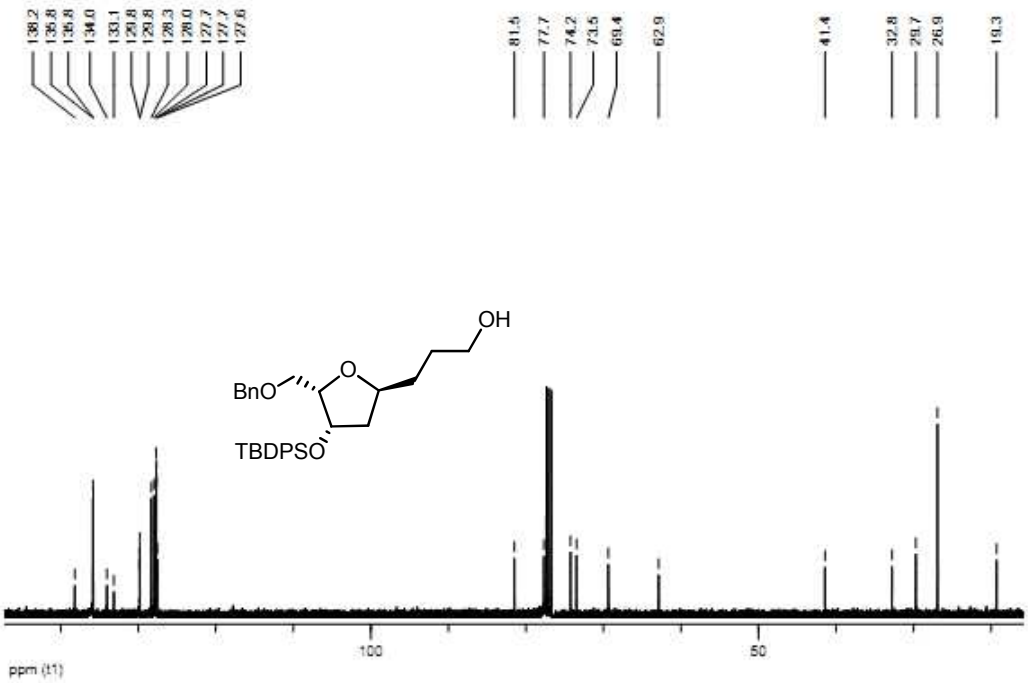
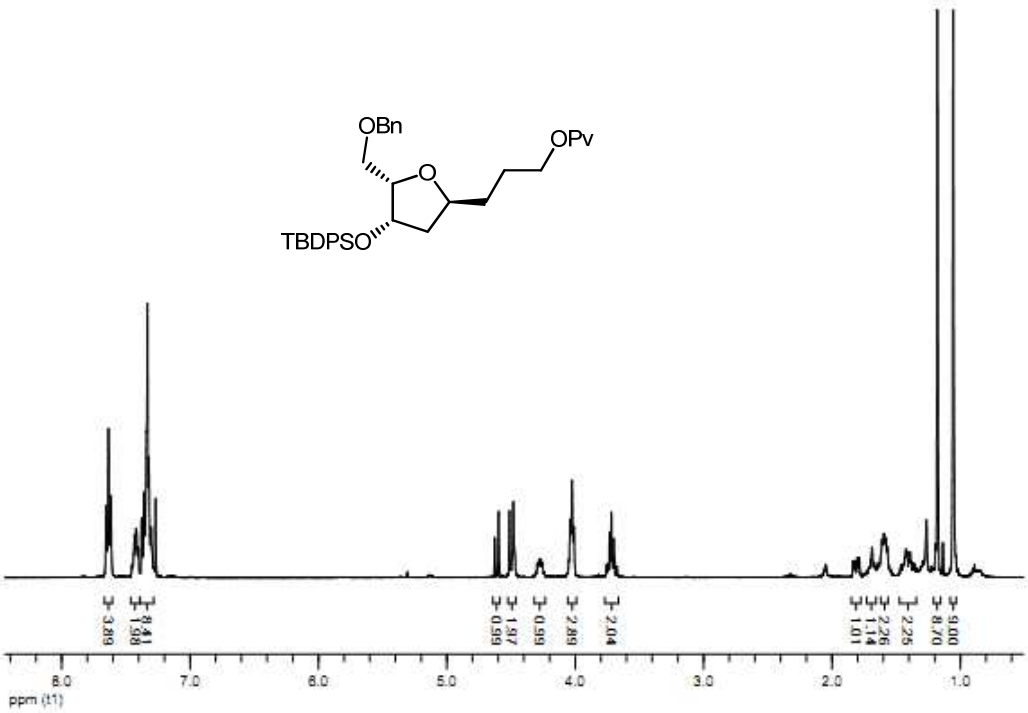


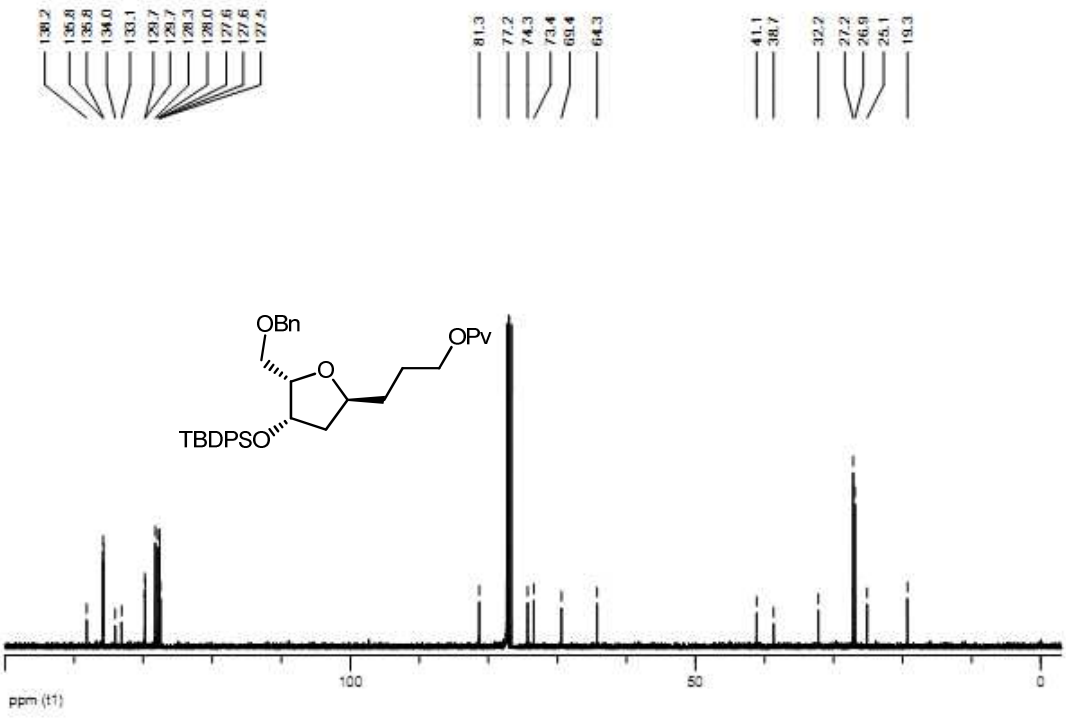
^{13}C NMR ($CDCl_3$, 100MHz)



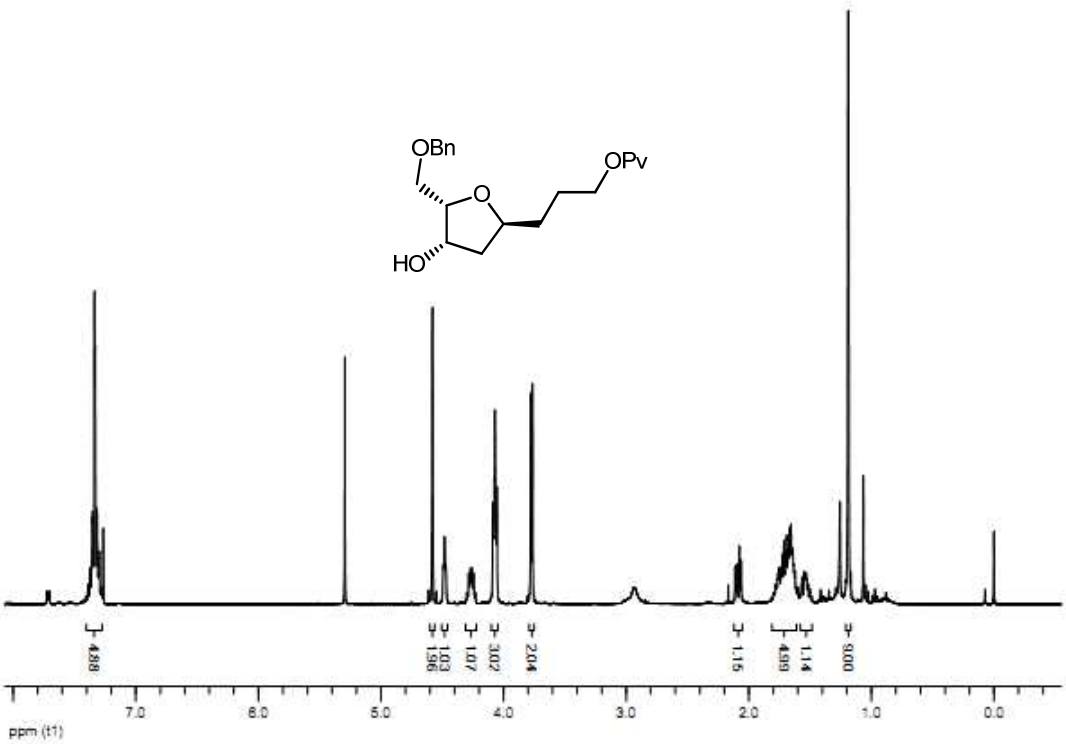
^{1}H NMR ($CDCl_3$, 400MHz)



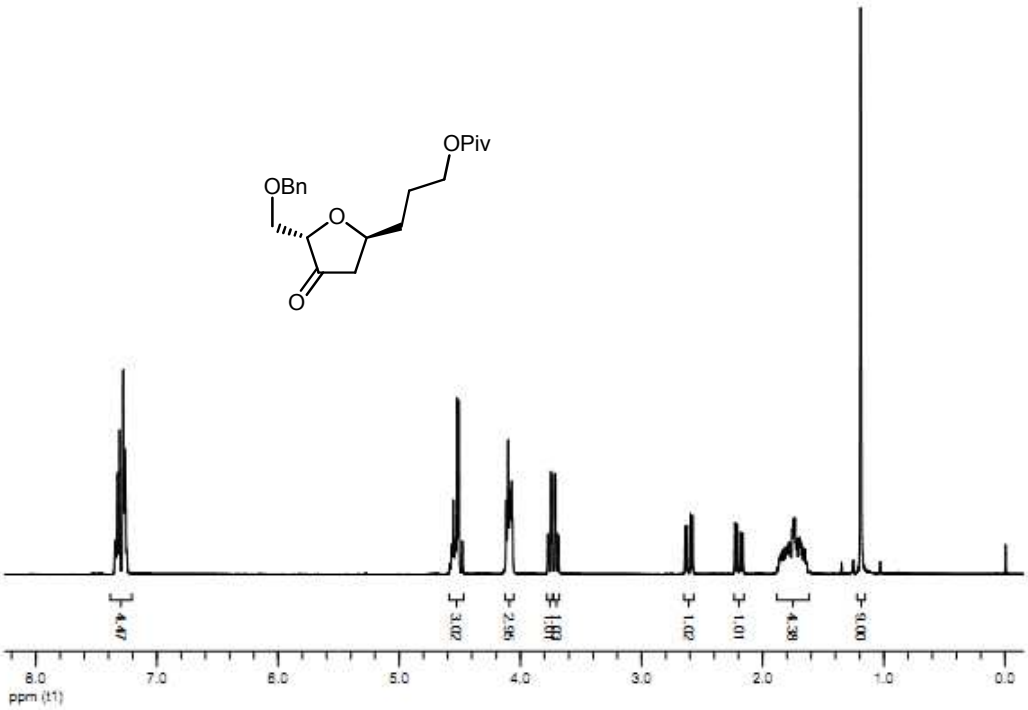
^{13}C NMR ($CDCl_3$, 100MHz)



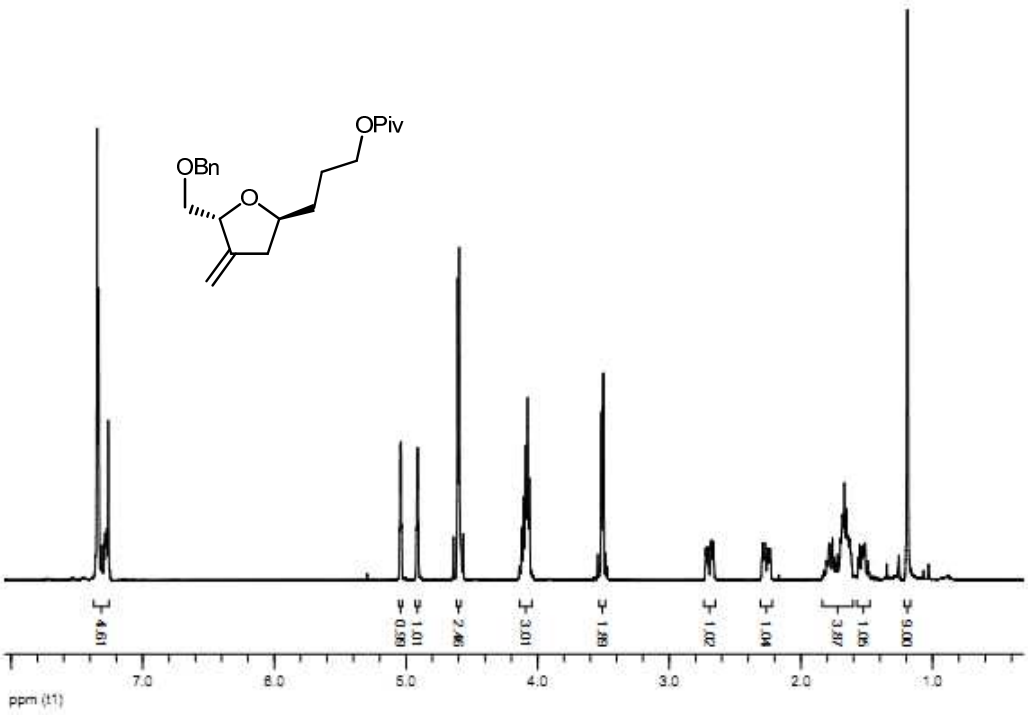
^{1}H NMR ($CDCl_3$, 400MHz)



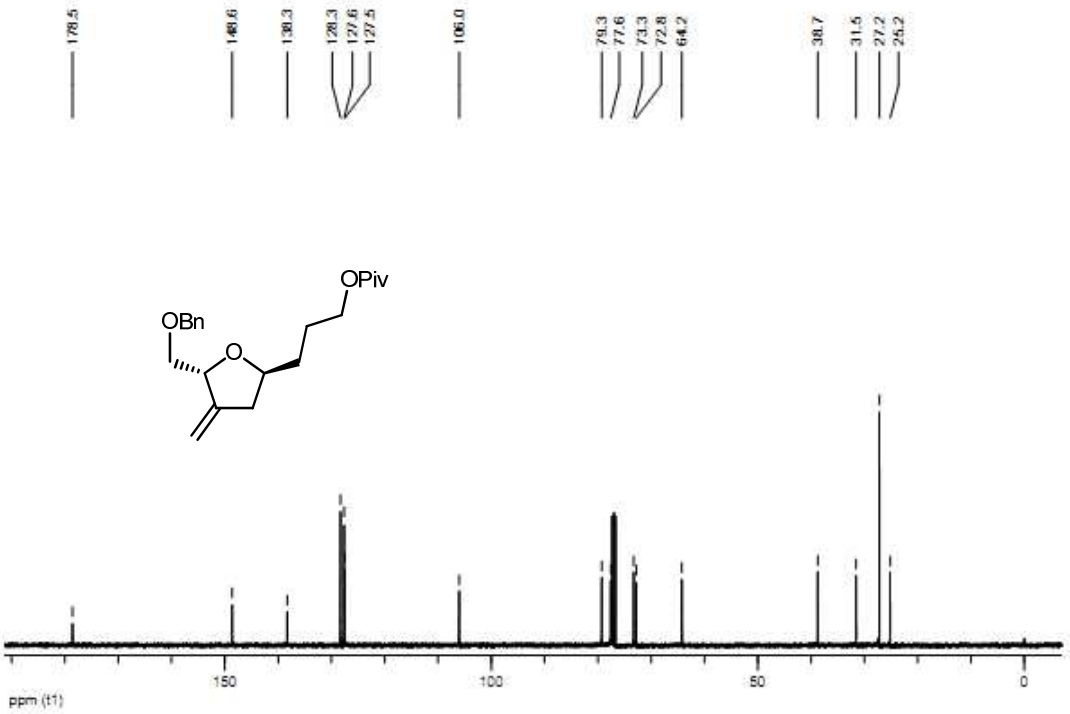
^{1}H NMR ($CDCl_3$, 400MHz)



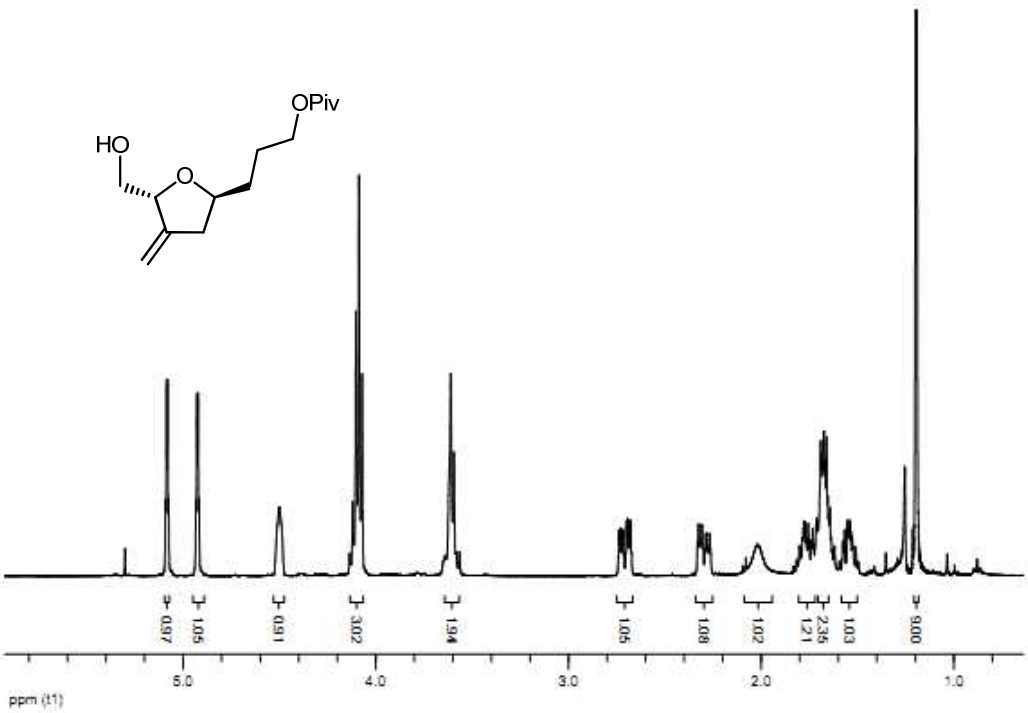
^{1}H NMR ($CDCl_3$, 400MHz)



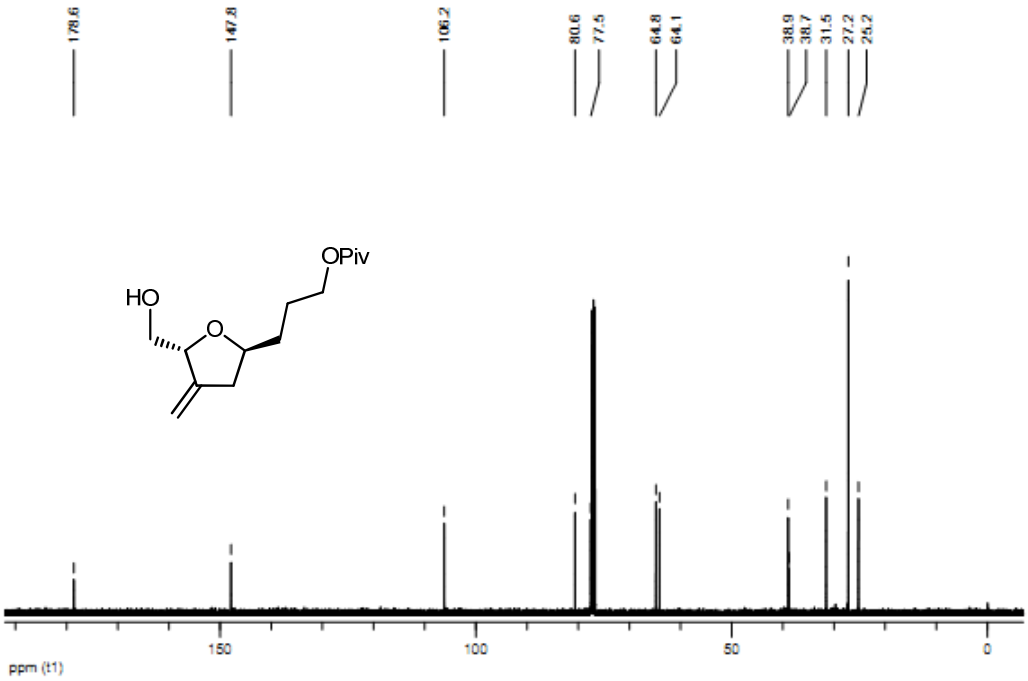
^{13}C NMR ($CDCl_3$, 100MHz)



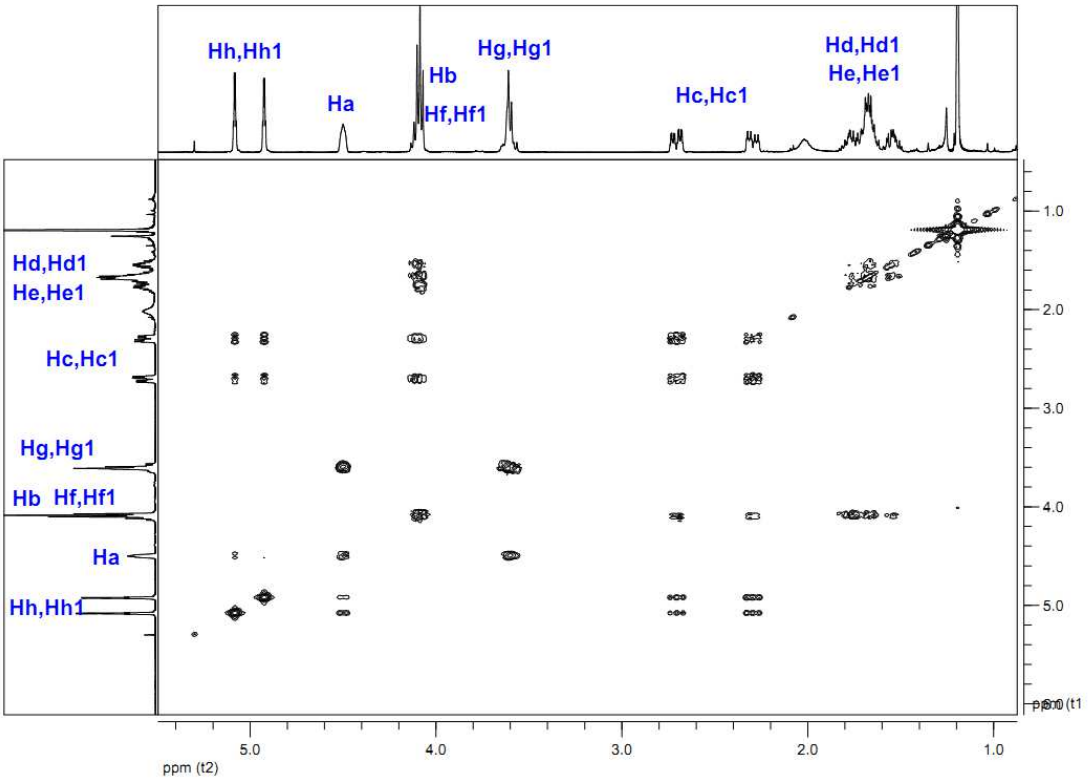
^{1}H NMR ($CDCl_3$, 400MHz)

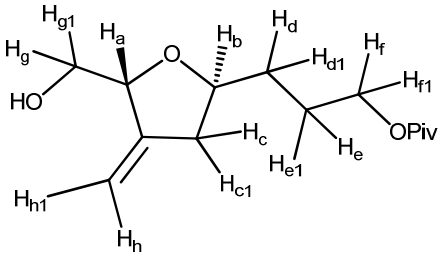


^{13}C NMR ($CDCl_3$, 100MHz)

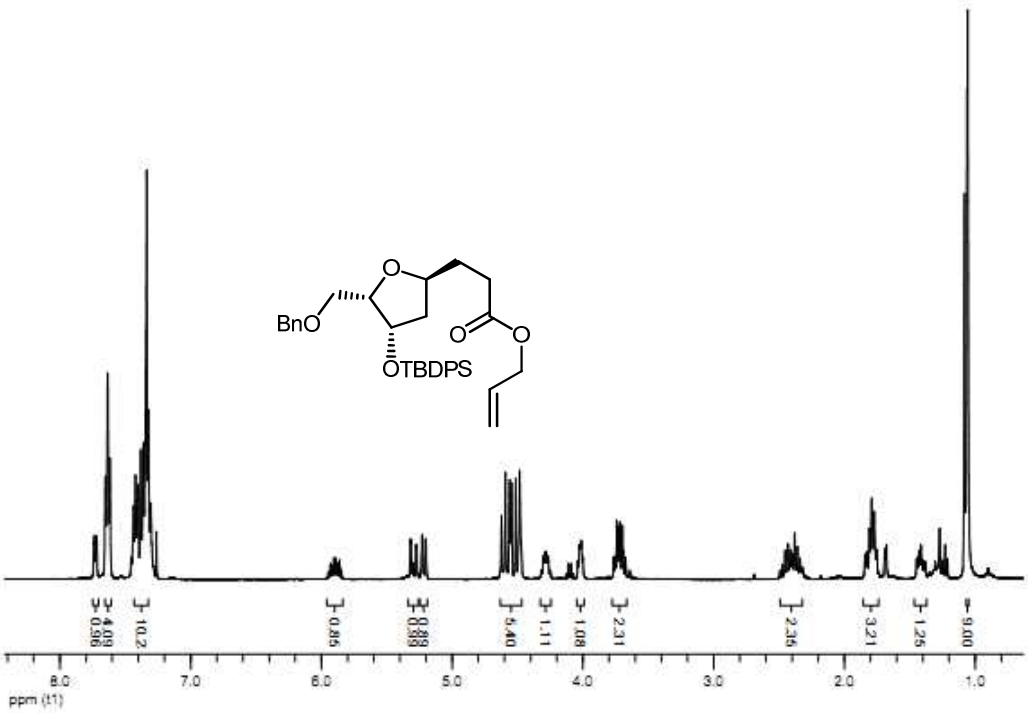


^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)

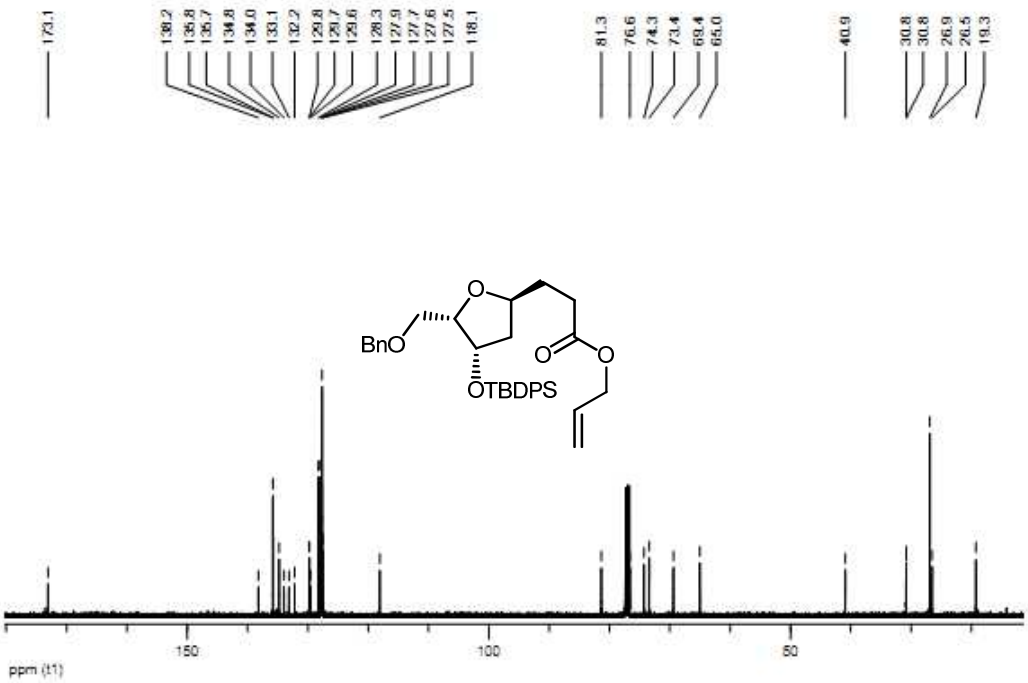




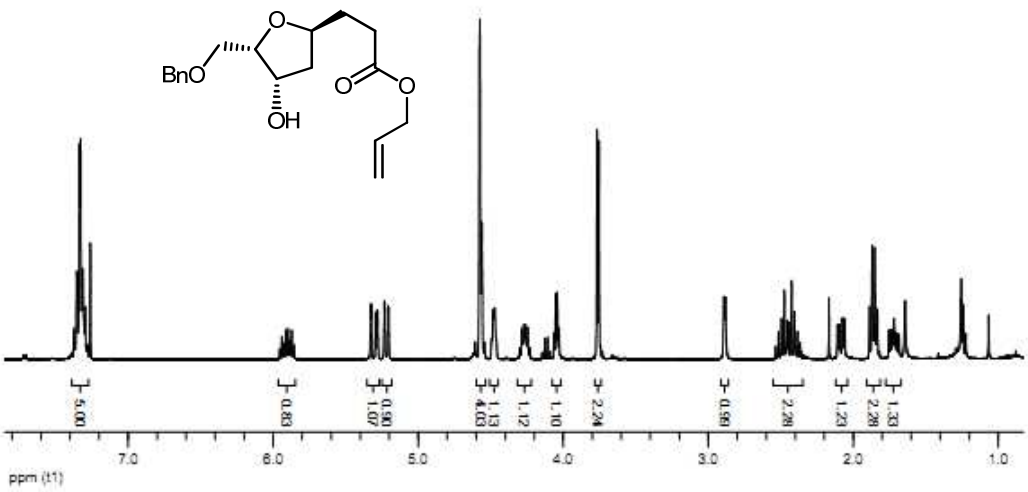
^{1}H NMR ($CDCl_3$, 400MHz)



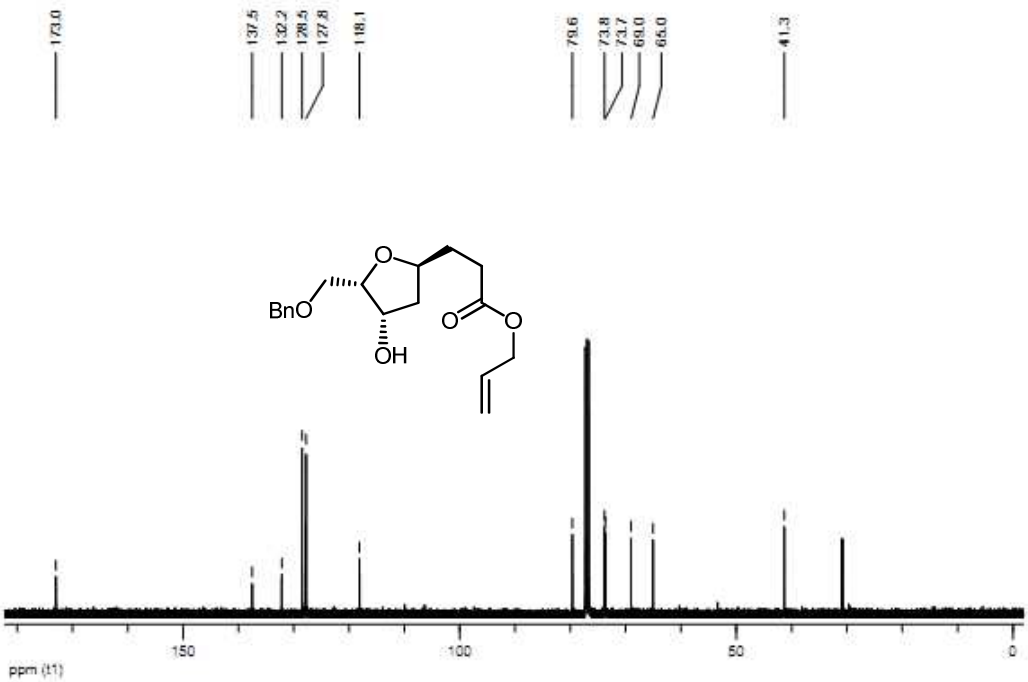
^{13}C NMR ($CDCl_3$, 100MHz)



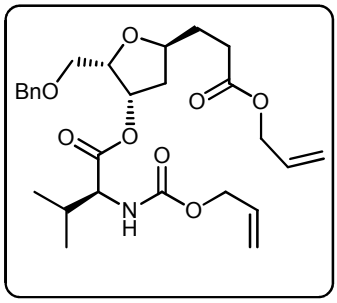
^{1}H NMR ($CDCl_3$, 400MHz)

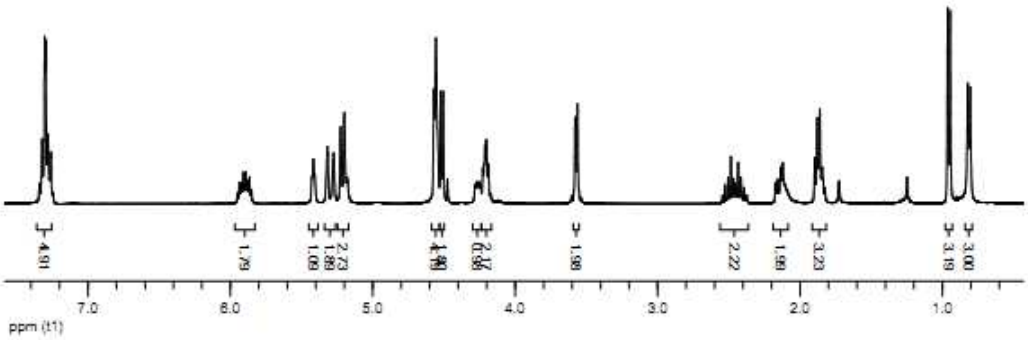


^{13}C NMR ($CDCl_3$, 100MHz)

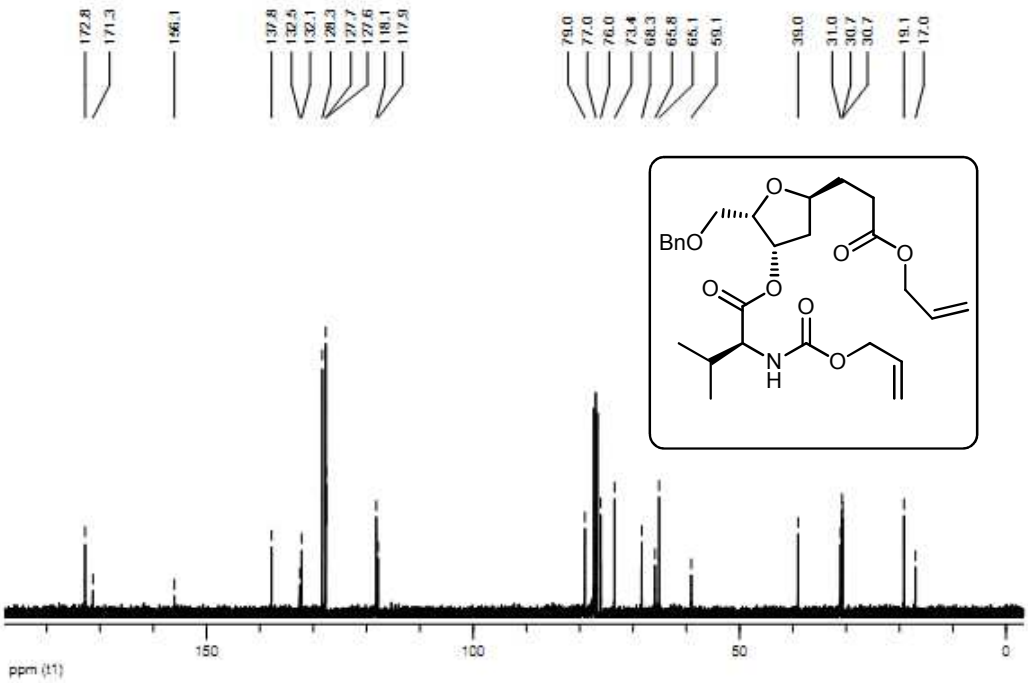


^{1}H NMR ($CDCl_3$, 400MHz)

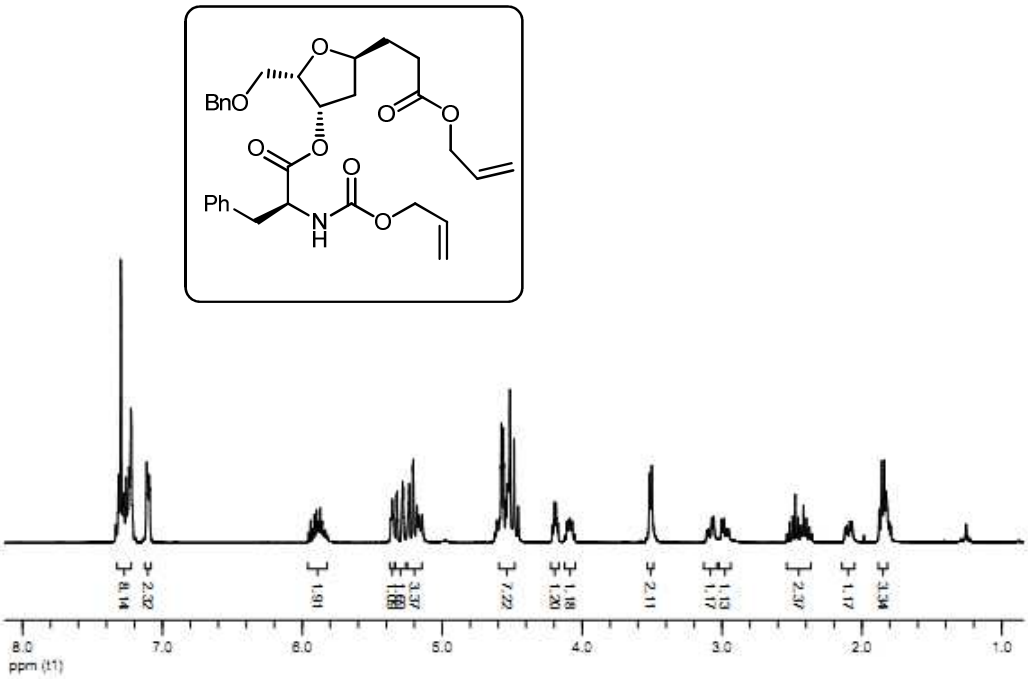




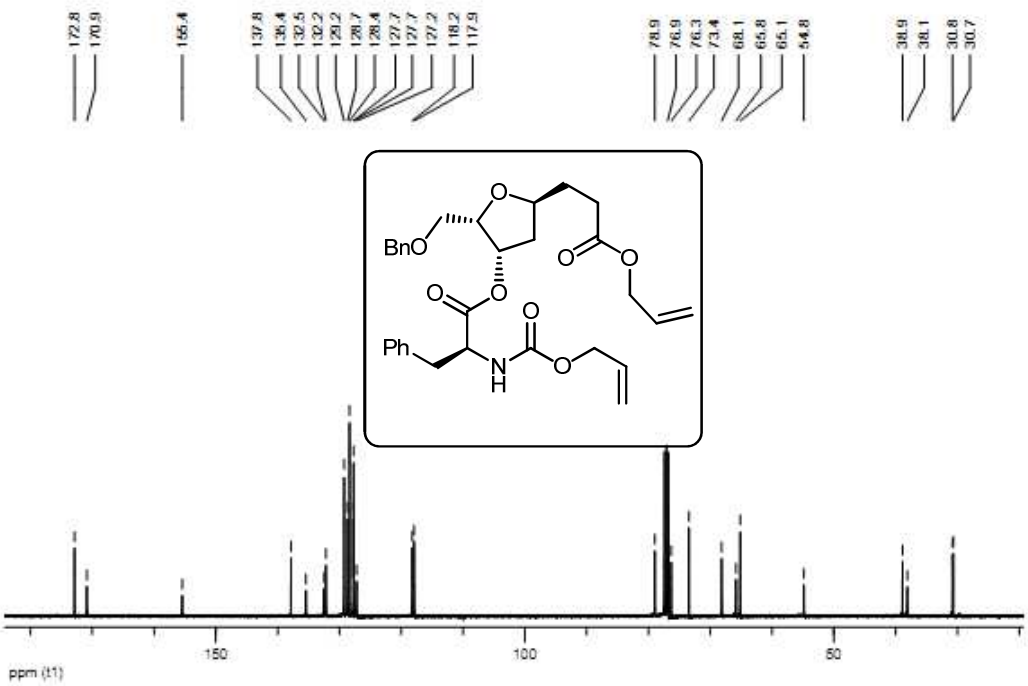
^{13}C NMR ($CDCl_3$, 100MHz)



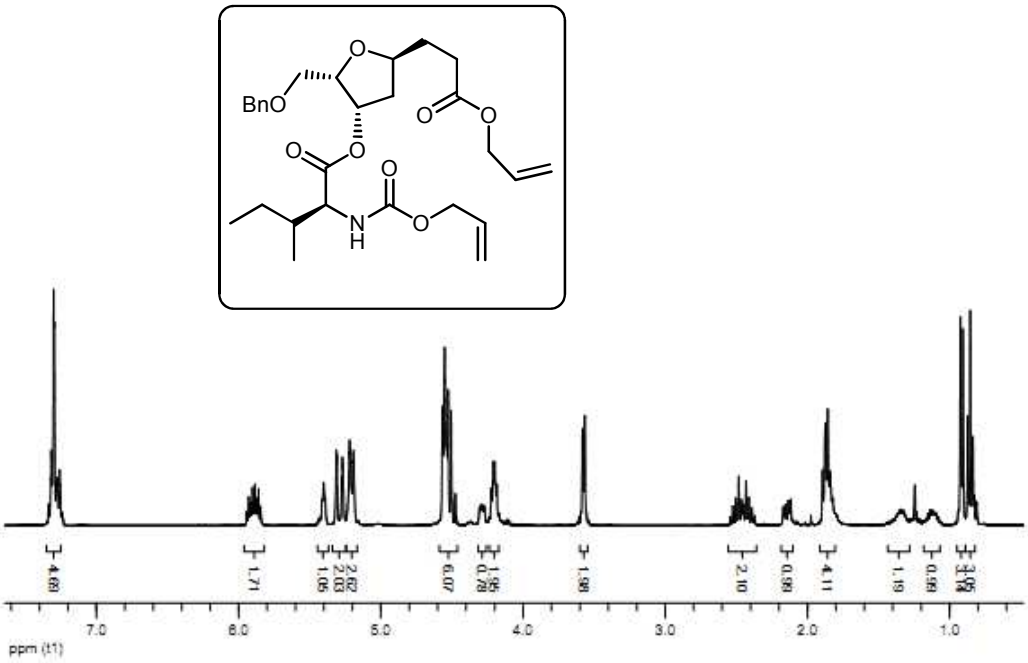
^{1}H NMR ($CDCl_3$, 400MHz)



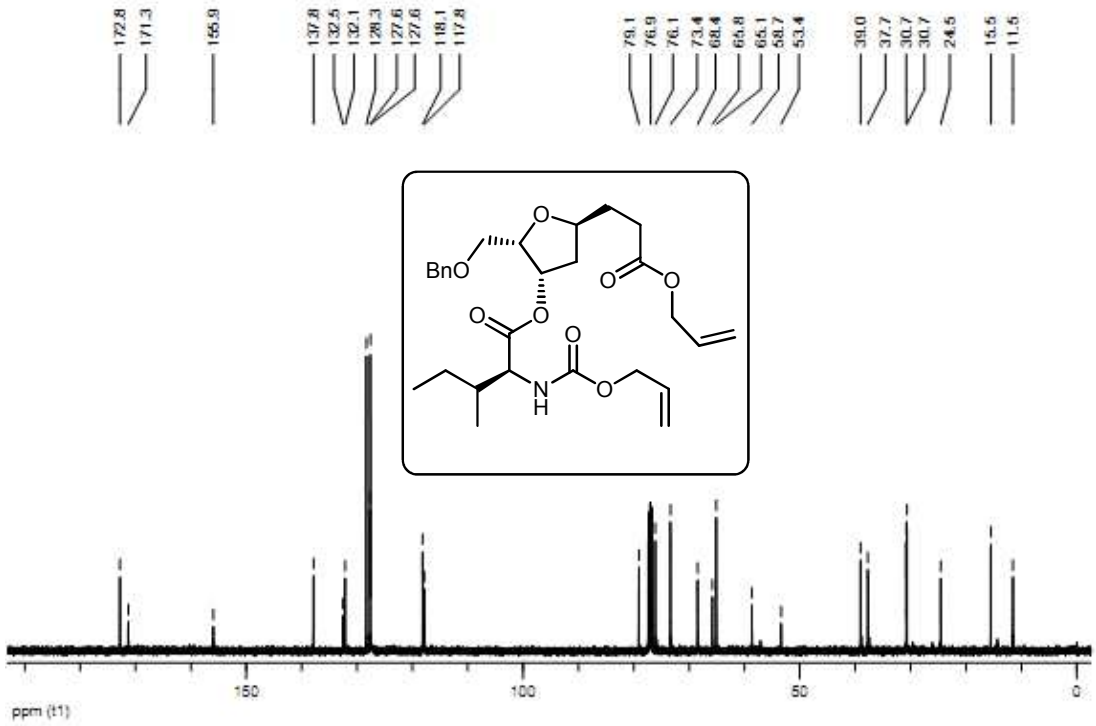
^{13}C NMR ($CDCl_3$, 100MHz)



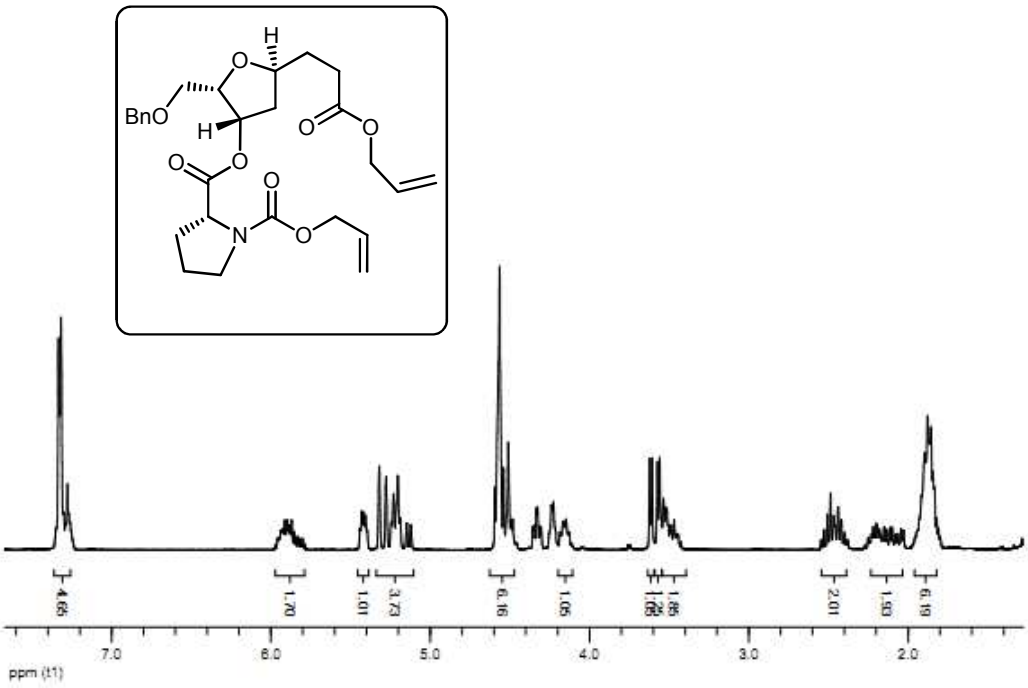
^{1}H NMR ($CDCl_3$, 400MHz)



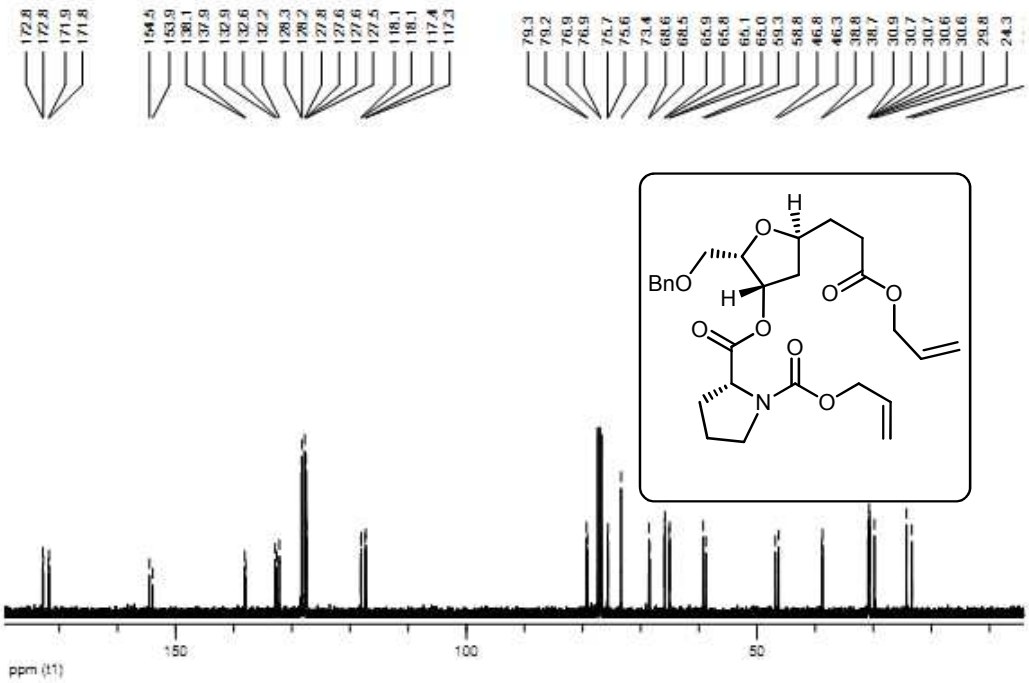
^{13}C NMR ($CDCl_3$, 100MHz)



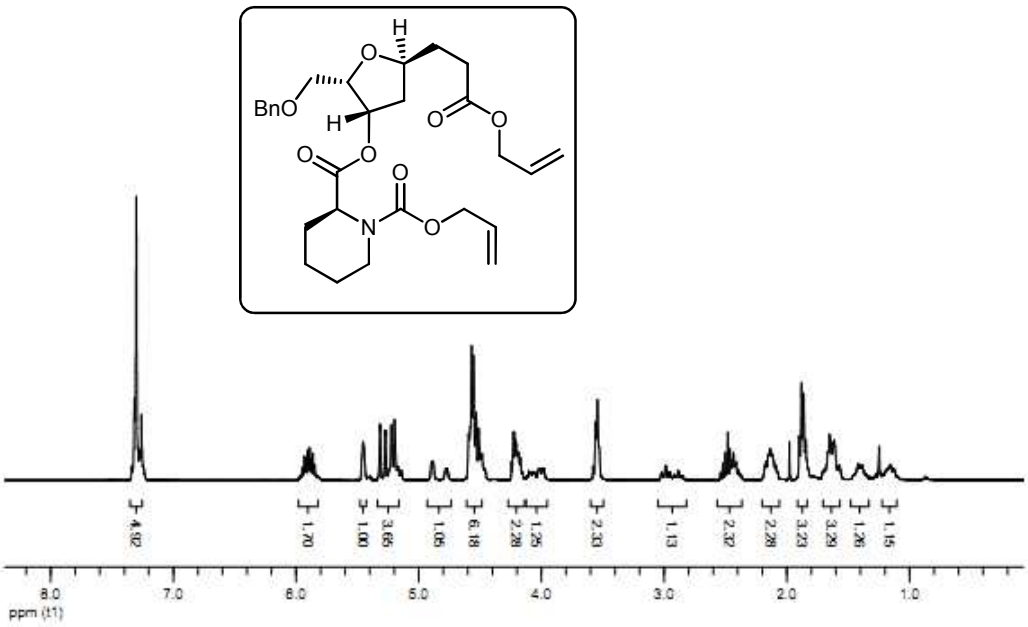
^{1}H NMR ($CDCl_3$, 400MHz)



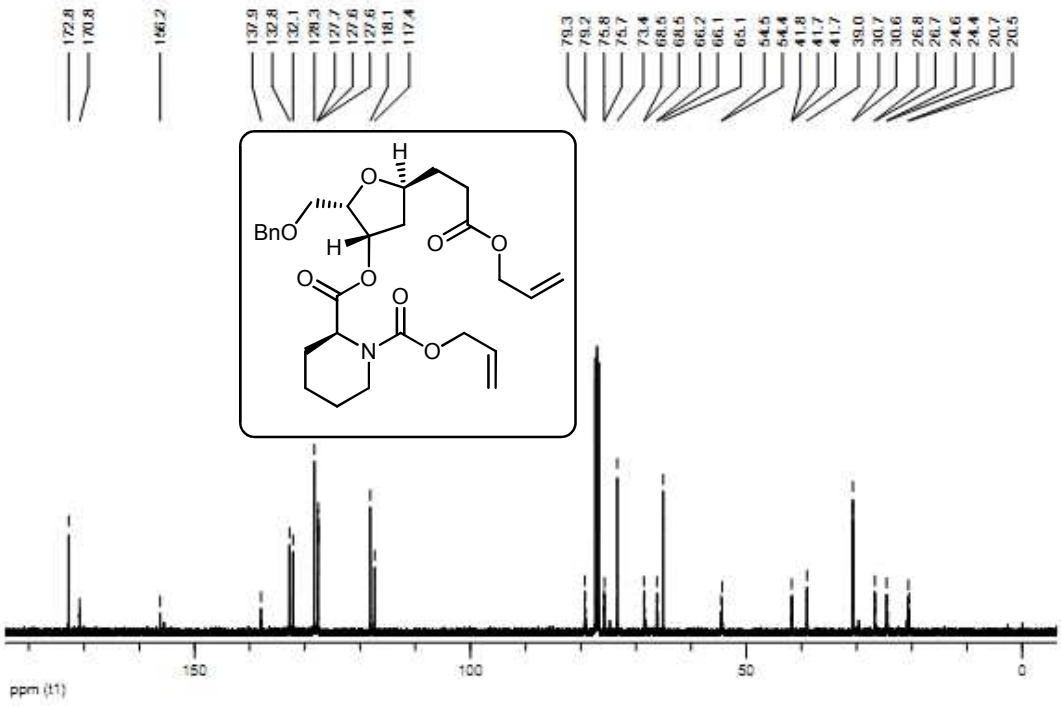
^{13}C NMR ($CDCl_3$, 100MHz)



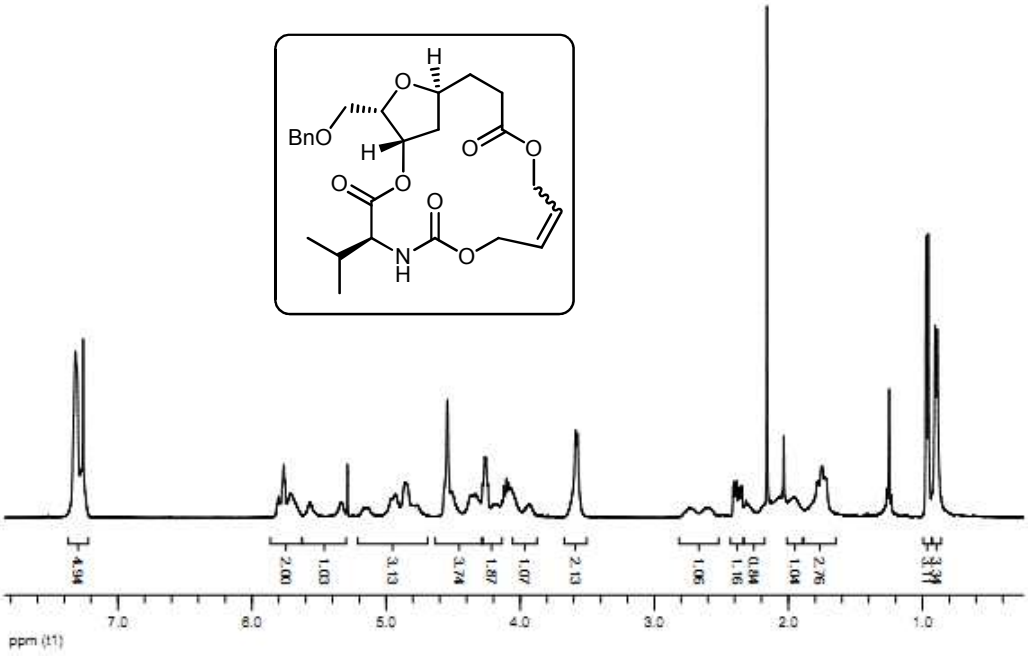
^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



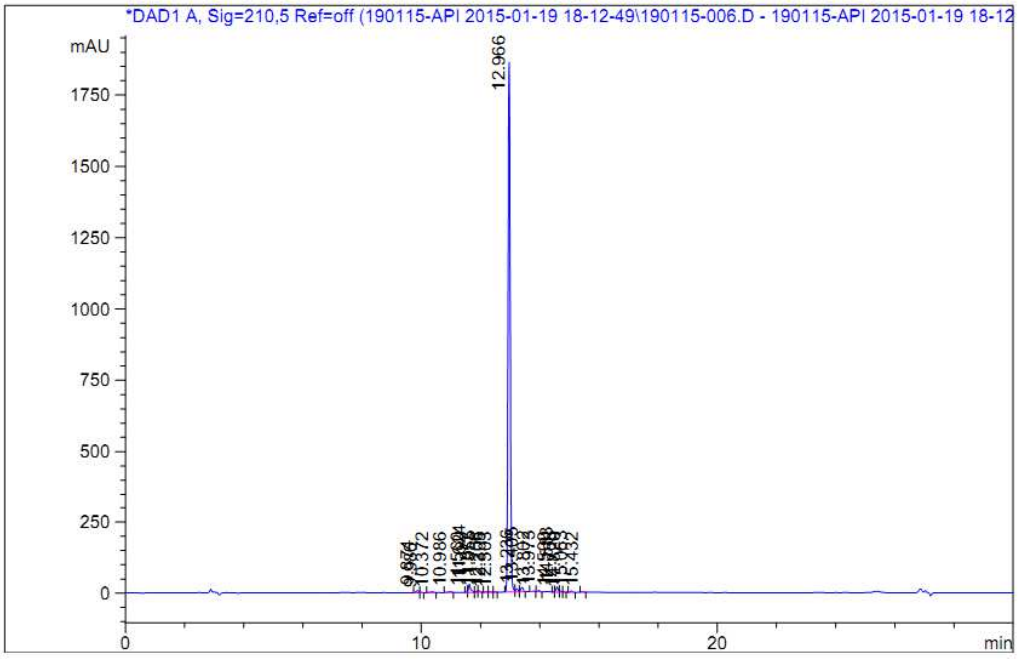
^{1}H NMR ($CDCl_3$, 400MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

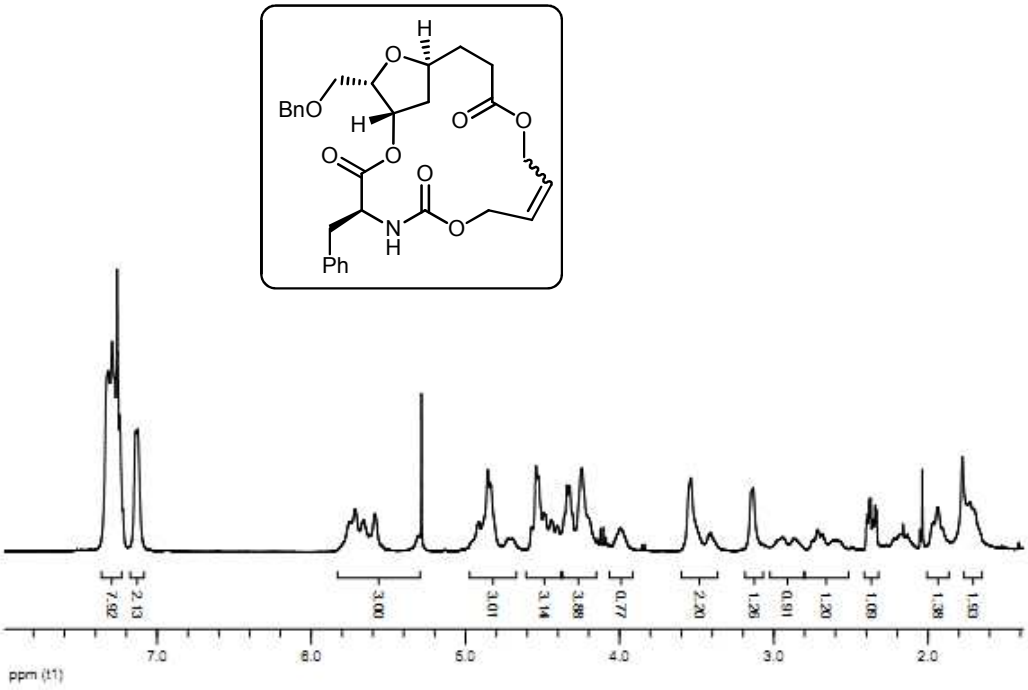
```
Inj Date        : Mon, 19. Jan. 2015          Acq Operator: VARMA
Sample Name     : ILS-MNK-ERB-C-205                         Vial 21
A R Number      : CM15A010                   ->Inj. Vol.    : 5µL
Acq. Method     : D:\chem32\1\DATA\190115-API 2015-01-19 18-12-49\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API CFZ.M
Method Info     : Column :  X-Terra RP18 250*4.6mm,5µm
                  Mobile phase: A)0.1%TFA in Water B) ACN (gradient )
                  T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: Water
```



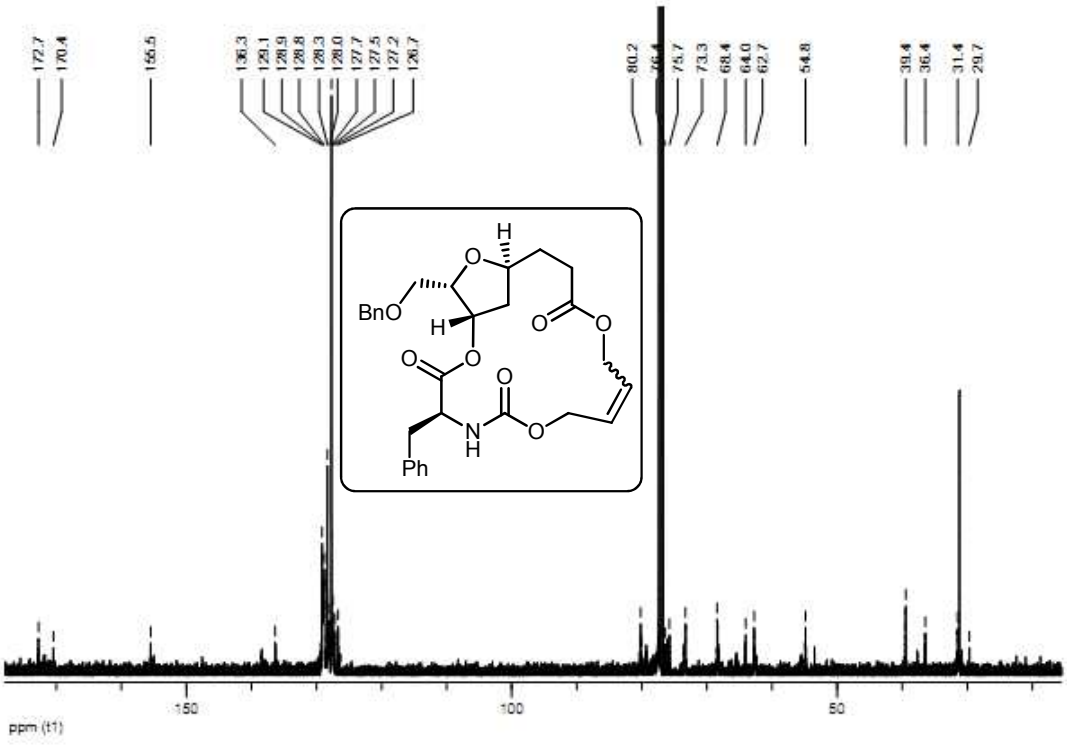
Signal 1: DAD1 A, Sig=210,5 Ref=off

Peak #	RT [min]	Area	Area %
1	9.874	45.783	0.456
2	9.986	5.685	0.057
3	10.372	19.718	0.196
4	10.986	21.143	0.211
5	11.560	9.252	0.092
6	11.624	147.255	1.467
7	11.872	20.775	0.207
8	11.955	21.265	0.212
9	12.206	10.371	0.103
10	12.305	6.614	0.066
11	12.503	3.363	0.034
12	12.966	9386.533	93.517
13	13.236	58.029	0.578
14	13.405	85.210	0.849
15	13.802	10.689	0.106
16	13.973	22.588	0.225
17	14.500	5.576	0.056
18	14.583	93.792	0.934
19	14.703	17.931	0.179

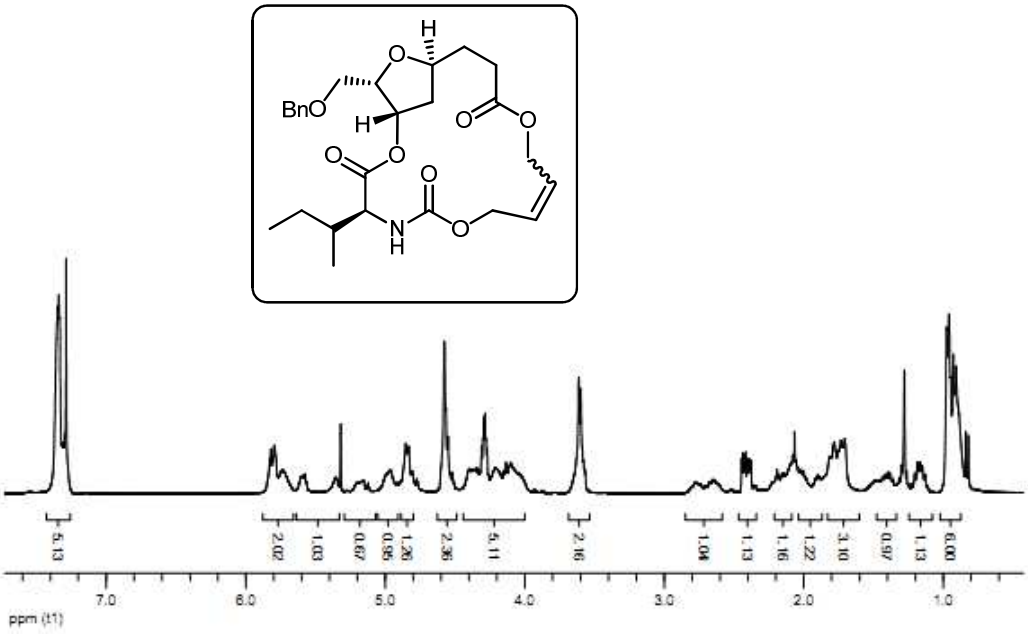
^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



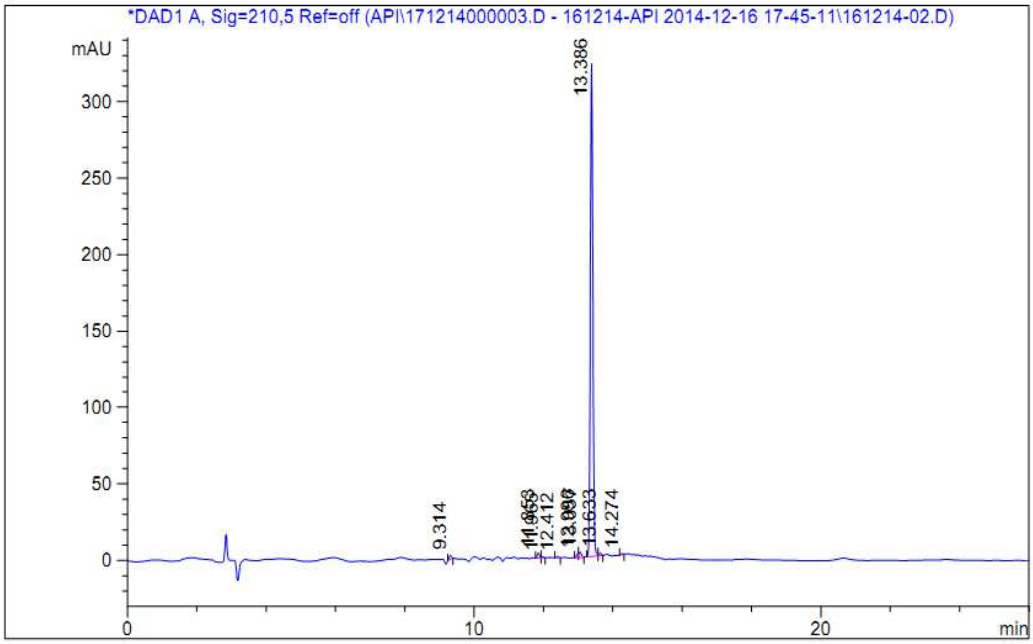
^{1}H NMR ($CDCl_3$, 400MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

```
Inj Date        : Wed, 17. Dec. 2014          Acq Operator: VARMA
Sample Name     : ILS-MNK-ERB-C-206                         Vial 31
A R Number      : CM14L015                  ->Inj. Vol.   : 5µL
Acq. Method     : D:\CHEM32_002\1\METHODS\API DCV-001.M
Analysis Method : D:\CHEM32_002\1\METHODS\API DCV-001.M
Method Info     : Column :  X-Terra RP18 250*4.6mm,5µm
                  Mobile phase: A)0.1%TFA in Water B) ACN (gradient )
                  T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```

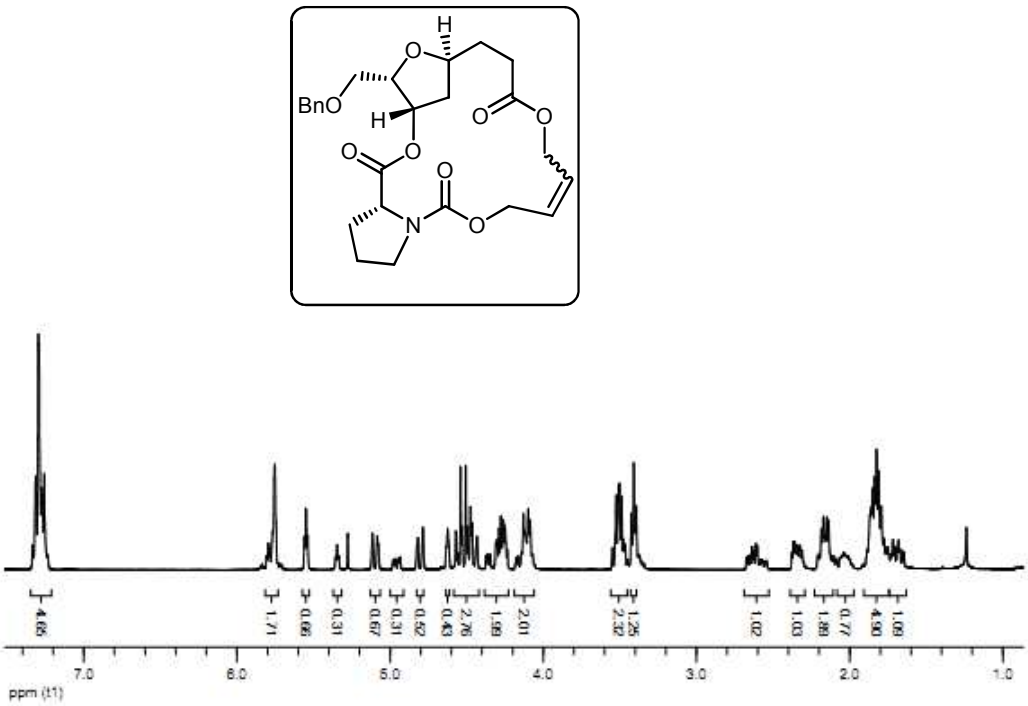


==========

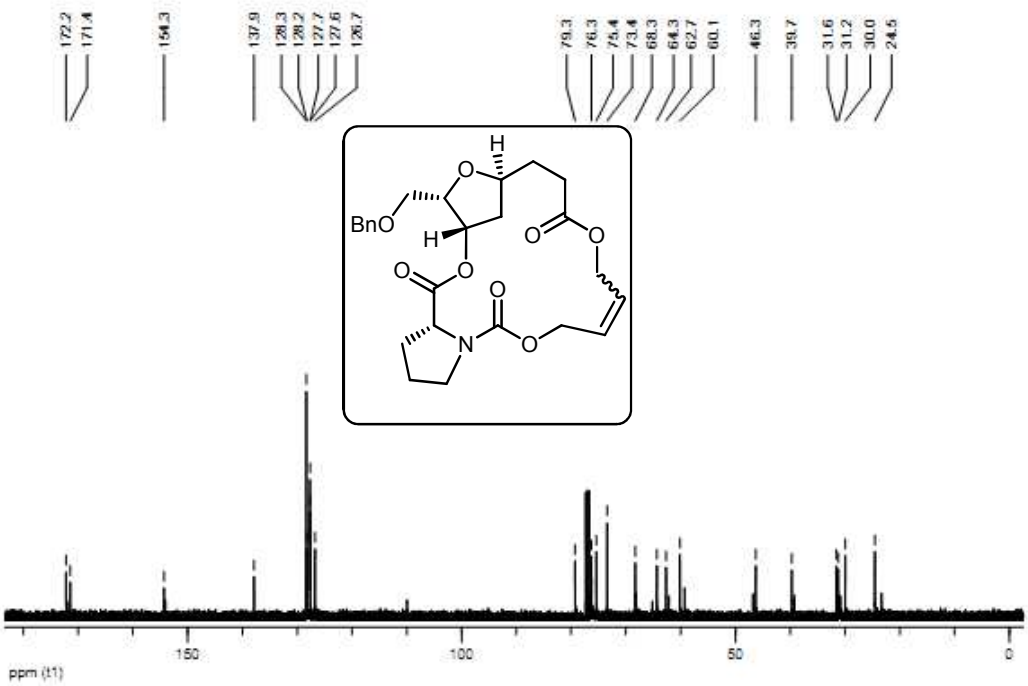
Signal 1: DAD1 A, Sig=210,5 Ref=off

Peak #	RT [min]	Area	Area %
1	9.314	12.825	0.754
2	11.853	15.241	0.896
3	11.965	3.015	0.177
4	12.412	4.152	0.244
5	12.996	10.655	0.627
6	13.057	17.570	1.033
7	13.386	1626.521	95.640
8	13.633	7.646	0.450
9	14.274	3.053	0.179

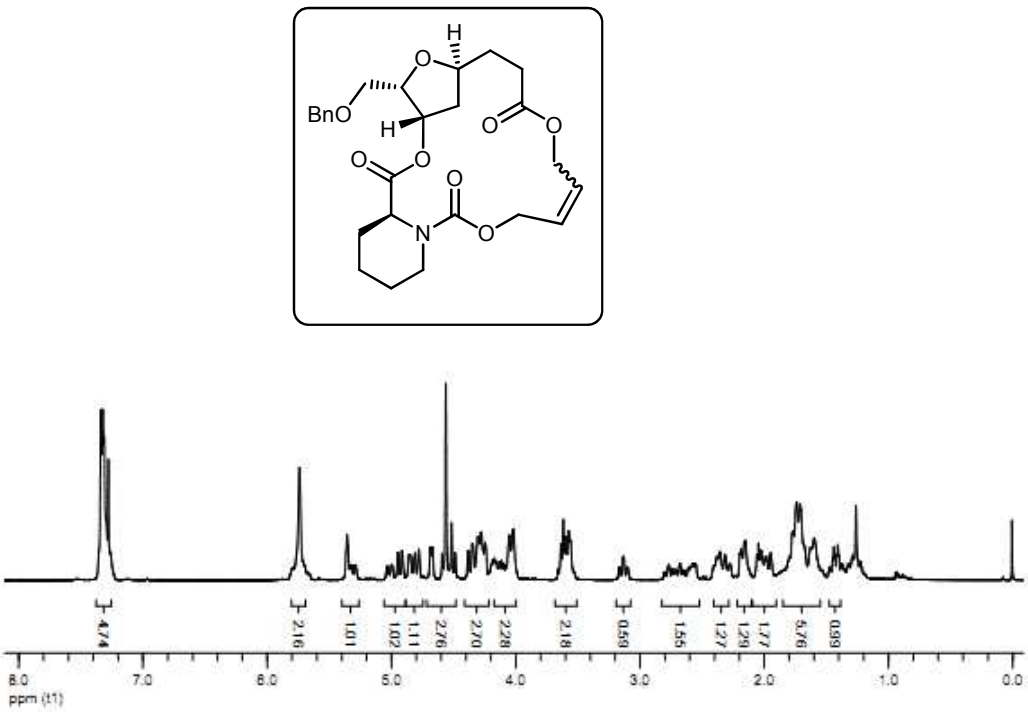
^{1}H NMR ($CDCl_3$, 400MHz)



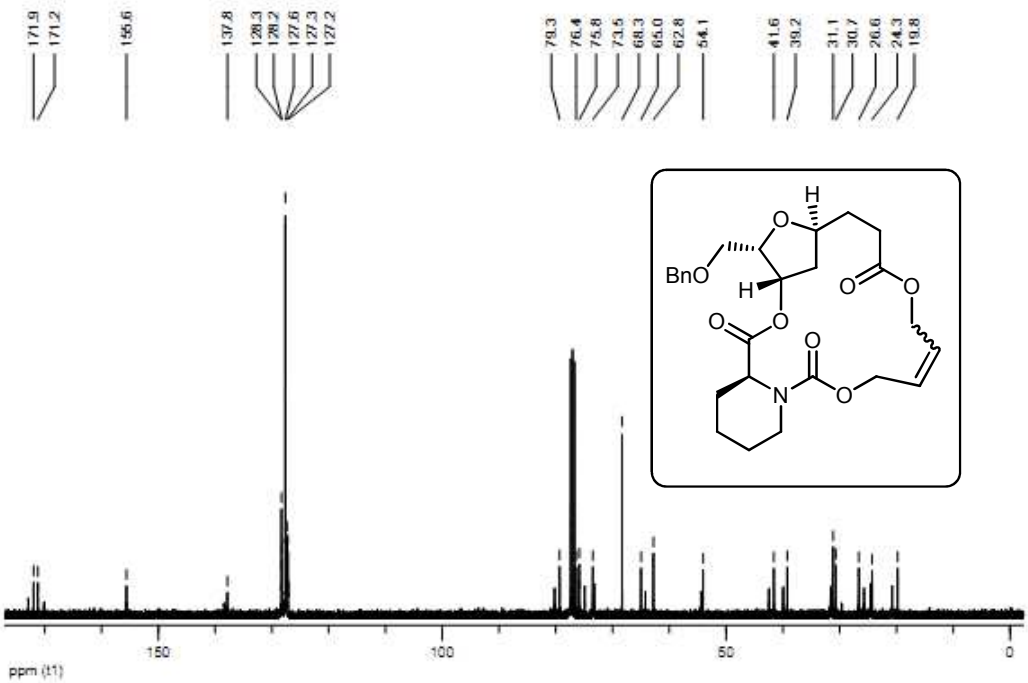
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



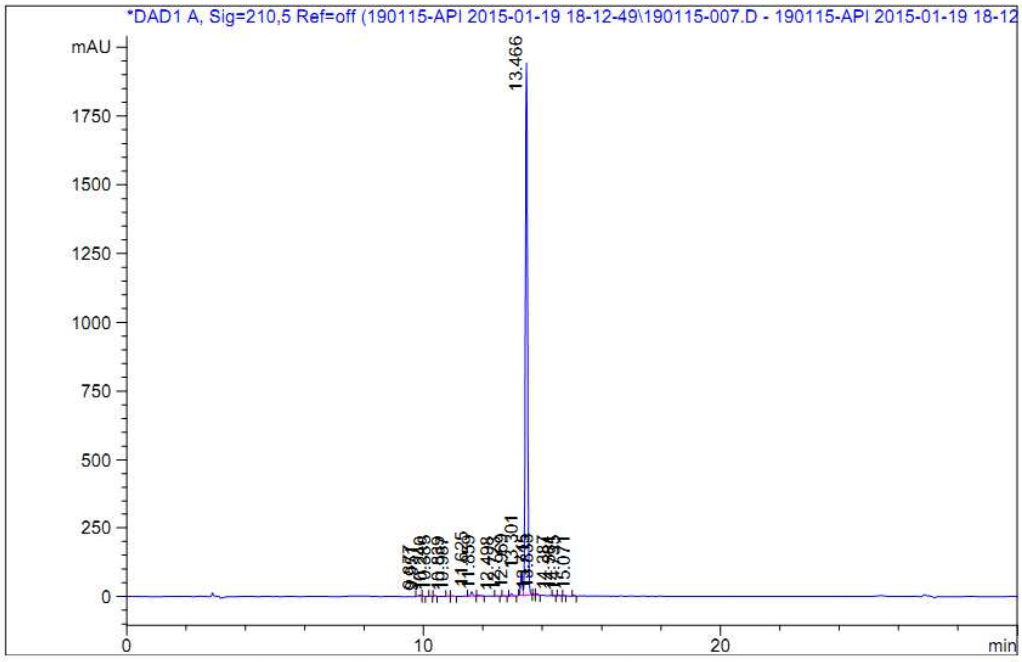
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

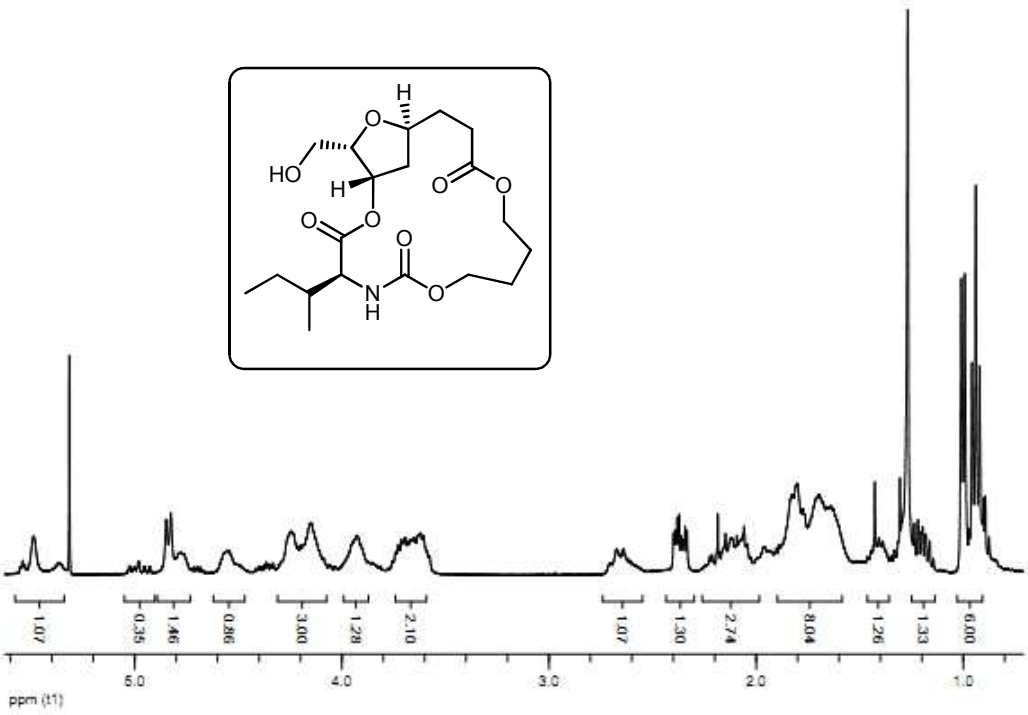
```
Inj Date        : Mon, 19. Jan. 2015          Acq Operator: VARMA
Sample Name     : ILS-MNK-ERB-C-213                         Vial 32
A R Number      : CM15A009                  ->Inj. Vol.    : 5µL
Acq. Method     : D:\chem32\1\DATA\190115-API 2015-01-19 18-12-49\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API CFZ.M
Method Info     : Column :  X-Terra RP18 250*4.6mm,5µm
                  Mobile phase: A)0.1%TFA in Water B) ACN (gradient )
                  T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: Water
```



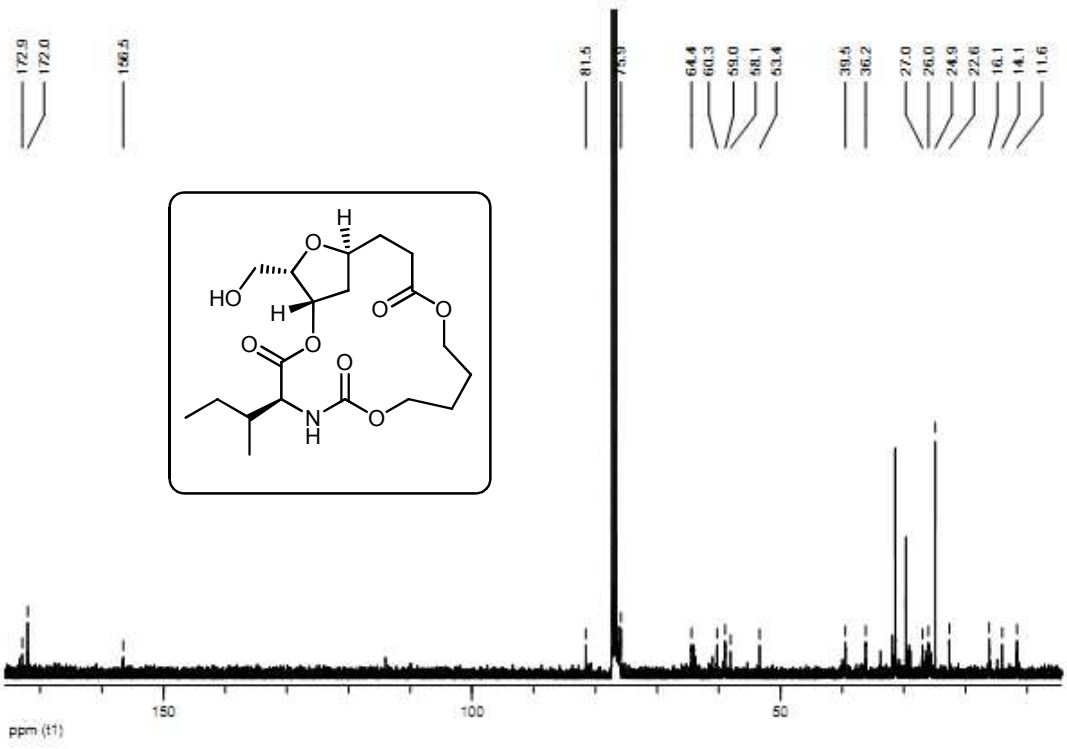
Signal 1: DAD1 A, Sig=210,5 Ref=off

Peak #	RT [min]	Area	Area %
1	9.877	29.570	0.281
2	9.951	4.981	0.047
3	10.240	4.004	0.038
4	10.385	11.366	0.108
5	10.839	4.120	0.039
6	10.987	8.823	0.084
7	11.625	87.252	0.829
8	11.859	28.582	0.272
9	12.498	3.553	0.034
10	12.723	3.744	0.036
11	12.969	42.374	0.403
12	13.301	345.811	3.287
13	13.466	9873.540	93.841
14	13.715	6.744	0.064
15	13.833	26.075	0.248
16	14.387	10.389	0.099
17	14.581	12.722	0.121
18	14.735	5.169	0.049
19	15.071	12.717	0.121

^{1}H NMR ($CDCl_3$, 400MHz)



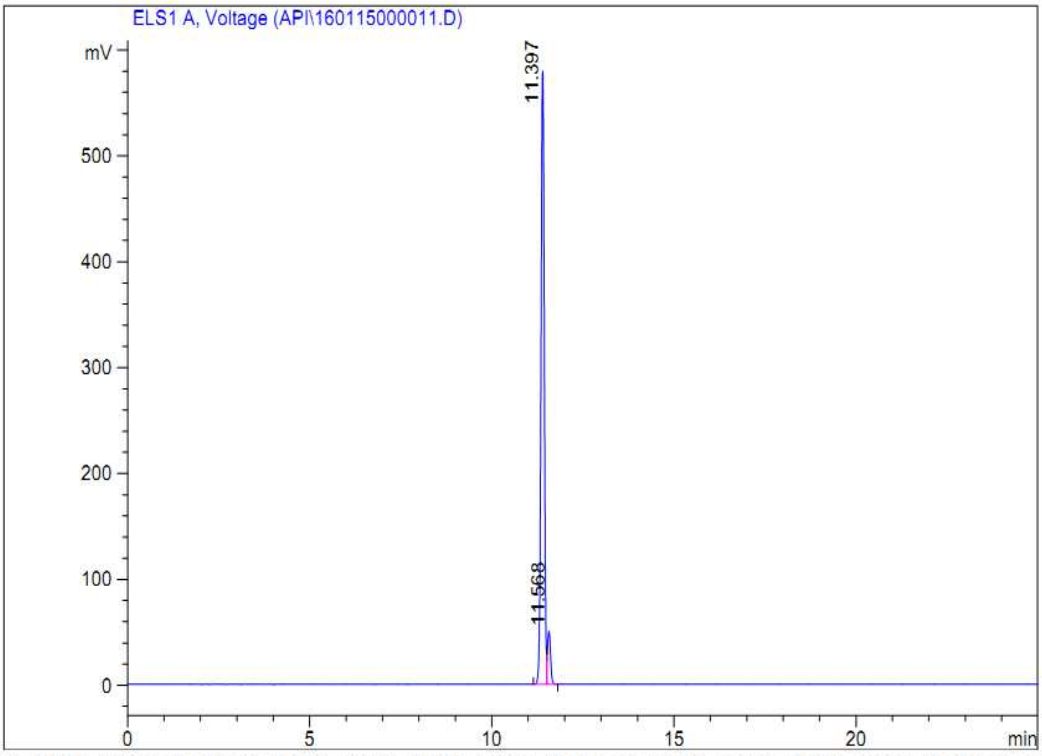
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

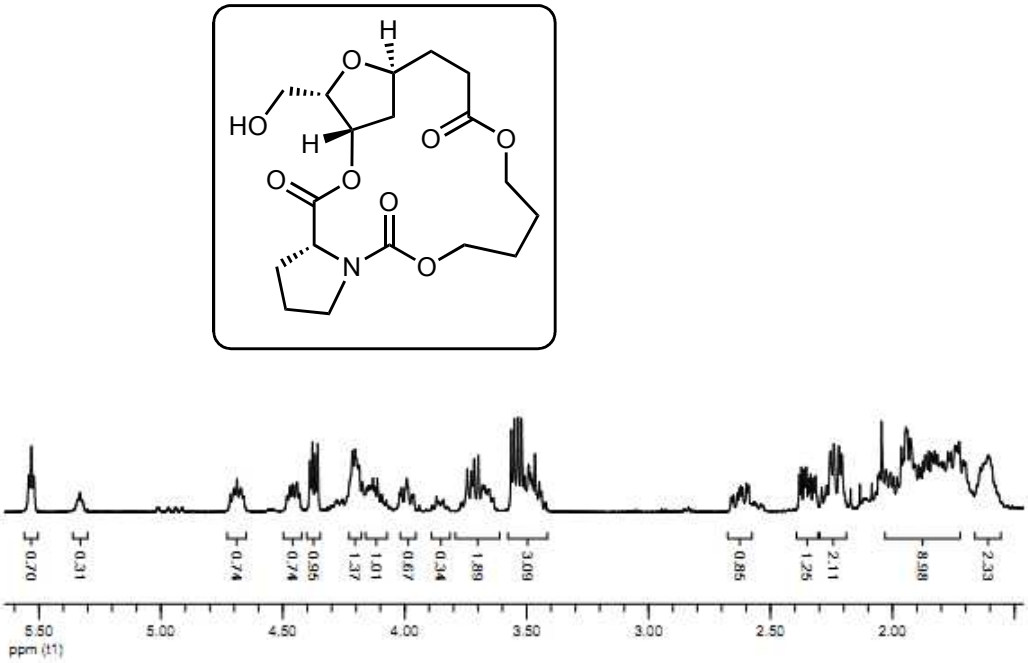
```
Inj Date        : Fri, 16. Jan. 2015          Acq Operator: VARMA
Sample Name     : ILS-MNK-ERB-C-208 (B)                     Vial 5
A R Number      : CM14L011                  ->Inj. Vol.   : 15µL
Acq. Method     : D:\CHEM32_002\1\METHODS\API DCV.M
Analysis Method : D:\CHEM32_002\1\METHODS\API DCV.M
Method Info     : Column: Sun fire C-8 250*4.6mm 5µm
                  Mobile phase: A) TFA in Water B) ACN
                  T/%B:0/10,3/10,12/95,20/95,22/10,25/10.
                  Flow:1.0ml/min, Diluent: WATER
```



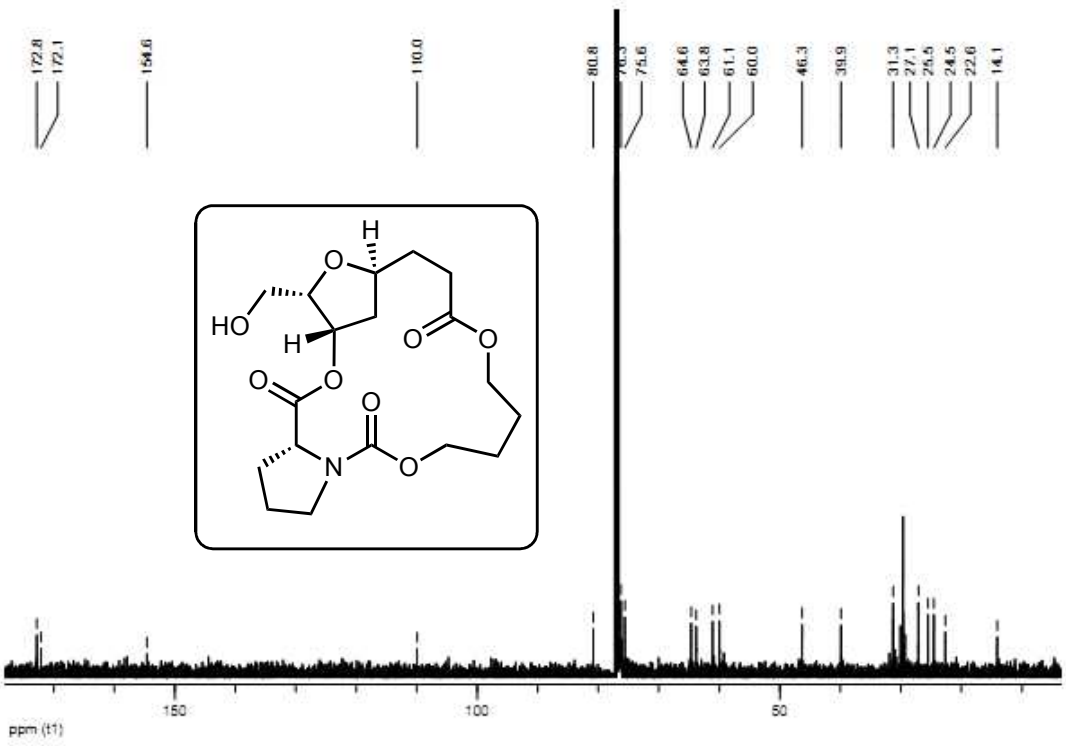
Signal 1: ELS1 A, Voltage

Peak #	RT [min]	Area	Area %
1	11.397	3625.322	92.558
2	11.568	291.470	7.442

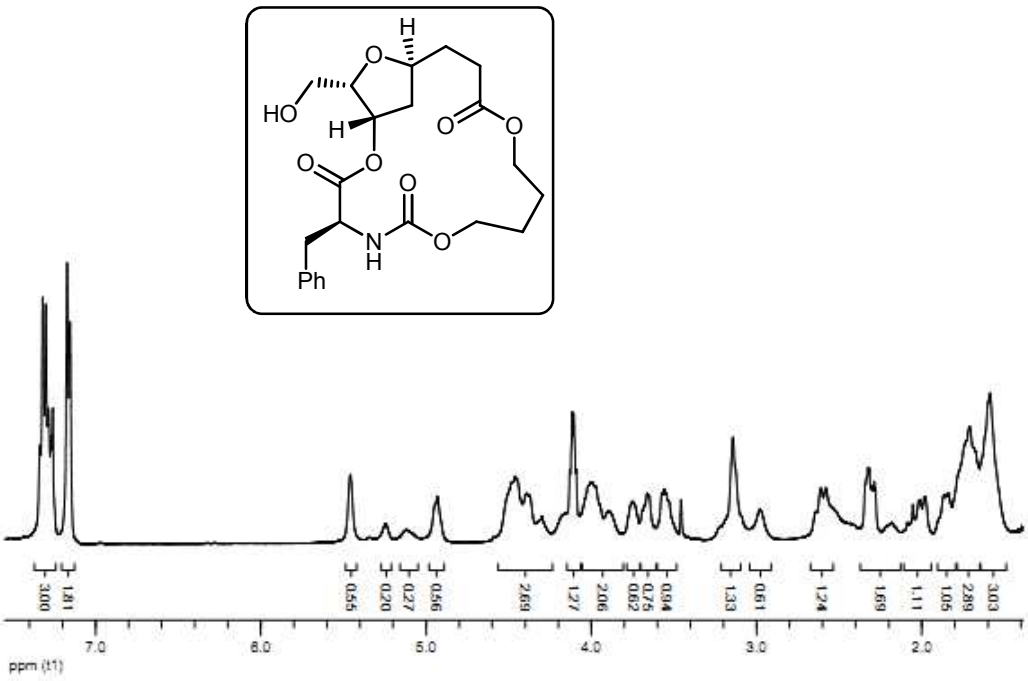
^{1}H NMR ($CDCl_3$, 400MHz)



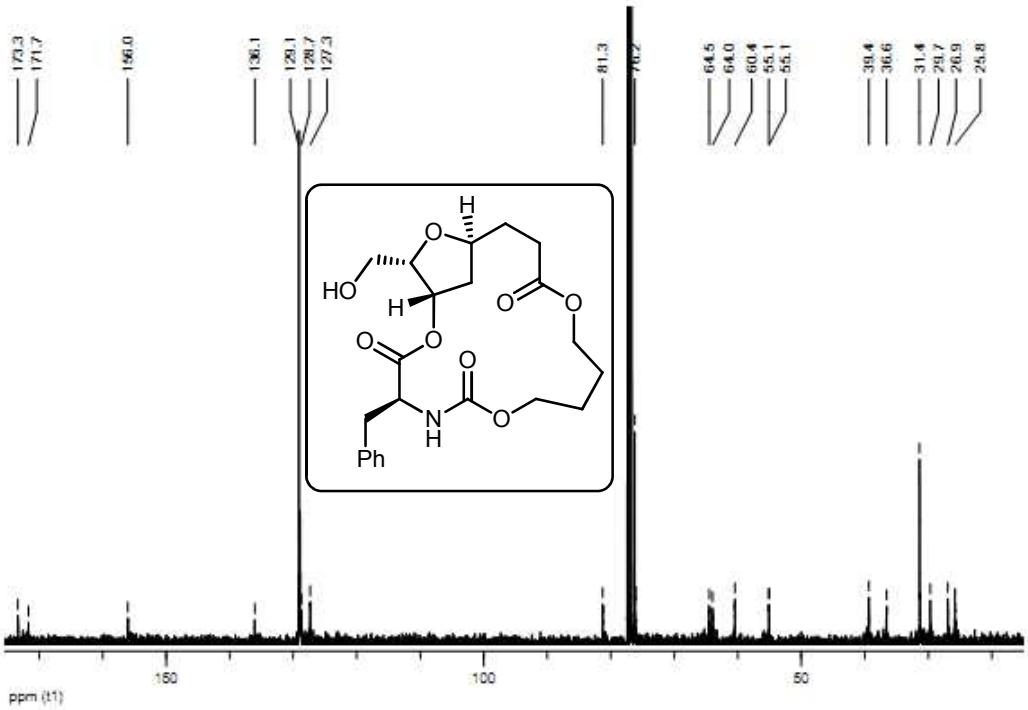
^{13}C NMR ($CDCl_3$, 100MHz)



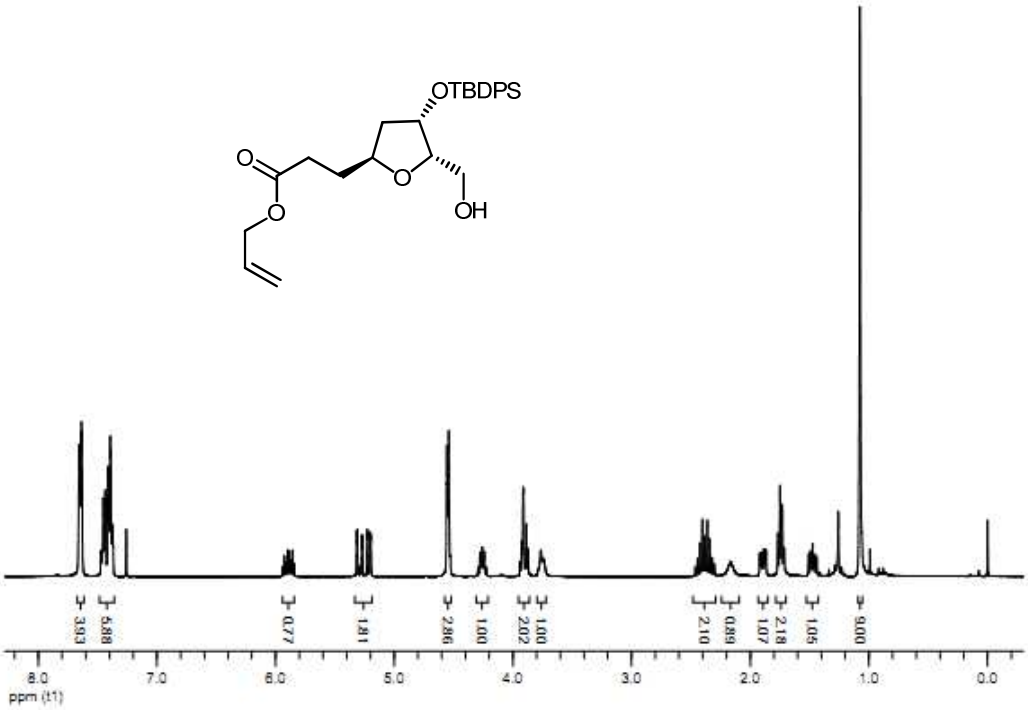
^{1}H NMR ($CDCl_3$, 400MHz)



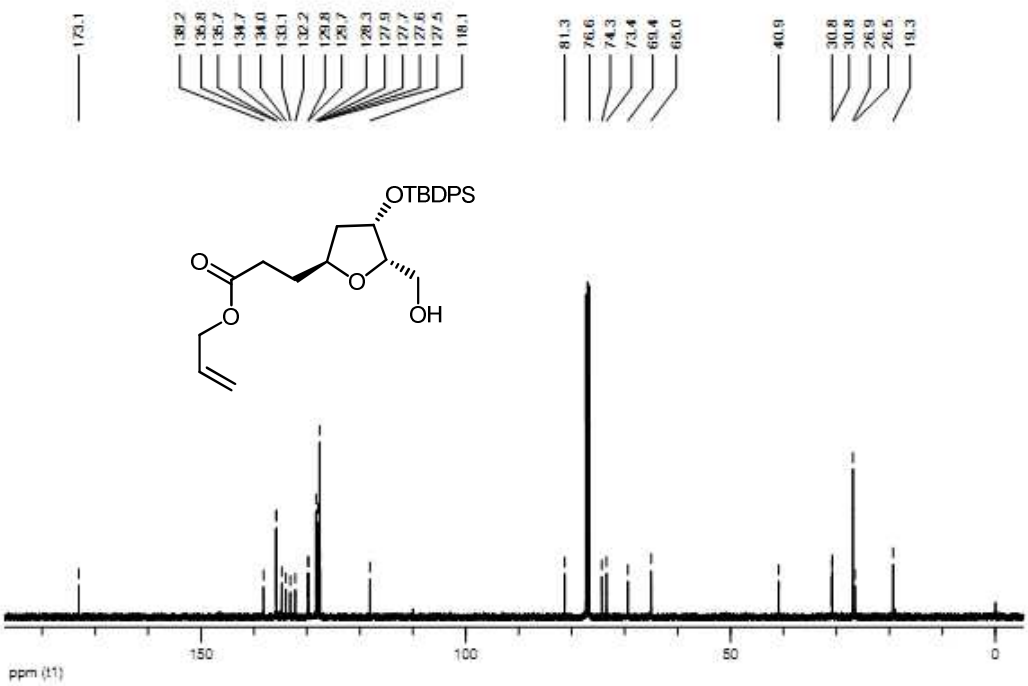
^{13}C NMR ($CDCl_3$, 100MHz)



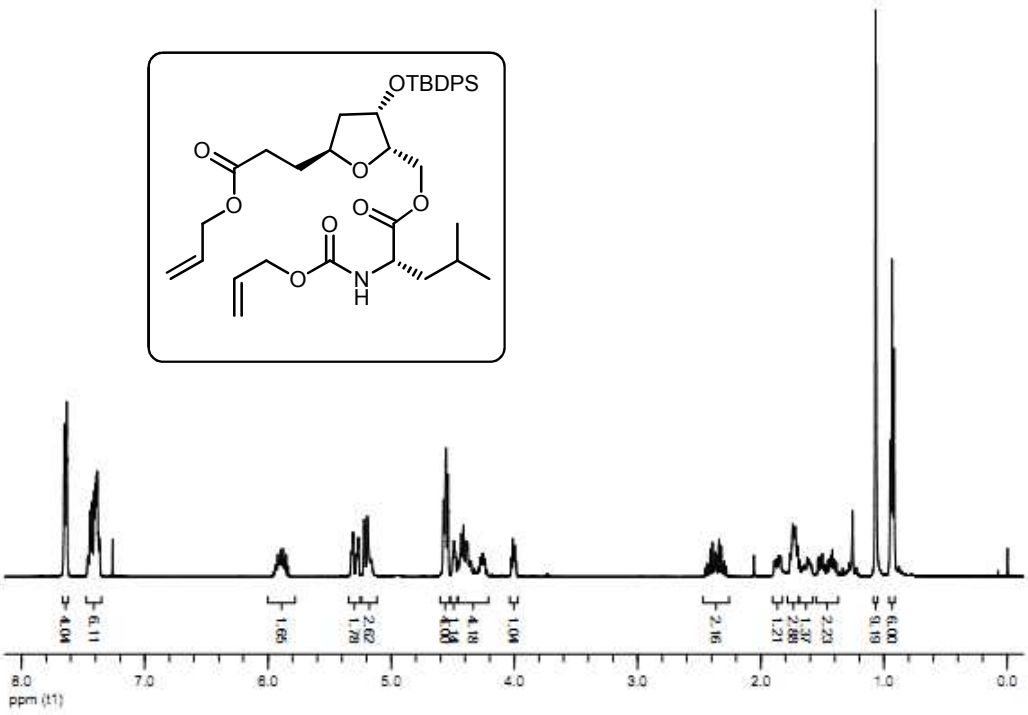
^{1}H NMR ($CDCl_3$, 400MHz)



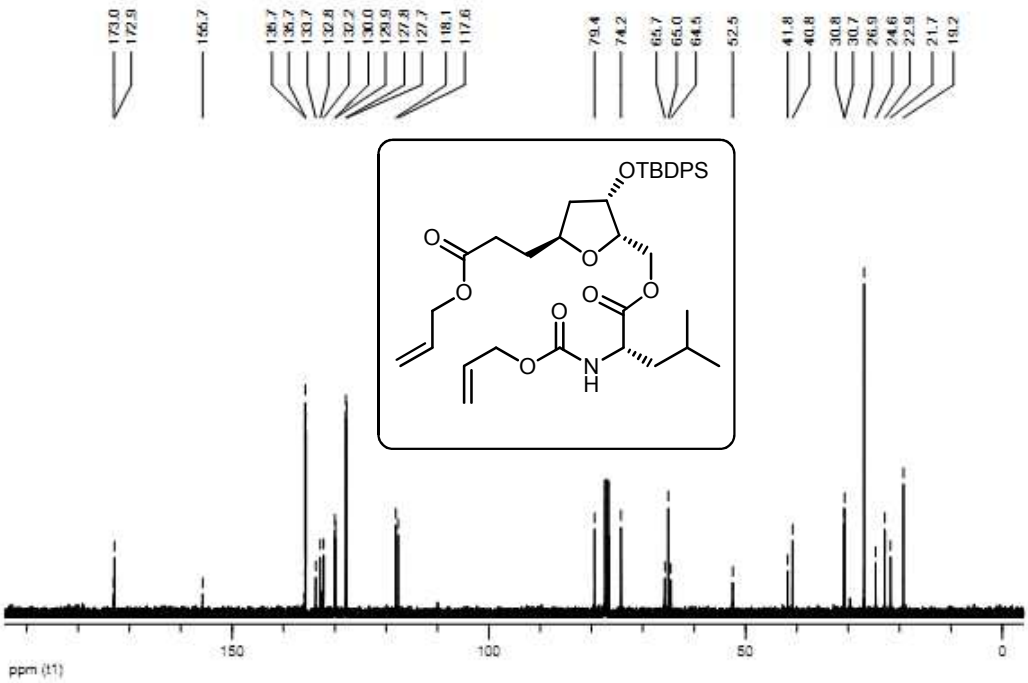
^{13}C NMR ($CDCl_3$, 100MHz)



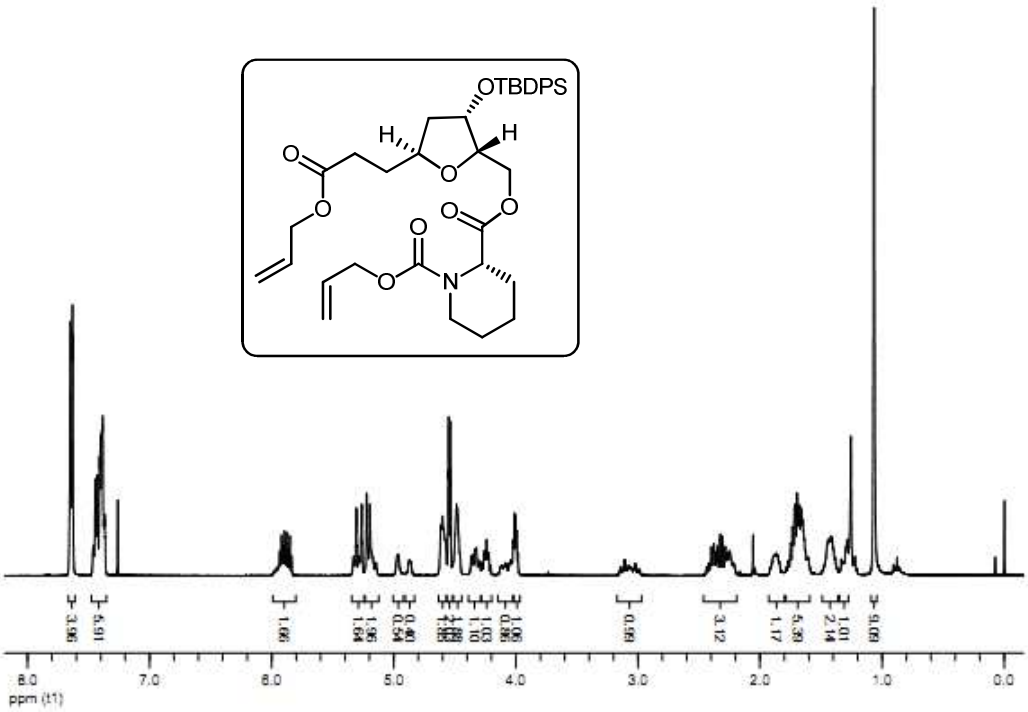
^{1}H NMR ($CDCl_3$, 400MHz)



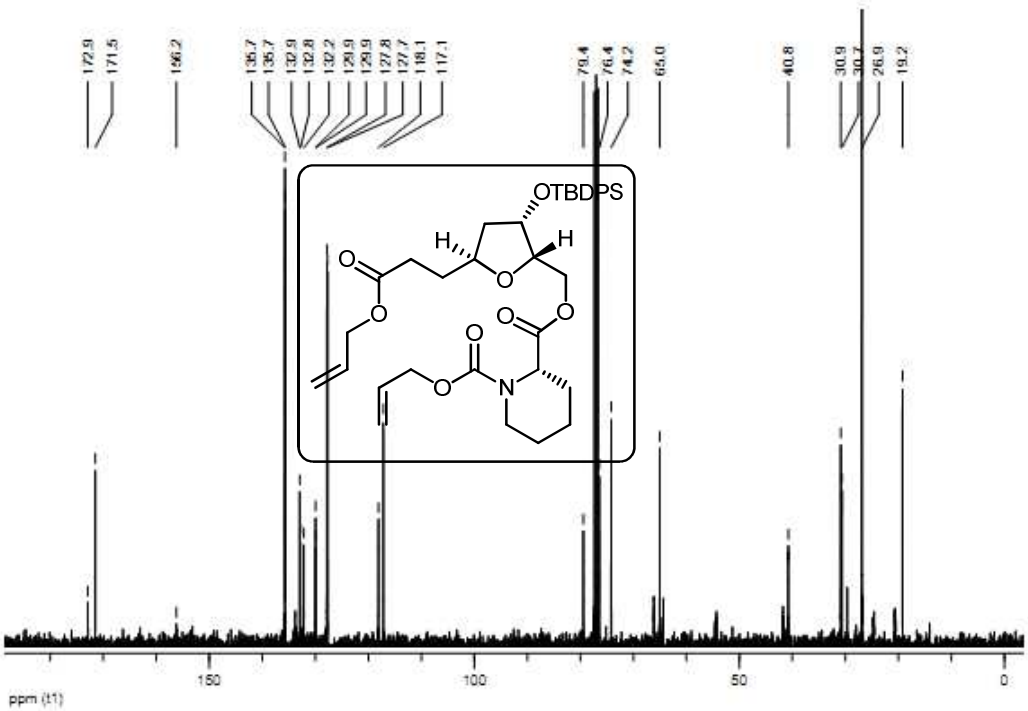
^{13}C NMR ($CDCl_3$, 100MHz)



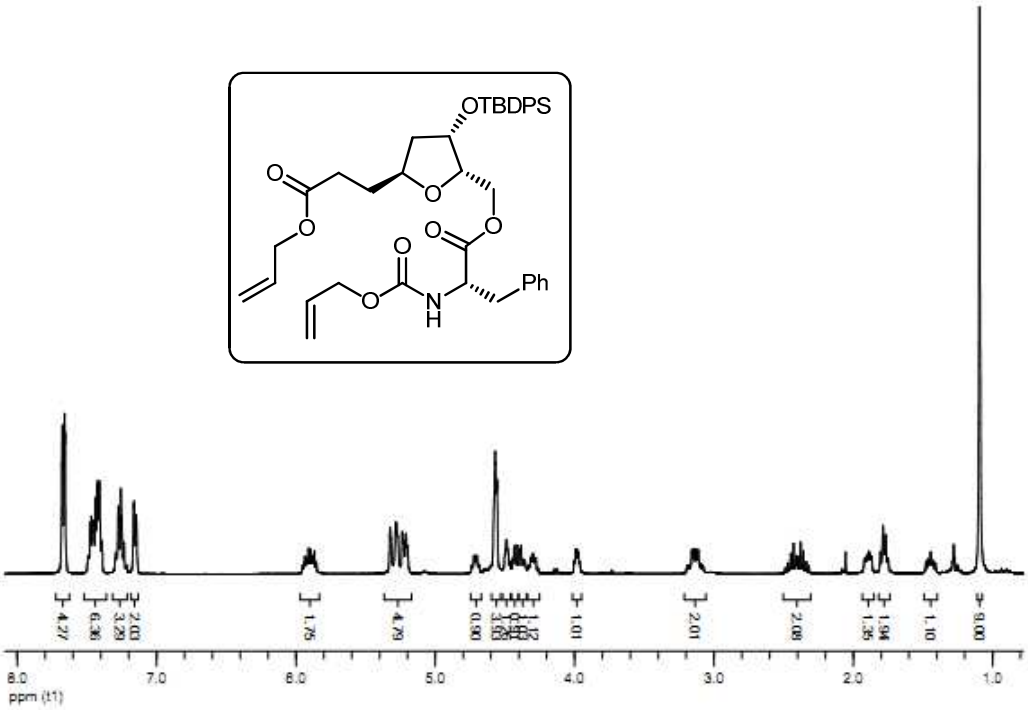
^{1}H NMR ($CDCl_3$, 400MHz)



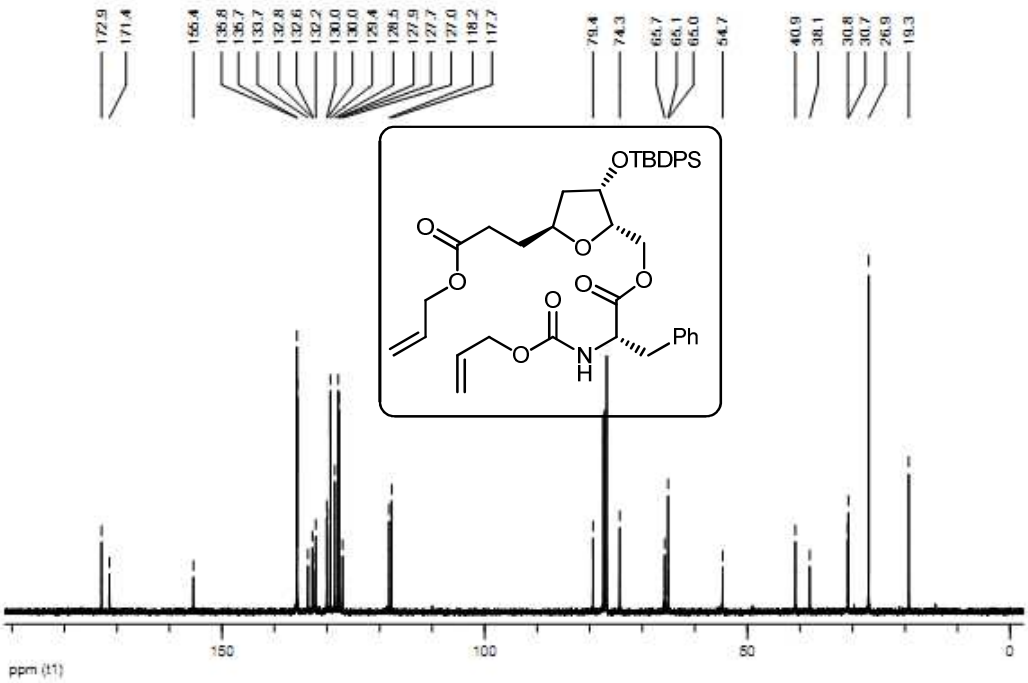
^{13}C NMR ($CDCl_3$, 100MHz)



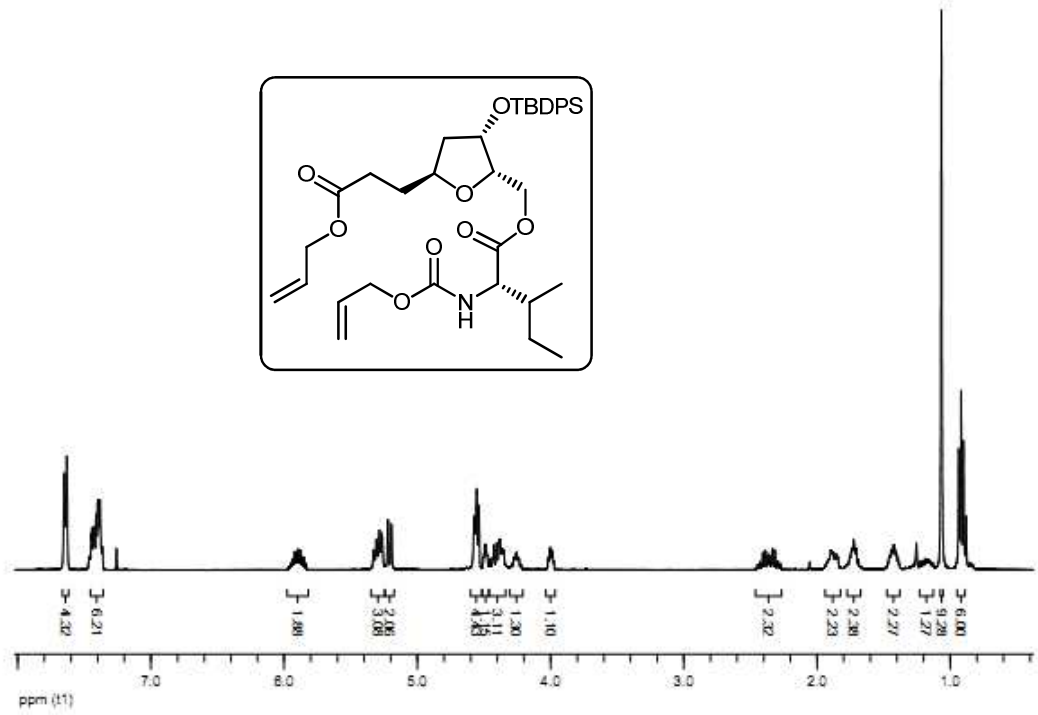
^{1}H NMR ($CDCl_3$, 400MHz)



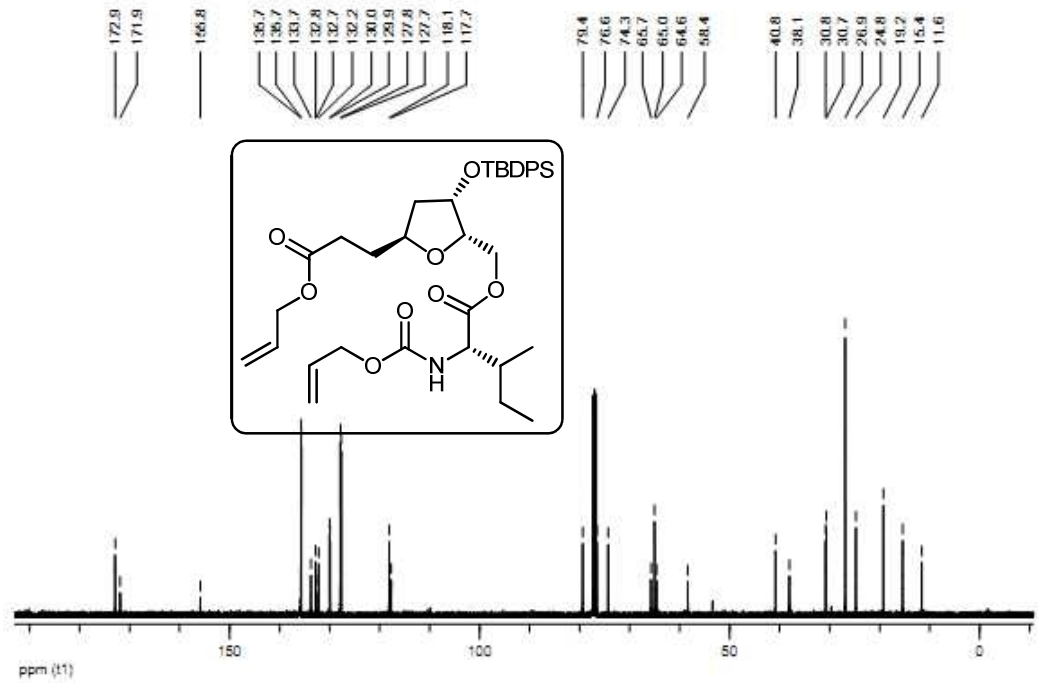
^{13}C NMR ($CDCl_3$, 100MHz)



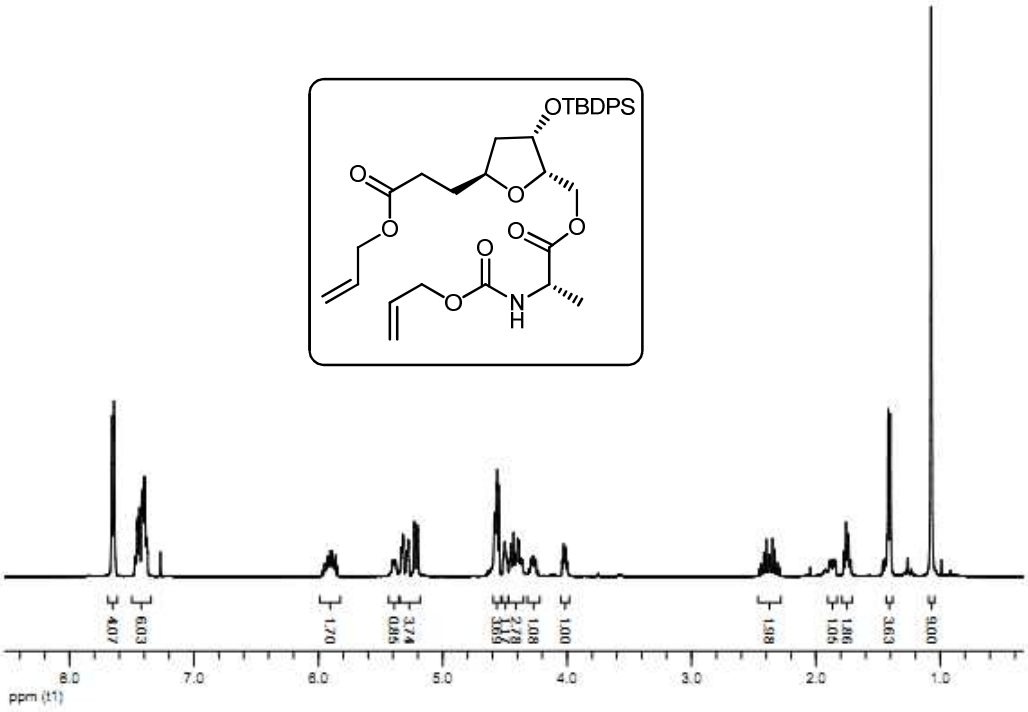
^{1}H NMR ($CDCl_3$, 400MHz)



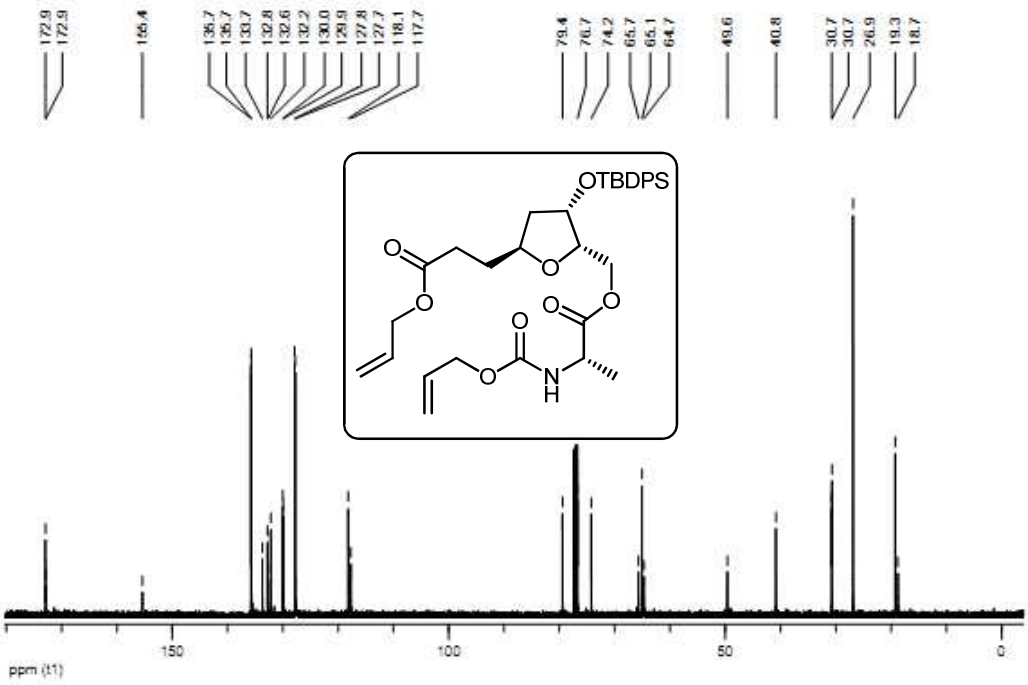
^{13}C NMR ($CDCl_3$, 100MHz)



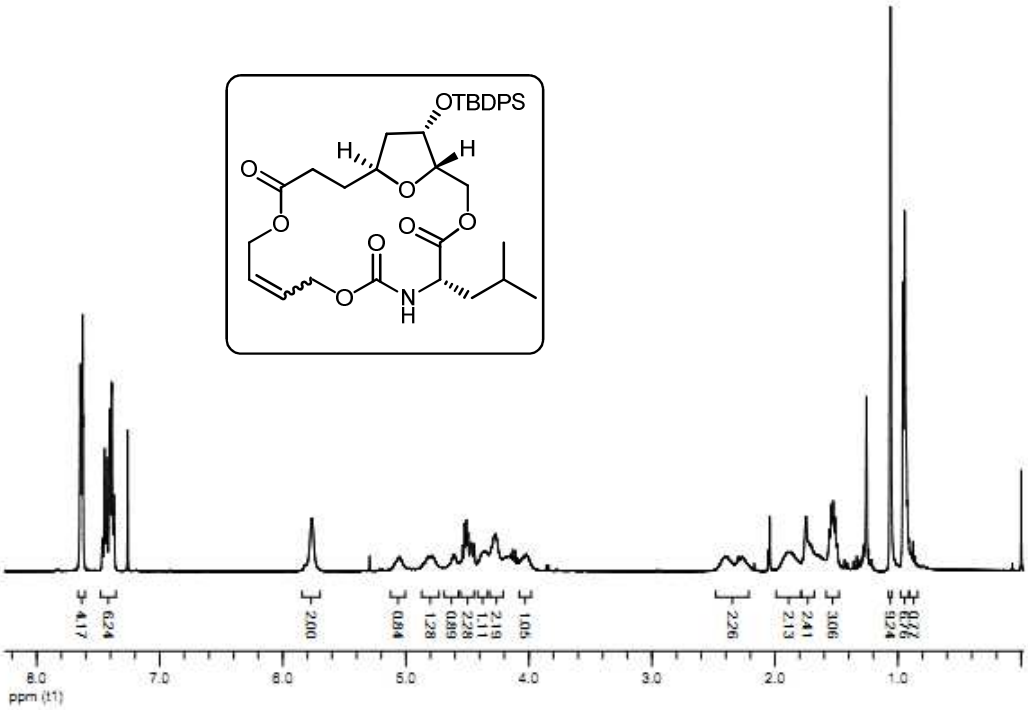
^{1}H NMR ($CDCl_3$, 400MHz)



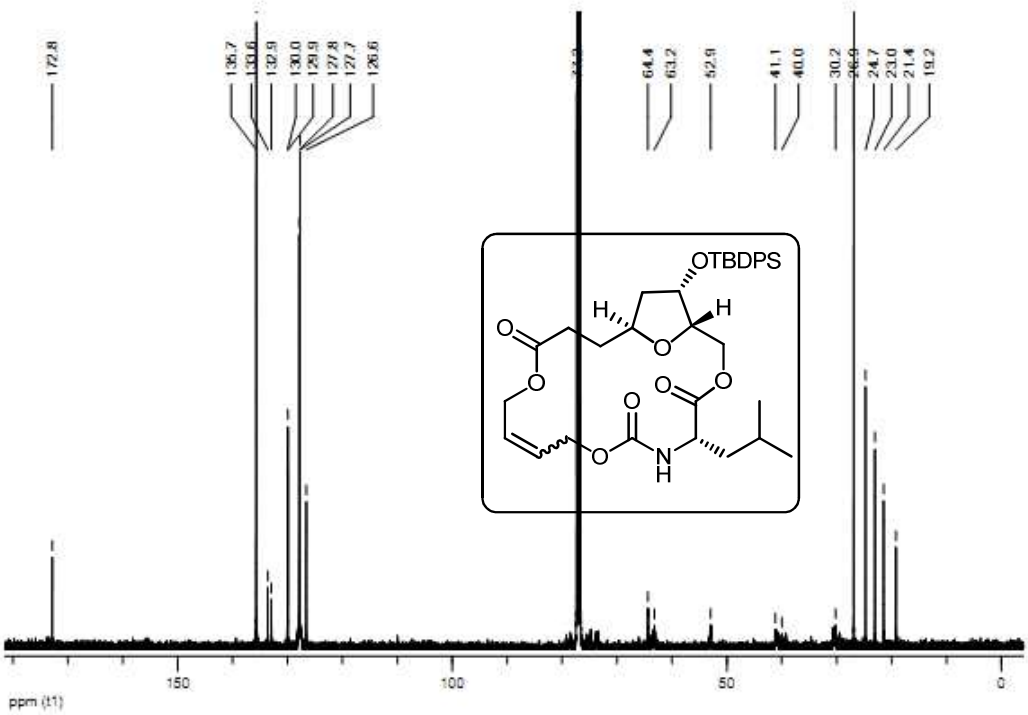
^{13}C NMR ($CDCl_3$, 100MHz)



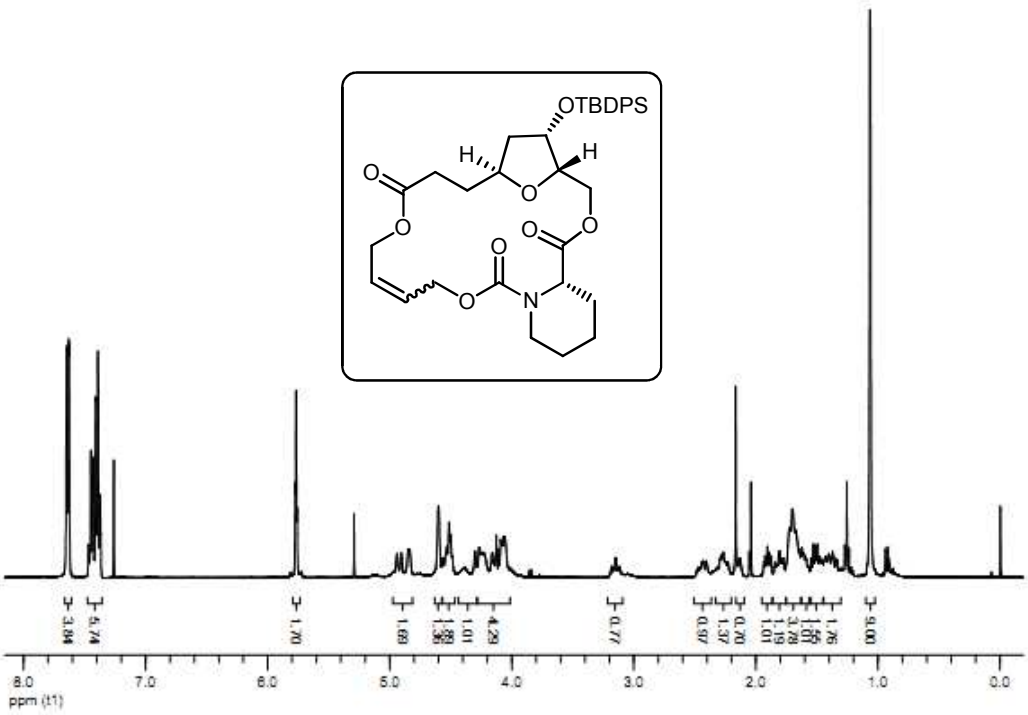
^{1}H NMR ($CDCl_3$, 400MHz)



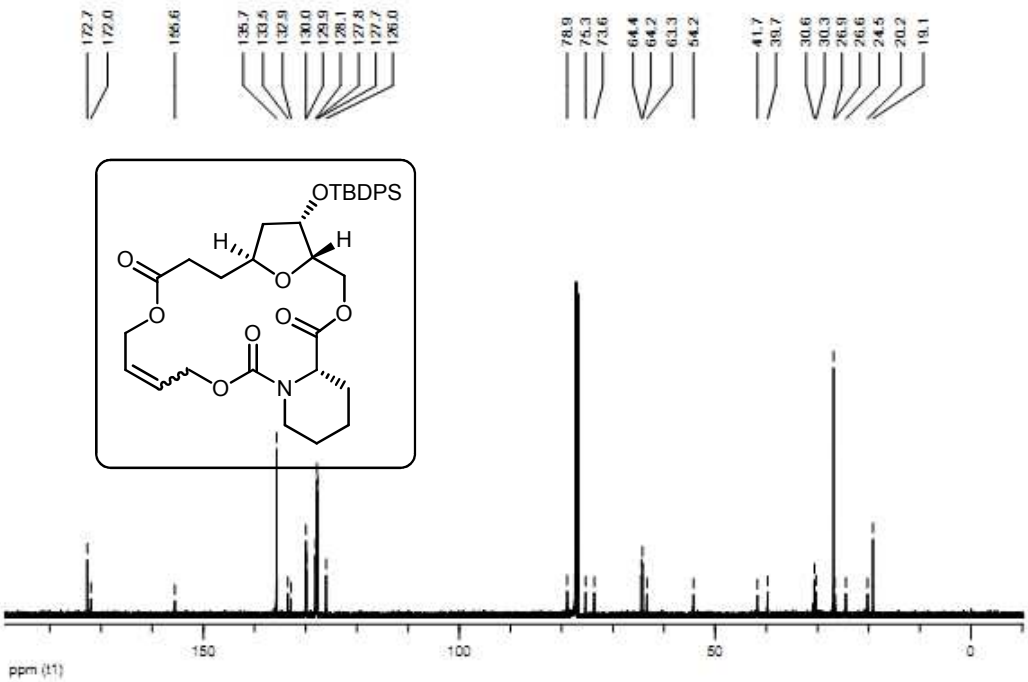
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



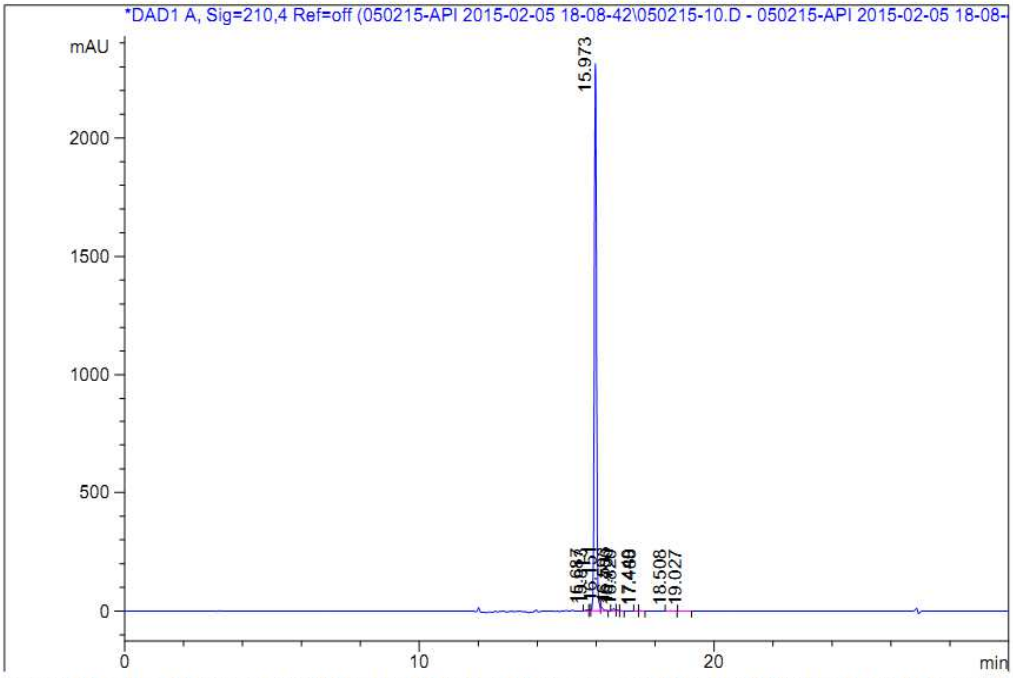
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

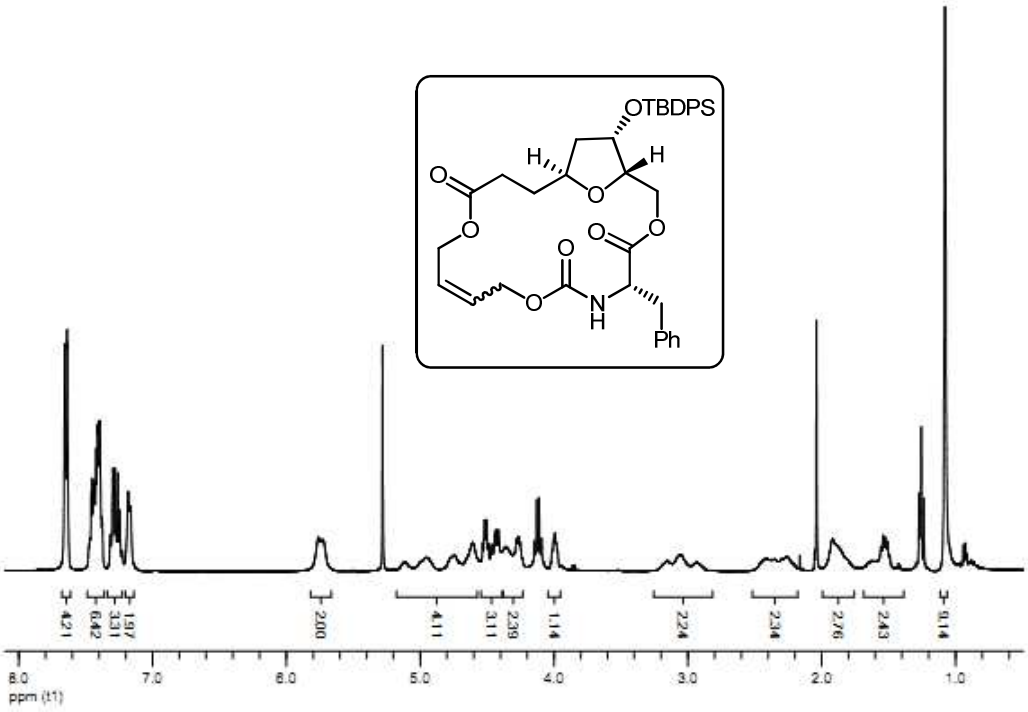
```
Inj Date        : Fri, 6. Feb. 2015          Acq Operator: SHASHIDHAR
Sample Name     : ILS-MNK-ERB-C-223                        Vial 20
A R Number      : CM15B005                   Inj. Vol.    : 5µL
Acq. Method     : D:\chem32\1\DATA\050215-API 2015-02-05 18-08-42\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API ASR.M
Method Info     : Column :  X-Terra RP18 250*4.6mm,5µm
                  Mobile phase: A) 5mm Ammonium Acetate in water B) ACN
                  A:B: 0/20,2/20,8/60,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```



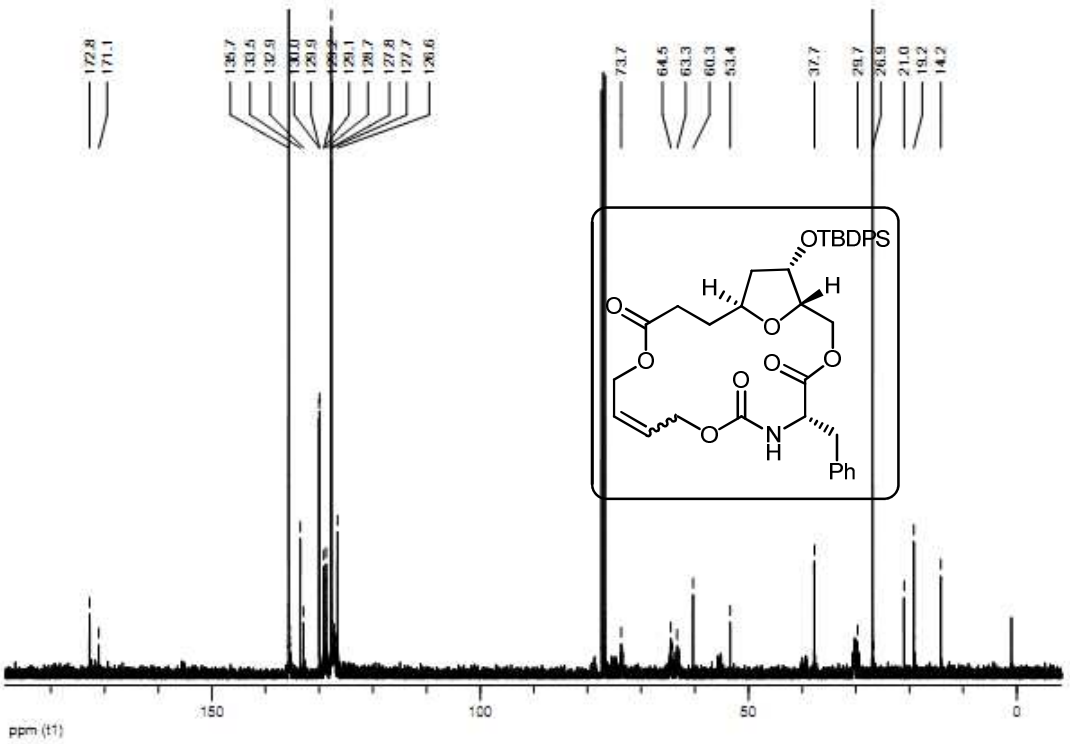

```
Signal  1: DAD1 A, Sig=210,4 Ref=off
```

Peak #	RT [min]	Area	Area %
1	15.687	26.587	0.219
2	15.813	10.848	0.090
3	15.973	11889.736	98.108
4	16.151	80.107	0.661
5	16.593	50.181	0.414
6	16.700	17.560	0.145
7	16.820	4.757	0.039
8	17.440	6.345	0.052
9	17.488	10.608	0.088
10	18.508	10.886	0.090
11	19.027	11.360	0.094

^{1}H NMR ($CDCl_3$, 400MHz)



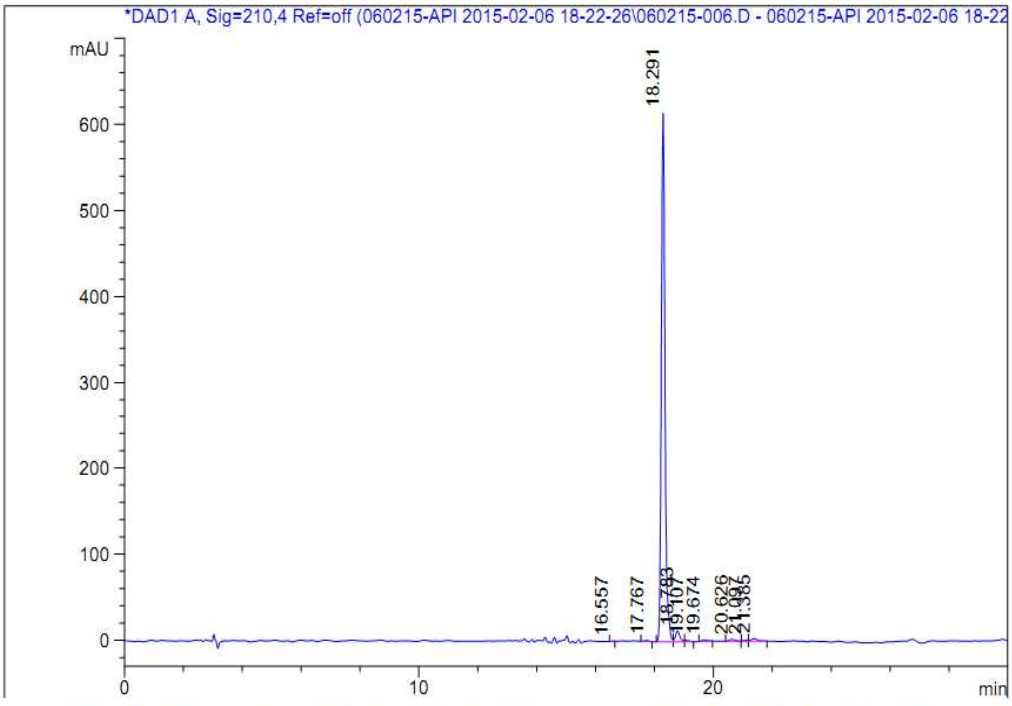
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

```
Inj Date        : Fri, 6. Feb. 2015            Acq Operator: SHASHIDHAR
Sample Name     : ILS-MNK-ERB-C-237                          Vial 19
A R Number      : CM15B006                   ->Inj. Vol.    : 5µL
Acq. Method     : D:\chem32\1\DATA\060215-API 2015-02-06 18-22-26\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API ASR.M
Method Info     : Column: X-Terra C-18 250*4.6mm, 5µm
                  Mobile phase: A) 5mm Ammonium Acetate in water B) ACN
                  A:B: 0/20,2/20,8/60,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```

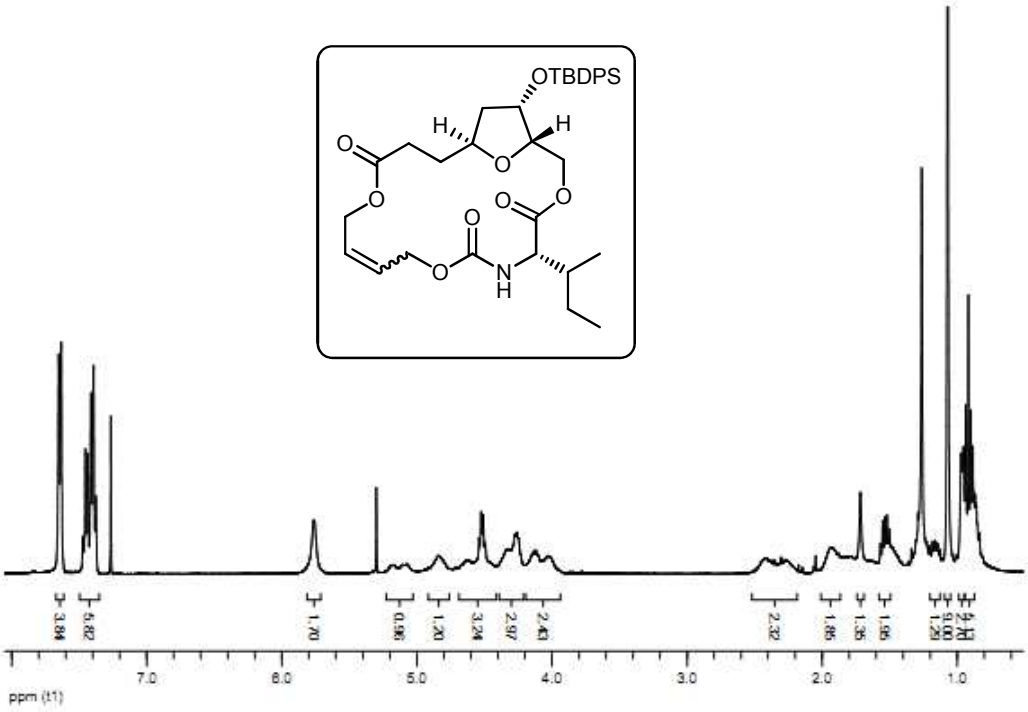



```
==================================================================================
```

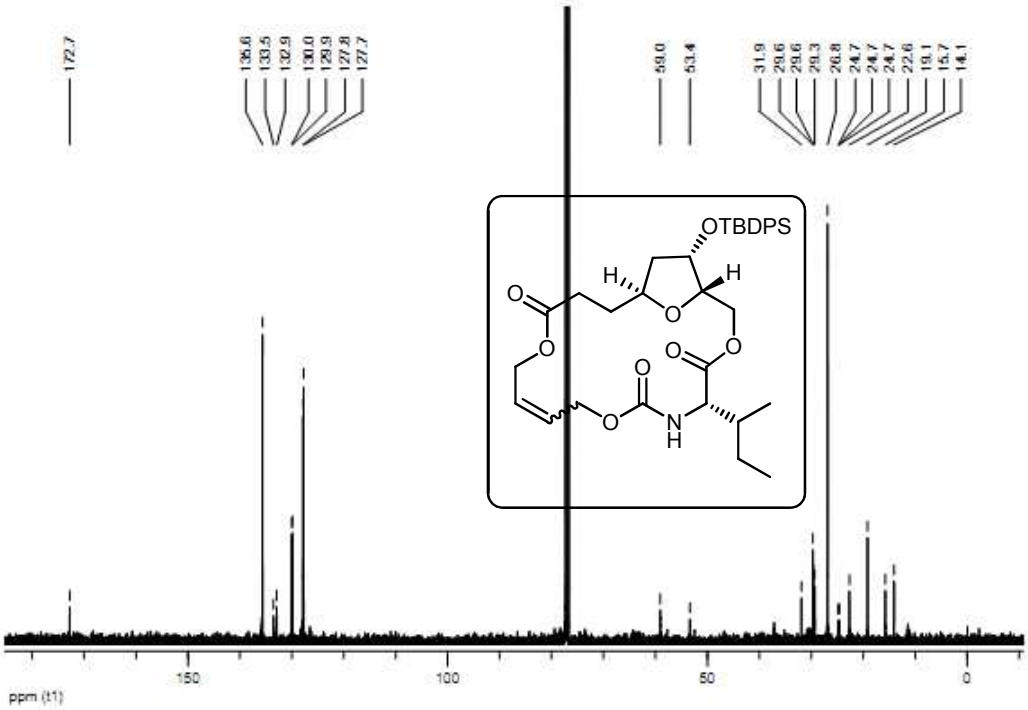
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RT [min]	Area	Area %
1	16.557	3.224	0.058
2	17.767	11.888	0.214
3	18.291	5302.142	95.275
4	18.783	131.052	2.355
5	19.107	17.196	0.309
6	19.674	21.494	0.386
7	20.626	25.032	0.450
8	21.097	7.683	0.138
9	21.385	45.369	0.815

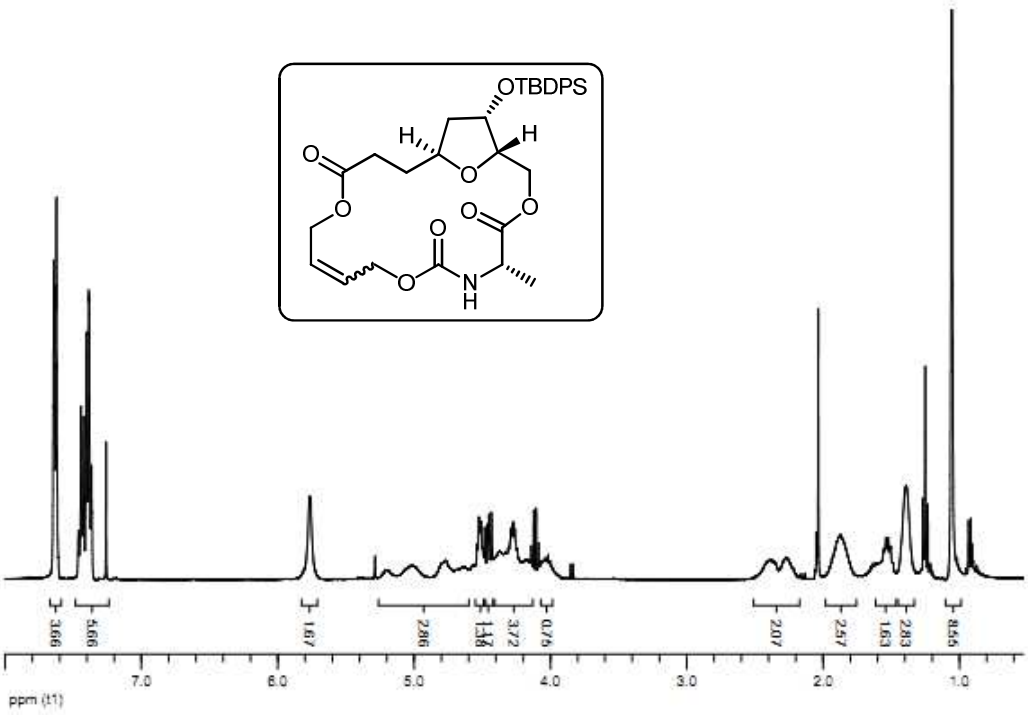
^{1}H NMR ($CDCl_3$, 400MHz)



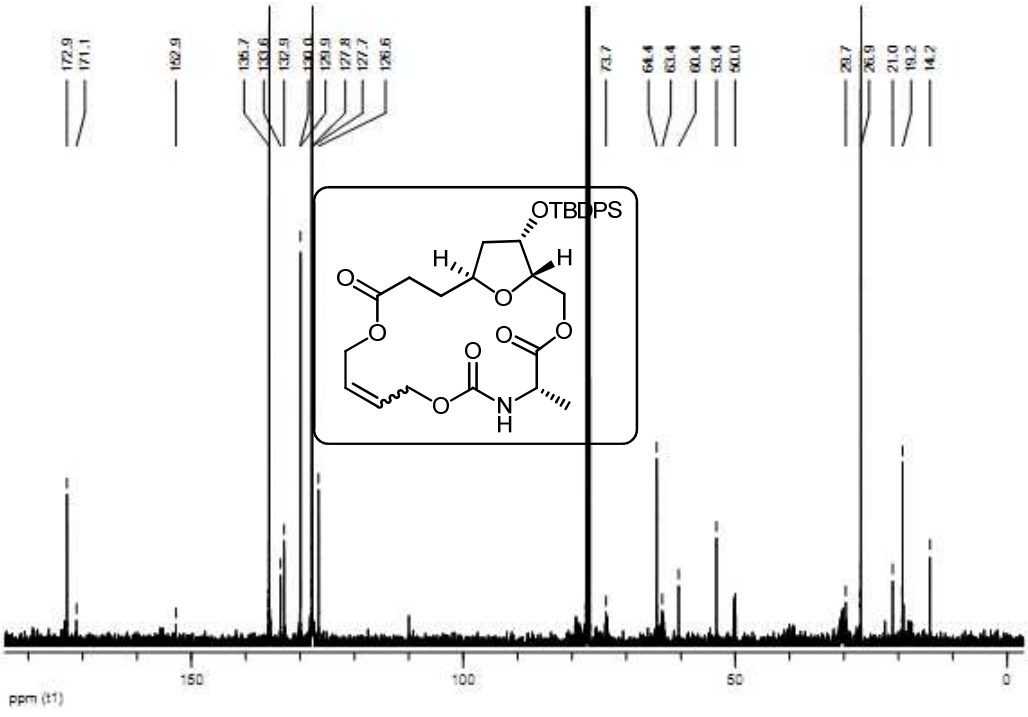
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



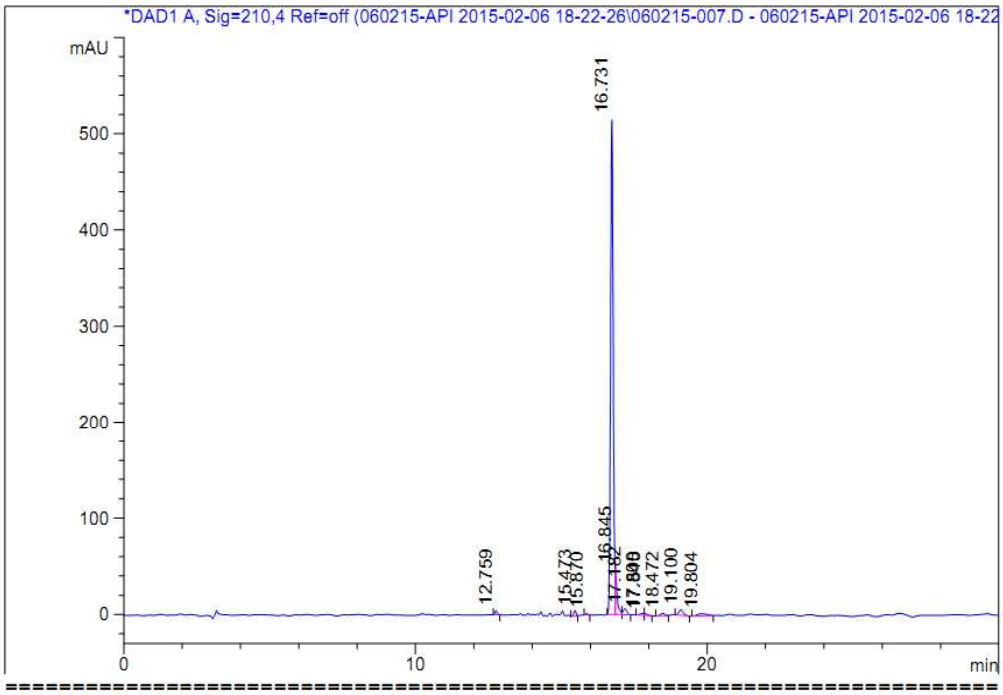
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

CPRI @ DRILS
HPLC ANALYSIS REPORT

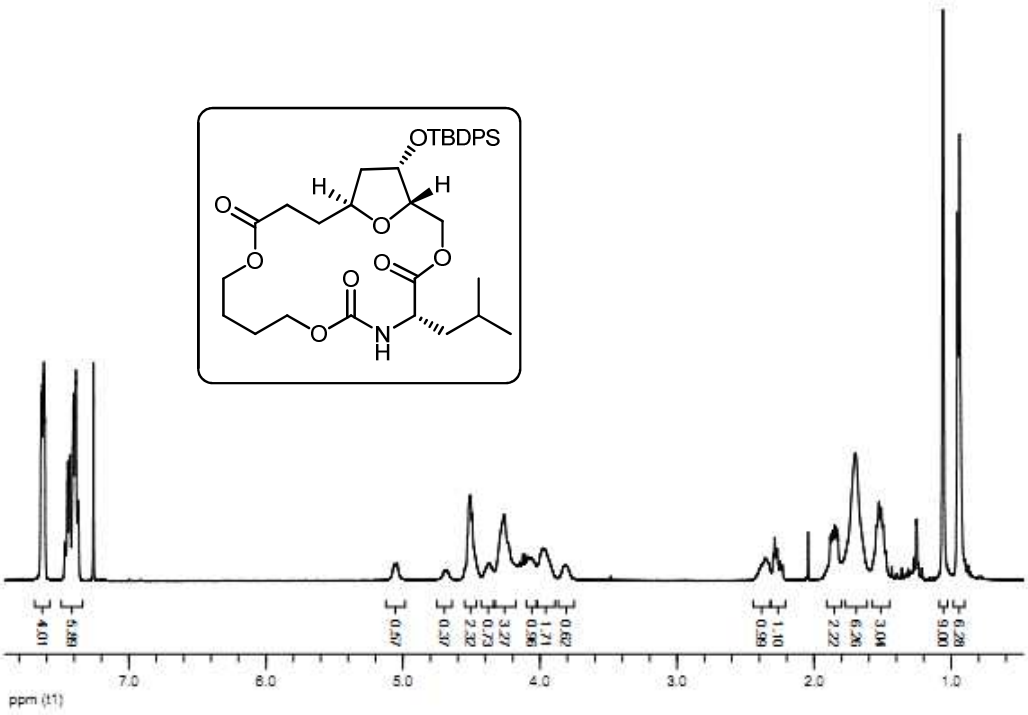
```
Inj Date        : Fri, 6. Feb. 2015          Acq Operator: SHASHIDHAR
Sample Name     : ILS-MNK-ERB-C-238                        Vial 28
A R Number      : CM15B007                  ->Inj. Vol.    : 5µL
Acq. Method     : D:\chem32\1\DATA\060215-API 2015-02-06 18-22-26\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API ASR.M
Method Info     : Column: X-Terra C-18 250*4.6mm, 5µm
                  Mobile phase: A) 5mm Ammonium Acetate in water B) ACN
                  A:B: 0/20,2/20,8/60,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```



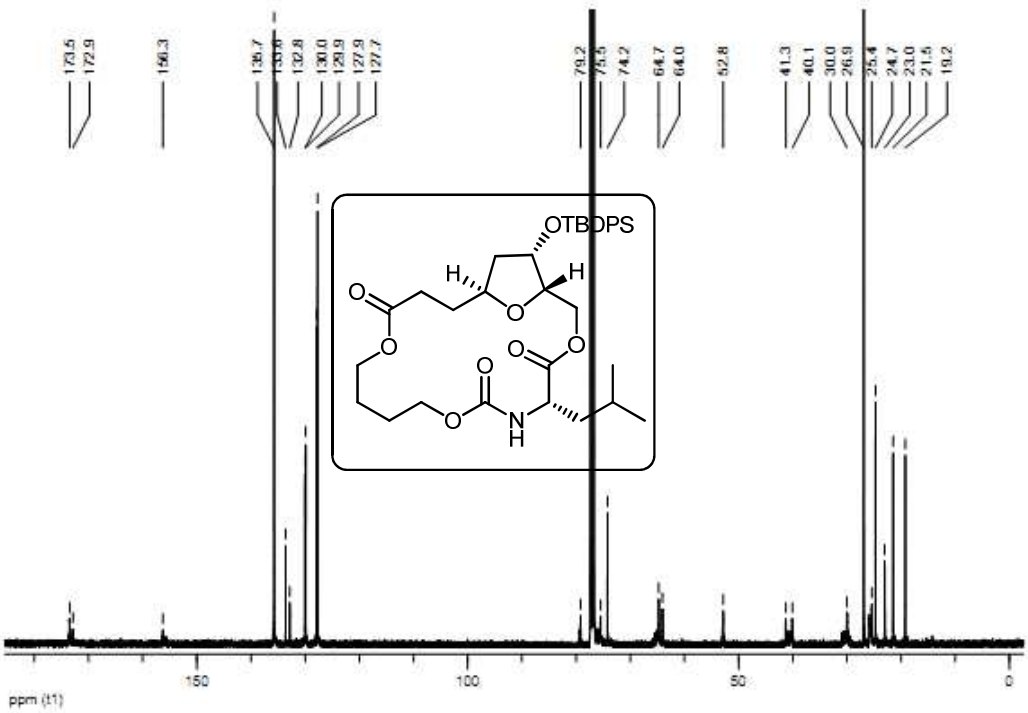
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RT [min]	Area	Area %
1	12.759	21.218	0.570
2	15.473	30.668	0.824
3	15.870	7.901	0.212
4	16.731	3253.234	87.433
5	16.845	195.957	5.266
6	17.182	54.133	1.455
7	17.800	14.877	0.400
8	17.845	10.970	0.295
9	18.472	24.495	0.658
10	19.100	60.032	1.613
11	19.804	47.349	1.273

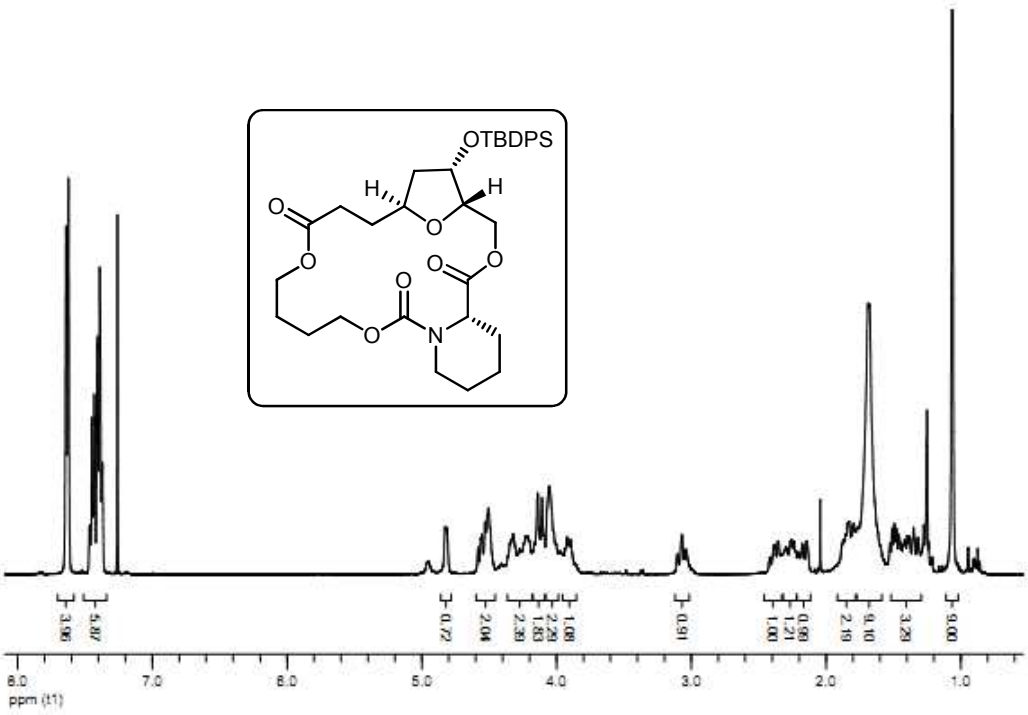
^{1}H NMR ($CDCl_3$, 400MHz)



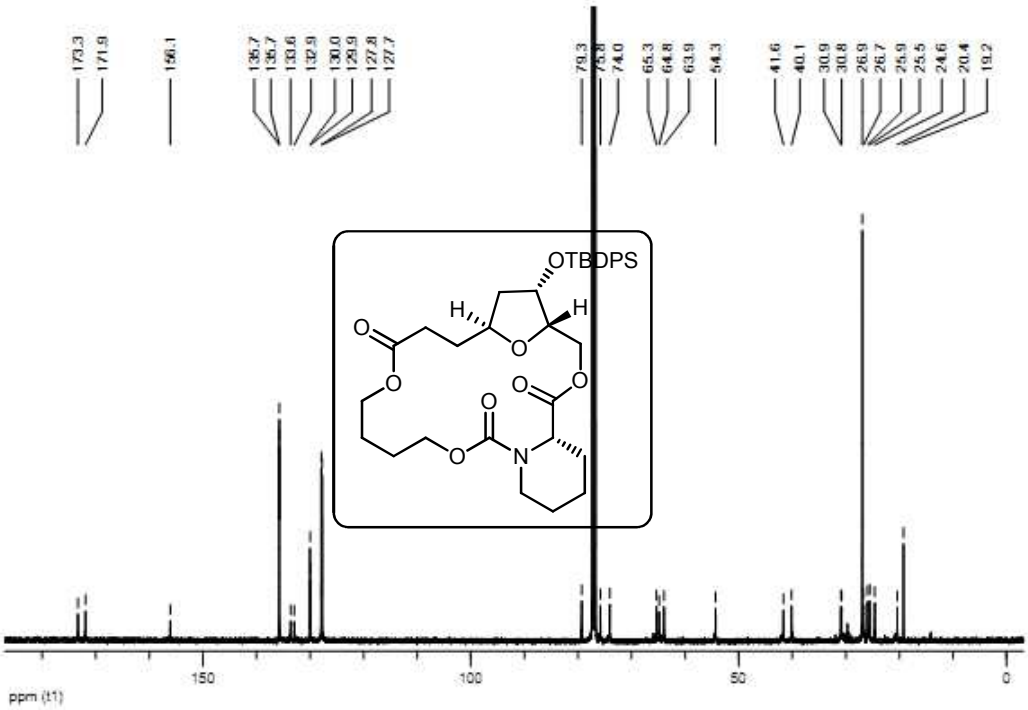
^{13}C NMR ($CDCl_3$, 100MHz)



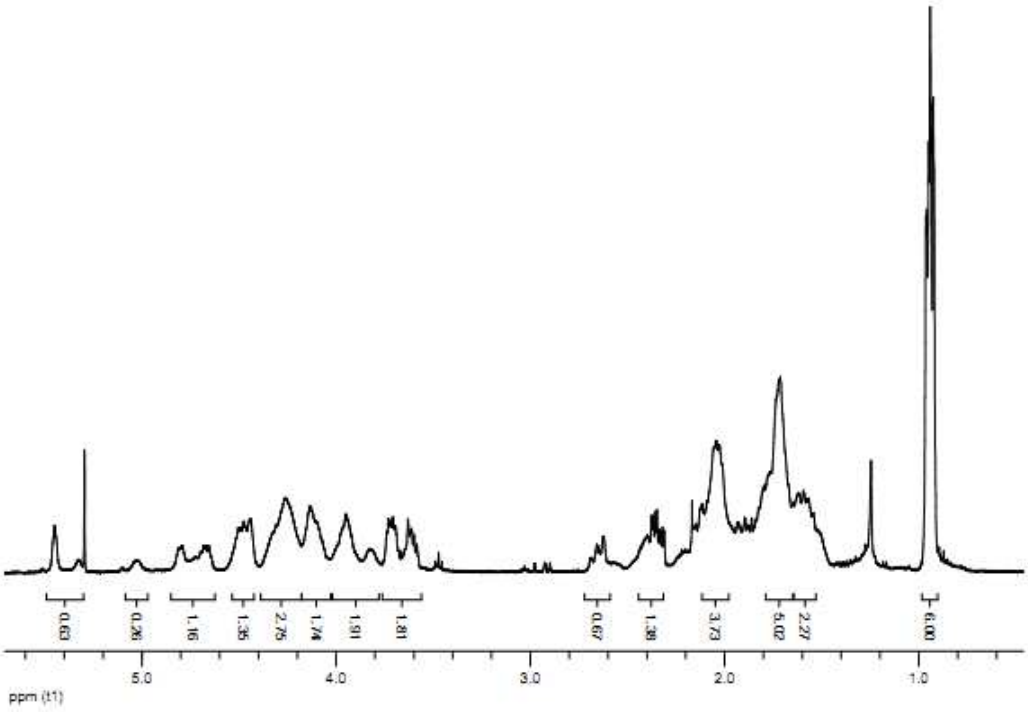
^{1}H NMR ($CDCl_3$, 400MHz)



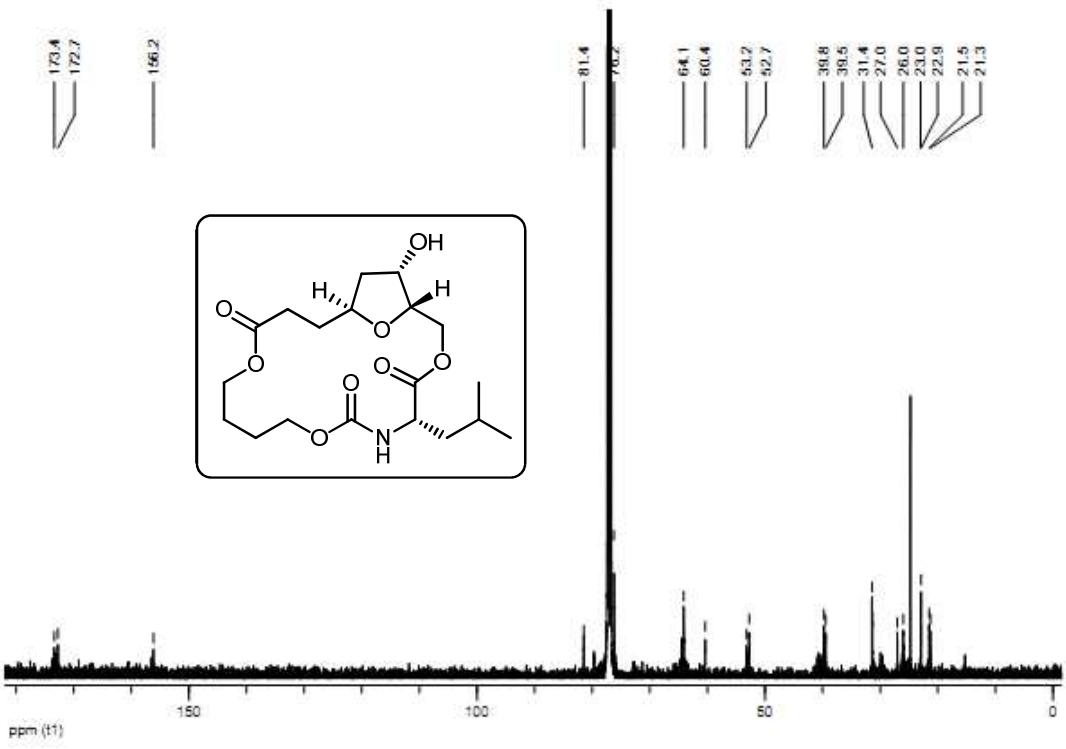
^{13}C NMR ($CDCl_3$, 100MHz)



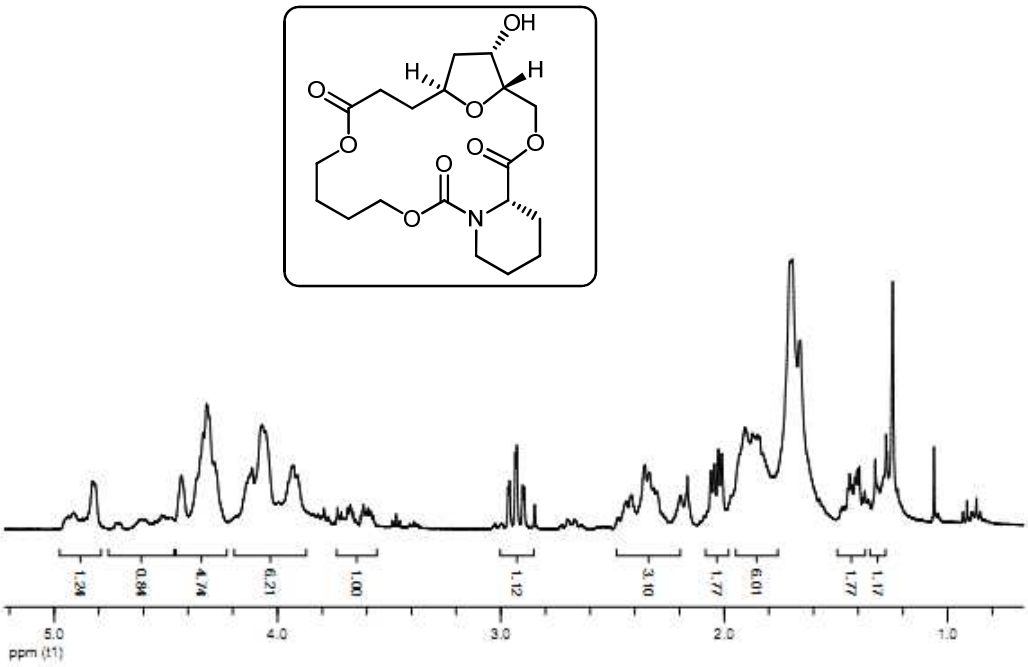
^{1}H NMR ($CDCl_3$, 400MHz)



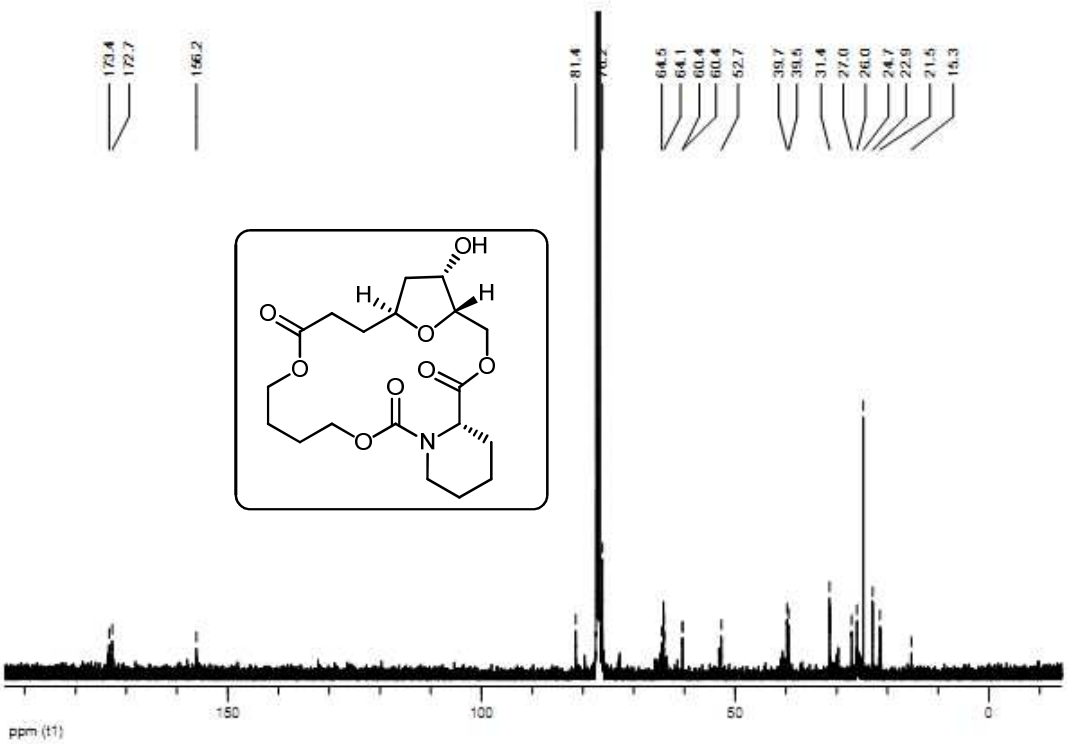
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



Chapter 3

Synthesis of C22-C28 Eribulin Fragment for Building A Diverse Set of Macrocycles

3.1. Introduction

As already discussed about eribulin (**F1.1**) and its biological importance in the second chapter, herein, with our continued interest in developing practical synthesis approaches to various sub-structures of eribulin and other bioactive natural products, I am going to discuss the synthesis of the C22-C28 (**F1.2**) fragment of eribulin. Many research groups have contributed their efforts towards the total synthesis of eribulin and its various key fragments.

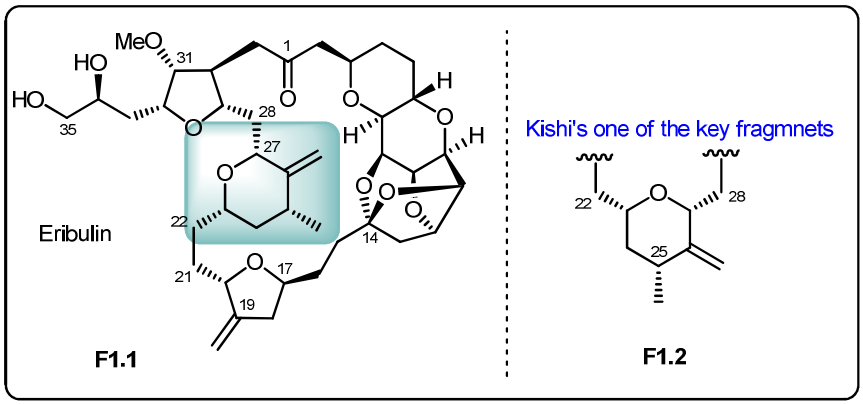


Figure 1: Eribulin (**F1.1**) and One of the Key Fragments of Eribulin **(F1.2)** Having 2,6 *cis*-Tetrahydropyran Ring

3.2. Working Hypothesis

With our continued interest in developing practical synthesis approaches to various sub-structures of eribulin and other bioactive natural products,[1,2] and their utilization in obtaining different sets of macrocyclic compounds, we focused our attention to the C22-C28 substituted tetrahydropyran fragment. This type of sub-structure along with the various types of macrocyclic rings are also commonly found in several other bioactive natural products. For example, peloruside A (**F2.1**),[3] *cis*-2,6- disubstituted tetrahydropyran is embedded in the functionalized 14-membered macrocyclic ring, and it is also known for its microtubule-stabilizing properties. Another family of natural products that contain this moiety along with the macrocyclic ring are (+)-dactylolide (**F2.2**),[4] and (-)-zampanolide (**F2.3**);[5] *cis*-2,6-disubstituted tetrahydropyran is embedded in the functionalized 18-membered macrocyclic ring in both, and they are known for their potent cytotoxicity. (-)-Zampanolide, in addition to having cytotoxic effect, it also possesses the potent microtubule–stabilizing properties.[6]

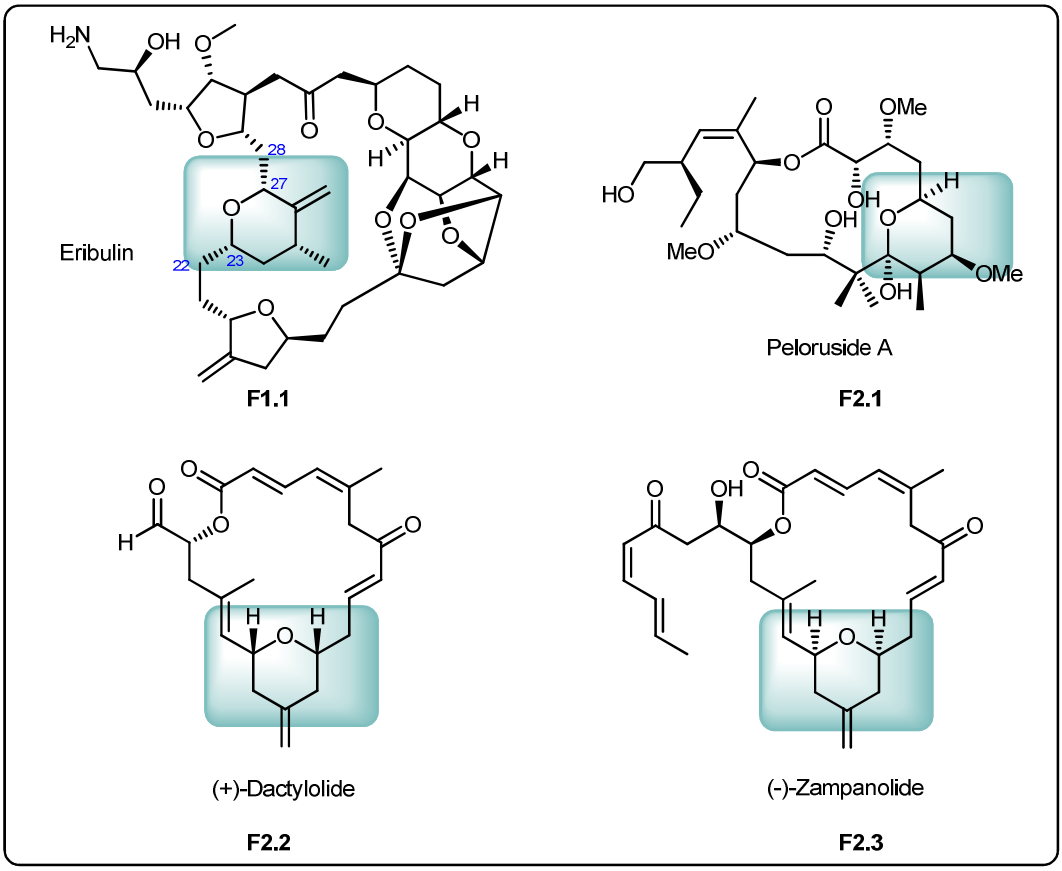
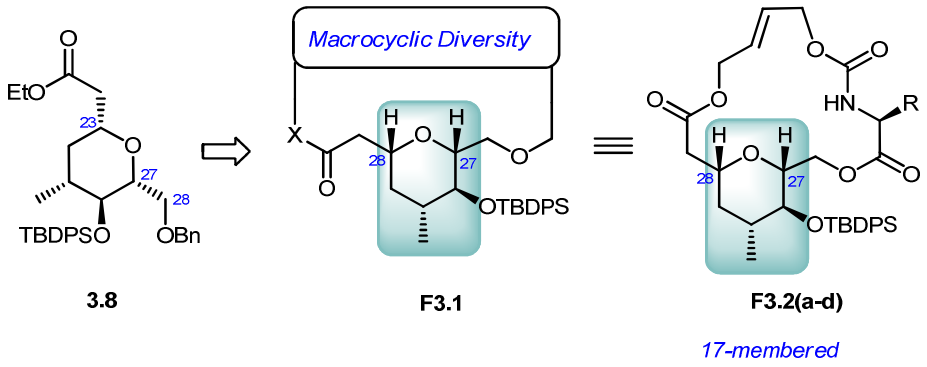


Figure 2: Erbulin and Other Bioactive Natural Products Containing the *cis* -2,6-Disubstituted Tetrahydropyran Moiety Embedded in the Macrocyclic Rings.

Due to the presence of *cis*-2,6-disubstituted tetrahydropyran moiety in eribulin and several bioactive natural products, we considered this as a privileged scaffold, and it can serve as a good starting point in building a chemical toolbox with a diverse set of macrocyclic compounds. With this goal in mind, we set three objectives, and these were: (i) to develop a practical and scalable synthesis of *cis*-2,6-disubstituted tetrahydropyran moiety as the key scaffold **3.8** (note: eribulin numbering is shown, see **Figure 2**), (ii) to complete the synthesis of the eribulin fragment having this moiety, and (iii) to develop a modular synthesis for obtaining macrocyclic compounds having a17- membered ring (see, **F3.2**). In our approach, the utilization of *cis*-2,6-disubstituted groups at C23 and C27 leads to a macrocycle with the 17-membered ring. The incorporation of an amino acid moiety in macrocyclic rings allow introducing a chiral diversity site for obtaining further structural analogs. The long-term goal of this study is accessing several different types of macrocyclic compounds having this privileged *cis*-2,6-disubstituted tetrahydropyran moiety.



3.8 **F3.1** **F3.2(a-d)**

Figure 3: Macrocyclic Target Having 17 Membered Ring (**F3.2**) from the Substituted Tetrahydropyran Moiety **(3.8)**

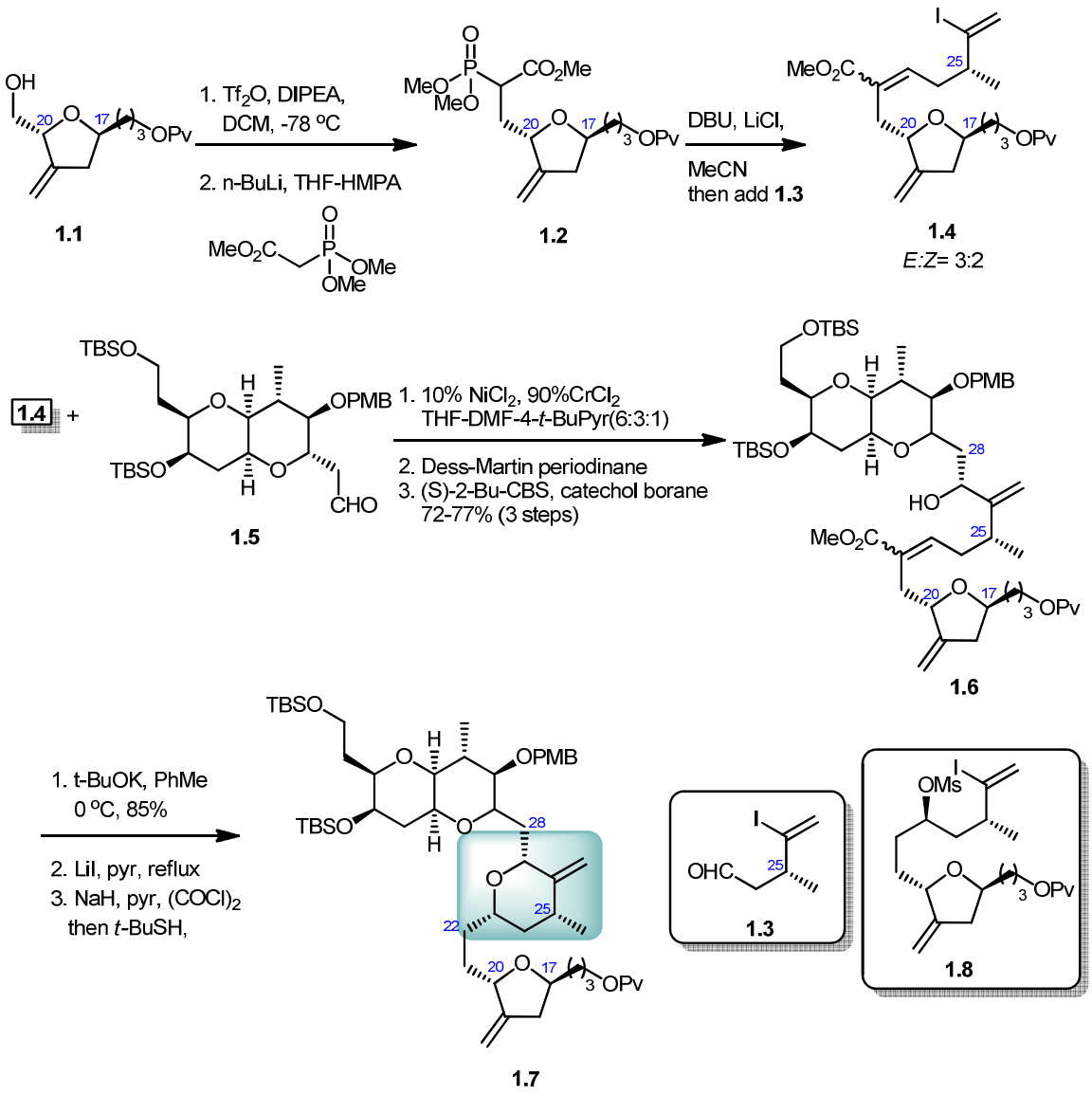
3.3 Literature Synthesis of C22-C28 Eribulin Fragment

In this section, the literature synthesis of C22-C28 eribulin fragment is covered.

3.3.1 Kishi's Approach

In 1992, Kishi and co-workers reported the synthesis of the C22-C28 fragment of eribulin from the coupling of two key sub-units, **1.5** with **1.8**. This was achieved using Ni(II)/Cr(II)-mediated coupling reaction, followed by a base induced cyclization, leading to an inversion, and this approach, furnished the synthesis of **1.7.**

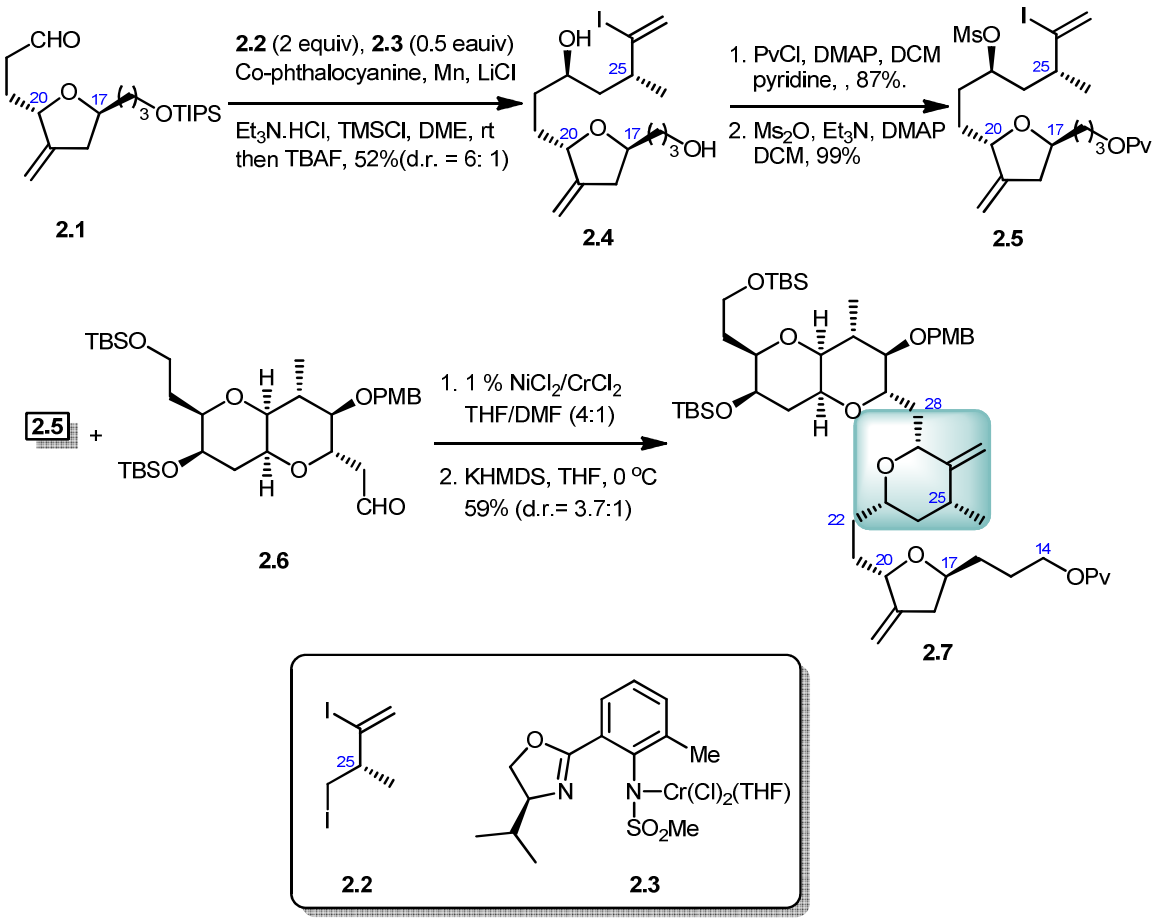
In 1997, the same group, further developed another approach to the synthesis C22-C28 fragment.[7] The synthesis was started with an activation of alcohol **1.1**[8] to its corresponding triflate derivative and the conversion to phosphonoester **1.2**. This reaction was carried-out in one pot. The modified Horner-Emmons reaction (Roush-Masamune conditions)[9] of **1.2** with an aldehyde **1.3**,[8] gave C14-C26 fragment as a 3:2 mixture of *E:Z* isomers. The NHK (Nozaki-Hiyama-Kishi) reaction between **1.4** and aldehyde **1.5**[8] produced the 4:1 diastereomeric mixture at the C27 center. The 4:1 diastereomeric mixture was then converted into a 17:1 diastereomeric mixture *via* Dess-martin oxidation[10] followed by the reduction approach with Corey's oxazaborolidine reagent.[11] The Michael reaction of **1.6** gave the cyclization product. The final step in this synthesis involved two steps, and these were: (i) the hydrolysis of the methyl ester into the corresponding acid under SN^2 conditions, and (ii) Barton's decarboxylation[7] for obtaining the desired product, **1.7** in 75% overall yield.



Scheme 1: The Synthesis of the C22-C28 Tetrahydropyran Fragment

3.3.2 Phillips' Approach

In 2009, Phillips and co-workers synthesized eribulin, by developing the novel synthetic routes to the subunits of eribulin and further their utilization in stitching technologies. This team synthesized the C22-C28 fragment of eribulin. The synthesis was started with an aldehyde **2.1**[12] (note: the synthesis of **2.1** is discussed in **Chapter 2**) and this was reacted with **2.2** in the presence of oxazoline/sulfonamide ligand **2.3** by using Kishi's protocol.[13] This approach furnished the synthesis of the diol **2.4** following the silyl group removal.



Scheme 2: The Synthesis of the C22-C28 Tetrahydropyran Fragment

The selective protection of the primary alcohol with pivaloyl chloride followed by mesylation gave compound **2.5.** The mesylated compound and an aldehyde **2.6** was further coupled by an established combination of Nozaki-Hiyama-Kishi reaction and the subsequent pyran ring formation[8] by SN^2 reaction. This approach produced **2.7** in 59% yield and the diastereoselectivity of this reaction was ~3.7:1.

3.4 Our Synthesis for the C22-C28 Eribulin Fragment

3.4.1 Retrosynthesis of C22-C28 Eribulin Fragment

The retrosynthetic analysis of our target **F4.1** is shown in **Figure 4.** Compound **F4.1** could be obtained from **F4.2** by an intramolecular oxy-Michael addition, protecting group removal, oxidation and terminal Wittig reactions. The α, β unsaturated ester **F4.2** could be easily obtained from **F4.3**. The lactone derivative, **F4.3** was planned from the cyclization and methylation of compound **F4.4.** The synthesis of **F4.4** was planned from iso-ascorbic acid in a few simple steps.

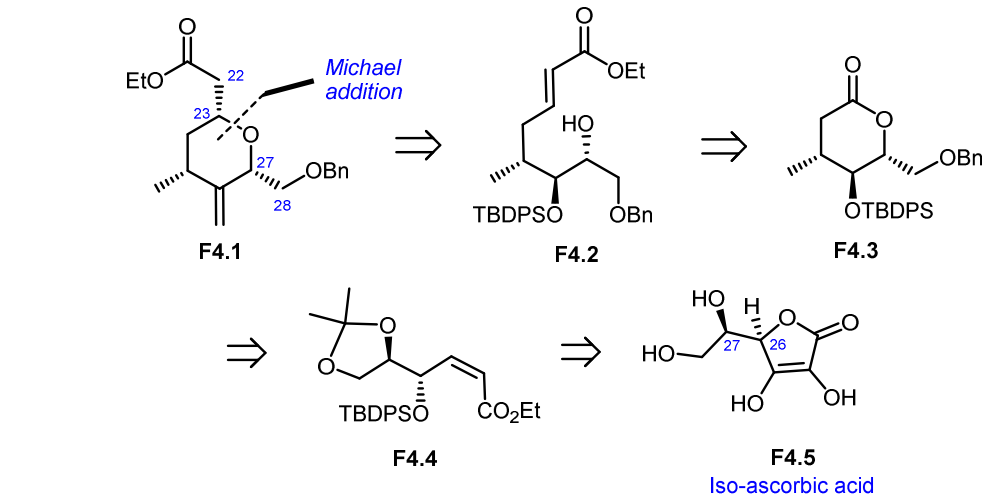
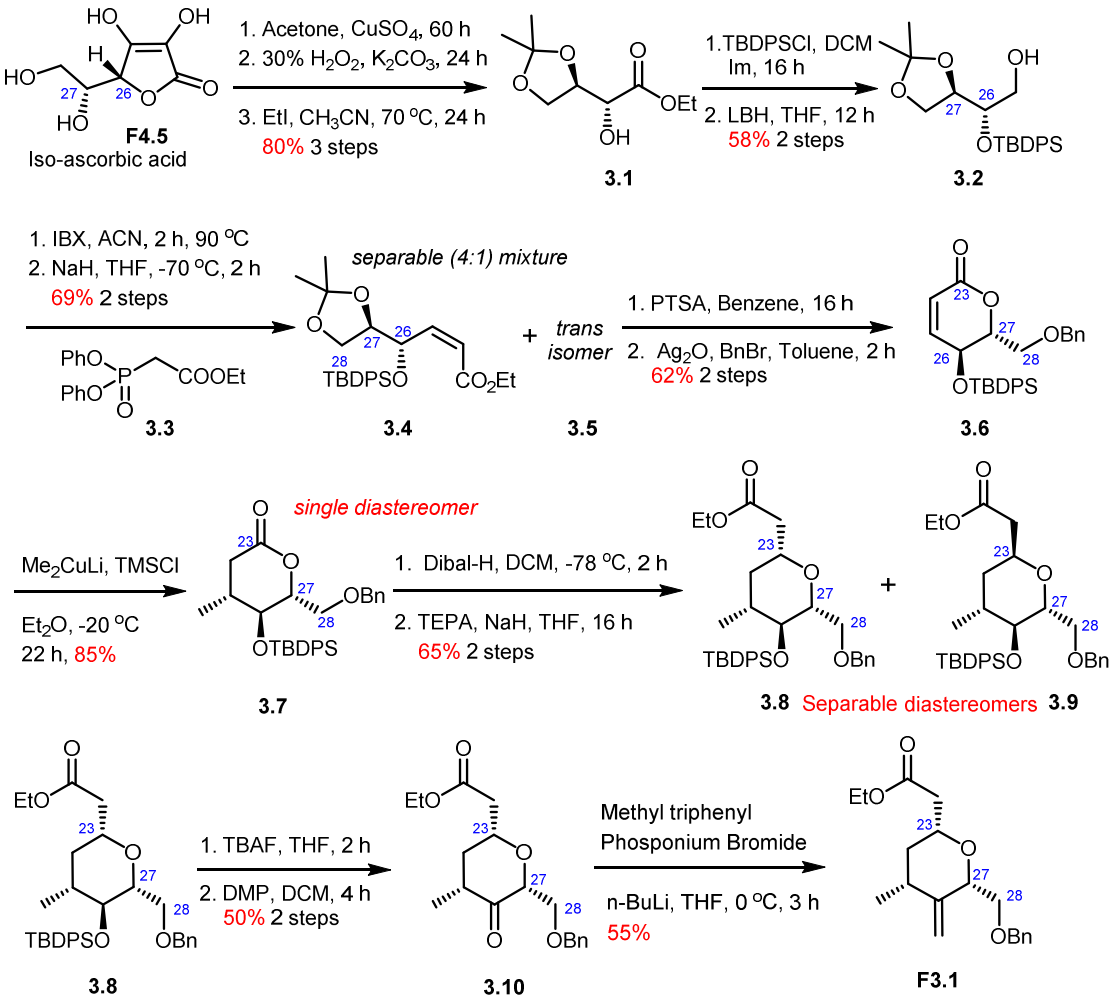


Figure 4: Retrosynthesis of C22-C28 Eribulin Fragment

3.4.2 Synthesis of C22-C28 Eribulin Fragment



Scheme 3: Synthesis of C22- C28 Fragment of Eribulin.

Our synthesis was started with a chiral starting material from Iso-ascorbic acid **F4.5,** and our plan is shown in **Scheme 3**. It was to converted to **3.1** in three simple steps.[14] This then led to producing the corresponding alcohol **3.2**, which following oxidation was then subjected to *cis* Wittig reaction,[15] thus giving the *cis*- (**3.4**) and *trans*- (**3.5**) products as two separable geometrical isomers in a 4:1 ratio. Compound **3.4** was then subjected for cyclization under the PTSA condition conditions,[16] and this approach gave α,β lactone, **3.6.** The diastereoselective conjugate addition[17] on **3.6** then produced compound **3.7**. The reduction followed by Horner-Wadsworth-Wittig reaction[18] furnished the synthesis of two separable diastereomers **3.8** and **3.9.** Finally, the required product **F3.1** was obtained from **3.8** in a series of steps that involved (i) the silyl group removal, (ii) oxidation and (iii) Wittig reaction.

Structural analysis

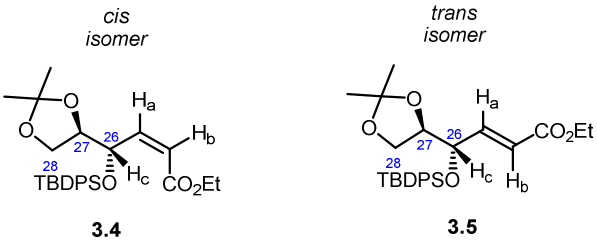


Figure 5: Assignment of **3.4** and **3.5**.

The compounds **3.4**, and **3.5** were assigned by using coupling constants. The coupling constant between H_a and H_b (J = 11.69), upfield of H_c chemical shift, downfield of H_a chemical shift in **3.4** when compared to **3.5** which indicated a *cis* relationship; whereas the coupling constant between H_a and H_b (J = 15.74), downfield of H_c chemical shift, upfield of H_a chemical shift in **3.5** when compared to **3.4,** indicated the H_a and H_b *trans* relationship in **3.5**

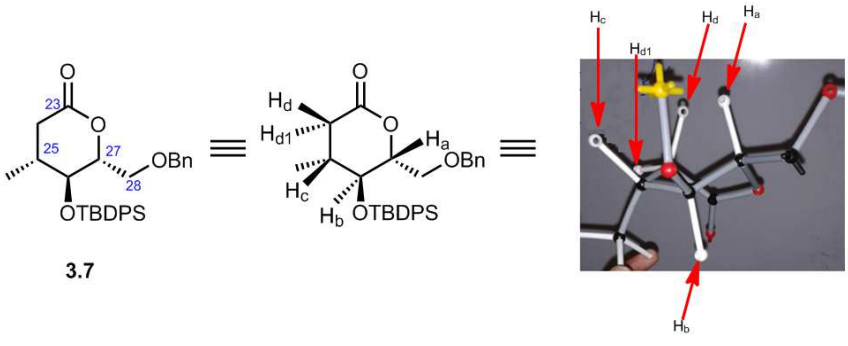


Figure 6: Assignment of **3.7**.

The structural assignment of compound **3.7** was carried-out by NMR, H^1-H^1 COSY experiments; stereochemistry at C25 carbon was assigned by 2D-NMR nOe analysis. *The 1,4 nOe between H_a and H_d indicated that it is not in the chair form!* The H_a showed nOe with H_d but not with H_{d1} and that H_{d1} showed nOe with methyl protons of C25 carbon, further indicated that the methyl group is below the plane.

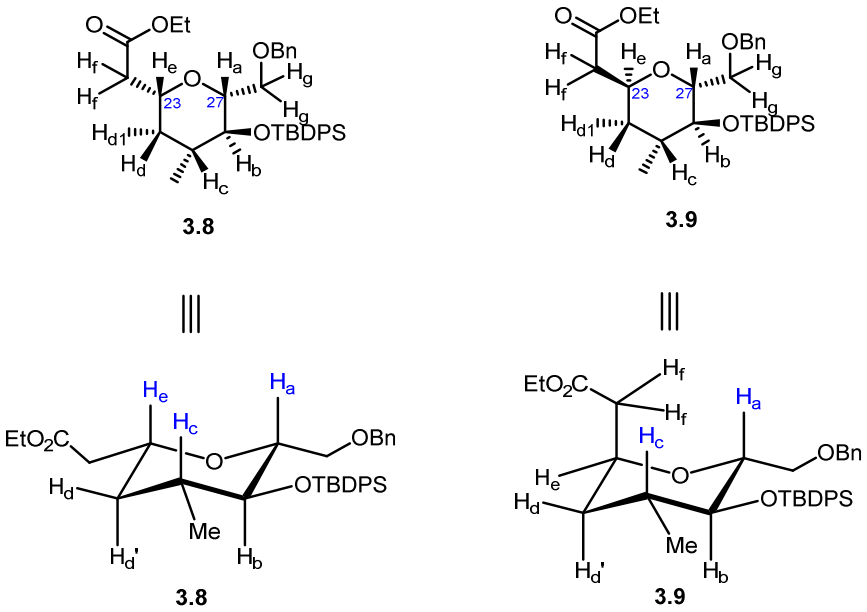
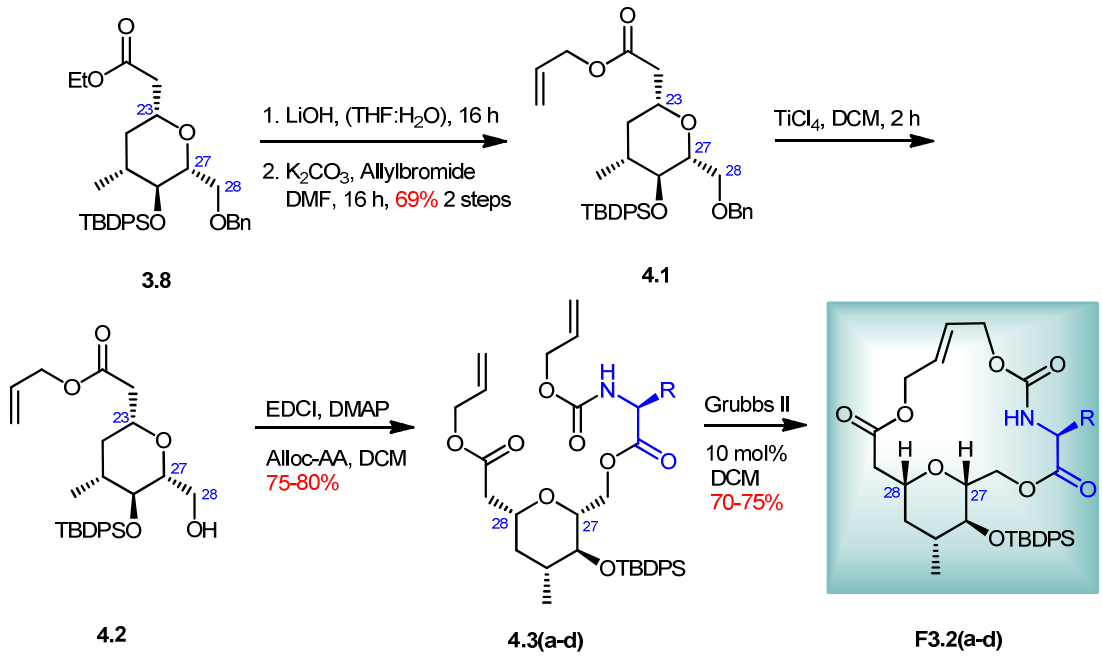


Figure 7: Assignment of **3.8** and **3.9**.

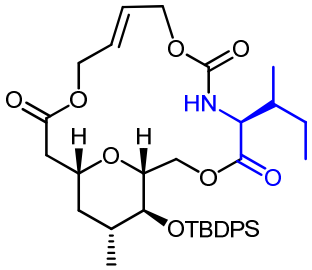
The compounds **3.8**, and **3.9** were assigned by 2D NMR nOe analysis. In compound **3.8**, the protons H_a, H_c and H_e showed nOe with each other. In compound **3.9**, protons H_a, H_c showed nOe with each other but both protons did not show any nOe with H_e proton, further indicating that the H_a, H_c are occupying an axial position whereas the H_e proton is present in equatorial orientation.

3.4.3 Synthesis of Macrocyclic Compounds

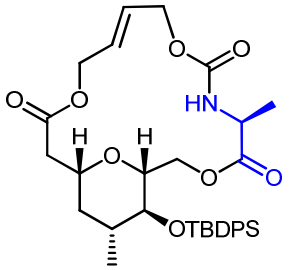
With this sufficient amount of the key intermediate **3.8,** we further developed a modular approach to the synthesis of macrocyclic compounds, **F3.2**. The **Scheme 4** shows our approach to the synthesis of a macrocyclic compound **F3.2.**



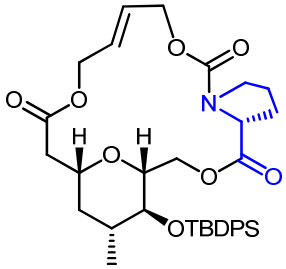
Scheme 4: 17-Membered Macrocyclic Compounds (**F3.2**) from *Cis*-2,5-Disubstituted Tetrahydropyran Fragment, **(3.8)**



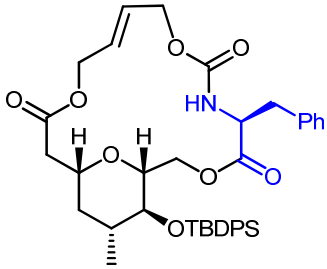
F3.2a



F3.2b



F3.2c



F3.2d

The hydrolysis of **3.8** produced free acid which was then allylated and protecting group removal, finally gave **4.2.** It was then coupled with four different amino acids for obtaining a precursor **4.3** for the crucial ring closing metathesis. The use of a second generation Grubbs catalyst successfully produced the 17-membered ring macrocycle with a single olefin geometry, **F3.2**. In one case, The olefin geometry was

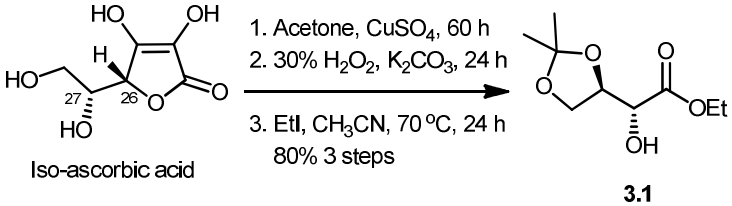
assigned by using NMR studies. In this study, four macrocyclic compounds were obtained by this approach. This further validated the feasibility of our ring formation that is independent of an amino acid utilized in the synthesis.

3.4.4 Conclusions

- To summarize, we succeeded in developing a practical and scalable synthesis of *cis*-2,6 substituted tetrahydropyran moiety which is present in eribulin as well as in several other important bioactive natural products.
- The privileged scaffold **3.8** was further utilized in obtaining 17-membered macrocyclic rings, and for completing the synthesis of C22-C28 fragment of eribulin.
- The incorporation of an amino acid moiety in macrocyclic rings allow introducing a chiral diversity site for obtaining further analogs with a variation in the chiral side chain.
- The key step involved in our synthesis was the diastereoselective conjugate addition, and Horner-Wadsworth-Wittig reaction and an advantage of our approach is the use of a cheap chiral starting material, Iso-Ascorbic acid
- The biological evaluation of all the compounds generated from this program is ongoing in collaboration with Dr. Satish Kitambi, Karolinska Institute, Sweden and Dr. Subhadra Dravida, Trans Cell Biologics, Hyderabad in various patient-derived cancer cells / cancer stem cells to search for novel selective cancer cell killers, for cell migration, and in general as cytoskeleton modulators.

3.4.5 Experimental Section

3.4.5a Synthesis of Key Intermediate 3.8



(R)-ethyl 2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxyacetate (3.1) :

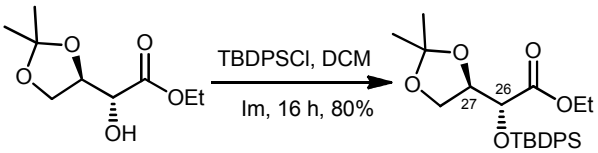
A mechanically stirred suspension of Iso-Ascorbic acid in (25 g, 142 mmol) in acetone (0.5 L) was treated with anhydrous $CuSO_4$ (37.5 g, 236.5 mmol). After the reaction was stirred at room temperature for 24 h, a second (37.5 g, 236.5 mmol) portion of $CuSO_4$ was added, and stirring was continued for an additional 24 h. The reaction was then filtered and concentrated, giving a near-quantitative yield of 3,4-isopropylidene-Iso-ascorbic acid. The iso-propylidene derivative was then dissolved in water (150 mL) containing 39 g of K_2CO_3. This solution was chilled in an ice bath and stirred while 30% H_2O_2 (35 mL) was added slowly till the color disappears and if not, further H_2O_2 was added till the color disappears. During the addition, the temperature was maintained below 20 ^oC. The solution was stirred overnight and then concentrated in vacuo. The moist solid was extracted with boiling absolute EtOH (6x200 mL). After filtration and evaporation, the salt was dried under vacuum to provide 30 g of material. Treatment of a mechanically stirred suspension of the salt with EtI (45 mL) in CH_3CN (150 mL) at reflux for 24 h gave, after concentration and removal of the inorganic salt by filtration gave the pure compound **3.1** (23 g, 80% 3 steps).

Molecular Formula: $C_9H_{16}O_5$

R_f (solvent system): 0.3 (30% ethyl acetate hexanes)

LRMS: (ES+) *m/z* = 205.0 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 4.38-4.25 (m, 4H), 4.04 (dd, *J* = 6.07, 3.18 Hz, 2H), 2.99-2.67 (m, 1H), 1.46 (s, 3H), 1.37 (s, 3H), 1.33 (t, *J* = 7.14 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*): 172.0, 77.0, 71.1, 65.0, 61.8, 26.3, 25.0, 14.1



3.1 **S1**

(R)-ethyl 2-((tert-butyldiphenylsilyl)oxy)-2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl) acetate (S_1) :

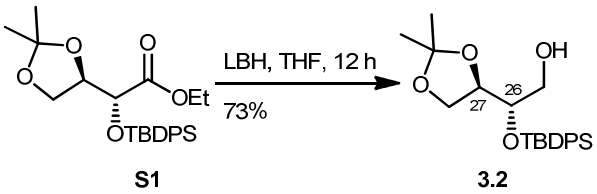
To a stirred of **3.1** (14.25 g, 69.8 mmol) in 80 mL of dry DCM, imidazole (9.4 g, 139.6 mmol) and TBDPSCl (21.7.mL, 83.76 mmol) were added sequentially under N_2 atmosphere at 0 ^oC. Stirred for 1 h at rt, 50 mL of H_2O was added and extracted with DCM (2x80 mL), combined organic layer was dried over Na_2SO_4, concentrated to get

the crude, which was purified by flash column chromatography with 5% ethyl acetate and hexanes gave **S_1** (24.8 g, 80%).

Molecular Formula: $C_{25}H_{34}O_5Si$

R_f (solvent system): 0.4 (5% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 443.2 (M+1).



S1 **3.2**

(S)-2-((tert-butyldiphenylsilyl)oxy)-2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanol (3.2) :

To a solution of **S1(** 24.8 g, 56.1 mmol) in 80 mL of dry THF at 0 ^oC and under N_2 atmosphere, lithium borohydride (3.19 g, 84.1 mmol) was added portion wise at 0 ^oC. After 12 h stirring, quenched carefully with 2 N NaOH solution, filtered through celite pad, the filtrate was dried over Na_2SO_4, concentrated, purified by flash column chromatography with 20% ethyl acetate and hexanes gave **3.2** (16.4 g, 73%).

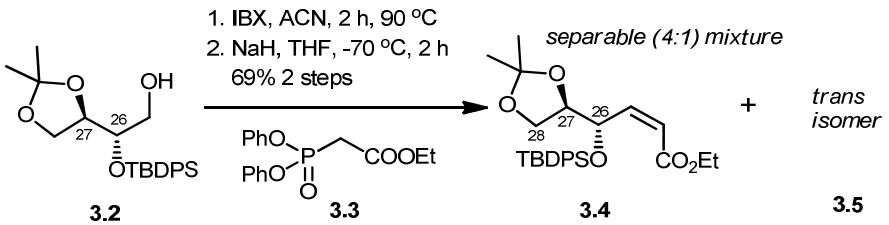
Molecular Formula: $C_{23}H_{32}O_4Si$

R_f (solvent system): 0.3 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 401.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.74-7.63 (m, 5H), 7.40 (dd, *J* = 8.58, 6.69 Hz, 5H), 4.18 (dd, *J* = 13.41, 6.42 Hz, 1H), 4.02 (dd, *J* = 8.33, 6.35 Hz, 1H), 3.71 (td, *J* = 8.50, 5.87 Hz, 2H), 3.60 (d, *J* = 4.09 Hz, 2H), 1.31 (s, 3H), 1.28 (s, 3H), 1.07 (s, 9H);

^{13}C NMR (100 MHz, *$CDCl_3$*) δ ppm : 135.9, 135.6, 134.8, 130.0, 129.6, 127.7, 109.2, 74.5, 67.5, 64.7, 27.0, 26.5, 25.3, 19.4.



3.2 **3.3** **3.4** **3.5**

The compound **3.2** (16.4 g, 40.97 mmol) was dissolved in CH_3CN (150 mL) and added IBX (34.41g, 122.9 mmol), then refluxed at 80 ^oC for 2 h. After consumption of total starting material (monitored by TLC) was filtered through celite, organic layer

washed with sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, concentrated to get the crude aldehyde (17g), which was subjected for next step directly.

To the wittig reagent **3.3** (20.5 g, 64.07 mmol) in 80 mL of dry THF, NaH (1.53 g, 64.07 mmol) was added at 0 ^oC, under N_2 atmosphere. Stirred for 30 minutes at 0 ^oC then cooled to -78 ^oC. The above aldehyde in 20 mL of dry THF was added slowly to this reaction mixture and stirred for 2 h at -78 ^oC. Reaction quenched with 60 mL sat. aq.NH_4Cl solution followed by 50 mL of sat.aq. NaCl solution was added and extracted with ethyl acetate (4x60 mL). The combined organic layer dried over Na_2SO_4, concentrated, purified by flash column chromatography with 5% ethyl acetate and hexanes gave separable (4:1) mixture of **3.4** (10.7 g) and **3.5** (2.6 g) 69% 2 steps)

Data for- **3.4 :**

Molecular Formula: $C_{27}H_{36}O_5Si$

R_f (solvent system): 0.2 (5% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 469.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72 (dd, *J* = 8.01, 1.46 Hz, 2H), 7.65 (dd, *J* = 8.04, 1.37 Hz, 2H), 7.44-7.31 (m, 6H), 6.00 (dd, *J* = 11.69, 8.72 Hz, 1H), 5.56 (td, *J* = 6.35, 2.93 Hz, 2H), 4.15 (dt, *J* = 6.73, 4.22 Hz, 1H), 3.97 (td, *J* = 13.88, 6.88 Hz, 3H), 3.92-3.86 (m, 1H), 1.39 (s, 3H), 1.35 (s, 3H), 1.16 (dd, *J* = 8.99, 5.31 Hz, 3H), 1.10 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 165.1, 147.1, 135.9, 133.1, 129.7, 127.4, 120.7, 109.2, 78.8, 69.4, 65.5, 60.0, 27.0, 26.2, 25.4, 19.3, 14.0.

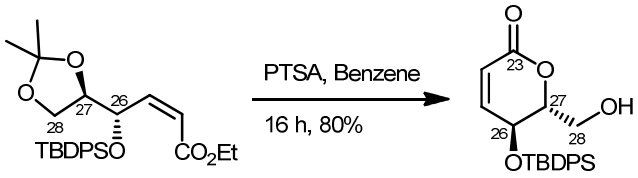
Data for- **3.5 :**

Molecular Formula: $C_{27}H_{36}O_5Si$

R_f (solvent system): 0.2 (5% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 469.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.71-7.68 (m, 2H), 7.65-7.61 (m, 2H), 7.45-7.35 (m, 6H), 6.76 (dd, *J* = 15.74, 6.54 Hz, 1H), 5.71 (dd, *J* = 15.74, 1.22 Hz, 1H), 4.31 (dt, *J* = 7.67, 7.30, 3.82 Hz, 1H), 4.17-4.10 (m, 2H), 4.05 (q, *J* = 6.21 Hz, 1H), 3.94 (td, *J* = 8.31, 4.85 Hz, 1H), 3.78 (dd, *J* = 8.34, 6.30 Hz, 1H), 1.31 (s, 3H), 1.26 (t, *J* = 7.13 Hz, 3H), 1.13 (s, 3H), 1.10 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 165.8, 146.1, 135.9, 133.1, 129.9, 127.6, 122.7, 109.6, 78.4, 73.6, 66.2, 60.3, 27.0, 26.4, 25.3, 19.4, 14.2.



3.4 **S_2**

(5S, 6R)-5-((tert-butyldiphenylsilyl)oxy)-6-(hydroxymethyl)-5,6-dihydro-2H-pyran-2-one (S_2) :

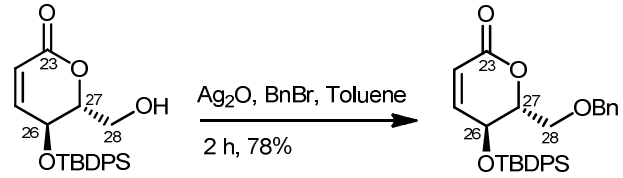
To a solution of **3.4** (10.7g, 22.8 mmol) in 50 mL of benzene, PTSA (3.93g, 22.8 mmol) was added ,After 3 h stirring at 30 ^oC, reaction mixture directly extracted with ethyl acetate (3x40 mL), combined organic layer dried over Na_2SO_4, concentrated, purified by flash column chromatography with 20% ethyl acetate and hexanes gave **S_2** (7 g, 80%).

Molecular Formula: $C_{22}H_{26}O_4Si$

R_f (solvent system): 0.2 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 383.1 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72-7.68 (m, 4H), 7.51-7.40 (m, 6H), 6.59 (dd, *J* = 10.04, 1.95 Hz, 1H), 5.79 (dd, *J* = 10.02, 1.98 Hz, 1H), 4.70 (td, *J* = 9.40, 1.95 Hz, 1H), 4.42 (ddd, *J* = 9.40, 4.05, 2.78 Hz, 1H), 3.92 (dd, *J* = 12.51, 2.37 Hz, 1H), 3.81 (dd, *J* = 12.48, 3.86 Hz, 1H), 1.87 (s, 1H), 1.10 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 162.5, 149.5, 135.7, 132.9, 130.4, 128.1, 119.3, 82.9, 64.0, 61.1, 26.8, 19.3.



S_2 **3.6**

(5S,6R)-6-((benzyloxy)methyl)-5-((tert-butyldiphenylsilyl)oxy)-5,6-dihydro-2H-pyran-2-one (3.6) :

To a solution of **S_2** (5.1g, 13.3 mmol) in 50 mL of dry Toulene, at 0 ^oC and under N_2 atmosphere, Ag_2O (9.25g, 40.05 mmol) was added followed by slow addition of BnBr (2.36 mL, 19.95 mmol). After 2 h stirring at 30 ^oC, reaction mixture directly extracted with ethyl acetate (3x40 mL), combined organic layer dried over Na_2SO_4,

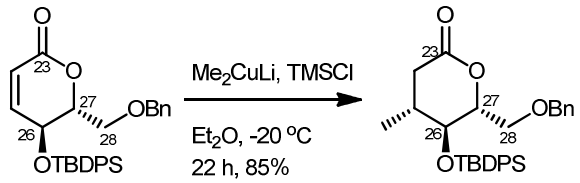
concentrated, purified by flash column chromatography with 8% ethyl acetate and hexanes gave **3.6** (5 g, 78%).

Molecular Formula: $C_{29}H_{32}O_4Si$

R_f (solvent system): 0.45 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 473.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64 (d, *J* = 7.90 Hz, 4H), 7.49-7.41 (m, 2H), 7.33 (ddd, *J* = 16.90, 11.29, 7.10 Hz, 7H), 7.26-7.19 (m, 2H), 6.51 (dd, *J* = 9.99, 2.57 Hz, 1H), 5.79 (dd, *J* = 9.99, 1.52 Hz, 1H), 4.69 (dt, *J* = 8, 2 Hz 1H), 4.55-4.47 (m, 1H), 4.46 (s, 2H), 3.71 (dq, *J* = 10.87, 3.58 Hz, 2H), 1.05 (s, 9H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 162.6, 147.9, 137.6, 135.7, 133.0, 132.1, 130.2, 128.3, 128.0, 127.9, 127.6, 119.9, 81.9, 73.5, 68.3, 64.0, 26.8, 19.3.



3.6 **3.7**

(4R, 5S, 6R)-6-((benzyloxy)methyl)-5-((tert-butyldiphenylsilyl)oxy)-4-methyl tetrahydro-2H-pyran-2-one (3.7) :

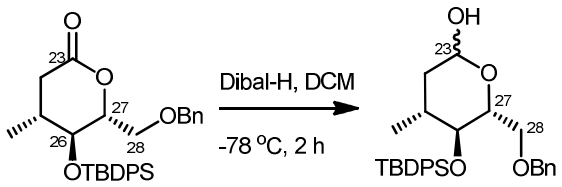
A solution of the Methylithium 1.6 M in ether (31.75 mL, 50.8 mmol) was added dropwise to a slurry of CuI (4.83 g, 25.4 mmol) in diethyl ether(45 mL) at 0 °C under argon atmosphere. After the mixture was stirred for 10 min, the copper reagent was treated with TMSCl (8.05 mL, 63.5 mmol). After the mixture was cooled at -20 ^{o}C, a solution of the α,β-unsaturated δ-lactone **3.6** (3g, 6.35 mmol) in diethyl ether (10 mL) was added. The reaction mixture was stirred at -20 °C for 22 h. After this time, the reaction was quenched by the addition of 30mL of a NH_3/NH_4Cl solution (pH 8). The mixture was diluted with diethyl ether, the phases were separated, the aqueous phase was extracted with diethyl ether (twice), and the combined organic extracts were washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent gave the crude material, which was further purified by flash chromatography, give pure compound **3.7** (2.63 g, 85%).

Molecular Formula: $C_{30}H_{36}O_4Si$

R_f (solvent system): 0.43 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 489.2 (M+1).

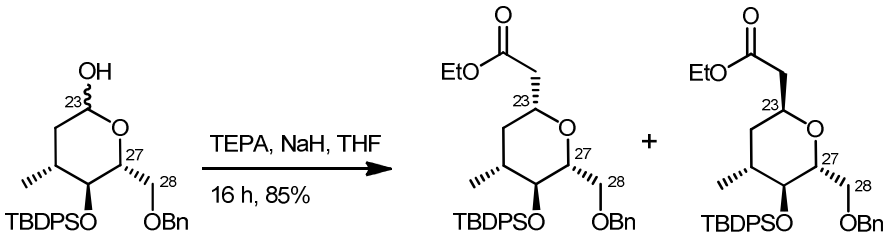
^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.64 (t, *J* = 7.06 Hz, 4H), 7.45 (t, *J* = 7.36 Hz, 2H), 7.40-7.27 (m, 7H), 7.20 (d, *J* = 6.41 Hz, 2H), 4.36 (t, *J* = 3.45 Hz, 3H), 3.74 (dd, *J* = 6.55, 4.56 Hz, 1H), 3.57 (dd, *J* = 10.78, 2.27 Hz, 1H), 3.42 (dd, *J* = 10.78, 4.63 Hz, 1H), 2.71 (dd, *J* = 15.22, 4.46 Hz, 1H), 2.19-2.10 (m, 2H), 1.03 (s, 9H), 0.65 (d, *J* = 6.87 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.0, 137.7, 135.9, 135.8, 133.1, 132.8, 130.1, 130.0, 128.3, 127.8, 127.6, 82.8, 73.3, 72.1, 69.6, 34.5, 34.2, 26.9, 19.3, 18.5.



3.7 **S_3**

(4R,5S,6R)-6-((benzyloxy)methyl)-5-((tert-butyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-ol (S_3) :

To a solution of **3.7** (0.924 g, 1.89 mmol) in 20 mL of dry Toulene at -78 °C, under N_2 atmosphere, 1.7M in toluene DIBAL-H (13.9 mL, 23.7 mmol) was added slowly. After 1 hour, reaction diluted with 60 mL ethyl acetate, quenched with 80 mL sat. aq. Na-K tartrate solution and stirred for 4 hours to get a clear separation. The organic layer dried over Na_2SO_4, concentrated to get the crude, which was purified by flash column chromatography with 40% ethyl acetate and hexanes gave (0.714 g, 77%) of **S_3**



S_3 **3.8** **3.9**

Separable diastereomers

Ethyl-2-((2R,4R,5S,6R)-6-((benzyloxy)methyl)-5-((tert-butyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-yl)acetate (3.8) :

To a suspension of 60% NaH (0.280 g, 11.65 mmol) in dry THF (10 mL), triethyl phosphonoacetate (3.26 g, 14.57 mmol) was added at 0 °C, and stirred under nitrogen atmosphere for 30 min. A solution of **S_3** (0.714 g, 1.457 mmol) in 5 mL THF was then

added drop wise to the reaction mixture and allowed to stir for 12 h at rt. After completion of the reaction (monitored by TLC), reaction mixture was quenched by the addition of saturated ammonium chloride solution (10 mL) and extracted with ethyl acetate. Combined organic layer was washed with 20 mL brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude oil, which was purified by flash chromatography (4% ethyl acetate/hexanes) to give the separable compounds **3.8** (0.56 g) **& 3.9** (0.14 g) as colourless liquid.

Data for- **3.8 :**

Molecular Formula: $C_{34}H_{44}O_5Si$

R_f (solvent system): 0.2 (4% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 561.2 (M+1).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.70 (dt, *J* = 8.12, 4.00 Hz, 5H), 7.44-7.38 (m, 2H), 7.38-7.27 (m, 7H), 7.24 (s, 1H), 4.40-4.28 (m, 2H), 4.12 (q, *J* = 7.14 Hz, 2H), 3.80-3.87 (m, 1H), 3.73 (d, *J* = 8.8 Hz 1H), 3.57-3.47 (m, 2H), 3.33 (t, *J* = 8.8 Hz 1H), 2.59 (dd, *J* = 15.2, 6.8 Hz 1H), 2.34 (dd, *J* = 15.2, 6.8 Hz 1H), 1.81-1.72 (m, 1H), 1.70-1.59 (m, 2H), 1.22 (t, *J* = 7.17 Hz, 3H), 0.98 (s, 9H), 0.65 (d, *J* = 6.39 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 171.2, 138.6, 136.1, 134.0, 133.5, 129.5, 128.2, 127.6, 127.4, 127.3, 81.6, 74.5, 73.3, 73.0, 70.5, 60.4, 40.9, 39.2, 37.7, 27.0, 20.0, 19.9, 14.2.

Data for- **3.9 :**

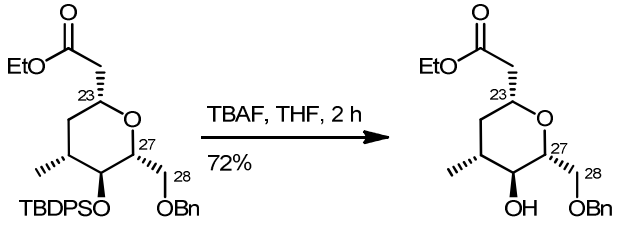
Molecular Formula: $C_{34}H_{44}O_5Si$

R_f (solvent system): 0.2 (4% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 561.2 (M+1).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.69 (ddd, *J* = 11.94, 8.00, 1.34 Hz, 4H), 7.45-7.21 (m, 11H), 4.37-4.27 (m, 3H), 4.12 (q, *J* = 7.14 Hz, 2H), 3.76-3.69 (m, 1H), 3.61 (dd, *J* = 10.41, 2.18 Hz, 1H), 3.53 (dd, *J* = 10.42, 5.54 Hz, 1H), 3.39 (t, *J* = 8.30 Hz, 1H), 2.83 (dd, *J* = 14.52, 7.65 Hz, 1H), 2.56 (dd, *J* = 14.53, 7.04 Hz, 1H), 1.90-1.79 (m, 1H), 1.57 (dt, *J* = 14, 4 Hz, 1H), 1.49-1.39 (m, 1H), 1.23 (t, *J* = 7.13 Hz, 3H), 0.97 (s, 9H), 0.65 (d, *J* = 6.56 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 171.3, 138.4, 135.9, 133.9, 133.5, 129.6, 128.1, 127.7, 127.5, 127.3, 74.5, 74.3, 73.0, 70.5, 68.5, 60.5, 37.1, 34.9, 32.7, 27.0, 19.8, 19.5, 14.2.

3.4.5b Synthesis of C22-C28 Fragment of Eribulin from 3.8



3.8 **S_4**

Ethyl2-((2R,4R,5S,6R)-6-((benzyloxy)methyl)-5-hydroxy-4-methyltetrahydro-2H-pyran-2-yl)acetate (S_4) :

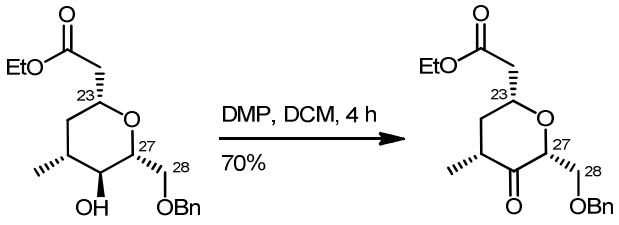
To a stirred solution of **3.8** (0.072 g, 0.128 mmol) in 5 mL of dry THF, 1M TBAF (0. 067 g, 0.257 mmol) was added. After 3 h, 10 mL of sat. aq. NaCl solution was added and extracted with ethyl acetate (2x15 mL), combined organic layer dried over Na_2SO_4, concentrated to get the crude which was purified by flash column chromatography with 20% ethyl acetate and hexanes gave **S_4** (0.03 g, 72%).

Molecular Formula: $C_{18}H_{26}O_5$

R_f (solvent system): 0.2 (20% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 323.1 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.37-7.25 (m, 5H), 4.60-4.52 (m, 2H), 4.13 (q, *J* = 7.14 Hz, 2H), 3.90-3.81 (m, 1H), 3.73 (dd, *J* = 9.70, 4.52 Hz, 1H), 3.61 (dd, *J* = 9.69, 6.48 Hz, 1H), 3.37 (ddd, *J* = 9.01, 6.45, 4.55 Hz, 1H), 3.15 (t, *J* = 9.35 Hz, 1H), 2.53 (dd, *J* = 15.28, 7.32 Hz, 1H), 2.37 (dd, *J* = 15.28, 5.84 Hz, 1H), 1.74 (ddd, *J* = 13.24, 4.09, 1.93 Hz, 1H), 1.68-1.60 (m, 1H), 1.24 (t, *J* = 7.13 Hz, 3H), 1.14 (dd, *J* = 15.70, 9.00 Hz, 1H), 1.05 (d, *J* = 6.41 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.1, 137.6, 128.5, 127.9, 127.8, 78.3, 75.3, 73.7, 73.7, 72.2, 60.4, 40.9, 38.6, 36.9, 18.0, 14.2.



S_4 **3.10**

ethyl 2-((2R,4R,6R)-6-((benzyloxy)methyl)-4-methyl-5-oxotetrahydro-2H-pyran-2-yl)acetate (3.10) :

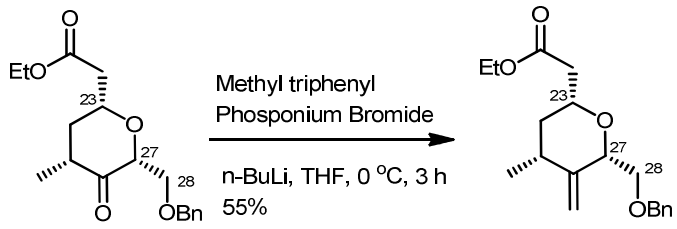
To a solution of **S_4** (0.03 g, 0.09mmol) in 5 mL of dry DCM and N_2 atmosphere, Dess-Martin periodinane (0.8 g, 0.186 mmol) was added at 0 ^oC. After 30 minutes reaction diluted with 20 mL DCM, quenched with 20 mL sat. aq.$Na_2S_2O_3$ solution. Separated organic layer washed with 20 mL sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4, concentrated to get the crude Ketone, which was purified by wash column with 15% ethyl acetate and hexanes gave the Ketone **3.10** (0.02 g, 70%).

Molecular Formula: $C_{18}H_{24}O_5$

R_f (solvent system): 0.2 (15% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 321.1 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.35-7.30 (m, 4H), 7.28-7.25 (m, 1H), 4.62-4.52 (m, 2H), 4.35 (dtd, *J* = 11.18, 6.61, 1.87 Hz, 1H), 4.15 (t, *J* = 5.44 Hz, 2H), 3.91 (dd, *J* = 10.99, 3.46 Hz, 1H), 3.63 (dd, *J* = 10.99, 6.31 Hz, 1H), 2.72 (dd, *J* = 15.65, 6.76 Hz, 1H), 2.59 (td, *J* = 12.65, 6.44 Hz, 1H), 2.49 (dd, *J* = 15.64, 6.39 Hz, 1H), 2.27 (ddd, *J* = 13.21, 6.29, 1.85 Hz, 1H), 1.70-1.60 (m, 2H), 1.25 (d, *J* = 7.15 Hz, 3H), 1.10 (d, *J* = 6.59 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 207.2, 170.7, 138.2, 128.3, 127.7, 127.5, 81.9, 73.5, 73.2, 68.4, 60.7, 42.5, 40.9, 40.5, 14.2.



3.10 **F3.1**

Ethyl-2-((2R,4R,6S)-6-((benzyloxy)methyl)-4-methyl-5-methylenetetrahydro-2H-pyran-2-yl)acetate (F3.1) :

A suspension of Methyltriphenylphosphonium bromide (446.2 mg, 1.25 mmol) in dry THF (3 mL) was treated with n-BuLi (0.5 mL, 1 mmol, 1.6 M solution in hexane) under N_2 at 0 ^oC. The resulting yellow solution was allowed to stir at room temperature for 30 min, then cooled to -78 °C. A solution of ketone **3.10** (40 mg, 0.125 mmol) in dry THF (2.5mL) was added slowly and the reaction mixture was allowed to warm up to room temperature. The stirring was continued for 4 hr before it was quenched by saturated aqueous NH_4Cl. The aqueous layer was extracted with

Et_2O three times, the combined organics were dried ($MgSO_4$), concentrated under reduced pressure and purified by flash chromatography (silica gel 60-120 mesh, 15% EtOAc in n-hexane, TLC: R_f = 0.3) gave pure **F3.1** (18 mg, 55%).

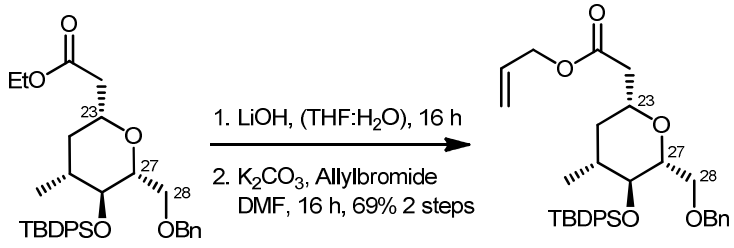
Molecular Formula: $C_{19}H_{26}O_4$

R_f (solvent system): 0.3 (15% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 319.1 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.37-7.26 (m, 5H), 4.89-4.83 (m, 2H), 4.65-4.53 (m, 2H), 4.13 (q, *J* = 7.11 Hz, 2H), 4.09-4.04 (m, 1H), 4.00 (t, *J* = 5.46 Hz, 1H), 3.81 (dd, *J* = 10.09, 5.28 Hz, 1H), 3.66 (dd, *J* = 10.09, 5.84 Hz, 1H), 2.60 (dd, *J* = 15.24, 7.00 Hz, 1H), 2.39 (dd, *J* = 15.23, 6.25 Hz, 1H), 2.35-2.28 (m, 1H), 1.91-1.84 (m, 1H), 1.25 (d, *J* = 7.08 Hz, 3H), 1.16 (dd, *J* = 14.07, 9.58 Hz, 1H), 1.09 (d, *J* = 6.48 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.2, 148.3, 138.2, 128.3, 127.9, 127.6, 105.5, 74.2, 73.4, 70.1, 60.5, 42.1, 41.2, 35.4, 17.6, 14.2.

3.4.5c Synthesis of 17-Membered Macrocycles F3.2 from 3.8



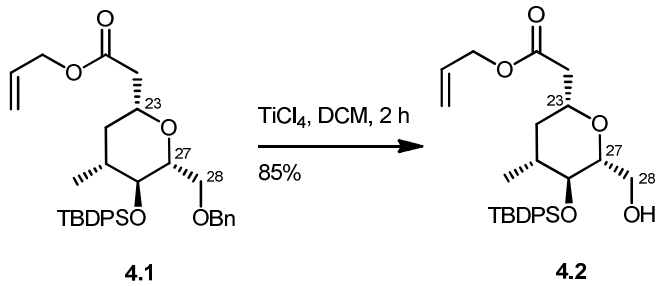
3.8 **4.1**

Allyl 2-((2R,4R,5S,6R)-6-((benzyloxy)methyl)-5-((tert-butyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-yl)acetate (4.1) :

To a solution of compound **3.8** (0.4 g, 0.714 mmol) in THF:H_2O mixture (4:1) added LiOH.H_2O (0.179 g, 4.28 mmol) allowed to stirred for 24 h at room temperature then added 5% HCl solution (5 mL) and the compound extracted twice with EtOAc. The organic phase was dried over Na_2SO_4, filtered and evaporated solvent afforded the carboxylic acid product as colourless oil which is subjected to Allylation reaction without further purification.

To the solution of above crude compound acid(1 eq) in dry DMF added K_2CO_3(4 eq), allylbromide (2 eq) at 0 ^oC then allowed stirred for 12 hours at room temperature under nitrogen atmosphere. Then reaction quenched with saturated NaCl and added cold water extracted twice with EtOAc. Combined organic layers were dried over

Na_2SO_4, filtered and evaporated. Purification of crude compound by flash column chromatography over silica gel (10% EtOAc/hexane) afforded the compound **4.1** as light yellow oil (0.283 g, 69% for two steps)



4.1 **4.2**

Allyl2-((2R,4R,5S,6R)-5-((tert-butyldiphenylsilyl)oxy)-6-(hydroxymethyl)-4-methyltetrahydro-2H-pyran-2-yl)acetate (4.2) :

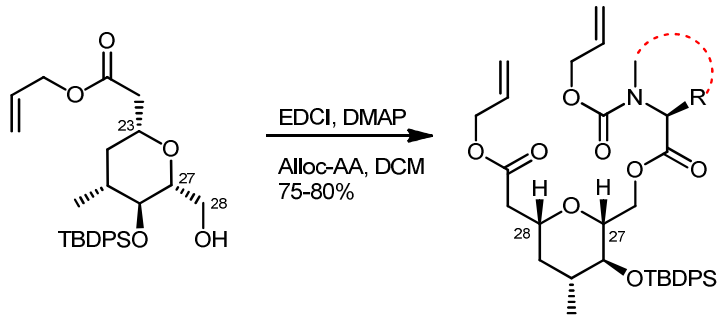
To a solution of **4.1** (0.28 g, 0.489 mmol) in dry DCM (10 mL) added $TiCl_4$ (181 mg, 0.97 mmol) a 0 ^oC under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at then quenched with saturated NH_4Cl solution. and two layers were separated and aqueous layer extracted with DCM, combined organic layers were washed with brine and dried over Na_2SO_4. Then organic layer was evaporated. Purification of crude compound by flash column chromatography over silica gel (20% EtOAc/hexane) afforded the compound **4.2** (0.2 g, 85%).

Molecular Formula: $C_{28}H_{38}O_5Si$

R_f (solvent system): 0.3 (20% ethyl acetate/hexanes)

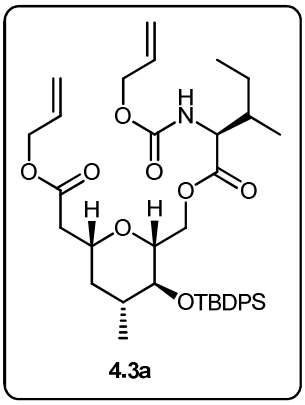
LRMS: (ES+) *m/z* = 483.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72 (dd, *J* = 7.89, 6.56 Hz, 4H), 7.44-7.36 (m, 6H), 5.96-5.82 (m, 1H), 5.26 (ddd, *J* = 13.78, 11.56, 1.28 Hz, 2H), 4.57 (d, *J* = 5.70 Hz, 2H), 3.92-3.84 (m, 1H), 3.81 (dd, *J* = 10.81, 1.70 Hz, 1H), 3.45 (dd, *J* = 11.2, 6 Hz, 1H), 3.41(td, *J* = 10.4, 2 Hz, 1H), 3.24 (t, *J* = 9.01 Hz, 1H), 2.49 (dd, *J* = 15.30, 7.76 Hz, 1H), 2.37 (dd, *J* = 15.30, 5.35 Hz, 1H), 1.84-1.74 (m, 1H), 1.67 (s, 2H), 1.00 (s, 9H), 0.71 (d, *J* = 6.41 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 170.7, 136.0, 136.0, 133.7, 133.1, 132.0, 129.7, 127.6, 118.4, 81.5, 74.5, 73.1, 65.2, 63.1, 40.7, 39.2, 37.6, 27.1, 20.0, 19.9.



4.2 **4.3 (a-d)**

To the solution of **4.2** (1 eq) in DCM solution added alloc amino acid building block (1 eq) and EDC.HCl (1.5 eq) at room temperature under nitrogen atmosphere and allowed to stirred for 2 hours. Then added saturated $NaHCO_3$ solution to this reaction mixture extracted twice with EtOAc. Combined organic layers were washed with brine solution and dried anhydrous Na_2SO_4, evaporated the solvent, Purification of crude compound by flash chromatography over silica gel (40% EtOAc/hexane) afforded the compound **4.3 (a-d)** as colourless oil.



4.3a

(2S,3S)-((2R,3S,4R,6R)-6-(2-(allyloxy)-2-oxoethyl)-3-((tertbutyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-yl)methyl 2-(((allyloxy)carbonyl)amino)-3-methylpentanoate (4.3a) :

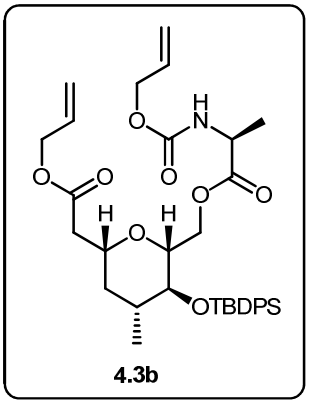
Molecular Formula: $C_{38}H_{53}NO_8Si$

R_f (solvent system): 0.3 (40% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 680.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72-7.67 (m, 4H), 7.44-7.36 (m, 6H), 5.96-5.87 (m, 2H), 5.36-5.28 (m, 3H), 5.27-5.19 (m, 2H), 4.58 (dd, *J* = 11.11, 5.61 Hz, 4H), 4.41 (dd, *J* = 11.49, 2.06 Hz, 1H), 4.33 (dd, *J* = 8.98, 4.62 Hz, 1H), 4.25 (dd, *J* = 10.27, 5.97 Hz, 1H), 3.99 (dd, *J* = 11.49, 7.11 Hz, 1H), 3.76 (dd, *J* = 10.81, 4.56 Hz, 1H), 3.24 (t, *J* = 7.90 Hz, 1H), 2.76 (dd, *J* = 14.68, 7.51 Hz, 1H), 2.57 (dd, *J* = 14.69,

6.98 Hz, 1H), 1.86-1.83 (m, 1H), 1.62 (dd, *J* = 12.55, 5.39 Hz, 2H), 1.48-1.34 (m, 2H), 1.18-1.08 (m, 1H), 1.02 (s, 9H), 0.90 (dd, *J* = 7.12, 3.28 Hz, 6H), 0.69 (d, *J* = 6.64 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.5, 170.6, 155.8, 135.8, 133.7, 133.0, 132.0, 129.9, 127.7, 118.3, 117.7, 74.4, 73.3, 68.0, 65.7, 65.2, 58.3, 38.2, 37.2, 34.4, 32.7, 27.0, 24.7, 19.7, 19.3, 15.4, 11.6.



4.3b

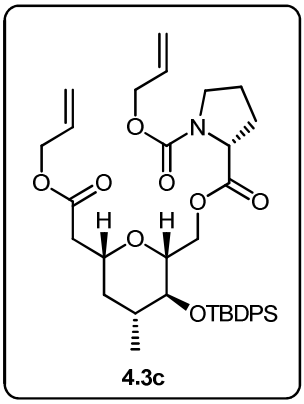
(S)-((2R,3S,4R,6R)-6-(2-(allyloxy)-2-oxoethyl)-3-((tert-butyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-yl)methyl 2-(((allyloxy)carbonyl) amino) propan oate (4.3b) :

Molecular Formula: $C_{35}H_{47}NO_8Si$

R_f (solvent system): 0.3 (40% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 638.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.69 (ddd, *J* = 9.72, 7.89, 1.54 Hz, 4H), 7.47-7.35 (m, 6H), 5.97-5.85 (m, 2H), 5.45-5.11 (m, 5H), 4.62-4.55 (m, 4H), 4.37 (td, *J* = 11.02, 5.48 Hz, 2H), 4.32-4.24 (m, 1H), 4.06 (dd, *J* = 11.51, 6.81 Hz, 1H), 3.80-3.74 (m, 1H), 3.26 (t, *J* = 7.90 Hz, 1H), 2.82 (dd, *J* = 14.66, 8.36 Hz, 1H), 2.52 (dd, *J* = 14.66, 6.17 Hz, 1H), 1.90-1.80 (m, 1H), 1.61 (dd, *J* = 9.87, 4.12 Hz, 1H), 1.48-1.39 (m, 1H), 1.35 (d, *J* = 7.13 Hz, 3H), 1.01 (s, 9H), 0.68 (d, *J* = 6.64 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.5, 170.6, 155.3, 135.9, 133.7, 133.0, 132.0, 129.9, 127.7, 118.3, 117.7, 74.3, 73.2, 68.1, 65.3, 49.6, 37.3, 34.5, 32.8, 27.0, 19.7, 19.3.



4.3c

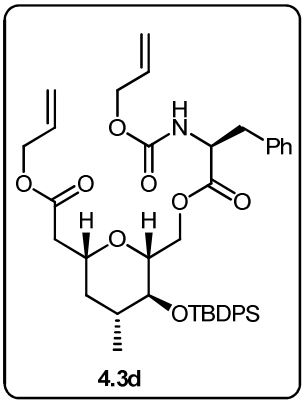
(R)-1-allyl 2-(((2R,3S,4R,6R)-6-(2-(allyloxy)-2-oxoethyl)-3-((tert-butyldiphenyl silyl)oxy) -4-methyltetrahydro-2H-pyran-2-yl)methyl) pyrrolidine-1,2-di carbox ylate (4.3c) :

Molecular Formula: $C_{37}H_{49}NO_8Si$

R_f (solvent system): 0.3 (40% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 664.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.70 (t, *J* = 6.14 Hz, 4H), 7.45-7.35 (m, 6H), 6.00-5.78 (m, 2H), 5.35-5.27 (m, 1H), 5.26-5.10 (m, 2H), 4.62-4.51 (m, 4H), 4.50-4.39 (m, 1H), 4.32 (ddd, *J* = 12.13, 8.51, 2.88 Hz, 1H), 4.04-3.95 (m, 1H), 3.76-3.69 (m, 1H), 3.62-3.53 (m, 1H), 3.46 (ddd, *J* = 17.57, 12.46, 7.31 Hz, 1H), 3.25 (dt, *J* = 7.88, 2.55 Hz, 1H), 2.79 (ddd, *J* = 14.66, 7.40, 4.42 Hz, 1H), 2.62-2.52 (m, 1H), 2.24-2.04 (m, 1H), 1.95-1.81 (m, 4H), 1.60 (td, *J* = 13.95, 3.90 Hz, 1H), 1.46-1.35 (m, 1H), 1.27 (d, *J* = 11.09 Hz, 2H), 1.01 (s, 9H), 0.68 (d, *J* = 6.55 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.2, 170.7, 170.6, 154.2, 135.8, 133.8, 132.8, 132.0, 129.8, 127.7, 118.3, 117.1, 74.3, 73.3, 68.1, 65.2, 64.8, 58.8, 46.8, 37.2, 34.6, 32.6, 30.9, 29.9, 27.1, 24.1, 23.3, 19.7, 19.4



4.3d

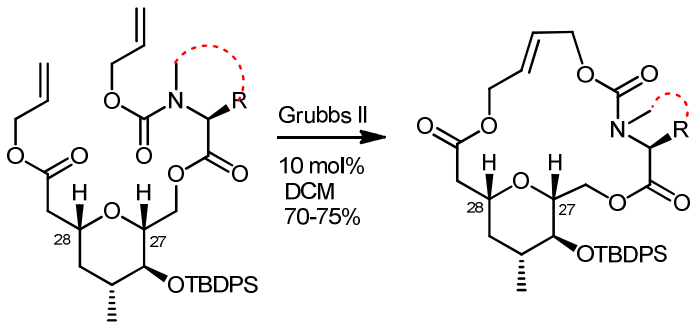
(S)-((2R,3S,4R,6R)-6-(2-(allyloxy)-2-oxoethyl)-3-((tert-butyldiphenylsilyl)oxy)-4-methyltetrahydro-2H-pyran-2-yl)methyl 2-(((allyloxy)carbonyl)amino)-3-phenyl propanoate (4.3d) :

Molecular Formula: $C_{41}H_{51}NO_8Si$

R_f (solvent system): 0.3 (40% ethyl acetate/hexanes)

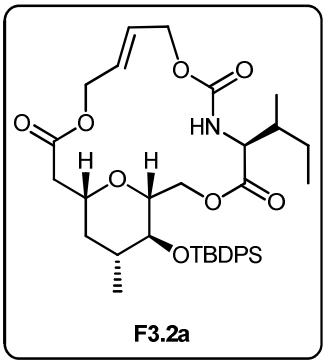
LRMS: (ES+) *m/z* = 714.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.74-7.64 (m, 4H), 7.46-7.35 (m, 6H), 7.26-7.20 (m, 3H), 7.11 (d, *J* = 6.72 Hz, 2H), 5.95-5.81 (m, 2H), 5.32-5.16 (m, 5H), 4.64 (d, *J* = 7.39 Hz, 1H), 4.54 (d, *J* = 4.76 Hz, 4H), 4.41 (d, *J* = 10.98 Hz, 1H), 4.30 (s, 1H), 4.00 (dd, *J* = 11.21, 7.38 Hz, 1H), 3.80 (t, *J* = 7.03 Hz, 1H), 3.24 (t, *J* = 7.81 Hz, 1H), 3.11 (dd, *J* =14, 5.2 Hz, 1H), 3.02 (d, *J* = 14.2, 5.70 Hz, 1H), 2.81 (dd, *J* = 14.65, 8.07 Hz, 1H), 2.53 (dd, *J* = 14.65, 6.17 Hz, 1H), 1.86 (d, *J* = 6.12 Hz, 1H), 1.45 (dd, *J* = 12.45, 7.83 Hz, 1H), 1.31 (d, *J* = 19.70 Hz, 1H), 1.03 (s, 9H), 0.70 (d, *J* = 6.61 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.0, 170.6, 155.4, 135.8, 133.7, 133.0, 132.0, 129.9, 129.5, 128.5, 127.7, 126.9, 118.3, 117.6, 74.4, 73.2, 68.1, 65.6, 65.3, 54.6, 38.1, 37.3, 34.5, 32.8, 27.1, 19.7, 19.3.



4.3 (a-d) **F3.2 (a-d)**

To a solution of **4.3 (a-d)** (1eq) in dry DCM under nitrogen atmosphere added Grubbs' 2nd generation catalyst (10 mol%) and reaction mixture was allowed to stirred for 2 h at 40 ^{o}C. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **F3.2(a-d).**



F3.2a

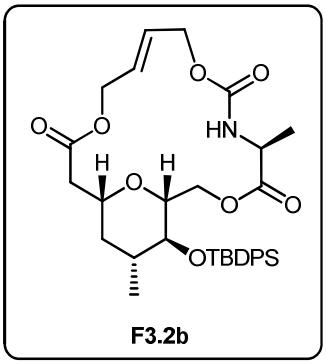
(1R,5S,16R,18R,19S,E)-5-((R)-sec-butyl)-19-((tert-butyldiphenylsilyl)oxy)-18-methyl-3,8,13,20-tetraoxa-6-azabicyclo[14.3.1]icos-10-ene-4,7,14-trione (F3.2a) :

Molecular Formula: $C_{36}H_{49}NO_8Si$

R_f (solvent system): 0.3 (30% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 652.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.72-7.65 (m, 4H), 7.45-7.34 (m, 6H), 5.88-5.66 (m, 2H), 4.97-4.73 (m, 2H), 4.70-4.56 (m, 1H), 4.48-4.20 (m, 3H), 3.98 (dd, *J* = 12, 6.4 Hz, 1H), 3.89-3.77 (m, 1H), 3.43-3.26 (m, 1H), 3.01-2.81 (m, 1H), 2.29 (d, *J* = 14 Hz, 1H), 1.90-1.75 (m, 3H), 1.69-1.57 (m, 1H), 1.50-1.36 (m, 2H), 1.19-1.06 (m, 1H), 1.04-1.00 (m, 9H), 0.93-0.86 (m, 6H), 0.76-0.57 (m, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 171.1, 170.5, 155.7, 135.9, 133.9, 133.1, 129.8, 127.7, 126.6, 77.2, 74.2, 60.4, 59.0, 53.4, 37.0, 34.0, 27.1, 27.0, 24.8, 21.0, 19.6, 15.7, 14.2, 11.4.



F3.2b

(1R,5S,16R,18R,19S,E)-19-((tert-butyldiphenylsilyl)oxy)-5,18-dimethyl-3,8,13,20-tetraoxa-6-azabicyclo[14.3.1]icos-10-ene-4,7,14-trione (F3.2b) :

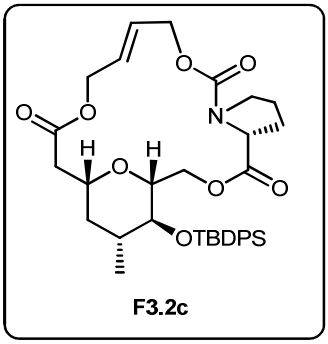
Molecular Formula: $C_{33}H_{43}NO_8Si$

R_f (solvent system): 0.3 (30% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 610.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.73-7.64 (m, 4H), 7.47-7.34 (m, 6H), 5.88-5.71 (m 2H), 4.87-4.71 (m, 2H), 4.70-4.62 (m, 1H), 4.48-4.31 (m, 2H), 4.28-4.17 (m, 1H),

3.96-3.87 (m, 1H), 3.79 (t, *J* = 6 Hz, 1H), 3.37-3.20 (m, 1H), 3.03-2.85 (m, 1H), 2.33-2.24 (d, *J* = 14 Hz, 1H), 1.92-1.80 (m, 1H), 1.69 (s, 3H), 1.50-1.41 (m, 1H), 1.31 (d, *J* = 7.15 Hz, 3H), 1.01 (s, 9H), 0.68 (d, *J* = 6.11 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 171.1, 170.5, 155.6, 135.9, 133.9, 133.1, 129.8, 127.7, 126.6, 74.2, 60.4, 59.0, 53.4, 37.0, 34.0, 27.0, 24.8, 21.0, 19.6, 15.7, 14.2, 11.4.



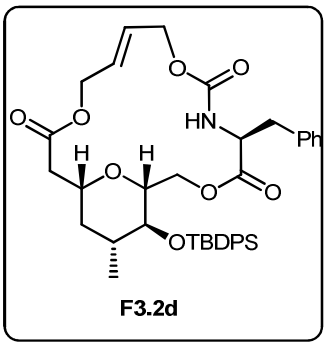
F3.2c

Molecular Formula: $C_{35}H_{45}NO_8Si$

R_f (solvent system): 0.3 (30% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 636.2 (M+1).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm : 7.70-7.62 (m, 4H), 7.46-7.33 (m, 6H), 5.85-5.77 (m, 1H), 5.74 (dt, *J* = 15.6, 3.2 Hz, 1H), 5.11-5.04 (dd, *J* = 13.2, 1.6 Hz ,1H), 4.58-4.42 (m, 2H), 4.32 (dd, *J* = 8.8, 3.2 Hz, 2H), 4.23 (dd, *J* = 13.13, 6.81 Hz, 1H), 4.14 (dd, *J* = 11.81, 7.20 Hz, 1H), 3.92 (dd, *J* = 13.05, 6.46 Hz, 2H), 3.57 (ddd, *J* = 10.51, 7.68, 4.96 Hz, 1H), 3.52-3.43 (m, 1H), 3.11 (t, *J* = 7.39 Hz, 1H), 2.92 (dd, *J* = 14.21, 11.99 Hz, 1H), 2.32 (dd, *J* = 14.26, 2.78 Hz, 1H), 2.22-2.13 (m, 1H), 1.95-1.80 (m, 4H), 1.65 (dd, *J* = 9.50, 4.65 Hz, 1H), 1.45-1.36 (m, 1H), 1.02 (s, 9H), 0.77 (d, *J* = 6.75 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm : 173.3, 170.3, 153.8, 135.9, 133.4, 133.3, 129.8, 127.6, 127.2, 124.9, 74.3, 73.7, 67.6, 66.5, 63.9, 58.6, 46.7, 38.7, 34.4, 32.7, 30.8, 27.1, 23.5, 19.6, 18.8.



F3.2d

(1R,5S,16R,18R,19S,E)-5-benzyl-19-((tert-butyldiphenylsilyl)oxy)-18-methyl-3,8,13,20-tetraoxa-6-azabicyclo[14.3.1]icos-10-ene-4,7,14-trione (F3.2d) :

Molecular Formula: $C_{39}H_{47}NO_8Si$

$\mathbf{R_f}$ (solvent system): 0.3 (30% ethyl acetate/hexanes)

LRMS: (ES+) *m/z* = 686.3 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.68 (dd, *J* = 10.95, 4.46 Hz, 4H), 7.46-7.34 (m, 6H), 7.33-7.25 (m, 3H), 7.16 (d, *J* = 6.82 Hz, 2H), 5.91-5.64 (m, 2H), 4.70 (d, *J* = 1.00 Hz, 3H), 4.50-4.23 (m, 3H), 4.06-3.92 (m, 1H), 3.80 (t, *J* = 5.79 Hz, 1H), 3.42-3.13 (m, 1H), 3.10-2.98 (m, 1H), 2.34-2.23 (m, 1H), 2.13-2.05 (m, 1H), 2.03-1.96 (m, 1H), 1.94-1.81 (m, 1H), 1.69-1.49 (m, 2H), 1.29 (dd, *J* = 7.82, 6.16 Hz, 1H), 1.03 (d, *J* = 5.02 Hz, 9H), 0.92-0.82 (m, 1H), 0.69 (d, *J* = 2.15 Hz, 3H); **^{13}C NMR** (100 MHz, *$CDCl_3$*) δ ppm : 172.2, 170.4, 155.0, 136.0, 133.8, 133.1, 129.9, 129.7, 129.1, 128.8, 127.6, 126.7, 77.2, 74.3, 55.3, 37.5, 34.5, 31.6, 29.7, 27.0, 25.8, 22.6, 19.7, 14.1, 11.4.

3.4.6 References

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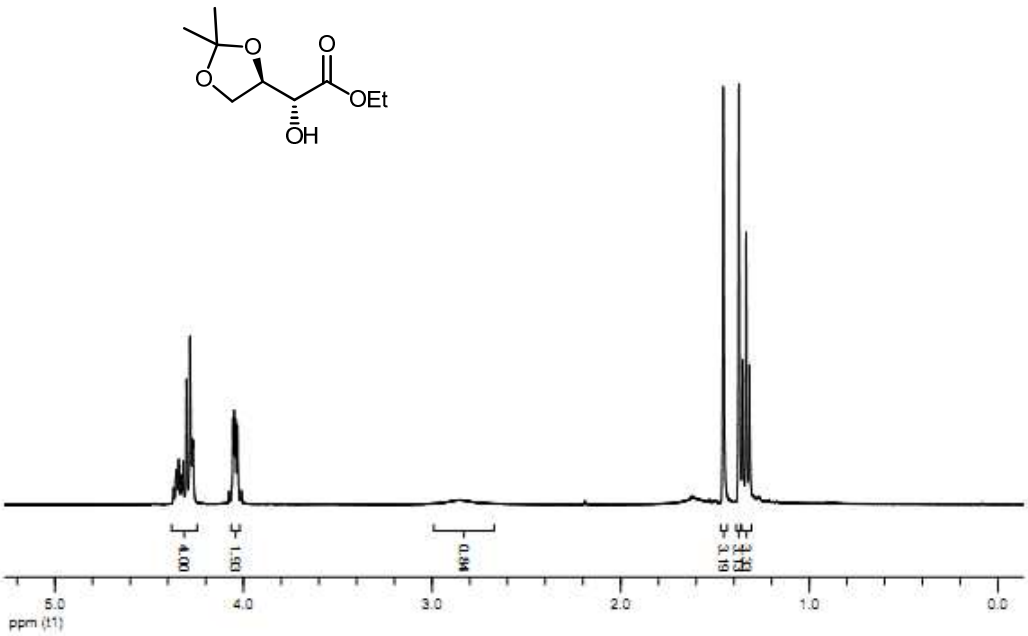
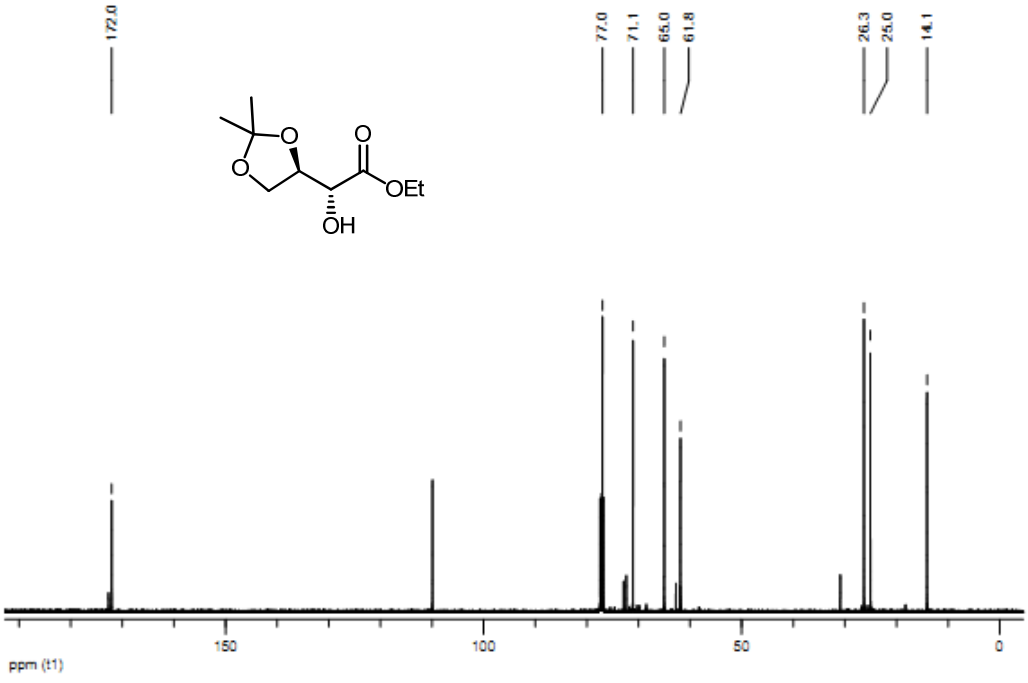
3.4.7 Spectral Data

^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



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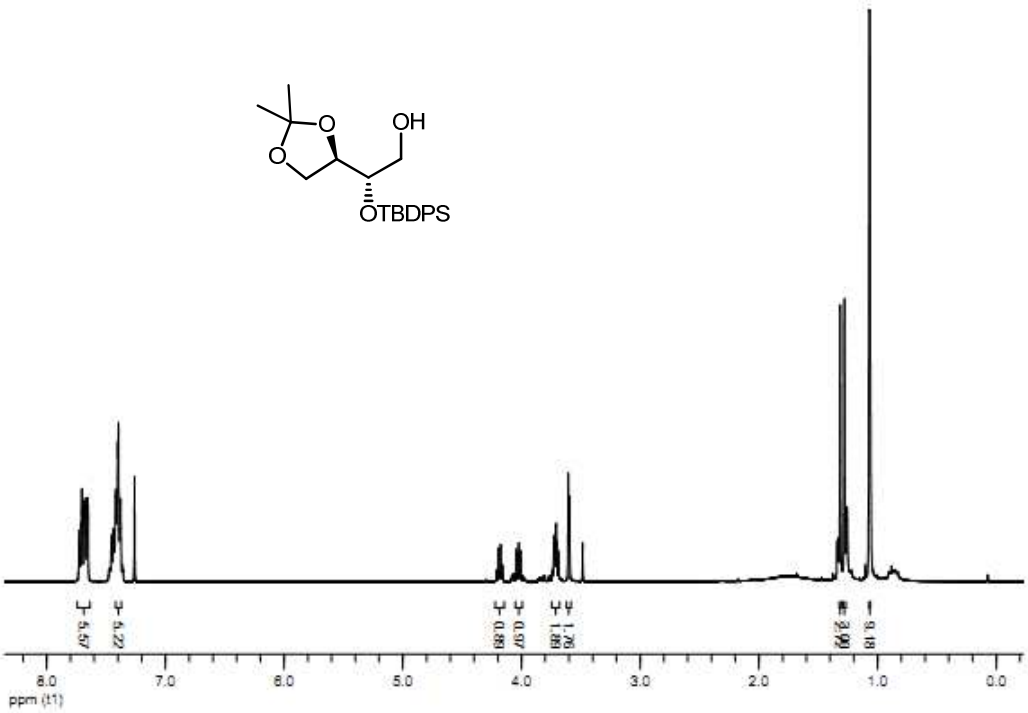

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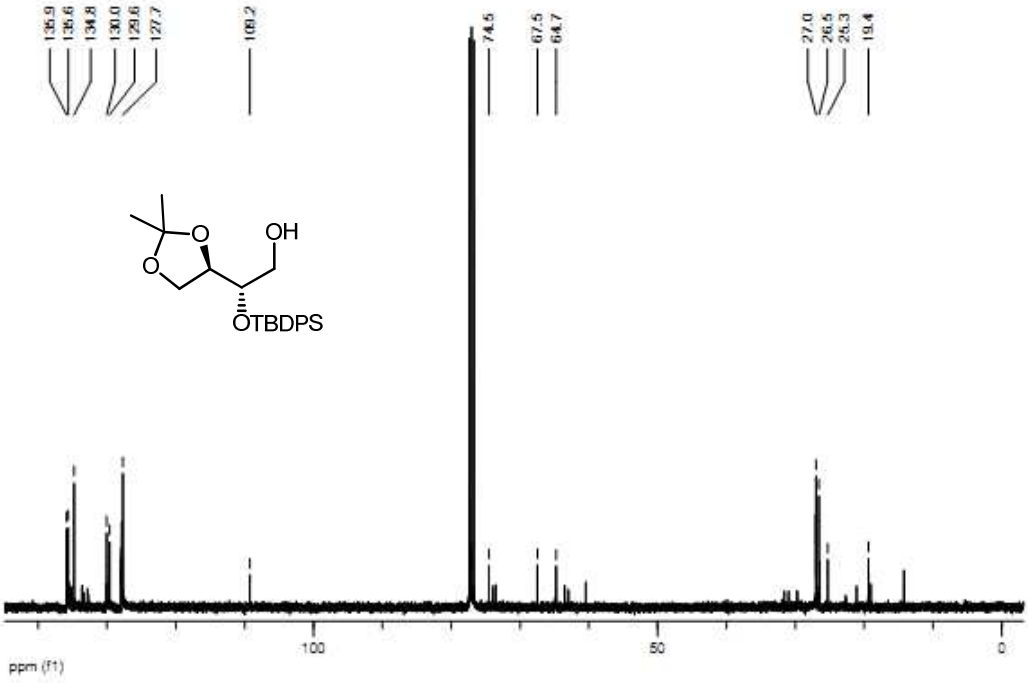

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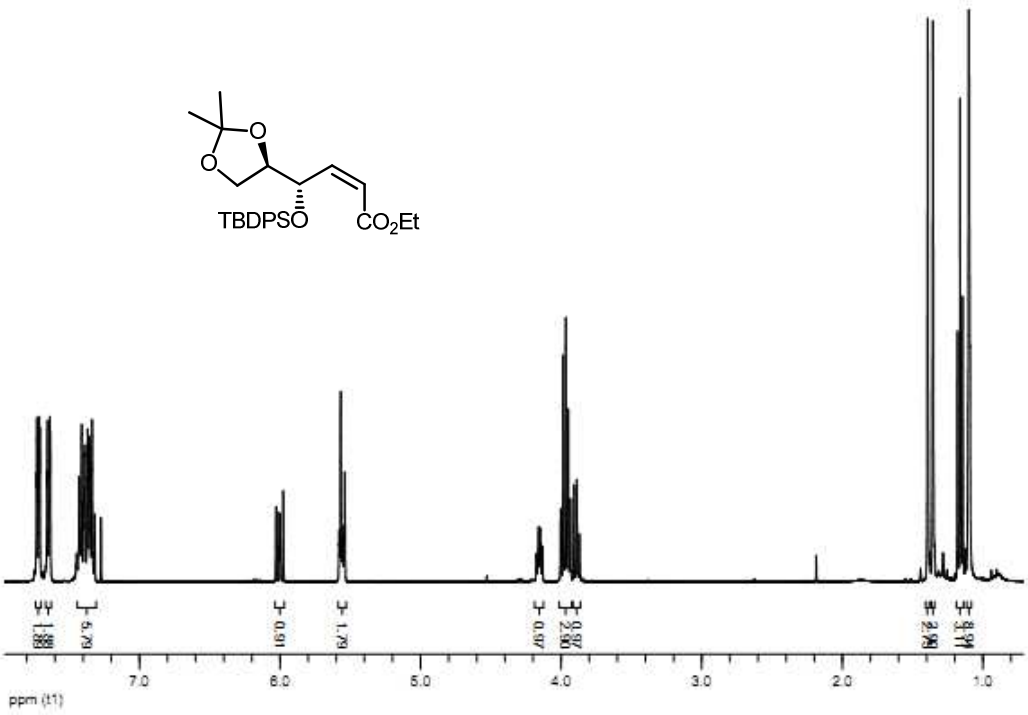

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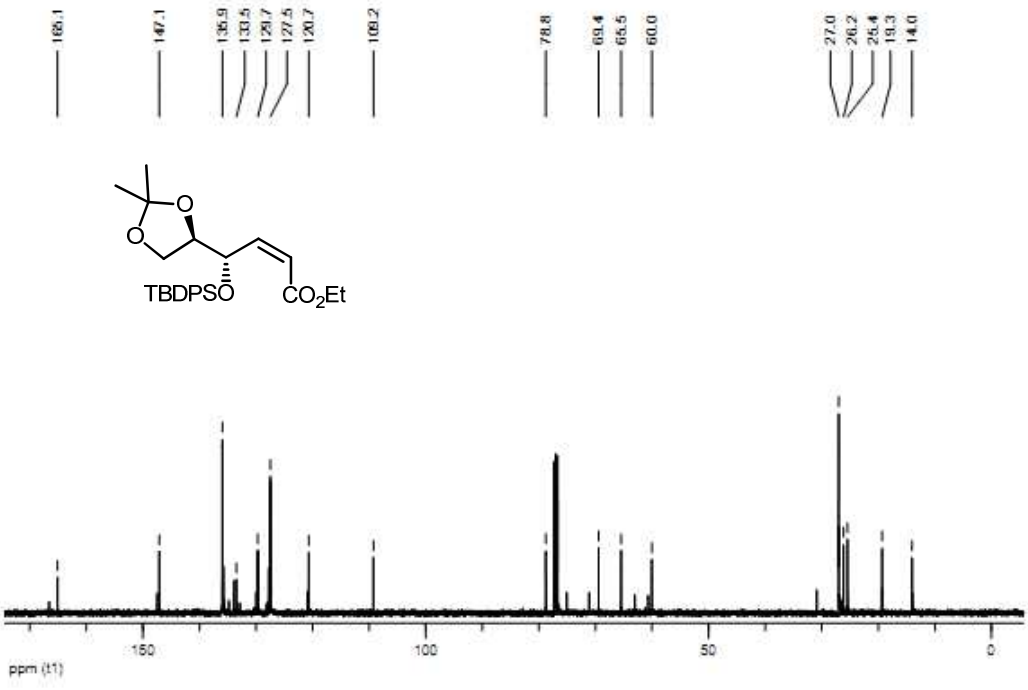

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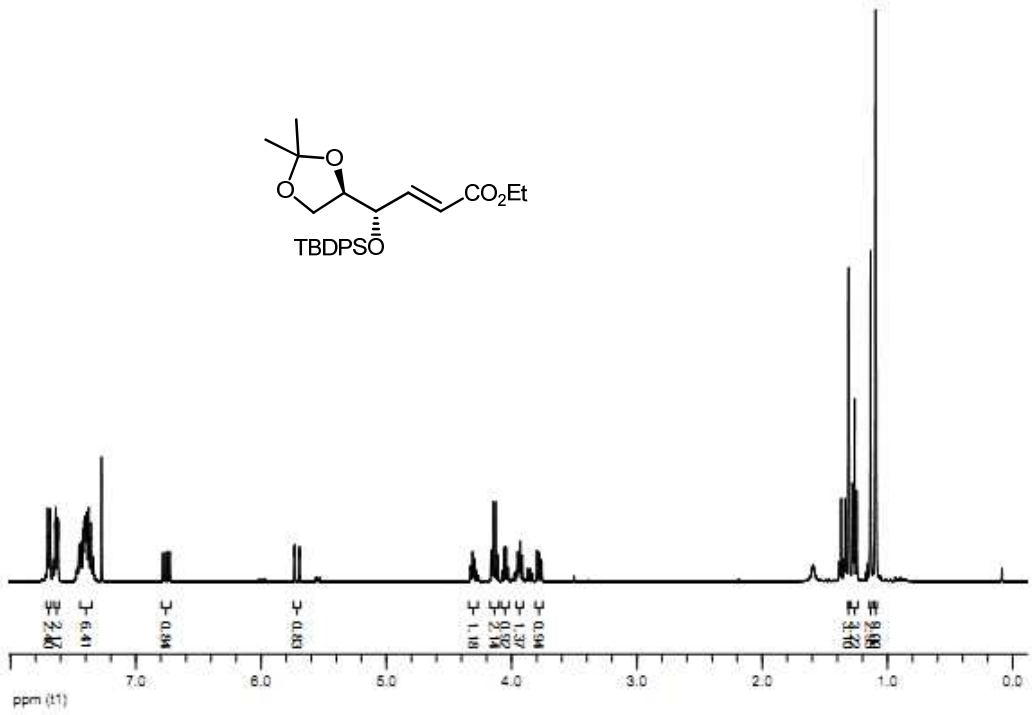

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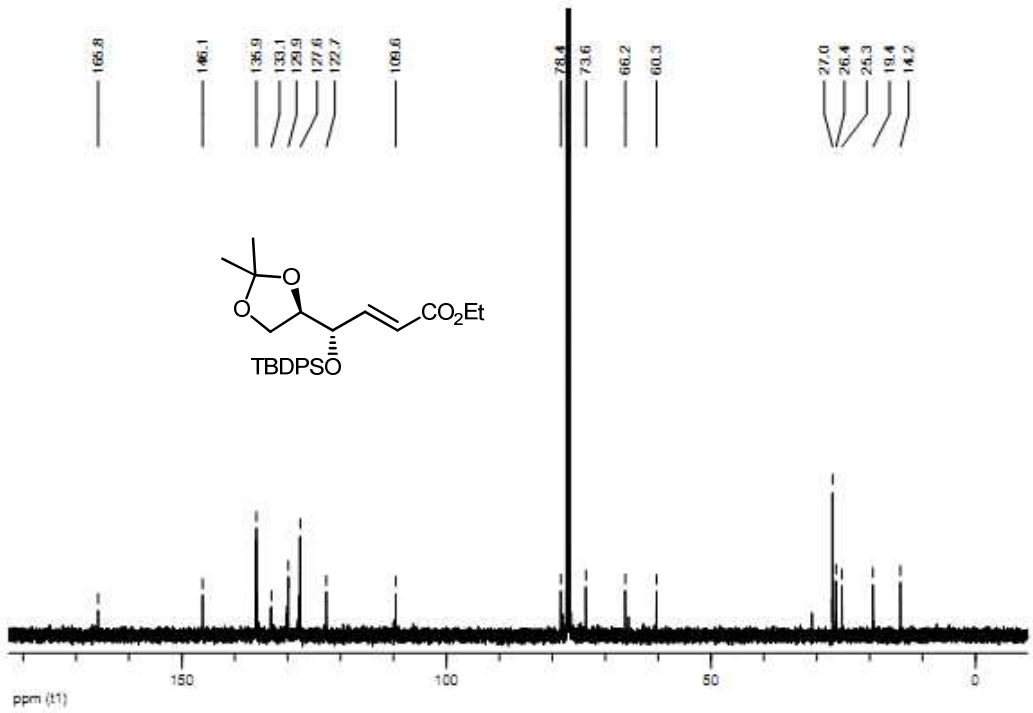

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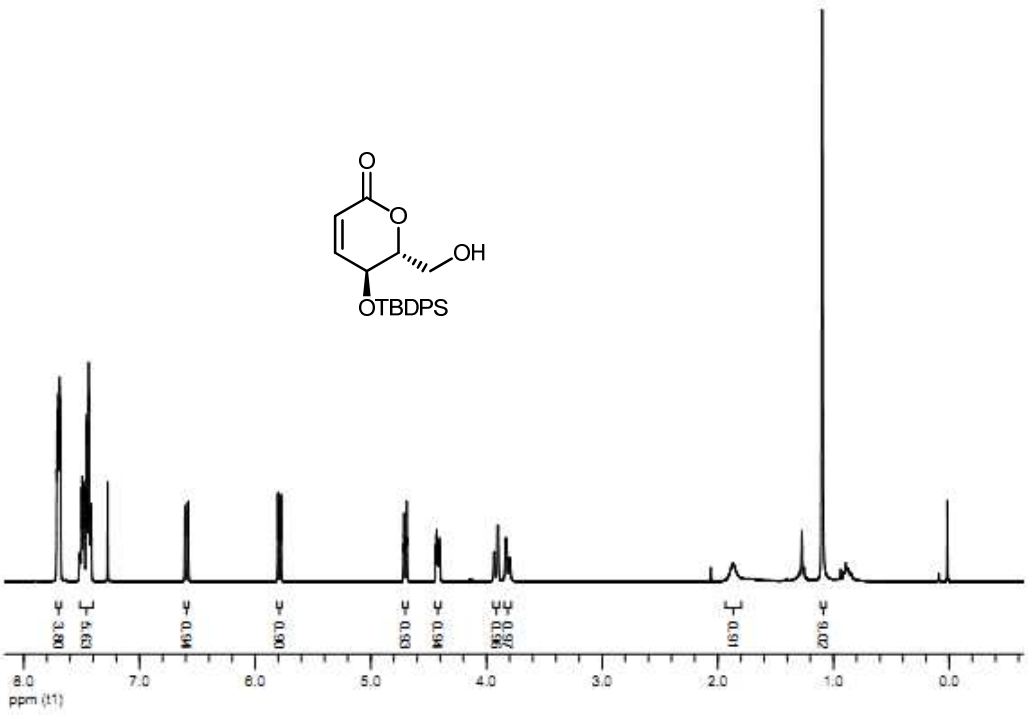
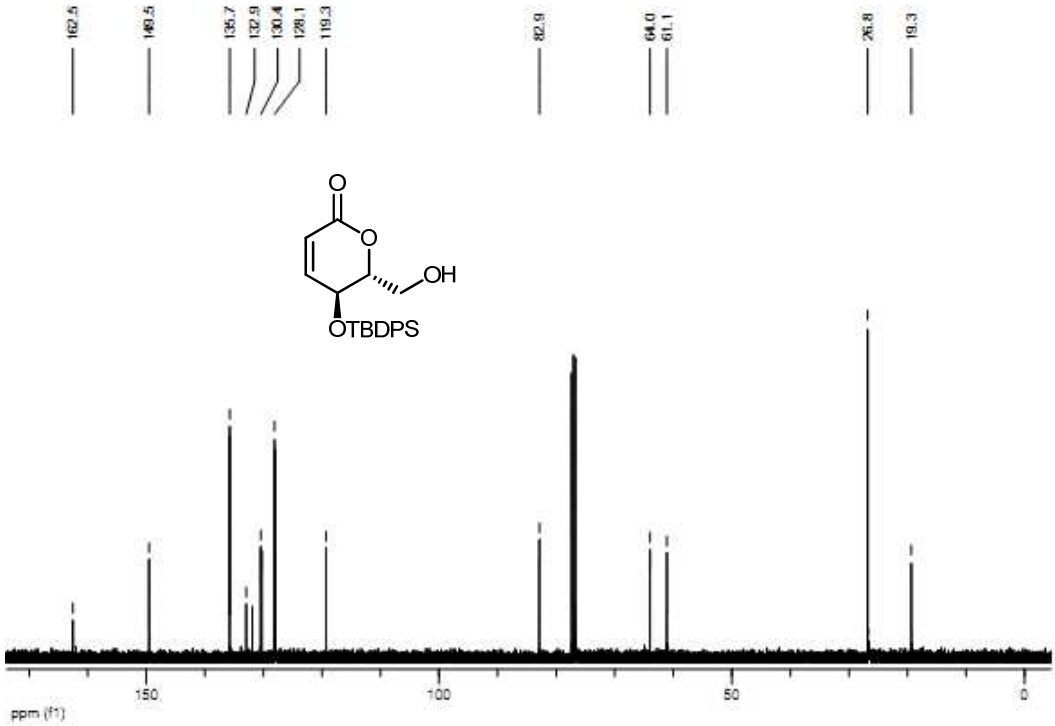
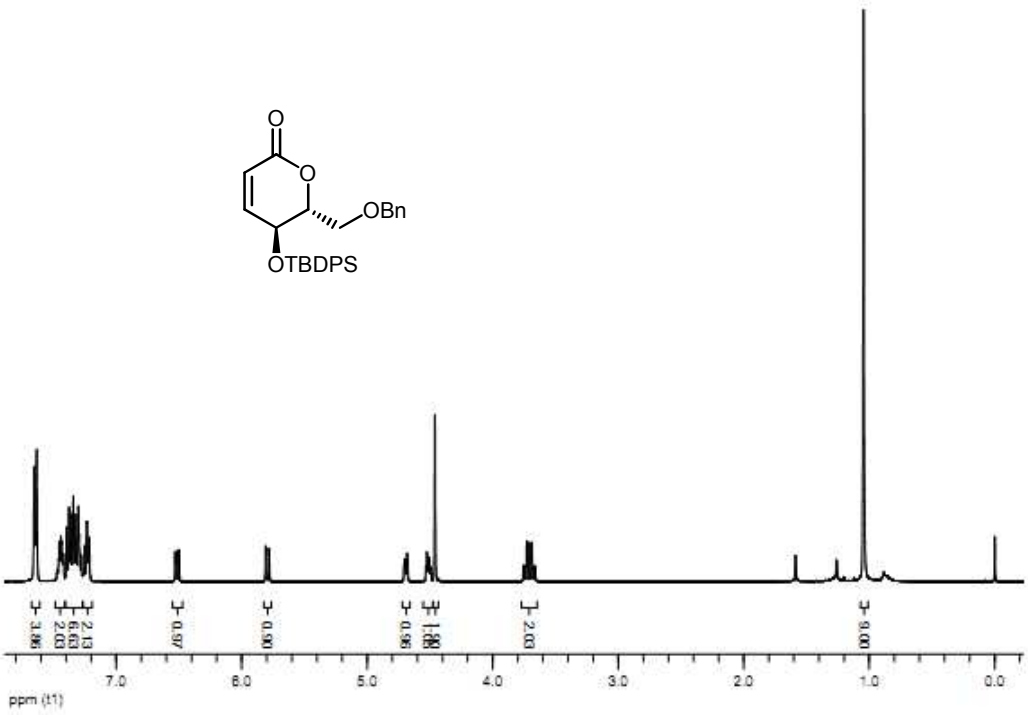


^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



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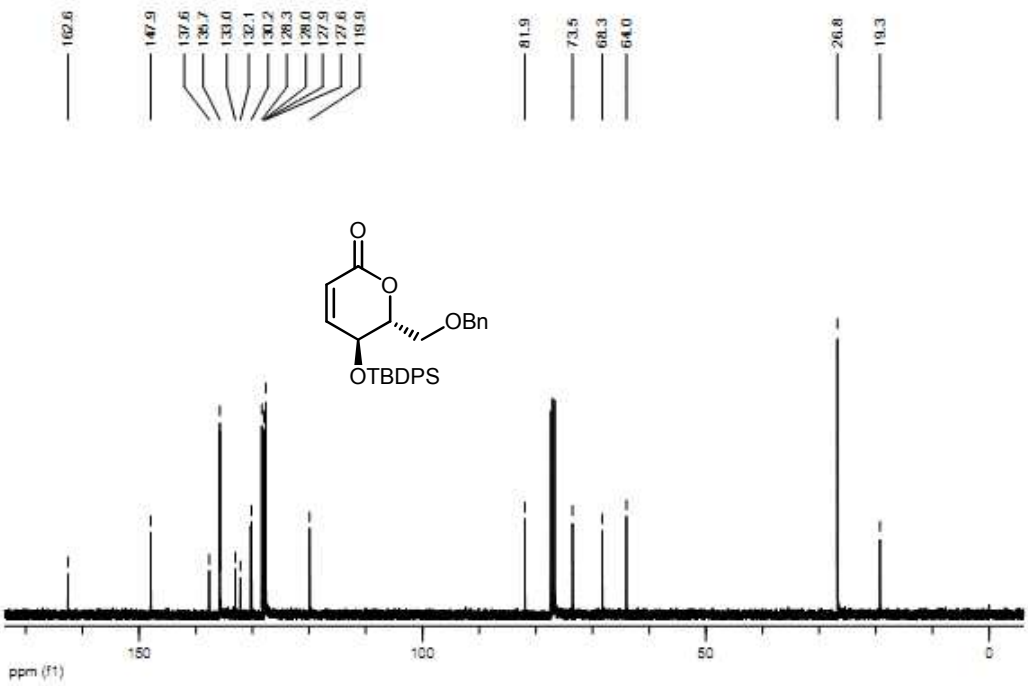

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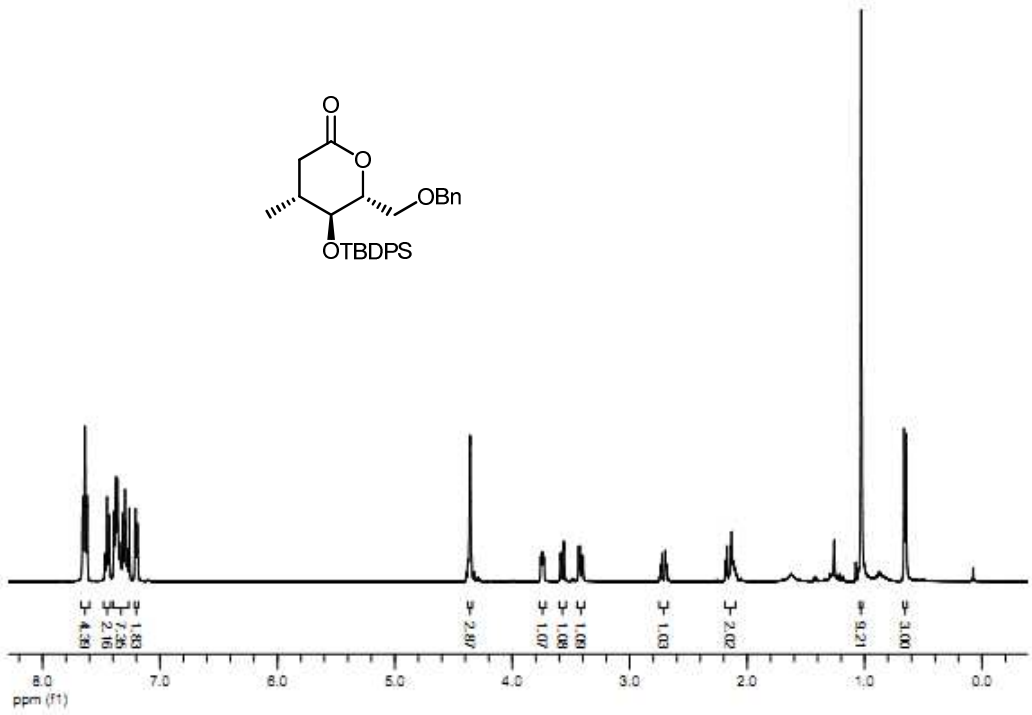
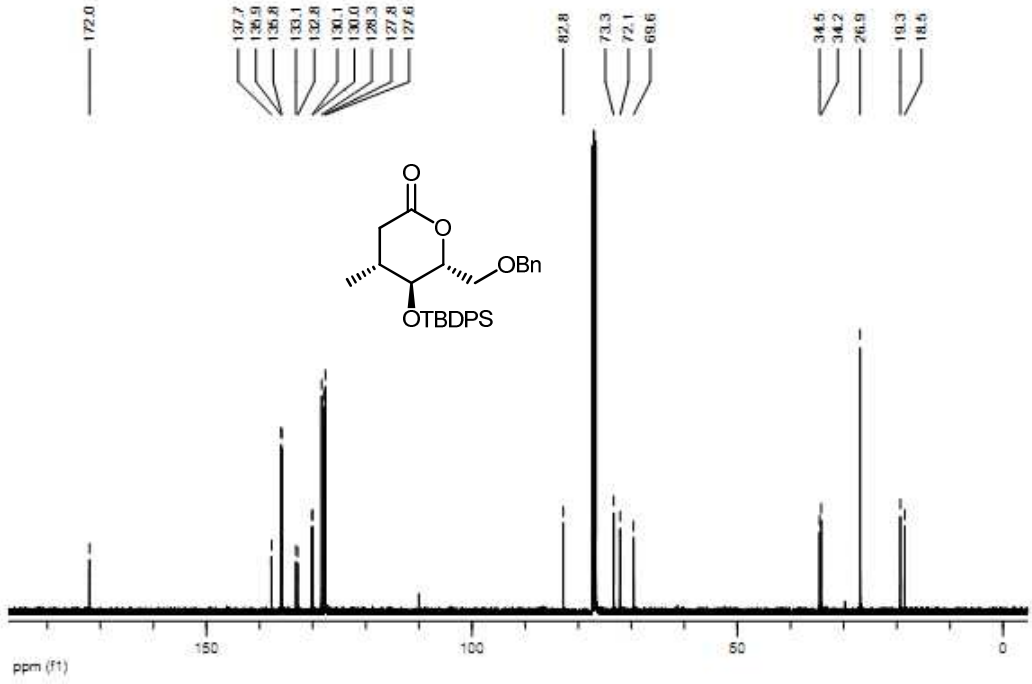
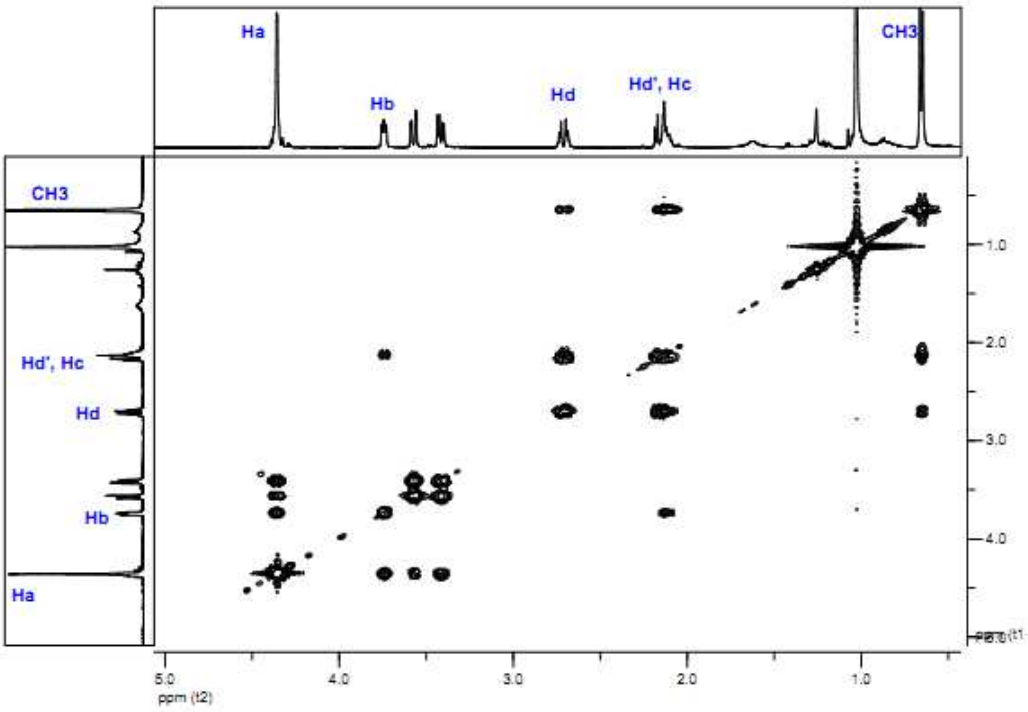
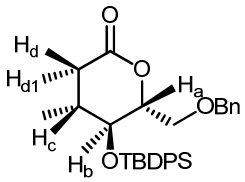


^{13}C NMR ($CDCl_3$, 100MHz)

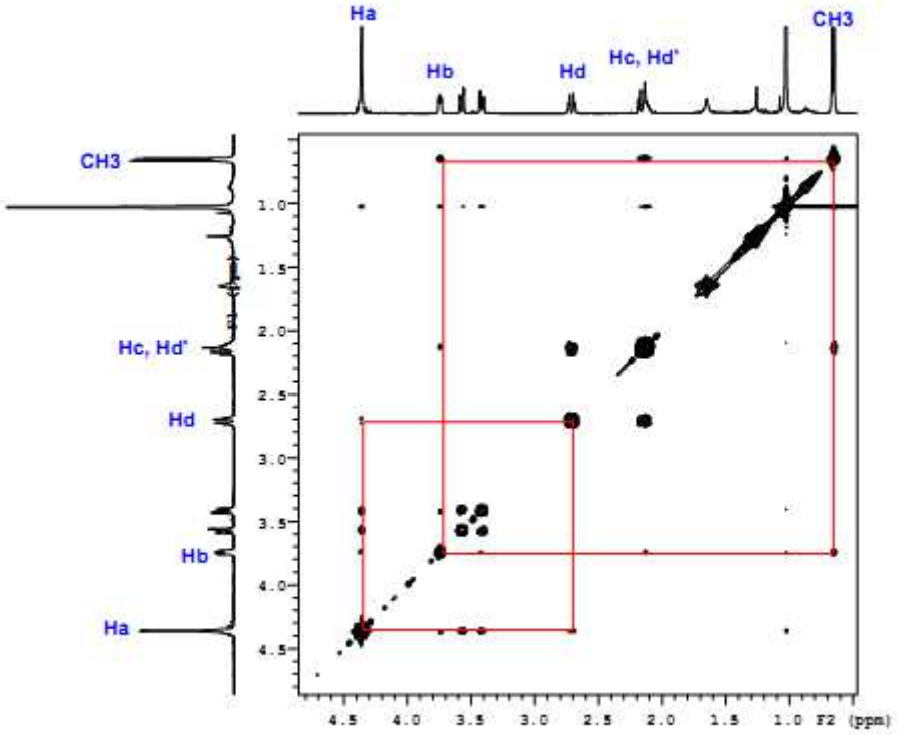


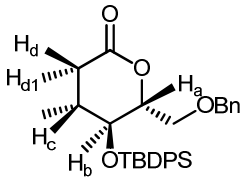
^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)

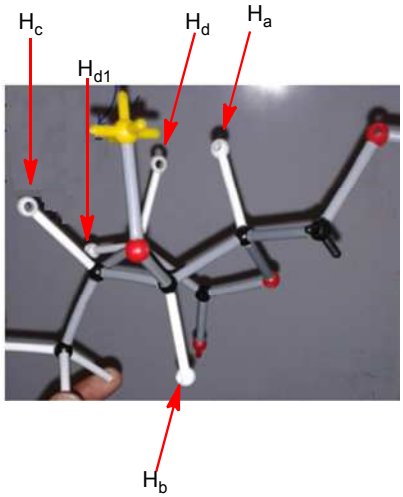




NOESY



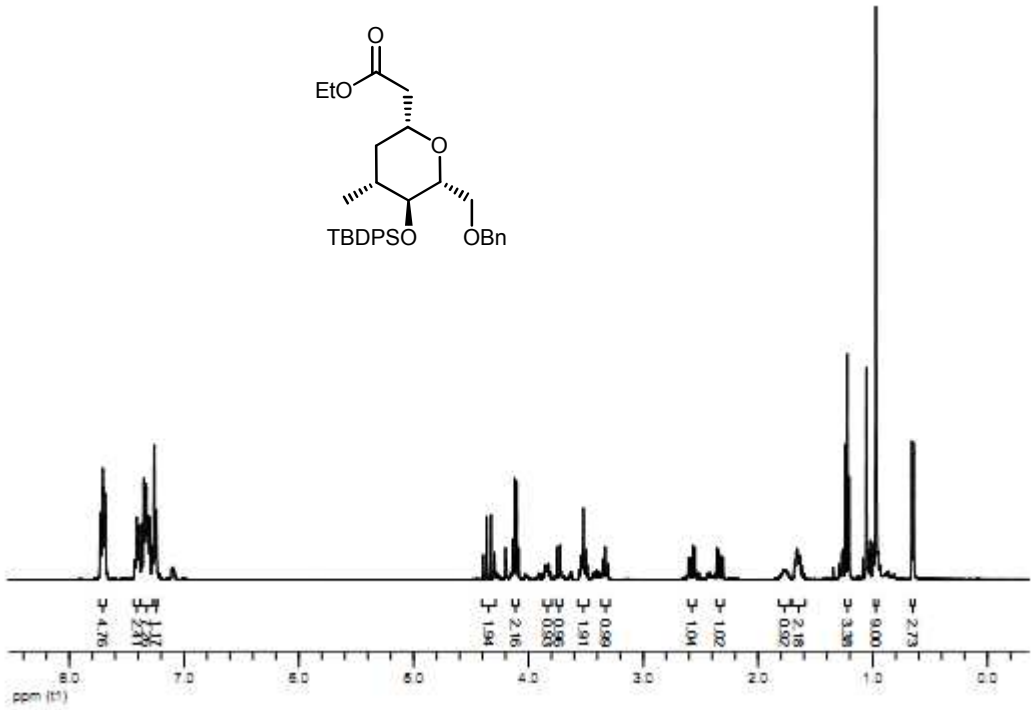




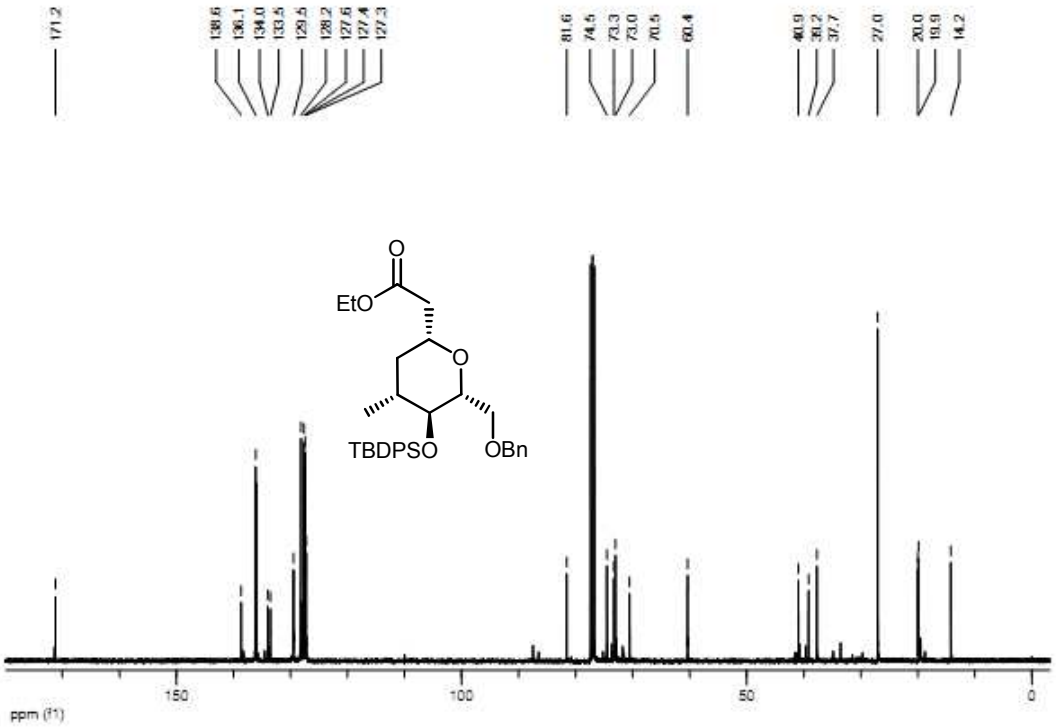
H_a-H_d – nOe **CH_3- H_b – nOe**

H_a-H_d[1]- No nOe **CH_3- H_d – No nOe**

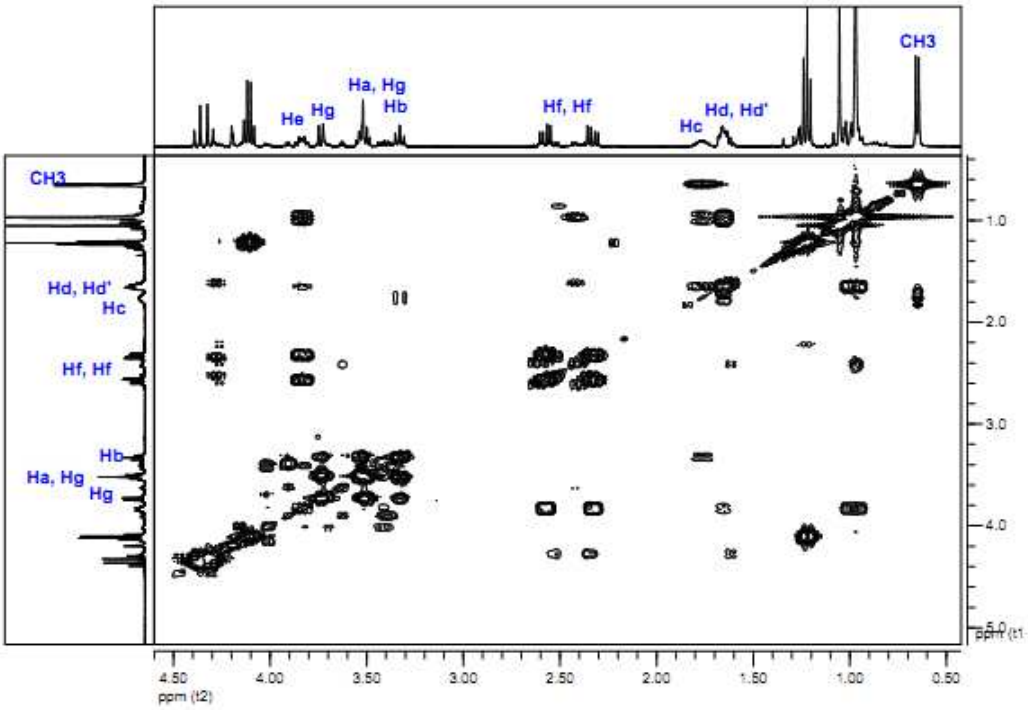
^{1}H NMR ($CDCl_3$, 400MHz)

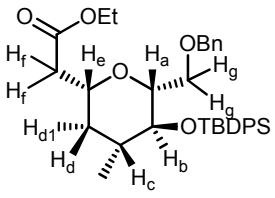


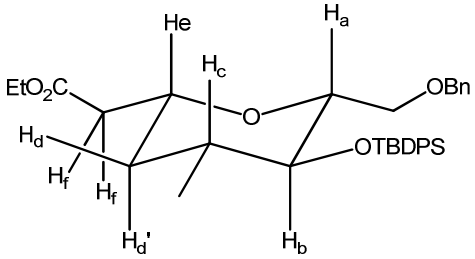
^{13}C NMR ($CDCl_3$, 100MHz)



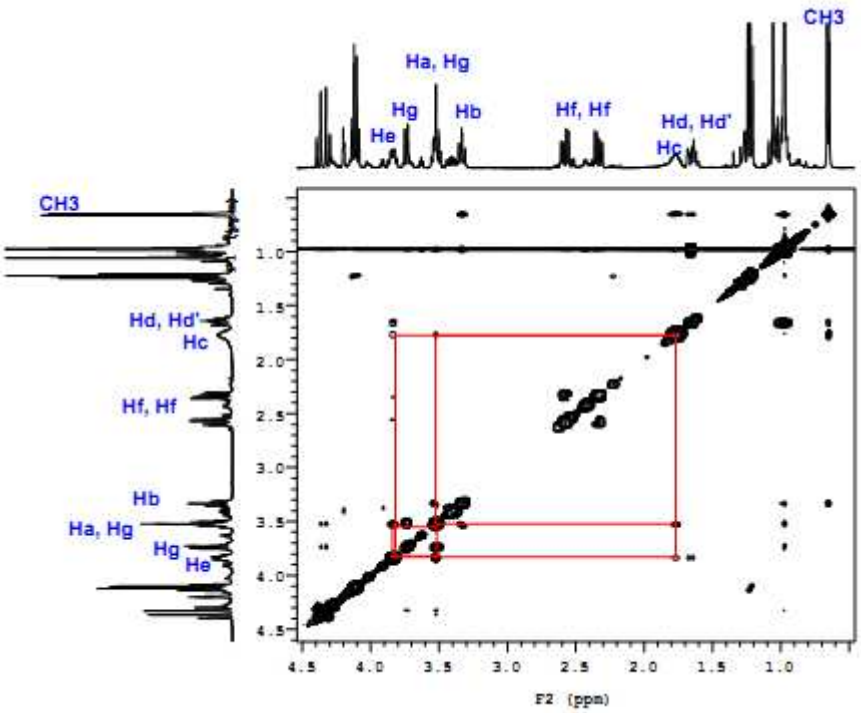
^{1}H- H^{1} COSY NMR ($CDCl_3$, 400MHz)

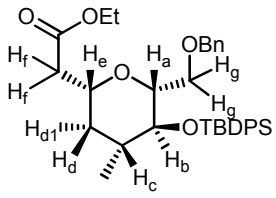






NOESY


CH3
Ha, Hg
Hg
Hb
He
Hf, Hf
Hd, Hd'
Hc
CH3
Hd, Hd'
Hc
Hf, Hf
Hb
Ha, Hg
Hg
He
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
F2 (ppm)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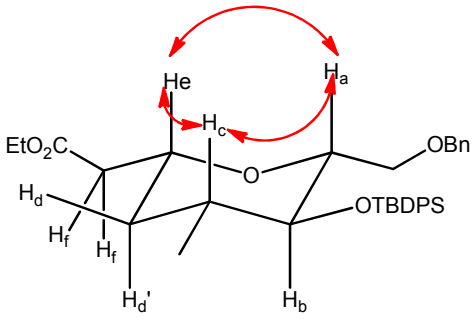
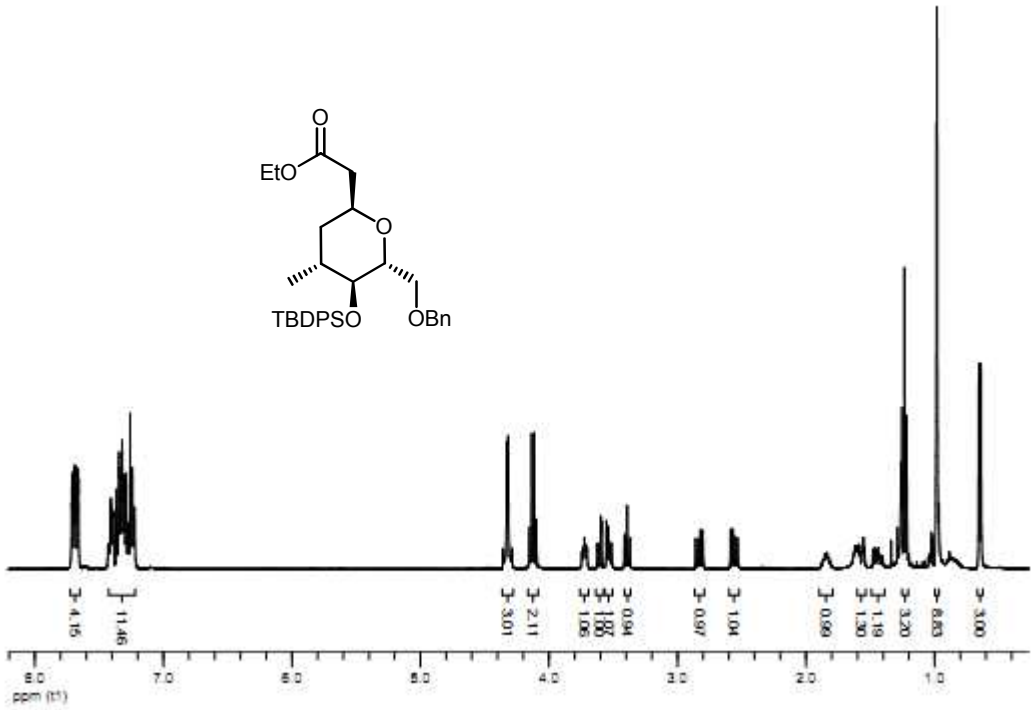
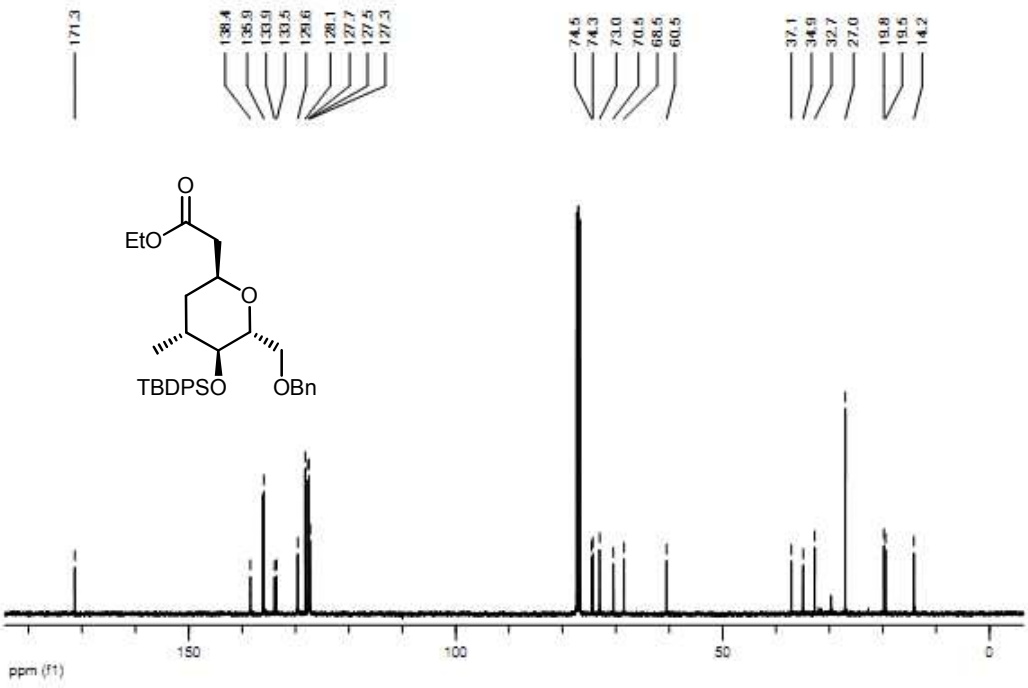
OEt
Hf
Hf
He
O
Ha
OBn
Hg
Hg
Hd1
OTBDPS
Hd
Hc
Hb


He
Ha
Hc
EtO2C
O
OBn
Hd
OTBDPS
Hf
Hf
Hd'
Hb

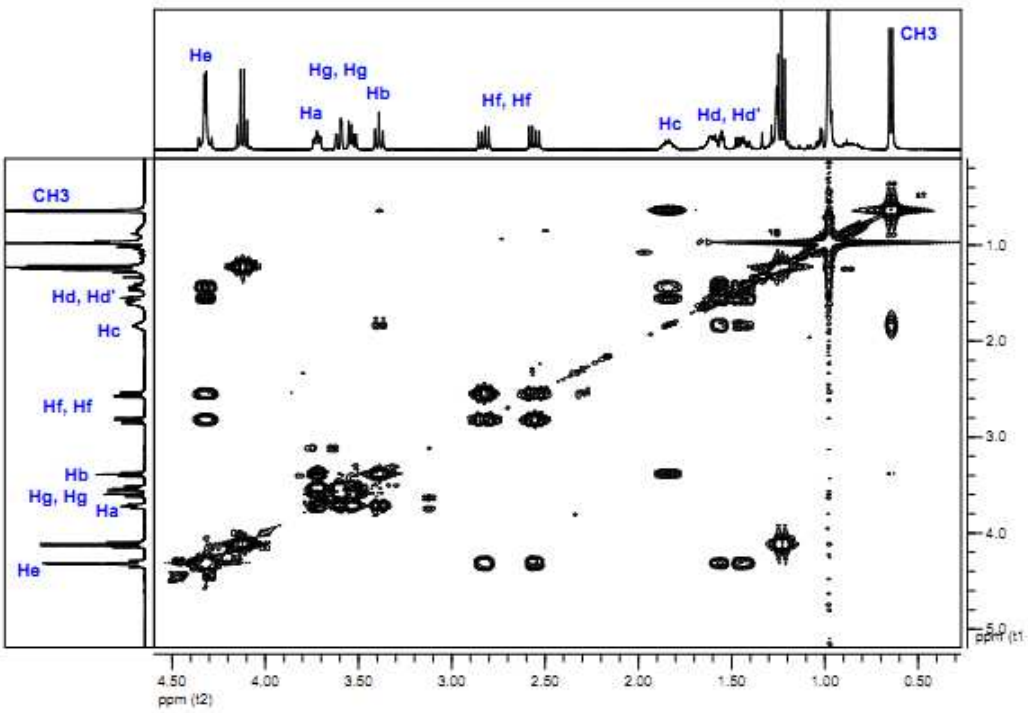
^{1}H NMR ($CDCl_3$, 400MHz)

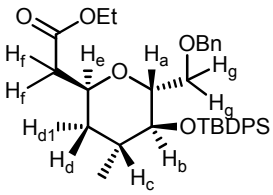


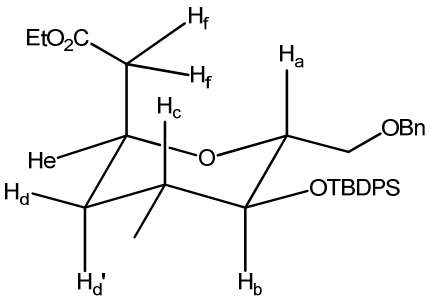
^{13}C NMR ($CDCl_3$, 100MHz)



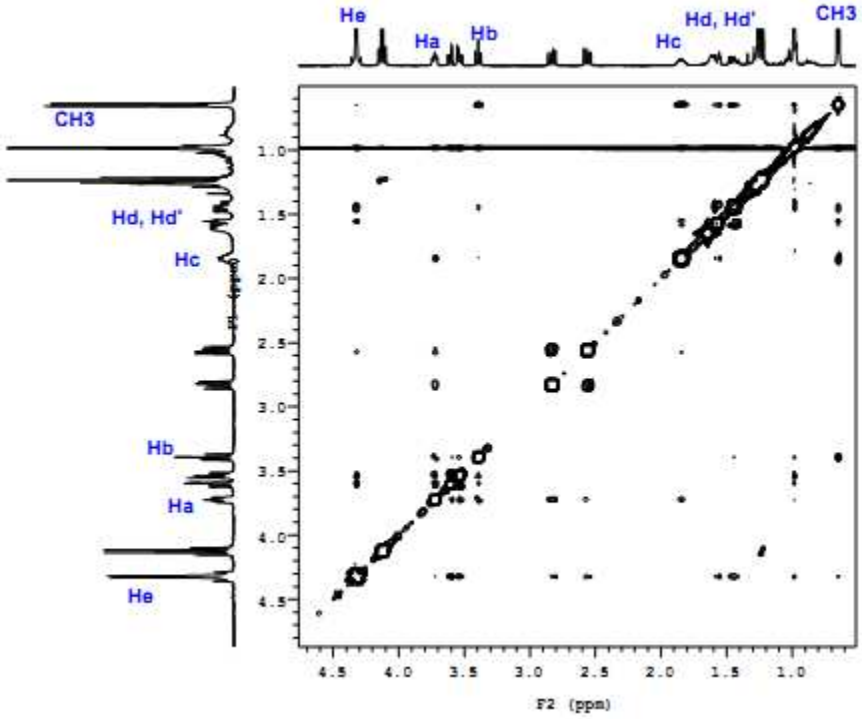
^{1}H- H^{1} COSY NMR ($CDCl_3$, 400MHz)

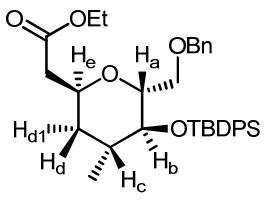






NOESY


He
Ha
Hb
Hd, Hd'
Hc
CH3
CH3
Hd, Hd'
Hc
Hb
Ha
He
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
F2 (ppm)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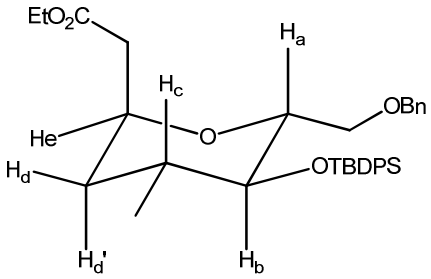
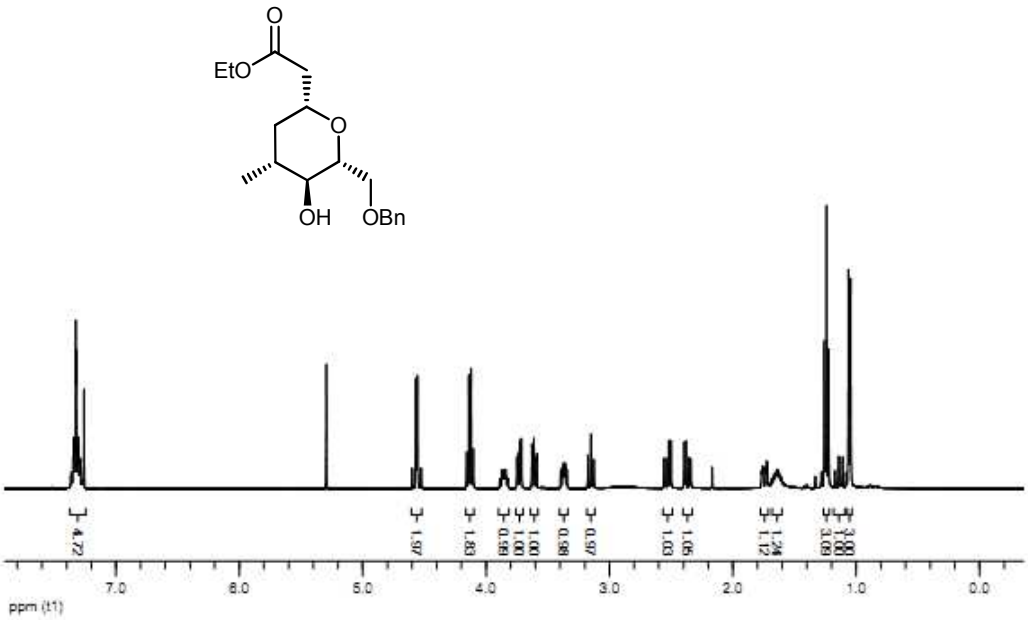
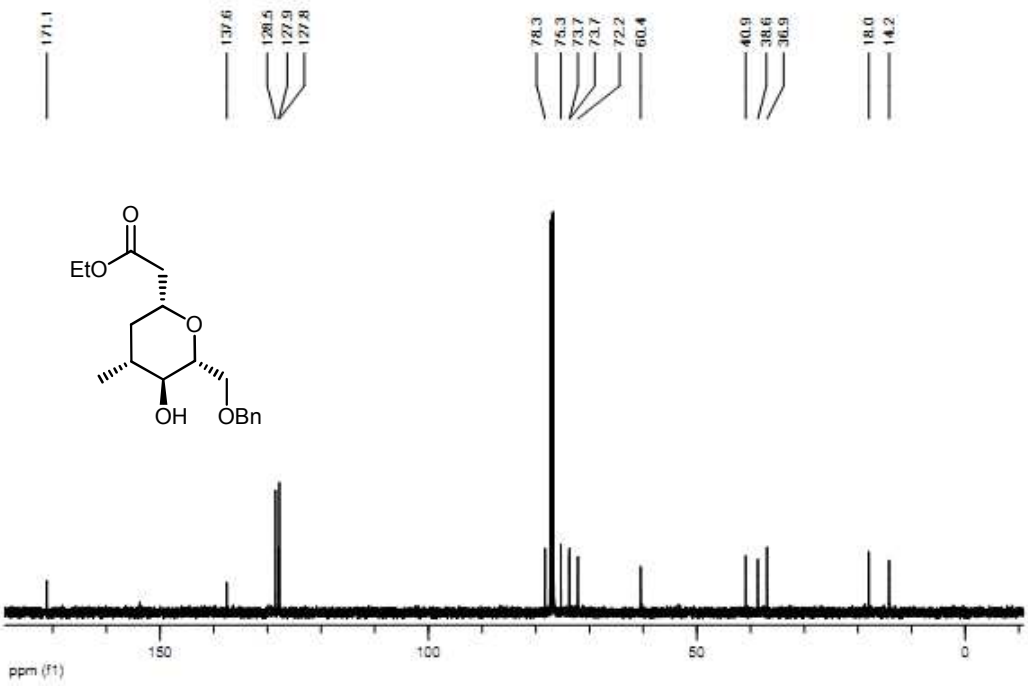
OBn
O
Hd1
Hd
OTBDPS
Hb
Hc


EtO2C
Ha
Hc
OBn
He
O
Hd
OTBDPS
Hd'
Hb

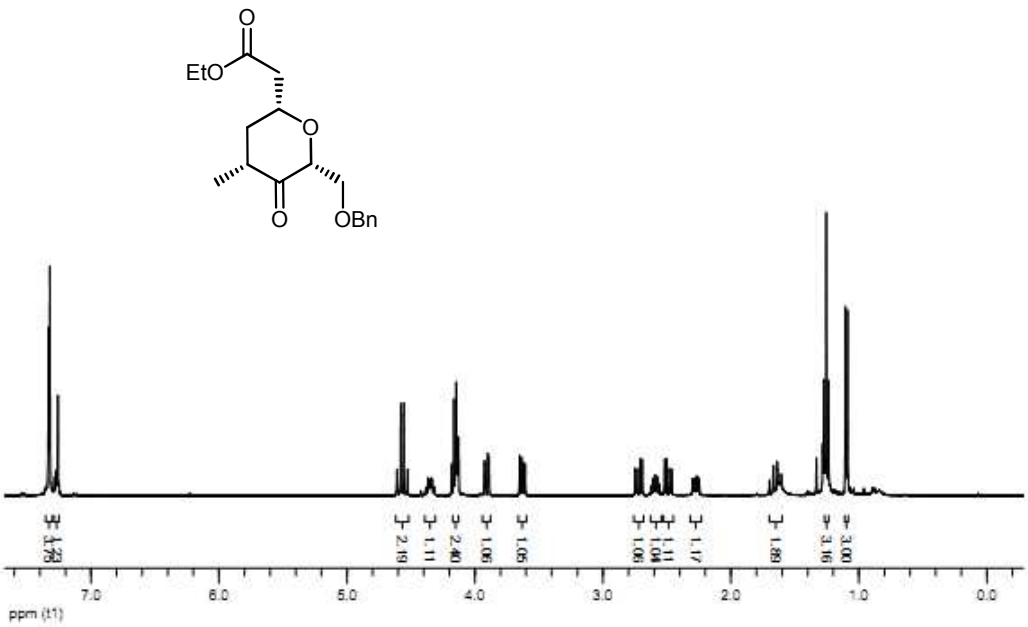
^{1}H NMR ($CDCl_3$, 400MHz)



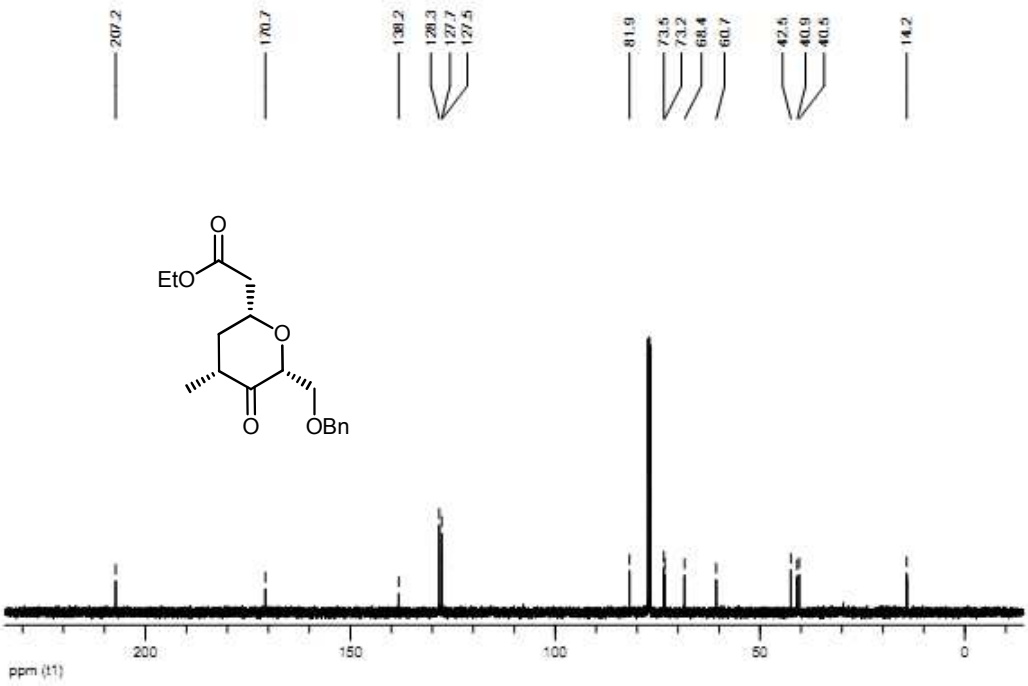
^{13}C NMR ($CDCl_3$, 100MHz)



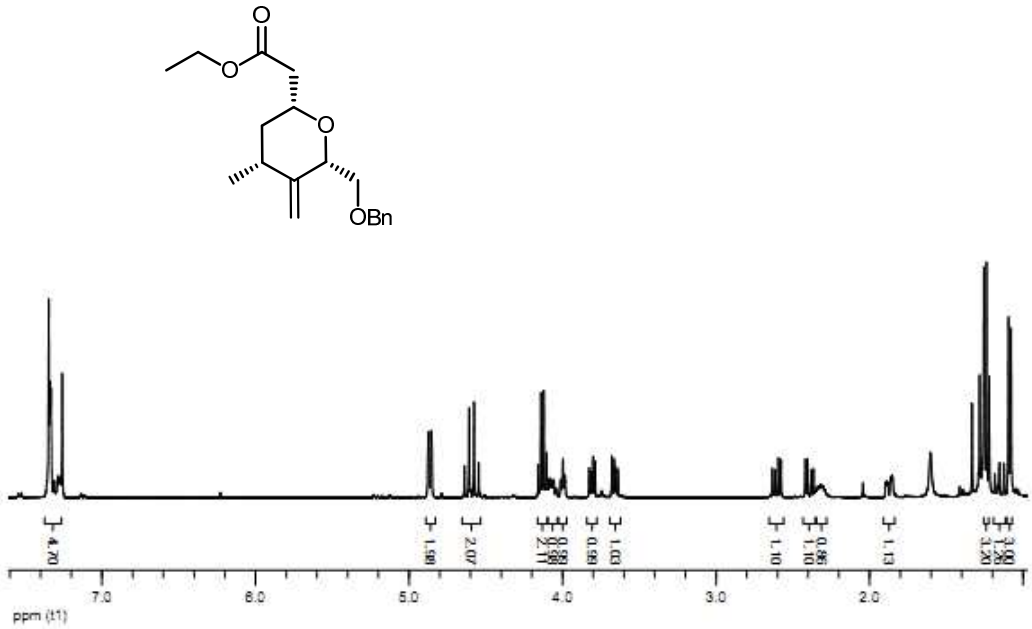
^{1}H NMR ($CDCl_3$, 400MHz)



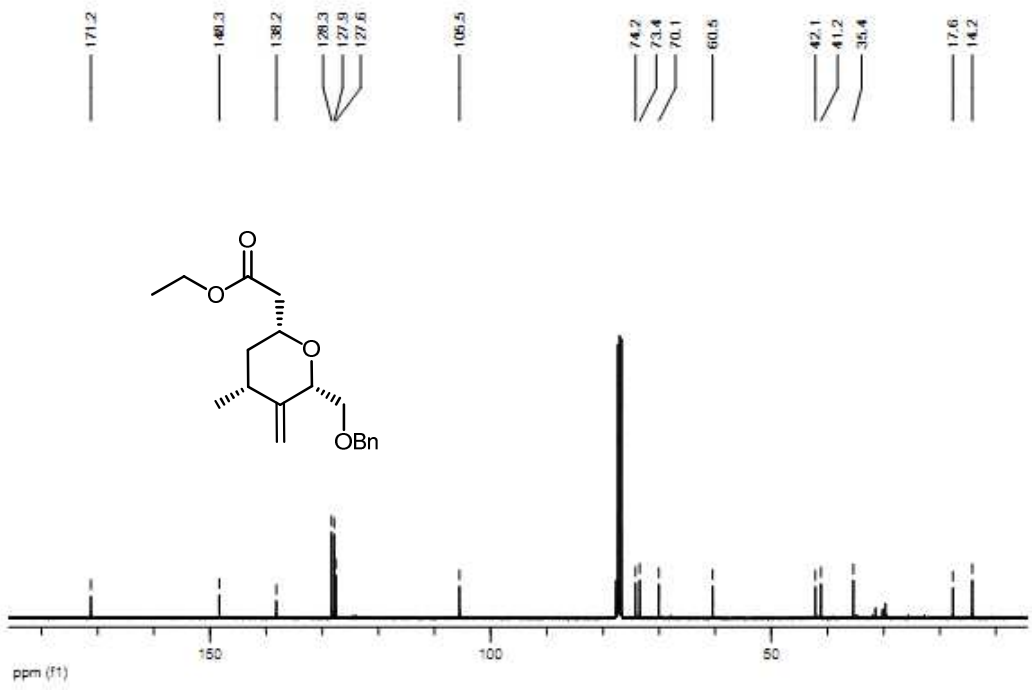
^{13}C NMR ($CDCl_3$, 100MHz)



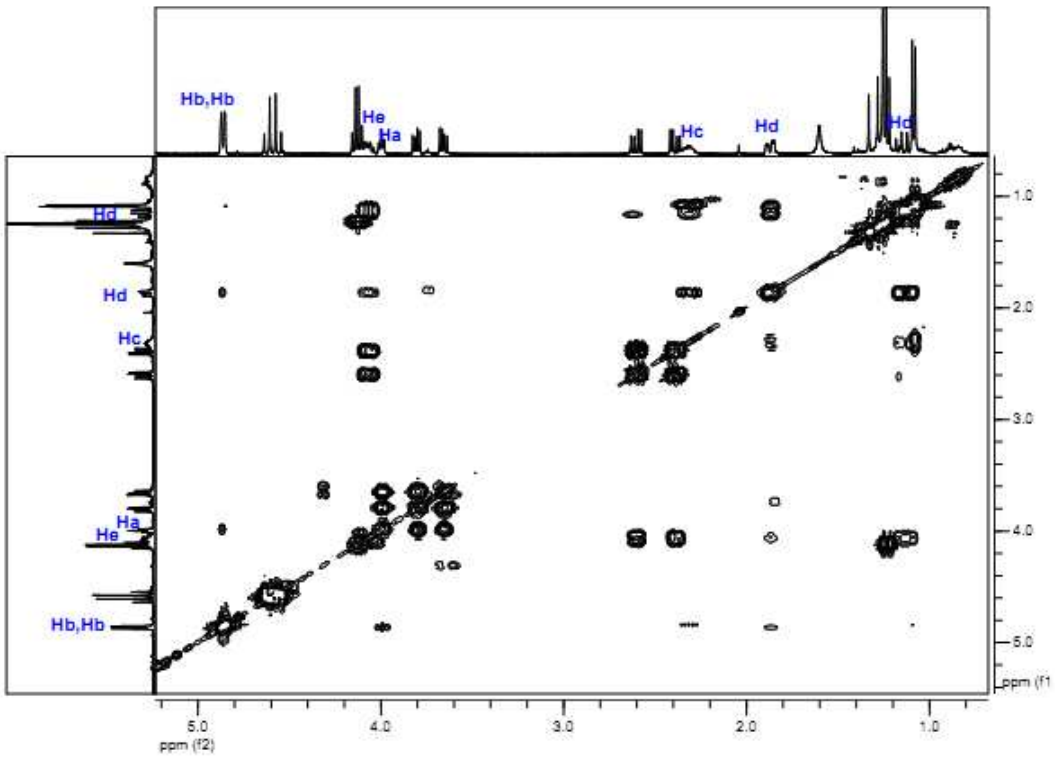
^{1}H NMR ($CDCl_3$, 400MHz)

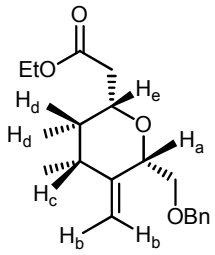


^{13}C NMR ($CDCl_3$, 100MHz)

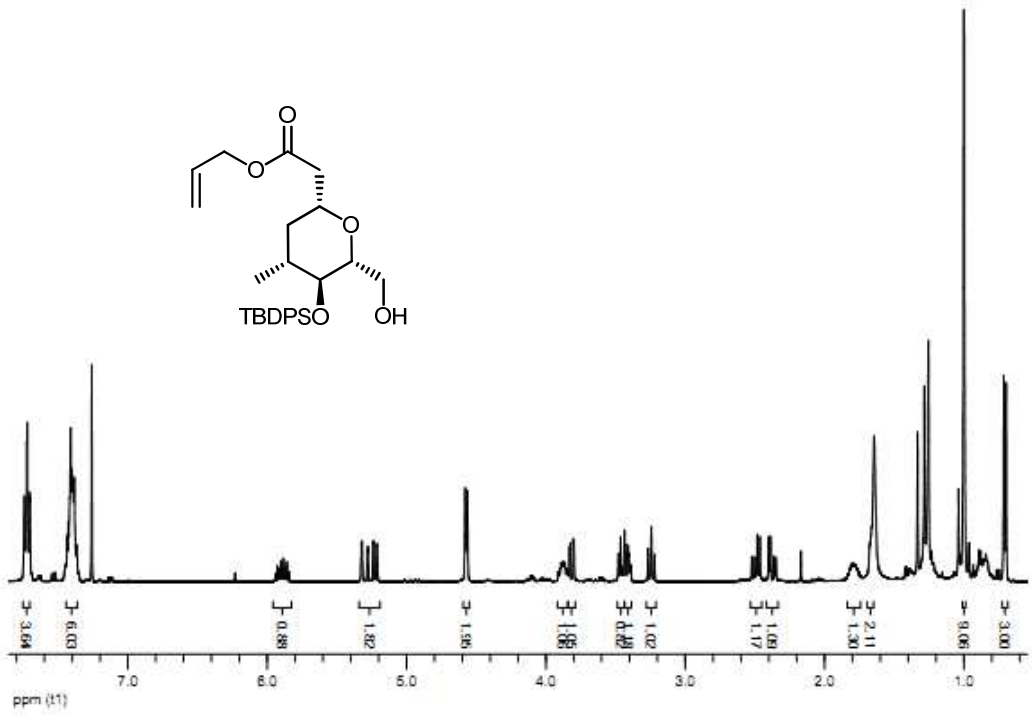


^{1}H- H^{1} COSY NMR ($CDCl_3$, 400MHz)

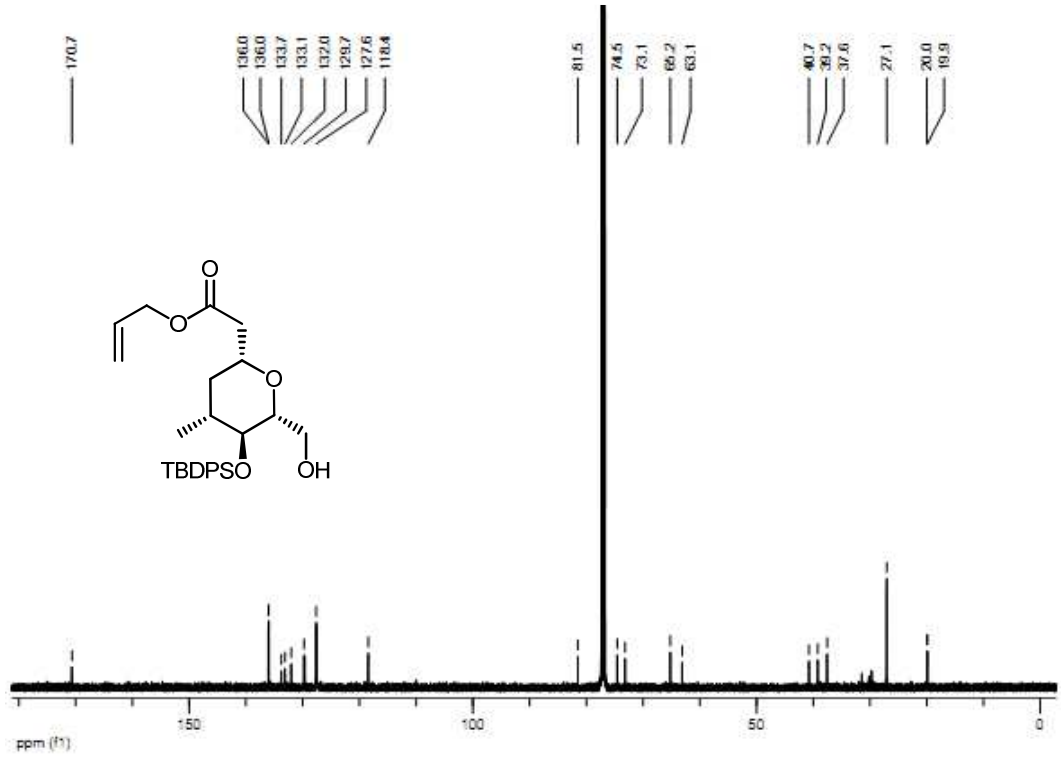




^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



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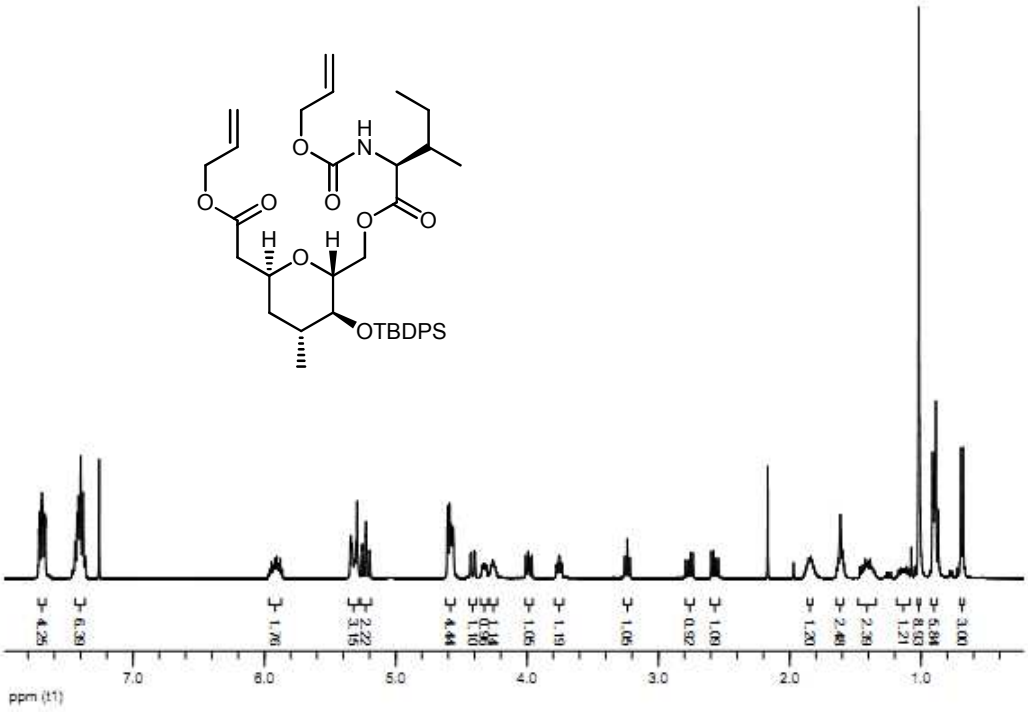

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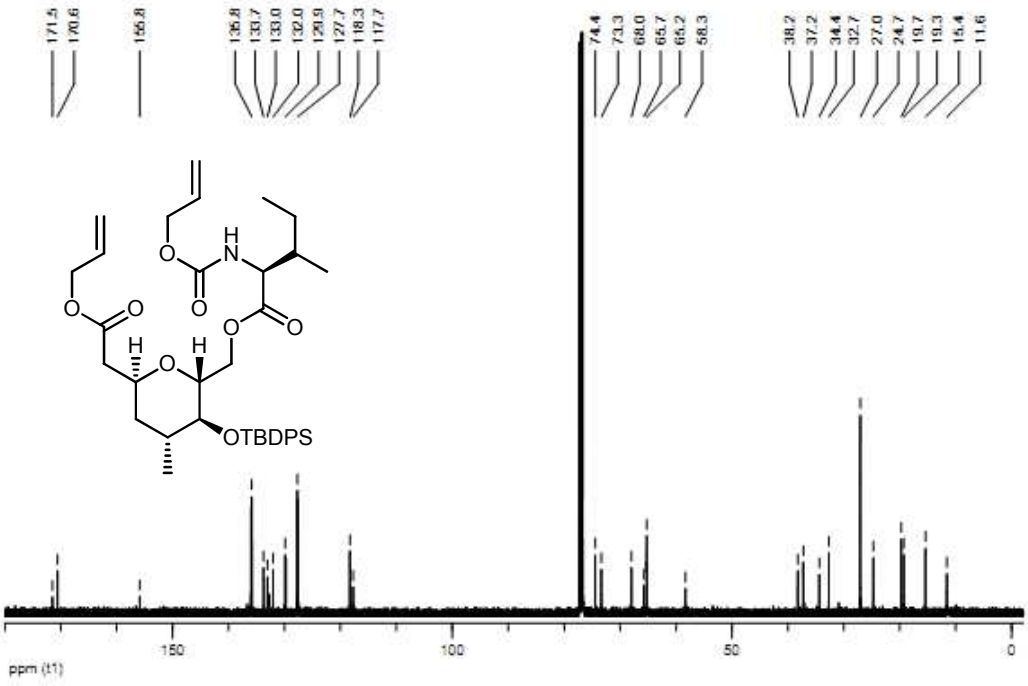

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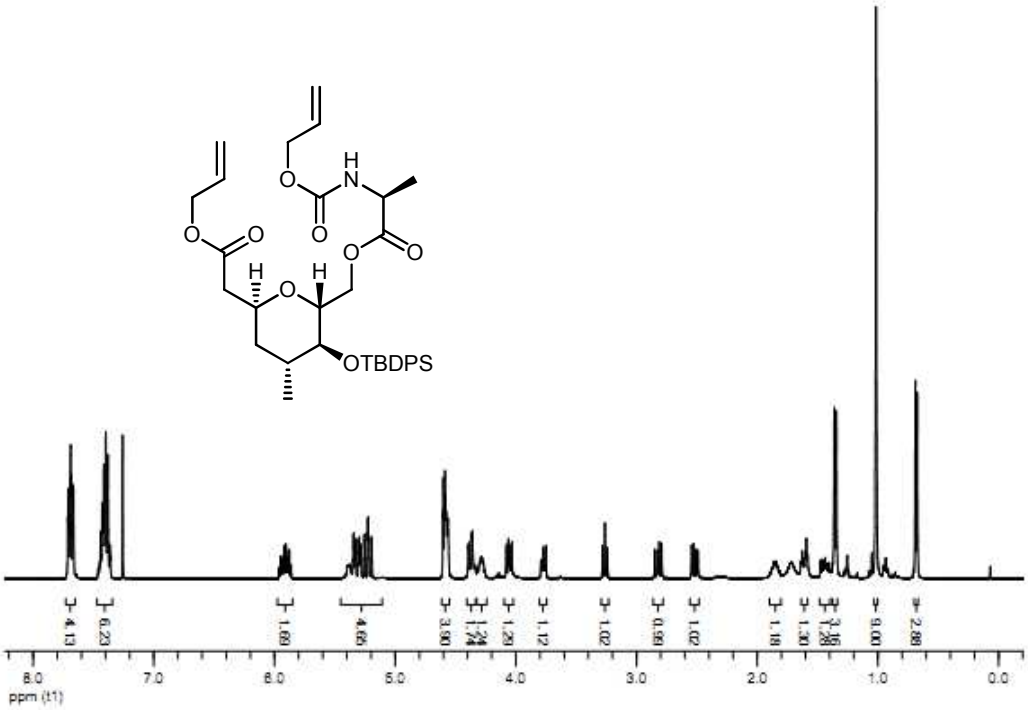

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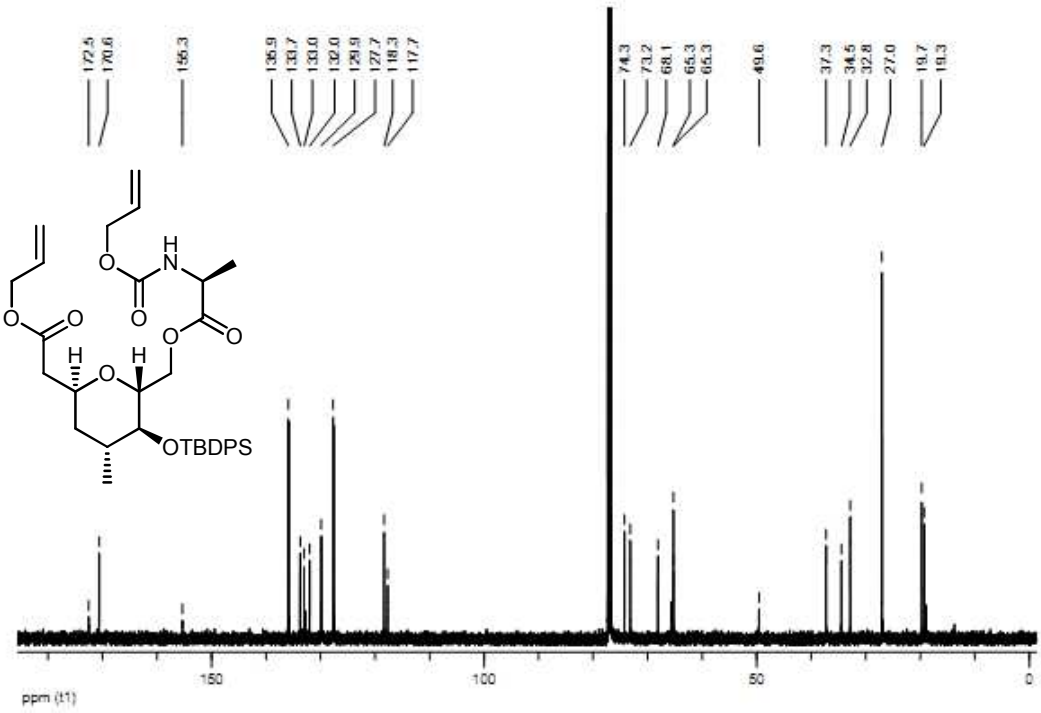

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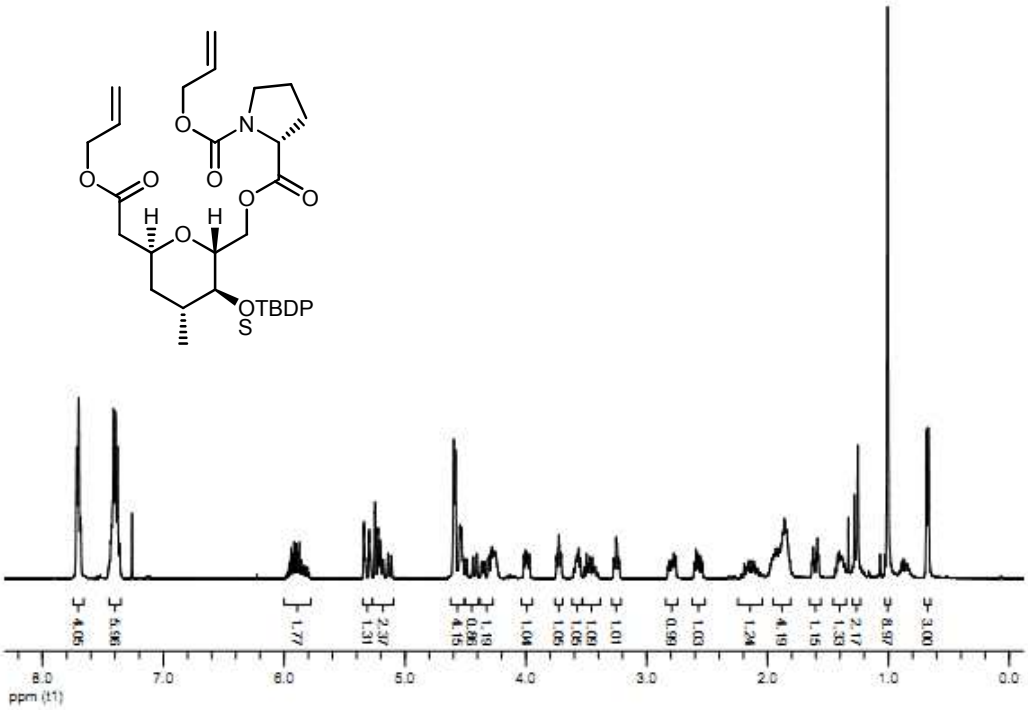

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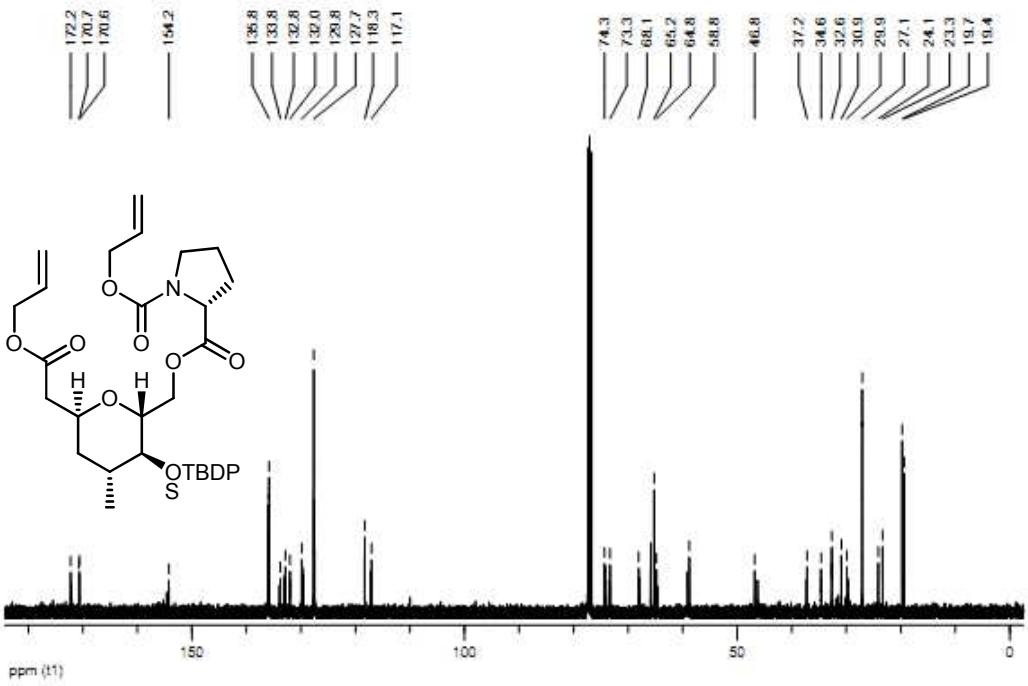

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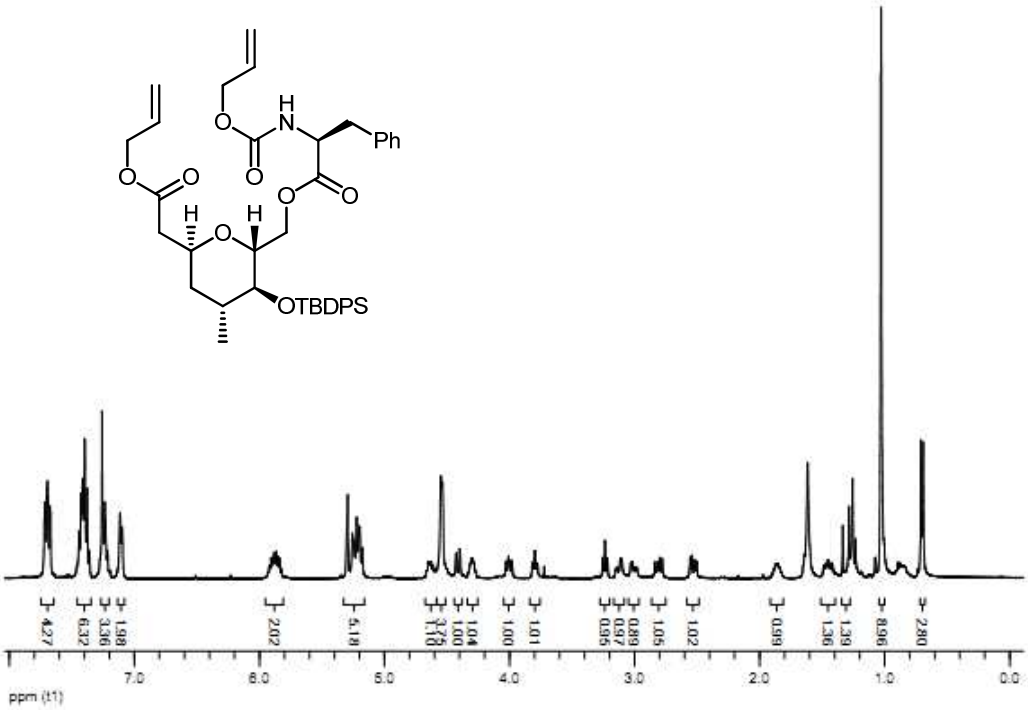

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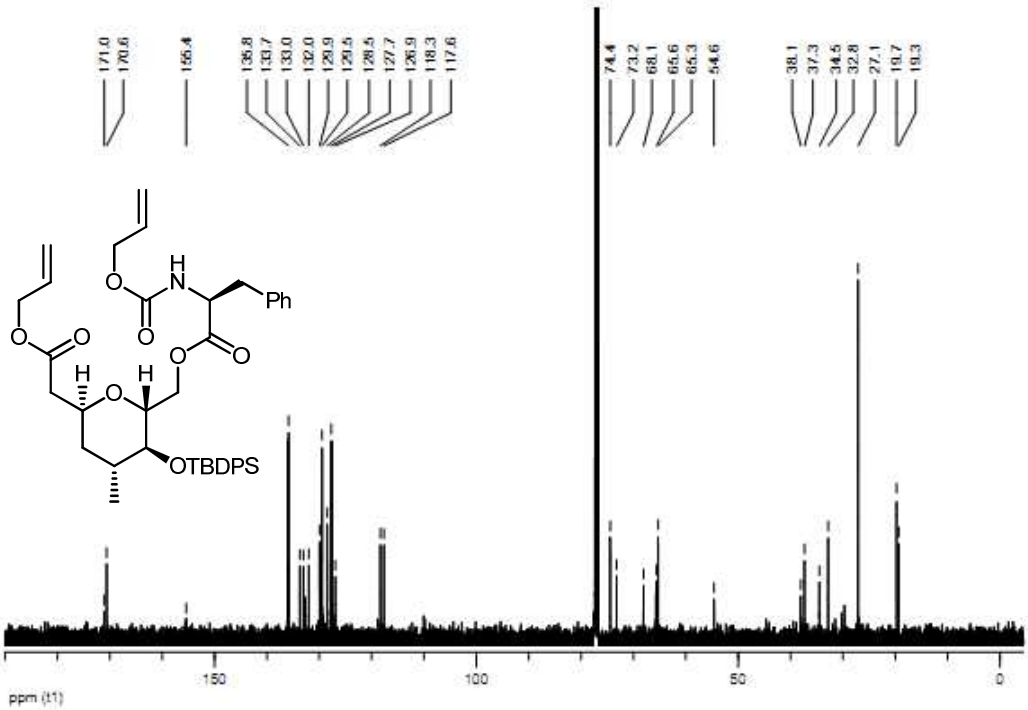

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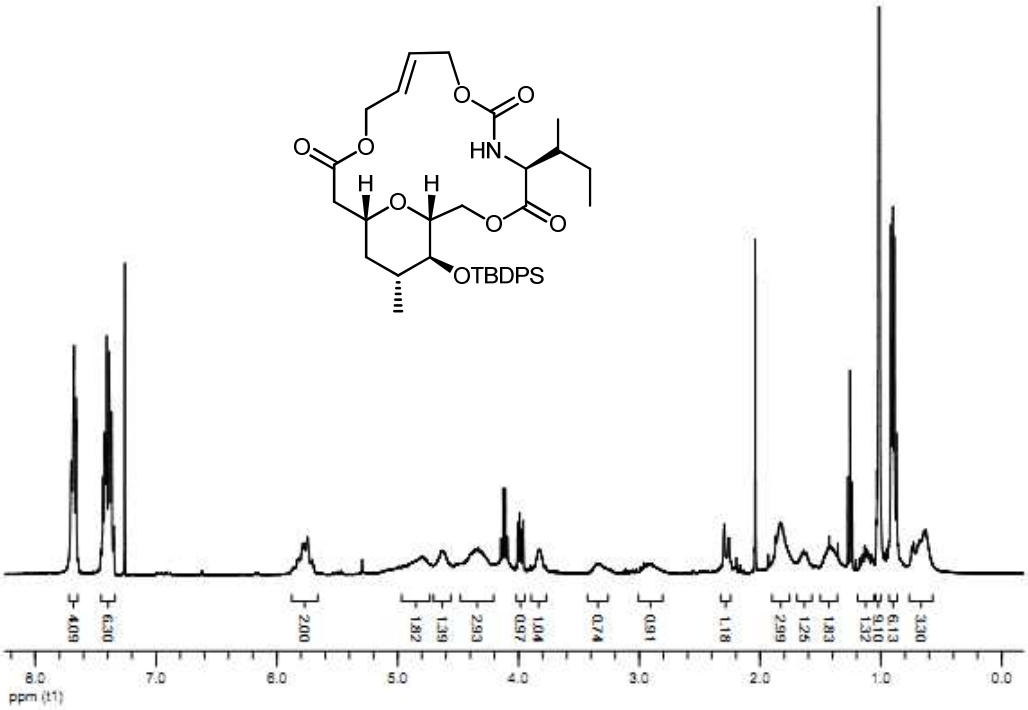

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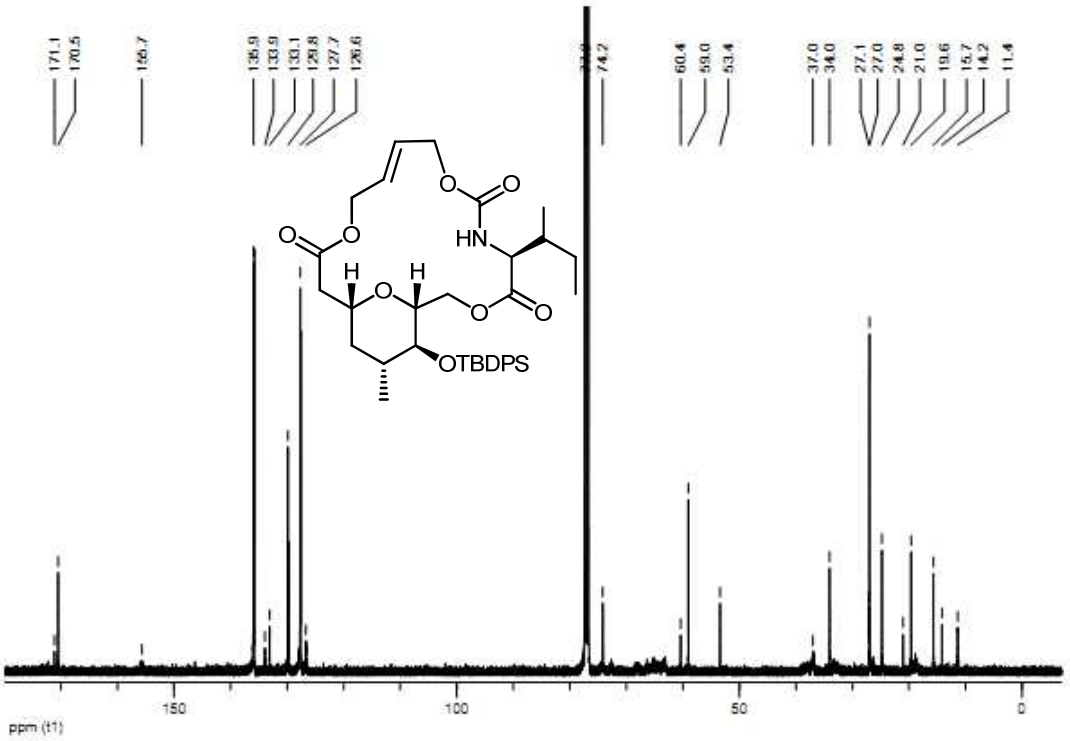

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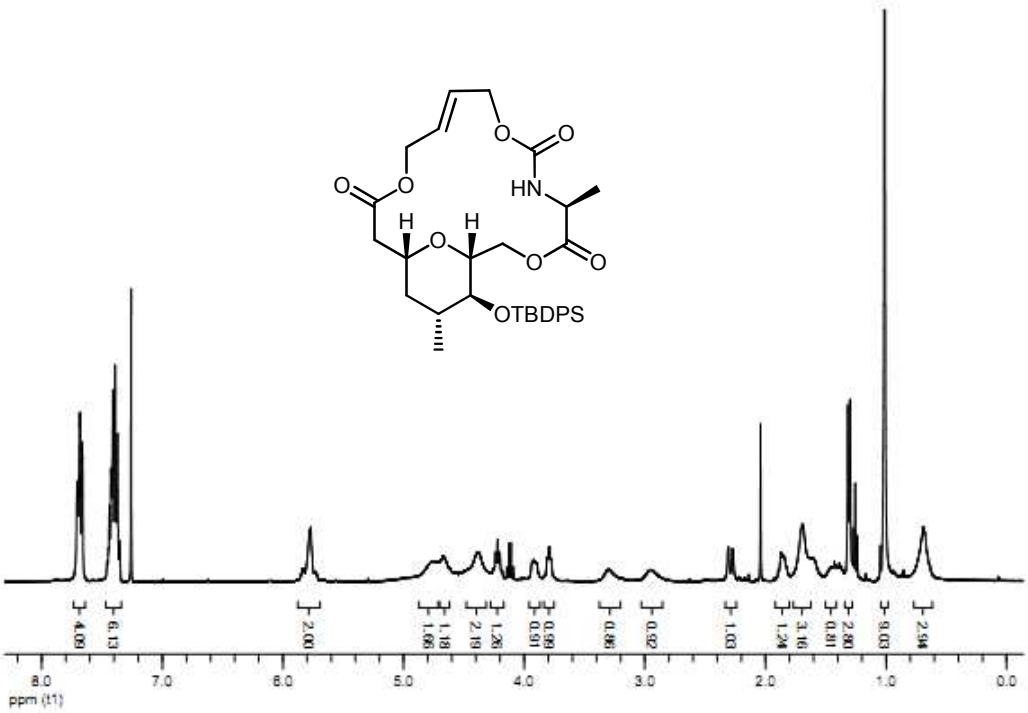
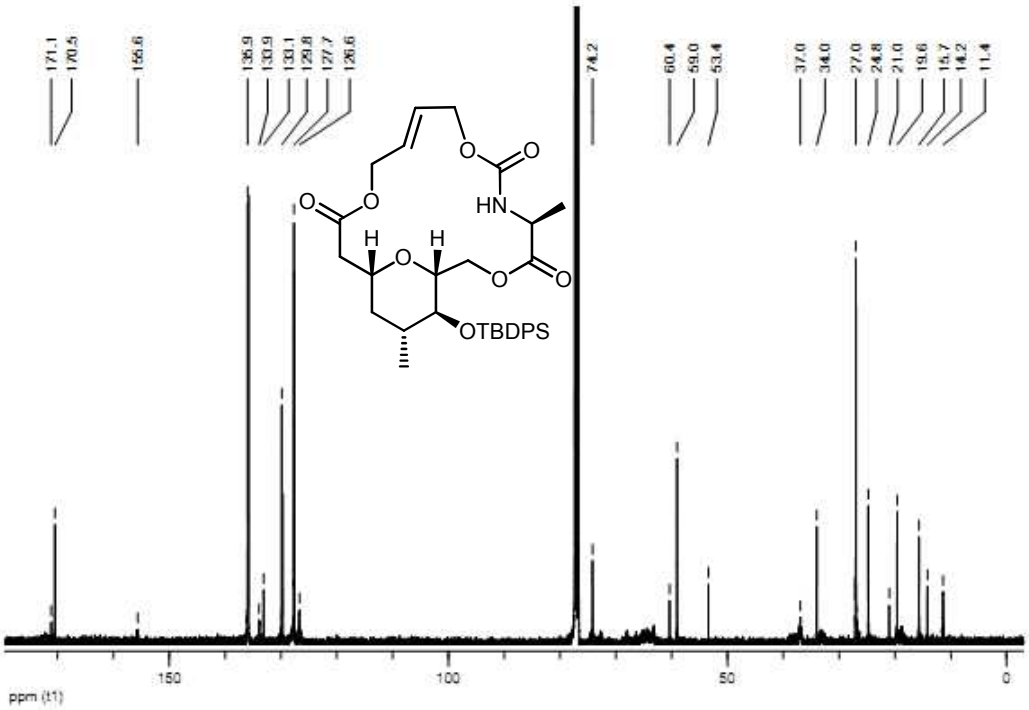
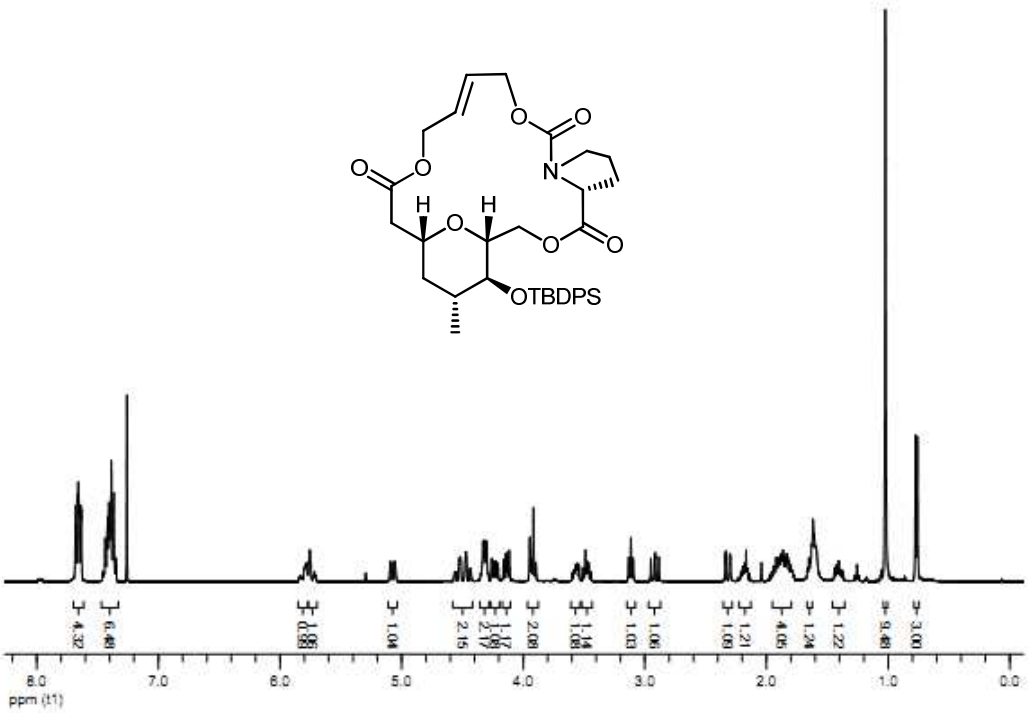


^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



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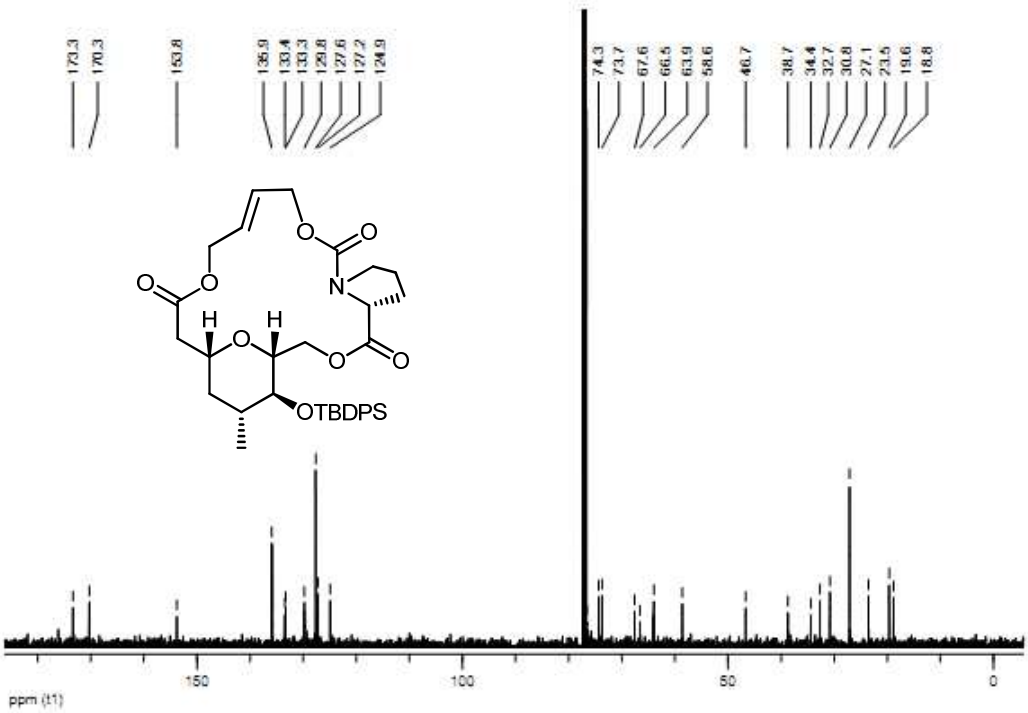

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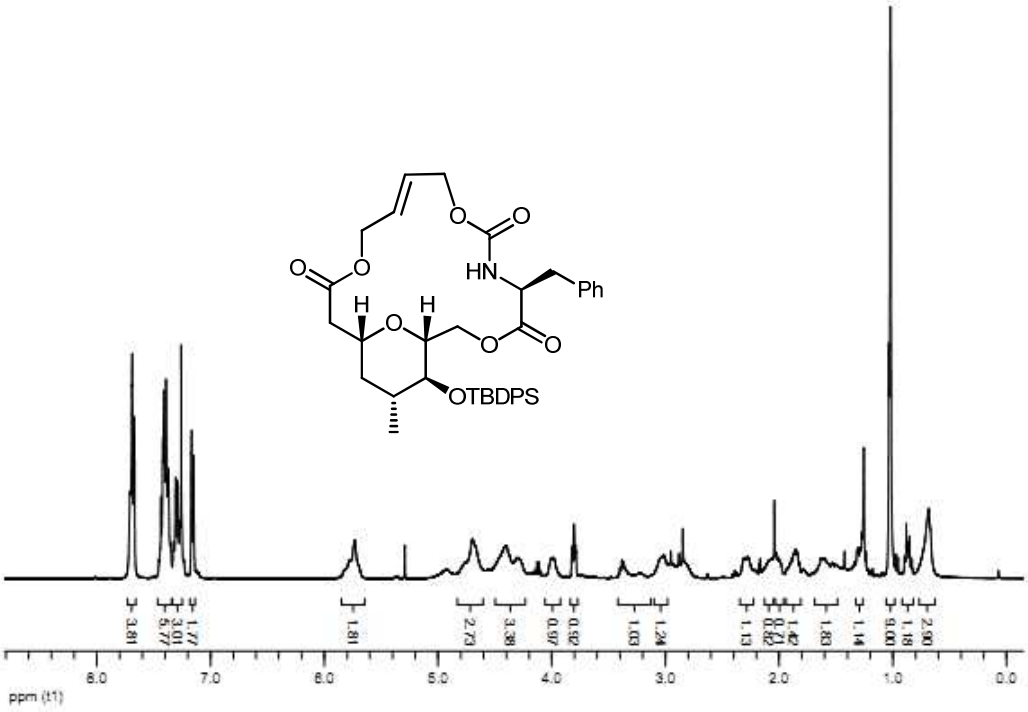

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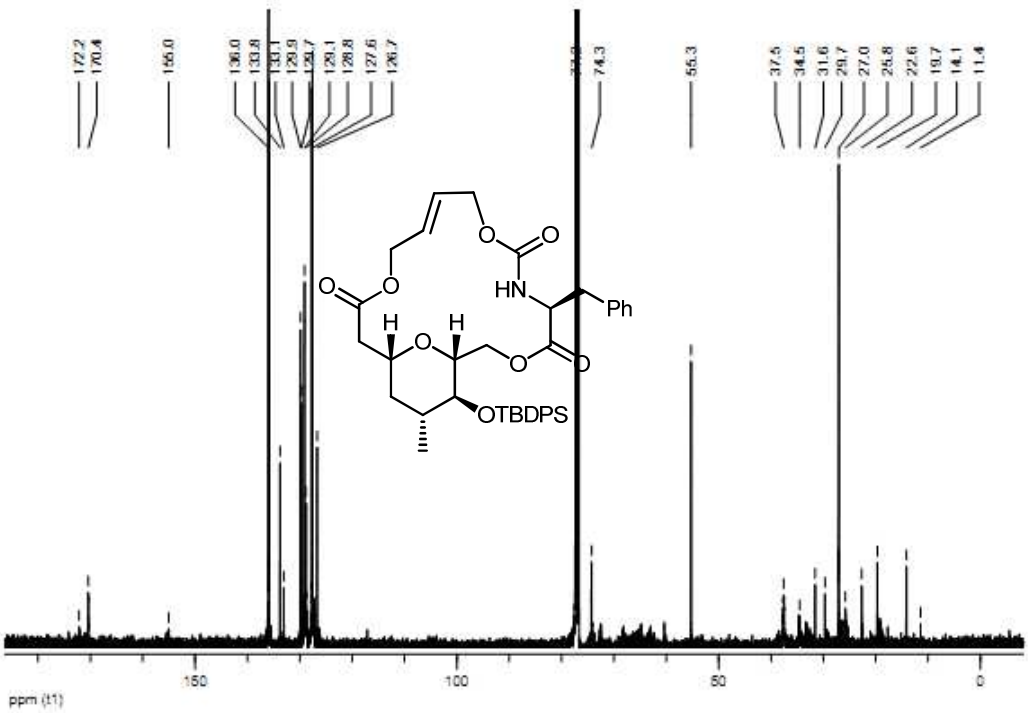


Chapter 4

Synthesis of Isatin-based Diverse Set of Macrocyclic Compounds

4.1 Synthesis of Isatin-based 13-Membered Macrocyclic Compounds

4.1.1 Isatin Introduction

Isatin (1H-indole-2, 3-dione, **Figure 1**) was first synthesized by Erdman[1] and Laurent[2] in 1841 as a product from the oxidation of indigo by chromic acid and nitric acid. It is an indole derivative, and in nature, it is found in many plants, such as *Calanthe discolor,*[3] *Isatis tinctoria,*[4] *and in Couroupita guianensis;*[5] moreover, it is a metabolic derivative of adrenaline[6] in humans.

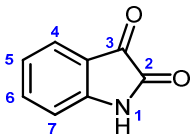


Figure 1: Structure of Isatin

Isatins have significant importance in medicinal chemistry[7] and are used as a key starting material in the synthesis of a large variety of heterocyclic compounds, which are biologically active.[7,8] Several pharmacological actions are associated with isatin, and some its derivatives include: anticancer, antimicrobial, antiviral, anti-inflammatory, anticonvulsant, and analgesic agents.

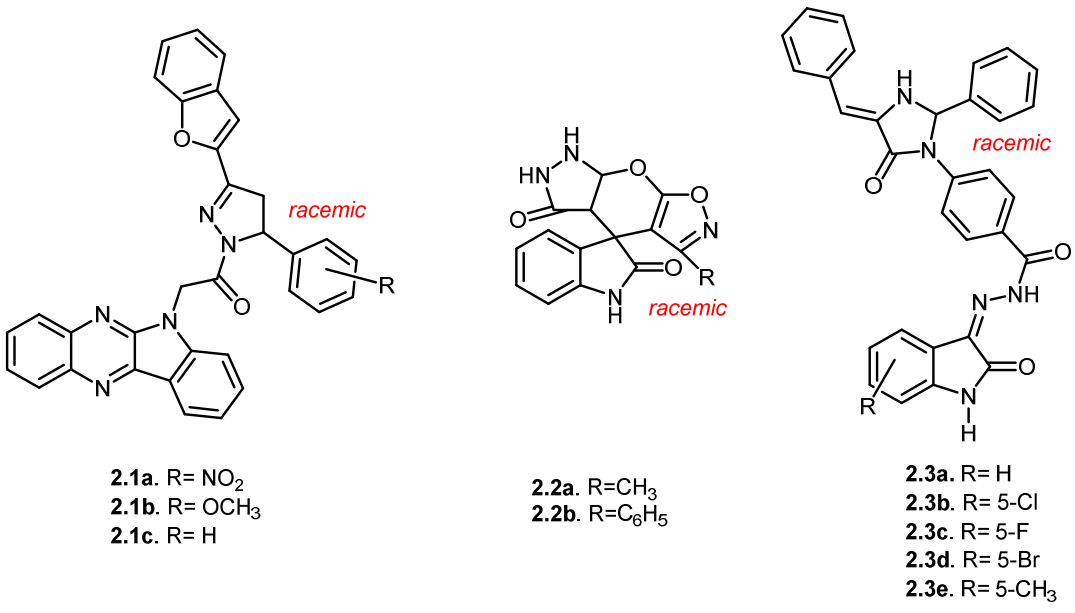
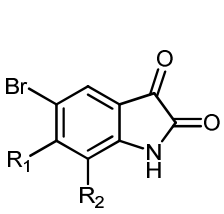


Figure 2: Isatin Derivatives with Antimicrobial Activity

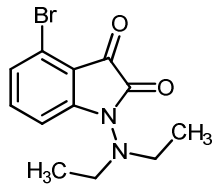
The synthesized compounds **2.1a**, **2.1b**, **2.1c** reported by Manna and co-workers[9] and showed antimicrobial activity. The MICs were found below 10 µg/ml against *P. aereuginos*a (5.0, 10.0, and 9.5), *E. coli* (10.0, 5.0, and 8.0), and *S. aureus* (7.5, 8.5 and 2.5).

Abdel-rahman and co-workers[10] reported the spiro-indoline–based heterocycles **2.2a** and **2.2b** which showed a very high activity against *E. coli* (54.0, 59.0), *A. niger* (70.0, 63.0) and *S. subtilis* (75.0, 66.0).

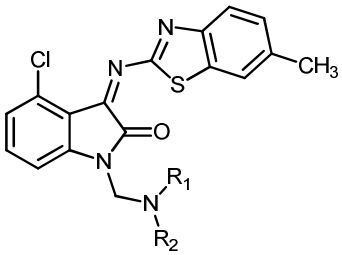
Bari and co-workers[11] reported some isatin derivatives **2.3(a-e)** which showed antimicrobial activities. Among several compounds, the derivative with 5-Br substitution showed the most favorable antimicrobial activity against *C. albicans*, *A. niger*.



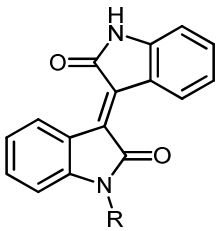
3.1a. R_1=H, R_2=Br
3.1b. R_1=H, R_2=NO_2
3.1c. R_1=Br, R_2=Br



3.2



3.3
R_1=R_2= Morpholinyl



3.4a. R=$(CH_2)_3C_6H_5$
3.4b. R=$(CH_2)_2C_6H_4$(p-OCH_3)

Figure 3: Isatin Derivatives with Anticancer Activity

The compounds **3.1(a-c)**reported by Vine and co-workers[12] showed the greater selectivity towards lymphoma and leukemia cells over breast, colorectal and prostate carcinoma cell lines.

Solomon and co-workers[13] reported the synthesis of compounds **3.2** and **3.3** which showed a good anticancer activity. For example, compound **3.2** showed 10-15 fold a higher cytotoxic effect on cancer compared to non-cancer cells.

Wee and co-workers[14] synthesized two compounds, **3.4a** and **3.4b**, and both of them were evaluated for antiproliferative activity on a panel of human cancer cells.

Shibinskya and co-workers[15] synthesized **4.1(a-e)** compounds. They showed the antiviral activity. The selective index (SI) value of antiviral effectiveness was determined as the ratio of the CC_{50} to the IC_{50} (SI =CC_{50}).

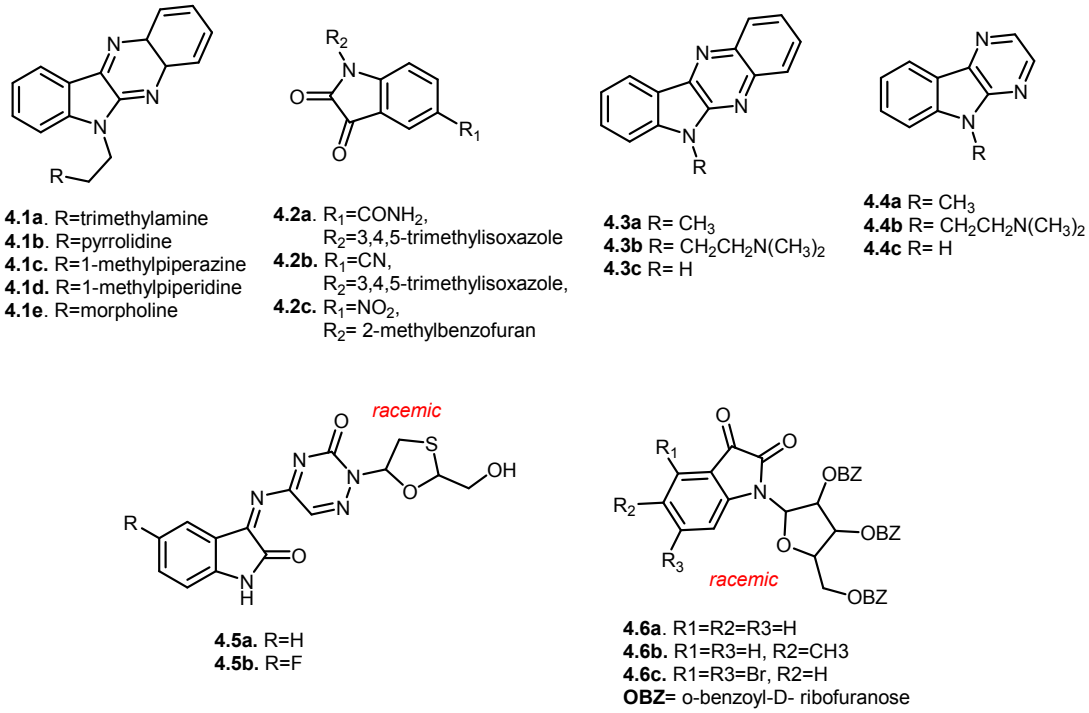


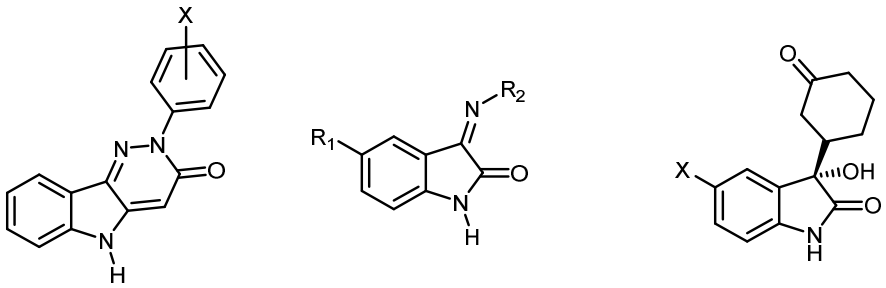
Figure 4: Isatin Derivatives with Antiviral Activity

Chen and co-workers[16] reported the synthesis of *N*-substituted isatin derivatives, **4.2(a-c)** and these compounds were found to be as potent and selective inhibitors against SARS Coronaviral 3CL Protease with IC_{50} values ranging from 0.95 to 17.50 µM.

Andrien and co-workers[17] synthesized **4.3(a-c)** and **4.4(a-c)** and these compounds showed antiviral activity and were also shown to interact with the minor groove of DNA. The synthesized compounds **4.5a** and **4.5b** by Sriram and co-workers[18] showed *in-vitro* anti-retroviral activities.

Oliveira and co-workers[19] synthesized the compounds **4.6(a-c)**, which showed antiviral activity on HSV-1 infected cells. The ribonucleoside derivative **4.6c** showed 66% inhibitory activity with the HIV-1 reverse transcriptase.

Synthesized compounds **5.1(a-c)** reported by Campagna and co-workers[20] were also evaluated for their ability to prevent the pentylenetetrazole (PTZ) caused seizures in mice model.



5.1a. X=Cl
5.1b. X=Br
5.1c. X=H

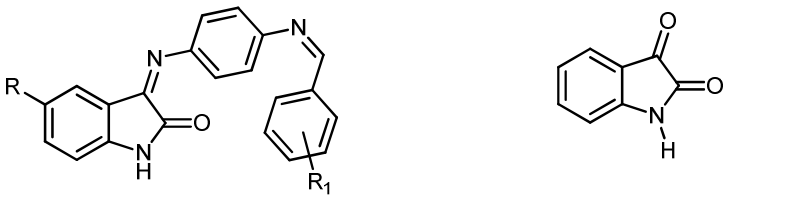
5.2a. R_1=H, R_2= 4-methylphenyl
5.2b. R_1=CH_3, R_2= 4-Chlorophenyl
5.2c. R_1=CH_3, R_2= 1-Naphthyl

5.3a. X=Br
5.3b. X=Cl

Figure 5: Isatin Derivatives with Anticonvulsant Activity

The synthesis of compounds **5.2(a-c)** was achieved by Sridhar and co-workers,[21] and they were active in MES (maximal electro shock) test. Among several of these compounds, **5.2b** was found to be much more active and showed 87% protection at 100 mg/kg dose level with an ED50 value of 53.61 mg/kg.

Compounds **5.3a** and **5.3b** were reported by Veerasamy and co-workers,[22] showed the PTZ (pentylenetetrazol) test and MES (maximal electro shock) test Compound **5.3a** was active in PTZ seizure threshold test (PTZ), thus act as a efficient anticonvulsant.



6.1a. R=H, R_1= Cl
6.1b. R=F, R_1=OCH_3
6.1c. R=Cl, R_1=NO_2
6.1d. R=OCH_3, R_1=OH

6.2a. R=5-F, **6.2b.** R=6-Cl
6.2c. R=7-Cl, **6.2d.** R=4-Br
6.2e. R=5-I, **6.2f.** R=-CH_3

Figure 5: Isatin derivatives with anti-inflammatory and analgesic activity

Compounds **6.1(a-d)** synthesized by Reddy and co-workers[23] were investigated for anti-inflammatory (i.e. carrageenan-induced paw oedema method)[24] and analgesic (tail-immersion method)[24] activity. Compounds **6.2(a-f)** reported by Mathues and co-workers[25] inhibited the cyclooxygenase (COX-2) enzymes in RAW 264.7 activated cells. The effect of isatin derivatives on COX-2 protein expression when compared with vehicle treated groups. The incubation of cells with isatin derivatives reduced the COX-2 protein expression.

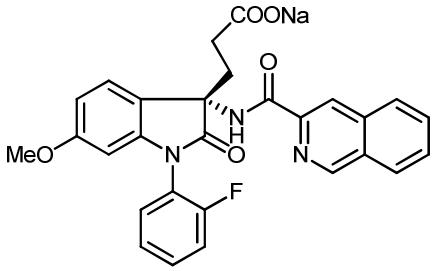


Figure 7: T-0632

The Glucagon-Like Peptide 1 Receptor (GLP-1R) is a member of class B GPCR family, that has been difficult to target by small molecule drugs. T-0632,[8] a non-peptide compound binds with the micromolar affinity to the human glucagon-like peptide 1 receptor, and further, blocks the GLP-1 induced cAMP production.

As I discussed in **Chapter 1**, due to the distinct structural features of macrocyclic compounds, they have less conformational flexibility than compared with their equivalent acyclic compounds and so suffer a trivial entropic loss upon binding to a receptor. In contrast to the smaller cyclic systems, macrocyclic compounds are not rigid and allowing them to potentially mould to a target surface for achieving an optimal binding. In addition to this, macrocycles offer the possibility for binding across the larger surface areas that are difficult to access with the traditional small molecules. From the chemistry point of view, if applied properly, macrocyclic compounds can offer the stereochemical complexity and a diverse functionality in a conformationally restricted manner. With this objective, we were interested in developing a practical synthesis method for developing the synthesis of different types of 13-membered macrocyclic compounds using isatin as the privileged starting scaffold. Our synthesis plan is shown in **Scheme 1**. The introduction of an amino acid moiety in the macrocyclic ring would allow introducing a chiral diversity site for obtaining further, several structural analogs.

4.1.2 Retrosynthesis

The retrosynthetic analysis of our target **F8.1** is shown in **Figure 8.** Compound **F8.1** could be obtained from an amino acid building block coupling followed by the ring closing metathesis of compound **F8.2**. Synthon **F8.2** could be obtained from the commercially available, isatin **F8.3**

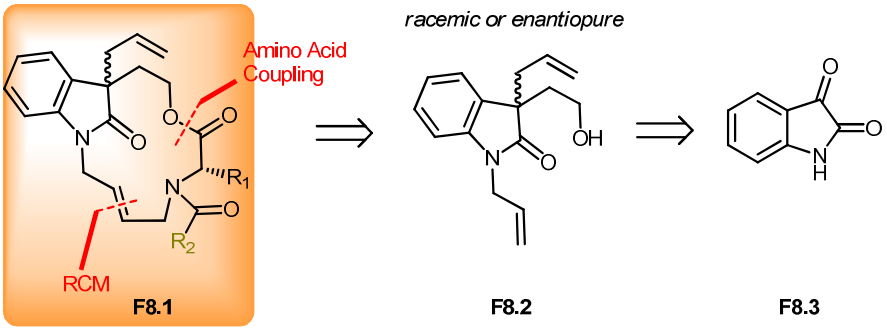
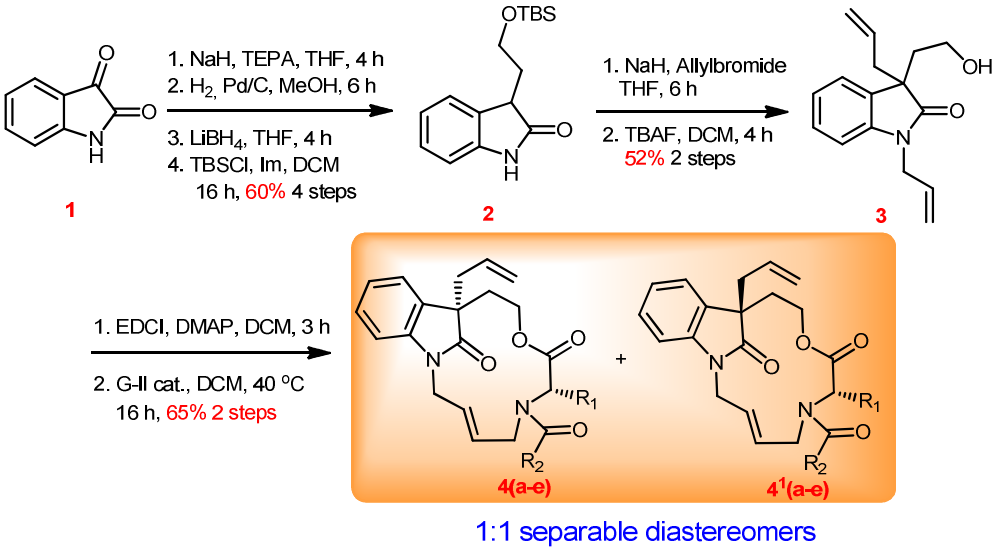


Figure 8: Retrosynthesis of Target 13-Membered Macrocycles.

4.1.3 Synthesis

Our synthesis started with the Horner-Wadsworth-Emmons reaction of commercially available isatin (**1**) which gave the Wittig product. The reduction of the double bond with H_2, Pd/C then gave the racemic mixture, followed by reduction of ester with lithium borohydride, protection of primary alcohol with silyl group furnished the synthesis of compound **2.** Allylation of compound **2** (racemic) gave *bis*-allylated product which on deprotection with TBAF produced compound **3.** It was then coupled with five different amino acids for obtaining the precursor for the ring closing metathesis. The use of the second generation Grubbs catalyst produced the 13-membered ring macrocyles with a *trans* olefin geometry **4** and **4^{I}** as two separable diastereomers (note - the exact stereochemistry of the chiral centre in both compounds is not known at this stage)

General Experimental Procedures of Macrocyclic Compounds

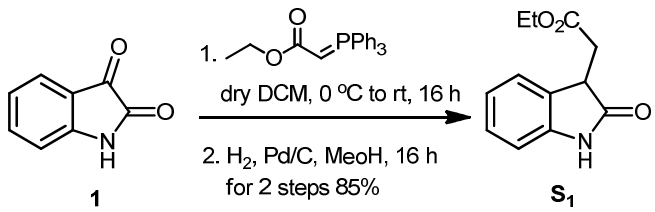


Scheme 1: Synthesis of 13-Membered Macrocycles

4.1.4 Conclusion

- Inspired by an active compound, **T-0632**, we successfully developed the synthesis of a new family of 13-membered macrocyclic compounds using isatin as the privileged starting sub-structure. Various compounds obtained in this approach allow bringing an amino acid moiety within their ring skeleton and this places various chiral groups on their macrocyclic ring architecture.
- The biological testing of all these compounds is ongoing in the research laboratory of Dr. Prasenjit Mitra, Biology Dept, DRILS, who has established several assays to search for novel small molecule activators or inhibitors of GLP1-R. This study will be made available when complete.

4.1.5 Experimental Section



Ethyl 2-(2-oxoindolin-3-yl) acetate (S_1) :

Isatin (0.068 mol, 10 g) was dissolved in 60 mL of dichloromethane at 0 ^{o}C. Ethyl (triphenylphosphoranylidene) acetate (0.081 mol, 28 g) in 40 mL dichloromethane was slowly added at 0 ^{o}C and the reaction was allowed to warm up to room temperature over night. After the reaction was completed, the solvent was removed gave crude product.

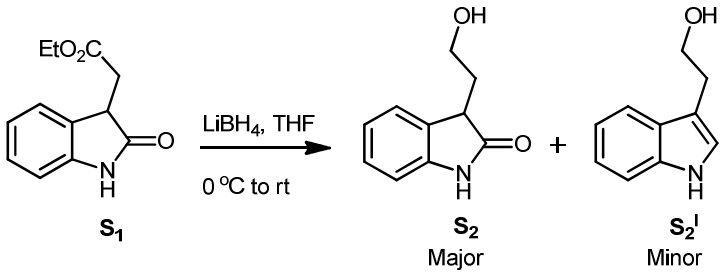
The above crude was dissolved in 80 mL of methanol and was allowed to react in the presence of 10% Pd/C (100mg) under a hydrogen atmosphere for overnight. After the starting material was consumed, the catalyst was removed via filtration through celite, followed by removal of the solvent by vacuum. The crude product was purified via flash chromatography on silica gel using (1:4 ethyl acetate/hexanes) after the solvents were removed compound **S_1** was obtained (11.91 g) as white solid.

Molecular Formula: $C_{12}H_{13}NO_3$

R_f (solvent system): 0.25 (25%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 219.0 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 9.17 (s, 1H), 7.27-7.19 (m, 2H), 7.01 (t, *J* = 7.55 Hz, 1H), 6.91 (d, *J* = 7.73 Hz, 1H), 4.14 (ttd, *J* = 10.77, 7.26, 3.68 Hz, 2H), 3.82 (dd, *J* = 7.48, 4.51 Hz, 1H), 3.08 (dd, *J* = 16.85, 4.49 Hz, 1H), 2.84 (dd, *J* = 16.85, 7.85 Hz, 1H), 1.20 (t, *J* = 7.13 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 179.7, 171.1, 141.8, 128.3, 124.0, 122.4, 110.0, 61.0, 42.4, 34.8, 14.1.



3-(2-hydroxyethyl) indolin-2-one (S_2) :

To a compound **S_1** (4.5 g, 0.02mol) in dry THF (120 mL), at 0 °C, $LiBH_4$ (0.89 g, 0.04 mol) was added, and reaction mixture was allowed to stir for 24 h at room temperature. After completion of the reaction, reaction mixture was quenched by the addition of ice cold water, and extracted with ethyl acetate (3 X 100 mL). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to give a solid, which was purified by column chromatography (7:3ethyl acetate/hexanes) to gave the compound **S_2** (2 g, 55.8% yield) and compound **S_2^I** (500 mg, 15% yield).

Molecular Formula: $C_{10}H_{11}NO_2$;

R_f (solvent system): 0.25 (50%, EtOAc/hexane);

LRMS: (ES+) *m/z* = 177.0 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 9.38-8.99 (m, 1H), 7.21 (t, *J* = 7.65 Hz, 2H), 7.04 (t, *J* = 7.45 Hz, 1H), 6.90 (d, *J* = 7.74 Hz, 1H), 3.88 (t, *J* = 5.67 Hz, 2H), 3.65-3.59 (m, 1H), 2.30-2.19(m, 1H), 2.10 (td, *J* = 14.33, 6.15 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 171.0, 141.7, 128.7, 128.3, 124.0, 122.4, 109.9, 61.0, 42.4, 34.8.

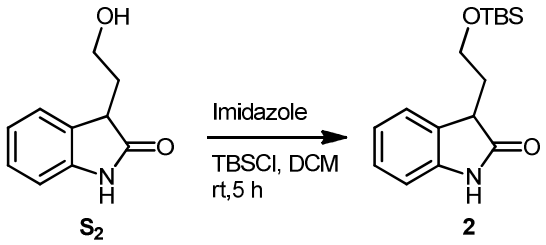
Molecular Formula: $C_{10}H_{11}NO$

R_f (solvent system): 0.25 (70%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 161.0 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 8.14 (s, 1H), 7.64 (d, *J* = 7.83 Hz, 1H), 7.37 (d, *J* = 8.12 Hz, 1H), 7.23 (dd, *J* = 14.66, 6.74 Hz, 1H), 7.15 (t, *J* = 7.46 Hz, 1H), 7.06 (s, 1H), 3.92 (t, *J* = 6.34 Hz, 2H), 3.05 (t, *J* = 6.35 Hz, 2H), 1.73 (broad singlet, 1H); **^{13}C**

NMR ($CDCl_3$, 100 MHz) δ ppm : 136.4, 127.4, 122.5, 122.2, 119.4, 118.8,112.2, 111.2, 62.6, 28.7.



S_2 2

3-(2-((tert-butyldimethylsilyl)oxy)ethyl)indolin-2-one (2) :

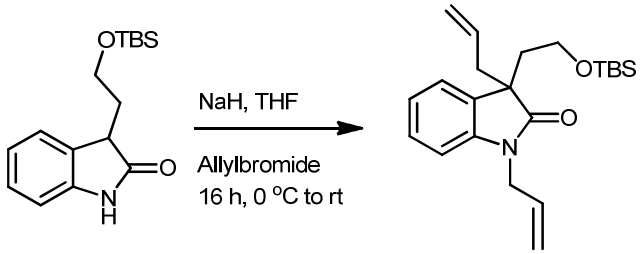
To a solution of S_2 (1.6 g, 0.009mol) and imidazole (0.918 g, 0.0135mol) in DCM (30 mL) was added TBS-Cl (20.3 g, 0.0135mol) at 0 ^oC. After stirring 3 h at room temperature, water (30 mL) was added. The organic layer was separated and the aqueous layer was extracted with DCM (50 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (10% EtOAc in n-hexane, TLC: R_f = 0.2) to afford the title compound **2** (2.1 g, 83%).

Molecular Formula: $C_{16}H_{25}NO_2Si$

R_f (solvent system): 0.2 (70%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 292.1 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 9.58-9.09 (m, 1H), 7.25-7.19 (m, 2H), 7.01 (t, *J* = 7.49 Hz, 1H), 6.92 (d, *J* = 7.70 Hz, 1H), 3.88.-3.76 (m, 2H), 3.62 (t, *J* = 6.43 Hz, 1H), 2.23 (qd, *J* = 12.43, 6.17 Hz, 1H), 2.10 (dt, *J* = 13.31, 6.54 Hz, 1H), 0.87 (s, 9H), 0.01 (d, *J* = 4.71 Hz, 6H)



2 S_3

1,3-diallyl-3-(2-((tert-butyldimethylsilyl)oxy)ethyl)indolin-2-one (S_3) :

To the solution of **2** (2.1 g, 0.0072 mol) in THF (30 mL) was added NaH (60%, 0.43 g, 0.018 mmol) and, allyl bromide (0.018 mol, 1.54 mL) was added at 0 ^oC and the reaction mixture was stirred for overnight and was quenched with aqueous NH_4Cl.

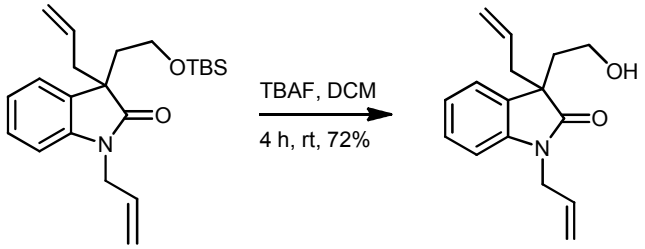
The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine. The resulting mixture was dried over Na_2SO_4 and concentrated under reduce pressure. Purification by column chromatography (10% EtOAc in n-hexane, TLC: R_f = 0.6) provided product **S_3** (1.92 g, 72%).

Molecular Formula: $C_{22}H_{33}NO_2Si$

R_f (solvent system): 0.2 (10%, EtOAc/hexane)

LRMS: (ES+) *m/z* = 372.2 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 7.25-7.17 (m, 2H), 7.04 (t, *J* = 7.47 Hz, 1H), 6.79 (d, *J* = 7.76 Hz, 1H), 5.80 (ddd, *J* = 15.6, 10.36, 5.22 Hz, 1H), 5.41 (tdd, *J* = 17.35, 10.02, 7.36 Hz, 1H), 5.19 (ddd, *J* = 7.79, 4.90, 1.39 Hz, 2H), 4.94 (dd, *J* = 15.4, 10.2 Hz, 2H), 4.42 (ddd, *J* = 16.35, 3.17, 1.74 Hz, 1H), 4.21 (dd, *J* = 16.36, 5.48 Hz, 1H), 3.45-3.28 (m, 2H), 2.55 (d, *J* = 7.36 Hz, 2H), 2.31-2.18 (m, 1H), 2.09-2.01 (m, 1H), 0.79 (d, *J* = 6.35 Hz, 9H), -0.11 (s, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.7, 142.9, 132.1, 131.7, 131.2, 127.6, 123.3, 122.1, 118.9, 117.2, 108.7, 59.3, 50.7, 42.8, 42.1, 39.3, 25.8, 18.2, -5.6



S_3 **3**

1,3-diallyl-3-(2-hydroxyethyl)indolin-2-one (3) :

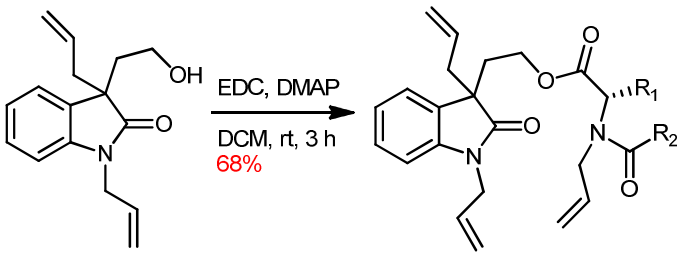
To a solution of **S_3** (1.92 g, 0.0051mol) in THF (25 mL) was added TBAF (1.62 g, 0.0062 mol,) at 0 ^oC. The mixture was allowed to stand at room temperature for 1 h, then EtOAc (10 mL), H_2O (5 mL) and saturated aqueous NaCl (10 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2×20 mL). The organic extracts were combined, dried (Na_2SO_4), concentrated under reduced pressure and purified by flash chromatography (30% EtOAc in n-hexane, TLC: R_f = 0.2) to give the title compound **3** (0.93 g, 70%).

Molecular Formula: $C_{16}H_{19}NO_2$

R_f (solvent system): 0.2 (30%, EtOAc/hexane)

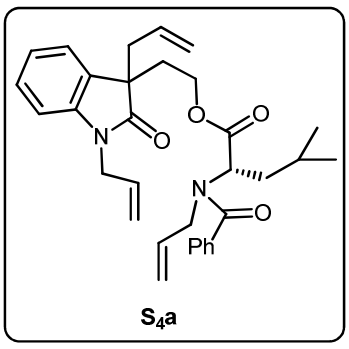
LRMS: (ES+) *m/z* = 258.2 (M+1).

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm : 7.27 (d, *J* = 7.55 Hz, 1H), 7.20 (d, *J* = 7.36 Hz, 1H), 7.08 (t, *J* = 7.48 Hz, 1H), 6.84 (d, *J* = 7.78 Hz, 1H), 5.81 (ddd, *J* = 15.6, 10.38, 5.24 Hz, 1H), 5.48-5.36 (m, 1H), 5.23 (dd, *J* = 20.38, 5.00 Hz, 2H), 4.98 (dd, *J* = 15.4, 10.2 Hz, 2H), 4.43-4.34 (m, 1H), 4.30 (dd, *J* = 16.35, 5.29 Hz, 1H), 3.61 (td, *J* = 11.86, 6.10 Hz, 1H), 3.50-3.40 (m, 1H), 2.60 (dq, *J* = 13.30, 7.32 Hz, 2H), 2.26 (td, *J* = 13.14, 6.49 Hz, 1H), 2.07 (td, *J* = 14.10, 6.10 Hz, 1H), 1.67 (s, 1H).



3 **S_4(a-e)**

To the solution of **3** (1 eq) in DCM solution added amino acid building block (1 eq) and EDC.HCl (1.5 eq) at room temperature under nitrogen atmosphere and allowed to stirred for 3 hours. Then added saturated $NaHCO_3$ solution to this reaction mixture extracted twice with EtOAc. Combined organic layers were washed with brine solution and dried anhydrous Na_2SO_4, evaporated the solvent, Purification of crude compound by flash chromatography (20% EtOAc/hexane TLC: R_f = 0.35) afforded the compound **S_4(a-e)** as colourless oil



S_4a

(2S)-2-(1,3-diallyl-2-oxoindolin-3-yl)ethyl 2-(N-allylbenzamido)-4-methyl pentan oate (S_4a) :

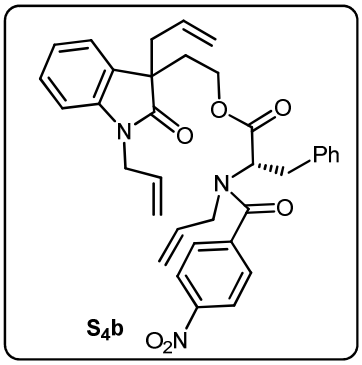
Molecular Formula: $C_{32}H_{38}N_2O_4$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 515.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.38 (d, *J* = 5.04 Hz, 5H), 7.25-7.18 (m, 2H), 7.08 (d, *J* = 7.43 Hz, 1H), 6.81 (d, *J* = 7.60 Hz, 1H), 6.00-5.70 (m, 2H), 5.43-5.32 (m, 1H), 5.23-5.17 (m, 2H), 5.11-4.90 (m, 4H), 4.42-3.60 (m, 7H), 2.57 (d, *J* = 6.53 Hz, 2H), 2.28-2.38 (m, 1H), 1.40-1.90 (m, 4H), 0.94 (s, 3H), 0.67 (d, *J* = 75.93 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.1, 172.3, 171.2, 142.7, 134.0, 131.4, 129.6, 128.3, 126.6, 123.2, 122.6, 119.3, 117.4, 109.1, 71.4, 61.4, 50.6, 44.3, 42.5, 35.0, 31.9, 29.6, 26.0, 22.6.



(2S)-2-(1,3-diallyl-2-oxoindolin-3-yl)ethyl 2-(N-allyl-4-nitrobenzamido)-3-phenyl propanoate (S_4b) :

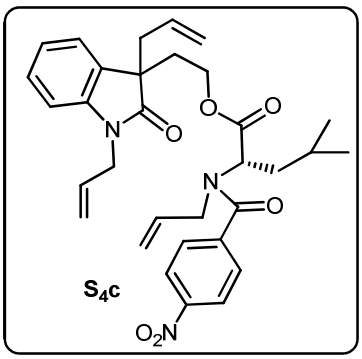
Molecular Formula: $C_{35}H_{35}N_3O_6$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 594.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.74 (d, *J* = 7.15 Hz, 1H), 7.55-7.29 (m, 5H), 7.23 (ddd, *J* = 22.22, 10.38, 6.09 Hz, 5H), 7.12-7.02 (m, 2H), 6.87-6.76 (m, 1H), 5.88-5.67 (m, 1H), 5.49-5.34 (m, 1H), 5.28-5.11 (m, 3H), 5.06-4.77 (m, 4H), 4.51-4.21 (m, 2H), 4.18-3.85 (m, 3H), 3.83-3.58 (m, 1H), 3.46-3.34 (m, 1H), 3.32-2.96 (m, 2H), 2.64-2.49 (m, 2H), 2.42-2.20 (m, 2H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.3, 171.0, 166.7, 142.8, 135.8, 131.4, 129.5, 129.4, 128.5, 128.3, 127.1, 126.7, 123.2, 122.6, 119.5, 117.5, 109.2, 61.9, 60.2, 54.1, 53.5, 50.7, 42.3, 37.8, 35.0.



(2S)-2-(1,3-diallyl-2-oxoindolin-3-yl)ethyl2-(N-allyl-4-nitrobenzamido)-4-methyl pentanoate (S_4c) :

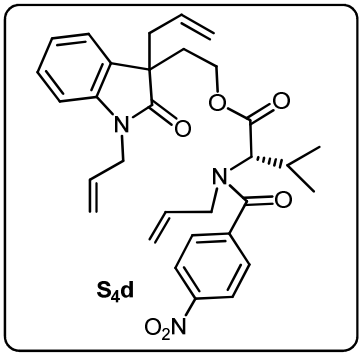
Molecular Formula: $C_{32}H_{37}N_3O_6$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 560.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 8.27 (d, *J* = 8.44 Hz, 2H), 7.57 (d, *J* = 10.43 Hz, 2H), 7.26-7.19 (m, 2H), 7.11-7.05 (m, 1H), 6.84 (d, *J* = 7.2 Hz, 1H), 5.99-5.64 (m, 2H), 5.46-5.32 (m, 1H), 5.10-4.90 (m, 4H), 5.01 (d, *J* = 16.95 Hz, 2H), 4.46-3.82 (m, 7H), 2.57 (d, *J* = 7.68 Hz, 2H), 2.44-2.32 (m, 2H), 2.29-2.19 (m, 1H), 2.01-1.72 (m, 2H), 0.96 (t, *J* = 6.13 Hz, 3H), 0.81-0.56 (m, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.1, 173.1, 170.1, 148.3, 142.8, 133.3, 131.5, 131.5, 128.2, 127.8, 123.7, 123.2, 122.6, 119.4, 117.5, 117.5, 109.2, 61.7, 52.4, 51.7, 50.6, 42.3, 37.7, 35.0, 31.9, 29.7, 28.3.



(2S)-2-(1,3-diallyl-2-oxoindolin-3-yl)ethyl2-(N-allyl-4-nitrobenzamido)-3-methyl butanoate (S_4d) :

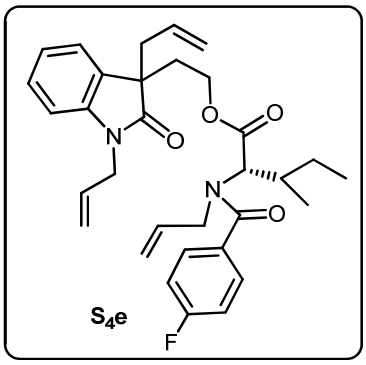
Molecular Formula: $C_{31}H_{35}N_3O_6$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 546.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 8.33-8.19 (m, 2H), 7.63-7.47 (m, 2H), 7.28-7.17 (m, 2H), 7.05 (dd, *J* = 13.78, 6.48 Hz, 1H), 6.81 (d, *J* = 7.78 Hz, 1H), 5.92-5.58 (m, 2H), 5.46-5.33 (m, 1H), 5.17 (dd, *J* = 11.18, 5.31 Hz, 2H), 5.12-4.88 (m, 4H), 4.49-4.34 (m, 1H), 4.31-4.11 (m, 2H), 3.97-3.67 (m, 4H), 3.61 (t, *J* = 10.59 Hz, 1H), 2.55 (d, *J* = 7.17 Hz, 2H), 2.40-2.31 (m, 1H), 2.23-2.16 (m, 1H), 1.03-0.91 (m, 3H), 0.78-0.65 (m, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.0, 170.1, 169.1, 148.2, 142.7, 132.4, 131.3, 130.1, 128.4, 127.6, 123.8, 123.1, 122.6, 119.7, 117.7, 117.6, 109.2, 67.0, 61.8, 50.5, 45.1, 42.3, 42.2, 34.7, 29.6, 27.6, 20.7.



S_4e

(2S,3R)-2-(1,3-diallyl-2-oxoindolin-3-yl)ethyl2-(N-allyl-4-fluorobenzamido)-3-methylpentanoate (S_4e) :

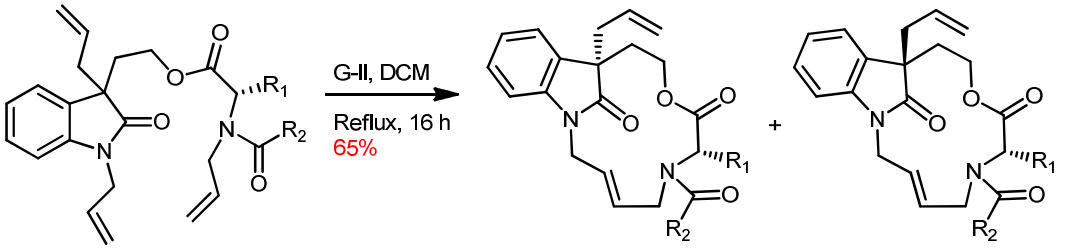
Molecular Formula: $C_{32}H_{37}FN_2O_4$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 533.2 (M+1).

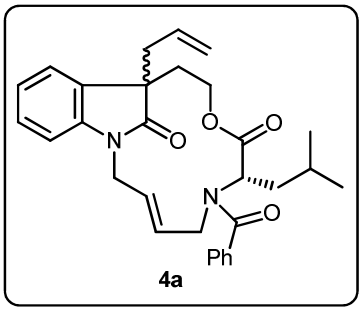
^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.51-7.32 (m, 2H), 7.26 (dd, *J* = 12.58, 4.82 Hz, 2H), 7.19-7.03 (m, 3H), 6.84 (d, *J* = 7.75 Hz, 1H), 5.95-5.57 (m, 2H), 5.50-5.34 (m, 1H), 5.21 (d, *J* = 13.52 Hz, 2H), 5.02 (d, *J* = 16.37 Hz, 4H), 4.51-4.38 (m, 1H), 4.30-4.16 (m, 1H), 3.99-3.70 (m, 4H), 2.58 (d, *J* = 6.41 Hz, 2H), 2.42-2.31 (m, 1H), 2.28-2.16 (m, 1H), 1.87-1.70 (m, 1H), 1.65-1.36 (m, 1H), 1.22-1.05 (m, 1H), 1.01-0.88 (m, 3H), 0.85-0.59 (m, 4H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 171.5, 164.4, 162.0, 142.7, 131.3, 130.2, 129.2, 129.1, 128.2, 123.2, 122.5, 119.5, 117.5, 115.6, 115.4, 109.2, 61.3, 50.5, 42.2, 34.8, 33.9, 25.8, 15.8, 10.9.



S_4(a-e) **4(a-e)** **4^1(a-e)**

1:1 seperable diastereomers

To a solution of **S_4 (a-e)** (1eq) in dry DCM under nitrogen atmosphere added Grubbs' 2^{nd} generation catalyst (10 mol%) and reaction mixture was allowed to stirred for 16 h at 40 ^oC. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (5% MeOH/DCM) afforded the products **4(a-e) and 4^1(a-e)**



4a

Molecular Formula: $C_{30}H_{34}N_2O_4$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

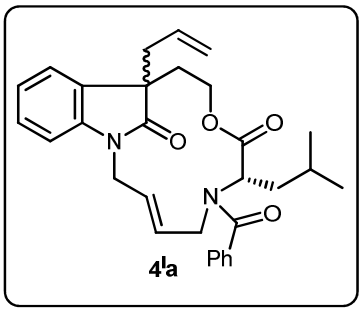
Yield: 75%

LRMS: (ES+) *m/z* = 487.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.44-7.27 (m, 5H), 7.21-7.15 (m, 2H), 7.11 (t, *J* = 7.35 Hz, 1H), 6.87 (d, *J* = 7.79 Hz, 1H), 5.90 (dd, *J* = 16, 5.2 Hz, 1H), 5.58 (t, *J* = 13.4 Hz, 1H), 5.43 (dd, *J* = 17.11, 9.92 Hz, 1H), 4.96 (t, *J* = 12.54 Hz, 2H), 4.63 (d, *J* = 11.90 Hz, 1H), 4.36 (dd, *J* = 16.67, 5.76 Hz, 1H), 4.21 (d, *J* = 14.17 Hz, 1H), 4.01 (dd, *J* = 16.59, 2.20 Hz, 1H), 3.74 (dd, *J* = 8.56, 5.45 Hz, 1H), 3.52 (dd, *J* = 14.12, 10.17 Hz, 1H), 3.29 (t, *J* = 11.98 Hz, 1H), 2.73 (t, *J* = 12.4, 1H), 2.50-2.42 (m, 2H), 1.98 (dd, *J* = 14.15, 9.76 Hz, 3H), 1.79-1.69 (m, 1H), 1.00 (t, *J* = 5.64 Hz, 6H);

^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm : 178.6, 170.4, 143.9, 135.7, 131.5, 129.6, 129.0,

128.2, 128.1, 126.8, 123.8, 122.2, 119.3, 107.6, 61.3, 56.0, 53.0, 50.7, 43.9, 38.9, 37.6, 34.8, 25.1, 23.3, 22.2.



4'a

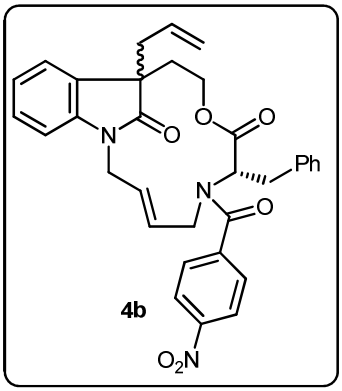
Molecular Formula: $C_{30}H_{34}N_2O_4$

R_f (solvent system): 0.5 (30%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 487.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.37 (d, *J* = 21.22 Hz, 6H), 7.16 (d, *J* = 7.2 Hz, 1H), 7.08 (d, *J* = 7.2 Hz, 1H), 6.90-6.81 (m, 1H), 6.02 (dd, *J* = 15.4, 6 Hz, 1H), 5.93 (dd, *J* = 16, 5.6 Hz, 1H), 5.51-5.36 (m, 1H), 5.04 (dd, *J* = 18.4, 10.4 Hz, 2H), 4.68 (dd, *J* = 15.2, 6 Hz, 1H), 4.03-3.94 (m, 2H), 3.92-3.85 (m, 1H), 3.78 (dd, *J* = 15.2, 4.8 Hz, 2H), 3.51 (dd, *J* = 14.8, 7.2 Hz, 1H), 2.64-2.48 (m, 3H), 2.12 (d, *J* = 15.2, 1H), 2.01-1.92 (m, 1H), 1.68-1.57 (m, 2H), 0.98 (t, *J* = 5.64 Hz, 6H).



4b

Molecular Formula: $C_{33}H_{31}N_3O_6$

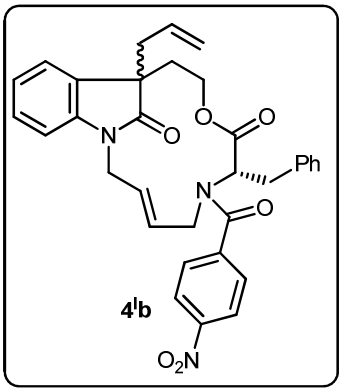
R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 566.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.40-7.25 (m, 9H), 7.16 (d, *J* = 6.70 Hz, 1H), 7.07 (dd, *J* = 17.20, 7.04 Hz, 3H), 6.81 (d, *J* = 7.78 Hz, 1H), 5.68 (dd, *J* = 15.6, 6.02 Hz, 1H), 5.35-5.46 (m, 2H), 4.96 (t, *J* = 12.53 Hz, 2H), 4.68 (dt, *J* = 11.64, 2.8 Hz,

1H), 4.26 (dd, *J* = 16.8, 6.40 Hz, 1H), 3.94 (dd, *J* = 16.48, 2.25 Hz, 1H), 3.87 (dd, *J* = 11.8, 4.80 Hz, 1H), 3.85 (d, *J* = 16.81 Hz, 1H), 3.41 (t, *J* = 11.65 Hz, 1H), 3.38 (dd, *J* = 14.01, 4.87 Hz, 1H), 3.29 (t, *J* = 11.65 Hz, 1H), 2.80-2.71 (m, 1H), 2.51-2.39 (m, 3H), 1.97 (d, *J* = 16.09 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.7, 170.2,169.7, 143.9, 138.7, 135.4, 131.5, 129.9, 129.6, 129.4, 129.1, 128.4, 128.2, 128.0, 126.9, 126.5, 123.7, 122.3, 119.4, 107.6, 61.5, 59.5, 53.3, 50.7, 43.9, 38.9, 34.7, 33.9, 29.6.



4^{l}b

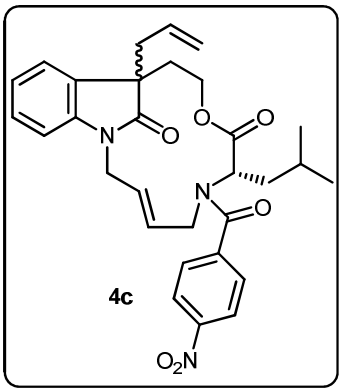
Molecular Formula: $C_{33}H_{31}N_3O_6$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 566.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.44-7.38 (m, 3H), 7.33 (d, *J* = 4.69 Hz, 5H), 7.26 (d, *J* = 6.88 Hz, 2H), 7.23-7.09 (m, 2H), 6.87 (d, *J* = 7.60 Hz, 1H), 6.06-5.94 (m, 1H), 5.73 (dt, *J* = 16.01, 6.4 Hz, 1H), 5.54-5.42 (m, 1H), 5.04 (dd, *J* = 18.4, 10.4 Hz, 2H), 4.63 (dd, *J* = 15.2, 7.6 Hz, 1H), 4.02 (t, *J* = 11.6 Hz, 1H), 3.91 (d, *J* = 12.4 Hz, 1H), 3.76 (d, *J* = 12.4 Hz, 1H), 3.72 (dd, *J* = 9.6, 4.8 Hz 1H), 3.67(d, *J* = 4.8 Hz, 1H), 3.36-3.21 (m, 2H), 2.69-2.61 (m, 1H), 2.61-2.48 (m, 3H), 2.15 (d, *J* = 15.8 Hz, 1H), 2.09-1.99 (m, 1H).



4c

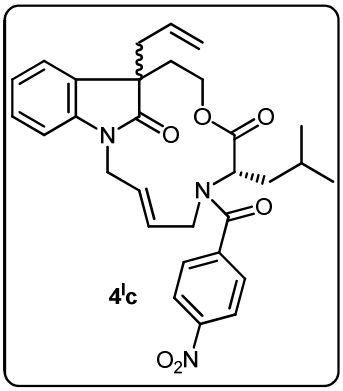
Molecular Formula: $C_{30}H_{33}N_3O_6$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 532.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 8.20 (d, *J* = 8.59 Hz, 2H), 7.34 (t, *J* = 7.05 Hz, 3H), 7.17 (dd, *J* = 19.52, 7.08 Hz, 2H), 6.88 (d, *J* = 7.82 Hz, 1H), 5.82 (dd, *J* = 16, 5.2 Hz, 1H), 5.54 (dd, *J* = 16, 8.4 Hz, 1H), 5.48-5.35 (m, 1H), 5.04 (dd, *J* = 18.4, 10.4 Hz, 2H), 4.61 (d, *J* = 12 Hz, 1H), 4.36 (dd, *J* = 16.2, 6.4 Hz, 1H), 4.02 (d, *J* = 15.32 Hz, 2H), 3.81 (dd, *J* = 8.0, 5.2 Hz, 1H), 3.54 (dd, *J* = 14.0, 10.0 Hz, 1H), 3.33 (t, *J* = 12.0 Hz, 1H), 2.72 (td, *J* = 12.4, 2.8 Hz, 1H), 2.46 (dd, *J* = 7.6, 2.8 Hz, 2H), 2.06-1.91 (m, 3H), 1.69 (d, *J* = 7.17 Hz, 1H), 1.01 (dd, *J* = 6.51, 2.21 Hz, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.6, 169.9, 168.3, 148.3, 143.8, 141.7, 131.4, 130.0, 129.8, 128.3, 127.8, 127.0, 123.8, 122.4, 119.5, 107.6, 61.6, 56.1, 52.8, 50.7, 43.9, 38.8, 37.4, 34.5, 25.1, 23.3, 22.1.



4'c

Molecular Formula: $C_{30}H_{33}N_3O_6$

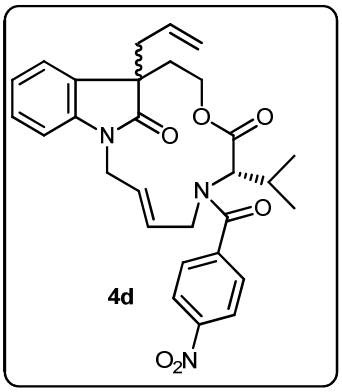
R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 532.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 8.24 (d, *J* = 8.55 Hz, 1H), 8.12 (d, *J* = 8.55 Hz, 1H), 7.48 (d, *J* = 8.53 Hz, 2H), 7.28-7.22 (m, 1H), 7.10 (t, *J* = 7.23 Hz, 1H), 7.02 (dd, *J* = 14, 7.2 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 5.86 (dd, *J* = 15.4, 6 Hz, 1H), 5.51-5.28 (m, 2H), 4.95 (dd, *J* = 18.4, 10.4 Hz, 2H), 4.52 (dd, *J* = 14.8, 5.6 Hz, 1H), 3.86-3.90 (m, 2H), 3.72-3.80 (m, 2H), 3.60 (t, *J* = 13.6 Hz, 1H), 3.45 (dd, *J* = 16, 8 Hz, 1H), 2.61-2.52 (m, 1H), 2.51-2.42 (m, 2H), 2.09-2.02 (m, 1H), 1.96-1.90 (m, 1H), 1.72-1.64 (m, 2H), 0.86 (d, *J* = 6.39 Hz, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 179.2,

170.3, 168.8, 148.2, 142.7, 142.0, 131.6, 130.0, 129.1, 128.2, 127.5, 124.2, 124.0, 122.6, 119.5, 107.5, 62.4, 58.8, 56.0, 50.5, 43.5, 38.7, 37.6, 34.4, 25.1, 22.8, 22.2.



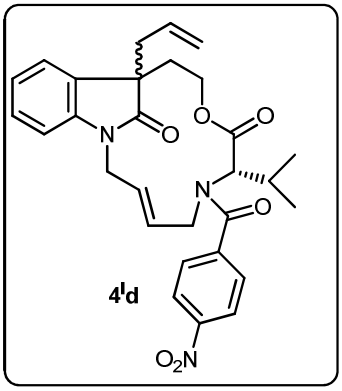
Molecular Formula: $C_{29}H_{31}N_3O_6$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 518.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 8.21 (d, *J* = 8.65 Hz, 2H), 7.35 (t, *J* = 7.42 Hz, 3H), 7.22-7.08 (m, 2H), 6.88 (d, *J* = 7.76 Hz, 1H), 5.91 (dd, *J* = 16.0, 6.42 Hz, 1H), 5.52 (dd, *J* = 14.82, 4.8 Hz, 1H), 5.46-5.38 (m, 1H), 4.98 (t, *J* = 12.37 Hz, 2H), 4.65 (d, *J* = 11.79 Hz, 1H), 4.37 (dd, *J* = 16.45, 5.88 Hz, 1H), 4.08-3.97 (m, 2H), 3.52 (dd, *J* = 14.40, 10.07 Hz, 1H), 3.40 (d, *J* = 9.17 Hz, 1H), 3.29 (t, *J* = 11.61 Hz, 1H), 2.80-2.61 (m, 2H), 2.48 (dd, *J* = 7.20, 4.30 Hz, 2H), 2.01 (dd, *J* = 15.36, 1.21 Hz, 1H), 1.19 (d, *J* = 6.52 Hz, 3H), 0.98 (d, *J* = 6.88 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.5, 168.8, 168.5, 148.2, 143.8, 141.9, 131.4, 129.8, 128.3, 127.6, 127.0, 123.8, 122.4, 119.4, 107.6, 63.6, 61.1, 53.7, 50.8, 43.8, 38.8, 34.8, 21.6, 19.2.



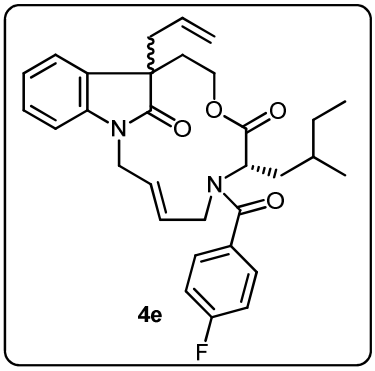
Molecular Formula: $C_{29}H_{31}N_3O_6$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 518.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 8.32 (d, *J* = 8.45 Hz, 1H), 8.24-8.15 (m, 1H), 7.56 (d, *J* = 8.55 Hz, 2H), 7.45-7.31 (m, 1H), 7.21-7.16 (m, 1H), 7.10 (t, *J* = 7.59 Hz, 1H), 6.83 (d, *J* = 7.83 Hz, 1H), 5.98 (dd, *J* = 15.62, 4.8 Hz, 1H), 5.91-5.81 (m, 1H), 5.50-5.41 (m, 1H), 5.01-4.98 (m, 3H), 4.65 (dd, *J* = 15.62, 6.4 Hz, 1H), 4.01 (td, *J* = 12.0, 4.0 Hz, 2H), 3.95-3.88 (m, 2H), 3.84-3.77 (m, 1H), 3.42 (dd, *J* = 14.0, 7.6 Hz ,1H), 2.57 (dd, *J* = 9.14, 4.62 Hz, 3H), 2.20-2.12 (m, 1H), 0.96 (dd, *J* = 10.09, 6.83 Hz, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 169.1, 169.0, 168.9, 148.3, 142.6, 142.1, 131.6, 130.1, 129.0, 128.1, 127.5, 124.0, 122.6, 119.5, 107.7, 63.8, 62.2, 53.0, 50.5, 43.6, 38.6, 34.3, 28.2, 21.8,19.3.



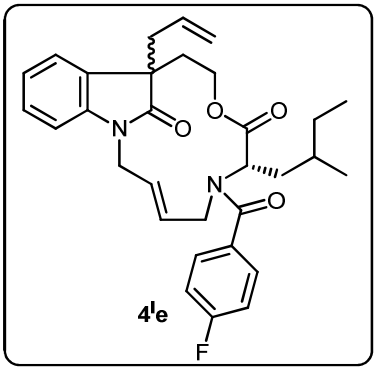
Molecular Formula: $C_{31}H_{35}FN_2O_4$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 519.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.32 (dt, *J* = 7.60, 1.2 Hz 1H), 7.19 (dd, *J* = 13.89, 6.19 Hz, 3H), 7.12 (d, *J* = 7.42 Hz, 1H), 7.02 (t, *J* = 8.46 Hz, 2H), 6.87 (d, *J* = 7.72 Hz, 1H), 5.92-5.82 (m, 1H), 5.57-5.48 (m, 1H), 5.46-5.35 (m, 1H), 4.98 (t, *J* = 18.03 Hz, 2H), 4.65 (dd, *J* = 11.68, 2.4 Hz, 1H), 4.39-4.33 (m, 1H), 4.21 (t, *J* = 12.0 Hz, 1H), 4.01 (d, *J* = 16.82 Hz, 1H), 3.56-3.48 (m, 1H), 3.44 (t, *J* = 8.75 Hz, 1H), 3.24 (t, *J* = 12.0 Hz, 1H), 2.74-2.61 (m, 1H), 2.52-2.48 (m, 3H), 1.98 (d, *J* = 15.10 Hz, 1H), 1.82-1.71 (m, 1 H), 1.64-1.54 (m, 1 H), 1.12 (d, *J* = 6.29 Hz, 2H), 1.03-0.88 (m, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 178.6, 164.6, 162.1, 144.0, 131.6, 129.2, 128.3, 127.9, 123.8, 122.4, 119.4, 115.6, 115.4, 107.7, 61.6, 54.2, 50.9, 43.9, 38.9, 34.1, 29.7, 25.2, 17.7, 15.2, 11.5.



Molecular Formula: $C_{31}H_{35}FN_2O_4$

R_f (solvent system): 0.5 (50%, EtOAc/hexane)

Yield: 75%

LRMS: (ES+) *m/z* = 519.2 (M+1).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm : 7.41-7.29 (m, 2H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 7.28 Hz, 1H), 7.14-7.03 (m, 3H), 6.84-6.79 (m, 1H), 6.13-5.93 (m, 1H), 5.93-5.84 (m, 1H), 5.50-5.40 (m, 1H), 4.99 (dd, *J* = 18.4, 10.4 Hz, 2H), 4.77-4.63 (m, 1H), 4.09-3.87 (m, 3H), 3.84-3.72 (m, 1H), 3.46-3.26 (m, 1H), 3.22-3.02 (m, 1H), 2.55 (d, *J* = 7.94 Hz, 4H), 2.40-2.10 (m, 3H), 1.55-1.43 (m, 1H), 1.43-1.31 (m, 1H), 0.89 (dd, *J* = 11.67, 4.57 Hz, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ ppm : 171.7, 164.4,161.9, 142.7, 132.0, 131.7, 128.6, 128.1, 122.5, 119.4, 115.7, 115.5, 107.7, 62.1, 50.5, 43.6, 38.5, 34.2, 28.1, 25.5, 17.6, 15.2, 11.5;

4.1.6 References

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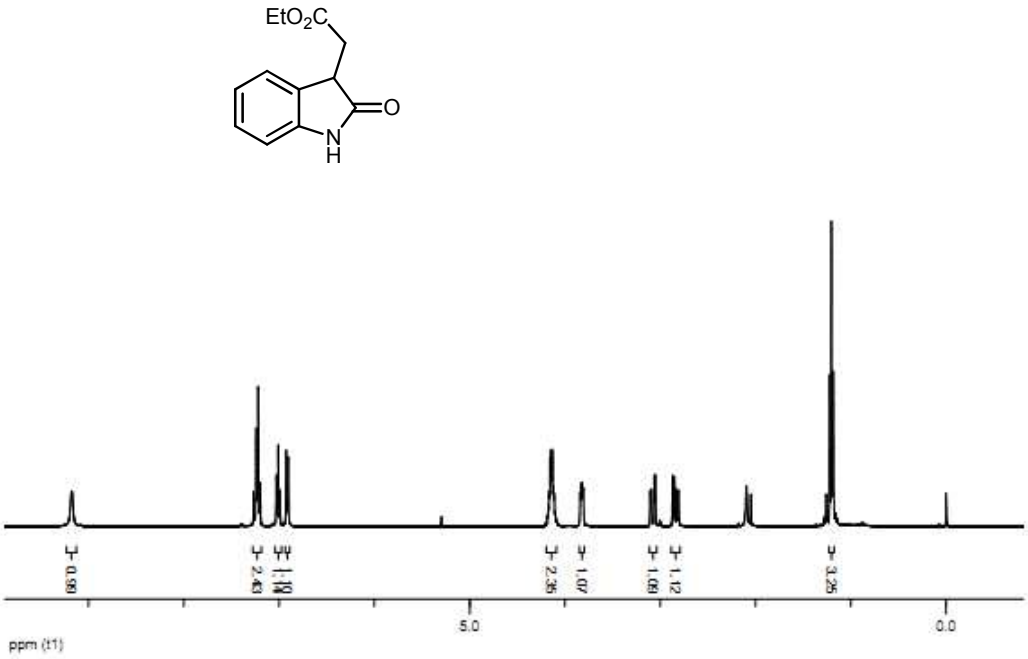
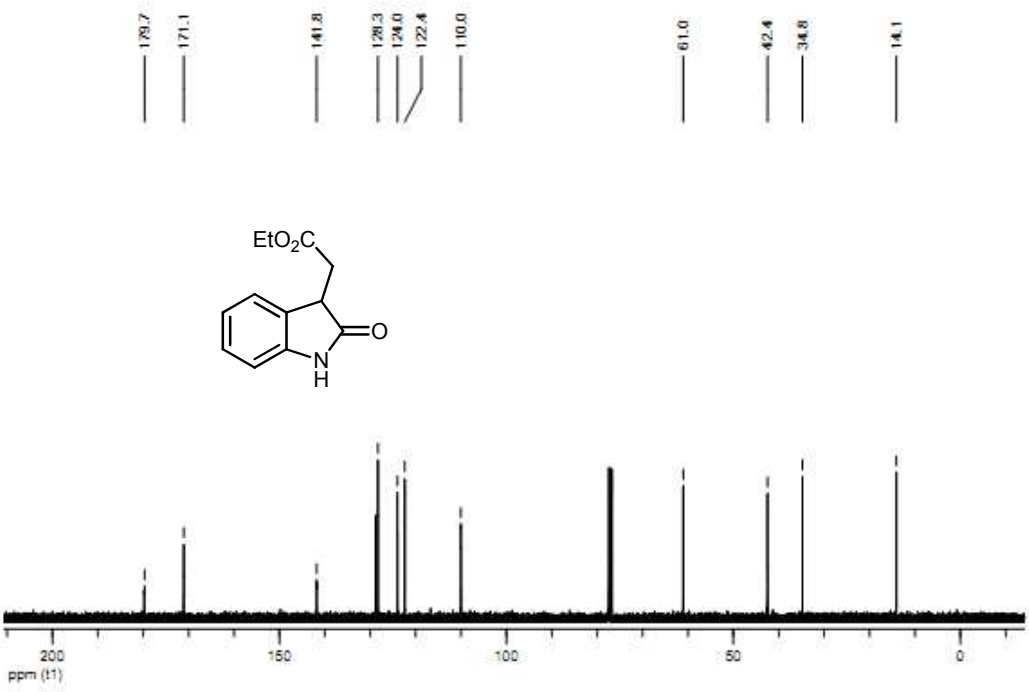
4.1.7 Spectral Data

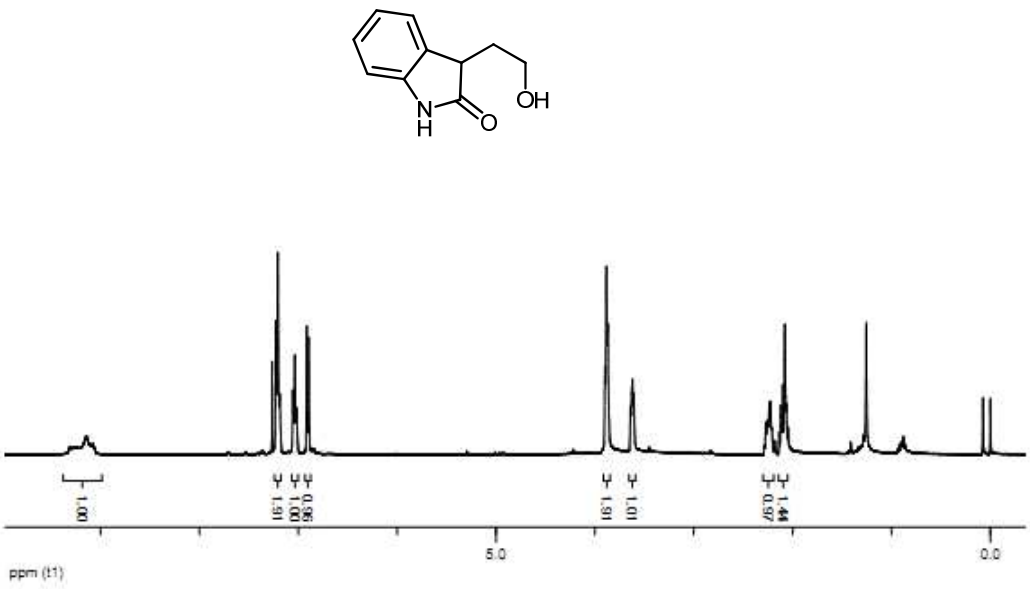
^{1}H NMR ($CDCl_3$, 400MHz)



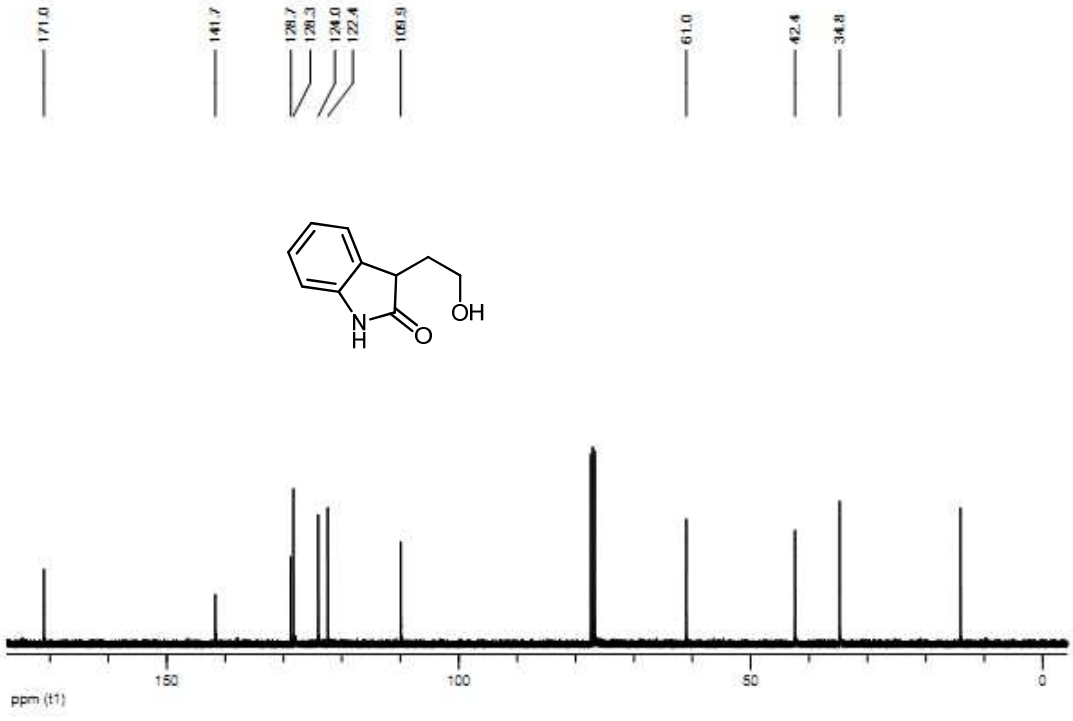
^{13}C NMR ($CDCl_3$, 100MHz)



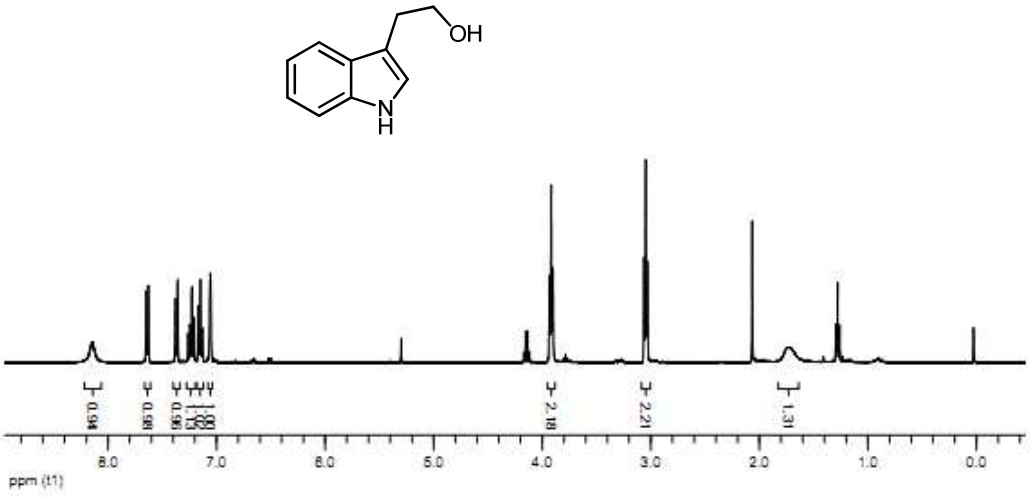
^{1}H NMR ($CDCl_3$, 400MHz)



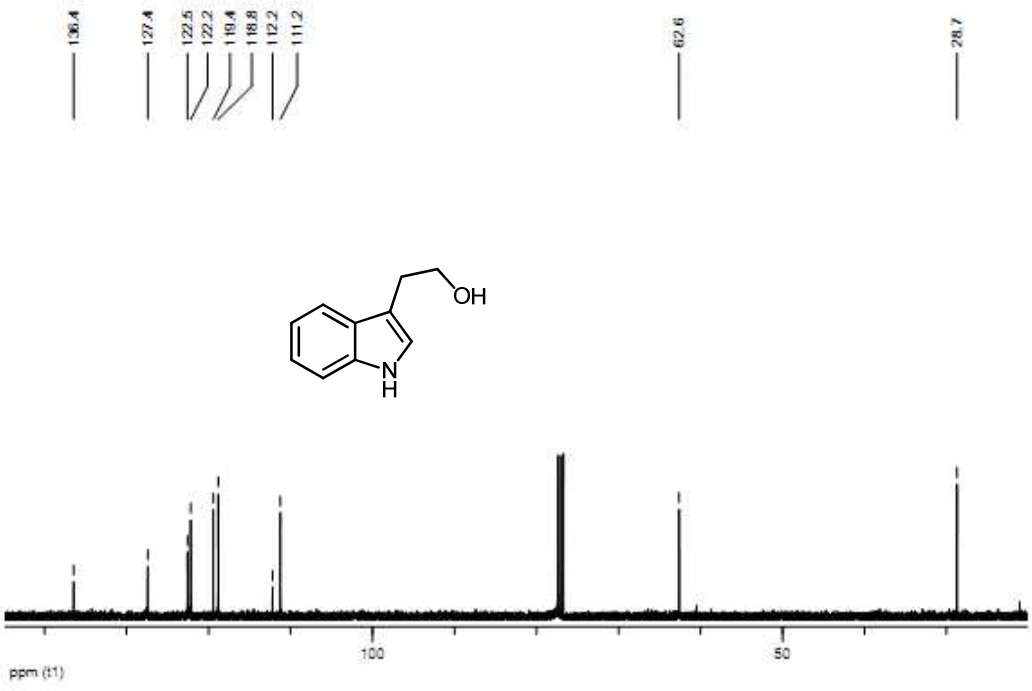
^{13}C NMR ($CDCl_3$, 100MHz)



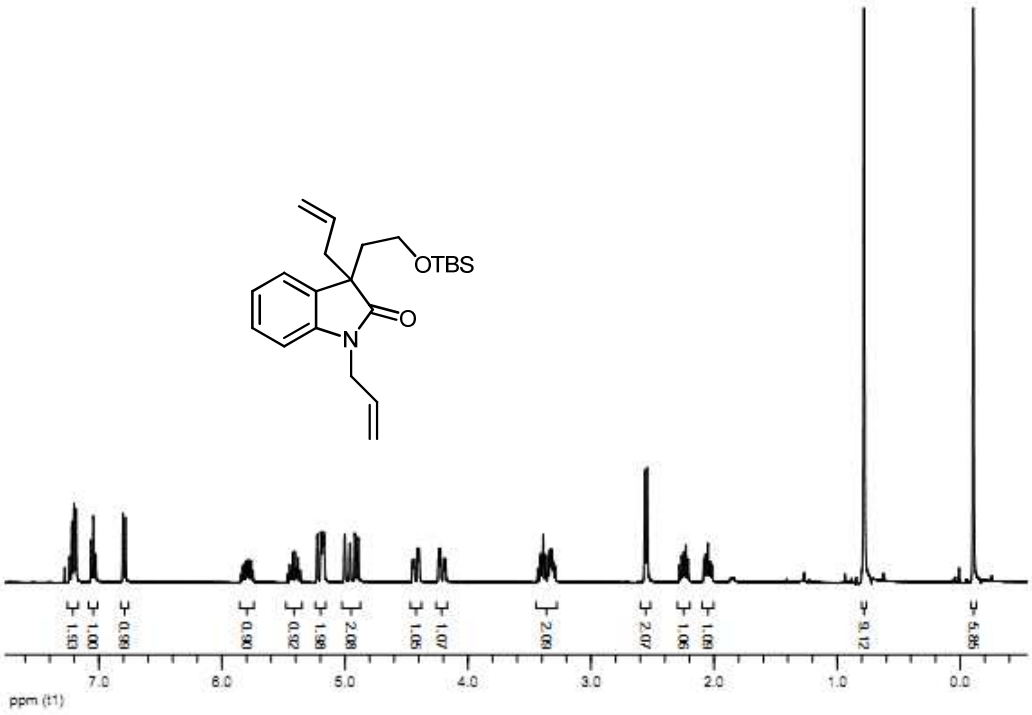
^{1}H NMR ($CDCl_3$, 400MHz)



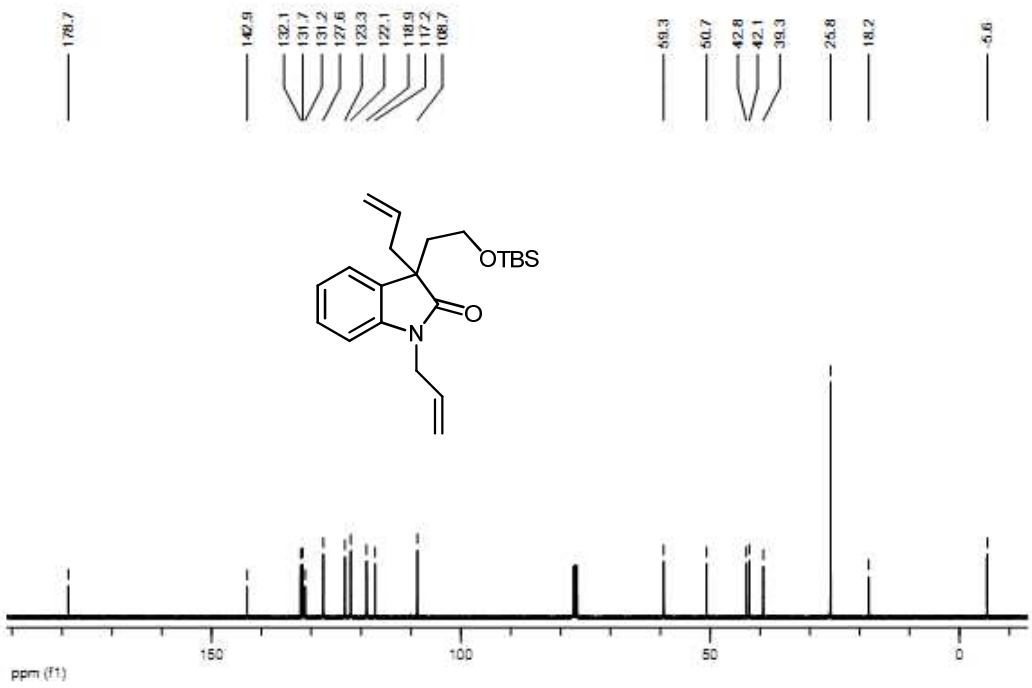
^{13}C NMR ($CDCl_3$, 100MHz)



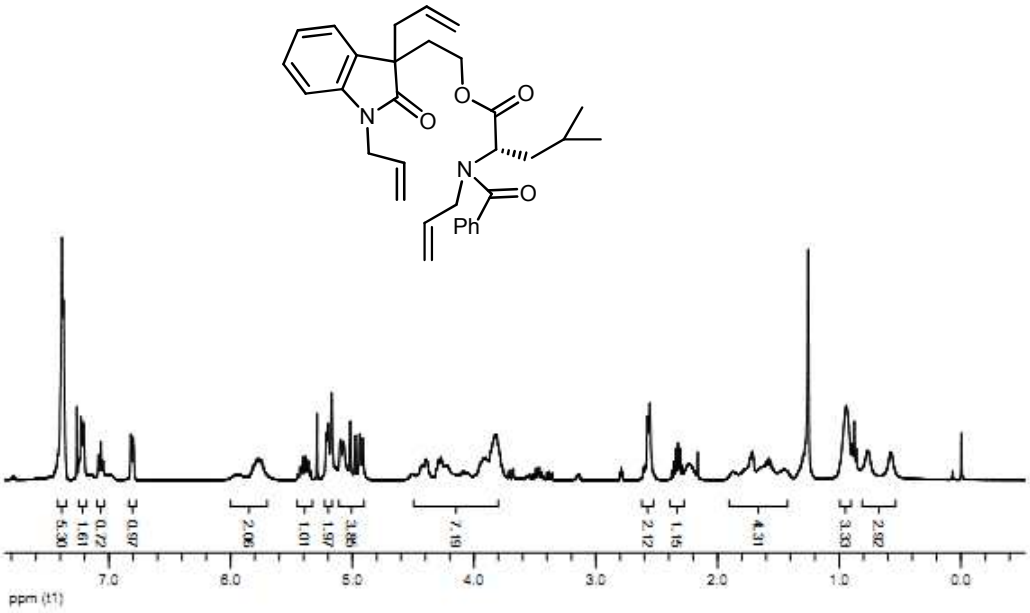
^{1}H NMR ($CDCl_3$, 400MHz)



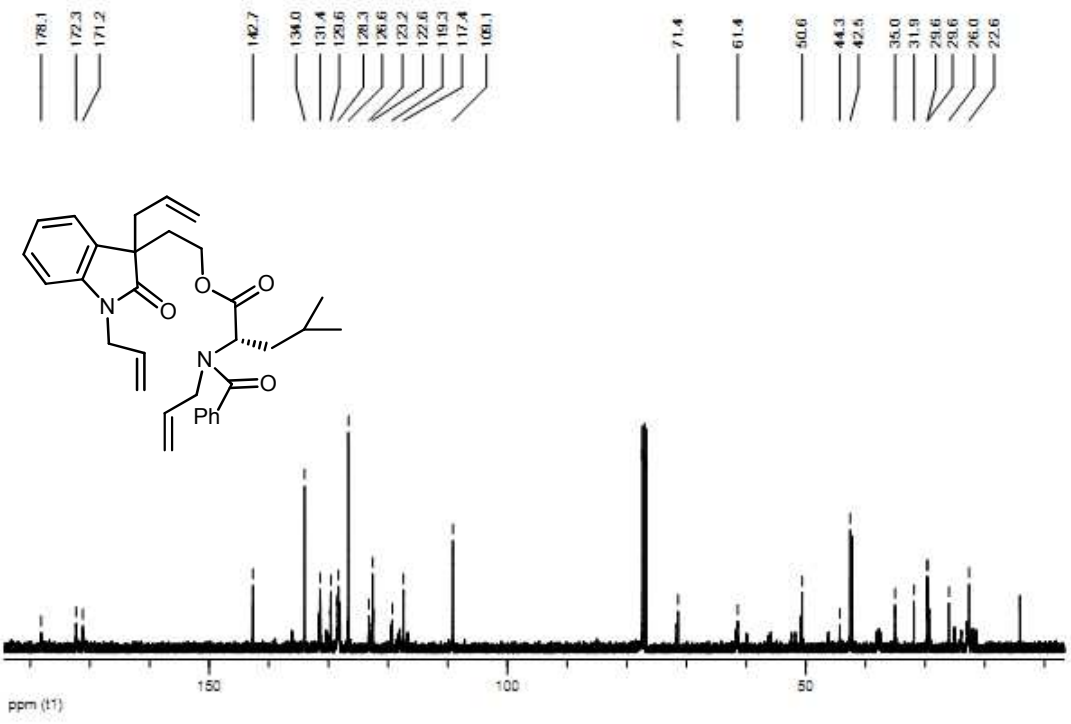
^{13}C NMR ($CDCl_3$, 100MHz)



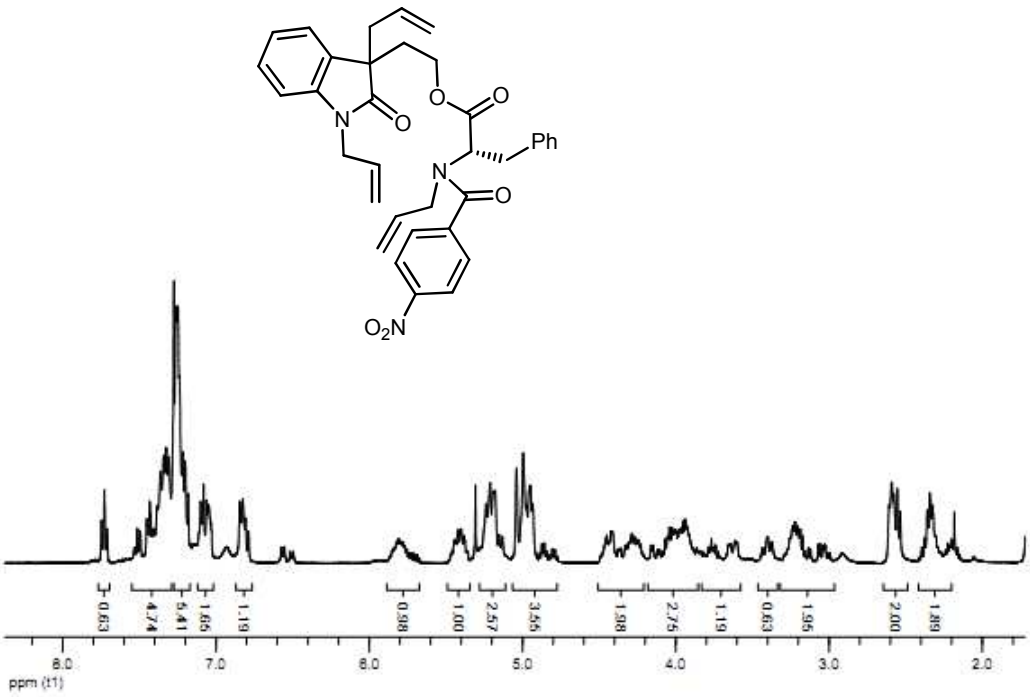
^{1}H NMR ($CDCl_3$, 400MHz)



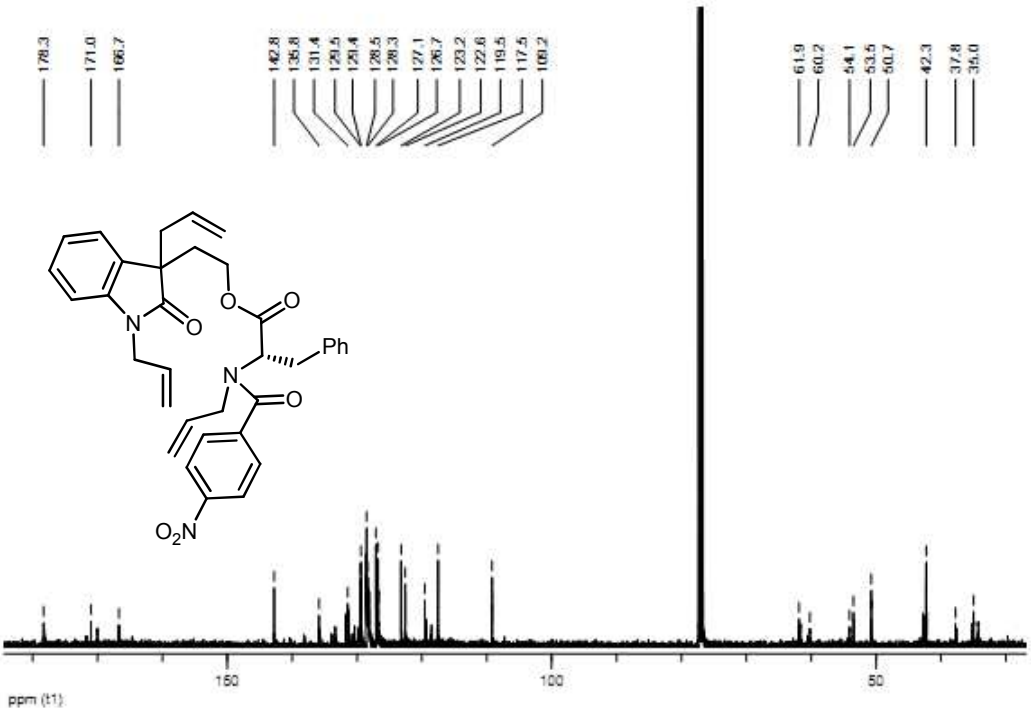
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)

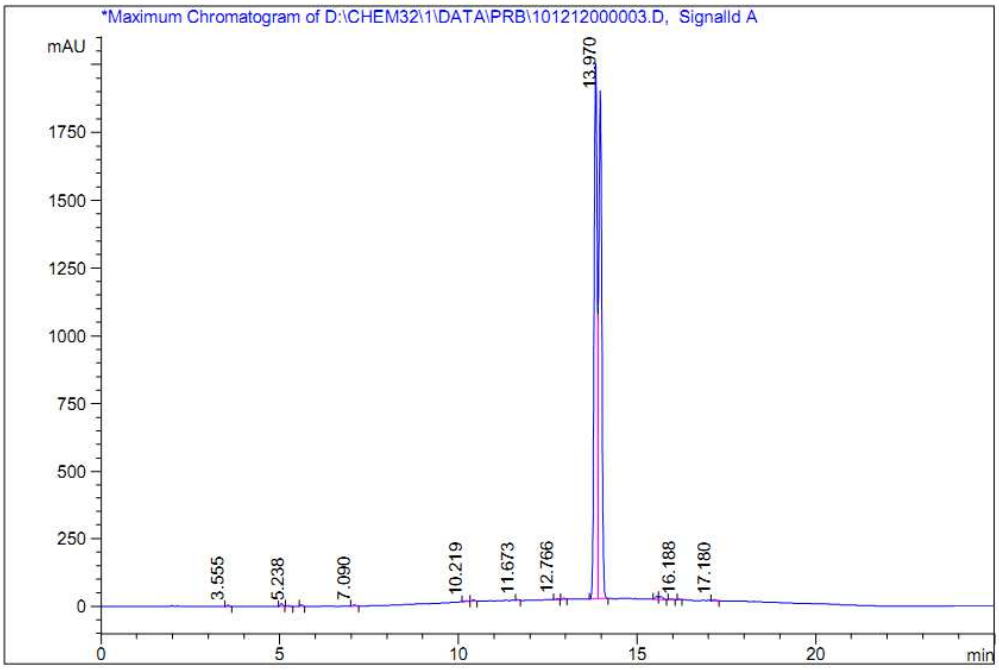


HPLC

HPLC ANALYSIS REPORT

		Seq Line :	0
Injection Date	: Mon, 10. Dec. 2012	Location :	Vial 51
Sample Name	: ILS-MNK-C63/33-B-22	Inj. No. :	0
Acq Operator	: VARMA	Inj. Vol. :	0.1 µl
Acq. Method	: D:\CHEM32\1\METHODS\CN-A50B50 MD.M		
Analysis Method	: D:\CHEM32\1\METHODS\CN-A50B50 MD.M		

Method Info : Column : Zorbax SB CN 150*4.6mm 5µ
Mobile phase: C) 001% HCOOH ,B) ACN (gradient)
T/B%:0/60,3/60,12/90,20/98,22/98,23/60,25/60.
Flow : 1.0ml/min Diluent : ACN:WATER(90:10) Temp:25



Signal 1: DAD1 A, Sig=200,4 Ref=360,100

Peak #	RT [min]	Area	Area %
1	3.555	21.057	0.089
2	5.051	46.598	0.198
3	5.238	11.947	0.051
4	5.622	26.005	0.110
5	7.090	27.079	0.115
6	10.219	23.634	0.100
7	10.426	30.564	0.130
8	11.673	12.015	0.051
9	12.766	19.683	0.083
10	12.953	21.989	0.093
11	13.843	12040.286	51.049
12	13.970	11120.896	47.151

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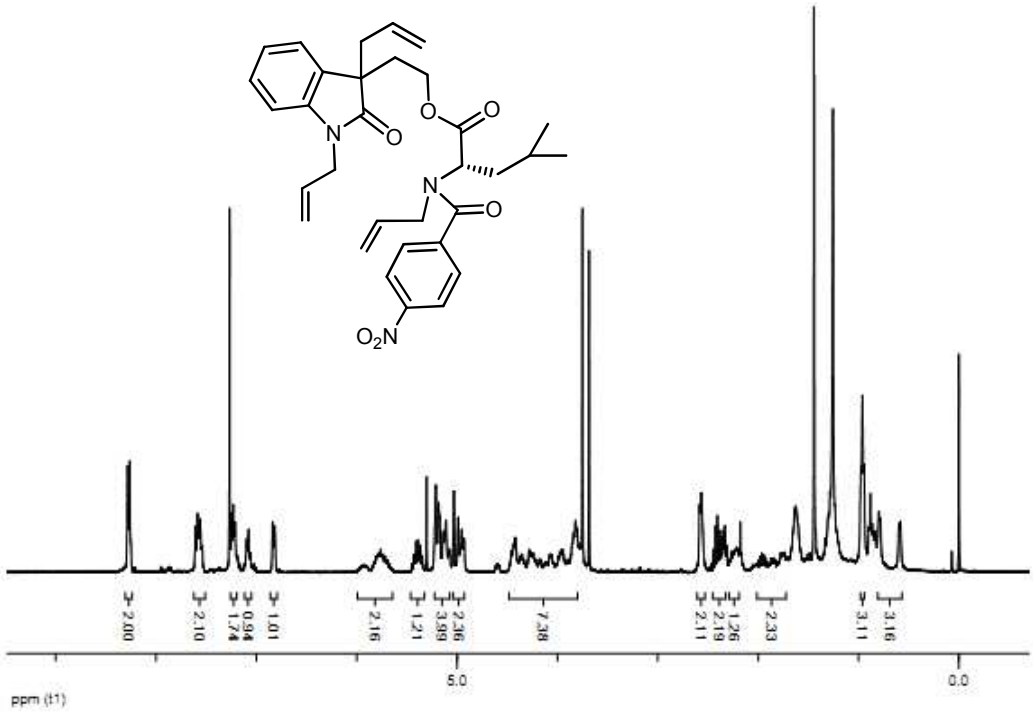
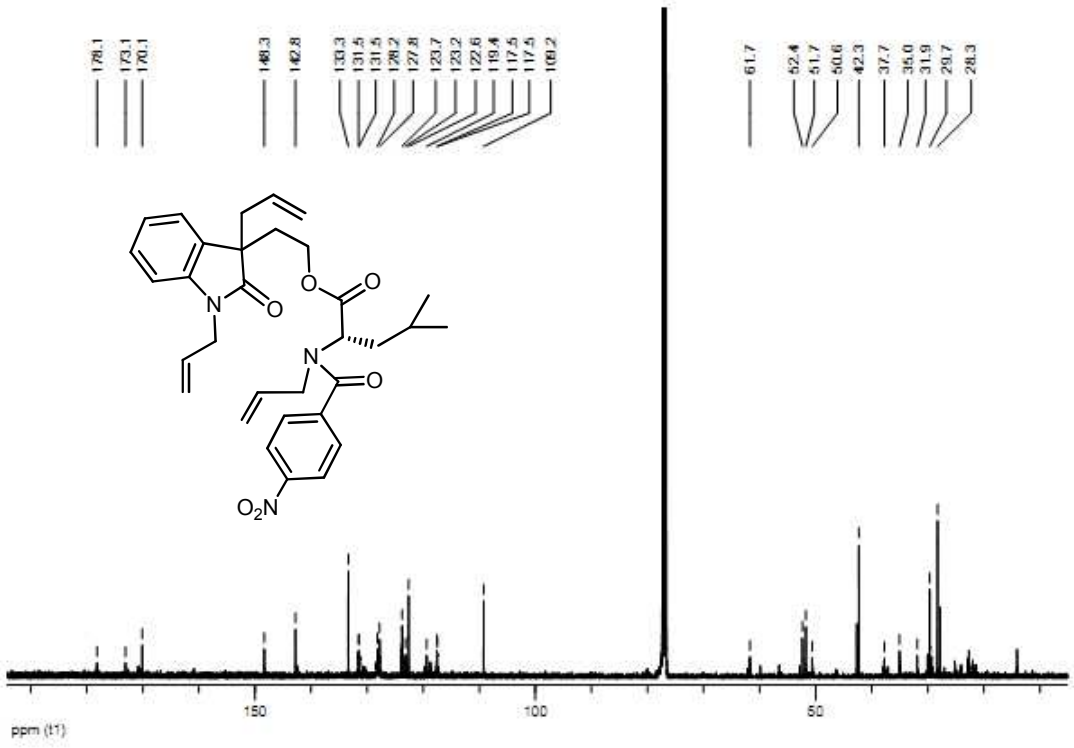
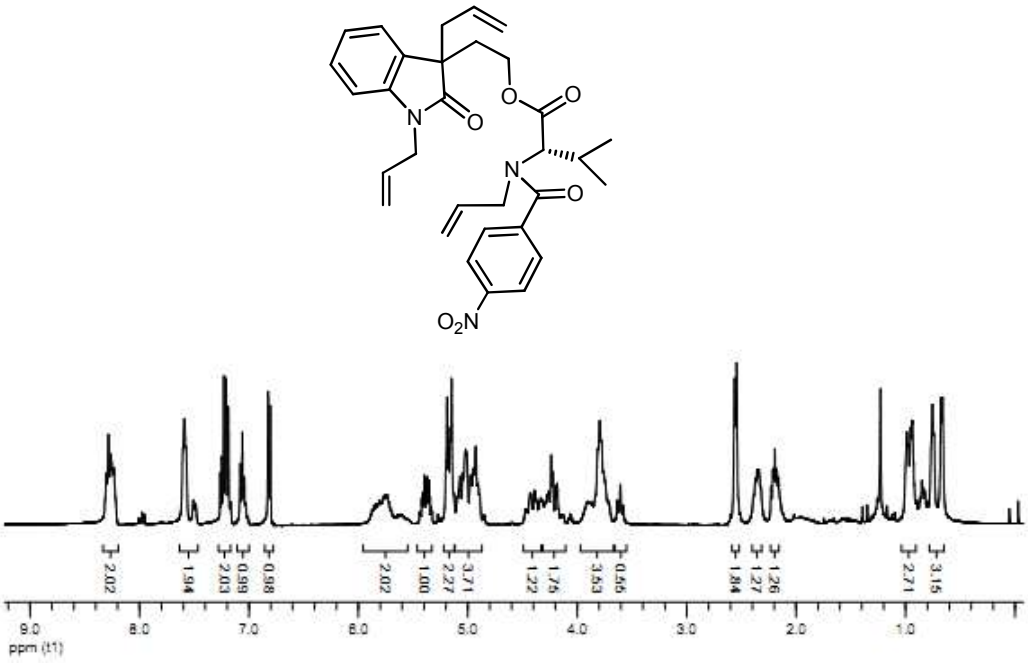
^{1}H NMR ($CDCl_3$, 400MHz)



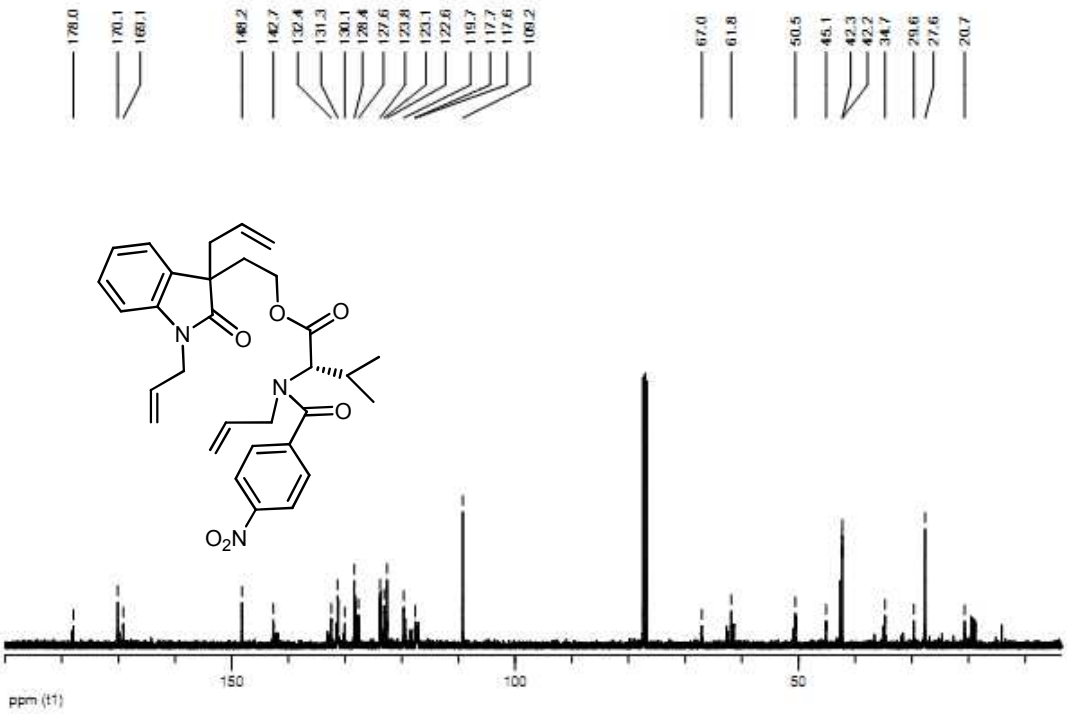
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



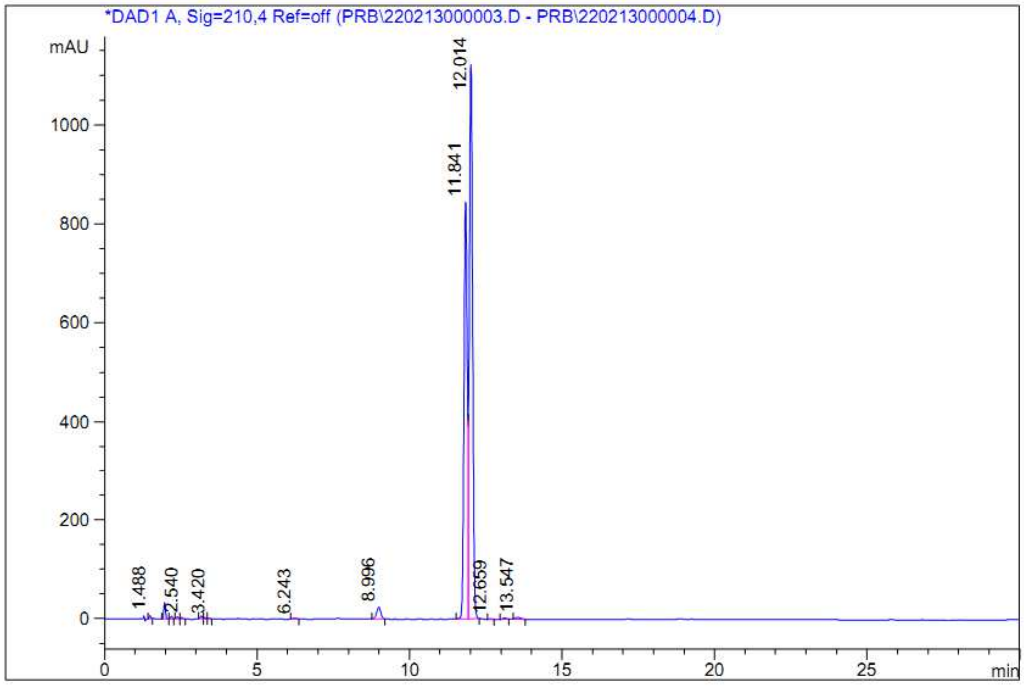
^{13}C NMR ($CDCl_3$, 100MHz)



HPLC

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line	: 0
Injection Date	: Fri, 22. Feb. 2013	Location	: Vial 4
Sample Name	: ILS-MNK-C63-33-B-40	Inj. No.	: 0
Acq Operator	: VARMA	Inj. Vol.	: 10 µl
Acq. Method	: D:\CHEM32\1\METHODS\AMD PRB.M		
Analysis Method	: D:\CHEM32\1\METHODS\AMD PRB.M		
Method Info	: Column: Zorbax XDB C-18 150*4.6mm,5µ		
	Mobile phase: A) 0.1% HCOOH in water,B) ACN (GRADIENT)		
	T/%B : 0/60,5/60,10/75,15/75,20/95,25/95,27/60,30/60		
	Flow:1.0 ml/min Diluent: ACN:Water(90:10)		



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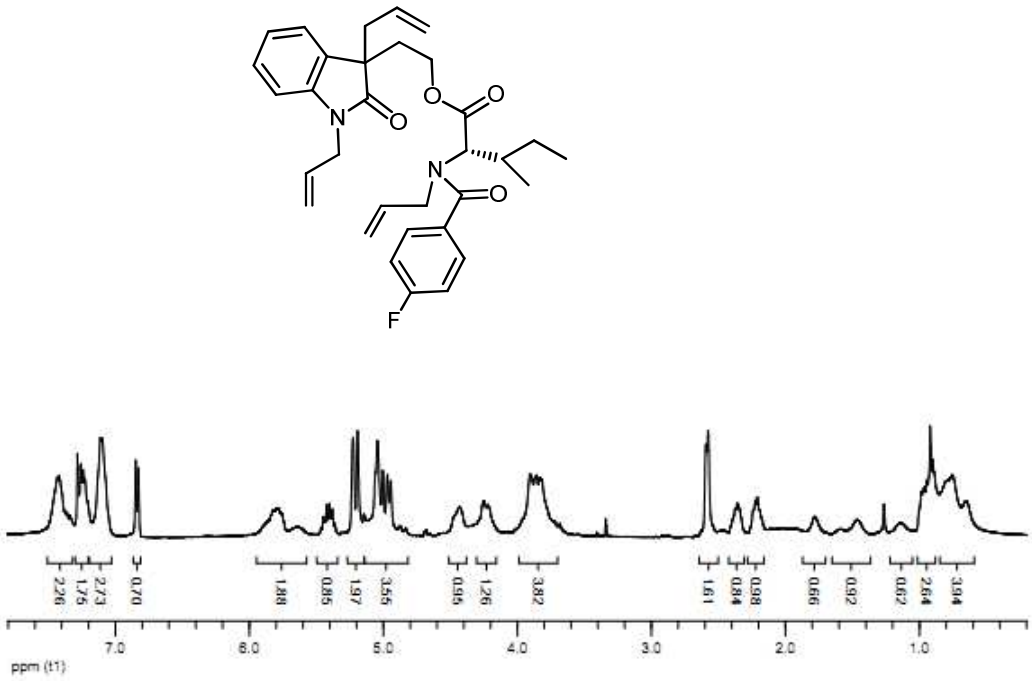
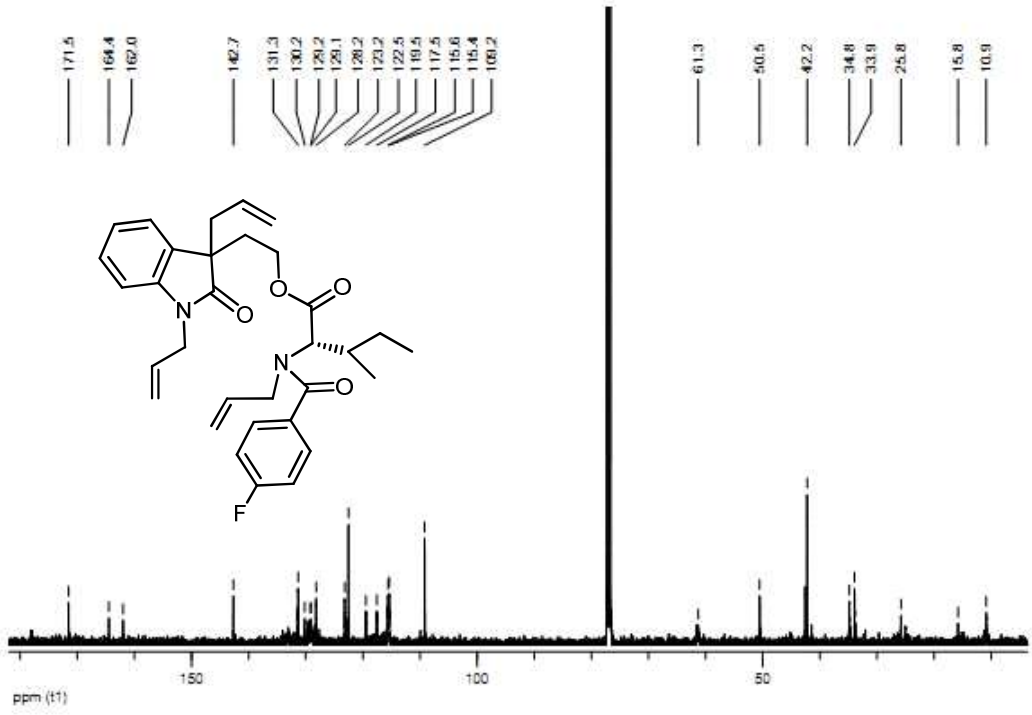
Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RT [min]	Area	Area %
1	1.488	22.513	0.148
2	1.970	128.313	0.844
3	2.198	22.366	0.147
4	2.386	15.492	0.102
5	2.540	12.251	0.081
6	3.171	25.366	0.167
7	3.296	11.976	0.079
8	3.420	6.707	0.044
9	6.243	10.048	0.066
10	8.996	225.671	1.484
11	11.841	6227.802	40.944
12	12.014	8436.233	55.463

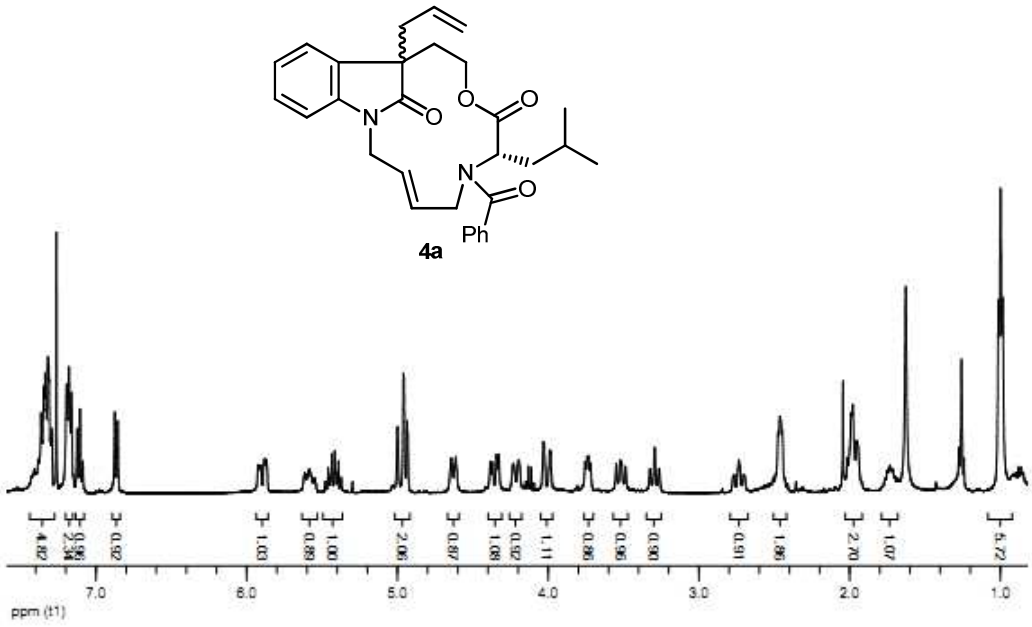
^{1}H NMR ($CDCl_3$, 400MHz)



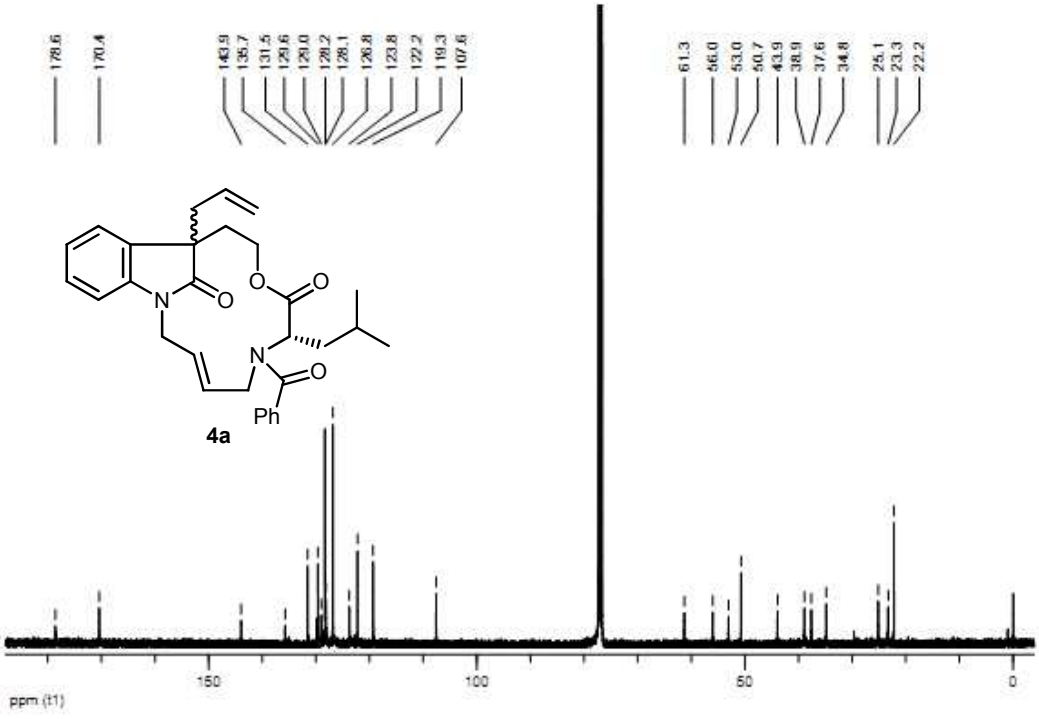
^{13}C NMR ($CDCl_3$, 100MHz)



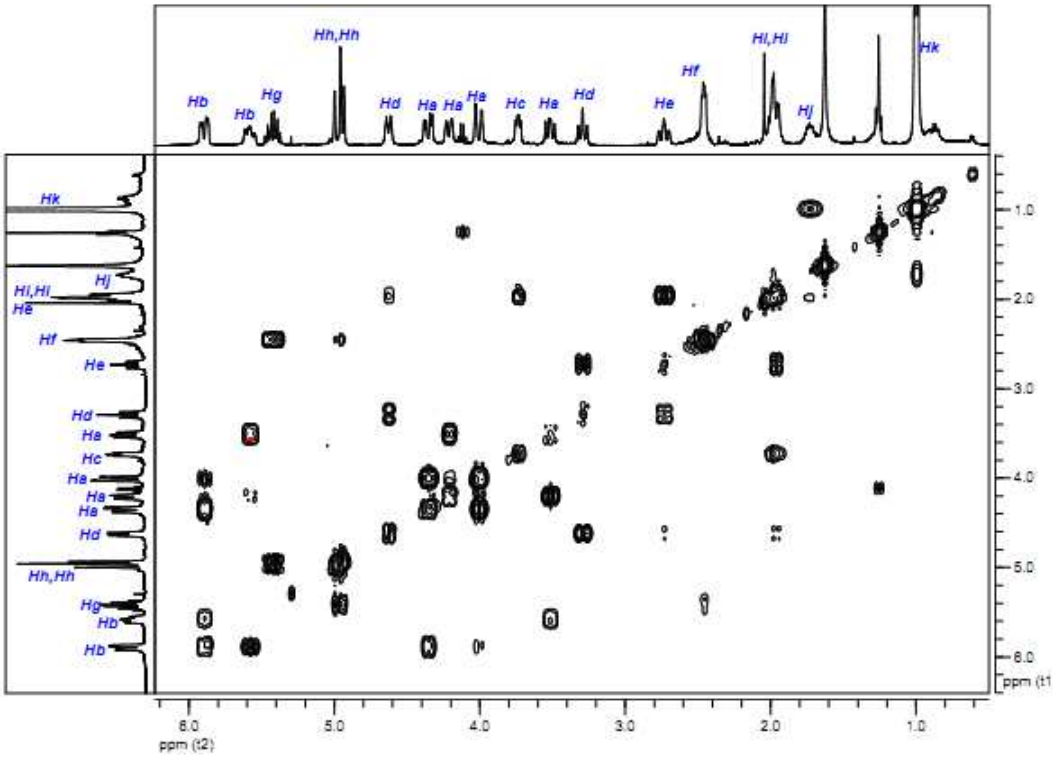
^{1}H NMR ($CDCl_3$, 400MHz)

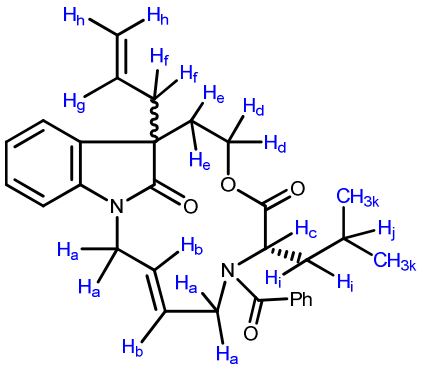


^{13}C NMR ($CDCl_3$, 100MHz)



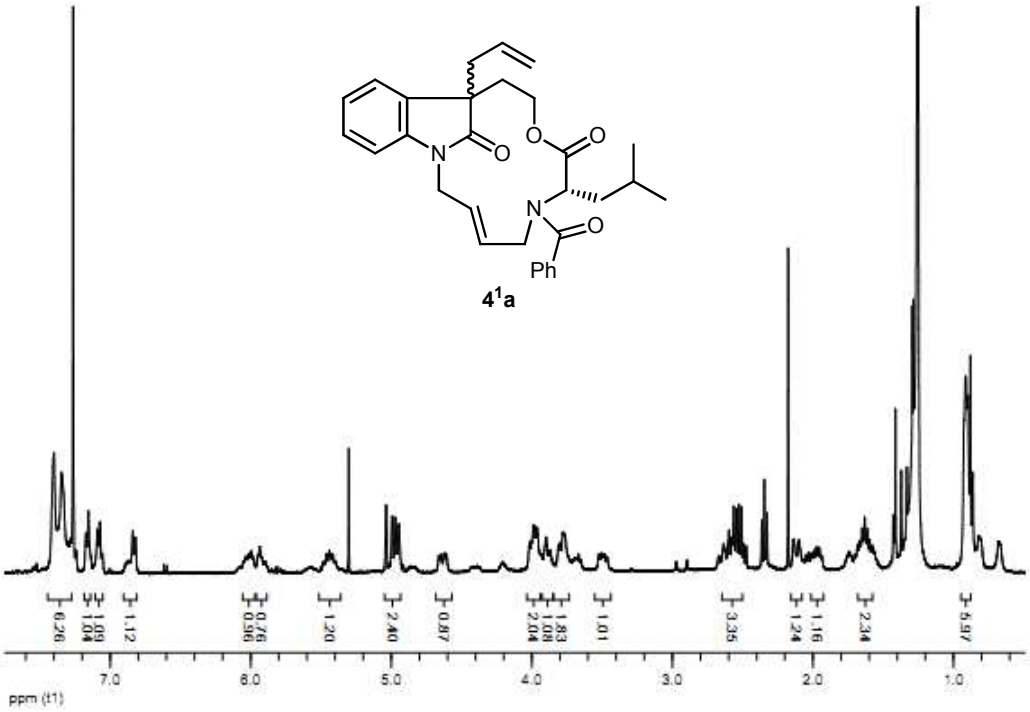
^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)





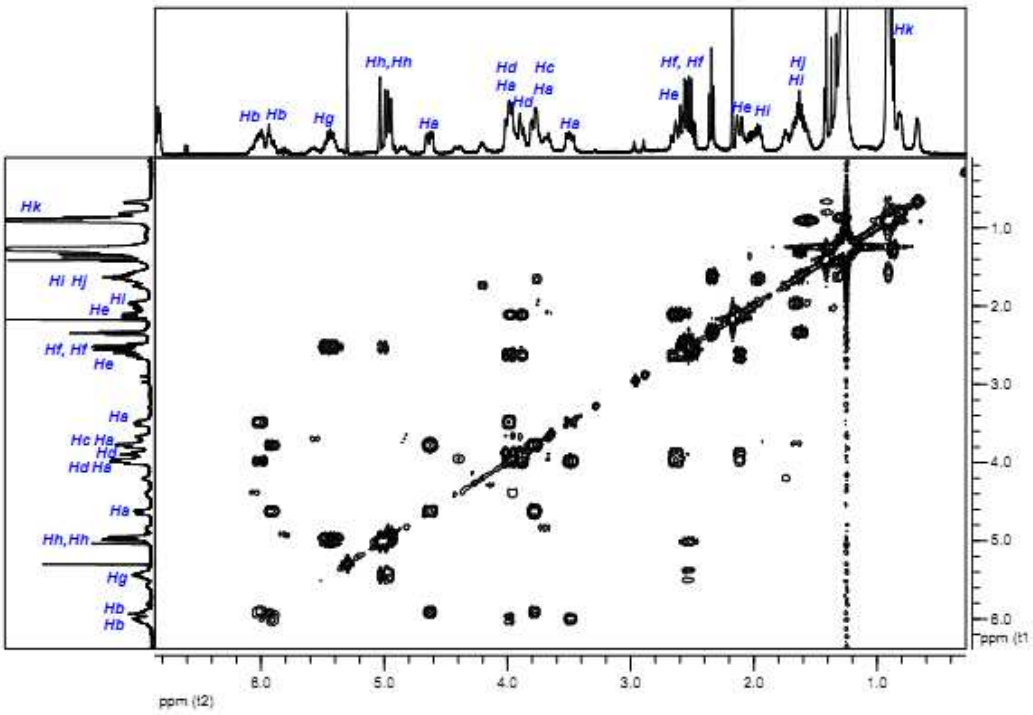
4a

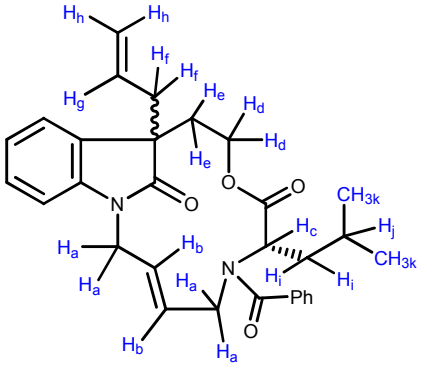
^{1}H NMR ($CDCl_3$, 400MHz)



4[1]a

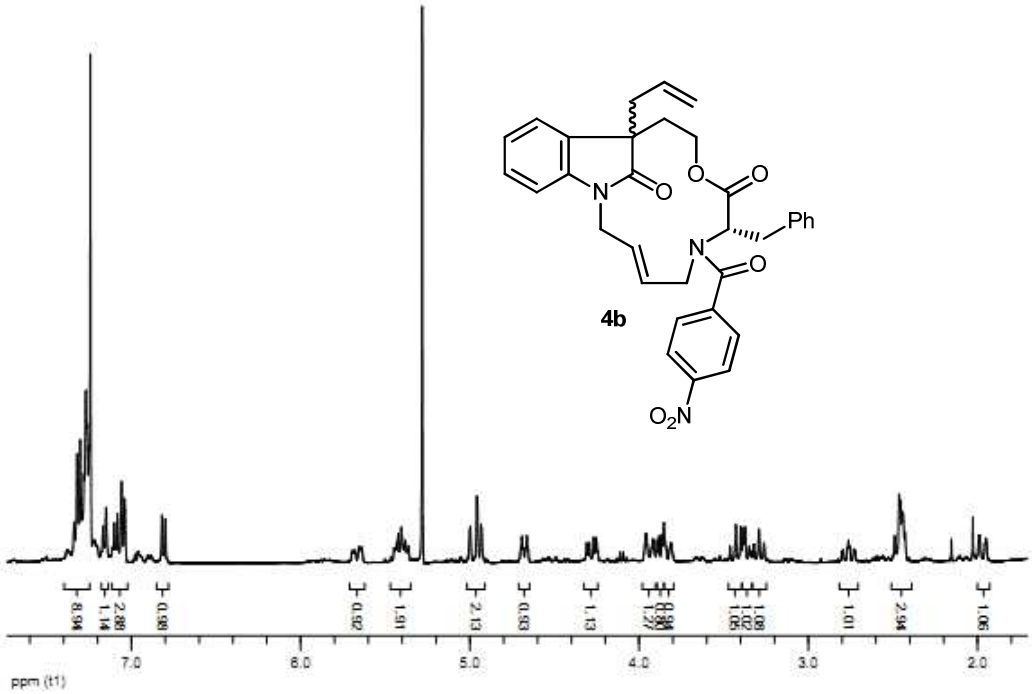
^{1}H- ^{1}H COSY NMR ($CDCl_3$, 400MHz)



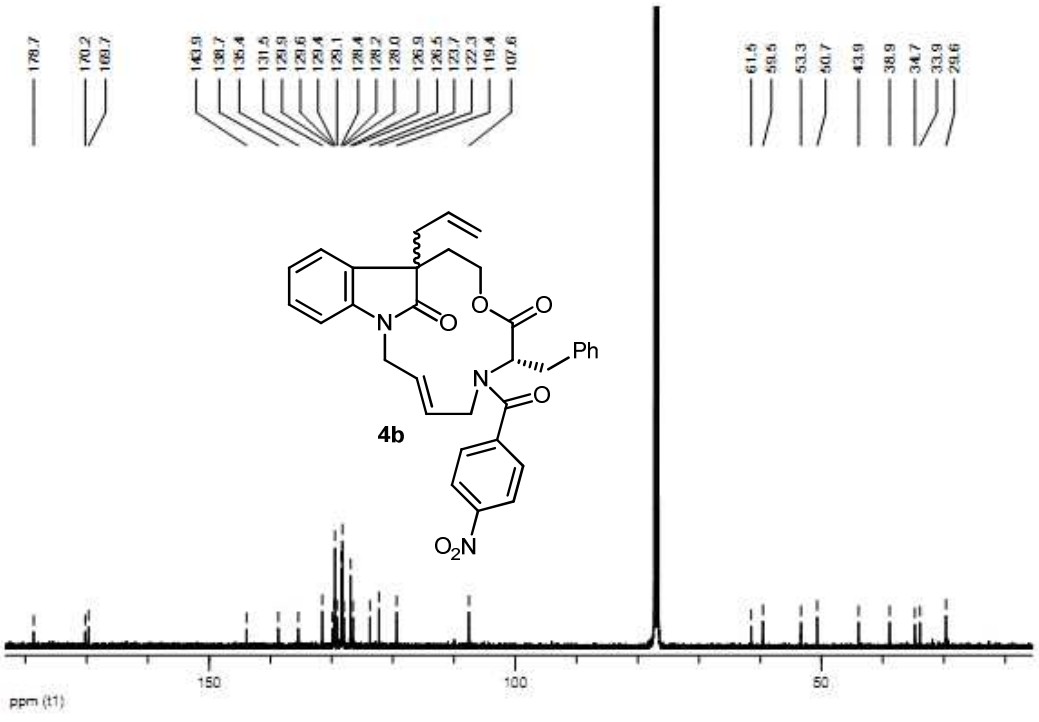


4[1]a

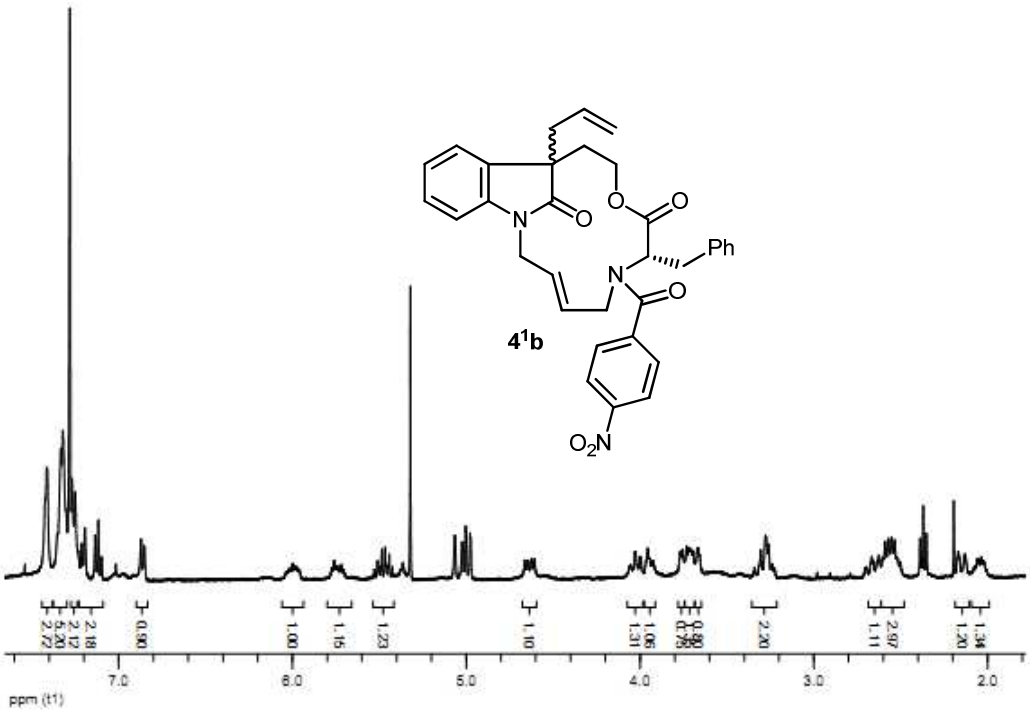
^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



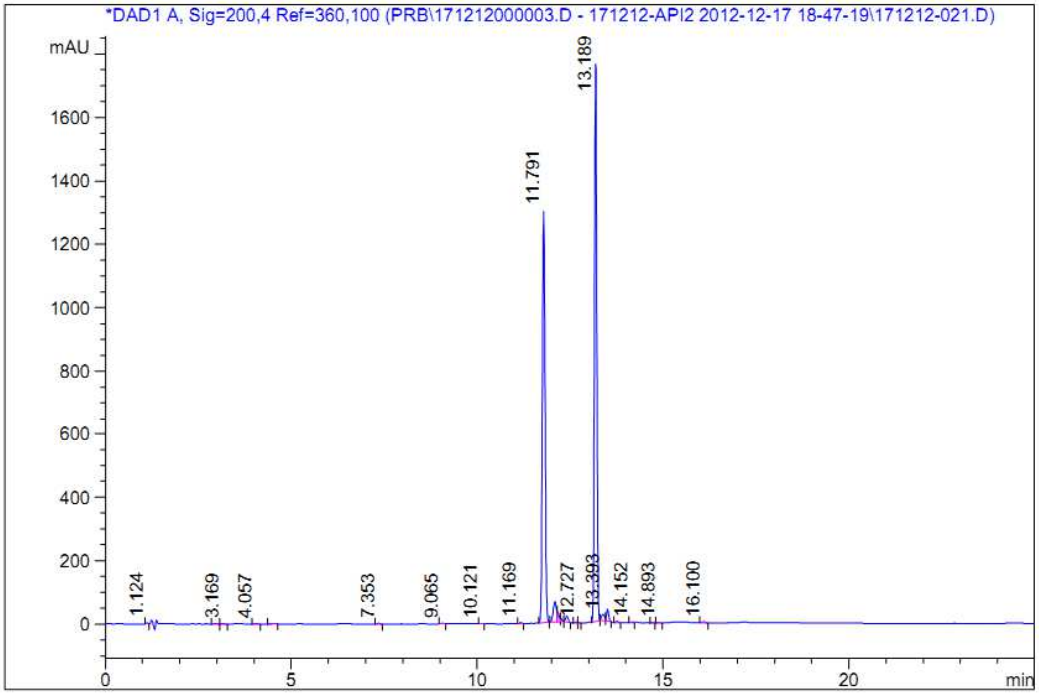
^{1}H NMR ($CDCl_3$, 400MHz)



HPLC (separation before) (4b & 4[1]b)

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line	: 0
Injection Date	: Mon, 17. Dec. 2012	Location	: Vial 21
Sample Name	: ILS-MNK-C63/33-B-24	Inj. No.	: 0
Acq Operator	: VARMA	Inj. Vol.	: 5 µl
Acq. Method	: D:\CHEM32\1\METHODS\CN-A20B80.M		
Analysis Method	: D:\CHEM32\1\METHODS\XDB A60B40 GM.M		
Method Info	: Column : Eclipse XDB C-18 150*4.6mm 5µ		
	Mobile phase: A) 0.1% Formic Acid in water ,B)ACN		
	T/%B: 0/40,3/40,12/95,20/95,22/40,25/40.		
	Flow:1.0ml/min Dil: ACN:Water(90:10)		



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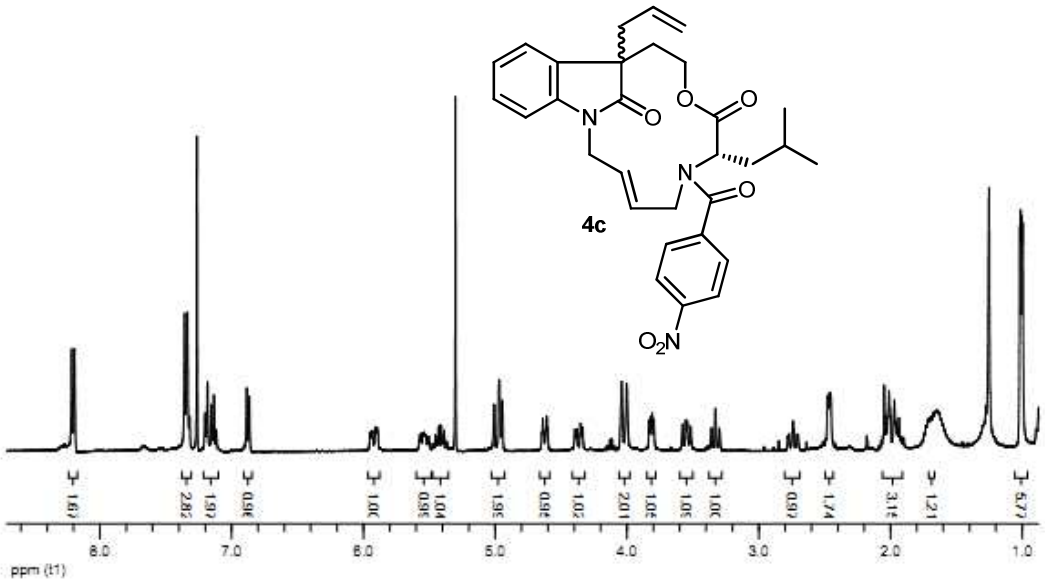
Signal 1: DAD1 A, Sig=200,4 Ref=360,100

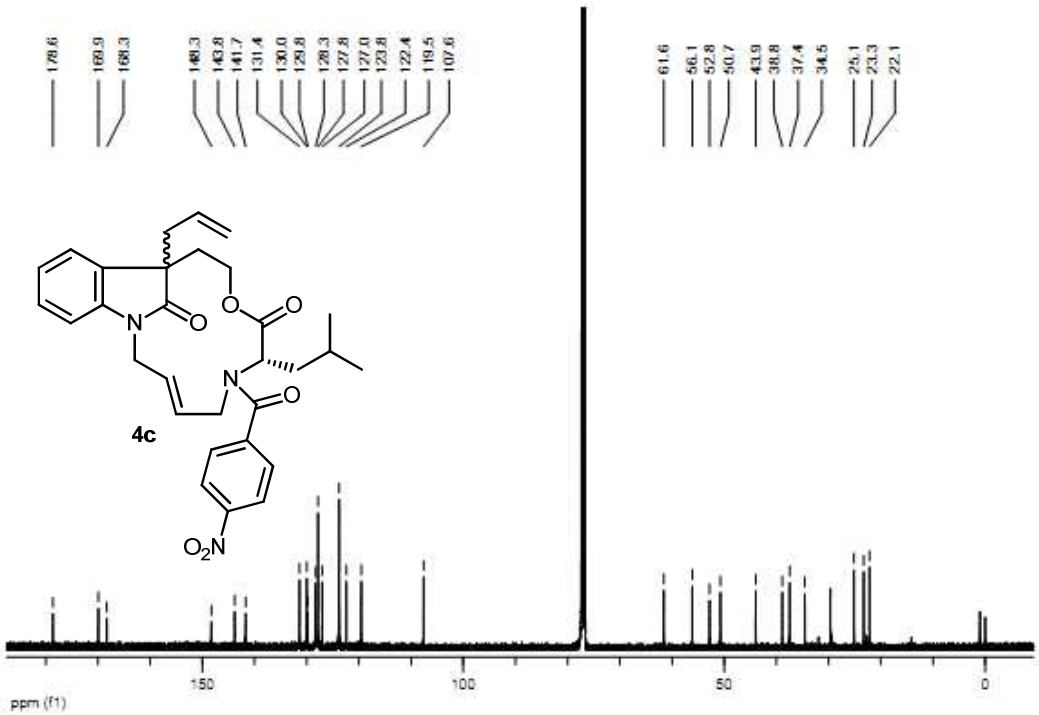
Peak #	RT [min]	Area	Area %
1	1.124	9.957	0.065
2	2.942	11.856	0.078
3	3.169	10.582	0.070
4	4.057	8.217	0.054
5	4.500	11.278	0.074
6	7.353	13.166	0.087
7	9.065	8.446	0.056
8	10.121	7.255	0.048
9	11.169	18.426	0.121
10	11.791	6569.448	43.197

Peak #	RT [min]	Area	Area %
11	12.094	390.964	2.571
12	12.157	103.544	0.681
13	12.259	24.953	0.164
14	12.413	88.638	0.583
15	12.655	15.500	0.102
16	12.727	5.820	0.038
17	13.189	7586.477	49.884
18	13.393	107.888	0.709
19	13.509	149.269	0.981
20	13.766	21.166	0.139
21	14.152	10.555	0.069
22	14.721	7.397	0.049
23	14.893	6.286	0.041
24	16.100	21.144	0.139

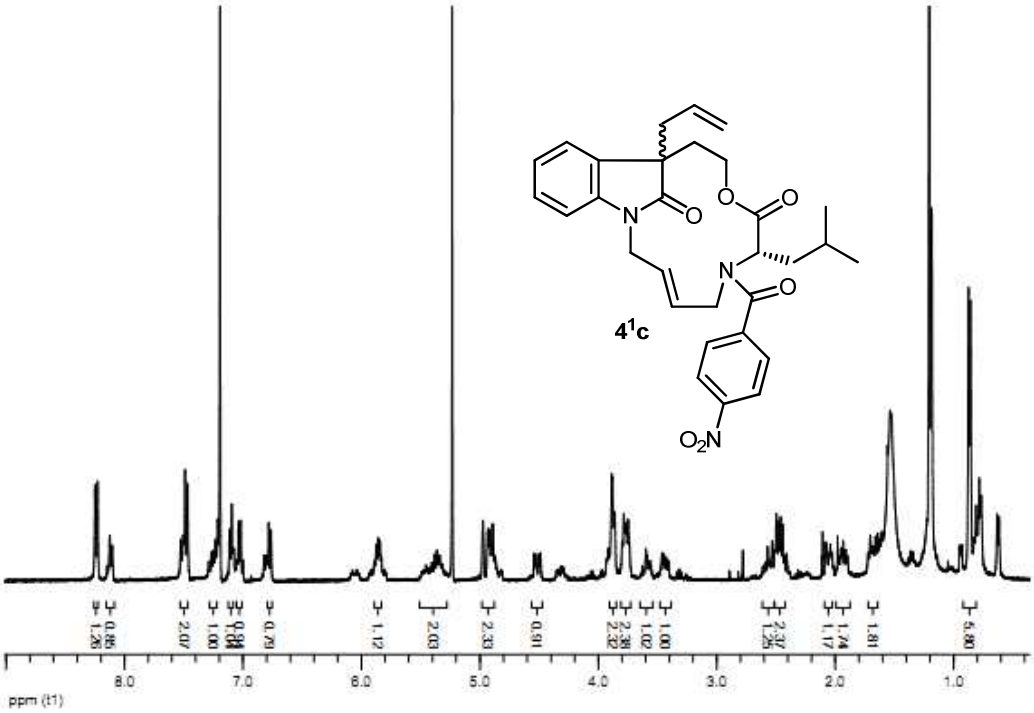
^{1}H NMR ($CDCl_3$, 400MHz)



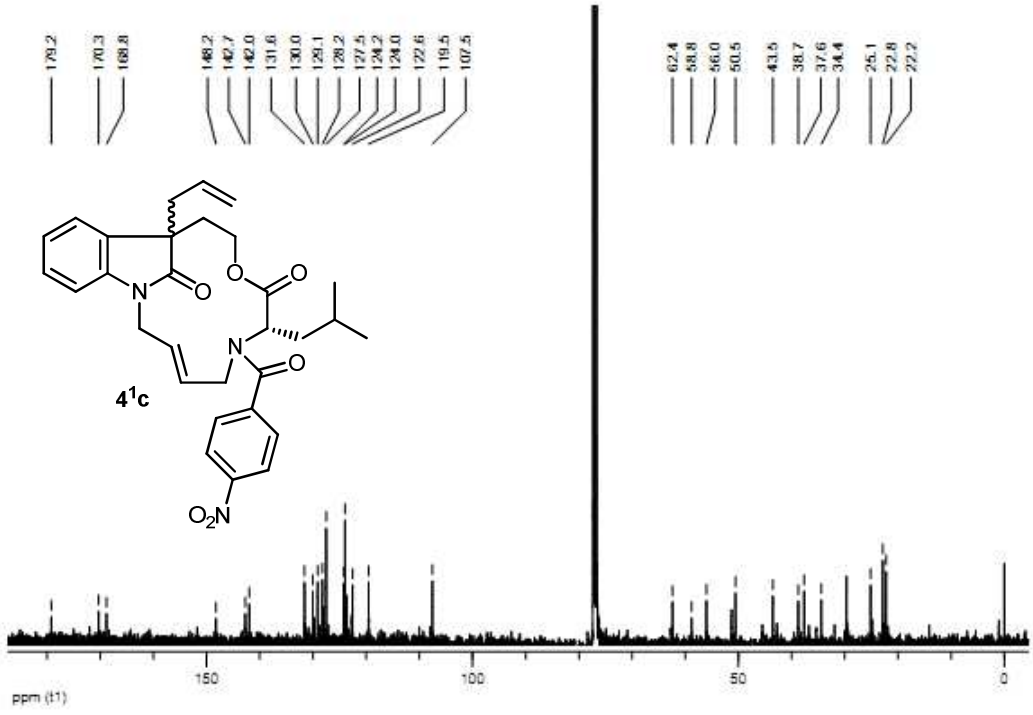
^{13}C NMR ($CDCl_3$, 100MHz)



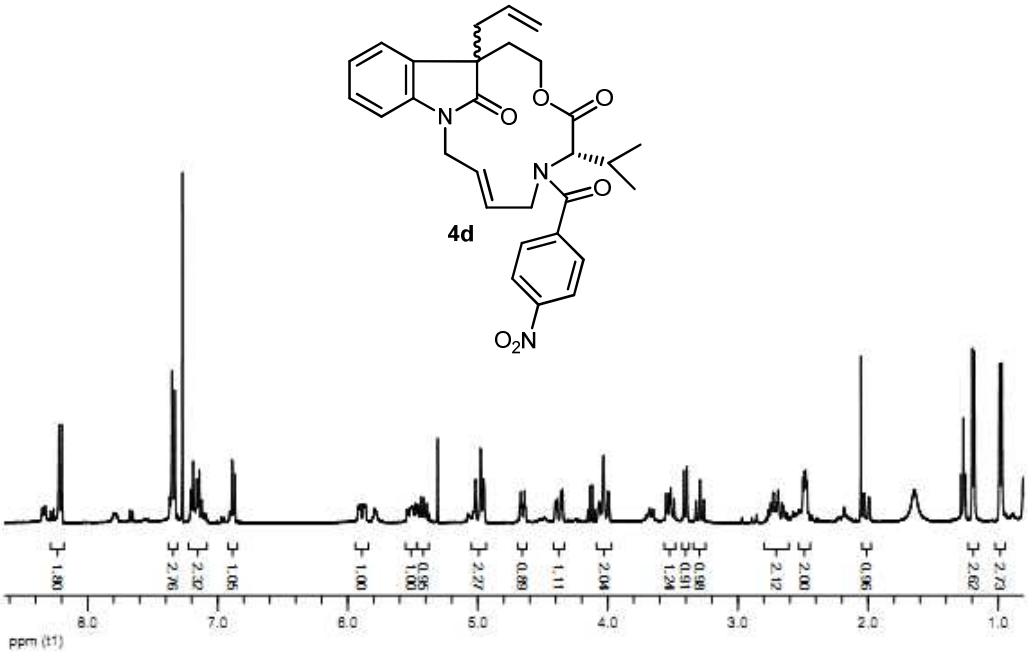
^{1}H NMR ($CDCl_3$, 400MHz)



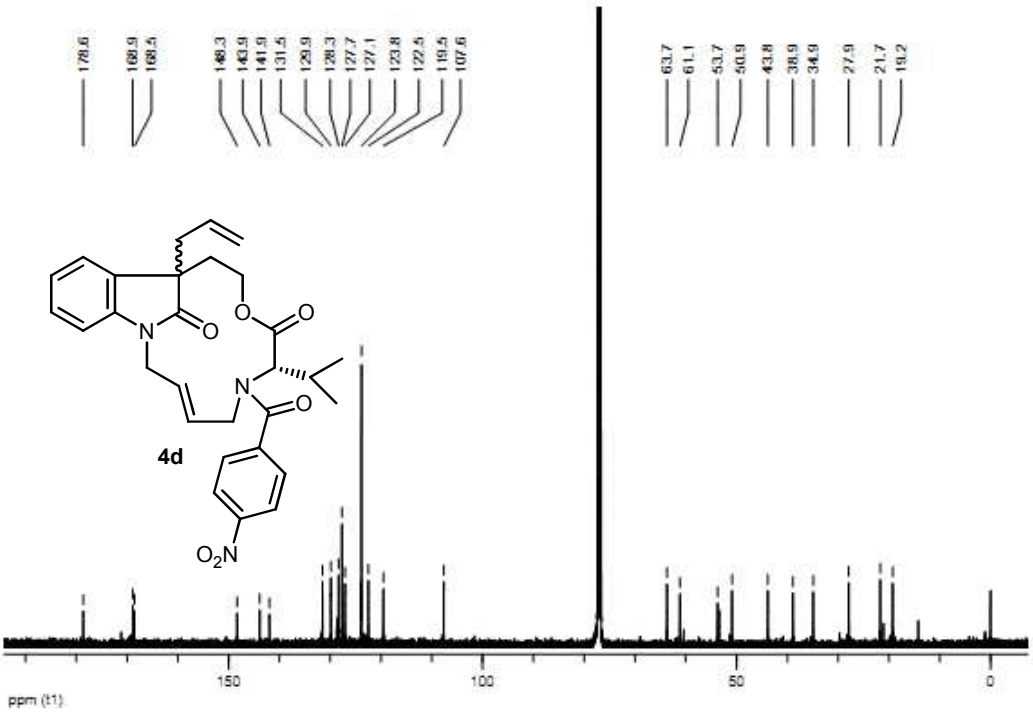
^{13}C NMR ($CDCl_3$, 100MHz)



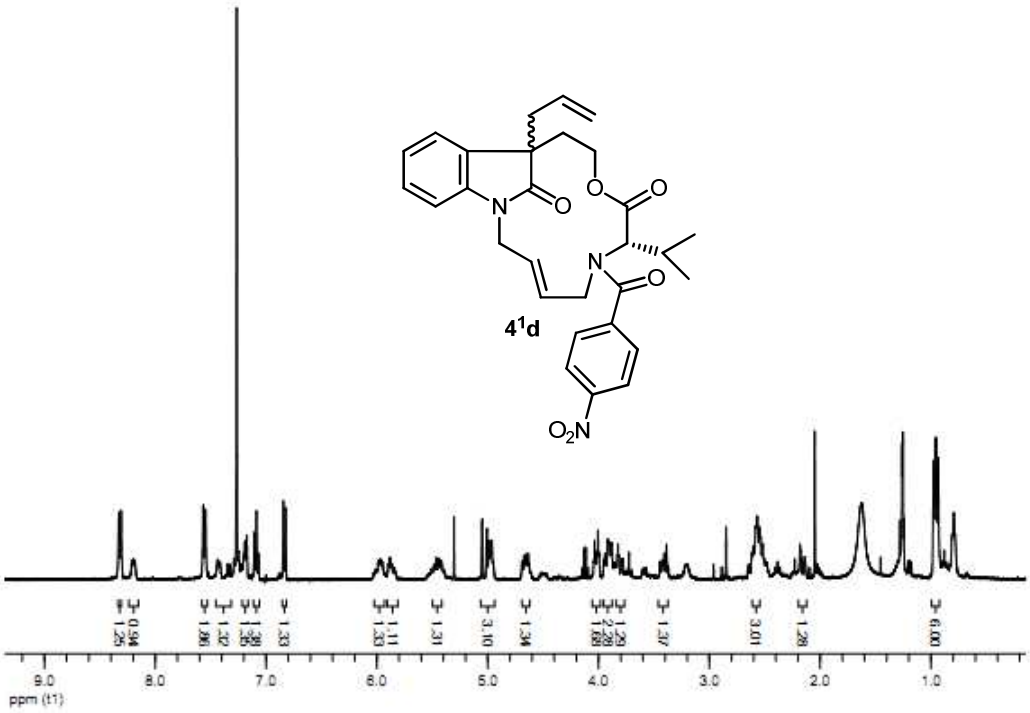
^{1}H NMR ($CDCl_3$, 400MHz)



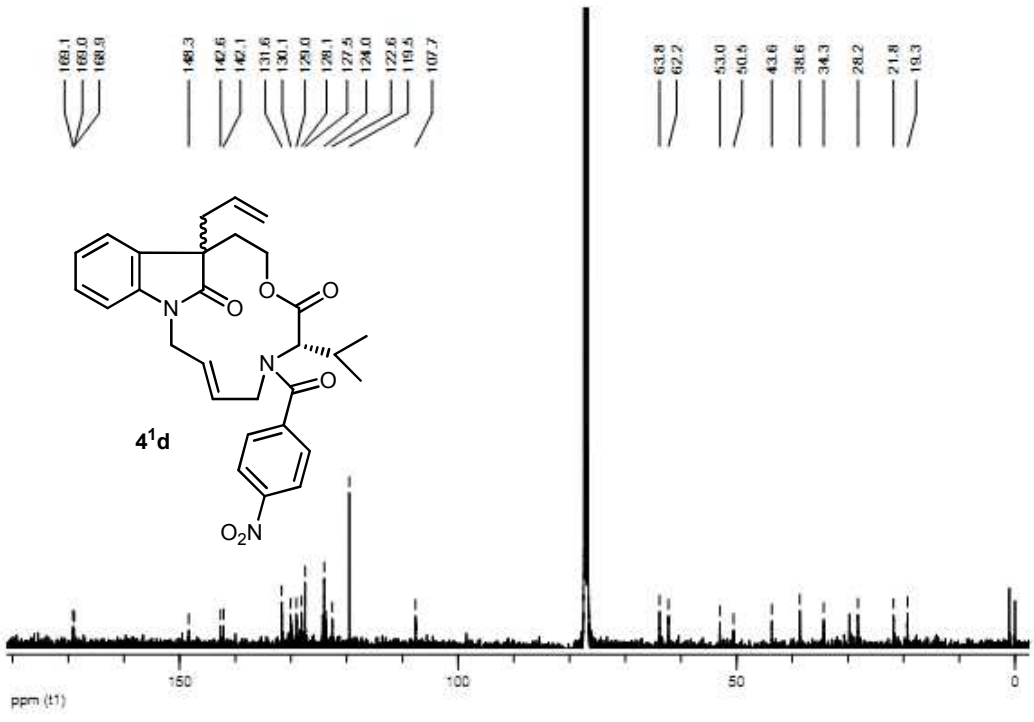
^{13}C NMR ($CDCl_3$, 100MHz)



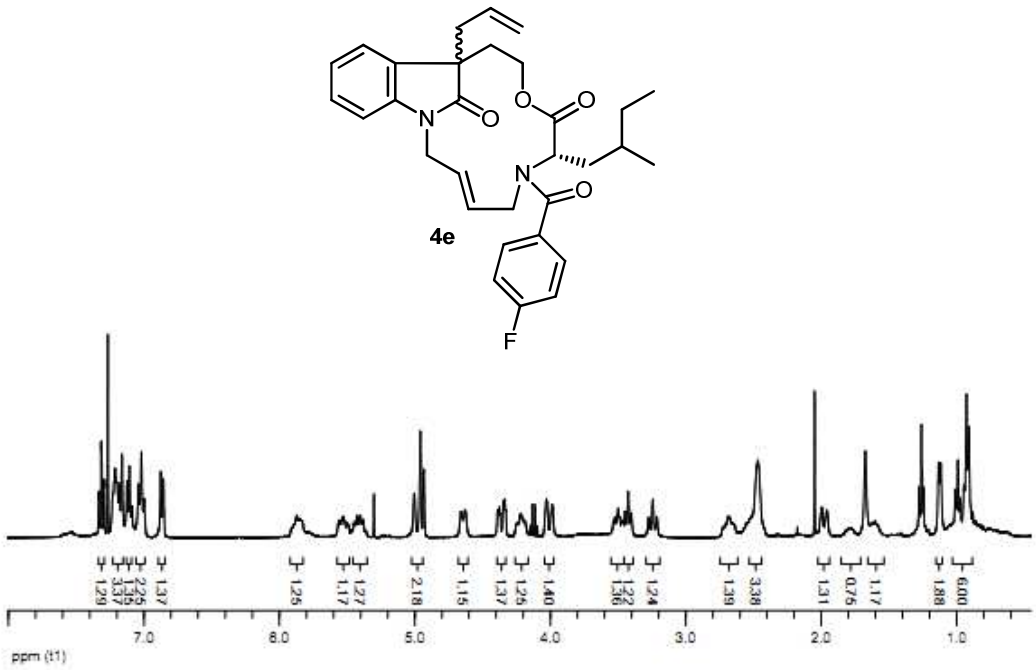
^{1}H NMR ($CDCl_3$, 400MHz)



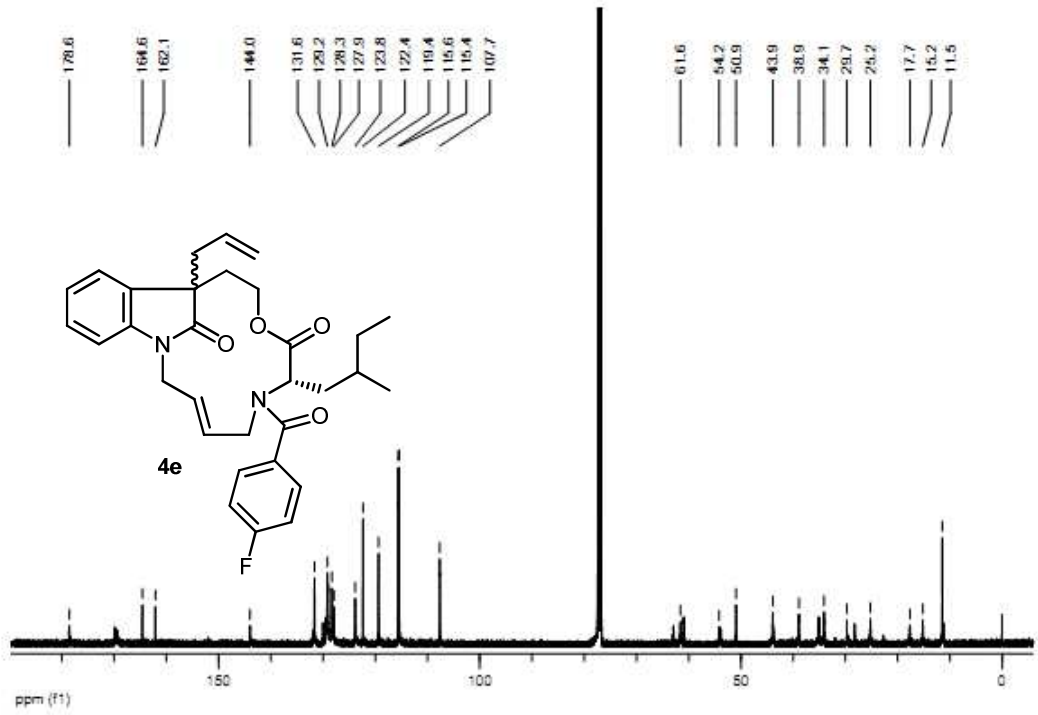
^{13}C NMR ($CDCl_3$, 100MHz)



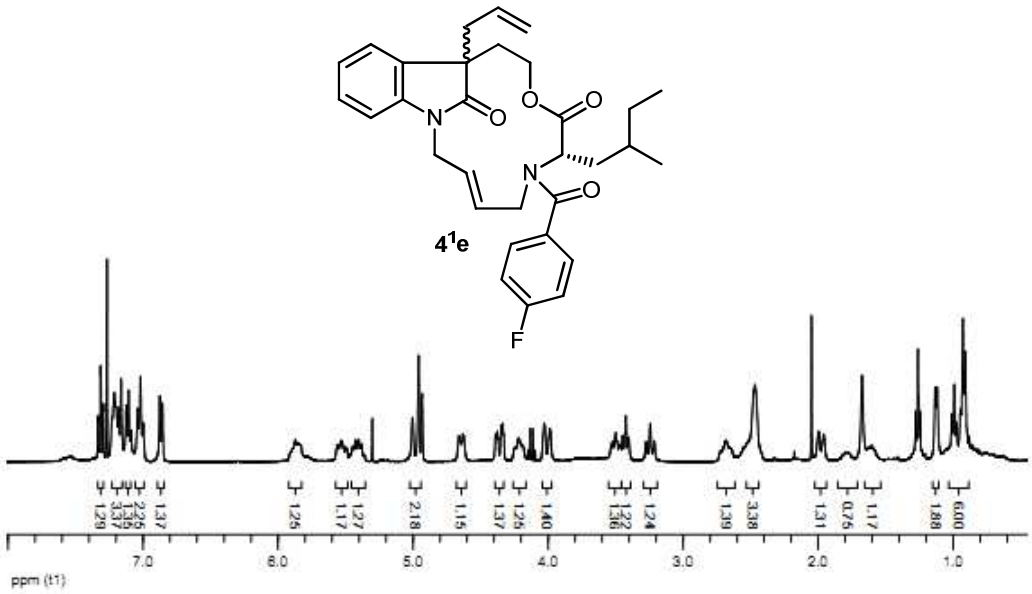
^{1}H NMR ($CDCl_3$, 400MHz)



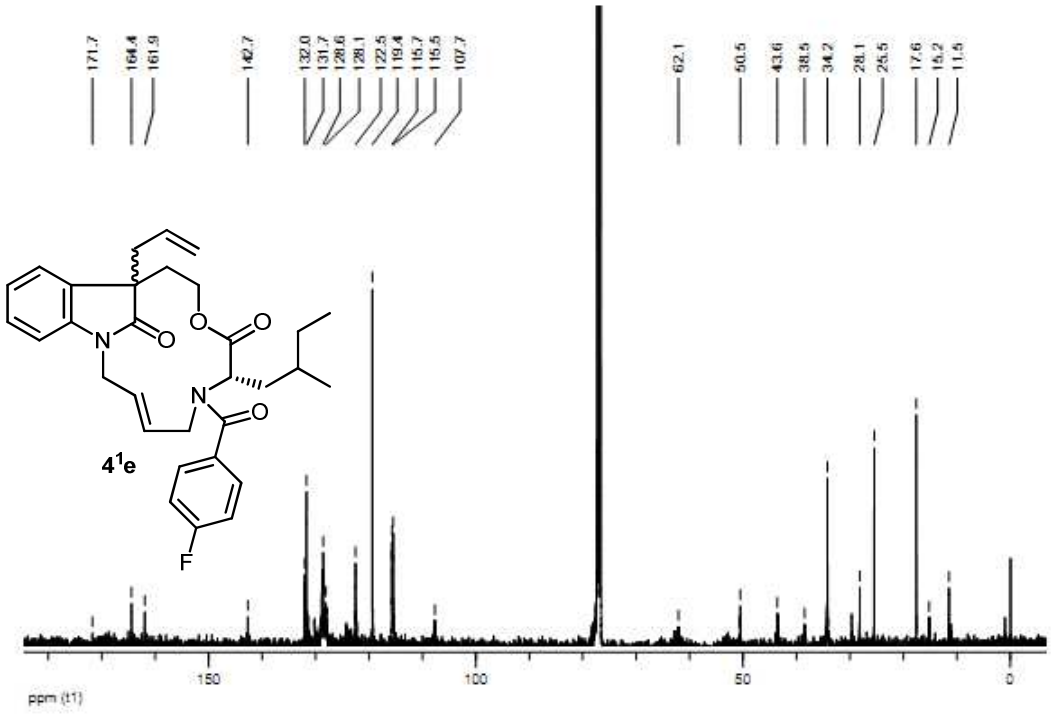
^{13}C NMR ($CDCl_3$, 100MHz)



^{1}H NMR ($CDCl_3$, 400MHz)



^{13}C NMR ($CDCl_3$, 100MHz)



Publications

Naveen Kumar Mallurwar Peer Reviewed Publications

1. 14-Membered macrocyclic ring-derived toolbox: the identification of small molecule inhibitors of angiogenesis and early embryo development in zebrafish assay. Aeluri, M., Pramanik, C., Chetia, L., **Mallurwar, N. K.,** Balasubramanian, S., Chandrasekar, G., Kitambi, S.S., Arya, P. *Org. Lett.* **2015**, *15*(3), 436-439.
2. A Modular Approach to Building 17- and 18-Membered Macrocyclic Diversity from Eribulin C14-C21 Fragment, **Naveen Kumar Mallurwar**, Saidulu Konda, Mahender Khatravath, Pallavi Rao, Shivashankar Sripally, Javed Iqbal and Prabhat Arya, **2016**, *submitted for publication.*
3. Synthesis of C1-C11 Eribulin Fragment its Analogs for Building A Diverse Set of Macrocycles, Mahender Khatravath, **Naveen Kumar Mallurwar**, Saidulu Konda, Pallavi Rao, Shivashankar Sripelly, Javed Iqbal and Prabhat Arya, **2016**, *accepted for publication.* in the journal, *Synthesis.*
4. Stereoselective Synthesis of C27-C35 Eribulin Fragment and Its Utilization in Building Structurally Diverse Macrocycles, Saidulu Konda, Mahender Khatravath, **Naveen Kumar Mallurwar**, Pallavi Rao, Shivashankar Sripelly, Javed Iqbal and Prabhat Arya, **2016**, *accepted for publication* in a special issue of the journal, *Synthesis*, "Target Oriented Synthesis of Complex Molecules".
5. Stereoselective Synthesis of C22-C28 Eribulin Fragment for Building A Diverse Set of 17-Membered Macrocyclic Compounds. *Manuscript under preparation, **2016.***
6. Synthesis of Isatin based Diverse set of Macrocyclic Compounds. *Manuscript under preparation, **2016.***

Seminars/Conferences Attended

2013 Presented a poster at DRILS, University of Hyderabad Campus, India

2014 Attended at the **X-JNOST** 2014 international conference, IIT, Madras, India

2015 Presented a poster at "Trend Setting Innovations in Chemical Sciences & Technology Applications in Pharmaceutical Industry" that was held at JNTUH, Hyderabad, India

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by Naveen Kumar M

STEREOSELECTIVE SYNTHESIS OF SUB-STRUCTURES

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