A Divergent Approach to Tetrahydroquinoline-Based Macrocycles and Rapamycin Fragment-Derived Hybrid Molecules

A Thesis
Submitted for the Degree of
DOCTOR OF PHILOSOPHY
In Chemistry

By

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Dedicated to

My Parents

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Declaration

I, hereby, declare that the matter embodied in the thesis is the result of investigation carried out by me at the Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad, India, under the supervision of **Professor Prabhat Arya**.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators. Any omission, which might have occurred by oversight or error, is regretted.

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
October 2014

Shiva Krishna Reddy Guduru

Certificate

This is to certify that the thesis entitled ***"A Divergent Approach to Tetrahydroquinoline-Based Macrocycles and Rapamycin Fragment-Derived Hybrid Molecules"*** being submitted by ***Mr. Shiva Krishna Reddy Guduru*** to 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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Usha, ***Vasavi***, ***Prathyusha***, ***Swathi***, ***Ravi***, ***Shuka***, ***Ashok***, ***Laxmikanth***, ***Mogilanna***, ***Ravinayak, Narender***, ***Sreenu***, ***Ramesh***, ***Waheed***, ***Jakeer, Rajashekar Reddy, Guddeti Chandrashekar Reddy, Jagadeesh and Sumantha***.

My family (dad- ***Pulla Reddy***, mom- ***Chandrakala***, brothers- ***Rama Krishna Reddy*** and ***Hari Krishna Reddy***, sister-in-law- ***Madhumita***) has been a great anchor in my life. They brought me up to become the person I am, and they have done a fantastic job. Although unfortunately we do not get to see each other very often, I always know that their unconditional love, support, and prayers always help me get through even my toughest times. My life is also enriched with my cousins, namely ***Kishore***, ***Praveen***, ***Kiran***, ***Shekar***, ***Jyothi***, ***Babu***, ***Ajay***, ***Giridhar***, ***Lohitha***, ***Padma***, ***Hemanth***, ***Hanmanth Reddy***, ***Keerthana***, ***Abhi***, ***Chintu*** and ***Chinni***; and, niece- ***Sonu***, ***Siri***, ***Laddu*** nephew- ***Yashu, Sidhu*** and my ***Uncles*** and ***Aunts***. I would like to express a special gratitude to my aunt- ***Mukthamma***, and uncle- ***Sai Reddy***.

I thank the Almighty for giving me the strength and patience to work through all these years so that today I can stand proud with my head held high.

Dr. Reddy's Institute of Life Sciences ***Shiva Krishna Reddy Guduru***

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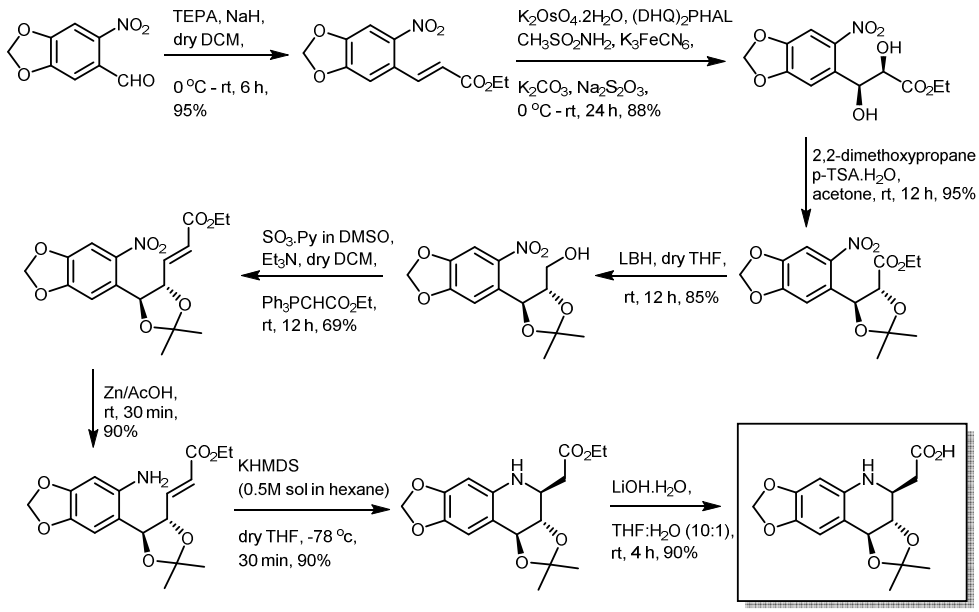
Synopsis

This thesis entitled**, "A Divergent Approach to Tetrahydroquinoline-Based Macrocycles and Rapamycin Fragment-Derived Hybrid Molecules"** contains four chapters

Chapter 1: Synthesis of Enantioenriched Tetrahydroquinoline Scaffold

(*J. Comb. Chem.* **2004**, *6,* 54-64), (*ACS Med. Chem. Lett.* **2013**, *4,* 666-670)

Tetrahydroquinoline is a privileged scaffold, widely found in bioactive natural products. Many synthetic tetrahydroquinolines are already used or have been tested as potential drugs. Due to importance of this scaffold bring our attention towards to synthesis of tetrahydroquinoline scaffold. We have developed a novel method to synthesis of *enantioenriched* tetrahydroquinoline scaffold. The presence of a β-amino acid functionality and three contiguous chiral functcional groups are the two attractive features of this scaffold. Our synthesis is practical and enantioselective in nature and allows us to access this scaffold in sufficient quantities in a short period that utilized a stereoselective aza-Michael reaction as the key step. The synthestic steps are shown in **Scheme 1**.



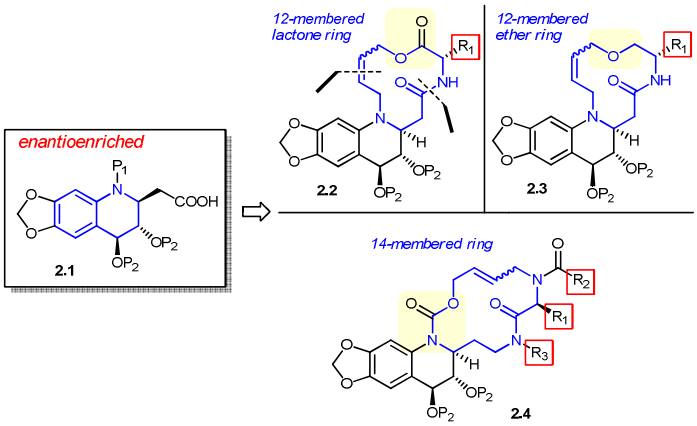
Scheme 1: Synthesis of *Enantioenriched* Tetrahydroquinoline Scaffold

Chapter 2: Synthesis of Natural Product-inspired Tetrahydroquinoline Macrocyclic Toolbox

(*ACS Med. Chem. Lett.* **2013**, *4*, 666-670)

Macrocyclic natural products are proven to exhibit remarkable biological responses when it comes to modulating protein-protein interactions (PPIs) through small molecules. There are several reasons associated with macrocyclic natural product derivatives, and these include: (i) an ability to map a large surface area, (ii) numerous binding interaction options, (iii) enhanced cell permeation properties when compared to their liner derivatives, and (iv) dynamic pre-organized structures to display various functional groups. Despite all these benefits that are associated with bioactive macrocyclic natural products, we have not seen a significant growth in building a chemical toolbox having diverse sets of different types of macrocyclic shapes available to explore their biological value. Due to the inherited challenges associated with complex bioactive natural products (i.e. macrocyclic or non-macrocyclic compounds), an interest in building a chemical toolbox having small molecules that are obtained by inspirational approaches is also rising.

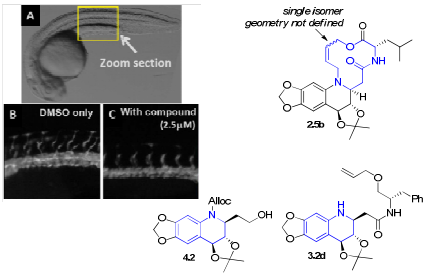
With this objective, we were interested in developing a modular synthesis method to access natural product-inspired tetrahydroquinoline macrocyclic toolbox by utilizing above enantioenriched tetrahydroquinoline scaffold (**Scheme 2**).



Scheme 2: Incorporation of Different Macrocyclic Rings onto an *Enantioenriched* Tetrahydroquinoline Scaffold

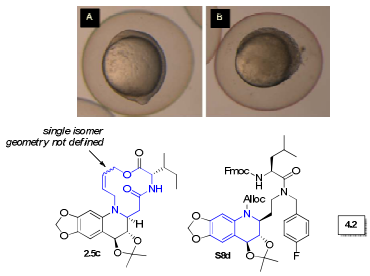
There are two main objectives in our design strategy, first, is to retain the functionalized privileged sub-structure, i.e. tetrahydroquinoline, and, second, is to map the macrocyclic chemical space with the additional functional groups. For example, target **2.2** has the additional 12-membered ring with an incorporation of an amino acid moiety in the skeleton. The ring closing metathesis reaction was the stitching technology to obtain the macrocyclic rings. Using a similar approach, one can also obtain compound **2.3** with a 12-membered ring having the connectivity through the ether linkage. In addition to these two compounds, we also plan to incorporate 14-membered macrocyclic ring **2.4** onto the tetrahydroquinoline scaffold having an amino acid moiety in the ring skeleton. Overall, our approach to building different types of large ring skeletons onto the tetrahydroquinoline scaffolds provides an excellent opportunity to accessing a chemical toolbox with a diverse set of functionalized large ring-based derivatives.

Figure 1: Zebrafish Screen for angiogenesis[a]



[a](A) zoom section of wild-type or vehicle treated embryo, and (B and C) zoom sections after treatment with three compounds. One macrocyclic derivative (2.5b) and two tetrahydroquinoline-based compounds (3.2d and 4.2) showed complete inhibition at 2.5μM.

Figure 2: Zebrafish Screen for an early embryo development[a]



[a](A) DMSO exposed embryos at 10 hpf of development, (B) small molecule exposed embryos causing a delay in epiboly. One macrocyclic derivative (2.5c), and two tetrahydroquinoline-based compounds (S8d and 4.2) exhibited the complete inhibition of an early embryo development at 2.5μM.

Having the chemical toolbox available to explore its biological value, we then decided to search for functional small molecules in three zebrafish screens and these are: (i) angiogenesis, (ii) an early embryonic development. Of all the compounds tested from this toolbox (60 compounds in total), we identified three compounds **(2.5b, 3.2d** and **4.2)** that exhibited the inhibition of angiogenesis at 2.5 μM. These results are shown in **Figure 1**. In another zebrafish screen to search for functional small molecules affecting an early embryonic development (see, **Figure 2**), we identified three compounds **(2.5c, S8d** and **4.2)** that inhibited at 2.5 μM. It is interesting to note that the functional macrocyclic compounds (**2.5b** and **2.5c**) in both assays are structurally-related. It would be excellent to find the exact mechanism of action of these compounds and to determine if there is any common mode of action in these two phenotype experiments.

A Few Key References:

(1) (a) Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Discov.* **2004**, *3*, 301. (b) Wells, J. A.; McClendon, C. L. *Nature* **2007**, *450*, 1001.

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Chapter 3: Synhtesis and Chemical Biology of Rapamycin and Rapamycin-Derived Hybrid Molecules.

This chapter deals with the detailed literature survey on rapamycin and rapamycin-derived hybrid molecules and their biological importance.

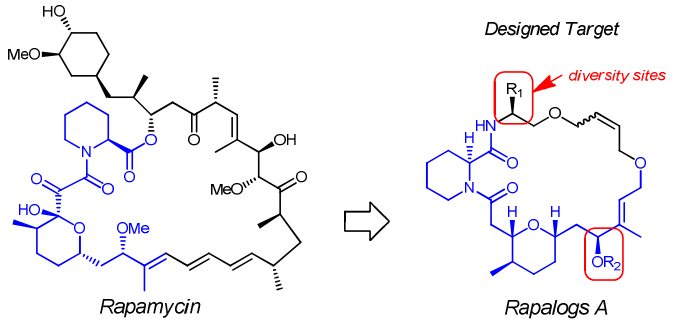
Chapter 4: Synthesis of Rapamycin Fragment-Derived Hybrid Molecules

(Manuscript under preparation)

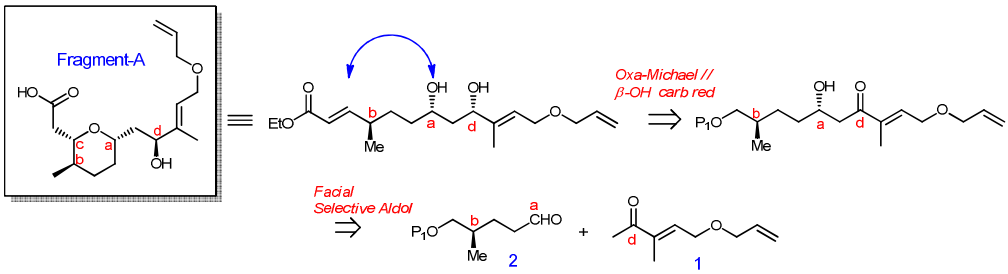
Due to remarkable biological properties of rapamycin and rapamycin-derived hybrid molecules, these small molecules are brought to our attention towards to synthesis of new class of rapamycin-derived hybrid natural products. Designed target molecules are shown in **Scheme 3**.

Our aim is to develop a novel convergent and stereoselective method to construct Fragment-A, and, by utilizing this key fragment, we plan to develop a synthesis route to access rapamycin-derived hybrid macrocycles. Retrosynthetic plan to access fragment-A is shown in **Scheme 4**. The key reactions involved are: (i)

facial selective aldol, (ii) stereoselctive β-hydroxy carbonyl reduction, and, (iii) oxa-Michael addition.

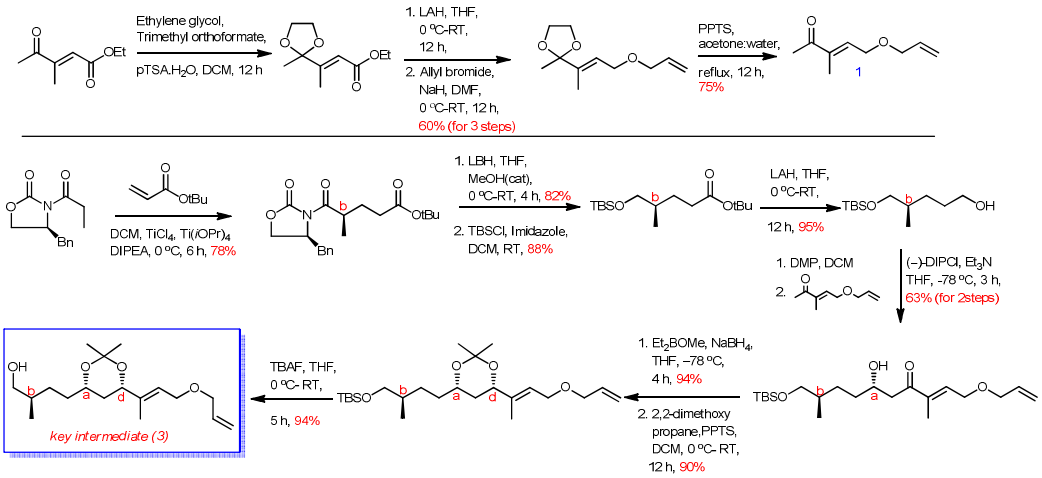


Scheme 3: Rapamycin-Derived Hybrid Molecules (Rapalogs A)



Scheme 4: Retrosynthesis of Fragment A

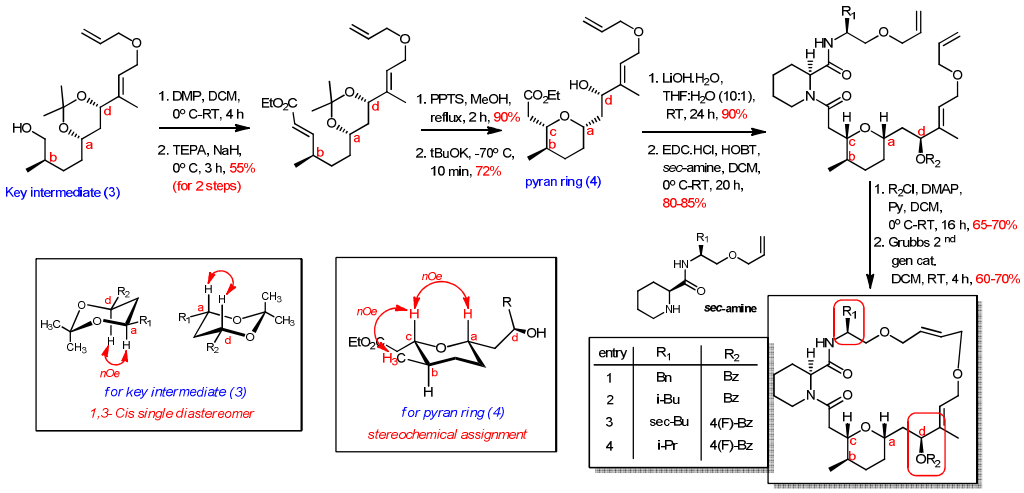
The synthetic plan of the keto fragment (**1**) and key intermediate (**3**) is shown in **Scheme 5**. For the keto fragment (**1**) we started with (E)-ethyl 3-methyl-4-oxopent-2-enoate and for key intermediate (**3**) started with Evans' chiral auxiliary.



Scheme 5: Synthesis of Keto Fragment (1) and Key Intermediate (3)

By utilizing the key intermediate (**3**), we achieved the synthesis of the pyran skeleton (**4**), and, the stereochemical assignment was thoroughly carried-out by

2D-NOESY experiments. Having this pyran ring moiety in hand, it was further modified to obtain the ring closing metathesis precursor that after the reaction produced 22-memberd macrocyclic rapamycin-derived hybrid molecules (Rapalogs-A) as shown in **Scheme 6**.



Scheme 5: Synthesis of Rapalogs-A and Assignments of Key Intermediate (3) and Pyran Ring (4)

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(1) (a) Sehgal, S. N.; Baker, H.; Vezina, C. *J. Antibiotics* **1975**, *28*, 727. (b) Vezina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiotics* **1975**, *28*, 721.

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Abbreviations

AA	:	Amino acid
Ac	:	Acetyl
Ac_2O	:	Acetic anhydride
AcOH	:	Acetic acid
aq.	:	Aqueous
Ar	:	Aryl
$BF_3 \cdot OEt_2$	:	Borontrifluoride-etherate complex
BnBr	:	Benzyl bromide
$(Boc)_2O$	:	Di-*tert*-butyldicarbonate
n-BuLi	:	*n*-Butyllithium
Cbz	:	Benzyloxy carbonyl
$CDCl_3$	:	Chloroform-d
CH_3CN or MeCN	:	Acetonitrile
CuCl	:	Copper(I) chloride
AllocCl	:	Allyl chloroformate
DBU	:	1,8-Diazabicycloundec-7-ene
DCM	:	Dichloromethane
DEAD	:	Diethyl azodicarboxylate
DIPEA or *i*-Pr_2NEt	:	*N*, *N'*-Diisopropylethylamine
DIPCl	:	B-Chlorodiisopinocamphenylborane
DIBAL-H	:	Diisobutyl aluminium hydride
DMF	:	*N, N*'-Dimethylformamide
DMAP	:	4-(Dimethylamino)pyridine
DMP	:	Dess-Martin periodinane
DMSO	:	Dimethyl sulfoxide
EDC.HCl	:	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
Et_3N or TEA	:	Triethylamine
Et_2O	:	Diethyl ether
EtOAc	:	Ethyl acetate
EtOH	:	Ethanol
Et_2BOMe	:	Diethylmethoxyborane

FmocCl	:	Fluorenylmethyloxycarbonyl chloride
FKBPs	:	FK506 binding proteins
G-II	:	Grubb's 2nd generation catalyst
GI_{50}	:	Growth inhibition of 50%
h	:	Hour(s)
H_2O_2	:	Hydrogen peroxide
HBTU	:	*O*-(Benztriazole-1yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HOBT	:	Hydroxy benzotriazole
HPLC	:	High-performance liquid chromatography
MHz	:	Mega hertz
I_2	:	Molecular iodine
IC_{50}	:	Half maximal inhibitory concentration
$InCl_3$	:	Indium(III) chloride
IR	:	Infrared
KBr	:	Potassium bromide
K_2CO_3	:	Potassium carbonate
kDa	:	Kilodalton
KHMDS	:	Potassium bis(trimethylsilyl)amide
K_2PtCl_4	:	Potassium tetrachloroplatinate(II)
KO^tBu or KTB	:	Potassium *tert*-butoxide
LAH or $LiAlH_4$	:	Lithium aluminiumhydride
LBH	:	Lithium borohydride
$LiOH.H_2O$	:	Lithium hydroxide monohydrate
Me	:	Methyl
MeOH	:	Methanol
MS	:	Mass spectrometry
MsCl	:	Methanesulfonyl chloride
mTOR	:	Mammalian target of rapamycin
mTORC1	:	mTOR complex 1
mTORC2	:	mTOR complex 2
$NaBH_4$	:	Sodium borohydride
$NaCNBH_3$	:	Sodium cyanoborohydride
NaH	:	Sodium hydride

$NaHCO_3$	:	Sodium bicarbonate
NaOH	:	Sodium hydroxide
Na_2SO_4	:	Sodium sulfate
NH_4Cl	:	Ammonium chloride
NMR	:	Nuclear Magnetic Resonance
NOESY	:	Nuclear Overhauser Effect Spectroscopy
$Pd(OH)_2$	:	Palladium hydroxide
$Pd(dba)_2$	:	Bis(dibenzylideneacetone)palladium(0)
$Pd(PPh_3)_4$	:	Tetrakis(triphenylphosphine)palladium(0)
$Pd(PPh_3)_2Cl_2$	:	Bis(triphenylphosphine)palladium(II) dichloride
Ph	:	Phenyl
PhCOOH	:	Bezoic acid
Ph_3PCHCO_2Et	:	Carbethoxymethylene triphenyl phosphorane
PI3K	:	Phosphoinositide3-kinase
PPI	:	Protein-protein interaction
PPTS	:	Pyridinium p-toluenesulfonate
PTSA or *p*-TsOH	:	*p*-Toluenesulfonic acid
Py	:	Pyridine
RT (or) rt	:	Room temperature
$[Rh(OH)(COD)]_2$	:	Hydroxy(cyclooctadiene)rhodium(I) dimer
SO_3.Py	:	Sulfur trioxide pyridine complex
TBAF	:	tetra-*n*-Butylammonium fluoride
TBAI	:	Tetrabutylammonium iodide
TBDMS or TBS	:	*tert*-Butyldimethylsilyl
TBDPS	:	*tert*-Butyldiphenylsilyl
TFA	:	Trifuoroacetic acid
THF	:	Tetrahydrofuran
$TiCl_4$	:	Titanium tetrachloride
TMEDA	:	Tetramethylethylenediamine
$Ti(O\text{-}i\text{-}Pr)_4$	:	Titanium(IV) isopropoxide
TPP or PPh_3	:	Triphenylphosphine
TsCl	:	*p*-Toluenesulfonyl chloride
UV	:	Ultra violet
Zn	:	Zinc

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 LCMS were recorded using electron impact, chemical ionisation or electrospray ionisation 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 aluminium 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 of *Enantioenriched* Tetrahydroquinoline Scaffold

1.1. Natural Products as Modulators of Protein-Protein Interactions (PPI):

Over the past several years, it has been shown that natural products are capable of acting as modulators (i.e. activators or inhibitors) of protein-protein interactions (PPI).[1] Natural products are highly complex and possess three-dimensional architectures with several chiral centres, and, a diverse range of protein-binding elements. Here in **Figure 1**, we have shown three bioactive natural products that are known to interfere with protein-protein interactions.

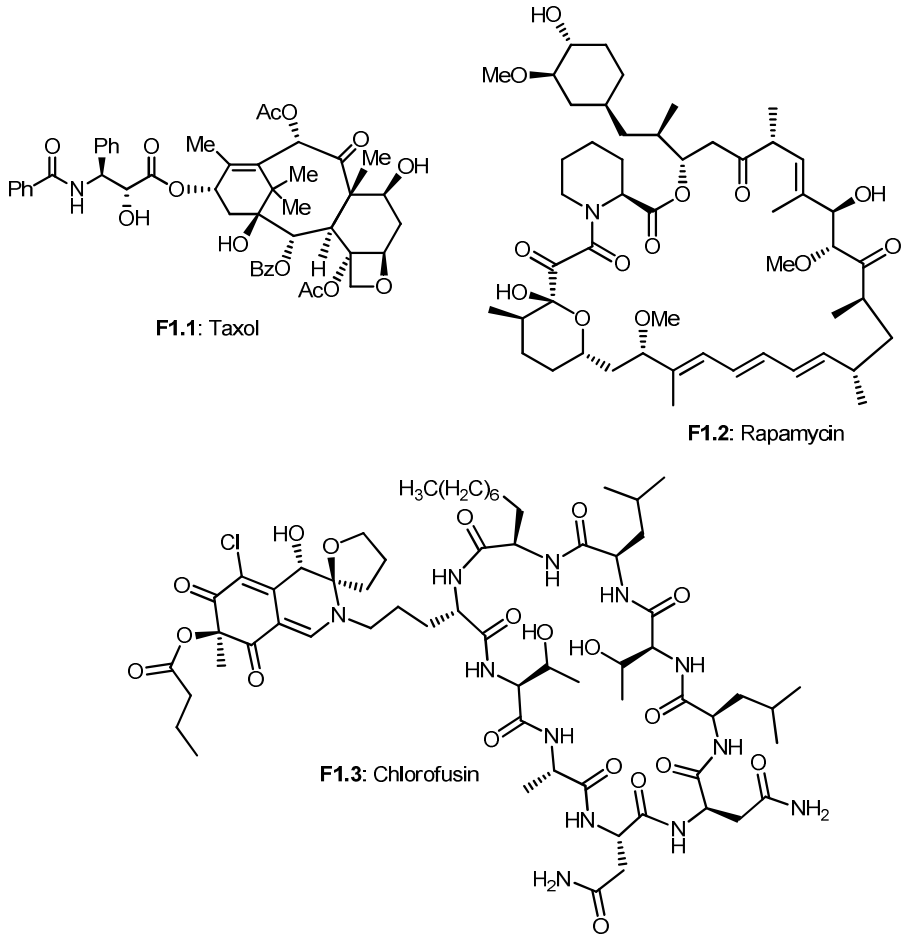


Figure 1: Bioactive Natural Products

Taxol (**F1.1**) and its analogues are known to bind to the β-subunit of the tubulin heterodimer, thus, stabilizing the heterodimer. These interactions enhance the polymerization of tubulin into microtubules, and, promote an arrest in the cell cycle, resulting in programmed cell death.[2] In recent years, several other natural products (i.e. epothilones[3], discodermolide[4] and laulimalide[5] - structures are not

shown) have been identified that also promote the stabilization of microtubules. As with taxol, these natural products also serve as interesting lead compounds in developing novel antitumour agents.

Rapamycin (**F1.2**) interacts with a protein known as FKBP12, and, this small molecule-protein complex can associate with FRAP, a critical downstream signalling component of the PI3K/Akt pathway. Hence, rapamycin behaves as a chemical inducer of dimerization (CID). Rapamycin and its derivatives have also served as useful probes as modulators of protein–protein interactions and, therefore, as a tool in understanding pathways that are involved in these interactions.[6]

Chlorofusin (**F1.3**), a novel fungal metabolite, was identified as an inhibitor of p53 binding to MDM2 during a screen of 5300 microbial extracts.[7] The p53 tumour suppressor protein is a short-lived protein due to its rapid proteasomal degradation. Upon exposure of cells to various stress stimuli, the levels of p53 rise as a consequence of reduced proteolytic activity. This results in induction of a program of gene expression that leads to the arrest of the cell growth and/or apoptosis.[8] MDM2 is an important regulator of the stability of p53, as it directly interacts with p53, and, promotes its ubiquitination and neddylation. Hence, a key function of MDM2 is to inhibit the growth-suppressing effects of p53. The co-crystal structure of p53 and MDM2 has shown that there is a hydrophobic cleft in MDM2 that acts as a 'hot spot' for the MDM2–p53 interaction, and, that targeting this site by a small-molecule intervention might be feasible.[9] Tumors often express elevated levels of MDM2. Thus, it was envisioned that tumor cells with high levels of MDM2 could be treated with a small molecule to interfere with p53–MDM2 interactions, and, this allow the cells to enter cell-cycle arrest or to be eliminated by apoptosis. Although complex in nature, chlorofusin was identified as an antagonist of MDM2 and is a promising lead compound.

The examples discussed above clearly indicate that natural products can interfere with biomacromolecular (for example, protein-protein) interactions. These features appear to be required when it comes to dissecting protein functions with the help of small molecules. Small-molecule chemical probes have tremendous potential to function in a highly selective and reversible manner on proteins.

Therefore, it is not surprising that an interest in developing new chemical approaches for creating architecturally complex compounds, leading to a wide variety of natural product-like compounds which could be helpful in understanding PPI-based cellular signalling pathways is growing. With this aim, our group embarked a program, to develop a method for the synthesis of natural product-inspired *enantioenriched* tetrahydroquinoline scaffold.

1.2. Tetrahydroquinoline Scaffold in Nature:

Tetrahydroquinoline is a privileged scaffold, and, is widely found in bioactive natural products.[10] Few bioactive natural products with the "tetrahydroquinoline" moiety as a core structure are shown in **Figure 2**.

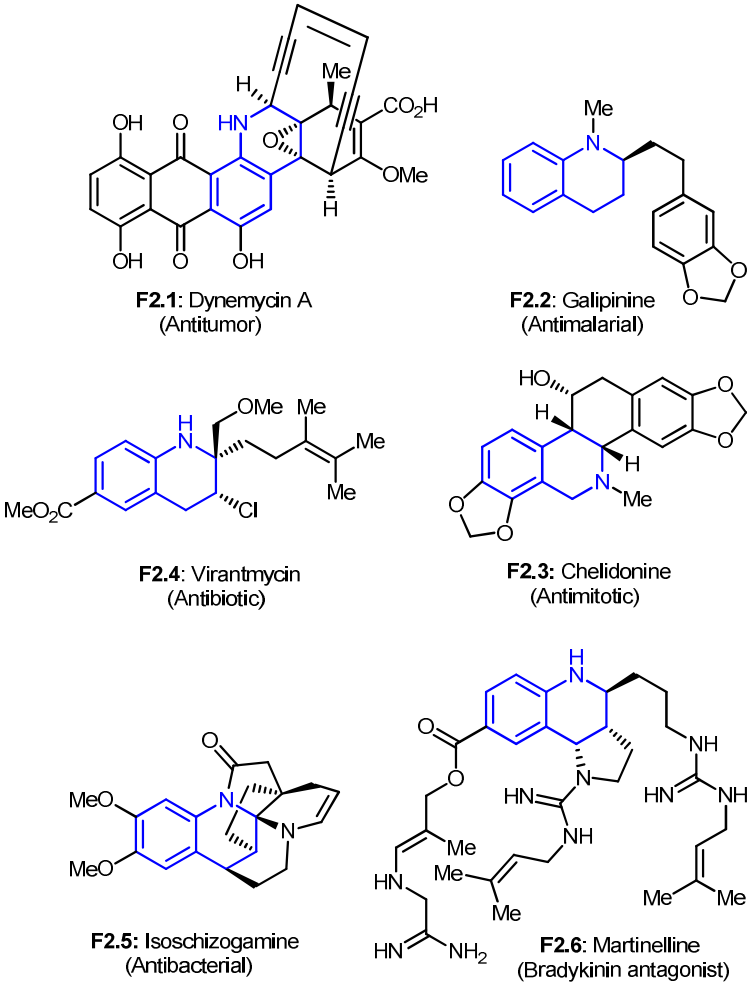


Figure 2: Tetrahydroquinoline Containing Bioactive Natural Products

Dynemycin A (**F2.1**) isolated from the *Micromonospora chersina* gram positive bacteria, has potent inhibitory activity against a wide range of bacteria and tumor

cell lines.[11] Galipinine (**F2.2**) was isolated from *Galipea officinalis* trunk bark, showed activity against *Plasmodium falciparum*.[12] Chelidonine (**F2.3**) is a tertiary hexahydro-benzophenanthridine alkaloid, isolated from *Chelidonium majus* (Papaveraceae), is a potent inhibitor of tubulin polymerization (IC_{50}= 24 μM).[13] (–)-Virantmycin (**F2.4**) is an unusual chlorinated tetrahydroquinoline that was isolated from a strain of *Streptomyces nitrosporeus*, and, it has shown a strong inhibitory activity against RNA and DNA viruses and antifungal activity.[14]

Isoschizogamine (**F2.5**) was isolated from *Schizozygia caffaeoides,* showed antibacterial activity.[15] Martinelline (**F2.6**) is an isolate of Martinella iquitosensis roots, exhibits activity as a bradykinin antagonist and with α-adrenergic, histaminergic and muscarinic receptors.[16]

1.3. Synthetic Bioactive Compounds Containing Tetrahydroquinoline Core:

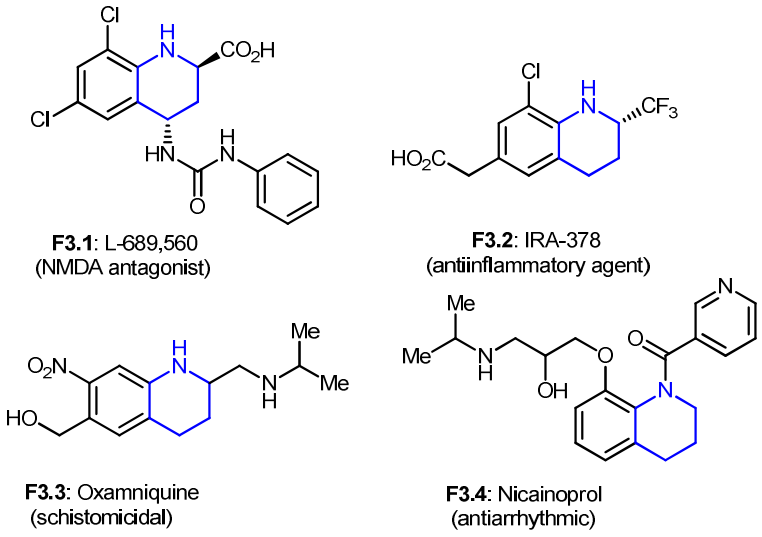


Figure 3: Tetrahydroquinoline Containing Synthetic Bioactive Compounds

Many synthetic tetrahydroquinolines are already used or have been tested as potential drugs. L-689,560 (**F3.1**) is a well known NMDA antagonist.[17] IRA-378 (**F3.2**) showed antirheumatic activity.[18] Oxamniquine (**F3.3**) possesses the schistomicide effect [19] and nicainoprol (**F3.4**) has shown antiarrhythmics property.[20] Hundreds of tetrahydroquinoline derivatives are known to have

interesting bio-chemical activity, and, some are considered as potential pharmaceutical agents from analgesic to anti-depressant.[10]

1.4. Synthesis of 1,2,3,4-Tetrahydroquinolines Involving the Generation of One Bond

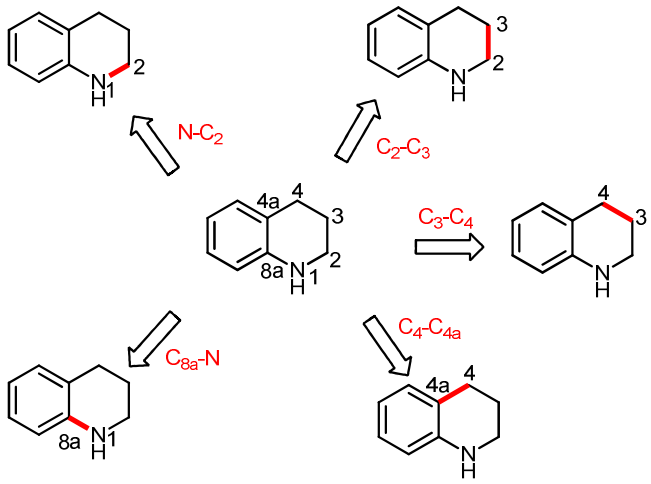


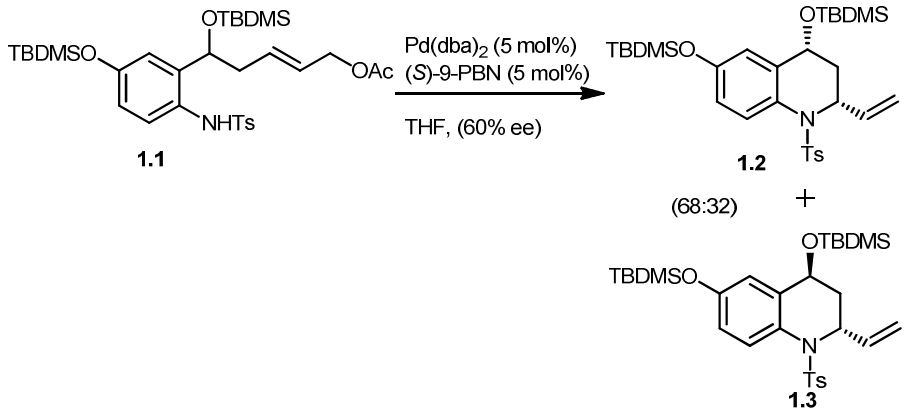
Figure 4: Disconnections of the Tetrahydroquinoline Framework

The 1,2,3,4-tetrahydroquinoline ring can be constructed by creating one new bond starting from precursors containing a benzene ring. In this section, I will discuss the synthetic methodologies involving the generation of one new bond as the key step. I have organized the section according to the numbers of the positions of the new bond in the final product, namely, N-C_2, C_2-C_3, C_3-C_4, C_4-C_{4a}, and C_{8a}-N.

1.4.1. Formation of the N-C_2 Bond:

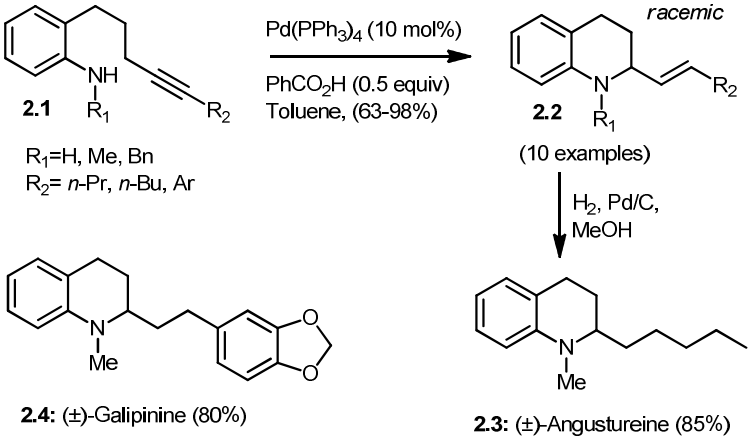
1.4.1a. Intramolecular Allylic Amination:

An intramolecular asymmetric allylic amination of allyl acetate **1.1** in the presence of a chiral phosphine (9-PBN) and $Pd(dba)_2$ afforded the diastereomeric mixture of tetrahydroquinolines **1.2** and **1.3** in moderate yields and enantioselectivity. The same reaction furnished the *racemic cis* product **1.2** in an excellent yield (92%) in a substrate controlled manner with tributylphosphine, which is an achiral phosphine (**Scheme 1**).[21]



Scheme 1: Synthesis of Tetrahydroquinolines via Pd-Catalyzed Intramolecular Asymmetric Allylic Amination Reaction

1.4.1b. Intramolecular Hydroamination:



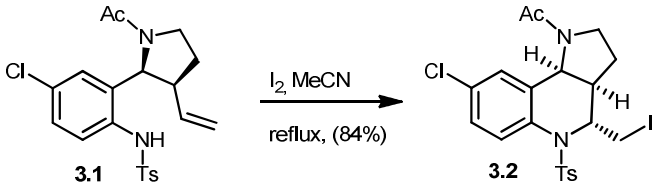
Scheme 2: Synthesis of (±)-Galipinine and (±)-Angustureine via Pd-Catalyzed Intramolecular Hydroamination of Anilinoalkynes

Yamamoto and co-workers reported the synthesis of 2-alkenyl-1,2,3,4-tetrahydroquinolines through an intramolecular hydroamination of anilinoalkynes catalyzed by $Pd(PPh_3)_4$ /PhCOOH. A wide variety of aminoalkynes, having a free amino group or electron-donating group on the *N*-atom, afforded the corresponding tetrahydroquinolines in good to excellent yields. However, the presence of electron-withdrawing groups, such as Boc, Ts, or Nf groups, on nitrogen led to failure of the reaction. The use of (R,R)-2,3-bis(diphenylphosphino)norbornane (RENORPHOS)/ $Pd_2(dba)_3$ catalytic system furnished the products with good enantioselectivities (up to 78% ee), and, the

methodology was subsequently applied to the synthesis of the alkaloids, such as angustureine **2.3** and galipinine **2.4** (**Scheme 4**).[22]

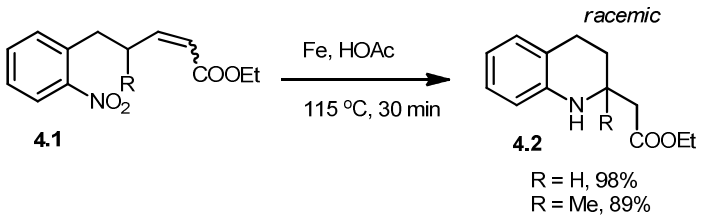
1.4.1c. Intramolecular Aminohalogenation:

Intramolecular aminohalogenation of *O*-allylanilines is of interest because it allows the construction of tetrahydroquinoline ring system bearing a halogen substituent, which could be useful for further functionalization to achieve complex derivatives. Frank and Aupe illustrated a novel intramolecular aminoiodination reaction to construct the pyrroloquinoline ring system during their study toward the synthesis of the tricyclic core of the martinelline natural products. Intermediate **3.1**, synthesized from the corresponding 2-aminoarylaldehyde in few steps, afforded pyrroloquinoline **3.2** diastereoselectively in 84% yield upon treatment with iodine. Similarly the *N*-unprotected analogue of **3.1** gave the aminoiodination product in moderate yield (**Scheme 7**).[23]



Scheme 3: Construction of the Martinelline Core Via Intramolecular Aminoiodination Reaction

1.4.1d. Intramolecular Aza-Michael Addition:



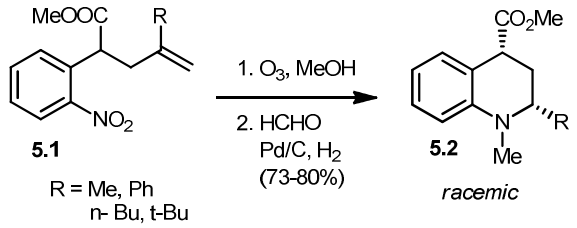
Scheme 4: Synthesis of Tetrahydroquinolines from Nitro Precursors

A tandem reduction-Michael addition reaction sequence was developed for the synthesis of tetrahydroquinoline-2-acetic esters. The 2-nitro-substituted substrates **4.1** reacted with 6 equivalents of iron powder in glacial acetic acid to afford

tetrahydroquinoline derivatives **4.2** in high yields through a reductive cyclization (**scheme 4**).[24]

1.4.1e. Reduction-Intramolecular Cyclization Sequences:

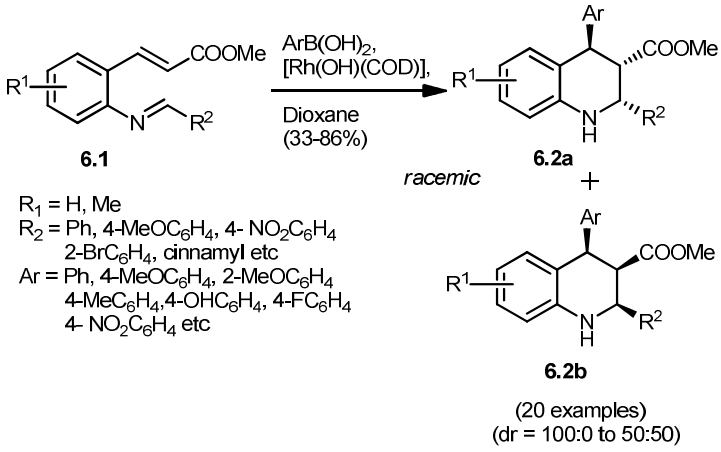
Bunce and co-workers also developed a tandem reduction-reductive amination procedure for the construction of tetrahydroquinolines from 2-nitroaryl compounds bearing a carbonyl group in the side chain.[25] The starting compounds **5.1** were simply prepared from methyl(2-nitrophenyl)acetate by alkylation with allyl halides. A one-pot ozonolysis-reduction-reductive amination reaction series provided the *N*-methyl-2-substituted-1,2,3,4-tetrahydroquinoline-4-carboxylic esters **5.2** in good yields (**Scheme 5**).



Scheme 5: Tandem Reduction-Reductive Amination Sequence Developed by Bunce and Co-workers

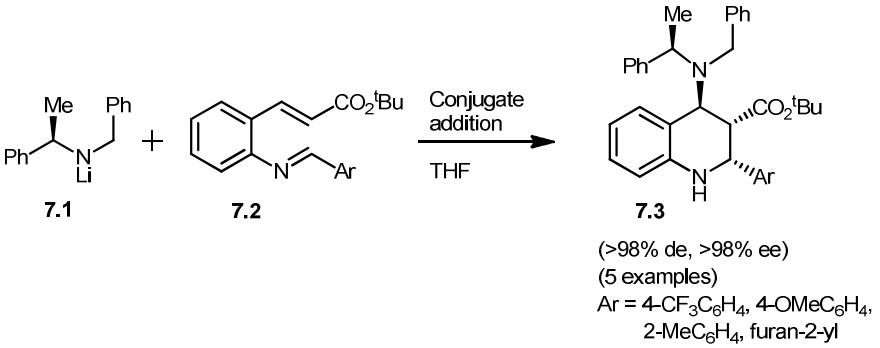
1.4.2. Formation of the C_2-C_3 Bond:

1.4.2a. Conjugate Addition-Cyclization Sequence:



Scheme 6: Rh-catalyzed Domino Conjugate Addition Mannich-Cyclization Sequence

The next method that involves the creation of the C2-C3 bond is an intramolecular cyclization of imine-substituted electron-deficient alkenes. Youn and co-workers demonstrated a rhodium-catalyzed domino conjugate addition-Mannich cyclization sequence of compound **6.1** for the synthesis of 2,3,4-trisubstituted-1,2,3,4-tetrahydroquinolines **6.2**. The starting materials reacted with arylboronic acids in the presence of $[Rh(OH)(COD)]_2$ to give diastereomeric mixtures of the tetrahydroquinolines in good yields (**Scheme 6**).[26]



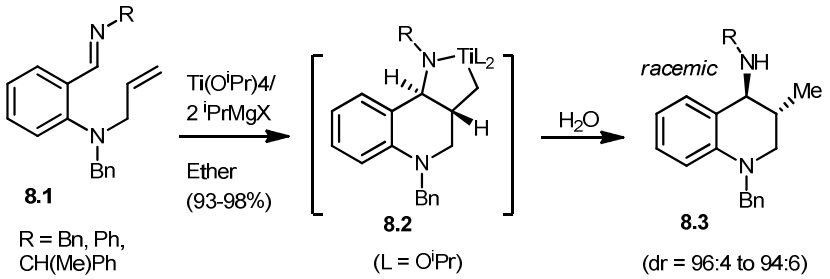
Scheme 7: Tandem Conjugate Addition-Cyclization Sequence

This method involves the tandem conjugate addition-cyclization of lithium (R)-*N*-benzyl-*N*-(R-methylbenzyl)amide **7.1** to aromatic imines, **7.2**, bearing an electron-deficient alkene substituent at the ortho position to give 2-aryl-4-aminotetrahydroquinoline-3-carboxylic acid derivatives **7.3** in excellent diastereo- and enantioselectivities (**Scheme 7**).[27]

1.4.3. Formation of the C_3-C_4 Bond:

1.4.3a. Intramolecular Cyclization of Ene-C=N Moiety:

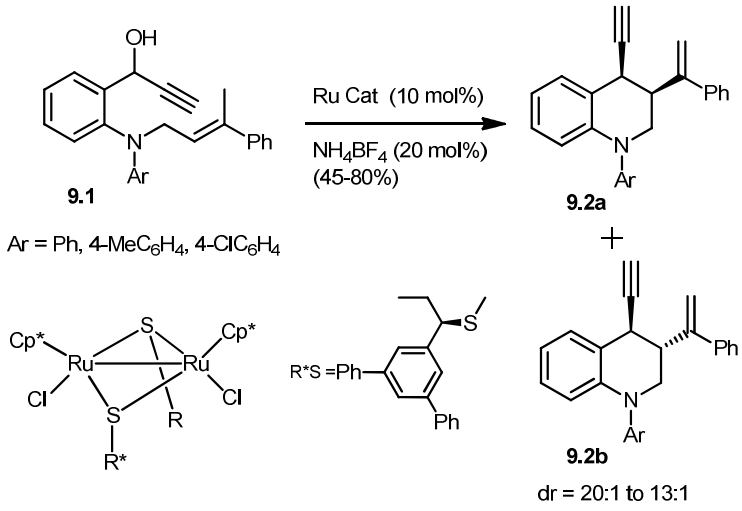
Okamoto and co-workers reported a novel synthesis of 1,2,3,4-tetrahydroquinoline derivatives through a cyclization of ω-vinylimines with Ti(O-i-Pr)$_4$/i-PrMgX.[28] Treatment of the eneimines **8.1** with the titanium reagent followed by addition of water afforded the 3,4-disubstituted tetrahydroquinolines **8.3** through intermediate **8.2** in excellent yields and diastereoselectivities (**Scheme 8**). The reaction allowed the introduction of other substituents, such as hydroxyl and iodo groups at the C-3 methyl group by the addition of oxygen or iodine, respectively, after treatment with the titanium reagent.



Scheme 8: Cyclization of ω-Vinylimines with Ti(O-i-Pr)$_4$/ i-PrMgX

1.4.3b. Miscellaneous Reactions:

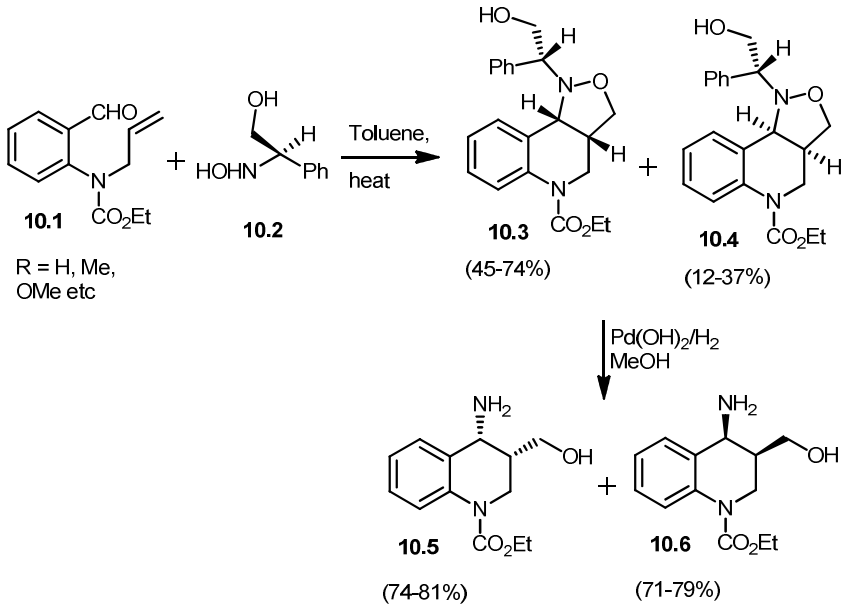
Nishibayashi and co-workers illustrated a ruthenium catalyzed enantioselective synthesis of chromane, thiochromane, and tetrahydroquinoline derivatives. For instance, the reactions of propargylic alcohols, **9.1**, bearing an allylic amine moiety with 10 mol% of an optically active thiolate-bridged diruthenium complex and 20 mol% of NH_4BF_4 furnished the corresponding tetrahydroquinolines **9.2** in good yields and excellent enantioselectivities (**Scheme 9**).[29]



Scheme 9: Ru-Catalyzed Enantioselective Synthesis of Tetrahydroquinolines

The enantioselective synthesis of 4-amino-3-hydroxymethyl-1,2,3,4-tetrahydro-quinolines **10.5** and **10.6** was attained through a strategy based on the intramolecular 1,3-dipolar cycloaddition of nitrones. The procedure involved the synthesis of 2-(*N*-allyl)-aryl aldehydes **10.1** using the Vilsmeier reaction followed by intramolecular cycloaddition of the *N*-allyl group with nitrone moiety, generated in situ from the reaction between the aldehyde and chiral hydroxylamine **10.2**. The final ring opening of the isoxazolidine intermediates

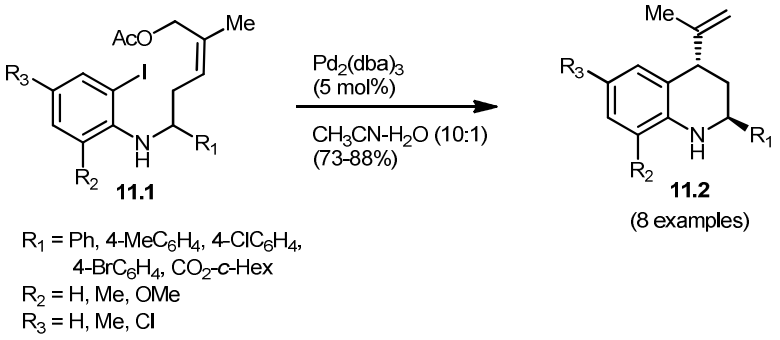
10.3 and **10.4** with $Pd(OH)_2/H_2$ system afforded the final products **10.5** and **10.6** (**Scheme 10**).[30]



Scheme 10: Synthesis of Tetrahydroquinolines via 1,3-Dipolar Cycloaddition of Nitrones

1.4.4. Formation of the C_4-C_{4a} Bond:

1.4.4a. Transition Metal-Catalyzed Reactions:

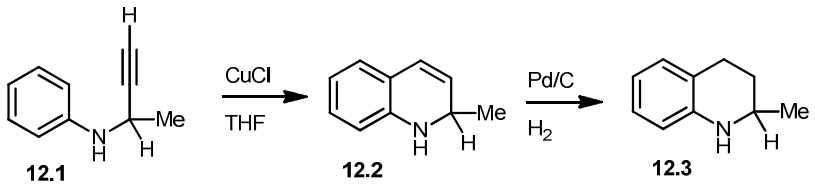


Scheme 11: Pd-catalyzed Synthesis of trans-2,4-Disubstituted Tetrahydroquinolines

A Pd-catalyzed intramolecular coupling between aryl iodides and allyl moieties was explored for the diastereoselective synthesis of 2,4-disubstitited 1,2,3,4-tetrahydro- quinolines. For instance, allyl acetates **11.1** underwent cyclization in the presence of 5 mol% of $Pd_2(dba)_3$ to afford *trans*-tetrahydroquinolines **11.2** in

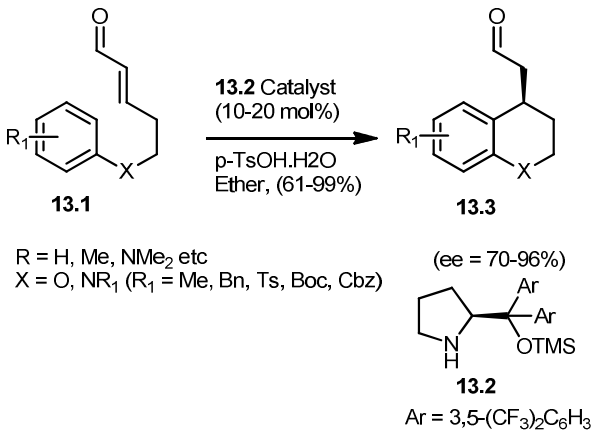
very good yields. The reaction tolerated both electron-donating and electron-withdrawing groups on the aryl ring (**Scheme 11**).[31]

A CuCl-catalyzed intramolecular hydroarylation of *N*-propargyl anilines was described by Hamann and co-workers for the synthesis of intermediates en route to biologically relevant nonsteroidal, peripherally selective tetrahydroquinoline androgen receptor (AR) antagonists.[32] The *N*-propargyl anilines **12.1** reacted with catalytic amount of CuCl in THF under reflux conditions to afford the dihydroquinolines **12.2**, which were subsequently hydrogenated to the corresponding tetrahydroquinolines **12.3**.



Scheme 12: CuCl-Catalyzed Intramolecular Hydroarylation of N-Propargyl Anilines

1.4.4b. Intramolecular Arylation:



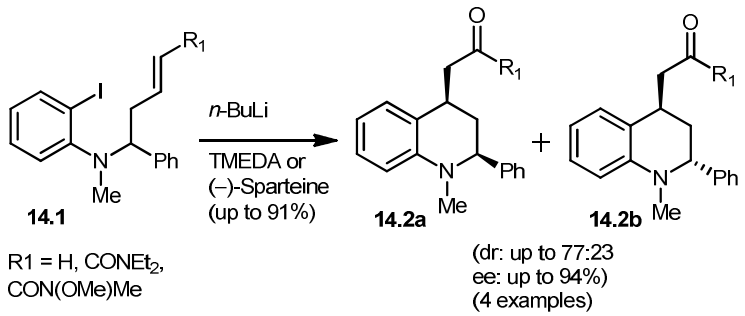
Scheme 13: Organocatalyzed Asymmetric Intramolecular Hydroarylation of **13.1**

The asymmetric intramolecular hydroarylation of electron rich phenol and aniline derivatives bearing an α,β-unsaturated aldehyde moiety afforded chroman and tetrahydroquinoline derivatives in high yields and enantioselectivities in the presence of a chiral organocatalyst.[33] The phenol and aniline-derived enals **13.1**,

in the presence of 10-20 mol % of the catalyst **13.2**, gave cyclized products **13.3** (**Scheme 13**).

1.4.4c. Miscellaneous Reactions:

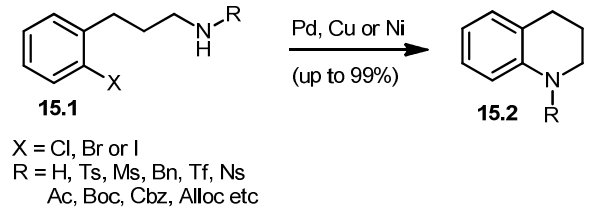
One of the best methods to synthesize functionalized six membered carbo- and heterocyclic systems include the carbolithiation of alkenes and alkynes. Lete and co-workers described the synthesis of 4-substituted-2-phenyltetrahydroquinolines **14.2** as mixtures of two diastereomers from *N*-alkenyl substituted 2-iodoanilines **14.1** using the carbolithiation process (**Scheme 14**).[34]



Scheme 14: Enantioselective Synthesis of Tetrahydroquinolines Involving Carbolithiation Process

1.4.5. Formation of the C_{8a}-N Bond:

1.4.5a. Metal-Catalyzed Intramolecular Amination:



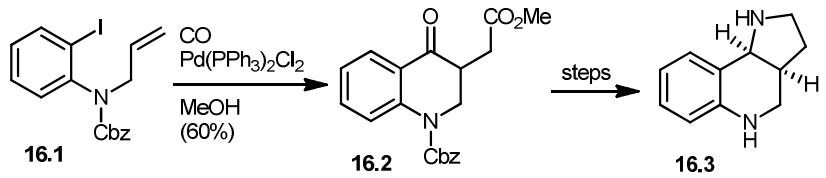
Scheme 15: Metal-Catalyzed Cyclization of N-Substituted Aryl Halides **15.1**

Reagents based on transition metals, including Pd, Cu, Ni, and others, effectively catalyzed intramolecular amination reactions that furnished tetrahydroquinolines by creating the C_{8a}-N bond. In the context of a general report on Pd-catalyzed *N*-arylation reactions in supercritical carbon dioxide, the cyclization of *N*-substituted aryl halide **15.1** was performed to afford the corresponding tetrahydroquinolines **15.2** in moderate to good yields (**Scheme 15**).[35] Other

reagents involved in the cyclization of substrates **15.1** with different N-substituents include CuI/CsOAc,[36] CuI,[37] CuI/Cs_2CO_3,[38] CuOAc/K_3PO_4,[39] and Ni(0).[40]

1.5. Synthesis of 1,2,3,4-Tetrahydroquinolines Involving The Generation of Multiple Bonds:

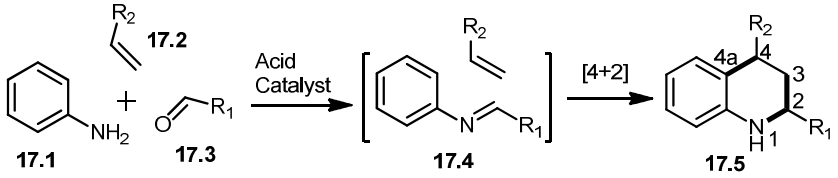
1.5.1. Formation of the C_3-C_4 and C_4-C_{4a} Bonds:



Scheme 16: Pd-Catalyzed Carbonylative Cyclization Reaction

The only available report for the synthesis of tetrahydroquinolin-4-one involving the creation of the C_3-C_4 and C_4-C_{4a} bonds is the palladium-catalyzed carbonylative cyclization of N-allyl-2-iodoaniline.[41] During the enantioselective synthesis of the pyrroloquinoline core of the martinelline alkaloids, Nieman and Ennis synthesized tetrahydroquinolin-4-one **16.2**, a precursor for the synthesis of pyrroloquinoline **16.3**, starting from the Cbz protected *N*-allyl-2-iodoaniline **16.1** and carbon monoxide in the presence of a palladium catalyst in methanol by employing a modified procedure developed by Negishi.[42] The tetrahydroquinolin-4-one **16.2** was subsequently transformed into pyrroloquinoline **16.3** in six additional steps (**Scheme 16**).

1.5.2. Formation of the N-C_2, C_2-C_3, and C_4-C_{4a} Bonds :



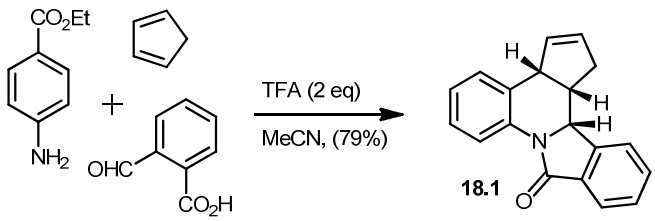
Scheme 17: Povarov Reaction

The acid-catalyzed inverse electron demand formal [4 + 2] cycloaddition reaction between *N*-arylimines and electron-rich dienophiles to give 1,2,3,4-tetrahydro-

quinolines, normally classified among aza-Diels-Alder or imino-Diels-Alder reactions, was developed by the Russian chemist Povarov in 1960s. It is now popularly known as the Povarov reaction.[43] The reaction can also be performed in a three component fashion using the *in situ* generated *N*-arylimines starting from suitable arylamines and aldehydes, and, a dienophile. The three-component Povarov reaction allows the creation of three bonds, that is, N-C_2, C_2-C_3 and C_4-C_{4a} bonds in a single operation. Although the two-component version of the reaction involving the use of isolated *N*-arylimines for the construction of tetrahydroquinolines by creating the C_2-C_3 and C_4-C_{4a} bonds in the key step is also widely used (**Scheme 17**).

1.5.2a. Brønsted Acid-Catalyzed Reactions:

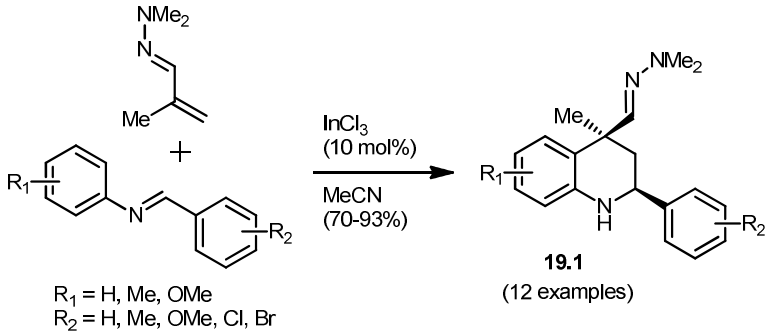
Khadem and co-workers developed a one-pot synthesis of isoindolo[2,1-a]quinoline derivative **18.1** based on a trifluoroacetic acid (TFA)-mediated reaction between ethyl-4-aminobenzoate, 2-carboxybenzaldehyde and cyclopentadiene as a single diastereomer through a Povarov-cyclocondensation domino sequence (**Scheme 18**).[44]



Scheme 18: Khadem's Synthesis of Isoindolo[2,1- a]-quinoline **18.4**

1.5.2b. Vinylogous Povarov Reactions:

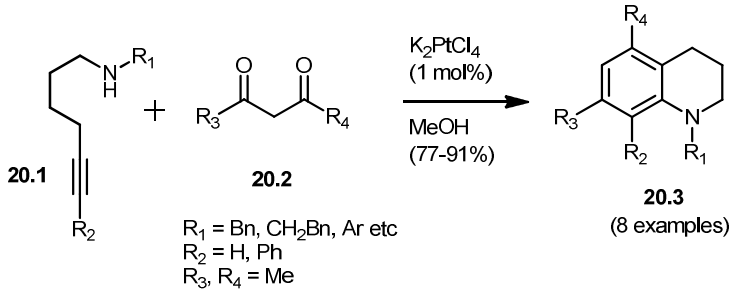
Vinylogous type-I Povarov reaction involves the use of (R)-β-unsaturated hydrazones as the dienophiles.[45] The treatment of a wide variety of *N*-arylimines with methacrolein N,N-dimethylhydrazone in the presence of 10 mol% of $InCl_3$ furnished 1,2,3,4-tetrahydroquinolines **19.1** bearing a hydrazone unit at the C-4 carbon as a single diastereomer in high yields (Scheme **19**). α,β-Unsaturated N,N-dimethylhydrazones are known to have a wide spread use as dienes in aza-Diels-Alder reactions,[46] but this was the first report of their use as dienophiles in this type of chemistry.



Scheme 19: Vinylogous Type-I Povarov Reaction Involving the Use of α,β-Unsaturated Hydrazones as the Dienophiles

1.6. Synthesis of 1,2,3,4-Tetrahydroquinolines Involving The Formation of The Aryl or Both Rings :

Liu and Che demonstrated a novel synthesis of indolines and tetrahydroquinolines starting from aminoalkynes and 1,3-diketones in the presence of a platinum(II) catalyst. A broad variety of 1,5-aminoalkynes **20.1** were treated with 1,3-diketones **20.2** under mild conditions with 1 mol% of K_2PtCl_4 to afford the corresponding tetrahydroquinolines **20.3** in high yields (**Scheme 20**).[47]

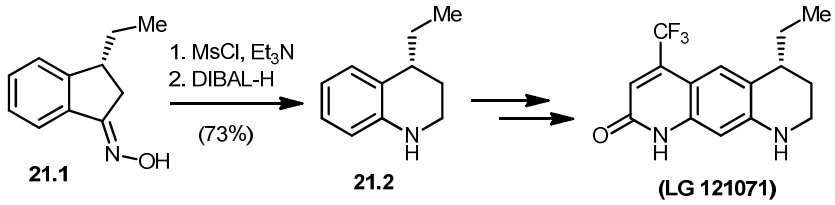


Scheme 20: Pt-Catalyzed Synthesis of Tetrahydroquinolines Developed by Liu and Che

1.7. Synthesis of 1,2,3,4-Tetrahydroquinolines Involving Rearrangement Reactions

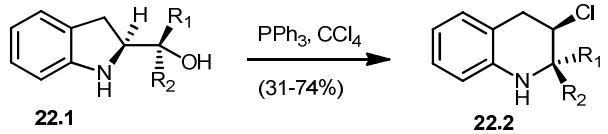
A modified Beckmann rearrangement was employed for the synthesis of chiral (R)-4-ethyl-1,2,3,4-tetrahydroquinoline **21.2**, which is a potential intermediate for the synthesis of the androgen receptor modulator LG 121071. The mesylate of the

enantiopure oxime **21.1**, derived from ethyl 3-phenylpent-2-enoate, was treated with DIBAL-H to afford the chiral tetrahydroquinoline derivative **21.2** in 73% yield (**Scheme 21**).[48]



Scheme 21: Synthesis of Chiral Tetrahydroquinoline **21.2** (Intermediate for **LG 121071**) via Beckmann Rearrangement

Kogen and co-workers illustrated a novel rearrangement of indoline-2-methanol derivatives for the synthesis of 3-chloro-1,2,3,4-tetrahydroquinolines bearing a quaternary C-2 carbon.[49] A broad variety of R,R-disubstituted indoline-2-methanols **22.1** were treated with triphenylphosphine (3 equiv) and CCl_4 (10 equiv) under reflux conditions to afford the corresponding 3-chloro tetrahydroquinolines **22.2** in good yields (**Scheme 22**).



Scheme 22: Rearrangement of Indoline-2-methanol Derivatives to 3-Chloro-1,2,3,4-tetrahydroquinolines

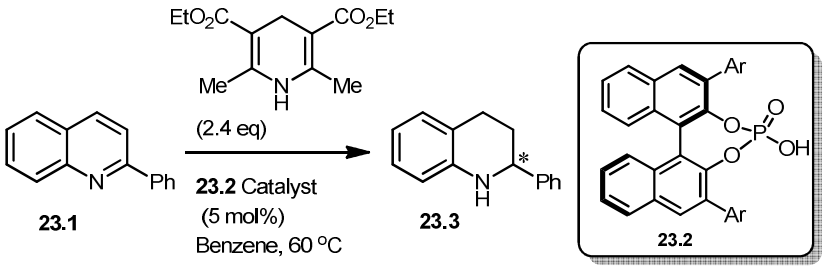
1.8. Asymmetric Hydrogenation of Quinolines:

Asymmetric synthesis remains a challenge to synthetic chemists as the demand for enantiomerically pure compounds continues to increase. Many groups working on chemical synthesis and drug discovery are striving to develop novel methods for asymmetric synthesis that allow the development of new chiral molecules. Asymmetric hydrogenation of quinoline derivatives is the best and easiest way to access an enantiopure 1,2,3,4-tetrahydroquinolines in a single operation. This chemistry was developed in the 2000 decade, mainly by Chinese groups (Zhou, Chan, and others), and, is based mainly on the use of iridium

catalysts, although other metal and Brønsted acid catalysts have also been developed simultaneously.

1.8.1. Brønsted Acid-Catalyzed Asymmetric Hydrogenation of Quinolines:

Chiral Brønsted acids are recently materialized as excellent catalysts for many synthetically important asymmetric transformations.[50] The first chiral Brønsted acid-catalyzed asymmetric transfer hydrogenation process of quinolines was again developed by Rueping.[51] After a systematic catalyst survey, the authors identified the chiral phosphoric acids **23.2** as the ideal choice for the asymmetric hydrogenation as shown in **Scheme 23**.

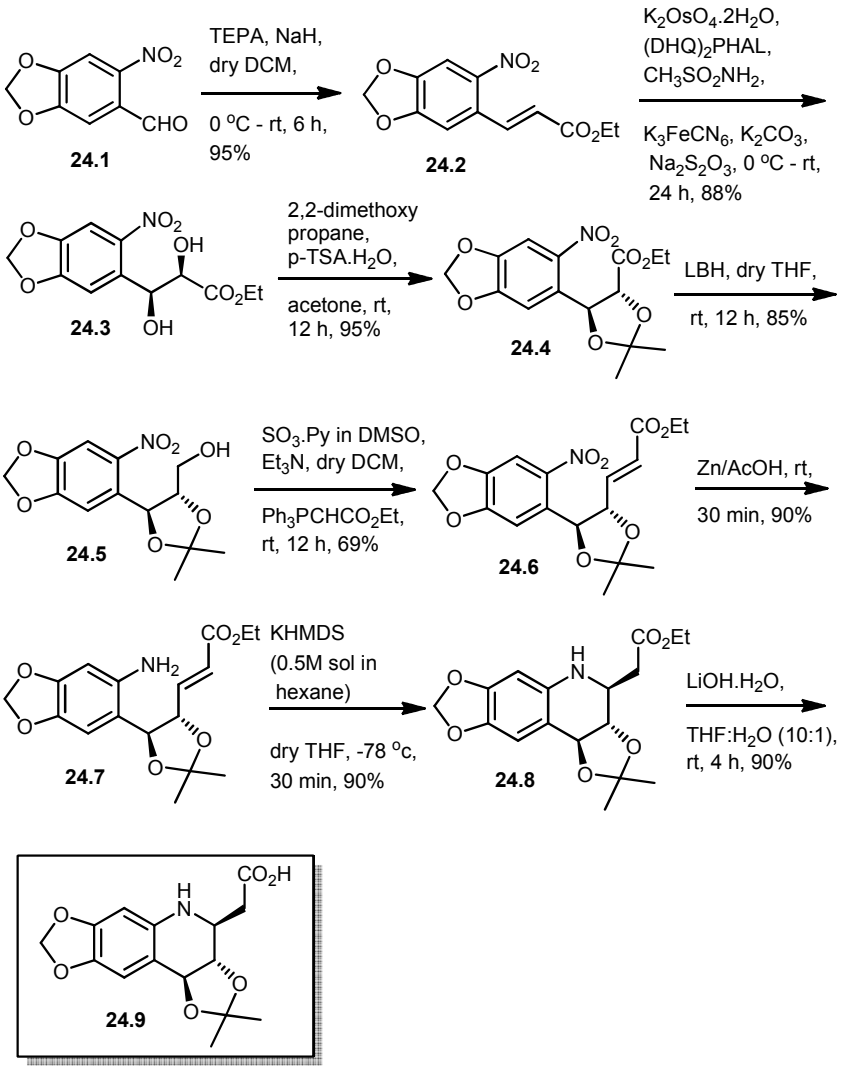


Scheme 23: First Chiral Brønsted Acid-Catalyzed Asymmetric Transfer Hydrogenation of Quinolines

1.9. Contribution from Arya Group to the Synthesis of *Enantioenriched* Tetrahydroquinoline Scaffold:

We have developed a novel method to the synthesis of an *enantioenriched* tetrahydroquinoline scaffold.[52] The presence of a β-amino acid functionality and three contiguous chiral functional groups are the two attractive features of this scaffold. Our synthesis is practical and enantioselective in nature and allows us to access this scaffold in sufficient quantities in a short period that utilized a stereoselective aza-Michael reaction as the key step. The synthesis of enantiopure tetrahydroquinoline β-amino acid **24.9** was carried out as follows.

1.9.1. Results and Discussion:



Scheme 24: Synthesis of Enantioenriched Tetrahydroquinoline Scaffold

Commercially available 6-Nitropiperonal **24.1** was converted to unsaturated carboxyl ester by Horner-Wittig[53] reaction (95%) and then subjected to Sharpless dihydroxylation reaction,[54] giving an enantiopure dihydroxyl derivative **24.3** (88%, >90% ee, determined by chiral HPLC). Following the acetonide protection, the carboxyl ester was then reduced by lithium borohydride to obtain alcohol **24.5**. This alcohol **24.5** was subjected to Parikh-Doering oxidation[55] then Wittig reaction in one pot to afford α,β-unsaturated ethyl ester **24.6** in a good yield. This was then subjected to nitro group reduction and then treatment with LDA or KHMDS to obtain the hetero Michael product, **24.8**, as a single diastereomer

which was subjected to $LiOH \cdot H_2O$ to obtain *enantioenriched* tetrahydroquinoline scaffold **24.9** containing β-amino acid functionality.

The stereochemistry of the new stereogenic center was assigned by nOe (H-2 and H-4). The reaction seems to be independent of the choice of the base and provides an easy access to enantiopure β-amino acid on a large scale. It appears that acetonide protection of vicinal hydroxyls at C_3 and C_4 is an important factor in the asymmetric hetero Michael reaction (see **Figure 5**).

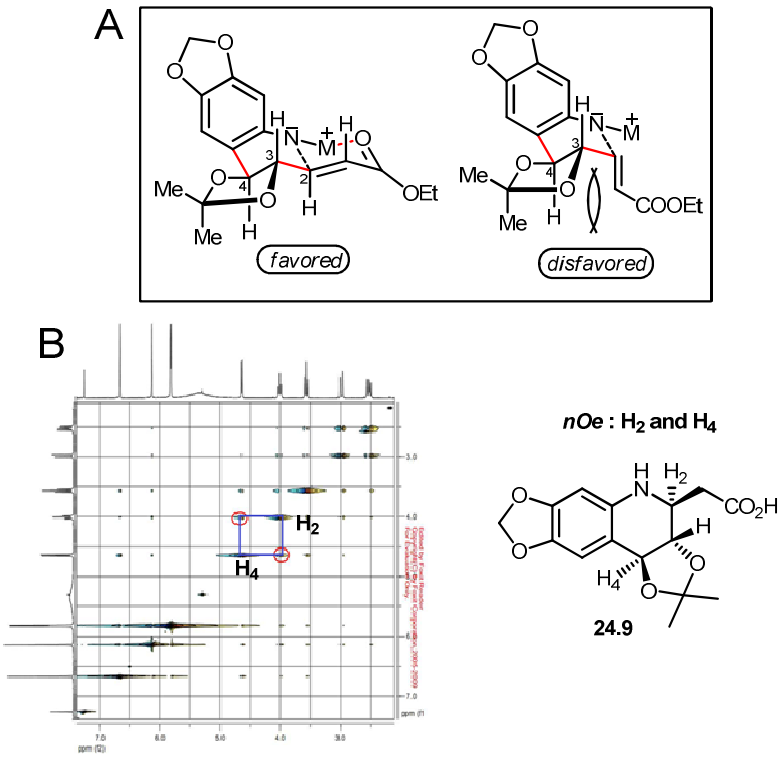
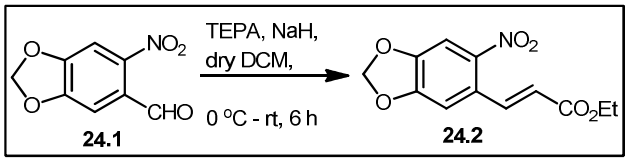


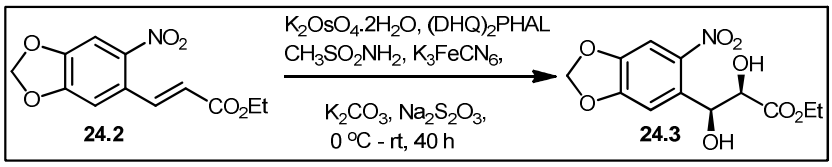
Figure 5: (A) Transition States for Aza-Michael Addition. (B) 2D-NOESY Data for Enantioenriched Tetrahydroquinoline Scaffold **24.9**

1.10. Experimental Procedure for Synthesis of Chiral Scaffold (24.9):



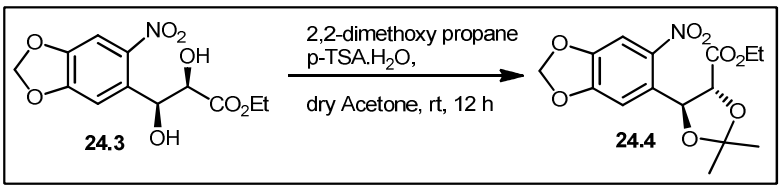
At 0 °C sodium hydride (1.84 g, 76.86 mmol) was added to dry THF (100 mL) followed by addition of triethylphosphonoacetate (7.62 mL, 38.4 mmol) carefully

in nitrogen atmosphere and allowed to stir for 20min. 6-nitropiperonal (**24.1**) (5g, 25.62 mmol) was added to the stirring solution. The reaction mixture was stirred until the starting material disappeared (TLC). The reaction was quenched with saturated NH_4Cl solution and the compound extracted twice with ethylacetate (2×100 mL) which was washed with water and brine. The organic phase was dried over anhydrous Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (3:1, hexane/ethyl acetate). The product **24.2** was obtained as a white solid (2.37 g, 95%). Molecular Name: (E)-ethyl 3-(6-nitrobenzo[d][1,3]dioxol-5-yl)acrylate; Molecular Formula: $C_{12}H_{11}NO_6$; R_f (solvent system): 0.5 (7:3, hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 8.09 (d, *J* = 15.74 Hz, 1H), 7.53 (s, 1H), 6.98 (s, 1H), 6.24 (d, *J* = 15.73 Hz, 1H), 6.15 (s, 2H), 4.27 (q, *J* = 7.13 Hz, 3H), 1.33 (t, *J* = 7.13 Hz, 4H); ^{13}C NMR (100 MHz, *CDCl*$_3$): 165.8, 152.0, 148.9, 143.0, 140.2, 127.2, 122.2, 107.3, 105.6, 103.4, 60.8, 14.2; LRMS: (ES+) m/z =265.9 (M+1).

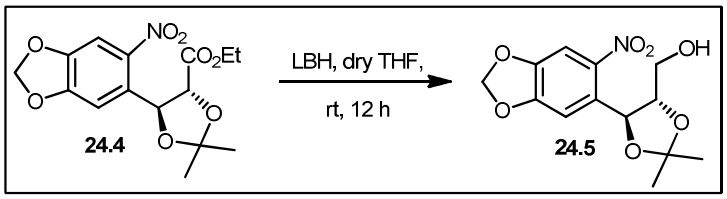


To a solution of the unsaturated carboxyl ester derivative **24.2** (14.0 g, 52.8 mmol) in *tert*-butylalcohol (300 mL) was added water (300 mL). The mixture was cooled to 0 ºC followed by the addition of methane sulfonamide (5.01 g, 52.7 mmol) and AD-mix-α (74 g).The reaction mixture was brought to room temperature and stirred for an additional 40 h. Sodium thiosulfate (14.5 g, 91.7 mmol) was added, and the mixture was stirred again for 30 min and then extracted with ethyl acetate. The organic phase was washed with 2M KOH (80 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by column chromatography (40:1 to 20:1, dichloromethane/methanol) afforded the product **24.3** (13.85 g, 88%) as a white solid. Molecular Name: (2R,3S)-ethyl 2,3-dihydroxy-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propionate; Molecular Formula: $C_{12}H_{13}NO_8$; R_f (solvent system): 0.24 (1:1,hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 7.55 (s, 1H), 7.28 (s, 1H), 6.14 (s, 1H), 6.11 (s, 1H), 5.74 (dd, *J* = 6.44, 1.73 Hz, 1H), 4.48 (dd, *J* = 5.90, 1.98 Hz, 1H), 4.35-4.30 (m, 2H), 3.29 (d, *J* = 5.99 Hz, 1H), 2.97 (d, *J* = 6.51 Hz, 1H), 1.34 (d, *J* = 7.14 Hz, 3H);

^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.5, 152.0, 147.0, 141.1, 135.1, 108.8, 104.6, 102.7, 73.6, 69.8, 61.6, 14.0; LRMS: (ES+) m/z = 300.1 (M+1).

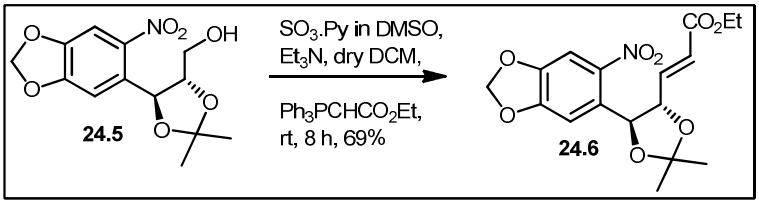


To a solution of the carboxyl ester **24.3** (2.6 g, 8.69 mmol) in DCM (52 mL) at room temperature was added 2,2-dimethoxypropane (52 mL, 44.04 g, 422.89 mmol) and *p*-toluene sulfonic acid monohydrate (260 mg, 1.37 mmol). The reaction mixture was stirred for 12 h. Following dilution with CH_2Cl_2 and saturated NH_4Cl, the organic layer was collected. It was then washed with water and dried over anhydrous Na_2SO_4. Purification by flash chromatography over silica gel (5:1, hexane/ethyl acetate) afforded the product **24.4** as colorless oil in 95% yield. Molecular Name: (4R,5S)-ethyl 2,2-dimethyl-5-(6-nitrobenzo[d][1,3]dioxol-5-yl)-1,3-dioxolane-4-carboxylate; Molecular Formula: $C_{15}H_{17}NO_8$; R_f (solvent system): 0.26 (5:1 hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.52 (s, 1H), 7.29 (s, 1H), 6.15 (d, *J* = 1.87 Hz, 2H), 5.90 (d, *J* = 7.25 Hz, 1H), 4.30-4.15 (m, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.26 (t, *J* = 7.14 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 169.6, 152.5, 147.7, 142.2, 130.9, 112.0, 106.8, 105.4, 103.2, 81.7, 76.2, 61.7, 27.0, 26.0, 13.9; LRMS:MS (ES+) m/z = 340.1 (M +1).

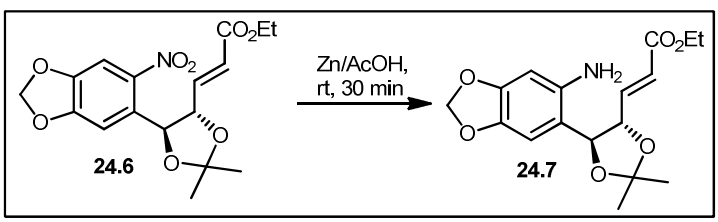


To a solution of the carboxyl ester **24.4** (8.6 g,26.36 mmol) in THF(250 mL) was added lithium borohydride (2.0 M solution in THF, 30 mL, 60 mmol) slowly at room temperature. The reaction mixture was stirred at room temperature for 12 h and cooled to 0 ºC and then quenched with saturated NH_4Cl. After solvent evaporation, the residue was dissolved in dichloromethane, washed with water and brine and then dried over Na_2SO_4. Purification by flash chromatography over silica gel (2:1, hexane/ethyl acetate) afforded the product **24.5** (7.3 g, 85%) as

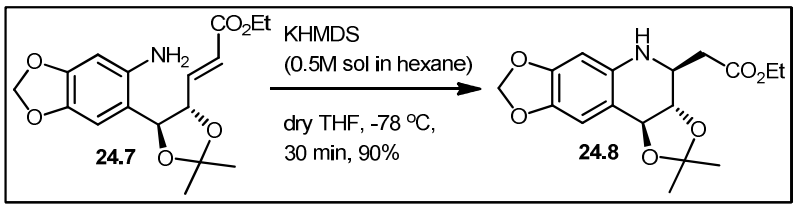
colorless oil; Molecular Name: ((4S,5S)-2,2-dimethyl-5-(6-nitrobenzo[d][1,3] dioxol-5-yl)-1,3-dioxolan-4-yl)methanol; Molecular Formula: $C_{13}H_{15}NO_7$; R_f (solvent system): 0.26 (2:1, hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.42 (s, 1H), 7.23 (s, 1H), 6.13 (d, *J* = 1.21 Hz, 2H), 5.48 (d, *J* = 7.93 Hz, 1H), 3.95-3.84 (m, 3H), 1.60 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 152.3, 147.6, 143.3, 130.5, 109.6, 107.6, 105.1, 103.1, 84.4, 74.3, 61.9, 27.1, 27.1; LRMS:MS (ES+) m/z = 298.1 (M +1).



To a solution of **24.5** (100 mg, 0.336 mmol) in 5.5mL DCM:DMSO (10:1) was added triethylamine (0.28 mL, 2 mmol) at room temperature followed by the addition of sulfur trioxide pyridine complex (213 mg,1.344 mmol) and Carbethoxymethylene triphenyl phosphorane (351 mg, 1 mmol).The reaction mixture was stirred for an additional 12 h at room temperature. The reaction was quenched with 5% HCl, extracted with DCM, dried over Na_2SO_4, and concentrated. Purification by coloumn chromatography over silica gel (7:1, hexane/ethylacetate) afforded the trans product **24.6** (86 mg, 70%) as a yellow oil; Molecular Name: (E)-ethyl 3-((4S,5S)-2,2-dimethyl-5-(6-nitrobenzo[d][1,3]dioxol-5-yl)-1,3-dioxolan-4-yl)acrylate; Molecular Formula: $C_{17}H_{19}NO_8$; R_f (solvent system): 0.29(5:1,hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.44 (s, 1H), 7.24 (s, 1H), 7.01 (dd, *J* = 15.58, 5.97 Hz, 1H), 6.13 (s, 2H), 6.02 (d, *J* = 15.59 Hz, 1H), 5.50 (d, *J* = 7.66 Hz, 1H), 4.32 (t, *J* = 6.79 Hz, 1H), 4.20 (q, *J* = 7.13 Hz, 2H), 1.61 (s, 3H), 1.52 (s, 3H), 1.27 (d, *J* = 7.14 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 165.6, 152.1, 147.7, 142.9, 142.6, 129.5, 122.9, 110.3, 107.1, 105.2, 103.1, 83.0, 60.4, 29.6, 26.9, 14.1; LRMS:MS (ES+) m/z = 366.0 (M +1).

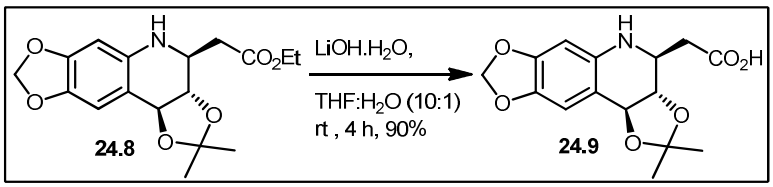


To a solution of **24.6** (3.48 g, 9.53 mmol) in ethanol (95 mL) was added zinc powder (6.23 g, 95.30 mmol) in one portion at room temperature. This was then followed by dropwise addition of aceticacid (5.45 mL, 95.20 mmol). The reaction mixture was stirred for 15 min, filtered, and concentrated. Purification by flash chromatography over silica gel (5:1, hexane/ethyl acetate) afforded the product **24.7** in quantitative yield as a yellow oil; Molecular Name: (E)-ethyl 3-((4S,5S)-5-(6-aminobenzo[d][1,3]dioxol-5-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate; Molecular Formula: $C_{17}H_{21}NO_6$; R_f (solvent system): 0.48 (4:1,hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.87 (dd, *J* = 15.53, 4.77 Hz, 1H), 6.58 (s, 1H), 6.25 (s, 1H), 6.09 (d, *J* = 15.55 Hz, 1H), 5.86 (s, 2H), 4.79-4.73 (m, 1H), 4.64 (d, *J* = 8.65 Hz, 1H), 4.19 (dd, *J* = 13.88, 6.85 Hz, 2H), 3.98 (s, 2H), 1.57 (s, 3H), 1.50 (s, 3H), 1.28 (t, *J* = 7.04 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 165.9, 148.6, 143.5, 141.0, 140.2, 122.8, 110.3, 109.6, 108.7, 100.9, 99.0, 82.3, 77.8, 60.6, 27.2, 26.4, 14.2; LRMS:MS (ES+) m/z = 336.1 (M +1).



To a solution of **24.7** (120 mg, 0.3578 mmol) in anhydrous THF (50 mL) was added KHMDS (0.5 M solution in hexane, 0.7 mL, 0.3578 mmol) dropwise at -78 ºC. After the mixture being stirred for an additional 30 min at -78 ºC, the reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel (5:1 hexane/ethylacetate) afforded the product **24.8** as a white solid (57%); Molecular Name: ethyl 2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1,3]dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetate; Molecular Formula: $C_{17}H_{21}NO_6$; R_f (solvent system): 0.55 (4:1 hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.12 (s, 1H), 5.81 (d, *J* = 3.31 Hz, 2H), 4.64 (d, *J* = 8.82 Hz, 1H), 4.36 (s, 1H), 4.19 (d, *J* = 6.67 Hz, 2H), 3.96 (d, *J* = 10.13 Hz, 1H), 3.53 (t, *J* = 9.30 Hz, 1H), 2.89 (d, *J* = 16.19 Hz, 1H), 2.47-2.38 (m, 1H), 1.50 (d, *J* = 12.56 Hz, 6H), 1.27 (m, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm:172.0, 147.4, 139.5, 136.9, 113.2, 112.0,

104.3, 100.5, 96.1, 79.4, 60.9, 52.0, 38.9, 29.7, 27.1, 27.0, 14.2; LRMS:MS (ES+) m/z = 336.1 (M +1).



To a solution of **24.8** (500 mg, 1.49 mmol) in THF (50 mL) : H_2O (5 mL) was added LiOH.H_2O (312.6 mg, 7.45 mmol) at room temperature and allowed to stir until starting material disappeared on TLC. The reaction mixture was acidified with 5% HCl and the compound extracted twice with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated afforded the product **24.9** as a white solid (412 mg, 90%); Molecular Name: ethyl2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1,3] di oxolo[4,5-c:4',5'-g]quinolin-4-yl)acetate Molecular Formula: $C_{15}H_{17}NO_6$; R_f (solvent system): 0.05 (1:1hexane/ethyl acetate); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.67 (s, 1H), 6.15 (s, 1H), 5.82 (d, *J* = 5.08 Hz, 2H), 4.65 (d, *J* = 8.88 Hz, 1H), 4.05-3.97 (m, 1H), 3.56 (t, *J* = 9.47 Hz, 1H), 3.02-2.95 (m, 1H), 2.52 (dd, *J* = 16.67, 10.15 Hz, 1H), 1.53 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 177.1, 147.4, 139.7, 136.6, 113.3, 112.3, 104.3, 100.5, 96.4, 79.2, 51.8, 38.8, 27.0, 26.9; LRMS:MS (ES+) m/z = 307.9 (M +1).

1.11. References:

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1.12. Spectra for Synthesis of Chiral Scaffold (24.9):

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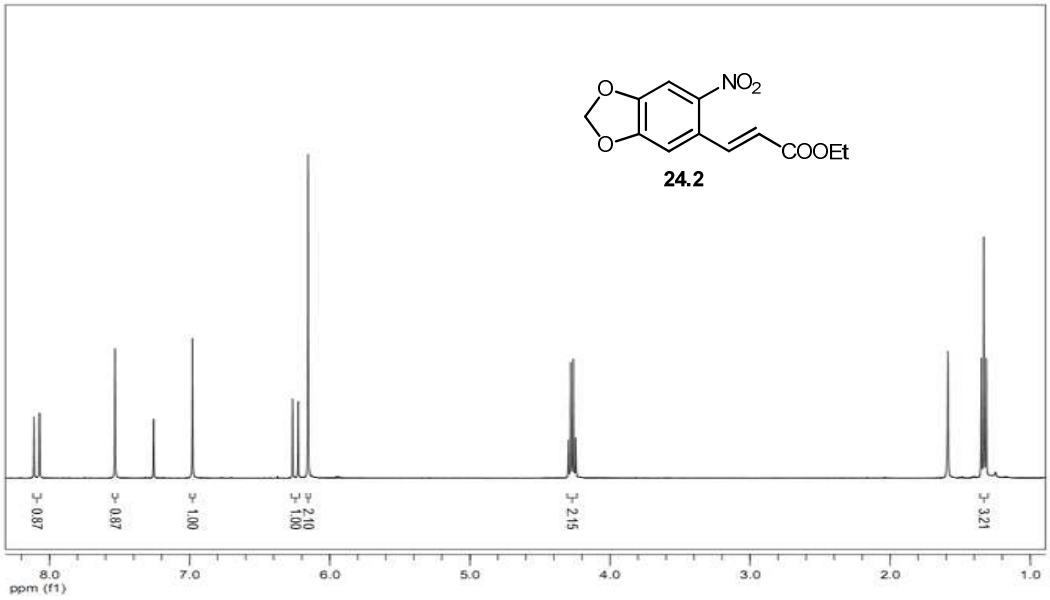

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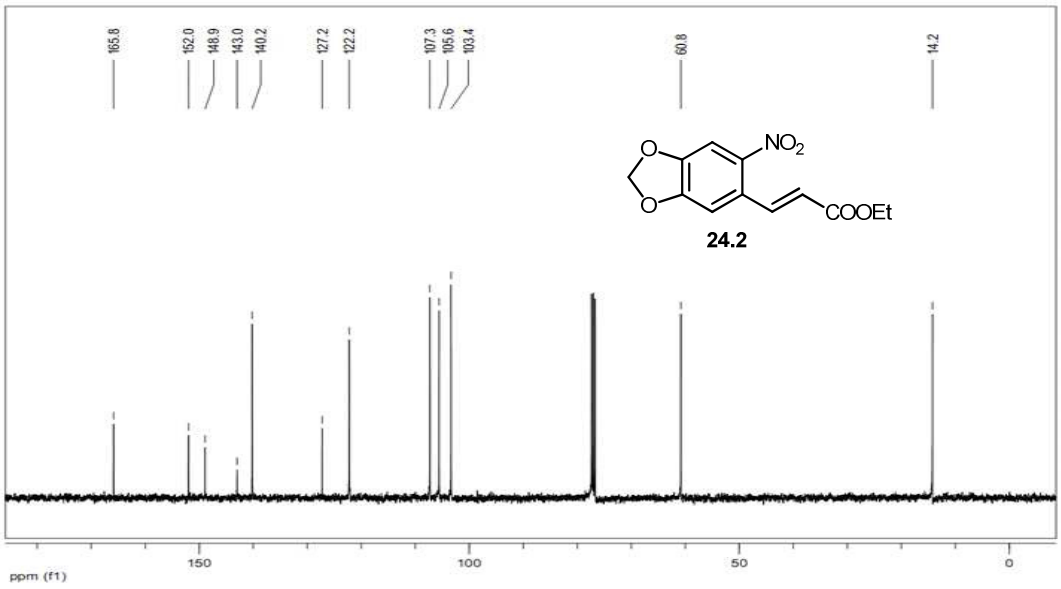

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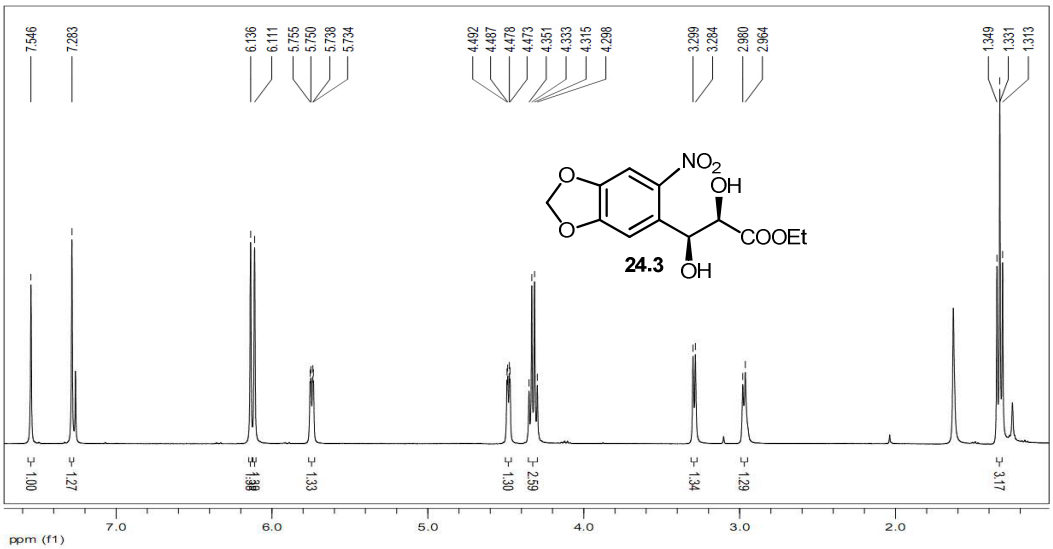

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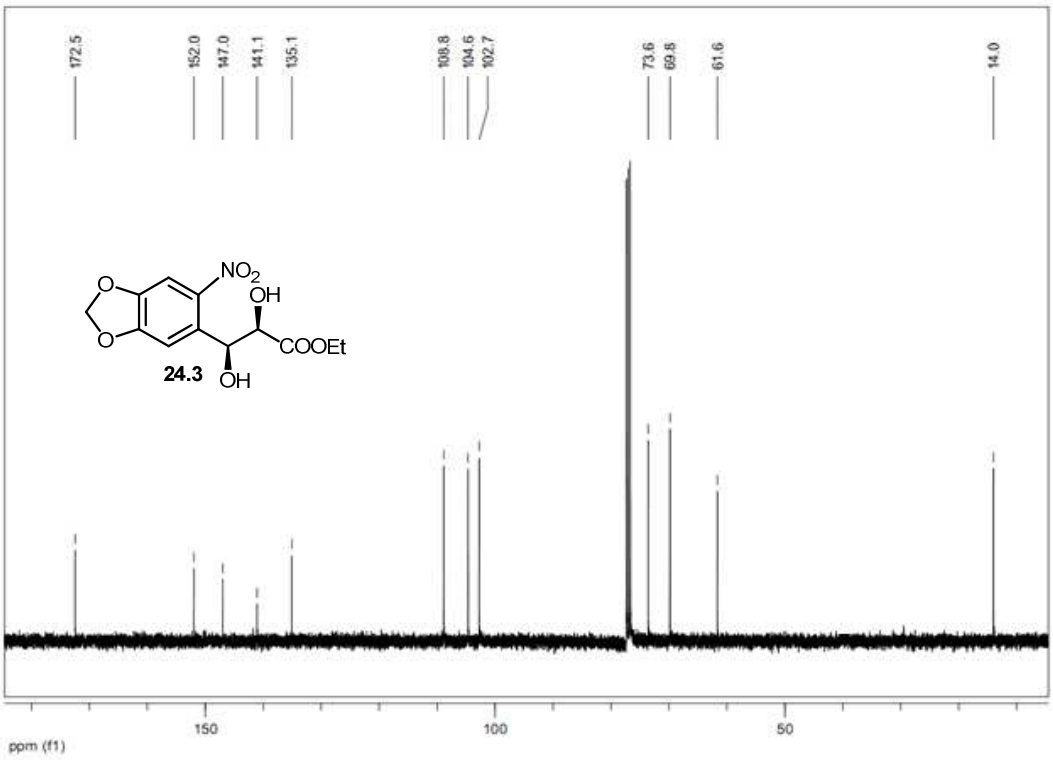

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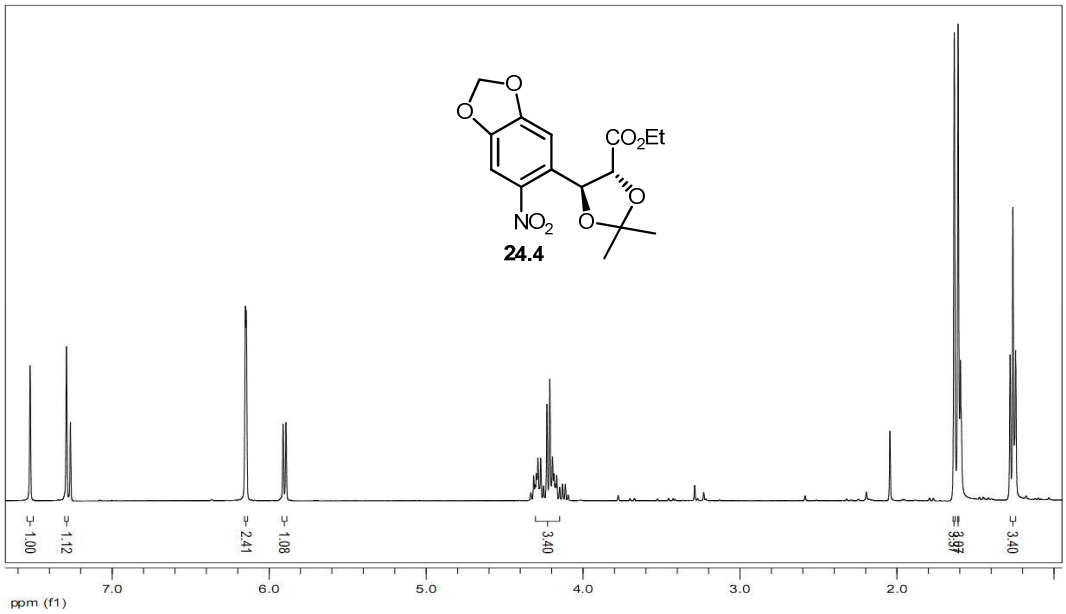

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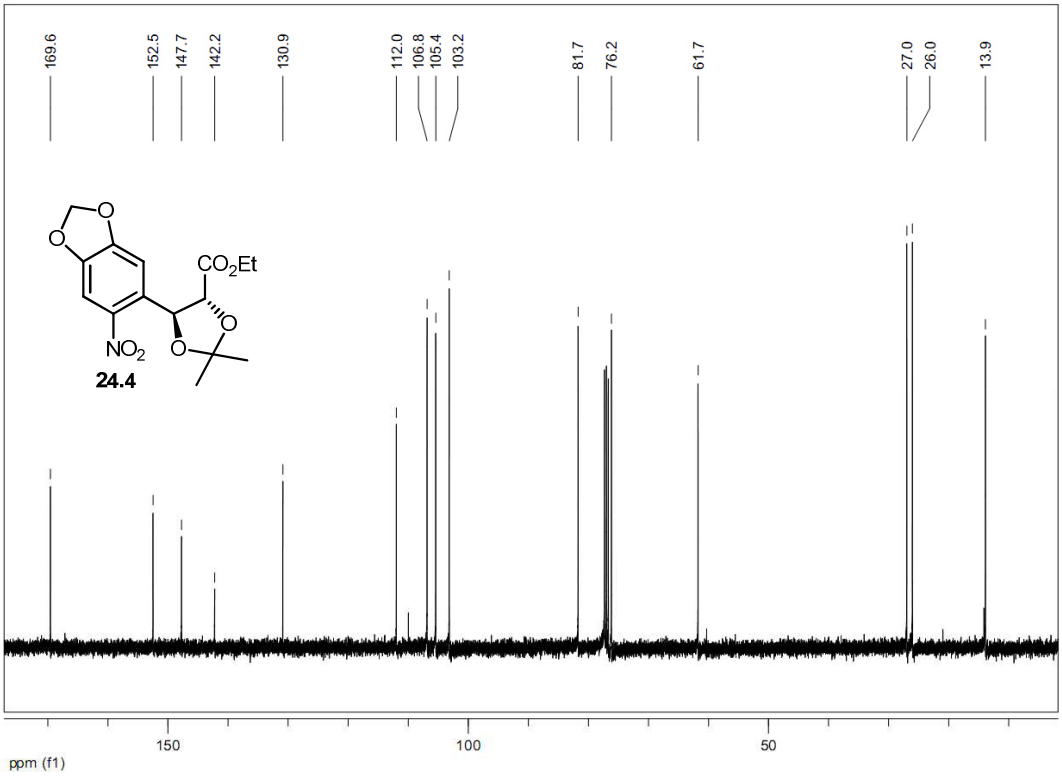

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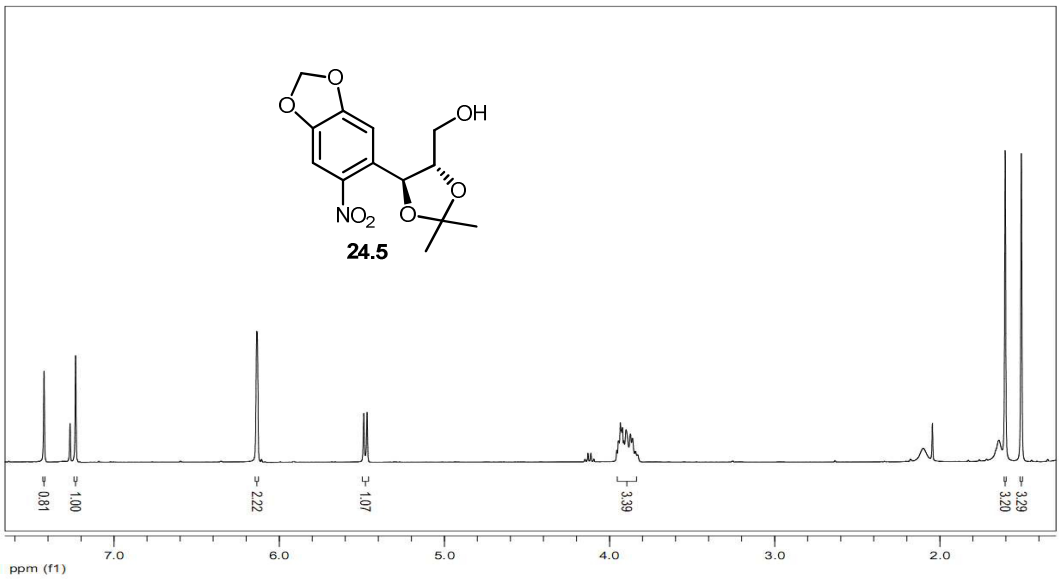

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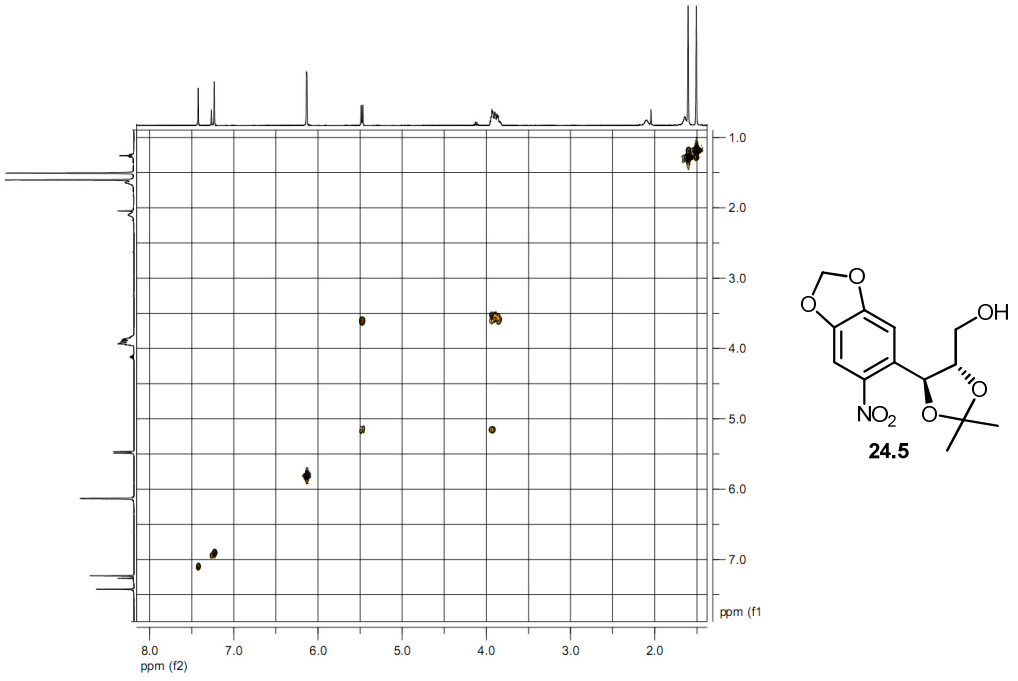

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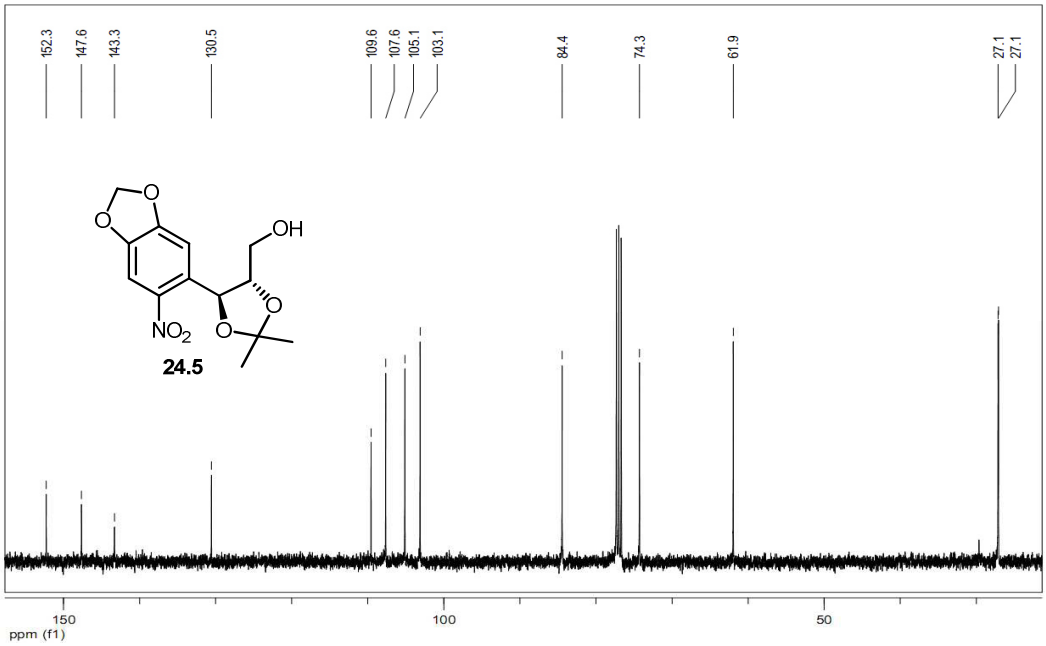

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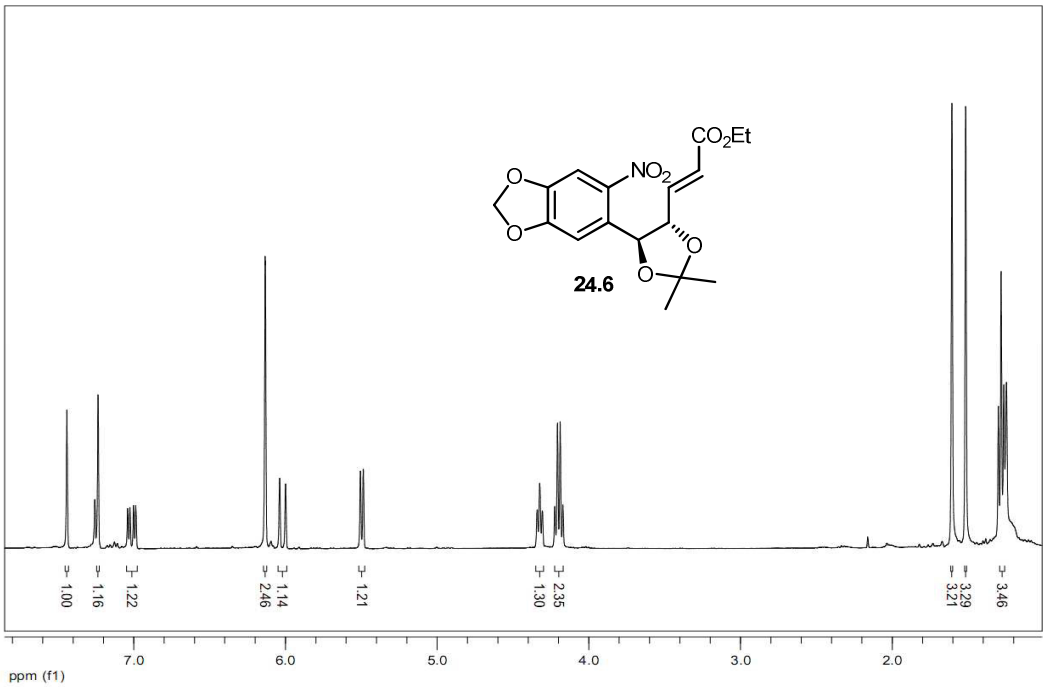

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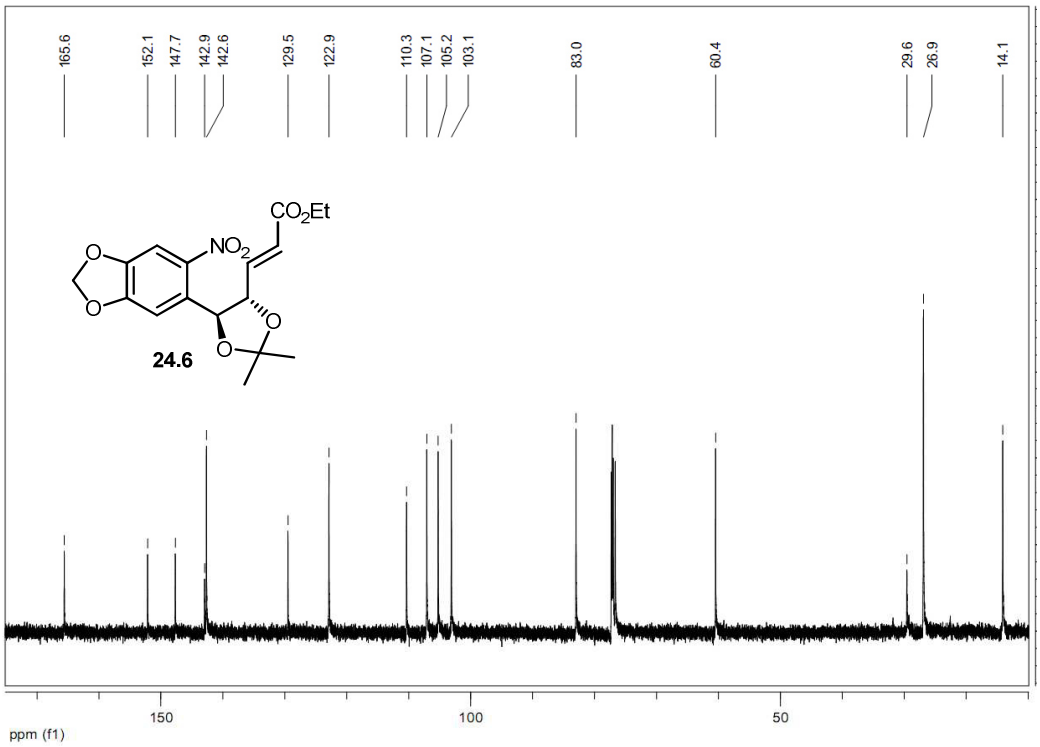

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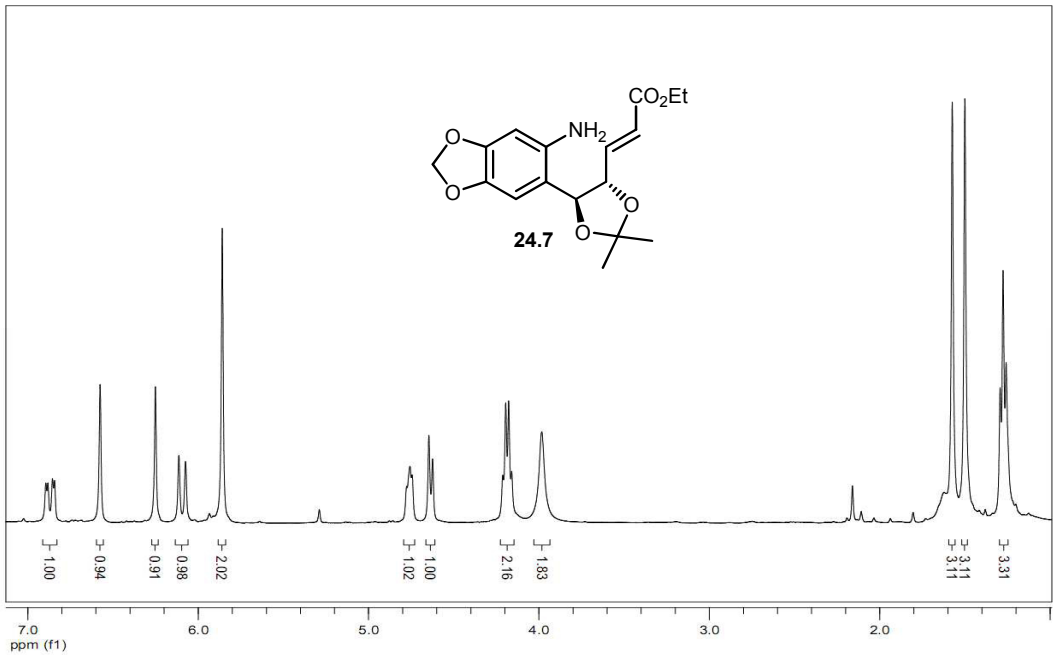

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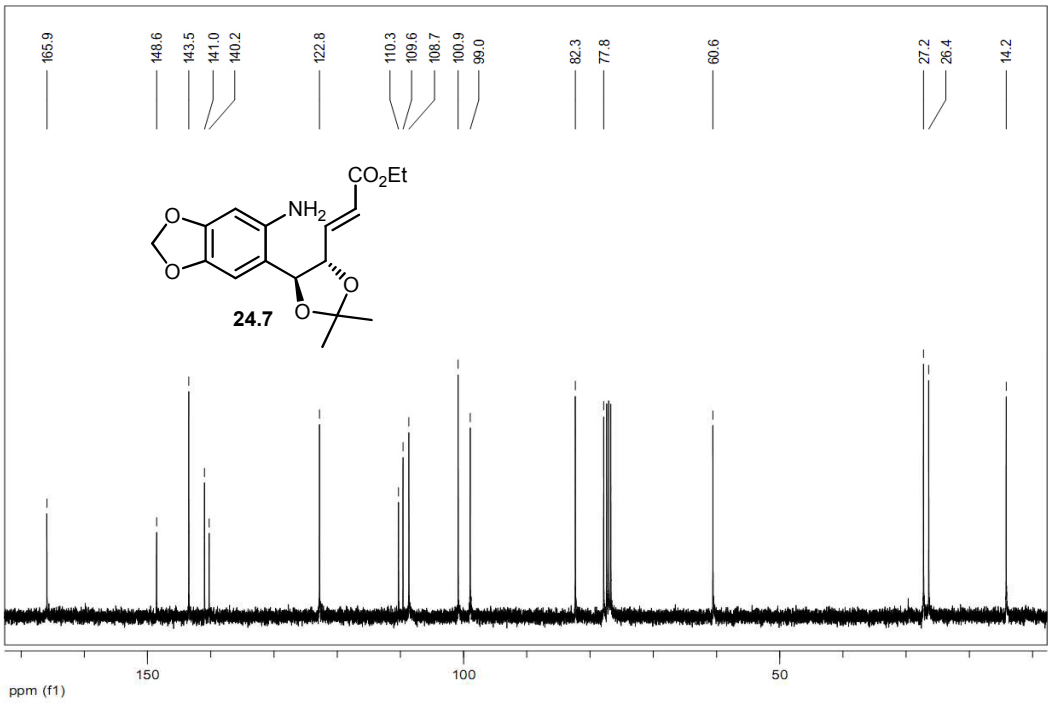

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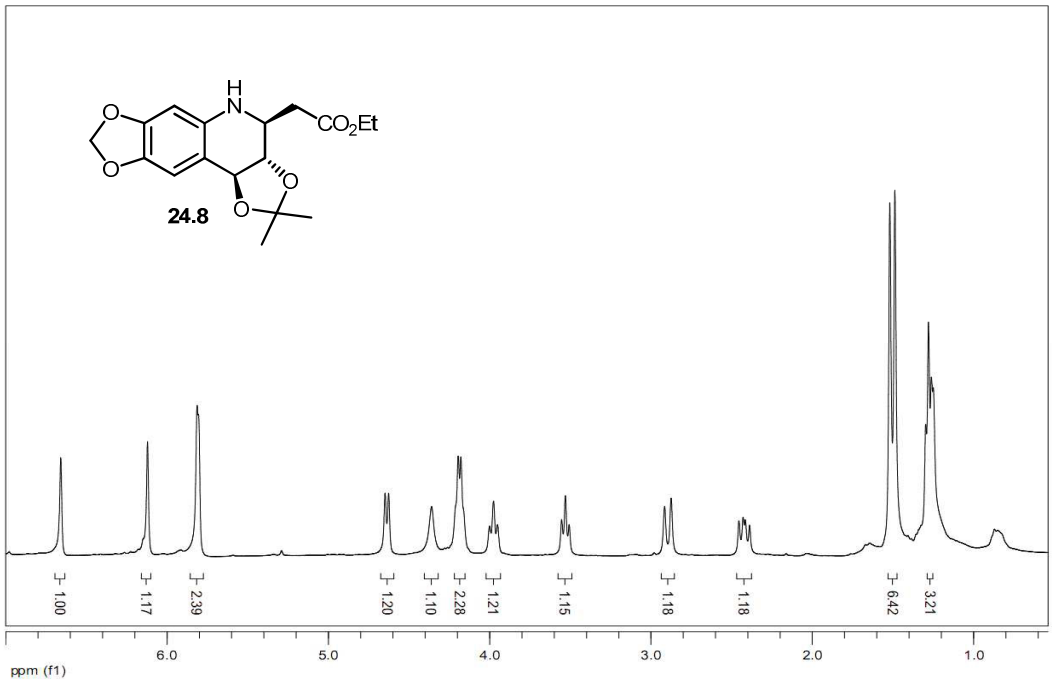

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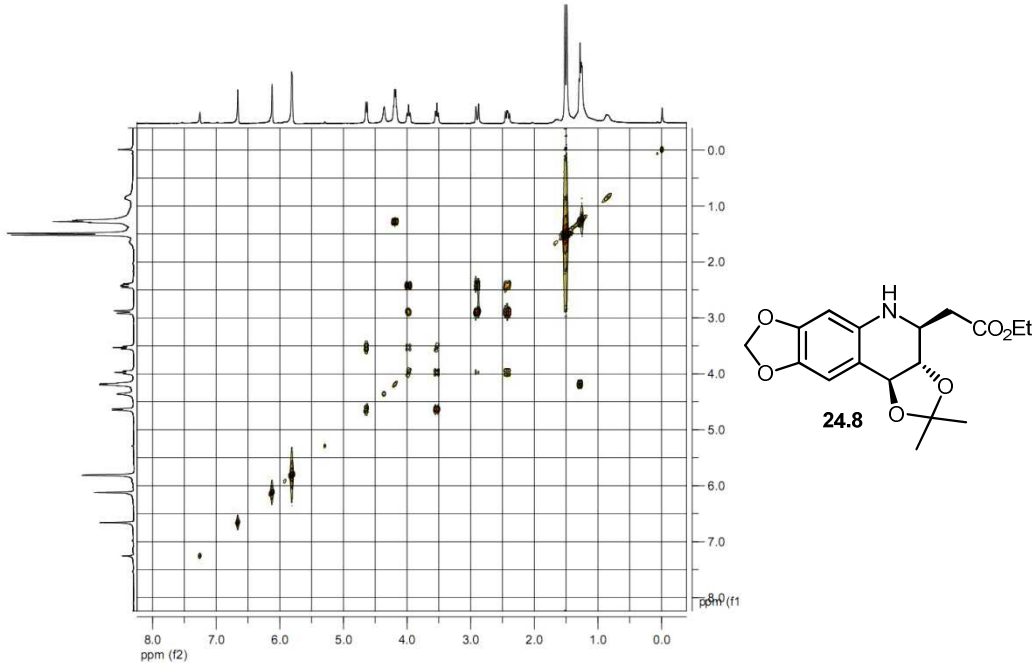

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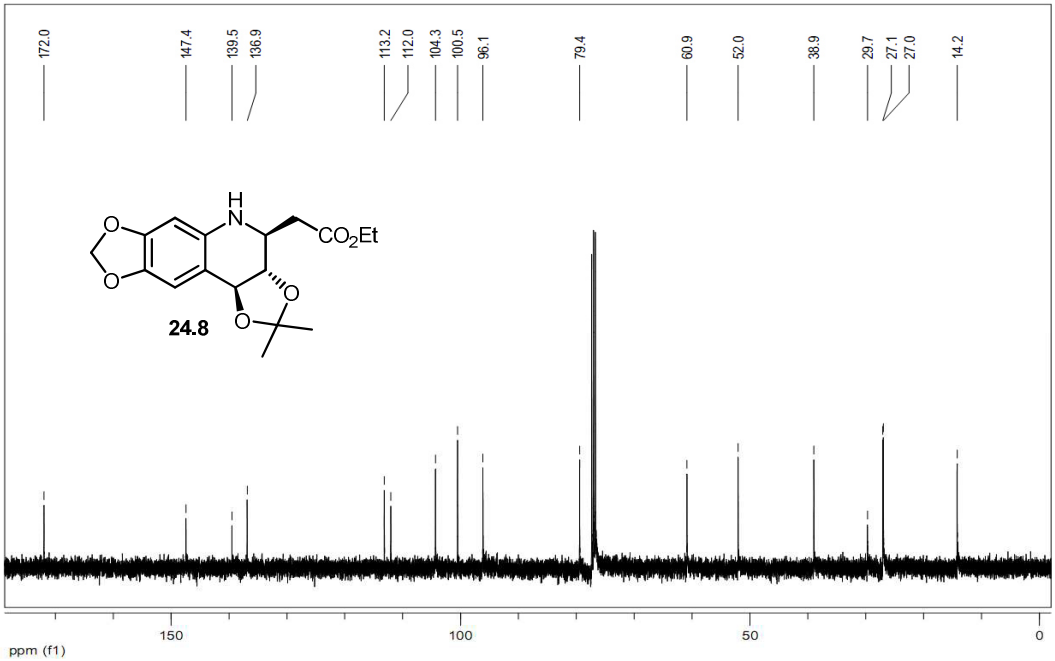

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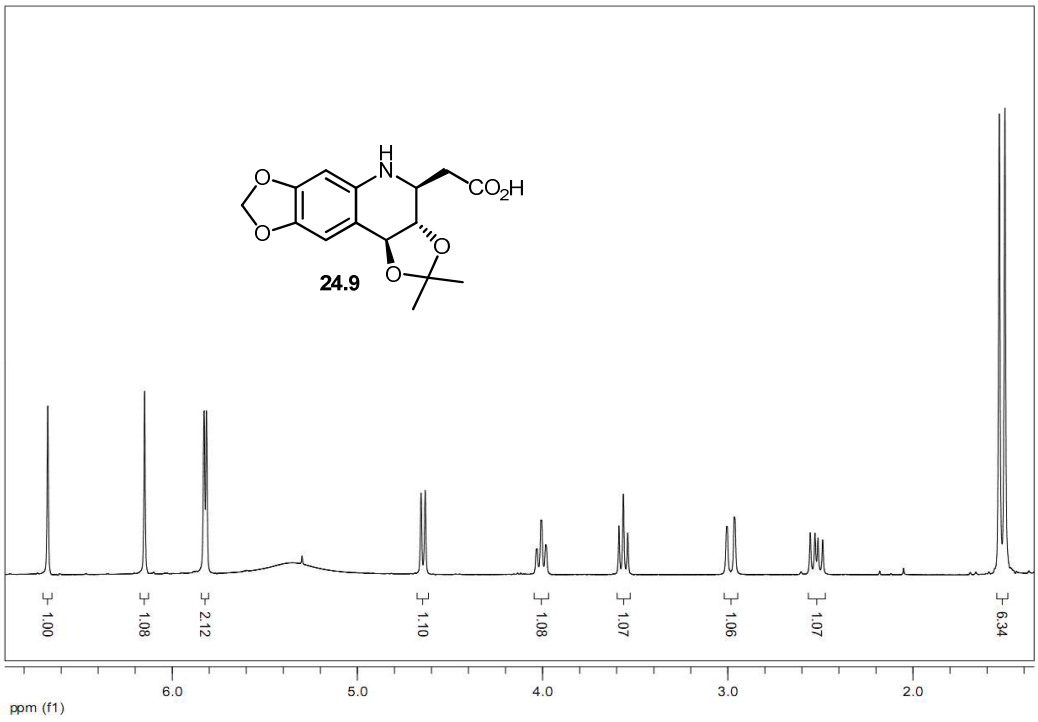

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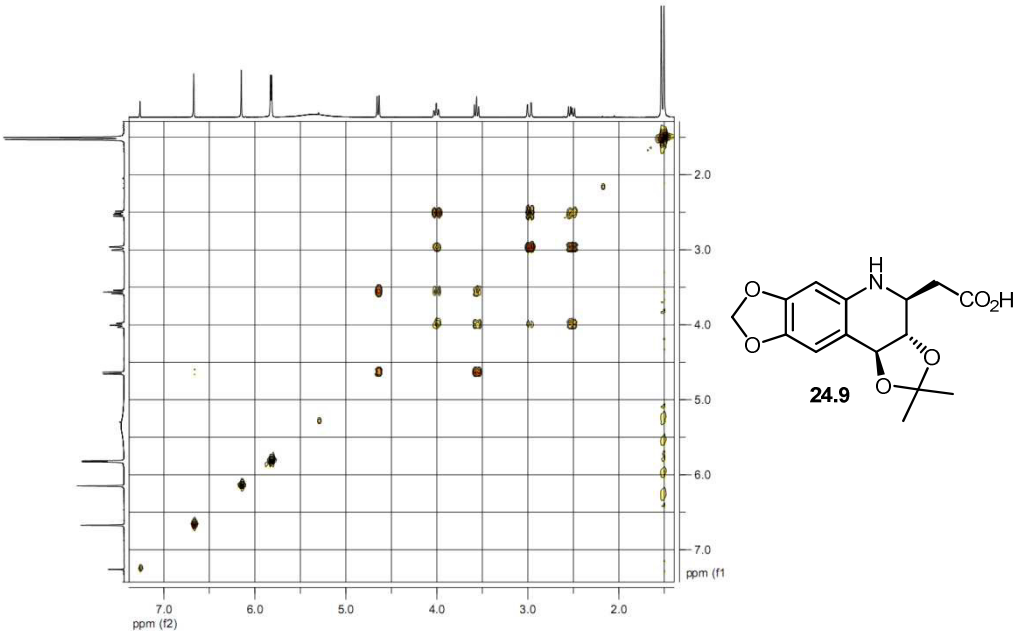

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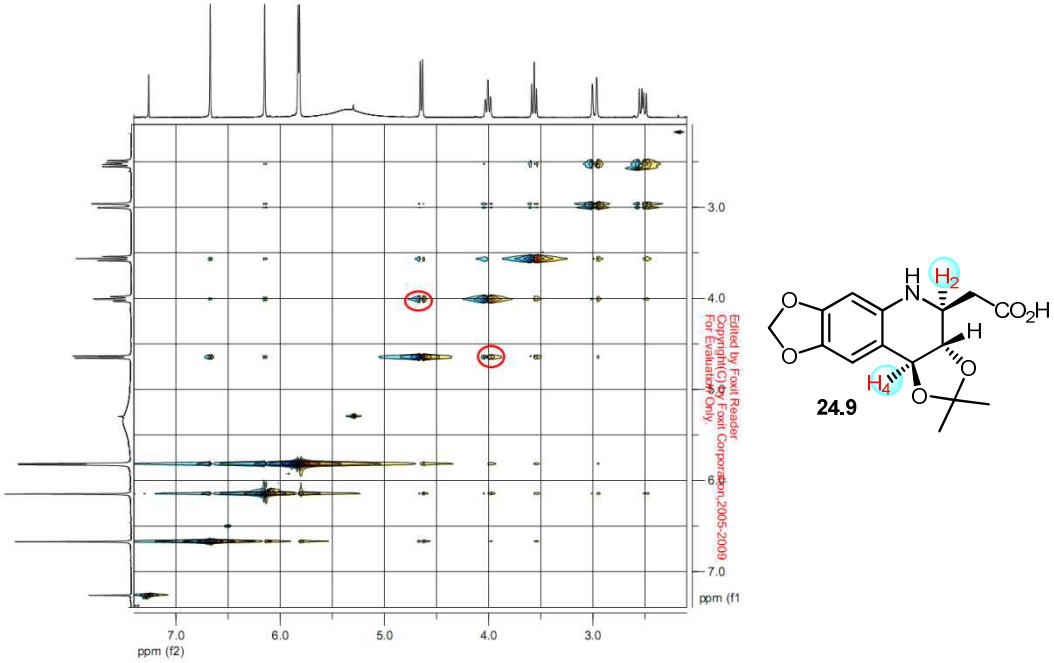

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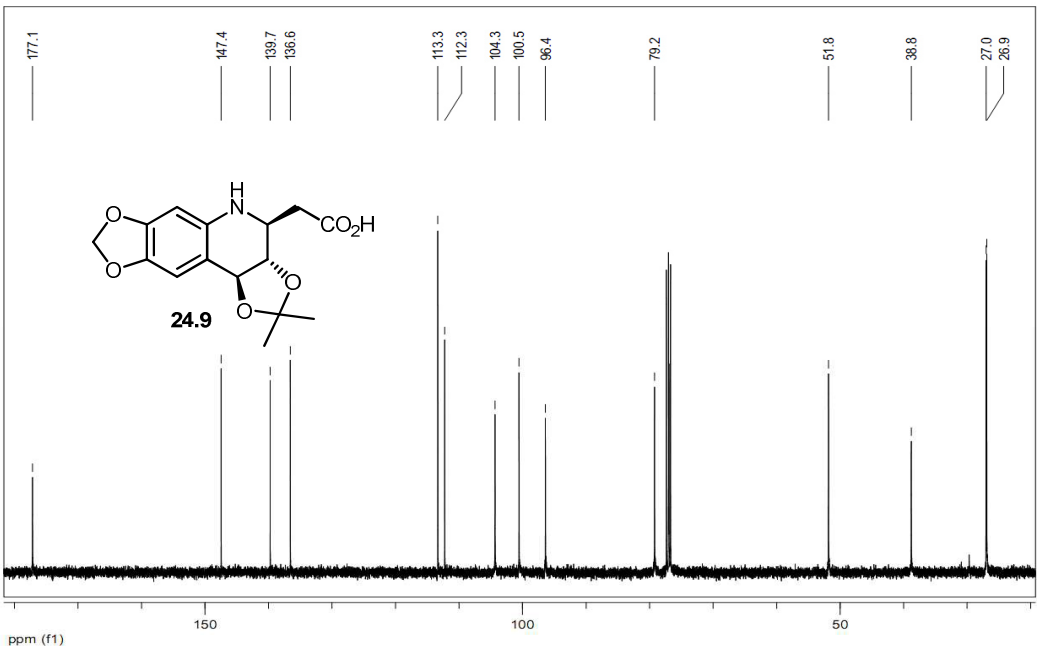


Chapter 2:
Synthesis of Natural Product-Inspired Tetrahydroquinoline Macrocyclic Toolbox

2.1. Introduction:

The pharmaceutical industry has focused on small, simple drug molecules for much of the past 100 years. These compounds are usually readily synthesized, and, have characteristics bounded by the Lipinski 'Rule of 5' guidelines.[1] These small molecules work by providing sufficient functionality in a concise package to bind to lipophilic pockets and polar functionality with concise binding sites, such as those found in an enzyme active site. In many cases, they replicate the interaction of the target protein molecule with its endogenous ligand or substrate.

However, there is a growing realization that such targets represent only a fraction of all disease relevant drug opportunities, and, that to find novel drugs for important medical needs, it is necessary to discover small molecules to disrupt protein-protein interactions (PPIs). These recognition events between proteins depend on many weak binding interactions spread over an extended surface area often exceeding 700 Å^2.[2] It has become apparent that 'Rule of 5' compliant small molecules are generally unable to make sufficient interactions to disrupt PPIs. The pharmaceutical industry has responded to this challenge by developing biological drugs – frequently derived from proteins or peptides – that gain sufficient potency by interacting over a large surface area. Antibodies or soluble receptors constitute a large part of this drug space, but increasingly, there are several new semi-synthetic biologicals such as Adnectins, avimers, and aptamers discovered and manufactured by biochemical means.

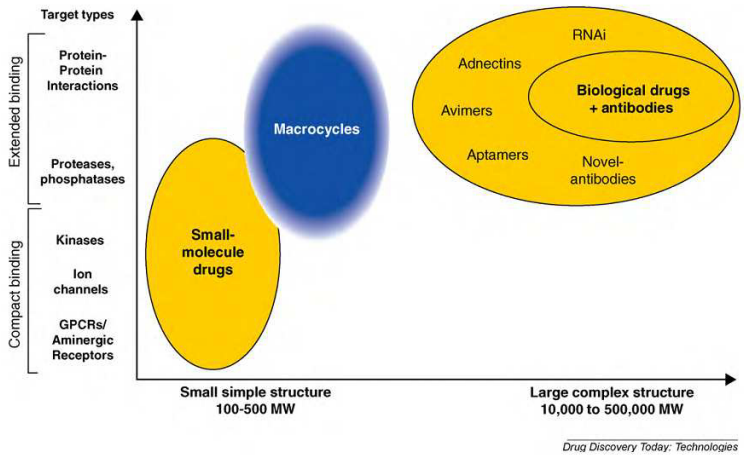


Figure 1: Macrocycles Space between Biological and Small Molecules

(Taken from *Drug Discovery Today: Technologies* **2010**, *7*, e97)

While providing high levels of potency, exquisite selectivity, and, in many cases long half-lives *in vivo*, molecule size significantly restricts membrane permeability. Thus, there remains a need for compounds that on one hand possess sufficient size and functionality to interact with protein surfaces, and, yet still maintain small molecule-like properties such as cell penetration and oral bioavailability. From amongst the range of pharmacologically active compounds, macrocycles offer the potential to sit in the 'middle space' between small molecules and biologicals, and furthermore are precedented by natural products (see **Figure 1**).

2.2. Macrocycles

The structural feature common across many larger natural products is a macrocycle ring structure of 12 or more atoms. Natural selection has clearly contributed to their design and function, and, specific target–ligand interactions in which these molecules participate, coupled with the advantages they confer in binding to their targets, and strongly support a history of selective optimization.

Macrocyclic natural products have evolved to fulfil numerous biochemical functions, and their profound pharmacological properties have led to their development as drugs. The macrocycle ring enables a molecule to achieve a degree of structural pre-organization, such that key functional groups can interact across extended binding sites in proteins without a major entropic loss on binding. Macrocycles can therefore be highly potent as well as selective. However, macrocycles are not rigid compounds. Instead, they provide a compromise between structural pre-organization and sufficient flexibility to mould to a target surface and maximize binding interactions. Furthermore, macrocycles are not just bigger versions of small molecules, but can be considered as the smallest examples of biomolecules that exhibit functional sub-domains. There is a growing evidence that despite having molecular weights above 'Rule of 5'-compliant small molecules, macrocycles can possess 'drug-like' physicochemical and pharmacokinetic properties, such as, good solubility, metabolic stability and bioavailability.[3]

2.3. Macrocycles in Nature

Current macrocyclic drugs are almost exclusively derived from natural sources (primarily microorganisms) and are either identical to or closely derived from naturally occurring macrocycles. For example, among the major classes of macrocyclic therapeutics, erythromycin (**F2.1**) was originally isolated from *Saccharopolyspora erythrea*[4] and the antituberculosis compound rifampicin (**F2.2**) from *Amycolatopsis rifamycinica*.[5] The immunosuppressant cyclic peptide cyclosporin A (**F2.3**) is produced by the fungus *Tolypocladium inflatum* Gams.[6] The cyclic glycopeptide antibiotic vancomycin (**F2.4**) was originally isolated from *Streptomyces orientalis*. (**Figure 2**).[7]

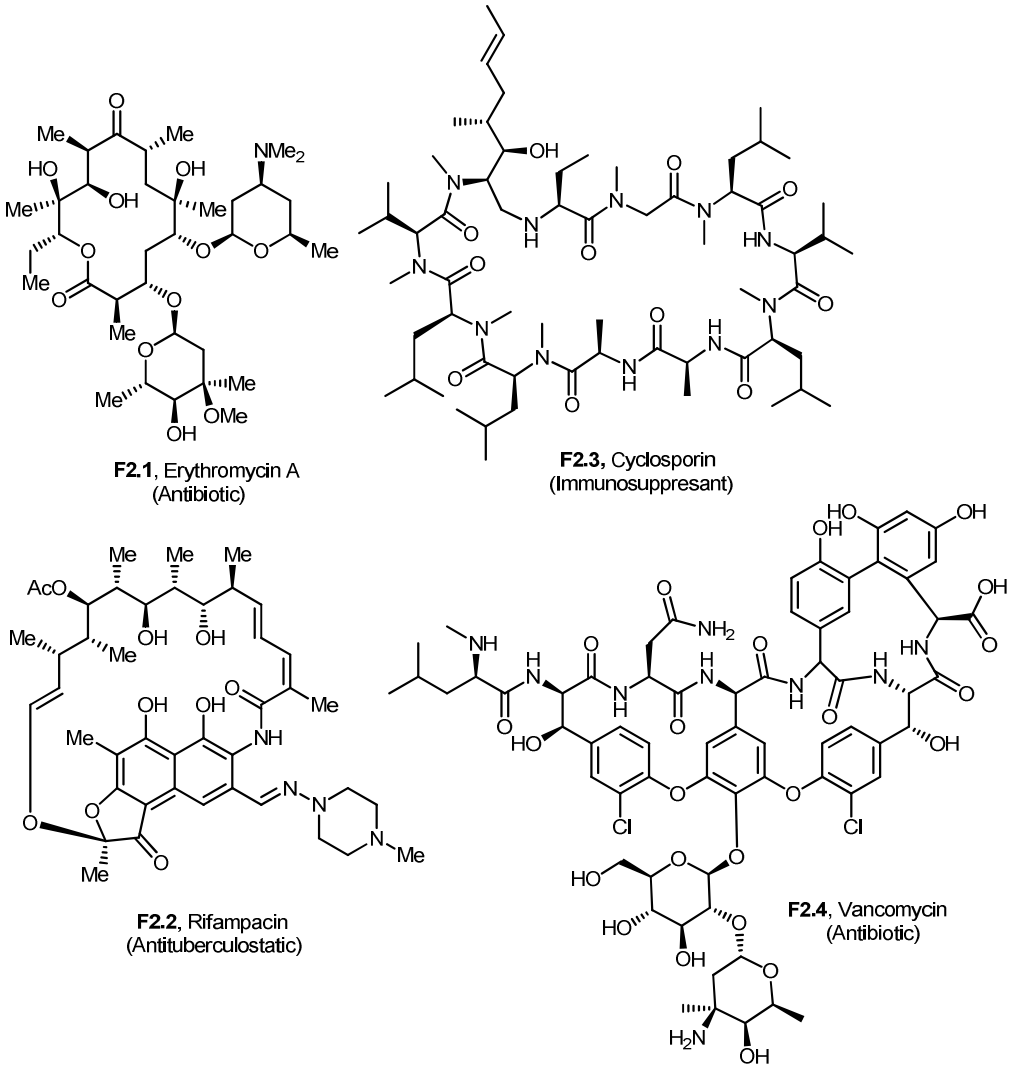


Figure 2: Macrocyclic Drugs Derived from Natural Sources

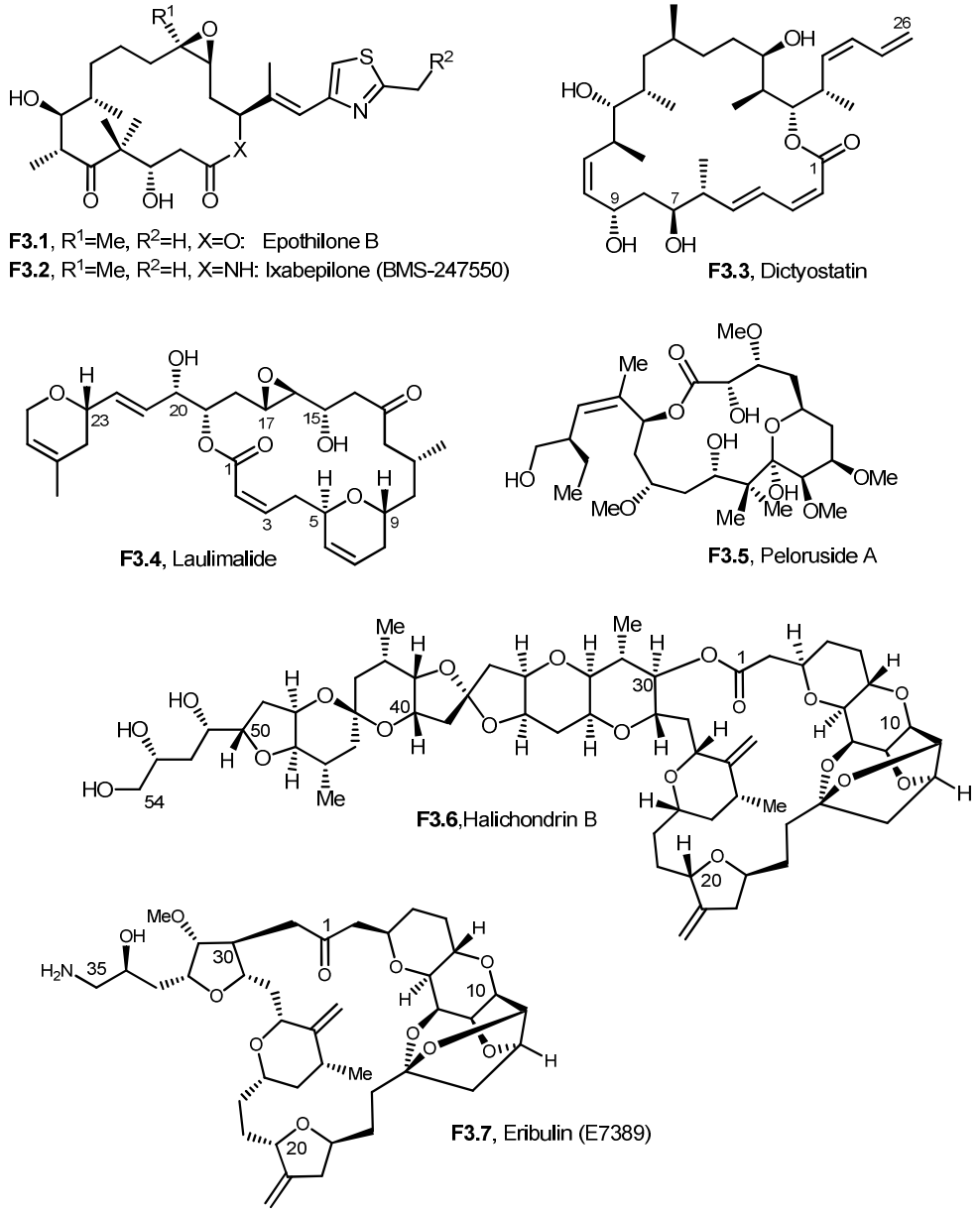


Figure 3: Macrocycles as Microtubule Modulators

Multiple classes of macrocyclic natural products are currently under investigation for their ability to modulate microtubule dynamics in mammalian cells, and thereby inhibit tumour growth.[8] Here again, these effects result from modulation of protein–protein interactions; in this case, between α and β subunits of the tubulin heterodimer. Epothilone B (**F3.1**), a macrocycle derived from myxobacteria, binds at the interface of the two tubulin subunits (a site that overlaps with the taxol binding site). Extensive re-configuration of the α–β interface stabilizes the dimer, and disturbs an overall microtubule dynamics.[9] Ixabepilone (**F3.2**), the lactam equivalent of epothilone B (**F3.1**), has been

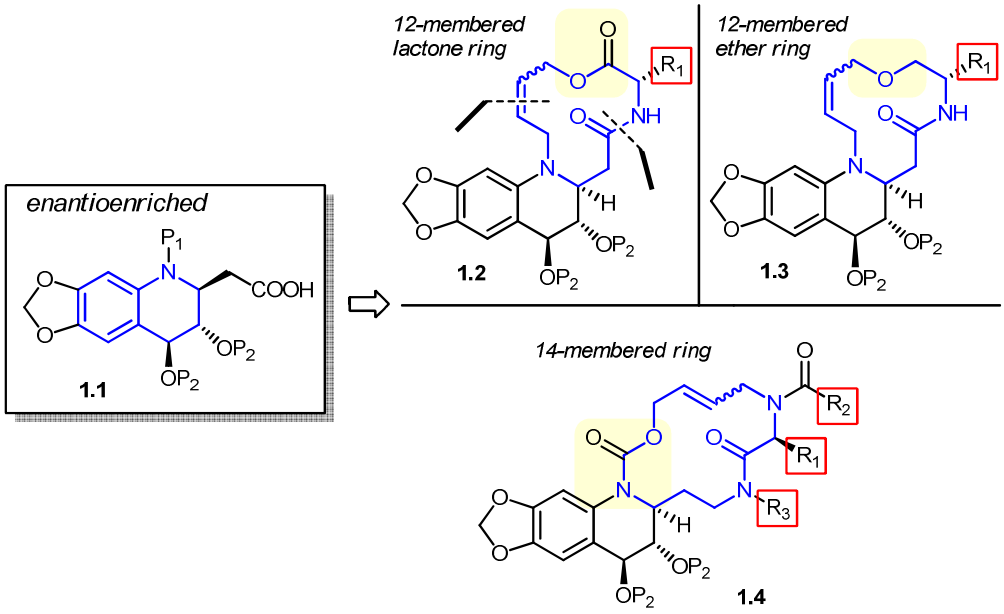
approved for the treatment of metastatic breast cancer.[10] Dictyostatin (**F3.3**) is a second macrocycle known to bind at the taxol site to stabilize α–β tubulin dimers.[11] Moreover, an additional site has been identified that is recognized by laulimalide (**F3.4**) and peloruside A (**F3.5**), macrocycles that have been found to be synergistic with taxol in stabilizing microtubules.[12] By contrast, binding at a different site by complex natural-product macrocycle halichondrin B (**F3.6**) [and its analogue eribulin, (e7389) (**F3.7**)] disrupts microtubule dynamics by destabilizing tubulin dimers.[13] While multiple examples of non-macrocyclic microtubule disrupters are also known, current knowledge indicates that nature has frequently identified macrocycles to modulate protein–protein interactions between microtubule subunits (**Figure 3**).

Despite the proven therapeutic potential of macrocyclic compounds, they have been under-explored and poorly exploited for the discovery of novel drug molecules. The reasons for this are many! There has been a growing reluctance in the pharmaceutical industry to investigate natural products because their structural complexity generates difficulties in analogue synthesis. Furthermore, preferential adoption of rule of 5-compliant compounds for screening has become widespread. However, several research groups are investigating the potential of synthetic macrocycles for drug discovery and have proved that such compounds can provide high target affinity and selectivity in structures that have acceptable drug-like properties. Several synthetic macrocycles, unrelated to natural products, are now under active preclinical and clinical development.[14]

2.4. Working Hypothesis and Targets

With this objective, we have developed a modular approach to obtain natural product-inspired tetrahydroquinoline macrocyclic toolbox. We explored a chemical space around an *enantioenriched* tetrahydroquinoline scaffold and the synthesis of this scaffold is explained in Chapter 1. There are two main objectives in our design strategy, first is to retain the functionalized privileged substructure, i.e., tetrahydroquinoline, and, second is to map the macrocyclic chemical space with the additional functional groups. Our synthesis is practical in nature and allows us to access 12- and 14-membered macrocycles in sufficient quantities in a short period. These macrocycles resembles the natural products in the sense of

3D-architectually, skeletal diversity and in dense functionality. So, we called these macrocycles as natural product-inspired macrocycles.[15]



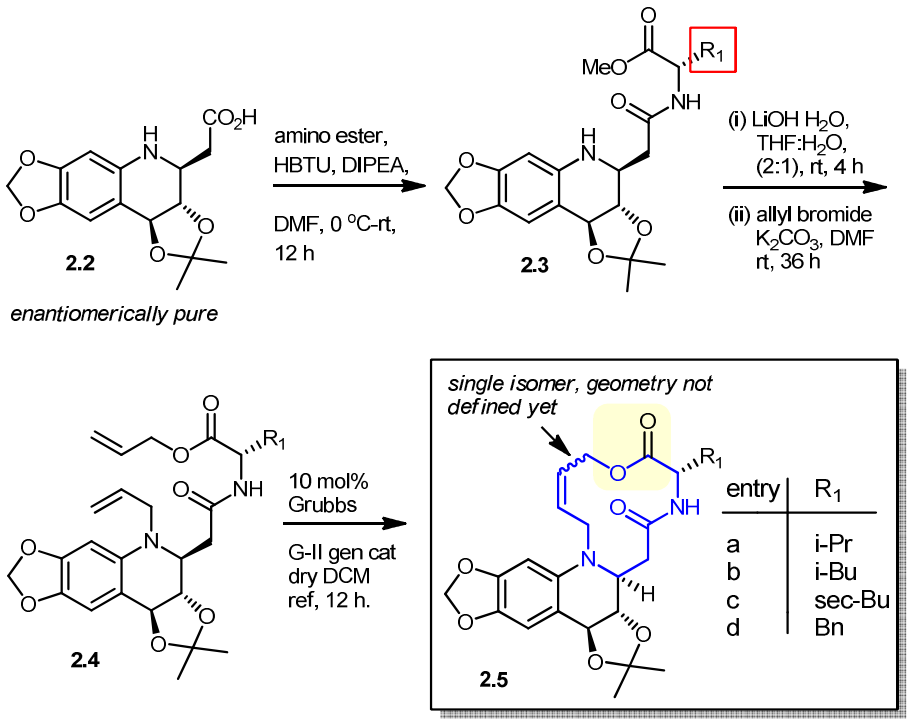
Scheme 1: Incorporation of Different Macrocyclic Rings onto an *Enantioenriched* Tetrahydroquinoline Scaffold

Target **1.2** has the additional 12-membered ring with an incorporation of an amino acid moiety in the skeleton. The ring-closing metathesis reaction was utilized as the stitching technology to obtain the macrocyclic rings. Using a similar approach, one can also obtain compound **1.3** with a 12-membered ring having the connectivity through the ether linkage. In addition to these two compounds, we also plan to incorporate 14-membered macrocyclic ring onto the tetrahydroquinoline scaffold having an amino acid moiety in the ring skeleton. Overall, our approach to building different types of large ring skeletons onto the tetrahydroquinoline scaffold provides an excellent opportunity to accessing a chemical toolbox with a diverse set of functionalized large ring-based derivatives (**Scheme 1**).

2.5. Results and Discussion:

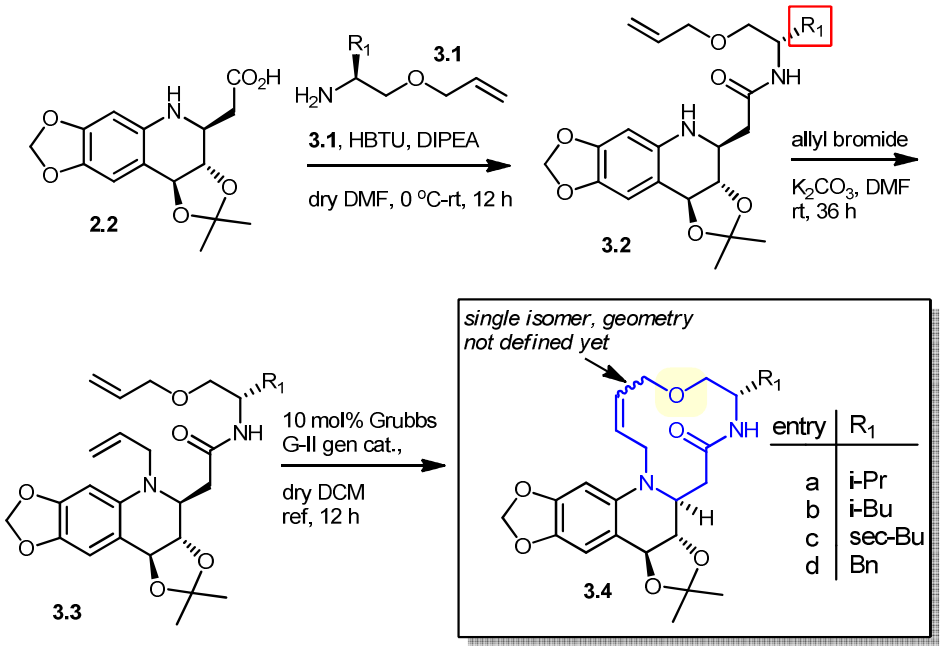
2.5.1. Synthesis of 12-Membered Macrocycles 2.5 and 3.4:

Our synthesis approach to incorporate a 12-membered ring onto an enantioenriched tetrahydroquinoline scaffold is shown in **Scheme 2**.



Scheme 2: Synthesis Route to Obtain a 12-Membered Macrocycle onto the Tetrahydroquinoline Ring

The free carboxylic acid group (**2.2**) was coupled with several amino esters to obtain compound **2.3** (R_1 as the diversity site). This was easily converted to *bis*-allyl derivative **2.4** needed for building the 12-membered macrocyclic ring. Upon subjection to the ring-closing metathesis stitching technology,[16] we successfully obtained the cyclic product **2.5** in good yields as the single isomer (olefin geometry is not defined yet). Our approach is general in nature, and, as a proof of concept studies, four macrocyclic compounds **2.5a-d** were further synthesized. All the products in this scheme are thoroughly purified and characterized using HPLC-MS and NMR.



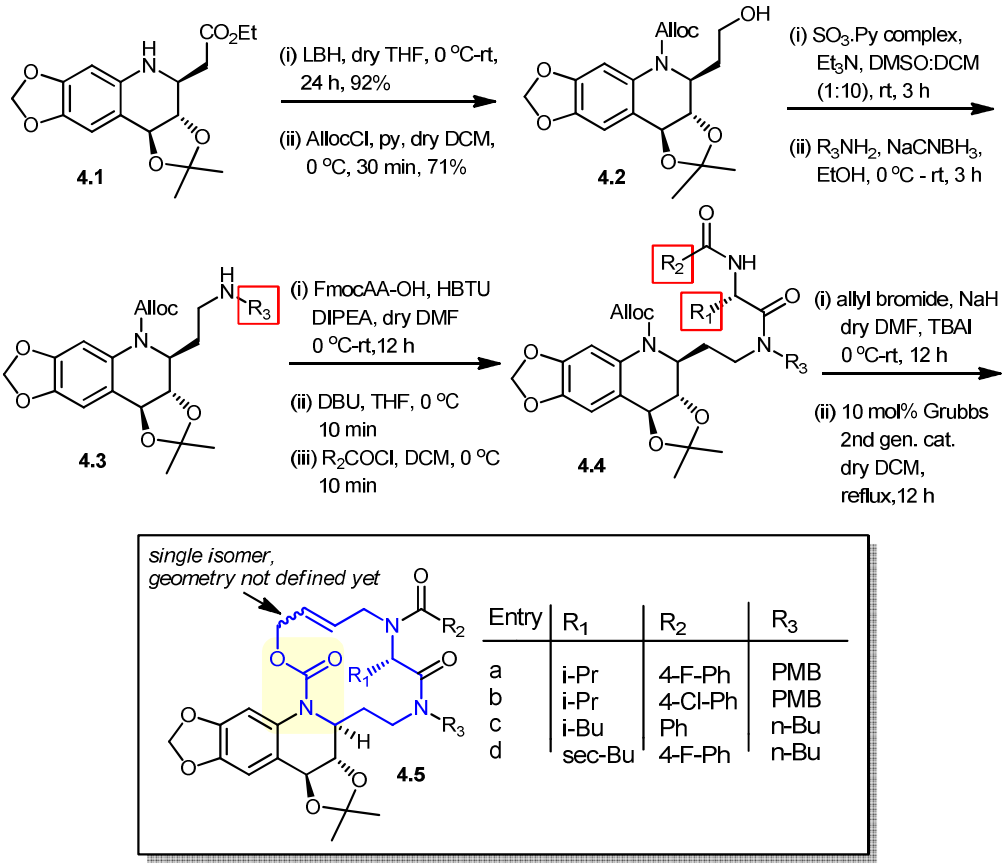
Scheme 3: Synthesis Route to Obtain a 12-Membered Macrocyclic Ring onto the Tetrahydroquinoline Ring that Utilizes Amino Alcohols

In another similar approach, we utilized the corresponding amino-alcohol derivatives **3.1** (see **Scheme 3**) to couple with the free carboxylic acids, and this successfully led to the synthesis of 4 examples (**3.4a-d**). Once again, the products are obtained as a single isomer and the olefin geometry is not defined yet.

2.5.2. Synthesis of 14-Membered Macrocycles 4.5a-d:

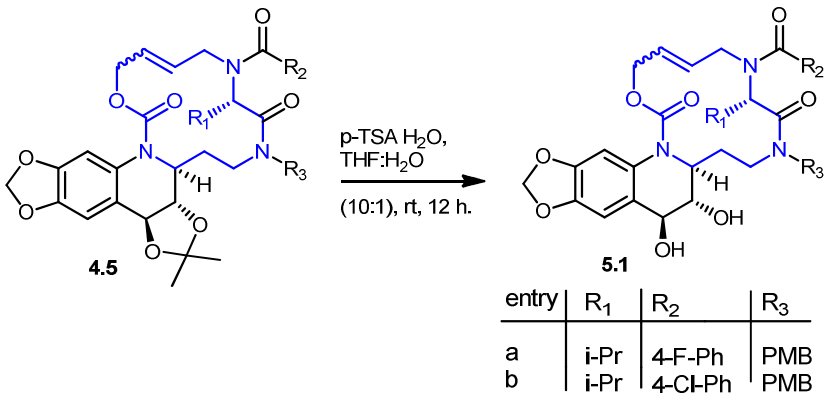
Our plans to incorporate a 14-membered ring onto the tetrahydroquinoline scaffold are shown in **Scheme 4**. Compound **4.1** as the starting material was utilized to obtain **4.2** in two steps that followed the carboxyl ester reduction and aromatic amine protection as –*N*Alloc. Following the oxidation of a primary hydroxyl group, it was then reductively alkylated to obtain the secondary amine (with the first diversity as R_3), and, then coupled with several amino acids and further amidation (second and third diversity sites, R_1 and R_2) to obtain **4.4**. This was then subjected to *N*-allylation followed by ring closing metathesis stitching technology using second generation Grubbs catalyst giving the 14-membered ring-derived compounds **4.5**. This reaction worked well and it was also utilized to obtain four test macrocyclic compounds **4.5a–d**. All the products were purified

over silica gel and thoroughly characterized by HPLC-MS and NMR (note: olefin geometry is not defined yet).



Scheme 4: Synthesis Route to Incorporate a 14-Membered Ring onto an *Enantioenriched* Tetrahydroquinoline Scaffold

As test studies, in two cases, the acetonide protection was removed under mild acidic conditions giving the macrocyclic compounds with two free hydroxyl groups and these areshown in **Scheme 5**.



Scheme 5: Acetonide Deprotection for 14-Membered Macrocycles

2.6. Biological Evaluation in Zebrafish:

Having a chemical toolbox available to explore its biological value, we then decided to search for functional small molecules in three zebrafish screens,[17] and these are (i) angiogenesis,[18] (ii) an early embryonic development,[19] and (iii) neurogenesis.[20] These assays are well- documented in the literature[21,22] and performed in collaboration with Satish Srinivas Kitambi from Karolinska Institute, Sweden.

2.6.1. Zebrafish Husbandry:

Animals were maintained separately under 14/10-hour light/dark cycle and embryos were obtained by natural mating and staged according to Kimmel et. al (1). Zebrafish embryos of stages older than 24 hpf were treated with 0.03% PTU (N-Phenylthiourea) when needed to inhibit pigment formation. Transgenic line Tg (fli:EGFP) was used to assess effects on angiogenesis, Islet1:GFP was used to assess trunk neurogenesis and axonal growth and AB wild type strain was used for studying epiboly effects of the compounds.

2.6.2. Zebrafish Embryo Collection and Small Molecule Screening:

Zebrafish embryos for small molecule screening experiments were collected via pair wise matings, cleaned and incubated in PTU treated E3 water at 28.3 ^{o}C. One to four cell stage embryos were then distributed into 96 well clear bottom plate (Corning). The compound exposure was done in 96 well plate (Corning) and three embryos were taken in each well contain 200 µl of (0.5 to 15 µM) compound in PTU treated egg water. The 96 well plates were incubated at 28.3 ^{o}C and the embryos were allowed to grow for 10 hpf to assess the effect on epiboly or up to 36 hpf to assess the effect on angiogenesis and trunk neurons. Phenotypes were scored using a Zeiss Axiovert 200 inverted microscope equipped with a cooled CCD camera. Photographs were processed and assembled using Photoshop software.

We exposed a total of 30 embryos per compound producing defects in angiogenesis and early embryo developmental defects. We then quantified the number of embryos exhibiting severe defects in each treatment. Embryos at 2.5 µM completely exhibited the severe phenotype, this percentage dropped drastically when the concentration was slightly lowered. This may indicate that the minimum concentration required to produce any significant effect on these biological processes is above 2.5 µM. The severe effect was seen as the complete inhibition of angiogenesis and epiboly, and the partial inhibition was characterized by the inhibition of angiogenesis of more than 50% of vessels.

Of all the compounds tested from this toolbox (60 compounds in total), we identified three compounds (**2.5b**, **3.2d**, and **4.2**) that exhibited the inhibition of angiogenesis at 2.5µM. These results are shown in **Figure 4**.

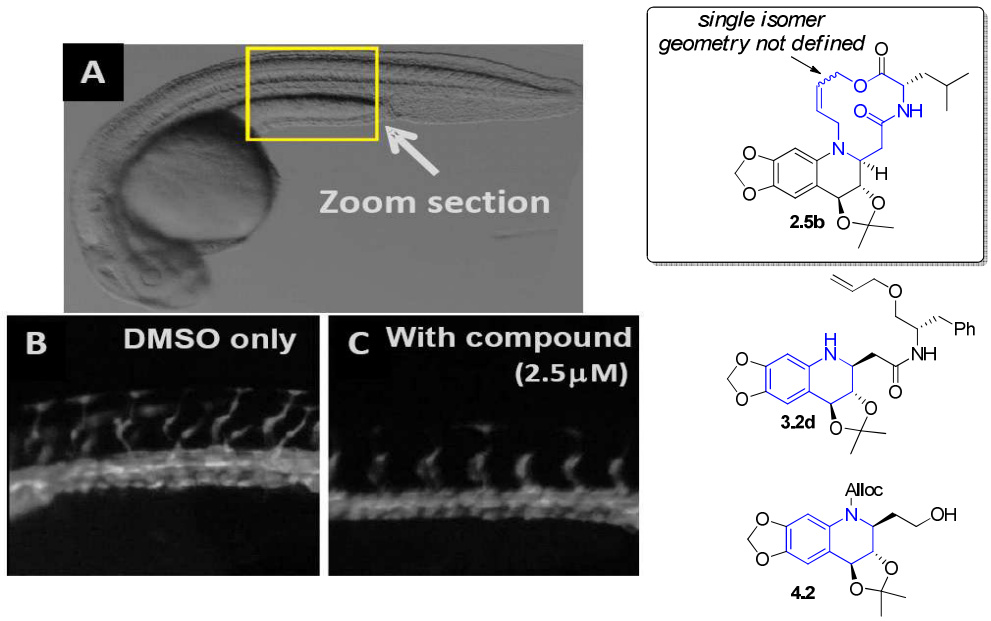
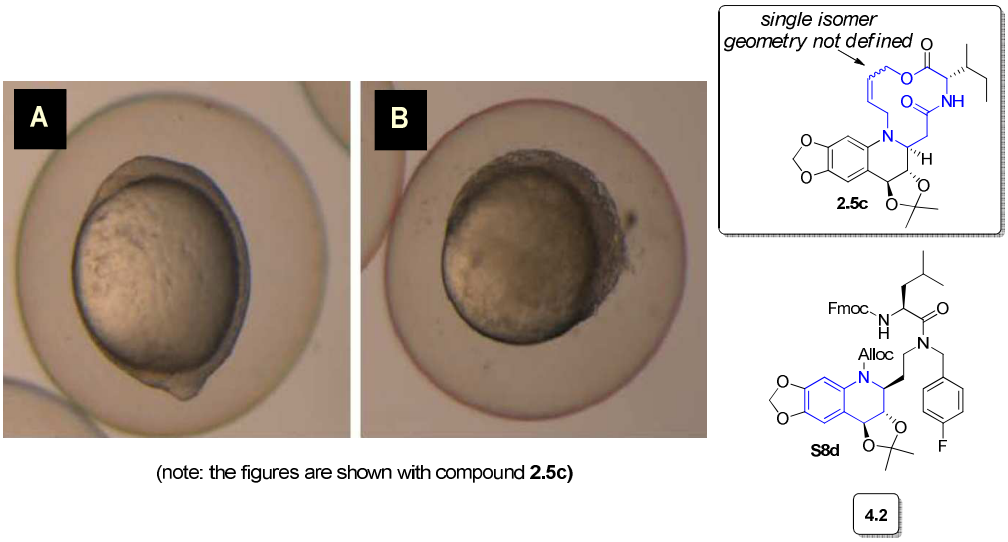


Figure 4: Zebrafish Screen for Angiogenesis: (**A**) zoom section of wildtype or vehicle treated embryo and (**B, C**) zoom sections after treatment with compound **2.5b**. One macrocyclic derivative (**2.5b**) and two tetrahydroquinoline-based compounds (**3.2d** and **4.2**) showed complete inhibition at 2.5 µM.

In another zebrafish screen to search for functional small molecules affecting an early embryonic development (see **Figure 5**), we identified three compounds (**2.5c**, **S8d**, and **4.2**) that inhibited at 2.5 µM.



(note: the figures are shown with compound **2.5c**)

Figure 5: Zebrafish Screen for an Early Embryo Development: (**A**) DMSO exposed embryos at 10 hpf of development and (**B**) small molecule **2.5c** exposed embryos causing a delay in epiboly. One macrocyclic derivative (**2.5c**) and two tetrahydroquinoline-based compounds (**S8d** and **4.2**) exhibited the complete inhibition of an early embryo development at 2.5 μM.

It is interesting to note that the functional macrocyclic compounds (**2.5b** and **2.5c**) in both assays are structurally related. It would be excellent to find the exact mechanism of action of these compounds and to determine if there is any common mode of action in these two phenotype experiments.

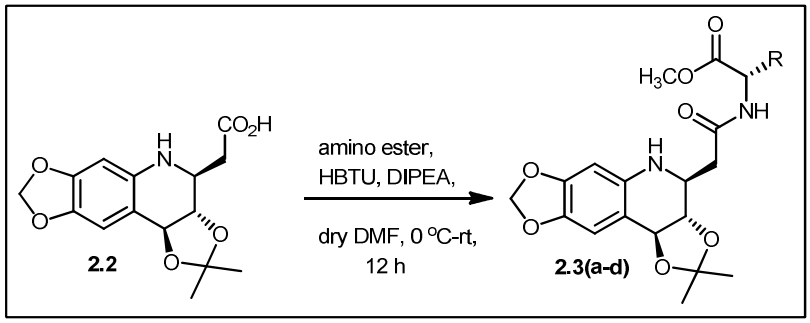
2.7. Conclusion:

With an objective to incorporate different macrocyclic rings onto the tetrahydroquinoline scaffold, we successfully developed several approaches. The presence of the privileged substructure, and, the additional macrocyclic rings (for example, functionalized 12- and 14-membered rings) are two unique features in our design strategy. Further, the incorporation of an amino acid in the large ring skeleton allows an opportunity to modulate the nature of the side chain (for example, chiral polar to nonpolar groups through utilizing natural and un-natural amino acids). Finally, when tested this toolbox in a zebrafish screen, we identified three functional small molecules active as antiangiogenesis agents at 2.5 μM and three as inhibitors of an early embryonic development. To understand

the precise mode of action of these compounds that are active in phenotypic screens, much work would be needed.

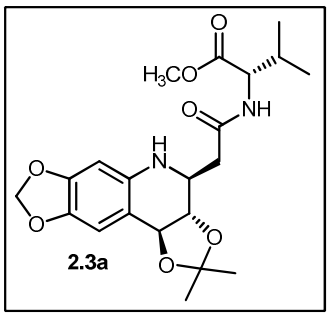
2.8. Experimental Procedures:

2.8.1. Synthesis of 12-Membered Macrocycles [2.5a-d]:



To a solution of **2.2** (1 eq) in dry DMF, was added amino ester (1.5 eq), HBTU (2 eq), and DIPEA (3 eq) at 0 ^{o}C. The reaction mixture was stirred for 12 h. The reaction was quenched with saturated $NaHCO_3$ solution, extracted twice with ethyl acetate and washed with brine. The organic phase was dried over anhydrous Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethyl acetate) afforded the product **2.3(a-d)** as a white solid.

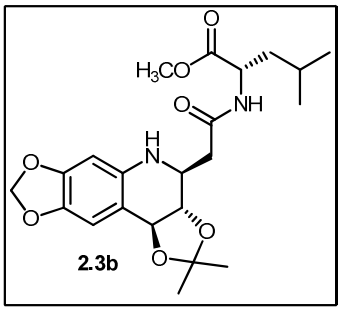
(S)-methyl 2-(2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetra hydrobis[1,3] dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetamido)-3-methylbutanoate (2.3a):



Molecular Formula: $C_{21}H_{28}N_2O_7$; R_f (solvent system): 0.35 (7:3, hexane/ethylacetate), Yield: 60%;^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 6.63 (s, 1H), 6.38-6.27 (m, 1H), 6.11 (s, 1H), 5.79 (dd, *J* = 2.44, 1.48 Hz, 2H), 4.72 (s, 1H), 4.63 (d, *J* = 8.89 Hz, 1H), 4.55 (dd, *J* = 8.70, 4.93 Hz, 1H), 3.93 (d, *J* = 2.19

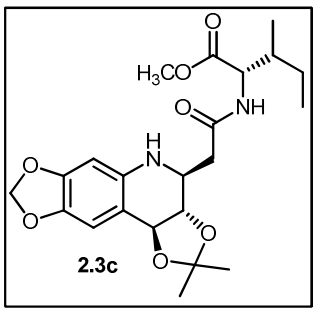
Hz, 1H), 3.74 (s, 3H), 3.53 (t, *J* = 9.42 Hz, 1H), 2.80 (dd, *J* = 14.90, 2.56 Hz, 1H), 2.43-2.33 (m, 1H), 2.21-2.11 (m, 1H), 1.51 (s, 3H), 1.47 (s, 3H), 0.93 (dd, *J* = 14.19, 6.87 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.3, 171.0, 147.4, 139.2, 137.0, 113.2, 111.7, 104.2, 104.2, 100.4, 96.1, 96.0, 79.7, 57.1, 52.5, 40.2, 31.1, 27.1, 26.9, 19.0, 17.8; LRMS: (ES+) m/z = 421.2 (M+1).

(S)-methyl 2-(2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetra hydrobis[1,3] dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetamido)-4-methylpentanoate (2.3b):



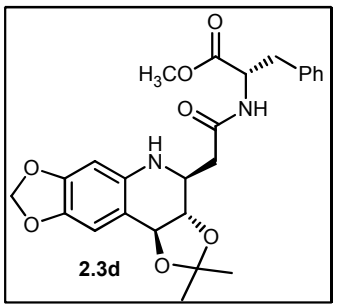
Molecular Formula: $C_{22}H_{30}N_2O_7$; R_f (solvent system): 0.35 (7:3, hexane/ethylacetate), Yield: 70%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.65 (s, 1H), 6.30 (d, *J* = 7.79 Hz, 1H), 6.13 (s, 1H), 5.81 (d, *J* = 2.72 Hz, 2H), 4.74 (s, 1H), 4.65 (d, *J* = 8.91 Hz, 1H), 4.61 (dd, *J* = 8.53, 4.94 Hz, 1H), 3.95 (dt, *J* = 9.82, 2.25 Hz, 1H), 3.75 (s, 3H), 3.55 (t, *J* = 9.43 Hz, 1H), 2.80 (dd, *J* = 14.92, 2.53 Hz, 1H), 2.39 (dd, *J* = 14.91, 9.80 Hz, 1H), 1.90 (tdd, *J* = 9.49, 6.95, 4.91 Hz, 1H), 1.53 (s, 3H), 1.49 (s, 3H), 1.48-1.39 (m, 1H), 1.20-1.12 (m, 1H), 0.92 (dd, *J* = 11.05, 4.16 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm:173.4, 170.9, 147.4, 139.2, 137.0, 113.2, 111.7, 104.2, 100.4, 96.1, 79.7, 52.5, 52.4, 52.4, 50.7, 41.4, 40.1, 27.1, 26.9, 24.9, 22.7, 21.9; LRMS: (ES+) m/z = 435.3 (M+1).

(2S,3S)-methyl2-(2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b tetrahydrobis[1,3] dioxolo [4,5-c:4',5'-g]quinolin-4-yl)acetamido)-3-methylpentanoate (2.3c):



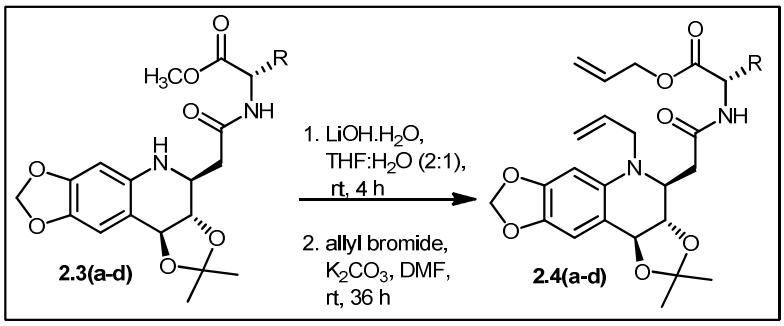
Molecular Formula: $C_{22}H_{30}N_2O_7$; R_f (solvent system): 0.35 (7:3, hexane/ethylacetate), Yield: 70%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.13 (s, 1H), 6.65 (s, 1H), 6.21 (s, 1H), 5.80 (d, *J* = 2.81 Hz, 2H), 4.74 (s, 1H), 4.67-4.58 (m, 2H), 3.96 (t, *J* = 9.88 Hz, 1H), 3.75 (s, 3H), 3.54 (t, *J* = 9.42 Hz, 1H), 2.79 (dd, *J* = 14.90, 2.48 Hz, 1H), 2.37 (dd, *J* = 14.90, 9.87 Hz, 1H), 1.71-1.61 (m, 2H), 1.56 (m, 4H), 1.49 (s, 3H), 0.95 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.3, 170.8, 147.4, 139.2, 137.0, 113.2, 111.7, 104.2, 100.4, 96.1, 79.7, 56.4, 52.5, 52.2, 40.2, 37.8, 27.1, 26.9, 25.2, 15.5, 11.5; LRMS: (ES+) m/z = 435.3 (M+1).

(S)-methyl 2-(2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetra hydrobis[1,3] dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetamido)-3-phenylpropanoate (2.3d):



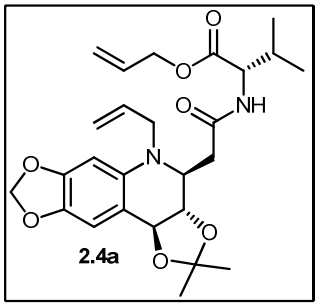
2.3d

Molecular Formula: $C_{28}H_{28}N_2O_7$; R_f (solvent system): 0.35 (7:3, hexane/ethylacetate), Yield: 58%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.32-7.22 (m, 3H), 7.12-7.06 (m, 2H), 6.64 (s, 1H), 6.19 (d, *J* = 8.14 Hz, 1H), 6.09 (s, 1H), 5.80 (d, *J* = 2.39 Hz, 2H), 4.89 (dd, *J* = 13.73, 6.11 Hz, 1H), 4.61 (d, *J* = 8.67 Hz, 2H), 3.91 (dt, *J* = 9.91, 1.89 Hz, 1H), 3.74 (s, 3H), 3.50 (t, *J* = 9.44 Hz, 1H), 3.18 (dd, *J* = 13.94, 5.65 Hz, 1H), 3.07 (dd, *J* = 13.88, 6.35 Hz, 1H), 2.73 (dd, *J* = 14.92, 2.35 Hz, 1H), 2.29 (dd, *J* = 14.92, 10.02 Hz, 1H), 1.50 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.8, 170.6, 147.4, 139.3, 137.0, 135.6, 129.1, 128.7, 127.3, 113.2, 111.7, 104.3, 100.4, 96.1, 79.6, 53.1, 52.4, 40.2, 37.8, 27.0, 26.9; LRMS: (ES+) m/z = 469.4 (M+1).



1. To a solution of **2.3(a-d)** (1 eq) in THF: H_2O (2:1) was added LiOH.H_2O (5 eq) at room temperature and allowed to stir until starting material disappeared on TLC. The reaction mixture was then acidified with 5% HCl and the compound extracted twice with ethylacetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated afforded the carboxylic acid product as light yellow oil which is subjected to allylation without further purification.
2. To a solution of the above carboxylic acid product (1 eq) in dry DMF was added allyl bromide (5 eq), K_2CO_3 (5 eq), at room temperature. The reaction mixture was allowed to stir for 30 h. Water was added to the reaction mixture, extracted twice with ethyl acetate and washed with brine. The organic phase was dried over anhydrous Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethyl acetate) afforded the bisallyl product **2.4(a-d)** as a light yellow oil.

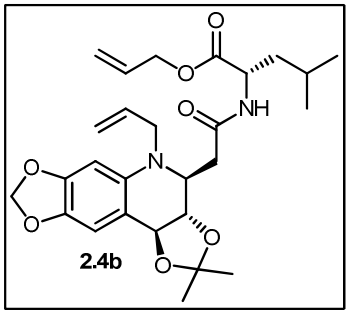
(S)-allyl 2-(2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1, 3] dioxolo [4, 5-c:4',5'-g]quinolin-4-yl)acetamido)-3-methylbutanoate (2.4a):



Molecular Formula: $C_{26}H_{34}N_2O_7$; R_f (solvent system): 0.4 (7:3, hexane/ethyl acetate),Yield: 80%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.70-6.63 (m, 2H), 6.39 (s, 1H), 5.91-5.77 (m, 4H), 5.34-5.17 (m, 4H), 4.60-4.49 (m, 4H), 4.03 (m, 1H), 3.88-3.73 (m, 2H), 3.68 (t, *J* = 9.49 Hz, 1H), 2.66 (m, 2H), 2.16 (m, 1H), 1.53 (s,

3H), 1.48 (s, 3H), 0.92 (dd, *J* = 11.98, 6.88 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.5, 170.1, 147.3, 140.3, 138.7, 134.7, 131.6, 118.9, 117.6, 117.5, 113.1, 103.3, 100.7, 99.1, 80.7, 76.3, 65.7, 57.5,57.1, 54.6, 39.7, 31.1, 27.1, 27.0, 19.0, 17.8; LRMS: (ES+) m/z = 487.4 (M+1).

(S)-allyl 2-(2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1, 3] dioxolo [4, 5-c:4',5'-g]quinolin-4-yl)acetamido)-4-methylpentanoate (2.4b):

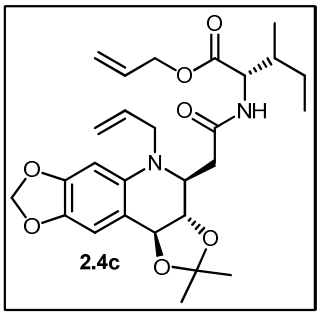


Molecular Formula: $C_{27}H_{36}N_2O_7$; R_f (solvent system): 0.4 (7:3, hexane/ethyl acetate); Yield: 50%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.67 (s, 1H), 6.57 (d, *J* = 7.87 Hz, 1H), 6.38 (s, 1H), 5.89-5.76 (m, 4H), 5.24 (m, 5H), 4.61-4.54 (m, 3H), 4.49 (d, *J* = 9.07 Hz, 1H), 4.00 (dd, *J* = 16.75, 4.67 Hz, 1H), 3.85-3.71 (m, 2H), 3.67 (t, *J* = 9.50 Hz, 1H), 2.62 (dq, *J* = 15.37, 4.68 Hz, 2H), 1.64-1.55 (m, 2H), 1.52 (s, 3H), 1.46 (s, 3H), 0.93-0.90 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.5, 170.8, 169.9, 147.4, 147.3, 139.3, 138.6, 137.0, 134.6, 131.6, 131.5, 119.0, 118.7, 117.8, 117.6, 113.2, 113.1, 111.7, 104.3, 104.3, 103.3, 103.3, 100.7, 100.5, 99.2, 99.2, 96.1, 96.1, 80.6, 79.7, 76.4, 66.0, 65.7, 57.4, 54.7, 52.6, 50.9, 50.8, 41.6, 41.5, 40.2, 39.5, 27.1, 27.0, 27.0, 25.0, 24.9, 22.8, 22.8, 22.0, 21.9; LRMS: (ES+) m/z = 501.5 (M+1).

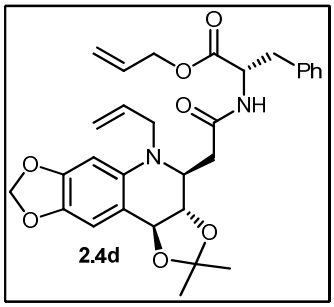
(2S,3S)-allyl 2-(2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b tetrahydro bis[1,3]dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetamido)-3-methylpentanoate (2.4c):

Molecular Formula: $C_{27}H_{36}N_2O_7$; R_f (solvent system): 0.4 (7:3, hexane/ethyl acetate),Yield: 50%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.70-6.64 (m, 2H), 6.39 (s, 1H), 5.87-5.77 (m, 4H), 5.34-5.20 (m, 4H), 4.65-4.56 (m, 4H), 4.53-4.48 (m, 1H), 4.02 (m, 1H), 3.85-3.78 (m, 1H), 3.68 (m, 1H), 2.65 (dd, *J* = 4.71, 1.73 Hz, 2H), 1.87 (m, 1H), 1.53 (s, 3H), 1.48 (s, 3H), 1.16(m, 2H), 0.93-0.88 (m,

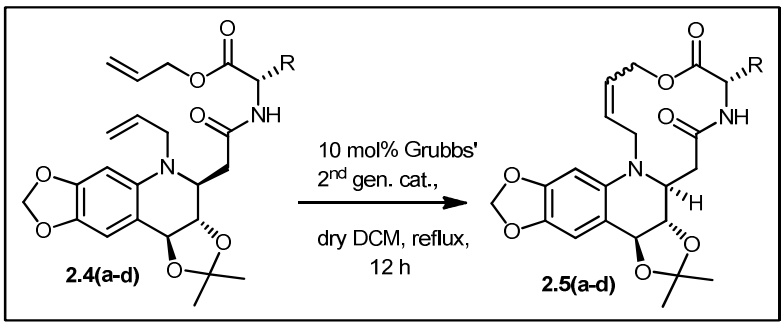
6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm:171.4, 169.9, 147.3, 140.4, 138.7, 134.7, 131.6, 118.9, 117.6, 113.1, 103.3, 100.7, 99.2, 80.7, 76.4, 65.7, 57.5, 56.5, 54.6, 39.7, 37.8, 27.1, 27.0, 25.3, 15.5, 11.6; LRMS: (ES+) m/z = 501.5 (M+1).



(S)-allyl2-(2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetrahydrobis [1,3]dioxolo[4,5-c:4',5'-g]quinolin-4-yl)acetamido)-3-phenylpropanoate (2.4d):

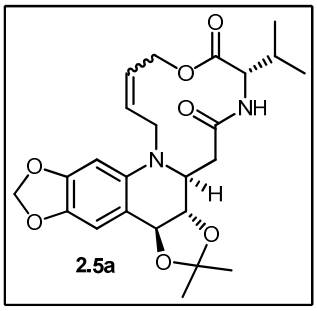


Molecular Formula: $C_{30}H_{34}N_2O_7$; R_f (solvent system): 0.45 (7:3, hexane/ethyl acetate),Yield: 70%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.31-7.23 (m, 3H), 7.14-7.08 (m, 2H), 6.71-6.63 (m, 2H), 6.17 (s, 1H), 5.84 (dd, *J* = 8.61, 1.27 Hz, 2H), 5.81-5.64 (m, 2H), 5.30-5.11 (m, 4H), 4.92 (td, *J* = 7.69, 5.91 Hz, 1H), 4.62-4.42 (m, 3H), 3.87 (dd, *J* = 16.65, 4.81 Hz, 1H), 3.76 (td, *J* = 9.42, 4.56 Hz, 1H), 3.63 (td, *J* = 15.13, 8.04 Hz, 2H), 3.09 (t, *J* = 8.06 Hz, 2H), 2.59 (dq, *J* = 15.32, 4.57 Hz, 2H), 1.44-1.38 (m, 3H), 1.49 (d, *J* = 6.53 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 170.9, 169.6, 147.2, 140.4, 138.3, 135.9, 134.2, 131.3, 129.3, 129.1, 128.5, 128.4, 127.0, 118.9, 117.9, 117.6, 112.9, 103.1, 100.6, 99.4, 79.9, 76.2, 65.8, 57.1, 54.4, 52.9, 38.8, 37.7, 26.8; LRMS: (ES+) m/z = 535.6 (M+1).



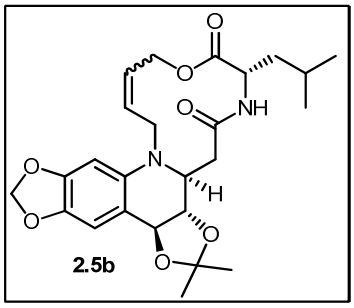
Bis allyl compound **2.4(a-d)** (1 eq) was taken in dry dichloromethane under nitrogen atmosphere and Grubbs' 2nd generation catalyst (0.1 eq) was added and reaction mixture was heated to 40 $^{\circ}$C for 12 h. The reaction mixture was concentrated and the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethyl acetate) afforded the product **2.5(a-d)**.

(3aS,3bS,7S,19bS,E/Z)-7-isopropyl-2,2-dimethyl-3b,4,6,7,13,19b-hexahydro bis[1,3]dioxolo[4,5-c:4',5'-g][1,4,8]oxadiazacyclododeca[8,7-a]quinoline-5,8 (3aH,10H)-dione (2.5a):



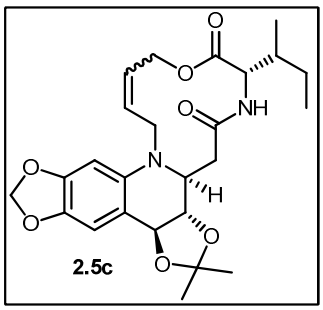
Molecular Formula: $C_{24}H_{30}N_2O_7$; R_f (solvent system): 0.3 (7:3, hexane/ethyl acetate); Yield: 23%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.58 (s, 1H), 5.99-5.92 (m, 1H), 5.88 (d, *J* = 11.66 Hz, 2H), 5.82-5.73 (m, 1H), 5.51-5.40 (m, 1H), 4.71-4.61 (m, 1H), 4.37 (d, *J* = 10.39 Hz, 3H), 4.26-4.14 (m, 1H), 3.93-3.85 (m, 1H), 3.67 (s, 1H), 3.39 (s, 1H), 2.80 (d, *J* = 13.13 Hz, 1H), 2.39-2.22 (m, 3H), 1.57 (s, 3H), 1.49 (s, 3H), 1.03 (dd, *J* = 12.83, 6.87 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.3, 170.8, 147.7, 139.6, 135.9, 125.4, 114.2, 107.8, 101.0, 99.8, 61.9, 59.1, 46.8, 43.2, 37.0, 29.7, 28.9, 22.7, 19.4, 18.4; LRMS: (ES+) m/z = 459.5 (M+1).

(3aS,3bS,7S,19bS,E/Z)-7-isobutyl-2,2-dimethyl-3b,4,6,7,13,19b-hexahydrobis [1,3]dioxolo[4,5-c:4',5'-g][1,4,8]oxadiazacyclododeca[8,7-a]quinoline-5,8 (3aH,10H)-dione (2.5b):



Molecular Formula: $C_{25}H_{32}N_2O_7$; R_f (solvent system): 0.3 (7:3, hexane/ethyl acetate),Yield: 22%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.58 (s, 1H), 5.88 (m, 3H), 5.51-5.42 (m, 1H), 5.81-5.74 (m, 1H), 4.65-4.60 (m, 1H), 4.58-4.51 (m, 1H), 4.38 (m, 2H), 4.30-4.21 (m, 1H), 3.94-3.87 (m, 1H), 3.68 (t, *J* = 9.6 Hz, 1H), 3.40 (t, *J* = 9.6Hz, 1H), 2.76 (d, *J* = 12.90 Hz, 1H), 2.34 (m, 1H), 2.07-1.99 (m, 1H), 1.75 (m, 1H), 1.57 (s, 3H), 1.48 (s, 3H), 1.28 (m, 1H), 0.97 (dd, *J* = 11.69, 6.04 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 173.6, 171.8, 147.5, 141.5, 138.1, 133.4, 124.0, 120.4, 112.8, 102.6, 100.9, 81.8, 60.5, 57.7, 51.9, 44.1, 39.4, 33.8, 31.9, 29.7, 27.1, 24.9, 23.0, 21.4, 14.1; LRMS: (ES+) m/z = 472.5 (M+1).

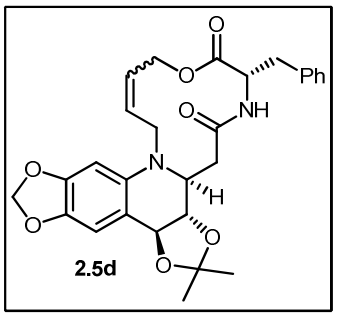
(3aS,3bS,7S,19bS,E/Z)-7-sec-butyl-2,2-dimethyl-3b,4,6,7,13,19b-hexahydro bis[1,3]dioxolo[4,5-c:4',5'-g][1,4,8]oxadiazacyclododeca[8,7-a]quinoline-5,8 (3aH,10H)-dione (2.5c):



Molecular Formula: $C_{25}H_{32}N_2O_7$; R_f (solvent system): 0.3 (7:3, hexane/ethyl acetate),Yield: 28%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.59 (s, 1H), 5.94-5.86 (m, 3H), 5.79 (m, 1H), 5.47 (m, 1H), 4.71-4.63 (m, 1H), 4.47 (m,

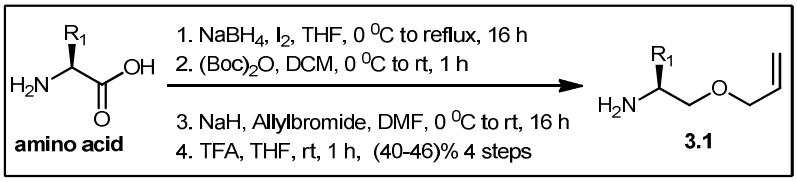
1H), 4.40-4.31 (m, 2H), 4.20 (m, 1H), 3.90 (m, 1H), 3.66 (t, *J* = 9.65 Hz, 1H), 3.39 (t, *J* = 9.60 Hz, 1H), 2.79 (d, *J* = 12.97 Hz, 1H), 2.34 (m, 1H), 2.04-1.96 (m, 1H), 1.57 (s, 3H), 1.55-1.48 (m, 5H), 1.02 (d, *J* = 6.86 Hz, 3H), 0.96 (m, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 173.8, 171.0, 147.5, 141.6, 138.1, 133.4, 124.1, 120.5, 112.8, 102.6, 100.9, 81.9, 76.1, 60.3, 58.1, 57.7, 51.2, 44.3, 35.6, 29.7, 27.1, 25.4, 16.0, 11.5; LRMS: (ES+) m/z = 472.5 (M+1).

(3aS,3bS,7S,19bS,E/Z)-7-benzyl-2,2-dimethyl-3b,4,6,7,13,19b-hexahydro bis[1,3]dioxolo[4,5-c:4',5'-g][1,4,8]oxadiazacyclododeca[8,7-a]quinoline-5,8 (3aH,10H)-dione (2.5d):



Molecular Formula: $C_{28}H_{30}N_2O_7$; R_f (solvent system): 0.3 (7:3, hexane/ethyl acetate),Yield: 29%;^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.55 (s, 1H), 6.65 (s, 1H), 7.36-7.19 (m, 6H), 5.98 (d, *J* = 8.03 Hz, 1H), 5.87 (dd, *J* = 13.28, 1.16 Hz, 2H), 5.72 (d, *J* = 5.17 Hz, 1H), 5.44 (d, *J* = 2.26 Hz, 1H), 4.80 (d, *J* = 4.57 Hz, 1H), 4.66-4.57 (m, 1H), 4.50 (dd, *J* = 13.59, 6.65 Hz, 1H), 4.35 (d, *J* = 9.15 Hz, 1H), 4.26 (dd, *J* = 16.53, 11.06 Hz, 1H), 3.90 (dd, *J* = 16.23, 1.58 Hz, 1H), 3.63 (t, *J* = 9.69 Hz, 1H), 3.40-3.24 (m, 2H), 3.10 (dd, *J* = 14.49, 9.16 Hz, 1H), 2.70 (d, *J* = 13.14 Hz, 1H), 2.25 (dd, *J* = 13.23, 9.53 Hz, 1H), 1.55 (s, 3H), 1.47 (d, *J* = 5.76 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm:173.4, 170.9, 147.5, 141.4, 138.0, 136.2, 132.9, 128.9, 128.8, 127.2, 123.9, 120.2, 112.8, 102.7, 102.2, 100.8, 81.6, 61.0, 57.6, 54.1, 50.8, 43.8, 36.2, 29.7, 27.1, 27.0; LRMS: (ES+) m/z = 507.5 (M+1).

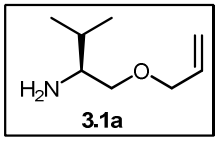
2.8.2. Synthesis of Amino Acid Building Block [3.1(a-d)]:



1. To a stirred solution of amino acid (18.18 mmol) and $NaBH_4$ (1.65 g, 43.6 mmol) in 50 mL THF, I_2 (4.59 g, 18.18 mmol) in 50 mL THF was added slowly at 0 ^{0}C for 30 min, reflux for 16 h, cool the reaction to 0 $^\circ$C and quenched with methanol cautiously, solvents were removed under reduced pressure, 150 mL 20% KOH solution was added, stirred for another 6 h. After completion of reaction (monitored by TLC), 50 mL brine solution was added and extracted with ethyl acetate (3×200 mL). Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude.
2. To the above crude in 60 ml DCM, $(Boc)_2O$ (26.45 mmol) was added slowly at 0 $^\circ$C, stirred at rt for 1 h. After completion of reaction (monitored by TLC), 50 mL saturated $NaHCO_3$ solution was added and extracted with ethyl acetate (2 × 150 mL). Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude.
3. To the above crude in 100 mL dry DMF, NaH (41.66 mmol) was added portionwise at 0 $^\circ$C, followed by allylbromide (41.66 mmol), stirred at rt for 16 h. After completion of reaction (monitored by TLC), 100 mL saturated ammonium chloride solution was added cautiously and extracted with ethyl acetate (2×100 mL). Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude , which was purified by flash chromatography giving the pure compound.
4. To the above pure compound in 10 mL dry THF, 10 mL TFA was added at 0 $^\circ$C, stirred at rt for 2 h. After completion of reaction (monitored by TLC), 100 mL saturated $NaHCO_3$ solution was added cautiously and extracted with ethyl acetate (2×100 mL). Purification of compound by

acid-base neutralisation technique, then organic layer was dried over anhydrous sodium sulfate, filtered and concentrated afforded the amino alcohol buildingblock **(3.1)**.

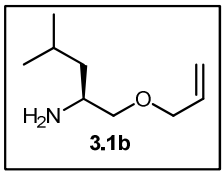
(S)-1-(allyloxy)-3-methylbutan-2-amine (3.1a):



3.1a

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 5.87 (m, 1H), 5.21 (d, *J* = 10.42 Hz, 1H), 5.28 (d, *J* = 17.76 Hz, 1H), 4.02 (d, *J* = 5.75 Hz, 2H), 3.65 (dd, *J* = 10.17, 3.51 Hz, 1H), 3.52 (t, *J* = 9.19 Hz, 1H), 3.14-3.06 (m, 1H), 2.03 (m, 1H), 1.06 (d, *J* = 6.81 Hz, 3H), 0.99 (d, *J* = 6.82 Hz, 3H),: ^{13}C NMR (100MHz, $CDCl_3$) δ ppm: 133.8, 117.9, 72.2, 67.4, 57.1, 28.3, 18.8, 18.2; LRMS: (ES+) m/z = 144.2 (M+1).

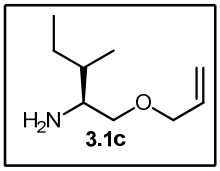
(S)-1-(allyloxy)-4-methylpentan-2-amine (3.1b):



3.1b

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 5.87 (m, 1H), 5.31-5.17 (m, 2H), 4.01 (d, *J* = 5.77 Hz, 2H), 3.60 (dd, *J* = 10.01, 3.09 Hz, 1H), 3.51-3.35 (m, 2H), 1.68 (m, 1H), 1.53 (m, 2H), 0.92 (dd, *J* = 6.26, 3.81 Hz, 6H); ^{13}C NMR (100MHz, *CDCl$_3$*) δ ppm: 133.7, 117.9, 72.2, 68.7, 50.0, 38.1, 21.7, 24.2, 22.5; LRMS: (ES+) m/z = 158.1 (M+1).

(2S,3S)-1-(allyloxy)-3-methylpentan-2-amine (3.1c):

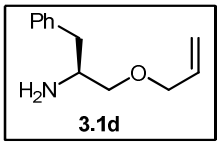


3.1c

^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 5.28 Hz, 1H), 5.86 (ddd, *J* = 16.31, 10.91), 5.27 (dd, *J* = 17.23, 1.29 Hz, 1H), 5.21 (d, *J* = 10.37 Hz, 1H), 4.01 (d, *J* = 5.66 Hz, 2H), 3.62 (dd, *J* = 10.21, 3.64 Hz, 1H), 3.56-3.49 (m, 1H), 3.27-3.20 (m,

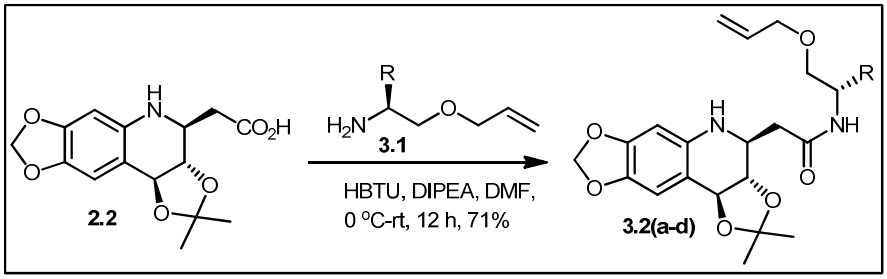
1H), 1.88-1.76 (m, 1H), 1.58-1.50 (m, 1H), 1.31-1.19 (m, 1H), 0.98-0.89 (m, 6H); ^{13}C NMR (100MHz, *CDCl$_3$*) δ ppm ^{13}C NMR (100MHz, CDCl$_3$) δ ppm: 133.7, 118.0, 72.2, 66.9, 55.5, 34.8, 25.6, 14.1, 10.9; LRMS: (ES+) m/z = 158.1 (M+1).

(S)-1-(allyloxy)-3-phenylpropan-2-amine (3.1d):



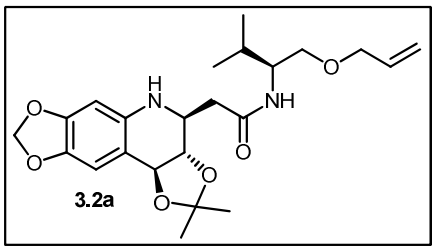
^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.25 (m, 5H), 5.56 Hz, 1H), 5.92 (ddd, *J* = 22.66, 10.78, 5.32-5.23 (m, 1H), 5.17 (d, *J* = 10.38 Hz, 1H), 3.99 (d, *J* = 5.45 Hz, 2H), 3.47-3.40 (m, 1H), 3.32-3.19 (m, 2H), 2.79 (dd, *J* = 13.34, 4.87 Hz, 1H), 2.55 (dd, *J* = 13.33, 7.83 Hz, 1H); ^{13}C NMR (100MHz, *CDCl$_3$*) δ ppm: 138.8, 134.7, 129.2, 40.7, 52.4, 72.1, 74.9, 116.9, 126.2, 128.4,; LRMS: (ES+) m/z = 192.1 (M+1).

2.8.3. Synthesis of 12-Membered Macrocycles [3.4a-d]:



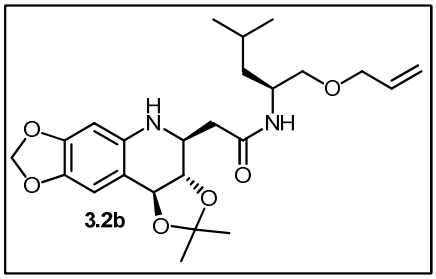
To a solution of Carboxylic acid **2.2** (1 eq) in dry DMF, were added primary amine **3.1** (1.5 eq), HBTU (2 eq), and DIPEA (3 eq) at 0 ^{o}C. The reaction mixture was stirred for 12 h. The reaction was quenched with saturated $NaHCO_3$ solution, extracted twice with ethyl acetate and washed with brine. The organic phase was dried over anhydrous Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethylacetate) afforded the product **3.2(a-d)**.

N-((S)-1-(allyloxy)-3-methylbutan-2-yl)-2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5, 10b-tetra hydrobis[1,3]dioxolo[4,5-c:4',5'-g] quinolin-4-yl)acetamide (3.2a):



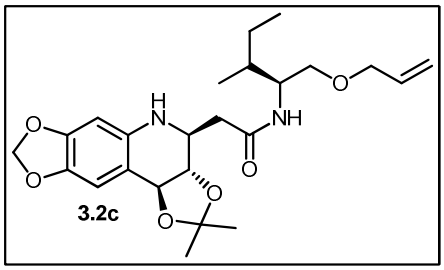
Molecular Formula: $C_{23}H_{32}N_2O_6$; R_f (solvent system): 0.25 (7:3, hexane/ethyl acetate), Yield: 70%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.14 (s, 1H), 5.95-5.83 (m, 2H), 5.81 (s, 1H), 5.80(s, 1H), 5.22 (m, 2H), 4.65 (d, *J* = 8.85 Hz, 1H), 4.03-3.90 (m, 3H), 3.89-3.81 (m, 1H), 3.55 (m, 2H), 3.40 (dd, *J* = 9.70, 3.80 Hz, 1H), 2.77-2.69 (m, 1H), 2.36 (dd, *J* =14.82, 10.04 Hz, 1H), 1.91 (m, 1H), 1.54 (s, 3H), 1.52 (s, 3H), 0.95 (t, *J* = 7.46 Hz, 6H)**;** ^{13}C NMR (101 MHz, *CDCl$_3$*) δ ppm: 170.57, 147.42, 139.18, 137.11, 134.39, 117.27, 113.15, 111.63, 104.22, 100.41, 96.05, 79.82, 72.14, 69.77, 54.21, 52.66, 40.39, 29.37, 27.08, 26.96, 19.49, 19.07**;** LRMS: (ES+) m/z = 433.2 (M+1).

N-((S)-1-(allyloxy)-4-methylpentan-2-yl)-2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetra hydrobis[1,3]dioxolo[4,5-c:4',5'-g] quinolin-4-yl)acetamide (3.2b):



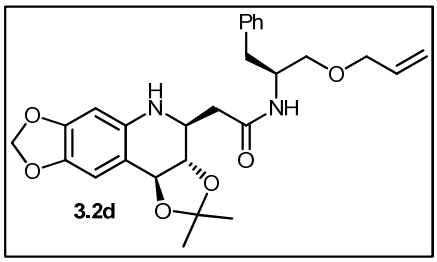
Molecular Formula: $C_{24}H_{34}N_2O_6$; R_f (solvent system): 0.5 (1:1, hexane/ethyl acetate); Yield: 50%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.65 (s, 1H), 6.14 (s, 1H), 5.94-5.78 (m, 4H), 5.22 (m, 2H), 4.86 (s, 1H), 4.64 (d, *J* = 8.84 Hz, 1H), 4.17 (m, 1H), 4.03-3.90 (m, 3H), 3.53 (t, *J* = 9.42 Hz, 1H), 3.43 (d, *J* = 3.49 Hz, 2H), 2.69 (dd, *J* = 14.74, 1.85 Hz, 1H), 2.32 (dd, *J* = 14.76, 10.02 Hz, 1H), 1.60 (m, 1H), 1.53 (s, 3H), 1.51 (s, 3H), 1.47-1.35 (m, 2H), 0.93 (m, 6H); ^{13}C NMR (101 MHz, *CDCl$_3$*) δ ppm: 170.32, 147.41, 139.15, 137.12, 134.42, 117.24, 113.14, 111.62, 104.21, 100.40, 96.02, 79.78, 72.18, 71.77, 52.69, 47.28, 40.88, 40.38, 27.07, 26.96, 24.96, 22.87, 22.37; LRMS: (ES+) m/z = 446.2 (M+1).

N-((2S,3R)-1-(allyloxy)-3-methylpentan-2-yl)-2((3aS,4S,10bS)-2,2-dimethyl-3a, 4,5,10b-tetrahydrobis[1,3]dioxolo[4,5-c:4',5'-g] quinolin-4-yl)acetamide (3.2c):



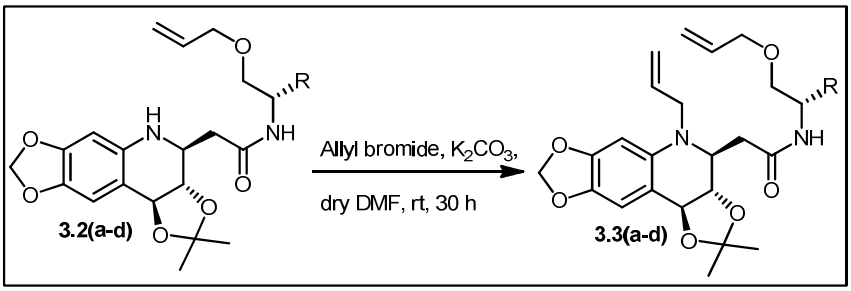
Molecular Formula: $C_{24}H_{34}N_2O_6$; R_f (solvent system): 0.5 (1:1, hexane/ethyl acetate); Yield: 58%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.66 (s, 1H), 6.14 (s, 1H), 5.95-5.83 (m, 2H), 5.81 (d, *J* = 2.90 Hz, 2H), 5.30-5.16 (m, 2H), 4.65 (d, *J* = 8.91 Hz, 1H), 4.02-3.87 (m, 4H), 3.60-3.51 (m, 2H), 3.41 (dd, *J* = 9.76, 3.63 Hz, 1H), 2.73 (dd, *J* = 14.83, 2.38 Hz, 1H), 2.36 (dd, *J* = 14.84, 9.98 Hz, 1H), 1.72-1.62 (m, 1H), 1.54 (s, 3H), 1.50 (s, 3H), 1.22-1.07 (m, 2H), 0.91 (m, 6H); ^{13}C NMR (400 MHz, *CDCl$_3$*) δ ppm: 170.51, 147.43, 139.19, 137.12, 134.39, 117.31, 113.16, 111.64, 104.22, 100.41, 96.05, 79.81, 72.16, 69.59, 53.05, 52.68, 40.42, 35.84, 27.08, 26.96, 25.68, 15.49, 11.36; LRMS: (ES+) m/z = 447.2 (M+1).

N-((S)-1-(allyloxy)-3-phenylpropan-2-yl)-2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1,3]dioxolo[4,5-c:4',5'-g]quinolin-4-yl) acetamide (3.2d):



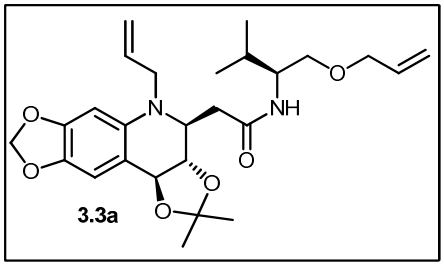
Molecular Formula: $C_{27}H_{32}N_2O_6$; R_f (solvent system): 0.5 (1:1, hexane/ethylacetate); Yield: 70%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.35-7.16 (m, 6H), 6.65 (s, 1H), 6.10 (s, 1H), 6.02-5.83 (m, 2H), 5.80 (d, *J* = 3.01 Hz, 2H), 5.24 (ddd, *J* = 13.80, 11.40, 1.24 Hz, 2H), 4.74-4.57 (m, 2H), 4.32 (ddd, *J* = 11.70, 7.70, 4.25 Hz, 1H), 4.03-3.88 (m, 3H), 3.50 (t, *J* = 9.43 Hz, 1H), 3.37 (d, *J*

= 3.53 Hz, 2H), 2.27 (dd, *J* = 14.82, 10.15 Hz, 1H), 1.52 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (101 MHz, *CDCl$_3$*) δ ppm: 170.28, 147.41, 139.17, 137.79, 137.07, 134.30, 129.30, 128.50, 126.56, 117.42, 113.14, 111.63, 104.23, 100.42, 96.03, 79.71, 72.19, 69.74, 52.60, 50.21, 40.38, 37.49, 27.08, 26.96; LRMS: (ES+) m/z = 480 (M+1).



To a solution of the coupling compound **3.2(a-d)** (1 eq) in dry DMF was added allyl bromide (5 eq), K_2CO_3 (5 eq), at room temperature. The reaction mixture was allowed to stir for 30 h. Water was added to the reaction mixture, extracted twice with ethyl acetate and later washed with brine. The organic phase was dried over anhydrous Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethyl acetate) afforded the product **3.3(a-d)**.

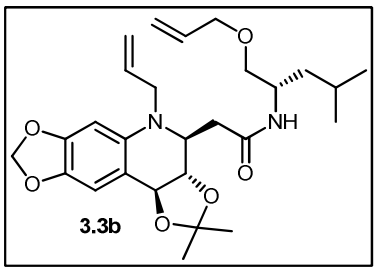
2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetra hydrobis[1,3]dioxolo [4,5-c:4',5'-g]quinolin-4-yl)-N-((S)-1-(allyloxy)-3-methylbutan-2-yl)acetamide (3.3a):



Molecular Formula: $C_{26}H_{36}N_2O_6$**;** R_f (solvent system): 0.26 (7:3, hexane/ethyl acetate)**;** Yield: 80%**;** ^{1}H NMR (400 MHz, *CDCl$_3$*, δ ppm: 6.69 (s, 1H), 6.40 (s, 1H), 6.30 (d, 1H), 5.89-5.74 (m, 4H), 5.20 (m, Hz, 4H), 4.50 (d, *J* = 9.08 Hz, 1H), 4.03 (dd, *J* = 16.76, 4.67 Hz, 1H), 3.90-3.73 (m, 5H), 3.64 (t, *J* = 9.52 Hz, 1H), 3.41 (dd, *J* = 9.61, 4.19 Hz, 1H), 3.31 (dd, *J* = 9.63, 4.15 Hz, 1H), 2.67-2.54

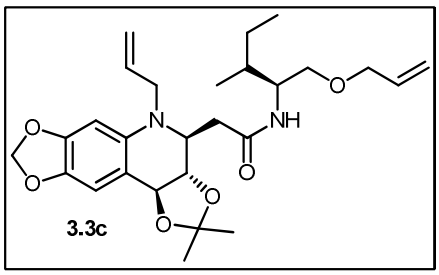
(m, 2H), 1.88 (m, 1H), 1.53 (s, 3H), 1.49 (s, 3H), 0.92 (t, *J* = 7.28 Hz, 6H)**;** ^{13}C NMR (101 MHz, *CDCl*$_3$, δ ppm: 169.67, 147.27, 140.34, 138.66, 134.61, 134.58, 117.69, 117.53, 116.95, 112.93, 103.31, 100.66, 99.14, 80.48, 72.00, 69.90, 57.60, 54.57, 53.91, 39.59, 29.20, 27.05, 27.02, 19.50, 18.79**;** LRMS: (ES+) m/z = 473.2 (M+1).

2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1,3]dioxolo [4,5-c:4',5'-g]quinolin-4-yl)-N-((S)-1-(allyloxy)-4-methylpentan-2-yl) acetamide (3.3b):



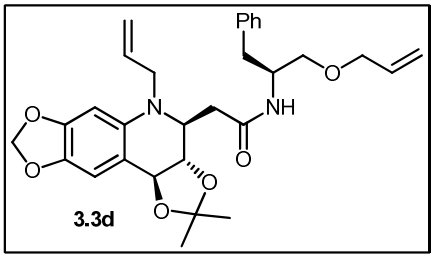
Molecular Formula: $C_{27}H_{38}N_2O_6$**;** R_f (solvent system): 0.5 (1:1, hexane/ethyl acetate)**;** Yield: 80%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 6.68 (s, 1H), 6.65 (s, 1H), 6.39 (s, 1H), 6.14 (s, 2H), 5.91-5.78 (m, 10H), 5.31-5.12 (m, 8H), 4.86 (s, 1H), 4.65 (d, *J* = 8.87 Hz, 1H), 4.50 (d, *J* = 9.08 Hz, 1H), 4.15 (ddd, *J* = 9.06, 7.39, 3.32 Hz, 3H), 4.05-3.72 (m, 11H), 3.62 (t, *J* = 9.51 Hz, 2H), 3.53 (t, *J* = 9.43 Hz, 1H), 3.43 (d, *J* = 3.55 Hz, 2H), 3.33 (d, *J* = 3.76 Hz, 3H), 2.69 (dd, *J* = 14.76, 2.04 Hz, 1H), 2.62-2.50 (m, 3H), 2.32 (dd, *J* = 14.74, 10.05 Hz, 1H), 1.59 (dd, *J* = 13.64, 6.96 Hz, 3H), 1.53 (s, 7H), 1.49 (s, 4H), 1.47 (s, 4H), 1.38 (ddd, *J* = 12.69, 9.59, 5.88 Hz, 5H), 0.95-0.90 (m, 12H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ ppm: 170.32, 169.42, 147.42, 147.27, 140.29, 139.16, 138.71, 137.12, 134.76, 134.61, 134.42, 117.64, 117.39, 117.24, 116.94, 113.15, 112.93, 111.63, 104.21, 103.28, 100.66, 100.41, 99.04, 96.02, 80.60, 79.79, 72.19, 72.08, 72.02, 71.78, 57.61, 54.52, 52.69, 47.29, 47.14, 41.00, 40.88, 40.39, 39.80, 29.67, 27.07, 27.04, 26.96, 24.96, 24.84, 23.08, 22.87, 22.37, 22.20; LRMS: (ES+) m/z = 446.2 (M+1).

2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10btetrahydro bis[1,3]dioxolo [4,5-c:4',5'-g]quinolin-4-yl)-N-((2S,3R)-1-(allyloxy)-3-methylpentan-2yl)acetamide (3.3c):



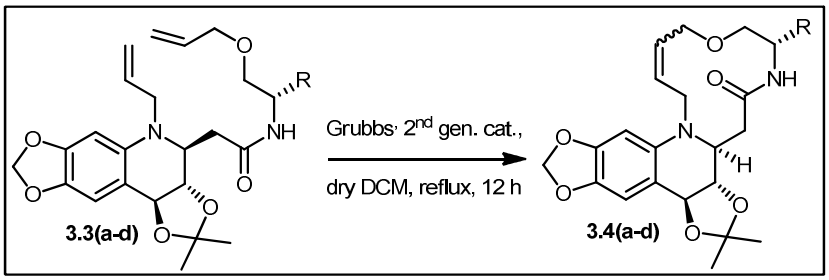
Molecular Formula: $C_{27}H_{38}N_2O_6$**;** R_f (solvent system): 0.5 (1:1, hexane/ethyl acetate)**;** Yield: 60%**;** ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.69 (s, 1H), 6.39 (s, 1H), 6.32 (dd, *J* = 11.21, 7.31 Hz, 1H), 5.88-5.74 (m, 5H), 5.28-5.10 (m, 4H), 4.50 (d, *J* = 9.12 Hz, 1H), 4.03 (dd, *J* = 16.70, 4.67 Hz, 1H), 3.91 (qd, *J* = 13.00, 4.41, 4.10 Hz, 1H), 3.87-3.73 (m, 4H), 3.65 (t, *J* = 9.52 Hz, 1H), 3.43 (dd, *J* = 9.63, 4.12 Hz, 1H), 3.33 (dd, *J* = 9.67, 3.75 Hz, 1H), 2.67-2.54 (m, 2H), 1.63 (ddd, *J* = 9.85, 7.29, 3.47 Hz, 1H), 1.53 (s, 3H), 1.48 (s, 3H), 1.19-1.05 (m, 2H), 0.88 (m, 6H); ^{13}C NMR (400 MHz, *CDCl$_3$*) δ ppm: 169.58, 147.28, 140.33, 138.70, 134.67, 134.60, 117.68, 117.49, 116.99, 112.94, 103.32, 100.67, 99.12, 80.48, 72.04, 69.73, 57.61, 54.55, 52.86, 39.61, 35.79, 27.07, 27.04, 25.50, 15.51, 11.37; LRMS: (ES+) m/z = 487.2 (M+1).

2-((3aS,4S,10bS)-5-allyl-2,2-dimethyl-3a,4,5,10b-tetra hydrobis [1,3]dioxolo [4,5-c:4',5'-g]quinolin-4-yl)-N-((S)-1-(allyloxy)-3-phenylpropan-2-yl)acetamide (3.3d):



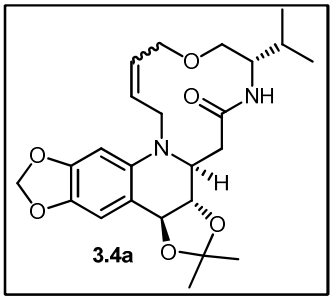
Molecular Formula: $C_{30}H_{36}N_2O_6$; R_f (solvent system): 0.5 (1:1, hexane/ethyl acetate); Yield: 80%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.25 (m, 6H), 6.66 (s, 1H), 6.43-6.34 (m, 1H), 6.23 (s, 1H), 5.82 (dt, *J* = 10.38, 1.97 Hz, 4H), 5.31-5.12 (m, 4H), 4.46 (d, *J* = 9.11 Hz, 1H), 4.37-4.27 (m, 1H), 3.98-3.89 (m, 1H), 3.89-3.83 (m, 2H), 3.83-3.75 (m, 1H), 3.70-3.61 (m, 1H), 3.58 (d, *J* = 9.47 Hz, 1H), 3.22 (t, *J* = 3.95 Hz, 2H), 2.86 (dd, *J* = 6.99, 2.52 Hz, 2H), 2.54 (dd, *J* = 16.82, 4.65 Hz, 2H), 1.44 (s, 3H), 1.51 (d, *J* = 3.40 Hz, 3H); ^{13}C NMR (101 MHz,

CDCl$_3$) δ ppm: 169.48, 147.27, 140.45, 138.53, 137.95, 134.49, 129.43, 129.30, 128.49, 128.42, 126.44, 117.93, 117.58, 117.09, 112.93, 103.28, 100.69, 99.32, 80.31, 72.03, 69.74, 57.48, 54.60, 49.79, 39.44, 37.25, 27.06, 27.03; LRMS: (ES+) m/z = 521.2 (M+1).



Bis allyl compound **3.3(a-d)** (1eq) was taken in dry dichloromethane under nitrogen atmosphere and Grubbs' 2nd generation catalyst (10 mol%) was added and reaction mixture was heated to 40 ^{o} C for 12 h. The reaction mixture was concentrated and the crude product was purified by flash chromatography on silica gel (4:1, hexane/ethylacetate) afforded the product **3.4(a-d)**.

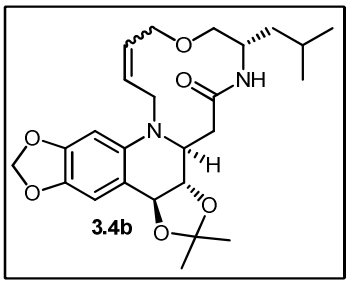
(3aS,3bS,7S,19bS,E/Z)-7-isopropyl-2,2-dimethyl-3b,4,6,7,8,10,13,19b-octa hydrobis[1,3]dioxolo[4,5-c:4',5'-g][1,4,8] oxadiaza cyclododeca[8,7-a]quinol in-5(3aH)-one (3.4a):



Molecular Formula: $C_{24}H_{32}N_2O_6$; R_f (solvent system): 0.3 (1:1, hexane/ethyl acetate); Yield: 80%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 6.69 (s, 1H), 6.23 (s, 1H), 6.08-5.96 (m, 1H), 5.94-5.79 (m, 3H), 5.40 (d, *J* = 8.90 Hz, 1H), 4.71 (t, *J* = 9.75 Hz, 1H), 4.50 (d, *J* = 9.37 Hz, 1H), 4.10-3.99 (m, 1H), 3.95-3.75 (m, 4H), 3.68 (m, *J* = 10.04, 1H), 3.58 (dd, *J* = 11.59, 3.17 Hz, 1H), 3.39 (m, 1H), 2.74 (dd, *J* = 16.03, 2.77 Hz, 1H), 2.45 (dd, *J* = 16.10, 3.63 Hz, 1H), 1.78 (m, 1H), 1.55 (d, *J* = 2.56 Hz, 6H), 0.91 (dd, *J* = 14.46, 6.68 Hz, 6H); ^{13}C NMR (101

MHz, *$CDCl_3$*) δ ppm: 169.51, 147.26, 140.77, 139.70, 133.81, 127.45, 115.97, 112.57, 103.57, 100.56, 97.00, 70.41, 66.84, 60.98, 29.67, 29.64, 28.99, 27.10, 27.05, 19.80, 19.66; LRMS: (ES+) m/z = 445; LRMS: (ES+) m/z = 444.3 (M+1).

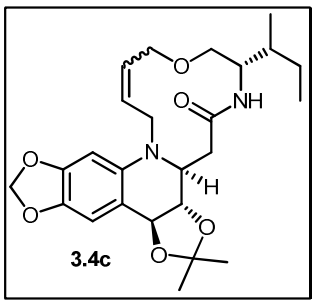
(3aS,3bS,7S,19bS,E/Z)-7-isobutyl-2,2-dimethyl-3b,4,6,7,8,10,13,19b-octahydro bis[1,3] dioxolo[4,5-c:4',5'-g][1,4,8]oxadiaza cyclododeca [8,7-a]quinolin-5(3aH)-one (3.4b):



3.4b

Molecular Formula: $C_{25}H_{34}N_2O_6$**;** R_f (solvent system): 0.4 (1:1, hexane/ethyl acetate)**;** Yield: 66%**;** ^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 6.24 (s, 1H), 6.69 (s, 1H), 6.05-5.97 (m, 1H), 5.83 (d, *J* = 8.77 Hz, 3H), 5.34-5.27 (m, 1H), 4.68-4.59 (m, 1H), 4.49 (d, *J* = 9.16 Hz, 1H), 4.02 (d, *J* = 9.41 Hz, 1H), 3.95 (s, 1H), 3.84 (s, 2H), 3.75-3.56 (m, 4H), 2.71-2.63 (m, 1H), 2.50-2.42 (m, 1H), 1.63-1.54 (m, 7H), 0.93 (d, *J* = 6.42 Hz, 2H), 0.89-0.85 (m, 6H).**;** ^{13}C NMR(400 MHz, *$CDCl_3$*) δ ppm: 169.56, 147.24, 140.78, 139.73, 134.27, 126.86, 116.10, 112.57, 103.48, 100.56,97.15, 70.34, 68.65, 60.79, 56.33, 48.75, 40.04, 37.54, 29.67, 27.09, 27.06, 26.95, 24.71, 22.97, 22.13; LRMS: (ES+) m/z = 459.3 (M+1).

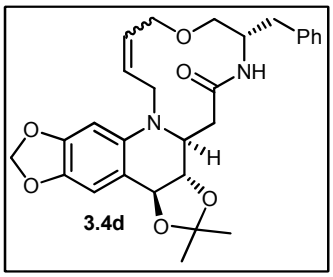
(3aS,3bS,7S,19bS,E/Z)-7-sec-butyl-2,2-dimethyl-3b,4,6,7,8,10,13,19b-octa hydro bis[1,3] dioxolo[4,5-c:4',5'-g][1,4,8]oxadiaza cyclododeca[8,7-a]quino lin-5(3aH)-one(3.4c):



3.4c

Molecular Formula: $C_{25}H_{34}N_2O_6$; R_f (solvent system): 0.48 (1:1, hexane/ethyl acetate); Yield: 50%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 6.64 (s, 1H), 6.47 (s, 1H), 5.95 (d, *J* = 5.70 Hz, 1H), 5.86 (s, 1H), 5.82 (s, 1H), 5.74 (d, *J* = 6.89 Hz, 1H), 4.66-4.59 (m, 1H), 4.49 (d, *J* = 9.14 Hz, 1H), 4.01 (t, *J* = 9.96 Hz, 1H), 3.89 (ddd, *J* = 22.76, 12.95, 6.61 Hz, 3H), 3.54 (d, *J* = 5.40 Hz, 1H), 3.46 (t, *J* = 9.54 Hz, 1H), 3.01 (s, 1H), 2.88 (d, *J* = 14.60 Hz, 1H), 2.47 (s, 1H), 2.30 (dd, *J* = 14.63, 10.06 Hz, 1H), 1.85 (dd, *J* = 6.08, 4.28 Hz, 1H), 1.72-1.67 (m, 1H), 1.57 (s, 3H), 1.48 (s, 3H), 1.17 (m, 2H), 0.92 (dd, *J* = 6.96, 2.05 Hz, 6H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ ppm: 172.76, 147.61, 146.40, 139.99, 138.05, 116.88, 112.76, 107.48, 103.33, 100.68, 98.95, 80.26, 70.24, 58.17, 54.18, 50.01, 43.33, 36.43, 29.67, 29.64, 27.12, 27.06, 25.89, 20.42, 14.99, 11.44; LRMS: (ES+) m/z =459.0 (M+1).

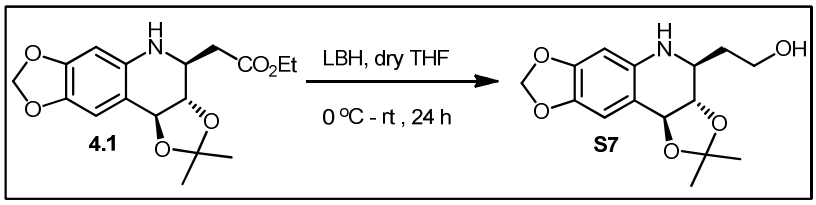
(3aS,3bS,7S,19bS,E/Z)-7-benzyl-2,2-dimethyl-3b,4,6,7,8,10,13,19b-octahydro bis[1,3] dioxolo[4,5-c:4',5'-g][1,4,8] oxadiaza cyclododeca[8,7-a]quinolin-5(3aH)-one (3.4d);



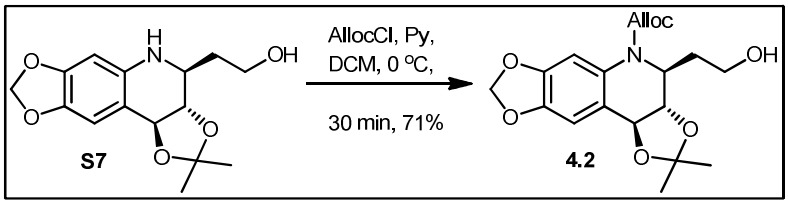
Molecular Formula: $C_{28}H_{32}N_2O_6$; R_f (solvent system): 0.48 (1:1, hexane/ethyl acetate); Yield: 50%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 7.33-7.18 (m, 6H), 6.47 (s, 1H), 6.65 (s, 1H), 5.89 (t, *J* = 4.83 Hz, 2H), 5.86 (d, *J* = 1.33 Hz, 1H), 5.82 (d, *J* = 1.33 Hz, 1H), 4.66 (td, *J* = 8.92, 6.72 Hz, 1H), 4.48 (d, *J* = 8.50 Hz, 1H), 4.22 (ddd, *J* = 11.58, 7.51, 4.09 Hz, 1H), 3.97 (t, *J* = 9.75 Hz, 1H), 3.75 (dq, *J* = 11.19, 6.66 Hz, 2H), 3.54 (ddd, *J* = 15.38, 10.89, 5.98 Hz, 1H), 3.44 (t, *J* = 9.53 Hz, 1H), 3.04 (dd, *J* = 13.70, 5.38 Hz, 1H), 2.96-2.79 (m, 4H), 2.57-2.47 (m, 1H), 2.30 (dd, *J* = 14.89, 10.15 Hz, 1H), 1.81 (dt, *J* = 12.49, 12.21, 6.29 Hz, 2H), 1.56 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ ppm: 172.55, 147.61, 145.94, 140.10, 138.09, 137.07, 129.29, 128.57, 126.72, 117.01, 112.82, 108.71,

103.33, 100.70, 99.04, 80.17, 70.67, 57.93, 51.25, 50.51, 43.13, 37.57, 27.10, 27.06, 20.33; LRMS: (ES+) m/z =492.9 (M+1).

2.8.4. Synthesis of 14-Membered Macrocycles [4.5a-d] & [5.1a-b]:

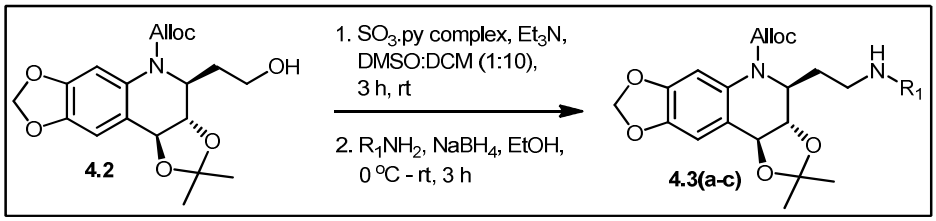


To a solution of **4.1** (500 mg, 1.49 mmol) in anhydrous THF (50 mL) was added LBH (81.13 mg, 3.72 mmol) at 0 ºC. After stirring the mixture for an additional 24 h at room temperature, the reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel (4:1 hexane/ethylacetate) afforded the product **S7** as a white solid (402.3 mg, 92%); Molecular Name: 2-((3aS,4S,10bS)-2,2-dimethyl-3a,4,5,10b-tetrahydrobis[1,3] dioxolo[4,5-c:4',5'-g]quinolin-4-yl)ethanol; Molecular Formula: $C_{17}H_{21}NO_5$; R_f (solvent system): 0.34 (hexane/ethylacetate1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.68 (s, 1H), 6.10 (s, 1H), 5.81 (d, *J* = 4.53 Hz, 2H), 4.64 (d, *J* = 8.61 Hz, 1H), 3.87 (dd, *J* = 6.59, 3.74 Hz, 3H), 3.71 (td, *J* = 10.54, 6.33 Hz, 1H), 3.58 (t, *J* = 9.33 Hz, 1H), 2.61 (s, 1H), 1.99-1.91 (m, 1H), 1.83 (ddd, *J* = 14.11, 10.45, 6.97 Hz, 1H), 1.55 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 147.4, 139.2, 137.1, 113.3, 111.6, 104.3, 100.5, 95.5, 80.2, 77.3, 60.7, 55.4, 37.7, 27.1, 27.0; LRMS:MS(ES+) m/z = 294.1 (M +1).



To a solution of **S7** (92 mg, 0.243 mmol) anhydrous dichloromethane (15 mL) at 0 ºC was added pyridine (25 µL) and allylchloroformate (33 µL). After stirring for 20 min at 0 ºC, the reaction was quenched with saturated aqueous ammonium

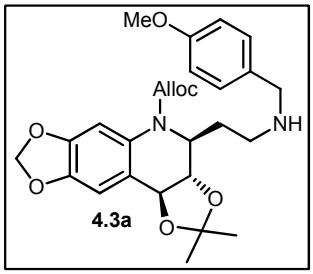
chloride. The aqueous layer was extracted twice with dichloromethane, and the combined organic layer was dried with anhydrous sodium sulfate, filtered, and then concentrated in vacuo. The residue was purified by flash chromatography over silica gel with 4:1 hexane and ethyl acetate giving 84 mg(71%) of the product **4.2** as a yellow oil; Molecular Name: (3aS,4S,10bS)-allyl 4-(2-hydroxyethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]dioxolo[4,5-c:4',5'-g]quinoline-5(10bH)-carboxylate; Molecular Formula: $C_{19}H_{23}NO_7$; R_f (solvent system): 0.51(hexane/ethyl acetate,1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.84 (d, *J* = 22.30 Hz, 2H), 5.96 (t, *J* = 10.37 Hz, 3H), 5.33-5.20 (m, 2H), 4.74-4.55 (m, 2H), 4.40 (dd, *J* = 17.56, 8.18 Hz, 2H), 3.74 (s, 2H), 3.26 (t, *J* = 8.78 Hz, 1H), 1.88 (d, *J* = 0.95 Hz, 2H), 1.63 (s, 1H), 1.55 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 146.2, 145.6, 132.1, 127.1, 125.2, 118.1, 114.0, 108.3, 101.8, 101.3, 84.4, 76.0, 66.9, 58.8, 54.7, 38.1, 29.6, 27.0, 26.9; LRMS:MS(ES+) m/z = 378.1 (M +1).



1. To a solution of the **4.2** (1 eq) in DCM:DMSO (1:10) at 0 ºC under nitrogen was added triethylamine (6 eq) and sulfur trioxide pyridine complex (6 eq). After stirring at room temperature for 3 h, the reaction was quenched with saturated aqueous ammonium chloride. The aqueous layer was extracted thrice with dichloromethane. The combined organic layer was dried with anhydrous sodium sulfate, filtered, and then concentrated in vacuo afforded aldehyde, which was subjected to reductive amination without further purification.
2. To a solution of aldehyde in the ethanol was added primary amine (R_1NH_2) (1 eq) at room temperature and stirred for 3 h. Sodium boro hydride was added at 0 ºC and stirred for 10min; the reaction was quenched with saturated aqueous ammonium chloride. The aqueous layer was extracted thrice with ethyl acetate. The combined organic layer was dried with anhydrous sodium sulfate, filtered, and then

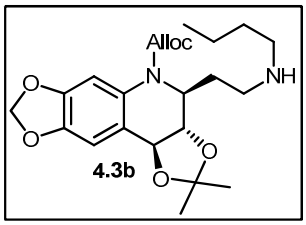
concentrated in vacuo. Purification of the residue by flash chromatography over silica gel with 1:1 hexane and ethyl acetate afforded secondary amine **4.3(a-c)**.

(3aS,4S,10bS)-allyl 4-(2-(4-methoxybenzylamino)ethyl)-2,2-dimethyl-3a,4-di hydrobis [1,3]dioxolo[4,5-c:4',5'-g]quinoline-5(10bH)-carboxylate (4.3a):



Molecular Formula: $C_{27}H_{32}N_2O_7$; R_f (solvent system): 0.2 (hexane/ ethyl acetate, 1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.21 (d, *J* = 8.38 Hz, 2H), 6.84 (dd, *J* = 22.78, 14.29 Hz, 4H), 5.96 (d, *J* = 8.37 Hz, 3H), 5.22 (d, *J* = 10.53 Hz, 2H), 4.67 (d, *J* = 5.32 Hz, 2H), 4.37 (d, *J* = 9.08 Hz, 1H), 4.35-4.26 (m, 1H), 3.79 (s, 3H), 3.71 (s, 2H), 3.25 (t, *J* = 8.79 Hz, 1H), 2.72 (t, *J* = 7.03 Hz, 2H), 2.14-2.04 (m, 1H), 1.83-1.72 (m, 2H), 1.54 (s, 3H), 1.43 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 158.6, 154.5, 146.2, 145.4, 132.3, 132.1, 129.3, 113.8, 113.7, 108.1, 101.8, 101.3, 76.1, 66.8, 55.4, 55.2, 53.1, 52.4, 44.8, 34.8, 31.9, 29.7, 29.6, 27.1, 27.0, 22.7, 14.1; LRMS:MS(ES+) m/z = 497.0 (M +1).

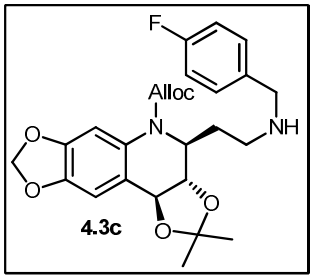
(3aS,4S,10bS)-allyl 4-(2-(butylamino)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]di oxolo [4, 5-c:4',5'-g]quinoline-5(10bH)-carboxylate (4.3b):



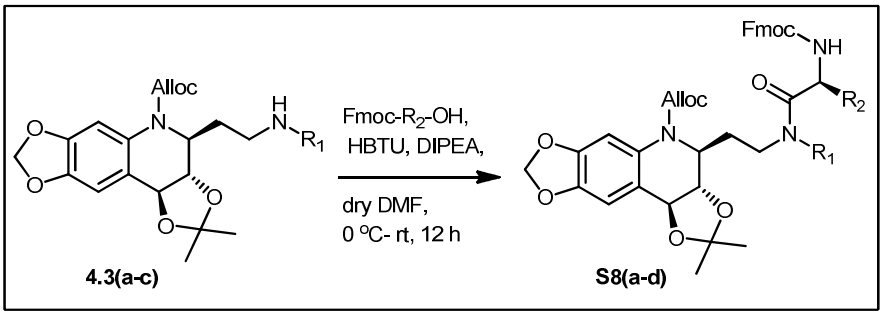
Molecular Formula: $C_{27}H_{32}N_2O_7$; R_f (solvent system): 0.2 (hexane/ ethyl acetate, 1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.80 (s, 1H), 6.65 (s, 1H), 5.95 (m, 3H), 5.86-5.77 (m, 1H), 5.65-5.58 (m, 1H), 5.37-5.31 (m, 1H), 5.26-5.22 (m, 1H), 4.65 (m, 2H), 4.39-4.35 (m, 1H), 3.24 (m, 1H), 2.71-2.68 (m, 1H), 2.57 (m, 4H), 1.62

(s, 3H), 1.55 (s, 3H), 1.34 (m, 4H), 0.91 (m, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 154.5, 146.2, 145.4, 135.0, 132.3, 126.8, 117.7, 113.8, 109.3, 108.1, 101.3, 76.1, 66.7, 55.4, 50.9, 49.5, 49.1, 45.6, 32.2, 29.7, 27.0, 20.4, 14.0; LRMS:MS(ES+) m/z = 433.1 (M +1).

(3aS,4S,10bS)-allyl 4-(2-(4-fluorobenzylamino)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3] dioxolo[4,5-c:4',5'-g]quinoline-5(10bH)-carboxylate (4.3c):



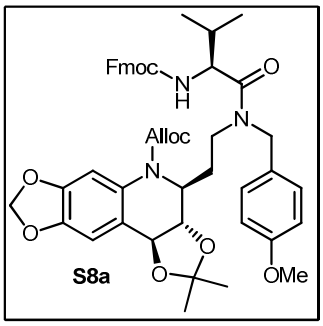
Molecular Formula: $C_{26}H_{29}FN_2O_6$; R_f (solvent system): 0.2 (hexane/ ethyl acetate, 1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.26 (t, *J* = 6.53 Hz, 2H), 6.98 (t, *J* = 8.53 Hz, 3H), 6.79 (s, 1H), 5.93 (dd, *J* = 15.60, 6.12 Hz, 3H), 5.38-5.18 (m, 2H), 4.74-4.55 (m, 2H), 4.34 (dd, *J* = 29.42, 6.84 Hz, 2H), 3.80-3.67 (m, 2H), 3.24 (s, 1H), 2.72 (t, *J* = 6.76 Hz, 2H), 2.15-2.01 (m, 1H), 1.79 (dd, *J* = 22.79, 15.49 Hz, 2H), 1.53 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 163.1, 160.6, 154.5, 146.2, 145.4, 135.9, 135.9, 132.3, 129.7, 129.6, 125.0, 118.0, 114.9, 113.8, 108.1, 101.8, 101.3, 84.0, 76.1, 66.8, 55.3, 53.0, 44.9, 34.9, 29.7, 27.0, 27.0, 22.7; LRMS:MS(ES+) m/z = 485.4 (M +1).



To a solution of **4.3(a-b)** (1 eq) in anhydrous DMF at 0 ºC was added Fmoc-R_2-OH (1.2 eq), HBTU (1.5 eq) and DIPEA (3 eq) and allowed stirred for 12 h. The reaction mixture was quenched with sodium bicarbonate solution, concentrated, and extracted thrice with ethyl acetate. Combined organic layer was washed with

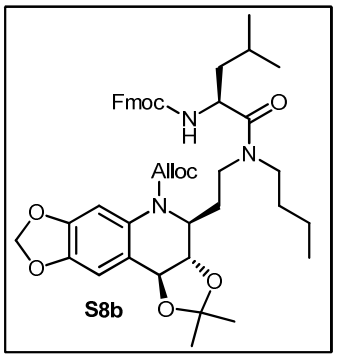
brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude oil, which was purified by column chromatography (1:4 ethyl acetate/hexanes) to give the compound **S8(a-d)** as a light yellow oil.

(3aS,4S,10bS)-allyl4-(2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-N-(4-methoxybenzyl)-3-methylbutanamido)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3] dioxolo [4,5-c:4',5'-g]quinoline-5(10bH)-carboxylate (S8a):



Molecular Formula: $C_{47}H_{51}N_3O_{10}$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.76 (d, *J* = 7.53 Hz, 2H), 7.65-7.58 (m, 2H), 7.39 (s, 2H), 7.35-7.27 (m, 2H), 7.14 (s, 2H), 6.83 (s, 2H), 6.77 (s, 1H), 5.93 (d, *J* = 1.43 Hz, 2H), 5.91-5.84 (m, 1H), 5.65-5.57 (m, 1H), 5.33-5.15 (m, 2H), 4.71-4.49 (m, 4H), 4.45-4.29 (m, 3H), 4.27-4.14 (m, 2H), 3.78 (s, 3H), 3.44-3.34 (m, 1H), 3.25-3.09 (m, 1H), 2.80 (s, 2H), 2.09-1.95 (m, 2H), 1.61 (m, 1H), 1.52 (d, *J* = 2.80 Hz, 3H), 1.46 (d, *J* = 6.15 Hz, 3H), 0.94-0.88 (m, 6H); ^{13}C NMR (400 MHz, *CDCl$_3$*) δ ppm: 171.9, 171.9, 159.3, 159.0, 156.4, 156.2, 154.4, 146.3, 146.2, 145.5, 145.5, 144.0, 144.0, 143.9, 143.9, 141.3, 132.2, 129.6, 129.5, 129.1, 128.5, 128.1, 127.7, 127.6, 127.4, 127.1, 127.0, 125.2, 125.2, 125.1, 124.9, 124.8, 119.9, 114.3, 114.3, 114.2, 114.0, 113.9, 108.1, 101.8, 101.7, 101.3, 83.9, 67.0, 67.0, 66.8, 55.9, 55.2, 55.1, 54.8, 47.2, 38.6, 31.9, 31.8, 31.7, 29.7, 29.6, 29.3, 27.0, 27.0, 26.9, 22.7, 19.9, 19.6, 17.4, 17.2, 14.1; LRMS:MS(ES+) m/z = 818.6 (M +1).

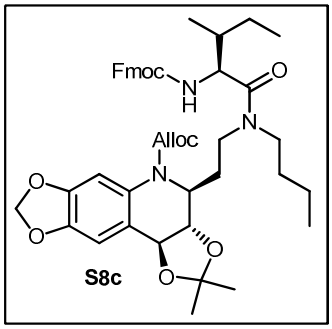
(3aS,4S,10bS)-allyl 4-(2-((S)-2-(((9H-fluoren-9-yl)methoxy) carbonylamino)-N-butyl-4-methylpentanamido)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]di oxolo [4,5-c:4',5'-g] quinoline-5(10bH)-carboxylate (S8b):



S8b

Molecular Formula: $C_{44}H_{53}N_3O_9$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.75 (m, 2H), 7.60 (m, 2H), 7.38 (m, 3H), 7.31 (m, 3H), 5.95 (m, 3H), 5.60-5.50 (m, 1H), 5.39-5.28 (m, 1H), 5.26-5.18 (m, 1H), 4.64 (m, 2H), 4.57-4.47 (m, 1H), 4.34-4.27 (m, 1H), 4.24-4.20 (m, 1H), 4.45-4.38 (m, 1H), 3.98-3.79 (m, 2H), 3.31-3.06 (m, 2H), 1.62-1.54 (m, 6H), 1.49 (m, 4H), 0.93 (m, 14H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.2, 156.1, 154.5, 147.3, 146.3, 143.8, 141.3, 137.6, 132.5, 132.2, 127.7, 127.0, 125.2, 119.9, 114.0, 113.0, 112.0, 109.6, 104.2, 101.8, 101.3, 100.4, 96.2, 80.3, 76.1, 67.0, 55.2, 52.6, 49.3, 49.2, 47.2, 43.1, 31.3, 29.7, 27.0, 24.6, 23.5, 22.7, 21.6, 20.0, 13.7; LRMS:MS(ES+) m/z = 767.6 (M +1).

(3aS,4S,10bS)-allyl-4-(2-((2S,3R)-2-(((9H-fluoren-9-yl)methoxy) carbonyl amino)-N-butyl-3-methylpentanamido)ethyl)-2,2-di methyl-3a,4-dihydrobis [1,3]dioxolo[4,5-c:4',5'-g]quinoline-5(10bH)-carboxylate (S8c):

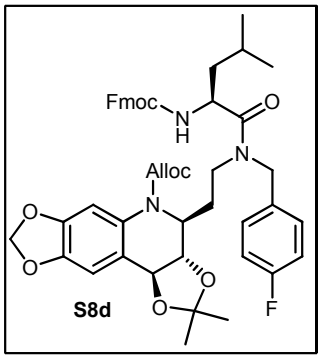


S8c

Molecular Formula: $C_{44}H_{53}N_3O_9$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.77-7.73 (m, 2H), 7.63-7.58 (m, 2H), 7.38 (m, 3H), 7.30 (m, 3H), 5.96 (m, 3H), 5.59-5.46 (m, 1H), 5.26-5.15 (m, 1H), 4.68-4.59 (m, 2H), 4.53-4.37 (m, 3H), 4.33-4.17 (m, 3H), 4.17-4.06 (m, 1H), 3.72-3.02 (m, 4H), 1.67 (m, 6H), 1.60-1.50 (m, 6H), 1.36-1.30 (m, 2H), 0.95-0.86

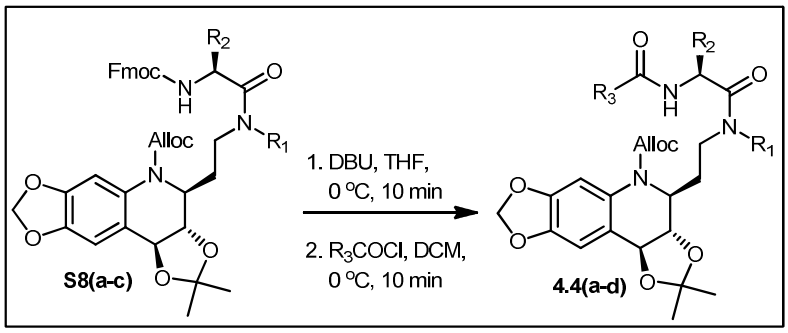
(m, 9H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.9, 156.3, 156.1, 143.8, 141.3, 132.5, 127.7, 127.0, 125.2, 119.9, 117.7, 109.6, 101.8, 101.5, 101.3, 76.1, 66.9, 66.0, 65.7, 55.1, 54.9, 54.9, 48.9, 47.2, 38.5, 38.3, 29.7, 29.4, 29.3, 27.1, 27.0, 26.7, 20.4, 20.1, 19.9, 16.0, 15.9, 15.8, 13.7, 11.3; LRMS:MS (ES+) m/z = 767.5 (M +1).

(3aS,4S,10bS)-allyl 4-(2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-N-(4-fluorobenzyl)-4-methylpentanamido)ethyl)-2,2-dimethyl-3a,4-dihydro bis [1,3]dioxolo [4,5-c:4', 5'-g]quinoline-5(10bH)-carboxylate (S8d):



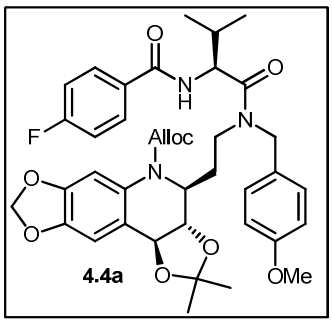
S8d

Molecular Formula: $C_{47}H_{50}FN_3O_9$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.76 (d, *J* = 7.18 Hz, 1H), 7.39 (t, *J* = 6.88 Hz, 2H), 7.35-7.08 (m, 5H), 7.08-6.90 (m, 3H), 6.77 (d, *J* = 2.35 Hz, 1H), 7.59 (t, *J* = 6.62 Hz, 2H), 5.92 (t, *J* = 18.91 Hz, 3H), 5.57-5.42 (m, 1H), 5.22 (dd, *J* = 21.20, 14.22 Hz, 2H), 5.03-4.80 (m, 1H), 4.76-4.52 (m, 4H), 4.44-4.28 (m, 3H), 4.21 (d, *J* = 7.78 Hz, 2H), 3.44-3.27 (m, 1H), 3.27-3.10 (m, 1H), 2.13-1.96 (m, 1H), 1.72-1.59 (m, 4H), 1.53 (d, *J* = 7.91 Hz, 3H), 1.45 (d, *J* = 8.69 Hz, 3H), 0.91 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 173.0, 163.4, 161.0, 156.1, 156.1, 154.4, 146.3, 146.2, 145.6, 143.9, 143.8, 141.3, 132.1, 129.8, 128.5, 127.6, 127.0, 125.1, 120.9, 119.9, 115.7, 115.4, 114.3, 114.0, 112.4, 108.1, 101.8, 101.7, 101.3, 84.0, 70.3, 66.9, 54.8, 47.2, 43.0, 34.1, 31.9, 29.7, 27.0, 27.0, 27.0, 24.6, 23.5, 22.7, 21.5, 14.1; LRMS:MS (ES+) m/z = 820.9 (M +1).



To a solution of **S8(a-c)** (1 eq) in little amount of dry THF at 0 ºC was added DBU (2 eq), then stirred for 10min then dry DCM was added to the reaction mixture followed by acid chloride (R_3COCl) carefully at 0 ºC. The reaction mixture was allowed to stir for 10 min. Saturated sodium bicarbonate solution was added to the reaction mixture and compound extracted thrice with ethylacetate. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Purification of the residue by flash chromatography over silica gel with 4:1 hexane and ethyl acetate afforded product **4.4(a-d)** as a light yellow oil.

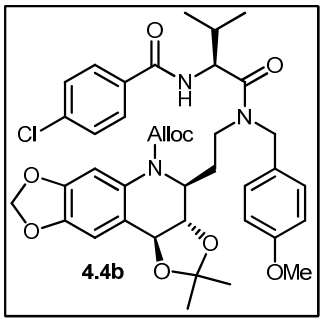
(3aS,4S,10bS)-allyl-4-(2-((S)-2-(4-fluorobenzamido)-N-(4-methoxybenzyl)-3-methyl butanamido)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]dioxolo[4,5-c:4', 5'-g]quinoline-5(10bH)-carboxylate (4.4a):



Molecular Formula: $C_{39}H_{44}FN_3O_9$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.86 (m, 2H), 7.20-7.04 (m, 5H), 6.92-6.75 (m, 3H), 6.00-5.83 (m, 3H), 5.26 (d, *J* = 27.80 Hz, 2H), 5.14-5.01 (m, 1H), 4.64 (d, *J* = 22.78 Hz, 3H), 4.41-4.11 (m, 2H), 3.79 (d, *J* = 7.41 Hz, 3H), 3.39 (s, 1H), 3.25-3.12 (m, 1H), 2.06 (s, 2H), 1.50 (dd, *J* = 21.33, 14.38 Hz, 6H), 0.94 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.8, 159.3, 156.2, 154.4, 146.2, 145.5, 143.9, 143.9, 141.3, 132.2, 129.5, 129.1, 128.5, 127.6, 127.6, 127.0, 127.0,

125.2, 119.9, 114.2, 114.2, 114.0, 113.9, 108.1, 101.7, 101.7, 101.3, 83.9, 76.0, 76.0, 67.0, 66.8, 55.8, 55.2, 55.2, 54.7, 47.2, 37.1, 36.6, 31.9, 29.7, 29.6, 29.3, 28.0, 27.0, 24.7, 22.7, 19.9, 17.3, 17.1, 14.1; LRMS:MS(ES+) m/z = 718.6 (M +1).

(3aS,4S,10bS)-allyl-4-(2-((S)-2-(4-chlorobenzamido)-N-(4-methoxybenzyl)-3-methyl butanamido)ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]dioxolo[4,5-c:4', 5'-g]quinoline-5(10bH)-carboxylate (4.4b):

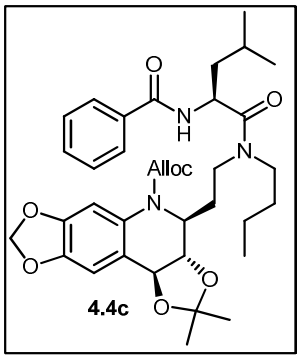


Molecular Formula: $C_{39}H_{44}ClN_3O_9$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.86-7.75 (m, 2H), 7.40 (d, *J* = 8.35 Hz, 2H), 7.24-7.04 (m, 3H), 6.92-6.74 (m, 3H), 5.95 (dd, *J* = 10.76, 9.50 Hz, 3H), 5.36-5.17 (m, 2H), 5.15-4.85 (m, 2H), 4.62 (s, 3H), 4.42-4.16 (m, 2H), 3.79 (d, *J* = 7.51 Hz, 3H), 3.52-3.35 (m, 1H), 3.24-3.13 (m, 1H), 2.19-1.99 (m, 2H), 1.56-1.37 (m, 6H), 0.92 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.8, 166.0, 159.3, 154.3, 146.3, 145.4, 137.7, 132.4, 132.1, 131.3, 129.5, 128.6, 128.0, 114.2, 114.0, 108.0, 101.7, 101.2, 83.9, 76.0, 66.7, 55.2, 54.7, 54.3, 47.4, 33.7, 31.8, 29.6, 27.0, 22.6, 19.7, 17.6, 14.0; LRMS:MS(ES+) m/z = 735.1 (M +1).

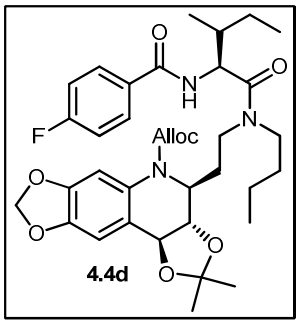
(3aS,4S,10bS)-allyl-4-(2-((S)-2-benzamido-N-butyl-4-methylpentanamido) ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]dioxolo[4,5-c:4',5'-g]quinoline-5 (10bH)-carboxylate (4.4c):

Molecular Formula: $C_{36}H_{47}N_3O_8$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.80 (m, 2H), 7.55-7.33 (m, 5H), 5.92 (m, 3H), 5.62-5.40 (m, 1H), 5.25-5.14 (m, 2H), 4.71-4.53 (m, 3H), 4.45-4.18 (m, 2H), 3.69-3.54 (m, 1H), 3.27-3.05 (m, 1H), 3.49-3.32 (m, 2H), 1.71 (m, 3H), 1.55 (m, 4H), 1.40 (m, 3H), 1.03-0.83 (m, 14H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 172.3, 166.7, 154.4, 146.2, 145.5, 134.0, 132.4, 131.5, 128.4, 128.0, 127.1, 117.7,

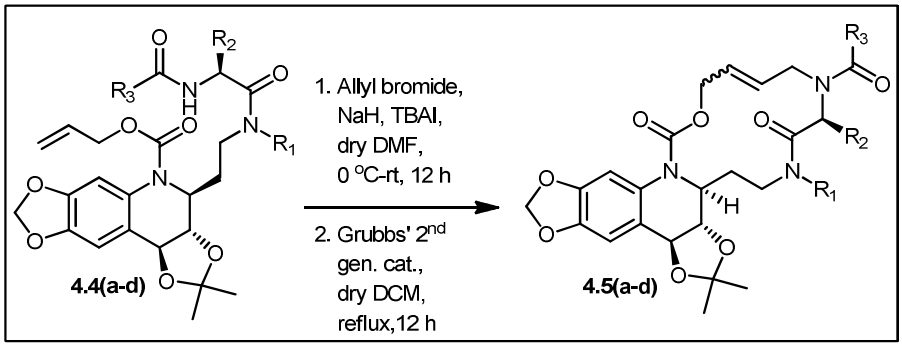
114.0, 109.6, 102.4, 101.3, 85.2, 76.1, 66.8, 65.8, 55.1, 47.7, 45.8, 43.2, 34.8, 31.9, 31.3, 29.7, 27.0, 24.8, 23.5, 23.5, 22.7, 21.8, 20.0, 13.8; LRMS:MS(ES+) m/z = 650.5 (M +1).



(3aS,4S,10bS)-allyl-4-(2-((2S,3R)-2-benzamido-N-butyl-3-methylpentan amido) ethyl)-2,2-dimethyl-3a,4-dihydrobis[1,3]dioxolo[4,5-c:4',5'-g]quinol ine-5 (10bH)-carboxylate (4.4d):



Molecular Formula: $C_{36}H_{46}FN_3O_8$; R_f (solvent system): 0.4 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 8.17-8.05 (m, 1H), 8.03-7.80 (m, 2H), 7.10 (m, 3H), 5.93 (m, 3H), 5.63-5.39 (m, 1H), 5.37-5.11 (m, 2H), 5.06-4.93 (m, 1H), 4.64 (s, 2H), 4.45-4.07 (m, 2H), 4.05-3.83 (m, 1H), 3.68-3.32 (m, 2H), 3.31-3.07 (m, 1H), 1.98-1.77 (m, 1H), 1.74-1.44 (m, 8H), 1.38 (m, 2H), 1.27 (m, 5H), 0.93 (m, 9H); ^{13}C NMR (400 MHz, *CDCl$_3$*) δ ppm: 172.3, 168.4, 166.0, 164.6, 163.5, 154.5, 132.6, 132.5, 129.7, 128.7, 127.0, 121.0, 119.7, 115.6, 115.3, 114.0, 109.6, 101.5, 76.0, 66.8, 65.7, 53.5, 49.2, 47.6, 38.2, 31.9, 31.4, 29.7, 29.4, 26.9, 26.7, 20.1, 19.9, 15.9, 13.8, 13.7, 11.1; LRMS:MS(ES+) m/z = 668.5 (M +1).

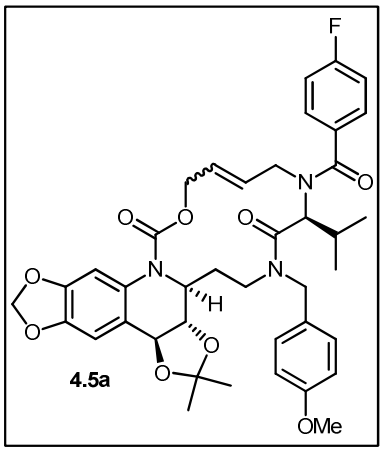


1. To a solution of **4.4(a-d)** (1 eq) in dry DMF added sodium hydride (3 eq) at 0 °C then added allylbromide (5 eq), tetra butyl ammonium iodide (0.5 eq) and stirred for 12 h at room temperature. The reaction mixture was quenched with saturated ammonium chloride and the compound extracted thrice with ethylacetate. The combined organic phase was washed with water, brine and dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Purification of the residue by flash chromatography over silica gel with 4:1 hexane and ethylacetate afforded allyl product as a light yellow oil.
2. To a solution of above allyl product (1 eq) in dry DCM added Grubbs' 2nd generation catalyst (0.1 eq) under nitrogen atmosphere. The reaction mixture was stirred for 12 h reflux and concentrated which was subjected to flash chromatography over silica gel with 4:1 hexane and ethylacetate afforded cyclised product **4.5(a-d)** as a light yellow oil.

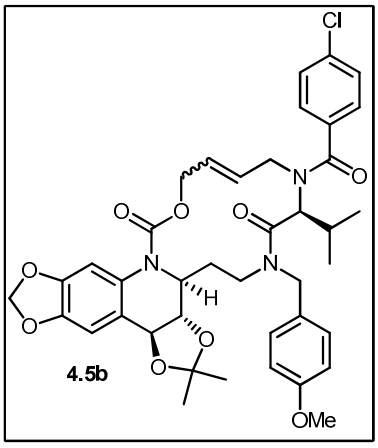
(3aS,3bS,8R,21bS,E/Z)-9-(4-fluorobenzoyl)-8-isopropyl-6-(4-methoxybenzyl) -2,2-dimethyl-3b,4,5,6,9,10,13,21b-octahydrobis [1,3]dioxolo[4,5-c:4',5'-g] [1,3,7, 10]oxatriaza cyclotetradeca[3,4-a] quinoline-7,15(3aH,8H)-dione (4.5a):

Molecular Formula: $C_{40}H_{44}FN_3O_9$; R_f (solvent system): 0.3 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.39 (dd, *J* = 8.41, 5.39 Hz, 2H), 7.17 (d, *J* = 8.47 Hz, 2H), 7.13-7.04 (m, 3H), 6.91-6.77 (m, 3H), 5.94 (d, *J* = 3.44 Hz, 3H), 5.29 (d, *J* = 10.57 Hz, 1H), 5.24-5.11 (m, 2H), 4.78-4.69 (m, 1H), 4.68-4.60 (m, 1H), 4.53 (d, *J* = 8.89 Hz, 1H), 4.41-4.31 (m, 2H), 4.13-3.98 (m, 2H), 3.89-3.83 (m, 1H), 3.80 (s, 3H), 3.18 (s, 1H), 3.03-2.93 (m, 1H), 2.92-2.81 (m, 1H), 2.61-2.50 (m, 1H), 2.08-1.98 (m, 1H), 1.60 (s, 3H), 1.37 (s, 3H), 0.88 (dd, *J* =

8.15, 4.10 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.5, 171.1, 164.4, 161.9, 158.9, 153.3, 146.4, 145.4, 139.2, 132.5, 132.5, 129.5, 128.8, 128.7, 127.6, 127.5, 127.2, 124.3, 115.4, 115.2, 114.0, 114.0, 107.9, 101.7, 101.3, 84.2, 76.1, 63.9, 57.9, 55.7, 55.3, 50.5, 45.9, 43.4, 33.8, 31.9, 29.7, 29.6, 29.3, 27.2, 27.1, 22.7, 20.0, 17.7, 14.1; LRMS:MS(ES+) m/z = 730.5 (M +1).



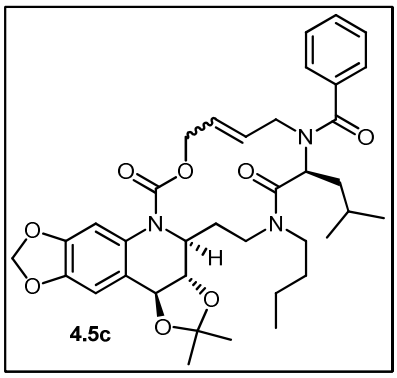
(3aS,3bS,8R,21bS,E/Z)-9-(4-chlorobenzoyl)-8-isopropyl-6-(4-methoxybenzyl)-2,2-di methyl-3b,4,5,6,9,10,13,21b-octahydrobis[1,3]dioxolo [4,5-c:4',5'-g][1,3,7,10]oxatriaza cyclotetradeca[3,4-a] quinoline-7,15(3aH, 8H)-dione (4.5b):



Molecular Formula: $C_{40}H_{44}ClN_3O_9$; R_f (solvent system): 0.3 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.39 (d, *J* = 8.06 Hz, 2H), 7.33 (d, *J* = 8.09 Hz, 2H), 7.17 (d, *J* = 8.17 Hz, 2H), 6.88 (d, *J* = 8.21 Hz, 2H), 7.05 (s, 1H), 6.79 (s, 1H), 5.91-5.82 (m, 1H), 5.94 (d, *J* = 3.44 Hz, 2H), 5.32-5.10 (m, 3H), 4.79-4.70 (m, 1H), 4.67 (s, 1H), 4.54 (d, *J* = 8.93 Hz, 1H), 4.38 (d, *J* = 12.35 Hz, 2H), 4.14-3.98 (m, 2H), 3.81 (s, 4H), 3.19 (s, 1H), 3.04-2.93 (m, 1H),

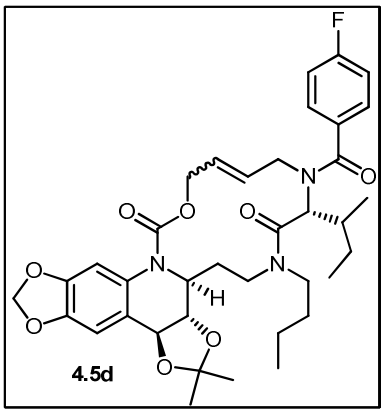
2.93-2.80 (m, 1H), 2.62-2.50 (m, 1H), 2.09-1.98 (m, 1H), 1.37 (s, 3H), 1.60 (s, 3H), 0.89 (dd, *J* = 12.51, 6.44 Hz, 6H); ^{13}C NMR (400 MHz, *CDCl$_3$*) δ ppm: 171.362, 171.1, 158.9, 153.3, 146.4, 145.4, 135.5, 134.8, 129.5, 128.5, 128.0, 127.6, 124.3, 114.0, 114.0, 107.9, 101.7, 101.7, 101.3, 84.2, 64.0, 57.8, 57.8, 55.7, 50.5, 45.8, 43.4, 34.2, 31.9, 29.7, 27.2, 22.7, 20.0, 17.7, 14.1; LRMS:MS(ES+) m/z = 747.1 (M +1).

(3aS,3bS,8R,21bS,E/Z)-9-benzoyl-6-butyl-8-isobutyl-2,2-dimethyl-3b,4,5,6, 9,10,13,21b-octahydrobis[1,3]dioxolo[4,5-c:4',5'-g][1,3,7,10]oxatriaza cyclotetradeca[3,4-a] quinoline-7,15(3aH,8H)-dione (4.5c):

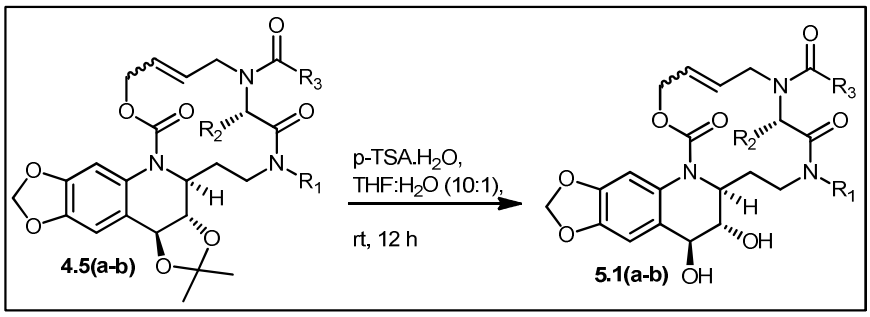


Molecular Formula: $C_{37}H_{47}N_3O_8$; R_f (solvent system): 0.3 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.42 (m, 5H), 7.17-7.12 (m, 1H), 6.65 (s, 1H), 5.98 (s, 3H), 5.89-5.83 (m, 1H), 5.60-5.46 (m, 2H), 5.28-5.11 (m, 3H), 4.92-4.82 (m, 1H), 4.79-4.61 (m, 2H), 4.41-4.34 (m, 1H), 4.33-4.25 (m, 1H), 3.82-3.65 (m, 3H), 3.49-3.38 (m, 1H), 3.05-2.96 (m, 1H), 2.59-2.47 (m, 1H), 1.88-1.75 (m, 1H), 1.52 (s, 3H), 1.49 (s, 3H), 1.45 (m, 4H), 1.02-0.93 (m, 9H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.7, 168.8, 154.8, 147.6, 136.4, 134.4, 133.0, 132.4, 129.4, 128.5, 126.3, 126.0, 119.2, 112.3, 111.7, 110.0, 109.4, 102.0, 85.0, 80.2, 64.1, 55.3, 53.4, 49.9, 46.0, 45.2,43.7, 39.0, 30.5, 29.7, 29.6, 27.2, 27.0, 24.8, 22.9, 22.9, 19.7, 13.9; LRMS:MS(ES+) m/z = 662.5 (M +1).

(3aS,3bS,8R,21bS,E/Z)-8-sec-butyl-6-butyl-9-(4-fluorobenzoyl)-2,2-dimethyl-3b,4,5,6,9, 10,13,21b-octahydrobis[1,3]dioxolo[4,5-c:4',5'-g][1,3,7,10]oxatri aza cyclotetradeca[3,4-a]quinoline-7,15(3aH,8H)-dione (4.5d):

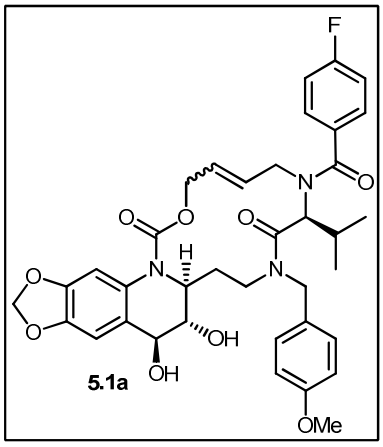


Molecular Formula: $C_{37}H_{46}FN_3O_8$; R_f (solvent system): 0.3 (hexane/ethyl acetate, 7:3); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.12 (m, 4H), 6.62 (s, 2H), 5.98 (s, 3H), 5.87 (m, 1H), 5.53-5.47 (m, 1H), 5.37-5.30 (m, 1H), 5.17 (m, 3H), 4.87-4.79 (m, 1H), 4.78-4.70 (m, 1H), 4.66-4.60 (m, 1H), 4.41-4.36 (m, 1H), 4.30-4.23 (m, 1H), 3.80-3.70 (m, 2H), 3.57-3.49 (m, 1H), 3.06-2.98 (m, 1H), 2.61-2.52 (m, 1H), 2.35-2.27 (m, 1H), 2.07-1.99 (m, 1H), 1.65-1.61 (m, 1H),1.50 (m, 6H), 1.41-1.35 (m, 4H), 1.00-0.88 (m, 9H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.4, 168.6, 164.3, 161.8, 154.8, 147.5, 146.8, 134.3, 132.7, 128.2, 128.2, 126.5, 119.2, 115.5, 112.3, 109.4, 102.0, 85.0, 80.2, 64.2, 56.4, 55.3, 45.9, 44.1, 33.6, 31.9, 30.9, 29.7, 29.3, 27.2, 23.8, 22.7, 19.8, 15.9, 14.1, 13.9, 11.0; LRMS:MS(ES+) m/z = 680.6 (M +1).



To a solution of **4.5(a-b)** in THF:H_2O (10:1) added p-toluene sulfonic acid monohydrate (5 eq) allowed to stir for 12 h at room temperature. Saturated sodium bicarbonate was added to the reaction mixture and compound extracted twice with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Purification of the residue by flash chromatography over silica gel with 1:1 hexane and ethyl acetate afforded product **5.1(a-b)** as a light yellow oil.

(8S,12aS,13S,14S,E/Z)-7-(4-fluorobenzoyl)-13,14-dihydroxy-8-isopropyl-10-(4-methoxybenzyl)-7,8,10,11,12,12a,13,14-octahydro-[1,3]dioxolo[4,5-g] [1,3,7,10] oxatri azacyclo tetradeca[3,4-a]quinoline-1,9(3H,6H)-dione (5.1a):

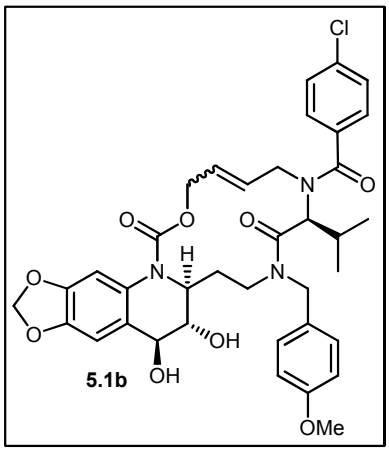


Molecular Formula: $C_{37}H_{40}FN_3O_9$; R_f (solvent system): 0.2 (hexane/ethyl acetate, 1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.36 (dd, *J* = 8.46, 5.33 Hz, 2H), 7.15-7.06 (m, 4H), 7.00 (s, 1H), 6.86 (d, *J* = 8.60 Hz, 2H), 6.77 (s, 1H), 5.96-5.91 (m, 2H), 5.88-5.78 (m, 1H), 5.19 (d, *J* = 10.59 Hz, 1H), 5.10 (d, *J* = 16.81 Hz, 2H), 4.87 (s, 2H), 4.28 (dd, *J* = 10.59, 5.98 Hz, 3H), 4.14-3.95 (m, 2H), 3.87-3.80 (m, 1H), 3.78 (s, 3H), 3.22-3.16 (m, 1H), 3.00-2.90 (m, 1H), 2.63-2.45 (m, 2H), 2.04 (s, 1H), 1.40 (s, 2H), 0.87 (dd, *J* = 13.62, 11.03 Hz, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*) δ ppm: 171.6, 171.4, 164.4, 161.9, 158.9, 153.2, 146.6, 145.6, 132.5, 132.5, 129.6, 128.8, 128.1, 127.4, 126.8, 126.2, 124.9, 115.5, 115.3, 114.2, 106.4, 102.9, 101.4, 78.7, 71.2, 63.7, 58.1, 57.4, 55.3, 50.5, 46.0, 43.8, 33.6, 31.9, 29.7, 29.7, 29.6, 29.4, 27.3, 22.7, 20.0, 17.8, 14.1; LRMS:MS(ES+) m/z = 690.6 (M +1).

(8S,12aS,13S,14S,E/Z)-7-(4-chlorobenzoyl)-13,14-dihydroxy-8-isopropyl-10-(4-methoxybenzyl)-7,8,10,11,12,12a,13,14-octahydro-[1,3]dioxolo[4,5-g] [1,3,7,10] oxatri azacyclo tetradeca[3,4-a] quinoline-1,9(3H,6H)-dione (5.1b):

Molecular Formula: $C_{37}H_{40}ClN_3O_9$; R_f (solvent system): 0.2 (hexane/ethyl acetate, 1:1); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.38 (d, *J* = 8.33 Hz, 2H), 7.30 (d, *J* = 8.34 Hz, 2H), 7.13 (d, *J* = 8.54 Hz, 2H), 7.02 (s, 1H), 6.87 (d, *J* = 8.54 Hz, 2H), 6.79 (s, 1H), 5.94 (d, *J* = 7.28 Hz, 2H), 5.88-5.77 (m, 1H), 5.15 (dd, *J* = 34.77, 13.72 Hz, 3H), 4.87 (s, 2H), 4.37-4.24 (m, 3H), 4.14-4.05 (m, 1H), 4.03-3.95 (m, 1H), 3.79 (s, 4H), 3.22 (d, *J* = 3.32 Hz, 1H), 3.02-2.93 (m, 1H), 2.65-

2.49 (m, 2H), 2.09-2.00 (m, 1H), 1.42 (d, J = 7.15 Hz, 2H), 0.86 (dd, J = 13.82, 9.47 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 171.2, 158.8, 153.0, 146.6, 145.5, 135.4, 134.8, 129.6, 128.4, 127.9, 127.3, 126.7, 126.0, 114.1, 106.4, 102.8, 102.8, 101.3, 78.7, 71.2, 63.6, 57.3, 55.2, 45.9, 31.9, 29.7, 29.6, 27.3, 22.7, 20.0, 17.7, 14.1; LRMS:MS(ES+) m/z = 707.0 (M +1).



5.1b

2.9. References

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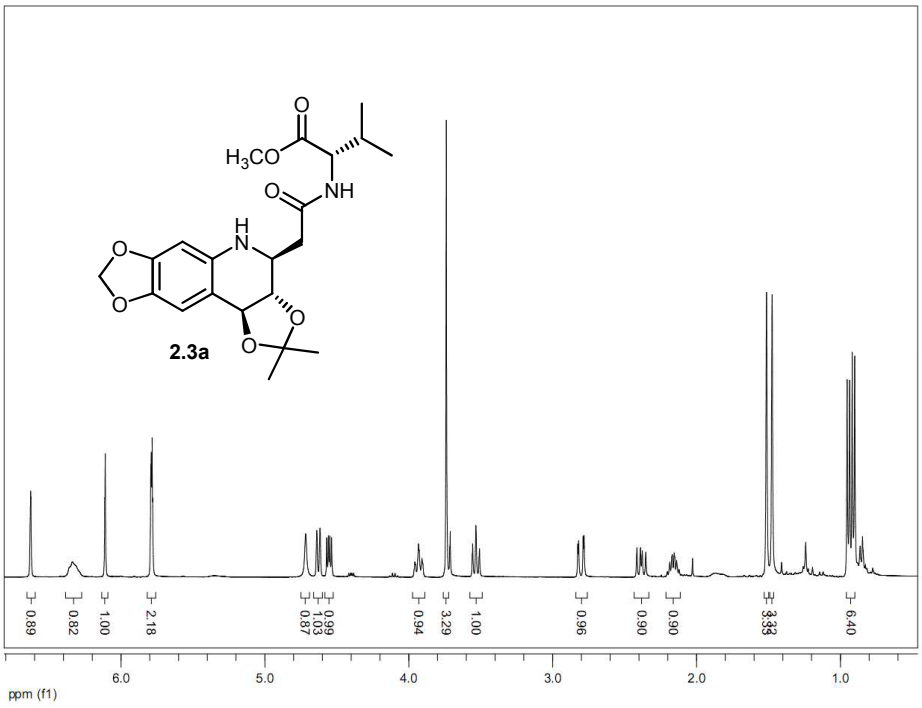
2.10. Spectral Data:

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



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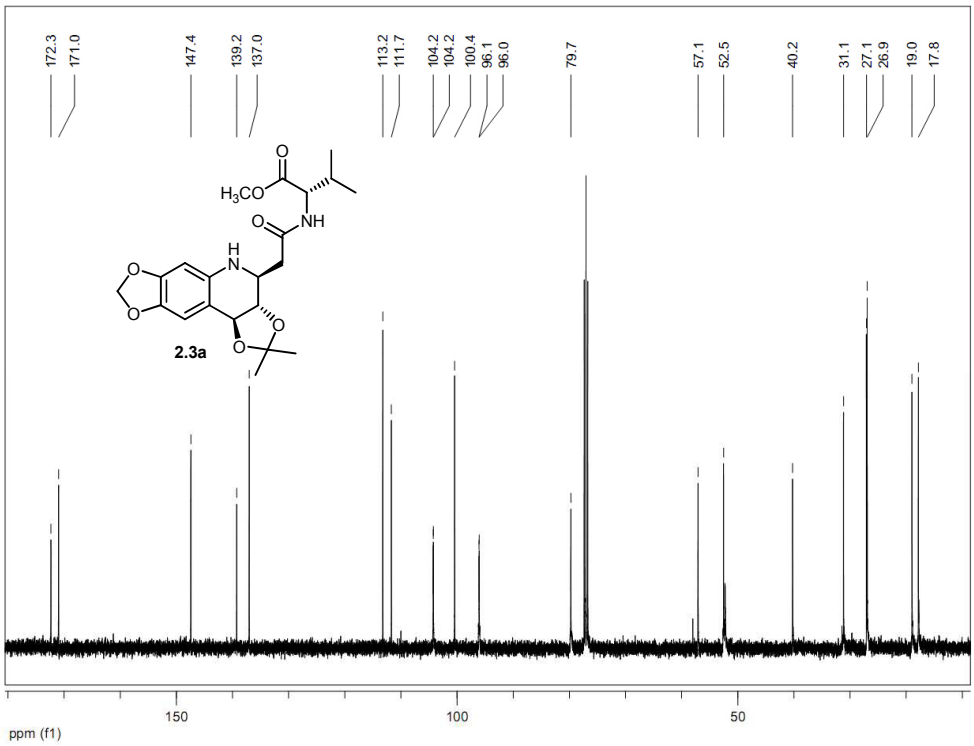

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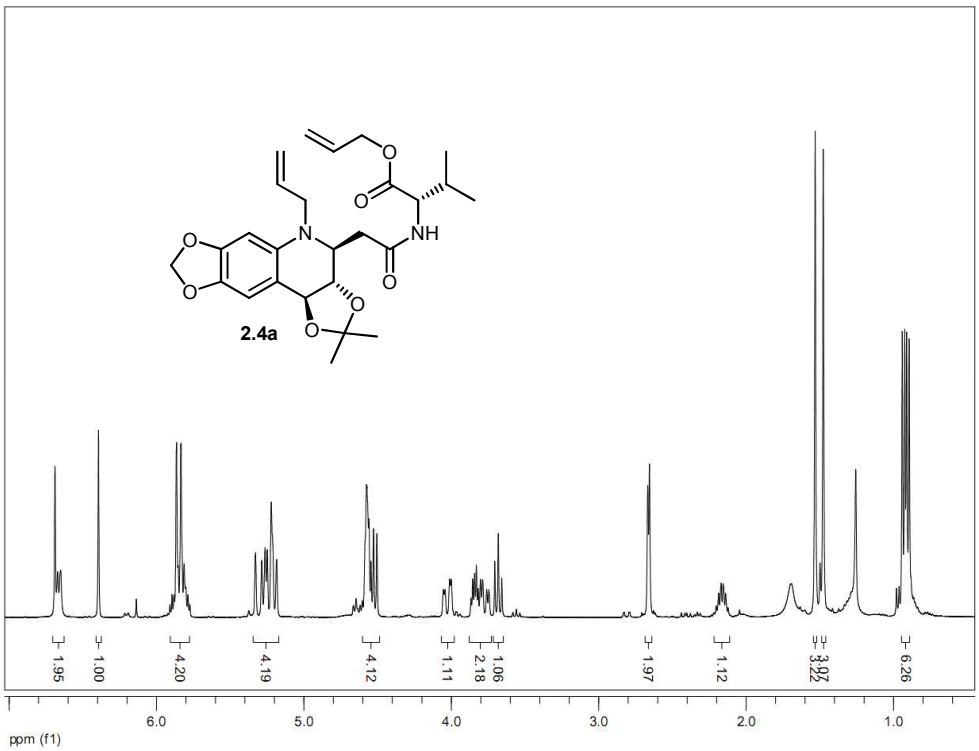

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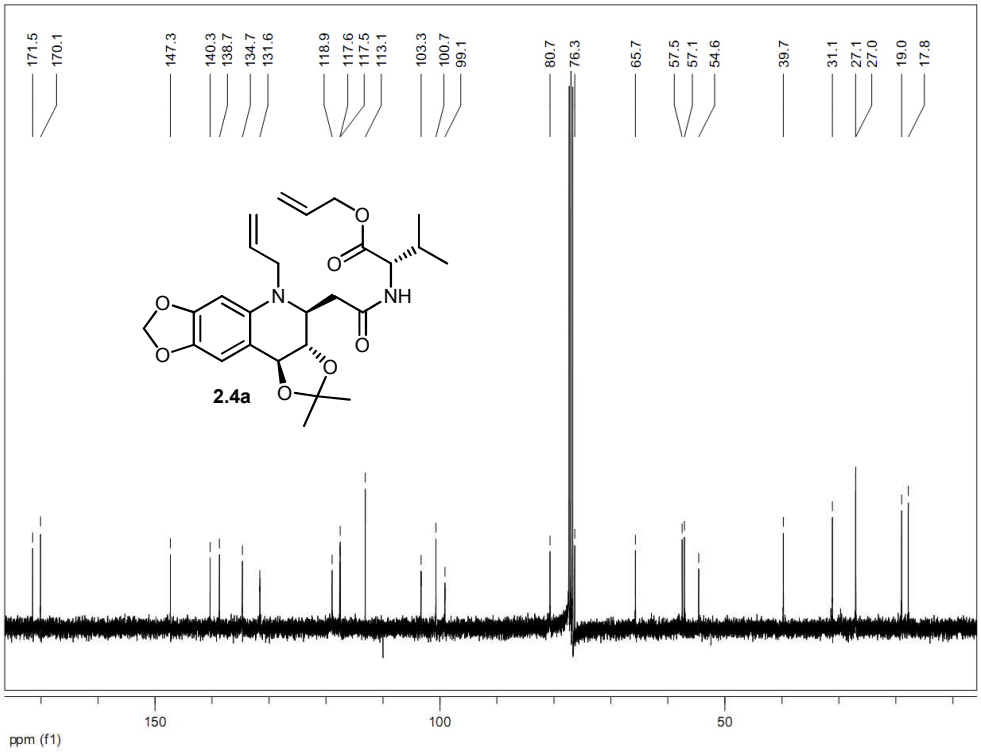

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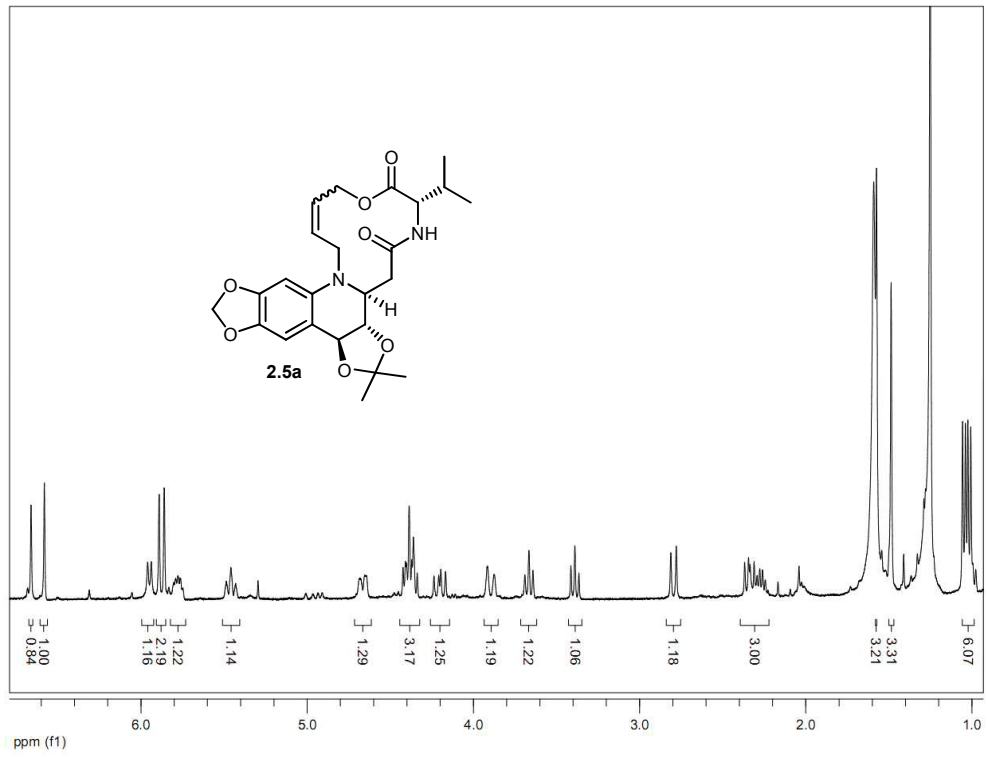

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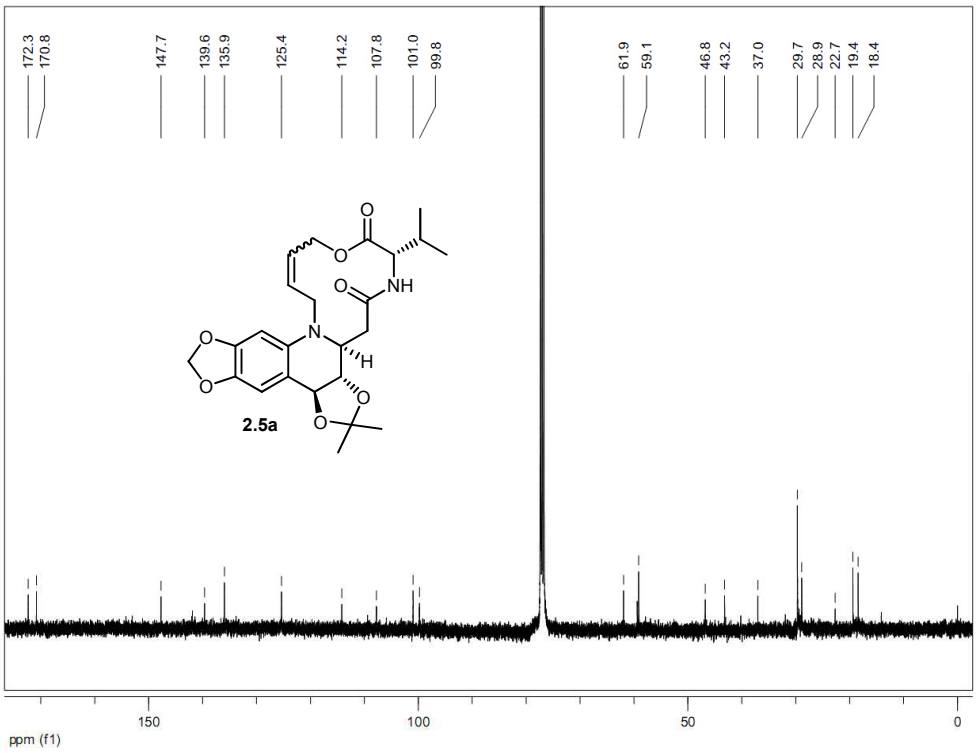

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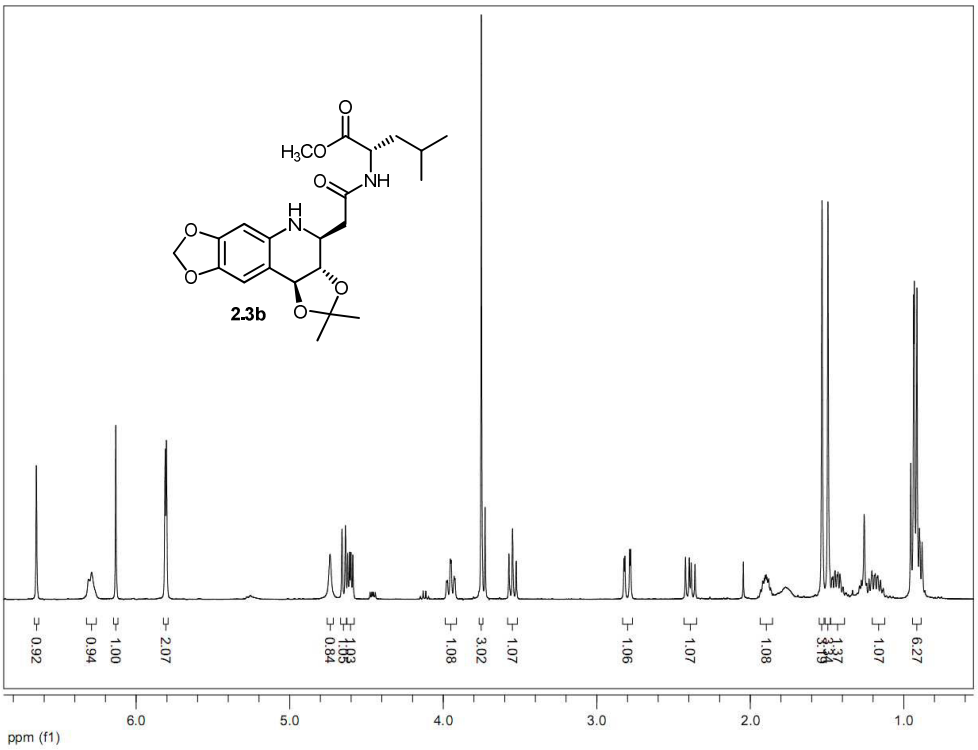

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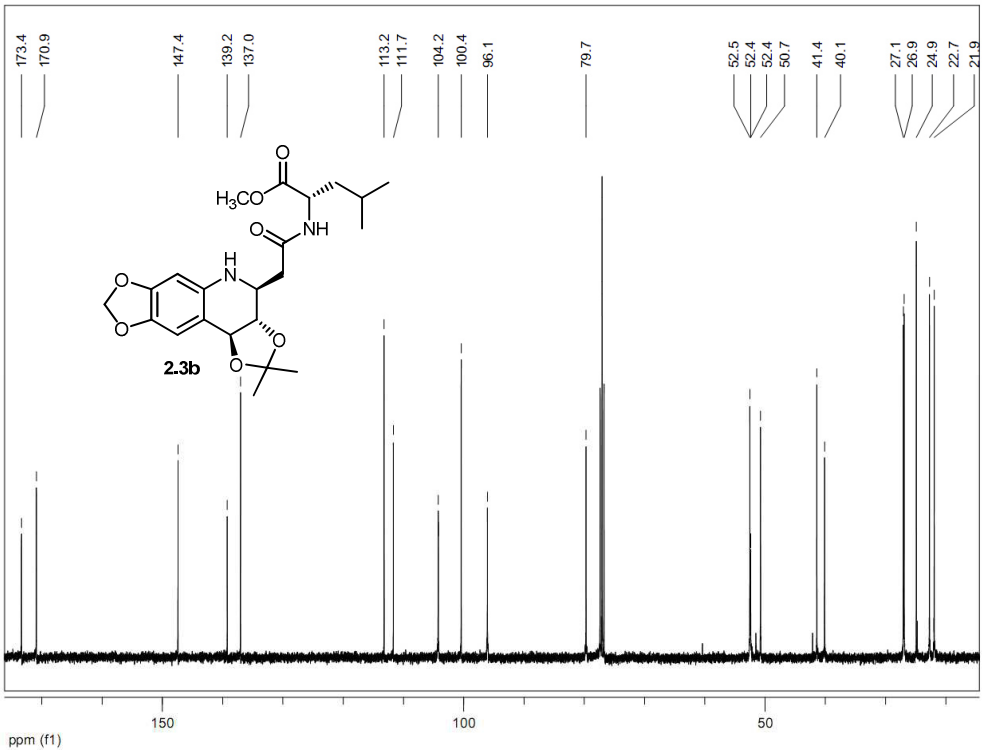

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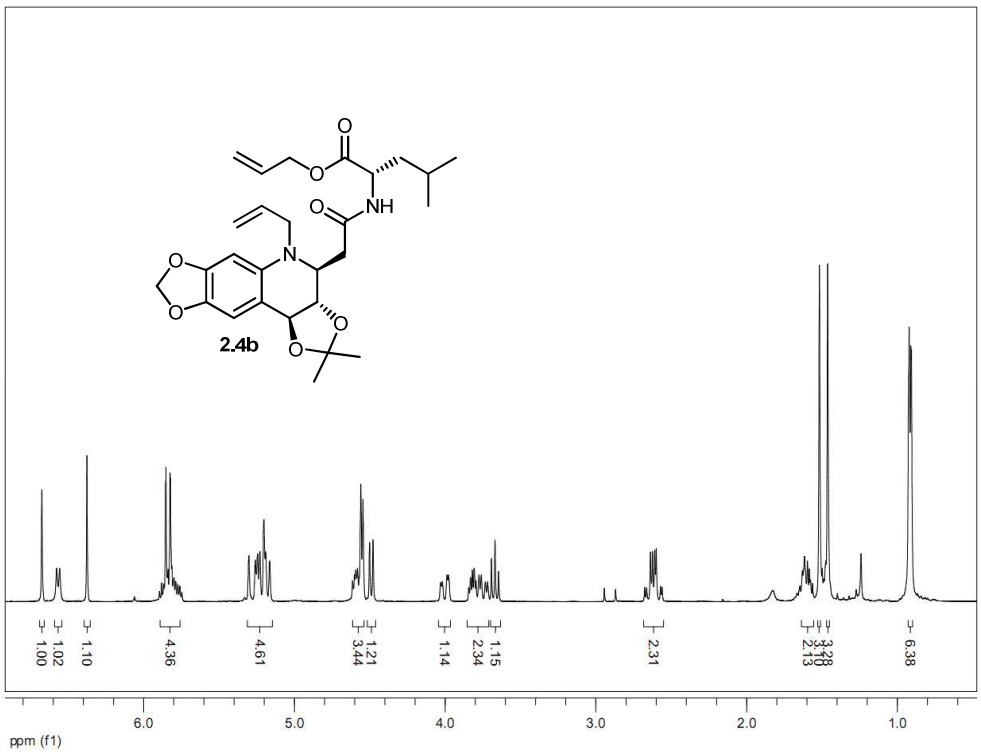

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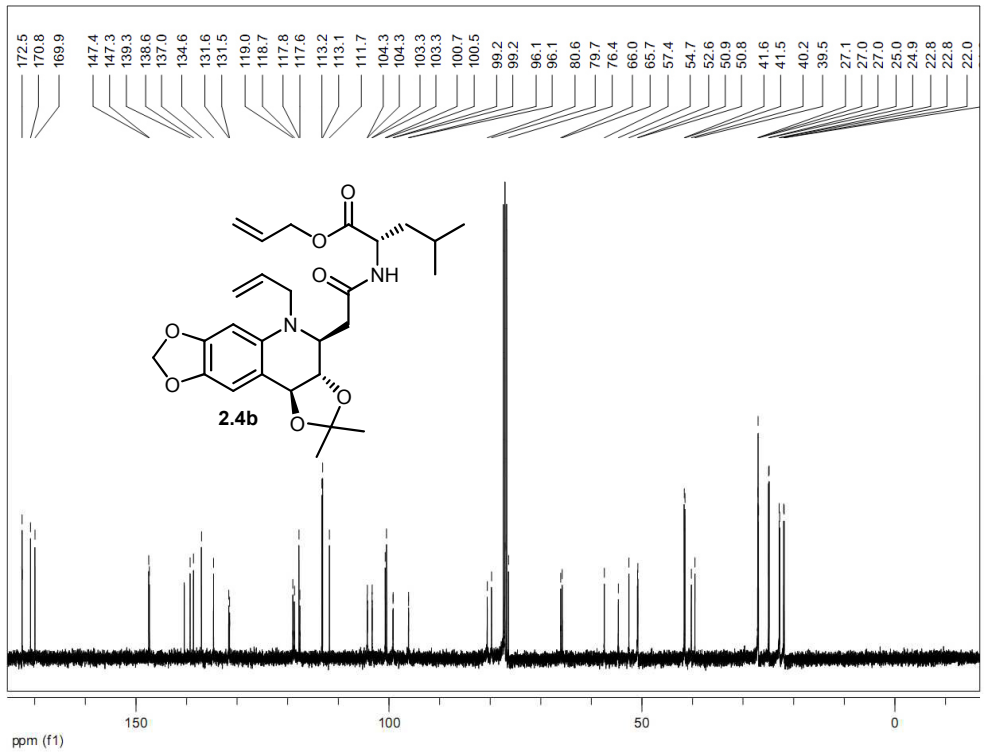

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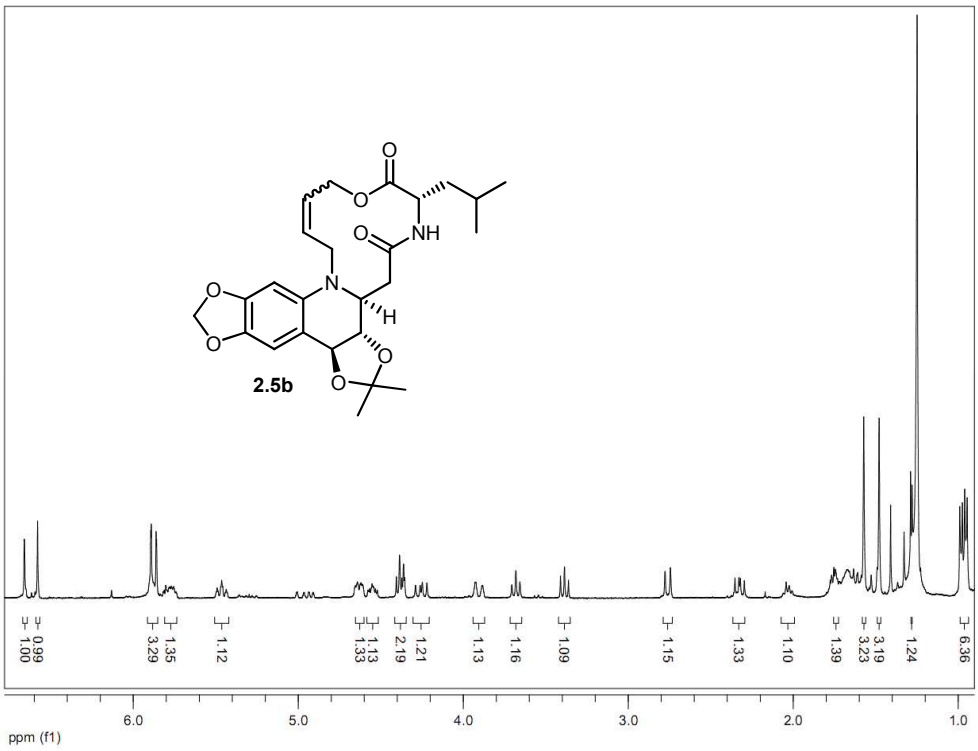

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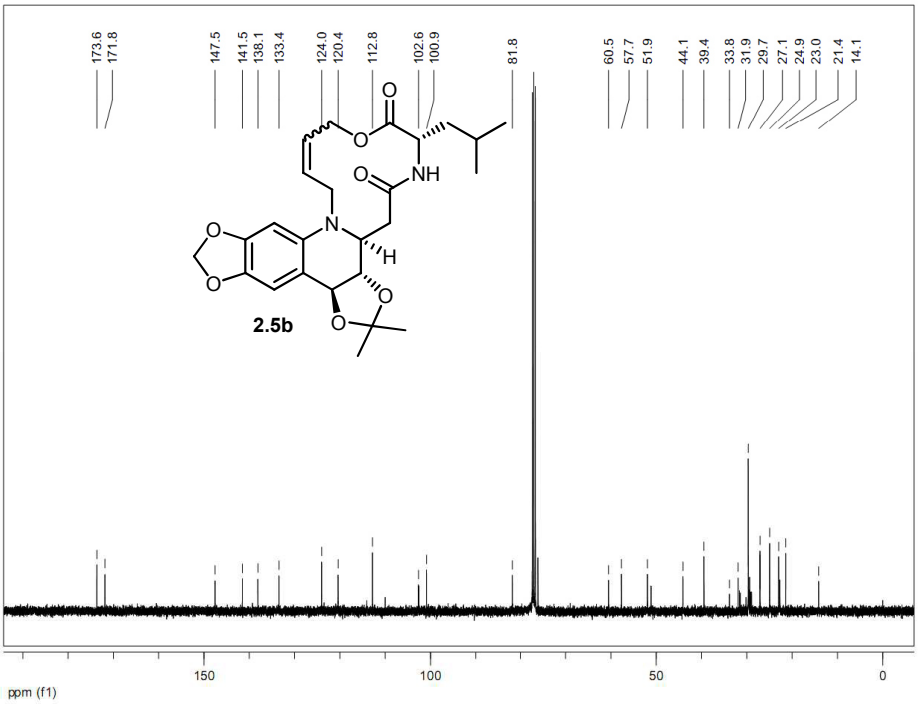

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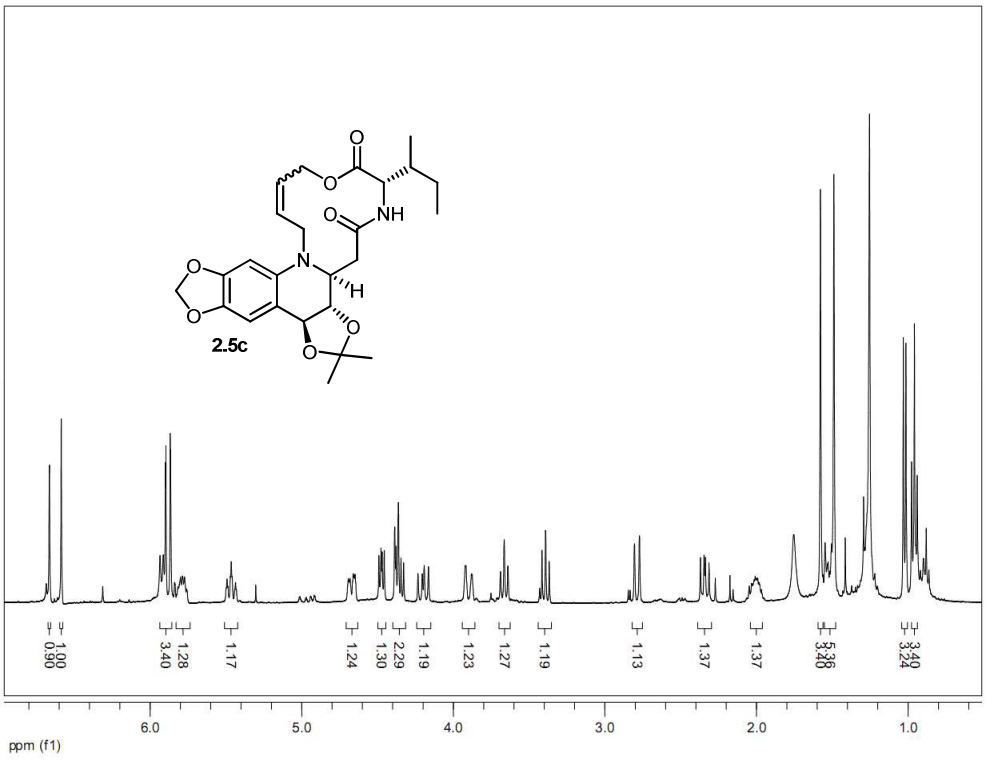

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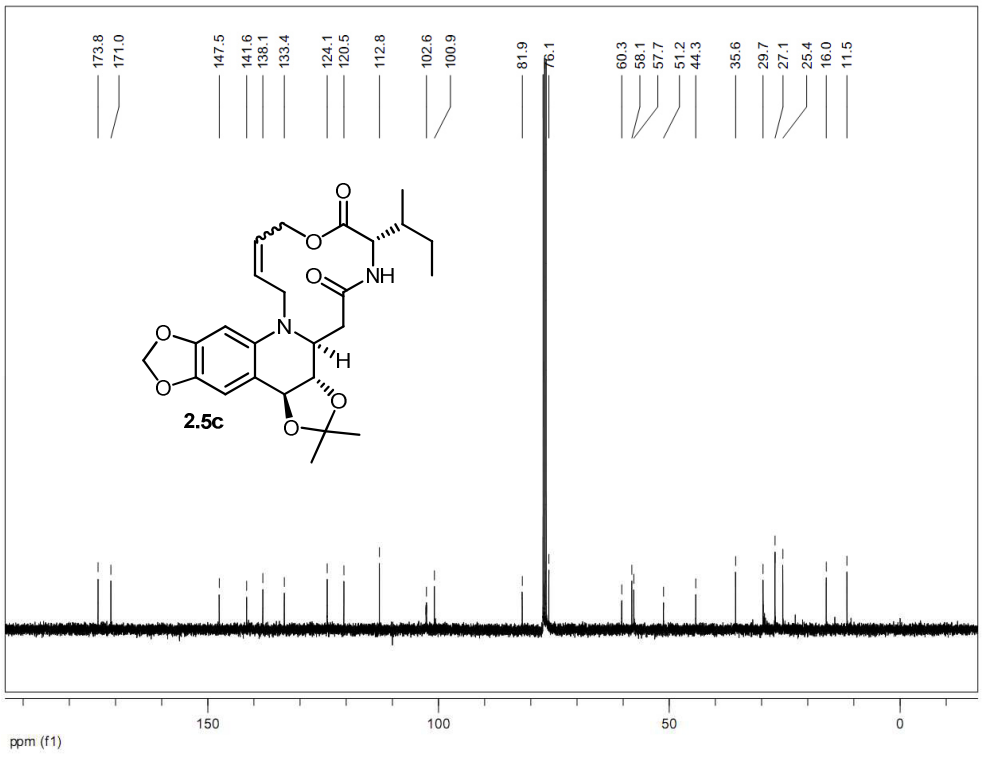

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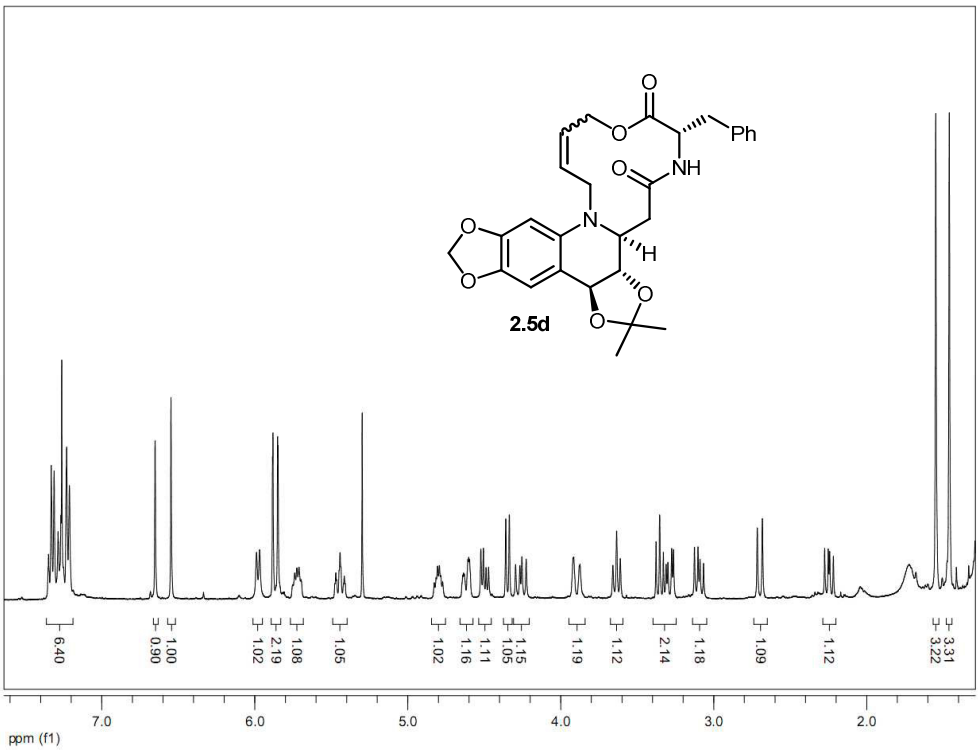

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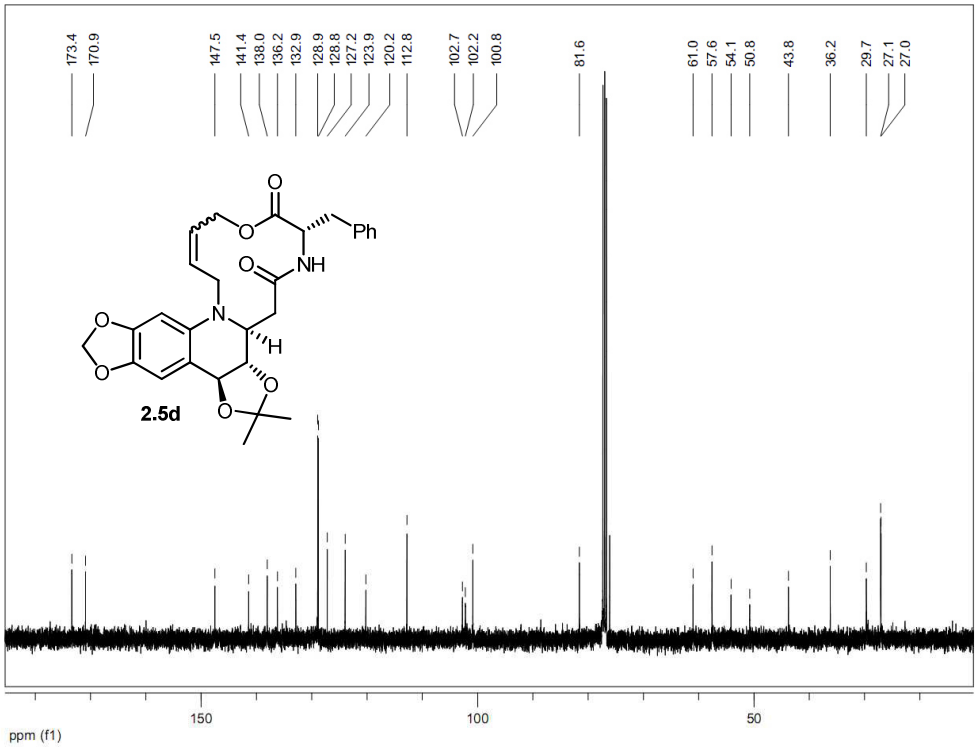
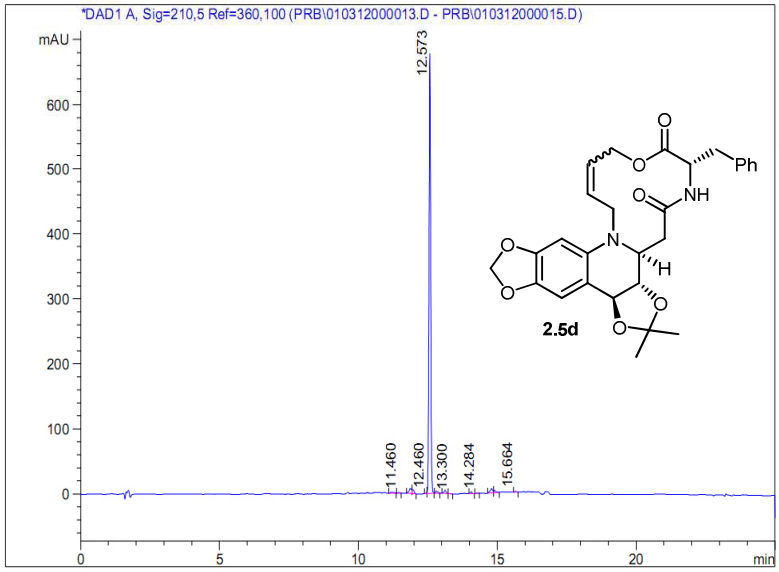


HPLC Analysis Report:

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line	: 0
Injection Date	: Thu, 1. Mar. 2012	Location	: Vial 4
Sample Name	: ILS-GSK-C87-40	Inj. No.	: 0
Acq Operator	: RADHA	Inj. Vol.	: 5 µl
Acq. Method	: D:\CHEM32\1\METHODS\C-18 A80B20.M		
Analysis Method	: D:\CHEM32\1\METHODS\C-18 A80B20.M		
Method Info	: Column : X Bridge C-18 150*4.6mm 5µm		
	Mobile phase: A) 0.1% HCOOH in water , B) ACN (gradient)		
	T/B%:0/20,3/20,14/98,20/98,22/20,25/20		
	Flow:1.0 ml/min Diluent: ACN:WATER (80:20)		



==========

Signal 1: DAD1 A, Sig=210,5 Ref=360,100

Peak #	RT [min]	Width [min]	Area	Area %	Name
1	11.220	0.125	7.107	0.236	
2	11.460	0.083	4.190	0.139	
3	11.873	0.103	45.974	1.525	
4	11.947	0.085	28.053	0.931	
5	12.460	0.043	4.659	0.155	
6	12.573	0.069	2806.642	93.114	
7	12.817	0.085	15.125	0.502	
8	13.121	0.087	23.646	0.784	
9	13.300	0.071	1.736	0.058	
10	14.062	0.070	11.932	0.396	
11	14.284	0.073	3.882	0.129	
12	14.795	0.107	40.968	1.359	

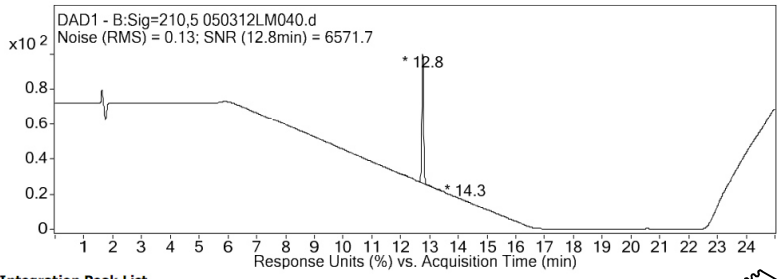
ILS-CSM-5001 Fri, 2. Mar. 2012 04:11:50 pm Page 1 of 2

COSMIC Discoveries@ILS

LC-MS Analysis Report

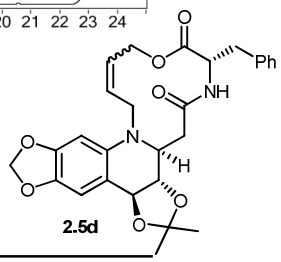
Data Filename	050312LM040.d	**Sample Name**	ILS-GSK-C87-40
Sample Type	Sample	**Position**	Vial 94
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-UNI.m	**Acquired Time**	3/5/2012 7:57:17 PM
IRM Calibration Status	Not Applicable	**DA Method**	Reserpine_Checkout.m
Comment	XBridge C18, 150*4.6mm 5um		

User Chromatograms



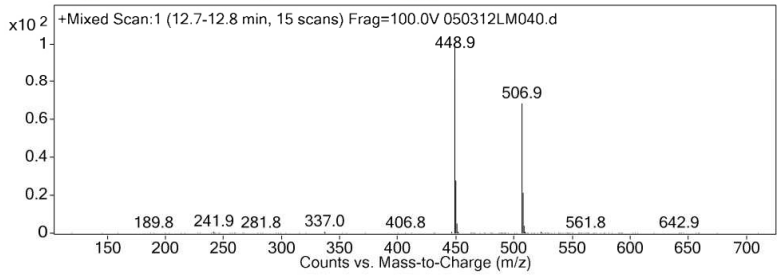
Integration Peak List

Peak	RT	Area	%Area
1	12	7.84	0.21
2	12.1	18.59	0.51
3	12.8	3608.86	98.2
4	12.9	8.68	0.24
5	13.2	22.41	0.61
6	14.3	8.6	0.23



User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed



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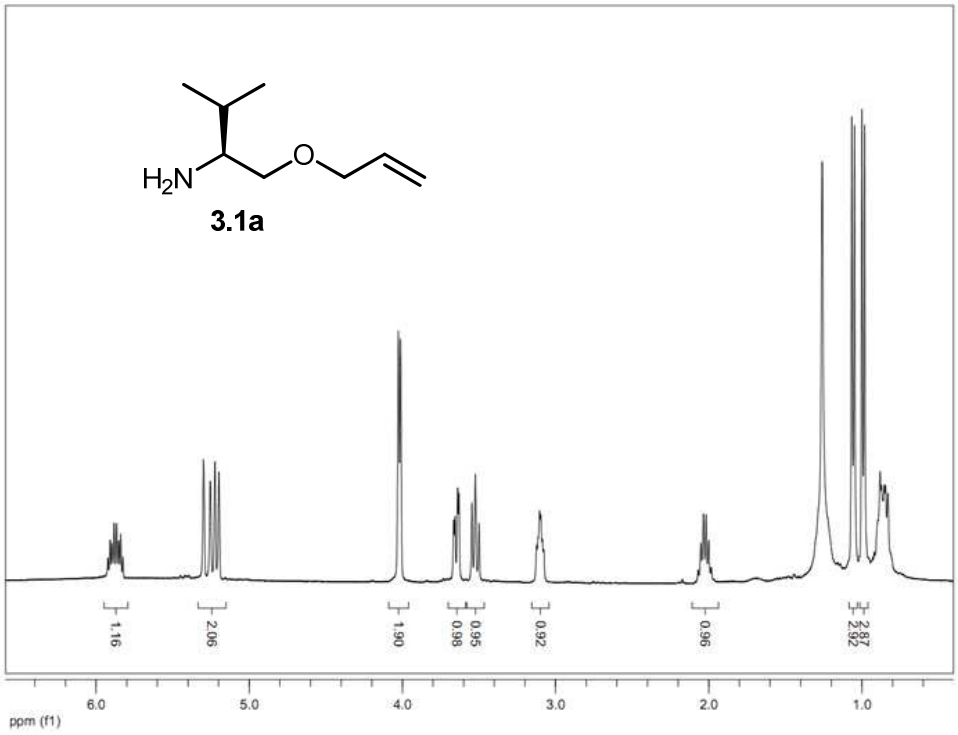
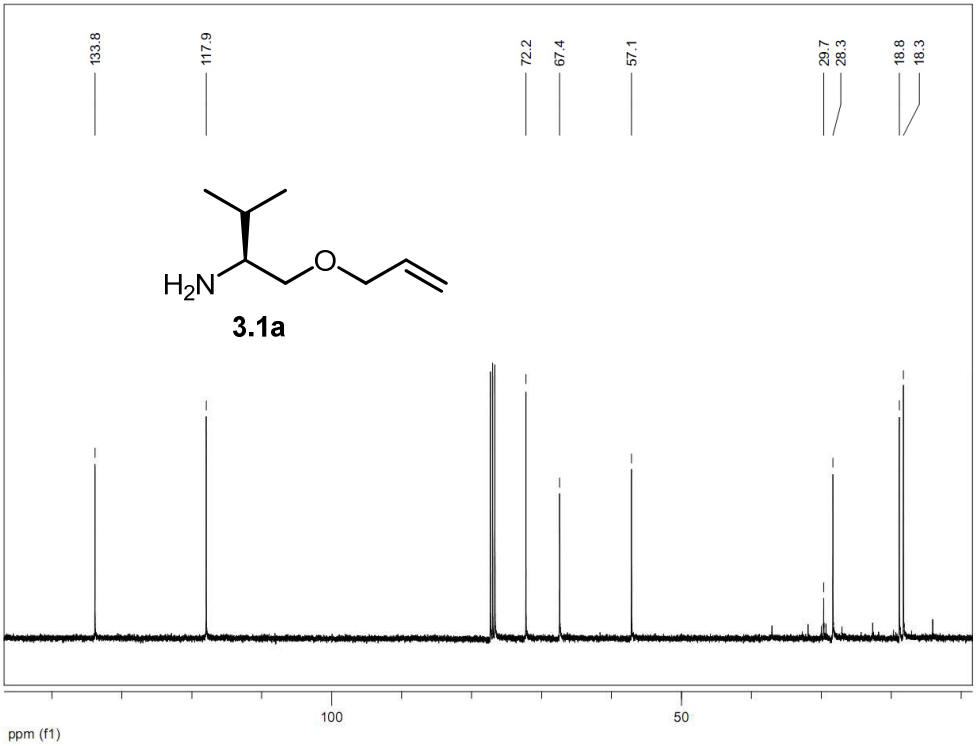
Printed at: 10:12 AM on: 3/6/2012

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



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



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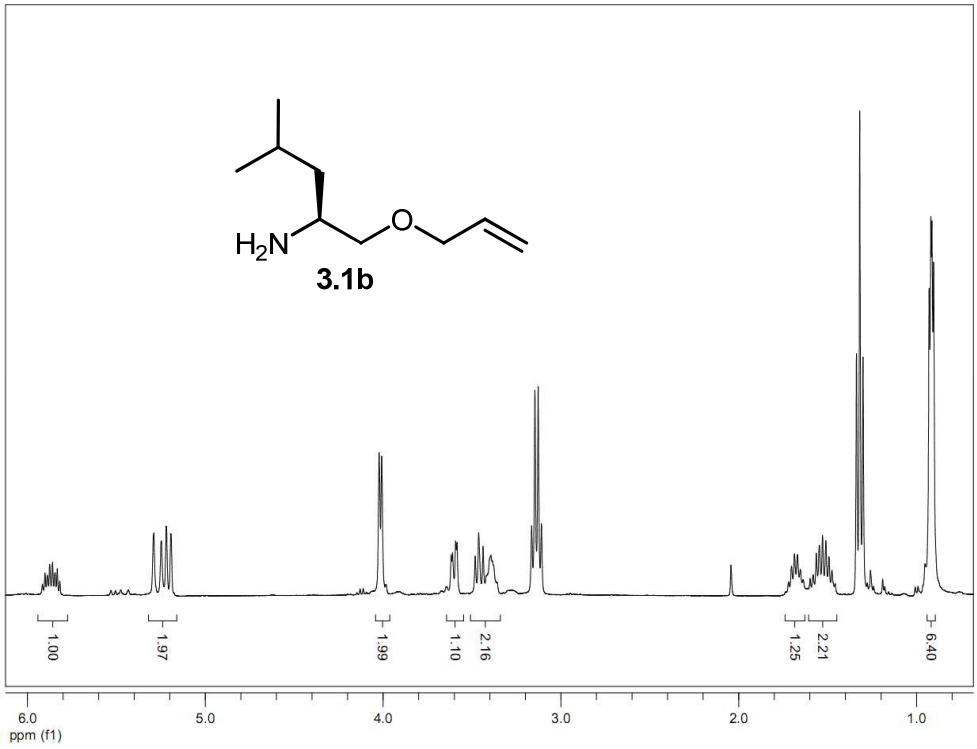
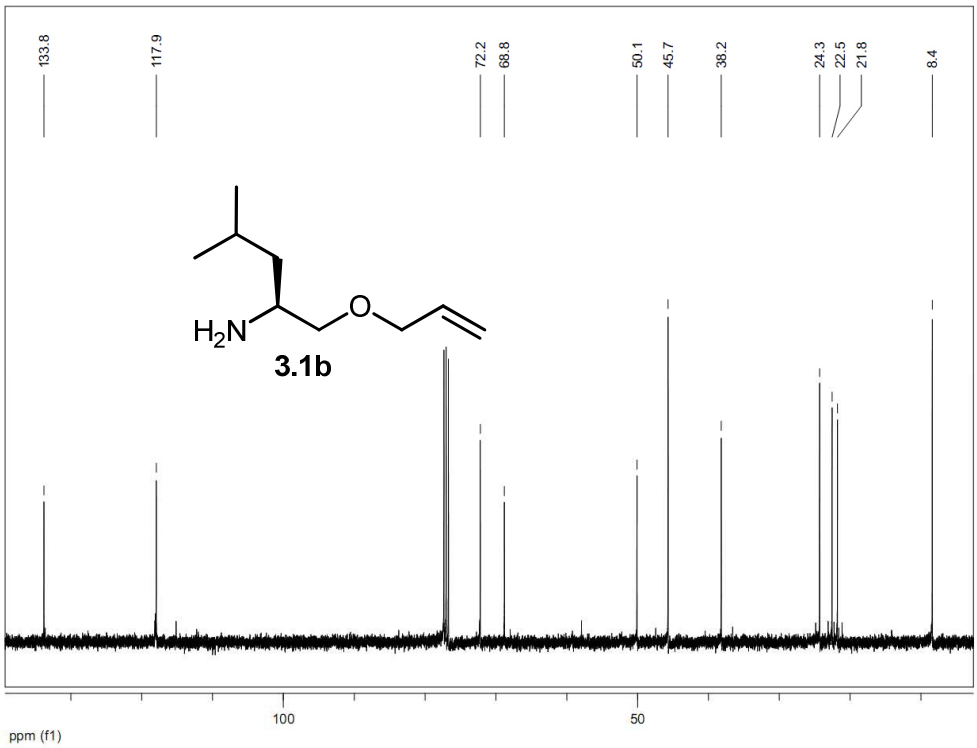


^{13}C-NMR (100 MHz, *$CDCl_3$*):



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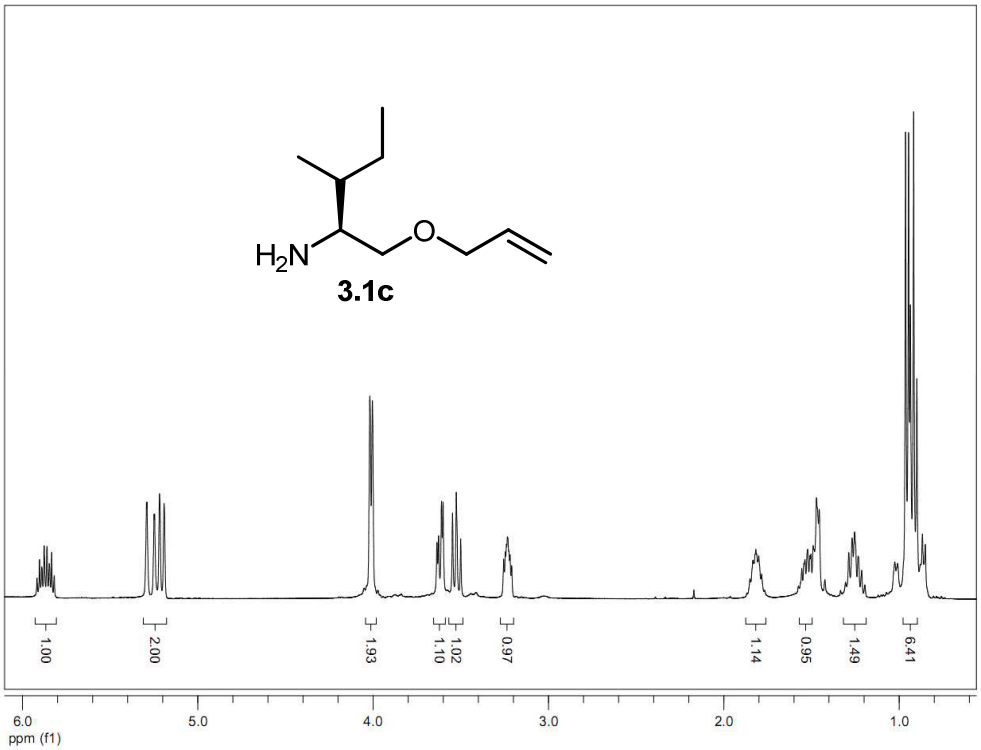

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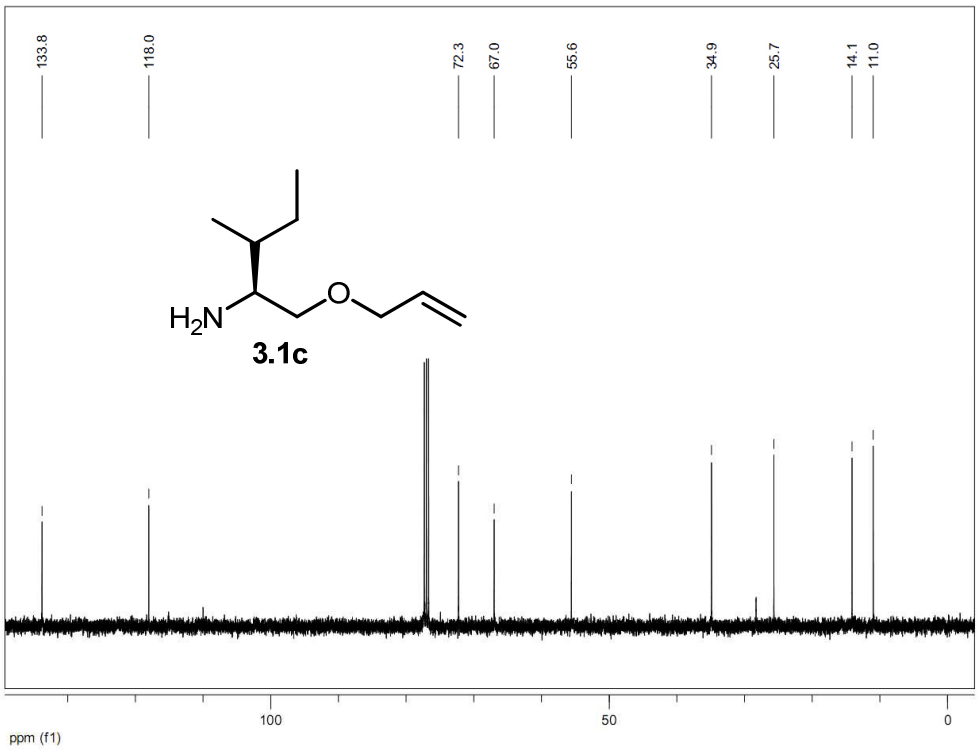

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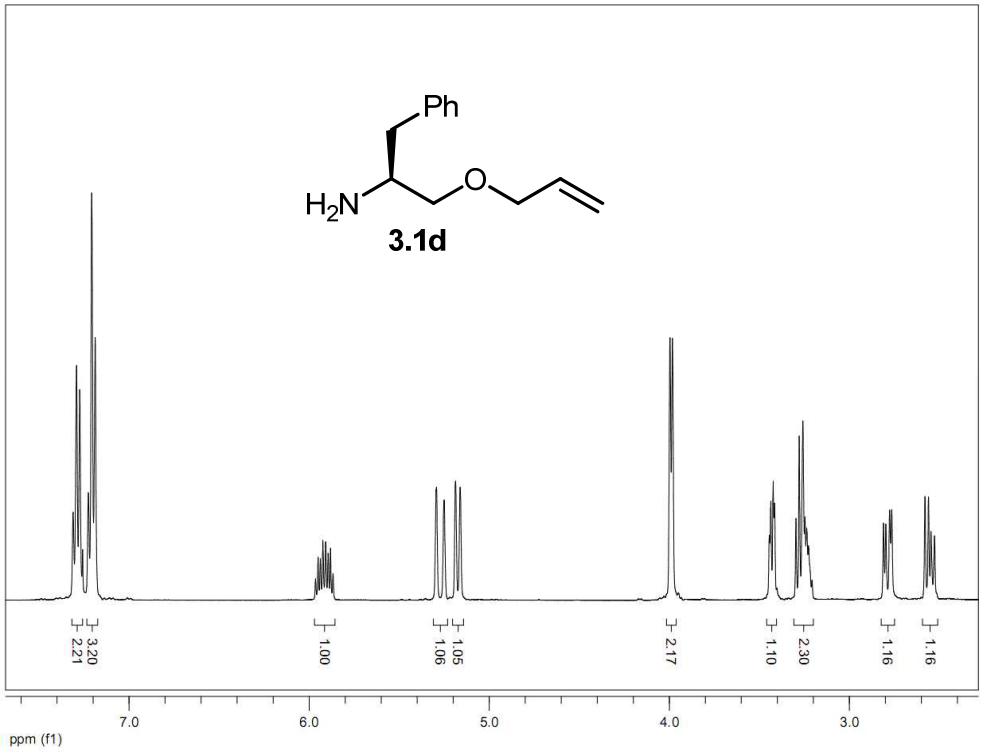

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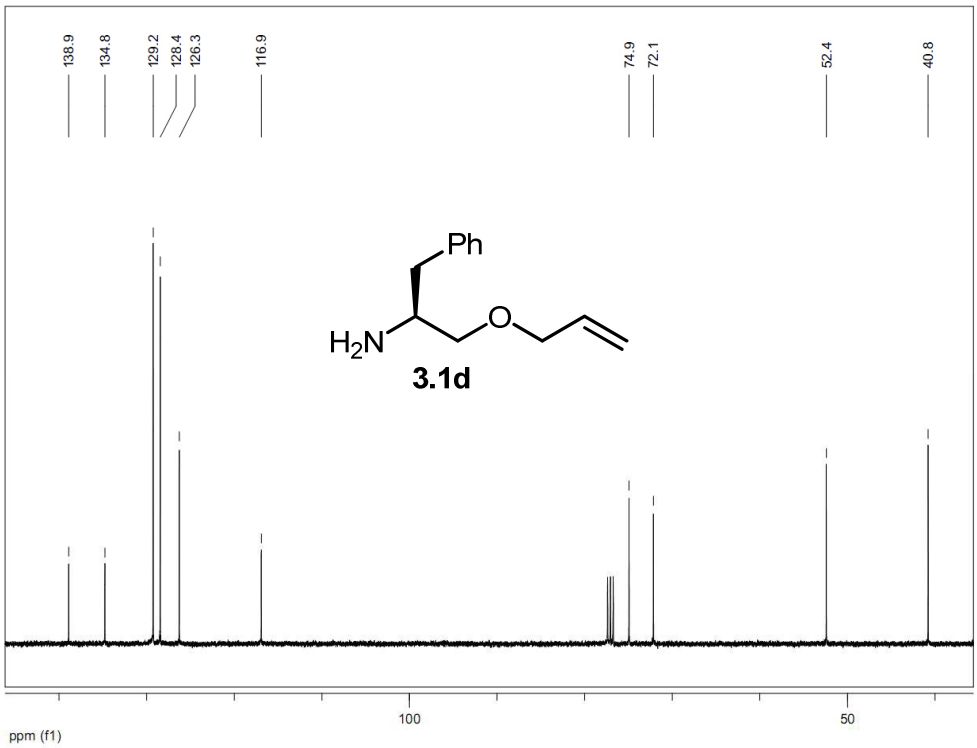

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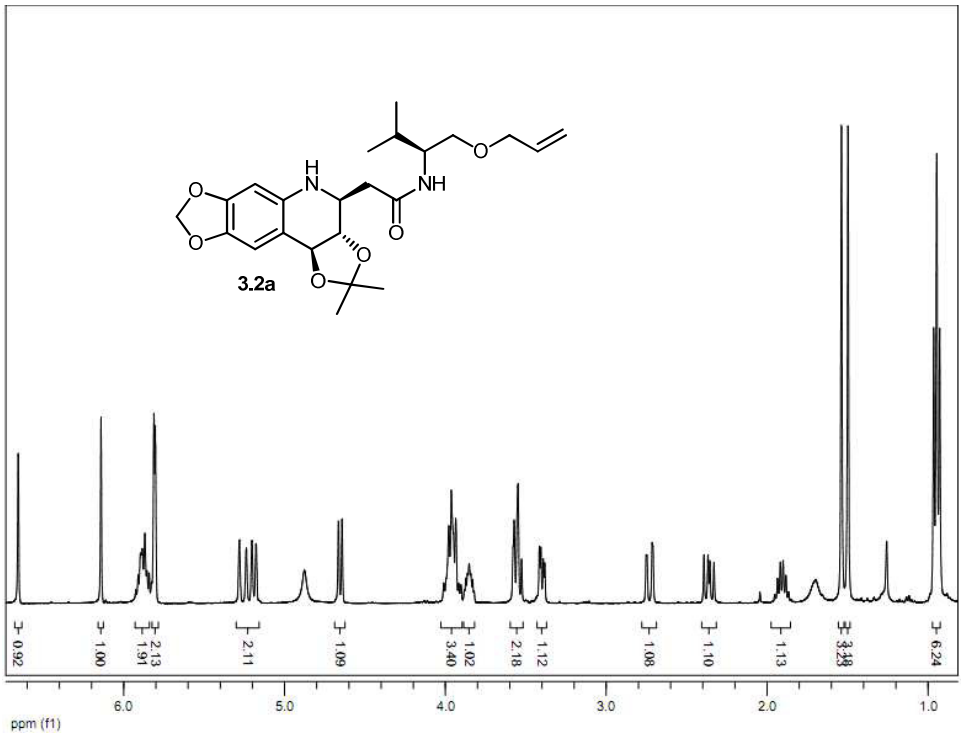

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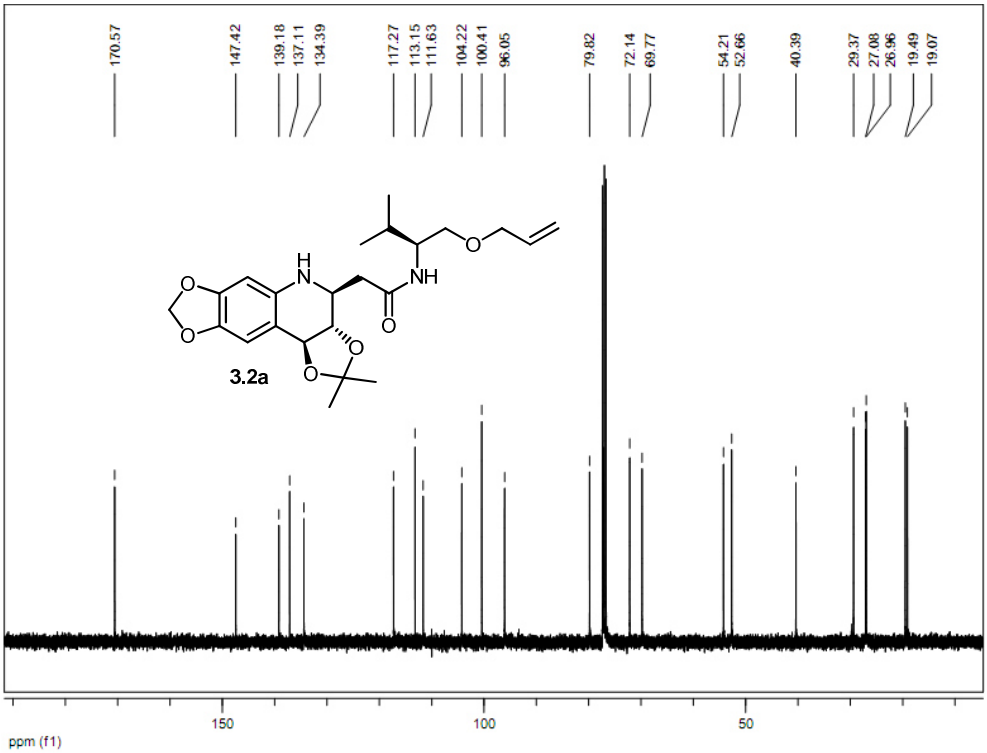

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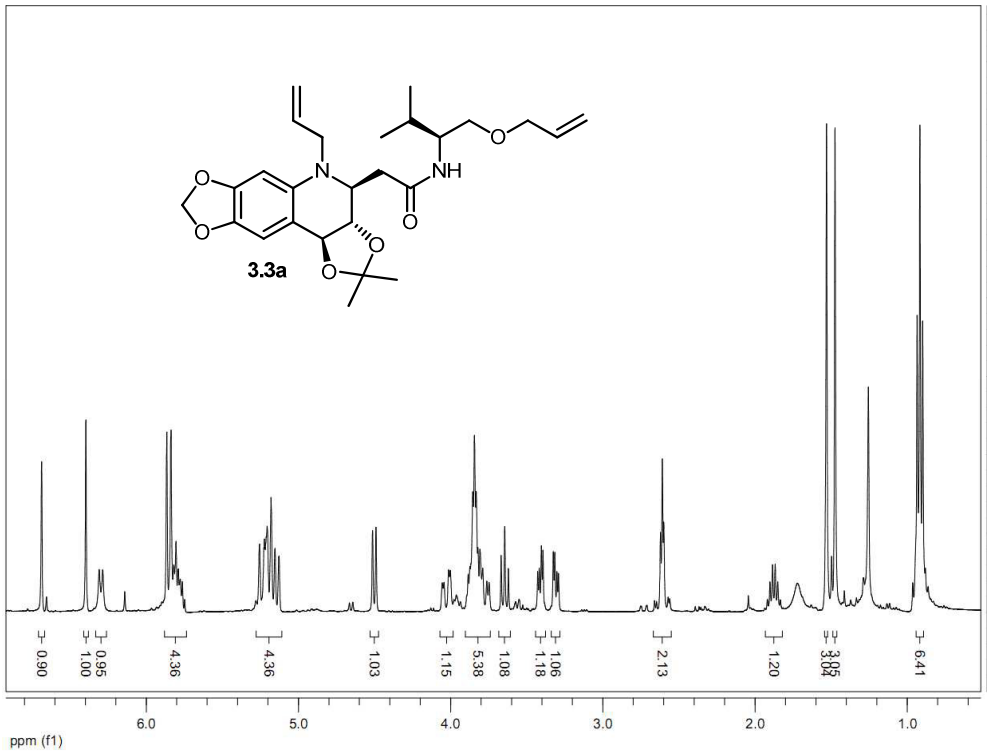

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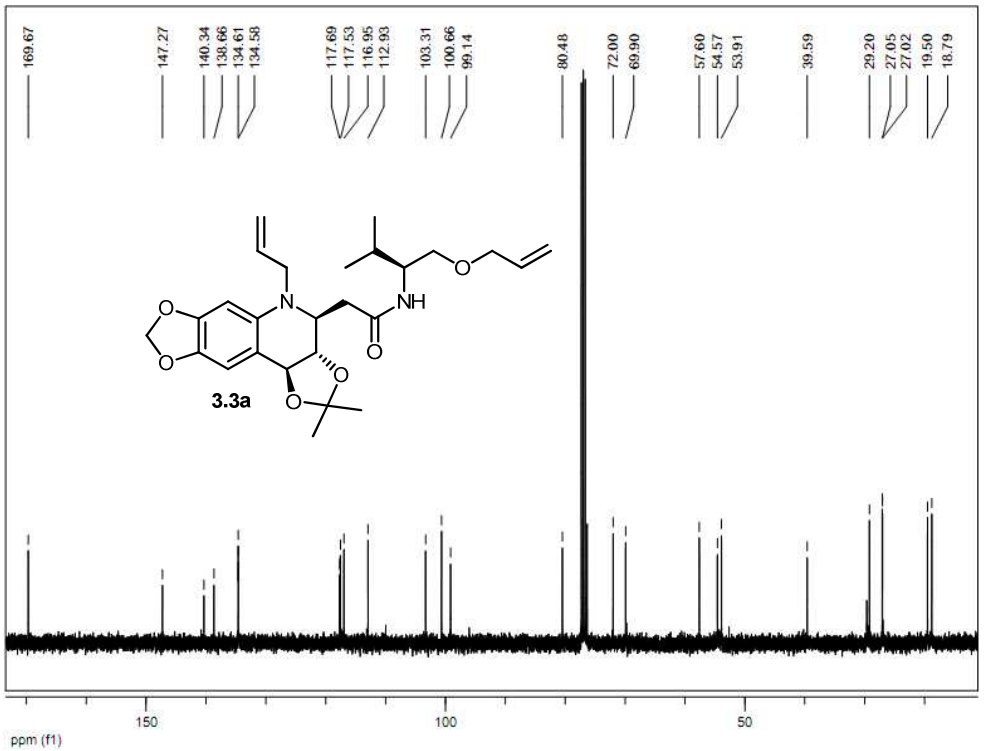

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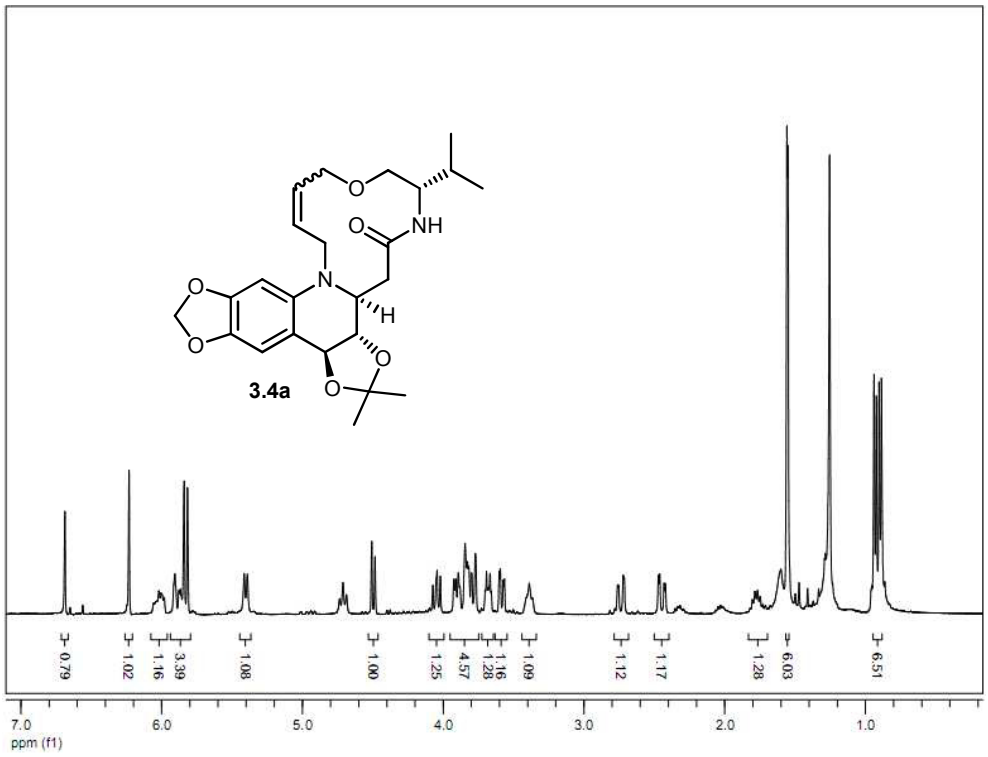

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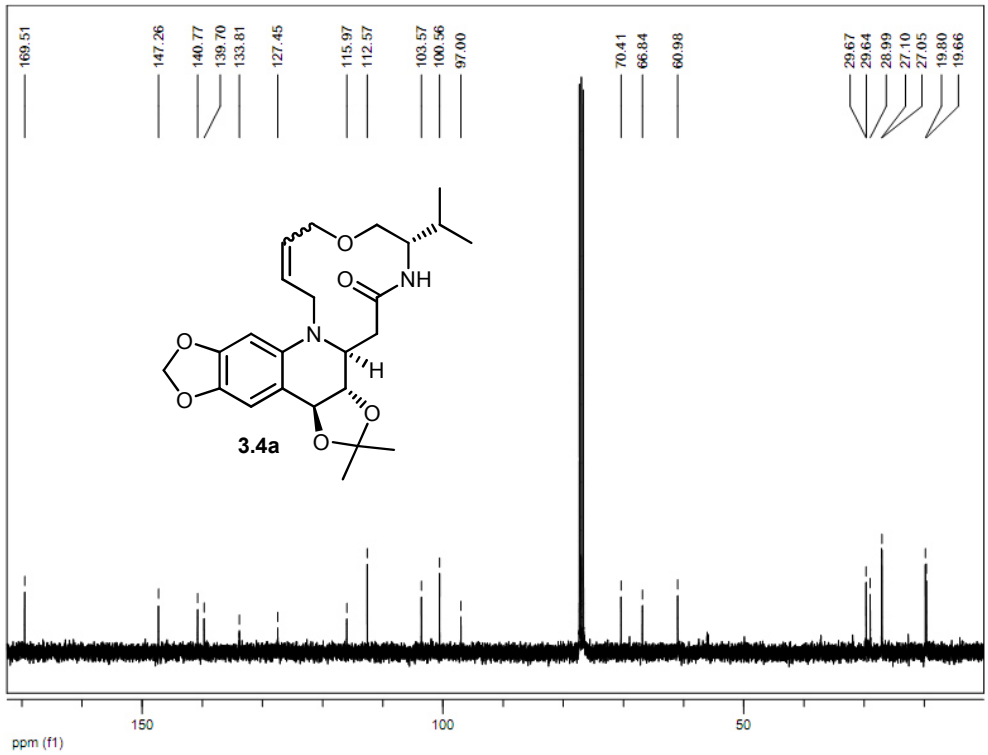

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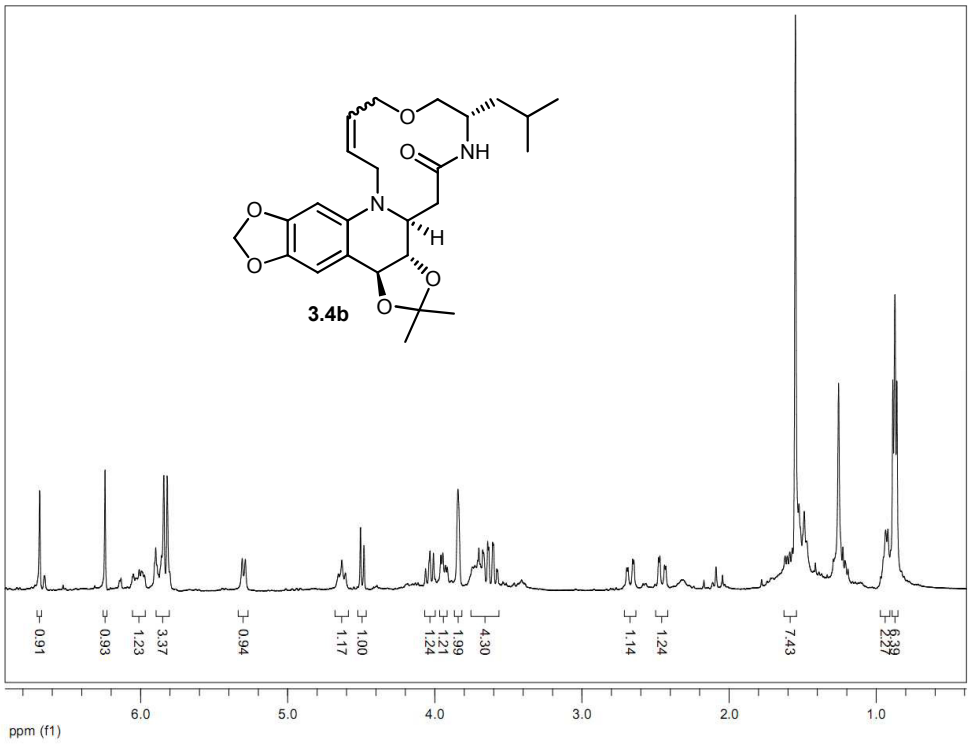

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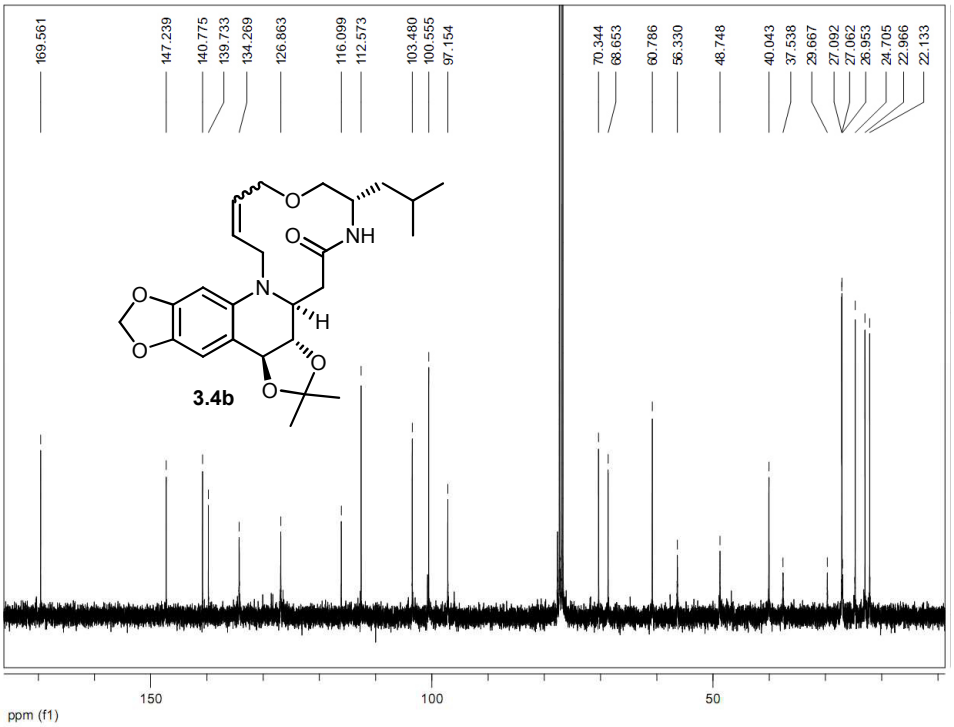

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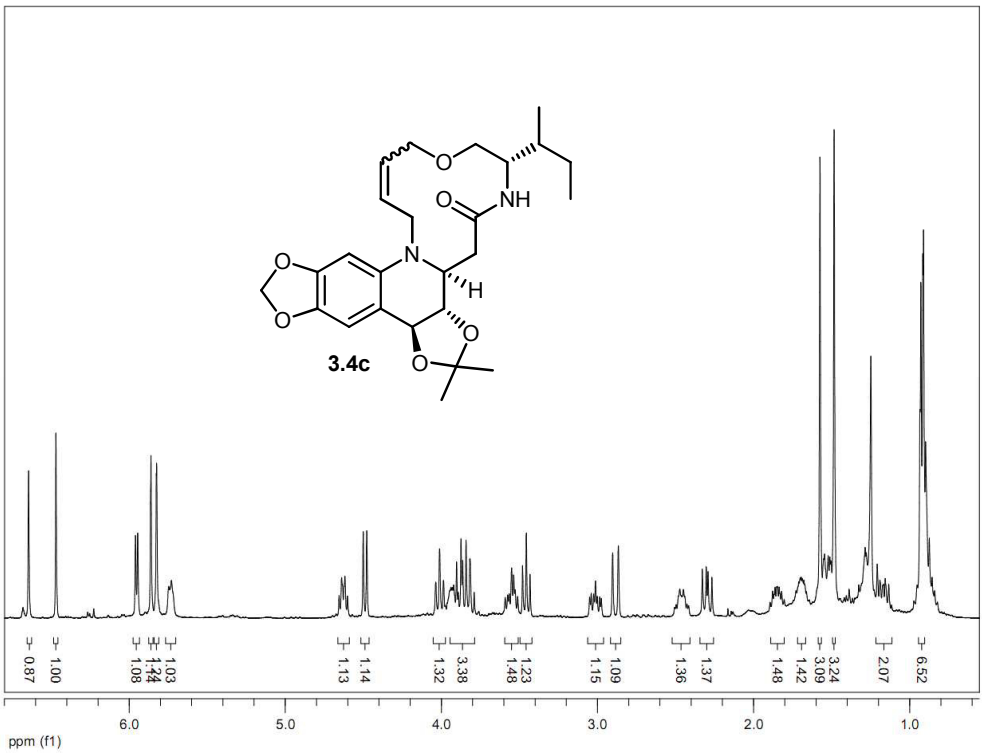

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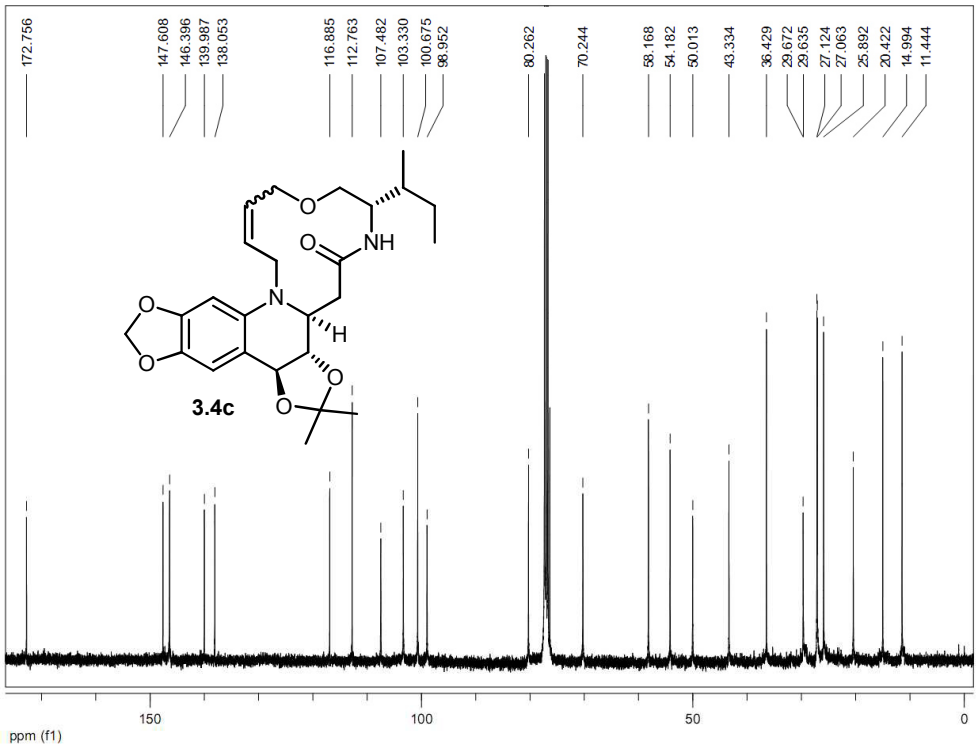

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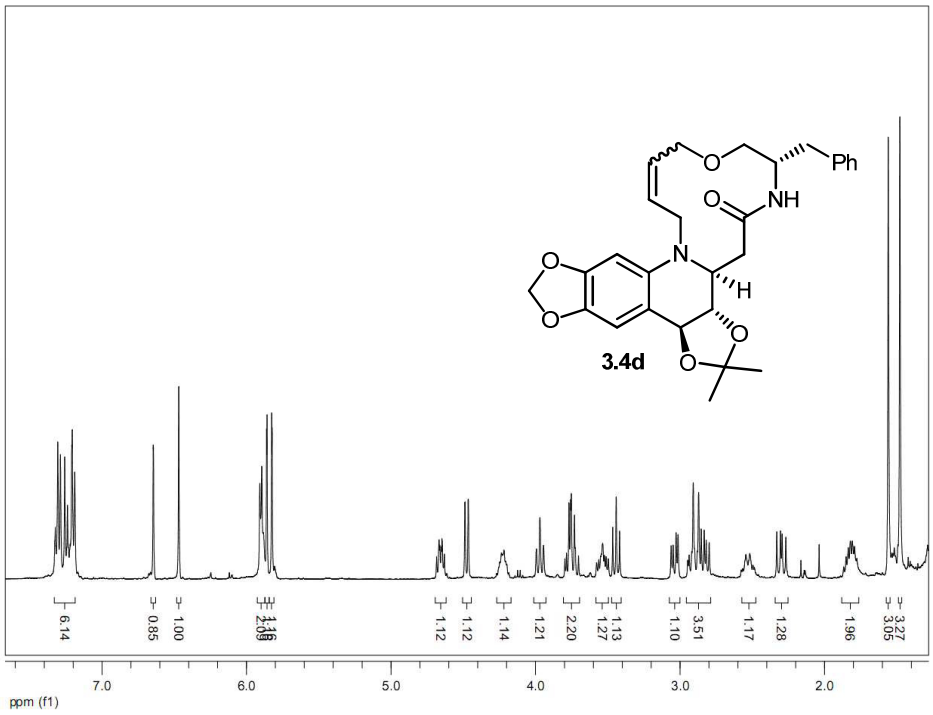

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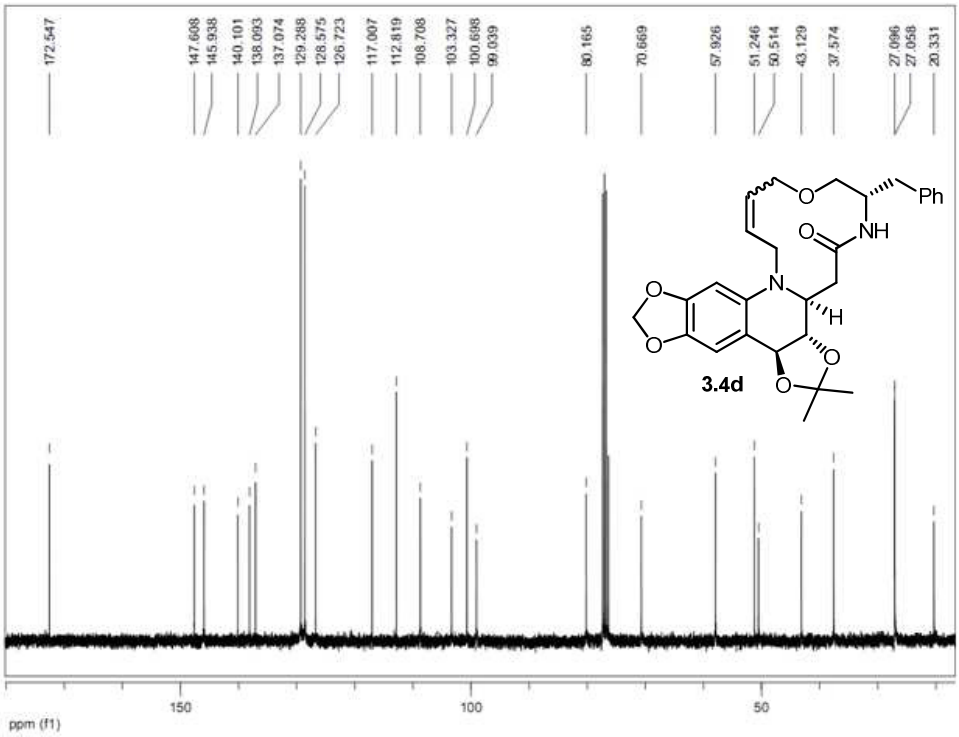

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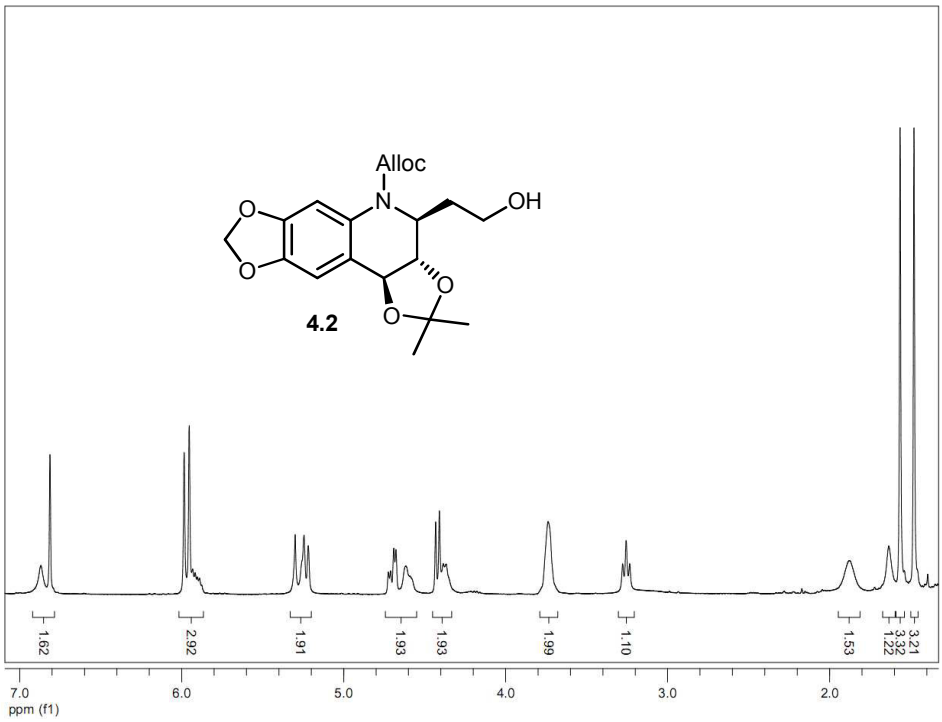

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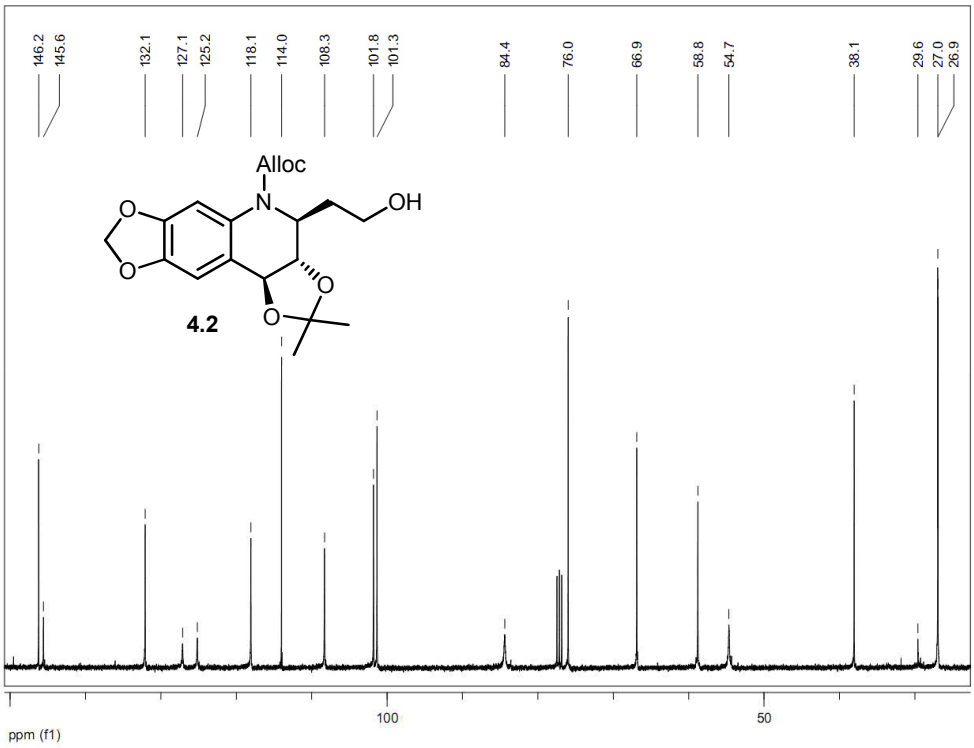

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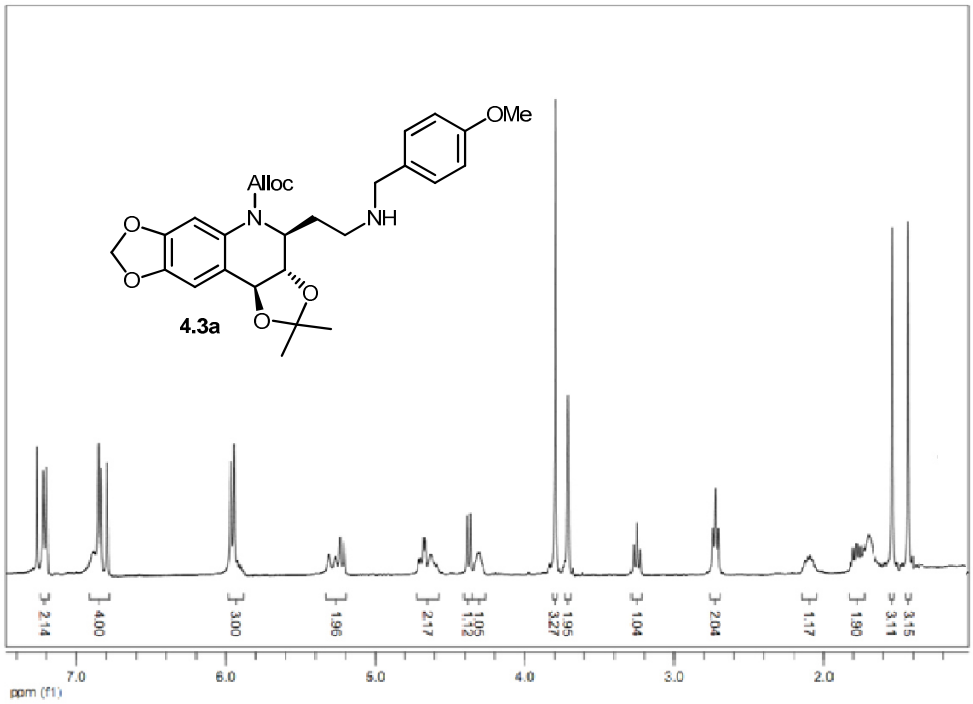

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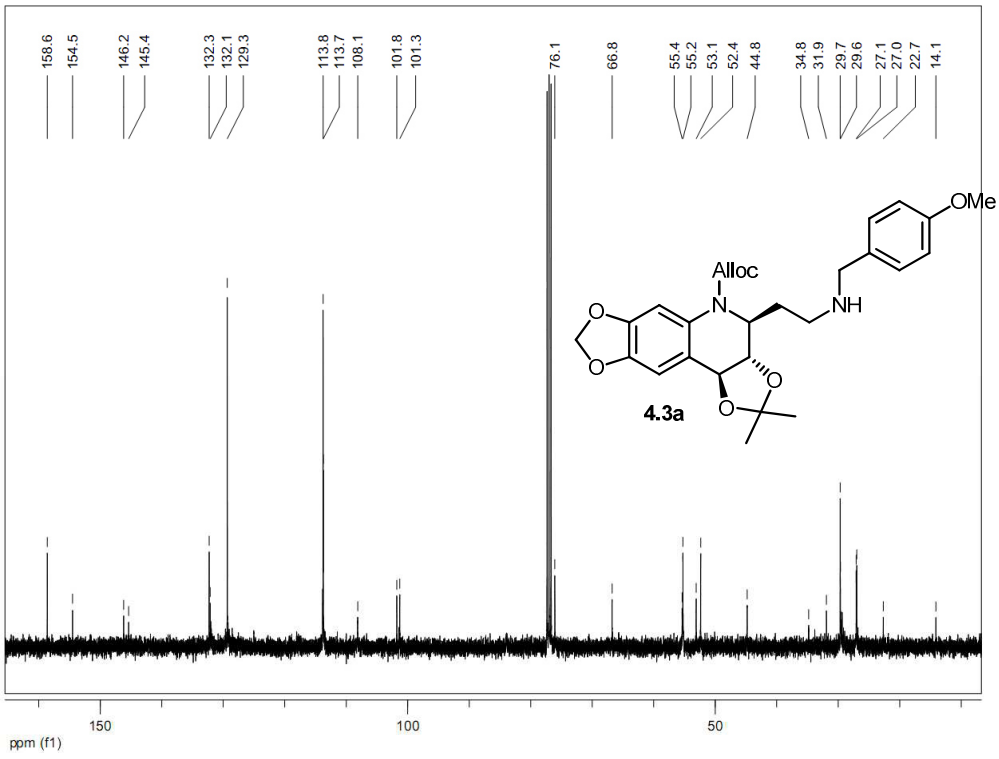

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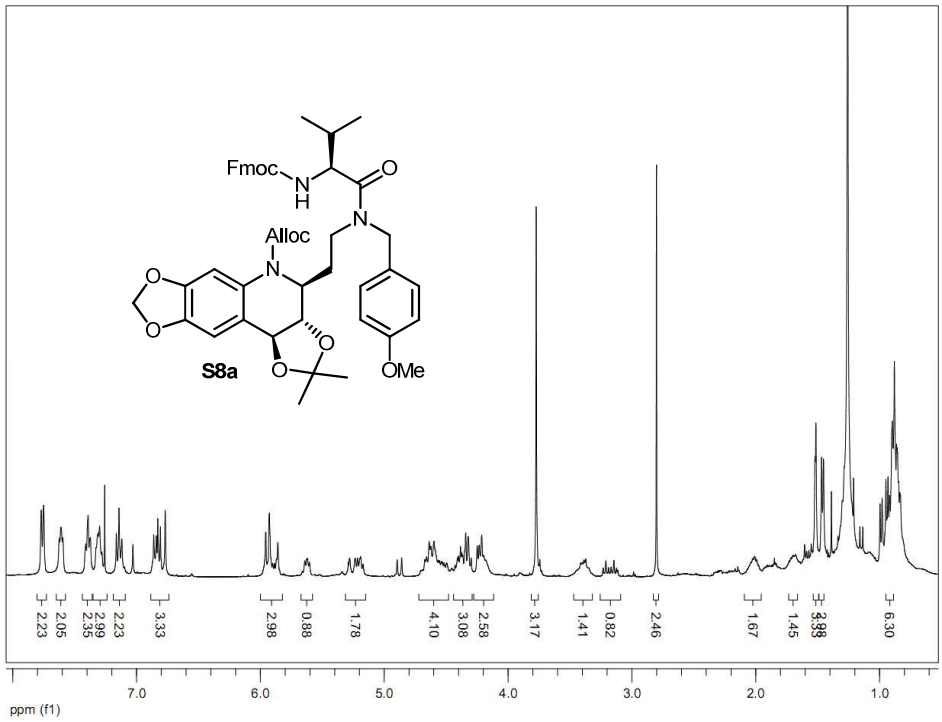

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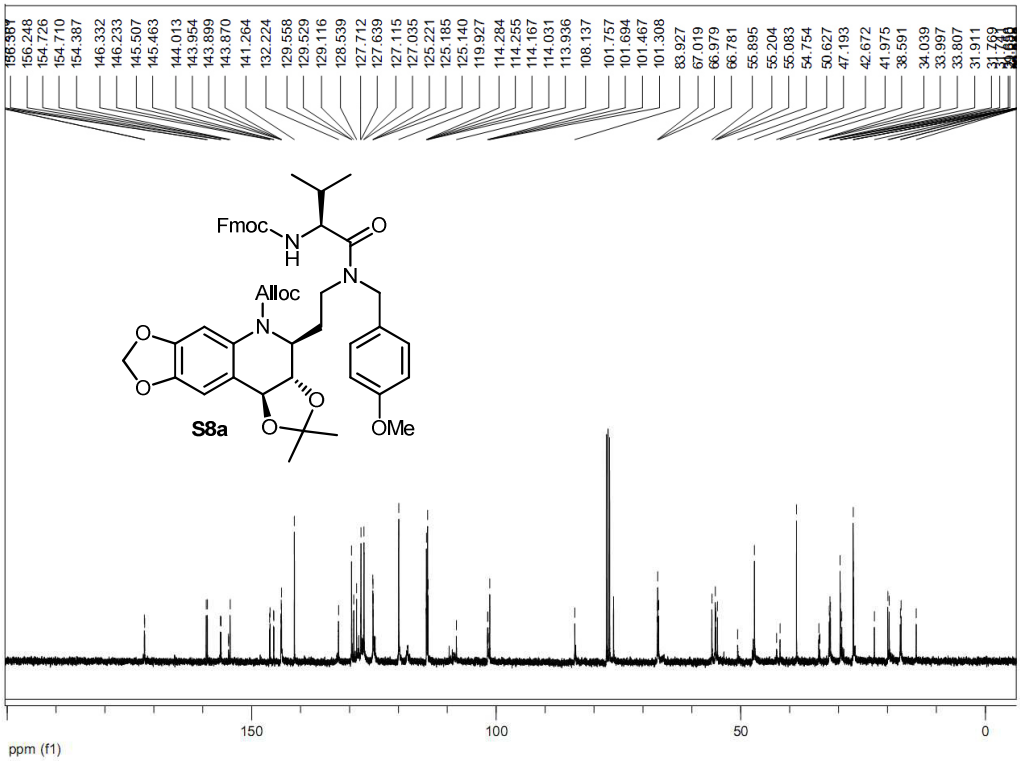

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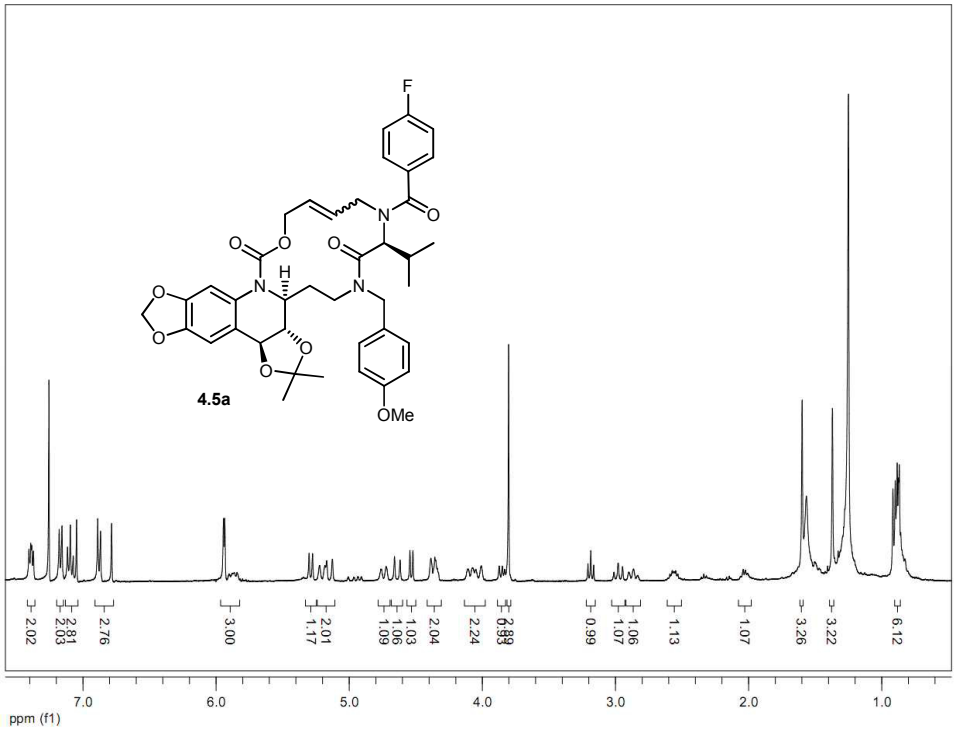

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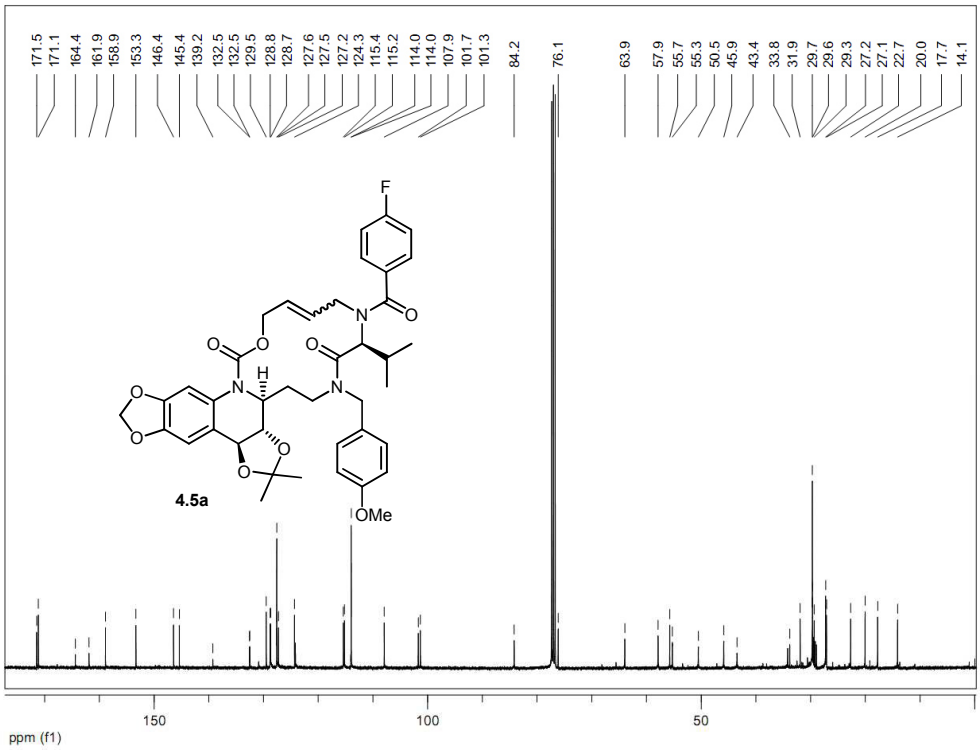

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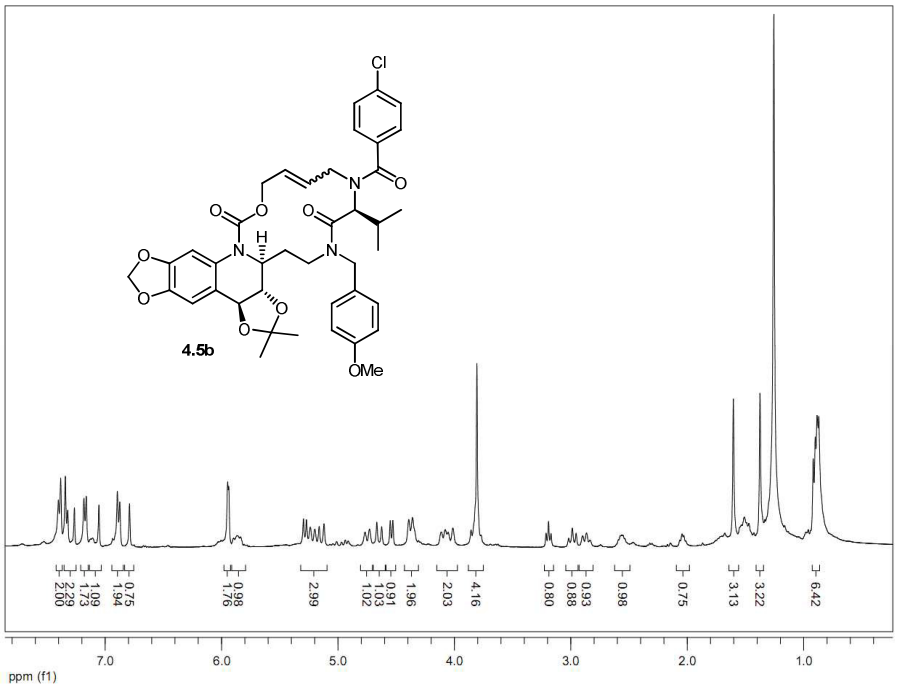

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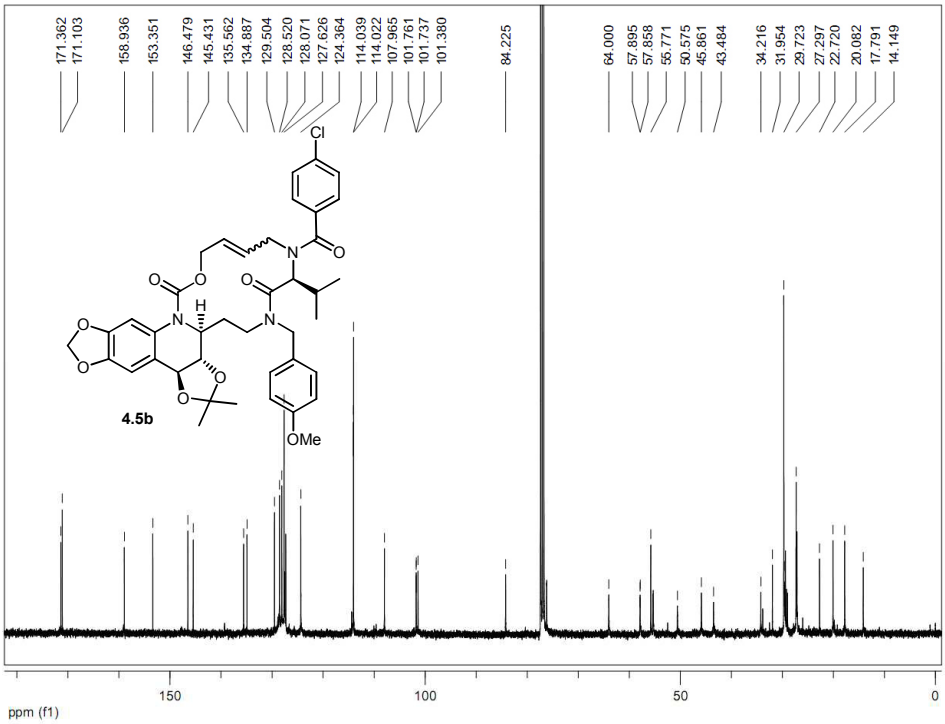

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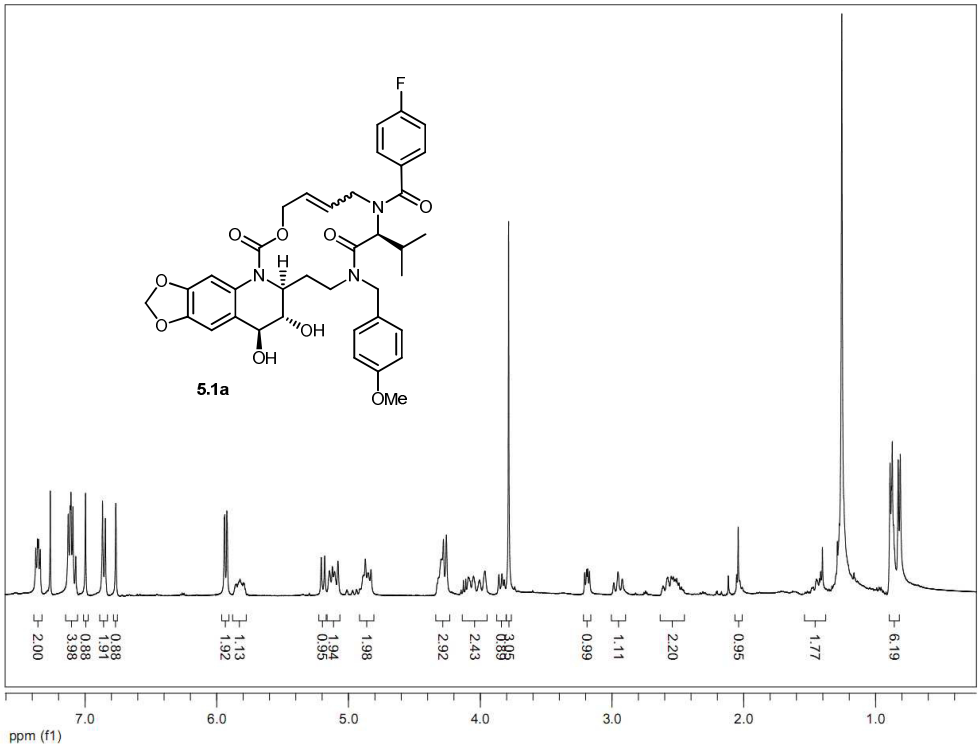

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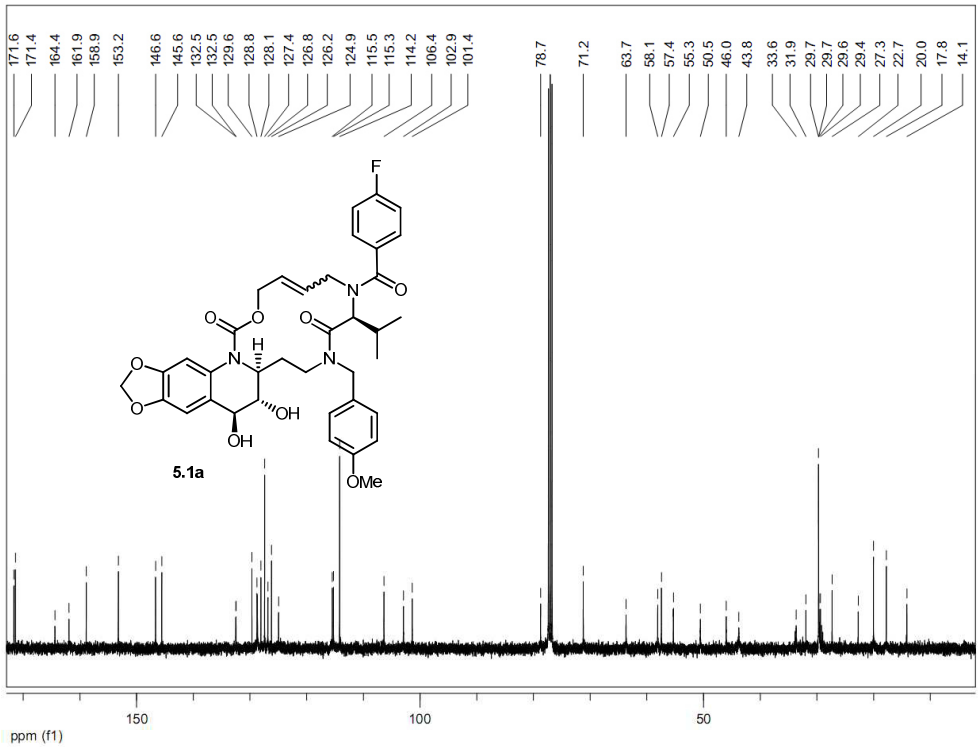

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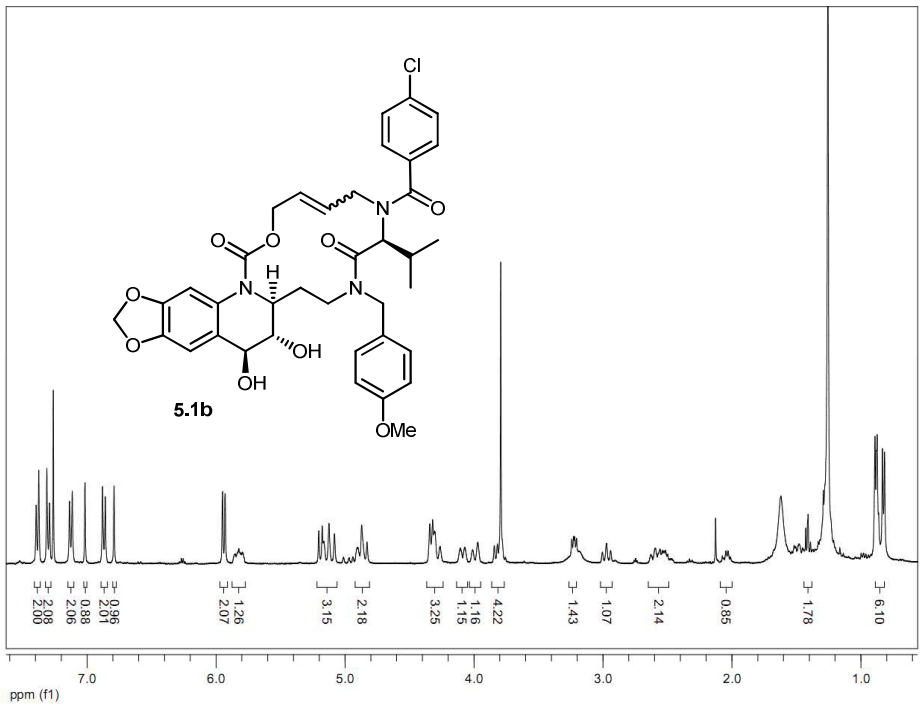

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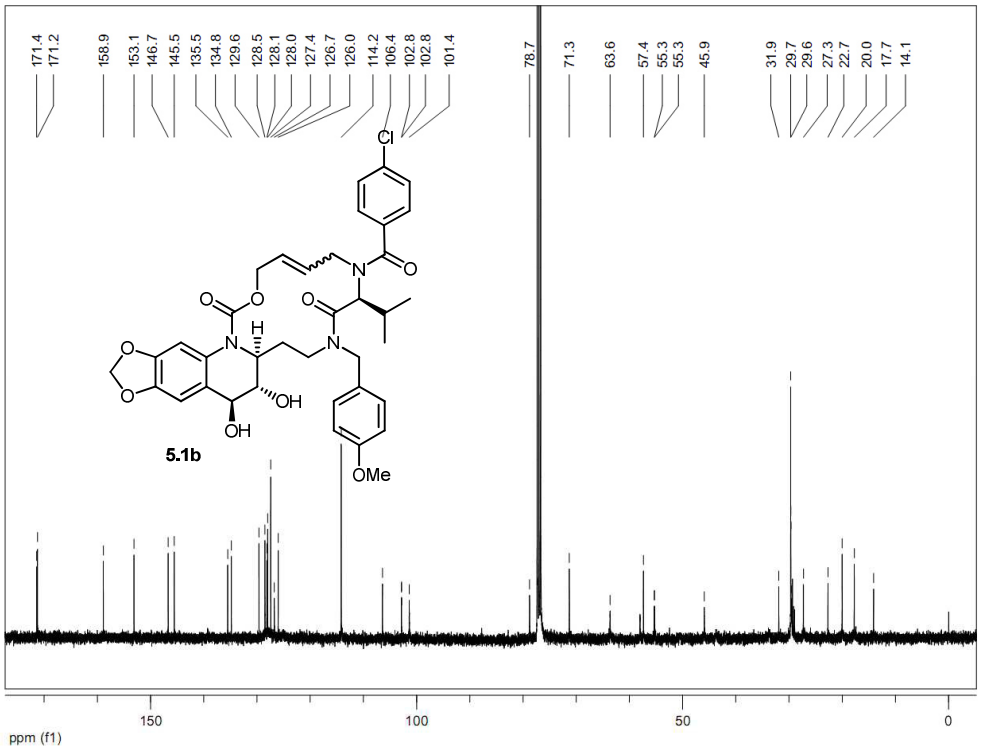


Chapter 3:
Synthesis and Chemical Biology of Rapamycin and Rapamycin-Derived Hybrid Molecules

3.1. Introduction:

The purpose of this chapter is to provide a detailed literature on the synthesis and chemical biology of rapamycin and rapamycin-derived hybrid molecules.

Rapamycin (also known as Sirolimus), a secondary metabolite produced by soil bacterium *Streptomyces hygroscopicus* was first isolated at Easter Island (Rapa Nui) in the 1970s.[1] Rapamycin is a 31-membered macrolactone polyketide featuring a cyclohexyl moiety derived from the shikimic acid pathway, a 6-membered hemiacetal, a pipecolinyl ring and a triene in its structure (see **Figure 1**). The biological activity of rapamycin was originally discovered in a screen for novel antifungal agents, mainly active against *Candida albicans*. Later, it was found to be an antibiotic and an immunosuppressant that has been used for several years to prevent rejection in organ transplantation and is currently approved for treatment in cardiovascular diseases by US Food and Drug Administration (FDA).[2]

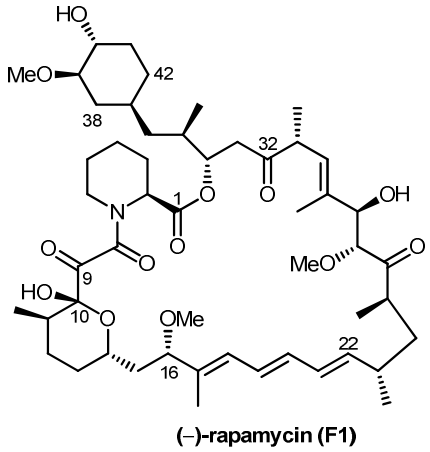


Figure 1: Structure of Rapamycin

3.2. mTOR Structure and Biological Function:

One of the key features of rapamycin is that it inhibits the activity of mammalian target of rapamycin (mTOR), 289-kDa, an intracellular serine/threonine protein kinase belonging to the phosphoinositide3-kinase (PI3K)-related kinase family that plays a central role in various cellular processes, including cell growth and proliferation, protein synthesis and autophagy.[3] mTOR constitutes the central regulatory catalytic core of at least two functionally distinct multi-protein

complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which can be distinguished from each other based on their unique compositions and substrates (see **Figure 2**). Initially mTORC1 and mTORC2 were identified on the basis of their differential sensitivity to the inhibitory effects of rapamycin, mTORC1 being originally considered as rapamycin-sensitive and mTORC2 as rapamycin-insensitive.[4] However, treatment with high doses of rapamycin as long in duration can also inhibit mTORC2 activity in a cell type specific manner.[5]

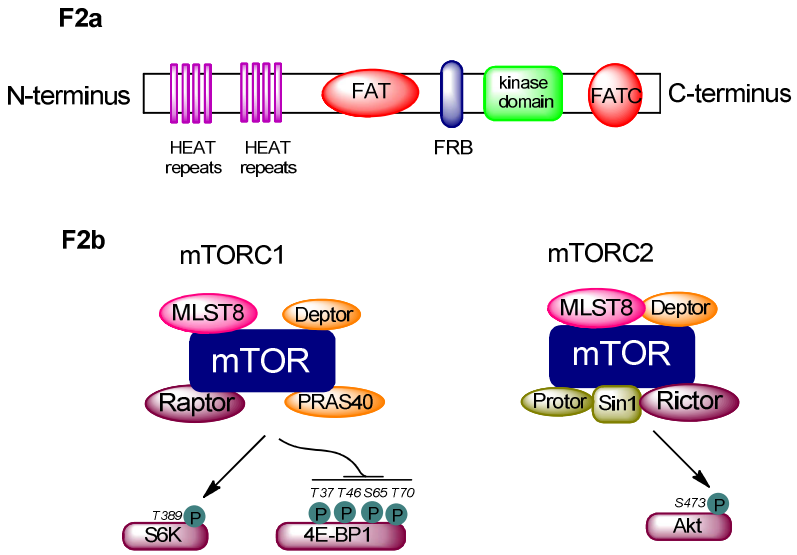


Figure 2: (a) The Domain Structure of mTOR. mTOR contains tandem HEAT repeats, central FAT domain, FRB domain, a catalytic kinase domain and the FATC domain. Rapamycin associates with its intracellular receptor, FKBP12, and the resulting complex interacts with the FRB domain of mTOR. Binding of rapamycin-FKBP12 to the FRB domain disrupts the association of mTOR with the mTORC1 specific component Raptor and thus uncouples mTORC1 from its substrates, thereby blocking mTORC1 signaling. **(b)** Composition of mTORC1 and mTORC2. mTORC1 consists of mTOR, Raptor, PRAS40, mLST8 and Deptor. mLST8 binds to the mTOR kinase domain in both complexes, where it seems to be crucial for their assembly. Deptor acts as an inhibitor of both complexes. Other protein partners differ between the two complexes. mTORC2 contains Rictor, mSIN1, and Protor1.

mTOR, especially mTORC1, has a key role as a nutrient and energy sensor at both cellular and whole-body levels. The result of mTORC1 signaling in

metabolically important organs such as the liver, adipose tissue, muscle and the hypothalamus coordinates whole-body metabolism during intervals of food intake and starvation. In the healthy state, glucose homeostasis is maintained by glucose storage as glycogen when nutrient levels are high and, conversely, by the mobilization of glycogen back to glucose when blood glucose levels drop. These responses are tightly regulated by insulin and its downstream signalling to mTORC2 and mTORC1. Excessive nutrients can be stored as triglycerides in a white adipose tissue (WAT). mTORC1 promotes adiposity[6] and lipid accumulation by up regulating the transcription factors peroxisome proliferator-activated receptor-γ (PPARγ) and sterol regulatory element-binding protein 1 (SREBP1) and SREBP2.[7] Chronic overfeeding results to continuously high levels of mTOR signaling and disturbing of whole-body metabolic regulation, eventually leads to obesity (excessive lipid accumulation), and, type 2 diabetes (loss of insulin responsiveness). Abnormal metabolic homeostasis and growth control leads to diabetes and cancer, respectively, and it is not surprising that mTOR has a role in their aetiology. However, an additional aspect in the TOR story is a role of TOR in lifespan extension. Long-term suppression of mTOR signaling (which is stimulated by nutrients) mimics the dietary restriction, a well-documented means of prolonging lifespan.[8] Adult mice treated with rapamycin have shown increased lifespan with better health indicators compared to an untreated control group.[9] Though TOR inhibition had shown to increase lifespan in yeast, flies and worms, proving lifespan extension in vertebrates opens an opportunity to a potentially new application for mTOR inhibition, the pharmacological extension of lifespan in humans. The major clinical benefits to be anticipated from mTOR inhibition are in the treatment of cancer, management of diabetes and associated complications,[10] an extension of lifespan and amelioration of age-related disorders.[11]

3.3. Rapamycin Biological Activity:

Rapamycin is ideal for binding to FK506 binding proteins (FKBPs), these proteins belong to specific family of immunophilins (cytosolic binding proteins). The most related protein of this family for the immunosuppressive effects of rapamycin is FKBP12, is a 12 kDa protein and functions as cis/trans

peptidylpropyl isomerases.[12] Rapamycin binds to FKBP12 and inhibits its isomerase activity, but only rapamycin-FKBP12 complex is insufficient to mediate immunosuppressive effect of rapamycin. Rapamycin becomes biologically active only when FKBP12-rapamycin complex binds with specific intracellular target, FRB domain, which is a small 11 kDa hydrophobic binding domain, present on the 289 kDa protein FRAP then it leads to the protein-protein interaction (PPI) stabilization.[13] Inhibition of this protein blocks the signal transduction pathways thus inhibiting the cell cycle progression from G1 to S phase in various cell types, allowing rapamycin to exploit its action.[14]

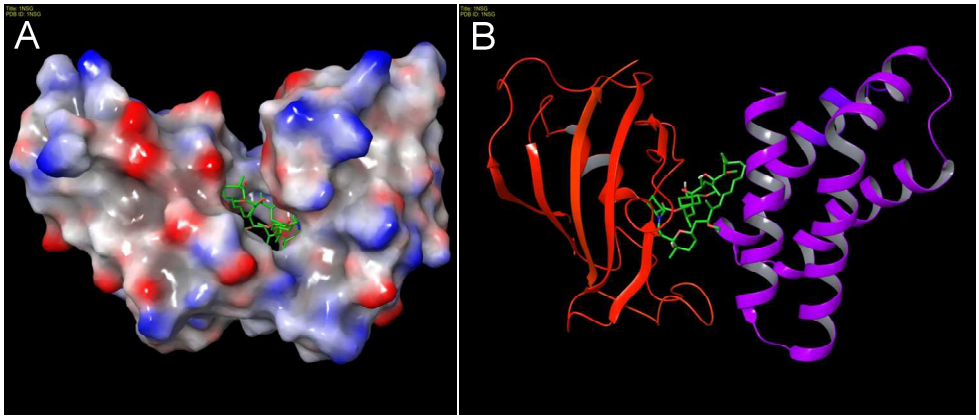


Figure 3: The Structure of The Ternary Complex of FKBP12–Rapamycin–FRB **(A)** Surface Model **(B)** Ribbon Model (PDB Code: 1NSG). [Molecular model images are taken through Maestro v9.6 software pakage (Schroedinger LLC)].

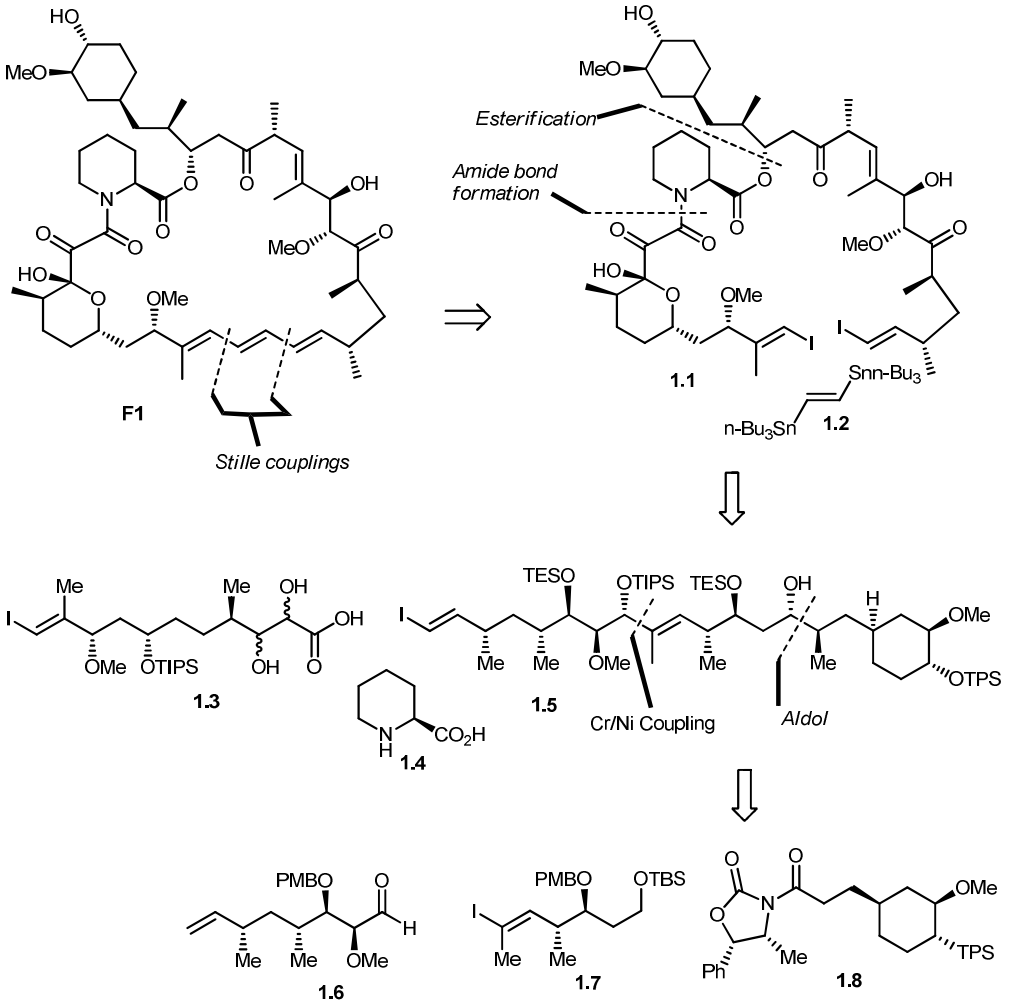
In 1996, Choi *et al.* solved the X-ray crystallographic structure of the ternary complex of FKBP12-rapamycin-FRB at 2.7 Å resolution. The structure of the complex is shown in **Figure 3**. Later, in 1999, Liang *et al.* crystallized the ternary complexes of rapamycin derivatives and their structures were solved at 1.85 and 2.2 Å resolution.[15] FKBP12 protein consists of α, β sheet made up of five anti-parallel β strands, and, also a short α helix is present. Rapamycin binds to the hydrophobic pocket formed between the α helix and β sheet. FRB domain of FRAP is composed of a bundle of four α helices with rapamycin binding to a hydrophobic pocket formed by helices α1 and α4. It is evident that rapamycin interacts with both (FKBP12 and FRB) receptor proteins. Moreover, experimental evidences are shown that the FKBP12 protein is unable to bind with FRB in the absence of rapamycin.[16] To get the deeper insight in the interaction network

responsible for the binding of rapamycin with FKBP12 and FRB, Maurizio Sironi group recently performed the molecular dynamics (MD) simulations and free energy calculations on this ternary system.[17] In particular, they have calculated binding free energies between the components of the FKBP12-rapamycin-FRB ternary system and performed computational alanine scanning (CAS) to evaluate the contribution of each of the amino acids at the protein–protein and protein–rapamycin interface to the binding energy.

Rapamycin powerfully suppresses interleukin-2 (IL-2)-stimulated T cell proliferation leads to an immunosuppression, which is desirable post-transplantation to prevent the allograft rejection. Rapamycin is in general tolerated well and it does not show the renal toxicity associated with an alternative immunosuppressant's such as cyclosporine or FK506.[18] In 1999, rapamycin was approved for the prevention of graft rejection in kidney transplant recipients. Another clinical application of rapamycin is that it inhibits the growth of vascular smooth muscle, approved in 2002, as an anti-restenosis agent following balloon angioplasty in coronary arterial stents.[19] Interest in recent years has been in the potential of rapamycin as an anticancer drug. This was surely one of its soonest recorded properties, but it has received renewed interest from the illumination of mTOR function and the preponderance of tumour suppressors or oncoproteins (PI3K, PTEN, phosphoinositide-dependent protein kinase 1 (PDK1), AKT, TSC1, TSC2 and LKB1) in the mTOR signaling network. Multiple studies have shown that rapamycin can also provide therapeutic benefit in experimental models of several age-linked neurodegenerative diseases,[20] including Alzheimer's disease,[21] Parkinson's disease[22] and Huntington's disease.[23] Other studies have demonstrated that rapamycin is able to extend the lifespan in various species, including mice.[9,24] Because of the multiplicity of mTOR downstream signaling pathways, different molecular mechanisms have been proposed to underlie rapamycin's effects in these studies.

By these extensive properties of rapamycin, it has brought much attention of the scientific community to synthesize rapamycin and its analogues and rapamycin-derived hybrid molecules by synthetically as well as semi- and biosynthetically with improved pharmacokinetic properties and an advantageous intellectual property position compared to the parent molecule. In this chapter, I am

describing few case studies on rapamycin analogs and derived hybrid molecules and their biological evaluation.

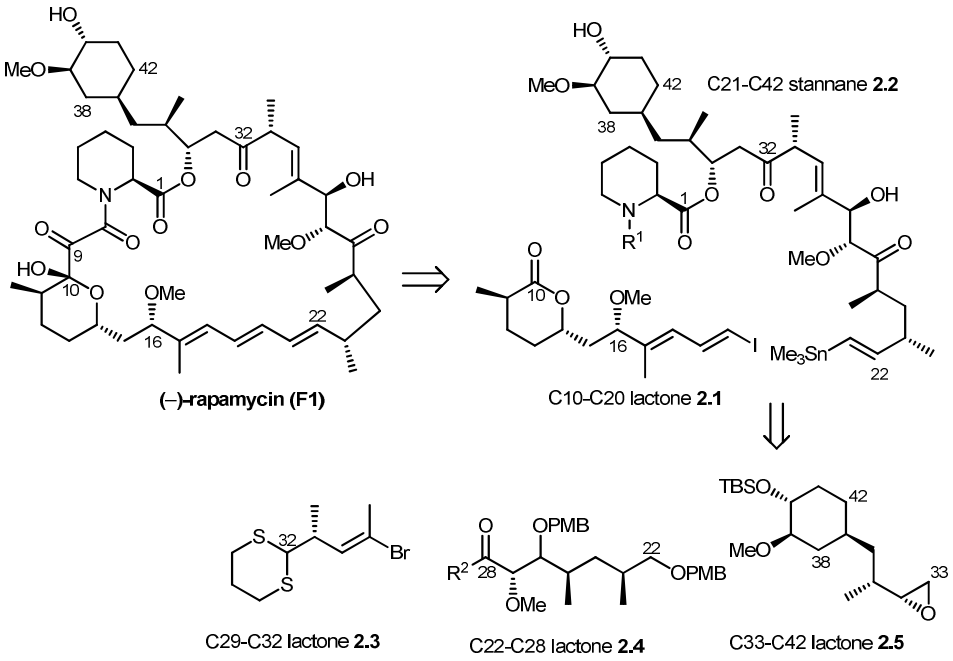


Scheme 1: Nicolaou's Approach to Synthesis of Rapamycin

3.4. Rapamycin Total Synthesis:

So far, five total syntheses of rapamycin have been published, and, these are from the following research groups: Nicolaou,[25] Schreiber,[26] Danyshefsky,[27] Smith,[28] and, more recently, Ley.[29] Nicolaou's team was the first to successfully synthesize rapamycin, and the retrosynthesis is shown in **Scheme 1**.[30] Disconnection of the triene system in rapamycin (**F1**) [Stille palladium-catalyzed coupling] suggested bis(vinyl iodide) **1.1** and distannylethene **1.2** as two potential precursors. Further, disconnection of the indicated amide and ester bonds in **1.1** and an opening of the lactol ring reveals, upon appropriate functional group

adjustments, compounds **1.3-1.5** as the advanced key intermediates. The most complex of the latter three fragments, compound **1.5** was then dissected (Evans aldol reaction for the C34-C35 bond and a chromium-nickel coupling for the C28-C29 bond) to afford, after functional group manipulations, compounds **1.6-1.8** as the potential building blocks. Thus, a strategy was devised entailing construction and coupling of intermediates **1.6-1.8**, and, the final elaboration to rapamycin.

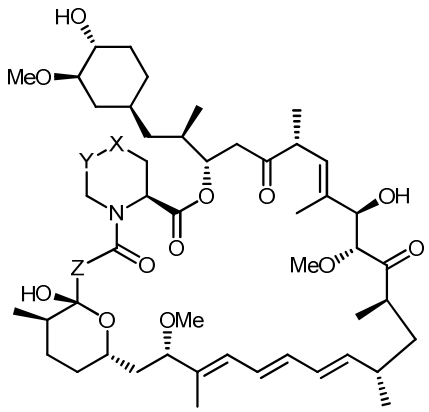


Scheme 2: Steve Ley's Approach to Synthesis of Rapamycin

Steven Ley's team synthesis is the most recent total synthesis of rapamycin and is less linear, and, the retrosynthesis is shown in **Scheme 2**.[31] The formation of the rapamycin macrocycle successfully by employing a transannular catechol-templated Dieckmann like reaction. Further, disconnection at the central olefin (C20-C21) of the triene through a Pd^0-catalyzed Stille coupling affords the simplified C10–C20 lactone **2.1** and C21-C42 vinyl stannane **2.2**. For the latter stannane (**2.2**), the researchers envisioned sequential carbanionic coupling of **2.3**, **2.4**, and **2.6**, whose synthesis were designed to highlight the chemistry that was developed by their group.

3.5. Rapamycin Analogues Synthesis and Biological Evaluation:

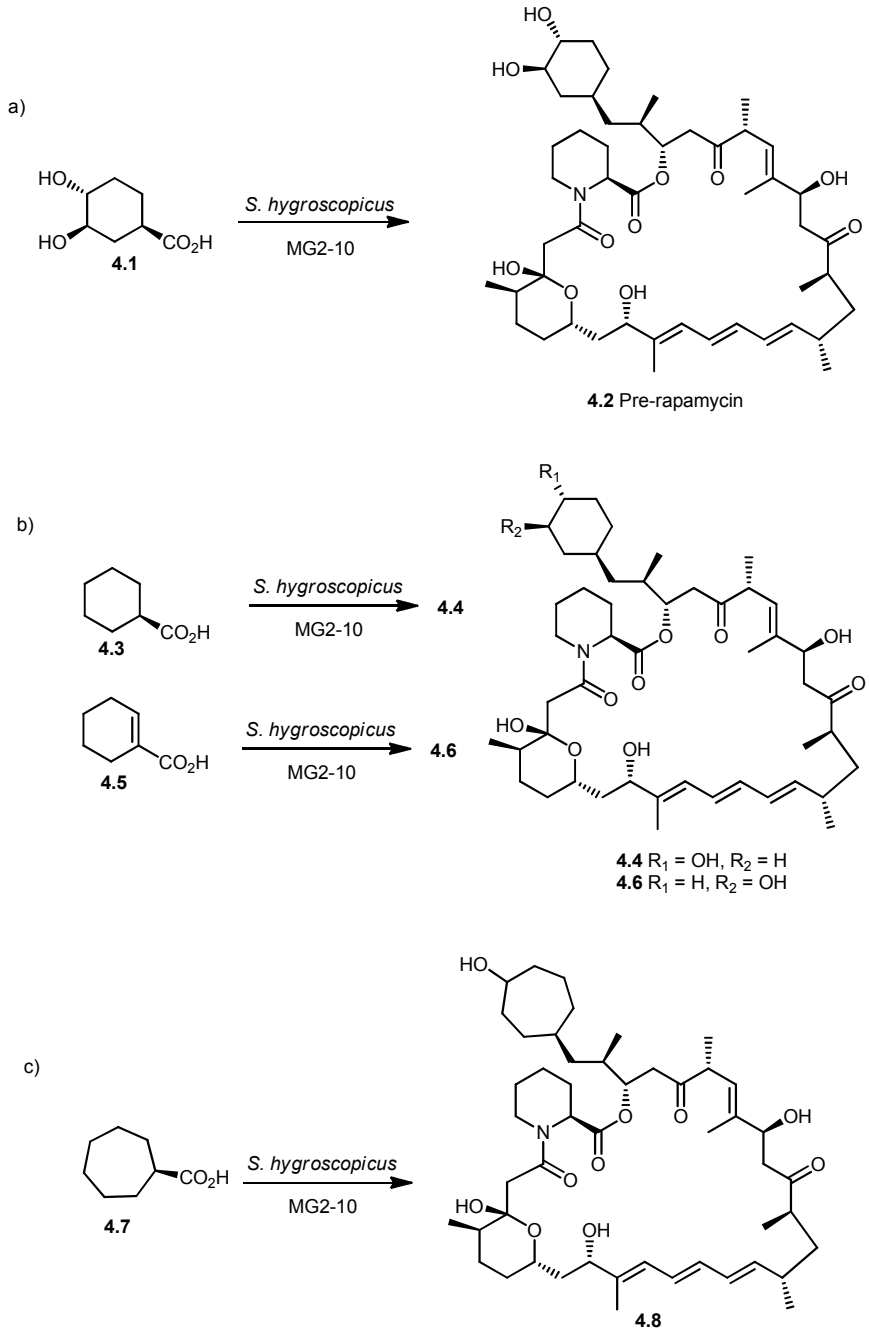
During the biosynthesis of rapamycin, the aminoacid L-pipecolate was incorporated into the rapamycin molecule. In 2004, from Oregon state university, Frank V. Ritacco and co-workers investigated the use of precursor-directed biosynthesis to create new rapamycin analogs by substitution of unusual L-pipecolate analogs in place of the normal amino acid.[32] Their results suggested that the L-pipecolate analog (±)-nipecotic acid inhibits the biosynthesis of L-pipecolate, thereby limiting the availability of this molecule for rapamycin biosynthesis. They used (±)-nipecotic acid for precursor-directed biosynthesis studies to reduce L-pipecolate availability, and, thereby enhance the incorporation of their pipecolate analogs into the rapamycin molecule. By using this method, they produced two new sulfur-containing rapamycin analogs and the yield of 20-thiarapamycin **(3.1)** was approximately 100mg/litre and of 15-deoxo-19-sulfoxylrapamycin **(3.2)** was approximately 10mg/litre (**Scheme 3**). These two analogs were tested in FKBP12 binding assay, expressed as percent inhibition of ^{3}H-labelled FK506-FKBP12 (control) complex formation. In each experiment, rapamycin was also tested for comparison of binding affinity. In this assay, 20-thiarapamycin **(3.1)** had a 50% inhibitory concentration (IC_{50}) of 53.6 nM, while rapamycin had an IC_{50} of 1.6 nM and 15-deoxo-19-sulfoxylrapamycin **(3.2)** had an IC_{50} of 800 nM, while rapamycin had an IC_{50} of 4.9 nM.



(**F1**) rapamycin	X : CH_2	Y : CH_2	Z : C=O
(**3.1**) 20-thiarapamycin	X : S	Y : CH_2	Z : C=O
(**3.2**) 15-deoxo-19-sulfoxylrapamycin	X : CH_2	Y : S=O	Z : CH_2

Scheme 3: Structures of Rapamycin, 20-Thiarapamycin and 15-Deoxo-19-sulfoxylrapamycin

In 2005, from Biotica Technology Ltd, Rose M. Sheridan and co-workers reported the mutasynthesis of rapamycin analogs through the manipulation of a gene governing starter unit biosynthesis.[33] Rapamycin, FK506, and F520 are biogenetically related natural products which are synthesized by mixed polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) systems.

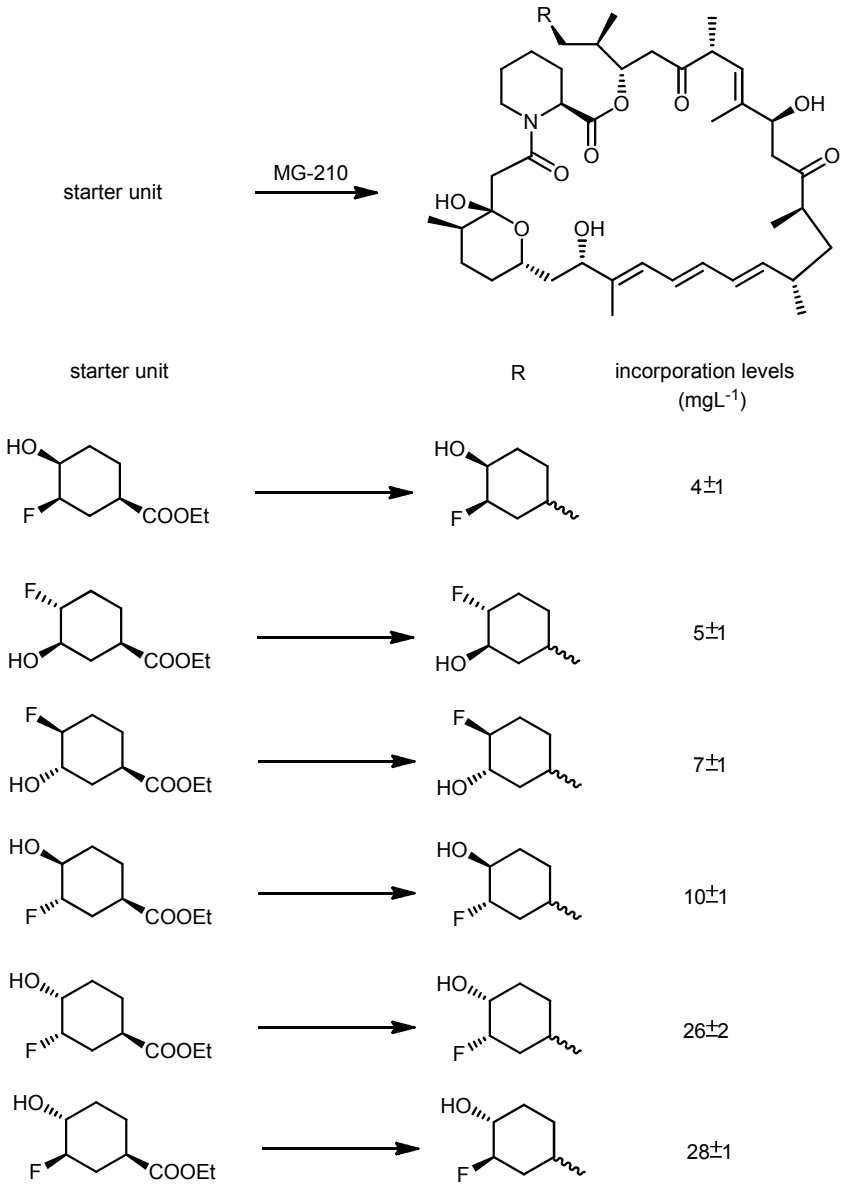


Scheme 4: (a) Reestablishment of Pre-rapamycin **4.2** Production in *S. hygroscopicus* MG2-10 by Feeding the Pseudostarter Unit **4.1 (b,c)** Exclusive Production of the Analogues **4.4-4.8** of Pre-rapamycin by Feeding, Respectively,

Cyclohexanecarboxylic Acid, Cyclohex-1-enecarboxylic Acid, and Cycloheptanecarboxylic Acid to *S. hygroscopicus* MG2–10.

The dihydroxycyclohexane moiety of rapamycin arises through the incorporation of a 4,5-dihydroxycyclohex-1-enecarboxylic acid (DHCHC) starter unit derived from shikimic acid. The immediate product of the rapamycin PKS is pre-rapamycin **4.2**, which is then modified by a series of two cytochrome P450 monooxygenases (RapJ and Rap N) and three *O*-methyltransferases (RapI, RapM and RapQ). When a region of DNA including the genes thought to encode these enzymes was excised (rapKIJMNOQL) from the rapamycin biosynthetic gene cluster, no production of rapamycins was observed from the resulting strain, Streptomyces hygroscopicus MG2-10. The mutant strain MG2-10 was then independently complemented with full-length copies of each of the genes which had been removed. Surprisingly, the production of pre-rapamycin **4.2** was only observed when rapK was reintroduced and expressed in the strain. They first carried out a series of experiments in an attempt to explain the lack of production of the rapamycin macrocycle by *S. hygroscopicus* MG2-10. These included feeding experiments in which exogenous pseudostarter carboxylic acid **4.1** was added to the fermentation medium to verify that the supply of this component was not a limiting factor. The addition of pseudostarter carboxylic acid **4.1** to the fermentation led to the efficient production of pre-rapamycin **4.2**. Furthermore, the addition of other carboxylic acids in place of pseudostarter carboxylic acid **4.1**, as reported for the wild-type organism, was found to lead to the specific production of pre-rapamycin analogues in which these non-natural starter acids had been incorporated, in most cases after prior hydroxylation. CHC **4.3**, cyclohex-1-enecarboxylic acid **4.5**, and cycloheptanecarboxylic acid **4.7** were all fed separately to *S. hygroscopicus* MG2–10, which led to the production of analogues **4.4, 4.6,** and **4.8** respectively (**Scheme 4**). Compounds **4.6** (22 mg) and **4.8** (77 mg) were isolated from the fermentation broth by preparative chromatography and their structures were confirmed by using high-resolution FT-ICR-MSn. Compound **4.4** (100 mg) was isolated and its structure confirmed by a combination of high-resolution FT-ICR-MSn and multidimensional NMR spectroscopy experiments. These analogues offer great potential as chemical

genetic probes of the molecular pathways involved in rapamycin action, and, as new agents with enhanced therapeutic properties.

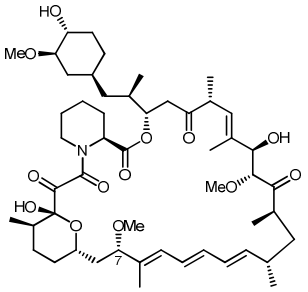


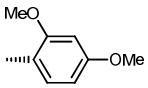
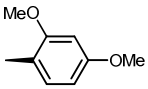
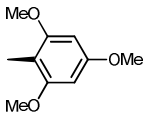
Scheme 5: Synthesized Fluorinated Starter Units, Incorporation Levels and Corresponding Pre-rapamycin Analogues

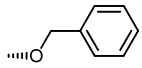
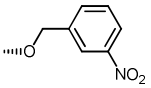
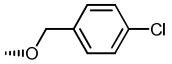
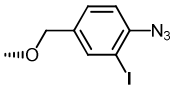
From the same company (Biotica Technology Ltd), Lanceron *et al* developed an expeditious route to fluorinated rapamycin analogues by utilizing mutasynthesis. They synthesized 6 flouorohydrins (fluorinated starter units) which were fed and then incorporated S. hygroscopicus MG-210 at various incorporation levels[34] (**Scheme 5**). The biological activity of the new fluorinated rapamycin analogs was then evaluated and the researchers came to know the importance of the

hydrogen bond of the hydroxyl group on carbon 40 with FKBP12, measuring the binding to FKBP12 and FRAP in the ternary complex and its impact on the biological activity of rapamycin.

Table 1: Biological Activities of Rapamycin C-7 Analogues



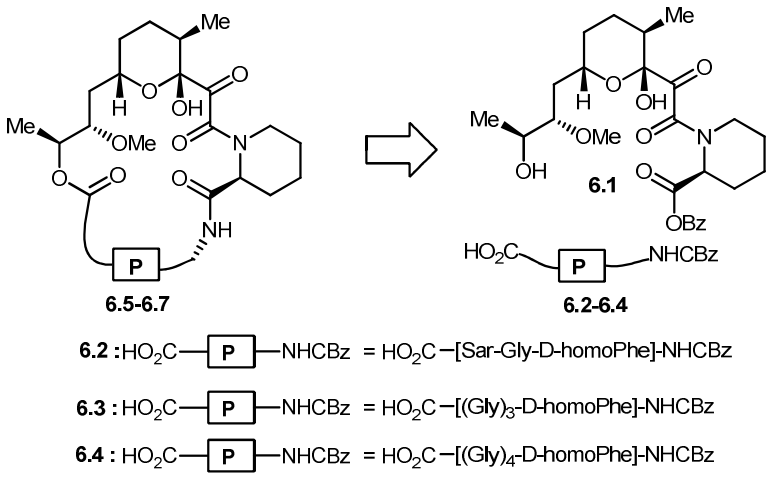
R	Compound	FKBP K_i (nM)	T Cell IC_{50} (nM)	Yeast IC_{12} (nM)
''''OMe	1	0.6	1	7
◄OMe	4	1	30	20
''''OEt	5a	3.5	10	10
◄OEt	5b	4	200	15
''''Sme	6a	1	4	4
◄Sme	6b	1	4	4
''''$NHCO_2Me$	7a	4.5	>10000	170
◄$NHCO_2Me$	7b	3.7	2500	220
	8a	9	1000	225
	8b	38	>1000	400
	9	7	>1000	>10000
	10a	3	20	12.5
	10b	6	20	3
	11a	10	11	28

	11b	1.5	70	28
—H	12	1	2	8
=O	13	29	300	210
	14a	5	6	6
	15a	7	50	20
	16a	1	45	6
	17a	30	90	60

Dennis A. Holt and his co-workers synthesized over 100 rapamycin analogues by using selective acid-catalyzed nucleophilic substitution reaction, at the C7 methoxy group of rapamycin.[35] This unique transformation allows the selective manipulation of the rapamycin effector domain which further effects on FKBP12 binding, and, on the biological activity. Rapamycin C-7-modified analogues of both *R* and *S* configurations were shown to have high affinities for FKBP12, yet, these analogues displayed a wide range of potencies in splenocyte and yeast proliferation assays as shown in Table 1. In the ternary complex, the C-7 methoxy group of rapamycin is situated in a close proximity to FRAP, at the interface between FRAP and FKBP12 (**Table 1**).

K. C. Nicolaou and his co-workers designed and synthesized rapamycin-based high affinity binding FKBP12 ligands, rapamycin-peptides (Rap-P), by using the peptide tether chemistry.[36] The designed small molecules contained the three peptide cassettes, D-homoPhe-Gly-Sar **6.2** cassette, and, this approach allows the formation of a 21-membred ring **6.5**, whereas the D-homoPhe-$(Gly)_3$ **6.3** and D-homoPhe-$(Gly)_4$ **6.4** cassettes lead to 24- and 27-membered rings **6.6** and **6.7**, respectively. Retrosynthesis of rapamycin-peptides is shown in **Scheme 6**. These hybrid molecules were then subjected to biological evaluation, and, exhibits powerful binding properties, but unlike rapamycin, show no activity in IL-6 dependant B-cells proliferation, and, in contrast to FK506, show no activity in the IL-2 reporter assay. The modular nature of these designed molecules should make it possible to generate a series of compounds with effector domains for targeting

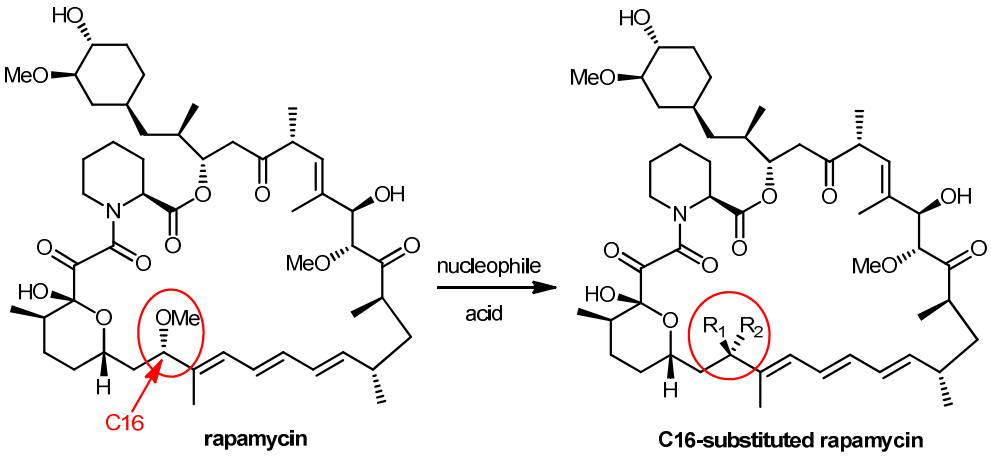
either calcineurin or FRAP (TOR/RAFT1) or both, as potential biological tools and immunosuppressive agents.



Scheme 6: Molecular Design and Retrosynthetic Analysis of Designed FKBP12 Ligands RAP-P6.2-6.4 (**6.5-6.7**)

Stuart L. Schreiber and co-workers developed a method for an inducible gene expression and translocation by using nontoxic derivatives of rapamycin.[37] But, rapamycin is an inhibitor of cell proliferation, the result of binding to FKBP12-rapamycin-associated protein (FRAP). To overcome this limitation, non-toxic derivatives of rapamycin bearing bulky substituent's at its C16-position were synthesized by using a known method of Luengo *et al* (**Scheme 7**). The isosteric isopropoxy and methallyl substituents with the non-natural C16-configuration abolish both binding to FRAP and inhibition of T cell proliferation. Binding proteins for these derivatives were identified from the libraries of cDNAs encoding mutants of the FRB domain of FRAP by using a mammalian three-hybrid transcription assay. The targeting of mutations was guided by the structure of the FKBP12-rapamycin-FRB ternary complex. Three compensatory mutations in the FRB domain, all along with one face of α-helix in a rapamycin-binding pocket, were identified that together restore binding of the rapamycin derivatives. Using this mutant FRB domain, these library of nontoxic rapamycin derivatives were screened, and, identified one nontoxic rapamycin derivative (3R) that induced the targeted gene expression in Jurkat T cells with an EC_{50} below 10 nM. Another derivative (9R) was used to recruit a cytosolic protein to the plasma membrane, mimicking a process involved in many signaling pathways.

The long half-life of rapamycin (62 to 82 hours in humans) has further complicated its use and the problem of its adverse side effects. In this context, Abott Laboratories initiated a program designed to identify rapamycin analogues with shorter *in vivo* half-lives and improved side effect profiles. Their efforts were mainly focused on the introduction of novel stable functionalities at C40 that would allow them to identify analogues with attractive physicochemical attributes for treating disorders due to excessive cell proliferation. This study led to the design of zotarolimus **F4.1**, which has a tetrazole substitution at C40 (**Figure 4**). The Abott team reported the *in vitro* antiproliferative activities and



rapamycin

C16-substituted rapamycin

Compound		R1	R2
F1	rapamycin	H	OMe
7.1R	C16-(R)-OMe	OMe	H
7.2R	C16-(R)-OEt	OEt	H
7.3R	C16-(R)-OiPr	OiPr	H
7.3S	C16-(S)-OiPr	H	OiPr
7.4R	C16-(R)-(R-O-2-butyl)	(R-O-2-butyl)	H
7.5R	C16-(R)-(S-O-2-butyl)	(S-O-2-butyl)	H
7.6R	C16-(R)-OBenzyl	OBenzyl	H
7.7R	C16-(R)-cyclohexylmethyl	CyMe	H
7.9R	C16-(R)-methallyl	methallyl	H
7.9S	C16-(S)-methallyl	H	methallyl

Scheme 7: Structure of Rapamycin and C16-Substituted Derivatives

in vivo immunosuppressive activities of zotarolimus, which has the potential for an improved safety profile by virtue of its shorter *in vivo* half-life. Zotarolimus was found to be mechanistically similar to rapamycin in having high affinity

binding to the immunophilin FKBP12 and comparable potency for inhibiting *in vitro* proliferation of both human and rat T cells.[38] Rat pharmacokinetic studies with intravenous dosing demonstrated terminal elimination half-lives of 9.4 hours and 14.0 hours for zotarolimus and rapamycin, respectively. Given orally, T1/2 values were 7.9 hours and 33.4 hours, respectively. Consistent with its shorter duration, zotarolimus showed a corresponding and statistically significant 4-fold reduction in potency for systemic immunosuppression in 3 rat disease models. Pharmacokinetic studies in cynomolgus monkey underpredicted the half-life difference between zotarolimus and rapamycin apparent from recent clinical data. The *in vitro* inhibition of human coronary artery smooth muscle cell proliferation by zotarolimus was comparable to rapamycin. Drug-eluting stents for the local delivery of zotarolimus to the vessel wall of coronary arteries are in clinical development.[39,40] The pharmacological profile of zotarolimus suggests that it may be advantageous for preventing restenosis with a reduced potential for causing systemic immunosuppression or other side effects.

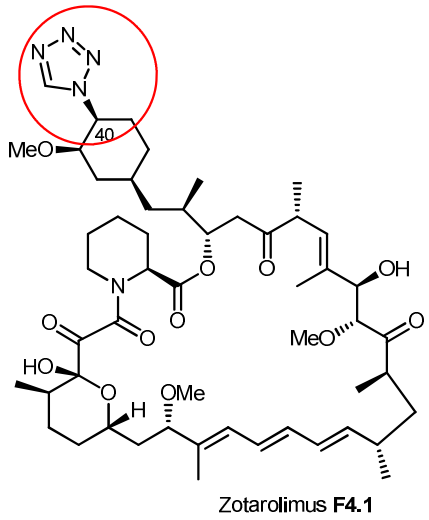
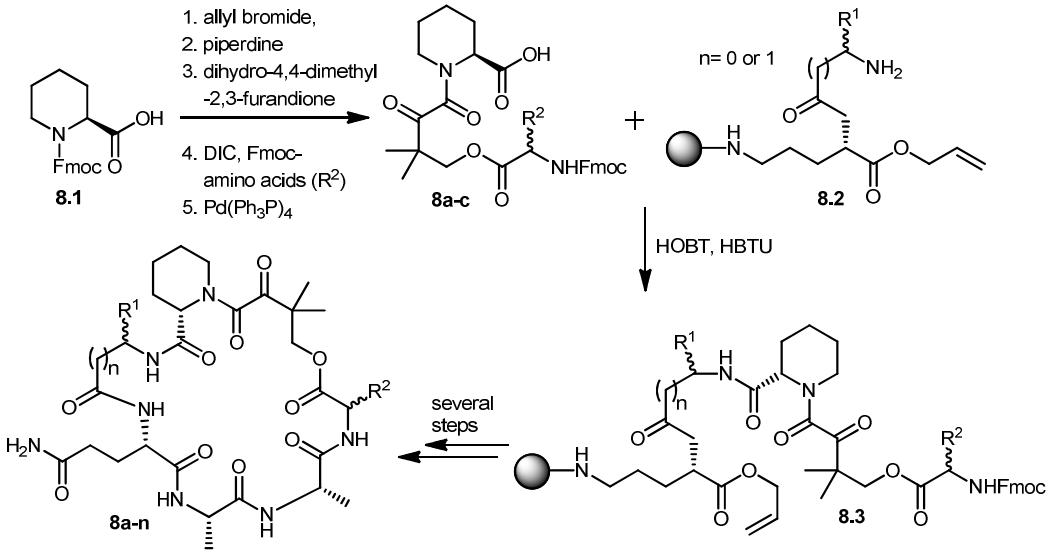


Figure 4: Chemical Structure of Zotarolimus

Wu *et al* developed a parallel synthesis method to generate a 200-member of bifunctional cyclic peptides as FK506 and rapamycin analogues, which were referred to as "rapalogs". Each rapalog consists of a common FKBP-binding moiety and a variable effector domain. They were chosen peptides as the effector domains, because peptides of diverse structures could be generated from a small set of amino-acid building blocks and are synthetically accessible, although other

types of structures were also used.[41] Synthesis of cyclic peptides **8a-n** began with the preparation of key building blocks **8a-c**, which was started from the



Scheme 8: Solid-Phase Synthesis of Rapalogs **8a-n**

commercially available *N*-Fmoc-L-pipecolinic acid **8.1**, its carboxyl group was protected as an allyl ester by treatment with allyl bromide under basic conditions. Removal of the Fmoc group with piperidine gave amine, which was acylated with dihydro-4,4-dimethyl-2,3-furandione to obtain alcohol. Coupling of alcohol with three different *N*-Fmoc amino acids followed by allyl removal with $Pd(PPh_3)_4$ afforded the building block **8a-c** in good yields. Next, the 15 cyclic peptides **(8a-n)** were synthesized in parallel on Rink amide resin. For synthesize building block **8.2**, *N*-Fmoc-Glu-α-allyl ester was coupled to the amino group of Rink resin using *O*-benzotriazole- N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) as the coupling agent. After removal of the Fmoc group, the *N*-terminal amine was acylated with 10 different *N*-Fmoc amino acids (R^1). Subsequent addition of building blocks **8a-c** and L-Ala-L-Ala were carried out using standard peptide chemistry that yielded a compound **8.3**. Prior to peptide cyclization, the *C*-terminal allyl group was removed by treatment with a catalytic amount of $Pd(PPh_3)_4$ in the presence of *N*-methylaniline, and the *N*-terminal Fmoc group was removed by piperidine. Peptide cyclization was achieved by using benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBop) as the coupling reagent. Finally, treatment with 50% trifluoroacetic acid (TFA) in dichloromethane released the peptides **(8a-n)** from the resin and deprotected the

amino acid side chains. The resulting crude peptides were then quickly passed through a silica gel column to remove the salts and used directly in various activity assays (**Scheme 8**).

These rapalogs (**8a-n**) were tested for binding to FKBP12 by a fluorescence polarization competition assay, and, the results showed that FKBP12 binds to most of the rapalogs with high affinity (K_I values in the nanomolar to low micromolar range), creating a large repertoire of composite surfaces for potential recognition of macromolecular targets such as proteins.[42]

3.6. Concluding Remarks:

Though rapamycin and its analogues help us understand mTOR pathway, thus serving as the potential immunosuppressive, anti-cancer and neuroprotective agents for age-related neurodegenerative diseases, there remains a need to synthesize rapamycin analogues further and derived hybrid molecules with better pharmacokinetic properties which enable us to know more about the downstream regulation of mTOR pathway. In the long run, these novel compounds may also serve as a potential clinical therapeutics, including an enhancement of human life span.

3.7. References:

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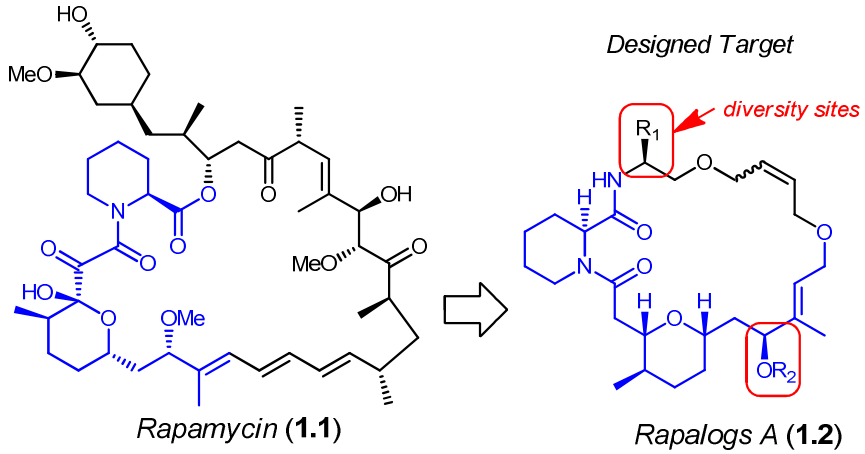
Chapter 4:
Synthesis of Rapamycin Fragment-Derived Hybrid Molecules

4.1. Design of Rapamycin Fragment-Derived Hybrid Molecules:

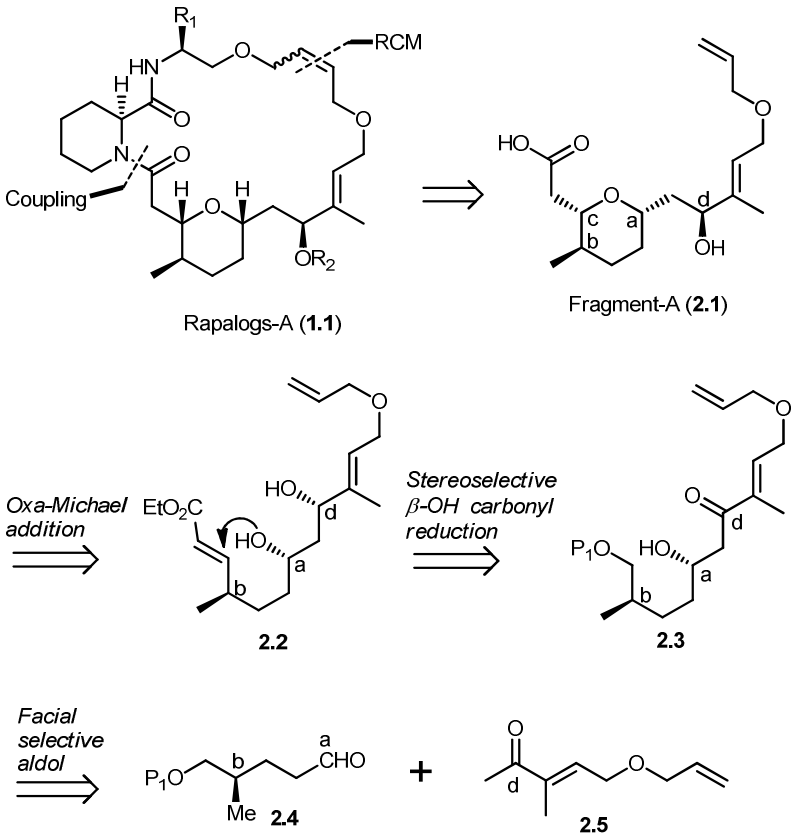
Due to remarkable biological properties of rapamycin (**1.1**) and rapamycin-derived hybrid molecules (as I explained in **Chapter 3**), these are brought to our attention to develop, further, the synthesis of a new class of rapamycin-derived hybrid small molecules. Our designed target, hybrid natural product (**1.2**) is shown in **Scheme 1**. The key features of our target are: (i) it contains R_1 and R_2 as two diversity sites, (ii) dense in stereochemical complexity, and, the stereochemistry of various chiral centres can be altered, and, (iii) the chemical toolbox from this approach can be readily made accessible.



Scheme 1: Design of Rapamycin-Derived Hybrid Molecules (Rapalogs A)

4.2. Working Hypothesis and Retrosynthesis:

Our aim is to develop a novel, convergent and stereoselective method to construct fragment-A (**2.1**). By utilizing this key fragment, we then plan to develop a synthetic route to access rapamycin-derived hybrid compounds. Our retrosynthetic plan to access rapalogs-A (**1.2**) is shown in **Scheme 2**. The coupling of a secondary amine to fragment-A (**2.1**), followed by a ring closing metathesis (RCM) using an *O*-allyl product can lead to rapalogs-A (**1.2**). Fragment-A (**2.1**) can be obtained from an oxa-Michael reaction of α,β-unsaturated ethyl ester (**2.2**). Compound 2.2 is planned to obtain from an *enantioselective* aldol reaction of **2.4** and **2.5**, and then, followed by a stereoselctive *β*-hydroxy carbonyl reduction of **2.3**.



Scheme 2: Retrosynthesis of Rapalogs-A (**1.1**)

4.3. Results and Discussion:

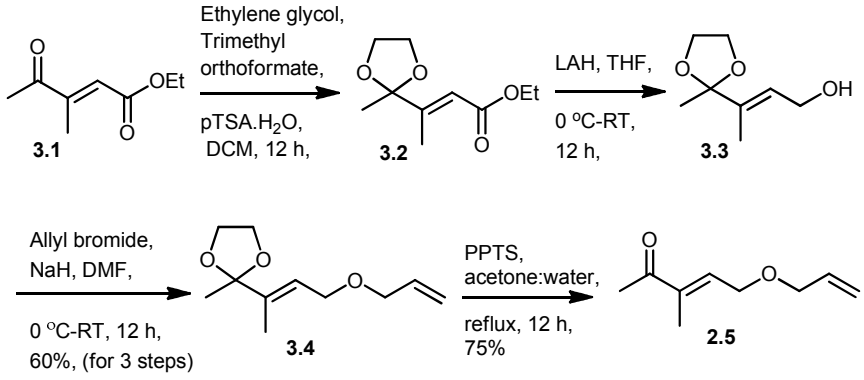
4.3.1. Develop a Novel Method to the Synthesis of Fragment-A (2.1):

We developed a novel method to the synthesis of fragment-A (**2.1**), and, the key reactions involved in our approach include: (i) an *enantioselective* aldol,[1] (ii) stereoselctive β-hydroxy carbonyl reduction,[2] and (iii) an intramolecular oxa-Michael reaction.[3]

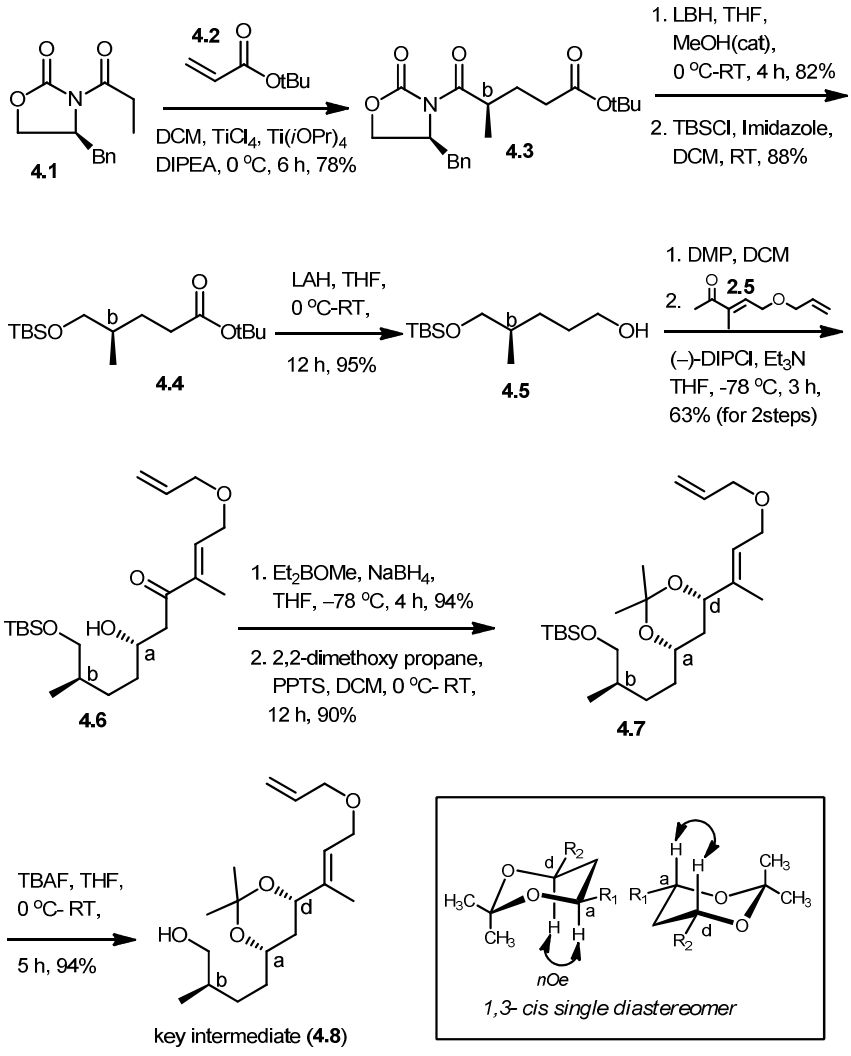
4.3.1a. Synthesis of Keto Fragment, 2.5:

For the synthesis of fragment **2.5**, we started with (E)-ethyl 3-methyl-4-oxopent-2-enoate **3.1**,[4] which was then subjected to protection of the keto functional group with an ethylene glycol. The reduction of an ester group with LAH led to access alcohol **3.3** which was then subjected to allylation yielding an -*O*-allyl product

3.4. This was then treated with PPTS to obtain the keto fragment **2.5** in good yields (**Scheme 3**).



Scheme 3: Synthesis of Keto Fragment **2.5**



Scheme 4: Synthesis of Key Intermediate **4.8**

4.3.1b. Synthesis of Key Intermediate 4.8:

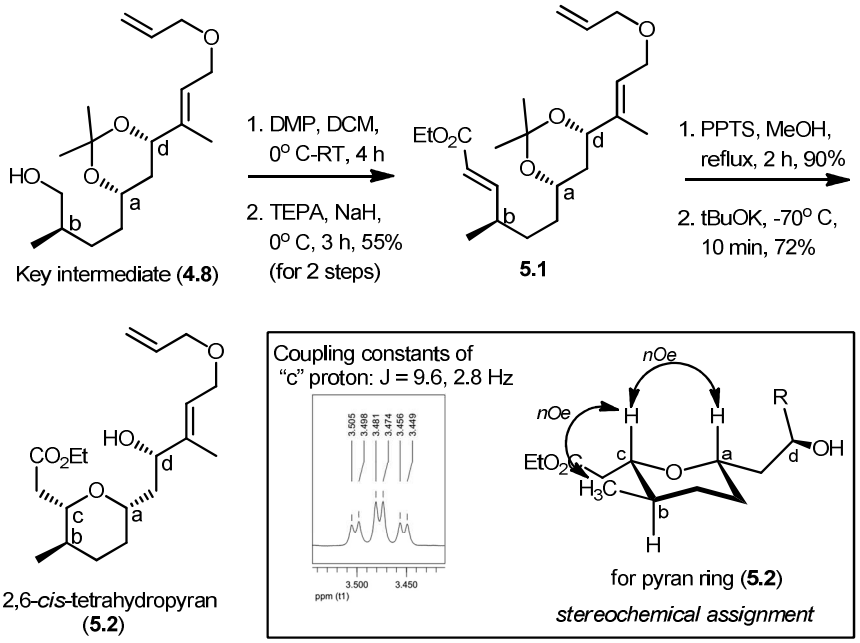
For the synthesis of fragment-A (**2.1**), we started with Evans' Michael addition-based approach[5] on *tert*-butyl acrylate **4.2** with oxazolidinone, **4.1**. The removal of an auxiliary led to the synthesis of a primary alcohol which was then protected with TBS to provide alcohol **4.4**. The *tert*-butyl ester was then subjected to reduction with LAH to obtain a primary alcohol **4.5**. This alcohol was then oxidized with DMP[6] to yield an aldehyde. With this freshly prepared aldehyde, we then subjected this to an *enantioselective* aldol reaction with the keto fragment **2.5** by using (−)-DIPCl (note: this approach is well-established in Ian Paterson's group at Cambridge University, UK, over the years). This reaction worked very-well and provided the *β*-hydroxyl carbonyl compound **4.6** in a good yield as the major diastereomer with the HPLC purity >95%. This β-hydroxyl carbonyl compound **4.6** was then treated with $NaBH_4$ in the presence of Et_2BOMe for *syn*-stereoselective β-hydroxyl carbonyl reduction yielding *syn*-1,3-diol which was then protected with 2,2-dimethoxy propane.[2] It was then treated with TBAF to yield the primary alcohol key intermediate (**4.8**). *Cis*-1,3-diol of **4.8** with the relative stereochemistry was confirmed with 2D-NOESY experiments (see **Scheme 4**).

4.3.1c. Synthesis of 2,6-*cis*-Tetrahydropyran (5.2) and the Stereochemical Assignment:

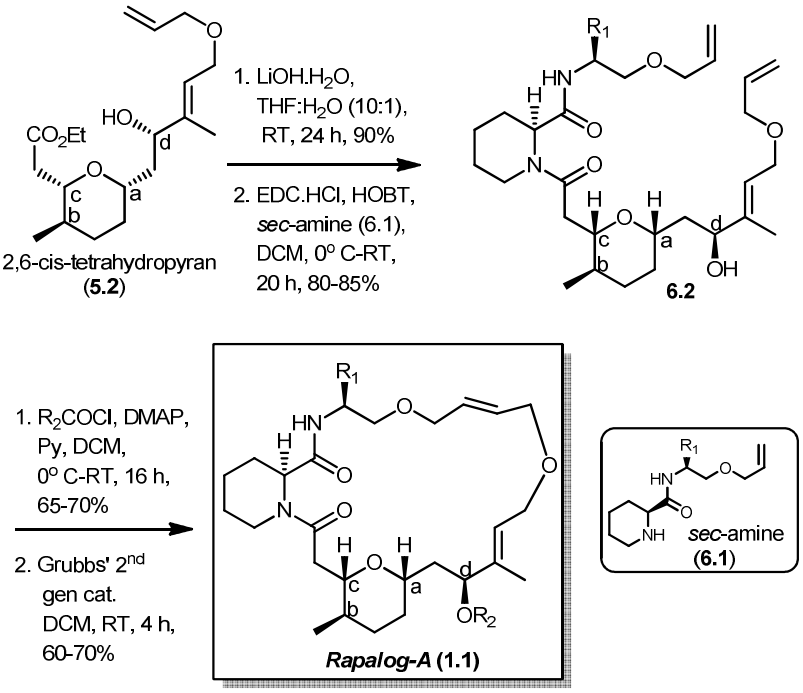
Key intermediate (**4.8**) alcohol functional group was then oxidized to aldehyde with DMP, and, then subjected to Horner-Wittig reaction[7] to obtain α,β-unsaturated ethyl ester **5.1** in a good yield. The 1,3-diol was deprotected with PPTS, and, further treated with potassium *tert*-butoxide which affected a smooth oxa-Michael cyclization (under thermodynamic conditions) to provide exclusively the 2,6-*cis*-tetrahydropyran moiety **5.2** (72%). An stereochemistry of 2,6-*cis*-tetrahydropyran (**5.2**) was assigned through a coupling constant of 'c' proton and 2D-NOESY experiments (see **Scheme 5**).

4.3.1d. Synthesis of Rapalogs-A (1.1):

Having 2,6-*cis*-tetrahydropyran moiety (**5.2**) in our hand, we then hydrolyzed the carboxyl ester to carboxylic acid with LiOH, and, then coupled with secondary



Scheme 5: Synthesis of 2,6-*cis*-Tetrahydropyran (**5.2**) and Stereochemical Assignment



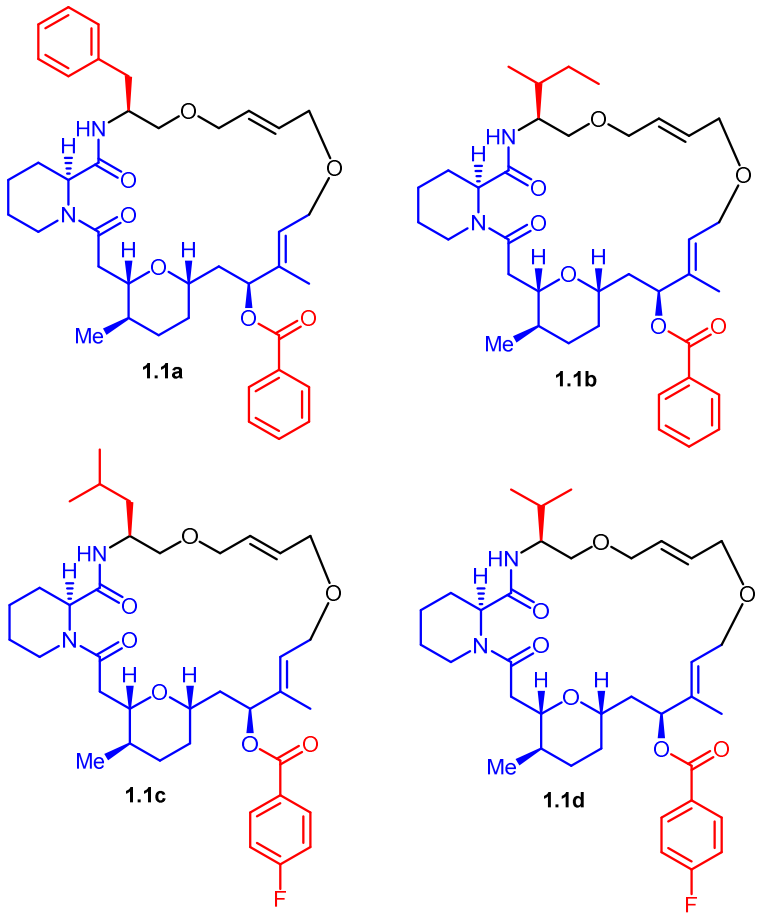
Scheme 6: Synthesis of Rapamycin-Derived Hybrids Rapalogs-A (**1.1**)

amine **6.1** by using EDC.HCl and HOBT as the coupling reagents. This was then treated with acid chloride (R_2COCl), and, finally, it was subjected to ring closing metathesis (RCM)[8] by using 10 mol% of Grubbs' second generation catalyst. To our delight, the RCM approach gave the product with *E*-olefin geometry

containing 22-membered macrocyclic rapamycin-derived hybrid molecules, called rapalog-A (**1.1**) as a major compound in a good yield (see **Scheme 6**).

4.3.2. Derivatives of Rapalog-A, 1.1:

By using various secondary amines and acid chlorides, we then synthesized four rapamycin-derived hybrid molecules as rapalog-A **1.1a-d**, and, all these derivatives are shown in **Scheme 7**.



Scheme 7: Derivatives of Rapalog-A (**1.1a-d**)

4.4. Molecular Modeling Studies:

4.4.1. Docking Method:

The docking analysis of rapamycin-based hybrid natural products that were synthesized by us was performed using a Maestro, version 9.8 implemented from Schrödinger molecular modeling suite-2014 in a collaborative study with Girdhar Singh Deora, The University of Queensland, School of Pharmacy, Australia. All

small molecules were sketched in 3D format using a build panel of maestro, and, LigPrep application was used to produce the low-energy conformers. In one collaborative study, the structural coordinates of human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complex with human immunophilin were taken from the protein data bank (PDB), id 1FKB and 1NSG respectively. PDB protein structures were prepared by giving a preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime, and, finally were minimized using OPLS-2005 force field. The grids for molecular docking simulations were generated with bound co-crystallized ligands. Our rapamycin-derived hybrid natural products were docked using Glide module, with up to three poses saved per molecule. The ligands were kept flexible, whereas, the receptors were kept rigid throughout the docking studies. The lowest energy conformations were selected and, the ligand interactions (H-bond and hydrophobic interactions) with target proteins were determined.

4.4.2. Results and Discussion:

Molecular docking studies were performed to predict the key binding interactions of synthesized molecules with rapamycin binding sites of human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complexed with human immunophilin. The docking studies predict good binding interactions of rapamycin analogues with both the target proteins.

All our hybrid natural products-based small molecules were well-occupied by the binding pockets of proteins (see **Figures 1A and 1B**). Molecule **1.1d** showed a good binding with both protein targets. It is interacting with Ile-56 (H-Bond), His-87 (π-π interaction) and A:Ile-56, A:Gln-53 (H-Bond) with human immunophilin FKBP-12 and FRAP-human immunophilin complex respectively (**Figure 1C and 1D**). In the case of human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complex, Ile-56 is a common hydrogen bonding residue except in the case of molecule **1.1b**. The binding pose of our molecules with human immunophilin FKBP-12 and human immunophilin FKBP12-FRAP complexes are shown in **Figures 2** and **3** respectively. **Table 1** represents a summary of the binding interaction of rapamycin analogues with both proteins targets used in our collaborative docking studies.

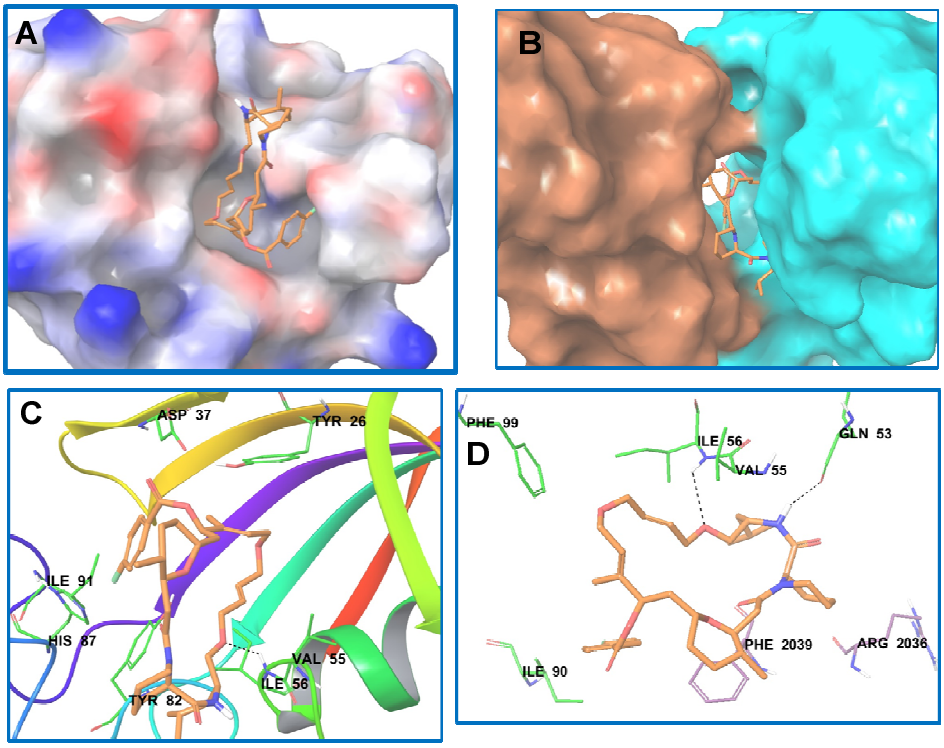


Figure 1: **(A)** Binding Orientation of **1.1d** at the Binding Pocket Human Immunophilin FKBP-12. **(B)** Binding Orientation of **1.1d** with Human Immunophilin FKBP-12 and FKBP12-Rapamycin Associated Protein Complex. **(C)** Binding Mode and Interactions of **1.1d** at the Rapamycin Binding Site of Binding Site of Human Immunophilin FKBP-12. **(D)** Binding Mode and Interactions of **1.1d** with Human Immunophilin FKBP-12 and FKBP12-Rapamycin Associated Protein Complex.

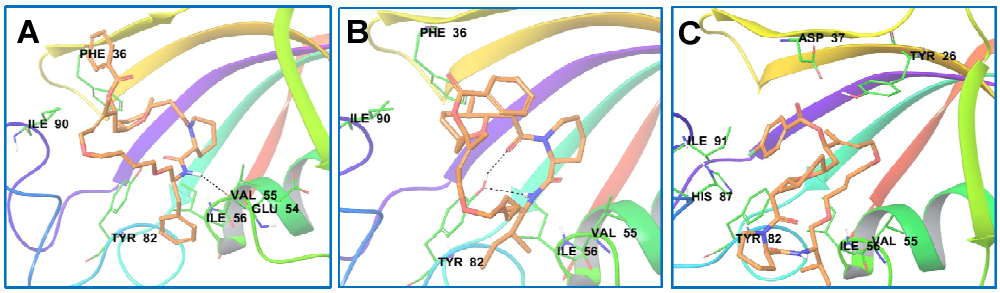


Figure 2: Binding Mode and Interactions of Rapalog-A at the Rapamycin Binding Site of Human Immunophilin FKBP-12. **(A)** with **1.1a (B)** with **1.1b (C)** with **1.1c**.

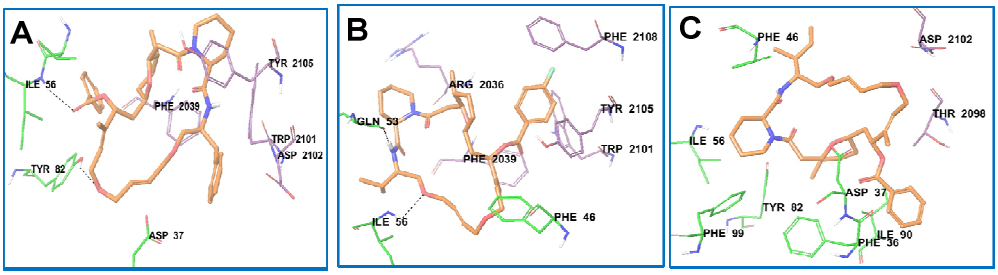


Figure 2: Binding Mode and Interactions of Rapalog-A with Human Immunophilin FKBP-12 and FKBP12-Rapamycin Associated Protein Complex. **(A)** with **1.1a (B)** with **1.1b (C)** with **1.1c**

Table 1: Docking Based Binding Interaction of Molecules with Target Proteins.

Compound Code	**Interacting Residues**	
	Human Immunophilin FKBP-12	**FRAP- Human Immunophilin FKBP-12 Complex**
1.1d	Ile-56 (H-Bond), His-87 (π- π)	A*:Ile-56, A:Gln-53 (H-Bond)
1.1c	His-87 (π- π)	A:Ile-56, A:Gln-53 (H-Bond), B#:Trp-2101
1.1a	Glu-54 (H-Bond)	A:Ile-56, A:Tyr-82 (H-Bond); A: Arg-42 and A:Tyr-82 (π- π)
1.1b	Tyr-82 (H-Bond)	-

Glide score and contributing XP parameters of rapalog-A docking with human immunophilin FKBP-12 and with FKBP12-rapamycin binding domain of FKBP12 and rapamycin associated protein complex is shown in **Table 2** and **Table 3** respectively.

Table 2: Glide Score and Contributing XP Parameters of Rapalogs-A Docking with Human Immunophilin FKBP-12.

Compound Code	GScore	Lipophilic EvdW	PhobEn	H-Bond	Electro
1.1d	-7.36	-4.77	-2.61	0.0	-0.09
1.1c	-6.83	-4.43	-2.32	0.0	-0.19
1.1a	-5.69	-3.87	-1.88	-0.66	-0.44
1.1b	-5.66	-3.26	-1.46	-0.65	-0.13

GScore: glide score
LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein-ligandvdw energy
H-Bond: Rewards for hydrogen bonding interaction between ligand and protein
PhobEn: Hydrophobic enclosure reward
Electro: Electrostatic reward

Table 3: Glide Score and Contributing XP Parameters of Rapalogs-A Docking with FKBP12-Rapamycin Binding Domain of FKBP12 and Rapamycin Associated Protein Complex.

Compound Code	GScore	Lipophilic EvdW	PhobEn	H-Bond	Electro
1.1d	-10.22	-6.40	-2.70	-0.66	-0.22
1.1c	-9.35	-6.36	-1.80	-0.67	-0.19
1.1a	-8.38	-5.38	-2.03	-0.67	-0.23
1.1b	-8.13	-6.22	-2.38	0.0	0.17

GScore: glide score
LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy
H-Bond: Rewards for hydrogen bonding interaction between ligand and protein
PhobEn: Hydrophobic enclosure reward
Electro: Electrostatic reward

4.5. Biological Evaluation:

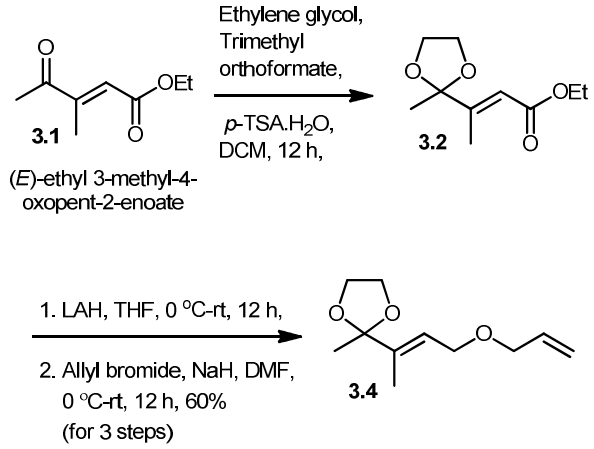
Finally, our chemical toolbox having hybrid natural products-based small molecules that are derived from the fragment of rapamycin (commonly known in our group as rapalog-A) evaluation is in progress with several collaborators in various biological assays, and, these are:

1. mTOR signaling-based assays in zebrafish with Satish Srinivas Kitambi (Karolinska Institutet, Sweden).

2. Cancer and neuronal stem cells with Subhadraw Dravida (Stem Cell Research, Tran-Scell Biologics Hyderabad)
3. Rapamycin-based cellular assays related to metabolic disorders and cancer with Prasenjit Mitra (DRILS, Hyderabad)

4.6. Experimental Procedures:

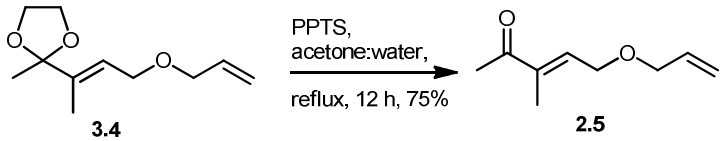
4.4.1. Procedures for Keto Fragment 2.5:



1. To a solution of (E)-ethyl 3-methyl-4-oxopent-2-enoate (**3.1**) (11g, 70 mmol, 1 eq) in DCM added p-toluenesulfonic acid mono-hydrate (2.67g, 14 mmol, 0.2 eq), ethylene glycol (79 mL,1408 mmol, 20 eq) and trimethyl orthoformate (77 mL, 704 mmol,10 eq) in DCM (150 mL). The mixture was stirred for 12 hours at ambient temperature before being washed sequentially with 1M sodium hydroxide solution and brine solution. The DCM phase was distilled to dryness to give the crude desired product as colourless liquid **3.2** which was subjected to further reaction.
2. To the solution of above dried crude liquid **3.2** (1 eq) in dry THF (150 mL) added Lithium aluminium hydride (1.2 eq) as a portionwise at 0 ^{o}C carefully under nitrogen atmosphere. Than the reaction mixture allowed to stirred at ambient temperature for 12 h then reaction quenched with 2M NaOH solution (20 mL) at 0 ^{o}C carefully and added water (50 mL) and EtOAc (50 mL) filtered through celite pad and two, layers were separated. Aqueous layer extracted twice with EtOAc (2x50 mL) and combine organic layers were washed with brine solution and dried over

Na_2SO_4, filterd and dried. Crude compound alcohol **3.3** got as a colourless liquid which was subjected to allylation reaction.

3. To the solution of above crude compound alcohol **3.3** (1 eq) in dry DMF added sodium hydride (3 eq), allylbromide (2 eq) and TBAI (0.1 eq) at 0 ^oC then allowed stirred for 12 hours at ambient temperature under nitrogen atmosphere. Then reaction quenched with saturated NH_4Cl at 0 ^oC 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 **3.4** as light yellow oil (8.3 g, 60% for three steps). Molecular Name: (E)-2-(4-(allyloxy)but-2-en-2-yl)-2-methyl-1,3-dioxolane; Molecular Formula: $C_{11}H_{18}O_3$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); ^1H NMR (400 MHz, *CDCl₃*) δ ppm: 5.93 (ddd, *J* = 22.88, 10.82, 5.68 Hz, 1H), 5.86-5.80 (m, 1H), 5.28 (dd, *J* = 17.19, 1.51 Hz, 1H), 5.22-5.16 (m, 1H), 4.05 (d, *J* = 6.36 Hz, 2H), 4.01-3.92 (m, 4H), 3.82 (dd, *J* = 8.53, 5.08 Hz, 2H), 1.47 (s, 3H), 1.67 (s, 3H); ^{13}C NMR (100 MHz, *CDCl₃*): 138.2, 134.7, 122.1, 117.1, 109.3, 71.2, 66.6, 64.3, 23.8, 12.5; LRMS: (ES+) m/z =198.9 (M+1).

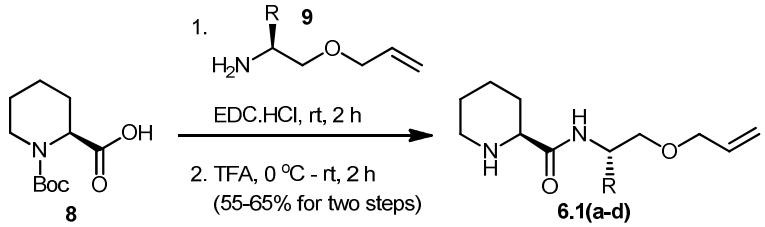


3.4 **2.5**

To the solution of **3.4** (10 g, 50.4 mmol) in (10:1) acetone: water (110 mL) added PPTS (2.53 g, 10 mmol) refluxed for 12 hours. Then evaporated the solvent at below 30 ^oC and added water extracted thrice with DCM. Combined organic layers were dried over Na_2SO_4, filtered and evaporated at below 30 ^oC. Purification of crude compound by flash column chromatography over silica gel (10% EtOAc/hexane) afforded the compound **2.5** as light yellow oil (5.8 g, 75%). Molecular Name: (E)-5-(allyloxy)-3-methylpent-3-en-2-one; Molecular Formula: $C_9H_{14}O_2$; R_f (solvent system): 0.25 (10%, EtOAc/hexane); ^1H NMR (400 MHz, *CDCl₃*) δ ppm: 6.70 (td, *J* = 5.60, 1.20 Hz, 1H), 5.95 (m, 1H), 5.34 (dd, *J* = 17.23, 1.53 Hz, 1H), 5.26 (dd, *J* = 10.35, 1.25 Hz, 1H), 4.28-4.23 (m, 2H), 4.06 (d, *J* = 5.72 Hz, 2H), 2.35 (s, 3H), 1.77 (d, *J* = 1.03 Hz, 3H); ^{13}C NMR (100

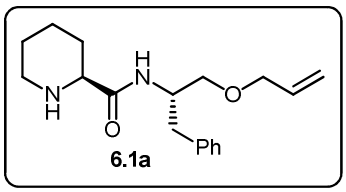
MHz, *CDCl$_3$*): 199.2, 139.4, 137.8, 134.1, 117.7, 71.9, 67.2, 25.3, 11.4 ;LRMS: (ES+) m/z =155.1 (M+1).

4.4.2. Procedures for Secondary Amine 6.1:



1. To the solution of (S)-1-(tert-butoxycarbonyl)piperidine-2-carboxylic acid **8** (1.2 eq) in acetontrile solution added amino acid building block amine **9** (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, and dried, afforded the crude colourless oil compound which was directly subjected to further reaction.
2. To the above crude oil compound (1 eq) in dry DCM added Trifluoroacetic acid (TFA) (5 eq) at 0 ^oC then allowed stirred for 2 hours then quenched with saturated $NaHCO_3$ solution carefully till the solution get basic pH then extracted twice with DCM. Combine organic layers were washed with brine solution and dried over anhydrous Na_2SO_4, evaporated. The purification of crude compound by flash column chromatography over neutralized silica gel with Et_3N (50% EtOAc/hexane) afforded the compound **6.1(a-d)**.

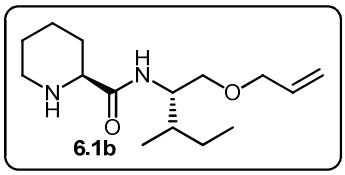
(S)-N-((S)-1-(allyloxy)-3-phenylpropan-2-yl)piperidine-2-carboxamide (22a):



Molecular Formula: $C_{18}H_{26}N_2O_2$; R_f (solvent system): 0.05 (50%, EtOAc/hexane); Yield: 65%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.22 (m, 6H), 6.99 (d, *J* = 8.69 Hz, 1H), 5.90 (m, 1H), 5.28 (dd, *J* = 17.20, 1.60 Hz, 1H), 5.18

(dd, *J* = 10.39, 1.56 Hz, 1H), 4.30 (m, 1H), 4.02 (m, 2H), 3.37 (d, *J* = 4.08 Hz, 2H), 3.15 (dd, *J* = 9.88, 3.22 Hz, 1H), 2.90 (m, 3H), 2.63 (m, 1H), 1.85 (m, 1H), 1.72 (m, 1H), 1.63 (m, 1H), 1.52 (m, 1H), 1.33 (m, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 173.6, 138.1, 134.6, 129.4, 128.3, 126.3, 117.0, 72.1, 70.2, 60.2, 49.5, 45.7, 37.5, 29.9, 26.0, 24.0; LRMS: (ES+) m/z =303.0 (M+1).

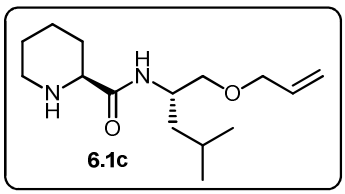
(S)-N-((2S,3R)-1-(allyloxy)-3-methylpentan-2-yl)piperidine-2-carboxamide (22b):



6.1b

Molecular Formula: $C_{15}H_{28}N_2O_2$; R_f (solvent system): 0.05 (50%, EtOAc/hexane); Yield: 60%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.95 (d, *J* = 9.39 Hz, 1H), 5.85 (m, 1H), 5.23 (d, *J* = 17.23 Hz, 1H), 5.15 (d, *J* = 10.38 Hz, 1H), 3.90 (m, 3H), 3.50 (dd, *J* = 9.79, 4.58 Hz, 1H), 3.38 (dd, *J* = 9.80, 3.93 Hz, 1H), 3.24 (dd, *J* = 9.43, 3.08 Hz, 1H), 3.04 (d, *J* = 11.67 Hz, 1H), 2.68 (dd, *J* = 15.52, 6.72 Hz, 2H), 1.96 (d, *J* = 8.62 Hz, 1H), 1.76 (dd, *J* = 9.59, 4.38 Hz, 1H), 1.65 (m, 1H), 1.57 (d, *J* = 9.43 Hz, 1H), 1.44 (m, 4H), 1.08 (m, 1H), 0.87 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 173.3, 134.6, 117.0, 72.0, 69.9, 60.3, 52.4, 45.6, 35.7, 30.1, 25.5, 25.3, 23.9, 15.5, 11.3; LRMS: (ES+) m/z =269.1 (M+1).

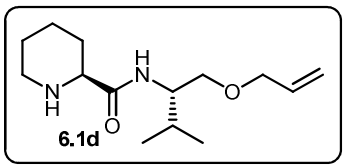
(S)-N-((S)-1-(allyloxy)-4-methylpentan-2-yl)piperidine-2-carboxamide (22c):



6.1c

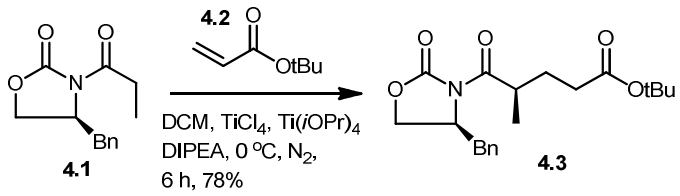
Molecular Formula: $C_{15}H_{28}N_2O_2$; R_f (solvent system): 0.05 (50%, EtOAc/hexane); Yield: 58%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.76 (d, *J* = 8.88 Hz, 1H), 5.84 (m, 1H), 5.22 (dd, *J* = 17.25, 1.19 Hz, 1H), 5.13 (d, *J* = 9.56 Hz, 1H), 4.11 (d, *J* = 3.84 Hz, 1H), 3.92 (m, 2H), 3.36 (s, 2H), 3.15 (d, *J* = 7.01 Hz, 1H), 2.99 (d, *J* = 11.16 Hz, 1H), 2.64 (t, *J* = 10.31 Hz, 1H), 1.93 (s, 2H), 1.74 (s, 1H), 1.56 (m, 2H), 1.37 (m, 5H), 0.88 (m, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 173.6, 134.7, 116.9, 72.2, 72.0, 60.4, 46.4, 45.8, 40.8, 30.2, 25.9, 24.9, 24.1, 23.1, 22.1; LRMS: (ES+) m/z =269.1 (M+1).

(S)-N-((S)-1-(allyloxy)-3-methylbutan-2-yl)piperidine-2-carboxamide (22d):



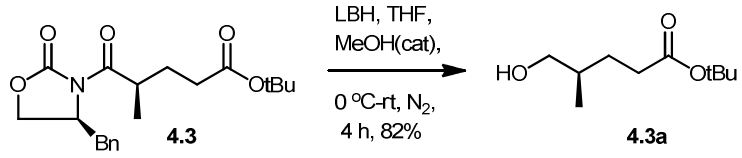
Molecular Formula: $C_{15}H_{28}N_2O_2$; R_f (solvent system): 0.05 (50%, EtOAc/hexane); Yield: 58%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.87 (d, *J* = 9.27 Hz, 1H), 5.78 (m, 1H), 5.15 (d, *J* = 17.23 Hz, 1H), 5.06 (d, *J* = 10.37 Hz, 1H), 3.86 (m, 2H), 3.74 (d, *J* = 2.04 Hz, 1H), 3.42 (m, 1H), 3.28 (m, 1H), 3.10 (d, *J* = 7.90 Hz, 1H), 2.93 (d, *J* = 11.47 Hz, 1H), 2.58 (t, *J* = 10.42 Hz, 1H), 1.84 (dd, *J* = 13.06, 4.18 Hz, 3H), 1.68 (s, 1H), 1.46 (s, 1H), 1.33 (s, 3H), 0.82 (m, 7H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 173.8, 134.6, 116.8, 71.9, 70.0, 60.4, 53.2, 45.7, 30.3, 29.2, 25.9, 24.1, 19.5, 18.7; LRMS: (ES+) m/z =255.1 (M+1).

4.4.3. Procedures for 2,6-*cis*-Tetrahydropyran 5.2:

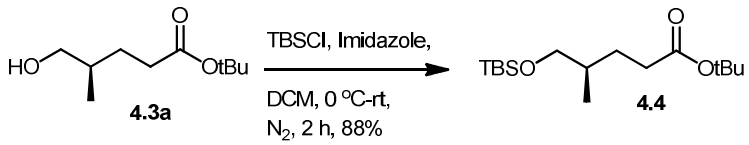


Titanium(IV) isopropoxide (3.87 mL, 13.0 mmol) and 8.66 mL (40 mmol) of titanium tetrachloride were added by syringe to 190 mL of dry DCM at 0 °C under nitrogen atmosphere. After 10 min, 9.4 mL (54 mmol) of diisopropylethylamine was added. The resulting brown solution was stirred for 10 min, and 11.65 g (50 mmol) of **4.1** was added as a solid. The resulting deep red solution was stirred at 0 °C for 1 h, and 11 mL (75 mmol) of tert-butyl acrylate **4.2** was added dropwise by syringe. The solution gradually turned brown. After 4 h at 0 °C, 500 mL of saturated aqueous NH_4Cl was added. The layers were separated, and the aqueous layer was extracted with 250 mL of DCM. The combined organic layers were washed with 500 mL of saturated aqueous $NaHCO_3$, dried on Na_2SO_4, filtered. After solvent evaporation, the crude product was purified by flash chromatography on silica gel (20%, EtOAc/hexane). The product **4.3** was obtained as viscous oil (14 g, 78%). Molecular Name: (R)-tert-butyl 5-((S)-4-benzyl-2-oxooxazolidin-3-yl)-4-methyl-5-oxopentanoate; Molecular Formula: $C_{20}H_{27}NO_5$; R_f (solvent system): 0.3 (20%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.40-7.20 (m, 5H), 4.69 (tdd, *J* = 10.53,

6.92, 3.36 Hz, 1H), 4.24-4.10 (m, 2H), 3.78 (td, *J* = 13.63, 6.82 Hz, 1H), 3.35 (dd, *J* = 13.31, 3.27 Hz, 1H), 2.75 (dd, *J* = 13.31, 9.89 Hz, 1H), 2.37-2.24 (m, 2H), 2.14-2.03 (m, 1H), 1.80 (m, 1H), 1.46 (s, 9H), 1.21 (d, *J* = 6.83 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl*$_3$): 176.5, 172.3, 153.0, 135.3, 129.4, 128.9, 127.3, 80.4, 66.0, 55.4, 38.1, 36.9, 33.1, 28.7, 28.1, 16.9; LRMS: (ES+) m/z =362.2 (M+1).

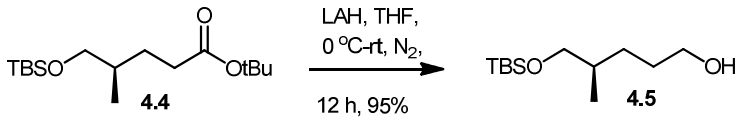


To a solution of **4.3** (4.2 g, 11.63 mmol) in dry THF (50 mL) and MeOH (0.5 mL) was added lithium borohydride (280 mg, 12.8 mmol) slowly at 0 ºC under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 4 h and cooled to 0 ºC and then quenched with 1N NaOH solution carefully. Then added 50 mL of water and extracted twice with EtOAc. The combined organic layers were washed with brine solution and then dried over Na_2SO_4. Purification by flash chromatography over silica gel (30%, EtOAc/hexane) afforded the product **4.3a** (1.8 g, 82%) as a colourless oil; Molecular Name: (R)-tert-butyl 5-hydroxy-4-methylpentanoate; Molecular Formula: $C_{10}H_{20}O_3$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 3.46 (d, *J* = 5.76 Hz, 2H), 2.37-2.16 (m, 2H), 1.78-1.59 (m, 3H), 1.45 (s, 9H), 0.93 (d, *J* = 6.68 Hz, 3H)); ^{13}C NMR (100 MHz, *CDCl*$_3$): 173.6, 80.3, 67.3, 35.3, 32.9, 28.0, 27.9, 16.4; LRMS: (ES+) m/z =189.1 (M+1).

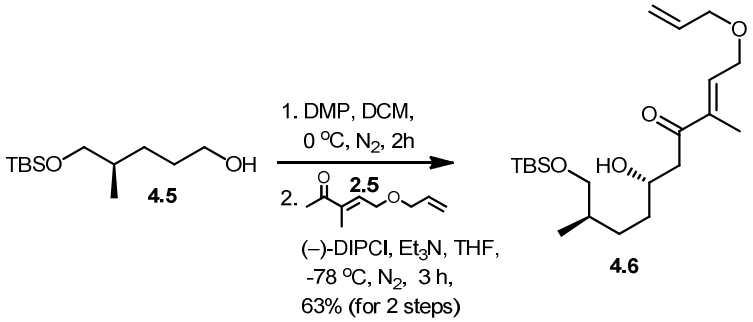


To a solution of **4.3a** (7.8 g, 41.75 mmol) in dry DCM (150 mL) was added TBSCl (9.4 g, 62.6 mmol) and imidazole (7.5 g, 125.2 mmol) at 0 ^{o}C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h then after starting material completed by monitoring with TLC poured water 20 mL and extracted twice with DCM. The combined organic layers were washed with brine solution and then dried over Na_2SO_4. Purification by flash chromatography over silica gel (5%, EtOAc/hexane) afforded the product **4.4** (11 g, 88%) as a colourless oil; Molecular Name: (R)-tert-butyl 5-((tert-

butyldimethylsilyl)oxy)-4-methylpentanoate; Molecular Formula: $C_{16}H_{34}O_3Si$; R_f (solvent system): 0.3 (5%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 3.41 (dq, *J* = 9.84, 6.04 Hz, 2H), 2.21 (m, 2H), 1.70 (m, 1H), 1.59 (tt, *J* = 12.76, 6.40 Hz, 1H), 1.43 (s, 9H), 0.88 (d, *J* = 6.67 Hz, 12H), 1.37 (m, 1H), 0.02 (s, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 173.3, 79.9, 67.9, 35.3, 33.3, 28.5, 28.1, 25.9, 18.3, 16.4, -5.4; LRMS: (ES+) m/z =303.5 (M+1).



To a solution of **4.4** (11 g, 36.36 mmol) in dry THF (150 mL) was added LAH (2 g, 54.54 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 12 h then after completion of starting material by monitoring with TLC quenched with 10% NaOH solution (10 mL) and ice cold water 20 mL then extracted twice with EtOAc. The combined organic layers were washed with brine solution and then dried over Na_2SO_4. Purification of compound by flash chromatography over silica gel (30%, EtOAc/hexane) afforded the product **4.5** (8 g, 95%) as a colourless oil; Molecular Name: (R)-5-((tert-butyldimethylsilyl)oxy)-4-methylpentan-1-ol; Molecular Formula: $C_{12}H_{28}O_2Si$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 3.63 (m, 2H), 3.41 (dq, *J* = 9.80, 6.21 Hz, 2H), 1.62 (m, 2H), 1.49 (m, 2H), 1.13 (ddt, *J* = 7.82, 6.29, 3.73 Hz, 1H), 0.88 (d, *J* = 6.67 Hz, 12H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 68.2, 63.3, 35.5, 30.1, 29.2, 25.9, 18.3, 16.7, -5.4; LRMS: (ES+) m/z =233.2 (M+1).

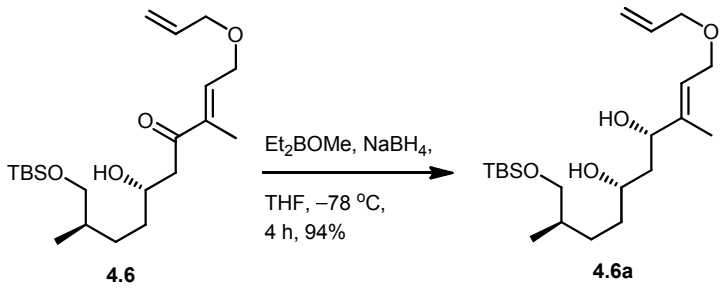


1. To a solution of alcohol **4.5** (1 g**,** 4.31 mmol) in dry DCM (15 mL) added DMP (2.2 g, 5.17 mmol) in one portion at 0 °C under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at 20 °C

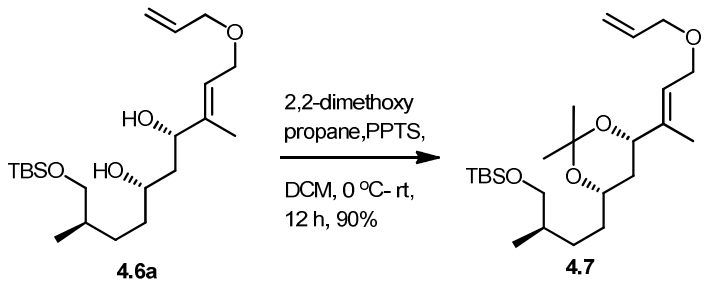
then completion of starting material by monitoring with TLC, poured a saturated solution of sodium thiosulfate ($Na_2S_2O_3$) (5 mL) and saturated $NaHCO_3$ solution (10 mL) allowed to stirred for 15 minutes. Two layers were separated and aqueous layer extracted with DCM, combined organic layers were washed with brine and dried over Na_2SO_4. Then organic layer was evaporated and obtained crude colourless liquid aldehyde (610 mg). This crude aldehyde was dried through high vaccum pump and without purification subjected to further step stereoselective Aldol reaction.

2. (−)-DIPCl (1.35 g, 4.22 mmol) taken into round bottom flask under nitrogen atmosphere and dried for 20 minutes through high vaccum then added dry DCM (10 mL) and cooled to -78 ^oC. To this mixture added Et_3N (0.73 mL, 5.28 mmol) dropwise and keto compound **2.5** (631 mg, 4.09 mmol) in 3 mL dry DCM then allowed to stirred for 3 h at same temperature. Then added above aldehyde (610 mg, 2.64 mmol)) in 1 mL dry DCM allowed to stirred for 2 h at -78 ^oC then brought -20 ^oC and allowed to stirred for 12 h at same temperature. To this reaction mixture added 14 mL MeOH: pH 7 buffer solution (1:1) followed by added 7 mL 30% H_2O_2 then temperature raised to 0 ^oC and stirred for 1 h. To this reaction mixture added 10 mL water and separated the two layers. Aqueous layer extracted thrice with DCM (3x10 mL) and combined organic layer was washed with brine solution, dried over $NaSO_4$ and evaporated solvent. Purification of compound by flash chromatography over silica gel (3%, EtOAc/hexane) afforded the β-hydroxy keto compound **4.6** (680 mg, 67%) as a colourless oil; Molecular Name: (6S,9R,E)-1-(allyloxy)-10-((tert-butyldimethylsilyl)oxy)-6-hydroxy-3,9-dimethyldec-2-en-4-one; Molecular Formula: $C_{21}H_{40}O_4Si$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); ^1H NMR (400 MHz, *$CDCl_3$*) δ ppm: 6.69 (t, *J* = 5.47 Hz, 1H), 5.98-5.86 (m, 1H), 5.36-5.19 (m, 2H), 4.22 (d, *J* = 5.47 Hz, 2H), 4.06-3.99 (m, 3H), 3.44 (m, 1H), 3.37 (m, 1H), 3.24 (bs, 1H), 2.89 (m, 1H), 2.76-2.66 (m, 1H), 1.75 (d, *J* = 1.01 Hz, 3H), 1.63-1.46 (m, 4H), 1.12-1.07 (m, 1H), 0.87 (d, *J* = 3.16 Hz, 12H), 0.02 (s, 6H); ^{13}C NMR (100 MHz, *$CDCl_3$*): 202.0, 139.7, 137.5, 134.1, 117.8,

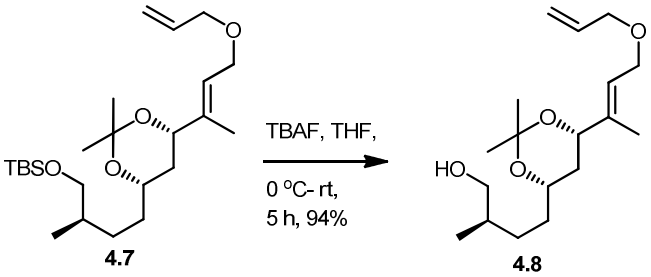
71.9, 68.1, 67.2, 43.4, 35.7, 33.9, 29.7, 29.0, 25.9, 18.3, 16.7, 11.4, -5.4; LRMS: (ES+) m/z =385.1 (M+1).



To a solution of β-hydroxy keto compound **4.6** (1 g, 2.6 mmol) in dry THF (24 mL) and MeOH (6 mL) added Et_2BOMe (1M in THF solution) (2.85 mL, 2.85 mmol) at -78 ^oC under nitrogen atmosphere and allowed to stirred for 30 minutes. To this reaction mixture added $NaBH_4$ (150 mg, 2.5 mmol) as one portion. After 3 h stirred at same temperature brought to 0 ^oC then added 3N NaOH solution (4 mL) dropwise and 30% H_2O_2 (2 mL) then allowed to stirred for 12 h at room temperature. Than after added 10 mL water and extracted thrice with EtOAc. Combined organic layers were washed with brine solution, dried over Na_2SO_4 and evaporated solvent. Purification of compound by flash chromatography over silica gel (30%, EtOAc/hexane) afforded the diol compound **4.6a** (940 mg, 94%) as a colourless oil; Molecular Name: (4S,6S,9R,E)-1-(allyloxy)-10-((tert-butyldimethylsilyl)oxy)-3,9-dimethyldec-2-ene-4,6-diol; Molecular Formula: $C_{21}H_{42}O_4Si$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); ^1H NMR (400 MHz, *CDCl₃*) δ ppm: 5.91 (m, 1H), 5.62 (t, *J* = 6.31 Hz, 1H), 5.23 (dd, *J* = 34.29, 13.78 Hz, 2H), 4.26 (dd, *J* = 7.33, 4.98 Hz, 1H), 4.02 (d, *J* = 6.49 Hz, 2H), 3.97 (d, *J* = 5.67 Hz, 2H), 3.83 (s, 1H), 3.40 (m, 3H), 1.66 (s, 3H), 1.58 (m, 3H), 1.45 (m, 3H), 1.08 (m, 1H), 0.87 (d, *J* = 8.54 Hz, 12H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, *CDCl₃*): 141.5, 134.7, 121.7, 117.3, 77.9, 73.1, 71.3, 68.2, 66.2, 41.0, 35.7, 35.3, 28.8, 25.9, 18.3, 16.8, 12.2, -5.4; LRMS: (ES+) m/z =369.1 (M–OH).



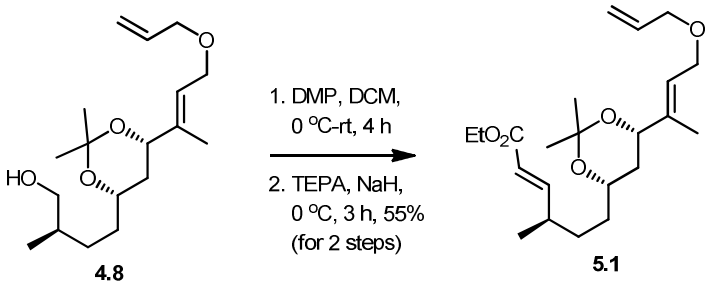
To a solution of diol **4.6a** (940 mg, 2.43 mmol) in dry DCM (15 mL) added pyridinium *p*-toluenesulfonate (PPTS) (61 mg, 0.243 mmol) and 2,2-dimethoxypropane (3 mL, 24.3 mmol) at 0 ^{o}C under nitrogen atmosphere. This reaction mixture allowed stirred for 12 h at room temperature then added saturated NaHCO3 solution (20 mL) and extracted twice with DCM, combined organic layers were washed with brine solution dried over Na_2SO_4 and evaporated solvent. Purification of compound by flash chromatography over silica gel (5%, EtOAc/hexane) afforded the compound **4.7** (900 mg, 90%) as a colourless oil; Molecular Name: ((R)-4-((4S,6S)-6-((E)-4-(allyloxy)but-2-en-2-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methylbutoxy)(tert-butyl)dimethylsilane; Molecular Formula: $C_{24}H_{46}O_4Si$; R_f (solvent system): 0.2 (5%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 5.94 (m, 1H), 5.68 (t, *J* = 6.42 Hz, 1H), 5.30 (dd, *J* = 17.25, 1.38 Hz, 1H), 5.20 (d, *J* = 10.34 Hz, 1H), 4.25 (d, *J* = 10.62 Hz, 1H), 4.06 (d, *J* = 6.43 Hz, 2H), 3.99 (d, *J* = 5.68 Hz, 2H), 3.83 (m, 1H), 3.46 (m, 1H), 3.48 (m, 1H), 1.69 (s, 3H), 1.58 (m, 3H), 1.48 (m, 5H), 1.43 (s, 3H), 1.31 (m, 1H), 1.06 (m, 1H), 0.90 (m, 12H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 139.1, 134.8, 122.4, 117.1, 98.5, 73.7, 71.3, 69.3, 68.2, 66.4, 35.7, 35.3, 33.8, 30.2, 28.3, 26.0, 19.8, 18.3, 16.6, 12.7, -5.3; LRMS: (ES+) m/z =427.5 (M+1).



4.7 **4.8**

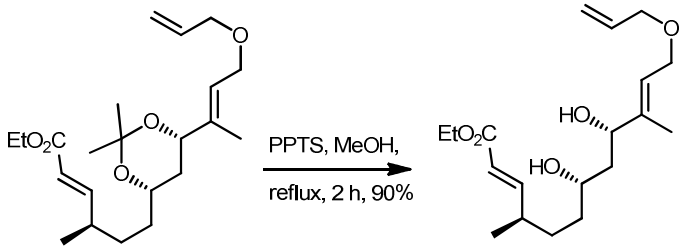
To a solution of compound **4.7** (474 mg, 1.1 mmol) in dry THF (10 mL) added TBAF at 0 ^{o}C under nitrogen atmosphere. This reaction mixture allowed to stirred for 5 h at room temperature then cooled to 0 ^{o}C added saturated NH_4Cl soltution (10 mL) and extracted twice with EtOAc. Combined organic layers were washed with brine solution dried over Na_2SO_4 and evaporated solvent. Purification of compound by flash chromatography over silica gel (30%, EtOAc/hexane) afforded the compound **4.8** (345 mg, 94%) as a colourless oil; Molecular Name: (R)-4-((4S,6S)-6-((E)-4-(allyloxy)but-2-en-2-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methylbutan-1-ol; Molecular Formula: $C_{18}H_{32}O_4$; R_f (solvent system): 0.2

(20%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 5.92 (m, 1H), 5.66 (t, *J* = 6.36 Hz, 1H), 5.28 (d, *J* = 17.21 Hz, 1H), 5.19 (d, *J* = 10.35 Hz, 1H), 4.23 (d, *J* = 11.20 Hz, 1H), 4.04 (d, *J* = 6.41 Hz, 2H), 3.98 (d, *J* = 5.66 Hz, 2H), 3.84 (m, 1H), 3.46 (m, 2H), 1.95 (s, 1H), 1.67 (s, 3H), 1.61 (m, 1H), 1.52 (m, 4H), 1.46 (s, 3H), 1.42 (s, 3H), 1.34 (m, 1H), 1.13 (m, 1H), 0.92 (d, *J* = 6.62 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 139.1, 134.8, 122.4, 117.1, 98.6, 73.6, 71.3, 69.4, 67.9, 66.3, 35.7, 35.3, 33.7, 30.2, 28.3, 19.8, 16.6, 12.7; LRMS: (ES+) m/z =313.3 (M+1).



1. To a solution of alcohol **4.8** (600 mg**,** 1.92 mmol) in dry DCM (15 mL) added DMP (1.3 g, 3 mmol) in one portion at 0 ^{o}C under nitrogen atmosphere. The reaction mixture was allowed stirred for 2 h at 20 ^{o}C 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. Then organic layer was evaporated and obtained crude colourless liquid aldehyde. This crude aldehyde was dried through high vaccum pump and without purification subjected to further step HWE reaction.
2. 15 ml dry THF taken into round bottom flask and cooled to 0 ^{o}C and added NaH (92 mg, 3.84 mmol) under nitrogen atmosphere. To this mixture added TEPA (0.76 ml, 3.84 mmol) carefully through syringe and allowed to stirred for 30 min at same temperature. Then added above aldehyde in 1 mL THF dropwise and allowed stirred for 2 h at 0 ^{o}C. After completion of starting material monitoring by TLC, reaction quenched with saturated NH_4Cl (15 mL) at 0 ^{o}C and extracted twice

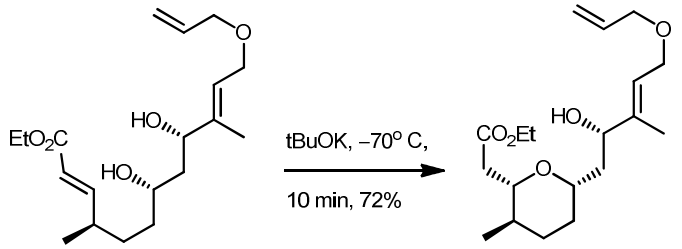
with EtOAc. Combined organic layers were washed with brine solution, dried over Na_2SO_4 and evaporated solvent. Purification of compound by flash chromatography over silica gel (5%, EtOAc/hexane) afforded α,β-unsaturated ethyl ester **5.1** (400 mg, 55% for 2 steps) as a colourless oil; Molecular Name: (R,E)-ethyl 6-((4S,6S)-6-((E)-4-(allyloxy)but-2-en-2-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-4-methylhex-2-enoate; Molecular Formula: $C_{22}H_{36}O_5$; R_f (solvent system): 0.2 (5%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl₃*) δ ppm: 6.86 (dd, *J* = 15.68, 7.94 Hz, 1H), 5.93 (m, 1H), 5.79 (d, *J* = 15.66 Hz, 1H), 5.67 (t, *J* = 6.18 Hz, 1H), 5.30 (d, *J* = 17.25 Hz, 1H), 5.20 (d, *J* = 10.26 Hz, 1H), 4.22 (m, 3H), 4.06 (d, *J* = 6.41 Hz, 2H), 3.99 (d, *J* = 5.62 Hz, 2H), 3.83 (m, 1H), 2.32 (m, 1H), 1.68 (s, 3H), 1.53 (m, 3H), 1.47 (s, 3H), 1.41 (m, 6H), 1.31 (t, *J* = 7.14 Hz, 3H), 1.08 (d, *J* = 6.72 Hz, 3H); ^{13}C NMR (100 MHz, *CDCl₃*): 166.8, 154.1, 139.0, 134.8, 122.4, 119.9, 117.0, 98.6, 73.6, 71.3, 68.8, 66.4, 60.2, 36.5, 35.3, 34.0, 31.2, 30.2, 19.8, 19.4, 14.3, 12.7; LRMS: (ES+) m/z =381.4 (M+1).



5.1 **5.1a**

To a solution of α,β-unsaturated ethyl ester **5.1** (400 mg, 1.0 mmol) in MeOH (20 mL) added pyridinium p-toluenesulfonate (PPTS) (75 mg, 0.30 mmol) and refluxed under nitrogen atmosphere. After 2 h, completion of starting material monitoring by TLC, solvent evaporated. Purification of crude compound by flash chromatography over silica gel (40%, EtOAc/hexane) afforded diol compound **5.1a** (325 mg, 90%) as a colourless oil; Molecular Name: (2E,4R,7S,9S,10E)-ethyl 12-(allyloxy)-7,9-dihydroxy-4,10-dimethyldodeca-2,10-dienoate; Molecular Formula: $C_{19}H_{32}O_5$; R_f (solvent system): 0.2 (50%, EtOAc/hexane); ^{1}H NMR (400 MHz, *CDCl₃*) δ ppm: 6.84 (dd, *J* = 15.70, 7.97 Hz, 1H), 5.91 (m, 1H), 5.77 (d, *J* = 15.67 Hz, 1H), 5.63 (t, *J* = 6.23 Hz, 1H), 5.28 (d, *J* = 17.24 Hz, 1H), 5.19 (d, *J* = 10.33 Hz, 1H), 4.27 (m, 1H), 4.18 (q, *J* = 7.15 Hz, 2H), 4.02 (d, *J* = 6.52 Hz, 2H), 3.98 (d, *J* = 5.67 Hz, 2H), 3.84 (m, 1H), 3.54-3.36 (bs, 1H), 3.06-2.87

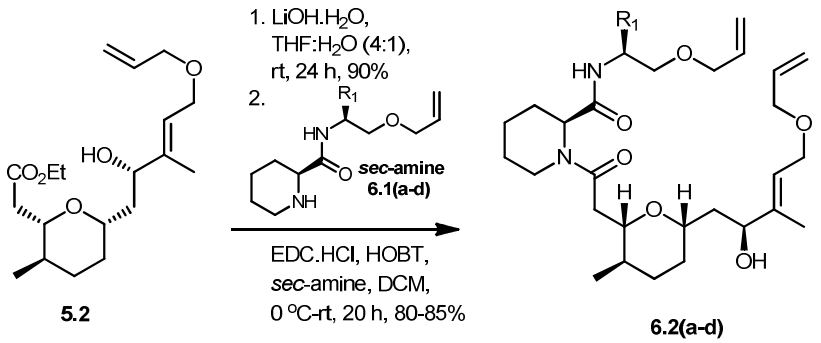
(bs, 1H), 2.30 (m, 1H), 1.67 (s, 3H), 1.59 (m, 2H), 1.45 (m, 4H), 1.29 (t, *J* = 7.13 Hz, 3H), 1.06 (d, *J* = 6.70 Hz, 3H); ^{13}C NMR (100 MHz, *$CDCl_3$*): 166.9, 154.2, 141.5, 134.6, 121.6, 119.9, 117.2, 77.7, 72.2, 71.3, 66.2, 60.2, 41.0, 36.4, 35.4, 31.5, 19.4, 14.2, 12.2; LRMS: (ES+) m/z =341.4 (M+1).



5.1a **5.2**

To a solution of diol **5.1a** (325 mg, 0.95 mmol) in dry THF (10 mL) added tBuOK (214 mg, 1.90 mmol) at -70 ^{o}C under nitrogen atmosphere. This reaction mixture was allowed stirred for 10 min then temperature raised to -30 ^{o}C then quenched with saturated NH_4Cl (5 mL). To this mixture added 10 mL water and extracted twice with EtOAc, combined organic layers were washed with brine solution dried over Na_2SO_4 and evaporated. Purification of crude compound by flash chromatography over silica gel (15%, EtOAc/hexane) afforded compound **5.2** (234 mg, 72%) as a colourless oil; Molecular Name: ethyl 2-((2S,3R,6S)-6-((S,E)-5-(allyloxy)-2-hydroxy-3-methylpent-3-en-1-yl)-3-methyltetrahydro-2H-pyran-2-yl)acetate; Molecular Formula: $C_{19}H_{32}O_5$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); ^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 5.91 (m, 1H), 5.62 (t, *J* = 6.52 Hz, 1H), 5.26 (dd, *J* = 17.23, 1.61 Hz, 1H), 5.16 (dd, *J* = 10.37, 1.17 Hz, 1H), 4.23 (m, 1H), 4.15 (m, 2H), 4.02 (d, *J* = 6.58 Hz, 2H), 3.95 (d, *J* = 5.64 Hz, 2H), 3.59 (m, 1H), 3.48 (m, 1H), 2.62 (m, 1H), 2.34 (m, 1H), 1.78 (m, 1H), 1.70 (m, 1H), 1.65 (s, 3H), 1.56 (m, 2H), 1.38 (m, 2H), 1.25 (t, *J* = 7.14 Hz, 3H), 0.94 (m, 1H), 0.84 (d, *J* = 6.53 Hz, 3H); ^{13}C NMR (100 MHz, *$CDCl_3$*): 171.8, 141.1, 135.0, 121.6, 116.9, 80.4, 79.2, 77.1, 71.0, 66.3, 60.8, 41.7, 39.1, 34.9, 32.4, 17.6, 14.1, 12.3; LRMS: (ES+) m/z =341.2 (M+1).

4.4.4. Procedures for Rapalogs 1.1:

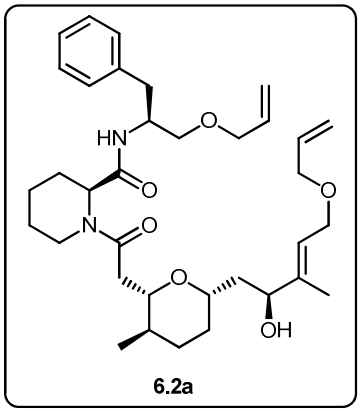


1. To a solution of compound **5.2** (1 eq) in THF:H_2O mixture (4:1) added $LiOH.H_2O$ (5 eq) allowed to stirred for 24 h at room temperature then added 5% HCl solution (5 mL) and the compound extracted twice with EtOAc. The organic phase was dried over Na_2SO_4, filtered and evaporated solvent afforded the carboxylic acid product as colourless oil which is subjected to coupling reaction without further purification.
2. To a solution of the above carboxylic acid (1 eq) in dry DCM added sec-amine **6.1(a-d)** (1.5 eq) at 0 ^oC then added $HOBT.H_2O$ (2 eq), EDC.HCl (2 eq) and DIPEA (2 eq) under nitrogen atmosphere. This reaction mixture allowed stirred for 20 h at room temperature then added $NaHCO_3$ solution stirred for 10 min. Then two layers were separated and aqueous layer was extracted twice with DCM . combined organic layers were washed with brine solution, dried over Na_2SO_4, filtered and evaporated the solvent. Purification of crude compound by flash chromatography over silica gel (40% EtOAc/hexane) afforded the compound **6.2(a-d)** as colourless oil.

(S)-1-(2-((2S,3R,6S)-6-((S,E)-5-(allyloxy)-2-hydroxy-3-methylpent-3-en-1-yl)-3-methyl tetrahydro-2H-pyran-2-yl)acetyl)-N-((S)-1-(allyloxy)-3-phenyl propan-2-yl)piperidine-2-carboxamide (6.2a):

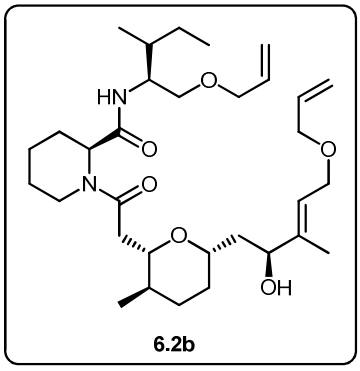
Molecular Formula: $C_{35}H_{52}N_2O_6$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 83%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 7.24 (m, 3H), 7.18 (m, 2H), 6.31 (m, 1H), 5.89 (m, 1H), 5.60 (m, 1H), 5.26 (m, 2H), 5.15 (m, 2H), 4.26 (m, 2H), 3.95 (m, 6H), 3.73 (m, 1H), 3.60 (m, 1H), 3.48 (m, 2H), 3.34 (dd, *J* = 6.46, 2.97 Hz, 1H), 2.96 (dd, *J* = 13.96, 6.44 Hz, 1H), 2.76 (d, *J* = 8.37 Hz, 1H), 2.56 (m, 1H), 2.43 (m, 1H), 2.11 (m, 2H), 1.78 (m, 2H), 1.66 (m, 4H), 1.52 (m, 4H), 1.36 (m, 4H), 1.01 (d, *J* = 6.93 Hz, 1H), 0.86 (m, 3H); ^{13}C NMR (100 MHz, *CDCl$_3$*):

171.2, 170.3, 141.3, 138.5, 134.6, 129.2, 128.3, 126.2, 121.5, 116.9, 76.9, 73.7, 72.1, 71.3, 71.0, 66.3, 52.3, 49.6, 44.0, 41.0, 37.6, 35.0, 32.7, 31.2, 29.7, 26.5, 25.9, 25.1, 20.3, 17.8, 16.9, 12.4; LRMS: (ES+) m/z =619.1 (M+Na).



6.2a

(S)-1-(2-((2S,3R,6S)-6-((S,E)-5-(allyloxy)-2-hydroxy-3-methylpent-3-en-1-yl)-3-methyl tetrahydro-2H-pyran-2-yl)acetyl)-N-((2S,3R)-1-(allyloxy)-3-methyl pentan-2-yl) piperidine-2-carboxamide (6.2b):

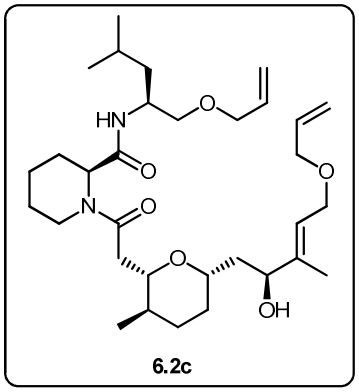


6.2b

Molecular Formula: $C_{32}H_{54}N_2O_6$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 85%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.41 (d, *J* = 9.11 Hz, 1H), 5.88 (m, 2H), 5.62 (t, *J* = 5.25 Hz, 1H), 5.25 (m, 2H), 5.16 (m, 2H), 4.23 (m, 2H), 4.02 (d, *J* = 6.34 Hz, 3H), 3.95 (dd, *J* = 9.06, 3.48 Hz, 4H), 3.86 (m, 1H), 3.76 (m, 1H), 3.61 (m, 1H), 3.49 (ddd, *J* = 14.87, 10.29, 4.80 Hz, 1H), 3.40 (dd, *J* = 9.65, 4.42 Hz, 1H), 3.23 (s, 1H), 2.85 (dd, *J* = 14.40, 10.85 Hz, 1H), 2.57 (m, 2H), 2.16 (m, 1H), 1.99 (m, 1H), 1.68 (m, 10H), 1.46 (m, 6H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.3, 171.2, 171.1, 170.8, 170.5, 169.3, 141.3, 134.9, 134.9, 134.7, 134.6, 121.5, 121.3, 117.0, 116.9, 116.9, 116.8, 80.1, 79.2, 73.7, 72.0, 72.0, 71.2, 71.1, 71.0, 70.9, 69.7, 66.3, 58.2, 53.4, 52.9, 52.8, 52.4, 44.4, 41.5, 41.4, 41.2, 40.1, 35.9, 35.5, 35.5, 34.9, 32.8, 31.6, 31.1, 29.7, 26.3, 26.0, 25.5,

25.3, 25.2, 20.4, 17.7, 16.9, 15.7, 15.6, 12.4, 11.2; LRMS: (ES+) m/z =585.3 (M+Na).

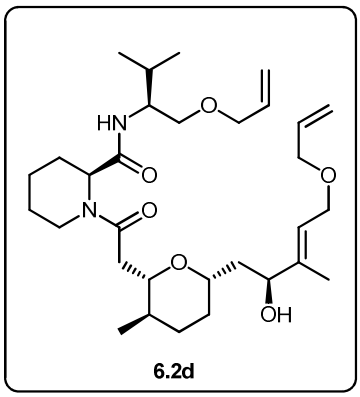
(S)-1-(2-((2S,3R,6S)-6-((S,E)-5-(allyloxy)-2-hydroxy-3-methylpent-3-en-1-yl)-3-methyl tetrahydro-2H-pyran-2-yl)acetyl)-N-((S)-1-(allyloxy)-4-methyl pentan-2-yl)piperidine-2-carboxamide (6.2c):



6.2c

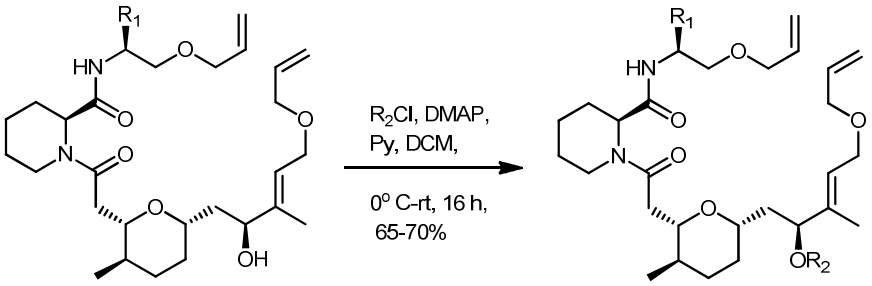
Molecular Formula: $C_{32}H_{54}N_2O_6$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 80%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.36 (d, *J* = 8.87 Hz, 1H), 5.86 (m, 2H), 5.60 (t, *J* = 6.21 Hz, 1H), 5.24 (dd, *J* = 17.00, 9.69 Hz, 2H), 5.15 (dd, *J* = 10.17, 4.33 Hz, 2H), 4.25 (m, 2H), 4.09 (m, 1H), 3.97 (dd, *J* = 25.35, 5.82 Hz, 6H), 3.76 (m, 1H), 3.60 (m, 1H), 3.36 (m, 2H), 3.16 (m, 1H), 2.84 (m, 1H), 2.50 (m, 2H), 2.15 (dd, *J* = 36.25, 15.07 Hz, 1H), 1.98 (m, 1H), 1.66 (m, 6H), 1.54 (m, 3H), 1.37 (m, 4H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.2, 170.3, 141.3, 134.9, 134.7, 121.4, 116.9, 116.9, 80.1, 79.2, 73.8, 72.0, 71.0, 66.3, 66.3, 58.2, 52.5, 47.2, 44.3, 41.0, 40.6, 32.7, 31.1, 29.7, 26.5, 26.0, 25.6, 25.0, 23.2, 22.1, 20.5, 17.8, 16.9, 12.3; LRMS: (ES+) m/z =585.3 (M+Na).

(S)-1-(2-((2S,3R,6S)-6-((S,E)-5-(allyloxy)-2-hydroxy-3-methylpent-3-en-1-yl)-3-methyl tetrahydro-2H-pyran-2-yl)acetyl)-N-((S)-1-(allyloxy)-3-methyl butan-2-yl)piperidine-2-carboxamide (6.2d):



6.2d

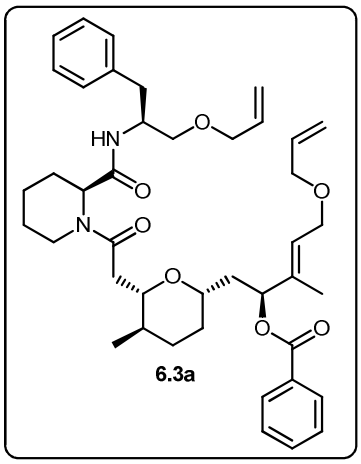
Molecular Formula: $C_{31}H_{52}N_2O_6$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 81%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 6.39 (d, *J* = 9.30 Hz, 1H), 5.88 (m, 2H), 5.60 (t, *J* = 5.42 Hz, 1H), 5.24 (dd, *J* = 16.37, 7.57 Hz, 2H), 5.14 (m, 2H), 4.21 (m, 2H), 4.00 (d, *J* = 6.34 Hz, 2H), 3.94 (d, *J* = 5.42 Hz, 3H), 3.79 (m, 2H), 3.60 (m, 1H), 3.48 (m, 1H), 3.35 (dd, *J* = 9.95, 4.89 Hz, 1H), 3.22 (m, 1H), 2.82 (m, 1H), 2.48 (m, 2H), 2.17 (m, 1H), 1.99 (m, 1H), 1.88 (m, 1H), 1.69 (m, 9H), 1.43 (m, 6H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.4, 170.7, 141.3, 134.9, 134.7, 134.6, 121.4, 116.9, 73.8, 72.0, 71.2, 71.0, 69.9, 66.3, 54.0, 52.4, 44.4, 41.2, 34.9, 32.8, 31.6, 31.2, 29.0, 26.4, 26.0, 25.5, 20.4, 19.8, 18.6, 16.9, 12.4; LRMS: (ES+) m/z =571.3 (M+Na).



6.2(a-d) **6.3(a-d)**

To a solution of **6.2(a-d)** (1 eq) in dry DCM at 0 ^{o}C added pyridine (6 eq) dropwise, DMAP (10 eq) and acid chloride (R_2Cl) (6 eq) under nitrogen atmosphere. Then reaction mixture allowed to stirred for 16 h at room temperature. To this mixture added 5 % HCl solution at 0 ^{o}C stirred for 10 min then added water and extracted twice with DCM. Combined organic layers were washed with brine solution, dried over Na_2SO_4, filtered and solvent evaporated. Purification of crude compound by flash column chromatography over silica gel (10% EtOAc/hexane) afforded the compound **6.3(a-d)** as a light yellow oil.

(S,E)-5-(allyloxy)-1-((2S,5R,6S)-6-(2-((S)-2-(((S)-1-(allyloxy)-3-phenylpropan-2-yl) carbamoyl)piperidin-1-yl)-2-oxoethyl)-5-methyltetrahydro-2H-pyran-2-yl)-3-methylpent-3-en-2-yl benzoate:



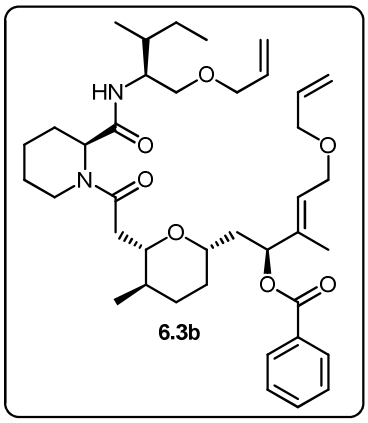
6.3a

Molecular Formula: $C_{42}H_{56}N_2O_7$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); Yield: 70%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 8.09 (d, *J* = 7.36 Hz, 2H), 7.55 (m, *J* = 7.29 Hz, 4H), 7.28 (m, 4H), 6.48 (m, 1H), 5.95 (m, 1H), 5.78 (m, 1H), 5.58 (m, 1H), 5.39-5.15 (m, 4H), 4.44 (m, 2H), 4.05 (dd, *J* = 30.21, 5.36 Hz, 6H), 3.82 (m, 1H), 3.47 (m, 3H), 3.00 (m, 1H), 2.82 (m, 1H), 2.53 (m, 3H), 2.15 (m, 2H), 1.93 (m, 3H), 1.77 (d, *J* = 12.66 Hz, 4H), 1.60 (m, 3H), 1.42 (m, 5H), 0.90 (m, 4H); ^{13}C NMR (100 MHz, *CDCl*$_3$): 171.3, 170.3, 165.6, 138.3, 136.9, 134.7, 134.6, 132.9, 130.4, 130.0, 129.6, 129.3, 129.2, 128.4, 128.3, 126.3, 124.5, 117.2, 117.1, 76.2, 72.8, 72.1, 71.2, 70.5, 68.1, 66.1, 52.1, 49.9, 43.7, 37.7, 33.3, 32.4, 31.9, 29.7, 26.3, 25.5, 25.0, 20.4, 15.6, 12.7; LRMS: (ES+) m/z =701.4 (M+1).

(S,E)-5-(allyloxy)-1-((2S,5R,6S)-6-(2-((S)-2-(((2S,3R)-1-(allyloxy)-methyl pentan-2-yl) carbamoyl)piperidin-1-yl)-2-oxoethyl)-5-methyltetra hydro-2H-pyran-2-yl)-3-methyl pent-3-en-2-yl benzoate (6.3b):

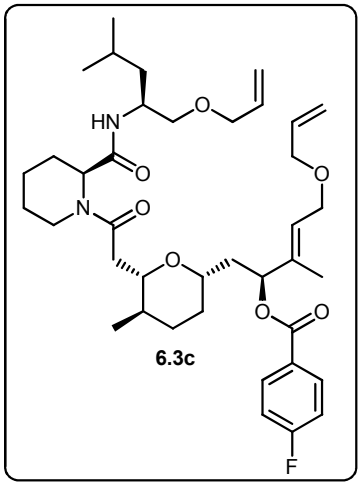
Molecular Formula: $C_{39}H_{58}N_2O_7$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); Yield: 67%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 8.08 (m, 1H), 8.03 (dd, *J* = 7.60, 3.42 Hz, 1H), 7.55 (dd, *J* = 16.52, 7.51 Hz, 1H), 7.44 (m, 2H), 6.40 (d, *J* = 9.23 Hz, 1H), 5.86 (m, 2H), 5.72 (t, *J* = 6.23 Hz, 1H), 5.50 (dd, *J* = 14.70, 6.88 Hz, 1H), 5.14 (m, 4H), 4.27 (d, *J* = 4.59 Hz, 1H), 4.02 (d, *J* = 6.16 Hz, 3H), 3.94 (tt, *J* = 12.28, 5.98 Hz, 5H), 3.72 (m, 1H), 3.40 (m, 3H), 3.11 (s, 1H), 2.56 (m, 2H), 2.26 (s, 1H), 2.13 (s, 1H), 1.72 (m, 10H), 1.44 (m, 5H), 1.05 (m, 1H), 0.85 (m,

10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.6, 170.7, 170.6, 169.6, 165.6, 165.4, 136.8, 134.7, 134.7, 134.7, 133.4, 132.9, 130.1, 129.7, 129.6, 129.6, 128.4, 128.4, 124.6, 117.2, 117.1, 117.0, 81.2, 76.2, 74.4, 73.1, 72.0, 71.9, 71.2, 71.2, 71.1, 69.6, 69.6, 67.9, 66.1, 66.0, 66.0, 57.9, 53.4, 53.4, 52.8, 52.8, 52.4, 44.1, 39.4, 37.8, 37.7, 37.2, 35.9, 35.5, 35.2, 33.2, 32.6, 29.7, 26.3, 25.6, 25.5, 25.3, 24.9, 22.7, 20.6; LRMS: (ES+) m/z =689.3 (M+Na).



6.3b

(S,E)-5-(allyloxy)-1-((2S,5R,6S)-6-(2-((S)-2-(((S)-1-(allyloxy)-4-methylpentan-2-yl)carbamoyl)piperidin-1-yl)-2-oxoethyl)-5-methyltetrahydro-2H-pyran-2-yl)-3-methylpent-3-en-2-yl 4-fluorobenzoate (6.3c):

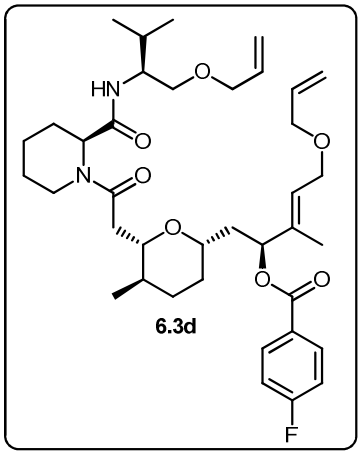


6.3c

Molecular Formula: $C_{39}H_{57}N_2O_7$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); Yield: 65%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 8.06 (m, 2H), 7.11 (m, 2H), 6.28 (d, *J* = 8.80 Hz, 1H), 5.85 (m, 1H), 5.71 (m, 1H), 5.49 (m, 1H), 5.24 (m, 4H), 4.26 (d, *J* = 4.30 Hz, 1H), 4.12 (m, 1H), 4.03 (t, *J* = 5.56 Hz, 2H), 3.96 (dd, *J* = 9.07, 3.59 Hz, 4H), 3.74 (m, 1H), 3.38 (ddd, *J* = 17.80, 9.52, 5.17 Hz, 2H), 3.10

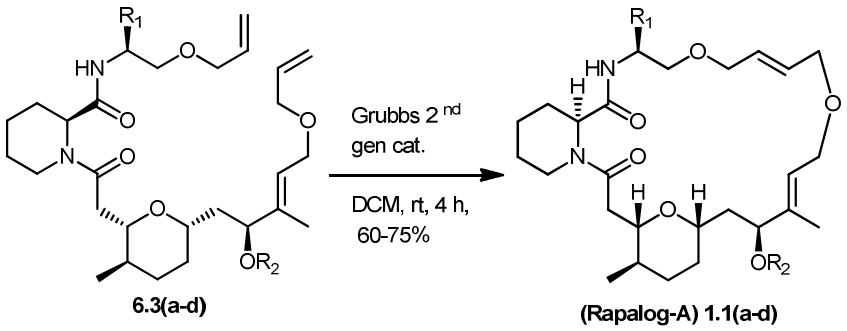
(m, 1H), 2.60 (m, 2H), 2.35 (m, 1H), 2.12 (m, 1H), 1.98 (m, 1H), 1.81 (ddd, *J* = 12.73, 9.76, 5.66 Hz, 2H), 1.64 (m, 7H), 1.40 (m, 8H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 165.7, 164.6, 158.9, 130.9, 129.0, 128.9, 127.0, 126.9, 126.6, 126.5, 126.5, 126.4, 126.4, 122.7, 119.0, 111.6, 111.5, 111.5, 111.3, 111.2, 110.0, 109.9, 109.8, 109.7, 67.3, 66.4, 66.3, 66.2, 66.1, 65.6, 65.5, 62.2, 60.4, 60.4, 60.3, 46.6, 41.5, 38.4, 35.1, 34.9, 26.8, 24.0, 24.0, 20.0, 19.7, 19.4, 19.3, 17.5, 17.4, 16.5, 16.4, 16.2, 14.9, 10.0, 8.4, 7.0, 6.9; LRMS: (ES+) m/z =707.3 (M+Na).

(S,E)-5-(allyloxy)-1-((2S,5R,6S)-6-(2-((S)-2-(((S)-1-(allyloxy)-3-methylbutan-2-yl) carbamoyl)piperidin-1-yl)-2-oxoethyl)-5-methyltetrahydro-2H-pyran-2-yl)-3-methyl pent-3-en-2-yl 4-fluorobenzoate (6.3d):

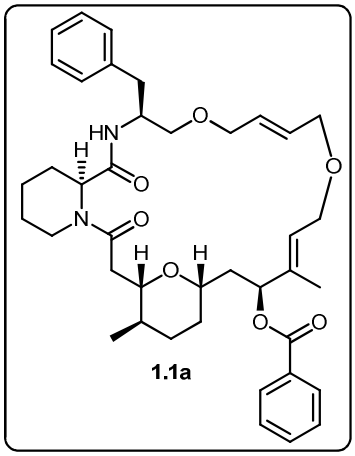


6.3d

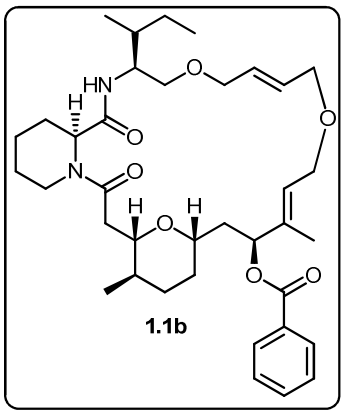
Molecular Formula: $C_{39}H_{57}N_2O_7$; R_f (solvent system): 0.2 (10%, EtOAc/hexane); Yield: 66%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 8.06 (m, 2H), 7.10 (dd, *J* = 10.31, 6.07 Hz, 2H), 6.35 (d, *J* = 9.20 Hz, 1H), 5.86 (m, 2H), 5.71 (m, 1H), 5.48 (m, 1H), 5.23 (m, 4H), 4.28 (m, 1H), 4.03 (d, *J* = 6.14 Hz, 2H), 3.95 (m, 4H), 3.78 (m, 2H), 3.46 (m, 3H), 3.10 (m, 1H), 2.62 (m, 1H), 2.48 (m, 1H), 2.26 (m, 1H), 2.07 (m, 1H), 1.84 (m, 5H), 1.68 (m, 7H), 1.43 (m, 4H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.5, 170.8, 170.7, 164.6 136.7, 134.8, 134.7, 134.6, 132.2, 132.1, 132.1, 124.8, 117.2, 117.0, 115.6, 115.4, 81.2, 74.4, 73.1, 72.0, 71.3, 69.8, 67.8, 66.0, 53.9, 52.3, 44.1, 37.8, 35.1, 32.5, 29.7, 29.7, 29.1, 26.3, 25.7, 25.3, 20.6, 19.8, 19.7, 18.8, 18.8, 18.6, 17.8, 12.6; LRMS: (ES+) m/z =693.3 (M+Na).



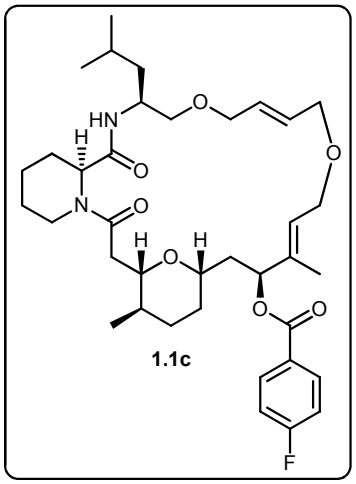
To a solution of **6.3(a-d)** (1eq) in dry DCM under nitrogen atmosphere added Grubbs' 2nd generation catalyst (0.1 eq) and reaction mixture was allowed to stirred for 2 h at room temperature. Then reaction mixture was concentrated after starting material disappeared monitoring with TLC and the crude product was purified by flash column chromatography over silica gel (30% EtOAc/hexane) afforded the product **1.1(a-d)** with trans-olefin geometry.



Molecular Formula: $C_{40}H_{52}N_2O_7$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 75%; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ ppm: 8.02 (m, 2H), 7.53 (m, 1H), 7.40 (t, *J* = 7.68 Hz, 2H), 7.23 (m, 5H), 6.65 (d, *J* = 8.80 Hz, 1H), 5.90 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.80 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.64 (m, 1H), 5.26 (m, 1H), 4.28 (m, 2H), 4.12 (m, 5H), 3.85 (m, 2H), 3.48 (d, *J* = 3.08 Hz, 2H), 2.88 (d, *J* = 8.23 Hz, 1H), 2.68 (m, 1H), 2.47 (d, *J* = 3.58 Hz, 2H), 2.28 (m, 1H), 1.98 (m, 3H), 1.73 (d, *J* = 5.26 Hz, 4H), 1.58 (m, 9H), 0.86 (m, 5H); ^{13}C NMR (100 MHz, *CDCl*$_3$): 171.2, 170.5, 165.2, 138.5, 135.8, 132.7, 130.7, 130.0, 129.5, 129.3, 129.0, 128.3, 128.2, 126.2, 125.9, 76.3, 73.0, 70.7, 70.4, 69.3, 66.6, 65.3, 52.4, 50.2, 44.0, 37.7, 32.9, 31.9, 29.7, 29.4, 26.2, 25.6, 24.7, 22.7, 20.6, 16.3, 14.1, 12.5; LRMS: (ES+) m/z =695.2 (M+Na).

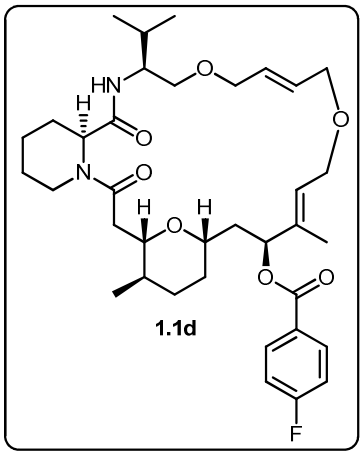


Molecular Formula: $C_{37}H_{54}N_2O_7$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 60%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 8.01 (d, *J* = 7.66 Hz, 2H), 7.53 (s, 1H), 7.42 (d, *J* = 7.81 Hz, 2H), 6.63 (d, *J* = 9.2 Hz, 1H), 5.92 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.77 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.62 (t, *J* = 6.4 Hz, 1H), 5.35 (m, 1H), 4.28 (m, 1H), 4.08 (m, 5H), 3.84 (m, 3H), 3.73 (m, 1H), 3.62 (m, 1H), 3.43 (m, 1H), 3.05 (m, 1H), 2.75 (m, 1H), 2.53 (m, 1H), 2.35 (m, 2H), 2.00 (m, 4H), 1.71 (m, 8H), 1.61 (m, 7.39 Hz, 5H), 1.10 (m, 1H), 0.89 (dd, *J* = 11.72, 6.44 Hz, 10H); ^{13}C NMR (100 MHz, *CDCl$_3$*): 171.2, 170.6, 165.2, 135.4, 132.7, 130.7, 129.7, 129.5, 128.8, 128.3, 126.3, 76.4, 73.4, 70.7, 69.6, 69.2, 66.3, 65.3, 53.1, 52.7, 44.5, 38.2, 35.7, 33.1, 32.4, 31.9, 29.7, 29.4, 26.1, 25.9, 24.8, 22.7, 20.8, 16.6, 15.7, 14.1, 12.3, 11.2; LRMS: (ES+) m/z =661.3 (M+Na).



Molecular Formula: $C_{37}H_{53}FN_2O_7$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 58%; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ ppm: 8.08 (dd, *J* = 8.77, 5.50 Hz, 2H), 7.15 (dd, *J* = 17.73, 9.02 Hz, 2H), 6.55 (d, *J* = 8.80 Hz, 1H), 5.95 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.84 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.68 (m, 1H), 5.40

(s, 1H), 4.36 (m, 1H), 4.15 (m, 5H), 3.96 (m, 1H), 3.82 (m, 1H), 3.53 (d, *J* = 3.28 Hz, 2H), 3.08 (m, 1H), 2.78 (m, 1H), 2.62 (m, 1H), 2.40 (m, 2H), 2.10 (m, 4H), 1.76 (m, 7H), 1.60 (m, 4H), 1.43 (m, 5H), 0.95 (m, 10H); ^{13}C NMR (100 MHz, *$CDCl_3$*): 171.1, 170.4, 164.3, 135.2, 132.7, 132.1, 132.0, 129.6, 128.9, 126.6, 115.5, 115.3, 73.3, 71.7, 70.8, 69.3, 66.4, 65.4, 52.8, 47.5, 44.4, 40.7, 38.1, 33.0, 31.9, 29.7, 26.2, 25.8, 25.2, 24.8, 23.0, 22.3, 20.8, 16.6, 14.1, 12.2; LRMS: (ES+) m/z =679.3 (M+Na).



1.1d

Molecular Formula: $C_{36}H_{51}FN_2O_7$; R_f (solvent system): 0.2 (30%, EtOAc/hexane); Yield: 60%; ^{1}H NMR (400 MHz, *$CDCl_3$*) δ ppm: 8.02 (dd, *J* = 8.81, 5.42 Hz, 2H), 7.08 (t, *J* = 8.66 Hz, 2H), 6.62 (d, *J* = 9.20 Hz, 1H), 5.88 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.78 (dt, *J* = 15.6, 5.2 Hz, 1H), 5.61 (m, 1H), 5.34 (m, 1H), 4.26 (m, 1H), 4.08 (m, 4H), 3.90 (m, 1H), 3.76 (m, 2H), 3.60 (m, 1H), 3.42 (m, 1H), 3.05 (m, 1H), 2.74 (m, 1H), 2.54 (m, 1H), 2.35 (m, 1H), 2.01 (m, 4H), 1.66 (m, 11H), 1.41 (m, 3H), 0.88 (m, 10H); ^{13}C NMR (100 MHz, *$CDCl_3$*): 171.2, 170.7, 166.8, 164.2, 135.2, 132.1, 132.0, 129.6, 128.8, 127.0, 126.6, 115.5, 115.3, 73.5, 70.7, 69.7, 69.2, 66.2, 65.3, 54.5, 52.7, 44.5, 38.4, 33.1, 32.3, 29.7, 29.5, 26.1, 25.8, 24.8, 22.7, 20.8, 19.8, 16.7, 14.1, 12.2; LRMS: (ES+) m/z =665.4 (M+Na).

4.5. References:

(1) (a) Paterson, I.; Goodman, J. M.; Anne Lister, M.; Schumann, R. C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663 (b) Paterson, I.; Lister, M. A.; McClure, C. K. *Tetrahedron Lett.* **1986**, *27*, 4787.

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(7) (a) Wadsworth, W. S. *Organic React.* **1977** (b) Wadsworth, W. S.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733.

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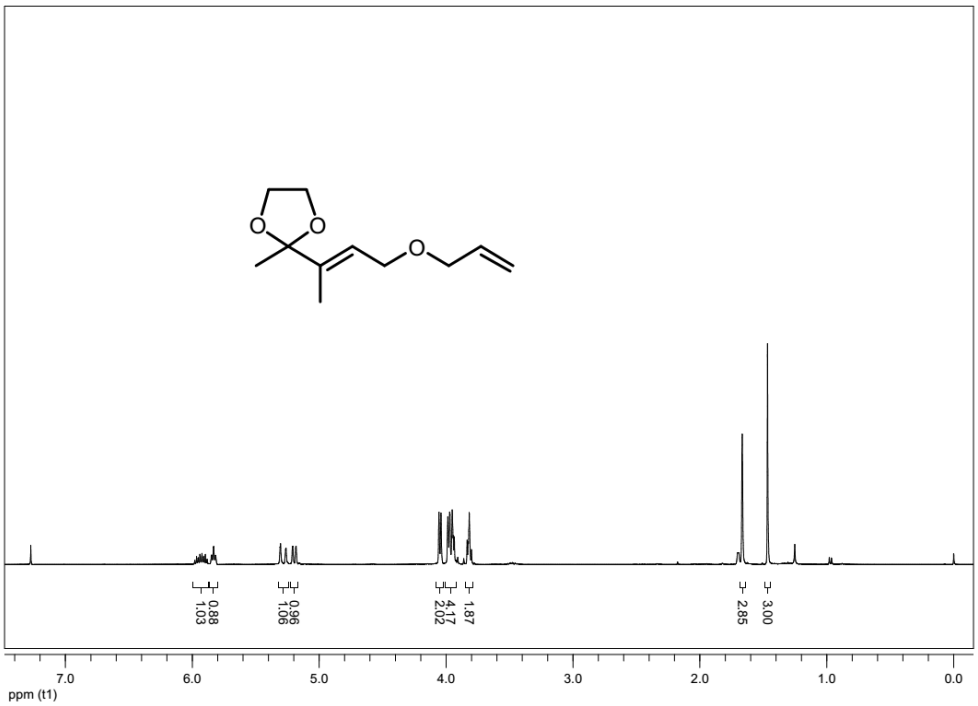
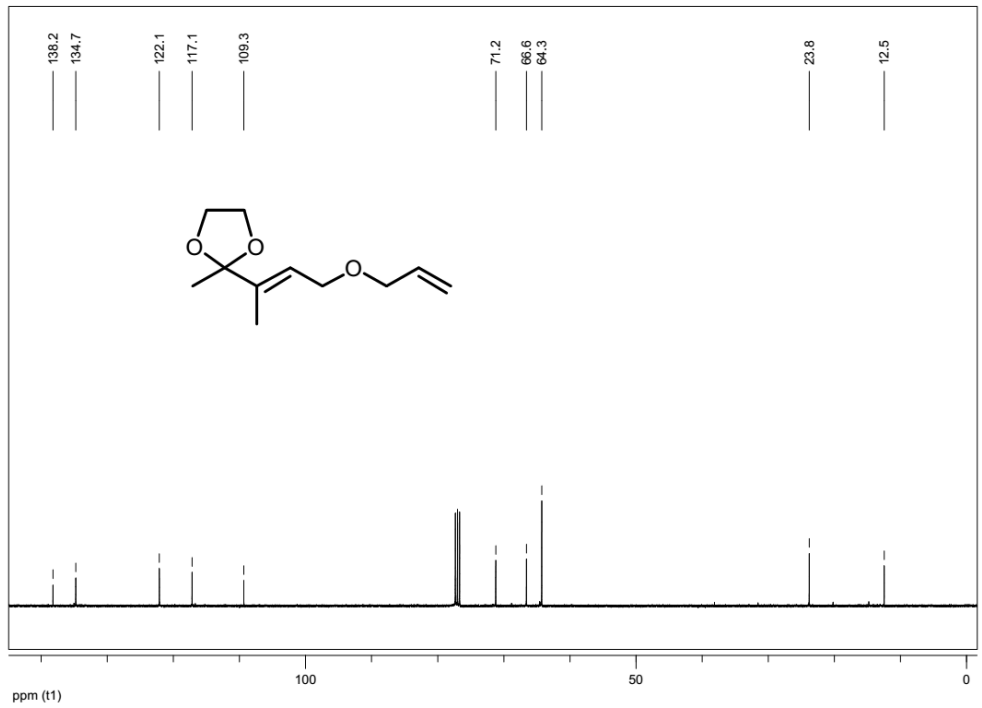
4.6. Spectral Data:

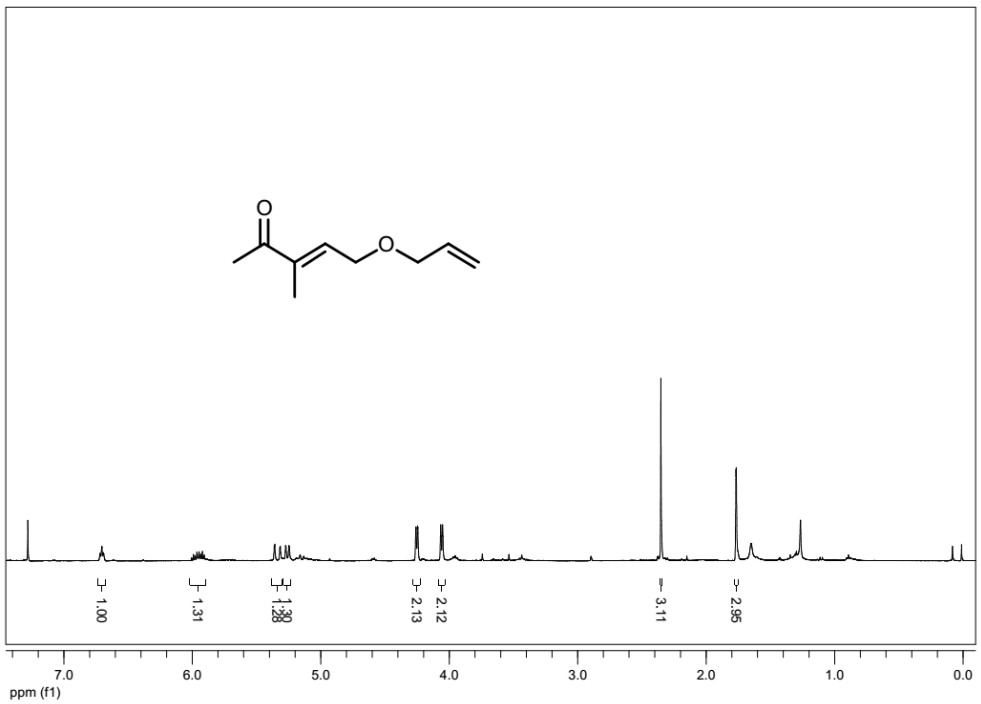
^{1}H NMR (400 MHz, $CDCl_3$):



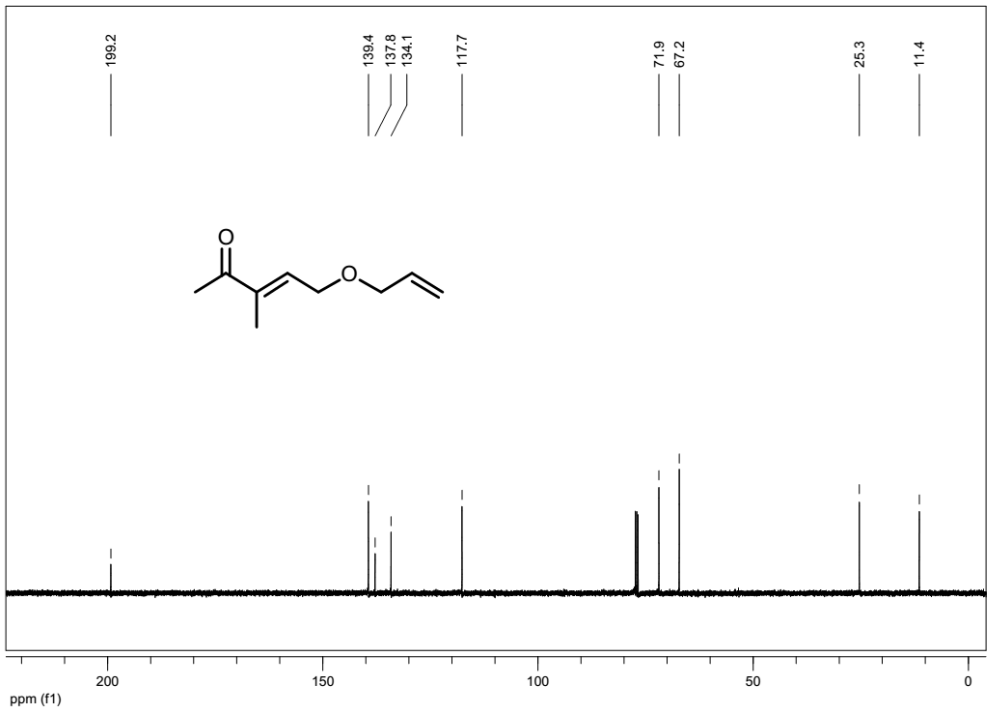
^{13}C NMR (100 MHz, $CDCl_3$):



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



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



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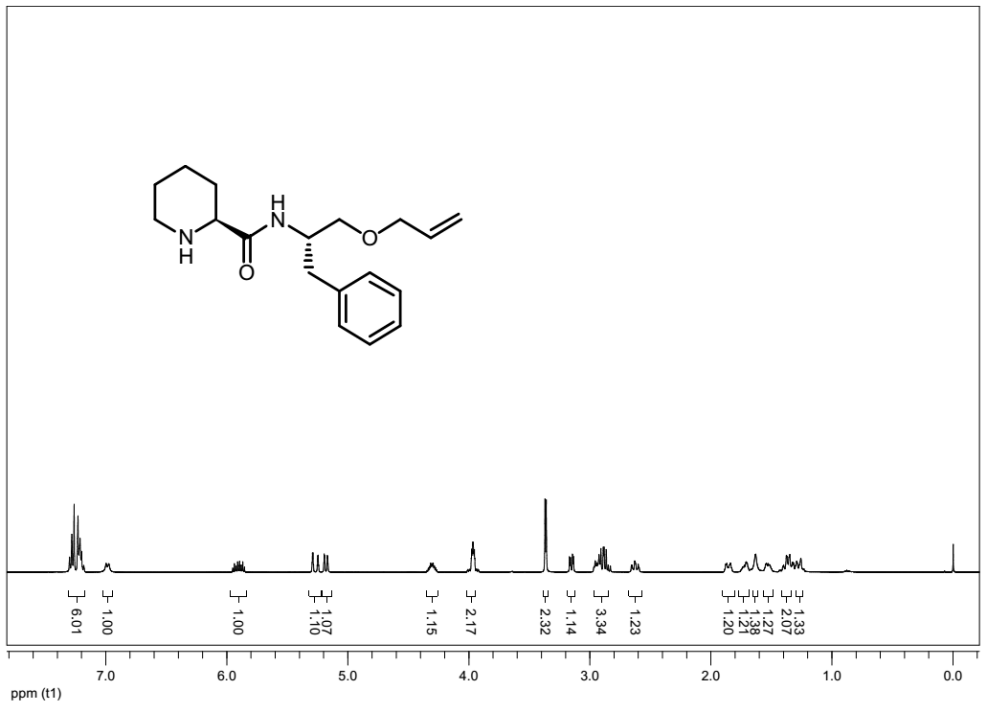

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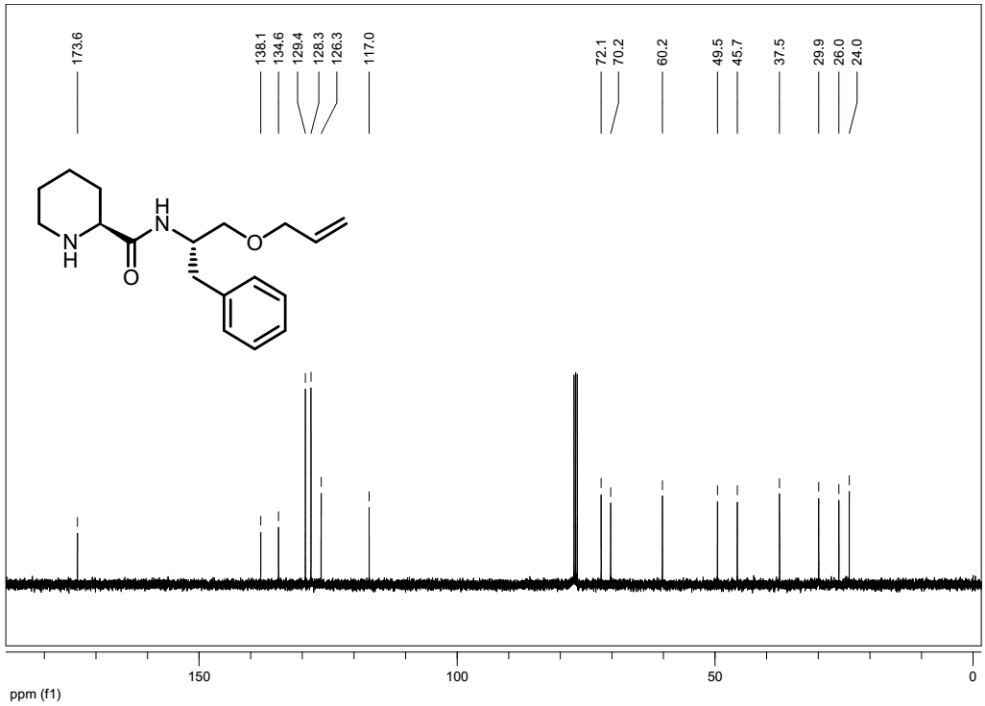
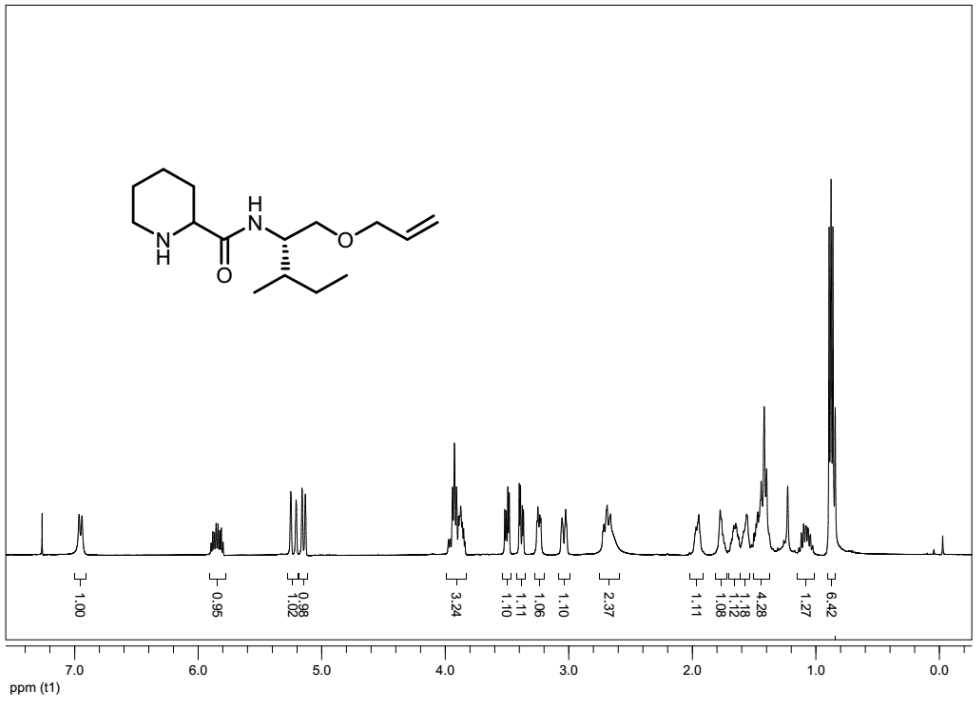
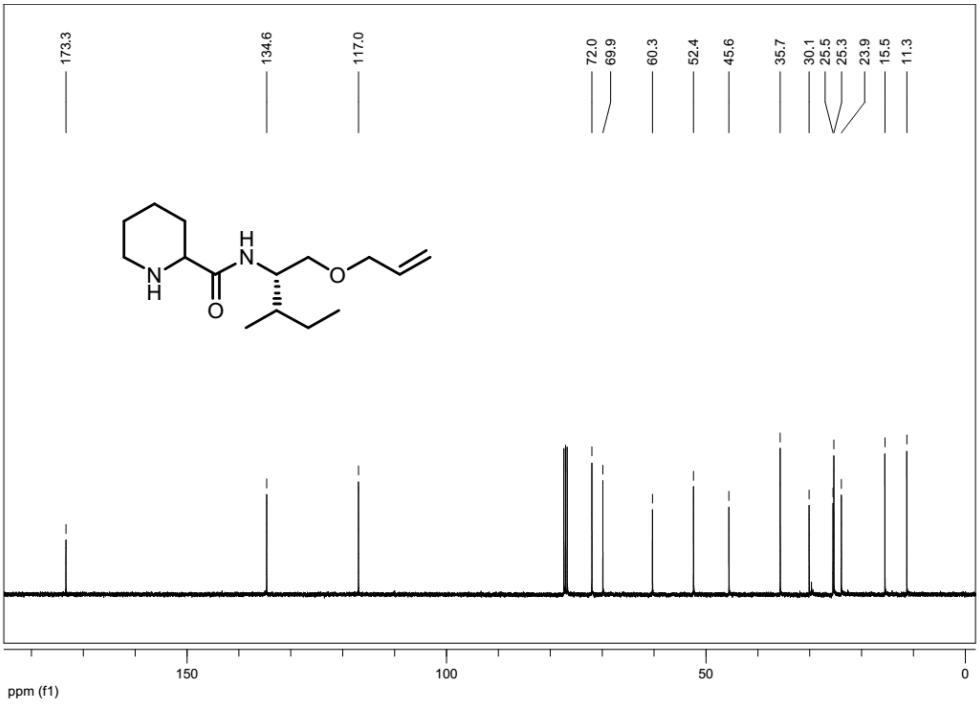


^{1}H NMR (400 MHz, $CDCl_3$):



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



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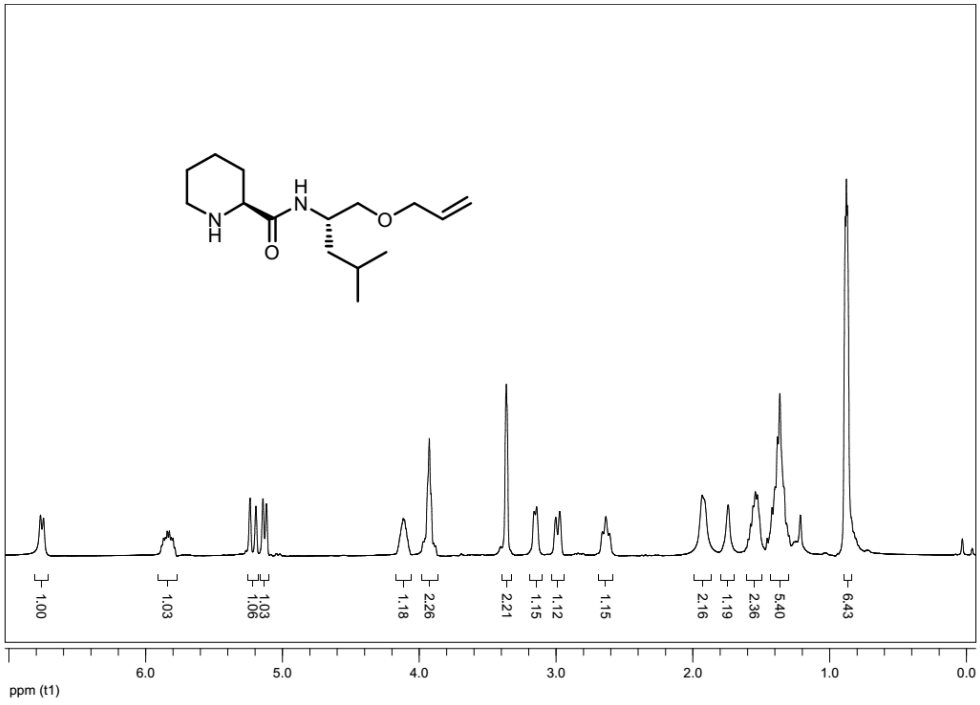

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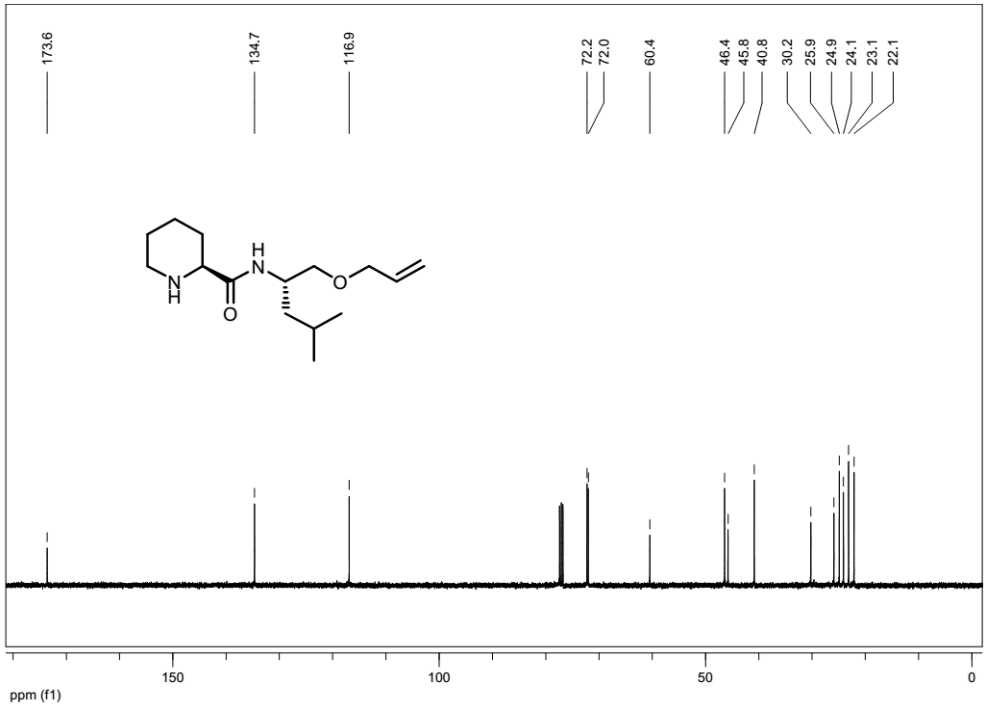

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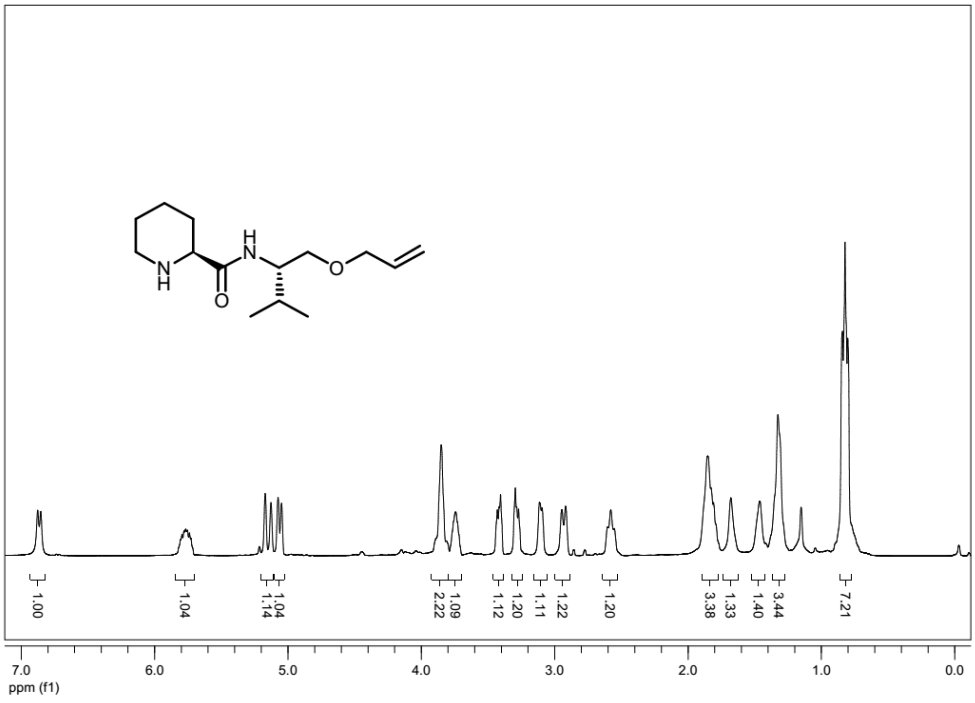
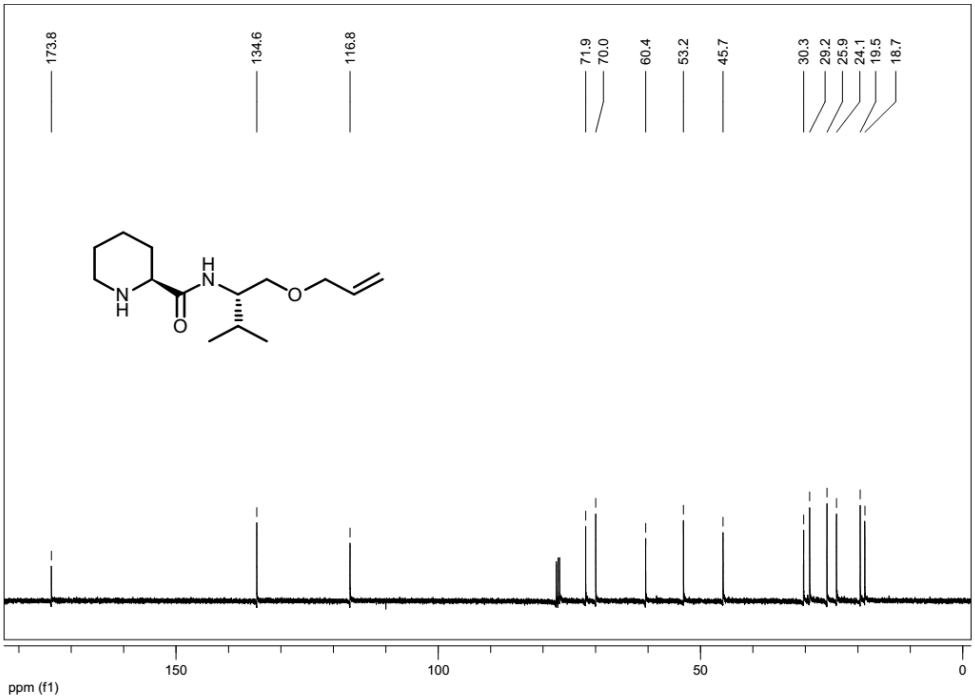
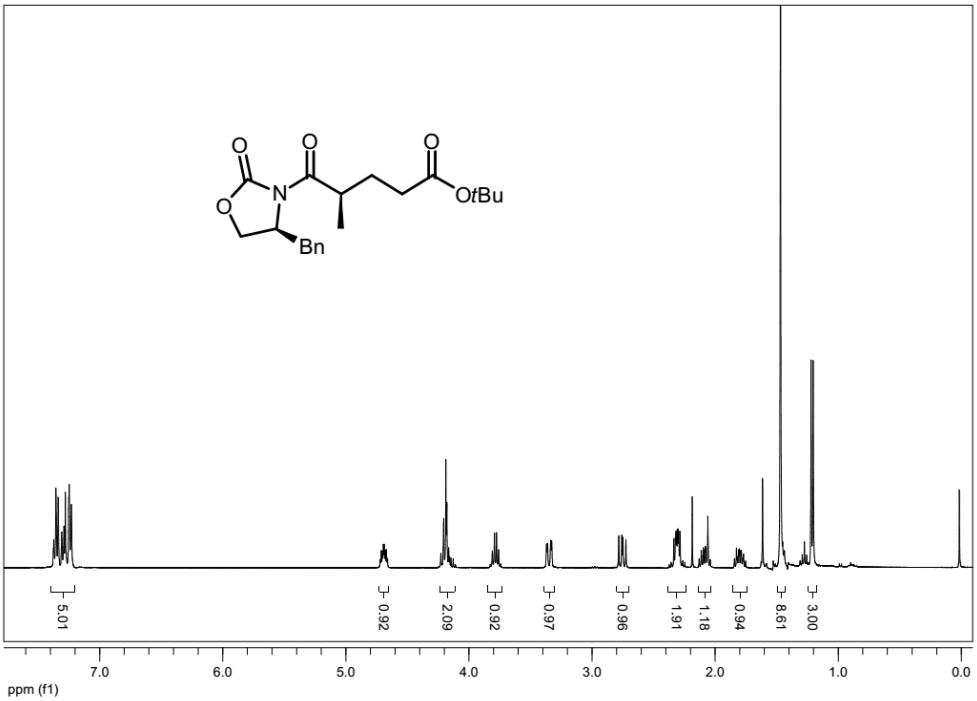


^{13}C NMR (100 MHz, *$CDCl_3$*):



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



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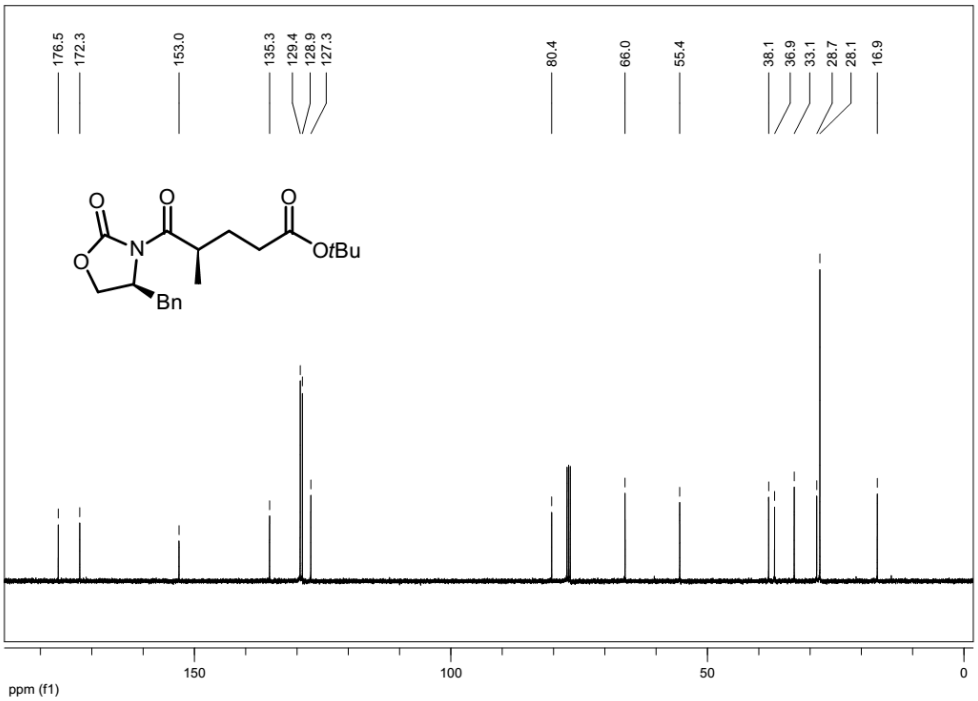

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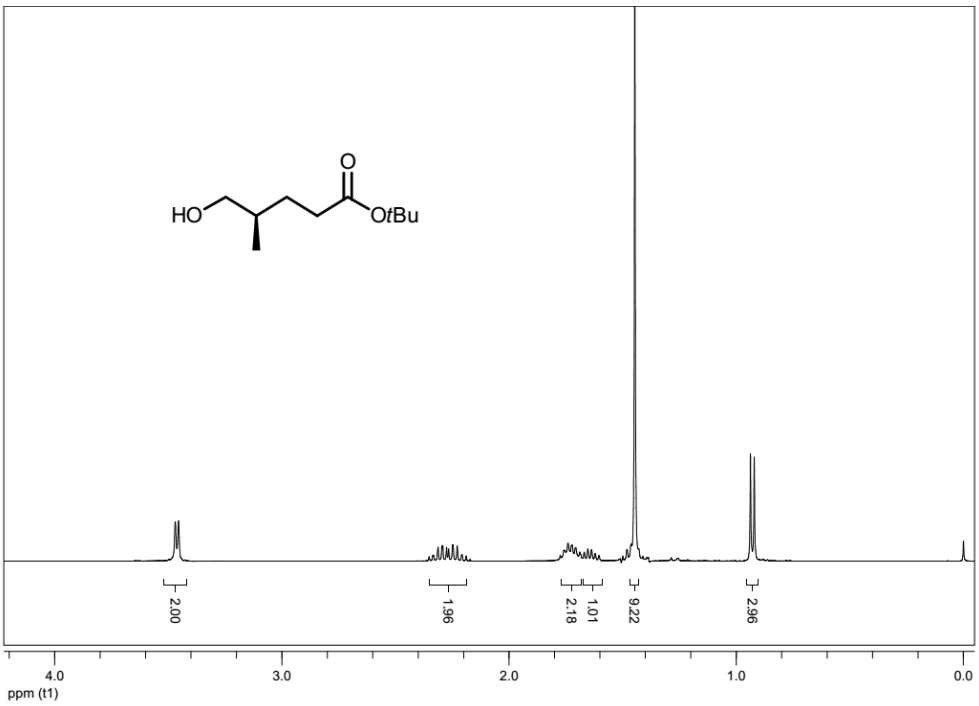

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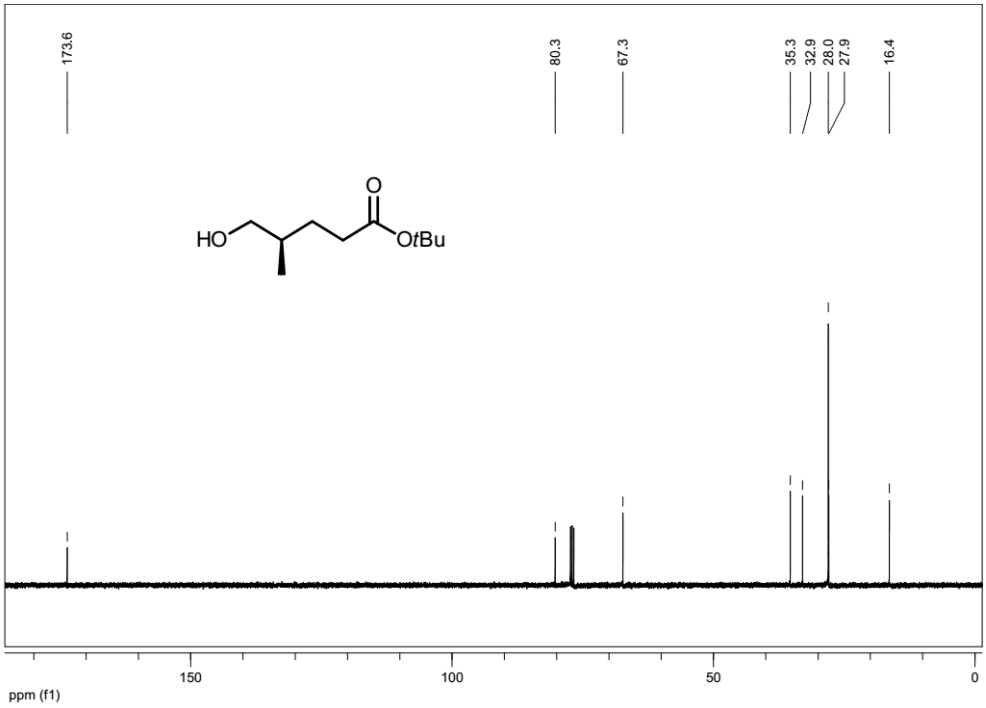

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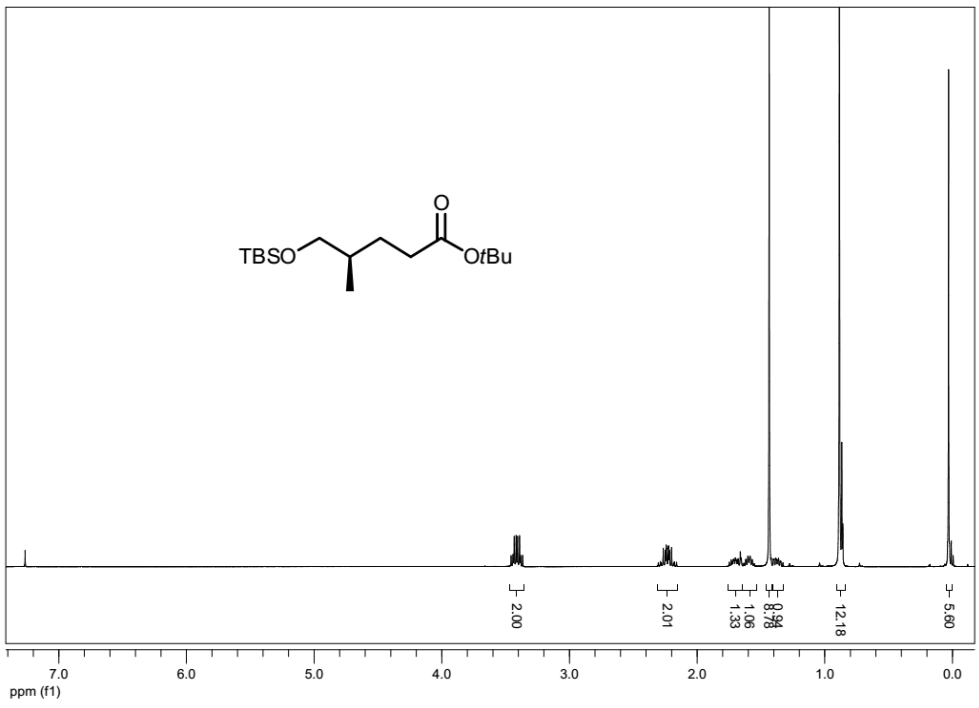
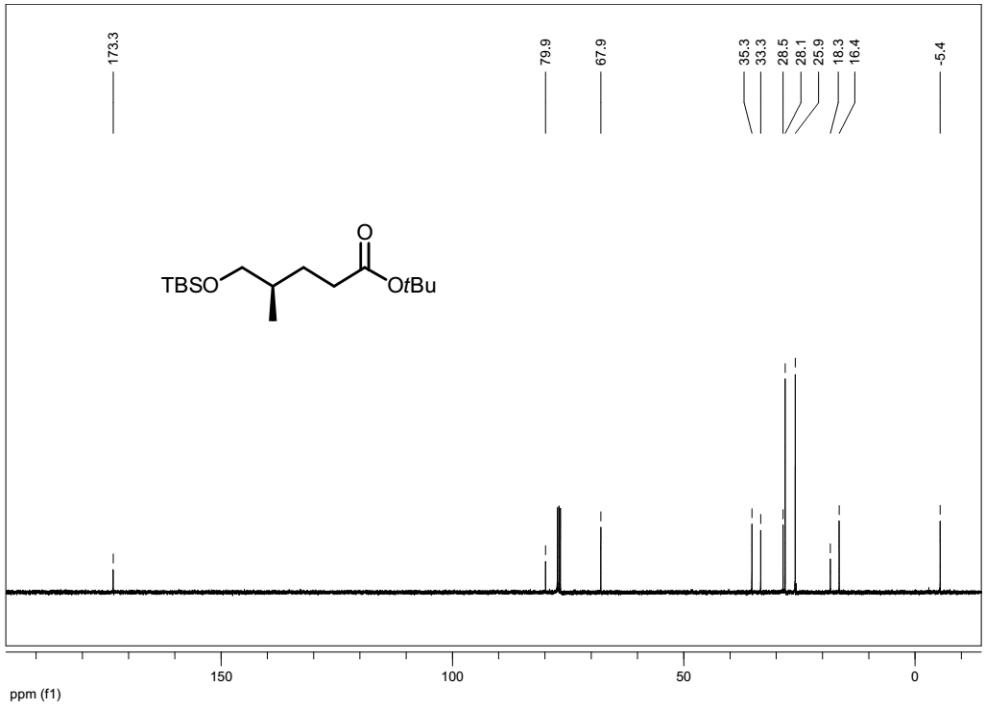
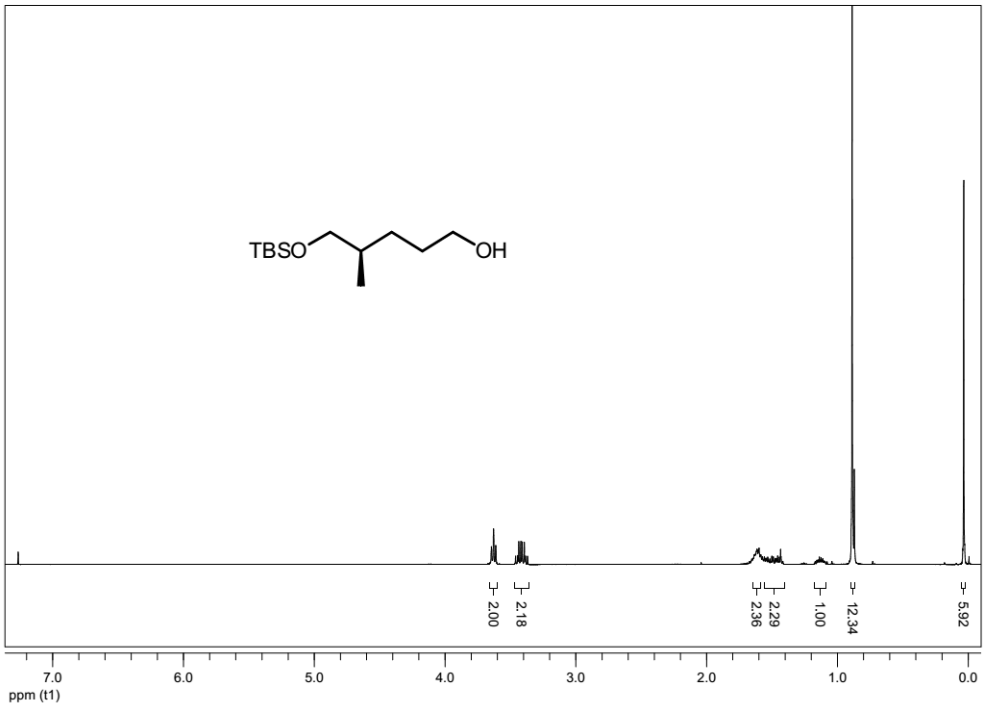


^{13}C NMR (100 MHz, $CDCl_3$):



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



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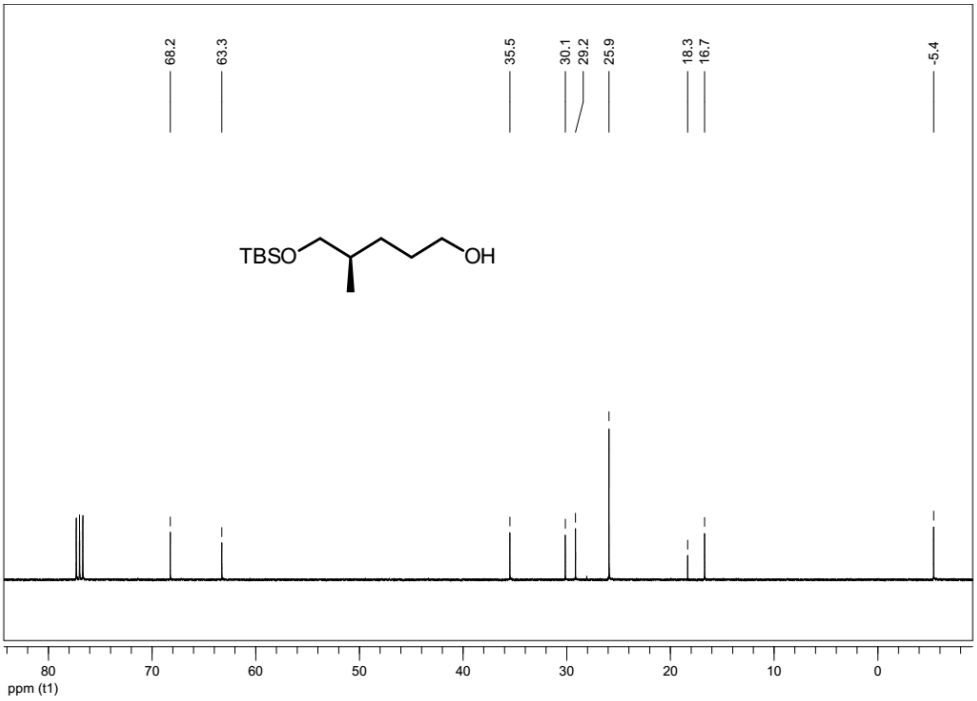

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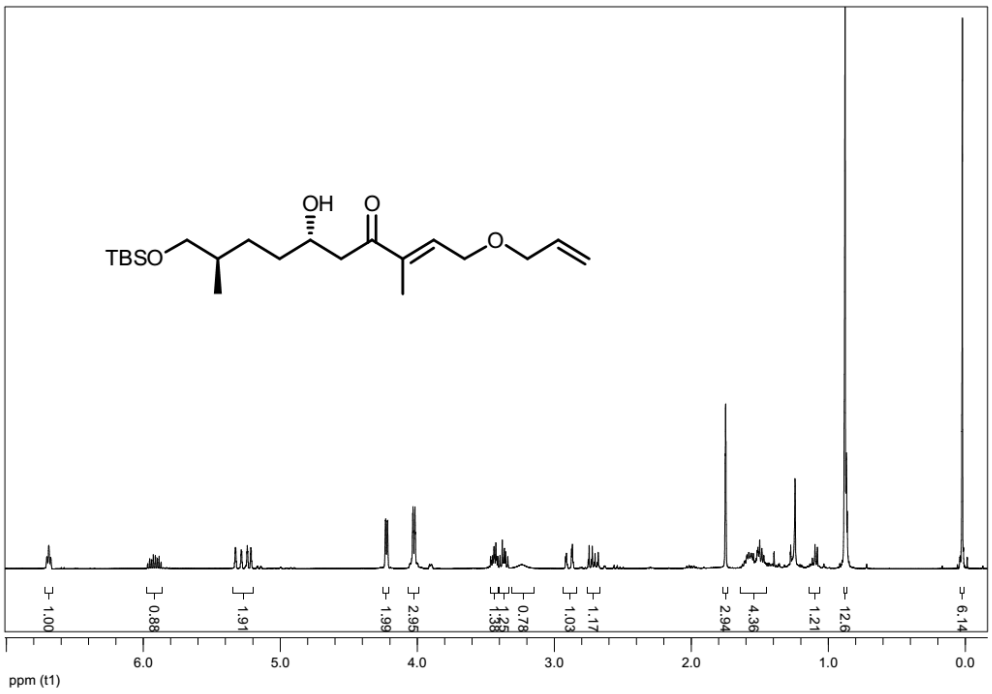

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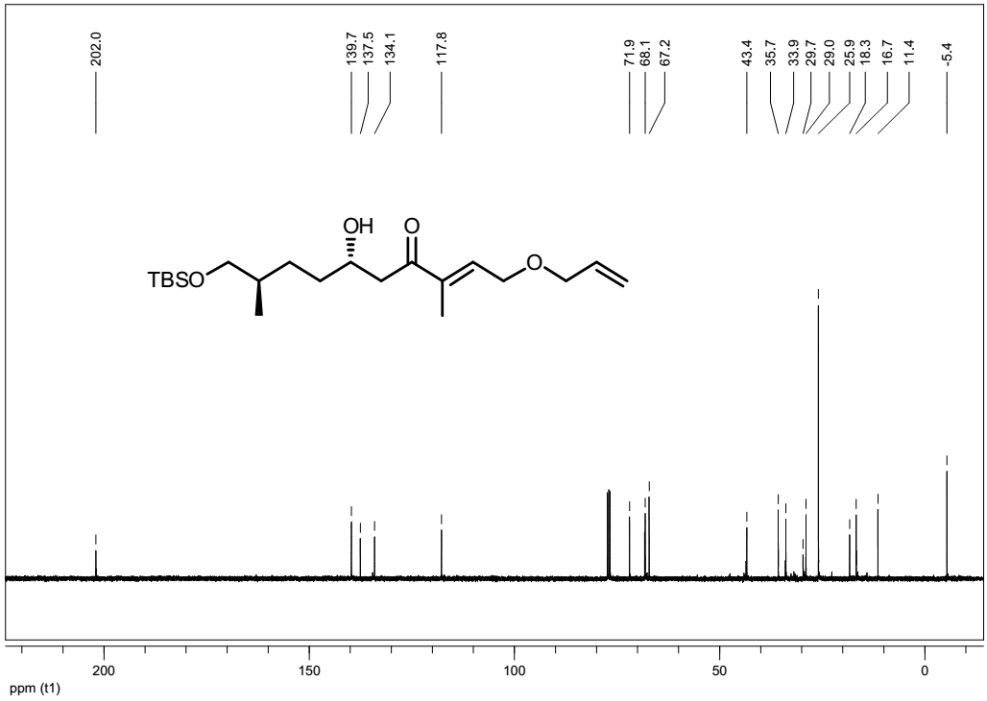

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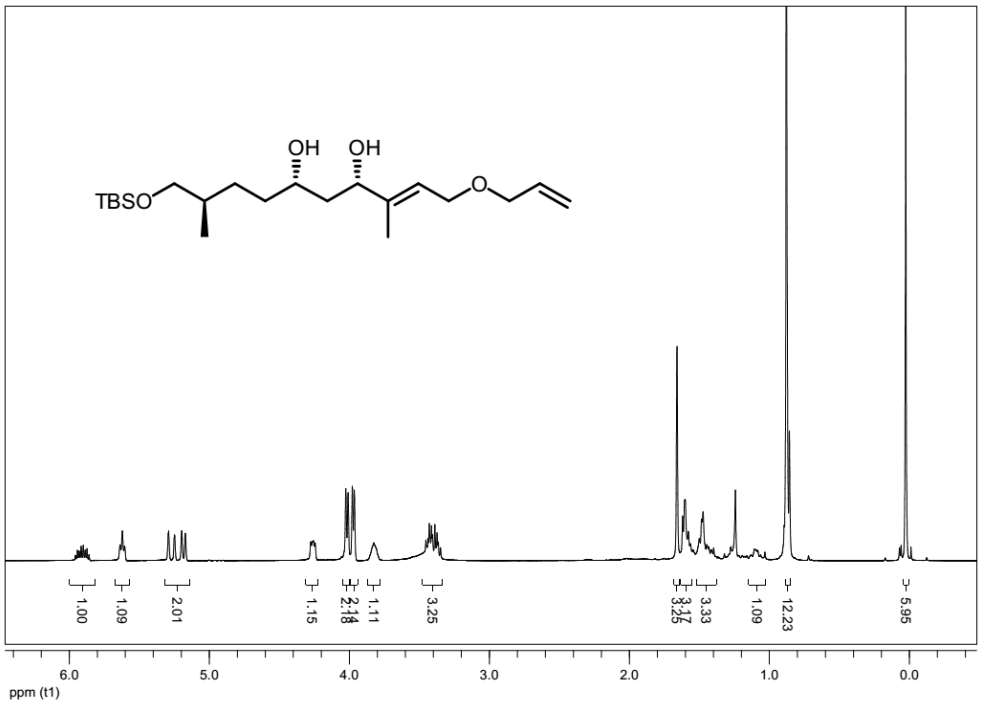

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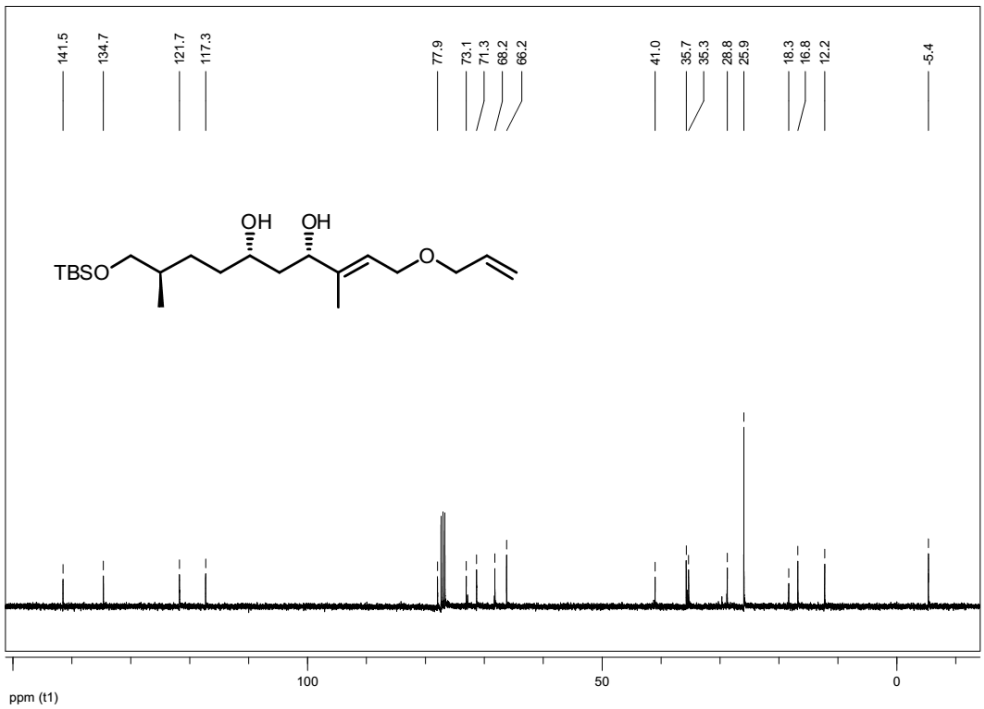

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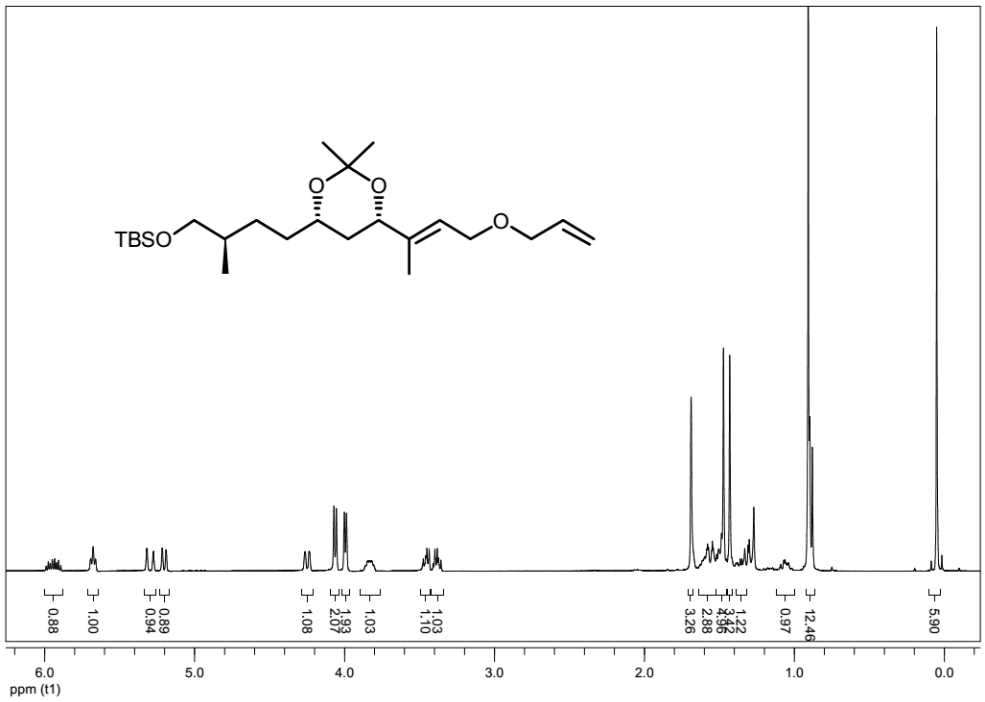

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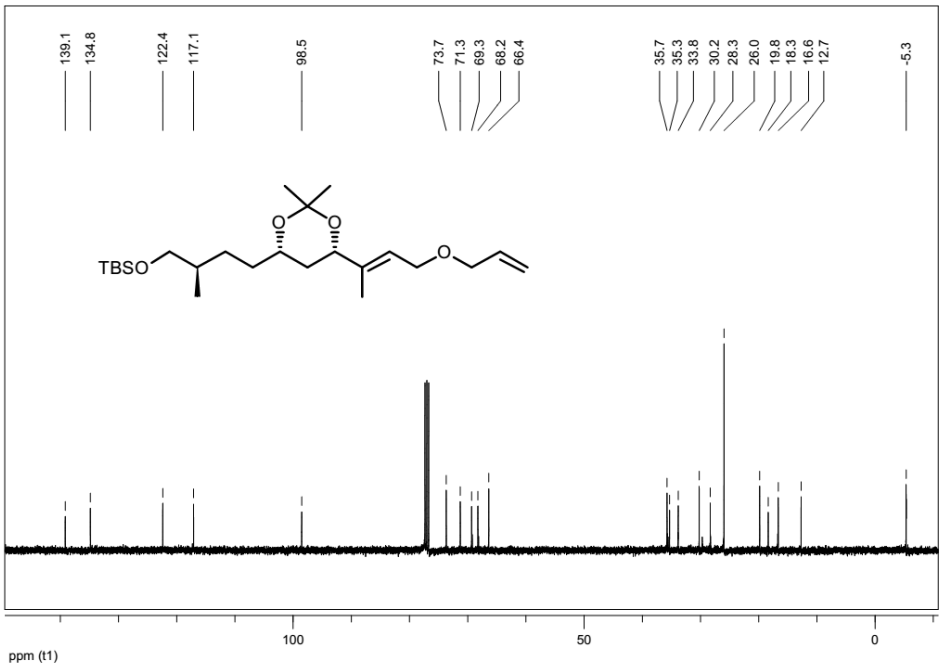

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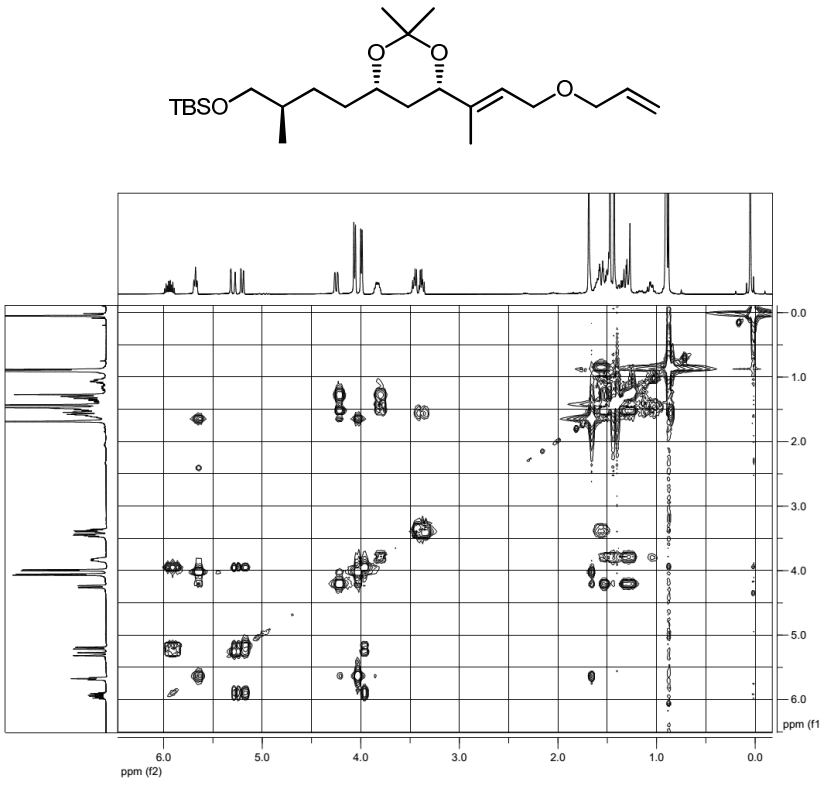

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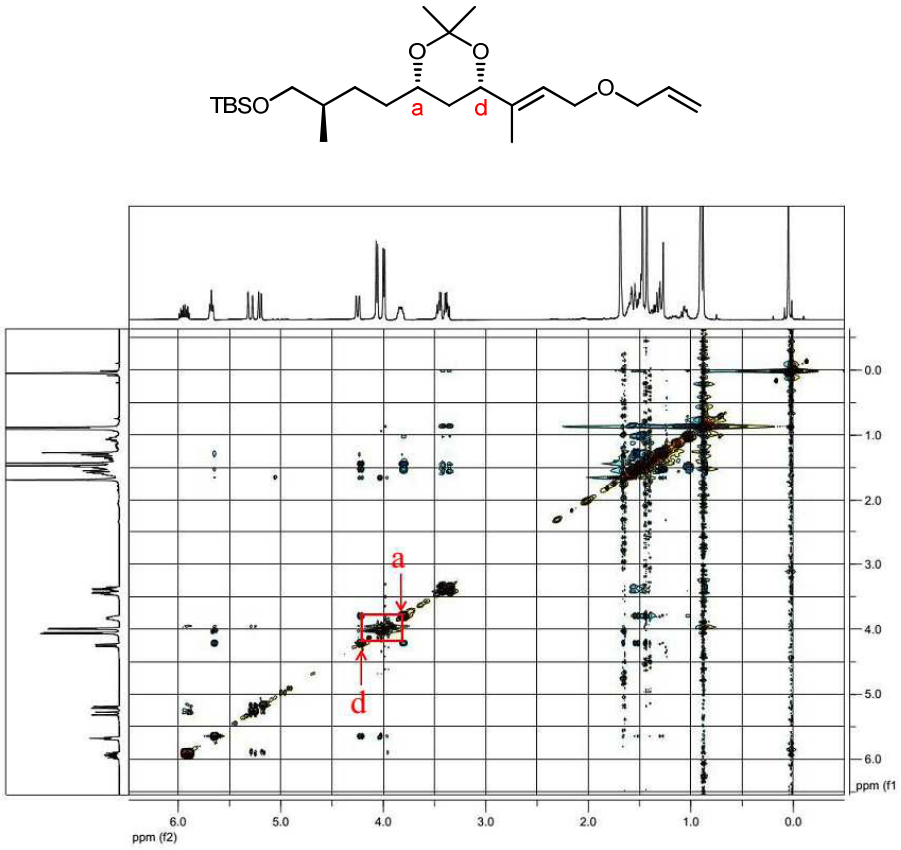
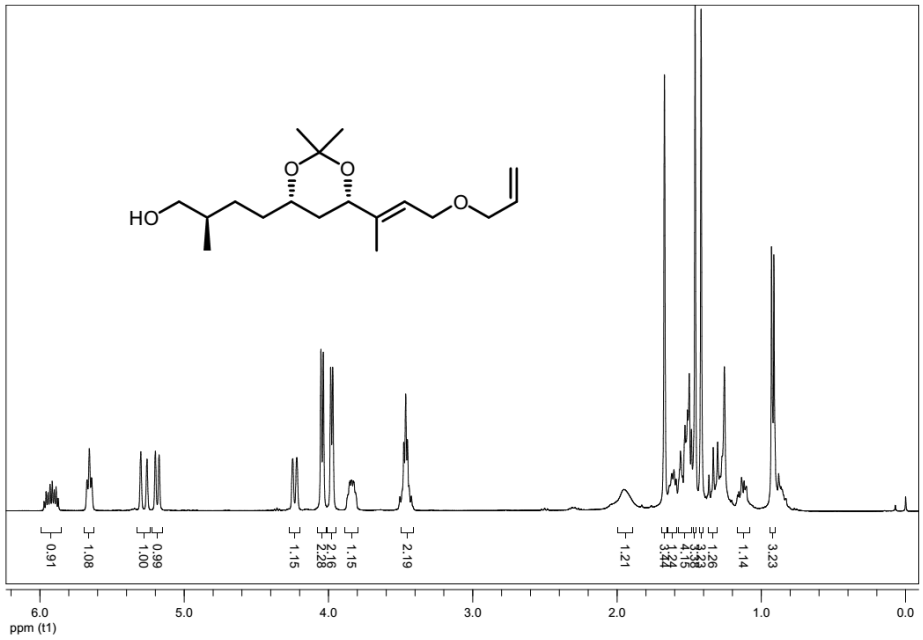


^{1}H NMR (400 MHz, *CDCl$_3$*):



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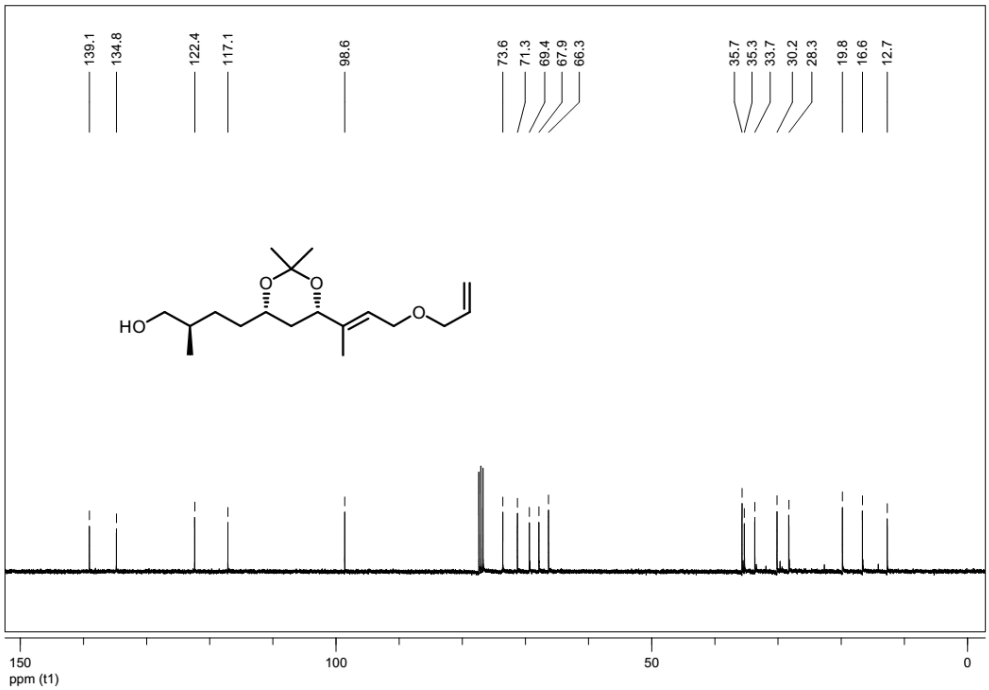

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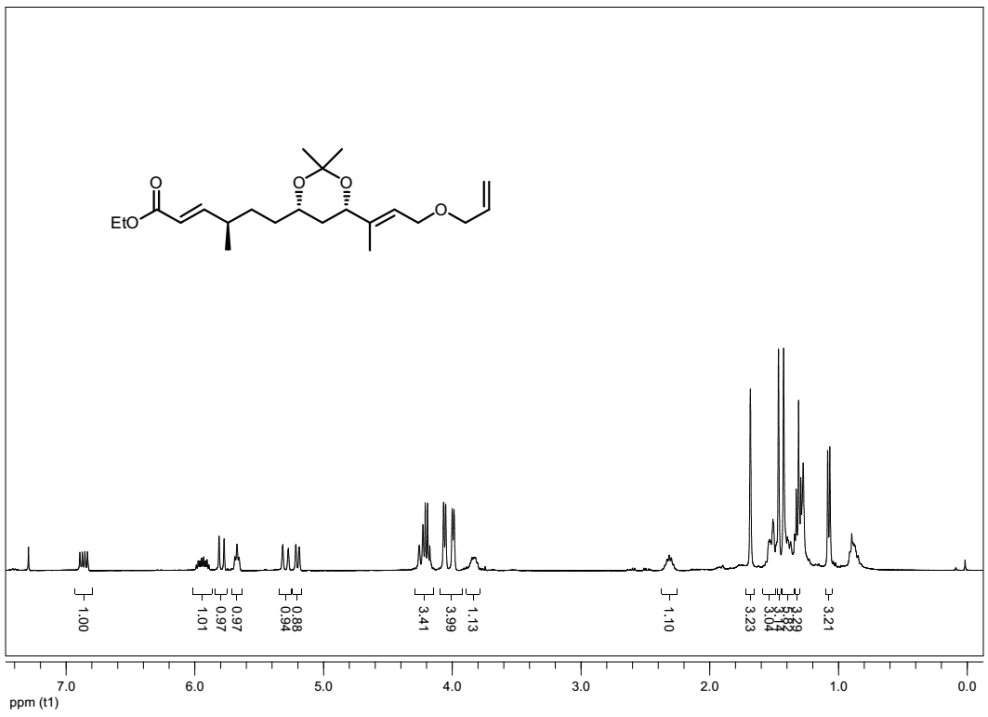
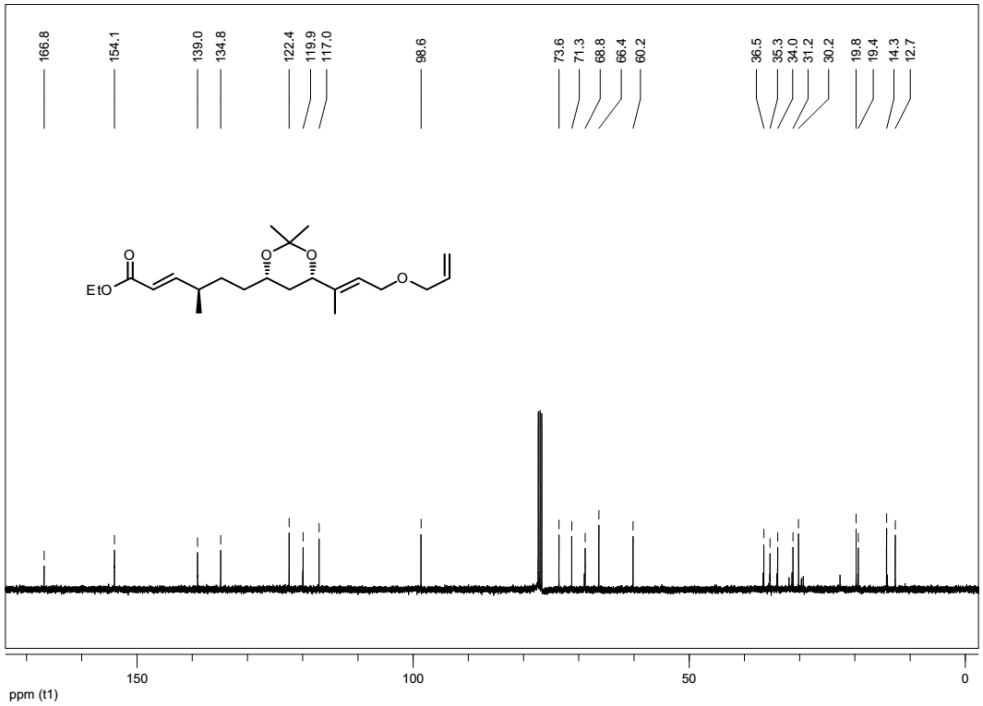


^{13}C NMR (100 MHz, *$CDCl_3$*):



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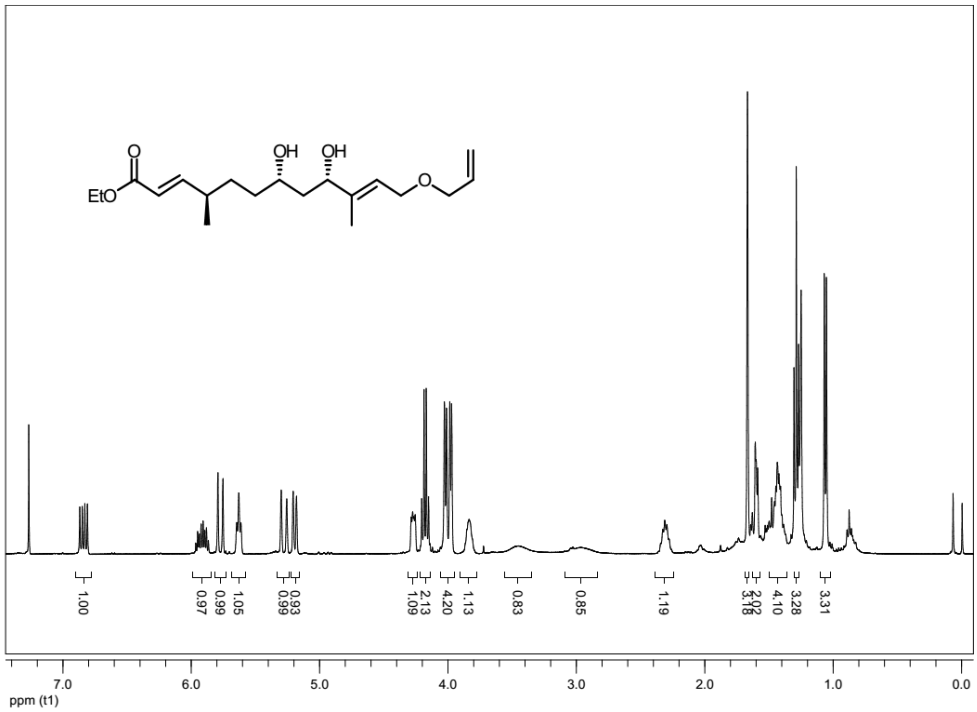

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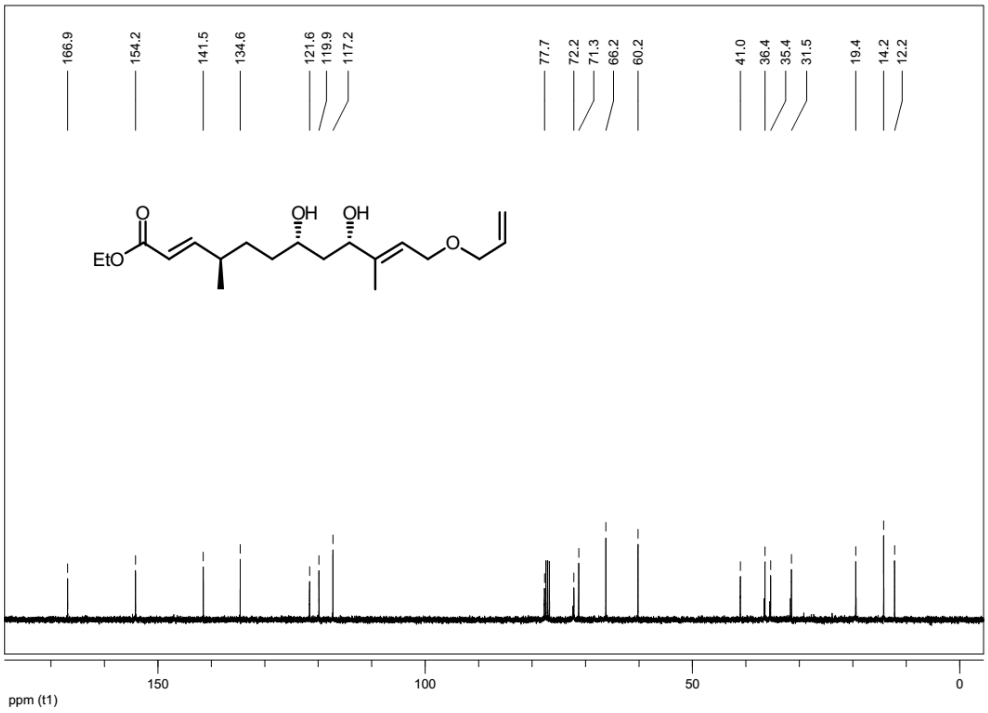

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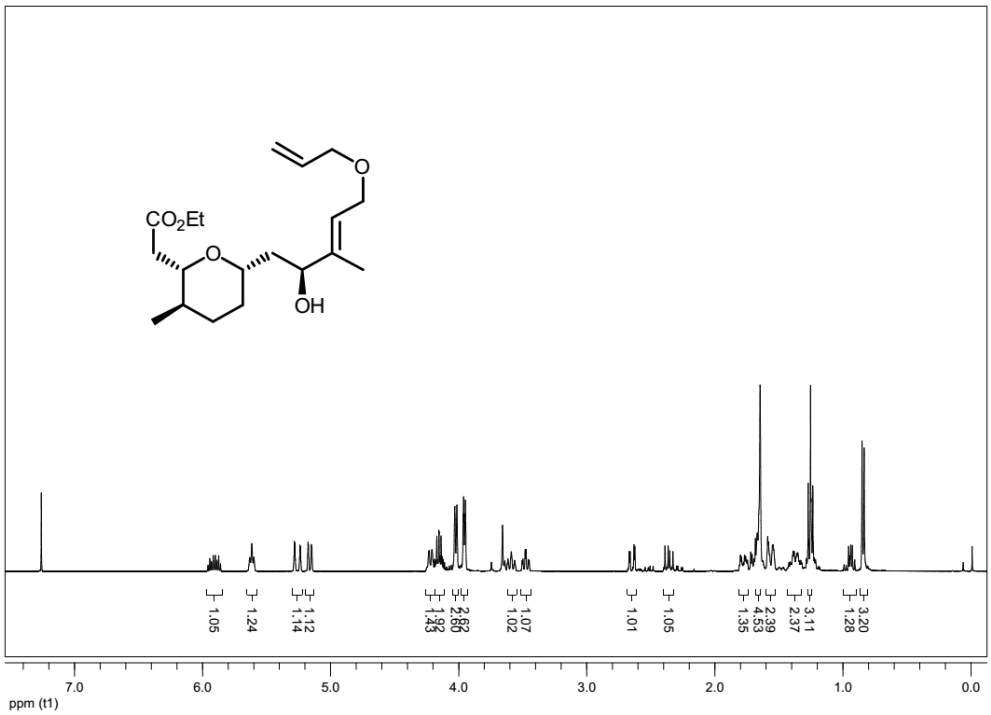
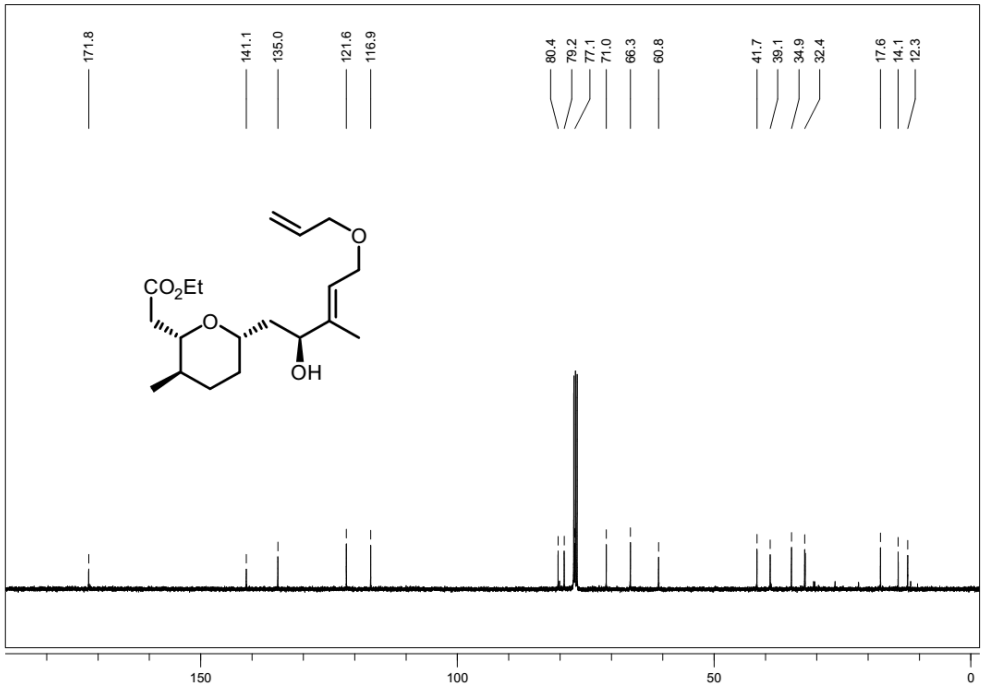


^{13}C NMR (100 MHz, *$CDCl_3$*):



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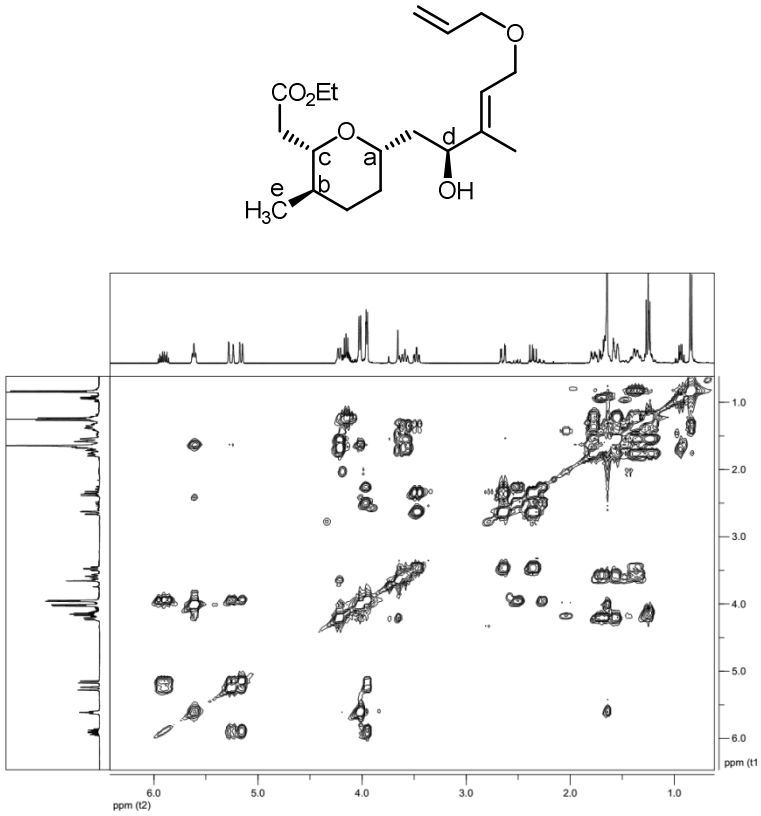

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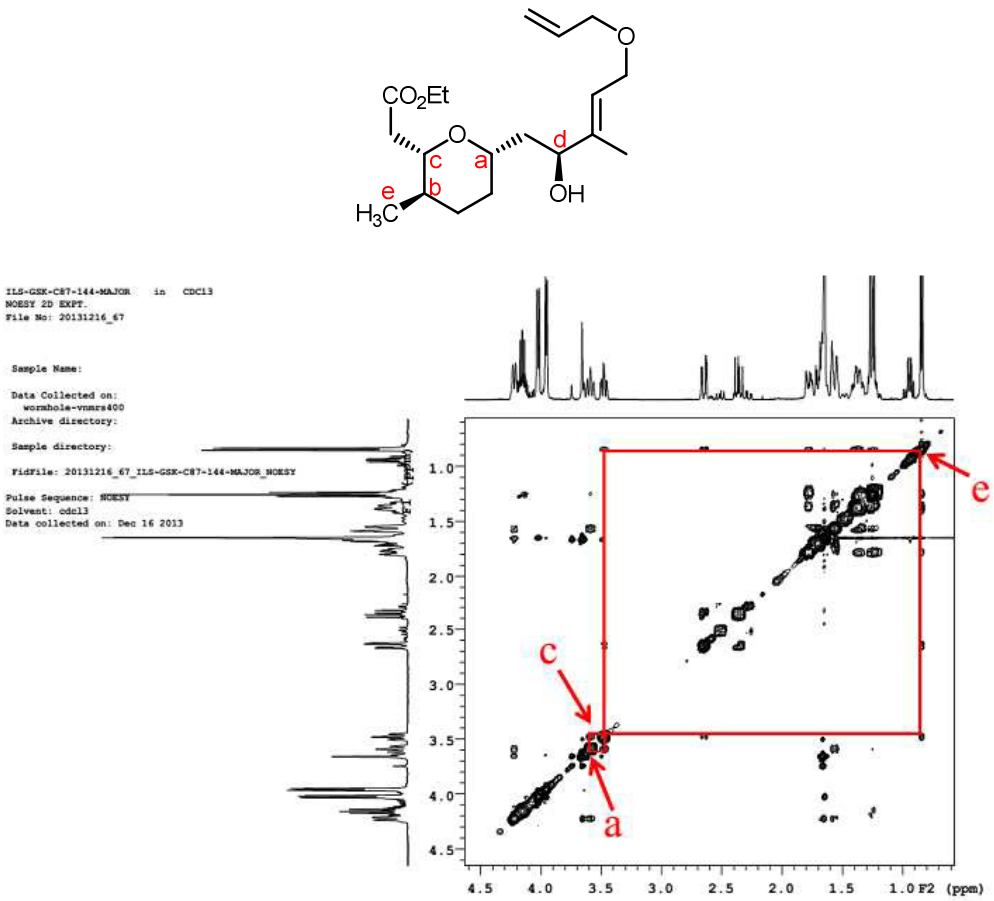
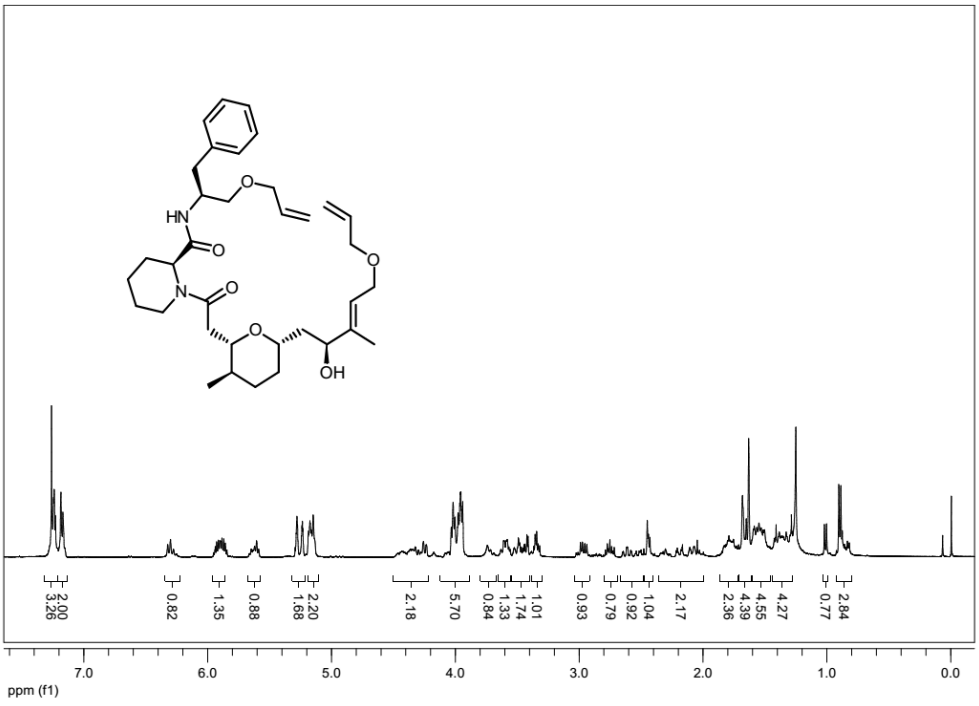


^{1}H NMR (400 MHz, *CDCl$_3$*):



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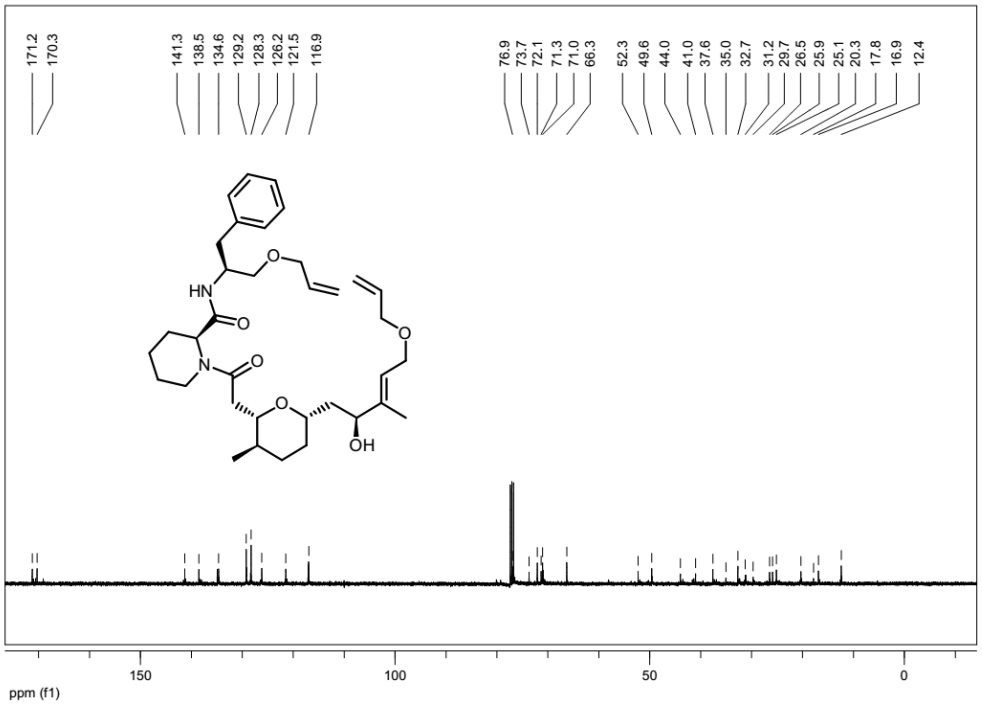

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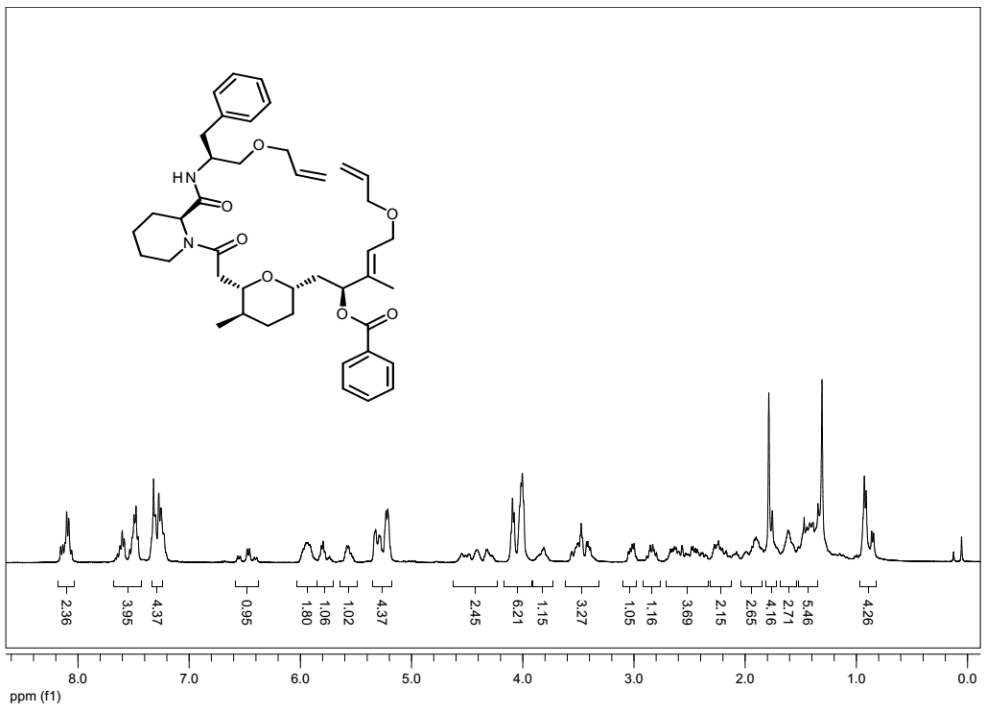

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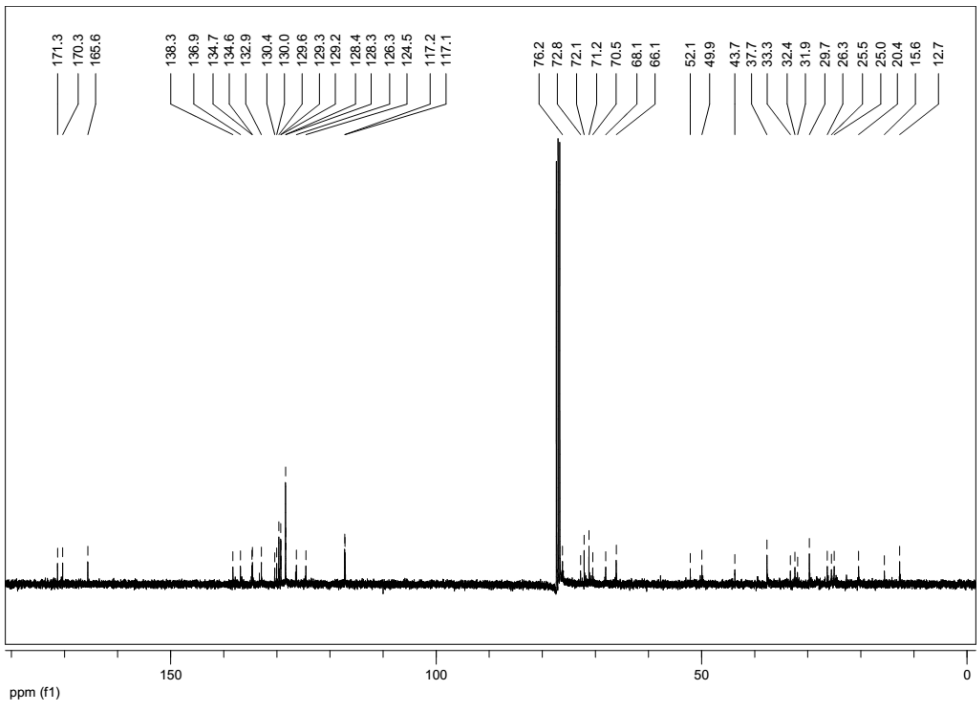

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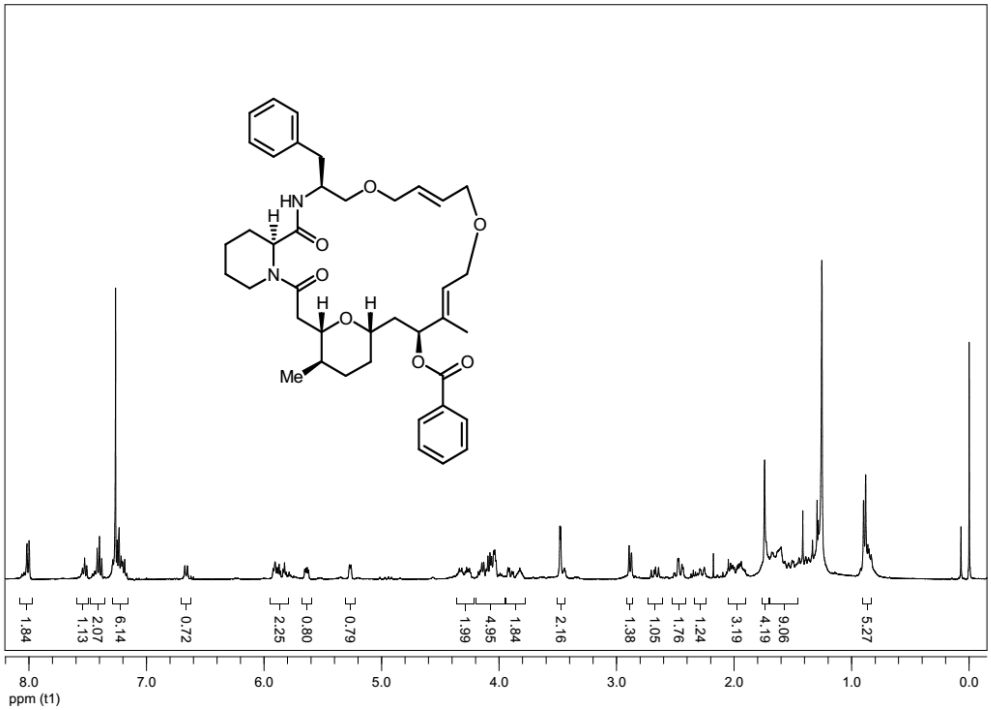

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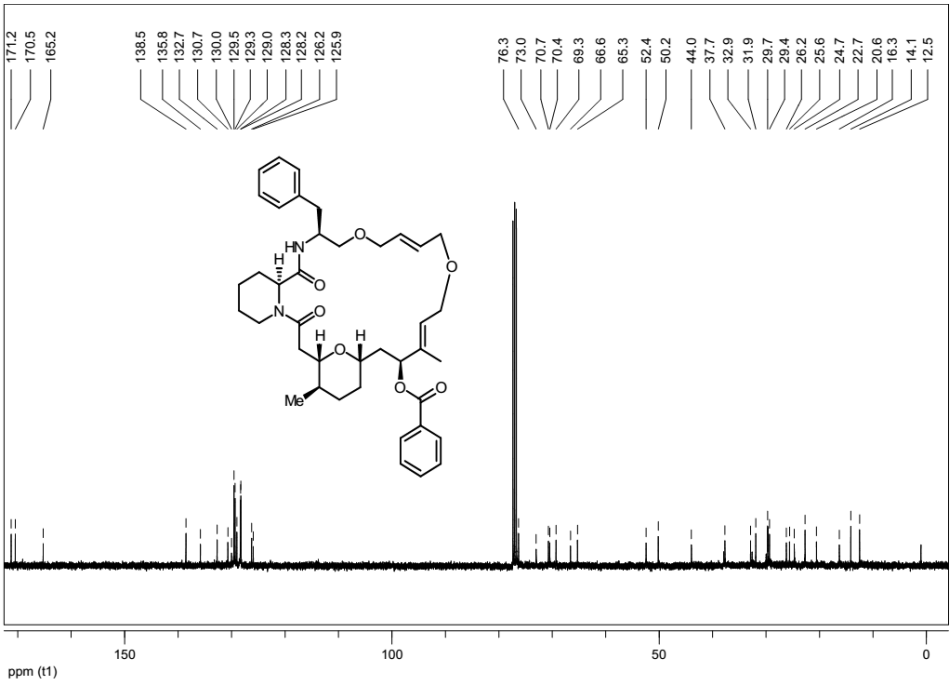

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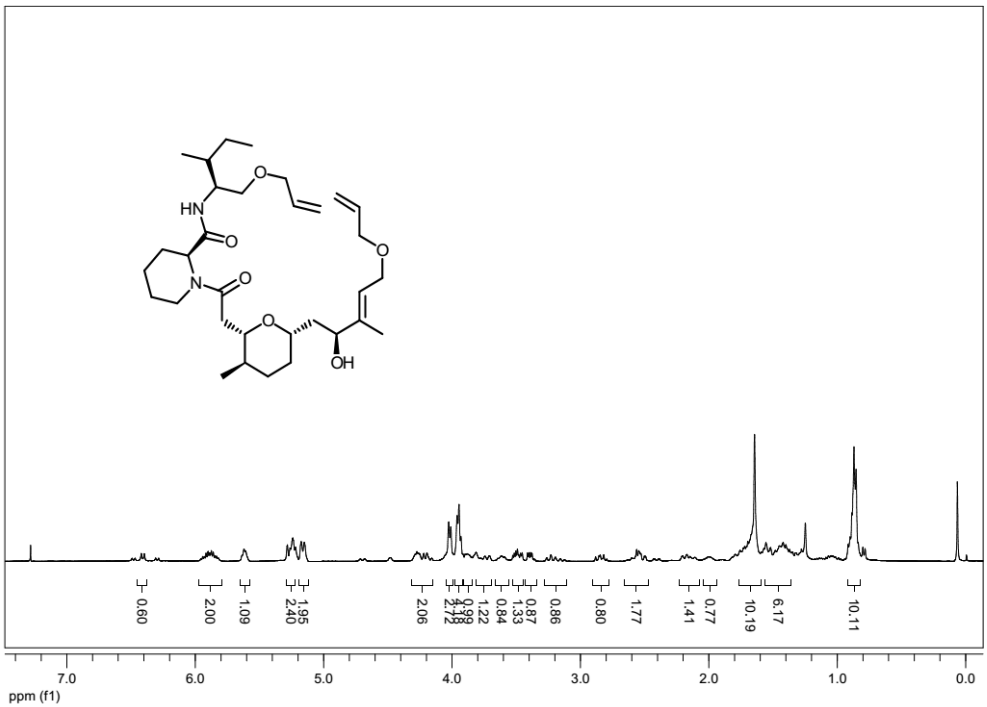

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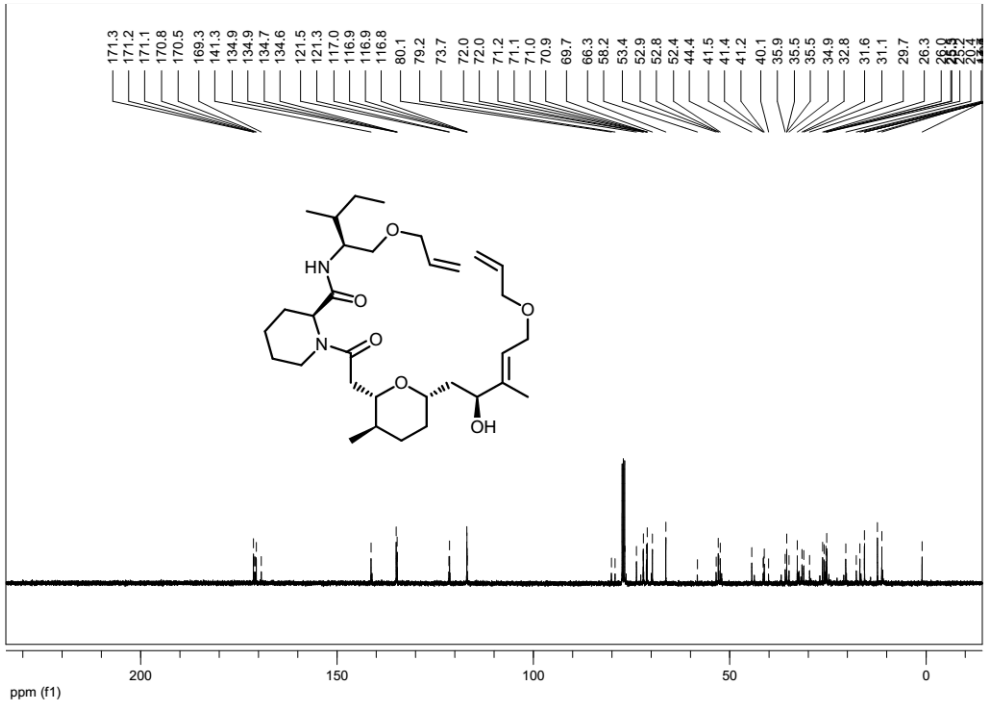

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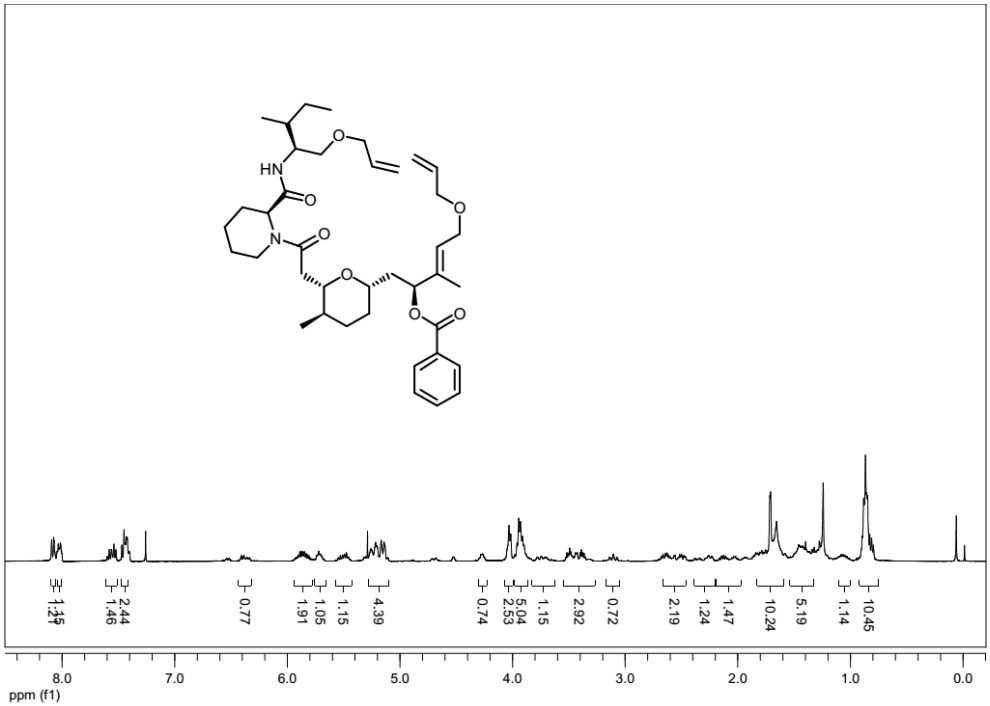

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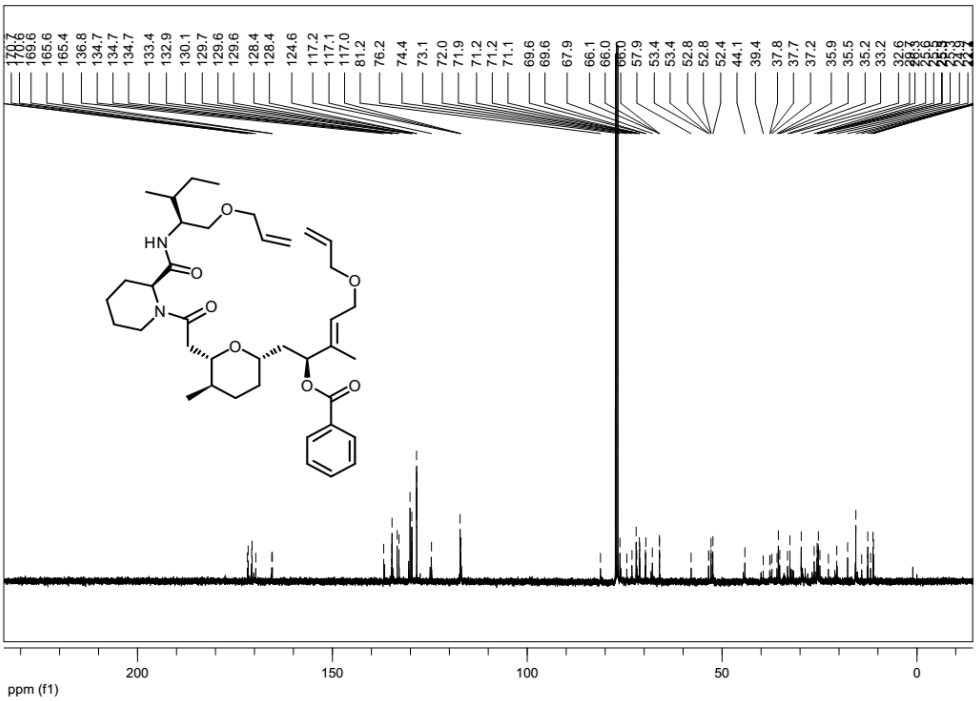

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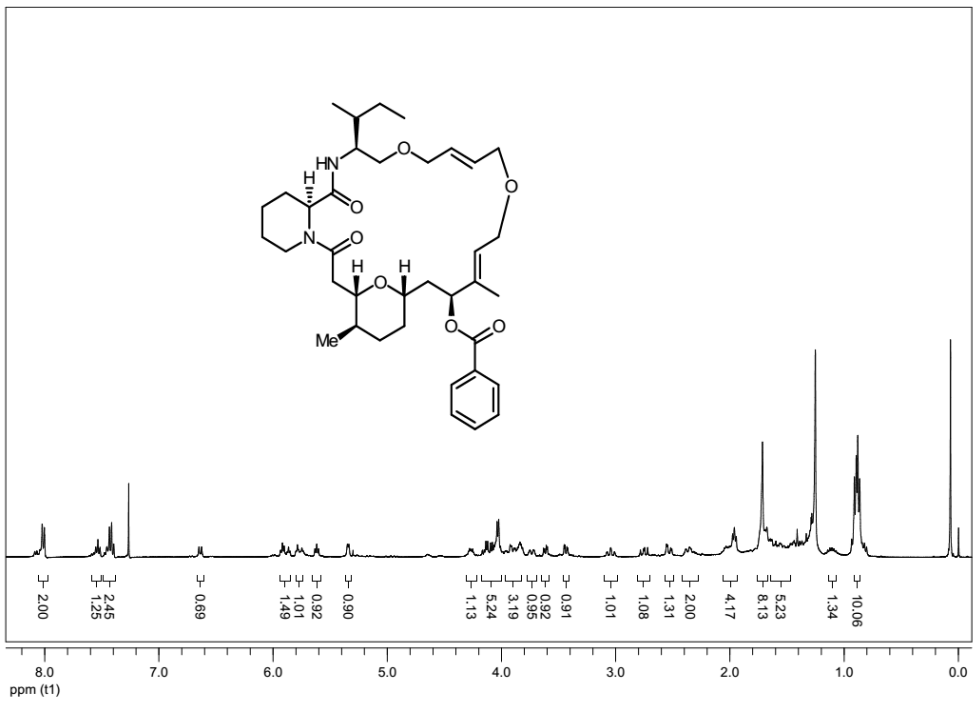

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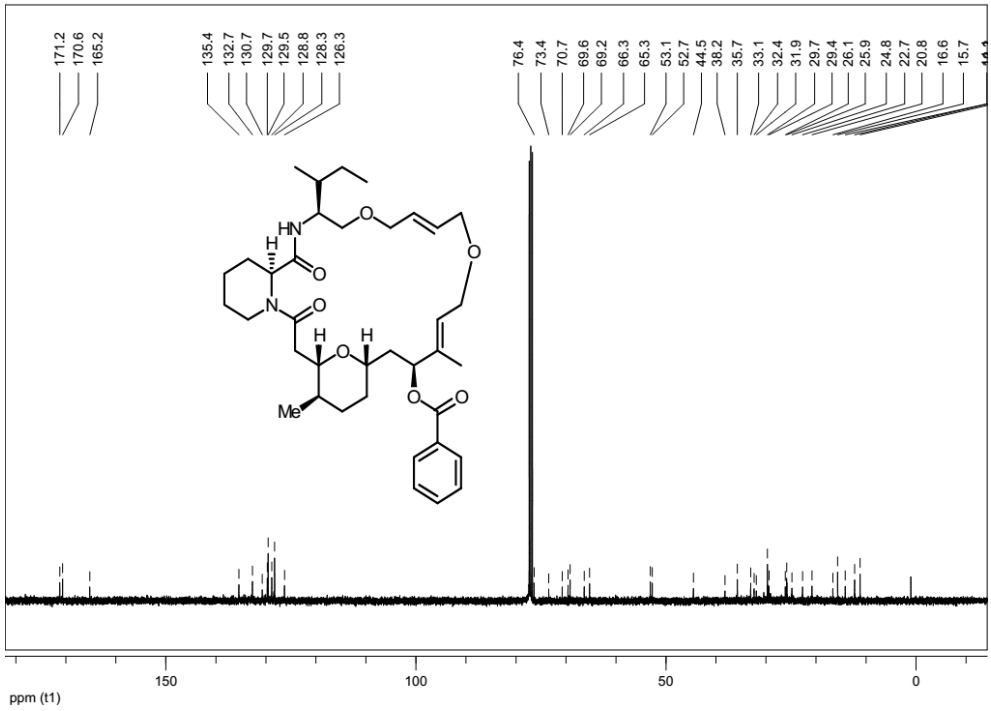

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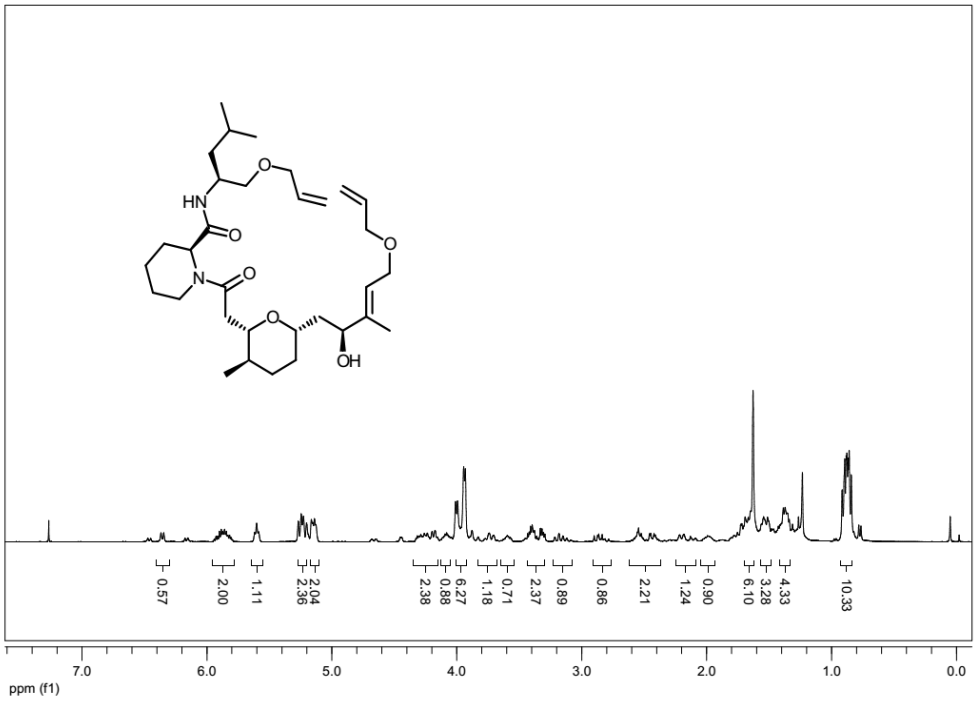

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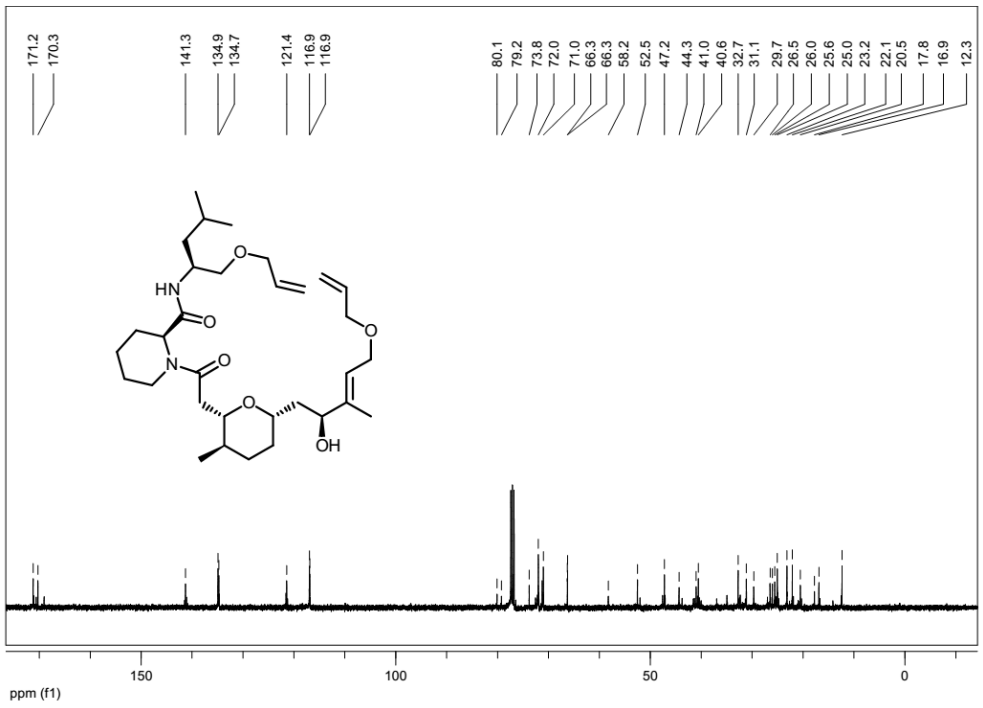

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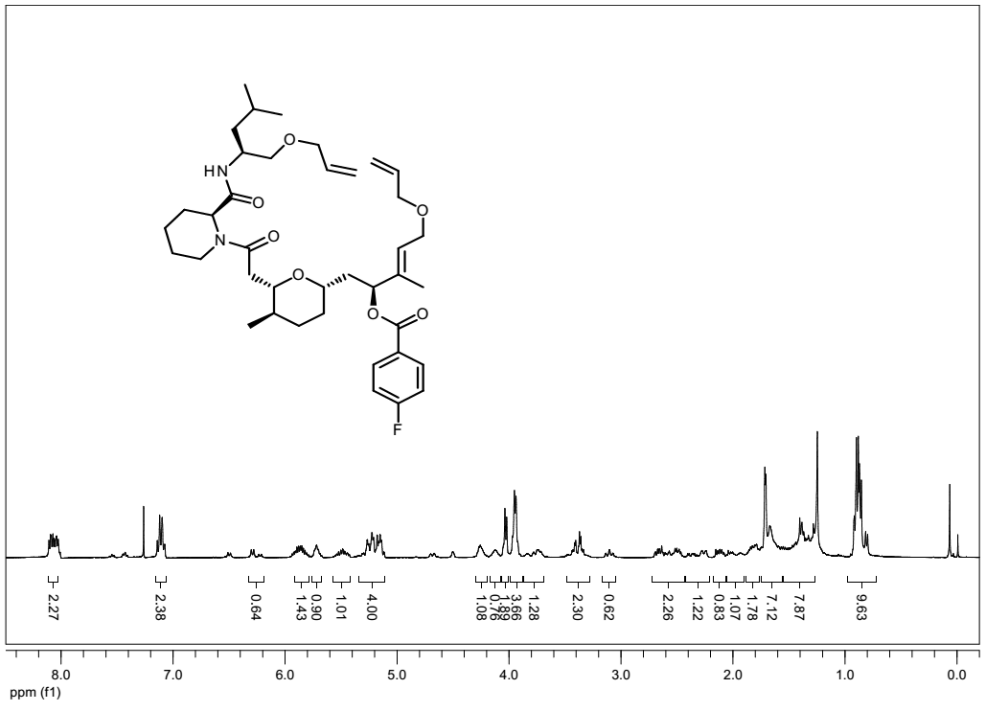

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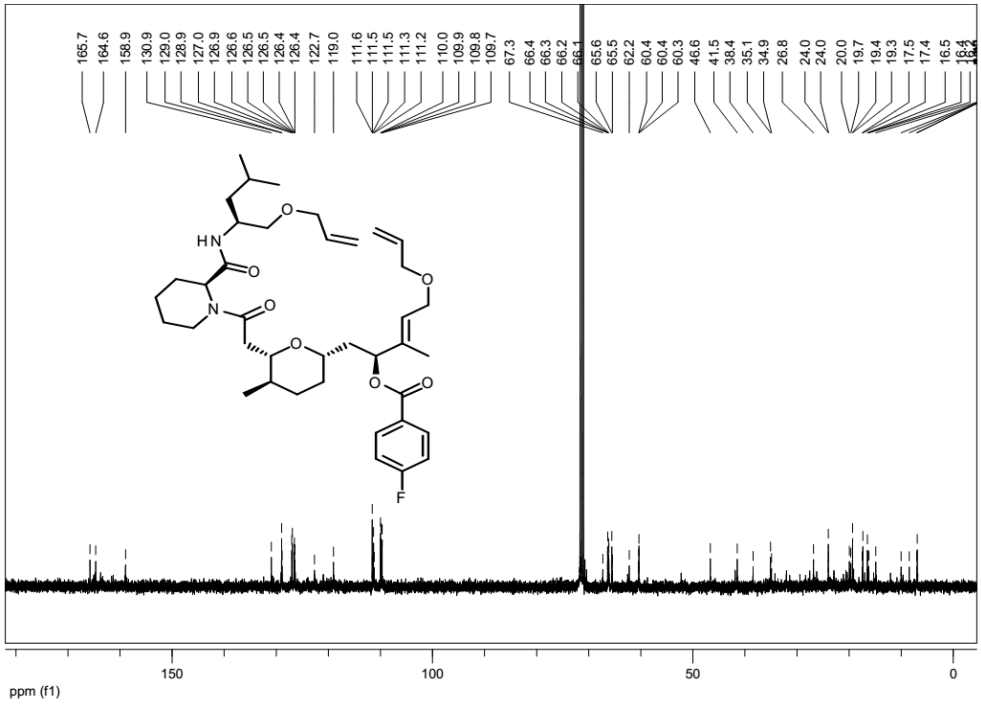

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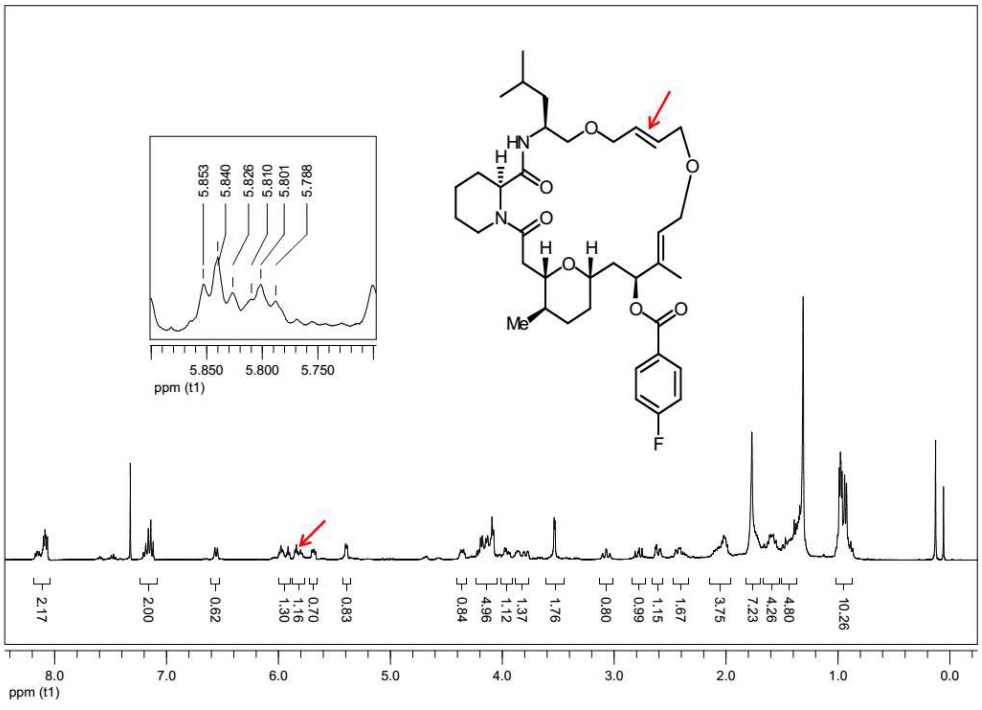
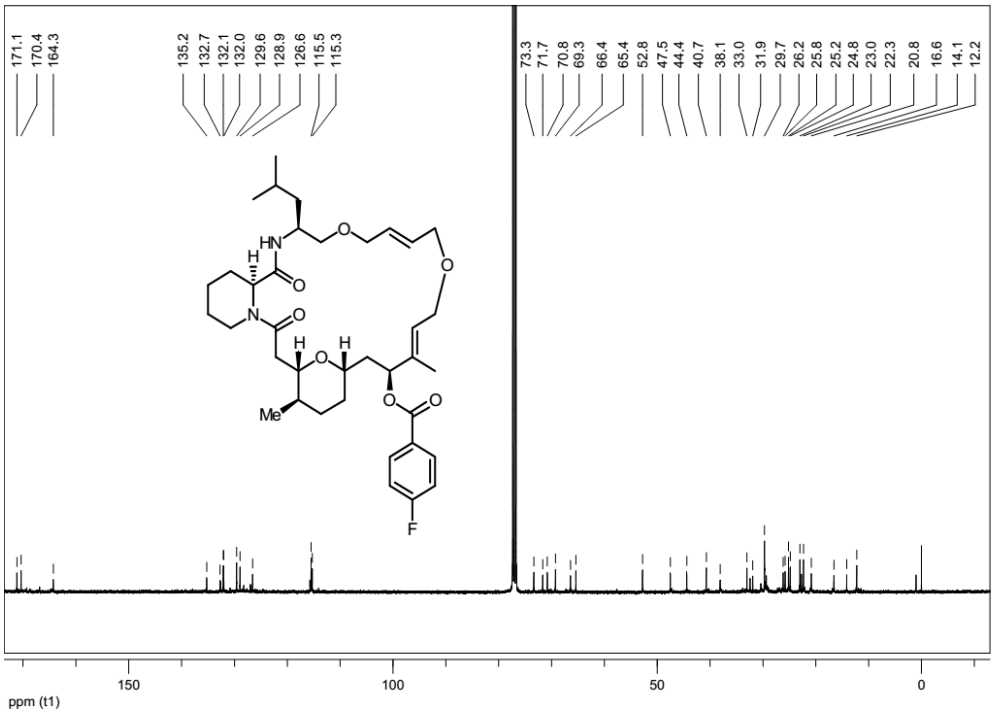


^{13}C NMR (100 MHz, *CDCl$_3$*):



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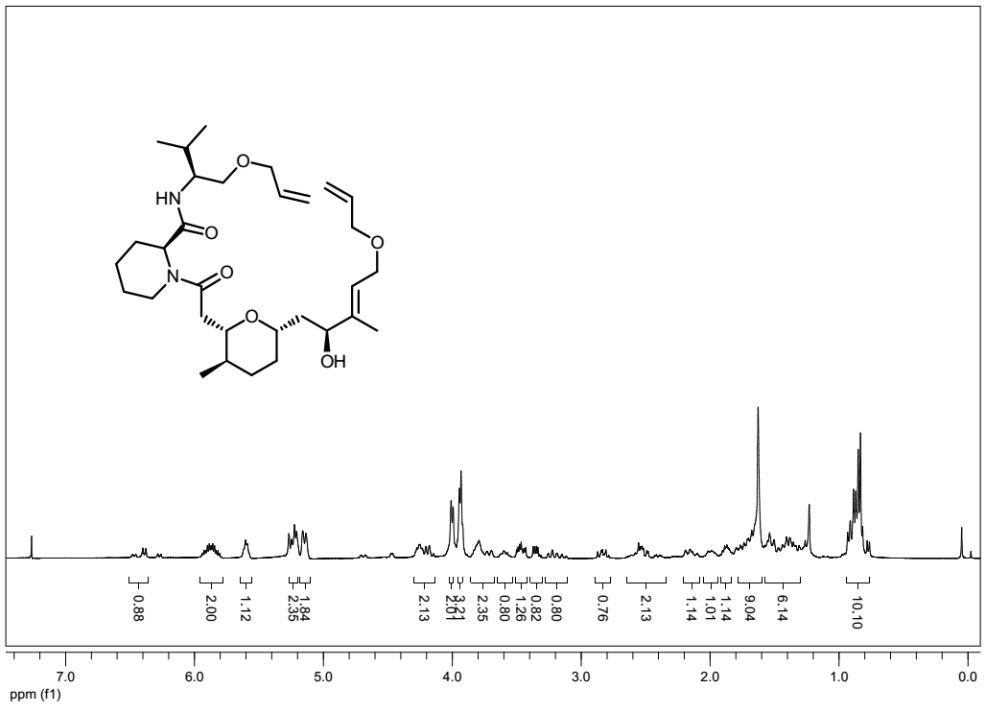

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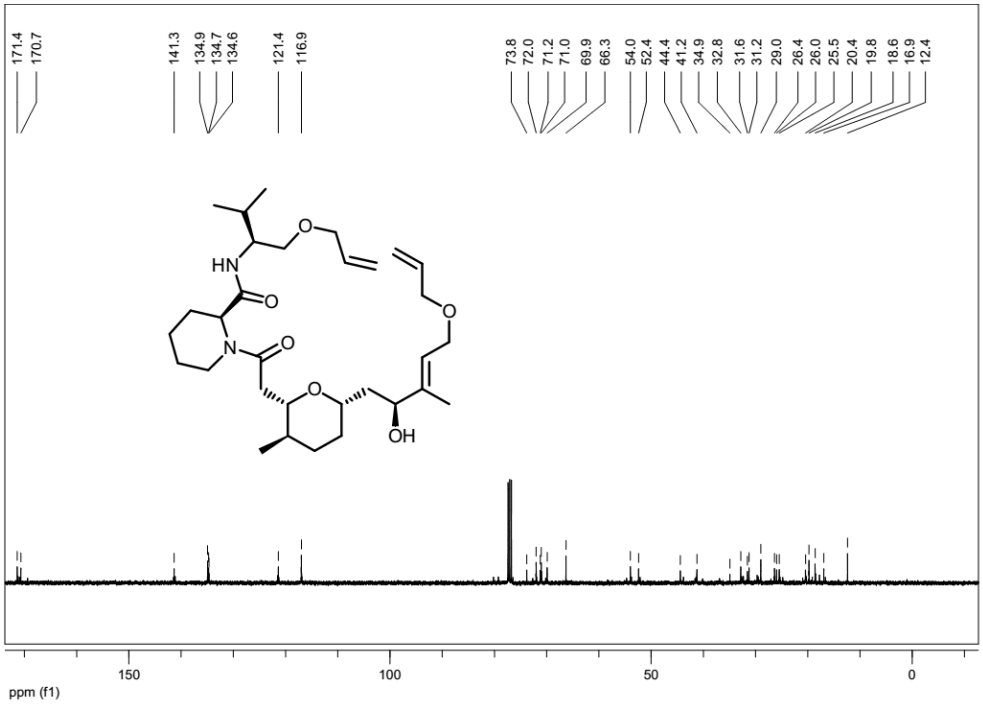

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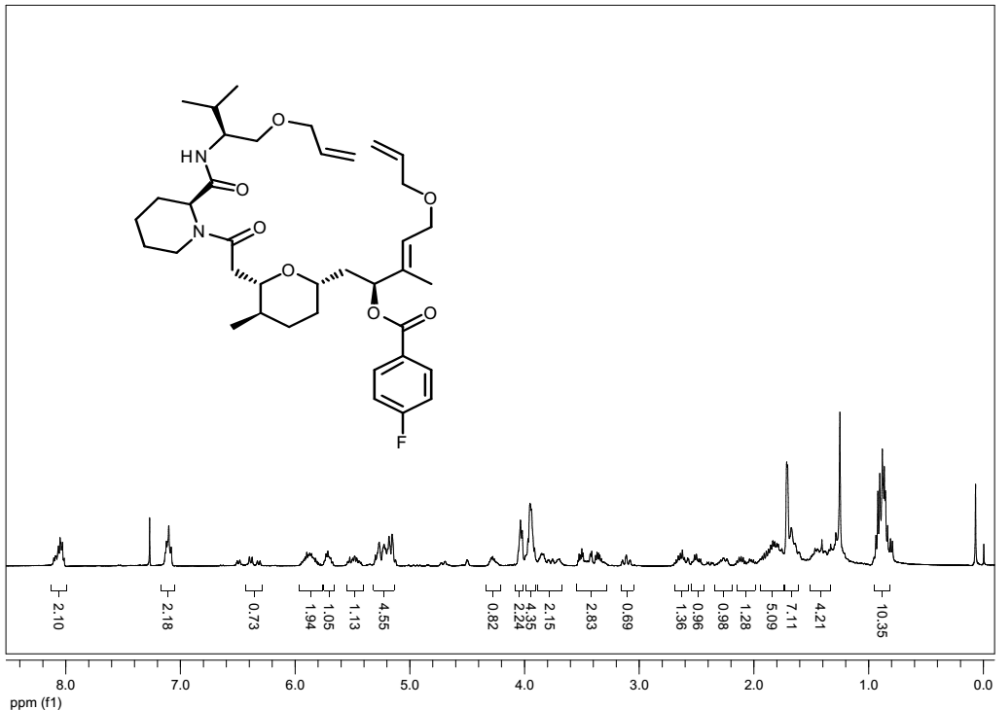

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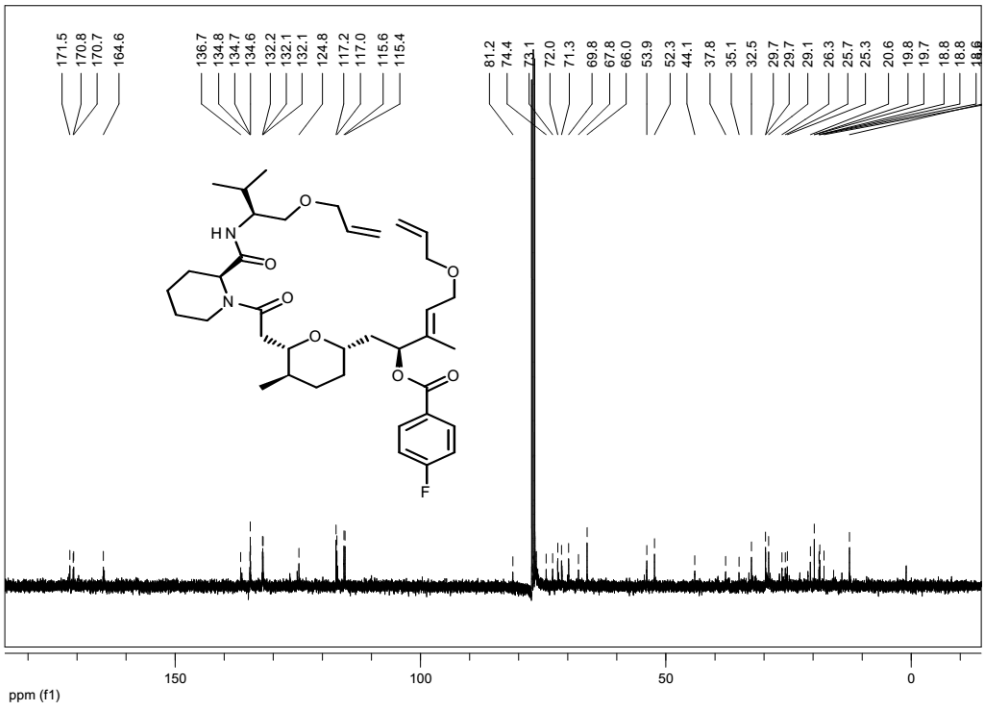
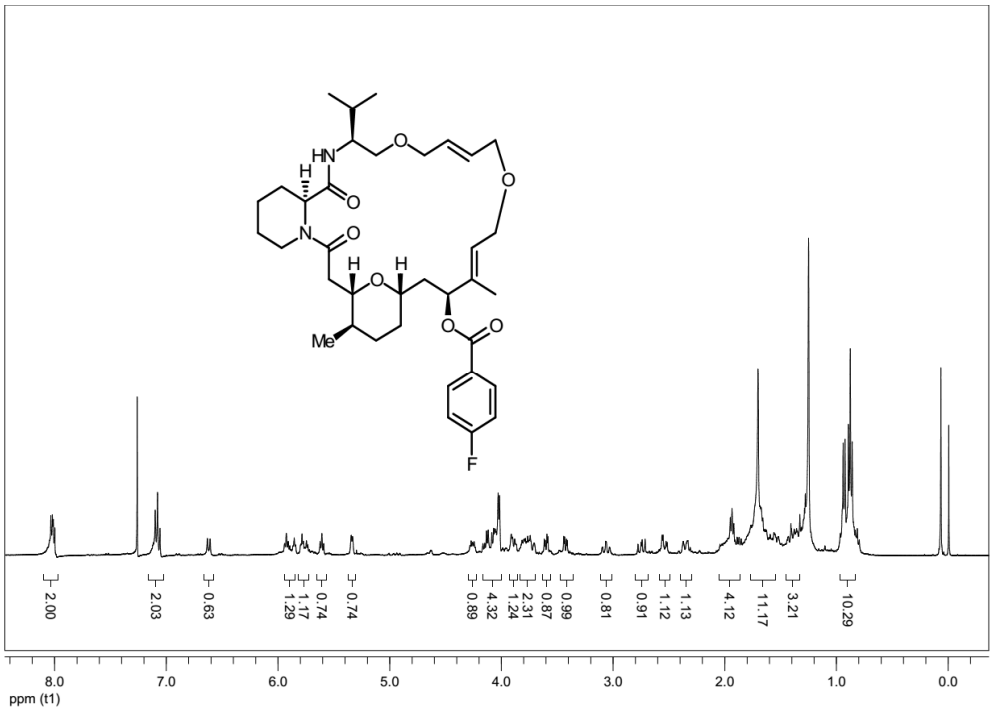
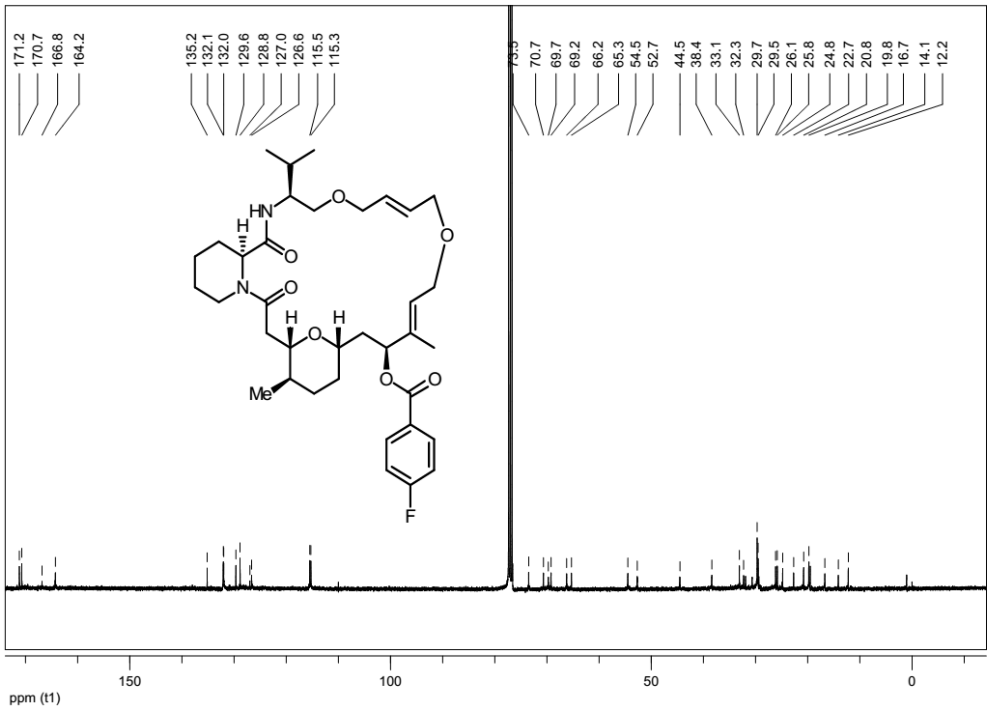


^{1}H NMR (400 MHz, $CDCl_3$):



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



Shiva Krishna Reddy Guduru Peer Reviewed Publications:

1. Tetrahydroquinoline-Derived Macrocyclic Toolbox: The Discovery of Anti angiogenesis Agents in Zebrafish Assay. Shiva Krishna Reddy Guduru, Srinivas Chamakuri, Gayathri Chandrasekar, Satish Srinivas Kitambi, and Prabhat Arya. *ACS Med. Chem. Lett*. **2013**, *4,* 666-670
2. A modular approach to build macrocyclic diversity in aminoindoline scaffolds identifies anti angiogenesis agents from a zebrafish assay. Srinivas Chamakuri, Shiva Krishna Reddy Guduru, Sreedhar Pamu, Gayathri Chandrasekar, Satish Srinivas Kitambi, and Prabhat Arya. *Eur. J. Org. Chem.* **2013**, *19*, 3959-3964
3. Small Molecule Modulators of Protein-Protein Interactions: Selected Case Studies. Madhu Aeluri, Srinivas Chamakuri, Bhanudas Dasari, Shiva Krishna Reddy Guduru, Ravikumar Jimmidi, Srinivas Jogula and Prabhat Arya. *Chem. Rev.* **2014**, *114*, 4640-4694
4. Synthesis of Rapamycin Fragment-Derived Hybrid Natural Products. Shiva Krishna Reddy Guduru and Prabhat Arya. **2014,** *(Manuscript under preparation)*
5. Building a Natural Product-inspired, Small Molecule Toolbox for Protein:Protein Interactions. Madhu Aeluri, Srinivas Chamakuri, Bhanudas Dasari, Shiva Krishna Reddy Guduru, Ravikumar Jimmidi, Srinivas Jogula and Prabhat Arya. *Acc. Chem. Res*. **2014**. *(submitted)*
6. Chemical Biology of Rapamycin-Derived, Next Generation Small Molecules. Shiva Krishna Reddy Guduru and Prabhat Arya. *Chem. Eur. J.* **2014,** *(Manuscript to be submitted shortly, Invited review article)*

Conferences/Workshops Participation

2014 Attended and presented an oral talk in international conference on "*Nature Inspired Initiatives in Chemical Trend*s" conducted by CSIR-IICT, India, during 2nd-5th March

2013 Attended and presented a poster at MEDCHEM 2013 conference on "*Advances in Anticancer Drug Discovery and Development*" organized by IIT Madras, India

2013 Participated in IX-JNOST international conference, IISER, Bhopal, India

2009 Participated in Two-Day National workshop on "*Green Chemistry*", conducted by Centre for Pharmaceutical Sciences, Institute of Science and technology and JNTU during 14th -15th February

2009 Participated in one day seminar held on 3rd February, at MNR P.G College, Hyderabad on "*New Perspectives in The Frontier of Drug Discovery*"

2009 Attended a lecture on *"Dorothy Hodgkin's Crystallographic Discoveries and their Relevance to Current Biology"* by Prof. Guy Dodson, University of York, UK on the 12th Foundation Day of Centre for DNA Fingerprinting and Diagnostics (CDFD) on 28th January

2008 Attended a two-day seminar on "*Spectroscopy"*, conducted by R.B.V.R.R. Women's College, Hyderabad, 20th-21st August

2008 Participated in one day seminar on "*Green Chemistry In Drug Synthesis*", conducted by MNR P.G. College, Hyderabad, 29th January