A Divergent Approach to Benzofuran-inspired, Macrocycles and Eribulin Fragment-based Hybrid Compounds

A THESIS
SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY

BY

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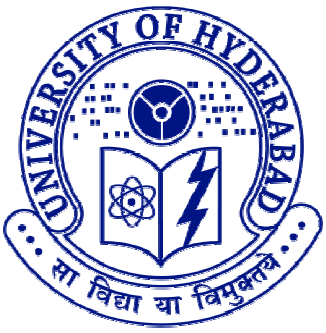

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September 2014

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.

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Dr. Reddy's Institute of Life Sciences *Ravikumar Jimmidi*

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ABBREVIATIONS

^{13}C NMR	:	carbon-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	:	hydrogen-1 nuclear magnetic resonance spectroscopy
AA	:	amino acid
Ac	:	acetyl
Ac_2O	:	acetic anhydride
Alloc	:	allyloxy carbonyl
Ar	:	aryl
aq	:	aqueous
BCl_3	:	boron trichloride
$BF_3 \cdot OEt_2$	:	borontrifluoride-etherate complex
Bn	:	benzyl
Boc	:	tert-butoxycarbonyl
Boc_2O	:	di-*tert*-butyldicarbonate
Bu_2BOTf	:	di-*n*-butylboryl trifluoromethanesulfonate
Cbz	:	benzyloxy carbonyl
DABCO	:	1,4-diazabicyclo[2.2.2]octane
DBU	:	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	:	dichloromethane
DCPE	:	1,2-Bis(dicyclohexylphosphino)ethane
DDQ	:	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	:	Diethyl azodicarboxylate
DIPEA or iPr$_2$NEt	:	*N*, *N*'-diisopropylethylamine
DIBAL-H	:	diisobutyl aluminium hydride
DMDO	:	dimethyldioxirane
DMF	:	N,N dimethylformamide
DMP	:	dess-Martin Periodinane
DMSO	:	dimethyl sulfoxide
DPPA	:	diphenylphosphoryl azide

EDC	:	3-(Ethyliminomethyleneamino)-*N*,*N*-dimethylpropan-1-amine
Et_3N or TEA	:	triethylamine
Et_2O	:	diethyl ether
EtOAc	:	ethyl acetate
EtOH	:	ethanol
Fmoc	:	fluornyloxy carbonyl
G-II	:	grubbs 2nd generation catalyst
h	:	hour(s)
H_2O_2	:	hydrogen peroxide
HOBT	:	hydroxy benzotriazole
Hz	:	hertz
IBX	:	2-Iodoxybenzoic acid
IC_{50}	:	half maximal inhibitory concentration
KBr	:	potassium bromide
K_2CO_3	:	potassium carbonate
KF	:	potassium flouride
KO^tBu or KTB	:	potassium *tert*-butoxide
LAH or $LiAlH_4$	:	lithium aluminiumhydride
LiOH	:	lithium hydroxide
mCPBA	:	meta-Chloroperoxybenzoic acid
Me	:	methyl
MeMgBr	:	methylmagnesium bromide
MeOH	:	methanol
MeI	:	methyl iodide
MEM	:	2-methoxyethoxymethyl
MOM	:	methoxymethyl
$NaBH_4$	:	sodium borohydride
NaH	:	sodium hydride
$NaHCO_3$	:	sodium bicarbonate
NaHMDS	:	Sodium bis(trimethylsilyl)amide
$NaIO_4$	:	Sodium periodate
NaOH	:	sodium hydroxide

nBuLi	:	*n*-butyl lithium
NH_4Cl	:	ammonium chloride
NOESY	:	Nuclear Overhauser Effect Spectroscopy
Ph	:	phenyl
$PhI(OAc)_2$	:	(Diacetoxyiodo)benzene
$POCl_3$	:	Phosphoryl chloride
PTSA or *p*-TsOH	:	*para*-toluenesulfonic acid
RCM	:	ring closing metathesis
RT (or) rt	:	room temperature
TBAF	:	tetra-*n*-butylammonium fluoride
TBDMS or TBS	:	*tert*-butyldimethylsilyl
TBDMSOTf	:	Trifluoromethanesulfonic acid tert-butyldimethylsilyl ester
TBDPS	:	*tert*-butyldiphenylsilyl
TEMPO	:	2,2,6,6-Tetramethylpiperidinyloxy
TFA	:	trifuoroacetic acid
THF	:	tetrahydrofuran
$TiCl_4$	:	titanium tetrachloride
TPP or PPh_3	:	triphenylphosphine
Triton-B	:	Benzyltrimethylammonium hydroxide
TsCl	:	*para*-toluenesulfonyl chloride
UV	:	ultra violet

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: Introduction to Benzofuran-Derived Natural Products and The Importance of Macrocycles in Protein-Protein Interactions

1.1. Introduction to Protein-Protein Interactions (PPIs)

There is a growing desire to undertake biological targets, within the drug discovery arena, that lie in the domain of protein-protein interactions (PPIs),[1] and, result from the de-regulation of intracellular signaling pathways.[2] Because, proteins play central roles in all aspects of cellular function including metabolism, information processing, decision making, transport, and, the structural organization,[3] it is becoming clear that signaling networks that are composed of several complex PPIs are central to both normal and dysfunctional cellular processes.[2e,2g,h,4] In a classical drug discovery approach, the focus was mainly on enzymes (such as kinases and phosphatases), and, depending upon the structural information of a given isolated target which would then aid in the design and synthesis of small molecules. In this era, the challenge was to discover novel small molecule that have the potential to hit only one target, i.e. the desired kinases or phosphatases. The post-genomic era taught us that this is not going to be an easy undertaking, keeping in mind that human genome encodes more than 600 kinases and ~250 phosphatases. So, the growing desire is to undertake biological targets that are focused on protein-protein interactions and on the de-regulation of dynamic signaling pathways.[5]

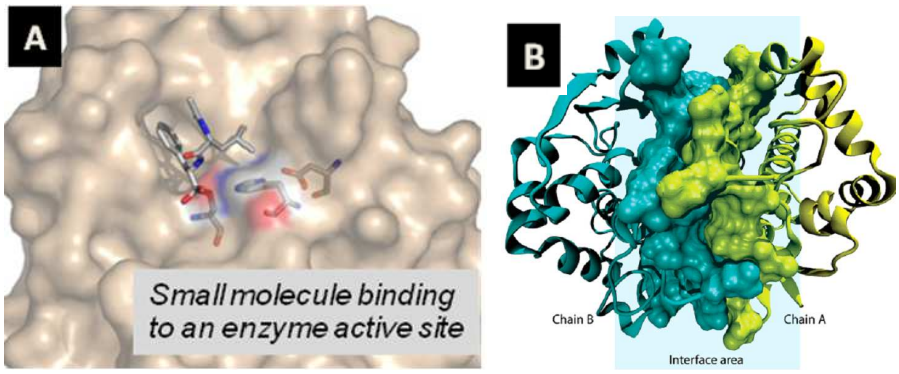


Figure 1. A. Small molecule binding to an enzyme active site. B. Illustration of protein-protein interfaces represents two interacting proteins (human glutathione S-transferase, PDB ID: 10gs, Chains A and B)

Unlike the deep and well-defined pockets that enzymes do offer, PPIs involve a shallow, large surface area with extensive hydrophobic interactions and a reference example is shown in **Figure 1**.[6] Protein-protein interactions are largely driven by the hydrophobic effect.[7] Hydrogen bonds and electrostatic interactions also play crucial

roles,[8] and, covalent bonds are also important. The discovery of small molecules that regulate protein-protein binding interactions is of great interest and practical importance.[9]

1.2. Protein-Protein Interactions: Challenges and Opportunities

Several factors have contributed to the limited success of small molecules as modulators of protein-protein interactions.[1a] In general, protein-protein interactions involve shallow surfaces and cover a relatively large surface area. Proteins also have areas known as "hot spots".[1d] In several cases, it has been observed that these "hot spots" contribute significantly to an overall binding, and, the targeting of these "focused surface areas" has successfully led to the design of small-molecule binders. There are a few examples in the literature where structural information of a protein-protein complex has led to the design of small molecules that exploit the "hot spots".[10] One such example of a rationally designed molecule involved the synthesis of small-molecule modulators capable of interfering with caspase 9-IAP BIR 3 domain interactions.[11] However, despite having structural information of the protein surface(s), there are very few cases where this information has successfully led to the rational design of small-molecule modulators of protein-protein interactions. Thus, by developing organic synthesis methods that allow building the chemical toolboxes having compounds belonging to natural product-inspired (or like) or even hybrid natural products remains an attractive strategy for the identification of small-molecule modulators of protein-protein interactions.[10,12]

Developing small molecules that modulate protein–protein interactions is difficult, owing to issues such as the lack of well-defined binding pockets. Nevertheless, there has been serious progress made in this endeavour in recent years.[1d] While the disruption of protein-protein interactions is a challenging undertaking, it has recently yielded several new compounds including small molecules that disrupt interactions important for cancer including the inhibitors for p53/mdm2, Bcl-2/BH3, XIAP/ caspase-9, XIAP/caspase-3, Rac/Tiam1, β-catenin/ TCF and Sur-2/ESX.[13]

1.3. Natural Products as Modulators of Protein-Protein Interactions

Natural products have served as the source and inspiration for a large fraction of the current pharmacopoeia.[14] Historically, natural products have been the source of many new drugs. Newman and Cragg analyzed the sources of new drugs from 1981 to

2006, and, using a fairly broad definition of what constitutes a "natural product-derived drug", it indicates that almost 50% of new drugs introduced during this period had a natural product origin.[15] Over the years, 3-dimensional (3-D), architecturally complex natural products have been used as small-molecule probes for understanding protein function(s). The search for novel natural products with interesting biological properties is still ongoing.[16,17] Natural products are a number of highly diverse, chiral functional groups, which are the potential sites for protein binding. Although natural products from a variety of sources (i.e. plants, soil, sea, etc.) are very useful candidates for identifying lead compounds, the major disadvantage with natural products is difficulty of the follow-up organic synthesis/medicinal chemistry efforts.

Natural products offer several advantages over the classical drug-like compounds in several ways, and, these are: (i) the presence of 3D shapes, (ii) the ability to present several chiral functional groups to provide specific binding interactions in the 3D space, and, (ii) multiple rings or macrocyclic shapes. The chemical properties of the small-molecule natural products that have recently been developed into drugs have been analyzed.[13] It appears that at least half of them were found to be closely compliant with Lipinski's rule of five for orally available compounds, but the remainder had higher molecular weights, more rotable bonds and more stereogenic centres, although they retained relatively low logP values. It is also well-accepted that, on average, natural products are more readily absorbed than synthetic drugs.

In many cases, there is insufficient material for the various desired biological assays, thereby limiting the exploration of their full potential. One solution to this, developing relatively simple structural analogs with comparative biological responses to the natural product, is a challenging undertaking, and, it is at this point that a diversity-oriented synthesis (DOS) program,[18,19] or biology-oriented synthesis (BIOS)[20] developed for the specific natural product class is extremely useful. Both approaches are aimed at populating the unexplored, natural product-based, chemical space that is currently unoccupied by conventional combinatorial chemistry.

Members of natural products including alkaloids,[21] flavonoids[22] and several of their derivatives are known to possess a diverse range of biological properties. Commonly

found in natural products, “benzofuran and derived substructures” that are ideal sub-structures for interactions with for protein targets.[23]

1.4. Examples of Benzofuran-Derived Natural Products as Modulators of Protein-Protein Interactions

In this section, I am going to discuss some of the benzofuran-derived natural products, and, two case studies of natural products, such as galantamine and rocaglaol.

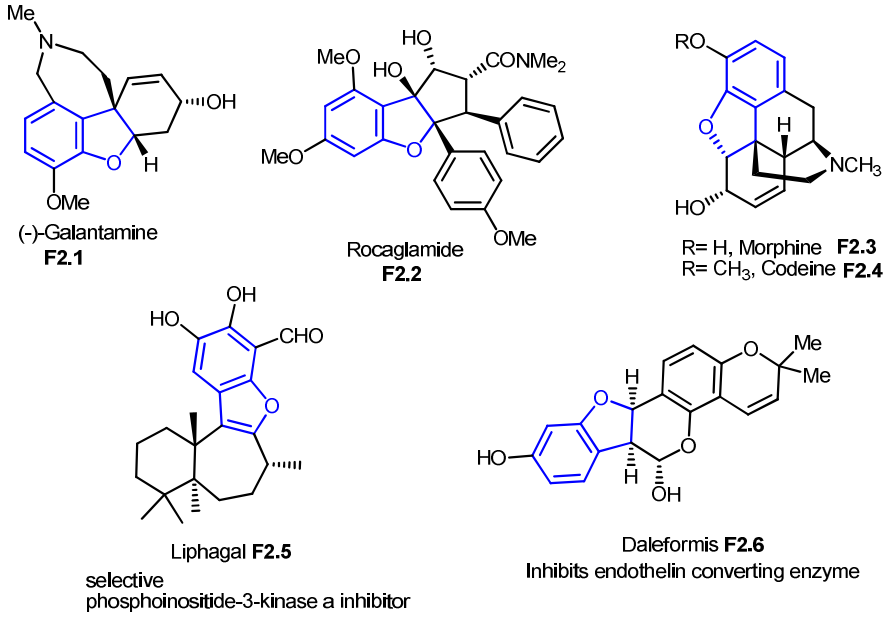


Figure 2. Benzofuran-derived bioactive natural products

Benzofuran and furan are two common structural elements present in numerous bioactive natural products as well as in pharmaceuticals, molecular electronic and functional polymers.[23]

1.4.1. Synthesis and Chemical Biology of (-)-Galantamine

(-)-Galantamine is an alkaloid isolated from the Caucasian snow-drop (Galanthus woronowii) and from the bulbs of different species of the *Amaryllidaceae* family and related genera like *Narcissus* (daffodil), *Leucojum* (snowflake), and *Lycoris* inclu ding *Lycoris radiate* (Red Spider Lily). It is a centrally acting, selective, reversible, and, competitive acetylcholinesterase (AChE) inhibitor,[24] as well as an allosteric modulator of the neuronal nicotinic receptor for acetylcholine.[25] (-)-Galantamine is the most recently approved AChE inhibitor in Europe by the European registration

bureau and in USA by FDA for the symptomatic treatment of Alzheimer's disease (AD) and it is commercially available as razadyne, galantamine hydrobromide. Some of galantamine-type amaryllidaceae alkaloids and their derivatives are shown in **Figure 3**.

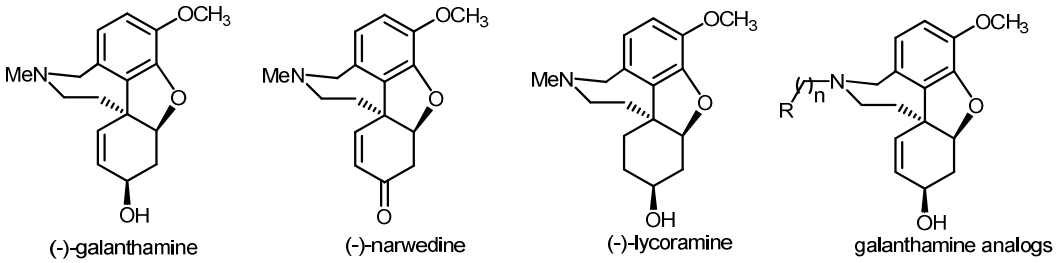
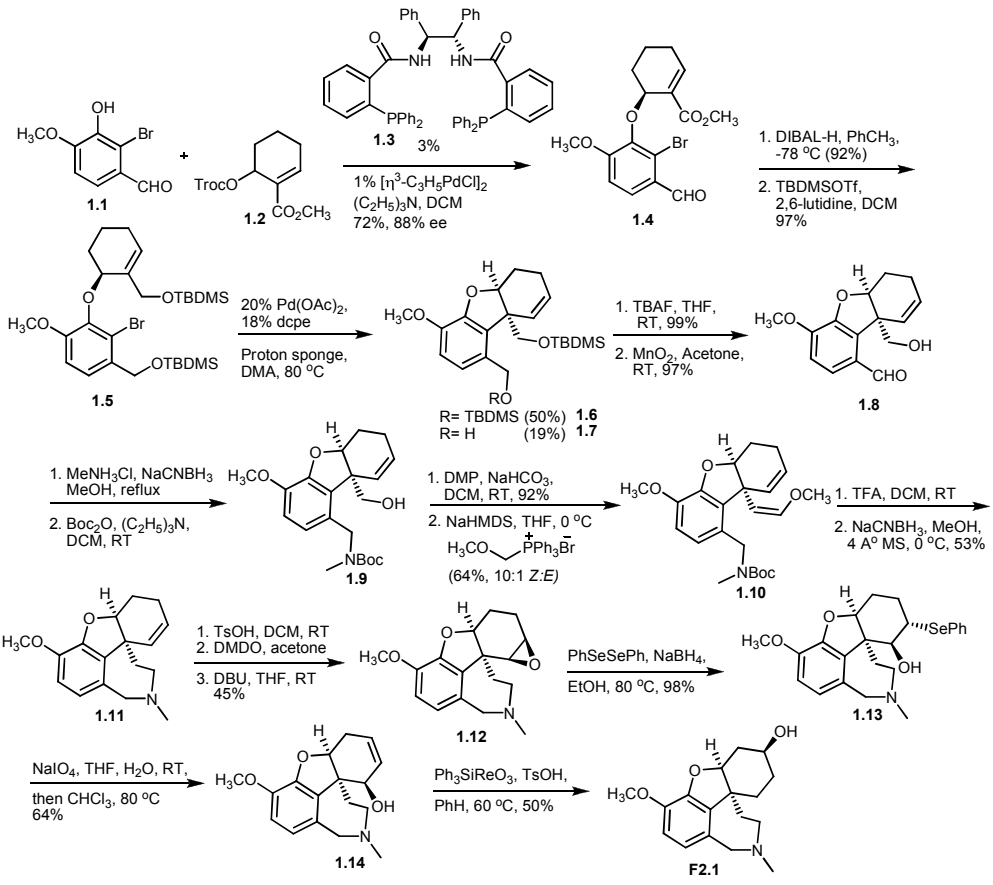


Figure 3. Naturally occurring galantamine-type amaryllidaceae alkaloids and their derivatives

Synthesis

Synthetic approaches to galantamine rely on two key reaction protocols: (a) a biomimetic approach via the phenolic oxidative coupling in the presence of metal oxidants,[26] and, (b) an intramolecular Heck reaction,[27] and, a very few reports have disclosed the asymmetric synthesis of this molecule. Synthesis of galantamine is shown in **Scheme 1**. The researchers planned first to form the O5-C4a bond by a palladium-catalyzed asymmetric allylic alkylation,[28] *ortho*-halophenol, and, then employ an intramolecular Heck reaction[29] to prepare the crucial quaternary center. Palladium catalyzed reaction of 2-bromovanillin **1.1**,[30] with carbonate **1.2**[31] which is available in two steps from glutaraldehyde and the Emmons-Wadsworth-Horner reagent in the presence of a ligand **1.3**[32] furnished the required aryl ether **1.4**. Next, **1.4** was reduced with DIBAL-H, and, the resulting diol protected with TBDMS-triflate to afford *bis*-TBDMS ether **1.5** in 89% yield from **1.4**. After several conditions 1,2-bis(dicylohexylphosphino)ethane (dcpe) proved to be an excellent ligand for an intramolecular Heck reaction, affording benzofuran **1.6** in 50% yield along with 19% of the monodeprotected product **1.7**. The TBDPS deprotection of **1.6** and **1.7** mixture followed by the chemoselective MnO_2 oxidation of the resulting diol, afforded aldehyde **1.8** and reductive amination with methylamine, followed by Boc protection gave **1.9** with 83% yield. Oxidation of **1.9** followed by Wittig reaction gave **1.10**. Concomitant de-blocking of the secondary amine and the

hydrolysis of the methoxy vinyl group gave a seven-membered ring hemiaminal which was not isolated, immediately treated with sodium cyanoborohydride to afford (-)-3-deoxygalanthamine, **1.11** in 53% overall yield. The reaction of the tosylammonium salt of tertiary amine **1.11** with dimethyl dioxirane[33] afforded a mixture of epoxide and the corresponding α-hydroxy tosylate. The treatment with DBU converted α-tosyl alcohol to an epoxide **1.12** which was isolated in 45% from **1.11**. Regioselective opening of an epoxide **1.12** with sodium phenyl selenide produced α-hydroxyselenide **1.13**, followed by a chemoselective oxidation with sodium periodate to produce 1:1 mixture of diastereomeric selenoxides. These selenoxides required heating to effect the desired conversion into (-)-isogalanthamine **1.14**. Reaction of **1.14** with Osborn's rhenium(VII) catalyst[34] produced (-)-galantamine.

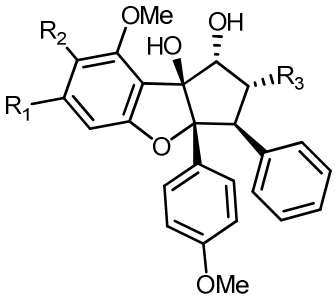


Scheme 1. Synthesis of (-)-galantamine[35]

1.4.2. Synthesis and Chemical Biology of (-)-Rocaglamide

Flavaglines are a family of natural compounds extracted from Asian plants of the genus Aglaia.[36] In 1982, King *et. al*., isolated the first flavagline, rocaglamide **F2.2**

from Aglaia elliptifolia, on the basis of its potent *in vivo* activity against murine P388 lymphocytic leukemia.[37] Since then, many other flavaglines, such as rocaglaol or silvestrol, have been isolated, mainly by the pharmacognosy laboratories of Kinghorn, Pezzuto, and Proksch. These cyclopenta[b]benzofurans have been shown to inhibit the proliferation of tumor cells in a low nanomolar range, either by cytostatic[38] or cytotoxic effects,[39] depending on the compounds as well as cell lines. Rocaglamide and different analogues are shown in **Figure 4.**

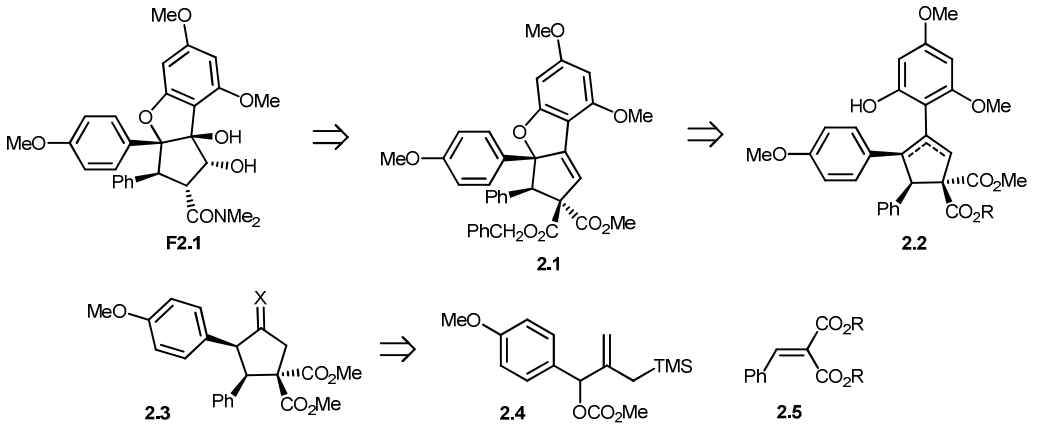


	R_1	R_2	R_3
rocaglaol (1)	OCH_3	H	H
aglafolin (2)	OCH_3	H	$COOCH_3$
pannellin (3)	$O-CH_2-O$		$COOCH_3$
rocaglamide (4)	OCH_3	H	$CON(CH_3)_2$
aglaroxin A (5)	$O-CH_2-O$		$CON(CH_3)_2$
desmethyl rocaglamide (6)	OCH_3	H	$CONHCH_3$

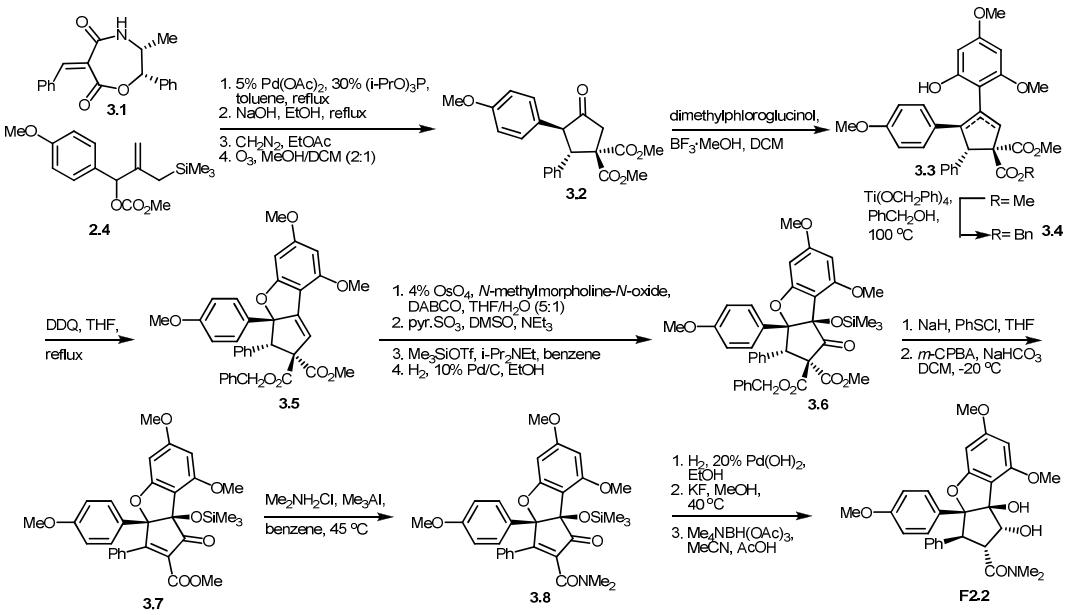
Figure 4. Rocaglamide and analogues

Synthesis

In 1987, Taylor and co-workers reported a number of synthetic approaches to the tricyclic rocaglamide skeleton,[40] and, in 1989, Kraus and co-workers reported the synthesis of a di-epi-analogue of rocaglamide.[41] Later, Trost *et. al.,* published a total synthesis of (-)-rocaglamide itself, which established the absolute configuration of the natural product.[42] Retrosynthesis of (-)-rocaglamide is shown in **Scheme 2**. The researchers developed a novel oxidative cyclization approach to create the dihydrobenzofuran ring (**2.2-2.1**).



Scheme 2. Retrosynthetic analysis of (-)-rocaglamide



Scheme 3. Synthesis of (-)-rocaglamide

Pd-catalyzed cycloaddition of the substituted TMM precursor **2.4** and an acceptor **3.1** under 5 mol% $Pd(OAc)_2$, 30 mol% $(iC_3H_7)_3P$, $PhCH_3$, reflux condition[43] gave the cycloadduct. This was followed by hydrolysis (NaOH, EtOH, reflux), esterification (CH_2N_2, C_2H_5OAc, room temperature), and, ozonolysis, which gave an optically pure adduct **3.2**. Condensation with dimethyl phloroglucinol yielded **3.3**, further transesterification[44] with benzyl alcohol [$Ti(OCH_2Ph)_2$, $PhCH_2OH$, 100 ^oC, 78%] afforded a single product **3.4** in which only one ester was exchanged. Oxidative cyclization of **3.4**, gave the complete nucleus **3.5** with 75% yield. Catalytic hydroxylation with 4 mol% OsO_4, 2 equiv of NMO, 5:1 THF/H_2O, room

temperature, along with DABCO[45,46] and Moffatt-Doering oxidation,[47] followed immediately by silylation and decarbobenzyloxylation gave the keto ester **3.6** as a 3:l keto/enol mixture, in an overall 60% yield. The introduction of the double bond into **3.6** by selenylation-dehydroselenylation[48] failed, whereas, sulfenylation with NaH, PhSCl followed by dehydrosulfenylation (MCPBA, $NaHCO_3$, CH_2Cl_2, -20 ^oC) produced enone **3.7** in 72% yield**.** The enone **3.7** smoothly underwent amidation under Weinreb's conditions[49] to obtain an amide **3.8**. The use of Pearlman's catalyst proved completely reproducible but generated a 2-3:1 diastereomeric mixture favouring **3.8**. The crude amide was directly desilylated and diastereoselectively reduced from the β-face by templating the reducing agent with the neighboring hydroxyl group[50] to provide (-)-rocaglamide **F2.2** in 50% overall yield for three steps.

1.4.3. Chemical Biology of Morphine

Morphine was isolated in 1804 by Friedrich Sertürner, which is the first isolation of a natural alkaloid in history. Sertürner originally named the substance *morphium* after the Greek god of dreams, Morpheus for its tendency to cause sleep. Morphine **F2.3** is an opioid analgesic drug, and, the most abundant opiate found in opium, the dried latex from unripe seedpods of *papaver somniferum* (the opium poppy). Like other opioids, such as hydromorphone and diacetylmorphine (heroin), morphine acts directly on the central nervous system (CNS) to relieve pain. The first synthesis of morphine was reported in 1952,[51] and, further interest in developing the improved asymmetric total synthetic routes continued because of the broad range of pharmacological properties.[52]

1.4.4. Chemical Biology of Liphagal

Liphagal is a selective inhibitor of PI3 kinase α and isolated from the sponge *Aka coralliphaga* in Dominica. The structural elucidation and biomimetic synthesis was reported in 2006.[53]

1.4.5. Chemical Biology of Daleformis

Daleformis, a new phytoalexin from the roots of *Dalea filiciformis* Snader (Fabaceae), was found to be active in endothelin converting enzyme (ECE) inhibitory screen. ECE is a membrane bound neutral metalloprotease that catalyzes the conversion of a 38-residue inactive intermediate bigendothelin (B-ET) to a 21-

residue potent vasoconstrictive peptide, endothelin-1(ET-1). The overproduction of ET is associated with numerous disorders including hypertension and renal failure. It is an ECE inhibitor and by interfering with the ET biosynthesis pathway it reduces the production of endothelin and this may have the therapeutic utility for hypertension or renal failure. Daleformis inhibited ECE with an IC_{50} of 9.0 μM.[54]

1.5. Importance of Macrocyclic Natural Products

One structural feature that is common in the several natural products is the presence of a macrocycle: a ring architecture of 12 or more atoms. Macrocyclic natural products have evolved to fulfil numerous biochemical functions and their profound pharmacological properties have led to their development as drugs.[55] Some examples of bioactive macrocyclic natural products are shown in **Figure 5**. A macrocycle provides diverse functionality and stereochemical complexity in a conformationally pre-organized ring structure. This can result in high affinity and selectivity for protein targets, while preserving sufficient bioavailability in reaching intracellular locations.[56] Despite the proven therapeutic potential of several macrocyclic compounds, they have been underexplored and poorly exploited for the discovery of novel drug molecules. Because of their structural complexity, it usually generates difficulties in an analogue synthesis and most macrocyclic compounds typically do not follow Lipinski's rule. Although several research groups are investigating the potential of synthetic macrocycles for drug discovery, and, have shown 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 an active preclinical and clinical developments.

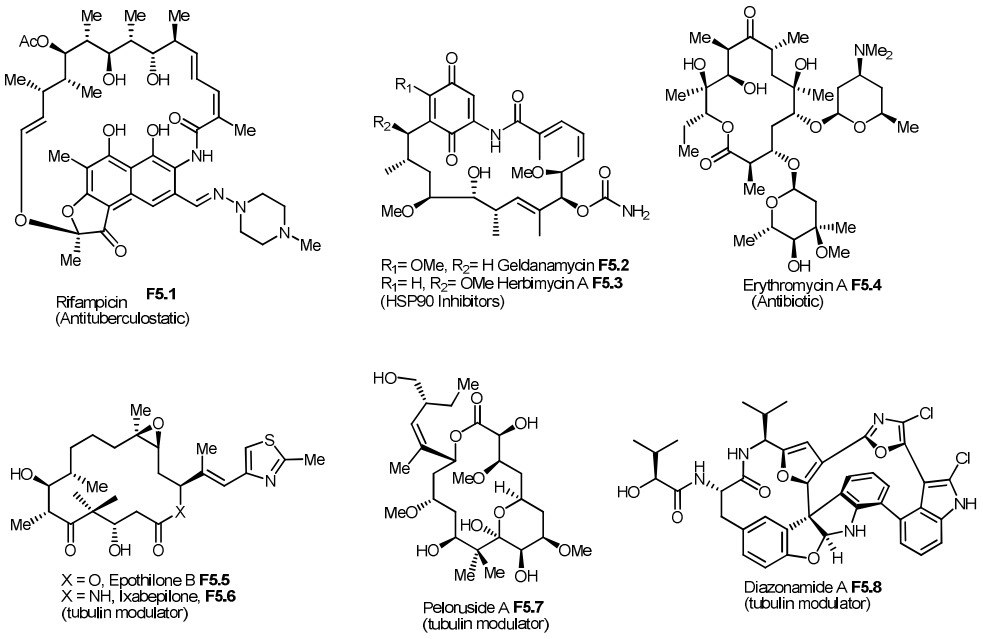


Figure 5. Examples of bioactive macrocyclic natural products

Current macrocyclic drugs are almost exclusively derived from natural sources (primarily microorganisms) and are either identical to or closely derived from naturally occurring macrocycles.

In this section, I am going to discuss about the importance of some macrocyclic natural products, and, a case study of diazonamide A, a benzofuran-derived 12-membered natural product.

1.5.1. Chemical Biology of Rifampicin

Rifamycins were first isolated in 1957 from a fermentation culture of *Streptomyces mediterranei* at the laboratory of Gruppo Lepetit SpA in Milan by two scientists named Piero Sensi and Maria Teresa Timbal, working with the scientist Pinhas Margalith. Rifampicin **F5.1** is a bactericidal antibiotic drug of the rifamycin group. Rifampicin inhibits the bacterial DNA-dependent RNA synthesis by inhibiting the bacterial DNA-dependent RNA polymerase.[57] A chemical process for the preparation of rifampicin is also reported.[58]

1.5.2. Chemical Biology of Geldanamycin

Geldanamycin was isolated by workers at Upjohn from *Streptomyces hygroscopicus* var. *geldanus* var. *nova* in 1970. Benzoquinoid ansamycins, such as geldanamycin **F5.2** and herbimycin A **F5.3**, are two antibiotics that exhibit the antitumor effects**.**[59] The Hsp90 client proteins can be destabilized when geldanamycin binds to the ATP-

binding site of Hsp90, and, it inhibits the chaperone activity of the protein. Geldanamycin competitively binds to the *N*-terminal ATP binding site of HSP90, and, this then prevents the ATP binding and disrupts the ATP-dependent conformational cyclization. The total synthesis of geldanamycin was carried-out, initially, by Andrus and co-workers[60] and, later, by several other researchers.[61]

1.5.3. Chemical Biology of Erythromycin A

In 1949, Eli Lilly's research team isolated erythromycin from the metabolic products of a strain of *Streptomyces erythreus* and the product was launched commercially in 1952. Erythromycin is an antibiotic useful for the treatment of a number of bacterial infections. In 1981, Robert B. Woodward along with a large number of members from his research group, reported the first stereocontrolled, asymmetric chemical synthesis of erythromycin A.[62]

1.5.4. Chemical Biology of Epothilone B

In 1996, Hofle and his co-workers isolated 16-memberd macrocyclic natural products called Epothilone A/B.[63] Like taxanes, epothilones were shown to interrupt the cell mitosis by stabilizing microtubules, and, thus exhibit excellent cytotoxic activity. Another interesting fact is that epothilones are known to bind the microtubule[64] at a site closer to that where taxol binds.

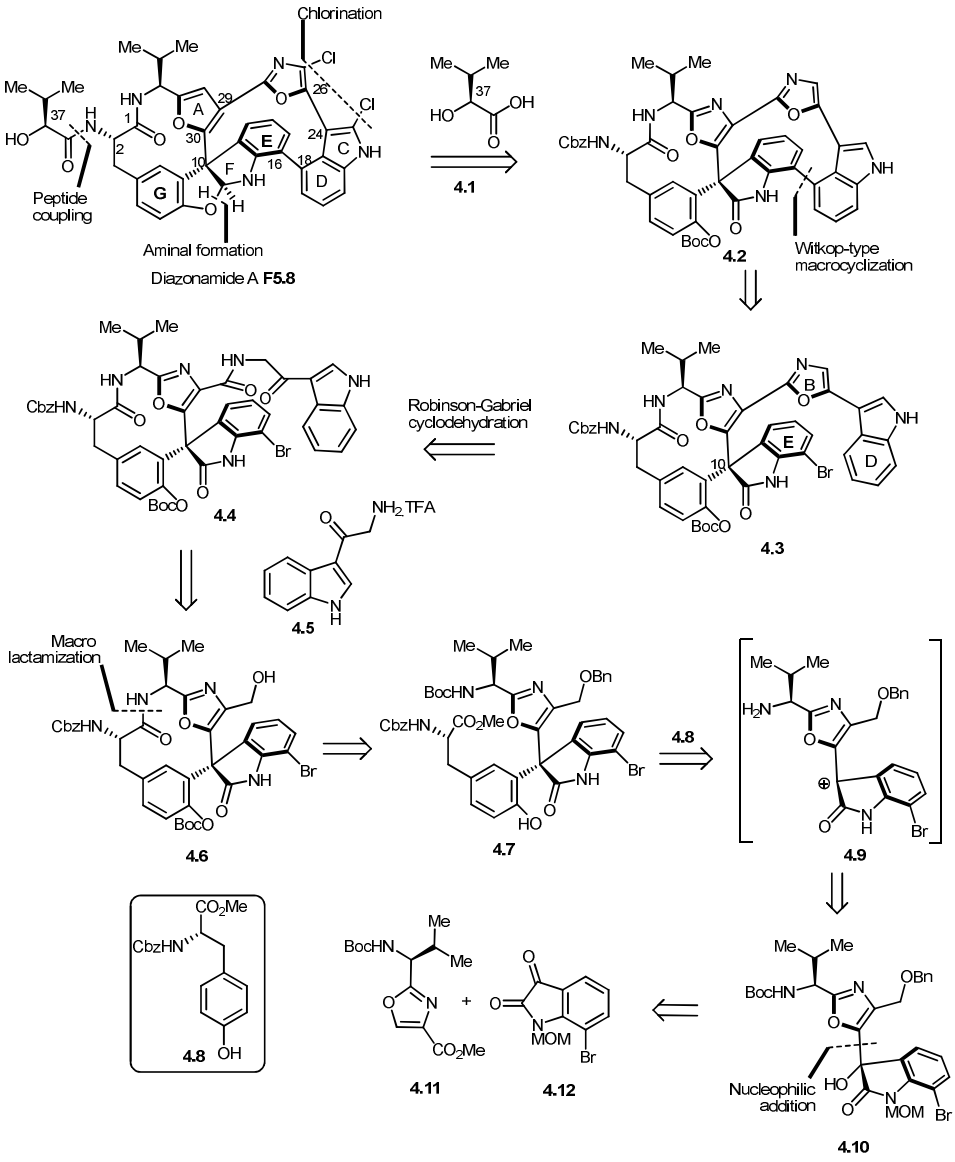
1.5.5. Chemical Biology of Peloruside A

Peloruside A was isolated from a marine sponge, *mycale*, and was reported by Northcote in 2000.[65] It is a potent microtubule stabilizer that acts in a manner synergistic to that of paclitaxel. The first total synthesis was reported by De Brabander group in 2003.[66] Peloruside A synthesis are also reported by several other groups.[67]

1.5.6. Synthesis and Chemical Biology of Diazonamide A

Diazonamide A **F5.8**, is a secondary metabolite isolated from the colonial ascidian *Diazona angulata.* In 1991, Fenical and Clardy, first disclosed the structure of diazonamide A, whose unprecedented molecular architecture included a cyclic polypeptide backbone, a strained halogenated hetero-aromatic core trapped as a single atropisomer, and, a lone quaternary center at the epicenter of its two major macrocyclic subunits. Later, despite unique challenges posed by this amazing

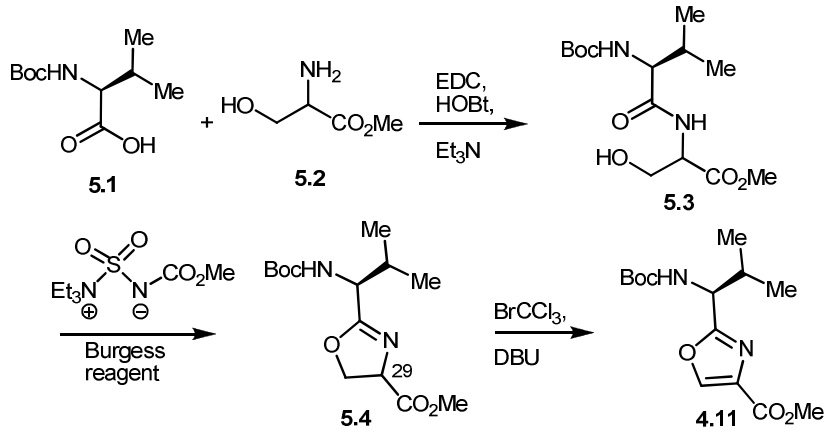
molecular framework, in concert with an impressive *in vitro* cytotoxicity (nanomolar activity against human colon carcinoma and B-16 murine melanoma cell lines,[68] the first successful synthesis of the proposed structure of diazonamide A was achieved by Harran group at the Southwestern Medical Center in Dallas, Texas.[69] Originally, the product was not matched with the proposed structure, and, later, the structure was revised which included an alteration of the terminal amino acid, exchange of the heteroatom in ring F, and, the addition of a tenth ring (ring H) to the natural product architecture. This further led to a series of new synthetic campaigns worldwide.[70]



Scheme 4. Retrosynthetic analysis of diazonamide A[71]

Retrosynthetic plan for diazonamide A **F5.8** is shown in **Scheme 4**. According to this plan, removal of the C-2 hydroxyisovaleric acid side, the excision of the two aryl

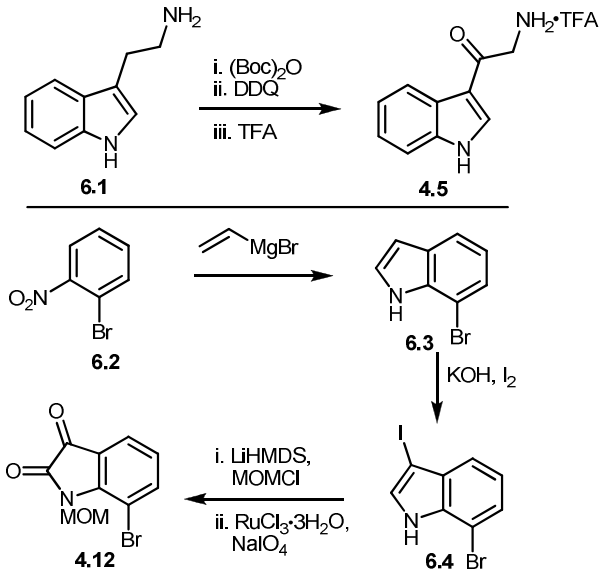
chlorines and the FH aminal ring on the basis of considerations of their stability to diverse reaction conditions. Later, two motifs could be generated in either order, with the chloro groups arising through a site selective electrophilic aromatic substitution reaction and the aminal ring resulting from the addition of the C-7 phenol onto an iminium species derived from the F-ring lactam in **4.2.** For the macrocyclic ring formation**,** the heterocycle-based ring system at its C16-C18 biaryl linkage was unfurled through the Witkop-type photocyclization transformation,[72] giving **4.3**. It was then simplified to **4.6** by unravelling its B-ring oxazole to a keto amide precursor **4.4** and then breaking apart the newly unveiled amide linkage to excise the CD-indole portion as amine **4.5**. The initial nucleophilic addition of a dianion was derived from oxazole **4.11** onto the more reactive C-3 carbonyl group of isatin **4.12**, a process that would lead to the requisite test compound **4.10** bearing a tertiary hydroxyl group. Subsequent exposure of this product **4.10** to acid in the presence of the electron-rich L-tyrosine-derived building block **4.8** was then expected to give rise to **4.7** by way of **4.9**.



Scheme 5. Synthesis of oxazole building block **4.11**

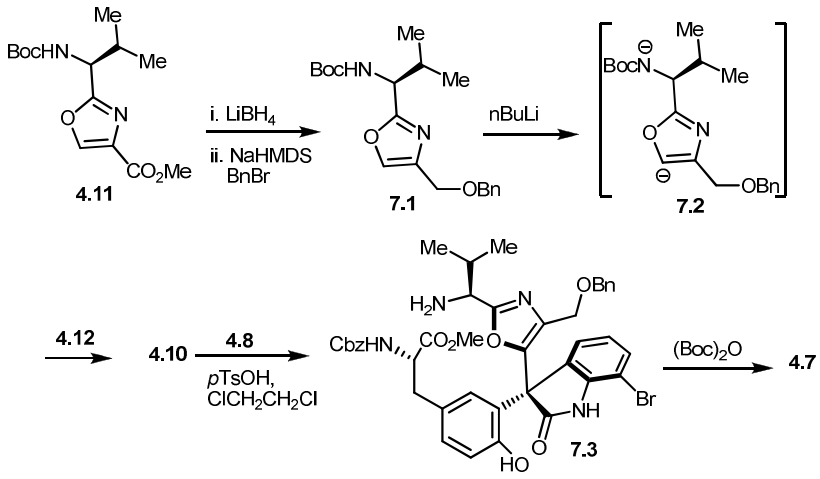
Synthesis of Diazonamide A

First, the construction of an oxazole fragment **4.11** is shown in **Scheme 5**. Starting with the merger of *Boc*-protected L-valine **5.1** and DL-serine methyl ester **5.2** led to obtain **5.3** in 92% yield through a standard peptide-coupling reaction as conducted by EDC and HOBt, and, following cyclization to oxazoline **5.4** through the action of Burgess reagent[73] in refluxing THF over the course of 4 h. Subsequent aromatization as effected by $BrCCl_3$ and DBU in DCM at 25 °C, and, this approach delivered the requisite fragment **4.11** in 72% overall yield.



Scheme 6. Synthesis of compounds **4.5** and **4.12**

As shown in **Scheme 6**, to access indole fragment **4.5** by selectively protecting the free primary amine of tryptamine **6.1**, a ketone carbonyl was installed through the action of DDQ in aqueous THF, and, then exposing the resultant product to TFA to concomitantly cleave the *NBoc* protecting group. This approach delivered the product as its stable TFA salt. The MOM-protected 7-bromoisatin **4.12** required four steps from 1-bromo-2-nitrobenzene **6.2**, by the way of 7-bromoindole **6.3**, in 43% yield.

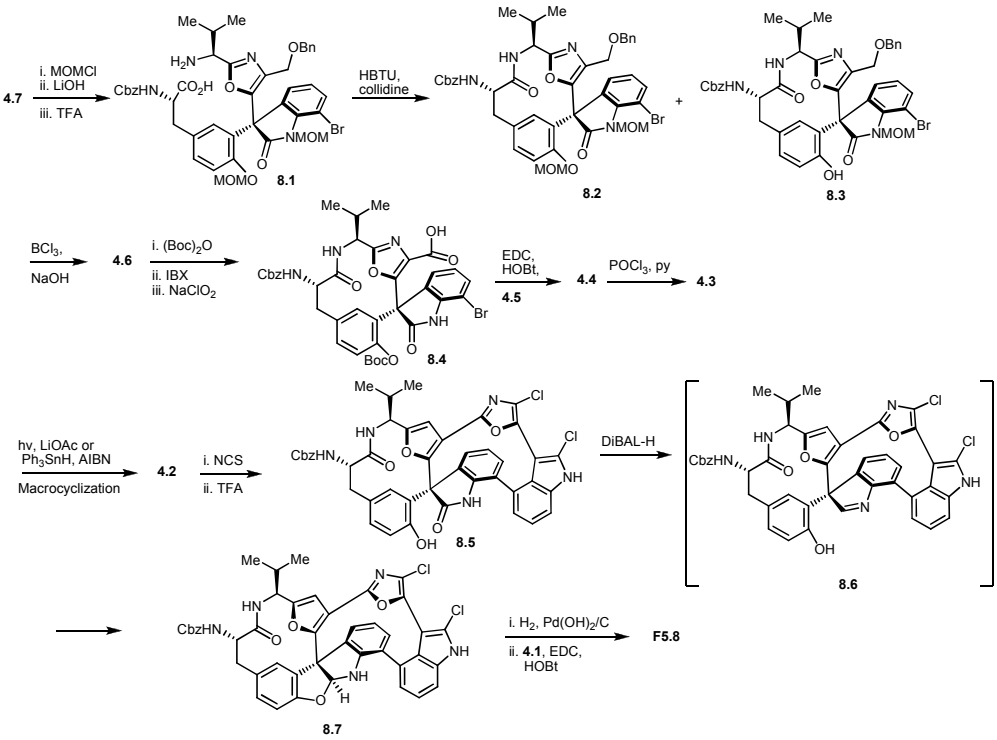


Scheme 7. Synthesis of compound **4.7**

Synthesis of the First Macrocyclic Unit

Compound **4.11** was converted into a protected primary alcohol **7.1**, and, then fragments were smoothly converted into **4.10** in 73% yield as shown in **Scheme 7**.

After extensive optimization conditions, the synthesis of **7.3** was accomplished in 47% yield by refluxing a solution containing **4.8** (4.0 equiv), **4.10** (1.0 equiv), and *p*-TsOH (4.0 equiv) in 1,2-dichloroethane followed by *NBoc* protection of free amine, which gave **4.7**, with a separable C-10 epimer. Finally, an intramolecular lactamization was achieved to obtain **8.2** along with a small amount of phenol **8.3** in 36% combined yield. The MOM and benzyl ethers were then removed through the action of BCl_3 in DCM at -78 °C, as shown in **Scheme 8**.



Scheme 8. Final stages and completion of the first total synthesis of diazonamide A

Completion of the Synthesis

Selectively protecting its H-ring phenol as a *NBoc* carbonate using $(Boc)_2O$ in a solvent mixture of aqueous $NaHCO_3$ and 1,4-dioxane (1:2) over the course of 24 h followed by the conversion of free hydroxyl group into a carboxylic acid through two iterative oxidations (IBX, $NaClO_2$), afforded **8.4**. Further, peptides coupling with **4.5** produced ketoamide **4.4**, followed by a Robinson-Gabriel cyclodehydration using pyridine-buffered $POCl_3$ at 25 °C afforded **4.3**. Compound **4.3** was then subjected to Witkop-type photocyclization conditions, and, this gave **4.2** with the desired stereochemistry. Alternatively, the conversion of **4.3** into **4.2** under free radical conditions by treating **4.3** with a slight excess of Ph_3SnH and catalytic amounts of

AIBN in refluxing benzene yielded 10% of **4.2**. After treating **4.2** with NCS at 60 °C for 2 h in a 1:1mixture of THF and CCl_4, this led to the site- and atropselective incorporation of the two requisite chlorines. This was then followed by *NBoc* deprotection to obtain **8.5**. After several optimization conditions, the synthesis of **8.7** was accomplished in 56% yield through the portion-wise addition of 100 equiv of DIBAL-H (5 x 20 equiv) to a solution of 50 in THF over 3 h, using a cooling and warming cycle between -78 °C and 25 °C after each addition. Finally, the *Cbz* group was removed chemoselectively, guarding the C-2 amine through a standard hydrogenation protocol using Pearlman's catalyst [20% $Pd(OH)_2/C$] in EtOH, followed by a peptide coupling with **4.1** gave diazonamide A in overall 21 steps.

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Chapter 2: Synthesis of *Enantioen-riched*, Benzofuran-Derived, Small Molecules: The Discovery of Novel Inhibitors of Pro-apoptotic Proteins, Bax and Bak

2.1. Introduction

Excess cell death via apoptosis is associated with auto-immune and degenerative disease.[1,2] Small molecules that interfere with the ordered series of protein:protein interactions by which the pro-apoptotic proteins Bax and Bak permeabilize mitochondria to kill cells would be useful as tools for studies in live cells and as leads for novel therapeutics.[3,4] The transient prevention of programmed cell death (PCD) is predicted to be of potential therapeutic benefit when cells are subjected to acute stress, such as, during ischemia, ischemia-reperfusion, inflammation as well as when exposed to other forms of stress, for example in normal tissues during cancer chemotherapy.[5,6,7,8,9] While there are many cellular strategies for avoiding apoptosis,[10,11,12] pharmacological inhibition of apoptosis has not been widely investigated. The multi-domain pro-apoptotic Bcl-2 family members Bax and Bak are theoretically attractive targets for the development of small molecule inhibitors due to their central importance in mitochondrial outer membrane permeabilization (MOMP), an event widely accepted as committing most cells to apoptosis.[13,14] MOMP results from an ordered series of steps beginning with activation of one or more Bcl-2 homology 3 proteins (BH3-proteins). Once activated, BH3-proteins bind to mitochondria, directly recruit and activate Bax and the constitutively membrane bound Bak.[15]

In some cases 'activation' involves releasing a previously activated Bax or Bak from inhibition by an anti-apoptotic protein of the Bcl-2 family.[16,17] The embedded together model suggests that membrane bound Bax recruits and activates both Bax and Bak by catalyzing insertion of the core helices 5-6 into the lipid bilayer. Oligomerization of integral membrane Bax and/or Bax culminates in membrane permeabilization.[8,9] Theoretically, MOMP could be prevented by inhibiting any one of the individual steps that lead to the oligomerization of Bax and Bak in the outer mitochondrial membrane. It is therefore not surprising that small molecules that alter the physical properties of membranes such as dibucaine, propranolol and cholesterol have all been shown to partially inhibit the insertion of Bax into membranes thereby reducing apoptosis.[18,19,20] **Figure 1** shows the detailed mechanisam of apoptosis from the perspective of the Bcl-2 family of proteins.[21] However, gross perturbation of cellular membranes is likely to have wide ranging effects on cellular physiology, meaning, such molecules are unlikely to be useful probes for studying the molecular

mechanism of Bax activation or as leads for the development of pharmaceuticals. Two other classes of Bax inhibitors have been reported; one of which is sufficiently hydrophobic that it is expected to partition into membrane.[18,22,20] While access to these molecules has been limited, it has been shown that they inhibit Bax but not Bak.[18] Furthermore, elegant electrophysiology studies suggested that they act as channel blockers rather than inhibiting Bax oligomerization.[23] However, permeabilization of mitochondria and liposomes by Bax releases large molecules like proteins. The molecular mechanism by which small molecule channel blockers prevent Bax mediated protein release from mitochondria is not clear.

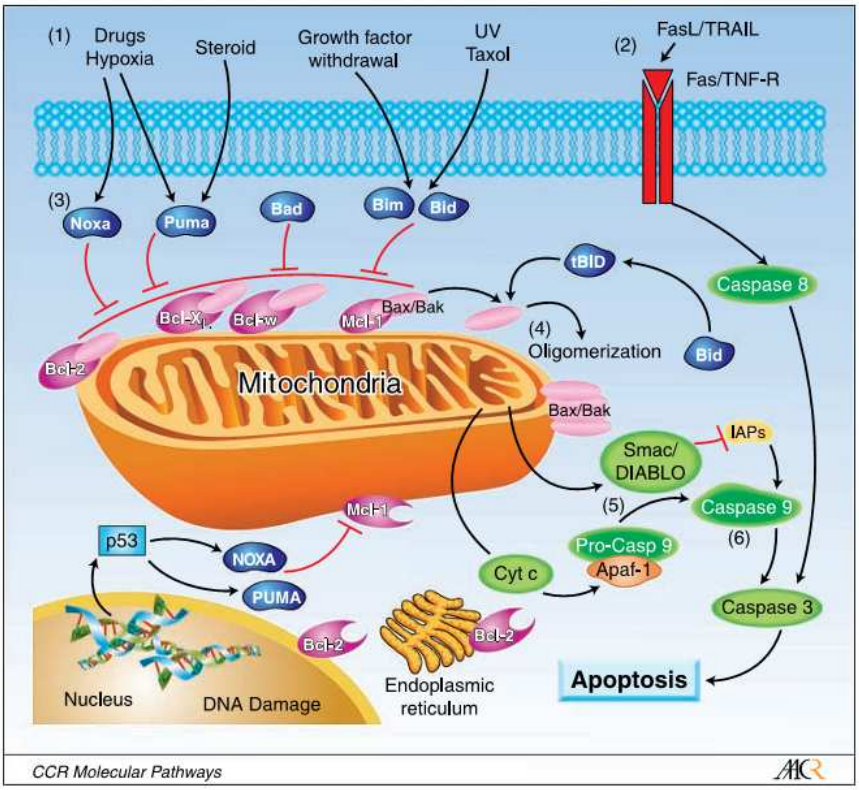
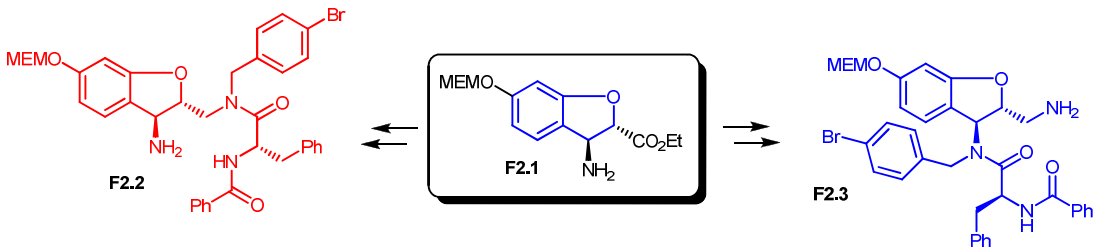


Figure 1. (This picture is taken from *Clin. Cancer Res.* **2009**, *15*, 1126) The apoptotic pathway to cell death from the perspective of the Bcl-2 family of proteins: (1), The intrinsic pathway is initiated by various signals, principally extracellular stimuli. (2), The extrinsic pathway is activated by Fas ligand or TRAIL, subsequently activa ting caspase-8. Caspase-8 transforms Bid into truncated Bid. In addition, caspase-8 initiates a cascade of caspase activation. (3), BH3-only proteins (Bim, Bid, Bad, Noxa, Puma) engage with anti-apoptotic Bcl-2 family proteins to relieve their inhibition of Bax and Bak to activate them Next, Bax and Bak are oligomerized and activated, leading to mitochondrial outer membrane permeabilization. (5), Once

mitochondrial membranes are permeabilized, cyto chrome c and/or Smac/DIABLO is released into the cytoplas m, where in they combine with an adaptor molecule, apoptosis protease-activating factor 1, and an inactive initiator caspase, procaspase-9, within a multiprotein complex called the apoptosome. Smac/DIABLO inhibits inhibitors of apoptosis proteins to activate caspase-9. (6), Caspase-9 activates caspase-3, which is the initiation step for the cascade of caspase activation. Intrinsic and extrinsic pathways converge on caspase-3. Bcl-2 family proteins are also found on the endoplasmic reticulum and the perinuclear membrane in hematopoietic cells, but they are predominantly localized to mitochondria.[21]

2.2. Working Hypothesis

With the objective, to accessing small molecules that are inspired by bioactive natural products having 3-dimensional architectures to explore their biological functions our group developed a modular method to access enantioenriched benzofuran derived 1,2-*trans* β–amino acid derivative **F2.1**.[24] Further explore chemical space around these scaffold, synthesized small libray of enantioenriched benzofuran derived compounds to explore their biological functions. Small library of our novel benzofuran derived natural product-inspired small molecules were screened our collaborator david andrew for identified inhibitors of tBid-Bax mediated liposome permeabilization. These results shown that, two of our compounds **F2.2 (MSN50)** and **F2.3 (MSN125)** shown in **Figure 2**, inhibited tBid-Bax mediated liposome permeabilization with IC_{50} 6 µM and 3 µM respectively.



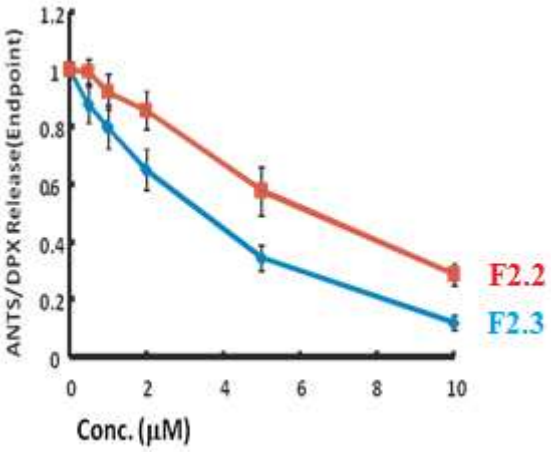


Figure 2. Novel Bax inhibitor from our lab **F2.2** and **F2.3**

With this object, I am interested in the synthesis of these two active compounds to undertake additional biological studies along with some molecules for structure–activity relationship (SAR) study as shown in **Scheme 3**. For the divesity sites, we varied R_1, R_2, R_3, and also instead of secondary amine, we selected the primary amine to coupled with different amino acid moieties.

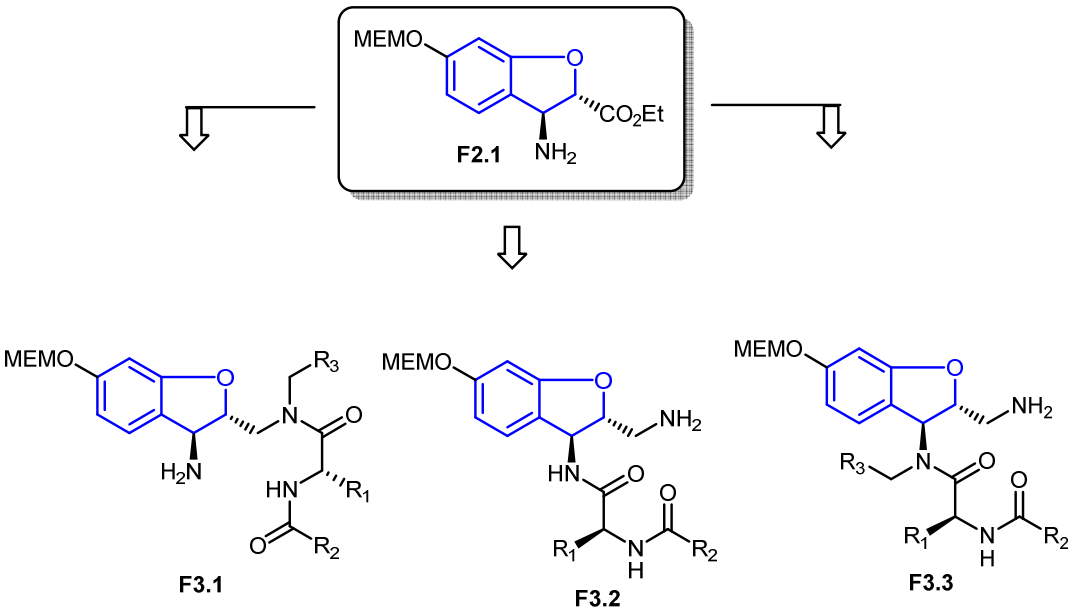
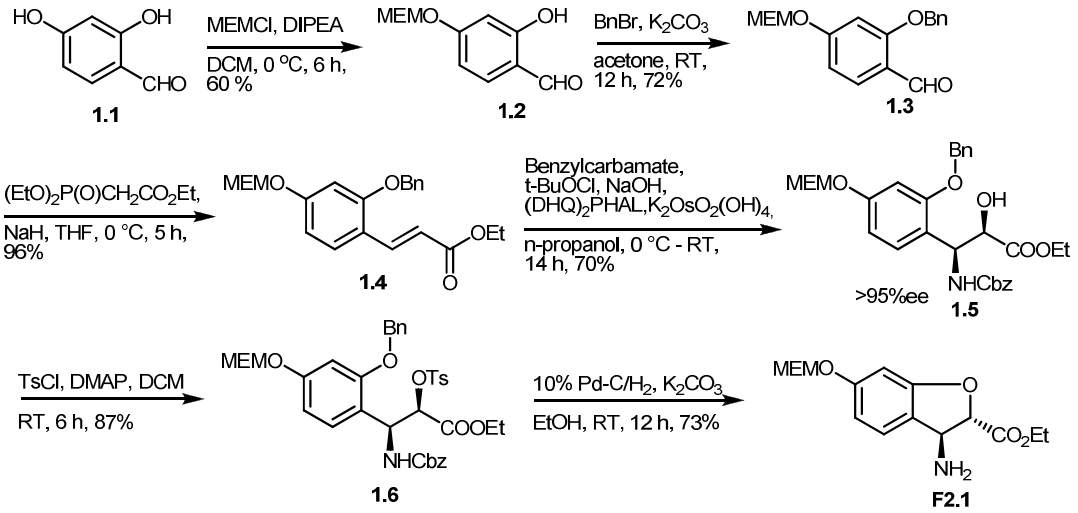


Figure 3. Designed *enantioenriched* benzofuran inspired targets

2.3. Results and Discussion

2.3.1. Synthesis of F2.1

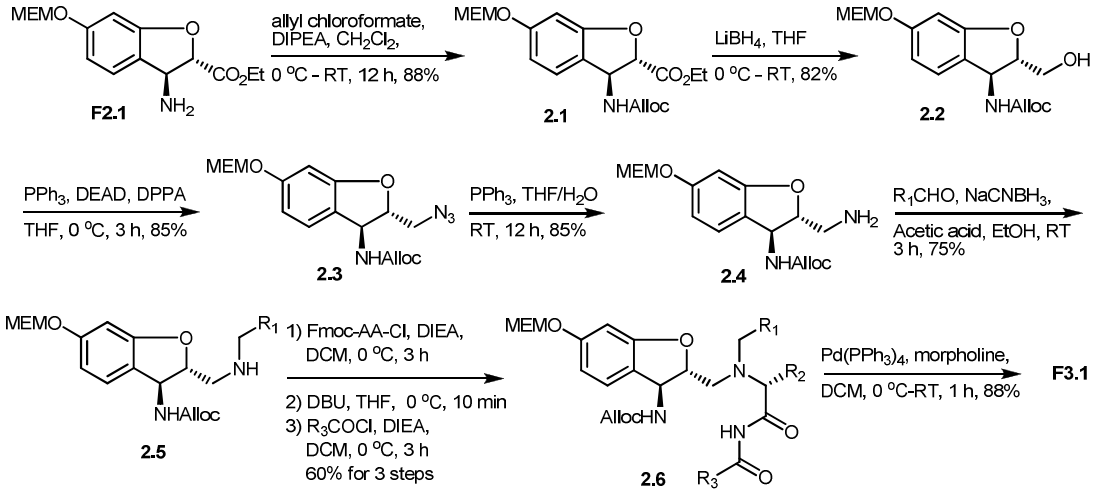
The synthesis of **F2.1** is shown in **Scheme 1**. To develop an enantioselective synthesis of benzofuran derived cyclic β-amino ester scaffold **F2.1**, 2,4-dihydroxy benzaldehyde **1.1** was subjected to a selective stepwise phenolic hydroxyl protections, as OMEM **1.2** and –OBn **1.3** (**Scheme 1**). Following the two carbon chain extension by Wittig-Horner reaction to obtain **1.4**, it was then subjected to Sharpless aminohydroxylation reaction,[25,26] which worked very-well even on a large scale and the *N*-protected aminohydroxyl product **1.5** was obtained in 75% yield (ee >95%). The hydroxyl group was then tosylated to obtain **1.6,** which to our delight, under hydrogenation conditions in the presence of a mild base (K_2CO_3), afforded the benzofuran derivative **F2.1**, following deprotection of the amino and the phenolic hydroxyl groups and the nucleophillic displacement of -OTs by the phenolic hydroxyl generated *in situ*. This process is highly simple, practical, and, both enantiomers of β-amino acids could be easily obtained in large quantities depending on the chirality of the ligand used for the aminohydroxylation reaction.



Scheme 1. Synthesis of *enantioenriched* benzofuran derived cyclic β-amino ester

2.3.2. Synthesis of F3.1

Having a large amount of *enantioenriched* benzofuran derived cyclic β-amino ester **F2.1** in hand, we then planed to synthesis of **F3.1**, **F3.2** and **F3.3** to explore the chemical space using ester and an amine functional group on the scaffold. In our plan the incorporation of amino acid was attractive to introduce a diverse array of chiral side chains having a variation in polarity.

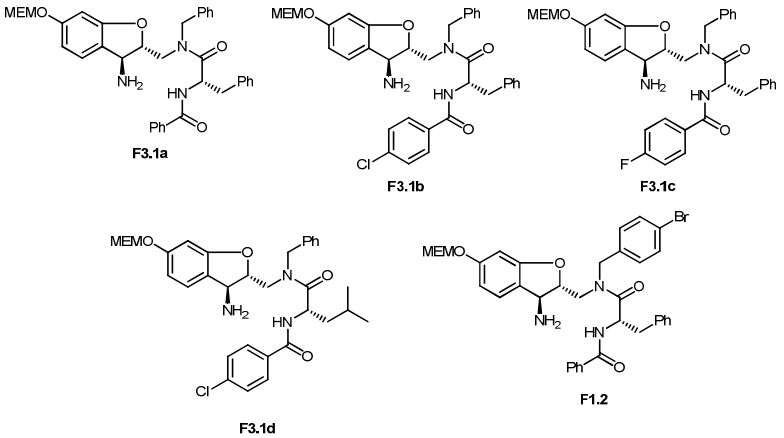


Scheme 2. Synthesis of compound F3.1

Synthesis of **F3.1** was started with -*N*alloc protection of amine **F2.1**, with allylchloro formate, DIPEA conditions gave **2.1** in good yield. Reduction of ester **2.1** with lithium borohydride provided an alcohol **2.2** in 82% yield. It was then subjected to Mitsunobu reaction under PPh_3, DEAD, DAAP conditions[27,28] that gave an azide with 85% yield. Azide **2.3** was reduced to amine **2.4** using Staudinger reaction (PPh_3, THF/H_2O condition),[29,30] and followed by a reductive imination of amine **2.4** under $NaCNBH_3$/AcOH condition yielded **2.5** with 75% yield. Coupling of -*N*Fmoc protected amino acid chloride with **2.5,** followed by the Fmoc removal under DBU condition, the subseqent amine was converted to amide with R_3COCl, DIEPA to obtain compound **2.6** with 60% overall yield in 3 steps. Deprotection of -*N*alloc of **2.6** with $Pd(PPh_3)_4$, morpholine condition gave the final product **F3.1** in a good yield.

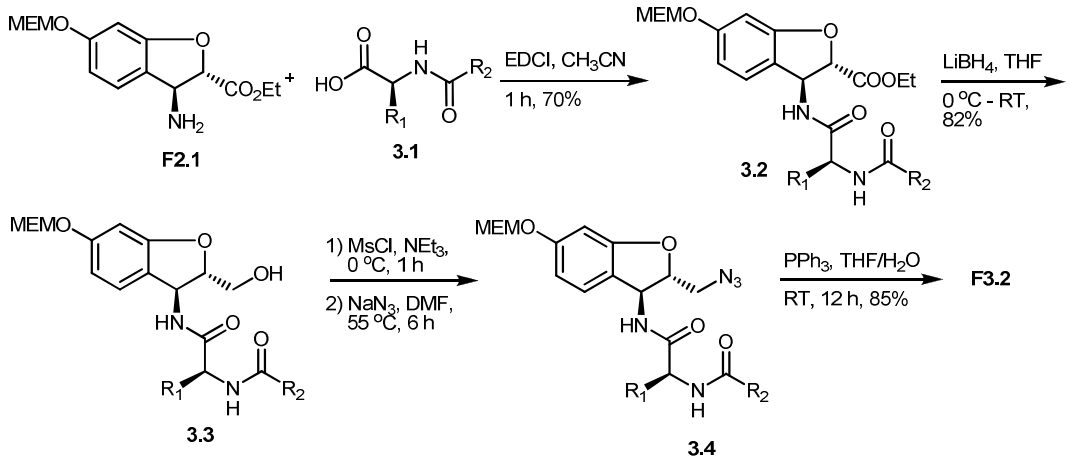
2.3.3. Derivatives of F3.1

We synthesized 5 derivatives by changing R_1, R_2, and R_3 and these are:



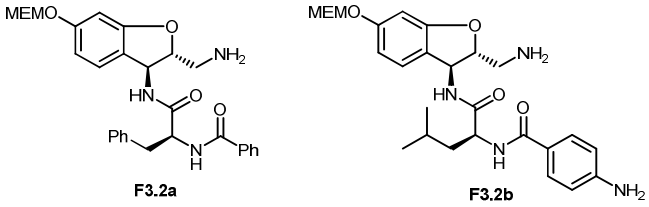
2.3.4. Synthesis of F3.2

Synthesis of **F3.2** is shown in **Scheme 3**. The coupling of modified amino acid **3.1** with benzylic amine **F2.1** under EDCI, acetonitrile conditions provided compound **3.2** with a good yield. Reduction of ester with lithium borohydride gave alcohol **3.3**. The alcohol **3.3** was then converted to mesylated product using $MsCl/NEt_3$ condition, and, subseqently the mesylated product was treated with NaN_3, at 55 ^oC in DMF solvent to obtain azide **3.4**. Finally, compound **F3.2** was synthesized from **3.4** under staudinger conditions in a good yield.



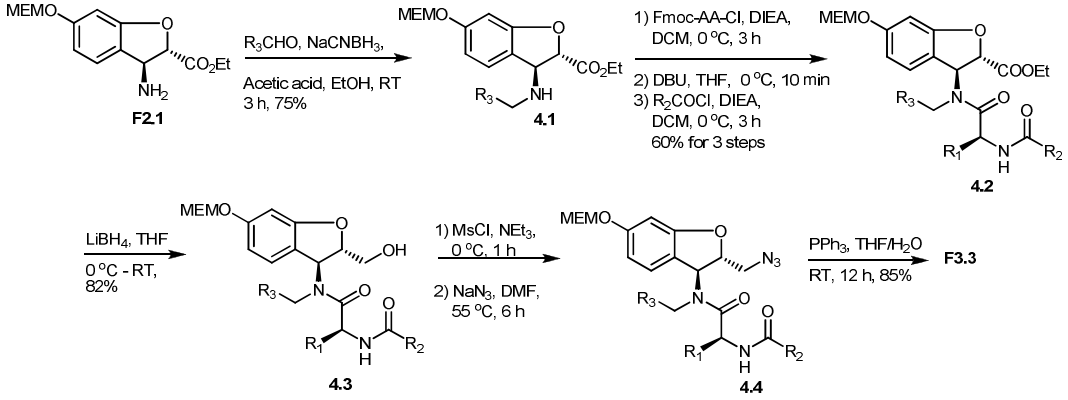
Scheme 3. Synthesis of compound F3.2

2.3.5. Derivatives of F3.2



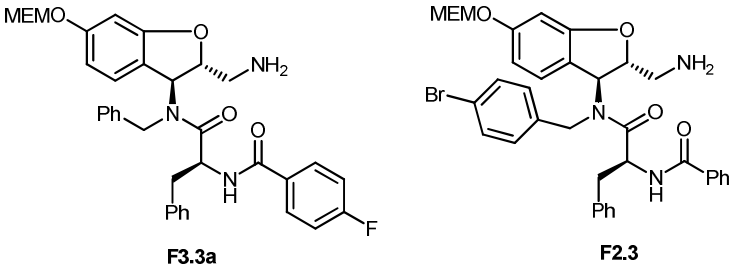
2.3.6. Synthesis of F3.3

In a similar manner, the synthesis of **F3.3** from an enantioenriched scaffold **F2.1** ws achieved and it is shown in **Scheme 4**. Reductive imination of **F2.1** under $NaCNBH_3$/AcOH condition[31] gave secondary amine **4.1** which was then coupled with *N*Fmoc amino acid chloride. It was then followed by *N*Fmoc deprotection with DBU, and the subseqent primary amine convertion to amide with R_2COCl/DIEA to provide **4.2**. Reduction of ester **(4.2)** with lithium borohydride conditions led the synthesis of alcohol **4.3.** The compound **4.3** was converted to **F3.3** using a similar procedure as described in the synthesis of **F2.3** from **3.3**.



Scheme 4. Synthesis of compound F3.3

2.3.7. Derivatives of F3.3

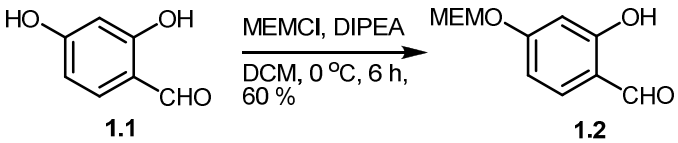


2.4. Conculsions

We identified two novel bax inhibitors **F2.2** and **F2.3** from our research group, and, further synthesized several *enantioenriched* benzofuran derivatives as analogs. The biological evalution of all our reported derivativesis ongoing and will be reported when available.

2.5. Experimental procedure

Synthesis of compound 1.2

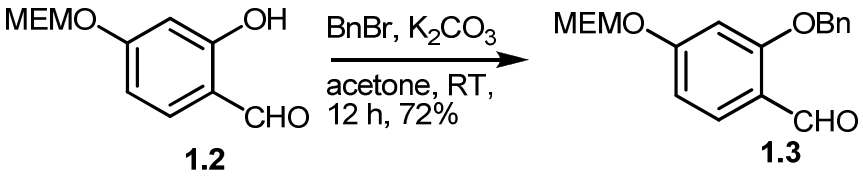


2-hydroxy-4-((2-methoxyethoxy)methoxy)benzaldehyde (1.2):

To a solution of 2,4-dihydroxybenzaldehyde **1.1**(10.0 g, 72.3 mmol) in DCM (100 mL) at -5 °C was added DIPEA (12.50 mL, 72.3 mmol) over a 5 minute period dropwise. The following mixture was stirred for 5 minutes followed by the addition of 2-methoxy ethoxymethyl chloride (8.20 mL, 72.3 mmol). The solution was stirred at -5 °C for 6 hours. The reaction was then quenched with water (50 mL) at 0 °C and

extracted with DCM (3 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to produce yellowish oil. Purification by flash chromatography to afforded **1.2**(14.9 g, 91%) as a colorless oil.

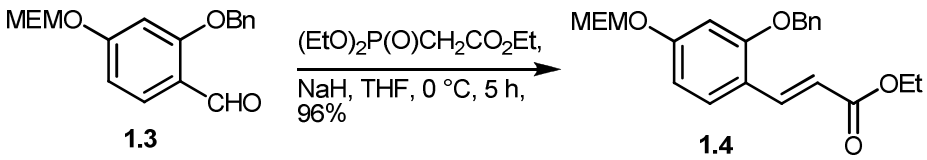
Synthesise of compound 1.3



1.2 **1.3**

To a solution of **1.2** (14.9 g, 69.08 mmol) in acetone (200 mL) at room temperature was added K_2CO_3 (38.19 g, 276.35 mmol). The suspension was stirred for 5 minutes followed by the subsequent addition of benzyl bromide (8.22 mL, 69.08 mmol). The reaction mixture was stirred for 12 hours at room temperature and then filtered through a 5 cm celite pad and concentrated in vacuo to produce yellowish oil. Purification by flash chromatography to afforded **1.3.**

Synthesis of compound 1.4

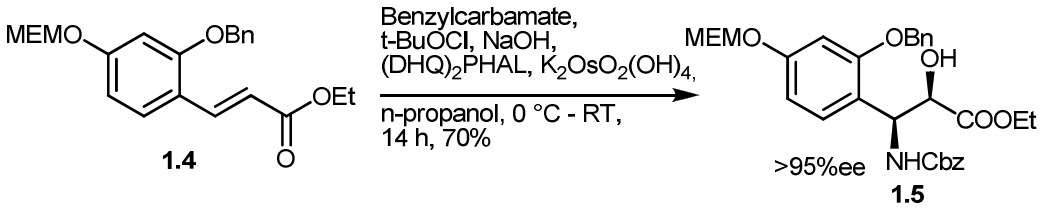


1.3 **1.4**

(E)-ethyl3-(2-(benzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl)acrylate (1.4):

To a suspension of NaH (2.87 g, 61.1 mmol) in dry THF (100 mL) at 0 °C was slowly added triphenylphosphinoethyl acetate (10.91 mL, 55.01 mmol) for 5 minutes. The reaction mixture was then allowed to stir for 30 minutes at 0 °C and then aldehyde **1.3** (15 g, 47.4 mmol) dissolved in dry THF (50 mL) was added to it slowly. After being stirred for 5 hours at 0 °C under the N_2 atmosphere the reaction mixture was quenched with water very slowly at 0 °C and extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography to afforded **1.4.**

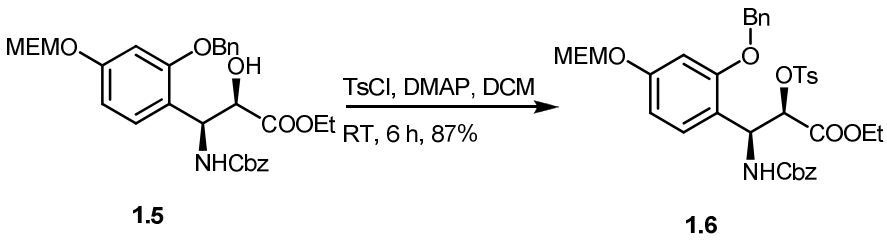
Synthesis of compound 1.5



(2R,3S)-ethyl3-(2-(benzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl)-3-(benzyl oxycarbonylamino)-2-hydroxypropanoate (1.5):

Benzyl carbamate (14.08 g, 93.3 mmol) was dissolved in n-propanol (124 mL). To this stirring solution a freshly prepared solution of NaOH (3.73 g, 93.3 mmol in 232 mL of distilled water) was added, followed by the subsequent additions of the freshly prepared tBuOCl (10.7 mL, 93.3 mmol), and the solution of $(DHQ)_2PHAL$ ligand (1.240 g, 1.59 mmol in 108 mL of n-propanol) at 0 °C. The reaction mixture was stirred at room temperature for 5 minutes, and then **1.4** (12 g, 31.1 mmol in 30mL of n-propanol) was added followed by the addition of the osmium catalyst ($K_2OsO_2(OH)_4$, 480 mg 1.30 mmol). The resulting mixture was stirred for 3 hours at room temperature. The solution turned from a dark green to a dark yellow over the period. Once reaction completed, the reaction mixture was extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dark brown oil. Purification by flash chromatography to obtained **1.5.**

Synthesis of compound 1.5

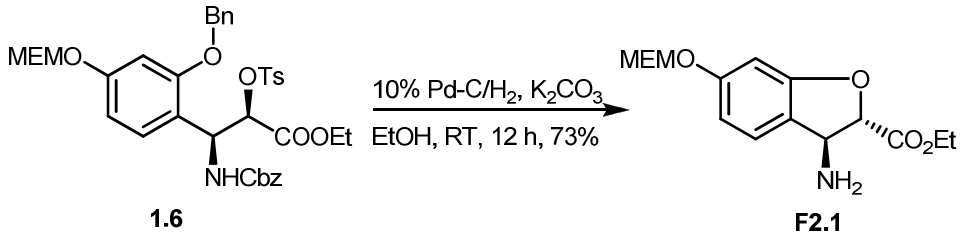


(2R,3S)-ethyl 3-(2-(benzyloxy)-4-((2-methoxyethoxy)methoxy)phenyl)-3-(benzyl oxycarbonylamino)-2-(tosyloxy)propanoate (1.6):

Tosyl chloride (4.55 g, 23.9 mmol) was added to a stirred solution of **1.5** (11 g, 19.9 mmol) and DMAP (3.64 g, 29.9 mmol) in DCM (100 mL) at room temperature. The reaction mixture was refluxed for 12 hours at 40 °C. After completion of the reaction the solution was washed with saturated aqueous $NaHCO_3$ solution (3 x 20 mL) and combined organic layer was extracted with DCM, washed with brine, dried over

anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography afforded **1.6.**

Synthesis of compound F2.1

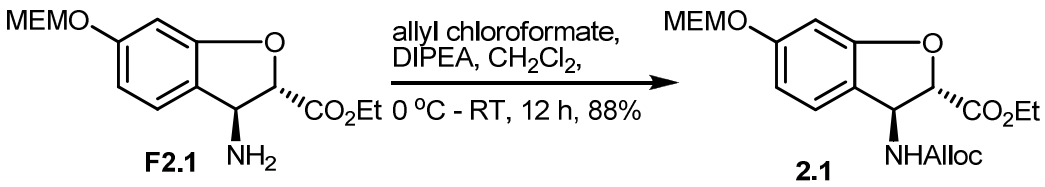


1.6 **F2.1**

(2S,3S)-ethyl 3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzo fura n-2- carboxylate (F2.1):

To a stirred solution of compound **1.6** (13 g, 18.4 mmol) in 95% ethanol (130 mL) was added 10% Pd/C (1.3 g) followed by the addition of anhydrous K_2CO_3 (5.1 g, 36.8 mmol). The solution was stirred under H_2 atmosphere for 48 hours at room temperature. The reaction mixture was then filtered through a celite pad (5 cm) and concentrated into pale yellow oil. Purification by flash chromatography to afforded **F2.1**.

Synthesis of compound 2.1



F2.1 **2.1**

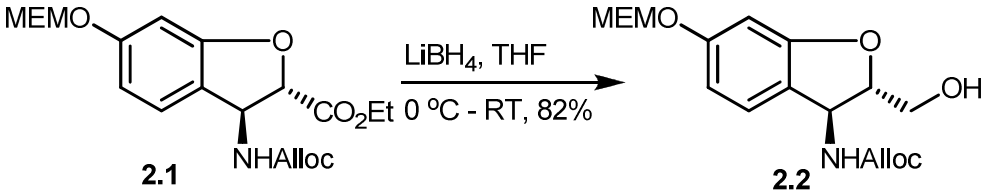
(2S,3S)-ethyl-3-(allyloxycarbonylamino)-6-((2-methoxyethoxy)methoxy)-2,3di hydrobenzofuran-2-carboxylate (2.1):

To a stirred solution of compound **F2.1 (**1.25 g, 4.01 mmol, 1eq) in dry DCM (15 mL) was added DIEA (1.5 ml, 6.02 mmol, 1.5 eq) at 0 °C under inert atmosphere. After 5 minute, AllocCl (4.83 mmol, 1.2 eq) was added. The reaction mixture was stirred for 4 h. The reaction mixture was quenched by the addition of a saturated *aq*. $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (30% ethyl acetate in hexane) afforded compound **2.1** (0.38 g, 80 %) as a white solid.

Molecular Formula: $C_{19}H_{25}NO_8$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 80%; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.30 (t, J = 7.1 Hz, 3H), 3.37 (s, 3H), 3.56 (d, J =

4.5 Hz, 2H), 3.80 (d, *J* = 4.5 Hz, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 4.58-4.65 (m, 2H), 4.93 (d, *J* = 3.8 Hz, 1H), 5.06-5.13 (m, 1H), 5.20-5.26 (m, 3H), 5.31 (d, *J* = 17.0 Hz, 1H), 5.47 (dd, *J* = 2.9, 1.3 Hz, 1H), 5.85-6.00 (m, 1H), 6.63-6.69 (m, 2H), 7.17 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 14.0, 56.8, 58.9, 61.8, 65.8, 67.7, 71.4, 86.5, 93.5, 99.1, 110.0, 117.9, 125.3, 132.4, 155.1, 159.7, 160.6, 169.1; LRMS: (ES+) m/z = 396 (m+1).

Synthesis of compound 2.2

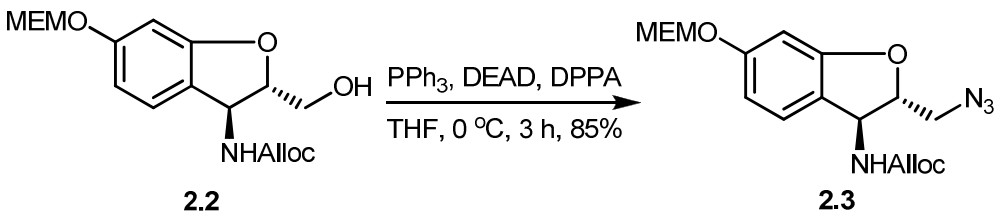


allyl(2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-ylcarbamate (2.2):

To a stirred solution of compound **2.1 (**0.5 g, 1.26 mmol) in dry THF (10 mL) was added a solution of 2M $LiBH_4$ in THF of (0.62 mL, 1.26 mmol) at 0 °C under inert atmosphere. The reaction was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was quenched by addition of a saturated *aq*.NH_4Cl, extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (40% ethyl acetate in hexane) afforded compound **2.2** (0.38 g) as a white solid.

Molecular Formula: $C_{17}H_{23}NO_7$; R_f (40% ethyl acetate/hexane): 0.2; Yield: 82%; ^{1}H NMR ($CDCl_3$, 400 MHz): 3.21 (s, 3H), 3.39 (m, 2H), 3.81-3.88 (m, 4H), 4.59-4.61 (m, 3H), 5.14-5.35 (m, 6H), 5.90-5.94 (m, 1H), 6.58 (s, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 7.16 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 56.0, 59.4, 63.9, 66.5, 68.1, 71.9, 91.8, 93.9, 98.4, 99.4, 109.9, 118.6, 118.7, 125.8, 132.7, 156.5, 160.0, 161.2; MS: (ES+) m/z = 354 (M+1).

Synthesis of compound 2.3

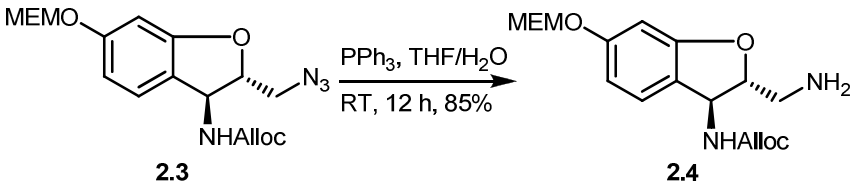


allyl(2R,3S)-2-(azidomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-ylcarbamate (2.3):

To a solution of alcohol **2.2** (0.047 g, 0.097 mmol) in 3.88 mL of THF was added triphenylphosphine (0.026 g, 0.099 mmol), DEAD (0.019 mL, 0.099 mmol), and diphenylphosphoryl azide (0.021 mL, 0.099 mmol) at 0 °C and stirred for 3 hours. The reaction was quenched with saturated $NaHCO_3$ and extracted with ethyl acetate (3 x 10 mL). The combined organics were dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by silica gel column chromatography to afford azide **2.3** (0.042 g) as brownish liquid.

Molecular Formula: $C_{17}H_{22}N_4O_6$; R_f (30% ethyl acetate/hexane): 0.2; Yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.39 (s, 3H), 3.50-3.60 (m, 3H), 3.70-3.74 (m, 1H), 3.81-3.84 (m, 2H), 4.61 (d, *J* = 5.52 Hz, 2H), 4.65-4.69 (m, 1H), 5.04-5.07 (m, 1H), 5.12-5.14 (m, 1H), 5.25-5.27 (m, 3H), 5.28-5.35 (d, *J* = 17.5 Hz, 1H), 5.87-5.98 (m, 1H) 6.62 (d, *J* = 2.0 Hz, 1H), 6.65-6.68 (m, 1H), 7.17-7.19 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 53.8, 56.1, 59.4, 66.3, 68.2, 71.9, 90.2, 94.0, 99.5, 110.1, 118.4, 125.7, 132.8, 156.0, 160.2, 161.1; MS: (ES+) m/z = 379 (M+1).

Synthesis of compound 2.4



2.3 **2.4**

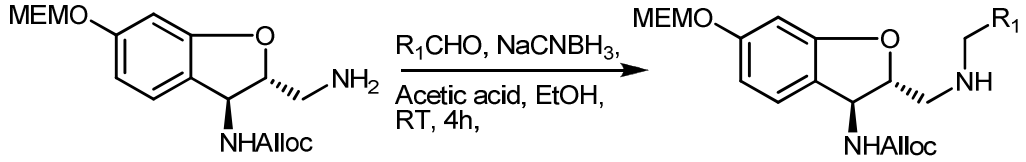
allyl(2R,3S)-2-(aminomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzo furan-3-ylcarbamate (2.4):

To a stirred solution of azide compound **2.3** (0.225 g, 0.59 mmol) in THF (3 mL) was added TPP (0.166 g, 0.63 mmol) and water (100 µL). The reaction mixture was left for stirring for 12 h at room temperature. After the completion of the reaction THF was removed under vacuo and purified by flash column using DCM, MeOH solvent system to afford pure amine **2.4** as colorless liquid.

Molecular Formula: $C_{17}H_{24}N_2O_6$; R_f (100% ethyl acetate): 0.2; Yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.96-3.03 (m, 1H), 3.09-3.14 (m, 1H), 3.40 (s, 3H), 3.56-3.59 (m, 2H), 3.81-3.84 (m, 2H), 4.48-4.53(m, 1H), 4.62 (d, *J* = 5.0 Hz, 2H), 5.05-5.12 (m, 1H), 5.25-5.28 (m, 3H), 5.31-5.38 (d, *J* = 16.5 Hz, 1H), 5.87-5.99 (m, 1H),

6.57 (d, *J* = 2.0 Hz, 1H), 6.64 (d, *J* = 2.5 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 45.4, 56.1, 59.4, 66.2, 68.1, 71.9, 93.2, 94.0, 99.3, 109.6, 118.4, 119.3, 125.9, 132.9, 156.0, 160.0, 161.3; MS: (ES+) m/z = 353 (m+1).

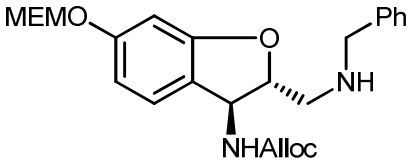
Synthesis of compound 2.5



2.4 **2.5**

The pure amine **2.4** (1eq) and R_1CHO (1.1 eq) dissolved in EtOH, and a solution of $NaCNBH_4$ (1.3 eq) in EtOH/AcOH (catalytic amount) was added to the above mixture at room temperature. The reaction mixture was stirred for 4 h. The reaction was quenched with saturated NH_4Cl solution and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to give the product **2.5.**

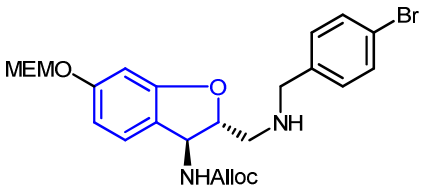
allyl(2R,3S)-2-((benzylamino)methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihyd robenzofuran-3-ylcarbamate (2.5a):



2.5a

Molecular Formula: $C_{24}H_{30}N_2O_6$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.97 (m, 1H), 3.36 (s, 3H), 3.48-3.57 (m, 2H), 3.80 (m, 4H), 4.52-4.66 (m, 3H), 5.08-5.37 (m, 6H), 5.87-5.89 (m, 1H), 6.49-6.65 (m, 2H), 7.13 (d, *J* = 8.2 Hz, 1H), 7.19-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 51.8, 53.8, 56.2, 58.9, 65.7, 67.7, 71.5, 90.5, 93.5, 98.9, 109.2, 117.9, 119.1, 125.5, 126.9, 128.1, 128.3, 132.5, 140.0, 155.6, 159.5, 160.7; LRMS: (ES+) m/z = 443 (M+1).

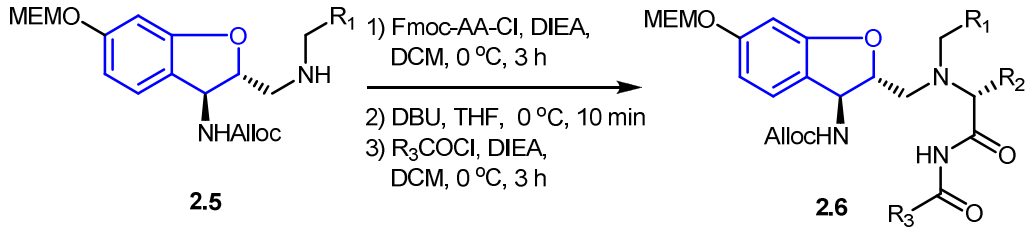
allyl(2R,3S)-2-((4-bromobenzylamino)methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl carbamate (2.5b):



2.5b

Molecular Formula: $C_{24}H_{29}BrN_2O_6$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 77%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.90-2.95 (m, 1H), 3.01-3.04 (m, 1H), 3.39 (s, 3H), 3.56-3.58 (m, 2H), 3.81-3.83 (m, 4H), 4.60-4.65 (m, 3H), 5.03 (d, *J* = 7.5 Hz, 1H), 5.14-5.17 (m, 1H), 5.23-5.26 (m, 3H), 5.31-5.35 (m, 1H), 5.89-5.99 (m, 1H), 6.56 (d, *J* = 2.0 Hz, 1H), 6.61 (d, *J* = 2.0 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.44 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 52.1, 53.5, 56.6, 59.4, 66.2, 68.1, 71.9, 91.0, 94.0, 99.3, 109.7, 118.4, 119.3, 121.1, 125.9, 130.2, 131.8, 132.9, 139.5, 156.0, 160.0, 161.2; MS: (ES+) m/z = 521 (M+1), 523 (M+3).

Synthesis of compound 2.6



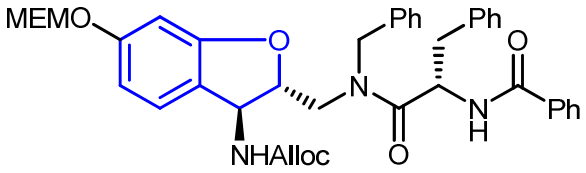
2.5 **2.6**

To the stirred solution of **2.5** (1eq) in dry DCM added DIEA (1.5eq) at 0 °C under inert atmosphere. After 5 min freshly prepared Fmoc protected amino acid chloride (1.5 eq) in DCM, was added slowly. Reaction mixture was stirred at room temperature and reaction monitored by TLC. The reaction mixture was quenched by the addition of a saturated *aq*. $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography. The pure Fmoc amino acid coupled product was subjected to Fmoc removal, using DBU (1.2 eq) in THF. This reaction completed in 10 min, then concentrated the solvent *in vacuo.*

To a suspension of above amine (1 eq) in DCM (10 mL), added DIEA (1.5eq) followed by acid chloride (R_2COCl) (1.5 eq) at 0 °C under inert atmosphere. After completion of the reaction, reaction mixture was quenched with sodium bicarbonate solution (5 mL), concentrated, and extracted with ethyl acetate (3 X 20 mL).

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 to give pure compound **2.6.**

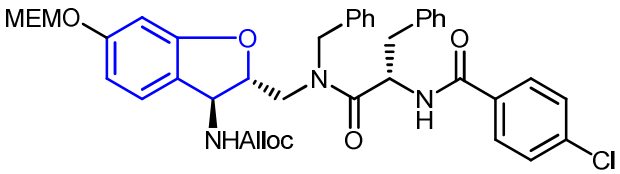
allyl(2R,3S)-2-(((S)-2-benzamido-N-benzyl-3-phenylpropanamido)methyl)-6-((2-meth oxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylcarbamate (2.6a):



2.6a

Molecular Formula: $C_{40}H_{43}N_3O_8$; R_f (30% ethyl acetate/hexane): 0.1; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.00-3.30 (m, 3H), 3.39 (s, 3H), 3.61-3.50 (m, 2H), 3.75-3.87 (m, 2H), 4.27-4.98 (m, 6H), 5.10-5.46 (m, 6H), 5.81-5.99 (m, 1H), 6.48-6.68 (m, 2H), 6.98 (bs, 1H), 7.11-7.57 (m, 14H), 7.73-7.84 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 694 (M+1).

allyl(2R,3S)-2-(((S)-N-benzyl-2-(4-chlorobenzamido)-3-phenylpropanamido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylcarbamate (2.6b):

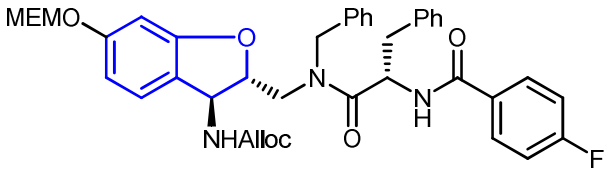


2.6b

Molecular Formula: $C_{40}H_{42}ClN_3O_8$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.96-3.30 (m, 4H), 3.36 (s, 3H), 3.52-3.54 (m, 3H), 3.74-3.83 (m, 2H), 4.51-4.57 (m, 3H), 4.66-4.87 (m, 2H), 5.10-5.42 (m, 6H), 5.77-5.94 (m, 1H), 6.58 (bs, 2H), 6.95 (s, 1H), 7.07-7.32 (m, 9H), 7.34-7.37 (m, 3H), 7.64-7.70

(m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 727 (M-1).

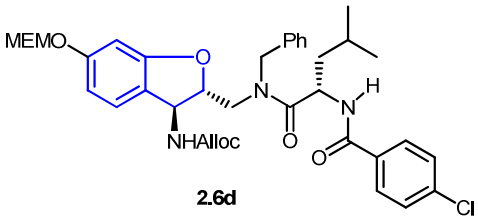
allyl(2R,3S)-2-(((S)-N-benzyl-2-(4-fluorobenzamido)-3-phenylpropanamido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylcarbamate (2.6c):



2.6c

Molecular Formula: $C_{40}H_{42}FN_3O_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.96-3.30 (m, 4H), 3.36 (s, 3H), 3.52-3.54 (m, 3H), 3.74-3.83 (m, 2H), 4.51-4.57 (m, 3H), 4.66-4.87 (m, 2H), 5.10-5.42 (m, 6H), 5.77-5.94 (m, 1H), 6.58 (bs, 2H), 6.95 (s, 1H), 7.07-7.32 (m, 9H), 7.34-7.37 (m, 3H), 7.64-7.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 710 (M-1).

allyl(2R,3S)-2-(((S)-N-benzyl-2-(4-chlorobenzamido)-4-methylpentanemido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylcarb amate (2.6d):

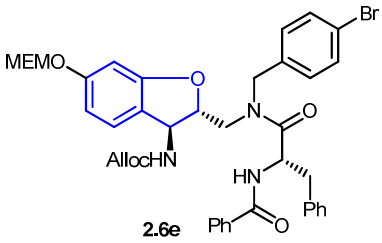


2.6d

Molecular Formula: $C_{37}H_{44}ClN_3O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 58%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.85-1.00 (m, 6H), 1.59 (dd, *J* = 14.3, 7.6 Hz, 2H), 1.72 (bs, 1H), 3.37 (s, 3H), 3.55-3.60 (m, 3H), 3.77-4.01 (m, 3H), 4.44-4.60 (m, 2H),

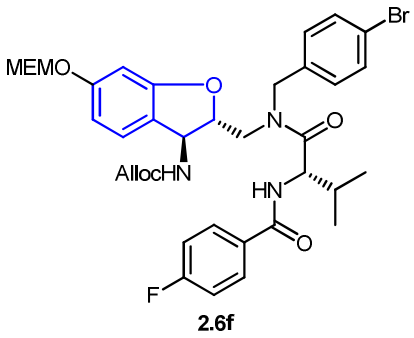
4.66-4.79 (m, 2H), 4.91-5.11 (m, 2H), 5.16-5.36 (m, 6H), 5.81-5.97 (m, 1H), 6.55-6.69 (m, 2H), 7.13-7.47 (m, 10H), 7.78 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.6, 23.2, 24.7, 29.6, 42.1, 48.2, 52.3, 56.0, 59.0, 65.7, 67.7, 71.5, 88.7, 93.5, 98.8, 109.5, 118.5, 126.6, 128.5, 128.6, 128.7, 128.7, 128.9, 131.3, 132.3, 132.4, 136.1, 137.7, 155.4, 159.6, 159.7, 160.5, 165.7, 165.9, 174.1; LRMS: (ES+) m/z = 695 (M+1).

allyl(2R,3S)-2-(((S)-2-benzamido-N-(4-bromobenzyl)-3-phenylpropanamido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylcarbama te (2.6e):



Molecular Formula: $C_{40}H_{42}BrN_3O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR ($CDCl_3$, 400 MHz, 58 ^{o}C) δ ppm 3.11-3.28 (m, 2H), 3.38-3.40 (m, 3H), 3.53-3.60 (m, 2H), 3.78-3.85 (m, 3H), 4.26-5.00 (m, 6H), 5.16-5.39 (m, 6H), 5.50 (bs, 1H), 5.83-6.00 (m, 1H), 6.49-6.76 (m, 3H), 6.83-7.00 (m, 2H), 7.11-7.28 (m, 4H), 7.35-7.54 (m, 6H), 7.71-7.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 772 (M+1), 774 (M+3).

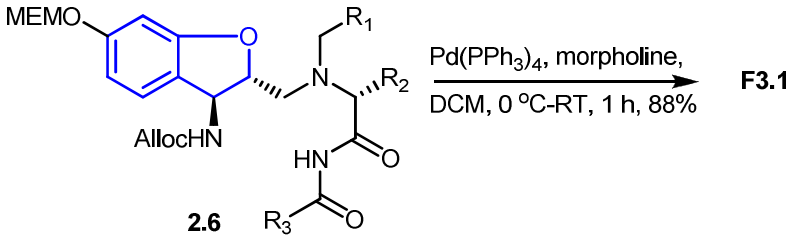
allyl(2R,3S)-2-(((S)-N-(4-bromobenzyl)-2-(4-fluorobenzamido)-3-methylbutan amido)methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl carbamate (2.6f):



2.6f

Molecular Formula: $C_{36}H_{41}BrFN_3O_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.80-1.00 (m, 6H), 2.07 (bs, 1H), 3.36 (s, 3H), 3.55 (d, *J* = 3.2 Hz, 3H), 3.79 (bs, 3H), 4.41-4.61 (m, 3H), 4.70-4.76 (m, 2H), 4.81-4.99 (m, 2H), 5.04 (bs, 1H), 5.09-5.39 (m, 6H), 5.79-5.97 (m, 1H), 6.50 (bs, 1H), 6.60-6.61 (m, 2H), 7.10 (m, 6H), 7.44-7.46 (m, 2H), 7.85 (d, *J* = 3.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 17.2, 19.7, 29.6, 31.5, 48.9, 51.4, 54.3, 58.9, 65.7, 67.6, 71.4, 88.2, 93.4, 98.8, 109.5, 115.3, 109.8, 117.8, 118.3, 121.4, 121.7, 125.7, 128.5, 129.4, 131.6, 132.0, 135.1, 135.9, 155.3, 159.6, 159.7, 163.4, 166.0, 172.8; LRMS: (ES+) m/z = 742 (M+1), 744 (M+3).

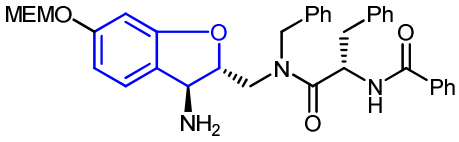
Synthesis of compound F3.1



2.6

To a solution of **2.6** (1 eq) in dry CH_2Cl_2 (5 mL) under nitrogen atmosphere at 0 °C, was added morpholine (1.5 eq) and tetrakis(triphenylphosphine) palladium (0) catalyst (0.1 eq). The round-bottom flask containing the mixture was covered with aluminum foil and stirred for 1 h. TLC showed the completion of the reaction. The reaction was quenched with saturated NH_4Cl solution and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give crude compound which upon flash column chromatography to afford amine.

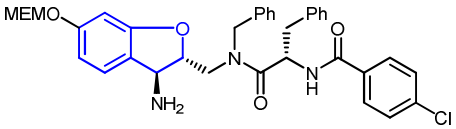
N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzo furan-2-yl)methyl)(benzyl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide (F3.1a):



F3.1a

Molecular Formula: $C_{36}H_{39}N_3O_6$; R_f (10% MeOH\DCM): 0.1; Purified by flash chromato graphy using 100% ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.99 (bs, 2H), 3.02-3.25 (m, 2H), 3.32-3.44 (m, 4H), 3.46-3.61 (m, 5H), 3.62 -3.71(m, 1H), 3.74-3.86 (m, 2H), 3.64-3.69 (m, 1H), 4.58-4.61 (bs, 1H), 4.75-4.91 (m, 1H), 5.25 (bs, 2H), 5.34-5.48 (m, 1H), 6.48-6.56 (m, 2H), 7.00-7.22 (m, 6H), 7.23-7.33 (m, 3H), 7.37-7.58 (m, 5H), 7.62-7.71 (m, 1H), 7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0, 136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 608 (M+1).

N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzo furan-2-yl)methyl)(benzyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-chlorobenzam ide (F3.1b):

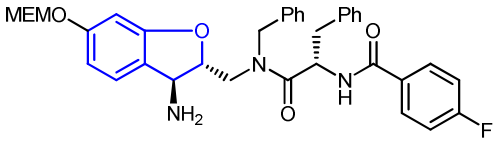


F3.1b

Molecular Formula: $C_{36}H_{38}ClN_3O_6$; R_f (10% MeOH\DCM): 0.1; Purified by flash chromatography using 100% ethyl acetate; ^{1}H NMR (400 MHz, CDCl3) δ ppm 2.86-2.95 (bs, 2H), 3.03-3.06 (m, 4H), 3.37 (s, 3H), 3.49-3.55 (m, 2H), 3.70-3.72 (m, 2H), 3.79-3.80 (m, 2H), 4.02-4.25 (m, 1H), 4.55-4.64 (m, 1H) 4.74-4.90 (m, 1H), 5.21 (bs, 2H), 6.46-6.70 (m, 2H), 7.04-7.07 (m, 2H), 7.13-7.41 (m, 9H), 7.40-7.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0,

136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 663 (M-1).

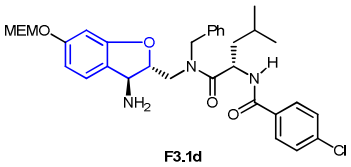
N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl)(benzyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-fluorobenzamide (F3.1c):



F3.1c

Molecular Formula: $C_{36}H_{38}FN_3O_6$; R_f (10% MeOH\DCM): 0.1; Purified by flash chromatography using 100% ethyl acetate; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.86-2.95 (bs, 2H), 3.03-3.06 (m, 4H), 3.37 (s, 3H), 3.49-3.55 (m, 2H), 3.70-3.72 (m, 2H), 3.79-3.80 (m, 2H), 4.02-4.25 (m, 1H), 4.55-4.64 (m, 1H) 4.74-4.90 (m, 1H), 5.21 (bs, 2H), 6.46-6.70 (m, 2H), 7.04-7.07 (m, 2H), 7.13-7.41 (m, 9H), 7.40-7.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0, 136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 650 (M+Na).

N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl)(benzyl)amino)-4-methyl-1-oxopentan-2-yl)-4-chlorobenzamide (F3.1d):

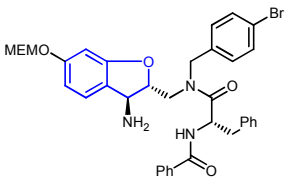


F3.1d

Molecular Formula: $C_{36}H_{38}BrN_3O_6$; R_f (10% MeOH/DCM): 0.2; Purified by flash chromato graphy using 100% ethyl acetate; Yield: 93%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.84-1.00 (m, 6H), 1.59 (bs, 2H), 2.03 (d, *J* = 5.3 Hz, 1H), 3.37 (s, 3H), 3.55-3.58 (m, 4H), 3.80-3.90 (m, 3H), 4.29 (d, *J* = 5.2 Hz, 1H), 4.42-4.63 (m, 1H), 4.78 (bs, 1H), 4.91 (bs, 1H), 5.14-5.29 (m, 3H), 6.60-6.65 (m, 2H), 7.12-7.45 (m, 8H), 7.74 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.4, 22.6, 23.3, 24.7,

29.6, 42.3, 52.2, 56.8, 59.0, 67.6, 71.5, 91.7, 93.6, 93.6, 98.8, 109.2, 124.9, 126.8, 127.8, 127.9, 128.6, 128.6, 128.7, 128.7, 128.9, 132.2, 136.1, 136.8, 137.8, 158.8, 158.9, 159.4, 159.8, 165.8, 165.9, 174.1; LRMS: (ES+) m/z = 611 (M+1).

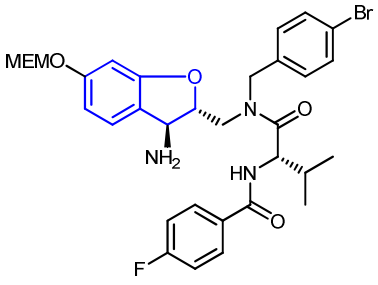
N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl)(4-bromobenzyl)amino)-1-oxo-3-phenylpropan-2yl)benzamide (F2.2):



F2.2

Molecular Formula: $C_{36}H_{38}BrN_3O_6$; R_f (100% ethyl acetate): 0.1; Purified by flash chromatography using 100% ethyl acetate; Yield: 95%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.02-3.31 (m, 2H), 3.37 (m, 3H), 3.47-3.48 (m, 0.5H), 3.54-3.61 (m, 2.5H), 3.73-3.86 (m, 2.5H), 3.94-3.99 (m, 0.5H), 4.05-4.10 (m, 1H), 4.14-4.24 (m, 1H), 4.36-4.49 (m, 1H), 4.51-4.63 (m, 1H), 4.70-4.81 (m, 0.5H), 5.02-5.06 (m, 0.5H), 5.19-5.31 (m, 2.5H), 5.36-5.59 (m, 1H), 6.49-6.55 (m, 1H), 6.61-6.65 (m, 1H), 6.79-7.02 (m, 3H), 7.09-7.16 (m, 2H), 7.20-7.34 (m, 3H), 7.39-7.54 (m, 5H), 7.73-7.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 39.2, 39.6, 48.1, 48.5, 49.3, 51.1, 56.1, 56.8, 58.9, 60.3, 67.6, 71.5, 73.3, 75.1, 75.3, 90.8, 91.2, 91.2, 93.6, 98.9, 99.2, 109.3, 109.5, 121.3, 121.5, 124.5, 124.9, 127.0, 127.1, 128.4, 128.5, 128.5, 129.4, 130.0, 131.9, 133.5, 133.7, 135.1, 135.6, 136.0, 136.1, 158.9, 159.3, 159.7, 166.6, 166.6, 172.5, 172.8; LRMS: (ES+) m/z = 671 (M-NH_3+1), 673 (M-NH_3+3).

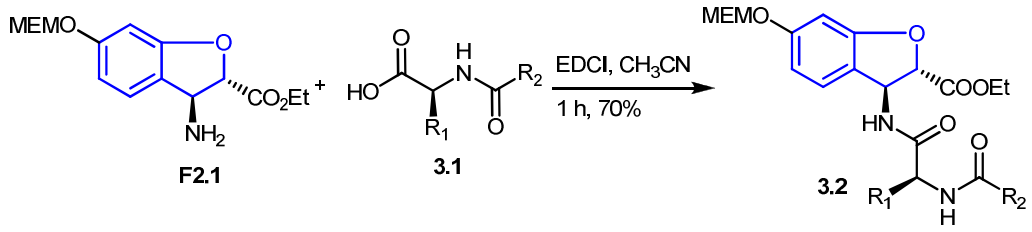
N-((S)-1-((((2R,3S)-3-amino-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2yl)methyl)(4-bromobenzyl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluorobenzamide (F3.1e):



F3.1e

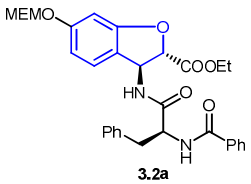
Molecular Formula: $C_{32}H_{37}BrN_3O_6$; R_f (100% ethyl acetate): 0.1; Purified by flash chromatography using 100% ethyl acetate; Yield: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.86-2.95 (bs, 2H), 3.03-3.06 (m, 4H), 3.37 (s, 3H), 3.49-3.55 (m, 2H), 3.70-3.72 (m, 2H), 3.79-3.80 (m, 2H), 4.02-4.25 (m, 1H), 4.55-4.64 (m, 1H) 4.74-4.90 (m, 1H), 5.21 (bs, 2H), 6.46-6.70 (m, 2H), 7.04-7.07 (m, 2H), 7.13-7.41 (m, 9H), 7.40-7.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0, 136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 682 (M+Na).

Synthesis of compound 3.2



To a stirred solution of compound **F2.1 (**1eq) and amino acid derivative **3.1** (1.2 eq) in CH_3CN was added EDCI (1.2 eq) at room temperature under an inert atmosphere. The reaction was allowed stirred for 1 hour. The reaction mixture was quenched by the addition of a saturated *aq*. $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product.

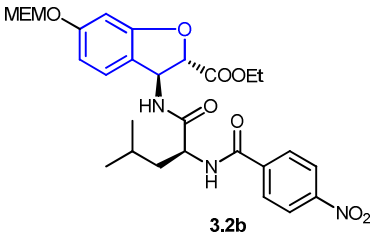
(2S,3S)-ethyl3-((S)-2-benzamido-3-phenylpropanamido)-6-((2-methoxyethoxy) methoxy)-2,3-dihydrobenzofuran-2-carboxylate (3.2a):



Molecular Formula: $C_{31}H_{34}N_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.26 (t, *J* = 7.1 Hz, 3H), 3.06-3.09 (m, 2H), 3.35 (s, 3H), 3.47-3.58 (m, 2H), 3.71-3.83 (m, 2H), 4.15-4.32 (m, 2H), 4.55 (d, *J* = 4.0 Hz, 1H), 4.84

(d, *J* = 4.1 Hz, 1H), 5.00-5.15 (m, 2H), 5.20 (t, *J* = 5.0 Hz, 1H), 5.57-5.63 (m, 1H), 6.44-6.61 (m, 2H), 6.76 (d, *J* = 8.1Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.11-7.25 (m, 4H), 7.42-7.52 (m, 1H), 7.37 (m, 3H), 7.55-7.57 (m, 2H), 7.65-7.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 39.2, 54.9, 58.9, 61.8, 67.6, 67.7, 71.4, 86.0, 93.4, 93.5, 98.8, 98.9, 109.8, 117.4, 117.7, 117.7, 125.3, 125.5, 127.1, 128.4, 128.5, 129.3, 129.4, 131.8, 133.1, 136.2, 159.4, 160.6, 167.1, 169.2, 169.2, 171.0; LRMS: (ES+) m/z = 563 (M+1).

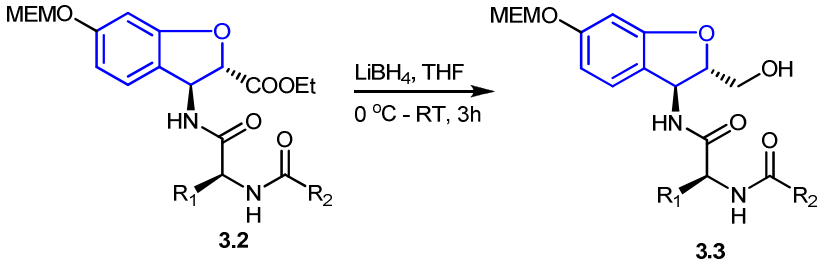
(2S,3S)-ethyl6-((2-methoxyethoxy)methoxy)-3-((S)-4-methyl-2-(4-nitrobenz amido)penta namido)-2,3-dihydrobenzofuran-2-carboxylate (3.2b):



3.2b

Molecular Formula: $C_{28}H_{35}N_3O_{10}$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (bs, 6H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.70-1.79 (m, 3H), 3.33-3.39 (m, 4H), 3.56 (s, 3H), 3.75-3.84 (m, 2H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.79 (d, *J* = 7.7 Hz, 1H), 4.90 (d, *J* = 4.1 Hz, 1H), 5.15-5.26 (m, 2H), 5.63 (dd, *J* = 6.9, 4.2 Hz, 1H), 6.54-6.61 (m, 2H), 7.06 (d, *J* = 8.2 Hz, 1H), 7.38-7.43 (m, 1H), 7.87.88 (m, 2H), 8.20-8.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 22.7, 22.1, 24.8, 41.4, 52.3, 55.3, 58.8, 61.9, 67.7, 71.4, 86.1, 93.4, 99.0, 110.1, 117.2, 123.5, 123.7, 125.2, 128.3, 128.3, 128.4, 138.7, 149.6, 159.5, 160.6, 165.6, 169.0, 172.5; LRMS: (ES+) m/z = 574 (M+1).

Synthesis of compound 3.3

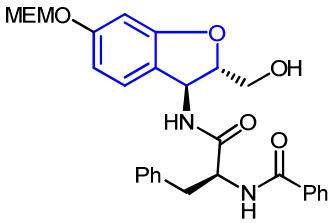


3.2 **3.3**

To a stirred solution of compound **3.2** (1eq) in dry THF was added $LiBH_4$ (1.2 eq) at 0 °C under inert atmosphere. The reaction was allowed to warm to room temperature

and stirred for 3 hours. The reaction mixture was quenched by the addition of a saturated *aq*. NH_4Cl, extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purified by flash chromatography to obtained pure product **3.3**.

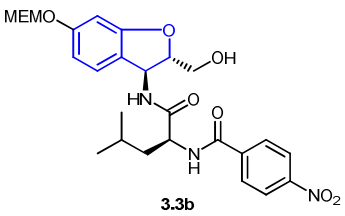
N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (3.3a):



3.3a

Molecular Formula: $C_{29}H_{32}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.03-3.15 (m, 2H), 3.22 (dd, *J* = 13.5, 6.1 Hz, 1H), 3.35 (s, 3H), 3.47-3.56 (m, 2H), 3.72-3.78 (m, 4H), 4.10-4.12 (m, 1H), 4.98-5.08 (m, 1H), 5.10-5.23 (m, 3H), 6.40-6.52 (m, 2H), 6.76 (d, *J* = 8.1Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.16-7.28 (m, 5H), 7.35 (td, *J* = 15.0, 7.6 Hz, 2H), 7.48 (td, *J* = 9.1, 6.2 Hz, 2H), 7.59 (dd, *J* = 17.3, 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 38.9, 39.1, 58.9, 58.9, 63.3, 67.6, 71.5, 90.7, 93.4, 98.7, 171.7, 109.2, 117.8, 125.4, 127.1, 128.5, 128.6, 128.7, 129.3, 131.8, 133.1, 133.2, 136.0, 136.3, 159.3, 160.7, 167.1, 171.6; LRMS: (ES+) m/z = 519(M-1).

N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)-4-nitrobenzamide (3.3b):

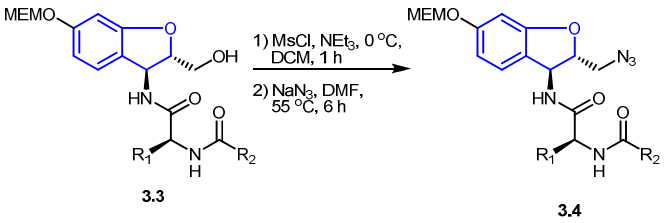


3.3b

Molecular Formula: $C_{26}H_{33}N_3O_9$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.88 (bs, 6H), 1.68-1.70 (m, 3H), 3.35 (s, 3H), 3.54 (dd, *J* =

7.3, 4.3 Hz, 2H), 3.75-3.82 (m, 4H), 4.52-4.53 (m, 1H), 4.89-4.91 (m, 1H), 5.08-5.28 (m, 3H), 6.46 (bs, 2H), 7.00 (d, *J* = 8.3 Hz, 1H), 7.76-7.58 (m, 1H), 7.85 (d, *J* = 8.7 Hz, 2H), 8.13-8.25(m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 22.1, 24.9, 29.6, 41.5, 52.1, 54.3, 58.9, 63.3, 67.7, 71.5, 91.1, 93.4, 98.7, 109.4, 117.6, 123.5, 125.3, 128.4, 138.8, 149.6, 159.5, 160.8, 165.6, 173.0; LRMS: (ES+) m/z = 530 (M-1).

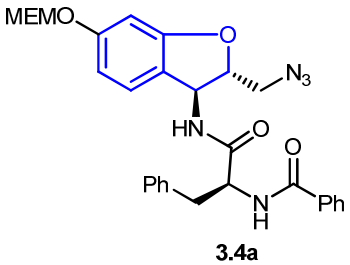
Synthesis of compound 3.4



To a solution of **3.3** (1eq) in dry dichloromethane, at 0 °C, Et_3N (1.2 eq) and methane sulfonyl chloride (1.5) were added, and reaction mixture was allowed to stir for 1 h at room temperature. After completion of the reaction, reaction mixture was quenched by the addition of sodium bicarbonate solution, and extracted with dichloromethane (3 X 10 mL). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude liquid.

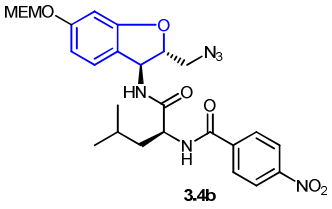
To a solution of above crude in dry DMF, NaN_3 (1.5 eq) was added, and reaction mixture was heated at 55 °C for 6 h. After completion of the reaction, reaction mixture was quenched by the addition of sodium bicarbonate solution, and extracted with dichloromethane (3 X 10 mL). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated to leave a crude liquid, which was purified by column chromatography to give the compound **3.4.**

N-((S)-1-((2R,3S)-2-(azidomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzo furan-3-ylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (3.4a):



Molecular Formula: $C_{29}H_{31}N_5O_6$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 62%; ^{1}H NMR (400 MHz, CDCl3) δ ppm 3.03-3.08 (m, 1H), 3.11-3.26 (m, 1H), 3.36 (s, 3H), 3.49 (bs, 2H), 3.62-3.64 (m, 1H), 3.72-3.83 (m, 2H), 4.10 (t, *J* = 6.8 Hz, 1H), 4.51-4.60 (m, 1H), 4.92-5.26 (m, 4H), 6.43-6.57 (m, 2H), 6.76 (d, *J* = 8.1Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.17-7.26 (m, 6H), 7.37 (m, 2H), 7.45-7.54 (m, 1H), 7.61 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 53.2, 59.0, 67.7, 67.7, 71.5, 88.9, 89.2, 93.5, 98.9, 109.4, 117.7, 125.3, 127.0, 127.0, 128.5, 129.3, 131.9, 133.1, 136.3, 159.6, 160.6, 166.9, 167.0, 171.1, 171.2; LRMS: (ES+) m/z = 546 (M+1).

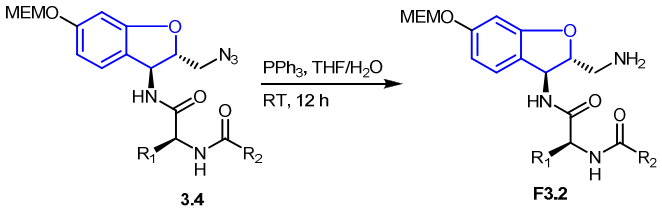
N-((S)-1-((2R,3S)-2-(azidomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)-4-nitrobenzamide (3.4b):



3.4b

Molecular Formula: $C_{26}H_{32}N_6O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.87-1.01 (m, 6H), 1.62-1.74 (m, 3H), 3.36 (bs, 3H), 3.54 (m, 3H), 3.65-3.65 (m, 1H), 3.74-3.80 (m, 2H), 4.60 (bs, 1H), 4.81 (d, *J* = 7.7 Hz, 1H), 5.16-5.20 (m, 3H), 6.49-6.57 (m, 2H), 7.04 (d, *J* = 8.1Hz, 1H), 7.58 (m, 2H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.93 (bs, 1H), 8.20 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.1, 22.8, 24.9, 31.4, 36.5, 41.5, 52.1, 53.3, 54.4, 58.9, 67.7, 71.5, 89.4, 93.5, 98.9,109.7, 117.5, 123.5, 123.6, 125.3, 128.3, 138.9, 149.6, 159.7, 160.7, 162.5, 165.4, 172.5; LRMS: (ES+) m/z = 557 (M+1).

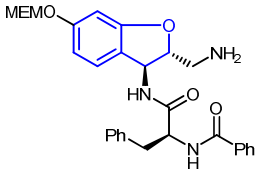
Synthesis of compound F3.2



3.4 F3.2

To a stirred solution of azide compound **3.4** (1 eq) in THF (3 mL) was added TPP (1.1 eq) and water (100 µL). The reaction mixture was left for stirring for 12 h at room temperature. After the completion of the reaction THF was removed under vacuo and purified by flash column using DCM, MeOH solvent system to afford pure amine **F3.2.**

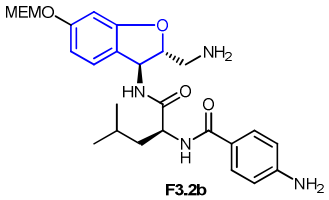
N-((S)-1-((2R,3S)-2-(aminomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzo furan-3-ylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (F3.2a):



F3.2a

Molecular Formula: $C_{29}H_{33}N_3O_6$; R_f (100% ethyl acetate): 0.2; Purified by flash chromatography using 100% ethyl acetate; Yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.04-3.25 (m, 2H), 3.35 (s, 3H), 3.49 (bs, 2H), 3.62-3.64 (m, 1H), 3.71-3.83 (m, 2H), 4.10 (t, *J* = 6.8 Hz, 1H), 4.51-4.60 (m, 1H), 4.93-5.25 (m, 4H), 6.43-6.57 (m, 2H), 6.74-6.76 (m, 1H), 6.96-6.97 (m, 1H),7.17-7.26 (m, 6H), 7.37 (m, 2H), 7.45-7.54 (m, 1H), 7.61 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.1, 53.2, 59.0, 67.7, 67.7, 71.5, 88.9, 89.2, 93.5, 98.9, 109.4, 117.7, 125.3, 127.0, 127.0, 128.5, 129.3, 131.9, 133.1, 136.3, 159.6, 160.6, 166.9, 167.0, 171.1, 171.2; LRMS: (ES+) m/z = 520 (M+1).

4-amino-N-((S)-1-((2R,3S)-2-(aminomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)benzamide (F3.2b):

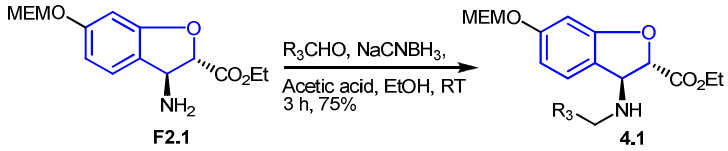


F3.2b

Molecular Formula: $C_{26}H_{36}N_4O_6$; R_f (10% MeOH\DCM): 0.1; Purified by flash chromato graphy using 100% ethyl acetate; Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.93 (bs, 6H), 1.69-1.70 (m, 3H), 2.19-2.49 (bs, 2H), 2.78-3.15 (m, 2H), 3.37 (s, 3H), 3.54 (bs, 2H), 3.78 (bs, 2H), 4.38-4.56 (m, 1H), 4.60-4.79 (m, 1H), 5.17-5.26 (m, 3H), 6.48-6.68 (m, 4H), 6.97-7.21 (m, 2H), 7.55 (d, *J* = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.0, 22.9, 24.9, 29.6, 31.8, 33.2, 37.6,

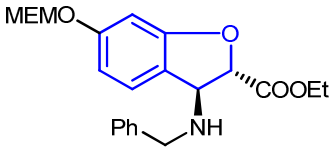
40.9, 58.9, 67.7, 71.5, 93.4, 98.7, 109.5, 109.5, 113.9, 118.2, 122.4, 125.7, 129.1, 150.2, 150.2, 159.3, 160.5, 167.5, 173.6; LRMS: (ES+) m/z = 500 (M+1), 523 (M+Na).

Synthesis of compound 4.1



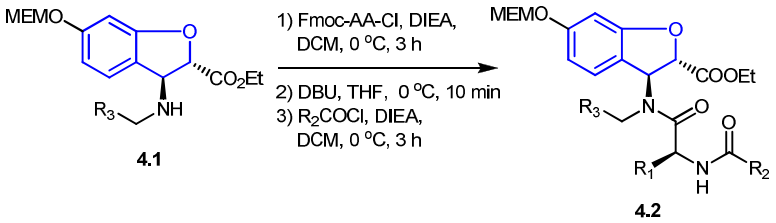
The pure amine **F2.1**(1eq) and R_1CHO (1.1 eq) dissolved in EtOH, and a solution of $NaCNBH_4$ (1.3 eq) in EtOH/AcOH (catalytic amount) was added to the above mixture at room temperature. The reaction mixture was stirred for 4 h. The reaction was quenched with saturated NH_4Cl solution and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to give the product **4.1.**

(2S,3S)-ethyl 3-(benzylamino)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-2 carboxylate (4.1):



Molecular Formula: $C_{22}H_{27}NO_6$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (t, *J* = 7.1 Hz, 3H), 3.29 (s, 3H), 3.48 (dd, *J* = 5.4, 3.8 Hz, 2H), 3.73 (dd, *J* = 5.4, 3.8 Hz, 2H), 3.84 (s, 2H), 4.15 (q, *J* = 7.1, 2H), 4.48 (d, *J* = 3.3 Hz, 1H), 4.98 (d, *J* = 3.39 Hz, 1H), 5.12-5.18 (m, 2H), 6.54-6.63 (m, 2H), 7.01 (s, 1H), 7.01 (s, 1H), 7.23-7.31 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 50.3, 59.0, 61.5, 63.9, 67.6, 71.5, 85.7, 93.6, 99.1, 109.5, 120.0, 125.4, 127.1, 128.1, 128.4, 139.5, 159.2, 160.6, 170.4; LRMS: (ES+) m/z = 411 (M+1).

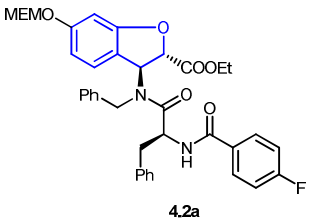
Synthesis of compound 4.2



To the stirred solution of **4.1**(1eq) in dry DCM added DIEA (1.5eq) at 0 °C under inert atmosphere. After 5 min freshly prepared Fmoc protected amino acid chloride (1.5 eq) in DCM, was added slowly. Reaction mixture was stirred at room temperature and reaction monitored by TLC. The reaction mixture was quenched by the addition of a saturated *aq*. $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography. The pure Fmoc amino acid coupled product was subjected to Fmoc removal, using DBU (1.2 eq) in THF. This reaction completed in 10 min, then concentrated the solvent *in vacuo.*

To a suspension of above amine (1 eq) in DCM (10 mL), added DIEA (1.5eq) followed by acid chloride (R_2COCl) (1.5 eq) at 0 °C under inert atmosphere. After completion of the reaction, reaction mixture was quenched with sodium bicarbonate solution (5 mL), concent -rated, and extracted with ethyl acetate (3 X 20 mL). 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 to give pure compound **4.2.**

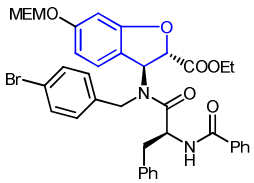
(2S,3S)-ethyl3-((S)-N-benzyl-2-(4-fluorobenzamido)-3-phenylpropanamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-carboxylate (4.2a):



Molecular Formula: $C_{38}H_{39}FN_2O_8$; R_f (30% ethyl acetate/hexane): 0.1; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25-1.33 (t, *J* = 7.1 Hz, 3H), 3.15-3.21 (m, 2H), 3.34-3.41 (s, 3H), 3.52-3.59 (m, 2H), 3.74-3.84 (m, 2H), 4.17-4.33 (m, 3H), 4.58 (d, *J* = 17.4 Hz, 1H), 4.84-5.06 (m, 2H), 5.11- 5.27 (m, 3H), 6.39-6.48 (dd, *J* = 8.3, 2.0 Hz, 1H),

6.62 (bs, 2H), 6.79-6.90 (m, 1H), 7.01-7.10 (m, 6H), 7.17-7.26 (m, 6H), 7.76 (m, 2H), 8.10 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0, 136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 671 (M+1).

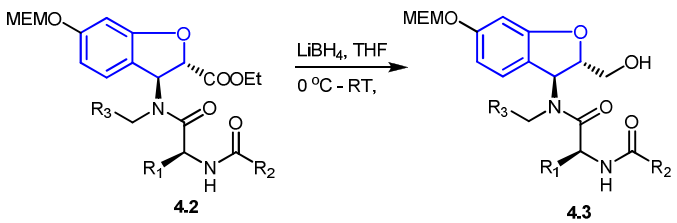
(2S,3S)-ethyl3-((S)-2-benzamido-N-(4-bromobenzyl)-3-phenylpropanamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-carboxylate (4.2b):



4.2b

Molecular Formula: $C_{38}H_{39}BrN_2O_8$; R_f (30% ethyl acetate/hexane): 0.1; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25-1.33 (t, *J* = 7.1 Hz, 3H), 3.15-3.21 (m, 2H), 3.34-3.41 (s, 3H), 3.52-3.59 (m, 2H), 3.74-3.84 (m, 2H), 4.17-4.33 (m, 3H), 4.58 (d, *J* = 17.4 Hz, 1H), 4.84-5.06 (m, 2H), 5.11- 5.27 (m, 3H), 6.39-6.48 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.62 (bs, 2H), 6.79-6.90 (m, 1H), 7.01-7.10 (m, 6H), 7.17-7.26 (m, 6H), 7.76 (m, 2H), 8.10 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 39.1, 50.9, 56.7, 58.8, 66.3, 67.0, 67.5, 71.4, 75.1, 75.3, 90.8, 93.5, 93.5, 98.8, 109.1, 126.6, 126.9, 128.3, 128.4, 128.4, 128.8, 129.3, 131.5, 131.8, 131.9, 132.0, 132.8, 133.6, 133.6, 133.8, 135.7, 136.0, 136.0, 136.4, 158.7, 159.3, 159.7, 160.7, 166.4, 171.2, 172.2, 172.7; LRMS: (ES+) m/z = 732 (M+1).

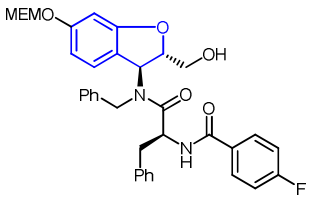
Synthesis of compound 4.3



4.2 4.3

Same experimental procedure as synthesis of **3.3**

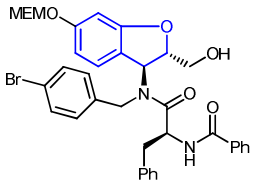
N-((S)-1-(benzyl((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)-4-fluorobenzamide (4.3a):



4.3a

Molecular Formula: $C_{36}H_{37}FN_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.98-2.99 (m, 4H), 3.38 (s, 3H), 3.54-3.58 (m, 2H), 3.68-3.74 (m, 2H), 3.79-3.83 (m, 3H), 4.35-4.36 (m, 2H), 5.25 (s, 2H), 6.37 (d, *J* = 6.9 Hz, 2H), 7.04-709 (m, 4H), 7.22-7.26 (m, 3H), 7.30-7.34 (m, 6H), 7.65-7.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 627 (M-1).

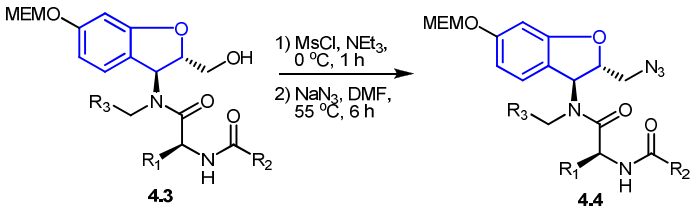
N-((S)-1-((4-bromobenzyl)((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy) methoxy)-2,3-dihydrobenzofuran-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)ben zamide (4.3b):



4.3b

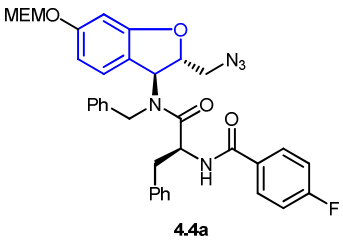
Molecular Formula: $C_{36}H_{37}BrN_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.98-2.99 (m, 4H), 3.38 (s, 3H), 3.54-3.58 (m, 2H), 3.68-3.74 (m, 2H), 3.79-3.83 (m, 3H), 4.35-4.36 (m, 2H), 5.25 (s, 2H), 6.37 (d, *J* = 6.9 Hz, 2H), 7.04-709 (m, 4H), 7.22-7.26 (m, 3H), 7.30-7.34 (m, 6H), 7.65-7.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 688 (M-1).

Synthesis of compound 4.4



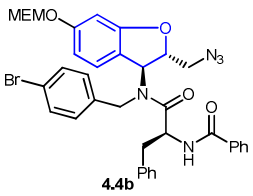
Same experimental procedure as synthesis of **3.4**

N-((S)-1-(((2R,3S)-2-(azidomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzo furan-3-yl)(benzyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-fluorobenza mide (4.4a):



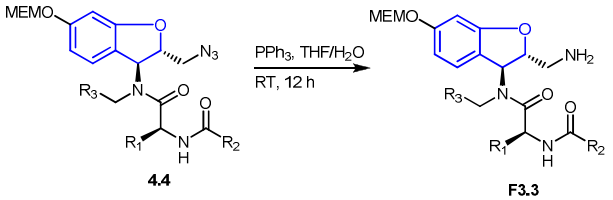
Molecular Formula: $C_{36}H_{36}FN_5O_6$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.98-2.99 (m, 4H), 3.38 (s, 3H), 3.54-3.58 (m, 2H), 3.68-3.74 (m, 2H), 3.79-3.83 (m, 3H), 4.35-4.36 (m, 2H), 5.25 (s, 2H), 6.37 (d, *J* = 6.9 Hz, 2H), 7.04-709 (m, 4H), 7.22-7.26 (m, 3H), 7.30-7.34 (m, 6H), 7.65-7.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 652 (M-1).

N-((S)-1-(((2R,3S)-2-(azidomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-yl)(4-bromobenzyl)amino)-1-oxo-3-phenylpropan-2-yl) benzamide (4.4b):



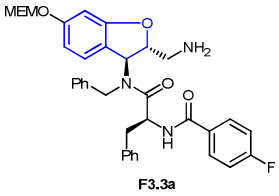
Molecular Formula: $C_{36}H_{37}BrN_2O_7$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.98-2.99 (m, 4H), 3.38 (s, 3H), 3.54-3.58 (m, 2H), 3.68-3.74 (m, 2H), 3.79-3.83 (m, 3H), 4.35-4.36 (m, 2H), 5.25 (s, 2H), 6.37 (d, *J* = 6.9 Hz, 2H), 7.04-709 (m, 4H), 7.22-7.26 (m, 3H), 7.30-7.34 (m, 6H), 7.65-7.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 713(M-1).

Synthesis of compound F3.3



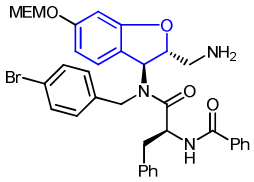
Same experimental procedure as synthesis of **F3.2**

N-((S)-1-(((2R,3S)-2-(aminomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)(benzyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-fluoro benzamide (F3.3a):



Molecular Formula: $C_{36}H_{38}FN_3O_6$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 100% ethyl acetate in hexane; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.98-2.99 (m, 4H), 3.38 (s, 3H), 3.54-3.58 (m, 2H), 3.68-3.74 (m, 2H), 3.79-3.83 (m, 3H), 4.35-4.36 (m, 2H), 5.25 (s, 2H), 6.37 (d, *J* = 6.9 Hz, 2H), 7.04-709 (m, 4H), 7.22-7.26 (m, 3H), 7.30-7.34 (m, 6H), 7.65-7.69 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 39.6, 48.3, 51.4, 51.9, 56.0, 58.9, 65.7, 65.8, 67.6, 67.7, 71.4, 87.6, 88.3, 88.6, 93.5, 98.8, 109.5, 118.4, 126.6, 127.1, 127.3, 127.5, 128.3, 128.4, 128.4, 128.5, 128.5, 128.8, 129.5, 129.5, 131.5, 132.4, 135.9, 155.4, 159.5, 159.7, 160.4, 166.8, 172.5; LRMS: (ES+) m/z = 626 (M-1).

N-((S)-1-(((2R,3S)-2-(aminomethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-3-yl)(4-bromobenzyl)amino)-1-oxo-3-phenylpropan-2-yl)benzamide (F2.3):



F2.3

Molecular Formula: $C_{36}H_{38}BrN_3O_6$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 100% ethyl acetate in hexane; Yield: 88%; ^{1}H NMR ($CDCl_3$, 400 MHz): 2.47-2.67 (m, 0.5 H), 2.83-3.03 (m, 1.5 H), 3.11-3.21 (m, 2H), 3.38-3.41 (m, 3H), 3.56-3.61 (m, 2H), 3.78-3.84 (m, 2H), 3.95-3.98 (m, 0.5), 4.14-4.27 (m, 2H), 4.36-4.47 (m, 0.5H), 5.19-5.28 (m, 2.5H), 5.69-5.83 (m, 1.5H), 6.46-6.54 (m, 2H), 6.69-7.02 (m, 4H), 7.14-7.16 (m, 1H), 7.25-7.35 (m, 4H), 7.45-7.57 (m, 4H), 7.77-7.81 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 39.2, 39.6, 48.1, 48.5, 49.3, 51.1, 56.1, 56.8, 58.9, 60.3, 67.6, 71.5, 73.3, 75.1, 75.3, 90.8, 91.2, 91.2, 93.6, 98.9, 99.2, 109.3, 109.5, 121.3, 121.5, 124.5, 124.9, 127.0, 127.1, 128.4, 128.5, 128.5, 129.4, 130.0, 131.9, 133.5, 133.7, 135.1, 135.6, 136.0, 136.1, 158.9, 159.3, 159.7, 166.6, 166.6, 172.5, 172.8; LRMS: (ES+) m/z = 671 (M-NH_3+1), 673 (M-NH_3+3).

2.6. References

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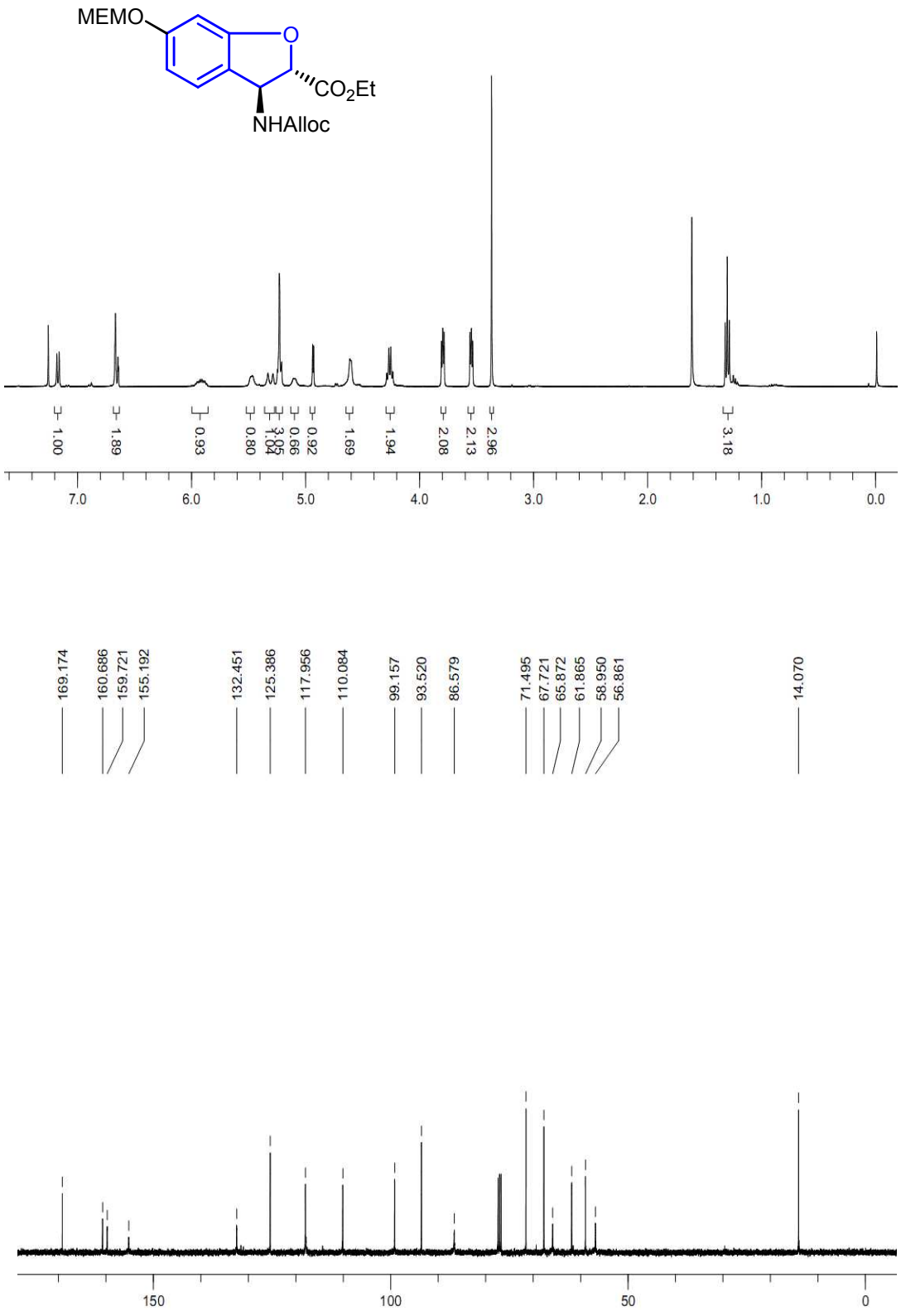
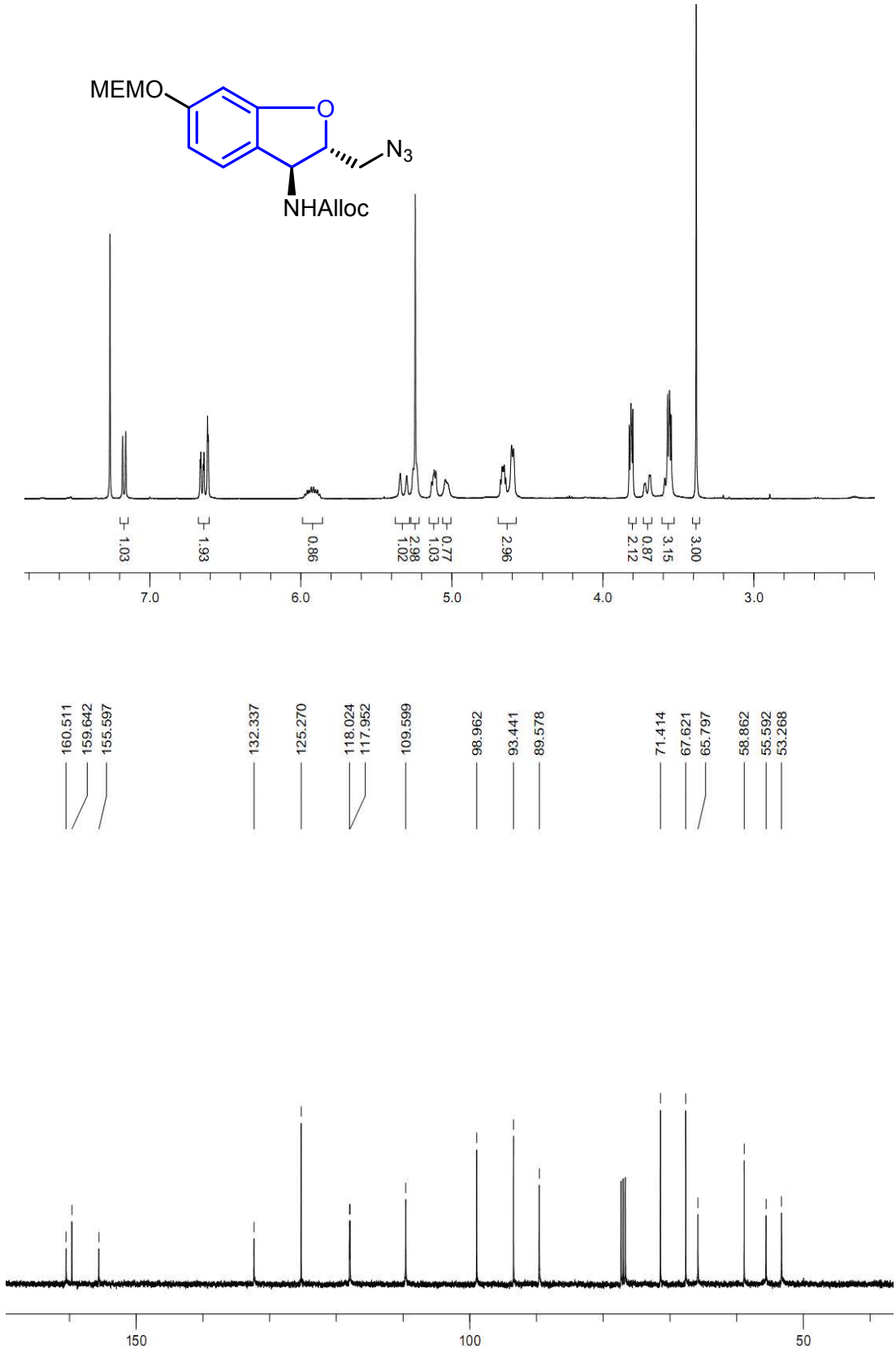
2.7. Spectra



^{1}H and ^{13}C spectra of compound **2.1**



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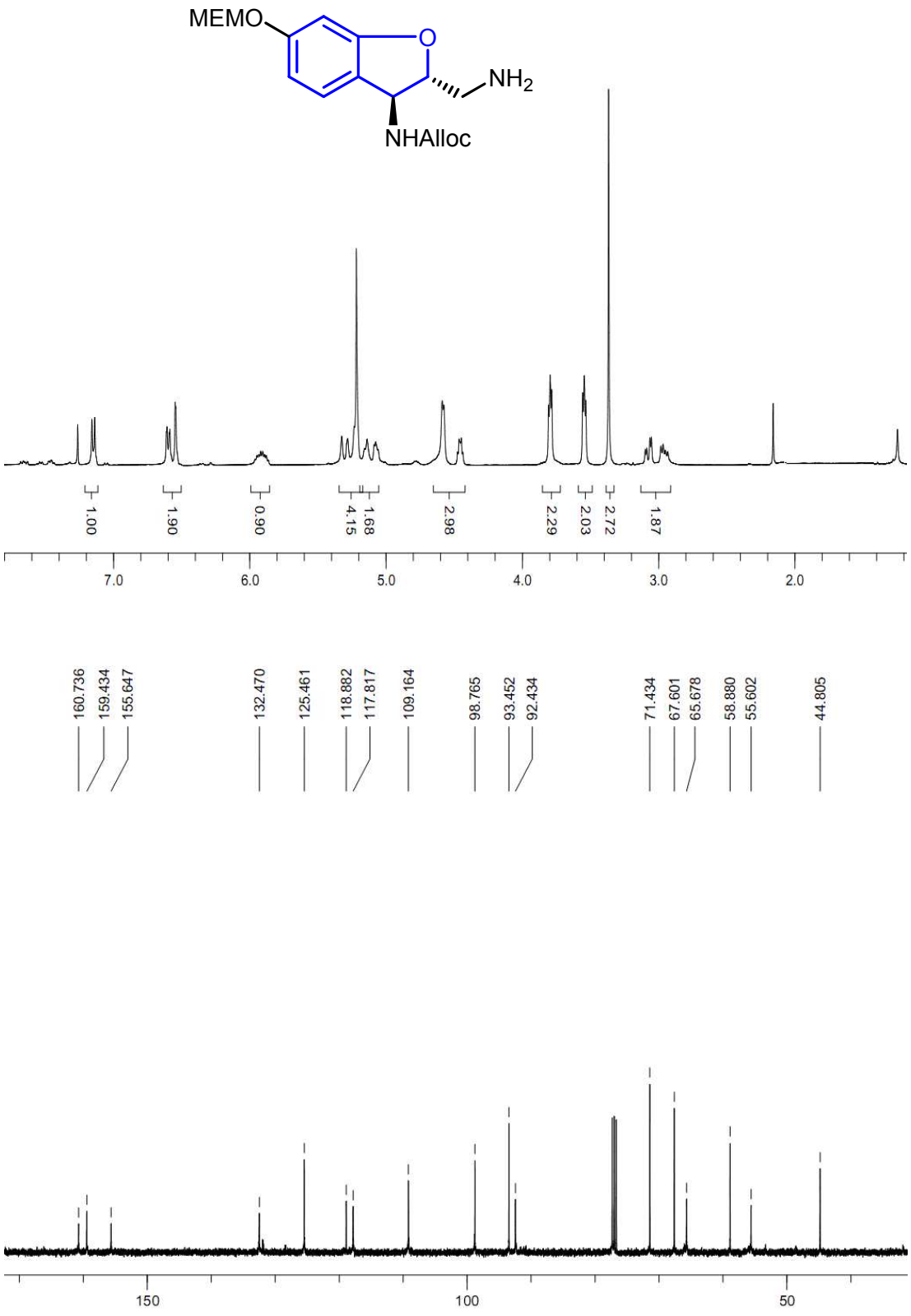

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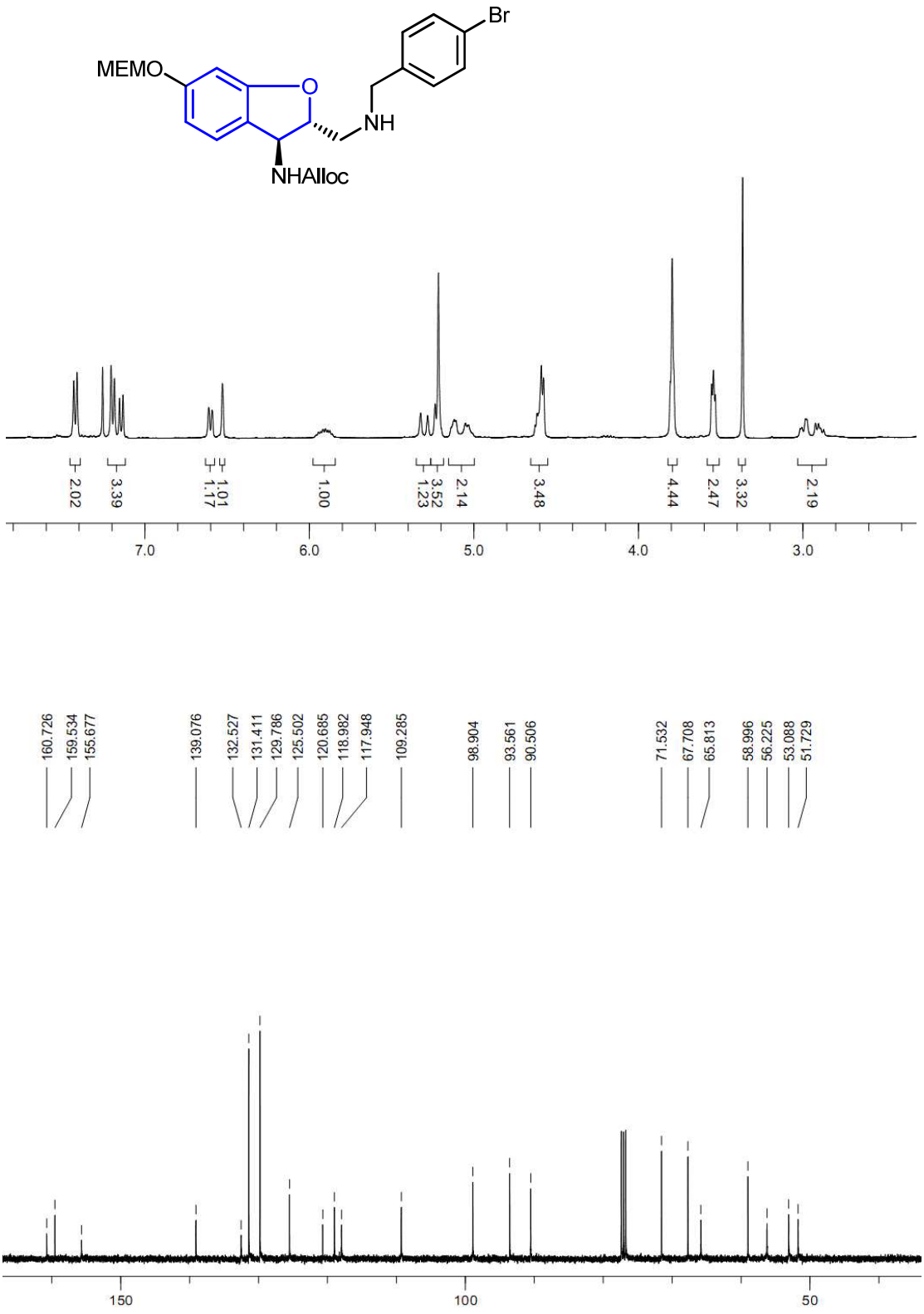

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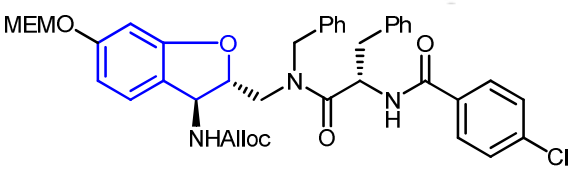
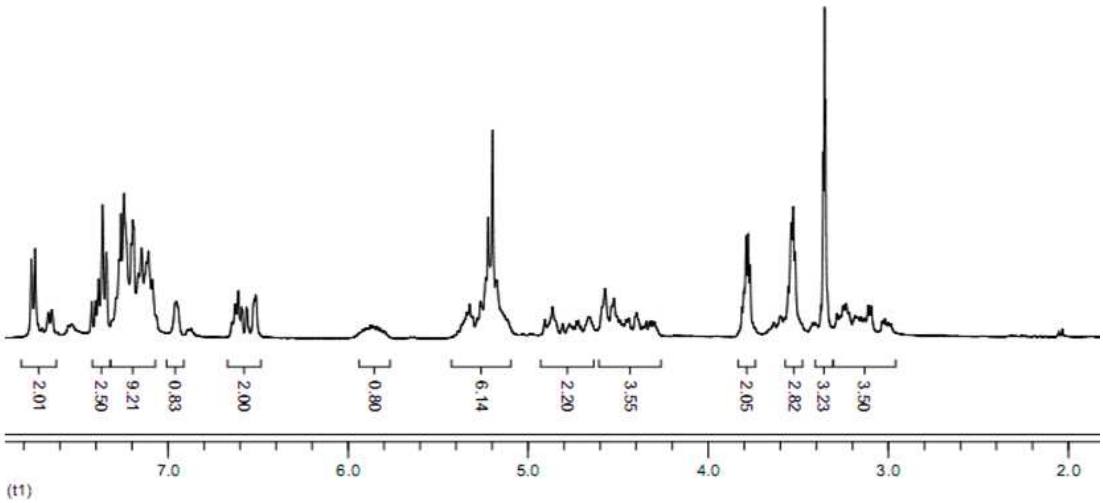
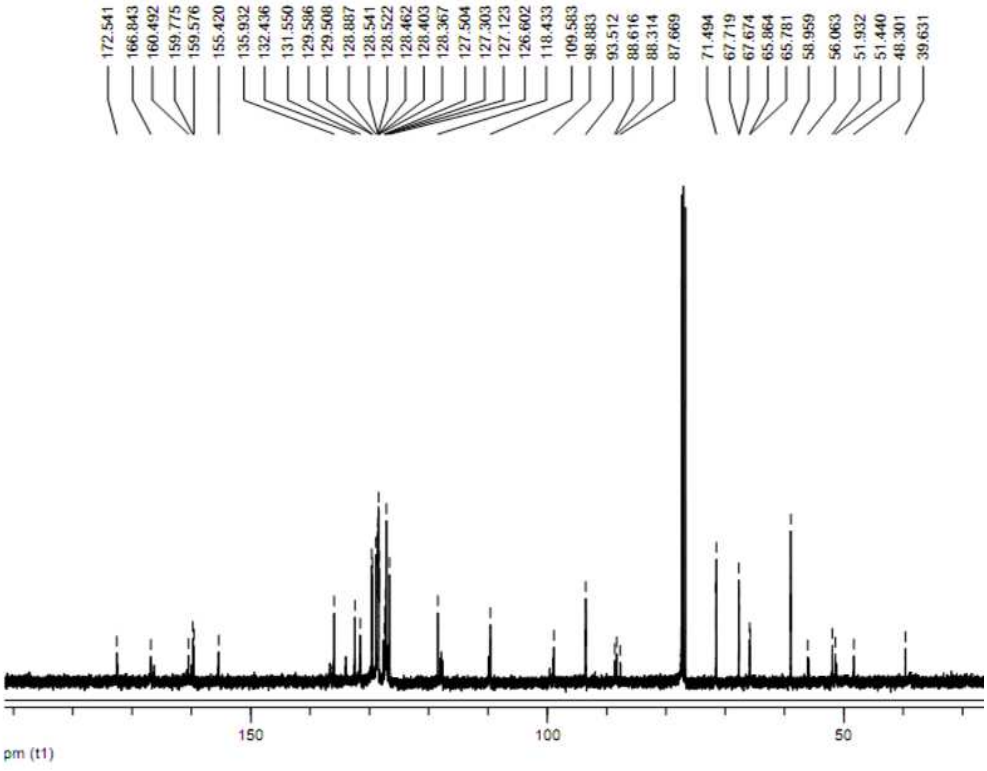
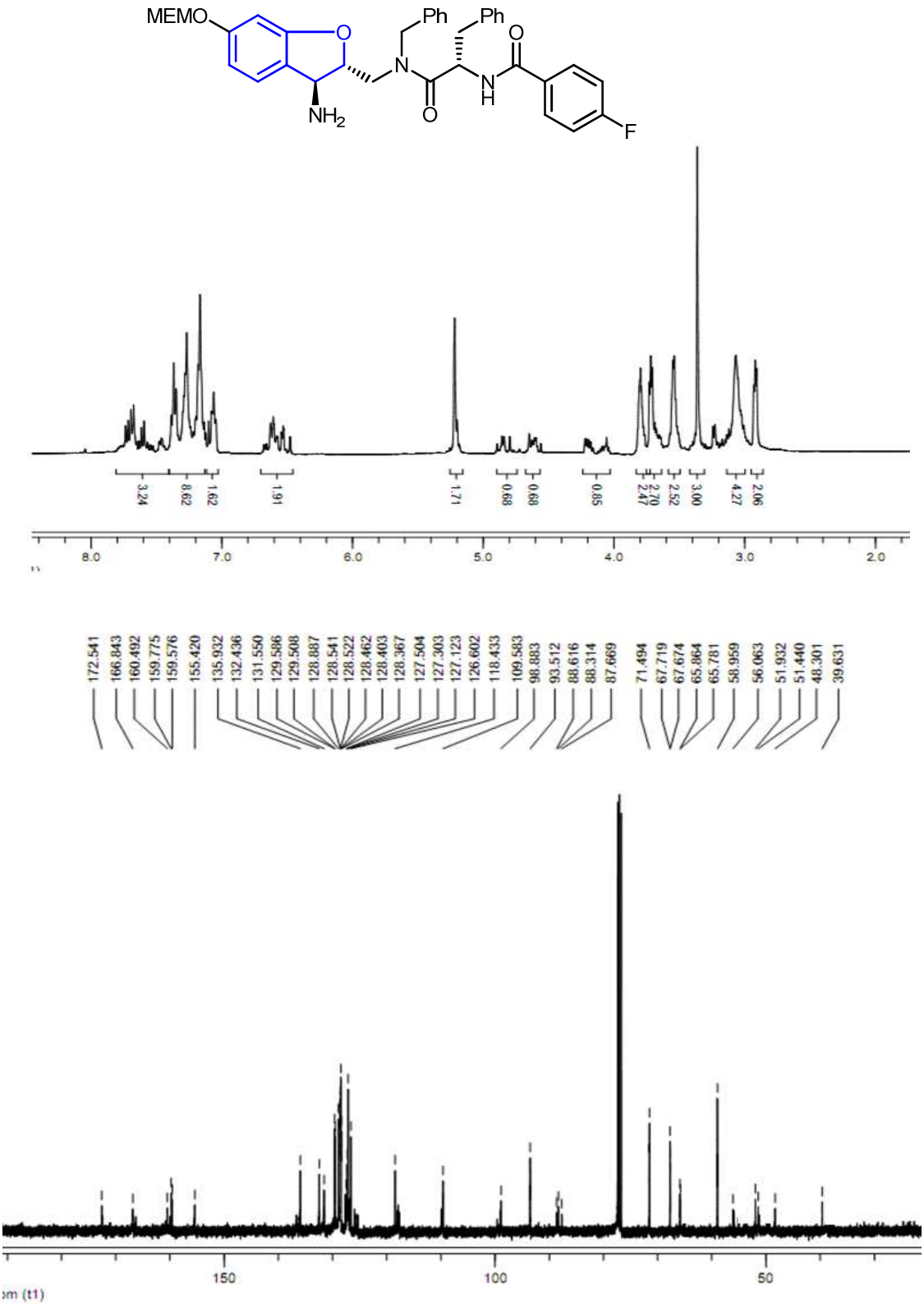


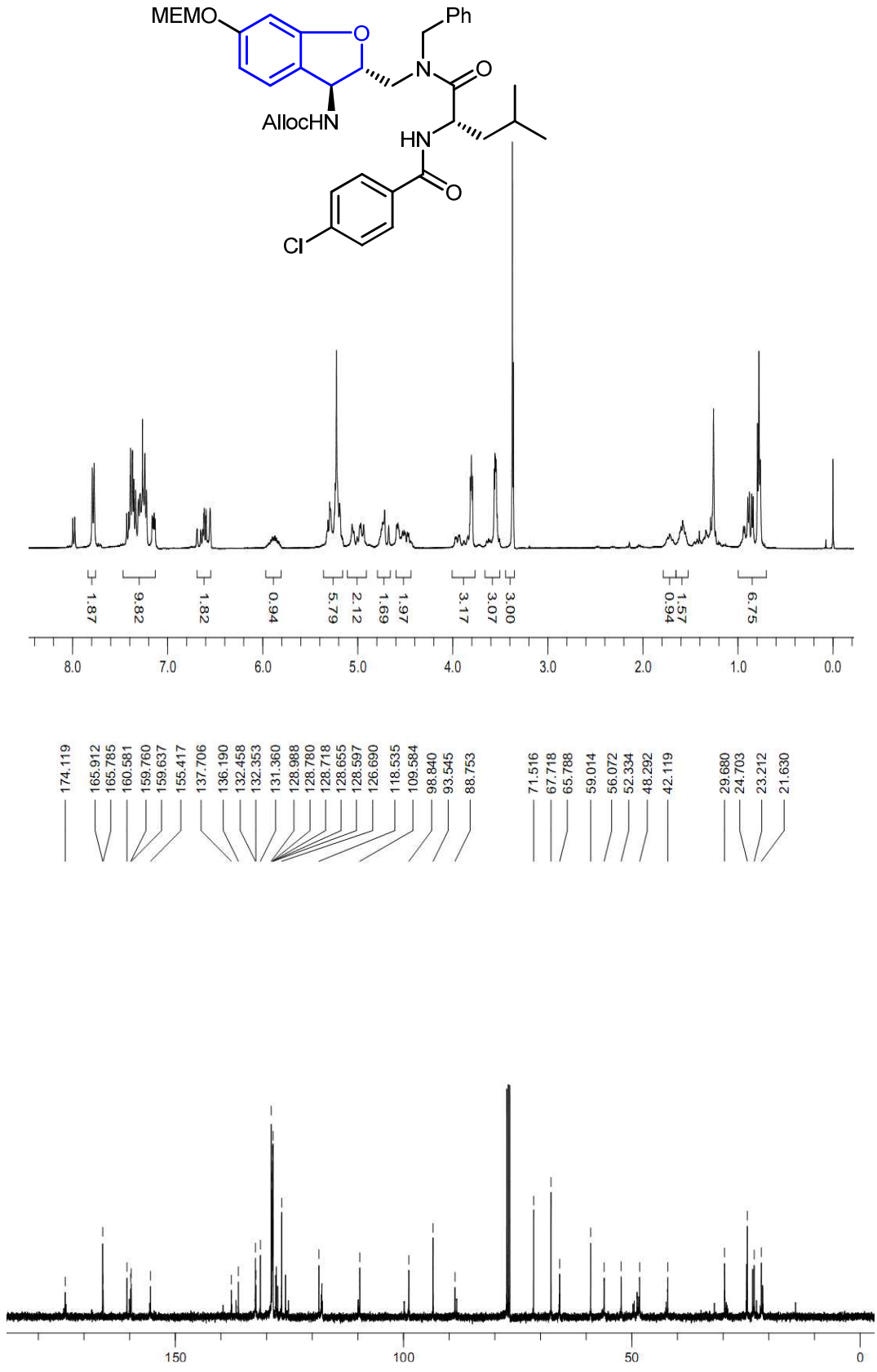




^{1}H and ^{13}C spectra of compound **2.6b**



^{1}H and ^{13}C spectra of compound **F3.1c**



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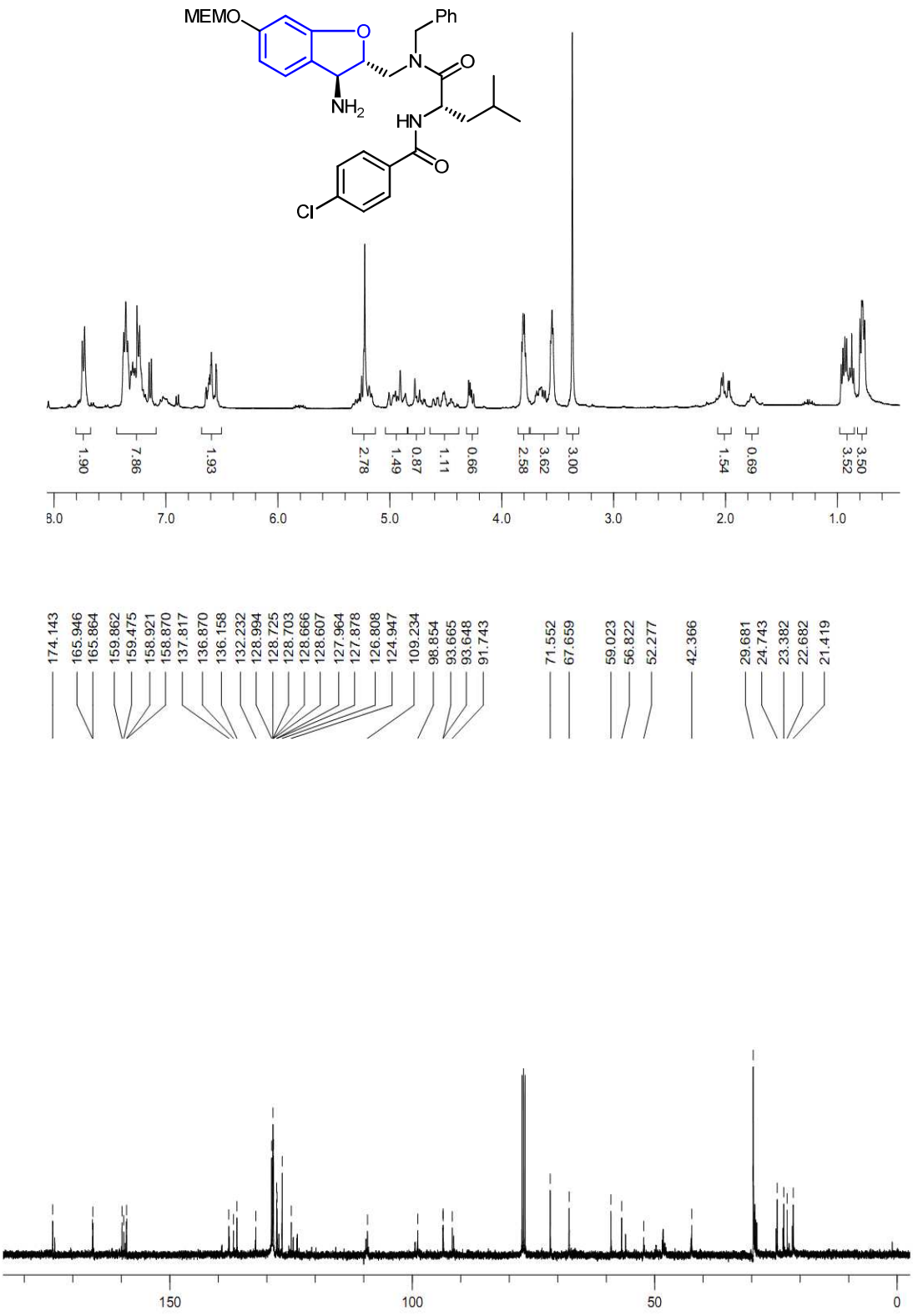

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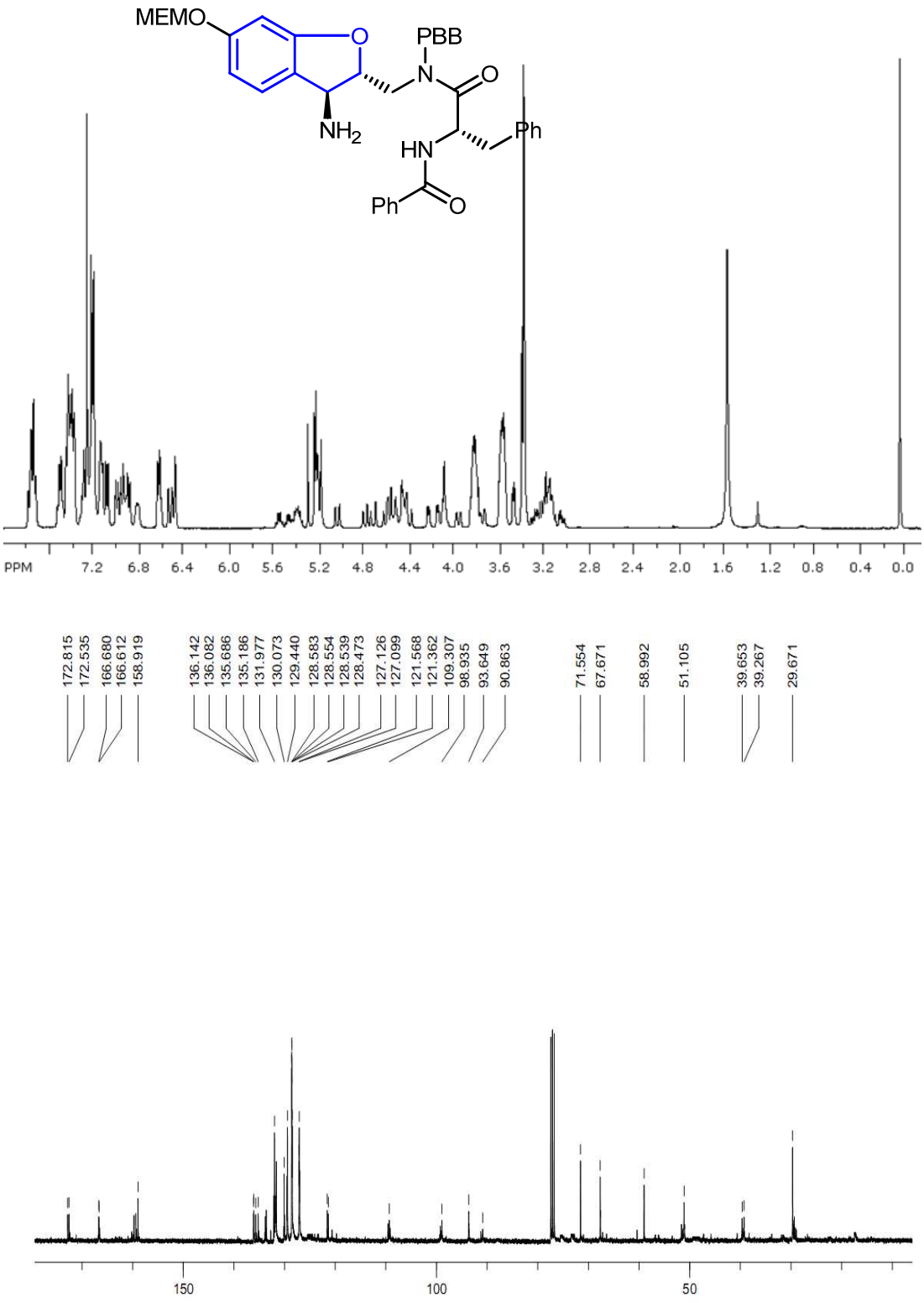
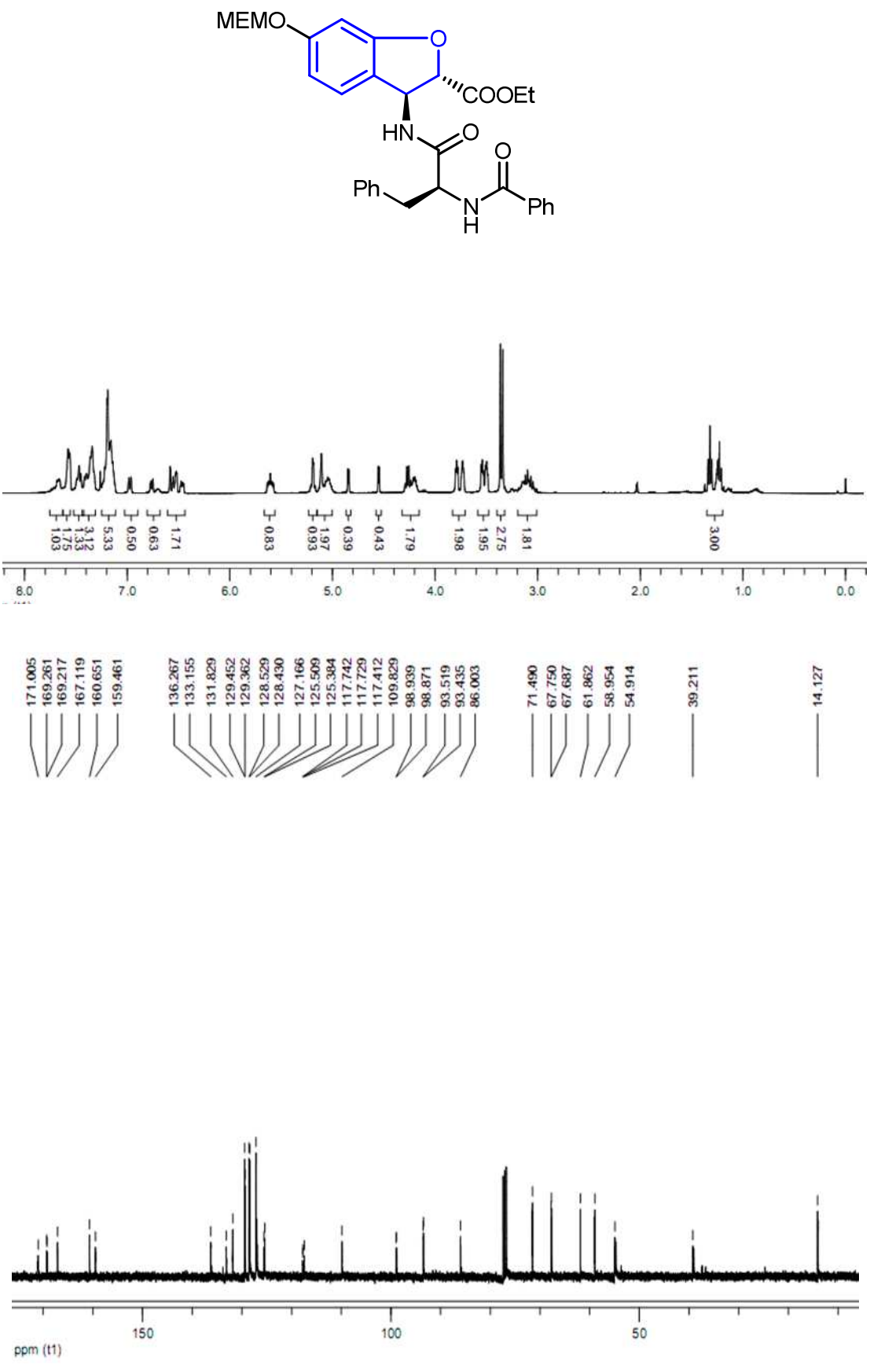
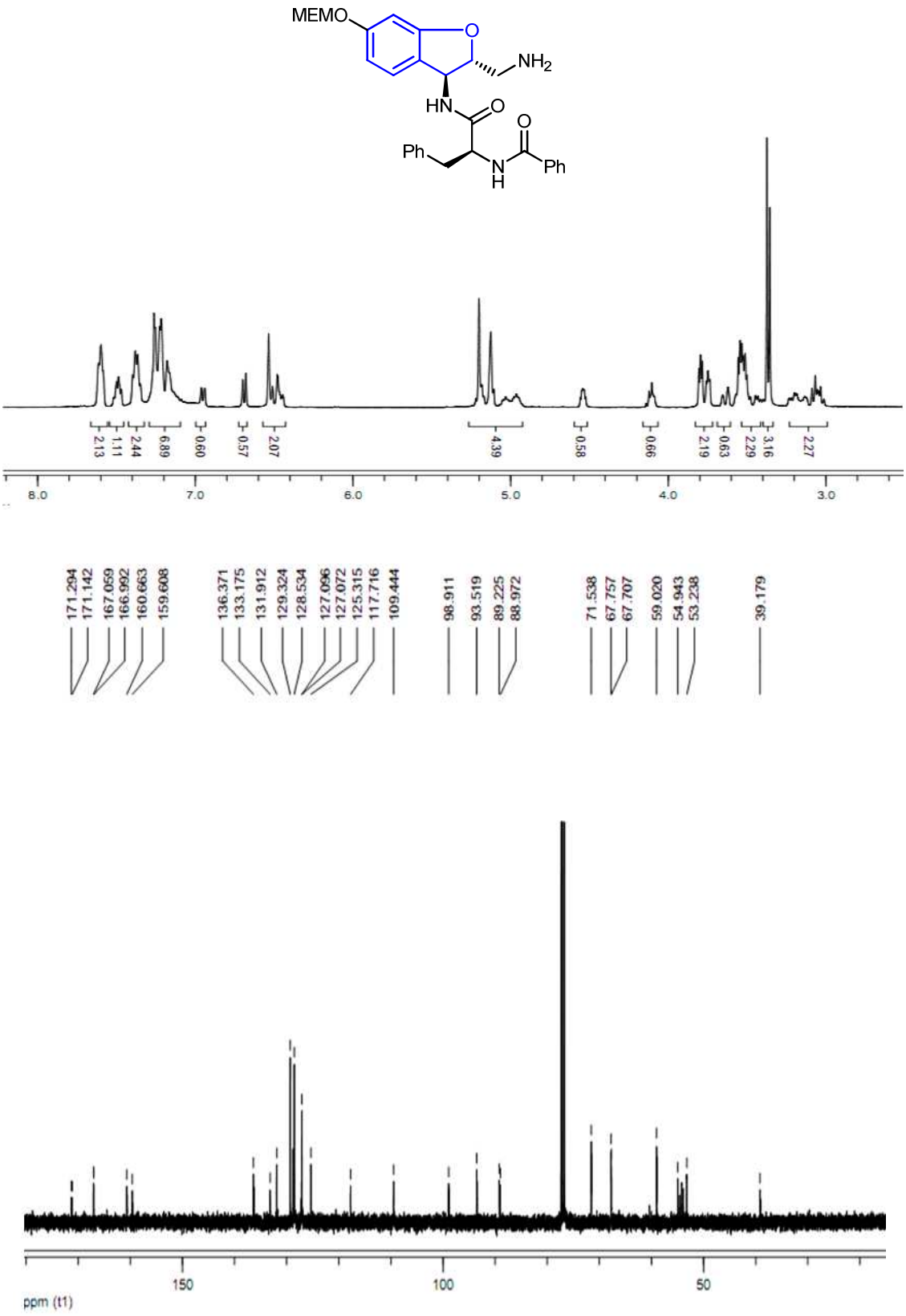


^{1}H and ^{13}C spectra of compound **F2.2**



^{1}H and ^{13}C spectra of compound **3.2a**



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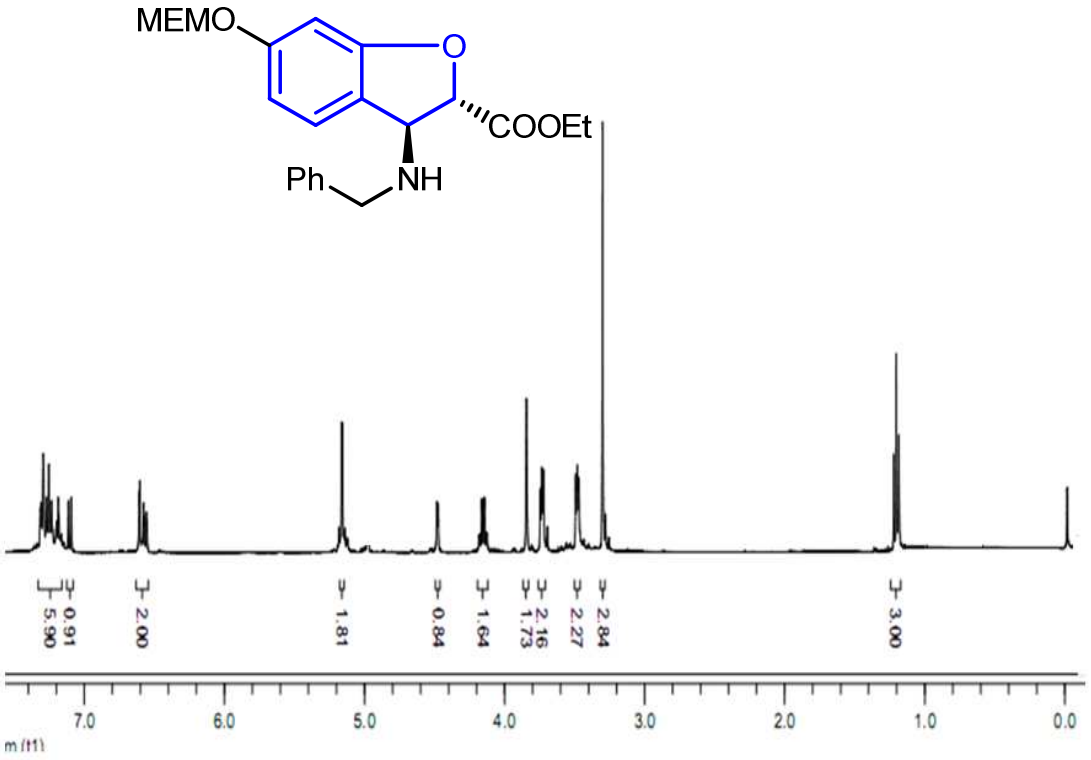
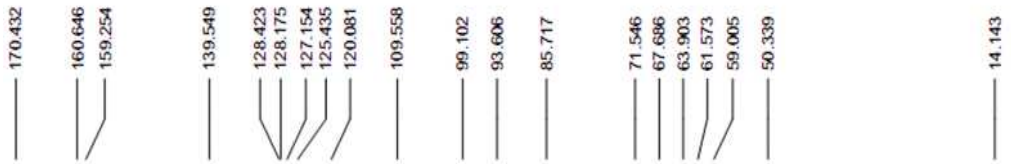
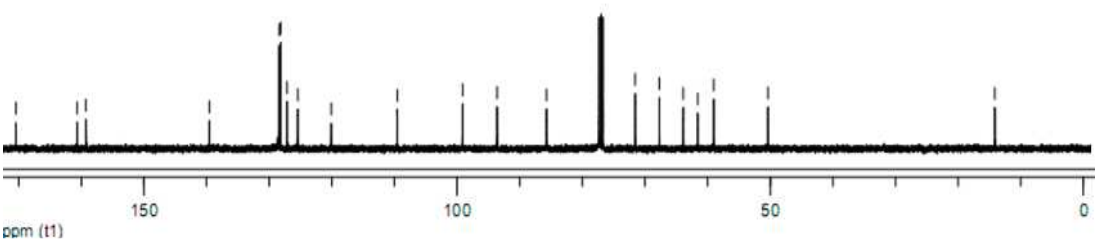
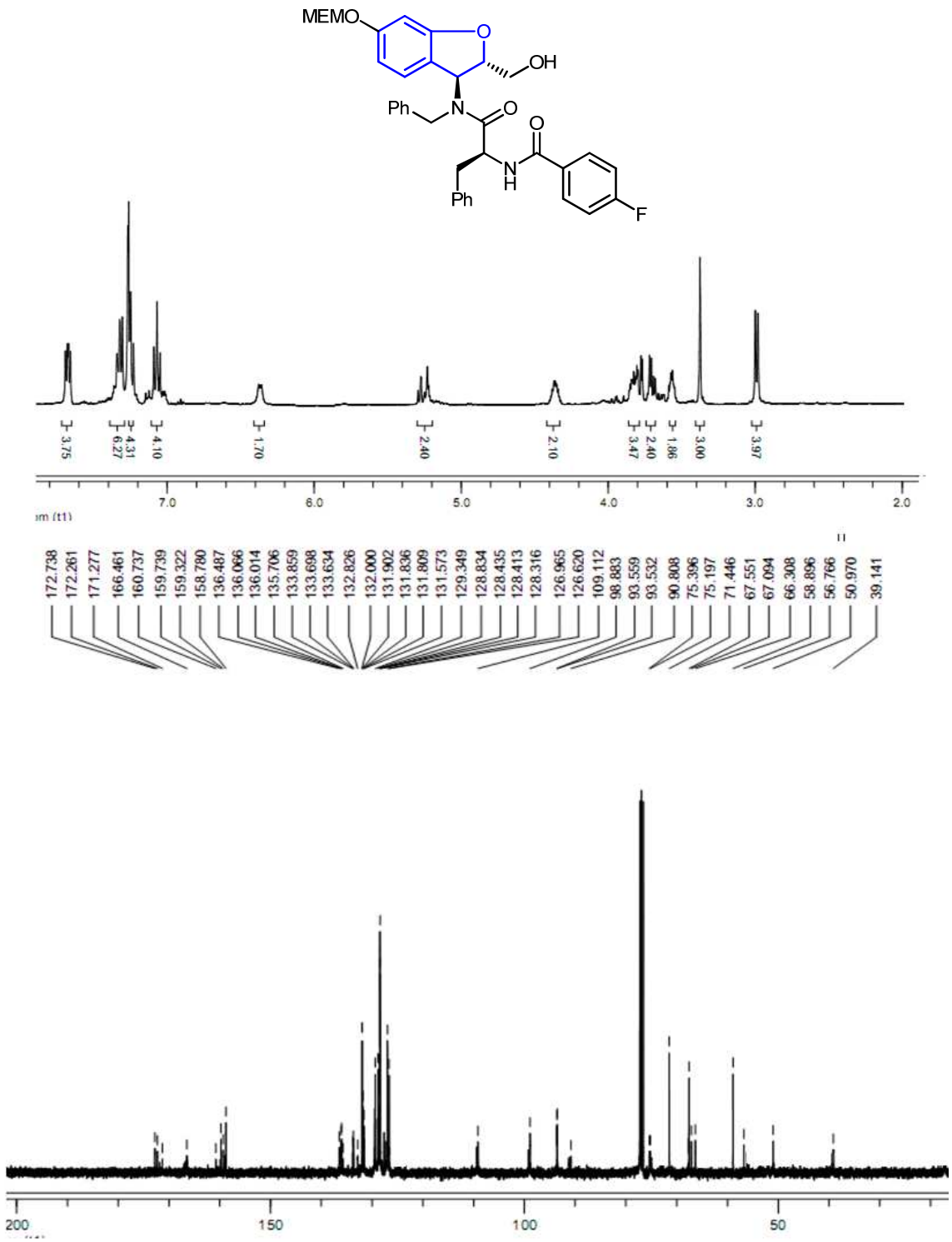


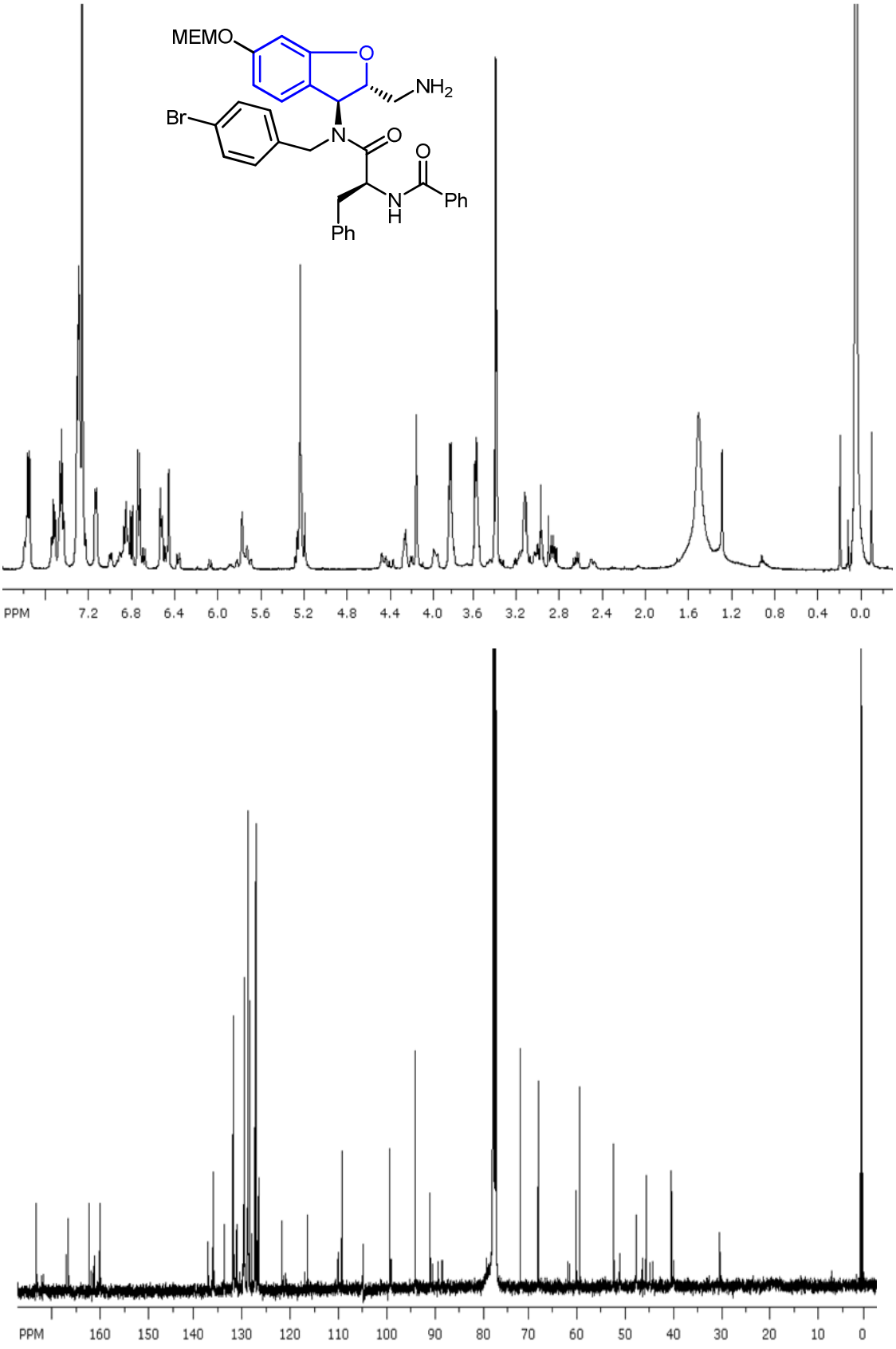




^{1}H and ^{13}C spectra of compound **4.1a**



^{1}H and ^{13}C spectra of compound **4.3a**



^{1}H and ^{13}C spectra of compound **F2.3**

Chapter 3: Synthesis of *Enantioen-riched* Benzofuran-Derived Macrocyclic Architectures

3.1. Introduction

There are numerous examples of complex macrocyclic natural products exhibiting a wide range of biological properties.[1] Due to several advantages that are associated with the macrocyclic rings, there is also a growing interest[2,3,4] in developing modular synthesis methods that allow to obtain a diverse of chemical toolbox having different-types of macrocyclic architectures. Some of these advantages include: (i) pre-organization, (ii) enhanced cell permeation properties, and, (iii) the possibility of having numerous binding interactions; a property that could be highly relevant to search for small molecule modulators of protein-protein,[5,6] and, other types of bio-macromolecular (e.g. DNA/RNA-protein)[7] interactions. Despite these valuable characteristics and the proven success of several marketed macrocyclic compounds as drugs derived from natural products, this structural class has been poorly explored within the drug discovery arena.[1] With these valuable features, we aimed at developing a practical and modular methods that allow us building a diverse set of macrocyclic toolbox to explore their biological functions.[4,8]

3.2. Working Hypothesis

With this objective, we were interested in developing a modular synthesis method to access different types of 12-membered ring macrocyclic compounds based on an *enantioenriched* benzofuran scaffold. Several bioactive natural products having 12-membered macrolides, such as, patulolides A **F1.1**, cladospolide B **F1.2**, 10,11-dehydrocurvularin **F1.3**, YC-17 **F1.4**, lasidilodin **F1.5**, diazonamide A **F1.6** and someothers are well-known in the literature.

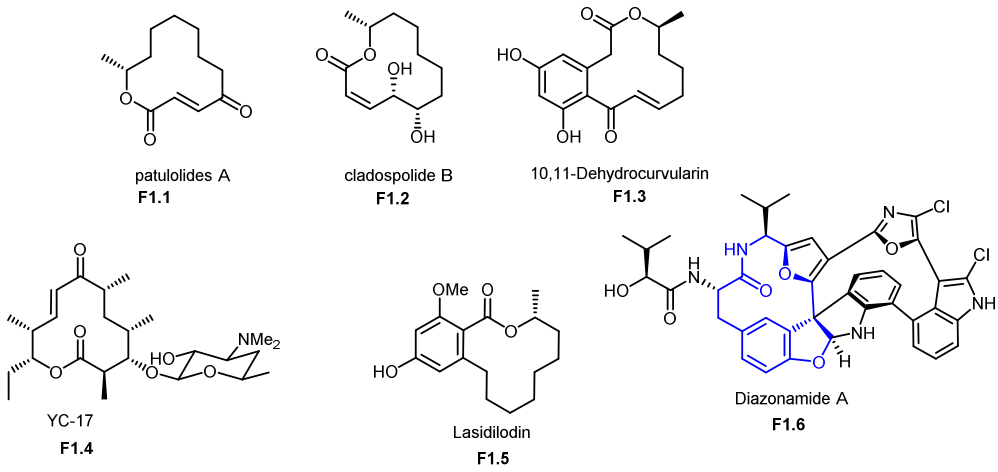


Figure 1. Examples of 12-membered macrocyclic natural products

Earlier, we reported an enantioselective synthesis of benzofuran-based, 1,2-trans-β-amino ester **F2.1**.[9] With an objective to explore further the additional large-ring chemical space, we developed a modular approach that allowed us to incorporate three different types of 12 membered macrocyclic rings onto this scaffold. Our designed 12-membered macrocycles **F2.2-F2.4** are shown in **Figure 2**. In our design strategy, we can introduce the skeletal diversity and various functional groups on the nitrogen atom. All the planned macrocycles are having an olefin functionality, and, the presence of an amino acid moiety within the macrocyclic architecture is an attractive feature to introduce a diverse array of chiral side chains having a variety of polar and non-polar groups.

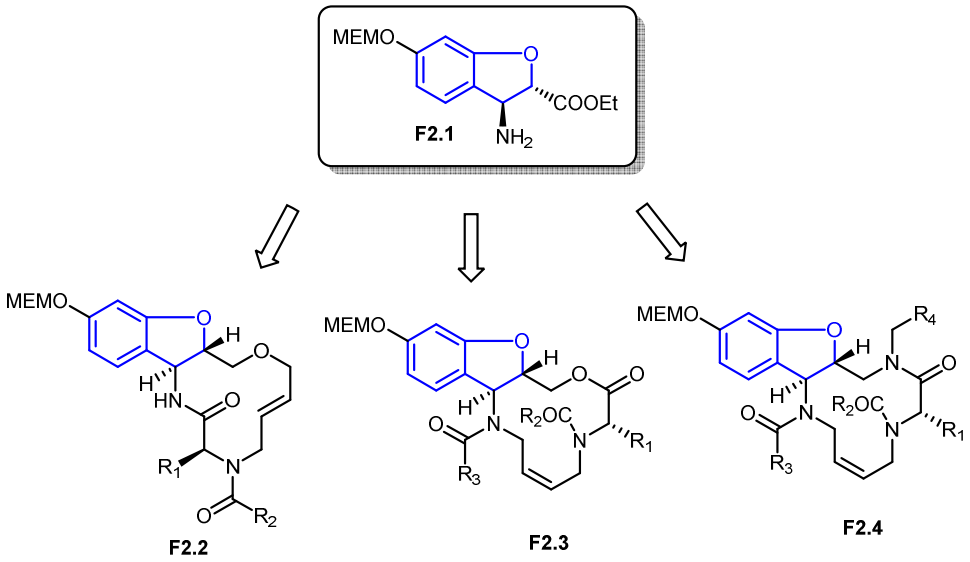


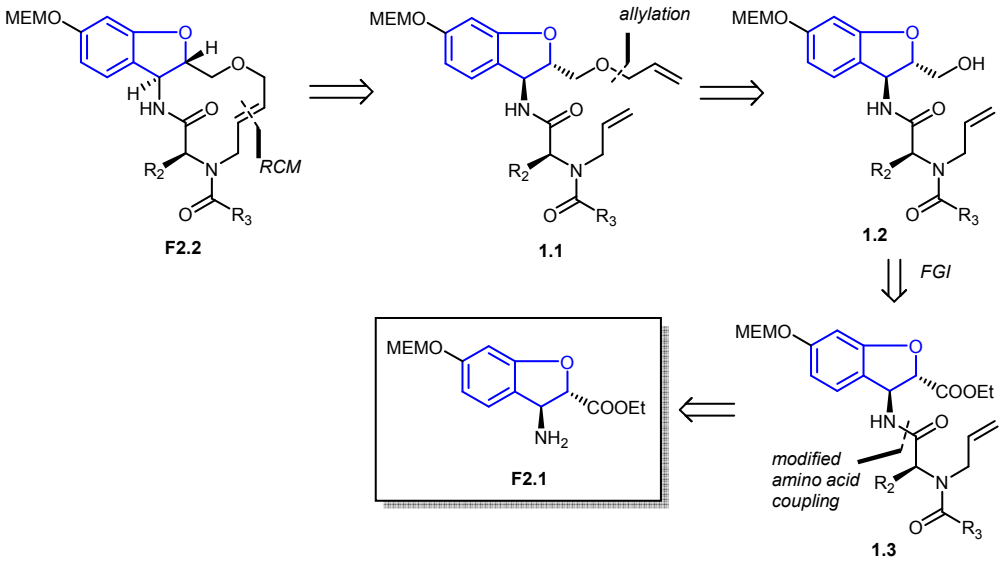
Figure 2. 12-membered ring-derived three different macrocycles

In our modular design strategy, we had the option to attach the modified amino acid functionality either from the benzylic nitrogen atom or oxygen atom; and, we can also convert the oxygen atom to nitrogen to obtain further different skeletally diverse macrocycles. Further, the variation in the side chain, i.e. R_3-R_4 on the macrocyclic skeleton can also be achieved through the selective amidation and alkylation, respectively.

3.3. Results and Discussion

3.3.1. Retrosynthesis of Macrocycle F2.2

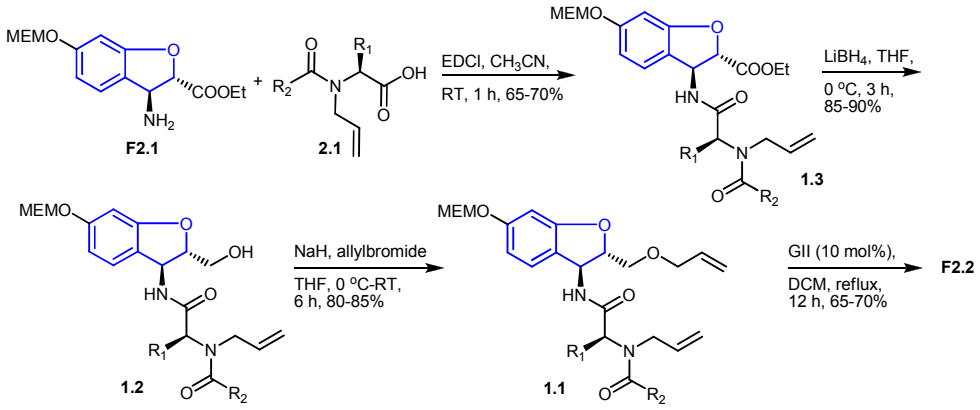
The retrosynthetic analysis of macrocycles **F2.2** is shown in **Scheme 1**. Macrocyclization would be carried-out by ring closing metathesis of *bis*-allylated compound **1.1**, which could be obtained from allylation of **1.2** with allyl bromide. Compound **1.2** could be obtained from compound **1.3** by an ester reduction, which would be obtained from coupling of the modified amino acid building blocks with an *enantioenriched* benzofuran derived β-amino ester **F2.1**.



Scheme 1. Retrosynthesis of macrocycles **F2.2**

3.3.2. Synthesis of Macrocycle F2.2

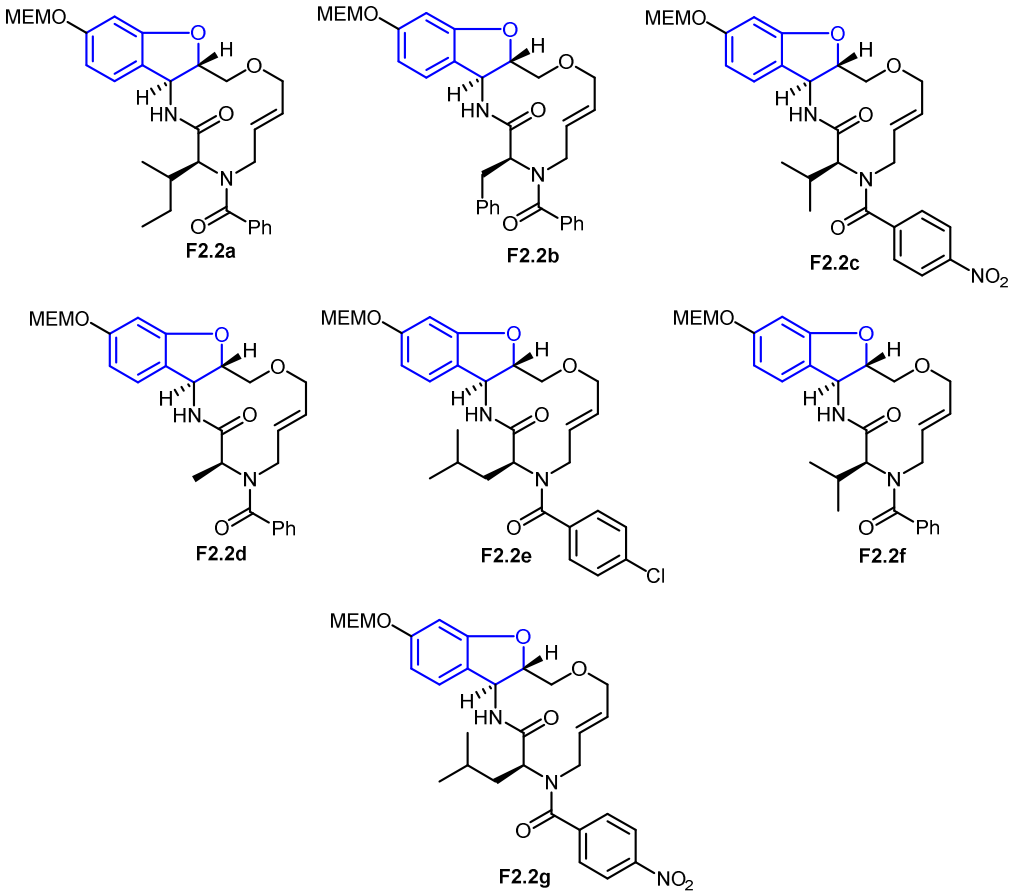
As shown in **Scheme 2**, we started our synthesis of macrocycle **F2.2**, with *enantioenriched* benzofuran scaffold **F2.1**. The coupling with modified amino acid **2.1** using EDC▪HCl and acetonitrile condition gave **1.3** with 65-70% yields. The carboxylester derivative of **1.3** was reduced with lithium borohydride, and, this yielded **1.2** with 85-90% yield. Allylation of primary hydroxyl group of **1.2** with allyl bromide and NaH condition gave **1.1** with a good yield. Finally, macrocyclization was achieved from the corresponding *bis*-allylated compound **1.1** by ring-closing metathesis using Grubbs 2nd generation catalyst (G-II)[10] in 65-70% yield. In this case, we synthesized seven macrocycles, all of them showed the *trans* geometry of the double bond which was confirmed by ^{1}H NMR experiments.



Scheme 2. Synthesis of macrocycle **F2.2**

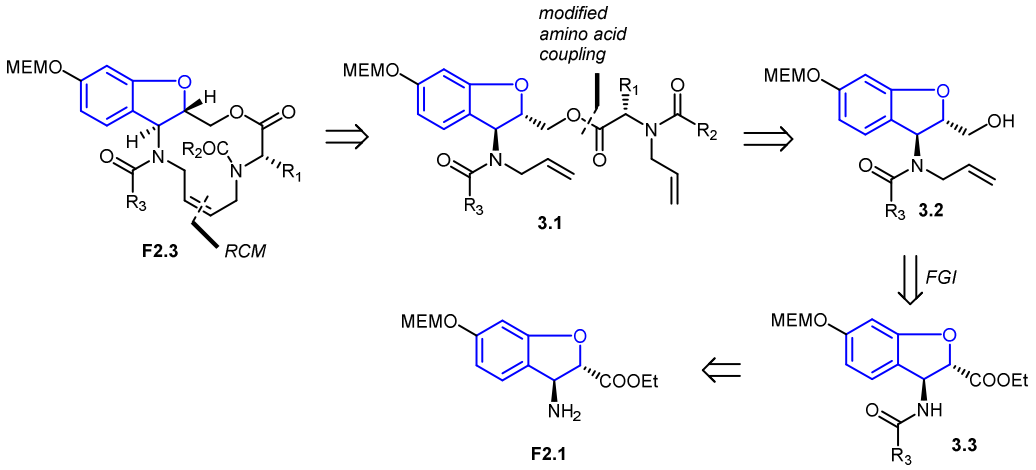
3.3.3. Derivatives of Macrocycle F2.2

We synthesized seven derivatives of macrocycles **F2.2**, by replacing R_1, R_2 groups, and, these compounds are shown below:



3.3.4. Retrosynthesis of Macrocycle F2.3

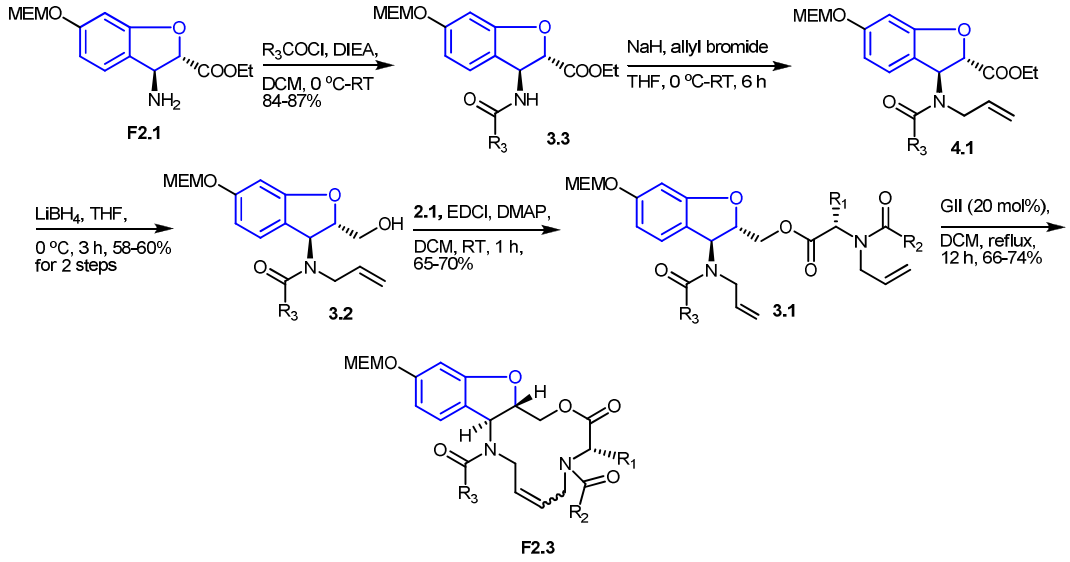
The retrosynthetic analysis of macrocycle **F2.3** is shown in **Scheme 3**. Macrocyclization would be undertaken by ring closing metathesis of *bis*-allyl compound **3.1**, which would be obtained from coupling of modified amino acid building blocks **2.1** with **3.2**. Compound **3.2** could be obtained from **3.3** by reduction of an ester functional group. This compound **3.3** could be obtained from allylation of **3.4,** which would be obtained from a simple benzoylation of an *enantioenriched* benzofuran derived β-amino ester **F2.1**.



Scheme 3: Retrosynthesis of macrocycle **F2.3**

3.3.5. Synthesis of Macrocycle F2.3

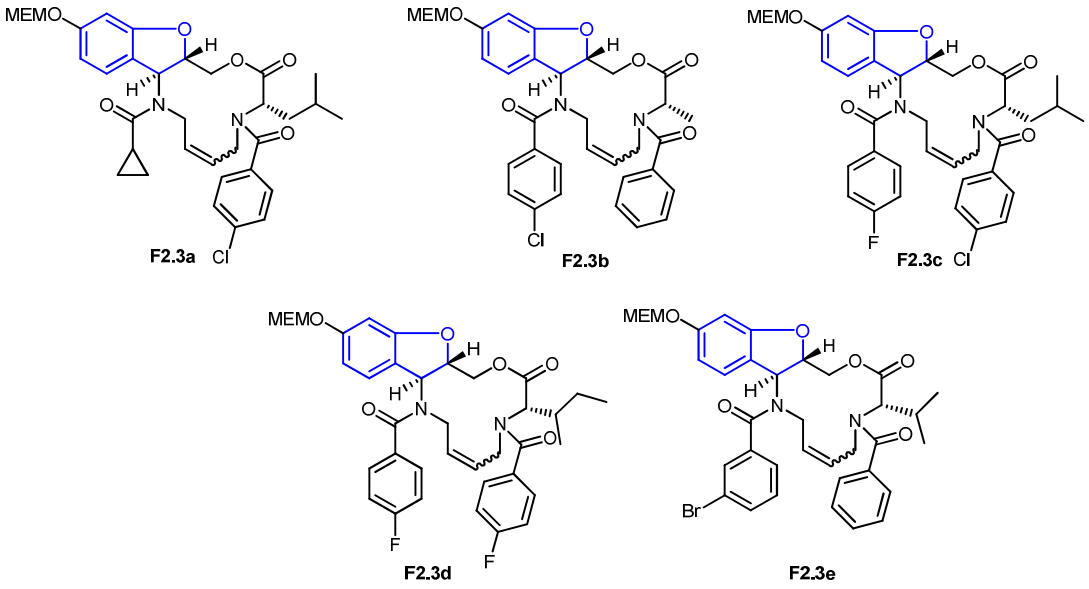
We started the synthesis of macrocycle **F2.3,** from an *enantioenriched* benzofuran scaffold **F2.1,** and, it is shown in **Scheme 4**. Primary amine **F2.1** was converted to an amide with benzoyl chloride and this gave compound **3.3**. It was then subjected to allylation with allyl bromide, NaH conditions to obtain compound **4.1**. Reduction of ester **4.1** with lithium borohydride led the synthesis of compound **5.2** with an overall 58-60% yield for two steps. This was then coupled with modified amino acid building blocks (**2.1**) using EDC▪HCl/DMAP conditions and this coupling reaction provided **3.1** with 65-70% yield. The *bis*-allylated compound **3.1** was then subjected to ring closing metathesis using 20 mol% Grubbs 2nd generation catalyst, and, this gave the macrocyclic compound **F2.3** with a moderate yield. In this series, we synthesized 5 macrocycles, and, in each compound, obtained 1:1 ratio of *cis/trans* geometric ratio, which was determined by HPLC-MS.



Scheme 4. Synthesis of macrocycle **F2.3**

3.3.6. Derivatives of Macrocycle F2.3

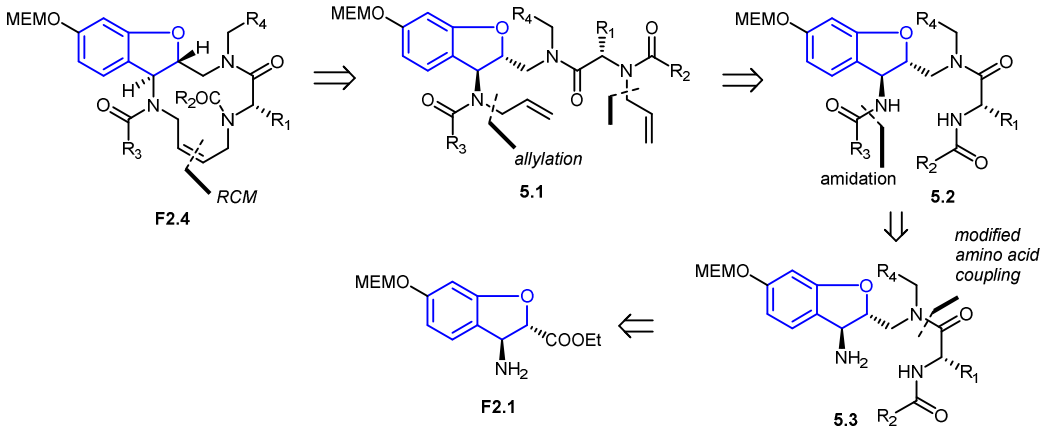
We synthesized five derivatives of macrocycle **F2.3**, by replacing R_1, R_2, R_3 groups and these are shown below:



3.3.7. Retrosynthesis of Macrocycle F2.4

The retrosynthetic analysis of macrocycle **F2.4** is shown in **Scheme 5**. Macrocycle **F2.4** would be obtained from *bis*-allyl product **5.1** by ring closing metathesis using Grubbs chemistry, which could be obtained from *bis*-allylation of **5.2** with allyl bromide. This Compound **5.2** could be obtained by benzoylation of benzylic amine

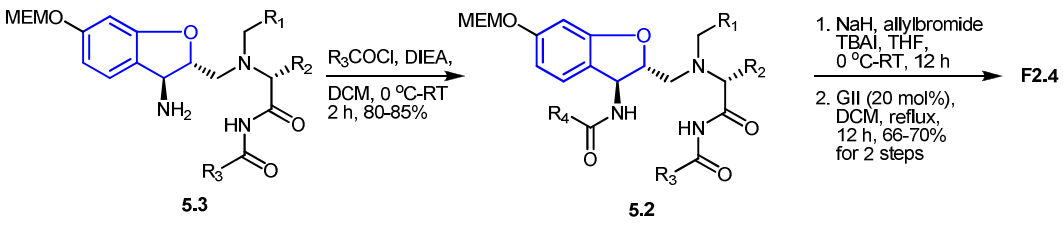
of **5.3,** which would be obtained from an *enantioenriched* benzofuran derived β-amino ester **F2.1** by using functional group transformations and then followed by a coupling reaction.



Scheme 5. Retrosynthesis of macrocycle **F2.4**

3.3.8. Synthesis of Macrocycle F2.4

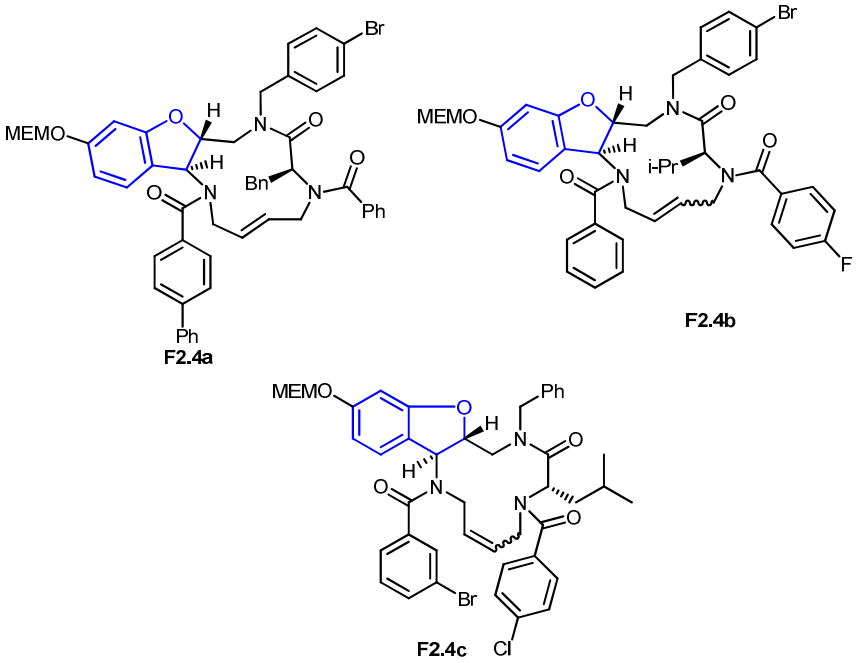
Synthesis of macrocycle **F2.4** follows the same approach as discussed with the synthesis of previous two macrocyles **F2.3** and **F2.2**, and, it starts from **F2.1** as shown in **Scheme 6**. The detailed synthesis of **7.3** from **F2.1** is shown in **Chapter 2** (synthesis of compound **F3.1)**. Benzoylation of **5.3** with benzoyl chloride/ DIEA condition gave **5.2** with 80-85% yield. The *bis*-allylation of **5.2** with allyl bromide/NaH/TBAI conditions, followed by macrocyclization, using 20 mol% Grubbs 2nd generation catalyst yielded the macrocycle compound **F2.4** with an overall 66-70% yield for 2 steps. In this case, we synthesized three macrocycles, all of them were obtained as the singal isomer as observed by NMR and HPLC-LC-MS. For example, **F2.5a** obtained with the *trans* geometry was assigned by ^{1}HNMR, but in other cases, we could not assign the olefine geometry due to the proton merging with other protons in ^{1}HNMR spectrum.



Scheme 6. Synthesis of macrocycle **F2.4**

3.3.9. Derivatives of Macrocycle F2.4

We synthesized three derivatives of macrocycle **F2.4**, by replacing R_1, R_2, R_3 groups and they are shown below:



3.4. Biological Evaluation

We screened a series of our *enantioenriched*, benzofuran-derived compounds with 12-membered macrocyclic toolbox for their ability to inhibit the mitochondrial membrane permeabilization, and, to prevent cytochrome c release during the endoplasmic reticulum stress in cultured pancreatic β-cells.

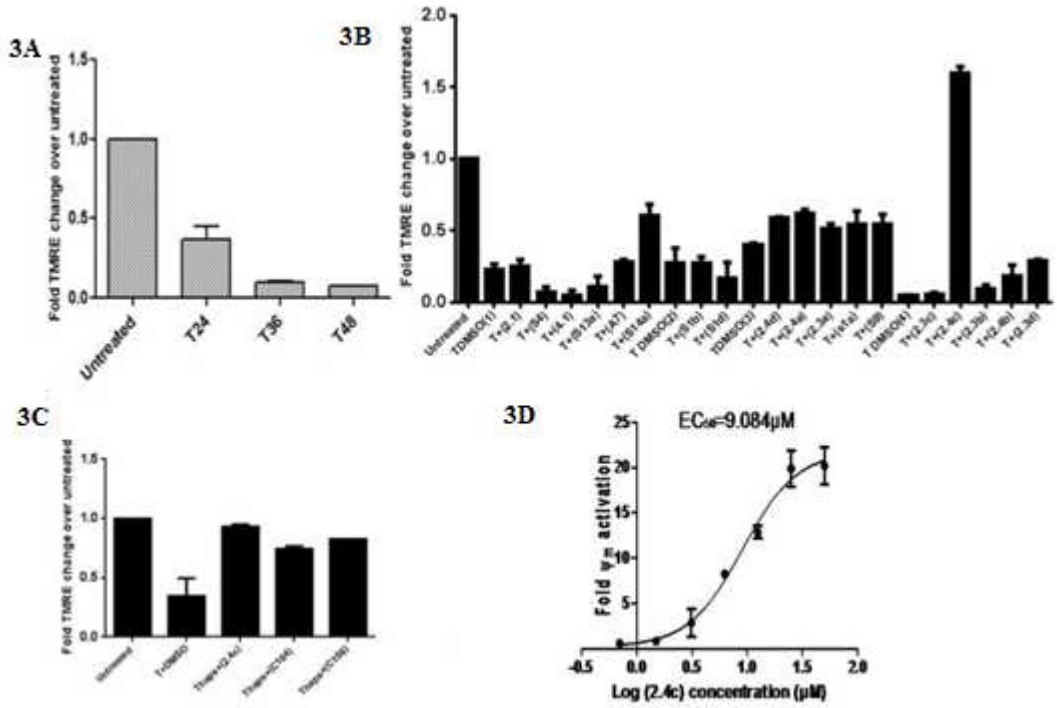
Mitochondria play an essential role in pancreatic β-cell homeostasis through their involvement in the modulation of stimulus-coupled insulin secretion,[11] and, in the regulation of cell survival.[12,13] The permeabilization of mitochondrial membranes under the influence of various cytokines results in the release of cytochrome c, which is known to activate the caspase cascade.[14] Most importantly, during the chronic endoplasmic reticulum (ER) stress, the accumulation of unfolded proteins in the ER results in leakage of calcium from the ER, which leads to calcium overload in the mitochondria,[15,16] and, the consequent opening of the mitochondrial permeability transition pore. The latter process plays a decisive role in the depolarization of the mitochondrial membrane potential and programmed cell death (commonly known as

PCD). In our present study, we induced chronic ER stress in cultured BRIN-BD11 pancreatic β-cells by treating them with the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) pump inhibitor thapsigargin. Thapsigargin treatment causes the depolarization of the mitochondrial inner membrane potential and compromises cell survival.[17] In our search to prevent this depolarization, we utilized a small molecule toolbox that are rich in 3D-architectures and can be considered in the broad family of natural product inspired benzofuran-based macrocycles compounds. Our data reveals that macrocycle ring **F2.2c** prevent the depolarization of mitochondrial membrane potential, inhibits cytochrome c release from mitochondria, preserves the mitochondrial function and also prevents thapsigargin induced death of cultured pancreatic β-cells.

Figure 3A shows the temporal effect of thapsigargin on the depolarization of the MMP. As the data reveals, a 36 h treatment of thapsigargin at a concentration of 5.0 μM caused a 10-fold reduction in the mitochondrial membrane potential. To prevent this depolarization of the MMP, we screened a library of benzofuran-derived compounds to study their efficacy to rescue the phenotype (**Figure 3B,C**) Compound **F2.2c** was found to prevent the depolarization of the MMP induced on thapsigargin treatment in cultured pancreatic β-cells. Compound **F2.2c** possessing a 12-membered macrocyclic ring and having an N-(4-nitrobenzoyl)valine amino acid moiety fused to the benzofuran scaffold showed the highest activity for the prevention of thapsigargin-induced depolarization of the mitochondrial membrane potential. To validate the structural features of this compound, we further synthesized two more related macrocyclic compounds, that is, **F2.2f** and **F2.2g**. In **F2.2f**, the N-(4-nitrobenzoyl)valine is replaced by an N-benzoylvaline unit (i.e., no NO_2 group), whereas the amino acid moiety in **F2.2c** is replaced by leucine to obtain **F2.2g**. A comparative account of the efficacy of all these macrocycles to prevent thapsigargin induced depolarization of the MMP is shown in **Figure 3**. Compounds **F2.2c**, **F2.2f** , and **F2.2g** showed comparable efficacy in the prevention of thapsigargin-induced depolarization of the MMP at a concentration of 10.0 μM. Interestingly, the acyclic precursor of **F2.2c** (i.e. **1.2c**) did not show any effect. In addition, replacement of the N-benzoylvaline amino acid moiety with an N-benzoyl-(phenylalanine) (i.e., **F2.2b**) group dramatically reduced the activity. A dose–response curve for the prevention of

the depolarization of the MMP by **F2.2c** is shown in **Figure 3d**, and the EC_{50} of the response was found to be 9.04 μM (**Figure 3D**).

In the next step, we evaluated the distribution of cytochrome c in untreated, thapsigargin treated, and **F2.2c** treated cells. As **Figure 2a** reveals, in normal cells, there was a complete overlap of cytochrome c staining with Mito-Tracker Red; this indicated its presence in the mitochondria. Treatment with thapsigargin for 18 h caused a marked loss of Mito-Tracker Red and a concomitant release of cytochrome c into the cytoplasm (**Figure 4b**), which was totally prevented with the use of **F2.2c** (**Figure 4c**). The data suggest the role of **F2.2c** in preventing the release of cytochrome c from the mitochondria, which is known to activate cell death in pancreatic β-cells.



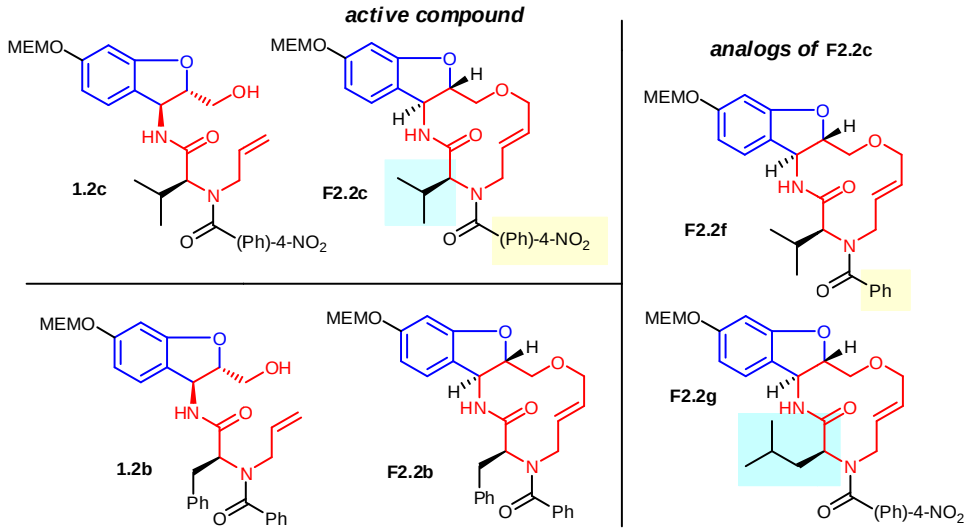


Figure 3. The prevention of thapsigargin induced mitochondrial depolarization: **3A**: Temporal depolarization of mitochondrial membrane potential ($\Delta\Psi_m$) in pancreatic β-cells; **3B** and **3C**: Screening potential of compounds that prevent the depolarization of MMP; 3D: Dose response curve for the rescue of Thapsigargin induced depolarization of $\Delta\Psi_m$ by compound **F2.2c**.

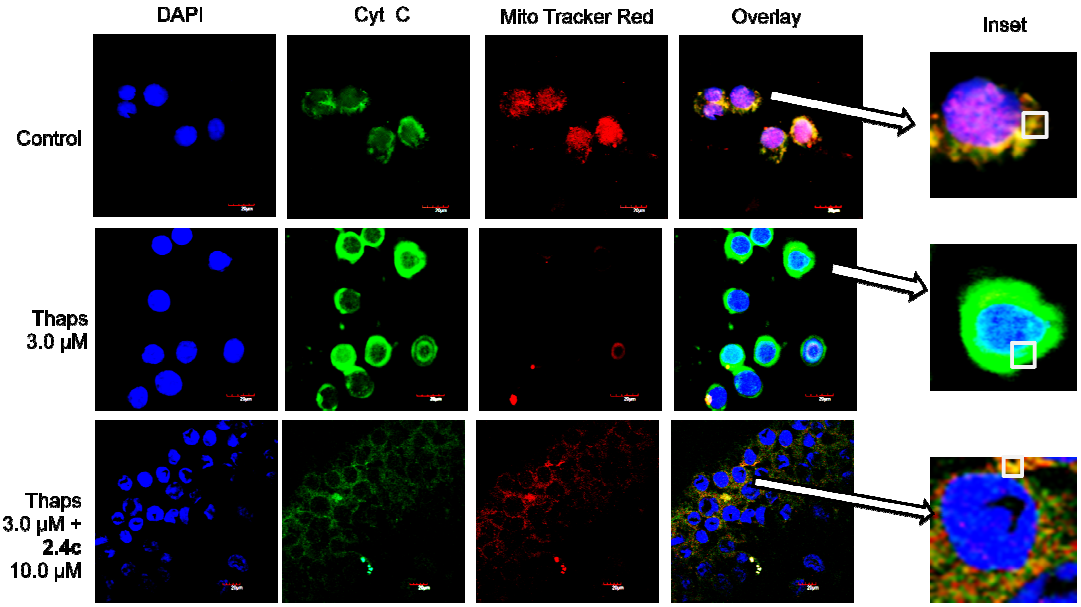


Figure 4. The protective effect of compound **F2.2c** from thapsigargin induced apoptosis: Confocal microscopy images of BRIN-BD11 cells immune-labeled with primary monoclonal mouse anti-cytochrome c antibody (Green) depicting release of cytochrome c from mitochondria. Mitochondria were labeled by Mito-tracker Red

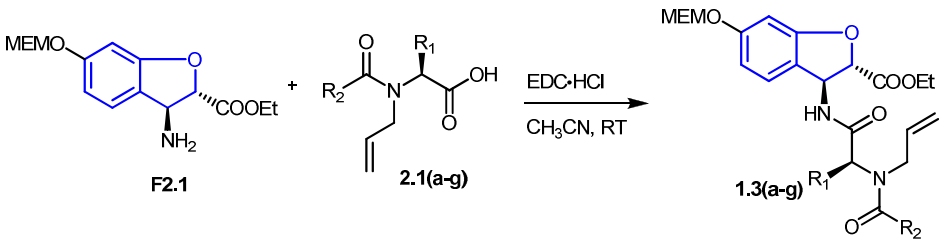
(Red) and nucleus was visualized by DAPI (Blue) staining. Yellow punctuates in control and **F2.2c** treated cells showed co-localization of cytochrome c in mitochondria.

3.5. Conclusions

We synthesized *enantioenriched* benzofuran-derived natural product inspired 12-membered macrocyclic toolbox. To the best of our knowledge, this is the first report of a macrocyclic small molecule that modulates the mitochondrial membrane potential (ΔΨm), and, the compound further prevents the release of cytochrome c from mitochondria from thapsigargin-induced ER stress in pancreatic β-cells. Given the role of small molecule **F2.2c** in preventing mitochondria from high cytosolic calcium insult, this compound may also have interesting applications related to neurological disorders, such as, cortical spreading depression (CSD). Whether **F2.2c** regulates protein misfolding and/or clustering in mitochondria or participates in chaperone-mediated regulation of mitochondrial membrane permeabilization is yet to be determined.

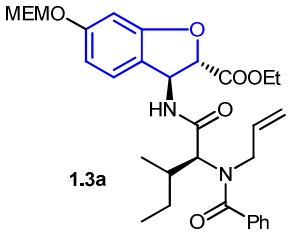
3.6. Experimental Procedure

Compound 1.3(a-g):



To a stirred solution of compound **F2.1 (**1eq) and amino acid derivative **2.1(a-g)** (1.2 eq) in CH_3CN was added EDC·HCl (1.2 eq) at room temperature under an inert atmosphere. The reaction was allowed stirred for 1 hour. The reaction mixture was quenched by the addition of a saturated *aq*. $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product **1.3(a-g)**.

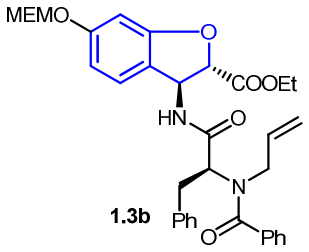
(2S,3S)-ethyl-3-((2S,3S)-2-(N-allylbenzamido)-3-methylpentanamido)-6-((2-methoxy ethoxy)methoxy)-2,3-di hydro benzofuran-2-carboxylate (1.3a):



1.3a

Molecular Formula: $C_{31}H_{40}N_2O_8$; R_f (50% ethyl acetate/hexane): 0.4; Purified by flash chromatography using 40% ethyl acetate in hexane; Yield: 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.86-1.02 (m, 6H), 1.12-1.18 (m, 3H),1.25-1.32 (m, 2H) 1.70-1.87 (m, 1H), 2.43-2.63 (m, 1H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.81-3.83 (m, 3H), 3.94-4.17 (m, 2H), 4.26-4.29 (m, 2H), 4.83-5.06 (m, 3H), 5.25 (s, 2H), 5.66-5.73 (m, 1H), 5.90-5.92 (m, 1H), 6.45-6.55 (m, 1H), 6.65-6.68 (m, 1H), 6.98-7.16 (m, 2H), 7.53-7.41 (m, 5H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 10.2, 10.9, 13.8, 15.5, 24.6, 26.0, 31.9, 32.4, 54.7, 50.3, 58.7, 61.5, 67.4, 71.3, 85.8, 93.3, 98.9, 109.6, 109.7, 118.1, 124.7, 124.9, 126.3, 126.4, 128.2, 129.6, 129.7, 132.9, 133.0, 135.9, 135.9, 159.2, 159.2, 160.4, 169.0, 169.1, 169.8, 169.9, 173.5, 173.7, 173.7; LRMS: (ES+) m/z = 569.3 (M+1).

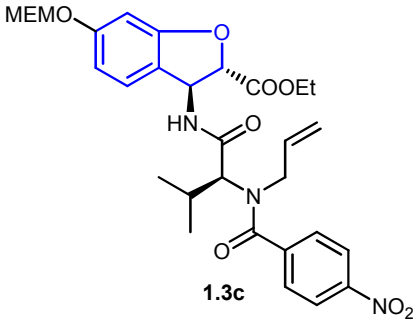
(2S,3S)-ethyl,3-((S)-2-(N-allylbenzamido)-3-phenylpropanamido)-6-((2methoxy ethoxy)methoxy)-2,3-dihydrobenzo furan-2-carboxylate (1.3b):



1.3b

Molecular Formula: $C_{34}H_{38}N_2O_8$; R_f (50% ethyl acetate/ hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 69%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.97 (m, 6H), 1.23-1.32 (m, 3H), 1.54-1.71 (m, 1H), 1.94-1.76 (m, 2H), 3.38 (s, 3H), 4.27 (t, *J* = 7.1 Hz, 2H), 3.54-3.60 (m, 2H), 3.80-3.85 (m, 2H), 3.86-4.01 (m, 2H), 4.91 (d, *J* = 4.2 Hz, 3H), 5.10 (m, 1H), 5.26 (s, 2H), 5.65-5.75 (m, 2H), 6.64-6.73 (m, 2H), 7.22 (m, 2H), 7.35 (dd, *J* = 8.3, 4.1 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.1, 34.3, 51.5, 55.2, 59.0, 61.9, 67.8, 71.5, 86.1, 93.6, 99.2, 99.3, 110.0, 118.4, 124.9, 125.3, 128.3, 128.7, 133.4, 134.1, 136.2, 159.7, 160.7, 160.8, 169.2, 169.2, 170.5; LRMS: (ES+) m/z = 603 (M+1), 625.3 (M+Na).

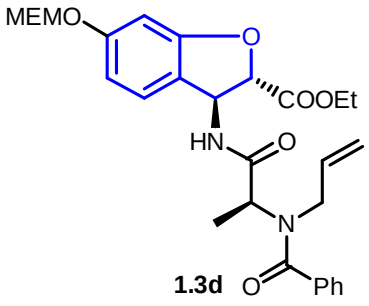
(2S,3S)-ethyl3-((S)-2-(N-allyl-4-nitrobenzamido)-3-methylbutanamido)-6-((2-methoxyethoxy) methoxy)-2,3-dihydrobenzofuran-2-carboxylate (1.3c):



1.3c

Molecular Formula: $C_{30}H_{37}N_3O_{10}$; R_f (30% ethyl acetate/hexane): 0.4; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.98 (m, 6H), 1.22 (d, *J* = 7.4 Hz, 3H), 2.52 (m, 1H), 3.29 (s, 3H), 3.50 (t, *J* = 4.8 Hz, 2H), 3.76 (t, *J* = 4.8 Hz, 2H), 3.83-4.02 (m, 2H), 4.19 (dd, *J* = 13.0, 6.1 Hz, 2H), 4.29-4.46 (m, 1H), 4.70-4.88 (m, 2H), 4.97 (t, *J* = 7.8 Hz, 1H), 5.21 (m, 3H), 5.50-5.69 (m, 2H), 6.57 (d, *J* = 6.6 Hz, 2H), 7.00-7.12 (m, 1H), 7.34-7.51 (m, 2H), 7.68 (d, *J* = 8.9 Hz, 1H), 8.17 (t, *J* = 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 18.9, 19.6, 29.5, 49.6, 55.0, 58.9, 61.8, 67.7, 71.4, 85.6, 85.9, 93.5, 99.0, 110.0, 118.1, 123.7, 125.0, 127.6, 128.2, 132.8, 142.1, 148.3, 159.5, 160.5, 165.2, 169.1, 169.2, 169.3, 169.3, 171.5; LRMS: (ES+) m/z = 600.3 (M+1).

(2S,3S)-ethyl3-((S)-2-(N-allylbenzamido)propanamido)-6-((2-methoxyethoxy) meth oxy)-2,3-dihydrobenzo furan-2-carboxylate (1.3d):

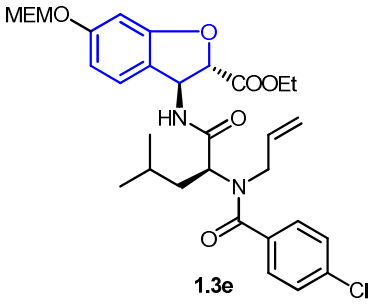


1.3d

Molecular Formula: $C_{28}H_{34}N_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25-1.30 (m, 6H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.78-3.87 (m, 2H), 3.94 (bs, 2H), 4.27 (q, *J* = 6.8 Hz, 2H), 4.87-4.94 (m, 2H), 5.04-5.15 (m, 2H), 5.24 (s, 2H), 5.67-5.72 (m, 2H), 6.65-6.68 (m, 2H), 7.09-7.17 (m, 1H), 7.27-7.50 (m, 5H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.1, 29.6, 49.8, 55.2, 59.0, 61.8, 67.7, 71.5, 86.2, 93.5, 93.5, 99.2, 110.1, 117.9, 125.0, 125.2, 126.5, 127.3,

128.5, 130.0, 131.9, 133.8, 135.6, 159.6, 159.7, 160.7, 160.8, 169.1, 171.0; LRMS: (ES+) m/z = 485.2 (M-1).

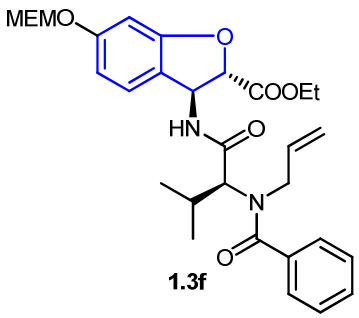
(2S,3S)-ethyl,3-((S)-2-(N-allyl-4-chlorobenzamido)-4-methylpentanamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-carboxylate (1.3e):



1.3e

Molecular Formula; $C_{31}H_{39}ClN_2O_8$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.97 (m, 6H), 1.23-1.32 (m, 3H), 1.54-1.71 (m, 1H), 1.76-1.94 (m, 2H), 3.38 (s, 3H), 4.27 (t, *J* = 7.1 Hz, 2H), 3.54-3.60 (m, 2H), 3.80-3.85 (m, 2H), 3.86-4.01 (m, 2H), 4.91 (d, *J* = 4.2 Hz, 3H), 5.10 (m,1H), 5.26 (s, 2H), 5.65-5.75 (m, 2H), 6.64-6.73 (m, 2H), 7.22 (m, 2H), 7.35 (dd, *J* = 8.3, 4.1 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.1, 22.7, 24.9, 25.0, 29.6, 36.8, 49.7, 55.1, 58.9, 61.8, 67.7, 71.5, 86.1, 93.6, 99.1, 99.2, 110.0, 118.4, 124.9, 125.3, 128.3, 128.7, 133.4, 134.1, 136.2, 159.7, 160.7, 160.8, 169.2, 169.2, 170.5; LRMS: (ES+) m/z = 601(M-1).

(2S,3S)-ethyl3-((S)-2-(N-allylbenzamido)-3methylbutanemido)-6-((2-methoxy ethoxy) methoxy)-2,3-dihydrobenzofuran-2-carboxylate (1.3f):

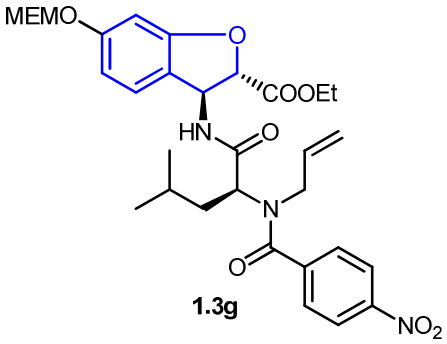


1.3f

Molecular Formula: $C_{30}H_{38}N_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.99 (m, 6H), 1.29 (t, *J* = 7.1 Hz, 3H), 2.59-2.77 (m, 1H), 3.34 (s, 3H), 3.58-3.51 (d, 2H), 3.74-3.89 (m, 4H), 3.92-4.05 (m, 1H), 4.08-4.32 (m, 3H),

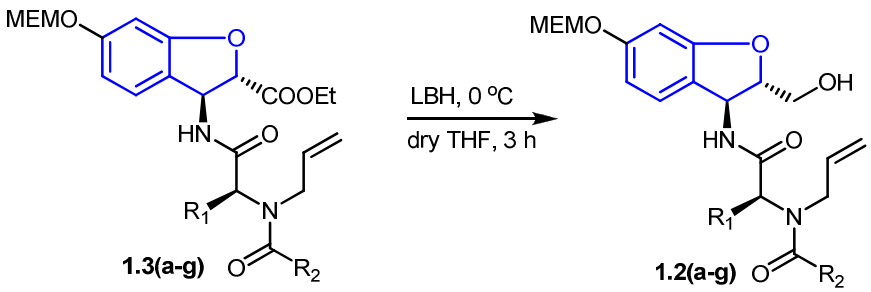
4.85-4.87 (m, 2H), 5.05 (t, *J* = 7.8 Hz, 1H), 5.16-5.28 (m, 3H), 5.68-5.70 (m, 2H), 6.65 (d, *J* = 11.5 Hz, 2H), 7.09 (s, 1H), 7.31-7.46 (m, 5H), 7.78-7.91 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 19.1, 19.8, 26.3, 54.9, 54.9, 59.0, 61.8, 67.6, 71.5, 86.1, 93.4, 99.1, 99.2, 109.9, 118.1, 118.7, 118.9, 125.0, 126.6, 128.4, 130.0, 132.9, 136.0, 136.0. 159.5, 160.6, 169.2, 170.1, 173.9; LRMS: (ES+) m/z = 555.6 (M+1).

(2S,3S)-ethyl3-((S)-2-(N-allyl-4-nitrobenzamido)-4-methylpentanamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-carboxylate (1.3g):



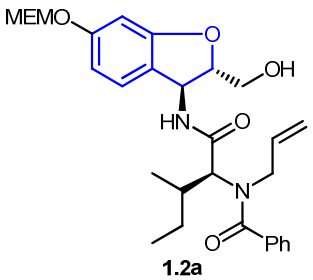
Molecular Formula; $C_{31}H_{39}N_3O_{10}$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.97 (m, 6H), 1.32-1.23 (m, 3H), 1.71-1.54 (m, 1H), 1.94-1.76 (m, 2H), 3.38 (s, 3H), 4.27 (t, *J* = 7.1 Hz, 2H), 3.54-3.60 (m, 2H), 3.80-3.85 (m, 2H), 3.86-4.01 (m, 2H), 4.91 (d, *J* = 4.2 Hz, 3H), 5.10 (m,1H), 5.26 (s, 2H), 5.65-5.75 (m, 2H), 6.64-6.73 (m, 2H), 7.22 (m, 2H), 7.35 (dd, *J* = 8.3, 4.1 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.1, 22.7, 25.0, 25.0, 36.8, 49.8, 55.2, 59.0, 61.9, 67.8, 71.5, 86.1, 93.6, 99.2, 99.3, 110.0, 118.4, 124.9, 125.3, 128.3, 128.7, 133.4, 134.1, 136.2, 159.7, 160.7, 160.8, 169.2, 169.2, 170.5; LRMS: (ES+) m/z = 612 (M-1).

Compound 1.2(a-g):



To a stirred solution of compound 1.3(a-g) (1eq) in dry THF was added $LiBH_4$ (1.2 eq) at 0 °C under inert atmosphere. The reaction was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was quenched by the addition of a saturated *aq*. NH_4Cl, extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purified by flash chromatography to obtained pure product 1.2(a-g).

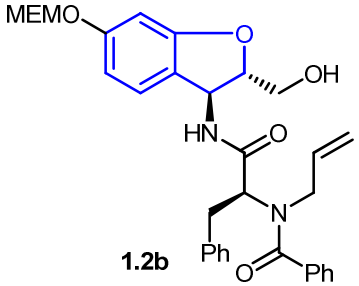
N-allyl-N-((2S,3S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxopentan-2-yl)benzamide (1.2a):



1.2a

Molecular Formula: $C_{29}H_{38}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91-0.98 (m, 6H), 1.14 (bs, 1H), 1.58 (bs, 1H), 2.45 (bs, 1H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.80-4.04 (m, 6H), 4.18-4.43 (m, 1H), 4.51 (d, *J* = 5.0 Hz, 1H), 4.87-4.94 (m, 1H), 5.03-5.08 (m, 1H), 5.24-5.31 (m, 3H), 5.63-5.67 (bs, 1H), 6.56-6.64 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.26-7.54 (m, 5H), 7.82-8.00 (m, 1H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 10.3, 11.1, 15.1, 15.9, 24.9, 26.4, 29.6, 31.9, 32.5, 50.8, 53.2, 58.9, 67.6, 70.8, 71.5, 89.6, 93.5, 99.1, 109.1, 117.3, 124, 125.0, 126.7, 126.7, 128.4, 130.0, 132.9, 133.1, 134.3, 136.1, 159.3, 161.0, 170.5, 174.0, 174.0; LRMS: (ES+) m/z = 525.3 (M-1), 599.1 (M+Na).

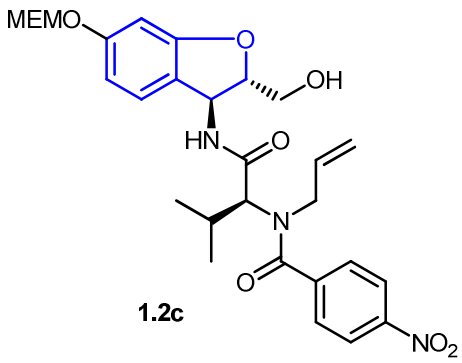
N-allyl-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (1.2b):



1.2b

Molecular Formula: $C_{32}H_{36}N_2O_7$; R_f (70% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 70% ethyl acetate in hexane; Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.34-3.35 (m, 5H), 3.51-3.61 (m, 2H), 3.64-3.90 (m, 6H), 4.46-4.55 (m, 1H), 4.81(bs, 1H), 4.97-5.09 (m, 2H), 5.21-5.31 (m, 3H), 5.42-5.60 (m, 1H), 6.56-6.69 (m, 2H), 7.00-7.11(m, 3H), 7.24-7.44 (m, 8H), 7.50-7.58 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 34.4, 52.0, 52.0, 54.6 54.7, 59.0, 63.4, 63.5, 67.7, 71.5, 90.9, 91.2, 93.5, 98.9, 98.9, 109.4, 109.4, 118.2, 118.8, 125.0, 125.1, 128.4, 128.6, 129.2, 129.2, 130.1, 130.1, 133.0, 133.1, 135.4, 135.4, 136.9, 159.5, 159.6, 160.8, 160.9, 171.5, 173.7; LRMS: (ES+) m/z = 561 (M+1), 583.3 (M+Na).

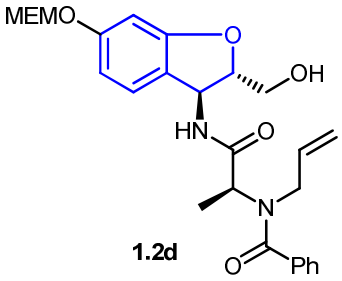
N-allyl-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxobutan-2-yl)-4-nitrobenzamide (1.2c):



1.2c

Molecular Formula: $C_{28}H_{35}N_3O_9$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.02 (m, 6H), 2.57-2.74 (m, 1H), 3.38 (s, 3H), 3.53-3.60 (m, 2H), 3.78-3.99 (m, 6H), 4.22-4.38 (m, 1H), 4.49-4.56 (m, 1H), 4.89 (m, 1H), 5.10 (m, 1H), 5.21-5.35 (m, 3H), 5.59-5.73 (m, 1H), 6.63 (m, 2H), 7.10 (t, *J* = 7.9 Hz, 1H), 7.51 (dd, *J* = 8.5, 3.4 Hz, 3H), 8.27 (d, *J* = 6.7 Hz, 2H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.0, 19.1, 19.7, 29.6, 50.8, 54.6, 59.0, 63.5, 67.7, 71.5, 90.9, 91.2, 93.5, 99.0, 109.5, 118.8, 119.2, 123.8, 125.1, 127.7, 128.3, 132.5, 141.9, 148.5, 159.6, 160.8, 170.6, 171.7; LRMS: (ES+) m/z = 522 (M+1).

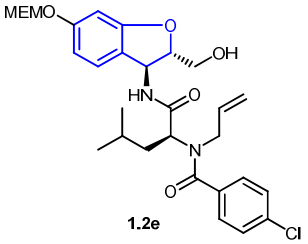
N-allyl-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihy drobenzofuran-3-yl amino)-1-oxopropan-2-yl)benzamide (1.2d):



1.2d

Molecular Formula: $C_{26}H_{32}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (bs, 3H), 3.31 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.72-3.75 (m, 6H), 4.41-4.51 (m, 1H), 4.77-4.81 (m, 1H), 5.07-5.11 (m, 2H), 5.17 (s, 2H), 5.19-5.25 (m, 3H), 5.73 (bs, 1H), 6.50-6.57 (m, 2H), 7.01-7.09 (m, 1H), 7.26-7.37 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 29.6, 49.8, 55.2, 59.0, 61.8, 67.7, 71.5, 86.2, 93.5, 98.9, 99.0, 109.4, 118.2, 125.0, 125.3, 126.5, 126.6, 128.5, 130.1, 133.7, 133.8, 135.5, 159.5, 159.6, 160.9, 172.1, 172.1; LRMS: (ES+) m/z = 485.2 (M+1).

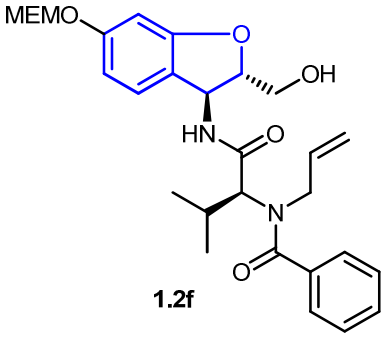
N-allyl-4-chloro-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy-2,3-dihydrobenzofuran-3-ylamino)-4-methyl-1oxopentan-2-yl)benzamide (1.2e):



1.2e

Molecular Formula: $C_{29}H_{37}ClN_2O_7$; R_f (50% ethyl acetate/hexane): 0.1; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.98 (s, 6H), 1.53-1.66 (m, 1H), 1.77-1.99 (m, 2H), 3.34-3.43 (m, 3H), 3.53-3.60 (m, 2H), 3.69-4.08 (m, 6H), 4.44-4.61 (m, 1H), 4.83-5.09 (m, 2H), 5.08-5.16 (m, 1H), 5.25 (s, 3H), 5.63-5.85 (m, 1H), 6.58 (s, 1H), 6.64 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.05-7.18 (m, 1H), 7.27-7.31 (m, 2H), 7.36-7.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.7, 22.8, 25.0, 36.7, 50.2, 54.7, 59.0, 59.0, 63.5, 67.7, 71.5, 91.2, 93.5, 99.0, 109.5, 118.0, 124.9, 125.3, 128.3, 128.4, 128.9, 133.3, 134.0, 136.4, 136.4, 159.7, 159.7, 160.8, 160.9, 171.9; LRMS: (ES+) m/z = 561 (M+1).

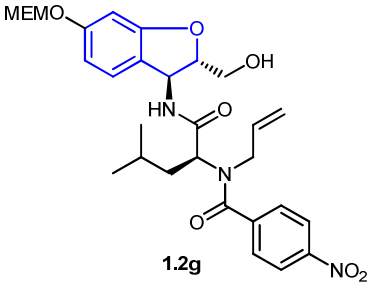
N-allyl-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxobutan-2-yl)benzamide (1.2f):



1.2f

Molecular Formula: $C_{28}H_{36}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.03 (m, 6H), 2.57-2.74 (m, 1H), 3.37-3.38 (m, 4H), 3.56 (m, 3H), 3.76-3.92 (m, 5H), 4.52 (d, *J* = 5.4 Hz, 1H), 4.89-5.03 (m, 1H), 5.06-5.32 (m, 4H), 5.61-5.75 (m, 1H), 6.53-6.68 (m, 2H), 7.10 (s, 1H), 7.39-7.40 (m, 5H), 7.83-7.96 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 19.1, 19.7, 22.6, 26.6, 26.6, 29.6, 31.8, 54.6, 59.0, 63.5, 67.7, 71.5, 90.9, 91.2, 93.5, 99.0, 109.5, 118.8, 119.2, 123.8, 125.1, 127.7, 128.3, 132.5, 141.9, 148.5, 159.6, 160.8, 170.6, 171.7; LRMS: (ES+) m/z = 513 (M+1).

N-allyl-N-((S)-1-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)-4-nitrobenz amide (1.2g):

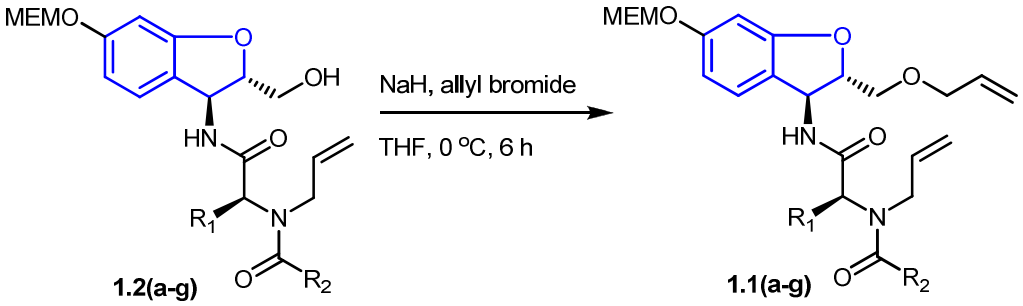


1.2g

Molecular Formula: $C_{29}H_{37}N_3O_9$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.98 (s, 6H), 1.53-1.66 (m, 1H), 1.77-1.99 (m, 2H), 3.34-3.43 (m, 3H), 3.53-3.60 (m, 2H), 3.69-4.08 (m, 6H), 4.44-4.61 (m, 1H), 4.83-5.09 (m, 2H), 5.08-5.16 (m, 1H), 5.25 (s, 3H), 5.63-5.85 (m, 1H), 6.58 (s, 1H), 6.64 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.05-7.18 (m, 1H), 7.27-7.31 (m, 2H), 7.36-7.40 (m, 2H); ^{13}C NMR

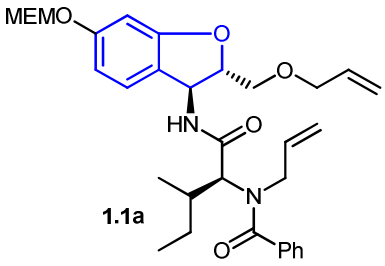
(100 MHz, $CDCl_3$) δ ppm 22.8, 22.8, 25.1, 29.7, 54.7, 59.0, 59.1, 63.5, 67.8, 71.6, 91.2, 93.6, 99.1, 109.6, 118.1, 124.9, 125.3, 128.3, 128.4, 128.9, 133.3, 134.0, 136.4, 136.4, 159.7, 159.7, 160.9, 161.0, 171.9; LRMS: (ES+) m/z = 572 (M+1).

Compound 1.1(a-g)



To a stirred solution of compound **1.2(a-g) (**1eq) in dry THF was added NaH (1.2 eq) at 0 °C under inert atmosphere. The reaction mixture was stirred for 5 minute, then allyl bromide (1.3eq) was added. The reaction mixture was stirred for 6 hours, then reaction mixture was quenched by ice water, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product **1.1(a-g)**.

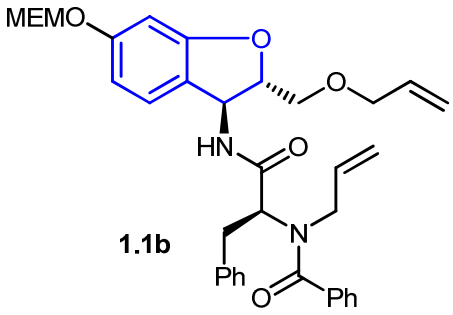
N-allyl-N-((2S,3S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxopentan-2-yl)benzamide (1.1a):



Molecular Formula: $C_{32}H_{42}N_2O_7$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91-0.98 (m, 6H), 1.14 (bs, 1H), 1.58 (bs, 1H), 2.45 (bs, 1H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.70-3.74 (m, 1H), 3.89-4.11(m, 3H), 4.23-4.36 (m, 1H), 4.61 (bs, 1H), 4.86-4.92 (m, 1H), 5.05 (d, *J* = 8.0 Hz, 1H), 5.15-5.18 (m, 1H), 5.24-5.28 (m, 3H), 5.31-5.33 (m, 1H), 5.67 (bs, 1H), 5.85-5.93(m, 1H), 6.60-6.63 (m, 2H), 6.99-7.17 (m, 1H), 7.31-7.54 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 10.3, 11.1, 15.1, 15.9, 24.9, 26.4, 29.6, 31.9, 32.5, 53.2, 58.9, 67.6, 70.8, 71.5, 72.5, 89.6, 93.5, 99.1, 109.1, 117.3, 124.9, 125.0, 126.7, 126.7, 128.4,

130.0, 130.0, 132.9, 133.1, 134.3, 136.1, 159.3, 161.0, 170.5, 174.0, 174.0; LRMS: (ES+) m/z = 567.3 (M+1), 589.3 (M+Na).

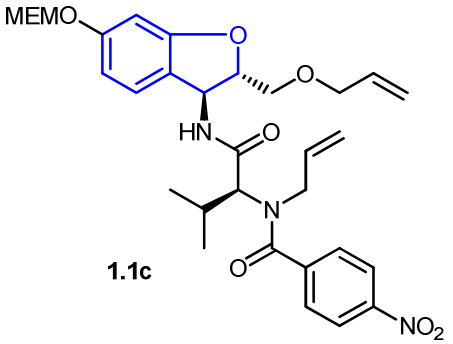
N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (1.1b):



1.1b

Molecular Formula: $C_{35}H_{40}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.4; Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.31-3.48 (m, 5H), 3.55-3.57 (t, *J* = 4.8 Hz, 2H), 3.69-3.73 (m, 2H), 3.76-3.86 (m, 4H), 4.06-4.09 (m, 2H), 4.51-4.66 (m, 1H), 4.79-4.90 (bs, 1H), 4.94-5.14 (m, 2H), 5.18 (d, *J* = 10.4 Hz, 1H), 5.24 (s, 2H), 5.27-5.40 (m, 2H), 5.42-5.59 (m, 1H), 5.86-5.98 (m, 1H), 6.59-6.62 (m, 2H), 7.01-7.11 (m, 3H), 7.26-7.41 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 29.6, 53.4, 59.0, 67.6, 70.8, 71.5, 72.5, 89.4, 89.6, 93.5, 99.1, 99.1, 109.2, 117.3, 117.3, 118.7, 125.0, 125.1, 126.6, 126.9, 128.4, 128.6, 129.1, 129.2, 130.0, 133.1, 134.4, 135.5, 137.0, 137.1, 159.4, 159.4, 161.0, 161.1, 170.6, 173.6; LRMS: (ES+) m/z = 601 (M+1), 623 (M+Na).

N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxobutan-2-yl)-4-nitrobenz amide (1.1c):

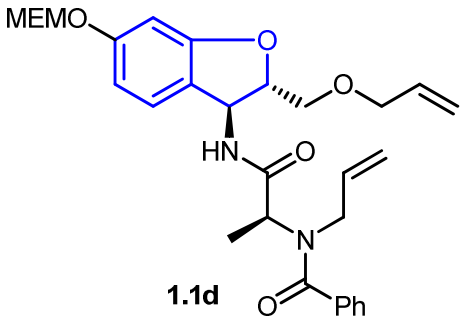


1.1c

Molecular Formula: $C_{31}H_{39}N_3O_9$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 83%; ^{1}H NMR (400

MHz, $CDCl_3$) δ ppm 0.93-1.12 (m, 6H), 2.56-2.70 (m, 1H), 3.38 (s, 3H), 3.54-3.60 (m, 2H), 3.73 (s, 1H), 3.77-3.86 (m, 3H), 3.93 (s, 2H), 4.08 (d, *J* = 5.3 Hz, 2H), 4.24-4.35 (m, 1H), 4.57-4.67 (m, 1H), 4.83-4.93 (m, 1H), 5.07 (m, 1H), 5.18 (m, 1H), 5.26 (m, 3H), 5.36 (bs, 1H), 5.56-5.73 (m, 1H), 5.84-5.98 (m, 1H), 6.63 (d, *J* = 9.8 Hz, 2H), 7.10 (s, 1H), 7.15-7.25 (m, 1H), 7.46-7.55 (m, 2H), 8.27 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 19.0, 19.8, 29.6, 50.5, 53.4, 59.0, 67.7, 71.5, 72.5, 89.5, 93.6, 99.1, 99.2, 109.3, 117.3, 118.7, 118.8, 118.9, 118.9, 123.8, 125.1, 127.7, 132.6, 134.3, 142.1, 148.4, 159.5, 161.0, 169.7; LRMS: (ES+) m/z = 596.2 (M-1).

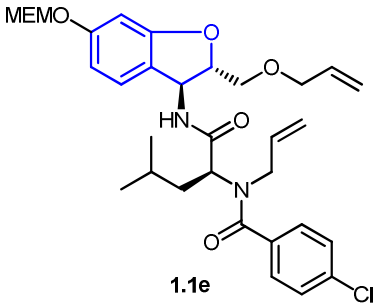
N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl amino)-1-oxopropan-2-yl)benzamide(1.1d):



1.1d

Molecular Formula: $C_{29}H_{36}N_2O_7$; R_f (30% ethyl acetate/hexane): 0.4; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 87%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (bs, 3H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.67-3.76 (m, 1H), 3.80-3.82 (m, 3H), 3.94 (bs, 2H), 4.07 (d, *J* = 4.30 Hz, 2H), 4.55-4.70 (m, 1H), 5.06-5.17 (m, 3H), 5.24 (s, 3H), 5.27 (bs, 1H), 5.34-5.36 (m, 1H), 5.77 (bs, 1H), 5.85-5.93 (m, 1H), 6.60-6.63 (m, 2H), 7.06-7.15 (m, 1H), 7.31-7.42 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 29.6, 50.0, 54.5, 58.9, 63.4, 67.7, 71.5, 91.1, 93.5, 98.9, 99.0, 109.4, 118.2, 125.0, 125.3, 126.5, 126.6, 128.5, 130.1, 133.7, 133.8, 135.5, 159.5, 159.6, 161.0, 161.1, 171.3; LRMS: (ES+) m/z = 546.9 (M+Na).

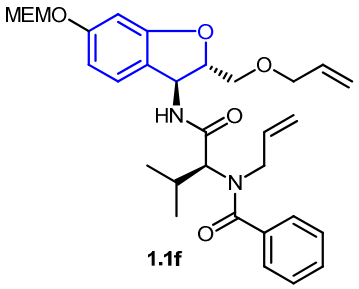
N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)-4-chlorobenzamide (1.1e):



1.1e

Molecular Formula: $C_{32}H_{41}ClN_2O_7$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.97 (bs, 6H), 1.64 (bs, 1H), 1.85 (bs, 2H), 3.38 (s, 3H), 3.52-3.60 (m, 2H), 3.71 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.75-3.92 (m, 4H), 3.90-4.14 (m, 3H), 4.52-4.69 (m, 1H), 4.82-5.04 (m, 2H), 5.06-5.39 (m, 6H), 5.60-5.75 (m, 1H), 5.83-5.99 (m, 1H), 6.57-6.68 (m, 2H), 7.03-7.13 (m, 2H), 7.25-7.43 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.2, 22.8, 25.0, 29.6, 36.6, 36.6, 49.9, 49.9, 53.3, 53.3, 53.4, 59.0, 59.0, 67.7, 70.8, 71.5, 72.5, 89.6, 93.5, 93.5, 99.2, 109.1, 109.2, 117.3, 117.4, 118.8, 124.8, 125.2, 128.3, 128.8, 133.4, 134.1, 134.3, 136.2, 159.5, 161.0, 161.1, 170.9, 172.9; LRMS: (ES+) m/z = 600 (M-1).

N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-3-methyl-1-oxobutan-2-yl)benzamide (1.1f):

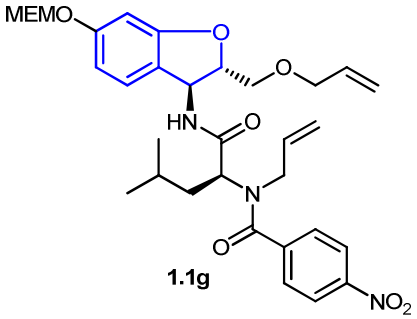


1.1f

Molecular Formula: $C_{31}H_{40}N_2O_7$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane ; yield 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.03 (m, 6H), 2.38-2.45 (m, 2H), 3.07 (d, *J* = 13.8 Hz, 2H), 3.18 (d, *J* = 13.8 Hz, 2H), 3.38 (s, 3H), 3.54-3.60 (m, 2H), 3.65-3.73 (m, 2H), 3.82 (dd, *J* = 4.9, 3.8 Hz, 3H), 4.06 -4.08 (m, 1H), 4.57-4.68 (m, 1H), 4.87-4.99 (m, 1H), 5.03-5.11 (m, 1H), 5.15-5.30 (m, 4H), 5.62-5.73 (m, 1H), 5.85-5.96 (m, 1H), 6.61 (s, 2H), 7.09 (d, *J* = 7.9 Hz, 3H), 7.20 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 14.0, 19.0, 19.8, 26.4, 26.5, 29.6, 53.4, 59.0, 67.7, 71.5, 72.5, 89.5,

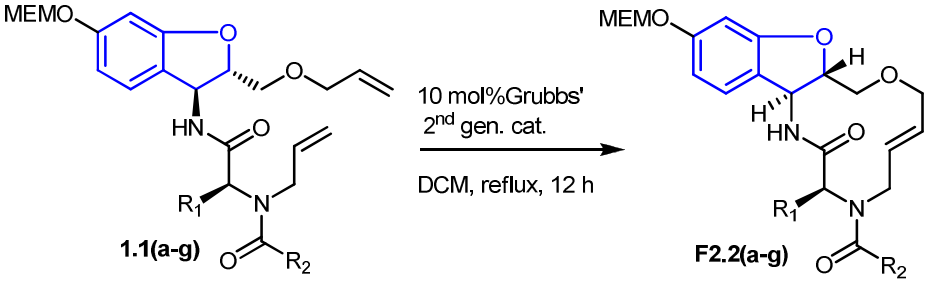
93.5, 93.6, 99.1, 99.2, 109.3, 117.3, 118.7, 118.8, 118.9, 118.9, 123.8, 125.1, 127.7, 132.6, 134.3, 142.1, 148.4, 159.5, 161.0, 169.7; LRMS: (ES+) m/z = 551.2 (M-1).

N-allyl-N-((S)-1-((2S,3S)-2-(allyloxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-ylamino)-4-methyl-1-oxopentan-2-yl)-4-nitrobenzamide (1.1g):



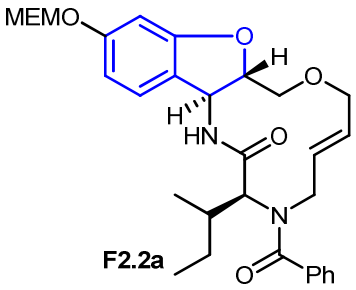
Molecular Formula: $C_{32}H_{41}N_3O_9$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.97 (bs, 6H), 1.64 (bs, 1H), 1.85 (bs, 2H), 3.38 (s, 3H), 3.52-3.60 (m, 2H), 3.71 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.75-3.92 (m, 4H), 3.90-4.14 (m, 3H), 4.52-4.69 (m, 1H), 4.82-5.04 (m, 2H), 5.06-5.39 (m, 6H), 5.60-5.75 (m, 1H), 5.83-5.99 (m, 1H), 6.57-6.68 (m, 2H), 7.03-7.13 (m, 2H), 7.25-7.43 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.2, 22.8, 25.0, 29.6, 36.6, 36.6, 49.9, 49.9, 53.3, 53.3, 53.4, 59.0, 59.0, 67.7, 70.8, 71.5, 72.5, 89.6, 93.5, 93.5, 99.2, 109.1, 109.2, 117.3, 117.4, 118.8, 124.8, 125.2, 128.3, 128.8, 133.4, 134.1, 134.3, 136.2, 159.5, 161.0, 161.1, 170.9, 172.9; LRMS: (ES+) m/z = 610 (M-1).

Compound F2.2(a-g)



To a stirred solution of compound **1.1(a-g) (**1eq) in dry DCM was added 10 mol% Grubbs' second generation catalyst at room temperature. The reaction mixture was refluxed for 12 hours. The reaction mixture concentrated *in vacuo* and purified by flash chromatography to obtain pure product **F2.2(a-g).**

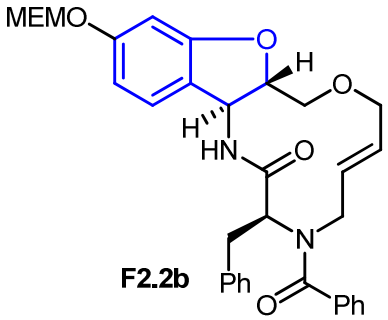
(3S,10aS,15bS,E)-4-benzoyl-3-sec-butyl-13-((2-methoxyethoxy)methoxy)-4,5,8, 10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiazacyclododecin-2(3H)-one (F2.2a):



F2.2a

Molecular Formula: $C_{30}H_{38}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91-0.98 (m, 6H), 1.14 (bs, 1H), 1.58 (bs, 1H), 2.45 (bs, 1H), 3.38 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.62 -3.87 (m, 6H), 4.07-4.16 (m, 2H), 4.46-4.50 (m, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 5.24 (s, 2H), 5.38-5.45 (m, 1H), 5.63-5.70 (m, 2H), 6.54 (s, 1H), 6.63 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.00 (d, *J* = 9.6 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 7.44-7.46 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 11.4, 14.4, 26.8, 29.6, 31.7, 46.1, 52.5, 59.0, 60.0, 67.6, 71.5, 89.2, 93.4, 98.8, 109.6, 118.5, 125.7, 127.0, 127.1, 128.7, 128.8, 129.0, 130.3, 131.6, 135.5, 159.2, 160.6, 169.6, 174.1; LRMS: (ES+) m/z = 539.3 (M+1).

(3S,10aS,15bS)-4-benzoyl-3-benzyl-13-((2-methoxyethoxy)methoxy)-4,5,8,10, 10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiazacyclododecin-2(3H)-one (F2.2b):

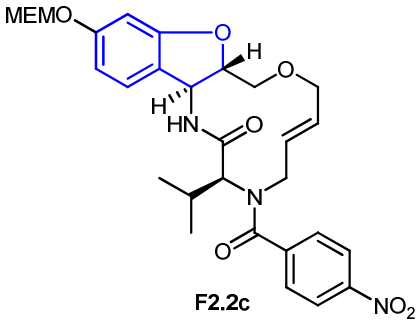


F2.2b

Molecular Formula: $C_{33}H_{36}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.22-3.46 (m, 5H), 3.52 (t, *J* = 4.8 Hz, 2H), 3.66-3.95 (m, 7H), 4.07-4.15 (m, 1H), 4.44 (d, *J* = 5.7 Hz, 1H), 5.24 (s, 2H), 5.45-5.51 (m, 1H), 5.59

(dd, *J* = 6.4, 3.6 Hz, 1H), 5.64-5.68 (m, 2H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.99-7.03 (m, 3H), 7.13-7.15 (m, 1H), 7.27-7.39 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 33.2, 46.2, 52.9, 55.7, 59.0, 67.6, 71.5, 71.8, 89.0, 93.5, 98.8, 109.6, 118.4, 125.8, 126.7, 127.0, 128.6, 128.6, 128.8, 129.0, 130.3, 132.1, 134.9, 136.9, 159.2, 160.5, 170.1, 174.1; LRMS: (ES+) m/z = 573 (M+1), 595.3 (M+Na); Note: the olefin geometry determined by coupling constant.

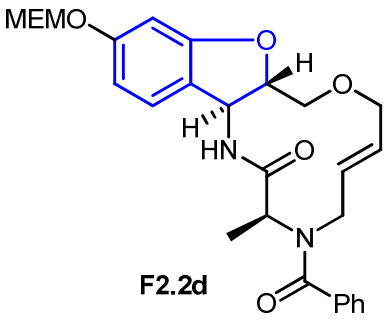
(3S,10aS,15bS,E)-3-isopropyl-13-((2-methoxyethoxy)methoxy)-4-(4-nitrobenzoyl)-4,5,8,10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiazacyclododecin-2(3H)-one (F2.2c):



F2.2c

Molecular Formula: $C_{29}H_{35}N_3O_9$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.06 (m, 6H), 2.63-2.50 (m, 1H), 3.39 (s, 3H), 3.54-3.61 (m, 2H), 3.79 (m, 5H), 3.86 (s, 2H), 4.11-4.20 (m, 1H), 4.49 (d, *J* = 7.3 Hz, 1H), 4.74 (d, *J* = 11.0 Hz, 1H), 5.24 (s, 2H), 5.33-5.47 (m, 1H), 5.67 (dd, *J* = 9.0, 5.6 Hz, 2H), 6.55 (d, *J* = 1.7 Hz, 1H), 6.64 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.73 (d, *J* = 9.7 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 8.32 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.3, 20.0, 29.6, 46.0, 52.6, 59.0, 61.6, 67.7, 71.5, 89.3, 93.5, 98.9, 109.7, 118.1, 124.1, 125.7, 128.0, 128.1, 132.1, 141.5, 148.6, 159.3, 160.5, 168.6, 171.7; LRMS: (ES+) m/z = 570 (M+1).

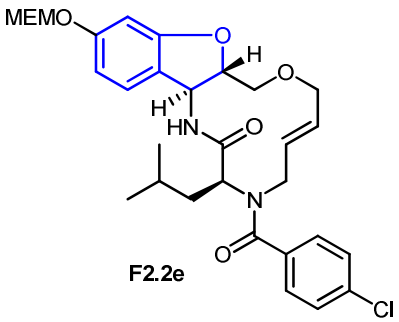
(3S,10aS,15bS,E)-4-benzoyl-13-((2-methoxyethoxy)methoxy)-3-methyl-4,5,8,10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiazacyclododecin-2(3H)-one (F2.2d):



F2.2d

Molecular Formula: $C_{27}H_{32}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (bs, 3H), 3.37 (s, 3H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.67-3.90 (m, 6H), 4.04-4.08 (m, 1H), 4.42 (d, *J* = 5.6 Hz, 1H), 5.23 (s, 2H), 5.29-5.31 (m, 1H), 5.52-5.63 (m, 3H), 6.53 (d, *J* = 2.0 Hz, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 7.12-7.20 (m, 2H), 7.41-7.45 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 29.6, 45.9, 50.9, 52.9, 59.0, 67.6, 71.5, 89.5, 93.5, 98.8, 98.8, 109.6, 118.6, 125.7, 127.1, 128.7, 129.0, 130.3, 132.2, 135.2, 159.2, 160.5, 171.1, 173.7; LRMS: (ES+) m/z = 518 (M+Na).

(3S,10aS,15bS,E)-4-(4-chlorobenzoyl)-3-isobutyl-13((2-methoxyethoxy)methoxy)-4,5,8,10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiaza cyclododecin-2 (3H)-one (F2.2e):

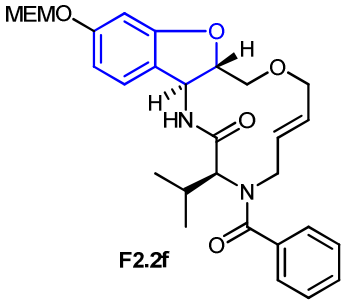


F2.2e

Molecular Formula: $C_{30}H_{37}ClN_2O_7$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (d, *J* = 6.4 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 1.63-1.66 (m, 1H), 1.80-1.92 (m, 2H), 3.39 (s, 3H), 3.59 (d, *J* = 4.4 Hz, 2H), 3.75-3.66 (m, 2H), 3.93-3.79 (m, 4H), 4.03-4.11 (m, 2H), 4.41 (q, *J* = 6.0 Hz, 1H), 5.17-5.23 (m, 3H), 5.441-5.51 (m, 1H), 5.61-5.68 (m, 2H), 6.54 (d, *J* = 1.9 Hz, 1H), 6.63 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.00 (d, *J* = 9.5 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.37-7.44 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.9, 23.3, 24.8, 35.6, 46.1, 52.8, 53.5, 59.0, 67.6, 71.5, 89.6, 93.4, 98.8, 109.6, 118.3 125.7, 128.5, 128.8, 129.1, 132.5,

133.5, 136.7, 159.2, 160.5, 170.7, 173.2; LRMS: (ES+) m/z = 572 (M+1), 594 (M+Na).

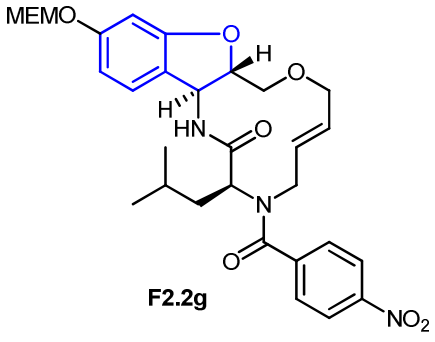
(3S,10aS,15bS,E)-4-benzoyl-3-isopropyl-13-((2-methoxyethoxy)methoxy)-4,5,8, 10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8]oxadiazacyclododecin-2(3H)-one (F2.2f):



F2.2f

Molecular Formula: $C_{29}H_{36}N_2O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.02 (d, *J* = 6.7 Hz, 3H), 1.12 (d, *J* = 6.3 Hz, 3H), 2.49-2.60 (m, 1H), 3.39 (s, 3H), 3.55-3.60 (m, 2H), 3.63-3.76 (m, 2H), 3.80-3.82 (m, 4H), 4.08-4.19 (m, 2H), 4.43-4.54 (m, 1H), 4.70-4.76 (m, 1H), 5.25 (s, 2H), 5.39-5.47 (m, 1H), 5.62-5.71 (m, 2H), 6.55 (d, *J* = 1.9 Hz, 1H), 6.61-6.66 (m, 1H), 6.87-6.95 (m, 1H), 7.10-7.19 (m, 1H), 7.42-7.50 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.0, 14.0, 18.4, 20.1, 25.4, 29.6, 46.0, 52.5, 59.0, 61.5, 67.6, 71.5, 89.4, 93.5, 98.9, 109.6, 118.4, 125.7, 127.1, 128.7, 128.9, 131.8, 135.4, 159.2, 160.6, 169.6, 174.2; LRMS: (ES+) m/z = 525 (M+1).

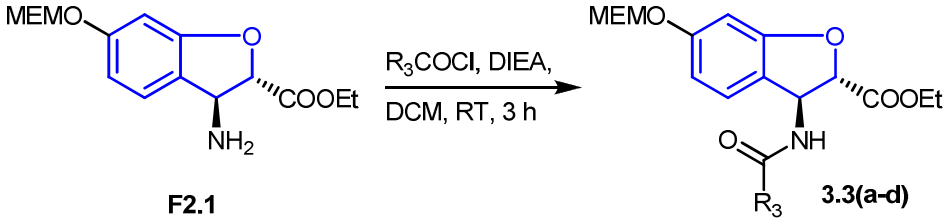
(3S,10aS,15bS,E)-3-isobutyl-13-((2-methoxyethoxy)methoxy)-4-(4-nitrobenzoyl)4,5,8,10,10a,15b-hexahydro-1H-benzofuro[2,3-c][1,5,8] oxadiaza cyclododecin-2 (3H)-one (F2.2g):



F2.2g

Molecular Formula: $C_{30}H_{37}N_3O_9$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (d, *J* = 6.4 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 1.63-166 (m, 1H), 1.80-192 (m, 2H), 3.39 (s, 3H), 3.59 (d, *J* = 4.4 Hz, 2H), 3.75-3.66 (m, 2H), 3.93-3.79 (m, 4H), 4.03-4.11 (m, 2H), 4.41 (q, *J* = 6.0 Hz, 1H), 5.17-5.23 (m, 3H), 5.51-5.44 (m, 1H), 5.61-5.68 (m, 2H), 6.54 (d, *J* = 1.9 Hz, 1H), 6.63 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.00 (d, *J* = 9.5 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 7.37-7.44 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.9, 23.3, 24.8, 29.6, 35.6, 46.1, 52.8, 53.5, 59.0, 67.6, 71.5, 89.6, 93.4, 98.8, 109.6, 118.3 125.7, 128.8, 128.5, 129.1, 132.5, 133.5, 136.7, 159.2, 160.5, 170.7, 173.2; LRMS: (ES+) m/z = 584 (M+1), 606 (M+Na).

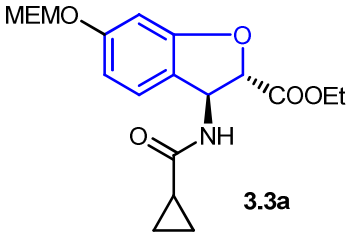
Compound 3.3(a-e)



F2.1 **3.3(a-d)**

To a stirred solution of compound **F2.1 (**1 eq) in dry DCM was added DIEA (1.3 eq) at 0 °C under inert atmosphere. The reaction mixture was stirred for 5 minute, Then R_3COCl (1.2 eq) was added and reaction mixture was stirred for 3 hours. The reaction mixture was quenched by the addition of a saturated $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtained pure product.

(2S,3S)-ethyl-3-(cyclopropanecarboxamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-carboxylate (3.3a):

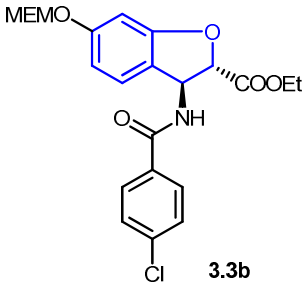


3.3a

Molecular Formula: $C_{19}H_{25}NO_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.75 (dd, *J* = 7.7, 2.9 Hz, 2H), 0.97-1.02 (m, 2H), 1.23-1.30 (m, 4H), 3.34 (s, 3H), 3.53 (d, *J* =4.8 Hz, 2H), 3.78 (d, *J* = 4.8 Hz, 2H), 4.24 (t, *J* = 7.1

Hz, 2H), 4.90 (d, *J* = 3.4 Hz, 1H), 5.21(s, 2H), 5.66 (d, *J* = 3.2 Hz, 1H), 6.18-6.25 (m, 1H), 6.65 (dd, *J* = 7.1, 4.9 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 7.31, 13.9, 14.1, 54.9, 58.7, 61.7, 67.6, 71.4, 86.1, 93.3, 98.7, 109.7, 118.4, 125.4, 159.3, 160.6, 169.3, 173.4; LRMS: (ES+) m/z = 380 (M+1).

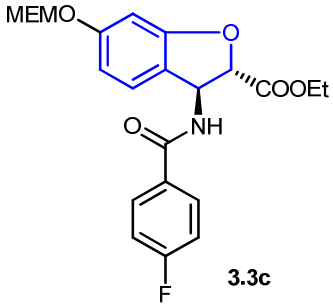
(2S,3S)-ethyl-3-(4-chlorobenzamido)-6-((2-methoxyethoxy)methoxy)-2,3dihydro benzofuran-2-carboxylate (3.3b):



3.3b

Molecular Formula: $C_{22}H_{24}ClNO_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.30 (t, *J* = 7.1 Hz, 3H), 3.33 (s, 3H), 3.51 (t, *J* = 4.8 Hz, 2H), 3.80 (d, *J* = 4.8 Hz, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.98 (d, *J* = 3.4 Hz, 1H), 5.21 (s, 2H), 5.85 (dd, *J* = 7.4, 3.3 Hz, 1H), 6.61-6.69 (m, 2H), 6.93 (d, *J* = 6.3 Hz, 1H), 7.18-7.23 (m, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 14.0, 55.4, 58.8, 61.9, 67.8, 71.5, 86.3, 93.4, 98.9, 110.0, 117.9, 117.9, 125.6, 128.6, 128.7, 131.7, 138.0, 138.0, 159.5, 159.5, 160.9, 165.8, 169.2; LRMS: (ES+) m/z = 500 (M+1).

(2S,3S)-ethyl-3-(4-fluorobenzamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-2-carboxylate (3.3c):

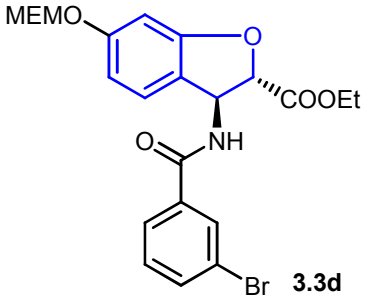


3.3c

Molecular Formula: $C_{22}H_{24}FNO_7$; R_f (30% ethyl acetate /hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 87%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 3.36 (s, 3H), 3.51 (t, *J* = 4.8 Hz,

2H), 3.80 (d, *J* = 4.8 Hz, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 5.01 (d, *J* = 3.3 Hz, 1H), 5.20 (s, 2H), 5.87 (dd, *J* = 7.4, 3.3 Hz, 1H), 6.65-6.68 (m, 3H), 7.07-7.11 (m, 2H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.78-7.82 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 13.8, 55.3, 58.6, 61.7, 67.6, 71.3, 86.1, 93.2, 98.7, 109.7, 115.0, 115.3, 118.0, 125.5, 129.4, 129.4, 129.5, 129.6, 159.3, 160.7, 163.4, 165.7, 169.2; LRMS: (ES+) m/z = 433.9 (M+1), 455.8 (M+Na).

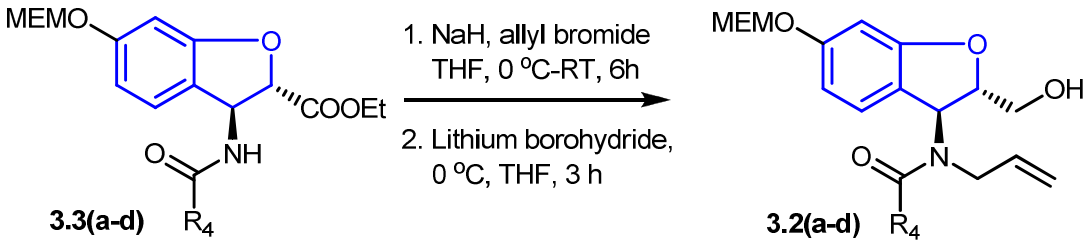
(2S,3S)-ethyl-3-(3-bromobenzamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-2-carboxylate (3.3d):



3.3d

Molecular Formula: $C_{22}H_{24}BrNO_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 84%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.32 (t, *J* = 7.1 Hz, 3H), 3.36 (s, 3H), 3.58-3.47 (m, 2H), 3.72-3.83 (m, 2H), 4.23 (q, *J* = 7.3 Hz, 2H), 4.98 (t, *J* = 3.9 Hz, 1H), 5.14-5.24 (m, 2H), 5.85 (dd, *J* = 7.1, 3.6 Hz, 1H), 6.61 (t, *J* = 9.4 Hz, 2H), 7.59 (t, *J* = 7.2 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.95 (bs, 1H), 7.26 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 13.9, 55.4, 58.6, 61.8, 67.6, 71.4, 86.1, 93.2, 98.7,109.8, 117.7, 117.8, 122.3, 122.4, 125.6, 125.8, 129.8, 130.3, 134.5, 135.1, 165.4, 165.4, 169.1,169.2; LRMS: (ES+) m/z = 495.8 (M+1), 517.8 (M+Na).

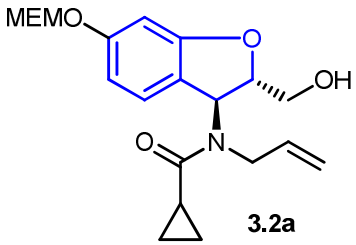
Compound 3.2(a-d)



To a stirred solution of compound **3.3(a-d)** (1 eq) in dry THF was added NaH (1.2 eq) at 0 °C under inert atmosphere. The reaction mixture was stirred for 5 minutes,

and then allyl bromide (1.3 eq) was added. The reaction mixture was stirred for 6 hours, then reaction mixture quenched by ice water, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. To the crude mixture of above product (1 eq) in dry THF was added lithium borohydride (1.3 eq) at 0 °C under inert atmosphere. The reaction mixture was stirred for 2 hours. The reaction mixture was quenched with ice water, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product.

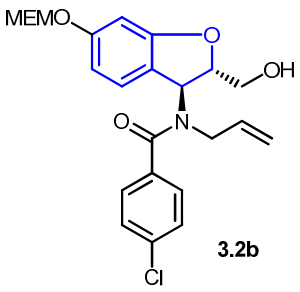
N-allyl-N-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3dihydrobenzofuran-3-yl)cyclopropanecarboxamide (3.2a):



3.2a

Molecular Formula: $C_{20}H_{27}NO_6$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.76-0.84 (m, 2H), 0.99 (dd, *J* = 7.1, 5.4 Hz, 1H), 1.11 (d, *J* = 5.8 Hz, 1H), 1.69-1.72 (m, 1H) 3.36 (s, 3H), 3.49-3.57 (m, 3H), 3.65-3.71 (m, 1H), 3.75-3.79 (m, 3H), 3.89 (d, *J* = 2.0 Hz, 1H), 4.47-4.55 (m, 1H), 5.07-5.20 (m, 2H), 5.22 (s, 2H), 5.70-5.80 (m, 1H), 5.90 (d, *J* = 4.3 Hz, 1H), 6.53-6.61 (m, 2H), 7.04 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 8.4, 9.0, 12.3, 46.5, 58.9, 59.0, 63.4, 67.7, 71.5, 89.2, 89.3, 93.5, 98.7, 109.1, 109.3, 116.3, 116.9, 117.0, 126.7, 134.4, 159.6, 161.6, 176.0; LRMS: (ES+) m/z = 377 (M-1).

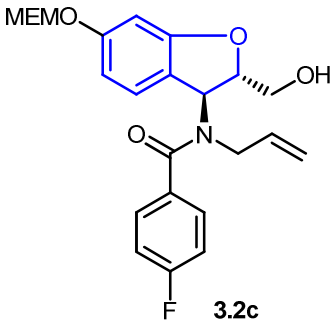
N-allyl-4-chloro-N-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)benzamide (3.2b):



3.2b

Molecular Formula: $C_{23}H_{26}ClNO_6$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 58%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.38 (s, 3H), 3.51 (t, *J* = 4.8 Hz, 2H), 3.67 (s, 1H), 3.81 (*J* = 4.8 Hz, 2H), 3.87-3.96 (m, 2H), 4.65 (d, *J* = 5.1 Hz, 1H), 4.72-4.76 (m, 1H), 4.94-5.04 (m, 1H), 5.24 (s, 2H), 5.48 (dd, *J* = 6.6, 4.8 Hz, 1H), 6.59 (dd, *J* = 9.8, 1.8 Hz, 1H), 7.09-7.16 (m, 1H), 7.39-7.45 (m, 3H), 7.72 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 55.2, 59.0, 63.5, 67.7, 71.5, 91.5, 93.5, 99.1, 109.5, 117.8, 125.4, 128.3, 128.4, 128.8, 128.9, 131.5, 133.4, 138.4, 159.8, 161.2, 166.7; LRMS: (ES+) m/z = 469.8 (M+Na).

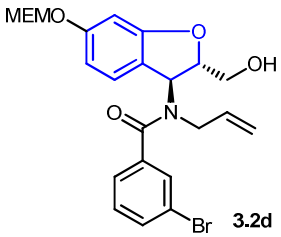
N-allyl-4-fluoro-N-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl) benzamide (3.2c):



3.2c

Molecular Formula: $C_{23}H_{26}FNO_6$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 55%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.35 (s, 3H), 3.51 (t, *J* = 4.8 Hz, 2H), 3.64 (m, 2H), 3.66-4.03 (m, 4H), 4.71-4.72 (m, 2H), 4.96 (d, *J* = 8.3 Hz, 1H), 5.22 (s, 2H), 5.37-5.69 (m, 1H), 5.71-5.97 (m, 1H), 6.54-6.62 (m, 2H), 7.05-7.09 (m, 3H), 7.42-7.45 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 49.2, 58.9, 63.4, 67.7, 71.5, 89.2, 93.4, 98.9, 109.3, 115.5, 115.7, 115.7, 129.1, 129.1, 131.9, 131.9, 133.5, 159.7, 162.1, 164.6; LRMS: (ES+) m/z = 433 (M+1).

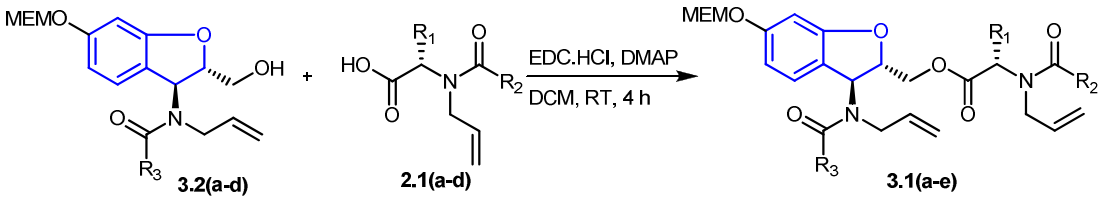
N-allyl-3-bromo-N-((2S,3S)-2-(hydroxymethyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)benzamide (3.2d):



3.2d

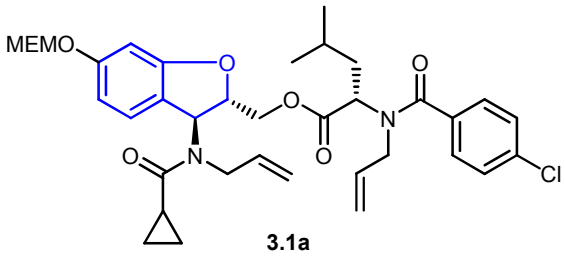
Molecular Formula: $C_{23}H_{26}BrNO_6$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.35 (bs, 4H), 3.46-3.55 (m, 2H), 3.55-3.61 (m, 2H), 3.88 (t, *J* = 4.8 Hz, 2H), 3.74-3.83 (m, 2H), 4.61 (d, *J* = 4.9 Hz, 1H), 4.68-4.77 (m, 1H), 4.92-5.05 (m, 1H), 5.22 (s, 2H), 5.43-5.50 (m, 1H), 5.77-5.97 (m, 1H) 6.54-6.68 (m, 2H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.31-7.44 (m, 1H), 7.53-7.74 (m, 2H), 7.93 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 55.1, 58.9, 63.4, 67.7, 71.5, 91.4, 93.5, 99.0, 109.5, 117.9, 122.8, 125.3, 125.5, 125.7, 129.8, 130.2, 130.3, 133.3, 135.0, 135.1, 159.7, 159.8, 161.1, 166.3; LRMS: (ES+) m/z = 487.8 (M-1).

Compound 3.1(a-e):



To a stirred solution of compound **3.2(a-d)** (1 eq) and 2 (1.2 eq) in dry DCM were added EDC.HCl (1.2 eq) and DMAP (1 eq) at room temperature. The reaction mixture was stirred for 4 hours, then the reaction mixture was quenched by the addition of a saturated $NaHCO_3$, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product.

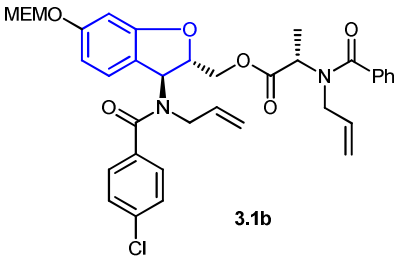
(S)-((2S,3S)-3-(N-allylcyclopropanecarboxamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl2-(N-allyl-4-chlorobenzamido)-4-methylpentano ate (3.1a):



Molecular Formula: $C_{36}H_{45}ClN_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 66%; ^{1}H NMR (400

MHz, $CDCl_3$) δ ppm 0.47-0.60 (m, 1H), 0.62-1.18 (m, 9H), 1.69-1.74 (m, 4H), 3.36 (s, 3H), 3.54 (t, *J* = 4.8 Hz, 2H), 3.67-3.90 (m, 5H), 4.20-4.53 (m, 3H), 4.61-4.71 (m, 1H), 4.97-5.18 (m, 4H), 5.20-5.25 (m, 3H), 5.65-5.82 (m, 1H), 5.92-6.11 (m, 1H), 6.55 (bs, 2H), 6.96-7.13 (m, 1H), 7.33 (dd, *J* = 8.7, 7.7 Hz, 4H); ^{13}C NMR(100 MHz, $CDCl_3$) δ ppm 8.3, 8.4, 8.4, 12.1, 22.4, 22.5, 22.5, 37.6, 46.3, 58.0, 58.0, 59.1, 67.7, 67.8, 71.5, 84.2, 85.5, 93.6, 98.6, 98.8, 109.3, 109.3, 116.7, 128.4, 134.4, 134.5, 134.6, 134.6, 159.7, 159.7, 161.7, 170.8, 170.9, 171.2; LRMS: (ES+) m/z = 670 (M+1).

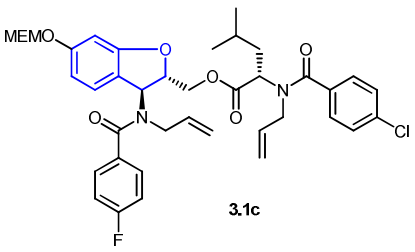
(S)-((2S,3S)-3-(N-allyl-4-chlorobenzamido)-6((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl-2-(N-allylbenzamido)propanoate (3.1b):



3.1b

Molecular Formula: $C_{36}H_{39}ClN_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.50 (bs, 3H), 3.37 (s, 3H), 3.54-3.64 (m, 3H), 3.79-3.96 (m, 5H), 4.23-4.31 (m, 1H), 4.41-4.60 (m, 2H), 5.02-4.74-5.00 (m, 2H), 5.13-5.39 (m, 4H), 5.48-5.53 (m, 1H), 5.71-5.96 (m, 2H), 6.51-6.73 (m, 2H), 7.37 (bs, 7H), 7.69 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 29.6, 54.3, 54.5, 59.0, 67.8, 71.5, 93.5, 93.5, 98.9, 109.3, 109.4, 109.4, 116.7, 117.9, 126.7, 128.2, 128.8, 128.8, 128.8, 129.8, 129.8, 133.6, 134.4, 135.9, 159.8, 159.8, 171.1, 171.7.; LRMS: (ES+) m/z = 684.9 (M+Na).

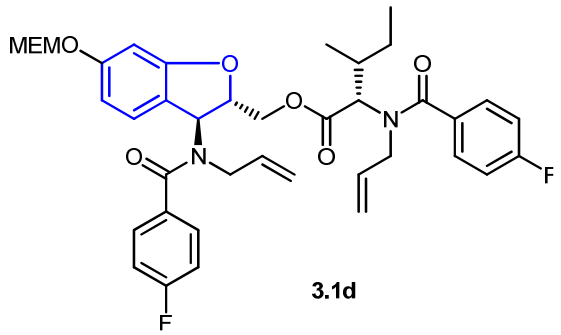
(S)-((2S,3S)-3-(N-allyl-4-fluorobenzamido)-6((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl2-(N-allyl-4-chlorobenzamido)-4-methylpentanoate (3.1c):



3.1c

Molecular Formula: $C_{39}H_{44}ClFN_2O_8$; R_f (30% ethyl acetate/hexane): 0.4; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 75%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.52-0.89 (m, 6H), 1.52-1.93 (m, 3H), 3.36 (s, 3H), 3.51 (t, *J* = 4.8 Hz, 2H), 3.57-3.70 (m, 2H), 3.73-3.85 (m, 3H), 4.25-4.51 (m, 3H), 4.88-5.09 (m, 5H), 5.21 (s, 2H), 5.42-6.07 (m, 3H), 6.55-6.62 (m, 2H), 7.06-7.10 (m, 3H), 7.33-7.43 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.4, 21.9, 22.0, 22.6, 23.9, 25.0, 25.0, 29.6, 37.6, 37.7, 47.9, 56.4, 59.0, 67.8, 71.5, 93.5, 93.6, 98.8, 98.8, 109.4, 115.6, 115.8, 115.8, 117.6, 117.6, 118.5, 128.2, 128.6, 128.6, 128.6, 128.7, 129.0, 129.0, 132.0, 132.0, 133.7, 134.3, 159.9, 159.9, 162.1, 170.8, 170.9, 170.9, 171.1; LRMS: (ES+) m/z = 744.9 (M+Na).

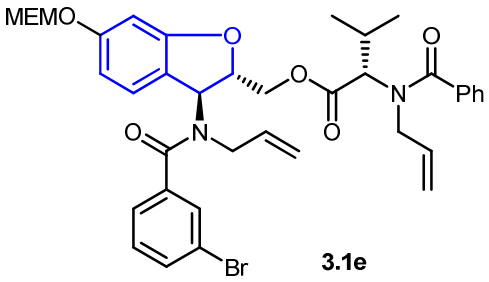
(2S,3S)-((2S,3S)-3-(N-allyl-4-fluorobenzamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl2-(N-allyl-4-fluorobenzamido)-3-methyl pentanoate (3.1d):



3.1d

Molecular Formula: $C_{39}H_{44}F_2N_2O_8$; R_f (30% ethyl acetate/hexane): 0.4; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 73%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm , 0.73-0.93 (m, 6H), 0.95-1.04 (m, 2H), 1.53-1.66 (m, 1H), 3.35 (s, 3H), 3.51 (t, *J* = 4.8 Hz, 2H), 3.61-3.71 (m, 1H), 3.75-3.93 (m, 3H), 4.31-4.70 (m, 2H), 4.76-5.05 (m, 3H), 5.23 (s, 2H), 5.46-6.15 (m, 2H), 6.54-6.73 (m, 2H), 6.98-7.23 (m, 5H), 7.41-7.45 (m, 2H), 7.77-7.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 11.3, 14.1, 16.3, 29.6, 33.9, 33.9, 58.9, 67.7, 71.5, 93.5, 99.0, 109.5, 115.3, 115.5, 115.7, 115.8, 116.6, 117.5, 125.7, 126.1, 126.2, 128.9, 129.0, 132.0, 132.1, 132.1, 132.2, 133.7, 159.9, 162.0, 164.5, 164.5, 165.0, 167.0, 171.4, 171.8, 172.7, 173.0; LRMS: (ES+) m/z = 728.9 (M+Na).

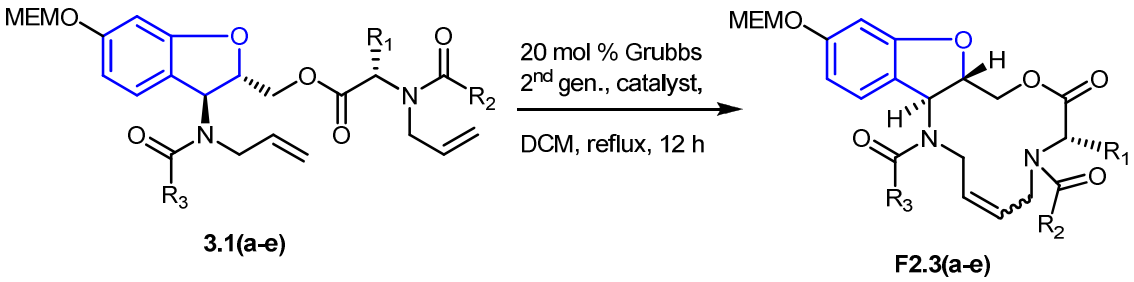
(S)-((2S,3S)-3-(N-allyl-3-bromobenzamido)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-2-yl)methyl2-(N-allylbenzamido)-3-methyl butanoate (3.1e):



3.1e

Molecular Formula: $C_{38}H_{43}BrN_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 76%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.18-1.36 (m, 6H), 1.68 (bs, 1H), 3.37 (s, 3H), 3.48-3.71 (m, 3H), 3.77-3.80 (m, 4H), 3.91-4.11 (m, 1H), 4.32-4.58 (m, 3H), 4.85-5.23 (m, 6H), 5.94 (bs, 1H), 6.61 (bs, 3H), 6.62 (s, 3H), 7.31-7.37 (m, 7H), 7.57-7.71 (m, 3H), 7.91 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 27.7, 27.7, 29.6, 44.9, 53.4, 59.0, 65.2, 67.7, 71.5, 93.4, 93.5, 94.5, 99.0, 109.6, 109.9, 125.6, 127.1, 127.1, 128.3, 130.2, 133.5, 134.8, 134.9, 135.3, 161.2, 161.3; LRMS: (ES+) m/z = 734 (M+1), 758.7 (M+Na).

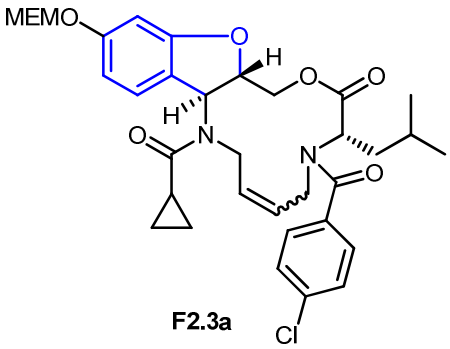
Compound F2.3(a-e):



3.1(a-e) F2.3(a-e)

To a stirred solution of compound **3.1(a-e)** (1eq) in dry DCM was added 20 mol% Grubbs' second generation catalyst at room temperature. The reaction mixture was refluxed for 12 hours. The reaction mixture concentrated *in vacuo* and purified by flash chromatography to obtained pure product **F2.3(a-e)** as a colorless liquid.

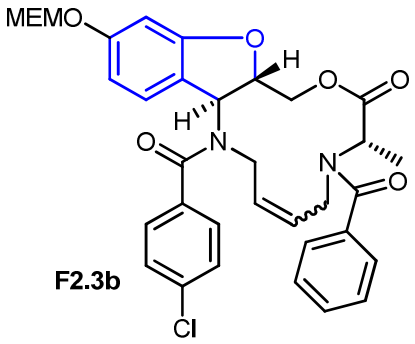
(7S,10aS,15bS)-6-(4-chlorobenzoyl)-1-(cyclopropanecarbonyl)-7-isobutyl-13-((2-methoxy)ethoxy)methoxy)-5,6,7,10,10a,15b-hexahydro-1H-benzofuro[3,2-j][1,4,9] oxadiazacyclododecin-8(2H)-one (F2.3a):



F2.3a

Molecular Formula: $C_{34}H_{41}ClN_2O_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.58-1.08 (m, 10H), 1.42-2.02 (m, 4H), 3.38 (s, 3H), 3.52-3.61 (m, 2H), 3.80 (bs, 3H), 3.96-4.42 (m, 3H), 4.48-4.77 (m, 2H), 4.92-5.35 (m, 4H), 5.50-5.94 (m, 2H), 6.55 (bs, 2H), 6.99-7.22 (m, 1H), 7.39-7.42 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 7.4, 7.6, 7.7, 7.8, 12.0, 21.6, 21.6, 29.6, 29.6, 36.7, 51.4, 51.7, 58.9, 59.0, 67.6, 71.5, 93.5, 98.5, 108.7, 108.8, 118.4, 123.6, 128.1, 128.1, 128.8, 128.8, 134.1, 135.9, 159.1, 159.5, 161.2, 170.9, 171.8, 174.4; Note: Inseparable mixture of E/Z isomers determined by HPLC(1:1.1 isomers); LRMS: (ES+) m/z = 656.8 (M+Na).

(7S,10aS,15bS)-6-benzoyl-1-(4-chlorobenzoyl)-13-((2-methoxyethoxy)methoxy)-7-methyl-5,6,7,10,10a,15b-hexahydro-1H-benzofuro[3,2-j][1,4,9]oxadiazacyclododecin-8(2H)-one (F2.3b):

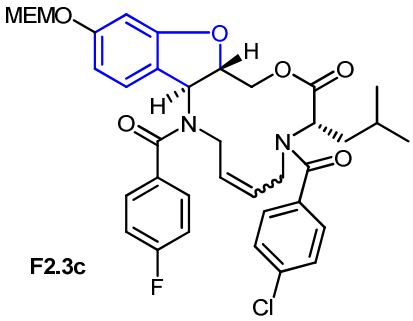


F2.3b

Molecular Formula: $C_{34}H_{35}ClN_2O_8$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.49 (bs, 3H), 3.39 (s, 3H), 3.57-3.60 (m, 3H), 3.80-3.82 (m, 3H), 4.10-4.46 (m, 4H), 4.68-4.83 (m, 2H), 4.90-5.02 (m, 2H), 5.20-5.29 (m, 3H), 5.75-5.88 (m, 1H), 6.57-6.64 (m, 2H), 7.05-7.11 (m, 1H), 7.26-7.35 (m, 4H), 7.43-7.46 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 14.1, 33.8, 59.0, 59.0, 64.2,

64.3, 67.7, 71.5, 93.5, 98.6, 109.9, 114.0, 115.8, 116.0, 123.4, 126.4, 128.7, 129.8, 135.6, 139.2, 159.5, 159.5, 159.5, 171.0, 171.7 Note: Inseparable mixture of E/Z isomers determined by HPLC(1:1.8 isomers); LRMS: (ES+) m/z = 656.8 (M+Na).

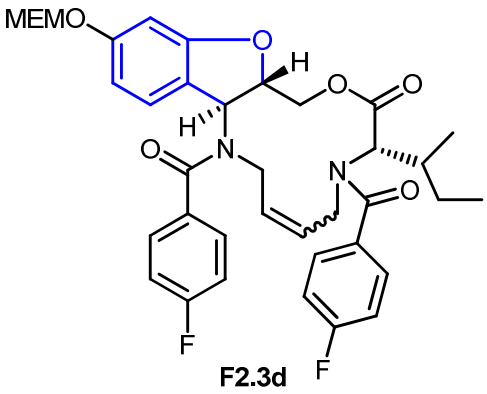
(7S,10aS,15bS)-6-(4-chlorobenzoyl)-1-(4-fluorobenzoyl)-7-isobutyl-13-((2-methoxyethoxy)methoxy)-5,6,7,10,10a,15b-hexahydro-1H-benzofuro[3,2-j][1,4,9] oxadiaza cyclododecin-8(2H)-one (F2.3c):



F2.3c

Molecular Formula: $C_{37}H_{40}ClFN_2O_8$; R_f (30% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 66%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.77-1.10 (m, 6H), 1.48-1.51(m, 1H), 1.79-1.98 (m, 1H), 1.36-1.60 (m, 1H), 3.37 (s, 3H), 3.51 (t, J = 4.8 Hz, 2H), 3.79-3.80 (m, 3H),4.07-4.11 (m, 1H), 4.13-4.46 (m, 3H), 4.53-4.86 (m, 2H), 5.14-5.29 (m, 3H), 5.47-5.82 (m, 2H), 5.90 (bs, 1H), 6.58-6.61 (m, 2H), 6.96-7.16 (m, 3H), 7.27-7.51 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm 14.1, 14.1, 21.7, 22.1, 22.6, 24.1, 29.6, 31.9, 36.9, 42.7, 59.0, 59.0, 60.3, 64.8, 64.8, 67.7, 71.5, 93.5, 98.6, 109.1,109.1, 115.3, 115.5, 117.6, 123.6, 128.8, 129.0, 129.0, 129.0, 130.7, 131.7, 134.0, 134.0, 135.9, 135.9, 136.0, 159.5, 162.2, 164.7, 171.0,171.7; Note: Inseparable mixture of E/Z isomers determined by HPLC(1:1.1 isomers); LRMS: (ES+) m/z = 716.9 (M+Na).

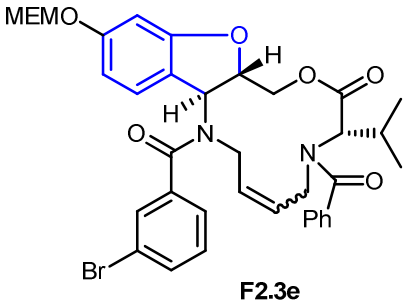
((7S,10aS,15bS)-7-sec-butyl-13-((2-methoxyethoxy)methoxy)-8-oxo-5,7,8,10, 10a, 15b-hexahydro-1H-benzofuro[3,2-j][1,4,9]oxadiazacyclododecine-1,6(2H)-diyl) bis((4-fluorophenyl) methanone) (F2.3d):



F2.3d

Molecular Formula: $C_{37}H_{40}F_2N_2O_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 74%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.75-0.92 (m, 6H), 0.95-1.10 (m, 2H), 1.54-1.65 (m, 1H), 3.38 (bs, 4H), 3.48-3.65 (m, 3H), 3.76-3.85 (m, 3H), 3.96-4.21 (m, 2H), 4.26-4.48 (m, 1H), 4.80-4.99 (m, 2H), 5.19-5.30 (m, 3H), 5.46-5.67 (m, 1H), 5.75-6.00 (m, 1H), 6.55-6.66 (m, 2H), 6.97-7.20 (m, 6H), 7.29-7.41 (m, 2H), 7.42-7.49 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 10.9,11.3, 14.1,14.8, 16.3, 22.6, 24.5, 26.8, 28.9, 29.3, 29.6, 31.6, 31.9, 33.8, 59.0, 64.7, 67.7, 71.5, 93.5, 98.6, 98.6, 109.3, 109.3, 115.3, 115.5, 117.6, 123.6, 123.6, 123.7, 123.7, 123.7, 124.0, 124.4, 129.1, 130.5, 131.7, 132.6, 159.4, 169.6. 169.6, 170.5, 171.5; Note: Inseparable mixture of E/Z isomers determined by HPLC (1:1.1 isomers); LRMS: (ES+) m/z = 700.9 (M+Na).

(7S,10aS,15bS)-6-benzoyl-1-(3-bromobenzoyl)-7-isopropyl-13-((2-methoxy ethoxy)methoxy)5,6,7,10,10a,15b-hexahydro-1H-benzofuro[3,2-j][1,4,9]oxadi azacyclodo decin-8(2H)-one (F2.3e):

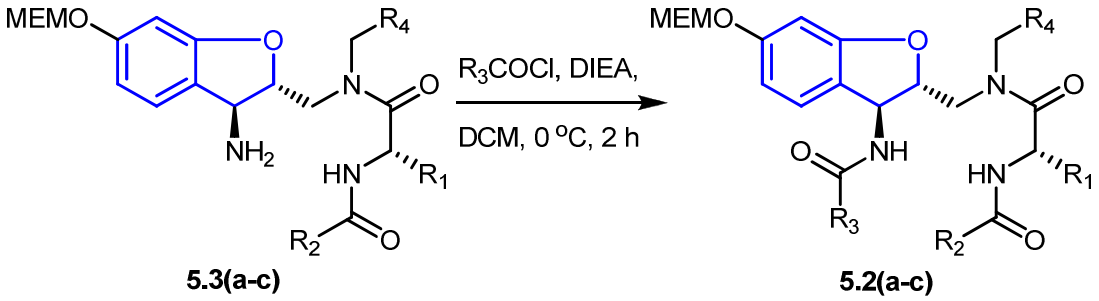


F2.3e

Molecular Formula: $C_{36}H_{39}BrN_2O_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 68%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.18-1.36 (m, 6H), 1.68 (bs, 1H), 3.39 (s, 3H), 3.57 (t, J = 4.8 Hz, 2H), 3.82 (t, J = 4.8 Hz, 2H), 3.96-4.16 (m, 3H), 4.30-4.60 (m, 2H), 4.80-5.01 (m, 2H), 5.19-5.37 (m, 3H), 5.45-5.68 (m, 1H), 5.76-6.07 (m, 1H), 6.54-6.74

(m, 2H), 7.14-7.70 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 22.6, 29.3, 31.9, 59.0, 64.0, 65.2, 66.3, 67.7, 71.5, 77.2, 93.5, 114.0, 127.1, 128.6, 129.6, 129.8, 129.9, 130.0, 130.0, 130.2, 130.3, 137.8, 137.9, 139.2, 169.9, 170.1, 170.5; Note: Inseparable mixture of E/Z isomers determined by HPLC (1:1.5 isomers); LRMS: (ES+) m/z = 730.7 (M+Na).

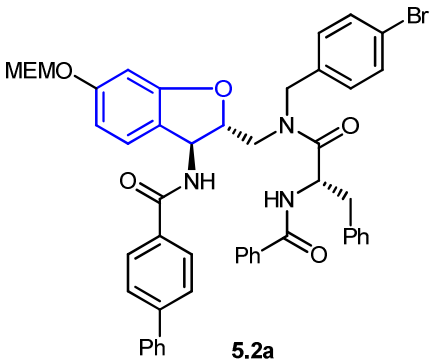
Compound 5.2(a-c):



5.3(a-c) **5.2(a-c)**

To a stirred solution of compounds **5.3(a-c)** (1.0 mmol) in dichloromethane under N_2 atmosphere at 0 °C was added DIEA (2.5 mmol) and corresponding acid chloride (1.5 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. After the completion of the reaction as indicated by TLC, the reaction solution was diluted with dichloromethane and was washed with water and brine. The organic layer was evaporated to give the crude product which was subjected to column purification to give pure products **5.2(a-c)**.

N-((2R,3S)-2-(((S)-2-benzamido-N-(4-bromobenzyl)3-phenylpropanamido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)biphenyl-4-carboxamide (5.2a):

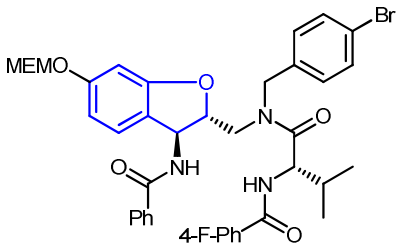


5.2a

Molecular Formula: $C_{49}H_{46}BrN_3O_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 85%; ^{1}H NMR

($CDCl_3$, 400 MHz, 58 ^oC) δ ppm 3.06-3.26 (m, 2H), 3.28-3.49 (m, 4H), 3.54-3.61 (m, 2H), 3.69-3.89 (m, 3H), 4.13-4.32 (m, 0.25H), 4.28 (d, *J* = 17.0 Hz, 0.25H), 4.43-4.60 (m, 1.5H), 4.68-4.87 (m, 1H), 5.00-5.07 (m, 0.5H), 5.12-5.32 (m, 3H), 5.33-5.60 (m, 1.5H), 5.75-5.81 (m, 0.25H), 6.29-6.35 (m, 0.25H), 6.44-6.49 (m, 0.25H), 6.53-6.70 (m, 1.5H), 6.75-6.86 (m, 0.75H), 6.90-7.09 (m, 1.5H), 7.11-7.29 (m, 10H), 7.31-7.54 (m, 8H), 7.59-7.75 (m, 5H), 7.80-7.93 (m, 2H), 8.06 (d, *J* = 8.3 Hz, 0.5H); LRMS: (ES+) m/z = 868 (M+1), 870 (M+3).

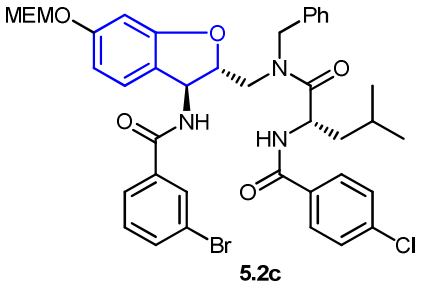
N-((S)-1-((((2R,3S)-3-benzamido-6-((2-methoxyethoxy)methoxy)-2,3-dihydro benzofuran-2-yl)methyl)(4-bromobenzyl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluorobenzamide (5.2b):



5.2b

Molecular Formula: $C_{39}H_{41}BrFN_3O_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 85%; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 0.92 (m, 6H), 2.05-2.19 (m, 1H), 3.37 (s, 3H), 3.50-3.59 (m, 3H), 3.77-3.85 (m, 2H), 4.02-4.05 (m, 1H), 4.68-4.70 (m, 1H), 4.74-4.80 (m, 1H), 4.81-4.89 (m, 1H), 4.95-5.06 (m, 1H), 5.19-5.26 (m, 2H), 5.25-5.29 (m, 1H), 5.62 (dd, *J* = 6.9, 2.7 Hz, 1H), 6.45 (d, *J* = 7.2 Hz, 1H), 6.54 (d, *J* = 1.6 Hz, 1H), 6.72-6.61 (m, 2H), 7.04 (t, *J* = 8.5 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 2H), 7.17-7.25 (m, 3H), 7.37-7.53 (m, 6H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 7.5 Hz, 1H), 7.86 (td, *J* = 8.3, 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 17.3, 19.8, 29.5, 31.4, 32.0, 47.8, 49.1, 50.0, 51.3, 54.1, 54.4, 54.9, 55.4, 58.8, 58.9, 67.6, 67.6, 71.4, 88.4, 88.8, 93.4, 98.8, 99.8, 109.6, 115.2, 115.4, 117.4, 118.1, 121.4, 121.7, 125.0, 125.9, 126.8, 126.9, 128.4, 128.5, 128.5, 130.0, 131.6, 131.9, 133.1, 135.1, 136.0, 159.6, 160.3, 160.7, 165.9, 172.6; LRMS: (ES+) m/z = 763 (M+1), 766 (M+3).

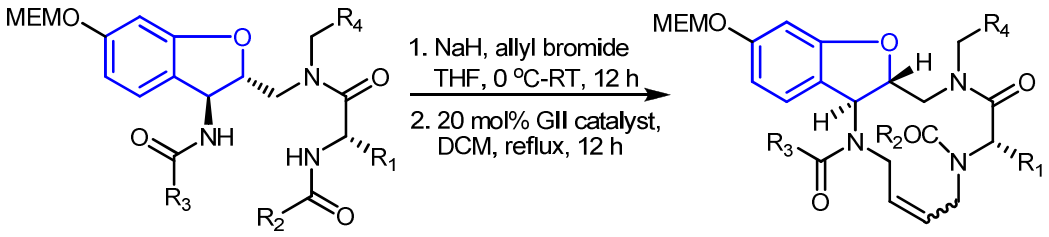
N-((2R,3S)-2-(((S)-N-benzyl-2-(4-chlorobenzamido)-4-methylpentanamido) methyl)-6-((2-methoxyethoxy)methoxy)-2,3-dihydrobenzofuran-3-yl)-3-bromo benzamide (5.2c):



5.2c

Molecular Formula: $C_{40}H_{43}BrClN_3O_7$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.81-1.00 (m, 6H), 1.36-1.51 (m, 2H), 1.52-1.63 (m, 1H), 1.70-1.81 (m, 1H), 3.37 (s, 3H), 3.54-3.57 (m, 3H), 3.72-3.85 (m, 2H), 4.11-4.21 (m, 1H), 4.65-4.74 (m, 1H), 4.78-4.89 (m, 1H), 4.99-5.07 (m, 1H), 5.20-5.22 (m, 3H), 5.61-5.66 (m, 1H), 6.55-6.57 (m, 2H), 7.21-7.47 (m, 15H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 21.6, 22.9, 24.7, 29.6, 41.6, 48.6, 49.0, 58.9, 59.0, 63.1, 67.7, 71.5, 93.5, 109.8, 118.2, 122.4, 122.4, 128.5, 122.9, 123.0, 129.0, 129.8, 130.3, 130.6, 131.7, 132.0, 132.9, 133.0, 133.3, 133.4, 135.4, 136.1, 136.2, 136.4, 137.5, 160.7, 165.6, 166.1, 169.0, 174.4; LRMS: (ES+) m/z = 792 (M+1), 794 (M+3).

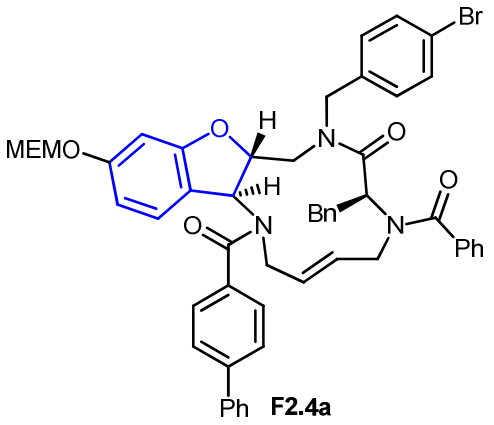
Compound F2.4(a-c):



5.2(a-c) **F2.4 (a-c)**

To a stirred solution of compound **5.2(a-c)** (1.0 mmol) in dry THF under N_2 atmosphere were added NaH (10 mmol) and allyl bromide (6 mmol) at 0 °C. The reaction mixture was allowed to warm rt and heated at 55 °C for overnight. After the completion of the reaction as indicated by LC-MS, the reaction mixture was quenched with aqueous ammonium chloride and extracted with ethyl acetate (3x 20 mL). The combined organic layers were washed with brine and evaporated to dryness to give crude bisallylated product purified by column chromatography. To the stirred solution of bisallylated compounds in DCM, was added 20 mol% Grubbs' second generation catalyst at room temperature and reflux the reaction mixture for 12 h. The organic layer was evaporated to give the crude product which was subjected to column purification to give pure products **F2.4(a-c)**.

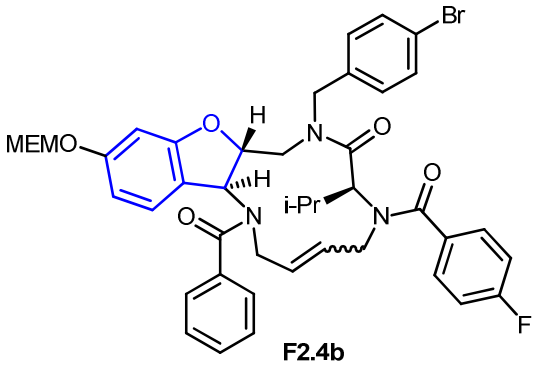
(7S,10aR,15bS,E)-6-benzoyl-7-benzyl-1-(biphenylcarbonyl)-9-(4-bromobenzyl)-13-((2-methoxyethoxy)methoxy)-1,2,6,7,9,10, 10a,15boctahydro benzofuro[2,3-f] [1,4,8] triazacyclododecin-8(5H)-one (F2.4a):



F2.4a

Molecular Formula: $C_{53}H_{50}BrN_3O_7$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield 80 %; ^{1}H NMR ($CDCl_3$, 400 MHz, 58 ^oC) δ ppm 2.82-2.91 (m, 2H), 3.01-3.07 (m, 1H), 3.24-3.33 (m, 4H), 3.51 (t, *J* = 5.4 Hz, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 4.07-4.20 (m, 4H), 4.34-4.43 (m, 1H), 4.65 (d, *J* = 17.1 Hz, 1H), 5.01-5.09 (m, 2H), 5.16-5.23 (m, 3H), 5.56-5.64 (m, 2H), 5.75-5.82 (bd, *J* = 14.8 Hz, 1H), 6.42 (s, 1H), 6.57 (d, *J* = 7.7 Hz, 1H), 6.92-7.03 (bs, 2H), 7.08-7.14 (bs, 2H), 7.19-7.30 (m, 6H), 7.33-7.51 (m, 10H), 7.66-7.72 (m, 4H); LRMS: (ES+) m/z = 921 (M+1), 923 (M+3); Note: the olefin geometry determined by coupling constant.

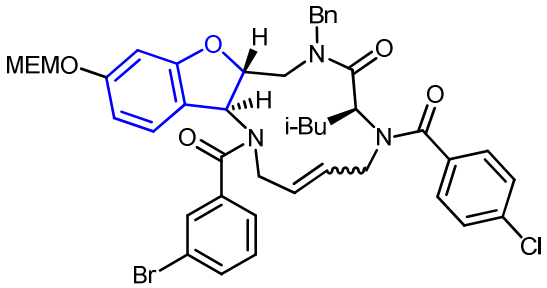
(7S,10aR,15bS)-1-benzoyl-9-(4-bromobenzyl)-6-(4-fluorobenzoyl)-7-isopropyl-13-((2-methoxyethoxy)methoxy)-1,2,6,7,9,10,10a,15b-octahydrobenzofuro[2,3-f][1,4,8] triazacyclododecin-8(5H)-one (F2.4b):



F2.4b

Molecular Formula: $C_{43}H_{45}BrFN_3O_7$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield 80 %; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.84-0.9 (m, 6H), 1.58-1.69 (m, 2H), 3.05-3.19 (m, 1H), 3.37 (s, 3H), 3.47-3.59 (m, 2H), 3.73-3.86 (m, 2H), 3.89-4.08 (m, 2H), 4.24-4.53 (m, 2H), 4.57-4.72 (m, 1H), 4.95-5.07 (m, 1H), 5.22 (d, *J* = 3.8 Hz, 4H), 5.35-5.72 (m, 2H), 6.53 (bs, 2H), 7.01-7.28 (m, 6H), 7.37 (d, *J* = 8.9 Hz, 7H), 7.52 (d, *J* = 7.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 14.1, 29.6, 40.1, 47.1, 53.2, 59.0, 62.0, 67.0, 67.7, 71.5, 83.7, 85.0, 93.5, 98.8, 99.3, 109.8, 114.3, 115.5, 120.0, 120.9, 121.3, 125.1, 127.0, 127.7, 128.7, 128.8, 129.6, 129.7, 131.0, 136.0, 136.6, 141.2, 143.7, 143.8, 155.4, 159.7, 160.1, 161.5, 169.0, 169.4, 172.9; LRMS: (ES+) m/z = 814 (M+1), 816 (M+3). Note: Single isomer (determined by HPLC); geometry not known at this stage.

(7S,10aR,15bS)-9-benzyl-1-(3-bromobenzoyl)-6-(4-chlorobenzoyl)-7-isobutyl-13-((2-methoxyethoxy)methoxy)-1,2,6,7,9,10,10a,15b-octahydrobenzofuro[2,3-f][1,4,8] triazacyclododecin-8(5H)-one (F2.4c):



F2.4c

Molecular Formula: $C_{44}H_{47}BrClN_3O_7$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield 80 %; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.85-1.00 (m, 6H), 1.59 (dd, *J* = 14.3, 7.6 Hz, 2H), 1.72 (bs, 1H), 3.08-3.15 (m, 1H), 3.38 (bs, 4H), 3.50-3.58 (m, 2H), 3.76-3.84 (m, 2H), 3.88-4.08 (m, 2H), 4.22-4.35 (m, 1H), 4.48-4.65 (m, 2H), 4.89-5.04 (m, 1H), 5.23 (bs, 3H), 5.40-5.57 (m, 2H), 5.65-5.74 (m, 2H), 6.51-6.63 (m, 2H), 7.44-7.62 (m, 3H), 7.06 (d, *J* = 8.2 Hz 1H), 7.34-7.40 (m, 11H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 23.1, 24.5, 24.5, 29.6, 38.9, 44.8, 49.4, 49.4, 58.9, 59.1, 67.7, 71.5, 93.5, 96.7, 98.3, 171.6, 100.0, 101.0, 101.3, 118.0, 122.4, 123.7, 127.6, 127.9, 128.7, 128.8, 128.9, 129.0, 129.2, 134.0, 134.0, 136.0, 136.2, 138.0, 159.3, 159.3, 162.0, 171.5;

LRMS: (ES+) m/z = 844 (M+1), 846 (M+3). Note: Single isomer (determined by HPLC); geometry not known at this stage.

3.7. References

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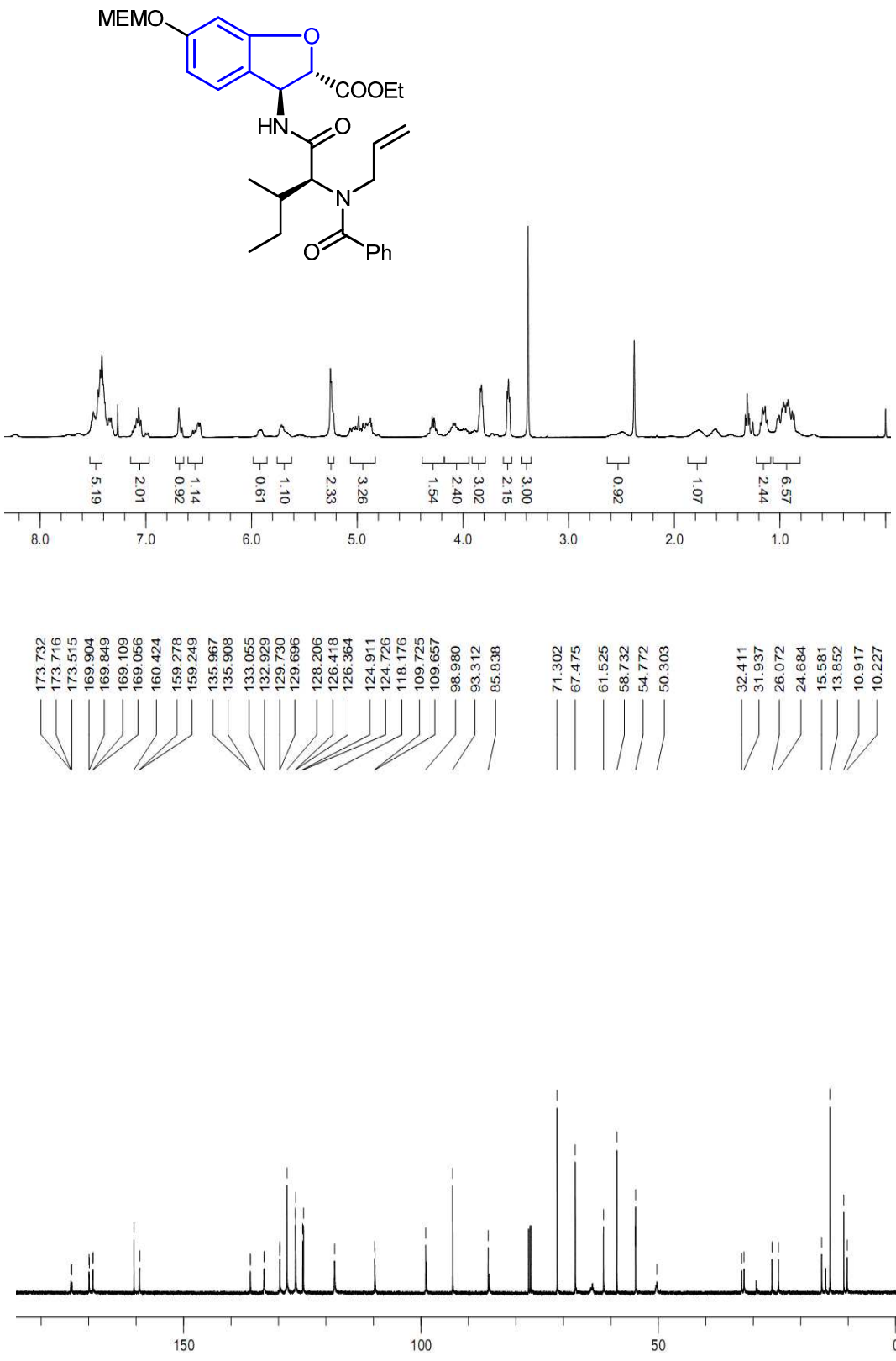
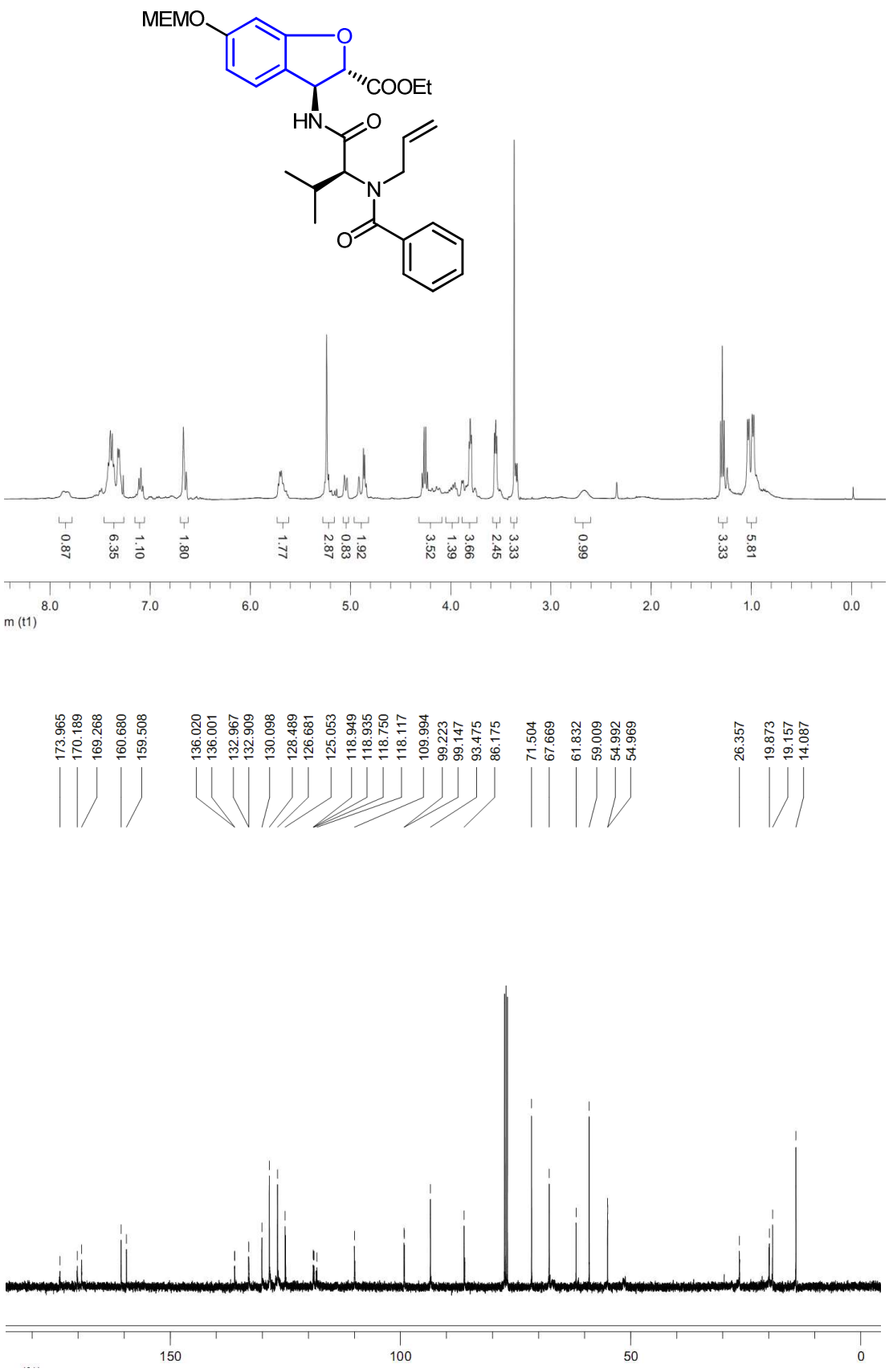
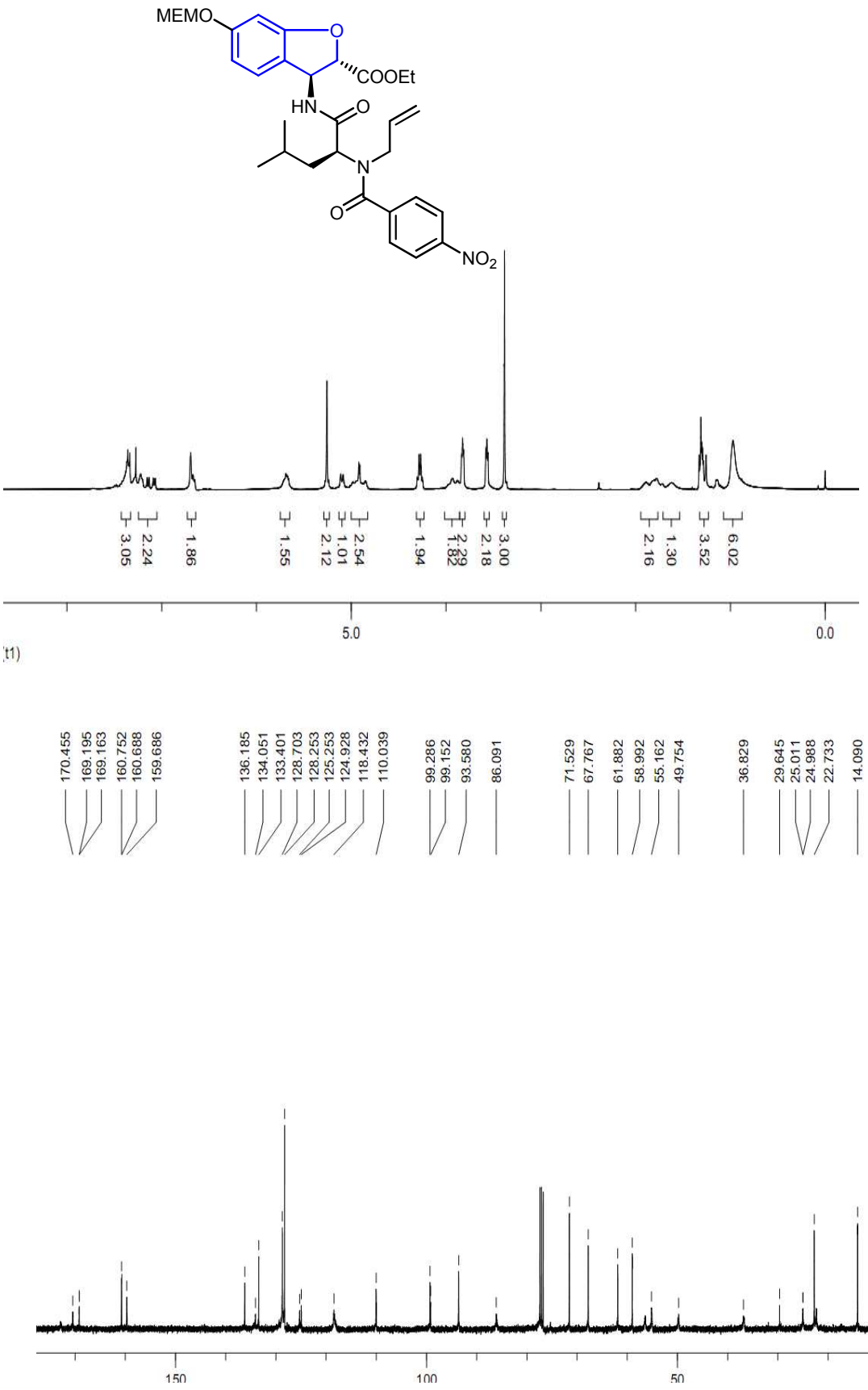
3.8. Spectra



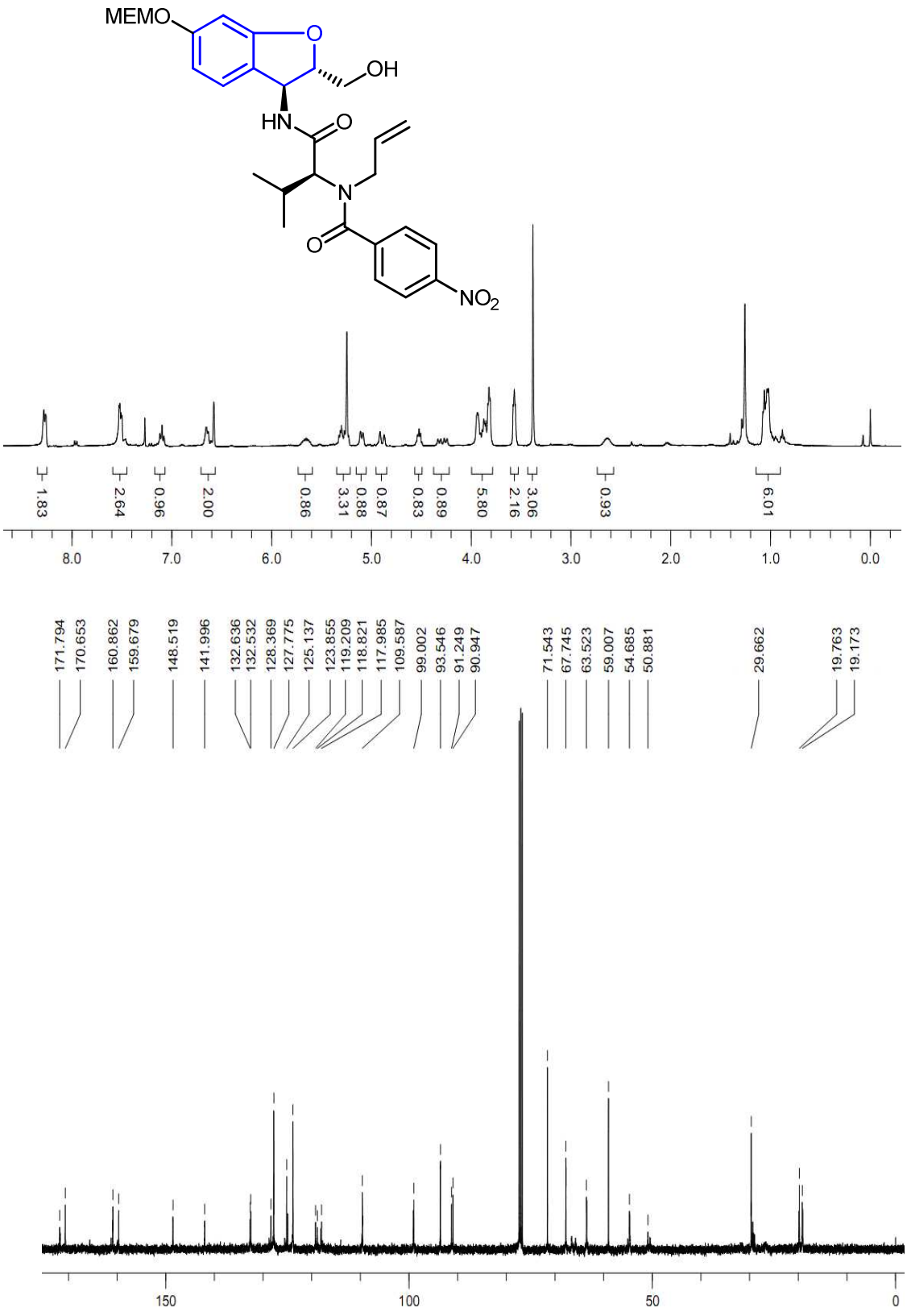
^{1}H and ^{13}C spectra of compound **1.3a**



^{1}H and ^{13}C spectra of compound **1.3c**



^{1}H and ^{13}C spectra of compound **1.3g**



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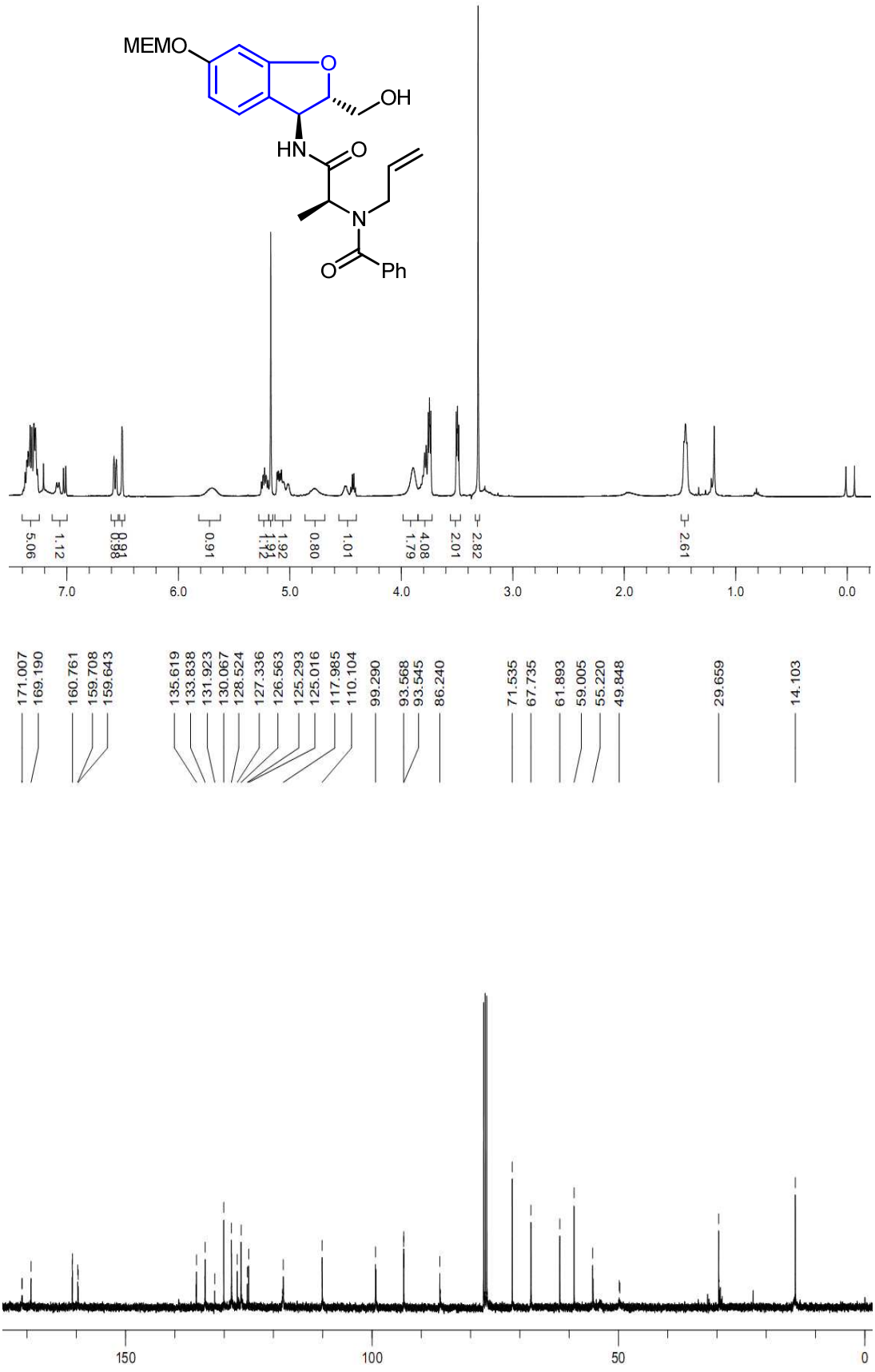
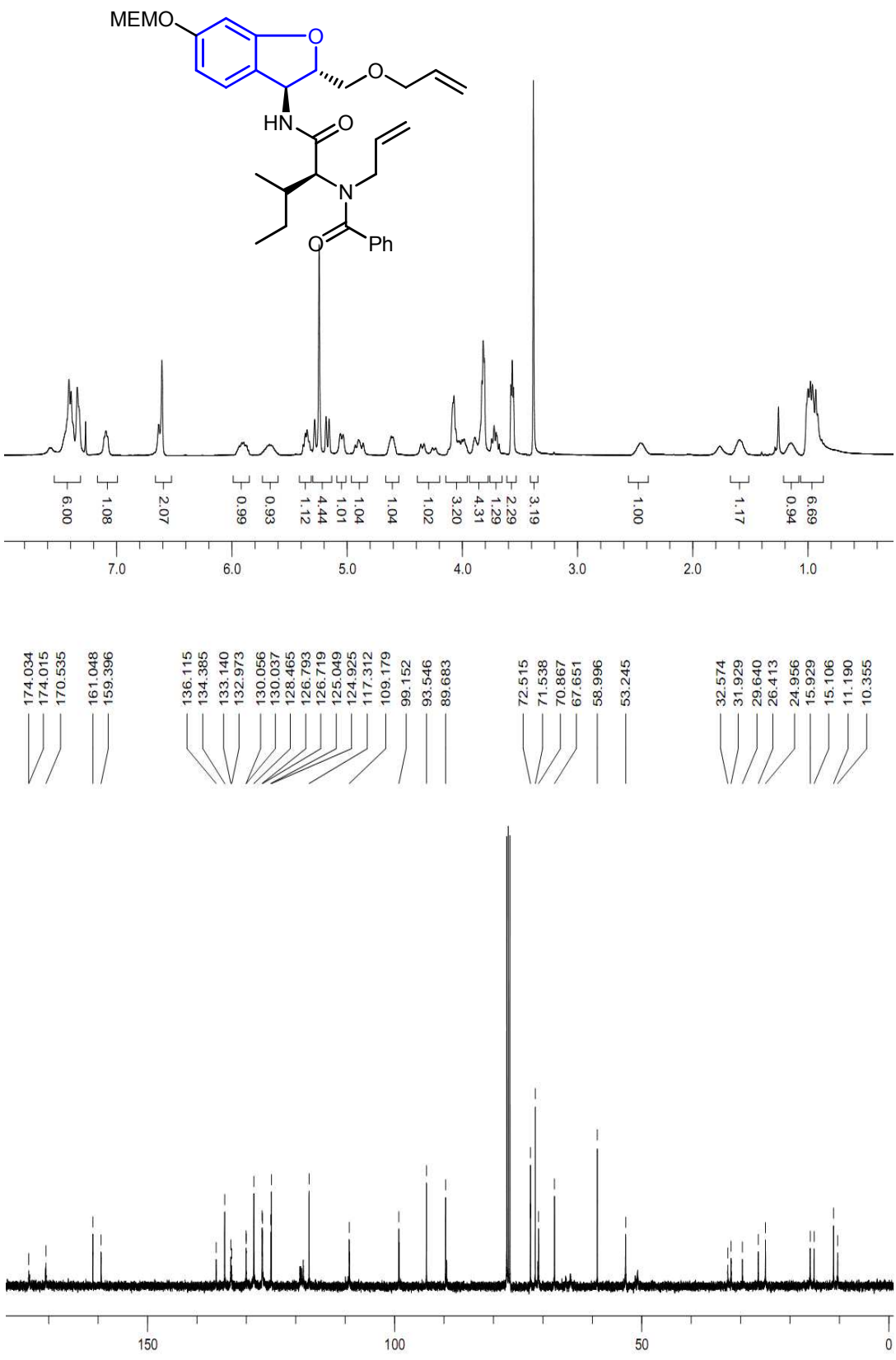
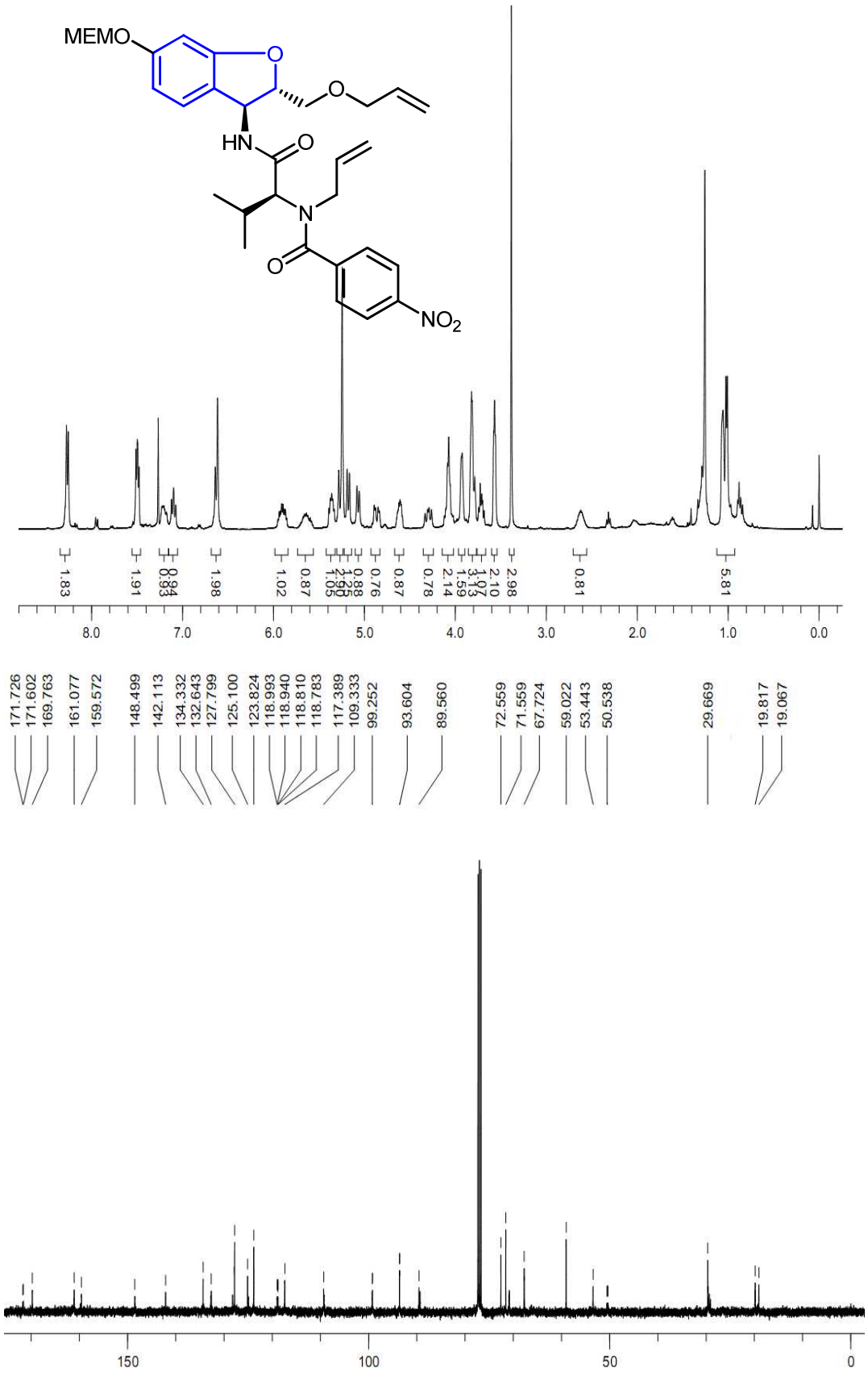


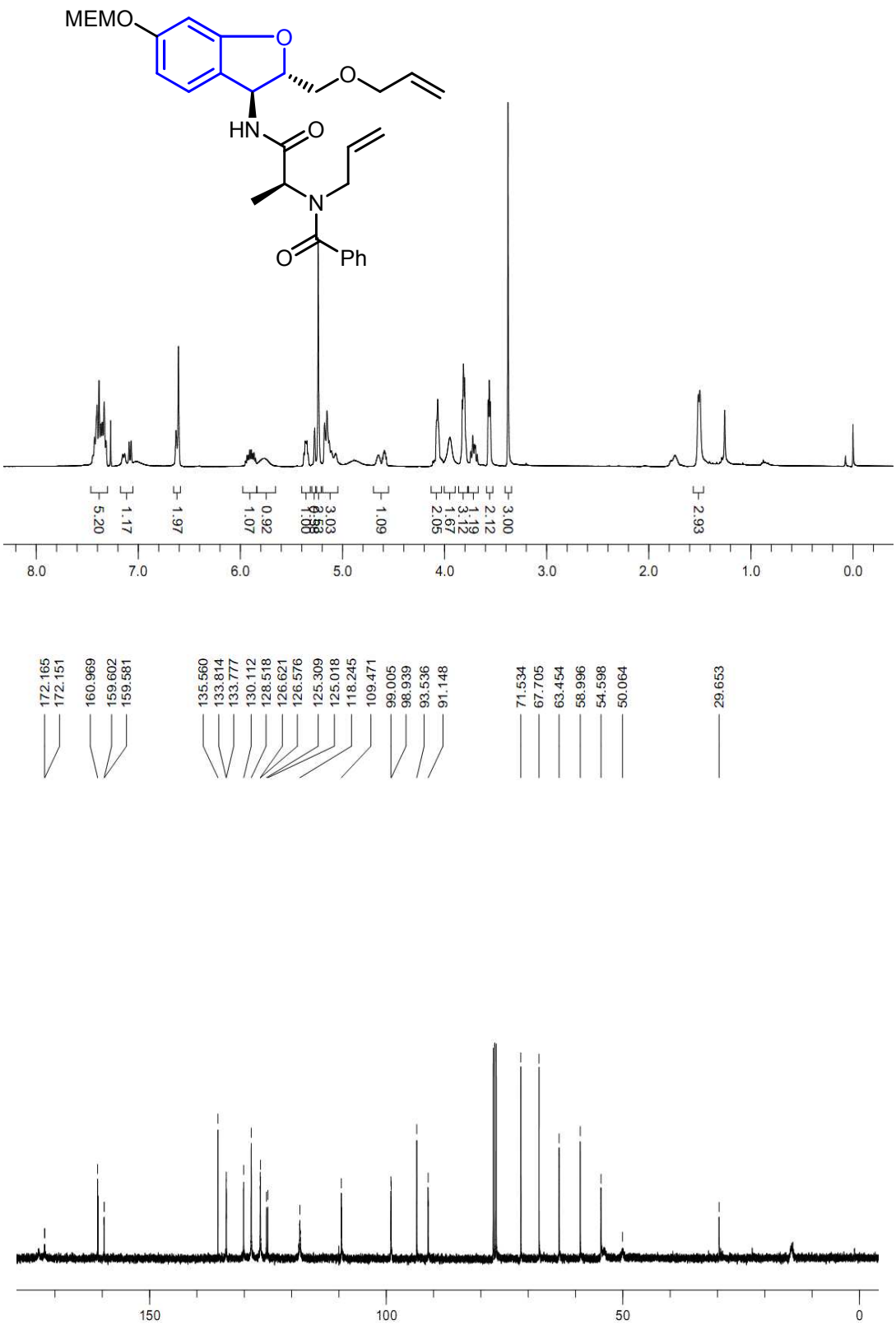
^{1}H and ^{13}C spectra of compound **1.2d**



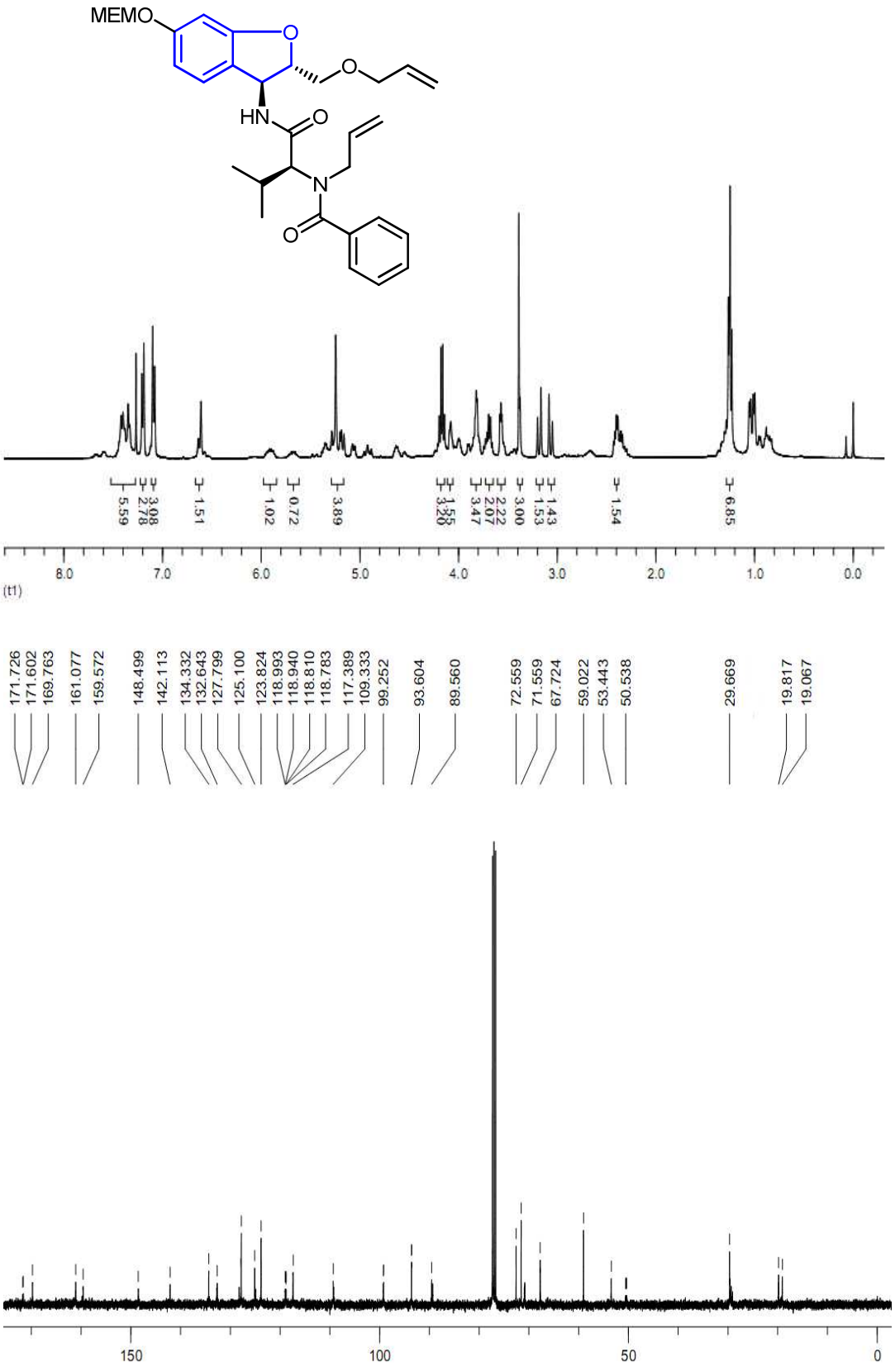
^{1}H and ^{13}C spectra of compound **1.1a**



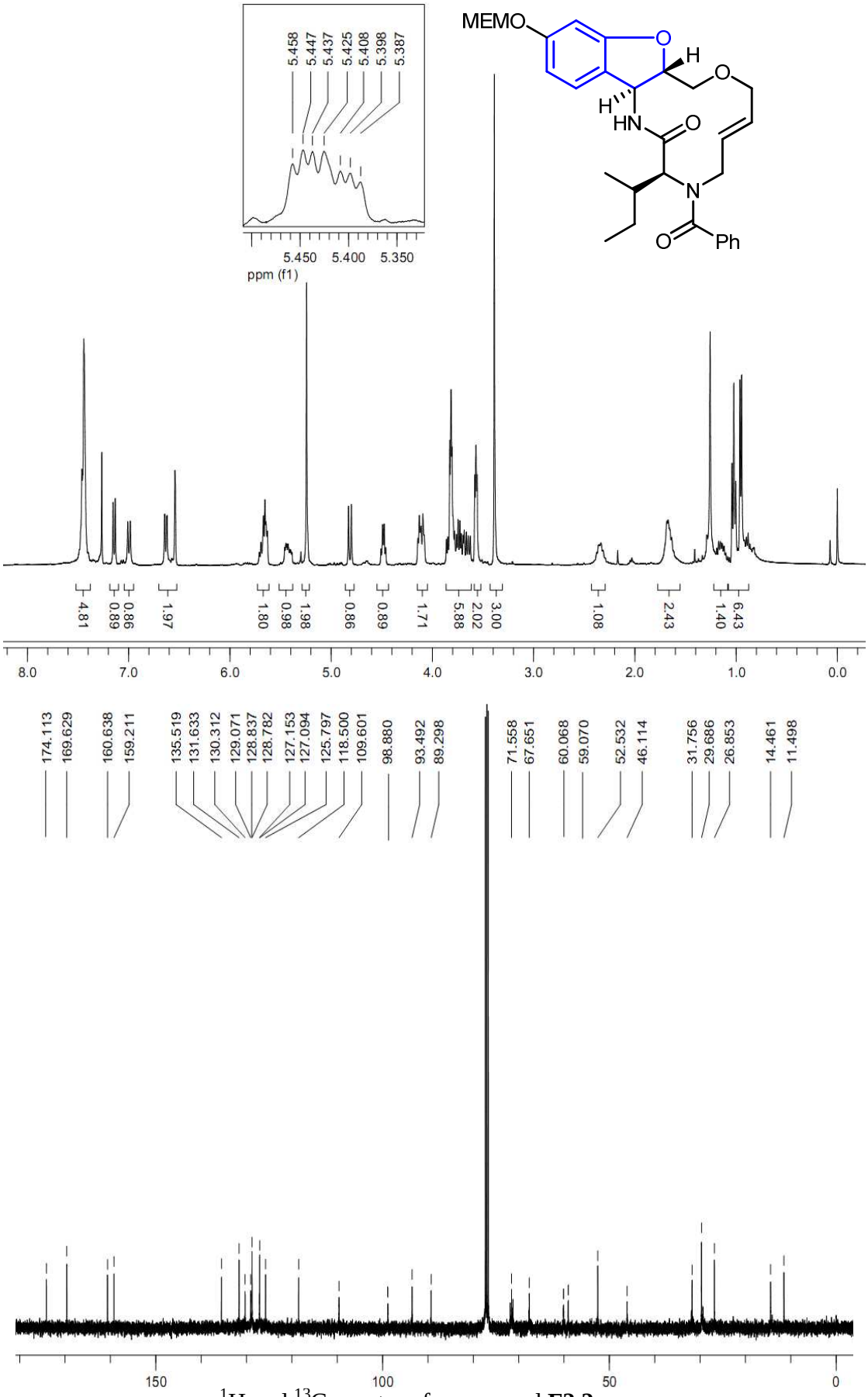
^{1}H and ^{13}C spectra of compound **1.1c**



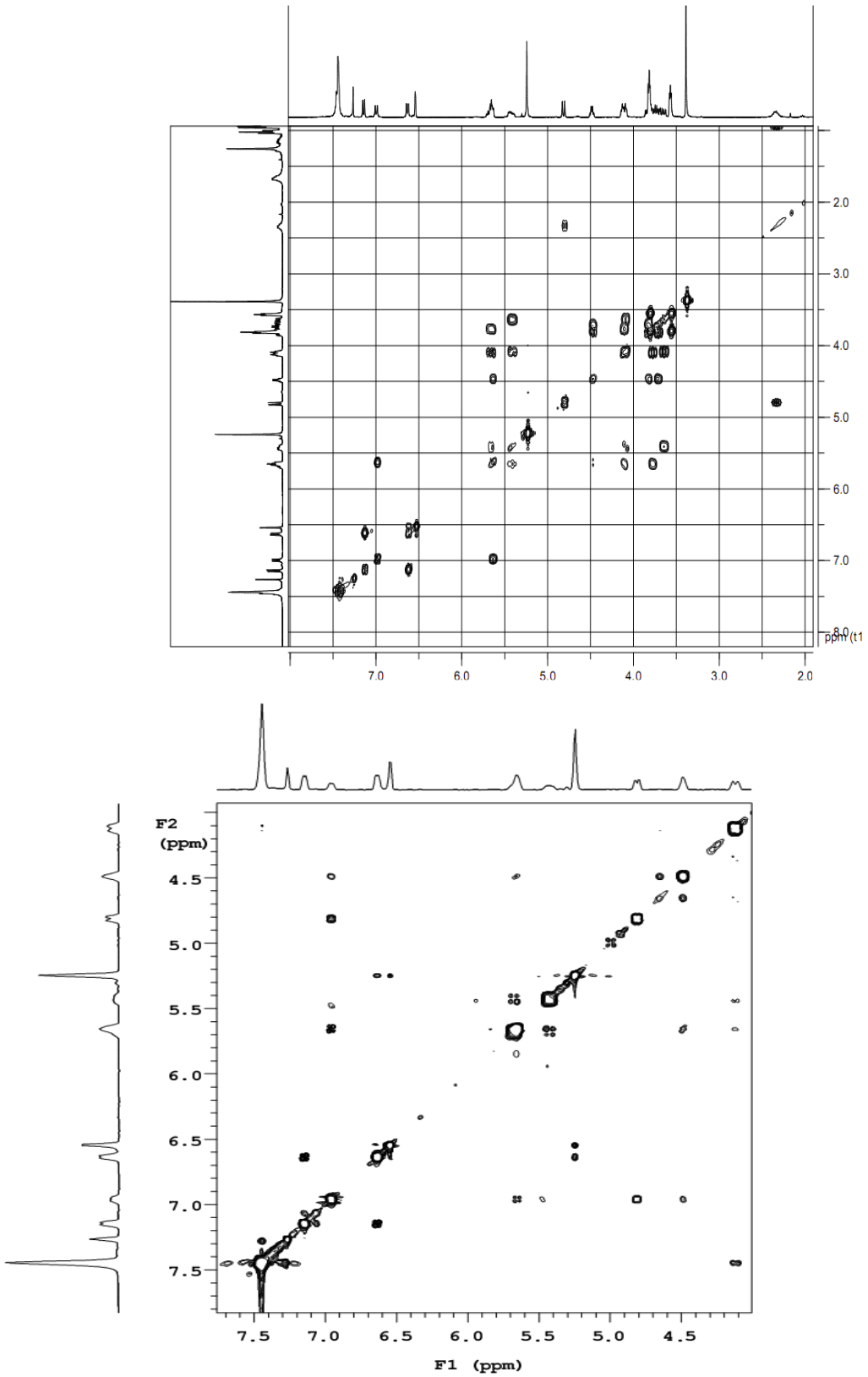
^{1}H and ^{13}C spectra of compound **1.1d**



^{1}H and ^{13}C spectra of compound **1.1f**



^{1}H and ^{13}C spectra of compound **F2.2a**

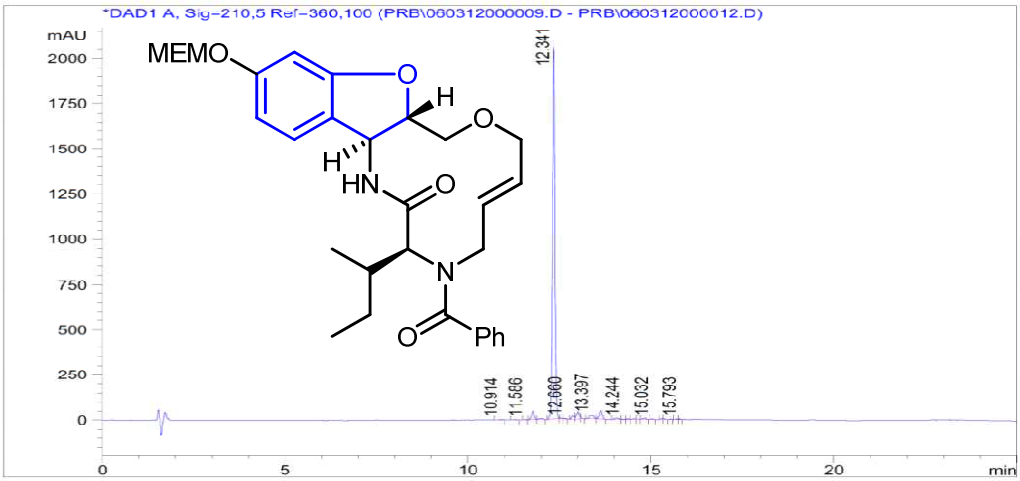


COSY and 2D NOESY of compound **F2.2a**

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line :	0
Injection Date	: Tue, 6. Mar. 2012	Location :	Vial 7
Sample Name	: ILS-JRK-C95-97(1)	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	10.914	0.110	15.763	0.138	
2	11.302	0.098	15.112	0.132	
3	11.586	0.099	4.257	0.037	
4	11.775	0.082	223.773	1.955	
5	11.987	0.130	42.204	0.369	
6	12.341	0.082	10107.292	88.310	
7	12.533	0.053	8.183	0.071	
8	12.660	0.067	11.290	0.099	
9	12.862	0.087	92.426	0.808	
10	13.000	0.087	205.521	1.796	
11	13.071	0.044	25.822	0.226	
12	13.397	0.151	173.464	1.516	

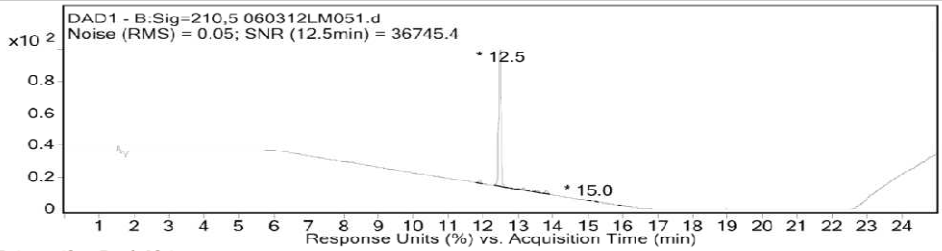
HPLC of compound **F2.2a**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	060312LM051.d	**Sample Name**	ILS-JRK-C95-97(1)
Sample Type	Sample	**Position**	Vial 92
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-UNI.m	**Acquired Time**	3/6/2012 6:17:11 PM
IRM Calibration Status	Not Applicable	**DA Method**	Reserpine_Checkout.m
Comment	XBridge C18, 150*4.6mm 5um.		

User Chromatograms

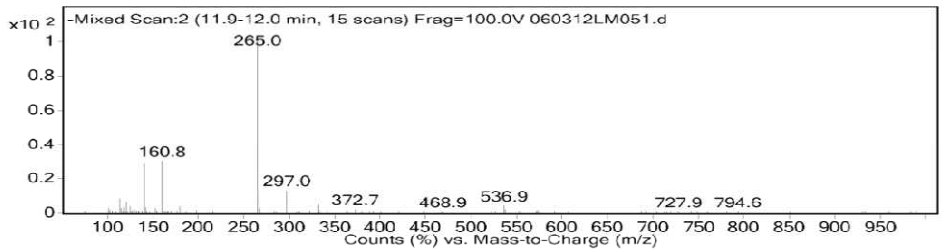


Integration Peak List

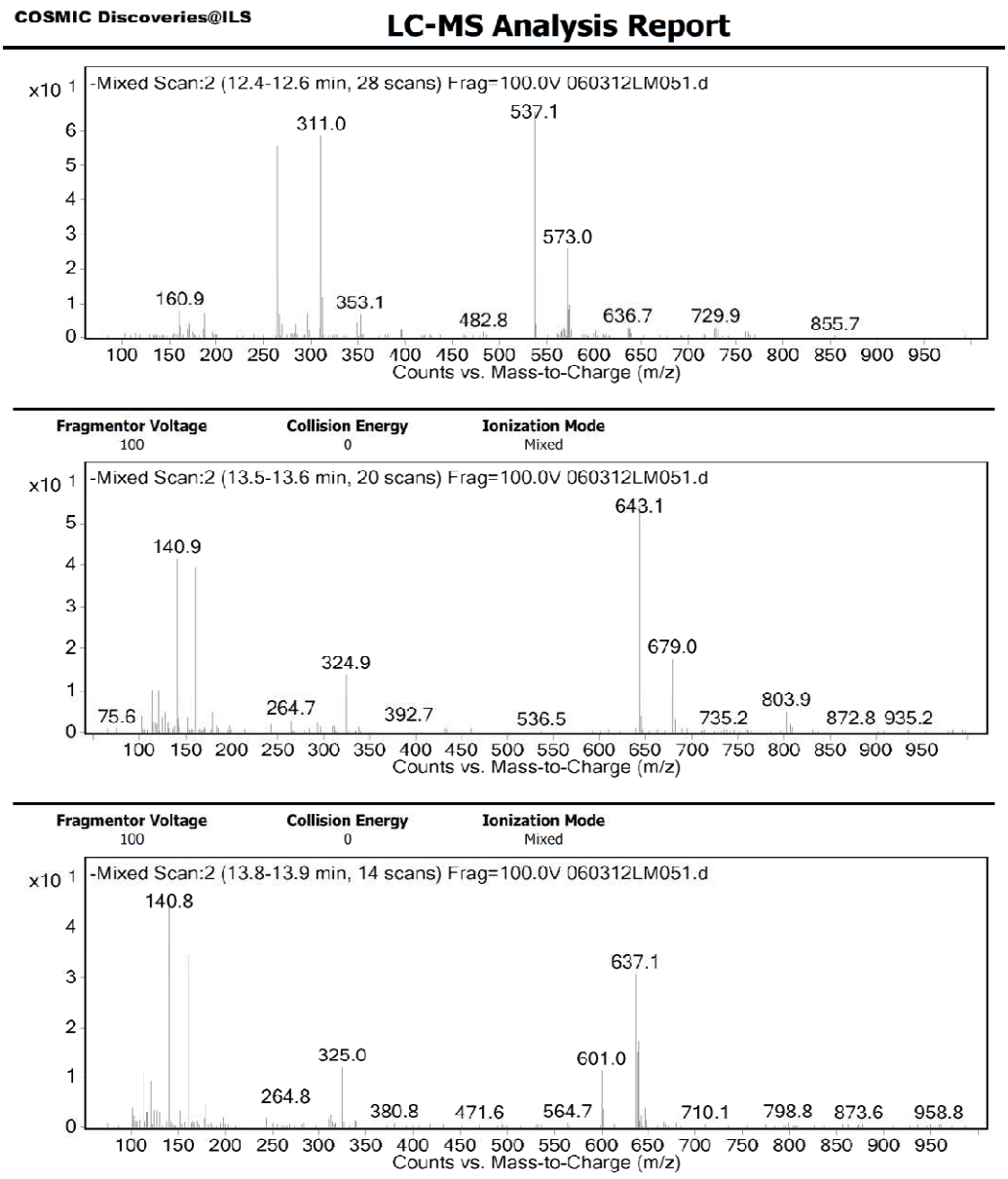
Peak	RT	Area	%Area
1	11.9	188.69	1.73
2	12.5	9949.94	90.97
3	12.6	14.1	0.13
5	13	70.72	0.65
6	13.2	148.02	1.35
7	13.2	15.7	0.14
8	13.5	214.83	1.96
9	13.8	241.04	2.2
10	15	41.93	0.38
11	15.2	17.33	0.16

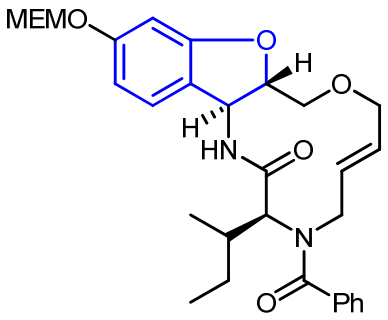
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed

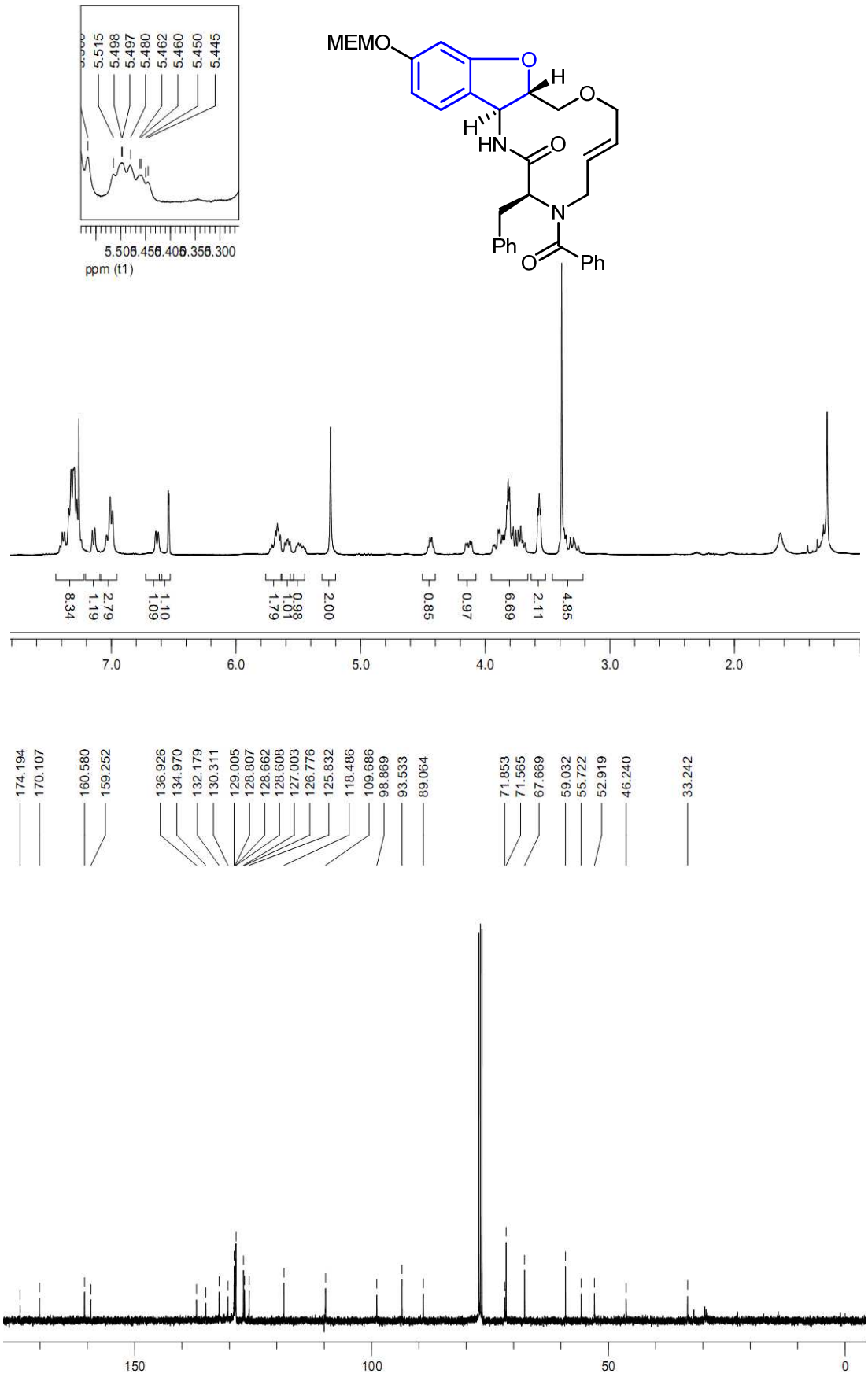


Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed





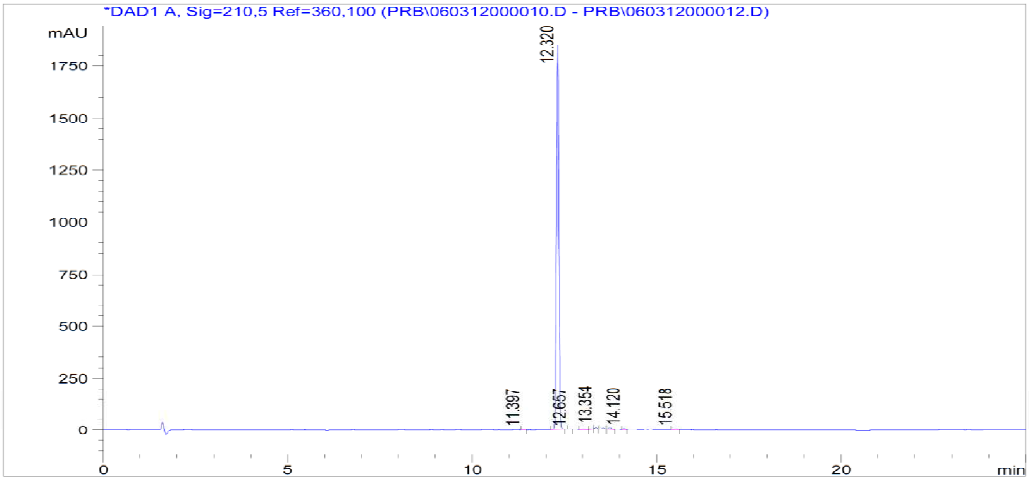
LC-MS of compound **F2.2a**



^{1}H and ^{13}C spectra of compound **F2.2b**

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line :	0
Injection Date :	Tue, 6. Mar. 2012	Location :	Vial 1
Sample Name :	ILS-JRK-C95-127	Inj. No. :	0
Acq Operator :	RADHA	Inj. Vol. :	7 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	11.397	0.066	9.197	0.108	
2	12.320	0.074	8195.257	96.655	
3	12.657	0.065	8.897	0.105	
4	13.021	0.104	23.285	0.275	
5	13.246	0.073	20.872	0.246	
6	13.354	0.073	67.364	0.794	
7	13.556	0.081	51.260	0.605	
8	13.734	0.076	52.114	0.615	
9	14.120	0.065	23.925	0.282	
10	15.518	0.075	26.743	0.315	

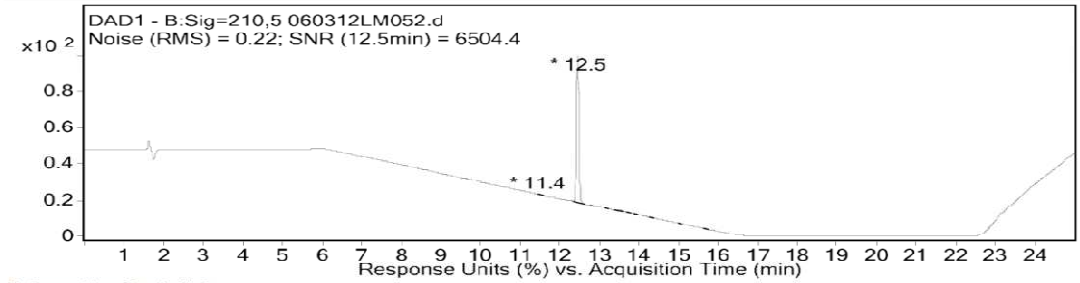
HPLC of compound **F2.2b**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	060312LM052.d	**Sample Name**	ILS-JRK-C95-127
Sample Type	Sample	**Position**	Vial 93
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-UNI.m	**Acquired Time**	3/6/2012 6:46:05 PM
IRM Calibration Status	Not Applicable	**DA Method**	Reserpine_Checkout.m
Comment	XBridge C18, 150*4.6mm 5um.		

User Chromatograms

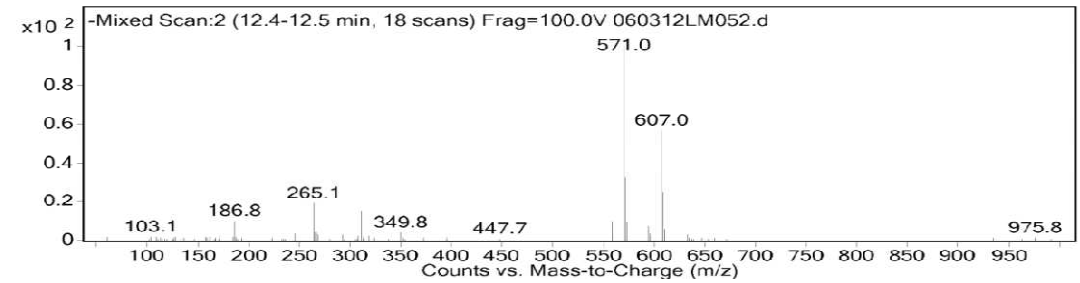


Integration Peak List

Peak	RT	Area	%Area
1	11.4	6.34	0.1
2	12.5	6119.87	97.23
4	13.1	4.73	0.08
5	13.3	12.34	0.2
6	13.5	63.1	1
7	13.7	20.72	0.33
8	13.9	23.99	0.38
9	14.2	14.11	0.22
10	15	12.85	0.2
11	15.6	13.76	0.22

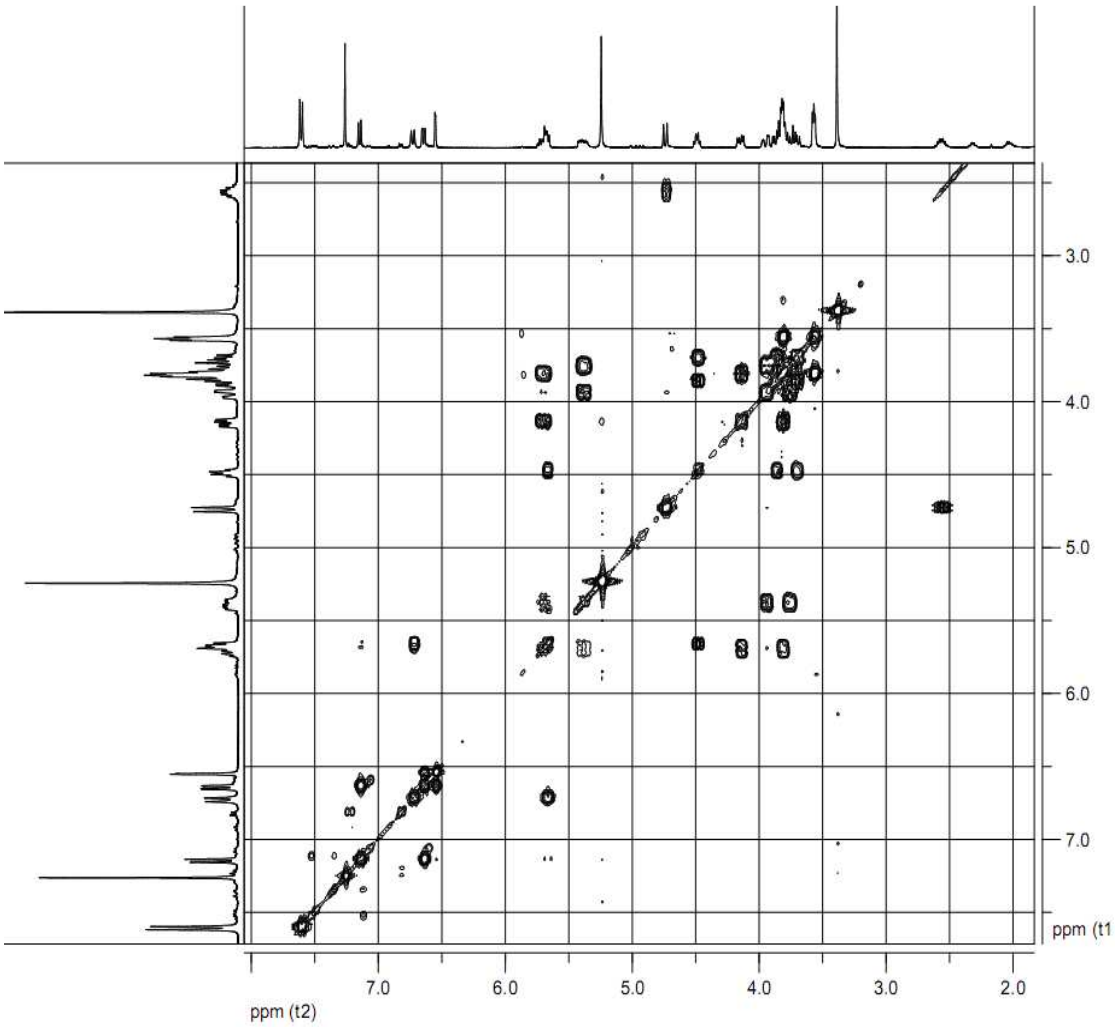
User Spectra

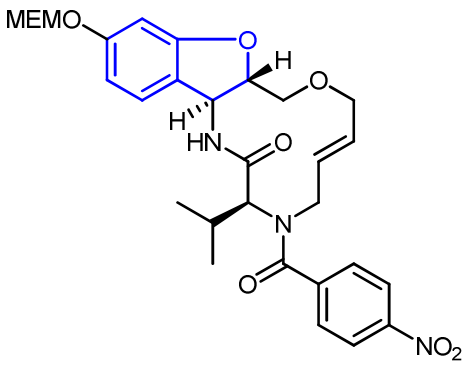
Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed



LC-MS of compound **F2.2b**

^{1}H and ^{13}C spectra of compound **F2.2c**



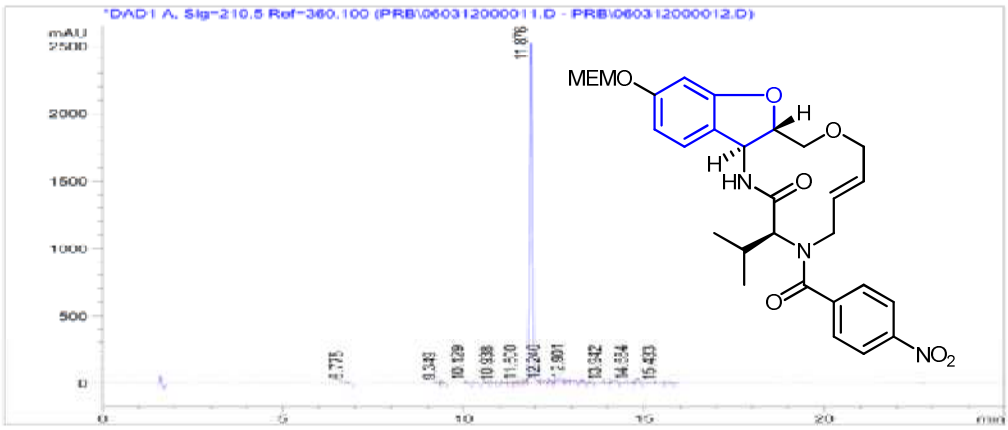


COSY of compound **F2.2c**

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line :	0
Injection Date	: Tue, 6. Mar. 2012	Location :	Vial 8
Sample Name	: ILS-JRK-C95-120	Inj. No. :	0
Acq Operator	: RADHA	Inj. Vol. :	7 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
13	11.715	0.072	25.510	0.178	
14	11.876	0.806	12960.711	90.512	
15	12.240	0.104	36.640	0.256	
16	12.420	0.076	83.294	0.581	
17	12.616	0.119	244.906	1.710	
18	12.901	0.067	38.360	0.268	
19	13.033	0.097	96.388	0.673	
20	13.313	0.070	40.600	0.283	
21	13.565	0.070	14.582	0.102	
22	13.942	0.077	6.900	0.048	
23	14.175	0.120	154.262	1.077	
24	14.684	0.107	38.388	0.268	
25	14.843	0.073	166.513	1.162	
26	15.433	0.088	9.338	0.065	
27	15.715	0.107	30.749	0.215	

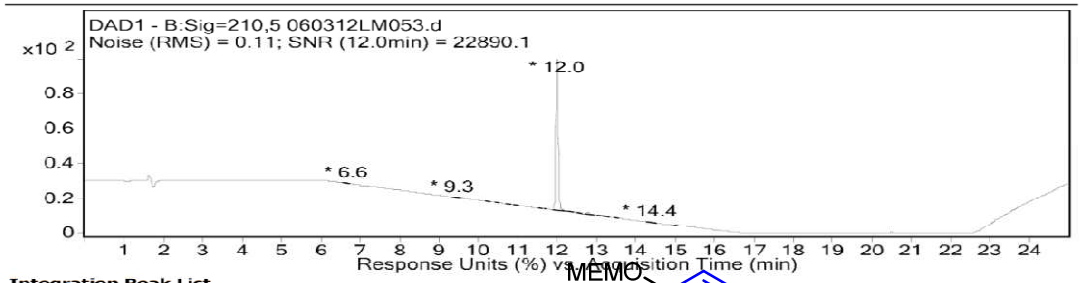
HPLC of compound **F2.2c**

COSMIC Discoveries@ILS

LC-MS Analysis Report

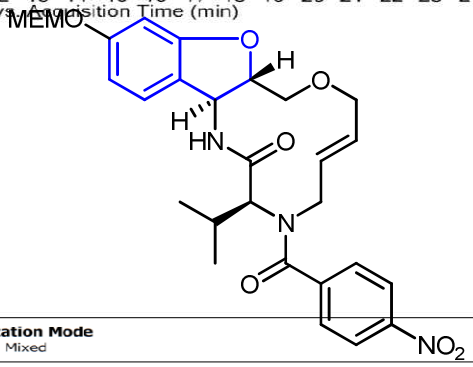
Data Filename	060312LM053.d	**Sample Name**	ILS-JRK-C95-128
Sample Type	Sample	**Position**	Vial 94
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-UNI.m	**Acquired Time**	3/6/2012 7:15:01 PM
IRM Calibration Status	Not Applicable	**DA Method**	Reserpine_Checkout.m
Comment	XBridge C18, 150*4.6mm 5um.		

User Chromatograms



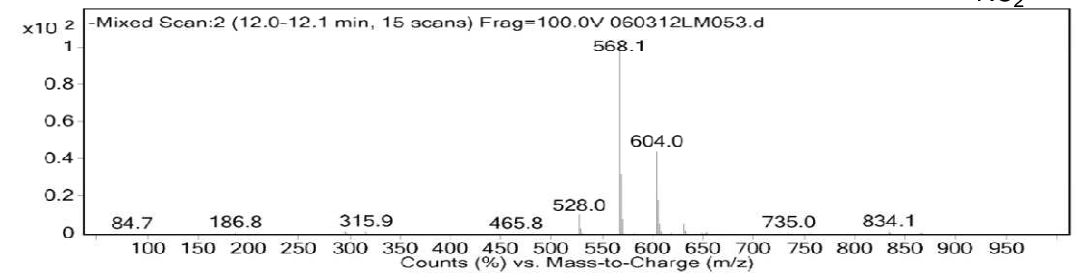
Integration Peak List

Peak	RT	Area	%Area
1	6.6	51.88	0.43
3	9.5	34.08	0.28
6	10.7	32.11	0.26
10	12	11399.82	93.91
12	12.6	52.89	0.44
13	12.8	151.93	1.25
16	13.2	79.06	0.65
17	13.5	25.57	0.21
18	14.4	106.71	0.88
19	15	91.19	0.75

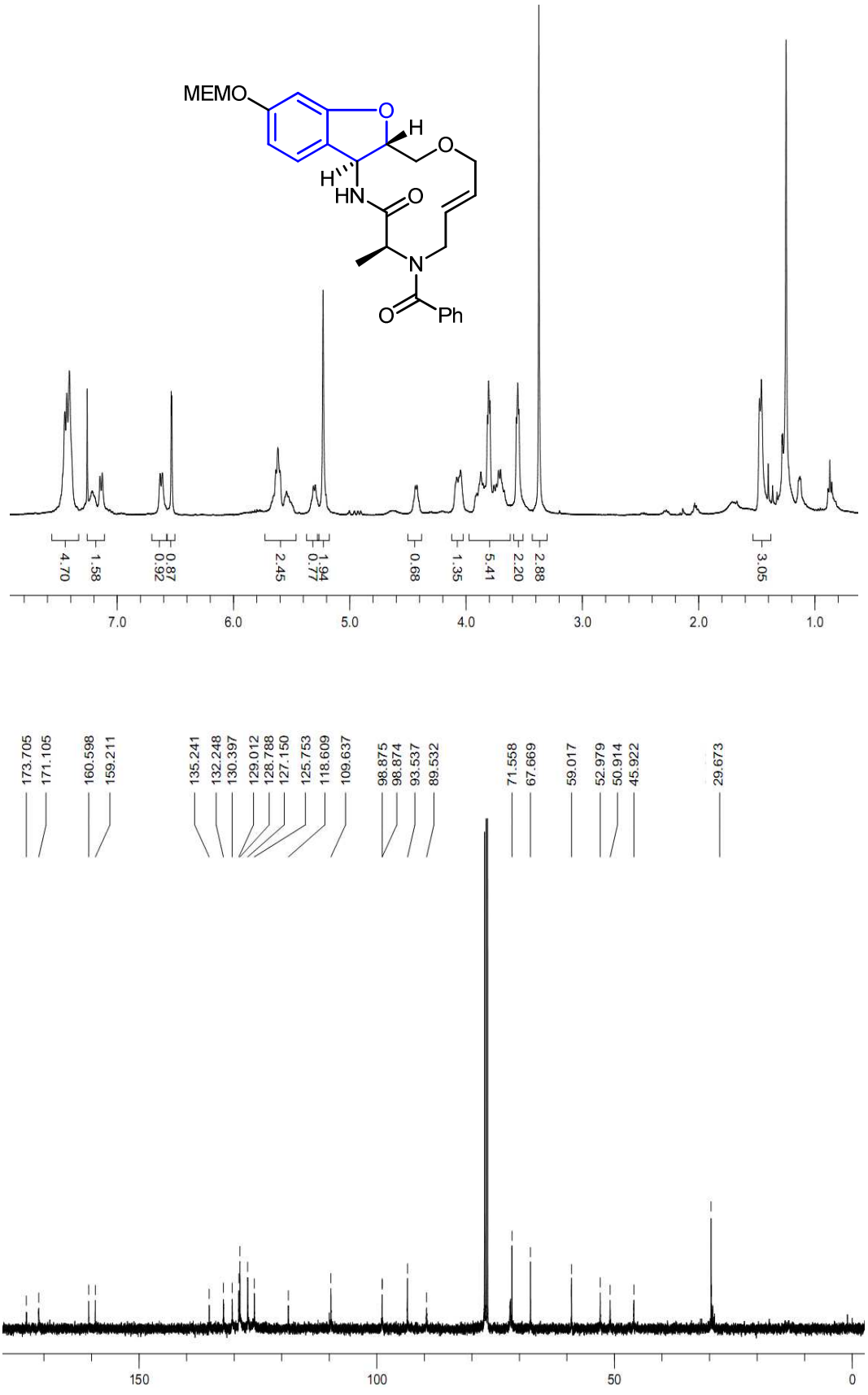


User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed



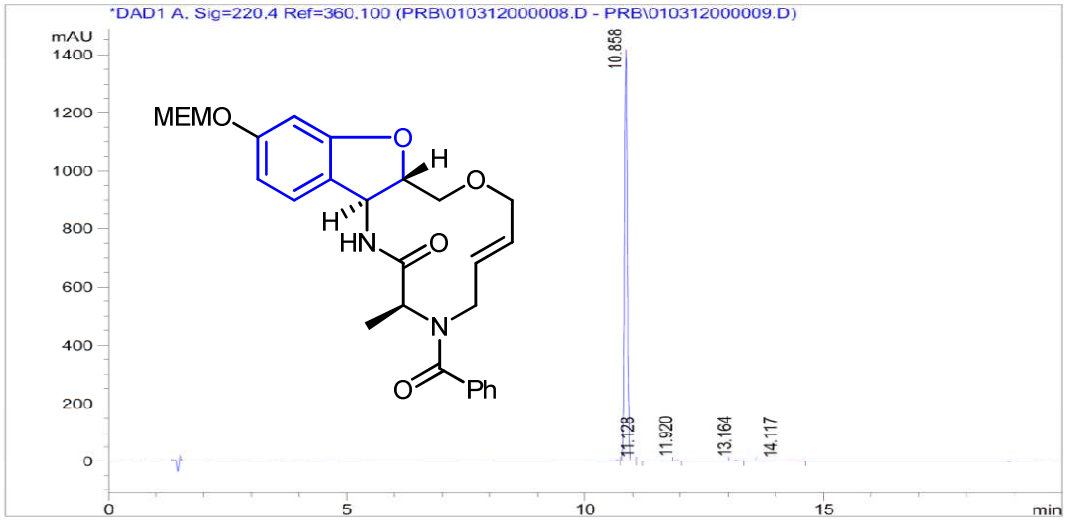
LC-MS of compound **F2.2c**



^{1}H and ^{13}C spectra of compound **F2.2d**

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line :	0
Injection Date	: Thu, 1. Mar. 2012	Location :	Vial 24
Sample Name	: ILSJRK-C95-167	Inj. No. :	0
Acq Operator	: RADHA	Inj. Vol. :	2 µl
Acq. Method	: D:\CHEM32\1\METHODS\C-18 A50B50.M		
Analysis Method	: D:\CHEM32\1\METHODS\C-18 A50B50.M		
Method Info	: Column: X Bridge C18 150*4.6mm 5µm		
	Mobile phase: A) 0.1% HCOOH in water ,B) ACN		
	(GRADIENT) T/%B:0/50,2/50,9/98,16/98,18/50,20/50		
	Flow :1.0 ml/min Diluent:ACN:Water(50:50)		



Signal 1: DAD1 A, Sig=220,4 Ref=360,100

Peak #	RT [min]	Width [min]	Area	Area %	Name
1	10.673	0.065	17.398	0.310	
2	10.858	0.065	5523.697	98.540	
3	11.128	0.072	1.782	0.032	
4	11.920	0.065	14.544	0.259	
5	13.164	0.090	21.862	0.390	
6	14.117	0.547	26.259	0.468	

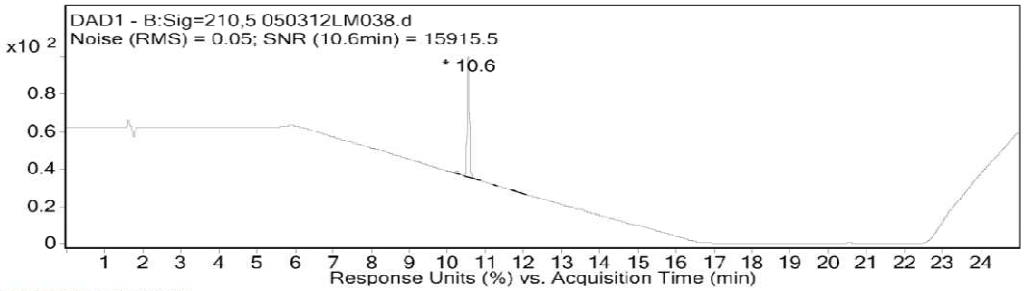
HPLC of compound **F2.2d**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	050312LM038.d	**Sample Name**	ILS-JRK-C95-167
Sample Type	Sample	**Position**	Vial 92
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-UNI.m	**Acquired Time**	3/5/2012 6:59:19 PM
IRM Calibration Status	Not Applicable	**DA Method**	Reserpine_Checkout.m
Comment	XBridge C18, 150*4.6mm 5um		

User Chromatograms

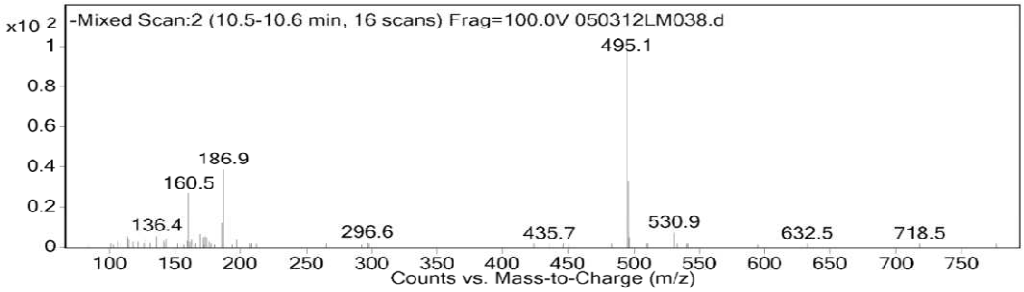


Integration Peak List

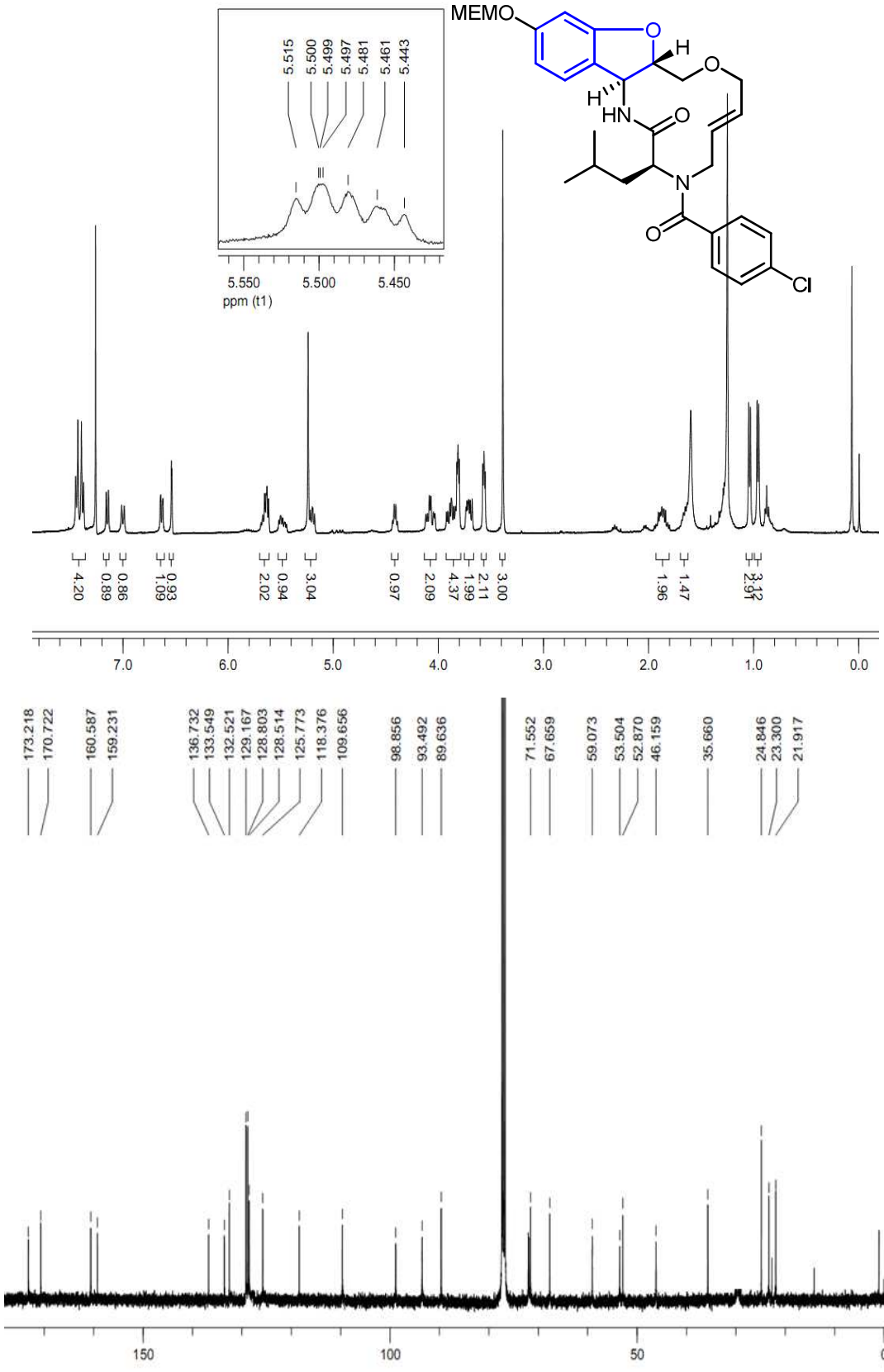
Peak	RT	Area	%Area
1	10.2	72.71	1.99
2	10.6	3487.17	95.5
3	10.7	18.98	0.52
4	10.8	4.41	0.12
5	11.2	5.08	0.14
6	11.7	33.86	0.93
7	11.9	20.14	0.55
8	12	9.04	0.25

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed



LC-MS of compound **F2.2d**

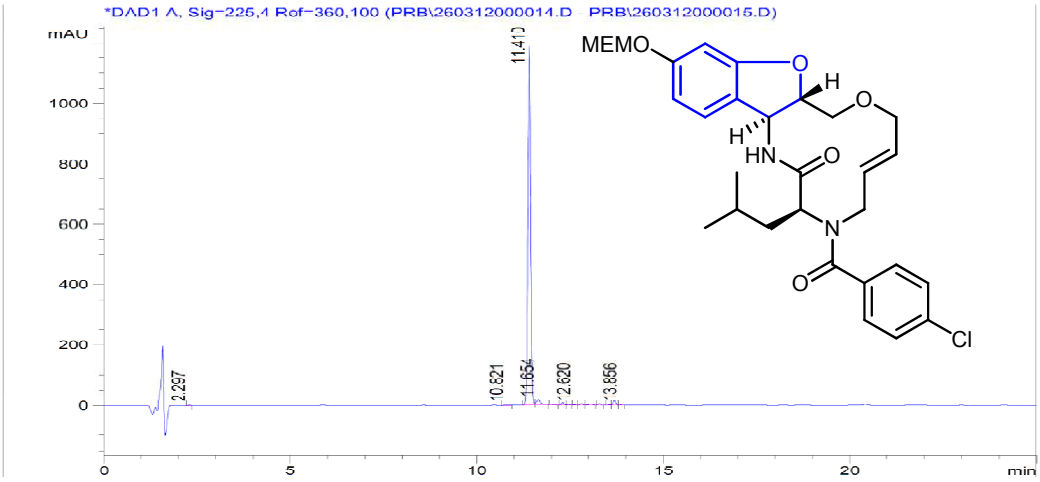


^{1}H and ^{13}C spectra of compound **F2.2e**

COSMIC DISCOVERIES @ ILS
HPLC ANALYSIS REPORT

		Seq Line	: 0
Injection Date	: Mon, 26. Mar. 2012	Location	: Vial 12
Sample Name	: ILS-JRK-C95-191	Inj. No.	: 0
Acq Operator	: RADHA	Inj. Vol.	: 4 µl
Acq. Method	: D:\CHEM32\1\METHODS\C-18 A60B40-M.M		
Analysis Method	: D:\CHEM32\1\METHODS\C-18 A60B40-M.M		
Method Info	: Column:X-Bridge C-18 150*4.6mm 5µ		

Mobile phase: A) 0.1% HCOOH in water,B) ACN
(GRADIENT) T/B%:0/40,3/40,14/95,20/95,22/40,25/40
Flow:1.0 ml/min Diluent: ACN:MeOH(80:20)



Signal 1: DAD1 A, Sig=225,4 Ref=360,100

Peak #	RT [min]	Width [min]	Area	Area %	Name
1	2.297	0.061	4.099	0.064	
2	10.821	0.123	9.560	0.149	
3	11.410	0.085	6086.069	94.703	
4	11.654	0.121	113.391	1.764	
5	12.053	0.118	9.471	0.147	
6	12.304	0.087	30.633	0.477	
7	12.458	0.079	10.026	0.156	
8	12.620	0.074	6.500	0.101	
9	12.803	0.086	19.807	0.308	
10	13.049	0.147	23.627	0.368	
11	13.283	0.091	21.810	0.339	
12	13.555	0.078	9.282	0.144	

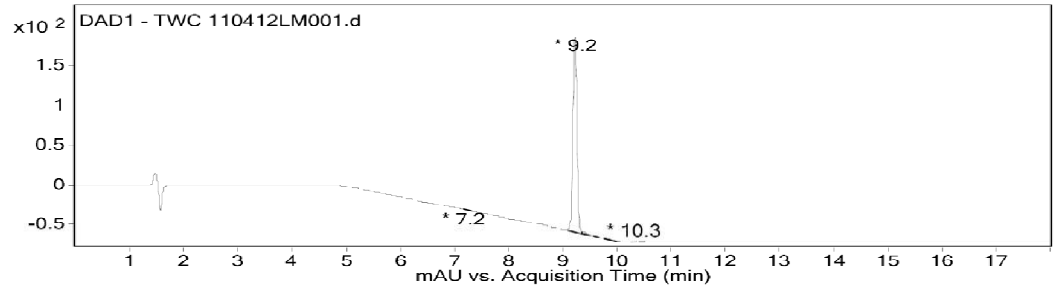
HPLC of compound **F2.2e**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	110412LM001.d	**Sample Name**	ILS-JRK-C95-191
Sample Type	Sample	**Position**	Vial 1
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	4/11/2012 9:55:22 AM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms

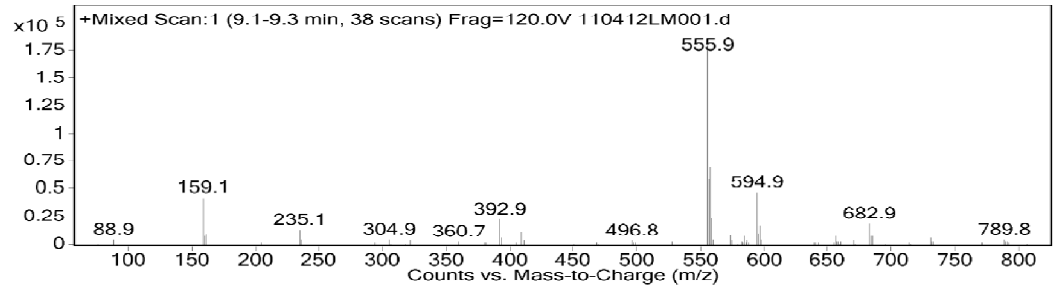


Integration Peak List

Peak	RT	Area	%Area
1	7.2	0.665	0.05
2	9.2	1273.943	98.11
3	9.4	12.688	0.98
4	9.6	0.871	0.07
5	9.8	8.791	0.68
6	10.3	0.873	0.07
7	10.5	0.684	0.05

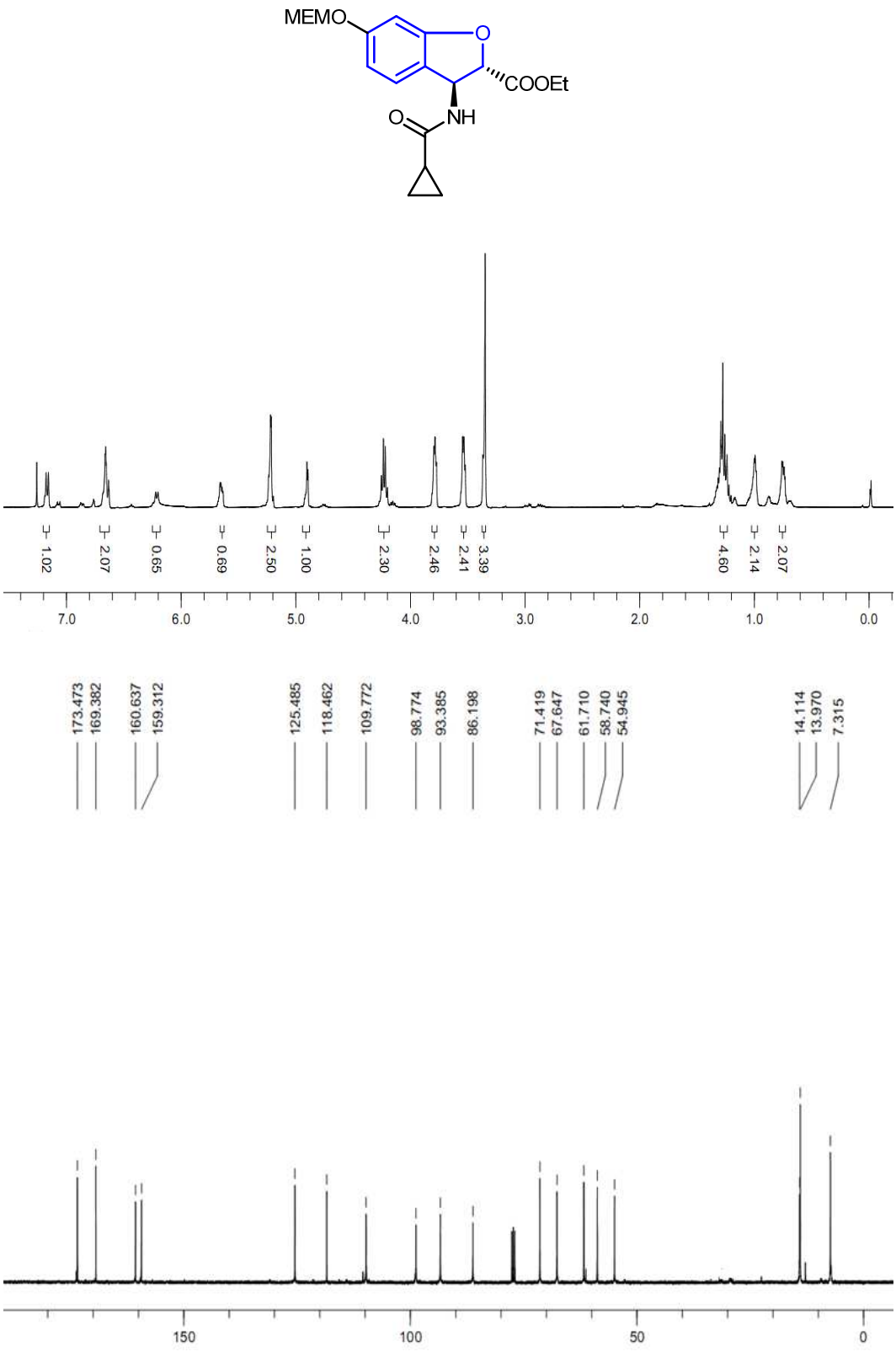
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

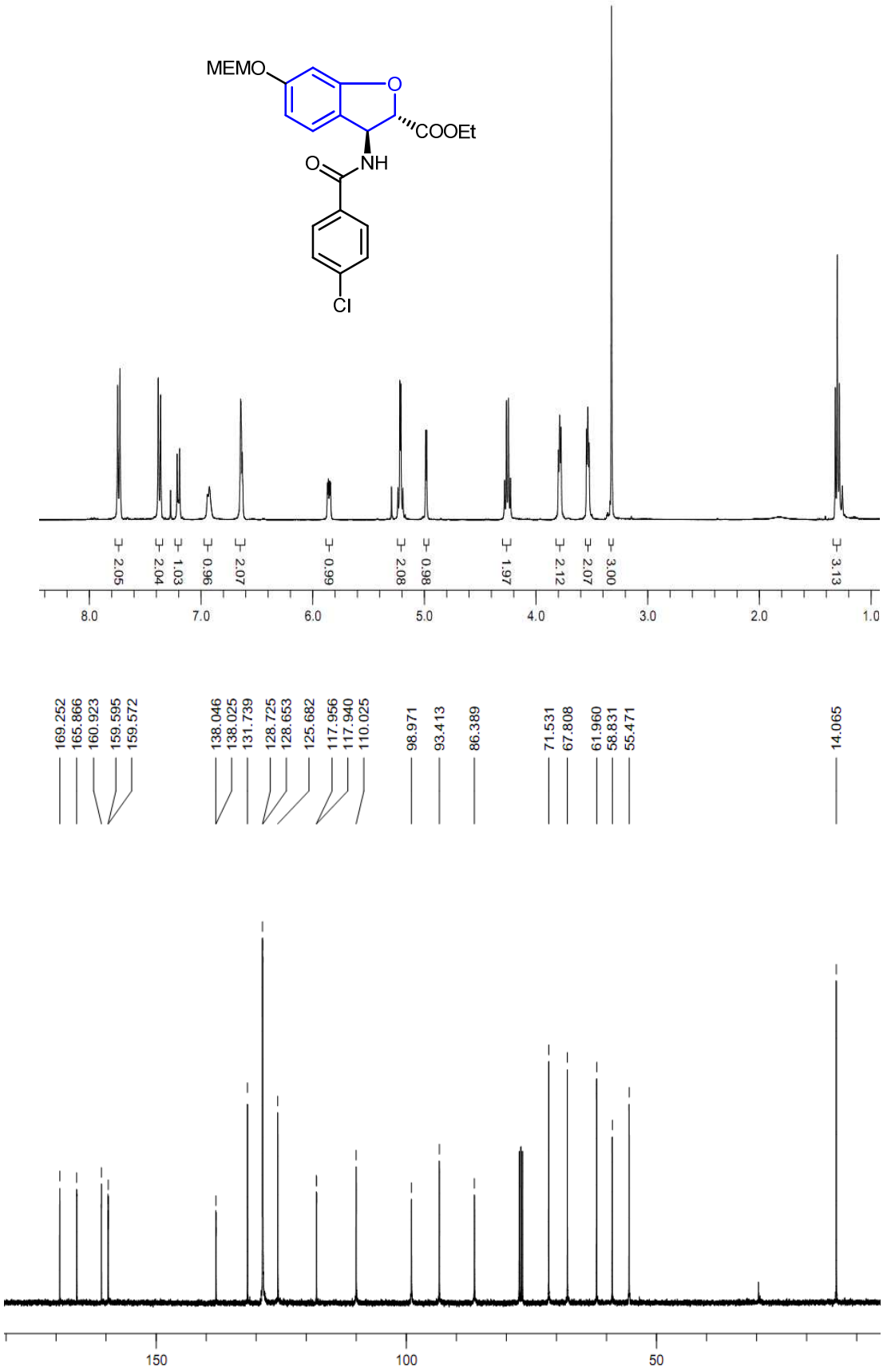


Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

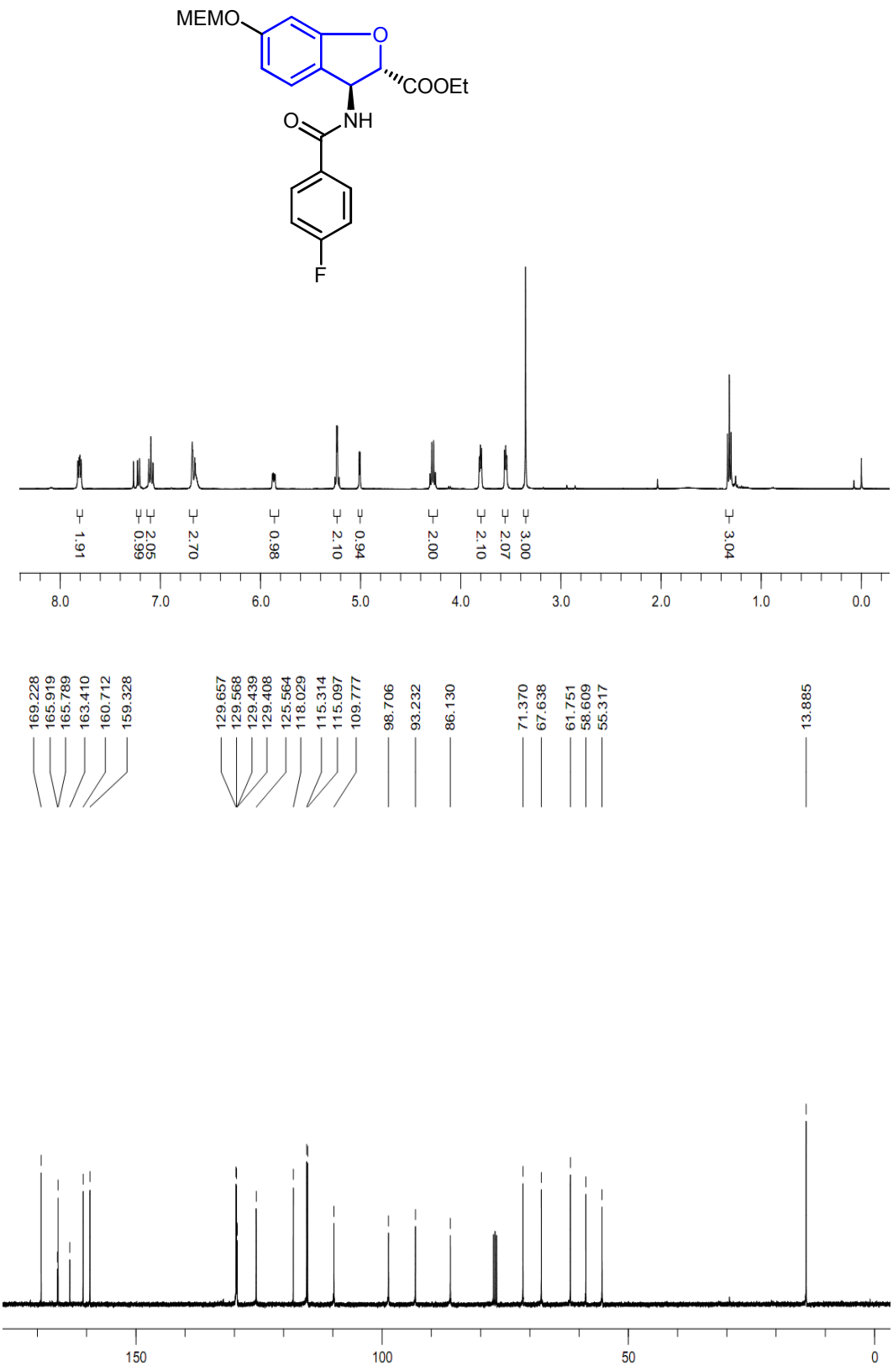
LC-MS of compound **F2.2e**



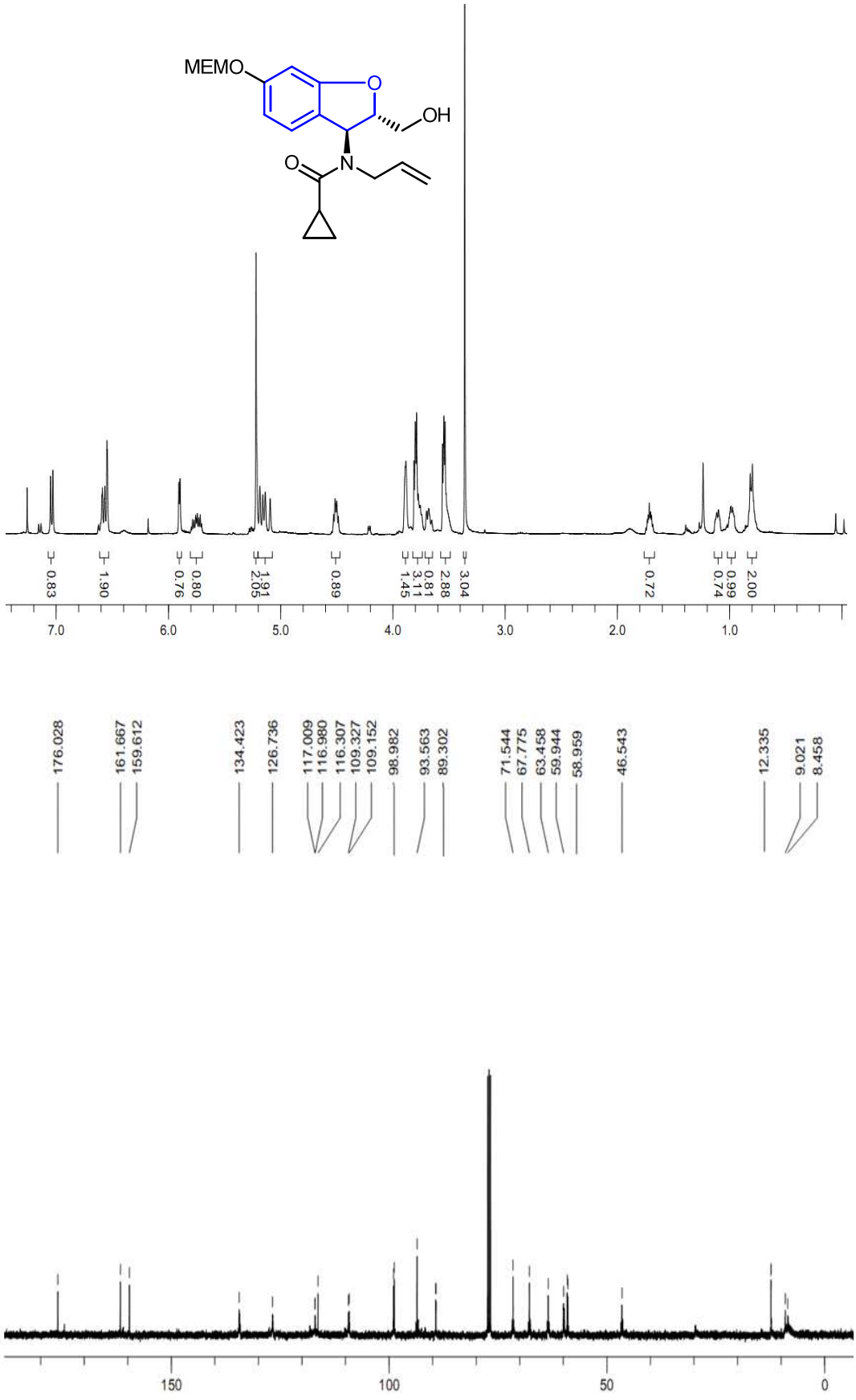
^{1}H and ^{13}C spectra of compound **3.3a**



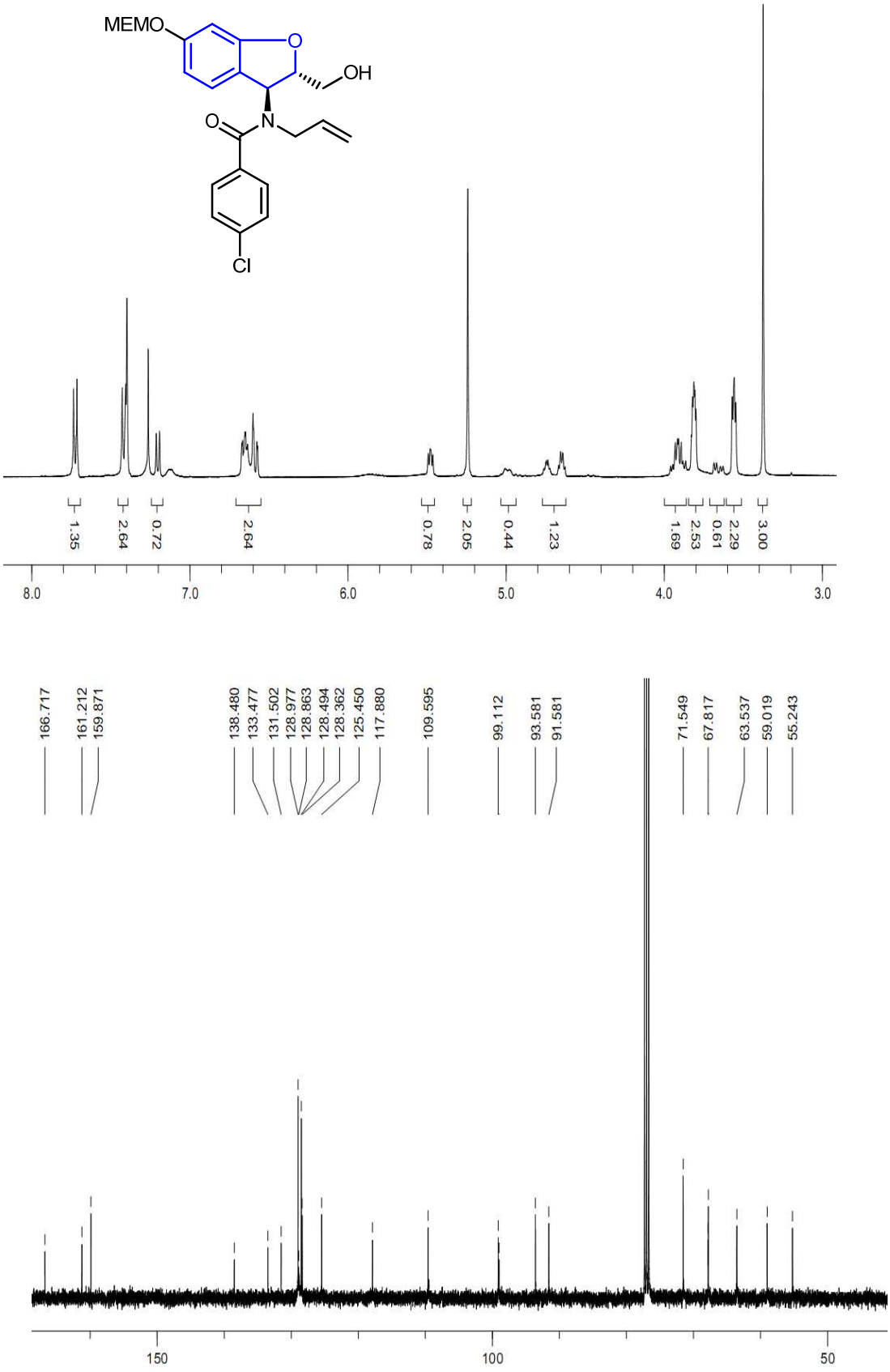
^{1}H and ^{13}C spectra of compound **3.3b**



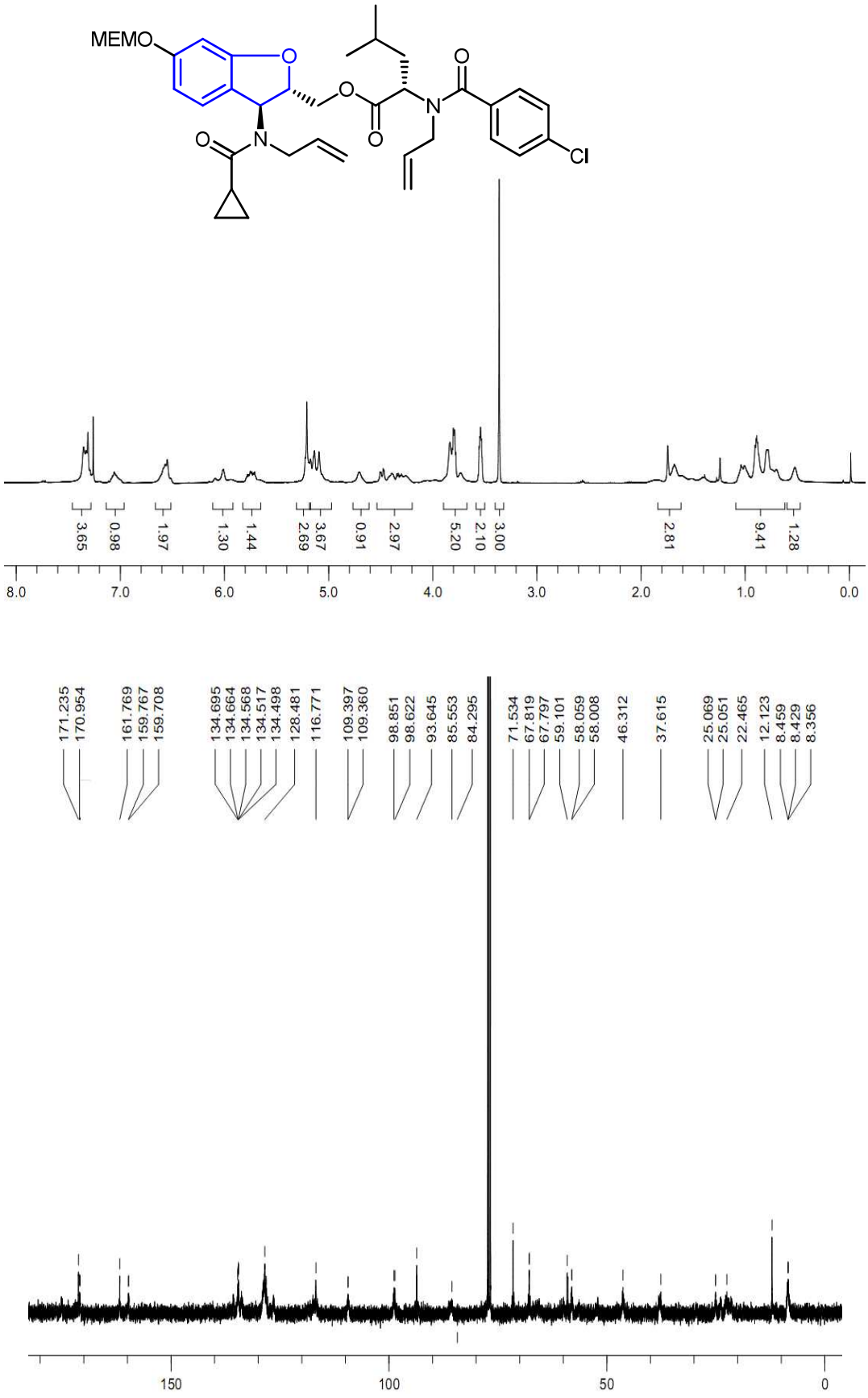
^{1}H and ^{13}C spectra of compound **3.3c**



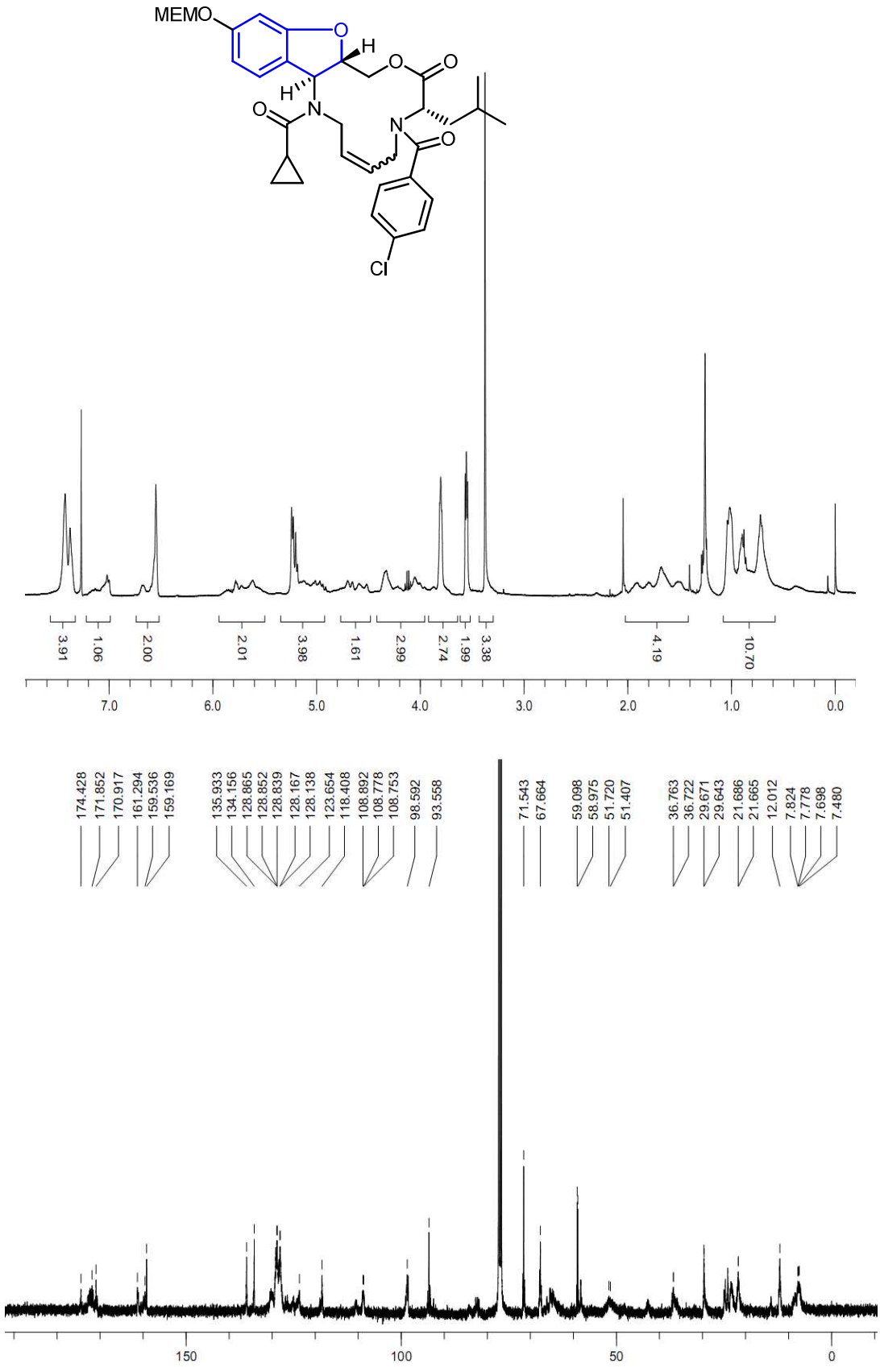
^{1}H and ^{13}C spectra of compound **3.2a**



^{1}H and ^{13}C spectra of compound **3.2b**



^{1}H and ^{13}C spectra of compound **3.1a**



^{1}H and ^{13}C spectra of compound **F2.3a**

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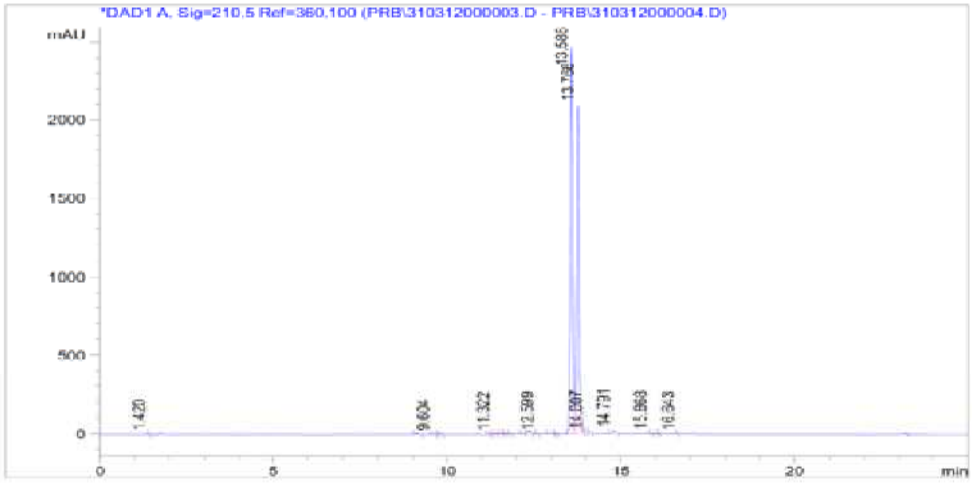
                    COSMIC DISCOVERIES @ ILS
                      HPLC ANALYSIS REPORT

                                                Seq Line   :            0
Injection Date   : Sat, 31. Mar. 2012           Location   :      Vial 21
Sample Name      : ILS-JRK-C95-197              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 5µ
                   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
13	12.599	0.050	5.823	0.027	
14	12.998	0.076	71.892	0.328	
15	13.129	0.071	16.192	0.074	
16	13.586	0.076	11285.745	51.459	
17	13.766	0.077	9705.541	44.253	
18	13.854	0.033	257.011	1.172	
19	14.007	0.056	10.374	0.047	
20	14.093	0.075	41.103	0.187	
21	14.791	0.113	137.372	0.626	
22	15.868	0.061	14.756	0.067	
23	16.033	0.069	10.819	0.049	
24	16.078	0.033	3.063	0.014	
25	16.643	0.055	3.528	0.016	

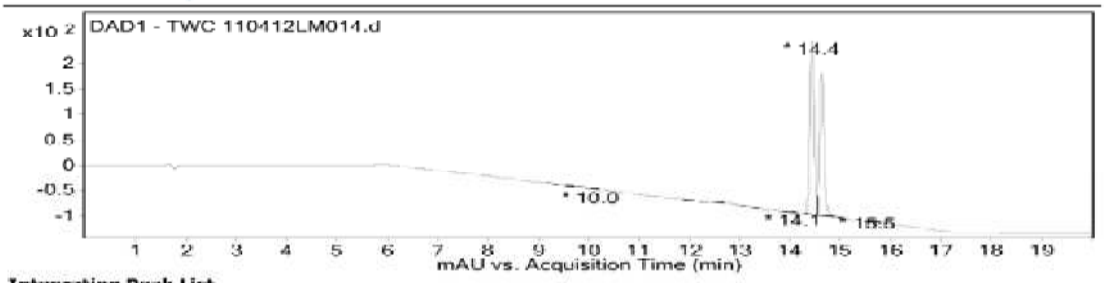
HPLC of compound **F2.3a**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	110412LM014.d	**Sample Name**	ILS-JRK-C95-197
Sample Type	Sample	**Position**	Vial 2
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	4/11/2012 1:14:12 PM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms

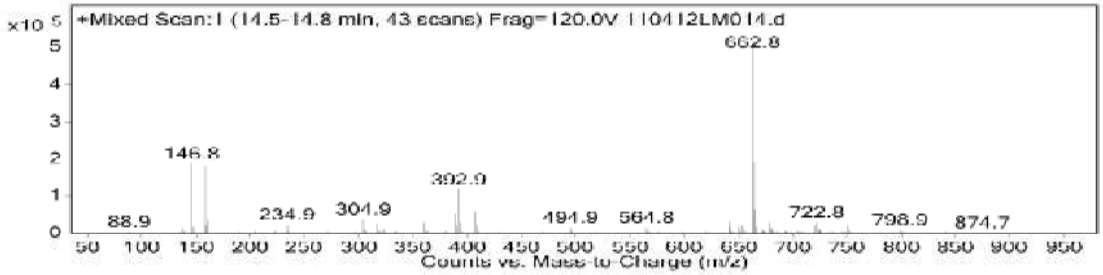


Integration Peak List

Peak	RT	Area	%Area
1	9.3	13.095	0.33
2	9.6	16.021	0.4
6	12.3	5.004	0.13
7	12.5	8.596	0.22
10	13.8	9.232	0.23
12	14.1	6.699	0.17
13	14.4	2094.162	52.73
14	14.6	1763.984	44.42
16	15.5	3.482	0.09
17	15.7	20.878	0.53

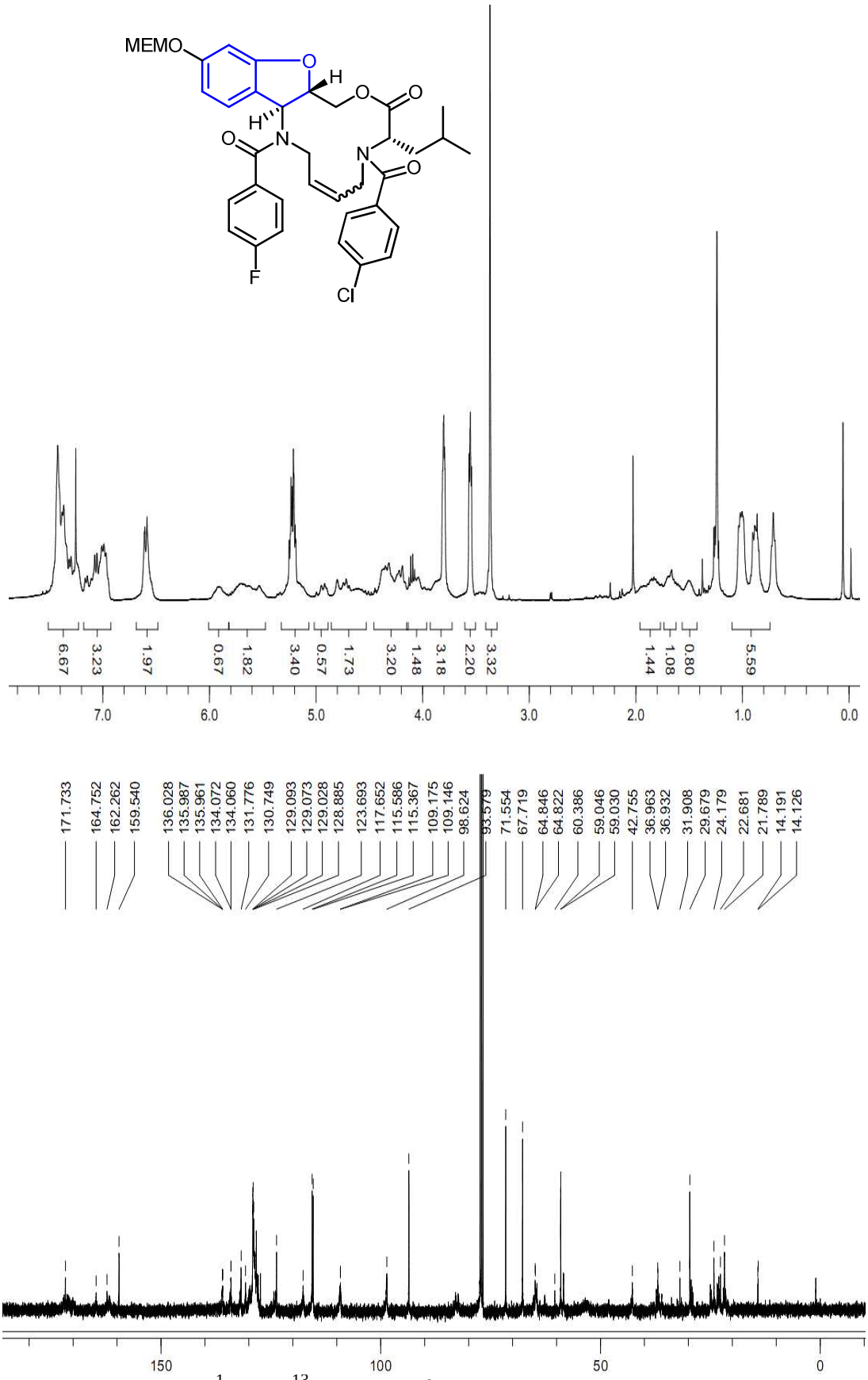
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed



--- End Of Report ---

LC-MS of compound **F2.3a**



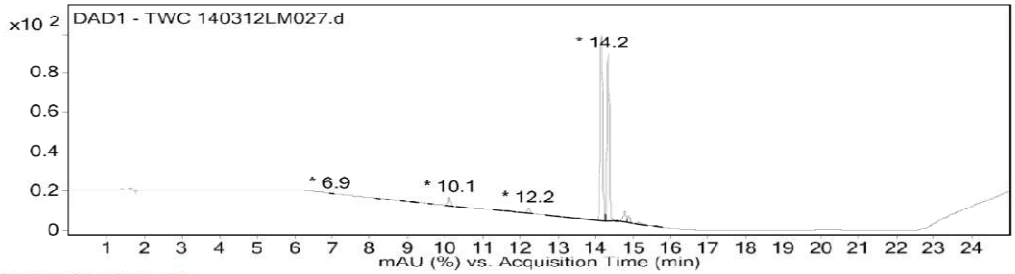
^{1}H and ^{13}C spectra of compound **F2.3c**

COSMIC Discoveries@ILS

LC-MS Analysis Report

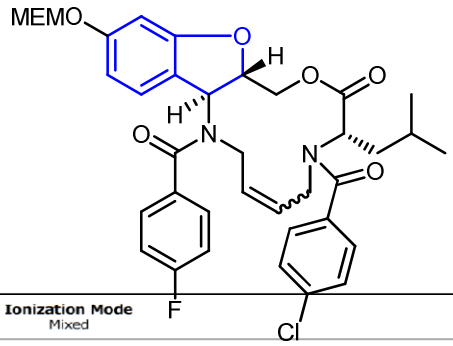
Data Filename	140312LM027.d	**Sample Name**	ILS-JRK-C95-189
Sample Type	Sample	**Position**	Vial 43
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	3/14/2012 7:34:23 PM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms



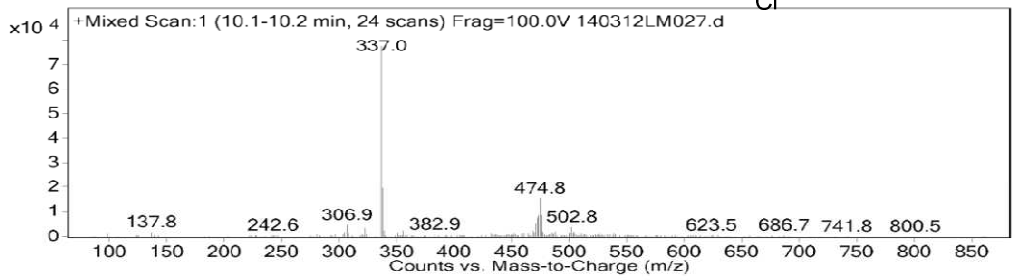
Integration Peak List

Peak	RT	Area	%Area
5	9.2	13.625	0.21
7	10.1	115.904	1.81
11	11.7	17.396	0.27
14	12.2	87.033	1.36
19	13.8	20.247	0.32
21	14.2	3021.13	47.11
22	14.4	2702.61	42.15
24	14.8	181.897	2.84
25	14.9	103.671	1.62
26	15.2	47.99	0.75



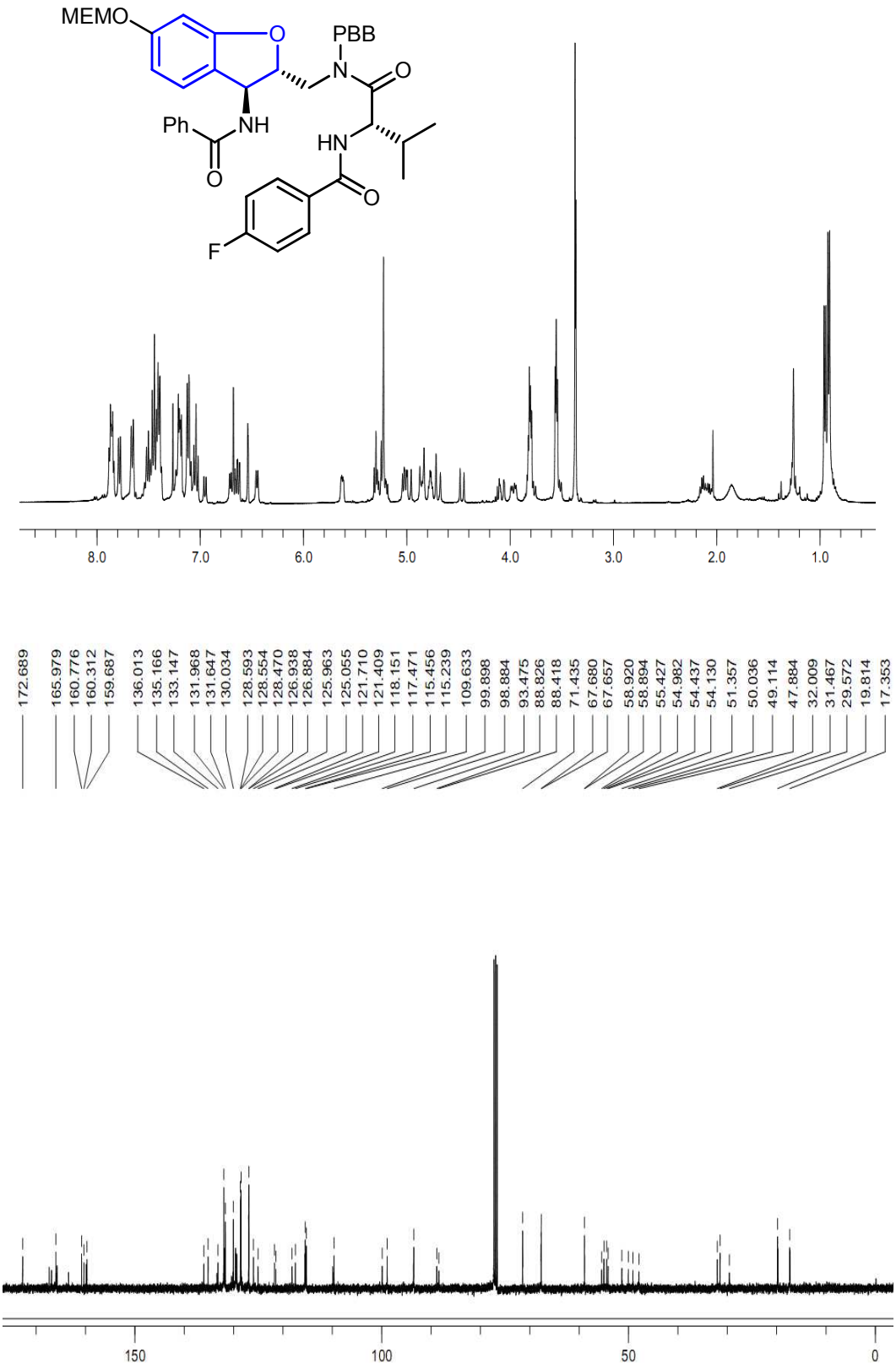
User Spectra

Fragmentor Voltage 100 **Collision Energy** 0 **Ionization Mode** Mixed

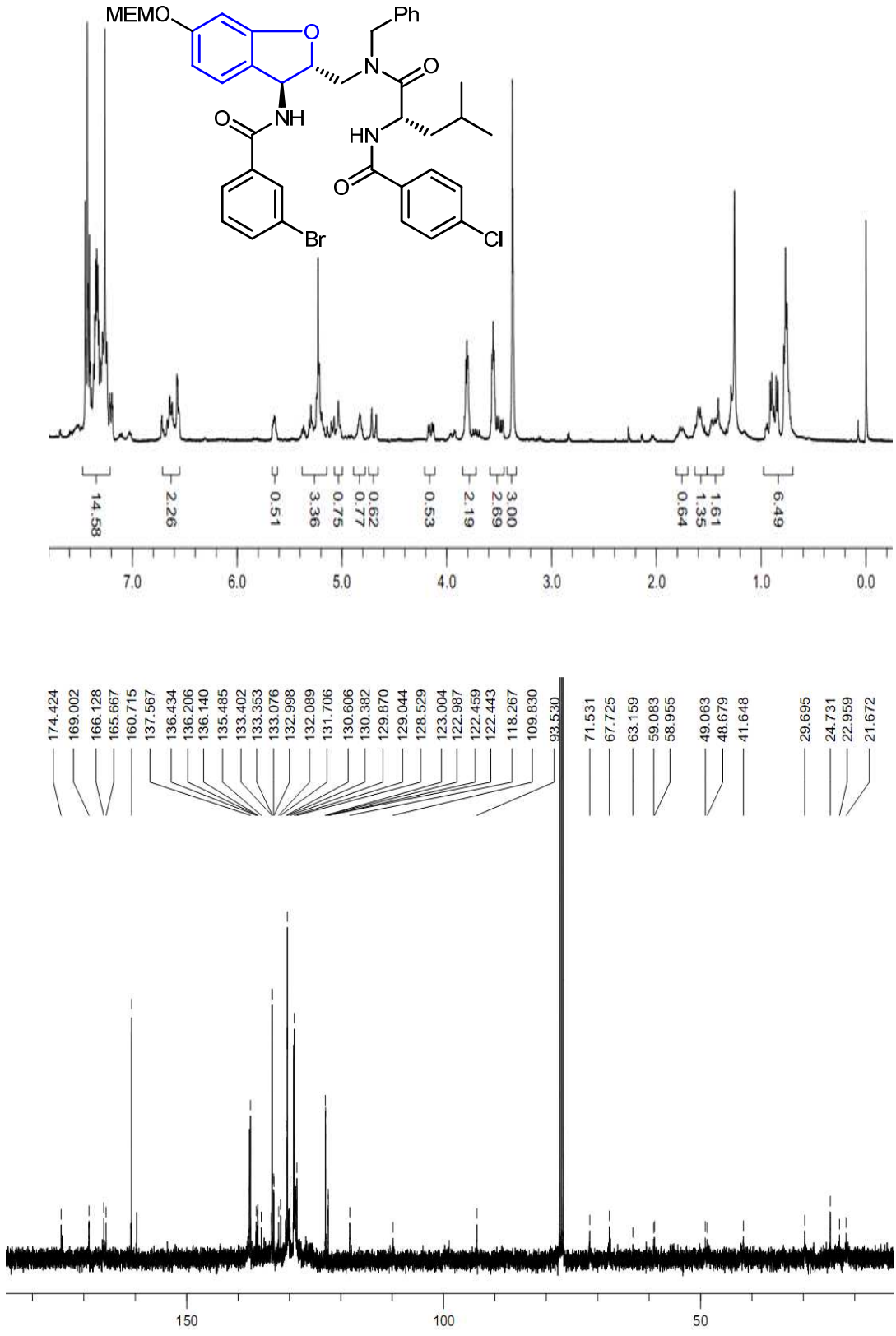


Fragmentor Voltage **Collision Energy** **Ionization Mode**

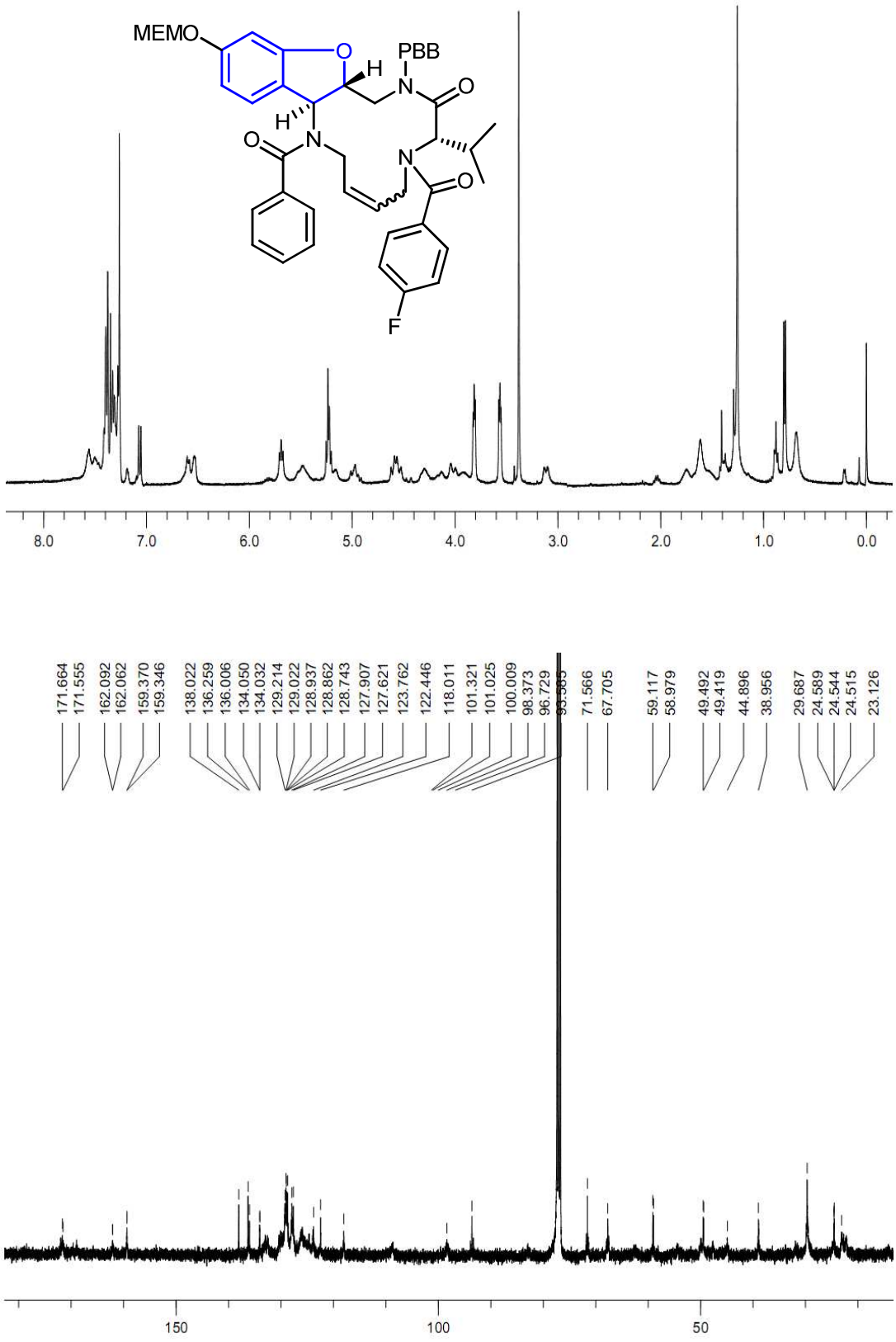
LC-MS of compound **F2.3c**



^{1}H and ^{13}C spectra of compound **5.2b**



^{1}H and ^{13}C spectra of compound **5.2c**



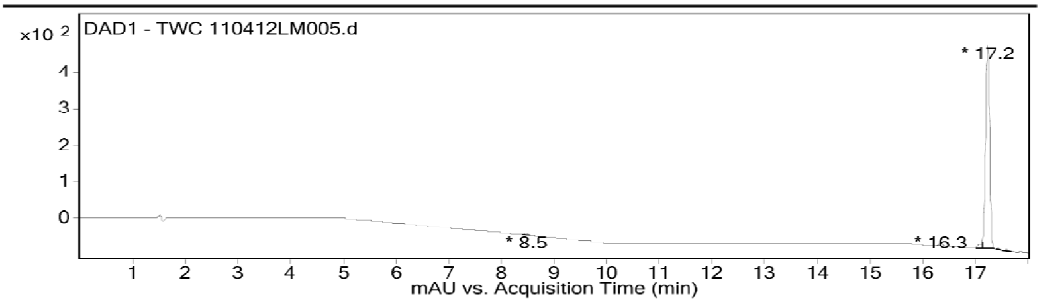
^{1}H and ^{13}C spectra of compound **F2.4b**

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	110412LM005.d	**Sample Name**	ILS-JRK-C95-198
Sample Type	Sample	**Position**	Vial 3
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	4/11/2012 11:55:16 AM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms

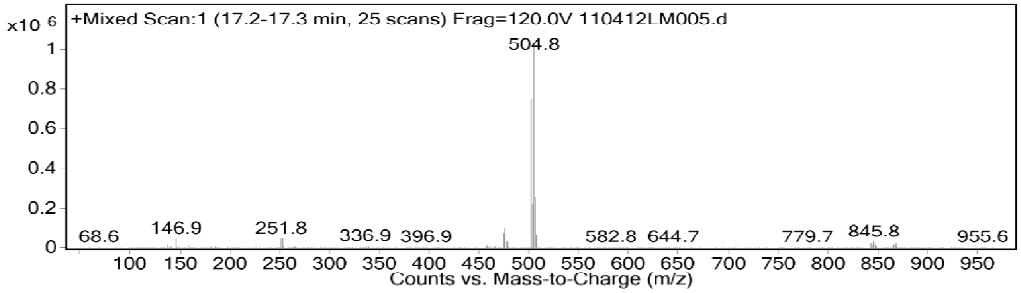


Integration Peak List

Peak	RT	Area	%Area
1	8.5	25.976	0.8
2	16.3	47.507	1.46
3	17.1	75.234	2.31
4	17.2	3086.337	94.69
5	17.4	10.327	0.32
6	17.6	14.126	0.43

User Spectra

Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** Mixed



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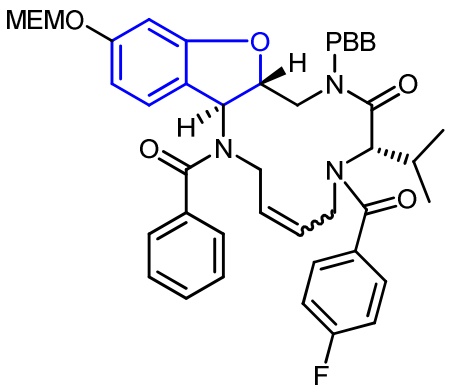


LC-MS of compound **F2.4b**

Chapter 4: Synthesis of C27-C35 Fragment of Eribulin and Its Derived Hybrid Macrocyclic Toolbox

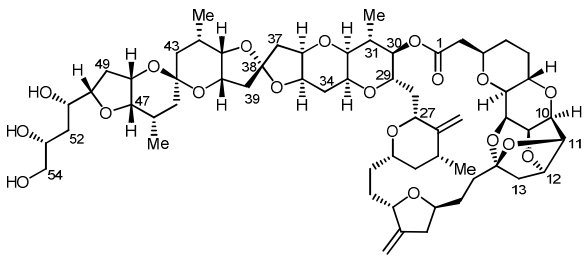
Section A: The Discovery of Eribulin

In this section, I am going to discuss about the discovery of eribulin, and, the literature synthesis of C27-C35 fragment of eribulin.

4.1. Introduction

Eribulin mesylate (Halaven®) **F2.1** is first-in-class, non-taxane microtubule dynamics inhibitor that is currently approved for clinical use in over 40 countries including Japan, USA, and European Union for the treatment of certain patients with late-stage metastatic breast cancer. To date, this agent is the only chemotherapeutic drug to have demonstrated an increase in overall survival in this patient population.[1] Eribulin is a macrocyclic ketone derivative that represent the most structurally complex, non-peptidic, and, a fully synthetic drug of halichondrin B.[2] Halichondrins are a class of polyether macrolides, first isolated from the marine sponge *Halichondria okadai* Kadota,[3] later, from *Axinella* sp. sponges.[4] Okadaic acid was isolated in 1981 by Schuer and Schmitz, two independent research groups from two sponges, *Halichondria okadai* Kadota, a black sponge (**F1.1** shown in **Figure 1**),[5] is commonly located along the pacific coast of Japan, and *Halichondria melanodocia*, a Caribbean sponge that was collected from Florida. The structure was elucidated in collaboration with Clardy's team.[6,7] Potent *in vivo* activity of crude extracts from *Halichondria okadai* Kadota, led the isolation and identification of norhalichondrin A with the cytotoxicity, IC_{50} = 5 ng/mL vs B16 melanoma by Uemura team.[8] Later, Uemera and co-workers collected 600 kg of *H. Okadai*, and, further, identified seven halichondrins.[3]

F1.1



F1.2

Figure 1. Black sea sponge *Halichondria okadai* (**F1.1**), Halichondrin B (**F1.2)**

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

The most exciting feature of halichondrins is their remarkable *in vitro* and *in vivo* antitumour activities. Among these compounds, especially halichondrin B **(F1.2)** and homohalichondrin, are shown to have an extraordinary activity. Previous biological studies have shown that halichondrin B is highly potent against B-16 melanoma cells, P-388 leukemia cells, and L-1210 leukemia cells, and, also result in a dramatically increased survival times in mice.[9] Later, studies have shown that halichondrin B act as a tubulin destabilizing agent with subtle differences in the mechanism of action from other antimitotics like vinca alkaloids.[10] Halichondrin is known to block cells at G_2/M phase of the cell cycle, and, it disrupts normal mitotic spindle architecture through the microtubule destabilization *in vitro*. It has also shown *in vivo* activity against various chemoresistant human solid tumor xenografts, including LOX melanoma, KM20L colon, FEMX melanoma, and, some other cell lines. Due to its impressive biological activity, National Cancer Institute (NCI), USA recommended halichondrin B for preclinical trials in March 1992. The Low abundance of this natural product from marine sources, and, a difficult to supply the large amounts needed for preclinical trials and further clinical investigation would require this sample on the order of 10 g.[11] One potential source of halichondrin B is an aquaculture of appropriated sponge genera, but unfortunately it was not succeeded. Due to these reasons, the total synthesis of halichondrin B has become a reliable source to supply the large quantity needed for further biological studies. However, the molecular architecture of this natural product present significant challenges to the synthetic community, although the total synthesis of halichondrin B[12] and norhalichondrin B[13] have been reported to date.

First, total synthesis of halichondrin and norhalichondrin was achieved by Kishi and co-workers in 1992.[12] Second, total synthesis of norhalichondrin B was reported by Phillips and co-workers in 2009.[13] Some other groups also worked on the total synthesis, and, on the fragments of halichondrin family natural products,[14,15,16,17]

Although the synthesis of halichondrin is reported so far, for any of the synthesis methods to become viable and cost-effective options, significant improvements are required further. This led to developing several alternative approaches leading to obtain the simplified analogues, and, finally, this resulted in eribulin as the clinical candidate.

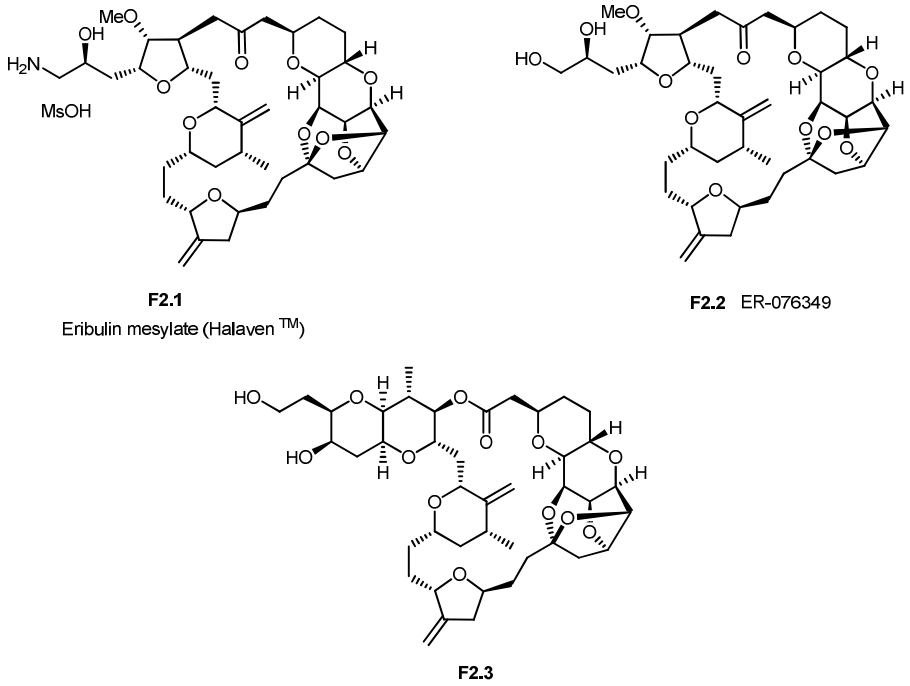


Figure 2. Chemical structure of eribulin (**F2.1)**, ER-076349 (**F2.2)** and diol (**F2.3)**

4.2. Discovery and Development of Eribulin

In 1992, Kishi's group synthesized halichondrin B, and, provided a sample for biological activity along with several intermediates to Eisai Research Institute for *in vitro* and *in vivo* activity evaluation. During this stduy, a macrocyclic macrolactone diol **F2.3** was discovered, as a more potent compound than halichondrin B against DLD-1 human colon cancer cells.[18] Compound **F2.3** and halichondrin B **F1.2** both blocked the cell cycle progression at G_2/M phase, and, further caused the microtubule destabilization. Compound **F2.3** is not active *in vivo* in a LOX human melanoma xenograft model due to the reversibility of action of **F2.3,** as revealed by flow cytometry, which was not the case with the natural product. A number of analogues of **F2.3** that were modified in the terminal C30-C38 region were then subsequently evaluated. All compounds showed similar abilities to inhibit the cell growth but they exhibited different and irreversible complete mitotic block due to the

variation in their structures.[19] In one case, a compound with a significant change in which C29-C36 pyranopyran domain was replaced by a monocyclic pyran and furan derivatives[20] was also tested in the LOX melanoma xenograft model. This derivative did not show any efficacy, and, this was attributed due to the stability of a macrolactone in these tetrahydronpyran and tetrahydrofuran analogues toward nonspecific esterases present in mouse serum. This then led to the preparation of non-hydrolyzable isosteres for ester derivatives, such as, ketone, ether, and amide functionalities. Among these several derivatives, compounds having the ketone moiety were the most promising, and, thus, **F2.2** (ER-076349) and **F2.1** showed prominence in activity (E7389, previously ER-086526).

4.3. Clinical Profile of Eribulin

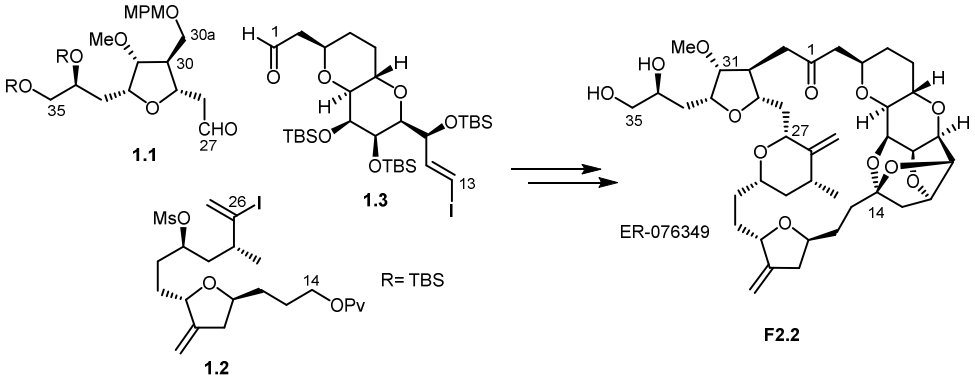
Halaven **F2.1** is a non-taxane, first-in-class microtubule dynamics inhibitor. The FDA approved this compound for use in patients who have previously received at least two prior chemotherapeutic regiments for the metastatic breast cancer. The novel mechanism of action of eribulin, differs from other known classes of tublin-targeted agents, such as, taxane (paclitaxel and docetaxel), vinca alkaloids (vinorelbine and vinblastine), and epothilones (ixabepilone). All of them binds to an interdimer interface or the β-tubulin subunit alone, and, inhibits the microtubular growth phase of microtubular dynamics instability in interphase cells without effect on shortening.[21,22]In addtion to this, moreover, it also promotes the centromere spindle relaxation without affecting the rate of stretching.[23] Several biochemical correlation of apoptosis are observed, such as, cytochrome c release from mitochondria, activation of caspase-3 and 9 cleavage of PARP, including phosphorylation of Bcl-2 in eribulin treated human lymphoma and prostate cancer cells.[24]

The preclinical study of eribulin showed a broad spectrum of anti-tumour activity against a wide variety of human cancer types.[25] Based on the phase I studies results, a number of advanced phase trials were conducted to evaluated the safety and efficacy of the drug and three phase II trials of eribulin in chemotherapy pretreated advanced breast cancer patients showed a manageable tolerability profile with most common drug-related adverse effect. Peripheral neuropathy is a common toxicity associated with tubulin-targeted chemotherapeutic agents, the phase II study compared the

incidence and severity of neuropathy associated with eribulin or ixabepilone in metastatic breast cancer was designed to detect a difference in neuropathy rate of 35% for eribulin versus 63% for ixabepilone. These studies have shown the incidence of neuropathy (any grade) to be 33.3 and 48.0%, and peripheral neuropathy as 31.4 and 44.0% for eribulin and ixabepilone respectively eventhough these results were not significant.[26] The phase 3 trial, 762 women with LABC or MBC were randomly allotted in 2:1 ratio to eribulin 1.4mg/m^2 over 2-5 min on days 1 and 8 of 21-day cycle (n=508) or treatment of physicians choice (TPC) (n=254), result showed a significant increase in OS for eribulin (13.1 months) compared with TPC (10.6 months).[27] Based on the result FDA has approved eribulin mesylate as a third-line treatment for MBC refractory to anthracyclines and taxanes.

4.4. Key Fragments of Eribulin

The Eisai synthesis of ER-076349 and E7389 utilized most of the technology that was adopted from Kishi's approaches. ER-076349 was readily synthesized from three fragments, and, they are shown in **Scheme 1**. A practical gram scale synthesis of eribulin was reported by Yu *et. al*.[28]



Scheme 1. Final assembly of ER-076349 from three key fragments

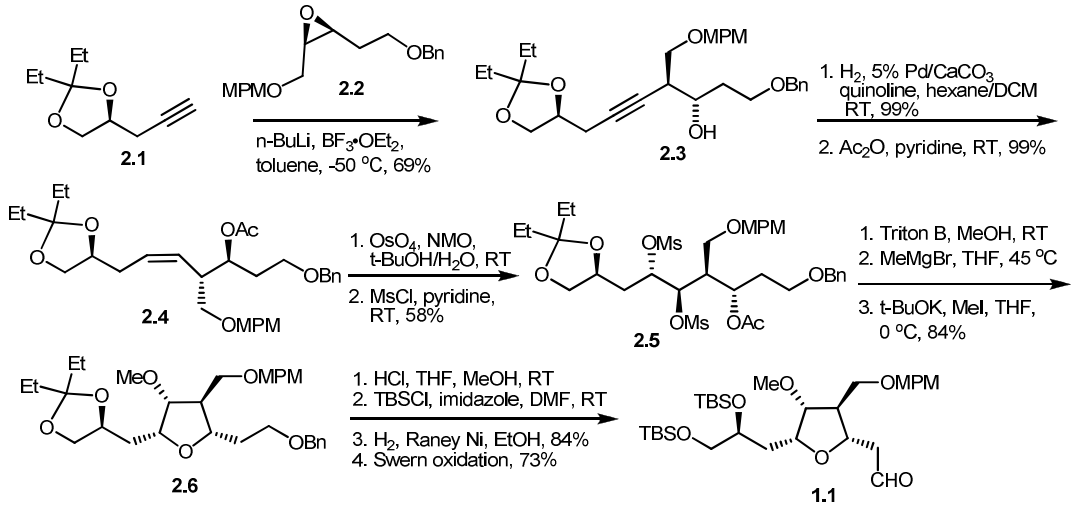
4.5. Literature Synthesis of C27-C35 Fragment of Eribulin 1.1

The first synthesis of the fragment **1.1** proceeded from the stock intermediate which was prepared from L-arabinose in 9 steps. It was converted to C30a modified derivative (structure not shown) in 14 synthetic transformations in 23 steps from the commercially available starting material, with an overall yield of 1.3%.[29] Later, Kishi's team further developed a practical synthesis of fragment **1.1** from a commercially available starting material, D-(+)-Glucurono-6,3-lactone.[30] In the

second generation synthesis of fragment **1.1**, the C30 $PhSO_2CH_2$ group was introduced stereoselectively (>100:1) via hydrogenation using the Crabtree catalyst, and, this synthesis was practically free from any chromatographic separation.[31]

Kishi approach 1

In 2004, Kishi and co-workers developed a new methodology leading to the synthesis of fragment **1.1**, and, it is outlined in **Scheme 2**.



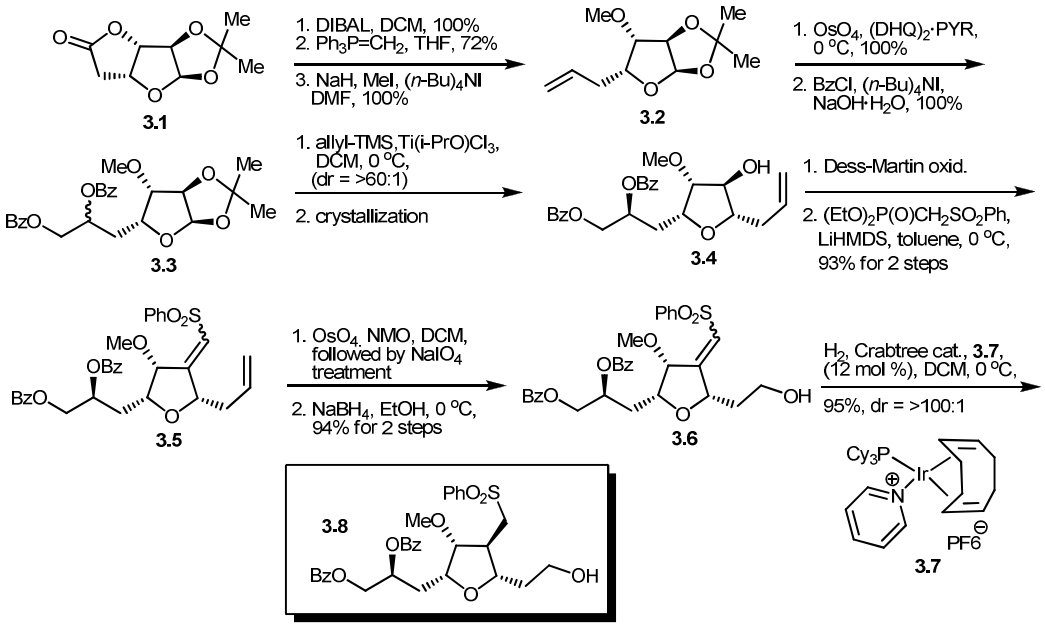
Scheme 2. The synthesis of the C27-C35 tetrahydrofuran fragment

Synthesis of fragment **1.1** was started with a regioselective opening of an epoxide **2.2** which was prepared from the corresponding allylic epoxy alcohol[32] protected with the MPM group, with the lithium anion of **2.1**[33] in the presence of $BF_3{\cdot}OEt_2$. This gave 3:1 mixture of structural isomers, favoring the desired product **2.3**. Hydrogenation using Lindlar's catalyst followed by acetylation furnished the *cis*-olefin **2.4**. Further, dihydroxylation with osmium tetraoxide afforded 8:1 mixture of diastereomers, which was directly converted to the corresponding dimesylates, **2.5.** The desired isomer **2.5** was separated by column chromatography and then subjected to cyclization in the presence of triton B. This was further subjected to desulfonylation with methyl magnesium bromide, followed by methylation of the corresponding alcohol, giving **2.6**. Through adjusting the protecting groups on the C32 side chain, a selective cleavage of the benzyl ether under Raney-Nickel condition and Swern oxidation, finally, afforded fragment **1.1**.[34]

Kishi approach 2

In 2003, Kishi's group synthesized C27-C35 fragment of eribulin from D-(+)-glucurono-6,3-lactone. D-Glucurono-6,3-lactone was converted to 1,2-*O*-isopropy lidene-α-D-5-deoxyglucurono-6,3-lactone **3.1** by a simple modification, and, DIBAL reduction of **3.1**, followed by Wittig reaction and then O-benzylation, provided an olefin derivative. An asymmetric dihydroxylation of terminal olefin is known to proceed with relatively low asymmetric induction. This was achieved with Sharpless approach $(DHQ)_2PYR$, yielding 3:1 mixture of the C-34 diastereomers. The C30 stereocenter was stereospecifically introduced via $NaBH(OAc)_3$ reduction under the influence of the C31 hydroxyl group, followed by protection as the benzyl ether.[30] Although the first-generation synthesis was long, it has attractive features, including a high overall yield and only one chromatographic purification. With the two major modifications, the second generation synthesis is more practical, and, it is outlined in **Scheme 3**.[31] The synthesis began with 1,2-*O*-isopropylidene-α-D-5-deoxyglucurono-6,3-lactone, DIBAL reduction followed by Wittig reaction and *O*-methylation with NaH/MeI conditions to obtain 3.2 with a good yield. Catalytic asymmetric dihydroxylation of **3.2** was best achieved with Sharpless approach $(DHQ)_2PYR$, to obtain a 3:1 mixture of the C34 diastereomers. This was directly subjected to benzoylation **3.3**, and, then *C*-allylation gave α-*C*-allylated product with dr >60:1, which was then separated by crystallization **3.4**.

Oxidation of secondary hydroxyl group with DMP, followed by sulfonyl Wittig-Horner-Emmons reaction with $PhSO_2CH_2P(O)(OEt)_2$/LiHMDS in toluene, furnished the corresponding α,β-unsaturated phenylsulfone as a 30:1 mixture of the Z/E-isomers. The terminal olefin **3.5** was selectively cleaved and followed by reduction, to furnish the primary alcohol **3.6** as a 30:1 Z/E-mixture. Hydrogenation of **3.6** in the presence of Crabtree catalyst **3.7** in DCM at 0 °C smoothly proceeded to furnish the C27-C35 building block **3.8** in 95% yield with >100:1 stereoselectivity.



Scheme 3. Second generation synthesis of the C27-C35 tetrahydrofuran fragment

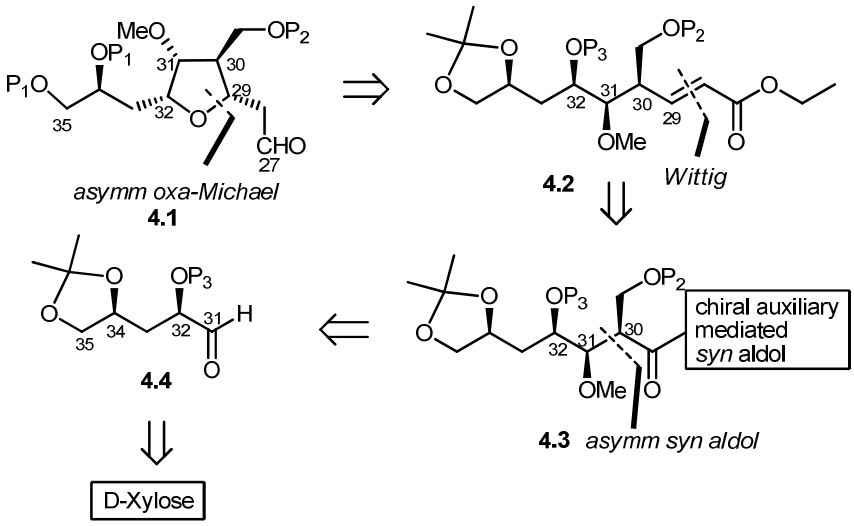
Section B: A Divergent Approach to Eribulin Fragment and Its Derived Hybrid Macrocyclic Toolbox

In this section, our approach to the synthesis of C27-C35 fragment of eribulin, the other diastereomer of eribulin fragment **1.1**, and, the synthesis of a diverse set of 12 and 14-membered eribulin fragment-derived hybrid macrocyclic architectures are covered.

Due to the clinical important of eribulin, our group is interested in the synthesis of eribulin fragments. In particular, we are interested in the eribulin fragment **1.1** because of the biological important of this scaffold in several bioactive natural products. Fragment **1.1** contains a highly functionalized tetrahydrofuran scaffold with the defined chiral functional groups. We aimed at developing a modular and practical synthesis of eribulin fragment **1.1**, along with other diastereomers of eribulin fragment **1.1.** As an extension to this, further, we planned the synthesis of a diverse set of hybrid macrocyclic toolbox using these chiral scaffolds. This is aimed at exploring the additional macrocyclic chemical space around the chiral furan scaffold.

4.6. Retrosynthesis of C27-C35 Fragment of Eribulin

We developed a modular approach to the synthesis of C27-C35 fragment of eribulin and its C29 and C32 diastereomer of eribulin fragment via Evans asymmetric *syn* aldol and asymmertic oxa-Michael reactions as the key steps.

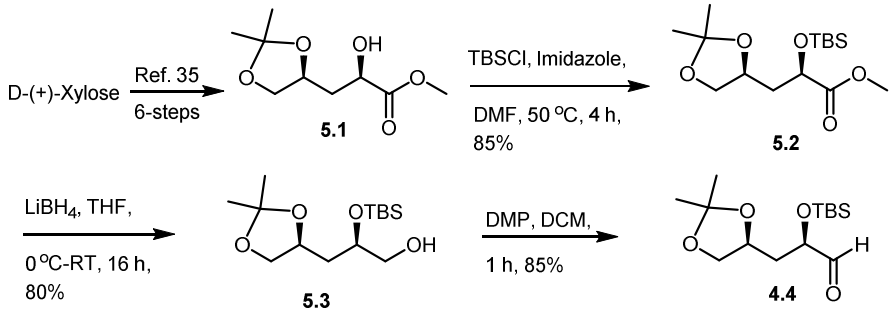


Scheme 4. Retrosynthesis of C27-C35 fragment of eribulin

The retrosynthetic analysis of our approach is shown in **Scheme 4**. We planned to obtain a highly functionalized tetrahydrofuran ring **4.1** by an asymmetric oxa-Michael reaction of **4.2**, as the key step, which can be synthesized from **4.3** by simple functional group transformations. We further planned to obtain C30 and C31 chiral centres through Evans asymmetric *syn* aldol reaction using a chiral aldehyde **4.4**. This *enantioenriched* aldehyde **4.4** could be synthesized from commercially available, inexpensive chiral source, D-xylose.

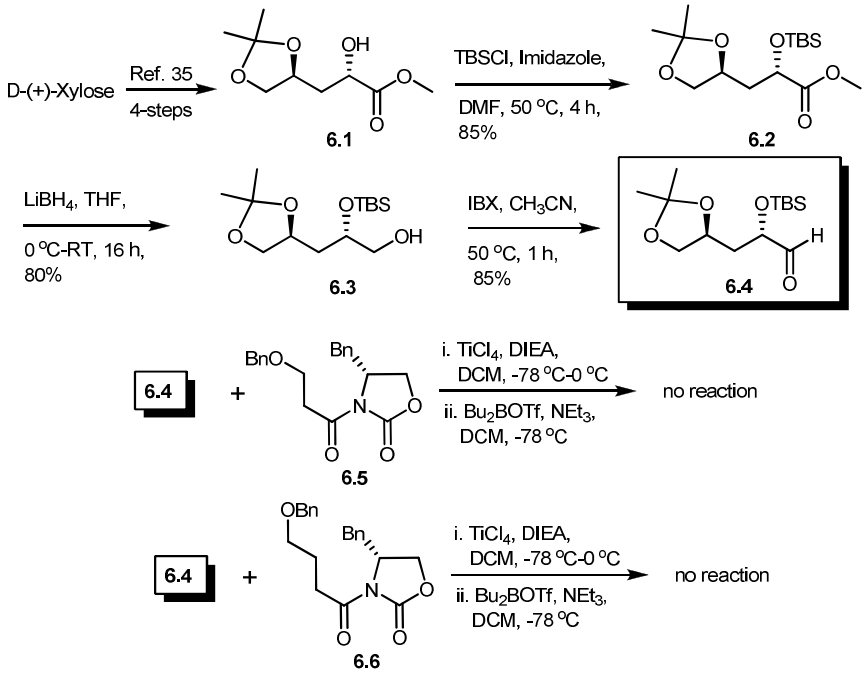
4.7. Synthesis of C27-C35 Fragment of Eribulin

The stereoselective synthesis of aldehyde **4.4** was started with a suitable chiral starting material, D-xylose. Initially, we synthesized (R)-α-hydroxyester **5.1** from D-xylose, in six steps, with an overall yield 27.5% as reported by Masami Okabe *et. al*.[35] α-Hydroxyester **5.1** was protected as its silyl ether **5.2** under TBSCl/imidazole, DMF solvent at 50 ^{o}C conditions with 85% yield. Reduction of ester **5.2** with lithium borohydride gave **5.3** in 80% yield and the additional 10% secondary alcohol product, in which, the TBS group had migrated to primary alcohol. Oxidation of alcohol **5.3** was achieved with DMP to obtain an aldehyde **4.4** in 85% yield after column chromatography.



Scheme 5. Synthesis of *enantioenriched* aldehyde **4.4**

To validated our strategy, we planned Evans asymmetric aldol reaction with (S)-(α)-silyl ether aldehyde **6.4** which was easily obtained from D-xylose in 7-steps. Similar to the synthesis of **4.6,** we started with an *enantioenriched* ester **6.1**, which was obtained in 4-steps with an overall yield 33.6%, from a natural chiral source, D-xylose.[35] The -OTBS protection of the hydroxyl group with TBSCl and imidazole gave TBS protected compound **6.2**. It was then reduced with lithium borohydride to yield the primary alcohol **6.3**, which was then subjected to oxidation with IBX condition to provide a chiral aldehyde **6.4** with 58% overall yield.

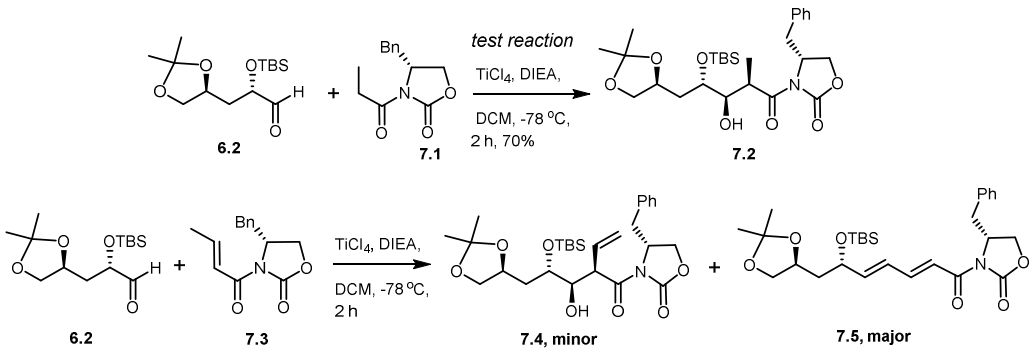


Scheme 6. Synthesis of *enantioenriched* aldehyde **6.4** and aldol reactions

Once chiral aldehyde **6.4** is in our hand in good quantities, we tried Evans asymmetric aldol reaction with (R)-4-benzyl-3-(3-(benzyloxy)propanoyl)oxazolidin-2-one auxiliary **6.5** to obtain an aldol product. To achieve this, we followed the

literature conditions, such as $TiCl_4$/DIEA,[36] -78 ^oC-0 ^oC, Bu_2BOTf/NEt_3,[37,38] -78 ^oC-0 ^oC but all of them did not work in our hands. Following this, we then synthesized one carbon extension-based chiral auxiliary, (R)-4-benzyl-3-(4-(benzyloxy)butanoyl) oxazolidin-2-one **6.6**, and, tried different conditions as shown in **Scheme 6**. In this case also, we did not succeed in obtaining the aldol product.

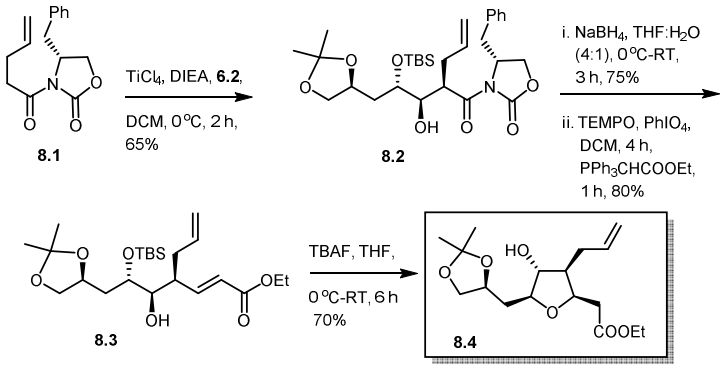
As a test study to validate the Evans approach in our hands, we took simple (R)-4-benzyl-3-propionyloxazolidin-2-one auxiliary **7.1** and achieved the aldol product **7.2** with a good yield without any difficulty.[36] With this encouraging result, we attempted Evans asymmetric aldol reaction with the crotolyl auxiliary (R,E)-4-benzyl-3-but-2-enoyloxazolidin-2-one **7.3.**[39] In this system, we obtained the desired product as a minor **7.4** with 10% yield, along with major product, which was γ-carbon of auxiliary attacked on aldehyde carbon followed by dehydration to obtain **7.5** with 45% yield as shown in **Scheme 7**. Further, functional group transformations of **7.4,** led to accessing the aldol product **4.3**.



Scheme 7. Evans *syn* aldol reaction

To avoid the enolization of auxiliary, we chose pentenoic acid derived auxiliary **8.1** (R)-4-benzyl-3-pent-4-enoyloxazolidin-2-one,[40] and, titanium-mediated aldol reaction conditions as our next attempt. This approach exclusively gave **8.2** as a single diastereomer with 65% yield with dr > 20:1. Due to the α-OTBS (dipolar interactions) and minimum *syn* pentane interaction favored the Zimmerman-Traxler **TS-1** desired aldol product as shown in **Figure 3**. The other Zimmerman-Traxler **TS-2** is disfavored due to the steric hindrance of auxiliary with the approach of an aldehyde.[41] Aldol product was subjected to *O*-methylation under MeI/Ag_2O[42] and MeOTf/DIEA[43] conditions, and, this approach did not provide any product due to a steric hinderane of the starting material **8.2**. So, next, we planned a reductive removal

of an auxiliary under sodium borohydride[44] to obtain an alcohol moiety. A selective oxidation of primary alcohol with TEMPO/PhI(OAc)$_2$[45] in DCM, followed by Wittig reaction gave **8.3** in 78% overall yield. The -OTBS deprotection of **8.3,** followed by an asymmetric oxa-Micheal reaction[46] with 1M TBAF solution provided the *cis* diastereomer as a single product **8.4** with 70% yield. The relative stereochemistry of this product **8.4** was thoroughly assigned by NMR, 2D-COSY and 2D-NOESY experiments. The proposed transition state (**TS-3**) that favors the *cis* diastereomer is shown in **Figure 4**. In this case, there appears to be no steric interaction of 1,3 substitutions, and, this leads to obtain the favored *cis* diastereomer **8.4**. 2D NOESY experiments also showed nOe between H_4 (3.74 ppm) - H_1 (4.15 ppm) protons H_4 (3.74 ppm) - H_2 (2.0 ppm) protons and between H_3 (3.63 ppm) -H_5 (1.7 ppm), and, they are shown in **Figure 7**.



Scheme 8. Synthesis of C29 and C32 diastereomer of eribulin fragment **1.1**

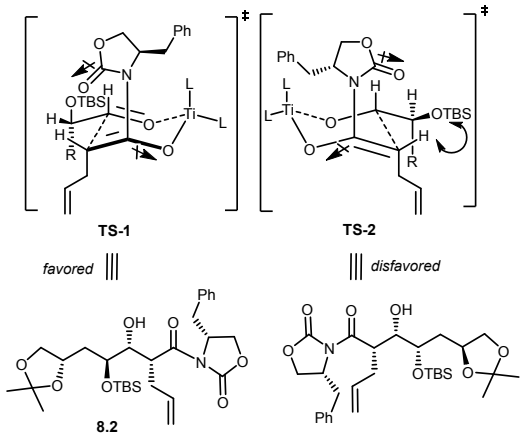


Figure 3. Proposed Zimmerman-Traxler transition states of *syn* aldol reaction

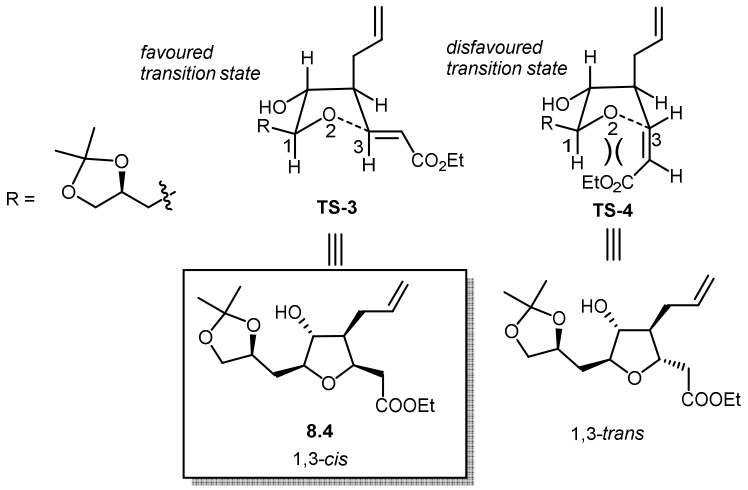
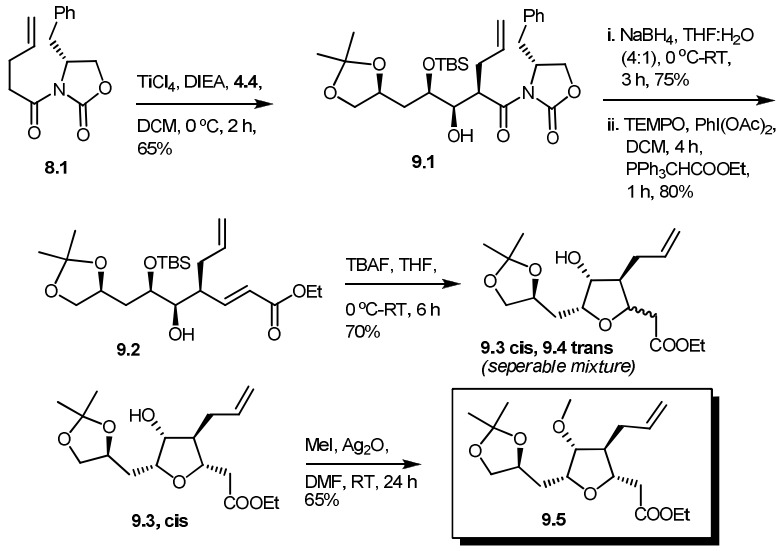


Figure 4. Proposed transition state for compound **8.4**

Now, we have taken our desired aldehyde and performed Evans' asymmetric titanium mediated *syn* aldol reaction with (R)-4-benzyl-3-pent-4-enoyloxazolidin-2-one **8.1**. Under $TiCl_4$/DIEA conditions, we obtained the desired product as a major diastereomer (dr >20:1) with 65% moderate yield **9.1** and 15% other diastereomers. Due to the disfavored α-OTBS group dipolar interactions and *syn* pentane interactions in the Zimmerman-Traxler **TS-5**, the desired aldol product is shown in **Figure 5**. The other Zimmerman-Traxler **TS-6** is disfavoured because of steric hindrance of an auxiliary to approach an aldehyde. Due to the disfavoring of α-OTBS group, low selectivity was observed compared to the first case with **8.2**. Similar to **8.3**, in this case also**,** the reductive removal of chiral auxiliary with sodium borohydride conditions and one pot selective oxidation of the primary alcohol with TEMPO/$PhI(OAc)_2$ followed by Wittig reaction gave **9.2** with 77.5% yield for an overall 2 steps. The -OTBS removal of **9.2** and an asymmetric oxa-Micheal reaction was attempted in one pot reaction with 1M TBAF solution and this yielded 1:1 separable mixture of *cis* **9.3** and *trans* **9.4** diastereomers. We assigned both products using 1D and 2D experiments. Due to the steric hindrance of 1,3 substitutions in oxa-Micheal reaction, it favored both products. The proposed transition states for *cis* and *trans* isomers are shown in **Figure 6**. 2D NOESY experiments showed nOe between H_5 (1.90 ppm) - H_9 (2.6 ppm) protons, H_3 (3.75 ppm) - H_7 (2.2 ppm) protons, and, we also observed nOe between H_3 (3.75 ppm) - H_8 (2.3 ppm) as shown in **Figure 7.** Following this, we selected our desired *cis* diastereomer **9.3**, and, further worked with this compound for the next steps. The protection of hydroxyl group with MeI/Ag_2O in DMF conditions, gave *O*-methyl product with 65% yield. By

performing simple transformations with **9.3**, we planned to obtain our desired target **1.1**.



Scheme 9. Synthesis of C27-C35 fragment of eribulin **9.5**

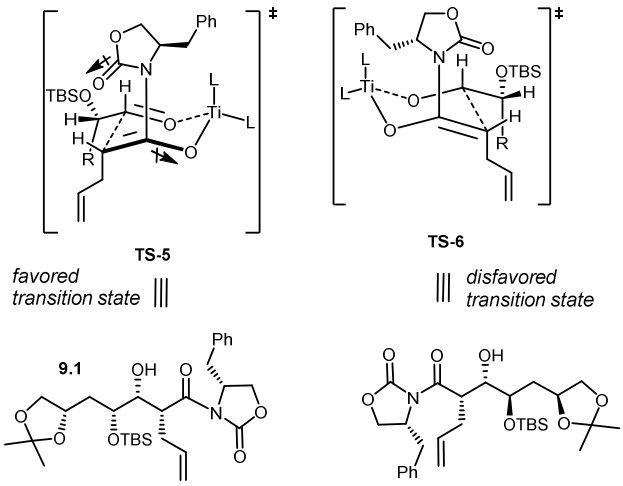


Figure 5. Proposed Zimmerman-Traxler transition states of *syn* aldol reaction

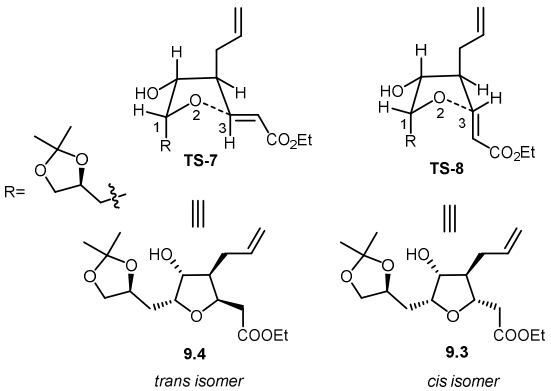


Figure 6. Proposed transition states for compounds **9.4** and **9.3**

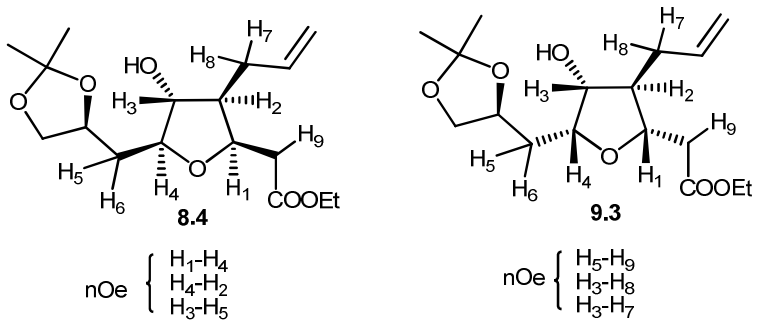


Figure 7. Stereochemical assignment of **8.4** and **9.3**

4.8. Synthesis of 14 and 12-Membered Eribulin Fragment Derived Macrocyclic Architectures

As an extension of my research project, the next milestone was to build a diverse set of macrocyclic chemical toolbox to search for small molecule modular of protein-protein interactions. In this project, we were interested in exploring further the additional 14-membered macrocyclic chemical space.[47] For this study, a highly functionalized enantioenriched tetrahydrofuran **9.5** was taken as the starting material.

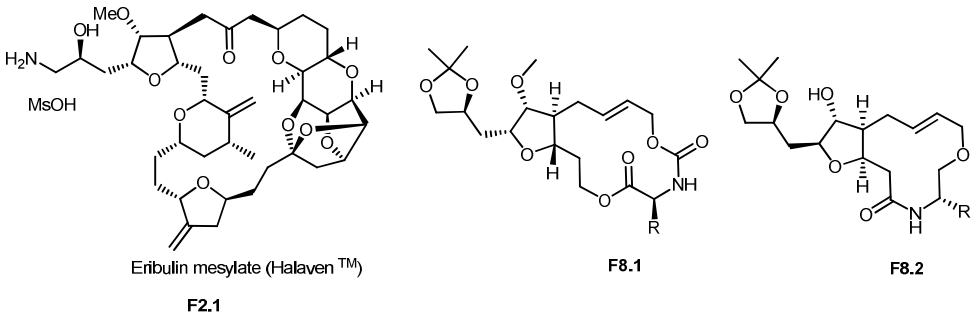


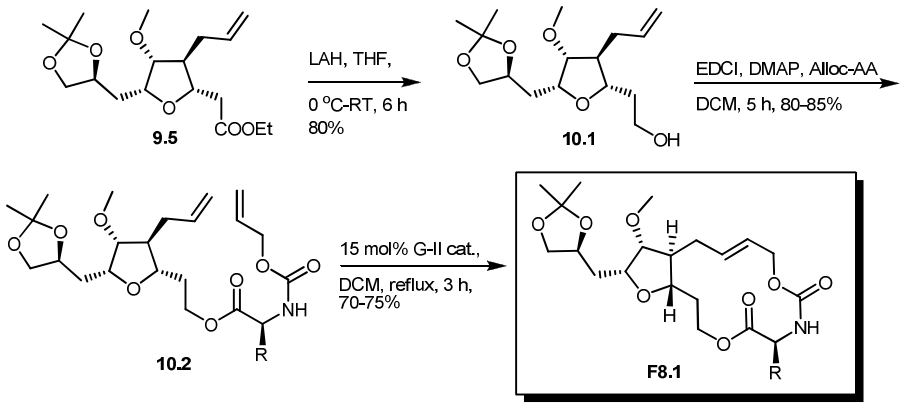
Figure 8. 14 and 12-membered eribulin fragment derived macrocycles

The attractive feature of macrocycle **F8.1** was the incorporation of an amino acid moiety to allow us introducing the diversity step to access various polar and non-polar natural and unnatural amino acids into the macrocyclic ring.

4.8.1. Synthesis of 14-Membered Eribulin Fragment Derived Macrocycle (F8.1)

Synthesis of 14-membered macrocyclic architecture **F8.1** is shown in **Scheme 10**. We started our synthesis with **9.5** as the starting material. Reduction of the ester with lithium aluminum hydride gave **10.1** with 80% yield. Coupling of different *N*-Alloc protected amino acids with **10.1**, in the presence of EDCI and DMAP reagent gave *bis*-allylated product **10.2** with 80-85% yield. To obtain the 14 membered

macrocycle, *bis*-allylated compound was then subjected to ring closing metathesis using 15 mol% Grubbs II catalyst in DCM solvent under reflux conditions. We were pleased to observe the successful synthesis of 14-membered macrocycle **F8.1** in 70-75% yield. The coupling constants of the olefinic protons in all the three cases, showed >14 Hz in ^{1}H NMR, proving a *trans* geometry across the double bond.



Scheme 10. Synthesis of 14-membered eribulin fragment derived macrocycle **F8.1**

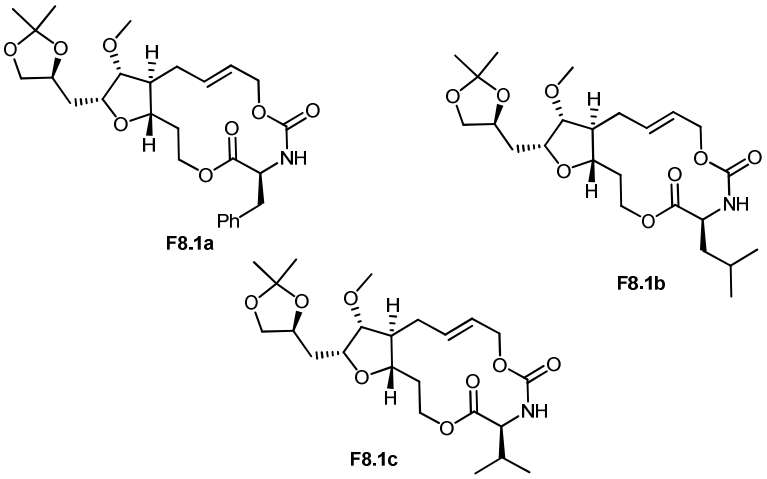
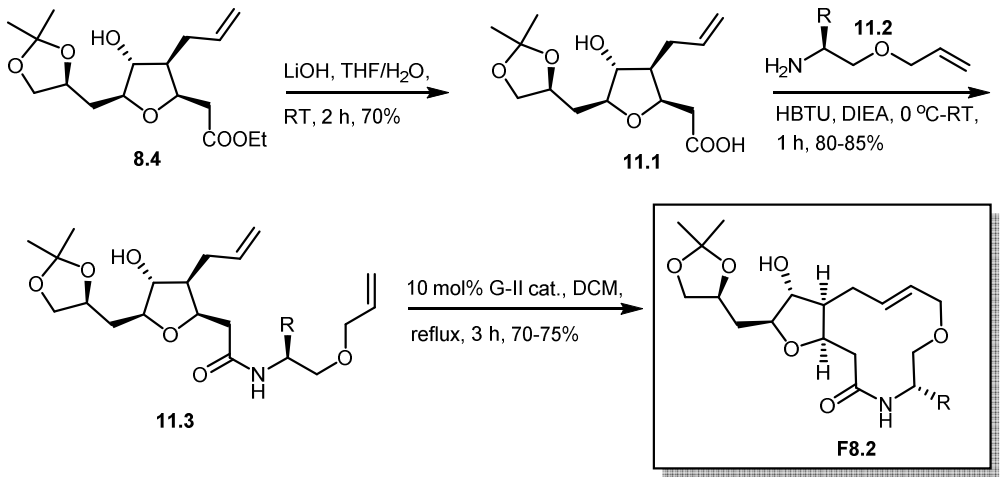


Figure 8. Derivatives of macrocycle **F8.1**

In our next approach, we were interested in accessing a different type of macrocyclic toolbox having the additional12-membered ring macrocycles, **F8.2**. For this study, we selected the other C29 and C32 diastereomers of eribulin fragment **8.4**. In this macrocycle **F8.2**, different modified amino acid building blocks were incorporated as the source of a chiral side chain as the diversity site.[48]

4.8.2. Synthesis of 12-Membered Eribulin Fragment Derived Macrocycle (F8.2)

For macrocycle **F8.2**, we hydrolyzed the carboxylester and coupled with modified amino acid derivative **11.2** having O-allyl functional group and free amine. Synthesis of macrocycle **F8.2** was started from the hydrolysis of ester with lithium hydroxide monohydrate condition, which gave the free carboxylic acid **11.1**. This was further utilized without purification and was coupled with different modified amino acid building blocks **11.2**, under HBTU/DIEA conditions to obtain the products **11.3** with 80-85% yield. Finally, we achieved, the synthesis of a 12-membered macrocycle ring using 10 mol% Grubbs II catalyst in DCM solvent under reflux conditions, as shown in **Scheme 11**. In this case also, we observed the *trans*-geometry of the double bond in all the three macrocycles (i.e. ^{1}H-NMR experiment showed >14 Hz coupling constant).



Scheme 11. Synthesis of 12-Membered Eribulin Fragment Derived Macrocycle **F8.2**

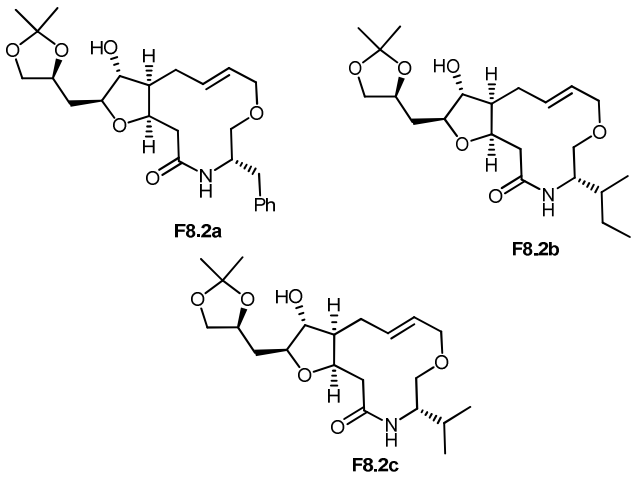
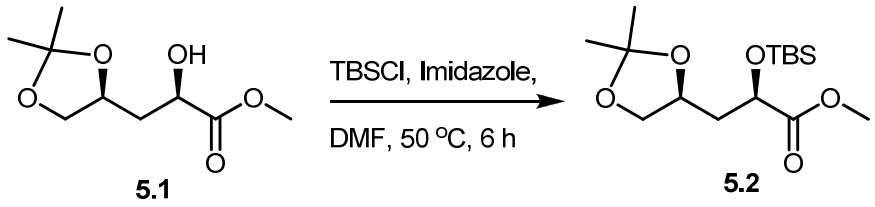


Figure 9. Derivatives of macrocycle **F8.2**

4.9. Conclusion

In conclusion, we developed a novel and efficient methodology to synthesize C27-C35 fragment of eribulin as a key fragment and C29 and C32 diastereomers of eribulin fragment via Evans *syn* aldol and oxa-Michael reaction. These fragments were further utilized in the successful synthesis of 14- and 12-membered macrocyclic architectures. The small molecule, macrocyclic chemical toolbox is under biological evaluation with our several biological collaborators in search for the modulators of protein-protein interactions and signaling pathways related to a variety of cancer and neuronal stem cells, and, these studies will be made available when complete.

4.10. Experimental Procedure

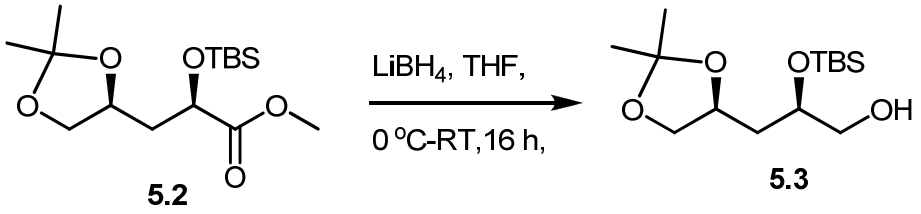


5.1 5.2

(R)-methyl2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl) propanoate (5.2):

To the stirred solution of secondary alcohol **5.1**(4 g, 19.6 mmol) in DMF solvent were added Imidazole (39.2 mmol, 2 eq) and TBSCl (29.4 mmol, 1.5 eq) at 0 ^{o}C. The reaction mixture was allowed to warm to room temperature then heated to 50 ^{o}C for 6 h. After the completion of the reaction as indicated by TLC, the reaction solution was diluted with dichloromethane and was washed with water and brine (2 times). The organic layer was evaporated to give the crude product which was subjected to column purification to give pure product **5.2** (5.3g, 85%) as a colorless liquid.

Molecular Formula: $C_{15}H_{30}O_5Si$; R_f: 0.2 (10% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.10 (s, 3H), 0.92 (s, 9H), 1.35 (s, 3H), 1.42 (s, 3H), 1.80-1.87 (m, 1H), 1.93-2.01 (m, 1H), 3.54 (t, *J* = 7.6 Hz, 1H), 3.73 (s, 3H), 4.06 (dd, *J* = 7.8, 5.9 Hz, 1H), 4.23-4.27 (m, 1H), 4.43 (dd, *J* = 10.2, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -5.5, -5.1, 18.2, 25.6, 25.7, 26.9, 38.8, 51.8, 69.4, 69.4, 71.9, 108.7, 174.1; MS: (ES+) m/z = 319 (M+1).

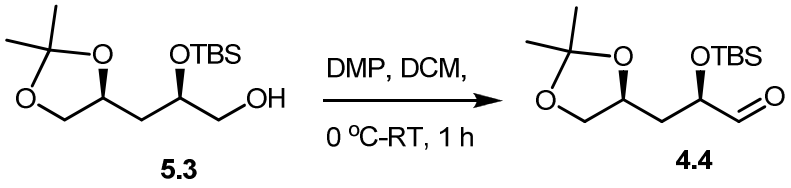


5.2 **5.3**

(R)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-1-ol (5.3):

To a stirred solution of compound **5.2 (**3 g, 9.4 mmol) in dry THF (10 mL) was added $LiBH_4$ (246 mg, 11.3 mmol) at 0 °C under inert atmosphere. The reaction was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was quenched by addition of a saturated *aq*.NH_4Cl, extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (30% ethyl acetate in hexane) afforded compound **5.3** (2.18g, 80%) as a semisolid.

Molecular Formula: $C_{14}H_{30}O_4Si$; R_f : 0.2 (30% ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.10 (s, 3H), 0.88 (s, 9H), 1.32 (s, 3H), 1.37 (s, 3H), 1.64 (m, 1H), 1.72-1.80 (m, 1H), 2.20 (bs, 1H), 3.47 (dt, *J* = 7.8, 1.7 Hz, 1H), 3.54-3.61 (m, 2H), 3.88-3.92 (m, 1H), 4.03 (ddd, *J* = 7.8, 5.9, 1.8 Hz, 1H), 4.14-4.17 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.81, -4.57, 17.9, 25.7, 26.9, 38.2, 67.0, 69.7, 70.2, 72.6, 108.8; MS: (ES+) m/z = 289 (M-1).



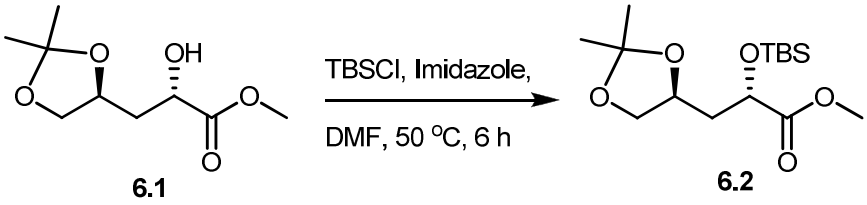
5.3 **4.4**

(R)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl) pro panal (4.4):

To the stirred solution of alcohol **5.3** (1.5g, 5.17 mmol, 1 eq) in DCM was added DMP (2.41g, 5.68 mmol, 1.1 eq) at room temperature and stirred for 1 h. After completion of reaction filtered the crude through celite and wash with $NaHCO_3$ (twice) and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

Purification by flash chromatography (10% ethyl acetate in hexane) afforded compound **4.4** (1.26g, 85%) as a colorless liquid.

Molecular Formula: $C_{14}H_{28}O_4Si$; R_f (10% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.10 (s, 3H), 0.88 (s, 9H), 1.32 (s, 3H), 1.38 (s, 3H), 1.68 (ddd, *J* = 13.7, 10.2, 3.6 Hz, 1H), 1.88 (ddd, *J* = 13.7, 10.2, 3.5 Hz, 1H), 3.55 (t, *J* = 7.7 Hz, 1H), 4.05 (dd, *J* = 7.8, 5.9 Hz, 1H), 4.21-4.25 (m, 1H), 9.62 (s, 1H);

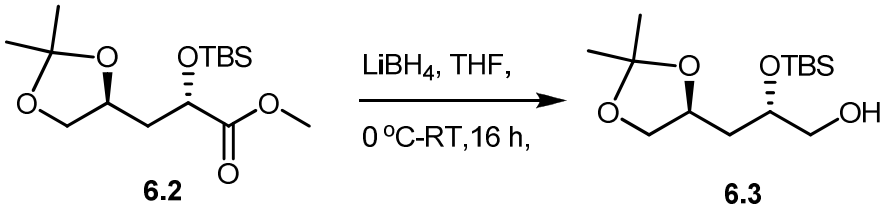


6.1 6.2

(S)-methyl 2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)propanoate (6.2):

Same experiment as synthesis of compound **5.2.**

Molecular Formula: $C_{15}H_{30}O_5Si$; R_f: 0.2 (10% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 1.34 (s, 3H), 1.42 (s, 3H), 1.80-1.86 (m, 1H), 1.93-2.00 (m, 1H), 3.55 (t, *J* = 7.6 Hz, 1H), 3.72 (s, 3H), 4.05 (dd, *J* = 7.8, 5.9 Hz, 1H), 4.23-4.26 (m, 1H), 4.42 (dd, *J* = 10.2, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -5.5, -5.1, 18.2, 25.6, 25.7, 26.9, 38.8, 51.8, 69.4, 69.4, 71.9, 108.7, 174.1; MS: (ES+) m/z = 319 (M+1).

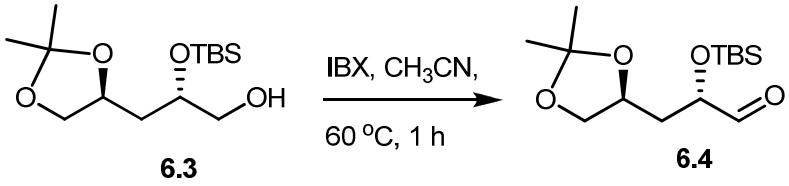


6.2 6.3

(S)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-1-ol (6.3):

Same experiment as synthesis of compound **5.3.**

Molecular Formula: $C_{14}H_{30}O_4Si$; R_f (30% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.10 (s, 3H), 0.89 (s, 9H), 1.33 (s, 3H), 1.36 (s, 3H), 1.65 (m, 1H), 1.73-1.81 (m, 1H), 2.21 (bs, 1H), 3.48 (dt, *J* = 7.8, 1.7 Hz, 1H), 3.54-3.62 (m, 2H), 3.88-3.91 (m, 1H), 4.02 (ddd, *J* = 7.8, 5.9, 1.8 Hz, 1H), 4.15-4.16 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.81, -4.57, 17.9, 25.7, 26.9, 38.2, 67.0, 69.7, 70.2, 72.6, 108.8; MS: (ES+) m/z = 289 (M-1).

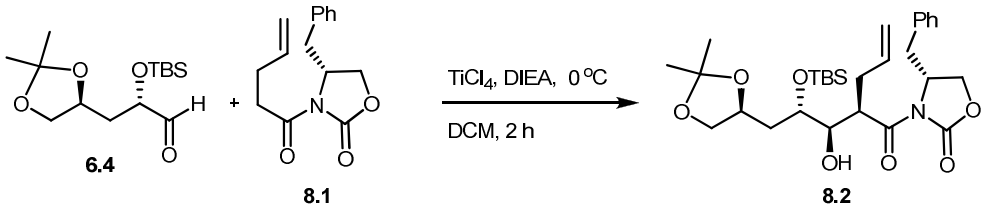


6.3 **6.4**

(S)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)propanal (6.4):

To the stirred solution of alcohol **6.3** (2g, 6.89 mmol, 1 eq) in CH_3CN was added IBX (2.12g, 7.58 mmol, 1.1 eq) at room temperature and stirred for 1 h. After completi on of reaction filtered the crude through celite and wash with $NaHCO_3$ (twice) and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography (10% ethyl acetate in hexane) afforded compound **6.4** (1.68 g, 85%) as a colorless liquid.

Molecular Formula: $C_{14}H_{28}O_4Si$; R_f (20% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H), 0.10 (s, 3H), 0.89(s, 9H), 1.31 (s, 3H), 1.37 (s, 3H), 1.68 (ddd, *J* = 13.7, 10.2, 3.6 Hz, 1H), 1.87 (ddd, *J* = 13.7, 10.2, 3.5 Hz, 1H), 3.54 (t, *J* = 7.7 Hz, 1H), 4.06 (dd, *J* = 7.8, 5.9 Hz, 1H), 4.22-4.26 (m, 1H), 9.61(s, 1H);

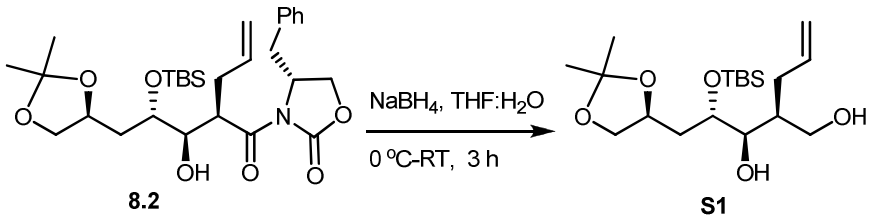


6.4 **8.1** **8.2**

(R)-4-benzyl-3-((R)-2-((1R,2S)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxypropyl)pent-4-enoyl)oxazolidin-2-one (8.2):

(R)-4-benzyl-3-pent-4-enoyloxazolidin-2-one **8.1** (1.51g, 5.83 mmol, 1 eq.) was dissolved in anhydrous DCM (20 mL) under nitrogen atmosphere and the solution was cooled to 0 °C. $TiCl_4$ (0.64 ml, 1.02 eq., 5.94 mmol) was added and the yellow solution was stirred 5 min. DIPEA (2.5 mL, 2.5 eq., 14.5 mmol) was added dropwise and the deep purple solution was stirred for 45 min at 0 °C. Freshly prepared aldehyde **6.4** (1.68g, 5.83 mmol, 1 eq.) in 4 mL of DCM was added dropwise and the solution was stirred for 2 h at 0 °C. The reaction mixture was quenched with NH_4Cl and stirred for overnight. Then reaction mixture was filtered through celite and the aqueous layer was extracted with DCM (3 times). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (30% EtOAc in Hexane) to gave **8.2** (2g, 65%) as a slightly yellow oil.

Molecular Formula: $C_{29}H_{45}NO_7Si$; R_f (30% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.14 (s, 3H), 0.18 (s, 3H), 0.93 (s, 9H), 1.33 (s, 6H), 1.56 (ddd, *J* = 14.1, 9.3, 2.3 Hz, 1H), 1.79 (ddd, *J* = 13.9, 9.3, 2.3 Hz, 1H), 2.57 (td, *J* = 13.9, 9.7 Hz, 2H), 2.78-2.82 (m, 1H), 3.34 (dd, *J* = 13.2, 3.2 Hz, 1H), 3.49 (t, *J* = 8.0 Hz, 1H), 3.90 (td, *J* = 9.7, 2.6 Hz, 1H), 3.97-4.05 (m, 2H), 4.09-4.22 (m, 4H), 4.66-4.72 (m, 1H), 5.03-5.18 (m, 1H), 5.85-5.95 (m, 1H), 7.24-7.30 (m, 3H), 7.33-7.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.7, -4.5, 17.9, 25.8, 25.8, 25.9, 26.8, 34.4, 37.9, 43.5, 55.6, 65.7, 69.8, 70.3, 72.3, 74.9, 108.5, 117.6, 127.3, 128.9, 129.4, 134.6, 135.4, 152.7, 174.0; MS: (ES+) m/z = 548 (M+1).

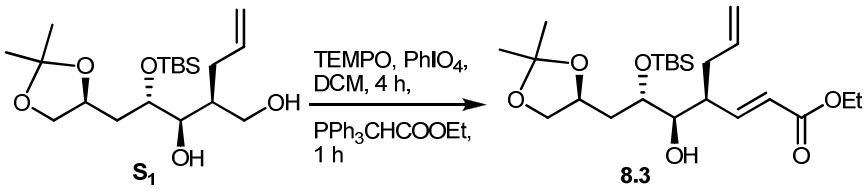


(2S,3R,4S)-2-allyl-4-(tert-butyldimethylsilyloxy)-5-((S)-2,2-dimethyl-1,3-dioxo lan-4-yl)pentane-1,3-diol (S_1):

To the stirred solution of **8.2** (1.5g, 2.74 mmol) in 4:1 THF and water was added sodium borohydride (2 eq) at 0 ^{o}C. The reaction mixture was stirred at room temperature for 3 h. After completion of starting material reaction was quenched with dil.HCl and extracted with ethyl acetate. The organic layer was dried over

Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (50% EtOAc in Hexane) to give **S_1** (0.77g, 75%) as a slightly yellow oil.

Molecular Formula: $C_{19}H_{38}O_5Si$; R_f (50% ethyl acetate/hexane): 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.11 (s, 6H), 0.87-0.93 (m, 9H), 1.36 (s, 3H), 1.41 (s, 3H), 1.63-1.72 (m, 1H), 1.72-1.78 (m, 1H), 1.80-1.85 (m, 1H), 2.06 (bs, 1H), 2.20-2.28 (m, 1H), 2.41-2.47 (m, 1H), 2.91 (bs, 1H), 3.54 (t, *J* = 8.0 Hz, 1H), 3.69-3.72 (m, 2H), 4.01-4.09 (m, 2H), 4.21-4.23 (m, 1H), 5.05-5.15 (m, 2H), 5.77-5.94 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.5, -4.4, 17.9, 25.7, 25.8, 26.9, 31.0, 41.1, 62.6, 69.8, 70.8, 72.7, 76.1, 108.8, 116.6, 136.9; LRMS: (ES+) m/z = 373 (M-1).

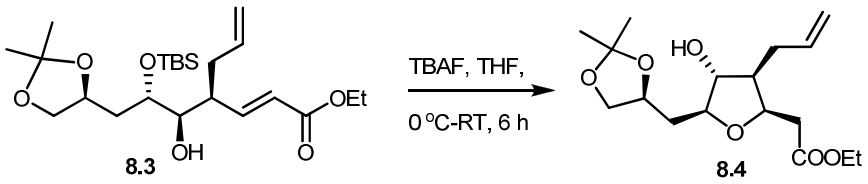


(S,E)-ethyl 4-((1R,2S)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxypropyl)hepta-2,6-dienoate (8.3):

To the stirred solution of starting material **S_1** (750 mg, 2.0 mmol) in DCM were added $PhI(OAc)_2$ (774.5mg, 2.4 mmol) and TEMPO reagent (31.2 mg, 0.2 mmol) at room temperature under nitrogen atmosphere. The round bottom flask was covered with aluminum foli to avoid light. The reaction mixture was stirred for 4 h, then added Wittig reagent (765 mg, 1.1 eq) and again stirred for 1 h. After completion of reaction, quenched with saturated $NaHCO_3$ solution and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (20% ethyl acetate in hexane) to gave the product **8.3** (710 mg, 80%)

Molecular Formula: $C_{26}H_{37}BrN_2O_6Si$; R_f (20% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.09 (S, 6H), 0.89 (s, 9H), 1.25-1.28 (t, *J* = 6.0, 3H), 1.29 (s, 3H), 1.32 (s, 3H), 1.50 (ddd, *J* = 13.8, 9.4, 2.5 Hz, 1H), 1.66-1.73 (m, 2H), 2.10-2.19 (m, 1H), 2.26-2.31 (dq, *J* = 9.6, 3.2 Hz, 1H), 2.56 (bs, 1H), 2.60-2.69 (m, 1H), 3.47-3.54 (m, 1H), 3.49-3.52 (t, *J* = 7.2 Hz, 1H), 3.55-3.58 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.82 (td, *J* = 10.4, 2.6 Hz, 1H), 4.05 (dd, *J* = 7.8, 6.0 Hz, 1H), 4.20 (q, *J* = 6.0 Hz,

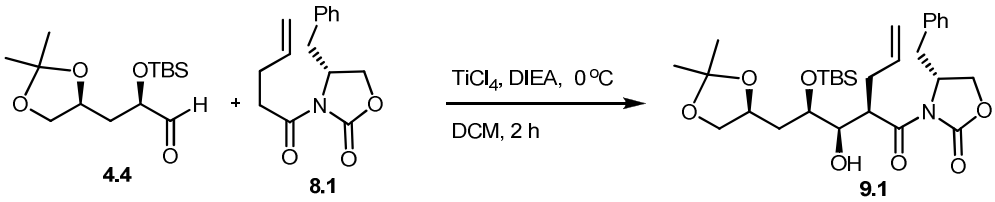
2H), 4.98-5.08 (m, 2H), 5.63-5.67 (m, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 6.65 (dd, *J* = 15.6, 10.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.7, -4.5, 114.2, 17.9, 25.6, 25.8, 26.8, 33.6, 44.9, 60.2, 69.8, 70.8, 72.3, 108.7, 117.1, 123.5, 135.1, 146.5, 166.0; LRMS: (ES+) m/z = 465 (M+23).



ethyl 2-((2R,3R,4R,5S)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4 hydroxytetrahydrofuran-2-yl)acetate (8.4):

To the stirred solution of wittig product **8.3** (700 mg, 1.58 mmol) in dry THF was added 1M TBAF solution (4.75 mmol, 4.75ml) at 0 ^{o}C under nitrogen atmosphere. The reaction mixture was stirred for 6 h at room temperature. After completion of reaction, quenched with saturated $NaHCO_3$ solution and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (20% ethyl acetate in hexane) to gave the product **8.4** (363 mg, 70%);

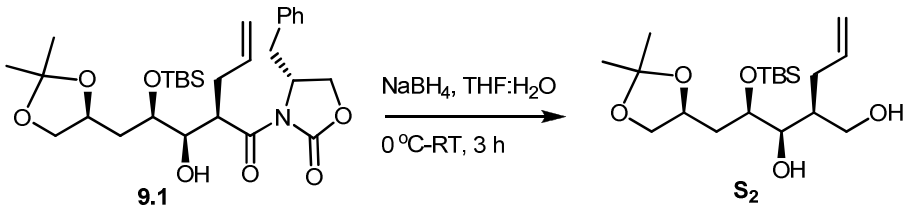
Molecular Formula: $C_{17}H_{28}O_6$; R_f (20% ethyl acetate/hexane): 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25 (t, *J* = 6.8, 3H), 1.34 (s, 3H), 1.45 (s, 3H), 1.65-1.80 (m, 1H), 1.93-2.08 (m, 2H), 2.26 (td, *J* = 14.9, 7.6 Hz, 1H), 2.33-2.44 (m, 1H), 2.56-2.58 (t, *J* = 2.4, 1H), 3.54-3.58 (m, 1H), 3.61-3.63 (m, 1H), 3.69-3.80 (m, 1H), 4.08-4.17 (m, 4H), 4.18-4.24 (m, 1H), 5.02-5.18 (m, 1H), 5.84-5.88 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2, 25.8, 26.8, 35.5, 37.8, 40.6, 50.7, 60.5, 69.7, 73.9, 77.9, 80.6, 81.4, 109.4, 117.0, 135.7, 171.1; LRMS: (ES+) m/z = 327 (M-1).



(R)-4-benzyl-3-((R)-2-((1R,2R)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimeth yl-1,3-dioxolan-4-yl)-1-hydroxypropyl)pent-4-enoyl)oxazolidin-2-one (9.1):

Same experiment as synthesis of compound **8.2**:

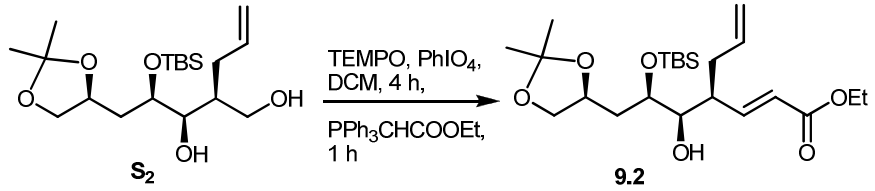
Molecular Formula: $C_{29}H_{45}NO_7Si$; R_f (30% ethyl acetate/hexane): 0.2; yield: 65% major product; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.13 (s, 3H), 0.19 (s, 3H), 0.94 (s, 9H), 1.34 (s, 6H), 1.57 (ddd, *J* = 14.1, 9.3, 2.3 Hz, 1H), 1.78 (ddd, *J* = 13.9, 9.3, 2.3 Hz, 1H), 2.56 (td, *J* = 13.9, 9.7 Hz, 2H), 2.77-2.82 (m, 1H), 3.36 (dd, *J* = 13.2, 3.2 Hz, 1H), 3.50 (t, *J* = 8.0 Hz, 1H), 3.91 (td, *J* = 9.7, 2.6 Hz, 1H), 3.98-4.04 (m, 2H), 4.09-4.21 (m, 4H), 4.66-4.72 (m, 1H), 5.02-5.19 (m, 1H), 5.86-5.96 (m, 1H), 7.24-7.30 (m, 3H), 7.33-7.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.7, -4.5, 17.9, 25.8, 25.8, 25.9, 26.8, 34.4, 37.9, 43.5, 55.6, 65.7, 69.8, 70.3, 72.3, 74.9, 108.5, 117.6, 127.3, 128.9, 129.4, 134.6, 135.4, 152.7, 174.0; LRMS: (ES+) m/z = 548 (M+1).



(2S,3R,4R)-2-allyl-4-(tert-butyldimethylsilyloxy)-5-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)pentane-1,3-diol (S_2):

Same experiment as synthesis of compound **S_1**:

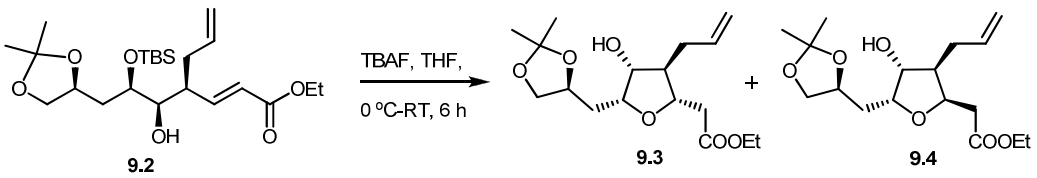
Molecular Formula: $C_{19}H_{38}O_5Si$; R_f (50% ethyl acetate/hexane): 0.3; yield: 75% ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.12 (s, 6H), 0.88-0.92 (m, 9H), 1.35 (s, 3H), 1.40 (s, 3H), 1.64-1.72 (m, 1H), 1.72-1.79 (m, 1H), 1.81-1.84 (m, 1H), 2.09 (bs, 1H), 2.22-2.28 (m, 1H), 2.41-2.49 (m, 1H), 2.90 (bs, 1H), 3.55 (t, *J* = 8.0 Hz, 1H), 3.69-3.70 (m, 2H), 4.01-4.10 (m, 2H), 4.20-4.22 (m, 1H), 5.06-5.14 (m, 2H), 5.78-5.95 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.4, -4.3, 18.0, 25.6, 25.8, 26.9, 32.5, 36.3, 44.7, 61.6, 71.5, 73.9, 75.4, 108.7, 116.2, 137.4; LRMS: (ES+) m/z = 373 (M-1).



(S,E)-ethyl4-((1R,2R)-2-(tert-butyldimethylsilyloxy)-3-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-hydroxypropyl)hepta-2,6-dienoate (9.2):

Same experiment as synthesis of compound **8.3**:

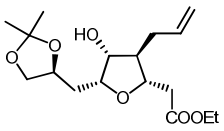
Molecular Formula: $C_{23}H_{42}O_6Si$; R_f (20% ethyl acetate/hexane): 0.2; yield: 80% ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.09 (S, 6H), 0.88 (s, 9H), 1.276-1.28 (t, *J* = 6.0, 3H), 1.29 (s, 3H), 1.31 (s, 3H), 1.50 (ddd, *J* = 13.8, 9.4, 2.5 Hz, 1H), 1.67-1.72 (m, 2H), 2.10-2.18 (m, 1H), 2.27-2.30 (dq, *J* = 9.6, 3.2 Hz, 1H), 2.56 (bs, 1H), 2.60-2.68 (m, 1H), 3.46-3.54 (m, 1H), 3.49-3.51 (t, *J* = 7.2 Hz, 1H), 3.54-3.58 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.83 (td, *J* = 10.4, 2.6 Hz, 1H), 4.03 (dd, *J* = 7.8, 6.0 Hz, 1H), 4.21 (q, *J* = 6.0 Hz, 2H), 4.97-5.09 (m, 2H), 5.63-5.67 (m, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 6.65 (dd, *J* = 15.6, 10.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm -4.7, -4.5, 114.2, 17.9, 25.6, 25.8, 26.8, 33.6, 44.9, 60.2, 69.8, 70.8, 72.3, 108.7, 117.1, 123.5, 135.1, 146.5, 166.0; LRMS: (ES+) m/z = 465 (M+23).



9.2 9.3 9.4

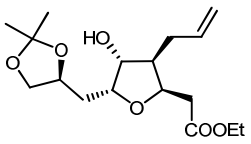
Same experiment as synthesis of compound **8.4**:

ethyl2-((2S,3R,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)acetate (9.3):

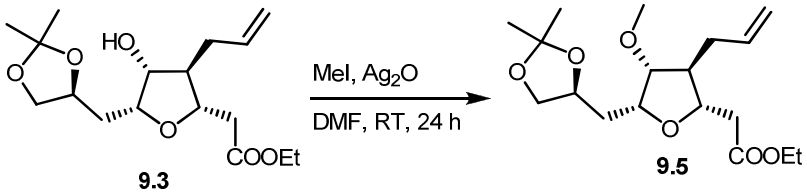


Molecular Formula: $C_{17}H_{28}O_6$; R_f (30% ethyl acetate/hexane): 0.2; yield: 35%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.27 (t, *J* = 6.8, 3H), 1.35 (s, 3H), 1.43 (s, 3H), 1.83-1.93 (m, 1H), 1.99-2.01 (m, 2H), 2.08-2.18 (m, 1H), 2.29-2.33 (m, 2H), 2.45-2.51 (m, 1H), 2.56-2.64 (m, 1H), 3.59 (t, *J* = 8.0 Hz, 1H), 3.78 (q, *J* = 4.8 Hz, 1H), 3.80-3.85(m, 1H), 4.06 (ddd, *J* = 8.0, 5.9, 2.3 Hz, 1H), 4.09-4.22 (m, 3H), 4.26-4.36 (m, 1H), 5.12 (m, 2H), 5.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 25.5, 26.7, 29.6, 31.8, 36.4, 38.6, 51.7, 60.6, 68.8, 72.6, 77.6, 78.3, 78.6, 108.9, 116.9, 135.5, 171.6; LRMS: (ES+) m/z = 328 (M-1).

ethyl 2-((2R,3R,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)acetate (9.4):



Molecular Formula: $C_{17}H_{28}O_6$; R_f (30% ethyl acetate/hexane): 0.2; yield: 35%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.27 (t, *J* = 6.8, 3H), 1.35 (s, 3H), 1.42 (s, 3H), 1.90-1.92 (m, 1H), 2.05 (m, 1H), 2.10-2.16 (m, 2H), 2.25-2.26 (m, 1H), 2.59 (dd, *J* = 16.5, 5.7 Hz, 1H), 2.76 (dd, *J* = 16.5, 4.5 Hz, 1H), 3.52 (d, *J* = 6.8 Hz, 1H), 3.63 (t, *J* = 8.0 Hz, 1H), 3.78 (q, *J* = 6.8 Hz, 1H), 3.86 (dd, *J* = 7.8, 4.7 Hz, 1H), 3.90 (bs, 1H), 4.06 (dd, *J* = 8.0, 6.1 Hz, 1H), 4.15 (q, *J* = 6.8 Hz, 1H), 4.26-4.35 (m, 1H), 5.10 (m, 2H), 5.79 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.1, 25.5, 26.7, 29.6, 31.8, 36.4, 38.6, 51.7, 60.6, 68.8, 72.6, 77.6, 78.3, 78.6, 108.9, 116.9, 135.5, 171.6; LRMS: (ES+) m/z = 328 (M-1).



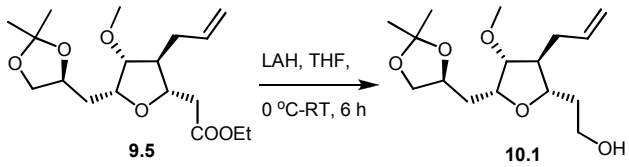
9.3 **9.5**

ethyl2-((2S,3S,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxytetra hydrofuran-2-yl)acetate (9.5):

To a stirred solution of compound **9.3 (**700 mg, 2.13 mmol**,** 1 eq) in DMF was added silver oxide (10.65 mmol, 5 eq) and methyl iodide (6.40 mmol, 3 eq) at room temperature under an inert atmosphere. The reaction was allowed stirred for 12 hour. The reaction mixture was filtered through celite and quenched by the addition of water, extracted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by flash chromatography to obtain pure product **9.5** (yield 65%) as a colorless liquid.

Molecular Formula: $C_{18}H_{30}O_6$; R_f : 0.2 (20% ethyl acetate/hexanes); Solvent system for column purification (20% ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 1.27 (t, *J* =7.2 Hz, 3H), 1.35 (s, 3H), 1.40 (s, 3H), 1.62 (bs, 1H), 1.73 (ddd, *J* =

13.7, 9.9, 6.5 Hz, 1H), 1.90-2.05 (m, 2H), 2.27-2.19 (m, 2H), 2.52-2.66 (m, 2H), 3.36 (s, 3H), 3.55 (t, *J* = 7.6 Hz, 1H), 3.99 (td, *J* = 9.9, 3.9 Hz, 1H), 4.04 -4.10 (m, 1H), 4.10-4.21 (m, 4H), 5.05-5.16 (m, 2H), 5.78 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 14.2, 25.7, 26.9, 36.3, 38.6, 39.8, 49.9, 57.6, 60.4, 70.0, 74.1, 78.8, 80.0, 90.9, 108.3, 117.0, 135.6, 171.1; LRMS: (ES+) m/z = 341 (M-1).

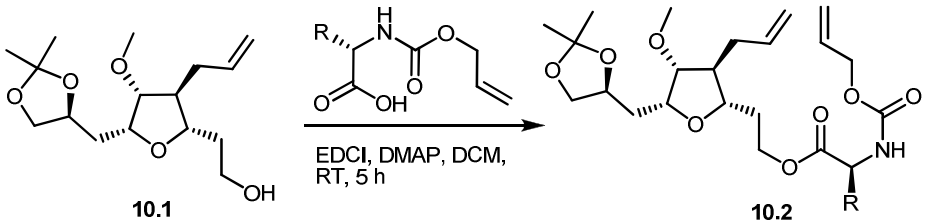


9.5 10.1

2-((2S,3S,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxytetrahy drofuran-2-yl)ethanol (10.1):

To a stirred solution of compound **9.5** (200 mg, 0.584 mmol, 1eq) in dry THF was added Lithium aluminum hydride (0.70 mmol, 1.2 eq) at 0 °C under inert atmosphere. The reaction was allowed to warm to room temperature and stirred for 6 hours. The reaction mixture was quenched by the addition of a saturated *aq*. NH_4Cl, extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purified by flash chromatography using 40% ethyl acetate in hexane gave **10.1** (140 mg, 80%)

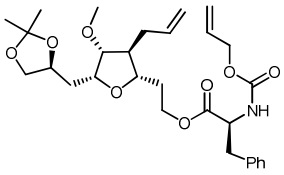
Molecular Formula: $C_{16}H_{28}O_5$; R_f (30% ethyl acetate/hexane): 0.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (s, 3H), 1.41 (s, 3H), 1.62 (bs, 1H), 1.65-1.82 (m, 3H), 1.84-1.94 (m, 2H), 1.96-2.01 (m, 1H), 2.23 (t, *J* = 7.2 Hz, 1H), 3.29 (bs, 1H), 3.32 (s, 3H), 3.52-3.58 (t, *J* = 7.2 Hz, 1H), 3.78 (m, 2H), 3.82-3.88 (m, 1H), 4.03-4.12 (m, 2H), 4.19 (t, *J* = 7.2 Hz, 1H), 5.04-5.14 (m, 2H), 5.78 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm; 25.2, 26.9, 29.6, 36.2, 36.2, 38.3, 50.5, 57.7, 61.3, 69.8, 73.8, 79.6, 81.9, 90.8, 108.5, 116.9, 135.7; LRMS: (ES+) m/z = 299 (M-1).



10.1 10.2

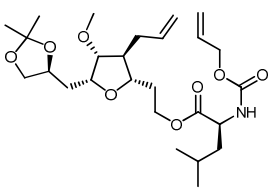
To a stirred solution of primary alcohol **10.1**(1 eq) in DCM (5 mL), alloc amino acid (1.5), EDCI (1.5 eq) and DMAP (2 eq) were added. The mixture was stirred at room temperature for 5 hours and then quenched with addition of saturated sodium bicarbonate solution. Aqueous layer was washed twice with dichloromethane (10 mL) and combined the organic layers washed with brine solution, dried over anhydrous sodium sulphate. Solvent was concentrated to leave crude solid, which was purified by the flash column chromatography to bis allyl product **10.2**.

(S)-2-((2S,3S,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxytetra hydrofuran-2-yl)ethyl 2-(allyloxycarbonylamino)-3-phenylpro panoate (10.2a):



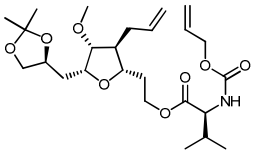
Molecular Formula: $C_{29}H_{41}NO_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.36 (s, 3H), 1.43 (s, 4H), 1.59 (bs, 2H), 1.68-177 (m, 1H), 1.78-189 (m, 2H), 1.92 (d, *J* = 6.3 Hz, 2H), 2.22 (t, *J* = 6.4 Hz, 2H), 3.12 (m, 2H), 3.34 (s, 3H), 3.57 (s, *J* = 7.2 Hz, 1H), 3.66-3.73 (m, 1H), 3.95-4.03 (m, 1H), 4.08-4.17 (m, 1H), 4.20-4.23 (m, 3H), 4.57 (d, *J* = 4.8 Hz, 3H), 5.07-5.11 (m, 2H), 5.21-5.32 (m, 2H), 5.70-5.83 (m, 1H), 5.85-6.00 (m, 1H), 7.15 (d, *J* = 6.7 Hz, 2H), 7.25-7.36 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 25.7, 27.0, 29.6, 33.3, 36.7, 38.8, 48.5, 55.8, 57.5, 61.6, 64.7, 69.6, 74.1, 79.6, 92.0, 108.5, 116.9, 118.3, 128.9, 135.8, 155.7, 171.4; LRMS: (ES+) m/z = 532 (M+1), 553 (M+23).

(S)-2-((2S,3S,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxytetrahydrofuran-2-yl)ethyl2-(allyloxycarbonylamino)-4-methylpentano ate (10.2b):

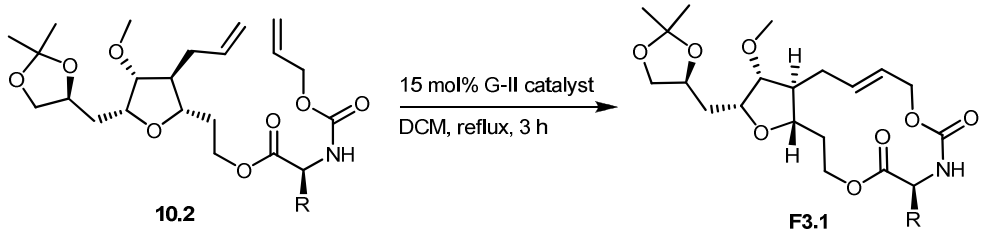


Molecular Formula: $C_{26}H_{43}NO_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (d, *J* = 3.1 Hz, 6H), 1.37 (s, 3H), 1.43 (s, 3H), 1.50-158 (m, 1H), 1.65 (bs, 2H), 1.67-1.78 (m, 3H), 1.89-1.93 (m, 3H), 2.04-2.08 (m, 1H), 2.23 (t, *J* = 7.1 Hz, 1H), 3.31 (d, *J* = 8.9 Hz, 1H), 3.33 (s, 3H), 3.54-3.62 (q, *J* = 7.1 Hz, 1H), 3.74 (td, *J* = 8.8, 5.0 Hz, 1H), 3.95-4.00 (m, 1H), 4.07-4.13 (m, 1H), 4.17-4.27 (m, 3H), 4.34-4.41 (m, 1H), 4.58 (d, *J* = 5.6 Hz, 2H), 5.06-5.15 (m, 2H), 5.23 (dd, *J* = 10.4, 1.0 Hz, 1H), 5.28-5.36 (m, 1H), 5.78 (ddd, *J* = 17.1, 7.4, 2.9 Hz, 1H), 5.87-5.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm; 21.7, 22.8, 24.7, 25.7, 26.9, 29.6, 33.4, 36.4, 41.8, 50.2, 52.4, 57.6, 62.5, 65.7, 69.8, 74.0, 78.7, 79.5, 91.0, 108.4, 116.9, 117.7, 132.6, 155.7, 155.7, 172.9; LRMS: (ES+) m/z = 498 (M+1), 520 (M+23).

(S)-2-((2S,3S,4R,5R)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxytetrahydrofuran-2-yl)ethyl2-(allyloxycarbonylamino)-3-methylbutanoate (10.2c):

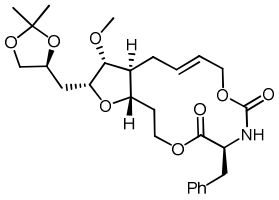


Molecular Formula: $C_{25}H_{41}NO_8$; R_f (30% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 30% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) ppm 0.91 (d, *J* = 7.2, 3H), 0.97 (d, *J* = 7.2, 3H), 1.34 (s, 3H), 1.36-1.45 (s, 3H), 1.69 (m, 1H), 1.90 (m, 3H), 2.00-2.07 (m, 1H), 2.10-2.25 (m, 2H), 3.27 (s, 1H), 3.29-3.38 (bs, 3H), 3.54 (t, *J* = 7.6 Hz, 1H), 3.69-3.77 (m, 1H), 3.96 (td, *J* = 9.9, 3.9, 3.9 Hz, 1H), 4.08 (dd, *J* = 8.0, 5.9 Hz, 1H), 4.12-4.31 (m, 3H), 4.57 (d, *J* = 5.2 Hz, 2H), 5.03-5.13 (m, 2H), 5.18-5.38 (m, 3H), 5.76 (dd, *J* = 17.0, 10.1 Hz, 1H), 5.84-5.98 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.9, 22.6, 25.7, 26.9, 29.6, 31.2, 31.8, 33.5, 36.4, 38.5, 50.2, 57.7, 58.9, 62.4, 65.7, 69.8, 74.0, 78.7, 79.5, 91.0, 108.4, 116.9, 117.7, 132.6, 135.6, 156.0, 171.9; LRMS: (ES+) m/z = 484 (M+1), 506 (M+23).



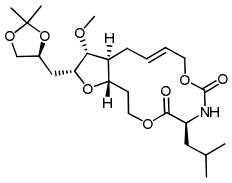
To a stirred solution of compound **10.2** (1eq) in dry DCM was added 15 mol% Grubbs' second generation catalyst at room temperature. The reaction mixture was refluxed for 3 h. The reaction mixture concentrated *in vacuo* and purified by flash chromatography to obtain pure product **F8.1**.

(2R,3R,3aS,11S,15aS,E)-11-benzyl-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3-methoxy-2,3,3a,4,10,11,15,15a-octahydrofuro[2,3-i][1,6,3]dioxaazacyclotetradecine-9,12(7H,14H)-dione (F8.1a):



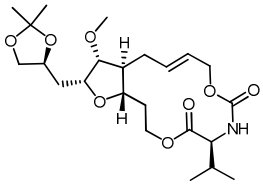
Molecular Formula: $C_{27}H_{37}NO_8$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.38 (s, 3H), 1.44 (s, 3H), 1.75-1.87 (m, 2H), 1.94 (d, *J* = 2.4 Hz, 2H), 2.01-2.08 (m, 1H), 2.26-2.44 (m, 2H), 2.94-3.03 (m, 1H), 3.12-3.24 (m, 1H), 3.27 (bs, 1H), 3.34 (s, 3H), 3.60-3.68 (t, *J* = 6.8 Hz, 1H), 3.93-3.96 (m, 2H), 4.01-4.11 (m, 2H), 4.22 (q, *J* = 6.8 Hz, 1H), 4.38-4.48 (m, 1H), 4.59-4.67 (d, *J* = 6.8 Hz, 1H), 4.83-4.92 (q, *J* = 9.2 Hz, 1H), 4.92-4.99 (m, 1H), 5.62 (ddd, *J* = 14.4, 8.8, 5.2 Hz, 1H), 5.72-5.80 (m, 1H), 7.20 (d, *J* = 6.8 Hz, 2H), 7.32-7.45 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm; 25.7, 27.0, 29.6, 33.3, 36.7, 38.8, 48.5, 55.8, 57.5, 61.6, 64.7, 69.6, 74.1, 79.6, 92.0, 108.5, 127.4, 128.8, 128.9, 135.8, 155.7, 171.4; LRMS: (ES+) m/z = 484 (M+1), 506 (M+23).

(2R,3R,3aS,11S,15aS,E)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-11-isobutyl-3-methoxy-2,3,3a,4,10,11,15,15a-octahydrofuro[2,3-i][1,6,3]dioxaazacyclotetradecine-9,12(7H, 14H)-dione (F8.1b):

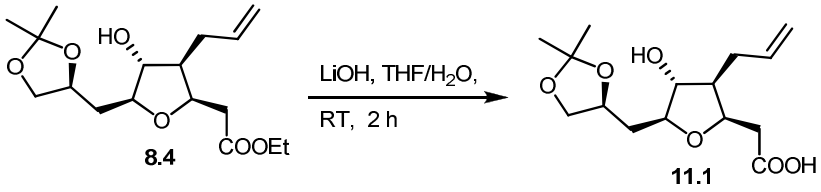


Molecular Formula: $C_{24}H_{39}NO_8$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.92-0.97 (m, 6H), 1.35 (s, 3H), 1.41 (s, 3H), 1.54 (m, 2H), 1.70 (m, 2H), 1.80 (m, 2H), 1.92 (m, 2H), 2.03 (d, *J* = 11.6 Hz, 1H), 2.25-2.35 (m, 1H), 3.22-3.29 (bs, 1H), 3.36 (s, 3H), 3.63 (d, *J* = 7.0 Hz, 1H), 3.89-4.00 (m, 3H), 4.02-4.09 (m, 2H), 4.17-4.24 (m, 1H), 4.51-4.59 (d, *J* = 11.2 Hz , 1H), 4.94 (m, 2H), 5.62 (ddd, *J* = 14.0, 7.6, 5.2 Hz, 1H), 5.74-5.80 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.8, 24.7, 25.6, 27.0, 29.6, 31.8, 33.3, 35.4, 38.7, 39.8, 48.5, 53.6, 57.4, 61.4, 64.6, 69.6, 74.2, 79.6, 92.1, 108.4, 127.6, 135.7, 155.8, 172.1; LRMS: (ES+) m/z = 470 (M+1), 492 (M+23).

(2R,3R,3aS,11S,15aS,E)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-11-isopropyl-3-methoxy-2,3,3a,4,10,11,15,15a-octahydrofuro[2,3-i][1,6,3]dioxaazacyclotetradecine-9,12(7H, 14H)-dione (F8.1c):



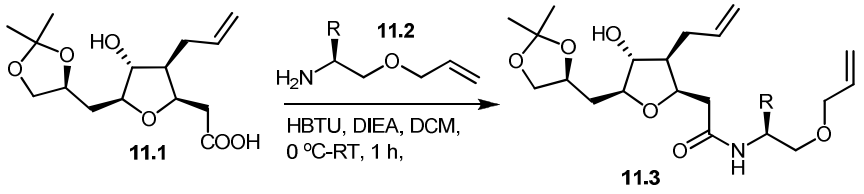
Molecular Formula: $C_{23}H_{37}NO_8$; R_f (50% ethyl acetate/hexane): 0.3; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.95 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 1.35 (s, 1H), 1.42 (s, 1H), 1.74-1.80 (m, 1H), 1.86-2.07 (m, 5H), 2.14 (d, *J* = 6.3 Hz, 1H), 2.27-2.34 (d, *J* = 12.0 Hz, 1H), 3.25 (dd, *J* = 3.7, 1.9 Hz, 1H), 3.32 (s, 3H), 3.56-3.64 (t, *J* = 7.6 Hz, 1H), 3.90-4.00 (m, 4H), 4.03-4.10 (m, 2H), 4.17-4.23 (q, *J* = 6.4 Hz, 1H), 4.57-4.64 (d, *J* = 11.6 Hz , 1H), 4.92-5.00 (m, 2H), 5.61 (ddd, *J* = 14.8, 8.8, 4.8 Hz, 1H), 5.75-5.80 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.2, 19.3, 22.6, 25.6, 27.0, 29.6, 31.8, 33.6, 35.8, 38.9, 48.4, 57.4, 60.8, 61.2, 64.6, 69.7, 74.1, 79.6, 92.2, 108.5, 127.4, 135.8, 156.1, 171.2; LRMS: (ES+) m/z = 456 (M+1), 478 (M+23).



2-((2R,3R,4R,5S)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)acetic acid (11.1):

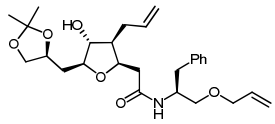
To the stirred solution of ester (500 mg, 1.52mmol, 1 eq) in 5:1 mixture of THF and Water solvent was added 3 eq of $LiOH.5H_2O$ at room temperature. Stirred the reaction mixture for 2 h, monitor by TLC. After completion of reaction, neutralized with 10% HCl, extracted with ethyl acetate (2 x) and dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Without Purification carried out further reactions.

Molecular Formula: $C_{15}H_{24}O_6$; R_f (50% ethyl acetate/hexane): 0.1; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H), 1.39 (s, 3H), 1.66-1.68 (s, 2H), 1.97-2.09 (m, 2H), 2.20-2.31 (m, 1H), 2.38-2.49 (m, 1H), 2.63 (dd, *J* = 10.2, 6.1 Hz, 1H), 3.56-3.71 (m, 2H), 3.74-3.83 (m, 1H), 4.12 (dd, *J* = 8.0, 5.8 Hz, 2H), 4.18-4.28 (m, 1H), 5.10 (dd, *J* = 19.2, 13.5 Hz, 2H), 5.75-5.89 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 25.8, 26.7, 29.6, 35.3, 37.8, 40.1, 50.6, 69.7, 73.8, 80.1, 81.5, 109.5, 109.9, 117.2, 135.4, 175.0.



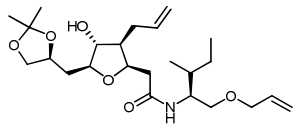
To the stirred solution of acid **11.1**(1eq) in dry DCM were added HBTU (1.2 eq) and DIEA (1.5 eq) at 0 ^oC. After 5 min 1.2 eq of amine **11.2** was added and the reaction mixture stirred for 1 h at room temperature, monitor the reaction by TLC. After completion of reaction quenched with aq. $NaHCO_3$ and extracted the aqueous layer with dichloromethane, dried over anhydrous sodium sulphate and concentrated *in vacuo.* Purified by flash chromatography to obtain pure product **11.3**.

2-((2R,3R,4R,5S)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-N-((S)-1-(allyloxy)-3-phenylpropan-2-yl)acetamide (11.3a):



Molecular Formula: $C_{27}H_{39}NO_6$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.36 (s, 3H), 1.40 (s, 3H), 1.73 (ddd, *J* = 13.8, 9.4, 8.3 Hz, 1H), 1.86-2.00 (m, 2H), 2.21-2.25 (m, 1H), 2.30-2.39 (m, 2H), 2.40-2.48 (m, 1H), 2.84-2.91 (m, 2H), 3.08-3.11 (m, 1H), 3.31-3.38 (m, 2H), 3.55-3.59 (t, *J* = 7.6, 1H), 3.61-3.64 (m, 1H), 3.73-3.78 (m, 1H), 3.91-3.99 (m, 2H), 4.07 (q, *J* = 1H), 4.09-4.14 (m, 1H), 4.15-4.22 (m, 1H), 4.30 (dt, *J* = 8.1, 7.9, 3.9 Hz, 1H), 5.20 (m, 4H), 5.77-5.98 (m, 2H), 6.65 (d, *J* = 8.5 Hz, 1H), 7.21 (td, *J* = 9.6, 4.7, 4.7 Hz, 3H), 7.27 (dd, *J* = 7.8, 3.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 25.8, 26.8, 29.6, 34.9, 37.4, 37.9, 41.8, 49.9, 50.6, 69.7, 69.8, 71.9, 73.7, 78.2, 80.3, 81.4, 109.4, 116.9, 117.1, 126.3, 128.3, 129.4, 134.5, 135.4, 138.0, 170; LRMS: (ES+) m/z = 474 (M+1).

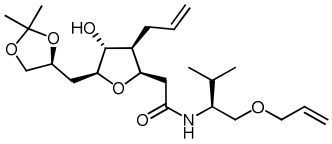
2-((2R,3R,4R,5S)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-N-((2S,3R)-1-(allyloxy)-3-methylpentan-2-yl)acetamide (11.3b):



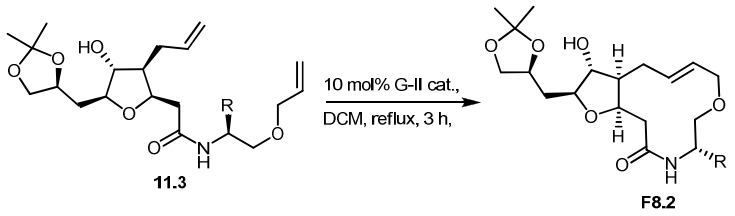
Molecular Formula: $C_{24}H_{41}NO_6$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.85-0.88 (m, 6H), 1.03-1.11 (m, 1H), 1.35 (s, 3H), 1.41 (s, 3H), 1.45-1.50 (m, 1H), 1.60-1.68 (m, 1H), 1.69-1.77 (m, 1H), 1.84-1.88 (m, 1H), 1.94-1.98 (m, 1H), 2.17-2.27 (m, 1H), 2.34 (dd, *J* = 14.2, 6.6 Hz, 1H), 2.38-2.46 (m, 1H), 2.52 (dd, *J* = 15.0, 3.0 Hz, 1H), 3.37-3.47 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.50-3.60 (m, 2H), 3.60-3.68 (t, *J* = 15.6, 1H), 3.76-3.82 (m, 1H), 3.88-4.02 (m, 4H), 4.11 (td, *J* = 8.0, 5.4, 5.4 Hz, 1H), 4.14-4.23 (m, 1H), 5.02-5.30 (m, 4H), 5.76-5.94 (m, 2H), 6.55 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 11.3, 15.4, 25.3, 25.8,

26.8, 34.9, 35.6, 37.9, 42.0, 50.7, 52.6, 69.7, 69.8, 71.9, 73.7, 78.4, 80.4, 81.4, 109.3, 116.9, 117.1, 134.6, 135.5, 170.2; LRMS: (ES+) m/z = 462 (M+23).

2-((2R,3R,4R,5S)-3-allyl-5-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-hydroxytetrahydrofuran-2-yl)-N-((S)-1-(allyloxy)-3-methylbutan-2-yl)aceta mide (11.3c):



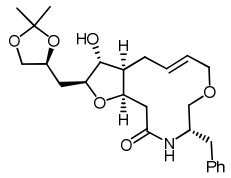
Molecular Formula: $C_{23}H_{39}NO_6$; R_f (50% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.89-0.92 (m, 6H), 1.36 (s, 3H), 1.43 (s, 3H), 1.69-1.77 (m, 1H), 1.89-1.91 (m, 2H), 1.95-2.02 (m, 1H), 2.25 (d, *J* = 7.1 Hz, 1H), 2.32-2.37 (m, 1H), 2.44 (dd, *J* = 15.1, 8.4 Hz, 1H), 2.54 (dd, *J* = 15.09, 2.9 Hz, 1H), 3.41 (dd, *J* = 9.7, 4.1 Hz, 1H), 3.52-3.61 (m, 2H), 3.62-3.69 (t, *J* = 8.0 Hz, 1H), 3.76-3.84 (m, 1H), 3.87 (d, *J* = 1.8 Hz, 1H), 3.91-4.06 (m, 3H), 4.11 (dd, *J* = 8.0, 5.9 Hz, 1H), 4.15-4.26 (m, 1H), 5.04-5.29 (m, 4H), 5.79-5.96 (m, 2H), 6.55 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.7, 19.5, 22.6, 25.8, 26.8, 29.2, 29.3, 29.6, 29.6, 34.9, 37.9, 42.0, 50.7, 53.7, 69.7, 71.9, 73.7, 78.4, 80.4, 81.4, 109.4, 116.8, 117.1, 134.6, 135.4, 170.3; LRMS: (ES+) m/z = 448 (M+23).



11.3 **F8.2**

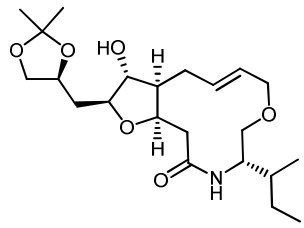
To a stirred solution of compound **11.3** (1eq) in dry DCM was added 10 mol% Grubbs' second generation catalyst at room temperature. The reaction mixture was refluxed for 3 h. The reaction mixture concentrated *in vacuo* and purified by flash chromatography to obtain pure product **F8.2**.

(2S,3R,3aR,10S,13aR,E)-10-benzyl-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl) methyl)-3-hydr oxy-3a,4,7,9,10,11,13,13a-octahydro-2H-furo[2,3-g][1,4] oxa azacyclododecin-12(3H)-one (F8.2a):



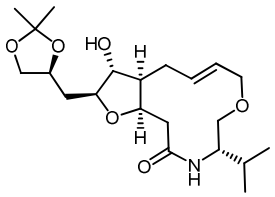
Molecular Formula: $C_{25}H_{35}NO_6$; R_f (70% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.39 (s, 3H), 1.43 (s, 3H), 2.08 (m, 4H), 2.19-2.39 (m, 2H), 2.60-2.67 (m, 1H), 2.68-2.77 (m, 1H), 2.81 (d, *J* = 7.7 Hz, 1H), 3.42-3.48 (m, 1H), 3.59 (s, 2H), 3.68 (dd, *J* = 12.4, 9.4 Hz, 2H), 3.81-3.90 (m, 1H), 3.94-4.05 (m, 1H), 4.12 (d, *J* = 5.8 Hz, 1H), 4.19-4.22 (m, 1H), 4.90-5.06 (m, 1H), 5.38-5.48 (m, 1H), 5.56-5.69 (m, 1H), 5.80 (ddd, *J* = 14.0, 7.0, 4.2 Hz, 1H), 7.15-7.26 (m, 2H), 7.29-7.30 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 22.6, 25.8, 26.7, 29.3, 29.6, 31.8, 37.0, 44.9, 67.5, 69.7, 70.6, 73.8, 80.7, 81.6, 126.2, 126.5, 129.1, 130.7, 133.5, 134.3, 174.6; LRMS: (ES+) m/z = 446 (M+1).

(2S,3R,3aR,10S,13aR,E)-10-sec-butyl-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl) methyl)-3-hydr oxy-3a,4,7,9,10,11,13,13a-octahydro-2H-furo[2,3-g][1,4] oxa azacyclododecin-12(3H)-one (F8.2b):



Molecular Formula: $C_{25}H_{35}NO_6$; R_f (70% ethyl acetate/hexane): 0.1; Purified by flash chromatography using 50% ethyl acetate in hexane; Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91 (m, 6H), 1.37 (s, 3H), 1.43 (s, 3H), 1.52 (m, 2H), 1.68 (dd, *J* = 16.4, 6.8 Hz, 2H), 1.97-2.01 (m, 2H), 2.08-2.10 (m, 1H), 2.26-2.37 (m, 1H), 2.57-2.72 (m, 1H), 2.76-2.85 (m, 1H), 3.56-3.62 (m, 2H), 3.62-3.70 (m, 2H), 4.02-4.10 (m, 1H), 4.11-4.14 (m, 1H), 4.21 (d, *J* = 2.95 Hz, 1H), 4.88-5.03 (m, 1H), 5.39-5.47 (m, 1H), 5.54-5.64 (m, 1H), 5.65-5.74 (m, 1H), 6.00 (ddd, *J* = 14.1, 8.1, 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 11.0, 11.3, 14.0, 15.3, 22.6, 26.7, 28.9, 29.3, 29.6, 30.8, 31.8, 33.7, 35.4, 38.1, 45.1, 52.4, 54.0, 69.7, 73.8, 79.9, 80.7, 81.6, 109.5, 114.0, 126.3, 134.0, 169.6; LRMS: (ES+) m/z = 412 (M+1).

2S,3R,3aR,10S,13aR,E)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-3-hydroxy-10-isopropyl-3a,4,7,9,10,11,13,13a-octahydro-2H-furo[2,3-g][1,4]oxaazacyclododecin-12(3H)-one (F8.2c):



Molecular Formula: $C_{21}H_{35}NO_6$; R_f (70% ethyl acetate/hexane): 0.2; Purified by flash chromatography using 70% ethyl acetate in hexane; Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.91 (m, 6H), 1.37 (s, 3H), 1.44 (s, 3H), 1.63-1.73 (m, 2H), 1.96-2.06 (m, 2H), 2.26-2.37 (m, 2H), 2.56-2.72 (m, 1H), 2.75-2.87 (m, 1H), 3.53-3.63 (m, 3H), 3.63-3.76 (m, 3H), 4.10-4.19 (m, 2H), 4.18-4.26 (m, 2H), 4.88-5.05 (m, 1H), 5.55-5.73 (m, 1H), 5.76-5.88 (m, 1H), 6.09 (ddd, *J* = 14.4, 8.4, 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 18.7, 19.5, 22.6, 26.7, 28.9, 29.3, 29.6, 30.8, 31.8, 33.7, 35.4, 38.1, 45.1, 52.4, 54.0, 69.7, 73.8, 79.9, 80.7, 81.6, 109.5, 114.0, 126.3, 134.0, 169.6; LRMS: (ES+) m/z = 398 (M+1).

4.11. References

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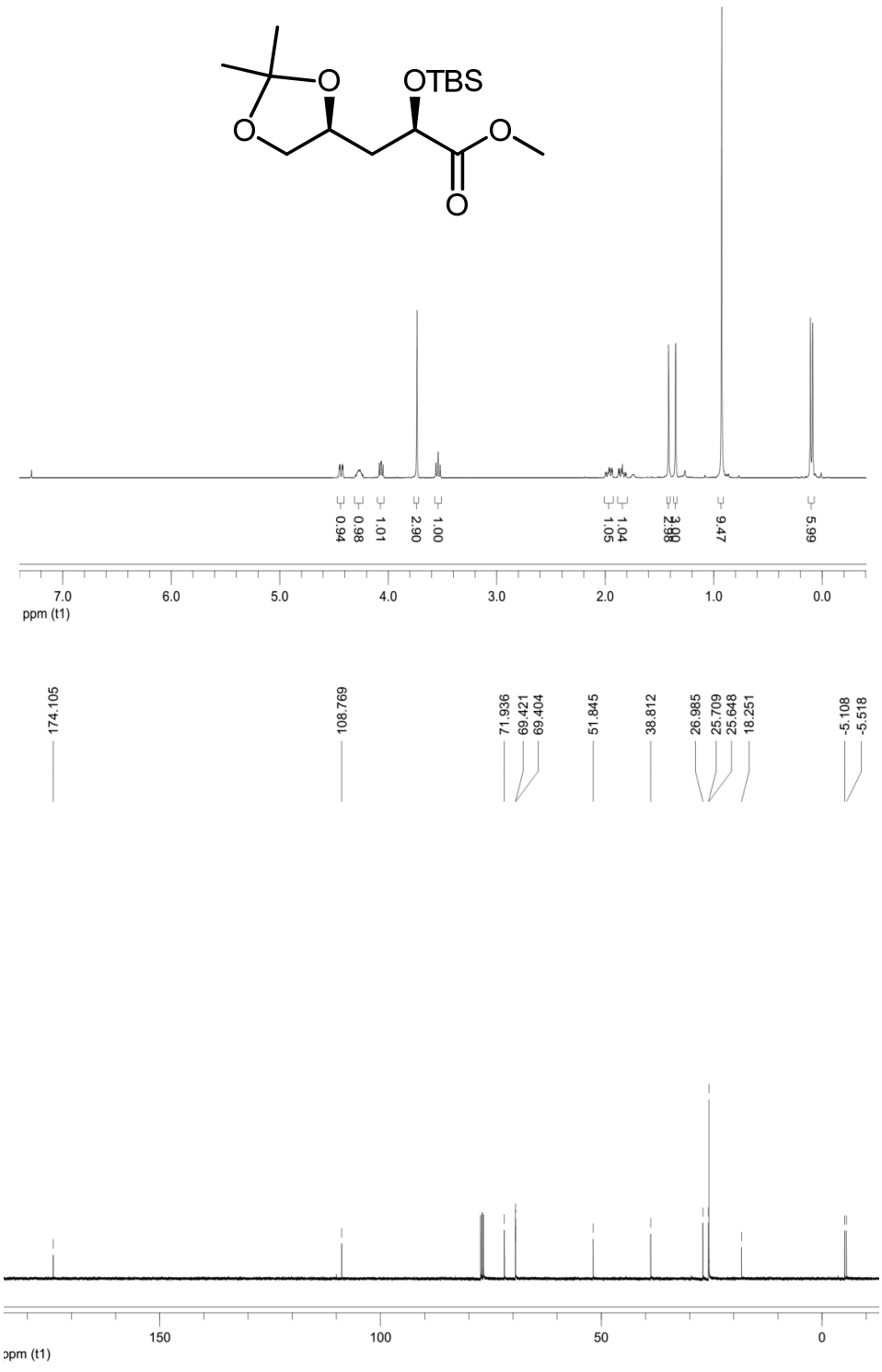
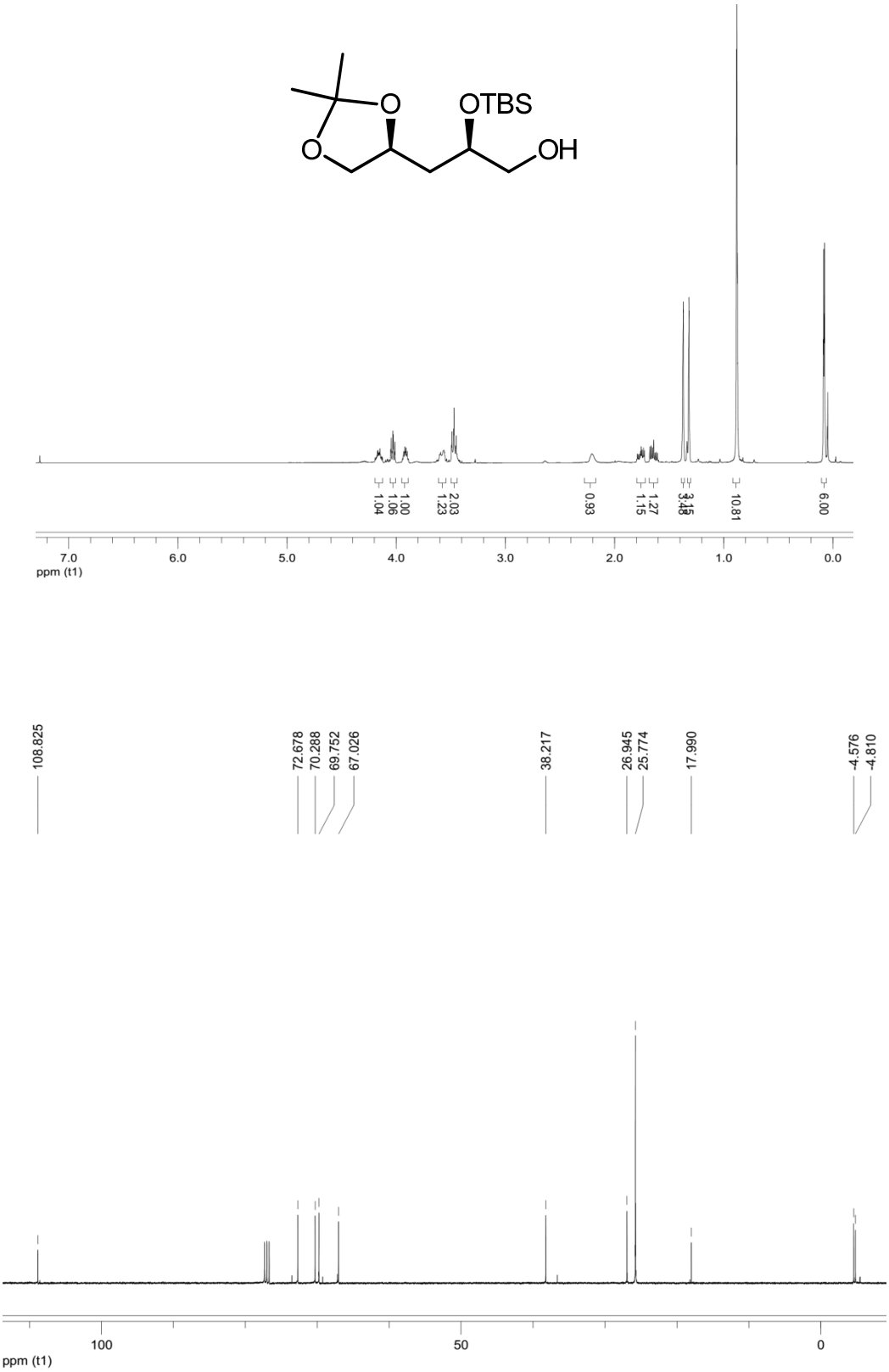
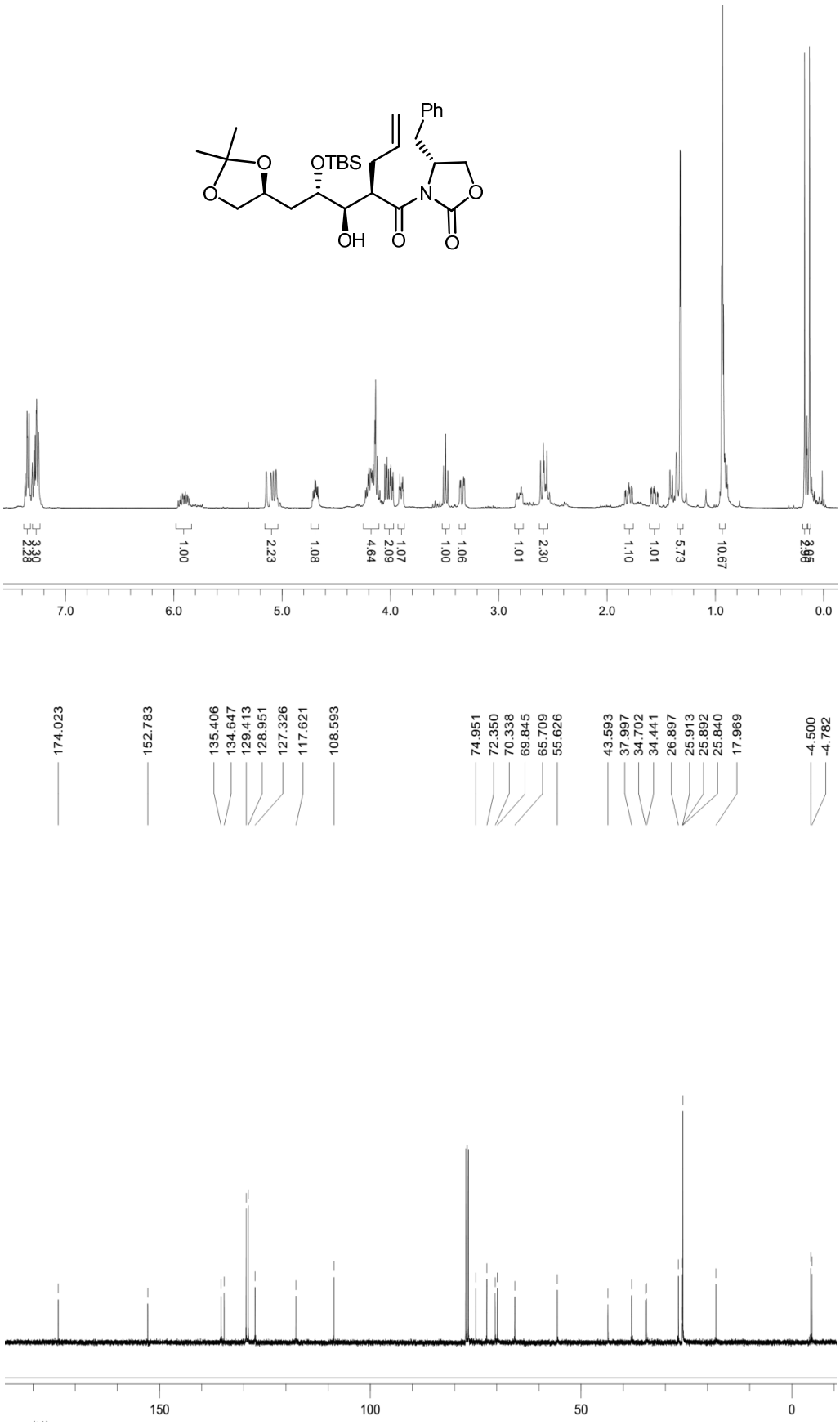
4.12. Spectra



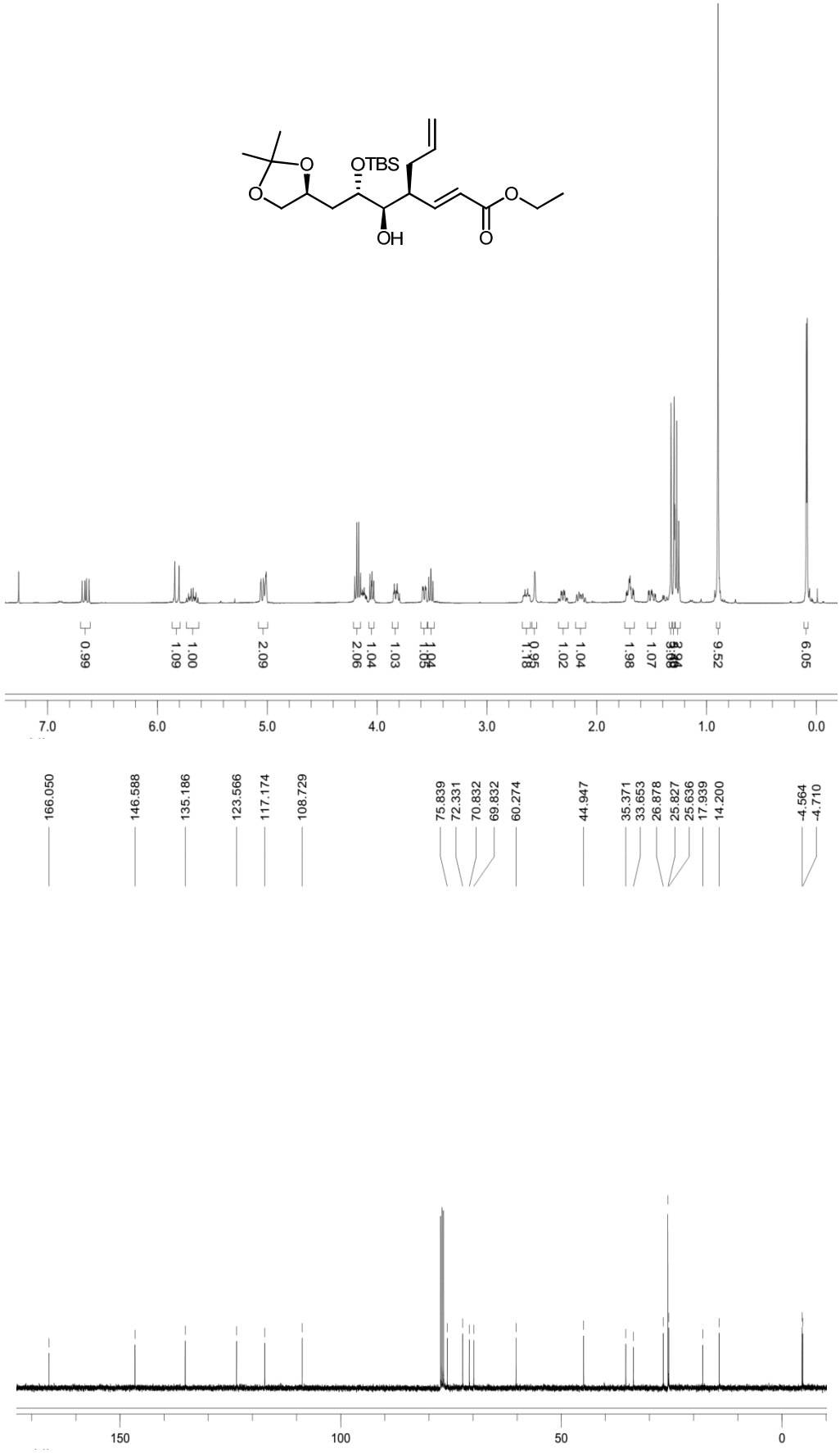
^{1}H and ^{13}C NMR of Compound **5.2**



^{1}H and ^{13}C NMR of Compound **5.3**



^{1}H and ^{13}C NMR of Compound **8.2**



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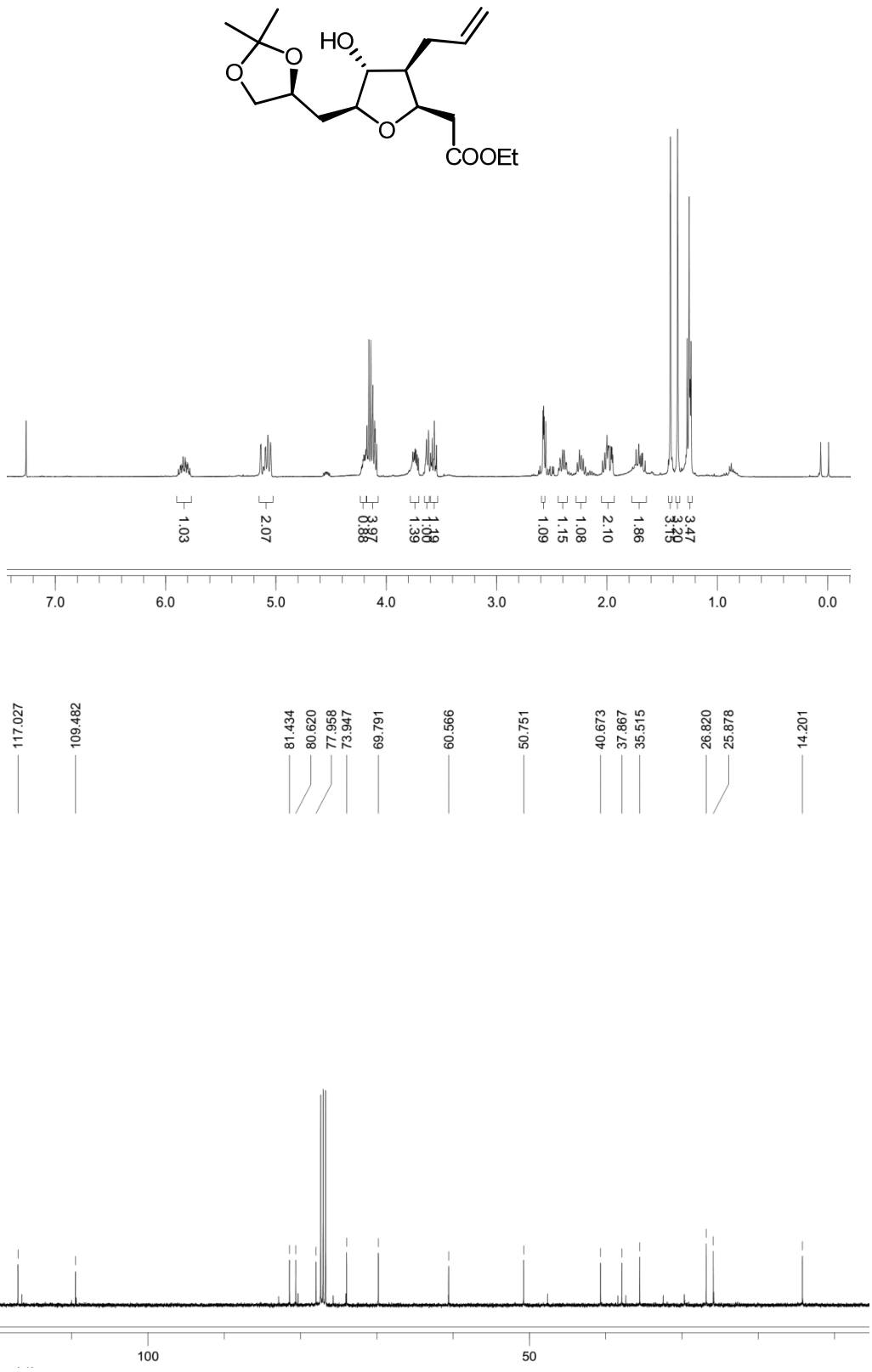
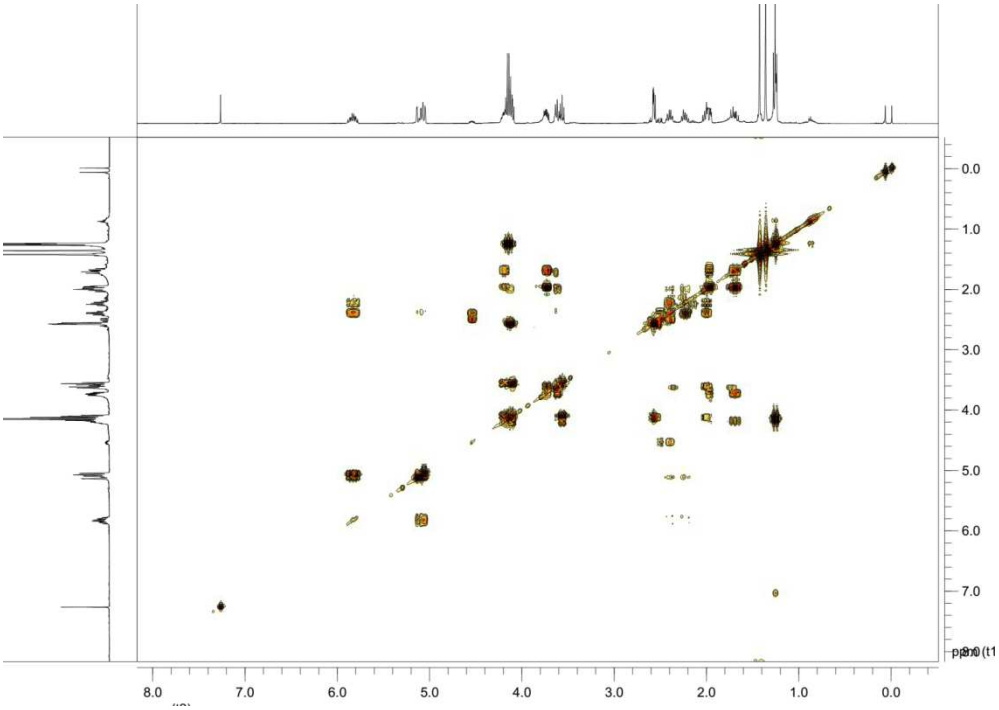
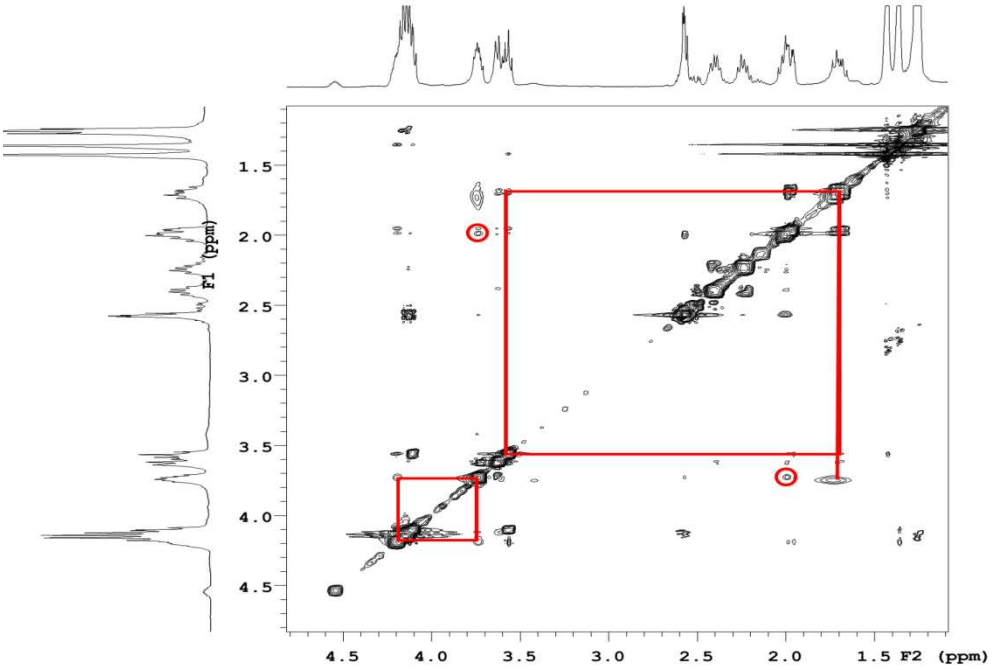
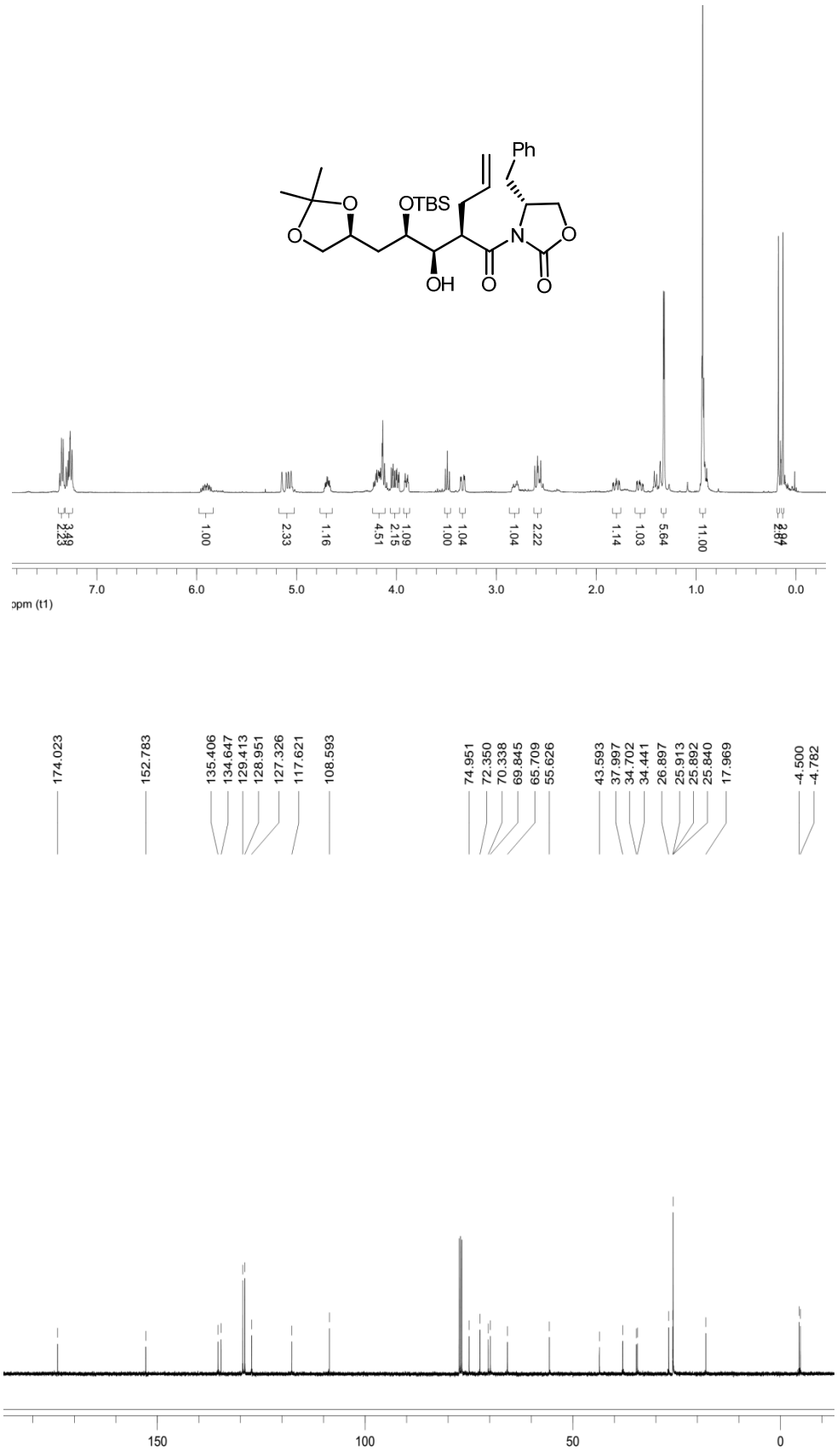


^{1}H and ^{13}C NMR of Compound **8.4**

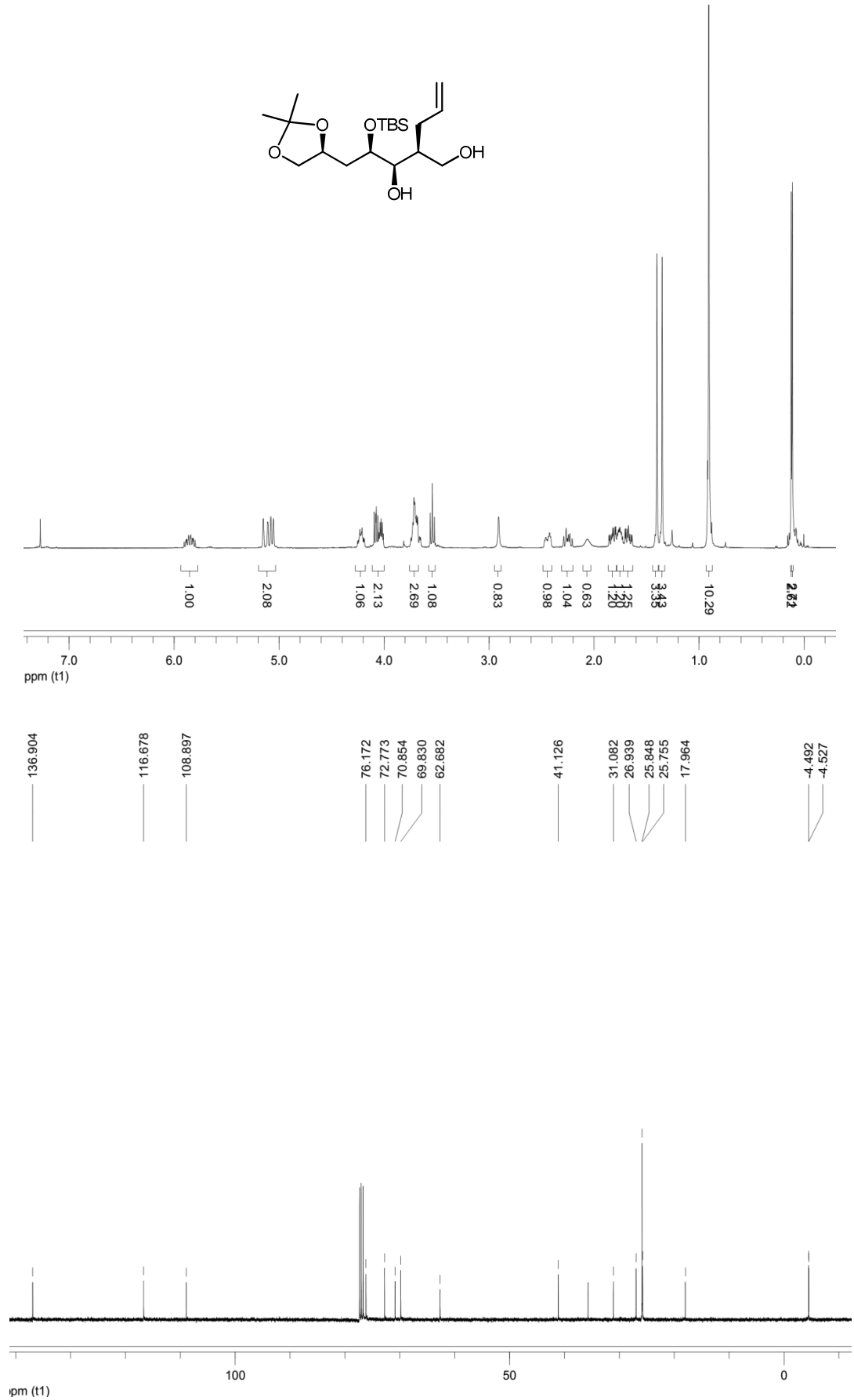




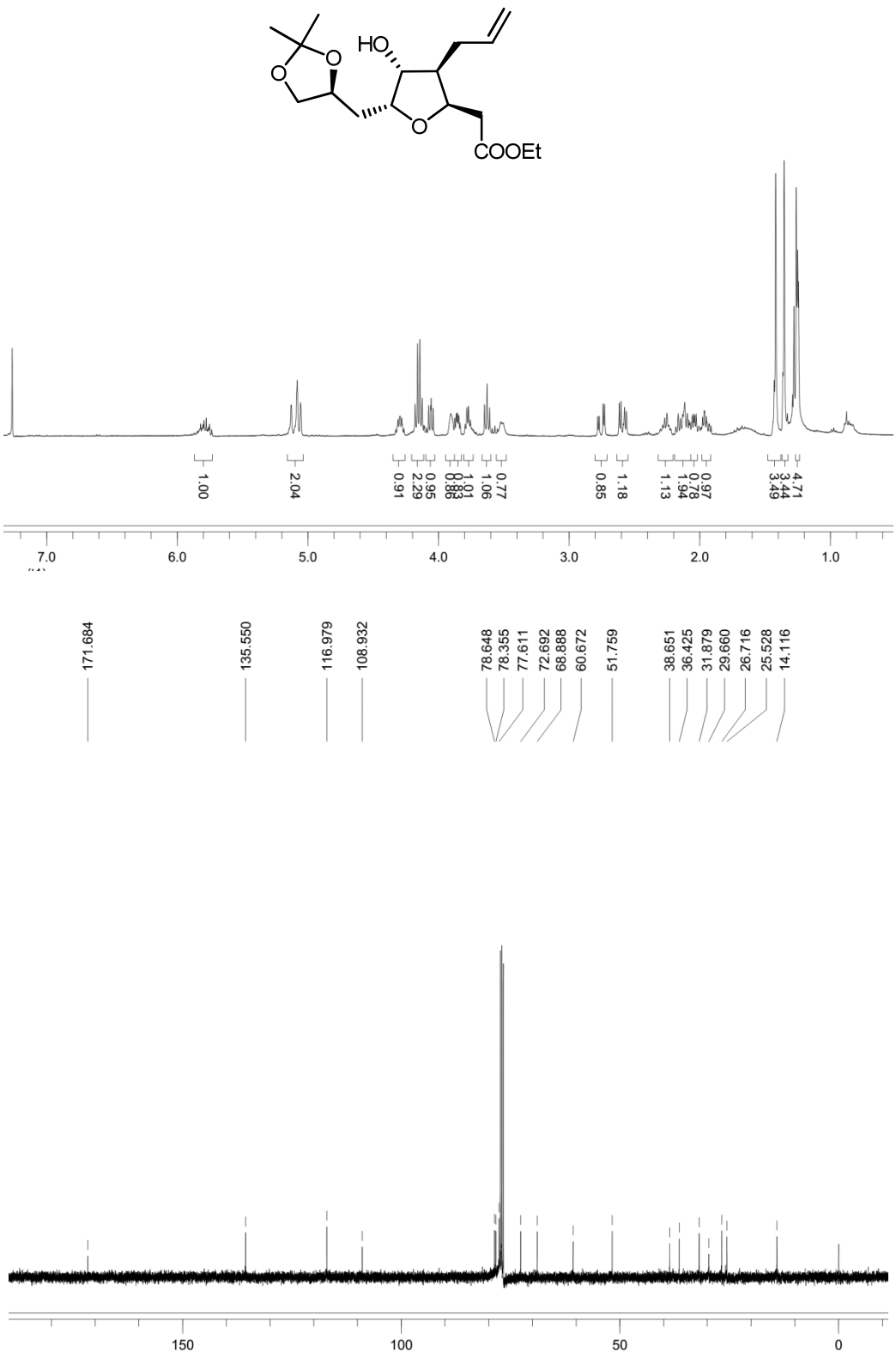
COSY and NOESY of compound **8.4**



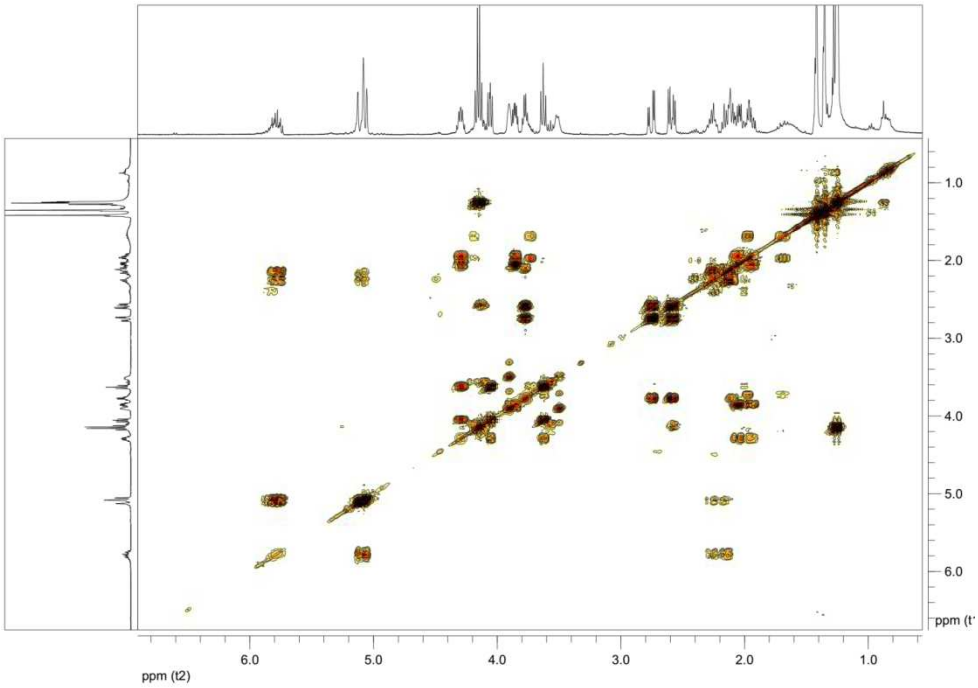
^{1}H and ^{13}C NMR of Compound **9.1**

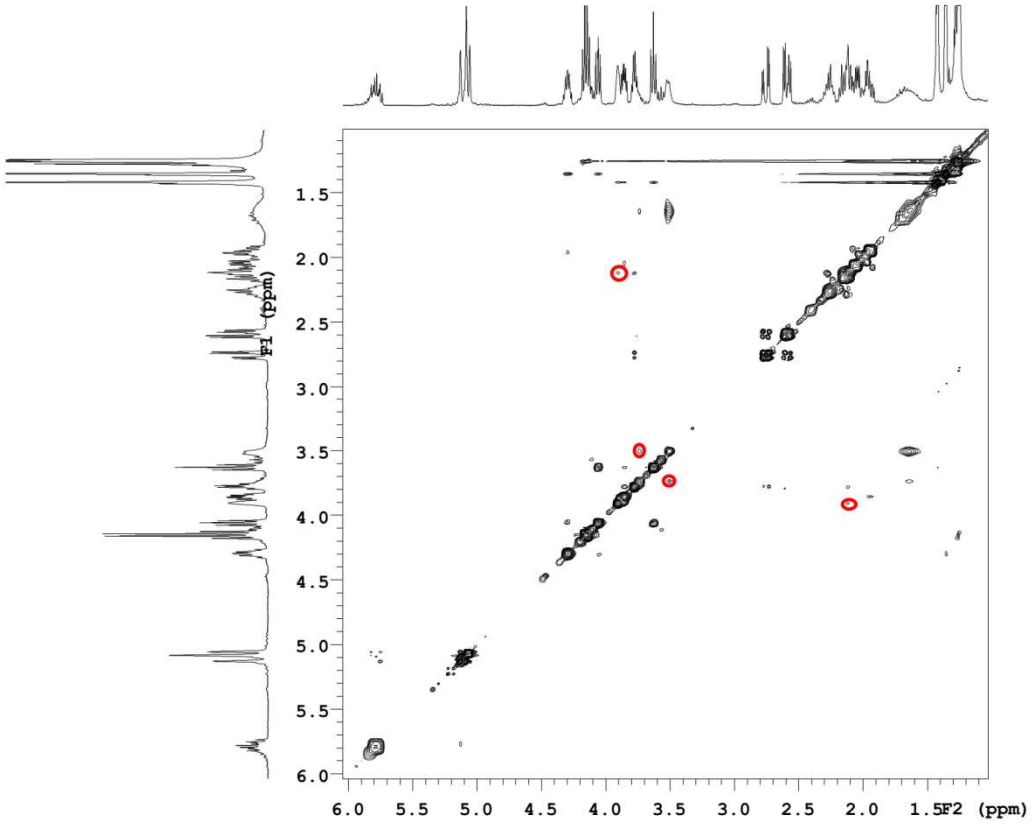


^{1}H and ^{13}C NMR of Compound $\mathbf{S_2}$

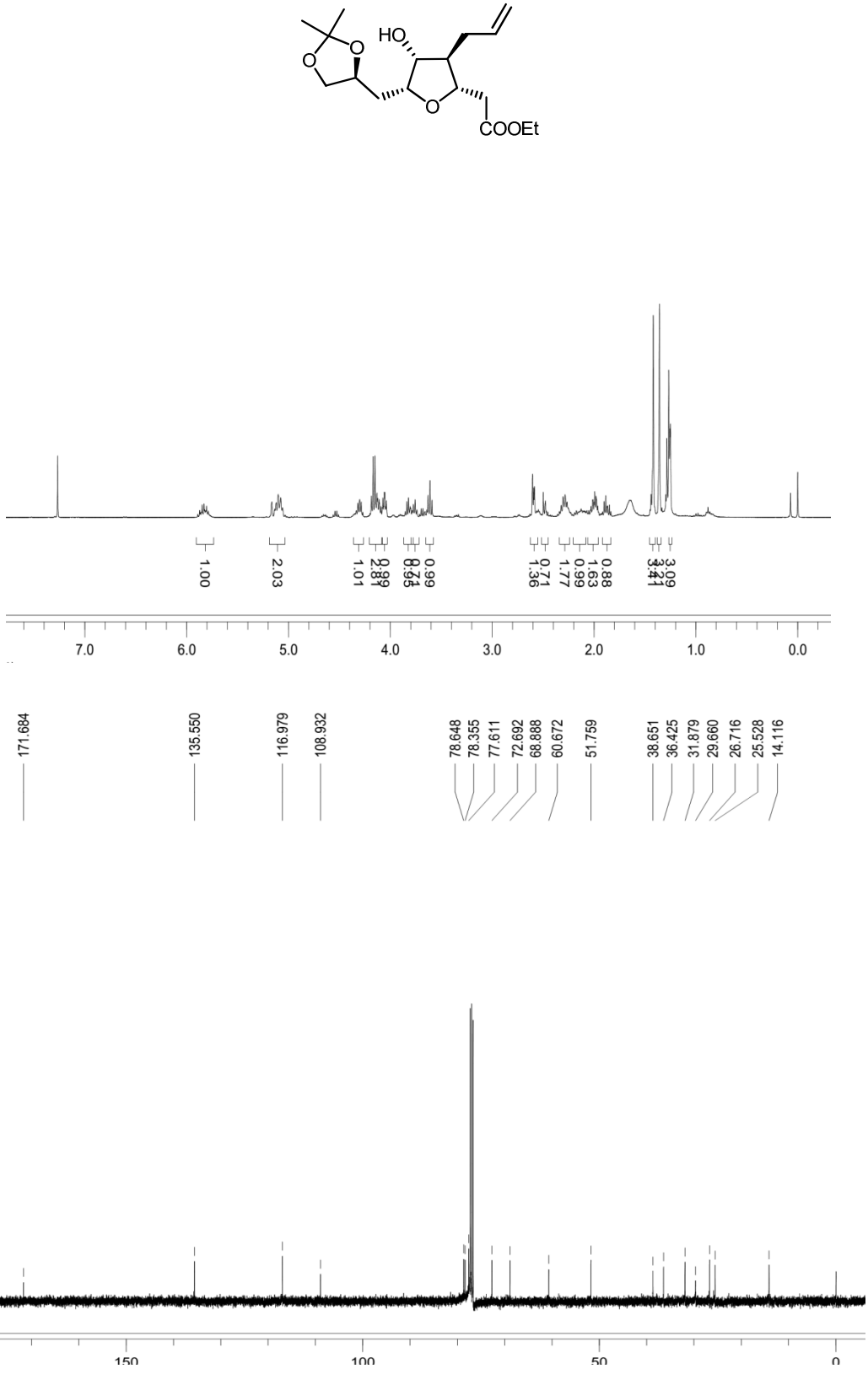


^{1}H and ^{13}C NMR of Compound **9.4**

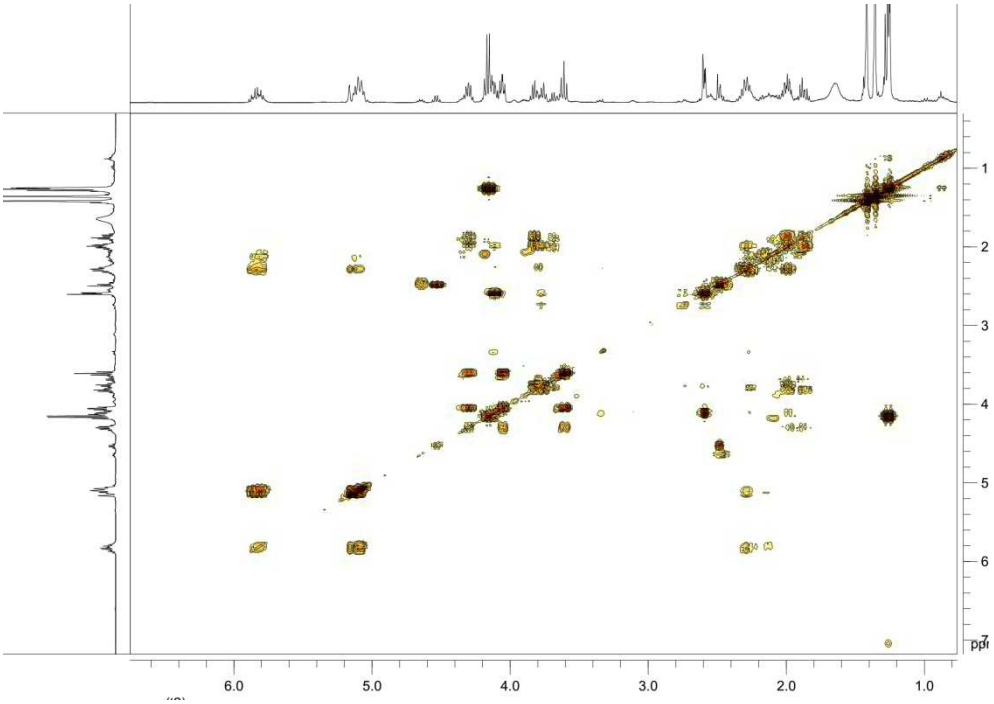


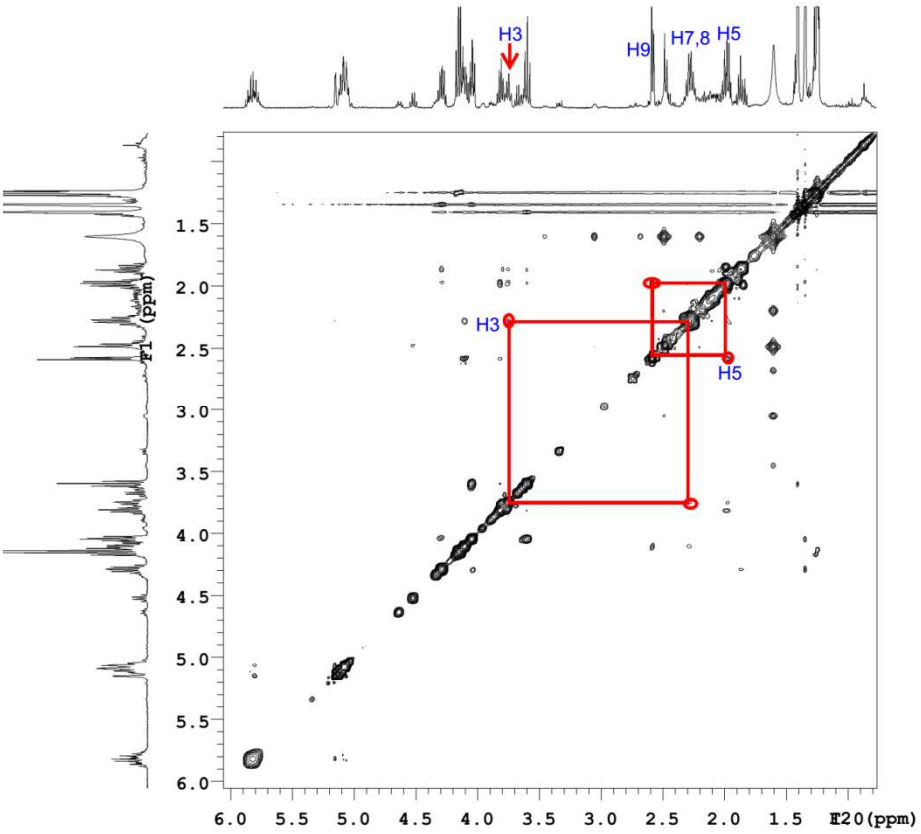


COSY and NOESY of compound **9.4**

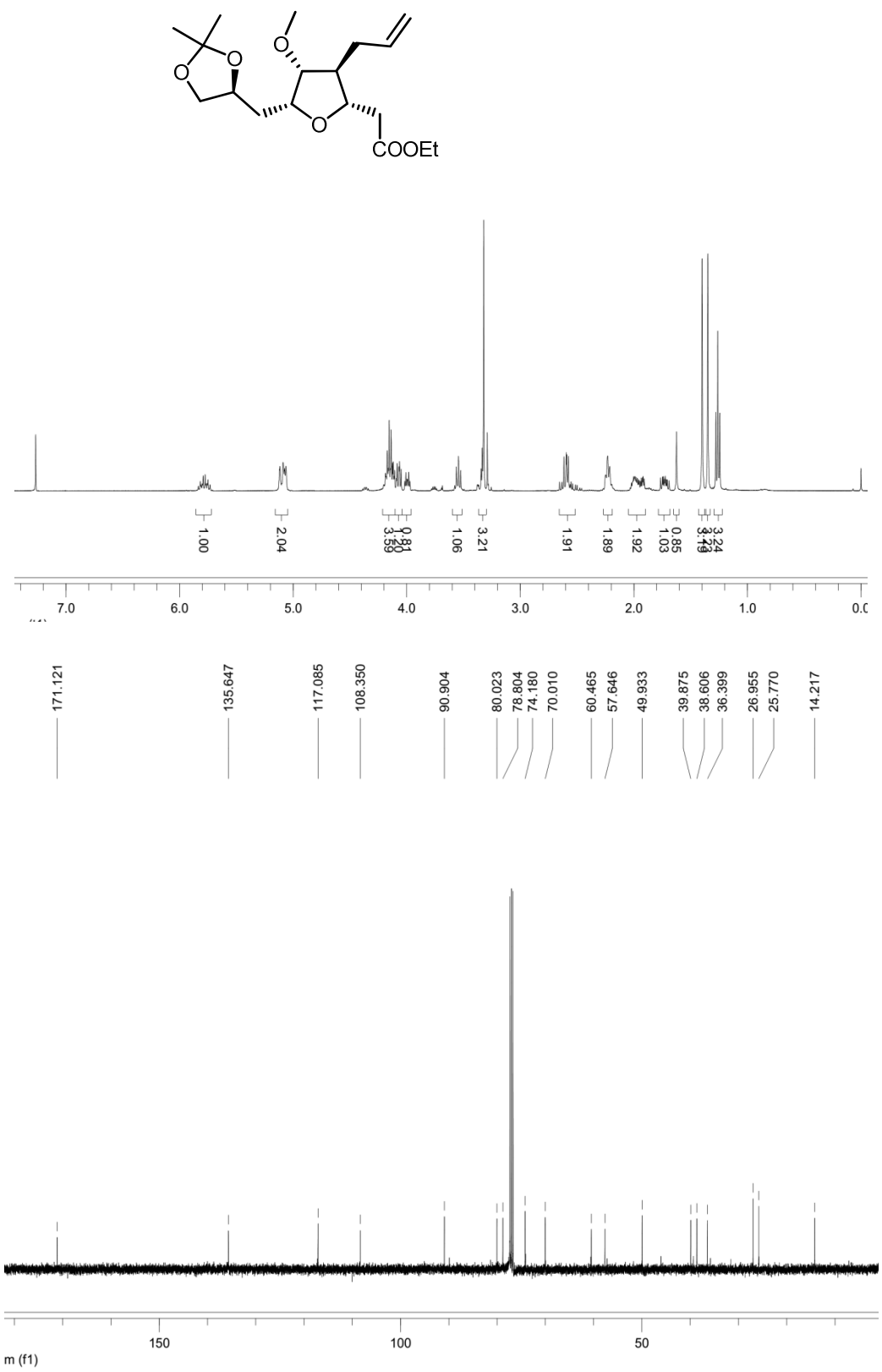


^{1}H and ^{13}C NMR of Compound **9.3**

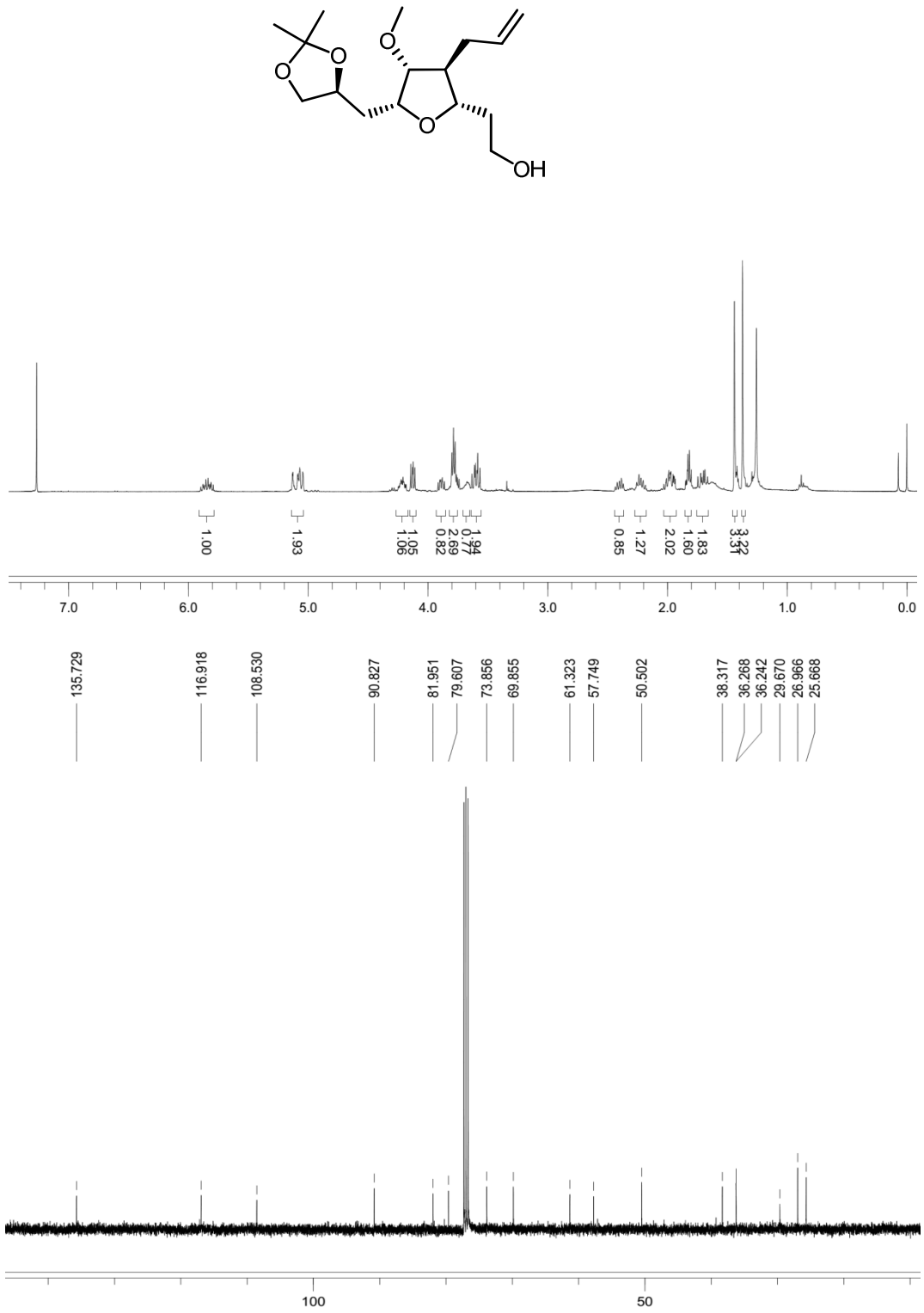




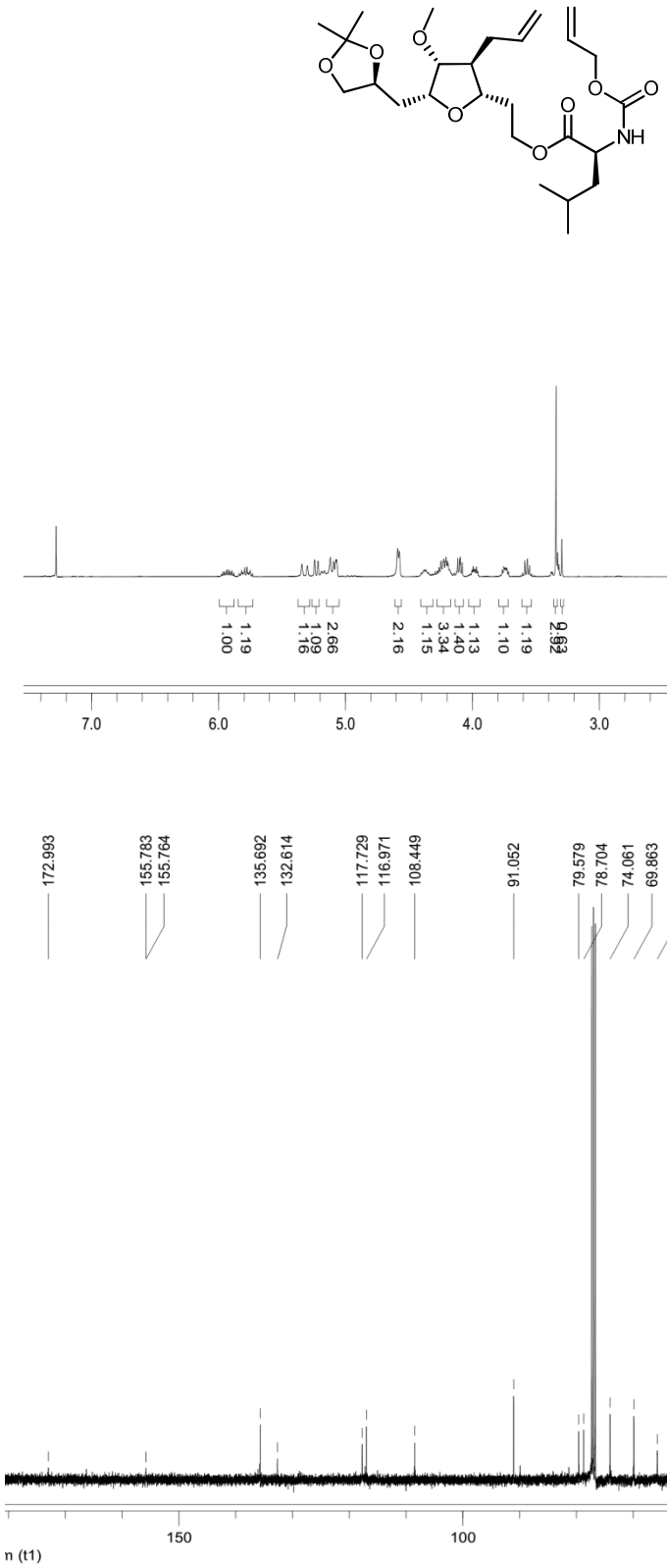
COSY and NOESY of compound **9.3**



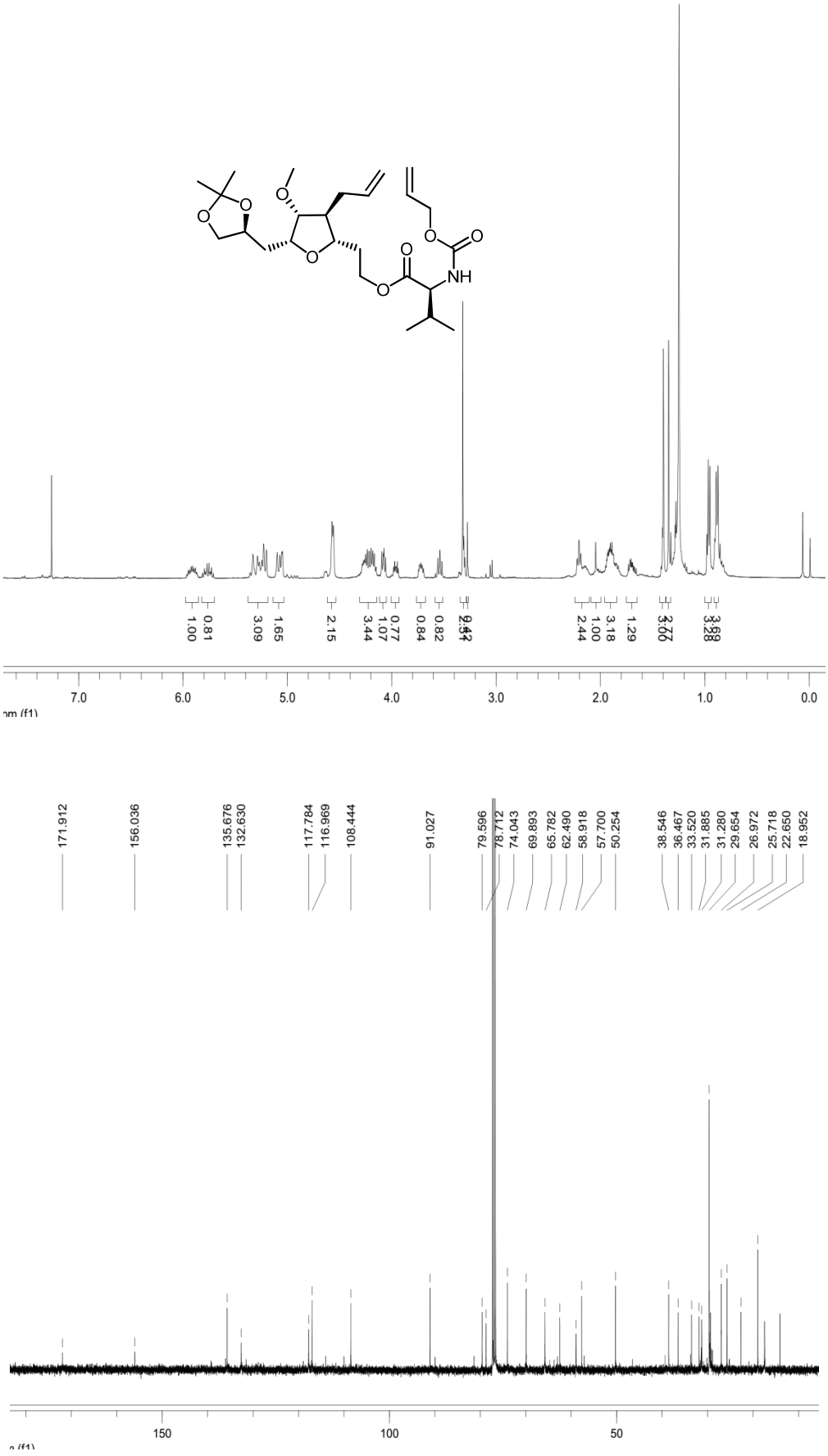
^{1}H and ^{13}C NMR of Compound **9.5**



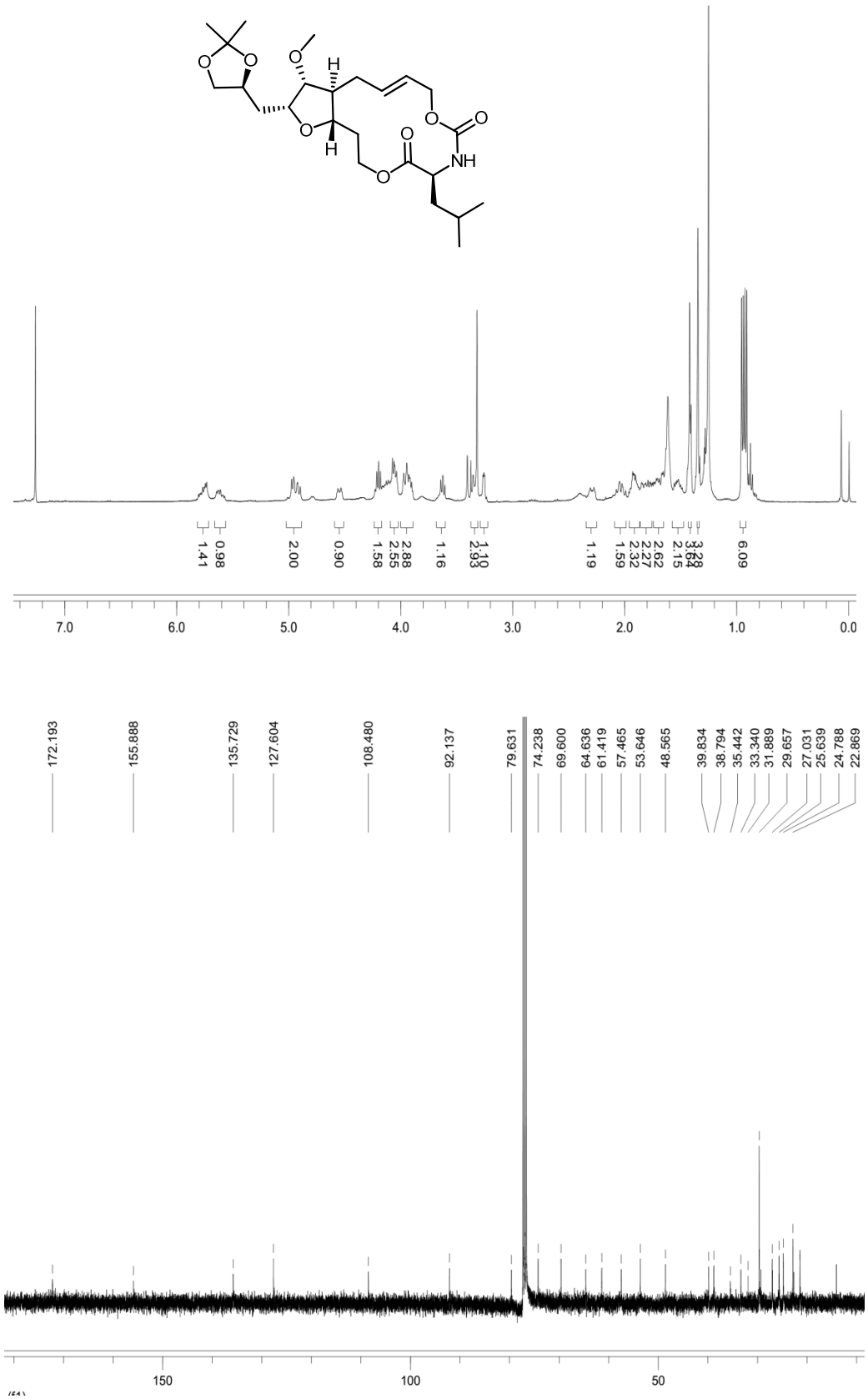
^{1}H and ^{13}C NMR of Compound **10.1**



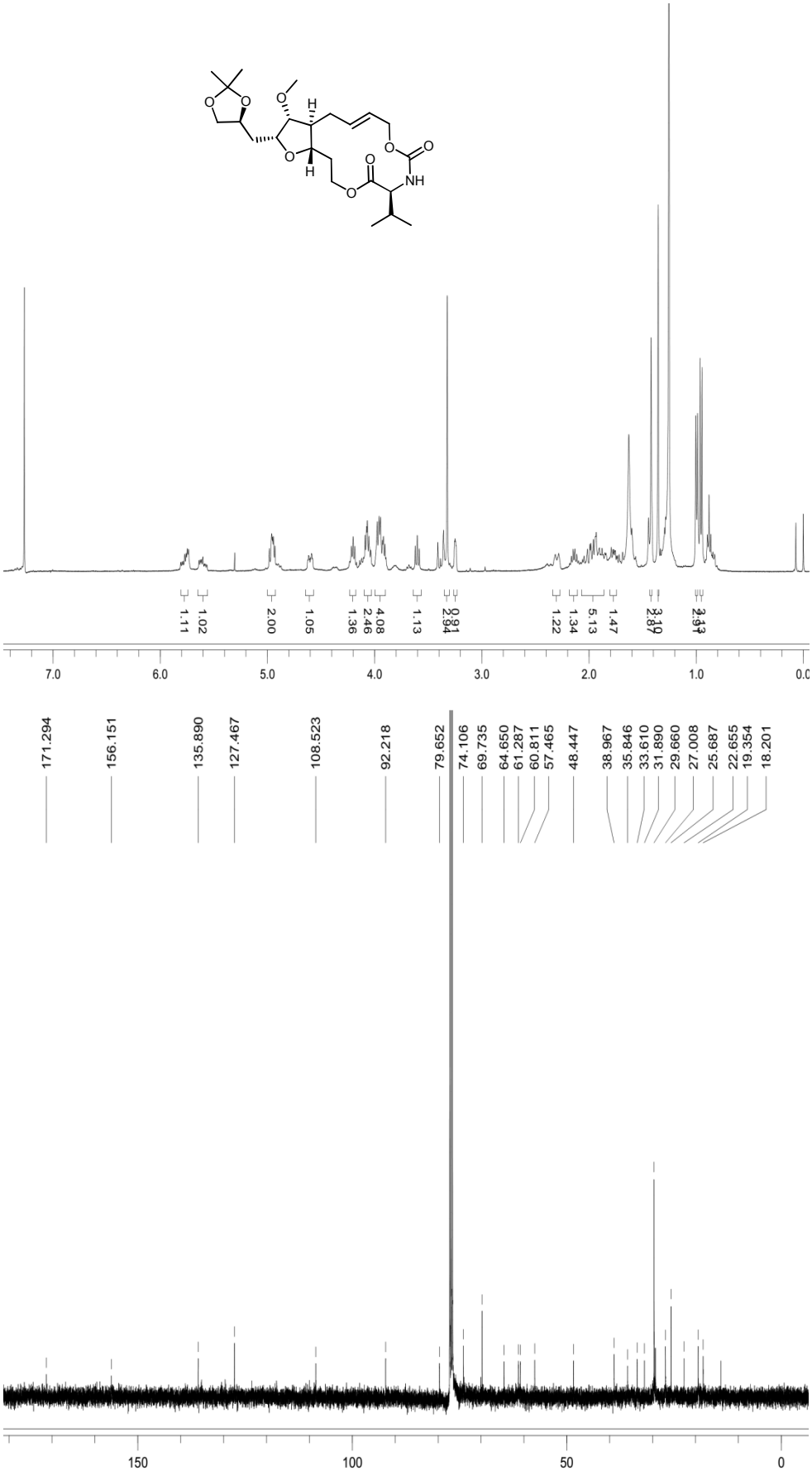
^{1}H and ^{13}C NMR of Compound **10.2b**



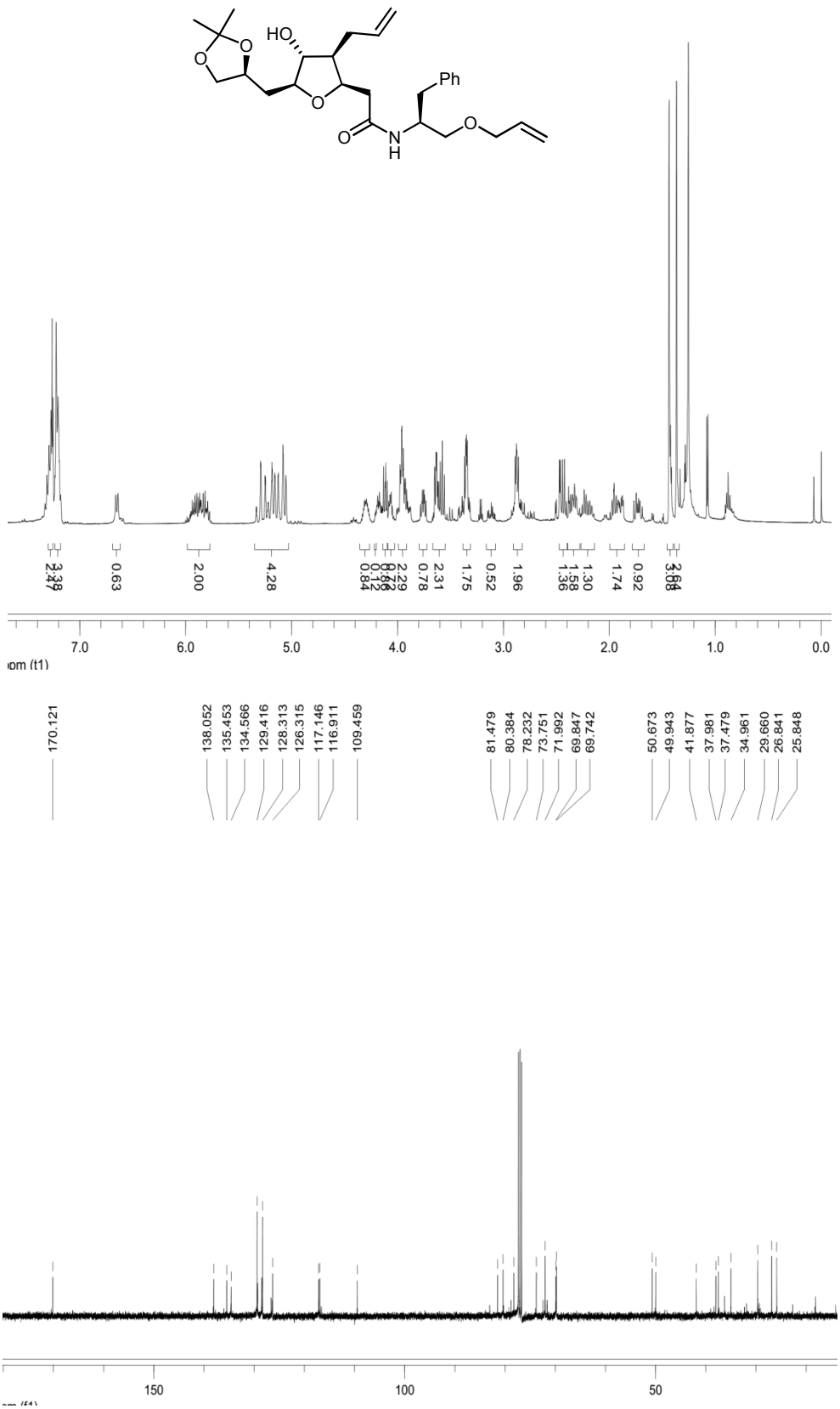
^{1}H and ^{13}C NMR of Compound **10.2c**



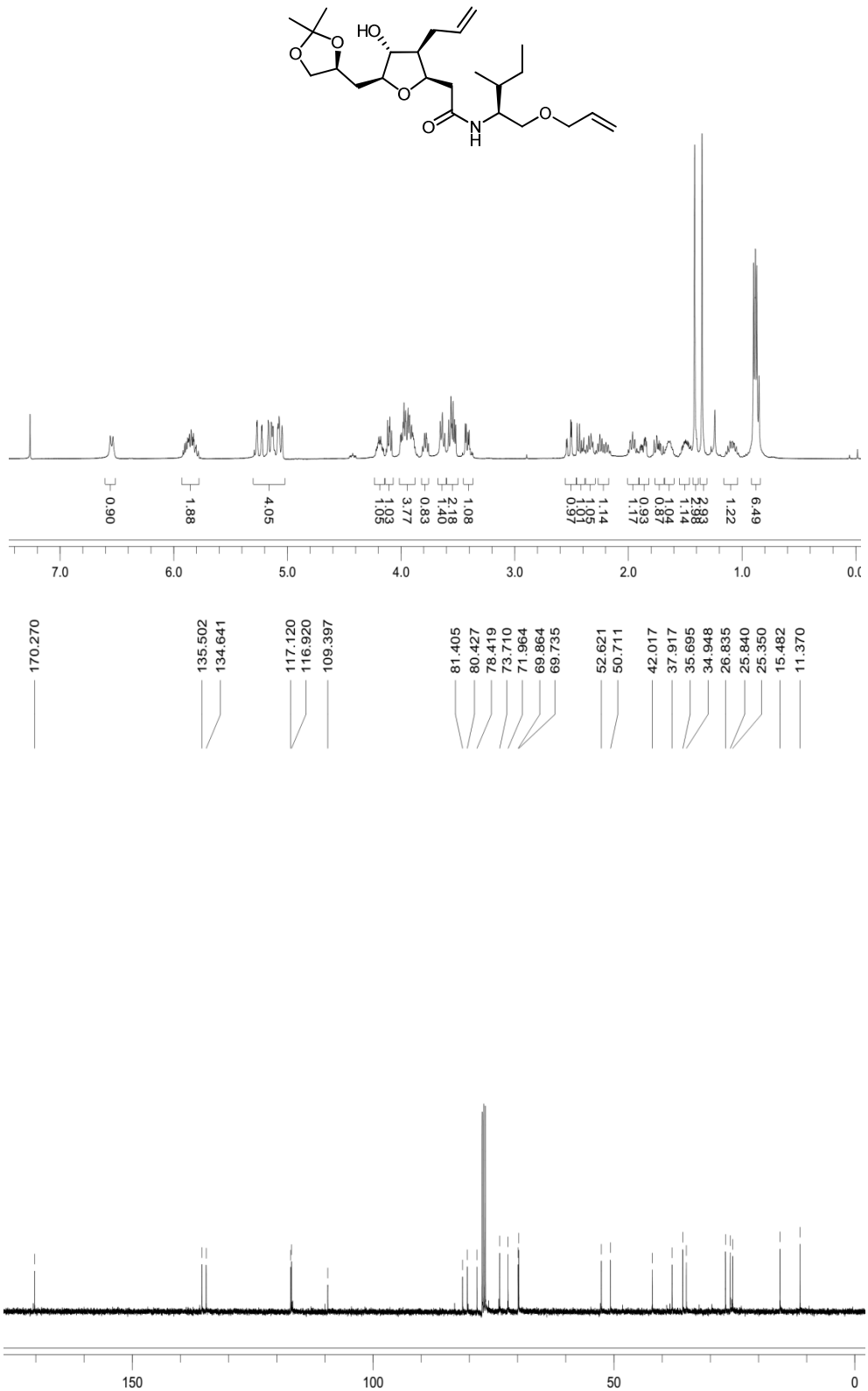
^{1}H and ^{13}C NMR of Compound **F8.1b**



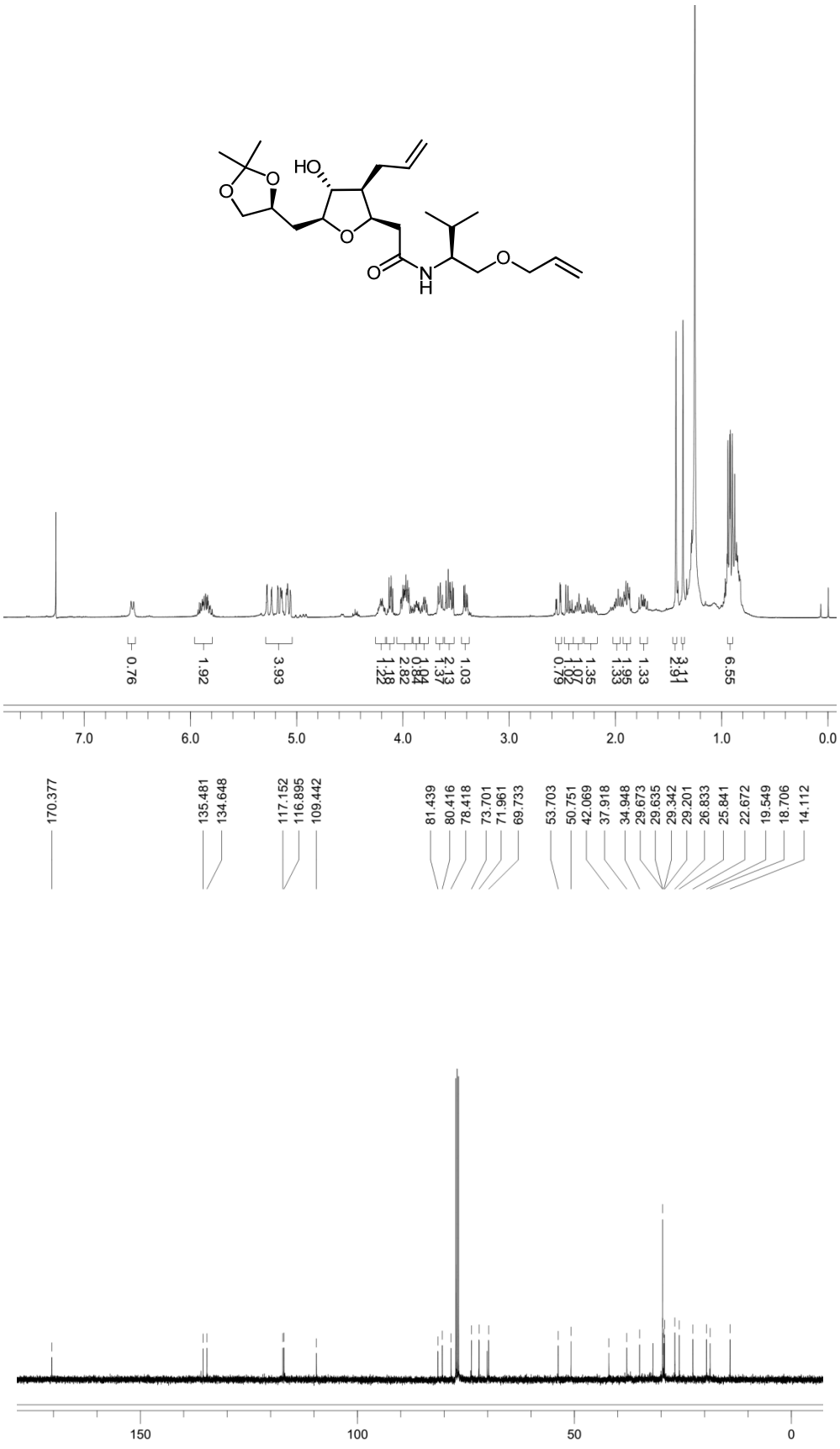
^{1}H and ^{13}C NMR of Compound **F8.1c**



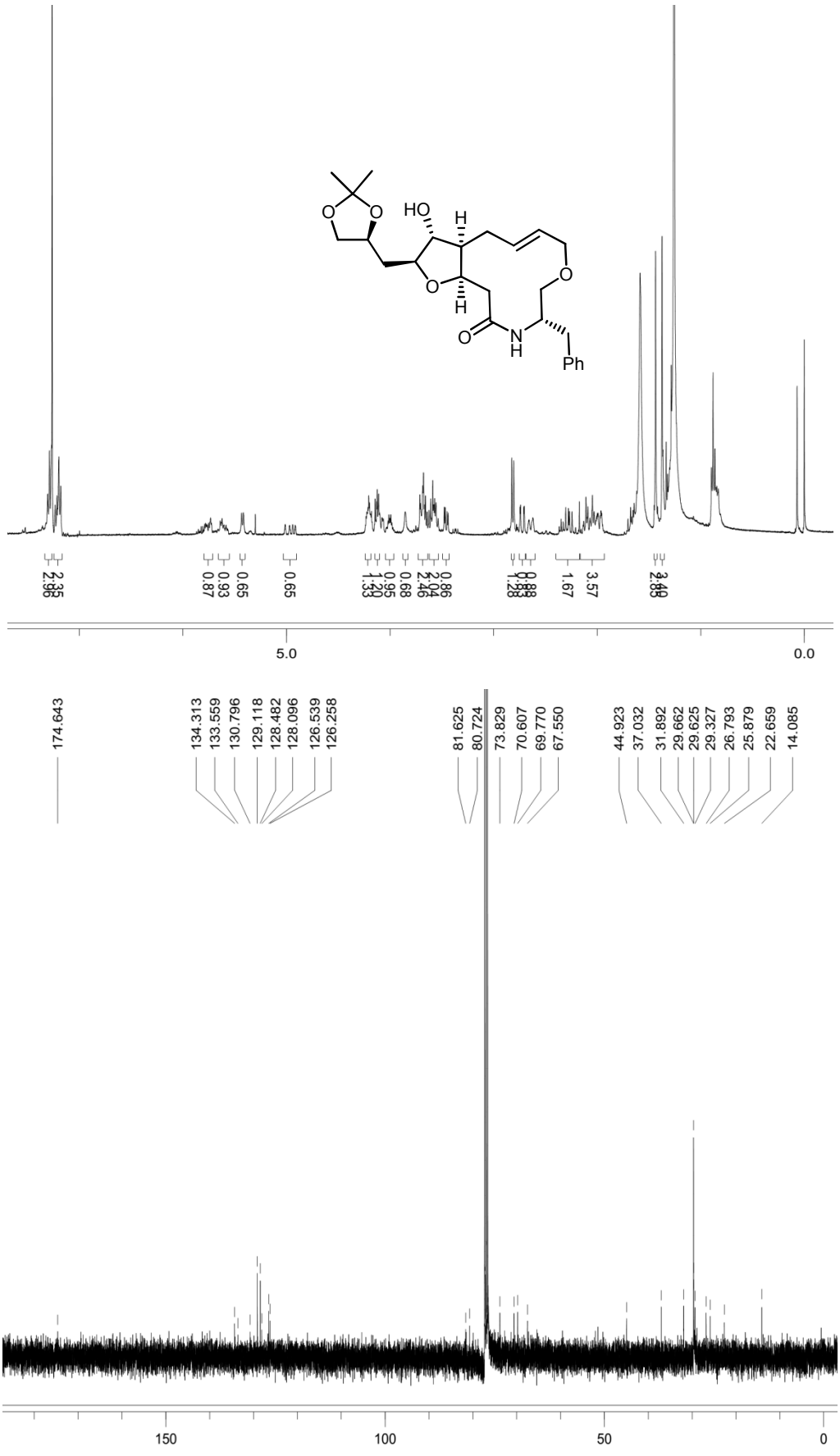
^{1}H and ^{13}C NMR of Compound **11.3a**



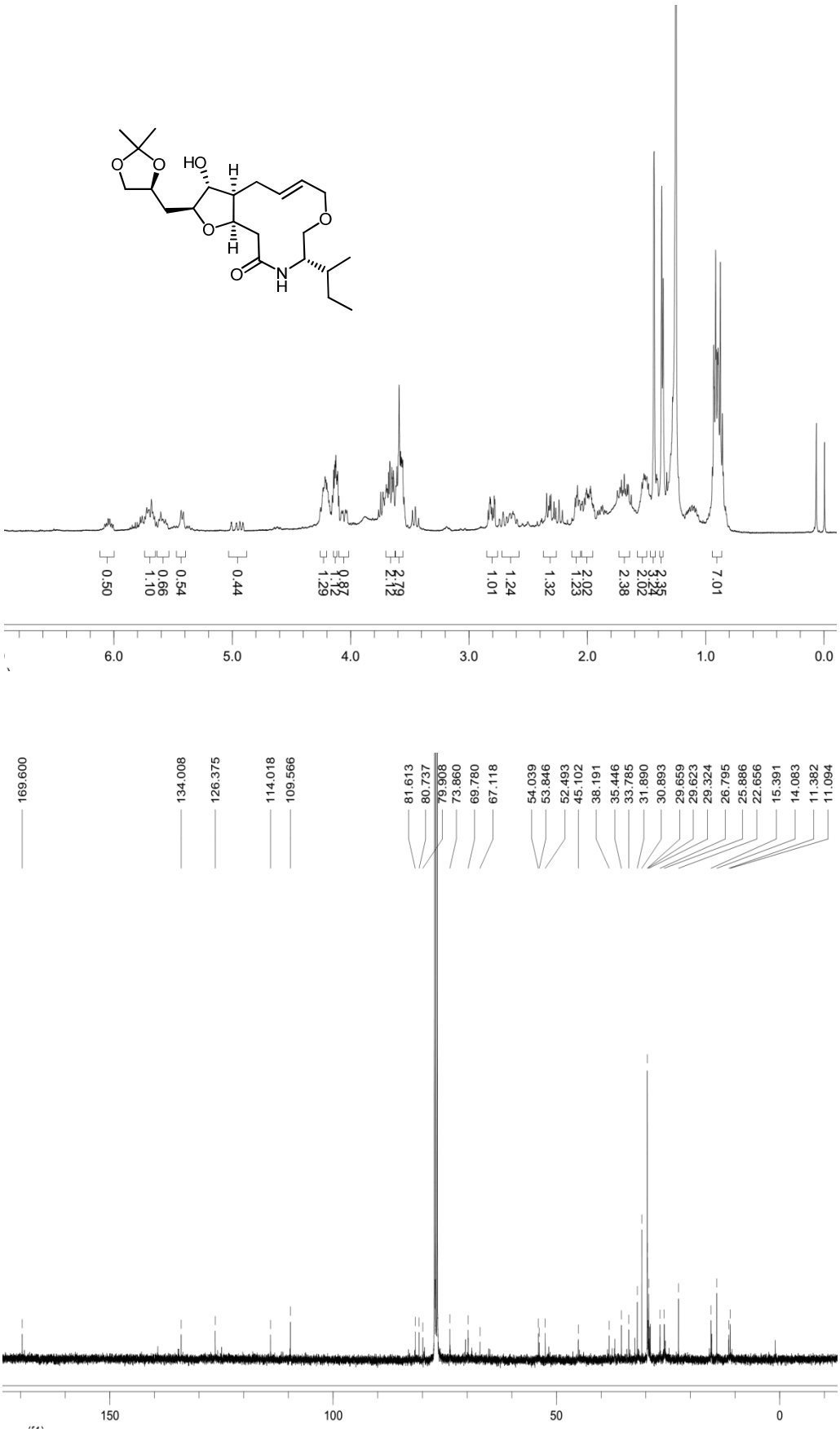
^{1}H and ^{13}C NMR of Compound **11.3b**



^{1}H and ^{13}C NMR of Compound **11.3c**



^{1}H and ^{13}C NMR of Compound **F8.2a**



^{1}H and ^{13}C NMR of Compound **F8.2b**

Ravikumar Jimmidi Peer Reviewed Publications

1. Small Molecule Modulators of Protein-Protein Interactions: Selected Case Studies. Aeluri, M.; Chamakuri, S.; Dasari, B.; Guduru, S. K. R.; Jimmidi, R.; Jogula, S.; Arya, P. *Chem. Rev*. **2014,** *114*, 4640-4694 (for a theme topic, May 2014 onwards: Chemical Biology of Protein-Protein Interactions; Guest Editor: Prabhat Arya)
2. Prevention of Mitochondrial Membrane Permeabilization and Pancreatic β-Cell Death by an Enantioenriched, Macrocyclic Small Molecule. Jimmidi, R.; Shroff, G. K.; Satyanarayana, M.; Reddy B. R.; Kapireddy, J.; Sawant, M. A; Sitaswad, S. L.; Arya, P.; Mitra, P. *Eur. J. Org. Chem.* **2014**, 1151-1156.
3. Identification and Characterization of Novel Inhibitors of the Pro-apoptotic Proteins Bax and Bak: Xin Niu, Hetal B., Eve Wong, Jamie M., Jyoti P. Nandy, Maragani S., Jimmidi, R., Jing Yi, Arya P., Brian L., and Andrews, D. W. (under review).
4. Synthesis of C25-C35 Eribulin Fragment and Its Derived Hybrid Macrocycles. Jimmidi, R.; Arya, P. 2**014** (Manuscript under preparation).
5. Building a Natural Product-inspired, Small Molecule Toolbox for Protein:Protein Interactions. Aeluri, M.; Chamakuri, S.; Dasari, B.; Guduru, S. R. K.; Jimmidi, R.; Jogula, S.; Arya, P. *Acc. Chem. Res*. **2014**. (submitted).
6. Selected Hybrid Natural Products as Tubulin Modulators. Dasari, B; Jimmidi, R; Arya. P. Invited mini-review article, *Eur. J. Med. Chem.* **2014**, (accepted).

Participation in Conferences

2014 Attended and presented a poster at the international conference, “Nature Inspired Initiatives in Chemical Trends” organized by CSIR-IICT, India, March 2-5.

2014 Participated in national symposium on “Shifting Paradigms on New Chemical Entities in India- Role of Process Chemistry” organized by C.K.M Art & Science College, Warangal, India, 22nd and 23rd February.

2013 Attended and presented a poster in conference, “Advances in Anticancer Drug Discovery and Development” organized by IIT Madras, India, October 25th and 26th.

2008 Participated in UGC sponsored National Symposium on "Emerging Trends In Medicinal Chemistry: India- A Global Hub" conducted by C.K.M Art & Science College, Warangal, India, 2nd and 3rd February.