Synthesis of Amino-indoline Based Macrocycles and Treprostinil Inspired Hybrid Compounds

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

BY
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August 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.

Dr. Reddy’s Institute of Life Sciences **Srinivas Chamakuri**
University of Hyderabad
August 2014

CERTIFICATE

This is to certify that the thesis entitled ***"Synthesis of Amino-indoline Based Macrocycles and Treprostinil Inspired Hybrid Compounds"*** being submitted by ***Mr. Srinivas Chamakuri*** to University of Hyderabad for the award of ***Doctor of Philosophy in Chemistry*** has been carried out by him under my supervision and the same has not been submitted elsewhere for a degree. I am satisfied that the thesis has reached to the standard of fulfilling the requirements of the regulations relating to the nature of the degree.

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

Dr. Reddy's Institute of Life Sciences **Srinivas chamakuri**
August 2014

Synopsis

The thesis entitled**, “Synthesis of Amino-indoline Based Macrocycles and Treprostinil Inspired Hybrid Compounds”** contain three chapters

Chapter 1: Introduction: Natural Products and Synthetic Macrocycles.

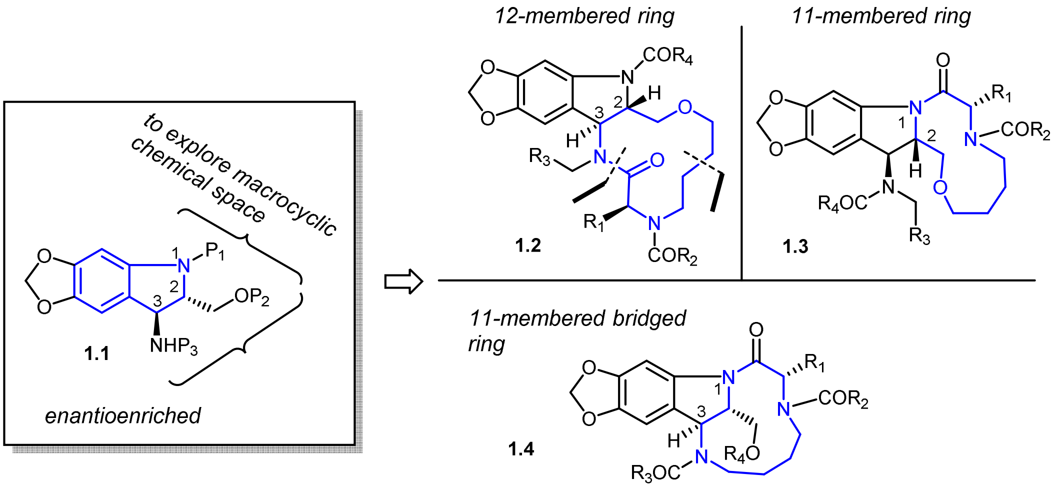
This chapter covers the importance of various natural product macrocycles and the synthetic macrocycles. In addition to discussing some of the selective synthesis example of bioactive natural products, their use in addressing biological questions related to portein-protein interactions is also covered

Chapter 2: Synthesis of Indoline Alkaloid Natural Product Inspired Macrocycles.

(*Eur. J. Org. Chem.* **2013,** *19,* 3959-3964)

With the goal of expanding the currently accepted chemical space to search for small molecule modulators of protein-protein, DNA/RNA-protein interactions and signaling pathways, the past few years has seen a growing interest in accessing small molecules that are more natural product-like, and, are obtained from the inspiration from bioactive natural products. Traditionally small molecules that are broadly available to medicinal chemistry community for an early biological evaluation are rich in sp^2 character, and in general, lack the features that are commonly found in bioactive natural products. Several leading review articles are being written on this topic that challenges the organic synthesis community to access small molecules for biological evaluation in the broad areas of protein-protein and other bio-macromolecular interactions.

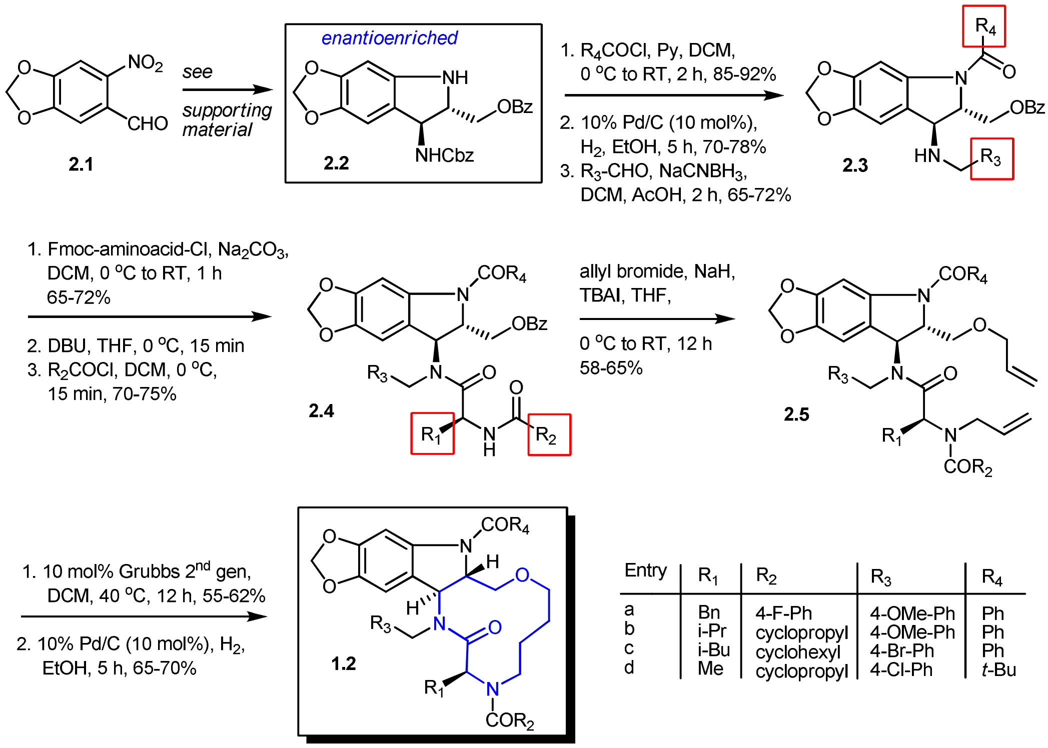
In particular, the interest in accessing functionalized large ring derivatives (i.e. macrocyclic compounds) is growing due to several reasons: (i) large ring structures present an opportunity to map a large surface area to interact with the protein targets, (ii) pre-organization in large rings allow to display several functional groups in specific orientations, and, (iii) in general, cyclic compounds possess better cell permeation properties. Despite all these attractive features that large rings offer and an excellent track record of several macrocyclic natural products as drugs, the full potential of this area remains to be explored thoroughly. One of the challenges in this-arena is the development of synthesis methods that are modular and practical in nature to allow obtaining different-types of functionalized medium to large rings. We initiated the synthesis program that was aimed at developing methods to allow us accessing functionalized, *enantioenriched* natural product-inspired scaffolds, a few years ago. In one study, we developed a practical synthesis of an aminoindoline scaffold having three orthogonally protected functional groups (**1.1**). As an extension to this work, with an objective to explore the macrocyclic chemical space, herein, we report a modular method that allow us to incorporate different-type of large ring skeletons (see, **1.2**, **1.3** and **1.4**, Scheme 1) onto an *enantioenriched* aminoindoline scaffold.



Scheme 1. The proposed plan to obtain three macrocyclic-derived compounds **1.2, 1.3** and **1.4** from an *enantioenriched*, aminoindoline scaffold **1.1**.

There are several attractive features in the aminoindoline scaffold (**1.1**) that makes it a highly attractive starting point to incorporate various large rings onto the skeleton. These include, (i) the presence of *trans* 2,3-aminoalcohol moiety that can further be utilized in building the additional *trans*-fused 12-membered ring (**1.2**), (ii) 1,2-aminoalcohol utilizing the indoline nitrogen to provide 11-membered ring (**1.3**), and (iii) 1,3-orthogonally protected di-amino functional groups that can lead to synthesize bridged 11-

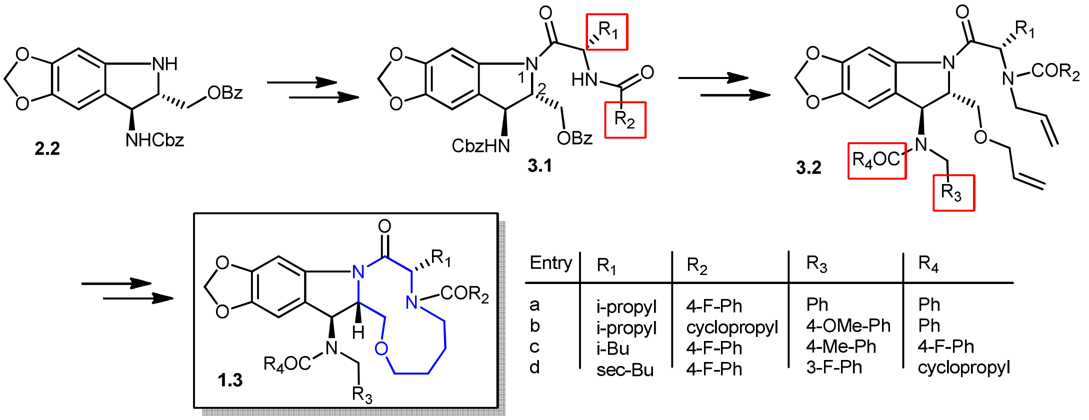
membered ring (**1.4**). In all the three strategies, we utilize a common approach to incorporate the amino acid functionality and the stitching technology to construct the macrocyclic rings.



Entry	R_1	R_2	R_3	R_4
a	Bn	4-F-Ph	4-OMe-Ph	Ph
b	i-Pr	cyclopropyl	4-OMe-Ph	Ph
c	i-Bu	cyclohexyl	4-Br-Ph	Ph
d	Me	cyclopropyl	4-Cl-Ph	*t*-Bu

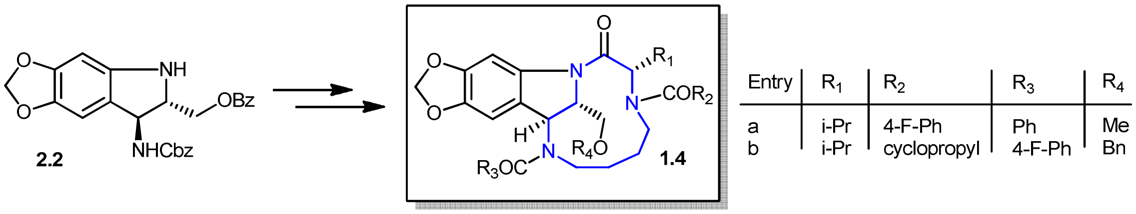
Scheme 2. The synthsis of **1.2** having a 12-membered macrocyclic ring on an aminoindoline scaffold

Our synthesis method to obtain 12-membered ring macrocyclic ring derivative **1.2** from an *enantioenriched* aminoindoline scaffold is outlined in Scheme 2. The free amine of **2.2** was acylated (i.e. R_4 as the first diversity), and following the -*N*Cbz removal (10% Pd over C), it was then subjected to reductive alkylation to obtain the secondary amine **2.3** in good yields (i.e. R_3, as the second diversity). The secondary amine was then coupled with different Fmoc amino acids that allowed us to introduce the diversity site as R_1 (i.e. the side chain of an amino acid). The -*N*Fmoc group was removed and the free amine was then acylated with acid chlorides to provide **2.4** with the fourth diversity (R_2). Upon treatment under basic conditions and with allyl bromide, it produced the *bis*-allylated product **2.5** in respectable yields. Under the reactions conditions that may have the traces of moisture, it also leads to benzoyl ester hydrolysis, which is then followed by *O*-allylation. The next crucial step was to explore the ring closing metathesis-based stitching technology on this highly functionalized substrate **2.5**. This reaction worked well, and as we proposed, the additional 12-membered macrocyclic ring was easily obtained. The cyclic product was then subjected to hydrogenation to obtain the saturated version as a clean product **1.2** that was well-characterized through various analytical techniques (HPLC-MS and NMR).



Entry	R_1	R_2	R_3	R_4
a	i-propyl	4-F-Ph	Ph	Ph
b	i-propyl	cyclopropyl	4-OMe-Ph	Ph
c	i-Bu	4-F-Ph	4-Me-Ph	4-F-Ph
d	sec-Bu	4-F-Ph	3-F-Ph	cyclopropyl

Scheme 3. The synthesis of **1.3** with an eleven membered ring



Entry	R_1	R_2	R_3	R_4
a	i-Pr	4-F-Ph	Ph	Me
b	i-Pr	cyclopropyl	4-F-Ph	Bn

Scheme 4. The incorporation of 11-membered bridged macrocycle

In similar manner 11 membered **(1.3)** and 11 membered bridged **(1.4)** macrocycles are synthesized. All the small molecules obtained from this project (in total 36 compounds) were tested in a zebrafish based assay to search for anti-angiogenesis agents and the inhibitors of an early embryo development (Figure 1). This was achieved in collaboration with Satish Kitambi team at the Karolinska Institute in Sweden. This study identified some novel small molecules as anti-angiogenesis agents and the inhibitors of early embryonic development

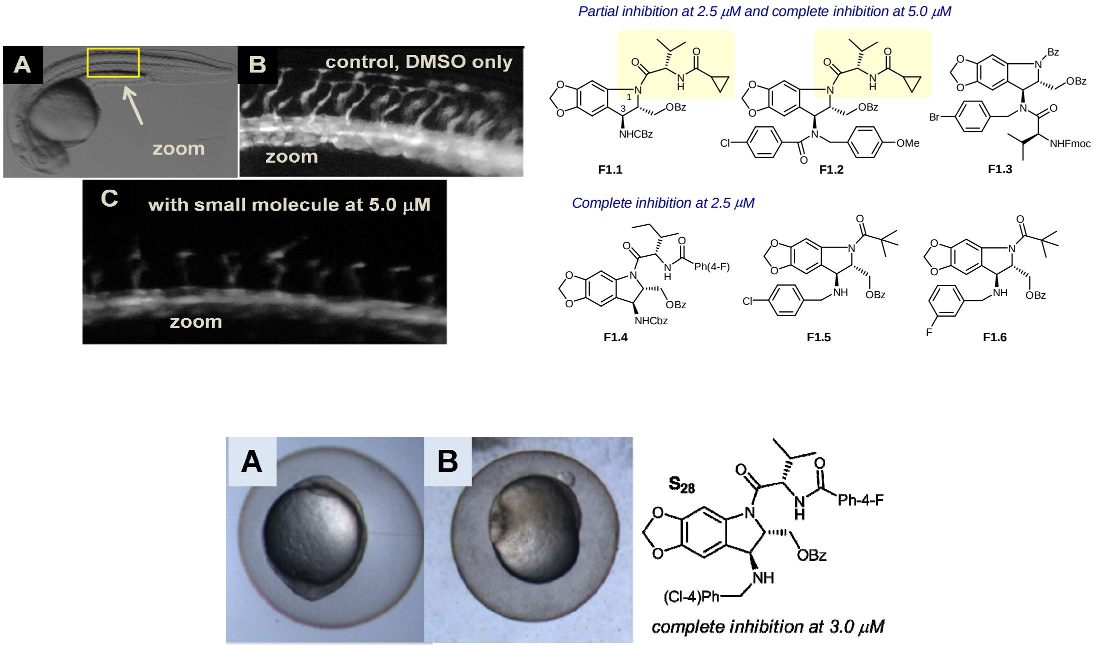


Figure 1. Zebrafish angiogenesis assay: (A) embryonic zebrafish, (B) control with DMSO, (C) anti-angiogenesis effect of **F1.1** at 5.0 µm; Embryo development assay: (A) normal development, (A) the effect of small molecule $\mathbf{S_{26}}$ at 3.0 µm.

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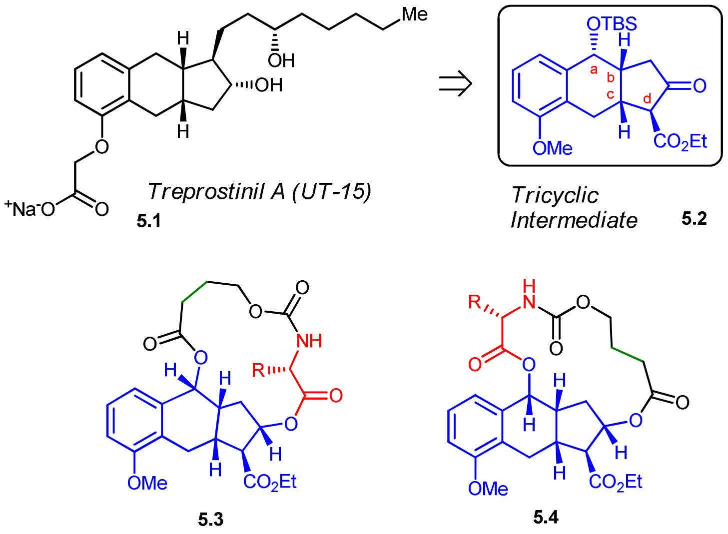
Chapter 3: Synthesis of Treprostinil Inspired Hybrid Macrocycles.

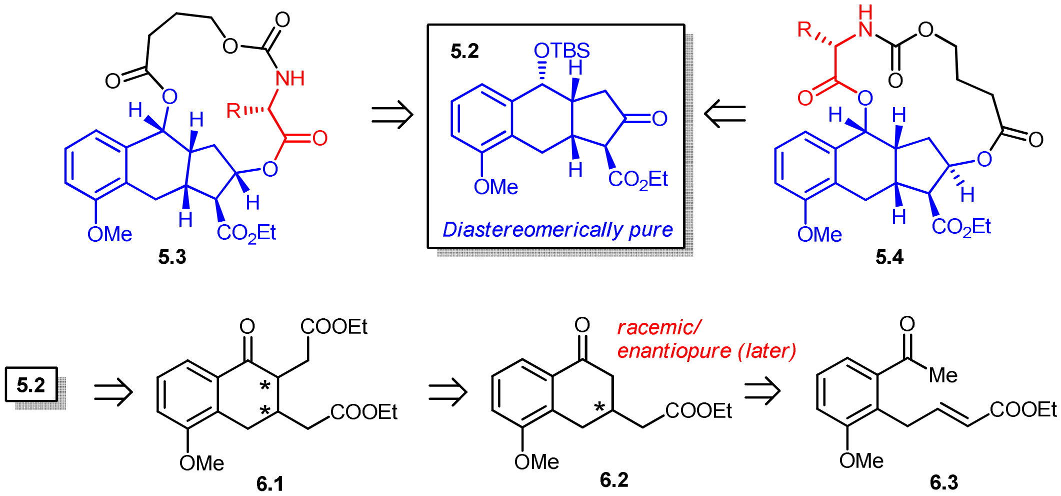
(*Manuscript under preparation*)

Treprostinil A is a FDA approved drug, known as UT-15. It is an effective drug in the treatment of pulmonary arterial hypertension. It is a part of stable synthetic analogue of prostacyclin (PGI_2). Prostacyclin is one of the important metabolic products of arachidonic acid produced in the vasculature of smooth muscle cell and endothelial cells. Prostacyclin is an effective vasodilator in both types of circulatory pathways. It was earlier used for the treatment of cardiovascular diseases. UT-15 retains all the biological activity of PGI_2. PGI_2 has a very short half-life (approx. 10 min) and is very unstable towards higher temperature. Whereas, UT-15 has a better half life of approx. 3 hrs. UT-15 is known for action towards the curing of severe congestive heart failure, severe intermittent claudication and immunosuppresion. Moreover, UT-15 has an anti proliferative effect on human pulmonary arterial smooth muscle cells. All these interesting features of UT-15 have attracted us to work on the synthesis of UT-15 and to develop methods to obtain hybrid macrocycles. Our main objective is to develop a novel route to key core of UT-15, and, further, the building of a macrocyclic chemical toolbox by exploring a new chemical space around the scaffold (key intermediate).



Our Proposed Treprostinil Inspired Hybrid Macrocycles

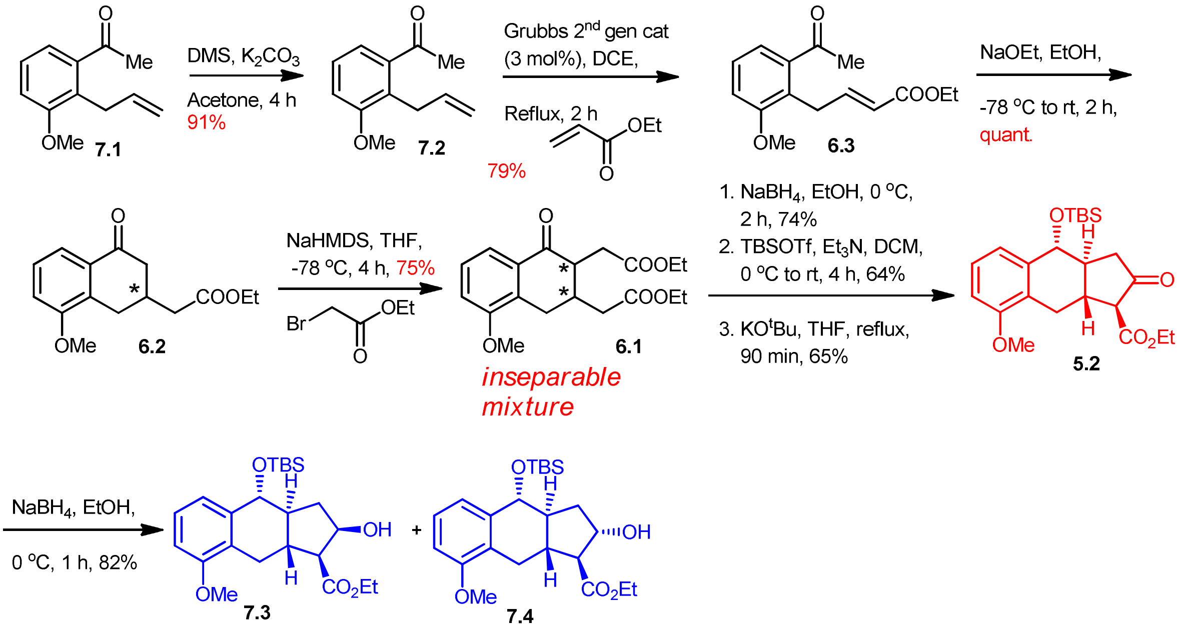
Scheme 5: Treprostinil A and Treprostinil Inspired Macrocycles



Scheme 6: Retrosynthesis of Treprostinil Inspired Macrocycles

Retrosynthesis of macrocycles (**5.3** and **5.4)** shown in **Scheme 6**. Macrocycles **5.3** and **5.4** can be synthesized from the key intermediate **5.2**, by incorporating the amino acid building block and subsequent RCM-based stitching technology. Intermediate **5.2** is synthesized from **6.1** by the functional group modification and Dieckmann cyclization.

Compound **6.1** could be derived from α–alkylation of carbonyl compound **6.2**, this bicyclic compound achieved from intermediate **6.3** by using Michael addition.



Scheme 7: Synthesis of key intermediate, **5.2** (racemic)

Our synthesis started from the phenolic derivative **7.1** which is derived from commercially available 3-hydroxyacetophenone. Protection of **7.1** followed by the cross metathesis gave the Michael precursor (**7.2**) in good yields. We then synthesized the bicyclic, racemic compound **6.2** from **6.3** by using NaOEt and this approach gave a quantitative yield of the bicyclic scaffold **6.2**. It was further subjected to enol ether alkylation condition giving compound **6.1** as the 6:1 mixture of inseparable diastereomers (note: the ratio was determined by NMR). Compound **6.1** as the diastereomeric mixture was then subjected to carbonyl reduction, silylation followed by Dieckmann cyclization to obtain the key intermediate **5.2**.

To our surprise, we obtained only one product that is a result of a region and stereoselective Dieckmann cyclization. The NMR of compound **5.2** is clean and further COSY and NOESY were helpful in assigning all the protons. Interestingly, it appears that the presence of a –OTBS group at the benzylic position is playing an important role in governing the regioselective deprotonation under the reaction conditions, and, this leads to having the –COOEt group at the site that is opposite to the -OTBS group. By having a key intermediate **5.2** in our hand, further, we then attempted the ketone reduction. At this stage, we are getting two isomers (**7.3** and **7.4**) which are easily separable. One of the isomer **7.3** is crystallised, it is further supported our assignment. Both the isomers were further coupled with various amino acid building blocks to get the macrocycles.

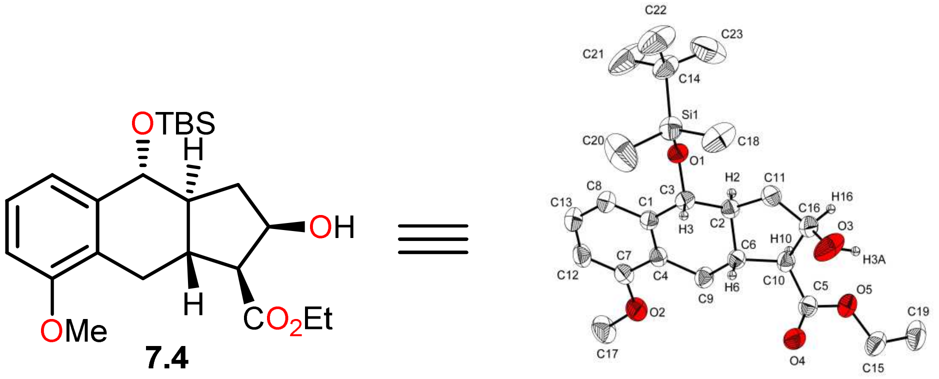
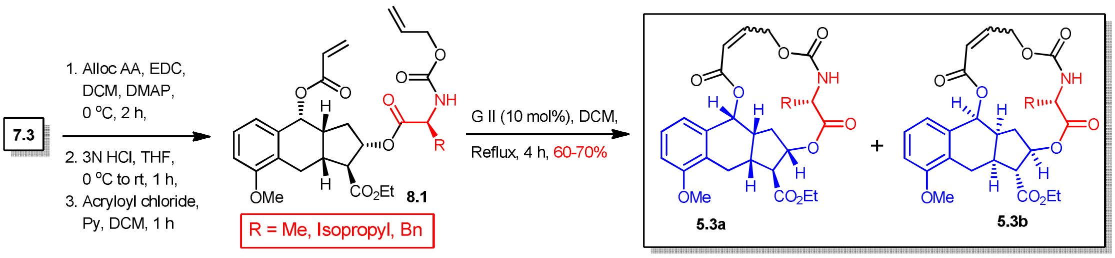


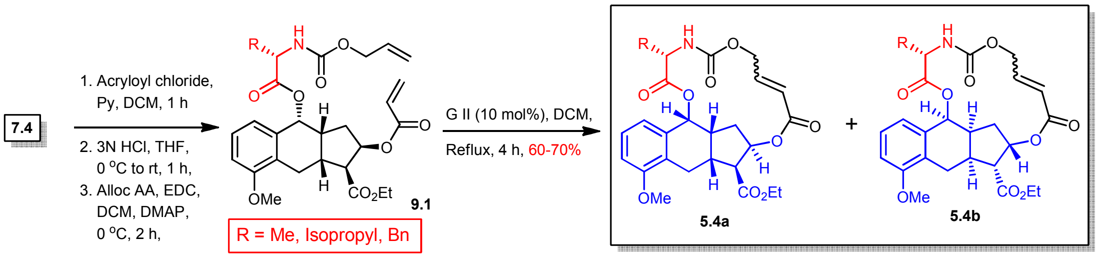
Figure 2: ORTEP diagram of compound **7.3**



Scheme 8: Synthesis of macrocycle **5.3** and derivatives

To synthesise macrocycle **5.3**, first we coupled the N-Alloc amino acid to **7.3**, and followed by the deprotection of silyl ether and acryloylation, gave intermediate **8.1**. This was further subjected to ring closing metathesis by using Grubbs 2nd generation catalyst gave macrocycles **5.3** with good yields (Scheme 8) as a mixture of two isomers.

In another approach, the synthesis of macrocycle **5.4** (**Scheme 9**) was achieved using **9.1** as the RCM precursor. Compound **9.1** was obtained by acryloylation of the compound **7.4 (**secondary -OH group**)**; the removal of the silyl group from the benzylic hydroxyl; and, then coupling with the N-Alloc amino acid moiety. Further subjected to ring closing metathesis by using Grubbs 2nd generation catalyst gave macrocycles **5.4** with good yields (Scheme 9).



Scheme 9: Synthesis of macrocycle **5.4** and derivatives

The biological evaluation of all these macrocycles and intermediates obtain in this project is under progress.

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ABBREVIATIONS

Ac	:	acetyl
Ac$_2$O	:	acetic anhydride
AcOH	:	acetic acid
aq.	:	aqueous
Ar	:	aryl
BF$_3$·OEt$_2$	:	borontrifluoride-etherate complex
BnBr	:	benzyl bromide
Boc	:	*t*-butoxycarbonyl
Boc$_2$O	:	di-*tert*-butyldicarbonate
Cbz	:	benzyloxy carbonyl
Alloc	:	allyloxy carbonyl
DCM	:	dichloromethane
DIPEA or $_i$Pr$_2$NEt	:	*N*, *N*’-diisopropylethylamine
DIBAL-H	:	diisobutyl aluminium hydride
DMSO	:	dimethyl sulfoxide
MeMgBr	:	methylmagnesium bromide
Et$_3$N or TEA	:	triethylamine
Et$_2$O	:	diethyl ether
EtOAc	:	ethyl acetate
EtOH	:	ethanol
Fmoc	:	fluornyloxy carbonyl chloride
G-II	:	grubb’s 2$_{nd}$ generation catalyst
GI$_{50}$	:	growth inhibition of 50%
h	:	hour(s)
H$_2$O$_2$	:	hydrogen peroxide
HOBT	:	hydroxy benzotriazole
Hz	:	hertz
IC$_{50}$	:	half maximal inhibitory concentration
IR	:	Infrared
KBr	:	potassium bromide
K$_2$CO$_3$	:	potassium carbonate
KO^{t}Bu or KTB	:	potassium *tert*-butoxide

LAH or $LiAlH_4$	:	lithium aluminiumhydride
LiOH	:	lithium hydroxide
Me	:	methyl
MeOH	:	methanol
EECl	:	ethoxyethylchloride
$NaBH_4$	:	sodium borohydride
NaH	:	sodium hydride
$NaHCO_3$	:	sodium bicarbonate
NaOH	:	sodium hydroxide
NH_4Cl	:	ammonium chloride
NMR	:	nuclear magnetic resonance
Ph	:	phenyl
PTSA or *p*-TsOH	:	*para*-toluenesulfonic acid
DEAD	:	Diethyl azodicarboxylate
RT (or) rt	:	room temperature
TBAF	:	tetra-*n*-butylammonium fluoride
TBDMS or TBS	:	*tert*-butyldimethylsilyl
TBDPS	:	*tert*-butyldiphenylsilyl
DMF	:	N,N dimethylformamide
TFA	:	trifuoroacetic acid
THF	:	tetrahydrofuran
$TiCl_4$	:	titanium tetrachloride
TPP or PPh_3	:	triphenylphosphine
TsCl	:	*para*-toluenesulfonyl chloride
UV	:	ultra violet
AA	:	amino acid

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 Indoline and Macrocyclic Natural Products and Their Inspired Analogous as Modulators of Protein-Protein Interactions (PPIs)

1.1. Introduction:

The post-genomic chemical biology age has brought challenges to the biomedical research community trying to develop a better understanding of protein-protein interaction (PPI)-based signaling networks.[1] It is becoming clear that these signaling networks are central to both normal and dysfunctional cellular processes.[2] In most cases, they involve multiple, dynamic, highly complex, protein-protein interactions. These confounding features have resulted in the lack of a thorough understanding of normal and disease-related cellular signaling networks, which has severely limited our ability to develop therapeutic approaches that exploit these networks. It is hoped that having a wide arsenal of relevant small-molecule chemical modulators of protein-protein interactions will result in a more informative probing of such dynamic processes, both in normal and dysfunctional cellular processes. In the long run, it will allow biomedical researchers to explore the biological space of proteins, something which has not currently been considered highly attractive.

1.2. Protein-Protein Interactions (PPIs):

Several factors have contributed to the limited success of small molecules as modulators of protein-protein interactions (PPIs). In general, protein-protein interactions involve shallow surfaces and cover a relatively large surface area. PPIs are largely driven by hydrophobic interfaces.[3] Hydrogen bonds and electrostatic interactions play crucial roles,[4] and, the covalent bonds are also considered very important for PPIs. The physical chemical principles of protein-protein interactions are general, and, many of the interactions observed *in vitro* are the outcome of experimental over-expression or of the crystal effects, complicating the functional prediction. Proteins also have areas known as "*hot spots*".[5] 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 the structural information of a protein-protein complex has led to the design of small molecules that exploit the "*hot spots*". 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.[6] Whereas a typical enzyme inhibition based approach involves seeking the structural information of an enzyme and then working closely with organic synthesis/medicinal and computational chemists. As in most cases, the enzymes present well-defined, often deep pockets and this information provides a good starting point to develop the medicinal chemistry program in the drug discovery arena.

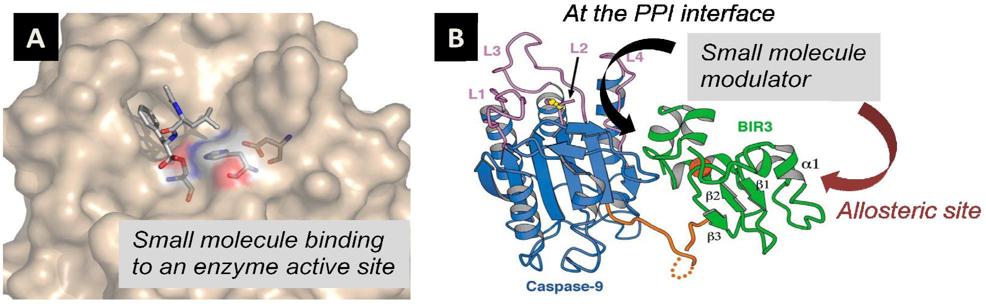


Figure 1: (A) An illustration of a small molecule binding to an enzyme active site; (B) small molecule modulators of protein-protein interactions via interface or an allosteric site

A practical way to predict protein function is through the identification of the binding partners. Since the vast majority of protein chores in living cells are mediated by protein-protein interactions, if the function of at least one of the components with which the protein interacts is identified, it is then expected to facilitate its functional and pathway assignment. Through the network of protein-protein interactions, we can map cellular pathways and their intricate cross-connectivity.[7] For example, two protein partners cannot simultaneously bind at the same (or overlapping) site, the discovery of the ways in which proteins associate should assist in inferring their dynamic regulation. Identification of protein-protein interactions is at the heart of functional genomics. The prediction of protein-protein interactions is also crucial for developing the drug discovery program. The knowledge of the pathway and its topology, length, and dynamics should provide useful information in forecasting the side effects.[8]

1.3. Natural Product Modulators of Protein-Protein Interactions:

Natural products are an attractive source of varied complex structures that exhibit potent biological activities, and, desirable pharmacological profiles. Historically, natural products have been the source of many new drugs. For example, most of the cancer drugs and infectious diseases-based drugs are derived from natural products.[9] 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.[10] Imbedded in these natural products are a number of highly diverse, chiral functional groups, which are the potential sites to exhibit specific interactions with different proteins. 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. In many cases, there is insufficient material for various desired biological assays, thereby limiting the exploration of their full potential. The development of relatively simple structural analogous 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[11,12,13] or Biology-Oriented Synthesis (BIOS)[14] 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 using conventional combinatorial chemistry approaches.[15]

Members of the diverse alkaloid natural products are known to interfere with protein surfaces involved in protein-protein interactions. Shown in Figure 2 are some examples of bioactive natural products that are obtained from different sources, and, function as PPIs modulators. Vinca alkaloids (vindoline **F2.1**, vinblastine **F2.2**) are two important anticancer agents known to disrupt microtubule dynamics, and, inhibit the assembly, resulting in the arrest of cell division at the metaphase.[16] TMC-95A (**F2.3**) is a potent proteasome inhibitor. Recently, it was shown that TMC-95A displays noncovalent and reversible inhibition of the proteasome. Macrocyclic natural products such as geldanamycin (**F2.6**) and herbimycin (**F2.7**) (commonly known as ansamycins) are potent and specific inhibitors of the ATPase activity of Hsp90. Cryptophycin (**F2.9**) is a well known, highly potent cytotoxin, and, is known for tubulin modulator to function as the inhibitor of cell division.[17] FK506[18] **(F2.4)** is an immunosuppressive agent. Rifamycin (**F2.8**) and kendomycin (**F2.5**) are known as antibiotics.

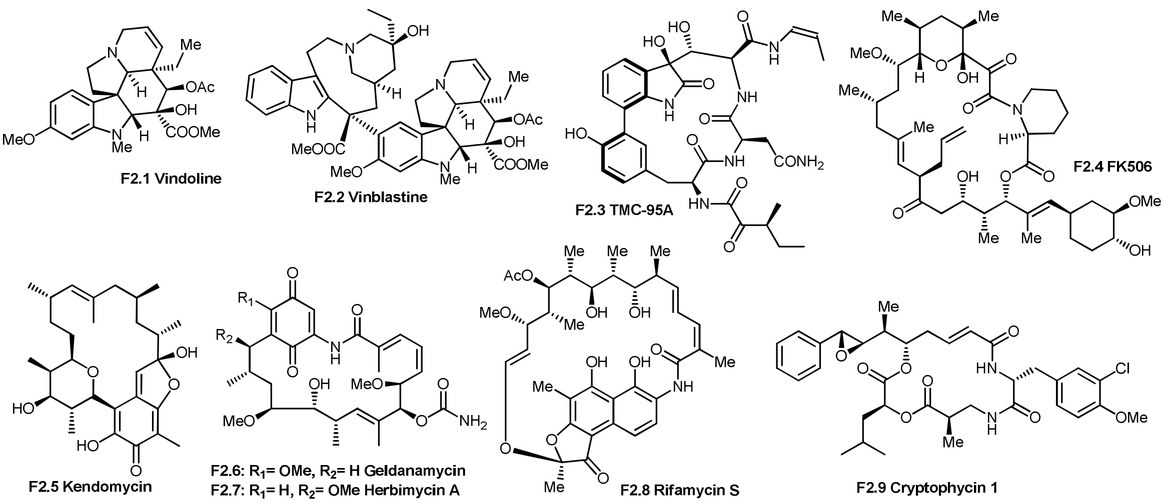


Figure 2: A few examples of bioactive natural products as the modulators of protein-protein interactions

1.3.1. Indoline and indole based natural products:

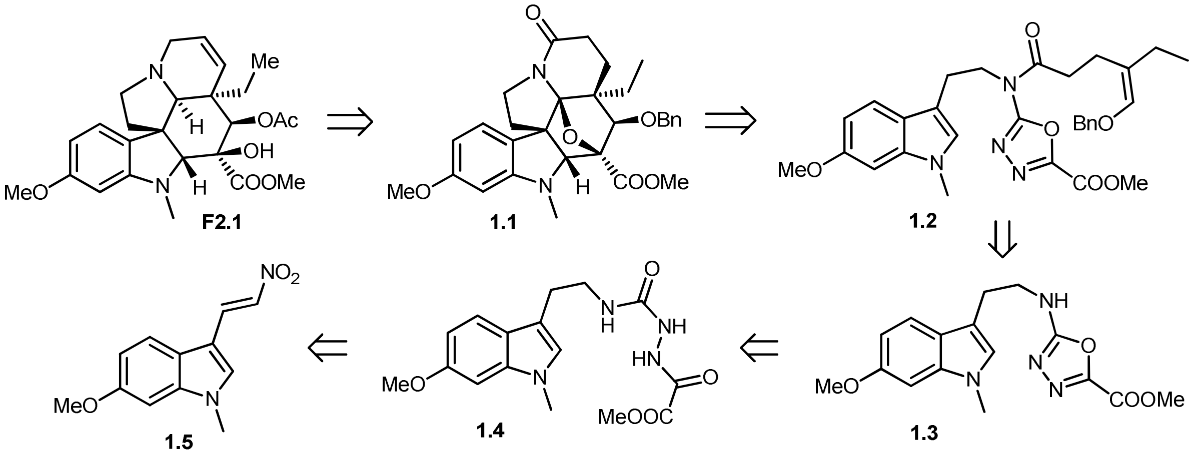
The indoline scaffold is ubiquitously present in many naturally bioactive alkaloids, such as vinblastine, strychnine, (–) - physostigmine, ajmaline, and, (+) - aspidospermidine. It is also the structural component of several important pharmaceutically active compounds, such as angiotensin converting enzyme (ACE) inhibitor and the antihypertensive drug pentopril. Indoline alkaloids constitute a large family of natural products. Their diverse and complex structures contribute to potent biological activities in a range of small molecules. In this section, I am going to discuss two case studies of natural products, and, these are vindoline and TMC-95A.

1.3.1.1. Vindoline:

Vinca alkaloids (vindoline **F2.1**) and vinblastine (**F2.2**), are two important anticancer agents. At sub-stoichiometric levels *in vitro*, vinca alkaloids are known to stabilize microtubules, possibly, by binding to microtubule ends, and, thus, inhibiting the hydrolysis of GTP.

Retrosynthesis:

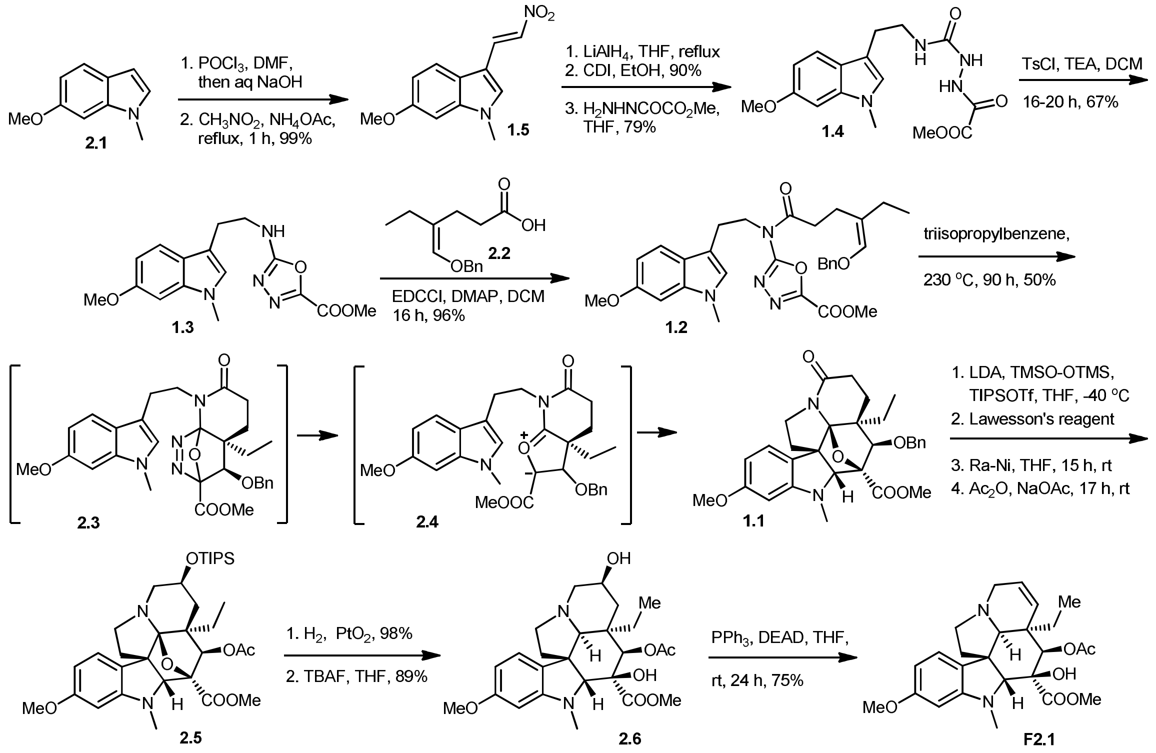
Boger *et al.*, synthesized (-)- and ent-(+)- vindoline (**F2.1**) through a diastereoselective tandem [4+2]/[3+2] cycloaddition of 1,3,4-oxadiazole (**1.2**). The unique reaction cascade assembles the fully functionalized pentacyclic ring system of vindoline in a single step that forms four C-C bonds and three rings while introducing all requisite functionality and setting all six stereocenters within the central ring including three contiguous and four quaternary centers.[19] An intermediate **1.2** was synthesized from **1.3** by attaching the side chain. Compound **1.3** was synthesized from **1.4** by the dehydrative cyclization.



Scheme 1: The retrosynthetic analysis of vindoline

Synthesis:

Synthesis of vindoline is shown in Scheme 2. The researchers started their synthesis with the indole derivative **2.1**, which was first subjected to Vilsmeier–Haack reaction followed by a Henry reaction to obtain an α, β-unsaturated nitro compound **1.5**. Further, an olefin reduction gave *N*-methyl-6-methoxy tryptamine, which was first treated with carbonyldiimidazole (CDI), and, then subsequently treated with methyl oxalylhydrazide to provide **1.4**. The intermediate **1.4** was further converted to **1.3** via the dehydrative cyclization by using TsCl and TEA. It was then coupled with isomerically pure acid, **2.2** to provide **1.2**, which was then subjected to a tandem [4+2]/[3+2] cycloaddition cascade leading to the pentacyclic product **1.1**.[20] This reaction sequence provides **1.1**, and, is initiated by an intramolecular inverse electron demand Diels-Alder cycloaddition of the 1,3,4-oxadiazole **1.2** with the tethered enol ether to obtain **2.3**. The loss of N_2 from the initial cycloadduct **2.3** provides the carbonyl ylide **2.4**, which then undergoes a subsequent established[21] 1,3-dipolar cycloaddition with the tethered indole moiety. Importantly, the diene and dienophile substituents complement and reinforce the [4+2] cycloaddition regioselectivity, that was dictated by the linking tether. The intermediate 1,3-dipole is stabilized by the complementary substitution at the dipole termini, and, the tethered 1,3-dipolarophile (indole) complements the [3+2] cycloaddition regioselectivity.



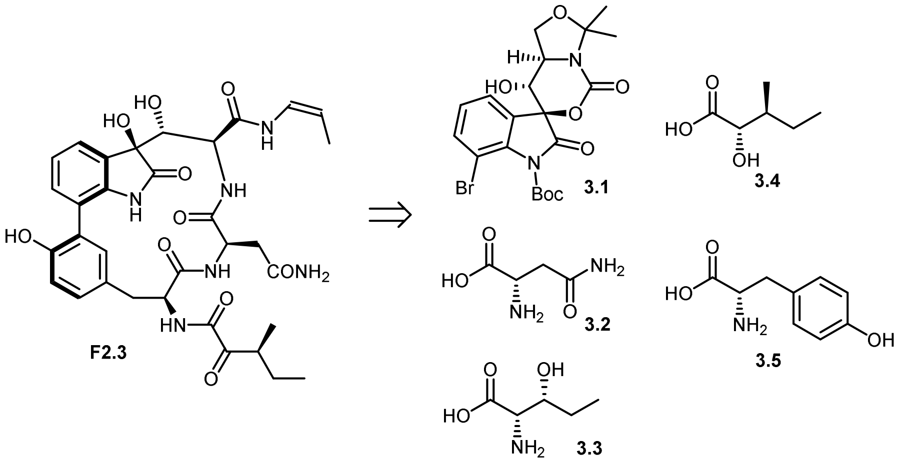
Scheme 2: Synthesis of vindoline

The relative stereochemistry in each cycloadduct is controlled by a combination of (i) the dienophile geometry, and, (ii) an exclusive endo indole [3+2] cycloaddition sterically directed opposite to the newly formed fused lactam. An α-hydroxylation of **1.1** was achieved by the treatment of the lactam enolate with TMSO-OTMS, and, this was then followed by a direct quench with TIPSOTf. It was further converted to thioamide with Lawesson's reagent, reductive desulfurization with raney Ni, and, subsequent acetylation of the resulting secondary alcohol afforded **2.5**. The diastereoselective reductive cleavage of the oxido-bridge upon catalytic hydrogenation (45 psi H_2, PtO_2, 50% MeOH-EtOAc) with reduction of the intermediate imminium ion from the α-face. Further, the silyl ether cleavage provided **2.6** and subsequent secondary alcohol activation and elimination provided either (-)- or *ent*- (+)- vindoline.

1.3.1.2. TMC-95A:

A proteasome is a multi-catalytic protease that is involved in a variety of biologically important processes, including immune responses, cell-cycle control, metabolic adaptation, stress response, and cell differentiation.[22] The selective inhibition of proteasomes may allow controlling these essential pathways.[23] TMC-95A (**F2.3**) and its C36 epimer TMC-95B are two potent proteasome inhibitors that bind to enzymes non-covalently with IC_{50} values at low nanomolar levels.[24] Recently, the X-ray crystallographic structure of a complex of TMC-95A with a proteasome was reported.[25] This study has allowed the design of novel TMC-95 analogous with tailored biological activities.

Retrosynthesis:

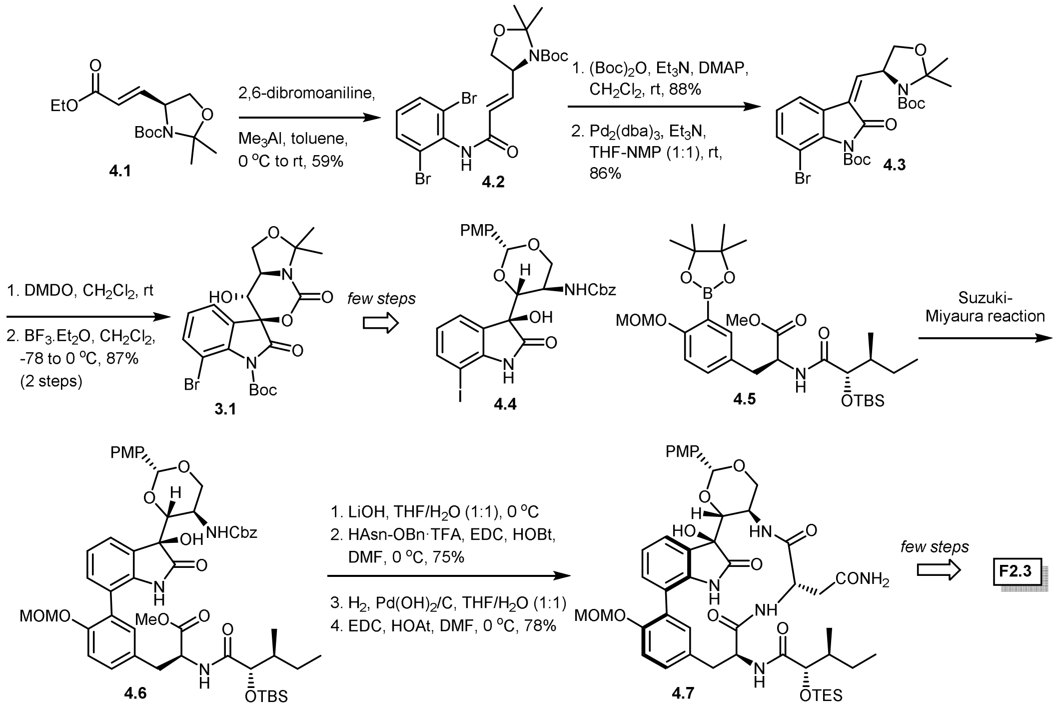


Scheme 3: The retrosynthetic analysis of TMC-95A

The macrolactam structure could be assembled by a Suzuki–Miyaura coupling reaction (C1-C20) and the peptide bond formation (N9-C10).[26] To avoid epimerization at the C-36 stereocenter, the researchers planned to introduce 3-methyl-2-oxopentanoic acid as the reduced form **3.4**, and, to generate the C35 ketone at the final stage of the synthesis. The northern part was synthesized by using the following key reactions (i) *Z*-selective Mizoroki-Heck reaction to construct the oxyindole portion, (ii) a diastereoselective epoxidation, (iii) 6-endo selective epoxide opening by Boc carbonyl group to establish the stereochemistry of C6, and, (iv) 1,3-elimination reaction of the L-allothreonine (**3.3**) derivative under Mitsunobu conditions to afford the (*Z*)-1-propenylamine.

Synthesis:

Synthesis of TMC-95A[27] was started from α, β unsaturated ester **4.1**. This compound was derived from Garner aldehyde. An amidation of the ester **4.1** with 2,6-dibromoaniline in the presence of Me_3Al resulted in **4.2**,[28] followed by protection of the N22-amide with a *N*Boc group. Further, an intramolecular Mizoroki-Heck reaction afforded the tri-substituted olefin **4.3**. The (*Z*)-selectivity of this facile intramolecular cyclization can be explained by the *syn* insertion of the alkyl palladium into (*E*)-olefin, followed by bond rotation, and *syn*-elimination of the C6-hydride to provide, predominantly, (*Z*)-oxyindole **4.3**. The bulky *N*Boc group seems to be protecting the *N*22-amide of **4.3** and this can explain the lack of further Pd(0) insertion into the C1-bromide of **4.3**, to cause undesirable side reactions, and, to maintain the catalytic cycle. The α, β–unsaturated olefin was further oxidized using 3,3-dimethyldioxirane (DMDO) to obtain an epoxide as a single diastereomer. An activation of the epoxide with $BF_3{\cdot}Et_2O$ selectively provided a six membered carbamate **3.1** with the concomitant loss of the *tertiary* butyl group. The *N*Boc group of **3.1** was selectively removed with $Mg(ClO_4)_2$ in acetonitrile, and, the C7 secondary alcohol was protected as the ethoxyethyl (EE) ether. For an effective biaryl coupling, the bromide **3.1** was converted into the corresponding iodide through the metalation reaction with *n*-BuLi, and, the subsequent addition of diiodoethane without affecting the reactive carbamate. The carbamate group of **3.1** was removed by using Gassman method,[29] and, further a few functional groups inter-conversions allowed to complete the synthesis of **4.4**.



Scheme 4: Synthesis of TMC-95A

The C1-C20 biaryl bond was constructed by Suzuki-Miyaura coupling of two intermediates, **4.4** and **4.5**. The basic hydrolysis of the methyl ester **4.6**, followed by an amidation reaction with L-asparagine benzyl ester, and, further hydrogenolysis of both the benzyloxycarbonyl (Z) and the benzyl groups gave the seco acid, which was then subjected to cyclization in the presence of EDC and HOAt to afford the macrolactam **4.7**. Further, the attachment of L-allothreonine benzyl ester, which was subjected to hydrogenolysis to provide the β-hydroxycarboxylic acid. The subsequent treatment with PPh_3 and DEAD in the presence of molecular sieves induced the dehydrative decarboxylation at room temperature, and, this led to obtain an isomerically pure (Z)- propenylamide. Followed by some functional group inter-conversions and de-protection, the synthesis of **F2.3** was then completed.

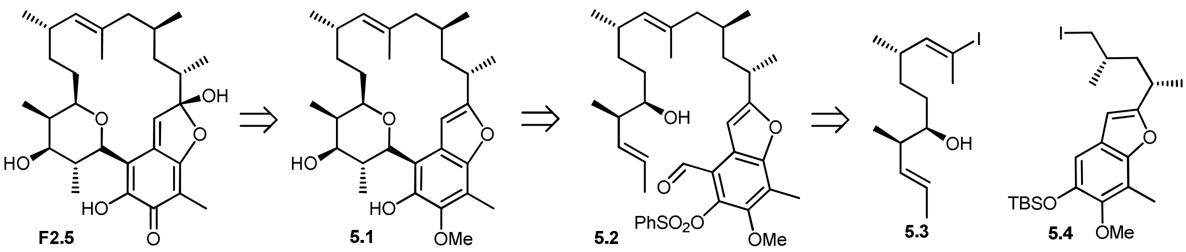
1.3.2. Macrocyclic natural products:

So far, a large number of macrocyclic natural products have been isolated and synthesized. The construction of macrocycles is generally considered a crucial and challenging step in the synthesis of macrocyclic natural products.[30] Over the last several decades, numerous efforts have been undertaken towards the synthesis of complex naturally occurring macrocycles, and, great progress has been made to advance the field of total synthesis. In this section, I am going to discuss two case studies of macrocyclic natural products, kendomycin (**F2.5**) and geldanamycin (**F2.6**).

1.3.2.1. Kendomycin:

(-)-Kendomycin (**F2.5**) has a diverse and highly fascinating pharmacological profile. It was isolated in 1996 from various *Streptomyces* bacteria. This natural product exhibits potent antagonism of the endothelin receptor, as well as, antiosteoporotic properties from calcitonin receptor agonism and potent cytotoxicity (GI_{50} < 100 nM) towards carcinoma cell lines. In addition to this, it has been shown to be a broad-spectrum antibacterial agent, and, active against even two biologically very improtant strains, MRSA and VRSA.[31] A recent report also described kendomycin's utility in probing the biological processes of the mammalian proteasome *in vitro.*[32]

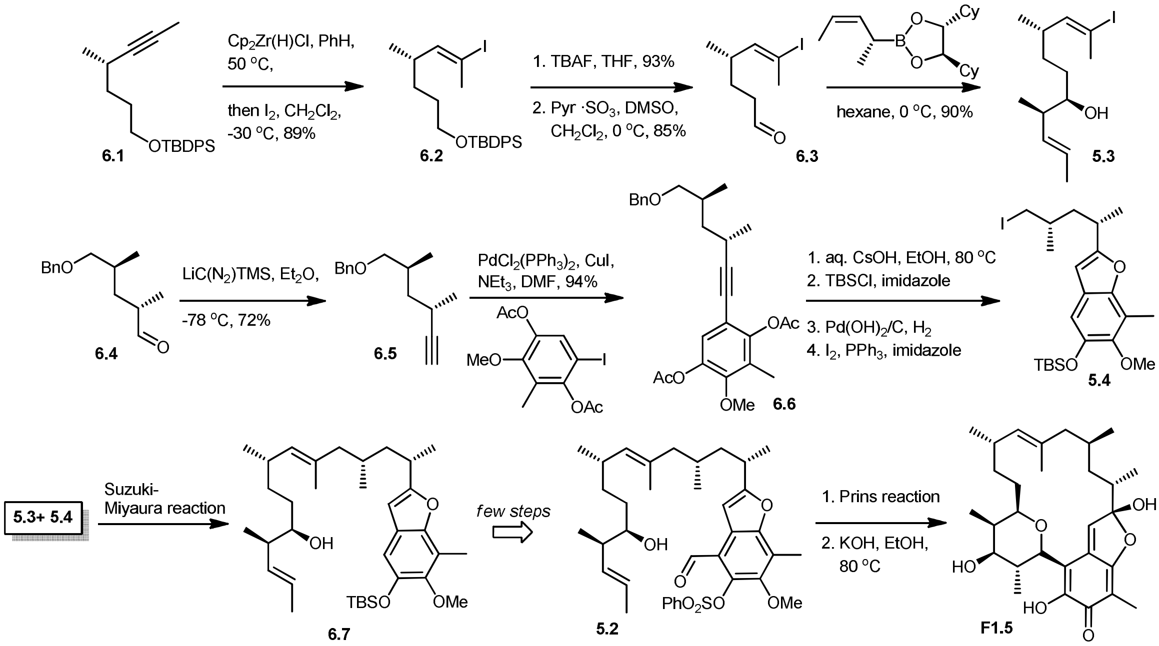
Retrosynthesis:



Scheme 5: The retrosynthetic analysis of (-)-Kendomycin

A retrosynthetic analysis of kendomycin is outlined in Scheme 5. The *p*-quinone methide of kendomycin can be prepared by an oxidation of hydroxybenzofuran **5.1**. The key Prins cyclization was utilized to generate the THP ring, three new stereogenic centers, and, the macrocycle simultaneously from the unsaturated hydroxy aldehyde **5.2**. Trisubstituted alkene **5.2** can be made available from the two major fragments **5.3** and **5.4**, which would be stitched via Suzuki-Miyaura coupling.[33] Importantly, this strategy allowed for the merger of equally complex fragments with a complete stereocontrol of the trisubstituted alkene.

Synthesis:



Scheme 6: Synthesis of (-)-Kendomycin

The synthesis (see Scheme 6) began with the construction of the cross-coupling partners to assemble the Prins-macrocyclization precursor **5.2**. Synthesis of vinyl iodide **5.3** was started from alkyne **6.1**. The one-pot treatment of alkyne **6.1** with freshly prepared Schwartz's reagent,[34] followed by treatment with iodine, afforded *E*-vinyl iodide **6.2** as a single regioisomer. The deprotection of silyl ether **6.2** and Parikh-Doering oxidation[35] to obtain aldehyde **6.3** which was further treated with Hoffmann's dimethallyl borane,[36] and, this produced the *E*-homoallylic alcohol **5.3** as a single diastereomer. Synthesis of the second partner of **5.2** was started from aldehyde **6.4**. The addition of aldehyde **6.4** to a cold solution of lithium trimethylsilyl diazomethane in Et$_2$O and subsequent Colvin rearrangement[37] generated terminal alkyne **6.5**. Sonogashira coupling of alkyne **6.5** and aryl iodide using catalytic PdCl$_2$(PPh$_3$)$_2$ delivered an aryl alkyne **6.6**. Treatment of aryl alkyne **6.6** with aqueous

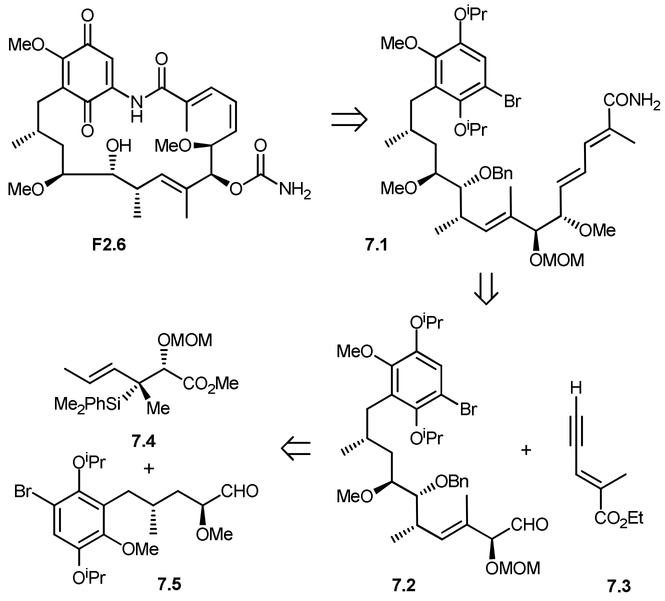
CsOH in EtOH at 80 °C deprotected the phenols and this induced a 5-*endo*-dig cyclization onto the alkyne to generate hydroxybenzofuran moiety. Further, deprotection of benzyl group and the conversion of hydroxy to iodide to obtain **5.4**. The synthesis of **6.7** was achieved by coupling of both the partners **5.3** and **5.4**. Finally, the treatment of **6.7** with TBAF removed the phenol-protecting group, and, then C5 aldehyde was introduced by using modified Duff ortho-formylation, followed by few functional group interconversions to access the precursor (**5.2)** of Prins macrocyclization.[38] The treatment of aldehyde **5.2** with $BF_3 \cdot OEt_2$ and HOAc in DCM at high dialution (15 mM) generated a mixture of the acetate and fluoride. The ethanolysis of macrocycle afforded the kendomycin.

1.3.2.2. Geldanamycin:

Heat shock protein 90 (Hsp90) has attracted much attention as a serious molecular target for cancer therapeutics. Geldanamycin (GDM) (**F2.6**) was the first small molecule inhibitor identified for Hsp90. GDM binds to the *N*-terminal domain ATP binding site of Hsp90, and, this leads to inhibiting the chaperone activity of the protein. This inhibition leads to the disruption of the Hsp90-client protein complex.[1d] The unchaperoned client proteins are subsequently ubiquitinated and degraded by the proteasome. Because many of the Hsp90 client proteins are important in signal transduction and transcription, GDM and its analogous have great potential in a cancer chemotherapy.

Retrosynthesis:

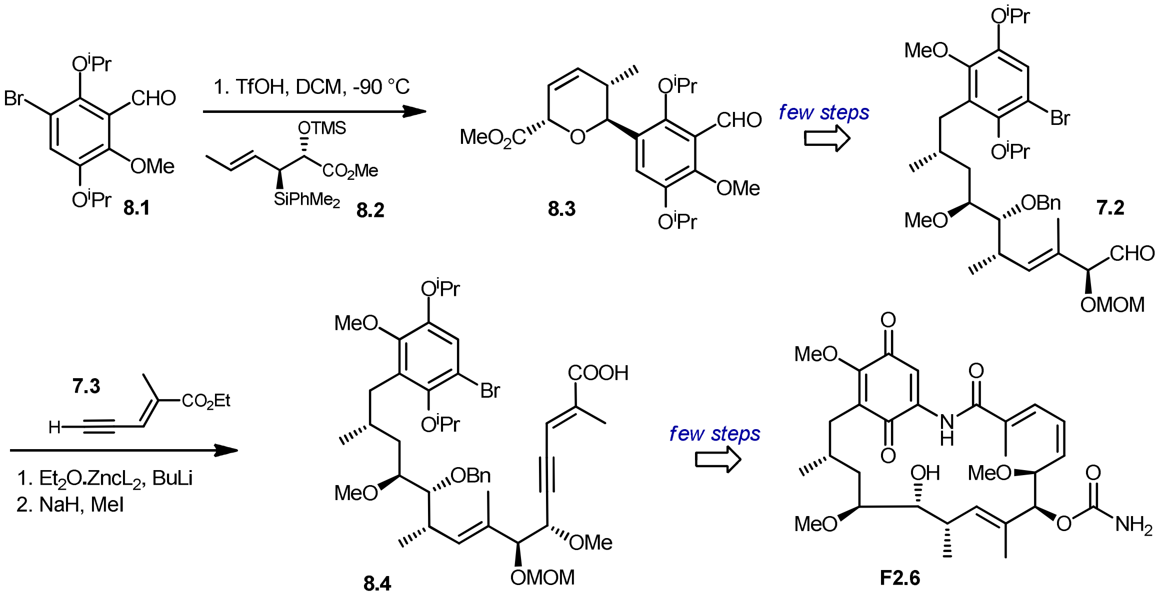
The 19-membered macrocycle (**F2.6**) was synthesized through copper(I)-mediated an intramolecular aryl amidation reaction, and, the synthesis of 11, 12, 14 stereocenters were achieved through a $Sc(OTf)_3/Et_3SiH$-mediated pyran ring-opening reaction. The (*E*,*Z*)-diene **7.1** was installed by reduction of the enyne from alkynylation of precursors **7.2** and **7.3** which could be generated from easily accessible aldehyde **7.5** and the chiral silane reagent **7.4**.[39]



Scheme 7: The retrosynthetic analysis of geldanamycin

Synthesis:

The synthesis of the C6-C21 fragment **7.2** (Scheme 8) was started from functionalized aromatic aldehyde **8.1**, which was easily obtained in three steps from commercially available 2-methoxyhydroquinone. [4+2]-Annulation of this aldehyde with silane **8.2** provided a mixture of dihydropyran **8.3**. The regio- and stereo-selective hydroboration of the pyran moiety double bond provided the secondary alcohol, which was subsequently methylated with Meerwein’s reagent to provide the tetrahydropyran skeleton. The reductive opening of the pyran moiety with $Sc(OTf)_3/Et_3SiH$ followed by a few functional group interconversions led the synthesis of aldehyde **7.2**. It was further homologated through the diastereoselective addition of a metallated acetylide and methylation of propargylic alcohol using NaH and MeI; this occurred with the simultaneous dealkylation of the ester to the corresponding acid **8.4**. which was subjected to Lindlar reduction, subsequently converted to the (*E*,*Z*)- unsaturated amide. The amide was then subjected to an intramolecular copper(I)-mediated aryl amidation reaction to provide the desired macrocyclic lactam. Deprotection of the protecting groups and the classical inter conversions to led the completion of synthesis of geldanamycin[39] (**F2.6**).



Scheme 8: Synthesis of geldanamycin

1.4. Natural Product Derived and Inspired Macrocyclic Compounds:

Most natural products are hardly synthesized by nature to aid the human health problems. In many cases, the real function of natural products is not even known; the evolutionary benefits are far from being fully exploited because the original targets often remain undefined. Also, many structures are highly complex in nature to access these compounds and their analogous in a time effiicent manner. Moreover, some structures still remains a serious challenge even for the directed total synthesis. Many of these total syntheses are far from being economical, for example, those of taxanes such as paclitaxel.[40] To overcome the problems with the synthetic availability of natural products and especially their derivatives required for quantitative structure–activity relationships (QSARs).

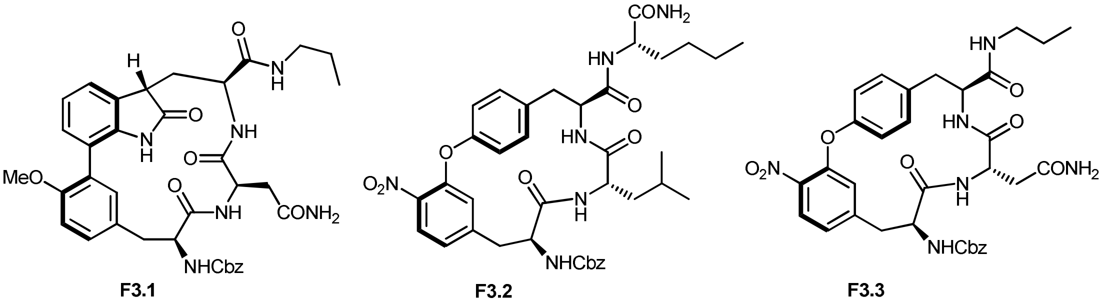
The development of relatively simple, structural analogous of natural products that also have comparative biological responses often proves to be a challenging undertaking. This is where the development of a natural product-inspired, diversity-oriented synthesis program could be extremely useful. Diversity-Oriented Synthesis

(DOS) aims at populating the unexplored, natural-product chemical space that is currently not occupied by conventional combinatorial chemistry.[12b,41] The combinatorial chemistry program in DOS utilizes modern (i.e. stereo- and enantio-selective reactions) organic synthesis reactions and is designed to provide small molecules that are rich in (i) stereochemically-defined polyfunctional groups, and (ii) conformationally diverse, natural product-inspired skeletons. On the contrary, with few exceptions, classical combinatorial chemistry efforts have led to simple compounds lacking rigid 3-dimensional architectures. These simple compounds do not populate the chemical space occupied by bioactive natural products and, typically, are not attractive as modulators of protein-protein interactions or as chemical probes for dissecting dynamic signaling networks. Macrocyclic compounds are attractive targets when searching for novel structures with biological activity due to the fact that, macrocyclic natural products typically include a 12 or more membered ring architectures and often do not possess the druglike “rule of five” properties. This unique structural feature and conformational flexibility of the macrocyclic ring can offer subsequent functional advantages, e.g., it has the potential of being highly potent as well as being selective when key functional groups interact with biological targets. In addition, from a chemistry point of view, macrocyclic compounds can offer diverse functionality and stereochemical complexity in a conformationally restricted manner. Moreover, macrocycles can demonstrate favorable drug-like properties, including good solubility, increased lipophilicity, enhanced membrane penetration, improved metabolic stability, and good oral bioavailability with desirable pharmacokinetic and pharmacodynamic properties.[42]

1.4.1. TMC-95A based analogous:

Among the natural proteasome inhibitors discovered so far, only TMC-95A, (a complex side chain and bridged tripeptide derivative) blocks all the active sites of the proteasome in a highly specific and noncovalent manner.[43] The TMC-95 is a cyclic tripeptide containing a biaryl system constrained into the 17-membered heterocycle, and its binding mode was characterized by X-ray crystallography.[25] In the TMC-95A/yeast proteasome complex the non-covalent interactions are mainly mediated by H-bonds between the extended peptide backbone and the protein counterpart in a manner similar to an antiparallel β sheet. The *C*-terminal (Z)-propenyl moiety acts as P1 and the asparagine as P3 residue, whereas the *N*-terminal keto-amide group is largely exposed to the surface without apparent interactions with the protein.[44]

From this structural information, Groll and co-workers designed and synthesized reversible inhibitors of the proteasome that is based on the lead structure of TMC-95A. The first generation analog **F3.1** of TMC-95A was synthesized by using the *N*-terminal 3-methyl-2-oxopentanoyl moiety. The synthetically challenging *C*-terminal (Z)- (prop-1-enyl) amino groups were replaced by (benzyloxy)carbonyl (Z) and propylamino groups, respectively, and, the highly oxidized form of the tryptophan was reduced to β-(2-oxoindol-3-yl)alanine. The ring closures of the biaryl-containing linear precursor via intramolecular side-chain Suzuki crosscoupling was achieved.[45] According to the X-ray analysis of the proteasome/TMC-95A complex, the main role of the phenol-oxindole biaryl system appears to be an induction and stabilization of an extended β type peptide-backbone conformation.[25] Biaryl ethers of the isodityrosine type are known to induce the identical conformational restriction when used as structural clamps in positions. Therefore, the replacement of the phenol-oxindole group in TMC-95A with an endocyclic biaryl ether could further facilitate the synthetic access of TMC-95A analogous as potential proteasome inhibitors. And, the resulting TMC-95A analogous **F3.2** and **F3.3** (2nd generation analogous) were found to exhibit promising inhibitory properties.

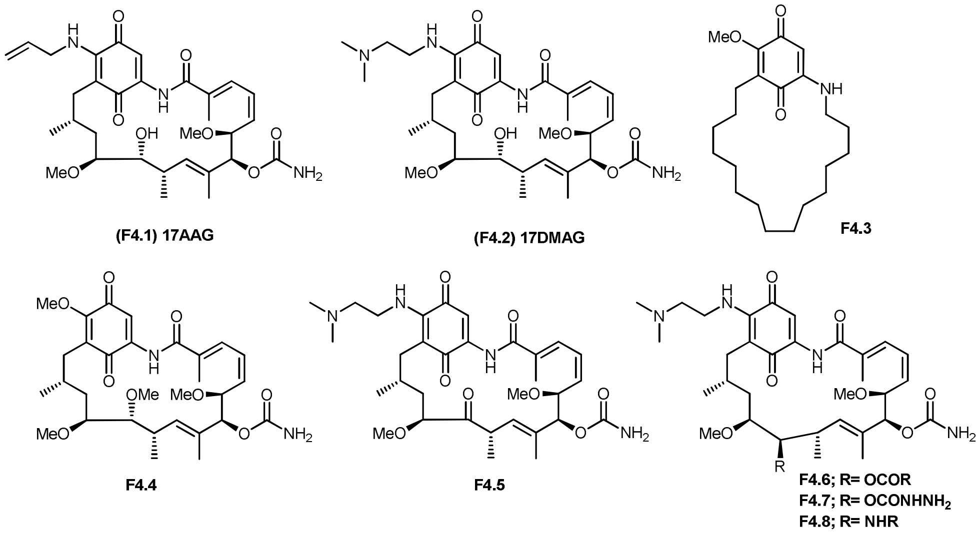


F3.1 **F3.2** **F3.3**

Figure 3: TMC-95-Based Proteasome Inhibitors

1.4.2. Geldanamycin-derived analogous:

Due to the high hepatotoxicity and poor solubility of geldanamycin, during preclinical studies in animals, there is a growing interest in developing geldanamycin analogous to overcome these hurdles.[46] The analogous of geldanamycin with alkylamino groups in place of the methoxy moiety at C-17 are shown to be less reactive toward nucleophiles, and, these compounds have excellent biological activity and the reduced hepatotoxicity.[46] These modifications at the C-17 position do not have an effect on formation of Hsp90 and geldanamycin complex. Geldanamycin analogous are also known to form similar complexes with Hsp90 as with the GDM.[47] 17-Allylamino geldanamycin (17-AAG, **F4.1**) and 17-desmethoxy-17-N,N dimethylaminoethylamino geldanamycin (17-DMAG, **F4.2**) are the 17-position derivatives of GDM, and, are shown to have lower *in vivo* toxicity than GDM. However, most of them exhibit less affinity toward Hsp90 than the parent natural product, GDM. 17-AAG has promising anticancer effects *in vitro* and *in vivo*, but it has a low solubility in water.



(F4.1) 17AAG

(F4.2) 17DMAG

F4.3

F4.4

F4.5

F4.6; R= OCOR
F4.7; R= OCONHNH$_2$
F4.8; R= NHR

Figure 4: Geldanamycin derived analogous

Recently, Hecht and co-workers synthesized geldanamycin analog[48] **F4.3**. This compound is a structurally simplified analog of geldanamycin and is shown to suppress the lipid peroxidation and to maintain cell viability in a dose dependent manner at low micromolar concentrations in an oxidative stressed environment for FRDA (Friedreich's ataxia) lymphocytes. In addition, **F4.3** suppressed the ROS (reactive Oxygen species) production and maintained the mitochondrial membrane potential in cultured FRDA lymphocytes and fibroblasts subjected to an oxidative stress. Compound **F4.3** was not cytotoxic and protected differentiated SH-SY5Y cells against Aβ-induced cell death. Interestingly, compound **F4.3** did not inhibit Hsp90, which may contribute to the cytotoxicity of geldanamycin, further illustrating its potential as a therapeutic agent for relieving the oxidative stress.[48]

Liu and co-workers synthesized C-11 modified derivatives[49] (**F4.4, F4.5, F4.6, F4.7** and **F4.8**) of GDM including ethers, esters, carbonates, ketones, and oximes, and, further, measured their affinity for Hsp90 and an ability to inhibit growth of human cancer cells. In accordance with the crystal structures reported for the complexes of GDMs with Hsp90, bulky groups attached to C-11 interfered with Hsp90 binding while smaller groups such as 11-*O*-methyl allowed Hsp90 binding. In addition, these analogous also showed *in vitro* cytotoxicity against human cancer cell lines. Esterification of the 11-OH of 17-AAG eliminated the Hsp90 binding *in vitro*. The readily hydrolyzed esters acted as pro-drugs during the measurement of cytotoxicity. Thus, during these experiments, the esters were hydrolyzed, releasing 17-AAG. Several 11-*O*-methyl-17-alkylaminogeldanamycin analogous were identified with improved potency relative to 17-AAG.

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Chapter 2: Synthesis of Indoline Alkaloid Natural Product Inspired Macrocyclic Toolbox

2.1. Introduction:

With the goal of expanding the currently accepted chemical space to search for small molecule modulators of protein-protein[1] and DNA/RNA-protein[2] interactions and signaling pathways in general,[3] the past few years have seen a growing interest in accessing small molecules[4] that are more natural product like, and many of these small molecules are obtained through the inspiration of bioactive natural products.[5] Traditionally, small molecules that are broadly available to the medicinal chemistry community for an early biological evaluation are rich in sp^2 character, and in general, they lack the features that are commonly found in bioactive natural products.[6] Several leading review articles have been written on this topic, and they challenge the organic synthesis community to access small molecules for biological evaluation in the broad areas of protein-protein interactions and other bio-macromolecular interactions.[4a,5a,5c,6b]

2.2. Working Hypothesis:

In particular, the interest in accessing functionalized large ring derivatives (i.e., macrocyclic compounds) is growing due to several reasons, and, these are: (i) medium-to-large ring structures present an opportunity to map a large surface area to interact with protein targets, (ii) the pre-organization in large rings allow several functional groups to display in specific orientations, (iii) cyclic compounds, in general, known to exhibit better cell permeation properties.[8] Despite all of these attractive features that large rings offer, and, having an excellent track record of several macrocyclic natural products as drugs, the full potential of this area remains to be explored thoroughly.[8-9] One of the challenges in this arena is the development of synthesis methods that are modular, and, practical in nature, and, that would allow an access to different types of functionalized medium to large rings.

Several indole and indoline alkaloid natural products are known to interfere with protein surfaces that involve in protein-protein interactions.[10] Moreover, it is a also considered a ubiquitous scaffold, and, commonly found in the structures of several naturally bioactive alkaloids such as vindoline, vinblastine, strychnine, staurosporin, tabersonine, stephatonic acid methyl ester, celogentin C, TMC-95, indolactam V (see

Figure 1). The synthesis of this "privileged structure" is meaningful in the design of new biologically active compounds.

As a result, we were interested in synthesizing alkaloid inspired compounds having the indoline scaffold containing an additional medium size cyclic ring to generate the skeletally diverse compounds.[11] Indoline-based compounds are quite abundant in nature, and, are considered highly privileged building blocks in medicinal chemistry as well.[12] Since, natural products containing this scaffold have led to interesting biological properties, this warranted further structural modifications to its backbone.

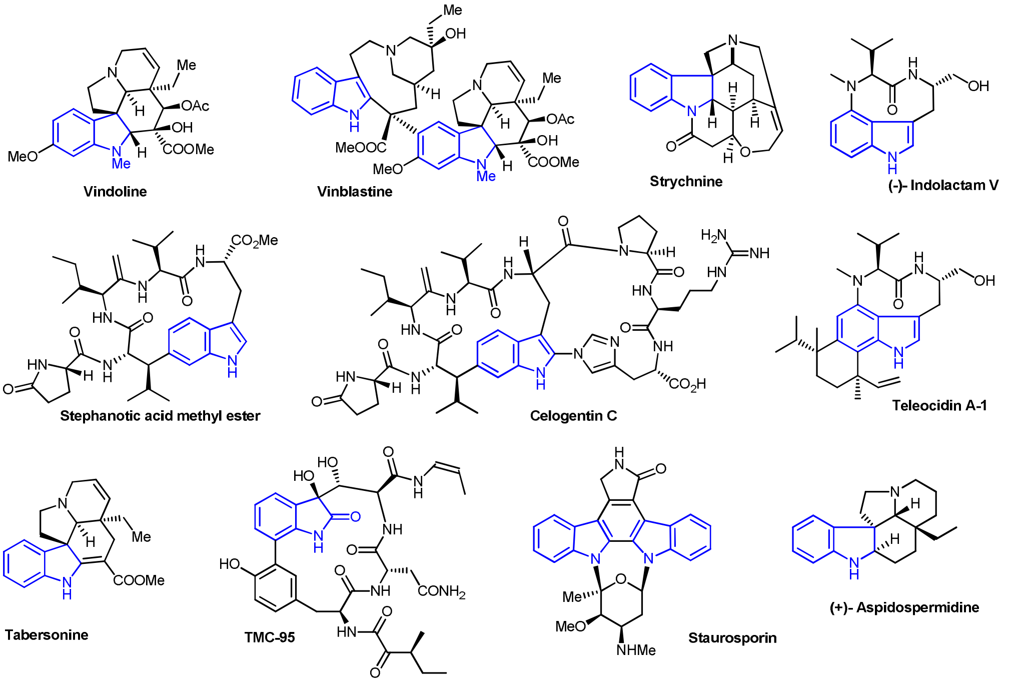
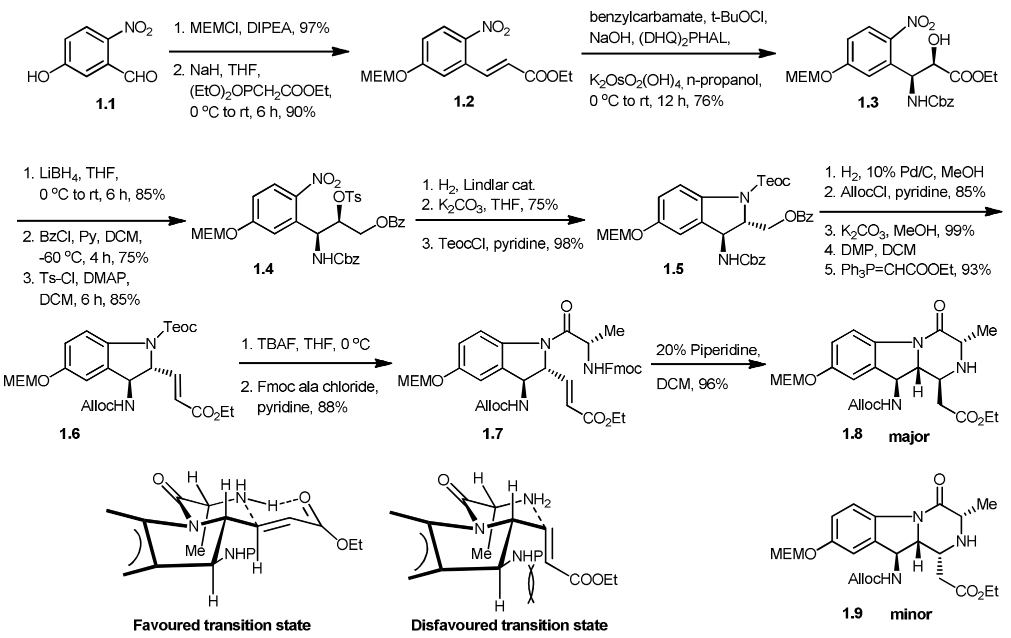


Figure 1: Indole and indoline alkaloid natural products as modulators of protein-protein interactions

A few years ago, we initiated the synthesis program, that was aimed at developing methods to allow us access to functionalized, enantioenriched natural product inspired scaffolds.[11a,13] In one study,[11a] we developed a practical synthesis of an amino indoline scaffold having three orthogonally protected functional groups. In this report, we demonstrated that this bicyclic scaffold can be converted into a tricyclic system through an *in situ*, stereocontrolled aza-Michael reaction. The enantioselective synthesis of amino indoline bicyclic scaffold **1.5** is shown in Scheme 1. 5-Hydroxy-2-nitro-benzaldehyde **1.1** was converted into **1.2** in two steps that involve the protection of the phenol and an elongation of the carbon chain. Compound **1.2** was then subjected to an asymmetric aminohydroxylation reaction to give compound **1.3** in 79% yield (>92% ee). The amino indoline **1.5** was obtained from **1.3** in several steps that involved (i) reduction of the carboxylate ester (70%), (ii) benzoyl-protection of the primary alcohol (OBz, 88%), (iii) tosylation of the secondary alcohol (OTs, 87%), (iv) selective reduction of the nitro group, and, (v) cyclization under mild basic conditions (75% for two steps), followed by protection of secondary amine.



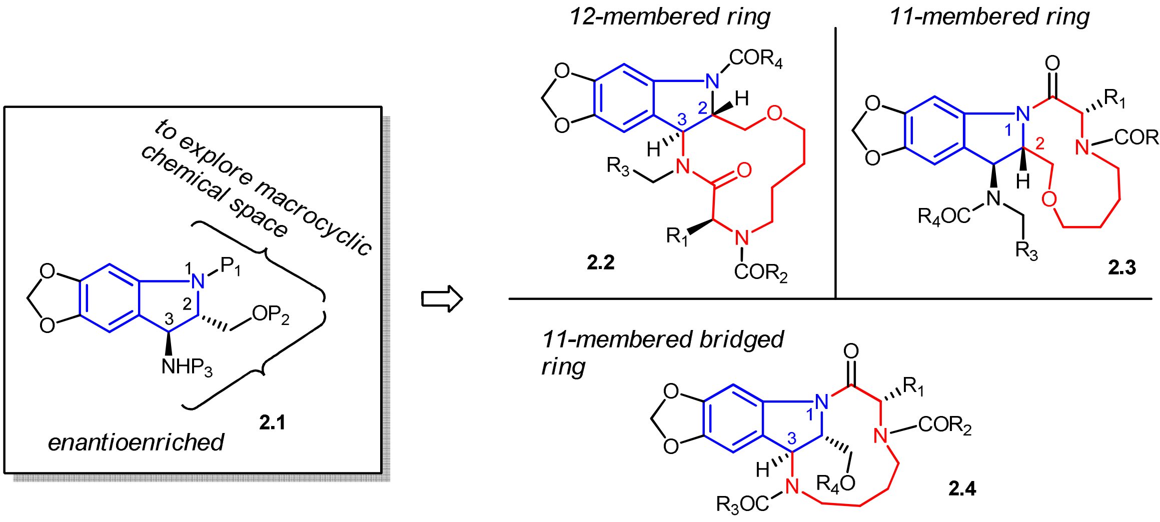
Scheme 1: Synthesis of indoline alkaloid natural product like complex polycyclic compounds

Compound **1.5** was then subjected to Cbz deprotection under hydrogenation conditions, and, the benzylic amine was protected with an Alloc group (85% for two steps). This further followed by a removal of the protecting group at the primary alcohol (99%), oxidation of the alcohol to the aldehyde, and a two carbon Wittig chain extension (93% for two steps) which finally gave **1.6**. Removal of the Teoc group, the indoline free amine was then coupled with the amino acid chloride to obtain *N*-Fmoc-protected derivative **1.7** (88% for two steps). Upon removal of the Fmoc group (20% piperidine), the six-membered ring was formed under these mild reaction conditions. The primary amine generated *in situ* was easily trapped by the conjugated carboxylate ester in a stereoselective manner to give the cyclic compounds

1.8 (major) and **1.9** (minor) in 96% overall yield (R_1=Me, >10:1 ratio of two diastereomers, 82% d.r. for the major product).

The stereochemistry of the new stereogenic center was assigned by NMR spectroscopy studies. The ease of an asymmetric, conjugate hetero-Michael reaction approach to yield the tricyclic product was a pleasant surprise, and, it opens-up a simple and very attractive methodology to the synthesis of a new family of cyclic β-amino acids. The stereochemical preference of this reaction was postulated by the proposed transition states (see Scheme 1) that may account for the attack of the nucleophile from the β face. The conjugate hetero-Michael reaction is highly reproducible, and, it gives the desired cyclic product upon use of different amino acid derivatives.

With an objective to explore macrocyclic chemical space around the amino indoline scaffold,[14] herein, we report another amino-indoline scaffold which can be synthesized from 6-nitropiperonal. We developed a modular approach that allowed us to incorporate different types of medium/large ring skeletons (see **2.2**, **2.3**, and **2.4**; Scheme 2) into an *enantioenriched* amino-indoline scaffold **2.1**.



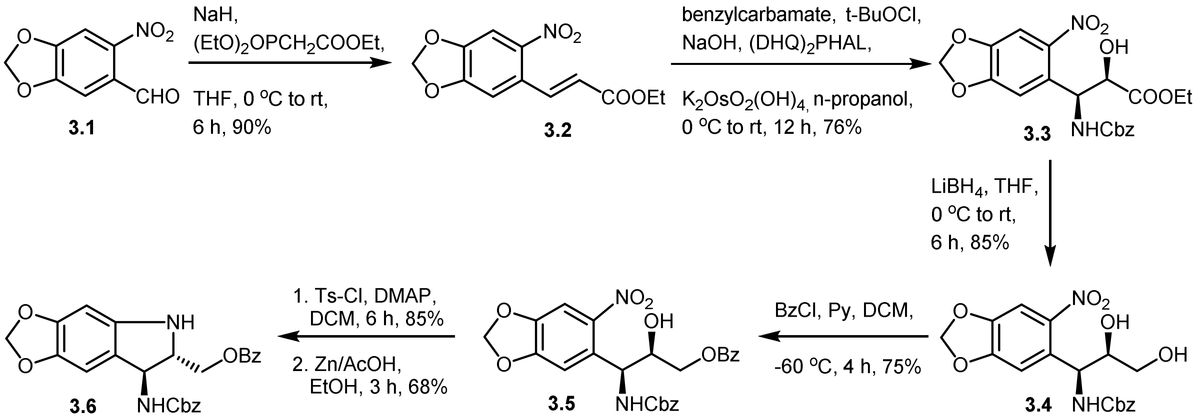
Scheme 2: The proposed plan to obtain macrocyclic-derived compounds 2.2, 2.3, and 2.4 from enantioenriched amino-indoline scaffold

There are several attractive features of amino-indoline scaffold **2.1** that makes it an attractive starting point to incorporate various medium/large rings into the skeleton. These include the presence of (i) a *trans*-2,3-amino alcohol moiety that can further be utilized to build *trans*-fused 12-membered ring, (ii) a 1,2-amino alcohol that utilizes the indoline nitrogen atom to provide 11-membered ring, and, (iii) 1,3-orthogonally protected diamino functional groups that can led to the additional bridged 11-membered ring. In all three strategies, we utilized a common approach to incorporate the amino acid functionality, and, ring-closing metathesis[15] was used as stitching technology to construct the macrocyclic ring. For example, the final target **2.2** presents several interesting features, including an enantioenriched amino indoline substructure, a *trans*-fused 12-membered ring, and the presence of an amino acid functionality in the macrocyclic skeleton to allow variation in the nature of the chiral side chain. Similarly, target **2.3** provides an amino indoline substructure and an additional 11-membered ring having an amino acid in the skeleton to allow variation in the chiral side chain. Finally, our third target, that is, compound **2.4**, contains an additional bridged 11-memebred ring that utilizes both nitrogen atoms of an amino indoline scaffold. Altogether, these three divergent strategies with a common objective provides an excellent opportunity to build a unique chemical toolbox having compounds that will represent the privileged substructure and additional different types of large-ring derivatives to explore their value in biology as modulators of protein-protein interactions and signaling pathways, in general.

2.3. Results and Discussion:

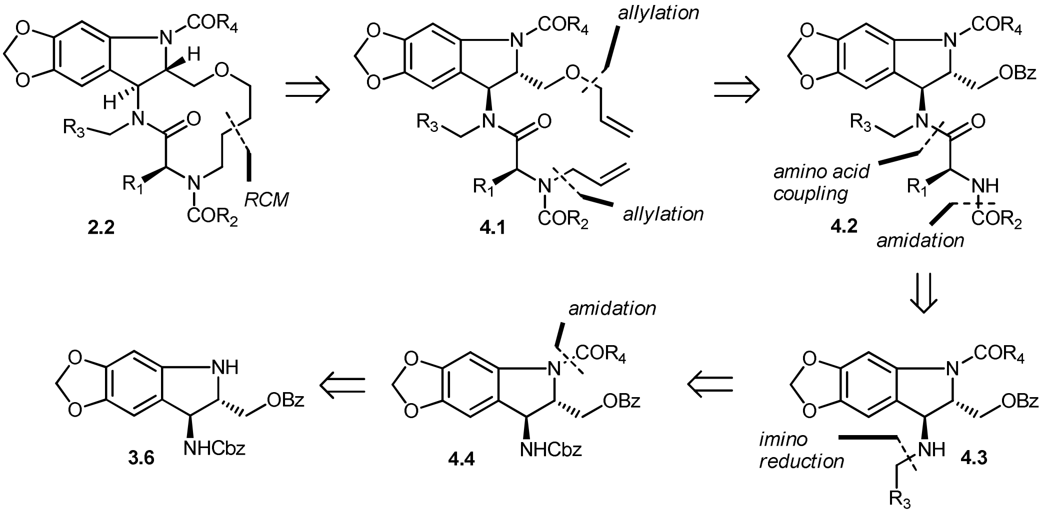
2.3.1. Synthesis of Amino Indoline Scaffold:

The enantioselective synthesis of amino indoline derivative **3.6** is shown in Scheme 3. 6-nitropiperonal **3.1** was converted into **3.2** by using Wittig-Horner reaction. Compound **3.2** was then subjected to an asymmetric aminohydroxylation reaction to give 1,2 amino alcohol compound **3.3** in 76% yield. This was further subjected to an ester reduction and followed by selective benzoyl protection of primary alcohol at low temperature gave **3.5** in good yields. The tosylation of secondary alcohol of **3.5**, followed by the nitro reduction using Zn/AcOH gave our required enantioenriched amino indoline scaffold **3.6**. In our apporach the cyclization took place *in situ*, with a good yield and high selectivity.



Scheme 3: Synthesis of amino indoline scaffold

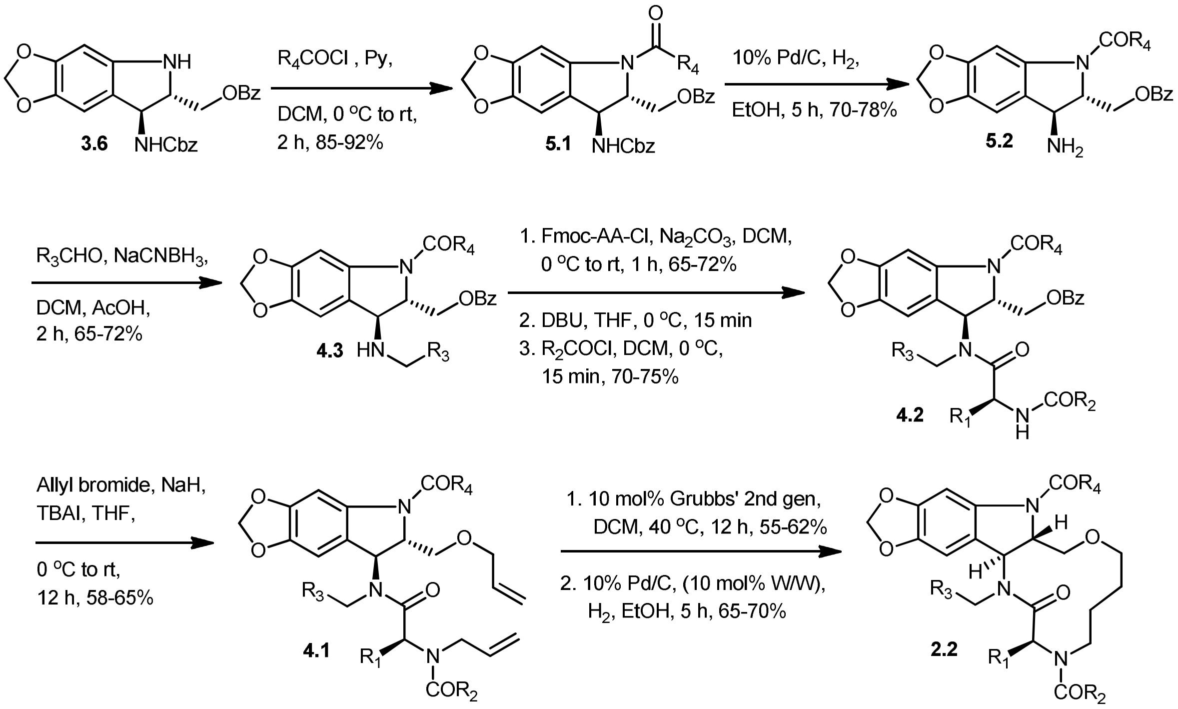
2.3.2. Retrosynthesis of 12 Membered Macrocycle:



Scheme 4: The retrosynthetic analysis of 12 membered macrocycle

The retrosynthetic analysis of 12-membered macrocyclic compound, **2.2** is shown in Scheme 4. As shown in this scheme, we planned the macrocyclization by a ring closing metathesis (RCM) of *bis*-allylated compound **4.1**, which could be synthesized from intermediate **4.2**, by a *bis*-allylation using allyl bromide. Compound **4.2** could be obtained from coupling of various amino acid chlorides with secondary amine **4.3**, which could be synthesized from an imino-reduction of the primary amine with various aldehydes. Compound **4.4** was synthesized from amino indoline scaffold **3.6**, by amide bond forming coupling using different acid chlorides.

2.3.3. Synthesis of 12-Membered Macrocycle, 2.2:

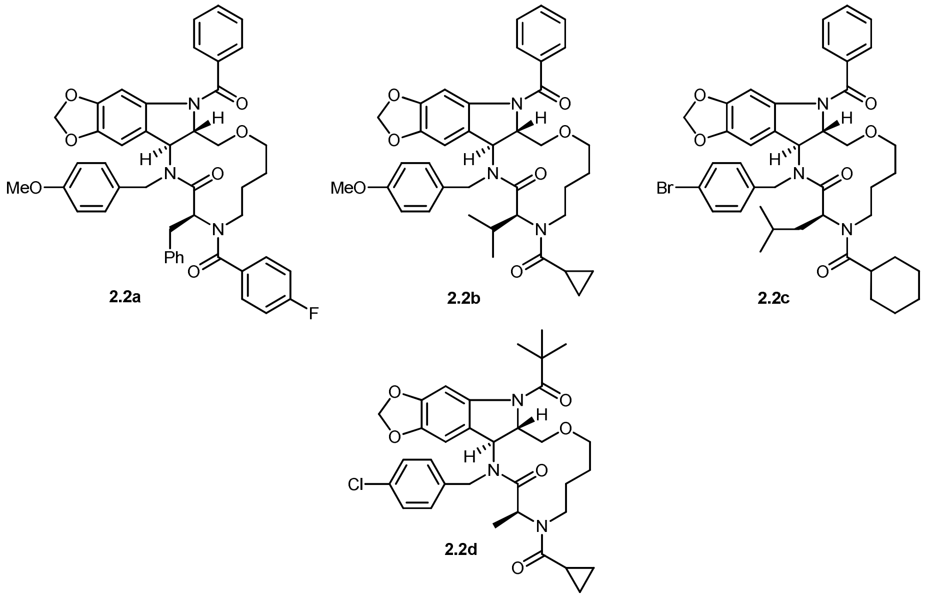


Scheme 5: Synthesis of 12 membered macrocycle

As shown in Scheme 5, synthesis of 12-membered macrocycles started with an acylation of free amine **3.6** (i.e., R$_4$ as the first diversity point). Following the removal of the benzyloxycarbonyl (Cbz) group (10% Pd over C), it was then subjected to a reductive alkylation to obtain secondary amine **4.3** in good yields (i.e., R$_3$ as the second diversity point). The secondary amine was then coupled with different 9-fluorenylmethoxycarbonyl (Fmoc) amino acid chlorides, which synthesized from their corresponding amino acids. This approach allowed us to introduce the diversity site as R$_1$ (i.e., the side chain of an amino acid). The NFmoc group was removed by using DBU,[16] and the free amine was then acylated with acid chlorides to provide **4.2** with the fourth diversity point (R$_2$). Upon treatment under basic conditions with allyl bromide, *bis*-allylated product **4.1** was produced in a respectable yield. Under reaction conditions that may have trace amounts of moisture, the hydrolysis of the benzoyl ester was also observed, which was then directly followed by an *O*-allylation. The next crucial step was to explore the ring-closing metathesis based stitching technology on highly functionalized substrate, **4.1**. This reaction worked-well, and, as we expected, the 12-membered macrocyclic ring was easily obtained. The cyclic product was then subjected to hydrogenation to obtain the saturated version as product **2.2**. In this series, four macrocyclic compounds were synthesized, and, all of them were thoroughly characterized using various analytical techniques (HPLC–MS and NMR spectroscopy).

2.3.4. Derivatives of 12 Membered Macrocycle, 2.2:

We synthesized 4 macrocyclic derivatives of **2.2** by changing the R_1, R_2, R_3 and R_4 groups, and, these compounds are shown in Figure 2.



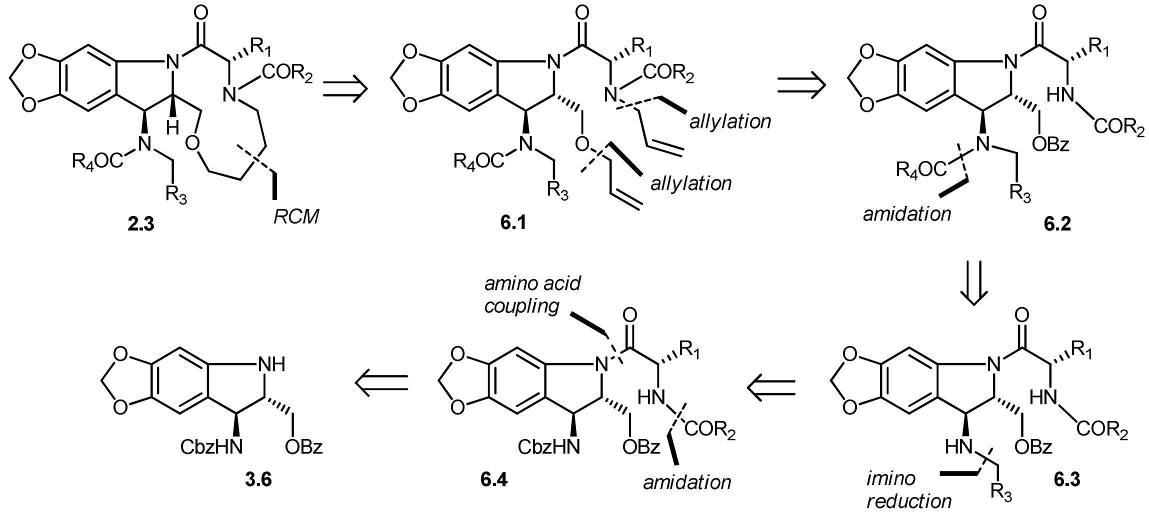
2.2a **2.2b** **2.2c** **2.2d**

Figure 2: Derivatives of 12 membered macrocycles

2.3.5. Retrosynthesis of 11 Membered Macrocycle:

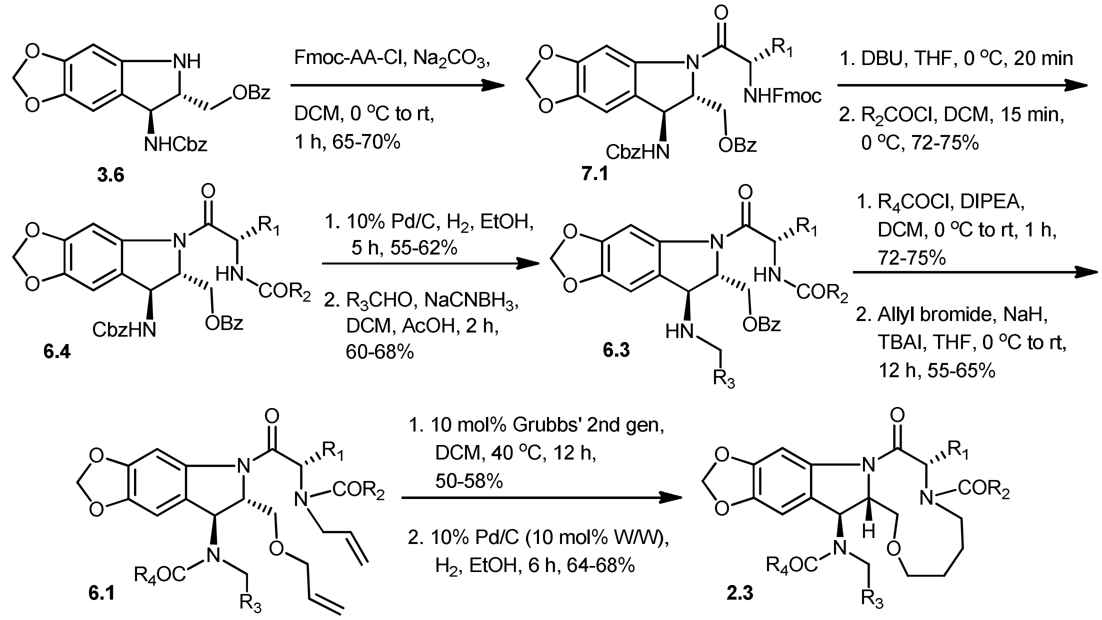
The retrosynthetic analysis of 11-membered macrocycle is shown in Scheme 6. As we have done in the previous series, we planned the key macrocyclic step by the ring closing metathesis (RCM) of *bis*-allylated compound **6.1**. *Bis*-allylated compound **6.1** can be easily synthesized from **6.2**, which can be obtained from secondary amine **6.3**. **6.3** can be obtained from **6.4** by the *N*-Cbz removal and followed by imino reduction.

Compound **6.4** is derived from an amino-indoline scaffold by coupling with different amino acid chlorides.



Scheme 6: The retrosynthetic analysis of 11 membered macrocycle

2.3.6. Synthesis of 11-Membered Macrocycle, 2.3:



Scheme 7: Synthesis of 11-membered macrocycle

Our next plan was to develop a synthetic method to access various 11-membered macrocyclic compounds as shown in Scheme 7. To achieve this objective, we again utilized compound **3.6** as the starting material. The direct coupling with various amino acid chlorides (i.e., to obtain the first diversity point as R_1) gave **7.1**. Following the NFmoc group removal, the amine was acylated with different acid chlorides to incorporate the second diversity point as R_2 and this produced **6.4** in good yields. In this series, we planned to utilize the amino indoline side chain oxygen atom to obtain our macrocyclic ring and the indoline amine was converted into a tertiary amide. This was achieved by removal of the Cbz group followed by imino reduction

with different aldehydes (third diversity point as R_3), which gave **6.3**. It was further converted into the tertiary amide by an acylation with various acid chlorides as the fourth diversity point. This compound was further subjected to *bis*-allylation with NaH and allylbromide to obtain **6.1**. As described earlier, under the reaction conditions of debenzoylation, and followed by *O*-allylation at the primary hydroxy group and allylation of amide nitrogen took place. The *bis*-allylated product **6.1** was then subjected to ring-closing metathesis by using Grubbs chemistry. This reaction worked-well and the 11-membered cyclic ring was easily obtained. To validate the generality of our approach, we synthesized four macrocyclic products. All of these macrocyclic products were then subjected to double bond reduction under hydrogenation conditions, and, their corresponding saturated derivatives (**2.3a, 2.3b, 2.3c, 2.3d**) were easily obtained as **2.3**. All the products were thoroughly purified and characterized by HPLC–MS and NMR spectroscopy.

2.3.7. Derivatives of 11-Membered Macrocycle, 2.3:

We synthesized four macrocyclic derivatives of **2.3** by changing the R_1, R_2, R_3 and R_4 groups, shown in Figure 3.

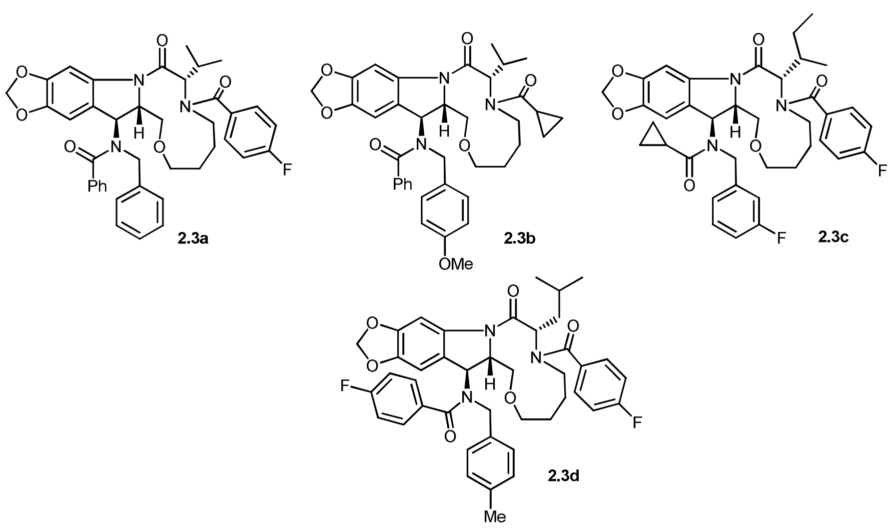
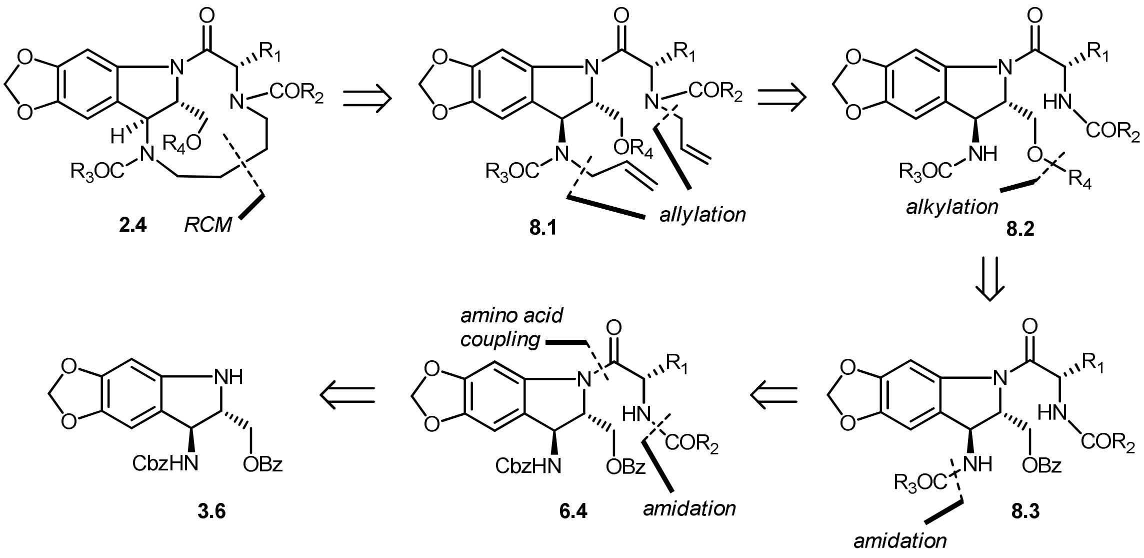


Figure 3: Derivatives of 11-membered macrocycles

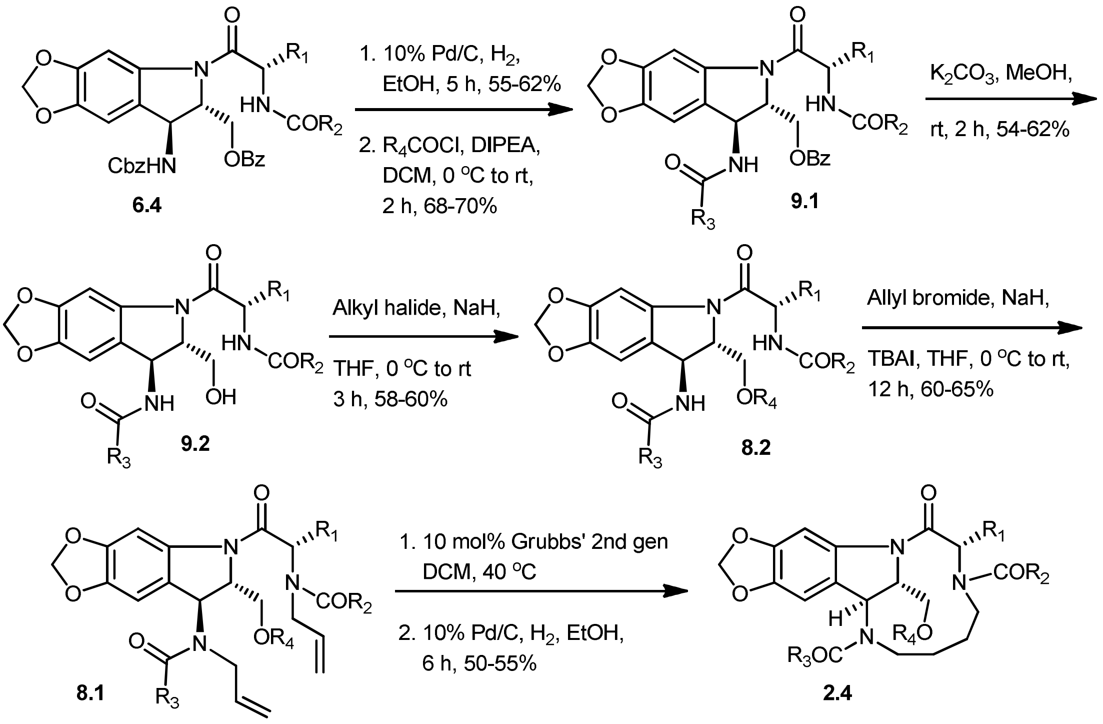
2.3.8. Retrosynthesis of 11-Membered Bridged Macrocycle, 2.4:



Scheme 8: Retrosynthesis of 11-membered bridged macrocycles

The retrosynthetic analysis of **11**-membered bridged macrocycles is shown in Scheme 8. In this series, the macrocyclic approach is developed using both the amine groups (i.e. aromatic secondary amine and benzylic amine) of an amino-indoline scaffold. Thus, the plan is to obtain macrocyclization by the ring closing metathesis (RCM) of *bis*-allylated compound **8.1**, which could be easily synthesized from compound **8.2**. Compound **8.2** is derived from **8.3** by a debenzoylation followed by an alkylation wth various alkyl halides. Intermediate **8.3** could be obtained from **6.4**, which could be synthesized from compound **3.6** by coupling with different amino acid chlorides.

2.3.9. Synthesis of 11-Membered Bridged Macrocycle, 2.4:

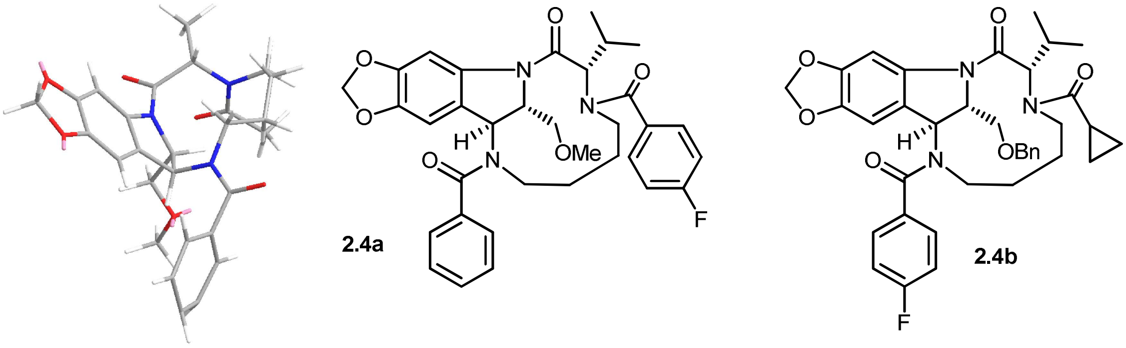


Scheme 9: Synthesis of 11-membered bridged macrocycles

Our final approach to develop a method to access 11-membered bridged macrocyclic derivatives is shown in Scheme 9. As in the previous case, we obtained compound **6.4** from **7.1** in easy transformations. At this stage, the amino group on the scaffold **6.4** was deprotected using Pd/C and H_2, and, further, acylated to obtain **9.1** (third diversity point as R_3). Following the hydrolysis of the side chain benzoyl ester to hydroxy group gave **9.2**. Then alkylation of **9.2** with different alkyl halides (fourth diversity point as R_4) gave **8.2** with good yields. It was then subjected to *bis*-allylation reaction conditions to obtain compound **8.1**. The *bis*-allylated product was then subjected to crucial ring closing metathesis chemistry. This reaction worked well and the final product **2.4** obtained after the double bond reduction. It was thoroughly purified and well-characterized. In this series, we synthesized two macrocycles. A careful analysis of the 3D model of **2.4a** (see Figure 4) indicated that the benzylic nitrogen occupies a pseudoaxial position, and, this brings the -CH_2OMe out of the plan due to a relative 1,2-*trans* orientation. This may be a crucial factor in bringing two olefinic groups in proximity to facilitate the 11-membered bridged macrocycle formation.

2.3.10. Derivatives of 11-Membered Bridged Macrocycle, 2.4:

We synthesized two macrocyclic derivatives of **2.4** by changing the R_2, R_3 and R_4 groups, shown in Figure 4.



2.4a

2.4b

Chem 3D Structure

Figure 4: Derivatives of 11 membered bridged macrocycles

2.4. Biological Evaluation:

All the small molecules obtained from this project (in total 39 compounds) were then subjected to an embryo zebrafish screen in three different assays: angiogenesis,[17] early embryonic development,[18] and, neurogenesis.[17c,19] These assays are well-documented in the literature, and, this study was carried out in collaboration with Satish Kitambi team at the Karolinska Institute, Sweden.[20] A major advantage with the use of the zebrafish technology is that it is close to the *in vivo* model, and, a cheap way to evaluate compounds in a high-throughput manner. Embryos were obtained by natural mating and staged according to the literature procedure[21] Zebrafish embryos of stages older than 24 hours post fertilization (hpf) were treated with 0.03% PTU (*N*-phenylthiourea) when needed to inhibit pigment formation. Wild type AB line, and transgenic lines Tg (fli:EGFP, islet1:GFP) were used to assess the effects on epiboly, angiogenesis and neurogenesis respectively. Zebrafish embryos for small molecule screening experiments were collected via pair-wise matings, cleaned and incubated in PTU treated E3 water at 28.3 ^{o}C. One to four cell stage embryos were then distributed into 96 well clear bottom plate (Corning).

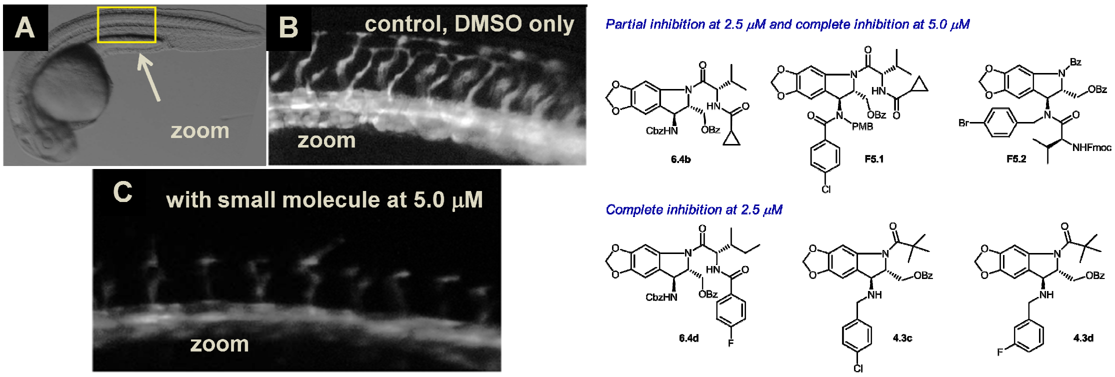
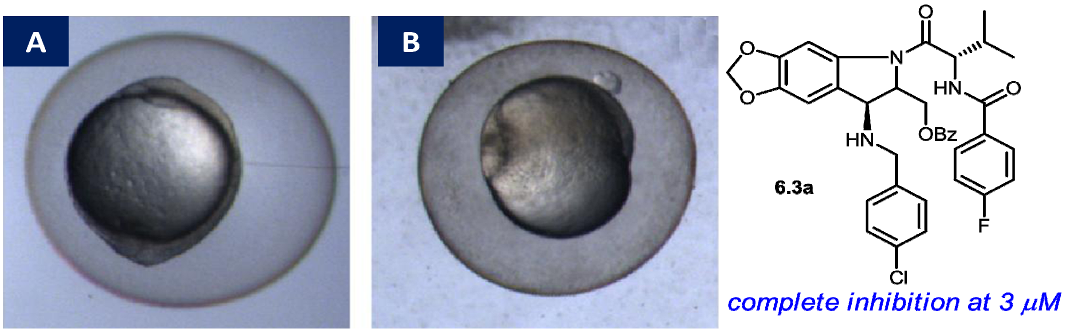


Figure 5: (**A**) Wild-type zebrafish embryo at 30 hpf of development, region zoomed in panels **B** and **C** is shown by a yellow box, (**B**) zoom section of wild-type or vehicle treated embryo, and (**C**) zoom section after treatment with compound **6.4b**.

The compound exposure was carry-out in a 96 well plate (Corning) and three embryos were taken in each well containing 200 µl of (0.5 to 15µM) compound in PTU treated egg water. The 96 well plates were incubated at 28.3 ^{o}C and the embryos were allowed to grow until 10 hpf or 30 hpf to assess the effect on epiboly, angiogenesis/neurogenesis respectively. Phenotypes were scored using a Zeiss Axiovert 200 inverted microscope equipped with a cooled CCD camera. Photographs were processed and assembled using Photoshop software (see Figure 5). The zebrafish screen identified **6.4b**, **F5.1**, and **F5.2** (Figure 5) as antiangiogenesis agents with partial inhibition at 2.5 µm and complete inhibition at 5.0 µm, and compounds **6.4d**, **4.3c**, and **4.3d** were found to exhibit complete inhibition at 2.5 µm.

In another parallel study, we identified another small molecule **6.3a**, that inhibited the epiboly cell movements during an early embryonic development. The delay in epiboly was clearly seen in embryos exposed to small molecule at 3.0 µM concentration. However these embryos did complete epiboly and developed normally without any visible effects on angiogenesis and neurogenesis. The mechanism underlying the inhibition of angiogenesis and epiboly will be characterized using both zebrafish and other cell based assays (Figure 6).



complete inhibition at 3 μM

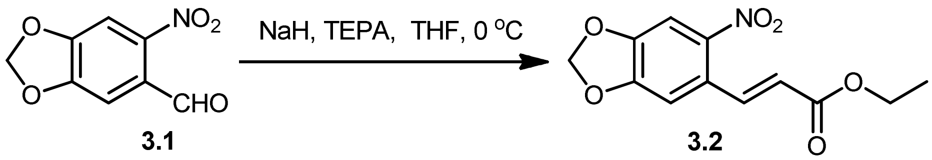
Figure 6: Zebrafish early embryonic development assay. (A) DMSO exposed embryos at 10 hpf of development, (B) small molecule exposed embryos causing a delay in epiboly.

2.5. Conclusion:

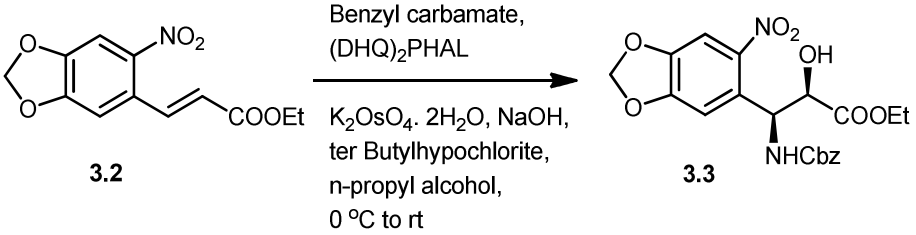
With the goal of building a diverse set of small molecules having a privileged indoline substructure, and, different types of medium to large ring derivatives, we developed a diversity based synthesis method. The first study utilized the primary hydroxy on the side chain and the benzylic amine to build a 12-membered macrocyclic ring. Using a similar strategy and through the use of the primary hydroxy group and the indoline amino group, 11-membered ring was synthesized. Finally, the use of both nitrogen atoms (i.e., primary benzylic and secondary aromatic amines) led to the successful synthesis of 11-membered bridged macrocycle. Upon evaluation of our small molecules in a zebrafish assays, we identified three novel compounds as antiangiogenesis agents. These rapid assay procedures allowed us to quickly identify the effects of small molecules on various biological processes. The effects produced by these novel small molecules provided a platform for using chemical biology approaches to understand the basic biological processes such as epiboly and angiogenesis. These findings are at an early stage, and, further work is required to gain deeper insight into the mode of action of these functional small molecules.

2.6. Experimental procedures:

2.6.1. Amino-indoline scaffold:

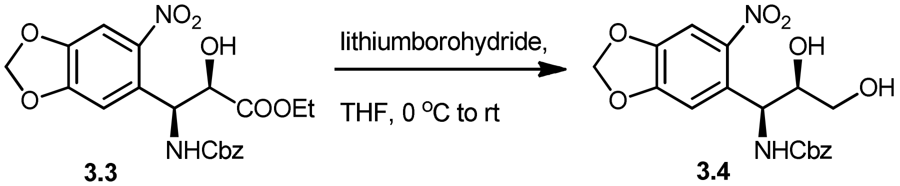


(E)-ethyl 3-(6-nitrobenzo[d][1,3]dioxol-5-yl)acrylate (3.2): To a stirred solution of 60% NaH (1.22 g, 50.83 mmol) in dry THF (100 mL), was added triethyl phosphonoacetate (TEPA) (7.62g, 38.43 mmol) at 0 °C, and stirred under nitrogen atmosphere for 30 min. A solution of 6-nitropiperonal **3.**1 (5g, 25.62 mmol) in THF was added drop wise to the reaction mixture and allowed to stirring for 4 h at 0 ^{o} C. After completion of the reaction, reaction mixture was quenched by the addition of saturated ammonium chloride solution (20 mL) and extracted with ethyl acetate (2 X 100 mL). Combined organic layers was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated, the crude product was purified by flash column chromatography (ethyl acetate/hexanes 5%) afforded The **3.2** as yellow crystalline solid. Molecular Formula: $C_{12}H_{11}NO_6$; R$_f$ (ethyl acetate/hexane 5%): 0.31; Yield: 90%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.09 (d, *J* = 15.74 Hz, 1H), 7.53 (s, 1H), 6.98 (s, 1H), 6.24 (d, *J* = 15.73 Hz, 1H), 6.15 (s, 2H), 4.27 (q, *J* = 7.13 Hz, 2H), 1.33 (t, *J* = 7.13 Hz, 4H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 165.8, 151.9, 148.9, 142.9, 140.1, 127.2, 122.2, 107.2, 105.5, 103.3, 60.8, 14.2; LRMS: (ES+) m/z = 266 (M+1).

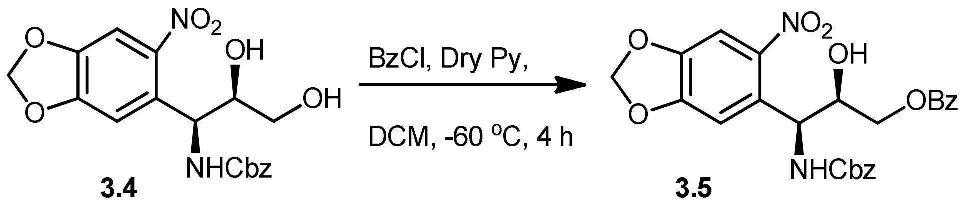


(2R,3S)-ethyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(6-nitro benzo[d][1,3]dioxol-5-yl)propanoate (3.3):

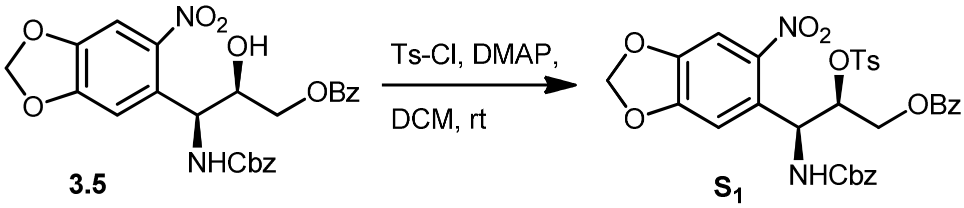
Benzyl carbamate (3.6 g, 23.8 mmol) was dissolved in n- propylalcohol (32 mL). To this stirred solution at 0 °C was added a freshly prepared solution of NaOH (.95g, 240 mmol) in 60 mL of water. Followed by a freshly prepared *ter*butylhypochlorite (2.6 g, 24 mmol,ca 2.8 mL). then a solution of the ligond (DHQ)$_2$PHAL (307 mg, 039 mmol, 5 mol%) in n-propylalcohol (28 mL) was added. The solution was stirred in an ice bath for a few minutes then the olefin **3.2** (2.1 g, 7.917 mmol) was added, followed by potassiumosmatedihydrate (116 mg, 0.314 mmol, 4 mol%). The reaction mixture was stirred for overnight, the dark green color of the solution turns to dark yellow color at the end. After TLC analysis confirmed the absence of starting material, extracted with ethyl acetate twice. The combined organic layers was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (ethyl acetate/hexane, 30%) afforded the **3.3** as yellow solid. Molecular Formula: $C_{20}H_{20}N_2O_9$; R_f (40% ethyl acetate/hexane): 0.3; Yield: 76%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.55 (s, 1H), 7.41-7.26 (m, 5H), 6.98 (s, 1H), 6.10 (d, *J* = 4.40 Hz, 3H), 5.96 (d, *J* = 8.74 Hz, 1H), 5.82 (d, *J* = 8.32 Hz, 1H), 5.07 (d, *J* = 12.28 Hz, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.63 (s, 1H), 4.30 (dd, *J* = 15.34, 7.80 Hz, 2H), 3.33 (s, 1H), 1.28 (t, *J* = 6.75 Hz, 4H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 172.6, 155.1, 152.0, 147.3, 142.1, 135.9, 132.5, 128.4, 128.3, 128.2, 128.0, 127.7, 108.2, 105.5, 103.0, 72.1, 67.1, 62.8, 52.6, 13.9; LRMS: (ES+) m/z = 433 (M+1).



benzyl (1S,2R)-2,3-dihydroxy-1-(6-nitrobenzo[d][1,3]dioxol-5-yl)propylcarbamate (3.4): To a solution of ester **3.3** (3.37g, 7.79 mmol) in Dry THF (75 mL) was added Lithium Borohydride (257mg, 11.6 mmol) under nitrogen at 0 °C. the solution was allowed to room temperature, and then stirred over night. The reaction was quenched by saturated ammonium chloride solution. The aqueous layer was extracted with ethyl acetate. The combined organic layers was washed with brine, dried over anhydrous sodiumsulfate, filtered and concentrated. The crude product was purified by flash column chromatography (ethyl acetate/hexane 30%) afforded the **3.4** as yellow solid. Molecular Formula: $C_{18}H_{18}N_2O_8$; R_f (50% ethyl acetate/hexane): 0.2; Yield: 85%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.59 (d, *J* = 8.26 Hz, 1H), 7.49 (s, 1H), 7.39-7.22 (m, 4H), 7.19 (s, 1H), 6.17 (s, 2H), 5.24 (d, *J* = 5.81 Hz, 1H), 5.04-4.88 (m, 3H), 4.67 (s, 1H), 3.68 (s, 1H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 156.1, 152.2, 147.0, 141.8, 136.0, 134.3, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.3, 107.8, 105.6, 103.0, 73.5, 67.1, 64.2, 52.0; LRMS: (ES+) m/z = 391 (M+1).

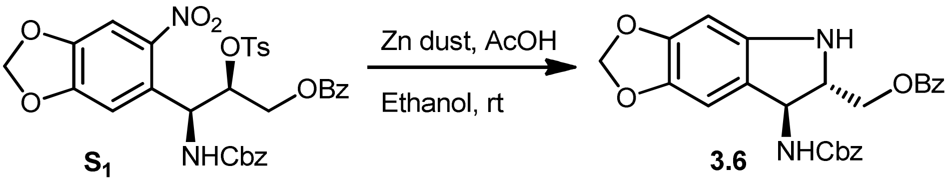


(2R,3S)-3-(benzyloxycarbonylamino)-2-hydroxy-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propyl benzoate (3.5): To a solution of diol **3.4** (1.66g, 4.25mmol) in dry DCM (50 mL) was added dry pyridine (373 µL, 4.63mmol). The solution was cooled to – 60 °C. Benzoylchloride (463 µL, 3.99mmol) in DCM was added to the solution drop wise. The solution was stirred at -60 °C for 4 h. The reaction mixture was diluted with DCM and washed with 1% HCl, brine and saturated $NaHCO_3$ solution. The organic layer was dried over sodiumsulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane 30%) afforded the **3.5** as yellow solid. Molecular Formula: $C_{25}H_{22}N_2O_9$; R_f (40% ethyl acetate/hexane): 0.3; Yield: 75%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 8.06 (d, *J* = 7.44 Hz, 2H), 7.63-7.51 (m, 2H), 7.43 (t, *J* = 7.56 Hz, 2H), 7.39-7.24 (m, 5H), 7.02 (s, 1H), 6.09 (s, 2H), 5.65 (d, *J* = 7.93 Hz, 1H), 5.03 (s, 2H), 4.64-4.46 (m, 2H), 4.37 (s, 1H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 167.0, 155.7, 152.1, 147.1, 141.9, 135.8, 134.1, 133.3, 129.7, 129.3, 128.5, 128.4, 128.1, 107.8, 105.6, 103.0, 71.5, 67.2, 66.7, 52.1; LRMS: (ES+) m/z = 495 (M+1).



(2R,3S)-3-(benzyloxycarbonylamino)-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)-2-(tosyloxy)propyl benzoate (S_1): To a solution of hydroxyl compound **3.5** (1.96g, 3.96 mmol) in dry DCM (30 mL) were added freshly purified (washed with $NaHCO_3$) Tosylchloride (1.12g, 5.96 mmol) and DMAP (0.966g, 7.92mmol) under inert conditions. The solution was stirred at room temperature for overnight. The solution was washed with brine and saturated sodiumbicarbonate solution. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane 30%) afforded the **S_1** as yellow solid. Molecular Formula: $C_{32}H_{28}N_2O_{11}S$; R_f (30%

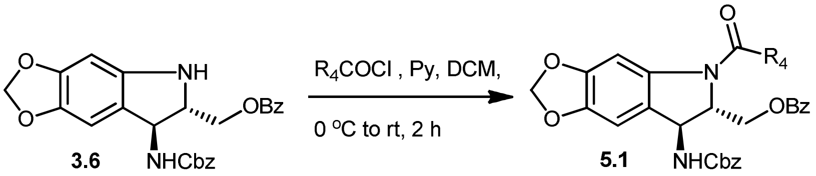
ethyl acetate/hexane): 0.26; Yield: 85%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 8.02 (d, *J* = 7.61 Hz, 2H), 7.60-7.51 (m, 3H), 7.47 (s, 1H), 7.41 (t, *J* = 7.64 Hz, 2H), 7.51-7.28 (m, 5H), 7.12 (t, *J* = 9.43 Hz, 2H), 6.80 (s, 1H), 6.11 (d, *J* = 31.87 Hz, 2H), 5.86 (dd, *J* = 15.75, 6.85 Hz, 2H), 5.27 (t, *J* = 5.17 Hz, 1H), 5.03 (s, 2H), 4.66 (dd, *J* = 11.78, 5.76 Hz, 1H), 4.52 (dd, *J* = 11.84, 6.27 Hz, 1H), 2.34 (s, 3H);); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 165.8, 155.3, 152.3, 147.5, 145.1, 141.6, 135.6, 133.2, 132.5, 131.8, 129.8, 129.7, 129.1, 128.5, 128.3, 128.1, 127.6, 107.9, 105.9, 103.2, 80.2, 67.5, 62.9, 52.0, 21.5; LRMS: (ES+) m/z = 649 (M+1).



S_1 **3.6**

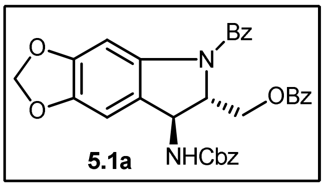
((6S,7S)-7-(benzyloxycarbonylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (3.6): The solution of Nitro compound **S_1** (2.3g, 3.54mmol) in ethanol (20mL) was added Zn powder (2.22g, 34.4mmol). Followed by AcOH (1.96mL) at room temperature. And allow to stirring for 2 hours. After TLC analysis confirmed the absence of starting material, reaction mixture was filtered through celite, solution was evaporated and the residue was diluted with ethyl acetate and washed with sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/hexane) afforded the **3.6** as white solid. Molecular Formula: $C_{25}H_{22}N_2O_6$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 68%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.99 (d, *J* = 7.64 Hz, 2H), 7.55 (t, *J* = 7.39 Hz, 1H), 7.42 (t, *J* = 7.70 Hz, 2H), 7.38-7.28 (m, 5H), 6.70 (s, 1H), 6.23 (s, 1H), 5.85 (d, *J* = 7.26 Hz, 2H), 5.18-5.06 (m, 4H), 4.57 (dd, *J* = 11.10, 3.77 Hz, 1H), 4.36 (dd, *J* = 10.62, 7.82 Hz, 1H), 4.02-3.86 (m, 2H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 166.4, 155.7, 148.9, 144.8, 141.3, 136.2, 133.0, 129.7, 129.6, 128.5, 128.3, 128.2, 128.1, 118.2, 105.3, 101.0, 93.6, 66.9, 66.5, 66.3, 56.3; LRMS: (ES+) m/z = 446 (M+1).

2.6.2. 12 Membered macrocycles:



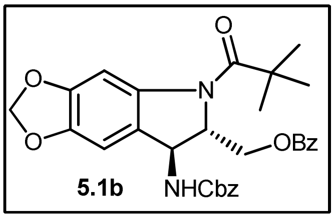
Compound 5.1:

To a stirred solution of secondary amine **(**1 mmol) in dry DCM was added Pyridine (4 mmol) at 0 °C under inert atmosphere. The reaction mixture was stirred for 5 minutes, Then acid chloride (1.2 mmol) was added and reaction mixture was stirred for 2 hours. After completion of the reaction, reaction mixture was quenched by the addition of saturated NaHCO$_3$ solution and extracted with DCM (2 X 10 mL), the combined organic layers was washed with 10% hydrochloric acid dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced Pressure. Purification by flash column chromatography to obtain pure product of **5.1**.



5.1a

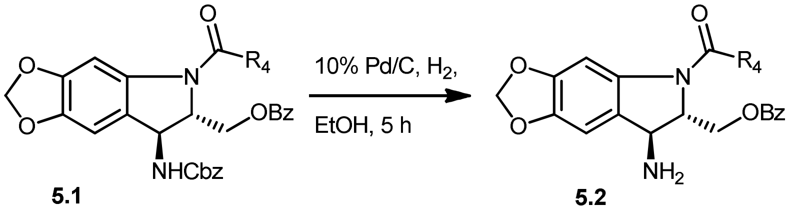
((6S,7S)-5-benzoyl-7-(benzyloxycarbonylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6yl)methyl benzoate (5.1a); Molecular Formula: C$_{32}$H$_{26}$N$_2$O$_7$; R$_f$ (30% ethyl acetate/hexane): 0.3; Yield: 92%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.72 (d, J = 7.31 Hz, 2H), 7.52-7.45 (m, 4H), 7.44-7.29 (m, 9H), 7.26 (s, 1H), 6.81 (s, 1H), 5.89 (d, J = 19.73 Hz, 2H), 5.21 (d, J = 6.86 Hz, 1H), 5.15 (s, 2H), 5.09-4.59 (m, 1H), 4.81 (s, 1H), 4.52-4.42 (m, 1H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 169.8, 168.7, 165.9, 155.4, 148.5, 144.4, 137.5, 135.7, 133.3, 133.0, 130.5, 130.0, 129.6, 129.3, 128.8, 128.5, 128.3, 128.2, 128.1, 127.1, 105.4, 101.6, 67.5, 67.1, 64.2, 54.3; LRMS: (ES+) m/z = 549 (M-1).



5.1b

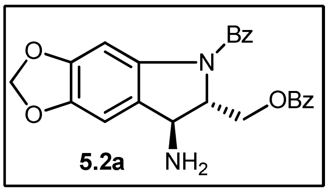
((6S,7S)-7-(benzyloxycarbonylamino)-5-pivaloyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (5.1b); Molecular Formula:

$C_{30}H_{30}N_2O_7$; R$_f$ (30% ethyl acetate/hexane): 0.4; Yield: 87%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.97 (d, *J* = 7.54 Hz, 2H), 7.62 (s, 1H), 7.56 (t, *J* = 7.37 Hz, 1H), 7.43 (t, *J* = 7.57 Hz, 2H), 7.39-7.25 (m, 6H), 6.79 (s, 1H), 5.93 (d, *J* = 9.13 Hz, 2H), 5.12-5.08 (m, 2H), 4.95-4.88 (m, 2H), 4.62-4.58 (m, 1H), 4.18-4.08 (m, 1H), 1.39-1.22 (m, *J* = 14.11 Hz, 9H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.7, 166.1, 155.2, 148.6, 144.6, 136.2, 136.1, 133.2, 129.7, 129.4, 128.5, 128.3, 128.2, 128.1, 128.1, 128.0, 121.0, 105.1, 102.8, 101.6, 66.8, 65.4, 63.9, 55.2, 40.6, 28.2; LRMS: (ES+) m/z = 553 (M+Na).

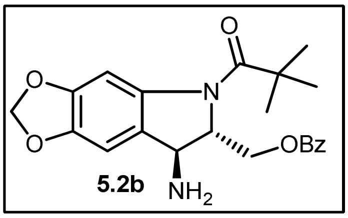


Compound 5.2:

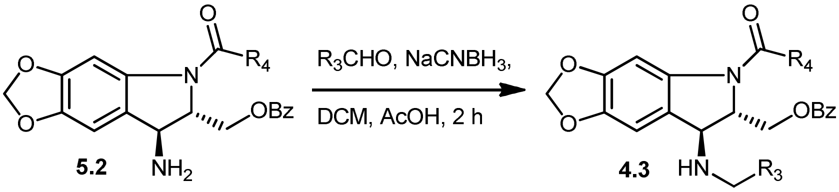
To a solution of the *N*-Cbz protected compound **5.1** (1 mmol) in ethanol was added Palladium catalyst (10 mol% (W/W), 10% palladium over activated carbon). The reaction mixture was then subjected to hydrogenation under atmospheric pressure for 5 h. The mixture was filtered through celite. The organic solution was concentrated under reduced pressure. Purification by flash column chromatography to obtain pure product of **5.2**.



((6S,7S)-7-amino-5-benzoyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (5.2a); Molecular Formula: $C_{24}H_{20}N_2O_5$; R$_f$ (ethyl acetate): 0.1; Yield: 75%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.73 (d, *J* = 7.57 Hz, 2H), 7.56-7.30 (m, 8H), 6.79 (s, 1H), 5.88 (d, *J* = 13.10 Hz, 2H), 4.38 (s, 2H), 4.20 (s, 1H), 4.66 (s, 1H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 168.6, 165.9, 147.7, 144.2, 136.1, 135.9, 133.0, 130.4, 129.4, 128.6, 128.1, 127.1, 104.9, 101.4, 69.7, 64.0, 55.0; LRMS: (ES+) m/z = 439 (M+Na).

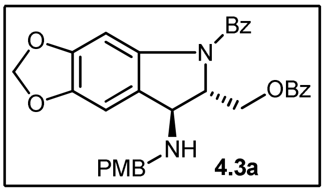

5.2b

((6S,7S)-7-amino-5-pivaloyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (5.2b); Molecular Formula: $C_{22}H_{24}N_2O_5$; R*f* (ethyl acetate): 0.1; Yield: 71%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.93 (d, *J* = 7.63 Hz, 2H), 7.68 (d, *J* = 4.90 Hz, 1H), 7.57 (t, *J* = 7.33 Hz, 1H), 7.43 (t, *J* = 7.72 Hz, 2H), 6.79 (s, 1H), 5.93 (d, *J* = 2.13 Hz, 2H), 4.84 (dd, *J* = 9.15, 3.69 Hz, 1H), 4.57 (dd, *J* = 11.2, 4.4 Hz, 1H), 4.14 (s, 1H), 4.04 (dd, *J* = 11.19, 9.33 Hz, 1H), 1.44 (s, 9H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.7, 170.3, 167.0, 166.0, 164.5, 145.0, 144.5, 133.2, 132.5, 132.4, 129.6, 129.6, 129.5, 128.5, 128.3, 128.2, 115.4, 115.2, 104.9, 101.7, 101.5, 100.8, 67.0, 66.1, 56.3, 40.8, 28.3, 28.1, 18.1; LRMS: (ES+) m/z = 395 (M-1).



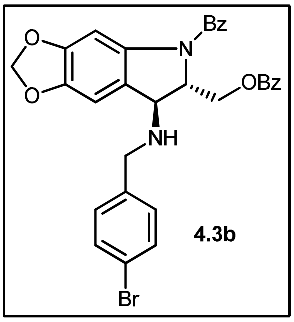
Compound 4.3:

The Primary amine **5.2** (1 mmol) was dissolved in dry DCM and added aldehyde (1.1 mmol). The reaction mixture was stirred for 30 minutes, then $NaCNBH_4$ (1.5 mmol) and AcOH (Catalytic amount) was added to the reaction mixture at room temperature. The reaction mixture was stirred for 2 h. then the reaction mixture was quenched by the addition of saturated $NaHCO_3$, and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography to give the product **4.3**.


4.3a

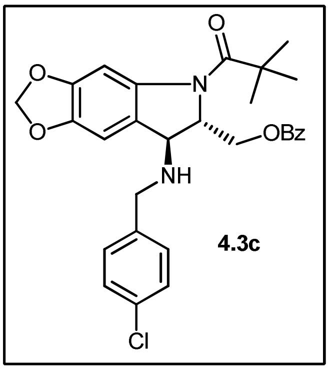
((6S,7S)-5-benzoyl-7-(4-methoxybenzylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.3a); Molecular Formula: $C_{32}H_{28}N_2O_6$; R*f* ((50%

ethyl acetate/hexane): 0.3; Yield: 68%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.72 (d, *J* = 7.65 Hz, 2H), 7.54-7.44 (m, 4H), 7.44-7.38 (m, 2H), 7.35 (t, *J* = 7.52 Hz, 2H), 7.29-7.21 (m, 3H), 6.84 (d, *J* = 7.93 Hz, 2H), 6.79 (s, 1H), 5.90 (d, *J* = 12.36 Hz, 2H), 4.93-4.77 (m, 1H), 4.34 (s, 2H), 4.05 (s, 1H), 3.85 (s, 2H), 3.79 (s, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 166.0**,** 158.8**,** 147.8**,** 144.2**,** 133.0**,** 131.5**,** 130.4**,** 129.5**,** 129.4**,** 129.2**,** 128.7**,** 128.2**,** 127.2**,** 113.9**,** 105.5, 101.4, 65.8, 65.8, 64.2, 60.3, 60.3, 55.2, 50.1, 29.6; LRMS: (ES+) m/z = 559 (M+Na).



4.3b

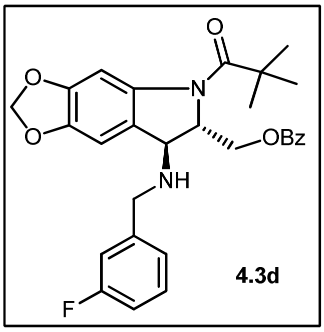
((6S,7S)-5-benzoyl-7-(4-bromobenzylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate(4.3b); Molecular Formula: $C_{31}H_{25}BrN_2O_5$; R$_f$ ((30% ethyl acetate/hexane): 0.3; Yield: 65%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.72 (d, *J* = 7.76 Hz, 2H), 7.57-7.33 (m, 10H), 7.26 (s, 1H), 7.22 (d, *J* = 8.17 Hz, 2H), 6.78 (s, 1H), 5.91 (d, *J* = 11.93 Hz, 2H), 4.94-4.74 (m, 1H), 4.45-4.32 (m, 2H), 4.04 (s, 1H), 3.86 (s, 2H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 168.6, 168.6, 165.9, 147.9, 144.2, 138.5, 136.6, 135.9, 133.1, 131.5, 130.4, 129.6, 129.5, 129.3, 128.7, 128.2, 127.1, 125.2, 120.9, 105.5, 101.5, 65.7, 64.0, 60.3, 49.9, 30.2, 29.6; LRMS: (ES+) m/z = 585 (M+1).



4.3c

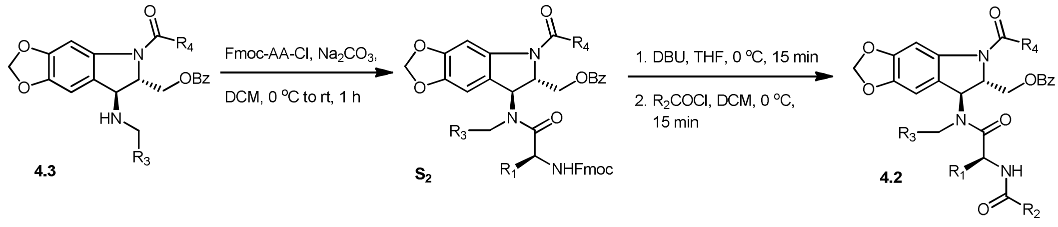
((6S,7S)-7-(4-chlorobenzylamino)-5-pivaloyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.3c); Molecular Formula: $C_{29}H_{29}ClN_2O_5$; R$_f$ ((30%

ethyl acetate/hexane): 0.4; Yield: 71%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.85 (d, *J* = 7.70 Hz, 2H), 7.69 (s, 1H), 7.59 (t, *J* = 7.43 Hz, 1H), 7.43 (t, *J* = 7.72 Hz, 2H), 7.23 (d, *J* = 8.32 Hz, 2H), 7.15 (d, *J* = 8.32 Hz, 2H), 6.77 (s, 1H), 5.92 (s, 2H), 5.00 (dd, *J* = 9.65, 3.81 Hz, 1H), 4.59 (dd, *J* = 10.97, 3.45 Hz, 1H), 3.97-3.78 (m, 4H), 1.45 (s, 9H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.6, 165.9, 147.7, 144.1, 138.3, 137.7, 133.3, 132.6, 129.4, 129.1, 129.0, 128.4, 128.3, 128.3, 128.0, 124.1, 105.1, 102.6, 101.3, 64.0, 63.8, 60.4, 49.9, 40.5, 28.2; LRMS: (ES+) m/z = 543 (M+Na).



4.3d

((6S,7S)-7-(3-fluorobenzylamino)-5-pivaloyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.3d); Molecular Formula: $C_{29}H_{29}FN_2O_5$; R$_f$ ((30% ethyl acetate/hexane): 0.4; Yield: 71%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.87 (d, *J* = 7.63 Hz, 2H), 7.69 (s, 1H), 7.58 (t, *J* = 7.41 Hz, 1H), 7.43 (t, *J* = 7.69 Hz, 2H), 7.17 (dd, *J* = 13.93, 7.86 Hz, 1H), 7.05 (d, *J* = 8.01 Hz, 2H), 6.89 (t, *J* = 8.78 Hz, 1H), 6.78 (s, 1H), 5.95 (s, 2H), 4.99 (dd, *J* = 9.64, 3.75 Hz, 1H), 4.58 (dd, *J* = 11.25, 3.75 Hz, 1H), 4.00-3.85 (m, 4H), 1.45 (s, 9H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.6, 166.0, 164.1, 161.7, 147.7, 144.1, 138.3, 137.7, 133.3, 132.6, 129.4, 129.1, 129.0, 128.4, 128.3, 128.3, 128.0, 124.1, 105.1, 102.6, 101.3, 64.0, 63.8, 60.4, 49.9, 40.5, 28.2; LRMS: (ES+) m/z = 505 (M+1).

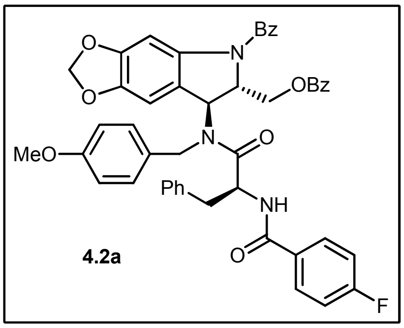


Compound 4.2:

To a mixture of the Secondary amine **4.3** (1 mmol) and Na_2CO_3 (3 mmol) in DCM, was added freshly prepared Fmoc Aminoacid chloride (2.5 mmol) at 0 °C under inert atmosphere. and reaction mixture was stirred for 1 hour. The reaction mixture was quenched by the addition of a saturated $NaHCO_3$, and washed with brine extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated under reduced Pressure. Purification by flash column chromatography to obtained pure product of **S_2**.

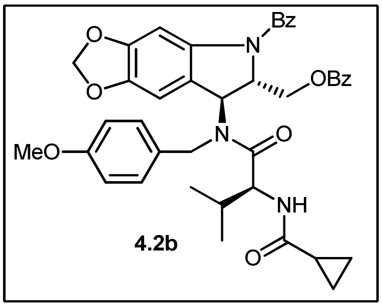
To the *N*-Fmoc compound **S_2** (1 mmol) in dry THF, DBU (2 mmol) was added at 0 °C, and stirred for 15 min. Following the complete removal of the NFmoc group that showed no starting material, THF was evaporated under reduced pressure.

To the above crude dry DCM (10 ml) was added followed by DIPEA (1.1 mmol), acid chloride (1.5 mmol) was added at 0 °C, and stirred for 15 min, then reaction mixture was quenched with saturated $NaHCO_3$, and washed with brine. The organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by flash column chromatography to give the product **4.2**.



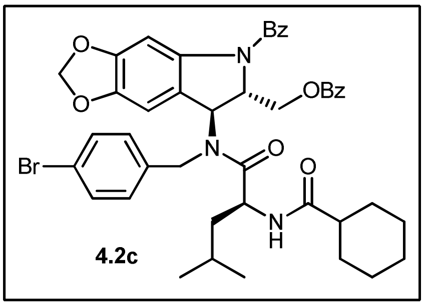
4.2a

((6S,7S)-5-benzoyl-7-((S)-2-(4-fluorobenzamido)-N-(4-methoxybenzyl)-3-phenyl propanamido)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.2a); Molecular Formula: $C_{48}H_{40}FN_3O_8$; R_f ((30% ethyl acetate/hexane): 0.3; Yield: 62%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.77 (dd, *J* = 8.63, 5.32 Hz, 2H), 7.72-7.40 (m, 5H), 7.39-7.19 (m, 10H), 7.16-7.07 (m, 3H), 7.03-6.92 (m, 1H), 6.81-6.71 (m, 4H), 6.45 (s, 1H), 5.84 (d, *J* = 17.57 Hz, 2H), 5.73-5.53 (m, 1H), 5.31-5.16 (m, 1H), 4.86-4.61 (m, 1H), 4.59-4.40 (m, 2H), 4.27-4.16 (m, 1H), 3.72 (d, *J* = 8.04 Hz, 3H), 3.21-2.98 (m, 2H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 172.9, 166.1, 165.9, 165.9, 165.4, 159.1, 136.0, 133.0, 130.4, 129.6, 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.1, 127.5, 127.2, 127.0, 126.8, 115.7, 115.5, 114.4, 101.5, 65.4, 64.6, 55.3, 51.8, 39.6; LRMS: (ES+) m/z = 804 (M-1).



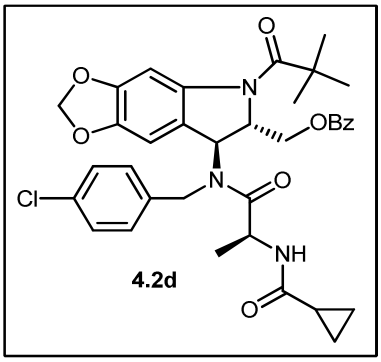
4.2b

((6S,7S)-5-benzoyl-7-((S)-2-(cyclopropanecarboxamido)-N-(4-methoxybenzyl)-3-ethylbutanamido)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.2b); Molecular Formula: $C_{41}H_{41}N_3O_8$; R_f ((40% ethyl acetate/hexane): 0.3; Yield: 62%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.67 (d, *J* = 7.83 Hz, 3H), 7.37-7.25 (m, 8H), 7.05 (d, *J* = 8.53 Hz, 2H), 6.82 (d, *J* = 8.50 Hz, 3H), 6.52 (s, 1H), 5.94-5.82 (m, 3H), 5.63-5.51 (m, 1H), 4.89-4.75 (m, 2H), 4.68 (s, 1H), 4.58-4.45 (m, 2H), 3.76 (s, 3H), 2.06-1.95 (m, 1H), 1.51-1.45 (m, 1H), 0.88 (d, *J* = 6.69 Hz, 3H), 0.82-0.71 (m, 7H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.9, 173.5, 165.8, 159.2, 135.7, 133.0, 130.4, 129.5, 129.4, 129.0, 128.6, 128.1, 128.1, 128.0, 128.0, 126.9, 126.7, 114.3, 113.8, 105.5, 101.5, 65.7, 64.7, 58.7, 55.2, 54.8, 31.6, 29.6, 19.6, 17.6, 14.5, 7.41, 7.37; LRMS: (ES+) m/z = 726 (M+Na).



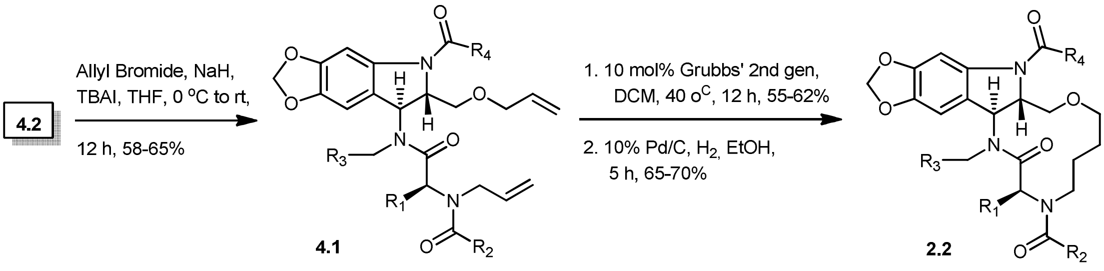
4.2c

((6S,7S)-5-benzoyl-7-((S)-N-(4-bromobenzyl)-2-(cyclohexanecarboxamido)-4-methylpentanamido)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.2c); Molecular Formula: $C_{41}H_{41}N_3O_8$; R_f ((30% ethyl acetate/hexane): 0.3; Yield: 68%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.69 (d, *J* = 7.33 Hz, 2H), 7.50-7.41 (m, 5H), 7.37-7.28 (m, 6H), 7.02 (d, *J* = 8.24 Hz, 2H), 6.51 (s, 1H), 5.90-5.83 (m, 3H), 4.87 (d, *J* = 18 Hz, 1H), 4.79-4.68 (m, 2H), 4.53-4.41 (m, 2H), 4.35-4.28 (m, 1H), 2.15-2.08 (m, 1H), 1.92-1.53 (m, 13H), 0.82 (d, *J* = 6.62 Hz, 3H), 0.58 (d, *J* = 6.47 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.1, 174.9, 165.8, 148.6, 144.5, 136.5, 135.5, 133.0, 132.0, 131.6, 130.5, 129.5, 129.4, 129.3, 128.8, 128.7, 128.2, 128.1, 127.7, 126.7, 121.5, 120.6, 101.6, 65.2, 64.6, 57.8, 47.8, 45.1, 41.7, 29.6, 29.4, 25.6, 25.6, 25.5, 24.5, 23.3, 21.1; LRMS: (ES+) m/z = 832 (M+Na). **Note:** Multiple signals of ^{1}H- and ^{13}C-NMR are due to the rotamers



4.2d

((6S,7S)-7-((S)-N-(4-chlorobenzyl)-2-(cyclopropanecarboxamido)propanamido)-5-pivaloyl-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (4.2d); Molecular Formula: $C_{36}H_{38}ClN_3O_7$; R_f ((30% ethyl acetate/hexane): 0.35; Yield: 65%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.87 (d, *J* = 7.68 Hz, 2H), 7.58-7.48 (m, 3H), 7.42-7.30 (m, 3H), 7.17 (d, *J* = 8.12 Hz, 2H), 6.84 (d, *J* = 8.22 Hz, 2H), 6.07 (s, 1H), 5.84-5.78 (m, 3H), 5.62 (s, 1H), 5.44 (dd, *J* = 7.88, 2.79 Hz, 1H), 4.90-4.86 (m, 1H), 4.64 (d, *J* = 11.40 Hz, 2H), 4.13-3.97 (m, 2H), 2.01-1.97 (m, 1H), 1.35 (s, 9H), 1.29-1.22 (m, 7H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 176.6, 176.2, 174.2, 172.7, 166.2, 148.6, 144.0, 140.7, 137.0, 135.5, 133.2, 133.2, 133.1, 129.7, 129.6, 128.6, 128.2, 128.2, 127.9, 127.2, 118.6, 106.5, 102.4, 101.5, 66.0, 64.6, 60.3, 58.6, 47.5, 46.4, 40.7, 28.3, 28.1, 20.9, 19.1, 14.5, 14.1, 7.3; LRMS: (ES+) m/z = 682 (M+Na).

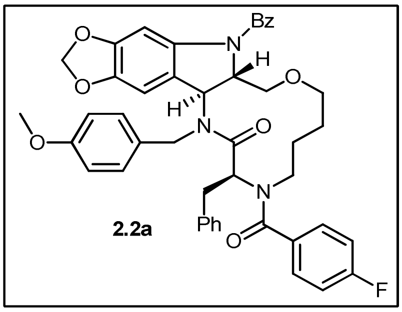


Compound 2.2:

To the solution of compound **4.2** (1 mmol) in THF at 0 °C, TBAI (0.5 mmol), NaH (6 mmol) followed by allyl bromide (8 mmol) was added under N_2 atmosphere. Then the reaction was stirred for12 hours at room temperature. After completion of the reaction, reaction mixture was quenched with ammoniumchloride solution and extracted with EtOAc. The combined organic layers were subsequently washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure and purified by flash column chromatography to obtain pure product **4.1**.

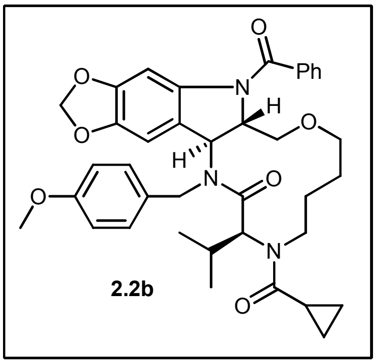
Bis-allyl compound (1mmol) was taken in dry dichloromethane (high dilution) under nitrogen atmosphere and Grubbs' 2nd generation catalyst (0.1mmol) was added and reaction mixture was heated to 40 ^{o}C for 12 h. The reaction mixture was concentrated under reduced pressure and the crude product was purified by flash column chromatography to obtain pure product.

To a solution of Macrocycle (RCM product) in ethanol, was added palladium catalyst (10 mol% (W/W), 10% palladium over activated carbon). The reaction mixture was then subjected to hydrogenation under atmospheric pressure for12 h. The mixture was filtered through celite. The organic solution was concentrated under reduced pressure and then purified by flash column chromatography to obtain pure product of **2.2**.

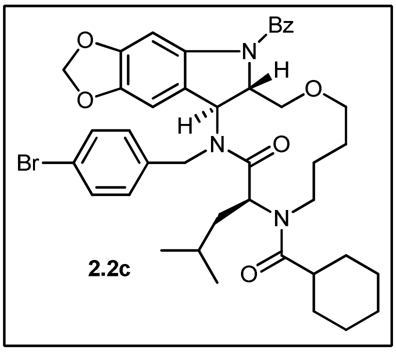


2.2a

(5aS,13S,15aS)-5-benzoyl-13-benzyl-12-(4-fluorobenzoyl)-15-(4-methoxybenzyl) 5a,6,8,9,10,11,12,13,15,15a-decahydro-[1,3]dioxolo[4,5-f][1,5,8]oxadiazacyclododeca[3,4-b]indol-14(5H)-one (2.2a); Molecular Formula: $C_{45}H_{42}FN_3O_7$; R$_f$ ((30% ethyl acetate/hexane): 0.30; Yield: 58%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.50-7.36 (m, 4H), 7.33-7.19 (m, 10H), 6.99 (t, *J* = 8.58 Hz, 2H), 6.80 (d, *J* = 8.25 Hz, 2H), 6.63 (d, *J* = 8.51 Hz, 2H), -6.055.75 (m, 5H), 4.67-4.57 (m, 1H), 4.50 (d, *J* = 14.4 Hz, 1H), 4.20 (d, *J* = 14.4 Hz, 1H), 3.73 (s, 3H), 3.51-3.41 (m, 2H), 3.40-3.35 (m, 2H), 3.34-3.26 (m, 1H), 3.23-3.09 (m, 1H), 2.88-2.71 (m, 1H), 1.59-1.47 (m, 2H), 1.32-1.26 (m, 2H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.5, 168.0, 164.2, 161.7, 158.6, 148.8, 144.8, 137.1, 136.0, 133.1, 133.1, 130.5, 129.7, 129.3, 128.7, 128.5, 126.9, 126.7, 115.2, 115.0, 113.5, 101.7, 55, 46, 29, 26; LRMS: (ES+) m/z = 756(M+1), 778 (M+Na).

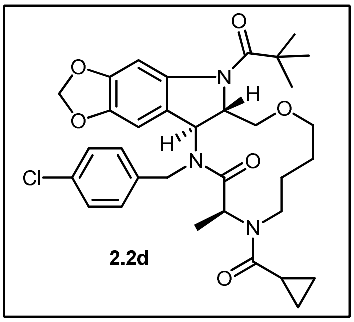


(5aS,13S,15aS)-5-benzoyl-12-(cyclopropanecarbonyl)-13-isopropyl-15-(4-methoxybenzyl)-5a,6,8,9,10,11,12,13,15,15a-decahydro-[1,3]dioxolo[4,5-f][1,5,8]oxadiazacyclododeca[3,4-b]indol-14(5H)-one(2.2b); Molecular Formula: $C_{38}H_{43}N_3O_7$; R*f* ((50% ethyl acetate/hexane): 0.30; Yield: 50%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.51-7.43 (m, 2H), 7.45-7.39 (m, 2H), 7.31 (d, *J* = 6.50 Hz, 2H), 7.05 (d, *J* = 8.58 Hz, 2H), 6.73 (d, *J* = 8.59 Hz, 2H), 6.64 (s, 1H), 5.92 (d, *J* = 4.52 Hz, 2H), 5.82 (s, 1H), 5.41 (d, *J* = 10.8 Hz, 1H), 5.04-4.89 (m, 1H), 4.72-4.37 (m, 3H), 4.17 (d, *J* = 14.4 Hz, 1H), 3.80-3.69 (m, 5H), 3.56-3.42 (m, 2H), 2.56-2.47 (m, 1H), 2.10-1.98 (m, 1H), 1.91-1.85 (m, 2H), 1.58-1.52 (m, 2H), 1.02-.91 (m, 4H), 0.85 (d, *J* = 6.27 Hz, 3H), 0.78 (d, *J* = 6.81 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 175.1**,** 171.2**,** 168.2, 158.6, 136.2, 130.5, 130.2, 129.8, 128.7, 128.6, 126.7, 114.6, 113.4, 101.6, 60.1, 55.2, 46.5, 43.7, 29.6, 29.6, 29.6, 29.3, 28.5, 27.3, 20.2, 17.8, 14.1, 12.0, 9.3, 8.6; LRMS: (ES+) m/z = 653 (M+1), 676 (M+Na).



(5aS,13S,15aS)-5-benzoyl-15-(4-bromobenzyl)-12-(cyclohexanecarbonyl)-13-isobutyl-5a,6,8,9,10,11,12,13,15,15a-decahydro-[1,3]dioxolo[4,5-f][1,5,8]oxadiazacyclododeca[3,4-b]indol-14(5H)-one(2.2c); Molecular Formula: $C_{41}H_{49}N_3O_6$; R*f* ((30% ethyl acetate/hexane): 0.32; Yield: 46%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.46-7.30 (m, 4H), 7.26-7.18 (m, 4H), 7.11-6.98 (m, 3H), 6.52 (d, *J* =

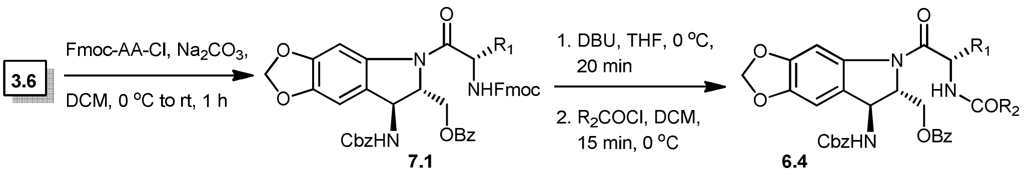
4 Hz, 1H), 6.09-5.91 (m, 3H), 5.71-5.57 (m, 1H), 5.06 (d, *J* = 17.6 Hz, 1H), 4.69-4.57 (m, 1H), 4.56-4.44 (m, 1H), 4.19-3.98 (m, 2H), 3.56-3.29 (m, 4H), 2.51-2.40 (m, 1H), 1.88-1.76 (m, 2H), 1.59-1.51 (m, 5H), 1.08-0.85 (m, 10H), 0.83 (d, *J* = 6.59 Hz, 3H), 0.69 (d, *J* = 6.44 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 176.8, 171.5, 168.6, 137.7, 137.3, 129.0, 128.6, 128.5, 127.8, 126.9, 126.7, 126.6, 101.8, 66.9, 50.5, 41.6, 39.0, 33.7, 29.9, 29.7, 29.6, 29.6, 29.6, 29.3, 25.9, 25.9, 25.7, 25.6, 25.5, 24.8, 24.3, 23.1, 22.6, 22.1, 20.3, 14.1, 13.8, 13.6; LRMS: (ES+) m/z = 704 (M+Na).



2.2d

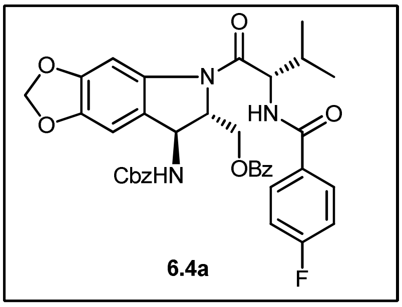
5aS,13S,15aS)-15-(4-chlorobenzyl)-12-(cyclopropanecarbonyl)-13-methyl-5-pivaloyl-5a,6,8,9,10,11,12,13,15,15a-decahydro-[1,3]dioxolo[4,5-f][1,5,8]oxadiazacyclododeca[3,4-b]indol-14(5H)-one (2.2d); Molecular Formula: $C_{33}H_{40}ClN_3O_6$; R_f ((25% ethyl acetate/hexane): 0.3; Yield: 52%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.23-7.15 (m, 2H), 7.08 (d, *J* = 7.40 Hz, 2H), 6.43 (s, 1H), 6.02 (s, 1H), 5.91-5.85 (m, 3H), 5.41-5.34 (m, 1H), 4.98 (d, J = 17.6 Hz, 1H), 4.35 (dd, J =11.2 , 4.8 Hz,1H), 4.15-4.05 (m, 2H), 3.61-3.49 (m, 5H), 1.72-1.65 (m, 3H), 1.62-1.58 (m, 2H), 1.13 (s, 9H), 1.02 (s, 3H), 0.86-0.78 (m, 4H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 178.2, 175.1, 171.7, 145.2, 142.8, 138.3, 132.0, 131.5, 128.5, 127.4, 127.2, 120.9, 104.2, 100.5, 99.4, 90.4, 58.4, 52.6, 44.5, 43.1, 38.4, 32.8, 29.6, 27.1, 27.0, 20.2, 15.3, 14.0, 13.7, 11.8, 8.7, 8.0; LRMS: (ES+) m/z = 608 (M-1).

2.6.3. 11 Membered macrocyles:



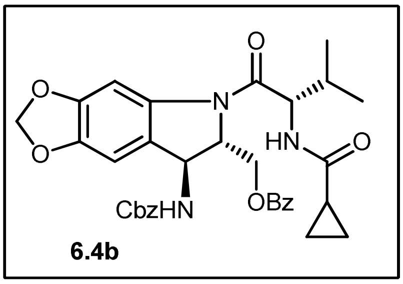
Compound 6.4:

Experimental procedure as per Ref. compound **4.2**.



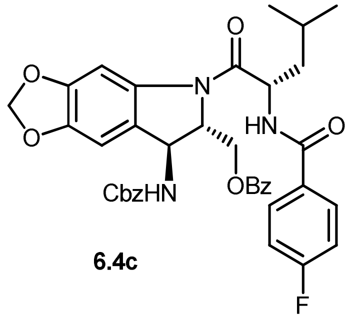
6.4a

((6S,7S)-7-(benzyloxycarbonylamino)-5-((S)-2-(4-fluorobenzamido)-3-methylbutanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.4a); Molecular Formula: $C_{37}H_{34}FN_3O_8$; R*f* (30% ethyl acetate/hexane): 0.25; Yield: 64%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.09 (dd, *J* = 8.71, 5.50 Hz, 2H), 7.89-7.80 (m, 3H), 7.78 (s, 1H), 7.36-7.29 (m, 6H), 7.14-7.05 (m, 3H), 6.79 (s, 1H), 5.93 (d, *J* = 15.83 Hz, 2H), 5.19-4.86 (m, 6H), 4.50 (s, 1H), 2.22-2.06 (m, 1H), 1.02 (dd, *J* = 13.56, 6.71 Hz, 6H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 176.7, **,** 170.2**,** 169.3**,** 166.0**,** 148.0**,** 144.4**,** 138.1**,** 133.3**,** 132.2**,** 132.2**,** 129.6**,** 129.5**,** 129.2**,** 128.5**,** 128.3, 128.2, 128.1, 125.3, 125.2, 115.2, 115.0, 104.8, 102.5, 101.4, 67.1, 64.2, 55.4, 40.8, 29.6, 28.3, 28.2, 28.1, 18.1; LRMS: (ES+) m/z = 668 (M+1), 690 (M+Na).



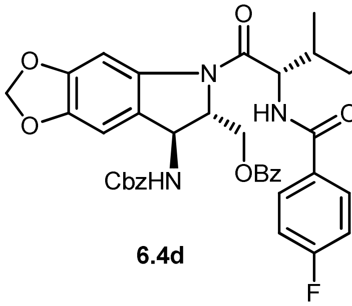
6.4b

((6S,7S)-7-(benzyloxycarbonylamino)-5-((S)-2-(cyclopropanecarboxamido)-3-methylbutanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.4b); Molecular Formula: $C_{34}H_{35}N_3O_8$; R*f* (40% ethyl acetate/hexane): 0.3; Yield: 64%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.85 (d, *J* = 7.62 Hz, 2H), 7.79 (s, 1H), 7.51 (t, *J* = 7.35 Hz, 1H), 7.38-7.29 (m, 7H), 6.78 (s, 1H), 6.45 (d, *J* = 2.39 Hz, 1H), 5.93 (d, *J* = 12.98 Hz, 2H), 5.19-5.04 (m, 2H), 5.05-4.89 (m, 3H), 4.85-4.78 (m, 1H), 4.47-4.33 (m, 1H), 2.05-1.95 (m, 1H), 1.48-1.40 (m, 1H), 1.04-0.91 (m, 8H), 0.74 (d, *J* = 6.97 Hz, 2H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.5, 170.5, 166.0, 155.1, 148.8, 144.8, 137.2, 136.0, 132.9, 129.7, 128.5, 128.2, 128.2, 121.6, 105.2, 101.7, 100.7, 67.0, 66.1, 63.8, 60.3, 55.8, 54.8, 32.5, 29.6, 19.2, 18.0, 14.5, 7.5, 7.4; LRMS: (ES+) m/z = 636 (M+Na).



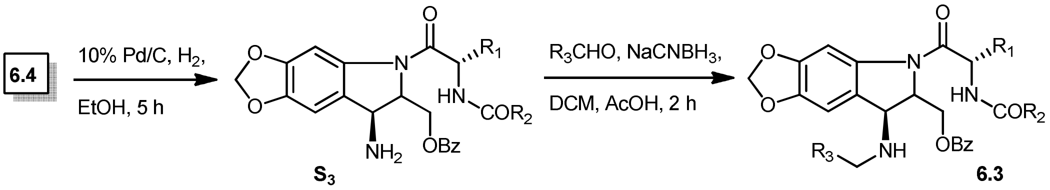
6.4c

((6S,7S)-7-(benzyloxycarbonylamino)-5-((S)-2-(4-fluorobenzamido)-4-methylpentanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.4c); Molecular Formula: $C_{38}H_{36}FN_3O_8$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 72%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.88 (d, *J* = 7.47 Hz, 2H), 7.82 (dd, *J* = 8.53, 5.30 Hz, 2H), 7.76 (s, 1H), 7.41-7.32 (m, 7H), 7.07 (t, *J* = 8.66 Hz, 3H), 6.81 (s, 1H), 5.96 (d, *J* = 12.35 Hz, 2H), 5.32-5.20 (m, 1H), 5.12 (s, 2H), 4.96-4.78 (m, 4H), 4.52-4.39 (m, 1H), 1.85-1.69 (m, 2H), 1.60-1.48 (m, 1H), 1.03 (d, *J* = 6.34 Hz, 3H), 0.88 (d, *J* = 5.6 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.4**,** 166.0**,** 165.9**,**163.4, 155.1, 148.9, 145.0, 137.3, 135.9, 133.0, 129.7, 129.6, 129.5, 128.5, 128.3, 128.2, 115.4, 115.2, 105.4, 101.8, 100.7, 67.0, 67.0, 66.1, 66.0, 63.7, 60.4, 54.8, 50.6, 42.8, 42.7, 42.7, 33.8, 31.9, 29.6, 29.6, 29.6, 29.3, 25.0, 23.5, 22.6, 21.9, 14.1; LRMS: (ES+) m/z = 680 (M-1), 716 (M+Na).

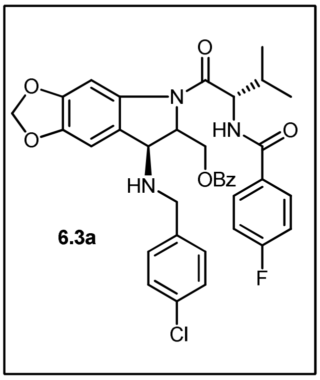


6.4d

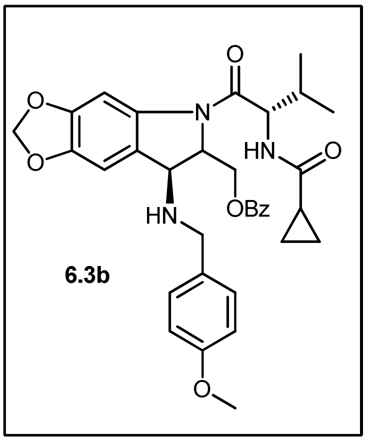
((6S,7S)-7-(benzyloxycarbonylamino)-5-((2S,3S)-2-(4-fluorobenzamido)-3-methyl pentanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.4d); Molecular Formula: $C_{38}H_{36}FN_3O_8$; R_f (30% ethyl acetate/hexane): 0.32; Yield: 74%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.89-7.81 (m, 4H), 7.78 (s, 1H), 7.43-7.29 (m, 7H), 7.10 (t, *J* = 8.56 Hz, 2H), 6.88 (d, *J* = 8.84 Hz, 1H), 6.80 (s, 1H), 5.99-5.91 (m, 2H), 5.21-5.14 (m, 1H), 5.09 (t, *J* = 8.30 Hz, 2H), 5.05-5.48 (m, 1H), 4.97-4.79 (m, 3H), 4.55-4.43 (m, 1H), 1.90-1.82 (m, 1H), 0.95 (d, *J* = 6.86 Hz, 3H), 0.91-0.894 (m, 5H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.9, 170.6, 166.1, 166.0, 165.9, 163.4, 155.2, 148.7, 144.9, 137.2, 136.1, 129.8, 129.5, 129.4, 128.3, 128.3, 128.0, 121.8, 115.5, 115.3, 115.3, 115.1, 101.7, 68.4, 66.9, 60.3, 38.9, 38.8, 38.6, 27.7, 24.9, 24.9, 24.8, 24.8, 24.8, 15.3, 15.1, 11.3, 11.2; LRMS: (ES+) m/z = 682 (M+1), 704 (M+Na).



Experimental procedure as per Ref. compound **5.2** and **4.3**.

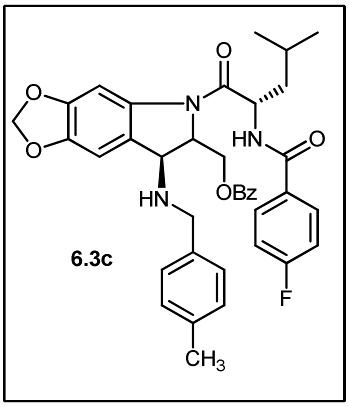


((6S,7S)-7-(4-chlorobenzylamino)-5-((S)-2-(4-fluorobenzamido)-3-methylbutanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.3a); Molecular Formula: $C_{36}H_{33}ClFN_3O_6$; R$_f$ (40% ethyl acetate/hexane): 0.3; Yield: 61%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.90-7.83 (m, 4H), 7.54 (t, *J* = 7.43 Hz, 1H), 7.37 (t, *J* = 7.76 Hz, 2H), 7.27-7.16 (m, 4H), 7.11 (t, *J* = 8.58 Hz, 2H), 6.92 (d, *J* = 8.14 Hz, 1H), 6.78 (s, 1H), 5.97 (d, d, *J* = 3.29 Hz, 2H), 5.23 (dd, *J* = 8.73, 6.37 Hz, 1H), 4.90-4.79 (m, 2H), 4.26-4.04 (m, 2H), 3.84 (q, *J* = 13.66 Hz, 2H), 2.28-2.25 (m, 1H), 1.16 (d, *J* = 6.76 Hz, 3H), 1.10 (d, *J* = 6.72 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 170.1, 166.1, 166.0, 163.5, 148.2, 144.8, 137.7, 136.2, 133.2, 132.8, 130.1, 130.1, 129.6, 129.6, 129.5, 129.2, 129.1, 128.6, 128.3, 124.8, 115.6, 115.4, 105.3, 101.6, 100.8, 64.4, 63.7, 60.6, 56.2, 50.1, 32.7, 19.8, 18.0; LRMS: (ES+) m/z = 680 (M+Na).



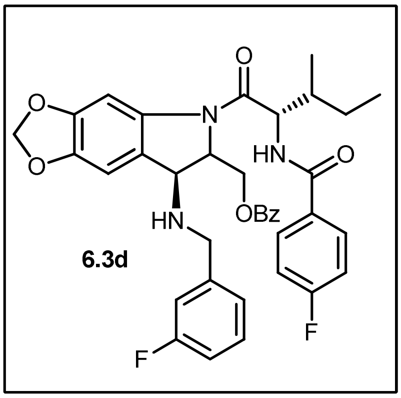
((6S,7S)-5-((S)-2-(cyclopropanecarboxamido)-3-methylbutanoyl)-7-(4-methoxybenzylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate

(6.3b); Molecular Formula: $C_{34}H_{37}N_3O_7$; R$_f$ (40% ethyl acetate/hexane): 0.2; Yield: 67%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.85 (d, *J* = 6.22 Hz, 3H), 7.55 (t, *J* = 7.39 Hz, 1H), 7.39 (t, *J* = 7.74 Hz, 2H), 7.15 (d, *J* = 8.52 Hz, 2H), 6.77-6.75 (m, 2H), 6.41 (d, *J* = 8.92 Hz, 1H), 5.95 (d, *J* = 3.29 Hz, 2H), 5.02 (dd, *J* = 8.98, 6.40 Hz, 1H), 4.81-4.68 (m, 2H), 4.22-4.09 (m, 1H), 4.06 (s, 1H), 3.79-3.73 (m, 4H), 2.19-2.09 (m, 1H), 1.48-1.41 (m, 1H), 1.10 (d, *J* = 6.76 Hz, 3H), 1.03 (t, *J* = 10.02 Hz, 3H), 0.98-0.91 (m, 2H), 0.78-0.71 (m, 2H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.6**,** 166.0**,** 158.7**,** 148.0, 144.6, 136.3, 133.1, 131.3, 129.7, 129.4, 129.0, 128.2, 125.1, 113.8, 105.4, 101.5, 100.7, 64.4, 63.8, 60.6, 55.9, 55.2, 55.2, 50.3, 32.4, 19.7, 17.9, 14.6, 7.51, 7.41; LRMS: (ES+) m/z = 600 (M+1), 622 (M+Na).



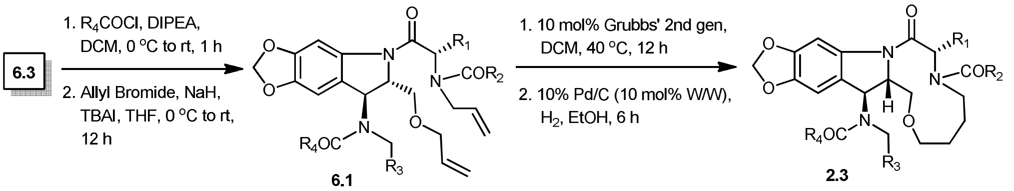
((6S,7S)-5-((S)-2-(4-fluorobenzamido)-4-methylpentanoyl)-7-(4-methylbenzylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.3c); Molecular Formula: $C_{38}H_{38}FN_3O_6$; R$_f$ (40% ethyl acetate/hexane):

0.3; Yield: 65%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.93-7.86 (m, 2H), 7.85-7.80 (m, 2H), 7.81 (s, 1H)7.52 (t, *J* = 7.44 Hz, 1H), 7.38 (d, *J* = 7.85 Hz, 2H), 7.16 (d, *J* = 7.88 Hz, 2H), 7.07 (dd, *J* = 13.74, 8.07 Hz, 4H), 6.78 (s, 1H), 5.97 (t, *J* = 6.59 Hz, 2H), 5.40-5.32 (m, 1H), 4.90 (dd, *J* = 11.41, 4.23 Hz, 1H), 4.74 (dd, *J* = 8.29, 4.76 Hz, 1H), 4.24 (dd, *J* = 11.33, 8.50 Hz, 1H), 4.10 (s, 1H), 3.91-3.75 (m, 2H), 2.30 (s, 3H), 1.16 (t, *J* = 7.13 Hz, 3H), 0.99 (d, *J* = 6.54 Hz, 3H), 1.27 (d, *J* = 7.53 Hz, 2H), 1.89-1.84 (m, 1H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 171.1, 166.0, 166.0, 163.5, 148.2, 144.7, 136.9, 136.4, 136.0, 133.1, 129.7, 129.6, 129.5, 129.3, 129.2, 128.3, 127.8, 124.7, 115.5, 115.3, 105.3, 105.3, 101.6, 100.8, 100.7, 64.4, 60.7, 50.6, 43.1, 29.6, 25.0, 23.6, 22.0, 21.0; LRMS: (ES+) m/z = 652 (M+1), 674 (M+Na).



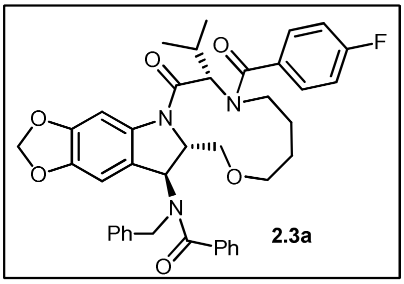
6.3d

((6S,7S)-5-((2S,3S)-2-(4-fluorobenzamido)-3-methylpentanoyl)-7-(3-fluorobenzylamino)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (6.3d); Molecular Formula: $C_{37}H_{35}F_2N_3O_6$; R_f (40% ethyl acetate/hexane): 0.30; Yield: 68%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.91-7.79 (m, 5H), 7.52 (t, *J* = 7.22 Hz, 1H), 7.42-7.34 (m, 2H), 7.19-7.15 (m, 1H), 7.12-6.98 (m, 4H), 6.90 (t, *J* = 7.96 Hz, 1H), 6.80 (s, 1H), 6.01-5.91 (m, 2H), 5.23 (t, *J* = 7.99 Hz, 1H), 4.98-4.80 (m, 2H), 4.29-4.22 (m, 1H), 4.12 (s, 1H), 3.87 (d, *J* = 3.10 Hz, 2H), 2.03 (s, 1H), 1.13 (d, *J* = 6.80 Hz, 3H), 0.98-0.85 (m, 5H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 170.4, 167.7, 166.1, 166.0, 164.2, 164.1, 163.5, 161.7, 161.7, 148.2, 144.8, 142.1, 136.2, 130.0, 130.0, 129.8, 129.7, 129.5, 129.4, 129.3, 129.3, 128.5, 128.0, 124.9, 105.3, 101.6, 101.0, 100.6, 68.1, 64.8, 64.3, 61.0, 60.8, 56.0, 55.7, 50.3, 39.1, 39.0, 15.9, 15.6,11.5, 11.2; LRMS: (ES+) m/z = 656 (M+1), 678 (M+Na).



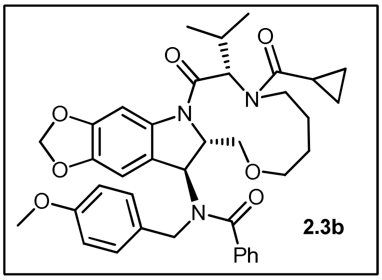
To a stirred solution of secondary amine **(**1 mmol) in dry DCM was added DIPEA (2 mmol) at 0 °C under inert atmosphere. The reaction mixture was stirred for 5 minutes, Then acid chloride (1.2 mmol) was added and reaction mixture was stirred for 1 hour. After completion of the reaction, reaction mixture was quenched by the addition of saturated $NaHCO_3$ solution and extracted with DCM (2 X 10 mL), the combined organic layers was washed with 10% hydrochloric acid dried over anhydrous Na_2SO_4 and concentrated under reduced Pressure. Purification by flash column chromatography to obtain pure product of **6.1**.

For **6.1** to **2.2** experimental procedure as per Ref. compound **2.2**.


2.3a

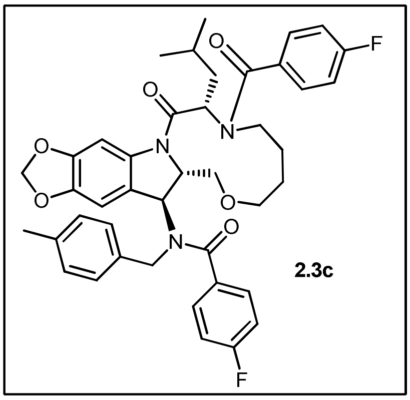
N-benzyl-N-((7S,14aS,15S)-8-(4-fluorobenzoyl)-7-isopropyl-6-oxo-6,7,8,9,10,11,12,14,14a,15-decahydro-[1,3]dioxolo[4,5-f][1,4,7]oxadiazacycloundeca[4,3-a]indol-15-yl)benzamide (2.3a);

Molecular Formula: $C_{40}H_{40}FN_3O_6$; R$_f$ (30% ethyl acetate/hexane): 0.3; Yield: 54%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.84 (s, 1H), 7.65-7.32 (m, 8H), 7.22-7.17 (m, 2H), 7.15-6.96 (m, 5H), 5.93 (d, *J* = 22.4 Hz, 2H), 5.52 (dd , *J* = 10.4, 2.8 Hz, 1H), 5.38 (d, *J* = 10.4, 1H), 4.11-3.99 (m, 1H), 3.76-3.48 (m, 4H), 2.90-2.52 (m, 4H), 2.09-1.99 (m, 1H), 1.82-1.60 (m, 4H), 0.88 (s, 6H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.6, 170.3, 163.9, 161.4, 148.8, 144.7, 136.1, 133.6, 133.6, 129.4, 129.3, 129.2, 129.1, 128.4, 128.3, 128.2, 126.7, 126.5, 115.5, 115.3, 115.2, 115.1, 101.6, 100.9, 100.7, 66.6, 66.6, 59.2, 59.1, 29.6, 29.6, 29.5, 27.9, 26.7, 22.6, 19.7, 18.1, 17.9, 14.1; LRMS: (ES+) m/z = 678 (M+1).



2.3b

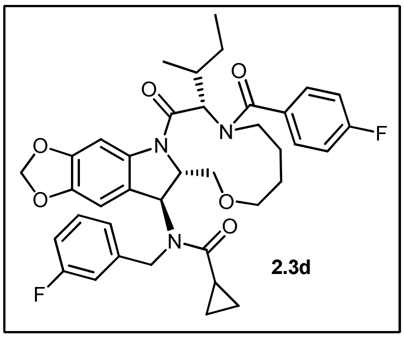
N-((7S,14aS,15S)-8-(cyclopropanecarbonyl)-7-isopropyl-6-oxo-6,7,8,9,10,11,12,14,14a,15-decahydro-[1,3]dioxolo[4,5-f][1,4,7]oxadiazacycloundeca[4,3-a]indol-15-yl)-N-(4-methoxybenzyl)benzamide (2.3b); Molecular Formula: $C_{38}H_{43}N_3O_7$; R_f (30% ethyl acetate/hexane): 0.35; Yield: 48%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.87-7.82 (m, 1H), 7.79-7.74 (m, 1H), 7.49-7.34 (m, 7H), 6.95-6.90 (m, 1H), 6.67-6.64 (m, 1H), 5.95-5.88 (m, 2H), 5.86-5.82 (m, 2H), 5.42-5.30 (m, 2H), 5.15-5.05 (m, 1H), 5.03-4.89 (m, 4H), 3.73 (s, 3H), 2.36-2.33 (m, 1H), 2.10-1.99 (m, 5H), 1.86-1.83 (m, 1H), 0.92-0.84 (m, 10H) ; **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 174.1, 160.0, 158.3, 139.2, 128.5, 128.2, 126.7, 123.4, 115.8, 114.0, 113.5, 101.7, 101.5, 100.6, 55.2, 33.8, 31.9, 29.6, 29.4, 29.3, 24.7, 22.6, 14.1; LRMS: (ES+) m/z = 654 (M+1)



2.3c

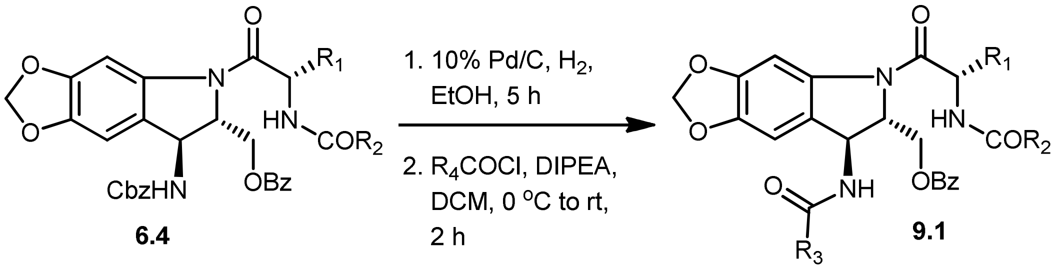
4-fluoro-N-((7S,14aS,15S)-8-(4-fluorobenzoyl)-7-isobutyl-6-oxo-6,7,8,9,10,11,12,14,14a, 15-decahydro-[1,3]dioxolo[4,5-f][1,4,7]oxadiazacycloundeca[4,3-a]indol-15-yl)-N-(4-methylbenzyl)benzamide (2.3c); Molecular Formula: $C_{42}H43F_2N_3O_6$; R_f (30% ethyl acetate/hexane): 0.4; Yield: 57%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.91-7.80 (m, 1H), 7.58-7.35 (m, 4H), 7.25-7.16 (m, 1H), 7.15-7.05 (m, 3H), 7.00 (d, *J* = 7.75 Hz, 3H), 6.89-6.67 (m, 2H), 6.01-5.84 (m, 2H), 5.65-5.48 (m, 1H), 5.39-5.19 (m, 1H), 5.05-4.85 (m, 1H), 4.54-

4.15 (m, 3H), 3.68-3.57 (m, 1H), 3.55-3.41 (m, 2H), 2.94-2.87 (m, 1H), 2.86-2.80 (m, 1H), 2.27 (s, 3H), 1.72-1.57 (m, 5H), 0.98-0.92 (m, 2H), 0.88 (t, *J* = 6.79 Hz, 6H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 164.5, 148.9, 129.3, 129.2, 129.0, 116.0, 115.7, 115.6, 115.6, 115.6, 115.6, 115.5, 115.4, 115.4, 101.6, 101.6, 31.9, 29.6, 29.5, 29.5, 29.3, 24.8, 24.6, 24.6, 22.6, 21.1, 20.8, 14.2, 14.0; LRMS: (ES+) m/z = 724 (M+1).

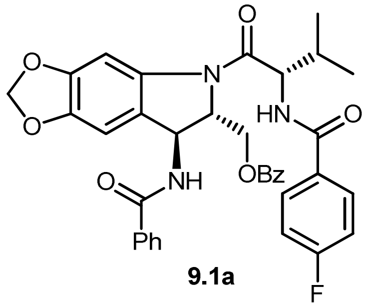


N-((7S,14aS,15S)-7-sec-butyl-8-(4-fluorobenzoyl)-6-oxo-6,7,8,9,10,11,12,14,14a,15-decahydro-[1,3]dioxolo[4,5-f][1,4,7]oxadiazacycloundeca[4,3-a]indol-15-yl)-N-(3-fluorobenzyl)cyclopropanecarboxamide (2.3d); Molecular Formula: $C_{38}H_{41}F_2N_3O_6$; R$_f$ (30% ethyl acetate/hexane): 0.3; Yield: 55%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.88 (s, 1H), 7.46-7.35 (m, 3H), 7.13-7.03 (m, 3H), 6.94-6.79 (m, 2H), 6.64 (s, 1H), 5.95 (s, 2H), 5.65 (s, 1H), 5.33-5.15 (m, 2H), 4.46-4.29 (m, 2H), 4.15-4.07 (m, 1H), 3.74-3.59 (m, 2H), 3.52-3.44 (m, 1H), 2.94-2.77 (m, 2H), 2.42-2.30 (m, 1H), 1.88-1.73 (m, 1H), 1.41 (s, 2H), 1.38-1.31 (m, 2H), 1.10-0.99 (m, 3H), 0.92-0.79 (m, 5H), 0.77-0.57 (m, 4H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 175.0, 175.0, 171.4, 171.4, 170.6, 170.5, 170.5, 148.7, 144.8, 144.8, 140.6, 137.7, 133.7, 128.9, 128.9, 128.9, 128.9, 128.8, 121.5, 115.7, 106.4, 106.4, 105.9, 101.7, 101.7, 101.6, 100.6, 100.5, 47.3, 29.6, 29.5, 24.0, 22.6, 15.5, 15.4, 14.1, 12.4, 12.2, 12.2, 10.9; LRMS: (ES+) m/z = 674 (M+1).

2.6.4. 11 Membered bridged macrocycle:

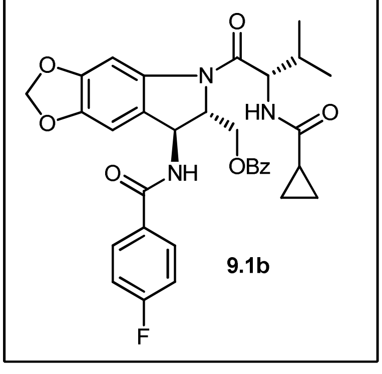


Experimental procedure as per Ref. compound **5.2** and **6.1**.



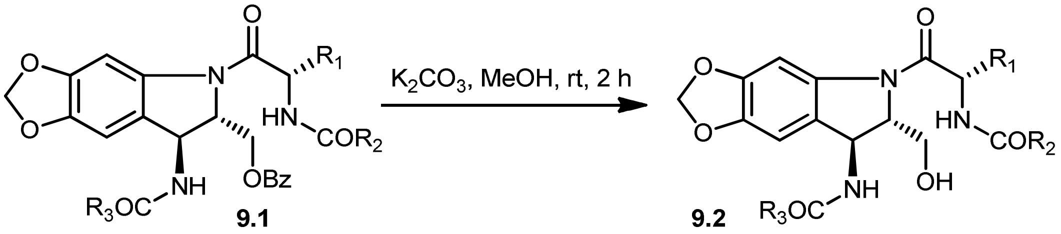
9.1a

((6S,7S)-7-benzamido-5-((S)-2-(4-fluorobenzamido)-3-methylbutanoyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (9.1a); Molecular Formula: $C_{36}H_{32}FN_3O_7$; R_f (30% ethyl acetate/hexane): 0.2; Yield: 68%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.90-7.81 (m, 5H), 7.54 (t, *J* = 7.43 Hz, 1H), 7.37 (t, *J* = 7.76 Hz, 2H), 7.11 (t, *J* = 8.58 Hz, 2H), 6.92 (d, *J* = 8.14 Hz, 1H), 6.78 (d, *J* = 5.77 Hz, 1H), 5.96 (d, *J* = 4.52 Hz, 2H), 7.20 (d, *J* = 9.16 Hz, 4H), 5.23 (dd, *J* = 8.73, 6.37 Hz, 1H), 4.87-4.80 (m, 2H), 2.23 (dd, *J* = 13.34, 6.70 Hz, 1H), 1.19-1.07 (m, 7H), 3.82 (t, *J* = 10.66 Hz, 2H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 170.1, 166.1, 166.0, 148.2, 144.8, 137.7, 136.2, 133.2, 132.8, 129.6, 129.6, 129.5, 129.2, 129.1, 128.6, 128.3, 124.8, 115.6, 115.4, 105.3, 101.6, 100.8, 64.4, 60.6, 56.2, 50.1, 32.7, 19.8, 18.0; LRMS: (ES+) m/z = 634 (M+1).



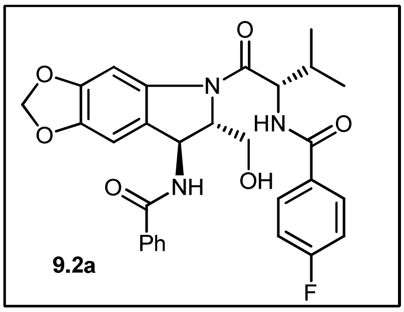
9.1b

((6S,7S)-5-((S)-2-(cyclopropanecarboxamido)-3-methylbutanoyl)-7-(4-fluoro benzamido)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-6-yl)methyl benzoate (9.1b); Molecular Formula: $C_{33}H_{32}FN_3O_7$; R*f* (30% ethyl acetate/hexane): 0.25; Yield: 70%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.89-7.81 (m, 3H), 7.71 (dd, *J* = 5.72, 3.30 Hz, 2H), 7.53 (d, *J* = 7.02 Hz, 1H), 7.38 (d, *J* = 7.71 Hz, 2H), 7.09 (t, *J* = 8.52 Hz, 2H), 6.83 (s, 1H), 5.96 (d, *J* = 19.37 Hz, 2H), 5.43-5.32 (m, 1H), 4.96-4.81 (m, 2H), 4.57-4.45 (m, 1H), 4.22 (s, 1H),), 2.04-1.92 (m, 1H), 1.74-1.68 (m, 1H), 1.01-0.83 (m, 10H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.4, 170.4, 170.4, 167.7, 167.7, 166.0, 166.0, 148.9, 145.0, 144.9, 137.6, 130.0, 129.5, 129.4, 121.6, 116.4, 116.3, 116.o, 68.1, 56.2, 38.6, 32.8, 30.3, 23.7, 22.9, 14.8; LRMS: (ES+) m/z = 602 (M+1).

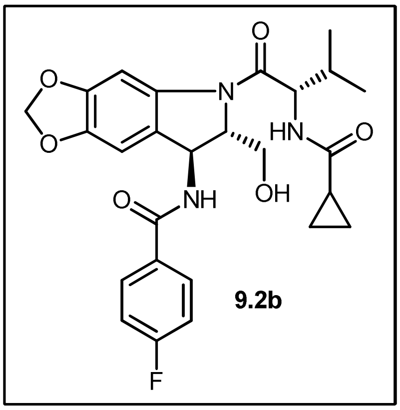


Compound 9.2:

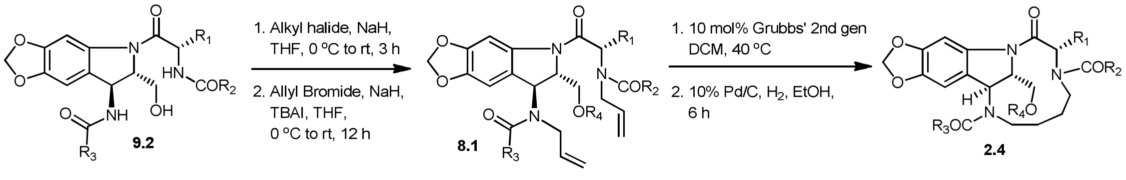
To a stirred solution of compound **9.1** (1 mmol) in methanol at room temperature was added potassium carbonate (2 mmol). And the reaction was allowed for stirring for 2 hours. After completion of the reaction, reaction mixture was quenched with ammonium chloride solution and extracted with EtOAc. The combined organic layers were subsequently washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure and purified by flash column chromatography to obtain pure product **9.2**.



N-((S)-1-((6S,7S)-7-benzamido-6-(hydroxymethyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-5-yl)-3-methyl-1-oxobutan-2-yl)-4-fluorobenzamide (9.2a); Molecular Formula: $C_{29}H_{28}FN_3O_6$; R*f* (50% ethyl acetate/hexane): 0.25; Yield: 62%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.94-7.82 (m, 3H), 7.52-741 (m, 5H), 7.13 (t, *J* = 8.57 Hz, 2H), 7.02-6.92 (m, 1H), 6.01 (s, 2H), 5.30-5.18 (m, 1H), 4.67-4.47 (m, 1H), 3.83-3.52 (m, 2H), 3.20-3.13 (m, 1H), 2.23-2.10 (m, 1H), 1.08-0.96 (m, *J* = 25.69, 13.13 Hz, 6H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 174.2, 163.5, 163.5, 148.9, 145.2, 129.5, 129.5, 129.3, 128.6, 128.4, 128.3, 127.3, 127.0, 115.8, 115.6, 115.5, 115.4, 101.8, 56.2, 29.7, 29.6, 22.6, 14.1, 14.0; LRMS: (ES+) m/z = 534 (-M+1).



N-((6S,7S)-5-((S)-2-(cyclopropanecarboxamido)-3-methylbutanoyl)-6-(hydroxymethyl)-6,7-dihydro-5H-[1,3]dioxolo[4,5-f]indol-7-yl)-4-fluorobenzamide (9.2b); Molecular Formula: $C_{26}H_{28}FN_3O_6$; R$_f$ (50% ethyl acetate/hexane): 0.3; Yield: 54%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.92-7.86 (m, 1H), 7.55-7.46 (m, 2H), 7.13 (s, 2H), 6.58-6.50 (m, 1H), 6.00 (s, 2H), 5.37-5.20 (m, 1H), 5.02-4.81 (m, 1H), 4.58-4.40 (m, 1H), 3.74-3.55 (m, 2H), 2.33-2.23 (m, 1H),), 1.52-1.43 (m, 1H), 1.05-0.88 (m, 10H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 170.8, 170.3, 150.3, 150.0, 148.9, 148.8, 148.8, 148.8, 148.7, 148.6, 146.2, 145.2,144.9, 142.5,129.0, 116.3, 116.1, 116.1, 116.1, 115.8, 101.8, 32.8, 32.8, 29.7, 29.6, 29.5, 29.4, 19.7, 19.6, 17.6, 14.7, 14.5, 14.5, 7.5, 7.3, 7.3; LRMS: (ES+) m/z = 498 (M+1).

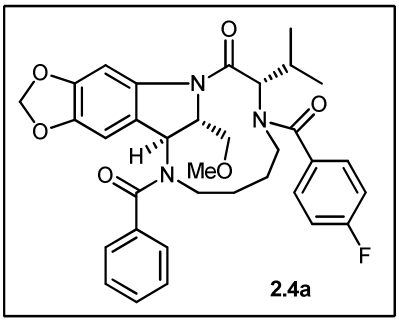


Compound 8.1:

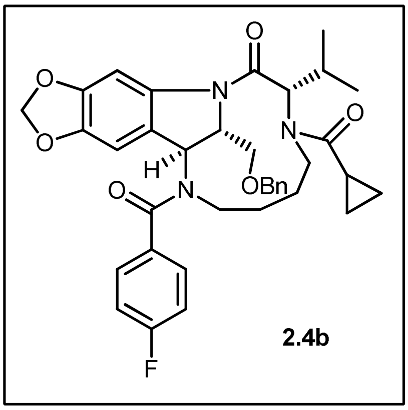
To the solution of compound **9.2** (1 mmol) in THF at 0 °C, NaH (1.5 mmol) followed by alkyl halide (2 mmol) was added under N_2 atmosphere. Then the reaction was stirred for 3 hours at room temperature. After completion of the reaction, reaction mixture was quenched with ammonium chloride solution and extracted with EtOAc. The combined organic layers were subsequently washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure and purified by flash column chromatography to obtain pure product **8.1**.

Compound 2.4:

Experimental procedure as per Ref. compound **2.2**.



(2.4a); Molecular Formula: $C_{34}H_{36}FN_3O_6$; R_f (30% ethyl acetate/hexane): 0.28; Yield: 50%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.38-7.36 (m, 1H), 7.33-7.28 (m, 1H), 7.16-7.00 (m, 4H), 6.88-6.76 (m, 1H), 6.10 (s, 2H), 5.55-5.44 (m, 1H), 5.42-5.28 (m, 1H), 4.92-4.78 (m, 1H), 3.89-3.70 (m, 2H), 4.50-4.34 (m, 1H), 4.10-3.92 (m, 1H), 3.43-3.25 (m, 3H), 2.87-2.60 (m, 2H), 2.07-1.97 (m, 2H), 1.70-1.57 (m, 4H), 1.17 (d, *J* = 5.88 Hz, 3H), 1.09 (d, *J* = 6.96 Hz, 4H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 172.1, 170.6, 167.2, 144.6, 139.2, 128.6, 127.7, 126.8, 115.9, 115.7, 114.0, 102.0, 60.3, 33.8, 31.9, 29.6, 29.6, 29.4, 29.3, 22.6, 20.7, 18.5, 14.1; LRMS: (ES+) m/z = 602 (M+1).



(2.4b); Molecular Formula: $C_{37}H_{40}FN_3O_6$; R_f (30% ethyl acetate/hexane): 0.3; Yield: 54%; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.87-7.82 (m, 1H), 7.79-7.74 (m, 1H), 7.49-7.34 (m, 7H), 6.95-6.90 (m, 1H), 6.67-6.64 (m, 1H), 5.95-5.88 (m, 2H), 5.86-5.82 (m, 2H), 5.42-5.30 (m, 2H), 5.15-5.05 (m, 1H), 5.03-4.89 (m, 4H),4.25 (s, 2H), 3.73 (s, 3H), 2.36-2.33 (m, 1H), 2.10-1.99 (m, 5H), 1.86-1.83 (m, 1H), 0.92-0.84 (m, 10H); LRMS: (ES+) m/z = 641 (M+1).

2.7. References:

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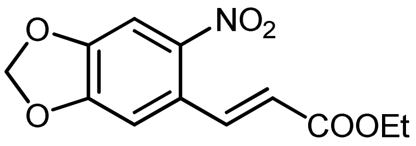
2.8. Spectra: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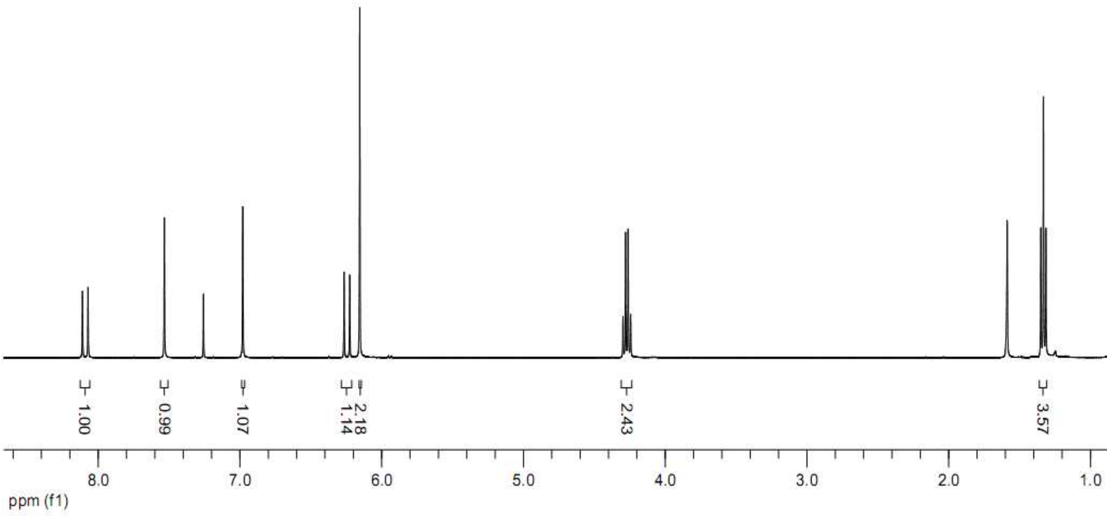
O
COOEt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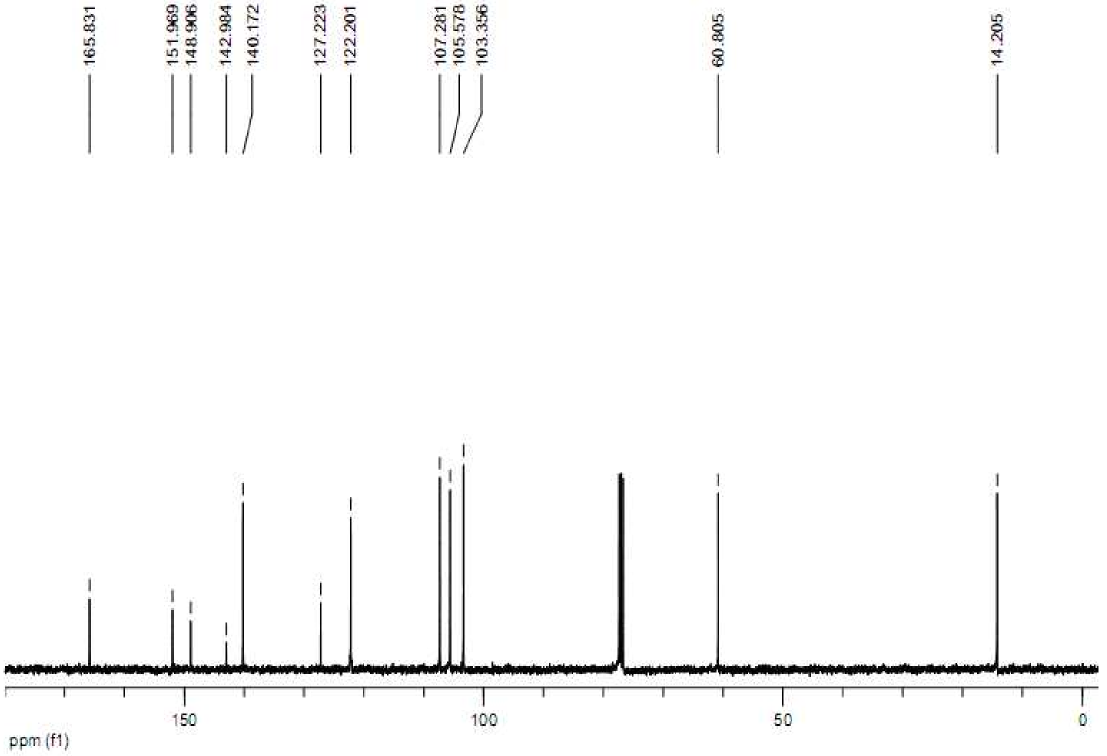
0.99
1.07
1.14
2.18
2.43
3.57
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


165.831
151.969
148.906
142.984
140.172
127.223
122.201
107.281
105.578
103.356
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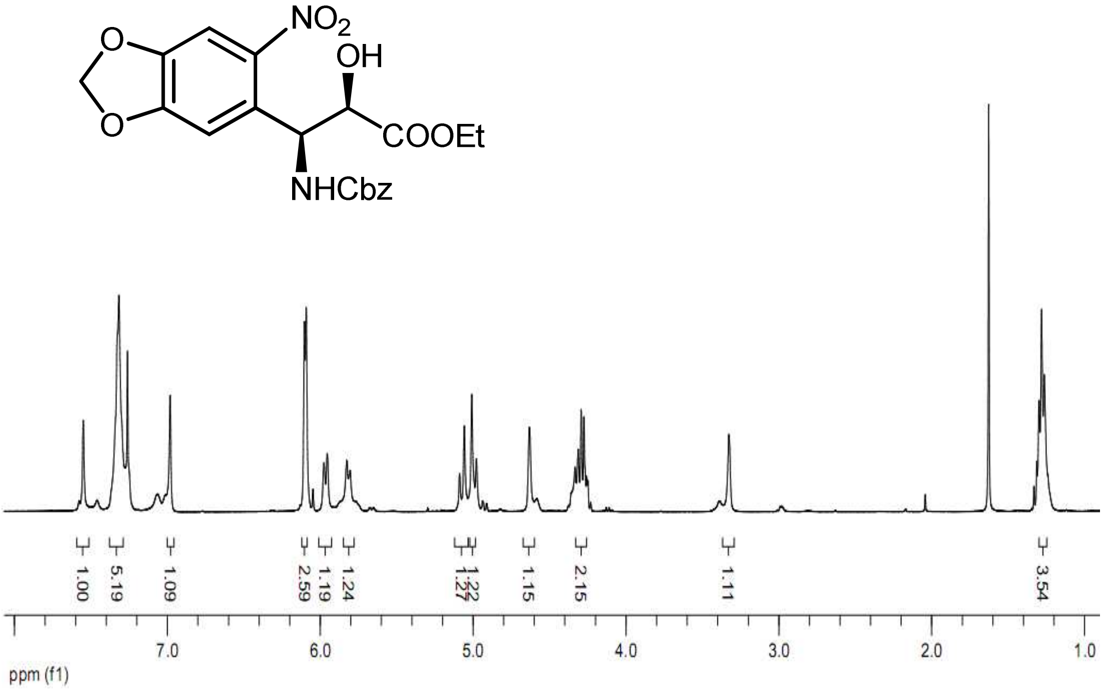
14.205
150
100
50
0
ppm (f1)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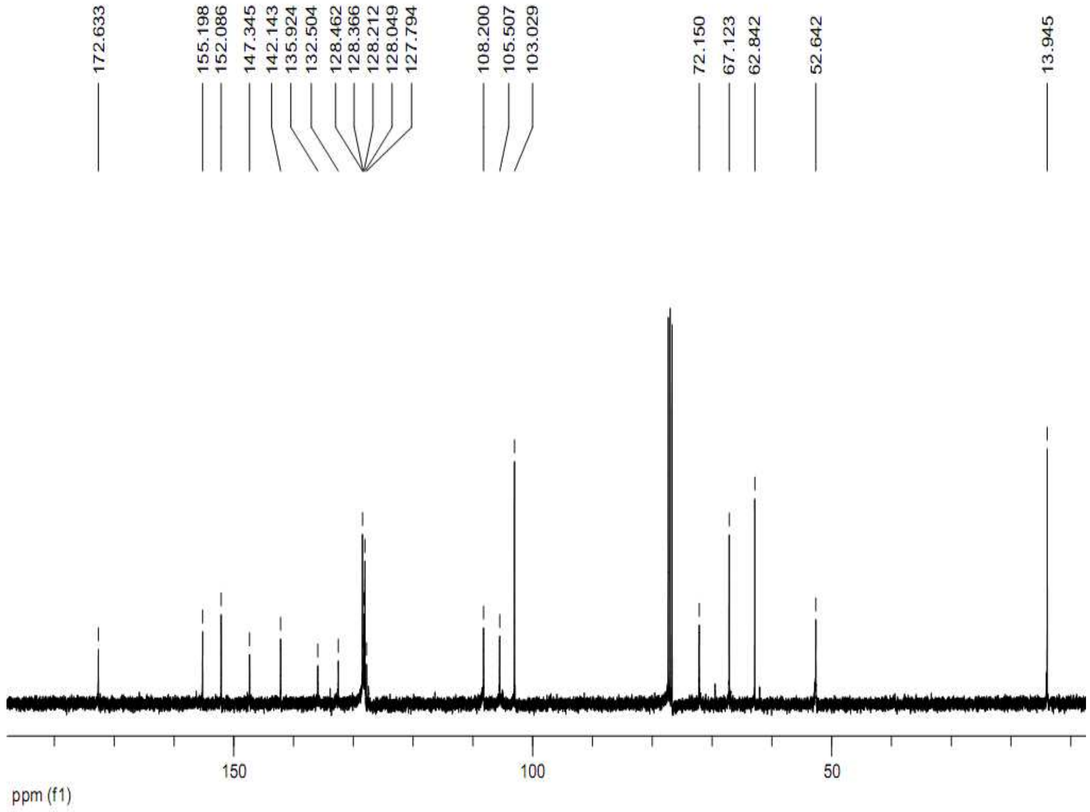
COOEt
NHCbz
O
O
1.00
5.19
1.09
2.59
1.19
1.24
1.27
1.22
1.15
2.15
1.11
3.54
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


172.633
155.198
152.086
147.345
142.143
135.924
132.504
128.462
128.366
128.212
128.049
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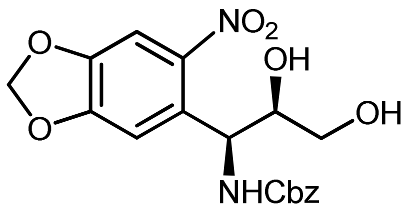
108.200
105.507
103.029
72.150
67.123
62.842
52.642
13.945
150
100
50
ppm (f1)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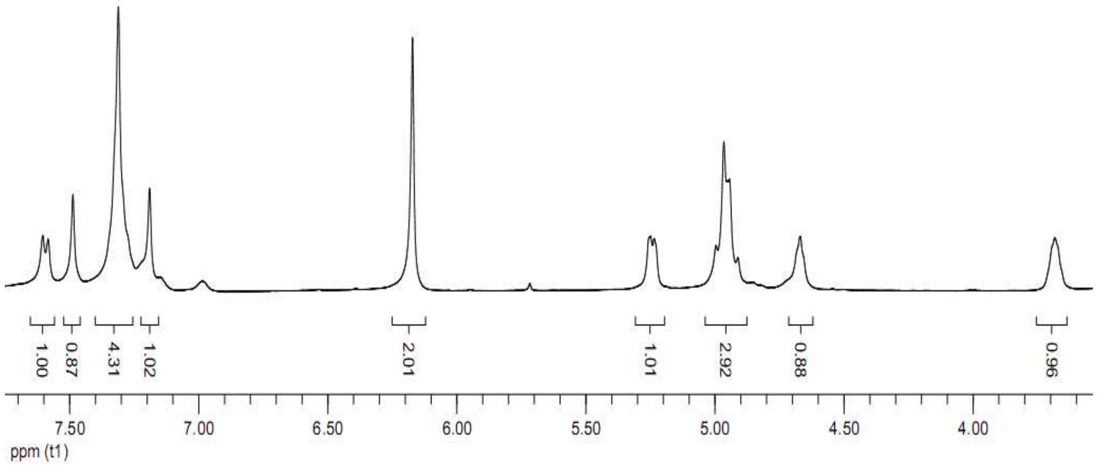
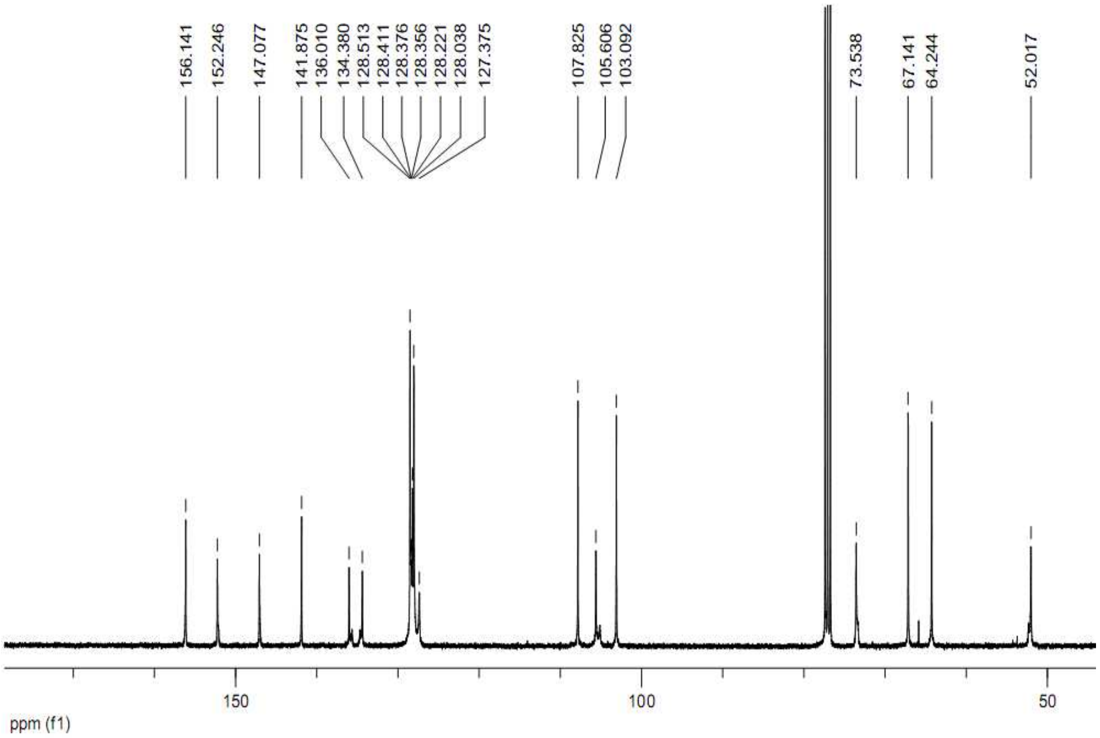
OH
OH
O
O
NHCbz


1.00
0.87
4.31
1.02
2.01
1.01
2.92
0.88
0.96
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
ppm (t1)


156.141
152.246
147.077
141.875
136.010
134.380
128.513
128.411
128.376
128.356
128.221
128.038
127.375
107.825
105.606
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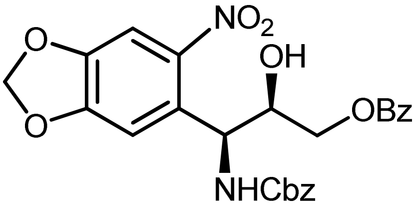
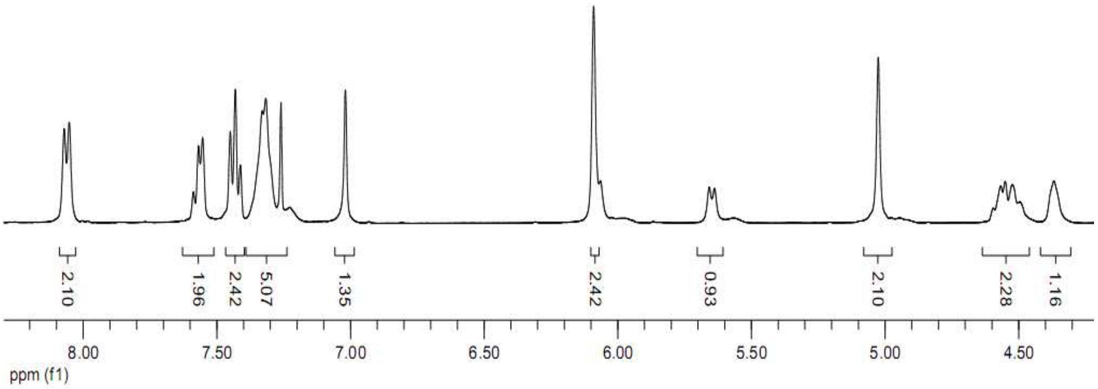
73.538
67.141
64.244
52.017
150
100
50
ppm (f1)


NO2
OH
O
O
OBz
NHCbz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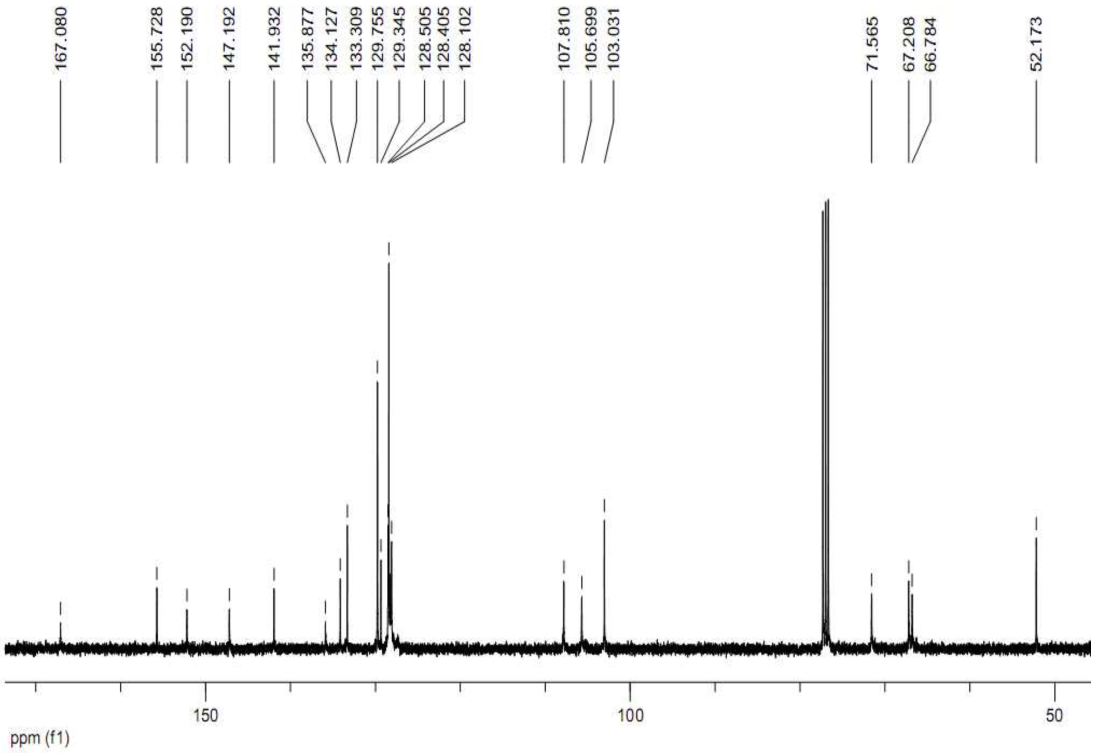
1.96
2.42
5.07
1.35
2.42
0.93
2.10
2.28
1.16
8.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
ppm (f1)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147.192
141.932
135.877
134.127
133.309
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129.345
128.505
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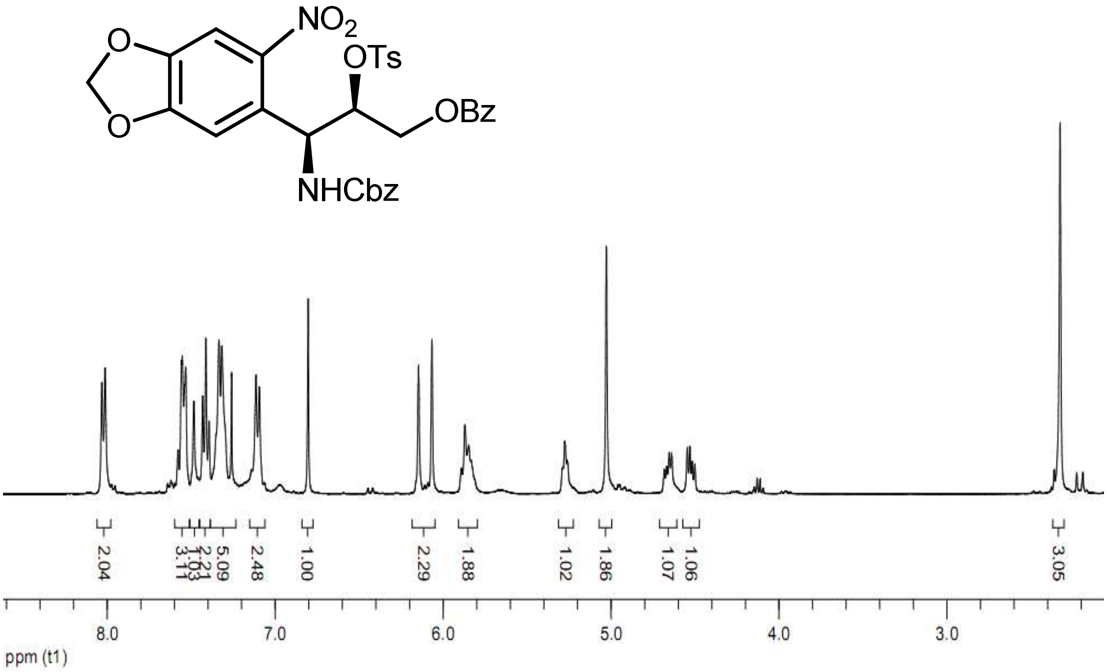
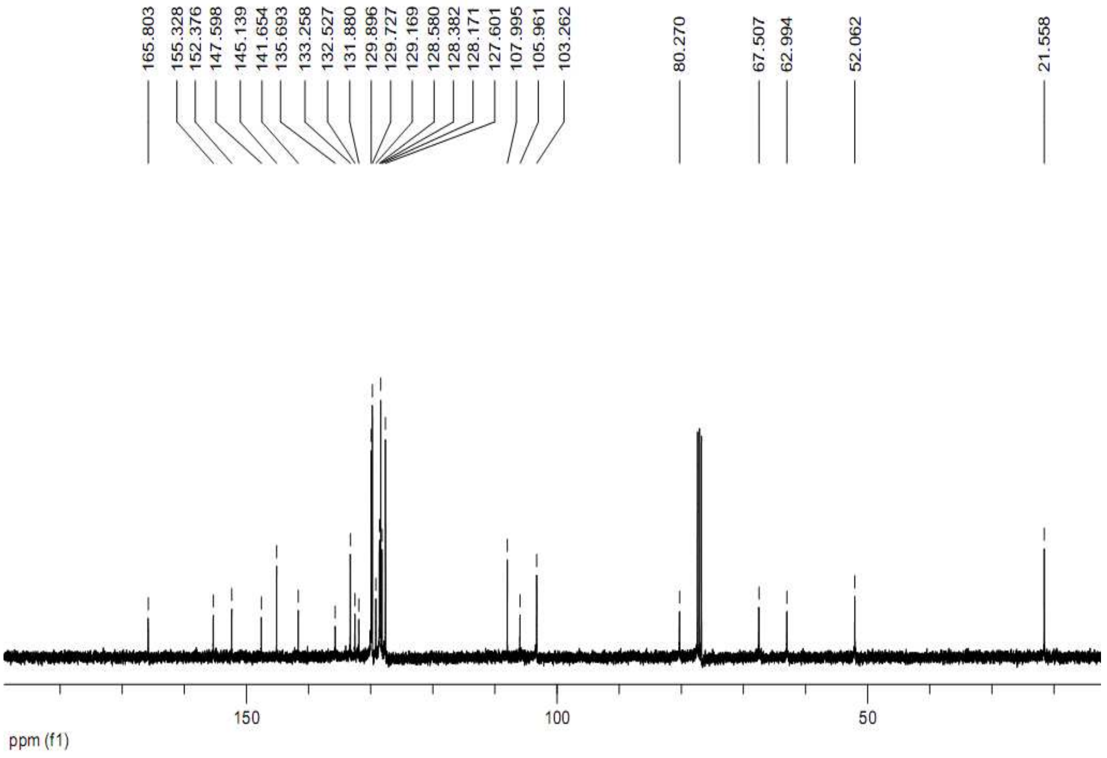
107.810
105.699
103.031
71.565
67.208
66.784
52.173
150
100
50
ppm (f1)


NO2
OTs
OBz
O
O
NHCbz
2.04
3.11
1.03
1.21
5.09
2.48
1.00
2.29
1.88
1.02
1.86
1.07
1.06
3.05
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)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152.376
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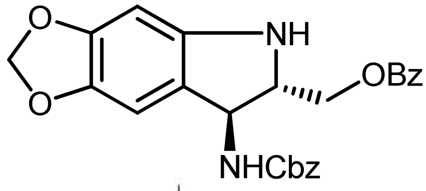
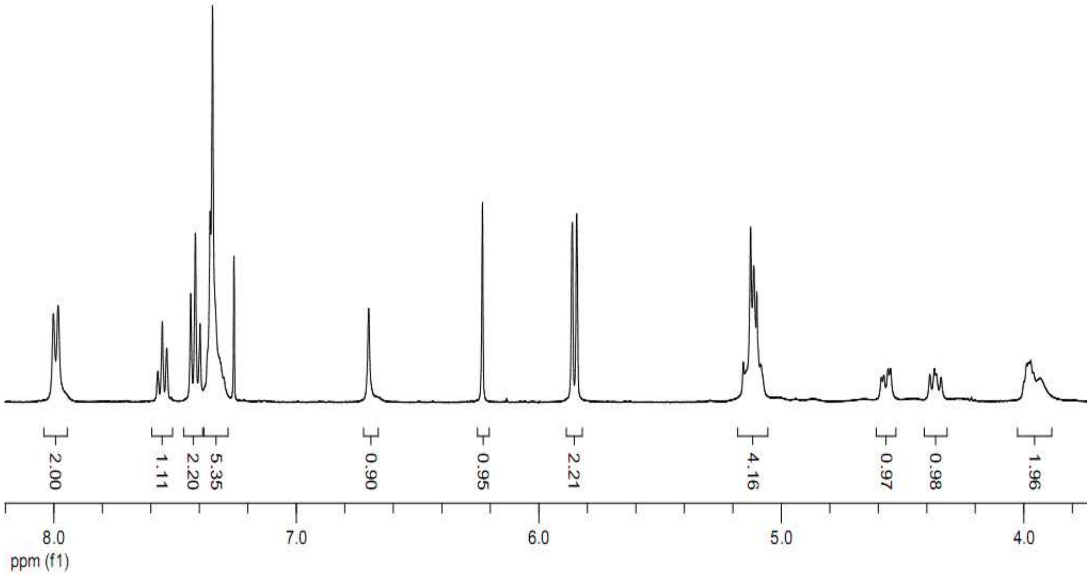
145.139
141.654
135.693
133.258
132.527
131.880
129.896
129.727
129.169
128.580
128.382
128.171
127.601
107.995
105.961
103.262
80.270
67.507
62.994
52.062
21.558
150
100
50
ppm (f1)


NH
OBz
NHCbz
O
O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0.90
0.95
2.21
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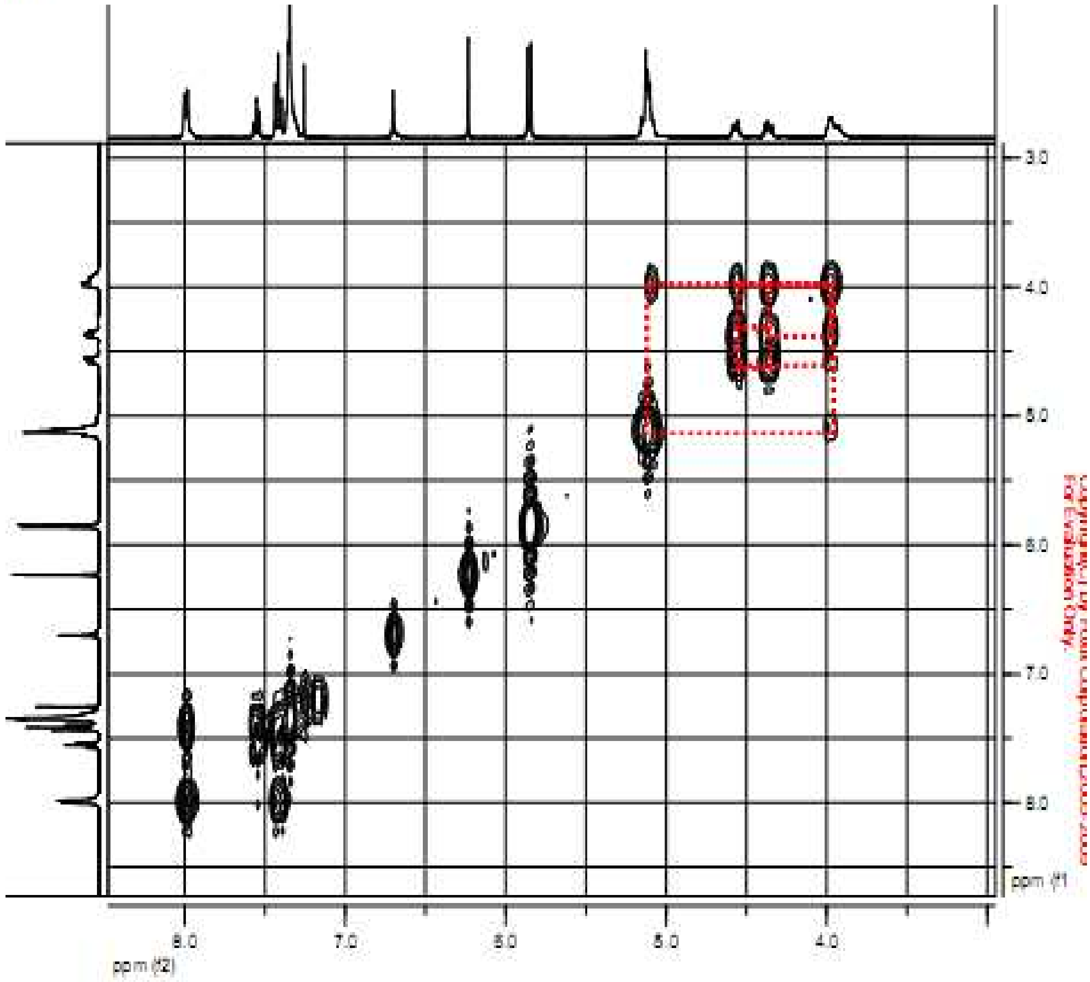
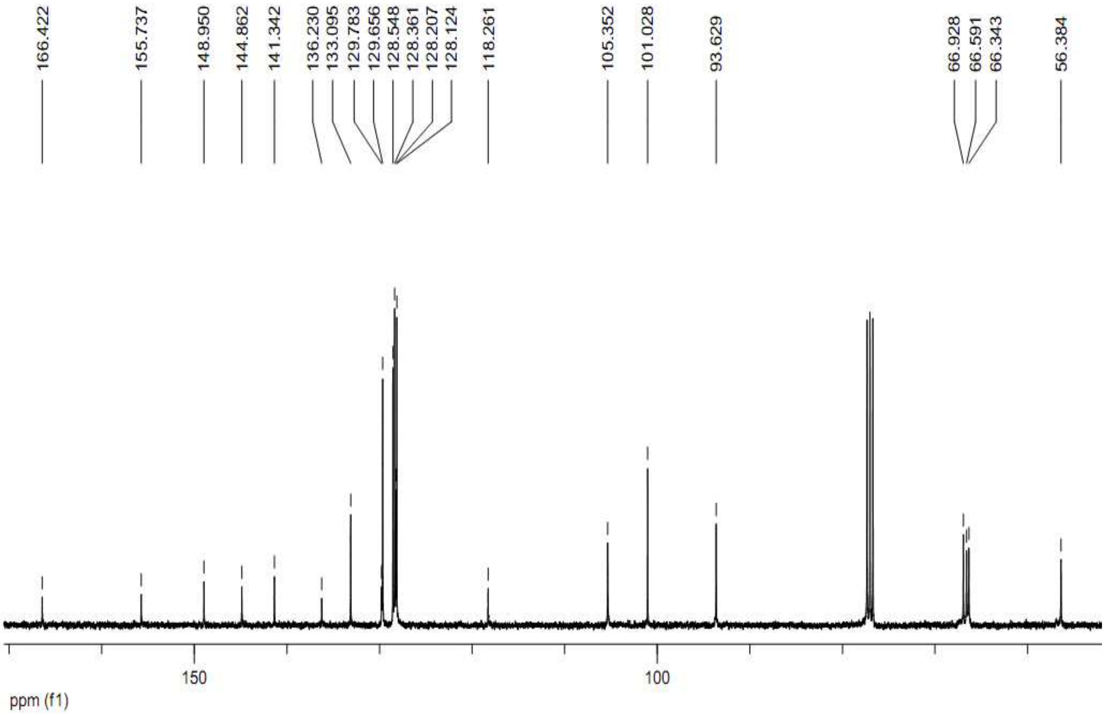
0.97
0.98
1.96
8.0
7.0
6.0
5.0
4.0
ppm (f1)


3.0
4.0
5.0
6.0
7.0
8.0
ppm (f1)
8.0
7.0
6.0
5.0
4.0
ppm (f2)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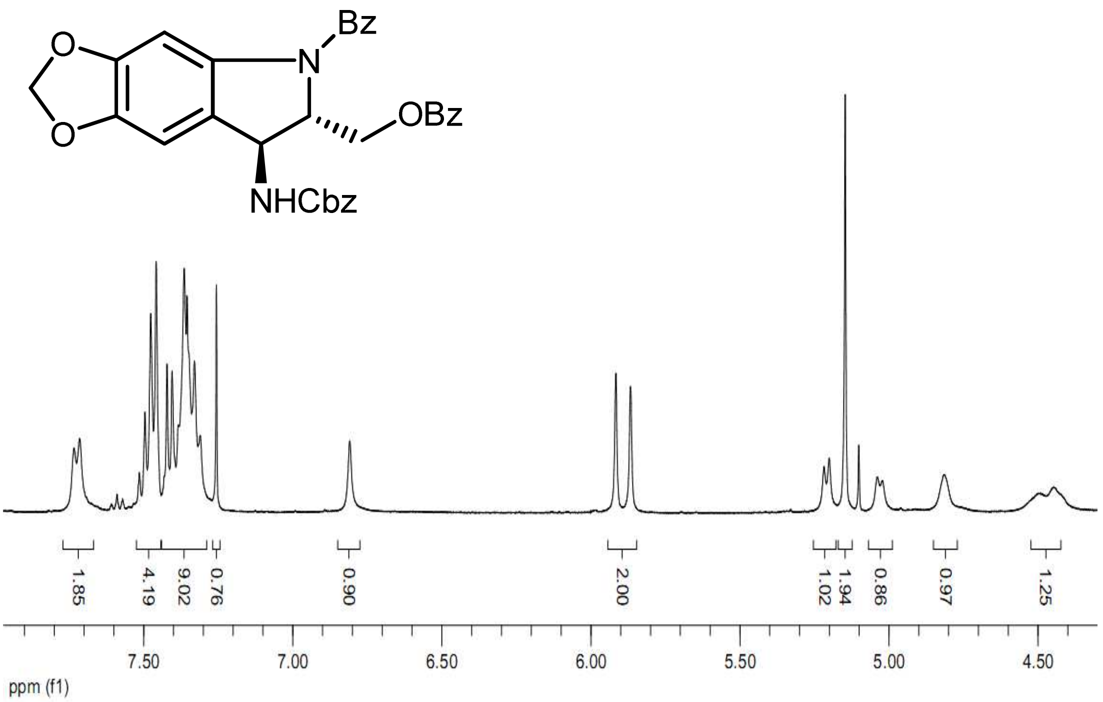
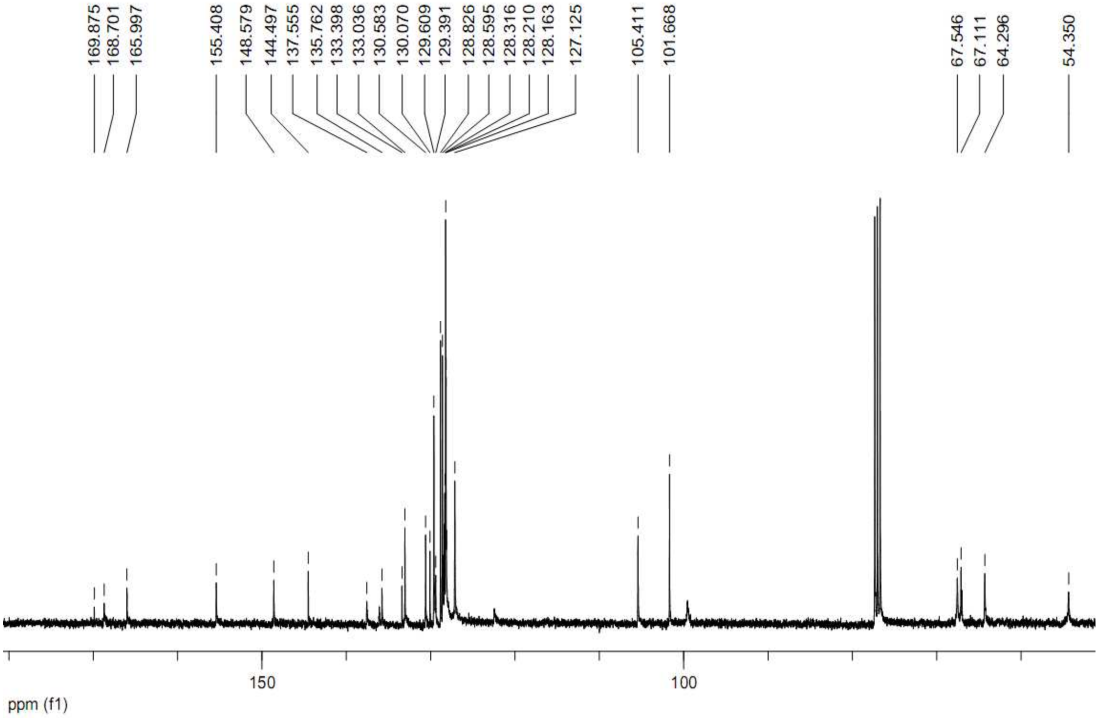
144.862
141.342
136.230
133.095
129.783
129.656
128.548
128.361
128.207
128.124
118.261
105.352
101.028
93.629
66.928
66.591
66.343
56.384
150
100
ppm (f1)


Bz
O
O
N
OBz
NHCbz
1.85
4.19
9.02
0.76
0.90
2.00
1.02
1.94
0.86
0.97
1.25
7.50
7.00
6.50
6.00
5.50
5.00
4.50
ppm (f1)


169.875
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155.408
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144.497
137.555
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133.398
133.036
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130.070
129.609
129.391
128.826
128.595
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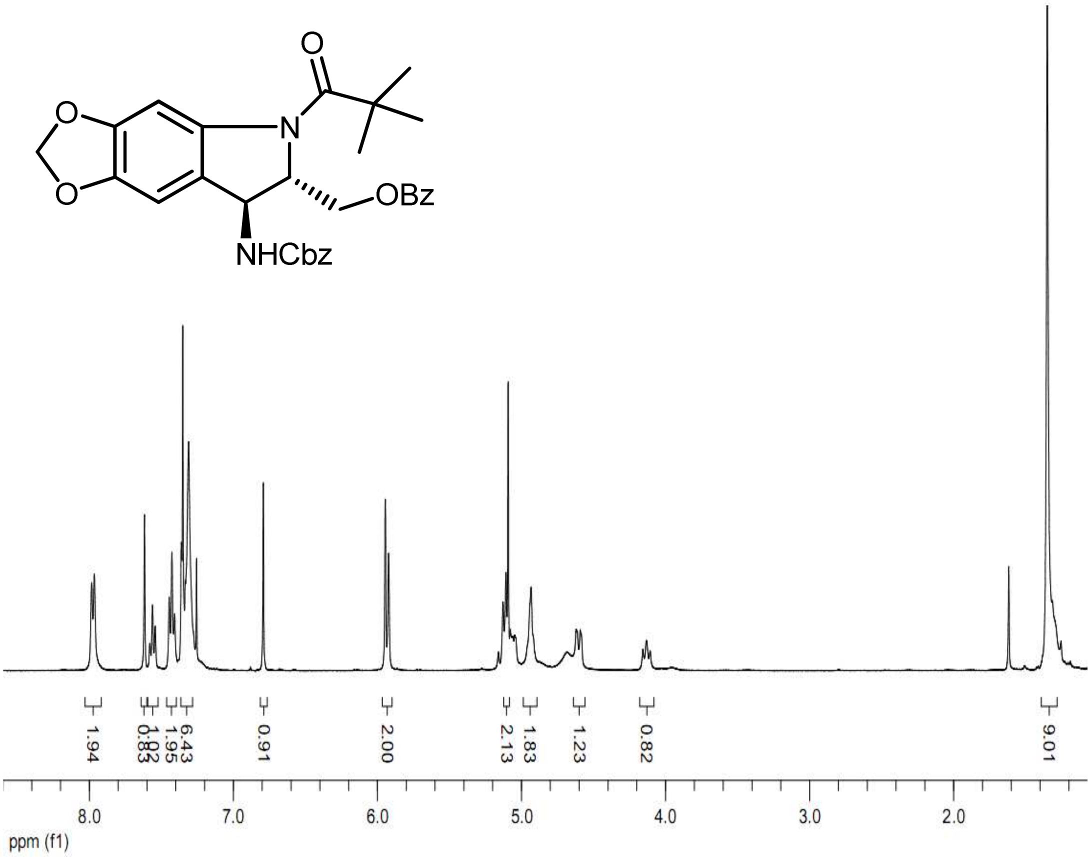
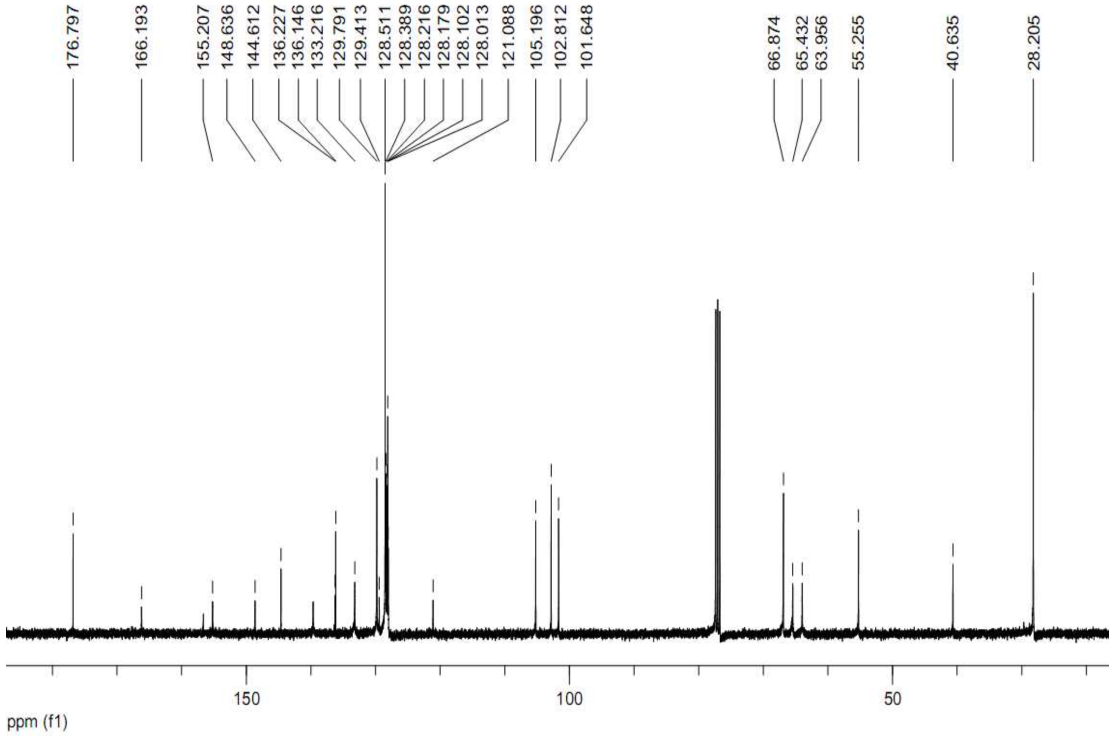
150
100
ppm (f1)


O
N
O
O
OBz
NHCbz
1.94
1.02
0.83
1.95
6.43
0.91
2.00
2.13
1.83
1.23
0.82
9.01
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)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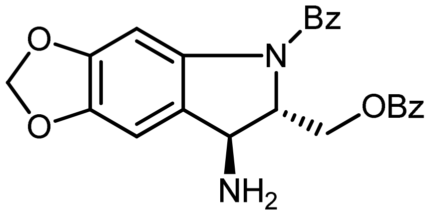
148.636
144.612
136.227
136.146
133.216
129.791
129.413
128.511
128.389
128.216
128.179
128.102
128.013
121.088
105.196
102.812
101.648
66.874
65.432
63.956
55.255
40.635
28.205
150
100
50
ppm (f1)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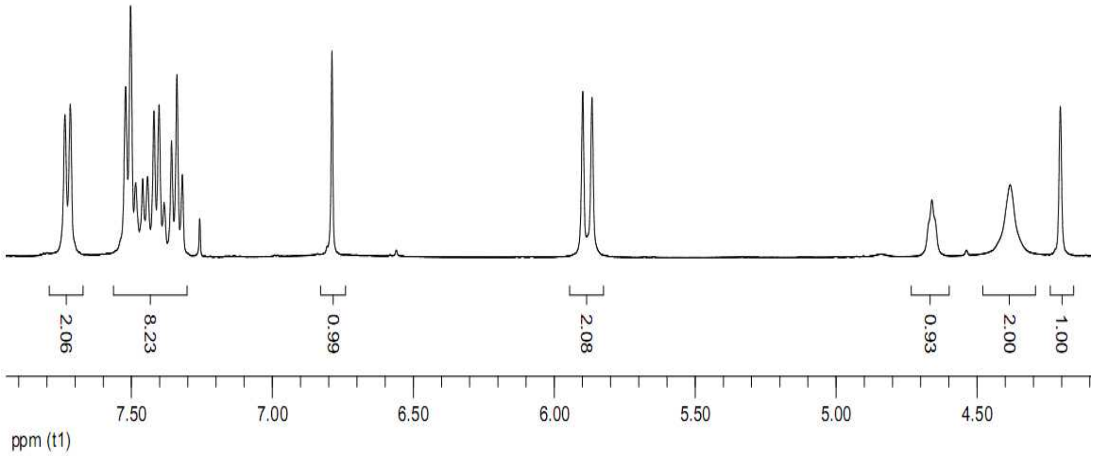
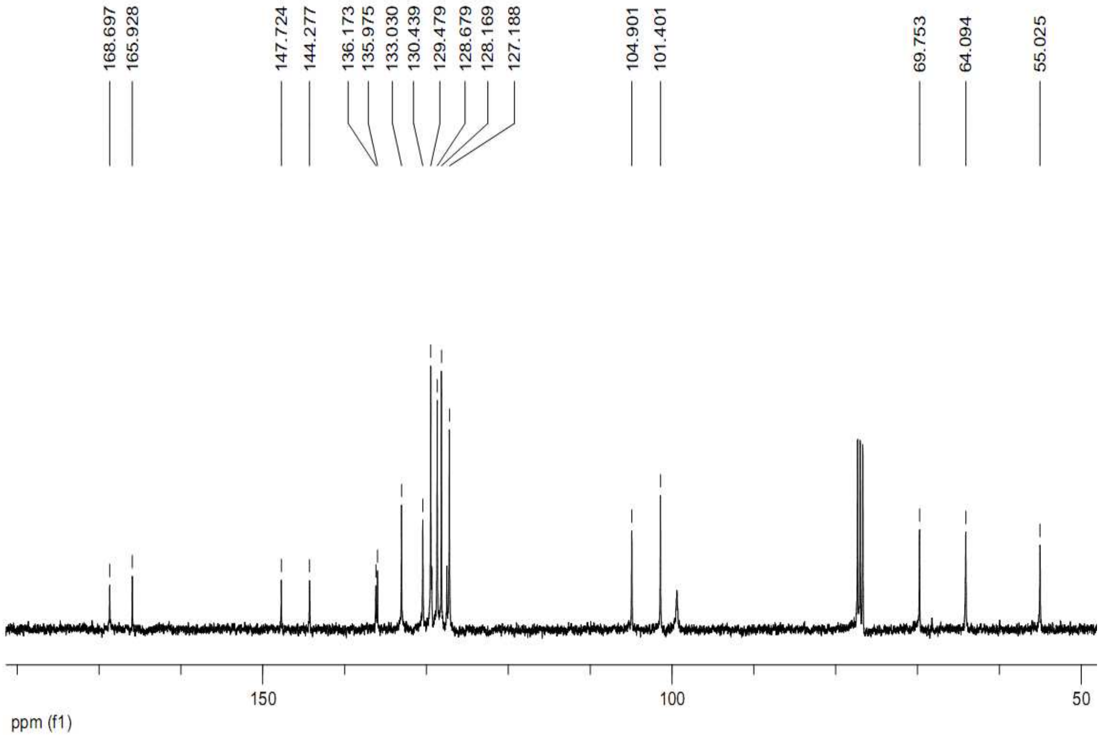
O
N
Bz
OBz
NH2


2.06
8.23
0.99
2.08
0.93
2.00
1.00
7.50
7.00
6.50
6.00
5.50
5.00
4.50
ppm (t1)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136.173
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133.030
130.439
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127.188
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101.401
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64.094
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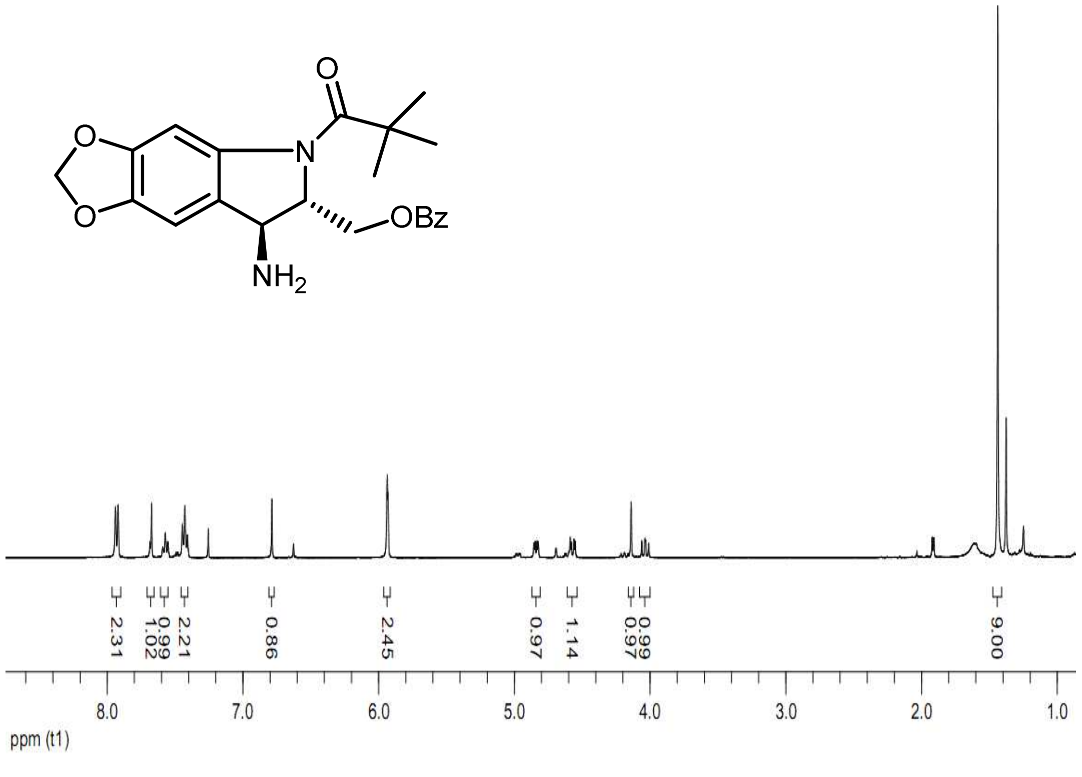
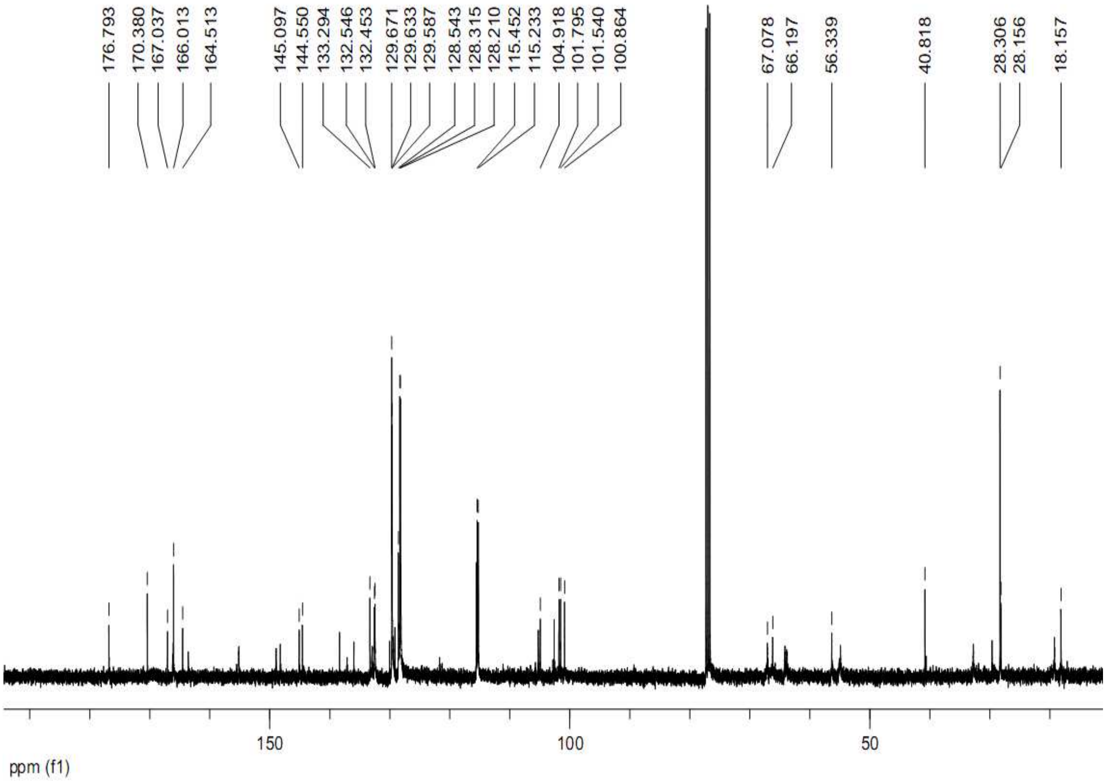
150
100
50
ppm (f1)


O
O
N
O
OBz
NH2
2.31
1.02
0.99
2.21
0.86
2.45
0.97
1.14
0.97
0.99
9.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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170.380
167.037
166.013
164.513
145.097
144.550
133.294
132.546
132.453
129.671
129.633
129.587
128.543
128.315
128.210
115.452
115.233
104.918
101.795
101.540
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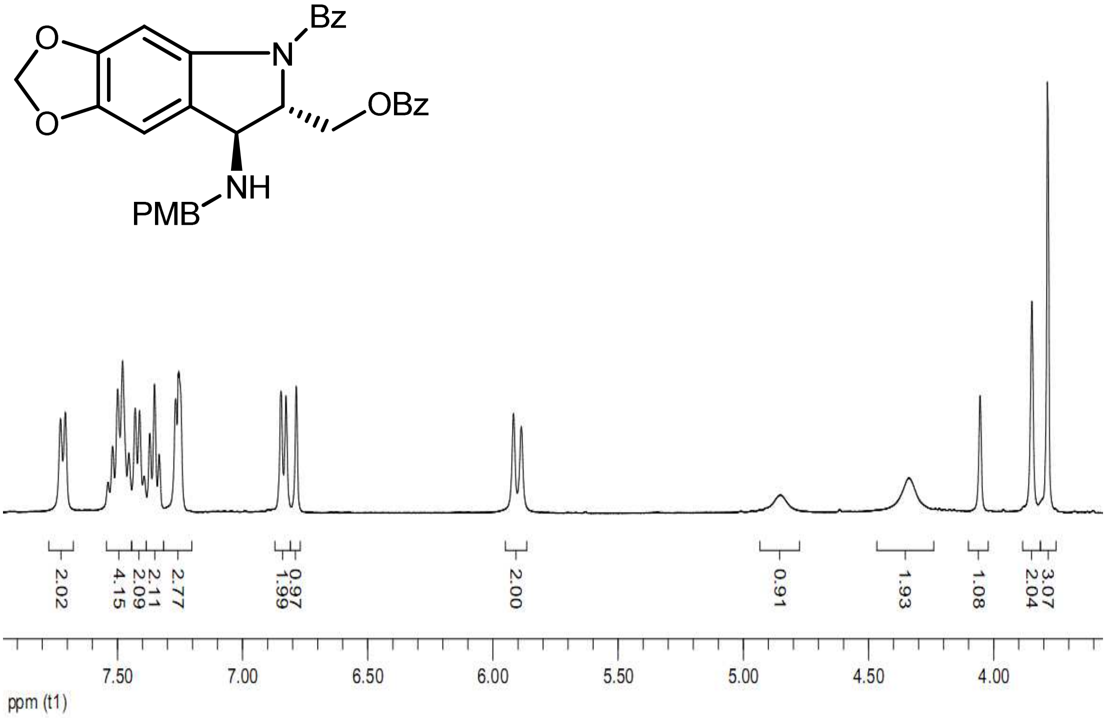
67.078
66.197
56.339
40.818
28.306
28.156
18.157
150
100
50
ppm (f1)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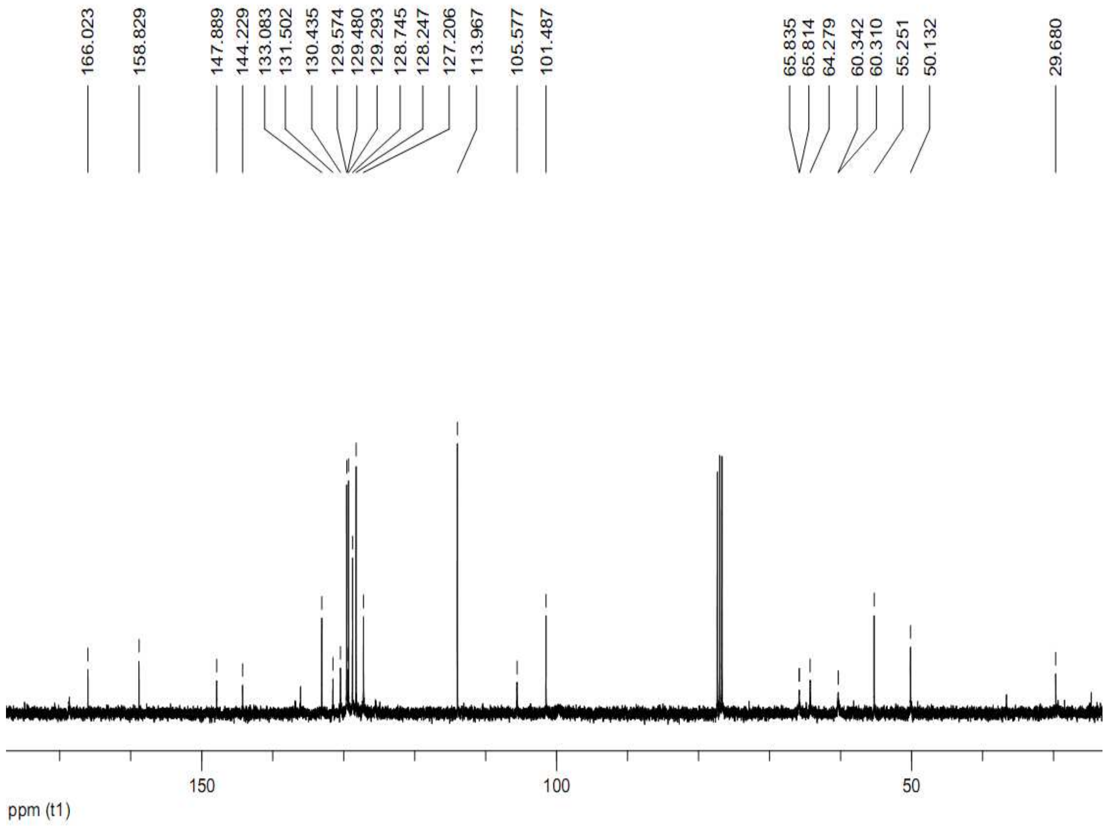
O
N
Bz
OBz
NH
PMB
2.02
4.15
2.09
2.11
2.77
1.99
0.97
2.00
0.91
1.93
1.08
2.04
3.07
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
ppm (t1)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147.889
144.229
133.083
131.502
130.435
129.574
129.480
129.293
128.745
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127.206
113.967
105.577
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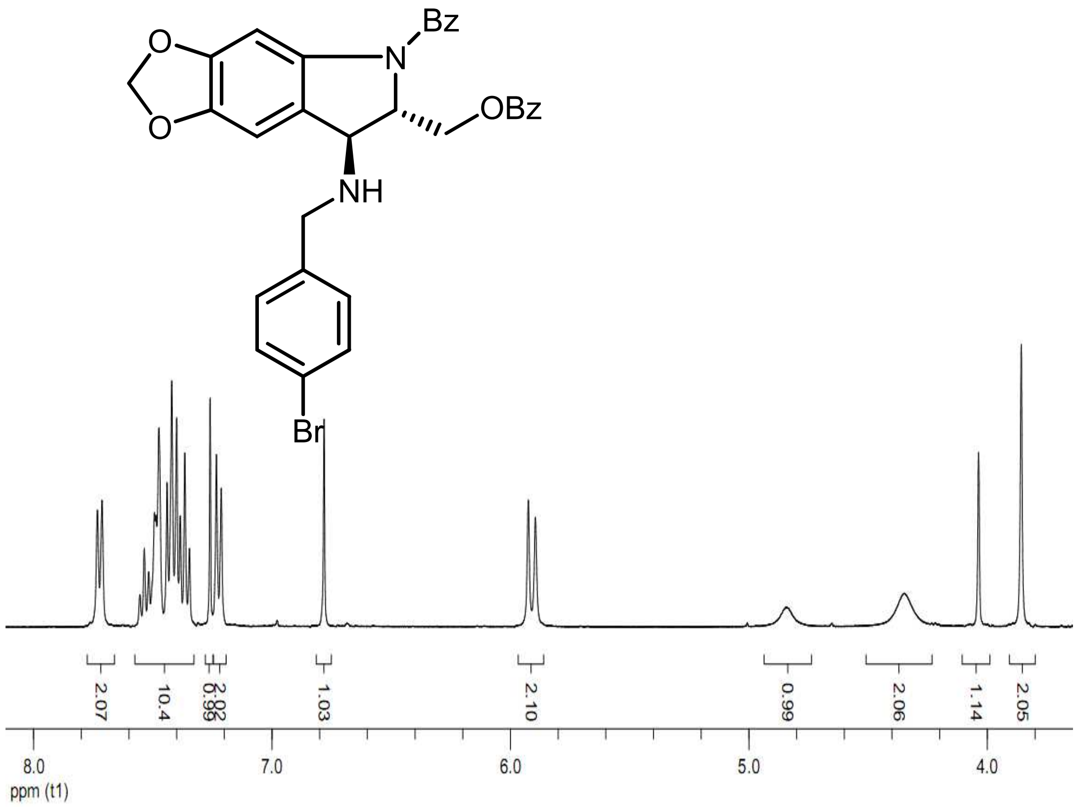
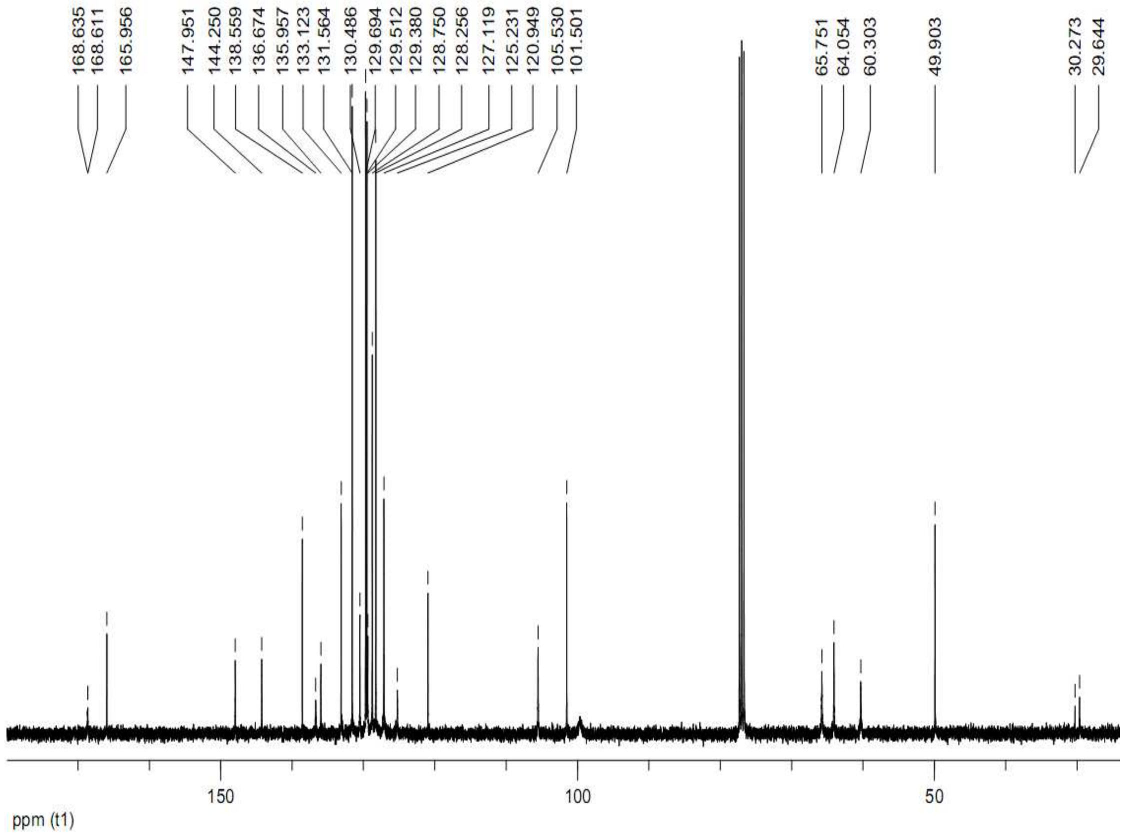
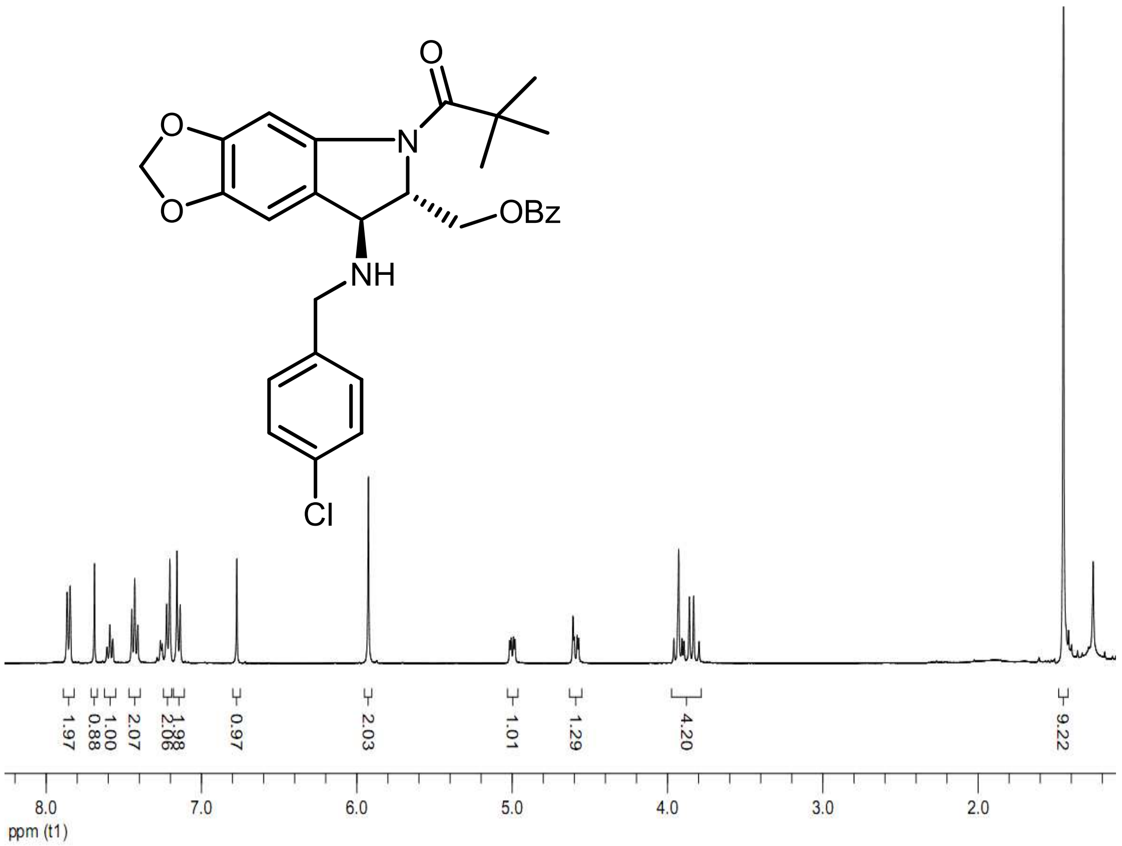
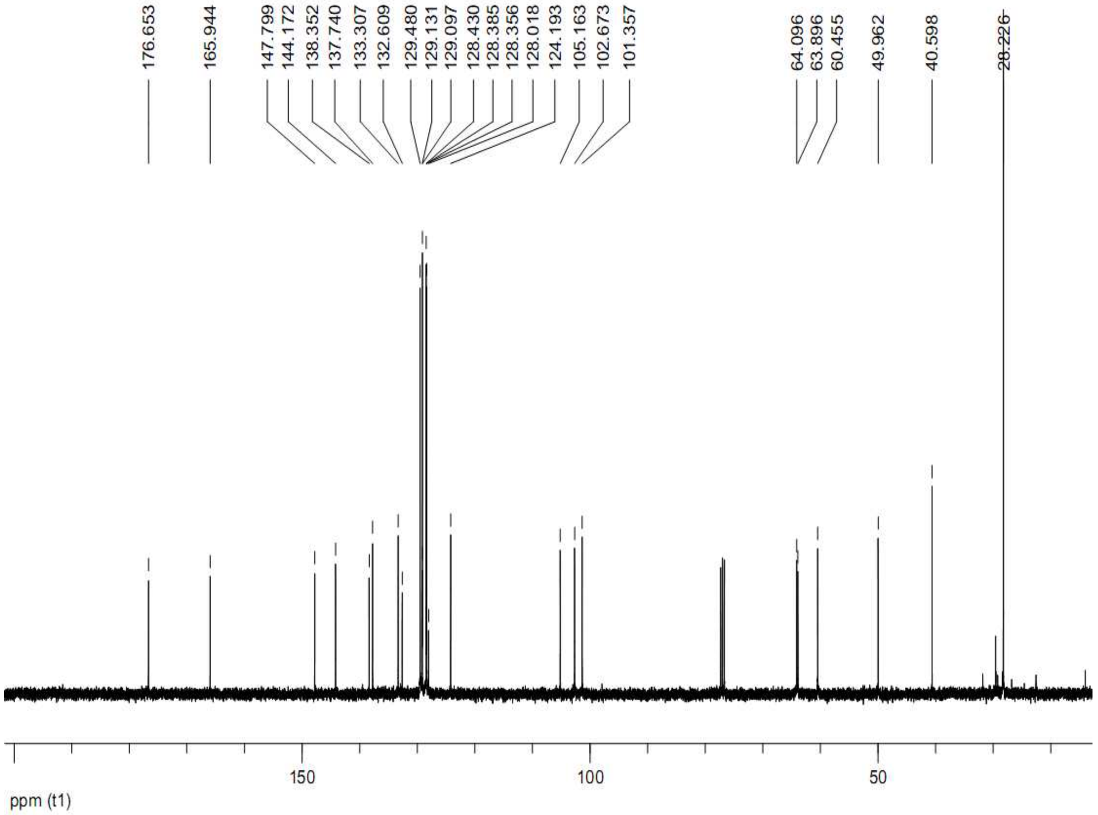
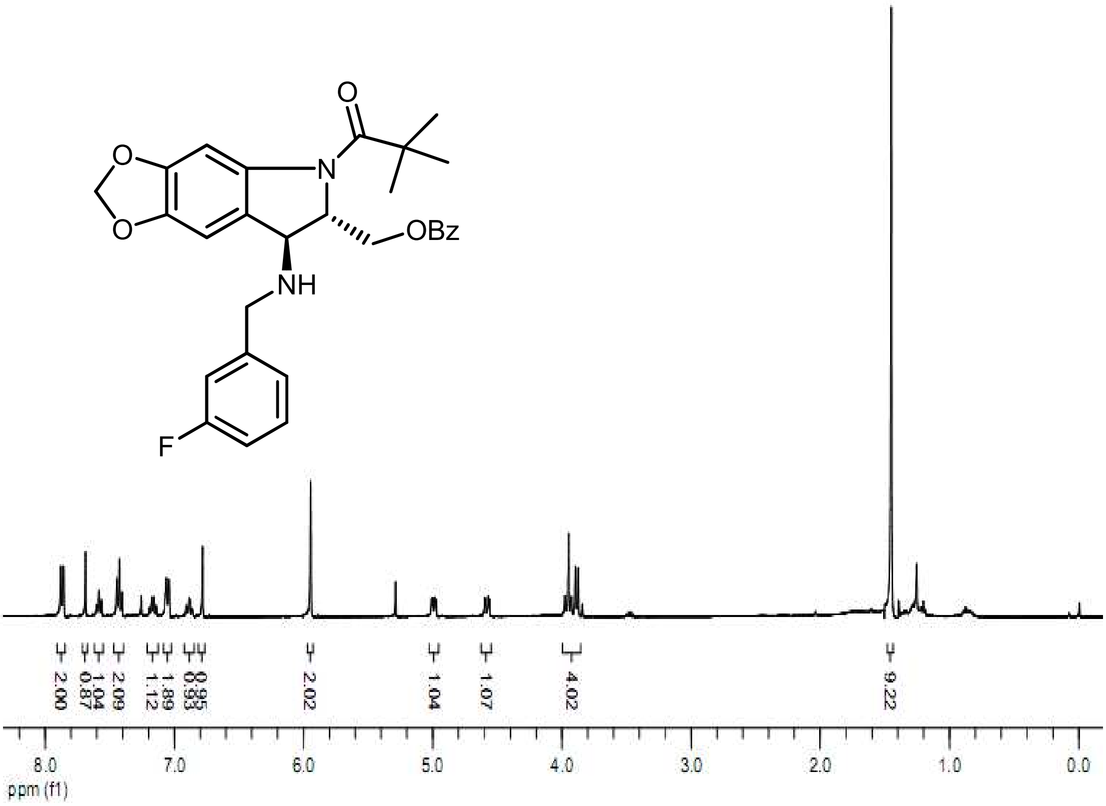
150
100
50
ppm (t1)

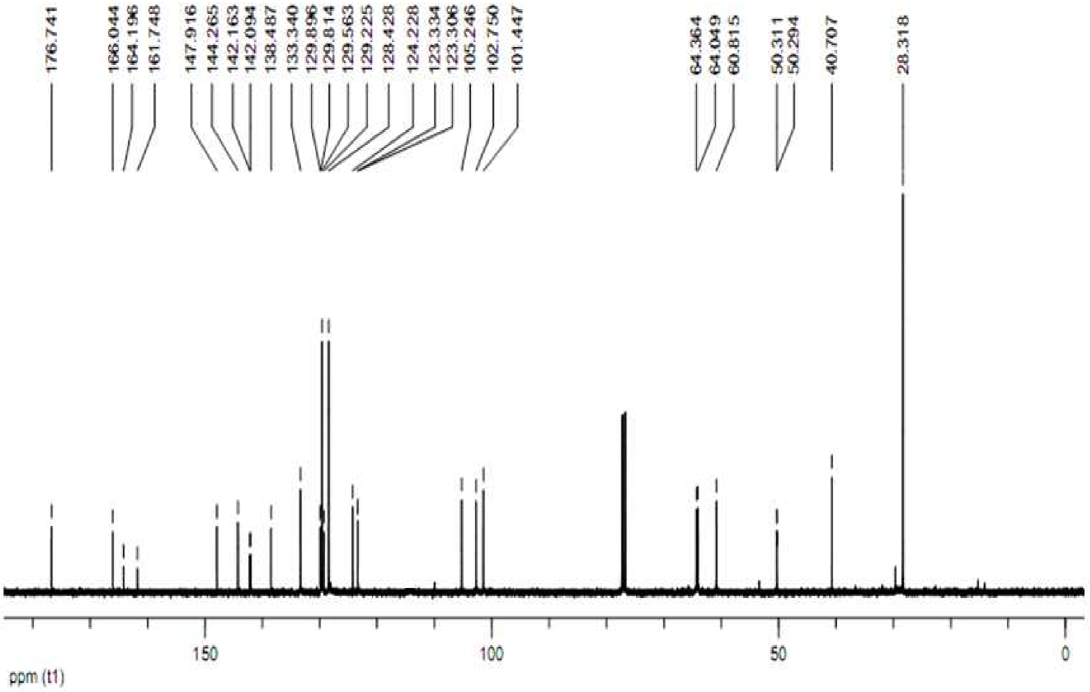

O
O
N
Bz
OBz
NH
Br
2.07
10.4
0.99
2.02
1.03
2.10
0.99
2.06
1.14
2.05
8.0
7.0
6.0
5.0
4.0
ppm (t1)

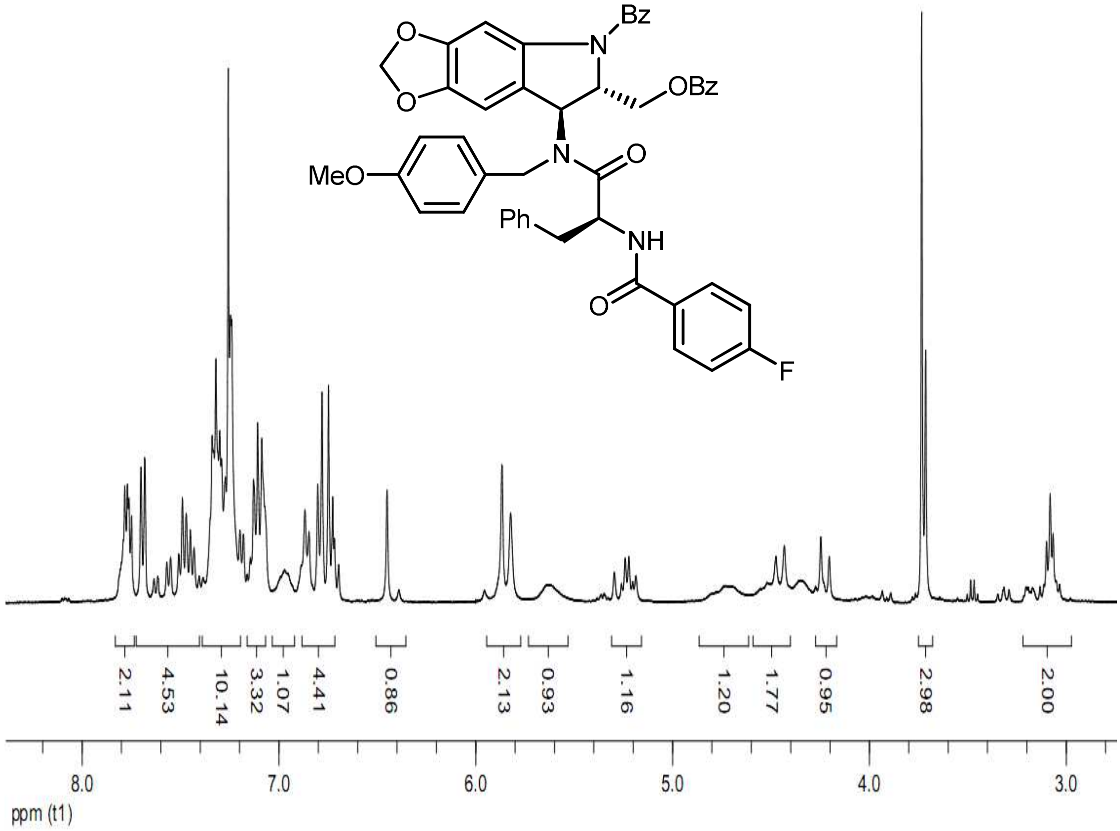

168.635
168.611
165.956
147.951
144.250
138.559
136.674
135.957
133.123
131.564
130.486
129.694
129.512
129.380
128.750
128.256
127.119
125.231
120.949
105.530
101.501
65.751
64.054
60.303
49.903
30.273
29.644
150
100
50
ppm (t1)

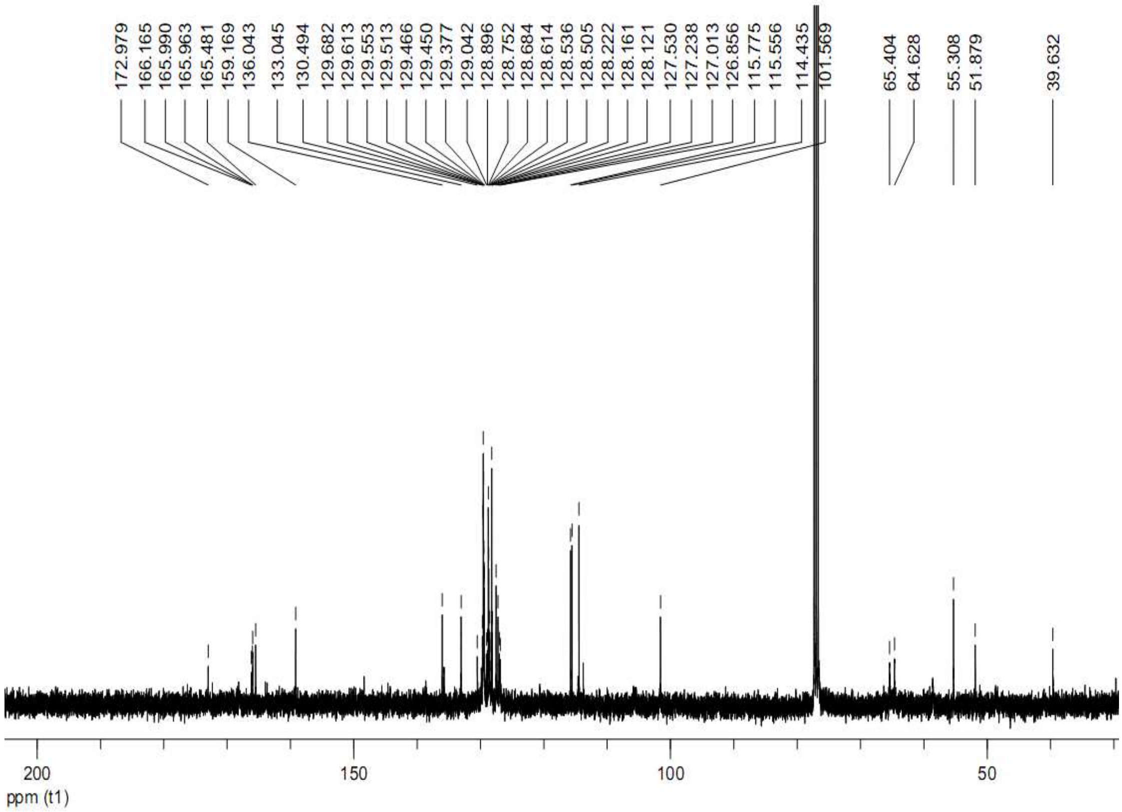

O
O
O
N
OBz
NH
Cl
1.97
0.88
1.00
2.07
2.06
1.88
0.97
2.03
1.01
1.29
4.20
9.22
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

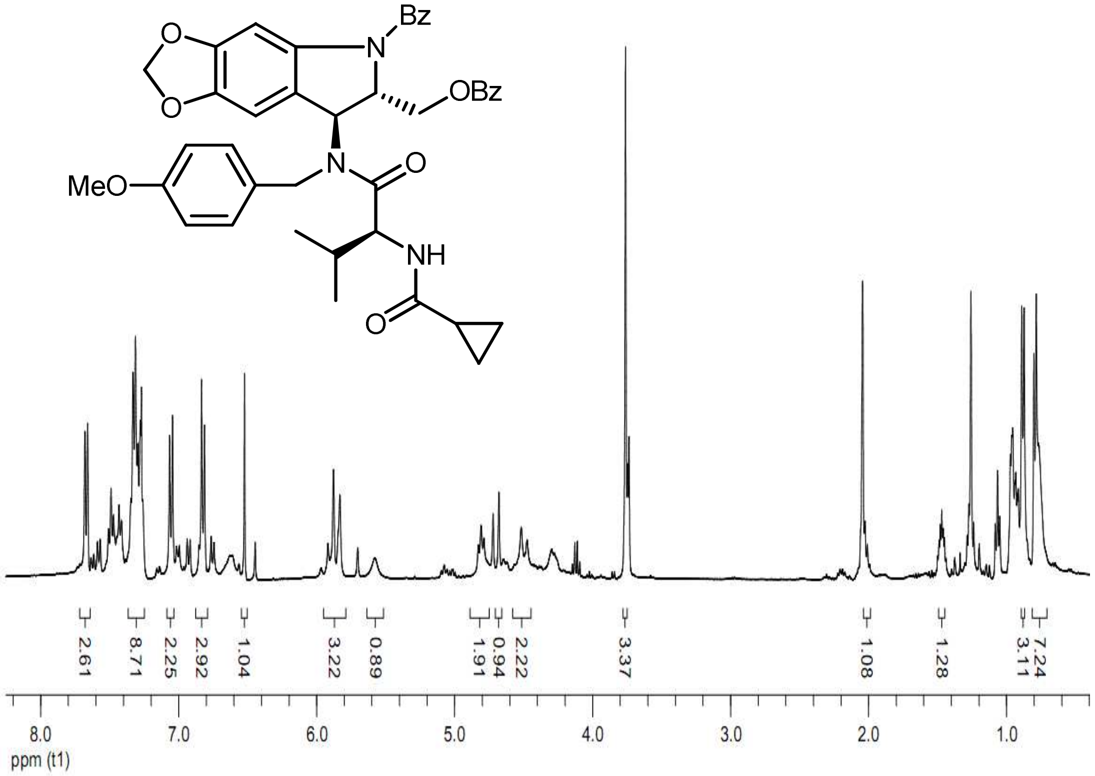

176.653
165.944
147.799
144.172
138.352
137.740
133.307
132.609
129.480
129.131
129.097
128.430
128.385
128.356
128.018
124.193
105.163
102.673
101.357
64.096
63.896
60.455
49.962
40.598
28.226
150
100
50
ppm (t1)

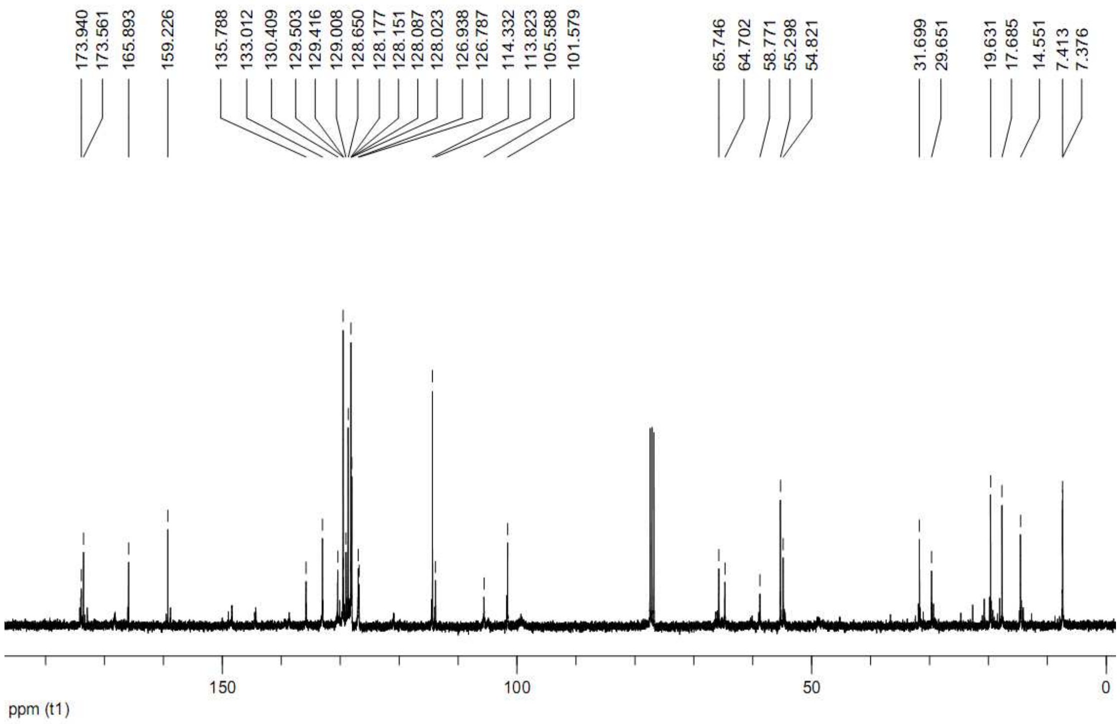

O
O
O
N
OBz
NH
F
2.00
0.87
1.04
2.09
1.12
1.89
0.93
0.95
2.02
1.04
1.07
4.02
9.22
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

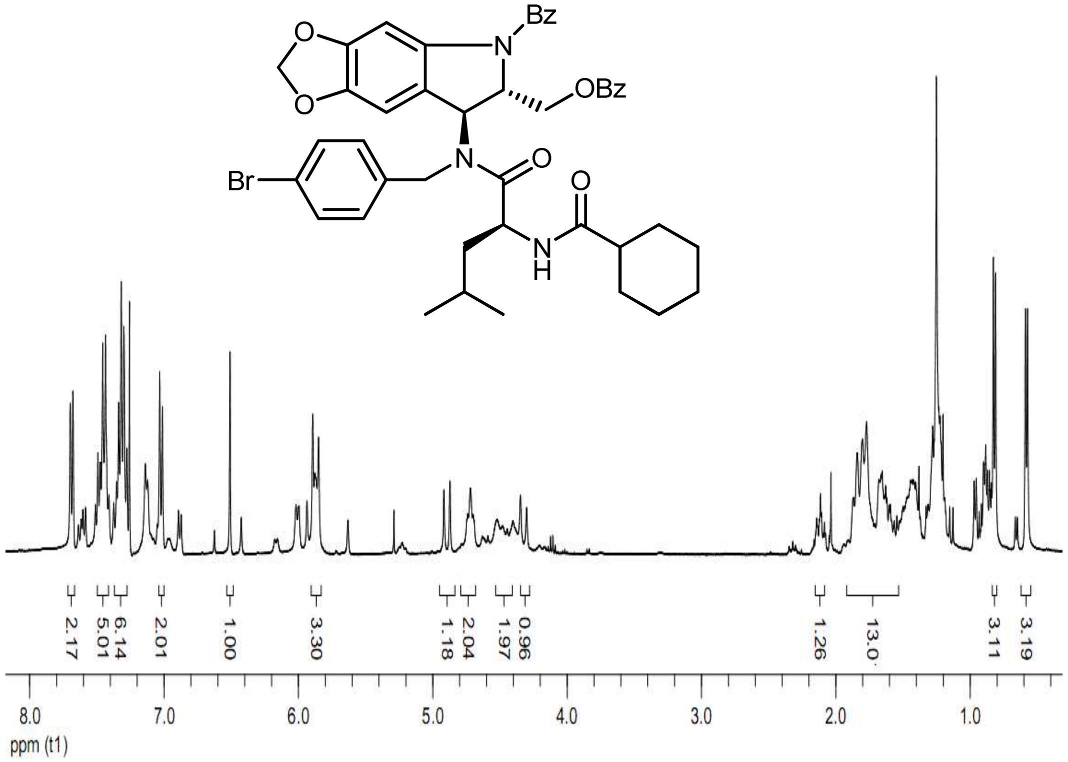

176.741
166.044
164.196
161.748
147.916
144.265
142.163
142.094
138.487
133.340
129.896
129.814
129.563
129.225
128.428
124.228
123.334
123.306
105.246
102.750
101.447
64.364
64.049
60.815
50.311
50.294
40.707
28.318
150
100
50
0
ppm (t1)

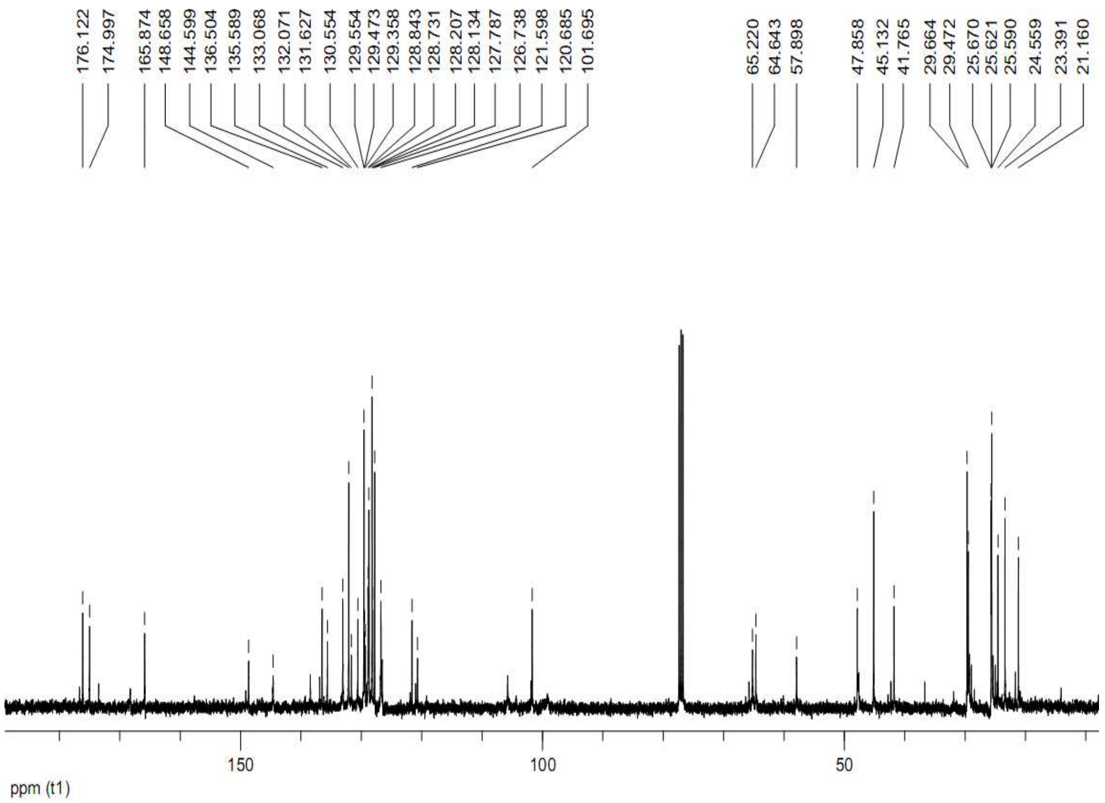

Bz
N
OBz
O
O
N
O
MeO
Ph
NH
O
F
2.11
4.53
10.14
3.32
1.07
4.41
0.86
2.13
0.93
1.16
1.20
1.77
0.95
2.98
2.00
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

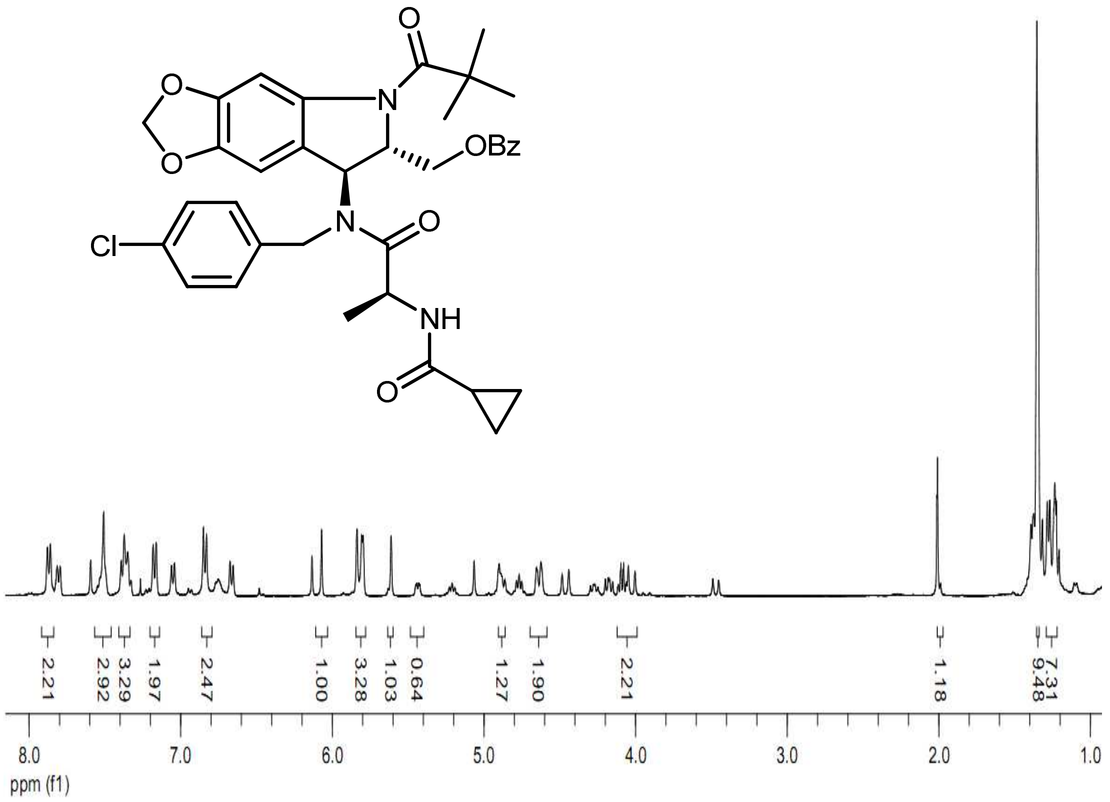

172.979
166.165
165.990
165.963
165.481
159.169
136.043
133.045
130.494
129.682
129.613
129.553
129.513
129.466
129.450
129.377
129.042
128.896
128.752
128.684
128.614
128.536
128.505
128.222
128.161
128.121
127.530
127.238
127.013
126.856
115.775
115.556
114.435
101.569
65.404
64.628
55.308
51.879
39.632
200
150
100
50
ppm (t1)

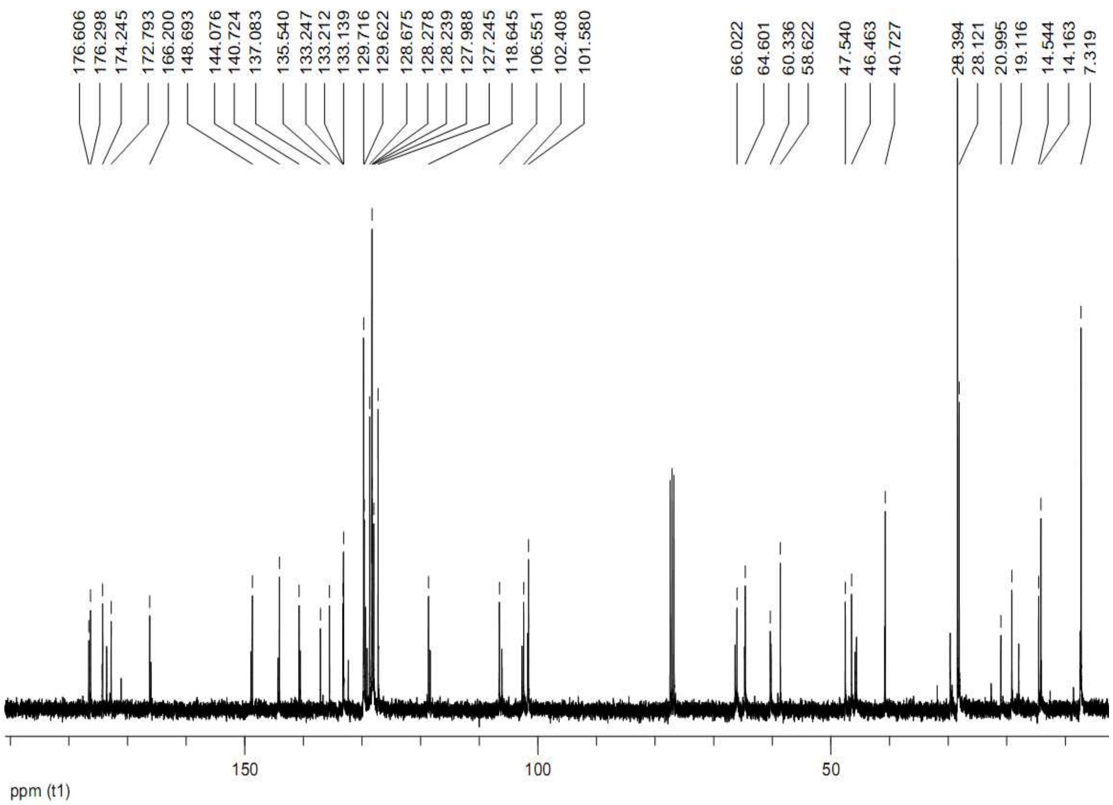

Bz
N
O
O
OBz
N
O
MeO
NH
O
2.61
8.71
2.25
2.92
1.04
3.22
0.89
1.91
0.94
2.22
3.37
1.08
1.28
3.11
7.24
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

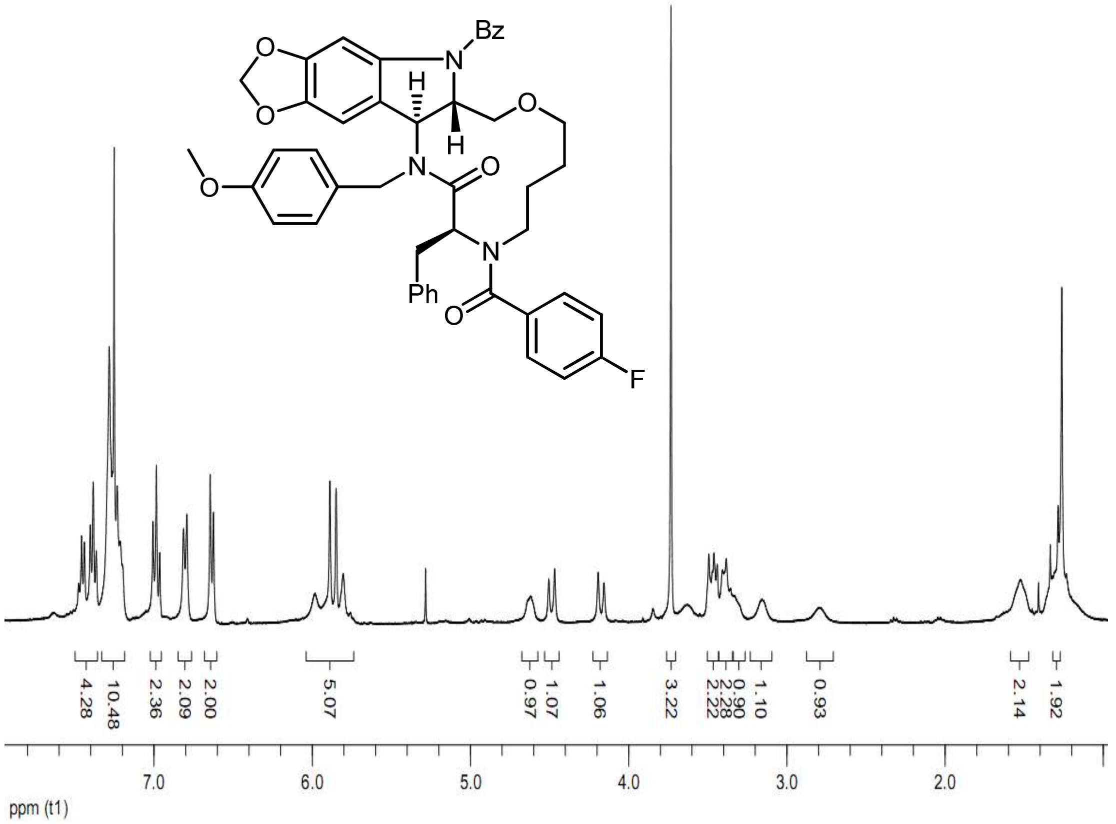

173.940
173.561
165.893
159.226
135.788
133.012
130.409
129.503
129.416
129.008
128.650
128.177
128.151
128.087
128.023
126.938
126.787
114.332
113.823
105.588
101.579
65.746
64.702
58.771
55.298
54.821
31.699
29.651
19.631
17.685
14.551
7.413
7.376
150
100
50
0
ppm (t1)

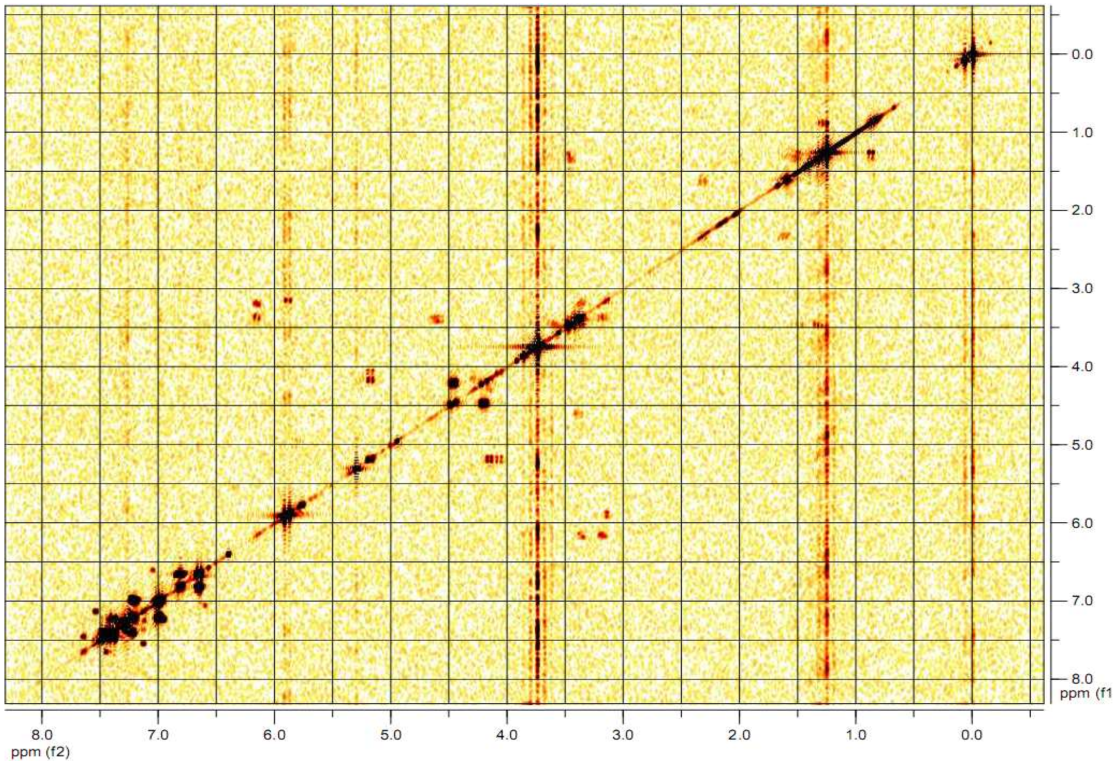

Bz
N
OBz
O
O
N
O
Br
O
N
H
2.17
5.01
6.14
2.01
1.00
3.30
1.18
2.04
1.97
0.96
1.26
13.0
3.11
3.19
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

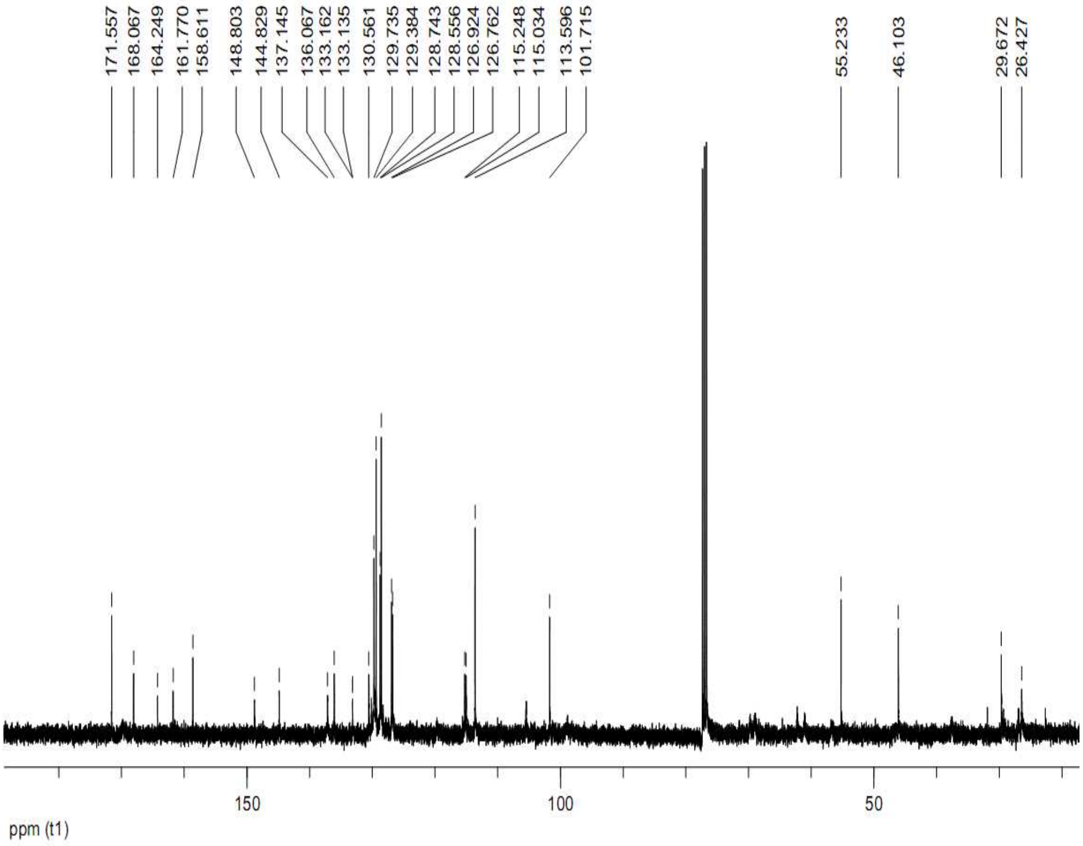

176.122
174.997
165.874
148.658
144.599
136.504
135.589
133.068
132.071
131.627
130.554
129.554
129.473
129.358
128.843
128.731
128.207
128.134
127.787
126.738
121.598
120.685
101.695
65.220
64.643
57.898
47.858
45.132
41.765
29.664
29.472
25.670
25.621
25.590
24.559
23.391
21.160
150
100
50
ppm (t1)


O
O
O
N
OBz
Cl
N
O
NH
O
2.21
2.92
3.29
1.97
2.47
1.00
3.28
1.03
0.64
1.27
1.90
2.21
1.18
9.48
7.31
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

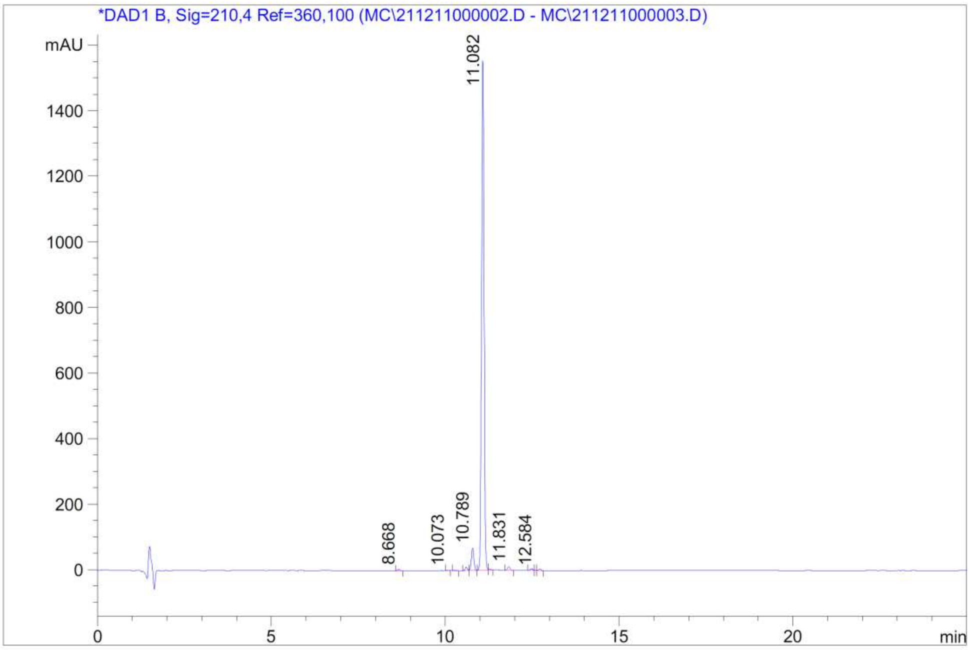

176.606
176.298
174.245
172.793
166.200
148.693
144.076
140.724
137.083
135.540
133.247
133.212
133.139
129.716
129.622
128.675
128.278
128.239
127.988
127.245
118.645
106.551
102.408
101.580
66.022
64.601
60.336
58.622
47.540
46.463
40.727
28.394
28.121
20.995
19.116
14.544
14.163
7.319
150
100
50
ppm (t1)


Bz
N
O
O
H
H
O
N
O
O
N
Ph
O
F
4.28
10.48
2.36
2.09
2.00
5.07
0.97
1.07
1.06
3.22
2.22
2.28
0.90
1.10
0.93
2.14
1.92
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

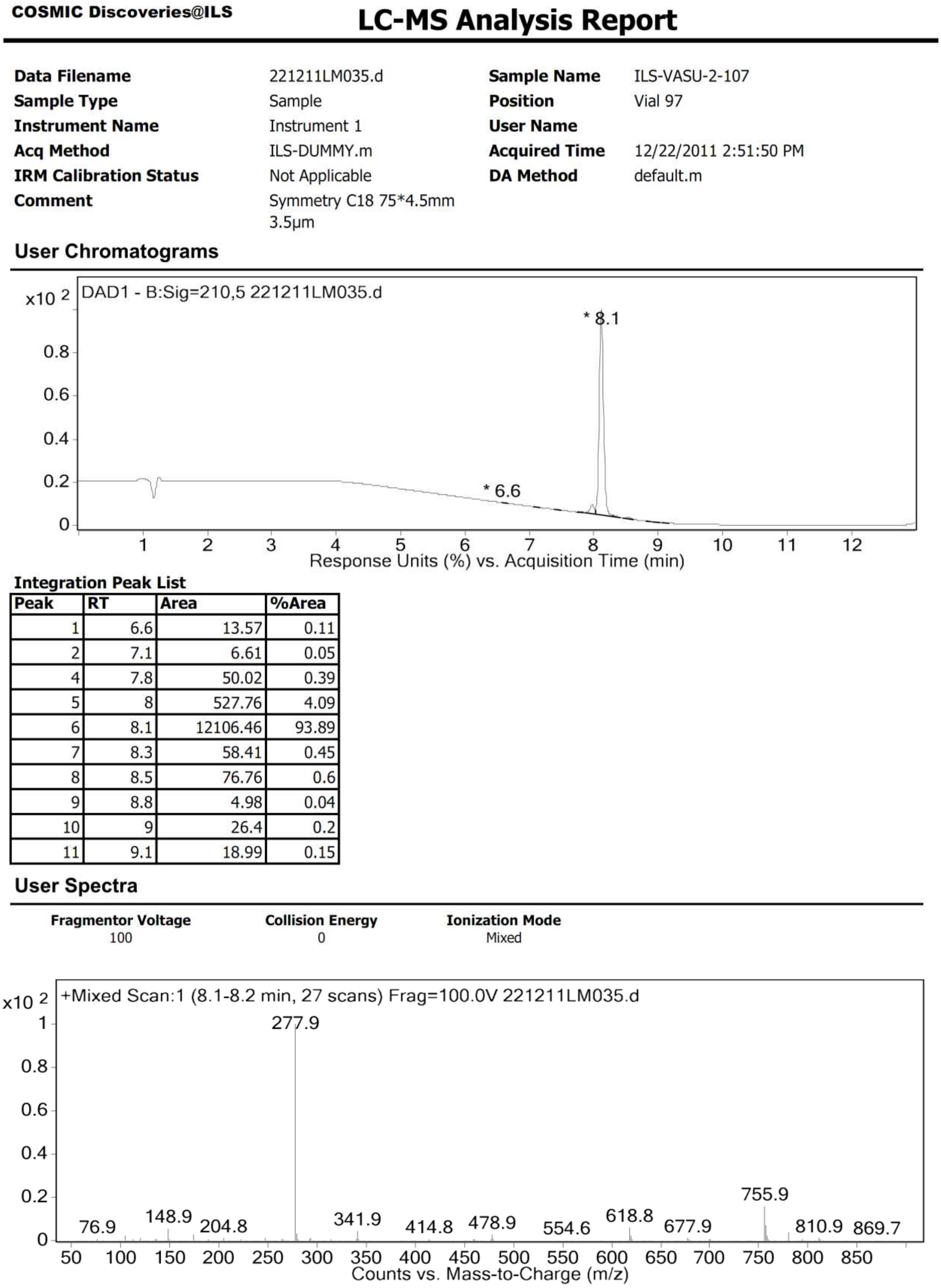

0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ppm (f1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f2)


171.557
168.067
164.249
161.770
158.611
148.803
144.829
137.145
136.067
133.162
133.135
130.561
129.735
129.384
128.743
128.556
126.924
126.762
115.248
115.034
113.596
101.715
55.233
46.103
29.672
26.427
150
100
50
ppm (t1)

```
                           COSMIC DISCOVERIES @ ILS
                              HPLC ANALYSIS REPORT
                                               Seq Line    :           0
Injection Date  : Wed, 21. Dec. 2011           Location    :     Vial 53
Sample Name     : ILS-VASU-2-107               Inj. No.    :           0
Acq Operator    : RADHA                        Inj. Vol.   :        5 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:0/50,3/50,12/98,19/98,22/50,25/50
                  Flow :1.0 ml/min  Diluent:ACN:WATER(50:50) Column Temp:23
```



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

Signal  1: DAD1 B, Sig=210,4 Ref=360,100

|Peak|    RT    | Width |   Area    | Area % |      Name      |
| #  |  [min]   | [min] |           |        |                |
|----|----------|-------|-----------|--------|----------------|
|   1|     8.668|  0.084|     20.162|   0.244|                |
|   2|    10.073|  0.072|      3.799|   0.046|                |
|   3|    10.289|  0.091|      9.194|   0.111|                |
|   4|    10.607|  0.074|     45.014|   0.545|                |
|   5|    10.789|  0.083|    338.876|   4.101|                |
|   6|    11.082|  0.083|   7724.214|  93.473|                |
|   7|    11.279|  0.076|     13.692|   0.166|                |
|   8|    11.831|  0.088|     61.797|   0.748|                |
|   9|    12.486|  0.079|     26.044|   0.315|                |
|  10|    12.584|  0.053|      2.495|   0.030|                |
|  11|    12.727|  0.078|     18.321|   0.222|                |
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	221211LM035.d	**Sample Name**	ILS-VASU-2-107
Sample Type	Sample	**Position**	Vial 97
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS-DUMMY.m	**Acquired Time**	12/22/2011 2:51:50 PM
IRM Calibration Status	Not Applicable	**DA Method**	default.m
Comment	Symmetry C18 75*4.5mm 3.5µm		

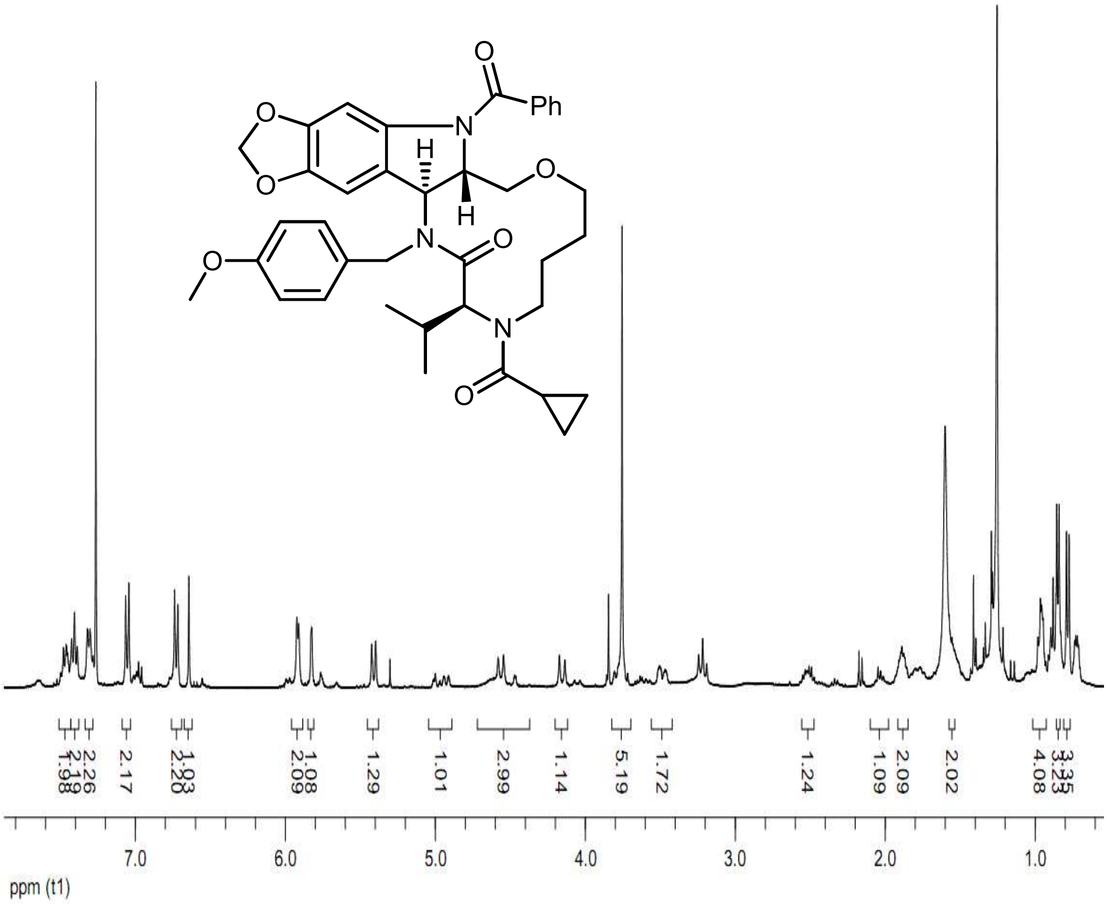
User Chromatograms

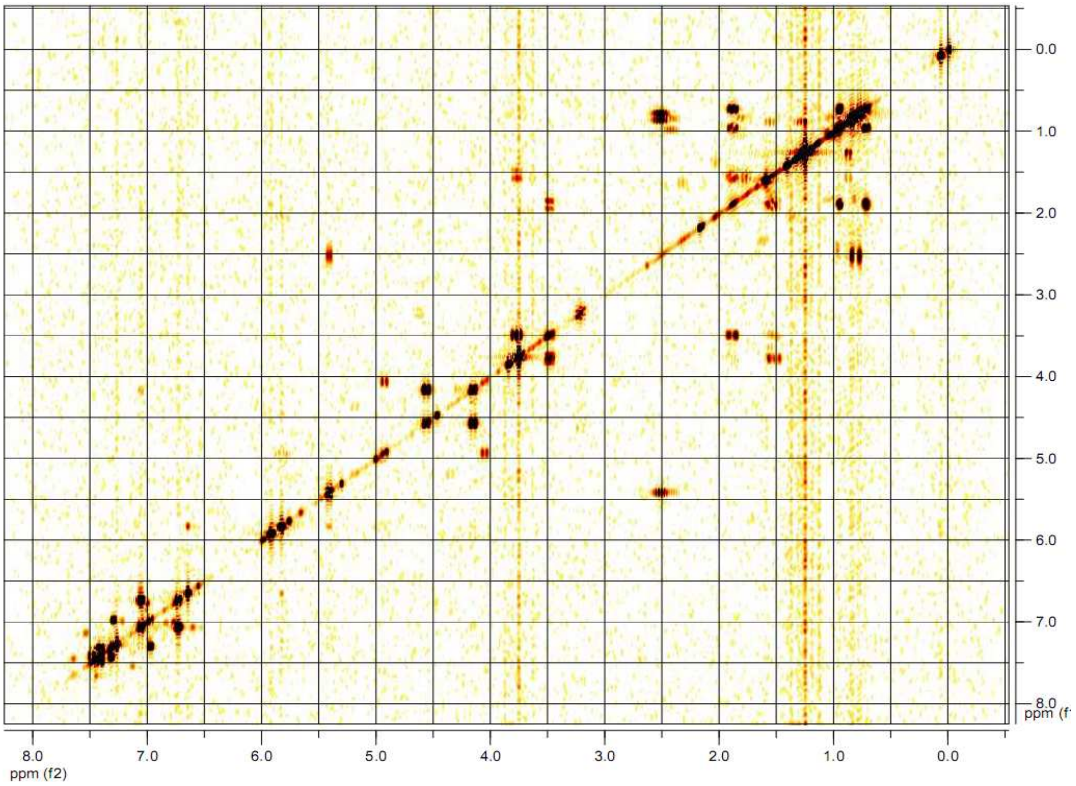
Integration Peak List

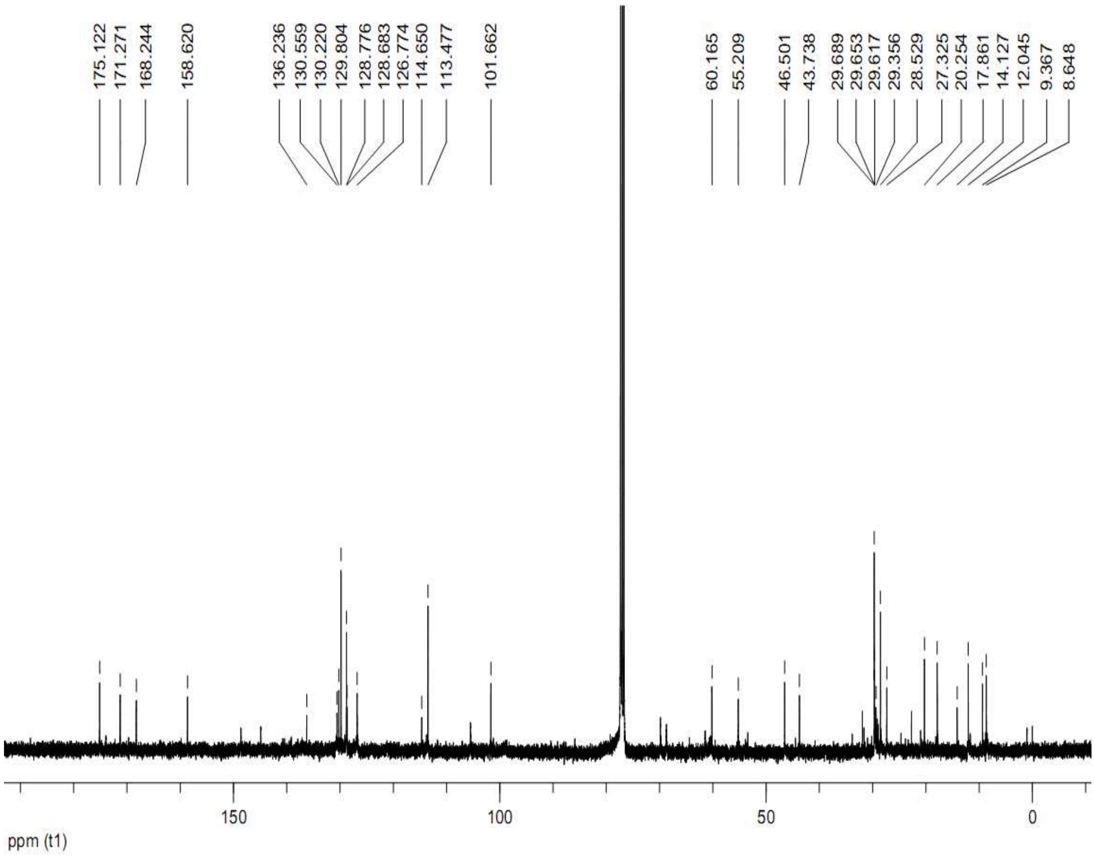
Peak	RT	Area	%Area
1	6.6	13.57	0.11
2	7.1	6.61	0.05
4	7.8	50.02	0.39
5	8	527.76	4.09
6	8.1	12106.46	93.89
7	8.3	58.41	0.45
8	8.5	76.76	0.6
9	8.8	4.98	0.04
10	9	26.4	0.2
11	9.1	18.99	0.15

User Spectra

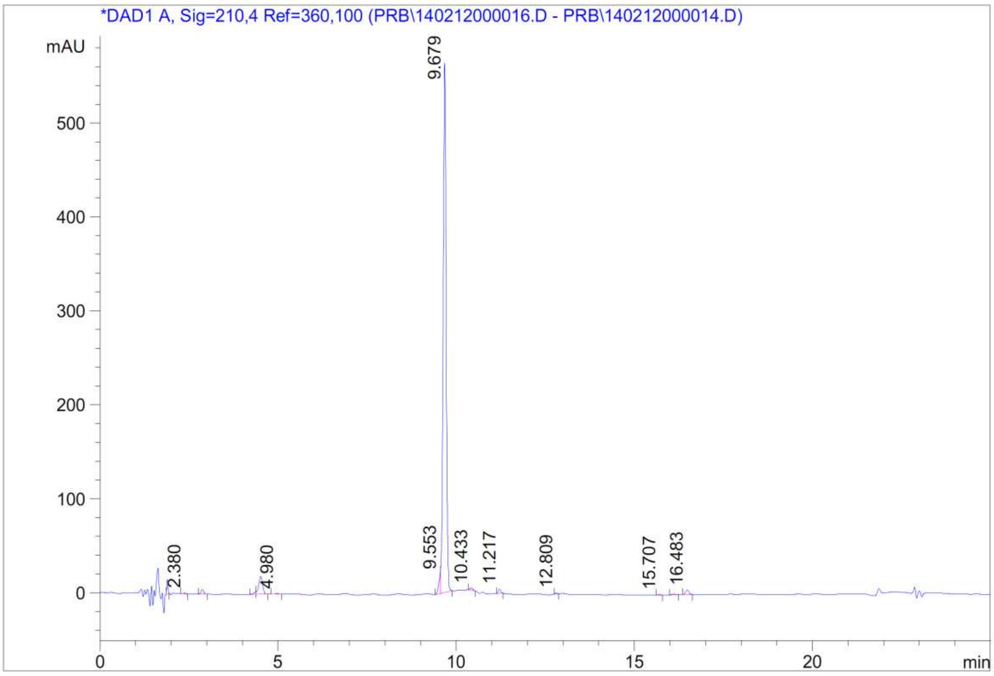
Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed


O
Ph
N
O
O
H
H
O
N
O
O
N
O
1.98
2.19
2.26
2.17
2.20
1.93
2.09
1.08
1.29
1.01
2.99
1.14
5.19
1.72
1.24
1.09
2.09
2.02
4.08
3.25
3.35
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

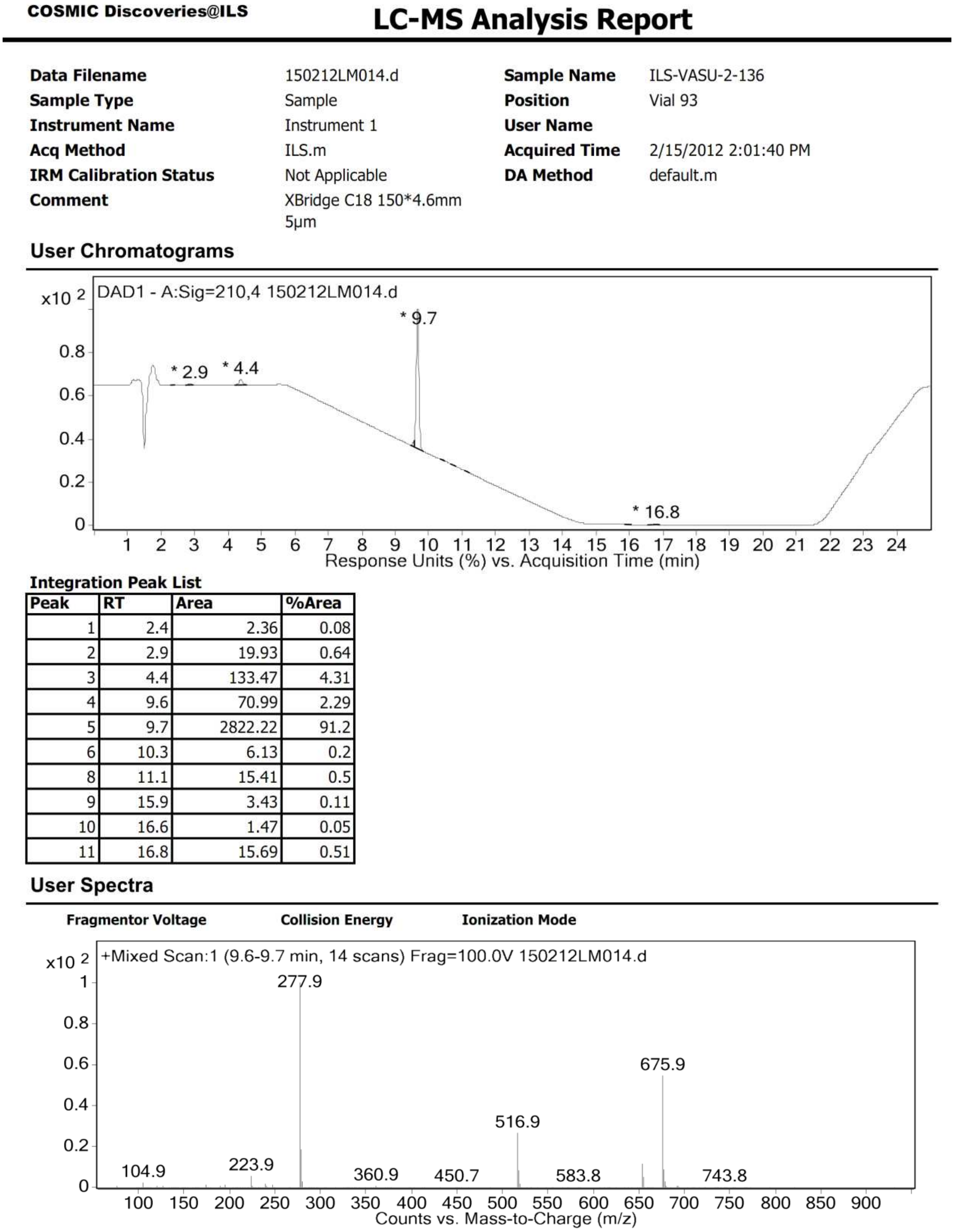

0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ppm (f
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f2)


175.122
171.271
168.244
158.620
136.236
130.559
130.220
129.804
128.776
128.683
126.774
114.650
113.477
101.662
60.165
55.209
46.501
43.738
29.689
29.653
29.617
29.356
28.529
27.325
20.254
17.861
14.127
12.045
9.367
8.648
150
100
50
0
ppm (t1)

```
                             HPLC ANALYSIS REPORT
                                                 Seq Line    :           0
Injection Date   : Tue, 14. Feb. 2012            Location    :     Vial 13
Sample Name      : ILS-VASU-2-136                Inj. No.    :           0
Acq Operator     : RADHA                         Inj. Vol.   :       12 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:0/50,3/50,12/98,19/98,22/50,25/50
                   Flow :1.0 ml/min Diluent:MeOH Column Temp:23°C
```



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

Signal   1: DAD1 A, Sig=210,4 Ref=360,100

|Peak|    RT     | Width |    Area    | Area % |      Name      |
| #  |  [min]    | [min] |            |        |                |
|----|-----------|-------|------------|--------|----------------|
|   1|     1.895|  0.038|    34.810|   0.945|                |
|   2|     2.380|  0.087|     2.342|   0.064|                |
|   3|     2.881|  0.081|    23.068|   0.626|                |
|   4|     4.373|  0.074|    10.716|   0.291|                |
|   5|     4.517|  0.130|   146.515|   3.978|                |
|   6|     4.980|  0.146|     6.127|   0.166|                |
|   7|     9.553|  0.056|    69.160|   1.878|                |
|   8|     9.679|  0.098|  3312.194|  89.930|                |
|   9|    10.433|  0.095|    14.341|   0.389|                |
|  10|    11.217|  0.079|    21.678|   0.589|                |
|  11|    12.809|  0.073|     5.263|   0.143|                |
|  12|    15.707|  0.097|     1.396|   0.038|                |
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	150212LM014.d	**Sample Name**	ILS-VASU-2-136
Sample Type	Sample	**Position**	Vial 93
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	2/15/2012 2:01:40 PM
IRM Calibration Status	Not Applicable	**DA Method**	default.m
Comment	XBridge C18 150*4.6mm 5μm		

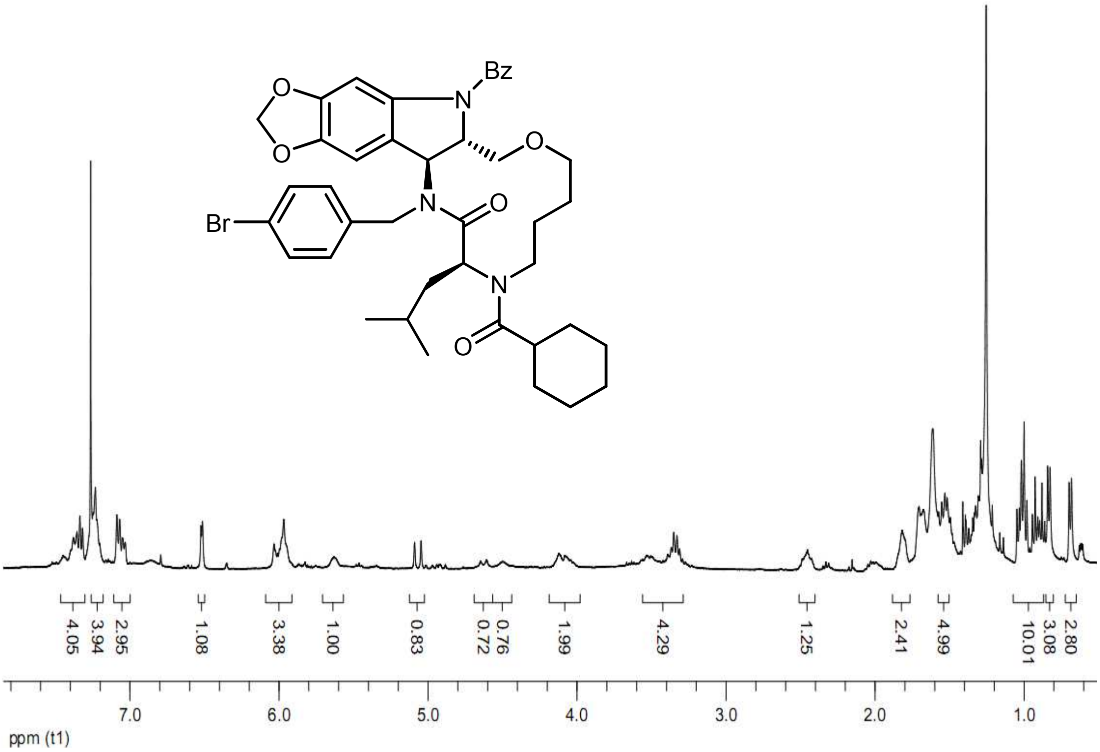
User Chromatograms

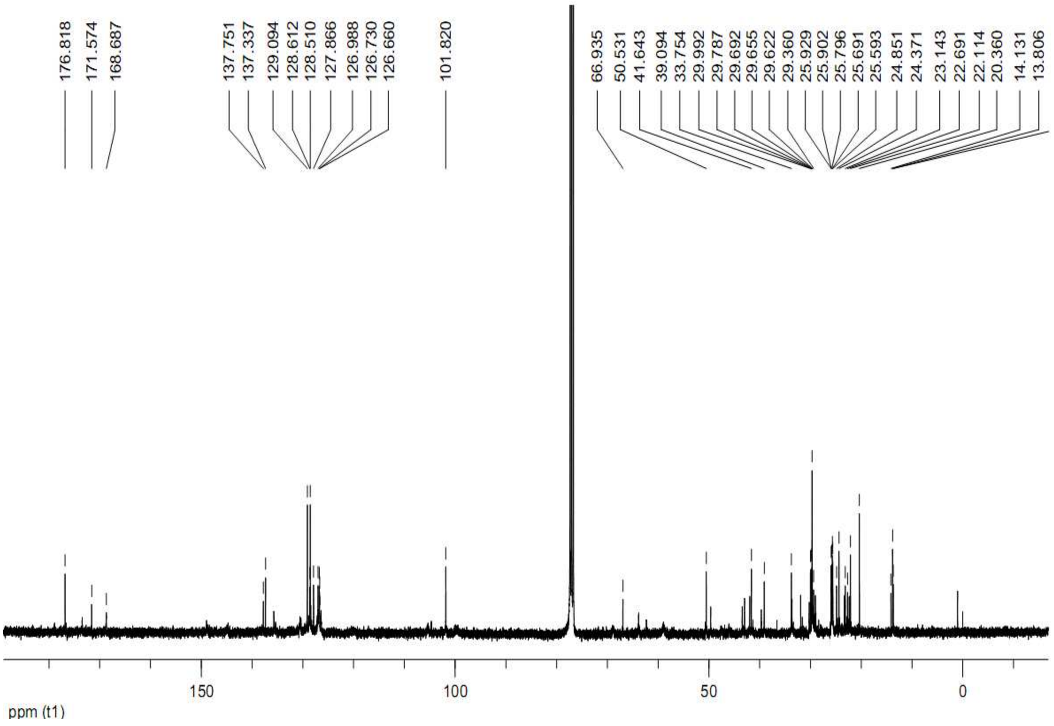
Integration Peak List

Peak	RT	Area	%Area
1	2.4	2.36	0.08
2	2.9	19.93	0.64
3	4.4	133.47	4.31
4	9.6	70.99	2.29
5	9.7	2822.22	91.2
6	10.3	6.13	0.2
8	11.1	15.41	0.5
9	15.9	3.43	0.11
10	16.6	1.47	0.05
11	16.8	15.69	0.51

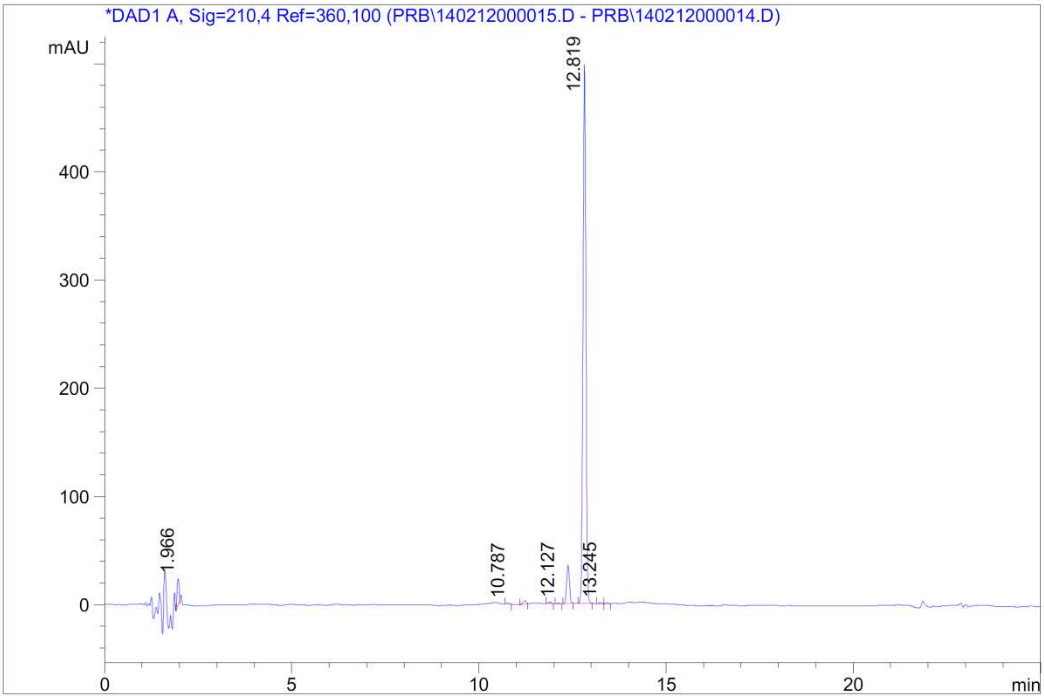
User Spectra

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

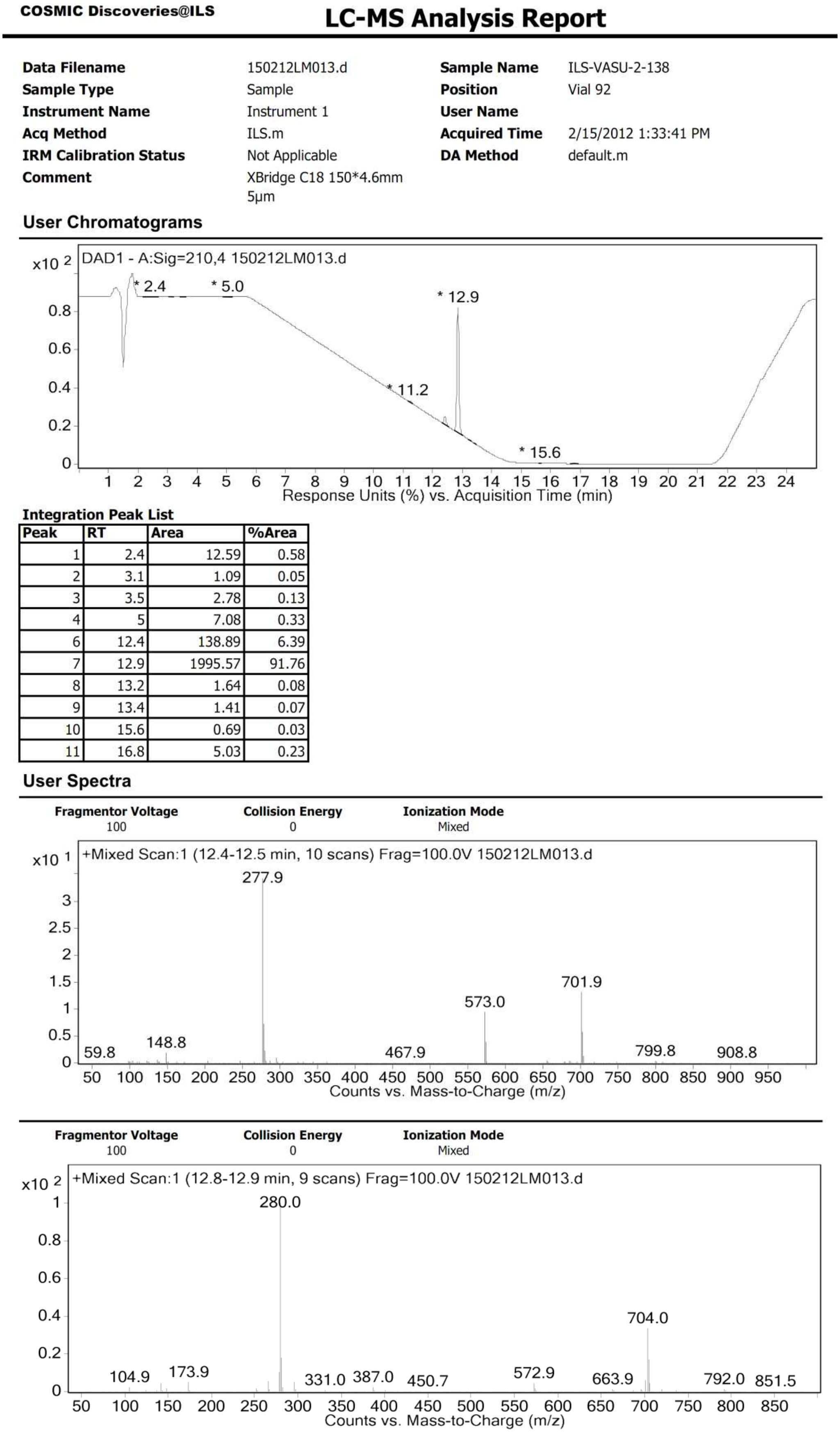

Bz
N
O
O
O
N
O
Br
N
O
4.05
3.94
2.95
1.08
3.38
1.00
0.83
0.72
0.76
1.99
4.29
1.25
2.41
4.99
10.01
3.08
2.80
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


176.818
171.574
168.687
137.751
137.337
129.094
128.612
128.510
127.866
126.988
126.730
126.660
101.820
66.935
50.531
41.643
39.094
33.754
29.992
29.787
29.692
29.655
29.622
29.360
25.929
25.902
25.796
25.691
25.593
24.851
24.371
23.143
22.691
22.114
20.360
14.131
13.806
150
100
50
0
ppm (t1)

```
                         HPLC ANALYSIS REPORT
                                                Seq Line   :          0
Injection Date  : Tue, 14. Feb. 2012            Location   :    Vial 11
Sample Name     : ILS-VASU-2-138                Inj. No.   :          0
Acq Operator    : RADHA                         Inj. Vol.  :      15 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:0/50,3/50,12/98,19/98,22/50,25/50
                  Flow :1.0 ml/min Diluent:MeOH Column Temp:23°C
```



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

Signal  1: DAD1 A, Sig=210,4 Ref=360,100

|Peak|    RT    | Width |   Area    | Area % |      Name      |
| #  |  [min]   | [min] |           |        |                |
|----|----------|-------|-----------|--------|----------------|
|   1|     1.966|  0.062|     91.981|   3.088|                |
|   2|    10.787|  0.089|      5.187|   0.174|                |
|   3|    11.223|  0.098|     17.754|   0.596|                |
|   4|    11.900|  0.090|      6.714|   0.225|                |
|   5|    12.127|  0.086|      4.549|   0.153|                |
|   6|    12.381|  0.088|    189.046|   6.348|                |
|   7|    12.819|  0.089|   2654.057|  89.114|                |
|   8|    13.245|  0.081|      4.270|   0.143|                |
|   9|    13.419|  0.083|      4.698|   0.158|                |
-------------------------------------------------------------
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	150212LM013.d	**Sample Name**	ILS-VASU-2-138
Sample Type	Sample	**Position**	Vial 92
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	2/15/2012 1:33:41 PM
IRM Calibration Status	Not Applicable	**DA Method**	default.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms

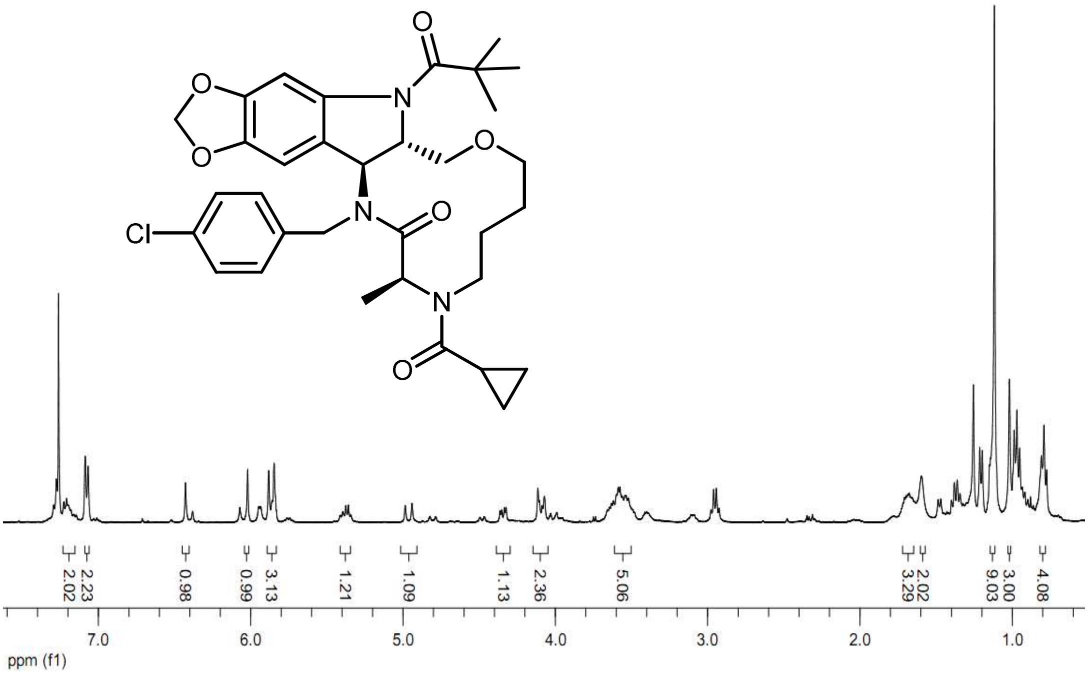
Integration Peak List

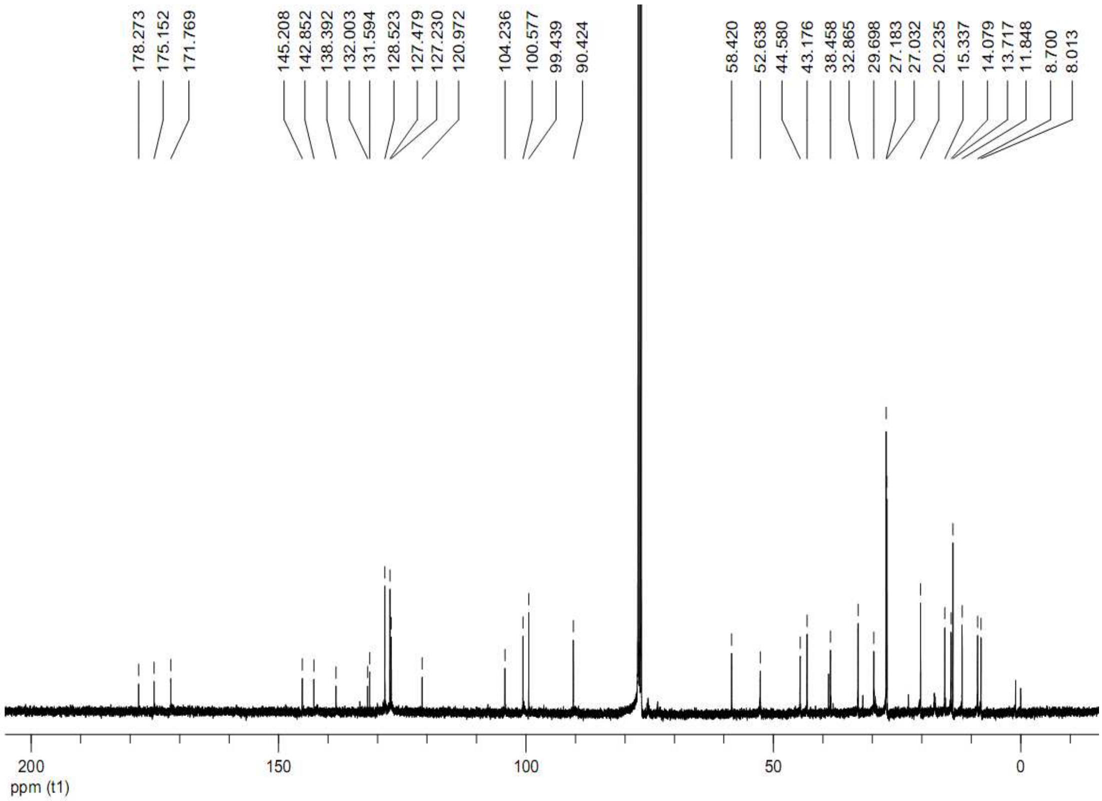
Peak	RT	Area	%Area
1	2.4	12.59	0.58
2	3.1	1.09	0.05
3	3.5	2.78	0.13
4	5	7.08	0.33
6	12.4	138.89	6.39
7	12.9	1995.57	91.76
8	13.2	1.64	0.08
9	13.4	1.41	0.07
10	15.6	0.69	0.03
11	16.8	5.03	0.23

User Spectra

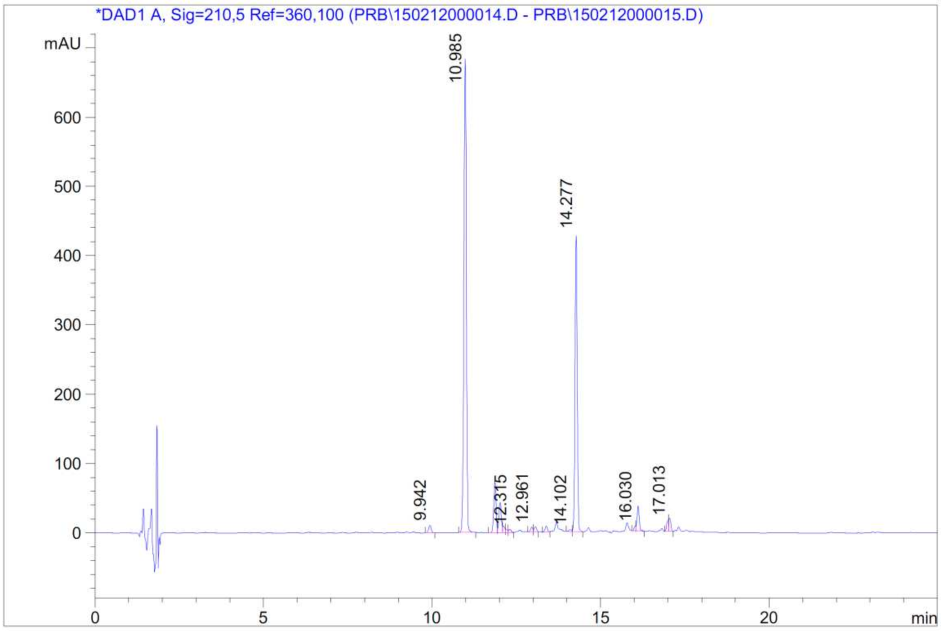
Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed


O
O
O
N
O
O
Cl
N
O
N
O
2.02
2.23
0.98
0.99
3.13
1.21
1.09
1.13
2.36
5.06
3.29
2.02
9.03
3.00
4.08
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


178.273
175.152
171.769
145.208
142.852
138.392
132.003
131.594
128.523
127.479
127.230
120.972
104.236
100.577
99.439
90.424
58.420
52.638
44.580
43.176
38.458
32.865
29.698
27.183
27.032
20.235
15.337
14.079
13.717
11.848
8.700
8.013
200
150
100
50
0
ppm (t1)

```
                            HPLC ANALYSIS REPORT
                                                Seq Line   :           0
Injection Date  : Wed, 15. Feb. 2012            Location   :      Vial 4
Sample Name     : ILS-VASU-2-137                Inj. No.   :           0
Acq Operator    : RADHA                         Inj. Vol.  :       15 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%:0/20,2/20,15/95,20/95,22/20,25/20
                  Flow : 1.0 ml/min Diluent :MeOH
```



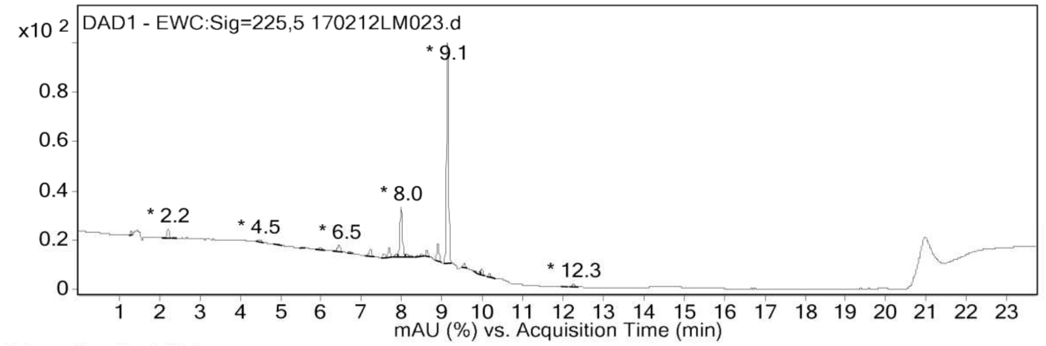
```
=========================================================================

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

|Peak|    RT     | Width |    Area    | Area % |      Name      |
| #  |   [min]   | [min] |            |        |                |
|----|-----------|-------|------------|--------|----------------|
|   1|     9.942|  0.089|     57.003|   0.875|                |
|   2|    10.985|  0.083|   3413.326|  52.387|                |
|   3|    11.869|  0.080|    356.224|   5.467|                |
|   4|    12.021|  0.080|    211.251|   3.242|                |
|   5|    12.124|  0.073|     42.198|   0.648|                |
|   6|    12.181|  0.053|     19.638|   0.301|                |
|   7|    12.315|  0.085|     23.690|   0.364|                |
|   8|    12.961|  0.073|     28.863|   0.443|                |
|   9|    13.070|  0.079|     36.842|   0.565|                |
|  10|    13.390|  0.083|     43.187|   0.663|                |
|  11|    14.102|  0.091|     15.696|   0.241|                |
|  12|    14.277|  0.075|   1944.455|  29.843|                |
```

COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	170212LM023.d	**Sample Name**	ILS-Vasu-2-137
Sample Type	Sample	**Position**	Vial 91
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS.m	**Acquired Time**	2/17/2012 3:06:52 PM
IRM Calibration Status	Not Applicable	**DA Method**	default.m
Comment	X-Bridge C18, 150*4.6mm,5um.		

User Chromatograms

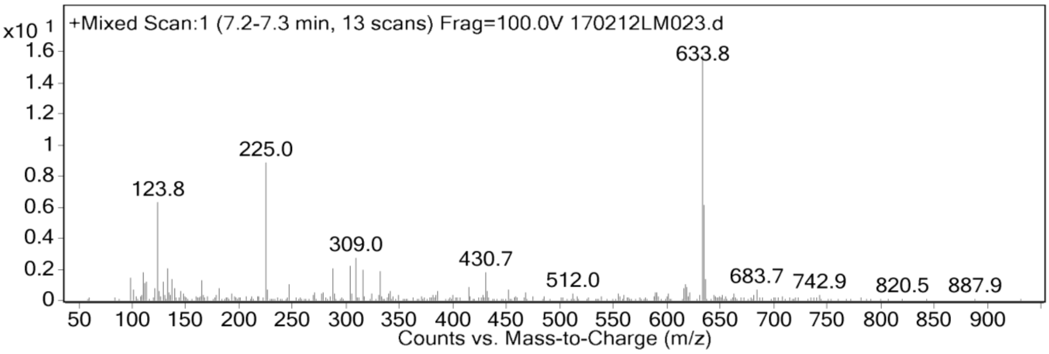


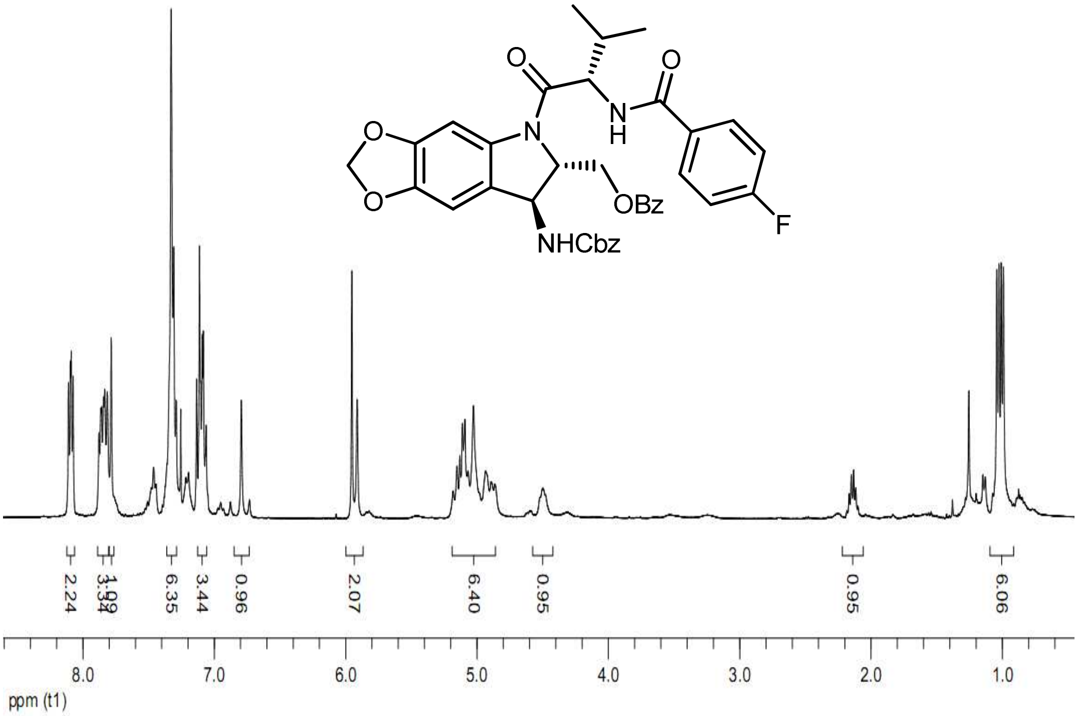
Integration Peak List

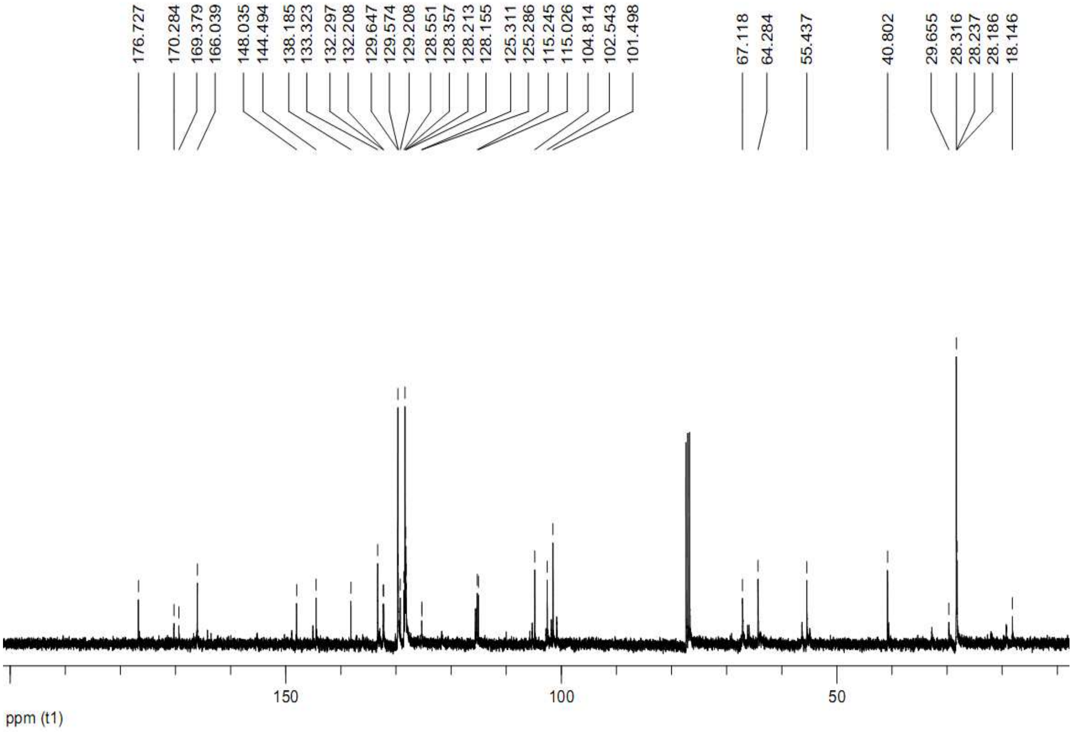
Peak	RT	Area	%Area
3	2.2	7.539	2.52
9	6.5	7.489	2.5
11	7.2	6.495	2.17
13	7.7	7.683	2.56
15	8	43.869	14.64
20	8.6	4.278	1.43
21	8.9	12.621	4.21
22	9.1	169.948	56.73
23	9.6	3.703	1.24
26	10	4.399	1.47

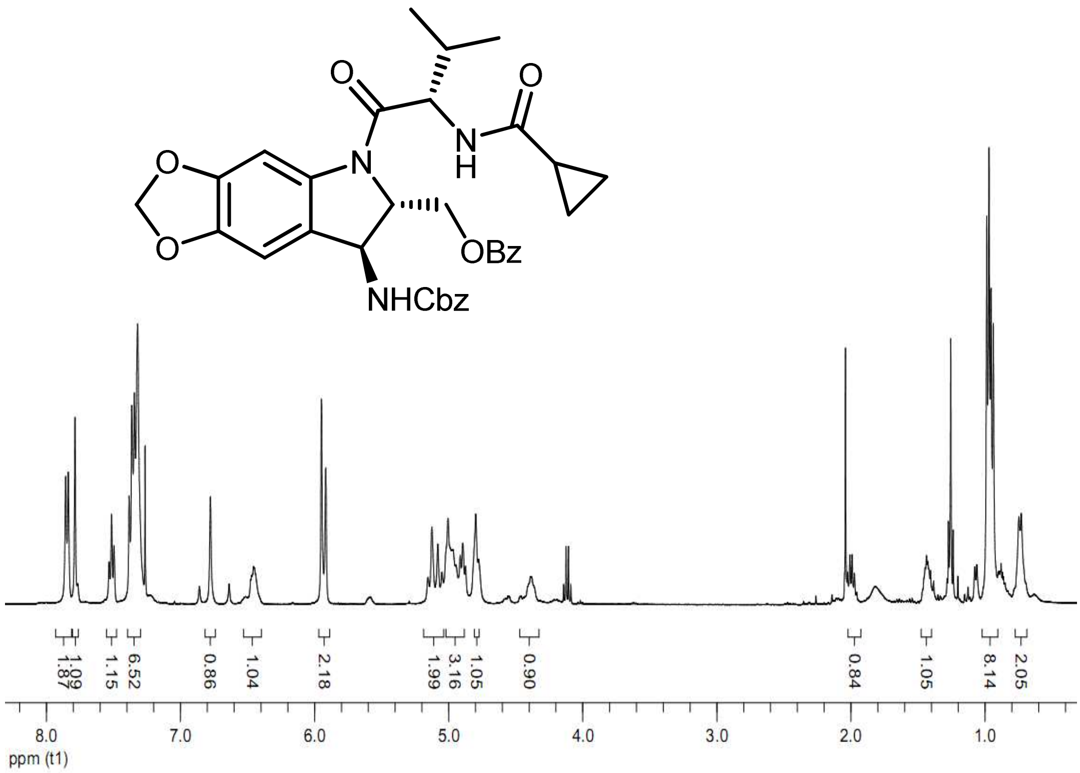
User Spectra

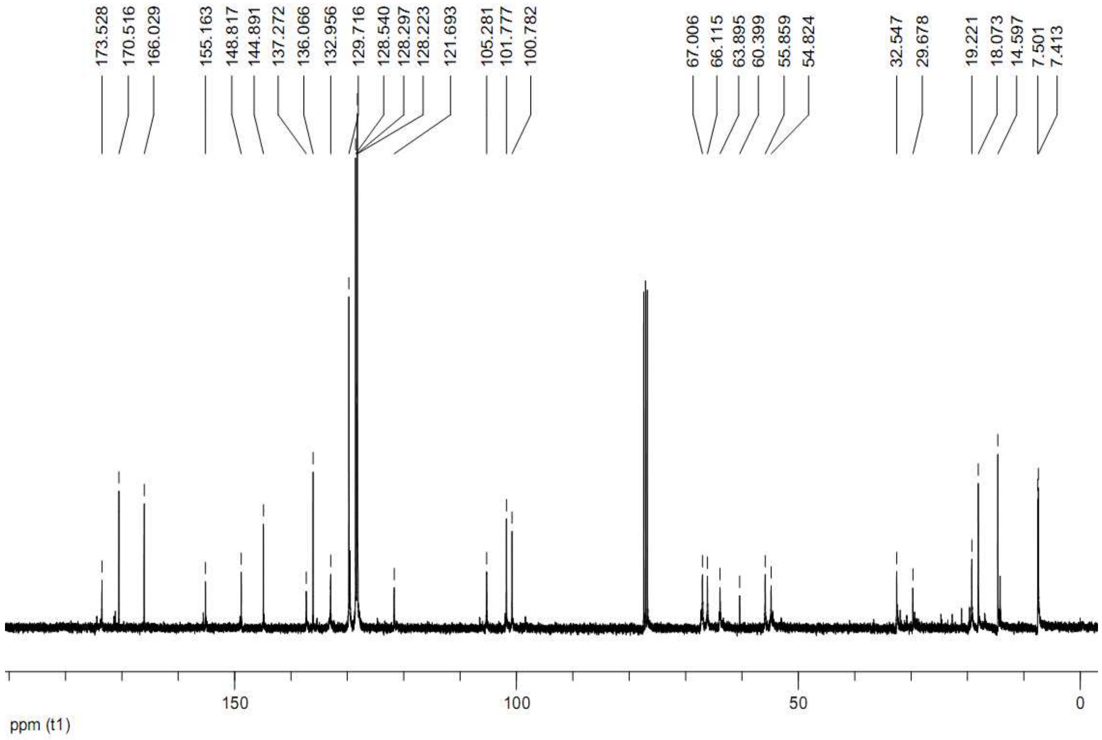
Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	Mixed

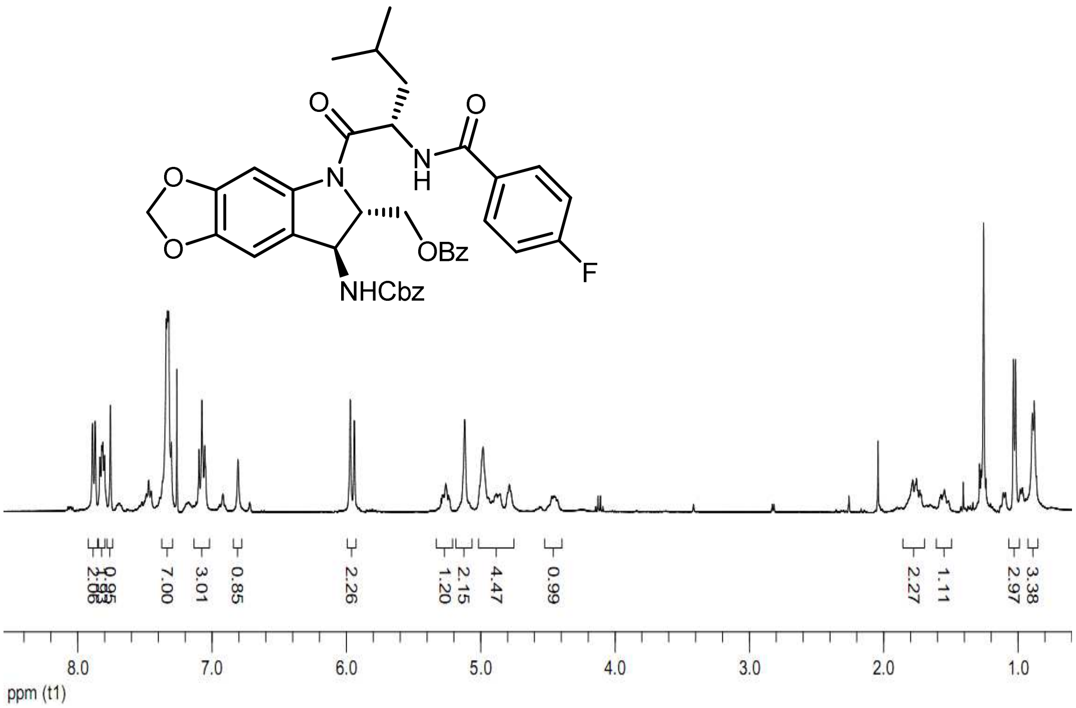


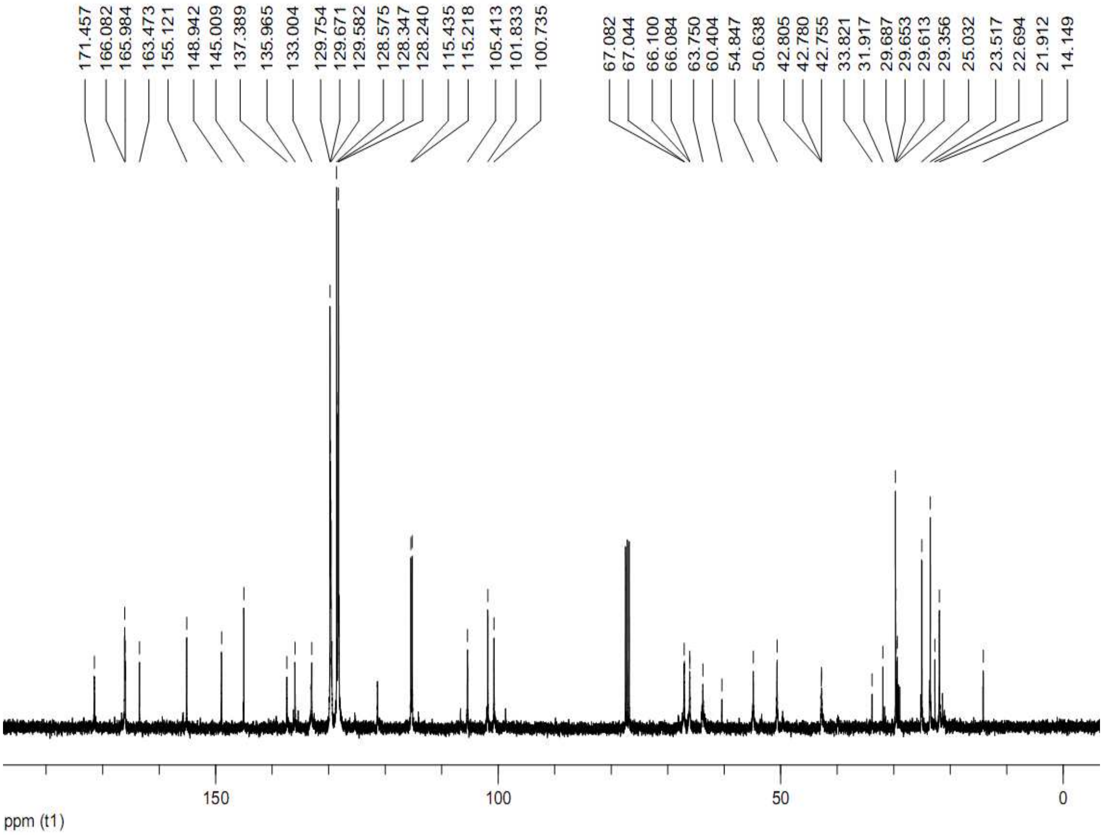

O
O
O
N
O
N
H
OBz
NHCbz
F
2.24
3.34
1.99
6.35
3.44
0.96
2.07
6.40
0.95
0.95
6.06
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

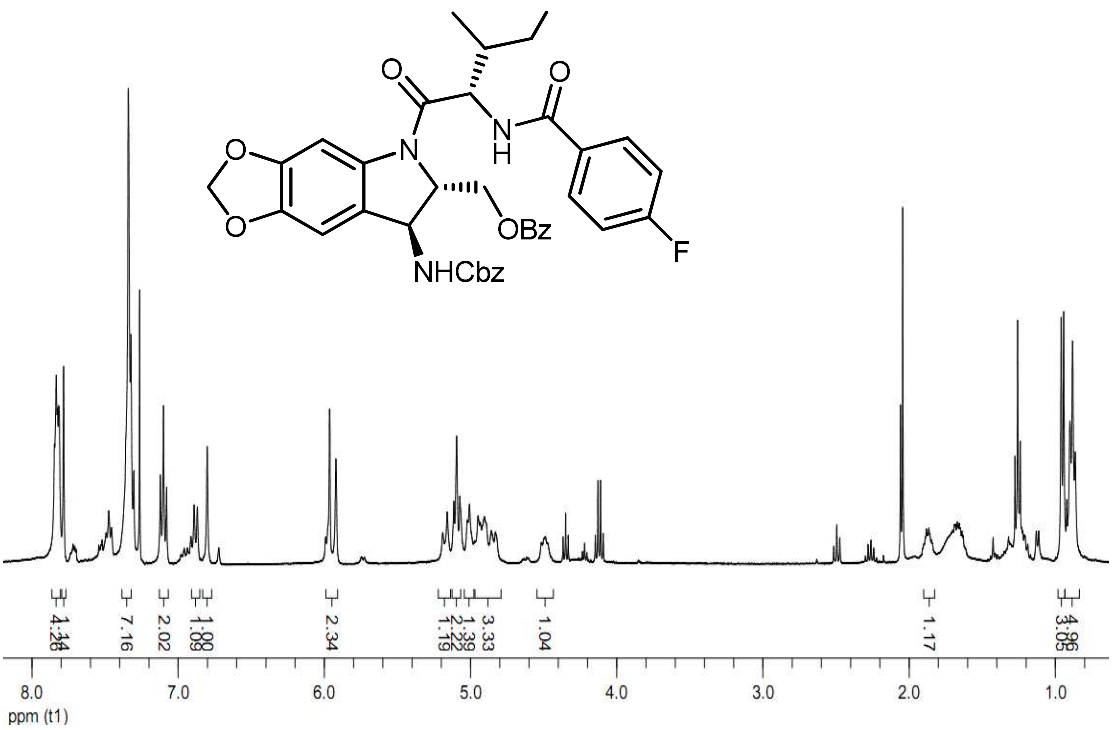

176.727
170.284
169.379
166.039
148.035
144.494
138.185
133.323
132.297
132.208
129.647
129.574
129.208
129.208
128.551
128.357
128.213
128.155
125.311
125.286
115.245
115.026
104.814
102.543
101.498
67.118
64.284
55.437
40.802
29.655
28.316
28.237
28.186
18.146
150
100
50
ppm (t1)

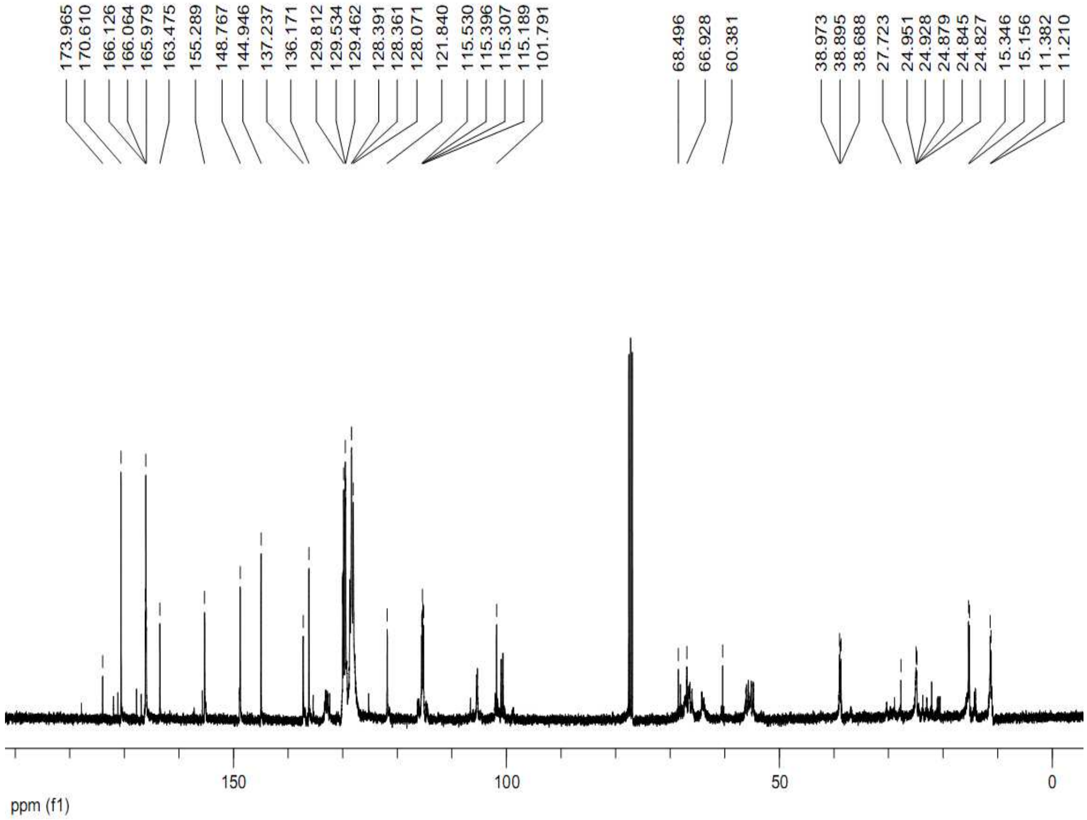

O
O
N
O
N
H
O
OBz
NHCbz
O
1.87
1.09
1.15
6.52
0.86
1.04
2.18
1.99
3.16
1.05
0.90
0.84
1.05
8.14
2.05
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

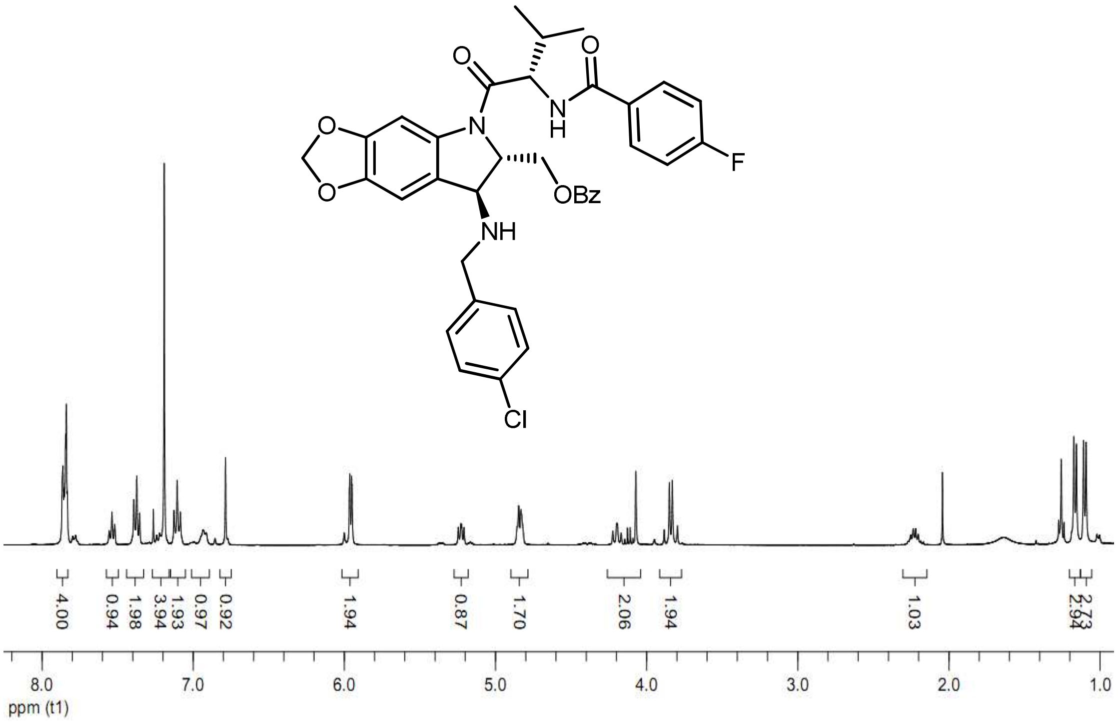

173.528
170.516
166.029
155.163
148.817
144.891
137.272
136.066
132.956
129.716
128.540
128.297
128.223
121.693
105.281
101.777
100.782
67.006
66.115
63.895
60.399
55.859
54.824
32.547
29.678
19.221
18.073
14.597
7.501
7.413
150
100
50
0
ppm (t1)


O
O
N
O
N
H
O
OBz
F
NHCbz
2.06
0.95
7.00
3.01
0.85
2.26
1.20
2.15
4.47
0.99
2.27
1.11
2.97
3.38
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

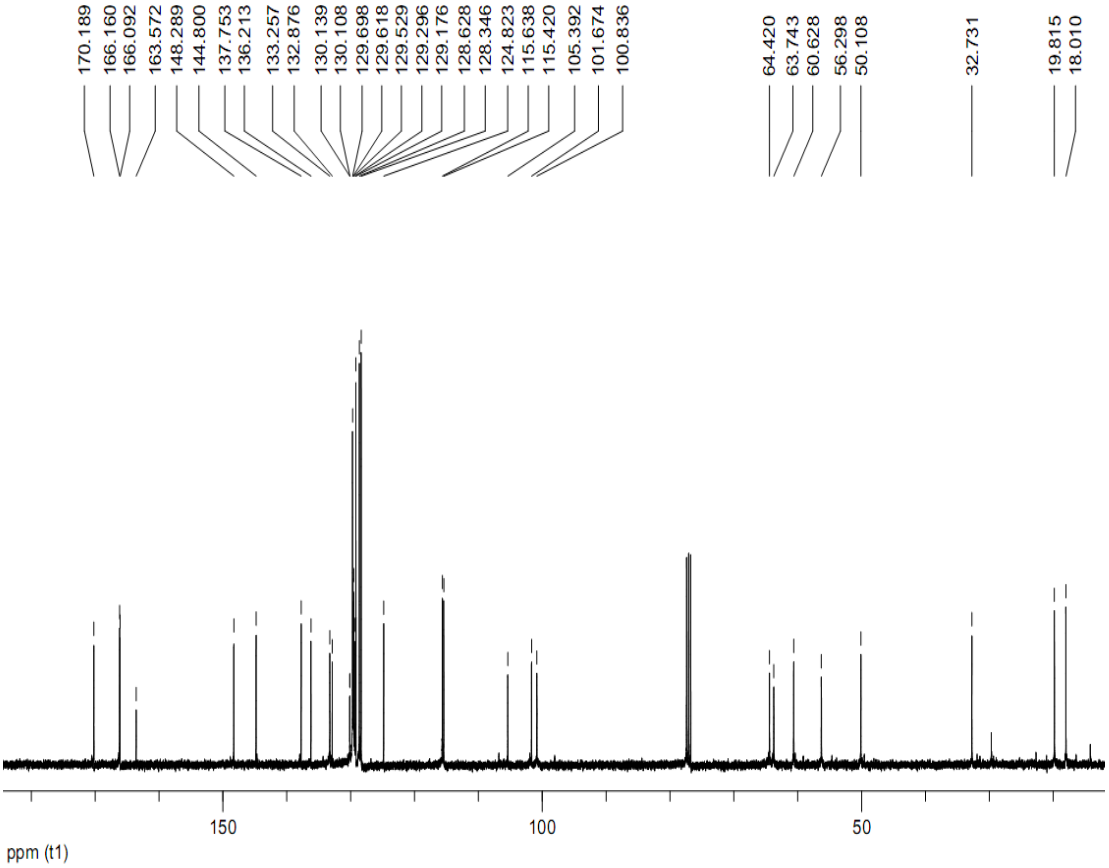

171.457
166.082
165.984
163.473
155.121
148.942
145.009
137.389
135.965
133.004
129.754
129.671
129.582
128.575
128.347
128.240
115.435
115.218
105.413
101.833
100.735
67.082
67.044
66.100
66.084
63.750
60.404
54.847
50.638
42.805
42.780
42.755
33.821
31.917
29.687
29.653
29.613
29.356
25.032
23.517
22.694
21.912
14.149
150
100
50
0
ppm (t1)

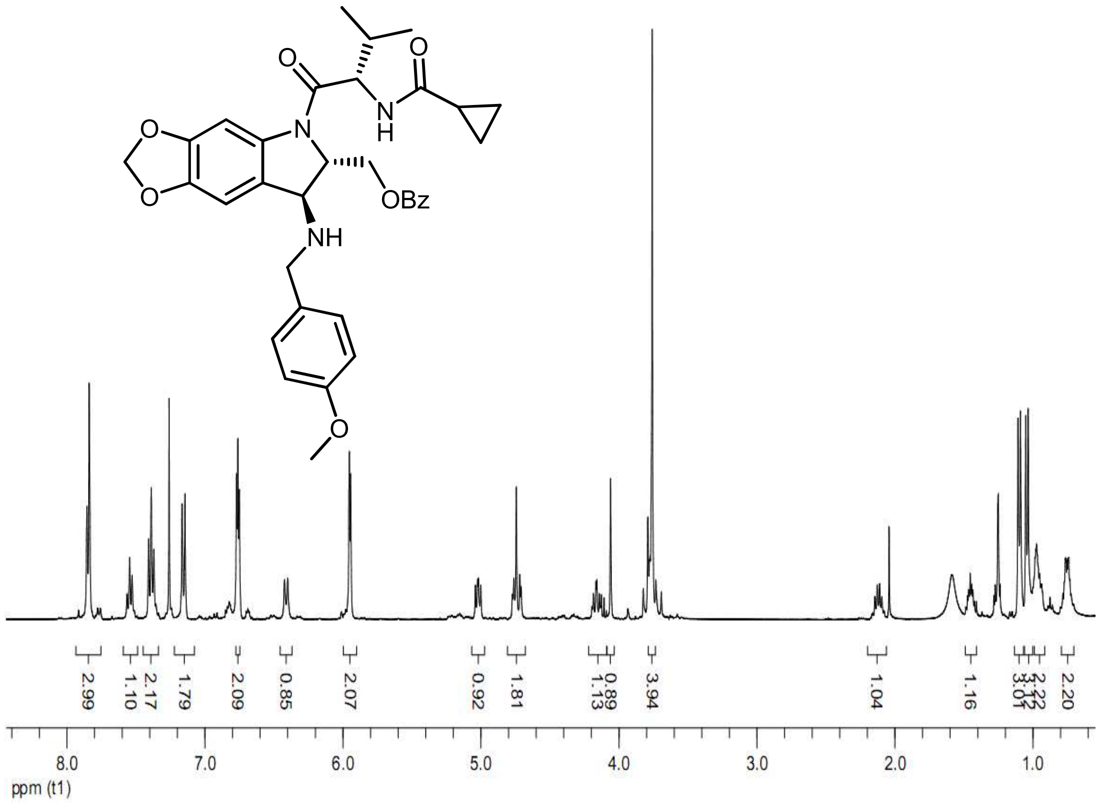

O
O
O
N
O
N
H
OBz
NHCbz
F
4.28
1.14
7.16
2.02
1.09
1.00
2.34
1.19
2.22
1.39
3.33
1.04
1.17
3.05
4.96
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

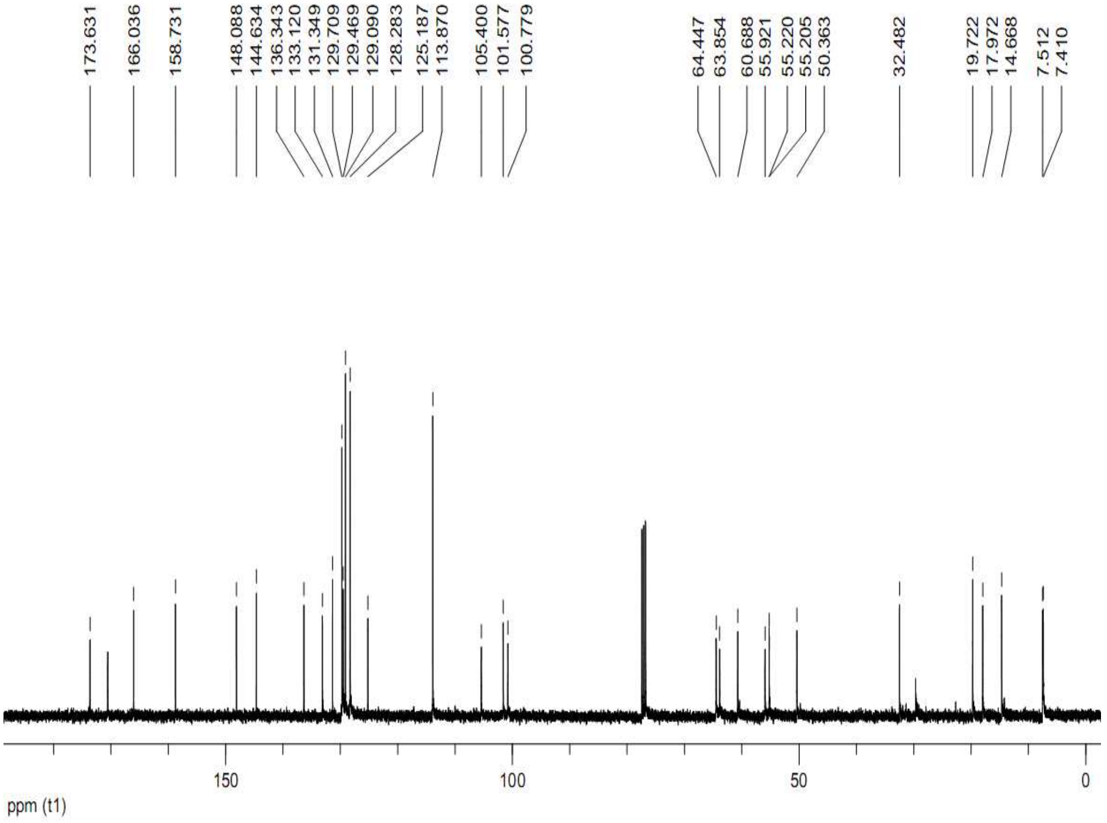

173.965
170.610
166.126
166.064
165.979
163.475
155.289
148.767
144.946
137.237
136.171
129.812
129.534
129.462
128.391
128.361
128.071
121.840
115.530
115.396
115.307
115.189
101.791
68.496
66.928
60.381
38.973
38.895
38.688
27.723
24.951
24.928
24.879
24.845
24.827
15.346
15.156
11.382
11.210
150
100
50
0
ppm (f1)

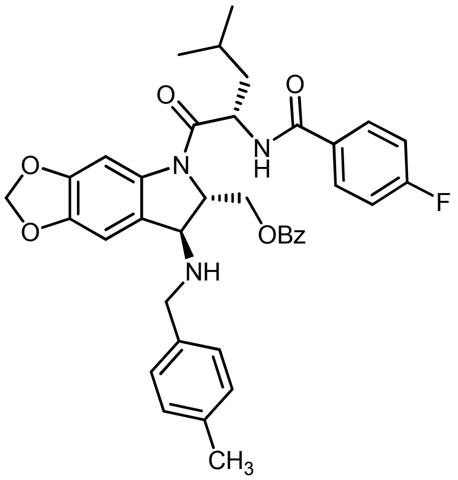

O
O
O
N
O
N
H
F
OBz
NH
Cl

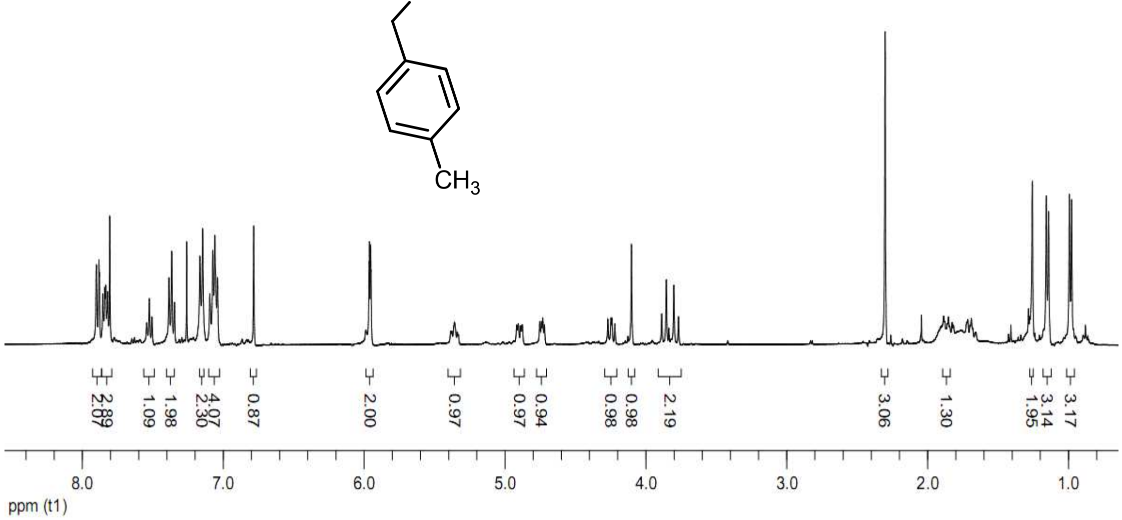
4.00
0.94
1.98
3.94
1.93
0.97
0.92
1.94
0.87
1.70
2.06
1.94
1.03
2.94
2.73
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

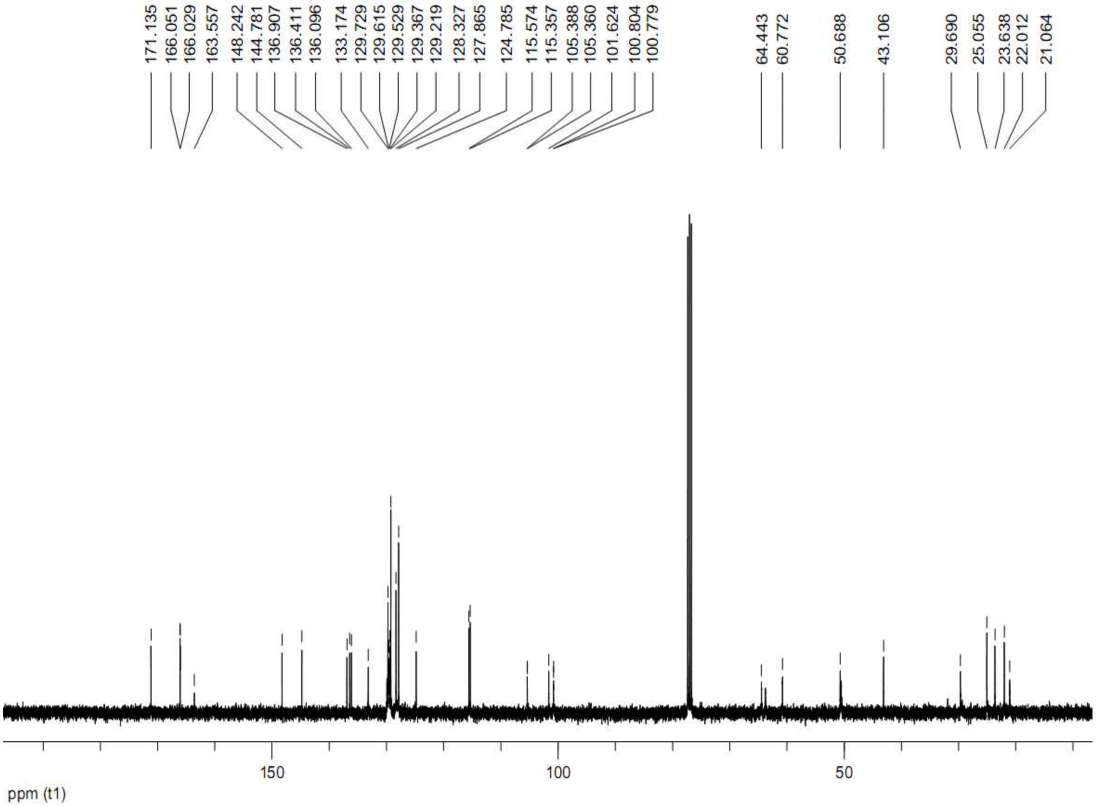

170.189
166.160
166.092
163.572
148.289
144.800
137.753
136.213
133.257
132.876
130.139
130.108
129.698
129.618
129.529
129.296
129.176
128.628
128.346
124.823
115.638
115.420
105.392
101.674
100.836
64.420
63.743
60.628
56.298
50.108
32.731
19.815
18.010
150
100
50
ppm (t1)

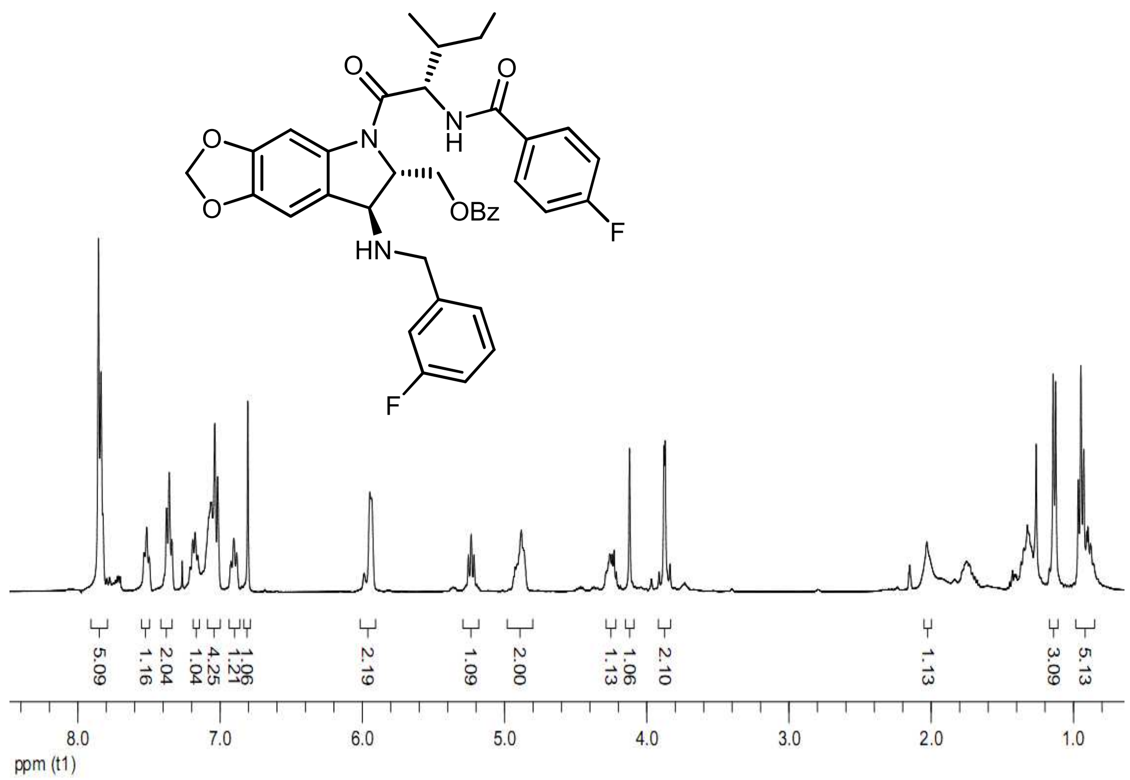

O
O
N
O
N
H
O
O
OBz
NH
O
2.99
1.10
2.17
1.79
2.09
0.85
2.07
0.92
1.81
1.13
0.89
3.94
1.04
1.16
3.01
3.12
2.22
2.20
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

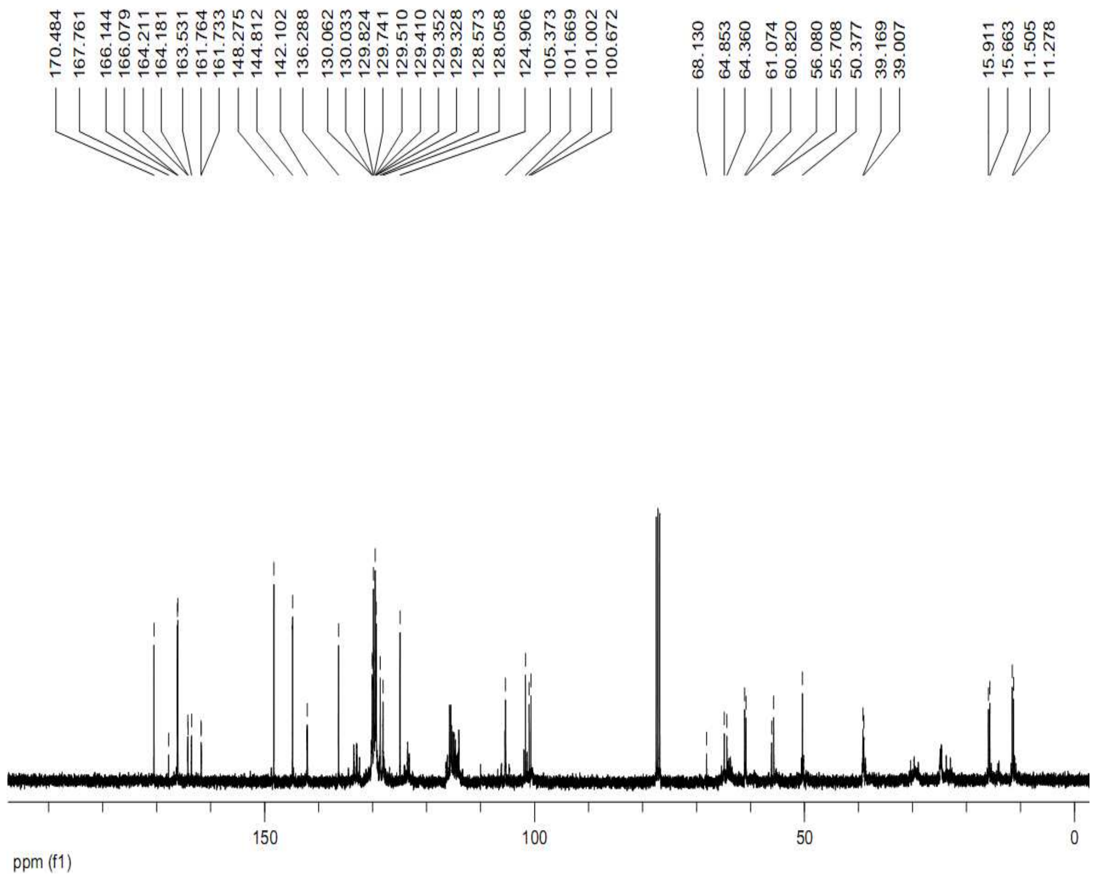

173.631
166.036
158.731
148.088
144.634
136.343
133.120
131.349
129.709
129.469
129.090
128.283
125.187
113.870
105.400
101.577
100.779
64.447
63.854
60.688
55.921
55.220
55.205
50.363
32.482
19.722
17.972
14.668
7.512
7.410
150
100
50
0
ppm (t1)

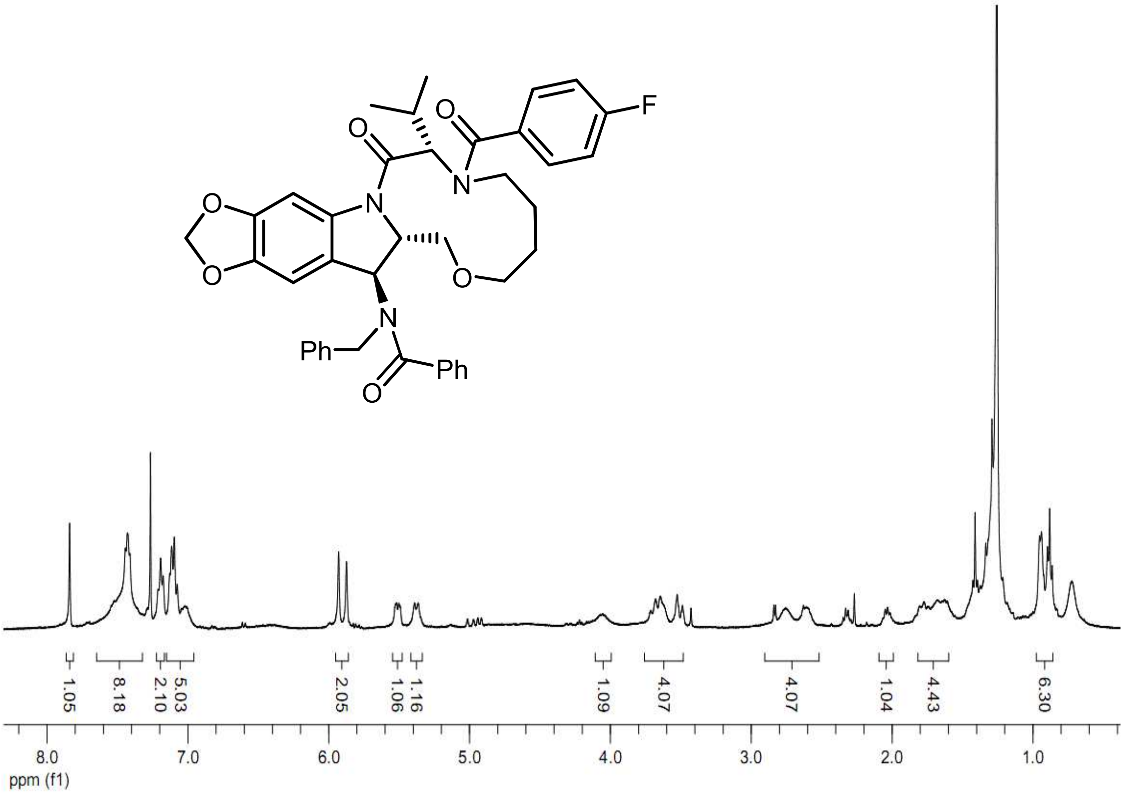

O
O
N
N
H
F
O
O
OBz
NH
CH3

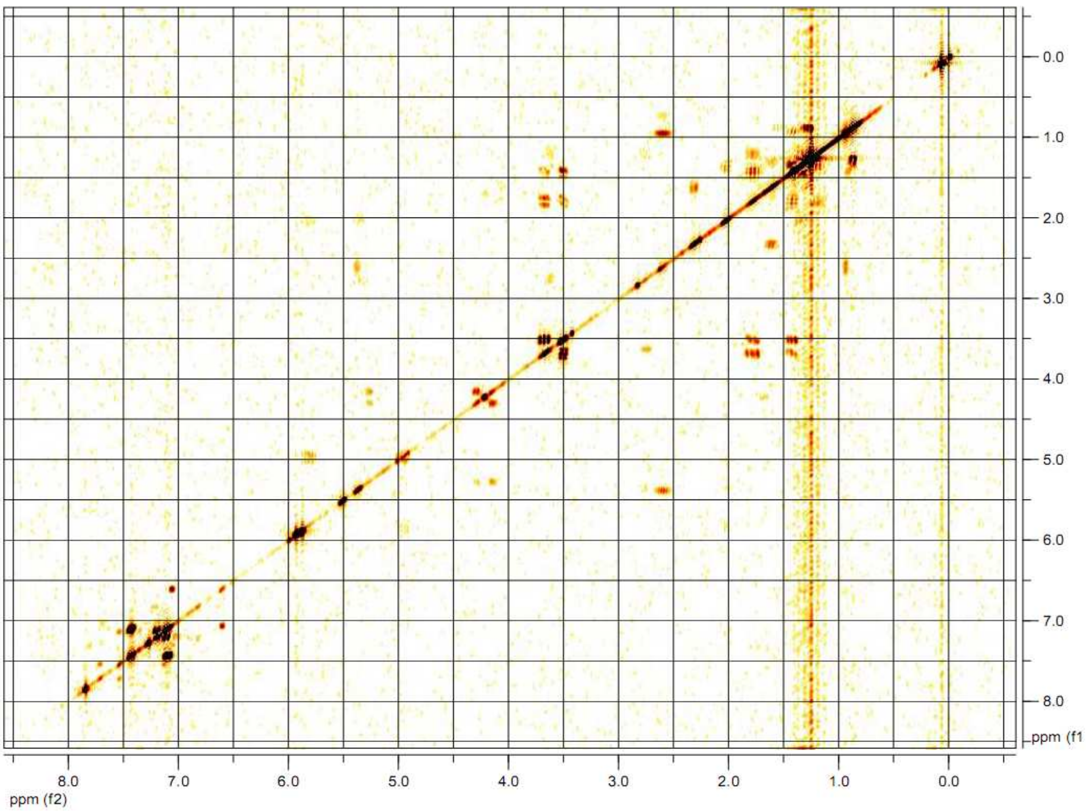

2.07
2.89
1.09
1.98
2.30
4.07
0.87
2.00
0.97
0.97
0.94
0.98
0.98
2.19
3.06
1.30
1.95
3.14
3.17
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

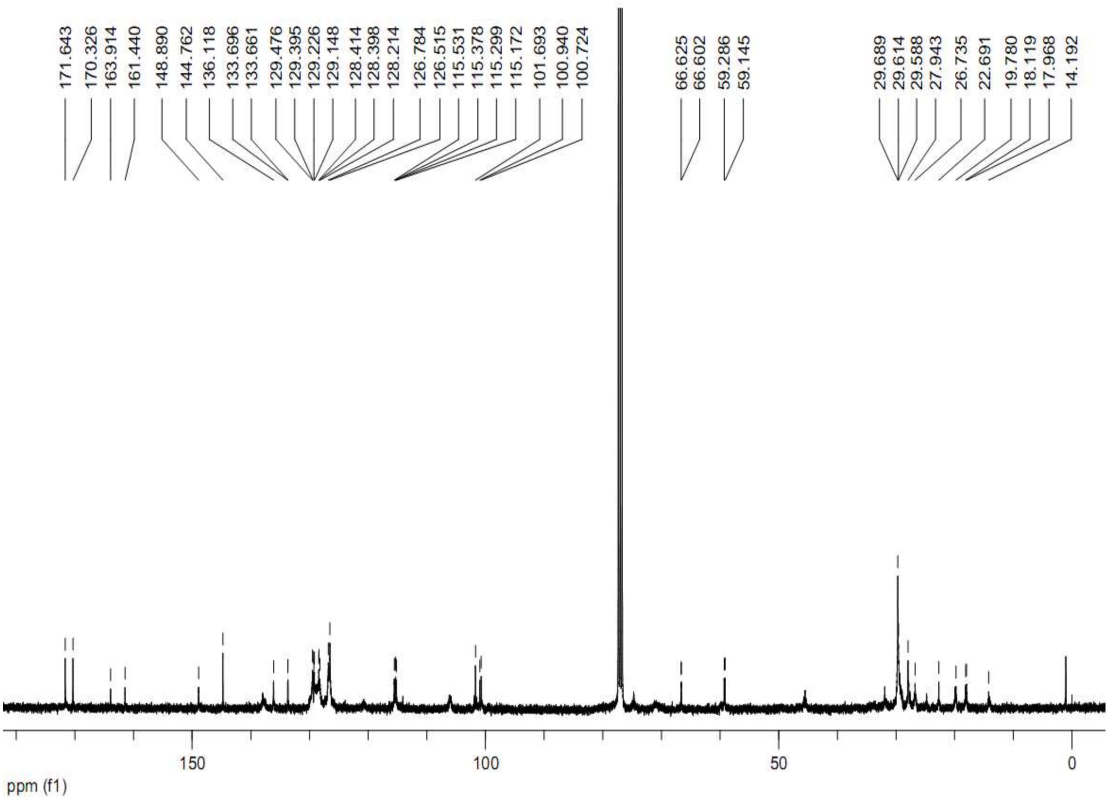

171.135
166.051
166.029
163.557
148.242
144.781
136.907
136.411
136.096
133.174
129.729
129.615
129.529
129.367
129.219
128.327
127.865
124.785
115.574
115.357
105.388
105.360
101.624
100.804
100.779
64.443
60.772
50.688
43.106
29.690
25.055
23.638
22.012
21.064
150
100
50
ppm (t1)


O
O
N
O
O
N
H
OBz
F
HN
F
5.09
1.16
2.04
1.04
4.25
1.21
1.06
2.19
1.09
2.00
1.13
1.06
2.10
1.13
3.09
5.13
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

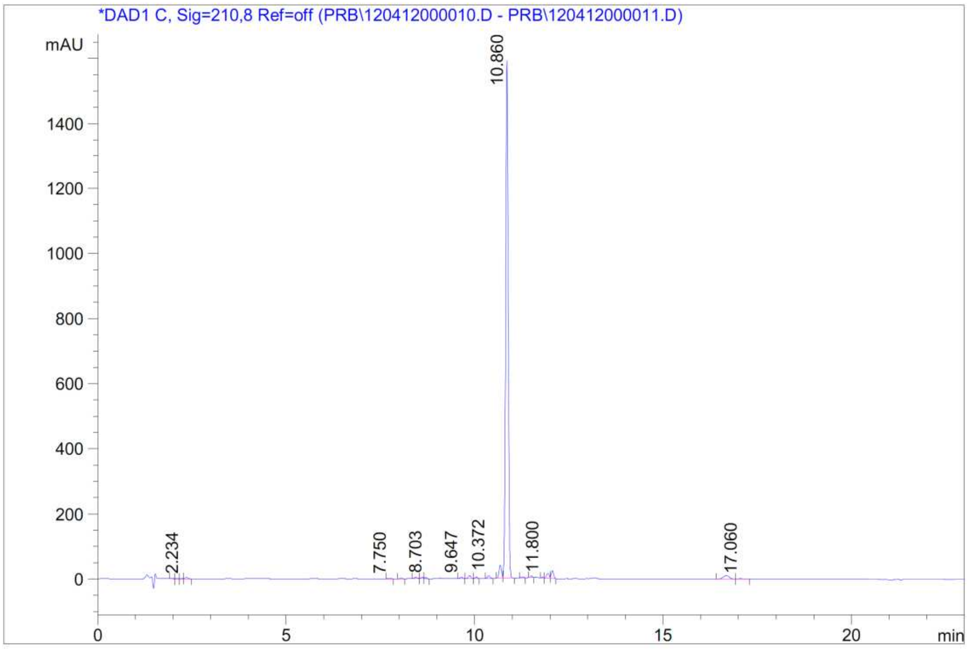

170.484
167.761
166.144
166.079
164.211
164.181
163.531
161.764
161.733
148.275
144.812
142.102
136.288
130.062
130.033
129.824
129.741
129.510
129.410
129.352
129.328
128.573
128.058
124.906
105.373
101.669
101.002
100.672
68.130
64.853
64.360
61.074
60.820
56.080
55.708
50.377
39.169
39.007
15.911
15.663
11.505
11.278
150
100
50
0
ppm (f1)


O
O
F
O
N
N
O
O
N
Ph
Ph
O
1.05
8.18
2.10
5.03
2.05
1.06
1.16
1.09
4.07
4.07
1.04
4.43
6.30
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

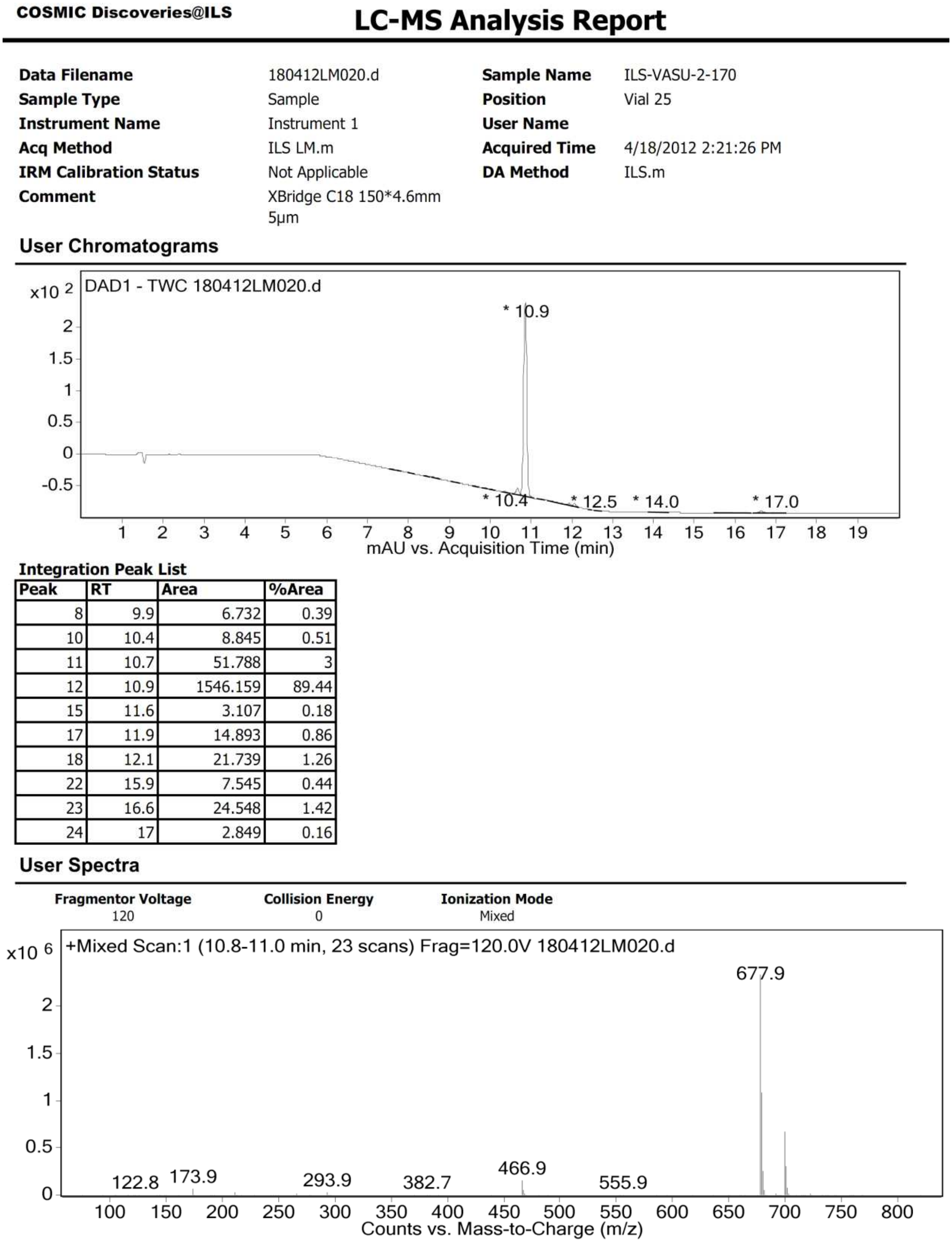

0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
ppm (f1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f2)


171.643
170.326
163.914
161.440
148.890
144.762
136.118
133.696
133.661
129.476
129.395
129.226
129.148
128.414
128.398
128.214
126.784
126.515
115.531
115.378
115.299
115.172
101.693
100.940
100.724
66.625
66.602
59.286
59.145
29.689
29.614
29.588
27.943
26.735
22.691
19.780
18.119
17.968
14.192
150
100
50
0
ppm (f1)

```
                         COSMIC DISCOVERIES @ ILS
                            HPLC ANALYSIS REPORT
                                                 Seq Line    :          0
Injection Date  : Thu, 12. Apr. 2012             Location    :    Vial 93
Sample Name     : ILS-VASU-2-170                 Inj. No.    :          0
Acq Operator    : VARMA                          Inj. Vol.   :      15 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 C-18 150*4.6mm 5µ
                  Mobile phase: A) 0.1% HCOOH in water,B) ACN
                  (GRADIENT)   T/B%:0/50,3/50,10/95,18/95,20/50,23/50
                  Flow:1.0 ml/min  Diluent: ACN:WATER(80:20)
```



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

Signal  1: DAD1 C, Sig=210,8 Ref=off

|Peak|   RT    | Width |   Area    | Area % |      Name      |
| #  |  [min]  | [min] |           |        |                |
|----|---------|-------|-----------|--------|----------------|
|   1|    1.975|  0.061|      3.955|   0.050|                |
|   2|    2.110|  0.059|      4.776|   0.060|                |
|   3|    2.234|  0.071|      2.982|   0.037|                |
|   4|    2.355|  0.076|     21.694|   0.272|                |
|   5|    7.750|  0.088|      8.186|   0.103|                |
|   6|    8.049|  0.081|     11.312|   0.142|                |
|   7|    8.440|  0.103|     20.114|   0.252|                |
|   8|    8.613|  0.087|     21.673|   0.271|                |
|   9|    8.703|  0.072|     15.599|   0.195|                |
|  10|    9.647|  0.075|     17.800|   0.223|                |
|  11|    9.866|  0.080|     43.883|   0.550|                |
|  12|   10.048|  0.068|     19.002|   0.238|                |


|  13|   10.372|  0.083|     44.568|   0.558|                |
|  14|   10.681|  0.073|    176.521|   2.211|                |
|  15|   10.860|  0.076|   7233.381|  90.604|                |
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	180412LM020.d	**Sample Name**	ILS-VASU-2-170
Sample Type	Sample	**Position**	Vial 25
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS LM.m	**Acquired Time**	4/18/2012 2:21:26 PM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

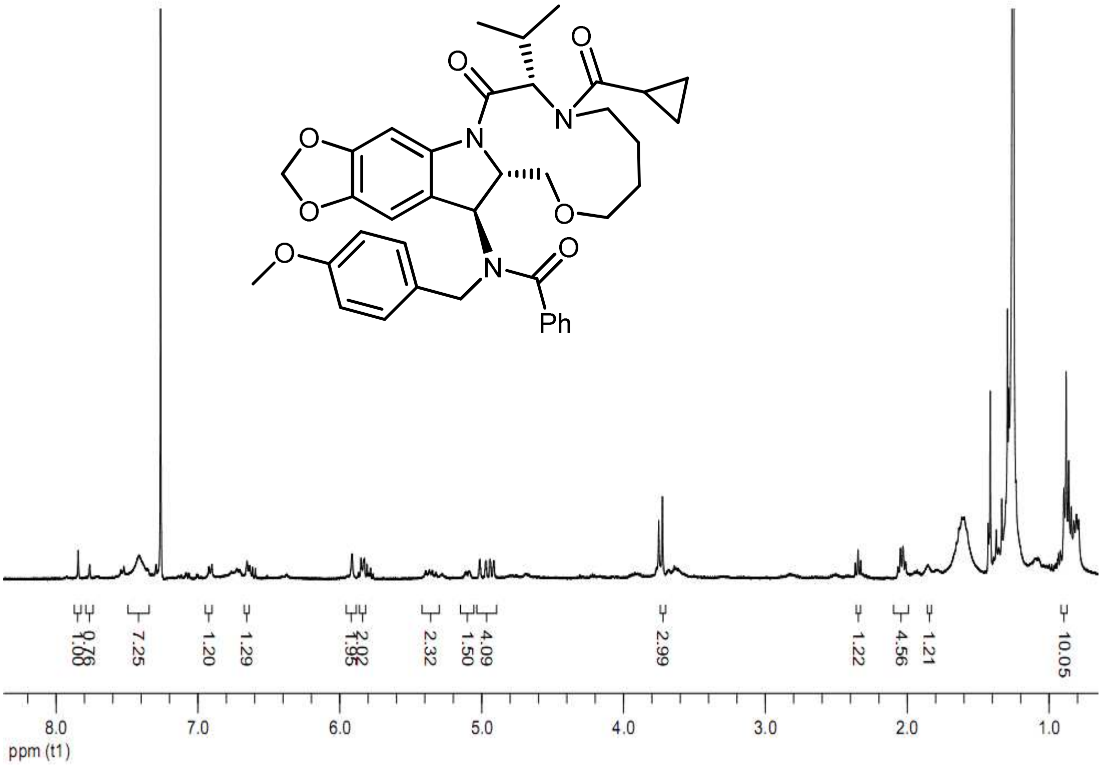
User Chromatograms

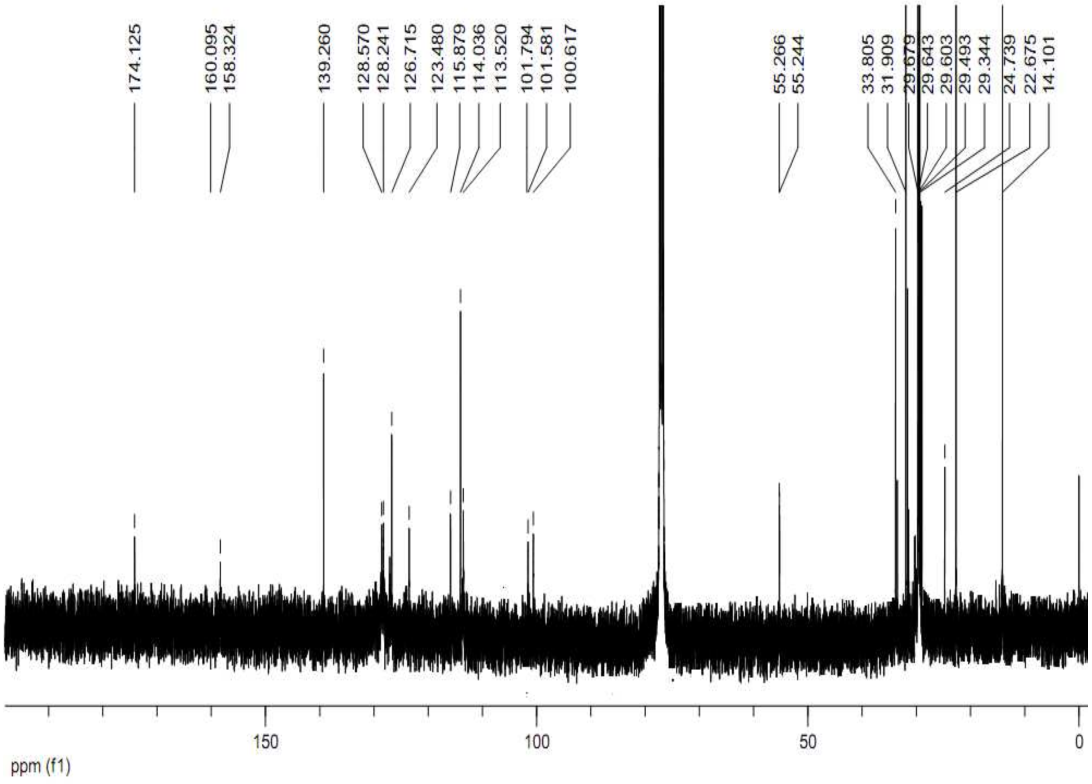
Integration Peak List

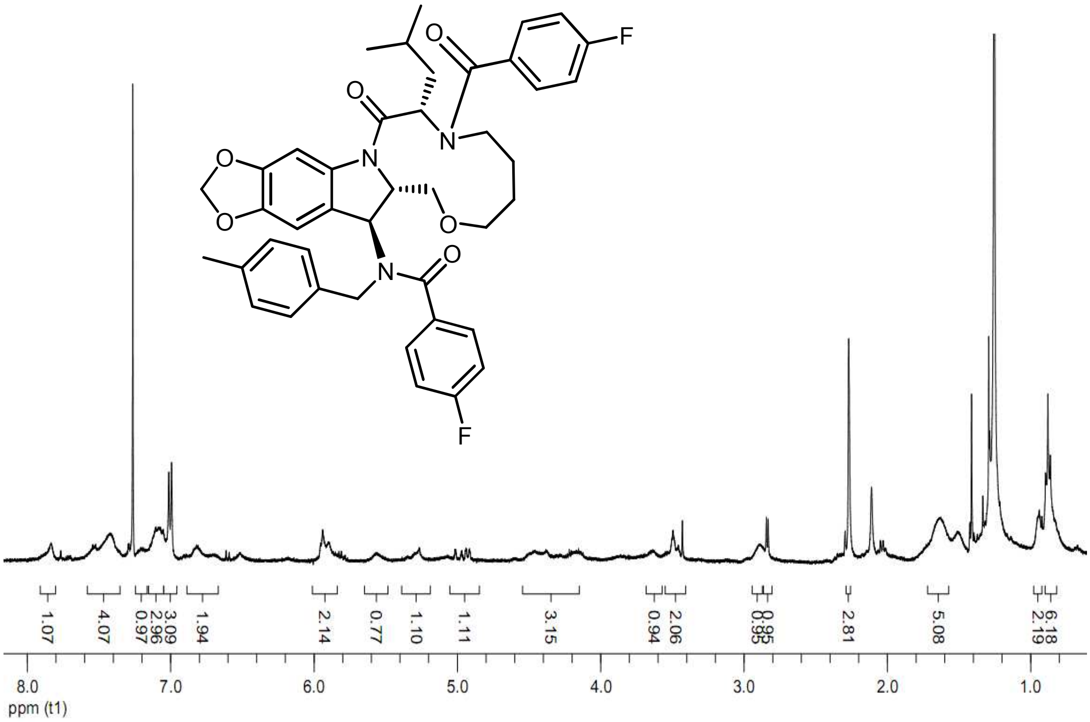
Peak	RT	Area	%Area
8	9.9	6.732	0.39
10	10.4	8.845	0.51
11	10.7	51.788	3
12	10.9	1546.159	89.44
15	11.6	3.107	0.18
17	11.9	14.893	0.86
18	12.1	21.739	1.26
22	15.9	7.545	0.44
23	16.6	24.548	1.42
24	17	2.849	0.16

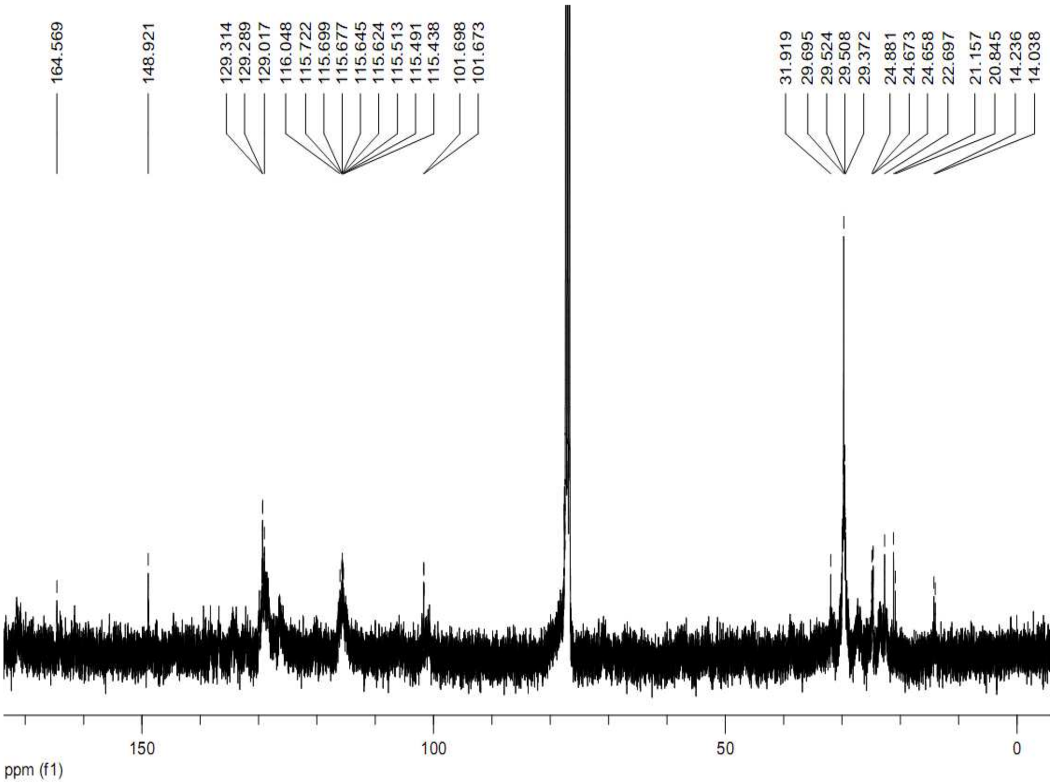
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

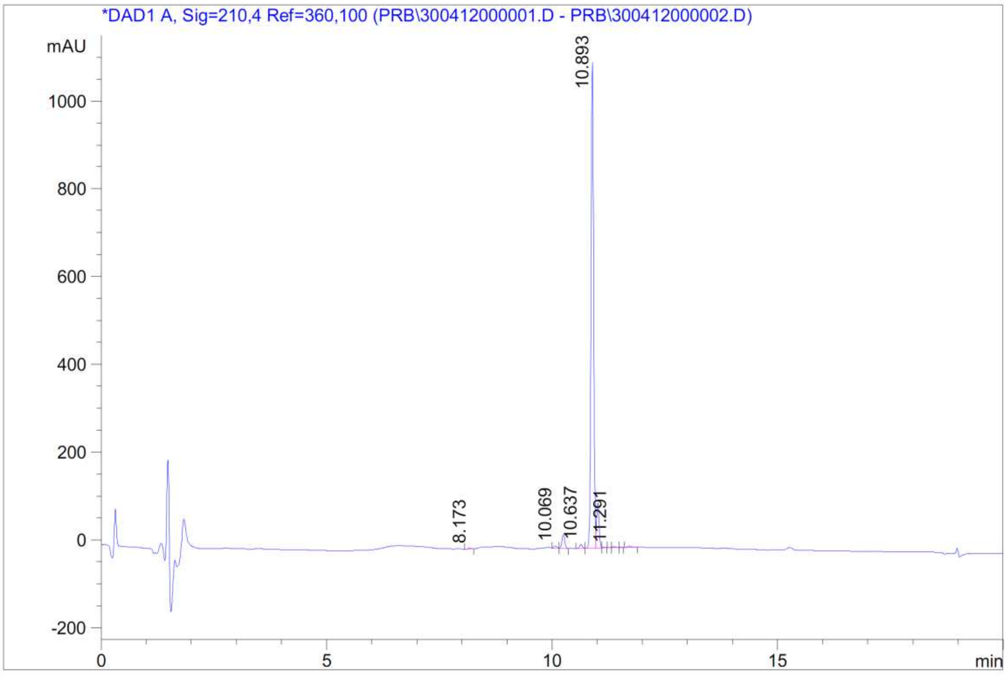

O
O
N
N
O
O
O
O
N
O
Ph
1.00
0.76
7.25
1.20
1.29
1.95
2.02
2.32
1.50
4.09
2.99
1.22
4.56
1.21
10.05
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


174.125
160.095
158.324
139.260
128.570
128.241
126.715
123.480
115.879
114.036
113.520
101.794
101.581
100.617
55.266
55.244
33.805
31.909
29.679
29.643
29.603
29.493
29.344
24.739
22.675
14.101
150
100
50
0
ppm (f1)

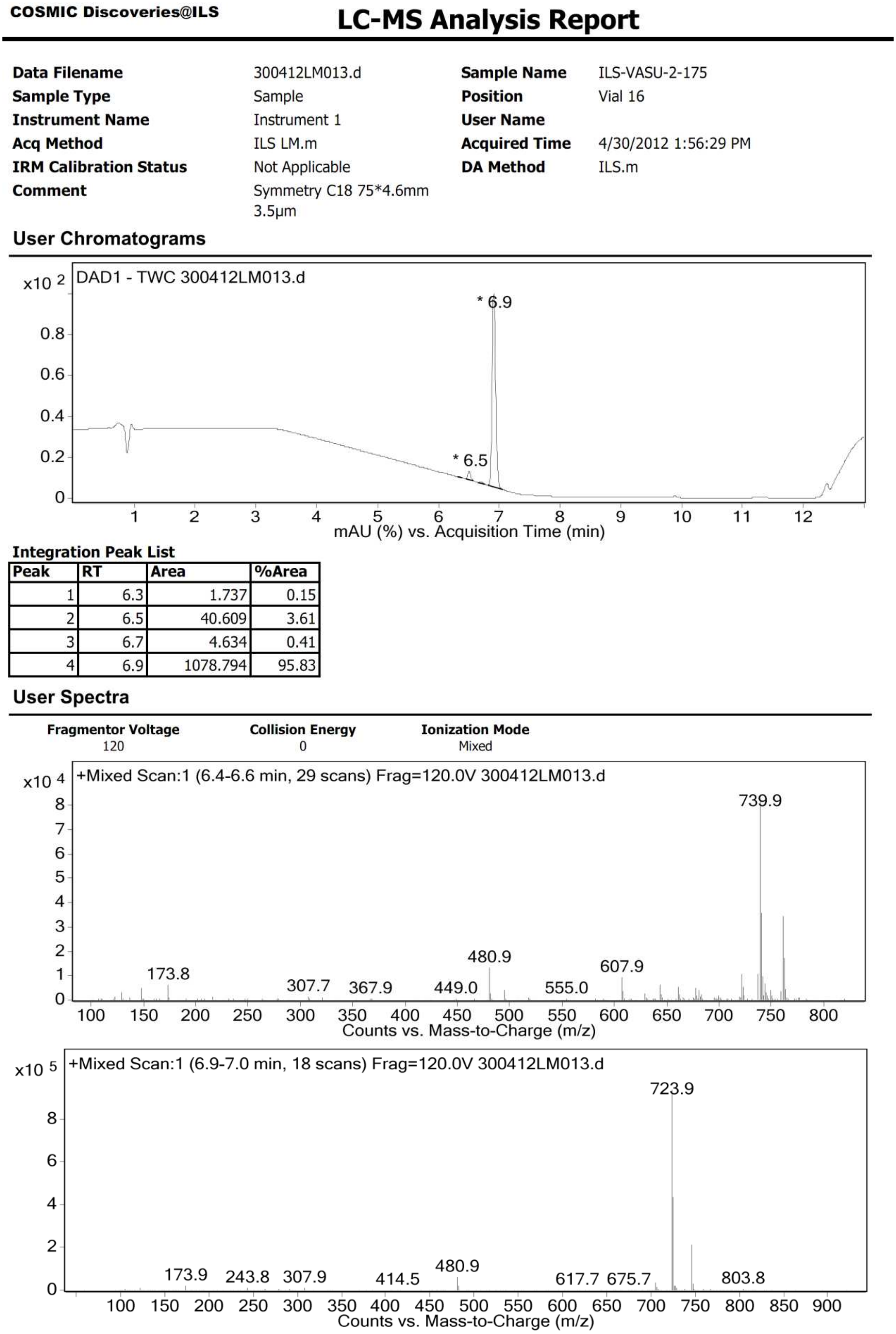

O
F
O
N
N
O
O
O
N
O
F
1.07
4.07
0.97
2.96
3.09
1.94
2.14
0.77
1.10
1.11
3.15
0.94
2.06
0.95
0.85
2.81
5.08
2.19
6.18
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


164.569
148.921
129.314
129.289
129.017
116.048
115.722
115.699
115.677
115.645
115.624
115.513
115.491
115.438
101.698
101.673
31.919
29.695
29.524
29.508
29.372
24.881
24.673
24.658
22.697
21.157
20.845
14.236
14.038
150
100
50
0
ppm (f1)

```
                         COSMIC DISCOVERIES @ ILS
                            HPLC ANALYSIS REPORT
                                                 Seq Line   :           0
Injection Date  : Mon, 30. Apr. 2012             Location   :     Vial 10
Sample Name     : ILS-Vasu-2-175                 Inj. No.   :           0
Acq Operator    : SRI KRISHNA VARMA              Inj. Vol.  :       20 µl
Acq. Method     : D:\CHEM32\1\METHODS\XB-A50B50.M
Analysis Method : D:\CHEM32\1\METHODS\XB-A50B50.M
Method Info     : Column :  XBridge C-18 150*4.6mm 5µm
                  Mobile phase: A) 0.1 % HCOOH in water , B) ACN (Gradient)
                  T/B% : 0/50,3/50,9/95,16/95,17/50,20/50.
                  Flow: 1.0 ml/min Diluent: ACN:Water (90:10)
```



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

Signal  1: DAD1 A, Sig=210,4 Ref=360,100

|Peak|    RT    | Width |    Area   | Area % |      Name      |
| #  |  [min]   | [min] |           |        |                |
|----|----------|-------|-----------|--------|----------------|
|   1|     8.173|  0.084|     12.884|   0.253|                |
|   2|    10.069|  0.081|     22.033|   0.432|                |
|   3|    10.252|  0.070|    134.648|   2.639|                |
|   4|    10.637|  0.074|     38.975|   0.764|                |
|   5|    10.893|  0.067|   4470.551|  87.636|                |
|   6|    11.015|  0.062|    382.896|   7.506|                |
|   7|    11.148|  0.059|      6.971|   0.137|                |
|   8|    11.291|  0.059|      2.432|   0.048|                |
|   9|    11.368|  0.081|      9.788|   0.192|                |
|  10|    11.534|  0.060|      2.815|   0.055|                |
|  11|    11.709|  0.097|     17.303|   0.339|                |
------------------------------------------------------------
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	300412LM013.d	**Sample Name**	ILS-VASU-2-175
Sample Type	Sample	**Position**	Vial 16
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS LM.m	**Acquired Time**	4/30/2012 1:56:29 PM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	Symmetry C18 75*4.6mm 3.5µm		

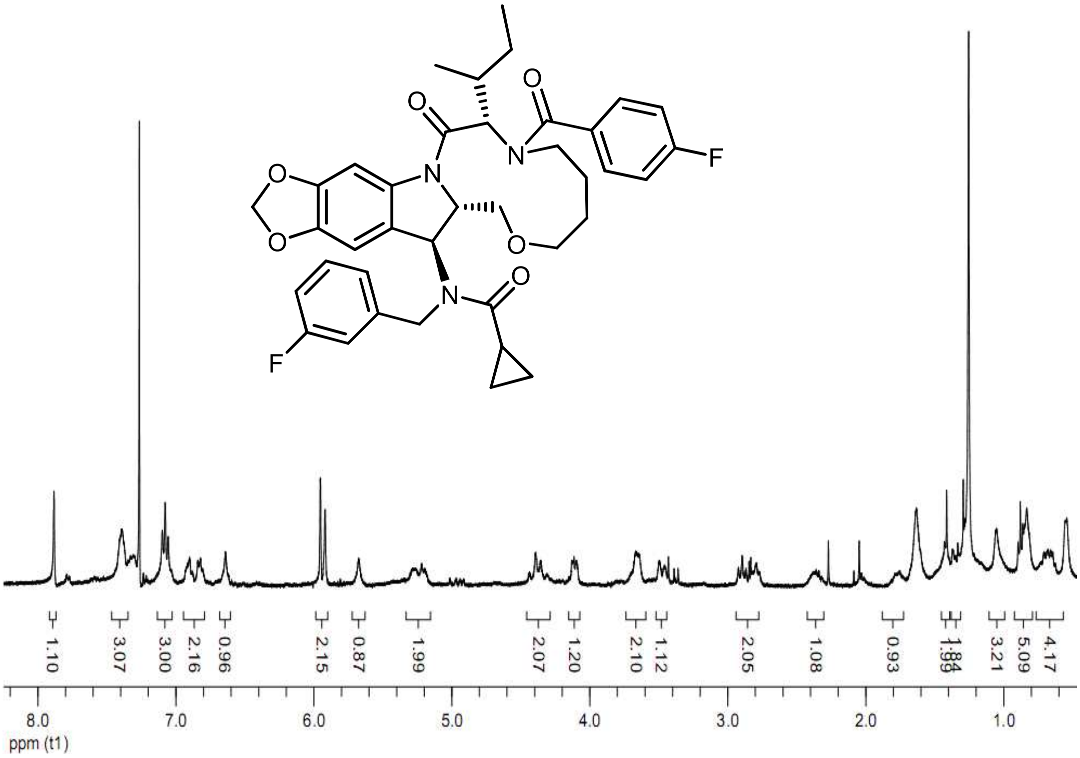
User Chromatograms

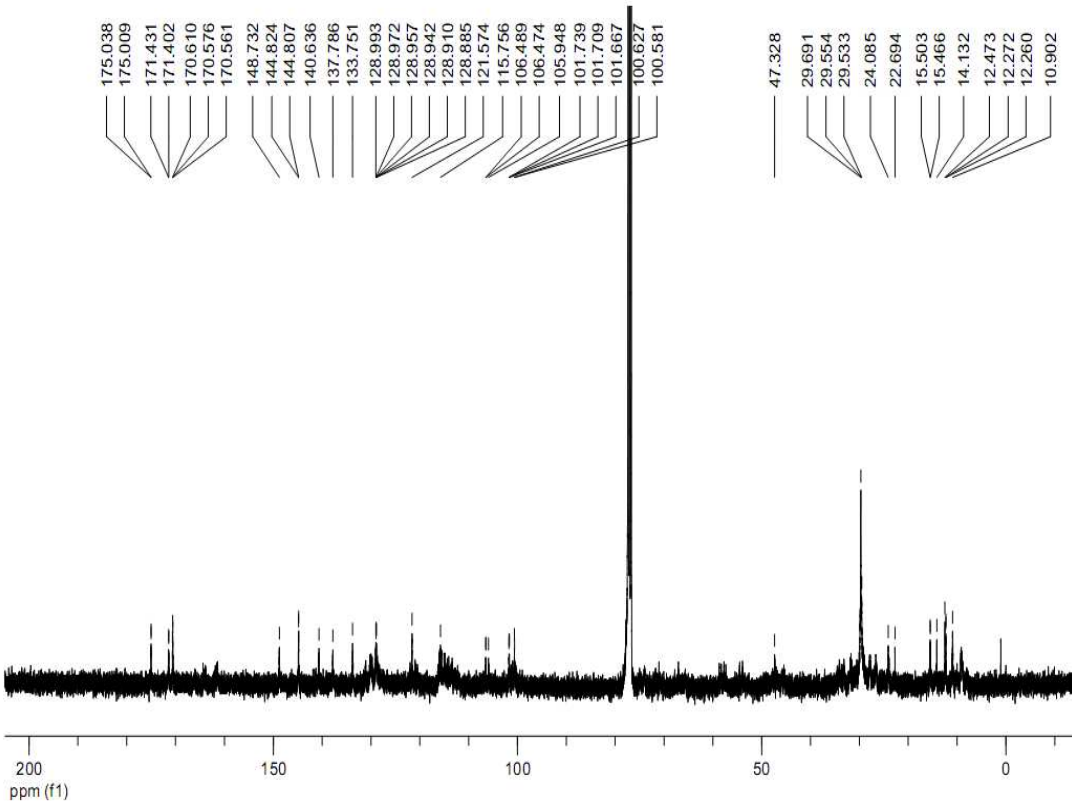
Integration Peak List

Peak	RT	Area	%Area
1	6.3	1.737	0.15
2	6.5	40.609	3.61
3	6.7	4.634	0.41
4	6.9	1078.794	95.83

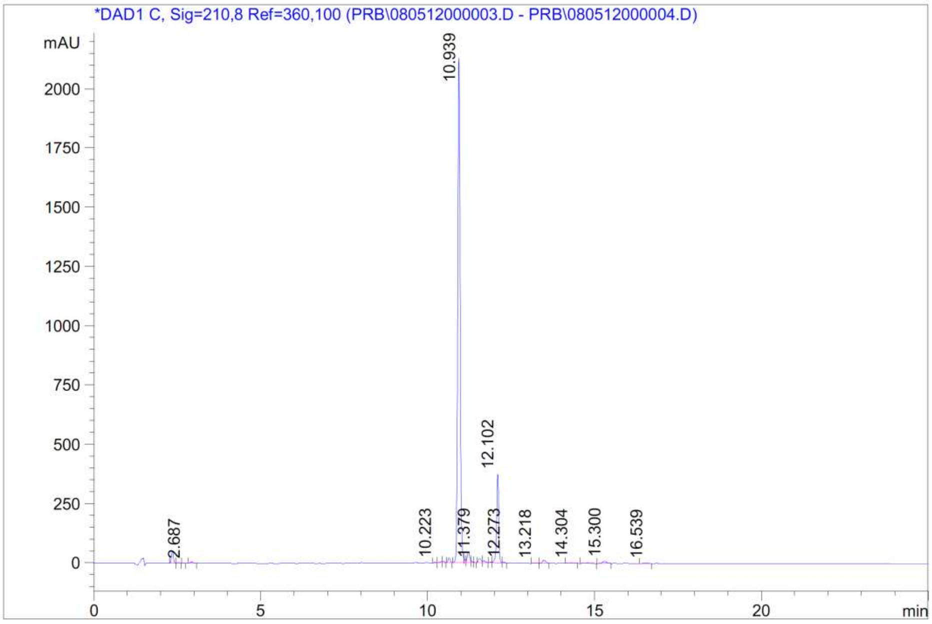
User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

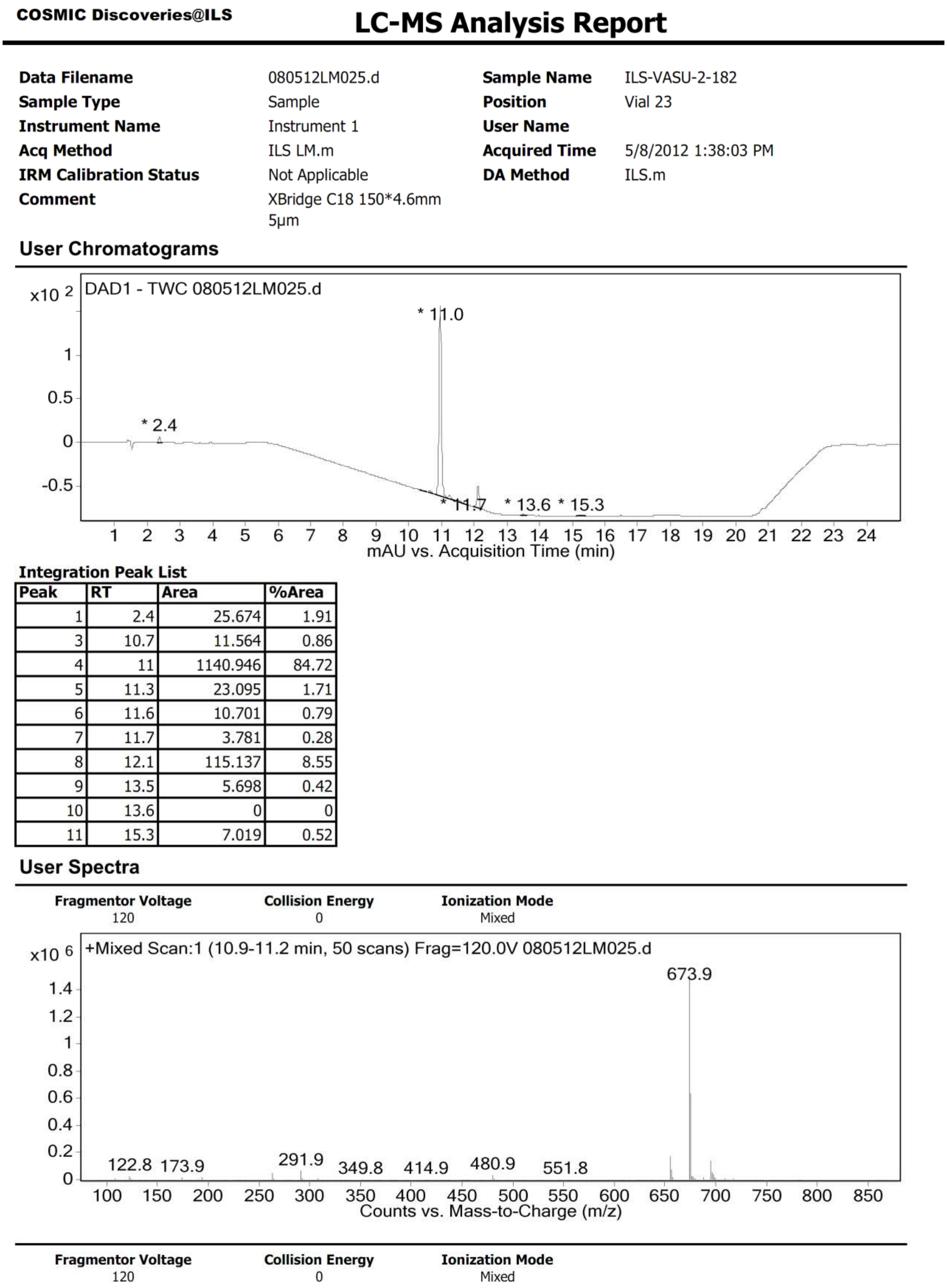

O
O
N
F
O
O
N
O
N
O
F
1.10
3.07
3.00
2.16
0.96
2.15
0.87
1.99
2.07
1.20
2.10
1.12
2.05
1.08
0.93
1.99
1.84
3.21
5.09
4.17
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


175.038
175.009
171.431
171.402
170.610
170.576
170.561
148.732
144.824
144.807
140.636
137.786
133.751
128.993
128.972
128.957
128.942
128.910
128.885
121.574
115.756
106.489
106.474
105.948
101.739
101.709
101.667
100.627
100.581
47.328
29.691
29.554
29.533
24.085
22.694
15.503
15.466
14.132
12.473
12.272
12.260
10.902
200
150
100
50
0
ppm (f1)

```
                         COSMIC DISCOVERIES @ ILS
                            HPLC ANALYSIS REPORT
                                                Seq Line    :           0
Injection Date  : Tue, 8. May. 2012             Location    :     Vial 23
Sample Name     : ILS-VASU-2-182                Inj. No.    :           0
Acq Operator    : SRI KRISHNA VARMA             Inj. Vol.   :       10 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 C-18 150*4.6mm 5µ
                  Mobile phase: A) 0.1% HCOOH in water,B) ACN
                  (GRADIENT)   T/B%:0/50,3/50,10/95,18/95,20/50,25/50
                  Flow:1.0 ml/min  Diluent: ACN:WATER(90:10)
```



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

Signal  1: DAD1 C, Sig=210,8 Ref=360,100

|Peak|    RT    | Width |   Area    | Area % |      Name      |
| #  |  [min]   | [min] |           |        |                |
|----|----------|-------|-----------|--------|----------------|
|   1|     2.354|  0.064|   197.902|   1.493|                |
|   2|     2.542|  0.066|    11.491|   0.087|                |
|   3|     2.687|  0.062|     1.845|   0.014|                |
|   4|     2.921|  0.095|    32.887|   0.248|                |
|   5|    10.223|  0.066|    10.645|   0.080|                |
|   6|    10.427|  0.073|    19.938|   0.150|                |
|   7|    10.470|  0.075|    27.095|   0.204|                |
|   8|    10.644|  0.071|    83.600|   0.631|                |
|   9|    10.939|  0.082| 10539.935|  79.513|                |
|  10|    11.088|  0.038|    44.043|   0.332|                |
|  11|    11.223|  0.083|   224.811|   1.696|                |
|  12|    11.307|  0.051|    19.803|   0.149|                |
```



COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	080512LM025.d	**Sample Name**	ILS-VASU-2-182
Sample Type	Sample	**Position**	Vial 23
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS LM.m	**Acquired Time**	5/8/2012 1:38:03 PM
IRM Calibration Status	Not Applicable	**DA Method**	ILS.m
Comment	XBridge C18 150*4.6mm 5µm		

User Chromatograms

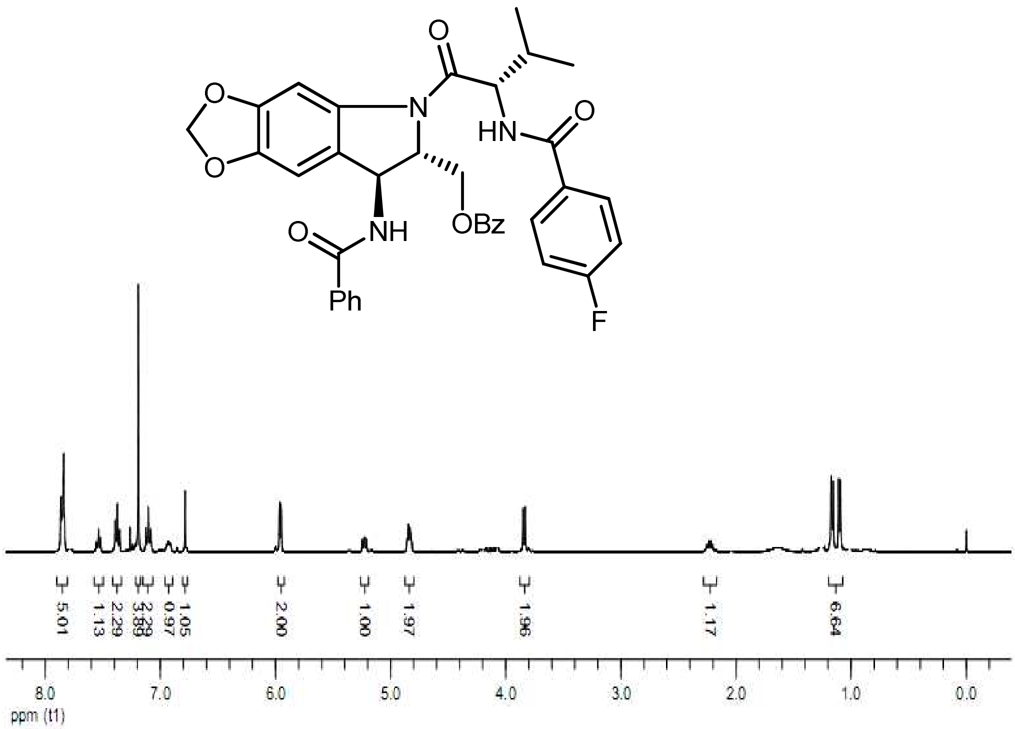
Integration Peak List

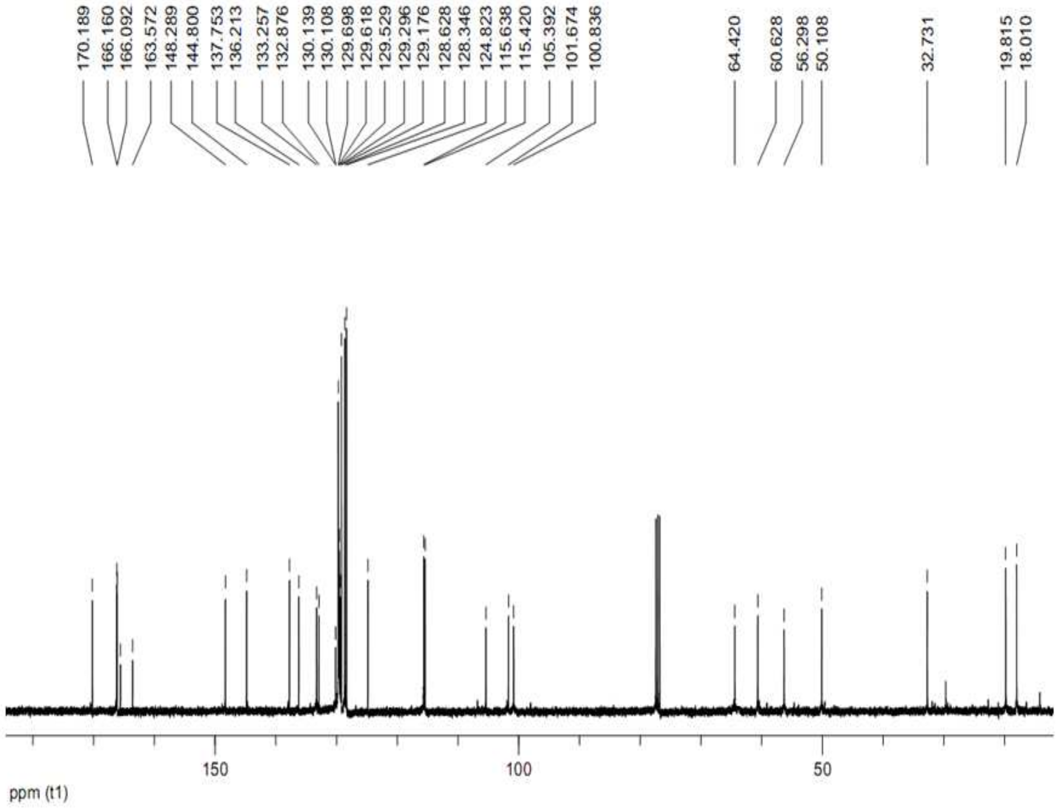
Peak	RT	Area	%Area
1	2.4	25.674	1.91
3	10.7	11.564	0.86
4	11	1140.946	84.72
5	11.3	23.095	1.71
6	11.6	10.701	0.79
7	11.7	3.781	0.28
8	12.1	115.137	8.55
9	13.5	5.698	0.42
10	13.6	0	0
11	15.3	7.019	0.52

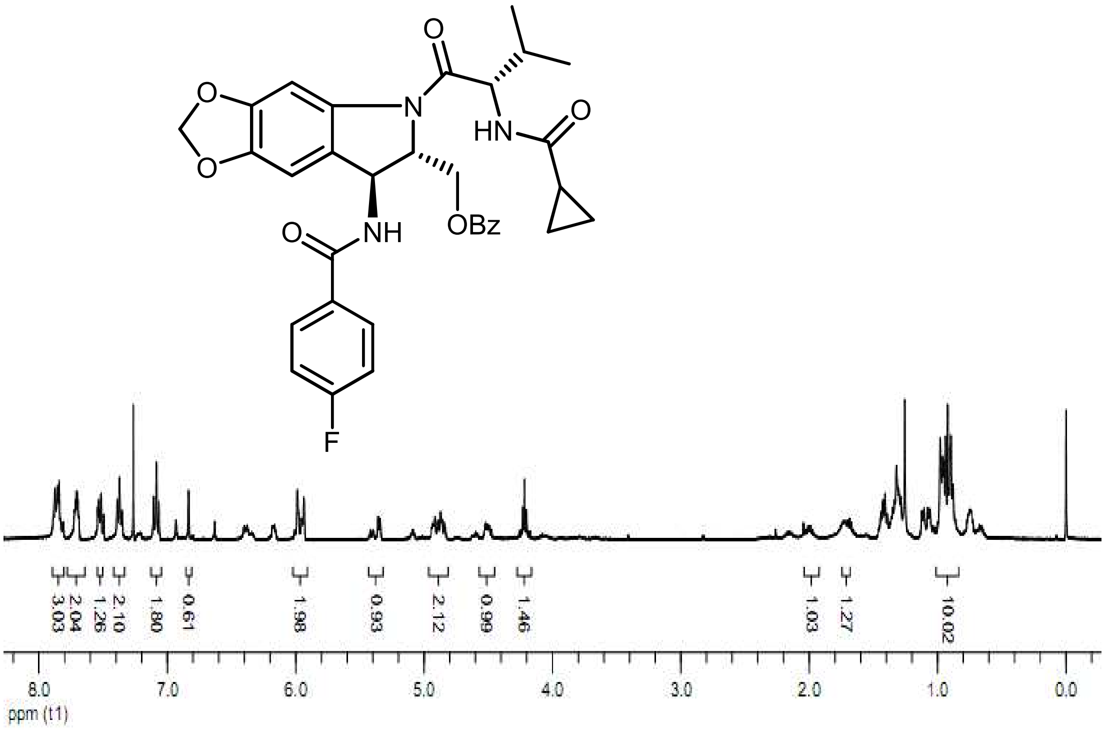
User Spectra

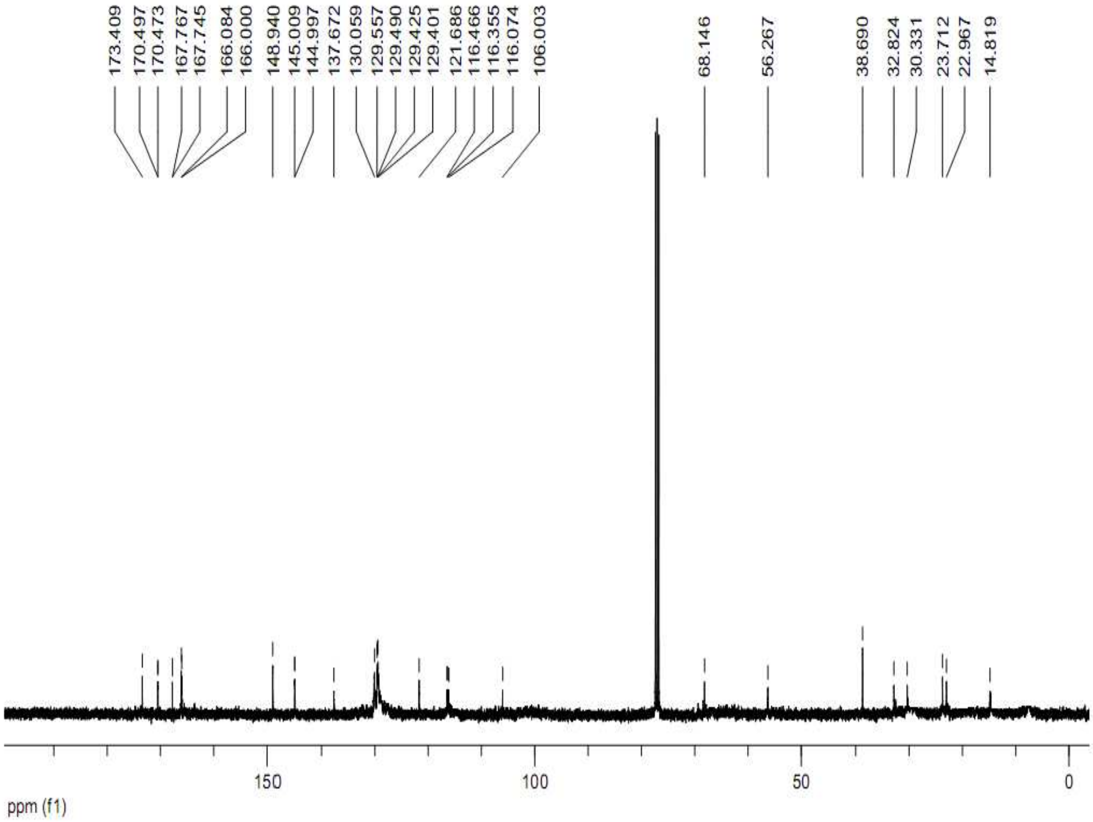
Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

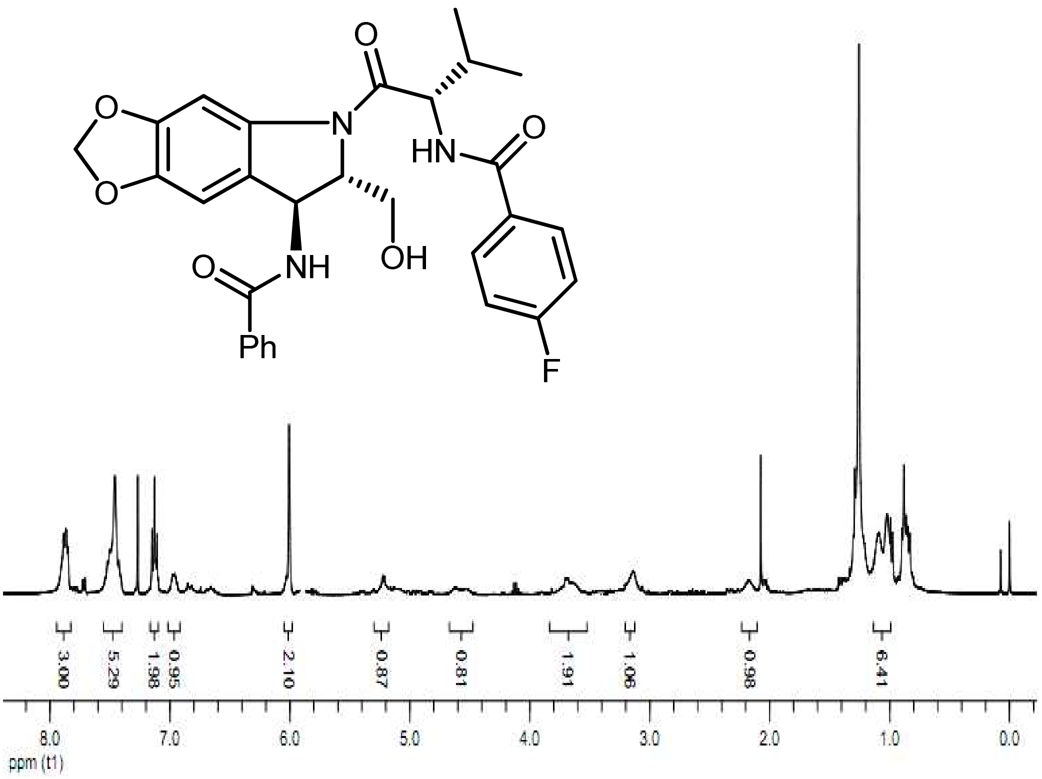
Fragmentor Voltage	Collision Energy	Ionization Mode
120	0	Mixed

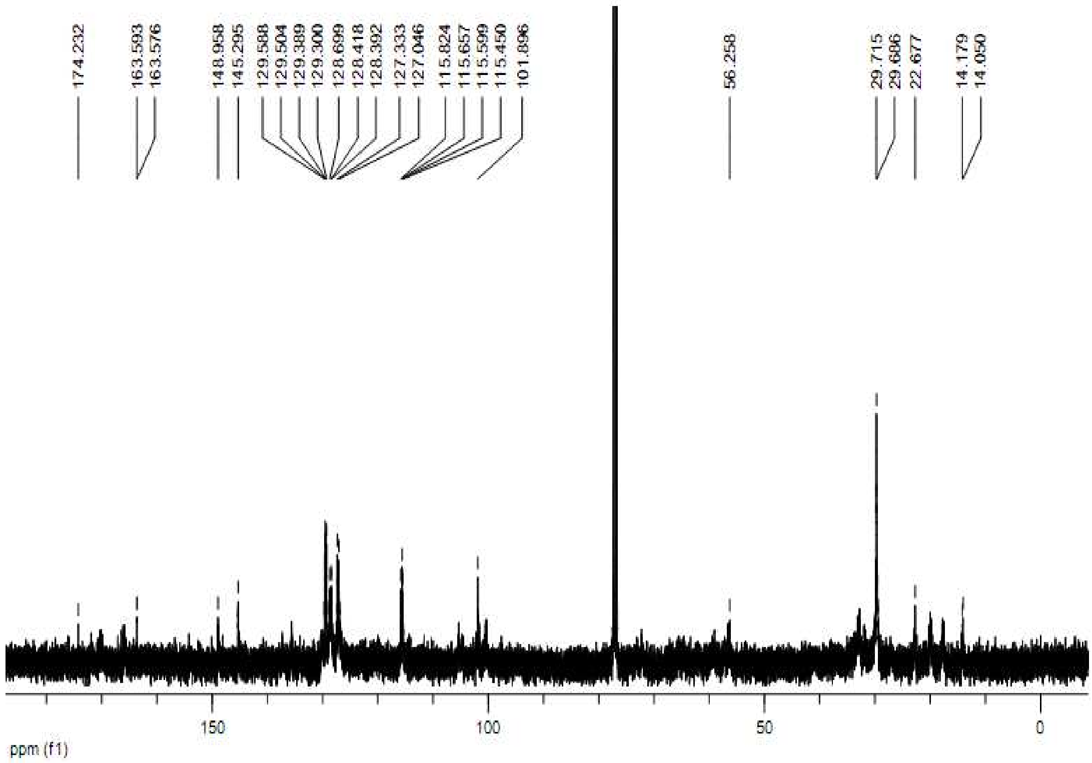

O
N
HN
O
O
O
NH
OBz
O
Ph
F
5.01
1.13
2.29
0.97
1.05
2.00
1.00
1.97
1.96
1.17
6.64
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

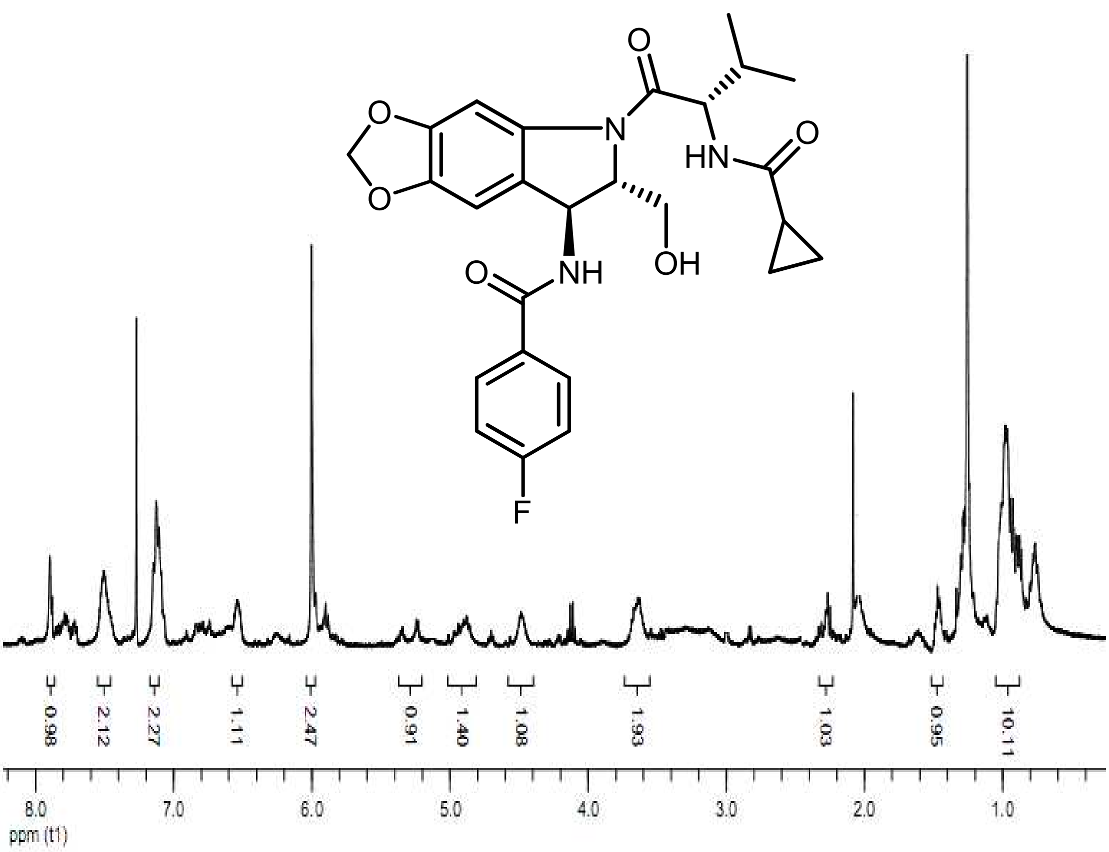

170.189
166.160
166.092
163.572
148.289
144.800
137.753
136.213
133.257
132.876
130.139
130.108
129.698
129.618
129.529
129.296
129.176
128.628
128.346
124.823
115.638
115.420
105.392
101.674
100.836
64.420
60.628
56.298
50.108
32.731
19.815
18.010
150
100
50
ppm (t1)

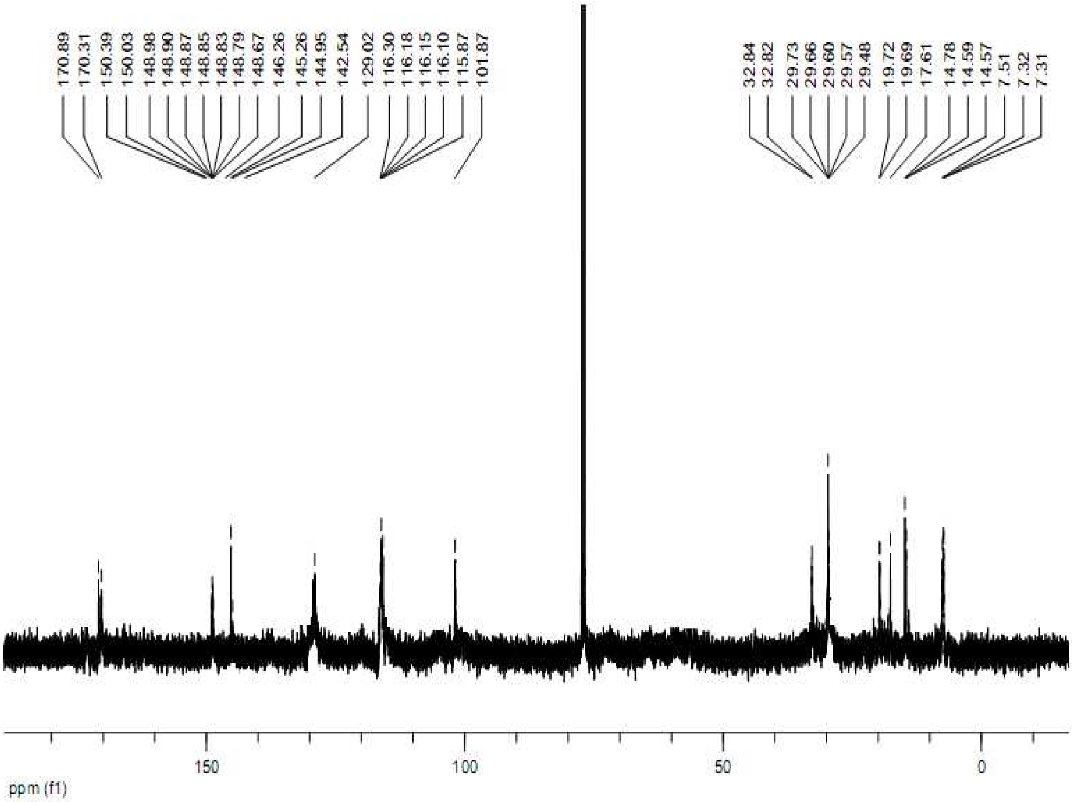

O
O
N
O
HN
O
O
NH
OBz
F
3.03
2.04
1.26
2.10
1.80
0.61
1.98
0.93
2.12
0.99
1.46
1.03
1.27
10.02
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

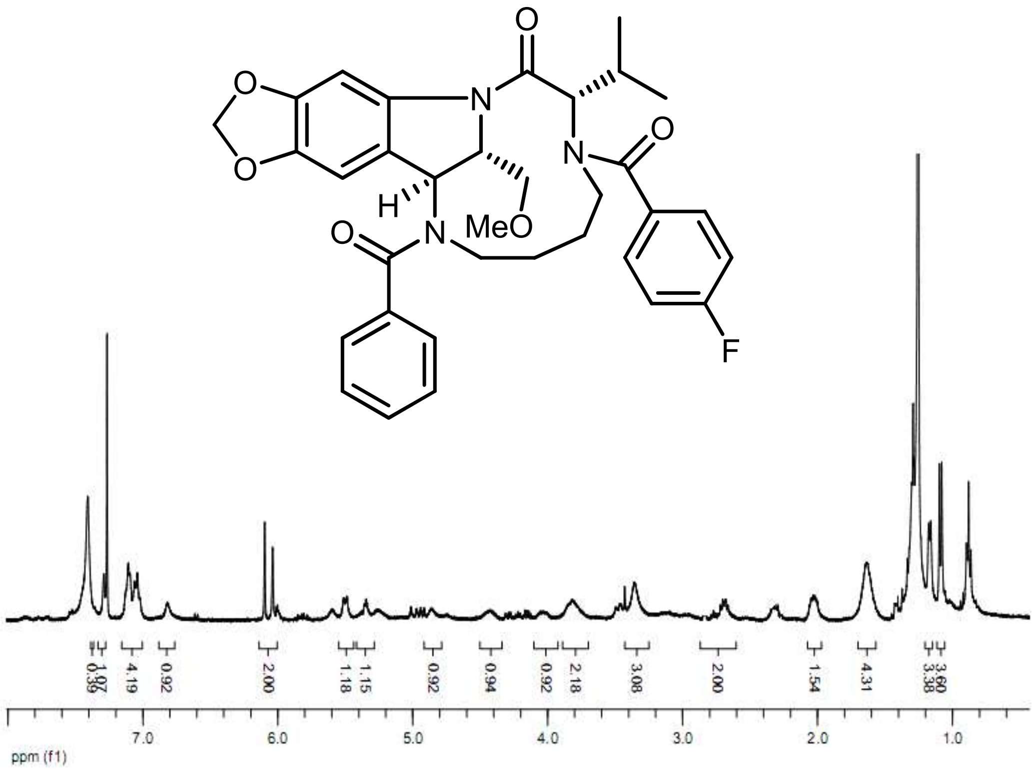

173.409
170.497
170.473
167.767
167.745
166.084
166.000
148.940
145.009
144.997
137.672
130.059
129.557
129.490
129.425
129.401
121.686
116.466
116.355
116.074
106.003
68.146
56.267
38.690
32.824
30.331
23.712
22.967
14.819
150
100
50
0
ppm (f1)

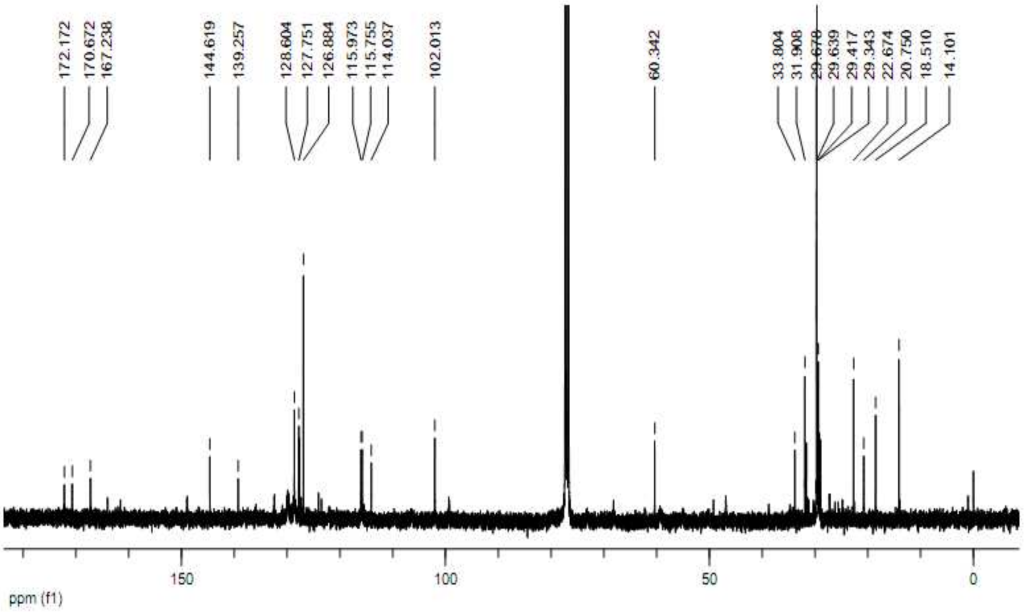

O
O
O
N
HN
O
NH
OH
O
Ph
F
3.00
5.29
1.98
0.95
2.10
0.87
0.81
1.91
1.06
0.98
6.41
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


174.232
163.593
163.576
148.958
145.295
129.588
129.504
129.389
129.300
128.699
128.418
128.392
127.333
127.046
115.824
115.657
115.599
115.450
101.896
56.258
29.715
29.686
22.677
14.179
14.050
150
100
50
0
ppm (f1)

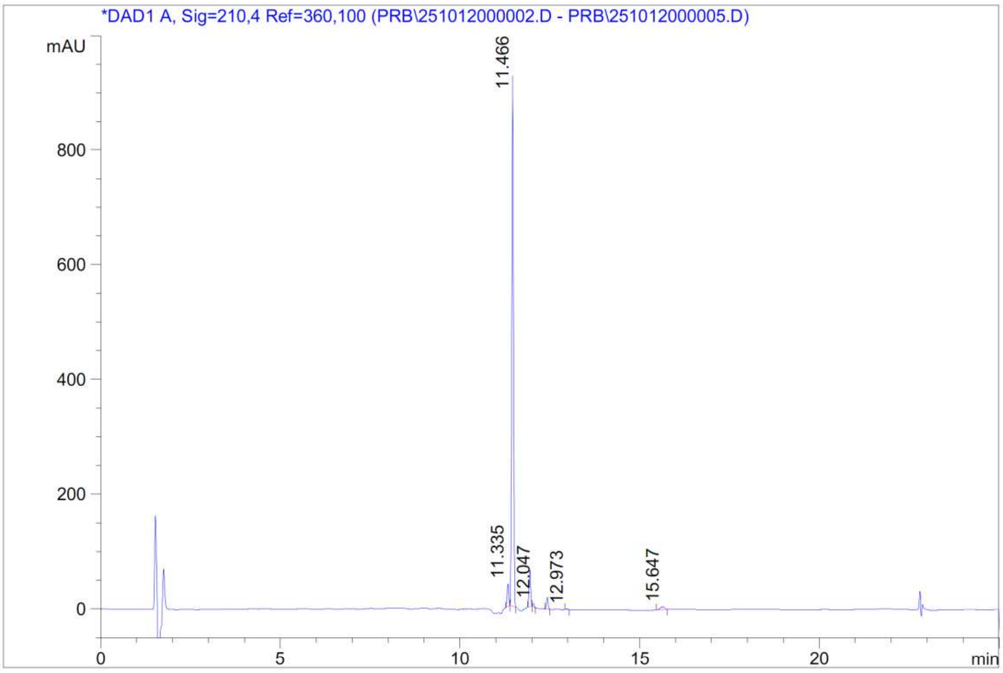

O
O
N
O
HN
O
NH
OH
O
F
0.98
2.12
2.27
1.11
2.47
0.91
1.40
1.08
1.93
1.03
0.95
10.11
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


170.89
170.31
150.39
150.03
148.98
148.90
148.87
148.85
148.83
148.79
148.67
146.26
145.26
144.95
142.54
129.02
116.30
116.18
116.15
116.10
115.87
101.87
32.84
32.82
29.73
29.66
29.60
29.57
29.48
19.72
19.69
17.61
14.78
14.59
14.57
7.51
7.32
7.31
150
100
50
0
ppm (f1)

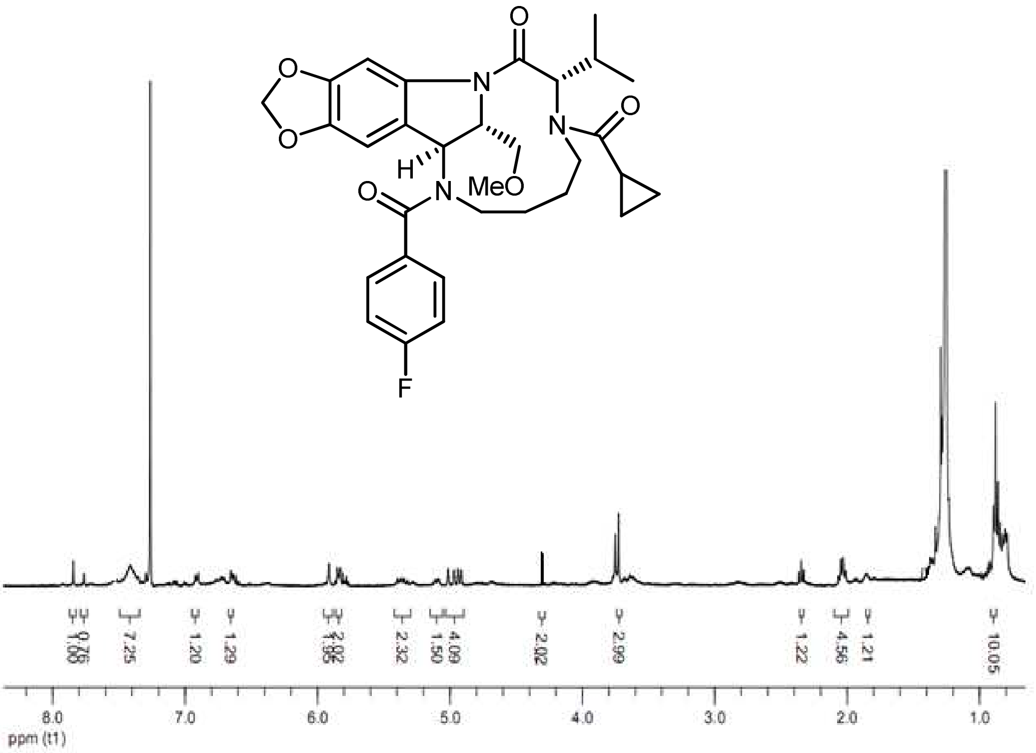

O
O
O
N
O
N
H
N
MeO
O
O
F
0.39
1.07
4.19
0.92
2.00
1.18
1.15
0.92
0.94
0.92
2.18
3.08
2.00
1.54
4.31
3.38
3.60
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


172.172
170.672
167.238
144.619
139.257
128.604
127.751
126.884
115.973
115.755
114.037
102.013
60.342
33.804
31.908
29.678
29.639
29.417
29.343
22.674
20.750
18.510
14.101
150
100
50
0
ppm (f1)

```
                        COSMIC DISCOVERIES @ ILS
                          HPLC ANALYSIS REPORT
                                               Seq Line    :          0
Injection Date   : Thu, 25. Oct. 2012          Location    :     Vial 1
Sample Name      : ILS-VASU-2-184              Inj. No.    :          0
Acq Operator     : VARMA                       Inj. Vol.   :      10 µl
Acq. Method      : D:\CHEM32\1\METHODS\C-18 A80B20G.M
Analysis Method  : D:\CHEM32\1\METHODS\C-18 A80B20G.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%:0/20,5/20,10/98,20/98,22/20,25/20
                   Flow:1.0 ml/min  Diluent: ACN:MeOH:Water(60:20:20)
```



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

Signal  1: DAD1 A, Sig=210,4 Ref=360,100

|Peak|    RT    |    Area   | Area % |
| #  |  [min]   |           |        |
|----|----------|-----------|--------|
|   1|    11.335|    120.397|   3.394|
|   2|    11.466|   3098.868|  87.353|
|   3|    11.944|    202.980|   5.722|
|   4|    12.047|     15.102|   0.426|
|   5|    12.432|     66.660|   1.879|
|   6|    12.973|      4.147|   0.117|
|   7|    15.647|     39.359|   1.109|
------------------------------------
```


O
O
O
N
O
N
O
H
N
MeO
O
F
1.00
0.76
7.25
1.20
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1.95
2.02
2.32
1.50
4.09
2.02
2.99
1.22
4.56
1.21
10.05
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

Chapter 3: Synthesis of Treprostinil Inspired Hybrid Compounds

3.1. Introduction:

As I discussed in the Chapter-1, there is a considerable interest in the synthesis of natural product-inspired compounds and their analogous for chemical biology and medicinal chemistry research. This need is arising due to the fact that natural products have shown a proven track record in exhibiting excellent biological responses in the area of protein-protein interactions, and, as the modulators of signaling pathways. Due to the high degree of complexity that is commonly found in most bioactive natural products, this seriously hampers placing them onto the drug discovery path. Moreover, in most cases, the availability of sufficient samples also limits their practicality. Keeping all this in mind, this is driving the need for the development of efficient synthesis methods to access natural product derived and inspired compounds. To meet the challenges that are associated in this arena of building novel chemical toolboxes to explore their functional biological value, the-

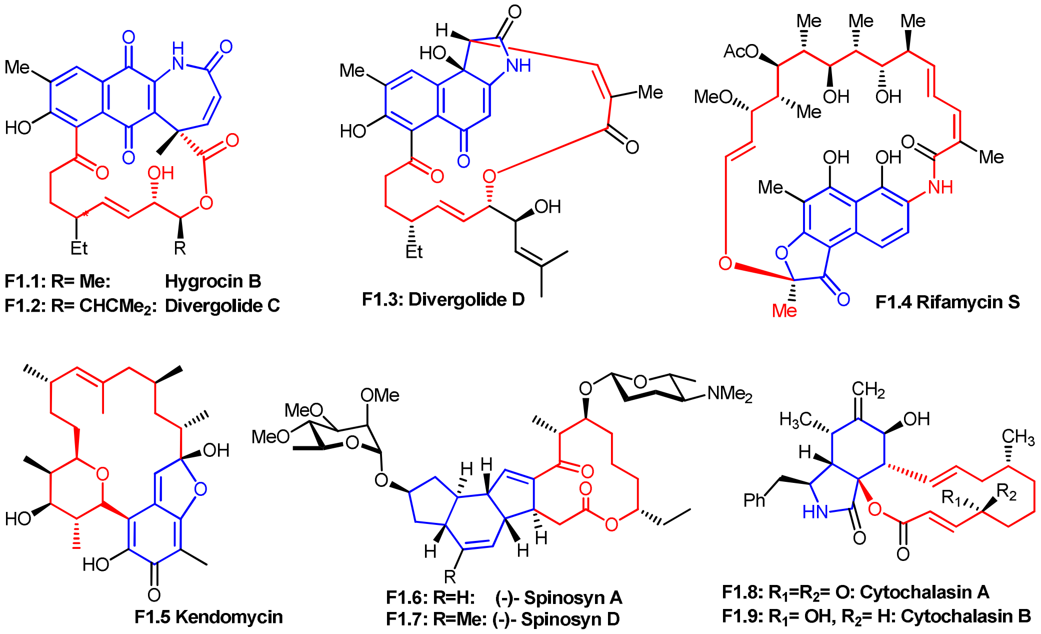

F1.1: R= Me: Hygrocin B
F1.2: R= $CHCMe_2$: Divergolide C
F1.3: Divergolide D
F1.4 Rifamycin S
F1.5 Kendomycin
F1.6: R=H: (-)- Spinosyn A
F1.7: R=Me: (-)- Spinosyn D
F1.8: R_1=R_2= O: Cytochalasin A
F1.9: R_1= OH, R_2= H: Cytochalasin B

Figure 1: A few natural products having polycyclic skeleton containing macrocyclic rings

-requirements of multi-steps stereoselective syntheses, and, the logic and methodology of natural product total synthesis need to be translated. In our group, we were interested in synthesizing natural product inspired compounds having the tricyclic scaffold and also containing an additional medium sized macrocyclic ring to generate skeletally diverse compounds. There are several natural products containing tricyclic and bicyclic ring skeletons, and, also having the additional macrocyclic rings. Examples in this category include: rifamycin,[1] kendomycin, hygrocin B,[2] divergolide C and D,[3] cytochalasin A and B,[4] spinosyn A and D.[5] Since, natural products containing these scaffolds have led to interesting biological properties, this further warranted the structural modifications to the backbone of the scaffold.

In particular, we are interested in treprostinil A[6] (**1**), which is contains the tricyclic skeleton having five stereocenters. Treprostinil A is commonly known as UT-15. It is an effective drug in the treatment of pulmonary arterial hypertension.[7] Treprostinil A is a synthetic analog of prostacyclin (PGI_2), which is one of the important metabolic products of arachidonic acid produced in the vasculature of smooth muscle cells and endothelial cells.[8] It is an effective vasodilator in both types of circulatory pathways. It was earlier used for the treatment of cardiovascular diseases.[9] UT-15 retains all the biological activity of PGI_2. PGI_2 has a very short half-life (approx. 10 min), and, is very unstable towards higher temperatures. Whereas, UT-15 has a better half life of approx. 3 hrs. UT-15 is known for an action towards the cure of severe congestive heart failure, severe intermittent claudication and an immune suppression. Moreover, UT-15 has an anti-proliferative effect on human pulmonary arterial smooth muscle cells.[10] All these interesting features of UT-15 attracted us to obtain the inspired compounds having the tricyclic skeleton that is found in UT-15. Our main objective is to develop a novel route to obtain the tricyclic core of UT-15, and, further, explore the chemical space around this scaffold by synthesizing different types of additional macrocyclic rings with few diversity points to obtain further analogous.

3.2. Literature synthesis:

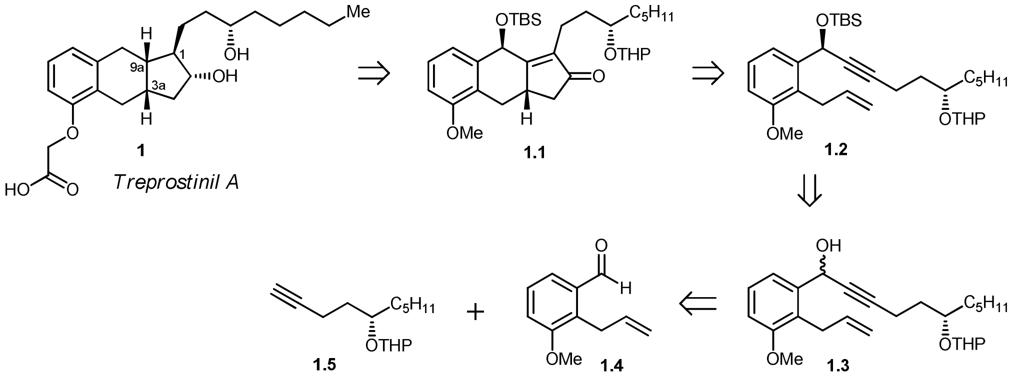
In this section, I am going to discuss two of the literature approaches to the synthesis of treprostinil.

3.2.1. Moriarty approach:

Moriarty *et. al.,* reported the synthesis of treprostinil A.[6a] The main challenge in the synthesis of this compound is the construction of the tricyclic intermediate. It was obtained via a stereoselective intramolecular Pauson-Khand cyclization (PKC).[11] The benzylic OTBS group, which serves as the temporary stereo-directing group that is conveniently removed by the benzylic hydrogenolysis concomitantly with the catalytic hydrogenation of the enone PKC product. Thus, the benzylic chiral center dictates the subsequent stereochemistry of the stereogenic centers at three carbon atoms (C_{3a}, C_{9a}, and C_1).

3.2.1.1. Retrosynthesis:

The retrosynthetic analysis of treprostinil A (**1**) is shown in Scheme 1. The key disconnections are, (i) to obtain **9a** stereochemistry from an enone moiety (**1.1**) by the catalytic hydrogenation of the double bond. Following this, cyclopentenone ring of **1.1** can be obtained from **1.2**, by an intramolecular asymmetric Pauson-Khand cyclization (PKC). The stereochemistry of the hydroxyl group of **1.2** can be achieved by the functional group inter-



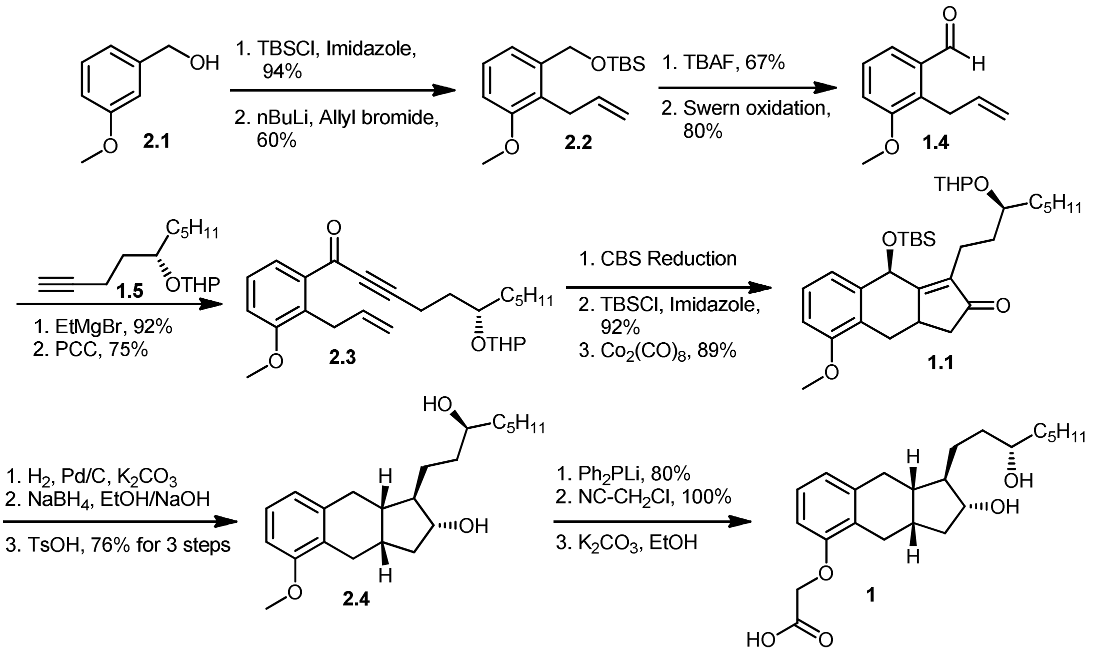
Scheme 1: The retrosynthetic analysis of treprostinil A (Moriarty et. al.)

-conversion of **1.3**. Further, the hydroxy group in **1.3** can be obtained from the stereoselective alkylation of aldehyde **1.4** by the Grignard addition of the side chain **1.5**. This side chain was synthesized from (*S*) -(-)- epichlorohydrin. Aldehyde **1.4** was synthesized from commercially available 3-hydroxy benzylalcohol.

3.2.1.2. Synthesis of Treprostinil A:

The detailed synthesis of treprostinil A is shown in Scheme 2. It was started from 3-methoxybenzyl alcohol **2.1**, which was protected as the -OTBS derivative, and, further *ortho*-allylated by using *n*BuLi to obtain an allyl product **2.2**. This was further subjected to deprotection of -OTBS ether and Swern oxidation to provide 2-allyl-3-

methoxybenzaldehyde **1.4**. The enyne **2.3** was synthesized by Grignard addition of the side chain **1.5** to aldehyde **1.4**, and, further oxidation with PCC to produce **2.3**. The diastereomeric side chain 5-*S*-tetrahydropropanoxy-1-decyne (**1.5**) was synthesized using Takano *et al.*, method.[12] For the subsequent stereoselective Pauson-Khand cyclization, the researchers required the *S*-configuration of the benzylic (propargylic) carbon bearing the hydroxyl group.

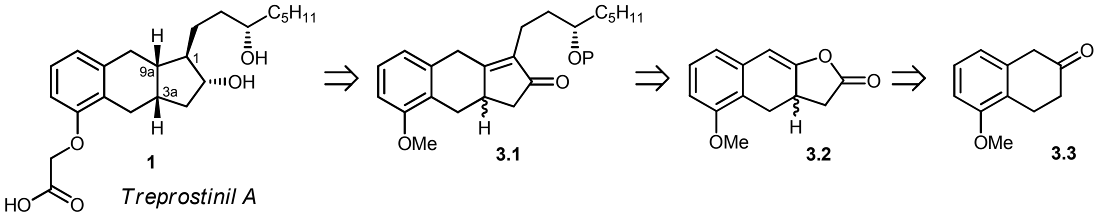


Scheme 2: Synthesis of treprostinil A

The stereochemistry was obtained by using a stoichiometric Corey-type asymmetric reduction[13] of **2.3** followed by the protection with TBS chloride reagent. Further, it was subjected to either stoichiometric or catalytic $Co_2(CO)_8$ mediated Pauson-Khand cyclization,[14] that provided the tricyclic enone **1.1** in 89% yield. The reductive elimination[15] of **1.1** by using catalytic hydrogenation, followed by reduction of ketone with sodium borohydride resulted in an alcohol moiety. Further, the removal of -OTHP protection led to the synthesis of **2.4**. Cleavage of the methoxyl group with lithium diphenylphosphine prepared *in situ* from diphenylphosphine and n-butyllithium.[16] This step afforded the crystalline triol for which an X-ray diffraction pattern confirmed the stereochemistry. The triol was then alkylated at the phenolic hydroxyl group with use of chloroacetonitrile, which was then hydrolyzed with ethanolic potassium hydroxide to obtain treprostinil A (**1**).

3.2.2. Aristoff and co-workers approach:

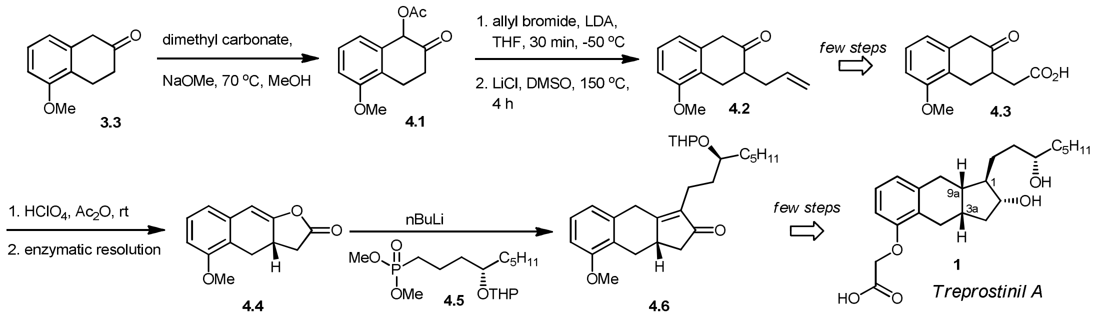
3.2.2.1. Retrosynthesis:



Scheme 3: The retrosynthetic analysis of treprostinil A (Aristoff and co-workers)

Aristoff and co-workers achieved the synthesis of treprostinil A from the tricycle enone moiety **3.1**, by subjecting to a selective reduction from the least hindered face. Ketone **3.1** can be derived from the reduction of an enone **3.2**. The key step in the synthesis involves an intramolecular coupling of the anion of phosphonate with an enol lactone **3.2**. Compound **3.2** is derived from an appropriate alkylation and the subsequent dehydration of 5-methoxy-2-tetralone (**3.3**).[6b]

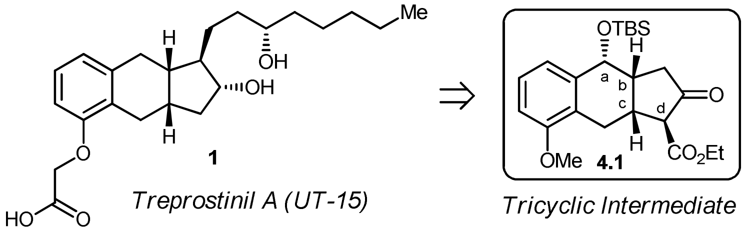
3.2.2.2. Synthesis of treprostinil A:



Scheme 4: Synthesis of treprostinil A

Aristoff and co-workers obtained the AB ring of treprostinil from tetralone **3.3**, which was then converted into the *racemic* version of **4.4** by following classical methods. Further, enzymatic resolution of the *racemic* compound to provide one of the isomer **4.4**. The key step in the synthesis involves the coupling of the phosphonate reagent **4.5** (note: the chirality was derived from a Sharpless resolution of an allylic alcohol precursor) with the enol lactone **4.4** to produce enone **4.6** via a modified intramolecular Wadsworth-Emmons-Wittig reaction. Hydrogenation of **4.6** followed by an unusual one-pot equilibration-reduction sequence, generated four chiral centers around the cyclopentane ring with the complete stereocontrol.[6b]

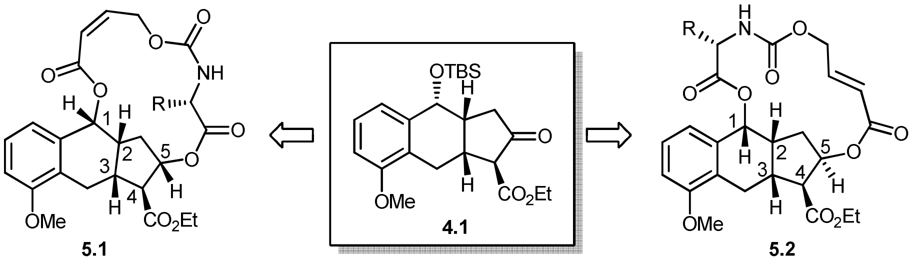
3.3. Synthesis of tricycle scaffold of treprostinil A (our approach):



Scheme 4: Retrosynthesis of Treprostinil A

As I discussed previously, our aim is to develop a novel method to obtain the tricyclic intermediate of treprostinil A. To generalize our synthetic methodology, initially, we started the project to obtain the *racemic* derivative. Having the complete sterecontrolled synthesis methodology of the tricyclic scaffold in hand, we then plan to explore the macrocyclic chemical space by incorporating various amino acid building blocks and the stitching technology.

In our synthesis palnning, we designed two macrocycles, based on the tricyclic scaffold. Both of them contain the additional fifteen membered macrocycles on the tricyclic scaffold. To obtain the additional 15-membered macrocycles, we planed to incorporate the amino acid functionality through the coupling reaction, and, then employ the ring-closing metathesis-based stitching technology.[17] The difference between two macrocycles (see Scheme 5) coupling of the amino acid moiety either through the benzylic -OH or via the secondary -OH group present on the tricyclic scaffold. Morevoer, further, we can also introduce different amino acids (D/L) in both approaches.

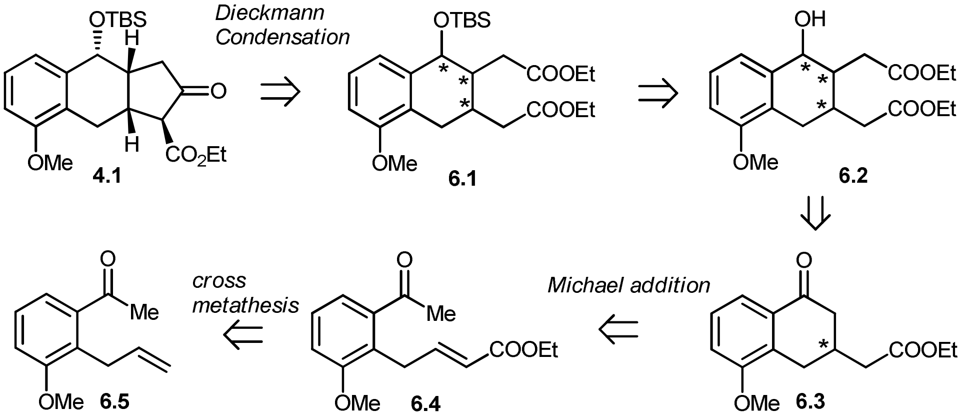


5.1 **4.1** **5.2**

Scheme 5: The proposed plan to obtain macrocyclic-derived compounds **5.1**, **5.2** from **4.1**

These two divergent strategies with a common objective provides an excellent opportunity to build a unique chemical toolbox having compounds that will represent the tricycle core and the additional 15-membered ring derivatives, further, to explore their value in chemical biology programs as the modulators of protein-protein interactions and signaling pathways.

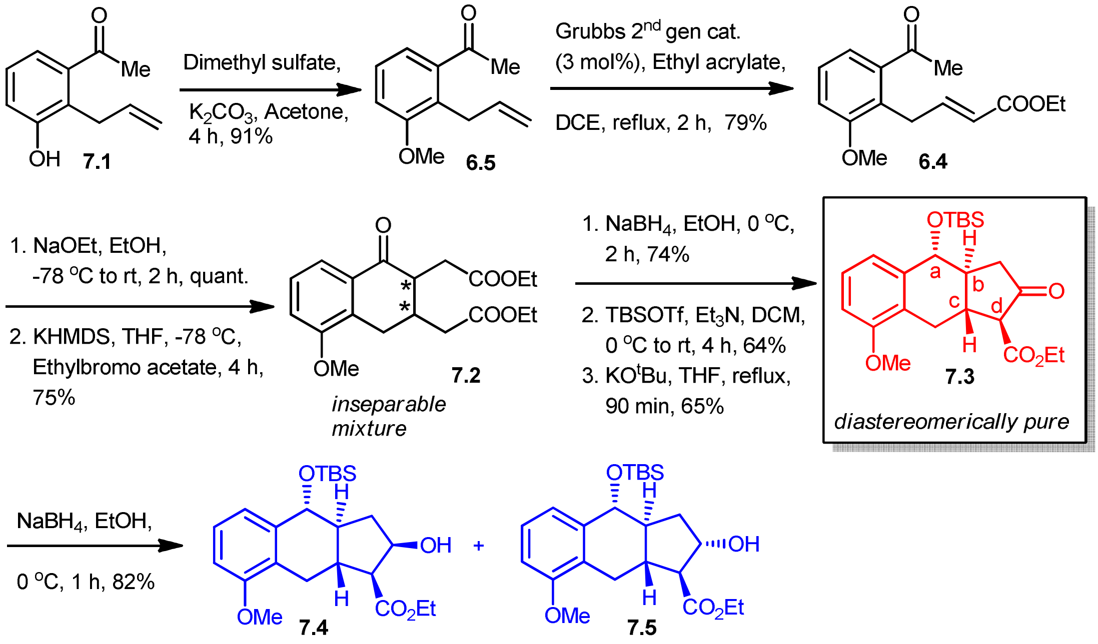
3.3.1. Retrosynthesis of tricyclic intermediate of treprostinil A:



Scheme 6: The retrosynthetic analysis of tricyclic intermediate

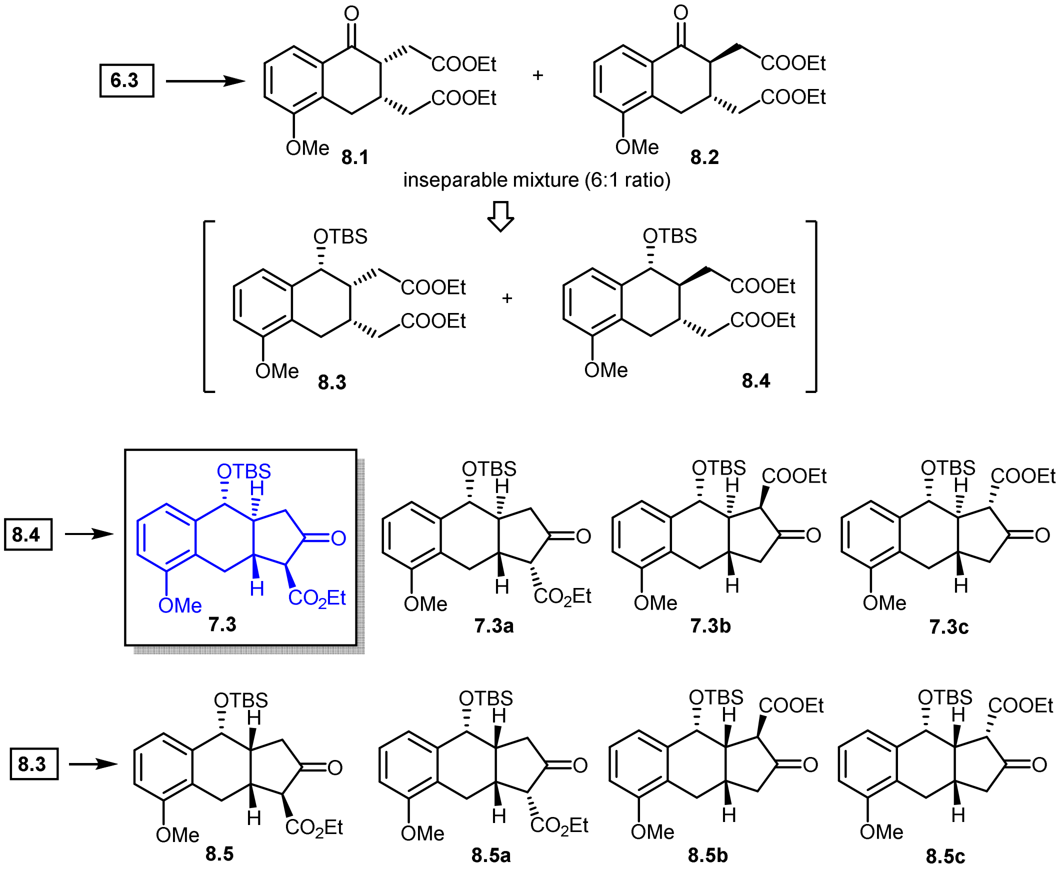
Retrosynthesis of the tricyclic scaffold is shown in Scheme 6. Compound **4.1** could be synthesized from -OTBS ether **6.1**, by using a Dieckmann condensation approach. Compound **6.1** is synthesized from alcohol **6.2**, which could be achieved from the bicyclic compound **6.3** by alkylating α to the carbonyl moiety followed by functional group inter-conversion. The new C-C bond formation approach of compound **6.3** is planned to be achieved by using Michael addition reaction of α, β-unsaturated ester **6.4**. Compound **6.4** is derived from an olefin **6.5** by cross metathesis reaction strategy.

3.3.2. Synthesis of tricyclic scaffold:



Scheme 7: Synthesis of tricyclic intermediate

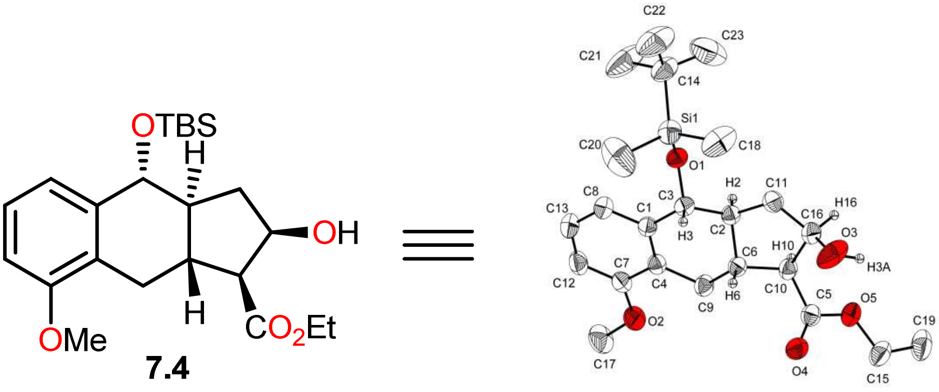
Synthesis of **4.1** is shown in Scheme 7. We started our synthesis with the phenolic derivative **7.1**, which was synthesized by a Claisen rearrangement of 1-(3-(allyloxy)phenyl) ethanone. Compound **7.1** was subjected to phenolic hydroxyl protection, and, this yielded **6.5** in 91%. The α, β-unsaturated compound **6.4** was synthesized from an olefin compound **6.5** by using Grubbs 2nd generation catalyst (3 mol%). α, β-Unsaturated compound **6.4** was further subjected to Michael addition[18] using sodium methoxide (NaOMe in ethanol at -78 °C and drop wise addition of the starting material in THF) to produce the bicyclic compound. This was further subjected to an enol ether alkylation condition using potassium hexamethyldisilazane (KHMDS) at -78 °C to obtain **7.2** as the inseparable diastereomeric mixture in 6:1 ratio. The diastereomeric mixture of **7.2** was further treated with sodium borohydride to reduce the keto moiety. The crude compound obtained following the carbonyl reduction was then subjected to -OTBS ether formation by using triethyl amine as base. At this stage, we moved with the major isomer that was obtained after the flash column chromatography. This major isomer was then subjected to Dieckmann cyclization[19] by using potassium *tertiary* butoxide as the base. To our pleasant surprise, we obtained only one diastereomer and the purity of this compound was confirmed by HPLC (99% purity). The product formed is a result of a regio- and stereo-selective Dieckmann cyclization. The NMR of compound **7.3** was very clean and further COSY and NOESY were helpful in assigning all the protons (note: the coupling constant between Ha and Hb is 8.8 Hz, and in NOESY experiment Ha is giving cross peak with Hc, so we confirmed that Ha and Hb are *trans* to each other and Ha and Hc are in *cis* relationship).



Scheme 8: The regio- and stereo-selective Dieckmann Cyclization

The formation of the regio and stereo-selective tricyclic compound **7.3** is shown in Scheme 8 (schematic representation). There is a possibility of formation of two diastereomers (**8.1** and **8.2**), during the alkylation reaction of *racemic* compound **6.3**. Further, the reduction of the ketone by using sodium borohydride (which can deliver the hydride anion from the less hindered face) to obtain the alcoholic compound, followed by the hydroxyl group protection to give **8.3** and **8.4**. The -OTBS ether compound was then subjected to Dieckmann cyclization reaction, and, in this reaction, there is a possibility to obtain 8 compounds, but we obtained only one compound **7.3**. Interestingly, it appears that the presence of a -OTBS group at the benzylic position is playing an important role in governing the regio-selective deprotonation under the reaction conditions. The deprotonation is taking place from the methylene carbon, which is away from the TBS ether. And, this also leads to having the -COOEt group at the site that is opposite to the -OTBS group. The β-keto ester **7.3** was further subjected to the carbonyl reduction, which yields two isomers (separable) in 1:1 ratio. We assigned both the isomers as **7.4** and **7.5,** by using various NMR techniques. Further, the final structures were obtained by having a crystal structure of one of the isomers (**7.4**).

3.3.3. The X-ray Crystal structure of 7.4:



7.4

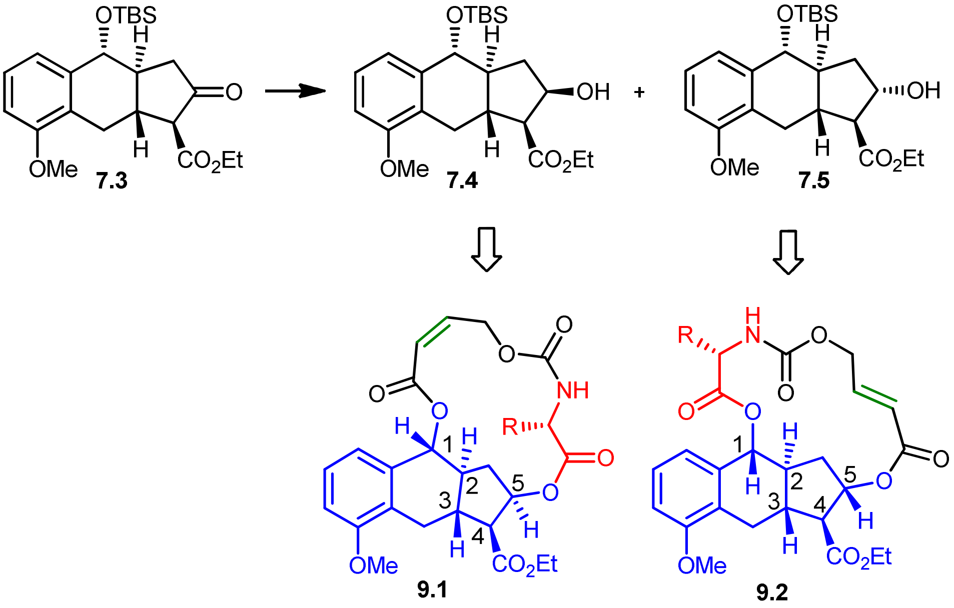
Figure 2: ORTEP diagram of compound **7.4**

Table 1: Crystal data and structure refinement for 7.4

Identification code	**7.4**
Empirical formula	$C_{23} H_{36} O_5 Si$

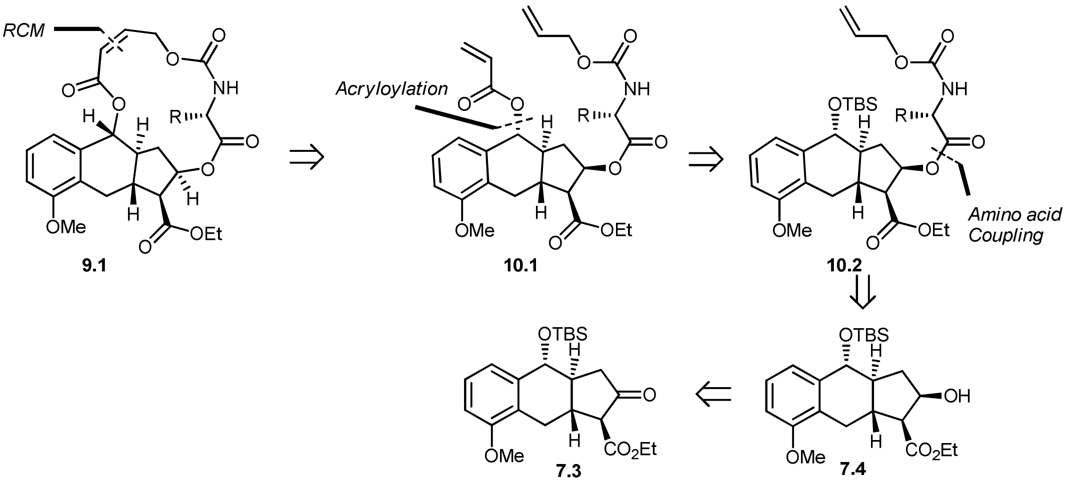
Formula weight	420.61	
Temperature	298(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 35.945(5) Å	α= 90°.
	b = 7.1685(10) Å	β= 130.561(2)°.
	c = 24.167(3) Å	γ = 90°.
Volume	4731.0(11) Å^{3}	
Z	8	
Density (calculated)	1.181 Mg/m^{3}	
Absorption coefficient	0.128 mm^{-1}	
F(000)	1824	
Crystal size	0.40 x 0.24 x 0.20 mm^{3}	
Theta range for data collection	1.49 to 26.08°.	
Index ranges	-44<=h<=44, -8<=k<=8, -29<=l<=29	
Reflections collected	23589	
Independent reflections	4677 [R(int) = 0.0388]	
Completeness to theta = 26.08°	99.8 %	
Absorption correction	None	
Max. and min. transmission	0.9748 and 0.9504	
Refinement method	Full-matrix least-squares on F^{2}	
Data / restraints / parameters	4677 / 0 / 270	
Goodness-of-fit on F^{2}	1.045	
Final R indices [I>2sigma(I)]	R1 = 0.0620, wR2 = 0.1570	
R indices (all data)	R1 = 0.0943, wR2 = 0.1771	
Largest diff. peak and hole	0.307 and -0.287 e.Å^{-3}	

3.4. Synthesis of macrocycles:



Scheme 9: Synthesis of macrocycles

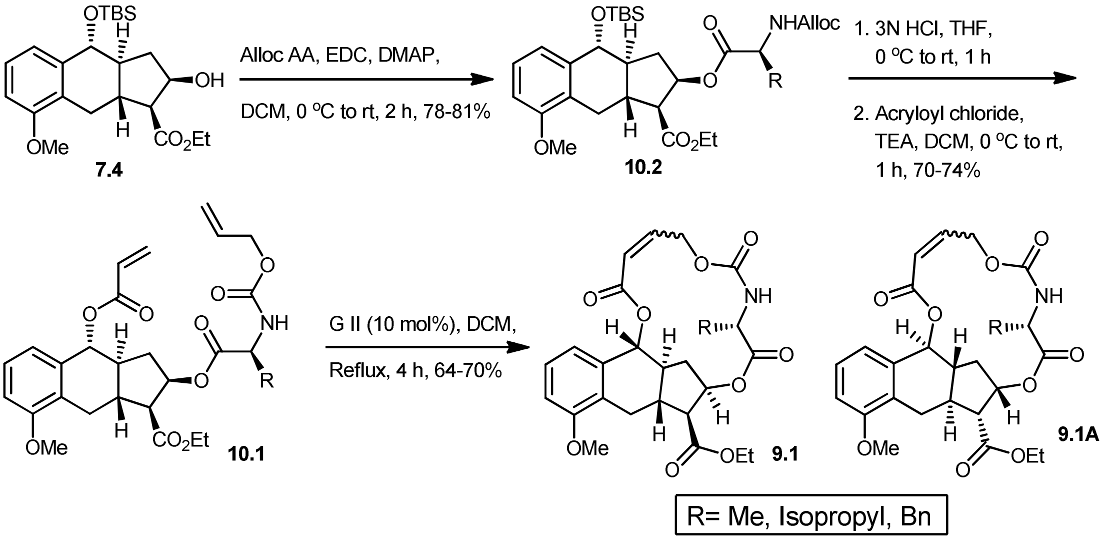
3.4.1. Retrosynthesis of macrocycle, 9.1:



Scheme 10: The retrosynthetic analysis of macrocycle **9.1**

The retrosynthesis of macrocycle **9.1** is shown in Scheme 10. Macrocycle **9.1** could be obtained from the *bis*-allyl compound **10.1** by ring closing metathesis, which is derived from the silyl ether **10.2**. Compound **10.2** was synthesized from the hydroxyl compound **7.4** by attaching the N-Alloc amino acid building blocks. Compound **7.4** having the -OH group can be derived from **7.3** by reducing the carbonyl moiety.

3.4.2. Synthesis of macrocycle 9.1:



Scheme 11: Synthesis of macrocycle **9.1**

Synthesis of macrocycle **9.1** was started from **7.4**. First, it was subjected to an amino acid coupling by using EDC, DMAP to obtain **10.2**. Compound **10.2** is diastereomeric mixture, which is further subjected silyl ether deprotection followed by an acryloylation to obtain the crucial RCM precursor **10.1**. For the silyl ether deprotection, initially, we used TBAF, which resulted in elimination by the proton removal from α to an ester moiety. Then, we switched to 3N HCl conditions. The *bis*-allyl compound **10.1** was further subjected to the ring closing metathesis reaction by using Grubbs 2nd generation catalyst, and, this resulted in 15-membered macrocycle as a mixture of two isomers (see Scheme 11). In one case, we carried out the double bond reduction to avoid any geometrical isomers. After the double bond reduction, the proton NMR spectrum is still showing the shouldering of signals and this may be due to the diastereomeric mixture nature of this compound.

3.4.3. Derivatives of 9.1:

As shown in Figure 3, we synthesized three macrocyclic derivatives of **9.1** by changing the amino acid building blocks and these compounds are shown in Figure 3.

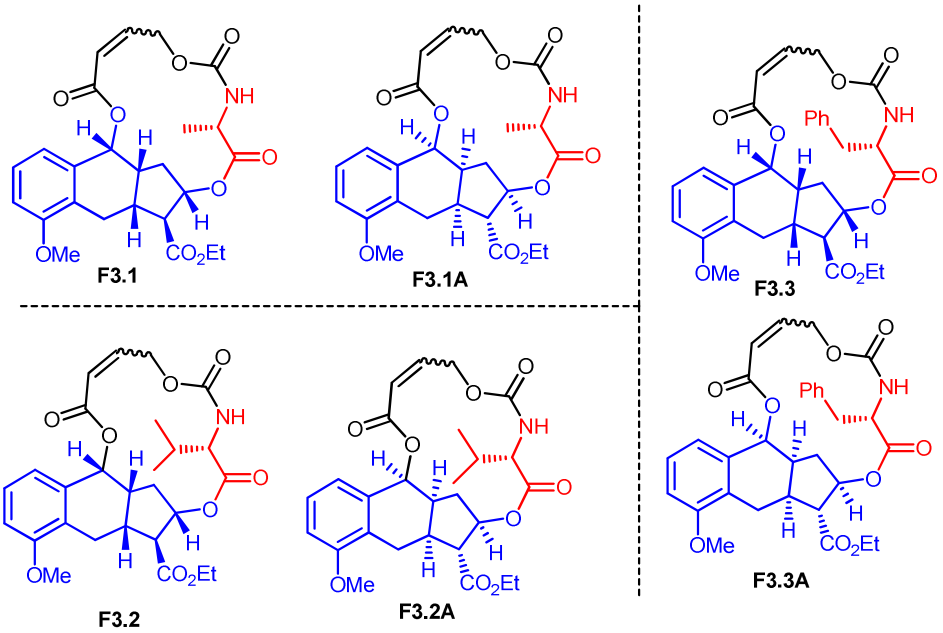
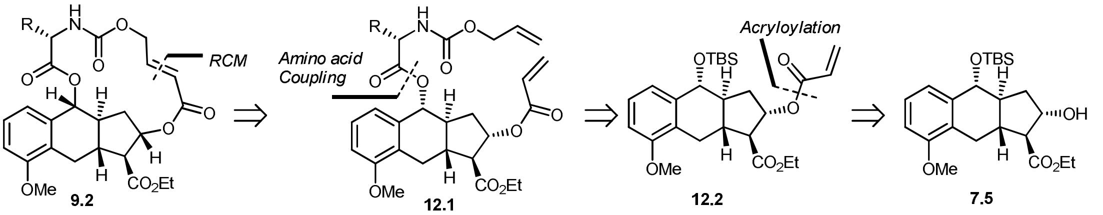


Figure 3: Derivatives of 15-membered macrocycles

3.4.4. Retrosynthesis of macrocycle, 9.2:

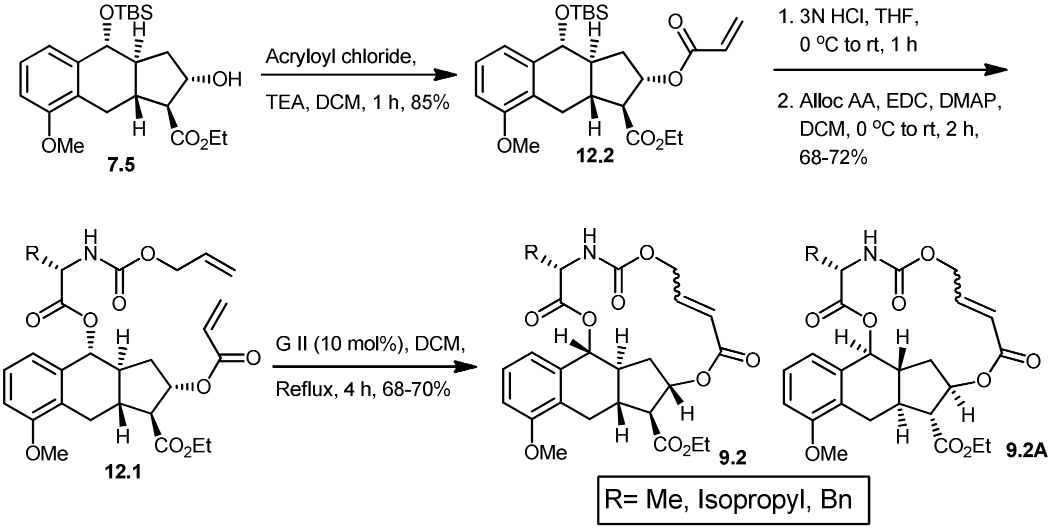


Scheme 12: The retrosynthetic analysis of macrocycle **9.2**

The first disconnection to obtain the 15-membered macrocycle **9.2** is the ring closing metathesis of **12.1**. Compound **12.1** can be synthesized from the corresponding silyl ether **12.2** by coupling with amino acid building blocks. The silyl compound **12.2** can be synthesized from **7.5** by an acryloylation of the hydroxyl group.

3.4.5. Synthesis of macrocycle 9.2:

Synthesis of macrocycle **9.2** was started from **7.5** (second diastereomer obtained from the β keto ester reduction reaction**)**. Acryloylation of **7.5** provided **12.2**, which was further subjected to silyl ether removal and then coupling with various *N*-Alloc amino acids. This led to the RCM precursor **12.1**. The *bis*-allyl compound **12.1** was further subjected to ring closing metathesis to obtain macrocycle **9.2** and we obtain macrocyclic compounds as a mixture of two diastereomers (see Scheme 13).



Scheme 13: Synthesis of macrocycle **9.2**

3.4.6. Derivatives of 9.2:

We synthesized 3 macrocyclic derivatives of **9.2** by changing the amino acid building blocks, and these are shown in Figure 4.

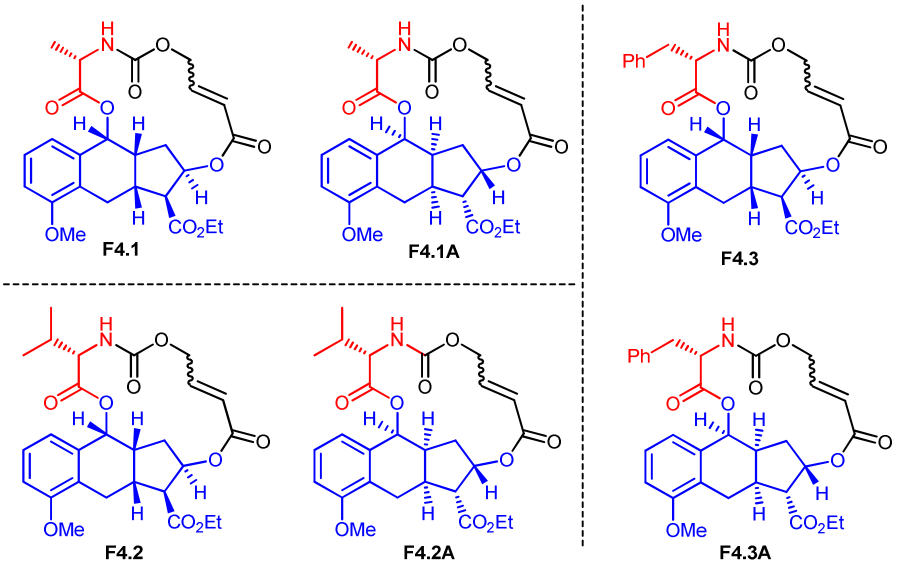


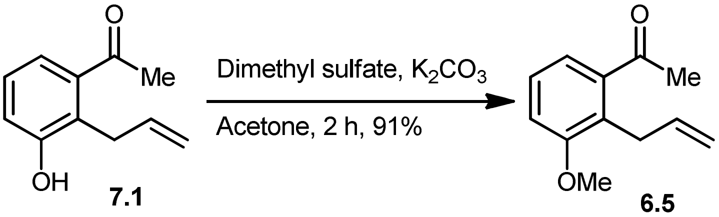
Figure 4: Derivatives of 15 membered macrocycle **9.2**

3.5. Conclusions:

In conclusion, we developed a novel and efficient methodology to synthesize the tricycle scaffold. By incorporating various amino acid building blocks, we further designed a modular approach to obtain treprostinil-derived macrocyclic compounds. The biological evaluation of these compounds is now under exploration in various cell signaling-based assays and these studies will be reported as they become available.

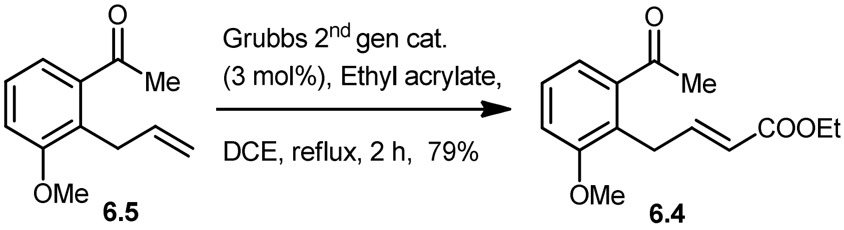
3.6. Experimental procedures:

3.6.1. Tricyclic intermediate:

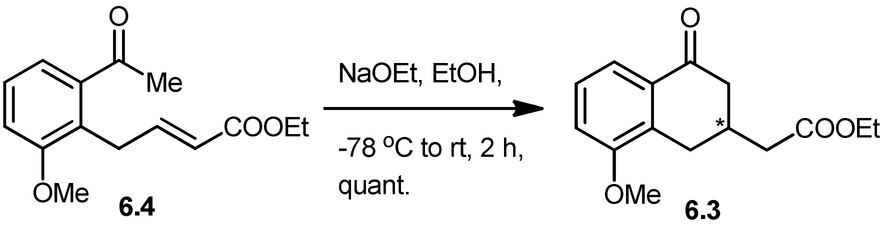


1-(2-allyl-3-methoxyphenyl)ethanone (6.5): To a stirred solution of phenolic compound **7.1** (1 mmol) in dry acetone was added potassium carbonate (2 mmol) at room temperature. The reaction mixture was stirred for 5 minutes, Then dimethyl sulfate (1.5 mmol) was added and reaction mixture was stirred for 2 hours. After completion of the reaction, reaction mixture was filtered by using centered funnel. concentrated under reduced Pressure. Purification by flash column chromatography (2-5% EtOAc/hexanes) to obtain pure product **6.5** as yellow oil. Molecular Formula:

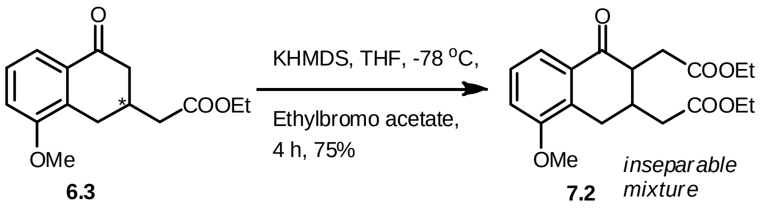
$C_{12}H_{14}O_2$; R_f (ethyl acetate/hexane 5%): 0.3; Yield: 91%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.27-7.23 (m, 1H), 7.15 (d, *J* = 6.93 Hz, 1H), 6.99 (d, *J* = 8.12 Hz, 1H), 6.01-5.91 (m, 1H), 4.97 (s, 1H), 4.96-4.91 (m, 1H), 3.85 (s, 4H), 3.59 (d, *J* = 6.14 Hz, 2H), 2.54 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 203.1, 158.0, 140.6, 137.0, 127.1, 126.9, 120.0, 114.7, 113.0, 55.8, 53.4, 30.5, 30.0; LRMS: (ES+) m/z = 191 (M+1).



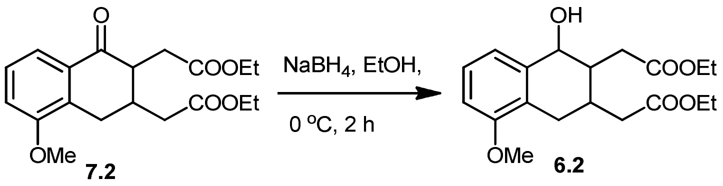
(E)-ethyl 4-(2-acetyl-6-methoxyphenyl)but-2-enoate (6.4): To a stirred solution of olefin **6.5 (**1 mmol) in dry DCE was added Grubbs 2nd gen catalyst (0.03 mmol) at room temperature and followed by ethyl acrylate (5 mmol). Then reaction mixture was allowed to reflux for 2 hours. After completion of the reaction, reaction mixture was allowed to room temperature and concentrated under reduced Pressure. Purification by flash column chromatography (5% EtOAc/hexanes) to obtain pure product **6.4** as yellow oil. Molecular Formula: $C_{15}H_{18}O_4$; R_f (ethyl acetate/hexane 10%): 0.3; Yield: 79%; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.33-7.23 (m, 2H), 7.12-7.05 (m, 1H), 7.01 (d, *J* = 7.95 Hz, 1H), 5.74-5.67 (m, 1H), 4.14 (q, *J* = 7.12 Hz, 2H), 3.84 (s, 3H), 3.77 (dd, *J* = 6.33, 1.43 Hz, 2H), 2.55 (s, 3H), 1.25 (t, *J* = 7.13 Hz, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 201.9, 166.9, 158.0, 147.5, 139.5, 127.5, 125.5, 121.2, 120.9, 113.4, 60.0, 55.8, 30.1, 28.7, 14.2; LRMS: (ES+) m/z = 262 (M+1).



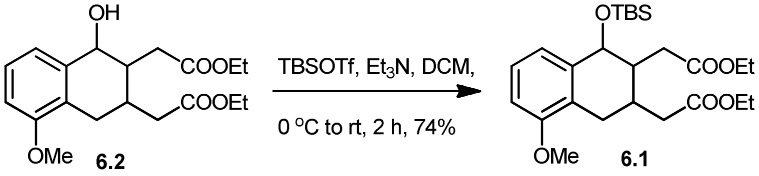
ethyl 2-(8-methoxy-4-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetate (6.3): To a dry MeOH solution (30 ml) of Sodium methoxide (2 mmol) was added a dry THF soln (10 ml) of α, β unsaturated ester **6.4** at -78 °C under Nitrogen atmosphere. The mixture was allowed to warm to room temp. After stirring for 2 hours at room temperature, reaction mixture was quenched with aqueous ammonium chloride solution.extracted with DCM. The extract was washed with brine and dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (5% EtOAc/hexanes) to obtain pure product **6.3** as yellow oil. Molecular Formula: $C_{15}H_{18}O_4$; R_f (ethyl acetate/hexane 10%): 0.3; Yield: quantitative yield; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.66 (d, *J* = 7.64 Hz, 1H), 7.33-7.27 (m, 1H), 7.05 (d, *J* = 7.99 Hz, 1H), 4.19 (q, *J* = 7.12 Hz, 2H), 3.87 (s, 3H), 3.30-3.22 (m, 1H), 2.81-2.76 (m, 1H), 2.74-2.61 (m, 1H), 2.55-2.37 (m, 4H), 1.29 (t, *J* = 7.14 Hz, 4H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 197.6, 171.7, 156.7, 133.0, 131.8, 127.0, 118.6, 114.5, 60.6, 55.6, 44.3, 40.4, 31.4, 28.7, 14.2; LRMS: (ES+) m/z = 262 (M+1).



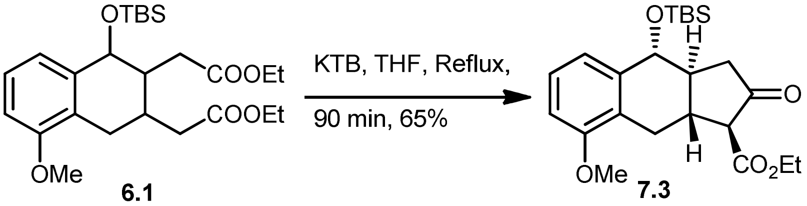
diethyl 2,2'-(5-methoxy-1-oxo-1,2,3,4-tetrahydronaphthalene-2,3-diyl)diacetate (7.2): To a solution **6.3** (1 mmol) in dry THF was allowed to -78 ^{o}C, added 1 molar KHMDS solution (1.1 mmol) dropwise. Then allowed further stirring at same temperature for 40 min. added ethylbromo acetate (1.5 mmol) and further allowed stirring at -78 ^{o}C for 3 hours. reaction mixture was quenched with aqueous ammonium chloride solution.extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (5% EtOAc/hexanes) to obtain pure product **7.2** as yellow oil. Molecular Formula: $C_{19}H_{24}O_6$; R_f (ethyl acetate/hexane 10%): 0.25; Yield: 75%;



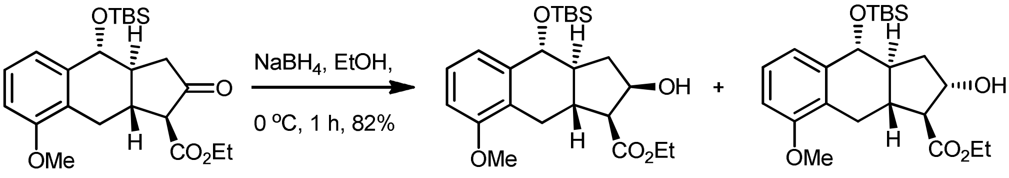
diethyl 2,2'-(1-hydroxy -5- methoxy-1,2,3,4- tetrahydronaphthalene- 2,3-diyl) diacetate (6.2): To a solution carbonyl compound **7.2** (1 mmol) in ethanol was allowed to 0 ^{o}C, added sodium borohydride (1 mmol). Then allowed further stirring at same temperature for 2 hours. After completion of the reaction, reaction mixture was quenched with 5% HCl, extracted with ethyl acetate (twise). The extract was dried over Na_2SO_4. After evaporating the solvent, the residue (colorless oil) was further subjected to TBS protection without purification.



Diethyl 2,2'-(1-((tert-butyldimethylsilyl)oxy) -5-methoxy- 1,2,3,4 -tetrahydronaphthalene-2,3-diyl) diacetate (6.1): To a solution hydroxyl compound **6.2** (1 mmol) in DCM was allowed to 0 ^{o}C, added triethyl amine (1.2 mmol). Then added TBS triflate (1.1 mmol) and allowed further stirring at room temperature for 2 hours. After completion of the reaction, reaction mixture was quenched with 5% HCl, extracted with DCM. The extract was washed with brine and dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (5% EtOAc/hexanes) to obtain pure product **6.1** as colorless oil. Molecular Formula: $C_{25}H_{40}O_6Si$; R_f (ethyl acetate/hexane 5%): 0.3; Yield: 74% (for two steps); **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.15 (t, *J* = 7.91 Hz, 1H), 6.86 (d, *J* = 7.58 Hz, 1H), 6.76 (d, *J* = 8.02 Hz, 1H), 4.60 (d, *J* = 3.30 Hz, 1H), 4.19-4.08 (m, 4H), 3.80 (s, 3H), 2.83-2.74 (m, 1H), 2.70-2.60 (m, 2H), 2.54-2.45 (m, 1H), 2.40-2.34 (m, 1H), 2.28 (m, 3H), 1.27-1.24 (m, 6H), 0.87 (s, 9H), 0.16 (s, 3H), 0.06 (s, 3H)

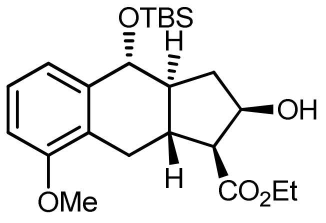


(1S,3aR,4R,9aR)-ethyl 4-((tert-butyldimethylsilyl)oxy)- 8-methoxy- 2-oxo-2,3,3a,4,9, 9a-hexahydro-1H-cyclopenta[b] naphthalene-1-carboxylate (7.3): To a solution diester compound **6.1** (1 mmol) in THF was added KO^{t}Bu (2 mmol). Then allowed to reflux for 90 minutes. After completion of the reaction, reaction mixture was quenched with 5% HCl, extracted with ethyl acetate twise. The combined organic layers was dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (5% EtOAc/hexanes) to obtain pure product **7.3** as white solid. Molecular Formula: $C_{23}H_{34}O_5Si$; R_f (ethyl acetate/hexane 5%): 0.3; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.22 (d, *J* = 8.01 Hz, 1H), 7.12 (d, *J* = 7.94 Hz, 1H), 6.77 (d, *J* = 8.01 Hz, 1H), 4.75 (d, *J* = 8.93 Hz, 1H), 4.29-4.21 (m, 2H), 3.82 (s, 3H), 3.25 (dd, *J* = 16.86, 5.15 Hz, 1H), 3.09 (d, *J* = 12.12 Hz, 1H), 2.80 (dd, *J* = 16.90, 5.77 Hz, 1H), 2.62 (dq, *J* = 11.77, 5.26 Hz, 1H), 2.44 (dd, *J* = 16.78, 11.39 Hz, 1H), 2.28-2.10 (m, 2H), 1.32 (t, *J* = 7.14 Hz, 3H), 0.96 (s, 9H), 0.24 (s, 3H), 0.13 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 209.2, 199.4, 168.5, 156.7, 141.1, 126.8, 124.0, 119.0, 108.3, 75.2, 62.3, 61.6, 55.4, 44.4, 44.3, 40.6, 28.9, 26.0, 18.2, 14.2, -3.3, -3.9; LRMS: (ES+) m/z = 419 (M+1).



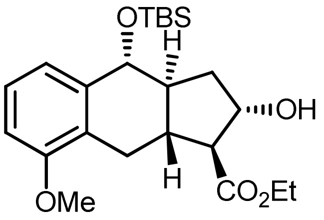
7.3 **7.4** **7.4**

To a solution carbonyl compound **7.3** (1 mmol) in ethanol was allowed to 0 °C, added sodium borohydride (1 mmol). Then allowed further stirring at same temperature for 1 hour. After completion of the reaction, reaction mixture was quenched with 5% HCl, extracted with ethyl acetate (twise). The extract was dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (0.3:5:4.7 EtOAc/DCM/hexane) to obtain pure products as white solids. Molecular Formula: $C_{23}H_{34}O_5Si$; R_f (ethyl acetate/hexane 10%): 0.15;



7.4

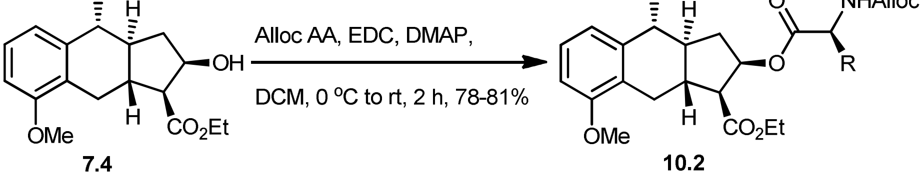
(1S,2S,3aR,4R,9aR)-ethyl 4-((tert-butyldimethylsilyl)oxy) -2-hydroxy-8-methoxy-2,3,3a,4,9,9a- hexahydro-1H-cyclopenta[b]naphthalene-1-carboxylate (7.4); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.20 (t, *J* = 7.96 Hz, 1H), 7.11 (d, *J* = 7.89 Hz, 1H), 6.74 (d, *J* = 7.91 Hz, 1H), 4.69 (d, *J* = 9.84 Hz, 1H), 4.62-4.58 (m, 1H), 4.29-4.19 (m, 2H), 3.80 (s, 3H), 3.23 (dd, *J* = 15.92, 3.99 Hz, 1H), 2.83 (s, 1H), 2.69-2.58 (m, 2H), 2.40-2.21 (m, 2H), 1.78-1.68 (m, 1H), 1.59-1.45 (m, 1H), 1.32 (t, *J* = 7.14 Hz, 3H), 0.95 (s, 9H), 0.22 (s, 3H), 0.16 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 173.1, 156.7, 142.0, 126.4, 125.1, 119.1, 108.0, 75.9, 72.2, 60.8, 56.1, 55.4, 47.3, 40.5, 40.3, 29.3, 26.1, 18.3, 14.3, -3.2, -3.9; LRMS: (ES+) m/z = 421 (M+1).



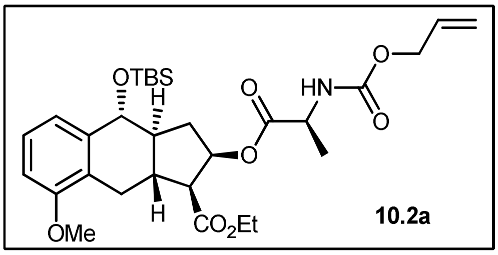
7.5

(1S,2R,3aR,4R,9aR)-ethyl 4-((tert-butyldimethylsilyl)oxy)-2-hydroxy -8-methoxy-2,3,3a,4,9,9a- hexahydro-1H-cyclopenta[b]naphthalene-1-carboxylate (7.5); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.20 (t, *J* = 7.97 Hz, 1H), 7.10 (d, *J* = 7.86 Hz, 1H), 6.73 (d, *J* = 7.95 Hz, 1H), 4.64-4.55 (m, 2H), 4.26-4.17 (m, 2H), 3.81 (s, 3H), 3.19 (dd, *J* = 16.96, 5.20 Hz, 1H), 2.60 (dd, *J* = 10.95, 5.34 Hz, 1H), 2.38 (dd, *J* = 16.95, 11.53 Hz, 1H), 2.24-1.80 (m, 5H), 1.31 (t, *J* = 7.13 Hz, 3H), 0.95 (s, 9H), 0.23 (d, *J* = 5.34 Hz, 3H), 0.13 (s, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 174.1, 156.7, 141.7, 126.4, 125.2, 119.2, 108.1, 75.8, 75.4, 60.8, 60.5, 55.4, 47.5, 42.6, 40.2, 29.2, 26.1, 18.3, 14.4, -3.2, -3.9; LRMS: (ES+) m/z = 421 (M+1).

3.6.2. Synthesis of 9.1:

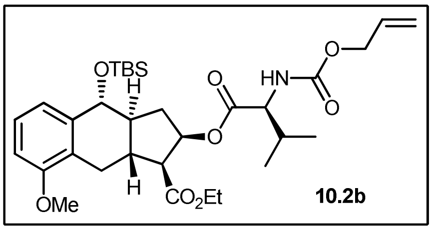


To a solution hydroxyl compound **7.4** (1 mmol) and Alloc protected amino acid (1.5 mmol) in DCM was allowed to 0 ^{o}C. Then added EDC chloride (1.1 mmol) and DMAP (1.2 mmol) at 0 ^{o}C. further allowed for stirring at room temperature for 2 hours. After completion of the reaction, reaction mixture was quenched with 5% HCl, dialuted with DCM and washed with aqueous $NaHCO_3$ solution, followed by brine solution. Then collected the organic phase and dried over Na_2SO_4. After evaporating the solvent, the residue was purified by flash column chromatography (10% EtOAc/hexanes) to obtain pure products.



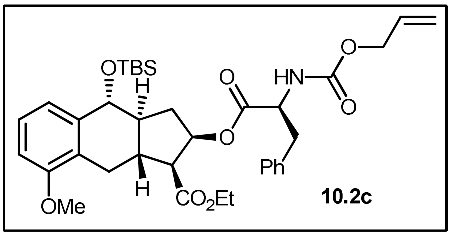
10.2a

(1S,2S,3aR,4R,9aR)-ethyl 2-(((S)-2-(((allyloxy) carbonyl) amino) propanoyl)oxy)-4-((tert-butyldimethylsilyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta [b] naphthalene-1-carboxylate (10.2a); Molecular Formula: $C_{30}H_{45}NO_8Si$; R_f (ethyl acetate/hexane 10%): 0.2; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.20 (t, *J* = 7.95 Hz, 1H), 7.10 (d, *J* = 7.86, 1H), 6.74 (d, *J* = 7.95 Hz, 1H), 5.96-5.85 (m, 1H), 5.58-5.48 (m, 1H), 5.40-5.16 (m, 4H), 4.73-4.53 (m, 4H), 4.40-4.28 (m, 1H), 4.25-4.13 (m, 2H), 3.84-3.77 (m, 3H), 3.27-3.20 (m, 1H), 2.84-2.57 (m, 2H), 2.53-2.04 (m, 3H), 1.81-1.76 (m, 1H), 1.65-1.48 (m, 1H), 1.47-1.37 (m, 3H), 0.98-0.91 (m, 9H), 0.27-0.19 (m, 3H), 0.15-0.05 (m, 3H); **^{13}C NMR (101 MHz, $CDCl_3$) δ ppm** 173.1, 170.1, 156.7, 155.3, 141.6, 141.3, 132.6, 126.6, 126.5, 124.8, 119.0, 117.8, 117.7, 108.1, 108.1, 78.3, 75.9, 75.4, 72.2, 65.7, 61.4, 61.0, 60.7, 55.3, 49.6, 49.5, 48.0, 47.1, 47.0, 43.7, 40.1, 40.0, 38.1, 29.1, 28.7, 26.0, 18.2, 14.1, -3.3, -3.9; LRMS: (ES+) m/z = 576 (M+1).



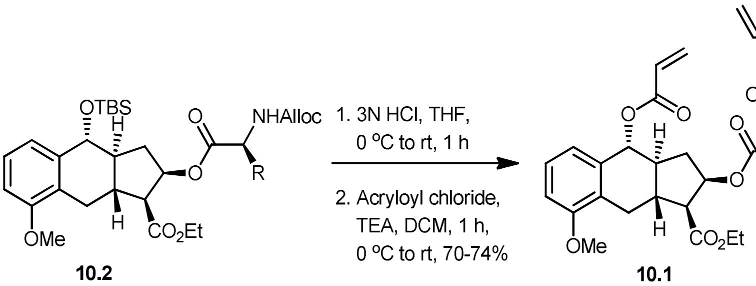
10.2b

(1S,2S,3aR,4R,9aR)-ethyl 2- (((S)-2- (((allyloxy)carbonyl) amino) -3-methylbutanoyl)oxy)-4-((tert-butyldimethylsilyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]naphthalene-1-carboxylate (10.2b); Molecular Formula: $C_{32}H_{49}NO_8Si$; R_f (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.20 (t, *J* = 7.97 Hz, 1H), 7.10 (dd, *J* = 7.81, 2.82 Hz, 1H), 6.74 (d, *J* = 7.99 Hz, 1H), 5.99-5.84 (m, 1H), 5.58-5.46 (m, 1H), 5.34-5.27 (m, 1H), 5.23-5.16 (m, 2H), 4.68 (t, *J* = 9.25 Hz, 1H), 4.56 (t, *J* = 5.65 Hz, 2H), 4.32-3.96 (m, 3H), 3.81 (s, 3H), 3.24 (d, *J* = 3.13 Hz, 1H), 2.84-2.69 (m, 2H), 2.45-2.35 (m, 1H), 2.25-2.08 (m, 2H), 1.87-1.72 (m, 1H), 1.54-1.47 (m, 1H), 1.28-1.25 (m, 3H), 0.96 (d, *J* = 9.64 Hz, 12H), 0.89-0.84 (m, 3H), 0.24-0.19 (m, 3H), 0.10 (d, *J* = 6.15 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.1, 171.0, 156.8, 156.8, 141.7, 132.7, 126.5, 124.9, 119.2, 119.0, 117.7, 108.2, 108.1, 76.0, 75.8, 65.8, 60.8, 60.7, 59.1, 58.7, 55.3, 54.8, 54.7, 47.3, 47.1, 40.1, 38.7, 38.5, 31.1, 29.7, 28.7, 26.0, 19.1, 18.9, 18.2, 17.4, 17.1,14.2, 14.1, -3.2, -3.3, -3.9; LRMS: (ES+) m/z = 604 (M+1).



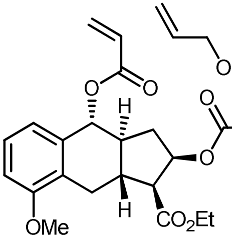
10.2c

(1S,2S,3aR,4R,9aR)-ethyl-2-(((S)-2-(((allyloxy)carbonyl)amino)-3-phenylpropanoyl) oxy) -4- ((tert-butyldimethylsilyl)oxy) -8- methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta [b] naphthalene-1-carboxylate (10.2c); Molecular Formula: $C_{36}H_{49}NO_8Si$; R_f (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.33-7.18 (m, 5H), 7.17-7.08 (m, 3H), 6.74 (d, *J* = 7.93 Hz, 1H), 5.95-5.81 (m, 1H), 5.55-5.46 (m, 1H), 5.23 (dd, *J* = 32.34, 13.81 Hz, 2H), 4.70-4.50 (m, 4H), 4.29-4.03 (m, 2H), 3.81 (s, 3H), 3.27-3.20 (m, 1H), 3.15-3.06 (m, 2H), 2.83-2.67 (m, 2H), 2.46-2.30 (m, 1H), 2.25-2.17 (m, 1H), 1.84-1.69 (m, 1H), 1.49-1.39 (m, 1H), 1.28-1.23 (m, 3H), 0.95 (s, 9H), 0.23 (s, 3H), 0.10 (s, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 170.5, 170.2, 170.1, 156.8, , 155.4, 141.6, 135.7, 132.6, 129.4, 128.6, 127.1, 126.5, 124.9, 119.1, 117.7, 108.2, 75.8, 75.7, 75.6, 65.8, 60.9, 55.3, 54.7, 54.5, 47.2, 47.1, 40.1, 38.6, 38.3, 38.0, 29.7, 28.7, 26.1, 26.0, 18.2, 14.3, 14.2, -3.1, -3.9; LRMS: (ES+) m/z = 652 (M+1).



To a solution compound **10.2** (1 mmol) in THF (5 mL) was added 3N HCl (5 mL) further allowed for stirring at room temperature for 2 hours. After completion of the reaction, reaction mixture was allowed to 0 °C and quenched with aqueous $NaHCO_3$ solution. and extracted with ethyl acetate (twise), combined the organic layer and dried over Na_2SO_4. After evaporating the solvent, the residue was further subjected next reaction without purification.

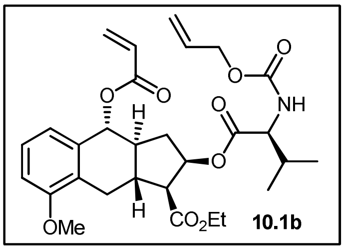
The alcoholic compound (1 mmol) was taken in DCM, allowed to 0 °C and added triethyl amine (2 mmol) followed by acryloyl chloride (1.5 mmol) at same temperature. After addition the reaction was allowed to room temperature for 1 h. after completion of the reaction added aqueous $NaHCO_3$ solution and extracted with DCM, evaporated under redused pressure. The residue was purified by flash column chromatography to obtain pure product.



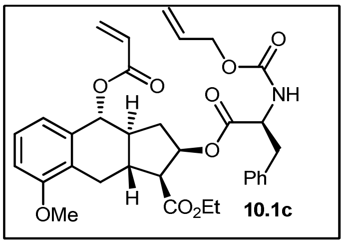
10.1a

(1S,2S,3aR,4R,9aR)-ethyl 4-(acryloyloxy)-2-(((S) -2- (((allyloxy)carbonyl) amino) propanoyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]naphthalene-1-carboxylate (10.1a); Molecular Formula: $C_{27}H_{33}NO_9$; R$_f$ (ethyl acetate/hexane 20%): 0.35; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.20 (t, *J* = 8.01 Hz, 1H), 6.79 (d, *J* = 8.08 Hz, 2H), 6.55-6.46 (m, 1H), 6.26-6.15 (m, 2H), 5.97-5.87 (m, 2H), 5.57-5,5 (m, 1H), 5.33-5.28 (m, 1H), 5.21 (d, *J* = 10.44 Hz, 1H), 4.58 (d, *J* = 4.73 Hz, 2H), 4.27-4.16 (m, 2H), 3.84 (s, 3H), 3.35-3.30 (m, 1H), 2.84-2.78 (m, 1H), 2.74-2.57 (m, 1H), 2.57-2.43 (m, 1H), 2.33-2.20 (m, 1H), 2.17-2.02 (m, 1H), 2.01-1.89 (m, 1H), 1.77-1.61 (m, 2H), 1.43-1.35 (m, 3H), 1.30-1.26 (m,3H); **^{13}C**

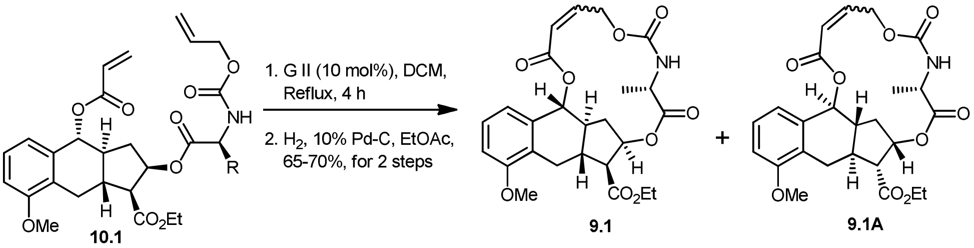
NMR (101 MHz, CDCl$_3$) δ ppm 172.6, 172.5, 171.9, 169.8, 169.7, 166.3, 166.2, 157.0, 157.0, 156.9, 156.9, 136.7, 131.6, 131.5, 128.2, 127.0, 126.9, 125.5, 118.5, 117.7, 117.7, 108.8, 78.2, 75.8, 65.7, 61.0, 60.8, 57.4, 55.3, 54.7, 54.5, 49.5, 45.6, 45.6, 44.8, 44.8, 43.5, 40.0, 39.9, 37.0, 37.0, 28.4, 25.6, 18.6, 14.2, 14.2; LRMS: (ES+) m/z = 516 (M+1).



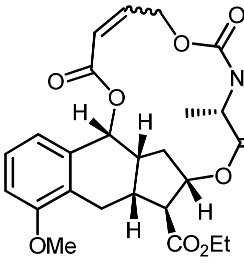
(1S,2S,3aR,4R,9aR)-ethyl 4-(acryloyloxy)-2-(((S)-2- (((allyloxy)carbonyl)amino) - 3- methylbutanoyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b] naphthalene -1-carboxylate (10.1b); Molecular Formula: $C_{29}H_{37}NO_9$; R$_f$ (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.18 (t, *J* = 7.98 Hz, 1H), 6.77 (d, *J* = 8.12 Hz, 2H), 6.47 (dd, *J* = 17.32, 1.39 Hz, 1H), 6.24-6.11 (m, 2H), 5.97-5.86 (m, 2H), 5.59-5.44 (m, 1H), 5.35-5.27 (m, 1H), 5.24-5.13 (m, 2H), 4.57 (s, 2H), 4.30-4.03 (m, 3H), 3.82 (s, 3H), 3.33 (dd, *J* = 16.89, 4.89 Hz, 1H), 2.83-2.76 (m, 1H), 2.71-2.47 (m, 2H), 2.29-2.22 (m, 1H), 2.19-2.06 (m, 1H), 1.98-1.88 (m, 1H), 1.69-1.62 (m, 2H), 1.30-1.26 (m, 3H), 0.96 (d, *J* = 6.85 Hz, 3H), 0.87 (dd, *J* = 14.75, 6.87 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 170.9, 169.8, 169.7, 166.2, 157.0, 136.7, 131.4, 128.3, 126.9, 125.6, 118.6, 117.8, 108.8, 75.8, 75.4, 60.9, 55.3, 54.6, 44.9, 44.8, 40.0, 39.9, 37.0, 29.7, 28.5, 19.1, 18.9, 17.3, 17.0, 14.1; LRMS: (ES+) m/z = 544 (M+1).



(1S,2S,3aR,4R,9aR)-ethyl4-(acryloyloxy)-2-(((S)-2-(((allyloxy)carbonyl)amino)-3-phenylpropanoyl) oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b] naphthalene-1-carboxylate (10.1c); Molecular Formula: $C_{33}H_{37}NO_9$; R$_f$ (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.35-7.28 (m, 2H), 7.26-7.11 (m, 4H), 6.78 (dd, *J* = 8.00, 2.67 Hz, 2H), 6.50 (ddd, *J* = 17.32, 2.83, 1.39 Hz, 1H), 6.21 (ddd, *J* = 17.32, 10.41, 2.94 Hz, 1H), 6.13 (d, *J* = 10.25 Hz, 1H), 5.96-5.83 (m, 2H), 5.51-5.45 (m, 1H), 5.27 (d, *J* = 17.19 Hz, 1H), 5.23-5.16 (m, 1H), 4.64-4.54 (m, 3H), 4.31-4.03 (m, 2H), 3.83 (s, 3H), 3.31 (td, *J* = 16.68, 4.84 Hz, 1H), 3.19-2.92 (m, 2H), 2.85-2.74 (m, 1H), 2.71-2.33 (m, 2H), 2.33-2.17 (m, 1H), 1.99-1.83 (m, 1H), 1.66-1.49 (m, 1H), 1.29-1.24 (m, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.2, 171.1, 167.6, 158.4, 158.4, 138.2, 130.7, 130.7, 130.1, 130.0, 129.7, 129.7, 128.6, 128.4, 126.9, 120.0, 119.1, 110.2, 77.2, 67.2, 62.4, 56.8, 56.1, 31.1, 29.9, 15.6; LRMS: (ES+) m/z = 592 (M+1).



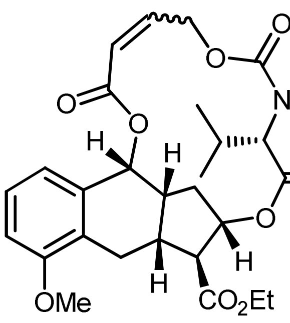
To the solution of *bis*-allyl compound **10.1** (1 mmol) in dry DCM (high dilution) under nitrogen atmosphere and Grubbs' 2nd generation catalyst (0.1 mmol) was added and reaction mixture was heated to 40 °C for 4 h. The reaction mixture was concentrated under reduced pressure and the crude product was purified by flash chromatography to obtain pure product.



9.1a

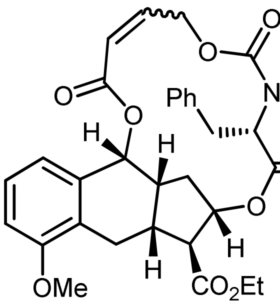
9.1: Molecular Formula: $C_{25}H_{29}NO_9$; R_f (ethyl acetate/hexane 40%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.18 (d, *J* = 3.44 Hz, 1H), 6.77 (d, *J* = 8.01 Hz, 2H), 6.56-6.44 (m, 1H), 6.27-6.09 (m, 1H), 5.97-5.86 (m, 1H), 5.82 (bs, 1H), 5.59-5.45 (m, 1H), 5.32-5.20 (m, 1H), 4.56 (bs, 2H), 4.38-4.26 (m, 1H), 4.25-4.13 (m, 2H), 3.82 (s, 3H), 3.38-3.20 (m, 1H), 2.87-2.75 (m, 1H), 2.73-2.57 (m, 1H), 2.54-2.38 (m, 1H), 2.34-

2.21 (m, 1H), 2.14-2.02 (m, 1H), 1.40-1.33 (m, 3H), 0.88 (t, *J* = 6.77 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.9, 169.7, 166.3, 157.0, 157.0, 145.6, 139.2, 136.7, 134.2, 131.5, 128.9, 128.3, 128.1, 126.9, 125.6, 118.6, 118.6, 117.7, 114.0, 108.8, 75.3, 64.5, 60.8, 55.4, 54.8, 54.5, 49.5, 44.9, 40.0, 37.0, 33.8, 31.9, 29.7, 29.6, 29.3, 28.5, 22.7, 14.2, 14.1; LRMS: (ES+) m/z = 488 (M+1).



9.1b

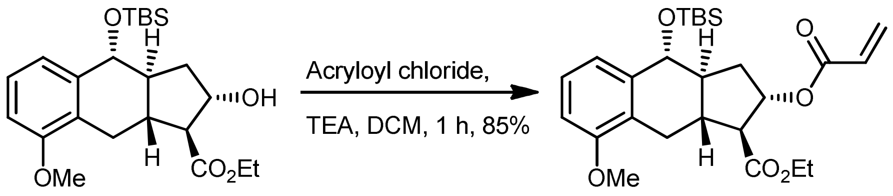
9.1b: Molecular Formula: $C_{27}H_{33}NO_9$; R$_f$ (ethyl acetate/hexane 40%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.18 (d, *J* = 3.61 Hz, 1H), 6.80-6.73 (m, 2H), 6.45 (d, *J* = 1.20 Hz, 1H), 6.19-6.09 (m, 1H), 5.95-5.81 (m, 2H), 5.58-5.44 (m, 1H), 5.25-5.12 (m, 1H), 4.56 (s, 2H), 4.27-4.17 (m, 2H), 3.82 (d, *J* = 3.55 Hz, 3H), 3.40-3.25 (m, 1H), 2.89-2.74 (m, 1H), 2.72-2.46 (m, 2H), 2.33-2.21 (m, 1H), 2.18-2.06 (m, 1H), 1.99-1.83 (m, 2H), 0.95 (d, *J* = 6.80 Hz, 3H), 0.91-0.81 (m, 6H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 171.1, 171.0, 169.7, 166.3, 157.0, 157.0, 155.9, 139.3, 136.7, 131.5, 128.9, 128.3, 128.2, 127.0, 125.6, 118.6, 114.1, 108.8, 75.8, 64.7, 61.0, 60.9, 58.9, 58.7, 55.4, 54.7, 45.0, 40.0, 39.9, 37.4, 37.1, 33.8, 31.9, 31.4, 31.2, 30.2, 29.7, 29.7, 29.6, 29.4, 29.2, 28.9, 28.5, 22.7, 19.2, 19.0, 18.1, 17.3, 17.0, 14.2, 14.1; LRMS: (ES+) m/z = 516 (M+1).



9.1c

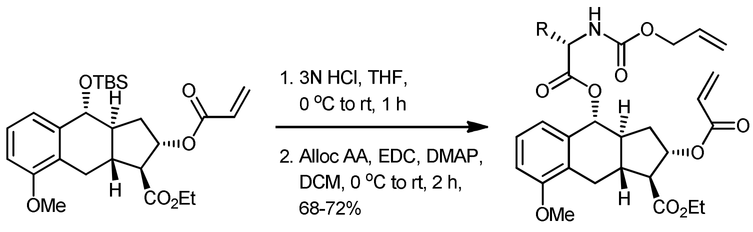
9.1c: Molecular Formula: $C_{31}H_{33}NO_9$; R$_f$ (ethyl acetate/hexane 40%): 0.25; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.36-7.08 (m, 6H), 6.78 (dd, *J* = 7.65, 2.76 Hz, 2H), 6.60-6.40 (m, 1H), 6.26-6.05 (m, 2H), 5.94-5.74 (m, 1H), 5.55-5.42 (m, 1H), 5.20-5.00 (m, 1H), 4.52 (s, 2H), 4.32-4.09 (m, 2H), 3.82 (s, 3H), 3.40-3.24 (m, 1H), 3.12-3.03 (m, 1H), 3.01-2.72 (m, 2H), 2.70-2.41 (m, 2H), 2.32-2.19 (m, 1H), 2.09-1.83 (m, 2H), 0.88 (t, *J* = 6.82 Hz, 3H).

3.6.3. synthesis of 9.2:

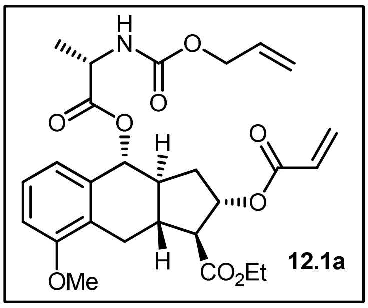


7.5 **12.2**

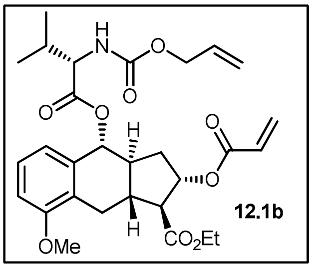
(1S,2R,3aR,4R,9aR)-ethyl 2-(acryloyloxy)-4-((tert-butyldimethylsilyl)oxy) -8-methoxy-2,3,3a,4,9,9a- hexahydro-1H-cyclopenta[b]naphthalene -1-carboxylate (12.2): The alcoholic compound **7.5** (1 mmol) was taken in DCM, allowed to 0 ^{o}C and added triethyl amine (2 mmol) followed by acryloyl chloride (1.5 mmol) at same temperature. After addition the reaction was allowed to room temperature for 1 h. after completion of the reaction added aqueous $NaHCO_3$ solution and extracted with DCM, evaporated under redused pressure. the residue was purified by flash column chromatography to obtain pure product **12.2** as white color solid. Molecular Formula: $C_{26}H_{38}O_6Si$; R$_f$ (ethyl acetate/hexane 10%): 0.4; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.21 (t, *J* = 7.97 Hz, 1H), 7.13 (d, *J* = 7.90 Hz, 1H), 6.75 (d, *J* = 7.93 Hz, 1H), 6.42 (dd, *J* = 17.33, 1.41 Hz, 1H), 6.13 (dd, *J* = 17.33, 10.41 Hz, 1H), 5.84 (dd, *J* = 10.43, 1.42 Hz, 1H), 5.55 (dd, *J* = 6.46, 4.70 Hz, 1H), 4.63 (d, *J* = 9.36 Hz, 1H), 4.23 (q, *J* = 7.11 Hz, 2H), 3.81 (s, 3H), 3.21 (dd, *J* = 17.05, 5.26 Hz, 1H), 2.73 (dd, *J* = 10.56, 4.56 Hz, 1H), 2.44 (dd, *J* = 16.98, 11.38 Hz, 1H), 2.29-2.20 (m, 1H), 2.17-2.10 (m, 1H), 2.09-1.89 (m, 2H), 1.31 (t, *J* = 7.13 Hz, 3H), 0.97 (s, 9H), 0.23 (s, 3H), 0.14 (s, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.3, 165.6, 156.7, 141.5, 130.9, 128.4, 126.5, 124.8, 119.1, 108.2, 77.5, 75.6, 60.9, 57.9, 55.3, 48.1, 43.7, 38.3, 29.1, 26.0, 18.2, 14.3, -3.3, -3.9; LRMS: (ES+) m/z = 475 (M+1).



Experimental procedure same as previous.

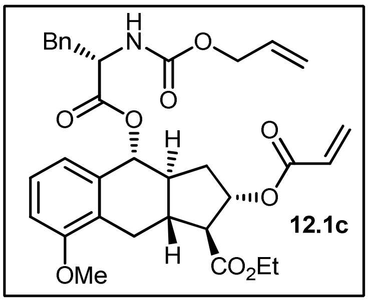

12.1a

(1S,2S,3aR,4R,9aR)-ethyl2-(acryloyloxy)-4-(((S)-2-(((allyloxy)carbonyl) amino) propanoyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]naphthalene-1-carboxylate (12.1a); Molecular Formula: $C_{27}H_{33}NO_9$; R$_f$ (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.21 (t, *J* = 7.97 Hz, 1H), 6.80-6.71 (m, 2H), 6.43 (dd, *J* = 17.33, 1.40 Hz, 1H), 6.19-6.09 (m, 1H), 6.06 (d, *J* = 9.63 Hz, 1H), 6.01-5.88 (m, 1H), 5.86 (dd, *J* = 10.44, 1.28 Hz, 1H), 5.58-5.50 (m, 1H), 5.40-5.19 (m, 3H), 4.61 (t, *J* = 5.25 Hz, 2H), 4.49-4.43 (m, 1H), 4.24 (q, *J* = 7.15 Hz, 2H), 3.85 (s, 3H), 3.26 (dd, *J* = 17.04, 4.84 Hz, 1H), 2.80-2.72 (m, 1H), 2.47 (dd, *J* = 17.05, 11.35 Hz, 1H), 2.30-2.20 (m, 1H), 2.18-2.03 (m, 3H), 1.54-1.48 (m, 3H), 1.31 (t, *J* = 7.13 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 173.1, 172.7,, 165.5, 157.0, 136.0, 135.9, 131.1, 131.1, 128.3, 128.2, 127.0, 125.7, 118.5, 117.8, 109.0, 108.9, 74.8, 67.9, 61.0, 61.0,57.5, 55.4, 48.5, 45.6, 45.5, 43.5, 43.4, 37.1, 36.9, 29.7, 28.8, 25.6, 18.7, 14.2; LRMS: (ES+) m/z = 516 (M+1).


12.1b

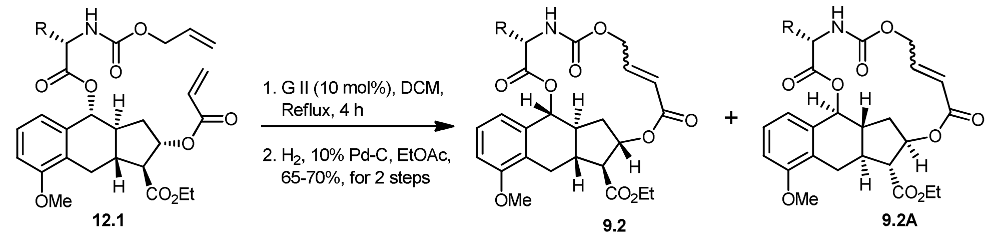
(1S,2S,3aR,4R,9aR)-ethyl 2-(acryloyloxy) -4-(((S)-2-(((allyloxy)carbonyl) amino)-3-methylbutanoyl)oxy) -8-methoxy-2,3,3a,4,9,9a- hexahydro -1H- cyclopenta [b]

naphthalene-1- carboxylate (12.1b); Molecular Formula: $C_{29}H_{37}NO_9$; R*f* (ethyl acetate/hexane 20%): 0.3; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.20 (q, *J* = 7.82 Hz, 1H), 6.84-6.70 (m, 2H), 6.41 (ddd, *J* = 17.33, 4.18, 1.35 Hz, 1H), 6.12 (ddd, *J* = 17.31, 10.42, 2.74 Hz, 1H), 6.05 (dd, *J* = 9.68, 3.66 Hz, 1H), 5.97-5.89 (m, 1H), 5.85-5.82 (m, 1H), 5.55-5.51 (m, 1H), 5.35-5.21 (m, 3H), 4.64-4.56 (m, 2H), 4.41-4.36 (m, 1H), 4.22 (q, *J* = 7.14 Hz, 2H), 3.83 (s, 3H), 3.24 (dd, *J* = 16.92, 4.64 Hz, 1H), 2.73 (dd, *J* = 10.65, 4.83 Hz, 1H), 2.44 (dd, *J* = 16.85, 11.41 Hz, 1H), 2.31-2.18 (m, 2H), 2.16-2.02 (m, 3H), 1.29 (t, *J* = 7.13 Hz, 3H), 1.04 (t, *J* = 7.09 Hz, 3H), 0.96 (dd, *J* = 16.11, 6.87 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 172.7, 165.5, 157.0, 156.9, 136.0, 131.1, 128.3, 128.2, 127.0, 117.9, 117.9. 109.0, 108.9, 65.9, 61.0, 61.0, 59.2, 57.5, 55.4, 45.8, 45.5, 43.5, 43.5, 37.3, 37.2, 31.0, 29.7, 28.7, 22.7, 19.4, 19.2, 17.3, 17.1, 14.2; LRMS: (ES+) m/z = 544 (M+1).

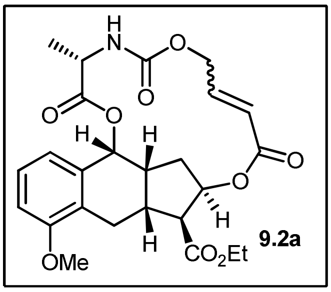


12.1c

(1S,2S,3aR,4R,9aR)-ethyl 2-(acryloyloxy) -4-(((S)-2- (((allyloxy)carbonyl) amino)-3-phenylpropanoyl)oxy)-8-methoxy-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta [b] naphthalene -1-carboxylate (12.1c); Molecular Formula: $C_{33}H_{37}NO_9$; R*f* (ethyl acetate/hexane 20%): 0.35; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.36-7.22 (m, 5H), 7.18-7.08 (m, 1H), 6.81-6.75 (m, 1H), 6.48-6.42 (m, 2H), 6.19-6.11 (m, 1H), 6.01 (d, *J* = 9.49 Hz, 1H), 5.94-5.78 (m, 2H), 5.57-5.49 (m, 1H), 5.33-5.18 (m, 3H), 4.82-4.70 (m, 1H), 4.57 (dd, *J* = 16.47, 5.57 Hz, 2H), 4.24 (q, *J* = 7.14 Hz, 2H), 3.84 (s, 3H), 3.29-3.19 (m, 2H), 3.17-3.08 (m, 1H), 2.74 (dd, *J* = 10.29, 4.69 Hz, 1H), 2.47-2.40 (m, 1H), 2.25-1.86 (m, 5H), 1.32 (t, *J* = 7.15 Hz, 3H); **^{13}C NMR (101 MHz, CDCl$_3$) δ ppm** 172.8, 165.5, 165.4, 156.9, 135.8, 131.1, 131.1, 129.4, 128.7, 128.3, 128.3, 127.2, 127.0, 125.7, 117.9, 108.9, 65.9, 61.0, 57.6, 57.5, 55.4, 45.7, 45.5, 43.5, 37.2, 29.7, 28.7, 14.2; LRMS: (ES+) m/z = 592 (M+1).

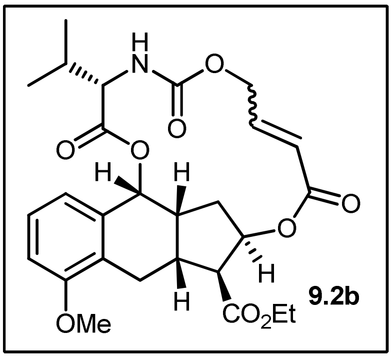


Experimental procedure same as previous.



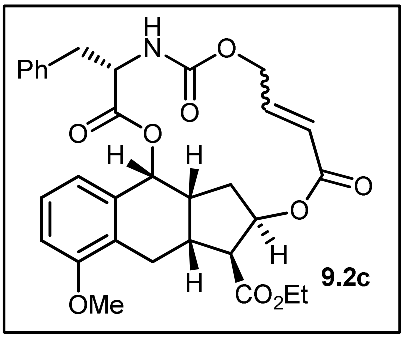
9.2a

9.2a: Molecular Formula: $C_{25}H_{29}NO_9$; R_f (ethyl acetate/hexane 40%): 0.3; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.23-7.15 (m, 1H), 6.98-6.86 (m, 1H), 6.82-6.64 (m, 2H), 6.08-5.92 (m, 2H), 5.55-5.43 (m, 1H), 4.84-4.66 (m, 1H), 4.64-4.53 (m, 1H), 4.49-4.33 (m, 1H), 4.21 (d, *J* = 7.13 Hz, 2H), 3.82 (s, 3H), 3.30-3.15 (m, 1H), 2.79-2.65 (m, 1H), 2.52-2.24 (m, 2H), 2.15-1.98 (m, 3H), 1.31 (d, *J* = 11.64 Hz, 3H), 0.88 (t, *J* = 5.36 Hz, 3H)



9.2b

9.2b: Molecular Formula: $C_{27}H_{33}NO_9$; R_f (ethyl acetate/hexane 40%): 0.3; **^{1}H NMR (400 MHz, $CDCl_3$) δ ppm** 7.24-7.11 (m, 1H), 6.99-6.86 (m, 1H), 6.77 (d, *J* = 8.21 Hz, 2H), 6.10-5.90 (m, 2H), 5.58-5.39 (m, 1H), 4.77-4.49 (m, 1H), 4.42-4.27 (m, 1H), 4.21 (d, *J* = 7.10 Hz, 2H), 3.82 (s, 3H), 3.32-3.16 (m, 1H), 2.81-2.63 (m, 1H), 2.57-2.14 (m, 4H), 2.04 (s, 3H), 1.13-0.95 (m, 6H), 0.88 (t, *J* = 6.80 Hz, 3H)



9.2c: Molecular Formula: $C_{31}H_{33}NO_9$; R_f (ethyl acetate/hexane 40%): 0.25; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.43-7.12 (m, 7H), 6.80-6.69 (m, 1H), 6.53-6.36 (m, 1H), 6.06-5.92 (m, 1H), 5.89-5.66 (m, 1H), 5.61-5.37 (m, 1H), 5.35-5.07 (m, 1H), 4.80-4.42 (m, 3H), 4.29-4.15 (m, 2H), 3.81 (s, 3H), 3.28-3.03 (m, 3H), 2.86-2.60 (m, 1H), 2.48-2.30 (m, 1H), 2.22-1.89 (m, 5H), 0.88 (t, *J* = 6.74 Hz, 3H)

3.7. References:

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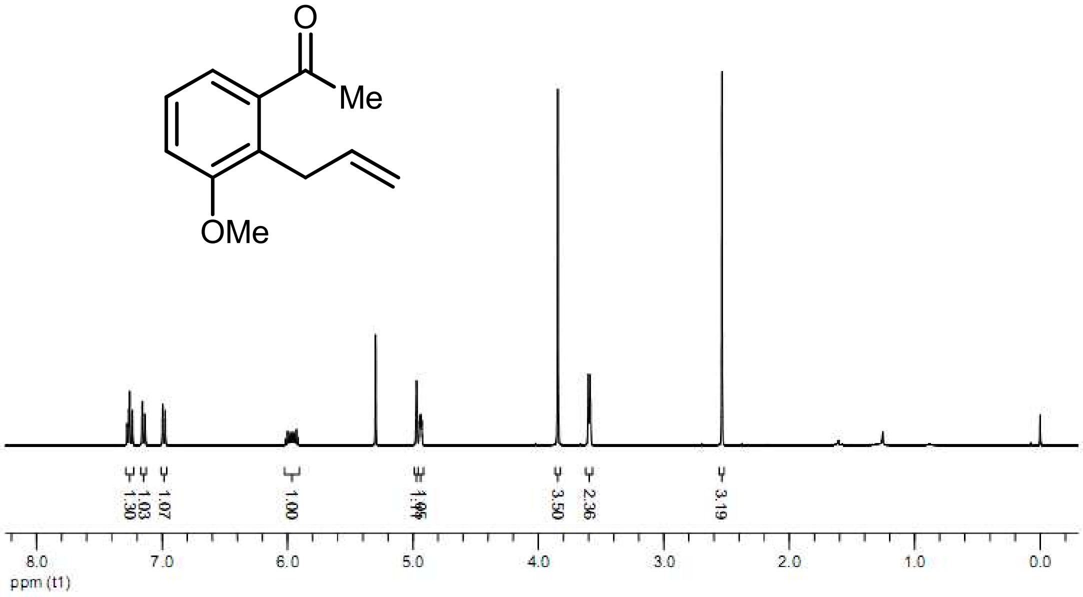
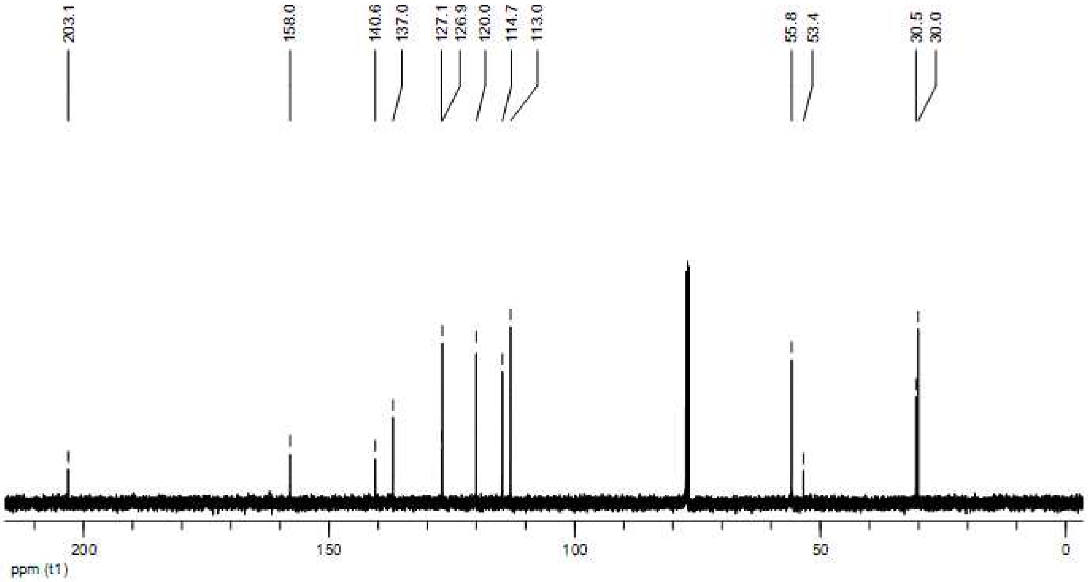
3.8. Spectra:

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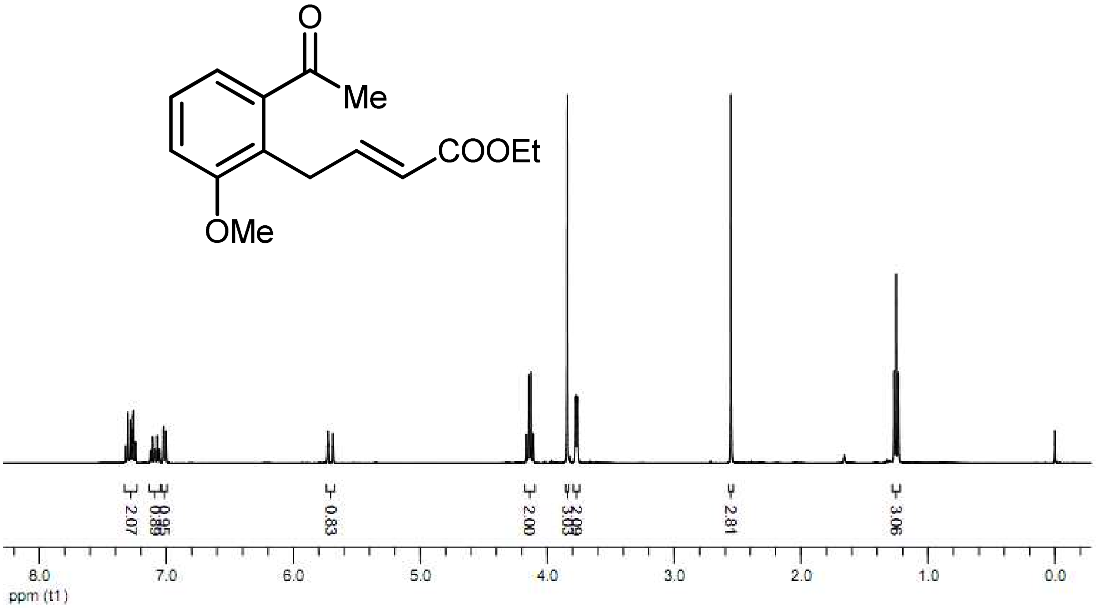
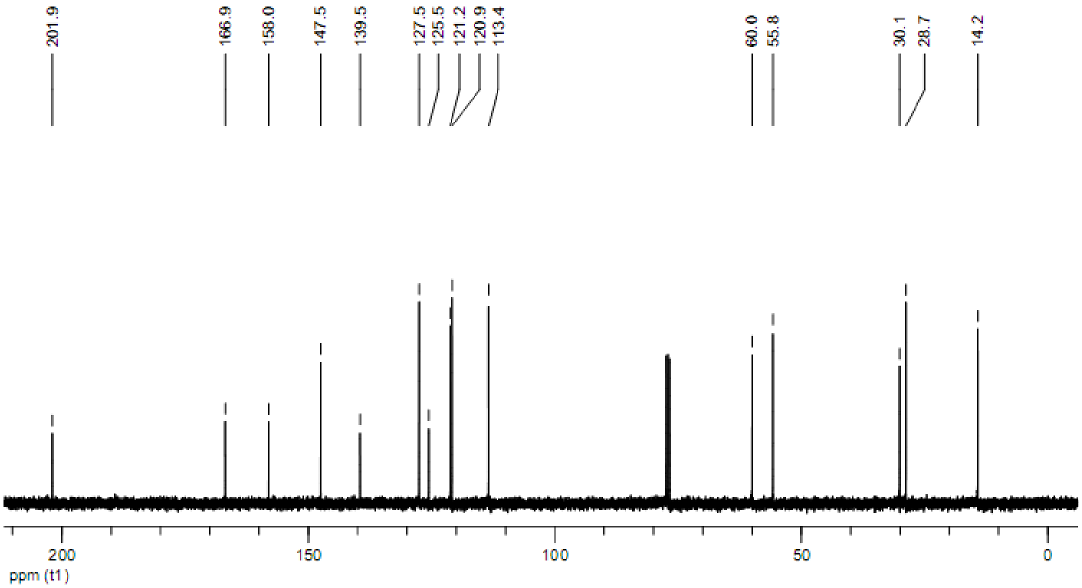
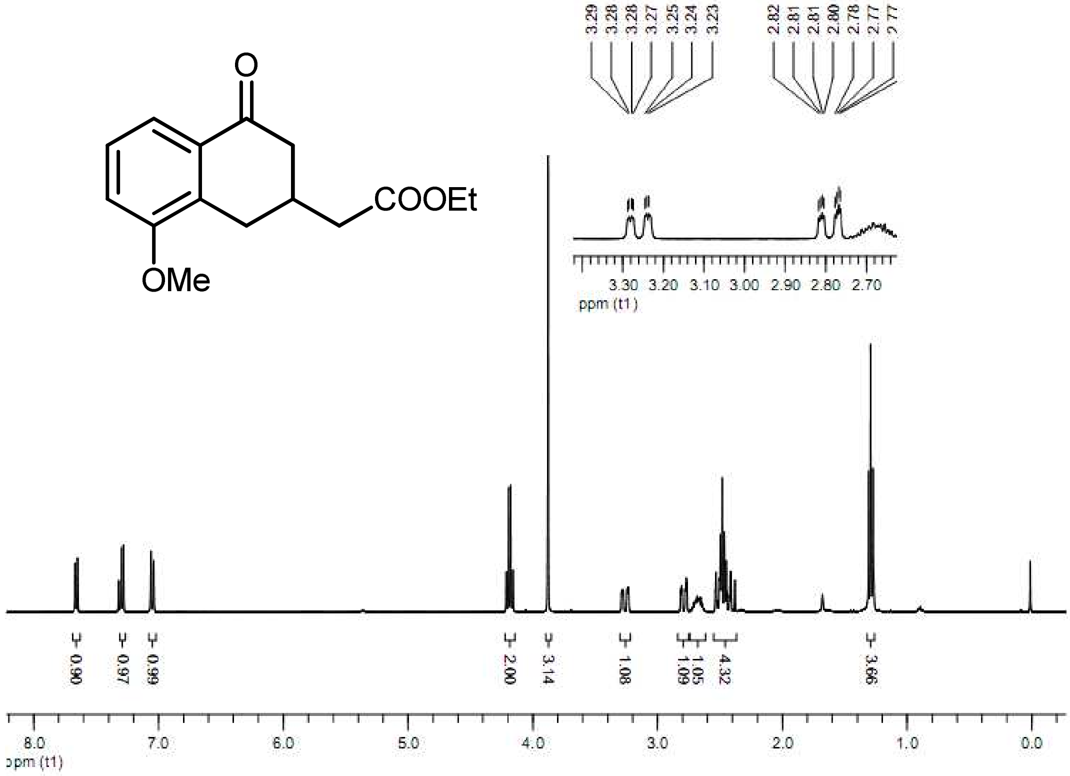
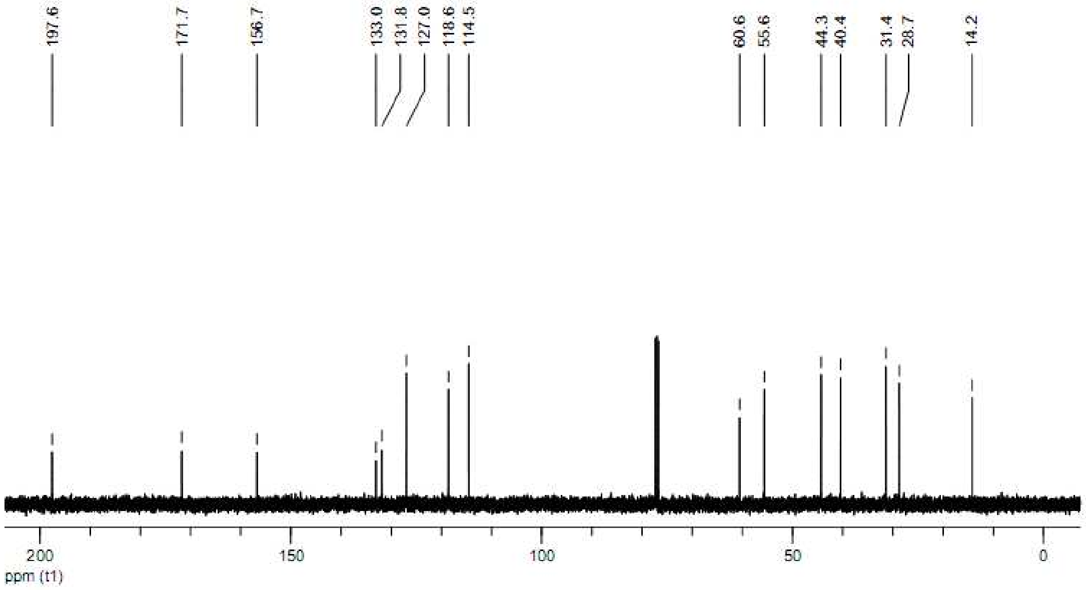
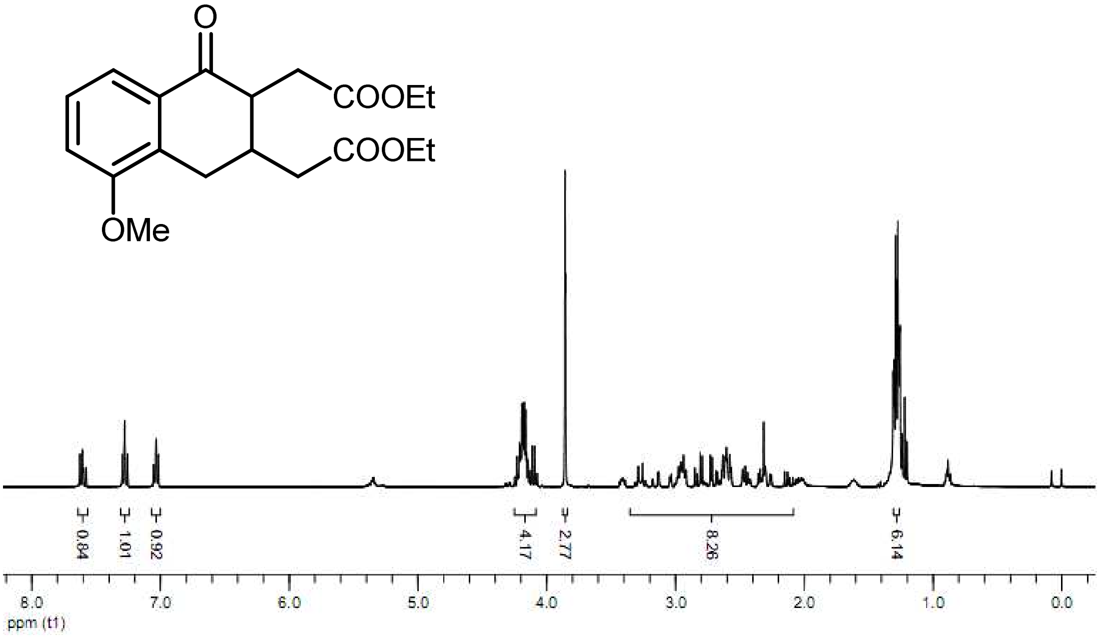
158.0
140.6
137.0
127.1
126.9
120.0
114.7
113.0
55.8
53.4
30.5
30.0
200
150
100
50
0
ppm (t1)

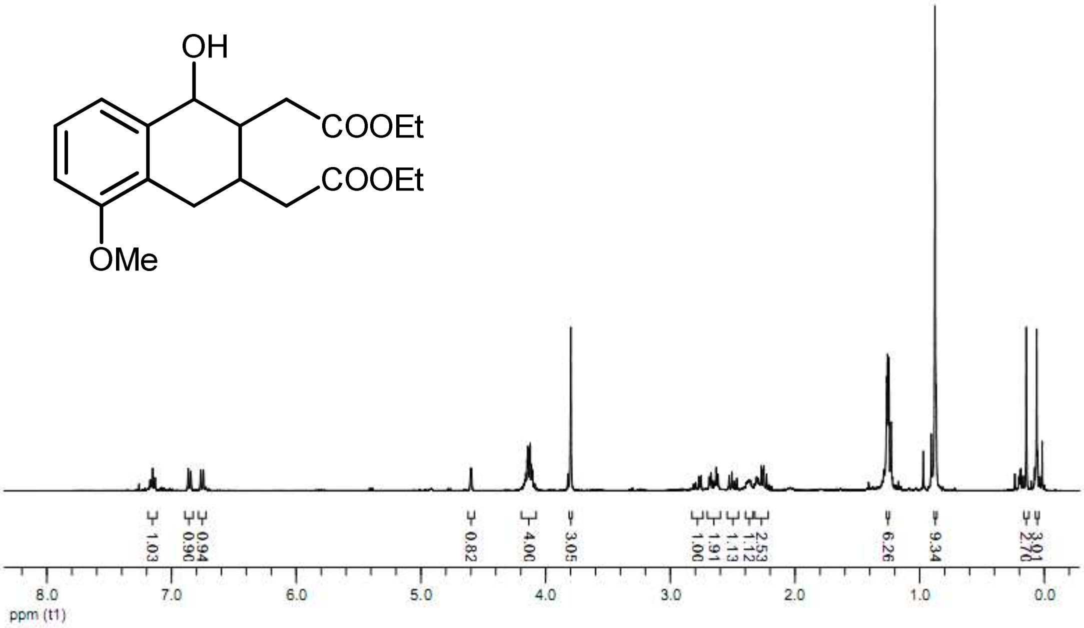

O
Me
COOEt
OMe
2.07
0.85
0.95
0.83
2.00
2.09
3.03
2.81
3.06
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

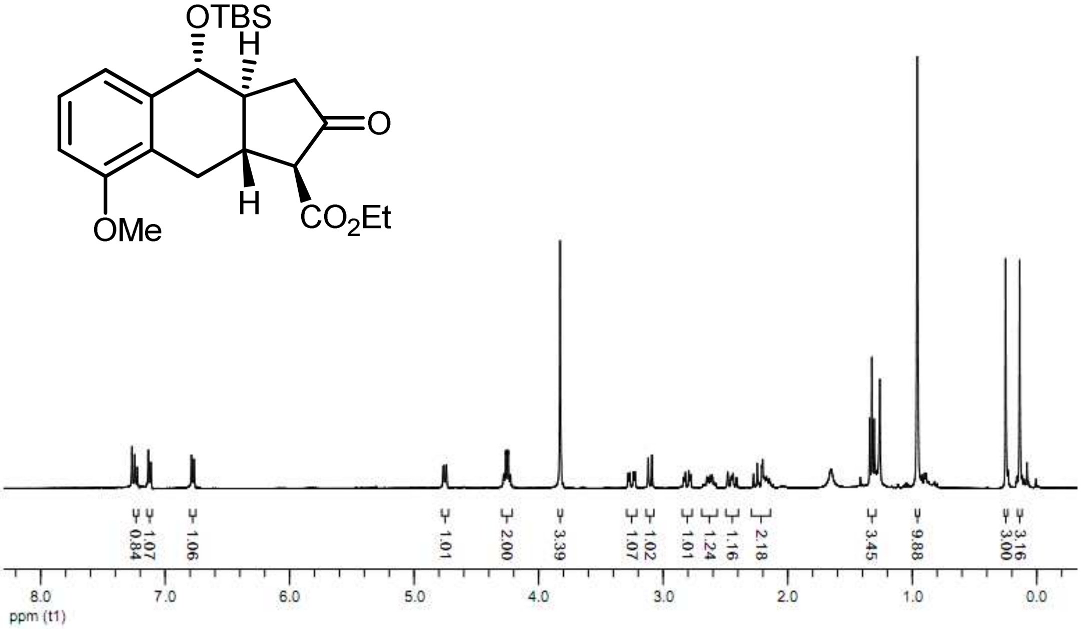

201.9
166.9
158.0
147.5
139.5
127.5
125.5
121.2
120.9
113.4
60.0
55.8
30.1
28.7
14.2
200
150
100
50
0
ppm (t1)


O
COOEt
OMe
3.29
3.28
3.28
3.27
3.25
3.24
3.23
2.82
2.81
2.81
2.80
2.78
2.77
2.77
3.30
3.20
3.10
3.00
2.90
2.80
2.70
ppm (t1)
0.90
0.97
0.99
2.00
3.14
1.08
1.09
1.05
4.32
3.66
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


197.6
171.7
156.7
133.0
131.8
127.0
118.6
114.5
60.6
55.6
44.3
40.4
31.4
28.7
14.2
200
150
100
50
0
ppm (t1)


O
COOEt
COOEt
OMe
0.84
1.01
0.92
4.17
2.77
8.26
6.14
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


OH
COOEt
COOEt
OMe
1.03
0.90
0.94
0.82
4.00
3.05
1.00
1.91
1.13
1.12
2.53
6.26
9.34
2.70
3.01
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)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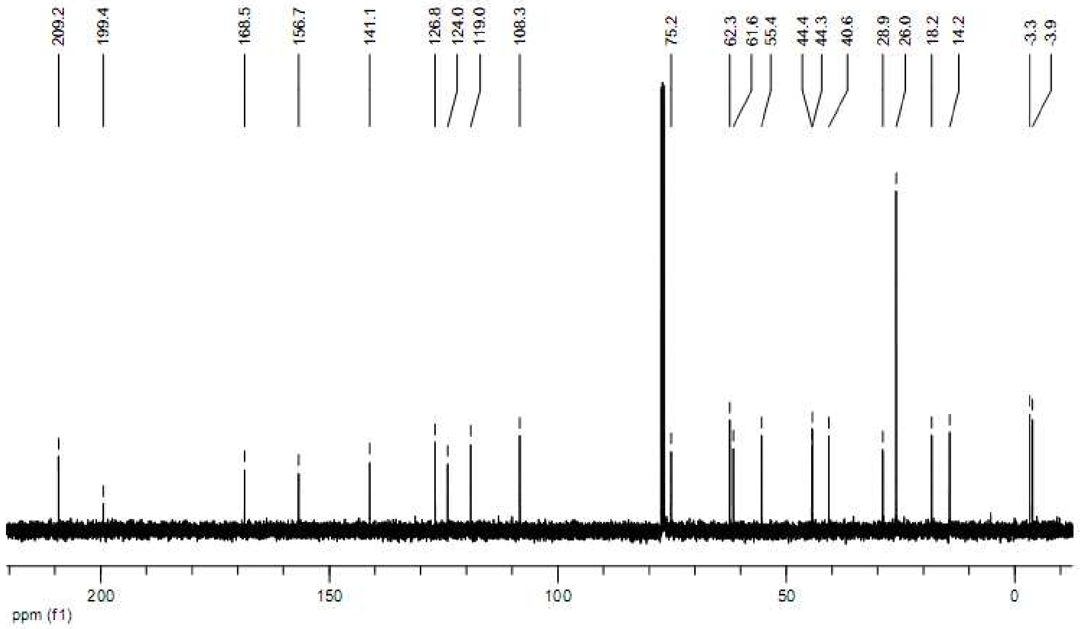
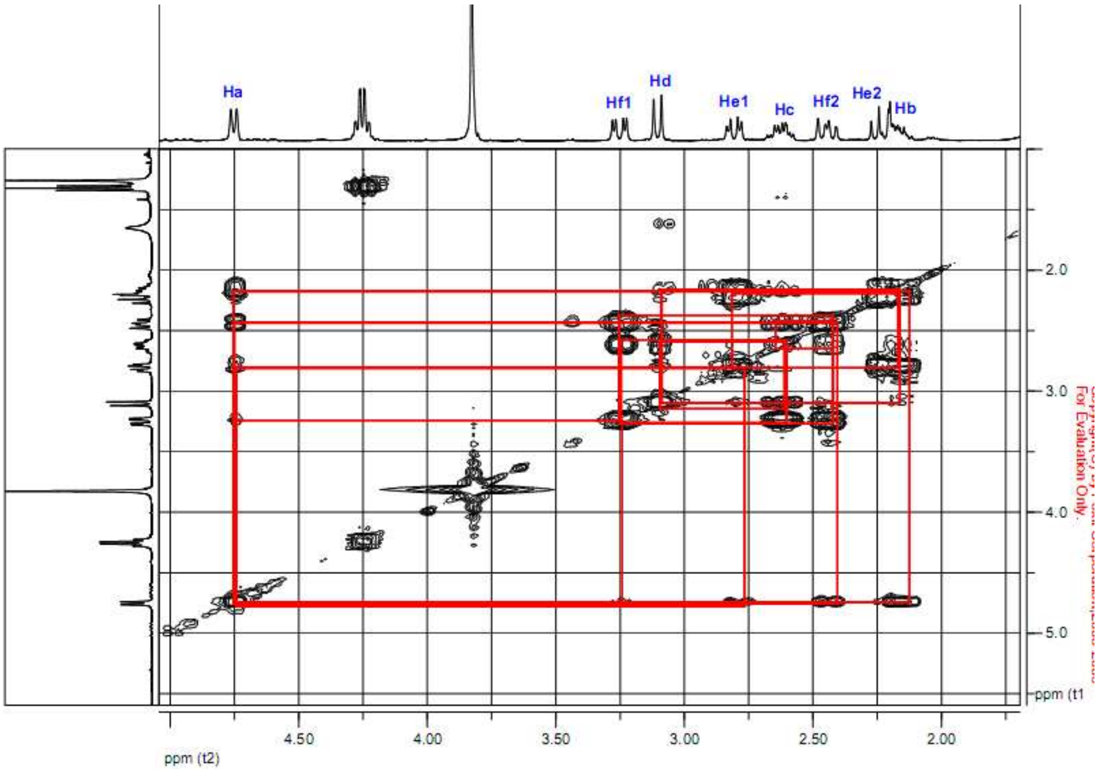
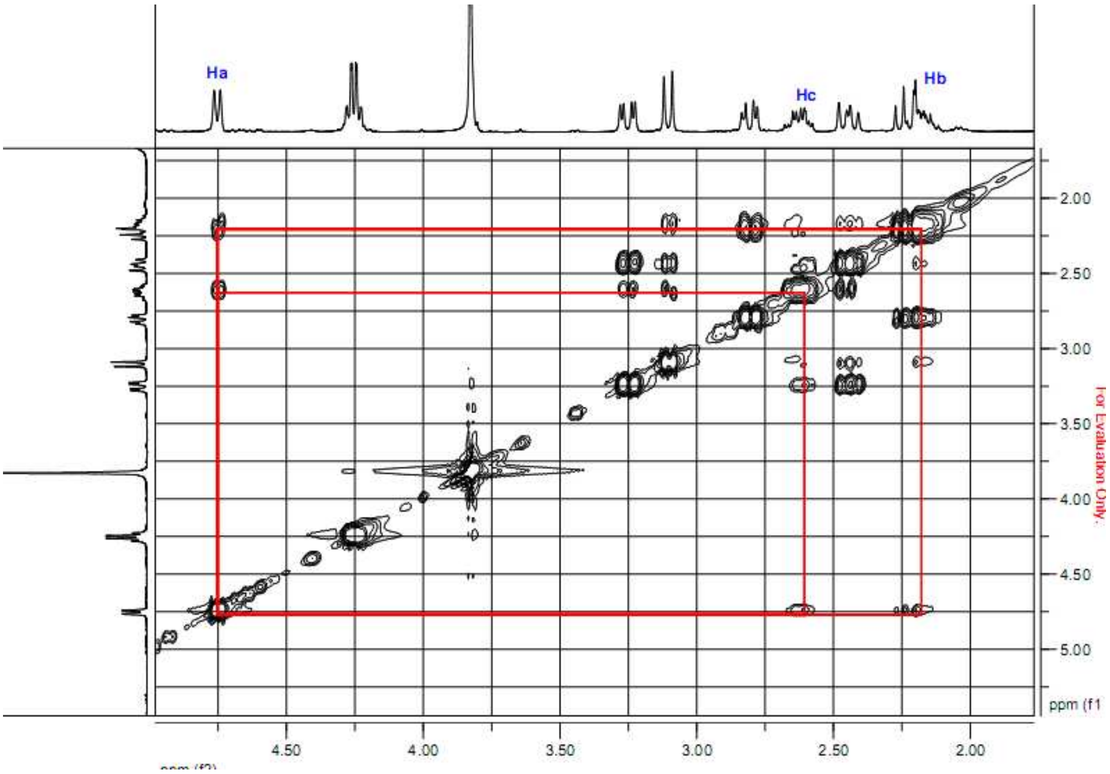
H
O
H
CO2Et
OMe
0.84
1.07
1.06
1.01
2.00
3.39
1.07
1.02
1.01
1.24
1.16
2.18
3.45
9.88
3.00
3.16
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


209.2
199.4
168.5
156.7
141.1
126.8
124.0
119.0
108.3
75.2
62.3
61.6
55.4
44.4
44.3
40.6
28.9
26.0
18.2
14.2
-3.3
-3.9
200
150
100
50
0
ppm (f1)

COSY


Ha
Hd
Hf1
He1
Hc
Hf2
He2
Hb
2.0
3.0
4.0
5.0
ppm (t1)
4.50
4.00
3.50
3.00
2.50
2.00
ppm (t2)
For Evaluation Only.

nOe


Ha
Hc
Hb
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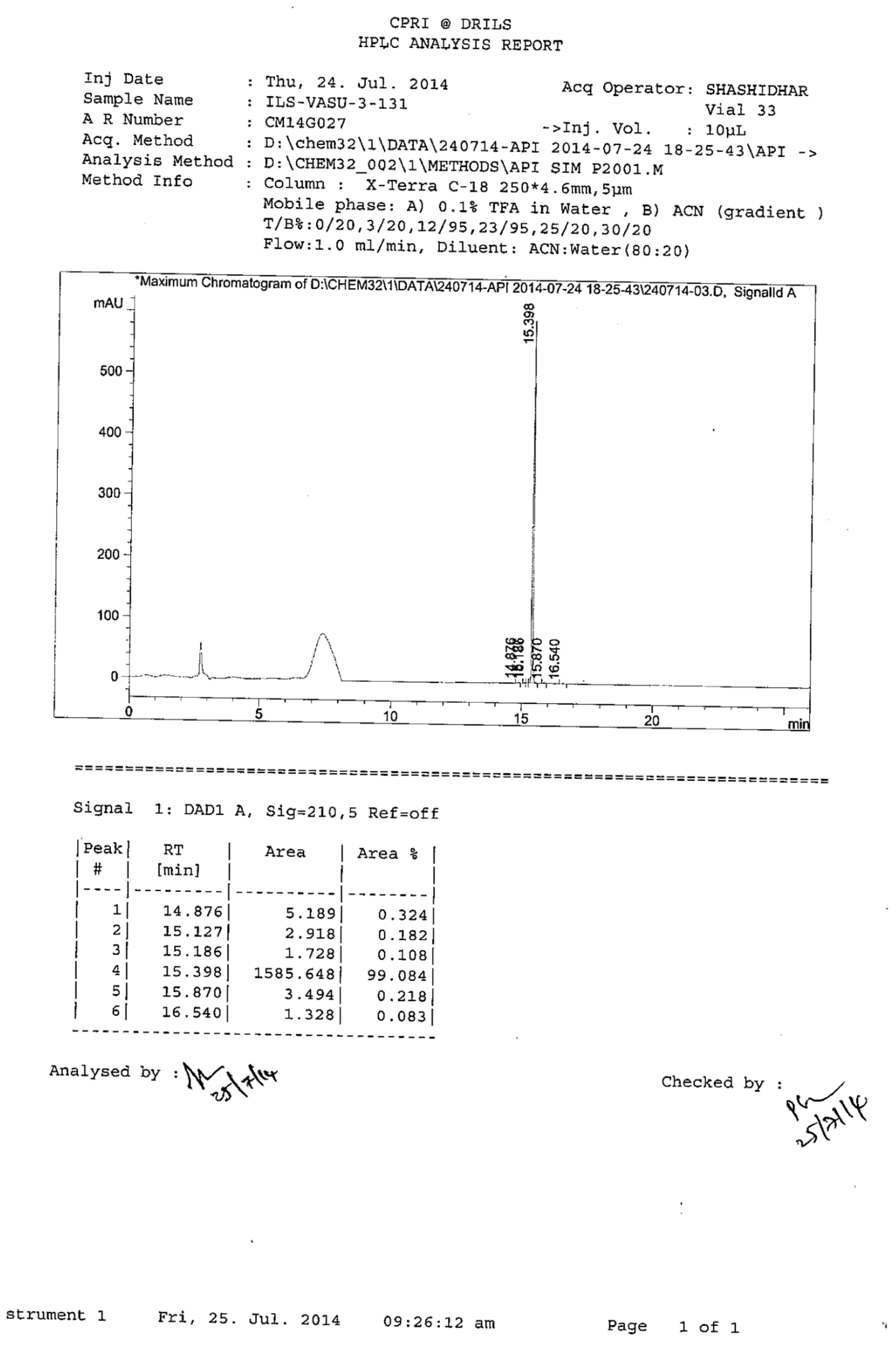
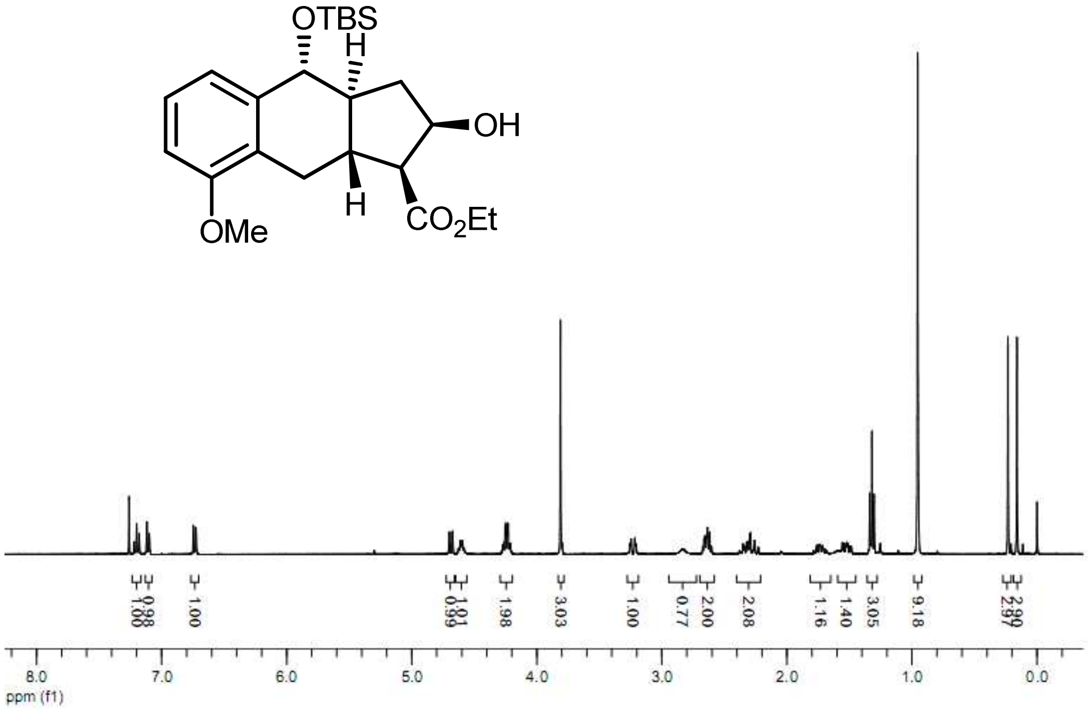
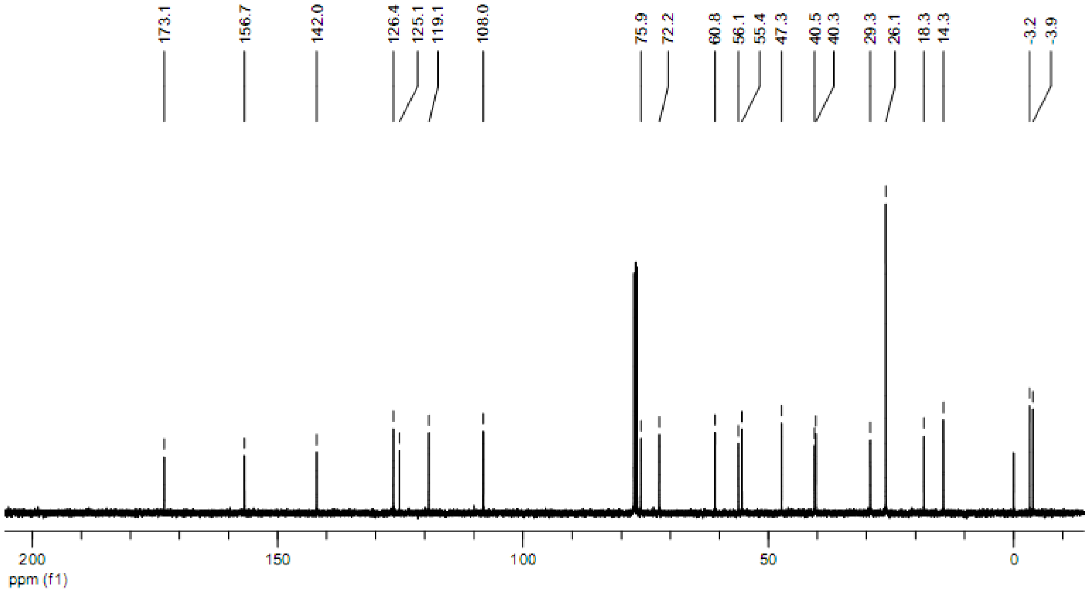
2.50
3.00
3.50
4.00
4.50
5.00
ppm (f1
4.50
4.00
3.50
3.00
2.50
2.00
ppm (f2)
For Evaluation Only.



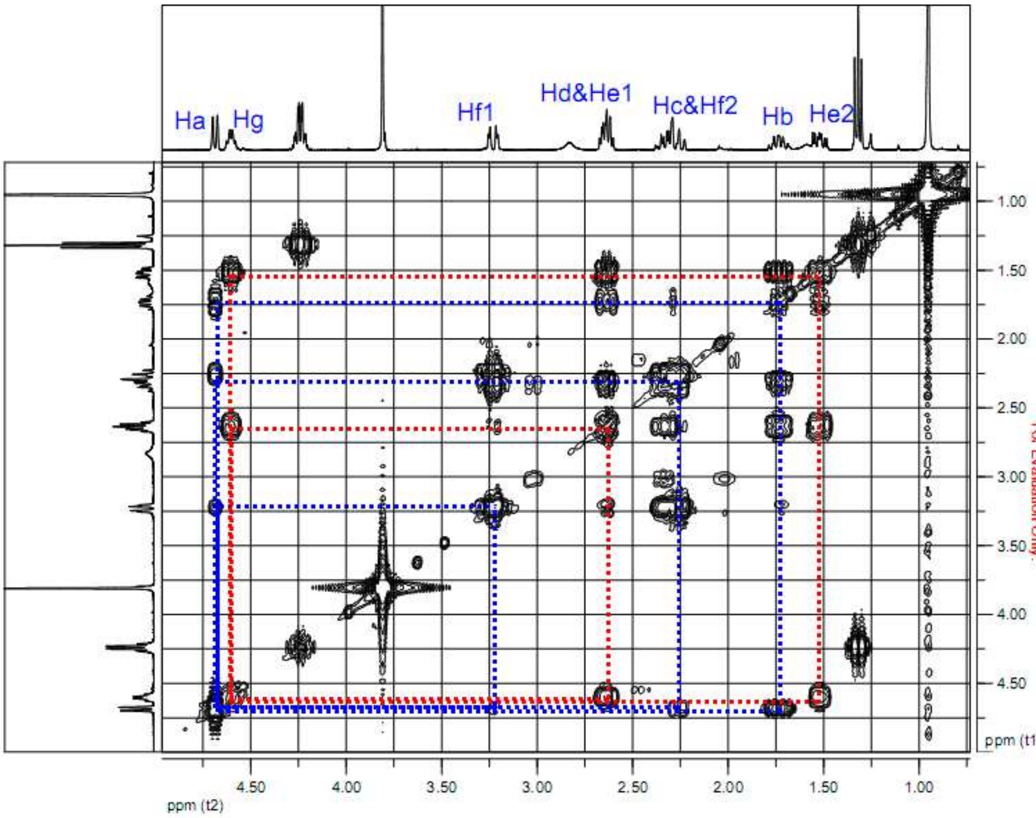
```
                         CPRI @ DRILS
                      HPLC ANALYSIS REPORT

Inj Date        : Thu, 24. Jul. 2014          Acq Operator: SHASHIDHAR
Sample Name     : ILS-VASU-3-131                            Vial 33
A R Number      : CM14G027                  ->Inj. Vol.   : 10µL
Acq. Method     : D:\chem32\1\DATA\240714-API 2014-07-24 18-25-43\API ->
Analysis Method : D:\CHEM32_002\1\METHODS\API SIM P2001.M
Method Info     : Column :  X-Terra C-18 250*4.6mm,5µm
                  Mobile phase: A) 0.1% TFA in Water , B) ACN (gradient )
                  T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                  Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```

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

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

|Peak|   RT    |   Area    | Area % |
| #  |  [min]  |           |        |
|----|---------|-----------|--------|
|   1|   14.876|      5.189|   0.324|
|   2|   15.127|      2.918|   0.182|
|   3|   15.186|      1.728|   0.108|
|   4|   15.398|   1585.648|  99.084|
|   5|   15.870|      3.494|   0.218|
|   6|   16.540|      1.328|   0.083|
-----------------------------------

Analysed by : 25/7/14                              Checked by :
                                                        25/7/14



strument 1     Fri, 25. Jul. 2014     09:26:12 am         Page   1 of 1
```


OTBS
H
OH
H
CO2Et
OMe
1.08
0.98
1.00
0.99
1.01
1.98
3.03
1.00
0.77
2.00
2.08
1.16
1.40
3.05
9.18
2.97
2.90
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


173.1
156.7
142.0
126.4
125.1
119.1
108.0
75.9
72.2
60.8
56.1
55.4
47.3
40.5
40.3
29.3
26.1
18.3
14.3
-3.2
-3.9
200
150
100
50
0
ppm (f1)

COSY


Ha
Hg
Hf1
Hd&He1
Hc&Hf2
Hb
He2
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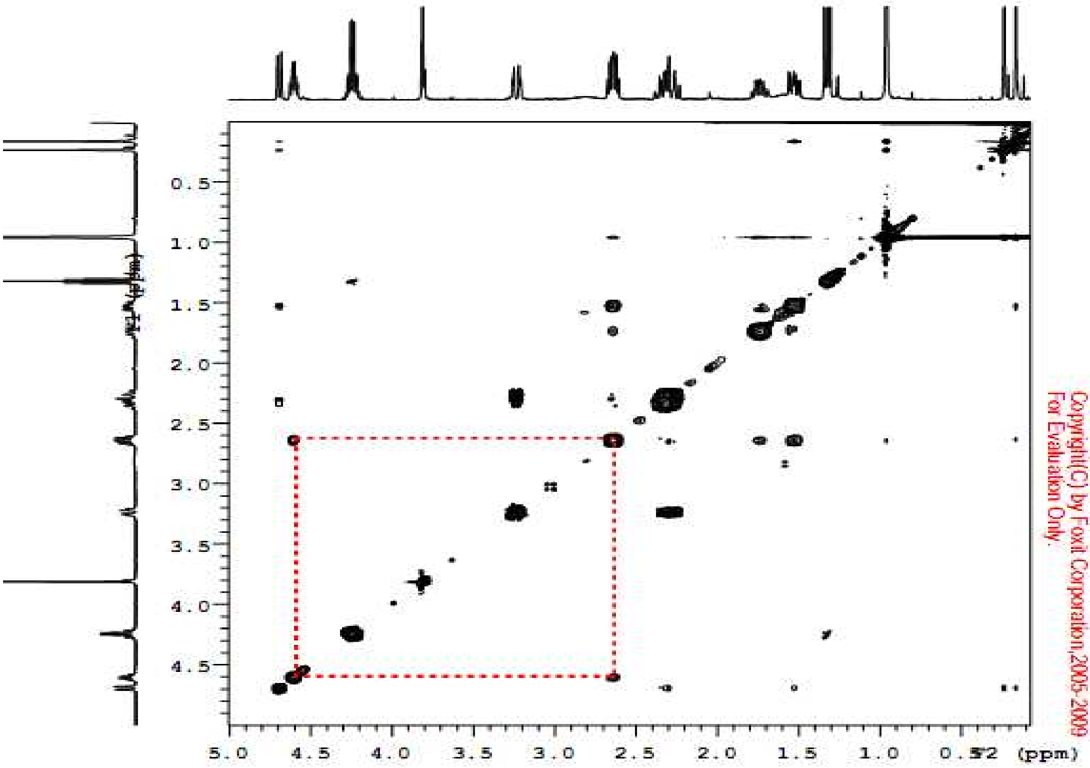
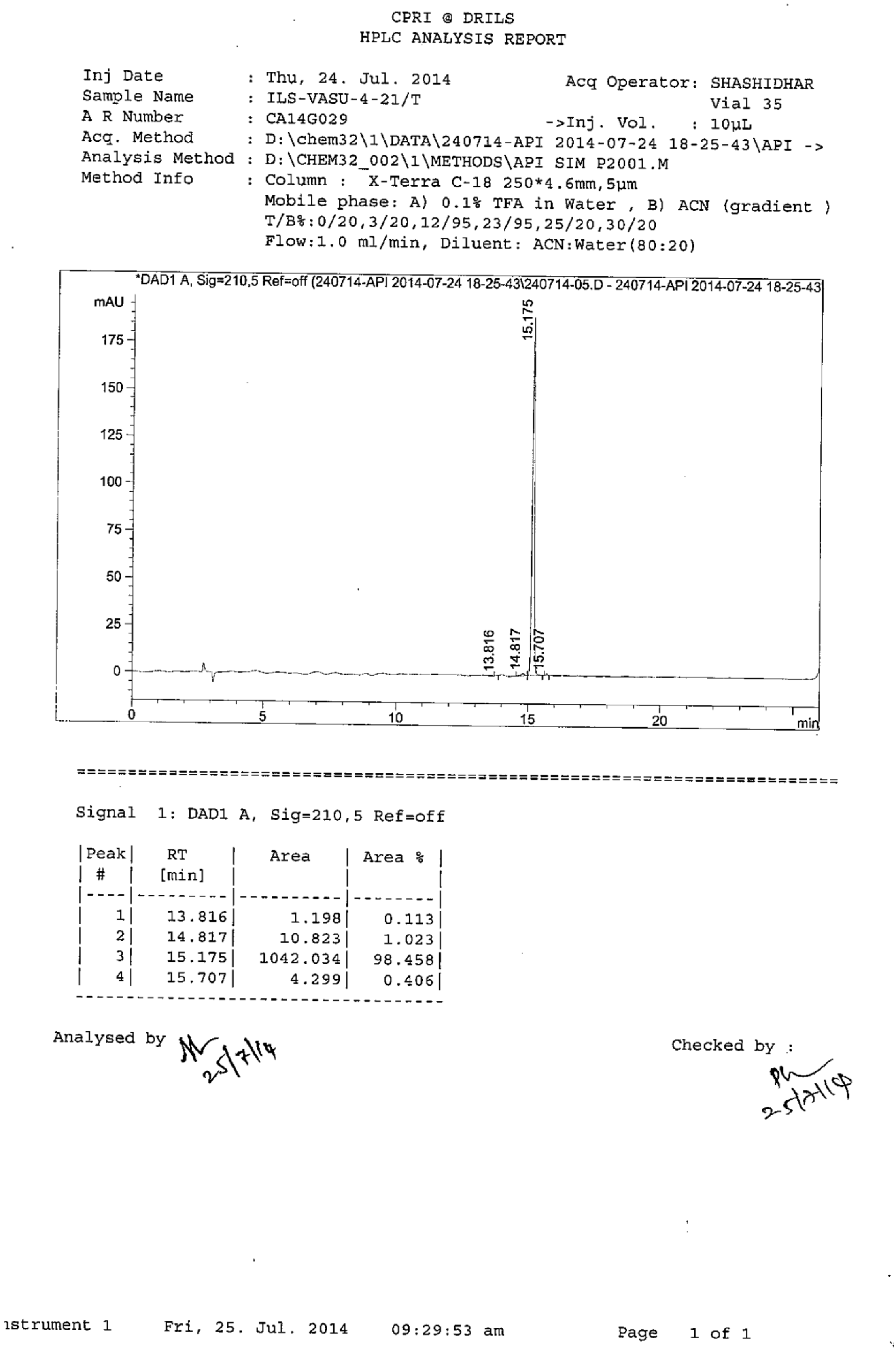
1.50
2.00
2.50
3.00
3.50
4.00
4.50
ppm (t1
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
ppm (t2)

nOe


0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)



```
                        CPRI @ DRILS
                    HPLC ANALYSIS REPORT

Inj Date         : Thu, 24. Jul. 2014          Acq Operator: SHASHIDHAR
Sample Name      : ILS-VASU-4-21/T                           Vial 35
A R Number       : CA14G029                  ->Inj. Vol.    : 10µL
Acq. Method      : D:\chem32\1\DATA\240714-API 2014-07-24 18-25-43\API ->
Analysis Method  : D:\CHEM32_002\1\METHODS\API SIM P2001.M
Method Info      : Column :  X-Terra C-18 250*4.6mm,5µm
                   Mobile phase: A) 0.1% TFA in Water , B) ACN (gradient )
                   T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                   Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```

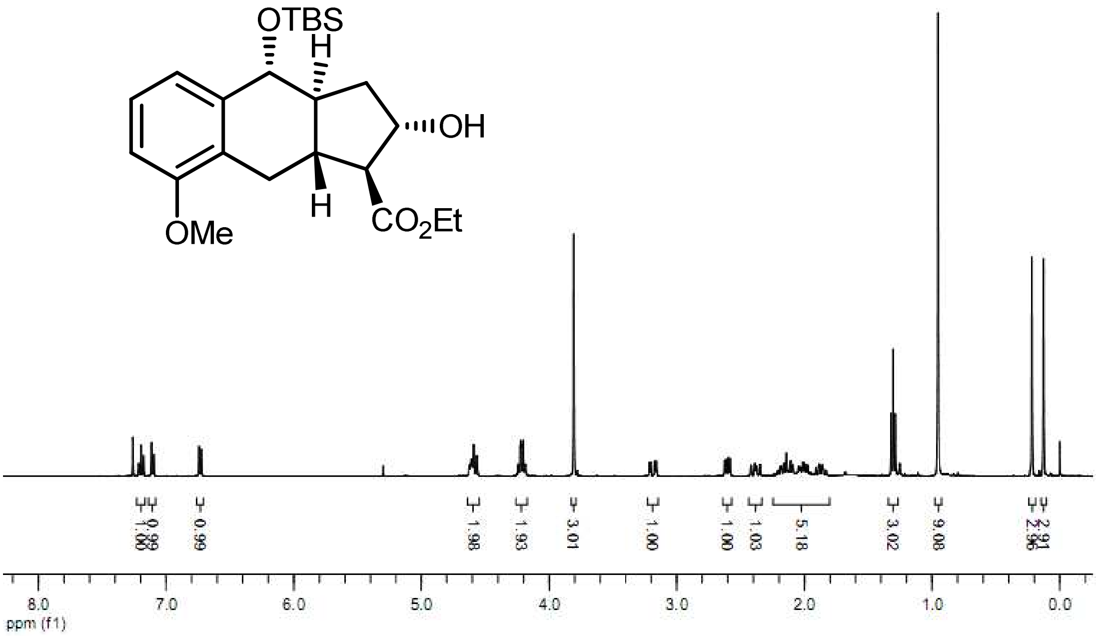
```
=========================================================================

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

|Peak|   RT    |   Area    | Area %  |
| #  |  [min]  |           |         |
|----|---------|-----------|---------|
|   1|   13.816|      1.198|    0.113|
|   2|   14.817|     10.823|    1.023|
|   3|   15.175|   1042.034|   98.458|
|   4|   15.707|      4.299|    0.406|
-------------------------------------
```

Analysed by 25/7/14   Checked by :
                                                             25/7/14

```
nstrument 1     Fri, 25. Jul. 2014     09:29:53 am          Page    1 of 1
```


OTBS
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OH
H
CO2Et
OMe
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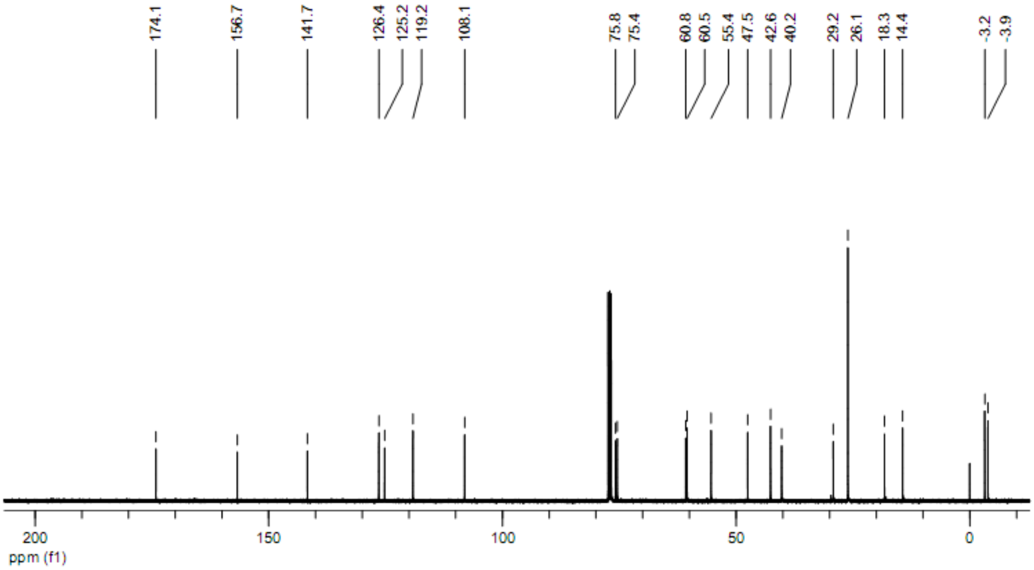
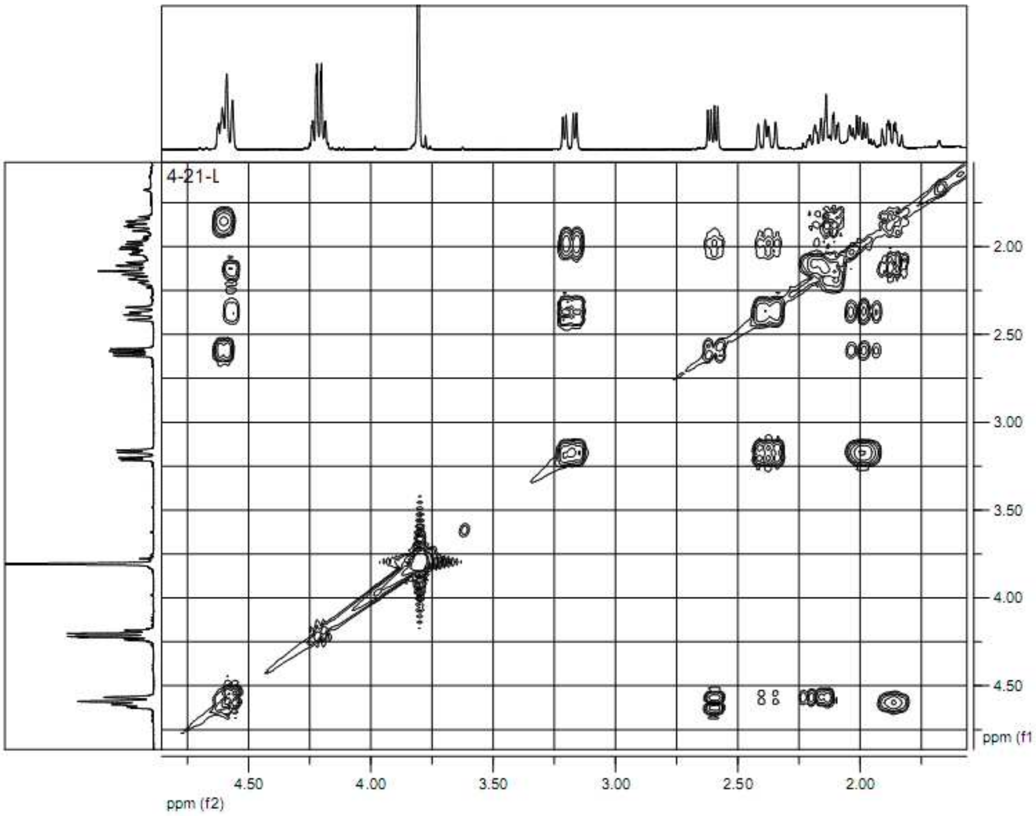
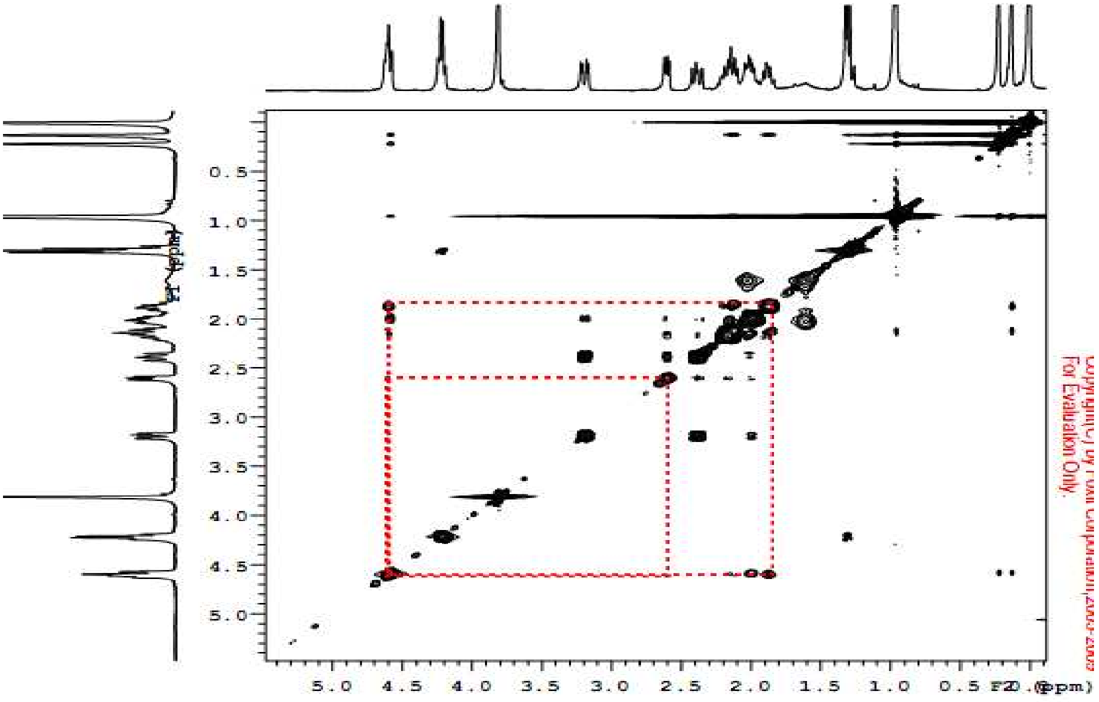
0.99
0.99
1.98
1.93
3.01
1.00
1.00
1.03
5.18
3.02
9.08
2.96
2.91
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

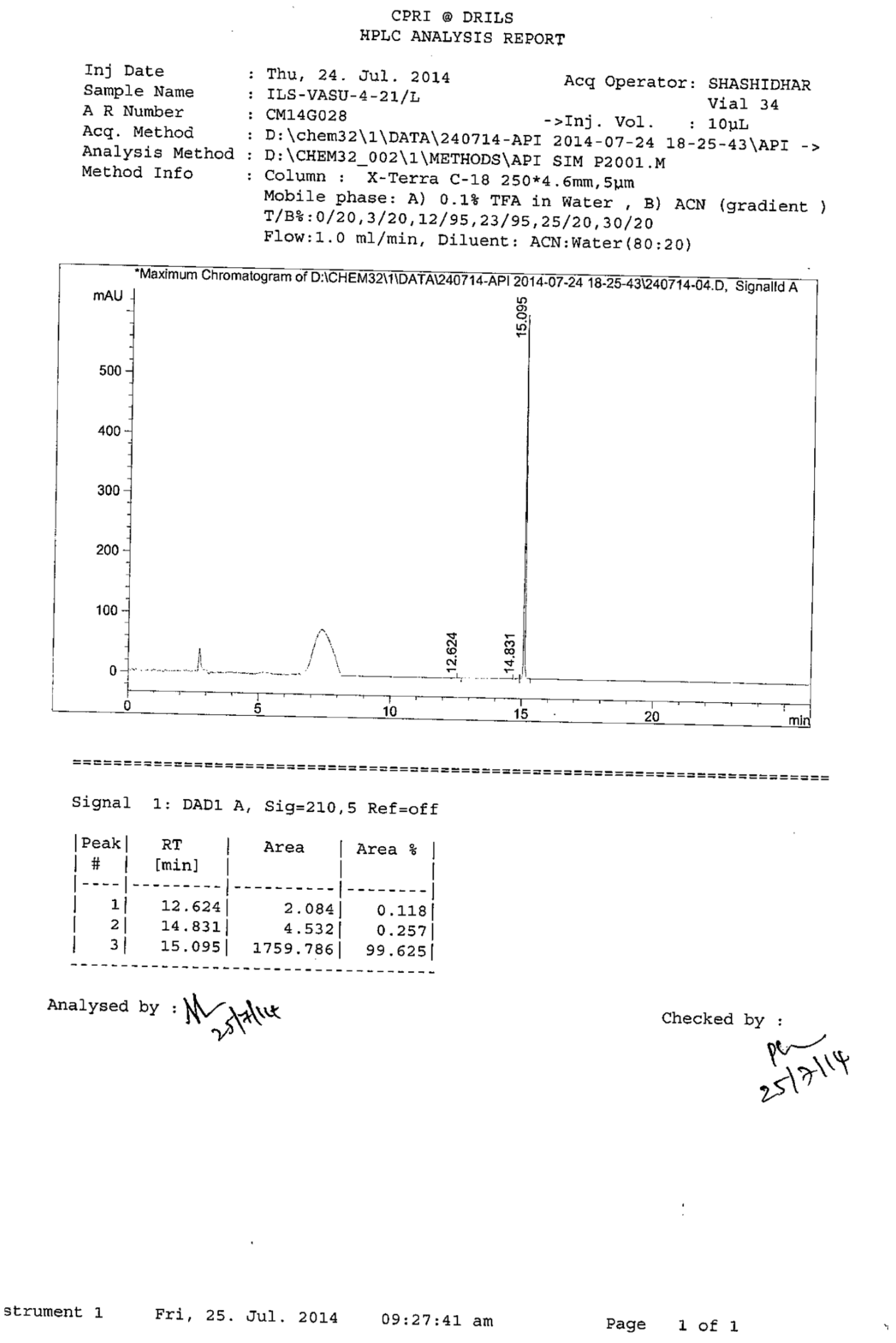

174.1
156.7
141.7
126.4
125.2
119.2
108.1
75.8
75.4
60.8
60.5
55.4
47.5
42.6
40.2
29.2
26.1
18.3
14.4
-3.2
-3.9
200
150
100
50
0
ppm (f1)

COSY


4-21-L
2.00
2.50
3.00
3.50
4.00
4.50
ppm (f1)
4.50
4.00
3.50
3.00
2.50
2.00
ppm (f2)

nOe


0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
F1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 (ppm)
For Evaluation Only.



```
                         CPRI @ DRILS
                     HPLC ANALYSIS REPORT

Inj Date          : Thu, 24. Jul. 2014          Acq Operator: SHASHIDHAR
Sample Name       : ILS-VASU-4-21/L                           Vial 34
A R Number        : CM14G028                   ->Inj. Vol.    : 10µL
Acq. Method       : D:\chem32\1\DATA\240714-API 2014-07-24 18-25-43\API ->
Analysis Method   : D:\CHEM32_002\1\METHODS\API SIM P2001.M
Method Info       : Column :  X-Terra C-18 250*4.6mm,5µm
                    Mobile phase: A) 0.1% TFA in Water , B) ACN (gradient )
                    T/B%:0/20,3/20,12/95,23/95,25/20,30/20
                    Flow:1.0 ml/min, Diluent: ACN:Water(80:20)
```

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

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

|Peak|   RT    |   Area    | Area %  |
| #  |  [min]  |           |         |
|----|---------|-----------|---------|
|   1|   12.624|      2.084|    0.118|
|   2|   14.831|      4.532|    0.257|
|   3|   15.095|   1759.786|   99.625|
-------------------------------------
```

Analysed by : 25/7/14

Checked by :
25/7/14

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strument 1    Fri, 25. Jul. 2014    09:27:41 am         Page   1 of 1
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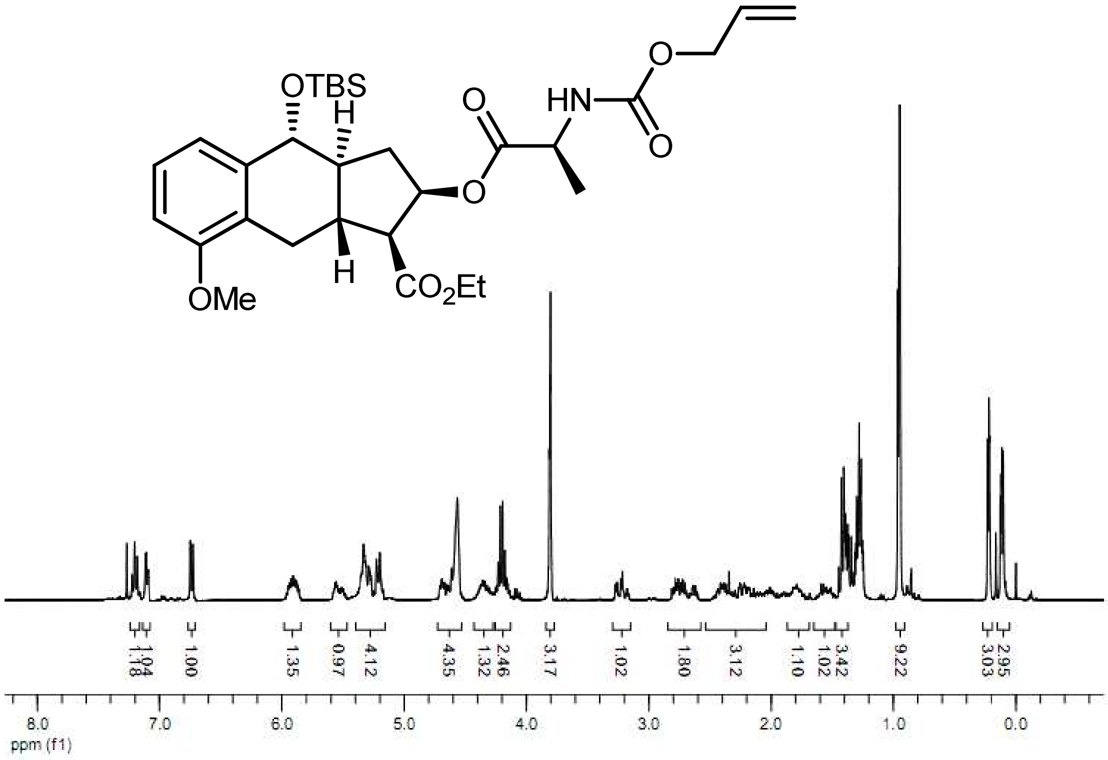
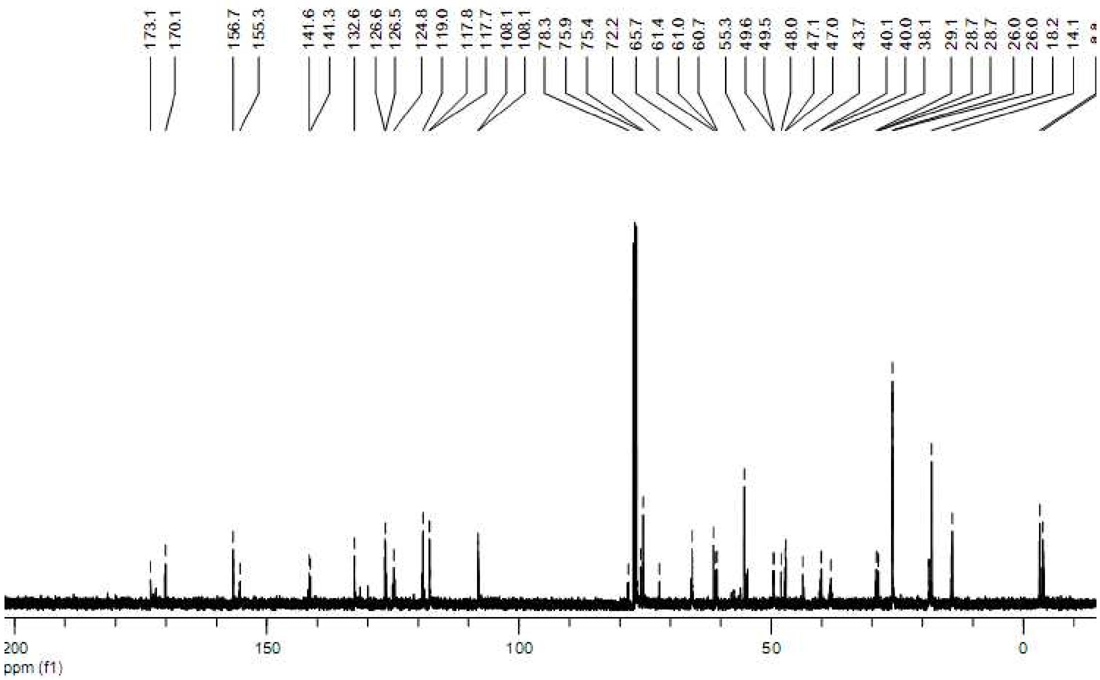

OTBS
H
O
HN
O
O
O
H
CO2Et
OMe
1.18
1.04
1.00
1.35
0.97
4.12
4.35
1.32
2.46
3.17
1.02
1.80
3.12
1.10
1.02
3.42
9.22
3.03
2.95
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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156.7
155.3
141.6
141.3
132.6
126.6
126.5
124.8
119.0
117.8
117.7
108.1
108.1
78.3
75.9
75.4
72.2
65.7
61.4
61.0
60.7
55.3
49.6
49.5
48.0
47.1
47.0
43.7
40.1
40.0
38.1
29.1
28.7
28.7
26.0
26.0
18.2
14.1
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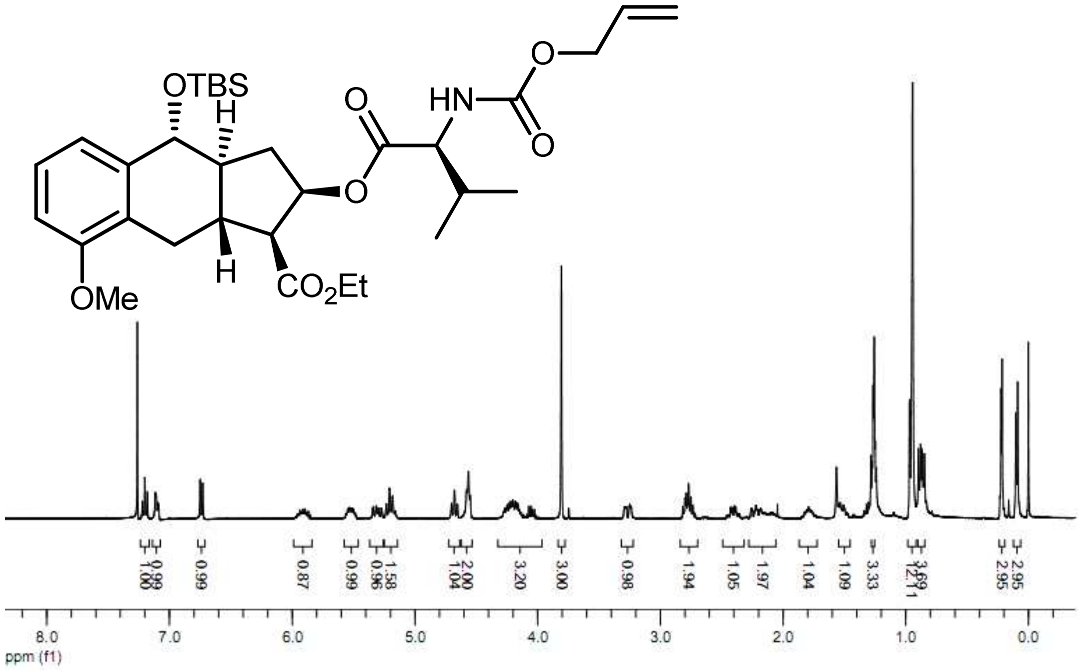
150
100
50
0
ppm (f1)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H
O
HN
O
O
O
H
CO2Et
OMe
1.00
0.99
0.99
0.87
0.99
0.96
1.58
1.04
2.00
3.20
3.00
0.98
1.94
1.05
1.97
1.04
1.09
3.33
12.71
3.69
2.95
2.95
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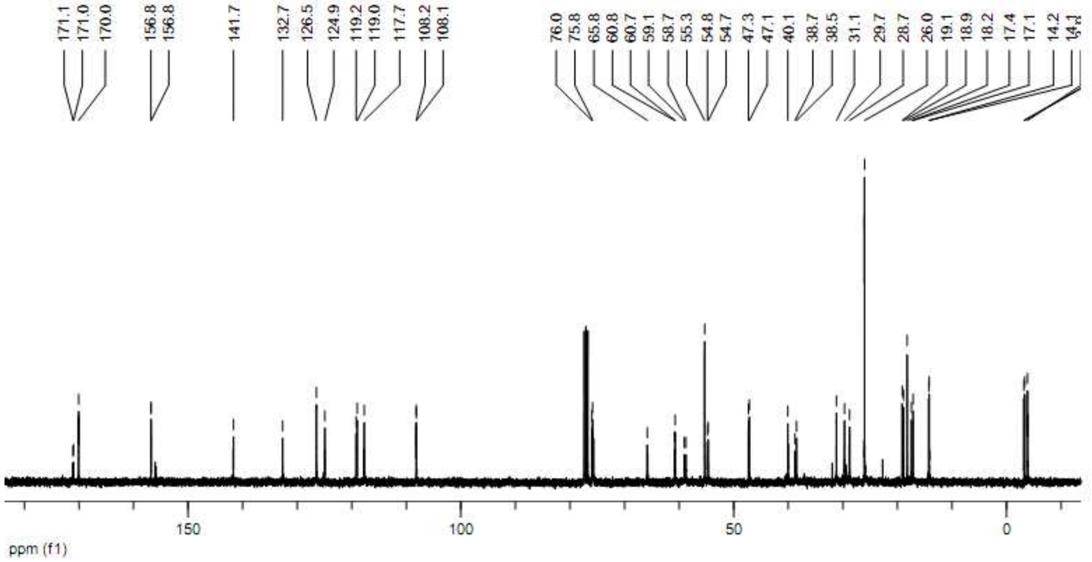
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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170.0
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156.8
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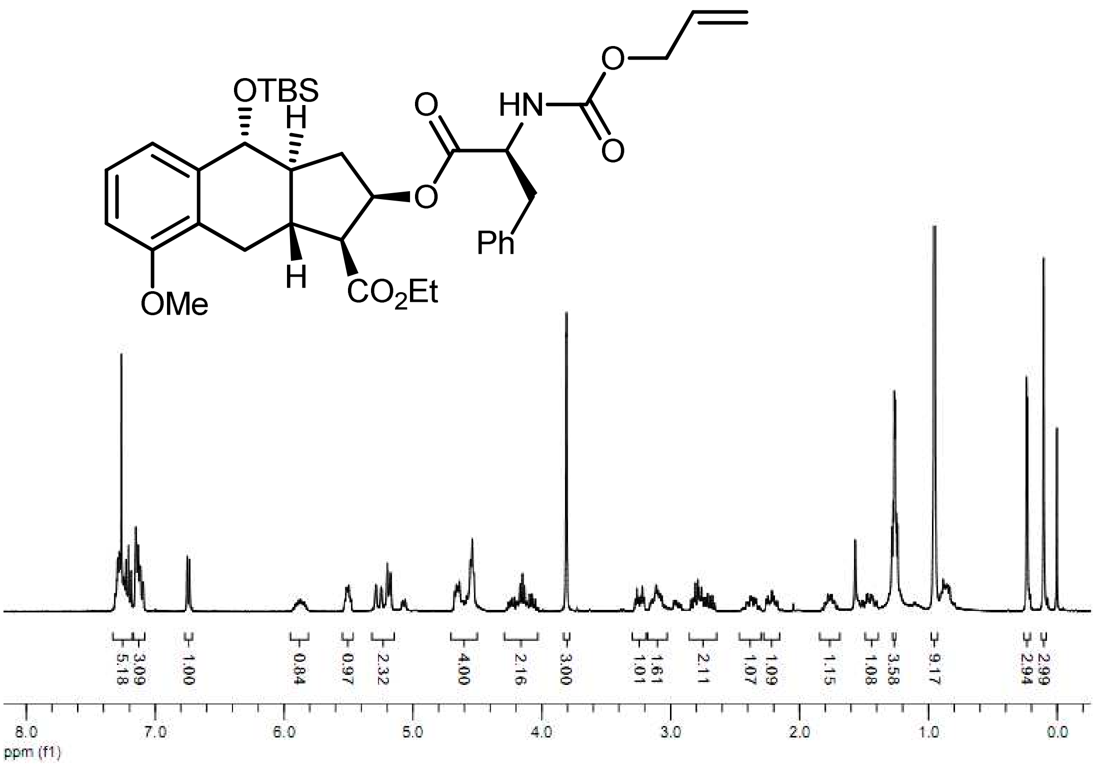
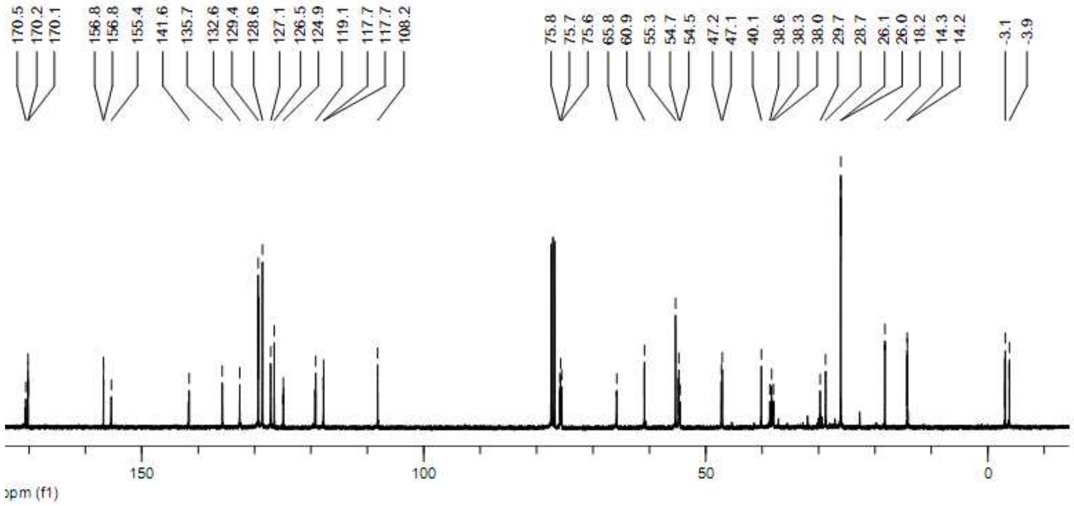
132.7
126.5
124.9
119.2
119.0
117.7
108.2
108.1
76.0
75.8
65.8
60.8
60.7
59.1
58.7
55.3
54.8
54.7
47.3
47.1
40.1
38.7
38.5
31.1
29.7
28.7
26.0
19.1
18.9
18.2
17.4
17.1
14.2
14.1
150
100
50
0
ppm (f1)


OTBS
H
O
HN
O
O
O
Ph
H
CO2Et
OMe
5.18
3.09
1.00
0.84
0.97
2.32
4.00
2.16
3.00
1.01
1.61
2.11
1.07
1.09
1.15
1.08
3.58
9.17
2.94
2.99
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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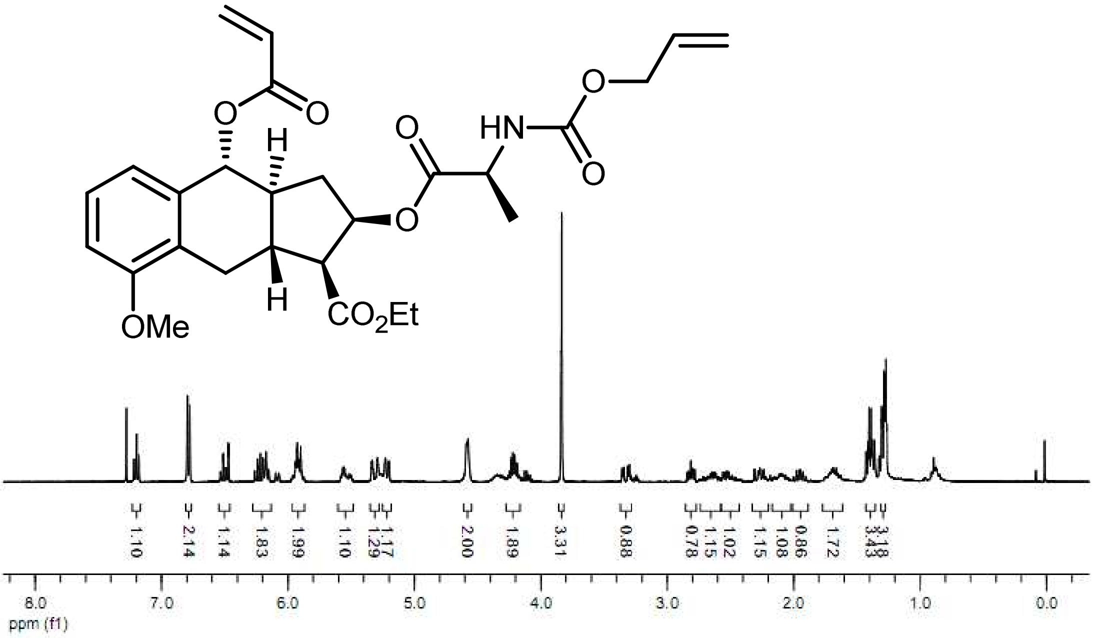
170.2
170.1
156.8
156.8
155.4
141.6
135.7
132.6
129.4
128.6
127.1
126.5
124.9
119.1
117.7
117.7
108.2
75.8
75.7
75.6
65.8
60.9
55.3
54.7
54.5
47.2
47.1
40.1
38.6
38.3
38.0
29.7
28.7
26.1
26.0
18.2
14.3
14.2
-3.1
-3.9
150
100
50
0
ppm (f1)


O
O
H
O
HN
O
O
O
OMe
H
CO2Et
1.10
2.14
1.14
1.83
1.99
1.10
1.29
1.17
2.00
1.89
3.31
0.88
0.78
1.15
1.02
1.15
1.08
0.86
1.72
3.43
3.18
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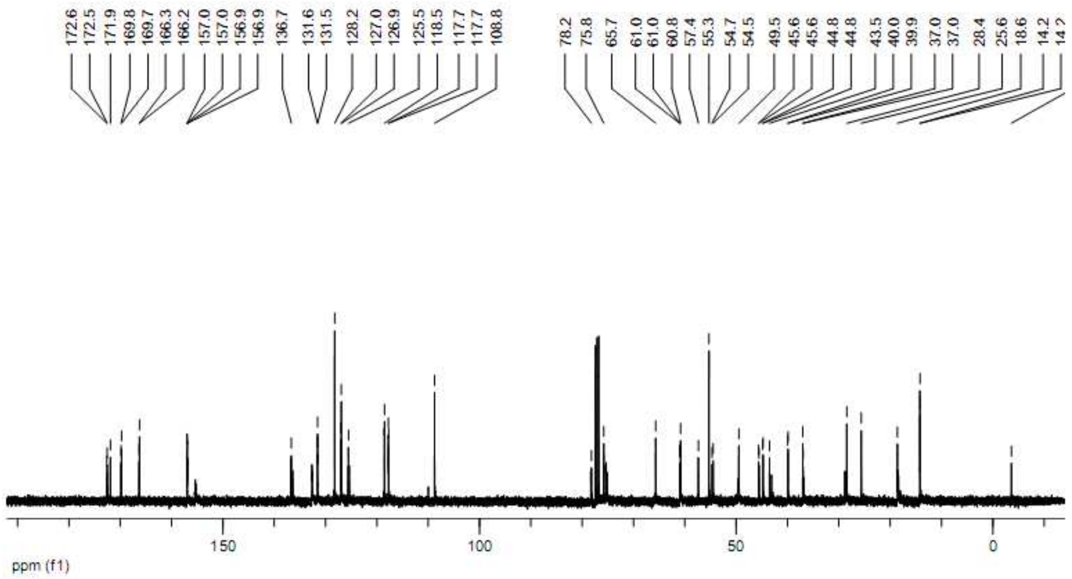
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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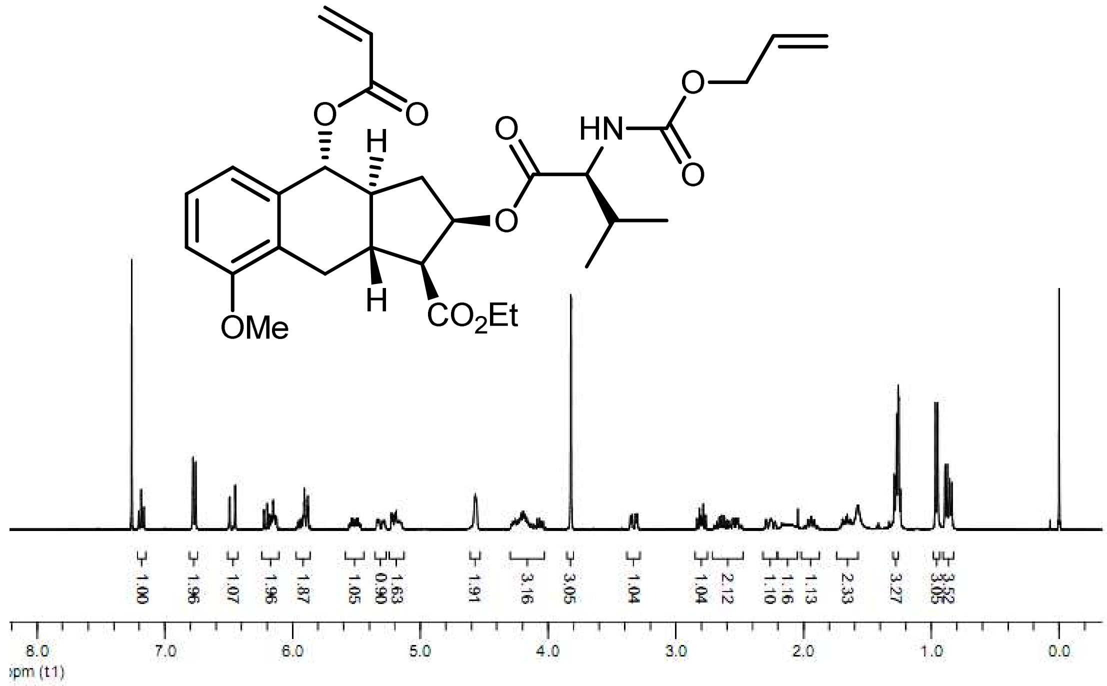
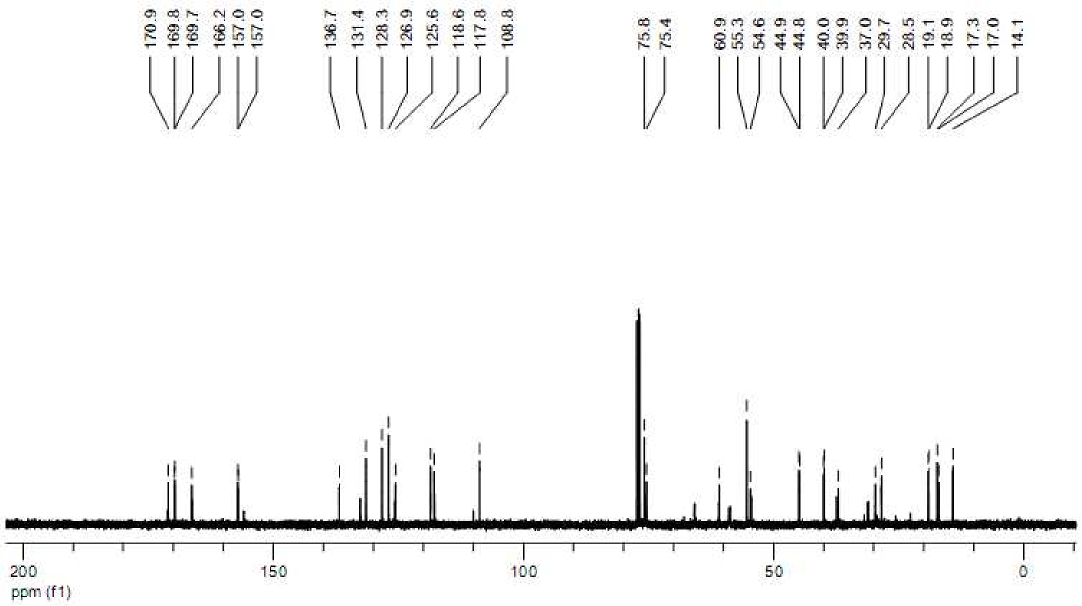
172.5
171.9
169.8
169.7
166.3
166.2
157.0
157.0
156.9
156.9
136.7
131.6
131.5
128.2
127.0
126.9
125.5
118.5
117.7
117.7
108.8
78.2
75.8
65.7
61.0
61.0
60.8
57.4
55.3
54.7
54.5
49.5
45.6
45.6
44.8
44.8
43.5
40.0
39.9
37.0
37.0
28.4
25.6
18.6
14.2
14.2
150
100
50
0
ppm (f1)


O
O
H
O
O
HN
O
O
O
H
OMe
CO2Et
1.00
1.96
1.07
1.96
1.87
1.05
0.90
1.63
1.91
3.16
3.05
1.04
1.04
2.12
1.10
1.16
1.13
2.33
3.27
3.05
3.52
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)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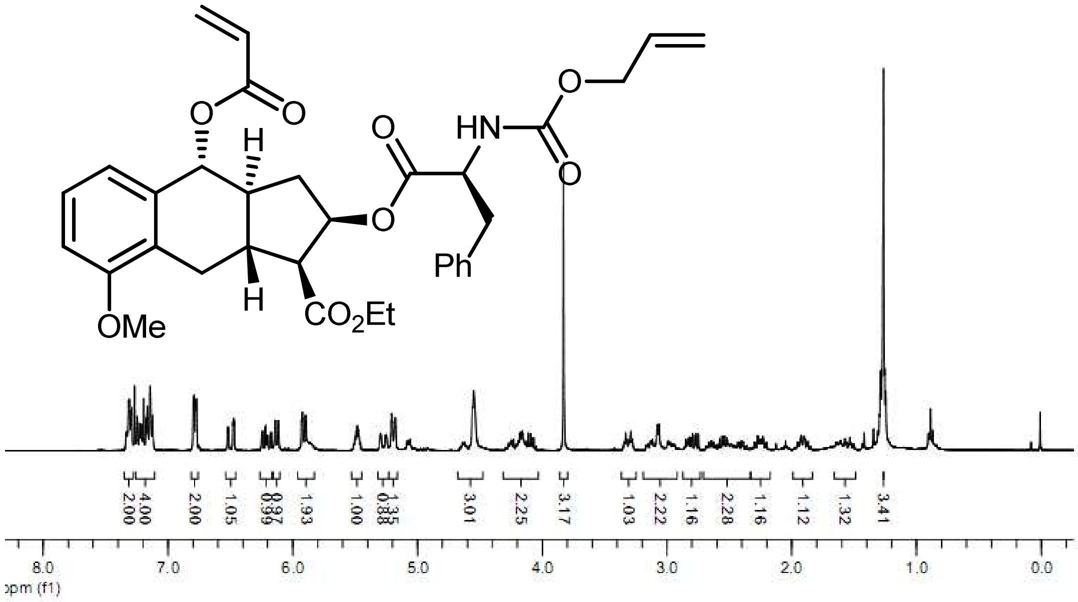
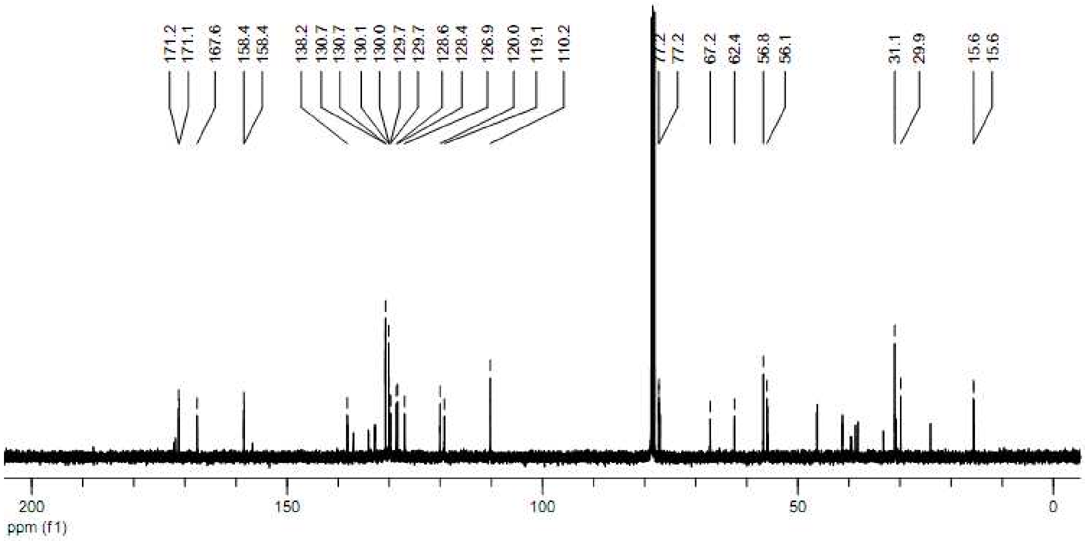
169.8
169.7
166.2
157.0
157.0
136.7
131.4
128.3
126.9
125.6
118.6
117.8
108.8
75.8
75.4
60.9
55.3
54.6
44.9
44.8
40.0
39.9
37.0
29.7
28.5
19.1
18.9
17.3
17.0
14.1
200
150
100
50
0
ppm (f1)


OMe
H
H
O
O
O
O
CO2Et
HN
O
O
Ph
2.00
4.00
2.00
1.05
0.99
0.87
1.93
1.00
0.88
1.35
3.01
2.25
3.17
1.03
2.22
1.16
2.28
1.16
1.12
1.32
3.41
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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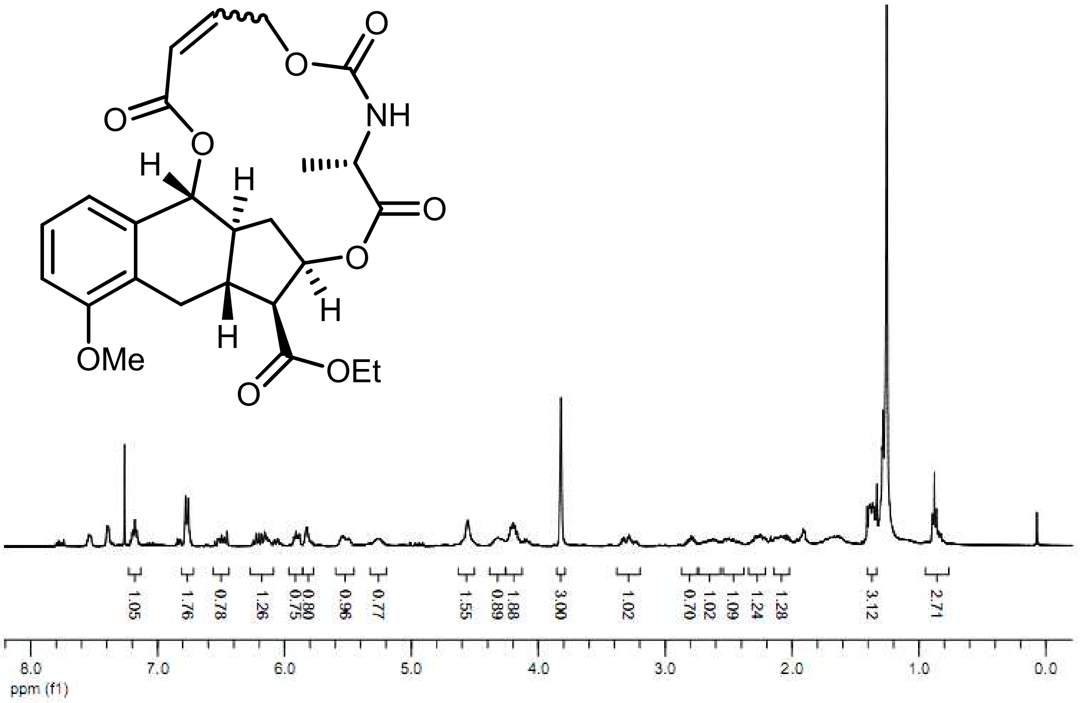
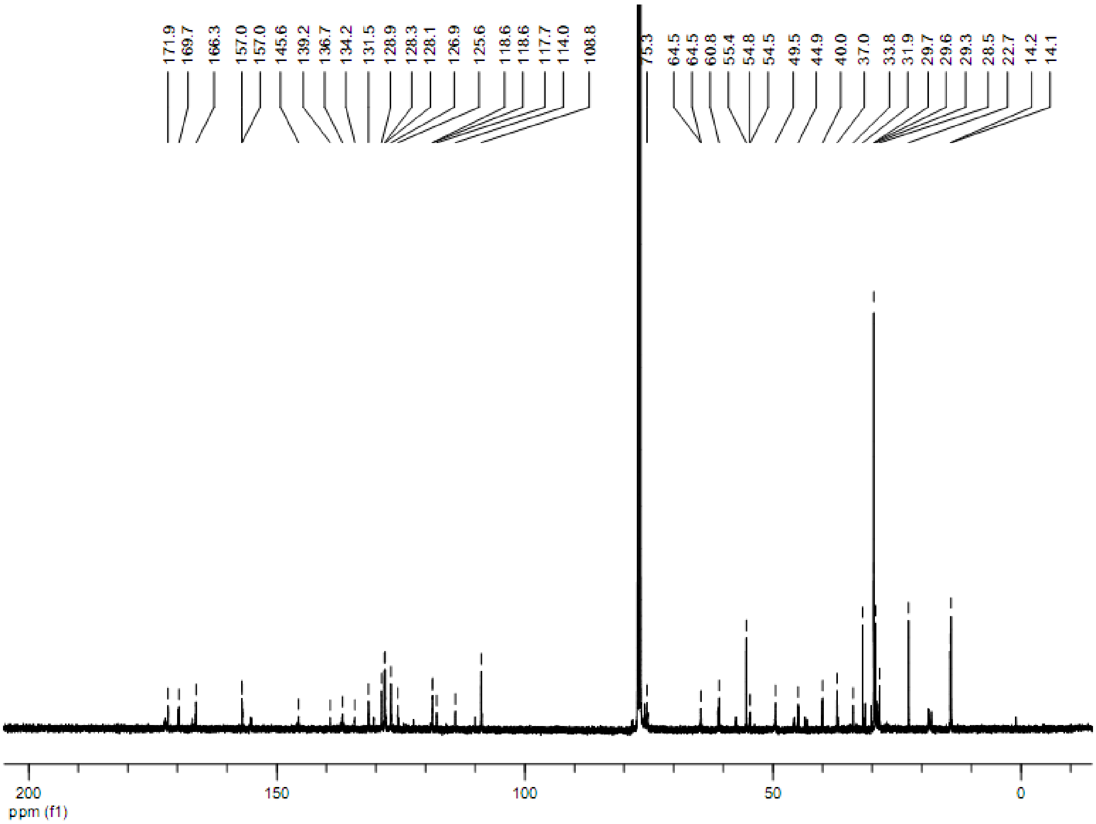
171.1
167.6
158.4
158.4
138.2
130.7
130.7
130.1
130.0
129.7
129.7
128.6
128.4
126.9
120.0
119.1
110.2
77.2
77.2
67.2
62.4
56.8
56.1
31.1
29.9
15.6
15.6
200
150
100
50
0
ppm (f1)


O
O
NH
O
H
H
O
O
H
H
OMe
O
OEt
1.05
1.76
0.78
1.26
0.75
0.80
0.96
0.77
1.55
0.89
1.88
3.00
1.02
0.70
1.02
1.09
1.24
1.28
3.12
2.71
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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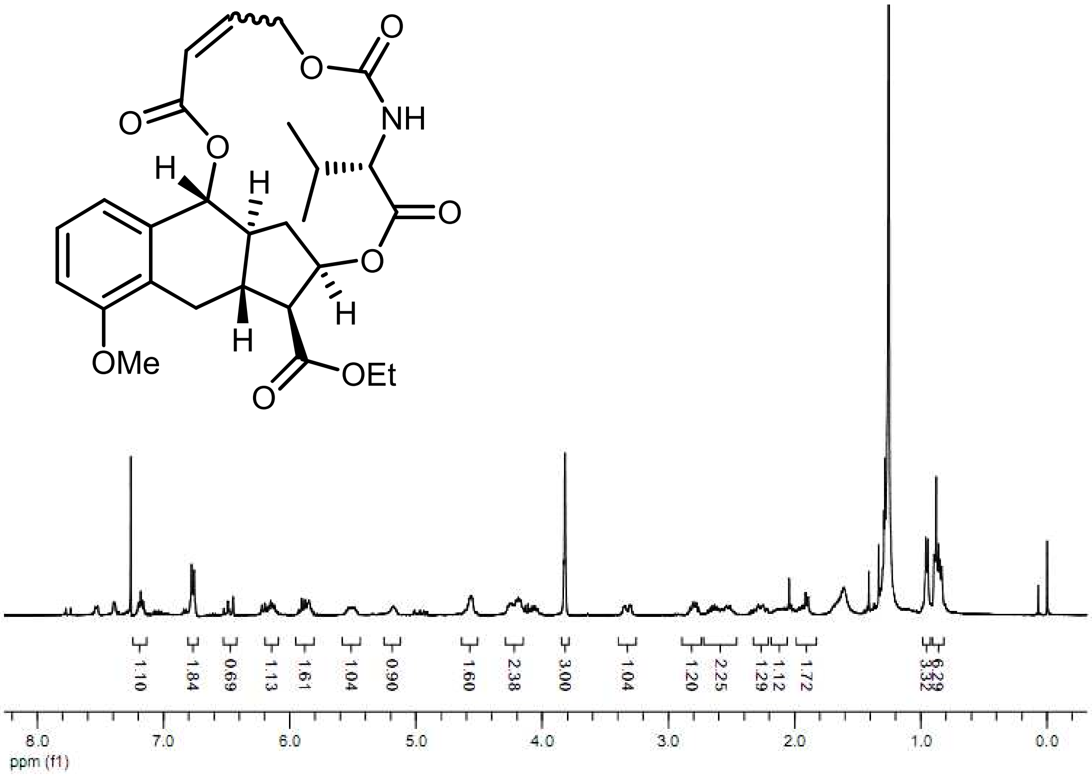
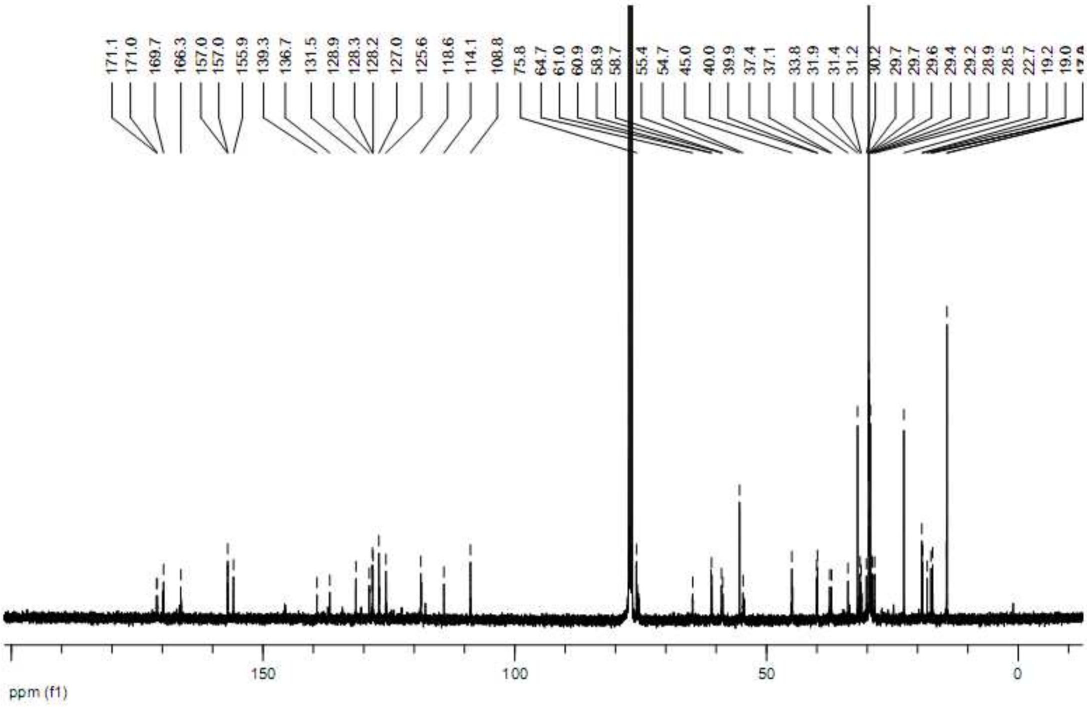
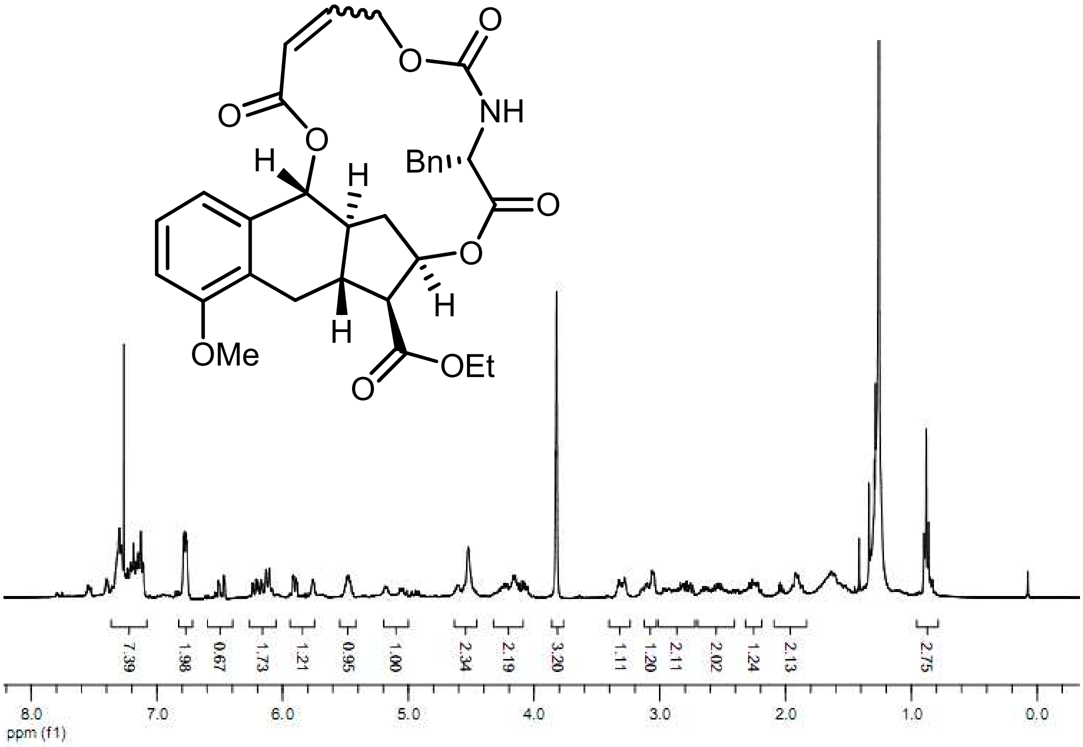
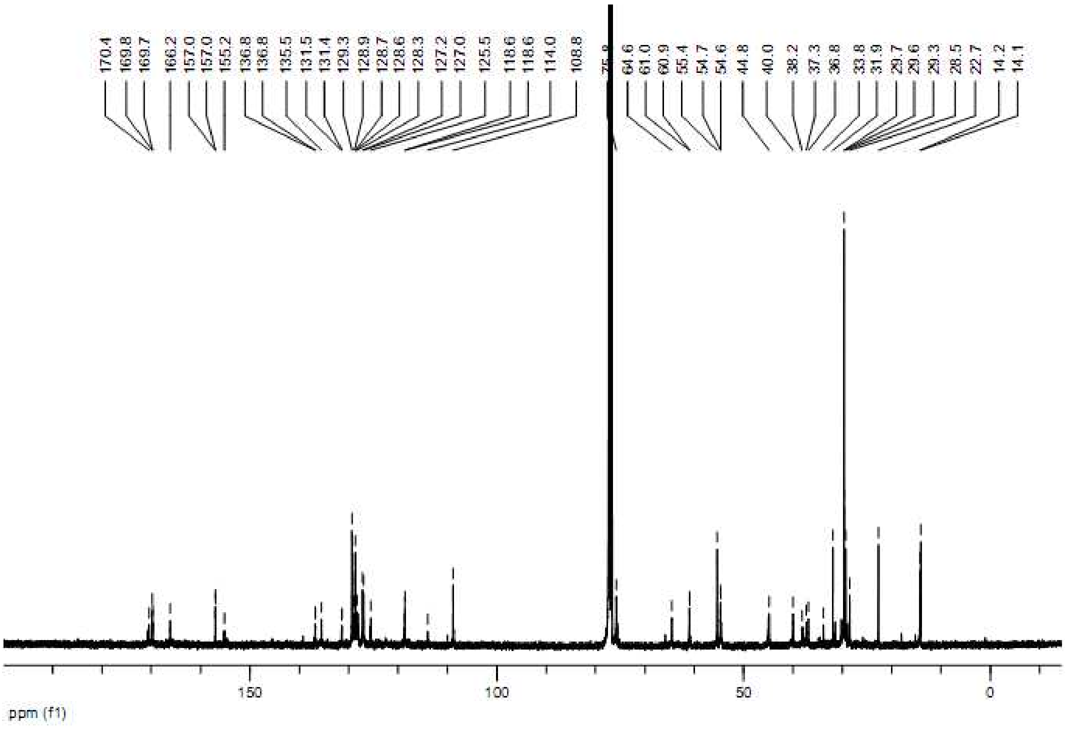
166.3
157.0
157.0
145.6
139.2
136.7
134.2
131.5
128.9
128.3
128.1
126.9
125.6
118.6
118.6
117.7
114.0
108.8
75.3
64.5
64.5
60.8
55.4
54.8
54.5
49.5
44.9
40.0
37.0
33.8
31.9
29.7
29.6
29.3
28.5
22.7
14.2
14.1
200
150
100
50
0
ppm (f1)


O
O
NH
O
H
H
O
O
H
H
OMe
O
OEt
1.10
1.84
0.69
1.13
1.61
1.04
0.90
1.60
2.38
3.00
1.04
1.20
2.25
1.29
1.12
1.72
3.32
6.29
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


171.1
171.0
169.7
166.3
157.0
157.0
155.9
139.3
136.7
131.5
128.9
128.3
128.2
127.0
125.6
118.6
114.1
108.8
75.8
64.7
61.0
60.9
58.9
58.7
55.4
54.7
45.0
40.0
39.9
37.4
37.1
33.8
31.9
31.4
31.2
30.2
29.7
29.7
29.6
29.4
29.2
28.9
28.5
22.7
19.2
19.0
150
100
50
0
ppm (f1)


O
O
NH
O
H
H
Bn
O
O
H
H
OMe
O
OEt
7.39
1.98
0.67
1.73
1.21
0.95
1.00
2.34
2.19
3.20
1.11
1.20
2.11
2.02
1.24
2.13
2.75
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)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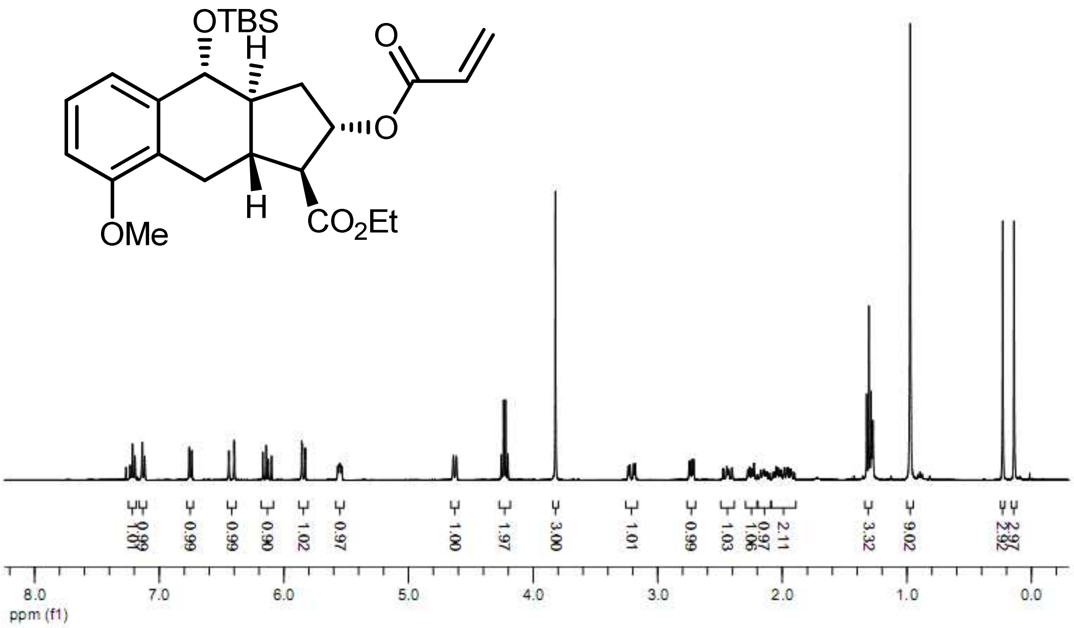
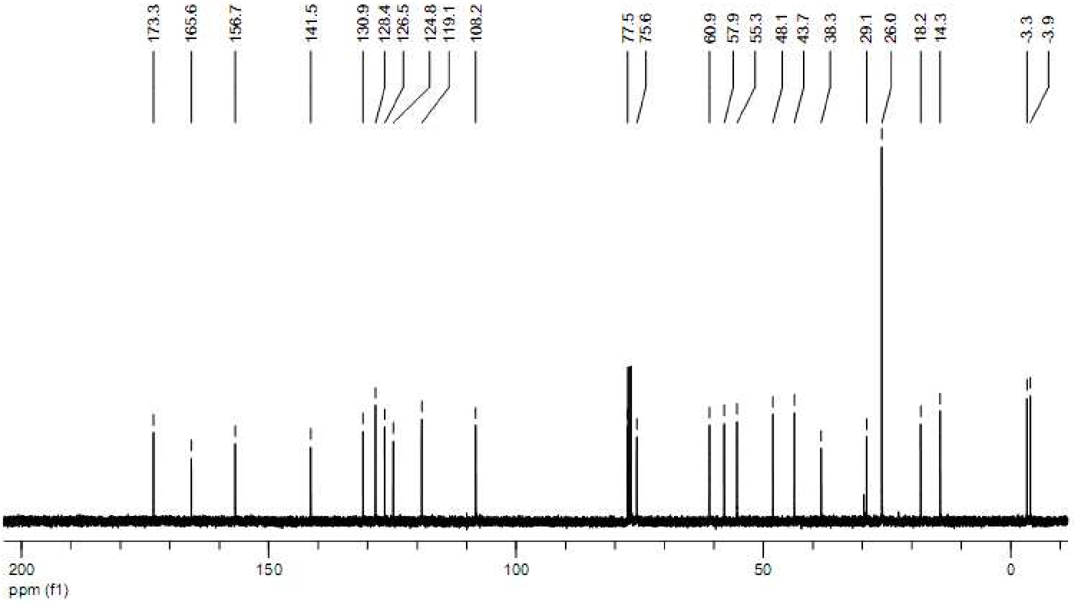
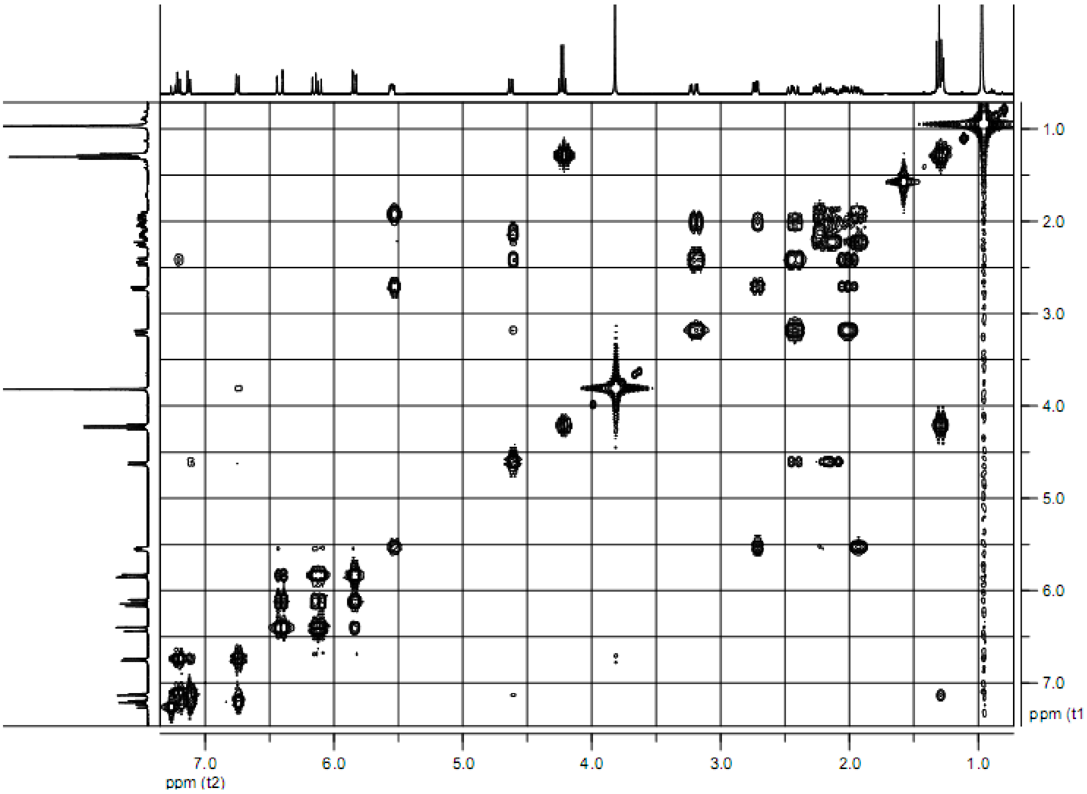
169.8
169.7
166.2
157.0
157.0
155.2
136.8
136.8
135.5
131.5
131.4
129.3
128.9
128.7
128.6
128.3
127.2
127.0
125.5
118.6
118.6
114.0
108.8
75.8
64.6
61.0
60.9
55.4
54.7
54.6
44.8
40.0
38.2
37.3
36.8
33.8
31.9
29.7
29.6
29.3
28.5
22.7
14.2
14.1
150
100
50
0
ppm (f1)

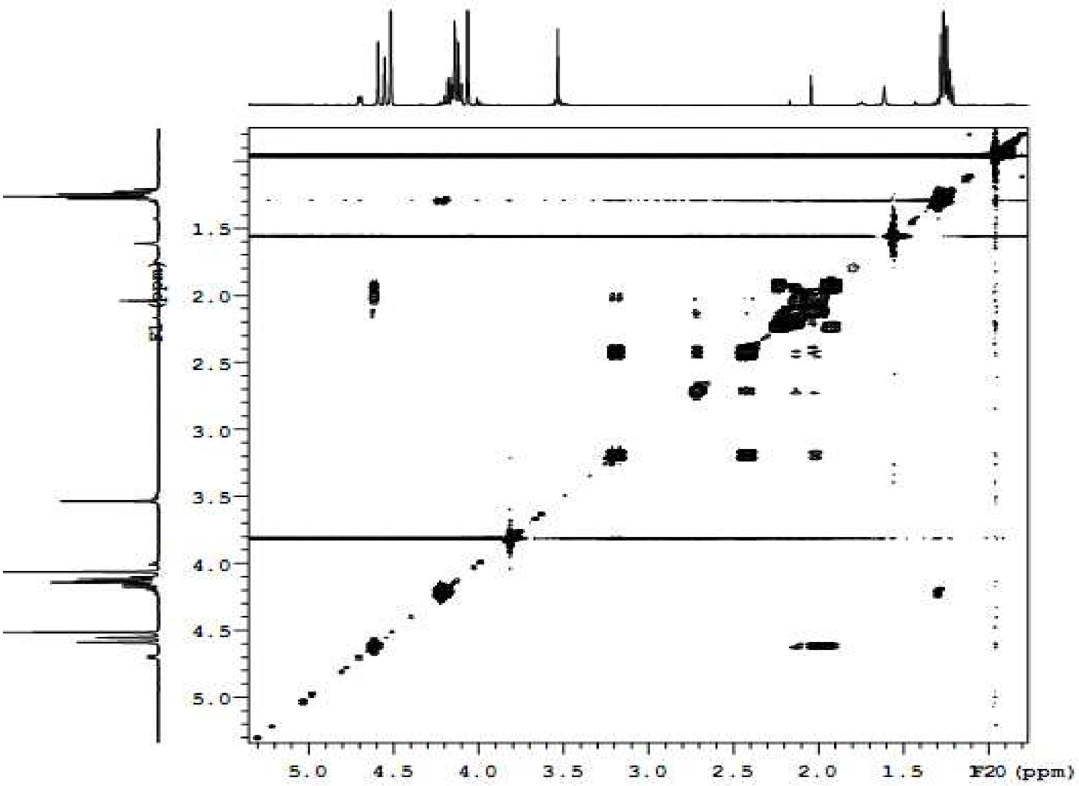

OTBS
H
O
O
H
CO2Et
OMe
1.07
0.99
0.99
0.99
0.90
1.02
0.97
1.00
1.97
3.00
1.01
0.99
1.03
1.06
0.97
2.11
3.32
9.02
2.92
2.97
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

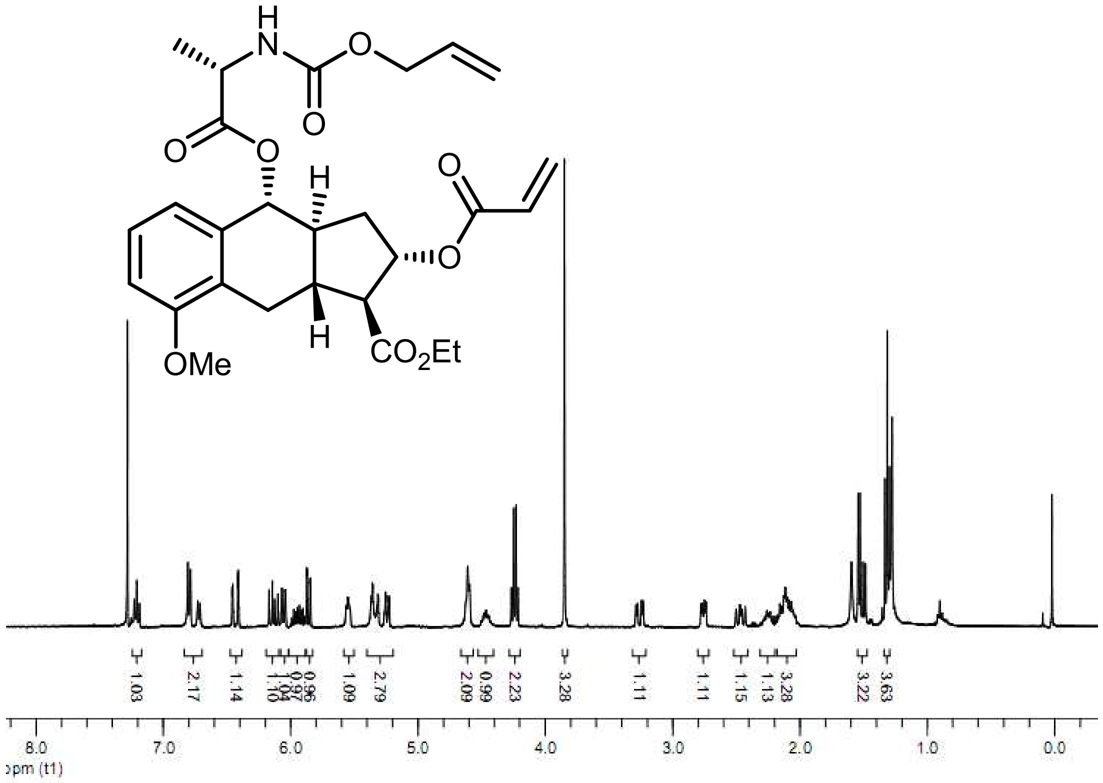

173.3
165.6
156.7
141.5
130.9
128.4
126.5
124.8
119.1
108.2
77.5
75.6
60.9
57.9
55.3
48.1
43.7
38.3
29.1
26.0
18.2
14.3
-3.3
-3.9
200
150
100
50
0
ppm (f1)

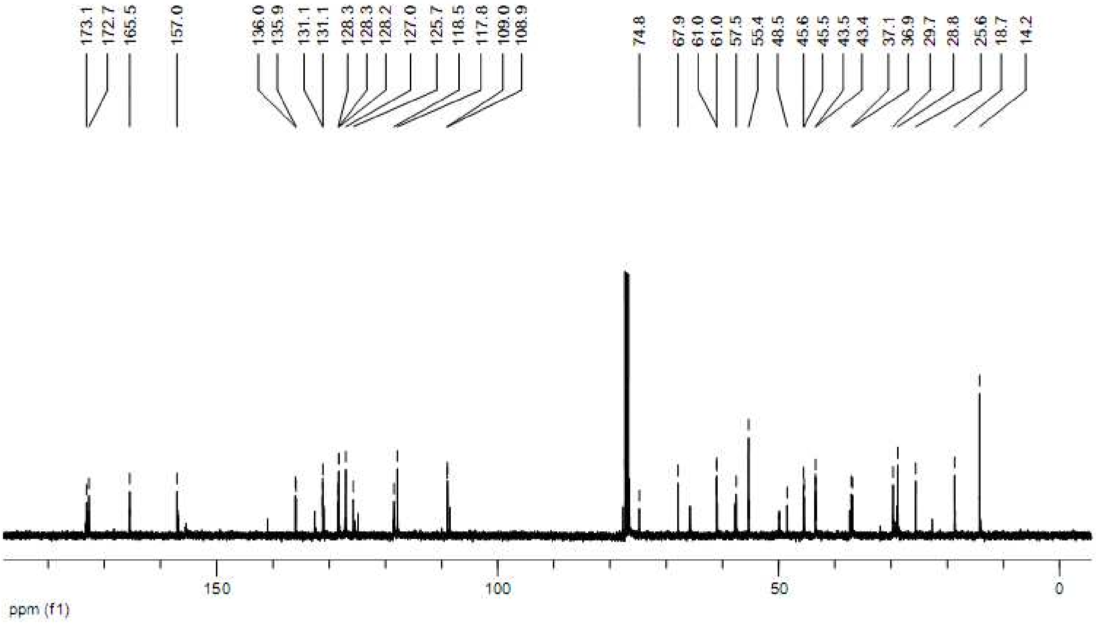
COSY


1.0
2.0
3.0
4.0
5.0
6.0
7.0
ppm (t1
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)

nOe


1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
F1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
F2 (ppm)


H
N
O
O
O
O
O
H
O
OMe
H
CO2Et
1.03
2.17
1.14
1.10
1.04
0.97
0.96
1.09
2.79
2.09
0.99
2.23
3.28
1.11
1.11
1.15
1.13
3.28
3.22
3.63
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


173.1
172.7
165.5
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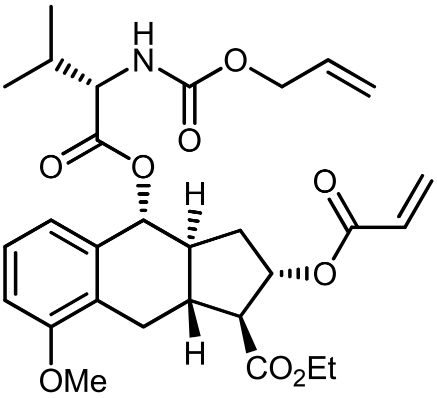
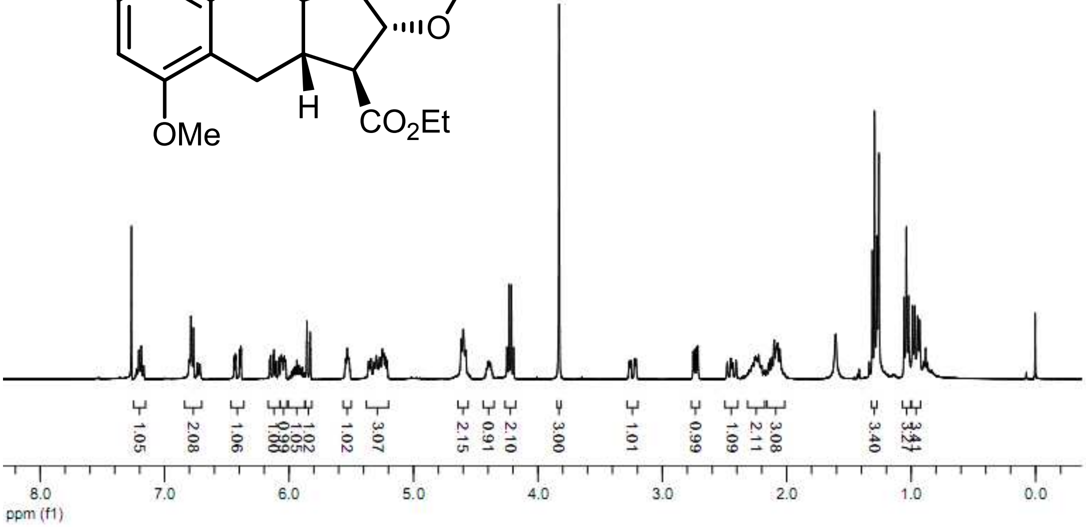
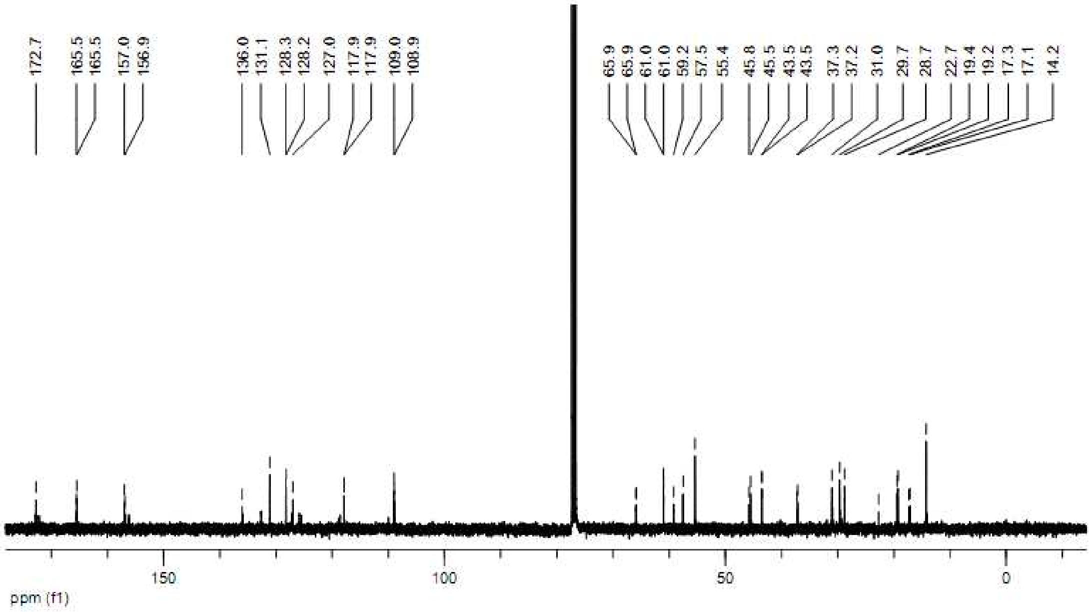
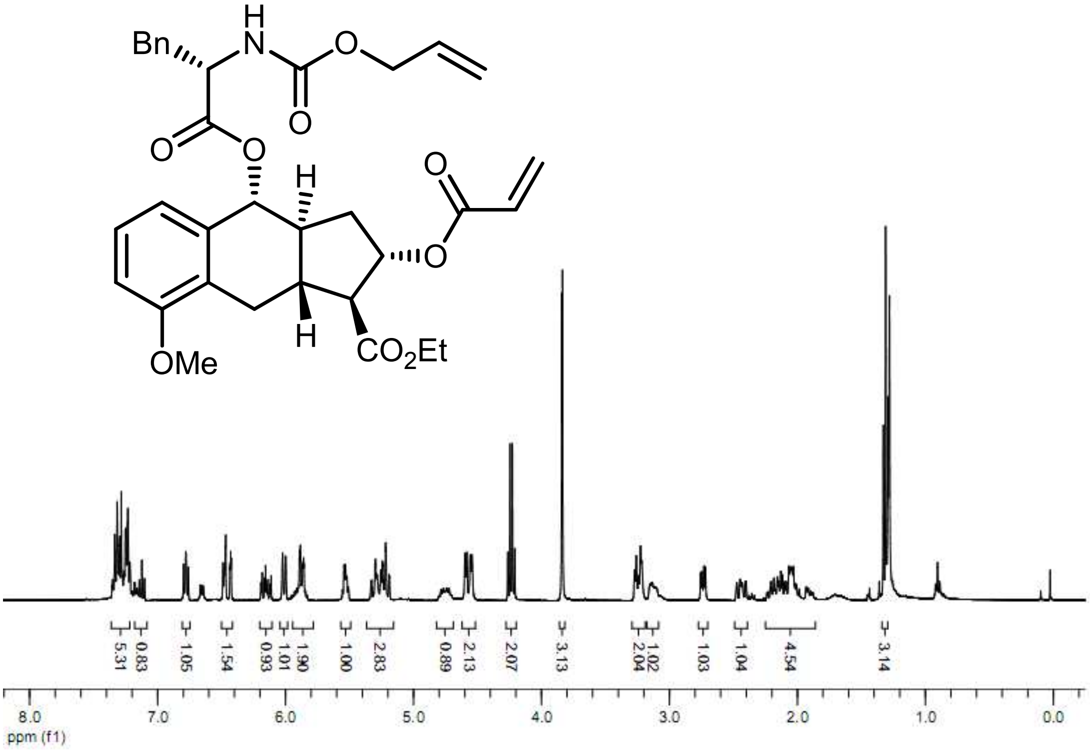
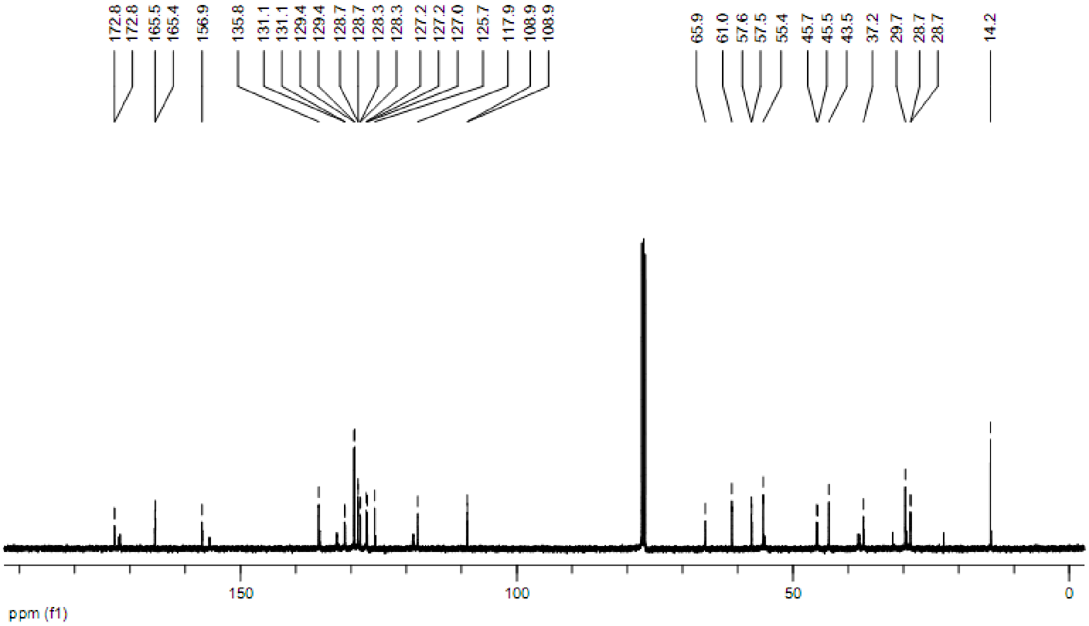
136.0
135.9
131.1
131.1
128.3
128.3
128.2
127.0
125.7
118.5
117.8
109.0
108.9
74.8
67.9
61.0
61.0
57.5
55.4
48.5
45.6
45.5
43.5
43.4
37.1
36.9
29.7
28.8
25.6
18.7
14.2
150
100
50
0
ppm (f1)

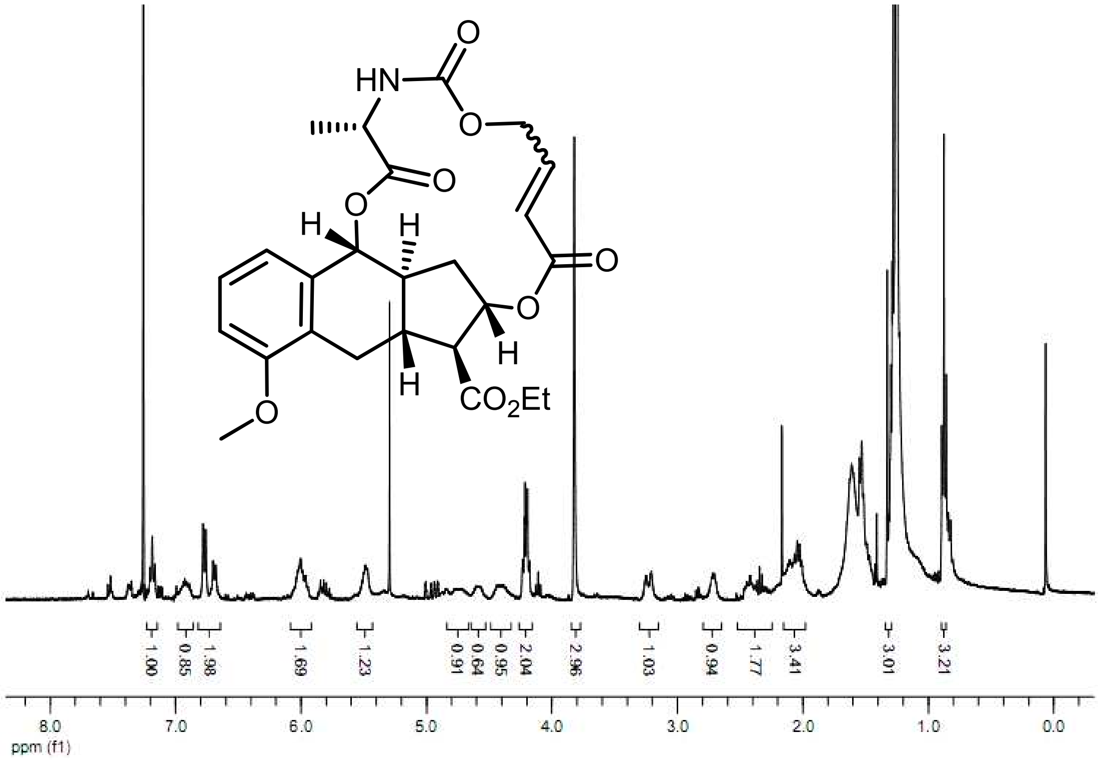

H
N
O
O
O
O
H
O
O
OMe
H
CO2Et

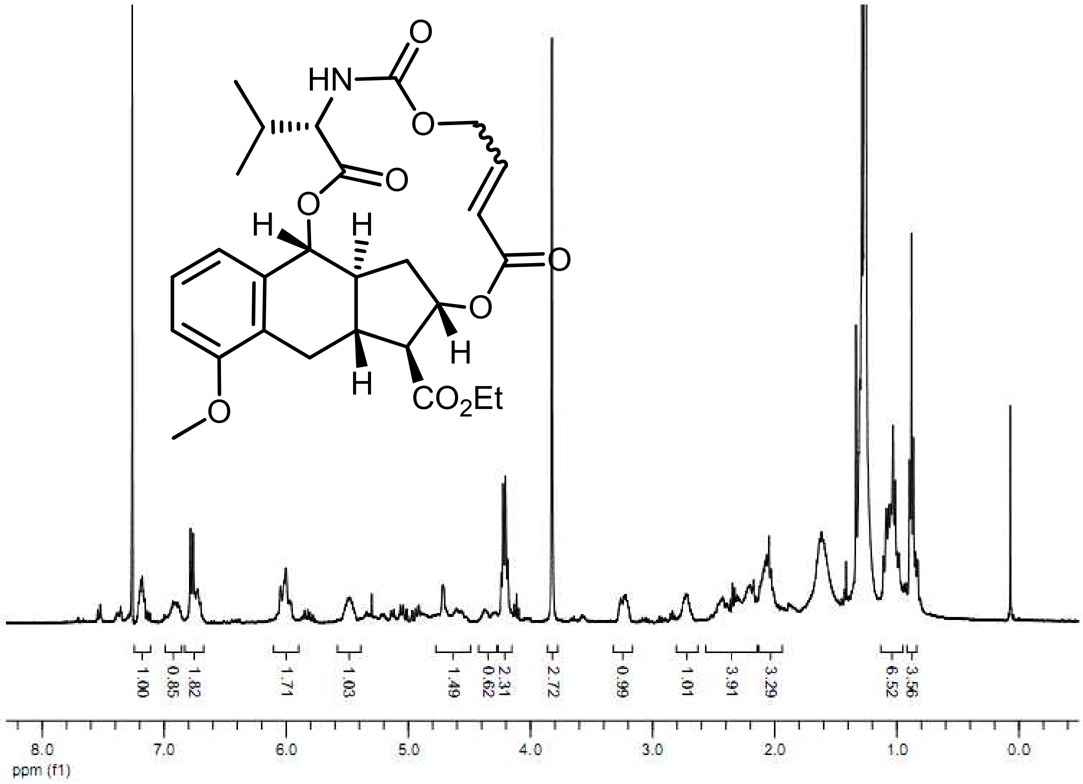

1.05
2.08
1.06
1.00
0.99
1.05
1.02
1.02
3.07
2.15
0.91
2.10
3.00
1.01
0.99
1.09
2.11
3.08
3.40
3.41
3.27
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

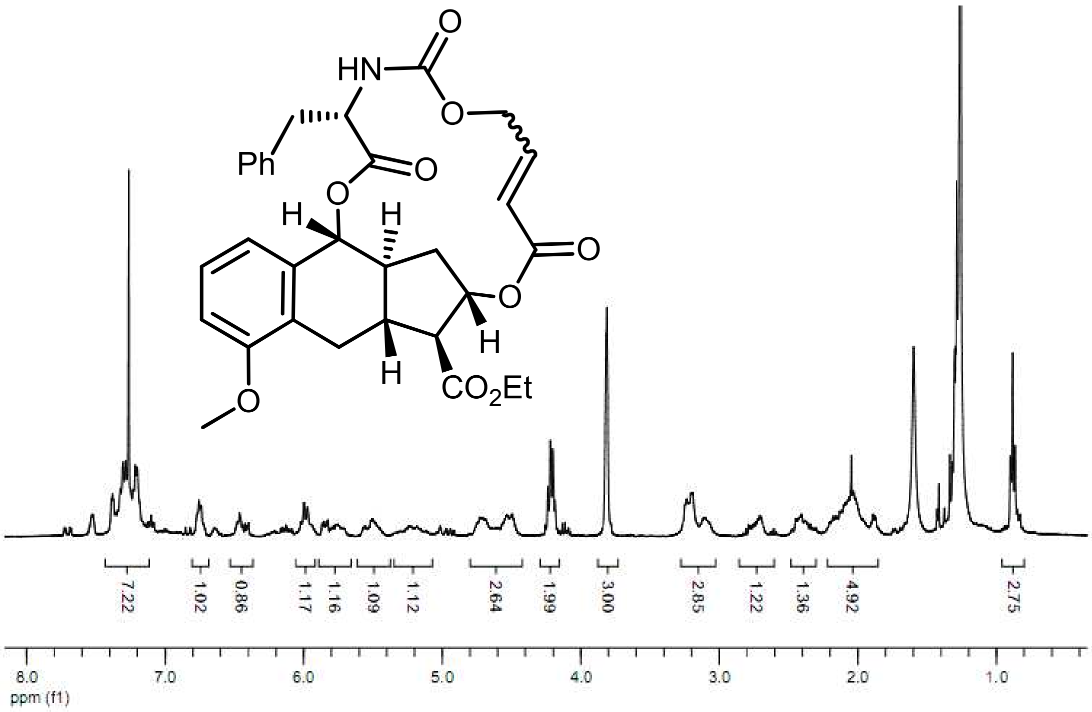

172.7
165.5
165.5
157.0
156.9
136.0
131.1
128.3
128.2
127.0
117.9
117.9
109.0
108.9
65.9
65.9
61.0
61.0
59.2
57.5
55.4
45.8
45.5
43.5
43.5
37.3
37.2
31.0
29.7
28.7
22.7
19.4
19.2
17.3
17.1
14.2
150
100
50
0
ppm (f1)


Bn
H
N
O
O
O
O
O
H
O
O
H
OMe
CO_2Et
5.31
0.83
1.05
1.54
0.93
1.01
1.90
1.00
2.83
0.89
2.13
2.07
3.13
2.04
1.02
1.03
1.04
4.54
3.14
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


172.8
172.8
165.5
165.4
156.9
135.8
131.1
131.1
129.4
129.4
128.7
128.7
128.3
128.3
127.2
127.2
127.0
125.7
117.9
108.9
108.9
65.9
61.0
57.6
57.5
55.4
45.7
45.5
43.5
37.2
29.7
28.7
28.7
14.2
150
100
50
0
ppm (f1)


O
HN
O
O
O
H
H
O
O
H
H
CO$_2$Et
O
1.00
0.85
1.98
1.69
1.23
0.91
0.64
0.95
2.04
2.96
1.03
0.94
1.77
3.41
3.01
3.21
8.0
ppm (f1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


O
HN
O
O
O
H
H
O
O
H
H
CO$_2$Et
O
1.00
0.85
1.82
1.71
1.03
1.49
0.62
2.31
2.72
0.99
1.01
3.91
3.29
6.52
3.56
8.0
ppm (f1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


O
HN
O
Ph
O
O
H
H
O
O
H
H
CO$_2$Et
O
7.22
1.02
0.86
1.17
1.16
1.09
1.12
2.64
1.99
3.00
2.85
1.22
1.36
4.92
2.75
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

Srinivas Chamakuri Peer Reviewed Publications:

Publications from the PhD Program

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 14, 2014 Issue : Chemical Biology of Protein:Protein Interactions; Guest Editor: Prabhat Arya)

2. A Modular Approach to Build Macrocyclic Diversity in Aminoindoline Scaffolds Identifies Antiangiogenesis Agents from a Zebrafish Assay. Chamakuri, S.; Guduru, S. R. K.; Pamu, S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P. *Eur. J. Org. Chem.* **2013,** *19*, 3959-3964.

3. Tetrahydroquinoline-Derived Macrocyclic Toolbox: The Discovery of Antiangiogenesis Agents in Zebrafish Assay. Guduru, S. R. K.; Chamakuri, S.; Chandrasekar, G.; Kitambi, S. S.; Arya, P.; *ACS Med. Chem. Lett.* **2013**, *4*, 666–670.

4. Synthesis of Treprostinil Inspired Hybrid Macrocycles. Srinivas Chamakuri, Prabhat Arya. **2014**, (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, under review)*

Other Publications

1. Acyl Homoserine Lactones Represent a Novel Scaffold for Killing Mycobacterium Tuberculosis under Biofilm Conditions. Ritta, M.; George, A.; Chamakuri, S.; Arya, P. **2014** (Submitted for publication).

2. $AlCl_3$-induced (hetero) arylation of thienopyrimidine ring: a new synthesis of 4-substituted Thieno [2,3-d] pyrimidines. Kumar, K. S.; Chamakuri, S.; Iqbal, J.; Pal, M.; *Tetrahedron Lett.* **2010,** *51*, 3269–3273.

Conferences Attended:

2013	Attended and presented a poster at the IX-JNOST international conference, IISER, Bhopal, India
2013	Attended and presented a poster in conference on “Advances in Anticancer Drug Discovery and Development” organized by IIT Madras, India
2008	Participated in a one day seminar on “Green Chemistry In Drug Synthesis”, conducted by MNR P.G.College, on 29th January
2007	Participated in a one day seminar on “Newer Facets of Pharmaceutical Chemistry”, conducted by MNR P.G.College, on 31st January