Antibacterial natural product Hunanamycin A: Total synthesis, lead optimization, and related studies

Thesis Submitted to AcSIR

For the Award of the Degree of

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In

CHEMICAL SCIENCES

By

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Under the guidance of
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December 2017

I dedicate all my efforts to

My family and Teachers

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Thesis Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Antibacterial natural product Hunanamycin A: Total synthesis, lead optimization, and related studies"** submitted by **Mr. Rahul Dilip Shingare** to Academy of Scientific and Innovative Research (AcSIR) in fulfilment of the requirements for the award of the Degree of Doctor of Philosophy, embodies original research work under my supervision. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

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Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Antibacterial natural product Hunanamycin A: Total synthesis, lead optimization, and related studies"** submitted to Academy of Scientific and Innovative Research for the award of degree of Doctor of Philosophy (Ph.D.) is the outcome of experimental investigations carried out by me under the supervision of **Dr. D. Srinivasa Reddy**, Senior Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

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Acknowledgement

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Above all, I thank the Almighty for His enormous blessings.

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General remarks

All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification. Solvents were dried using standard protocols or dried using Mbraun (MB SPS-800) instrument. Reactions were carried out in oven-dried glassware under a positive pressure of argon unless otherwise mentioned with magnetic stirring. Air sensitive reagents and solutions were transferred *via* syringe or cannula and were introduced to the apparatus *via* rubber septa. The progress of reactions was monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec. Column chromatography was performed on silica gel (100-200 or 230-400 mesh size). All the melting points are uncorrected and were recorded using a scientific melting point apparatus (Buchi B-540). Deuterated solvents for NMR spectroscopic analyses were used as received. All ^{1}H NMR and ^{13}C NMR analysis were obtained using a 200 MHz, 400 MHz, 500 MHz spectrometer. Coupling constants were measured in Hertz. All chemical shifts are quoted in ppm, relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS (ESI) were recorded on ORBITRAP mass analyser (Q Exactive). Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films in chloroform using NaCl plates. Optical rotations were recorded on a P-2000 polarimeter at 589 nm (sodium D-line). Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra. All microwave reactions were carried out in anton-paar monowave 300 instrument. The purity of products was determined by reverse phase HPLC analysis using Agilent technologies 1200 series; column: ZORBAX Eclipse XBD-C-18 (4.6 X 250 mm, 5 μ). Flow rate 8.00 mL/min, UV 254 nm; using mobile phases, Method 95/5 CH_3OH/H_2O for 20 min;

Abbreviations

Ac	Acetyl
AcOH	Acetic acid
ACN	Acetonitrile
ADME	Absorption, distribution, metabolism, and excretion
Bn	Benzyl
brsm	based on recovery of starting material
br.s	broad singlet
^{t}BuOH	*tert*-butanol
Cat.	Catalytic
$CDCl_3$	Deuterated chloroform
cm^{-1}	1/centimeter
CuI	Copper Iodide
Cs_2CO_3	Caesium carbonate
$CuSO_4$	Copper Sulfate
°C	degree celcius
DCM	Dichloromethane
DCE	1,2-Dichloroethane
DIBAL-H	Diisobutylaluminiumhydride
DIPEA	N,N-Diisopropylethylamine
DMF	*N, N'*-Dimethylformamide
DMAP	*N*,*N*-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
equiv.	Equivalents
EtOH	Ethanol
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
Et_3N	Triethylamine
g	gram
HSAB	Hard soft acid base
Hz	Hertz

Abbreviations

HRMS	High resolution mass spectroscopy
HPLC	High pressure liquid chromatography
HCl	Hydrchloric acid
H_2	Hydrogen gas
IR	Infrared
i.e.	that is
in vitro	outside a living organism
in vivo	inside a living organism
J	coupling constant
K_2CO_3	Potassium carbonate
LiOH	Lithium hydroxide
Me	Methyl
MeOH	Methanol
mg	Milligram
min	Minutes
MIC	Minimum inhibitory concentration
mL	Millilitre
µmol	Micromolar
mmol	Millimole
m/z	mass to charge ratio
MP	Melting point
Me	Methyl
MeI	Methyl iodide
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
$NaIO_4$	Sodium meta-periodate
NaOH	Sodium hydroxide
NA	no activity
NMR	Nuclear Magnetic Resonance
ORTEP	Oak Ridge Thermal-Ellipsoid Plot
OsO_4	Osmium tetraoxide

Abbreviations

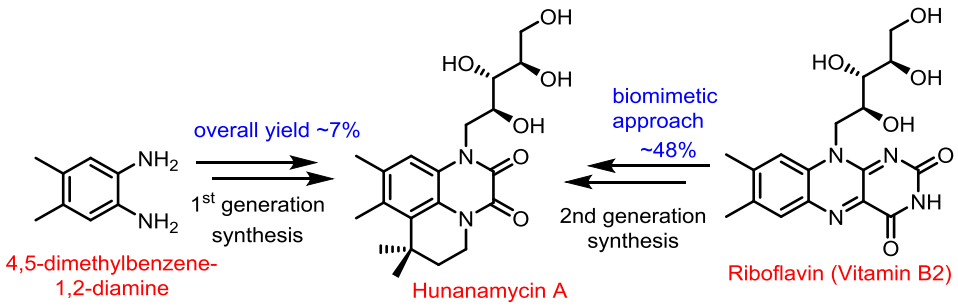
Ph	Phenyl
Pd	Palladium
PK	Pharmacokinetics
SAR	Structure Activity Relationship
TBAF	Tetra-*n*-butylammonium fluoride
TBSCl	*tert*-Butyldimethyl silyl chloride
TEA	Triethyl amine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TsCl	*p*-Toluenesulfonyl chloride
TLC	Thin Layer Chromatography
US-FDA	United States- Food and Drug Administration
vs	versus

Synopsis

Synopsis of the Thesis to be submitted to the Academy of Scientific and Innovative Research for Award of the Degree of Doctor of Philosophy in Chemistry	
Name of the Candidate	Rahul Dilip Shingare
Degree Enrolment No. & Date	Ph. D in Chemical Sciences (10CC12A26029); August 2012
Title of the Thesis	Antibacterial natural product Hunanamycin A: Total synthesis, lead optimization, and related studies
Research Supervisor	Dr. D. Srinivasa Reddy

The thesis is divided into three sections. Section **1** deals with the introduction and total synthesis of antibacterial natural product Hunanamycin A. Section **2** describes the design, synthesis, and structure–activity relationship studies on Hunanamycin scaffold towards finding the lead candidate against bacterial strains of *Salmonella enterica.* Section **3** is related to new chemistry developed around during the Hunanamycin A project execution. It includes synthesis of novel tetracyclic NHCs and their application in cross-coupling reaction. An interesting methodology was also developed for the conversion of quinoxaline-2,3-diones to benzimidazol-2-ones through decarbonylative ring contraction which and applied for synthesis of marketed drug Flibanserin.

Section 1: Introduction and total synthesis of an antibacterial natural product Hunanamycin A

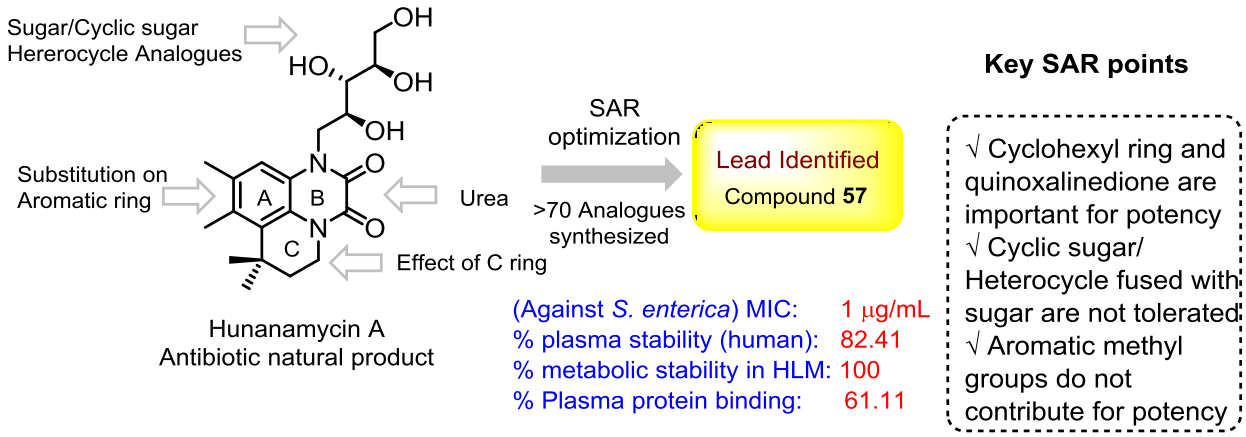


Scheme 1: Synthetic approaches towards Hunanamycin A

Hunanamycin A was recently isolated from a marine-derived *Bacillus hunanensis*.[1] It showed antimicrobial activity against bacterial strains (*Salmonella enterica*) that lacked riboflavin transport mechanism with an MIC of 4.8 μg/mL. *Salmonella enterica* is known to cause foodborne illness in human population in both developed and developing countries. Interesting biological activity with drug-like properties prompted us to initiate and execute a medicinal chemistry program around natural product Hunanamycin A. We started our studies

with accomplishment of first total synthesis of Hunanamycin A. *N*-prenylation, Friedel-Craft alkylation and *N*-alkenylation followed by dihydroxylation in a highly diastereoselective manner led us to achieve the total synthesis of Hunanamycin A.[2] In second generation approach, we have developed much improved practical synthesis (overall yield to 47%), which mimics proposed biosynthesis.[3] This approach allowed us to synthesize Hunanamycin A in multi-gram scale (up to 10g) and conduct all further studies using the material.

Section 2: Synthesis, SAR studies, and lead optimization of Hunanamycin scaffold.



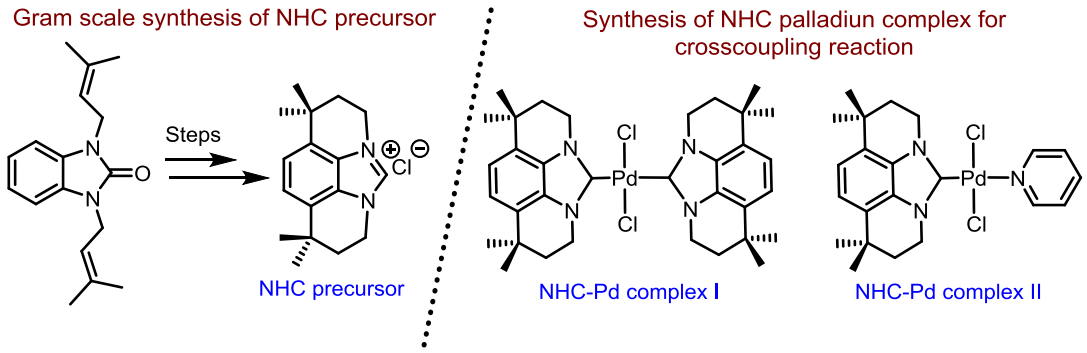
Scheme 2: Analogues planned around Hunanamycin scaffolds and key SAR points

The successful total synthesis and interesting biology of natural product encouraged us to plan and systematically synthesize a library of >70 close analogs of Hunanamycin A with different substitutions around the scaffold. Our main aim was to obtain simplified structure with desirable druggable properties towards lead identification. Systematically we have replaced the ribose sugar moiety with different sugars, cyclic sugars and heterocycles. Similarly, quinoxaline-2,3-diones ring was replaced with benzoyleneurea, benzimidazol-2-one etc. Finally, few analogues were synthesized with/without C ring to understand it's effect on potency. In collaboration with Prof. Macmillan, UT southwestern Medical Center, USA, all the compounds were subjected for biological screening to establish structure activity relationship. Selected compounds were also tested for initial pharmacokinetics, which displayed good in-vitro ADME. Now, after proper biological evaluation we identified compound **57** (MIC of 1 μg/ml against *Salmonella enterica*) with good in-vitro ADME properlies as a lead for further evaluation.[4]

Section 3: New chemistry related to Hunanamycin scaffold.

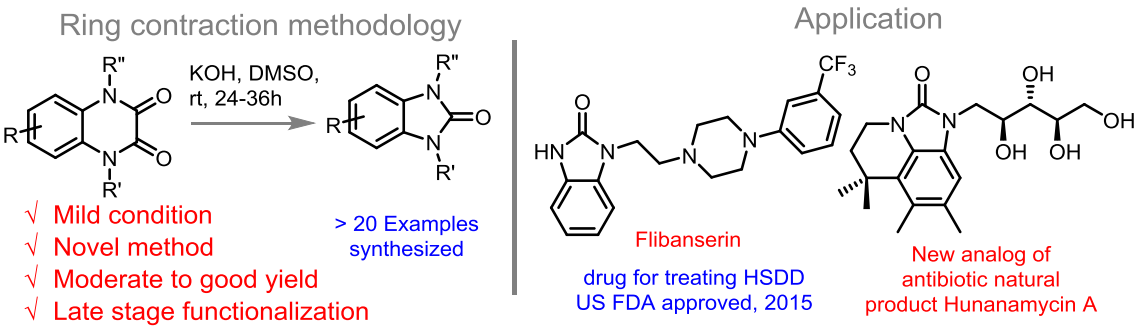
During the execution of Hunanamycin project, we got the opportunity to develop new chemi-

-stry which is described in chapter-3. The first subsection (3.1) deals with general approach to *N*-heterocyclic carbenes (NHCs) with a fused tetracyclic core that can be used as ligand for cross-coupling reactions. During the synthesis of Hunanamycin A and its analogues we encountered the *N*-dialkylated compound as the major side product.[4] Here, we used it for the



Scheme 3.1: Synthesis of tetracyclic NHC precursor and NHC palladium complexes

synthesis of tetracyclic NHC. To demonstrate the utility of the present tetracyclic NHC, we prepared NHC–Pd–NHC complex **I** and NHC–Pd–Py complex **II** from carbene precursor. Then the catalytic activity of NHC palladium complexes was investigated in the cross-coupling reactions and it is found to be superior to existing protocols. The second subsection (3.2) describes a route to benzimidazol-2-ones through decarbonylative ring contraction of quinoxaline-2,3-diones. A simple and practical method to access a variety of benzimidazol-2-ones was developed using potassium hydroxide and dimethyl sulfoxide.[5]



Scheme3.2: Decarbonylative ring contraction of quinoxaline-2,3-diones and application

A series of *N*-alkyl substituted benzimidazol-2-ones were synthesized by decarbonylative ring contraction starting from corresponding quinoxaline-2,3-diones. The present method is useful in medicinal chemistry and natural product synthesis particularly the late stage functionalization of quinoxaline-2,3-diones. The utility of the method has been demonstrated by synthesizing recently approved controversial drug Flibanserin (Addyi) and a urea analogue of marine antibiotic natural product Hunanamycin-A.

Noteworthy Findings:

- Achieved first total synthesis, followed by efficient biomimetic synthesis of Hunanamycin A from riboflavin in gram scale.
- Synthesized >70 analogs of Hunanamycin A and evaluated for antibacterial activity against *S. enterica*. The best compound **57** showed an MIC of 1 μg/mL with excellent PK profile.
- Developed general approach to *N*-heterocyclic carbenes with a fused tetracyclic core that can be used as ligands for Suzuki–Miyaura cross-coupling reaction.
- Developed a methodology for heterocyclic skeletal rearrangement of quinoxaline-2,3-diones to benzimidazol-2-one by decarbonylative ring contraction. Later this methodology was applied for the synthesis of Flibanserin drug.

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3. **Shingare, R. D**.; Sa ada Farhana.; Reddy, D. S. *Tetrahedron Lett.* **2016,** 57, 3662.
4. Sutar, R. L.; Kumar, V.; **Shingare, R. D**.; Thorat, S.; Gonnade, R.; Reddy, D. S. *Eur. J. Org. Chem*. **2014**, 4482.
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6. **Shingare, R. D.;** MacMillan J. B.; Reddy, D. S. Riboflavin synthase inhibitor Hunanamycin A; Lead identification towards anti-salmonella agents. *Manuscript under preparation*.

Contents

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Section 1

Introduction and total synthesis of an antibacterial natural product Hunanamycin A

Section 1

Introduction and total synthesis of an antibacterial natural product Hunanamycin A

1.1 Introduction

Natural products are "chemical substances produced by living organisms". They have been playing a pivotal role in traditional medicines, remedies from ancient times and still continuing. Natural products offer more advantages over the synthetic compounds because of their wide-ranging skeletal diversity, chemical complexity and other molecular properties owing to which they interact very specifically with biological targets, thus making them valuable as lead structures for drug discovery.[1-5] A Recent literature review by Newman and Cragg states that ~ 50% of the approved drugs belong to the class of natural products directly or indirectly.[6] The oceans or marines covering two third of the total earth's crust has been a valuable and priceless source of several biological and chemical diversities; but yet unexplored.[7-9] Although most of the currently available drugs derived from nature come from terrestrial species, contributions from marine sources are very significant. Adaptation to extreme environments of marine world renders the marine organisms to produce structurally diverse and interesting metabolites which may have impressive biological activity and unique mechanism of action.[10-11] Majority of the isolated marine natural products possess impressive anticancer activity (56%) and antibacterial activity (16%) (Figure **1.1**).[12-13]

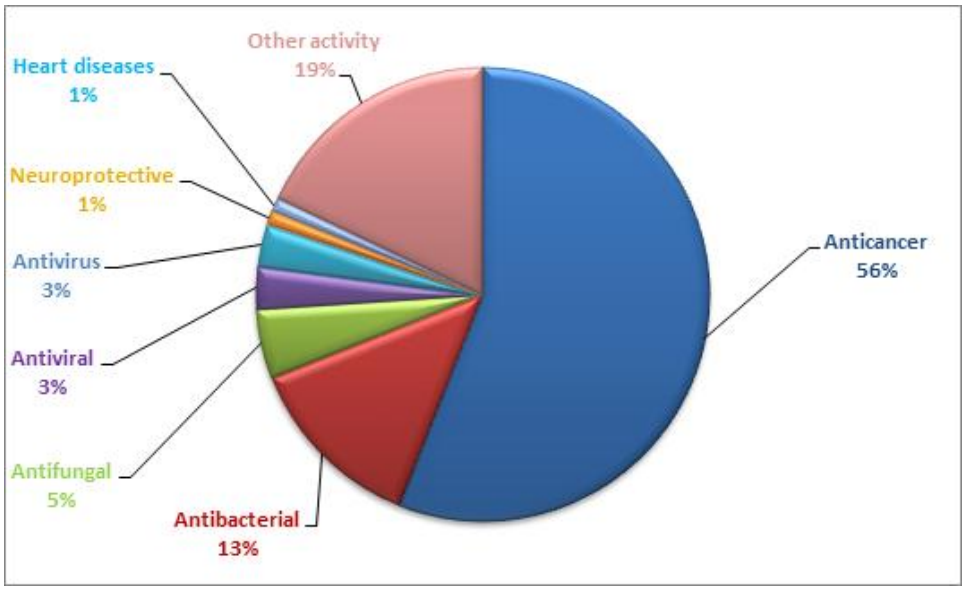


Figure 1.1: Bioactivities of marine natural products

Ziconotide (SNX-111; Prialt) an atypical analgesic agent for treatment of chronic pain was the first marine natural product approved by the FDA in 2004.[14] Till date, 7 marine natural products were approved as drugs, and 25 are in different phases of clinical trials. Some of the examples are captured in Figure **1.2**.[15]

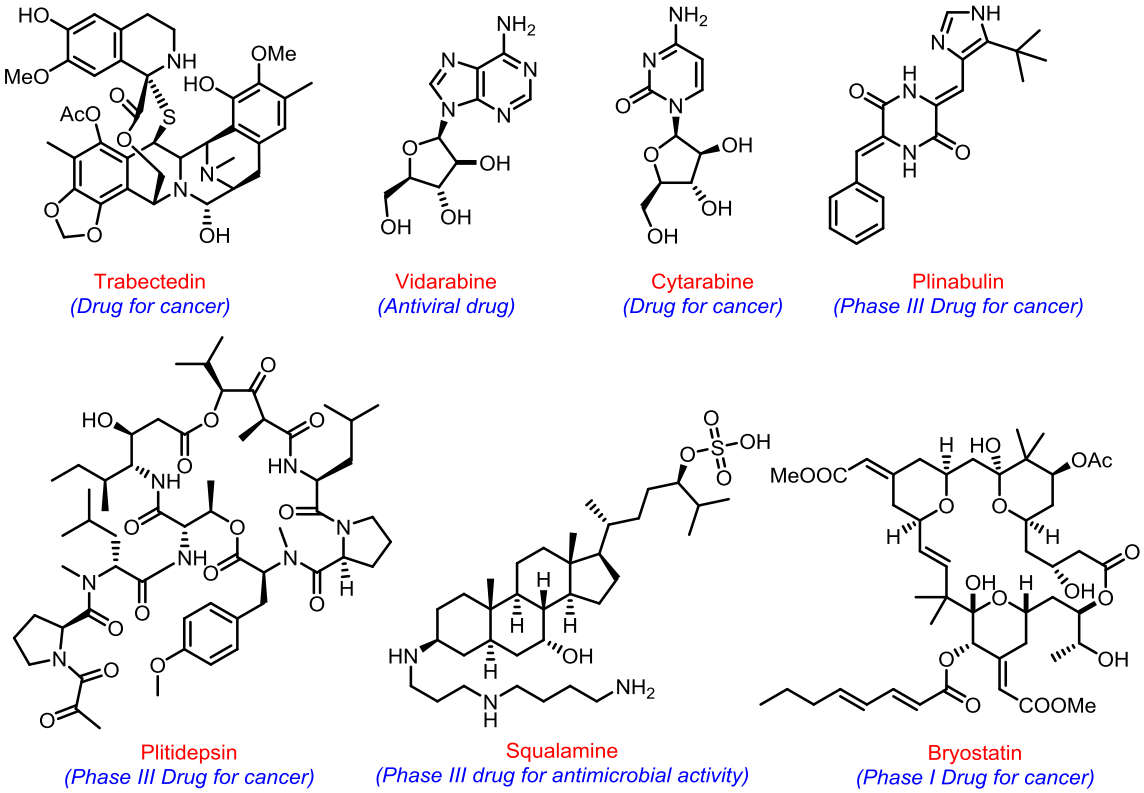


Figure 1.2: Selected marketed drugs and compounds in clinical trials from marine source

Although several natural products from the marine sources were isolated and characterized, the paucity of isolated material have been an unavoidable issue for complete biological profiling. To address the same, various research groups across the world are engaged in total synthesis of bioactive natural products. Besides, synthetic efforts to access the natural products, it also helps in producing fragments, compounds with simplified structural complexity and analogues related to the natural product, which may have improved biological properties.

In this context, our group has continued interest in the synthesis of biologically active natural products as well as their analogues towards identifying lead molecules.[16-20] In the course of doing so; we got attracted towards an antibacterial natural product Hunanamycin A. In 2013, MacMillan and co-workers isolated a new compound that had similar structural features of riboflavin from *Bacillus hunanensis* strain. Detailed spectroscopic analysis revealed the novel compound called Hunanamycin A (Figure **1.3**).[21-22] It can be considered

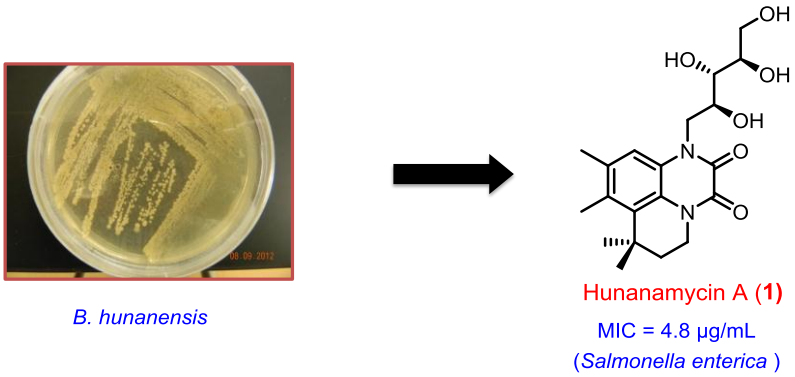
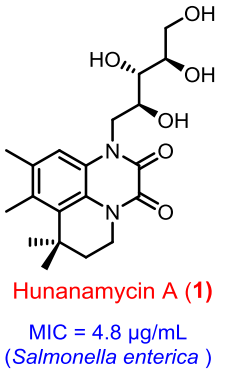


Figure 1.3: Antibiotic natural product Hunanamycin A

as the first example of *N*-prenylated riboflavin analogue. Owing to the structural similarities of Hunanamycin A with riboflavin degradation product, MacMillan group screened Hunanamycin A against various bacterial strains, in particular, that are lacking riboflavin transport mechanisms.[23] The results showed a MIC of 4.8 µg/mL against *Salmonella enterica* which probably acting through inhibition of riboflavin synthase. Riboflavin synthase enzyme catalyses the last step in the biosynthesis of riboflavin.[24] In disease models of salmonella infection, knockout of the riboflavin synthase was found to be lethal to the pathogen, and hence it is an interesting antibiotic target, as humans lack this riboflavin synthase enzyme.[25] Humans acquire riboflavin through food whereas bacteria synthesize endogenously using riboflavin synthase. At this stage, it is worth highlighting Cushman group's contribution towards identifying synthetic inhibitors for the riboflavin synthase and lumazine synthase (discussed in the next section).



Lipinski rule of five	Calculated parameters for **1**
Mol.Wt. < 500	Mol.Wt. = 392
cLogP < 5	cLogP = 1.38
H-bond donors < 5	H-bond donors = 4
H-bond acceptors <10	H-bond acceptors = 8

Figure 1.4: Comparison of Hunanamycin A on Lipinski parameter

Salmonella enterica, a prominent pathogenic species of *Salmonella*, causes foodborne illness and accounts for significant damage to humans in both developed and developing countries. Another interesting feature of Hunanamycin A molecule is that it perfectly fits in to

Lipinski's rule of five, a well-accepted and practiced rule in the field of medicinal chemistry (Figure **1.4**).[26]

Thus, we were interested in taking up the project with the following aims

i) The total synthesis of Hunanamycin A in sufficient quantity.

ii) Confirmation of the assigned structure along with its absolute configuration.

ii) Preparation of a diverse library of compounds around this scaffold.

1.2 Total synthesis

By considering attractive biological activity and novel chemical scaffold, we made several efforts in making the target molecule and its analogs. We have accomplished the first total synthesis of Hunanamycin A (**1**) in five longest linear reaction sequence. During the first total synthesis, the substrate controlled diastereoselective dihydroxylation was used as a key step and the relative stereochemistry was established by applying Kishi empirical rule. Later, in second generation approach; we undertook a green, practical and biomimetic route starting from readily accessible, inexpensive riboflavin and obtained Hunanamycin A with excellent overall yield. All the details about both synthetic routes are discussed in the following sections.

1.2.1 First total synthesis of Hunanamycin A

1.2.1.1 Initial attempts towards the total synthesis of Hunanamycin A

Our initial strategy to access the natural product Hunanamycin is shown in Figure **1.5**. We have envisioned that the tricyclic core of the natural product **1** could be derived from the known dihydroquinoxaline-2,3-dione moiety **2** by *N*-prenylation followed by Friedel–Crafts

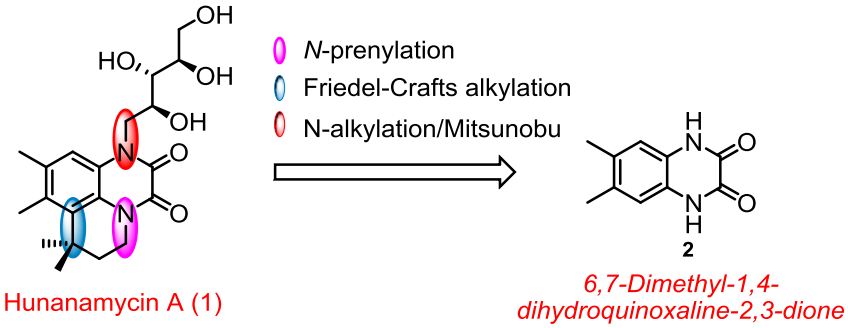
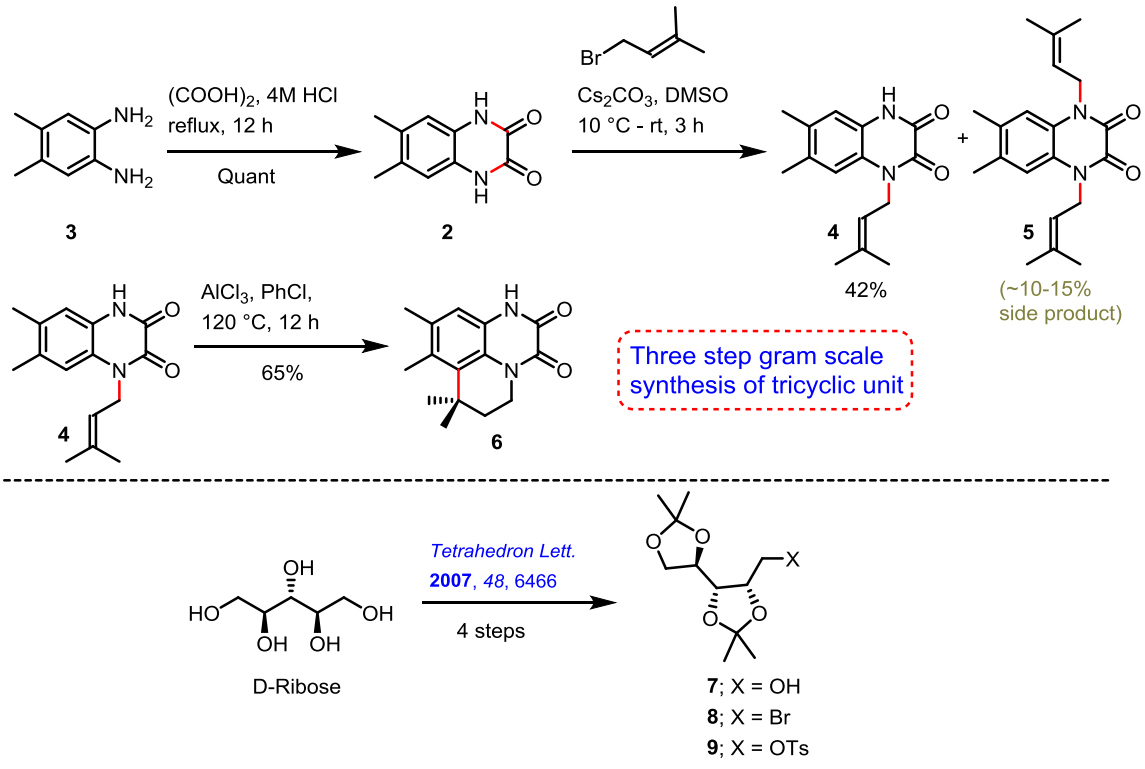


Figure 1.5: Key disconnections for the planned synthesis

cyclization. Finally, we planned to install readily available ribose unit on the tricyclic core by base-mediated *N*-alkylation, or Mitsunobu reaction. Initially, the construction of the tricyclic

part and attachment of a sugar unit to the tricyclic unit were planned. This sequence becomes convenient to synthesize related analogues of natural product Hunanamycin A (**1**).

Our planned synthesis began with a reaction of 4,5-dimethylbenzene-1,2-diamine **3** and oxalic acid by refluxing in 4M HCl to produce brown solid of 6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione **2** by following literature protocol.[27] The next step was to install the prenyl group selectively on one of nitrogen although, it is a symmetrical compound, selective mono alkylation proved to be a challenging task. We have screened several bases such as K_2CO_3, NaH, Cs_2CO_3 and solvents like THF, DMF, and DMSO and varying temperatures to have best conditions for the synthesis of mono alkylated product **4**. The mono-alkylation of **2** was achieved using prenyl bromide and Cs_2CO_3 as a base in DMSO at 10 °C to rt which furnished the desired product **4** in moderate yield (42%) along with undesired diprenylated compound **5**. The formation of mono prenylated product **4** was primarily indicated by staining TLC in iodine as well as in $KMnO_4$. In the ^1H NMR of compound **4** signals at δ 5.11 (br. s, 1H) corresponds to C-H of the internal olefin, and CH_2 from the prenyl unit appears at δ 4.69 (d, J = 6.8 Hz, 2 H). Further, it was confirmed

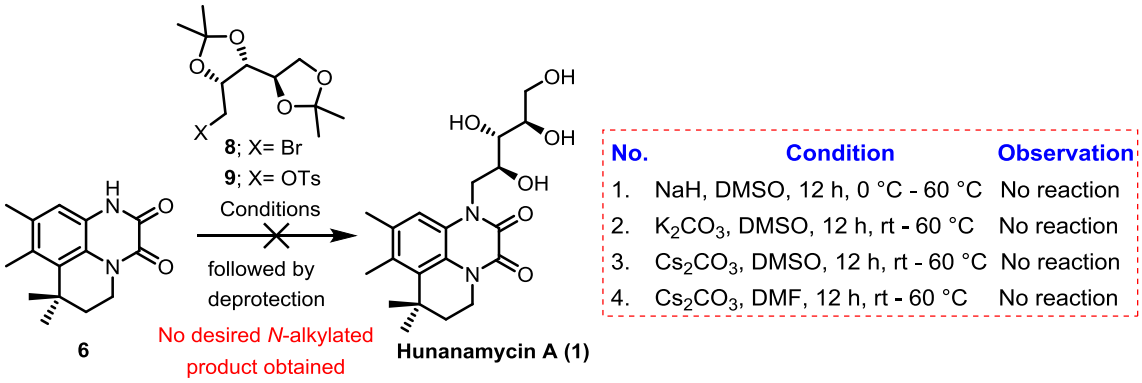


Scheme 1.1: Synthesis of tricyclic aglycon unit and protected ribose sugar

by HRMS analysis showed a peak at 281.1261 for the molecular formula $C_{15}H_{18}O_2N_2Na$ $[M+Na]^+$. The diprenylated compound **5** was confirmed by all the spectroscopic technique

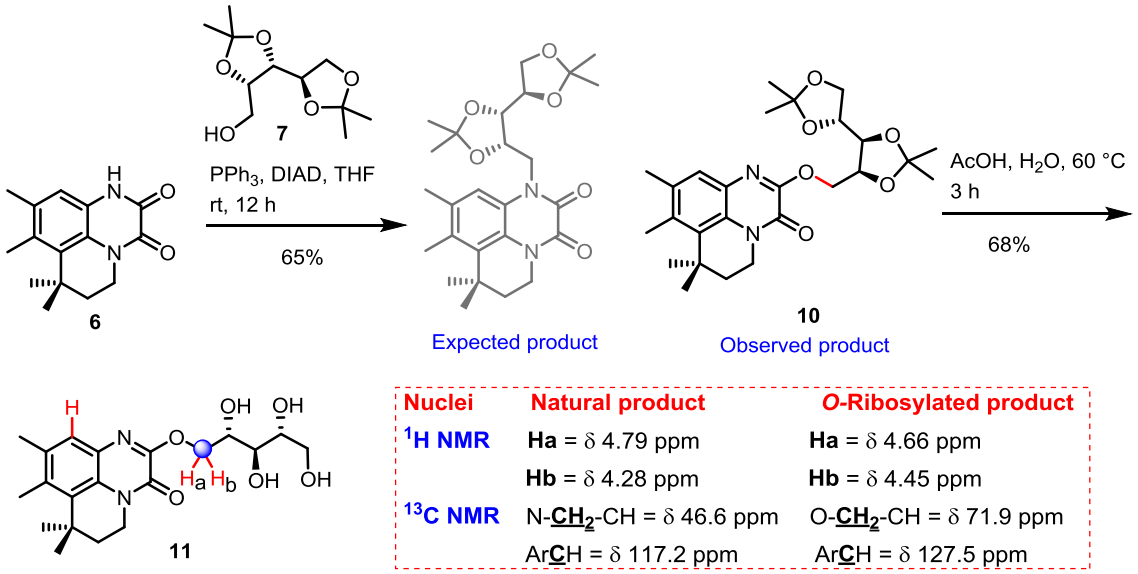
(^{1}H, ^{13}C NMR, IR, and HRMS) and found to be in agreement with the structure. During the scale-up of this reaction along with the product, we have isolated considerable amount of dialkylated side product **5** which was utilized to generate synthetically valuable intermediates and developed interesting chemistry, which will be discussed in section **3**. The gram scale synthesis of tricyclic aglycon **6** was achieved using intra-molecular Friedel-Crafts alkylation of compound **4** using Lewis acid $AlCl_3$, and chlorobenzene as a solvent in excellent yield (Scheme **1.1**).[28] Formation of the tricyclic compound was confirmed by the disappearance of olefin signals and appearance of the peak for methylene in ^{1}H NMR at δ 1.92 (t, J = 5.2 Hz, 2 H). It was again confirmed by HRMS, which showed a peak at 281.1258 corresponding to molecular formula $C_{15}H_{18}O_2N_2$ $[M+Na]^+$.

To install ribose sugar moiety on tricyclic core, we have synthesized desired derivatives **7**, **8** and **9** starting from readily available D-(-) ribose using well established procedures in the literature. Next, a *C-N* bond formation of tricyclic intermediate **6** with known ribose sugar derivative **8** or **9**,[29] using base mediated *N*-alkylation was attempted. To our surprise, all the conditions failed to provide desired *N*-alkylated product. Instead we observed decomposition of the sugar unit and most of the tricyclic intermediate **6** was recovered (Scheme **1.2**).



Scheme 1.2: Attempts toward base-mediated *N* alkylation

As an alternative approach, we attempted Mitsunobu reaction condition (TPP, DIAD)[30] for the formation of *C-N* bond between tricyclic core **6** and protected ribose derivative **7**.[29] This time we could see the formation of a new compound from TLC, along with mass the of desired product. We took forward this compound for deprotection of acetonide using aqueous acetic acid at 60 °C and the spectral data of the product **11** obtained was compared with the target natural product and indicated the formation of *O*- alkylated product **11** (Scheme **1.3**). The critical differences are shown in the Scheme **1.3** which clearly confirms the formation of



Scheme 1.3: Formation of *O*-ribosylated product under Mitsonobu reaction

O-alkylated product. In the case of ^{13}C NMR, the differences were very significant, particularly, in the aromatic region. Also, the methylene attached to nitrogen appears at δ 46.6 ppm whereas in *O*- ribosylated product methylene appears at δ 71.9 ppm which made us to conclude that there was formation of only *O*-alkylated product **11** (Figure **1.6**).[31-32]

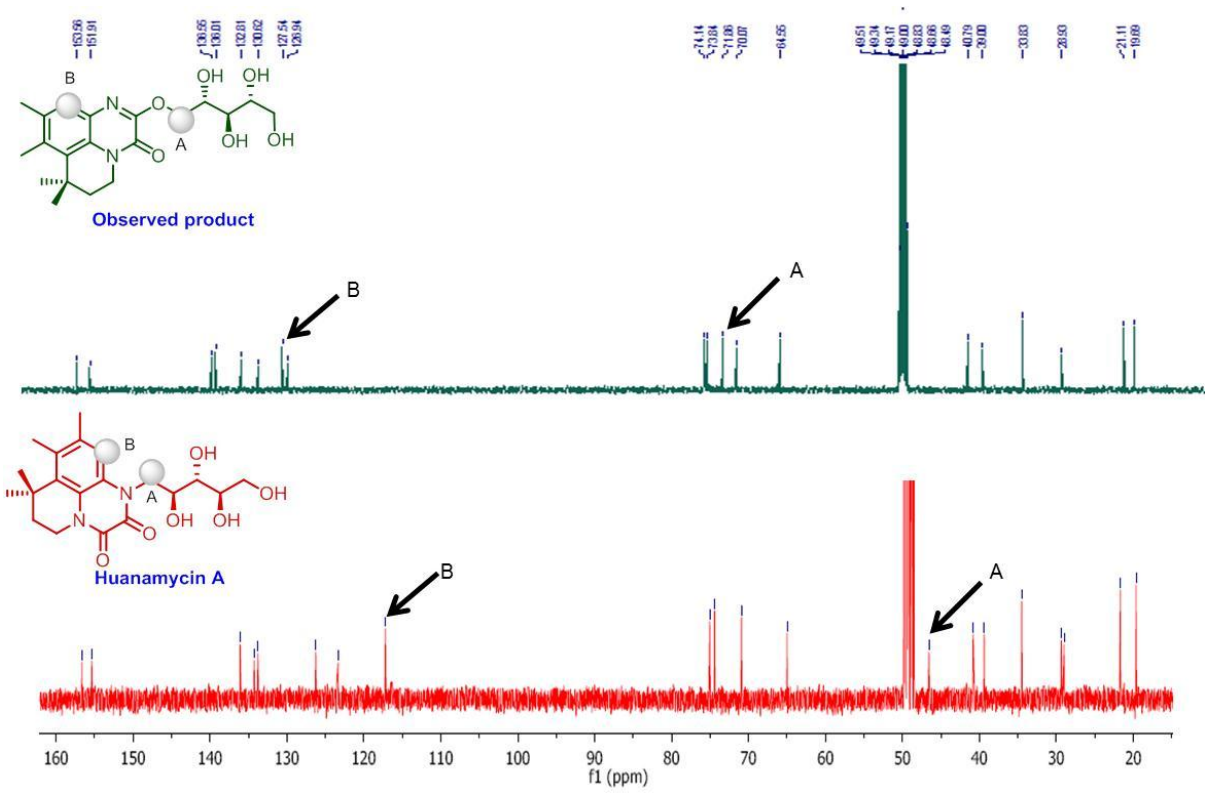


Figure 1.6: Comparison of ^{13}C spectrum of compounds **1**, and **11**

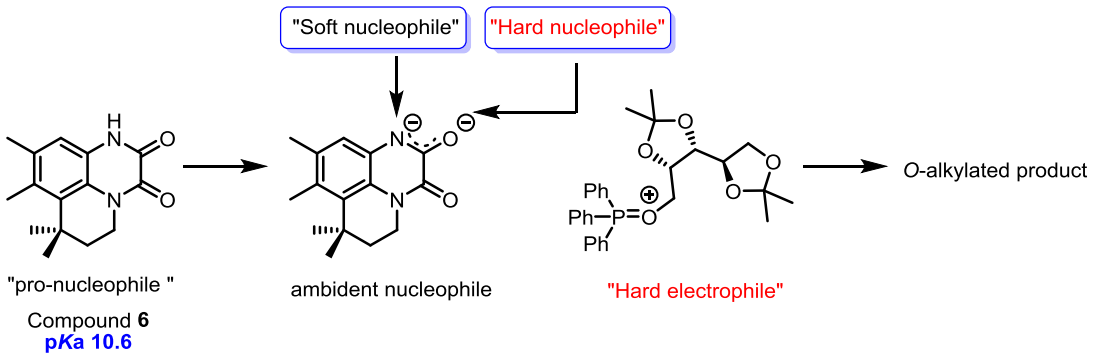
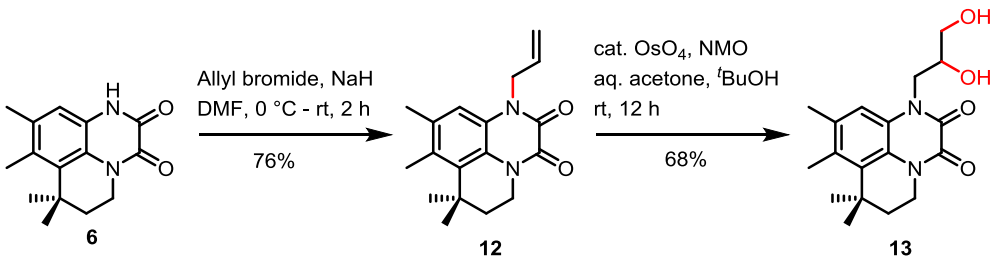


Figure 1.7: Explanation for *O*-alkylated product under Mitsunobu reaction

In the tricyclic quinoxaline-2,3-dione **6** the calculated p*K*a of anilide moiety is 10.6 which is considerably lower, thereby suggesting it as a pro-nucleophile in Mitsunobu reaction conditions (TPP/DIAD system).[33-34] The formation of the *O*-alkylated product **11** could be best explained based on the HSAB principle. The compound **6** contains amide functional group which is an ambident nucleophile; oxygen is more electronegetive than that of nitrogen atom hence it is considered to be a hard nucleophile. As per the understanding of Mitsunobu reaction mechanism, the intermediate alkoxyphosphonium formed is positively charged therefore it is considered as hard electrophile. So, according to Pearson's HSAB principle the harder, more electronegative atom i.e. oxygen of the ambident nucleophile should react with the hard electrophile which leads to the formation of *O*-alkylated product exclusively (figure **1.7**).[35-37] However, we did not make any attempts to confirm these by experimental evidences.

These results encouraged us to explore alternate options such as a base mediated *N*-alkylation with more reactive alkyl halide. Compound **6** on reacting with allyl bromide, in presence of sodium hydride (60% in mineral oil) and DMF as solvent gave *N* allylated intermediate **12**, in good yield (Scheme **1.4**). The formation of **12** was indicated by the presence of signals in ^{1}H NMR at δ 4.85-4.86 (m, 2 H) characteristics to the methylene group flanked in between olefin and nitrogen atom of amide, and δ 5.21-5.29 (m, 2 H), 5.90-5.96 (m, 1 H) for the terminal olefin. The ^{13}C NMR also confirmed the formation of **12** which displayed olefin peaks at δ 115, δ 130.8, and peak at δ 45.5 corresponds to methylene originating from the newly introduced allyl functionality. The double bond present in **12** was dihydroxylated, using a cat. OsO_4 and *N*-methyl morpholine oxide (Upjohn Dihydroxylation condition) to furnished the diol **13** in 68% yield (Scheme **1.4**). Compound **13** was confirmed by the disappearance of olefin signals and appearance of methylene proton in ^{1}H NMR at δ 3.63 (d, J = 5.5 Hz, 2 H). Besides the peak at δ 4.03 (m, 1 H) was assigned to the CH attac-



Scheme 1.4: Model reaction towards *N*-alkylation reaction

-hed to a newly generated hydroxyl group. It was further confirmed by HRMS, which showed a peak at 333.1802 for the molecular formula $C_{18}H_{25}O_4N_2$ $[M+H]^+$, with a calculated value of 333.1809. We also compared the ^{13}C NMR spectrum of compound **13** with natural product **1** and found that all aromatic carbon peaks and the signals appearing below 50 ppm match closely (Figure **1.8**).

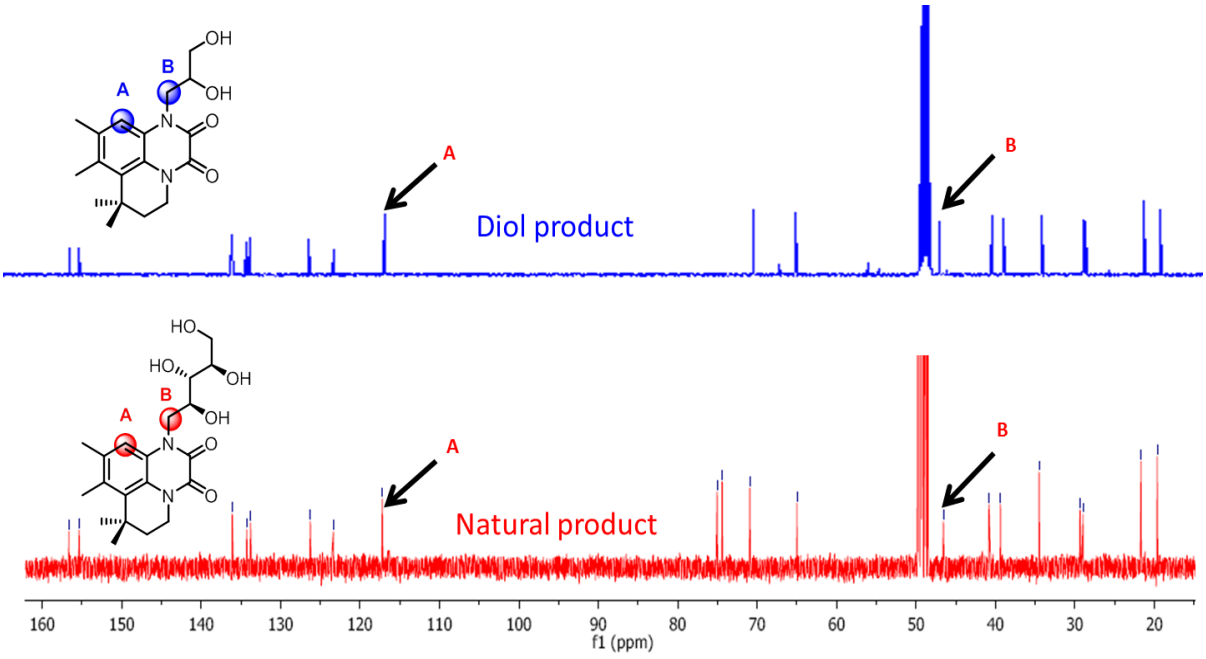


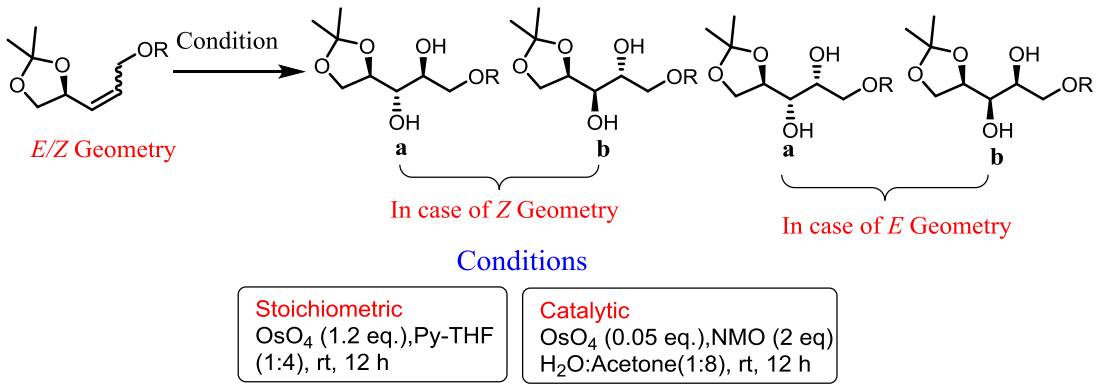
Figure 1.8: Comparison of ^{13}C spectrum of compounds **1** and **13**

1.2.1.2 Revised strategy and completion of the synthesis of Hunanamycin A

Excited with the above results, we came to the conclusion that the direct *N*-alkylation of the tricyclic core with ribose is difficult or not possible and for successful *N*-alkylation, we must use activated alkyl derivative. Accordingly, we have modified the strategy to install sugar moiety on the tricyclic core and opted Kishi's well-established model in redesigning the strategy.[38] Kishi and co-workers have extensively studied the dihydroxylation of olefins bearing suitably protected chiral hydroxyl group at one terminal position (Table **1.1**). Based on the observation of the selectivity obtained in the product Kishi formulated empirical rules

for the prediction of the relative stereochemistry of the product.

1. The stoichiometric procedure gave higher yield over catalytic.
2. Hydroxy or alkoxy group plays a key role in stereoselectivity.
3. High degree of Stereoselectivity is observed in the case of *Z* olefin over *E* olefin.
4. The relative stereochemistry between the pre-existing hydroxyl or alkoxy group and the adjacent newly introduced hydroxyl group of the major product in all cases is erythro.



No	R	Geometry	Condition	Selectivity (a : b)
1.	H	*Z*	Stoichiometric	6.3 : 1.0
			Catalytic	6.0 : 1.0
2.	$COC(Me)_3$	*Z*	Stoichiometric	6.3 : 1.0
			Catalytic	6.1 : 1.0
3.	$Si(Ph)_2(tBu)$	*Z*	Stoichiometric	8.0 : 1.0
			Catalytic	7.2 : 1.0
4.	H	*E*	Stoichiometric	6.3 : 1.0
			Catalytic	6.0 : 1.0
5.	$COC(Me)_3$	*E*	Stoichiometric	6.3 : 1.0
			Catalytic	6.1 : 1.0
6.	$Si(Ph)_2(tBu)$	*E*	Stoichiometric	8.0 : 1.0
			Catalytic	7.2 : 1.0

Table 1.1: Distereoselective dihydroxylation of allylic alcohol[35]

Keeping in mind the stereochemical outcome of dihydroxylation reaction of literature precedence,[38] we redesigned our substrate in such a way so as to tune it further to the desired stereochemistry in the Hunanamycin A. So here, we envisioned that the intermediate **14** with

the suitable protecting group would be ideal for dihydroxylation to induce the desired stereochemistry in natural product (Figure **1.9**). The necessary olefin coupling partner **17** was easily synthesized form D-mannitol in simple five steps according to literature protocol.

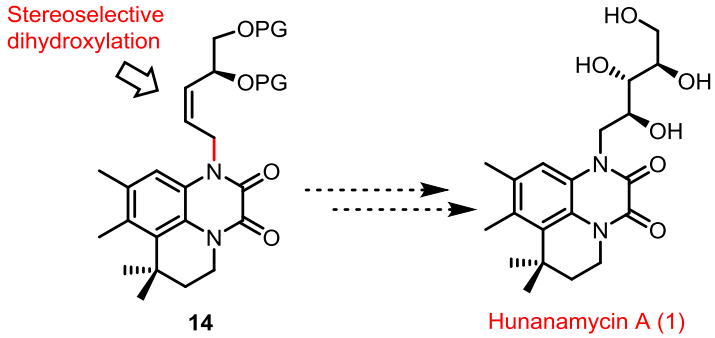
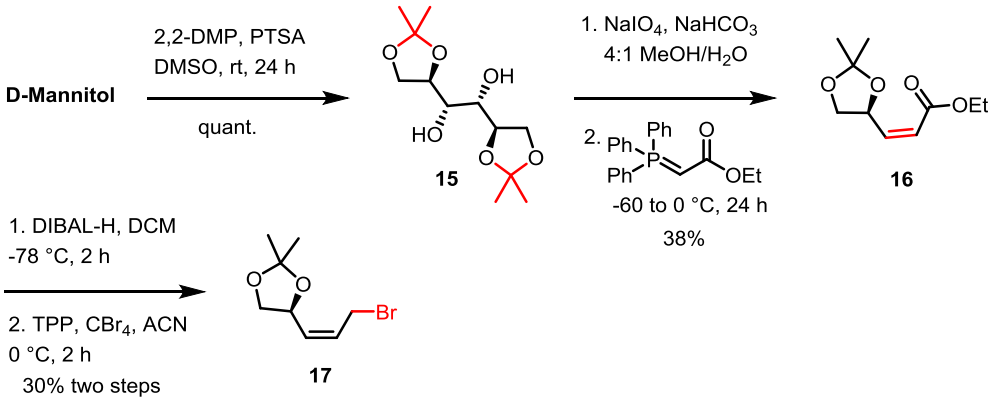


Figure 1.9: Revised strategy for the completion of the synthesis

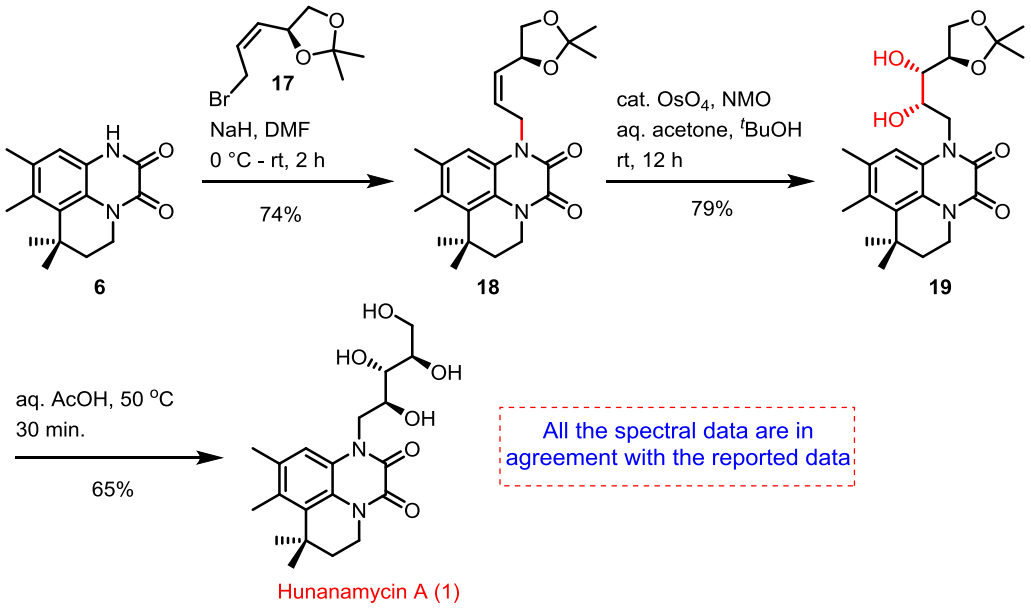
The synthesis started with the protection of mannitol using 2,2-Dimethoxypropane, catalytic PTSA in DMSO as a solvent to afford 1,2:5,6-di-*O*-isopropylidene D-mannitol **15** as a white solid in quantitative yields. One-pot periodate cleavage and successive Wittig olefination with ethyl 2-(triphenyl-λ^5-phosphaneylidene) acetate under well-established condition afforded the desired compound **16** with 16:1 *Z*:*E* selectivity. Further, ester moiety was reduced using DIBAL-H, the allylic alcohol obtained from the reaction on treatment with triphenylphosphine and carbon tetrabromide in acetonitrile solvent was converted to corresponding allyl bromide **17**.[39] The desired product **17** was further confirmed by comparing with ^{1}H NMR reported in the literature. (Scheme **1.5**)



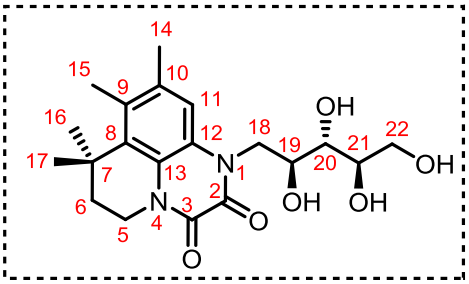
Scheme 1.5: Synthesis of allyl bromide key fragment for coupling **17**.

After having compound **17** in hand, we embarked on the total synthesis of Hunanamycin A. Here, we initially performed the *N*-alkylation of compound **6**, with sugar derived bromide **17**, using NaH (60% in mineral oil) in DMF to furnish the required compound **18** in good yield. The reaction was monitored by staining the TLC ($KMnO_4$ Solution). The formation of **18** was

indicated by the presence of signals in ^{1}H NMR at δ 5.70-5.69 (m, 1 H) and δ 5.67-5.61 (m, 1 H) for the internal olefin proton, and δ 4.13 (t, *J* = 5.7 Hz, 2 H) corresponds to CH_2 attached to amide nitrogen of tricyclic ring, and the methyl group form the acetonide resonate at δ 1.46 (s, 3 H) and 1.45 (s, 3 H). The ^{13}C NMR also confirmed the formation of **18** through the appearance of olefin peaks at δ 131.6, 127.5 along with the other characteristic peaks from both tricyclic skeletons and sugar unit further confirmation was obtained by HRMS analysis. The newly introduced *Z*-olefin was oxidized under previously optimized Upjohn dihydroxylation condition (OsO_4/NMO)[40] which resulted in the formation of compound **19** in highly diastereoselective fashion, which was the very pleasing outcome for us. These practical results are in accordance with the "Kishi's empirical rule" as explained above. The disappearance of olefin peaks was also observed when comparing the ^{1}H NMR and ^{13}C NMR of compound **19** and **18.** Besides, the diol formation could also be clearly seen in the IR spectrum which showed a characteristic broad peak at 3429 cm^{-1} which is absent in olefin intermediate **18** (IR: 1677, 1745, 2400, 2925, 3020 cm^{-1}). It was further confirmed by HRMS, which showed a peak at 455.2143 corresponding to $C_{23}H_{32}N_2O_6Na$ $[M+Na]^+$. Finally, the acetonide protecting group was removed in aqueous acetic acid by heating at 50 °C for 30 min to furnish PMA active new polar spot on TLC at R_f 0.2 (10% MeOH:DCM). Then it was purified by silica gel column chromatography to afford the bright yellow solid product which was fully characterized by spectroscopic techniques (Scheme **1.6**). The ^{1}H and ^{13}C NMR spectrum of the synthetic Hunanamycin A (**1**) were compared with the data of natural sample and found to be identical (See comparison table **1.2**).



Scheme 1.6: Completion of the total synthesis of Hunanamycin A



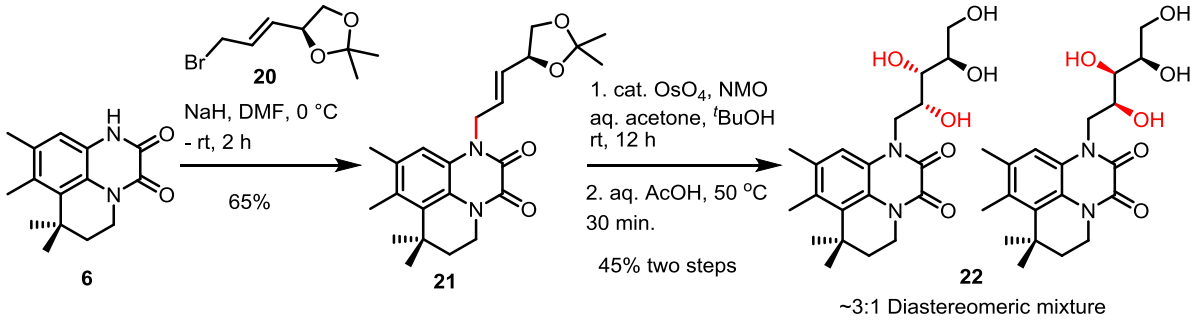
Hunanamycin A (1)

Natural sample in CD_3OD			Synthesis sample in CD_3OD	
No	^1H, mult, (*J* in Hz)	^{13}C	^1H	^{13}C
2	-	156.7	-	156.7
3	-	155.3	-	155.4
5	4.12, m	39.3	4.14, m	39.4
6	1.94, t (5.9)	40.8	1.94, t (5.8)	40.8
7	-	34.5	-	34.5
8	-	134.3	-	134.3
9	-	133.8	-	133.8
10	-	136.0	-	136.1
11	7.47, s	117.2	7.46, s	117.2
12	-	126.2	-	126.3
13	-	123.3	-	123.4
14	2.33, s	21.6	2.34, s	21.6
15	2.45, s	19.6	2.46, s	19.6
16	1.55, s	29.3	1.55, s	29.4
17	1.54, s	29.0	1.55, s	29.0
18	4.79, dd (9.8, 14.3) 4.28, dd (2.9, 14.3)	46.6	4.78, dd (9.9, 14.1) 4.29, m	46.6
19	4.25, ddd (9.9, 2.9, 4.0)	70.6	4.25, overlapped	71.0
20	3.76, dd (4.0, 7.2)	75.0	3.78, overlapped	75.1
21	3.77, ddd (7.2, 5.0, 2.9)	74.4	3.79, broad singlet	74.4
22	3.81, dd (2.9, 11.2) 3.67, dd (5.0, 11.2)	65.0	3.81, overlapped 3.67, dd (4.6, 11.9)	65.0

Table 1.2: Comparison of spectral data of natural and synthetic Hunanamycin A

The absolute stereochemistry of Hunanamycin A was confirmed by using optical rotation values of synthetic and natural Hunanamycin A samples. Following this route, we have prepared a good quantity (~80 mg) of the natural product Hunanamycin A (>100-fold with respect to natural sample) for the first time.

After successful synthesis of the natural product, our next aim was to study the diastereoselectivity in the dihydroxylation reaction of the olefin having *E* geometry and to access stereochemical analogues of the Hunanamycin A. Accordingly, we have synthesized bromide derivative **20** having *E* geometry as per the reported literature protocol.[41-42] The *N*-alkylation of aglycon **6** with *E*-olefin **20** was performed using NaH in DMF to afford compound **21** in good yields. The *E* geometry of the internal olefin in *N*- alkylated product **21** was confirmed by the coupling constant observed in ^{1}H NMR with δ 5.89 (dt, *J* = 15.6, 5.3 Hz 1 H) and δ 5.72 (dd, *J* = 15.6, 6.5 Hz 1 H), signals at δ 4.50 corresponds to CH attached to olefin and oxygen. ^{13}C NMR spectra showed the presence of olefin carbons at δ 131.7 and 126.0 whereas, two amide carbonyl also showed a resonance at δ 153.5, 153.4. Finally, OsO_4/NMO mediated dihydroxylation of **21** under similar condition as mentioned above, followed by deprotection of acetonide using aqueous AcOH in heating at 50 °C for 30 minutes furnished the Hunanamycin analogues **22** as a mixture of diastereomers (~3:1 ratio based on ^{1}H NMR) (Scheme **1.7**).



Scheme 1.7: Synthesis of Hunanamycin A analogue by dihydroxylation of *E*- olefin

As this product was a diastereomeric mixture, the ^{1}H NMR showed multiplets for all the peaks whereas the ^{13}C NMR spectrum showed peaks at δ 156.5, and 155.3 for amide carbonyl group. Four characteristic peaks were observed for carbons attached to the hydroxyl group at 72.7, 72.6, 68.6 and 65.2 along with other peaks from the tricyclic part as well. The IR spectrum showed a characteristic broad peak at 3363 cm^{-1} due to a hydroxyl group and peak at 1668 cm^{-1} which correspond to the amide carbonyl stretching. This was further confirmed by HRMS, which showed a peak at 415.1833 for the molecular formula

$C_{20}H_{28}N_2O_6Na$ $[M+Na]^+$. All the attempts to separate both the diastereomers went in vain. Thus, in the case of *E*-olefin containing compound, we have observed less diastereoselectivity with respect to *Z*-olefin. This observation was consistent with the results in the literature report by the Kishi's group (Table **1.1**).

1.2.2 Improved biomimetic synthesis of Hunanamycin A (2nd generation)

Although we have accomplished the first total synthesis of Hunanamycin A for the first time, It was not the best route which has some drawbacks such as poor yields, expensive starting material and multiple steps. Another aspect of natural product synthesis is to provide an uninterrupted and continuous supply of materials for various biological evaluations. Promising biological activity of Hunanamycin A and drawbacks associated with our previous synthetic approach motivated us to develop a convenient and efficient method for the synthesis, by using biomimetic approach (Figure **1.10**)

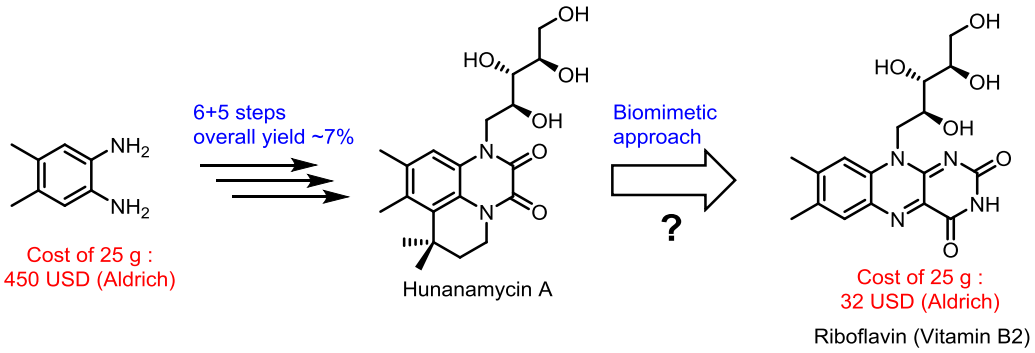


Figure 1.10: New strategy for the synthesis of Hunanamycin A

1.2.2.1 Brief introduction of biomimetic total synthesis

The biomimetic synthesis is a chemical synthesis that is specifically biologically inspired. The term "biomimetic synthesis" was coined by Nobel laureate Sir Robert Robinson in 1917, when he prepared the alkaloid tropinone, starting from simple materials in a one-pot procedure (Figure **1.11**). Biomimetic synthesis provides smarter solutions to long-standing challenges in the total synthesis, a novel disconnection approaches, an opportunity to develop new reaction to achieve an efficient and green synthesis.

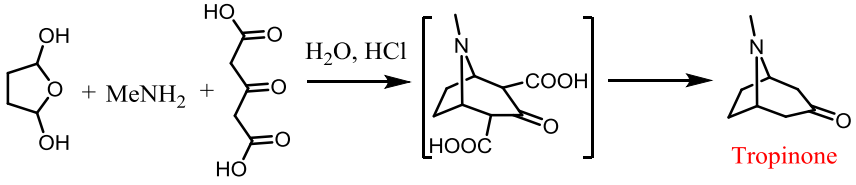


Figure 1.11: Robinson's biomimetic synthesis of tropinone

In recent times, biomimetic synthesis is getting more attention because emulating nature's methods have proved to be a useful strategy in natural product synthesis and often leads to very efficient synthetic protocols.[43]

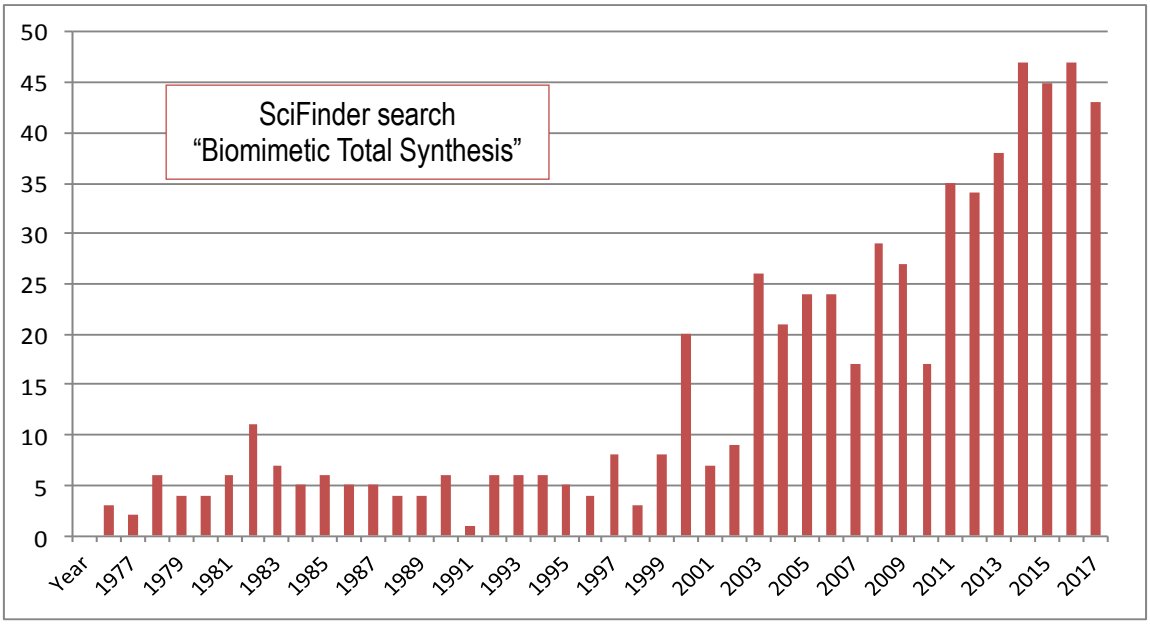
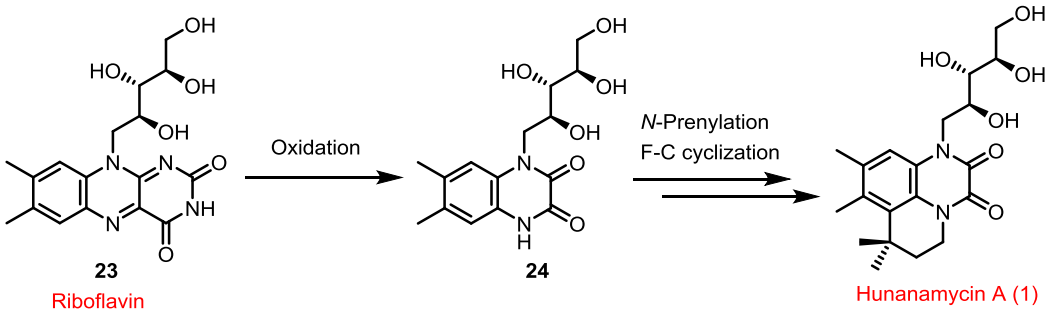


Figure 1.12: Biomimetic total synthesis publications over last 40 years

Our SciFinder search for 'Biomimetic total synthesis' notably shows the increase in a number of research articles published in recent years. It clearly highlights the growing importance of this approach and is attracting the focus of many research groups around the world (Figure **1.12**). Along these lines, it is worth to mention here, that we have used this strategy in one of the recent project.[44]

1.2.2.2 Proposed biomimetic synthesis of Hunanamycin A

MacMillan and co-workers' have proposed biosynthesis of Hunanamycin A based on the literature reported closest compound **24** which is the degradation product obtained from the riboflavin **23**.[22] This compound **24** undergoes biosynthetic *N*-prenylation, followed by



Scheme 1.8: Proposed biosynthesis by Macmillan and co-workers

the electrophilic attack on the π-system of the prenyl group by the aromatic ring in a manner similar to a Friedel-Crafts alkylation and would provide Hunanamycin A (Scheme **1.8**).

Inspired by proposed biogenesis by MacMillan group, we planned new strategy starting from riboflavin to overcome the problems in the previous synthesis and to access sufficient quantity of natural product. Accordingly, we have carried out a detailed literature review on the degradation of riboflavin, highlights of which are described below.

1.2.2.3 Alkaline hydrolysis of riboflavin

In 1951, Surrey and co-workers stabilized the method for alkaline hydrolysis of riboflavin **23**.[45] Treatment of riboflavin with 1 N NaOH solution at 90 °C for 1 h to obtain sodium salt of 6,7-dimethyl-3-oxo-4-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-3,4-dihydroquinoxaline-2-carbox -ylic acid which on acidification with excess of dilute H_2SO_4 gave yellow needles of riboflavin degraded keto acid product **25** in gram scale (Figure **1.13**).

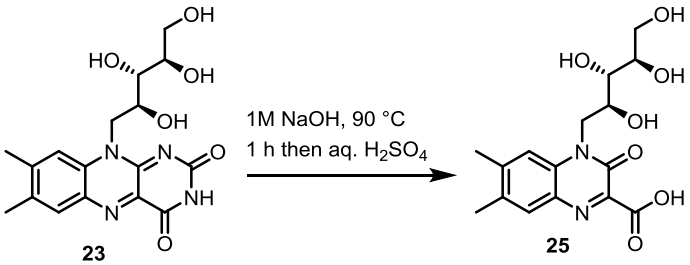


Figure 1.13: Hydrolysis of riboflavin using NaOH

1.2.2.4 Bacterial degradation of riboflavin

In the late 1950s, Harkness and co-workers studied a strain of *Pseudomonas riboflavinus* (*Pseudomonas RF*), an aerobic soil bacteria that uses riboflavin as a source of energy degrades riboflavin *via* 2,3-quinoxalinedione derivatives **24** and to 3,4- dimethyl-6- carboxy-α-pyrone **26**. However, the mechanism of this microbial degradation of riboflavin has not yet been known till date (Figure **1.14)**.[46]

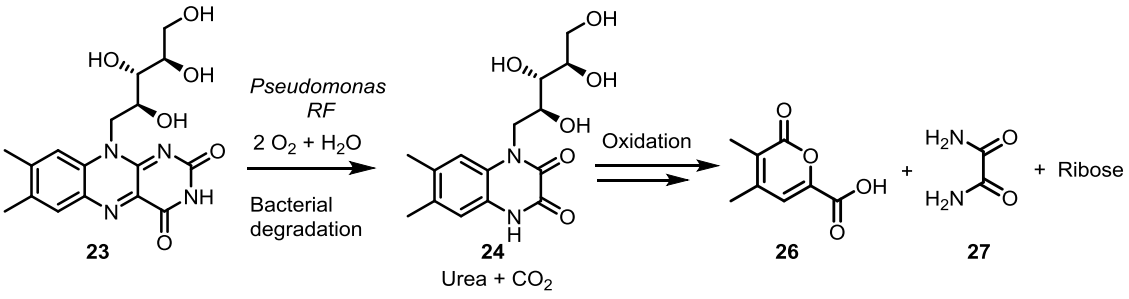
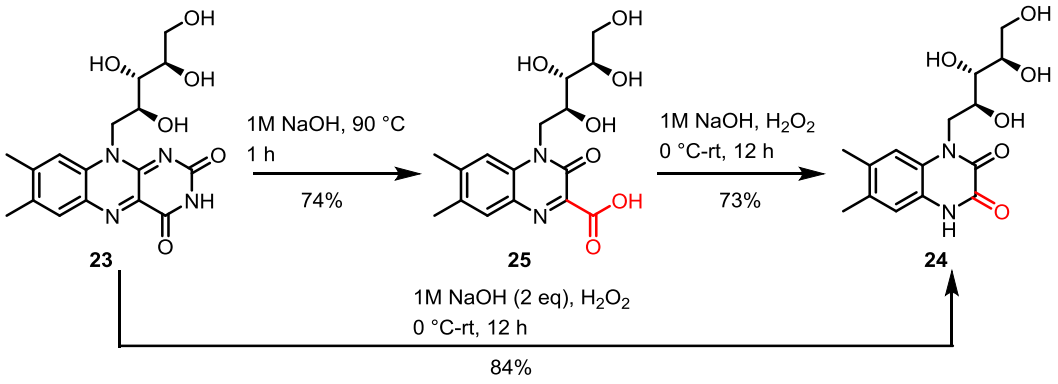


Figure 1.14: Bacterial degradation of riboflavin

1.2.2.5 Biomimetic synthesis of Hunanamycin A

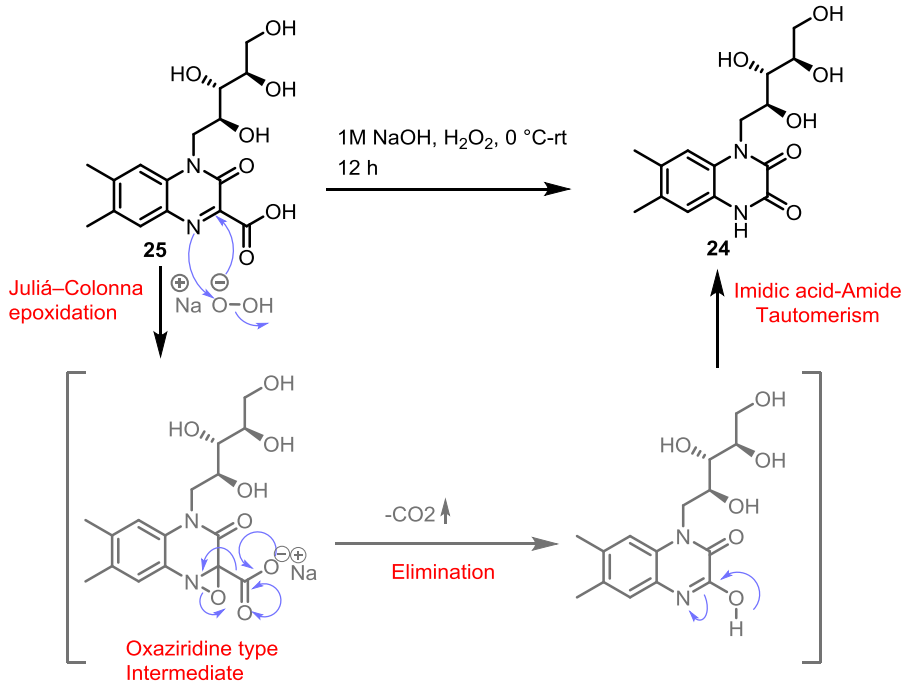
Accordingly, second synthesis commenced with a reaction on commercially available starting material riboflavin, which on hydrolysis using 1M NaOH under heating conditions (90 °C for 1 h) gave carboxylic acid **25** in good yield. The formation of **25** was indicated by the presence of characteristic signals in ^{13}C NMR at δ 165.5, 154.0 and 147.9 corresponding to acid carbonyl, amide carbonyl and imine carbon respectively. The IR spectrum also displayed characteristic stretching at 1711 cm^{-1} for acid and 1665 cm^{-1} for amide functional group. It was further confirmed by HRMS, which showed a peak at 353.1338 for the molecular formula $C_{16}H_{21}N_2O_7$ $[M+H]^+$. Although the synthesis **24** from riboflavin or acid **25** is reported in the literature, but the procedure are tedious, low yielding and cumbersome. Taking clue from literature the acid **25** was subjected to oxidative decarboxylation using H_2O_2 in NaOH to obtain 1-ribityl-2,3-diketo-1,2,3,4-tetrahydro-6,7-dimethyl-quinoxaline **24** in 73% yields which is the proposed biosynthetic precursor for Hunanamycin A (Scheme **1.9**). The product formed was confirmed by ^1H NMR with appearance of broad peak at δ 11.84 corresponding to NH of the amide, two well-separated singlets at δ 7.31 and 6.91 for the two aromatic CH along with the other characteristics peaks from the sugar parts and ^{13}C NMR clearly shows the disappearance of acid and imine carbon peak and appearance of signal at 155.8 and 153.8 for amide carbonyl of **24** when compared to **25.** At the same time, IR spectrum showed peaks at 1687 cm^{-1} and 1660 cm^{-1} for the two amide carbonyl. It was further confirmed by HRMS, which showed a peak at 325.1390 corresponding to molecular



No	Conditions (Acid 25 to Amide 24)	Observation
1	1M NaOH, 100 °C, 12h	SM Recovered
2	1M (3eq) NaOH, (40 eq)H_2O_2, rt, 12h	66% yield
3	1M AcOH, (40 eq)H_2O_2, rt, 12h	58% yield
4	1M (2eq) NaOH, (10 eq)H_2O_2, rt, 12h	73% yield

Scheme 1.9: Synthesis of proposed biogenetic precursor **24**

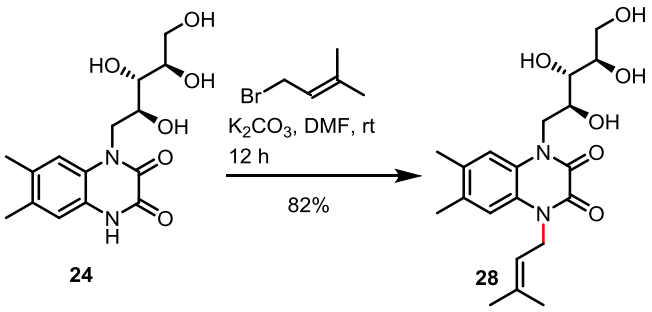
formula $C_{15}H_{21}N_2O_6$ $[M+H]^+$. The plausible mechanism for oxidative decarboxylation of acid **25** to amide **24** is depicted in scheme **1.10**. First, the hydroperoxide anion attacks the electron deficient imine to form intermediary peroxyhemiaminal which on the ring closure to form very reactive oxaziridine type intermediate,[47] which then rapidly undergoes decarboxylation to generate imidic acid tautomer which immediately collapses into amide **24**.



Scheme 1.10: Plausible mechanism for oxidative decarboxylation

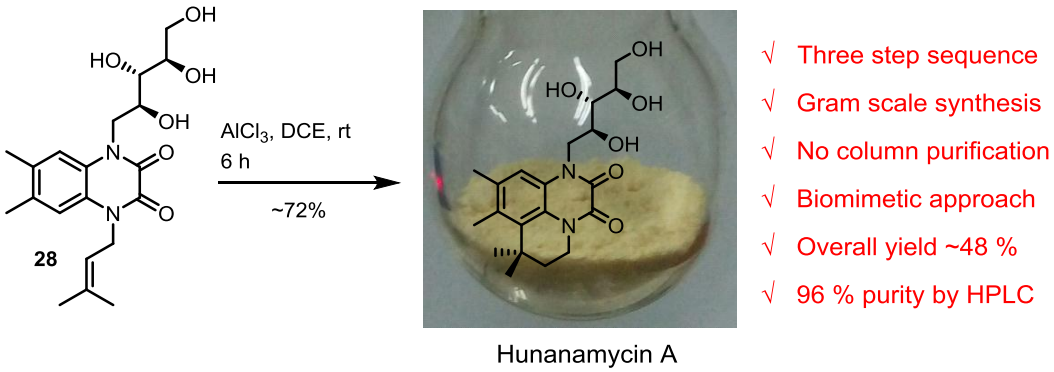
Having both steps optimized independently, we decided to combine both of them in single-pot operation. Accordingly, the synthesis of **24** by treatment of riboflavin **23** in 1M NaOH (2 eq.) at 90 °C for 1 h followed by treatment with 30% H_2O_2 (10 eq.) at room temperature for 12 h underwent oxidative decarboxylation smoothly and produced desired compound **24** in excellent yields (84%). We were pleased to observe that the product was precipitated from the reaction mixture at the end of the reaction, to obtain yellow coloured solid was then filtered and washed with water to have pure compound **24**. All the spectroscopic data for this one-pot procedure was in complete agreement with the previously synthesized product using two-step protocol. Although compound **24** has been reported as a riboflavin degradation product and it was also synthesized earlier, the synthetic procedures are tedious, low yielding, and all details are not available.[48-52] It is worth mentioning here that we were able to synthesize this intermediate in gram scale without much difficulty. After the optimization of one-pot protocol to access **24**, the next task was *N*-prenylation. It was successful using K_2CO_3 and prenyl bromide to have *N*-prenylated product **28** with excellent chemoselectivity and

good yield. The reaction also worked well with Cs_2CO_3 (yield of ~75%). But we preferred K_2CO_3 because of cost and ready availability as bench-top chemical (Scheme **1.11**).



Scheme 1.11: Chemoselective *N*-prenylation

The disappearance of the broad peak at δ 11.84 in ^{1}H NMR corresponding to NH of the amide and appearance of the peaks at δ 5.12 (br, s, 1 H) for CH of olefin, 4.74 (br, s, 2 H) methylene group from the prenyl unit confirms the formation of **28**.In ^{13}C NMR, the appearance of a signal at δ 136.6 and δ 119.1 corresponded to olefinic carbons, and in the DEPT-135 NMR showed three negative signals at δ 63.5, 44.6 and 40.6 for the terminal CH_2 attached to a hydroxyl group, CH_2 flanked between nitrogen and sugar moiety and CH_2 from the prenyl unit respectively. It was further confirmed by HRMS, which showed a peak at 393.2015 corresponding to molecular formula $C_{20}H_{29}N_2O_6$ $[M+H]^+$. In the base mediated *N*-alkylation, a high degree of chemoselectivity was observed over the *O*-alkylation which could be best explained using HSAB concept. Now, in this case prenylbromide is used as the alkylating agent which is a soft electrophile, and after deprotonation of amide using K_2CO_3, nitrogen of the amide behave as soft nucleophile which leads to the *N*- alkylated product in excellent yield.[35-37] Next, to complete the total synthesis, the *N*-prenylated intermediate **28** was subjected for Friedel–Crafts cyclization under different conditions (Table **1.3**). Treatment with methane sulfonic acid in DCM, hydrochloric acid in dioxane failed to give the desired product. We also tried our previous cyclization condition, i.e. $AlCl_3$ in chlorobenzene or nitrobenzene, which resulted in decomposition of the starting material. To our surprise, the change of solvent from chlorobenzene to dichloromethane worked well to afford cyclized product in moderate yields (41%). The possible reason could be the solubility of the compound **28** in the chlorobenzene or nitrobenzene is less compared to the dichloromethane. Lastly, best yield of the cyclized product was achieved by treatment of **28** with $AlCl_3$ in dichloroethane at room temperature for 6 h to obtain Hunanamycin A in 72 % yield (Scheme **1.12**). It is worth to highlight here that we did not carry any column chromatographic



Scheme 1.12: Completion of the synthesis of Hunanamycin A

No	Condition	Observation or yield
1.	$AlCl_3$, PhCl, 110 °C, 8 h	Complex RM
2.	4 M HCl in dioxane, rt, 12 h	No desired product observed
3.	Conc. HCl, reflux, 12 h	Complex RM
4.	CH_3SO_3H, CH_2Cl_2, rt, 12 h	No desired product observed
5.	$AlCl_3$, $PhNO_2$, rt - 80 °C	Complex RM
6.	$AlCl_3$, CH_2Cl_2, rt, 12 h	41% yield
7.	$AlCl_3$, DCE, 50 °C, 2 h	69% yield
8.	$AlCl_3$, DCE, rt, 6 h	72% yield

Table 1.3: Optimization table for cyclization condition

purification throughout the synthesis. All the spectral data (^{1}H NMR, ^{13}C NMR, IR, optical rotation) were compared with previously obtained/reported spectral data and found to be they are identical. Using this protocol, we have prepared the target molecule in multi-gram quantities, and it is a straightforward and practical method.

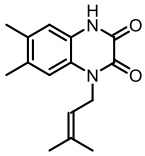
1.3 Conclusion

Thus, we have successfully accomplished the total synthesis of Hunanamycin A, a marine antibiotic natural product with novel chemotype in ~7% overall yield. Construction of ribose unit by diastereoselective dihydroxylation of protected allyl derivative was used as the key step in the synthesis. The relative stereochemistry of the newly generated hydroxyl groups was established using Kishi empirical model. In the second generation synthesis, we made an attempt towards ideal synthesis by eliminating multiple purification steps and thus we

achieved a practical and expeditious synthesis of Hunanamycin A. The second route, three-step protocol starting from readily available and less expensive riboflavin is much superior with respect to the previous synthesis developed by us, in terms of number of steps, economy and low yields. It is interesting to note that natural product was originally isolated in very small quantities (0.8 mg) and now one can access the same in multi-gram quantities using bench-top chemicals in present approach. We have generated more than 10 grams of the natural product Hunanamycin A within a short time span. In continuation of this work, we have prepared several analogues around the Hunanamycin A scaffold toward lead optimization. Details are captured in the following sections.

1.4 Experimental procedures

6, 7-dimethyl-1-(3-methylbut-2-en-1-yl)-1, 4dihydroquinoxaline-2, 3-dione (4)



To a solution of 6, 7-dimethyl-1, 4-dihydroquinoxaline-2,3-dione reaction **2** (600 mg, 3.15 mmol) in dry DMSO (60 mL) was added caesium carbonate (1.53 g, 4.73 mmol), followed by 3,3-dimethylallyl bromide (0.363 mL, 3.15 mmol) diluted in 5mL of DMSO and kept stirring for 3 h at room temperature reaction. The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 50 mL). The combined organic layer was washed water (50 mL) with brine (50 mL) and dried over Na_2SO_4, concentrated under reduced pressure, and subjected to flash chromatography over silica gel (60% EtOAc:PE) to afford mono-alkylated compound **4** (342 mg, 42%) as brown solid.

Melting point 271-273 °C;

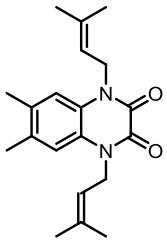
^{1}H NMR (200 MHz, DMSO-d_6) δ 1.68 (s, 3 H), 1.83 (s, 3 H), 2.19 (s, 3 H) 2.23 (s, 3 H), 4.69 (d, *J* = 6.8 Hz, 2 H), 5.11 (s, 1 H), 6.93 (s, 1 H), 7.02 (s 1 H), 11.93 (s, 1 H);

^{13}C NMR (100 MHz, DMSO-d_6) δ 18.2, 18.8, 19.4, 25.3, 40.5, 115.8, 116.3, 118.7, 123.4, 123.9, 131.3, 131.7, 136.1, 153.6, 154.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution)1452, 1600, 1626, 1694, 2854, 3353 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}O_2N_2$ $[M+Na]^+$ 281.1260, found 281.1261.

6,7-dimethyl-1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione (5)



Dialkylated product **5** as brown solid.

Melting point 255-258 °C;

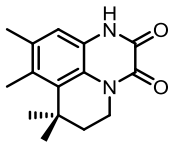
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69 (s, 6 H), 1.85 (s, 6 H), 2.27 (s, 6 H), 4.77 (t, *J* = 6.2 Hz, 4 H), 5.12 (d, *J* = 6.2 Hz, 2 H), 6.93 (s, 2 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 19.7, 25.7, 41.4, 116.3, 117.9, 124.5, 132.5, 137.2, 154.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1223, 1461, 1678, 2855, 2926, 3393 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}O_2N_2$ $[M+Na]^+$ 326.1994, found 326.1987.

7, 7, 8, 9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (6)



To a cooled solution of 6, 7-dimethyl-1-(3-methylbut-2-en-1-yl)-1, 4dihydroquinoxaline-2, 3-dione **4** (200 mg, 0.775 mmol) in chlorobenzene (8 mL), $AlCl_3$ (613 mg, 4.65 mmol) was added and stirred the reaction mixture at 120 °C for 12 h. After the starting material was completely consumed, reaction mixture poured on crushed ice water (10 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with saturated water, brine (30 mL), dried over sodium sulphate, filtered and concentrated *in vacuo* and purified by flash column chromatography over silica gel (10% EtOAc:DCM) to afford compound **6** (130 mg, 65%) as brown solid product.

Melting point 205-208 °C

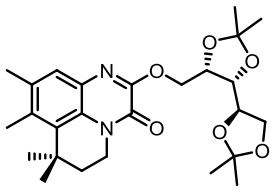
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 6 H), 1.92 (t, *J* = 5.9 Hz, 2H), 2.29 (s, 3 H), 2.41 (s, 3 H), 4.18 (m, 2 H), 7.13 (s, 1 H), 12.08 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 20.7, 28.5 (2C), 32.9, 38.1, 39.7, 116.4, 121.7, 122.4, 132.2, 132.4, 134.9, 154.6, 155.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1377, 1462, 1680, 1662, 2855, 2929 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}O_2N_2$ $[M+Na]^+$ 281.1260, found 281.1258.

7,7,8,9-tetramethyl-2-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methoxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one(10)



To a solution of 7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **6** (130 mg, 0.50 mmol) in dry THF (5 mL) was added triphenylphosphine (303 mg, 1.15 mmol), ((4*S*,4'*R*,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methanol **7** (140 mg, 0.60 mmol) and DIAD (0.225 mL, 1.15 mmol) sequentially and stirred for 12 h at room temperature. The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 X 40 mL), and the organic layer was washed successively with water and brine, dried over sodium sulphate, and concentrated in *vacuo.* The residue was purified by flash silica gel column chromatography (40% EtOAc:DCM) to afford *O*-alkylated product **10** (155 mg, 65%) as brown coloured oil.

Specific rotation $[\alpha]_D^{25} = -6.1$ (*c* 5.2, $CHCl_3$).

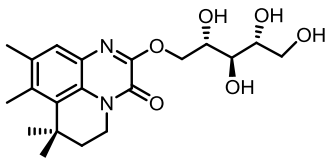
^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (s, 3 H), 1.35 (s, 3 H), 1.36 (s, 3 H), 1.43 (s, 3 H), 1.53 (s, 6 H), 1.93 (t, *J* = 5.9 Hz, 2H), 2.31 (s, 3 H), 2.48 (s, 3 H), 3.92 (dd, *J* = 8.2, 5.5 Hz, 1 H), 4.13 (m, 4 H), 4.21 (m, 1 H), 4.54 - 4.59 (m, 1 H), 4.68 - 4.69 (m, 1 H), 4.74 - 4.79 (m, 1 H), 7.30 (s, 1 H);

^{13}C NMR (100 MHz, CD_3OD) δ 19.8, 21.3, 25.7 (2C), 27.2, 28.3, 29.1, 34.0, 39.1, 41.0, 66.7, 69.0, 75.0, 76.9, 79.4, 110.5, 111.1, 127.2, 127.7, 130.5, 132.8, 135.9, 136.6, 151.6, 153.6.

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1067, 1371, 1618, 1630, 2934, 2985 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{26}H_{36}O_6N_2$ $[M+Na]^+$ 495.2466, found 495.2463.

Synthesis of 7,7,8,9-tetramethyl-2-(((2S,3R,4R)-2,3,4,5-tetrahydroxypentyl)oxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (11)



A solution of 7,7,8,9-tetramethyl-2-(((4*S*,4'*R*,5*S*)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methoxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one **10** (50 mg, 0.10 mmol) in acetic acid : water (1:1; 1 mL) was stirred for 3 h at 60 °C. The reaction mixture was concentrated under reduced pressure, and purified by flash column chromatography (10% MeOH: DCM) to give compound **11** (28 mg, 68%) as colourless sticky solid.

Specific rotation $[\alpha]_D^{25}$ = +5.7° (*c* 1.4, MeOH);

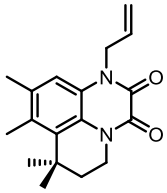
^{1}H NMR (500 MHz, CD_3OD) δ 1.53 (s, 6 H), 1.93 (t, *J* = 5.8 Hz, 2H), 2.31 (s, 3 H), 2.48 (s, 3 H), 3.70 - 3.71 (m, 1 H), 3.75 - 3.77 (m, 1 H), 3.78 - 3.83 (m, 2 H), 4.15 - 4.17 (m, 3 H), 4.45 (dd, *J* = 11.2, 7.0 Hz, 1 H), 4.64 (dd, *J*= 11.2, 2.7 Hz, 1 H), 7.30 (s, 1 H);

^{13}C NMR (125 MHz, CD_3OD) δ 19.8, 21.3, 29.1, 34.0 (2C), 39.1, 40.9, 64.7, 70.2, 72.0, 73.9, 74.3, 127.1, 127.7, 130.8, 132.9, 136.2, 136.7, 152.1, 153.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1066, 1299, 1614, 1646, 2924, 3366 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{28}O_6N_2$ $[M+Na]^+$ 415.1840, found 415.1836.

1-Allyl-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (12)



A solution of 7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **6** (30 mg, 0.11 mmol) in dry DMF (2 mL) was added drop wise to a pre-cooled suspension of sodium hydride (60% dispersion in mineral oil, 9.2 mg, 0.23 mmol) in 3 mL of DMF. Then cooling bath was removed, and the flask was allowed to warm to room temperature and stirred for 20 min. Allyl bromide (12 µl, 0.139 mmol) was then added drop wise at 0 °C and stirred for 2 h at room temperature. The reaction was quenched by the addition of H_2O (5 mL) and extracted with ethyl acetate (3 X 10 mL). The combined organics were dried over sodium sulphate, filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography over silica gel (30% EtOAc:DCM) to afford compound **12** (26 mg, 76%) as colourless oil.

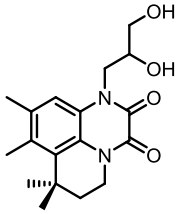
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (s, 6 H), 1.90 (t, *J* = 6.1 Hz, 2H), 2.30 (s, 3 H), 2.42 (s, 3 H), 4.16 (t, *J* = 5.8 Hz, 2H), 4.85 - 4.86 (m, 2 H), 5.21 - 5.29 (m, 2 H), 5.90 - 5.96 (m, 1 H), 6.94 (s, 1 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 19.1, 21.6, 28.7 (2C), 33.3, 38.0, 39.7, 45.5, 115.2, 117.9, 121.9, 124.4, 130.8, 132.0, 132.8, 134.1, 153.5, 153.55;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1462, 1680, 1662, 2875, 2925 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{22}O_2N_2$ $[M+Na]^+$ 321.1579, found 321.1577.

1-(2,3-dihydroxypropyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (13)



A solution of 1-allyl-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **12** (20 mg, 0.067 mmol) in acetone: water: ^{t}BuOH (2 mL, 7:2:1) was treated with NMO (31 mg, 0.268 mmol), osmium tetraoxide (2.5 % sol. in ^{t}BuOH) (0.136 mL, 0.013 mmol) stirred for 12 h at room temperature. The reaction mixture was quenched with ice cold solution of $NaHSO_4$ (5 mL) and extracted with ethyl acetate (3 X 10 mL) and washed with water and brine. The combined organic layer were dried over Na_2SO_4, filtered and concentrated in *vacuo*, and purified by column chromatography (10 % MeOH:DCM) to afford compound **13** (15 mg, 68%) as colourless oil.

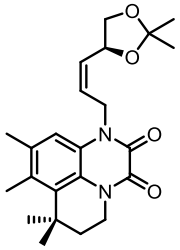
^{1}H NMR (400 MHz, CD_3OD) δ 1.54 (s, 3 H), 1.55 (s, 3 H), 1.92 (t, *J* = 6.4 Hz, 2 H), 2.34 (s, 3 H), 2.45 (s, 3 H), 3.63 (d, *J* = 5.5 Hz, 2 H), 4.05 - 4.13 (m, 3 H), 4.32 - 4.36 (m, 2 H), 7.35 (s, 1 H).

^{13}C NMR (100 MHz, CD_3OD) δ 19.6, 21.7, 29.0, 29.3, 34.5, 39.3, 40.8, 47.4, 65.2, 70.7, 116.9, 123.4, 126.3, 133.8, 134.3, 136.0, 155.2, 156.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1398, 1595, 1668, 2855, 2924, 3418 cm^{-1};

HRMS (ESI): *m/z* calculated for $C_{18}H_{24}O_4N_2$ $[M+H]^+$ 333.1809, found 333.1802

(*S*,*Z*)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (18)



A solution of 7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **6** (70 mg, 0.27 mmol) in dry DMF (2 mL) was added drop wise to pre-cooled (0 °C) suspension of sodium hydride (60% mineral oil, 27 mg, 0.67 mmol) in 3 mL of DMF. The reaction mixture was allowed to warm to room temperature and stirred for 20 min. (*S*,*Z*)-4-(3-bromoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolane **17** (119 mg, 0.54 mmol), dissolved in dry DMF (1 mL) was then added drop wise at 0 °C and stirred for 2 h at room temperature. The reaction mixture was added to cold water (10 mL) and extracted with ethyl acetate (3 X 15 mL) and the combined organic layers were washed with water, brine, then dried over sodium sulphate, filtered and concentrated in *vacuo*, and purified by column chromatography (30% EtOAc: CH_2Cl_2) to afford compound **18** (80 mg, 74%) as colourless oil.

Specific rotation $[\alpha]_D^{25}$ = +11 (*c* 1.1, $CHCl_3$).

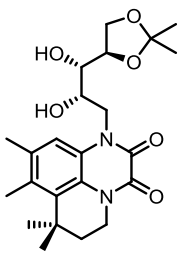
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3 H), 1.46 (s, 3 H), 1.52 (s, 6 H), 1.90 (t, *J* = 5.2 Hz, 2 H), 2.31 (s, 3 H), 2.41 (s, 3 H), 3.63 (t, *J* = 7.5 Hz, 1 H), 4.12 - 4.15 (m, 2 H), 4.25 (dd, *J* = 8.1, 6.1 Hz, 1 H), 4.73 (dd, *J* = 15.7, 6.7 Hz, 1 H), 5.12 (q, *J* = 7.2 Hz, 1 H), 5.25 - 5.29 (m, 1 H), 5.59 - 5.64 (m, 1 H), 5.67 - 5.70 (m, 1 H), 7.03 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 21.4, 25.8, 26.7, 28.6, 28.7, 33.2, 37.9, 39.6, 40.8, 69.4, 71.9, 109.6, 114.7, 121.9, 124.2, 127.5, 131.6, 132.1, 132.9, 134.3, 153.4, 153.5.

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1215, 1677, 1745, 2400, 2925, 3020 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{23}H_{31}O_4N_2$ $[M+H]^+$ 399.2278, found 399.2273

1-((2*S*,3*S*)-3-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (19)



A solution of (*S*,*Z*)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **18** (80 mg, 0.20 mmol) in acetone: water: ^{t}BuOH (5 mL, 7:2:1) was treated with *N*-methylmorpholine oxide (94 mg, 1.30 mmol), osmium tetraoxide (2.5% sol. in ^{t}BuOH) (0.4 mL, 0.040 mmol) and stirred for 12 h at room temperature. The reaction mixture was added to cold solution of $NaHSO_4$ and extracted with ethyl acetate (3 X 30 mL) and washed with water, brine and the combined organic layers were dried over sodium sulphate, filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography on silica (5% MeOH:DCM) to afford hydroxylated compound **19** (70 mg, 79%) as colourless oil.

Specific rotation $[\alpha]_D^{25}$ = -57 (*c* 0.7, $CHCl_3$).

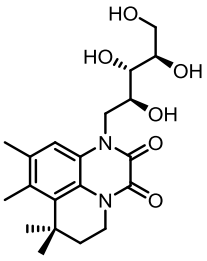
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 3 H), 1.40 (s, 3 H), 1.51 (s, 6 H), 1.88 (t, *J* = 5.2 Hz, 2 H), 2.31 (s, 3 H), 2.41 (s, 3 H), 3.78 (br. s., 1 H), 4.05 - 4.25 (m, 8 H), 4.73 (br. s., 1 H), 4.92 (dd, *J* = 14.3, 7.7 Hz, 1 H), 7.36 (s, 1 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 21.4, 25.3, 26.7, 28.6 (2C), 33.2, 38.0, 39.5, 45.4, 68.0, 71.8, 73.7, 76.7, 109.6, 115.8, 122.0, 124.7, 132.4, 132.6, 134.4, 153.4, 155.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1062, 1217, 1674, 2925, 3429 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{23}H_{32}O_6N_2$ $[M+Na]^+$ 455.2153, found 455.2143

7,7,8,9-tetramethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (Hunanamycin A) (1)



Hunanamycin A (**1**)

A solution of 1-((2*S*,3*S*)-3-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **19** (68 mg, 0.15 mmol) in acetic acid : water (1:1; 2 mL) was stirred for 30 min at 50 °C. The reaction mixture was then concentrated in *vacuo*, and purified by flash chromatography on over silica gel (10% MeOH:DCM) to afford Hunanamycin A **1** (45 mg, 65%) as yellow solid.

Specific rotation $[\alpha]_D^{25}$ = +15 (*c* 0.4, MeOH) is in consistent with natural Hunanamycin A $[\alpha]_D^{25}$= +20 (*c* 0.06, MeOH)

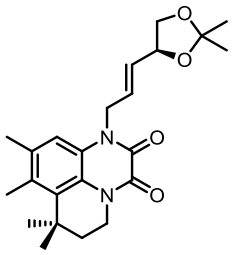
^{1}H NMR (500 MHz, CD_3OD) δ 7.46 (s, 1 H), 4.78 (dd, *J* = 9.9, 14.1 Hz, 1 H), 4.31 - 4.21 (m, 2 H), 4.14 (m, 2 H), 3.86 (m, 1 H), 3.81 (br. s., 2 H), 3.67 (dd, *J* = 11.9, 4.6 Hz, 1 H), 2.46 (s, 3 H), 2.34 (s, 3 H), 1.94 (t, *J* = 5.8 Hz, 2 H), 1.55 (s, 6 H);

^{13}C NMR (125 MHz, CD_3OD) δ 156.7, 155.4, 136.1, 134.3, 133.7, 126.3, 123.4, 117.2, 75.1, 74.4, 71.0, 65.0, 46.6, 40.8, 39.4, 34.5, 29.4, 29.0, 21.6, 19.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1038, 1424, 1669, 2865, 2925, 3428 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{28}O_6N_2$ $[M+Na]^+$ 415.1840, found 415.1832.

(*S*,*E*)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (21)



A solution of 7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **6** (60 mg, 0.23 mmol) in dry DMF (2 mL) was added dropwise to pre-cooled suspension of sodium hydride (60% mineral oil, 23 mg, 0.58 mmol) in 2 mL of DMF. The cooling bath was then removed, and the flask was allowed to warm to room temperature and stirred for 20 min. (*S*,*E*)-4-(3-bromoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolane **20** (113 mg, 0.51 mmol), dissolved in dry DMF, was added drop wise at 0 °C and stirred for 2 h at room temperature, then reaction mixture was added to cold water and extracted with ethyl acetate (3 X 10 mL). The combined organics were dried over sodium sulphate, filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography on over silica gel (30 % EtOAc: DCM) to afford compound **21** (52 mg, 65 %) as brown sticky mass.

Specific rotation $[\alpha]_D^{25}$= +12.5 (*c* = 0.8, $CHCl_3$)

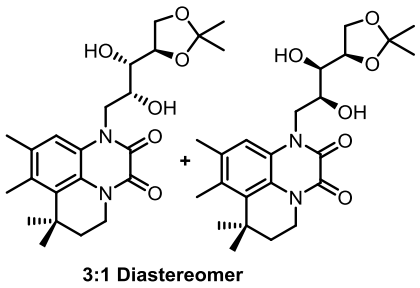
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 1 H), 5.89 (td, *J* = 5.4, 15.6 Hz, 1 H), 5.72 (dd, *J* = 6.7, 15.7 Hz, 1 H), 4.84 (t, *J* = 5.0 Hz, 2 H), 4.50 (q, *J* = 6.8 Hz, 1 H), 4.19 - 4.11 (m, 2 H), 4.05 (dd, *J* = 6.3, 8.3 Hz, 1 H), 3.55 (t, *J* = 7.8 Hz, 1 H), 2.41 (s, 3 H), 2.29 (s, 3 H), 1.93 - 1.87 (m, 2 H), 1.53 (s, 6 H), 1.39 (s, 3 H), 1.35 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 21.6, 25.8, 26.6, 28.7, 29.6, 33.2, 37.9, 39.6, 44.2, 69.2, 76.0, 109.4, 114.9, 121.9, 124.3, 126.0, 131.7, 132.0, 132.8, 134.1, 153.4, 153.42.

IR ν_{max} (thin film applied as $CHCl_3$ solution) 1215, 1677, 1745, 2400, 2925, 3020 cm^{-1};

HRMS (ESI): *m/z* calculated for $C_{23}H_{30}O_4N_2$ $[M+Na]^+$ 421.2098, found 421.2089

1-((2R,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (22i)



3:1 Diastereomer

A solution of (*S*,*E*)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **21** (50 mg, 0.12 mmol) in acetone: water:^{t}BuOH (2 mL, 7:2:1) was treated with *N*-methyl morpholine oxide (58 mg, 0.502 mmol), osmium tetraoxide (2.5% sol. in ^{t}BuOH) (0.255 mL, 0.0251 mmol) and stirred for 12 h at room temperature. The reaction mixture was added to cold sol of $NaHSO_4$ and extracted with ethyl acetate (3 X 10 mL) and washed with water, brine. The combined organics were dried over sodium sulphate, filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography on over silica gel (5% MeOH: DCM) to afford compound **22i** (32 mg, 66%, 4:1 inseparable diastereomers) as yellow sticky solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (s, 3 H), 1.32 (s, 3 H), 1.53 (s, 6 H), 1.91 (t, *J* = 6.2 Hz, 2 H), 2.33 (s, 3 H), 2.44 (s, 3 H), 3.41 (d, *J* = 7.7 Hz, 1 H), 3.95 - 4.01 (m, 1 H), 4.01 - 4.32 (m, 8 H), 4.49 - 4.55 (m, 1 H), 7.15 (s, 1 H)

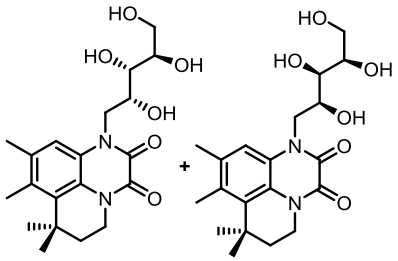
^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1, 21.6, 25.2, 26.7, 28.6, 28.7, 33.3, 38.1, 39.6, 46.4, 67.5, 68.3, 71.6, 75.1, 109.2, 114.8, 122.1, 124.2, 132.9, 133.1, 134.7, 153.1, 155.4.

IR ν_{max} (thin film applied as $CHCl_3$ solution) 770, 1216, 1675, 2925, 3429 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{23}H_{33}O_6N_2$ $[M+H]^+$ 433.2333, found 433.2328.

Note: Minor peaks in NMR (^{1}H and ^{13}C) which could not be cleanly distinguished, corresponds to other diastereomer.

7,7,8,9-tetramethyl-1-((2*R*,3S,4R)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (22)



3:1 Diastereomer

A solution of 1-((2*R*,3*S*)-3-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7,8,9-tetramethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **22i** (25 mg, 0.057 mmol) in Acetic acid:Water (1:1) 1mL and kept stirring for 3 h at 60 °C reaction was monitored by TLC. The reaction mixture concentrated under reduced pressure, and purified by column chromatography (10 % MeOH: DCM) to afford compound **22** (15 mg, 68%, 4:1 inseparable diastereomers) as colourless gum.

^{1}H NMR (500 MHz, CD_3OD) δ 1.54 (s, 6 H), 1.93 (t, *J* = 5.8 Hz, 2 H), 2.35 (s, 3 H), 2.46 (s, 3 H), 3.48 (d, *J* = 8.5 Hz, 1 H), 3.59 - 3.76 (m, 2 H), 3.76 - 3.85 (m, 1 H), 4.08 - 4.20 (m, 2 H), 4.29 - 4.58 (m, 3 H), 7.39 and 7.43 (s, 1 H);

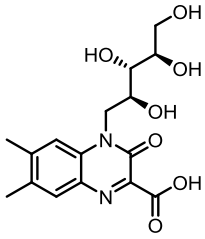
^{13}C NMR (125 MHz, CD_3OD) δ 19.6, 21.7, 29.1, 29.3, 34.5, 39.4, 40.8, 47.5, 65.2, 68.6, 72.6, 72.7, 117.0, 123.4, 126.4, 133.9, 134.4, 136.2, 155.3, 156.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 754, 1036, 1404, 1668, 2925, 3363 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{28}O_6N_2$ $[M+Na]^+$ 415.1840, found 415.1833.

Note: Minor peaks in NMR (^{1}H and ^{13}C) which could not be cleanly distinguished, corresponds to other diastereomer.

6,7-dimethyl-3-oxo-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-3,4-dihydroquinoxaline-2-carboxylic acid (25)



A solution of riboflavin **23** (6.0 g, 15.9 mmol) in 1 M sodium hydroxide (47.0 mL, 47.8 mmol) was heated at 90 °C for one hour. After cooling, the solution was neutralized with acetic acid (pH = 6) and allowed to stand for 12h at 0 °C. The solid formed was filtered off,

washed with minimum amount of water (~15 mL) and dried under vacuum. The obtained sodium salt of acid was re-dissolved in 60 mL of hot water and acidified (pH =3) with dilute sulfuric acid at 0 °C. The precipitate obtained was filtered, washed with minimum amount of water (~15 mL) and dried under reduced pressure to obtain product **25** as yellow solid (4.15 g, 74%);

Melting point: 178-182 °C;

Specific rotation: $[\alpha]_D^{25}$ = +16 (c 0.5, DMSO);

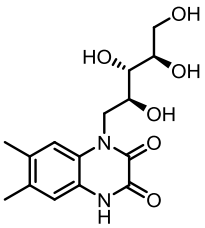
^{1}H NMR (400MHz, DMSO-d$_6$) δ 7.45 (s, 1 H), 7.42 (s, 1 H), 5.26 (br. s., 1 H), 5.13 (br. s., 1 H), 4.83 (br. s., 1 H), 4.72 (br. s., 1 H), 4.58 - 4.52 (m, 1 H), 4.09 (d, *J* = 11.7 Hz, 2 H), 3.65 - 3.60 (m, 4 H), 2.29 (s, 3 H), 2.25 (s, 3 H);

13C NMR (100MHz, DMSO-d$_6$) δ 168.4, 157.0, 153.7, 138.4, 131.5, 130.9, 130.7, 128.8, 115.6, 73.7, 72.8, 69.2, 63.6, 43.9, 20.0, 18.7;

IR ν_{max} (thin film applied in Nujol) 3362, 2898, 1711, 1665, 1598, 1461, 1377 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{21}N_2O_7$ $[M+H]^+$ 353.1343, found 353.1338.

6,7-dimethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (24):



To the cooled solution of 6,7-dimethyl-3-oxo-4-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-3,4-dihydroquinoxaline-2-carboxylic acid **25** (1.0 g, 2.8 mmol) in 1M NaOH (7.1 mL, 7.1 mmol) was added 30% aq. H_2O_2 (8.1 mL, 71.0 mmol) dropwise over a period of fifteen minutes. The reaction mixture then was allowed to warm to room temperature and stirred for an additional 12 h. The solution was cooled and neutralized with acetic acid (pH = 6), allowed to stand for 12 h at 0 °C. The bright yellow precipitate thus obtained was filtered and washed with cold water (5 mL). The solid obtained was dried under reduced pressure to afford **24** as pure yellow solid (0.68 g, 73%);

Melting point: 227-230 °C,

Specific rotation: $[\alpha]_D^{25}$ = -59 (c 0.15, MeOH:H_2O (1:1),

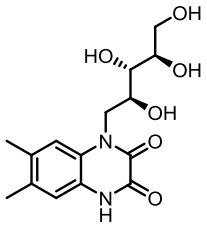
^{1}H NMR (400MHz, DMSO-d_6) δ 11.84 (br. s, 1 H,), 7.31 (s, 1 H), 6.91 (s, 1 H), 4.92 (br. s, 1 H), 4.78 (br. s, 1 H), 4.63 (d, *J* = 5.4 Hz, 1 H), 4.45 (br. s, 2 H), 4.15 - 3.92 (m, 2 H), 3.50 - 3.62 (m, 3 H) 3.44 (br. s, 1 H), 2.21 (s, 3 H), 2.18 (s, 3 H);

^{13}C NMR (100 MHz, DMSO-d_6) δ 155.8, 153.8, 131.3, 131.0, 125.0, 123.4, 116.6, 116.0, 73.6, 72.7, 68.2, 63.5, 44.6, 19.3, 18.7;

IR ν_{max} (thin film applied in Nujol) 3549, 2848, 1687, 1660, 1461, 1377, 1307 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{15}H_{21}N_2O_6$ $[M+H]^+$ 325.1394, found 325.1390.

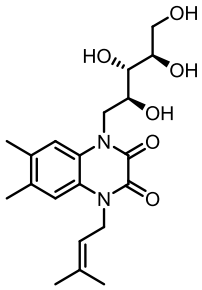
6,7-dimethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (24):(One pot synthesis of 24 from riboflavin)



7,8-dimethyl-10-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)benzo[g]pteridine-2,4(3H,10H)-dione (Riboflavin or Vitamin B2, **23**) (6.0 g, 15.0 mmol) was dissolved in 1M NaOH (48 mL, 48.0 mmol) and heated at 90 °C for 1 h. The reaction mixture was then cooled to 0 °C and 30% aq. H_2O_2 (18 mL, 159 mmol) was added dropwise and allowed to warm to room temperature with additional 12 h of stirring. The solution was then neutralized with acetic acid (until pH = 6) and allowed to stand for 12 h at 0 °C. The bright yellow precipitate thus obtained was filtered, washed with minimum amount of water (40 mL) and dried under reduced pressure to afford **24** as yellow solid (4.3 g, 84%).

Note: All the spectral data obtained here were found to be identical to the compound prepared by two step approach above.

6,7-dimethyl-1-(3-methylbut-2-en-1-yl)-4-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (28):



6,7-dimethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **24** (2.0 g, 6.2 mmol) was dissolved in DMF (30 mL), and potassium carbonate (3.4 g, 24.6 mmol), 3,3-dimethylallyl bromide (1.44 mL, 13.5 mmol) were added sequentially and stirred reaction mixture at room temperature for 12 h. The excess DMF was then removed in *vacuo*, and cold water (50 mL) was added to obtain precipitate, which was filtered, washed with cold water (30 mL) and dried under reduced pressure. The crude residue obtained was again washed several times with 2% MeOH:DCM (5 x 20 mL) to afford **28** as pure off-white solid (1.99 g, 82%);

Melting point 193-196 °C;

Specific rotation $[\alpha]_D^{25}$= +75 (c 0.2, MeOH);

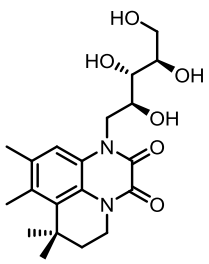
^{1}H NMR (400MHz ,DMSO-d$_6$) δ 7.40 (s, 1 H), 7.06 (s, 1 H), 5.12 (br. s, 1 H), 4.93 (br. s, 1 H), 4.80 (br. s, 1 H), 4.74 (br. s, 2 H), 4.64 - 4.50 (m, 2 H), 4.47 (br. s, 1 H), 4.15 - 3.94 (m, 2 H), 3.57 (br. s, 3 H), 3.43 (br. s, 1 H), 2.24 (br. s, 6 H), 1.85 (s, 3 H), 1.69 (s, 3 H);

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 154.3, 153.5, 136.1, 131.6, 131.4, 125.0, 124.0, 118.6, 117.0, 115.9, 73.6, 72.8, 68.1, 63.5, 44.6, 40.6, 25.3, 19.1, 18.2;

IR ν_{max} (thin film applied in Nujol) 3365, 2846, 1676, 1623, 1463, 1377, 1197 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{20}H_{29}N_2O_6$ $[M+H]^+$ 393.2020, found 393.2015.

7,7,8,9-tetramethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1*H*,5*H*-pyrido[1,2,3-de]quinoxaline-2,3-dione (Hunanamycin A, 1):



Hunanamycin A (**1**)

6,7-dimethyl-1-(3-methylbut-2-en-1-yl)-4-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **28** (3.7 g, 9.4 mmol) was taken in 1,2-dichloroethane (60 mL) and aluminum chloride (12.3 g, 94.4 mmol) was added portion wise and stirred for 6 h at room temperature. The reaction mixture was then added to crushed ice (~50 g), extracted with 10% methanol in dichloromethane (4 x 100 mL). The combined organic layer was then washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude product which was then triturated with diethyl ether (3 x 40 mL) to afford product as yellow solid (2.6 g, 72%); All the spectral data is in consistence with that of natural product prepared using first generation approach.

1.5 References:

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1.6 Copies of ^{1}H and ^{13}C NMR spectra

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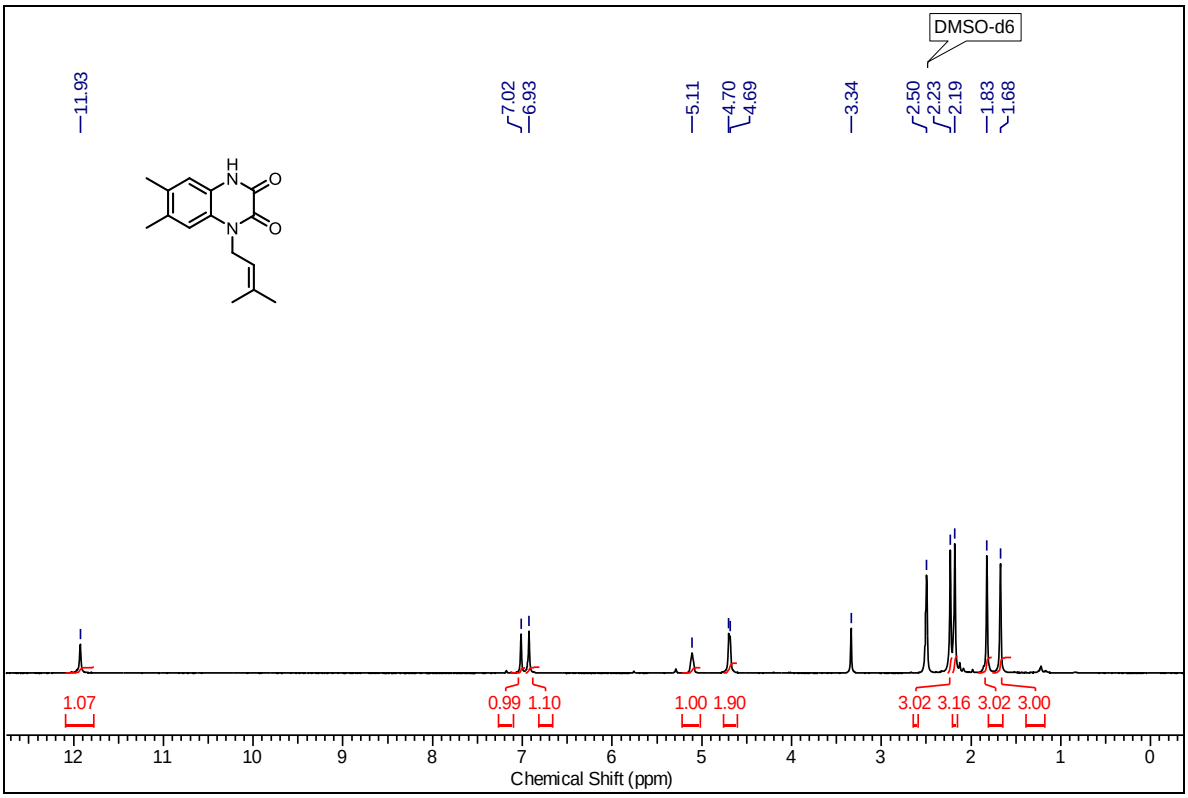

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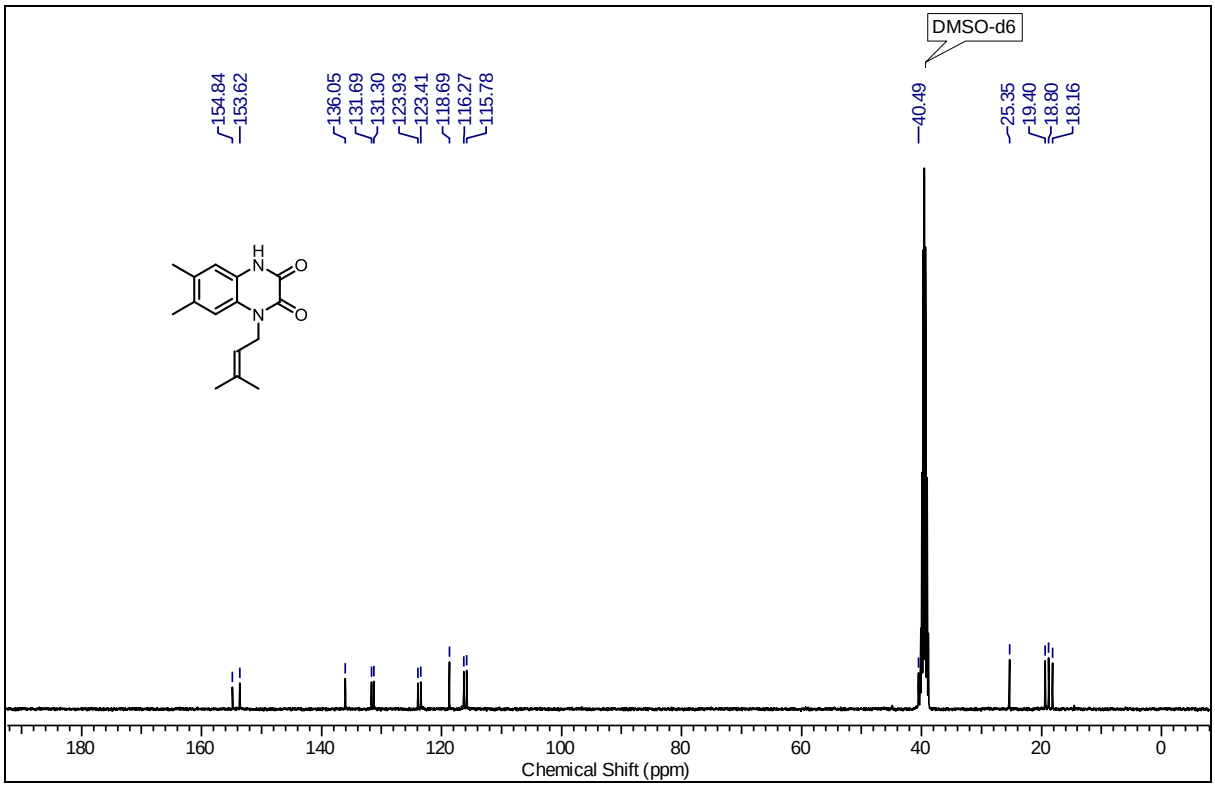

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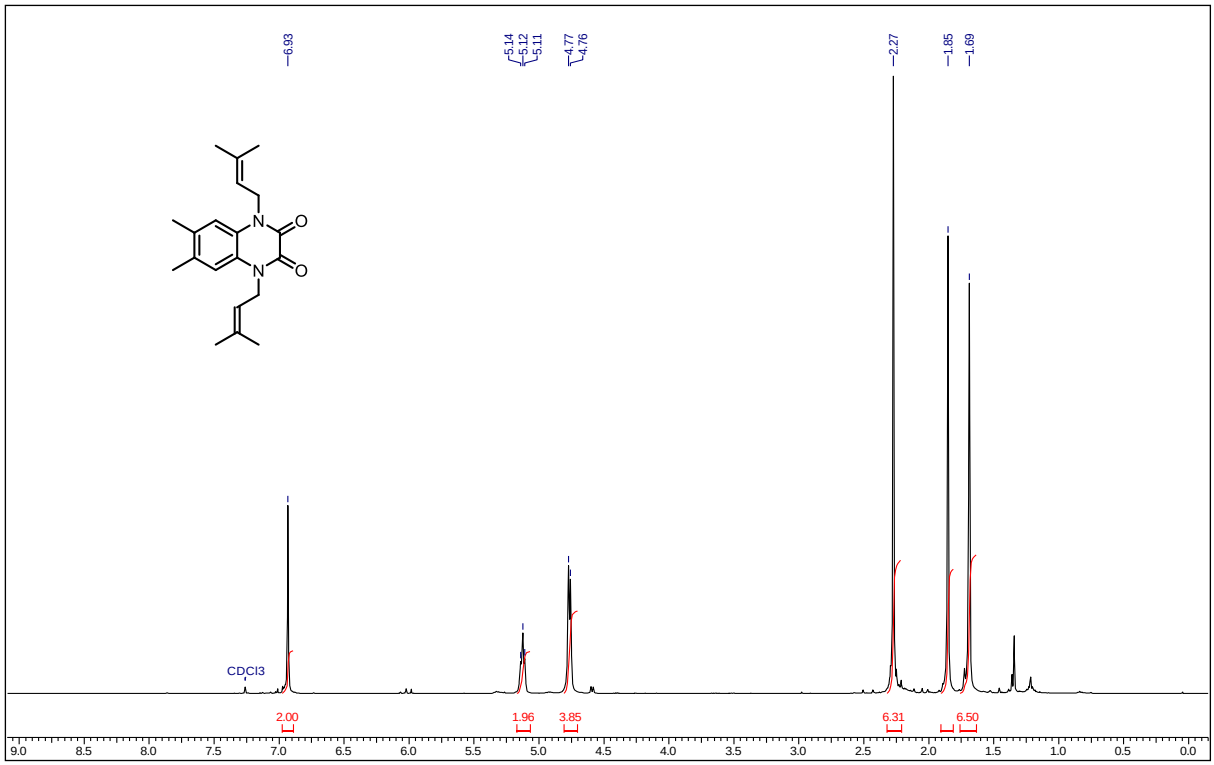

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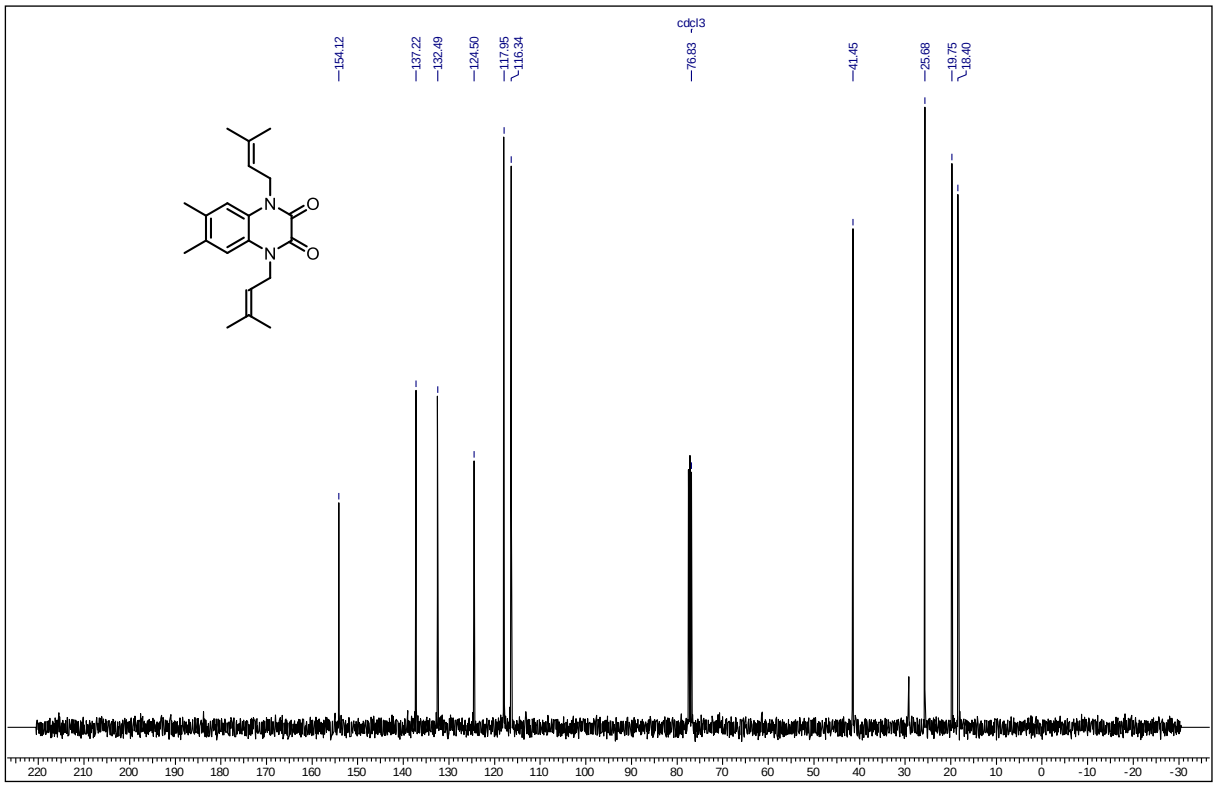

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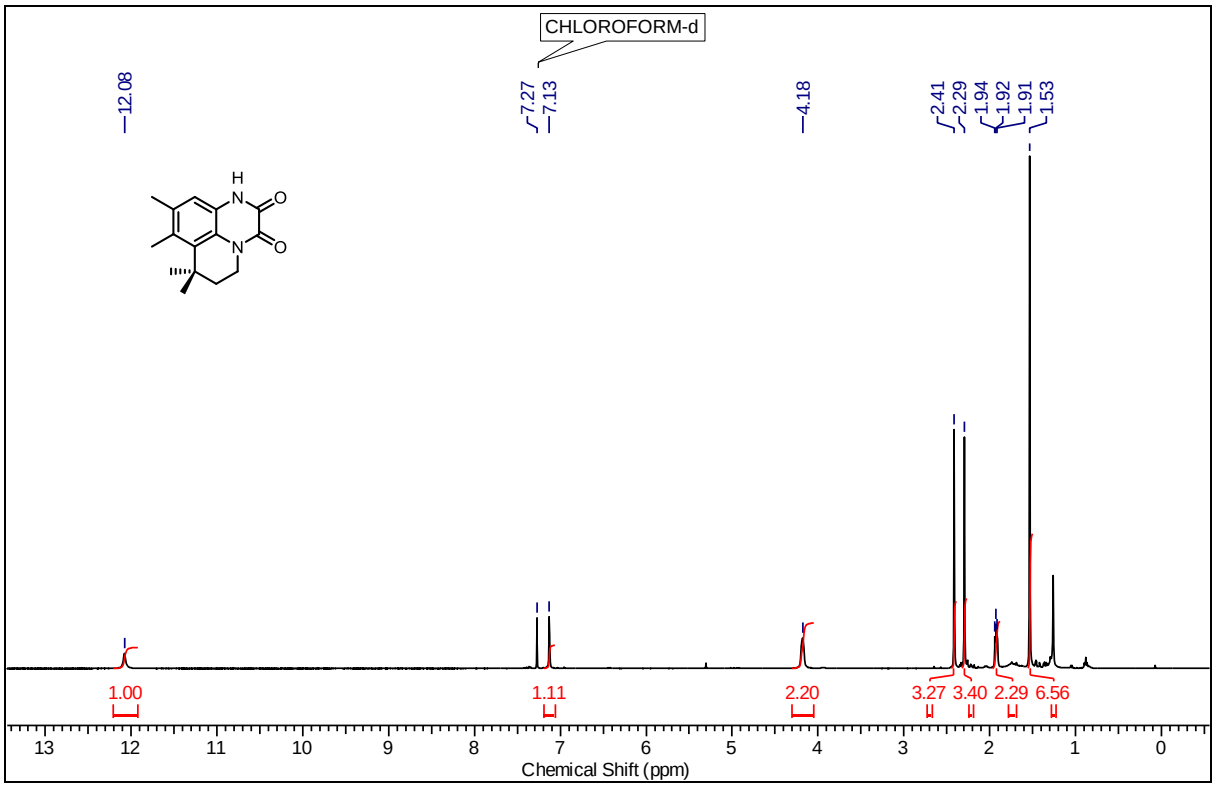

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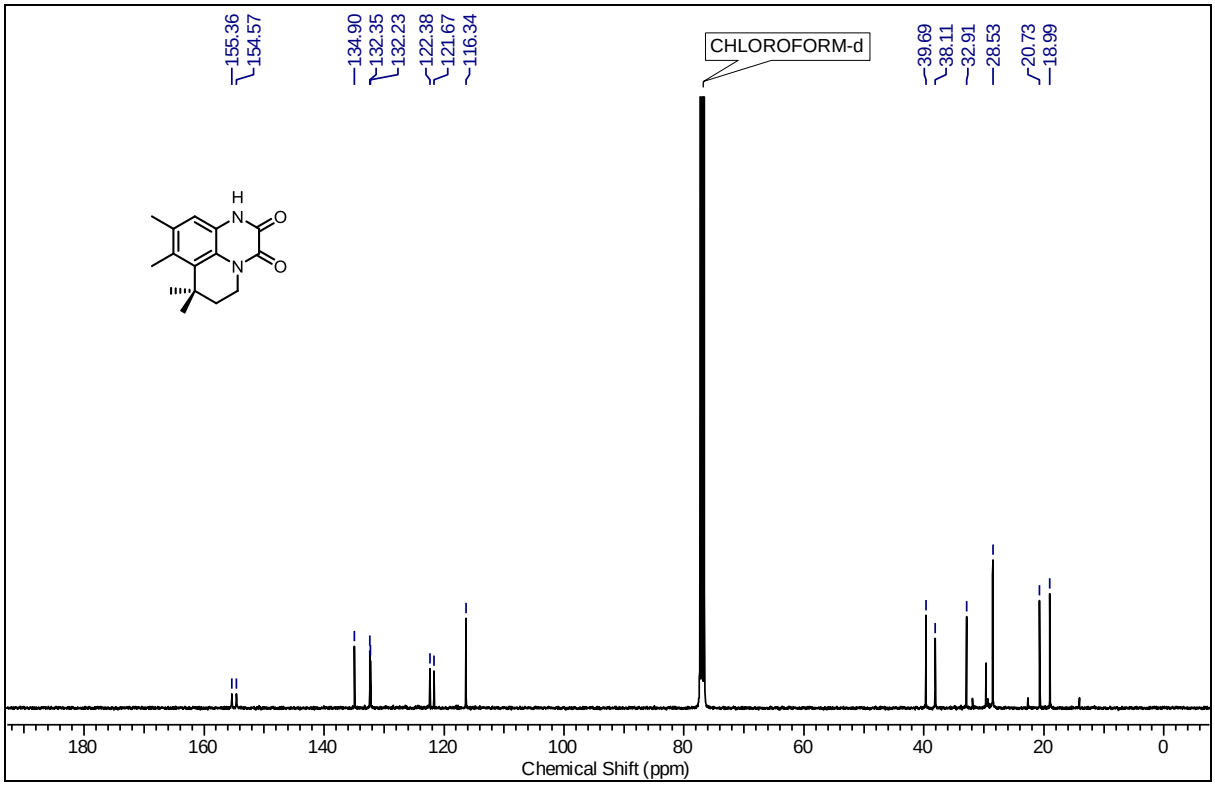

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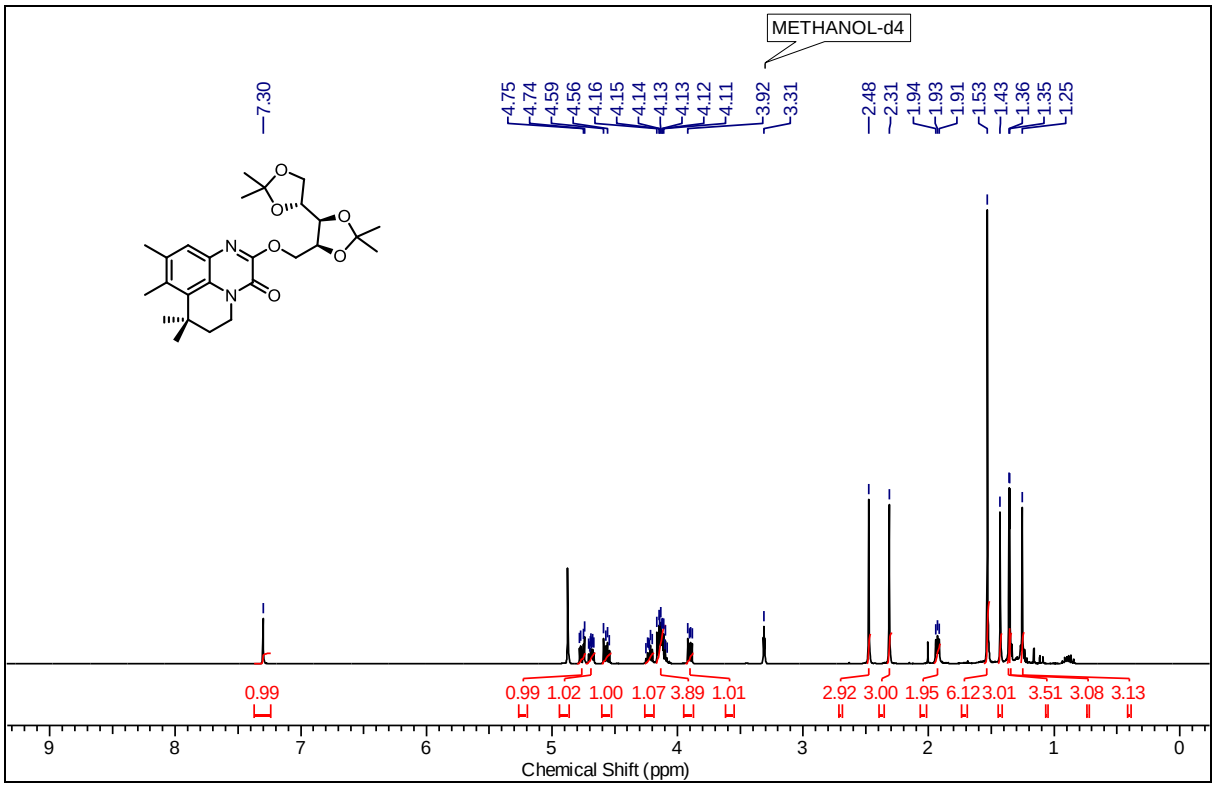

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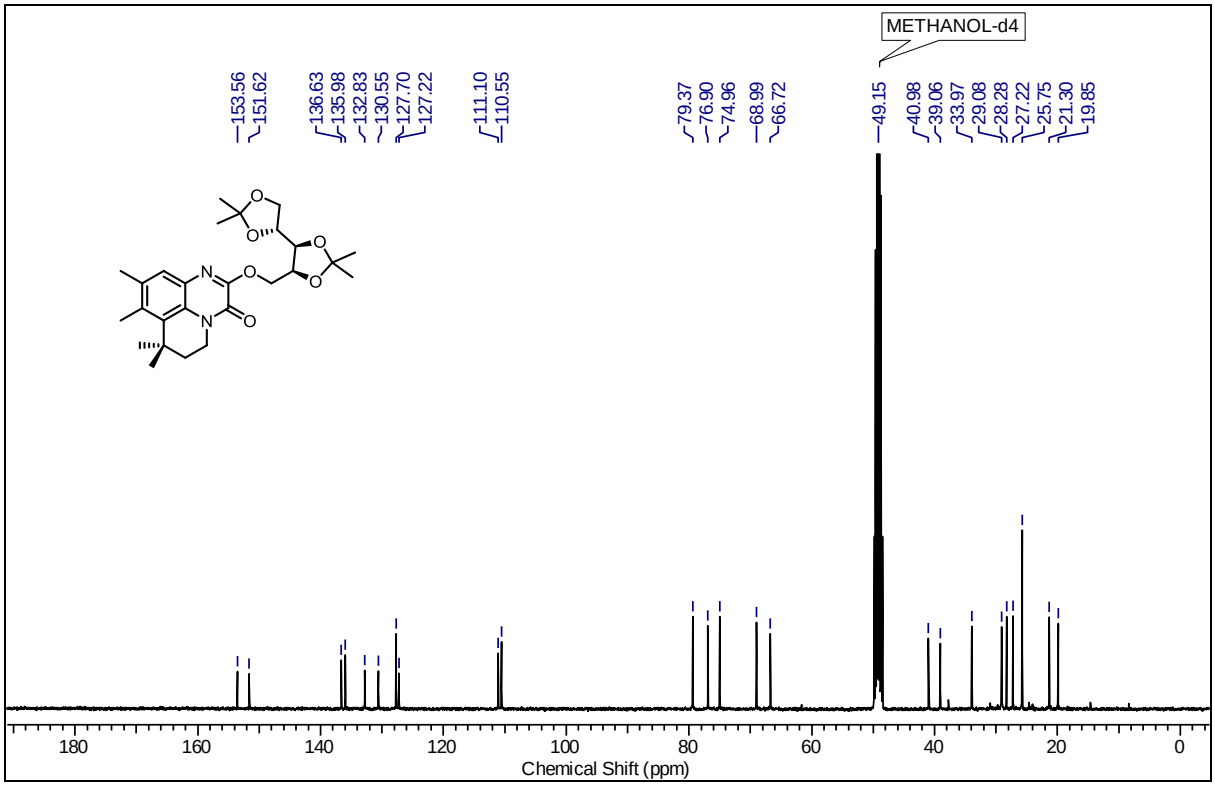

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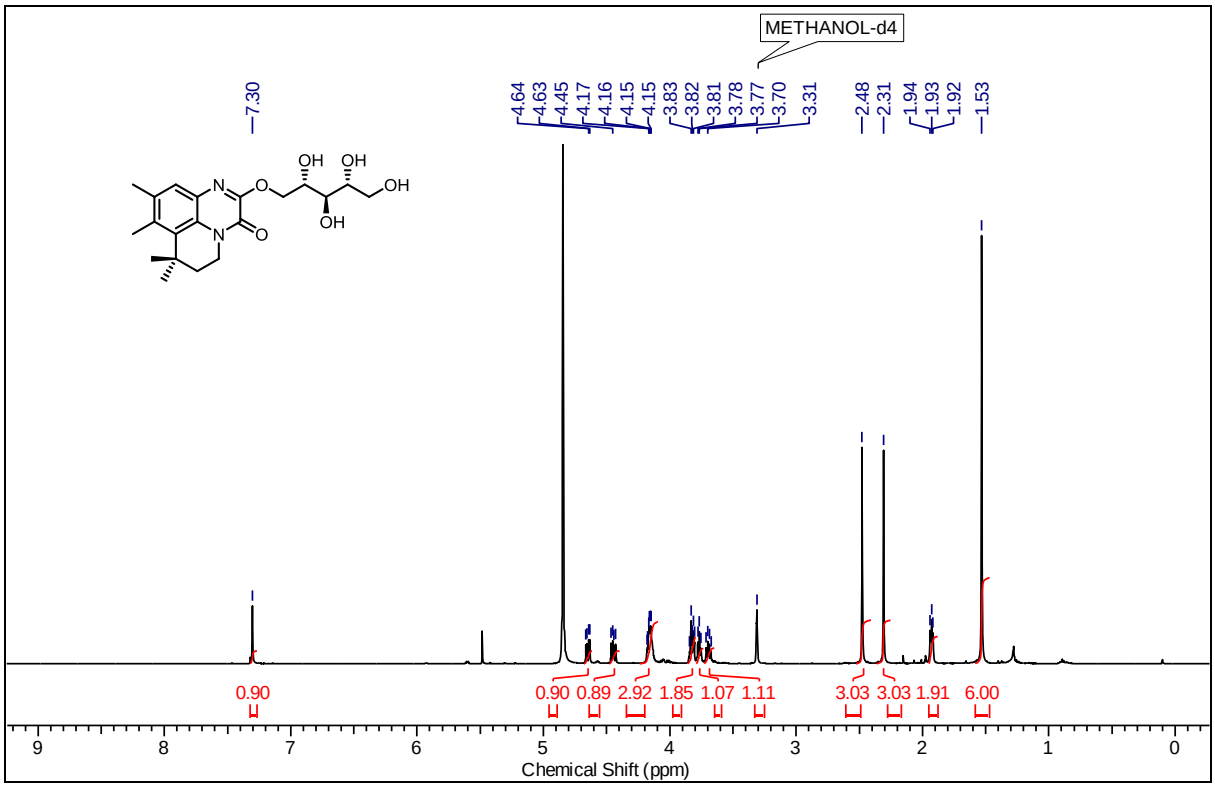

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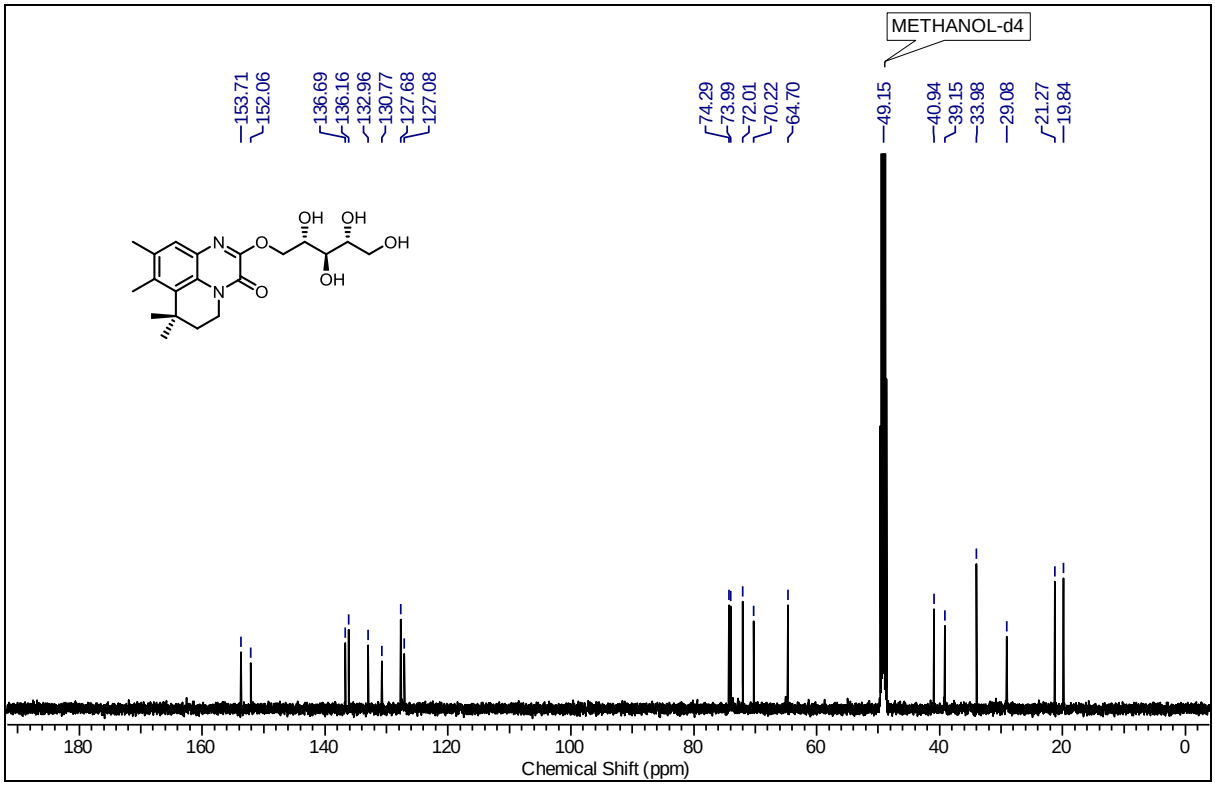

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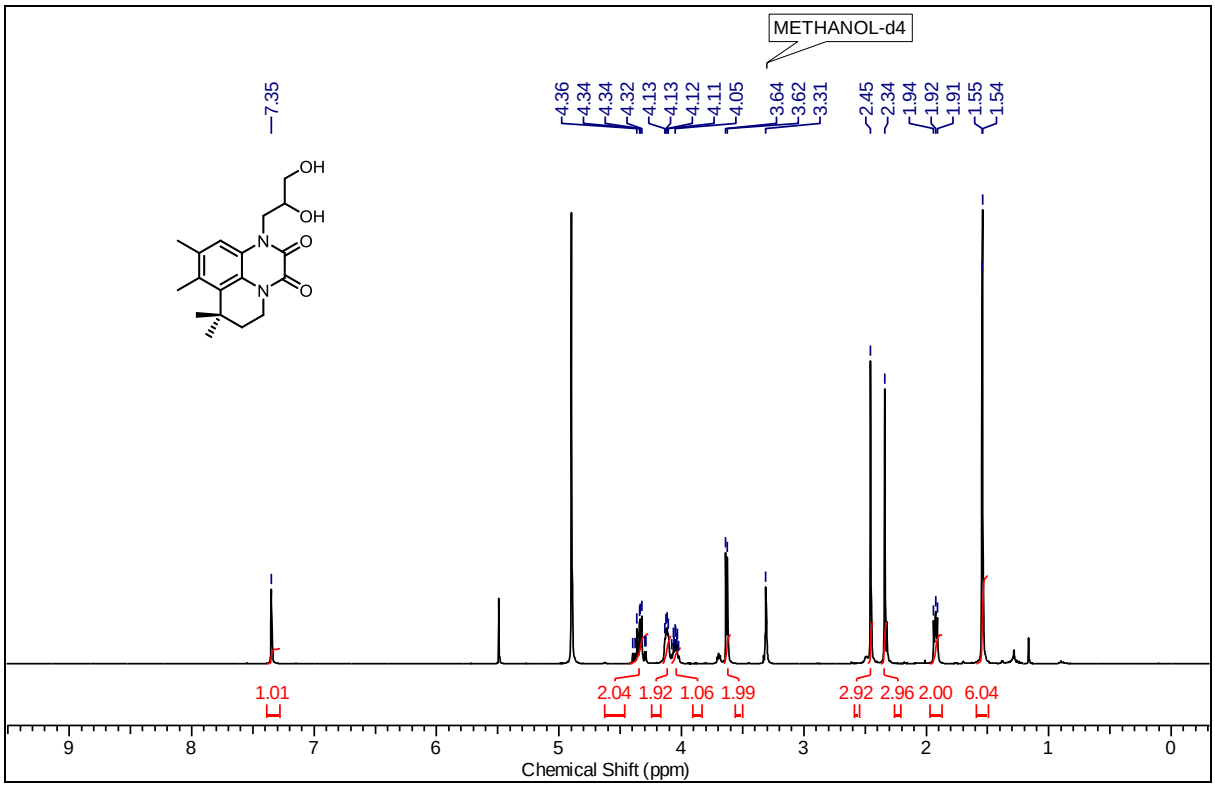

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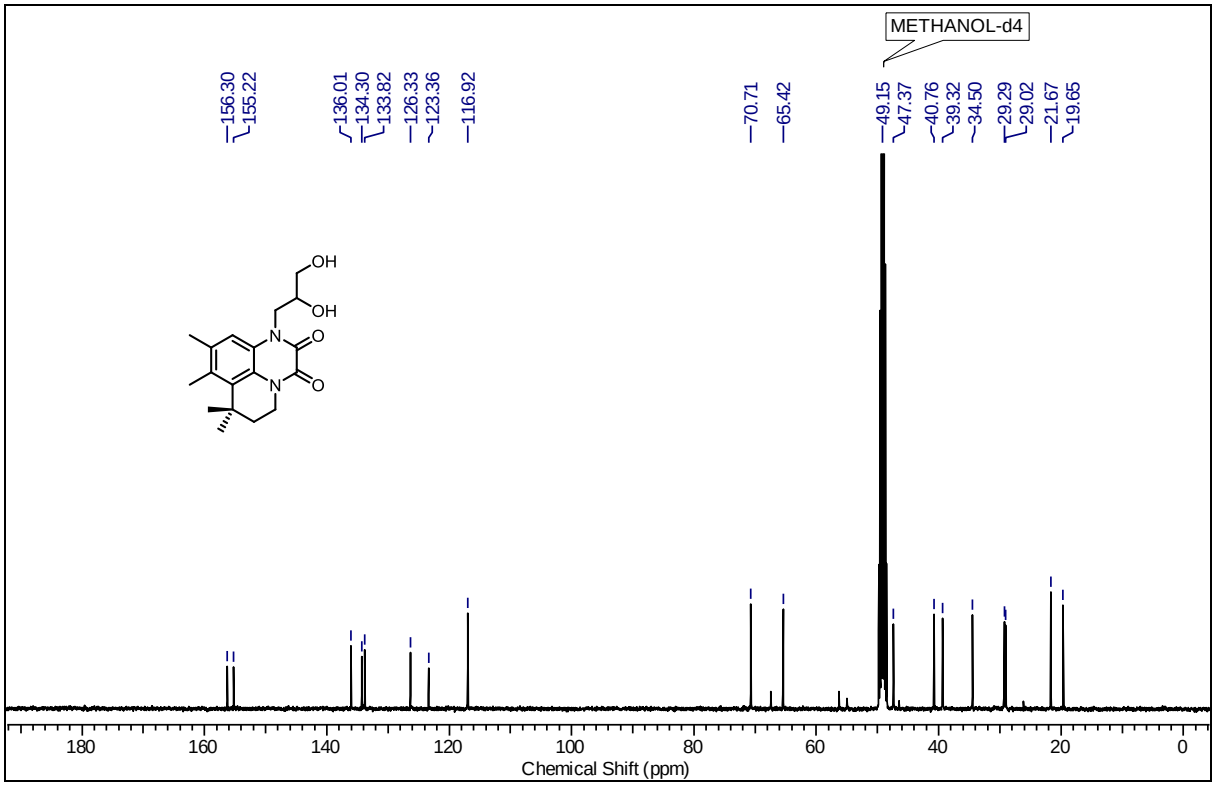

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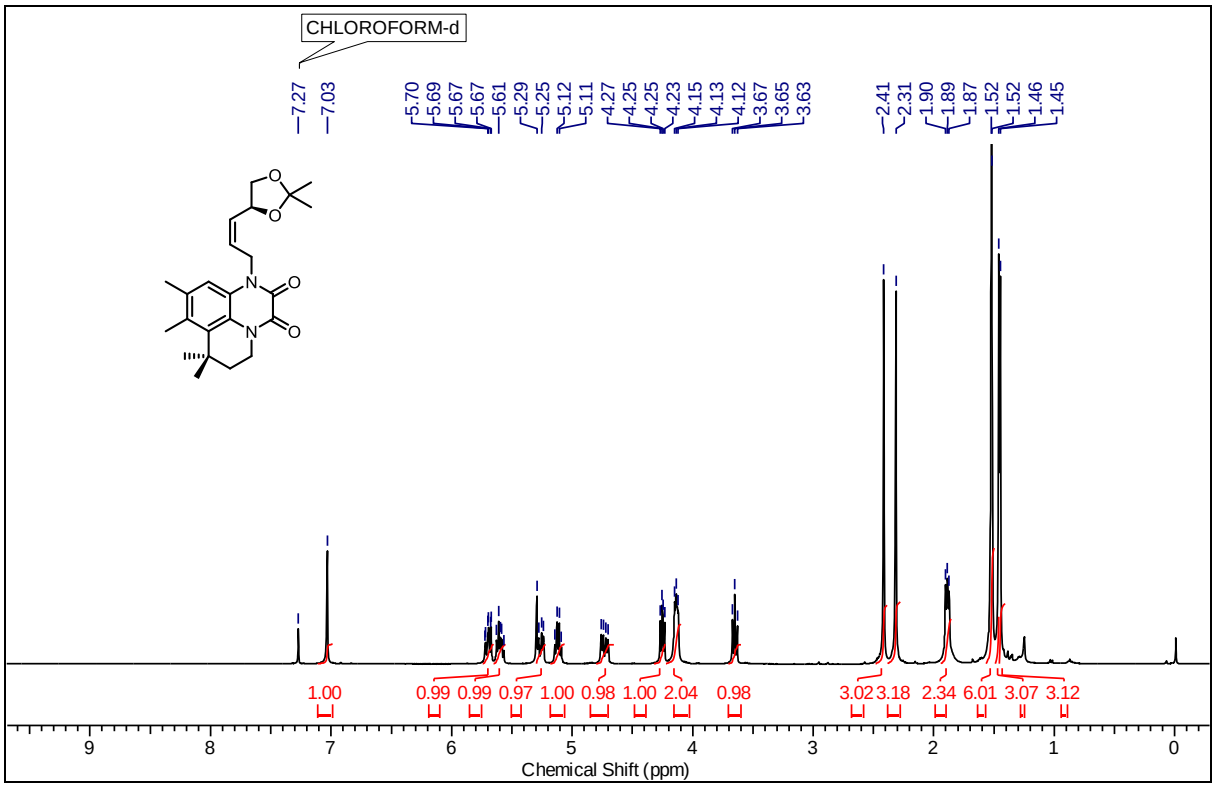

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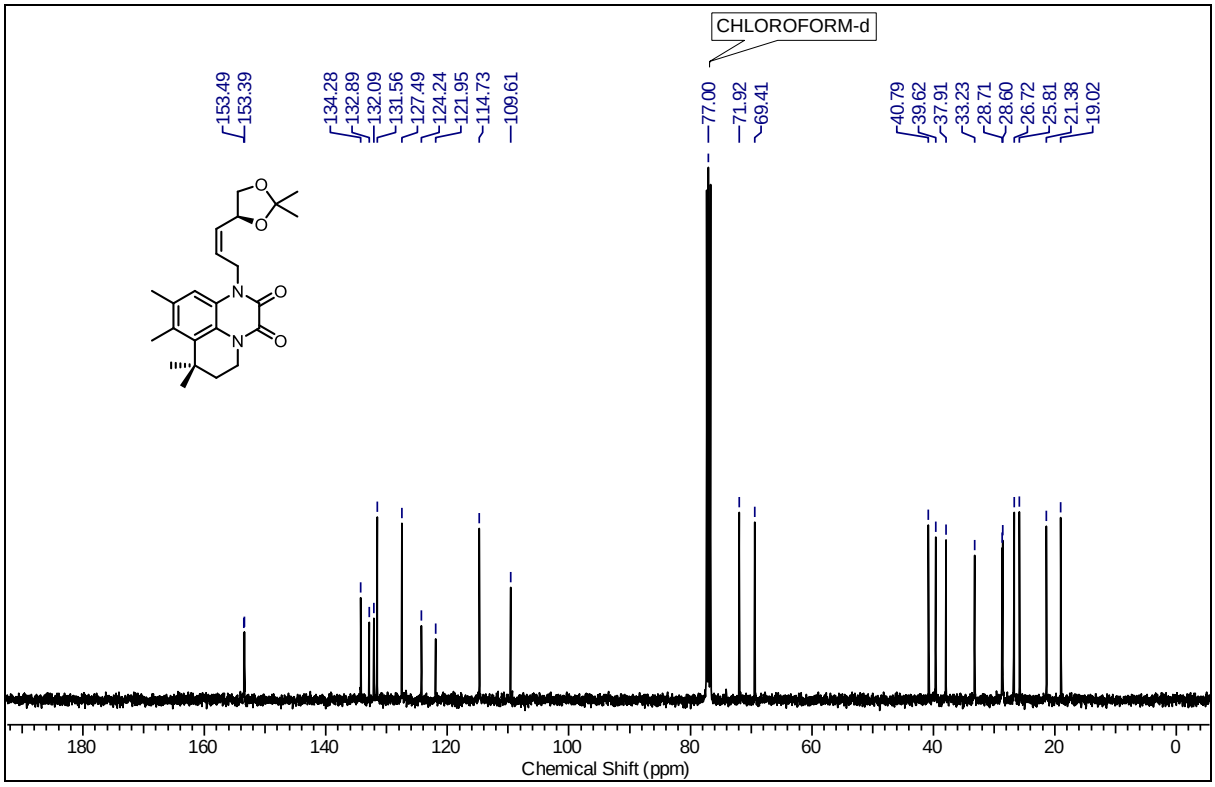

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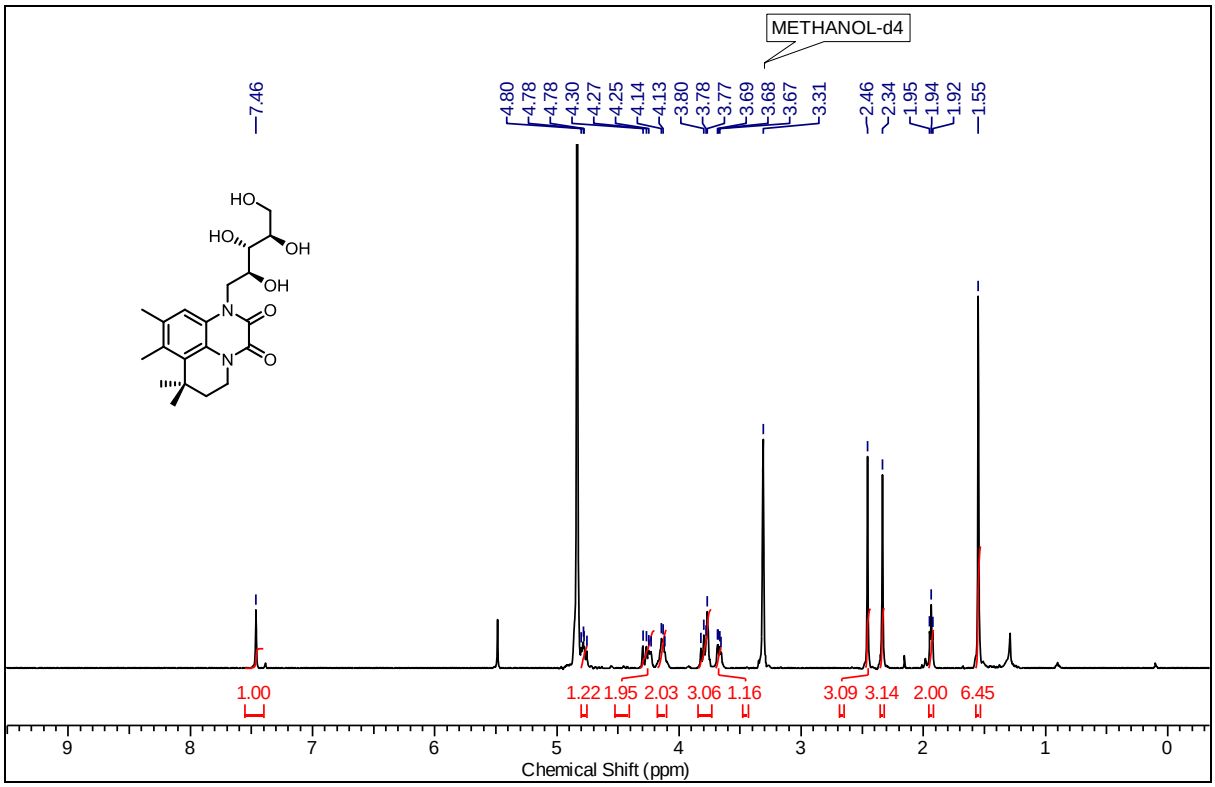

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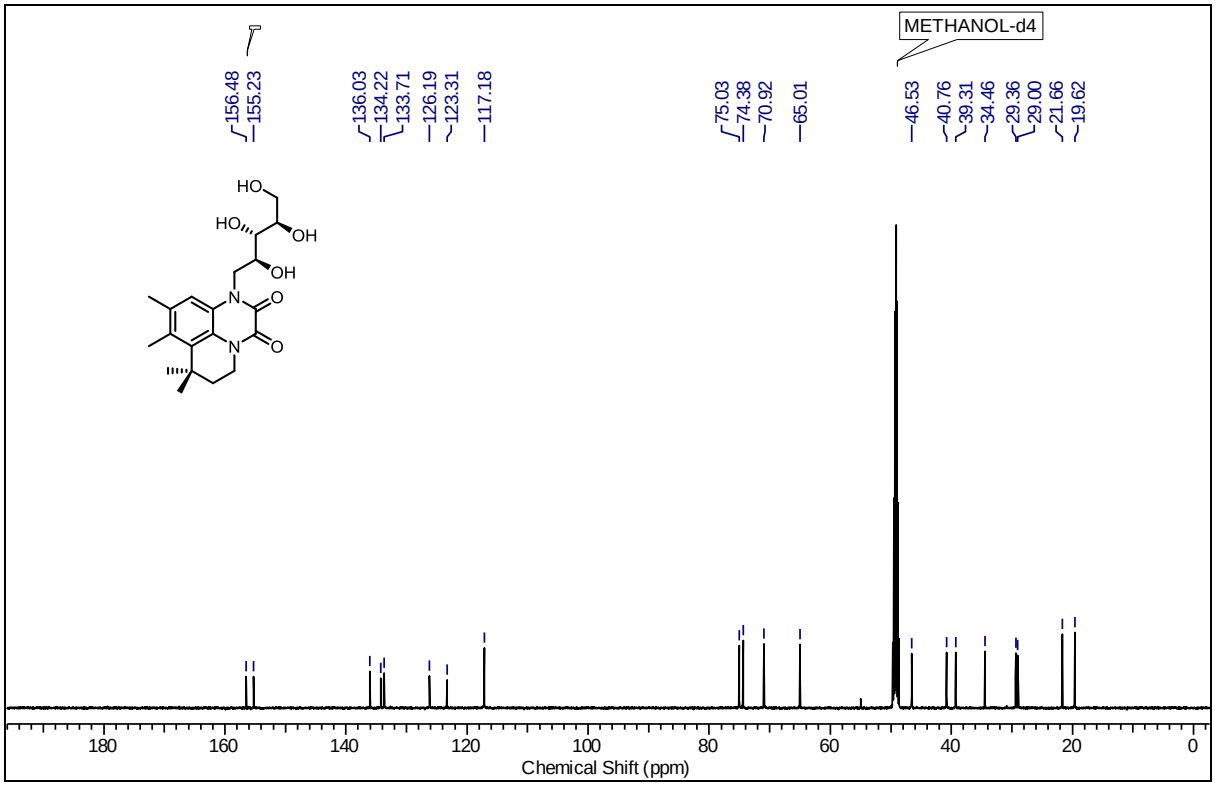

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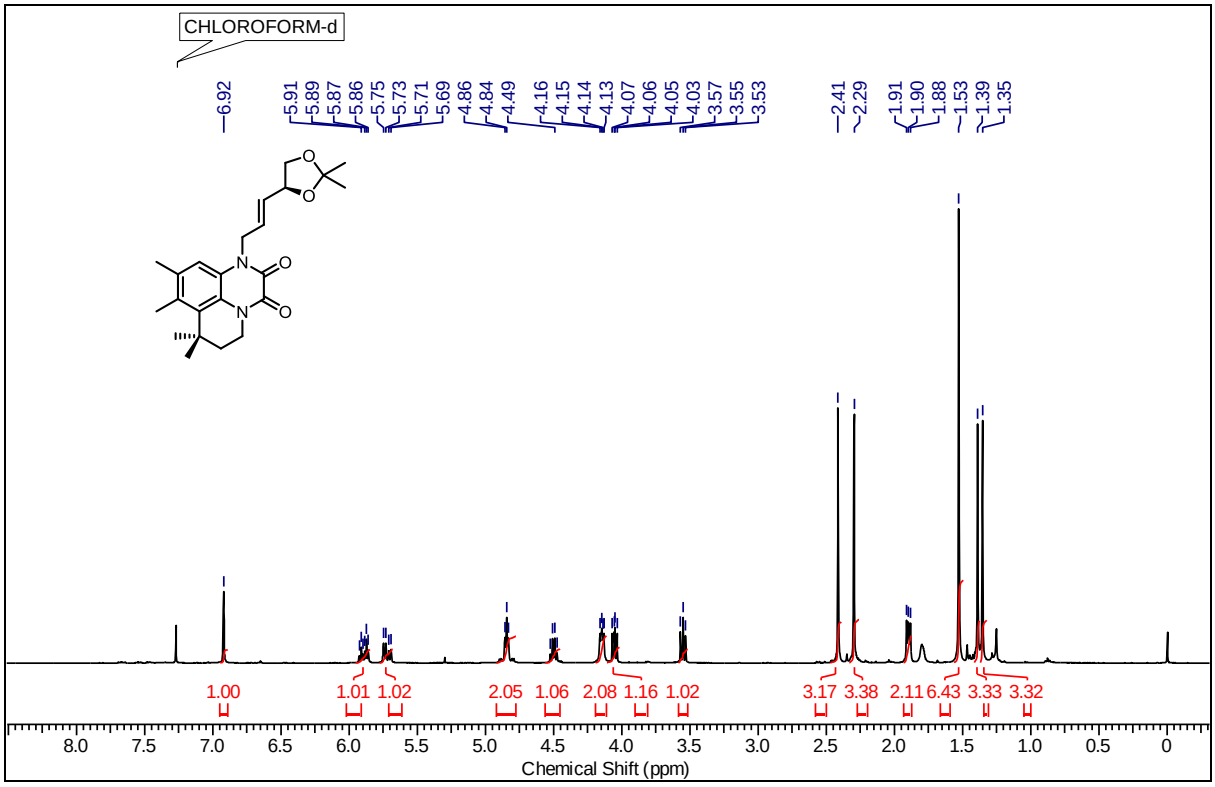

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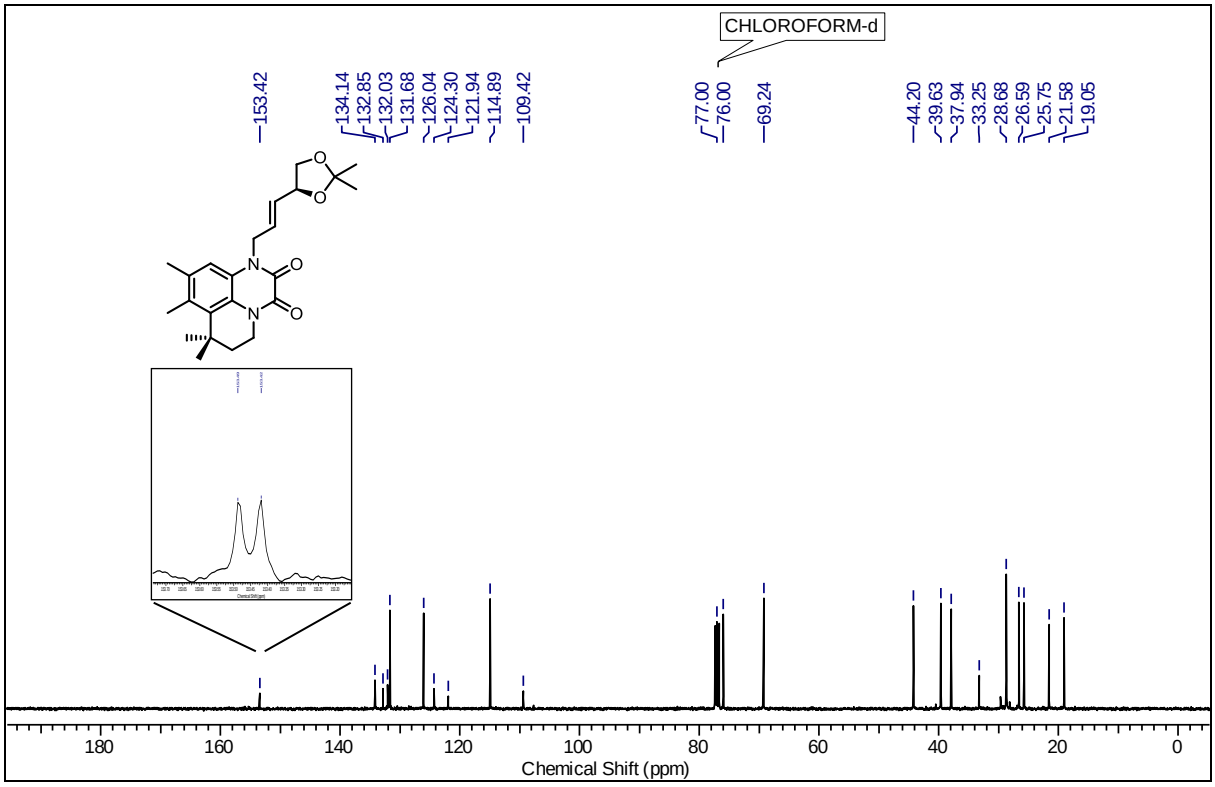

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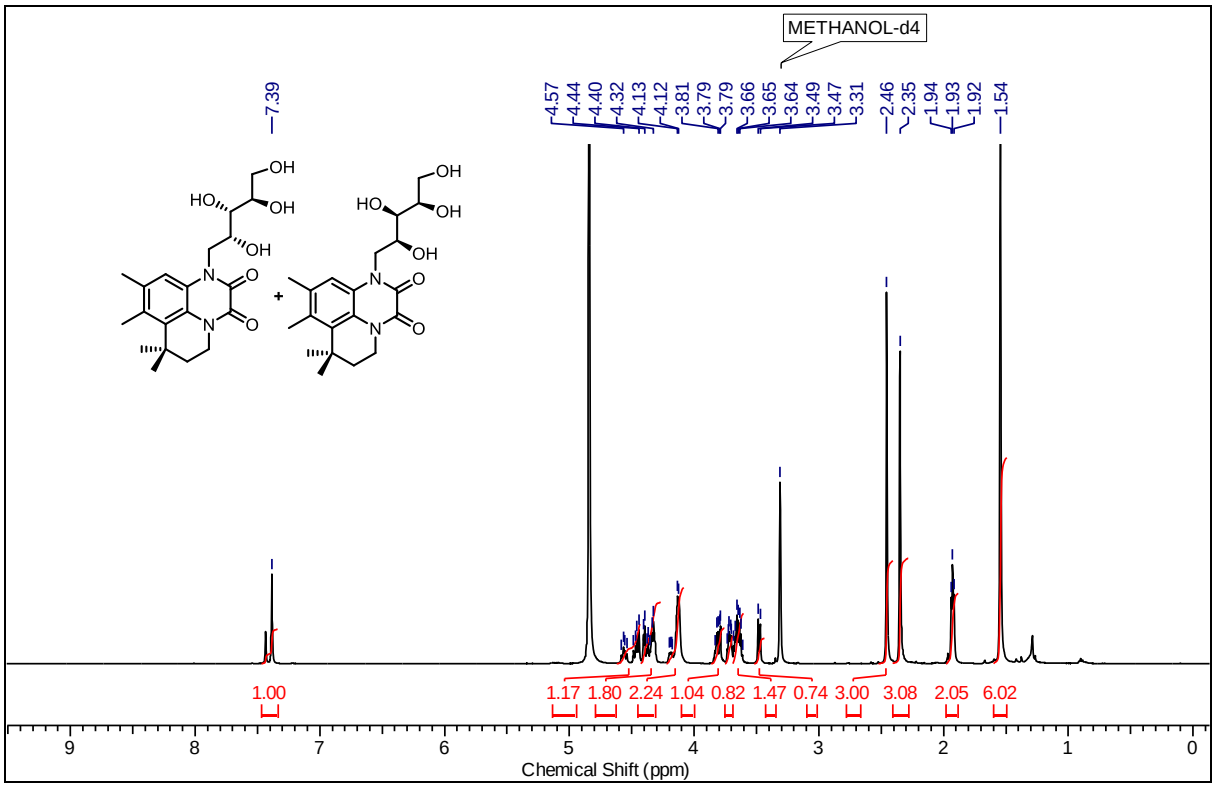

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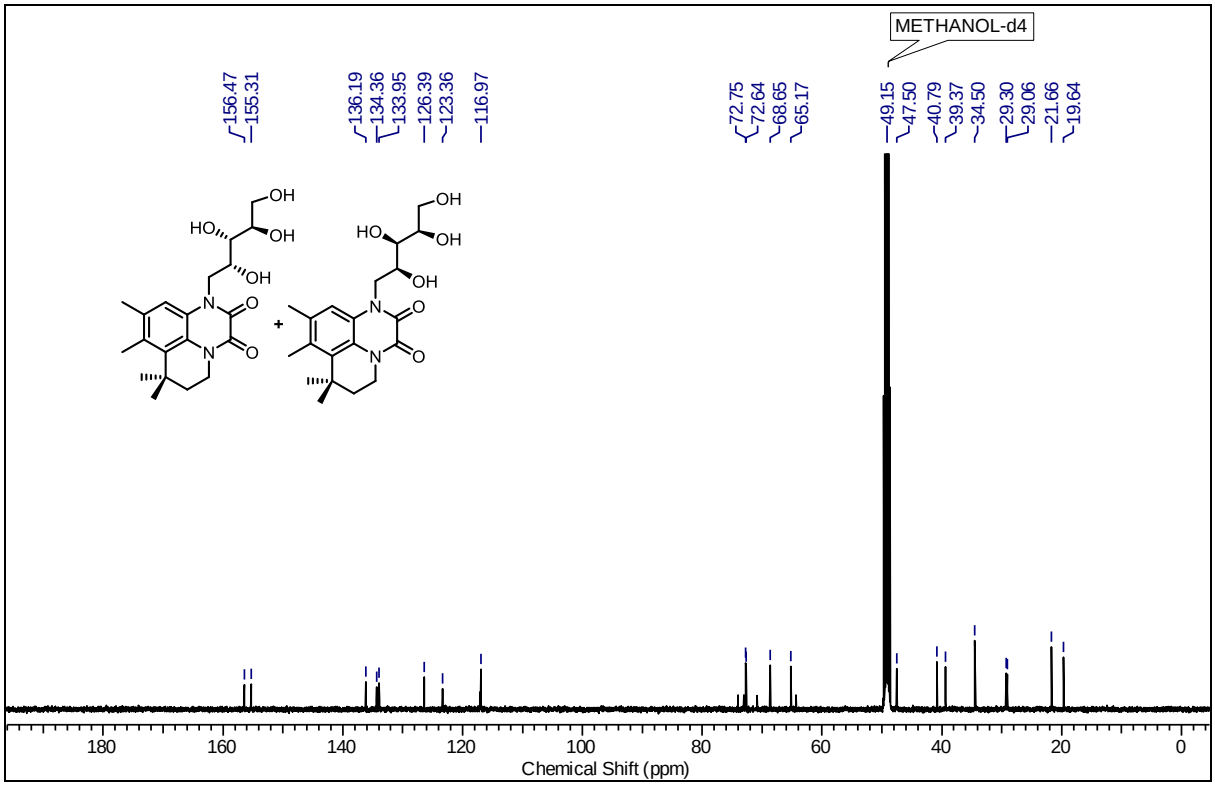

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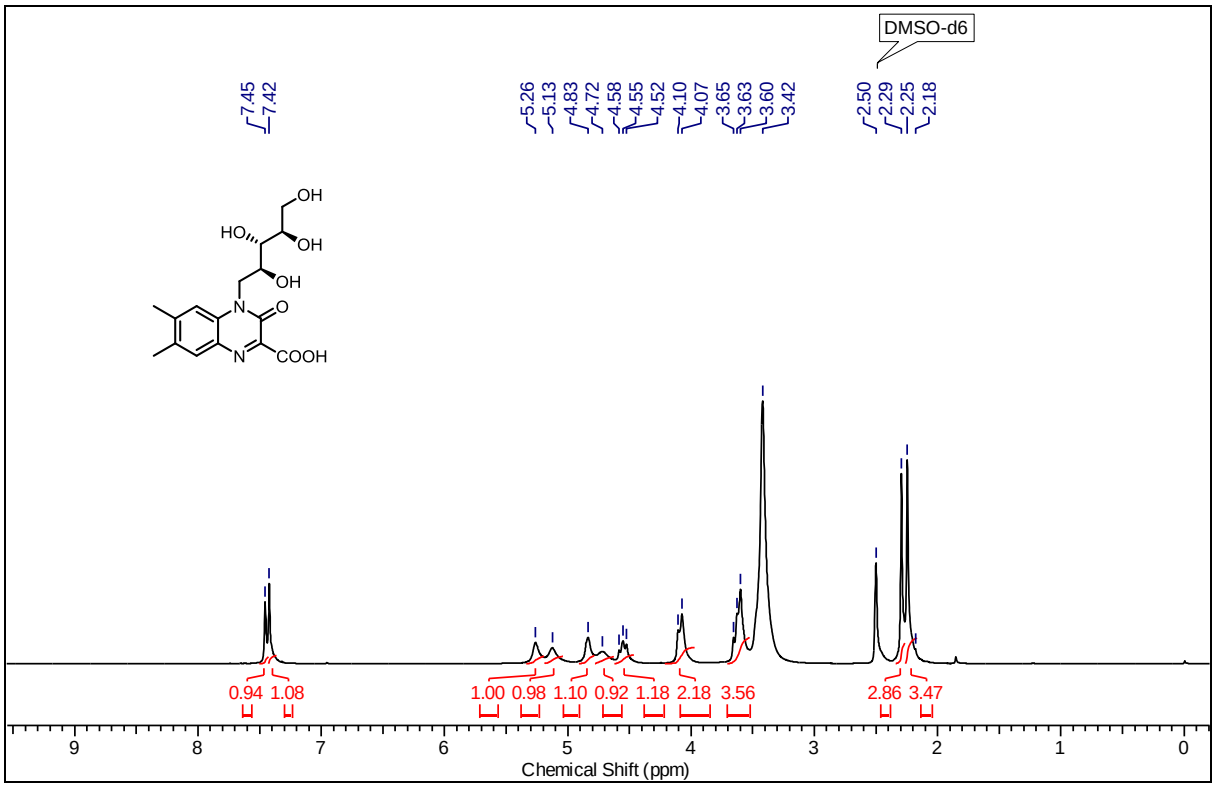

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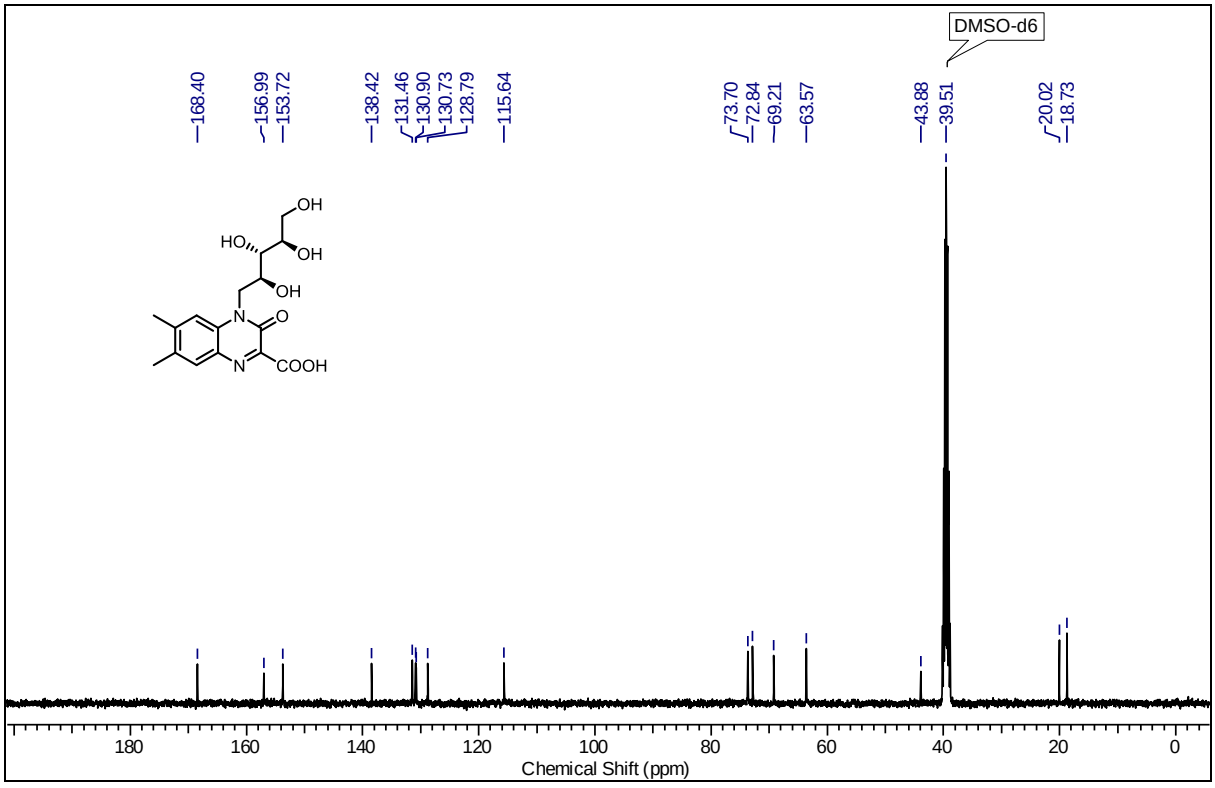

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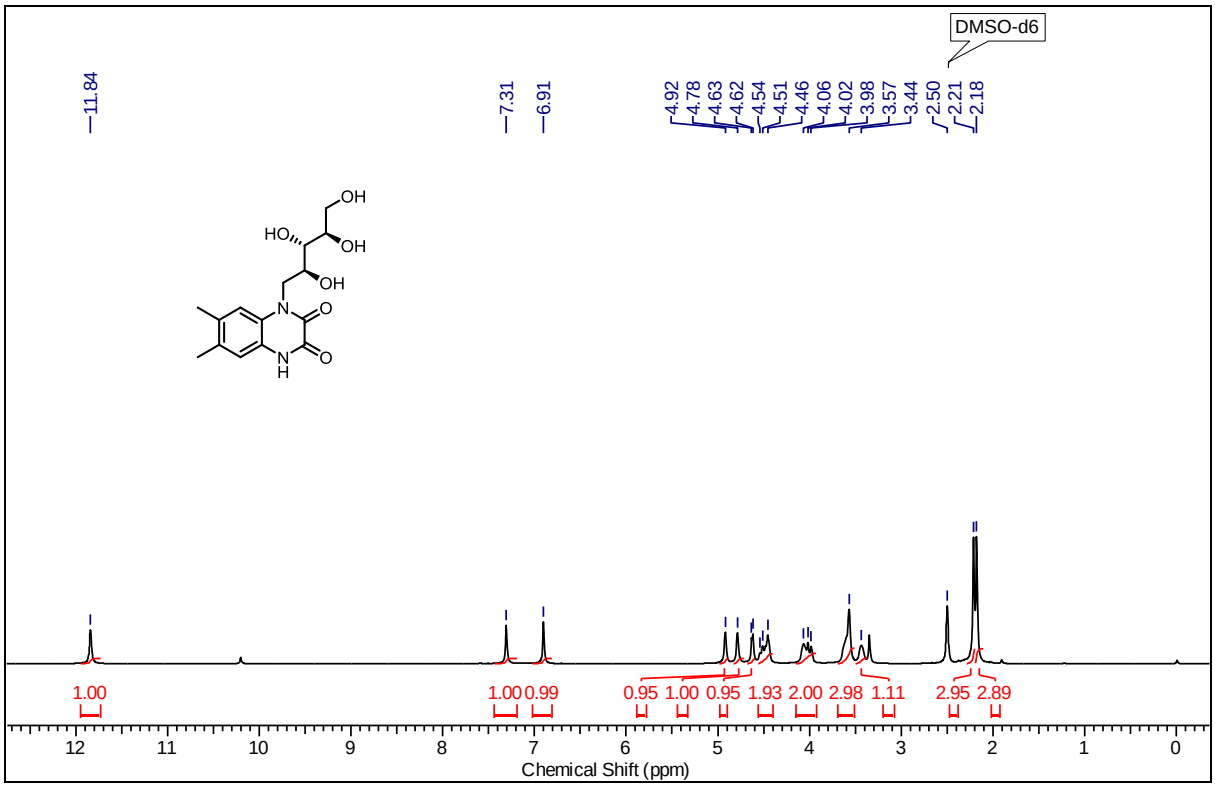

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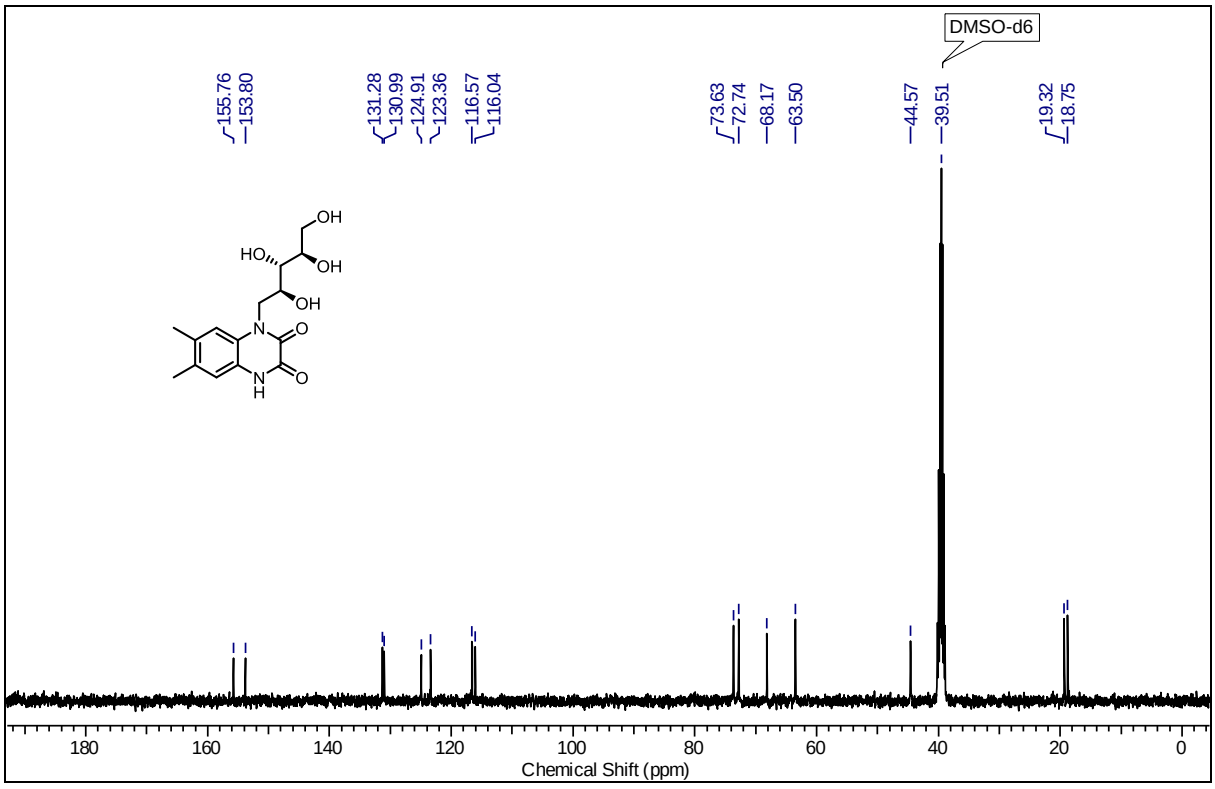

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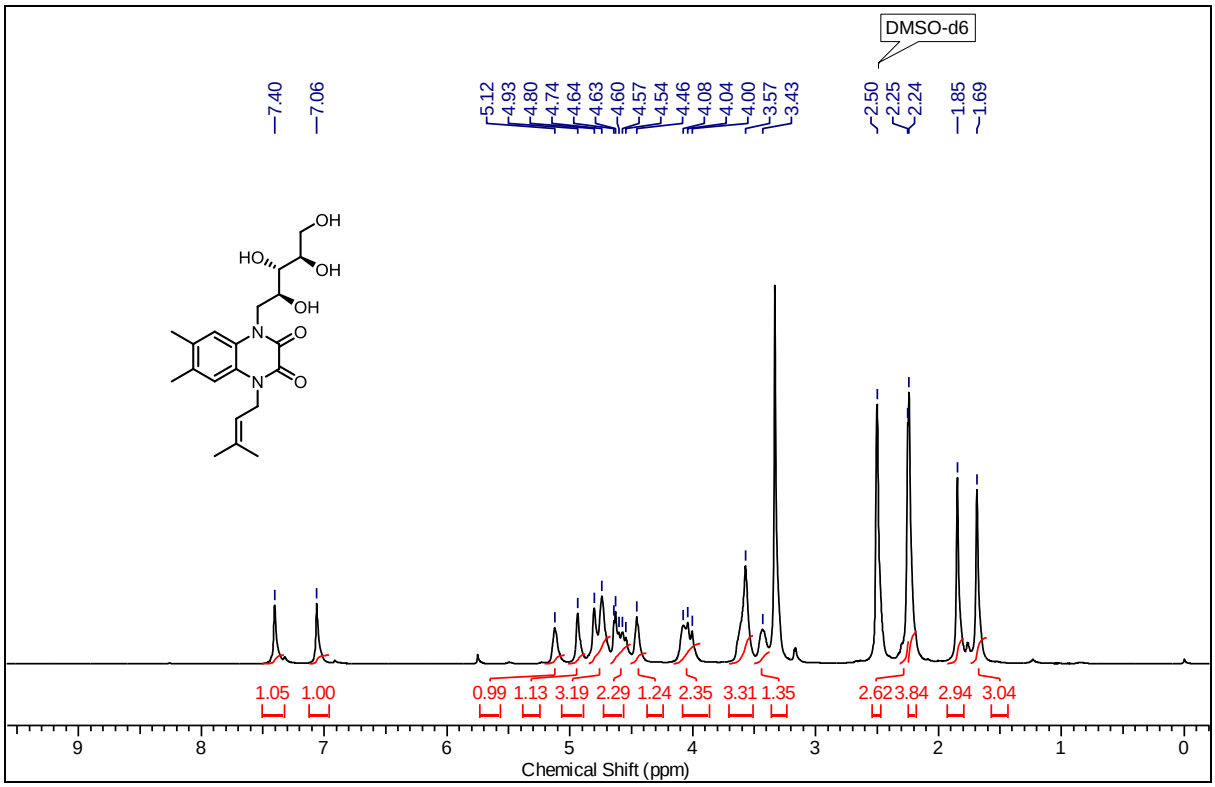

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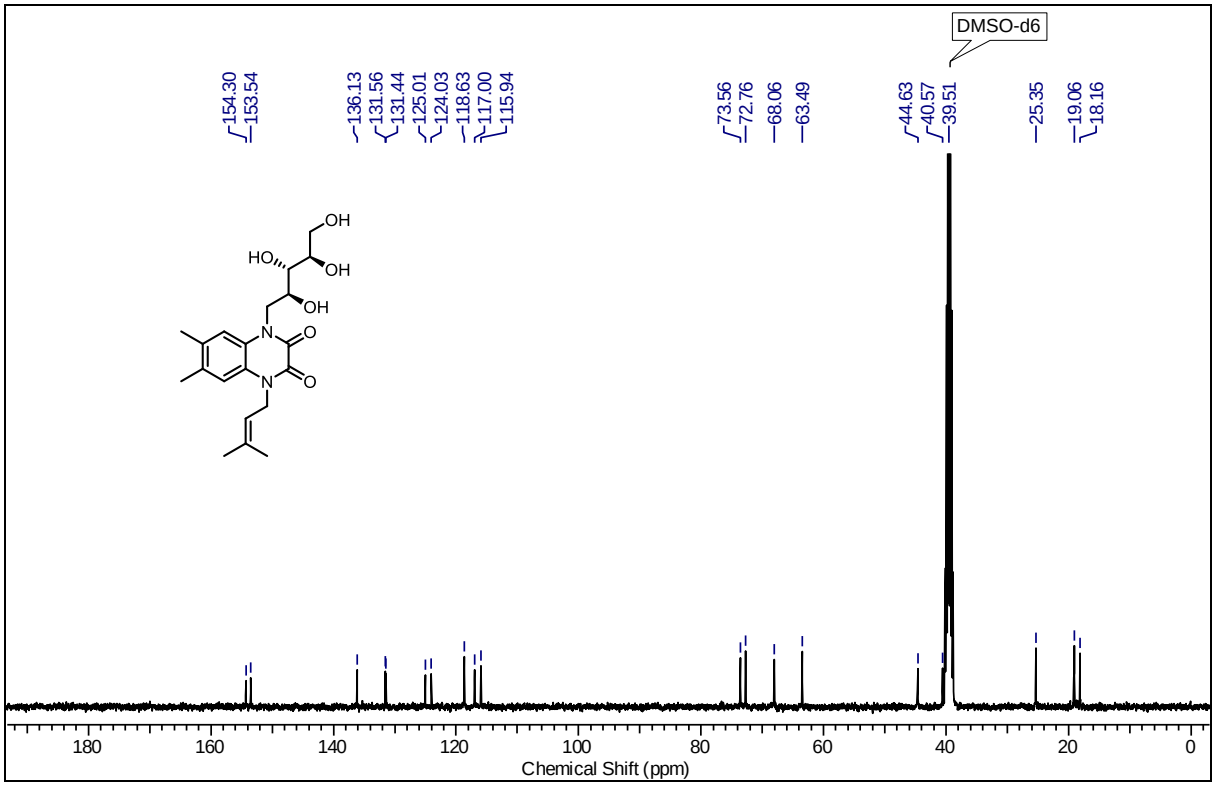


Section 2

Synthesis, SAR studies and lead optimization of Hunanamycin scaffold

Section 2

Synthesis, SAR studies and lead optimization of Hunanamycin scaffold

2.1 Introduction

Foodborne illnesses results from the consumption of contaminated food by pathogenic bacteria, viruses or parasites. They usually arise from improper handling, preparation or storage of food. Among the bacteria which cause foodborne illness, *salmonella* bacteria is the second highest cause. *Salmonella spp*. is rod-shaped, predominantly motile, gram-negative, invasive bacteria and belong to the *Enterobacteriaceae* family. The two species: *S. bongori.* and *S. enterica* are divisions of the genus *Salmonella.*[1-2] Nontyphoidal salmonellae are the major cause for bacterial diarrhoea worldwide; Almost 153 million cases of gastroenteritis are diagnosed and 57,000 deaths occur each year worldwide.[3] Most *Salmonella* infections don't require medication and oral rehydration therapy is recommended. However, in the case of elder people, young children, pregnant women and immunocompromised patients, it may lead to severe complications and death. According to one report, *Salmonella's* resistance to first-line drugs ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole is increasing in sub-Saharan Africa.[4] In addition, the current situation is alarming because the resistance to fluoroquinolones among *salmonella* strains is increasing worldwide.[4] In fact, overall scenario of infectious diseases are a leading cause of death worldwide, and the quick development of bacterial resistance to an available antibiotic is one of the world's most critical health problems.[5-6] Nearly all antibiotics that are in use today were discovered long ago (between 1940 to 1960) and bacteria have evolved and developed resistance against the current antibiotics.[7] In general, the antibacterial are classified based on their mode of action into five different groups as captured below (Figure **2.1**).

1. Cell wall synthesis inhibitors
2. Inhibitors of cell membrane functions
3. Protein synthesis inhibitors
4. Nucleic acid synthesis inhibitors
5. Inhibitors of other metabolic processes

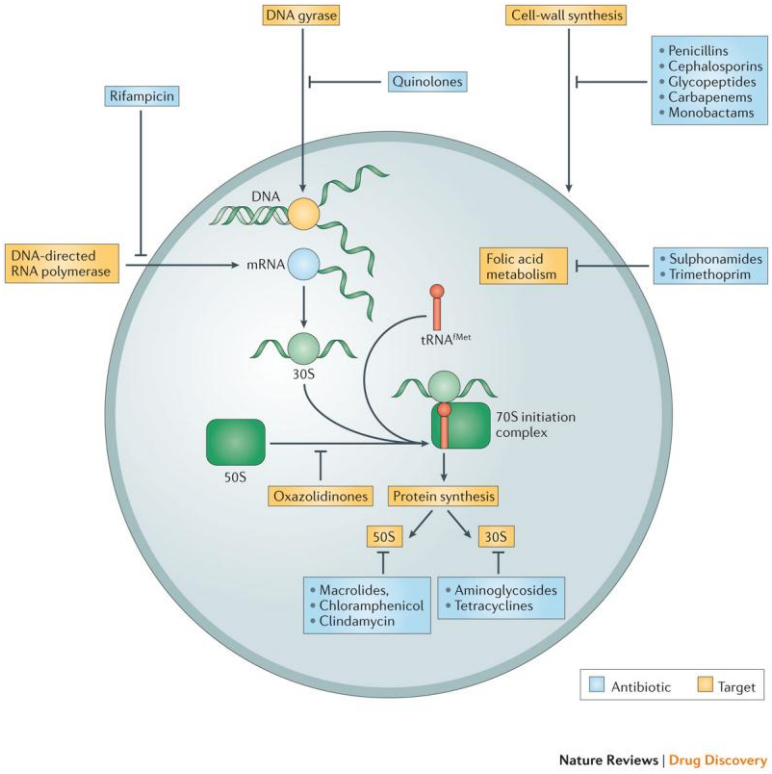


Figure 2.1: Antibacterial drugs and their targets (Source: Nature Review Drug Discovery)[8]

In view of the increase in drug resistance, the discovery of a new chemical class of drug with a different mode of action is urgently needed. Although, different approaches are being tried around the globe, a very few drugs with novel mechanisms crossed early clinical stages. Considering alarming drug resistance situation in the near future, more and more efforts are needed in this direction. Having synthesized antibacterial natural product which is expected to work through riboflavin synthase inhibition, an interesting target, we have made efforts in this project which resulted in interesting outcomes.

Riboflavin synthase is an attractive target to develop a novel antibacterial drug for the treatment of infectious diseases.[9-10] Riboflavin also known as vitamin B2, is very important and essential for all living organisms, it is especially involved in multiple extracellular processes in bacteria, such as quorum sensing signalling, extracellular electron transfer and the establishment of symbiotic associations with plants.[11-13] Animals including human beings obtain riboflavin through their diets, and most bacteria synthesize riboflavin *de novo*. Riboflavin synthase catalyzes the final reaction during riboflavin biosynthesis and hence inhibiting the same can reduce the growth of bacteria. As the enzyme riboflavin synthase is not present in humans, we can expect them to be safe for human use. Therefore, it is an attractive target for the development of potential antibacterial drugs with new mechanism.[9-10] Certain gram-negative bacteria that cannot transport riboflavin, are dependent upon riboflavin

biosynthesis.[14] Thus, a drug that can block riboflavin biosynthesis by targeting this riboflavin synthase should be lethal to the pathogen and not to the human beings. Potential antibiotics inhibiting riboflavin synthase and lumazine synthase enzyme would have a potential benefit over those that inhibit only one enzyme because microorganisms have to alter both enzymes in order to acquire drug resistance.[15]

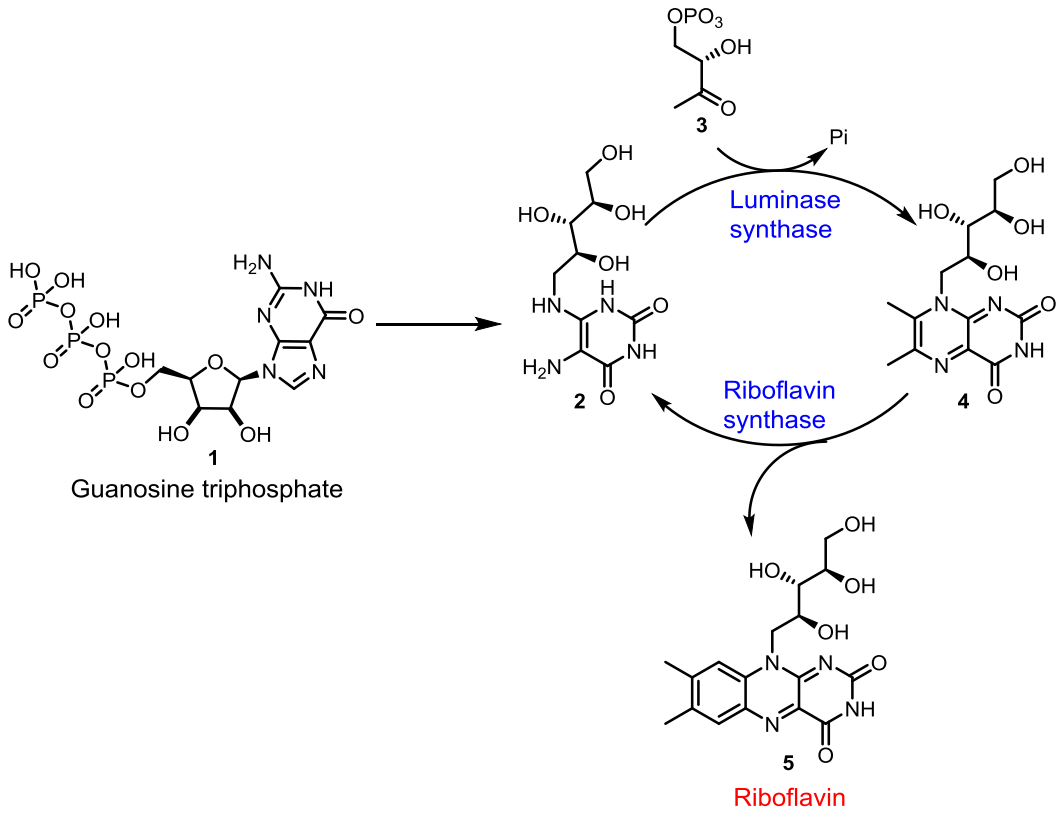


Figure 2.2: Biosynthesis of riboflavin[15]

Before going into synthetic details, it is worth discussing about the biosynthesis of riboflavin. As shown in figure **2.2** the biosynthesis of riboflavin starts from guanosine triphosphate **1** which on ring opening, deamination, reduction and dephosphorylation reaction gets transformed to the pyrimidinedione **2**. Lumazine synthase catalyzes the condensation of 3,4-dihydroxy-2-butanone 4-phosphate **3** with 5-amino-6-D-ribitylamino-2,4(1H,3H)-pyrimidinedione **2** yielding 6,7-dimethyl-8-D-ribityllumazine **4**. Finally, riboflavin synthase catalyses an unusual dismutation of two molecules of 6,7-dimethyl-8-D-ribityllumazine **4** which results in the formation of one molecule of riboflavin **5** and one molecule of the pyrimidinedione **2** (Figure **2.2**). Last two steps in the biosynthesis of riboflavin, lumazine synthase and riboflavin synthase play an important role. Hence both the lumazine synthase and riboflavin synthase are promising therapeutic targets for discovering new antibiotics.

2.2 Previous work towards identification of Riboflavin synthase inhibitors

The deliberate alteration in the natural substrates of enzyme yields many analogues, named antimetabolites.[16] The structural differences compared with the natural substrate, responsible for activities are highlighted in different colour; Blue in case of lumazine synthase and red in riboflavin synthase inhibitor (Figure **2.3**). Cushman and co-workers developed compound **16,** and **17** that inhibit both enzymes. Molecular modelling divulge that the phosphate moieties were found to bind *Mtb* lumazine synthase, resulting in very potent inhibitors and the additional carbonyl in the dioxolumazine system versus the purinetrione system was found to be binding to riboflavin synthase *of E. coli*.[15] Zhao *et al*.

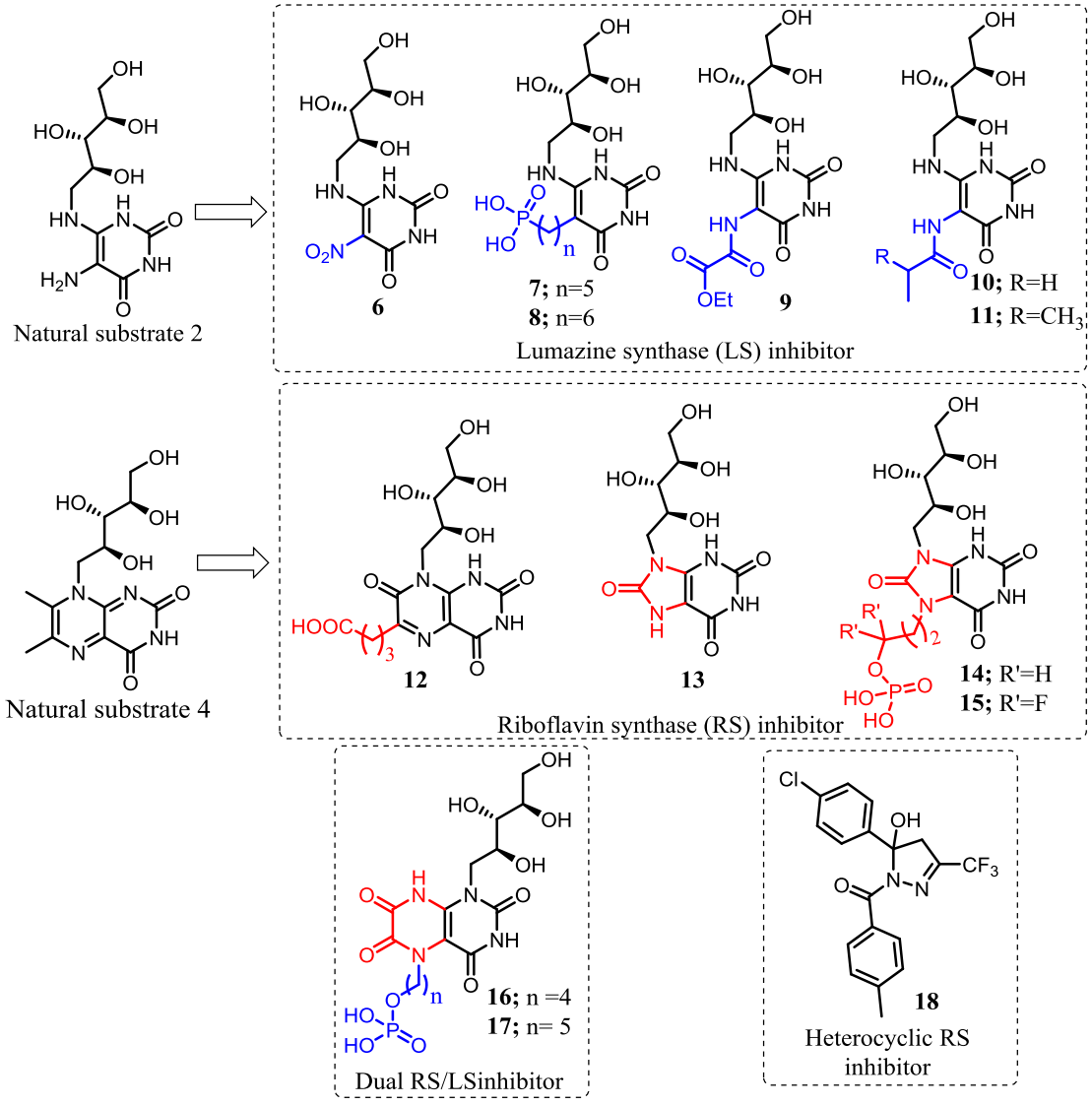


Figure 2.3: Structure-based Riboflavin synthase and Lumazine synthase inhibitors from the literature

reported structurally different dihydro-1H-pyrazole derivative **18** obtained by high throughput screening (HTS), which is inhibiting riboflavin synthase.[17] Literature search also reveals that there is no drug in the market till date which targets riboflavin synthase; it is worth to highlight the pioneering work by Mark Cushman towards identifying the 'novel chemical entities' for the inhibition of riboflavin synthase.[18-22]

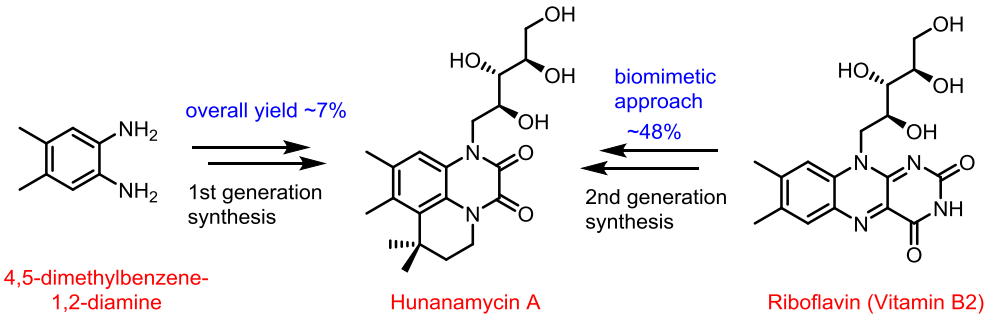
2.3 Present work

The successful total synthesis and interesting biology of natural product encouraged us to plan and systematically synthesize a library of analogues of Hunanamycin A with different substitutions around the scaffold. Our primary aim was to obtain a simplified structure with desirable druggable properties towards lead identification. Systematically we have replaced the ribose sugar moiety with different sugars, cyclic sugars and heterocycles. Similarly, the quinoxalinedione ring was replaced with benzoyleneurea, benzimidazol-2-one etc. Finally, a few analogues were synthesized with/without C ring to understand its effect on potency. In collaboration with Prof. MacMillan, UT Southwestern Medical Center, Dallas, Texas, U.S.A, all the compounds were subjected to biological screening to establish a structure-activity relationship. Selected compounds were also tested for initial pharmacokinetics, and displayed good in-vitro absorption, distribution, metabolism, and excretion (ADME). Now we have identified lead compound with MIC of 1 μg/mL against *Salmonella enterica* which possess good ADME properties. This work has been discussed in details in following sub-sections.

2.3.1 Hunanamycin A as Riboflavin synthase inhibitor

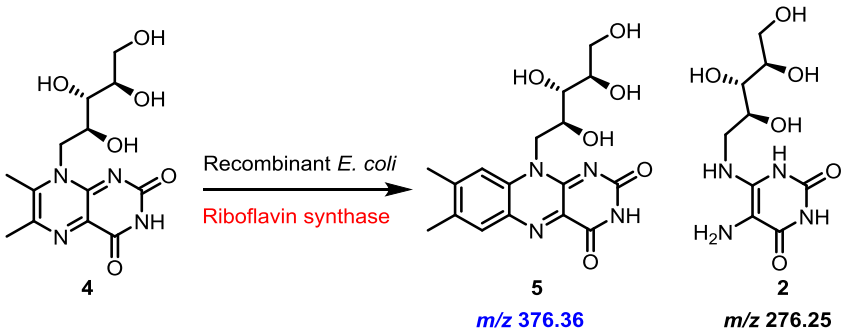
As discussed in section **1** the natural product/ natural product scaffold have potential to serve as hit which can be optimized further to a lead compound for the treatment of disease. The newly isolated marine natural product Hunanamycin A having potent antibiotic activity against the *salmonella enterica* with a minimum inhibitory concentration (MIC) of 4.8 μg/mL is expected to work through riboflavin synthase inhibition. Owing to its interesting structure and impressive biological activity, we have initiated a program on the natural product Hunanamycin A towards identifying a novel class of riboflavin synthase enzyme inhibitor. We have achived the total synthesis of natural product Hunanamycin A in an efficient and scalable manner (discussed in section **1**). It was hypothesized because Hunanamycin A has structural similarity with the riboflavin and also intermediates involved in its biosynthetic pathway. In order to prove the mechanism of action (MOA) and activity of the analogues

synthesized, we have collaborated with Prof. John MacMillan (University of Texas South western Medical Center, Dallas, Texas, U.S.A).



Scheme 2.1: Total syntheses of Hunanamycin A

In the experiments, it was planned to estimate the production of metabolites using LC-MS method in the last step of riboflavin biosynthesis in *E. coli*. The graph was plotted between the concentrations of inhibitor versus relative intensity of metabolites produced. Findings of



Monitor for production of metabolites using LC-MS

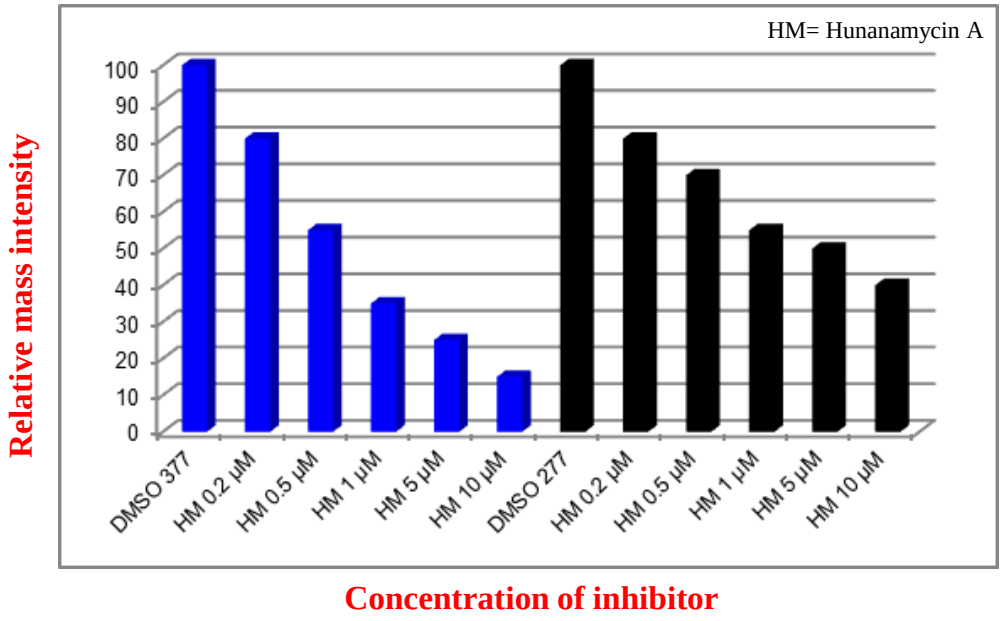


Figure 2.4: Proof for inhibition of Riboflavin synthase by Hunanamycin A

these experiments are exciting and are summarized below: In control, the relative intensity for the mass of riboflavin **5** and compound **2** is 100%, so in the absence of riboflavin synthase inhibitor (in this case Hunanamycin A) the bacteria can produce riboflavin efficiently. At 0.2 μM concentration of Hunanamycin A the relative intensity for riboflavin and compound **1** decrease by 20%, further at 0.5 μM concentration the relative intensity decrease up to 50% that clearly indicate the activity of the riboflavin synthase enzyme reduced nearly up to 50%. Finally at 10 μM concentration of inhibitor, activity of the enzyme is reduced to a minimum, now at this point, bacteria are not able to produce riboflavin efficiently which in turn led to the death (Figure **2.4**). The observed dose response correlations undoubtedly confirmed that Hunanamycin A is acting as a competitive inhibitor for the natural substrate **4** and ceases the natural process for riboflavin biosynthesis.

2.3.2 Synthesis of analogues of Hunanamycin A

After understanding of the mechanism of action of Hunanamycin A as riboflavin synthase inhibitor, we planned for the synthesis of analogues around the Hunanamycin scaffold. The total synthesis route developed was amenable for the synthesis of structurally close analogues of the natural product. Our efforts were dedicated on structure simplification and understanding of structure/substituent relationship with respect to its activity. Planned structural modifications are depcieted below (figure **2.5**):

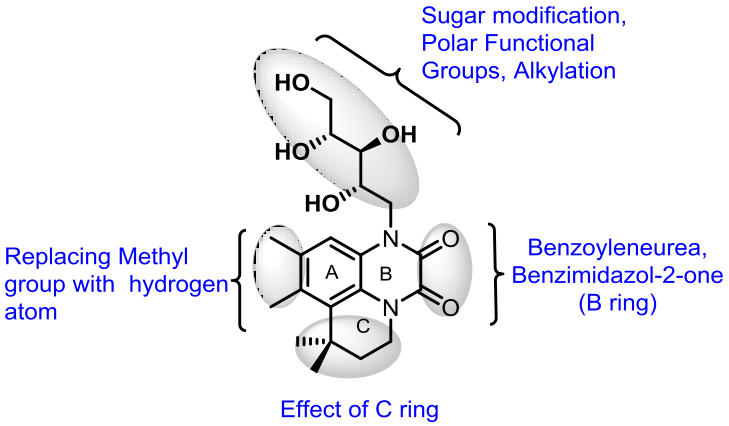
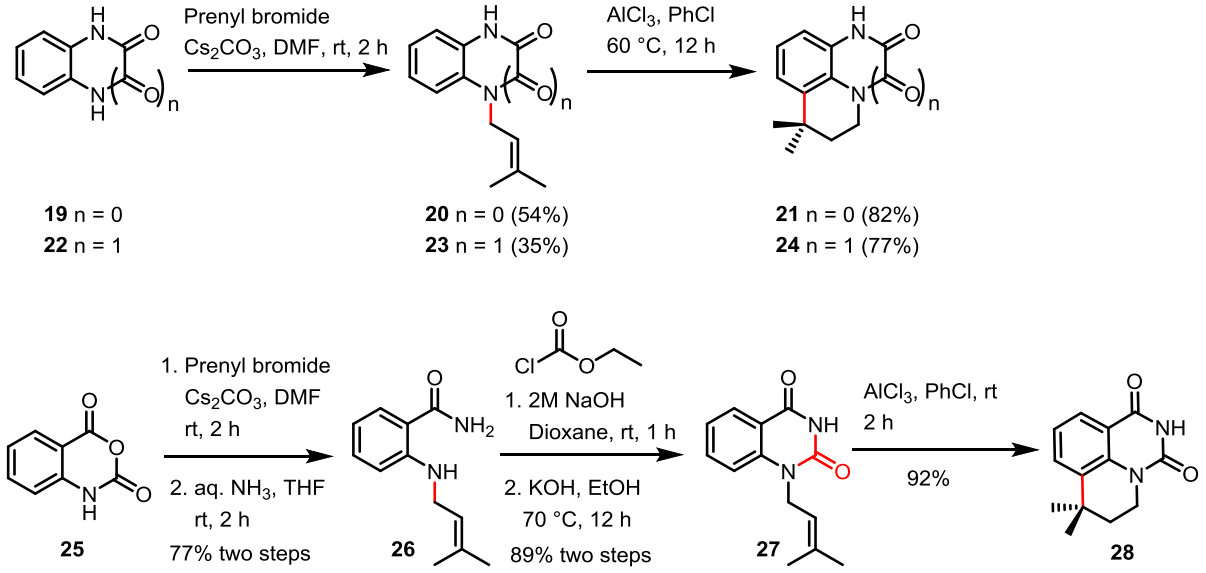


Figure 2.5: Modification planned around Hunanamycin scaffold

i) Replacing the ribose, with other cyclic sugar, heterocycles or aliphatic polar groups
ii) Changing the quinoxalinedione with benzoyleneurea, benzimidazol-2-ones (B ring)
iii) Removal of the aromatic methyl group and
iv) Removal of the C ring.

2.3.2.1 Gram-scale Synthesis of tricyclic building blocks

Accordingly, we planned three tricyclic building blocks **21**, **24**, and **28** for the synthesis of new analogues of Hunanamycin A. Our synthesis commenced with the preparation of compound **21** and **24** from known compound **19** and **22** by using our own developed protocol.[23] Whereas, the synthesis of **28** started with reaction of isatoic acid anhydride **25** with prenyl bromide in the presence of Cs_2CO_3 converted to the corresponding *N*-prenylated isatoic acid anhydride which on treatment with aq. ammonia in THF produced **26** in excellent yield (77% for two steps). The compound formed was confirmed by ^{1}H NMR showed signals at δ 5.33 (t, *J* = 6.4 Hz, 1 H) corresponds to C-H of the internal olefin, and δ 5.89 (br. s., 2 H), for the CH_2 from the prenyl unit, ^{13}C NMR spectrum clearly showed primary amide carbonyl at δ 172 ppm. Compound **26** was cyclized using ethyl chloroformate in NaOH, followed by treatment with KOH/EtOH for 12 h to afford benzoyleneurea derivative **27** in good yield (89%). The desired product formed was confirmed by ^{13}C NMR spectrum which



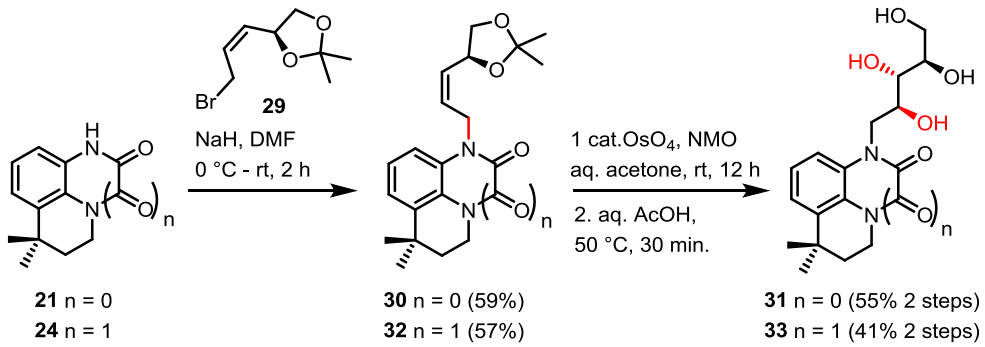
Scheme 2.2: Synthesis of building block for Hunanamycin analogues

showed amide carbonyl peaks at δ 163 and urea carbonyl at δ 151 ppm. Finally, the desired tricyclic product **28** was obtained in excellent yield (92%) through intramolecular Friedel-Crafts alkylation of **27** using $AlCl_3$ in chlorobenzene at room temperature. The ^{1}H NMR spectrum of **28** showed peak at δ 4.08 (t, *J* = 6.1 Hz, 2 H), 1.92 (t, *J* = 6.1 Hz, 2 H), which corresponds to the newly formed methylene after the cyclization reaction. It was further confirmed by HRMS, which showed a peak at 253.0946 corresponding to molecular formula

$C_{13}H_{14}N_2O_2Na$ $[M+Na]^+$. After the successful synthesis of these building blocks in gram scale, the next task was synthesis of analogues.

2.3.2.2 Synthesis of *N*-substituted sugar analogues

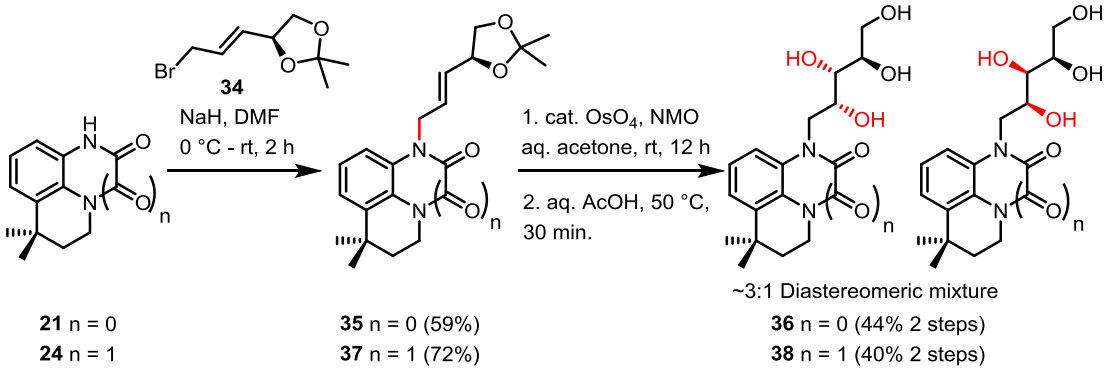
We have synthesized a series of different sugar analogues with these three scaffolds (**21**, **24** and **28**), to explore the SARs of Hunanamycin. To optimize the sugar chain length and the number of hydroxyl group tolerability, we systematically designed and synthesized tetraol, triol, diol and mono hydroxyl analogues. The results of these endeavours are depicted in schemes **2.3** to **2.7.** Synthesis of tetraol analogues started from *N*-alkylation of tricyclic benzimidazol-2-one **21** and tricyclic scaffold quinoxalinedione **24** and with the known *Z* bromide **29** by reacting with NaH (60% in mineral oil) in DMF at room temperature to produce **30** and **32** in moderate yields. The desired product **30** and **32** formed was indicated by stainning TLC in iodine as well as in $KMnO_4$. In case of compound **30** ^{1}H NMR showed peak at δ 5.77 - 5.63 (m, 2 H), corresponds to two internal olefin CH, whereas in case of **32** ^{1}H NMR showed two separate peak for olefin CH at δ 5.70 - 5.65 (m, 1 H) and δ 5.65 - 5.55 (m, 1 H) with all other characteristic peaks for tricyclic compound and olefin partner. Further, olefin was oxidized using Upjohn dihydroxylation (OsO_4, NMO) condition to afford diol intermediate in good yields the relative stereochemistry of the diol was assigned with the help of 'Kishi empirical rule' as explained in section 1. The product formed was characterized and confirmed with the help of ^{1}H, ^{13}C NMR and HRMS and was found in well agreement with the structure drawn in scheme **2.3**. We took forward this diol compound for



Scheme 2.3: Synthesis of desmethyl urea **31** and desmethyl **33** analogues of Hunanamycin A

deprotection of acetonide using aqueous acetic acid at 60 °C to furnish tetraol products **31** and **33** respectively in good overall yields (Scheme **2.3**). Structure of compound **31** was confirmed by ^{13}C NMR which showed four signals at 74.44, 74.41, 72.5, and 64.8

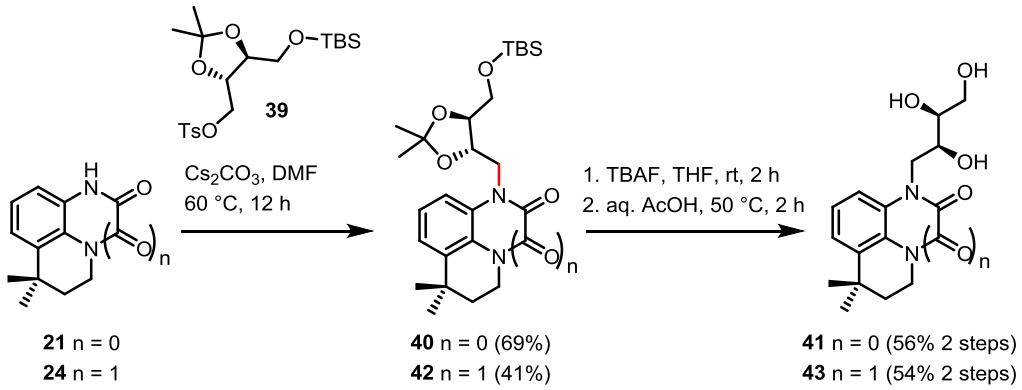
corresponding to the carbon attached to the hydroxyl group, it was further confirmed by HRMS value observed at 359.1570 for the molecular formula $C_{17}H_{24}N_2O_5Na$ $[M+Na]^+$. The product **33** also showed all the characteristic signals for tricyclic as well as sugar moiety in ^1H NMR, ^{13}C NMR, it was further confirmed by HRMS value observed at 387.1520 corresponding to molecular formula $C_{18}H_{24}N_2O_6Na$ $[M+Na]^+$. Also, we have synthesized *E* bromide **34**[24] and performed the same transformation as explained above to furnish tetrol product **36** and **38** in good overall yields. **A**s expected we observed poor diastereoselectivity (3:1) and these results are consistent with the literature report (Table **1.1** in section **1**). The ^1H, ^{13}C NMR spectrum was well in agreement with the structure drawn in scheme **2.4**.



Scheme 2.4: Systematic evaluation of sugar unit of Hunanamycin A: Tetraol derivative

Then, we moved to the synthesis of triol analogue of Hunanamycin, the synthesis which started from the *N* alkylation of compound **21** and known tosyl derivative **39** (prepared from diethyl L-tartrate)[25] in the presence of Cs_2CO_3 to furnish the product **40**, in 69 % yield. The formation of **40** was confirmed by appearance of signals in ^1H NMR spectrum at δ 0.88 (s, 9 H), 0.07 (s, 3 H), 0.03 (s, 3 H) corresponding to TBS group protons also, peak at δ 4.16 (dd, J = 3.7, 14.2 Hz, 1 H), 4.03 (dd, J = 6.4, 14.7 Hz, 1 H), corresponds to methylene flanked in nitrogen of the tricyclic ring and protected triol moiety. Similarly, the ^{13}C NMR also supported the formation of **40** with the appearance of additional two new negative signals in DEPT 135 at δ 62.8 for the oxygen attached CH_2 and at δ 43.6 corresponds to CH_2 flanked in nitrogen of the tricyclic ring and protected triol moiety. The HRMS analysis showed a peak at 483.2643 for the molecular formula $C_{25}H_{40}N_2O_4SiNa$ $[M+Na]^+$. The silyl group was cleaved using TBAF, and the acetonide protection was removed by heating in aq. AcOH to afford triol **41** in 56 % for two steps, as shown in Scheme **2.5**. The formation of **41** was primarily indicated by TLC which showed new polar spot at 0.2 R_f in 8% MeOH:DCM solvent system. The ^{13}C NMR also confirmed the formation of **41** with characteristic urea

carbonyl peaks at δ 155.4 ppm and signals at δ 73.2, 70.4, 64.3 ppm corresponds to the three carbons attached to hydroxyl group along with all the characteristics peaks for the tricyclic ring. It was further confirmed by HRMS, which showed a peak at 329.1469 corresponding to molecular formula $C_{16}H_{22}N_2O_4Na$ $[M+Na]^+$. In a similar way the synthesis of triol derivative

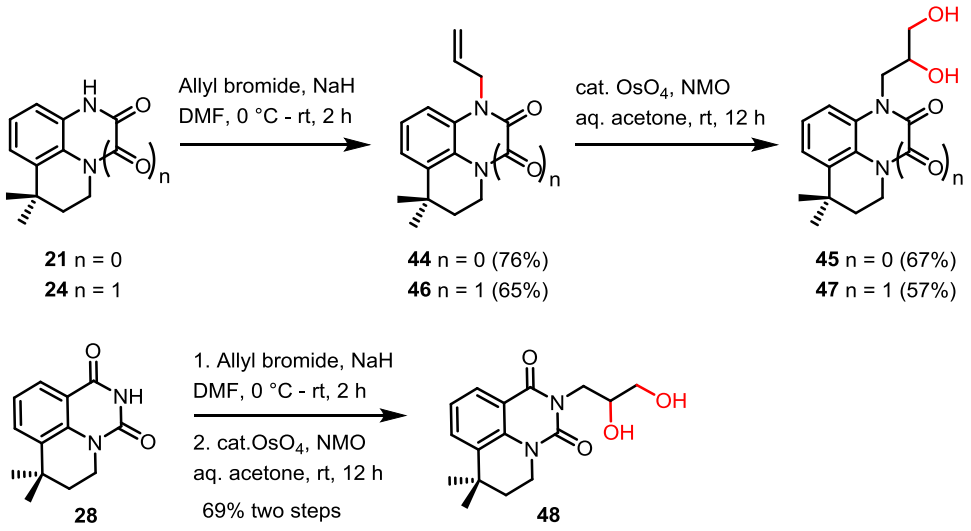


Scheme 2.5: Systematic evaluation of sugar unit of Hunanamycin A: Triol derivative

of tricyclic quinoxaline-2,3-dione compound **43** was achieved in 54 % overall yield starting from **24**. The formation of **43** was indicated by the presence of signals in ^{1}H NMR at δ 4.56 (dd, *J* = 8.6, 14.2 Hz, 1 H) and δ 4.37 (dd, *J* = 4.4, 14.2 Hz, 1 H) corresponds to the two diastereotopic protons attached to the nitrogen of the tricyclic ring and triol group along with the other characteristic signals for the tricyclic unit and triol moiety. The HRMS analysis also confirmed the formation of products. It was also observed that we did isolate small amounts of *O*-alkylated products and characterized in few cases. This can be due poor reactivity of electrophiles.

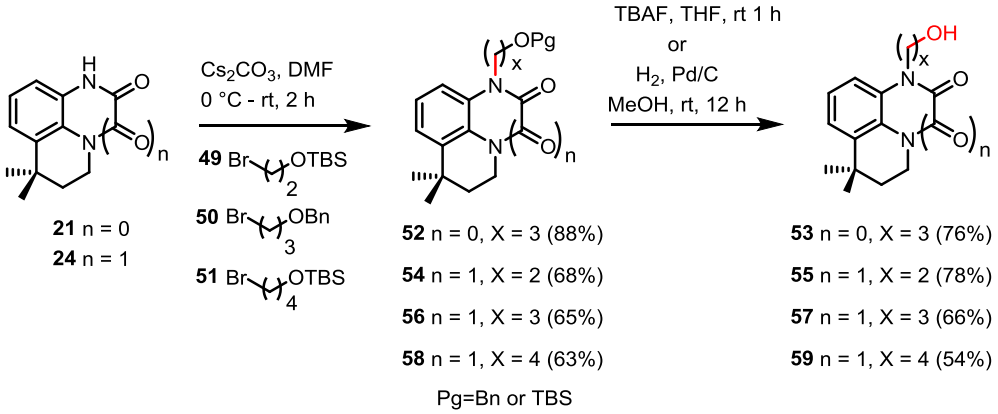
Further, we have planned to synthesize some analogues with less number of hydroxyl groups and shorter carbon chain compared to the natural product. Synthesis of analogues possessing the diol started with the reaction between the corresponding tricyclic core, allyl bromide and sodium hydride (60 % in mineral oil) to afford *N*- allylated product **44** (76 % yield) and **46** (65 % yield) respectively. The formation of *N*-allylated products was primarily indicated by staining TLC in Iodine as well as in $KMnO_4$. The product formed was further confirmed by ^{1}H NMR showed that typical terminal monosubstituted olefin pattern. Thus, *N*-allylated compounds under Upjohn dihydroxylation (OsO_4, NMO condition) furnish the target diol products **45** and **47** in moderate overall yields (57 % – 68 % yield). The formation of dihydroxylated products was primarily indicated by IR spectroscopy all the diol products showed a broad peak in between 3600 - 3400 cm^{-1} corresponding to a hydroxyl group. Further diol was confirmed by the disappearance of olefin signals and appearance of signals

for CH and CH_2 attached to hydroxyl group in ^1H NMR and ^{13}C NMR. It was again confirmed by HRMS for compound **45**, which showed a peak at 299.1366 corresponding to molecular formula $C_{15}H_{20}N_2O_3Na$ $[M+Na]^+$, with calculated value 299.1366. In case of **47** HRMS showed a peak at 327.1316 corresponding to molecular formula $C_{16}H_{20}N_2O_4Na$ $[M+Na]^+$, with calculated value 327.1315. Following the similar strategy, i.e. *N* allylation of **28** followed by dihydroxylation of the newly introduced olefin gave compound **48** in 69 % overall yields the desired product obtained was further confirmed by ^1H NMR, ^{13}C NMR clearly showed distinct signal at δ 163.1 and δ 151.5 ppm for the amide and urea carbonyl respectively, in addition it showed four methylene peaks at δ 70.6, 63.5, 43.9 and 39.6 ppm. The HRMS analysis showed a peak at 327.1311 for $C_{16}H_{20}N_2O_4Na$ $[M+Na]^+$ which is also in agreement with the structure (Scheme **2.6**). Unlike previous compounds (tetraols and triols), present compounds were prepared in racemic form. Based on the biological activity, they can be prepared in enantiomerically pure form.



Scheme 2.6: Systematic evaluation of sugar unit of Hunanamycin A: Diol derivative

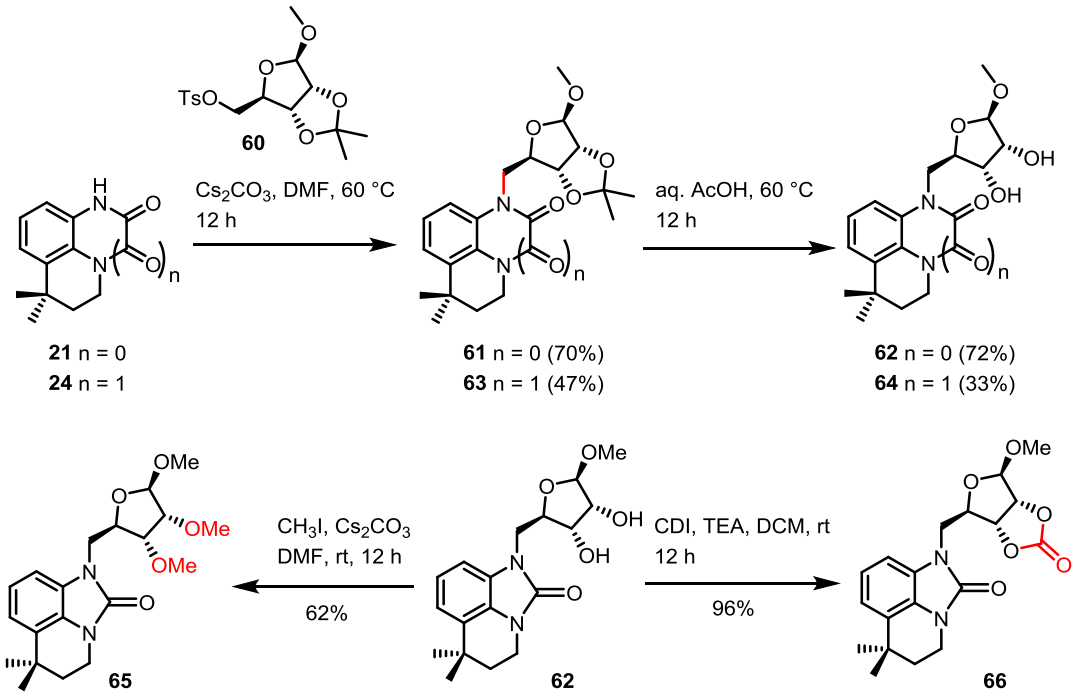
Finally, synthesis of mono-hydroxyl tricyclic derivatives (**53**, **55**, **57** and **59**) was accomplished *via* *N*-alkylation of the tricyclic core using *O*- protected alkyl bromides of varying chain length (**49**, **50** and **51**) in the presence of Cs_2CO_3 and DMF as solvent to afford **52**, **54**, **56**, and **58** in good yields. Finally the protecting group was then removed, in case of TBS protection TBAF was used whereas, benzyl group was removed using H_2, Pd/C condition to afford the free hydroxyl products in 54-78% yields. All the compounds were purified by silica gel column chromatography, the products were fully characterized with the help of spectroscopic techniques (^1H, ^{13}C NMR, IR, and HRMS) and it was found to be in good agreement with the structure as drawn in scheme **2.7**.



Scheme 2.7: Systematic evaluation of Hunanamycin A: Monohydroxyl derivative

2.3.2.3: Synthesis of *N*-alkylated ribofuranose analogues

To reduce the flexibility and understand the effect of cyclic *vs* acyclic we have synthesized ribofuranose derivative possessing the rigid cyclic structure. The synthesis began with the Cs_2CO_3 mediated *N*- alkylation of **21** and **24** with protected ribofuranose derivative **60**[26] to obtain compound **61** and **63** respectively in good yield. In case of alkylation of compound **24** was have isolated *N*-alkylated product in major amount and the *O*-alkylated product in minor amount. Then acetonide was deprotected using previously optimized condition to afford **62** (72 %) and **64** (33 %) ribofuranose analogues of Hunanamycin A (Scheme **2.8**). The formation of desired product **62** was confirmed by ^{1}H NMR showed a peak at δ 7.02 (s, 3H) corresponds to three aromatic protons, the hydrogen attached to the anomeric carbon of the ribofuranose appears at δ 4.67 (s, 1 H). The ^{13}C NMR showed a peak at δ 155.3 corresponds to urea carbonyl; DEPT 135 clearly showed a peak at δ 122.6, 117.7, 110.2 for aromatic CH, peak at δ 108.8 for anomeric carbon and signal at δ 82.0, 76.2, 74.1 ppm corresponds to the three CH from the ribofuranose ring. Finally, desired product formed was confirmed by HRMS showed a peak at 349.1755 corresponding tomolecular formula $C_{18}H_{25}N_2O_5$ $[M+H]^+$ with calculated value 349.1758. Similarly, compound **64** was fully characterized with the help of spectroscopic techniques (^{1}H, ^{13}C NMR, IR, and HRMS). Further, to study the importance of free hydroxyl group on potency, the hydroxyl group on compound **62** was masked as methyl ether **65** analogue using Cs_2CO_3 and methyl iodide formation of the desired product confirmed by ^{1}H NMR displayed two additional peaks for OCH_3 at δ 3.47 and δ 3.44. Next compound **62** on treatment of CDI, TEA in DCM yielded its carbonate derivative **66** in excellent yield 96 % the product formed was confirmed by ^{1}H NMR showed characteristic peak at δ 5.21 (s, 1H) for the proton attached to anomeric carbon,



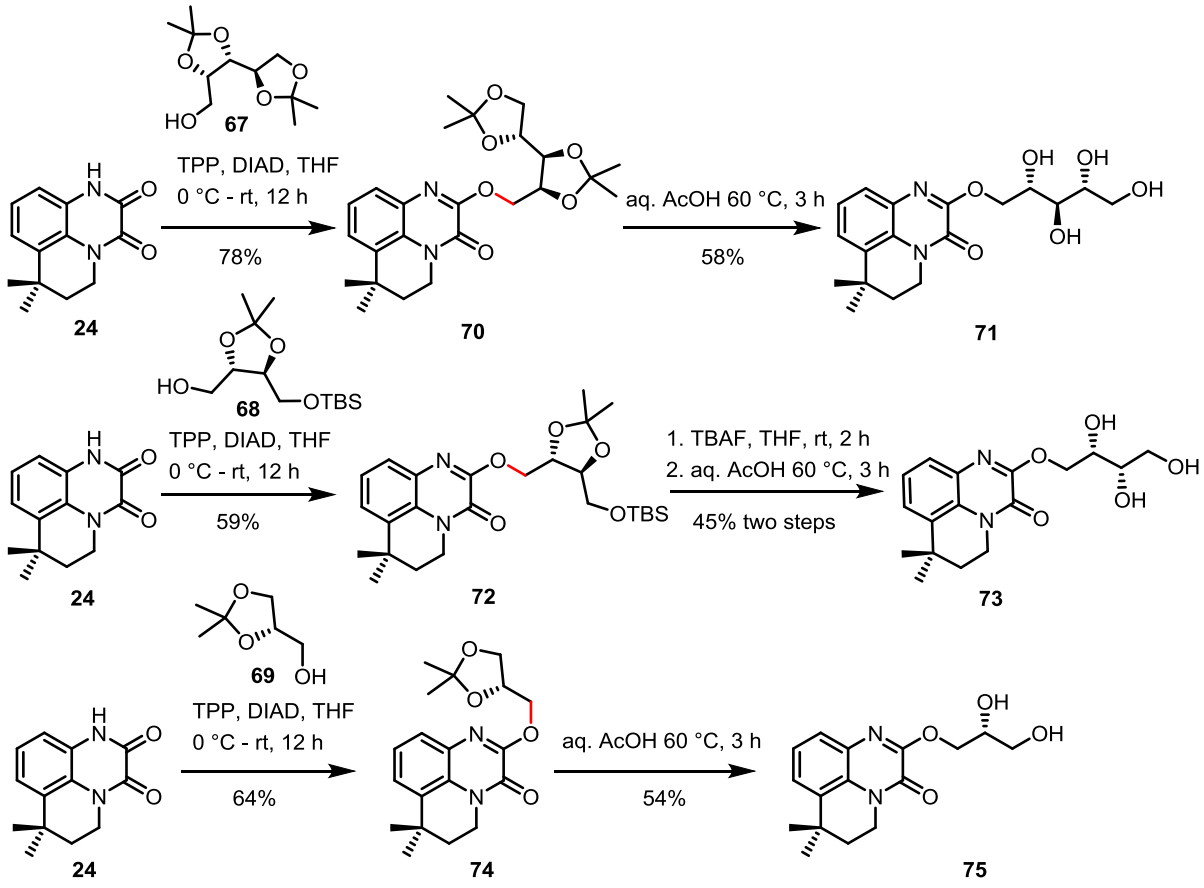
Scheme 2.8: Optimizing the effect of rigidity and free hydroxyl group on potency

signal at δ 5.48 (d, *J* = 6.6 Hz, 1 H), 5.17 (d, *J* = 6.8 Hz, 1 H) corresponds to proton attached to C2 and C3 carbon, signal at δ 4.28 (dd, *J* = 11.1, 14.5 Hz, 1 H) corresponds to proton attached to C4 carbon, finally very clean two doublets of doublet appear at δ 4.65 (dd, *J* = 4.0, 11.1 Hz, 1 H) and δ 3.84 (dd, *J* = 4.2, 14.7 Hz, 1 H), for the two protons attached to C5 carbon of the ribofuranose ring. The ^{13}C NMR showed signal at δ 153.4 and δ 153.2 for the amide carbonyl and newly introduced carbonate group respectively. Further, it was supported by HRMS showed a peak at 375.1544 molecular formula $C_{19}H_{23}N_2O_6$ $[M+H]^+$.

2.3.2.4 Synthesis of *O*-alkylated sugar analogues

After synthesis of *N*-alkylated Hunanamycin analogues, we turned our attention to the construction of designed *O*- alkylated Hunanamycin A analogues using Mitsunobu reaction as a key step. Tricyclic scaffold on reaction with sugar unit **67**,[27] **68**[25]and *S*-Solketal **69** under TPP, DIAD in THF furnished products **70**, **72**, and **74** (in 78, 59, and 64 % yield respectively) which after the removal of protecting group (Silyl group deprotected using TBAF, whereas acetonide by aq. AcOH at 60 °C) yielded compound **71**, **73**, and **75** respectively in moderate yield. The final products were undoubtedly confirmed by all the spectroscopic techniques (^{1}H, ^{13}C NMR, IR, HRMS). The IR spectrum of all the final compounds showed a broad peak at 3600-3400 cm^{-1} corresponding to a hydroxyl group. ^{1}H NMR showed typical peaks of two

doublets of doublets between δ 4.70 – 4.40 which corresponds to methylene group attached in between tricyclic ring and sugar moiety. Additionally, it was further confirmed by the ^{13}C NMR that showed typical peak for CH_2 attached to the oxygen of the tricyclic ring and sugar moiety at δ 64.5, 64.4 and 62.7 correspond to product **71**, **73** and **75** respectively. The possible reason for exclusive formation of *O*-alkylated product under Mitsunobu reaction was



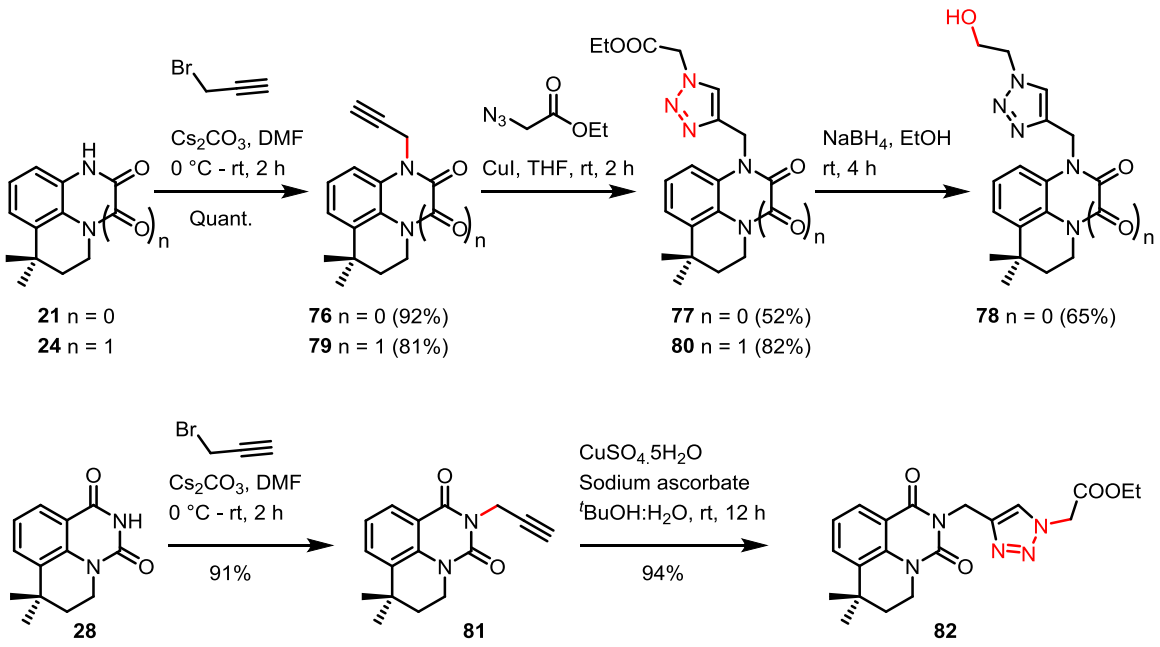
Scheme 2.9: Synthesis of *O*-alkylation derivative

explained in section **1**. During the acetonide deprotection, we have observed the removal of the sugar moiety to afford tricyclic unit in minor amount as a side product; this suggests that *O*- alkylated analogues are less stable in acidic medium (Scheme **2.9**).

2.3.2.5 Replacement of sugar unit with polar triazole/tetrazole derivative

As per the plan ribose sugar on the tricyclic ring was replaced with different azole unit. Triazole and tetrazoles are important five-membered heterocycles, can bind to a different enzymes and receptors and thus display a broad spectrum of bioactivities.[28-29] Another, interesting fact that the easy access for the synthesis of azole ring by click or cycloaddition

reaction. Click reaction, is the powerful tool in the organic chemistry for rapid synthesis of diverse library of heterocyclic scaffolds.[30-31] Here, we planned to use a triazole / tetrazole as a replacement for sugar functionality. Towards this effort, compound **21** was alkylated with propargyl bromide to afford **76** in quantitative yield and performed the click reaction with ethyl azidoacetate in the presence of CuI in THF to furnish triazole derivative **77** in good yield (52 %). The formation of **77** was confirmed by ^{1}H NMR showed signal at δ 7.73 (s, 1 H) corresponds to CH of the azole ring, two singlet at δ 5.16 and 5.07 for the methylene group attached to nitrogen of the azole ring and methylene group flanked between nitrogen of the tricycle and azole ring. The ester moiety was then reduced under the $NaBH_4$/EtOH condition to afford compound **78** in 65 % yields. The formation of the desired product **78** was primarily confirmed by IR spectroscopy the ester carbonyl peak (1750 cm^{-1}) was absent whereas, broad peak at 3394 cm^{-1} was found corresponding to hydroxyl group. Further the desired product was fully characterized by other spectroscopic technique (^{1}H, ^{13}C NMR, HRMS) and found to be in agreement with the structure drawn in scheme **2.10**.

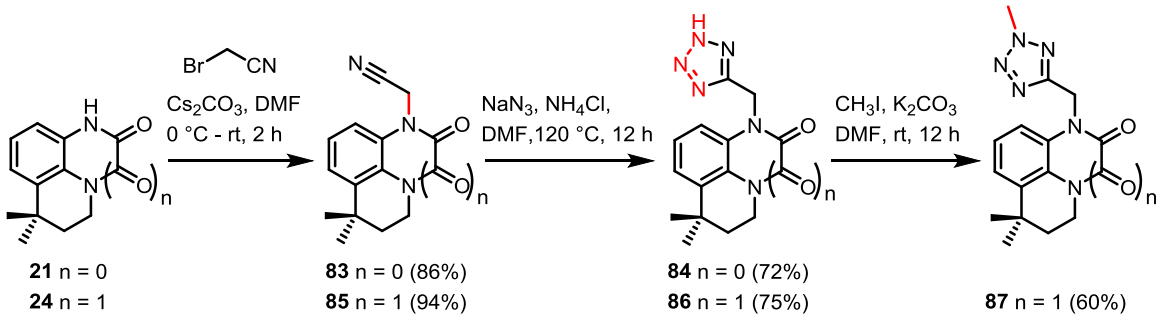


Scheme 2.10: Synthesis of heterocyclic derivative of Hunanamycin A

Following the same reaction sequence, we also have achieved the synthesis of compounds **80** and **82** in excellent overall yield from starting compound **24** and **28** respectively. The formation of **80** was confirmed by appearance of signals in ^{1}H NMR spectrum at δ 7.90 (s, 1 H) corresponding to proton from triazole ring additionally two singlet at δ 5.48 and δ 5.12 for the methylene attached to nitrogen of the triazole ring and methylene flanked between

tricyclic ring and azole ring. ^{13}C NMR displayed peak for ester at δ 166 ppm and other characteristic peaks for tricyclic ring and azole ring. It was further confirmed by HRMS, which showed a peak at 420.1636 corresponding to molecular formula $C_{20}H_{23}N_5O_4Na$ $[M+Na]^+$, with calculated value 420.1642. Similarly the product **82** was confirmed by ^1H NMR, ^{13}C NMR and HRMS (Scheme **2.10**).

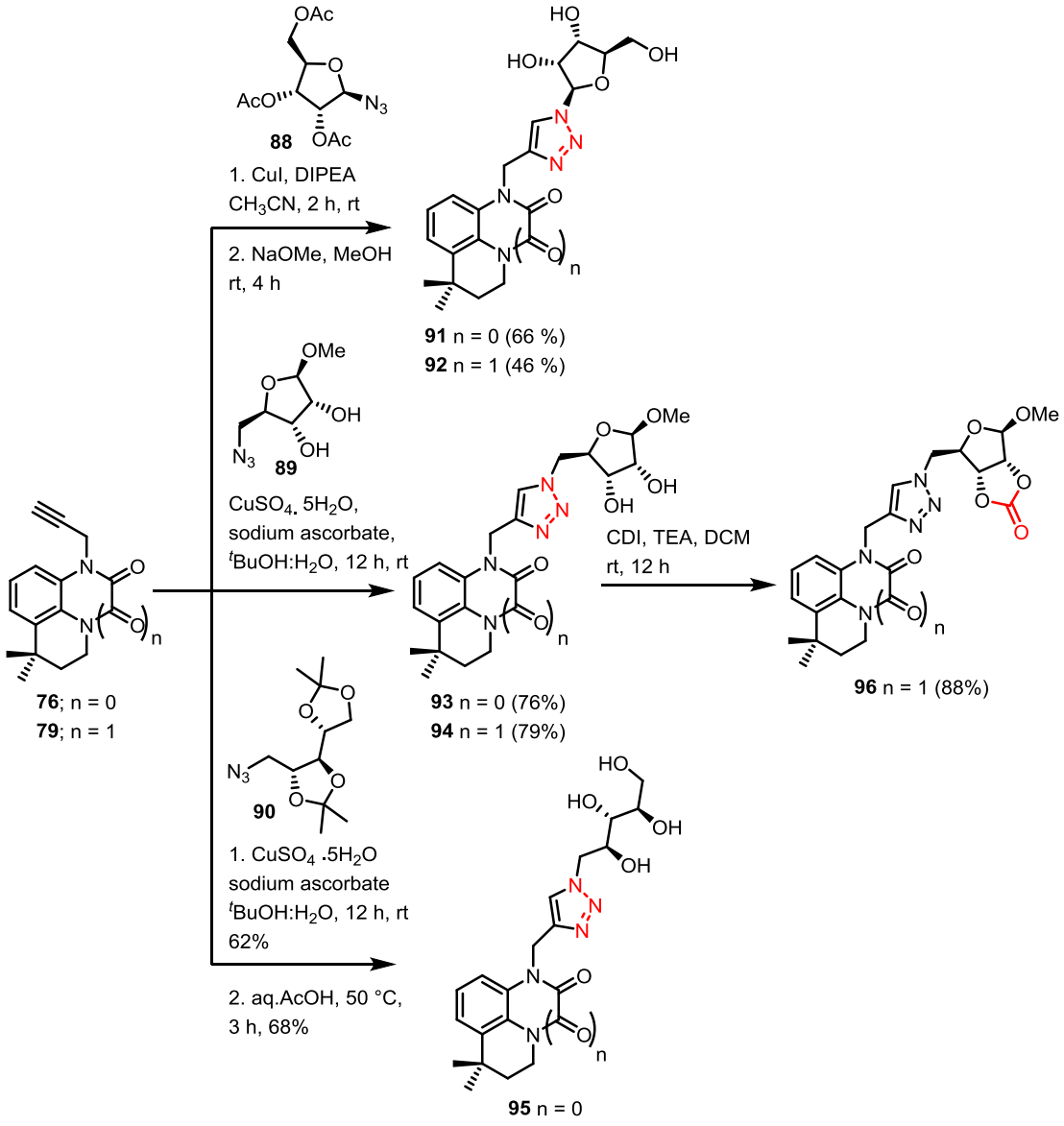
Tetrazole derivative **84** and **86** were synthesized in excellent yield by performing cycloaddition reaction of nitrile intermediate **83** and **85** with sodium azide in DMF by heating at 120 °C for 12 h. The product **84** obtained as white powdery solid, ^1H NMR showed signal at δ 5.47 (s, 2 H) corresponds to CH_2 attached between tetrazole and tricyclic ring, two mutually coupled triplets at δ 3.94 (t, *J* = 5.9 Hz, 2 H), δ 1.90 (t, *J* = 5.9 Hz, 2 H) of the tricyclic ring, and peak at δ 1.33 (s, 6 H) for the germinal dimethyl of the tricyclic ring. The assigned structure further justified by HRMS, which showed a peak at 285.1468 for the molecular formula $C_{14}H_{17}N_6O$ $[M+H]^+$, with calculated value 285.1463. Similarly the structure of product **86** was confirmed by ^1H NMR and ^{13}C NMR spectroscopy and well supported by HRMS. Further, the compound **86** was *N*-methylated using K_2CO_3, MeI in DMF to afford product 2-methyl derivative **87** as white solid powder in moderate yield along with we isolated its minor 1-methyl regioisomer (scheme **2.11**). The product **87** obtained was confirmed by comparing the ^1H NMR chemical shift of N-CH_3 with the literature reports.[32-33] The methyl attached to the nitrogen of tetrazole **87** resonate at δ 4.29 (s, 3 H) and in ^{13}C NMR this newly introduced methyl group appears at δ 39.6, further it is well supported by HRMS which showed a peak at 349.1379 for molecular formula $C_{16}H_{18}O_2N_6Na$ $[M+Na]^+$, with calculated value 349.1383.



Scheme 2.11: Synthesis tetrazole analogues of Hunanamycin A

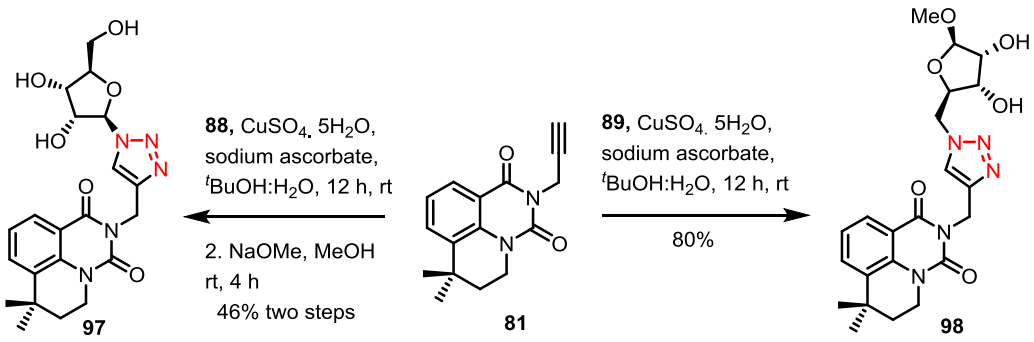
Furthermore, we have utilized triazole ring as a linker in between tricyclic moiety (**76,** and **79**) and sugar part (**88,**[34]**89**[35] **and 90**) using click reaction as the key step. The click reaction of tricyclic alkyne derivative and protected azidosugar performed to obtain click adduct

which was cleanly confirmed by ^{1}H NMR showed typical singlet observed for one proton in the range of δ 7.9 to δ 8.3 ppm for the hydrogen attached to triazole ring. Finally, removal of protecting group using suitable condition furnished compound **91-95** in moderate yield (Scheme **2.12**). Formation of the product was extensively confirmed by all the spectroscopic technique (IR, ^{1}H NMR, ^{13}C NMR, HRMS) and found well in agreements with the structure drawn in scheme **2.12,** the purity was determined by HPLC analysis. Further diol group in compound **94** was masked as carbonate using CDI, TEA in DCM to afford **96** in excellent yield (88%). The ^{1}H NMR spectrum of product **96** displayed signal at δ 8.02 (s, 1H) formed carbonate along with all the other characteristic peaks for the tricyclic ring and ribofuranose



Scheme 2.12: Synthesis of mixed analogues (Click reaction with C1and C5 azide sugar)

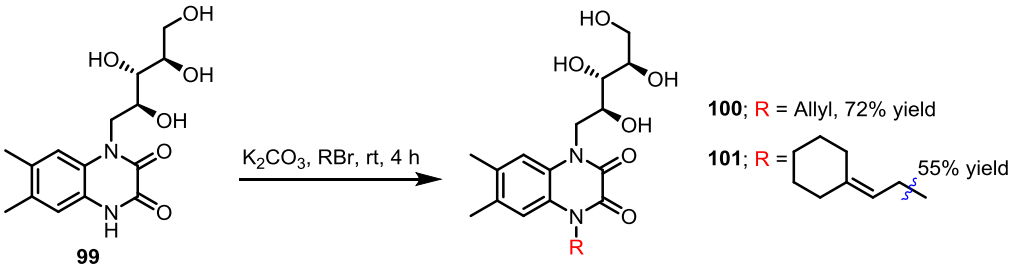
ring. Further, it was confirmed by HRMS which showed a peak at 506.1642 corresponding to molecular formula $C_{23}H_{25}N_5O_6Na$ $[M+Na]^+$, with calculated value 506.1646. As discussed above we also have prepared compound **97** and **98** by following the similar steps as mentioned in the scheme **2.12**. The formation of **97** and **98** were clearly evident in its ^1H NMR, where δ 8.20 (s, 1H), δ 7.92 (s, 1H) corresponds to the proton attached to triazole ring respectively. Further the click products have been confirmed by number of signals in ^{13}C NMR as well (Scheme **2.13**).



Scheme 2.13: Synthesis of mixed analogues of benzoyleneurea **97** and **98**

2.3.2.6 Removal of C ring in Hunanamycin A

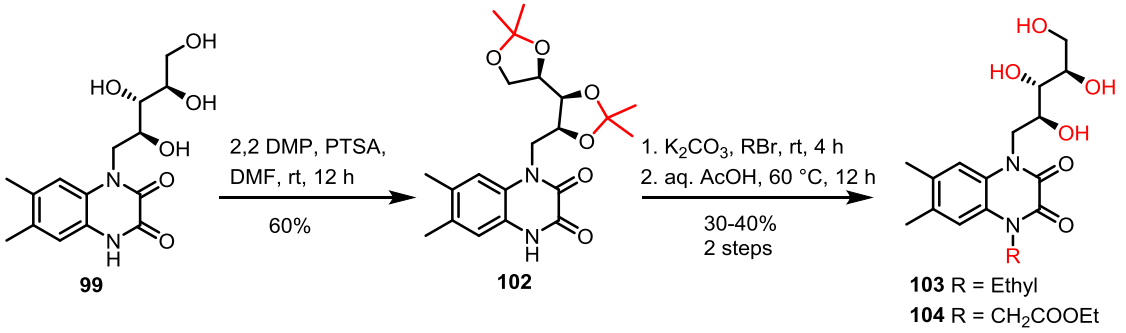
As the final part of our modification and to broaden the structure-activity relationship (SAR) study, we have removed C ring from Hunanamycin tricyclic scaffold. For this purpose, we have used the one of the advanced intermediate **99** from our second generation synthesis of Hunanamycin A. The successful *N*-alkylation of compound **99** (it was prepared from readily available riboflavin, see Section **1**; Scheme **1.9** for details) was achieved using K_2CO_3, alkyl halide in DMF to obtain compound **100** and **101** (Scheme **2.14**). The product **100** formed was primarily confirmed by TLC staining in iodine and $KMnO_4$, further ^1H NMR showed typical terminal olefin pattern, δ 5.92 (dt, *J* = 5.4, 11.0 Hz, 1 H) at δ 5.19 - 5.12 (m, 2 H). This was further confirmed by HRMS which showed a peak at 365.1704 corresponding to



Scheme 2.14: Synthesis of Hunanamycin analogues without C ring

molecular formula $C_{18}H_{25}N_2O_6$ $[M+H]^+$, with calculated value 365.1707. Similarly, the structure of the compound **101** was confirmed by ^1H NMR, ^{13}C NMR and HRMS. Further, in case of some other alkyl bromide, we failed to obtain *N*-alkylated product in good yields.

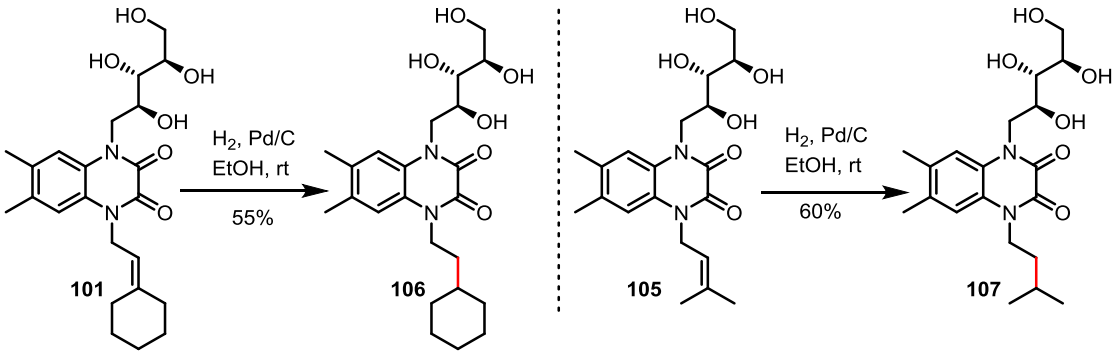
So we decided to protect hydroxyl group as acetonide then alkylation followed by deprotection to obtain the final alkylated product. To put this plan into action, the free hydroxyl group was protected as acetonide. This was achieved by using 2,2-dimethoxypropane, cat. *p*TSA in DMF to afford the product **102** in good yield (60% yield). The product formed was confirmed by mass spectroscopy and forwarded further without extensive characterization. Compound **102** was then treated with K_2CO_3 and different alkylating reagents ethyl bromide, ethyl bromoacetate, to produce the corresponding *N*-alkylated compounds. Finally, acetonide protections were removed using aq. AcOH at 60 °C to furnish the final free sugar unit **103,** and **104** in 30% - 40% yield for two steps (Scheme **2.15**). Desired product **103** formed was confirmed by ^1H NMR showed a peak at δ 4.20 - 4.11 (m, 2 H) corresponds to CH_2, and the signal at δ 1.22 (t, J = 6.8 Hz, 3 H) corresponds to CH_3 for the newly introduced ethyl group. Further ^{13}C NMR in DEPT 135 showed a negative peak at δ 37.3 corresponds to CH_2, and the positive signal at δ 12.2 corresponds to CH_3 from the ethyl group. The product **104** was primarily confirmed by the appearance of ester peak at 1740 cm^{-1} in IR spectrum. Further, ^1H NMR spectrum showed typical ethyl ester peaks. Finally, it was confirmed by HRMS which showed a peak at 433.1577 corresponding to molecular formula $C_{19}H_{26}N_2O_8Na$ $[M+Na]^+$ (Scheme **2.15**).



Scheme 2.15: Three steps synthesis of Hunanamycin A analogues without C ring.

The intermediates **101** and **105** containing olefin moiety was hydrogenated using 10% palladium on carbon and hydrogen to afford its saturated analogues **106** and **107** respectively (Scheme **2.16**). The product formed was primarily confirmed by the disappearance of tri-substituted olefin CH in ^1H NMR and ^{13}C NMR analysis displayed negative peak in DEPT 135 at δ 33.9 and δ 35.3 for the newly generated methylene group in product **106** and **107**

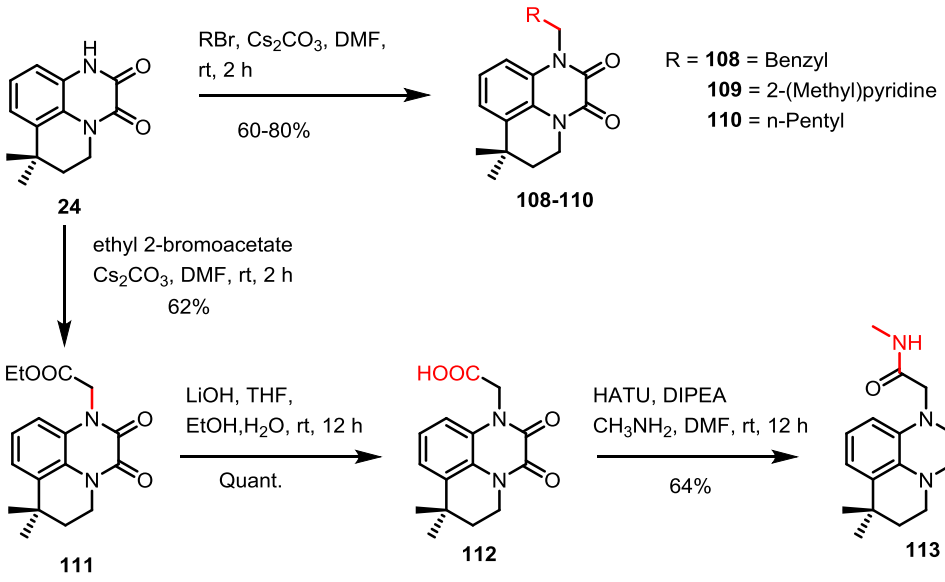
respectively. Both the structures of compounds **106** and **107** were further confirmed by HRMS analysis.



Scheme 2.16: Hydrogenation of olefin intermediate

2.3.2.7 Synthesis of amide/aliphatic derivative of Hunanamycin A

Due to our interest and to understand SAR in detail, we synthesized some of the miscellaneous analogues of the Hunanamycin A. We have prepared benzyl **108**, 2-(methyl)pyridine **109**, and n-pentyl **110** derivatives by *N*-alkylation of the tricyclic scaffolds **24** using Cs_2CO_3 and corresponding alkyl/aryl bromide in DMF. The entire products were formed was confirmed by IR, NMR, and HRMS and it was found well in agreement with the structures drawn (Scheme **2.17**). Next, ribose sugar we replaced with the aliphatic amide our synthesis started with the *N*-alkylation of the tricyclic compound **24** with ethyl bromoacetate in the presence of Cs_2CO_3 to afford ester derivative **111** in good yield (62 %). The newly for-



Scheme 2.17: Hunanamycin A analogs withN-alkyl side chains moiety

-med nonpolar spot on TLC showed PMA charring, in addition, IR showed a characteristic signal at 1747 cm^{-1} corresponds to the ester group. The structure of **111** was further confirmed by ^{1}H, ^{13}C NMR and HRMS. The ester group was hydrolyzed in LiOH, THF/H_2O condition to afford **112** as colourless solid in quantitative yield which was converted to corresponding *N*-methyl amide derivative **113** using standard amide coupling condition (HATU, DIPEA in DMF). The formation of amide compound **113** was confirmed by ^{1}H NMR shows a signal at δ 2.73 (d, J = 4.8 Hz, 3 H) for methyl attached to the nitrogen of amide, ^{13}C NMR showed a signal at δ 167.0 corresponds to *N*-methyl amide carbonyl along with the other characteristic peaks for tricyclic ring

2.4 Biological studies

2.4.1 *In vitro* antibacterial activity and structure activity relationships (SARs)

After generating a library of Hunanamycin analogues with various structural features, we carried out a biological evaluation of all the synthesized compounds and studied their structure-activity relationships. The MICs against *salmonella enterica* were determined using the Promega bac titer-glo microbial cell viability assay and ciprofloxacin was used as the standard. The summary of the results obtained for all the synthesized compounds has been captured in Figure **2.7**. The conclusions of these studies of the effect of structural modifications in the four portions, *i.e.* sugar modification, B ring modification, removal of the methyl group on the aromatic ring, and removal of C ring (Figure **2.5**). As can be seen from these results, the aromatic methyl (ring A) doesn't contribute much to potency, so can be replaced with hydrogen which is tolerated well (see, e.g., the MIC values for **13**$^{\#}$ *vs* **47** in Table 1). Removal of the cyclohexyl ring compound **99** or replacing with ethyl **103** led to complete loss of activity, whereas allyl **100**, cyclohexyl **101** and prenyl **105** displayed moderate activity. This shows cyclohexyl, i.e. C ring counterpart allowed the molecule to retain its potency (see the IC_{50} values for Hunanamycin A *vs* **99** in Figure **2.6**). Quinoxaline-2,3-dione moiety contributes significantly towards the antibacterial activity of Hunanamycin A, most of the tricyclic benzimidazol-2-one analogues showed inferior activity with respects to corresponding quinoxaline-2,3-dione analogues (see, e.g., the MIC values **47** *vs* **45** or **57** *vs* **53** Figure **2.6**). To our surprise, in some of the cases the benzimidazol-2-one analogues displayed better activity than that of quinoxaline-2,3-dione analogues (see, e.g., MIC value for **33** *vs* **31** and **64** *vs* **62**) whereas, all the benzoyleneurea analogues displayed moderate

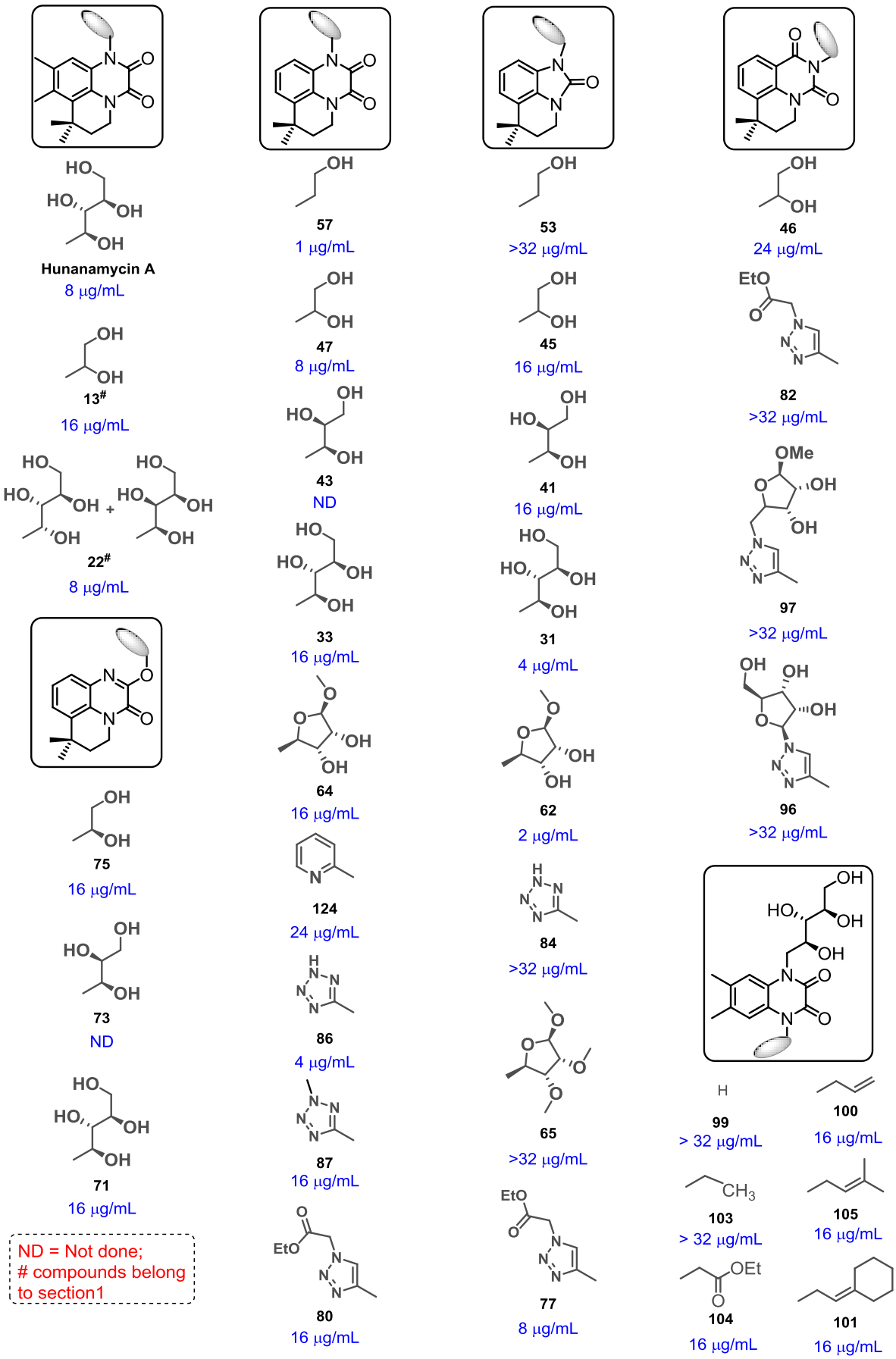


Figure 2.6. *In-vitro* screening results of selected compound against *S. enterica*.

activity (compound no **46, 82**, **96**, and **97**). Though *O*-ribosylated analogues of quinoxaline-2,3-dione exhibited moderate antibacterial activity but these are not good leads to take forward owing to the fact that *O*-ribosylated analogues are not stable in acidic condition. Reducing the flexibility of acyclic sugar to making it rigid cyclic structure doesn't observe the improvement in activity see, e.g. **64**, **66** and **65**. The synthesis of hybrid compound (**91 - 98**) containing azole as well as sugar moieties did not prove to be very useful as there is a four-fold decrease in activity compared to Hunanamycin A. These results clearly indicate that the bulkier substitution on tricyclic core is not fitting to the active site of enzyme. The tetraol **33** and diol **47** analogues of the quinoxaline-2,3-dione displayed moderate activity. Our strategy of decreasing the number of hydroxyl group of the sugar unit had been proven beneficial; compound **57** is the most active compound of the series displayed eight-fold more potency than the parent natural product. Three carbon chain bearing the hydroxyl group at the terminal end is optimum for retaining the potency (see, e.g., the MIC values **53**, **55** and **59** in Table Figure **2.6**). Whereas, simple aliphatic long chain/aromatic (**108**-**110**) and the amide (**113**) substitution in place of sugar was found to be completely inactive. More surprisingly compound **86** where ribose sugar replaced by tetrazole moiety is active whereas its *N*-methylated counterpart is fourfold less potent this proves the role of acidic *NH* for the desired activity. (For head to head comparison see Figure **2.6**).

2.4.2 *In vitro* pharmacokinetics (PK) studies

Identification and optimization of the properties of a lead compound in the early stage of the drug discovery process and they are of crucial. A successful drug-lead compound must be potent, selective to the biological target; good stability and pharmacokinetic properties.[36] Thus, upon completion of *in vitro* testing of antibacterial activities and clear understanding of the SAR, five best compounds of the series and Hunanamycin A were selected for further profiling. Specifically, solubility across the pH range, stability in plasma and liver microsomes, and plasma protein binding (PPB) were evaluated. Solubility is an essential parameter in drug discovery, and poor solubility can lead to less absorption of drugs from the gastrointestinal tract. In the solubility assays, a solution of the compound is added to buffers of varying pH and then centrifuged. The supernatant solution is subjected to HPLC analysis to determine the solubility at a particular pH. Hunamaycin A and selected analogues possess a perfect balance between the hydrophilic and hydrophobic groups, hence it showed good solubility at across all the pH range tested (Table **2.1**).

S. No	Compound	Solubility in μM				
		pH 1.2	pH 2.2	pH 4.5	pH 7.4	pH 10.4
1	Hunanamycin A	50.78	50.39	50.04	49.42	49.28
2	31	48.16	47.99	47.76	47.40	47.63
3	62	41.25	48.35	48.75	48.55	48.65
4	57	51.21	50.84	50.11	50.14	50.17
5	86	51.83	51.24	51.53	51.84	51.83

Table 2.1 *In-vitro* solubility of Hunanamycin A and selected analogues.

Degradation of a compound by plasma enzymes can potentially alter the bioavailability of the active compound, hence it is essential to have compound with better plasma stability.[37] All the compounds displayed good plasma stability; compound **57** (82.41% plasma stability in human) is relatively less stable compared to Hunanamycin A (Table **2.2**). Majority of marketed compounds are cleared by hepatic CYP-mediated metabolism. Several enzymes present in the body biochemically modify the drug. This process called drug metabolism introduces polar functionalities into the drug, and the hydrophilic metabolites are quickly cleared from the body. The liver is the primary site of metabolism and is rich in metabolising enzymes such as cytochrome P450. Drugs get absorbed from the gut into the blood and go to the liver before reaching the systemic circulation. The rate of metabolism is a critical factor in determining the bioavailability, half-life and dosage of the drug. In laboratory assays, liver microsomes which are rich in cytochrome P450 are used to determine the metabolic stability.[38-39] The metabolic stability of selected active compounds was evaluated using rodent and human liver microsomes after 30 min incubation at 1μM concentration. Therefore, metabolic stability is vital for determining the efficacy of the parent compound. The natural product Hunanamycin, as well as other compounds, does not have very reactive or oxidizable functional groups. Hence these compounds are stable and not prone for metabolism in human and mouse liver microsomes.All the selected compounds displayed excellent metabolic stability in human as well as mouse liver microsome. The compound **62** exhibited lower metabolic stability i.e. 74.3% and 90% in mouse and human liver microsomes respectively this may be due to the fact that ribofuranose ring present in **62** is prone for metabolism (Table **2.2**). Compounds with higher metabolic stability are expected to have higher half-life, which can translate into greater efficacy *in vivo*.

S. No	Compound	% Plasma stability in human	% Metabolic stability in liver microsomes (After 30 min.)	
			Mouse	Human
1	Hunanamycin	100.0	99.60	100.0
2	31	99.89	96.40	100.0
3	62	98.00	74.30	90.0
4	57	82.41	96.10	100.0
5	86	99.94	96.10	98.9

Table 2.2 *In-vitro* Data of Hunanamycin A and selected analogues.

Next we studied the plasma protein binding for Hunanamycin A and selected compounds. The compound **86** structurally different analogue of Hunanamycin A containing tetrazole moiety displayed higher plasma protein binding compared to other analogues. High PPB is associated with a lower clearance as well as the low concentration of drug fraction available for desired pharmacologic effects hence it is desirable to have a moderate PPB. All the selected compound except **86** are displayed moderate plasma protein binding (Table **2.3**).

Sr. No	Compound	% Human PPB
1	Hunanamycin	58.84
2	31	42.16
3	62	66.18
4	57	61.11
5	86	97.95

Table 2.3 % plasma protein binding of Hunanamycin A and selected compounds.

Based on structure activity relationships (SAR) we arrived at following conclusions.

1. Tricyclic ring is necessary for the activity.
2. Bulkier substitution on tricyclic ring is not favorable and three carbon chain bearing the hydroxyl group at terminal end is optimum.
3. Methyl group on aromatic ring does not contribute much towards the potency
4. Quinoxaline-2,3-dione moiety is well tolerated than the benzimidazol-2-one.

After studying all the parameters like structural simplicity, potency and *in vitro* PK profile we selected compound **57** as the optimized lead for further *in-vivo* study (Figure **2.7**)

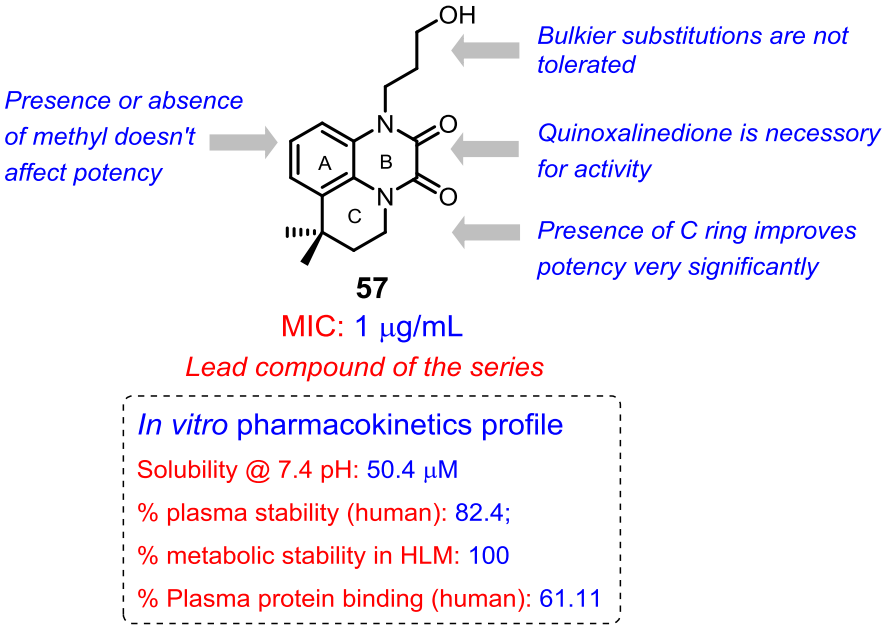
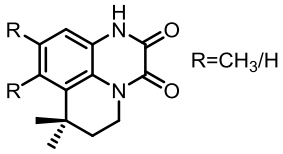


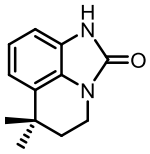
Figure 2.7. Optimized lead compound **57** from the series.

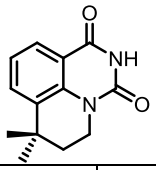
2.5 Conclusion

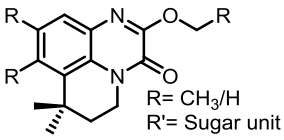
The impressive structural and biological properties of Hunanamycin A inspired us to develop new synthetic routes and synthesize diverse analogues of this natural product towards the identification of a novel riboflavin synthase inhibitor. We have synthesized > 70 close analogues of Hunanamycin A and screened against *salmonella enterica*. In collaboration with Prof J. B. MacMillan, we have established the mechanism of action of Hunanamycin A. Biological evaluation of synthesized compounds led to the identification of five potent riboflavin synthase inhibitor and established valuable structure-activity relationships (SARs). These investigations led to the identification of compound **57** as a lead candidate for further *in-vivo* studies by virtue of its potency, promising chemical and pharmacokinetic profiles.

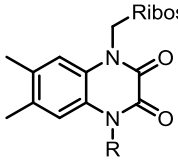
2.6 MIC values of all the compounds synthesized

	MIC value
Quinoxalinedione series	
	
Hunanamycin A[#]	8 µg/mL
13[#]	>16 µg/ mL
22[#]	8 µg/mL
33	>16 µg/ mL
38	>16 µg/ mL
43	ND
47	8 µg/mL
55	24 µg/mL
57	1 µg/ml
59	32 µg/mL
64	16 µg/ mL
80	16 µg/ mL
86	4 µg/mL
87	16 µg/mL
92	>32 µg/mL
94	>32 µg/mL
98	>32 µg/mL
108	>32µg/mL
109	24 µg/mL
110	>32 µg/mL
113	24 µg/mL

Compound No	MIC value
Benzimidazol-2-one series	
	
31	4 µg/mL
36	ND
41	>16 µg/ mL
45	>16 µg/ mL
53	>32 µg/mL
62	2 µg/mL
65	>32 µg/mL
66	>32 µg/mL
77	8 µg/mL
78	>16 µg/ mL
84	>32 µg/mL
91	>32 µg/mL
93	>32 µg/mL
95	>32 µg/mL

Benzoyleneurea series	
	
48	24 µg/mL
82	>32 µg/mL
96	>32 µg/mL
97	>32 µg/mL

***O*- alkylation of quinoxalinedione**	
	
11[#]	8 µg/mL
71	16 µg/mL
73	ND
75	>16 µg/ mL

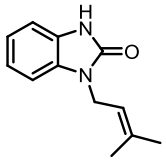
Without C ring series	
	
99	>32 µg/mL
100	16 µg/mL
101	>32 µg/mL
103	>32 µg/mL
104	16 µg/mL
105	16 µg/mL

Compound from section 1

ND-Not done; Standard compound: Ciprofloxacin

2.7 Experimental procedures

1-(3-methylbut-2-en-1-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (20)



To a solution of 1,3-dihydro-2H-benzo[d]imidazol-2-one **19** (2 g, 0.014 mol) in dry DMF (40 mL) was added caesium carbonate (5.84 g, 0.017 mol), followed by 3,3-dimethylallyl bromide (1.89 mL, 0.016 mmol) diluted in 5 mL of DMF and kept stirring for 2 h at room temperature reaction. The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 100 mL). The combined organic layer was washed with brine and dried over Na_2SO_4, concentrated under reduced pressure, and subjected to flash chromatography over silica gel (20 % EtOAc:PE) to afford mono-alkylated compound **20** (1.6 g, 55%) as white solid.

Melting point 168-171 °C;

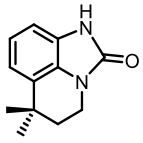
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.62 (br. s., 1 H), 7.15 - 7.13 (m, 1 H), 7.08 - 7.01 (m, 2 H), 7.06 - 6.98 (m, 1 H), 5.30 (t, *J* = 6.7 Hz, 1 H), 4.53 (d, *J* = 6.5 Hz, 2 H), 1.88 (s, 3 H), 1.75 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 136.4, 130.2, 128.2, 121.4, 121.0, 118.9, 109.7, 108.2, 38.8, 25.6, 18.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 1694, 1488, 1444, 1216, 923 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{12}H_{14}N_2ONa$ $[M+Na]^+$ 225.0998, found 225.0999.

6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (21)



To a cooled solution of 1-(3-methylbut-2-en-1-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one **20** (500 mg, 2.475 mmol) in chlorobenzene (8 mL), $AlCl_3$ (987 mg, 7.425 mmol) was added and stirred the reaction mixture stirred at room temperature for 2 h. After the starting material was completely consumed, reaction mixture poured on crushed ice water (10 mL). The aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic extracts were

washed with saturated water, brine (60 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* and purified by flash column chromatography over silica gel (10 % EtOAc:DCM) to afford compound **21** (410 mg, 82%) as brown solid product.

Melting point 167-169 °C;

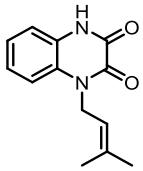
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.75 (br. s., 1 H), 7.02 - 6.96 (m, 3 H), 3.94 (t, *J* = 5.6 Hz, 2 H), 1.92 (t, *J* = 5.6 Hz, 2 H), 1.36 (s, 6 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.1, 128.4, 126.5, 126.1, 121.2, 115.9, 107.2, 36.6, 36.0, 31.6, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3065, 3019, 1692, 1496, 1355 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{12}H_{14}N_2ONa$ $[M+Na]^+$ 225.0998, found 225.0999.

1-(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione (23)



The title compound was synthesized from **22** using similar procedure employed for **20**.

Yield 990 mg, 35%;

Melting point 154-157 °C;

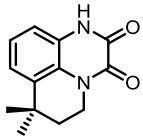
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (br. s., 1 H), 7.20 - 7.17 (m, 4 H), 5.11 (br. s, 1 H), 4.73 - 4.72 (m, 2 H), 1.82 (br. s., 3 H), 1.67 (br. s., 3 H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 154.9, 153.6, 136.1, 126.2, 125.8, 123.5, 123.2, 118.7, 115.7, 115.0, 40.6, 25.3, 18.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3022, 2919, 1681, 1512, 1385, 1217 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{13}H_{14}N_2O_2Na$ $[M+Na]^+$ 253.0947, found 231.0944.

7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (24)



The title compound was synthesized from **23** using similar procedure employed for **21**.

Yield 310 mg, 77%;

Melting point 272-275 °C;

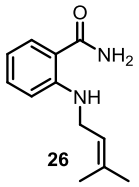
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.18 (br. s., 1 H), 7.30 (d, *J* = 8.1 Hz, 1 H), 7.28 (d, *J* = 7.8 Hz, 1 H), 7.15 (dt, *J* = 2.3, 7.8 Hz, 1 H), 4.20 (t, *J* = 5.5 Hz, 2 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.37 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 154.8, 134.3, 124.4, 124.0, 122.4, 121.3, 115.0, 39.1, 34.6, 31.6, 29.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 2404, 1694, 1600, 1395 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{13}H_{15}N_2O_2$ $[M+H]^+$ 231.1128, found 231.1127.

Synthesis of 2-((3-methylbut-2-en-1-yl)amino)benzamide (26)



26

The *N*-prenylated intermediate was synthesized from **25** using similar procedure employed for **20**. The intermediate (1.5 g, 6.49 mmol) was then dissolved in THF (7 mL) and treated excess of Ammonium hydroxide solution i.e. 28% NH_3 in H_2O (~ 4 mL) at room temperature for 2 h. The reaction mixture was then diluted with H_2O (10 mL) and extracted with ethyl acetate (3 X 20 mL). The combined organic layer was then washed with water, brine, dried over sodium sulphate and concentrated in *vacuum* to obtained **26** (1.3 g, 98% yield) as colourless solid product

Melting point 115-118 °C;

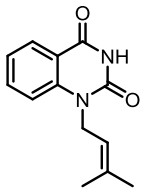
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (br. s., 1 H), 7.39 (d, *J* = 7.0 Hz, 1 H), 7.32 (t, *J* = 7.8 Hz, 1 H), 6.69 (d, *J* = 8.2 Hz, 1 H), 6.57 (t, *J* = 7.5 Hz, 1 H), 5.89 (br. s., 2 H), 5.33 (t, *J* = 6.4 Hz, 1 H), 3.75 (br. s., 2 H), 1.75 (s, 3 H), 1.73 (s, 3 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 150.2, 135.2, 133.4, 128.3, 121.4, 114.3, 113.1, 111.9, 41.0, 25.6, 18.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3413, 3018, 1655, 1580, 1516, 1381 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{12}H_{17}N_2O$ $[M+H]^+$ 205.1335, found 205.1335.

1-(3-methylbut-2-en-1-yl)quinazoline-2,4(1H,3H)-dione (27)



Mixture of the 2-((3-methylbut-2-en-1-yl)amino)benzamide **26** (1.2 g, 5.88 mmol), Ethyl chloroformate (0.66 mL, 7.06 mmol) and 4M NaOH (3.2 mL, 12.9 mmol) in dioxane (10 mL) was stirred for 1 h at room temperature. The reaction mixture was partitioned between Ethyl acetate (40 mL) and water (20 mL). The organic layer was worked up to give a residue which was dissolved in absolute ethanol (10 mL), KOH (0.71 g, 12.7 mmol) was added and stirred for 4 h at 78 °C. The reaction mixture was then cooled to room temperature, diluted with water (20 mL) and extracted with ethyl acetate (40 mL). The combined organic layer was washed with water, brine, dried over sodium sulphate and concentrated in vacuum to obtained pure compound **27** (1.0 g, 89% yield) as a brown solid product.

(Desired product formed was confirmed by ^{1}H NMR and Mass and forwarded for next step)

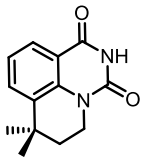
Melting point 164-167 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (br. s., 1 H), 7.55 (br. s., 1 H), 7.14 (br. s., 1 H), 7.04 (br. s., 1 H), 5.07 (br. s., 1 H), 4.63 (br. s., 2 H), 1.79 (br. s., 3 H), 1.68 (br. s., 3 H);

IR ν_{max}(thin film applied as $CHCl_3$ solution) 3399, 3022, 1693, 1605, 1488, 1400 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{13}H_{14}N_2O_2Na$ $[M+Na]^+$ 253.0947, found 253.0945.

7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione (28)



The title compound was synthesized from **27** using similar procedure employed for **21**.

Yield 920 mg, 92%

Melting point 197-200 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (br. s., 1 H), 8.07 (t, *J* = 6.8 Hz, 1 H), 7.67 (d, *J* = 7.8 Hz, 1 H), 7.21 (t, *J* = 7.7 Hz, 1 H), 4.08 (t, *J* = 6.1 Hz, 2 H), 1.92 (t, *J* = 6.1 Hz, 2 H), 1.39 (s, 6 H);

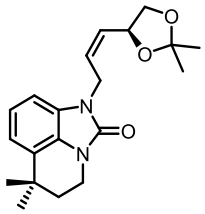
^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2, 150.1, 136.9, 133.8, 132.0, 126.5, 122.8, 115.6, 38.6, 34.6, 32.0, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3396, 3021, 1688, 1600, 1496, 1408 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{13}H_{14}N_2O_2Na$ $[M+Na]^+$ 253.0947, found 253.0946;

HPLC analysis t_R 4.6 min, purity 97.4%.

(S,Z)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (30)



A solution of 6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **21** (80 mg, 0.396 mmol) in dry DMF (2 mL) was added drop wise to pre-cooled (0 °C) suspension of sodium hydride (60% mineral oil, 39 mg, 0.990 mmol) in 3 mL of DMF. The reaction mixture was allowed to warm to room temperature and stirred for 20 min. (*S,Z*)-4-(3-bromoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolane **29** (140 mg, 0.633 mmol), dissolved in dry DMF (1 mL) was then added drop wise at 0 °C and stirred for 2 h at room temperature. The reaction mixture was added to cold water (10 mL) and extracted with ethyl acetate (3 X 15 mL) and the combined organic layers were washed with water, brine, then dried over Na_2SO_4, filtered and concentrated in *vacuo*, and purified by column chromatography (20% EtOAc: DCM) to afford compound **30** (135 mg, 59%) as colourless oil.

Specific rotation $[\alpha]_D^{25}$ = - 0.83° (*c* 0.2, $CHCl_3$);

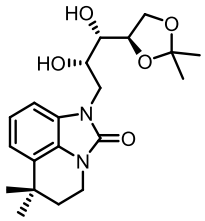
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 - 6.97 (m, 2 H), 6.85 (dd, *J* = 1.8, 6.9 Hz, 1 H), 5.71 - 5.67 (m, 2 H), 5.10 - 5.08 (m, 1 H), 4.71 - 4.54 (m, 1 H), 4.56 - 4.53 (m, 1 H), 4.20 (dd, *J* = 6.4, 8.2 Hz, 1 H), 3.90 (t, *J* = 5.95 Hz, 2 H), 3.62 (t, *J* = 7.8 Hz, 1 H), 1.90 (t, *J* = 5.95 Hz, 2 H), 1.46 (s, 3 H), 1.45 (s, 3 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 130.9, 128.5, 128.4, 127.4, 125.1, 120.9, 116.3, 109.5, 105.5, 71.7, 69.4, 38.3, 36.6, 36.3, 31.8, 28.5, 26.7, 25.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3393, 2928, 2358, 1684, 1500, 1418 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}N_2O_3Na$ $[M+Na]^+$ 365.1836, found 365.1833.

1-((2S,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (31i)



A solution of (S,Z)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **30** (90 mg, 0.263 mmol) in acetone: water: ^{t}BuOH (5 mL, 7:2:1) was treated with *N*-methylmorpholine oxide (123 mg, 1.052 mmol), osmium tetraoxide (2.5 % sol. in ^{t}BuOH) (0.26 mL, 0.026 mmol) and stirred for 12 h at room temperature. The reaction mixture was added to cold solution of $NaHSO_4$ and extracted with ethyl acetate (3 X 30 mL) and washed with water, brine and the combined organic layers were dried over sodium sulphate filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography on silica (5% MeOH: DCM) to afford compound **31i** (80 mg, 81%) as colourless gum.

Specific rotation $[\alpha]_D^{25}$ = -51° (*c* 6.0, $CHCl_3$);

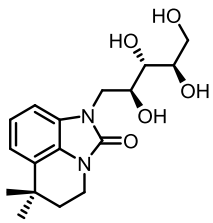
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 – 7.02 (m, 3 H), 5.33 (br. s., 1 H), 4.33 - 4.28 (m, 1 H), 4.22 - 4.16 (m, 2 H), 4.03 - 3.84 (m, 6 H), 3.32 (t, *J* = 8.1 Hz, 1 H), 1.97 - 1.86 (m, 2 H), 1.39 - 1.34 (m, 12 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.0, 129.2, 128.6, 124.9, 121.5, 116.6, 109.8, 107.3, 78.6, 75.0, 70.6, 68.2, 44.1, 36.5, 36.5, 31.7, 28.6, 28.5, 26.5, 25.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3393, 2958, 1681, 1497, 1424, 1378 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{28}N_2O_5Na$ $[M+Na]^+$ 399.1890, found 399.1885.

6,6-dimethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (31)



A solution of 1-((2S,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **31i** (50 mg, 0.132 mmol) in acetic acid : water (1:1; 2 mL) was stirred for 30 min at 50 °C. The reaction mixture was then

concentrated in *vacuo*, and purified by flash chromatography on over silica gel (10% MeOH: DCM) to afford **31** (30 mg, 68%) as yellow gum.

Specific rotation $[\alpha]_D^{25}$ = -46.5 (*c* = 0.4, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.09 – 7.02 (m, 3 H), 4.11 - 4.08 (m, 3 H), 3.89 (t, *J* = 5.5 Hz, 2 H), 3.82 - 3.74 (m, 2 H), 3.66 - 3.61 (m, 2 H), 1.92 (t, *J* = 5.5 Hz, 2 H), 1.34 (s, 6 H);

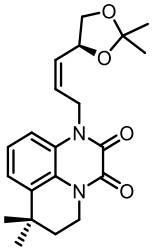
^{13}C NMR (100 MHz, CD_3OD) δ 155.8, 130.1, 129.9, 126.3, 122.7, 117.6, 108.0, 74.4, 72.5, 64.8, 45.4, 37.9, 37.6, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3404, 2960, 1674, 1497, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{24}N_2O_5Na$ $[M+Na]^+$ 359.1577, found 359.1570;

HPLC analysis t_R 4.2 min, purity 97.84%.

(S,Z)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (32)



The title compound was synthesized from **24** using similar procedure employed for **30**.

Yield 55 mg, 57%;

Specific rotation $[\alpha]_D^{25}$ = -1.0 (*c* = 0.2, $CHCl_3$);

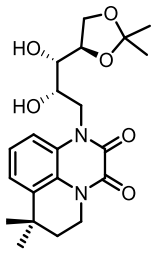
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.16 (m, 3 H), 5.70 - 5.65 (m, 1 H), 5.65 - 5.55 (m, 1 H), 5.32 - 5.20 (m, 1 H), 5.16 - 5.05 (m, 1 H), 4.80 - 4.70 (m, 1 H), 4.28 - 4.21 (m, 1 H), 4.17 (t, *J* = 5.5 Hz, 2 H), 3.63 (t, *J* = 8.0 Hz, 2 H), 1.91 (t, *J* = 5.5 Hz, 2 H), 1.45 (s, 3 H), 1.44 (s, 3 H), 1.37 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 153.6, 134.9, 131.9, 127.0, 126.2, 123.8, 122.6, 121.4, 113.0, 109.6, 72.1, 69.4, 40.9, 39.0, 34.3, 31.9, 29.9, 26.7, 25.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 2930, 1679, 1599, 1397, 1217 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{21}H_{26}N_2O_4Na$ $[M+Na]^+$ 393.1785, found 393.1780.

1-((2S,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (33i)



The title compound was synthesized from **30** using similar procedure employed for **31i**.

Yield 35 mg, 64%;

Specific rotation $[\alpha]_D^{25}$ = -29.2° (*c* = 2.5, $CHCl_3$);

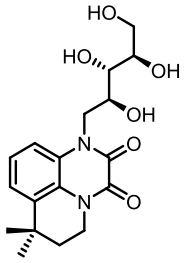
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 1.5, 8.3 Hz, 1 H), 7.34 - 7.20 (m, 2H), 4.77 (dd, *J* = 6.1, 14.9 Hz, 1 H), 4.45 - 4.32 (m, 2 H), 4.30 - 4.16 (m, 3 H), 4.16 - 4.09 (m, 1 H), 4.05 - 3.97 (m, 2 H), 3.68 - 3.62 (m, 1 H), 1.93 (t, *J* = 6.1 Hz, 3 H), 1.41 (s, 3 H), 1.39 (s, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 153.5, 134.7, 127.0, 123.9, 122.8, 121.9, 114.6, 109.7, 73.4, 72.5, 67.6, 46.4, 39.2, 34.3, 32.0, 29.9, 26.6, 25.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3450, 1682, 1602, 1402, 955 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{21}H_{28}N_2O_6Na$ $[M+Na]^+$ 427.1840, found 427.1836.

7,7-dimethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (33)



The title compound was synthesized from **33i** using similar procedure employed for **31**.

Yield 20 mg, 64%;

Specific rotation $[\alpha]_D^{25}$ = -16.2 (*c* = 0.8, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, *J* = 8.2 Hz, 1 H), 7.36 (d, *J* = 8.2 Hz, 1 H), 7.26 (t, *J* = 8.0 Hz, 1 H), 4.77 (dd, *J* = 9.8, 14.4 Hz, 1 H), 4.61 (s, 1 H), 4.33 (dd, *J* = 2.7, 14.2 Hz, 1 H), 4.27 - 4.21 (m, 1 H), 4.19 - 4.14 (m, 2 H), 3.83 - 3.73 (m, 3 H), 3.70 - 3.61 (m, 1 H), 1.96 (t, *J* = 6.41Hz, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H);

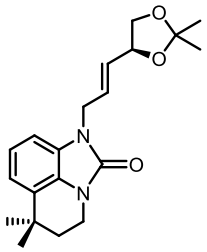
^{13}C NMR (100 MHz, CD_3OD) δ 156.9, 155.7, 136.4, 128.5, 125.2, 124.1, 122.9, 115.6, 75.1, 74.3, 71.0, 64.9, 46.9, 40.4, 35.6, 33.2, 30.4, 30.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3390, 2928, 1665, 1607, 1455, 1217 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{24}N_2O_6Na$ $[M+Na]^+$ 387.1527, found 387.1520;

HPLC analysis t_R 4.3 min, purity 95.92%.

(S,E)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (35)



The title compound was synthesized from **21** using similar procedure employed for **30**.

Yield 55 mg; 59%;

Specific rotation $[\alpha]_D^{25}$ = +1.98 (*c* = 0.2, $CHCl_3$);

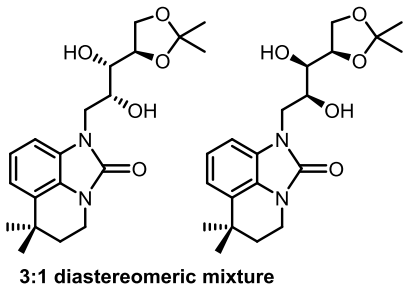
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 - 6.94 (m, 2 H), 6.80 (dd, *J* = 2.0, 6.6 Hz, 1 H), 5.90 (td, *J* = 5.5, 15.6 Hz, 1 H), 5.73 (dd, *J* = 7.0, 15.6 Hz, 1 H), 4.56 - 4.41 (m, 3 H), 4.07 (dd, *J* = 6.3, 8.1 Hz, 1 H), 3.94 - 3.85 (m, 2 H), 3.57 (t, *J* = 7.8 Hz, 1 H), 1.94 - 1.87 (m, 2 H), 1.41 (s, 3 H), 1.37 (s, 3 H), 1.36 (s, 6 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 153.0, 130.7, 128.4, 128.0, 127.6, 125.1, 120.9, 116.2, 109.4, 105.7, 76.1, 69.3, 42.4, 36.6, 36.3, 31.8, 28.5, 26.6, 25.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3396, 3022, 1692, 1622, 1422, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}N_2O_3Na$ $[M+Na]^+$ 365.1836, found 365.1833.

1-((2R,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (36i)



3:1 diastereomeric mixture

The title compound was synthesized from **35** using similar procedure employed for **31i**.

Yield 38 mg, 63%;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 - 6.89 (m, 3 H), 4.35 - 4.25 (m, 1 H), 4.25 - 3.96 (m, 6 H), 3.96 - 3.81 (m, 2 H), 3.60 - 3.37 (m, 1 H), 3.37 - 3.24 (m, 1 H), 1.96 - 1.88 (m, 2 H), 1.37 (s, 3 H), 1.36 (s, 3 H), 1.32 (s, 3 H), 1.29 (s, 3 H);

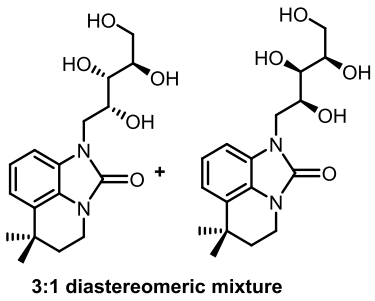
^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 128.9, 127.8, 125.0, 121.5, 116.7, 109.2, 105.6, 75.4, 71.5, 69.0, 67.2, 44.2, 36.5, 36.5, 31.7, 28.6, 28.5, 26.7, 25.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3382, 3020, 1679, 1497, 1422, 1356 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{28}N_2O_5Na$ $[M+Na]^+$ 399.1890, found 399.1888.

Note: Minor peaks in NMR (^{1}H and ^{13}C) which could not be cleanly distinguished, corresponds to another diastereomer.

6,6-dimethyl-1-((2R,3S,4R)-2,3,4,5-tetrahydroxypentyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (36)



3:1 diastereomeric mixture

The title compound was synthesized from **36i** using similar procedure employed for **31**.

Yield 20 mg, 58%;

^{1}H NMR (400 MHz, CD_3OD) δ 7.08 – 7.03 (m, 3 H), 4.26 (t, *J* = 6.8 Hz, 1 H), 4.15 - 3.97 (m, 2 H), 3.89 (t, *J* = 5.9 Hz, 2 H), 3.84 - 3.77 (m, 1 H), 3.75 - 3.56 (m, 2 H), 3.43 (d, *J* = 8.5 Hz, 1 H), 1.93 (t, *J* = 5.9 Hz, 2 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, CD_3OD) δ 155.5, 130.2, 129.7, 126.3, 122.8, 117.7, 107.6, 72.8, 72.5, 69.4, 65.2, 45.7, 37.9, 37.6, 32.9, 29.0;

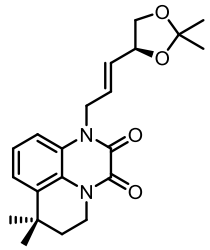
IR ν_{max} (thin film applied as $CHCl_3$ solution) 3412, 3017, 1694, 1644, 1496, 1421 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{24}N_2O_5Na$ $[M+Na]^+$ 359.1577, found 359.1577;

HPLC analysis t_R 4.3 min, purity 96.08%.

Note Minor peaks in NMR (^{1}H and ^{13}C) which could not be cleanly distinguished, corresponds to another diastereomer.

(S,E)-1-(3-(2,2-dimethyl-1,3-dioxolan-4-yl)allyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (37)



The title compound was synthesized from **24** using similar procedure employed for **30**.

Yield 70 mg, 72%;

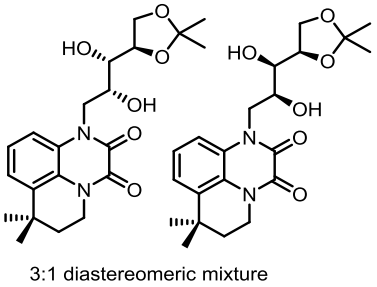
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 - 7.22 (m, 1 H), 7.18 (t, *J* = 8.0 Hz, 1 H), 7.08 - 7.06 (m, 1 H), 5.92 (td, *J* = 5.4, 15.6 Hz, 1 H), 5.75 (ddd, *J* = 1.3, 6.8, 15.6 Hz, 1 H), 4.86 (t, *J* = 4.8 Hz, 2 H), 4.49 (q, *J* = 6.8 Hz, 1 H), 4.22 - 4.13 (m, 2 H), 4.05 (dd, *J* = 6.1, 8.2 Hz, 1 H), 3.54 (t, *J* = 7.9 Hz, 1 H), 1.92 (t, *J* = 6.27Hz, 2 H), 1.38 (s, 9 H), 1.34 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 153.7, 134.9, 131.7, 126.3, 125.9, 123.9, 122.6, 121.4, 113.2, 109.4, 76.0, 69.2, 44.3, 39.0, 34.4, 32.0, 29.9, 26.6, 25.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3404, 2960, 1673, 1401, 1351, 1076 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{22}N_2O_4Na$ $[M+Na]^+$ 353.1472, found 353.1471.

1-((2R,3S)-3-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,3-dihydroxypropyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (38i):



3:1 diastereomeric mixture

The title compound was synthesized from **37** using similar procedure employed for **31i**.

Yield 28 mg; 73%;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 - 7.23 (m, 3 H), 4.51 - 4.46 (m, 1 H), 4.39 - 4.27 (m, 2 H), 4.27 - 4.09 (m, 5 H), 4.02 - 3.87 (m, 2 H), 3.48 - 3.39 (m, 1 H), 1.93 (t, *J* = 6.4 Hz, 2 H), 1.39 (s, 6 H), 1.31 (s, 3 H), 1.26 (s, 3 H);

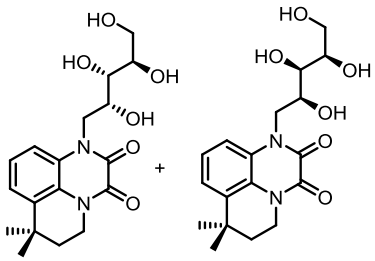
^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 153.4, 135.1, 126.3, 124.2, 122.7, 122.0, 113.3, 109.2, 75.2, 71.6, 68.2, 67.3, 46.4, 39.2, 34.3, 32.0, 29.9, 29.9, 26.7, 25.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3413, 3020, 1675, 1403, 1313, 1216 cm^{-1};

HRMS (ESI): *m/z* calculated for $C_{21}H_{28}N_2O_6Na$ $[M+Na]^+$ 427.1840, found 427.1837.

Note: Minor peaks in NMR (^{1}H and ^{13}C) which could not be cleanly distinguished, corresponds to another diastereomer.

7,7-dimethyl-1-((2R,3S,4R)-2,3,4,5-tetrahydroxypentyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (38)



3:1 diastereomeric mixture

The title compound was synthesized from **38i** using similar procedure employed for **31**.

Yield 15 mg, 55%;

^{1}H NMR (400 MHz, CD_3OD) δ 7.52 - 7.49 (m, 1 H), 7.38 - 7.34 (m, 1 H), 7.29 - 7.27 (m, 1 H), 4.58 - 4.45 (m, 2 H), 4.45 - 4.29 (m, 2 H), 4.24 - 4.11 (m, 2 H), 3.86 - 3.58 (m, 2 H), 1.96 (t, *J* = 6.41Hz, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H);

^{13}C NMR (100 MHz, CD_3OD) δ 156.7, 155.7, 136.5, 128.5, 125.3, 124.1, 123.0, 115.5, 72.7, 72.7, 68.6, 65.2, 47.7, 40.4, 35.6, 33.2, 30.3;

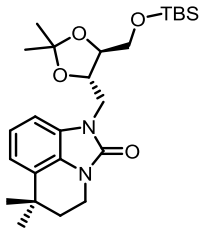
IR ν_{max} (thin film applied as $CHCl_3$ solution): 3411, 3020, 1671, 1406, 1315 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{24}N_2O_6Na$ $[M+Na]^+$ 387.1527, found 387.1526;

HPLC analysis t_R 4.0 min, purity 99.0%.

Note: Minor peaks in NMR (^{1}H and ^{13}C) which could not be clearly distinguished, corresponds to another diastereomer.

1-(((4S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (40):



To a solution of 6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **21** (120 mg, 0.59 mmol) in dry DMSO (5 mL) was added caesium carbonate (250 mg, 0.77 mmol), followed by ((4S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-

yl)methyl 4-methylbenzenesulfonate **39** (306 mg, 0.71 mmol) diluted in of DMSO (2 mL) and kept stirring for 12 h at 60 °C. The reaction mixture was added to cold water (10 mL) and extracted with ethyl acetate (40 mL). The combined organic layer was washed with brine and dried over Na_2SO_4, concentrated under reduced pressure, and subjected to flash chromatography over (100-200) silica gel to afford desired product **40** (190 mg, 69% yield) as yellow sticky product.

Specific rotation $[\alpha]_D^{25}$ = +4.7 (*c* = 2.8, $CHCl_3$);

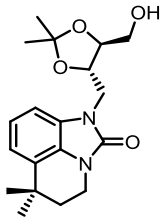
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 – 6.95 (m, 3 H), 4.13 (dt, *J* = 4.1, 6.9 Hz, 1 H), 4.32 (dd, *J* = 3.7, 14.2 Hz, 1 H), 4.03 (dd, *J* = 6.4, 14.7 Hz, 1 H), 3.96 - 3.87 (m, 3 H), 3.75 (d, *J* = 4.1 Hz, 2 H), 1.87 (t, *J* = 6.0 Hz, 2 H), 1.34 (s, 6 H), 1.33 (s, 6 H), 0.86 (s, 9 H), 0.05 (s, 3 H), 0.01 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4, 128.3, 128.1, 125.0, 120.8, 116.2, 109.4, 106.6, 79.0, 76.7, 62.8, 43.6, 36.6, 36.3, 31.7, 28.6, 27.1, 26.9, 25.8, 18.3, -5.5, -5.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3403, 2943, 1703, 1645, 1499, 1378 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{25}H_{40}N_2O_4SiNa$ $[M+Na]^+$ 483.2650, found 483.2643.

1-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (41i)



A solution of 1-(((4S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij] quinolin-2(1H)-one **40** (130 mg, 0.28 mmol) in THF (5 mL) was added tetra-n-butyl ammonium fluoride TBAF(1M Solution in THF) (0.56 mL, 0.56 mmol) and kept stirring at room temperature for 2 h the reaction was monitored by TLC. The reaction mixture diluted with water (5 mL) and extracted with ethyl acetate (20 mL). Organic layer was then concentrated under reduced pressure, and purified by column chromatography over (100-200) silica gel to afford desired product **41i** (70 mg, 72%) as colourless gum.

Specific rotation $[\alpha]_D^{25}$ = -17.2 (*c* = 1.4, $CHCl_3$);

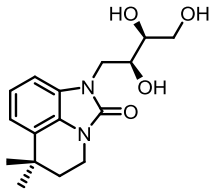
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 – 7.00 (m, 3 H), 4.26 - 4.20 (m, 1 H), 4.15 - 4.11 (m, 2 H), 3.96 - 3.86 (m, 3 H), 3.86 - 3.70 (m, 2 H), 2.95 (br. s., 1 H), 1.92 (t, *J* = 5.95 Hz, 2 H), 1.39 (s, 3 H), 1.37 (s, 3 H), 1.35 (s, 3 H), 1.21 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.9, 128.5, 128.4, 124.9, 121.1, 116.4, 109.3, 107.0, 77.9, 77.6, 62.3, 42.9, 36.6, 36.4, 31.7, 28.6, 28.5, 26.9, 26.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3406, 2954, 1691, 1497, 1422 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{26}N_2O_4Na$ $[M+Na]^+$ 369.1785, found 369.1774.

6,6-dimethyl-1-((2S,3S)-2,3,4-trihydroxybutyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (41)



The title compound was synthesized from **41i** using similar procedure employed for **31**.

Yield 35 mg, 79%;

Specific rotation $[\alpha]_D^{25}$ = +30.9 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.07 – 7.02 (m, 3 H), 4.02 (br s, 3 H), 3.92 - 3.79 (m, 2 H), 3.72 - 3.51 (m, 3 H), 1.91 (t, *J* = 6.0 Hz, 2 H), 1.34 (s, 6 H);

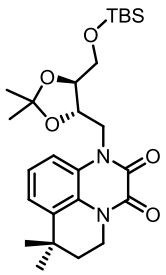
^{13}C NMR (100 MHz, CD_3OD) δ 155.4, 130.1, 129.7, 126.2, 122.7, 117.7, 107.7, 73.2, 70.4, 64.3, 45.6, 37.9, 37.6, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3413, 3021, 1679, 1499, 1422, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{22}N_2O_4Na$ $[M+Na]^+$ 329.1472, found 329.1469;

HPLC analysis t_R 4.3 min, purity 97.94%.

1-(((4S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (42)



The title compound was synthesized from **24** using similar procedure employed for **40**.

Yield 132 mg; 41%;

Specific rotation $[\alpha]_D^{25}$ = +10.8 (*c* = 1.3, $CHCl_3$);

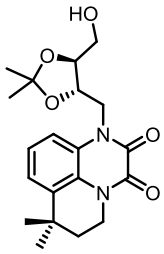
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 1.1, 8.4 Hz, 1 H), 7.25 (dd, *J* = 1.1, 7.6 Hz, 1 H), 7.19 (t, *J* = 8.0 Hz, 1 H), 4.50 (d, *J* = 5.3 Hz, 2 H), 4.44 - 4.33 (m, 1 H), 4.18 (t, *J* = 6.48 Hz, 2 H), 3.99 (ddd, *J* = 4.0, 5.5, 7.6 Hz, 1 H), 3.85 (dd, *J* = 3.8, 10.7 Hz, 1 H), 3.77 (dd, *J* = 5.7, 10.7 Hz, 1 H), 1.92 (t, *J* = 6.5 Hz, 2 H), 1.45 (s, 3 H), 1.39 (s, 3 H), 1.38 (s, 3 H), 1.35 (s, 3 H), 0.86 (s, 9 H), 0.08 (s, 3 H), 0.04 (s, 3 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 154.3, 153.6, 134.7, 126.9, 123.6, 122.7, 121.4, 113.8, 109.8, 79.6, 76.0, 63.1, 45.9, 39.1, 34.4, 32.0, 30.0, 30.0, 26.9, 25.9, 18.3, -5.4, -5.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3428, 3013, 1682, 1602, 1402 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{26}H_{40}N_2O_5SiNa$ $[M+Na]^+$ 511.2599, found 511.2593.

1-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (43i)



The title compound was synthesized from **42** using similar procedure employed for **41i**.

Yield 52mg, 76%;

Specific rotation $[\alpha]_D^{25}$ = -5.2 (*c* = 0.7, $CHCl_3$);

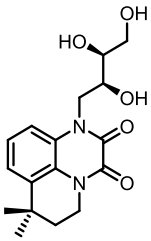
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.8 Hz, 1 H), 7.39 (d, *J* = 7.3 Hz, 1 H), 7.28 - 7.23 (m, 1 H), 4.62 (dd, *J* = 5.3, 10.8 Hz, 1 H), 4.54 - 4.48 (m, 1 H), 4.36 - 4.30 (m, 1 H), 4.22 - 4.11 (m, 3 H), 4.04 (dd, *J* = 4.1, 11.9 Hz, 1 H), 3.84 (dd, *J* = 3.7, 11.9 Hz, 1 H), 2.58 (br s, 1H) 1.93 (t, *J* = 5.5 Hz, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H), 1.37 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 153.6, 134.8, 126.9, 124.0, 122.8, 121.9, 114.3, 109.8, 79.2, 62.4, 46.2, 39.3, 34.5, 32.1, 30.1, 27.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3434, 3020, 1678, 1605, 1312 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}N_2O_5Na$ $[M+Na]^+$ 397.1734, found 397.1723.

7,7-dimethyl-1-((2S,3S)-2,3,4-trihydroxybutyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (43)



The title compound was synthesized from **43i** using similar procedure employed for **31**.

Yield 25 mg, 71%;

Specific rotation $[\alpha]_D^{25}$ = +13.1 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (d, *J* = 7.8 Hz, 1 H), 7.37 (d, *J* = 7.8 Hz, 1 H), 7.32 - 7.26 (t, *J* = 8.0 Hz, 1 H), 4.54 (dd, *J* = 8.6, 14.2 Hz, 1 H), 4.37 (dd, *J* = 4.4, 14.2 Hz, 1 H), 4.18 - 4.13 (m, 3 H), 3.74 - 3.63 (m, 3 H), 1.96 (t, *J* = 6.11 Hz, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H);

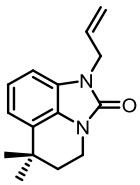
^{13}C NMR (100 MHz, CD_3OD) δ 156.7, 155.6, 136.4, 128.6, 125.3, 124.1, 122.9, 115.5, 73.6, 69.7, 64.2, 47.7, 40.4, 35.6, 33.2, 30.4, 30.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3396, 3015, 1672, 1602, 1456, 1404 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{22}N_2O_5Na$ $[M+Na]^+$ 357.1421, found 357.1416;

HPLC analysis t_R 4.1 min, purity 99.29%.

1-allyl-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (44)



The title compound was synthesized from **21** using similar procedure employed for **30**.

Yield 36 mg, 76%;

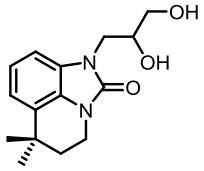
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 - 6.99 (m, 2 H), 6.81 (dd, *J* = 2.1, 6.5 Hz, 1 H), 5.95 - 5.88 (m, 1 H), 5.24 (t, *J* = 12.0 Hz, 2 H), 4.50 (d, *J* = 6.8 Hz, 2 H), 3.91 (t, *J* = 5.87 Hz, 2 H), 1.90 (t, *J* = 5.95 Hz, 2 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 132.3, 128.2, 127.6, 125.1, 120.7, 117.4, 116.1, 105.7, 43.5, 36.6, 36.2, 31.7, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3427, 1691, 1499, 1437, 1215;

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}N_2ONa$ $[M+Na]^+$ 265.1311, found 265.1311.

1-(2,3-dihydroxypropyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (45)



The title compound was synthesized from **44** using similar procedure employed for **31**.

Yield 23 mg, 67%;

Melting point 132-135 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 7.05 – 7.02 (m, 3 H), 4.00 - 3.86 (m, 5 H), 3.57 - 3.56 (m, 2 H), 1.91 (t, *J* = 5.8 Hz, 2 H), 1.34 (s, 6 H);

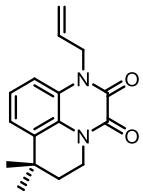
^{13}C NMR (100 MHz, CD_3OD) δ 155.5, 130.1, 129.8, 126.2, 122.7, 117.7, 107.7, 71.7, 65.0, 45.5, 37.9, 37.6, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3677, 3016, 1681, 1497, 1354 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{20}N_2O_3Na$ $[M+Na]^+$ 299.1366, found 299.1366;

HPLC analysis t_R 4.5 min, purity 97.73%.

1-allyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (46)



The title compound was synthesized from **24** using similar procedure employed for **30**.

Yield 38 mg, 65%;

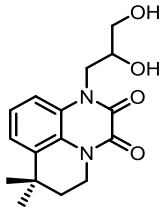
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 - 7.22 (m, 1 H), 7.18 (t, *J* = 7.9 Hz, 1 H), 7.13 - 7.10 (m, 1 H), 5.92 (tdd, *J* = 5.1, 10.4, 17.3 Hz, 1 H), 5.27 (m, 2 H), 4.88 - 4.86 (m, 2 H), 4.20 (t, *J* = 6.0 Hz, 2 H), 1.93 (t, *J* = 6.3 Hz, 2 H), 1.39 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 153.76, 134.8, 130.6, 126.4, 123.7, 122.6, 121.3, 118.2, 113.4, 45.6, 39.0, 34.4, 32.0, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3405, 3003, 1680, 1605, 1449, 1229 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{18}N_2O_2Na$ $[M+Na]^+$ 293.1260, found 293.1258.

1-(2,3-dihydroxypropyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (47)



The title compound was synthesized from **46** using similar procedure employed for **31**.

Yield 38 mg, 57%;

^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (br. s, 3 H), 4.97 - 3.88 (m, 5 H), 3.56 (br.s, 2 H), 1.91 (br. S, 2 H), 1.34 (m, 6 H);

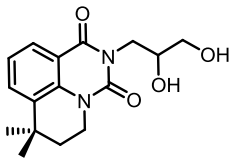
^{13}C NMR (100 MHz, CD_3OD) δ 155.5(2C), 130.2, 129.8, 126.3, 122.7, 117.7, 107.7, 71.7, 65.0, 45.5, 37.9, 37.6, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3420, 3018, 1676, 1606, 1402, 1215 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{20}N_2O_4Na$ $[M+Na]^+$ 327.1315, found 327.1316;

HPLC analysis t_R 4.2 min, purity 98.17%.

2-(2,3-dihydroxypropyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione (48)



Compound **48** was prepared by *N*-allylation reaction of **28** using the same procedure as mentioned for the synthesis of **30** followed by dihydroxylated using the same procedure as mentioned for the synthesis of **31** to afford compound **48** (92 mg, 69% yield) for two steps as a colourless solid product.

Melting point 94-97 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.8 Hz, 1 H), 7.66 (d, *J* = 7.6 Hz, 1 H), 7.21 (t, *J* = 7.8 Hz, 1 H), 4.34 - 4.29 (m, 2 H), 4.09 - 4.05 (m, 3 H), 4.12 - 4.02 (m, 3 H), 3.59 (br. s., 3 H), 3.30 (br. s., 1 H), 1.91 (t, *J* = 6.1 Hz, 2 H), 1.37 (s, 6 H);

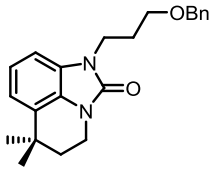
^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 151.5, 135.7, 133.5, 131.9, 126.7, 123.1, 114.8, 70.6, 63.5, 43.9, 39.6, 34.6, 31.8, 29.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3022, 1693, 1644, 1497, 1313, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{20}N_2O_4Na$ $[M+Na]^+$ 327.1315, found 327.1311;

HPLC analysis t_R 4.4 min, purity 97.17%.

1-(3-(benzyloxy)propyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (52)



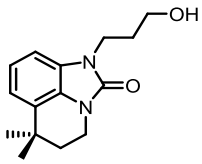
The title compound was synthesized from **21** using similar procedure employed for **20**.

Yield 228 mg, 88%;

HRMS (ESI) *m/z* calculated for $C_{22}H_{27}N_2O_2$ $[M+Na]^+$ 351.2067, found 351.2061.

Product obtained was forwarded to next step without further characterizations.

1-(3-hydroxypropyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (53)



To a solution of **52** (160 mg, 0.45 mmol) in Methanol (5 mL) was added palladium on carbon 10 wt. % (20 mg). The atmosphere in the flask was replaced with hydrogen gas using hydrogen bladder and stirred vigorously at room temperature for 12 h before being filtered through a celite pad. After rinsing several times with MeOH, the filtrate was concentrated and purified via column chromatography (30-40% EtOAc/PE) to afford compound **38** (90 mg, 76% yield) as a colourless solid product.

Melting point 109-112 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 – 7.00 (m, 2 H), 6.88 (dd, *J* = 1.0, 7.3 Hz, 1 H), 4.05 (t, *J* = 6.1 Hz, 2 H), 3.91 (t, *J* = 5.6 Hz, 2 H), 3.81 - 3.68 (m, 1 H), 3.59 (m, 2 H), 1.95 - 1.90 (m, 4 H), 1.36 (s, 6 H);

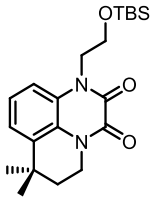
^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 128.6, 127.5, 125.1, 121.1, 116.3, 105.2, 58.2, 37.2, 36.6, 36.3, 31.7, 31.0, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3406, 3019, 1681, 1499, 1420 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{21}N_2O_2$ $[M+H]^+$ 261.1598, found 261.1596;

HPLC analysis t_R 4.7 min, purity 95.80%.

1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (54)

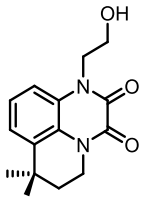


The title compound was synthesized from **24** using similar procedure employed for **20**.

Yield 220 mg, 65%;

Product was purified by column chromatography, confirmed by mass spectroscopy and forwarded for next step without extensive characterization.

1-(2-hydroxyethyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (55)



The title compound was synthesized from **54** using similar procedure employed for **41i**.

Yield 122 mg, 78%;

Melting point 204-207 °C;

^{1}H NMR (200 MHz, CD_3OD) δ 7.44 - 7.26 (m, 3 H), 4.39 (t, *J* = 6.1 Hz, 2 H), 4.14 (t, *J* = 6.1 Hz, 2 H), 3.87 (t, *J* = 6.1 Hz, 2 H), 1.94 (t, *J* = 6.2 Hz, 2 H), 1.38 (s, 6 H);

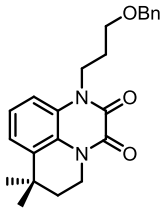
^{13}C NMR (50 MHz, CD_3OD) δ 156.2, 155.6, 136.5, 128.2, 125.3, 124.0, 122.9, 115.0, 59.5, 46.4, 40.4, 35.5, 33.1, 30.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3426, 2724, 1670, 1459, 1374, 1309 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{18}N_2O_3Na$ $[M+Na]^+$ 297.1210, found 297.1210;

HPLC analysis t_R 4.3 min, purity 98.5%.

1-(3-(benzyloxy)propyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (56):



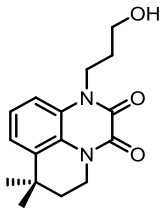
The title compound was synthesized from **24** using similar procedure employed for **20**.

Yield 214 mg, 65%;

HRMS (ESI) *m/z* calculated for $C_{23}H_{27}N_2O_3$ $[M+H]^+$ 379.2016, found 379.2013.

Desired product was obtained confirmed by Mass spectroscopy and forwarded for next step.

1-(3-hydroxypropyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (57)



The title compound was synthesized from **56** using similar procedure employed for **53**.

Yield 60 mg, 66%;

Melting point 152 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 - 7.23 (m, 3 H), 4.41 (t, *J* = 6.5 Hz, 2 H), 4.22 - 4.18 (m, 2 H), 3.64 (t, *J* = 5.5 Hz, 2 H), 3.30 (br. s., 1 H), 2.03 (quin, *J* = 6.0 Hz, 2 H), 1.95 - 1.92 (m, 2 H), 1.40 (s, 6 H);

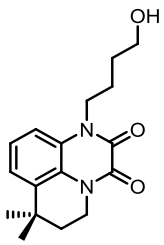
^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 153.4, 135.0, 126.0, 124.0, 122.8, 121.6, 112.9, 58.5, 40.2, 39.1, 34.3, 32.0, 29.9, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3452, 3010, 1676, 1606, 1400, 1367 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{20}N_2O_3Na$ $[M+Na]^+$ 311.1366, found 311.1364;

HPLC analysis t_R 4.4 min, purity 98.9%.

1-(4-hydroxybutyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (59)



Compound **59** was prepared from compound **24** following the same procedure as mentioned for the synthesis of **20** to afford compound **58** (230 mg, 63% yield) as colourless gum product. TBS protection was then removed using TBAF as mentioned in the synthesis of **41i** to afford **59** (75 mg, 54% yield) as colourless solid product

Melting point 110-113 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.25 - 7.14 (m, 3 H), 4.26 (t, *J* = 7.8 Hz, 2 H), 4.16 (t, *J* = 6.2 Hz, 2 H), 3.73 (t, *J* = 6.0 Hz, 2 H), 2.15 (br. s., 1 H), 1.93- 1.72 (m, 4 H), 1.69 - 1.66 (m, 2 H), 1.36 (s, 6 H);

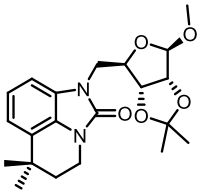
^{13}C NMR (50 MHz, $CDCl_3$) δ 153.9, 153.7, 134.9, 126.1, 123.9, 122.7, 121.3, 112.9, 62.0, 43.0, 39.0, 34.3, 31.9, 29.9, 29.4, 23.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3456, 3019, 1677, 1599, 1402, 1311 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{23}N_2O_3$ $[M+H]^+$ 303.1703, found 303.1701;

HPLC analysis t_R 4.4 min, purity 95.4%.

1-(((3aR,4R,6R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (61)



The title compound was synthesized from **21** using similar procedure employed for **40**.

Yield 135 mg, 70%;

Specific rotation $[\alpha]_D^{25}$ = -70.9 (*c* = 1.4, $CHCl_3$);

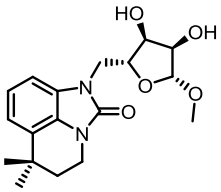
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 - 6.97 (m, 2 H), 6.87 (d, *J* = 7.3 Hz, 1 H), 5.02 (s, 1 H), 4.90 (d, *J* = 6.0 Hz, 1 H), 4.74 (d, *J* = 6.0 Hz, 1 H), 4.51 (dd, *J* = 4.6, 10.1 Hz, 1 H), 4.18 (dd, *J* = 10.5, 14.2 Hz, 1 H), 3.88 (t, *J* = 5.7 Hz, 2 H), 3.79 (dd, *J* = 4.6, 14.2 Hz, 1 H), 3.40 (s, 3 H), 1.90 (t, *J* = 5.9 Hz, 2 H), 1.43 (s, 3 H), 1.35 (s, 3 H), 1.34 (s, 3 H), 1.28 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 128.4, 127.4, 125.2, 121.0, 116.4, 112.3, 109.8, 105.1, 85.2, 84.1, 82.2, 55.2, 43.9, 36.5, 36.2, 31.7, 28.5, 26.3, 24.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3413, 3008, 1699, 1644, 1499, 1454 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{21}H_{28}N_2O_5Na$ $[M+Na]^+$ 411.1890, found 411.1885.

1-(((2R,3S,4R,5R)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (62)



The title compound was synthesized from **61** using similar procedure employed for **31**.

Yield 45 mg, 72%;

Specific Rotation $[\alpha]_D^{25}$ = -8 (*c* = 0.3, $CHCl_3$);

Melting Point 161-164 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 7.02 (s, 3 H), 4.67 (s, 1 H), 4.27 - 4.21 (m, 1 H), 4.20 - 4.07 (m, 2 H), 4.05 - 3.97 (m, 1 H), 3.88 - 3.84 (m, 3 H), 3.19 (s, 3 H), 1.89 (t, *J* = 5.7 Hz, 2 H), 1.33 (s, 6 H);

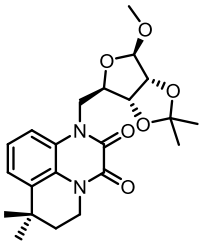
^{13}C NMR (100 MHz, CD_3OD) δ 155.3, 130.1, 129.7, 126.2, 122.6, 117.7, 110.2, 107.8, 81.9, 76.2, 74.1, 55.9, 45.4, 37.9, 37.6, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3386, 3012, 1686, 1498, 1424, 1217 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{25}N_2O_5$ $[M+H]^+$ 349.1758, found 349.1755;

HPLC analysis t_R 4.5 min, purity 96.2%.

1-(((3aR,4R,6R,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (63)



The title compound was synthesized from **24** using similar procedure employed for **40**.

Yield 130 mg, 47%;

Specific Rotation $[\alpha]_D^{25}$ = -16.6 (*c* = 0.7, $CHCl_3$);

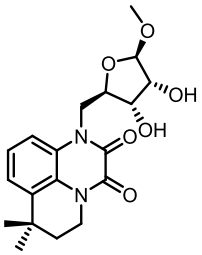
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 - 7.19 (m, 3 H), 5.01 (s, 1 H), 4.98 - 4.92 (m, 2 H), 4.76 (d, *J* = 7.6 Hz, 1 H), 4.46 (dd, *J* = 3.1, 10.1 Hz, 1 H), 4.18 (t, *J* = 6.2 Hz, 2 H), 3.87 (dd, *J* = 3.4, 14.4 Hz, 1 H), 3.42 (s, 3 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.39 (s, 3 H), 1.37 (s, 6 H), 1.24 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2, 153.4, 135.2, 125.9, 124.0, 122.8, 121.6, 112.8, 112.3, 110.0, 85.3, 83.3, 82.3, 55.3, 45.1, 39.0, 34.3, 32.0, 29.9, 26.2, 24.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3406, 3020, 1683, 1605, 1457, 1395 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{22}H_{28}N_2O_6Na$ $[M+Na]^+$ 439.1840, found 439.1835.

1-(((2R,3S,4R,5R)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (64)



The title compound was synthesized from **63** using similar procedure employed for **31**.

Yield 18 mg, 33%;

Specific Rotation $[\alpha]_D^{25}$ = -23.1 (*c* = 0.2, $CHCl_3$);

Melting Point 195-198 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 7.43 (d, *J* = 8.3 Hz, 1 H), 7.38 (d, *J* = 8.8 Hz, 1 H), 7.26 (t, *J* = 8.1 Hz, 1 H), 4.68 (s, 1 H), 4.59 - 4.52 (m, 2 H), 4.35 - 4.29 (m, 1 H), 4.28 - 4.21 (m, 1 H), 4.21 - 4.13 (m, 2 H), 3.89 (d, *J* = 4.4 Hz, 1 H), 3.29 (s, 3 H), 1.95 (t, *J* = 6.3 Hz, 2 H), 1.39 (s, 3 H), 1.39 (s, 3 H);

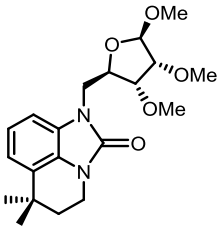
^{13}C NMR (100 MHz, CD_3OD) δ 156.4, 155.6, 136.5, 128.2, 125.3, 125.2, 124.1, 123.0, 115.4, 110.4, 80.7, 76.3, 74.9, 56.0, 47.5, 40.4, 35.6, 33.2, 30.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3393, 3020, 1667, 1603, 1454, 1308 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{24}N_2O_6Na$ $[M+Na]^+$ 399.1527, found 399.1523;

HPLC analysis t_R 4.2 min, purity 99.7%.

6,6-dimethyl-1-(((2R,3R,4R)-3,4,5-trimethoxytetrahydrofuran-2-yl)methyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (65)



To a solution of 1-(((2R,3S,4R)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **62** (30 mg, 0.086 mmol) in dry THF (5 mL) was added caesium carbonate (69 mg, 0.21 mmol), followed by methyl iodide (27 µl, 0.43 mmol) and kept stirring for 3 at 50°C. The reaction mixture was added to cold water and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4, concentrated under reduced pressure, and subjected to flash chromatography over (100-200) silica gel to afford desired product **65** (20 mg, 62% yield) as colourless gum product.

Specific Rotation $[\alpha]_D^{25}$ = +22.7 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 - 6.91 (m, 3 H), 4.85 (s, 1 H), 4.35 (td, *J* = 5.1, 7.6 Hz, 1 H), 4.09 (dd, *J* = 1.3, 5.0 Hz, 2 H), 3.95 (dd, *J* = 4.4, 7.6 Hz, 1 H), 3.90 (t, *J* = 5.6 Hz, 2 H), 3.70 (d, *J* = 4.6 Hz, 1 H), 3.47 (s, 3 H), 3.44 (s, 3 H), 3.22 (s, 3 H), 1.90 (t, *J* = 6.1 Hz, 2 H), 1.35 (s, 3 H), 1.34 (s, 3 H);

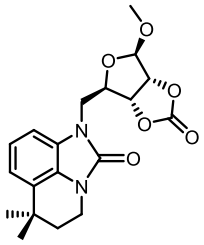
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6, 128.3, 128.1, 125.1, 120.8, 116.1, 106.0, 105.6, 81.4, 81.3, 79.0, 58.3, 58.2, 55.3, 44.1, 36.7, 36.3, 31.7, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3424, 3019, 1694, 1644, 1215 cm^{-1};

HRMS (ESI): *m*/*z* calculated for $C_{20}H_{29}N_2O_5$ $[M+H]^+$ 377.2071, found 377.2064;

HPLC analysis: t_R 5.2 min, purity 95.7%.

1-(((3aR,4R,6aR)-6-methoxy-2-oxotetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (66)



To a solution of 1-(((2R,3S,4R)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **62** (50 mg, 0.14 mmol) in dry DCM (4 mL) was added carbonyldiimidazole (27 mg, 0.17 mmol), followed by triethyl amine (8 µl, 0.05 mmol) and kept stirring for 12 at room temperature. The reaction mixture was added to cold water (5 mL) and extracted with ethyl acetate (20 mL). The combined organic layer was washed with brine and dried over sodium sulphate, concentrated under

reduced pressure, and subjected to flash chromatography over (100-200) silica gel to afford desired product **66** (52 mg, 96% yield) as colourless solid product.

Specific Rotation $[\alpha]_D^{25}$ = -47.0 (*c* = 0.2, $CHCl_3$);

Melting Point 85-88 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 - 7.03 (m, 2 H), 6.87- 6.86 (m, 1 H), 5.48 (d, *J* = 6.6 Hz, 1 H), 5.21 (s, 1 H), 5.15 (d, *J* = 6.8 Hz, 1 H), 4.65 (dd, *J* = 4.0, 11.1 Hz, 1 H), 4.28 (dd, *J* = 11.1, 14.5 Hz, 1 H), 3.92 (t, *J* = 6.1 Hz, 2 H), 3.84 (dd, *J* = 4.2, 14.7 Hz, 1 H), 3.46 (s, 3 H), 1.92 (t, *J* = 6.1 Hz, 2 H), 1.38 (s, 3 H), 1.36 (s, 3 H);

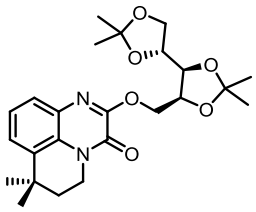
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4, 153.2, 129.0, 127.0, 125.2, 121.4, 117.0, 108.1, 104.9, 83.5, 83.3, 81.0, 55.7, 42.8, 36.5, 36.4, 31.8, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3405, 1813, 1698, 1498, 1380, 1102, cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{23}N_2O_6$ $[M+H]^+$ 375.1551, found 375.1544;

HPLC analysis t_R 4.7 min, purity 96.10%.

7,7-dimethyl-2-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methoxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (70)



To a solution of 7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **24** (100 mg, 0.43 mmol) in dry THF (5 mL) was added triphenylphosphine (262 mg, 1.00 mmol), ((4*S*,4'*R*,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methanol **67** (121 mg, 0.52 mmol) and DIAD (194 µL, 1.00 mmol) sequentially and stirred for 12 h at room temperature. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 X 20 mL), and the organic layer was washed successively with water and brine, dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by flash silica gel column chromatography (40% EtOAc: DCM) to afford *O*-alkylated product **70** (152 mg, 78 %) as brown coloured oil.

Specific Rotation $[\alpha]_D^{25}$ = +6.3 (*c* = 1.0, $CHCl_3$);

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.3 Hz, 1 H), 7.38 (d, *J* = 8.3 Hz, 1 H), 7.26 (d, *J* = 8.3 Hz, 1 H), 4.79 (m, 1 H), 4.77 - 4.63 (m, 2 H), 4.26 - 4.09 (m, 5 H), 4.00 - 3.93 (m, 1 H),

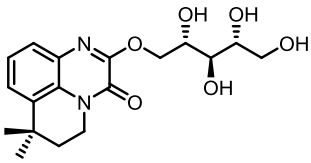
1.93 (t, *J* = 6.4 Hz, 2 H), 1.49 (s, 3 H), 1.41 (s, 3 H), 1.38 (s, 6 H), 1.37 (s, 3 H), 1.31 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 150.3, 133.4, 130.7, 126.8, 125.3, 123.5, 123.3, 109.7, 109.4, 77.7, 75.5, 73.5, 68.0, 65.7, 38.7, 34.7, 31.4, 27.7, 26.8, 25.5, 25.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3309, 2983, 1720, 1664, 1608, 1509 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{24}H_{32}N_2O_6Na$ $[M+Na]^+$ 467.2153, found 467.2146.

7,7-dimethyl-2-(((2S,3R,4R)-2,3,4,5-tetrahydroxypentyl)oxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (71)



The title compound was synthesized from **70** using similar procedure employed for **31**.

Yield 38 mg, 58%;

Specific Rotation $[\alpha]_D^{25}$ = +2.3 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.48 - 7.46 (m, 2 H), 7.45 - 7.26 (m, 1 H), 4.68 (dd, *J* = 3.0, 11.2 Hz, 1 H), 4.48 (dd, *J* = 7.3, 11.4 Hz, 1 H), 4.26 - 4.16 (m, 3 H), 3.83 - 3.77 (m, 4 H), 1.97 (t, *J* = 6.41Hz, 2 H), 1.39 (s, 6 H);

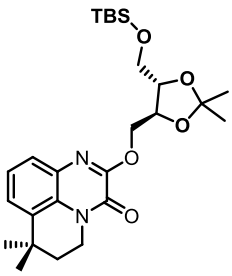
^{13}C NMR (100 MHz, CD_3OD) δ 154.3, 152.3, 135.3, 132.4, 127.5, 126.3, 125.3, 124.9, 79.1 74.1, 73.7, 71.8, 70.3, 64.5, 40.1, 35.7, 32.5, 30.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3386, 2929, 1654, 1607, 1455, 1299 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{24}N_2O_6Na$ $[M+Na]^+$ 387.1532, found 387.1527;

HPLC analysis t_R 3.2 min, purity 98.21%.

2-(((4S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-7,7-dimethyl-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (72)



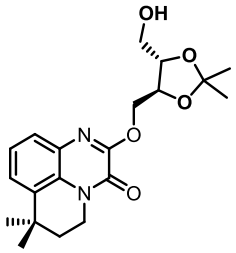
The title compound was synthesized from **24** using similar procedure employed for **70**.

Yield 186 mg, 59%;

The desired product formed was confirmed by HRMS and forwarded for next reaction without further extensive characterization.

HRMS (ESI) *m/z* calculated for $C_{26}H_{40}N_2O_5SiNa$ $[M+Na]^+$ 511.2599, found 511.2596.

2-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-7,7-dimethyl-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (73i)



The title compound was synthesized from **72** using similar procedure employed for **41i**.

Yield 30 mg, 66%;

Specific Rotation $[\alpha]_D^{25}$ = +5.6 (*c* = 2.0, $CHCl_3$);

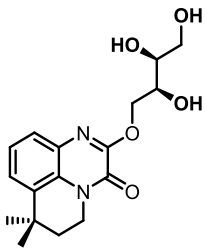
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 - 7.46 (m, 1 H), 7.40 - 7.38 (m, 1 H), 7.27 - 7.25 (m, 1 H), 4.74 (dd, *J* = 4.6, 11.2 Hz, 1 H), 4.51 (dd, *J* = 5.9, 11.2 Hz, 1 H), 4.36 - 4.29 (m, 1 H), 4.23 - 4.17 (m, 2 H), 4.14 (td, *J* = 4.3, 8.3 Hz, 1 H), 3.95 - 3.77 (m, 2 H), 2.63 (br. s., 1 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.48 (s, 3 H), 1.45 (s, 3 H), 1.38 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 150.3, 133.6, 130.6, 126.8, 125.3, 123.7, 109.7, 79.8, 75.8, 66.9, 62.6, 38.7, 34.6, 31.4, 29.7, 27.0, 26.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3443, 3013, 1661, 1610, 1476, 1445 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}N_2O_5Na$ $[M+Na]^+$ 397.1734, found 397.1727.

7,7-dimethyl-2-((2S,3S)-2,3,4-trihydroxybutoxy)-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (73)



The title compound was synthesized from **73i** using similar procedure employed for **31**.

Yield 18 mg, 69%;

Specific Rotation $[\alpha]_D^{25}$ = +4.5 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (d, *J* = 7.8 Hz, 2 H), 7.29 (t, *J* = 7.8 Hz, 1 H), 4.56 - 4.49 (m, 2 H), 4.23 - 4.20 (m, 2 H), 4.19 - 4.08 (m, 1 H), 3.82 - 3.65 (m, 3 H), 1.98 (t, *J* = 6.1 Hz, 1 H), 1.42 (s, 6 H);

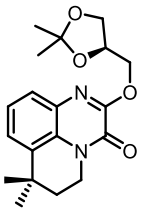
^{13}C NMR (100 MHz, CD_3OD) δ 154.5, 152.4, 135.6, 132.6, 127.9, 126.4, 125.3, 125.1, 73.2, 70.6, 70.3, 64.4, 40.2, 35.9, 32.7, 30.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3405, 3021, 1654, 1609, 1299 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{22}N_2O_5Na$ $[M+Na]^+$ 357.1421, found 357.1417;

HPLC analysis t_R 4.3 min, purity 96.65%.

(R)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-7,7-dimethyl-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (74)



The title compound was synthesized from **24** using similar procedure employed for **70**.

Yield 96 mg, 64%;

Specific Rotation $[\alpha]_D^{25}$ = +8.8 (*c* 0.6, $CHCl_3$);

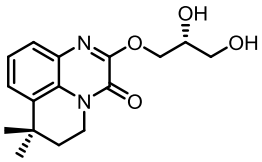
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 - 7.46 (m, 1 H), 7.37 (dd, *J* = 1.3, 7.7 Hz, 1 H), 7.26 (t, *J* = 7.8 Hz, 1 H), 4.60 - 4.57 (m, 2 H), 4.43 - 4.42 (m, 1 H), 4.21 - 4.18 (m, 3 H), 3.99 (dd, *J* = 5.5, 8.7 Hz, 1 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.49 (s, 3 H), 1.39 (s, 3 H), 1.38 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 150.2, 133.5, 130.6, 126.8, 125.3, 123.6, 123.5, 109.7, 73.3, 67.4, 67.1, 38.7, 34.7, 31.4, 29.7, 26.8, 25.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3425, 3014, 1663, 1610, 1477, 1377 cm^{-1};

HRMS (ESI): *m/z* calculated for $C_{19}H_{24}N_2O_4Na$ $[M+Na]^+$ 367.1628, found 367.1631.

(S)-2-(2,3-dihydroxypropoxy)-7,7-dimethyl-6,7-dihydro-3H,5H-pyrido[1,2,3-de]quinoxalin-3-one (75)



The title compound was synthesized from **74** using similar procedure employed for **31**.

Yield 38 mg, 54%;

Specific Rotation $[\alpha]_D^{25}$ = +1.0 (*c* 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (t, *J* = 8.7 Hz, 2 H), 7.26 (t, *J* = 7.8 Hz, 1 H), 4.51 (dd, *J* = 3.7, 11.0 Hz, 1 H), 4.39 (dd, *J* = 6.9, 11.0 Hz, 1 H), 4.18 - 4.17 (m, 2 H), 4.10 - 4.00 (m, 1 H), 3.69 (d, *J* = 6.0 Hz, 2 H), 1.95 (t, *J* = 5.9 Hz, 2 H), 1.38 (s, 6 H);

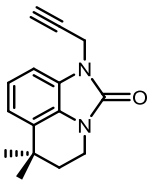
^{13}C NMR (100 MHz, CD_3OD) δ 152.9, 151.0, 134.0, 131.0, 126.3, 125.2, 124.1, 123.7, 69.9, 68.8, 62.7, 38.9, 34.5, 31.3, 29.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3402, 3019, 2358, 1655, 1608, 1299 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{21}N_2O_4$ $[M+H]^+$ 305.1496, found 305.1492;

HPLC analysis t_R 4.5 min, purity 99.43%.

6,6-dimethyl-1-(prop-2-yn-1-yl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (76)



The title compound was synthesized from **21** using similar procedure employed for **20**.

Yield 550 mg, 92%;

Melting Point 143-145°C;

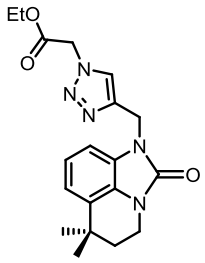
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.01 (m, 3 H), 4.68 (s, 2 H), 3.90 (t, *J* = 6.6 Hz, 2 H), 2.29 (s, 1 H), 1.90 (t, *J* = 6.6 Hz, 2 H), 1.36 (s, 6 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 152.4, 128.4, 126.8, 125.1, 121.0, 116.6, 106.0, 77.3, 72.5, 36.6, 36.3, 31.7, 30.5, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3372, 2955, 1693, 1631, 1494, 1344 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{16}N_2ONa$ $[M+Na]^+$ 263.1155, found 263.1152.

Ethyl 2-(4-((6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (77)



A solution of 6,6-dimethyl-1-(prop-2-yn-1-yl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **76** (50 mg, 0.20 mmol) in acetonitrile (5 mL) was treated with Ethyl azidoacetate (54 mg, 0.22 mmol), DIPEA (111 µL, 0.62 mmol) and cat. CuI (4 mg, 0.02 mmol) and stirred for 12 h at room temperature. The reaction mixture was added to water (10 mL) and extracted with ethyl acetate (3 X 20 mL) and washed with water, brine and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The resultant residue was purified by flash chromatography on silica (50% Ethyl acetate: DCM) to afford compound **77** (40 mg, 53%) as colourless solid product

Melting Point 120-123 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1 H), 6.99 - 6.95 (m, 3 H), 5.16 (s, 2 H), 5.07 (s, 2 H), 4.20 (q, *J* = 7.0 Hz, 2 H), 3.86 (t, *J* = 5.9 Hz, 2 H), 1.86 (t, *J* = 5.9 Hz, 2 H), 1.31 (s, 6 H), 1.23 (t, *J* = 7.1 Hz, 3 H);

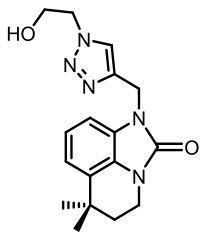
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 152.8, 143.7, 128.2, 127.1, 124.9, 124.0, 121.0, 116.3, 106.1, 62.2, 50.7, 36.4, 36.4, 36.2, 31.6, 28.4, 13.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3457, 2959, 1750, 1699, 1496, 1349 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{23}N_5O_3Na$ $[M+Na]^+$ 392.1693, found 392.1687;

HPLC analysis: t_R 4.9 min, purity 96.90%.

1-((1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one(78)



To a pre-cooled solution of Lithium aluminium hydride (10 mg, 0.27 mmol) in THF (5 mL) was added drop wise solution of Ethyl 2-(4-((6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate **77** **(**100 mg, 0.27

mmol) in THF at 0°C and stirred for 1 h at same temperature. Quench the reaction mixture with a saturated solution of ammonium chloride (5 mL) and extract with ethyl acetate (3 X 10 mL) and washed with water, brine and the combined organic layers were dried over sodium sulphate, filtered and concentrated in vaccume. The resultant residue was purified by flash chromatography on silica (5% MeOH:DCM) to afford compound **78** (58 mg, 65%) as a white solid product.

Melting Point 88-91 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1 H), 7.05 - 6.96 (m, 3 H), 5.07 (s, 2 H), 4.43 - 4.40 (m, 2 H), 4.00 - 3.99 (m, 2 H), 3.92 (br. s, 1H), 3.86 - 3.83 (m, 2 H), 1.87 (t, *J* = 5.9 Hz, 2 H), 1.32 (s, 6 H);

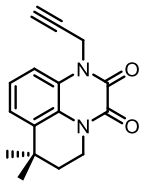
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 143.1, 128.4, 127.1, 124.8, 123.8, 121.3, 116.5, 106.2, 60.8, 52.7, 36.5, 36.4, 36.3, 31.7, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3394, 3006, 1689, 1495, 1423, 1284 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{21}N_5O_2Na$ $[M+Na]^+$ 350.1587, found 350.1583;

HPLC analysis t_R 4.3 min, purity 95.24%.

7,7-dimethyl-1-(prop-2-yn-1-yl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (79)



The title compound was synthesized from **24** using similar procedure employed for **20**.

Yield 480 mg, 81%;

Melting Point 142-145 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.22 (m, 3 H), 4.98 (d, *J* = 1.8 Hz, 2 H), 4.14 (, *J* = 6.3 Hz, 2 H), 2.27 (s, 1 H), 1.89 (t, *J* = 6.3 Hz, 2 H), 1.35 (s, 6 H);

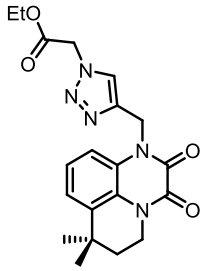
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4, 153.3, 134.9, 125.6, 123.9, 122.6, 121.7, 113.3, 76.7, 73.4, 39.1, 34.3, 32.7, 31.9, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3575, 3351, 2957, 1681, 1600, 1471 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{16}N_2O_2Na$ $[M+Na]^+$ 291.1104, found 291.1099;

HPLC analysis t_R 4.4 min, purity 97.27%.

Ethyl 2-(4-((7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (80)



The title compound was synthesized from **79** using similar procedure employed for **77**.

Yield 183 mg, 82%;

Melting Point 157-160 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1 H), 7.73 (dd, *J* = 3.2, 6.4 Hz, 1 H), 7.24 - 7.22 (m, 2 H), 5.48 (s, 2 H), 5.12 (s, 2 H), 4.23 (q, *J* = 7.2 Hz, 2 H), 4.17 - 4.12 (m, 2 H), 1.90 (t, *J* = 6.4 Hz, 2 H), 1.35 (s, 6 H), 1.25 (t, *J* = 7.1 Hz, 3 H);

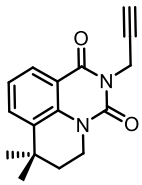
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 154.0, 153.6, 142.5, 134.7, 126.2, 125.5, 124.1, 122.5, 121.6, 113.8, 62.4, 50.8, 39.0, 34.3, 31.9, 29.8, 13.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3474, 3141, 2969, 1751, 1681, 1475 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_5O_4Na$ $[M+Na]^+$ 420.1642, found 420.1636;

HPLC analysis t_R 4.3 min, purity 96.26%.

7,7-dimethyl-2-(prop-2-yn-1-yl)-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione (81)



The title compound was synthesized from **28** using similar procedure employed for **20**.

Yield 425 mg, 91%;

Melting Point 169-172 °C;

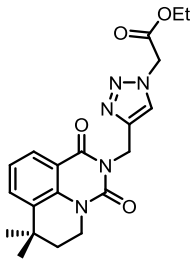
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.6 Hz, 1 H), 7.63 (d, *J* = 7.3 Hz, 1 H), 7.19 (t, *J* = 7.7 Hz, 1 H), 4.86 (d, *J* = 1.7 Hz, 2 H), 4.11 (t, *J* = 6.1 Hz, 2 H), 2.19 (br. s., 1 H), 1.91 (t, *J* = 6.1 Hz, 2 H), 1.37 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 149.8, 135.7, 133.4, 131.7, 126.8, 122.8, 115.0, 78.4, 70.6, 39.4, 34.7, 31.9, 30.7, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3415, 3021, 1701, 1658, 1602, 1495 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{16}N_2O_2Na$ $[M+Na]^+$ 291.1104, found 291.1102.

Ethyl 2-(4-((7,7-dimethyl-1,3-dioxo-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazolin-2(3H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (82)



7,7-dimethyl-2-(prop-2-yn-1-yl)-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione **81** (100 mg, 0.37 mmol) and Ethyl azidoacetate (52 mg, 0.41 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (5 mL). Sodium ascorbate (14 mg, 0.07 mmol) was added, followed by copper sulphate pentahydrate (9.2 mg, 0.037 mmol). The heterogeneous mixture was stirred vigorously overnight. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (30 mL); the organic layer was washed successively with water and brine, dried over sodium sulphate, and concentrated in *vaccuo.* The residue was purified by flash silica gel column chromatography to afford desired product **82** (140 mg, 94% yield) as colourless gum product.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.8 Hz, 1 H), 7.81 (s, 1 H), 7.60 (d, *J* = 7.6 Hz, 1 H), 7.17 (t, *J* = 7.7 Hz, 1 H), 5.42 (s, 2 H), 5.10 (s, 2 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 4.08 (t, *J* = 6.1 Hz, 2 H), 1.88 (t, *J* = 6.1 Hz, 2 H), 1.35 (s, 6 H), 1.27 (t, *J* = 7.2 Hz, 3 H);

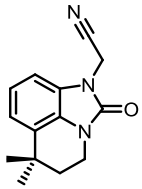
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 161.6, 150.2, 143.7, 135.8, 133.3, 131.5, 126.7, 124.9, 122.6, 115.1, 62.2, 50.7, 39.4, 36.3, 34.7, 31.9, 29.8, 14.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3016, 1751, 1700, 1654, 1601,1493 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{23}N_5O_4Na$ $[M+Na]^+$ 420.1642, found 420.1643;

HPLC analysis t_R 4.5 min, purity 99.72%.

2-(6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)acetonitrile (83)



The title compound was synthesized from **21** using similar procedure employed for **20**.

Yield 510 mg, 86%;

Melting Point 142-145 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (br. s, 2 H), 6.97 - 6.96 (m, 1 H), 4.81 (s, 2 H), 3.90 (t, *J* = 6.0 Hz, 2 H), 1.91 (t, *J* = 6.1 Hz, 2 H), 1.36 (s, 6 H);

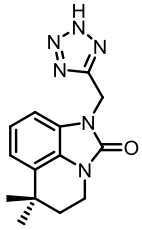
^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 129.1, 125.9, 125.2, 121.7, 117.8, 114.0, 105.5, 36.6, 36.5, 31.8, 29.0, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 2935, 2210, 1646, 1512, 909 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{14}H_{15}N_3ONa$ $[M+Na]^+$ 264.1107, found 264.1108;

HPLC analysis t_R 4.5 min, purity 99.06%.

1-((2H-tetrazol-5-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (84)



A mixture of the 2-(6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)acetonitrile **83** (50 mg, 0.20 mmol), sodium azide (16 mg, 0.24 mmol) and ammonium chloride (13 mg, 0.24 mmol) in dry DMF (2 mL) was heated under 120 °C for 12 h. Then the reaction mixture was then cooled to room temperature and added to crushed ice white solid precipitated obtained was collected by filtration and dried under *vaccue* to afford **84** (42 mg, 72 % yield) as colourless solid product

Melting Point 220-223 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, *J* = 7.6 Hz, 1 H), 7.11 (t, *J* = 7.8 Hz, 1 H), 7.05 (d, *J* = 7.6 Hz, 1 H), 5.47 (s, 2 H), 3.94 (t, *J* = 5.9 Hz, 2 H), 1.90 (t, *J* = 5.9 Hz, 2 H), 1.33 (s, 6 H);

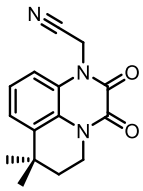
^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 153.3, 129.4, 126.5, 124.7, 122.3, 117.7, 106.5, 36.8, 36.4, 34.2, 31.7, 28.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3583, 3020, 1672, 1421, 1068 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{14}H_{16}N_6O$ $[M+Na]^+$ 307.1278, found 307.1274.

HPLC analysis t_R 4.4 min, purity 95.78%.

2-(7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)acetonitrile (85)



The title compound was synthesized from **24** using similar procedure employed for **20**.

Yield 551 mg, 94%;

Melting Point 171-174 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 7.27 (m, 2 H), 7.15 - 7.13 (m, 1 H), 5.19 (br. s., 2 H), 4.16 (t, *J* = 6.2 Hz, 2 H), 1.92 (t, *J* = 6.2 Hz, 2 H), 1.38 (s, 6 H);

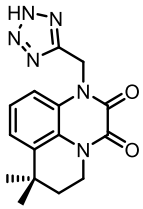
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.3, 152.7, 135.5, 124.9, 124.4, 122.6, 122.5, 113.6, 112.2, 39.2, 34.2, 32.0, 30.6, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3343, 3011, 2250, 1688, 1599, 1395 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{15}N_3O_2Na$ $[M+Na]^+$ 292.1056, found 292.1057;

HPLC analysis t_R 4.2 min, purity 97.16%.

1-((2H-tetrazol-5-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxal--ine-2,3-dione72 (86)



The title compound was synthesized from **85** using similar procedure employed for **84**.

Yield 43 mg, 75%;

Melting Point 262-265 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.1 Hz, 1 H), 7.28 - 7.22 (m, 1 H), 7.20 - 7.17 (m, 1 H), 5.67 (s, 2 H), 4.09 (t, *J* = 5.1 Hz, 2 H), 3.84 (br. s., 1 H), 1.87 (t, *J* = 5.1 Hz, 2 H), 1.31 (s, 6 H);

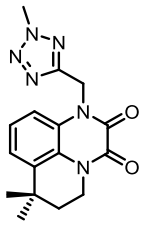
^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 153.5, 151.6, 135.2, 125.5, 124.6, 122.3, 113.1, 39.2, 36.7, 34.1, 31.8, 29.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3575, 3066, 1671, 1600, 1418, 1337 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{16}N_6O_2Na$ $[M+Na]^+$ 335.1227, found 335.1228;

HPLC analysis t_R 4.1 min, purity 99.21%.

7,7-dimethyl-1-((2-methyl-2H-tetrazol-5-yl)methyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (87)



To a solution of 1-((2H-tetrazol-5-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **86** (250 mg, 0.80 mmol) in dry DMF was added Potassium carbonate (132 mg, 0.96 mmol), followed by Methyl iodide (60 μL, 0.096 mmol) and kept stirring for 12 h at room temperature reaction. The reaction mixture was added to cold water and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4, concentrated under reduced pressure, and subjected to flash chromatography over silica gel (30 % EtOAc: DCM) to afford desired product **87** (170 mg, 60% yield) as yellow solid product

Melting Point 133-136 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28 - 7.25 (m, 1 H), 7.14 (d, *J* = 5.1 Hz, 2 H), 5.70 (s, 2 H), 4.29 (s, 3 H), 4.20 (t, *J* = 6.1 Hz, 2 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.37 (s, 6 H);

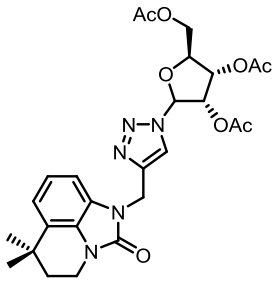
^{13}C NMR (50 MHz, $CDCl_3$) δ 161.4, 153.9, 153.4, 134.9, 126.1, 123.9, 122.6, 121.6, 113.0, 39.6, 39.1, 38.3, 34.3, 31.9, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3463, 3015, 1685, 1599, 1486, 1401 cm^{-1};

HRMS (ESI) *m/z* calculated for C16H18N6O2Na $[M+Na]^+$ 349.1383, found 349.1379.

HPLC analysis t_R 4.2 min, purity 97.18%.

(2S,3S,4S)-2-(acetoxymethyl)-5-(4-((6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-3,4-diyldiacetate (91i)



intermediate

To a solution of 6,6-dimethyl-1-(prop-2-yn-1-yl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one **76** (20 mg, 0.083) and (2R,3R,4R,5R)-2-(acetoxymethyl)-5-azidotetrahydrofuran-3,4-diyl diacetate (25 mg, 0.083) in acetonitrile (3 mL) was added CuI (31 mg, 0.166 mmol), DIPEA (0.044 μL, 0.249 mmol) at room temperature. The resultant mixture was stirred for for 2 h. The reaction mixture diluted with water (5 mL) and extracted with ethyl acetate (10 mL X 2). The combined organic layer dried over sodium sulphate concentrated under reduced pressure, and purified by column chromatography over (100-200) silica gel to afford desired product **91i** (34 mg, 75%) as colourless gum

Specific Rotation $[\alpha]_D^{25}$ = -33.0 (*c* = 1.3, $CHCl_3$);

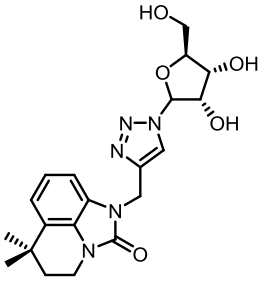
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1 H), 7.06 - 6.99 (m, 3 H), 6.09 (d, *J* = 3.9 Hz, 1 H), 5.80 (t, *J* = 4.9 Hz, 1 H), 5.61 (t, *J* = 5.3 Hz, 1 H), 5.18 (s, 2 H), 4.48 - 4.43 (m, 1 H), 4.38 (dd, *J* = 3.1, 12.3 Hz, 1 H), 4.21 (dd, *J* = 4.3, 12.3 Hz, 1 H), 3.91 (t, *J* = 5.9 Hz, 2 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.01 (s, 3 H), 1.91 (t, *J* = 6.0 Hz, 2 H), 1.34 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.4, 169.2, 152.9, 144.0, 128.4, 127.2, 125.0, 122.1, 121.2, 116.5, 106.1, 90.0, 80.9, 74.3, 70.7, 62.8, 36.6, 36.3, 31.7, 28.5, 20.5, 20.4, 20.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3403, 3018, 1751, 1696, 1499, 1222 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{26}H_{31}N_5O_8Na$ $[M+Na]^+$ 564.2065, found 564.2069.

1-((1-((3S,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (91)



To a solution of (2S,3S,4S)-2-(acetoxymethyl)-5-(4-((6,6-dimethyl-2-oxo-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate **91i** (70 mg, 0.13 mmol) in MeOH (3 mL) was added NaOMe (21 mg, 0.39 mmol) in MeOH (1 mL) at 4° C, then the mixture was stirred at 50 °C for 3h. The mixture was poured into a saturated aqueous ammonium chloride (10 mL), and the whole was extracted with CH_2Cl_2 (20 mL x 2), dried over sodium sulphate, and concentrated in *vacuo*. The crude product was purified by column chromatography on silica gel (10 % MeOH:DCM) to afford **91** as colourless solid (48 mg, 88%).

Specific Rotation $[\alpha]_D^{25}$ = -19.9 (*c* = 0.3, $CHCl_3$);

Melting Point 80 - 83 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1 H), 7.02 - 6.98 (m, 3 H), 5.99 (d, *J* = 3.9 Hz, 1 H), 5.15 (s, 2 H), 4.46 (t, *J* = 4.5 Hz, 1 H), 4.28 (t, *J* = 5.0 Hz, 1 H), 4.10 (q, *J* = 4.6 Hz, 1 H), 3.87 (t, *J* = 6.0 Hz, 2 H), 3.77 (dd, *J* = 3.2, 12.2 Hz, 1 H), 3.66 (dd, *J* = 4.4, 12.2 Hz, 1 H), 1.89 (t, *J* = 6.0 Hz, 2 H), 1.32 (s, 6 H);

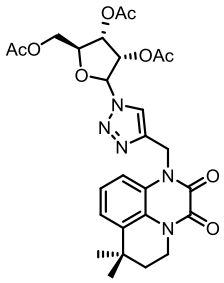
^{13}C NMR (100 MHz, CD_3OD) δ 154.7, 144.7, 130.3, 128.5, 126.3, 123.6, 122.8, 118.0, 107.5, 94.5, 87.3, 77.1, 72.0, 62.9, 37.8, 37.6, 37.3, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3368, 3019, 1687, 1497, 1349 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{25}N_5O_5Na$ $[M+Na]^+$ 438.1748, found 438.1741;

HPLC analysis t_R 4.2 min, purity 97.38%.

(2S,3S,4S)-2-(acetoxymethyl)-5-(4-((7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate (92i)



The title compound was synthesized from **79** using similar procedure employed for **91i**.

Yield 130 mg, 61%;

Specific Rotation $[\alpha]_D^{25}$ = -23.9 (*c* = 1.4, $CHCl_3$);

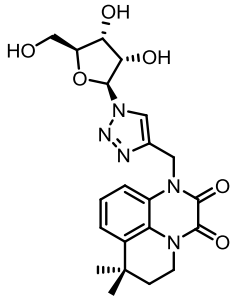
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1 H), 7.77 (dd, *J* = 3.2, 6.4 Hz, 1 H), 7.28 - 7.25 (m, 2 H), 6.10 (d, *J* = 3.4 Hz, 1 H), 5.79 (dd, *J* = 3.8, 5.0 Hz, 1 H), 5.59 (t, *J* = 5.4 Hz, 1 H), 5.59 - 5.48 (m, 2 H), 4.35 - 4.23 (m, 1 H), 4.23 - 4.20 (m, 1 H), 4.19 - 4.16 (m, 3 H), 2.11 (s, 6 H), 2.08 (s, 3 H), 1.92 (t, *J* = 6.1 Hz, 2 H), 1.38 (s, 3 H), 1.37 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.4, 169.2, 154.1, 153.6, 142.6, 134.8, 126.2, 124.2, 123.7, 122.5, 121.7, 113.8, 90.1, 80.9, 74.3, 70.6, 62.7, 39.1, 38.9, 34.4, 31.9, 29.9, 20.6, 20.4, 20.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3403, 3021, 1751, 1680, 1281 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{27}H_{32}N_5O_9$ $[M+H]^+$ 570.2195, found 570.2191.

1-((1-((3S,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (92)



The title compound was synthesized from **92i** using similar procedure employed for **91.**

Yield 38 mg, 69%;

Melting Point 149-152 °C;

Specific Rotation $[\alpha]_D^{25}$ = -29.5 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (s, 1 H), 7.46 (d, *J* = 8.3 Hz, 1 H), 7.34 (d, *J* = 7.8 Hz, 1 H), 7.22 (t, *J* = 8.1 Hz, 1 H), 5.97 (d, *J* = 3.9 Hz, 1 H), 5.53 (s, 2 H), 4.44 (t, *J* = 4.5 Hz, 1 H), 4.26 (t, *J* = 5.0 Hz, 1 H), 4.21 - 4.13 (m, 2 H), 4.13 - 4.07 (m, 1 H), 3.76 (dd, *J* = 3.2, 12.2 Hz, 1 H), 3.64 (dd, *J* = 4.2, 12.2 Hz, 1 H), 1.94 (t, *J* = 6.1 Hz, 1 H), 1.37 (s, 6 H);

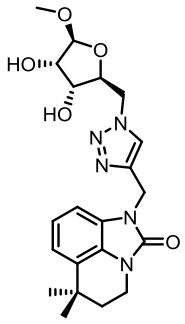
^{13}C NMR (100 MHz, CD_3OD) δ 156.1, 155.7, 143.9, 136.6, 127.7, 125.4, 124.1, 124.0, 123.1, 115.0, 94.5, 87.3, 77.1, 72.0, 62.9, 40.4, 40.0, 35.6, 33.1, 30.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3412, 3022, 669, 1619, 1407, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{21}H_{25}N_5O_6Na$ $[M+Na]^+$ 466.1697, found 466.1691;

HPLC analysis t_R 4.3 min, purity 97.32%.

1-((1-(((2S,3R,4S,5S)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (93)



The title compound was synthesized from **76** using similar procedure employed for **82.**

Yield 68 mg, 76%;

Specific Rotation $[\alpha]_D^{25}$ = -13.8 (*c* = 0.3, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (s, 1 H), 7.01 - 6.98 (m, 3 H), 5.15 (s, 2 H), 4.70 - 4.68 (m, 2 H), 4.66 (dd, *J* = 6.6, 14.2 Hz, 1 H), 4.19 (dt, *J* = 3.4, 7.1 Hz, 1 H), 4.02 (dd, *J* = 4.5, 7.5 Hz, 1 H), 3.88 (t, *J* = 5.9 Hz, 2 H), 3.80 (d, *J* = 4.4 Hz, 1 H), 3.14 (s, 3 H), 1.88 (t, *J* = 5.9 Hz, 2 H), 1.31 (s, 6 H);

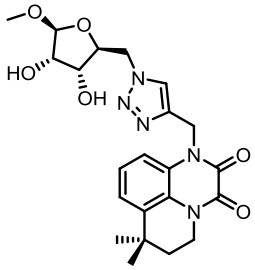
^{13}C NMR (100 MHz, CD_3OD) δ 154.7, 144.4, 130.3, 128.5, 126.3, 125.9, 122.8, 118.0, 110.2, 107.5, 81.8, 76.0, 73.3, 55.8, 54.1, 37.8, 37.6, 37.3, 32.8, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3387, 3017, 1690, 1498, 1352 cm^{-1};

HRMS (ESI): *m/z* calculated for $C_{21}H_{27}N_5O_5Na$ $[M+Na]^+$ 452.1904, found 452.1905;

HPLC analysis: t_R 4.3 min, purity 95.36%.

1-((1-(((2S,3R,4S,5S)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (94)



The title compound was synthesized from **79** using similar procedure employed for **82**.

Yield 135 mg, 79%;

Specific Rotation $[\alpha]_D^{25}$ = -14.6 (*c* = 0.3, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (s, 1 H), 7.47 (d, *J* = 7.6 Hz, 1 H), 7.34 (d, *J* = 7.8 Hz, 1 H), 7.21 (t, *J* = 8.1 Hz, 1 H), 5.52 (d, *J* = 3.2 Hz, 2 H), 4.66 - 4.62 (m, 2 H), 4.46 (dd, *J* = 6.6, 14.4 Hz, 1 H), 4.21 - 4.13 (m, 3 H), 4.00 (dd, *J* = 4.6, 7.3 Hz, 1 H), 3.78 (d, *J* = 4.6 Hz, 1 H), 3.19 (s, 3 H), 1.93 (t, *J* = 6.4 Hz, 2 H), 1.36 (s, 6 H);

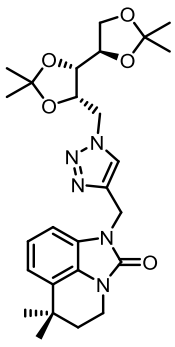
^{13}C NMR (100 MHz, CD_3OD) δ 156.1, 155.6, 143.5, 136.6, 127.7, 126.3, 125.3, 124.1, 123.1, 115.1, 110.3, 81.9, 76.0, 73.3, 55.9, 54.2, 40.4, 40.0, 35.6, 33.1, 30.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3405, 3016, 1676, 1605, 1403, 1311 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{22}H_{27}N_5O_6Na$ $[M+Na]^+$ 480.1854, found 480.1851;

HPLC analysis: t_R 4.1 min, purity 99.99%.

6,6-dimethyl-1-((1-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (95i)



The title compound was synthesized from **76** using similar procedure employed for **82.**

Yield 130 mg, 62%;

Specific Rotation $[\alpha]_D^{25}$ = -27.0 (*c* = 0.8, $CHCl_3$);

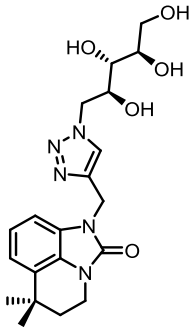
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (br. s., 1 H), 7.06 - 6.96 (m, 3 H), 5.25 - 5.17 (m, 2 H), 4.91 (d, *J* = 14.2 Hz, 1 H), 4.55 - 4.46 (m, 1 H), 4.32 (dd, *J* = 9.8, 14.2 Hz, 1 H), 4.19 - 4.12 (m, 1 H), 4.10 - 4.01 (m, 2 H), 3.97 - 3.84 (m, 3 H), 1.90 (t, *J* = 5.7 Hz, 2 H), 1.44 (s, 3 H), 1.37 (s, 3 H), 1.34 (s, 6 H), 1.32 (s, 3 H), 1.29 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 128.2, 127.3, 125.0, 121.1, 116.4, 110.2, 109.8, 106.4, 101.3, 77.9, 76.1, 73.0, 68.0, 50.6, 36.6, 36.3, 31.8, 28.5, 28.0, 26.7, 25.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3242, 2950, 1680, 1488, 1215, 1016 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{26}H_{36}N_5O_5$ $[M+H]^+$ 498.2711, found 498.2714.

6,6-dimethyl-1-((1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1H-1,2,3-triazol-4-yl)methyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (95)



The title compound was synthesized from **95i** using similar procedure employed for **31.**

Yield 68 mg, 68%;

Melting Point: 120 - 123 °C;

Specific Rotation $[\alpha]_D^{25}$ = -9.2 (*c* = 0.3, $CHCl_3$);

^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (br. s., 1 H), 7.00 (br. s, 3 H), 5.15 (s, 2 H), 4.67 - 4.64 (m, 1 H), 4.41 (dd, *J* = 8.8, 13.9 Hz, 1 H), 4.12 - 4.03 (m, 1 H), 3.89 (t, *J* = 5.9 Hz, 2 H), 3.79 - 3.71 (m, 2 H), 3.64 (dd, *J* = 5.3, 10.6 Hz, 1 H), 3.54 (t, *J* = 6.1 Hz, 1 H), 1.90 (t, *J* = 5.9 Hz, 2 H), 1.32 (s, 6 H);

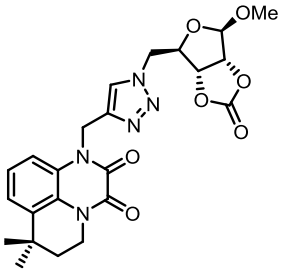
^{13}C NMR (100 MHz, CD_3OD) δ 154.8, 130.3, 128.6, 126.3, 126.0, 122.8, 118.0, 107.5, 74.4, 74.1, 72.8, 64.7, 54.2, 37.9, 37.6, 37.3, 32.9, 29.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3391, 3022, 1687, 1508, 1426, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{27}N_5O_5Na$ $[M+Na]^+$ 440.1904, found 440.1895;

HPLC analysis t_R 4.2 min, purity 95.13%.

1-((1-(((3aR,4R,6R,6aR)-6-methoxy-2-oxotetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (96)



Carbonyldiimidazole (19 mg, 0.120 mmol) was added to the mixture of 1-((1-(((2S,3R,4S,5S)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione **94** (50 mg, 0.109 mmol) and triethyl amine (0.012 mL, 0.087 mmol) in dry DCM (2 mL). The reaction mixture was stirred vigorously overnight. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 X 20 mL). The organic layer was washed successively with water and brine, dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by flash silica gel column chromatography to afford **96** (46 mg, 88% yield) as colour less solid product**.**

Specific Rotation $[\alpha]_D^{25}$ = -37.0 (*c* = 0.2, $CHCl_3$);

Melting point 237-240 °C;

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1 H), 7.80 (dd, *J* = 3.4, 6.4 Hz, 1 H), 7.31 - 7.29 (m, 2 H), 5.52 - 5.44 (m, 2 H), 5.37 (d, *J* = 6.7 Hz, 1 H), 5.20 (s, 1 H), 5.11 (d, *J* = 7.0 Hz, 1 H), 4.81 - 4.77 (m, 1 H), 4.67 (dd, *J* = 7.6, 14.0 Hz, 1 H), 4.54 (dd, *J* = 6.9, 14.2 Hz, 1 H), 4.20 - 4.18 (m, 2 H), 3.47 (s, 3 H), 1.95 (t, *J* = 6.4 Hz, 2 H), 1.40 (s, 6 H);

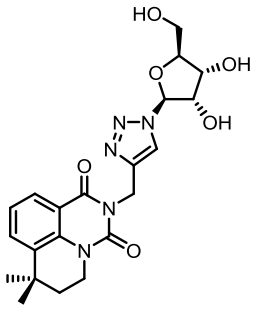
^{13}C NMR (125 MHz, $CDCl_3$) δ 154.2, 153.7, 153.1, 142.6, 134.8, 126.2, 125.0, 124.2, 122.5, 121.8, 113.8, 108.4, 84.2, 83.2, 80.6, 56.2, 51.8, 39.1, 34.4, 32.0, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3389, 3022, 1816, 1677, 1405, 1156 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{22}H_{25}N_5O_7Na$ $[M+Na]^+$ 506.1646, found 506.1642;

HPLC analysis t_R 4.1 min, purity 97.82%.

2-((1-((3S,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione (97)



The title compound was synthesized from **81** using similar two step procedure employed for the synthesis of **91.**

Yield 62 mg, 46% for two steps

Specific Rotation $[\alpha]_D^{25}$ = -37 (*c* = 0.2, $CHCl_3$);

^{1}H NMR (500 MHz, CD_3OD) δ 8.20 (s, 1 H), 7.98 (dd, *J* = 1.1, 7.8 Hz, 1 H), 7.75 (dd, *J* = 1.2, 7.6 Hz, 1 H), 7.22 (t, *J* = 7.6 Hz, 1 H), 5.99 (d, *J* = 4.0 Hz, 1 H), 5.33 (s, 2 H), 4.47 (t, *J* = 4.4 Hz, 1 H), 4.29 (t, *J* = 5.0 Hz, 1 H), 4.14 - 4.10 (m, 1 H), 4.08 (t, *J* = 6.1 Hz, 2 H), 3.80 (dd, *J* = 3.4, 12.2 Hz, 1 H), 3.68 (dd, *J* = 4.3, 12.2 Hz, 1 H), 1.91 (t, *J* = 6.4 Hz, 2 H), 1.37 (s, 6 H);

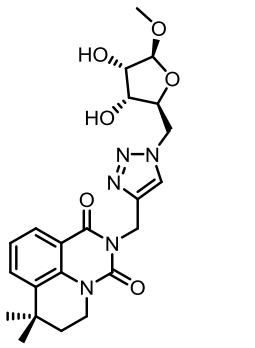
^{13}C NMR (125 MHz, CD_3OD) δ 163.4, 151.8, 145.0, 137.5, 135.4, 133.3, 127.4, 124.1, 123.9, 116.4, 94.5, 87.3, 77.1, 72.0, 63.0, 40.9, 37.5, 35.9, 33.1, 30.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3412, 3023, 1654, 1607, 1514, 1426 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{21}H_{25}N_5O_6Na$ $[M+Na]^+$ 466.1697, found 466.1693;

HPLC analysis t_R 4.1 min, purity 97.02%.

2-((1-(((2S,3R,4S,5S)-3,4-dihydroxy-5-methoxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinazoline-1,3(2H)-dione (98)



The title compound was synthesized from **81** using similar procedure employed for **82**.

Yield 110 mg, 80%;

Specific Rotation $[\alpha]_D^{25}$ = -20.4 (*c* = 0.2, $CHCl_3$);

Melting point 169-172 °C;

^{1}H NMR (400MHz, CD_3OD) δ 7.97 - 7.95 (m, 2 H), 7.73 (d, *J* = 7.3 Hz, 1 H), 7.21 (t, *J* = 7.7 Hz, 1 H), 5.32 (s, 2 H), 4.72 - 4.58 (m, 2 H), 4.45 (dd, *J* = 6.5, 14.3 Hz, 1 H), 4.18 (dt, *J* = 3.4, 6.8 Hz, 1 H), 4.12 - 3.97 (m, 3 H), 3.78 (d, *J* = 4.4 Hz, 1 H), 3.21 (s, 3 H), 1.90 (t, *J* = 6.1 Hz 2 H), 1.36 (s, 6 H);

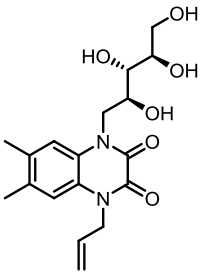
^{13}C NMR (100 MHz, CD_3OD) δ 163.4, 151.8, 137.4, 135.4, 133.3, 127.4, 126.3, 124.1, 116.4, 110.3, 82.0, 76.1, 73.4, 55.9, 54.1, 40.8, 37.5, 35.9, 33.1, 30.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3385, 3022, 1699, 1652, 1421, 1216 cm^{-1}.

HRMS (ESI): *m/z* calculated for $C_{22}H_{27}N_5O_6Na$ $[M+Na]^+$ 480.1854, found 480.1849;

HPLC analysis t_R 4.3 min, purity 99.11%.

1-allyl-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (100)



6,7-dimethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **99** (0.2 g, 0.61 mmol) was dissolved in DMF (10 mL), potassium carbonate (0.25 g, 1.85 mmol) and allyl bromide (0.16 mL, 1.85 mmol) was added sequentially at 30°C. Reaction mixture was stirred for 12 h at same temperature. All the solvent was removed on vacuum, cold water was added and the white solid obtained was filtered and washed with minimum water and dried over rota vapour. This solid was again washed with DCM to afford the pure solid product **100** (0.16 g, yield 72%)

Specific rotation $[\alpha]_D^{25}$ = -15 (c 0.2, DMF);

Melting point 77-80 °C;

1H NMR (400 MHz, DMSO-d_6) δ 7.40 (s., 1 H), 7.09 (s., 1 H), 5.92 (dt, *J* = 5.4, 11.0 Hz, 1 H), 5.19 - 5.12 (m, 3 H), 4.76 (br. s., 2 H), 4.67 - 4.48 (m, 3 H), 4.14 - 3.98 (m, 3 H), 3.61 (d, *J* = 11.2 Hz, 2 H), 3.43 (d, J = 6.8 Hz, 2 H), 2.23 (s., 6 H);

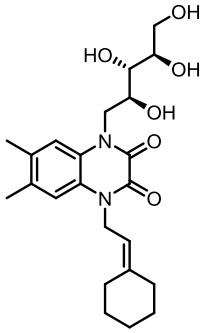
^{13}C NMR (100 MHz, DMSO-d_6) δ 154.3, 153.7, 131.6, 131.5, 125.0, 124.1, 116.9, 116.8, 116.3, 73.6, 72.8, 68.0, 63.5, 44.7, 44.5, 19.1, 19.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3377, 2925, 1666, 1518, 1405, 1214 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{25}N_2O_6$ $[M+H]^+$ 365.1707, found 365.1704;

HPLC analysis t_R 4.1 min, purity 98.03%.

1-(2-cyclohexylideneethyl)-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (101)



6,7-dimethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **99** (0.5 g, 1.54 mmol) was dissolved in DMF (20 mL), potassium carbonate (0.320 g, 2.31 mmol) and (2-bromoethylidene)cyclohexane (0.435 g, 2.31 mmol) was added sequentially at 30°C. Reaction mixture was stirred for 12 h at same temperature. All the solvent was removed on vacuum, cold water was added and the white solid obtained was filtered and washed with minimum water and dried over rota vapour. This solid was again washed with DCM to afford the pure solid product **101** (0.370 g, yield 55%)

Melting point 94-97 °C;

Specific rotation $[\alpha]_D^{25}$ = - 17 (c 0.2, DMF);

^{1}H NMR (200 MHz, DMSO-d_6) δ 7.41 (s, 1 H), 7.06 (s, 1 H), 5.05 (d, *J* = 15.2 Hz, 2 H), 4.82 (d, *J* = 12.5 Hz, 2 H), 4.65 - 4.53 (br. s., 3 H), 4.05 (br. s., 3 H), 3.58 (br. s., 4 H), 2.40 (br. s., 2 H), 2.33 - 2.18 (br. s, 6 H), 2.05 (br. s., 2 H), 1.58 (br. s., 4 H), 1.46 (br. s., 2 H);

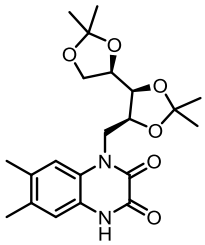
^{13}C NMR (100 MHz, DMSO-d_6) δ 154.3, 153.6, 143.6, 132.1, 131.6, 131.5, 131.4, 128.8, 128.7, 125.0, 124.0, 117.1, 115.9, 115.4, 73.5, 72.9, 68.1, 63.5, 44.7, 36.2, 28.6, 27.9, 27.3, 26.1, 19.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3365, 1676, 1623, 1463, 1377 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{23}H_{32}N_2O_6Na$ $[M+Na]^+$ 455.2158, found 455.2154;

HPLC analysis t_R 4.1 min, purity 98.03%.

6,7-dimethyl-1-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione (102)



2-Methoxypropene (1.77 mL, 24.6 mmol) and p-Toluenesulfonic acid (117 mg, 6.1 mmol) were added in sequence to a stirred solution of the 6,7-dimethyl-1-((2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **99** (2 g, 6.17 mmol) in *N*,*N*-dimethylformamide (18 mL) at 24 °C. The reaction mixture was stirred for 12 h at 24 °C. The reaction mixture was treated with saturated aqueous sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (20 mL X 2). The organic layers were combined and dried over sodium sulphate. The dried solution was filtered, and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (15% EtOAc:DCM) to afford the acetonide **102** as a yellow solid (2.1 g, 83%).

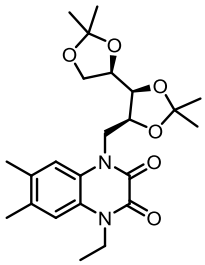
Melting point 154-157 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 11.86 (d, *J* = 16.5 Hz, 1 H), 7.23 - 7.04 (m, 2 H), 4.71 - 4.46 (m, 2 H), 4.42 - 3.92 (m, 3 H), 3.87 - 3.58 (m, 2 H), 2.30 (d, *J* = 4.9 Hz, 6 H), 1.65 (s, 1 H), 1.59 - 1.23 (m, 12 H);

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3017, 1688, 1385, 1215 1077 cm^{-1};

HRMS (ESI): *m*/*z* calculated for $C_{21}H_{28}N_2O_6Na$ $[M+Na]^+$ 427.1840, found 427.1835;

1-ethyl-6,7-dimethyl-4-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione (103i)

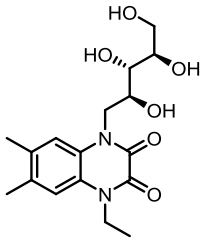


6,7-dimethyl-1-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)] 5-yl)methyl)-

-1,4-dihydroquinoxaline-2,3-dione **102** (200 mg, 0.49 mmol) was taken in dry DMF (5 mL) then potassium carbonate (102 mg, 0.74 mmol) and ethyl bromide (79 μL, 0.74 mmol) was added sequentially. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then diluted with water (10 mL) and extracted with ethyl acetate (20 mL X 2). The organic layers were combined and dried over sodium sulphate. The dried solution was filtered, and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (10% EtOAc:DCM) to afford the *N*-alkylated product **103i** as a yellow oil (135 mg, 62%).

The obtained product forwarded for next step without further characterization.

1-ethyl-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (103)



A solution of 1-ethyl-6,7-dimethyl-4-(((4S,4'R,5S)-2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methyl)-1,4-dihydroquinoxaline-2,3-dione **103i** (100 mg, 0.231 mmol) in AcOH:H_2O (1:1 1 mL) was stirred for 12 h at 60 °C. The reaction mixture was concentrated under reduced pressure, and purified by flash column chromatography (10% MeOH: DCM) to give compound **103** (58 mg, 71%) as colourless solid.

Specific rotation $[\alpha]_D^{25}$= + 5.6° (c 0.24, DMF);

Melting point 242-245 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (s, 1 H), 7.23 (s, 1 H), 4.95 (br. s., 1 H), 4.64 (d, *J* = 5.9 Hz, 1 H), 4.57 (dd, *J* = 10.3, 13.2 Hz, 1 H), 4.47 (t, *J* = 5.1 Hz, 1 H), 4.20 - 4.11 (m, 2 H), 4.08 (br. s., 1 H), 4.01 (d, *J* = 14.2 Hz, 1 H), 3.68 - 3.57 (m, 3 H), 3.43 (m, 1 H), 2.28 (s, 3 H), 2.24 (s, 3 H), 1.22 (t, *J* = 6.8 Hz, 3 H);

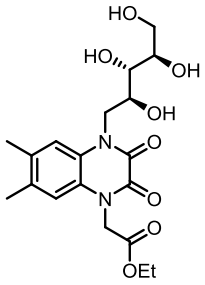
^{13}C NMR (100 MHz, DMSO-d_6) δ 154.3, 153.4, 131.8, 131.5, 125.0, 123.8, 117.1, 115.6, 73.6, 72.8, 68.1, 63.5, 44.6, 37.3, 19.1, 18.9, 12.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3395, 2724, 1667, 1457, 1374 cm^{-1};

HRMS (ESI) *m*/*z* calculated for $C_{19}H_{23}N_2O_6$ $[M+H]^+$ 375.1551, found 375.1558;

HPLC analysis t_R 4.1 min, purity 98.03%.

Ethyl2-(6,7-dimethyl-2,3-dioxo-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-3,4-dihydroquinoxalin-1(2H)-yl)acetate (104)



The title compound was synthesized from **102** using similar two step procedure employed for **103**.

Yield 108 mg, 73%;

Specific rotation $[\alpha]_D^{25}$= -207 (*c* = 0.3, $CHCl_3$);

Melting point 254-257 °C;

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.45 (s, 1 H), 7.13 (s, 1 H), 4.97 (s, 2 H), 4.89 - 4.79 (m, 1 H), 4.67 (d, *J* = 6.4 Hz, 1 H), 4.60 (dd, *J* = 10.3, 13.2 Hz, 1 H), 4.48 (t, *J* = 5.4 Hz, 1 H), 4.19 (q, *J* = 6.8 Hz, 2 H), 4.14 - 3.99 (m, 2 H), 3.69 - 3.53 (m, 3 H), 3.44 (dd, *J* = 5.1, 10.5 Hz, 1 H), 2.24 (br. s., 3 H), 2.24 (br. s., 3 H), 1.23 (t, *J* = 7.1 Hz, 3 H);

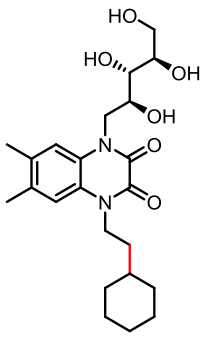
^{13}C NMR (100 MHz, DMSO-d$_6$) δ 167.6, 153.9, 153.9, 132.0, 131.9, 124.7, 124.3, 117.1, 115.7, 73.6, 72.8, 68.1, 63.5, 61.3, 44.7, 44.4, 19.1, 18.9, 14.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3555, 2726, 1740, 1675, 1593, 1518 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{26}N_2O_8Na$ $[M+Na]^+$ 433.1581, found 433.1577;

HPLC analysis t_R 4.1 min, purity 98.03%.

1-(2-cyclohexylethyl)-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione (106)



1-(2-cyclohexylideneethyl)-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquinoxaline-2,3-dione **101** (100 mg, 0.23 mmol) was taken in ethanol (10mL) and

10% palladium on carbon (20 mg) was added. The reaction mixture was stirred at room temperature for 12 h. After all the starting material was consumed, filter the reaction mixture through celite pad wash with more ethanol (30 mL). The filtrate was then concentrated to obtain the crude product which was then washed with 1:1 mixture of DCM:PE to obtain pure compound 91 as a white solid product **107** (56 mg, yield 55%).

Specific rotation $[\alpha]_D^{25}$ = -275 (*c* = 0.3, DMF);

Melting point 217 - 219 °C;

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.41 (s, 1 H), 7.13 (s, 1 H), 4.95 (br. s., 1 H), 4.82 (br. s., 1 H), 4.64 (d, *J* = 5.4 Hz, 1 H), 4.62 - 4.52 (m, 1 H), 4.47 (t, *J* = 5.1 Hz, 1 H), 4.18 - 4.07 (m, 4 H), 4.02 (d, *J* = 14.2 Hz, 1 H), 3.66 - 3.51 (m, 3 H), 3.48 - 3.37 (m, 1 H), 2.27 (s, 3 H), 2.24 (s, 3 H), 1.80 (d, *J* = 11.7 Hz, 2 H), 1.76 - 1.58 (m, 3 H), 1.56 - 1.44 (m, 2 H), 1.40 (br. s., 1 H), 1.31 - 1.11 (m, 3 H), 1.07 - 0.96 (m, 2 H);

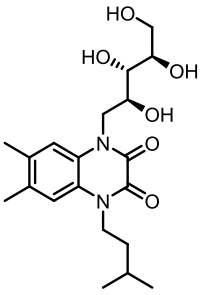
^{13}C NMR (100 MHz, DMSO-d$_6$) δ 154.2, 153.5, 131.7, 131.5, 125.0, 123.9, 117.1, 115.6, 73.6, 72.8, 68.0, 63.5, 44.6, 35.0, 33.9, 32.6, 26.0, 25.7, 19.1;

IR ν_{max} (thin film applied as CHCl$_3$ solution) 3477, 3327, 2726, 1683, 1650, 1456 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{23}H_{35}N_2O_6$ $[M+H]^+$ 435.2490, found 435.2491;

HPLC analysis t_R 3.8 min, purity 95.60%.

1-isopentyl-6,7-dimethyl-4-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-1,4-dihydroquino--xaline-2,3-dione (107)



The title compound was synthesized from **105** using similar procedure employed for **106**.

Yield 120 mg, 60%;

Specific rotation $[\alpha]_D^{25}$ = +137 (c 0.2, DMF);

Melting point 104-107 °C;

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.43 (br. s., 1 H), 7.13 (br. s., 1 H), 5.12 (br. s., 1 H), 4.99 (br. s., 1 H), 4.73 (br. s., 1 H), 4.68 - 4.47 (m, 2 H), 4.32 - 4.07 (m, 2 H), 4.02 (d, *J* = 14.2 Hz,

2 H), 3.62 (br. s., 1 H), 3.57 (br. s., 2 H), 3.45 (br. s., 1 H), 2.27 (br. s., 3 H), 2.24 (br. s., 3 H), 1.69 (d, *J* = 5.9 Hz, 1 H), 1.56 - 1.42 (m, 2 H), 0.95 (d, *J* = 5.9 Hz, 6 H);

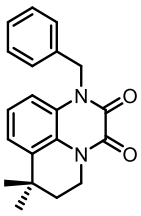
^{13}C NMR (100 MHz, DMSO-d_6) δ 154.2, 153.6, 131.7, 131.6, 125.0, 123.9, 117.2, 115.6, 73.6, 72.9, 68.1, 63.5, 44.7, 40.6, 35.4, 25.7, 22.4, 19.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3408, 2725, 1677, 1459, 1374 1211 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{31}N_2O_6$ $[M+H]^+$ 395.2177, found 395.2170;

HPLC analysis t_R 3.3 min, purity 95.11%.

1-benzyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (108)



The title compound was synthesized from **24** using similar procedure employed for **20**

Yield 172 mg, 82%;

Melting Point 168-171 °C;

^{1}H NMR (400MHz, $CDCl_3$) δ 7.34 - 7.23 (m, 6 H), 7.21 – 7.07 (m, 2 H), 5.48 (s, 2 H), 4.24 (t, *J* = 6.1 Hz, 2 H), 1.95 (t, *J* = 6.3 Hz, 2 H), 1.39 (s, 6 H);

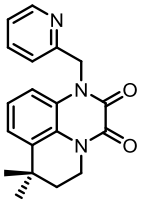
^{13}C NMR (50 MHz, $CDCl_3$) δ 154.3, 153.6, 134.9, 134.6, 128.7, 127.5, 126.6, 126.3, 123.7, 122.4, 121.3, 113.5, 46.9, 38.9, 34.2, 31.8, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3351, 3018, 1681, 1598, 1484, 1162 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{20}N_2O_2Na$ $[M+Na]^+$ 343.1417, found 343.1417;

HPLC analysis t_R 5.0 min, purity 99.43%.

7,7-dimethyl-1-(pyridin-2-ylmethyl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dion (109)



The title compound was synthesized from **24** using similar procedure employed for **20**

Yield 192 mg, 65%;

Melting Point 133-135 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 4.9 Hz, 1 H), 7.63 (dt, *J* = 1.7, 7.7 Hz, 1 H), 7.30 (t, *J* = 8.1 Hz, 2 H), 7.23 - 7.21 (m, 2 H), 7.13 - 7.11 (m, 1 H), 5.57 (s, 2 H), 4.22 (t, *J* = 6.4 6.4 Hz, 2 H), 1.93 (t, *J* = 6.1 Hz, 2 H), 1.37 (s, 6 H);

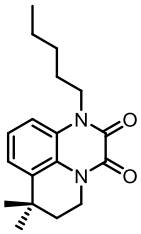
^{13}C NMR (50 MHz, $CDCl_3$) δ 155.2, 154.3, 153.7, 149.2, 137.1, 134.6, 126.5, 123.8, 122.7, 122.5, 122.0, 121.4, 114.0, 49.0, 39.0, 34.3, 31.9, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3416, 3018, 1680, 1398, 1313, 1216 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{20}N_3O_2$ $[M+H]^+$ 322.1550, found 322.1543;

HPLC analysis t_R 4.5 min, purity 96.01%.

7,7-dimethyl-1-pentyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (110)



The title compound was synthesized from **24** using similar procedure employed for **20**

Yield 191 mg, 76%;

Melting Point 104-107 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28 - 7.23 (m, 2 H), 7.21 - 7.07 (m, 1 H), 4.23 - 4.14 (m, 4 H), 1.91 (t, *J* = 6.3 Hz, 2 H), 1.81 - 1.62 (m, 2 H), 1.50 - 1.30 (m, 10 H), 0.91 (t, *J* = 6.8 Hz, 3 H);

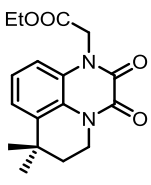
^{13}C NMR (50 MHz, $CDCl_3$) δ 153.7, 153.6, 134.8, 126.3, 123.7, 122.6, 121.1, 112.7, 43.2, 38.9, 34.3, 31.9, 29.9, 28.9, 26.5, 22.3, 13.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3007, 2959, 1679, 1598, 1470 1398 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{25}N_2O_2$ $[M+H]^+$ 301.1911, found 301.1909;

HPLC analysis t_R 5.7 min, purity 98.45%.

Ethyl 2-(7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)acetate (111)



The title compound was synthesized from **24** using similar procedure employed for **20**

Yield 250 mg, 62%;

Melting Point 123-126 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.26 - 7.12 (m, 2 H), 6.81 (dd, *J* = 1.5, 8.0 Hz, 1 H), 4.97 (s, 2 H), 4.21 (q, *J* = 7.1 Hz, 2 H), 4.16 - 4.08 (m, 2 H), 1.90 (t, *J* = 6.3 Hz, 2 H), 1.35 (s, 6 H), 1.26 (t, *J* = 7.1 Hz, 3 H);

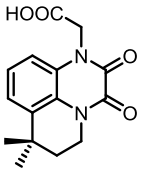
^{13}C NMR (50 MHz, $CDCl_3$) δ 166.9, 153.8, 153.3, 134.9, 126.2, 123.9, 122.4, 121.6, 112.1, 61.9, 44.4, 38.9, 34.1, 31.8, 29.7, 13.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 1747, 1685, 1600, 1477, 1403 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{20}N_2O_4Na$ $[M+Na]^+$ 339.1315, found 339.1315;

HPLC analysis t_R 4.4 min, purity 96.03%.

2-(7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)acetic acid (112)



Lithium hydroxide monohydrate (77 mg, 1.89 mmol) was added to a solution of ester **111** (150 mg, 0.47 mmol) in THF:MeOH:H_2O (3:2:1) 6 mL at 0 °C, and the resulting solution was stirred at room temperature for 12 h. The reaction mixture was evaporated in *vacuo*, and diluted with H_2O (4 mL) was added. The reaction mixture was washed with diethyl ether (2 × 5 mL) and acidified with 2N HCl up to pH 4 white precipitate obtained was filtered and washed with water and dried under vacuum to afford pure **112** (110 mg, 90 % yield) as a white solid product.

Melting Point 264-267 °C;

^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (d, *J* = 7.3 Hz, 1 H), 7.25 (t, *J* = 8.1 Hz, 1 H), 7.11 (d, *J* = 7.8 Hz, 1 H), 5.02 (s, 2H), 4.17 (t, *J* = 6.1 Hz, 2 H), 1.96 (t, *J* = 6.1 Hz, 2 H), 1.39 (s, 6 H);

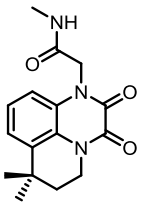
^{13}C NMR (100 MHz, CD_3OD) δ 170.6, 155.9, 155.3, 136.6, 127.9, 125.4, 123.8, 123.1, 114.1, 45.6, 40.4, 35.5, 33.1, 30.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution): 3022, 1739, 1678, 1632, 1414, 1215 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{16}N_2O_4Na$ $[M+Na]^+$ 311.1002, found 311.1003;

HPLC analysis t_R 4.1 min, purity 98.25%.

2-(7,7-dimethyl-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-1-yl)-N-methylacetamide (113)



To a solution of **112** (100 mg, 0.34 mmol) and (2M in THF) Methylamine (0.381 mL, 0.76 mmol) in dry DMF (5 mL) HATU (158 mg, 0.41 mmol) and DIPEA (136 µL, 0.76 mmol), were added and stirred for 12 h at room temperature. The reaction mixture was diluted with ethyl acetate (20 mL), washed with 1N HCl (10 mL), saturated aq. $NaHCO_3$ solution (10 mL) and the organic layer dried over anhydrous sodium sulphate. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100-200, 5% MeOH:DCM) to afford **113** (35 mg, 42%) as a colourless solid product.

Melting Point 171-173 °C;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.25 - 7.15 (m, 3 H), 7.01 (br. s., 1 H), 4.84 (s, 2 H), 4.08 (t, *J* = 6.2 Hz, 2 H), 2.73 (d, *J* = 4.8 Hz, 3 H), 1.88 (t, *J* = 6.3 Hz, 2 H), 1.34 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 154.6, 153.7, 134.9, 126.4, 124.4, 122.4, 122.0, 113.4 47.2, 39.2, 34.2, 31.9, 29.8, 26.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3323, 3014, 1673, 1600, 1404, 1316 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{16}H_{19}N_3O_3Na$ $[M+Na]^+$ 324.1319, found 324.1312;

HPLC analysis t_R 4.2 min, purity 97.21%.

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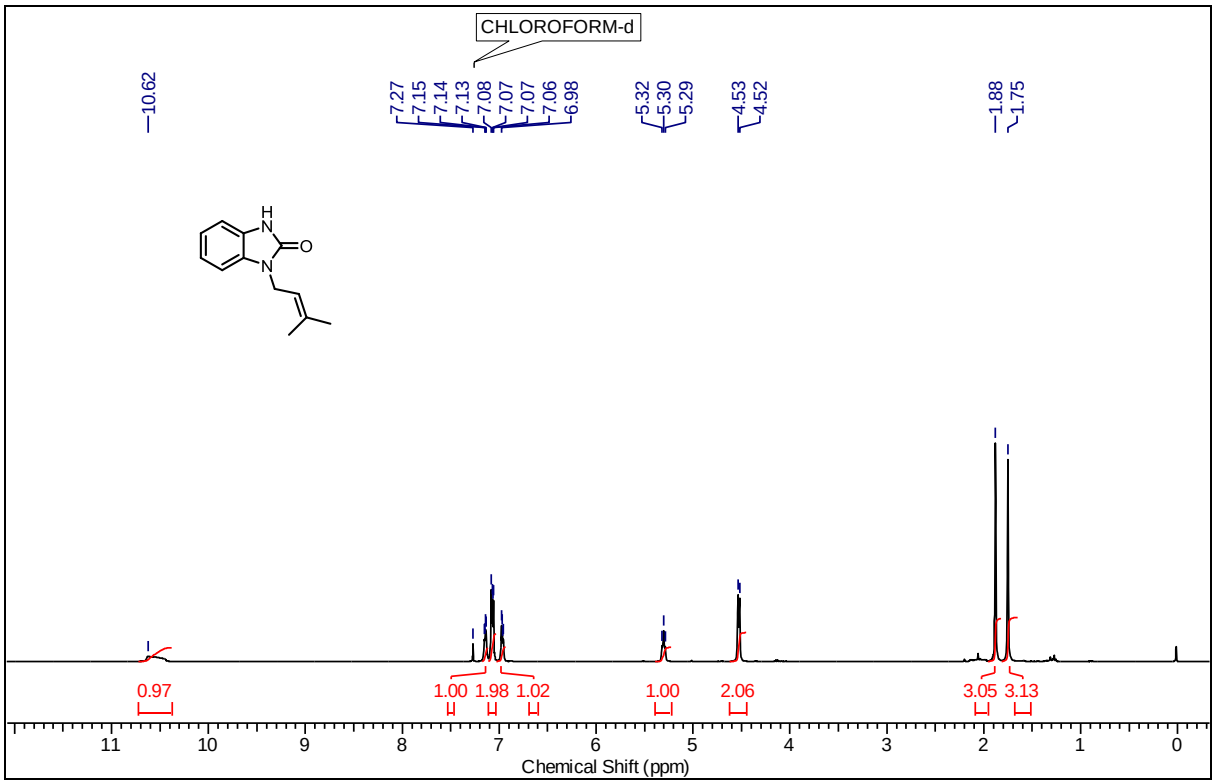
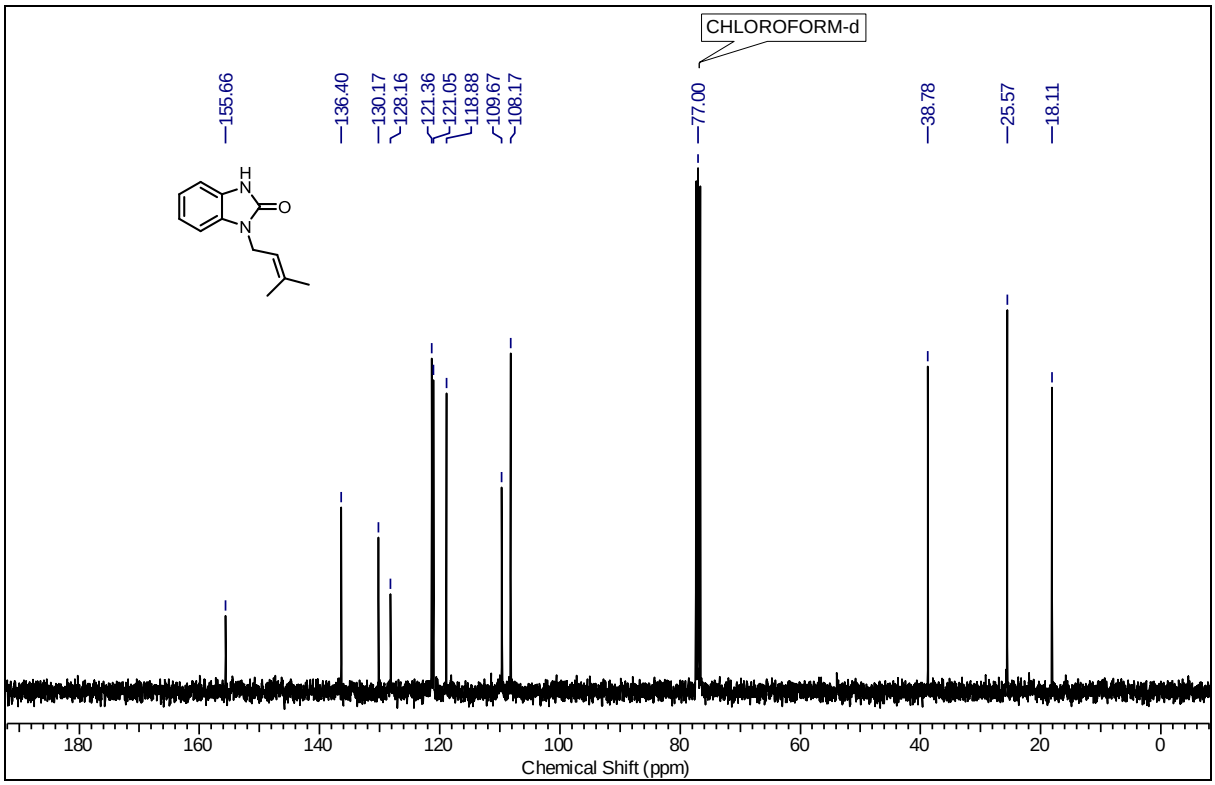
2.8 Copies of ^{1}H and ^{13}C NMR spectra

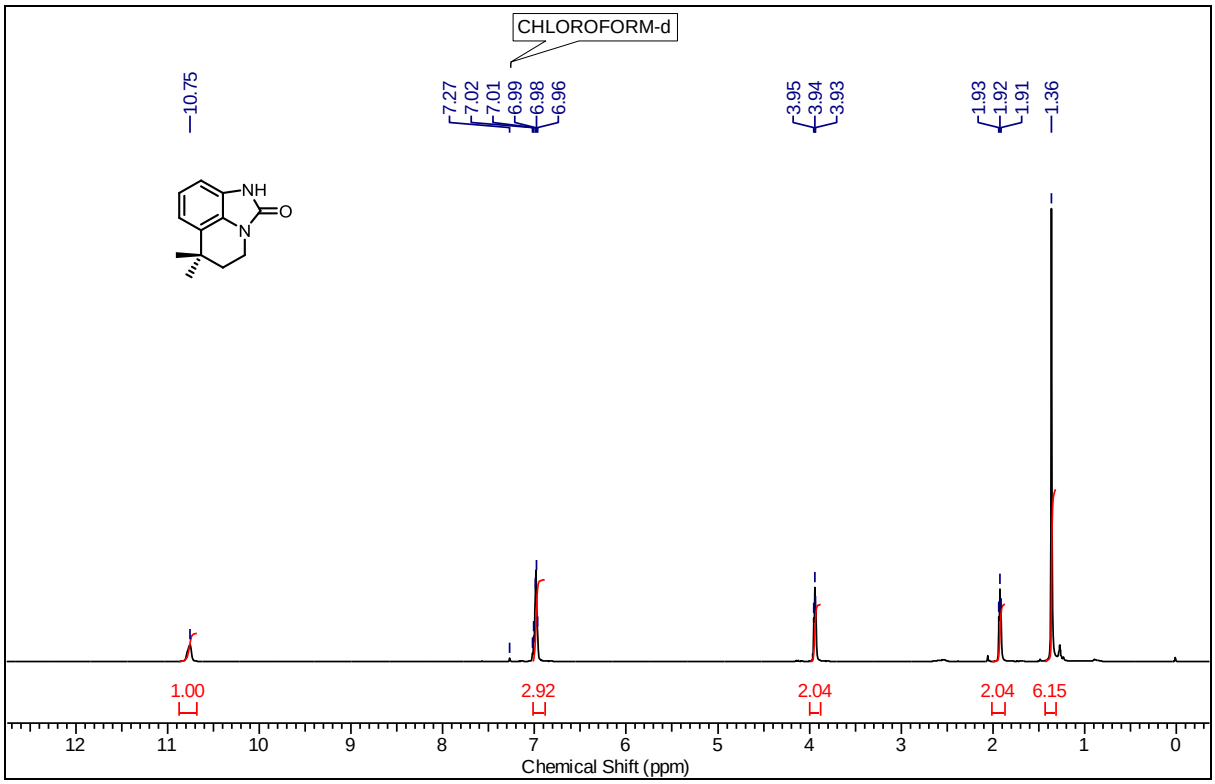
^{1}H NMR of Compound 20 at 400 MHz in $CDCl_3$



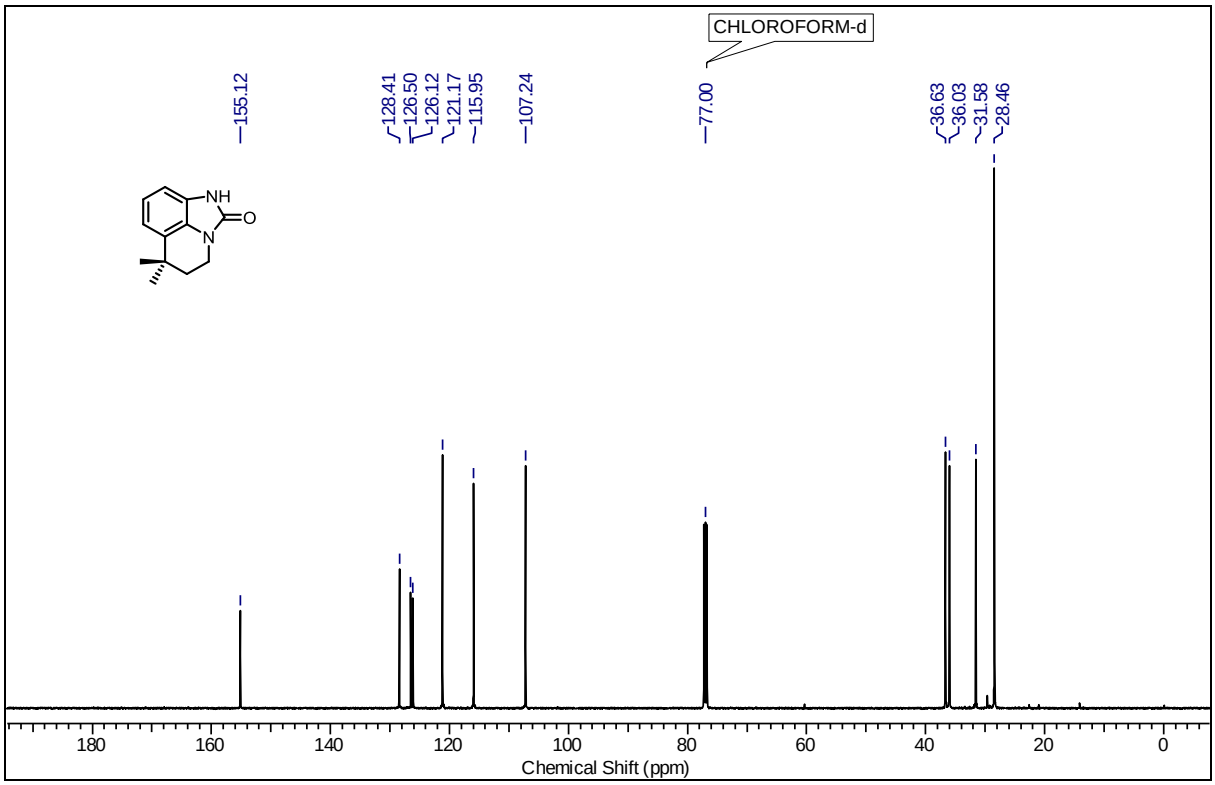
^{13}C NMR of Compound 20 at 100 MHz in $CDCl_3$



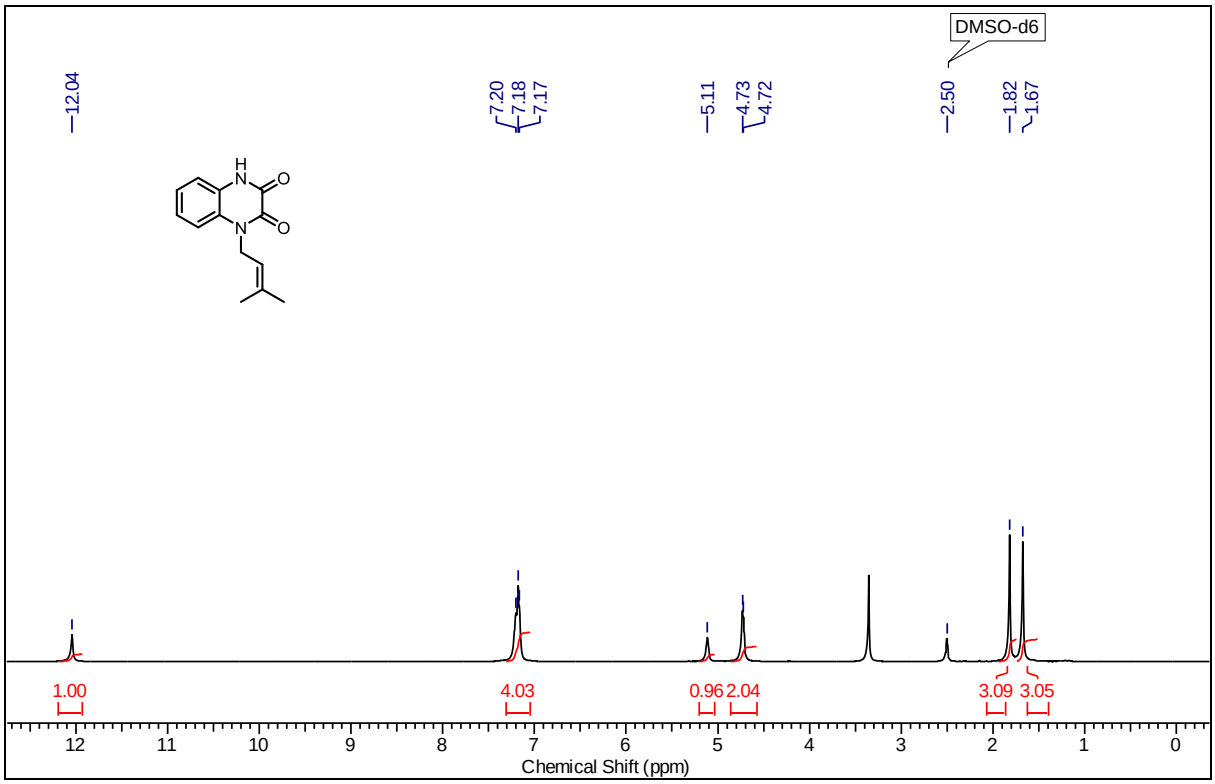
^{1}H NMR of Compound 21 at 400 MHz in $CDCl_3$



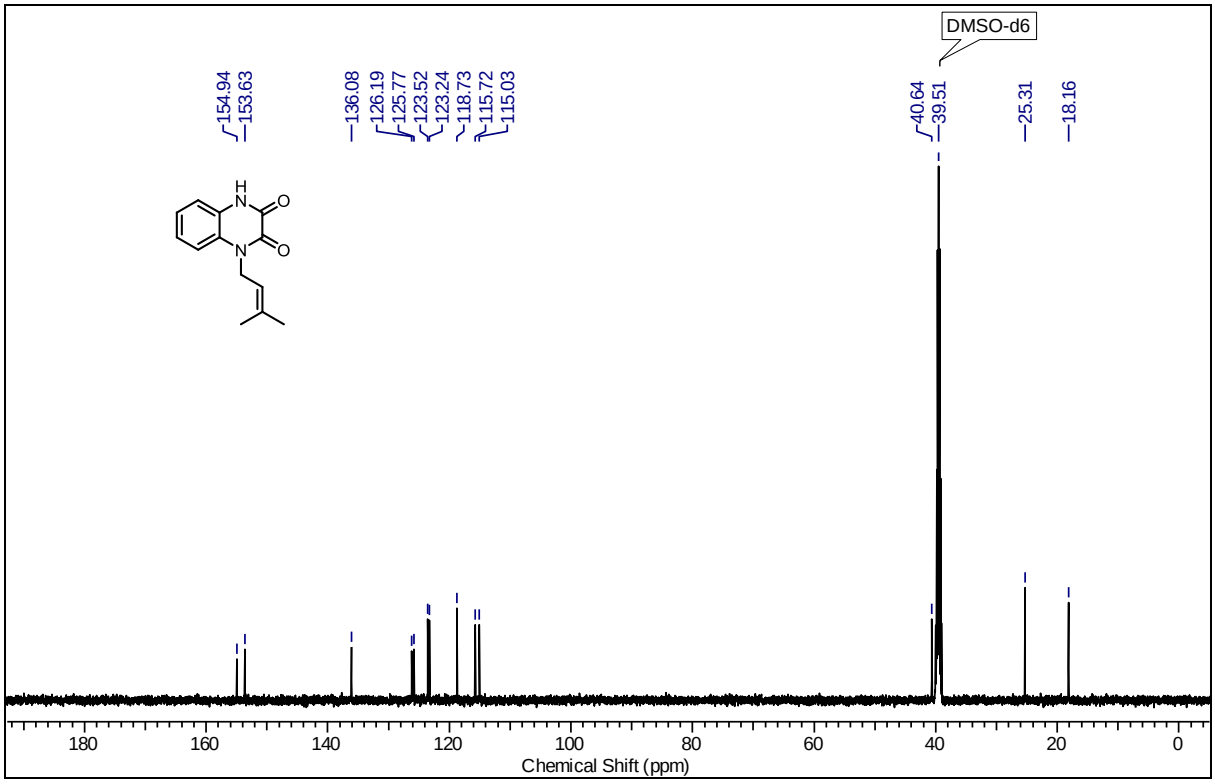
^{13}C NMR of Compound 21 at 100 MHz in $CDCl_3$



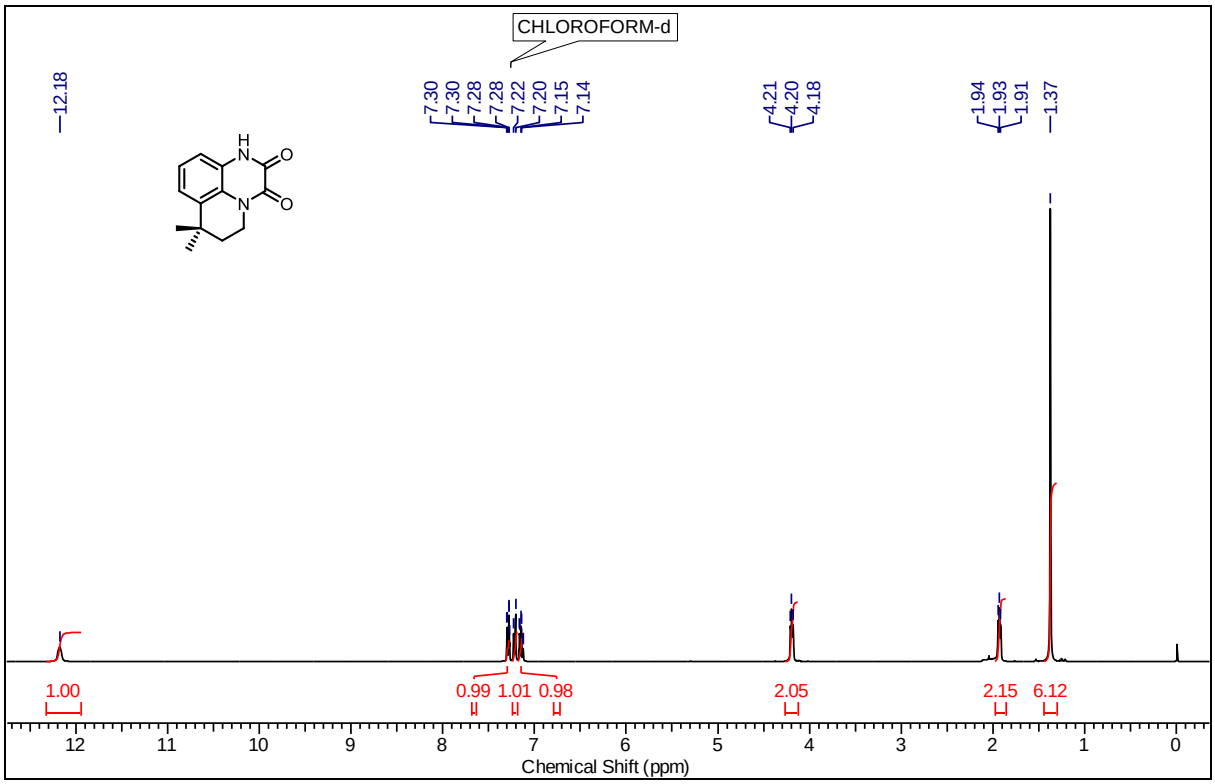
^{1}H NMR of Compound 23 at 500 MHz in DMSO-d_6



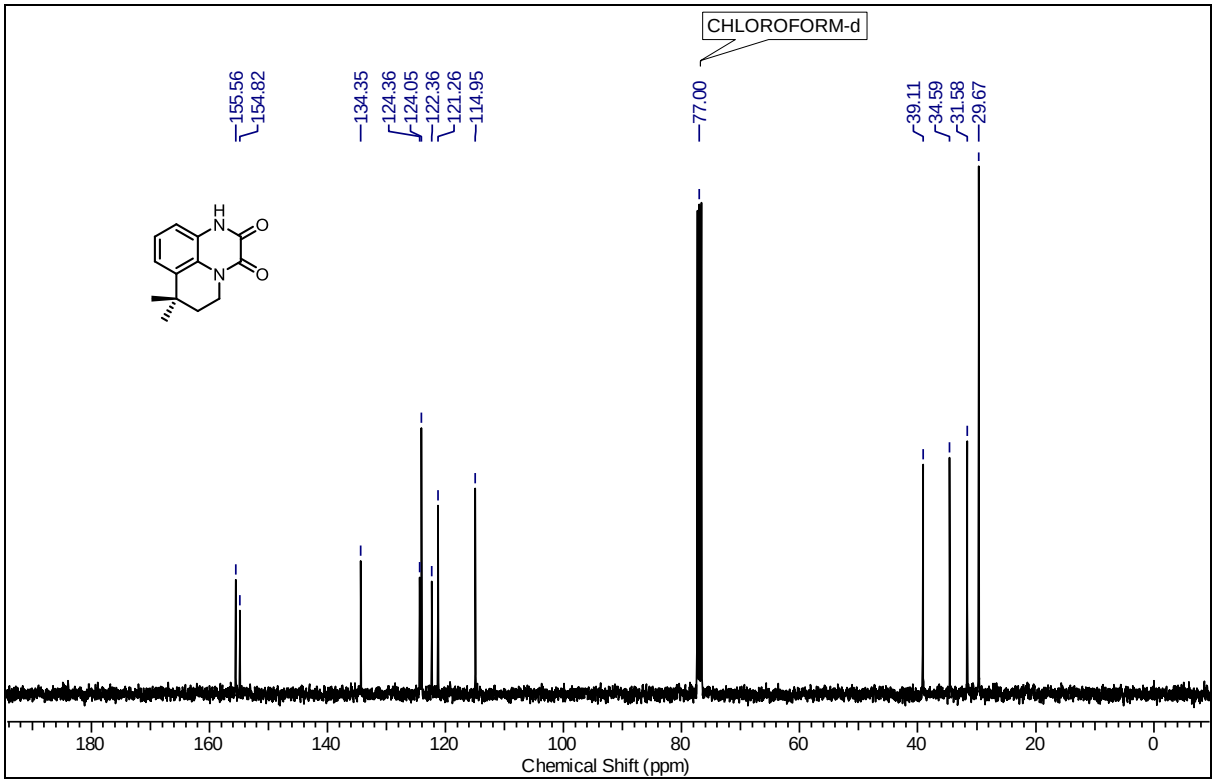
^{13}C NMR of Compound 23 at 125 MHz in DMSO-d_6



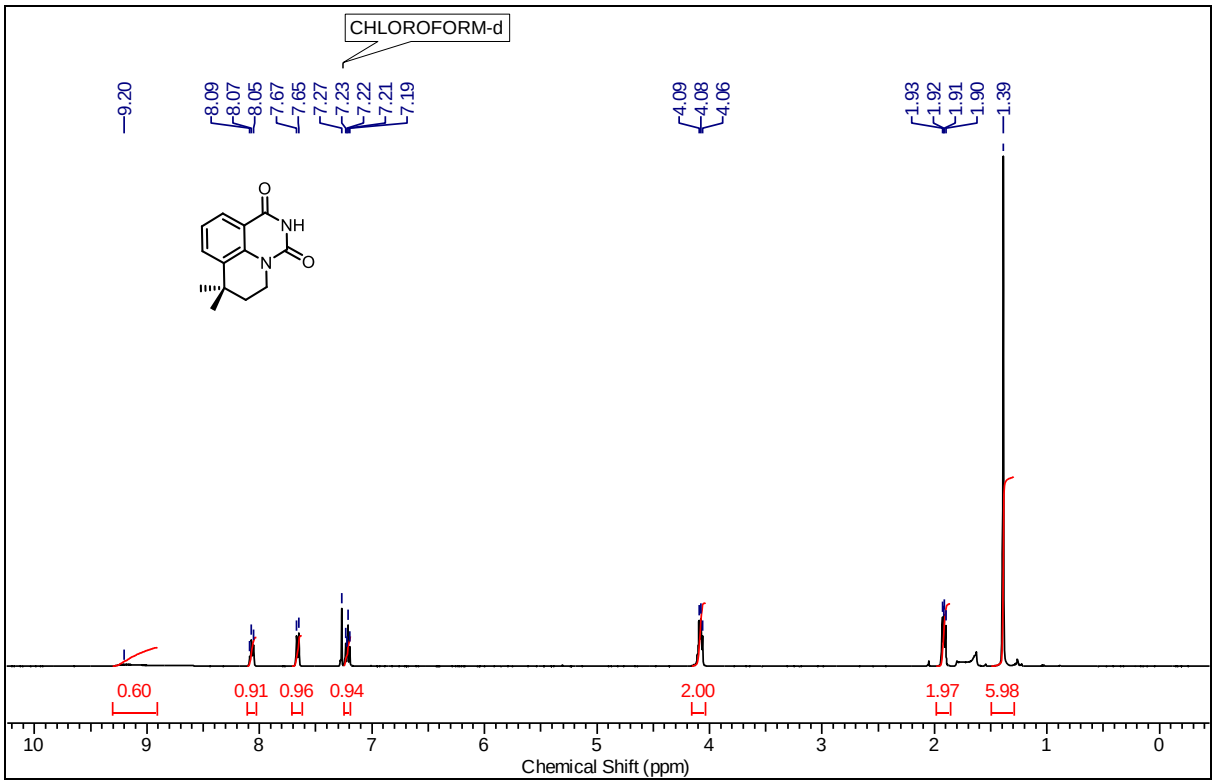
^{1}H NMR of Compound 24 at 400 MHz in $CDCl_3$



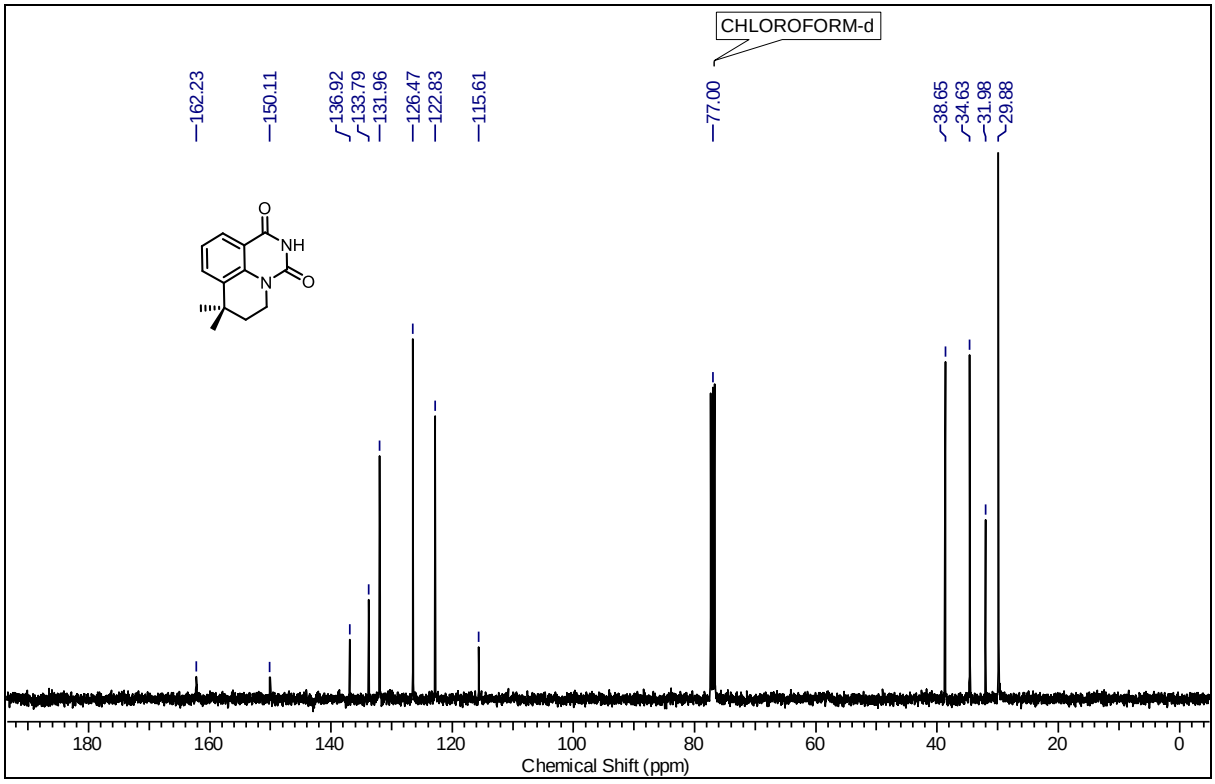
^{13}C NMR of Compound 24 at 100 MHz in $CDCl_3$



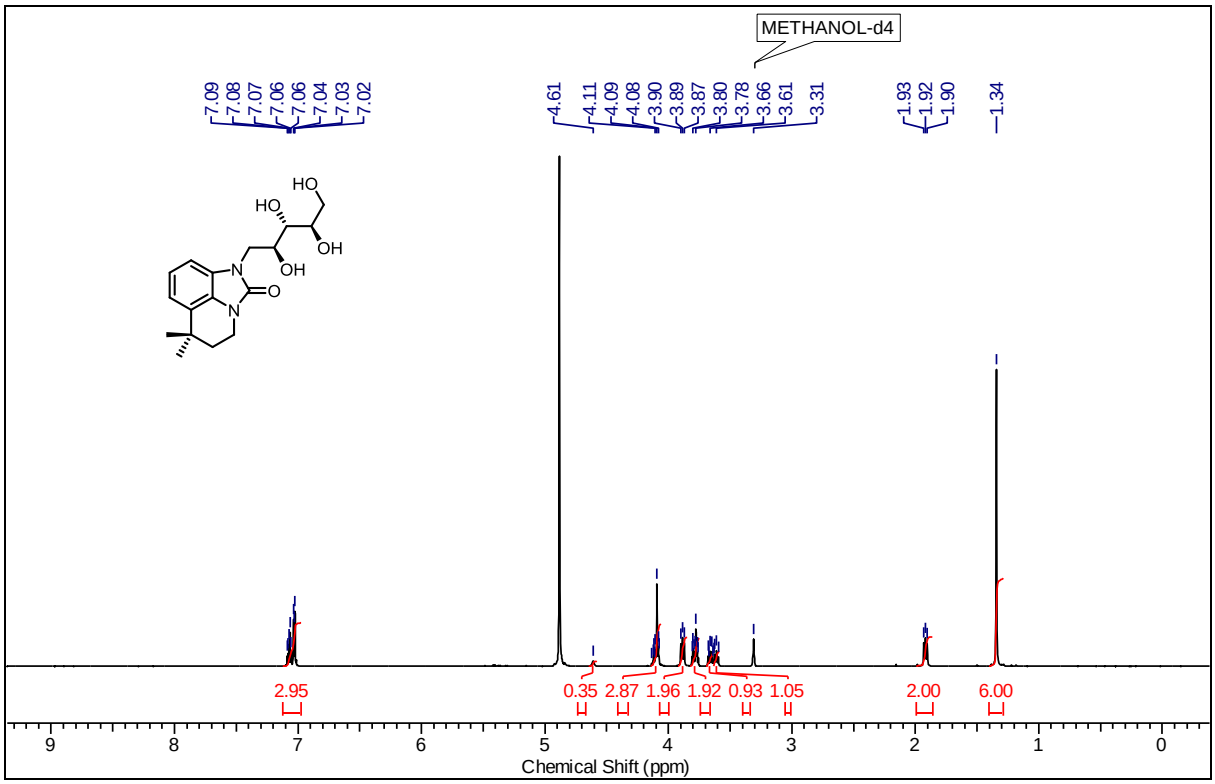
^{1}H NMR of Compound 28 at 400 MHz in $CDCl_3$



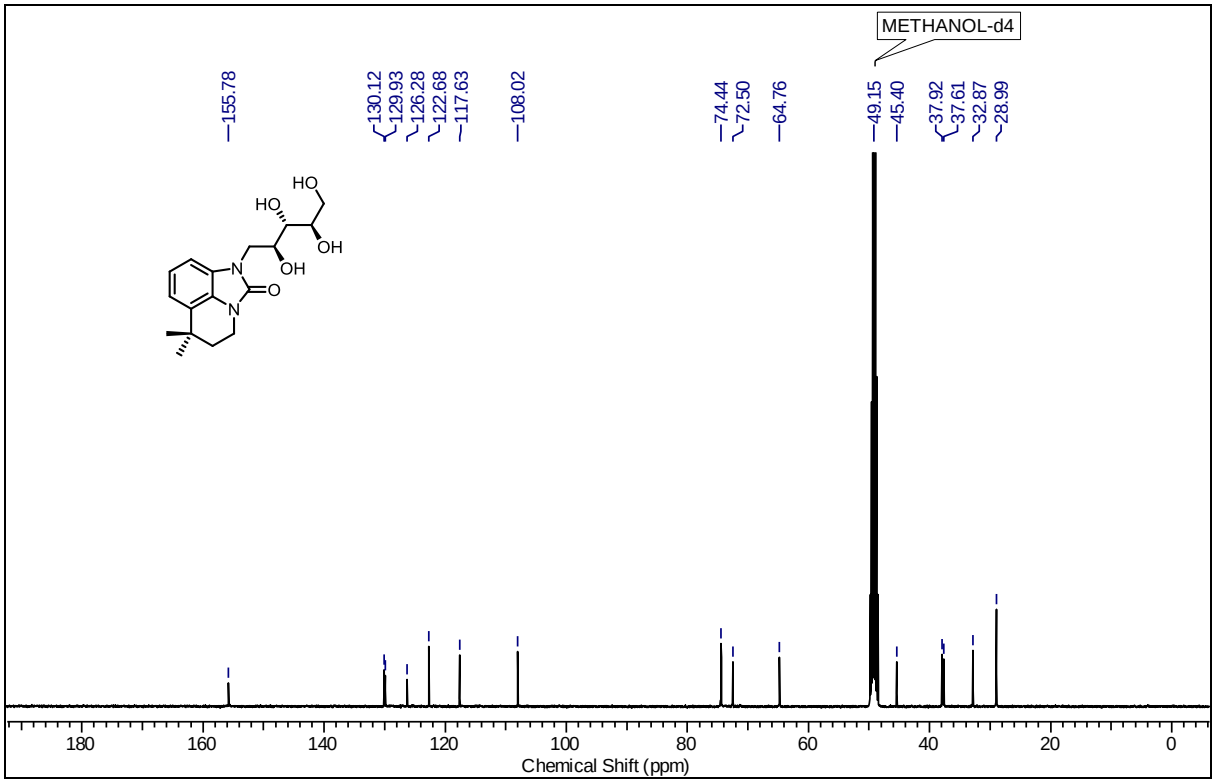
^{13}C NMR of Compound 28 at 100 MHz in $CDCl_3$



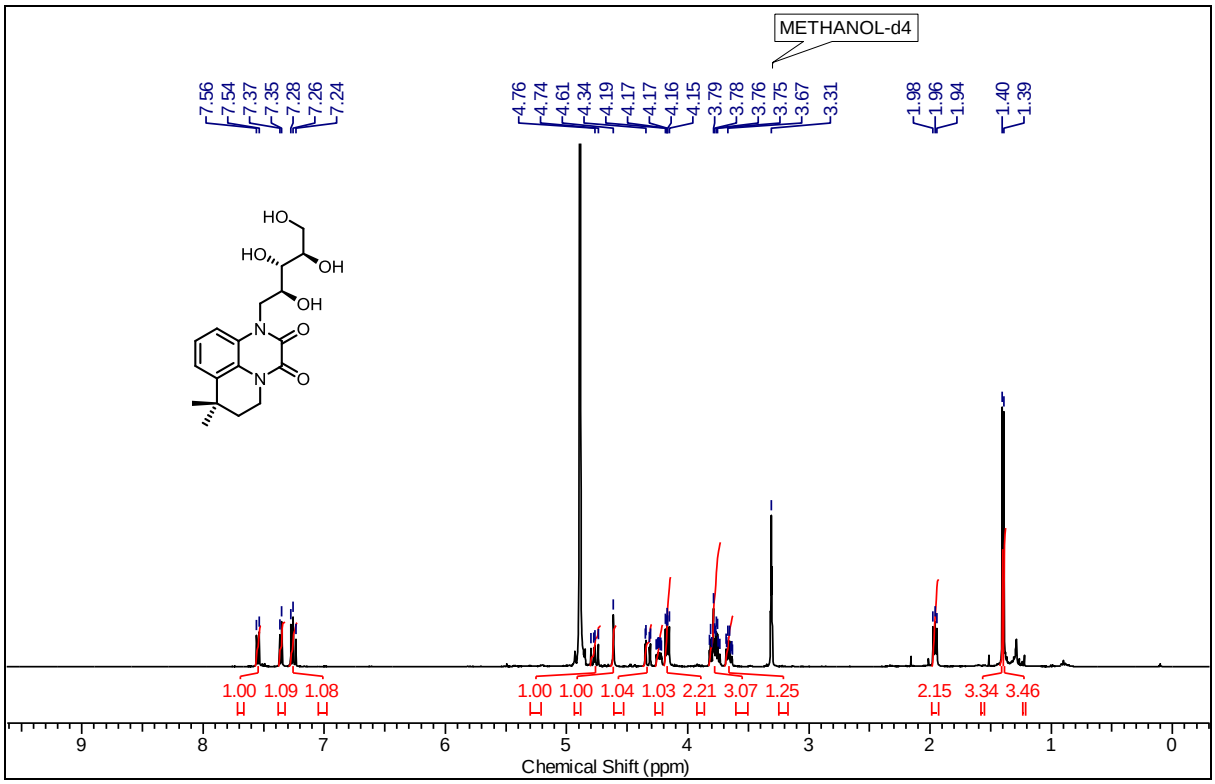
^{1}H NMR of Compound 31 at 400 MHz in CD_3OD



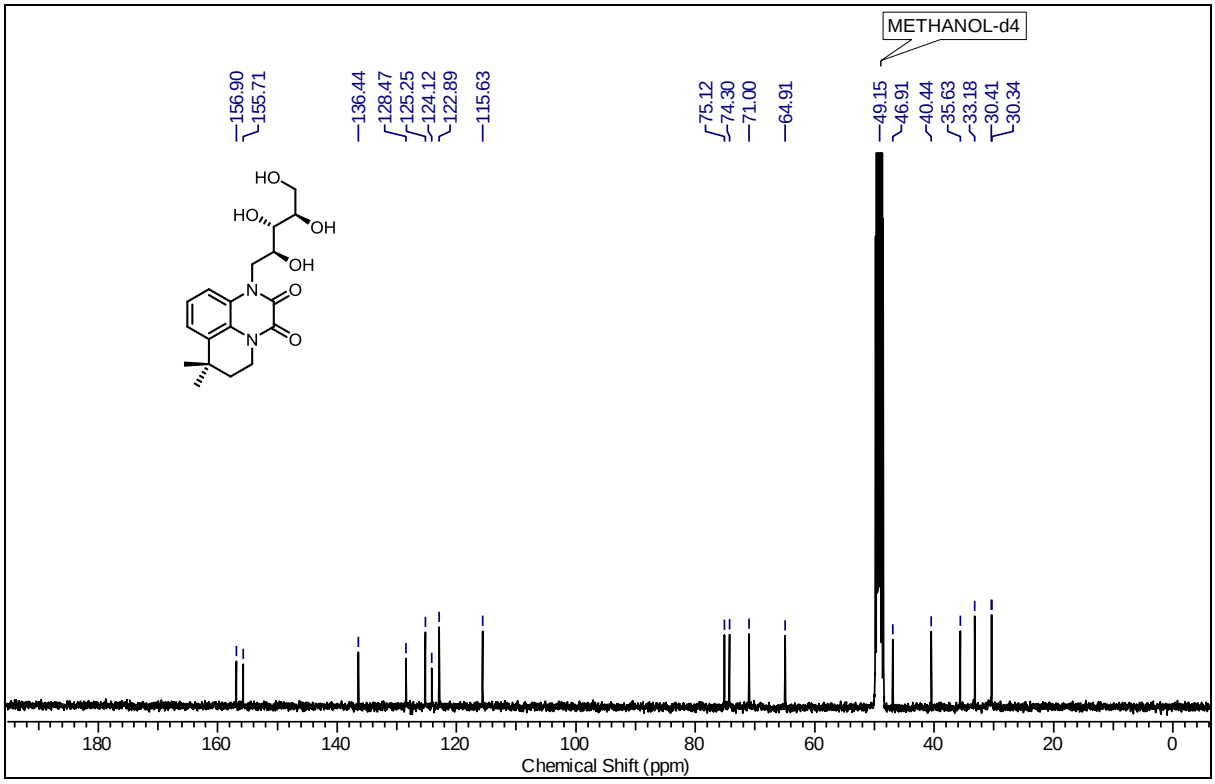
^{13}C NMR of Compound 31 at 100 MHz in CD_3OD



^{1}H NMR of Compound 33 at 400 MHz in CD_3OD



^{13}C NMR of Compound 33 at 100 MHz in CD_3OD



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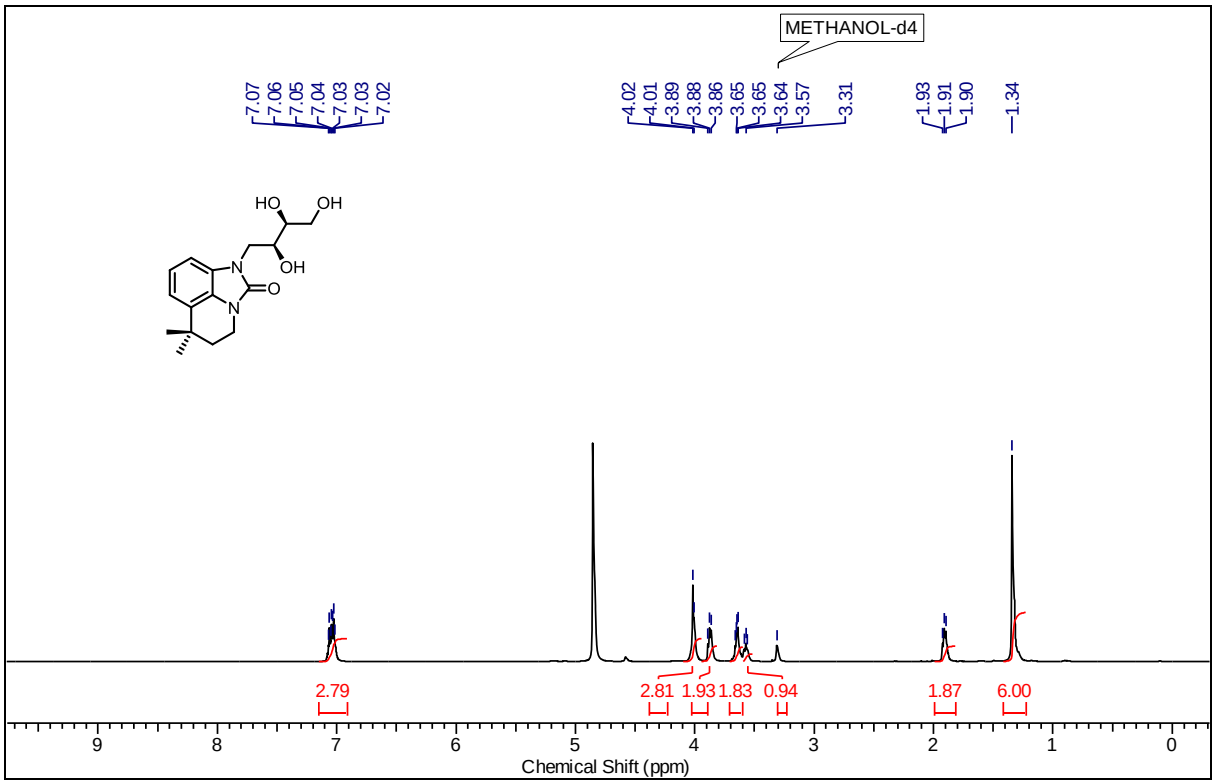
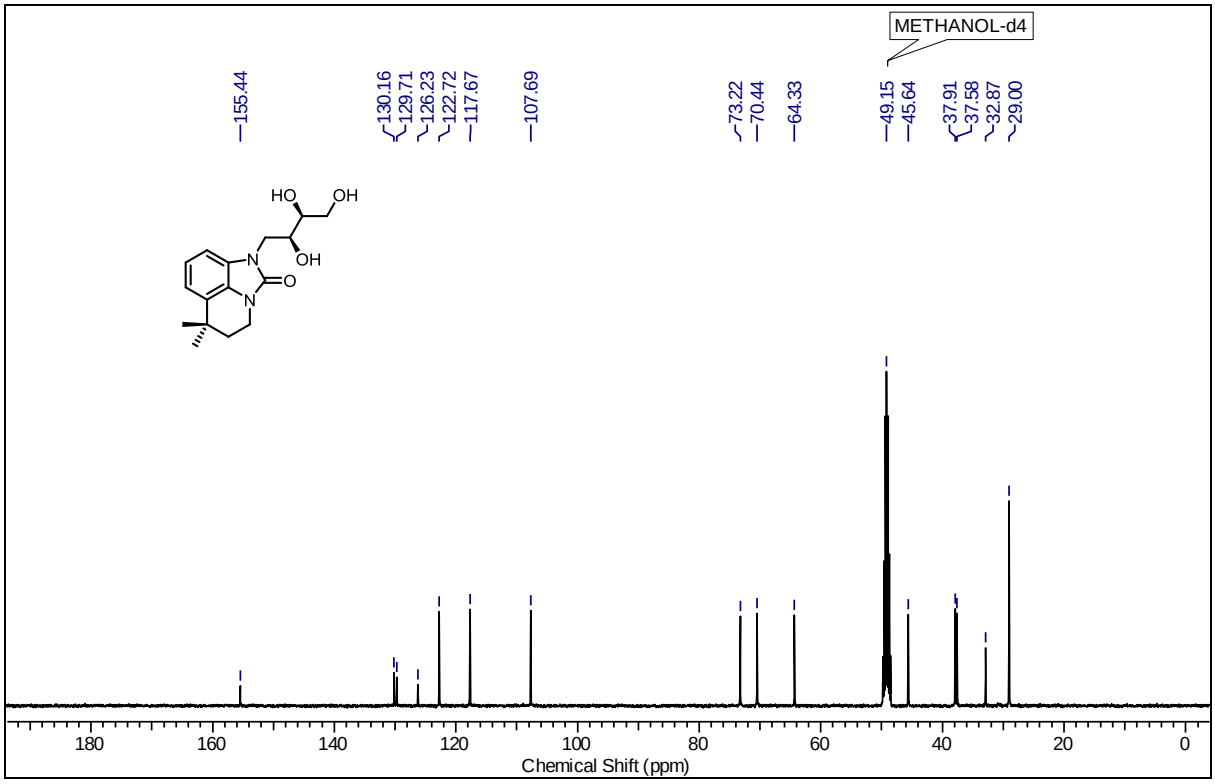
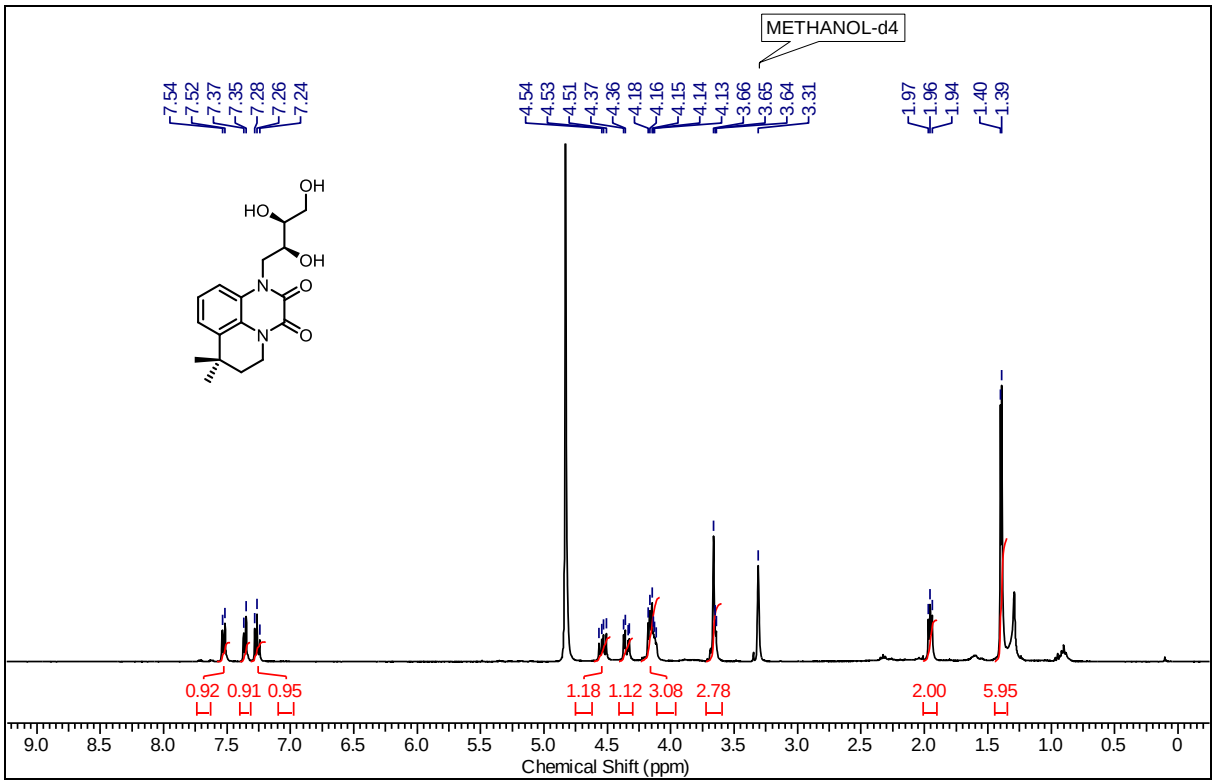


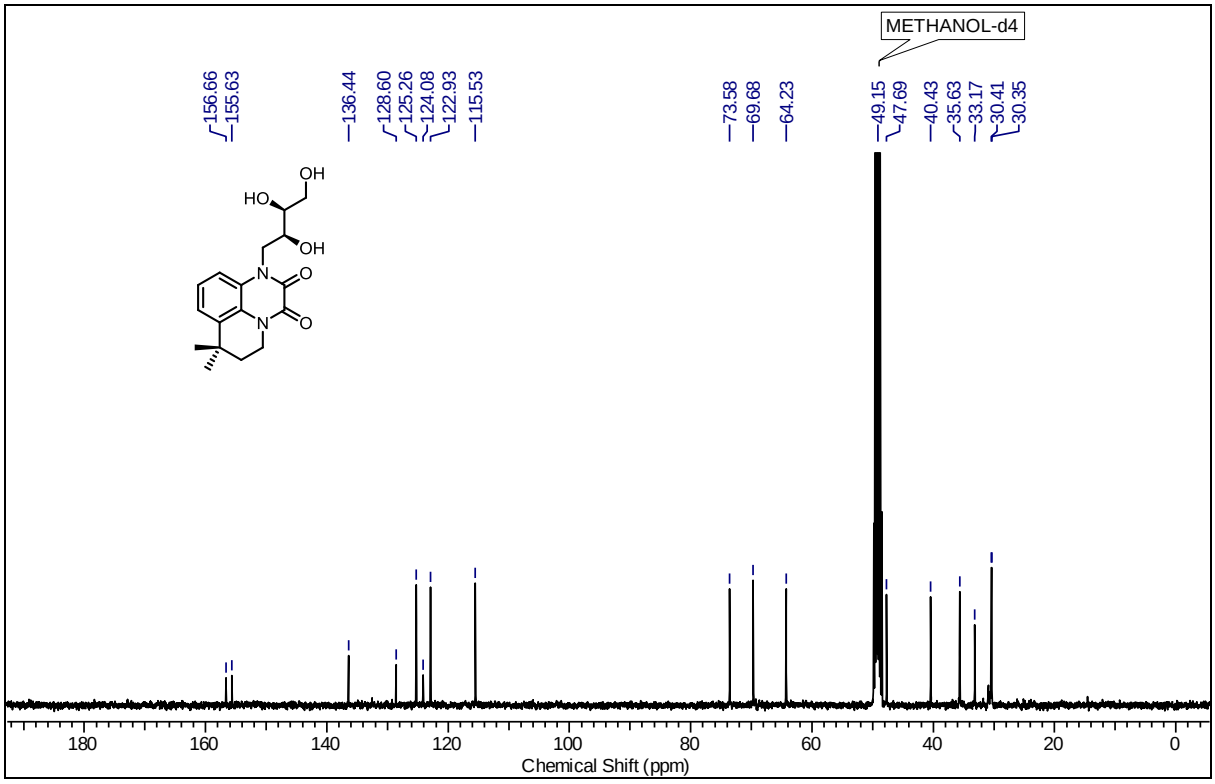
^{13}C NMR of Compound 41 at 100 MHz in CD_3OD



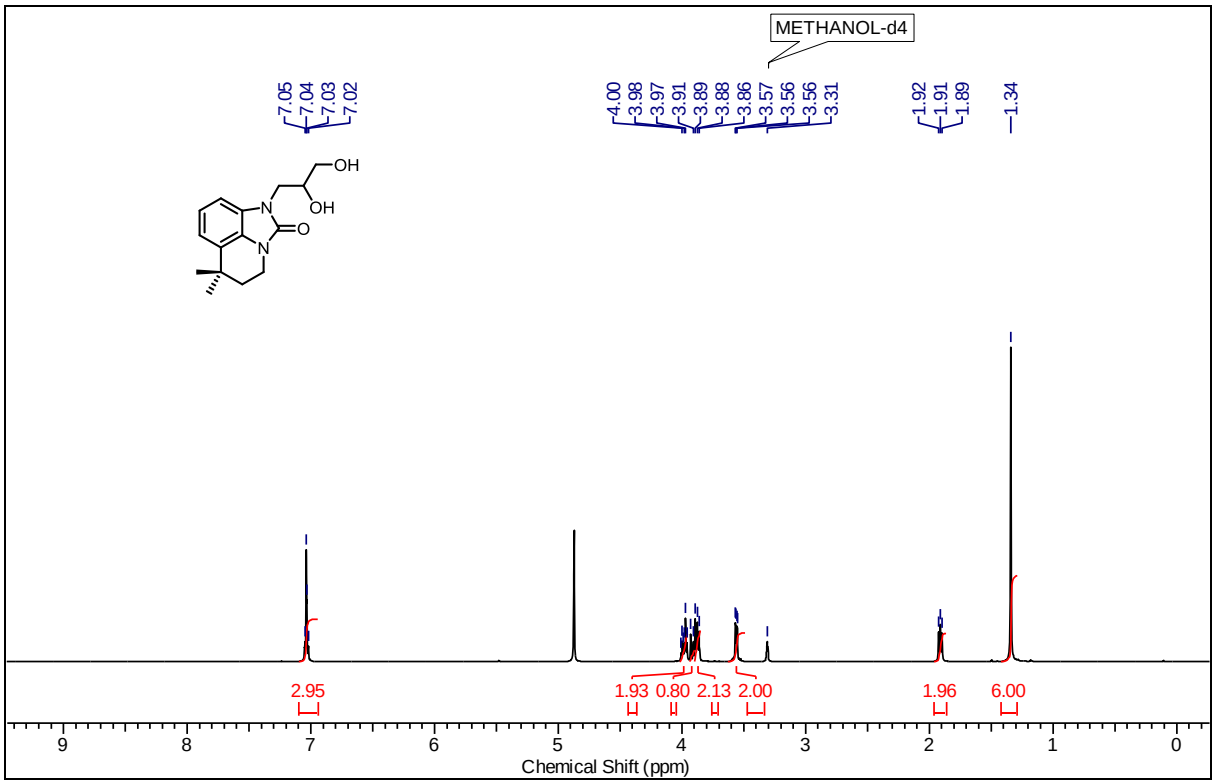
^{1}H NMR of Compound 43 at 400 MHz in CD_3OD



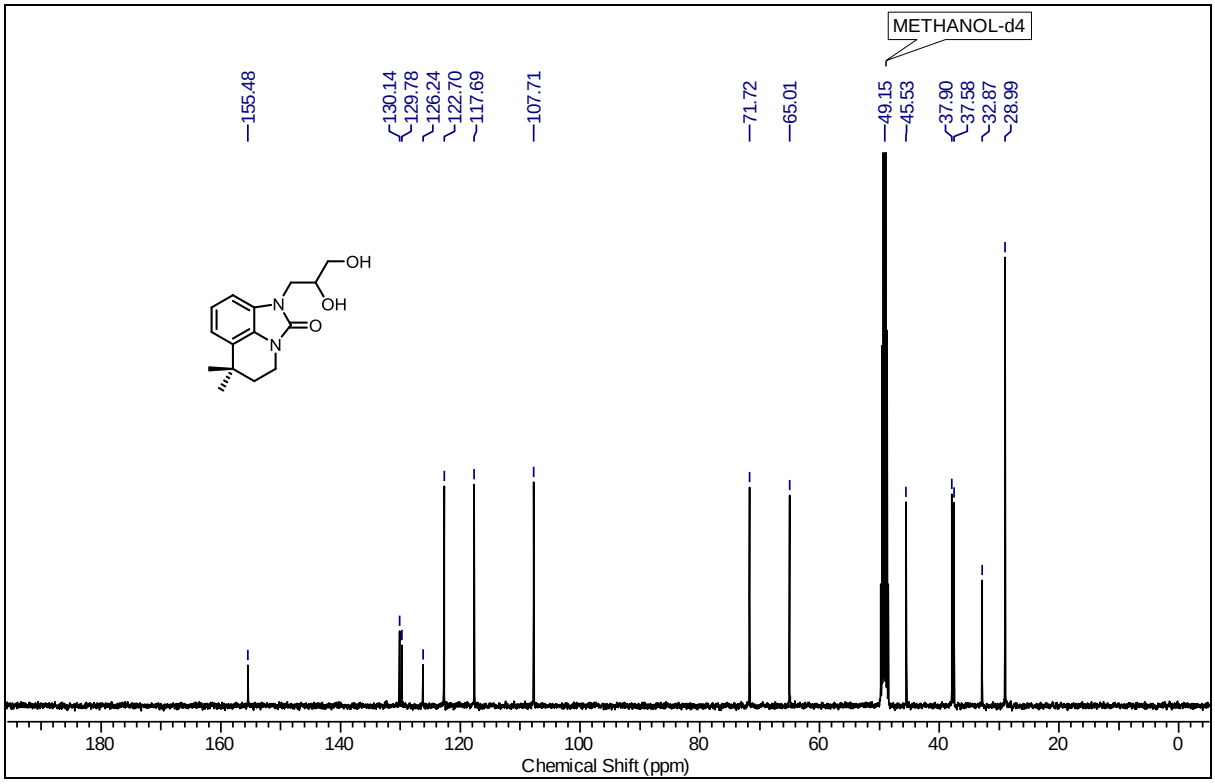
^{13}C NMR of Compound 43 at 100 MHz in CD_3OD



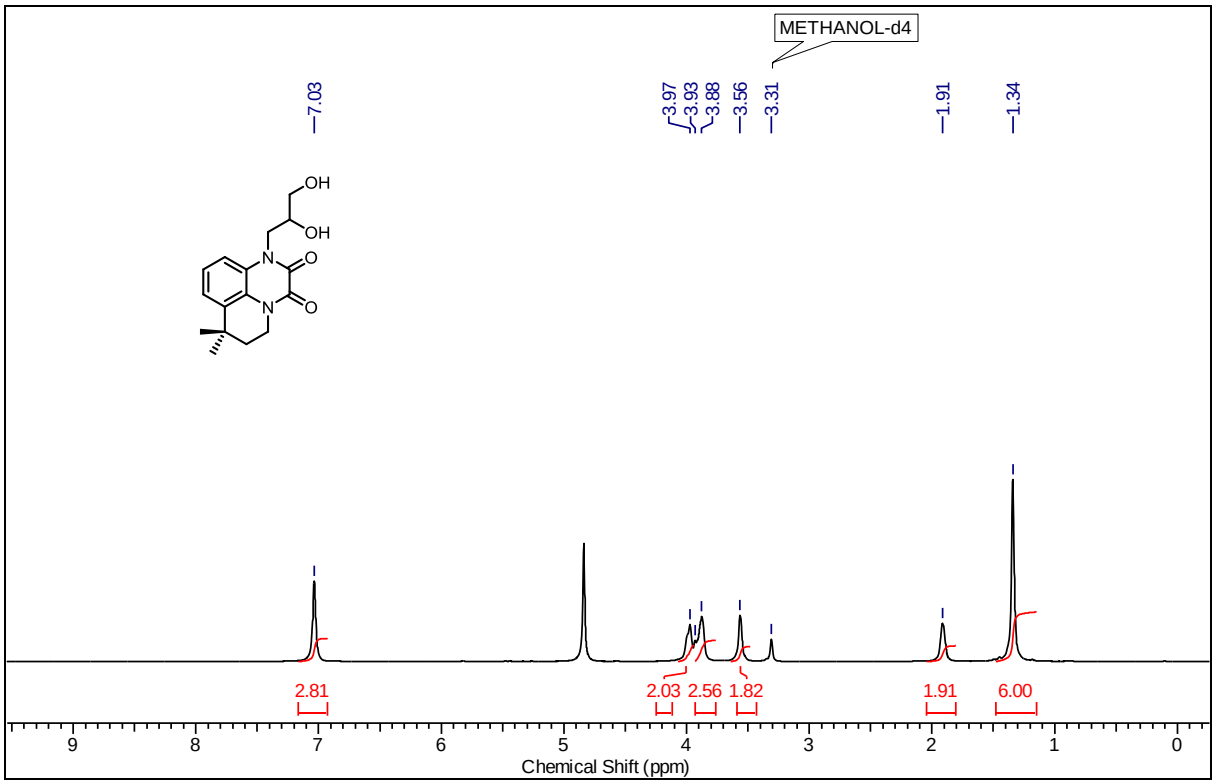
^{1}H NMR of Compound 45 at 400 MHz in CD_3OD



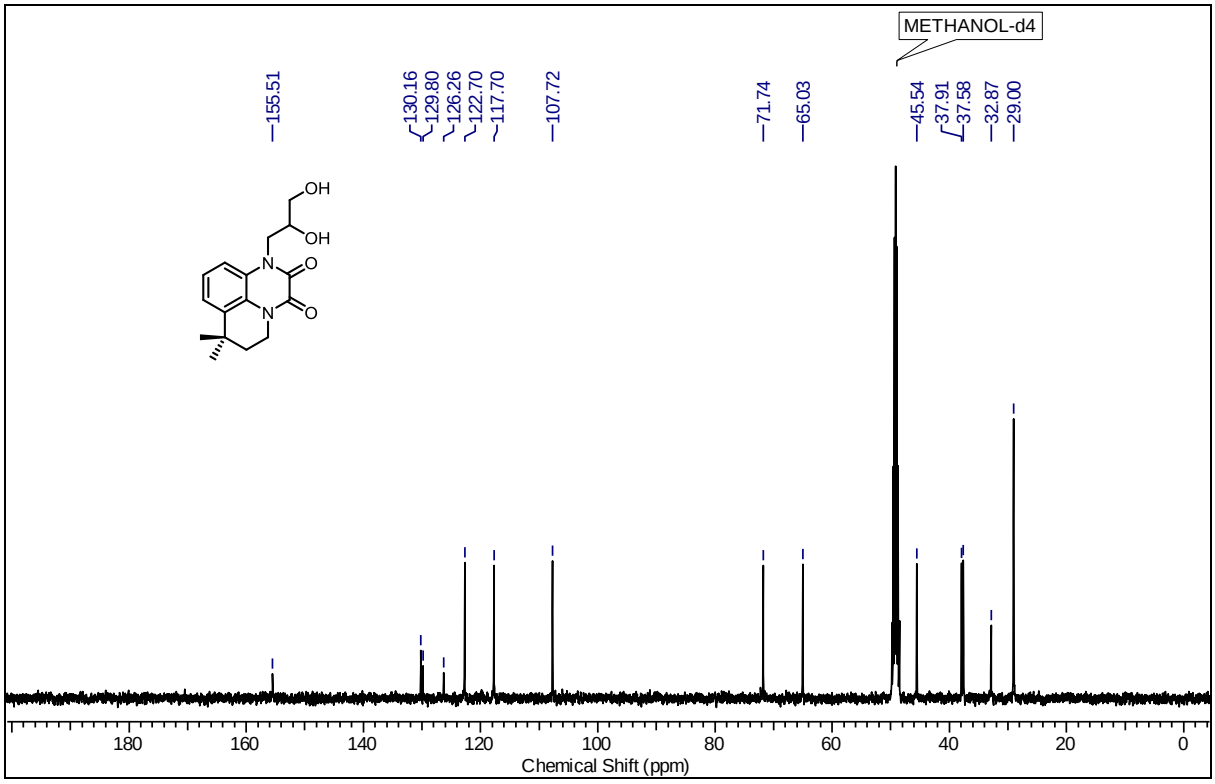
^{13}C NMR of Compound 45 at 100 MHz in CD_3OD



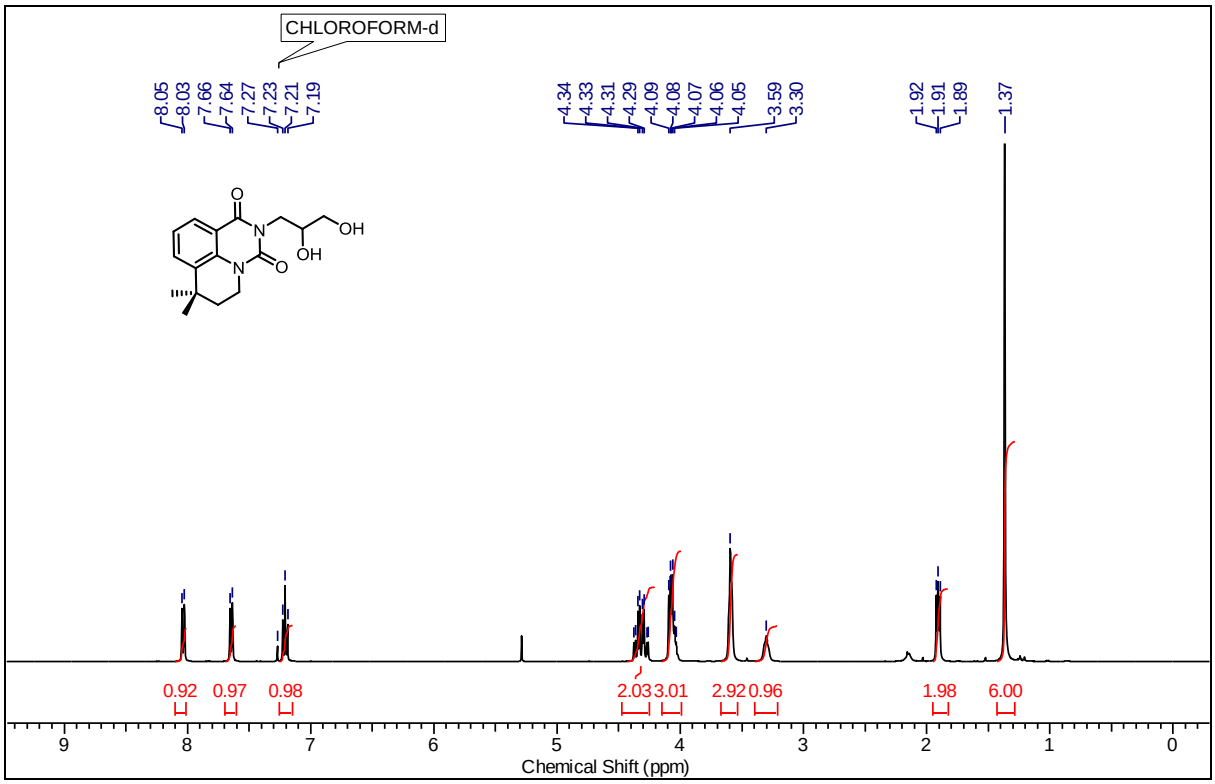
^{1}H NMR of Compound 47 at 400 MHz in CD_3OD



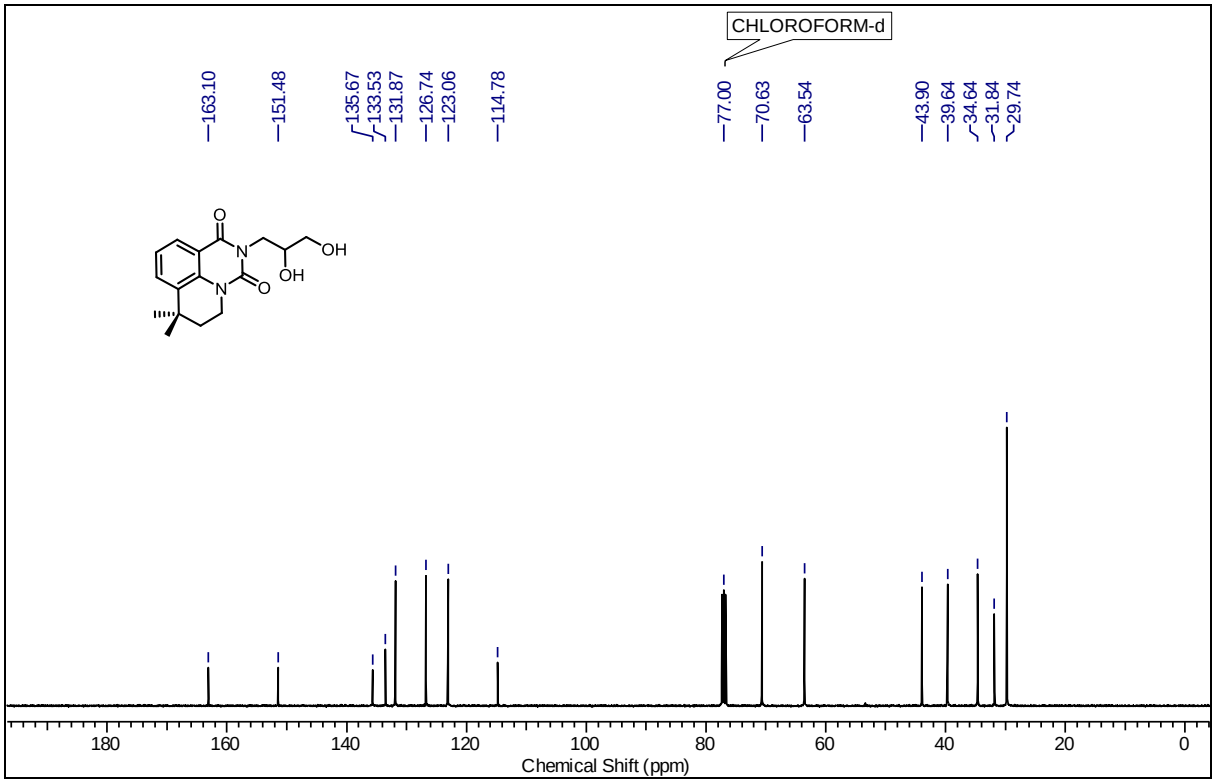
^{13}C NMR of Compound 47 at 100 MHz in CD_3OD



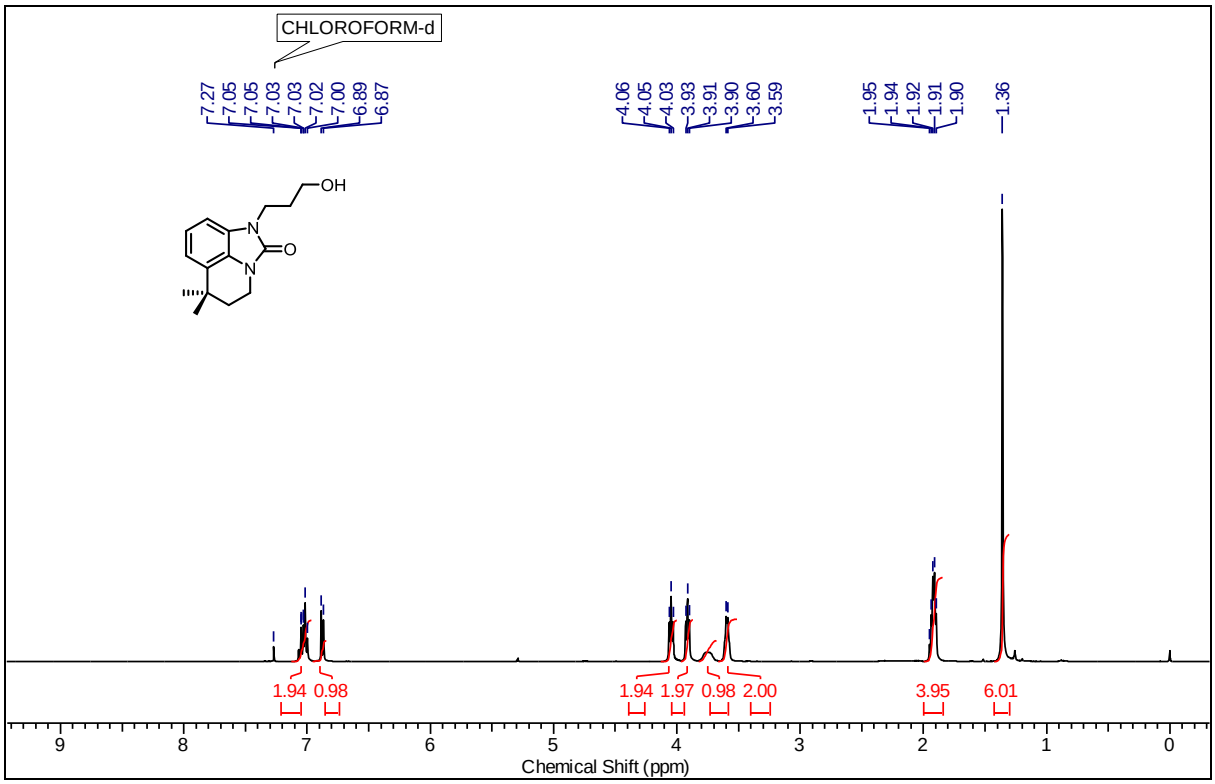
^{1}H NMR of Compound 48 at 400 MHz in $CDCl_3$



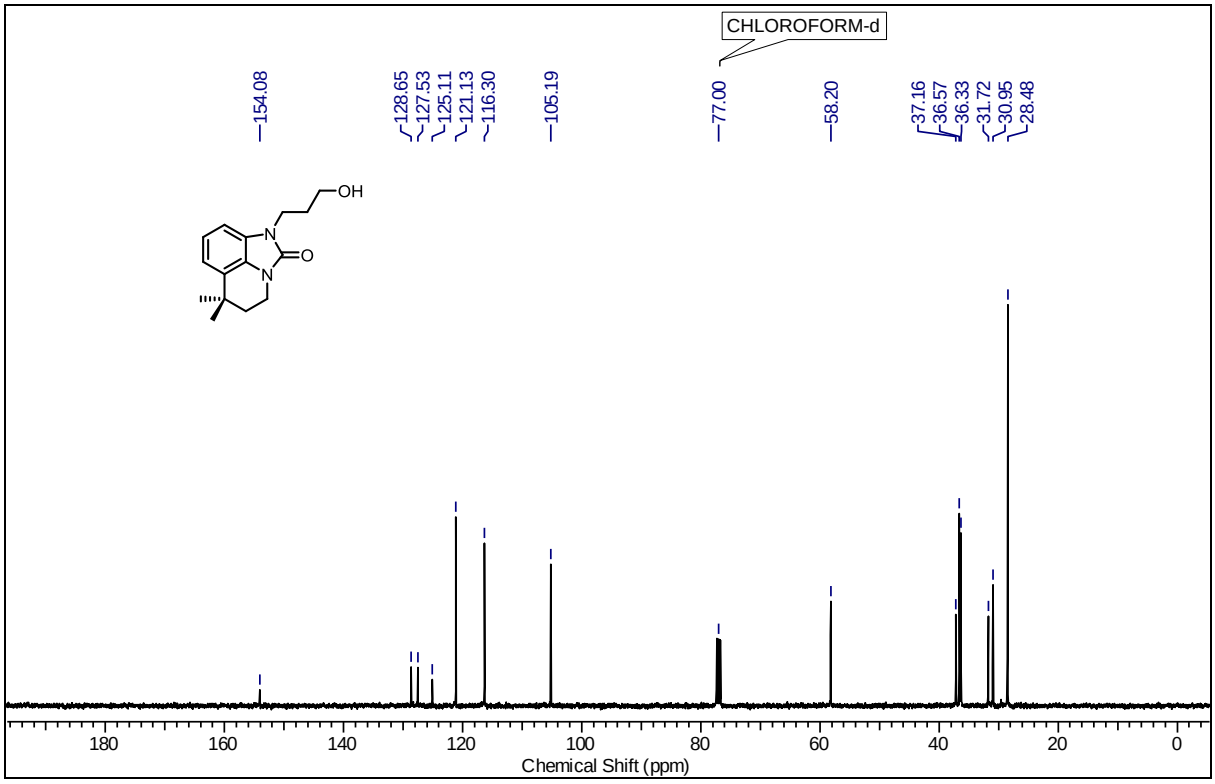
^{13}C NMR of Compound 48 at 100 MHz in $CDCl_3$



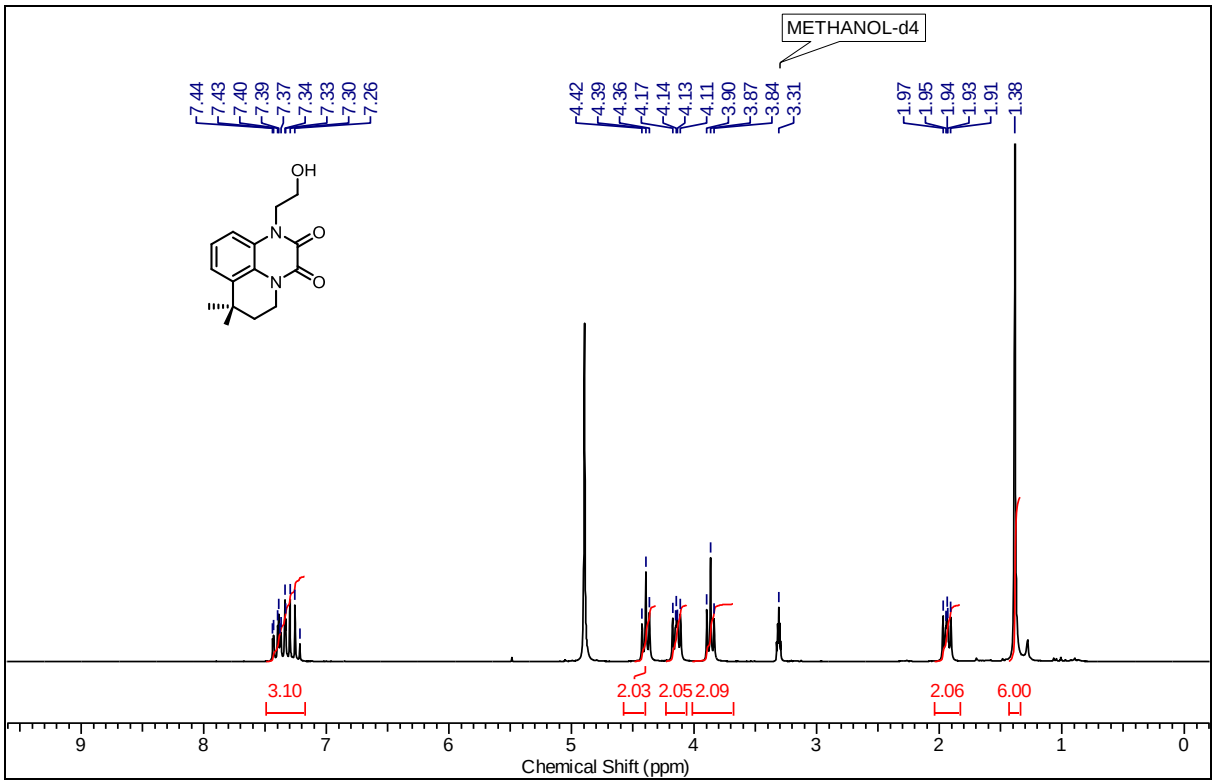
^{1}H NMR of Compound 53 at 400 MHz in $CDCl_3$



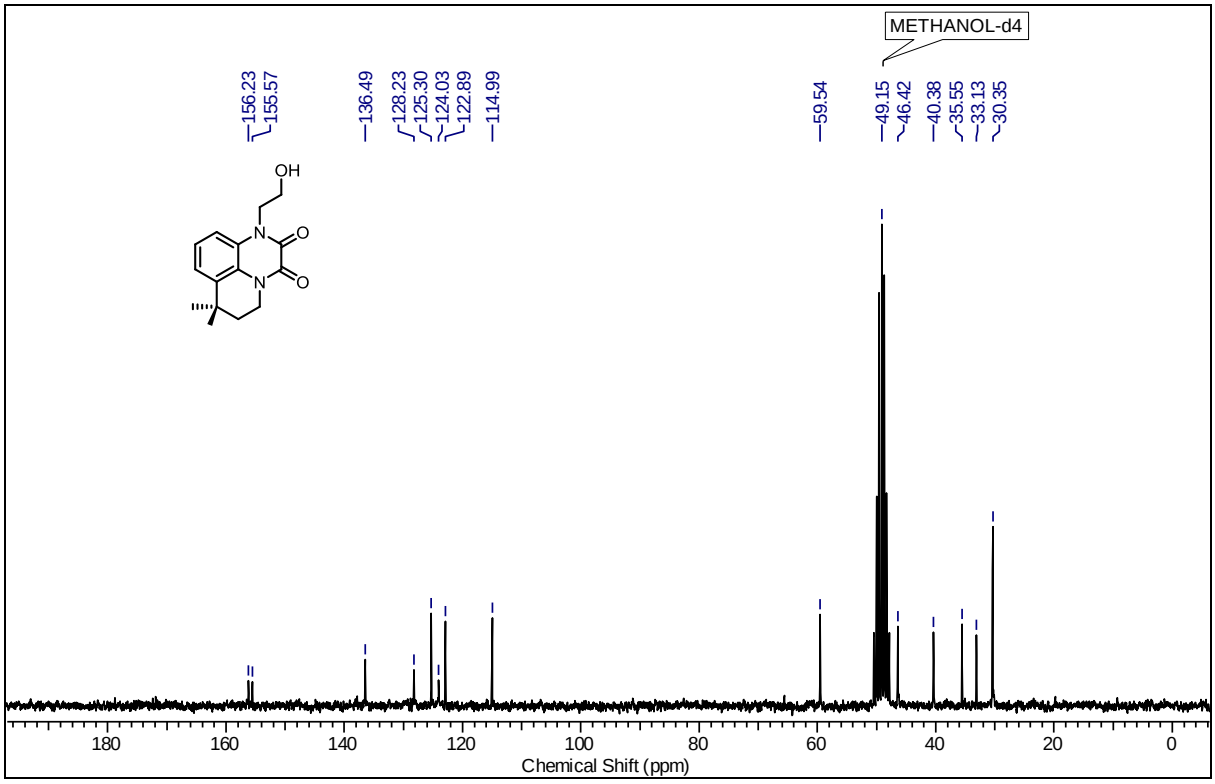
^{13}C NMR of Compound 53 at 100 MHz in $CDCl_3$



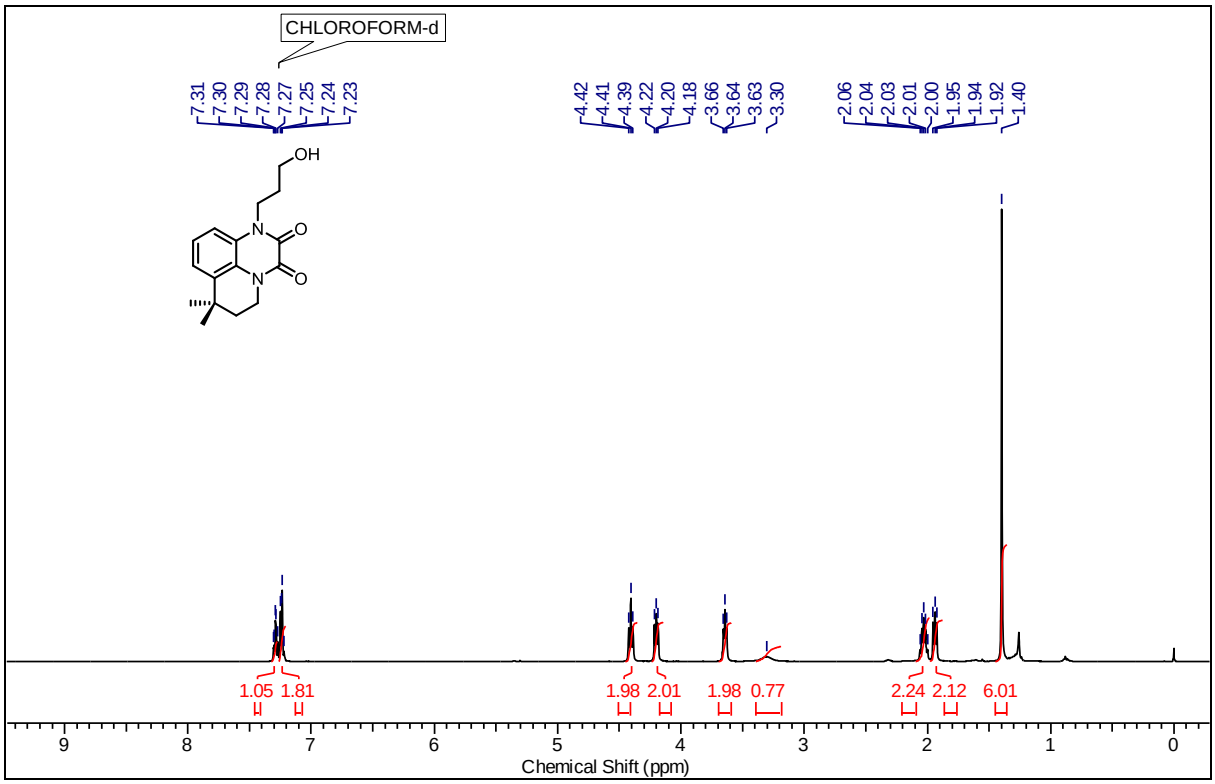
^{1}H NMR of Compound 55 at 400 MHz in CD_3OD



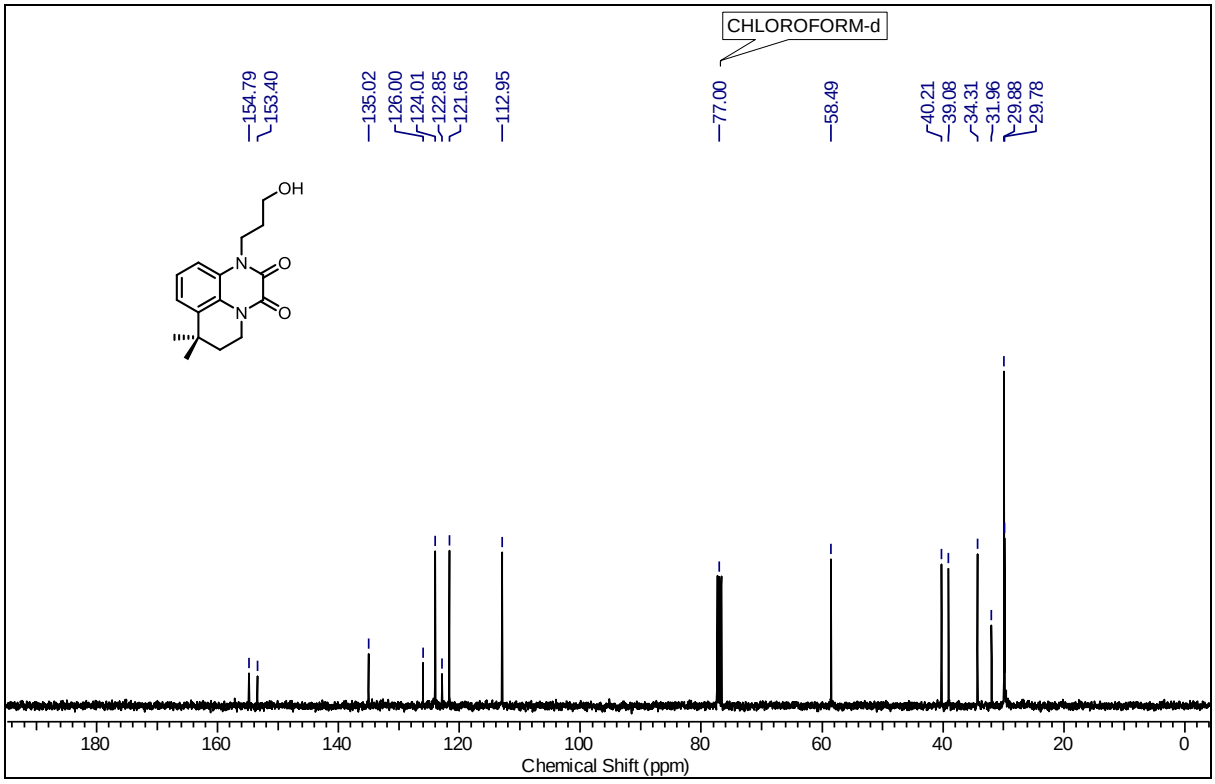
^{13}C NMR of Compound 55 at 100 MHz in CD_3OD



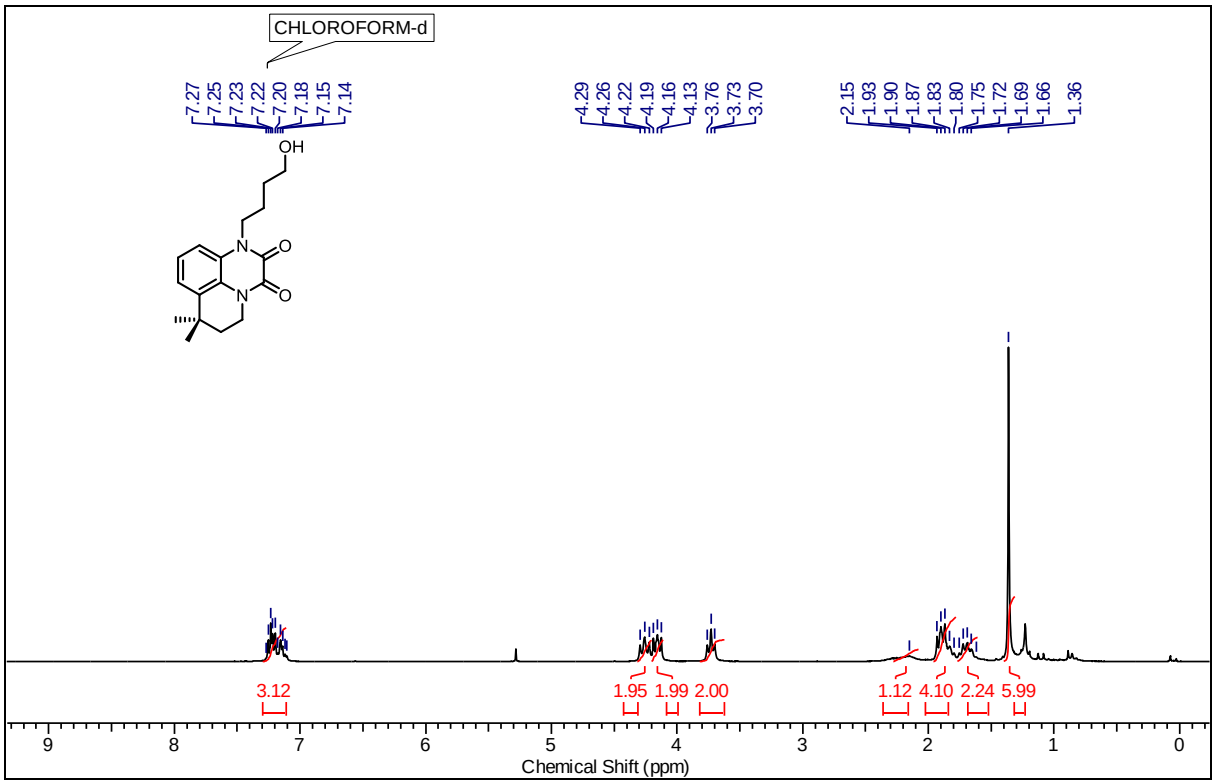
^{1}H NMR of Compound 57 at 400 MHz in $CDCl_3$



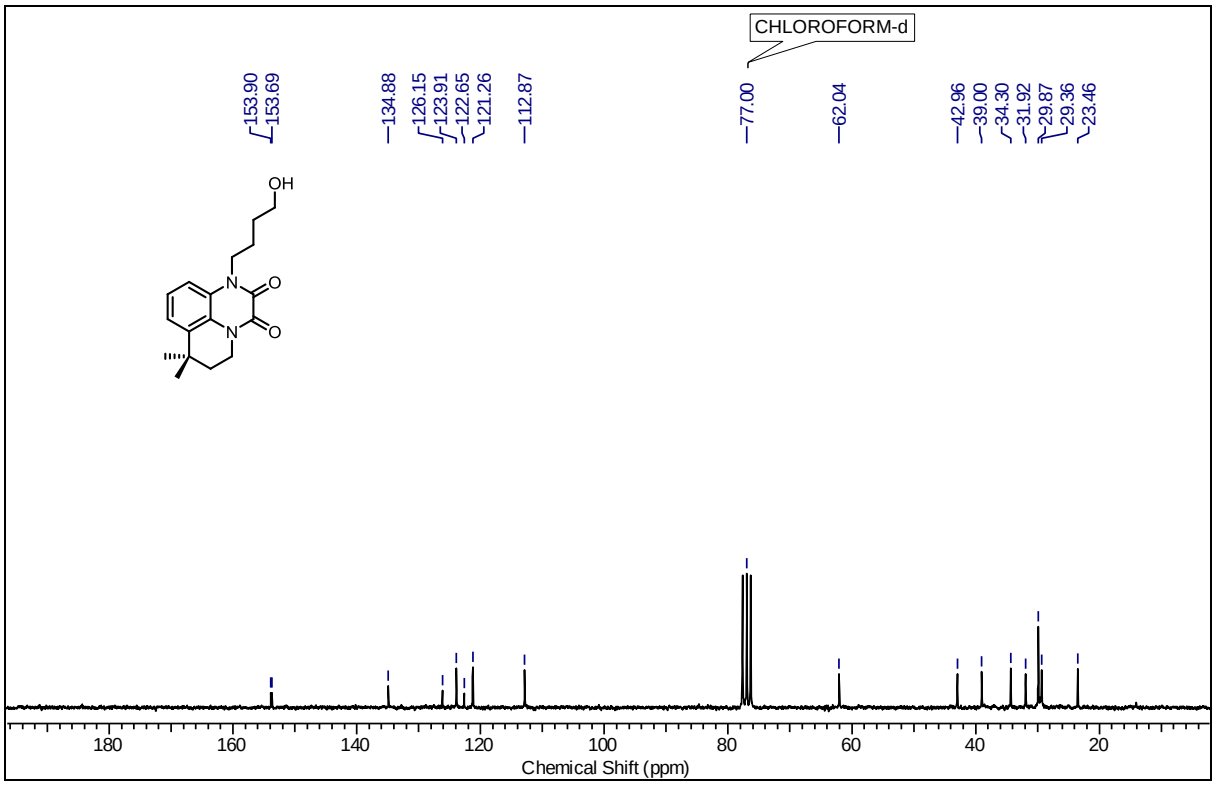
^{13}C NMR of Compound 57 at 100 MHz in $CDCl_3$



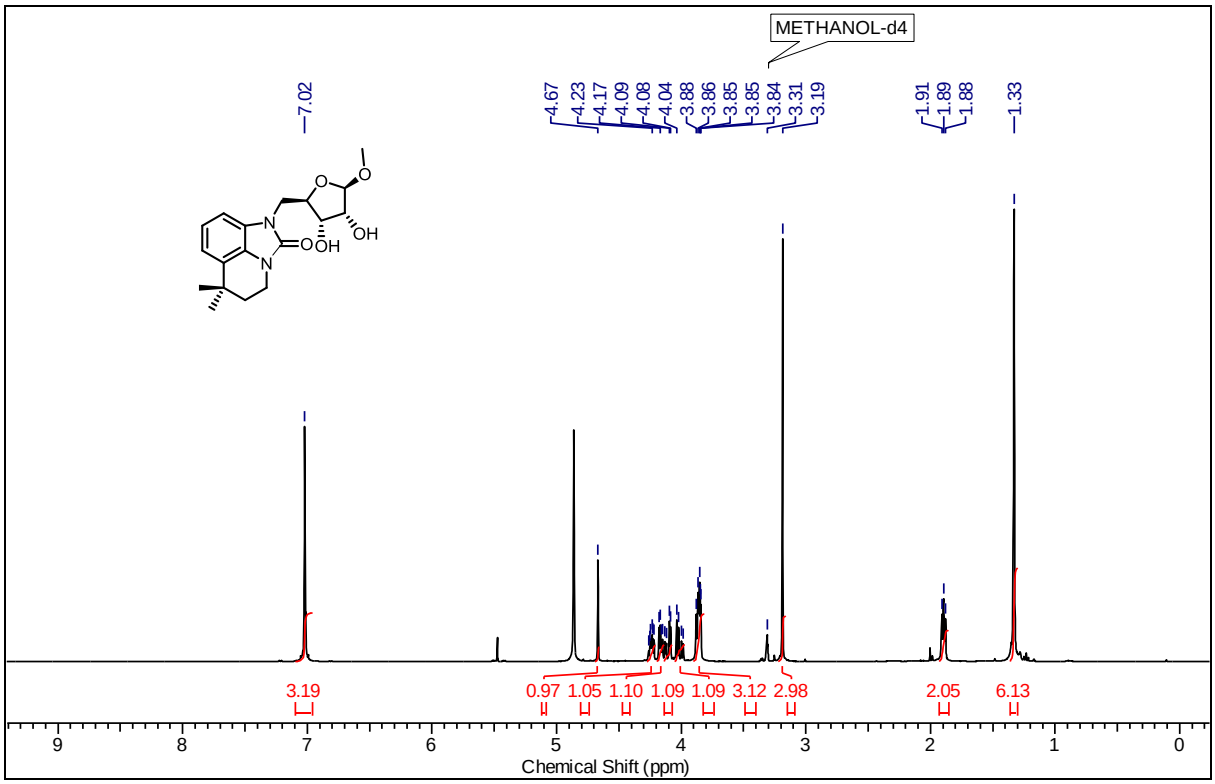
^{1}H NMR of Compound 59 at 400 MHz in $CDCl_3$



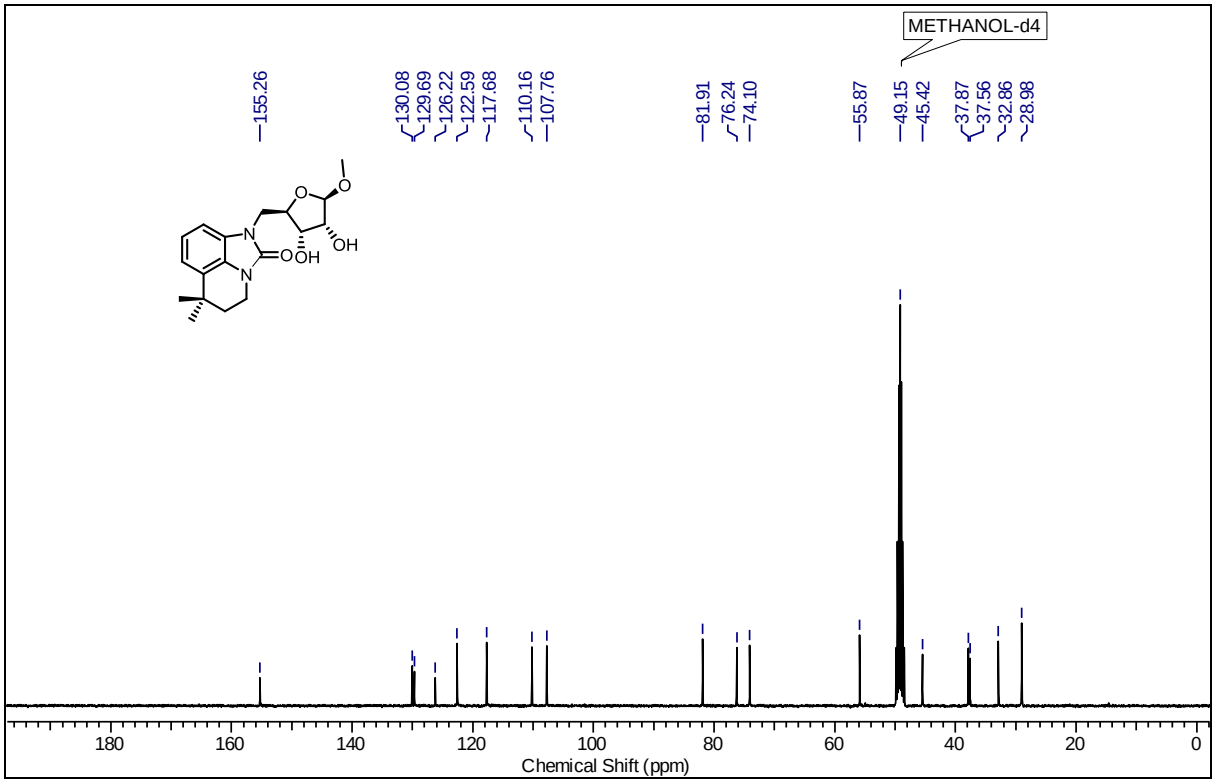
^{13}C NMR of Compound 59 at 100 MHz in $CDCl_3$



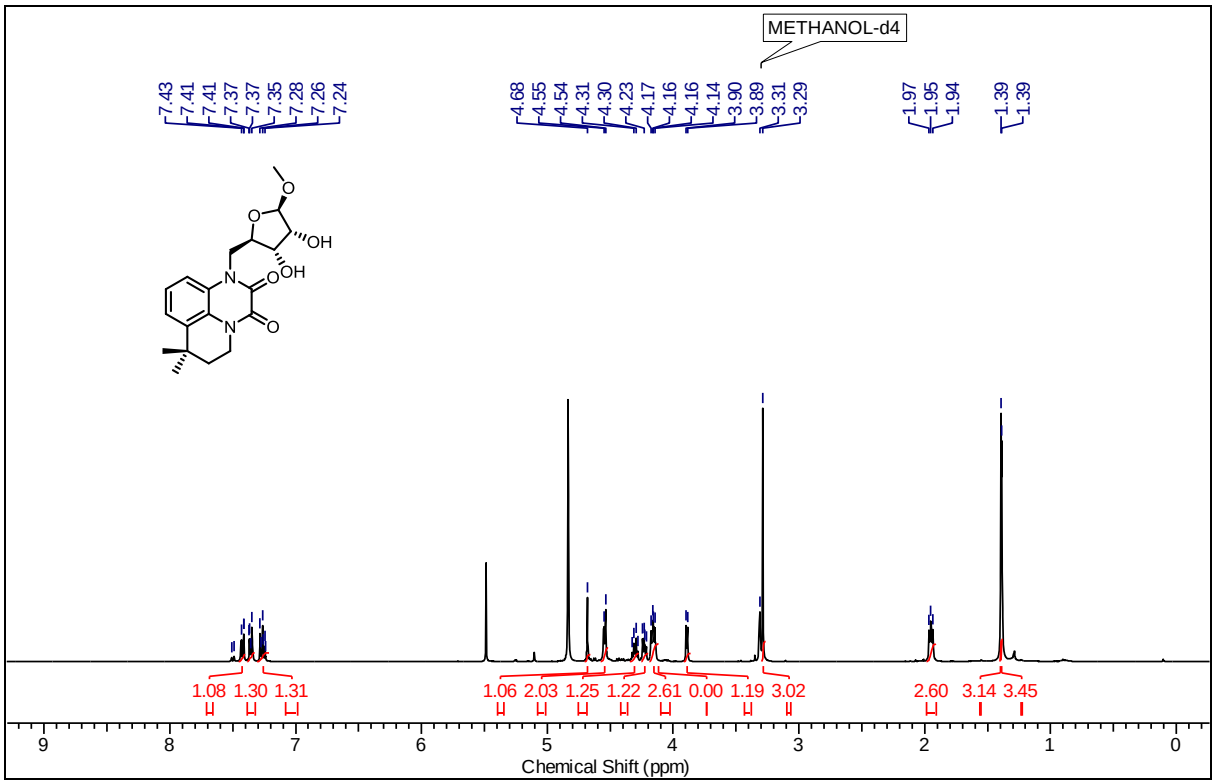
^{1}H NMR of Compound 62 at 400 MHz in CD_3OD



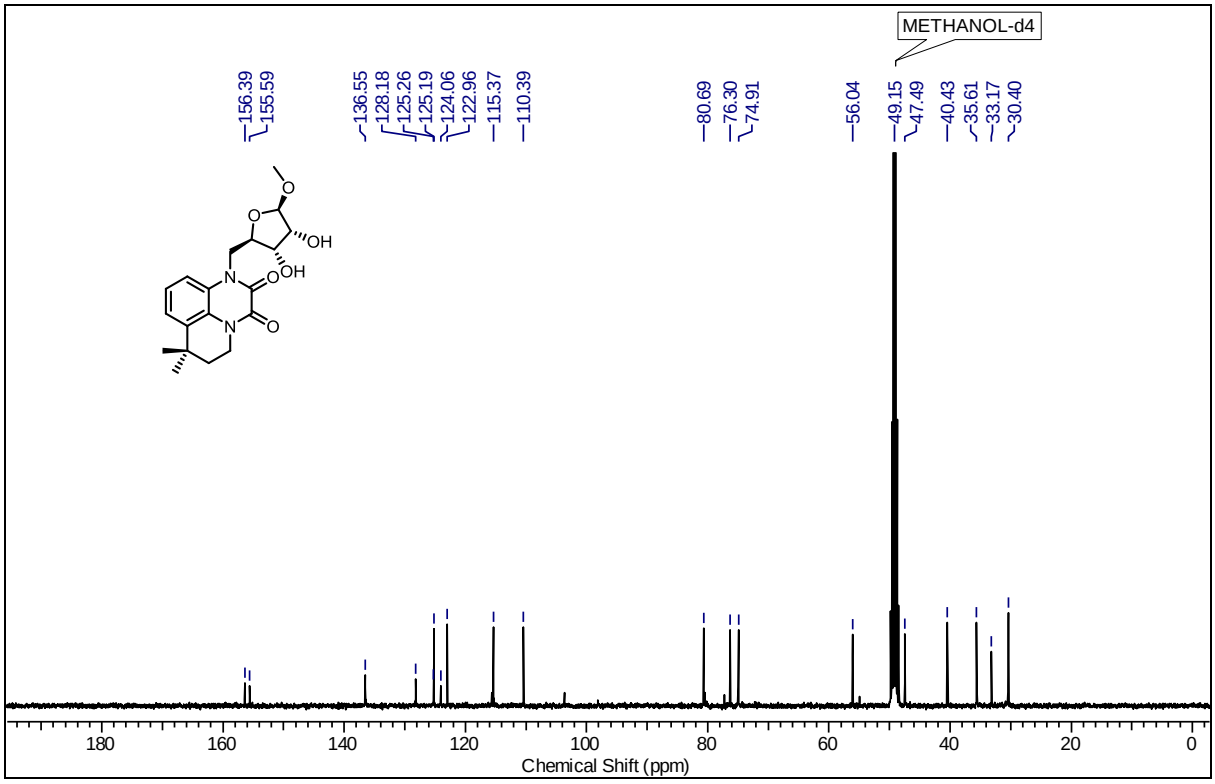
^{13}C NMR of Compound 62 at 100 MHz in CD_3OD



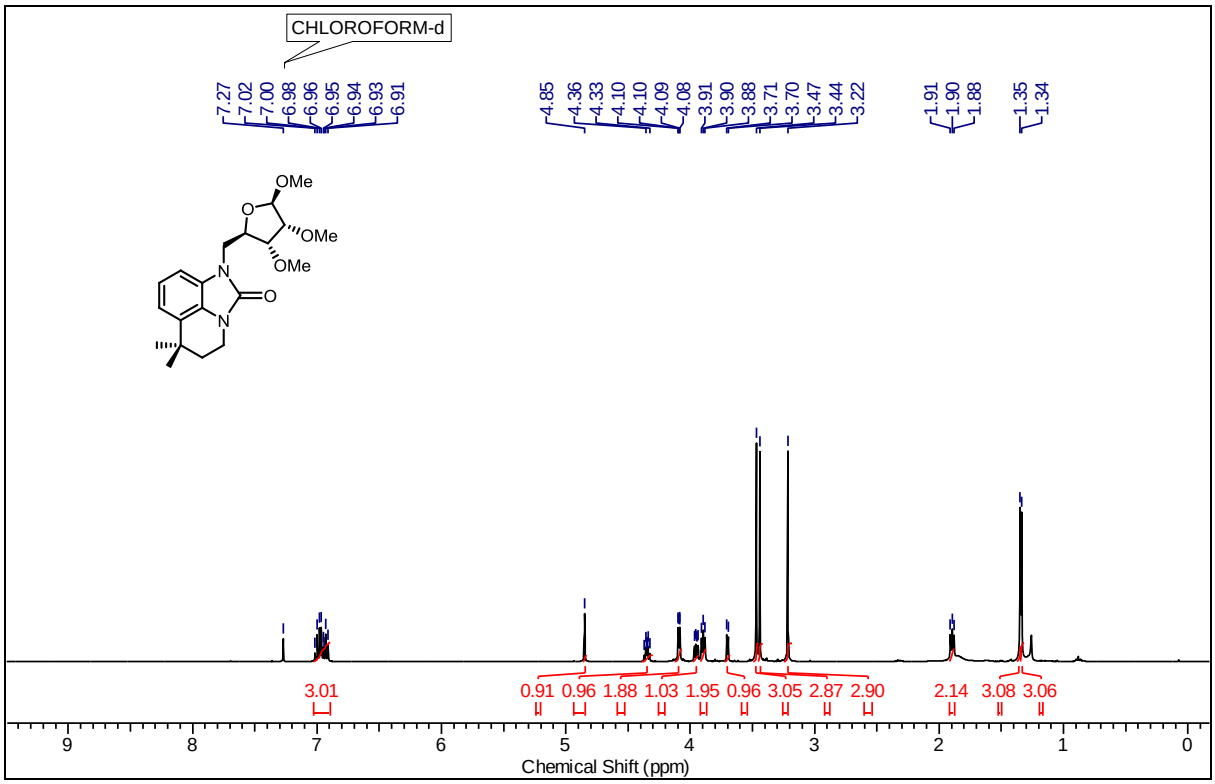
^{1}H NMR of Compound 64 at 400 MHz in CD_3OD



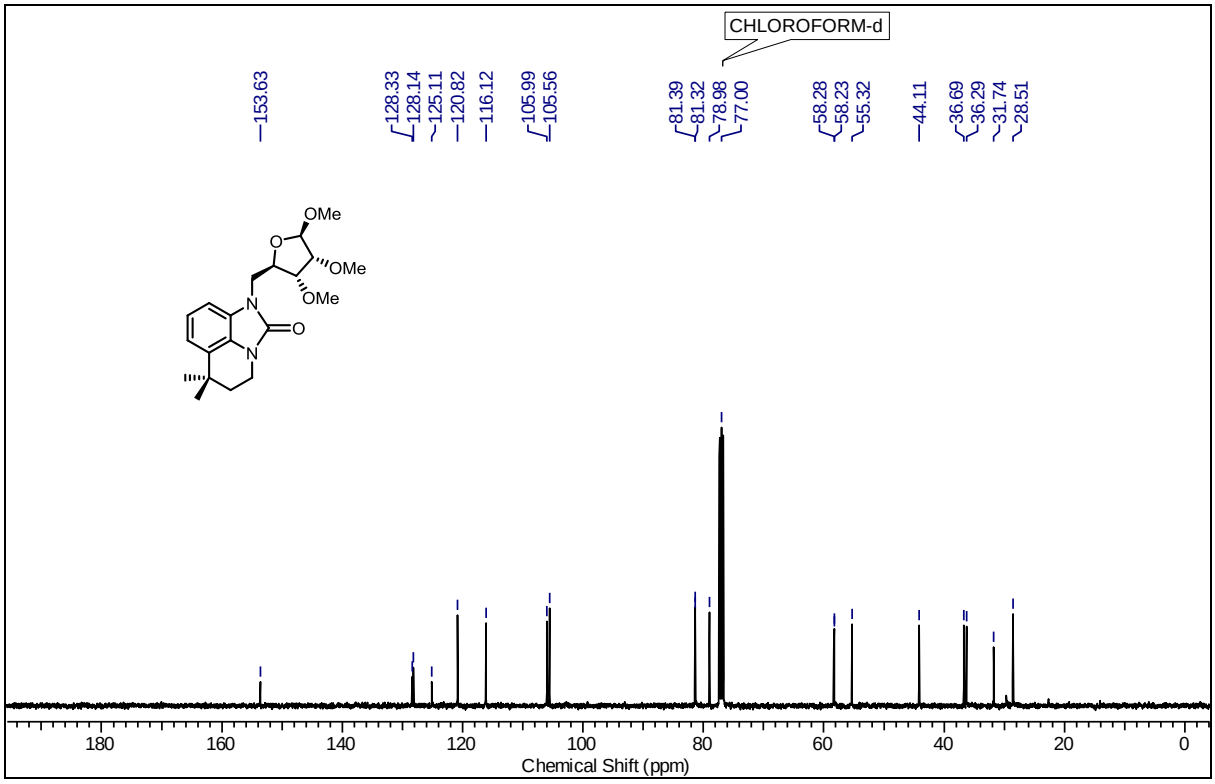
^{13}C NMR of Compound 64 at 100 MHz in CD_3OD



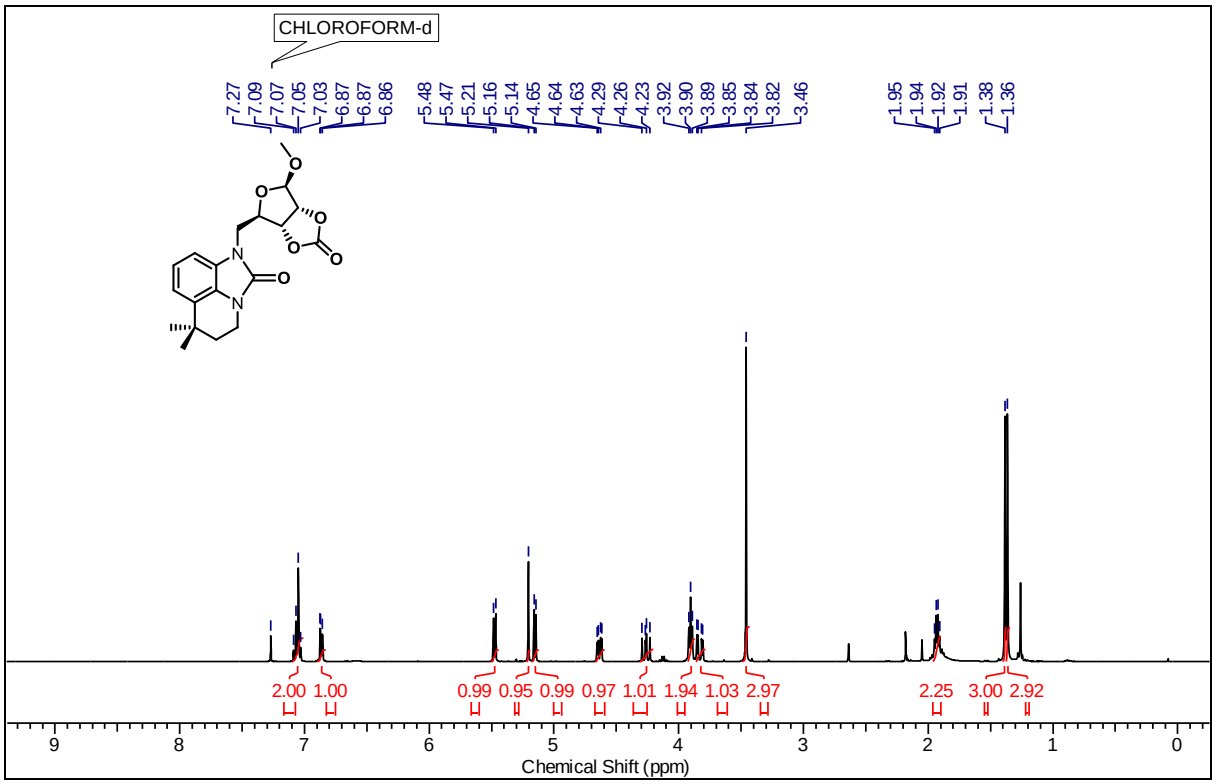
^{1}H NMR of Compound 65 at 400 MHz in $CDCl_3$



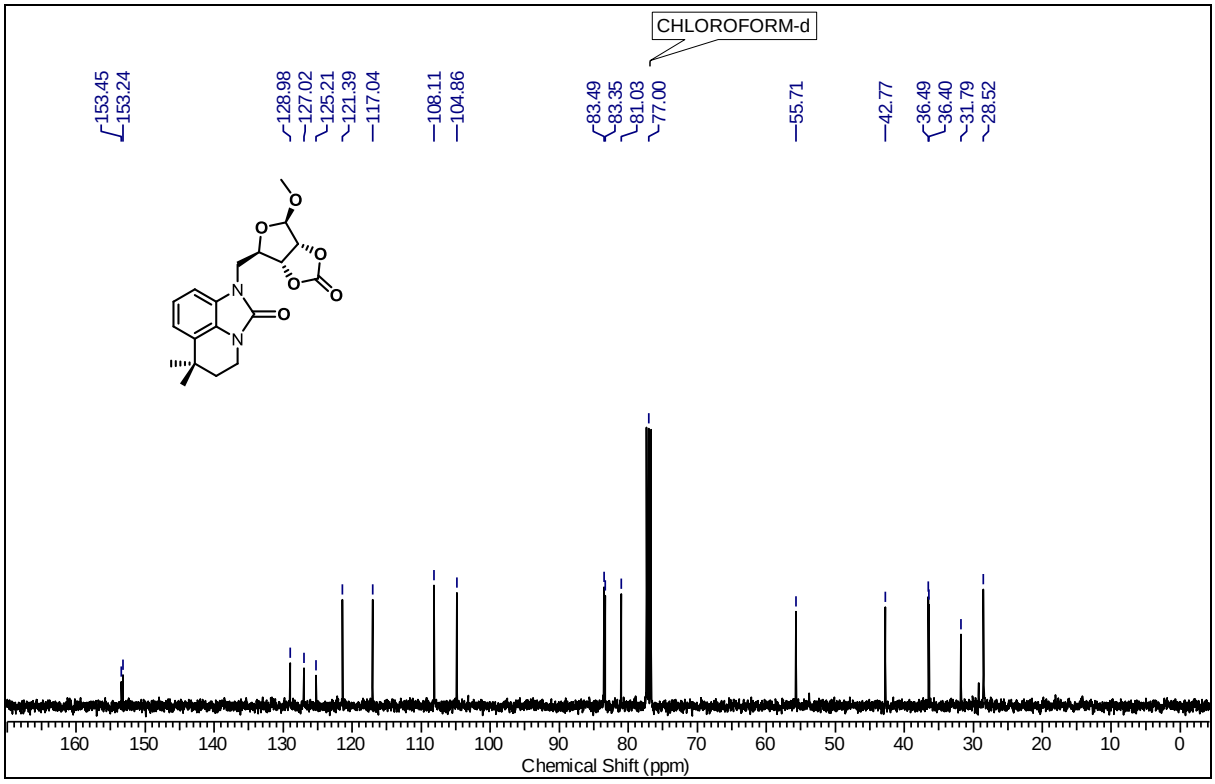
^{13}C NMR of Compound 65 at 100 MHz in $CDCl_3$



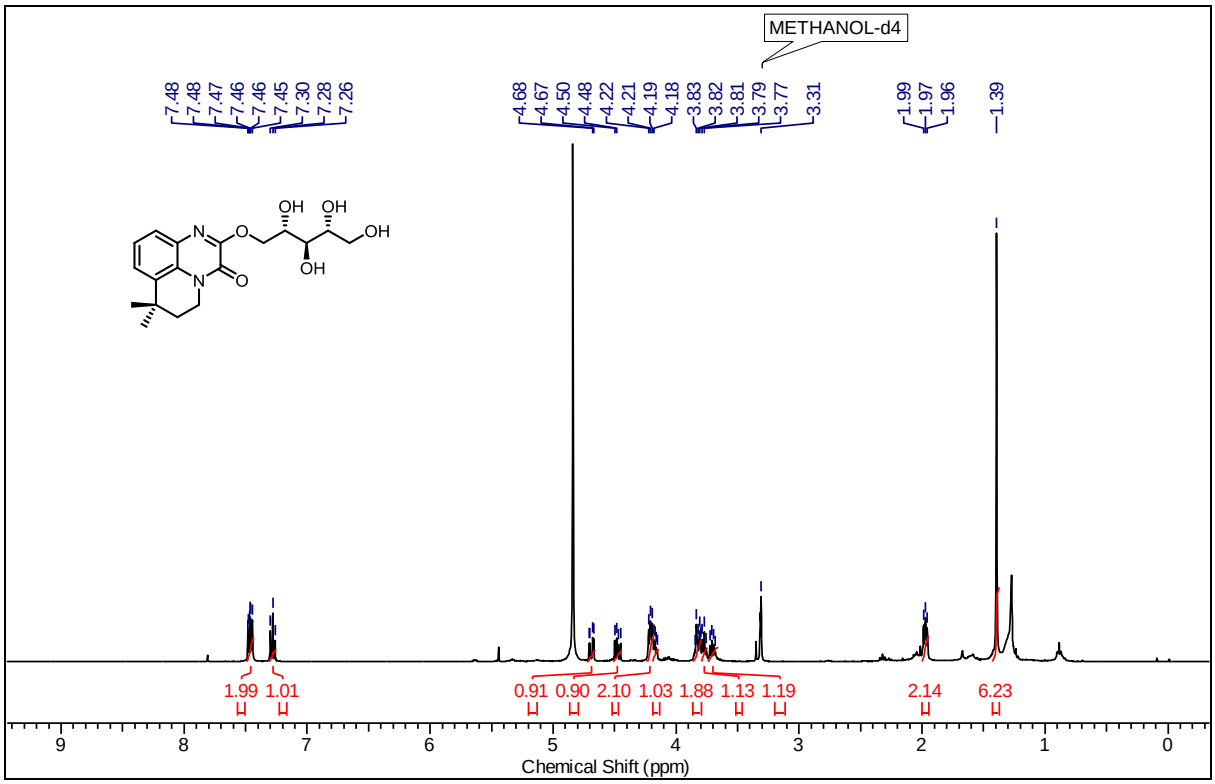
^{1}H NMR of Compound 66 at 400 MHz in $CDCl_3$



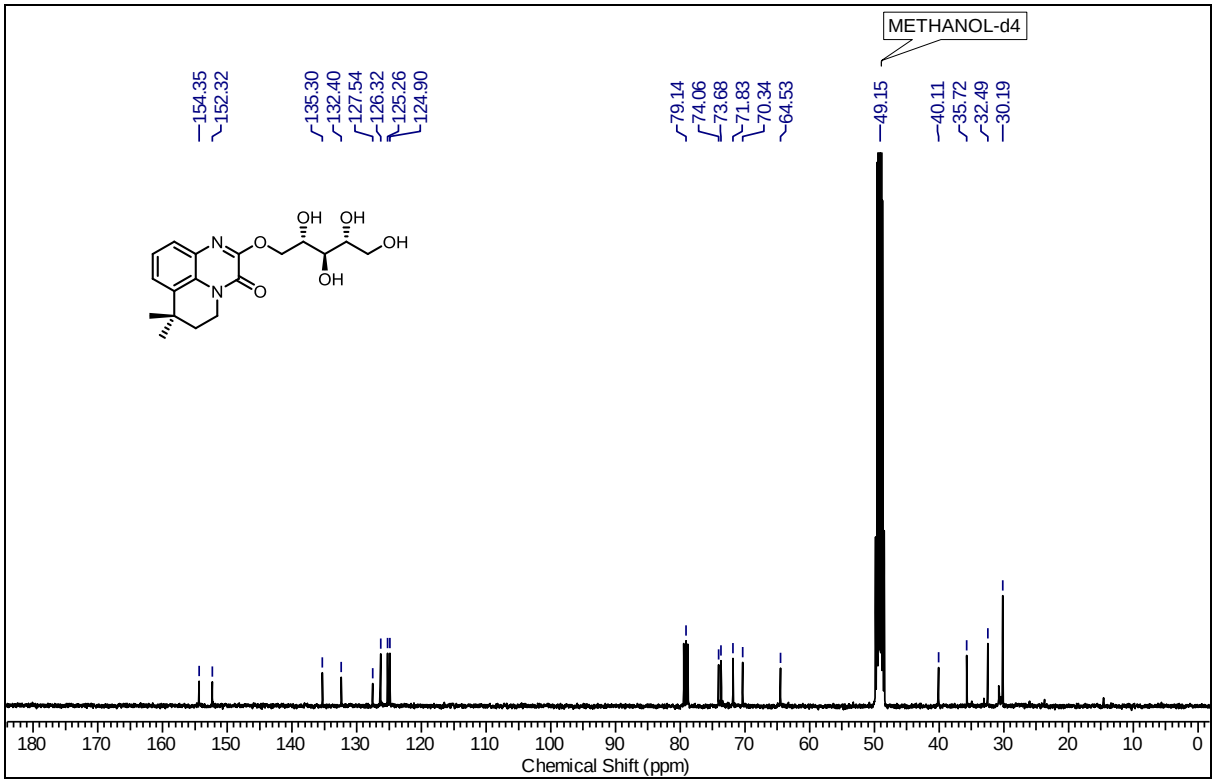
^{13}C NMR of Compound 66 at 100 MHz in $CDCl_3$



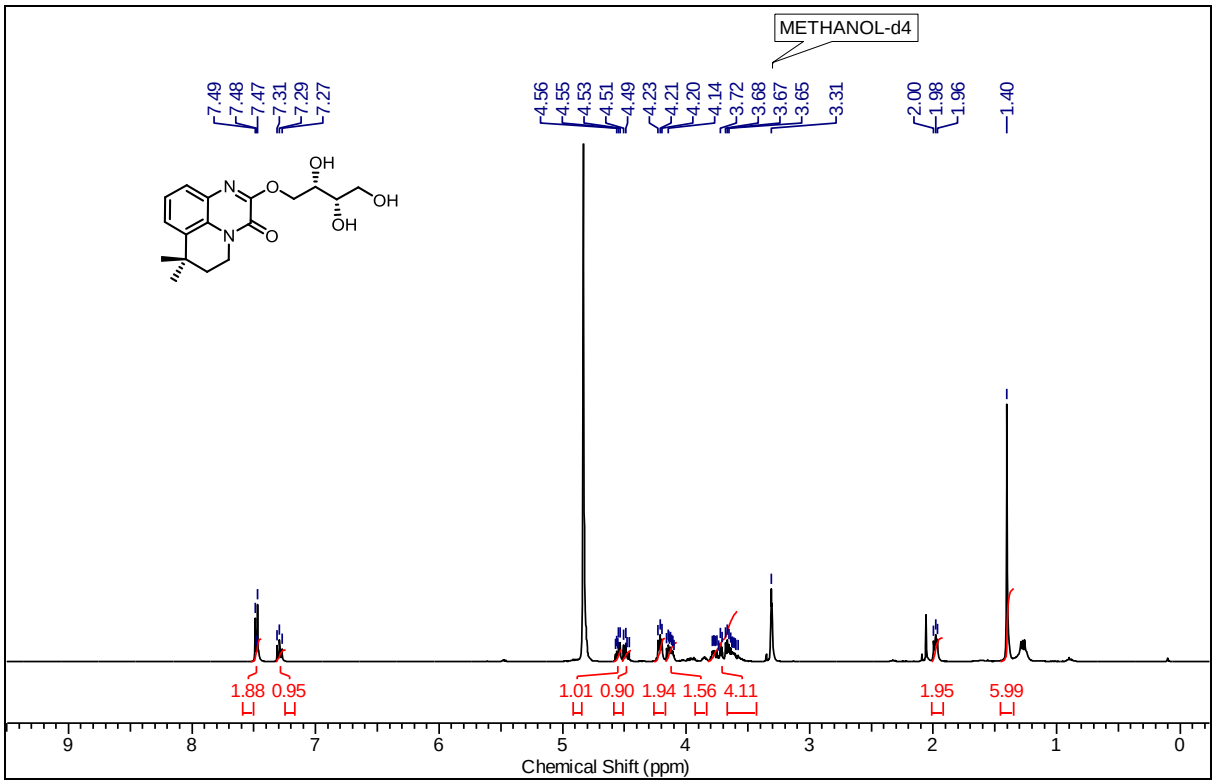
^{1}H NMR of Compound 71 at 400 MHz in $CD_3OD+CDCl_3$



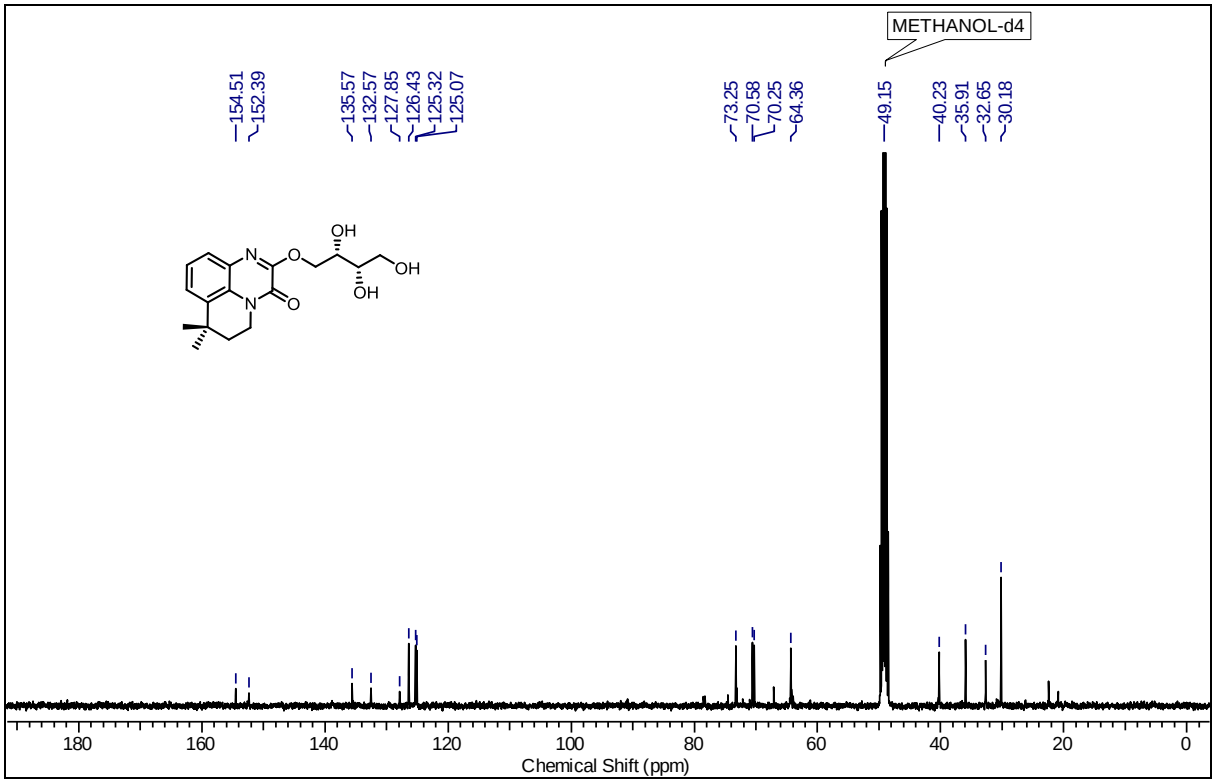
^{13}C NMR of Compound 71 at 100 MHz in $CD_3OD+CDCl_3$



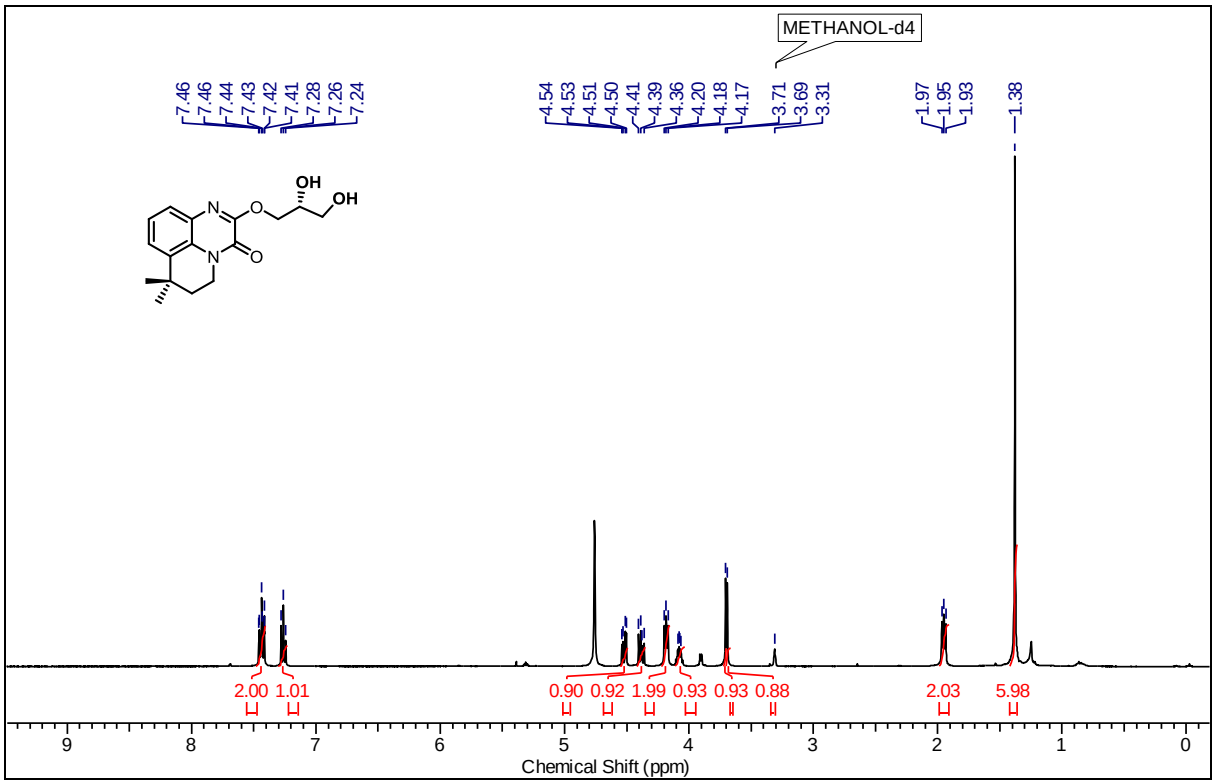
^{1}H NMR of Compound 73 at 400 MHz in CD_3OD



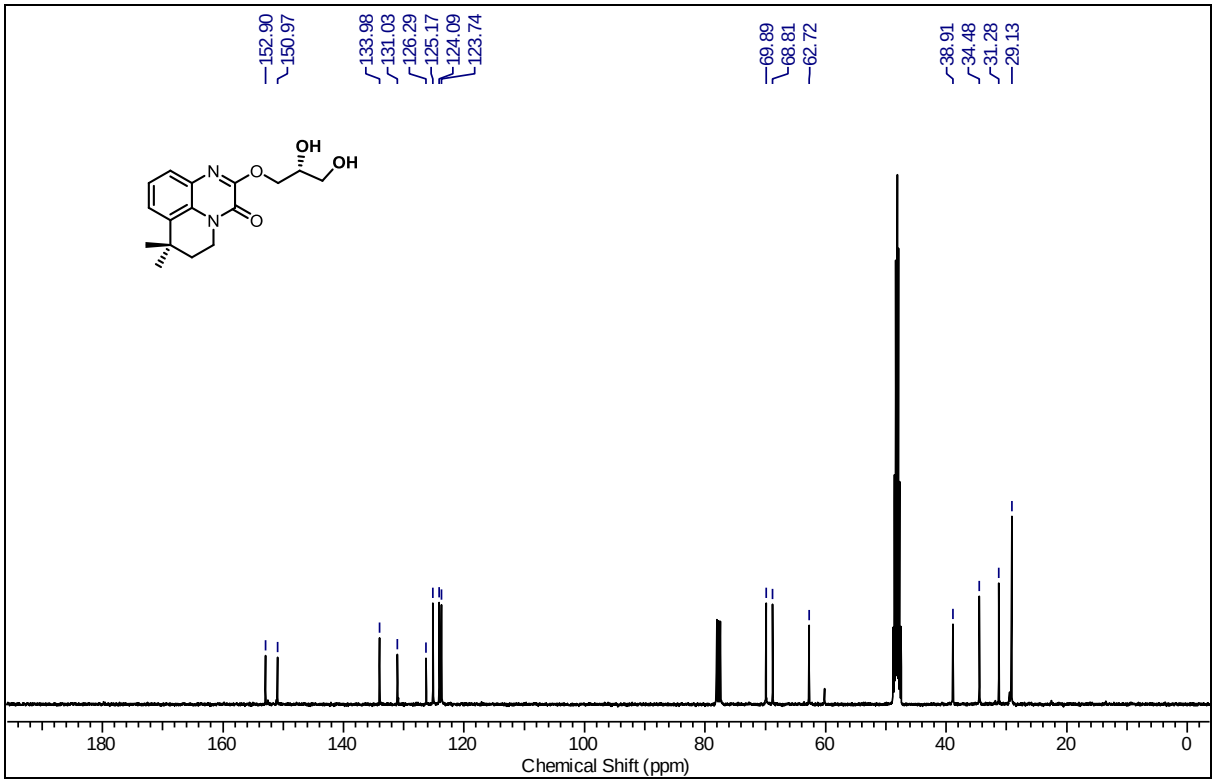
^{13}C NMR of Compound 73 at 100 MHz in CD_3OD



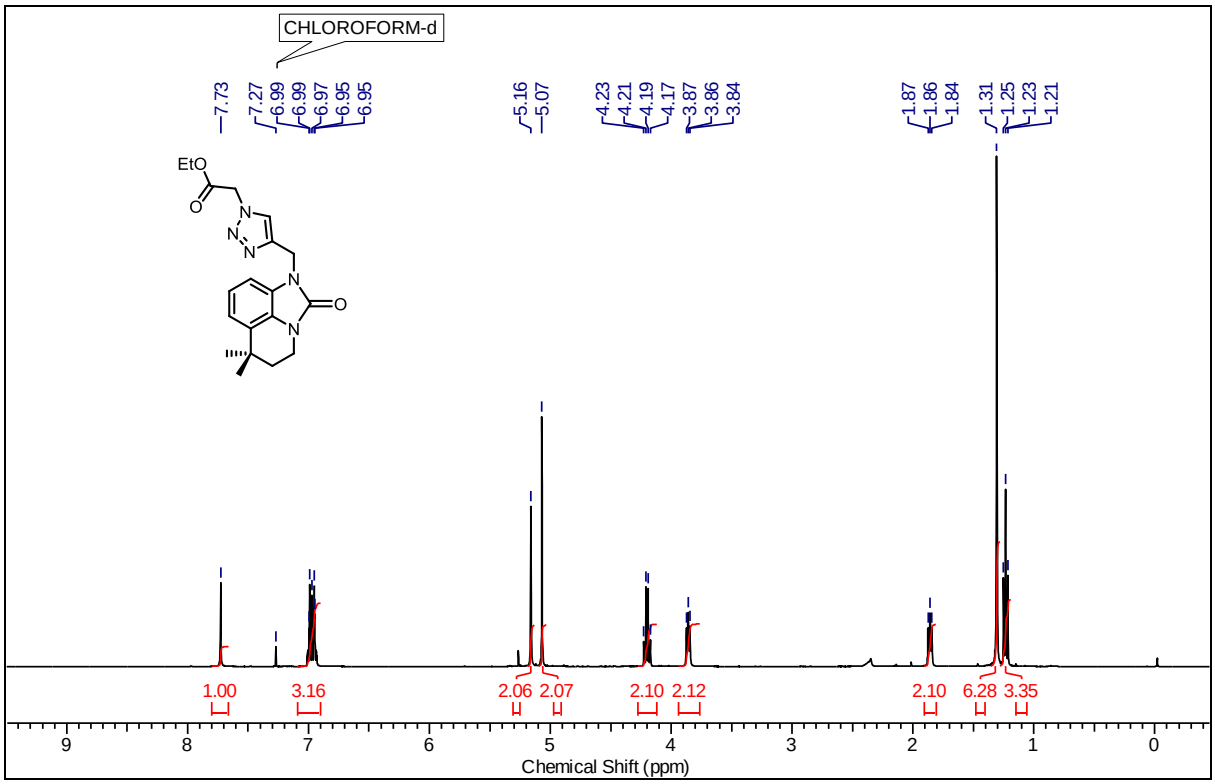
^{1}H NMR of Compound 75 at 400 MHz in $CD_3OD+CDCl_3$



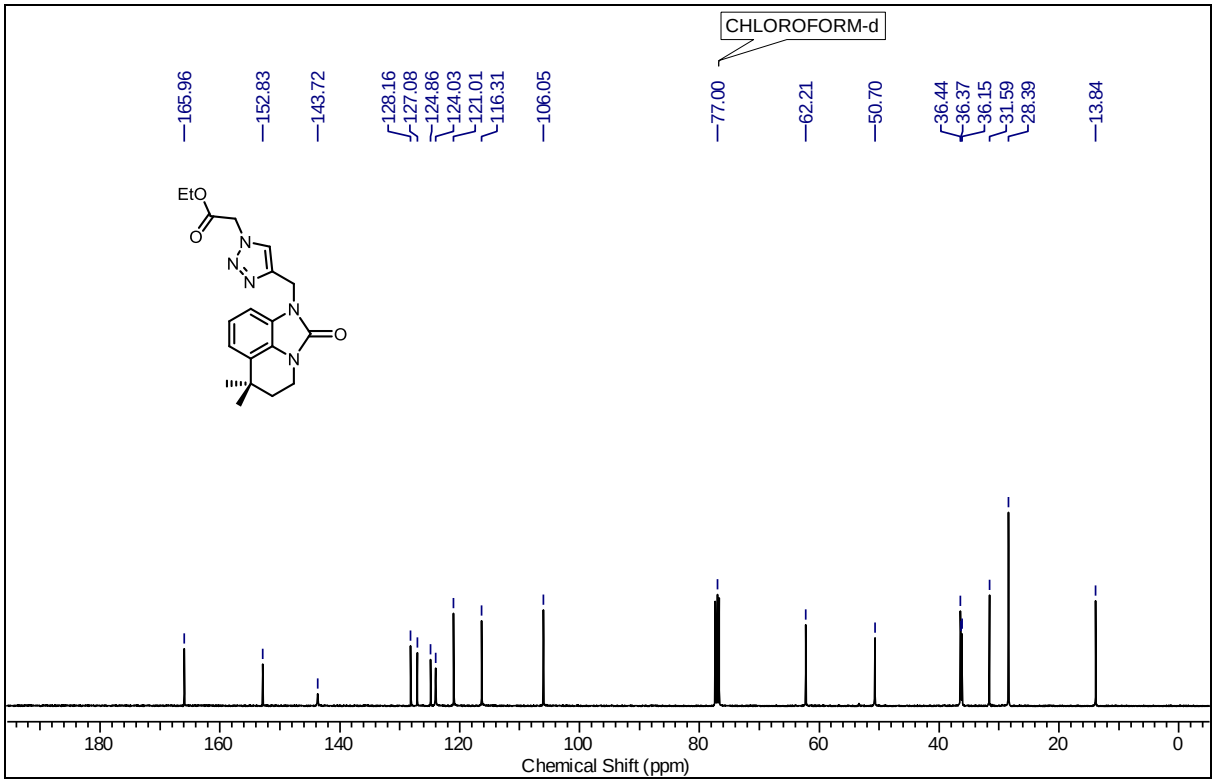
^{13}C NMR of Compound 75 at 100 MHz in $CD_3OD+CDCl_3$



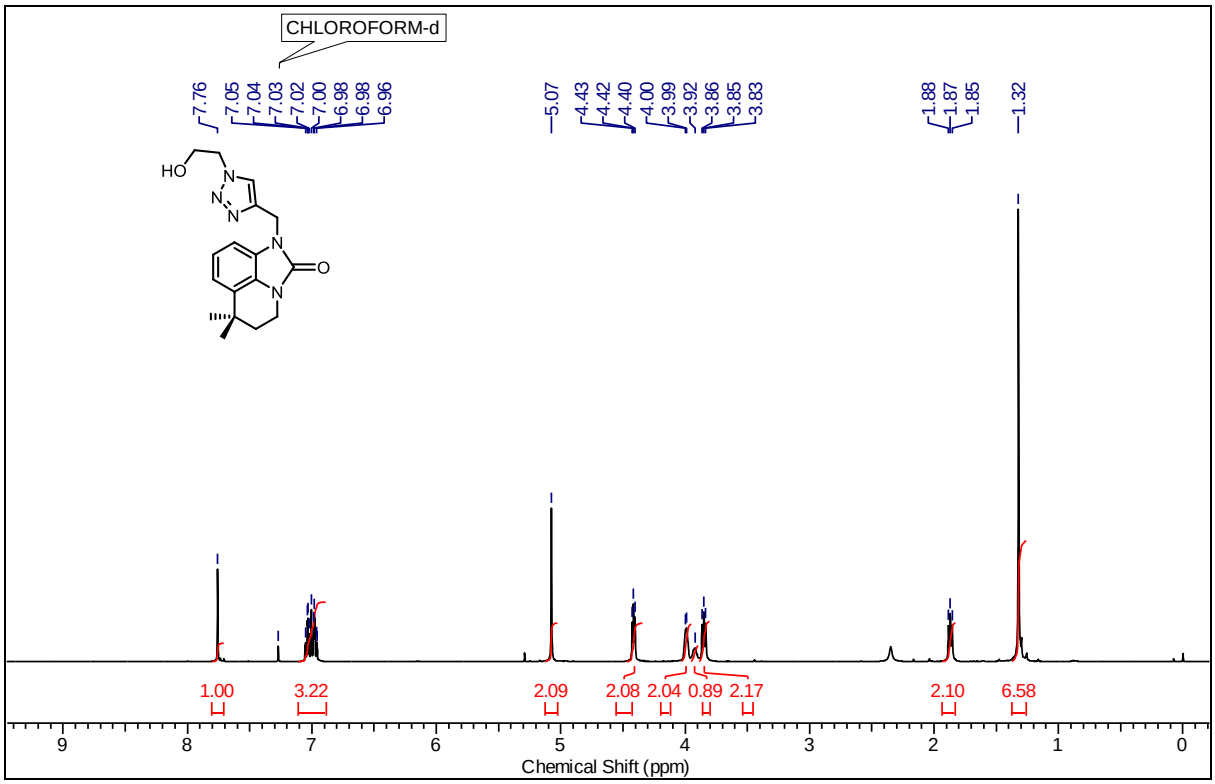
^{1}H NMR of Compound 77 at 400 MHz in $CDCl_3$



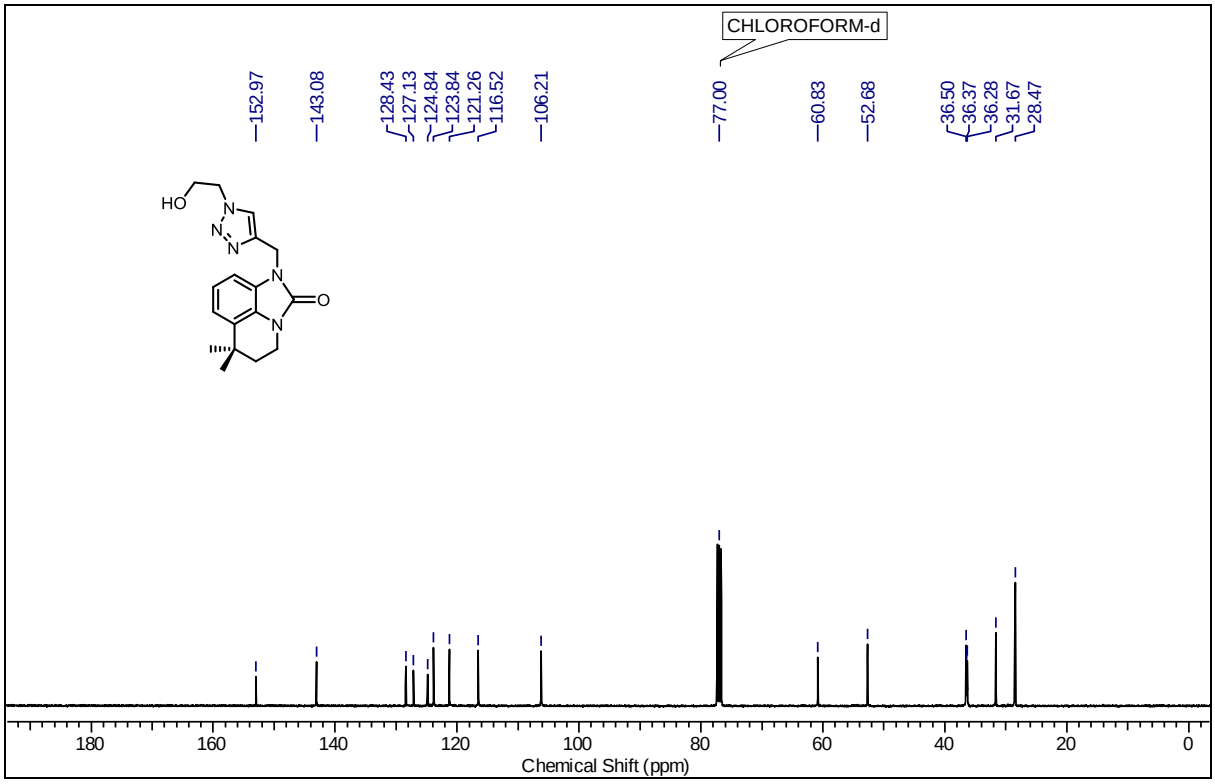
^{13}C NMR of Compound 77 at 100 MHz in $CDCl_3$



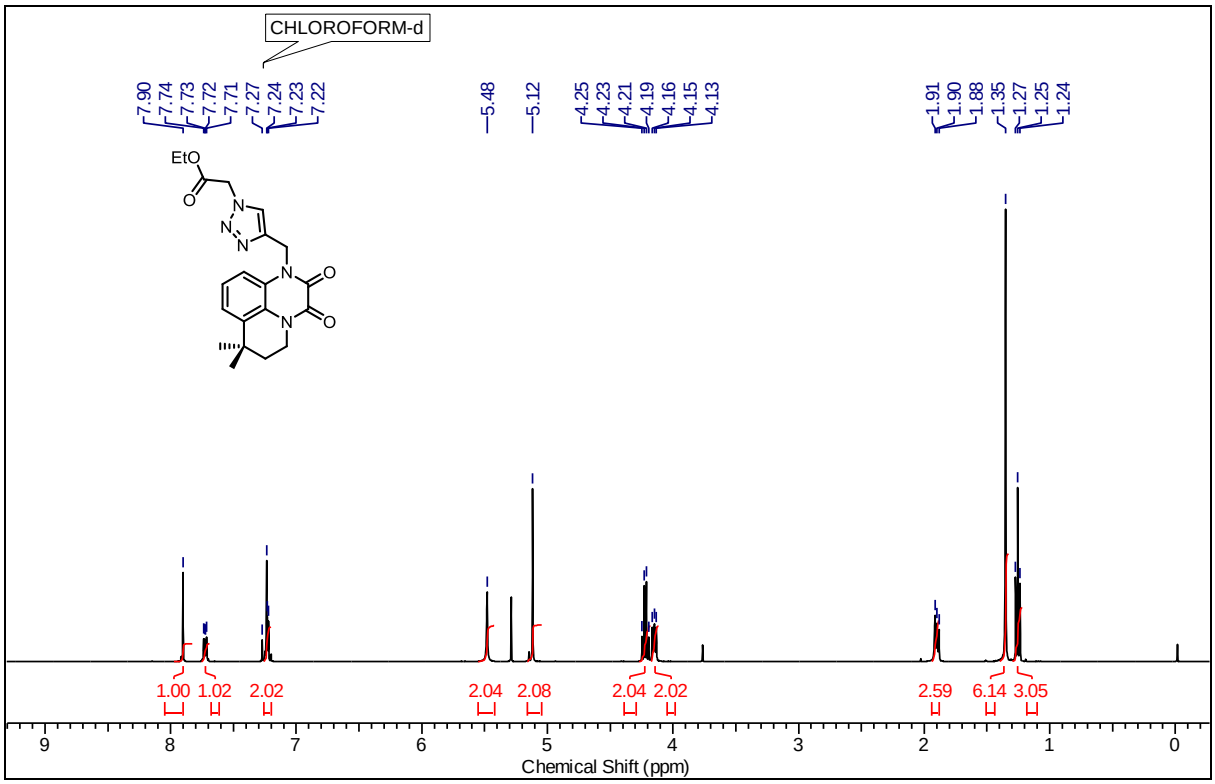
^{1}H NMR of Compound 78 at 400 MHz in $CDCl_3$



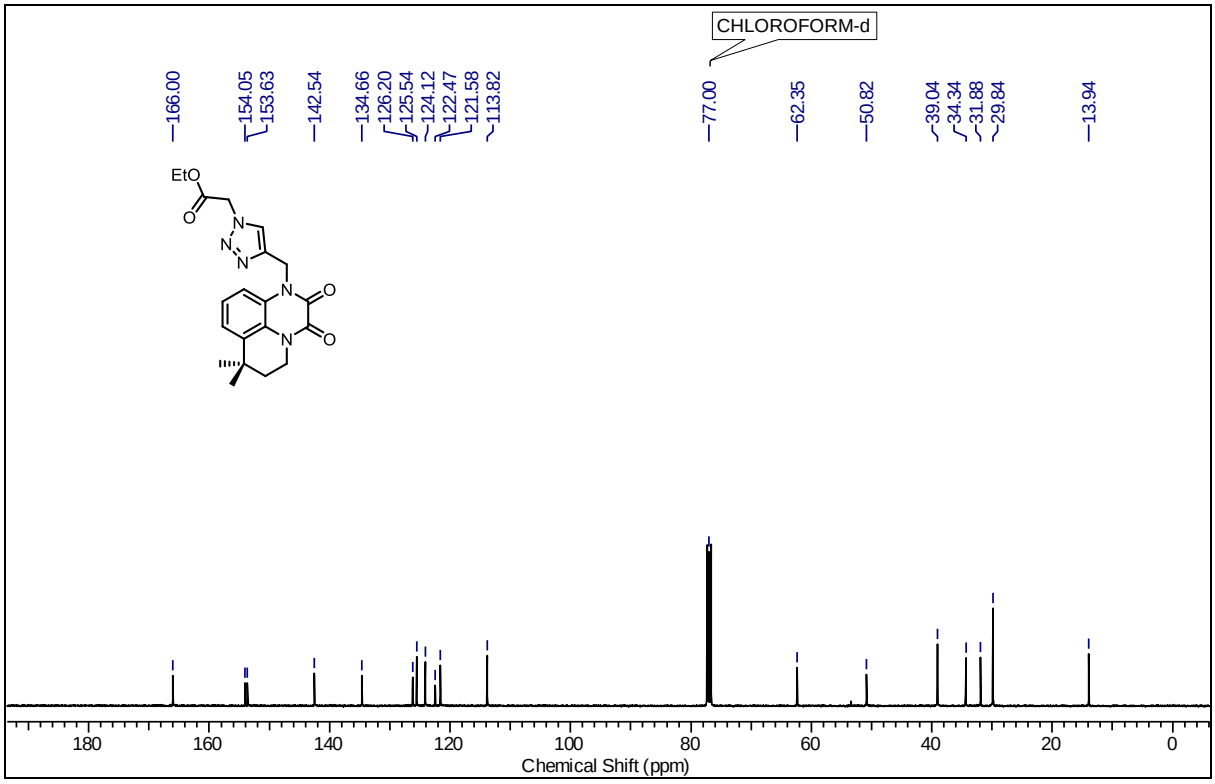
^{13}C NMR of Compound 78 at 100 MHz in $CDCl_3$



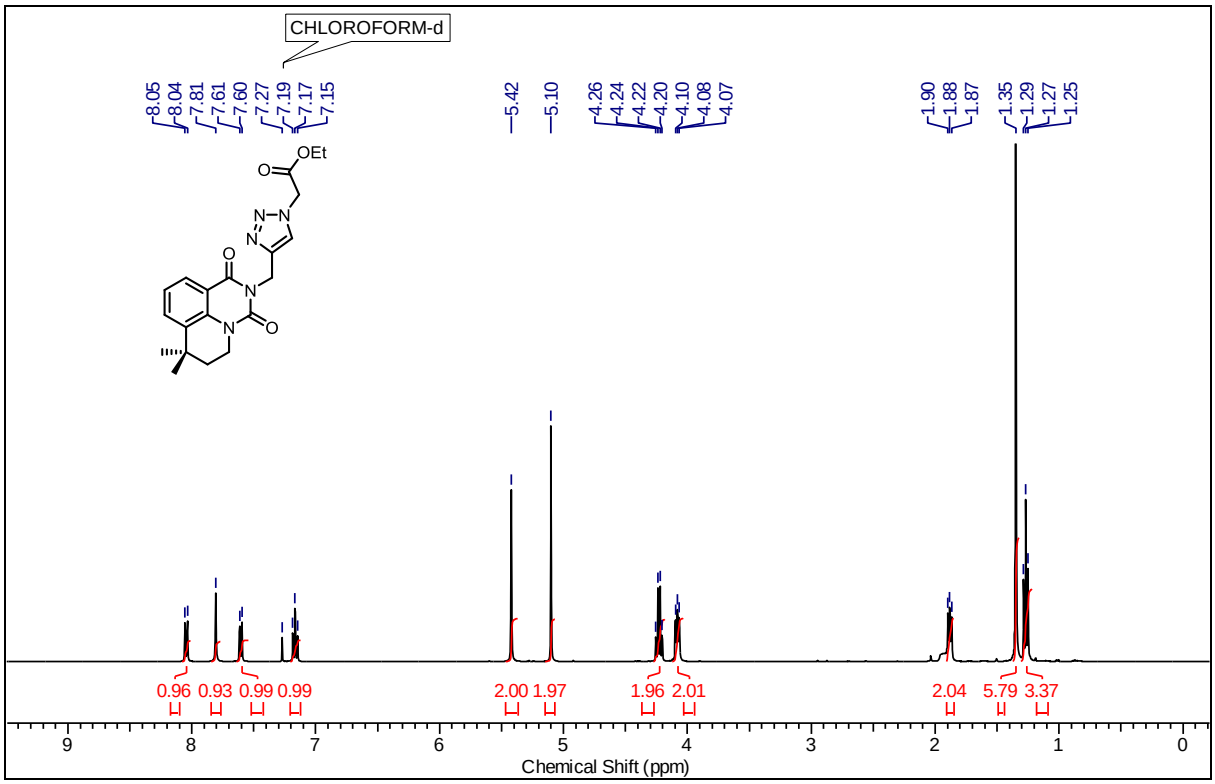
^{1}H NMR of Compound 80 at 400 MHz in $CDCl_3$



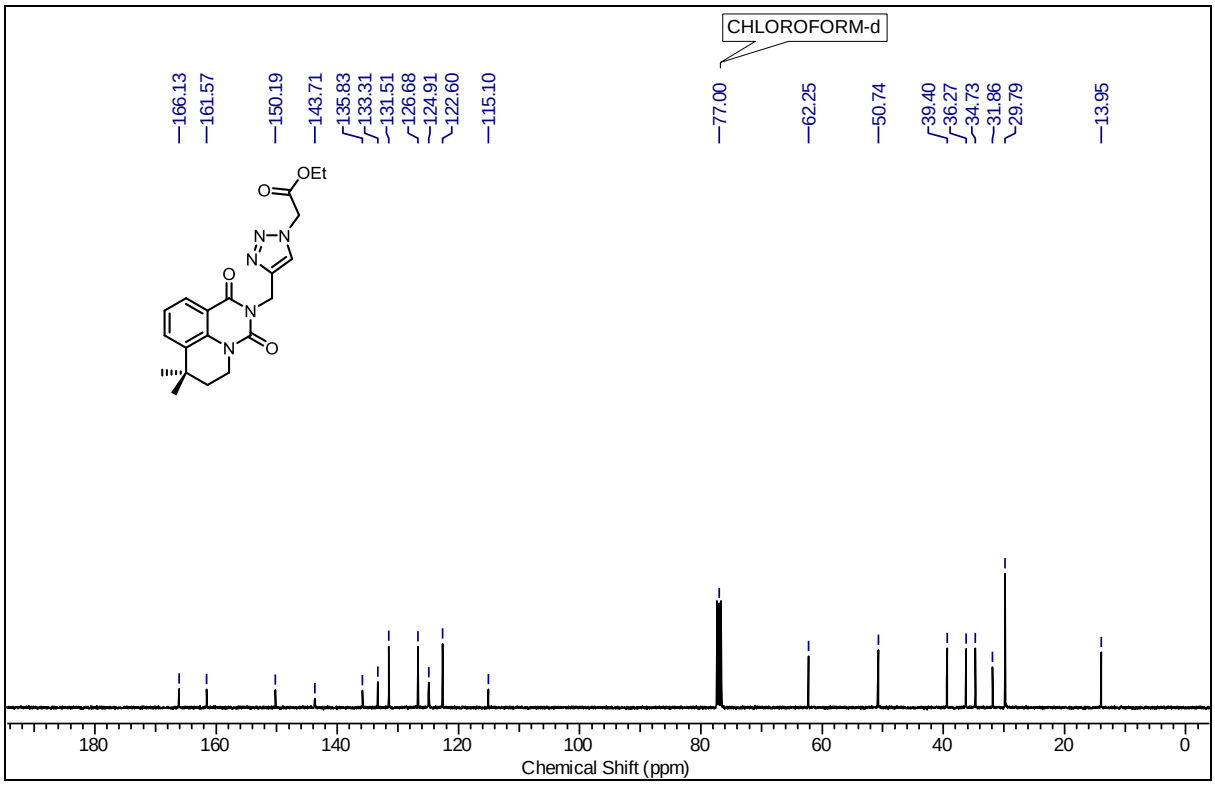
^{13}C NMR of Compound 80 at 100 MHz in $CDCl_3$



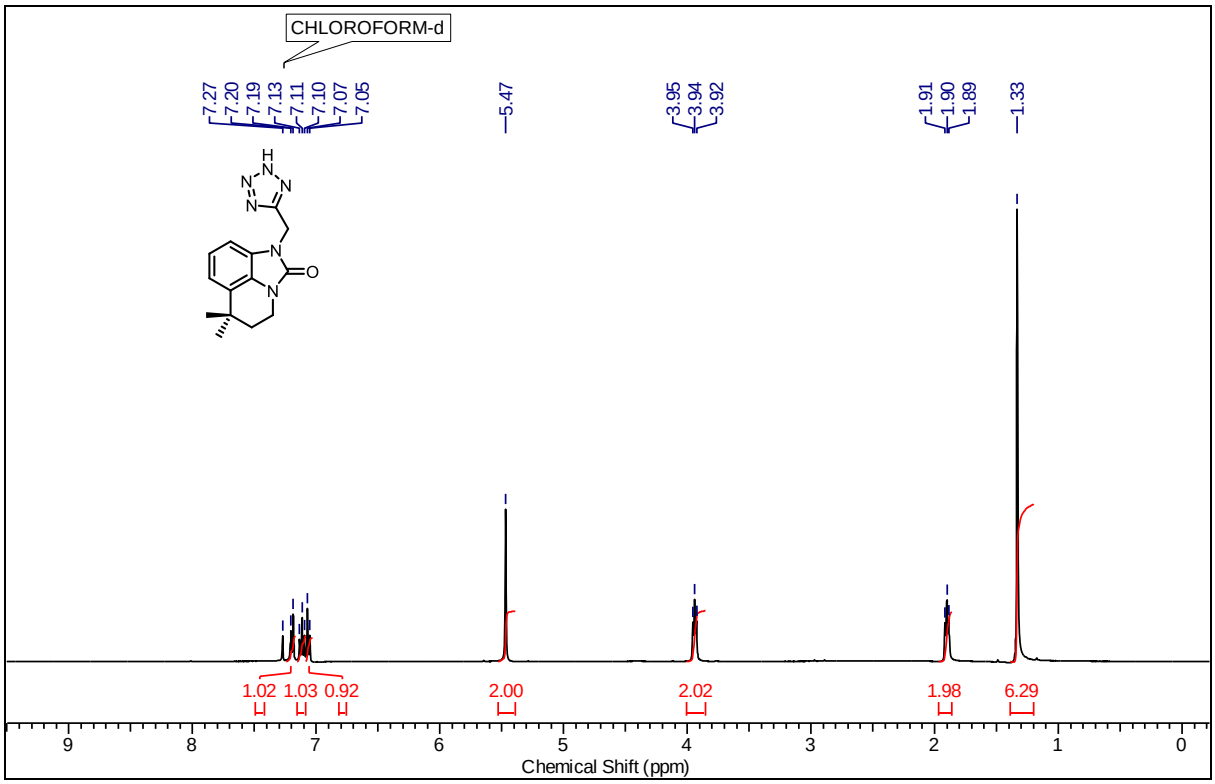
^{1}H NMR of Compound 82 at 400 MHz in $CDCl_3$



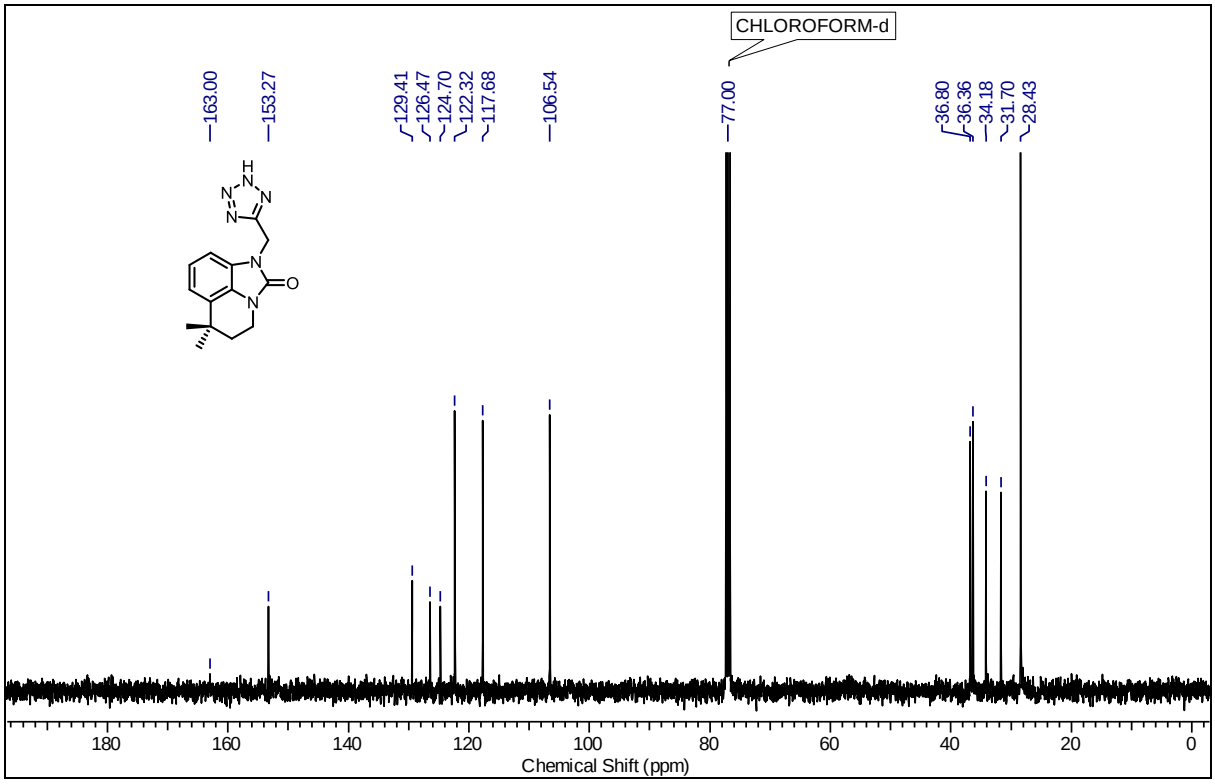
^{13}C NMR of Compound 82 at 100 MHz in $CDCl_3$



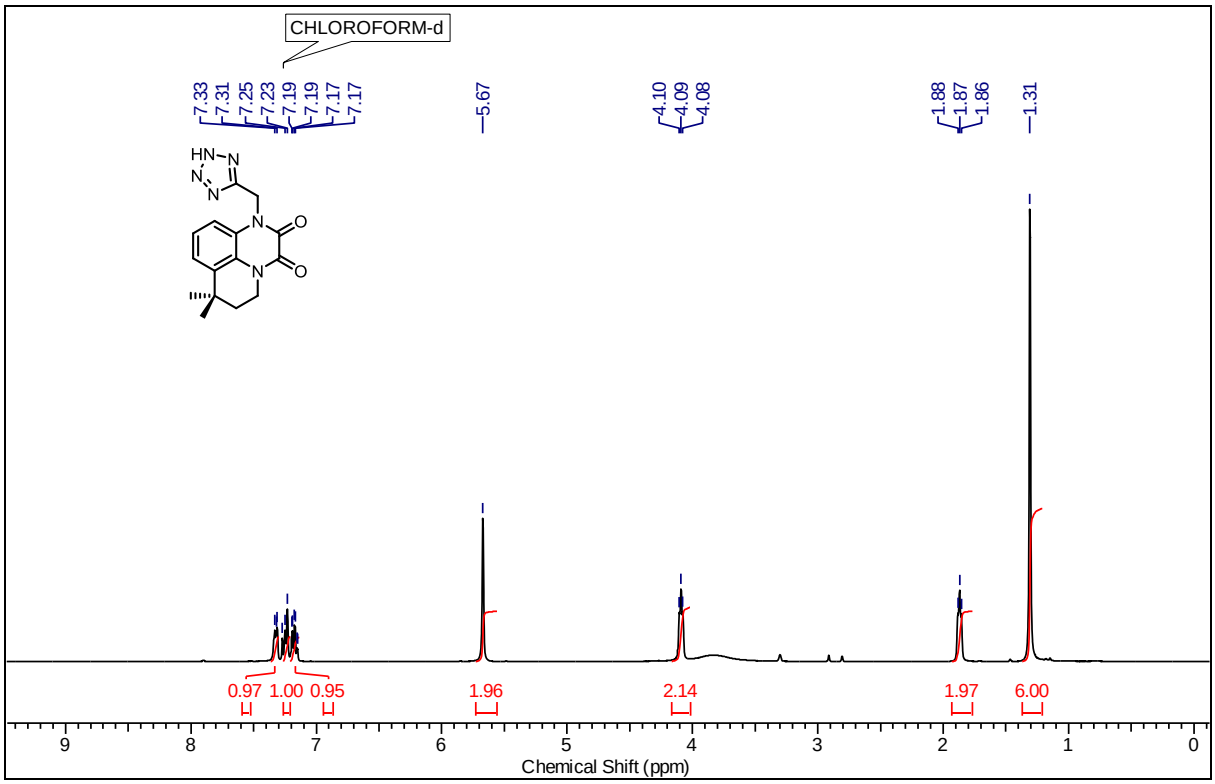
^{1}H NMR of Compound 84 at 400 MHz in $CDCl_3$



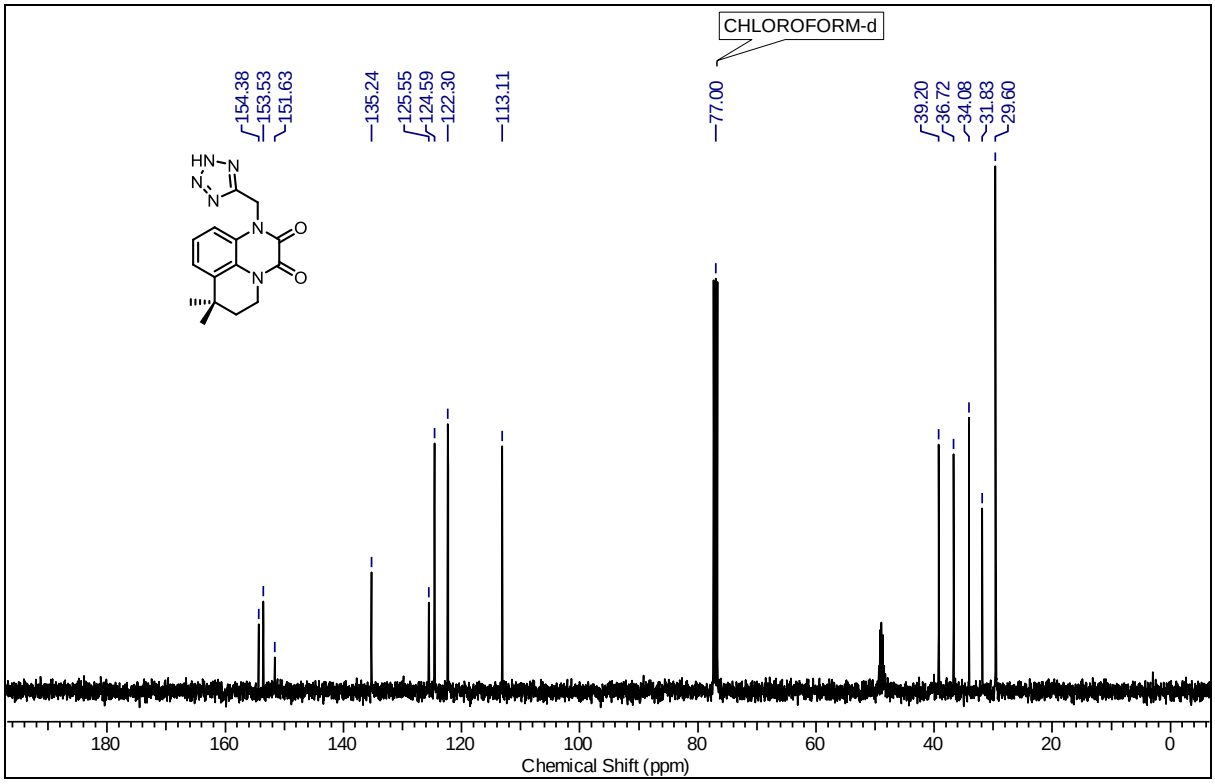
^{13}C NMR of Compound 84 at 100 MHz in $CDCl_3$



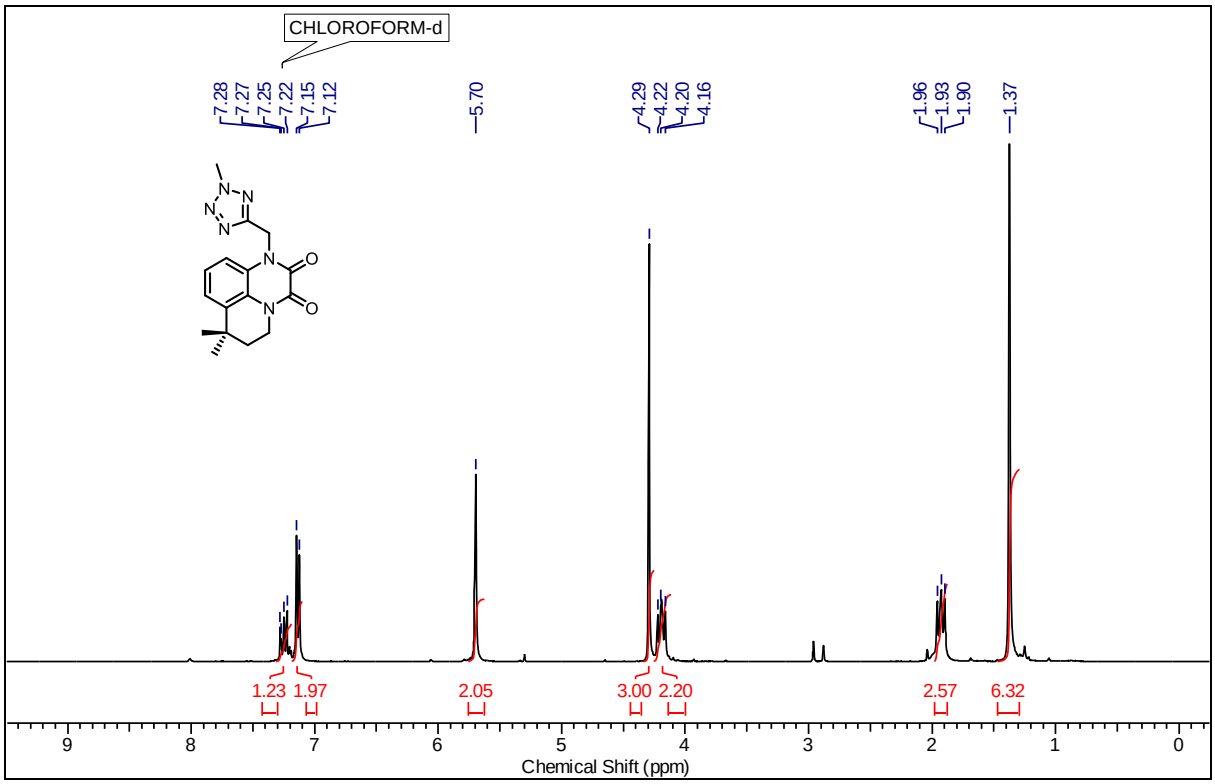
^{1}H NMR of Compound 86 at 400 MHz in $CDCl_3$+CD_3OD



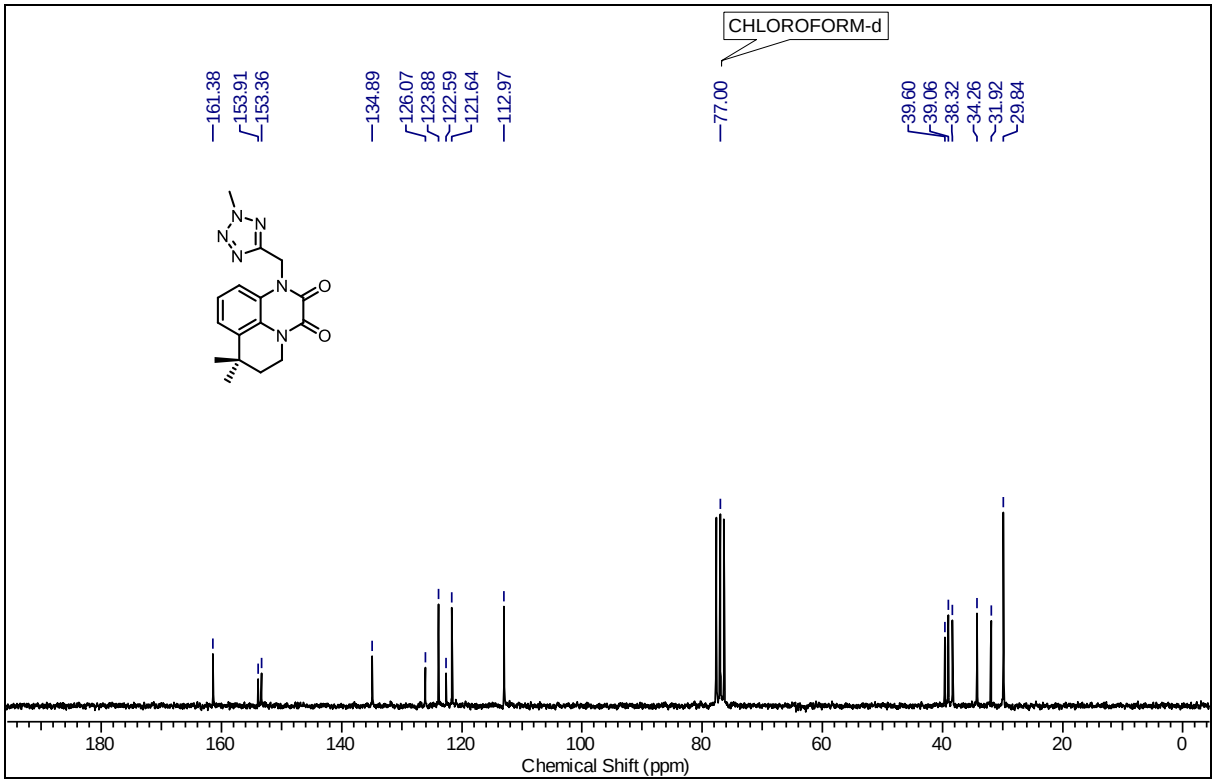
^{13}C NMR of Compound 86 at 100 MHz in $CDCl_3$+CD_3OD



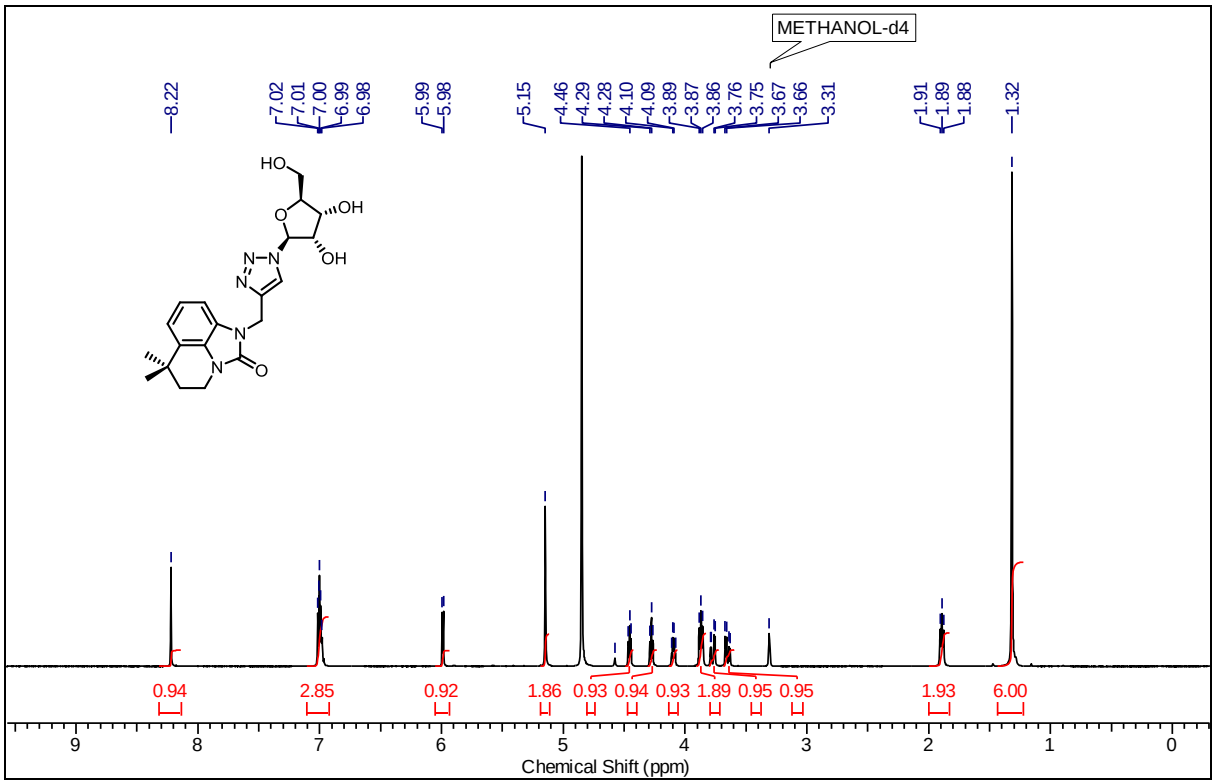
^{1}H NMR of Compound 87 at 400 MHz in $CDCl_3$



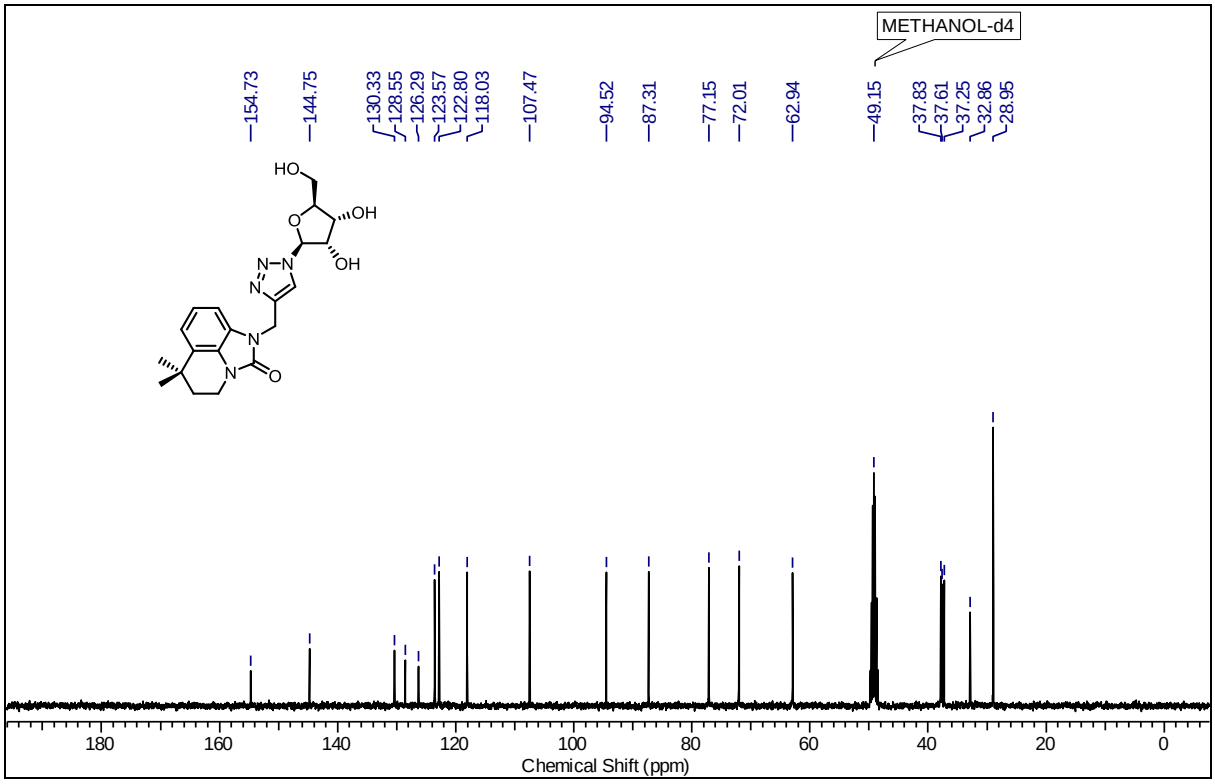
^{13}C NMR of Compound 87at 100 MHz in $CDCl_3$



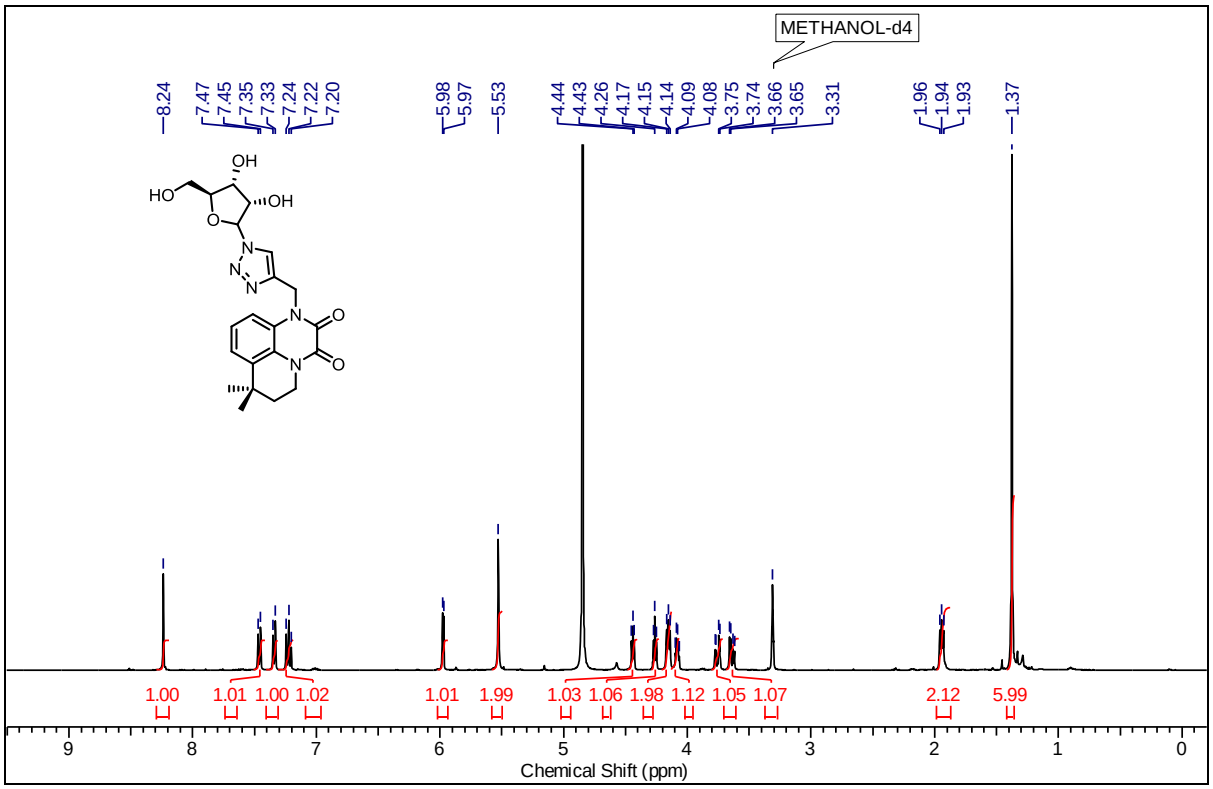
^{1}H NMR of Compound 91 at 400 MHz in CD_3OD



^{13}C NMR of Compound 91 at 100 MHz in CD_3OD



^{1}H NMR of Compound 92 at 400 MHz in CD_3OD



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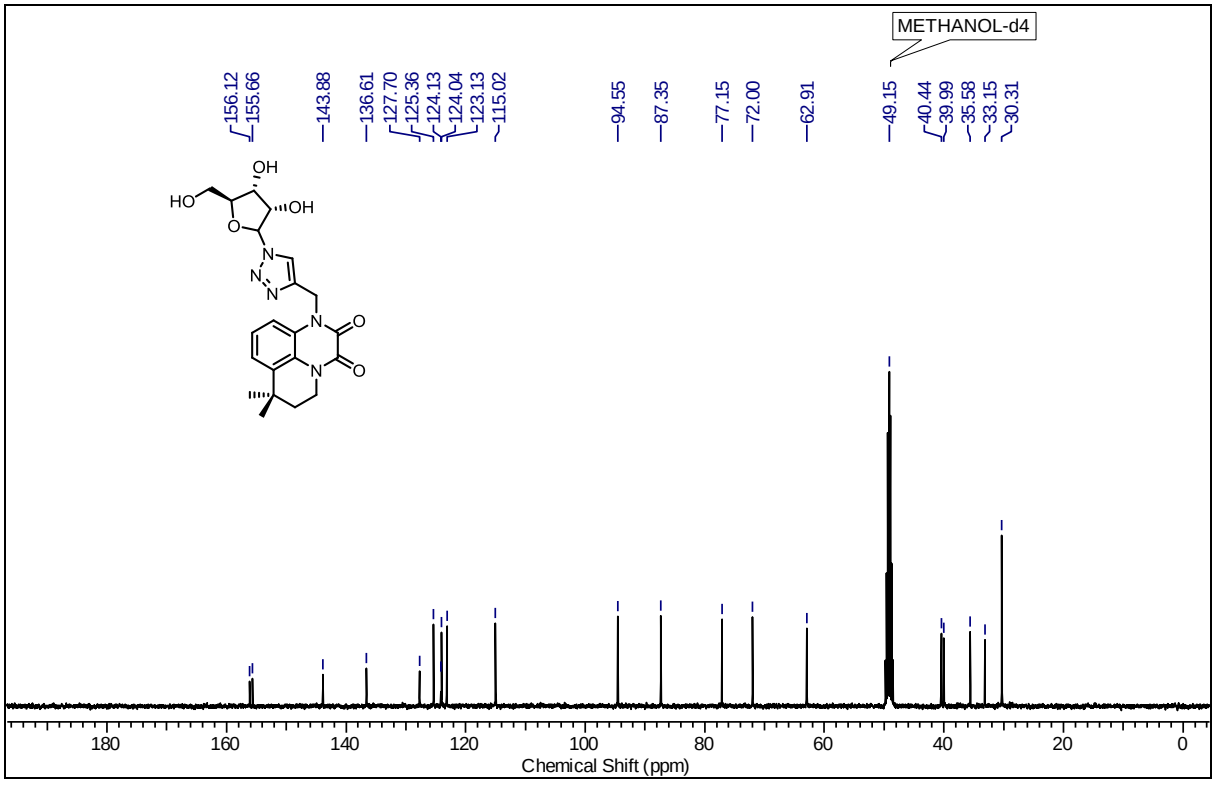

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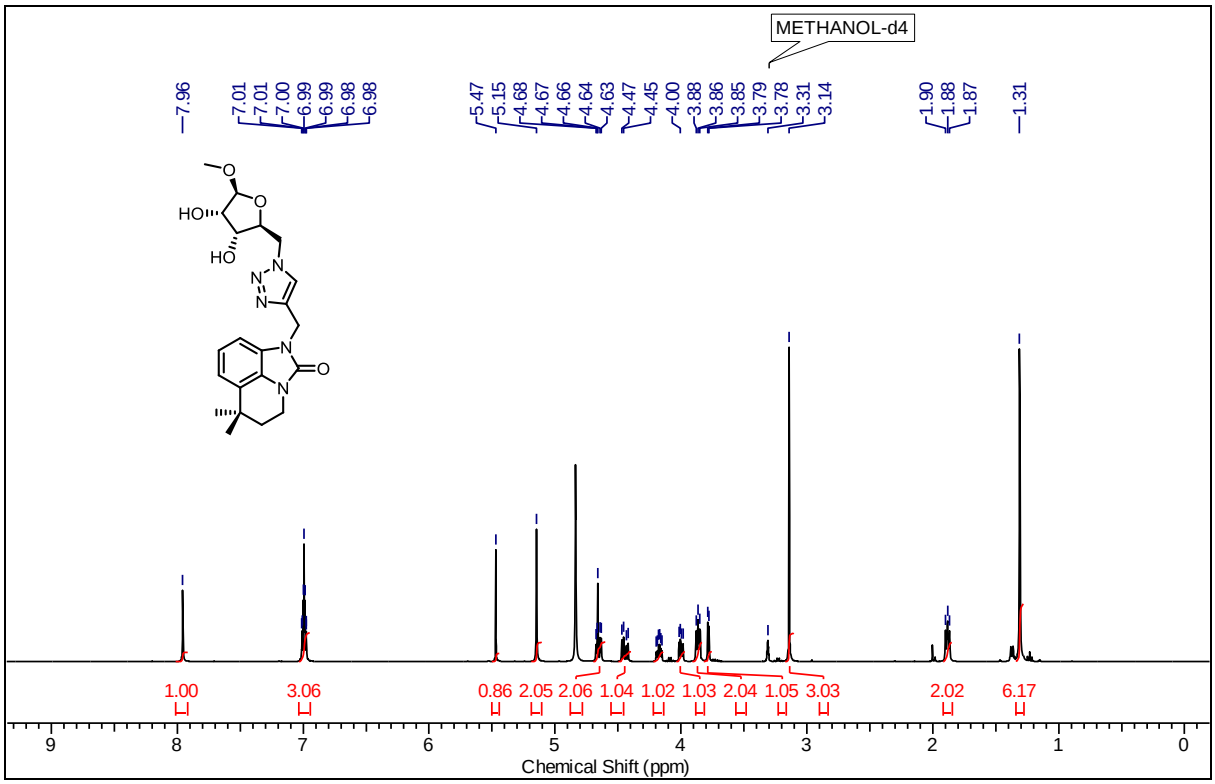

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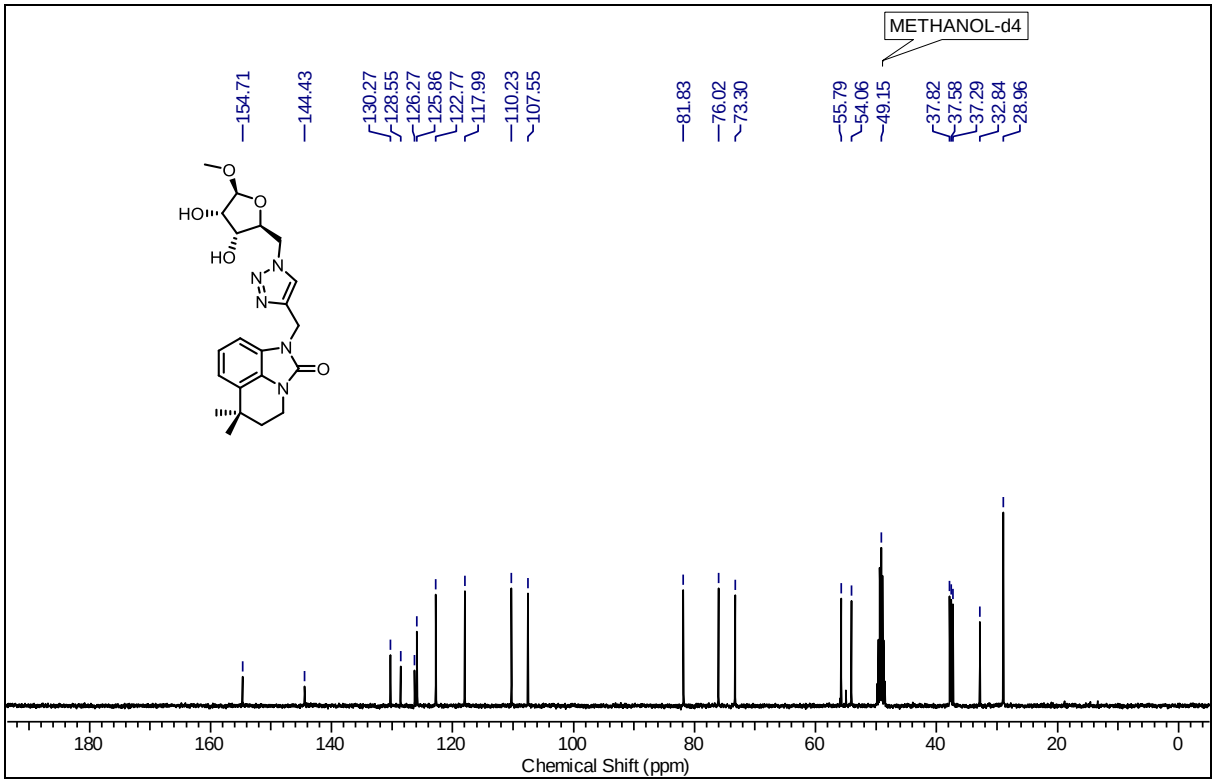
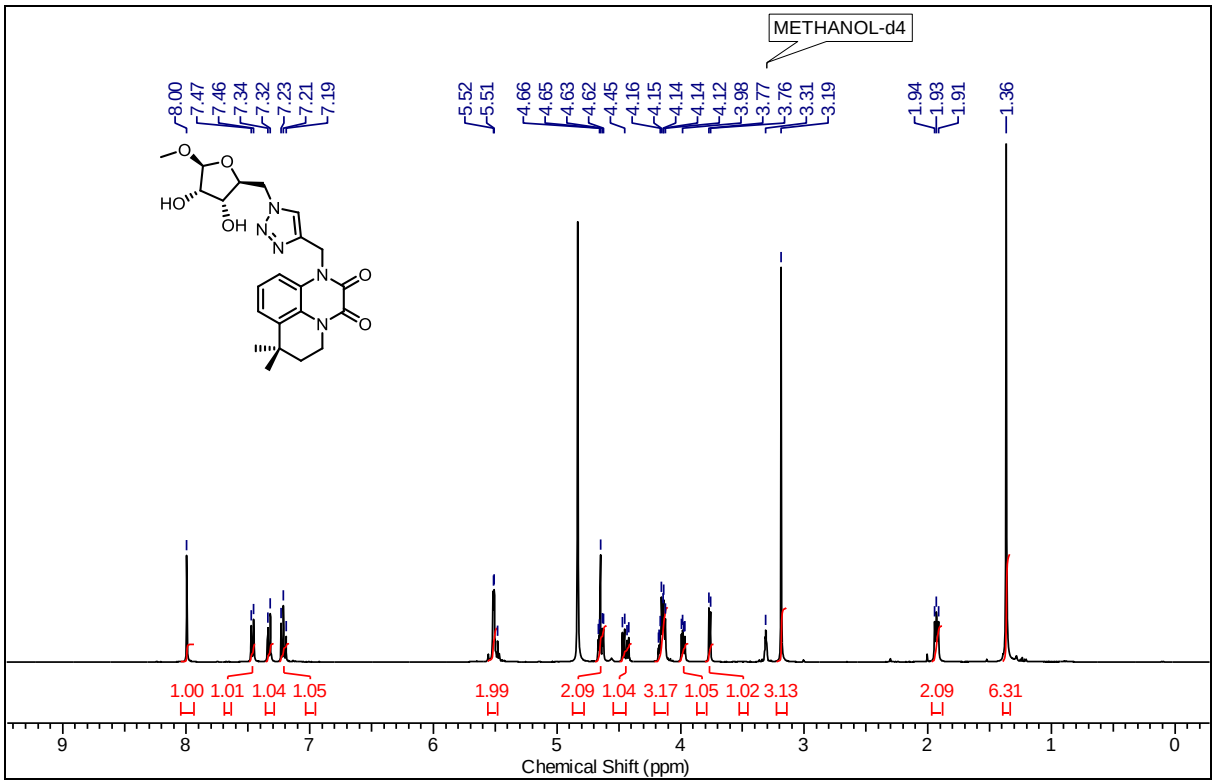
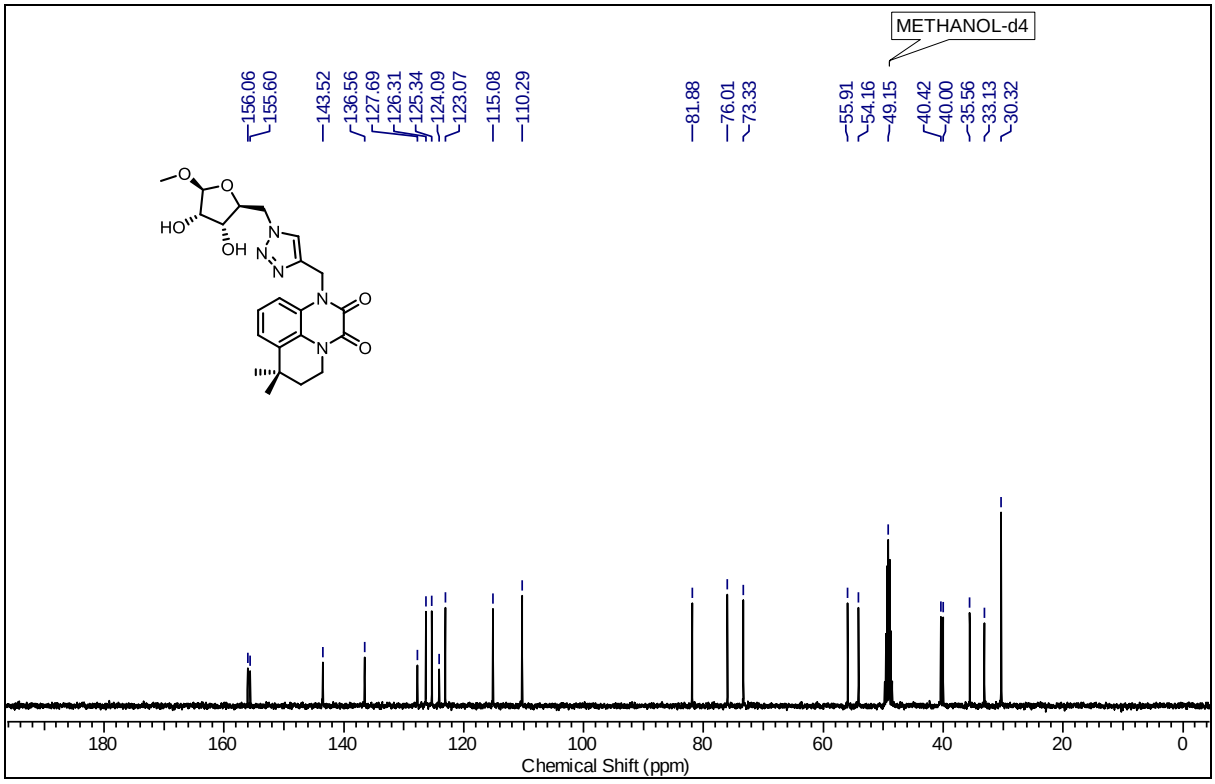


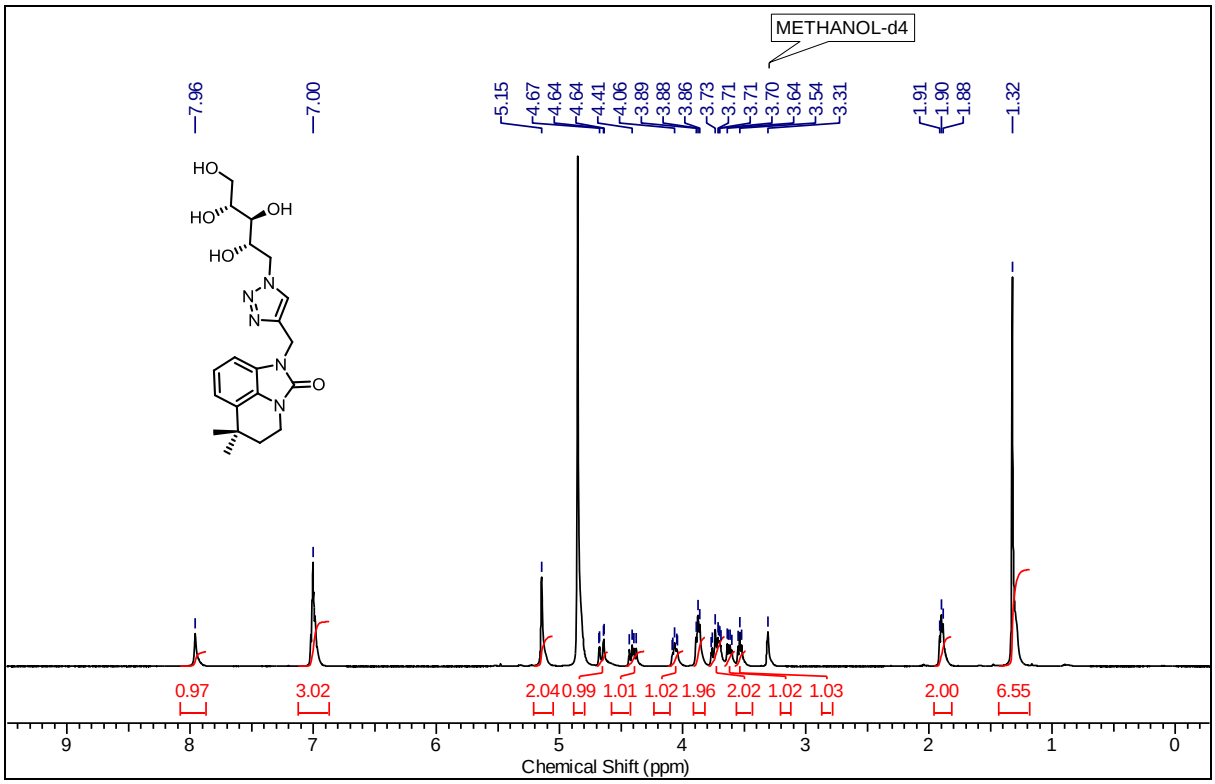
^{1}H NMR of Compound 94 at 400 MHz in CD_3OD



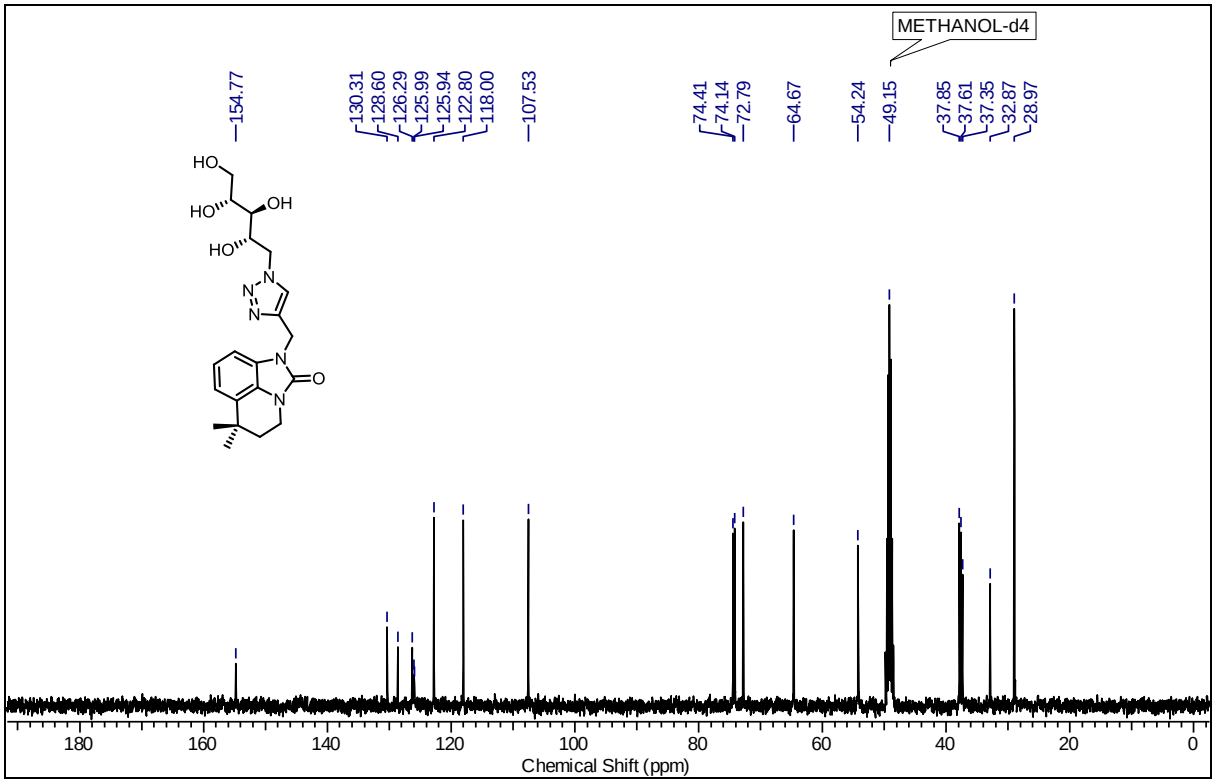
^{13}C NMR of Compound 94 at 100 MHz in CD_3OD



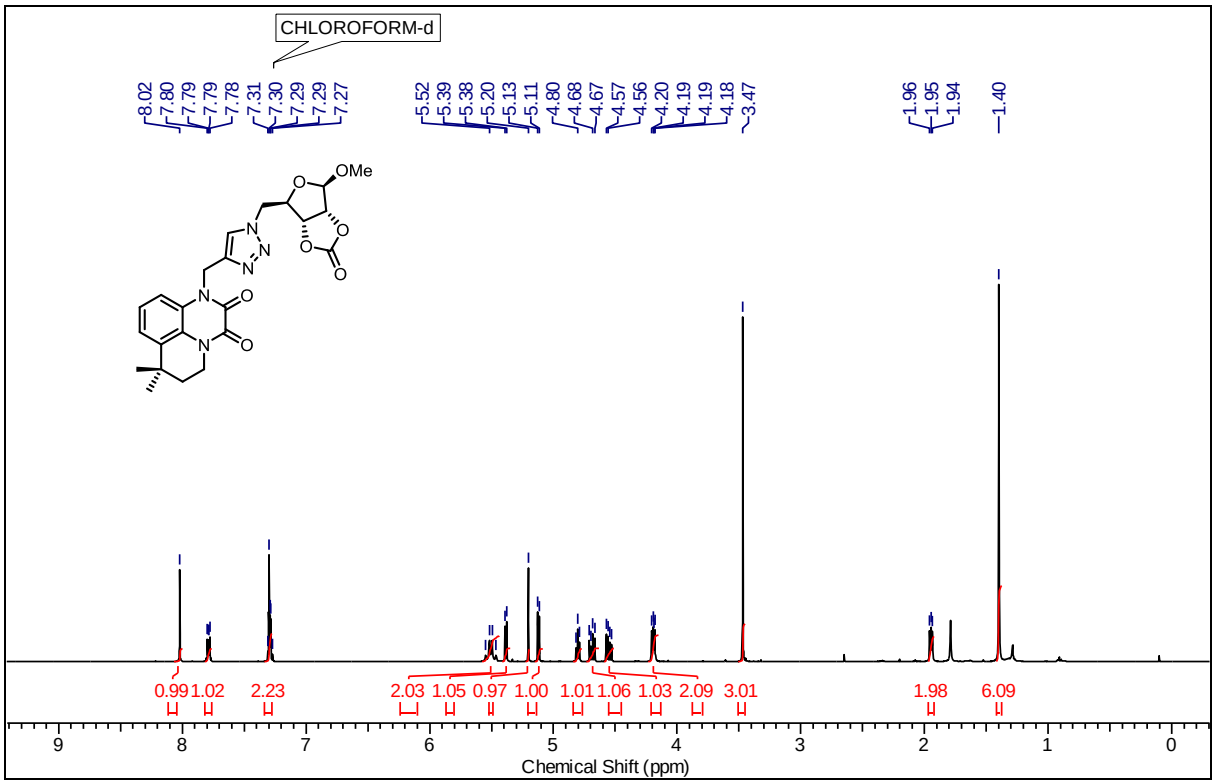
^{1}H NMR of Compound 95 at 400 MHz in CD_3OD



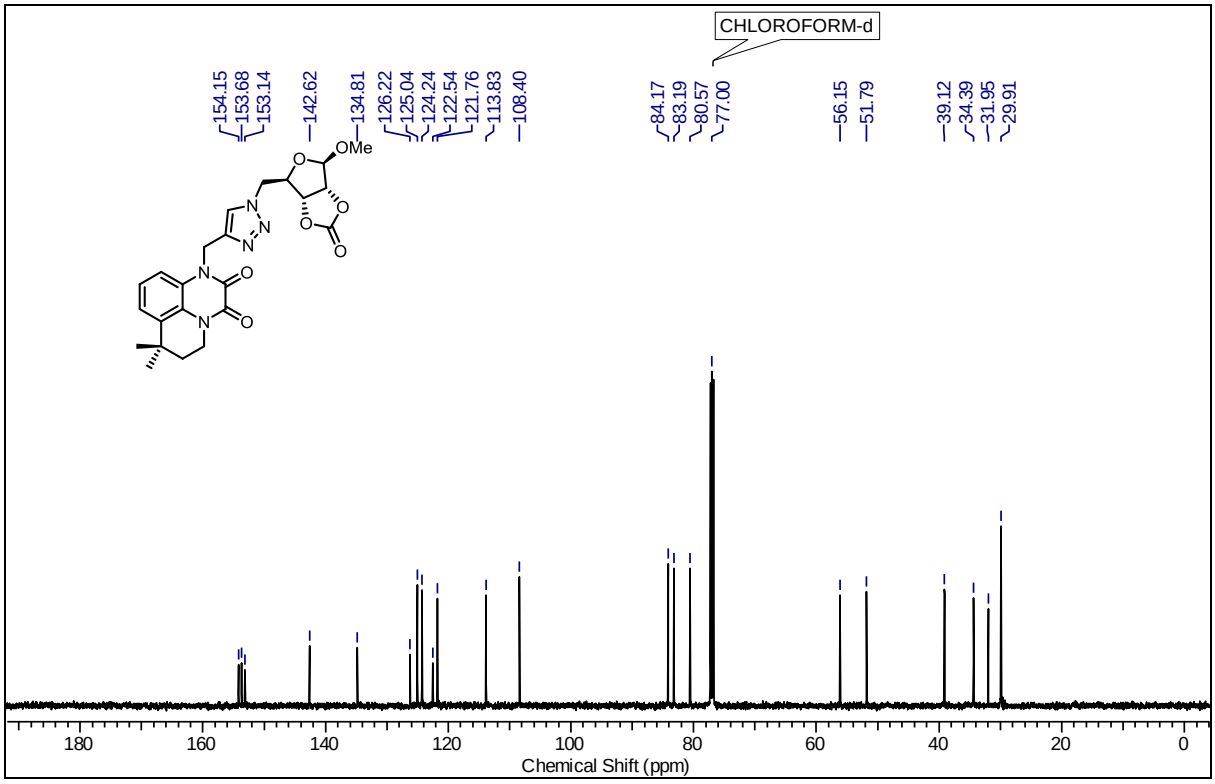
^{13}C NMR of Compound 95 at 100 MHz in CD_3OD



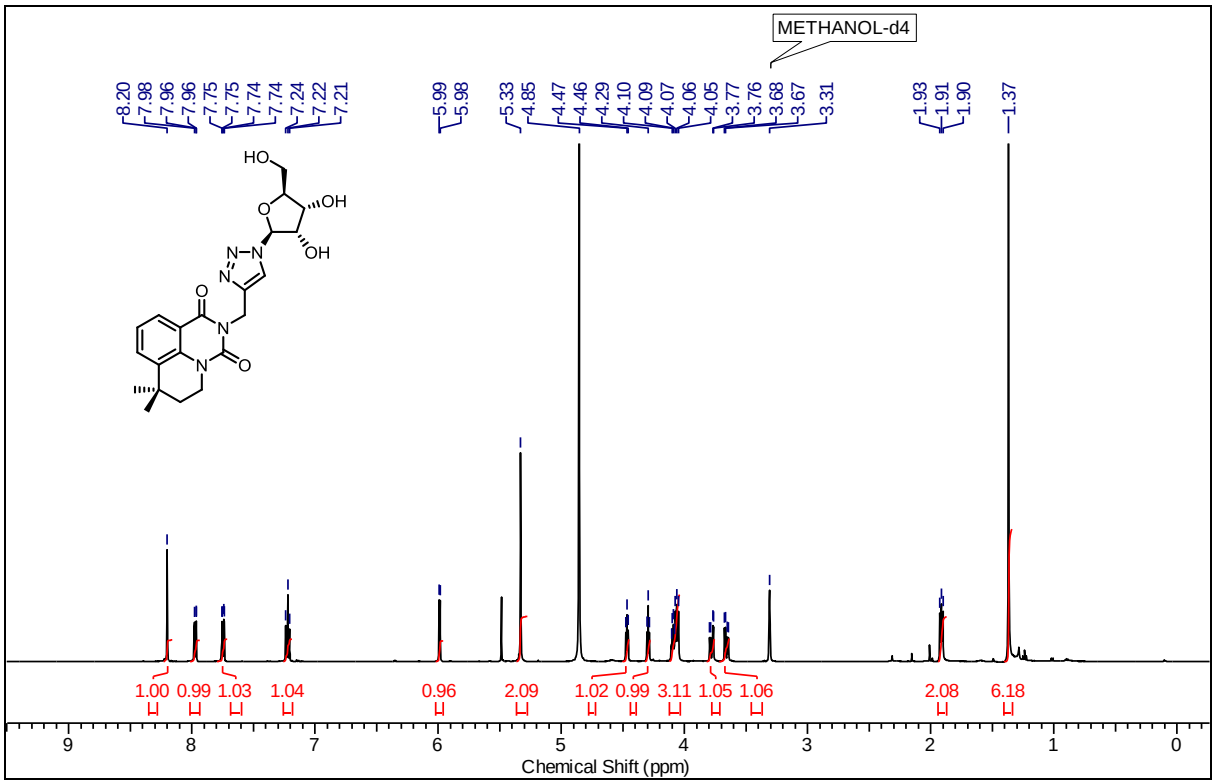
^{1}H NMR of Compound 96 at 400 MHz in $CDCl_3$



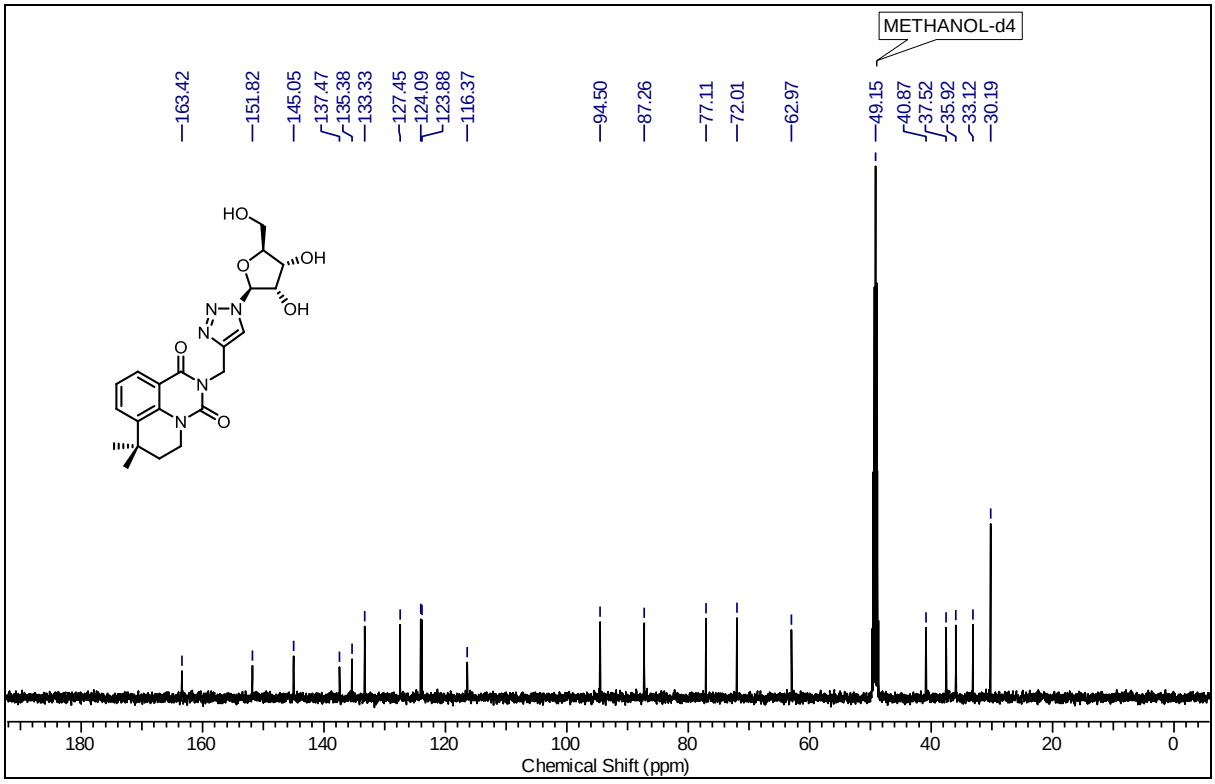
^{13}C NMR of Compound 96 at 100 MHz in $CDCl_3$



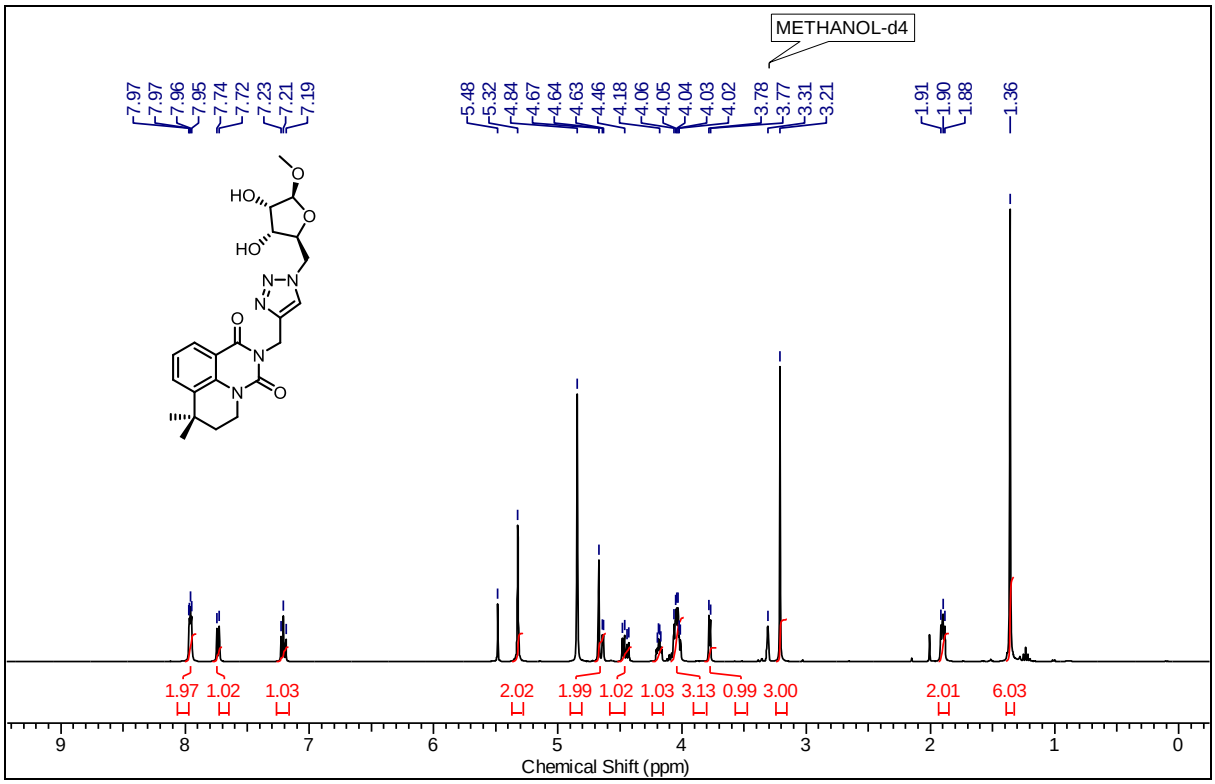
^{1}H NMR of Compound 97 at 500 MHz in CD_3OD



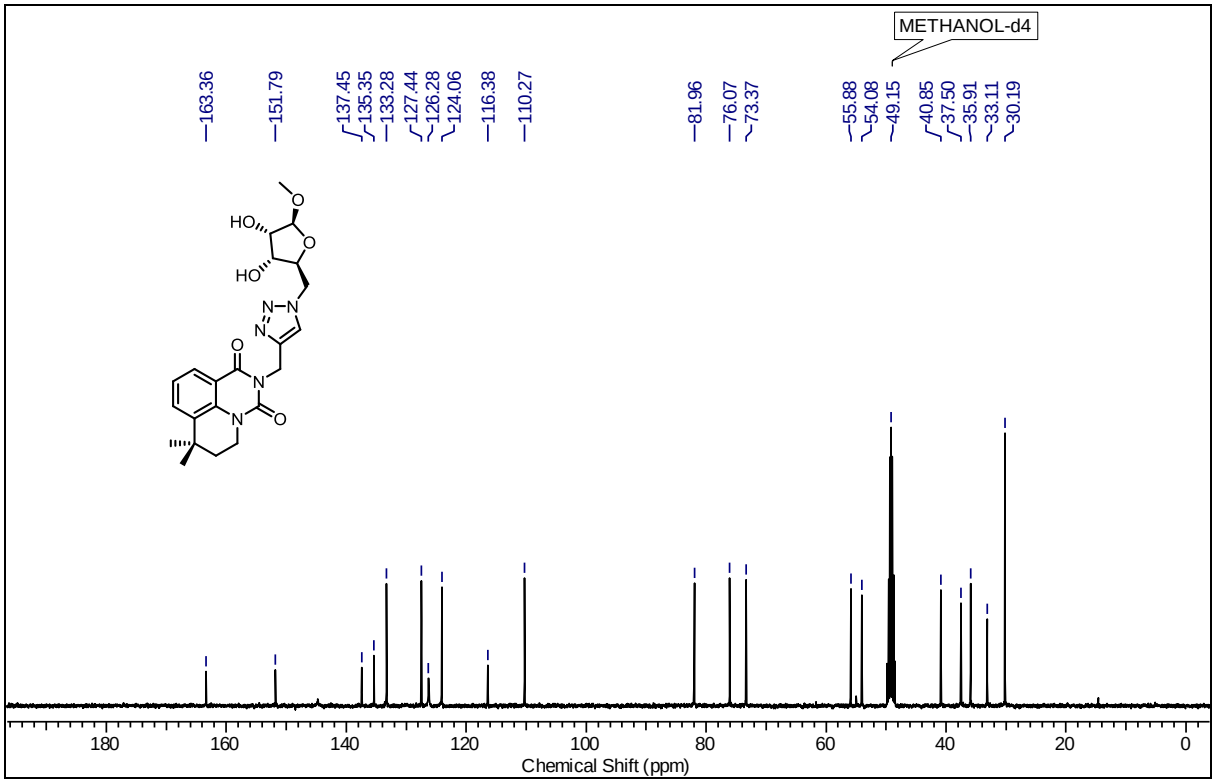
^{13}C NMR of Compound 97 at 125 MHz in CD_3OD



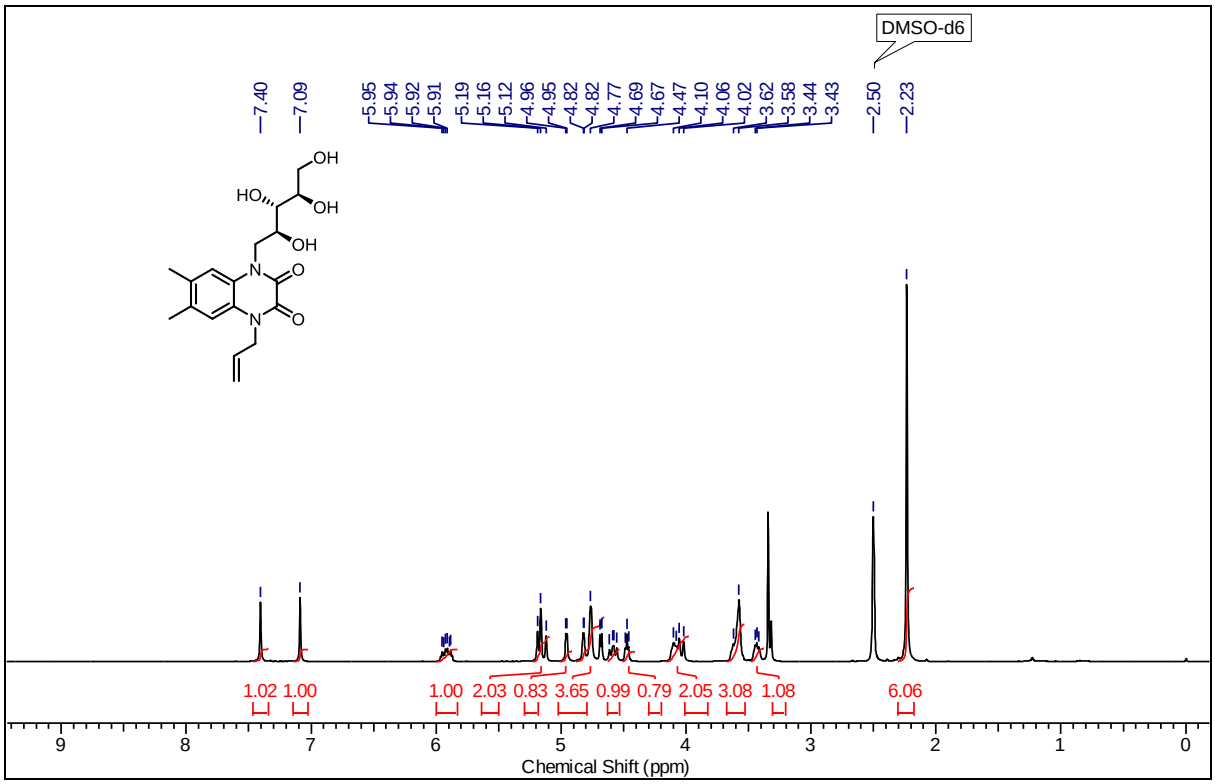
^{1}H NMR of Compound 98 at 400 MHz in CD_3OD



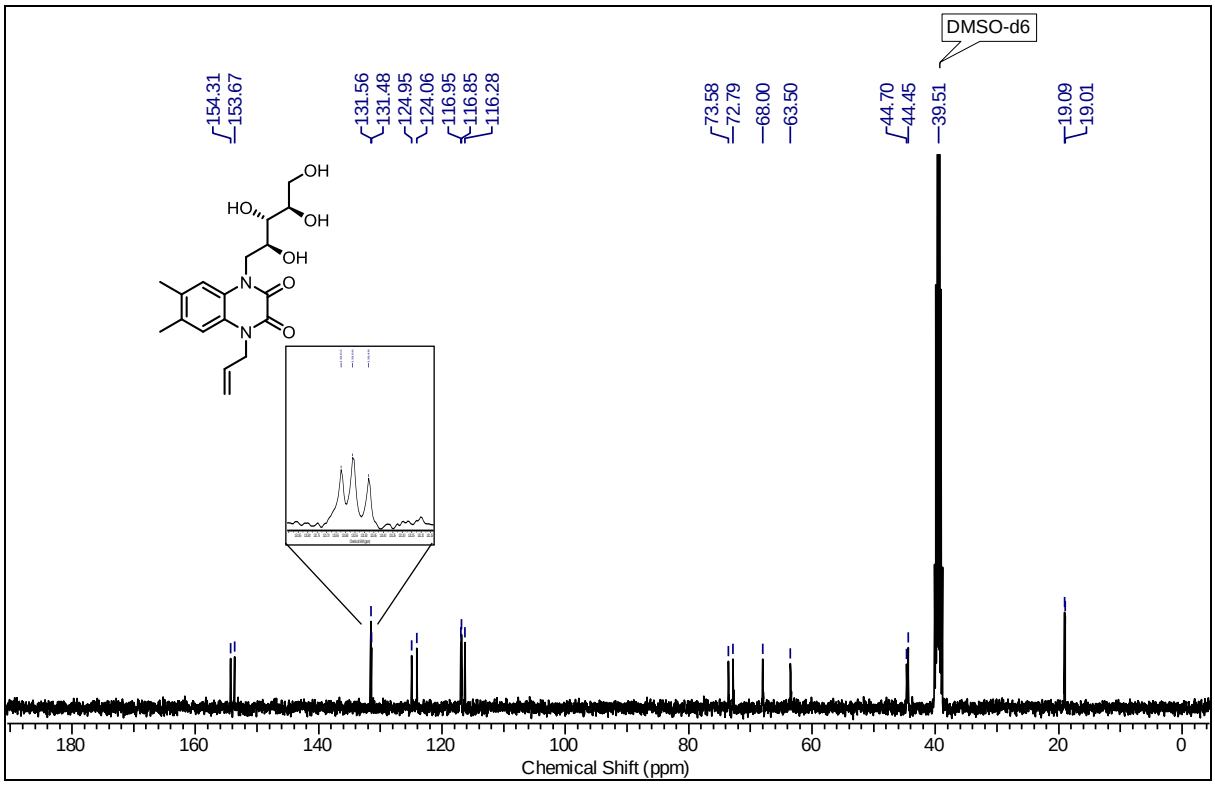
^{13}C NMR of Compound 98 at 100 MHz in CD_3OD



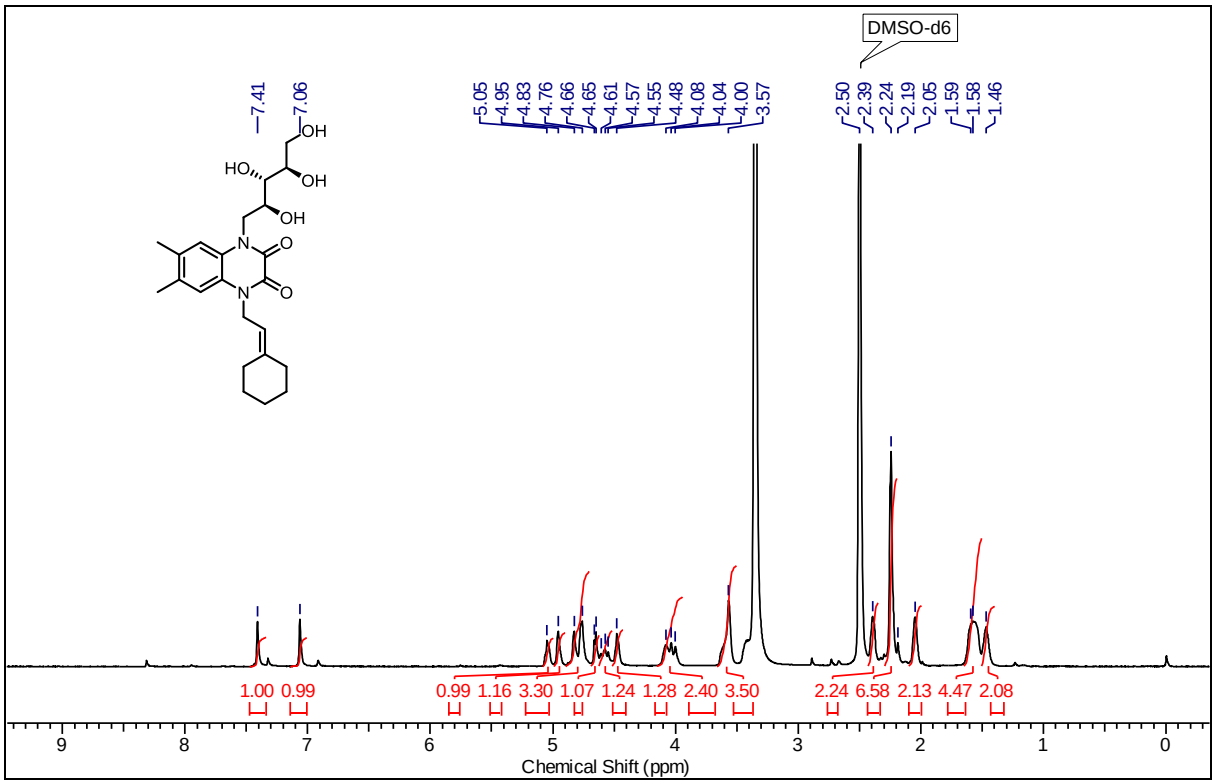
^{1}H NMR of Compound 100 at 400 MHz in DMSO-d_6



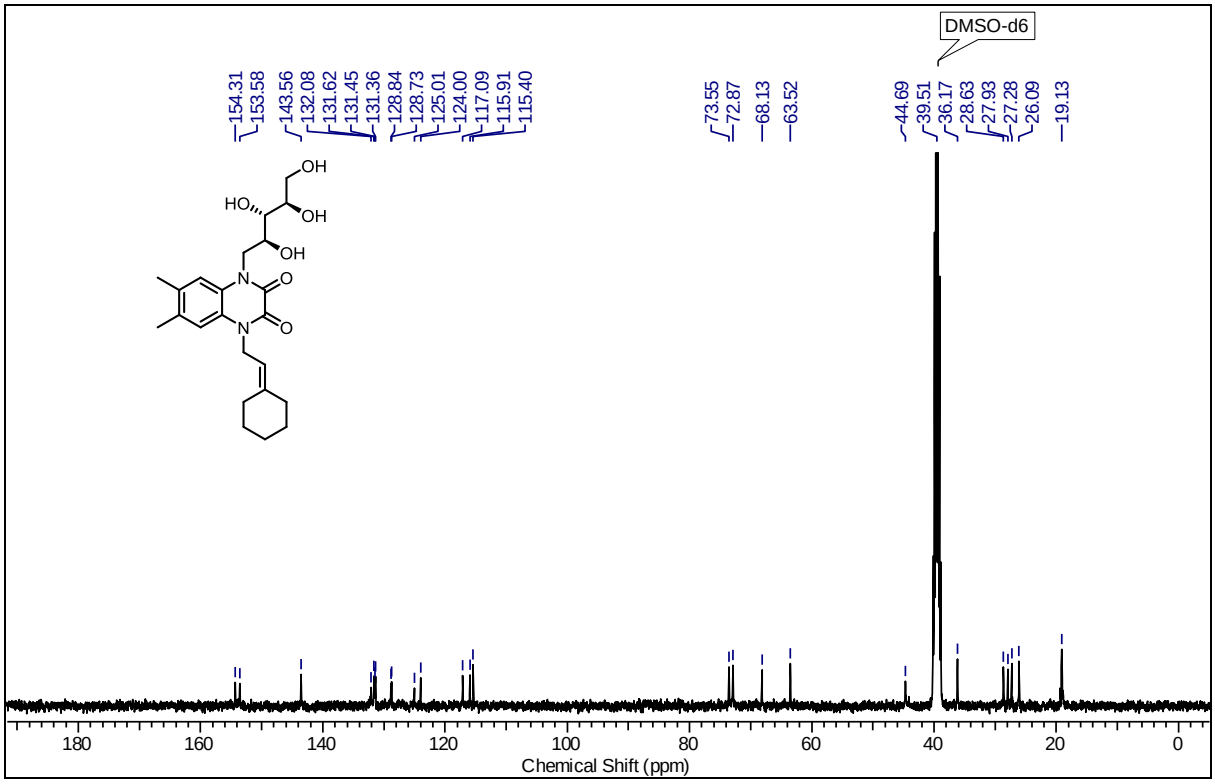
^{13}C NMR of Compound 100 at 100 MHz in DMSO-d_6



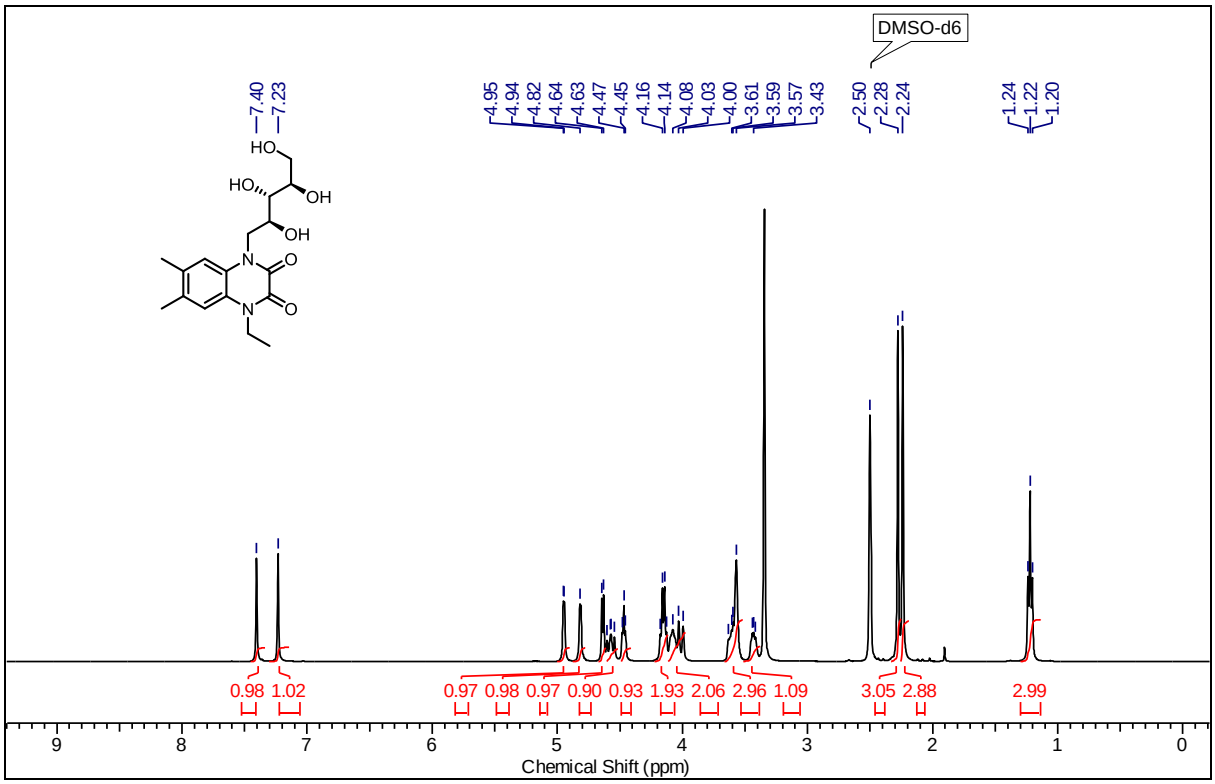
^{1}H NMR of Compound 101 at 400 MHz in DMSO-d_6



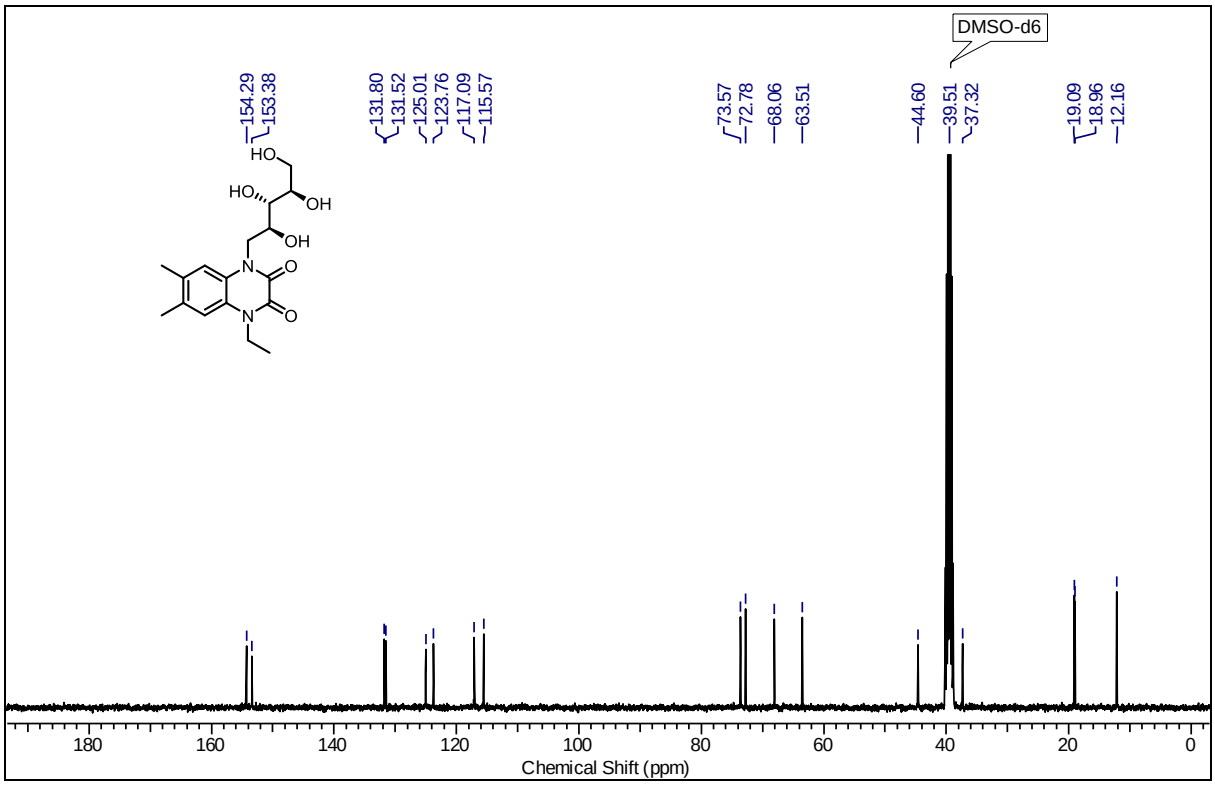
^{13}C NMR of Compound 101 at 100 MHz in DMSO-d_6



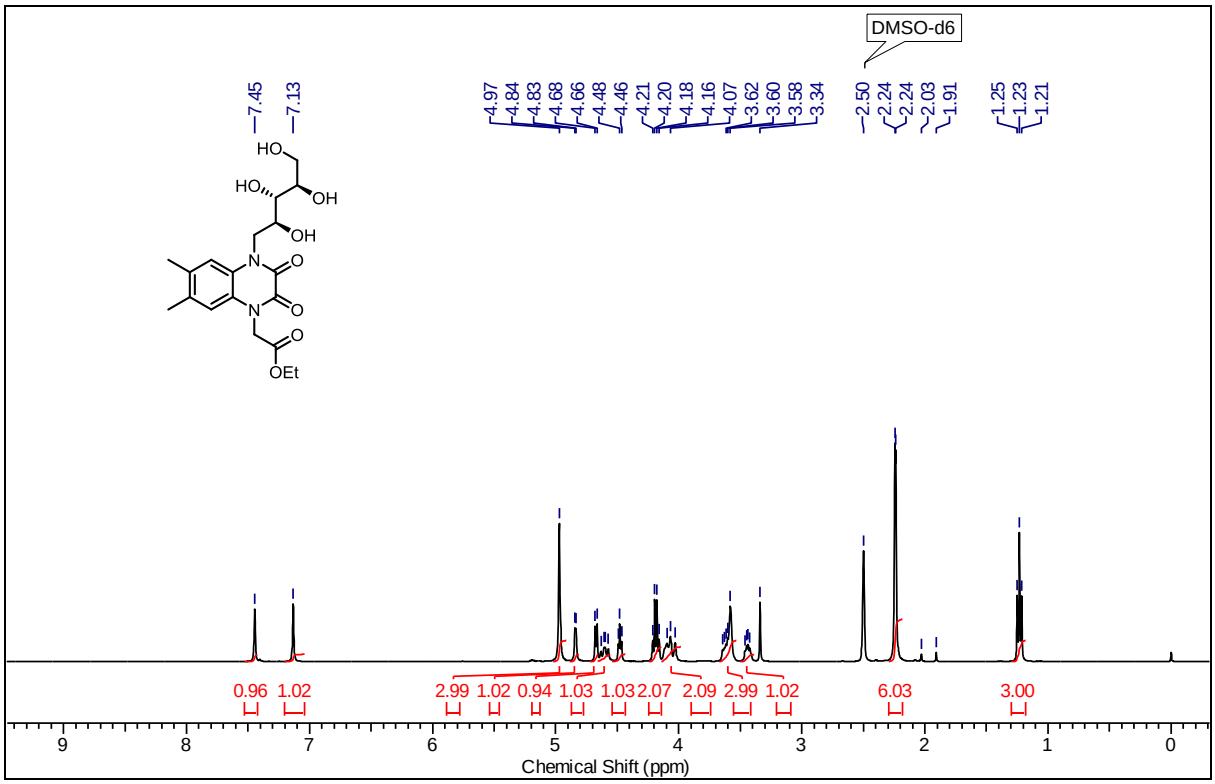
^{1}H NMR of Compound 103 at 400 MHz in DMSO-d_6



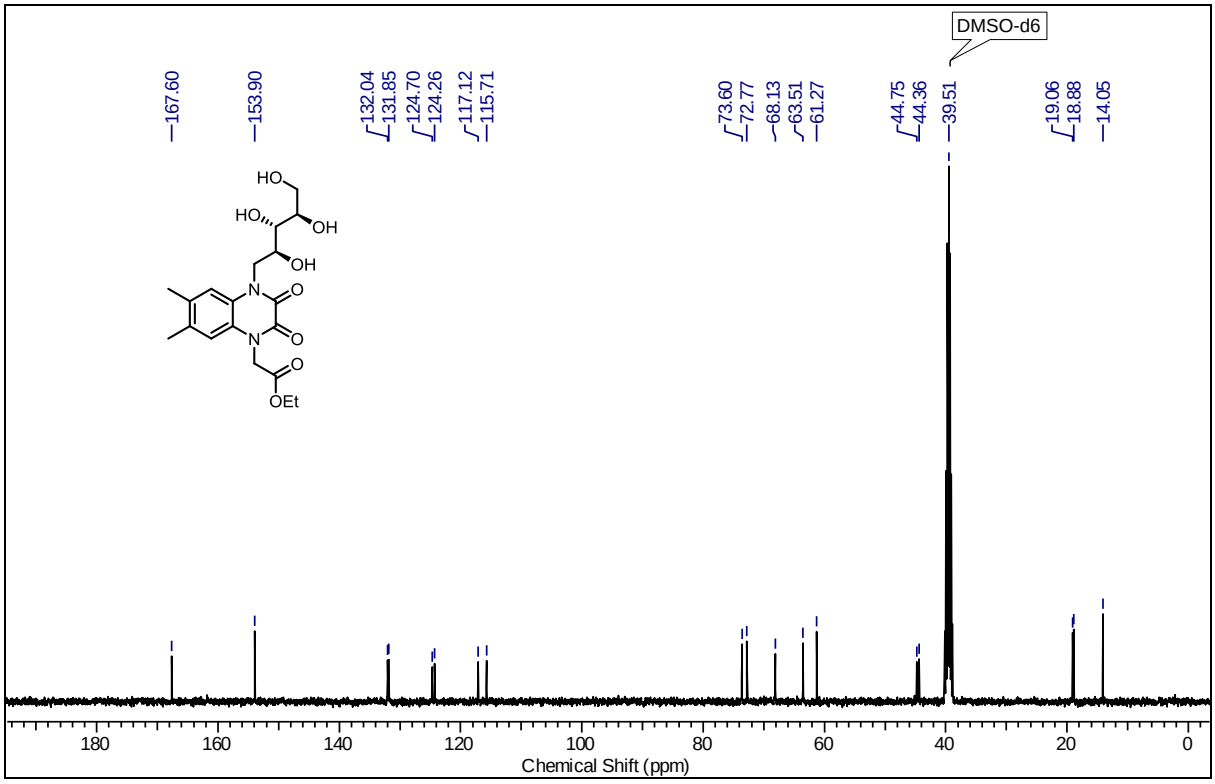
^{13}C NMR of Compound 103 at 100 MHz in DMSO-d_6



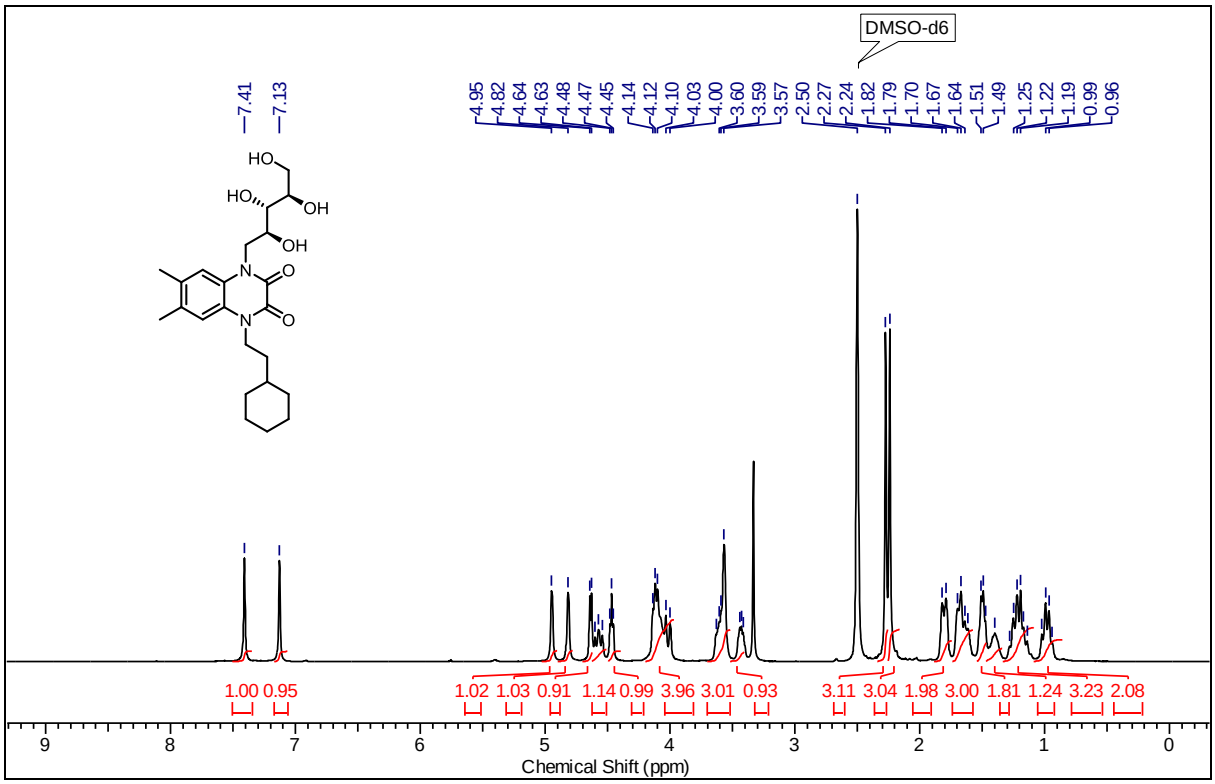
^{1}H NMR of Compound 104 at 400 MHz in DMSO-d_6



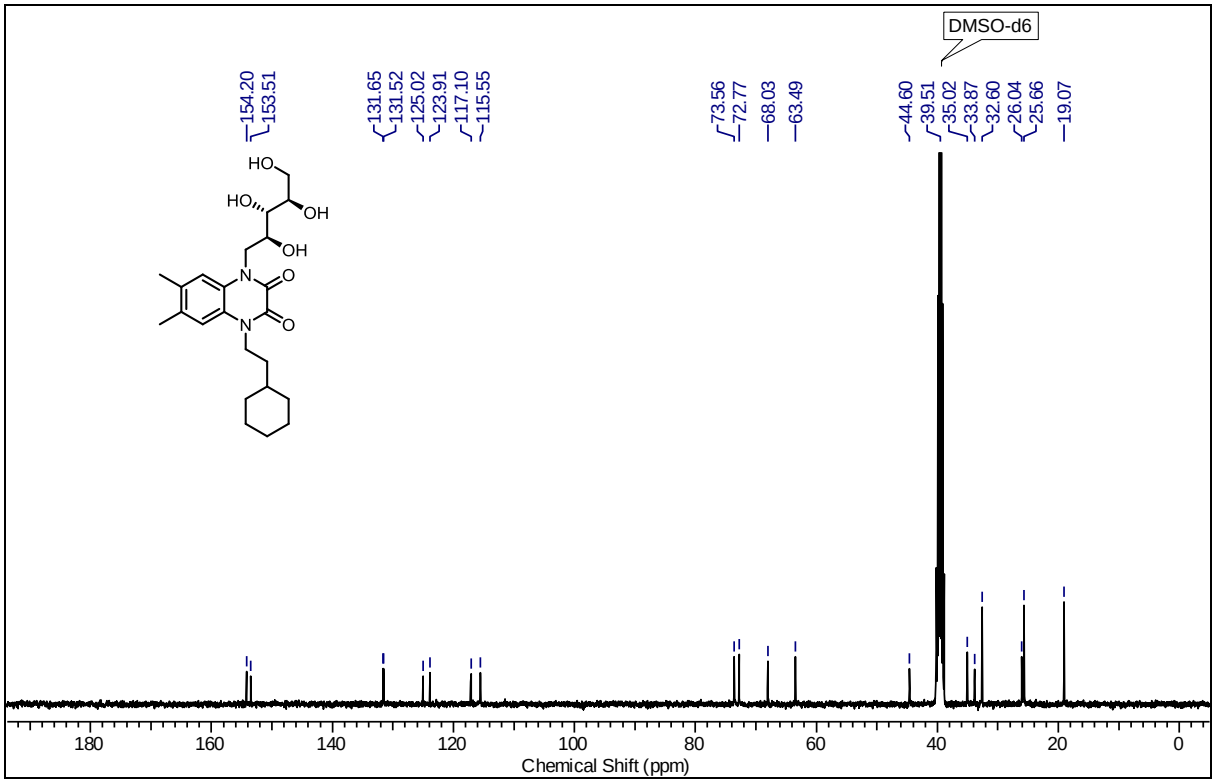
^{13}C NMR of Compound 104 at 100 MHz in DMSO-d_6



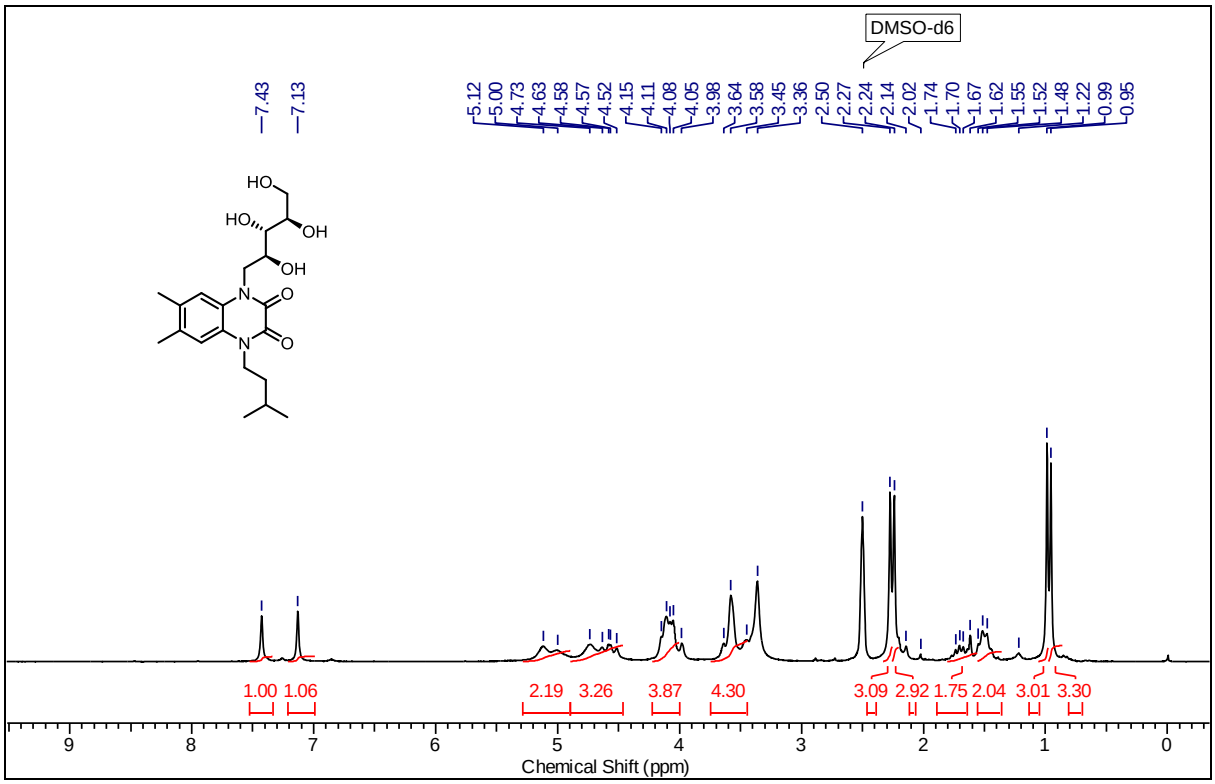
^{1}H NMR of Compound 106 at 400 MHz in DMSO-d_6



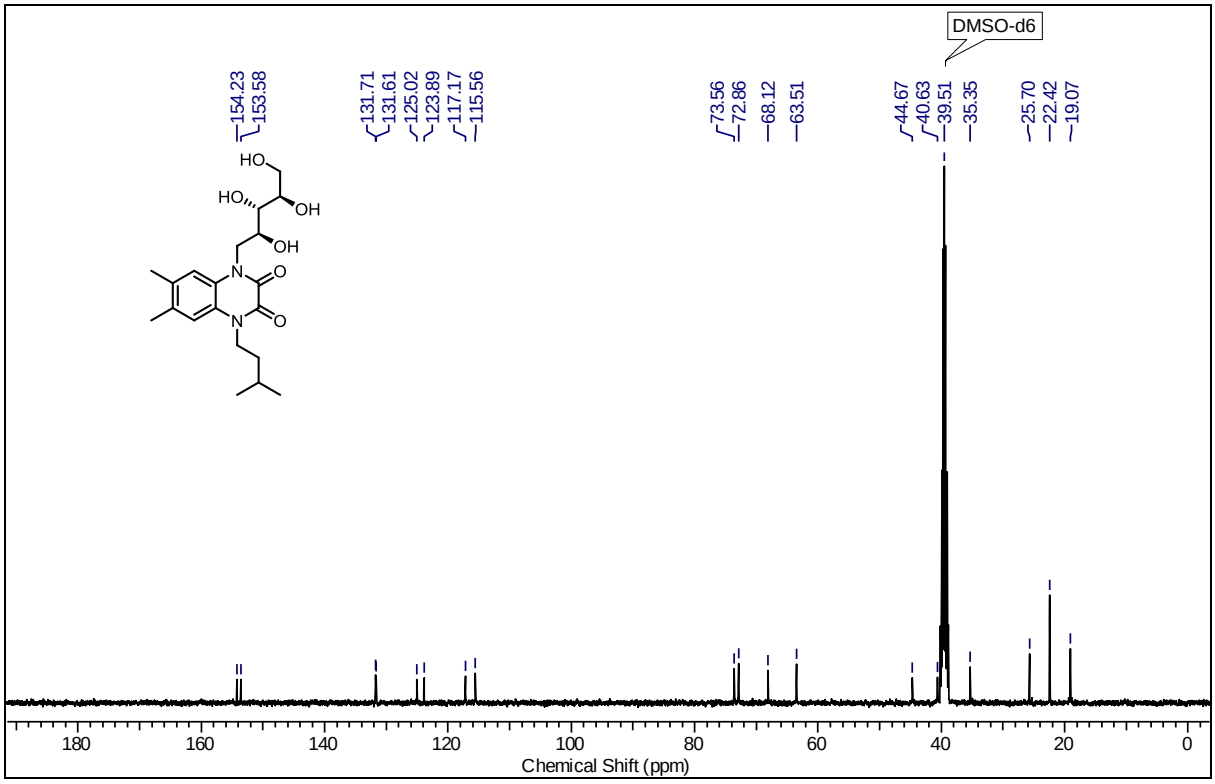
^{13}C NMR of Compound 106 at 100 MHz in DMSO-d_6



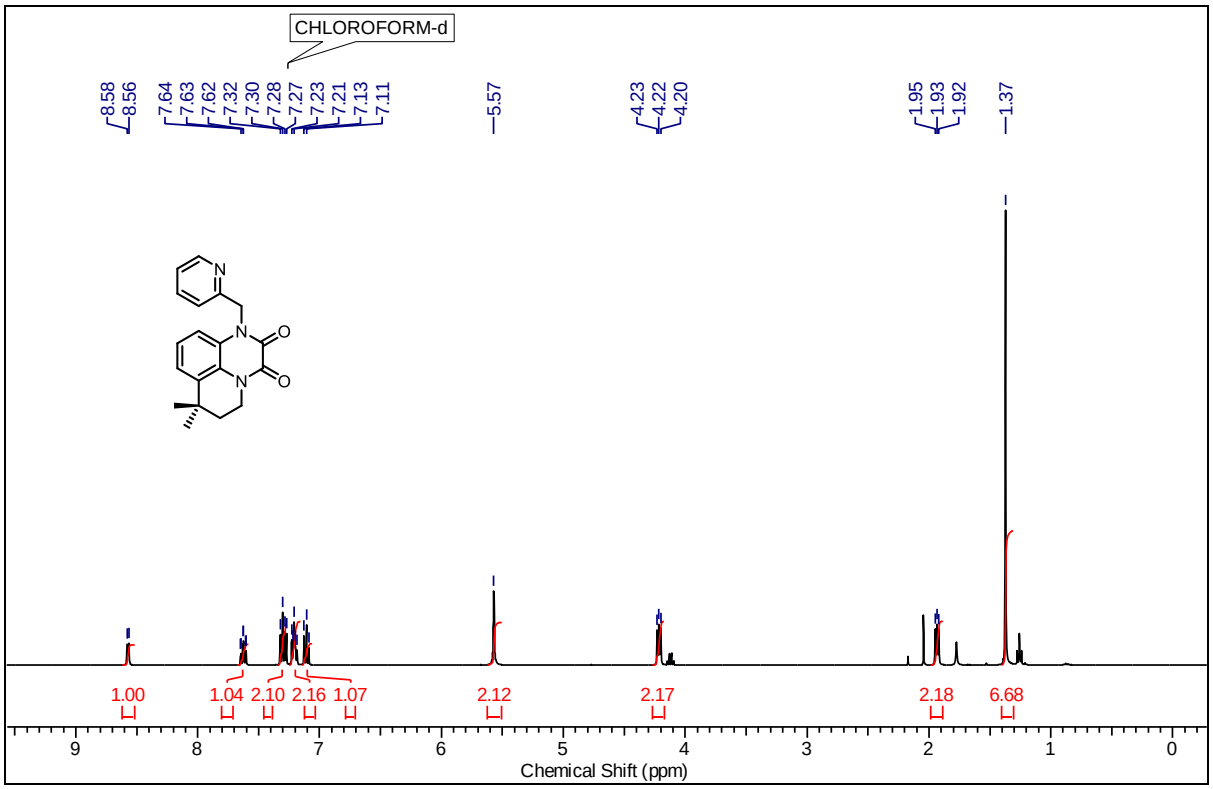
^{1}H NMR of Compound 107 at 400 MHz in DMSO-d_6



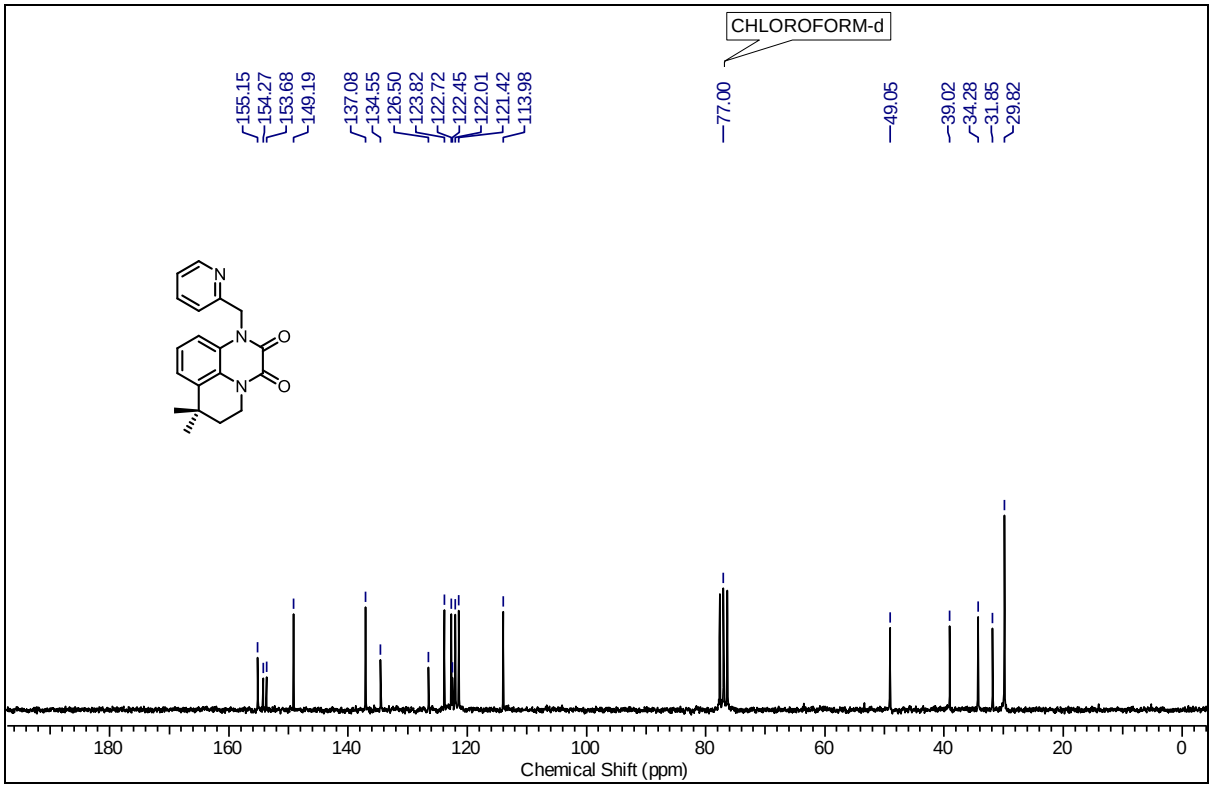
^{13}C NMR of Compound 107 at 100 MHz in DMSO-d_6



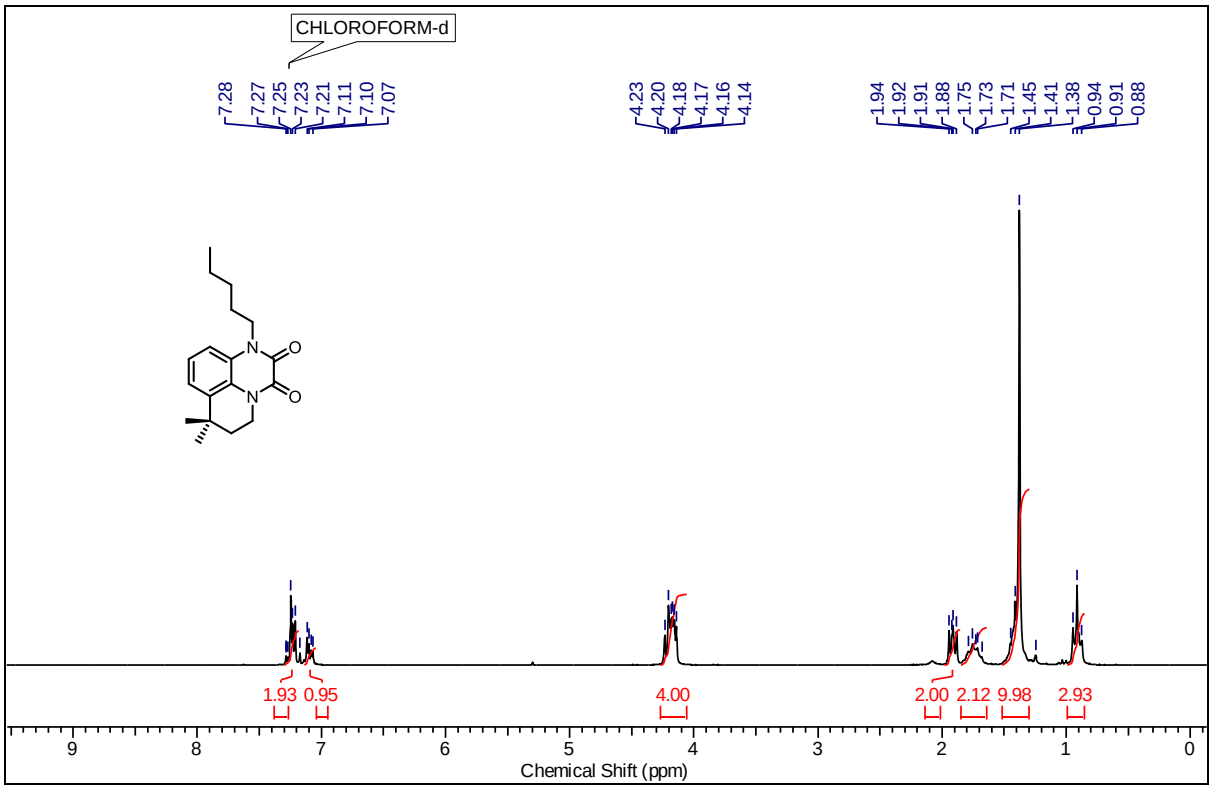
^{1}H NMR of Compound 109 at 400 MHz in $CDCl_3$



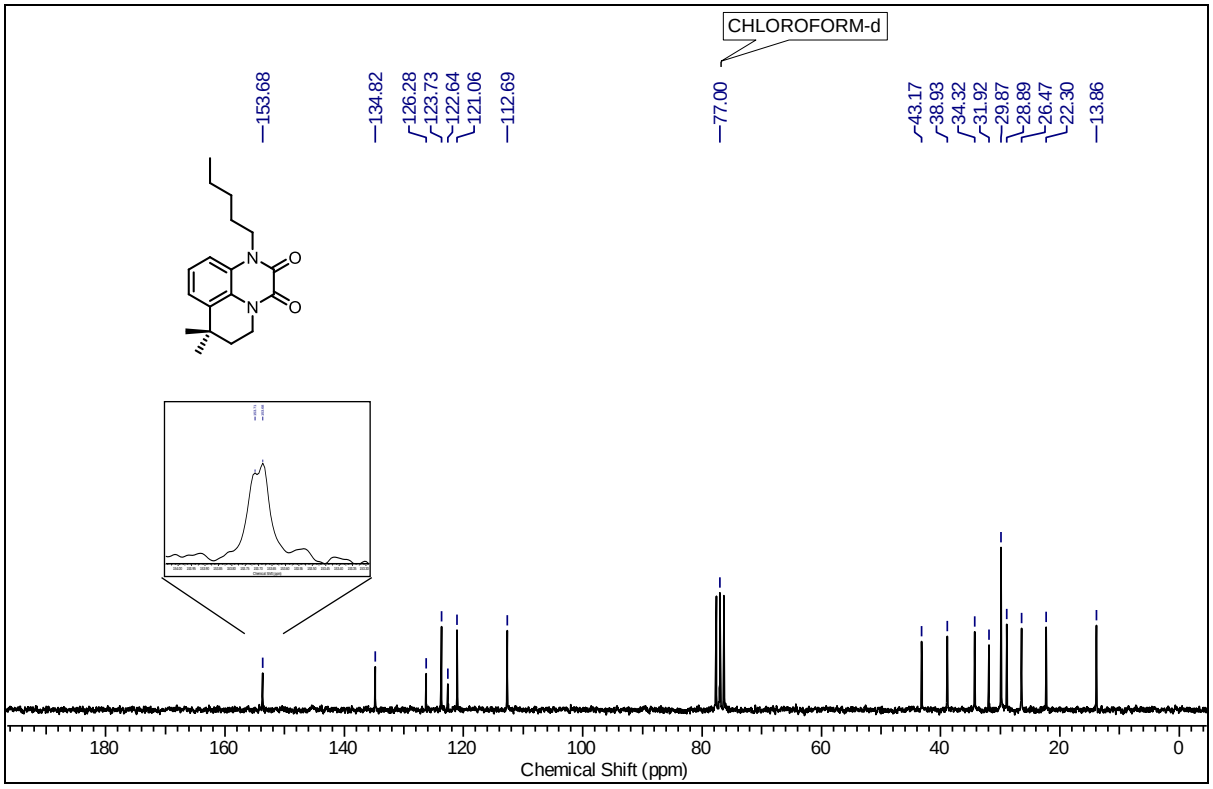
^{13}C NMR of Compound 109 at 50 MHz in $CDCl_3$



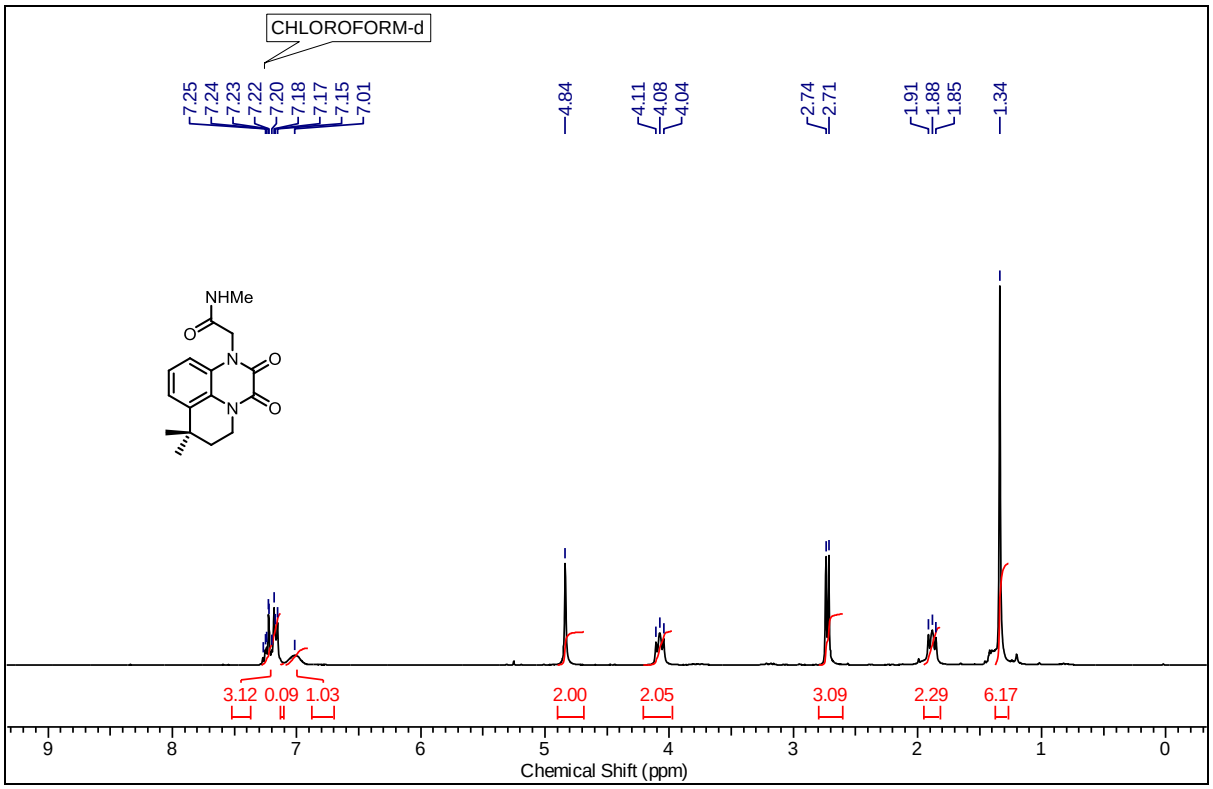
^{1}H NMR of Compound 110 at 200 MHz in $CDCl_3$



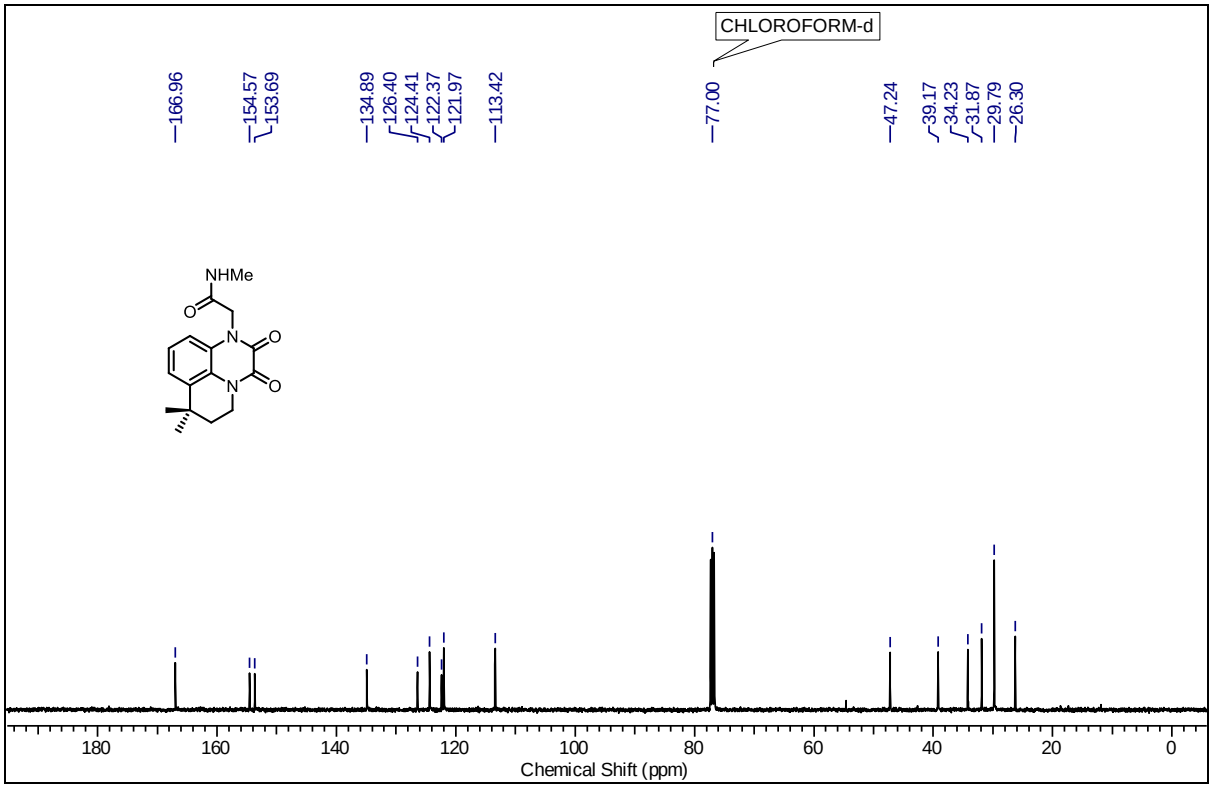
^{13}C NMR of Compound 110at 50 MHz in $CDCl_3$



^{1}H NMR of Compound 113 at 200 MHz in $CDCl_3$



^{13}C NMR of Compound 113 at 100 MHz in $CDCl_3$



Section 3

New chemistry related to Hunanamycin scaffold

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New chemistry related to Hunanamycin scaffold

In the course of Hunanamycin A total synthesis, we encountered exciting intermediates that presented us an opportunity to develop novel synthetic methodologies, which are described in this section. The first subsection (3.1) deals with a general approach to *N*-heterocyclic carbenes (NHCs) with a fused tetracyclic core that can be used as a ligand for cross-coupling reactions. We developed a synthesis of *N*-heterocyclic carbene (NHC) based on a tetracyclic scaffold by using simple and scalable chemistry. To demonstrate the utility of our work, we prepared NHC–Palladium complex, following which the catalytic activity of NHC palladium complexes were investigated in the cross-coupling reactions. The second subsection (3.2) describes a route to benzimidazol-2-ones through decarbonylative ring contraction of quinoxalinediones. A mild and practical method to access a variety of benzimidazol-2-ones was developed using potassium hydroxide and dimethyl sulfoxide. The present method is useful in drug discovery and natural product synthesis particularly for the late stage functionalization of quinoxalinediones. Following subsections will provide details of these projects.

Section 3.1 A general approach to tetracyclic *N*-heterocyclic carbenes (NHCs) and its utility in the Suzuki-Miyaura cross-coupling reaction.

3.1.1 Introduction

The chemistry of stable carbenes has grown expeditiously over the course of last two decades. The NHCs are an important class of ligands and catalysts because of their several attractive features and received the significant attention.[1-11] NHCs are defined as heterocyclic compound containing a carbene carbon and at least one nitrogen atom within the ring.[12] They are versatile and extensively used for a variety of applications. The effective uses of these species are as organocatalysts,[4] and as ligands for a variety of main group, transition metal[3] and f-block elelments,[9] metallopharmaceuticals both in homogeneous and heterogeneous applications. The most frequently encountered NHCs are those based on five-membered heterocycles and are captured in Figure **3.1**.[5] The versatile nature, reactivity and broad appli-

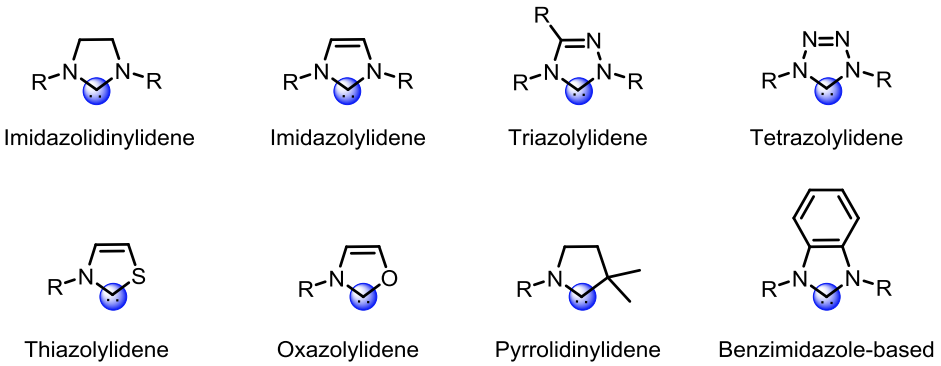


Figure 3.1: Common five-membered *N*-heterocyclic carbenes (NHC)

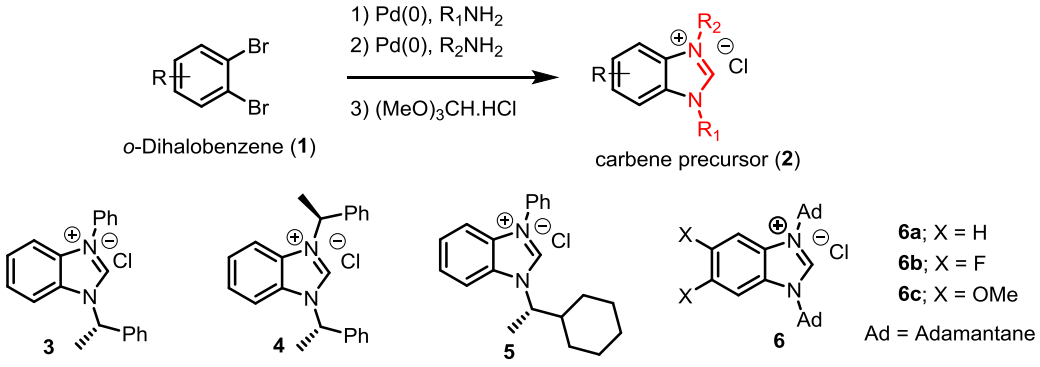
-cation of *N*-heterocyclic carbenes in synthetic chemistry led to the development of a variety of NHCs. However, only NHCs derived from Imidazolidinylidene or Imidazolylidene salts have found wide-spread use in homogeneous catalysis whereas, their benzannulated counterparts are relatively unexplored. The probable reasons are:

1. Intermediate reactivity and less stability.
2. Lack of a versatile method for preparation of differentially substituted chiral benzimidazoles.

3.1.2 Methods for the synthesis of benzimidazole NHC precursor

3.1.2.1 From *o*-dihalobenzene

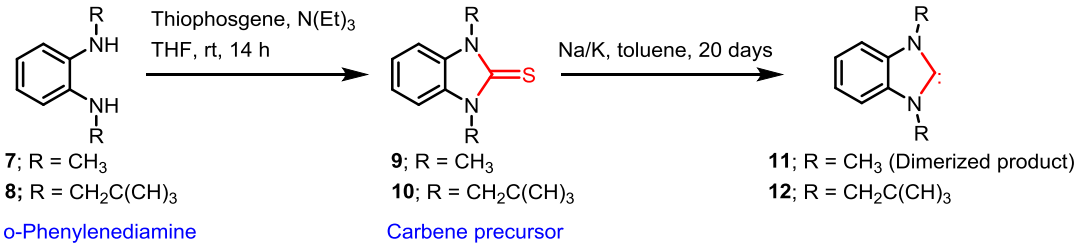
Diver and co-workers synthesized chiral *N*-substituted benzimidazolium salts (**3-5**) by a three-component condensation method.[13] The method involves subsequent Pd-catalyzed Buchwald-Hartwig amination of substituted dibromobenzene followed by subsequent ring closure step. Later, Organ and co-workers synthesized electronically different NHC ligands derived from *N*,*N*-diadamantylbenzimidazolium salts **6a-6c** using a similar strategy (Scheme **3.1**).[14]



Scheme 3.1: Synthesis of benzimidazole salts via 3-step protocol.

3.1.2.2 From *o*-Phenylenediamine

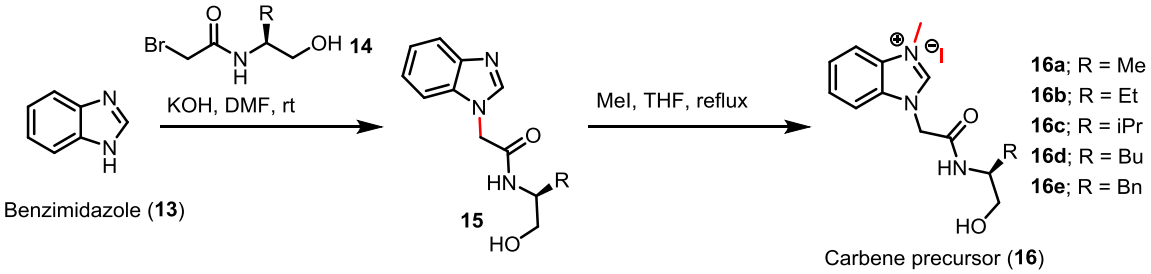
In 1999, Hann *et al.* reported the first stable free benzimidazolecarbene **12** derived from the corresponding benzoimidazole-2-thione **10**, together with the crystal structure of its tungsten complex.[15] Substituted aniline **7** and **8** which on reaction with thiophosgene gave thione **9** and **10** respectively. Reduction of **10** with sodium/potassium underwent desulfurization leading to the formation of carbene **12** while compound **9** under similar condition yielded dimerized product **11**. This result clearly indicates the steric bulk is essential for the stability of the *N*-heterocyclic carbenes (Scheme **3.2**). But this method was not frequently used for the synthesis of benzimidazolecarbene due to long reaction time.



Scheme 3.2: Desulfurization of thiones to generate carbene.

3.1.2.3 From benzimidazole

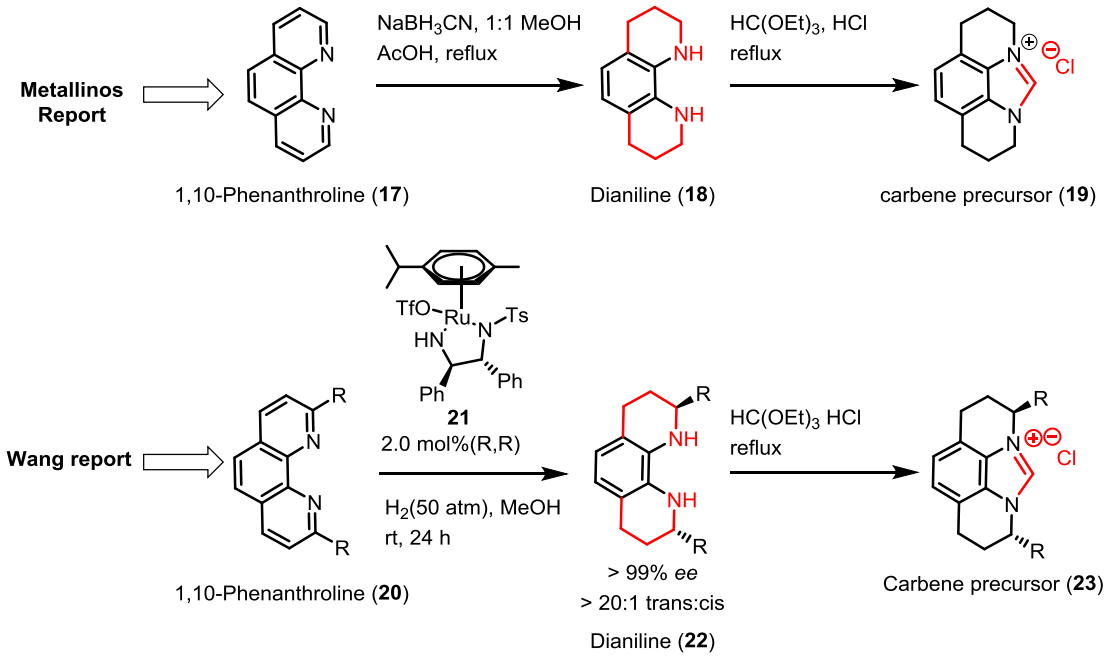
Another general method for synthesis of benzimidazole derived NHC is the successive *N-N'* dialkylation of benzimidazole using the same strategy Sakaguchi *et al*. synthesized a huge variety of azolium compounds by *N* alkylation of benzimidazole **13** with various amino acid derived alkyl bromides. This is a versatile method for the preparation of differently substituted carbene precursor (Scheme **3.3**). The chiral azolium compound **16a – 16e** were used for studying the enantioselective conjugate addition reaction.[16-17]



Scheme 3.3: Sequential alkylation of benzimidazole.

3.1.2.4 From 1,10-Phenanthrolines

Metallinos and co-workers reported the synthesis of benzimidazole embedded tetracyclic NHC precursor starting from 1,10-phenanthroline.[18] This is closely related to our work to be discussed in the following sections. The compound 1,10-phenanthroline **17** on reaction with $NaBH_3CN$ in 1:1 MeOH and glacial acetic acid at reflux yielded dianiline which on treatment with neat triethyl orthoformate and 1 equivalent of HCl provided the critical tetracyclic benzimidazolium chloride **19**.



Scheme 3.4: Three step synthesis of a novel class of benzimidazole salt.

Later in 2013, Wang *et al.* have developed ruthenium based chiral catalyst **21** and used for the chiral reduction of phenanthroline bearing the substitution on 2nd and 9th position to obtain corresponding optically pure diamine **22** with excellent enantioselectivity, and diastereoselectivity which was later transformed into chiral benzimidazolium chloride salt **23**.[19]

3.1.3 Present work

3.1.3.1 Synthesis of tetracyclic NHC precursor

As discussed in section 1 during the execution of first total synthesis of Hunanamycin A, in the scale-up of the tricyclic compound precursor, we have obtained the unwanted dialkylated compound **24** in considerable amount. To utilize this diprenylated product efficiently, we looked into literature and found that, this could be transformed into some synthetically useful

material *N*-heterocyclic carbene (NHC) by a few simple, functional group transformations. Some of the interesting NHCs along with their inventor names are captured in Figure **3.2**. Inspired by the work of Metallinos group,[18] and to take advantage of dialkylated side product formed, we became interested in synthesis and applications of tetracyclic NHCs.

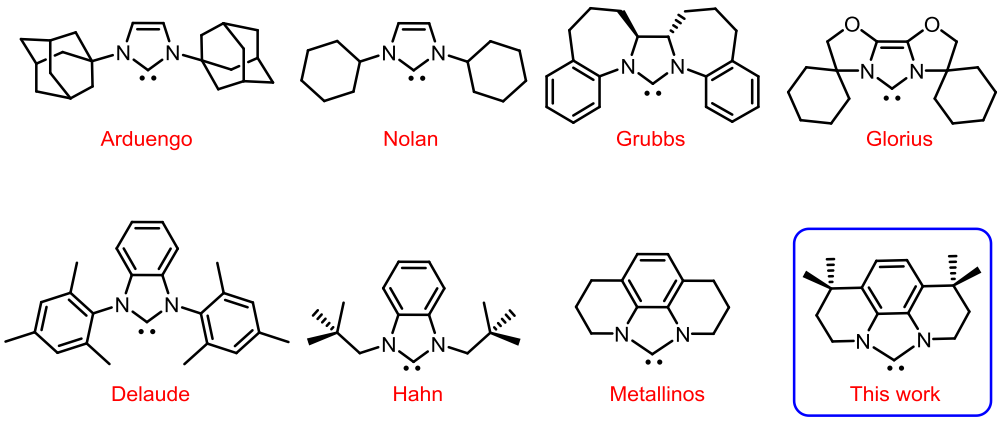
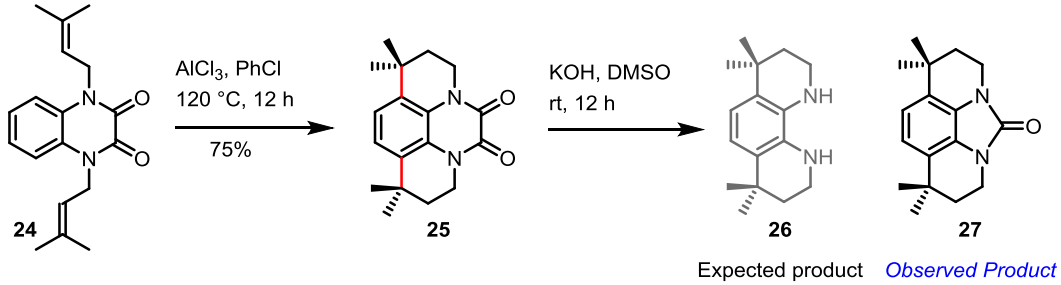


Figure 3.2: Selected NHCs documented in the literature.

Accordingly, our synthesis commenced with the dialkylated compound **24** which under previously developed Friedel-Craft condition ($AlCl_3$, PhCl) gave symmetric tetracyclic product **25** in quantitative yields. The compound **25** formed was confirmed by ^{1}H NMR showed signals at δ 7.18 (s, 2 H) corresponds to two aromatic C-H, newly formed methylene appeared at δ 1.88 (t, J = 5.8 Hz, 4 H). The symmetry pattern indicated by NMR signals in ^{1}H and ^{13}C NMR further confirmed the assigned structure. The next task was to prepare tricyclic aniline **26** for this we attempted the hydrolysis of tetracyclic quinoxalinedione **25** by the treatment of KOH in DMSO at room temperature for 12 h. To our surprise, the major product isolated did not show characteristic peaks for the desired product in NMR as well as in mass, instead we observed skeletally rearranged benzimidazol-2-one product **27** (Scheme **3.5**). The characterization of the newly formed product and its application will be discussed in the section **3b**.



Scheme 3.5: Initial approach toward tetracyclic NHC from quinoxalinedione

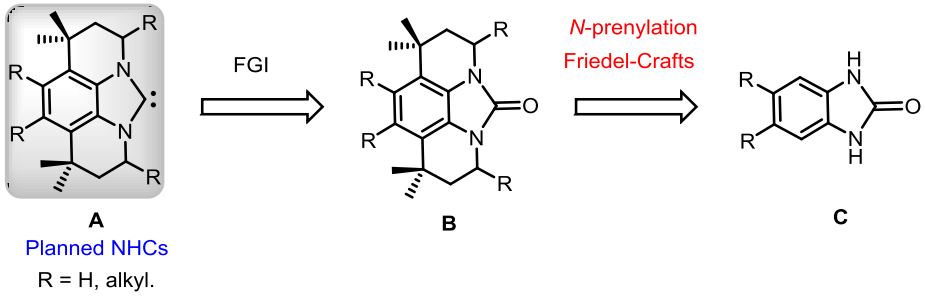
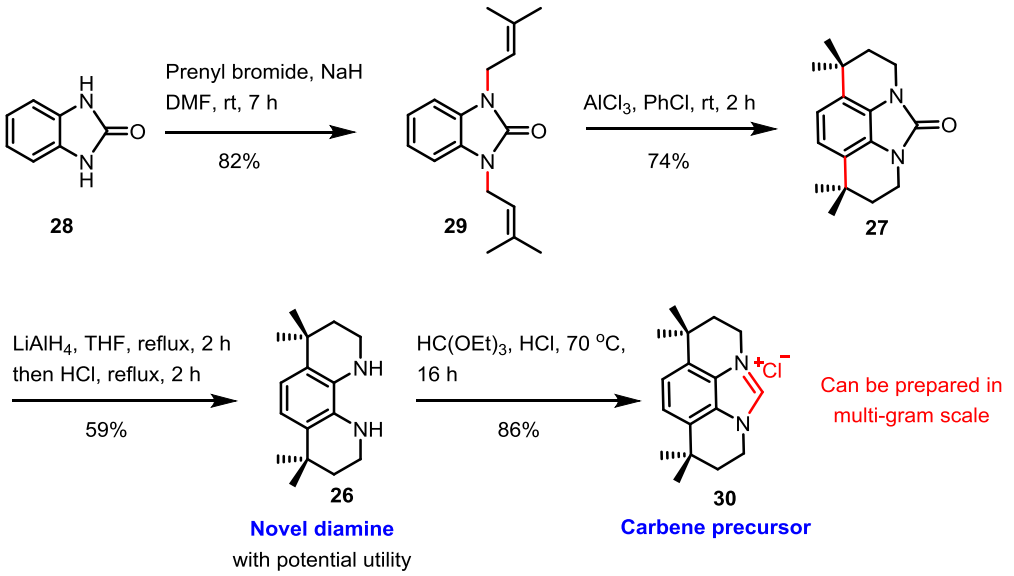


Figure 3.3: Revised retrosynthesis of planned NHCs

To move forward, we redesigned our scheme from corresponding dihydrobenzimidazol-2-ones as shown in Figure **3.3**. The desired NHCs **A** are visualized from a readily available dihydrobenzimidazol-2-ones **C** through urea derivatives **B** intermediate as shown in Figure **3.3**.

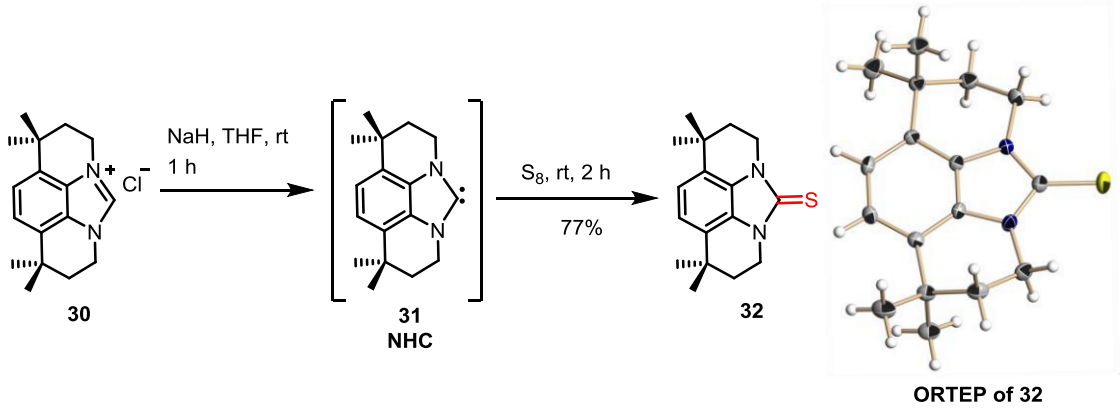
Based on this strategy, we planned to access benzimidazole-based NHCs with substituents decorated around the tetracycle including chiral substituents. Accordingly, dihydrobenzimidazol-2-one **28** was diprenylated with prenyl bromide and sodium hydride (60% in mineral oil), to provide compound **29** with excellent yield (82%). The compound formed was indicated by staining TLC in Iodine as well as in $KMnO_4$. The ^1H NMR showed signals at δ 5.27 (dd, J = 6.72 Hz, 2H) corresponds to C-H of the internal olefin; peak at δ 4.48 (d, J = 6.4 Hz, 4 H) for the methylene group from prenyl unit. Further, it was confirmed by HRMS value observed at 271.1805 corresponding to molecular formula $C_{17}H_{23}N_2O$ $[M+H]^+$. The compound **29** was then subjected to cyclization to give highly symmetric structure, tetracyclic urea **27** using $AlCl_3$ in chlorobenzene stirring at room temperature for 2 hours. The structure of the product was assigned with the help of ^1H NMR the two aromatic CH appeared at δ 6.89 (s, 2H), also signal at δ 1.91 (t, J = 5.80 Hz, 4H) indicating the CH_2 group of the cyclohexyl ring, ^{13}C NMR clearly show negative peak in DEPT 135 at δ 37.5 and δ 36.5 ppm corresponds to the two CH_2 from cyclohexyl ring. The carbonyl group from benzimidazol-2-one was then cleaved by reduction with $LiAlH_4$ to hydro aminal intermediate, followed by refluxing with aq. HCl, to produce tricyclic diamine **26**, in 59% overall yield.[20] The product formed was undoubtedly confirmed by the disappearance of the peak from δ 152.7 ppm corresponding to the urea carbonyl. It was further confirmed by HRMS signal observed at 245.2006 for the molecular formula $C_{16}H_{25}N_2$ $[M+H]^+$ with calculated value 245.2012. It is worth mentioning here that compound **26** is a constrained diamine, which has great potential, particularly when it is made is chiral form. Finally, the benzimidazolium salt **30** was prepared from **26** using triethyl orthoformate and HCl at 70 °C

(Scheme **3.6**).[18] The CH of the benzimidazole NHC precursor **30** in ^{1}H NMR resonate at δ 11.07 ppm whereas, in ^{13}C NMR at 138.8 ppm clearly confirm the product structure.



Scheme 3.6: Synthesis of tetracyclic NHC precursor

The treatment of **30** using NaH (60% in mineral oil) in THF generated free carbene **31** which was trapped in-situ using cyclo-octasulfur (S_8) to obtain thiourea **32** in good yield. The product formed was characterized and confirmed with the help of spectroscopic techniques (^{1}H, ^{13}C NMR, HRMS) and also by single crystal X-ray structure (Scheme **3.7**)



Scheme 3.7: Synthesis of compound **32** by trapping free carbene.

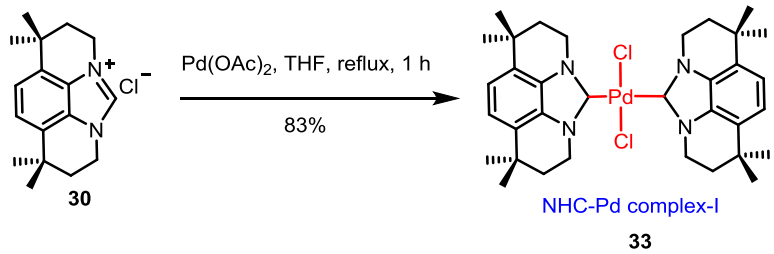
3.1.3.2 Synthesis of Pd-NHC complex:

Palladium catalysed cross coupling reactions are one of the powerful tool in the organic synthesis for the C-N and C-C bond formation reaction. In 1995 Herrmann and co-workers used *N*-heterocyclic carbene as a ligand in the Heck coupling reaction for the first time.[21] After this discovery scientific community got attracted towards the use NHC as a potential ligand in palladium mediated cross coupling reaction due to its added advantages over the

traditional phosphine ligand are as follows. [22-27]

1. High turnover number of the catalyst.
2. Stability of the catalyst to the moisture, oxygen and elevated temperature.
3. Commercially availability of NHC–Pd precatalysts, and user-friendly.

To demonstrate the usefulness of synthesized tetracyclic NHC, we have prepared NHC-Pd-NHC complex **33** by the reaction of compound **30** and $Pd(OAc)_2$ in THF refluxing for 1 h to obtain light grey coloured solid product (Scheme **3.8**). The product **33** was confirmed by ^1H NMR broad peak at δ 4.93 (br. s, 4 H), and δ 4.07 (br. s, 4 H) corresponds to CH_2 attached to the nitrogen whereas, the signal at δ 2.09 (br. s, 4 H) and δ 1.92 (br. s, 4 H) ppm for the other two methylene group. ^{13}C NMR showed a signal at 166.0 ppm corresponds to the carbene carbon attached to palladium. The product **33** was further confirmed by C, H, N elemental analysis: $C_{34}H_{44}Cl_2N_4Pd$ calculated: C, 59.52; H, 6.46; N, 8.17; experimentally found: C, 59.22; H, 6.17; N, 8.13 %.



30 33

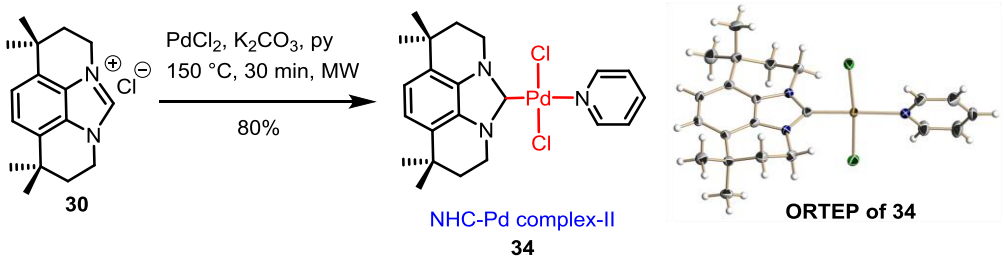
Scheme 3.8: Synthesis of NHC-Pd complex **33**.

In 2005 Prof. Organ and co-workers developed a new elegant palladium *N*-heterocyclic-carbene (NHC) catalyst system known as PEPPSI which stands for **P**yridine-**E**nhanced **P**recatalyst **P**reparation **S**tabilization and **I**nitiation.[28-31] The catalyst developed offered many advantages over the other existing catalysts, some of which are as follows:

1. Stable to air and moisture.
2. Easy access to multi gram scale and low cost.
3. Most of the reactions occur at room temperature.
4. No additional ligand requires.

Encouraged by this literature reports, the carbene precursor **30** was treated with $PdCl_2$, K_2CO_3 in pyridine as a solvent in the microwave at 150 °C for 30 minutes to obtain desired PEPPSI catalyst **34** in excellent yield (80%). The product **34** was confirmed by the disappearance of benzimidazole CH signal from δ 11.07 and appearance of the typical pyridine peaks in the ^1H NMR whereas, ^{13}C NMR showed carbene carbon resonate at δ 155.1 along with the other typical signals for the tetracycle and pyridine. The elemental analysis of

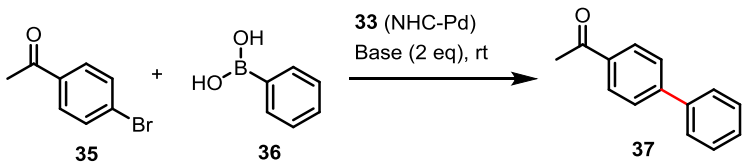
the also justifies the assigned structure and it was unambiguously confirmed by a single crystal X-ray diffraction studies (Scheme **3.9**)



Scheme 3.9: Synthesis of PEPPSI catalyst **34**.

3.1.3.3 Application of Pd-NHC complex in Suzuki cross coupling reaction

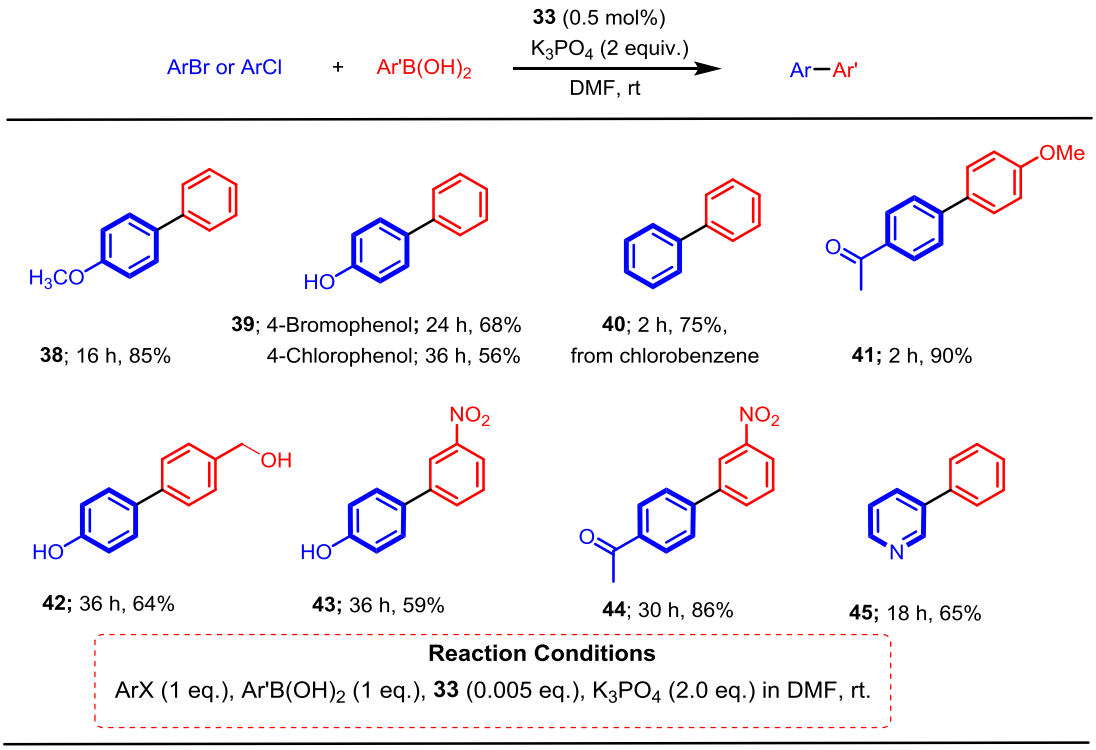
The catalytic activity of **33** and **34** were employed in the cross-coupling reaction of 4-bromoacetophenone **35** with phenyl boronic acid **36**. We screened several conditions varying the solvent, bases and found that the compound **33** showed exceptional catalytic activity at 0.5 mol% catalyst loading, K_3PO_4 base, in DMF at room temperature with the maximum yield of product **37** (94%, Table **3.1**). It is worth noting that related Metallinos' NHC-Pd complex found to be reactive at higher temperatures (>100 °C). We also attempted the Suzuki cross coupling reaction of **35** and **36** by the combination of compound **30** and $Pd(OAc)_2$



No	Base	(mol%) of 33	Solvent	Time (h)	% Isolated yield
1	K_2CO_3	0.7	Toluene	30	60
2	K_2CO_3	0.7	NMP	24	92
3	K_2CO_3	0.5	DMF	24	82
4	K_3PO_4	0.5	NMP	15	90
5	K_3PO_4	0.5	Toluene	30	84
6	**K_3PO_4**	**0.5**	**DMF**	**2**	**94**
7	K_3PO_4	0.5 mol% $Pd(OAc)_2$ + **30**	DMF	2.5	90
8	K_3PO_4	0.5 mol% $Pd(OAc)_2$	DMF	24	21
9	K_3PO_4	0.5 mol% $PdCl_2$	DMF	24	17

Table 3.1: Optimization reaction condition for the Suzuki-Miyaura cross-coupling reaction.

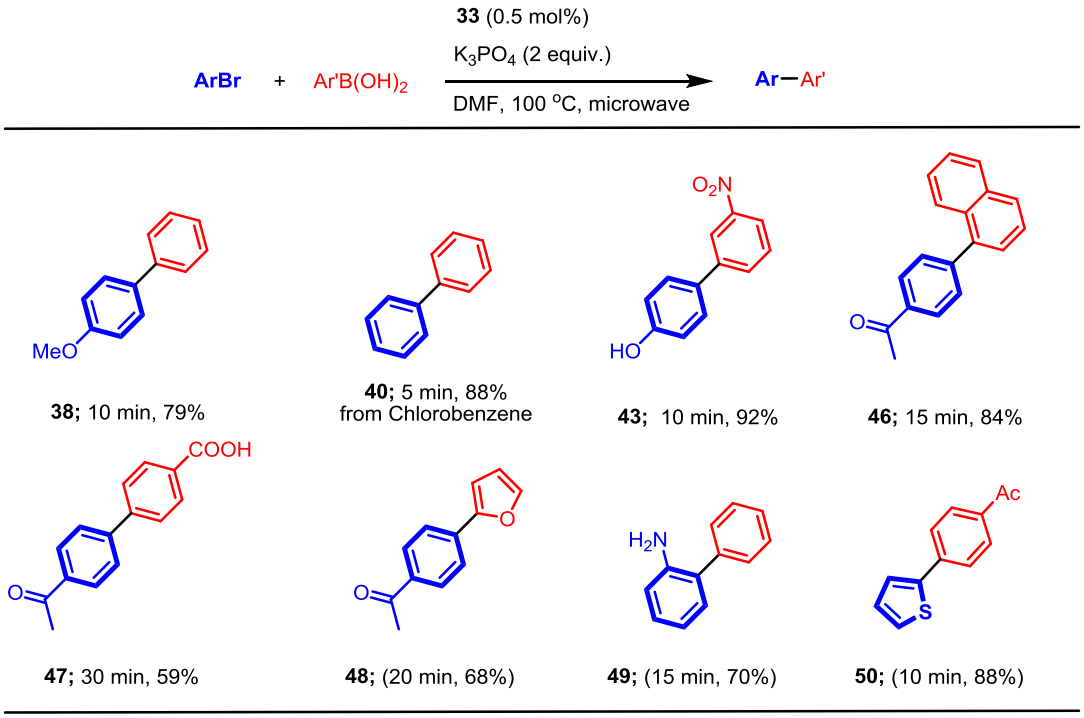
through the in-situ generation of active catalyst **33** to obtain **37** in 90% yield (entry **7**). While under the identical reactions using $Pd(OAc)_2$ and $PdCl_2$ without the addition of NHC precursor **30** gave poor yields (entries **8** and **9** respectively). After successful optimization protocol in hand, the scope of cross coupling reaction was tested with different aryl halides and aryl boronic acids (see details in Scheme **3.10**). Arylboronic acids with different substitution underwent a coupling reaction with different aryl halides at room temperature to furnished products **38**–**45** in good to moderate yields. The new products formed were confirmed with the help of spectroscopic techniques and reported compounds were confirmed by comparing the ^1H NMR with reported spectra. The electron rich aryl boronic acids, e.g. phenyl and 4-methoxyphenyl boronic acid undergo fast reaction with aryl bromides having electron withdrawing substituents, while those having the opposite functionalities require a longer time (Scheme **3.10**). The reaction also works well with aryl chlorides at room temperature, though the rate becomes slower in most cases.



Scheme 3.10: The scope of the Suzuki-Miyaura coupling reaction.

Further to improve the process, we have attempted the reaction under microwave conditions. As expected, the results were promising and the reaction times got reduced significantly (Scheme **3.10** vs Scheme **3.11**). All the results for cross coupling reaction obtained under microwave conditions are compiled in Scheme **3.11**. The new coupling product obtained were characterized with the help of spectroscopic techniques (IR, ^1H, ^{13}C NMR and HRMS)

and found well in agreements with the structure drawn in the scheme **3.11**. The cross coupling reaction of 2-bromoaniline with phenyl boronic acid underwent smoothly in the presence of catalyst **33** with good yield (product **49**). In addition products **48** and **50** were also prepared, which increased the scope of the reaction with heteroaryl halides. We have performed some of the cross-coupling reaction with catalyst **34,** and it was found to give comparable results.



Scheme 3.11: The scope of the reaction under microwave conditions.

3.1.4 Conclusion

We have prepared NHCs based on 1,1,9,9-tetramethyl-1,2,3,7,8,9-hexahydro-5*H* imidazo[1,5,4,3*lmn*][1,10]phenanthroline using an efficient and scalable synthetic route. The developed route is general and it will be useful for making a variety of diamines and NHCs which are potentially useful in organic synthesis. During this work, we have observed an interesting skeleton rearranged product which will be discussed in next subsection. We have accessed new NHC-Pd complexes **33** and **34** for the first time which are well characterized and it was further demonstrated their use in Suzuki-Miyaura cross-coupling reactions as catalysts at very low loading.

Section 3.2 A route to benzimidazol-2-ones via decarbonylative ring contraction of quinoxalinediones: Application to the synthesis of Flibanserin, and urea analogue of Hunanamycin A.

3.2.1 Introduction

Heterocycles play a central role in drug design and discovery process. Heterocycles are common fragments of the majority of marketed drugs as they can function as useful tools to modify some of the druggable properties like lipophilicity, polarity, solubility and hydrogen bonding capability which in turns may lead to improved toxicological, and physicochemical properties of the molecule.[32] Recent analysis by Njardarson and co-workers showed that 59% of FDA approved small-molecule drugs contains a nitrogen heterocycle.[33-34] Among various nitrogen-containing heterocyclic compounds, benzimidazol-2-ones occupy an important position and are considered to be privileged scaffolds in medicinal chemistry. Benzimidazol-2-one can interact with enzymes and receptors by various non-covalent interactions like π–π stacking, Van der Waals force and hydrogen bonding. Benzimidazol-2-one derivatives display a wide range of biological activities including CNS disorders, type II diabetes, cancer, infectious disease and pain management.[35-45] Selected molecules containing benzimidazol-2-

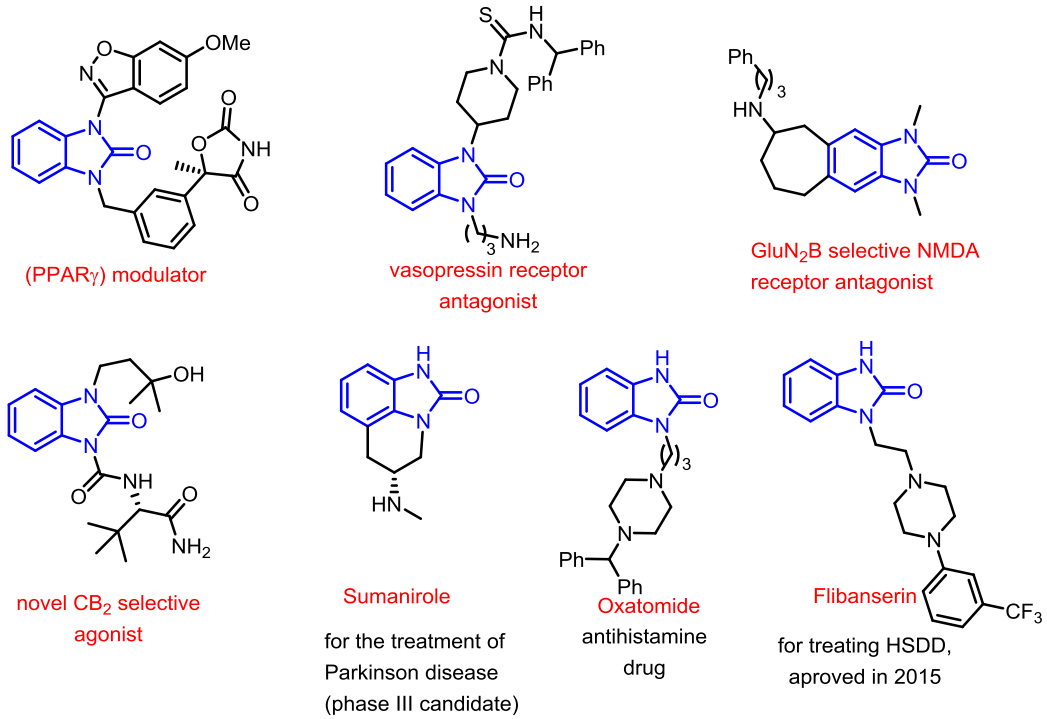


Figure 3.4: Examples of biologically important molecules with benzimidazol-2-one moiety

-one moiety are captured in Figure **3.4**. The sumanirole, a highly selective D_2 receptor full agonist containing benzimidazol-2-one core, was the phase III clinical candidate for the Parkinson's disease (PD) and restless leg syndrome.[46] The oxatomide drug is a first-generation anti-histamine was approved for use a few years ago.[47] In 2015, a new drug called Flibanserin was approved by the U S Food and Drug Administration (US-FDA) for the treatment of hypoactive sexual desire disorder (HSDD) in womens.[48]

3.2.2 Literature methods for the synthesis of benzimidazol-2-one

Considering the impressive biological activity and importance of this scaffold, several routes to access benzimidazol-2-one are documented in the literature. However, they broadly fall under two methods:

1. Carbonylation of substituted 1,2-diaminobenzene
2. Intramolecular or intermolecular cyclization of appropriately substituted urea.

3.2.2.1 Carbonylation of substituted 1,2-Diaminobenzene

Carbonylation reaction of benzene-1,2-diamines is the most frequently used reaction for the synthesis of benzimidazol-2-ones and require the use of phosgene, triphosgene, or carbonyl diimidazole (CDI).[49-51] Although several carbonylating reagents are available and they are used in preparing variety of industrial chemicals, some of these reagents possess inherent toxicity and safe handling issues (Figure **3.5**).

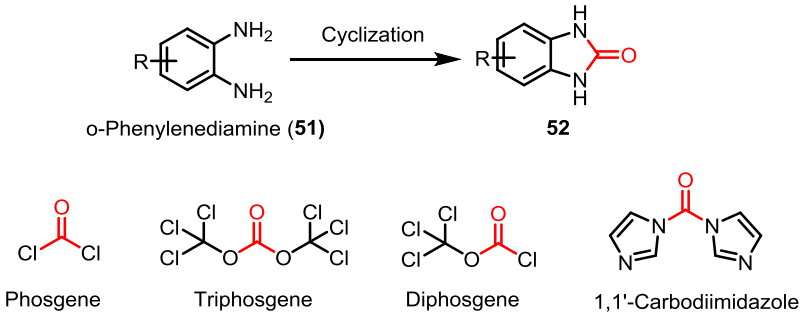


Figure 3.5: Synthesis of benzimidazol-2-one using carbonylation reagent

There are a few other methods available for making urea derivatives; selected methods are discussed following subsections.

3.2.2.2 Intramolecular or intermolecular cyclization of appropriately substituted urea

To avoid the use of hazardous chemicals listed in Figure 3.5, alternative procedures involving transition metal catalyzed cyclization were developed. McLaughlin and coworkers reported the synthesis of benzimidazol-2-one in excellent yields.[52] They have used chlorosulfonyl isocyanate **54** that converts amines into the corresponding primary ureas in excellent yields. In the case of **55b** due to the activated nature at the 2-position author conducted the cyclization reaction under acidic, basic and also in heating conditions failed to give the cyclized product. Later, this primary urea derivative which on palladium-catalyzed intramolecular cyclization furnished corresponding benzimidazol-2-one **56a** or imidazopyridinones **56b**. (Figure **3.6**).

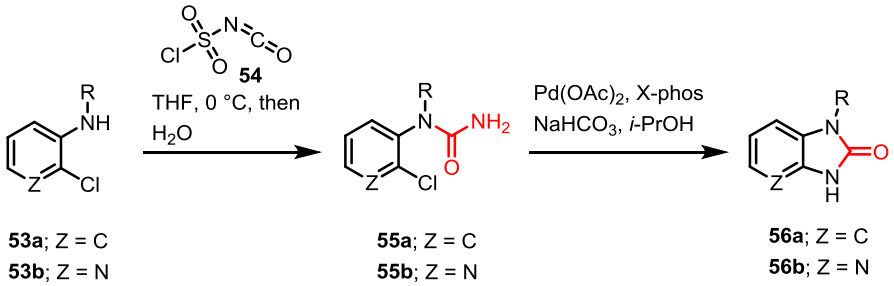


Figure 3.6: Palladium-catalyzed intramolecular cyclization

Dawai Ma and co-workers developed method for the coupling of 2-iodophenylcarbamates **57** and aqueous ammonia using CuI/L-proline as catalyst to afford corresponding aniline derivative at room temperature, which undergo in situ cyclization to give substituted 1,3-dihydrobenzimidazol-2-ones **58**. When NH_4Cl was used as an amine source instead of aqueous ammonia, relatively low yield was observed, and this approach has less substrate scope (Figure **3.7**).[53]

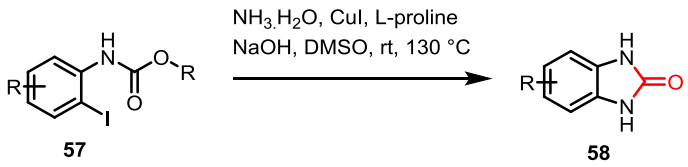


Figure 3.7: Copper-catalyzed amination followed by intramolecular cyclization

In 2015, Jipan Yu *et al.* published a paper which discussed the synthesis of *N,N*-disubstituted benzimidazol-2-one starting from *N,N'*-diarylurea **59** and $PhI(OAc)_2$ as oxidant without any external catalyst or ligand.[54] The *N,N'*-diaryl urea containing the electron releasing groups gave better yield in $PhI(OAc)_2$ mediated oxidative C-H amidation reaction, but this reaction failed to give product when R is hydrogen (Figure **3.8**).

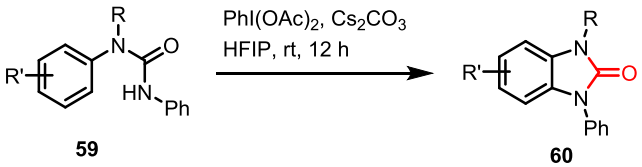


Figure 3.8: Oxidative C-H amidation followed by intramolecular cyclization

In continuation of the above, it is noteworthy to highlight the efforts by Bolm and co-workers for the development of a mild condition to access such heterocycles. In the presence of KOH and DMSO at 40 °C, intramolecular *N*-arylations of ureas to produced benzimidazol-2-ones was developed (Figure **3.9**).[55]

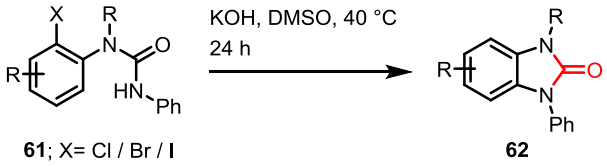


Figure 3.9: Synthesis of benzimidazole by KOH/DMSO mediated cyclization.

Jui and Buchwald together reported a direct regioselective construction of benzimidazol-2-one using one-pot palladium catalyzed sequential coupling of 1,2-dihaloaromatic **63** and monosubstituted urea **64**, but this protocol has less substrate scope (Figure **3.10**).[56]

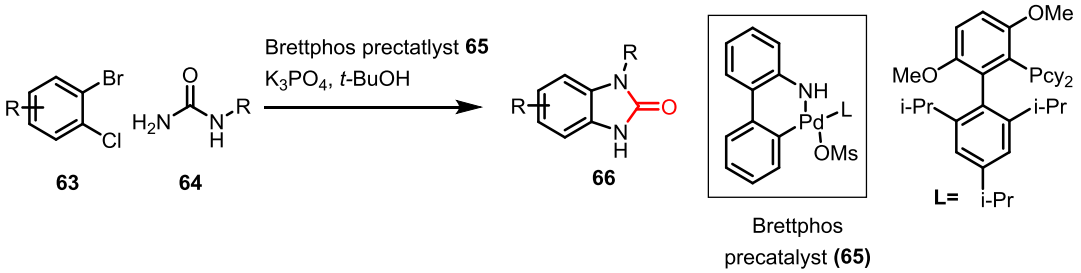
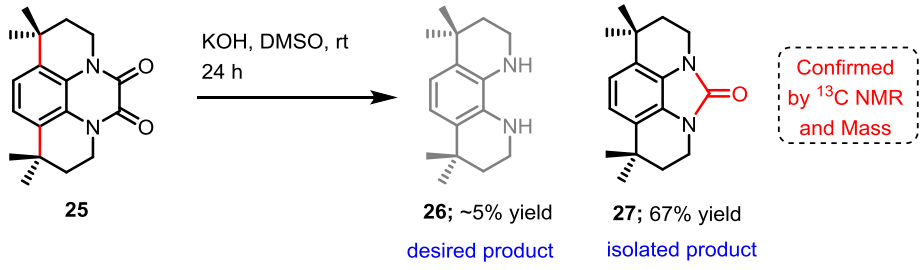


Figure 3.10: Palladium-catalyzed intermolecular cyclization.

3.2.3 Optimization of the reaction condition and substrate scope

As discussed in section 3.1, we were interested in the synthesis of tetracyclic *N*-heterocyclic carbenes (NHC) from the corresponding tetracyclic compound. we attempted hydrolysis of compound **25** using KOH in DMSO at room temperature to affords the corresponding diamine. Unexpectedly, a new spot formed was isolated from the reaction, which showed



Scheme 3.12: Unexpected formation of tetracyclic urea derivative **27**

similar ^{1}H NMR spectrum with ~0.3 ppm difference in all the signals with respect to starting dicarbonyl compound **25** (Scheme **3.12**). ^{13}C NMR spectrum showed difference in most of the peaks, to our surprise it also displayed one peak at δ 152.6 ppm for carbonyl carbon (Figure **3.11**). In addition the IR spectra of the product obtained showed signal at 1687 cm^{-1} thus confirming the presence of carbonyl group. After detailed analysis of all the spectral data, we confirmed the formation of tetracyclic benzimidazol-2-one **27**, assigned structure confirmed by HRMS showed peak at 271.1798 for the molecular formula $C_{17}H_{23}ON_2$ $[M+H]^+$ which corresponds to ring contracted product i.e. benzimidazol-2-one. Further the structure **27** was confirmed by comparing its spectral data with the compound synthesized by a different route.

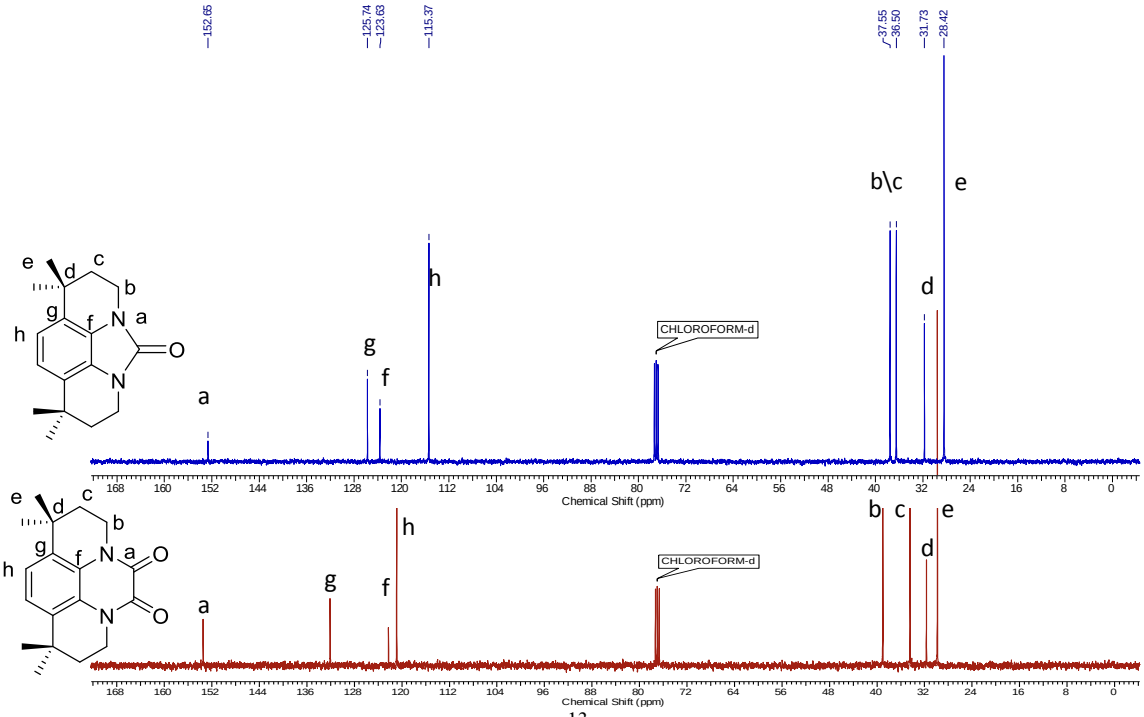
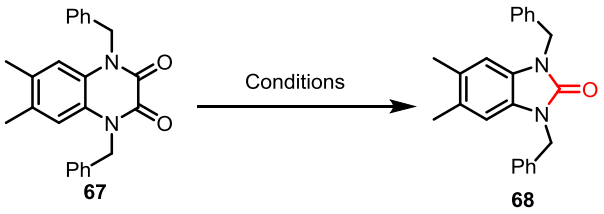


Figure 3.11: Comparision of ^{13}C NMR spectrum **25** and **26**

Here, we have established a mild method for decarbonylation of quinoxalinediones to form benzimidazol-2-ones using KOH in DMSO, based on our literature search this transformation has not been documented. Having made this interesting observation, compound **67** was chosen for further study and subjected to various conditions listed in Table **3.2**. Compound **67** which on hydrolysis followed by decarbonylative cyclisation in the presence of NaOH (2 eq.) gave poor conversion. Increasing the NaOH (5 eq.) equivalent does not affect greatly (entry 2). By switching to stronger base KOH (2 eq.) TLC showed better conversion; the desired product could be isolated. Excited with this observation, increasing

the equivalents of KOH (5 eq.) gave 57% of benzimidazol-2-one (entry 4). Finally, doubling the equivalent of KOH (10 eq) and stirring the reaction at room tempperature for 24 h gave



67 68

Entry	Base	Equivalent	Temp	Yield[a/b]
1	NaOH	2	rt	poor conversion [ab]
2	NaOH	5	rt	18%[ab]
3	KOH	2	rt	22%[ab]
4	KOH	5	rt	57%[ab]
5	KOH	5	60 °C	decomposed[c]
6	**KOH**	**10**	**rt**	**64%**[a]
7	KOH	10	rt	34%[bd]

[a] Reaction preformed on 50 mg scale in DMSO solvent, stirred for 24 h and Isolated yield;

[b] Starting material recovered; [c] Stirred for 12 h; [d] DMF solvent

Table 3.2: Optimization of decarbonylative ring contraction

product **68** in 64% yield (entry 6). The product **68** formed was primarily confirmed by TLC showed the new PMA active nonpolar spot, ^{1}H NMR displayed singlet at δ 6.69 (s, 2H) for two aromatic CH of benzimidazol-2-one, ^{13}C NMR showed a signal at δ 109.4 corresponds to the aromatic CH of the benzimidazol-2-one whereas in case of quinoxalinedione the aromatic CH appeared at δ 116.7 ppm. Further, it was confirmed by HRMS signal observed at 343.1800 corresponding to molecular formula $C_{23}H_{23}ON_2$ $[M+H]^+$ with calculated value 343.1805. In order to reduce reaction time and improve reaction yield we have carried reaction at elevated temperature (60 °C) which resulted in decomposition of starting material; increasing the temperature had an negative effect on the reaction (entry 5). Eventually, we arrived at the optimized condition for the decarbonylative ring conraction as 10 equivalents of KOH in DMSO and stirring at room temperature for 24 h. After the succesful optimization of the condition, we prepared several substituted quinoxalinediones substrates **69-89** by simple *N* alkylation reaction and the products formed were characterized with the help of spectroscopic technique (^{1}H, ^{13}C NMR, IR and HRMS) and it is found to be well in agreement with the structure drawn in table **3.3**. All the synthesized substrates were then submitted to the optimized condition (KOH, DMSO, rt, 24 h) and the results obtained along

with the yields of the products are summarized in Table **3.3**. Initially, we subjected different *N*-alkylated 6,7-dimethyl 1,4- dihydroquinoxaline-2,3-dione (compounds **70** - **74**) for the ring contraction and found that all of them are well tolerated and good yields of corresponding prodcuts obtained. To our surprise, the compound **69** and **81** failed to give desired product, this noticeably suggest that the tertiary amide is necessary for the reaction to proceed. It may be due to the fact that secondary amide exists in its tautomeric form, i.e. imidic acid, which probably hindered the hydrolysis step. Aromatic ring without any substitution gave a reasonable yield of products (compounds **76** and **24**). Methoxy substitution on the aromatic ring (compound **77** - **80**) was also tolerated giving a moderate yield of the product along with

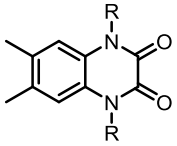
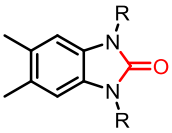
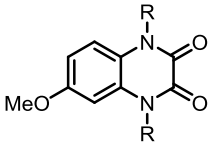
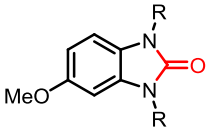
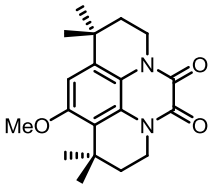
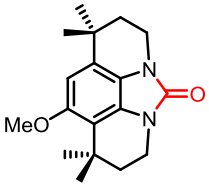
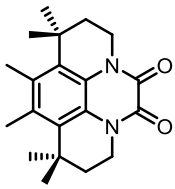
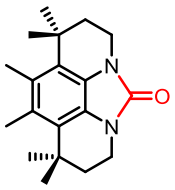
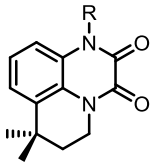
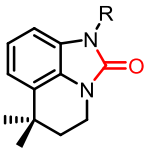
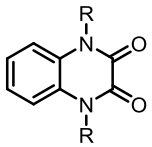
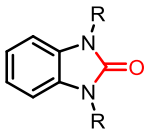
Substrate	Product	Yield	Substrate	Product	Yield
					
69; R= H	**69 a**; R= H	0%	**77**; R= Prenyl	**77 a**; R= Prenyl	43%
70; R= Methyl	**70 a**; R= Methyl	50%	**78**; R= Crotyl	**78 a**; R= Crotyl	36%
71; R= Ethyl	**71 a**; R= Ethyl	61%	**79**; R= Benzyl	**79 a**; R= Benzyl	55%
72; R= Allyl	**72 a**; R= Allyl	41%			46%
73; R= Prenyl	**73 a**; R= Prenyl	53%	**80**	**80 a**	
74; R= 3-Methylbutyl	**74 a**; R= 3-Methylbutyl	49%			
		62%			
75	**75 a**		**81**; R= H	**81 a**; R= H	0%
			82; R= Methyl	**82 a**; R= Methyl	57%
			83; R= Ethyl	**83 a**; R= Ethyl	62%
			84; R= Allyl	**84 a**; R= Allyl	53%
			85; R= Prenyl	**85 a**; R= Prenyl	59%
			86; R= Benzyl	**86 a**; R= Benzyl	64%
			87; R= 3-Methylbutyl	**87 a**; R= 3-Methylbutyl	68%
			88; R= 3-Butenyl	**88 a**; R= 3-Butenyl	58%
			89; R= *O*-Bromobenzyl	**89 a**; R= *O*-Bromobenzy	70%
					
76; R= Methyl	**76 a**; R= Methyl	55%			
24; R= Prenyl	**24 a**; R= Prenyl	45%			

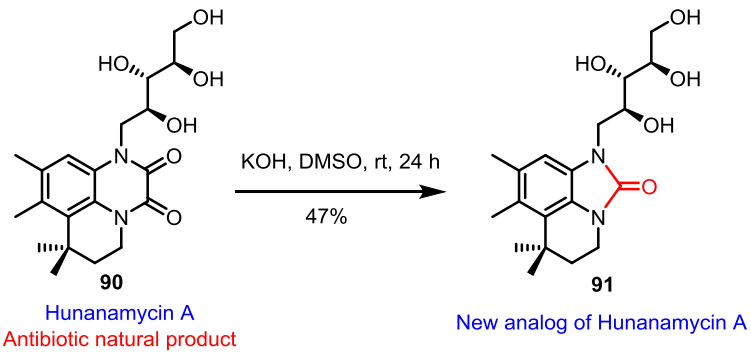
Table 3.3: Scope of the decarbonylative ring contraction to access benzimidazol-2-ones

the recovery of the starting material. No improvement in the yield observed by increasing the reaction time up to 36 h. Further, to increase the substrate scope, we have synthesized unsymmetrical *N*, *N*, di-substitution (**82 - 89**) by the base mediated *N* alkylation of the tricyclic quinoxaline-2,3-dione core which are part of our antibacterial research interest (discussed in section 2). All these compounds with unsymmetrical substitution were subjected to previously optimized condition and resulted in better yields of the desired products (compound **82a - 89a**). As these unsymmetric ring contracted product (**77a - 89a**) formed effortlessly, confirmed by ^{13}C NMR which showed only one signal for urea carbonyl group along with all other characteristic peaks. Almost in all the cases, we have observed the formation ~5-10% of corresponding dianiline (by the complete hydrolysis of the quinoxaline-2,3-dione).

3.2.4 Application of the decarbonalative ring contraction reaction

3.2.4.1 The synthesis of urea analogue of Hunanamycin A

As a direct application and to demonstrate the usefulness of the method developed, we decided to perform a late-stage transformation on natural product Hunanamycin A **90** which contains quinoxaline-2,3-dione core. Hunanamycin A was treated with KOH in DMSO at room temperature pleasingly, the reaction went smoothly to furnish benzimidazol-2-ones **91**, a new analog urea of Hunanamycin A in one step (scheme **3.13**). The compound **91** was confirmed by ^{1}H NMR peak at δ 6.95 (s, 1H) for the aromatic CH whereas in case of Hunanamycin A it was at δ 7.46 (s, 1H). Similarly, the signals in ^{13}C NMR also supported the formation of **91** with the appearance of one urea carbonyl at δ 155.4 along with the other characteristic peaks for the sugar unit and tricycle. It was further confirmed by HRMS, which showed a peak at 365.2068 corresponding to molecular formula $C_{19}H_{29}O_5N_2$ $[M+H]^+$. Thus, we have synthesized a close analogue **91** of natural product product Hunanamycin A using

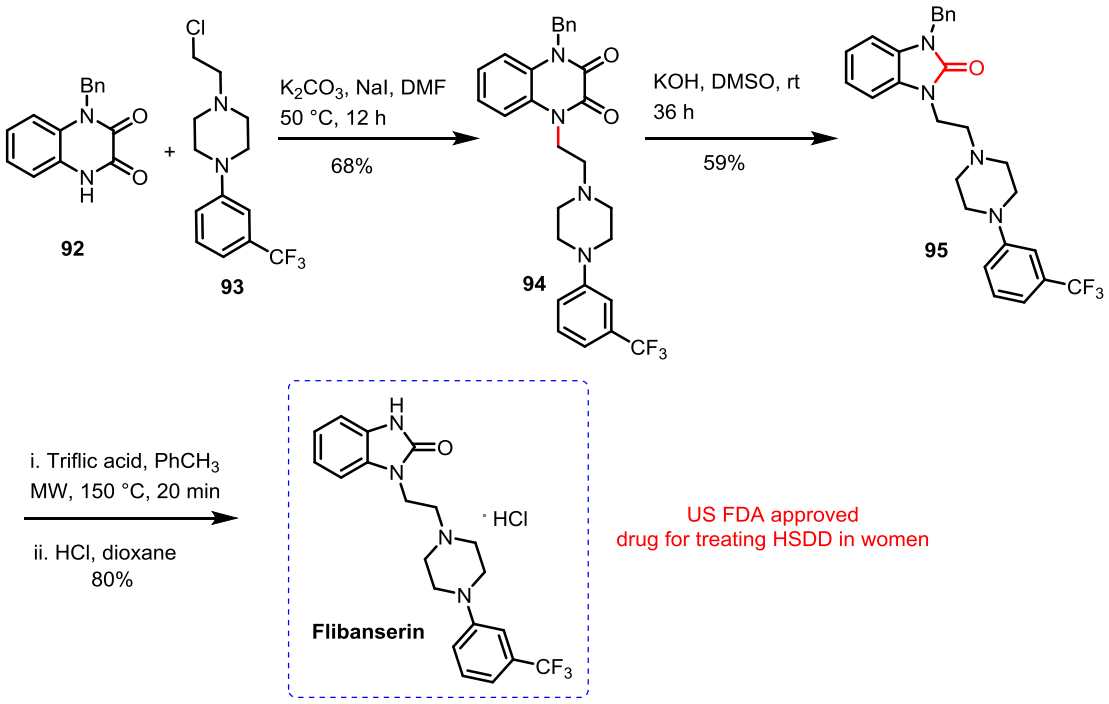


Scheme 3.13: Late-stage functionalization of Hunanamycin A

late-stage functionalization. This urea analogue of Hunanamycin A, showed MIC of 8 µg/mL against the *salmonella enterica* which is comparable to that of the Hunanamycin A.

3.2.4.2 New synthesis of Flibanserin drug

The above method was used for the synthesis of recently approved drug Flibanserin also popularly known as 'female viagra' for treatment of HSDD in women. The original preparation of flibanserin involves the harsh conditions, like the high temperature (200 °C) rearrangement it required special equipment and tedious operations.[57-58] Very recently, Jingshan Shen and group reported the five-step route for making flibanserin drug starting from *o*-phenylenediamine.[59] Our synthesis of flibanserin commenced with a benzyl protected quinoxalinedione **92**,[60] which was reacted with **93**[61] in presence of K_2CO_3 in DMF to give the desired product **94** in 68% yield. The product **94** was confirmed by ^1H NMR peak at δ 4.46 (t, J = 7.0 Hz, 2 H) for the methylene from the piperazine unit attached to the nitrogen of the amide. Similarly, the signals in ^{13}C NMR also supported the formation of **94** along with the all characteristic peaks for both coupling partner it showed the typical fluorine coupling pattern i.e. δ 124.2 (q, $^1J_{CF}$ = 272.8 Hz,) for the CF_3, δ 131.2 (q, $^2J_{CF}$ = 31.6 Hz) for the aromatic C attached to CF_3, δ 115.7 (q, $^3J_{CF}$ = 3.8 Hz) and δ 112.1 (q, $^3J_{CF}$ J = 3.8 Hz) value corresponds



Scheme 3.14: Synthesis of Flibanserin through ring contraction

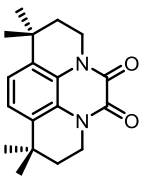
to two ortho C to the CF_3 attached carbon. It was further confirmed by HRMS, which showed a peak at 509.2166 corresponding to molecular formula $C_{28}H_{28}O_2N_4F_3[M+H]^+$ with calculated value 509.2159. Compound **94** was reacted using optimized condition (KOH, DMSO, rt, 36 h) to afford required product **95** in 59% yield. The formation of **95** was confirmed by ^{13}C NMR displayed a peak at δ 154.4 ppm corresponds to the urea carbonyl. It was further undoubtedly confirmed by HRMS, which showed a peak at 481.2215 corresponding to molecular formula $C_{27}H_{28}ON_4F_3[M+H]^+$ with calculated value 481.2210. In the end, triflic acid (TfOH) in toluene was used for benzyl group deprotection to afford flibanserin in better yield (Scheme **3.14**).[62] The product obtained was treated with 4M HCl in dioxane and isolated as HCl salt. The desired product obtained was confirmed by compairing the ^1H NMR with the reported data.

3.2.5 Conclusion

A new method for the synthesis of benzimidazol-2-ones starting from corresponding quinoxalinediones was developed under mild conditions. The present decarbonylative ring contraction method can be an addition to the other methods to access *N,N'* disubstitutated benzimidazol-2-ones. It will be useful for the functionalization of natural products, drug scaffolds, or intermediate containing quinoxaline-2,3-dione core. We have successfully synthesized a urea analog of antibiotic natural product Hunanamycin A using late-stage functionalization and also developed a novel bench-scale route for the synthesis of drug flibanserin.

3.2.6 Experimental procedure

1,1,10,10-tetramethyl-1,2,3,8,9,10-hexahydropyrazino[1,2,3,4-lmn][1,10]phenanthroline-5,6-dione (25)



A flame dried round bottom flask charged with 1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione **24** (200 mg, 0.671 mmol) and $AlCl_3$ (446 mg, 3.355 mmol) in chlorobenzene (10 mL) and stirred at room temperature for 4 h. The reaction was quenched by the addition of cold H_2O (20 mL). The reaction mixture was filtered through a pad of

Celite® and washed with ethyl acetate. The resulting filtrate was partitioned with ethyl acetate (3 X 100 mL) and water. The combined organic layers were dried over sodium sulphate, concentrated *in vacuo* and purified by flash column chromatography over silica gel (30% EtOAc:DCM) to afford compound **25** (150 mg, 75%) as brown solid.

Melting Point 285-288 °C;

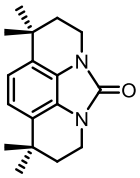
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 2 H), 4.17 (t, *J* = 6.1 Hz, 4 H), 1.88 (t, *J* = 6.1 Hz, 4 H), 1.34 (s, 12 H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 153.5, 132.1, 122.3, 120.9, 39.0, 34.4, 31.7, 29.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3010, 2966, 1683, 1498, 1399 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{18}H_{23}O_2N_2$ $[M+H]^+$ 299.1754 found 299.1753.

1,1,9,9-tetramethyl-1,2,3,7,8,9-hexahydro-5H-imidazo[1,5,4,3-lmn][1,10]phenanthrolin-5-one (27)



To a solution of 1,1,10,10-tetramethyl-1,2,3,8,9,10-hexahydropyrazino[1,2,3,4-lmn][1,10]phenanthroline-5,6-dione **25** (100 mg, 0.31 mmol) in dry DMSO (4 mL) was added powdered potassium hydroxide (172 mg, 3.1 mmol) and stirred under a positive pressure of argon for 24 h at room temperature. The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was washed water (2 X 10 mL), brine (1 X 10 mL) and dried over sodium sulphate, concentrated under reduced pressure. The crude product obtained was subjected to flash chromatography over silica gel to afford pure off white solid product **27** (57 mg, 67% yield)

Melting Point 185-189 °C;

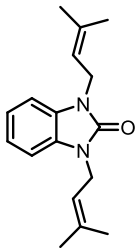
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 2 H), 3.90 (t, *J* = 6.1 Hz, 4 H), 1.90 (t, *J* = 5.8 Hz, 4 H), 1.34 (s, 12 H);

^{13}C NMR (101 MHz, $CDCl_3$) δ 152.6, 125.7, 123.6, 115.4, 37.5, 36.5, 31.7, 28.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2964, 1687, 1508, 1418, 1340 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{23}ON_2$ $[M+H]^+$ 271.1805 found 271.1800.

1,3-bis(3-methylbut-2-en-1-yl)-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one (29)



In an oven dried 100 mL side armed flask, a suspension of 60% sodium hydride (2.0 g, 50 mmol) in anhydrous DMF (10 mL) was cooled to 0 ^{o}C. The solution of 1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one **28** (2.68 g, 20 mmol) in anhydrous DMF (15 mL) was added dropwise under argon atmosphere. After vigorous stirring for 30 minutes at the same temperature the solution of 90% 3,3-dimethylallyl bromide (5.3 mL, 45 mmol) was added and the reaction mass was allowed to warm to room temperature. Following the completion of starting material (7 h) by TLC, Reaction was carefully quenched by drop wise addition of ice cold solution of ammonium chloride and extracted with ethyl acetate (3 x 30 mL). The organic layer was washed with brine (30 mL), dried over anhydrous sodium sulphate and concentrated. The residue obtained was purified by column chromatography using 20% EtOAc:PE as the eluent to get **29** (4.42 g, 82%) as white solid.

Melting Point 74−75 ^{o}C;

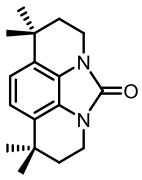
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (dd, *J* = 5.80, 3.3 Hz, 2 H), 6.94 (dd, *J* = 5.80, 3.3 Hz, 2 H), 5.27 (dd, *J* = 6.7 Hz, 2 H), 4.48 (d, *J* = 6.7 Hz, 4 H), 1.86 (s, 6 H), 1.73 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 136.2, 129.3, 120.9, 119.0, 107.9, 39.1, 25.6, 18.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 2926, 2852, 1670, 1632 cm^{-1};

HRMS (ESI) for $C_{17}H_{22}N_2O$; Calculated: 271.1810 $[M+H]^+$; Found: 271.1805.

1,1,9,9-tetramethyl-1,2,3,7,8,9-hexahydro-5*H*-imidazo[1,5,4,3 *lmn*] [1,10]phenanthrolin-5-one (27):



To the ice cold suspension of $AlCl_3$ (5.6 g, 42 mmol) in anhydrous chlorobenzene (35 mL), the solution of **29** (3.78 g, 14 mmol) in the same solvent (15 mL) was added under argon atmosphere. The wine-red solution was stirred at room temperature and monitored by TLC. After completion of the reaction (2 h), it was poured on ice cold water (20 mL) containing 1N HCl (10 mL). White emulsion was extracted with ethyl acetate (3 × 25 mL) and organic layer was washed with saturated aqueous $NaHCO_3$ (30 mL) followed by brine (30 mL). It was

dried over anhydrous sodium sulphate and concentrated to get crude product, which was purified by column chromatography using EtOAc: PE (1:3) as the eluent to result in **27** (2.8 g, 74%) as white solid.

Melting Point 197−198 ^{o}C;

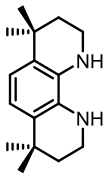
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 2 H), 3.90 (t, *J* = 5.8 Hz, 4 H), 1.90 (t, *J* = 5.8 Hz, 4 H), 1.34 (s, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 125.7, 123.6, 115.4, 37.6, 36.5, 31.8, 28.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 2948, 2922, 2856, 1712, 1628, 1134 cm^{-1};

HRMS (ESI) for $C_{17}H_{22}N_2O$; Calculated: 271.1810 $[M+H]^+$; Found: 271.1805.

4,4,7,7-tetramethyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (26):



The solution of **27** (2.7 g, 10 mmol) in anhydrous THF (30 mL) was dropwise added to the ice-cold suspension of 95% Lithium aluminium hydride (2.38 g, 50 mmol) in THF under argon atmosphere. The reaction mass was refluxed and monitored by TLC (2 h). It was cooled to 0 ^{o}C and quenched by cautious addition of cold water followed by 2N NaOH (30 mL). The white precipitate was filtered off and the filtrate and washings were dried over anhydrous sodium sulphate. The evaporation of solvent resulted in white solid which was redissolved in 5N HCl (30 mL) and refluxed for 2 h. After cooling to room temperature, the reaction mass was washed with diethyl ether (30 mL) and aqueous layer was basified with 1N NaOH up to pH 11. It was extracted with ethyl acetate (3 × 20 mL) and organic layer was washed with brine (30 mL). After drying over anhydrous sodium sulphate and evaporation of the solvent, the crude product obtained was purified by column chromatography using 25% EtOAc:PE as the eluent to get **26** (1.44 g, 59%) as light red solid.

Melting Point 136−137 ^{o}C;

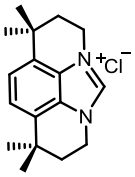
^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD) δ 6.85 (s, 2 H), 3.26 (t, *J* = 5.8 Hz, 4 H), 1.69 (t, *J* = 5.31 Hz, 4 H), 1.25 (s, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 132.3, 129.8, 117.1, 39.6, 38.9, 32.4, 31.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3409, 3348, 2958, 2922, 1608, 1568 cm^{-1};

HRMS (ESI) for $C_{16}H_{24}N_2$; Calculated: 245.2012 $[M+H]^+$; Found: 245.2006.

Preparation of benzimidazolium chloride (30):



The diamine **26** (1.22 g, 5.0 mmol) was dissolved in triethyl orthofomate (85 mL) and treated with conc. HCl (0.42 mL, 5.0 mmol). It was heated at 70 ^{o}C for 18 h under argon and for 2 h at the same temperature in air. The solution was cooled to room temperature and the salt was precipitated by addition of diethyl ether (50 mL). It was filtered through the Bückner funnel and dried under vacuum to yield **30** (1.25 g, 86%) as a white powder.

Melting Point > 295 ^{o}C;

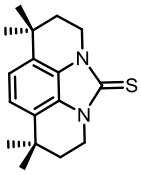
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.07 (s, 1 H) 7.36 (s, 2 H), 4.76 (t, *J* = 5.9 Hz, 4 H), 2.13 (t, *J* = 5.95 Hz, 4 H), 1.44 (s, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 131.6, 126.3, 121.2, 42.6, 37.3, 31.9, 28.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3323, 3242, 3115, 2953, 1641, 1623 cm^{-1};

HRMS (ESI) for $C_{17}H_{23}ClN_2$; Calculated: 255.1856 $[M\text{-}Cl]^+$; Found: 255.1853.

1,1,9,9-tetramethyl-1,2,3,7,8,9-hexahydro-5*H*-imidazo[1,5,4,3-*lmn*][1,10]phenanthroline-5-thione (32):



The benzimidazolium chloride **30** (58 mg, 0.2 mmol) and 60% sodium hydride (8.0 mg, 0.2 mmol) were suspended in anhydrous THF (6.0 mL) and stirred at room temperature for 2 h under inert atmosphere. During this time, the suspension becomes yellow-orange. To this the suspension of S_8 (51 mg, 0.2 mmol) in anhydrous THF (5.0 mL) was added drop wise to get almost a colorless mixture. After stirring at room temperature for additional 2 h, ice-cold water was added and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with brine (5.0 mL) and dried over anhydrous sodium sulphate. Evaporation of solvent, followed by column chromatographic purification of the crude product using EtOAc:PE (1:4) as the eluent yielded **32** (44 mg, 77%) as white solid.

Melting Point 236−237 ^{o}C;

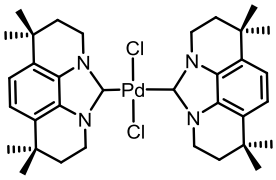
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (s, 2 H), 4.15 (t, *J* = 5.8 Hz, 4 H), 1.99 (t, *J* = 5.7 Hz, 4 H), 1.36 (s, 12 H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 127.2, 126.9, 117.1, 39.3, 37.7, 32.0, 28.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2963, 3298, 2922, 1608, 1568 cm^{-1};

HRMS (ESI) for $C_{17}H_{22}N_2S$; Calculated: 287.1576 $[M+H]^+$; Found: 287.1572.

Preparation of palladium complex (33):



NHC-Pd complex-I

The benzimidazolium chloride **30** (290 mg, 1 mmol) and $Pd(OAc)_2$ (112 mg, 0.5 mmol) were charged in an oven dried two neck round bottom flask equipped with reflux condensor. The whole assembly was flushed with argon and anhydrous THF (15 mL) was added to get white suspension. It was then heated at reflux for 1 h; during which color changes to pale gray. It was cooled to room temperature and volatiles were removed. The residue was re-dissolved in THF (10 mL) and the product was precipitated by addition of petroleum ether (40 mL). It was filtered through the Whatmann filter paper and the solid obtained was again dissolved in THF and the same procedure was repeated. The light gray solid thus obtained was dried under vacuum to get **33** (285 mg, 83%).

Melting Point 190 ^oC (with decomposition);

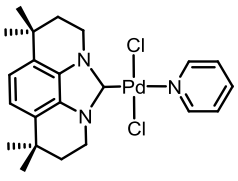
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 4 H), 4.93 (br.s, 4 H), 4.07 (br.s, 4 H), 2.09 (br.s, 4 H), 1.92 (br.s, 4 H), 1.40−1.30 (m, 24 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 129.2, 129.0, 118.1, 43.2 38.1, 31.9, 28.5, 28.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3014, 2974, 1596, 1475, 1403 cm^{-1};

Elemental analysis: $C_{34}H_{44}Cl_2N_4Pd$ requires: C, 59.52; H, 6.46; N, 8.17; found: C, 59.22; H, 6.17; N, 8.13 %.

Preparation of palladium complex (34):



NHC-Pd complex-II

The benzimidazolium chloride **30** (100 mg, 0.34 mmol), $PdCl_2$ (60 mg, 0.34 mmol) and potassium carbonate (190 mg, 1.37 mmol) in pyridine (2.0 mL) were charged in an oven dried microwave vial. It was then heated in microwave at 150 °C for 30 min. It was cooled to room temperature and the mixture was filtered through silica gel washing it with dichloromethane (3 mL). The volatiles were removed and the crude product was purified by column chromatography through a small pad of silica gel using dichloromethane as the eluent to yield **34** (140 mg, 80%) as a light yellow solid.

Melting Point 175 °C (with decomposition);

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (dd, *J* = 6.6, 1.7 Hz, 2 H), 7.81 (tt, *J* = 7.5, 1.4 Hz, 1 H), 7.40 (ddd, *J* = 7.5, 6.3, 1.4 Hz, 2 H), 7.04 (s, 2 H), 4.78 (t, *J* = 5.8, 4 H), 2.10 (t, *J* = 6.1, 4 H), 1.39 (s, 12 H);

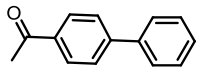
^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 151.2, 138.0, 129.6, 128.9, 124.5, 117.6, 42.9, 38.1, 31.9, 28.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3014, 2915, 1596, 1491, 1350 cm^{-1};

Elemental analysis $C_{22}H_{27}Cl_2N_3Pd$ requires: C, 51.73; H, 5.33; N, 8.23; found: C, 51.36; H, 5.04; N, 8.02 %.

Procedure for the conventional Suzuki-Miyaura cross-coupling reaction (A)

Synthesis of 1-(biphenyl-4-yl)ethanone (37):[63]

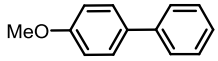


The 10 mL round bottom flask was charged with phenylboronic acid **36** (122 mg, 1 mmol), 4-bromoacetophenone **35** (199 mg, 1 mmol), K_3PO_4 (424 mg, 2 mmol) and palladium complex **33** (3.5 mg, 0.005 mmol) under argon. DMF (2.0 mL) was added to form the white suspension which was stirred vigorously at room temperature and monitored by TLC for the consumption of 4-bromoacetophenone. The reaction mass turned yellow-red suspension after 1 h. After completion of the reaction (2 h), ice cold water was added to the reaction mass and it was extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL) and dried over anhydrous sodium sulphate. The crude product was purified by column chromatography to yield **37** (185 mg, 94%) as white solid.

Melting Point 121−123 °C;

^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.05 (d, *J* = 8.4 Hz, 2 H), 7.57−7.69 (m, 4 H), 7.35−7.48 (m, 3H), 2.64 (s, 3H).

Synthesis of 4-methoxy-1,1'-biphenyl (38)[63]

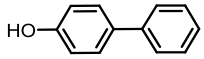


The compound **38** was synthesized by following conventional cross-coupling reaction (A)

Yield 85%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49−7.61 (m, 4 H), 7.37−7.48 (m, 2 H), 7.29−7.36 (m, 1 H), 6.98 (d, *J* = 8.0 Hz, 2 H), 3.86 (s, 3 H).

Synthesis of [1,1'-biphenyl]-4-ol (39)[63]

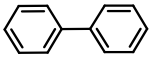


The compound **39** was synthesized by following conventional cross-coupling reaction (A)

Yield 68%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.29−7.61 (m, 7 H), 6.90 (d, *J* = 7.9 Hz, 2 H), 4.93 (s, 1 H).

Synthesis of 1,1'-biphenyl (40) [63]

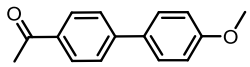


The compound **40** was synthesized by following conventional cross-coupling reaction (A)

Yield 75%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.1 Hz, 4 H), 7.30−7.52 (m, 6 H).

Synthesis of 1-(4'-methoxy-[1,1'-biphenyl]-4-yl)ethan-1-one (41) [63]

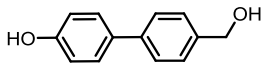


The compound **41** was synthesized by following conventional cross-coupling reaction (A)

Yield 90%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.4 Hz, 2 H), 7.54−7.71 (m, 4 H), 7.00 (d, *J* = 8.8 Hz, 2 H), 3.86 (s, 3 H), 2.63 (s, 3 H).

Synthesis of 4'-(hydroxymethyl)-[1,1'-biphenyl]-4-ol (42) [64]

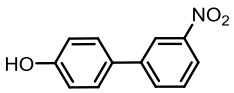


The compound **42** was synthesized by following conventional cross-coupling reaction (A)

Yield 64%;

^{1}H NMR (200 MHz, CD_3OD) δ 7.44−7.56 (m, 3 H), 7.33−7.44 (m, 3 H), 6.85 (dd, *J* = 6.8, 1.92 Hz, 2 H), 4.61 (s, 2 H).

Synthesis of 3'-nitro-[1,1'-biphenyl]-4-ol (43) [65]

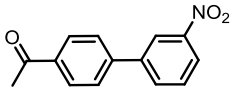


The compound **43** was synthesized by following conventional cross-coupling reaction (A)

Yield 59%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 8.15 (d, *J* = 7.7 Hz, 1 H), 7.87 (d, *J* = 7.5 Hz, 1 H), 7.41−7.68 (m, 3 H), 6.97 (d, *J* = 8.4 Hz, 2 H), 5.67 (br s, 1 H).

Synthesis of 1-(3'-nitro-[1,1'-biphenyl]-4-yl)ethan-1-one (44) [66]

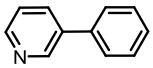


The compound **44** was synthesized by following conventional cross-coupling reaction (A)

Yield 86%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.50 (s, 1 H), 8.27 (d, *J* = 8.2 Hz, 1 H), 8.03−8.19 (m, 2 H), 7.97 (d, *J* = 7.7 Hz, 1 H), 7.59−7.81 (m, 3 H), 2.67 (s, 3 H).

Synthesis of 3-phenylpyridine (45) [67]



The compound **45** was synthesized by following conventional cross-coupling reaction (A)

Yield 65%;

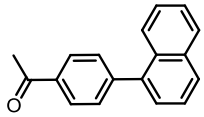
^{1}H NMR (200 MHz, $CDCl_3$) δ 8.86 (d, *J* = 4.7 Hz, 1 H), 8.55−8.68 (m, 1 H), 7.84−7.97 (m, 1 H), 7.35−7.64 (m, 6 H).

Procedure for Microwave Irradiation Experiments (B)

All the reactions involving microwave dielectric heating were performed with a microwave reactor (Anton Paar Monowave 300) under mono-mode irradiation in a 10-mL glass tube. The microwave heating was carried out at 100 °C for 5 minute to 30 minute depending on substrates. The reaction mixtures were stirred with a magnetic stir bar during the irradiation. During the course of reactions, the internal temperature was monitored through an IR sensor (standard infrared temperature sensor). The maximal internal pressure was monitored and maintained under the value of 300 psi using the provided software. At the end of the reaction, the tube was cooled to room temperature with air-compressed jet cooling. After completion

of the reaction, ice cold water was added to the reaction mass and it was extracted with ethyl acetate (2 × 10 mL). The organic layer was washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL) and dried over anhydrous sodium sulphate. The crude product was purified by column chromatography to obtain desired product.

Synthesis of 1-(4-(naphthalen-1-yl)phenyl)ethan-1-one (46) [68]

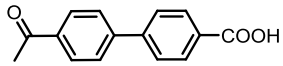


The compound **46** was synthesized by following general microwave irradiation procedure (B)

Yield 84%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.2 Hz, 2 H), 7.79−8.03 (m, 3 H), 7.41−7.63 (m, 6 H), 2.70 (s, 3 H).

Synthesis of 4'-acetyl-[1,1'-biphenyl]-4-carboxylic acid (47) [69]

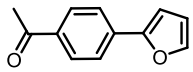


The compound **47** was synthesized by following general microwave irradiation procedure (B)

Yield 59%;

^{1}H NMR (200 MHz, DMSO-d_6) δ 8.00−8.10 (m, 4 H), 7.83−7.93 (m, 4 H), 2.63 (s, 3 H).

Synthesis of 1-(4-(furan-2-yl)phenyl)ethan-1-one (48)[70]

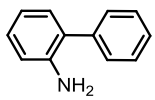


The compound **48** was synthesized by following general microwave irradiation procedure (B)

Yield 68%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 2 H), 7.74 (d, *J* = 8.3 Hz, 2 H), 7.53 (d, *J* = 1.6 Hz, 1 H), 6.80 (dd, *J* = 3.41, 0.63 Hz, 1 H), 6.52 (dd, *J* = 3.41, 1.77 Hz, 1 H), 2.61 (s, 3 H).

Synthesis of [1,1'-biphenyl]-2-amine (49) [71]

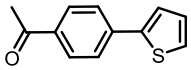


The compound **49** was synthesized by following general microwave irradiation procedure (B)

Yield 70%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42−7.51 (m, 4 H), 7.35−7.41 (m, 1 H), 7.10−7.24 (m, 2 H), 6.83 (dd, *J* = 8.0, 4.3 Hz, 2 H), 3.75 (br s, 2 H).

Synthesis of 1-(4-(thiophen-2-yl)phenyl)ethan-1-one (50) [67]

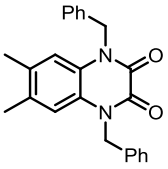


The compound **50** was synthesized by following general microwave irradiation procedure (B)

Yield 88%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.9 Hz, 2 H), 7.70 (d, *J* = 7.8 Hz, 2 H), 7.32−7.48 (m, 2 H), 7.05−7.18 (m, 1 H), 2.61 (s, 3 H).

1,4-dibenzyl-6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (67)



To a solution of 6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (300 mg, 1.577 mmol) in dry DMF (10 mL) was added potassium carbonate (654 mg, 4.736 mmol), followed by benzyl bromide (0.577 mL, 4.736 mmol) diluted in 2 mL of DMF and stirred for 12 h at room temperature. The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 25 mL). The combined organic layer was washed water (50 mL), brine (50 mL) and dried over sodium sulphate, concentrated under reduced pressure. The crude product obtained was subjected to flash chromatography over silica gel to afford pure product **67** (467 mg, 80% yield).

Melting Point 220-223 °C;

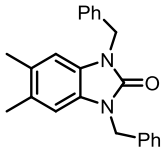
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 - 7.27 (m, 10 H), 6.97 (s, 2 H), 5.47 (s, 4 H), 2.15 (s, 6 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 154.7, 135.1, 132.8, 128.9, 127.6, 126.8, 124.5, 116.7, 46.9, 19.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1681, 1595, 1497 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{24}H_{23}O_2N_2$ $[M+H]^+$ 371.1754 found 371.1745;

1,3-dibenzyl-5,6-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (68)



To a solution of 1,4-dibenzyl-6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione **67** (80 mg, 0.325 mmol) in dry DMSO (2.5 mL) was added powdered potassium hydroxide (175 mg, 3.25 mmol) under a positive pressure of argon and stirred for 24 h at room temperature. The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was washed water (2 X 10 mL), brine (1 X 10 mL) and dried over sodium sulphate, concentrated under reduced pressure. The crude product obtained was subjected to flash chromatography over silica gel to afford pure product **68** (45 mg, 64%) as brown solid.

Melting Point 148-151 °C

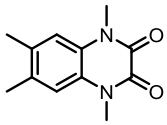
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.28 (m, 10 H), 6.69 (s, 2 H), 5.11 (s, 4 H), 2.21 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 136.5, 129.5, 128.7, 127.5, 127.4, 127.3, 109.4, 44.8, 19.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 2925, 1694, 1502, 1440, 1410 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{23}H_{23}ON_2$ $[M+H]^+$ 343.1805 found 343.1800;

1,4,6,7-tetramethyl-1,4-dihydroquinoxaline-2,3-dione (70)



The title compound was synthesized using similar procedure employed for **67**.

Yield 240 mg, 70%;

Melting Point 292-295 °C;

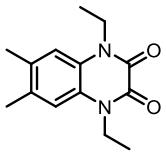
^{1}H NMR (200 MHz, $CDCl_3$) δ 6.99 (s, 2 H), 3.63 (s, 6 H), 2.33 (s, 6 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 154.3, 132.8, 125.1, 115.7, 30.0, 19.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1763, 1549, 1215 cm^{-1};

HRMS (ESI) *m/z* calculated for; $C_{12}H_{15}O_2N_2$ $[M+H]^+$ 219.1128 found 219.1126;

1,4-diethyl-6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (71)



The title compound was synthesized using similar procedure employed for **67**.

Yield 295 mg, 76%;

Melting Point 195-198 °C;

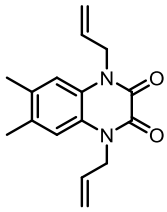
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 2 H), 4.25 (q, *J* = 7.1 Hz, 4 H), 2.34 (s, 6 H), 1.36 (t, *J* = 7.3 Hz, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.9, 132.7, 124.2, 115.8, 38.0, 19.6, 12.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3411, 1676, 1628, 1404, 772 cm^{-1};

HRMS (ESI) *m/z* calculated for; $C_{14}H_{19}O_2N_2$ $[M+H]^+$ 247.1441 found 247.1446.

1,4-diallyl-6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (72)



The title compound was synthesized using similar procedure employed for **67**.

Yield 319 mg, Yield 75%;

Melting Point 184-187 °C;

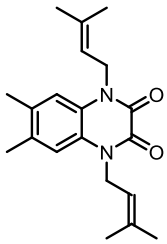
^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (s, 2 H), 6.11 - 5.75 (m, 2 H), 5.37 - 5.11 (m, 4 H), 4.84 (d, *J* = 5.1 Hz, 4 H), 2.29 (s, 6 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 153.9, 132.7, 130.5, 124.3, 118.0, 116.5, 45.3, 19.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1682, 1595, 1301 cm^{-1};

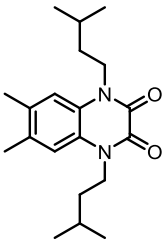
HRMS (ESI) *m/z* calculated for $C_{16}H_{19}O_2N_2$ $[M+H]^+$ 271.1441 found 271.1441.

6,7-dimethyl-1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione(73)



The title compound was synthesized using similar procedure employed for **67**.

1,4-diisopentyl-6,7-dimethyl-1,4-dihydroquinoxaline-2,3-dione (74)



6,7-dimethyl-1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione **73** (120 mg, 0.368 mmol) was taken in ethanol (5 mL) and 10% palladium on carbon (20 mg) was added. The reaction mixture was stirred at room temperature for 12 h. After all the starting material was consumed, filter the reaction mixture through celite pad wash with more ethanol (30 mL). The filtrate was then concentrated to obtain the crude product was then purified by column chromatography to afford pure compound **74** as a white sticky solid product

Yield 108 mg, 88%;

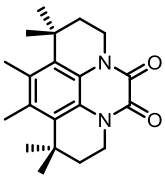
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 2 H), 4.15 (t, *J* = 7.9 Hz, 4 H), 2.31 (s, 6 H), 1.73 (sep, *J* = 6.7 Hz, 2 H), 1.59 (q, *J* = 7.3 Hz, 4 H), 1.00 (d, *J* = 6.1 Hz, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 132.4, 124.3, 115.8, 41.4, 35.3, 26.2, 22.3, 19.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2960, 2931, 1685, 1504, 1413 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{31}O_2N_2$ $[M+H]^+$ 331.2380 found 331.2377

1,1,10,10,11,12-hexamethyl-1,2,3,8,9,10-hexahydropyrazino[1,2,3,4-lmn][1,10]phenant--hroline-5,6-dione (75)



A flame dried round bottom flask charged with 6,7-dimethyl-1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione **73** (200 mg, 0.6134 mmol) and $AlCl_3$ (326 mg, 2.4939 mmol) in dichloroethane (10 mL) and stirred at room temperature for 1 h. The reaction was quenched by the addition of cold H_2O (20 mL). The reaction mixture was filtered through a pad of Celite® and washed with ethyl acetate. The resulting filtrate was partitioned with ethyl acetate (3 X 30 mL) and water. The combined organic layers were dried over Na_2SO_4, concentrated *in vacuo* and purified by flash column chromatography over silica gel (30% EtOAc:DCM) to afford compound **75** (180 mg, 90%) as brown solid.

Melting Point 224-227 °C

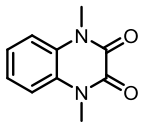
^{1}H NMR (200 MHz, $CDCl_3$) δ 4.17 (t, *J* = 5.9 Hz, 4 H), 2.41 (s, 6 H), 1.87 (t, *J* = 5.9 Hz, 4 H), 1.53 (s, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 133.4, 130.7, 122.2, 39.9, 38.2, 33.1, 29.0, 20.0

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3000, 2967, 1735, 1470, 1395 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{27}O_2N_2$ $[M+H]^+$ 327.2067, found 327.2069.

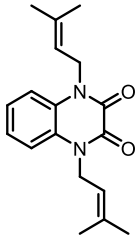
Synthesis of 1,4-dimethyl-1,4-dihydroquinoxaline-2,3-dione (76)



The title compound was synthesized using similar procedure employed for **67**.

Spectral data of the compound **19** was compared with the literature reported values and it is in well agreement.

1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione (24)



The title compound was synthesized using similar procedure employed for **67**.

Yield 380 mg, 68%;

Melting Point 143-147 °C;

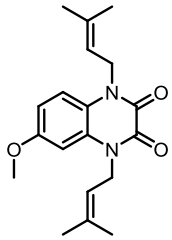
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 - 7.19 (m, 4 H), 5.23 - 5.10 (m, 2 H), 4.84 (d, *J* = 6.1 Hz, 4 H), 1.88 (s, 6 H), 1.73 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 137.4, 126.8, 123.9, 117.8, 115.4, 41.6, 25.6, 18.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3178, 2855, 1678, 1377, 1223 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{26}O_2N_2$ $[M+Na]^+$ 299.1754, found 299.1759.

6-methoxy-1,4-bis(3-methylbut-2-en-1-yl)-1,4-dihydroquinoxaline-2,3-dione (77)



The title compound was synthesized using similar procedure employed for **67**.

Yield 381 mg, 74%;

Melting Point 122-125 °C;

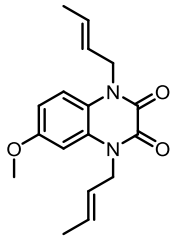
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 9.2 Hz, 1 H), 6.81 - 6.76 (d, *J* = 8.5 Hz, 1 H), 6.75 (s, 1 H), 5.15 (d, *J* = 6.1 Hz, 2 H), 4.80 (d, *J* = 6.1 Hz, 4 H), 3.83 (s, 3 H), 1.87 (s, 3 H), 1.86 (s, 3 H), 1.72 (br. s., 3 H), 1.72 (br. s., 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 154.4, 153.4, 137.3, 137.2, 127.8, 120.6, 117.9, 117.8, 116.1, 108.8, 101.8, 55.6, 41.7, 41.6, 25.6, 18.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3013, 1682, 1616, 1597, 1469 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{25}O_3N_2$ $[M+H]^+$ 329.1860 found 329.1859.

1,4-di((E)-but-2-en-1-yl)-6-methoxy-1,4-dihydroquinoxaline-2,3-dione (78)



The title compound was synthesized using similar procedure employed for **67**.

Yield 300 mg, 64%;

Melting Point 76-78 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, *J* = 9.2 Hz, 1 H), 6.84 - 6.63 (m, 2 H), 5.73 (dt, *J* = 6.7, 14.0 Hz, 2 H), 5.50 (d, *J* = 15.3 Hz, 2 H), 4.83 - 4.71 (m, 4 H), 3.80 (s, 3 H), 1.84-1.65 (br. s., 6 H);

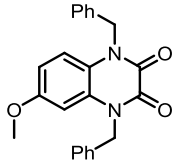
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 154.2, 153.2, 130.1, 129.9, 128.8, 127.6, 123.2, 123.1, 116.2, 108.7, 101.9, 55.6, 44.9, 44.7, 17.6, 17.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3014, 1683, 1617, 1596, 1517, 1215 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{21}O_3N_2$ $[M+H]^+$ 301.1547 found 301.1547.

Note: 1H NMR shows 1:4 Mixture of the cis-trans mixture.

1,4-dibenzyl-6-methoxy-1,4-dihydroquinoxaline-2,3-dione (79)



The title compound was synthesized using similar procedure employed for **67**.

Yield 360 mg, 62%;

Melting Point 216-219 °C;

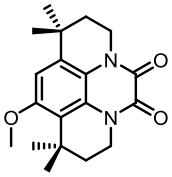
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 - 7.20 (m, 10 H), 7.09 (d, *J* = 8.5 Hz, 1 H), 6.74 (s, 1 H), 6.62 (d, *J* = 8.5 Hz, 1 H), 5.46 (br. s., 4 H), 3.66 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 155.0, 154.0, 134.9, 134.8, 129.0, 128.9, 127.8, 127.8, 127.7, 126.9, 126.8, 120.5, 116.7, 109.2, 102.3, 55.5, 47.2, 47.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 1680, 1616, 1593, 1495 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{23}H_{21}O_3N_2$ $[M+H]^+$ 373.1547 found 373.1547.

11-methoxy-1,1,10,10-tetramethyl-1,2,3,8,9,10-hexahydropyrazino[1,2,3,4-lmn][1,10]phenanthroline-5,6-dione (80)



The title compound was synthesized from **77** using similar procedure employed for **75**.

Yield 91 mg, 82%;

Melting Point 215-218 °C;

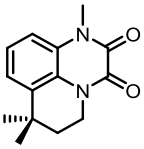
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (s, 1 H), 4.23 - 4.07 (m, 4 H), 3.88 (s, 3 H), 1.89 (t, *J* = 6.1 Hz, 2 H), 1.84 (t, *J* = 6.1 Hz, 2 H), 1.44 (s, 6 H), 1.37 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 153.6, 152.8, 132.6, 124.4, 120.1, 116.8, 105.1, 55.6, 38.9, 38.1, 38.0, 34.6, 32.3, 32.1, 29.9, 27.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3016, 2962, 1675, 1610, 1464 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{25}O_3N_2$ $[M+H]^+$ 329.1860 found 329.1860.

1,7,7-trimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (82)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 120 mg, 75%;

Melting Point: 148-151 °C;

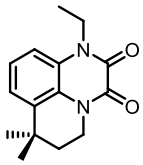
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 - 7.16 (m, 2 H), 7.07 (d, *J* = 7.3 Hz, 1 H), 4.12 (t, *J* = 6.1 Hz, 2 H), 3.61 (s, 3 H), 1.87 (t, *J* = 6.1 Hz, 2 H), 1.33 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.9, 153.5, 134.4, 127.1, 123.7, 122.2, 121.2, 112.6, 38.8, 34.2, 31.7, 30.2, 29.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 2970, 1679, 1593, 1446, 1399 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{14}H_{17}O_2N_2$ $[M+H]^+$ 245.1285 found 245.1285.

1-ethyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (83)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 132 mg, 59%);

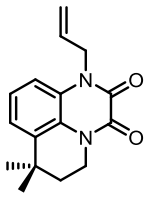
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31 - 7.19 (m, 2 H), 7.19 - 7.07 (m, 1 H), 4.29 (q, *J* = 7.2 Hz, 2 H), 4.18 (t, *J* = 6.3 Hz, 2 H), 1.92 (t, *J* = 6.4 Hz, 2 H), 1.44 - 1.29 (m, 9 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 153.7, 153.4, 134.8, 125.9, 123.7, 122.6, 121.0, 112.5, 38.8, 38.2, 34.3, 31.8, 29.8, 12.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3013, 2967, 1681, 1593, 1393 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{19}O_2N_2$ $[M+H]^+$ 259.1441 found 259.1441;

1-allyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (84)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 145 mg, 62%;

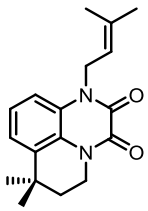
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 - 7.22 (m, 1 H), 7.18 (t, *J* = 7.9 Hz, 1 H), 7.13 - 7.07 (m, 1 H), 5.92 (tdd, *J* = 5.1, 10.4, 17.3 Hz, 1 H), 5.36 - 5.17 (m, 2 H), 4.95 - 4.79 (m, 2 H), 4.27 - 4.08 (m, 2 H), 1.93 (t, *J* = 6.27Hz, 2 H), 1.39 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 153.76, 134.8, 130.6, 126.4, 123.7, 122.6, 121.3, 118.2, 113.4, 45.6, 39.1, 34.4, 32.0, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3405, 3003, 1680, 1605, 1449, 1229 cm^{-1};

HRMS (ESI) *m/z* calculated for C16H18N2O2 $[M+Na]^+$ 293.1260, found 293.1258 cm^{-1}.

7,7-dimethyl-1-(3-methylbut-2-en-1-yl)-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (85)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 155 mg, 60%;

Melting Point 90-93 °C;

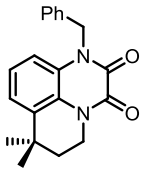
^{1}H NMR (400MHz , $CDCl_3$) δ 7.26 - 7.16 (m, 2 H), 7.06 (d, *J* = 7.9 Hz, 1 H), 5.15 (br. s., 1 H), 4.83 (d, *J* = 6.1 Hz, 2 H), 5.15 (br. s., 1 H), 4.83 (d, *J* = 6.1 Hz, 2 H), 4.16 (t, *J* = 5.5 Hz, 2 H), 1.90 (t, *J* = 5.8 Hz, 2 H), 1.85 (s, 3 H), 1.70 (s, 3 H), 1.36 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 153.7, 137.2, 134.7, 126.4, 123.7, 122.6, 121.1, 117.9, 113.1, 41.7, 38.9, 34.4, 31.9, 29.8, 25.5, 18.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3008, 2966, 1684, 1593, 1464, 1393 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{18}H_{23}O_2N_2$ $[M+H]^+$ 299.1754 found 299.1753;

1-benzyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione(86)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 178 mg, 64%;

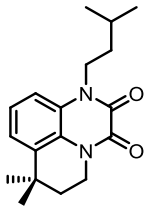
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.38 - 7.17 (m, 6 H), 7.10 - 7.04 (m, 2 H), 5.47 (s, 2 H), 4.23 (t, *J* = 6.1 Hz, 2 H), 1.94 (t, *J* = 6.1 Hz, 2 H), 1.38 (s, 6 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 154.4, 153.8, 135.0, 134.7, 128.9, 127.6, 126.8, 126.5, 123.8, 122.6, 121.4, 113.6, 47.1, 39.1, 34.4, 31.9, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3010, 2964, 1660, 1609, 1483, 1292 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{20}H_{21}O_2N_2$ $[M+H]^+$ 321.1598 found 321.1597.

1-isopentyl-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione(87)



The title compound was synthesized from **84** using similar procedure employed for **74**.

Yield 130 mg, 86%;

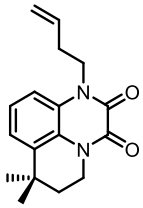
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 2 H), 7.07 (d, *J* = 7.3 Hz, 1 H), 4.19 (t, *J* = 7.9 Hz, 2 H), 4.14 (t, *J* = 6.1 Hz, 2 H), 1.89 (t, *J* = 6.1 Hz, 2 H), 1.74 (sep, *J* = 6.7 Hz 1 H), 1.35 (s, 6 H), 0.98 (d, *J* = 6.7 Hz, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6, 153.6, 134.8, 126.2, 123.7, 122.6, 121.0, 112.6, 41.7, 38.9, 35.3, 34.3, 31.9, 29.8, 26.3, 22.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3017, 2961, 2926, 1680, 1594, 1464 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{18}H_{25}O_2N_2$ $[M+H]^+$ 301.1911 found 301.1911;

1-(but-3-en-1-yl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione (88)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 145 mg, 59%;

Melting Point 141-144 °C;

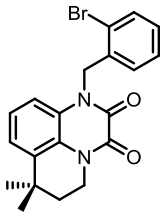
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 - 7.19 (m, 2 H), 7.10 (d, *J* = 7.9 Hz, 1 H), 5.89 (tdd, *J* = 6.8, 10.2, 17.1 Hz, 1 H), 5.17- 5.10 (m, 2 H), 4.29 (t, *J* = 6.1 Hz, 2 H), 4.18 (t, *J* = 6.1 Hz, 2 H), 2.52 (q, *J* = 7.3 Hz, 2 H), 1.92 (d, *J* = 6.7 Hz, 2 H), 1.39 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 153.7, 135.0, 133.9, 126.3, 123.8, 122.8, 121.2, 117.6, 112.7, 42.5, 39.0, 34.4, 32.0, 31.1, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3015, 2965, 2924, 1680, 1594 cm^{-1}.

HRMS (ESI) *m/z* calculated for; $C_{17}H_{21}O_2N_2$ $[M+H]^+$ 285.1598 found 285.1595;

1-(2-bromobenzyl)-7,7-dimethyl-6,7-dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-dione(89)



The title compound was synthesized from **81** using similar procedure employed for **67**.

Yield 188 mg, 54%;

Melting Point 191-194 °C;

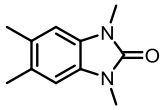
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.3 Hz, 1 H), 7.22 (d, *J* = 7.9 Hz, 1 H), 7.19 - 7.10 (m, 2 H), 7.07 (t, *J* = 7.9 Hz, 1 H), 6.87 (d, *J* = 6.7 Hz, 1 H), 6.77 (d, *J* = 7.9 Hz, 1 H), 5.48 (s, 2 H), 4.23 (t, *J* = 5.5 Hz, 2 H), 1.95 (t, *J* = 5.5 Hz, 2 H), 1.38 (s, 6 H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 153.6, 134.8, 133.4, 133.0, 129.1, 127.9, 127.0, 126.1, 124.0, 122.6, 122.3, 121.6, 113.6, 47.4, 39.1, 34.3, 32.0, 29.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2966, 1660, 1610, 1444 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{20}H_{20}O_2N_2$ Br$[M+H]^+$ 399.0703 found 399.0698;

1,3,5,6-tetramethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (70a)



The title compound was synthesized from **70** using similar procedure employed for **68**.

Yield 43 mg, 50%;

Melting Point 150-153 °C;

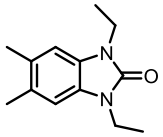
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 2 H), 3.38 (s, 6 H), 2.31 (s, 7 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 129.2, 128.1, 108.5, 27.1, 19.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2926, 1687, 1510, 1467 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{11}H_{15}ON_2$ $[M+H]^+$ 191.1179 found 191.1180;

1,3-diethyl-5,6-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (71a)



The title compound was synthesized from **71** using similar procedure employed for **68**.

Yield 48 mg, 61%;

Melting Point 89-92 °C;

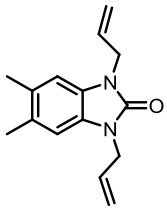
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 2 H), 3.91 (q, *J* = 7.3 Hz, 4 H), 2.32 (s, 6 H), 1.33 (t, *J* = 7.3 Hz, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6, 128.9, 127.2, 108.7, 35.6, 19.9, 13.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3008, 1687, 1504, 1414 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{13}H_{19}ON_2$ $[M+H]^+$ 219.1492 found 219.1492.

1,3-diallyl-5,6-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (72a)



The title compound was synthesized from **72** using similar procedure employed for **68**.
Yield 29 mg, 41%;

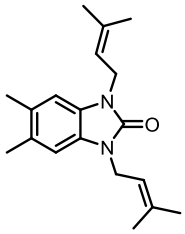
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (s, 2 H), 5.99 - 5.84 (m, 2 H), 5.35 - 5.14 (m, 4 H), 4.50 (d, *J* = 5.5 Hz, 4 H), 2.29 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 132.2, 129.3, 127.4, 117.2, 109.4, 43.4, 19.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2924, 1704, 1604, 1502, 1407 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{15}H_{19}ON_2$ $[M+H]^+$ 243.1492 found 243.1493.

5,6-dimethyl-1,3-bis(3-methylbut-2-en-1-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (73a)



The title compound was synthesized from **73** using similar procedure employed for **68**.
Yield 48 mg, 53 %;

Melting Point 136-139 °C;

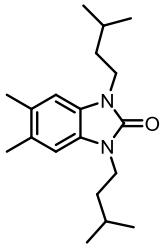
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 2 H), 5.16 (t, *J* = 6.1 Hz, 2 H), 4.80 (d, *J* = 6.4 Hz, 4 H), 2.30 (s, 6 H), 1.88 (s, 6 H), 1.72 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 137.2, 132.4, 124.5, 117.9, 116.3, 41.4, 25.6, 19.7, 18.3;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2921, 1678, 1593, 1516, 1446 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{27}ON_2$ $[M+H]^+$ 299.2118 found 299.1753.

1,3-diisopentyl-5,6-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (74a)



The title compound was synthesized from **74** using similar procedure employed for **68**.

Yield 34 mg, 49 %;

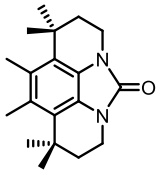
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (s, 2 H), 3.86 (t, *J* = 7.3 Hz, 4 H), 2.32 (s, 6 H), 1.71 - 1.58 (m, 6 H), 0.99 (d, *J* = 6.1 Hz, 12 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 128.9, 127.5, 108.8, 39.4, 37.1, 25.8, 22.4, 19.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3015, 2961, 2930, 1677, 1593, 1466 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{31}ON_2$ $[M+H]^+$ 303.2431 found 303.2432.

Synthesis of 1,1,9,9,10,11-hexamethyl-1,2,3,7,8,9-hexahydro-5H-imidazo[1,5,4,3-lmn][1,10]phenanthrolin-5-one (75a)



The title compound was synthesized from **75a** using similar procedure employed for **68**.

Yield 110 mg, 62%;

Melting Point 198-201 °C;

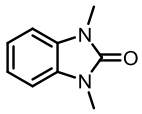
^{1}H NMR (200 MHz, $CDCl_3$) δ 3.81 (t, *J* = 5.8 Hz, 4 H), 2.36 (s, 6 H), 1.92 (t, *J* = 5.8 Hz, 4 H), 1.46 (s, 12 H);

^{13}C NMR (50 MHz, $CDCl_3$) δ 151.9, 127.3, 124.2, 122.6, 41.0, 35.6, 33.2, 28.2, 17.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2999, 1681, 1496, 1422 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{19}H_{27}ON_2$ $[M+H]^+$ 299.2118 found 299.2117.

1,3-dimethyl-1,3-dihydro-2H-benzo[d]imidazol-2-one (76a)



The title compound was synthesized from **76** using similar procedure employed for **68**.

Yield 38 mg, 55%;

Melting Point 108-111 °C

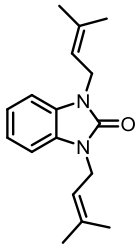
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 - 7.06 (m, 2 H), 7.06 - 6.90 (m, 2 H), 3.55 - 3.32 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 130.0, 121.2, 107.3, 27.1;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2939, 1697, 1502, 1324 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_9H_{11}ON_2$ $[M+H]^+$ 163.0866 found 163.0868.

Synthesis of 1,3-bis(3-methylbut-2-en-1-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (24a)



The title compound was synthesized from **24** using similar procedure employed for **68**.

Yield 41 mg, 45%.

Melting Point 136-139 °C;

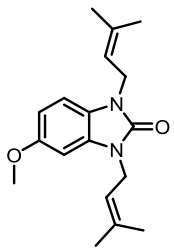
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 - 7.03 (m, 2 H), 6.98 - 6.91 (m, 2 H), 5.28 (t, *J* = 6.1 Hz, 2 H), 4.50 (d, *J* = 6.1 Hz, 4 H), 1.86 (s, 6 H), 1.73 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 136.2, 129.3, 120.9, 119.0, 107.9, 39.1, 25.6, 18.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3090, 1666, 1593, 1508, 1312 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{17}H_{23}ON_2$ $[M+H]^+$ 271.1805 found 271.1808;

5-methoxy-1,3-bis(3-methylbut-2-en-1-yl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (77a)



The title compound was synthesized from **77** using similar procedure employed for **68**.

Yield 40 mg, 43%;

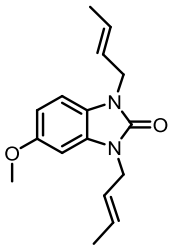
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (d, *J* = 8.5 Hz, 1 H), 6.61 (dd, *J* = 1.8, 8.5 Hz, 1 H), 6.56 (d, *J* = 2.4 Hz, 1 H), 5.26 (t, *J* = 6.1 Hz, 2 H), 4.46 (d, *J* = 6.7 Hz, 4 H), 3.81 (s, 3 H), 1.86 (s, 3 H), 1.84 (s, 3 H), 1.73 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.2, 154.1, 136.3, 136.1, 130.2, 123.6, 119.1, 118.9, 108.0, 105.8, 95.6, 55.9, 39.1, 25.6, 18.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2634, 1691, 1610, 1500, 1410 cm^{-1}.

HRMS (ESI): *m/z* calculated for $C_{18}H_{25}O_2N_2$ $[M+H]^+$ 301.1911 found 301.1911;

1,3-di((E)-but-2-en-1-yl)-5-methoxy-1,3-dihydro-2H-benzo[d]imidazol-2-one (78a)



The title compound was synthesized from **78** using similar procedure employed for **68**.

Yield 33 mg, 36%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 6.92 - 6.77 (m, 1 H), 6.67 - 6.49 (m, 2 H), 5.82 - 5.62 (m, 2 H), 5.61 - 5.43 (m, 2 H), 4.40 (d, *J* = 5.7 Hz, 4 H), 3.80 (s, 3 H), 1.68 (d, *J* = 6.2 Hz, 6 H);

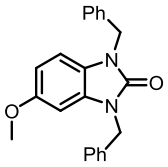
^{13}C NMR (50 MHz, $CDCl_3$) δ 155.1, 153.9, 130.2, 129.0, 128.9, 125.0, 124.8, 123.5, 108.2, 105.8, 95.7, 55.8, 42.8, 37.8, 17.5, 13.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1692, 1609, 1500, 1464 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{16}H_{21}O_2N_2$ $[M+H]^+$ 273.1598 found 273.1598;

Note: ^{1}H NMR shows 1:4 Mixture of the cis-trans mixture.

1,3-dibenzyl-5-methoxy-1,3-dihydro-2H-benzo[d]imidazol-2-one (79a)



The title compound was synthesized from **79** using similar procedure employed for **68**.

Yield 51 mg, 55%;

Melting Point 122-125 °C;

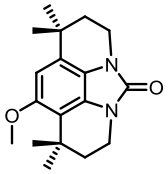
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.23 (m, 10 H), 6.75 (d, *J* = 8.5 Hz, 1 H), 6.53 (dd, *J* = 1.8, 8.5 Hz, 1 H), 6.51 - 6.47 (m, 1 H), 5.10 (s, 4 H), 3.71 (s, 3 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 154.8, 136.4, 136.2, 130.2, 128.7, 128.7, 127.7, 127.6, 127.4, 127.4, 123.4, 108.5, 106.1, 96.0, 55.8, 45.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2930, 1699, 1633, 1500, 1410 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{22}H_{21}O_2N_2$ $[M+H]^+$ 345.1598 found 345.1599;

10-methoxy-1,1,9,9-tetramethyl-1,2,3,7,8,9-hexahydro-5H-imidazo[1,5,4,3-lmn][1,10]phenanthrolin-5-one (80a)



The title compound was synthesized from **80** using similar procedure employed for **68**.

Yield 30 mg, 46%;

Melting Point 142-145 °C;

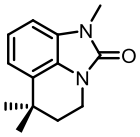
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.46 (s, 1 H), 3.86 (t, *J* = 6.1 Hz, 2 H), 3.83 (s, 3 H), 3.80 (t, *J* = 6.1 Hz, 2 H), 1.89 (br. s., 4 H), 1.41 (s, 6 H), 1.33 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 152.9, 125.8, 125.1, 118.4, 113.9, 100.3, 56.6, 39.3, 37.7, 36.4, 36.2, 32.2, 31.8, 28.4, 27.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2962, 1688, 1504, 1418 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{18}H_{25}O_2N_2$ $[M+H]^+$ 301.1911 found 301.1912;

1,6,6-trimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (82a)



The title compound was synthesized from **82** using similar procedure employed for **68**.

Yield 51 mg, 57%;

Melting Point 146-149 °C;

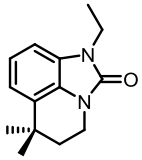
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 - 6.98 (m, 2 H), 6.82 (d, *J* = 7.3 Hz, 1 H), 3.90 (t, *J* = 6.1 Hz, 2 H), 3.42 (s, 3 H), 1.90 (t, *J* = 6.1 Hz, 2 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6, 128.5, 128.3, 125.1, 120.9, 116.1, 105.0, 36.7, 36.3, 31.8, 28.5, 27.2;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2965, 1690, 1502, 1467, 1435 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{13}H_{17}ON_2$ $[M+H]^+$ 217.1335 found 217.1336;

1-ethyl-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (83a)



The title compound was synthesized from **83** using similar procedure employed for **68**.

Yield 66 mg, 62%;

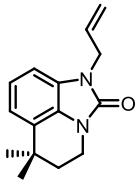
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 - 6.95 (m, 2 H), 6.85 (d, *J* = 7.4 Hz, 1 H), 3.98 - 3.87 (m, 4 H), 1.90 (t, *J* = 6.0 Hz, 2 H), 1.40 - 1.32 (m, 9 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 128.3, 127.5, 125.2, 120.8, 115.9, 105.1, 36.7, 36.2, 36.0, 31.8, 28.6, 13.9;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 2966, 1689, 1499, 1465 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{14}H_{19}ON_2$ $[M+H]^+$ 231.1492 found 231.1488;

Synthesis of 1-allyl-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (84a)



The title compound was synthesized from **84** using similar procedure employed for **68**.

Yield 43 mg, 60%;

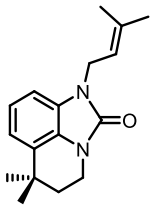
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 - 6.89 (m, 2 H), 6.81 (dd, *J* = 2.1, 6.5 Hz, 1 H), 5.98 - 5.75 (m, 1 H), 5.33 - 5.12 (m, 2 H), 4.50 (d, *J* = 6.8 Hz, 2 H), 3.91 (t, *J* = 5.87 Hz, 2 H), 1.90 (t, *J* = 5.95 Hz, 2 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.0, 132.3, 128.2, 127.6, 125.1, 120.7, 117.4, 116.1, 105.7, 43.5, 36.6, 36.2, 31.7, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3427, 3016, 2965, 1691, 1499, 1437 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{15}H_{19}N_2O$ $[M+H]^+$ 243.1492, found 243.1488;

6,6-dimethyl-1-(3-methylbut-2-en-1-yl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (85a)



The title compound was synthesized from **85** using similar procedure employed for **68**.

Yield 58 mg, 65%;

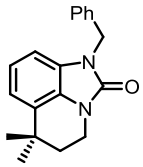
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (m, 2 H), 6.79 (d, *J* = 6.7 Hz, 1 H), 5.30 (t, *J* = 6.7 Hz, 1 H), 4.48 (d, *J* = 6.7 Hz, 2 H), 3.90 (t, *J* = 6.1 Hz, 2 H), 1.92 - 1.88 (m, 2 H), 1.86 (s, 3 H), 1.73 (s, 3 H), 1.35 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 136.2, 128.2, 127.7, 125.1, 120.7, 119.1, 115.9, 105.6, 39.1, 36.6, 36.2, 31.7, 28.5, 25.6, 18.0;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3012, 2966, 1689, 1499, 1412 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{17}H_{23}ON_2$ $[M+H]^+$ 271.1805 found 271.1807;

1-benzyl-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one(86a)



The title compound was synthesized from **86** using similar procedure employed for **68**.

Yield 47 mg, 64%;

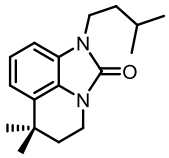
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.27 (m, 5 H), 7.04 - 6.93 (m, 2 H), 6.77 - 6.72 (m, 1 H), 5.09 (s, 2 H), 3.97 (t, *J* = 5.9 Hz, 2 H), 1.94 (t, *J* = 5.9 Hz, 2 H), 1.38 (s, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4, 136.6, 128.6, 128.3, 127.7, 127.6, 125.2, 120.8, 116.2, 105.8, 77.0, 45.0, 36.6, 36.3, 31.7, 28.5;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 2960, 1712, 1498, 1437, 1387 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{19}H_{21}ON_2$ $[M+H]^+$ 293.1648 found 293.1644;

1-isopentyl-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (87a)



The title compound was synthesized from **87** using similar procedure employed for **68**.

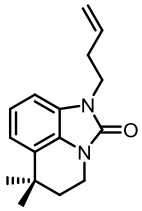
Yield 56 mg, 68%;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 - 6.92 (m, 2 H), 6.83 (d, *J* = 7.3 Hz, 1 H), 3.91 - 3.87 (m, 4 H), 1.89 (t, *J* = 5.8 Hz, 2 H), 1.71 - 1.64 (m, 3 H), 1.35 (s, 6 H), 0.99 (d, *J* = 6.1 Hz, 6 H);
^{13}C NMR (100 MHz, $CDCl_3$) δ 153.3, 128.2, 127.8, 125.1, 120.6, 115.8, 105.1, 39.5, 37.3, 36.6, 36.1, 31.7, 28.5, 25.7, 22.4;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3027, 1735, 2883, 1275, 1410 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{17}H_{25}ON_2$ $[M+H]^+$ 273.1961 found 273.1955.

1-(but-3-en-1-yl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (88a)



The title compound was synthesized from **88** using similar procedure employed for **68**.

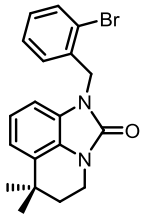
Yield 20 mg, 58%;

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.12 - 6.91 (m, 2 H), 6.91 - 6.78 (m, 1 H), 5.97 - 5.75 (m, 1 H), 5.18 - 5.01 (m, 2 H), 3.98 - 3.87 (m, 4 H), 2.52 (q, *J* = 7.0 Hz, 2 H), 1.90 (d, *J* = 6.1 Hz, 2 H), 1.36 (s, 6 H);
^{13}C NMR (50 MHz, $CDCl_3$) δ 153.3, 134.6, 128.3, 127.8, 125.1, 120.7, 117.3, 116.0, 105.3, 40.8, 36.7, 36.2, 32.9, 31.8, 28.6;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3018, 2965, 1690, 1499, 1414 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{16}H_{21}ON_2$ $[M+H]^+$ 257.1648 found 257.1644;

1-(2-bromobenzyl)-6,6-dimethyl-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (89a)



The title compound was synthesized from **89** using similar procedure employed for **68**.

Yield 65 mg, 70%;

Melting Point 165-168 °C;

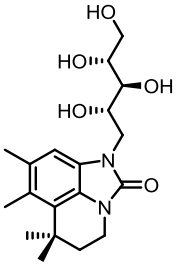
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.6 Hz, 1 H), 7.21 (t, *J* = 7.2 Hz, 1 H), 7.13 (t, *J* = 7.6 Hz, 2 H), 7.00 (d, *J* = 7.6 Hz, 1 H), 6.96 (t, *J* = 7.2 Hz, 1 H), 6.71 (d, *J* = 7.2 Hz, 1 H), 5.17 (s, 2 H), 3.97 (t, *J* = 5.3 Hz, 2 H), 1.95 (t, *J* = 5.3 Hz, 2 H), 1.38 (s, 6H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 153.5, 135.5, 132.8, 129.0, 128.5, 128.4, 127.7, 127.6, 125.2, 122.7, 121.0, 116.5, 106.1, 44.9, 36.7, 36.5, 31.8, 28.6**;**

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3013, 2964, 1696, 1498, 1466 cm^{-1} .

HRMS (ESI) *m/z* calculated for $C_{19}H_{20}ON_2$ Br$[M+H]^+$ 371.0754 found 371.0749.

6,6,7,8-tetramethyl-1-((2S,3S,4R)-2,3,4,5-tetrahydroxypentyl)-5,6-dihydro-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (91)



The title compound was synthesized from **Hunanamycin A** using similar procedure employed for **68**.

Yield 15 mg, 47%;

Specific rotation $[\alpha]_D^{25}$= -83 (*c* 0.8, MeOH);

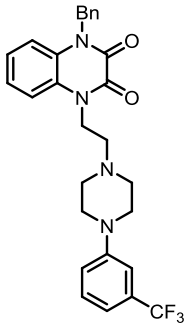
^{1}H NMR (400 MHz, CD_3OD) δ 6.95 (s, 1 H), 4.08 (t, *J* = 6.7 Hz, 1 H), 4.04 (d, *J* = 6.1 Hz, 2 H), 3.82 - 3.75 (m, 4 H), 3.70 - 3.62 (m, 1 H), 3.59 (t, *J* = 6.1 Hz, 1 H), 2.36 (s, 3 H), 2.31 (s, 3 H), 1.93 (t, *J* = 6.1 Hz, 1 H), 1.47 (s, 6 H);

^{13}C NMR (100 MHz, CD_3OD) δ 155.4, 132.2, 128.3, 128.1, 127.8, 125.3, 109.9, 74.5, 74.4, 72.6, 64.8, 45.2, 41.7, 37.0, 34.4, 28.6, 21.9, 17.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3575, 3374, 3019, 2963, 1667, 1466 cm^{-1}.

HRMS (ESI) *m/z* calculated for $C_{19}H_{29}O_5N_2$ $[M+H]^+$ 365.2071 found 365.2068;

1-benzyl-4-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)-1,4-dihydroquinoxa--line-2,3-dione (94)



A mixture of 1-benzyl-1,4-dihydroquinoxaline-2,3-dione (**92**) (150 mg, 0.595 mmol), 1-(2-chloroethyl)-4-(3-(trifluoromethyl)phenyl)piperazine (**93**) (235 mg, 0.714 mmol), potassium carbonate (205 mg, 1.488 mmol), Sodium Iodide (106 mg, 0.714 mmol), and DMF (5 mL) was heated at 50 °C overnight. The reaction mixture was cooled to room temperature and extracted with ethyl acetate (30 × 2 mL) and water (20 mL). The organic layer was dried and evaporated under reduced pressure to obtain crude **94**, which was further purified by column chromatography to afford pure compound **94** as white solid (160 mg, 68%)

Melting Point 150-135 °C;

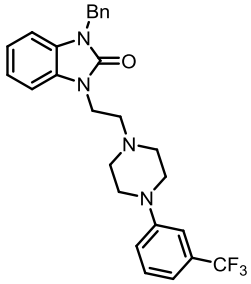
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.19 (m, 9 H), 7.18 - 7.01 (m, 4 H), 5.50 (s, 2 H), 4.48 (t, *J* = 7.0 Hz, 2 H), 3.28 (br. s., 4 H), 2.85 - 2.80 (m, 6 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 154.1, 151.2, 134.7, 131.2 (q *J* = 31.6 Hz), 129.5, 128.9, 127.7, 126.7, 126.5, 124.3, 124.2, 124.2 (q, *J* = 272.8 Hz,), 118.6, 116.1, 115.7 (q, *J* = 3.8 Hz), 114.9, 112.1 (q, *J* = 3.8 Hz), 54.3, 53.2, 48.5, 46.9, 40.8;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1737, 1606, 1513, 1475 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{28}H_{28}O_2N_4F_3[M+H]^+$ 509.2159 found 509.2166.

1-benzyl-3-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one (95)



To a solution of 1-benzyl-4-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)-1,4-dihydroquinoxaline-2,3-dione **94** (150 mg, 0.295 mmol) in dry DMSO (4 mL) was added powdered potassium hydroxide (165 mg, 2.952 mmol) stirred for 36 h at room temperature.

The reaction mixture was added to cold water and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was washed water (2 X 10 mL), brine (1 X 10 mL) and dried over Na_2SO_4, concentrated under reduced pressure. The crude product obtained was subjected to flash chromatography over silica gel to afford pure product **95** as yellow sticky solid product (85 mg, 59% yields).

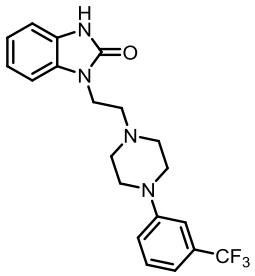
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 - 7.29 (m, 6 H), 7.11- 7.04 (m, 6 H), 6.91 (d, *J* = 7.9 Hz, 1 H), 5.09 (s, 2 H), 4.12 (t, *J* = 6.7 Hz, 2 H), 3.22 - 3.20 (m, 4 H), 2.80 (t, *J* = 6.7 Hz, 2 H), 2.77 - 2.72 (m, 4 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 151.3, 136.3, 131.3 (q, *J* = 31.6 Hz), 129.5, 129.3, 129.3, 128.7, 127.6, 127.4, 124.3 (q, *J* = 272.8 Hz), 121.2, 121.2, 118.7, 115.7 (q, *J* = 3.8 Hz), 112.1 (q, *J* = 3.8 Hz), 108.3, 107.7, 55.6, 53.0, 48.6, 44.8, 38.7;

IR ν_{max} (thin film applied as $CHCl_3$ solution) 3019, 1737, 2963, 1475, 1416 cm^{-1};

HRMS (ESI) *m/z* calculated for $C_{27}H_{28}ON_4F_3[M+H]^+$ 481.2210 found 481.2215.

1-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)-1,3-dihydro-2H-benzo[d]imida--zol-2-one (Flibanserin)



Trifluoromethanesulfonic acid (36 μL, 0.416 mmol) was added to a mixture of the synthesis of 1-benzyl-3-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)-1,3-dihydro-2H-benzo[d]imidazol-2-one **95** (50 mg, 0.104 mmol) and toluene (1 mL). The resulting mixture was stirred for 20 min at 150 °C under microwave irradiation. Reaction mixture was then diluted with cold saturated solution of $NaHCO_3$ (10 mL) and then extracted with ethyl acetate (20 mL x 2). Combined organic layer was then washed with water, brine, dried over Na_2SO_4, and concentrated to afford crude product. The crude product was dissolved in Dioxane (3 mL), and 4M HCl in dioxane (1 mL) was added reaction mixture was stirred at room temperature for 30 min. All the solvent was removed under vacuum to afford crude product which on trituration with diethyl ether (2 mL x 2) gave 32 mg (80% yield) pure Flibanserin hydrochloride as white solid.

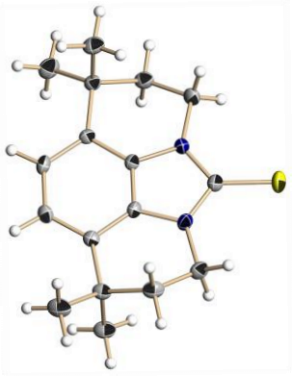
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1 H), 10.93 (br. s., 1 H), 7.49 - 7.45 (t, *J* = 7.9 Hz, 1 H), 7.34 - 7.29 (m, 3 H), 7.15 (d, *J* = 7.6 Hz, 1 H), 7.05 - 7.04 (m, 3 H), 4.30 (t, *J* = 6.7 Hz, 2 H), 4.01 (d, *J* = 11.6 Hz, 2 H), 3.75 (d, *J* = 10.4 Hz, 2 H), 3.48 (d, *J* = 4.2 Hz 2 H), 3.25 - 3.17 (m, 4 H);

HRMS (ESI) *m/z* calculated for $C_{20}H_{22}ON_4F_3[M+H]^+$ 391.1740 found 391.1743;

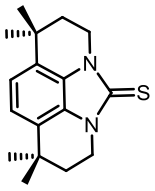
Crystal structure data of 32 and 34

A single crystal of compounds **32** was obtained from dichloromethane/ Pet ether and of **34** was obtained from ethanol. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at temperatures 297(2) K and 150(2) K respectively. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from three sets of 12 frames (total 36 frames). Diffraction data were collected with a ω scan width of 0.5° and at different settings of φ and 2θ. The sample-to-detector distance was fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX II program suite. All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX II program package. The structures were solved by direct method and refined by full matrix least squares, based on F^2, using SHELX-97. Molecular diagrams were generated using XSHELL program integrated in SHELXTL package.

Crystallographic data for 32



ORTEP of 32

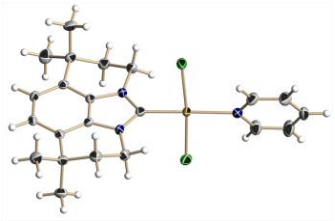


32

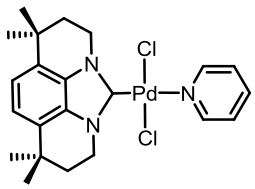
($C_{17}H_{22}N_2S$): *M* = 286.43, Crystal dimensions 0.70 x 0.26 x 0.12 mm^3, monoclinic, space group *P* $2_1/n$, *a* = 15.231(3), *b* = 5.7022(12), *c* = 18.793(4) Å, β = 108.491(14)°, *V* = 1547.9(6) $Å^3$, *Z* = 4, ρ_{calcd} = 1.229 gcm^{-3}, μ (Mo-K_α) = 0.202 mm^{-1}, *F(000)* = 616, $2\theta_{max}$ =

52.00°, *T* = 297(2) K, 14602 reflections collected, 2996 unique reflections (R_{int}=0.0263), 2361 observed ($I > 2\sigma(I)$) reflections, 185 refined parameters, *R* value 0.0684, *wR*2 = 0.1783, (all data *R* = 0.0894, *wR*2 = 0.1918), *S* = 1.117, minimum and maximum transmission 0.872 and 0.976; maximum and minimum residual electron densities +0.35 and –0.28 e Å^{-3}. All the H-atoms were placed in geometrically idealized position (C-H = 0.93 Å for the phenyl H-atom, C-H = 0.97 Å for the methylene H-atom and C-H = 0.96 Å for the methyl H-atom) and constrained to ride on their parent atoms [U_{iso}(H) = 1.2U_{eq}(C) for the phenyl and methylene groups and U_{iso}(H) = 1.5 U_{eq}(C) for the methyl group].

Crystallographic data for 34



ORTEP of 34



NHC-Pd complex-II

34

($C_{22}H_{27}C_{l2}N_3Pd$): *M* = 510.77, Crystal dimensions 0.68 x 0.32 x 0.11 mm^3, triclinic, space group *P-1*, *a* = 5.6847(6), *b* = 14.0640(14), *c* = 14.3961(14) Å, α = 108.211(4)°, β = 95.732(4)°, γ = 95.371(4)°, *V* = 1078.32(19)Å^3, *Z* = 2, ρ_{calcd} = 1.573 gcm^{-3}, μ (Mo-K$_\alpha$) = 1.121 mm^{-1}, *F(000)* = 520, 2θ_{max} = 50.00°, *T* = 150(2) K, 17520 reflections collected, 3781 unique reflections (R_{int}=0.0214), 3732 observed ($I > 2\sigma(I)$) reflections, 258 refined parameters, *R* value 0.0198, *wR*2 = 0.504, (all data *R* = 0.0202, *wR*2 = 0.0507), *S* = 1.149, minimum and maximum transmission 0.516 and 0.887; maximum and minimum residual electron densities +0.44 and –0.37 e Å^{-3}. All the H-atoms were placed in geometrically idealized position (C-H = 0.95 Å for the phenyl H-atom, C-H = 0.99 Å for the methylene H-atom and C-H = 0.98 Å for the methyl H-atom) and constrained to ride on their parent atoms [U_{iso}(H) = 1.2U_{eq}(C) for the phenyl and methylene groups and U_{iso}(H) = 1.5 U_{eq}(C) for the methyl group].

3.2.7 References

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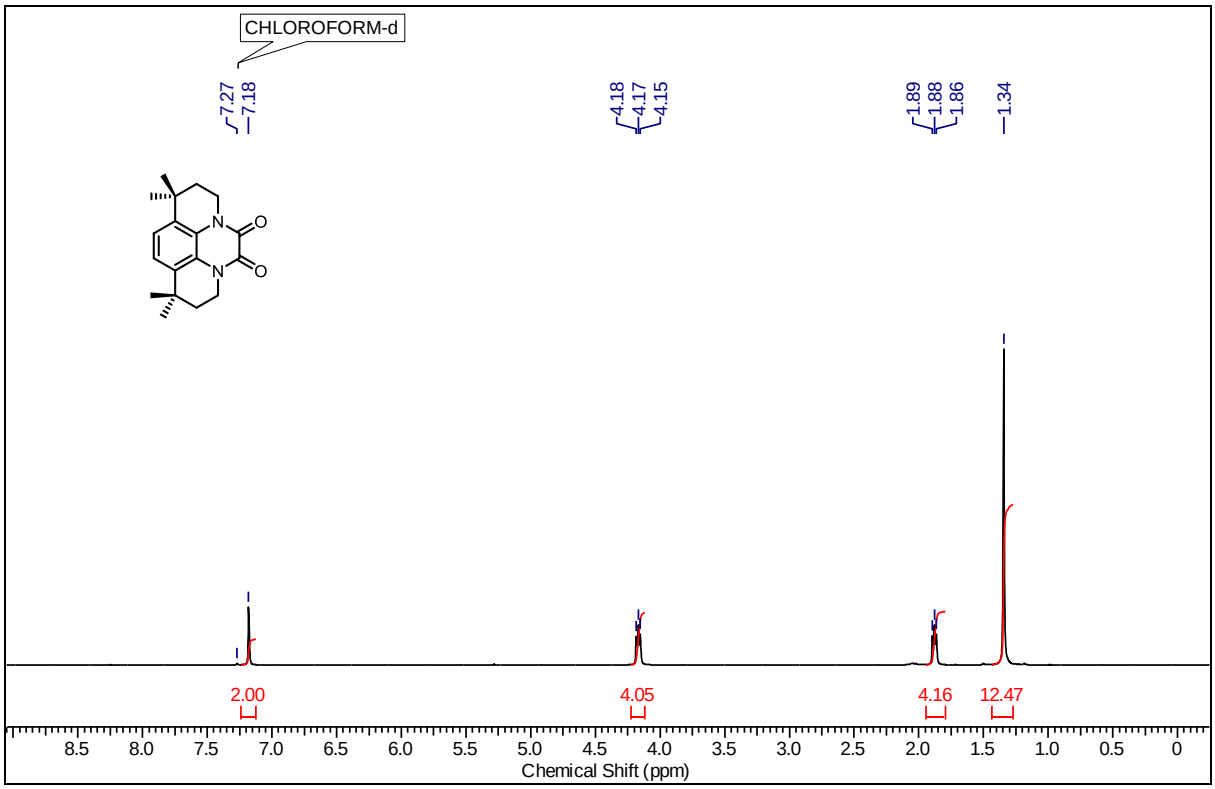
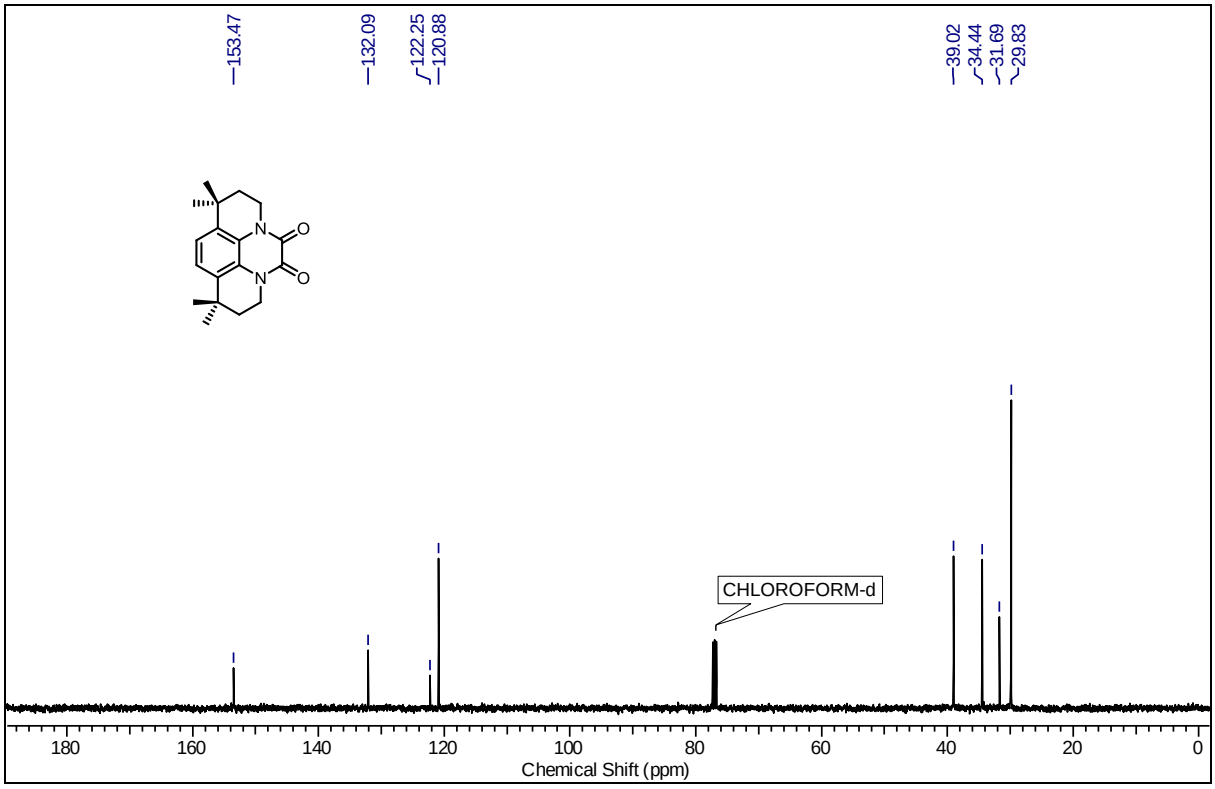
3.2.8 Copies of ^{1}H and ^{13}C NMR spectra

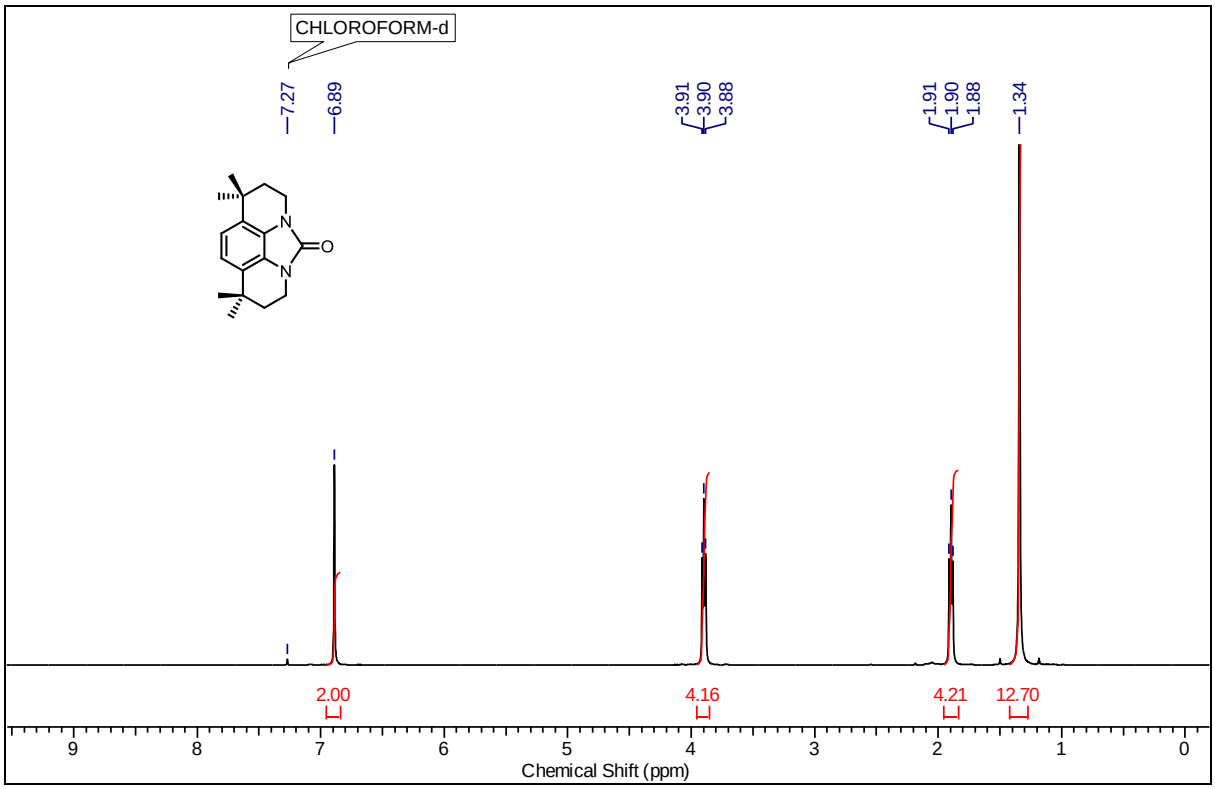
^{1}H NMR of Compound 25 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 25 at 400 MHz in $CDCl_3$



^{1}H NMR of Compound 27 at 400 MHz in $CDCl_3$



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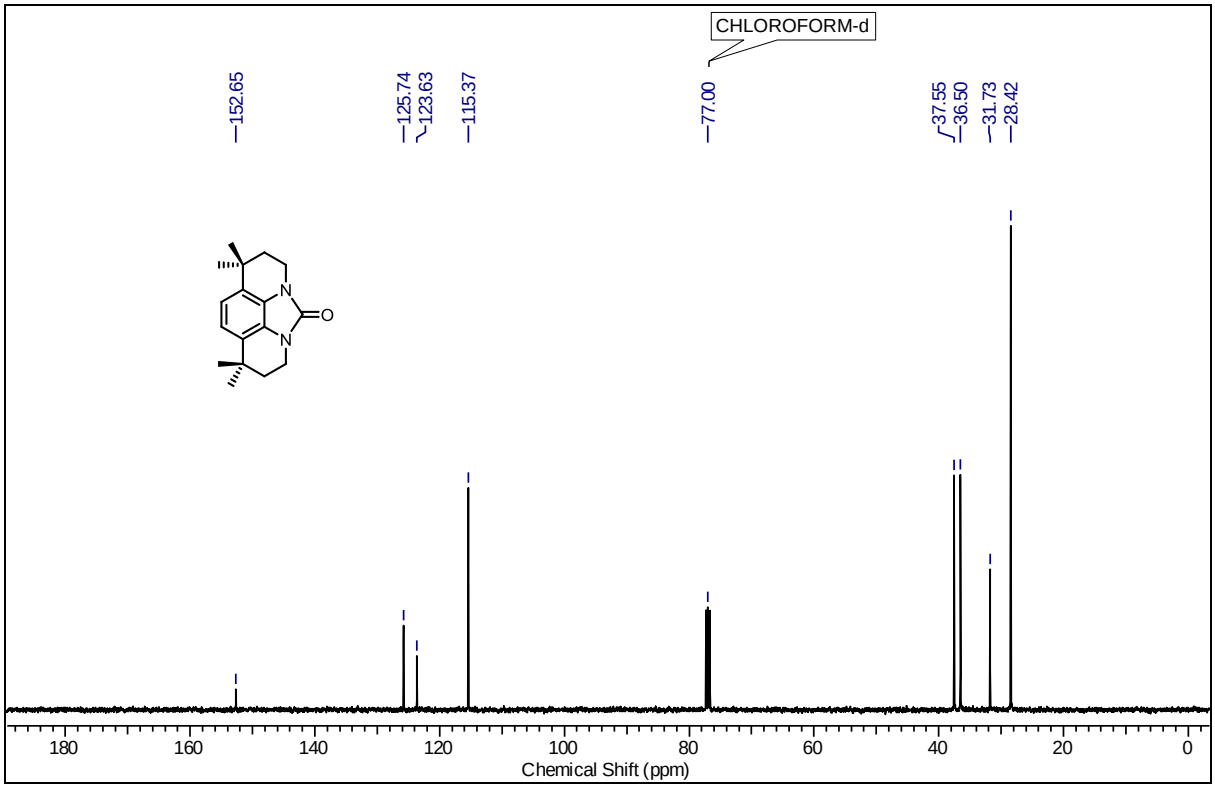
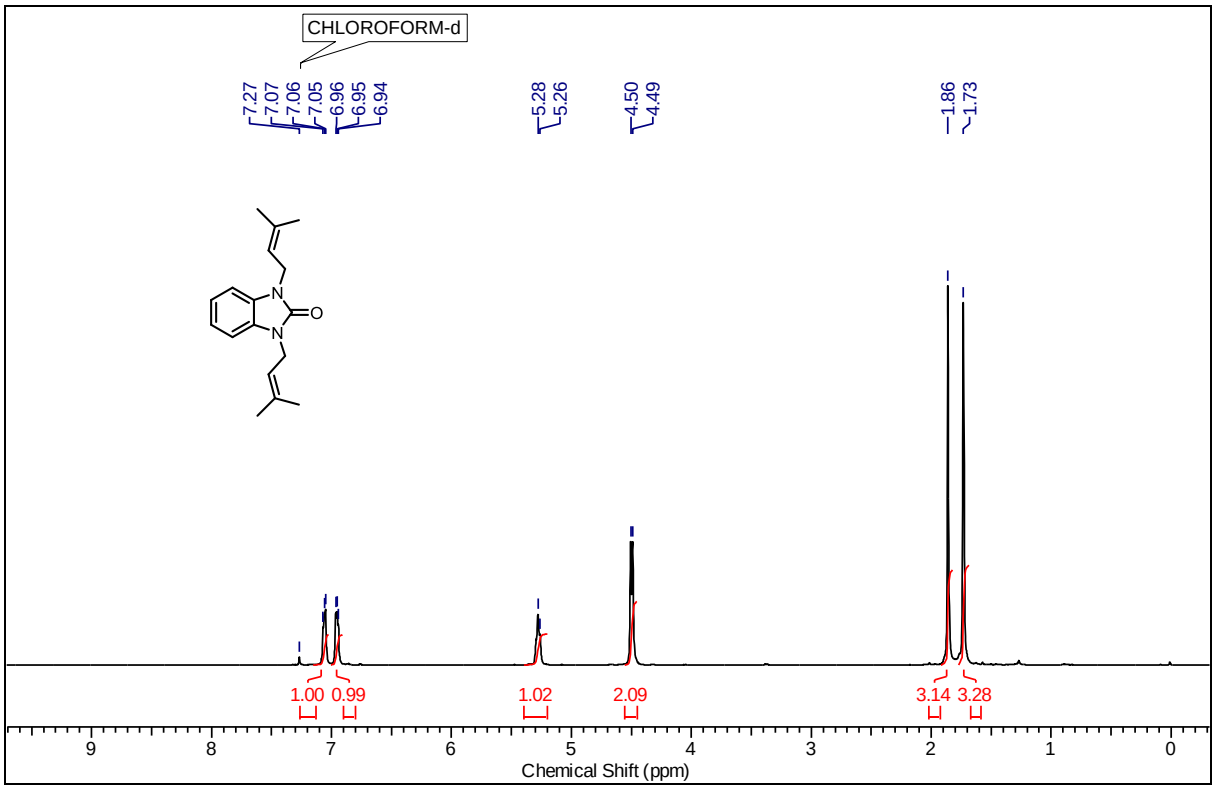
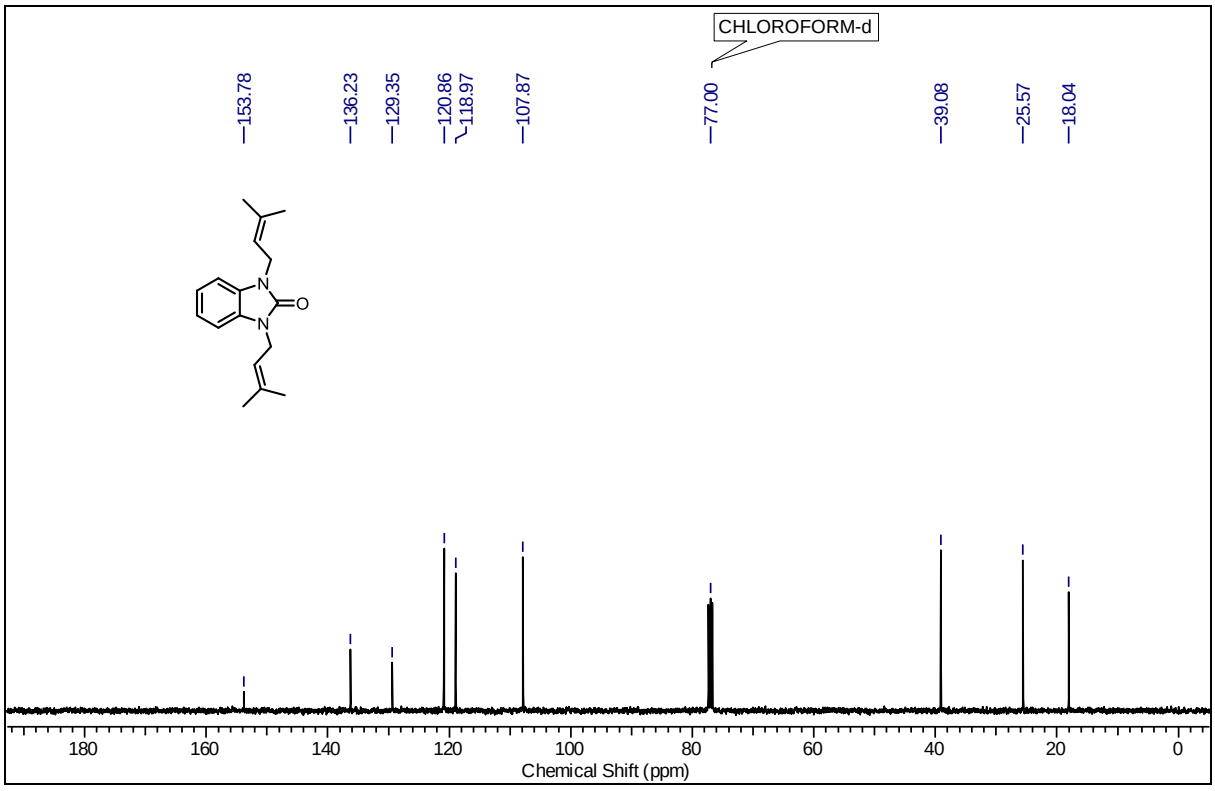


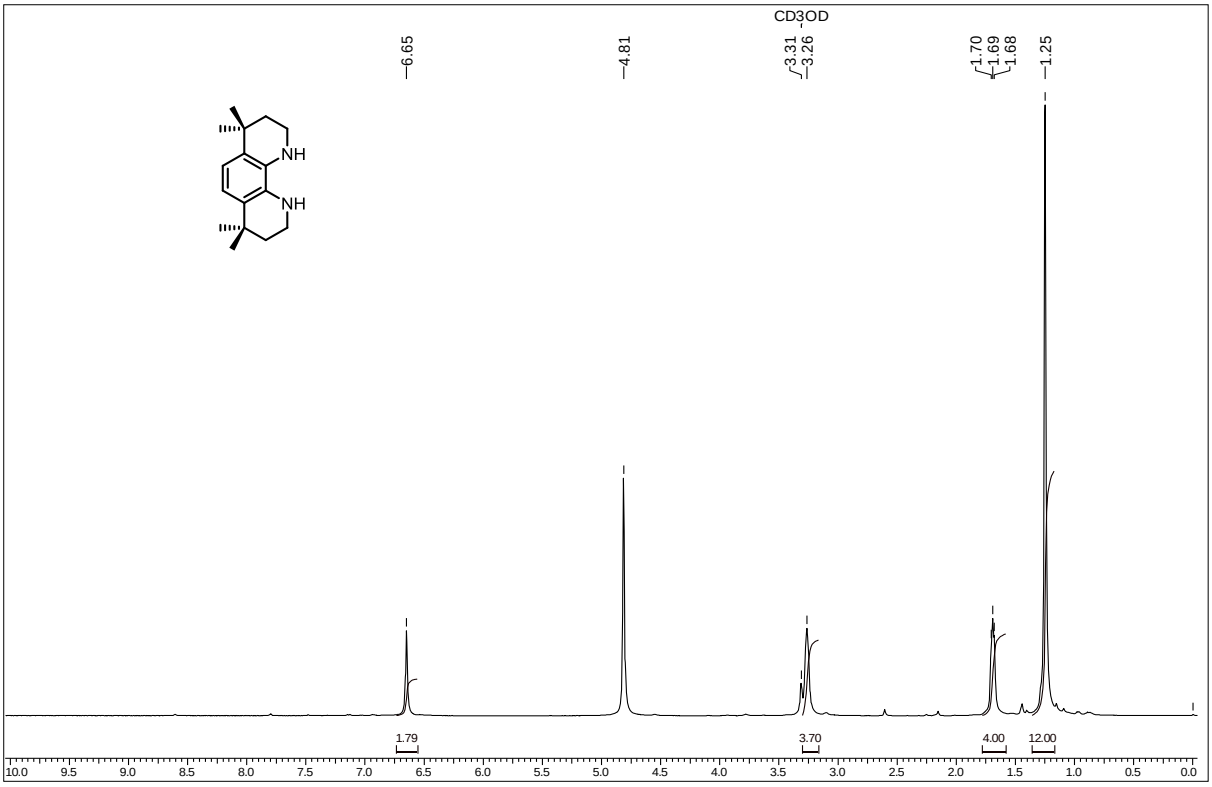
^{1}H NMR of Compound 29 at 400 MHz in $CDCl_3$



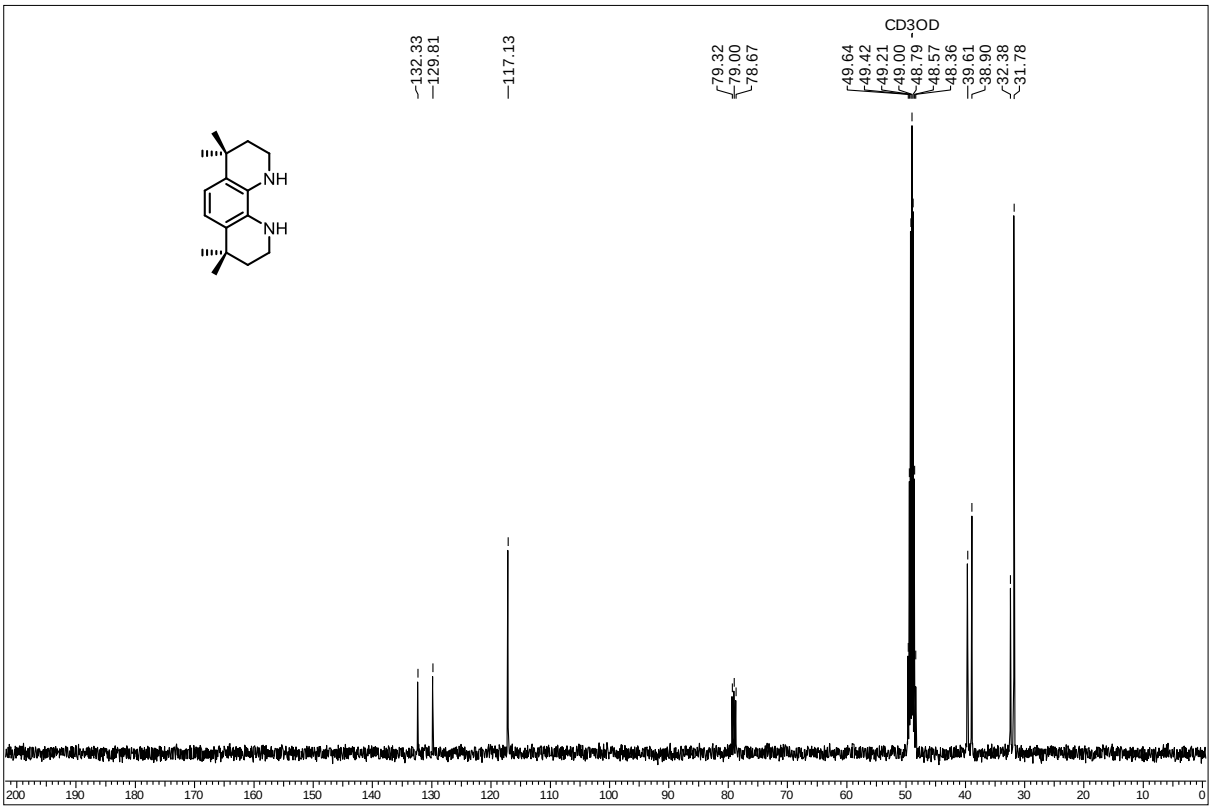
^{13}C NMR of Compound 29 at 400 MHz in $CDCl_3$



^{1}H NMR of Compound 26 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 26 at 400 MHz in $CDCl_3$



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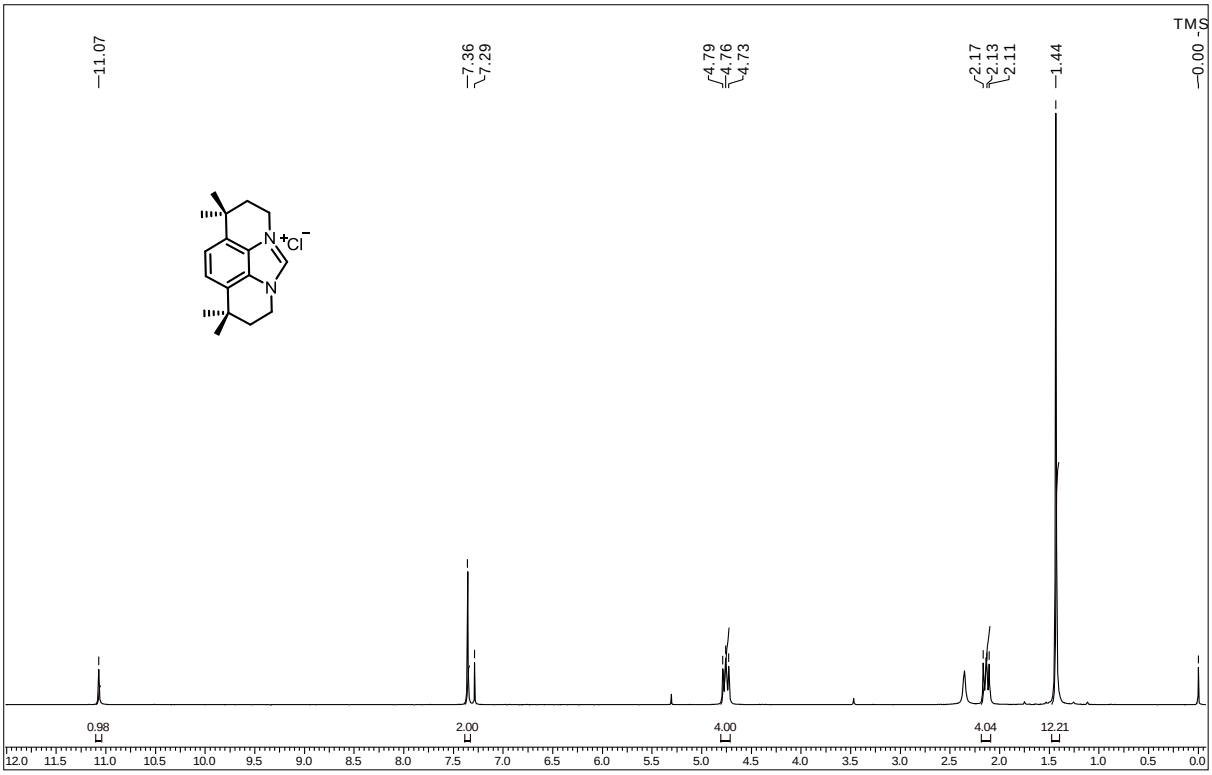

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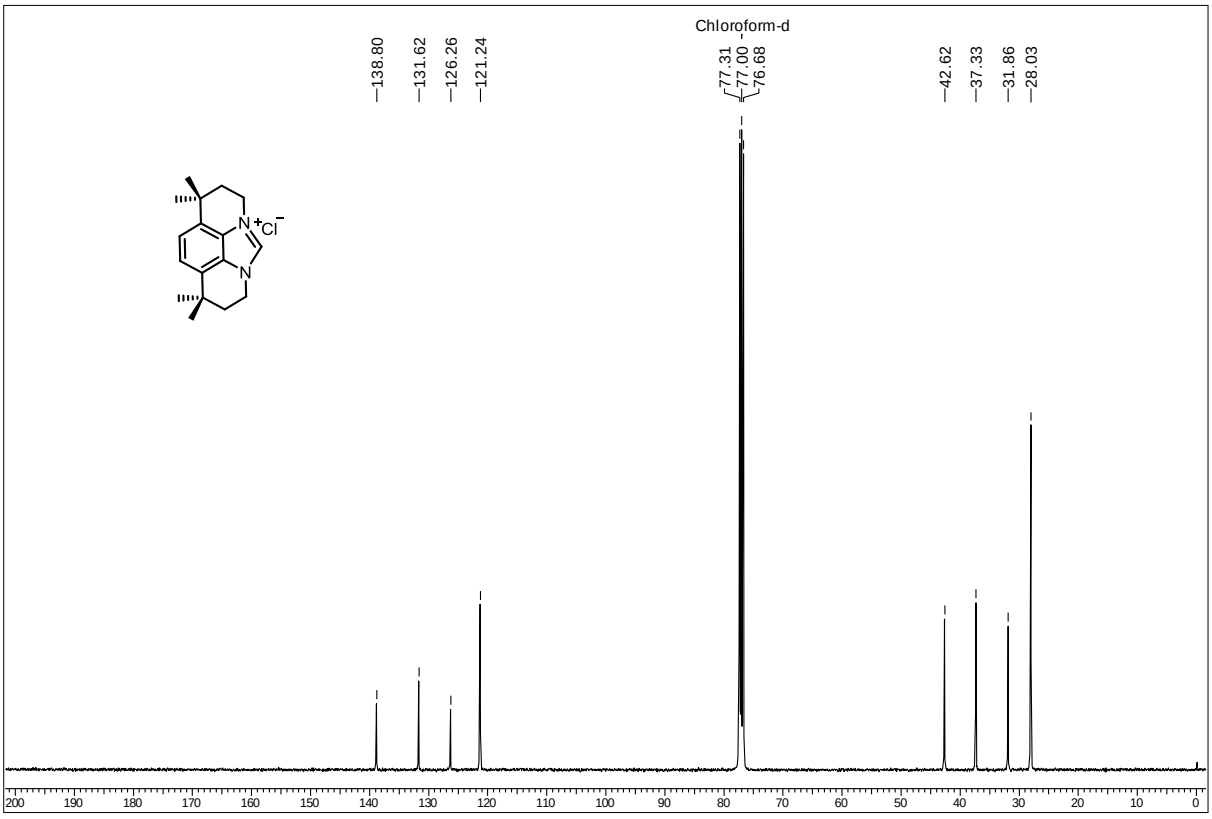
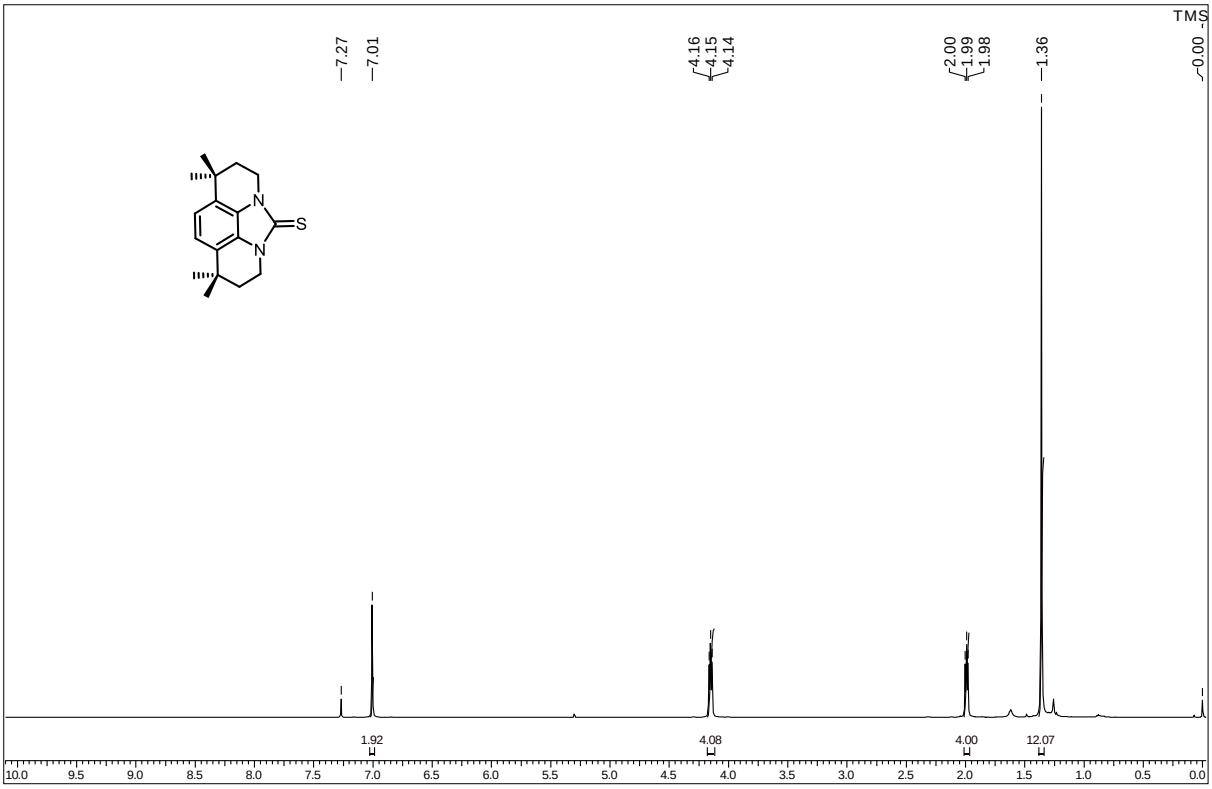
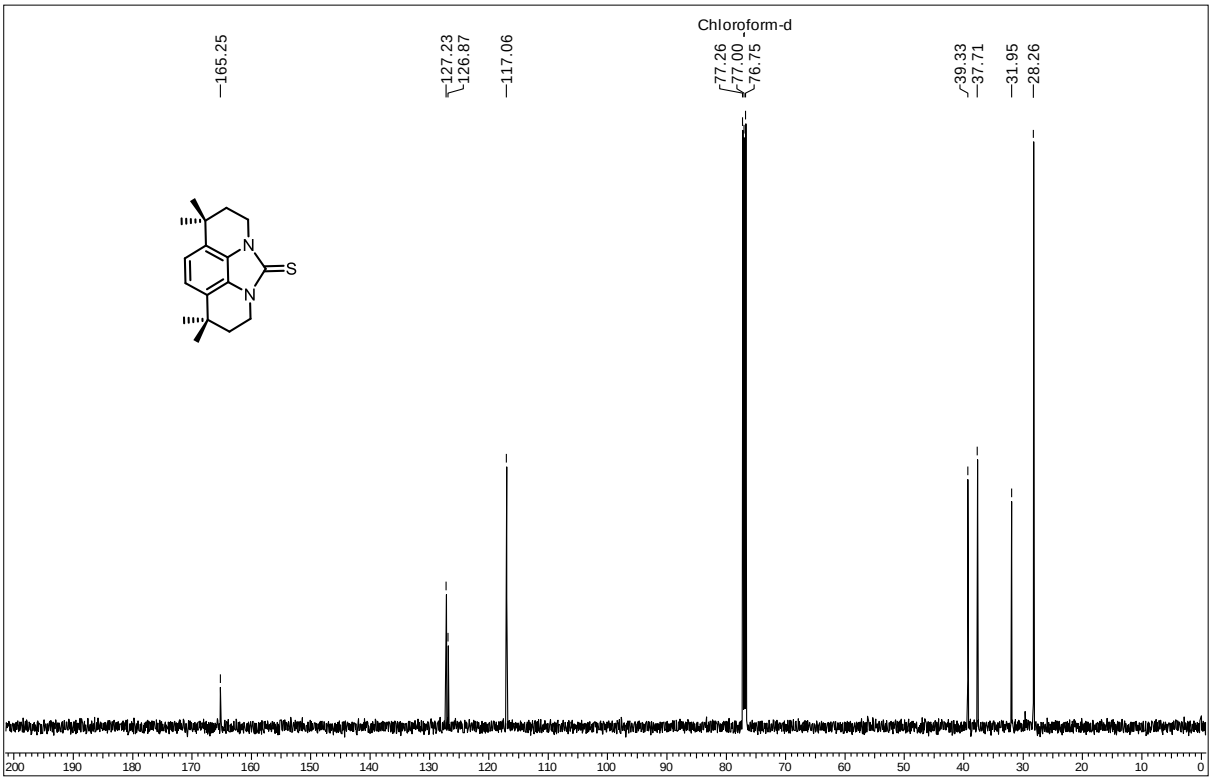


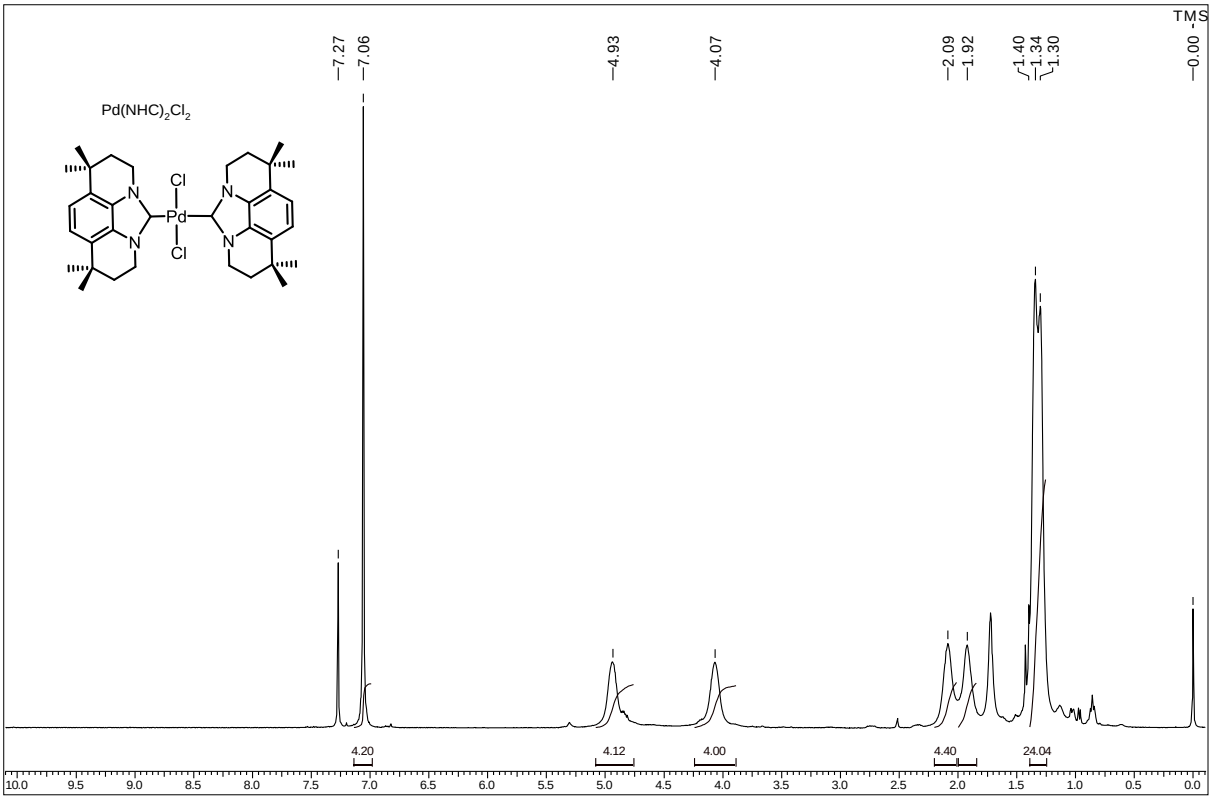
^{1}H NMR of Compound 32 at 400 MHz in $CDCl_3$



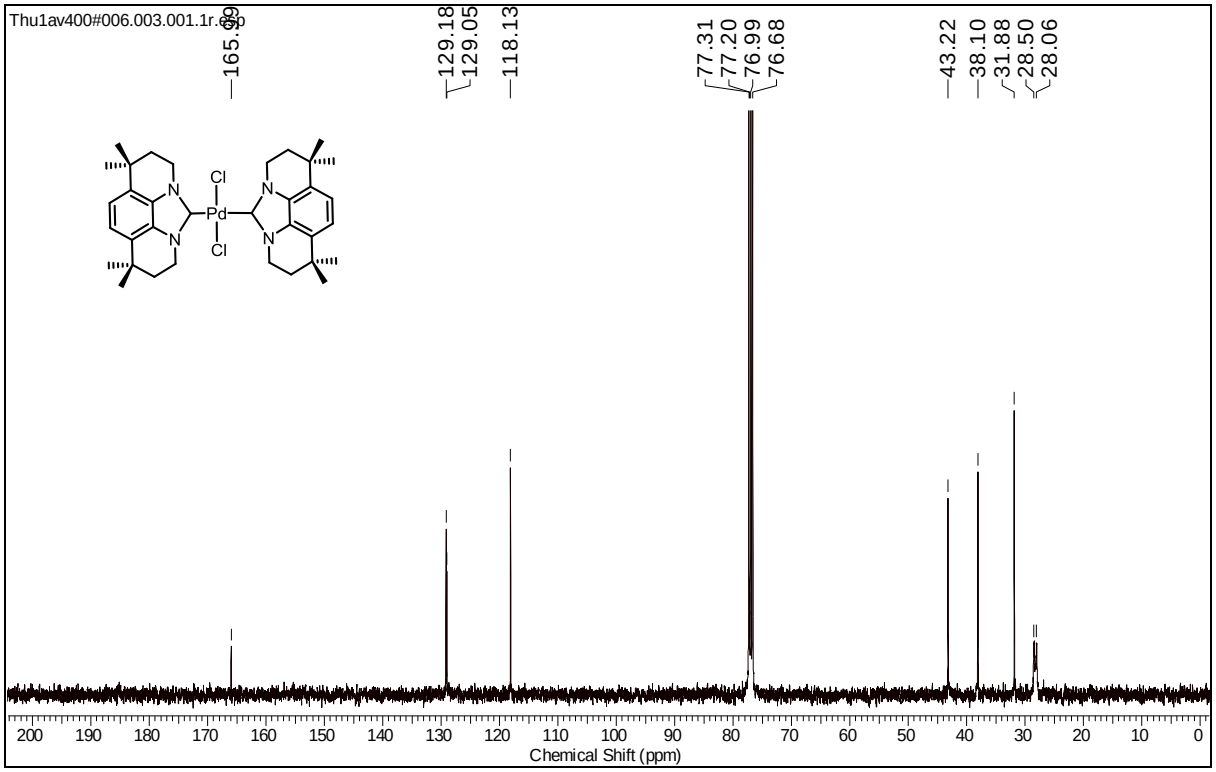
^{13}C NMR of Compound 32 at 400 MHz in $CDCl_3$



^{1}H NMR of Compound 33 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 33 at 400 MHz in $CDCl_3$



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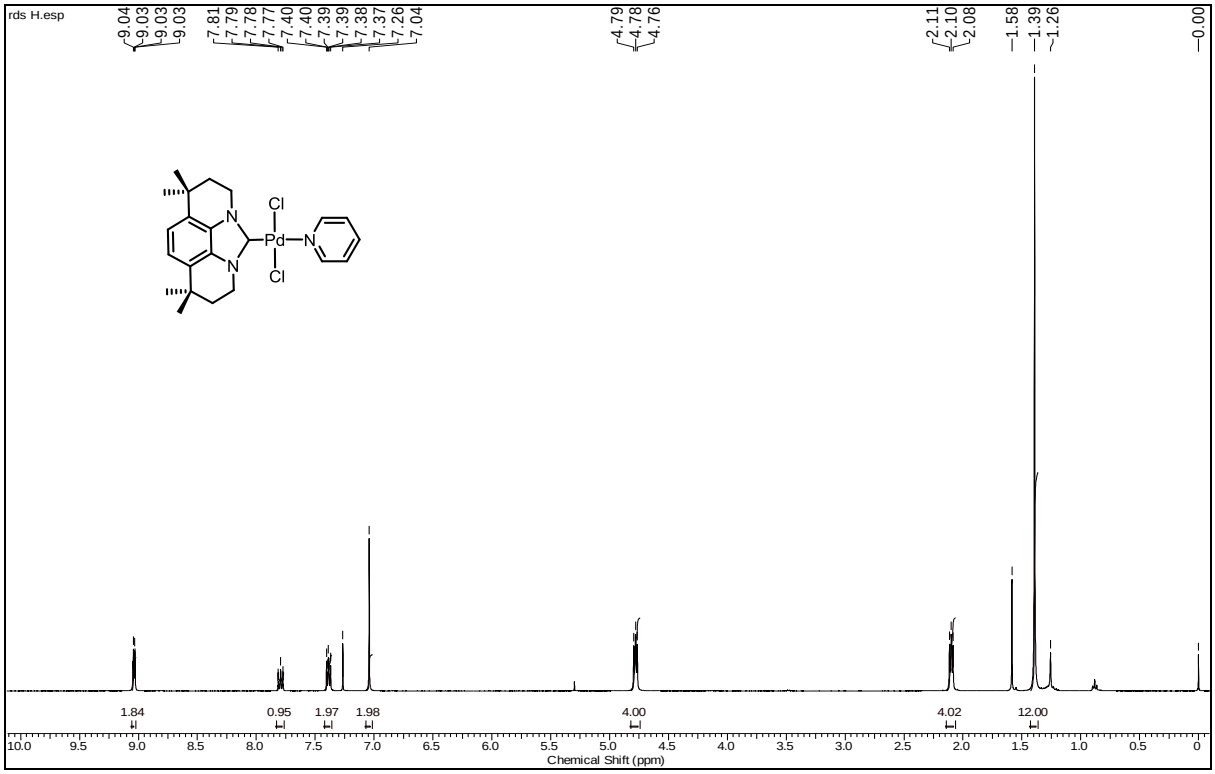
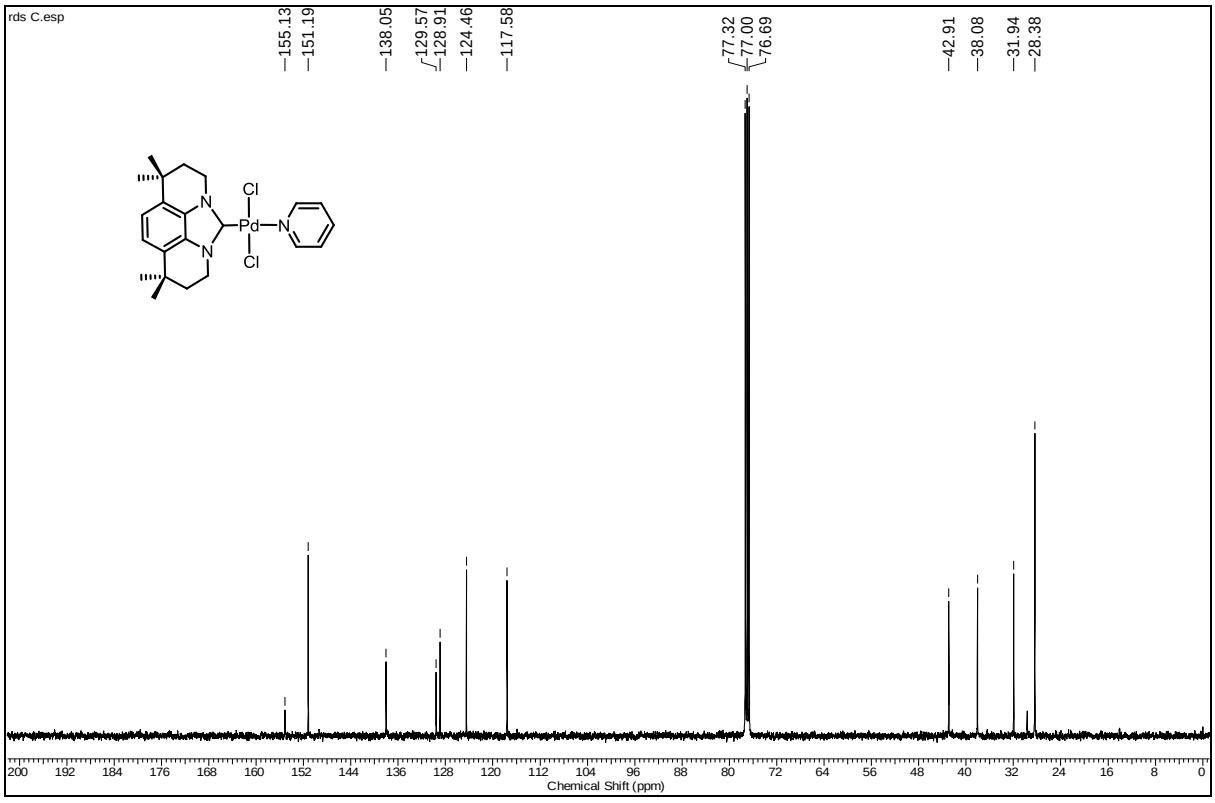
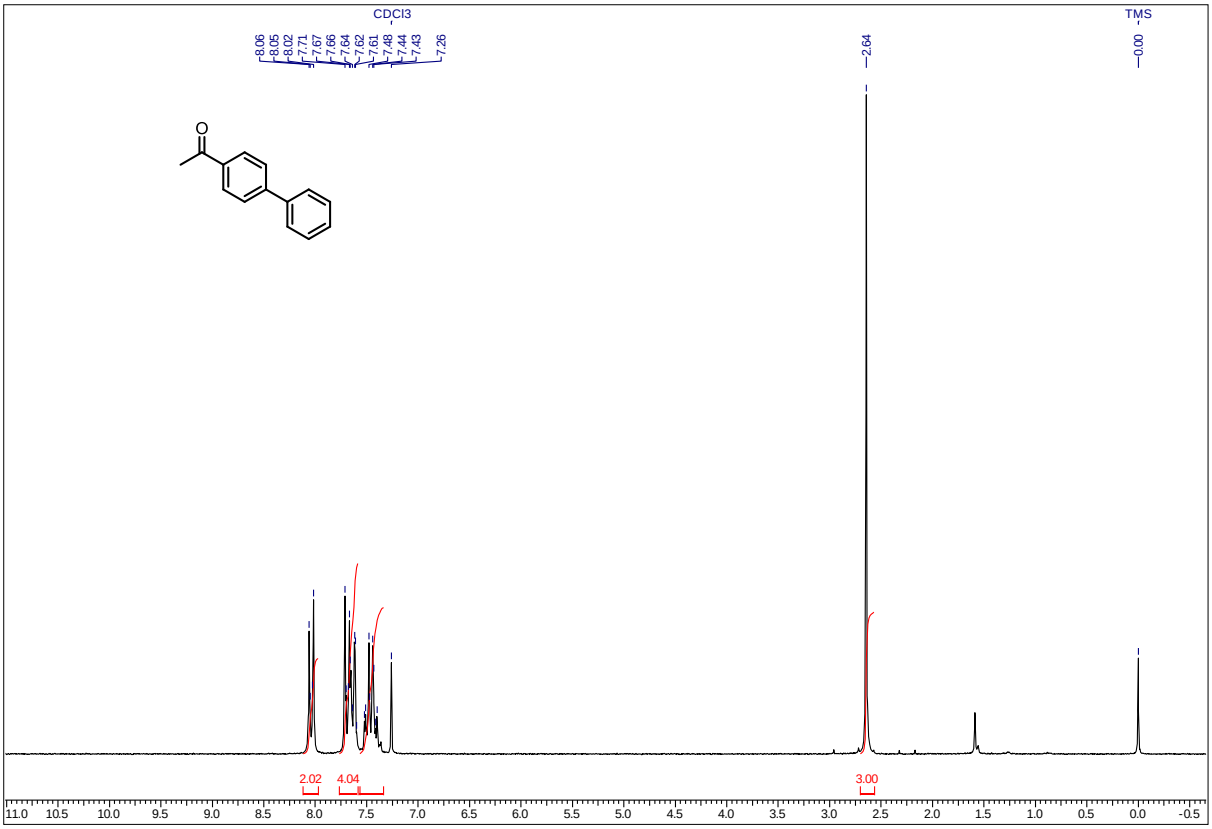


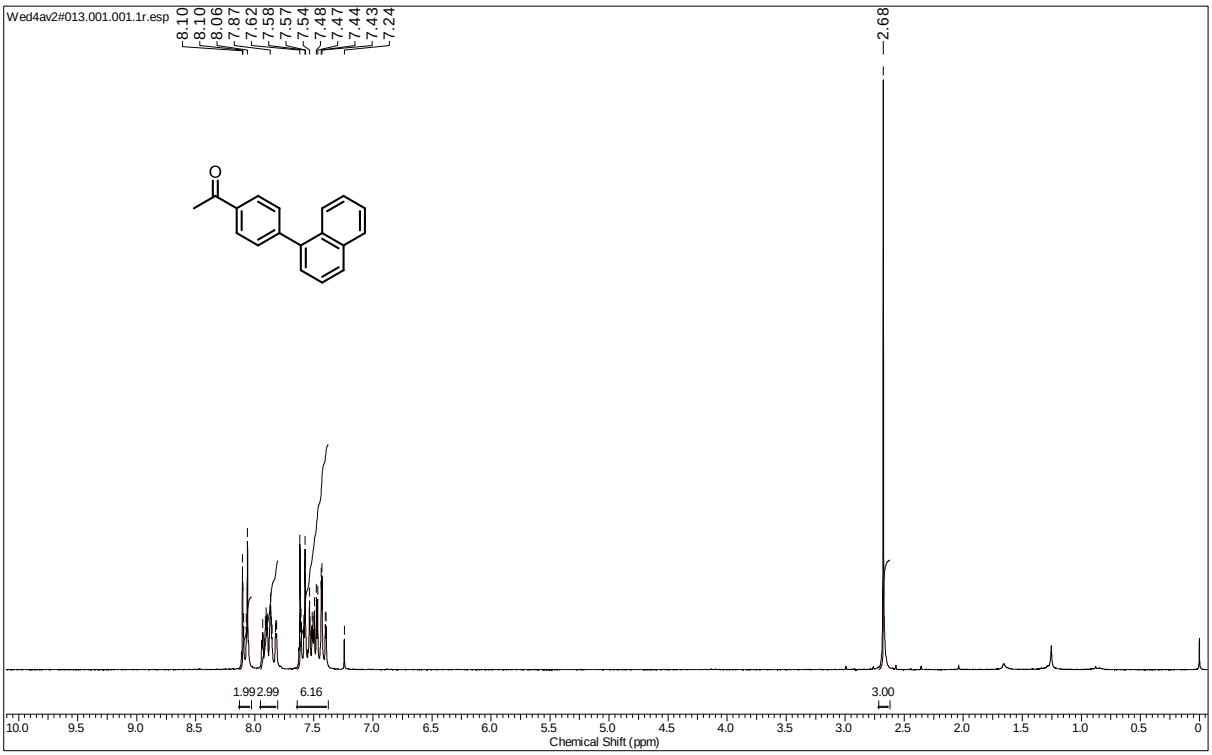
^{13}C NMR of Compound 34 at 400 MHz in $CDCl_3$



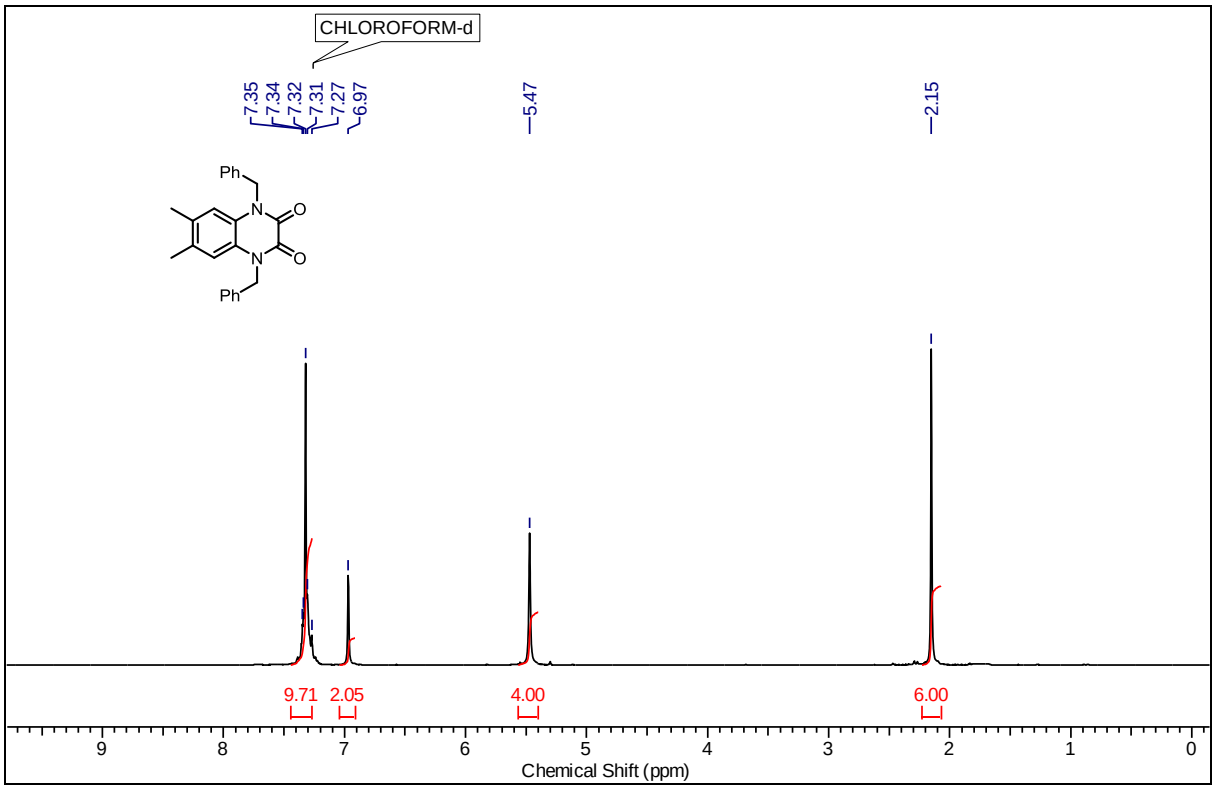
^{1}H NMR of Compound 37 at 200 MHz in $CDCl_3$



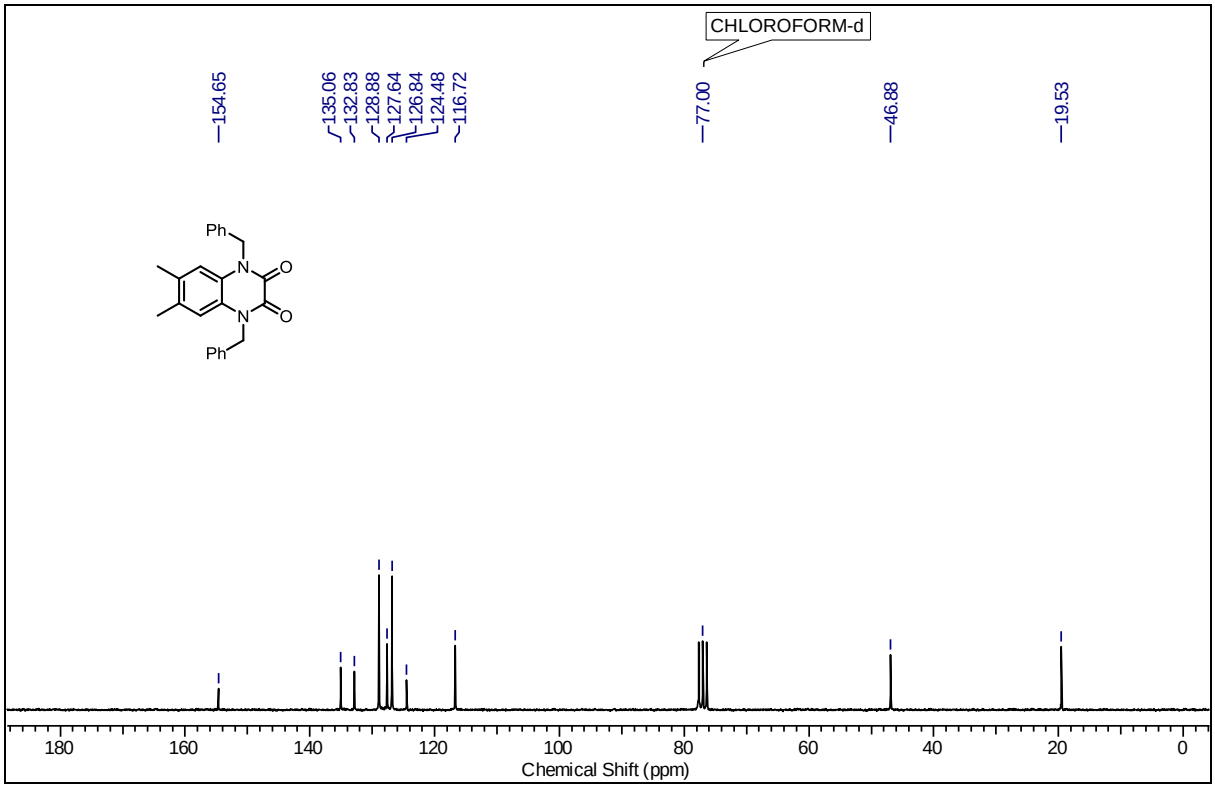
^{1}H NMR of Compound 46 at 200 MHz in $CDCl_3$



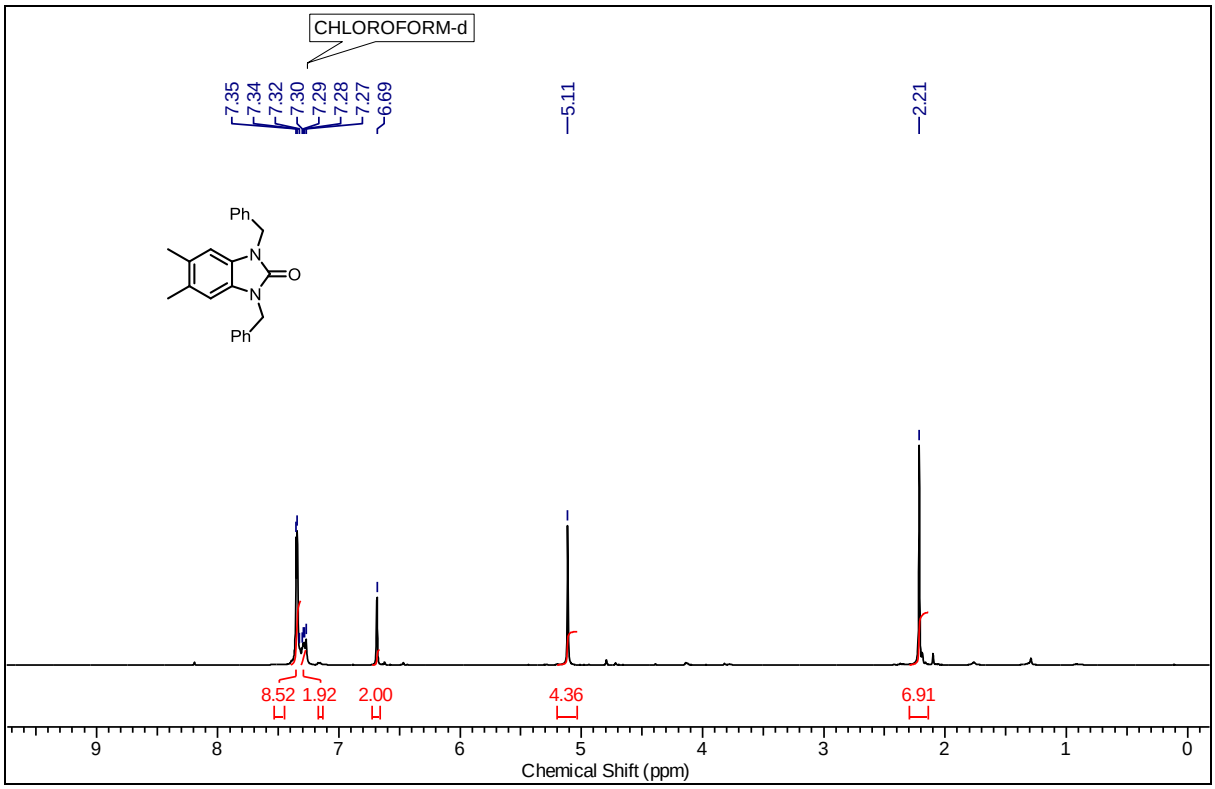
^{1}H NMR of Compound 67 at 200 MHz in $CDCl_3$



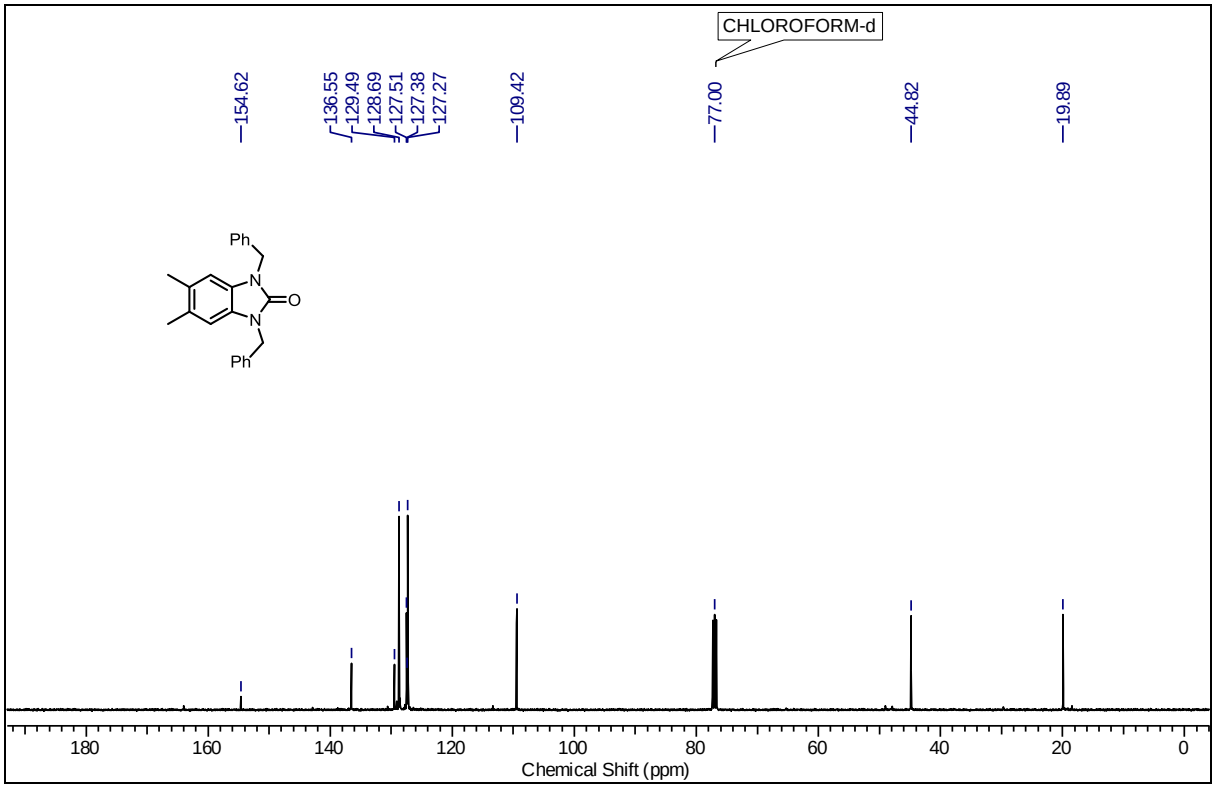
^{13}C NMR of Compound 67 at 50 MHz in $CDCl_3$



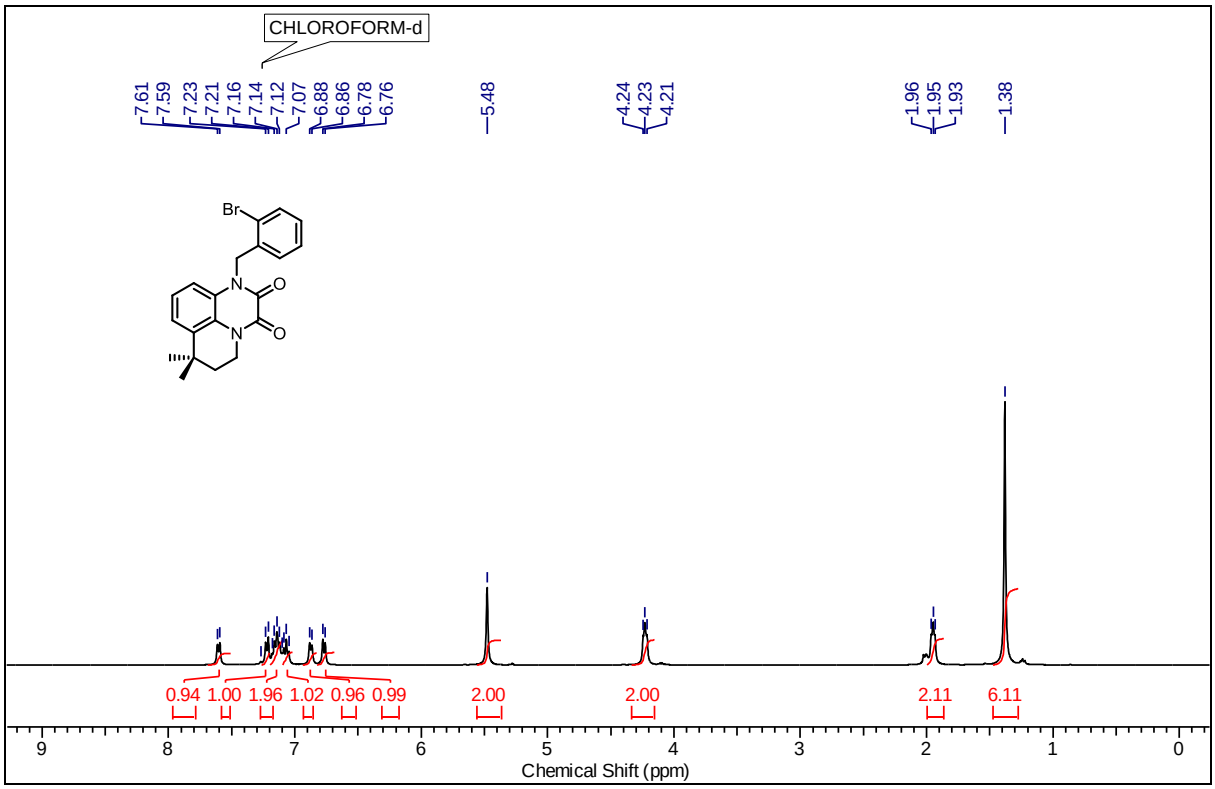
^{1}H NMR of Compound 68 at 400 MHz in $CDCl_3$



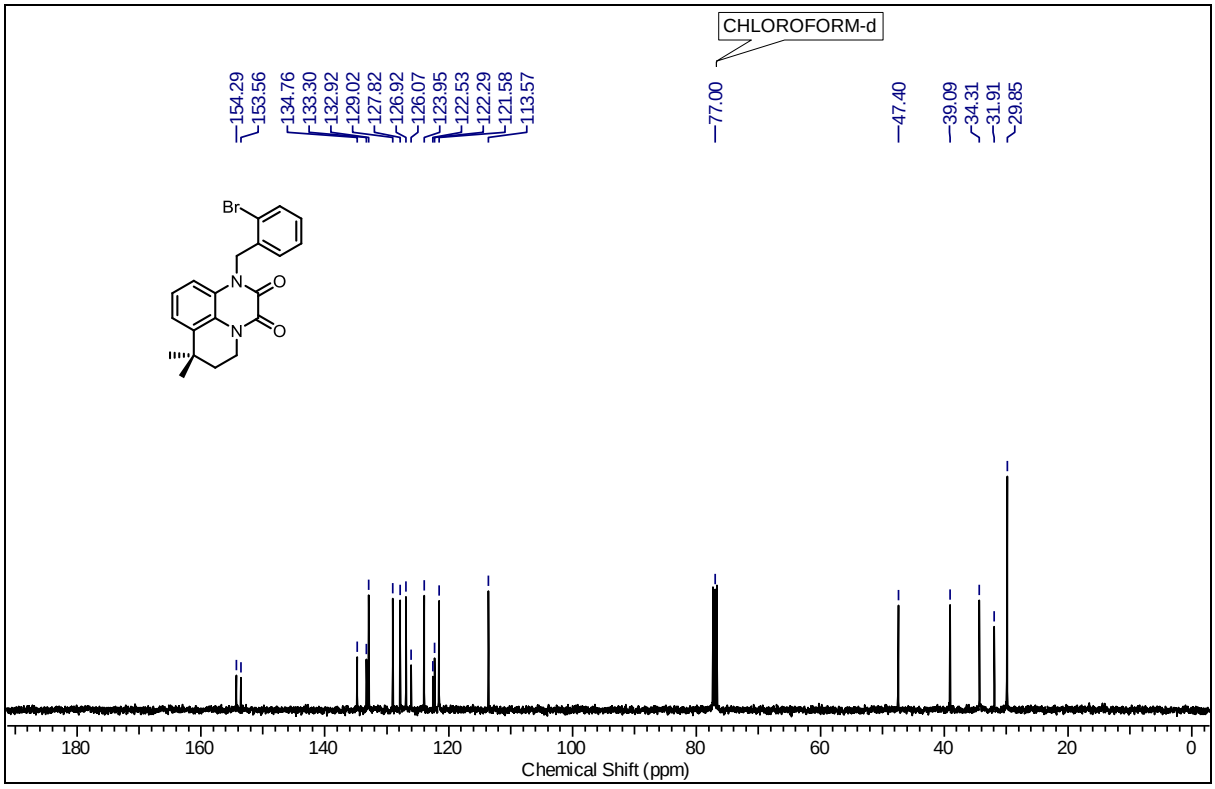
^{13}C NMR of Compound 68 at 100 MHz in $CDCl_3$



^{1}H NMR of Compound 89 at 400 MHz in $CDCl_3$



^{13}C NMR of Compound 89 at 100 MHz in $CDCl_3$



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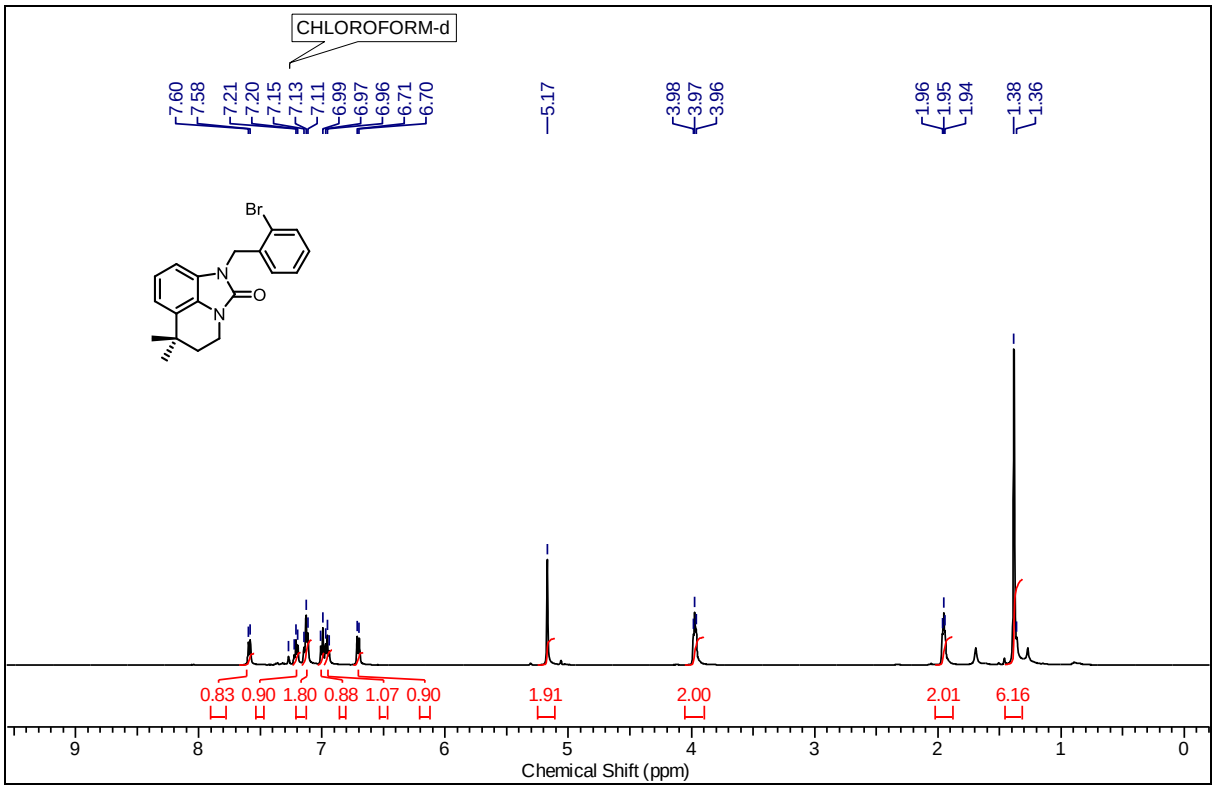

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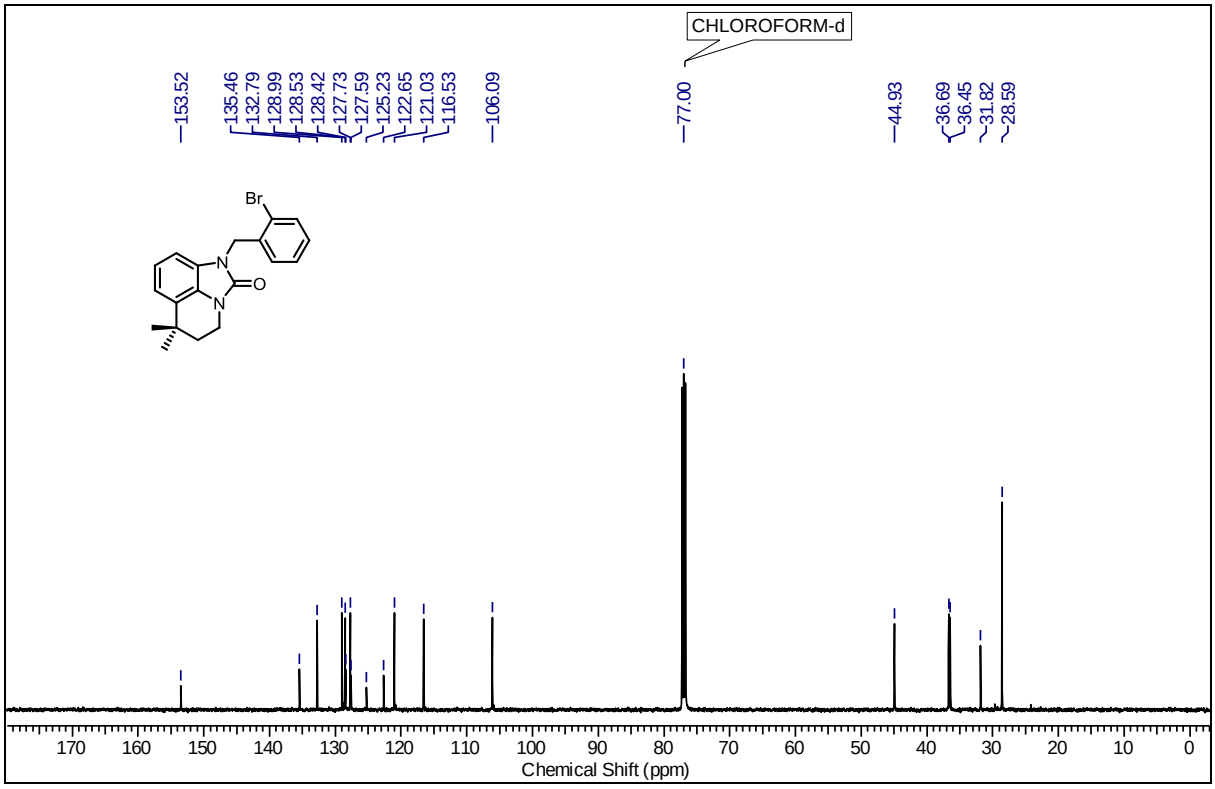

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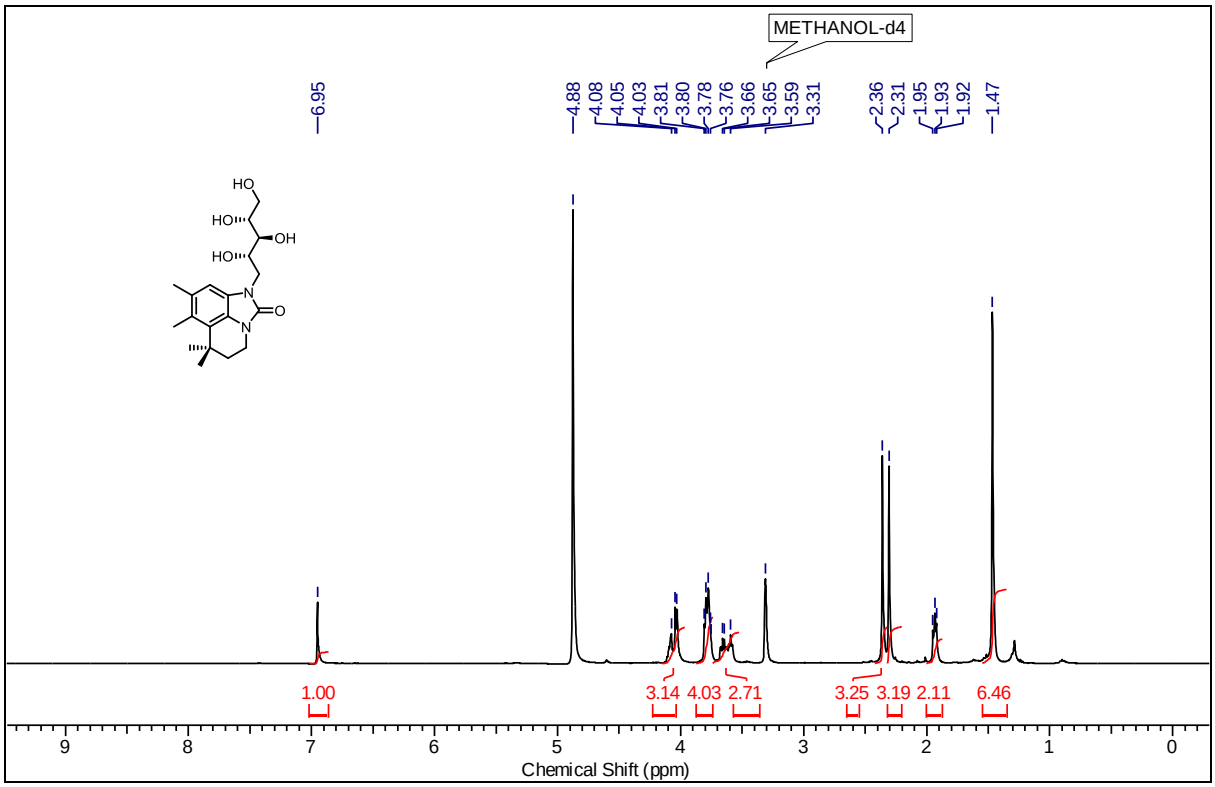

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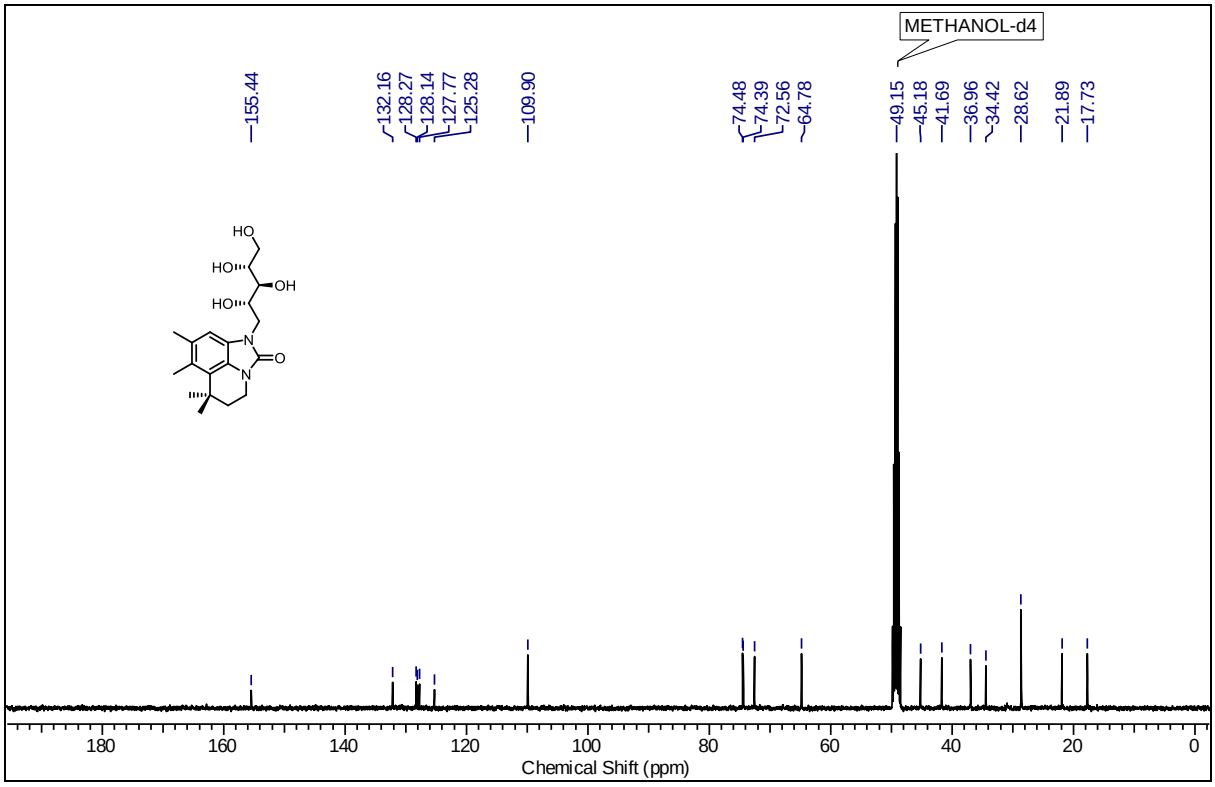

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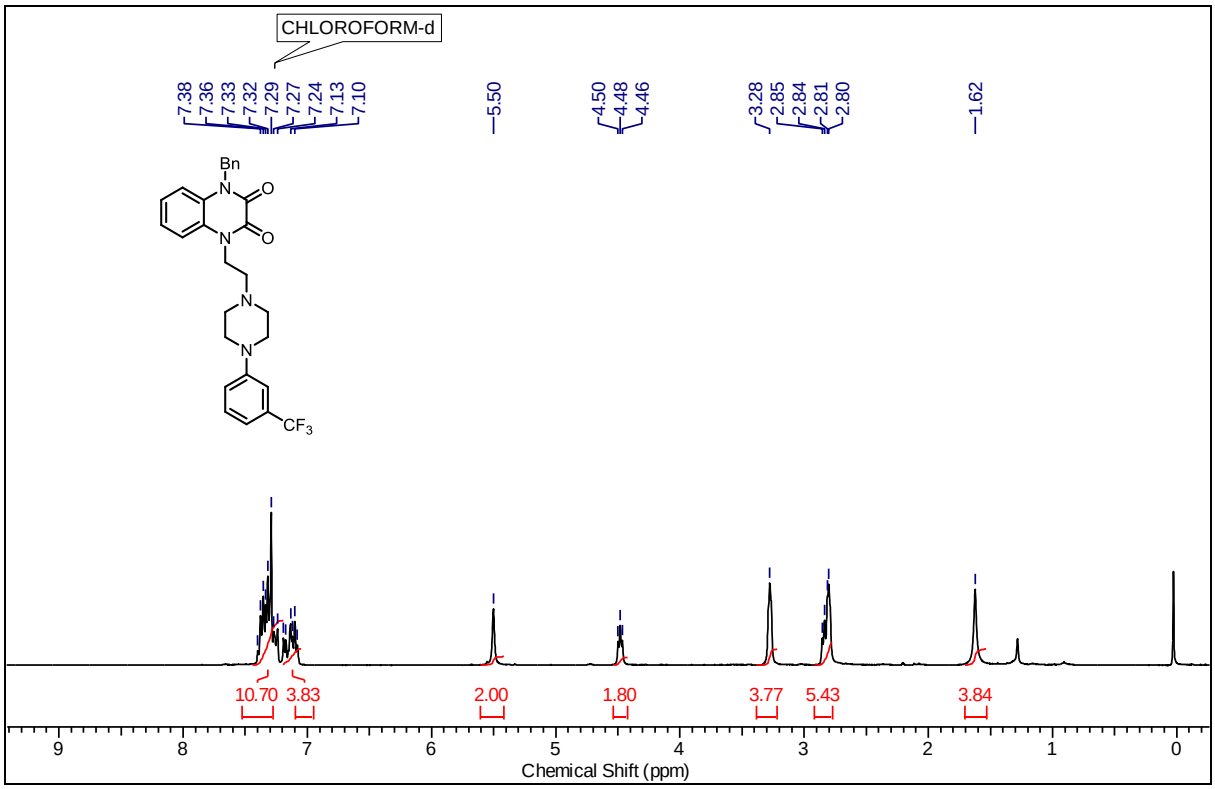

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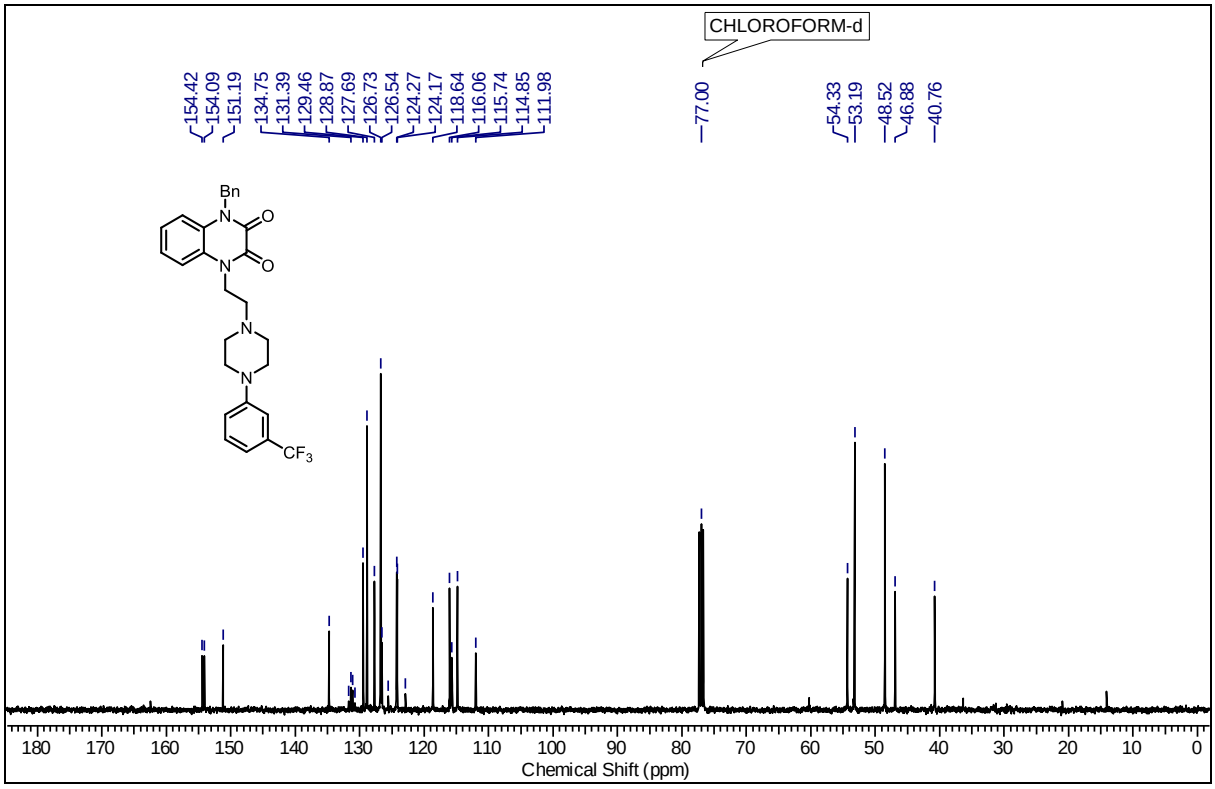

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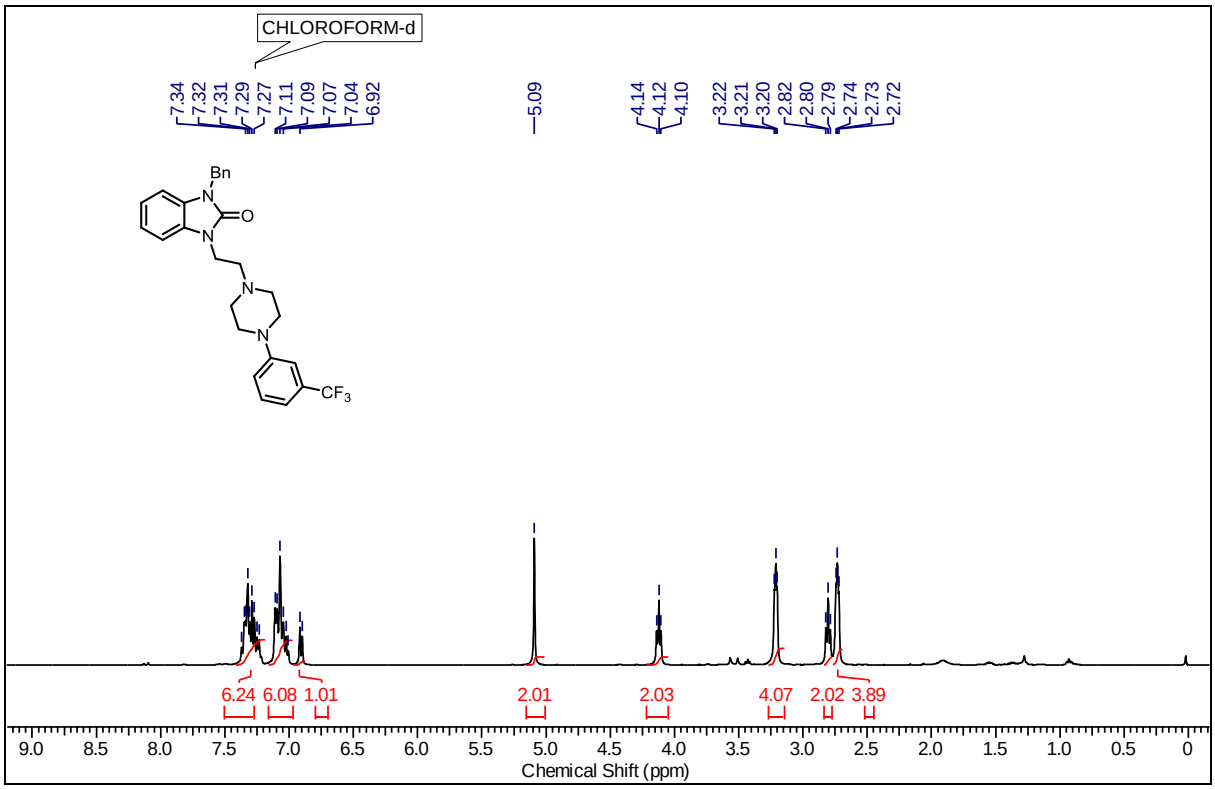

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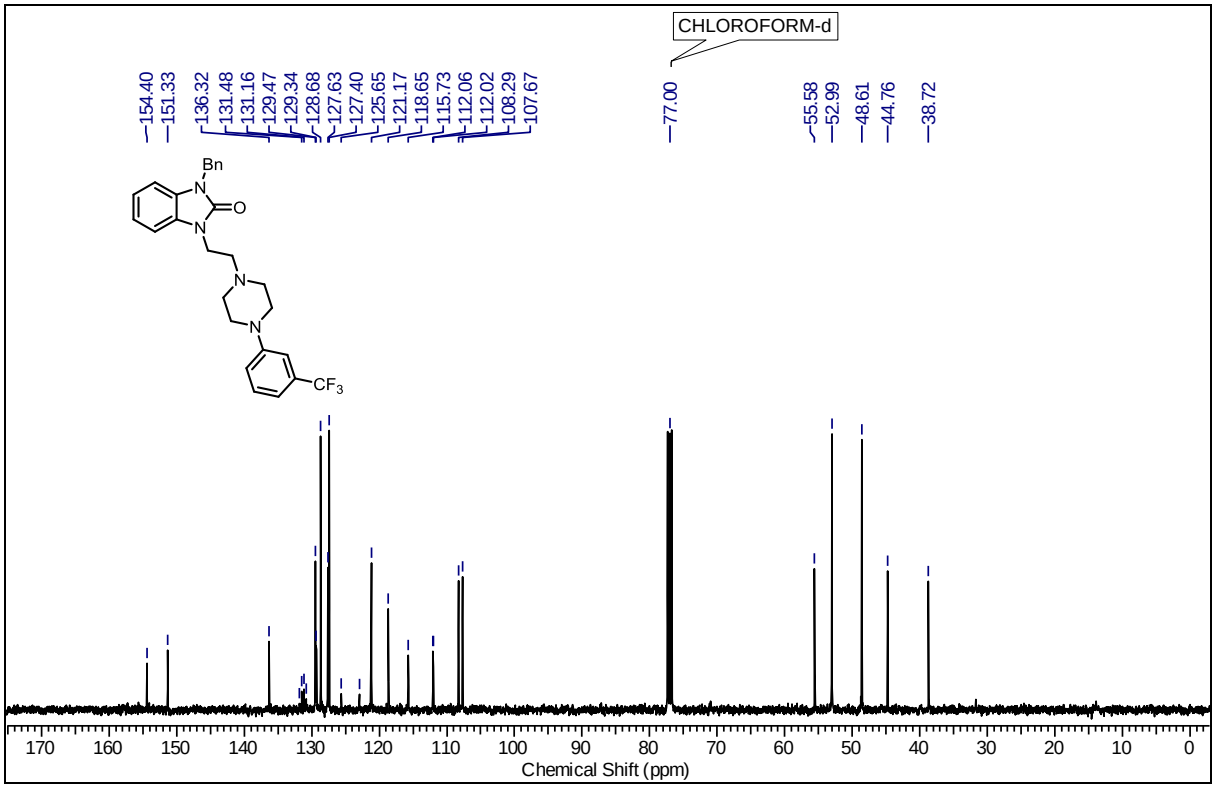

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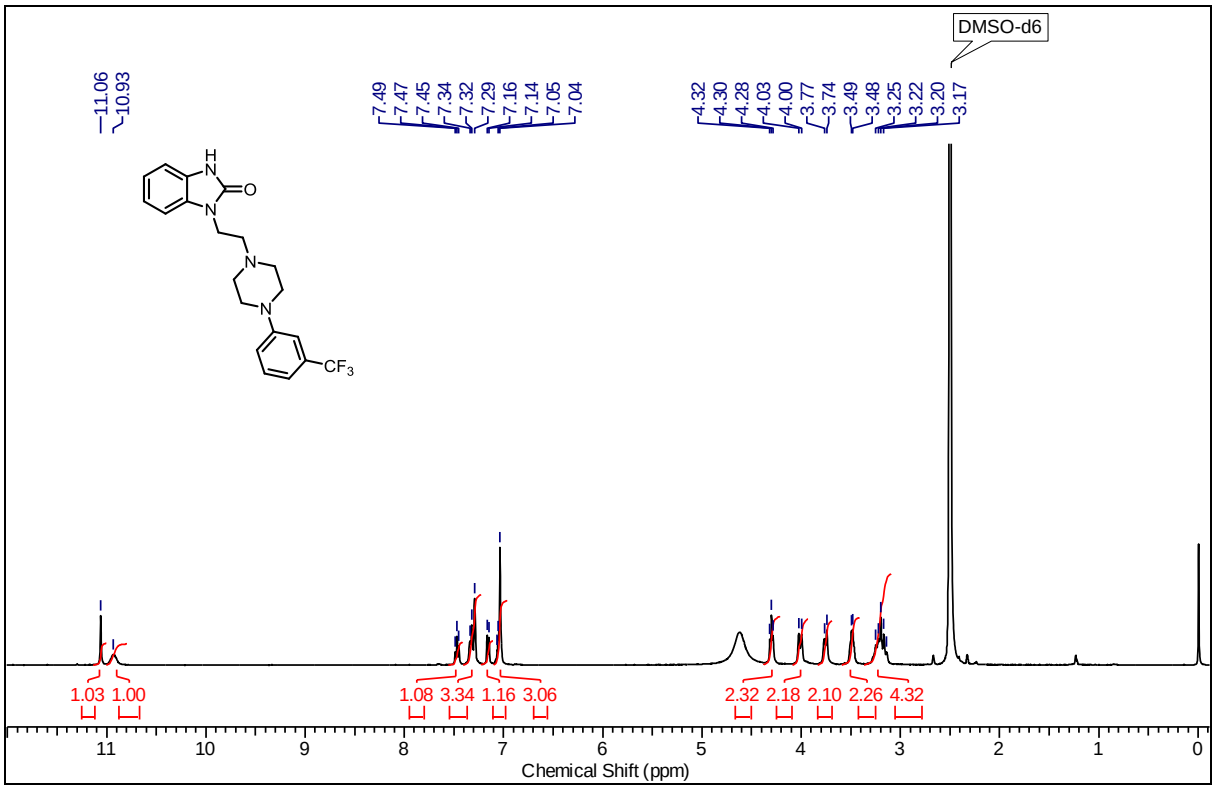

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1. **Shingare, R. D**.; Velayudham, R.; Gawade, J. R.; Reddy, D. S. "First total synthesis of Hunanamycin A" *Org. Lett*. **2013**, *15*, 4556.
2. Sutar, R. L.; Kumar, V.; **Shingare, R. D**.; Thorat, S.; Gonnade, R.; Reddy, D. S. "A general approach to *N*-heterocyclic carbenes with a fused tetracyclic core: Ligands for Suzuki–Miyaura cross-coupling reaction" *Eur. J. Org. Chem*. **2014**, *2014*, 4482.
3. **Shingare, R. D.**; Sa ada Farhana.; Reddy, D. S. "Multi-gram scale synthesis of Hunanamycin A, an antibiotic natural product from the marine source" *Tetrahedron Lett.* **2016**, *57*, 3662.
4. Ramesh R.; **Shingare, R. D**.; Kumar, V.; Anand, A.; Swetha B.; Veeraraghavan, S.; Viswanadha, S.; Ummanni, R.; Gokhale, R.; Reddy, D. S. "Repurposing of a drug scaffold: Identification of novel sila analogues of rimonabant as potent antitubercular agents" *Eur. J. Med. Chem*. **2016**, *2014*, 723.
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6. **Shingare, R. D.**; MacMillan, J. B; Reddy, D. S "Riboflavin synthase inhibitor Hunanamycin A; Lead identification towards anti-*salmonella* agents" *Manuscript under preparation.*
7. **Shingare, R. D**.; Velayudham, R.; Reddy, D. S. US 20160152616 A1 "Novel tricyclic compounds and process for preparation thereof"
8. Ramesh R.; **Shingare, R. D**.; Reddy, D. S. WO 2014181357 A1 "Novel pyrazole derivatives with silicon incorporation"
9. Sutar, R. L.; Kumar, V.; **Shingare, R. D**.; Reddy, D. S. WO 2015102020 A1 "Novel *N*-heterocyclic carbene compounds, their preparation and use"

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First Total Synthesis of Hunanamycin A

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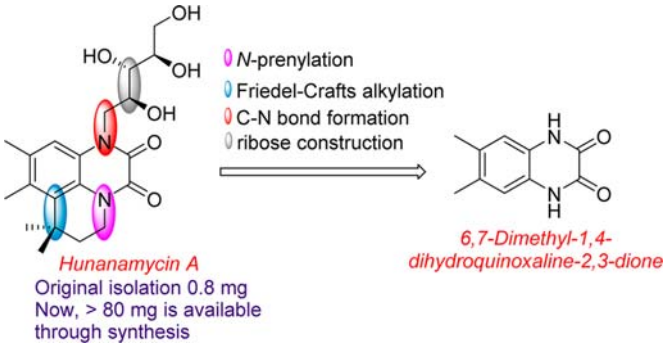
ABSTRACT



The first total synthesis of hunanamycin A, an antibiotic natural product with a pyrido[1,2,3-*de*]quinoxaline-2,3-dione core from a marine-derived *Bacillus hunanensis*, is disclosed. The present effort provides access to sufficient amounts of scarce hunanamycin A for further biological evaluation and confirmation of the assigned absolute configuration. In addition, four new analogues of the natural product are reported.

Very recently, the title compound hunanamycin A (**1**), a natural product, was isolated from a marine-derived *Bacillus hunanensis* by MacMillan et al.[1] The gross structure and stereochemical assignments (Figure 1) of the compound **1** were determined using extensive spectroscopic data analysis and published in this journal.[1] As the structure of hunanamycin A was related to riboflavin degradation products, the MacMillan group screened compound **1** for antimicrobial activity against bacterial strains that lacked riboflavin transport mechanisms. The results showed a minimum inhibitory concentration (MIC) of 12.4 μM against *Salmonella enterica* suggesting it is an inhibitor of riboflavin synthase (*ribB*).[1] Riboflavin synthase is an enzyme that catalyzes the final step in the biosynthesis of riboflavin.[2] In disease models of *Salmonella* infection, knockout of the riboflavin synthase was found to be lethal to the pathogen.[3] The *ribB* has shown to be an attractive antibiotic target, as humans lack this enzyme.[4] Significant efforts from Cushman's group along these lines are worth highlighting.[4] *Salmonella enterica*, the most pathogenic species of *Salmonella*, causes foodborne illness and accounted for considerable damage to humans in both developed and developing countries. For instance, in the United States it was estimated that *Salmonella* ranks number one, accounting for 35% of total foodborne illnesses in the country. About 3000 deaths in the United States are recorded annually owing to foodborne illnesses.[5] Therefore, any step toward addressing these problems are rewarding and satisfying. Another interesting aspect of the hunanamycin A molecule is that it does not violate the Lipinski rule of five.[6]

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Multi-gram scale synthesis of hunanamycin A, an antibiotic natural product from the marine source

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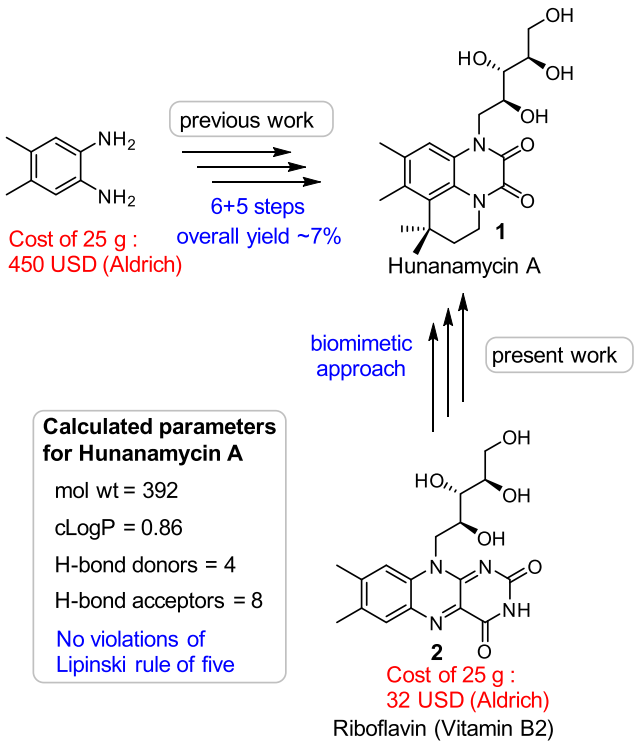
ABSTRACT

A simple, practical, and biomimetic approach to access antibiotic natural product hunanamycin A starting from readily available inexpensive material Riboflavin is disclosed here. The present synthesis consists of three operationally simple, protecting group free steps and it is far superior when compared with the previous route. Using this route one can make multi-gram quantities of the natural product which will help in further biological assays, in particular exploring the potential of treating food infections.

Hunanamycin A (**1**), was isolated (0.8 mg) from a marine-derived *Bacillus hunanensis* by MacMillan et al.[1] It is the first natural product with a pyrido[1,2,3-*de*]quinoxaline-2,3-dione core related to a degradation product of riboflavin (vitamin-B2). Hunanamycin A was reported to have shown interesting antimicrobial activity against bacterial strains (*Salmonella enterica*) that lacked riboflavin transport mechanisms.[1] It was shown to have minimum inhibitory concentration (MIC) of 4.8 μg/mL against *Salmonella enterica*. It is estimated that Salmonella bacteria is one of the major causes for outbreaks associated with hospitalizations.[2] According to very recent WHO report, the global burden of foodborne diseases shows that almost 420,000 people die every year by eating contaminated food.[3] The same also estimated that the African and South-East Asia Regions have the highest burden of foodborne diseases.[3] Hence there is a need for finding new drugs with novel mechanisms. In addition to interesting antibacterial activity, hunanamycin A also complies all the rules of Lipinski, frequently practiced in medicinal chemistry suggesting that this is a promising lead for further studies. Interesting biological activity, drug-like properties, and scarcity of the natural material prompted our group[4] and others[5] to initiate total synthesis programs. Recently, we have accomplished the first total synthesis of the hunanamycin A starting from 4,5-dimethyl-benzene-1,2-diamine in ~7% overall yield (Scheme 1).[4] Although



Scheme 1. Synthetic approaches to hunanamycin A.

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Article

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Route to Benzimidazol-2-ones via Decarbonylative Ring Contraction of Quinoxalinediones: Application to the Synthesis of Flibanserin, A Drug for Treating Hypoactive Sexual Desire Disorder in Women and Marine Natural Product Hunanamycin Analogue

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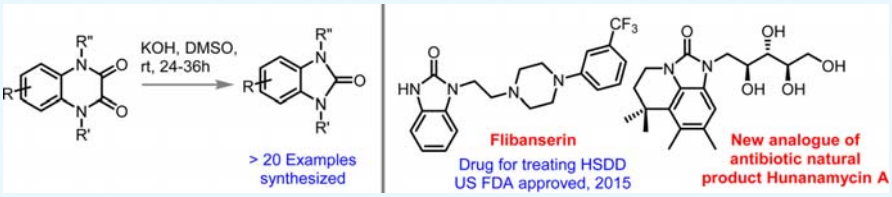
Supporting Information



ABSTRACT: A simple and practical method to access a variety of benzimidazol-2-ones is reported here. A series of *N*-alkyl-substituted benzimidazol-2-ones were synthesized by decarbonylative ring contraction starting from corresponding quinoxalinediones for the first time. The utility of the method has been demonstrated by synthesizing recently approved controversial drug flibanserin (Addyi) and a urea analogue of marine antibiotic natural product hunanamycin-A.

INTRODUCTION

Benzimidazol-2-ones **1** are an important class of heterocycles and a privileged scaffold in medicinal chemistry. They consist of cyclic urea fused with the aromatic backbone, which can potentially interact in a biological system by various non-covalent interactions such as hydrogen bonding and π stacking. Benzimidazolone derivatives exhibit a wide range of biological activities, and they are useful in treating various diseases including cancer, type II diabetes, central nervous system disorders, pain management, and infectious disease.[1] Selected compounds embedded with a benzimidazol-2-one moiety along with their use are captured in Figure 1. It is worth mentioning that oxatomide drug with a benzimidazol-2-one core was approved for marketing a few years ago.[2a] Very recently, US Food and Drug Administration approved a new drug called flibanserin for the treatment of hypoactive sexual desire disorder (HSDD) in females, which contains benzimidazol-2-one motif.[2b]

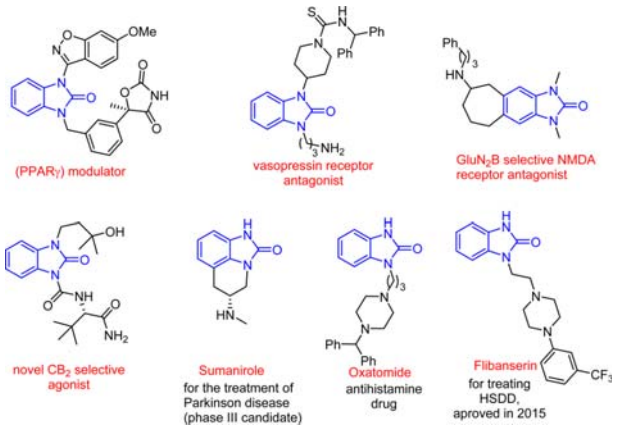


Figure 1. Examples of biologically important benzimidazolones.

RESULTS AND DISCUSSION

Considering interesting biological activity and the importance of this scaffold, we sought to develop a new and efficient method for the preparation of benzimidazolones. Literature search revealed that most of the methods for the synthesis of a benzimidazol-2-one core rely on carbonylation of benzene-1,2-diamines **2** or cyclization of appropriately substituted phenyl urea **3**. The carbonylation reaction of benzene-1,2-diamines requires the use of phosgene, triphosgene, or carbonyl diimidazole.[3] To avoid the use of such hazardous chemicals, alternative procedures using transition metals (palladium[4] and copper[5])-catalyzed intramolecular **3** or intermolecular **4** cyclization or C−H oxidation[6] reactions were developed. Some of the interesting interconversions of heterocyclic rings for the synthesis of benzimidazol-2-ones are also reported. According to Zhou's work, aminomethylene benzimidazoles **5** and alkyl halide in the presence of a base produced

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Short communication

Repurposing of a drug scaffold: Identification of novel sila analogues of rimonabant as potent antitubercular agents

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ABSTRACT

The structural similarity between an MmpL3 inhibitor BM212, and a cannabinoid receptor modulator rimonabant, prompted us to investigate the anti-tubercular activity of rimonabant and its analogues. Further optimization, particularly through incorporation of silicon into the scaffold, resulted in new compounds with significant improvement in anti-tubercular activity against *Mycobacterium tuberculosis* (H37Rv). The sila analogue **18a** was found to be the most potent antimycobacterial compound (MIC, 31 ng/mL) from this series with an excellent selectivity index.

1. Introduction

Drug discovery has become an increasingly tough endeavor. The average cost of bringing a drug into market is estimated to be about US$ 2600 million with a timeline of up to 15 years [1]. Identification of entirely novel compounds with unknown pharmacokinetic and safety profile poses more risk apart from being expensive and time consuming. The discovery of new indications for an existing drug termed "drug repurposing or drug reprofiling" is a lower risk strategy with increased probability of success within a short span of time. According to a recent report, 24 drugs had been remarketed for new uses and more than 15 are currently in the various developmental stages [2–4]. Also, molecules that failed in the clinic for a particular indication can be successfully repurposed for treatment of other conditions. Thalidomide, the most controversial drug of all time, is now used for pain relief in certain cancers and leprosy [5]. Pfizer's blockbuster drug sildenafil citrate (Viagra®), which was originally intended for hypertension was serendipitously repurposed after the Phase I clinical trials for erectile dysfunction [6].

Tuberculosis (TB) is an infectious disease caused by various strains of mycobacteria; the most common one being *Mycobacterium tuberculosis* (Mtb). Almost one-third of the total world population is asymptomatically infected by Mtb and it is the second leading cause of death due to an infectious agent [7]. Medications are known to treat TB; however, the response rate is slow with development of antibiotic resistance posing a serious threat. In view of these challenges, there is a need to develop new drug candidates with novel mechanisms for treating tuberculosis. The pre-clinical candidate BM212, a 1,5-aryl substituted pyrrole was

Abbrevations: MIC, Minimum Inhibitory Concentration; ADME, absorption, distribution, metabolism, excretion; CB1, Cannabinoid receptor Type 1; Mtb, *Mycobacterium tuberculosis*; CNS, Central nervous system; TBAF, Tetrabutylammonium fluoride; INH, Isoniazid; DMPK, Drug metabolism and pharmacokinetics; PPB, Plasma protein binding; A549 cells, human alveolar adenocarcinoma cell line; HepG2 cells, human liver hepatocellular carcinoma cell line.

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SHORT COMMUNICATION

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A General Approach to N-Heterocyclic Carbenes with a Fused Tetracyclic Core: Ligands for Suzuki–Miyaura Cross-Coupling Reaction

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Keywords: Synthetic methods / Homogeneous catalysis / N ligands / C–C coupling / Carbenes

The synthesis of an N-heterocyclic carbene (NHC) based on a tetracyclic scaffold by using simple, general, and scalable chemistry is disclosed. The developed route is suitable for introducing multiple substitutions on the tetracyclic scaffold. The utility of the present NHC as a ligand in the Suzuki–Miyaura cross-coupling reaction is demonstrated with a low catalyst loading.

Introduction

Recently N-heterocyclic carbenes (NHCs) have emerged as an important class of ligands and catalysts because of their several attractive features, and they have thus received significant attention from the scientific community.[1] However, of the imidazolylidenes,[2] benzimidazole-derived carbenes,[3] more particularly constrained ones with a tetracyclic scaffold,[4] have been less explored (Figure 1). Only the group of Metallinos has reported the synthesis of fused tetracyclic NHCs and showed their application.[4] Considering the importance of NHCs in several important reactions,[1] there is a need to search for new NHCs with varying structural motifs by using simple and scalable chemistry.[5,6] Inspired by the work of Metallinos, we became interested in the synthesis and applications of tetracyclic NHCs. Herein, we disclose the first synthesis of an NHC based on a tetracyclic scaffold by using a simple and general method, and we further report on its utility.

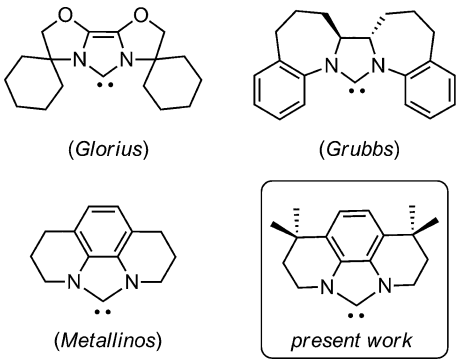

Figure 1. Selected NHCs with constraints documented in the literature.

Results and Discussion

We envisioned the synthesis of the desired NHCs of type **A** from commercially available dihydrobenzimidazol-2-ones **D** through the intermediacy of tetracyclic urea derivatives **B** and dialkylated benzimidazol-2-ones **C**, as shown in Scheme 1. On the basis of this general strategy, we hoped to access NHCs with multiple substituents including chiral ones. With this plan in mind, dihydrobenzimidazol-2-one **1** was diprenylated by using prenyl bromide and NaH as the base, which provided **2** in 82% yield.

Diprenylated **2** was then cyclized to give highly symmetric, tetracyclic urea **3** by using $AlCl_3$ in anhydrous chlorobenzene at room temperature.[7,8] The urea linkage was then cleaved by reduction with $LiAlH_4$ to the hydroaminal, which was followed by heating at reflux with 6 N aqueous HCl to furnish tricyclic diamine **4** in overall good yields.[4c] Notably, constrained diamine **4** has tremendous potential, particularly if it is chiral.[9] Diamine **4** was transformed into benzimidazolium salt **5** by using triethyl orthoformate and HCl under heating at 70 °C.[4a] Efforts to prepare this salt from the hydroaminal intermediate directly always resulted in low yields. We were able to generate the NHC directly from chloride salt **5** in THF solvent. We were not successful in isolating the NHC as such, but it was trapped in situ as thiourea **6**. Compound **6** was unambiguously characterized by spectral means and also by single-crystal X-ray structure analysis (Scheme 2). In addition, we prepared borane com-

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(54) **NOVEL TRICYCLIC COMPOUNDS AND PROCESS FOR PREPARATION THEREOF**

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(72) Inventors: **Dumbala Srinivasa Reddy**, Pune, Maharashtra (IN); **Rahul Dilip Shingare**, Pune, Maharashtra (IN); **Velayudham Ramadoss**, Pune, Maharashtra (IN)

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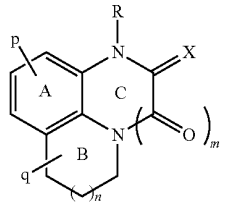
(57) **ABSTRACT**

The present invention relates to novel tricyclic compounds of formula (I) and (II) More particularly, the present invention relates to novel tricyclic compounds of formula (I) and (II) and process of preparation of these compounds from 4,5-dimethyl-o-phenylinediamine. Further, the present invention relates to a process for preparation of tricyclic compound hunanamycin A.

I



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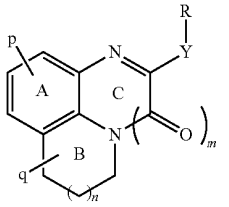

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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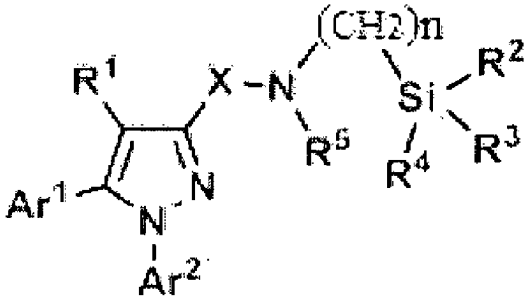
(54) Title: NOVEL PYRAZOLE DERIVATIVES WITH SILICON INCORPORATION



Formula-I

(57) Abstract: Disclosed herein, novel sila analogs of pyrazole compounds of Formula-I and process for preparation thereof. Further the invention relates to pharmaceutical compositions comprising of novel sila analogs of pyrazole compounds of formula-I, which show better activity than other analogs as antiobesity molecules and also show antitubercular activities.

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(71) Applicant: **COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH** [IN/IN]; Anusandhan Bhawan, Rafi Marg, 110 001 New Delhi (IN).

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(74) Agent: **CHOPRA, Priyanka**; K & S Partners, Intellectual Property Attorneys 109, Sector 44, National Capital Region, 122003 Gurgaon (IN).

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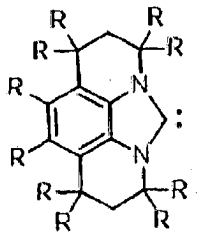
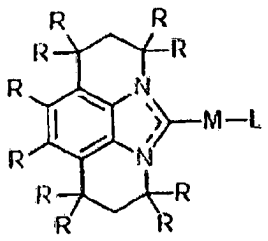
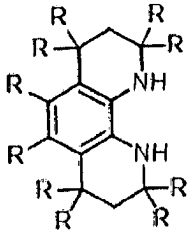
(54) Title: NOVEL N-HETEROCYCLIC CARBENE COMPOUNDS, THEIR PREPARATION AND USE



Formula (I)



Formula (II)



Formula (IV)

(57) Abstract: The present invention relates to novel N-heterocyclic carbenes compounds and process of the preparation thereof. The present invention further relates to the use of use of these novel N-heterocyclic carbenes as ligand in the Suzuki-Miyaura cross coupling reactions.

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