DESIGN SYNTHESIS AND BIOEVALUATION OF BILE ACID β-LACTAM CONJUGATES, STUDIES DIRECTED TOWARDS THE SYNTHESIS OF SQUALAMINE AND ENANTIOSELECTIVE REDUCTION OF PROCHIRAL KETONES USING CHIRAL AMINO ALCOHOLS

THESIS
SUBMITTED TO THE
UNIVERSITY OF PUNE
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

BY
Mr. NAMDEV S. VATMURGE

Dr. BRAJA G. HAZRA
(RESEARCH GUIDE)

DIVISION OF ORGANIC CHEMISTRY
NATIONAL CHEMICAL LABORATORY
PUNE 411 008, INDIA
AUGUST 2009

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Dedicated to my Parents and Brother....

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August 04, 2009

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "*Design Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates, Studies Directed Towards the Synthesis of Squalamine and Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols*" which is being submitted to the *University of Pune* for the award of *Doctor of Philosophy* in *Chemistry* by *Mr. Namdev Sangram Vatmurge* was carried out by him under my supervision at the National Chemical Laboratory, Pune. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

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CANDIDATE'S DECLARATION

I hereby declare that the thesis entitled "*Design Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates, Studies Directed Towards the Synthesis of Squalamine and Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols*" submitted by me for the degree of *Doctor of Philosophy* in *Chemistry* to the *University of Pune* is the record of work carried out by me during the period *August, 2003 to July, 2009* and has not been submitted by me for a degree to any other University or Institution. This work was carried out at Division of Organic Chemistry, National Chemical Laboratory, Pune, India.

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August 2009

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Special thanks to Sonali, my wife, for her love, affection and support extended to me during this work. I thank our beloved son "Arnav" for making our life more beautiful than it was.

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Namdev

CONTENTS

GENERAL REMARKS

- Independent reference and compound numbering have been employed for abstract, as well as each chapter (Chapter 1-3).
- All the solvents used were purified using the known literature procedures.
- Petroleum ether used in the experiments was of 60-80 °C boiling range.
- Column chromatographic separations were carried out by gradient elution using silica gel (60-120 mesh/230-400 mesh) or neutral deactivated alumina with light petroleum ether-ethyl acetate mixture, unless otherwise mentioned.
- TLC was performed on E-Merck pre-coated silica gel 60 F_{254} plates and the spots were rendered visible by exposing to UV light, iodine, charring or staining with ninhydrin, *p*-anisaldehyde or phosphomolybdic acid solutions in ethanol.
- Microwave irradiation was carried out in an open glass vessel using a domestic microwave oven (800 watt, BPL-make).
- Usual work up: organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*.
- Crystallization: Single crystals of the compounds were grown from a hot saturated filtered solution of these compounds in particular solvent. Suitable crystals were obtained by slow evaporation of the solvent at room temperature (RT).
- All the melting points reported are uncorrected and were recorded using Yanco electro-thermal melting point apparatus.
- Ultraviolet (UV) spectra were performed using Perkin-Elemer instrument, Lambda 35 UV/VIS Spectrometer.
- IR spectra were recorded on Shimadzu FTIR instrument, for solid either as nujol mull or in chloroform solution and neat in case of liquid compounds.
- NMR spectra were recorded on Bruker ACF 200 and AV200 (200.13 MHz for ^{1}H NMR and 50.03 MHz for ^{13}C NMR), MSL 300 (300.13 MHz for ^{1}H NMR and 75.03 MHz for ^{13}C NMR), AV 400 (400.13 MHz for ^{1}H NMR and 100.03 MHz for ^{13}C NMR) and DRX 500 (500.13 MHz for ^{1}H NMR and 125.03 MHz for ^{13}C NMR) spectrometers. Chemical shifts (δ) reported are referred to internal reference tetramethylsilane (TMS). The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = doublet of doublet, dt = doublet of triplet and ddd = doublet of doublet of doublet.

- Mass spectra were recorded on Finnigan-Mat 1020C mass spectrometer and were obtained at an ionization potential of 70 eV or on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using Electrosprey Ionization Technique. EI and CI mass spectra were recorded on an AEI MS-50 and AEI MS-9 spectrometer, respectively.
- Micro analytical data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyzer. Elemental analyses observed for all the newly synthesized compounds were within the limits of accuracy (± 0.4%).
- Optical rotations were obtained on Bellingham & Stanley ADP-220 Polarimeter. Specific rotations ($[\alpha]^{D}$) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent.
- All the compounds previously known in the literature were characterized by comparison of their R_f values on TLC, IR and NMR spectra as well as melting point with authentic samples.
- Starting materials were obtained from commercial sources or prepared using known procedures.

ABBREVIATIONS

Ac	Acetate
AIDS	Acquired Immunodeficiency Syndrome
Amp B	Amphotericin B
Aq.	Aqueous
Ar	Aryl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Cat.	Catalytic
Cbz	Benzyloxy
CCDC	Cambridge Crystallographic Data Centre
CSD	Cambridge Structural Database
CSA	Camphoresulfonic acid
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DHP	Dihydropyran
DMAP	4-(Dimethylamino)pyridine
DMEM	Dulbecco/Vogt modified Eagle's Minimal Essential Medium
DMF	Dimethylformamide
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-Tetraacetic acid
16-DPA	16-Dehydropregnenolone acetate
DTPA	Diethylenetriamine Pentaacetic acid
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ee	Enantiomeric Excess
Et	Ethyl
EtOAc	Ethyl acetate

EtOH	Ethanol
equiv.	Equivalent(s)
GR	Glucocorticoid Receptor
g	Grams
h	Hour(s)
HEK293	Human embryonic kidney cells
HGO	Hepatic Glucose Output
Hz	Hertz
HIV	Human immunodeficiency virus
HOBt	1-Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HSV	Herpes Simplex Virus
IBX	2-Iodoxybenzoic acid
IC	Inhibitory Concentration
i-Pr	isopropyl
IR	Infra Red
LAH	Lithium Aluminum Hydride
M	Molar
MCF-7	Human Mammary Adenocarcinoma Cells
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
min.	Minute(s)
mL	Millilitre(s)
µM	Micromolar
mmol	Millimole(s)
MOMCl	Methoxymethyl chloride
Mp	Melting Point
MS	Mass Spectrum

MS 4Å	Molecular Sieves (4Å)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Microwave
NCCLS	National Committee for Clinical Laboratory Standard
NCIM	National Collection of Industrial Micro-Organisms
NMR	Nuclear Magnetic Resonance
ORTEP	Oak Ridge Thermal Ellipsoid Plots
PCC	Pyridinium Chlorochromate
PDA	Potato Dextrose Agar
Ph	Phenyl
PMP	*p*-Methoxyphenyl
PPTS	Pyridinium *para*-tolune sulfonate
p-TSA	*p*-Toluenesulfonic acid
Py	Pyridine
rt	Room Temperature
SAR	Structure Activity Relationships
TBDMS	*t*-Butyldimethylsilylyl
TBDMSCl	*t*-Butyldimethylsilyl chloride
TBDPS	*t*-Butyldiphenylsilylyl
TBDPSCl	*t*-Butyldiphenylsilyl chloride
Temp.	Temperature
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin Layer Chromatography
TMSCl	Trimethylchlorosilane

ABSTRACT

The thesis entitled **"Design Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates, Studies Directed Towards the Synthesis of Squalamine and Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols."** has been divided into three chapters.

Chapter 1: Design Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates and is further divided into three sections.

Chapter 2: Studies Directed Towards the Synthesis of Squalamine.

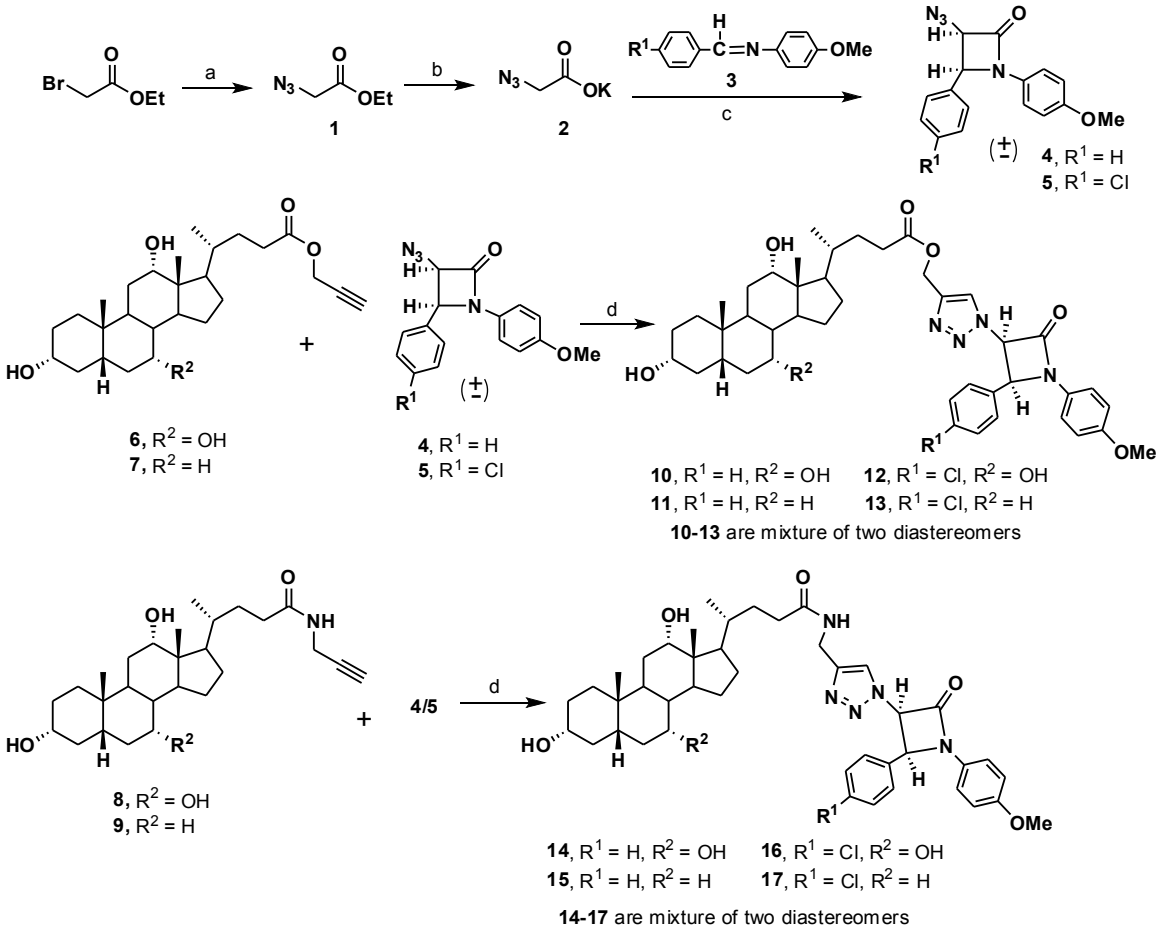
Chapter 3: Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols.

Chapter 1: Design, Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates.

Bile acids have been considered very useful in the preparation of new pharmaceutical drugs because of their inherent chemical and biological properties.[1] They are pharmacologically interesting as potential carriers of liver specific drugs, absorption enhancers and cholesterol lowering agents.[2] A common feature of bile acid derived antimicrobials is their potential to exhibit facially amphiphilic nature, due to polar hydroxyl groups on one face and nonpolar hydrophobic methyl group on the other. This type of amphiphilicity can also be exhibited by polyene macrolide amphotericin B, peptide antimicrobial agent polymixin B, and squalamine in the cyclic form which functions as an inophores. Several cholic acid derived facial amphiphiles have been reported,[3] that improve the permeability of membranes including bacterial cell wall. Furthermore, bile acids are imperative building blocks for the synthesis of dimers, oligomers and colaphanes[4, 5] due to their rigid framework with multiple chiral centers. The dimers, oligomers and colaphanes were synthesised from bile acids have a wide range of potential applications exists in pharmacology,[5] membrane bilayer probes, and ion complexation. Isolation of two naturally occurring steroidal β-lactams from the plants *Pachysandra terminalis*[6] and *Pachysandra procumbens*[7] has been reported. Steroidal β-lactam, isolated from *P. procumbens* is known as antiestrogen binding site inhibitory agent. There are two reports for synthetically prepared β-lactam on steroid and the biological activity data of these compounds has not been revealed.

Section A: Design, Synthesis and Antimicrobial Activity of β-Lactam-Bile Acid Conjugates Linked *via* 1,2,3-Triazole.

Azoles are the largest class of antifungal agents in clinical use.[8] 1,2,3-Triazole moieties are attractive connecting units, as they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and solubility. The β-lactam ring system in combination of 1,2,3-triazole moiety is present in a number of drugs such as the β-lactam antibiotic Tazobactam and the cephalosporine Cefatrizine.[9] Herein, we have designed and synthesized novel 1,2,3-triazole linked β-lactam-bile acid conjugates **10-17** (Scheme 1) using 1,3-dipolar cycloaddition reaction of azido β-lactams **4**, **5** and terminal alkynes of bile acids **6-9** in the presence of Cu(I) catalyst (click chemistry)[10].



Scheme: 1 Reagent and conditions: (a) NaN_3, Bu_4NBr, CH_2Cl_2/H_2O (1:1), 25 °C, 36 h, 98%. (b) KOH, MeOH, 25 °C, 4 h, 94%. (c) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, **4** (81%), **5** (77%). (d) Sodium ascorbate, $CuSO_4.5H_2O$, DMF/H_2O (7:3), microwave (385 watt), 5 min, 95-97%.

Accordingly, the synthesis of azido β-lactams **4** and **5** was started from ethyl bromoacetate. Ethyl bromoacetate on treatment with sodium azide furnished the azide compound **1**, saponification of ester functionality with KOH in methanol gave the potassium salt of azidoacetic acid **2.** The ketene-imine cycloaddition (Staudinger)[11] reaction of compound **2** and imines **3** in the presence of triphosgene and triethylamine in anhydrous dichloromethane afforded the azido β-lactams **4** and **5**.

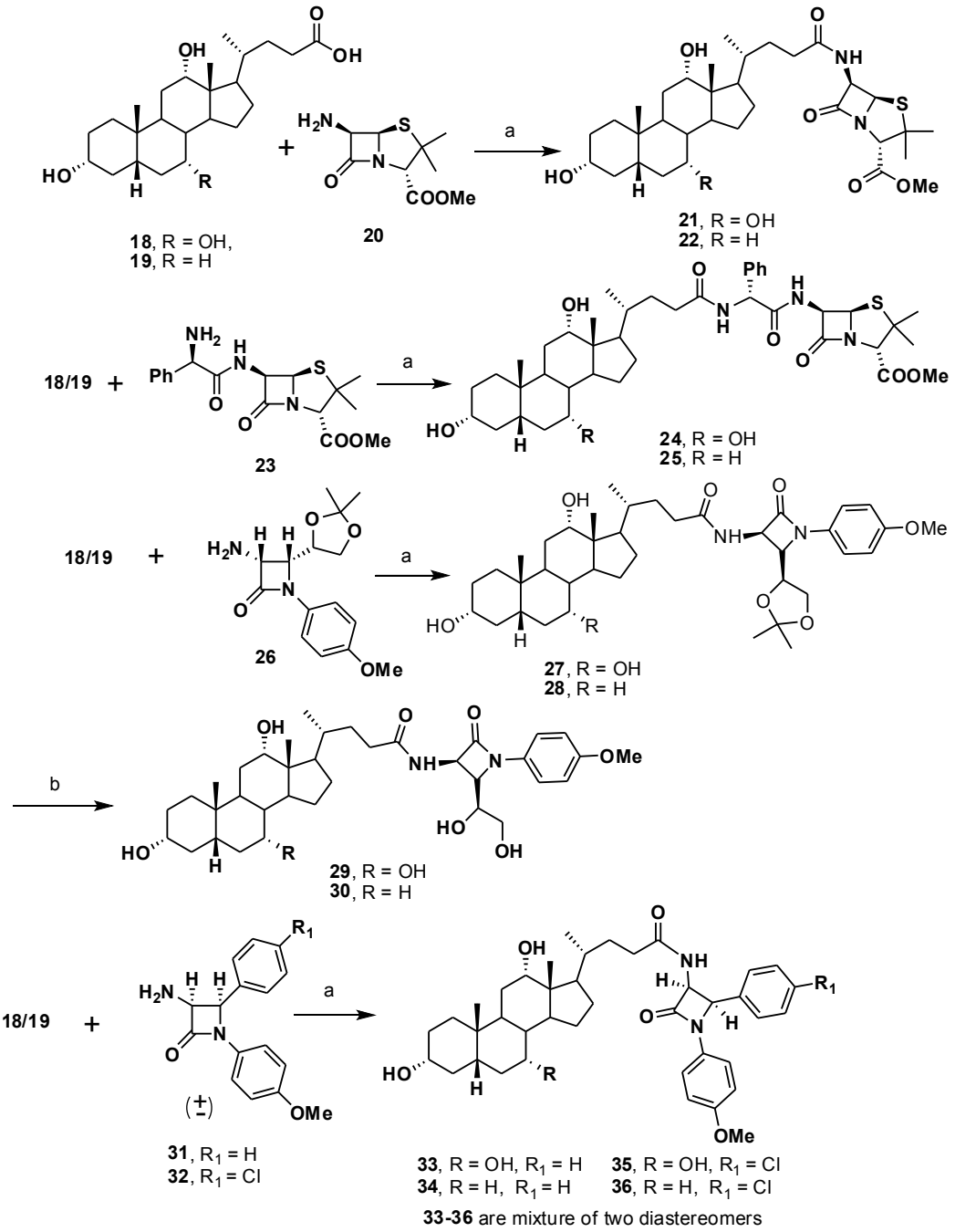
Our next target was to synthesise 1,2,3-triazole linked β-lactam-bile acid conjugates **10-17**. The cycloaddition reaction of propargyl esters **6** and **7** (derived from cholic acid and deoxycholic acid) with azido β-lactams **4** and **5** in the presence of Cu(I) catalyst (click chemistry) under microwave irradiation furnished diastereomeric mixture of novel β-lactam-bile acid ester conjugates linked via triazole **10-13** in excellent yields.

In a similar way, exposure of propargyl amides **8** and **9** with azido β-lactams **4** and **5** afforded diastereomeric mixture of hitherto unknown β-lactam-bile acid amide conjugates linked via triazole **14-17** in very good yields. The newly synthesized conjugate molecules **10-17** were evaluated in vitro for their antifungal and antibacterial activities. Most of the compounds exhibited significant antifungal and moderate antibacterial activity against all the tested strains.

Section B: Design and Synthesis of Bile Acid-β-Lactam Conjugates Using Amide and Ether Linkage, Study Their Biological Activity.

Due to their amphiphilic nature, rigid steroidal backbone, availability, and low cost, bile acids have become attractive tool in designing pharmacological hybrid molecules and prodrugs.[1] These molecules have tremendous transport capacity and organ specificity of enterohepatic circulation. Several bile acid drug conjugates have been reported in the literature.[2]

This section comprises with the formation of amide bond at C-24 of cholic acid **18**, deoxycholic acid **19** with methyl ester of 6-aminopenicillanic acid **20** and methyl ester of ampicillin **23** to obtain novel conjugates **21**, **22** , **24** and **25** (Scheme 2). In addition to this, we have also synthesized different cholic acid/deoxycholic acid-β-lactam conjugates **29**, **30**, **33-36** using 3-amino β-lactams **26**, **31** and **32**. 3-Amino β-lactams **26**, **31** and **32** have been synthesized in the laboratory. The acid functionality of 6-aminopenicillanic acid was converted to its methyl ester **20** by treating with diazomethane followed by acid

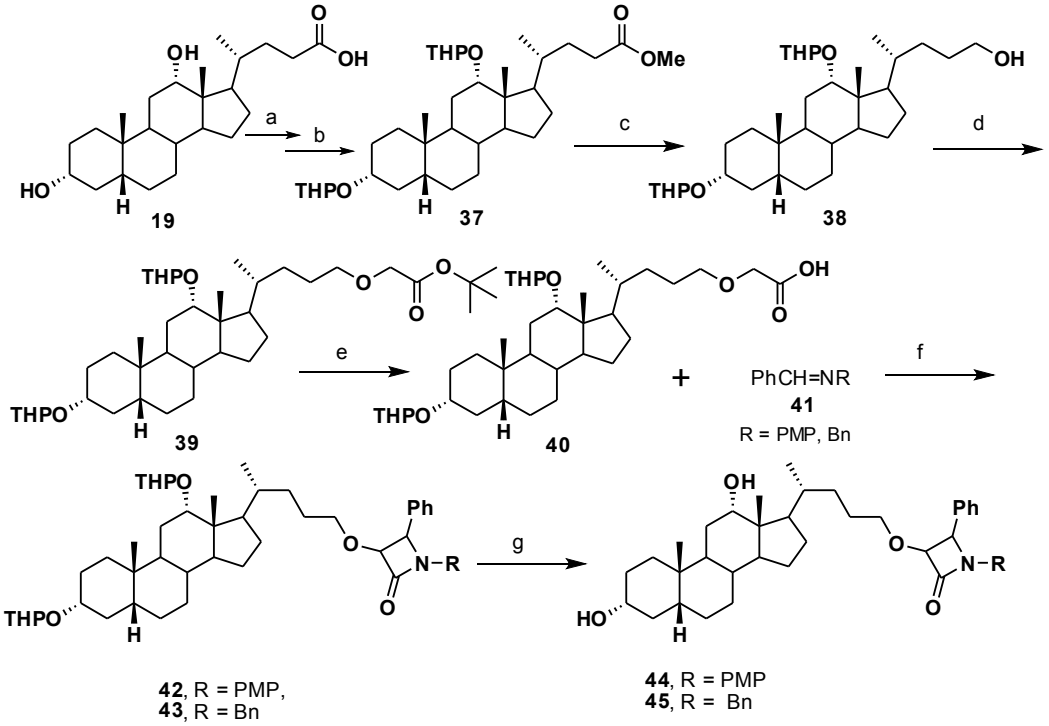


Scheme: 2 Reagent and conditions: (a) EDC·HCl, HOBt, DMF, 0-25 °C, 11 h, 90-95%. (b) *p*-TSA, THF-H_2O, reflux, 24 h, 92%.

amine coupling of cholic acid/deoxycholic acid (**18/19**) with compound **20** using EDCI as coupling agent afforded the conjugate molecules **21** and **22** in very good yields. In the similar way treatment of ampicillin with diazomethane gave the methyl ester of ampicillin **23**. The amine functionality in compound **23** was coupled with cholic acid/deoxycholic acid using EDCI as coupling agent to obtained bile acid-ampicillin conjugates **24** and **25**. The chiral amino β-lactam **26** was prepared following literature procedure and coupled

with the bile acids **18, 19** to get the compounds **27** and **28** which on acetonide deprotectin with *p*-TSA in THF-H_2O furnished β-lactam conjugates **29** and **30**. The synthesis of 3-amino β-lactams **31** and **32** were achieved by the reduction of azide functionality of β-lactam **4** and **5** with Raney nickel in hydrogen atmosphere. The cholic acid/deoxycholic acid conjugates **33-36** were prepared by coupling the β-lactams **31** and **32** with cholic acid **18** and deoxycholic acid **19** using EDCI as coupling agent in excellent yields.

This section also deals with the synthesis of β-lactams on deoxycholic acid side chain **44** and **45** using ether linkage. The synthesis of molecules **44** and **45** were depicted in Scheme 3.



Scheme: 3 Reagent and conditions: (a) *p*-TSA, MeOH, 22 h, 25 °C, 96%. (b) DHP, PPTS, CH_2Cl_2, 10 h, 93%. (c) LAH, THF, 1 h, 86%. (d) *tert*-butyl bromoacetate, 50% aq. NaOH, benzene, 1 h, 61%. (e) LiOH, MeOH-H_2O, 26 h, 30 °C, 95%. (f) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, **42** (45%) or **43** (48%). (g) *p*-TSA, MeOH, 30 °C, 5 h, 96-97%.

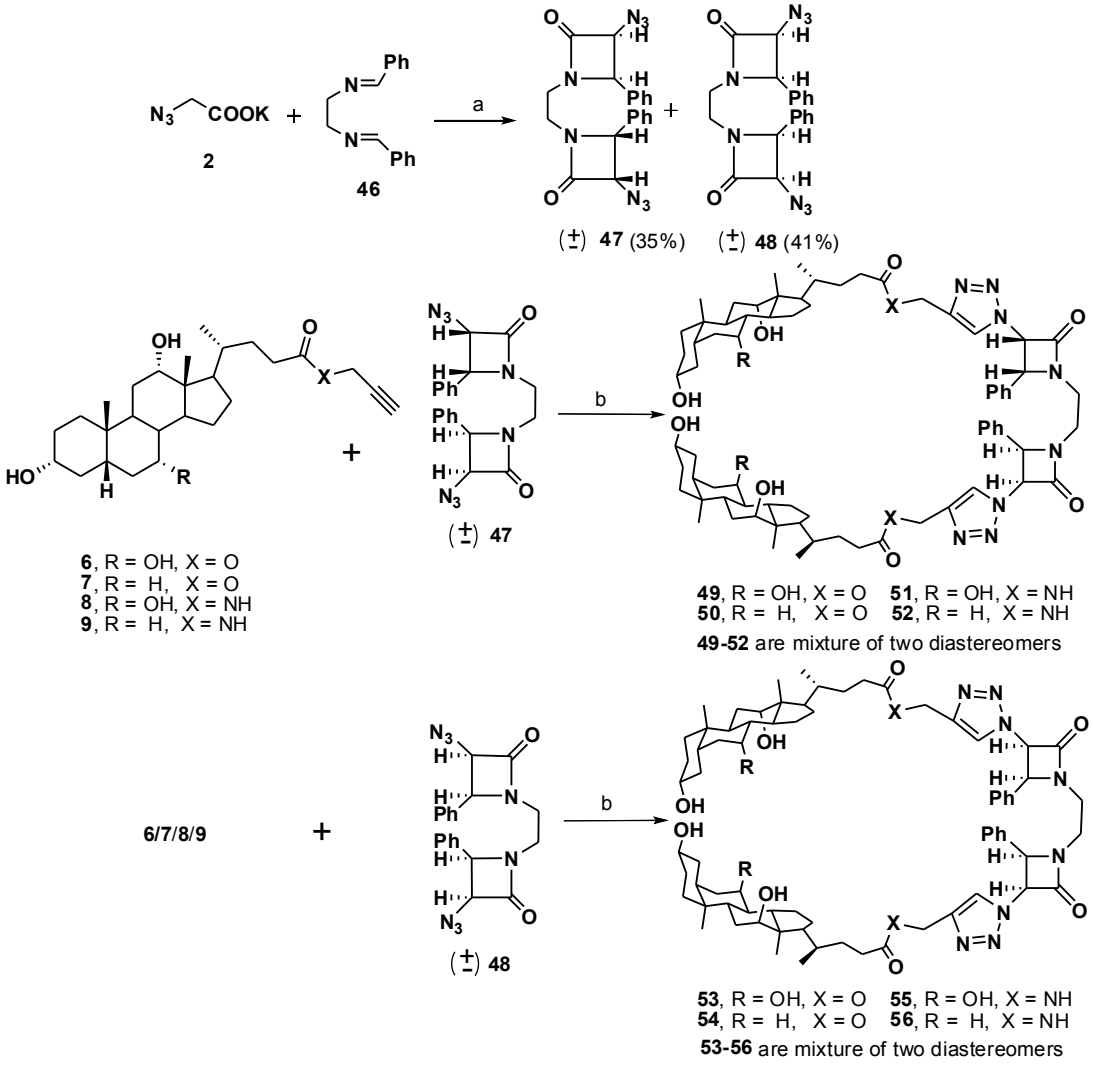
Deoxycholic acid **19** was converted to its methyl ester, protection of both the hydroxy group as THP ethers followed by LAH reduction provided C-24 alcohol **38**. The alcohol **38** was then treated with *tert*-butyl bromoacetate to give the corresponding *tert*-butyl ester **39** and this on hydrolysis furnished the acid **40**. Finally, the acid **40** was subjected to keten-imine cycloaddition (Staudinger) reaction to afford β-lactams **42** and **43** in moderate yields. Deprotection of THP led to targeted ether linked β-lactam bile acid

conjugates **44** and **45** in excellent yields. All these newly synthesized amide and ether linked bile acid-β-lactam conjugates are under biological evaluation for antibacterial activity.

Section C: Design, Synthesis and Biological Evaluation of Bile Acid Dimers Linked with 1,2,3-Triazole and bis-β-Lactam.

Bile acids are imperative building blocks for the synthesis of dimers, oligomers and colaphanes due to their rigid framework with multiple chiral centers. The dimers, oligomers and colaphanes were synthesised from bile acids have a wide range of potential applications exists in pharmacology, membrane bilayer probes, and ion complexation. The synthesis of novel bile acid dimers containing 1,2,3-triazole as a linker have been reported from our laboratory.[12] More recently, Regen *et al.* have reported bile acid derived molecular umbrellas as anti-HIV and anti-HSV agents and molecular umbrella-assisted transport of an oligonucleotide across cholesterol-rich phospholipids bilayers.[13]

In continuation of our work on bile acid dimers, we report herein the synthesis of eight novel cholic acid and deoxycholic acid dimers **49-56** using both 1,2,3-triazole and bis-β-lactam as a linker and studied their antimicrobial and cytotoxic activity. The targeted molecules **49-56** were synthesized using 1,3-dipolar cycloaddition reaction of bis-β-lactams **47, 48** containing azide and cholic acids **6, 8** and deoxycholic acids **7, 9** containing terminal alkyne, in the presence of Cu(I) catalyst (click chemistry). Thus, treatment of potassium salt of azidoacetic acid **2** and imine **46** (prepared by using literature procedure) in the presence of triphosgene and triethylamine in anhydrous dichloromethane afforded the diastereomeric mixture of two diazido bis-β-lactams **47** and **48** in 35% and 41% yields respectively (Scheme 4). The structure for bis-β-lactams **47** and **48** were assigned from spectral analysis data and it was further confirmed unambiguously by X-ray crystal analysis (Figure 1). Finally, the cycloaddition reaction of propargyl esters **6**, 7 and propargyl amides **8, 9** derived from cholic acid/deoxycholic acid with diazido bis-β-lactam **47** in DMF/H_2O (7:3) and $CuSO_4{\cdot}5H_2O$, sodium ascorbate under microwave irradiation for five minutes furnished diasteriomeric mixture of novel dimers **49-52** in 93-95% yields.



Scheme: 4 Reagent and conditions: (a) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, **3** (35%) and **4** (41%). (b) Sodium ascorbate, $CuSO_4{\cdot}5H_2O$, DMF/H_2O (7:3), microwave (385 watt), 5 min, 92-95%.

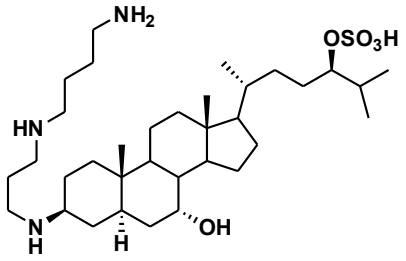
In a similar way, microwave irradiation of propargyl esters **6**, **7** and amides **8, 9** with diazido bis-β-lactam **48** afforded diasteriomeric mixture of dimeric compounds **53-56** in 92 to 94% yields.

Figure 1. ORTEP view for compounds **47** and **48**.

These novel dimers **49-56** were evaluated *in vitro* for their antifungal and antibacterial activity. Most of the compounds exhibited significant antifungal as well as antibacterial activity against all the tested fungal and bacterial strains. Moreover, their *in vitro* cytotoxicities towards HEK-293 and MCF-7 cells were also established.

Chapter 2: Studies Directed Towards the Synthesis of Squalamine.

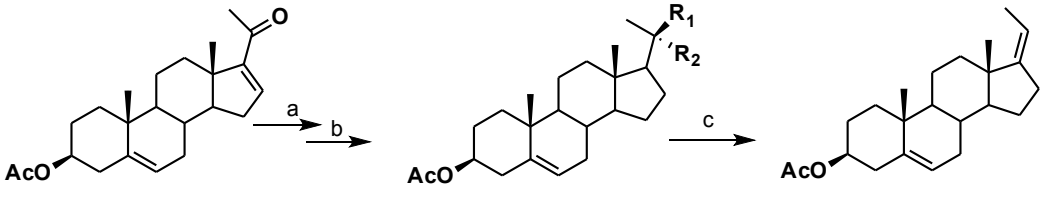
Squalamine **57** (Figure 2) is the first sterol-spermidine conjugate that has been isolated from tissues of the dogfish shark, *Squalus acanthias* in 1993.[14] This unusual natural product has attracted considerable attention because of its potent antimicrobial activity against a broad spectrum of microbes. Moreover, squalamine also found to possess antiangiogenic and antitumor activity.[15]



57

Figure 2

Our synthesis started from commercially available 16-dehydropregnenelone acetate (16-DPA) **58**. The compound **58** was subjected to chemoselective hydrogenation using 10% Pd/C in ethyle acetate followed by $NaBH_4$ reduction in methanol/THF furnished the 20*R* and 20*S* alcohols **59** and **60** in 9:1 ratio (Scheme 5). The dehydration of tertiary alcohol **59** with phosphorous oxychloride and pyridine afforded the Z olefine **61**.

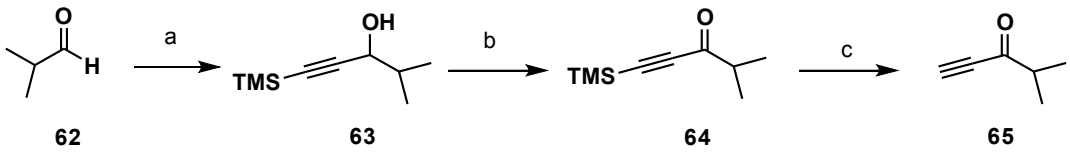


58 **59** R_1 = OH, R_2 = H; **60** R_1 = H, R_2 = OH **61**

Scheme: 5 Reagent and conditions: (a) 10% Pd/C, H_2, EtOAc, 45 psi, 30 ºC, 12 h, 98%. (b) $NaBH_4$, MeOH/THF, 0-25 °C, 2 h, 94%. (c) $POCl_3$, pyridine, 0-27 °C, 30 h, 89%.

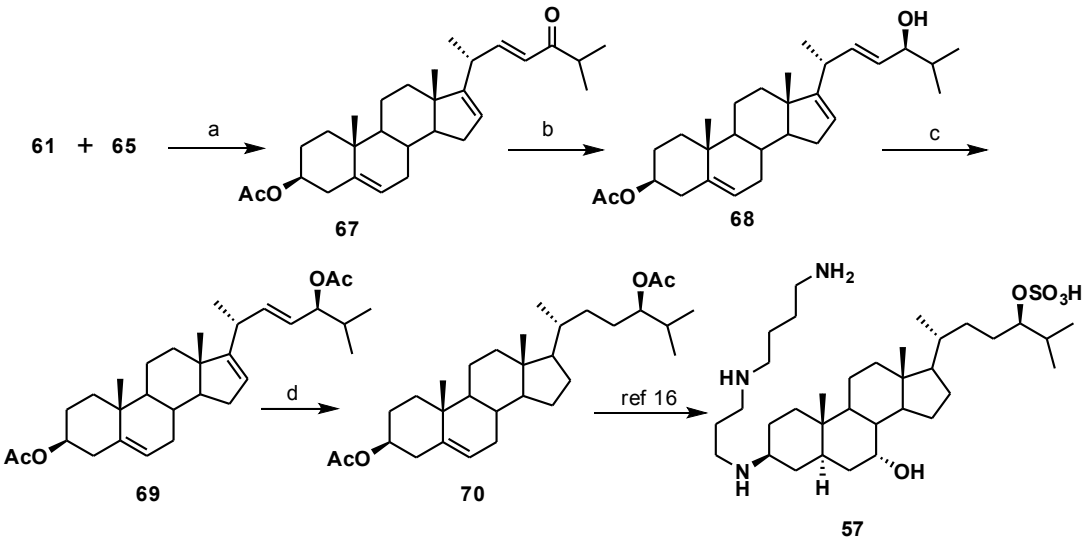
The synthesis of side chain fragment of squalamine **57** was started from isobutyraldehyde **62**. The treatment of aldehyde **62** with TMS-acetylene in the presence of *n*-BuLi obtained

the alcohol **63**, which was further subjected to PCC oxidation to furnish the ketone **64** (Scheme 6). Phase-transfer catalysed removal of TMS group of **64** with NaF and *n*-Bu_4NBr afforded ethynyl ketone **65**.



Scheme: 6 Reagent and conditions: (a) TMS-acetylene, *n*-BuLi, THF, - 78 to 25 °C, 6 h, 93%. (b) PCC, molecular sieves, CH_2Cl_2, 0-25 °C, 4 h, 96%. (c) NaF, *n*-Bu_4NBr, CH_2Cl_2/H_2O, 7 h, 72%.

The steroidal Z olefine **61** and ethynyl ketone **65** was subjected to ene reaction in the presence of borontrifluoride etherate to afford α,β-unsaturated ketone **67** (Scheme 7). The ketone **67** on CBS reduction followed by acetylation of C-24 alcohol gave the diacetate compound **69**. The chemoselective hydrogenation of C-16 and C-22 double bonds of **69** using Pt/C afforded compound **70**. Transformation of the compound **70** to squalamine 57 is well documented in literature.[16]



Scheme: 7 Reagent and conditions: (a) $BF_3.O_2Et_2$, CH_2Cl_2, 0-25 °C, 7 h, 74%. (b) (*R*)-MeCBS, $BH_3{\cdot}Me_2S$, THF, -30 °C, 0.5 h, 87%. (c) Ac_2O, DMAP, pyridine, 25 °C, 14 h, 96%; (d) Pt/C, H_2 (1 atm), EtOAc, 9 h, 94%.

Chapter 3: Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols.

The design of asymmetric transformation reaction is a great challenge in organic chemistry. Particularly, the development of enantioselective homogeneous catalysts in which a small amount of an optically active ligand can induce asymmetry for a given

reaction. Enantioselective oxazaborolidine catalysed borane reduction of prochiral ketones to chiral secondary alcohols provides a convenient access to a wide variety of optically active secondary alcohols, which are valuable chiral building blocks for the synthesis of natural products and bioactive compounds.[17] Oxazaborolidines prepared from chiral amino alcohols give excellent ee values and often have wide substrate scope. Itsuno and co-workers[18] have carried out pioneering work on asymmetric reduction of alkyl aryl ketones with the reagent prepared from (*S*)-(-)-2-amino-3-methyl-1,1- diphenylbutane-1-ol and borane. The secondary alcohols were obtained in 94-100% enantiomeric excess and in 100% chemical yields. The catalytic behaviour of the more sterically hindered oxazaborolidine based on (*S*)-(-)-2-diphenylhydroxymethyl-pyrrolidine was introduced[19] in borane reduction of ketones by Corey, Bakshi and Shibata (CBS). Optically active amino alcohols[20] have been documented recently as catalysts in enantioselective reduction of various ketones.

This chapter describes syntheses of six new chiral 1, 2-amino alcohols **71-76** (Figure 3) and their applications for oxazaborolidine catalysed enantioselective reduction of aromatic ketones. Accordingly commercially available and inexpensive chiral amino alcohol (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **77**, a precursor for the preparation of chloramphenicol has been utilized by us for the synthesis of all the six new chiral auxiliaries.

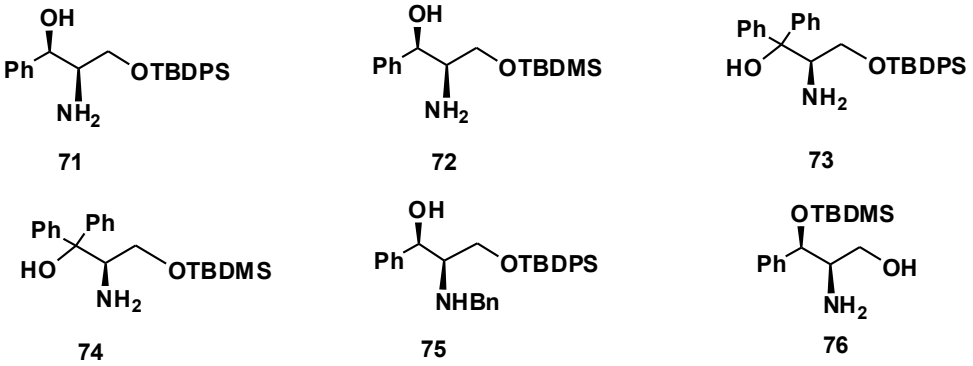
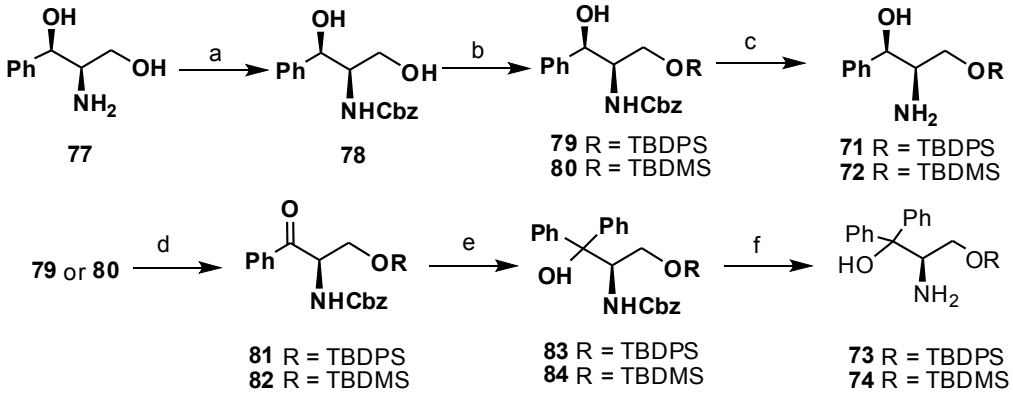


Figure 3

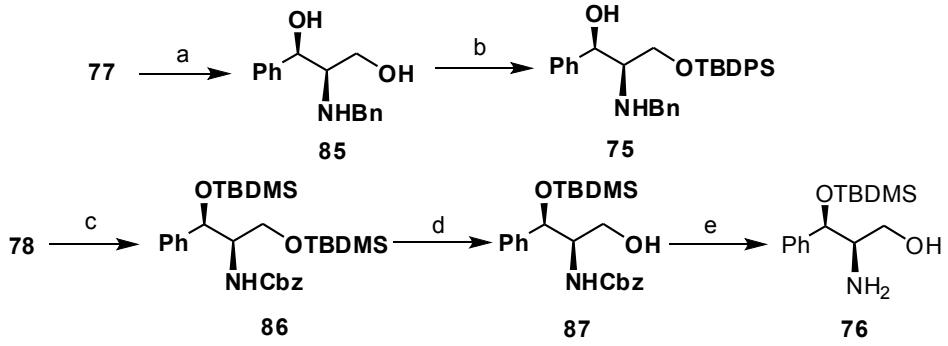
Amino group in compound **77** was protected as carbamate **78** and then primary alcohol at C-3 was converted into TBDPS ether 7**9** or TBDMS ether 8**0** (Scheme 8). Deprotection of carbamate by hydrogenation gave the required amino alcohol derivatives **71** and **72** in excellent yield. The secondary hydroxy group in compound 7**9** and **80** were subjected to oxidation with IBX in ethyl acetate to afford ketones **81** and **82**. Grignard reaction of

ketones **81** and **82** followed by carbamate deprotection furnished amino alcohols **73** and **74**.



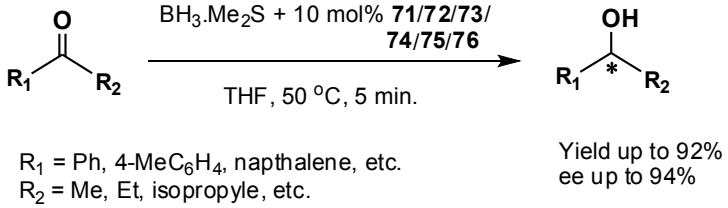
Scheme: 8 Reagent and conditions: (a) $PhCH_2OCOCl$, Na_2CO_3, water-dioxane, 30 °C, 2 h, 99%. (b) Compound-**79**: TBDPSCl, imidazole, DMF, 30 °C, 10 min., 92%; Compound-**80**: TBDMSCl, imidazole, DMF, 25 °C, 0.5 h, 90%; (c) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, **71** (98%) and **72** (99%). (d) IBX, EtOAc, reflux, 5 h, **81** (98%) and **82** (97%). (e) Bromobenzene, Mg, THF, 25 °C, 1 h, **83** (84%) and **84** (87%). (f) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, 99%.

Secondary amino alcohol **75** has been synthesized from amino diol **77** (Scheme 9). Compound **77** was converted into an imine with benzaldehyde which was reduced with sodium borohydride to give N-benzylated compound **85**. Primary hydroxyl group in compound **85** was selectively converted into TBDPS ether to furnish **75** in good yield. Synthesis of secondary hydroxyl group protected amino alcohol **76** is presented in (Scheme 9). Initially both the primary and secondary hydroxy group in compound 7**8** was protected as TBDMS ether followed by selective deprotection of primary O-TBDMS group with camphorsulfonic acid (CSA) in methanol afforded alcohol **87**.



Scheme 9. Reagents and conditions: (a) (i) PhCHO, anhydrous $MgSO_4$, DCM/MeOH (3:1), reflux, 2 h; (ii) $NaBH_4$, MeOH, 0-25 °C, 3 h, 76% in two steps; (b) TBDPSCl, imidazole, DMF, 25 °C, 0.5 h, 83%; (c) TBDMSCl, imidazole, DMF, 30 °C, 13 h, 99%; (d) CSA, MeOH, 10 min., 20 °C, 96%; (e) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, 98%.

Removal of carbamate group in **87** by catalytic hydrogenation gave the amino alcohol **76** in excellent yield. The newly synthesized amino alcohols **71-76** have been used for *in situ* generated oxazaborolidine catalysed enantioselective reduction of a variety of prochiral ketones using $BH_3{\cdot}Me_2S$ as BH_3 source (Scheme 10). The secondary alcohols were obtained in fair to excellent enantiomeric excess (43 to 94%)



Scheme 10. Oxazaborolidine catalysed enantioselective reduction of prochiral ketones.

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Chapter 1

Design, Synthesis and Bioevaluation of Bile Acid β-Lactam Conjugates

Introduction

Introduction to bile acids

Bile salts are biosynthesized from cholesterol in the liver and stored in the gall bladder (Figure1). The human liver produces 600-800 mL of bile per day. After food intake and subsequent gall bladder emptying, bile is secreted into the small intestine,

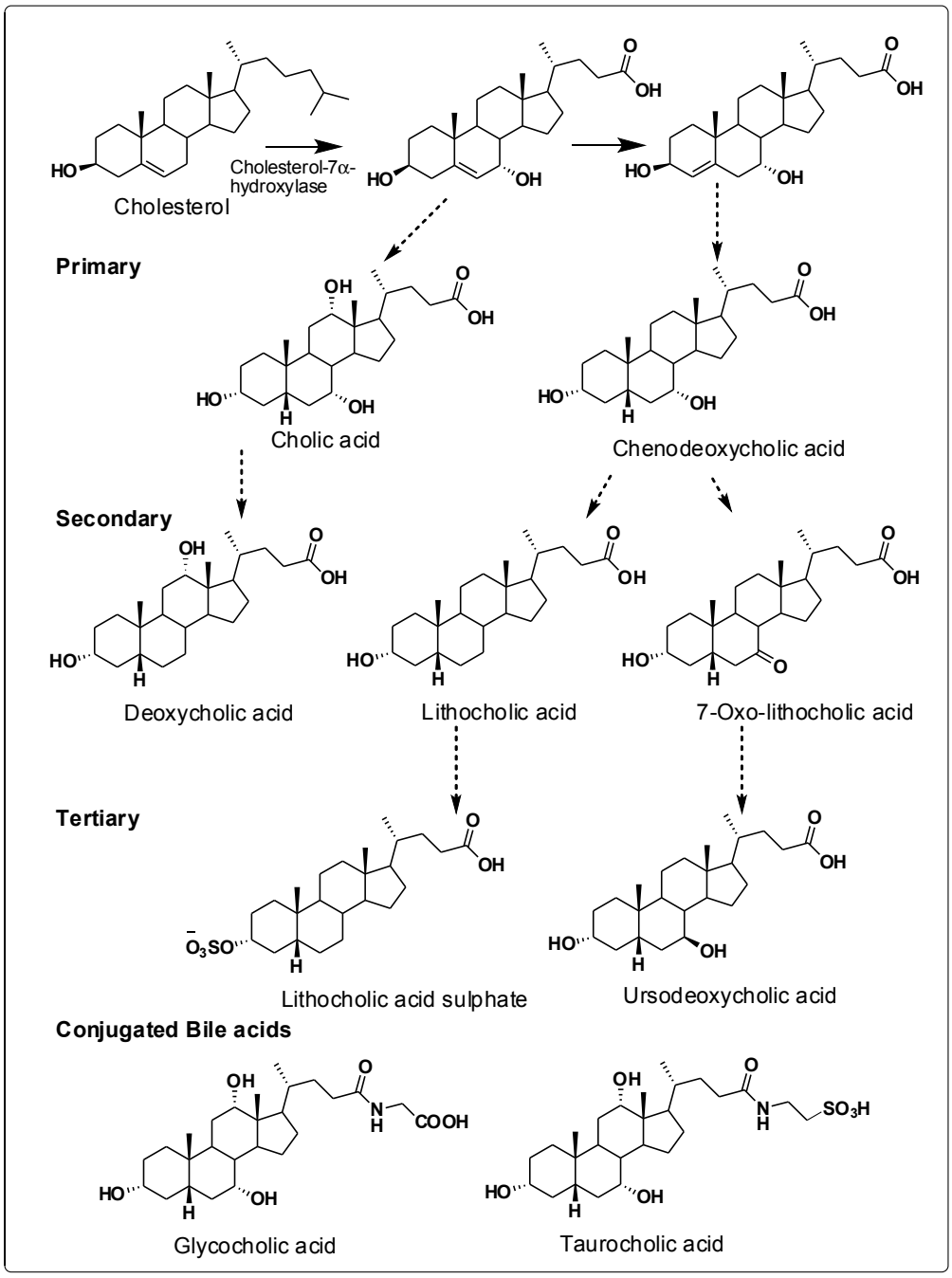


Figure 1: Biosynthesis of bile acids: Bile acid metabolism-production of primary, secondary and tertiary bile acids occurs in the liver and by intestinal bacteria. Structures of conjugated bile acids: glycocholic acid and taurocholic acid.

where the bile acids perform their essential function in the digestion and resorption of fat, fatty acids and lipid-soluble vitamins. These nutrients are insoluble in water which dispersed in micelles of bile acids and lipids. The bile acids recirculate to the liver *via* the portal vein, undergoing this enterohepatic circulation 6-15 times per day. Bile acids are synthesized from cholesterol in hepatocytes. This catabolic pathway represents the major metabolic fate of cholesterol (Figure 1). The most abundant primary bile salts in humans are cholate, chenodeoxycholate and deoxycholate, and they are normally conjugated with either glycine (75%) or taurine (25%). Conjugation increases the aqueous solubility of bile salts under physiological conditions. All primary bile acids appear to have three features in common: (i) they are the major end-products of cholesterol metabolism, (ii) they are secreted into the bile largely in a conjugated form, and (iii) these conjugates are membrane-impermeable, water-soluble, amphiphilic molecules having a powerful ability to transform lamellar arrays of lipids into mixed micelles.[1,2]

Chemical structures of bile acids

All bile acids consist of two connecting units, a rigid steroid nucleus and a short aliphatic side chain (Figure 2).[2] The steroid nucleus of bile acids has the saturated tetracyclic hydrocarbon perhydrocyclopentanophenanthrene, containing three six-member rings (A, B and C) and a five member ring D. In addition, there are angular methyl groups C-18 and C-19 at positions 13 and 10. The bile acid nucleus in higher vertebrates is curved (beaked) because rings A and B are *cis* fused.

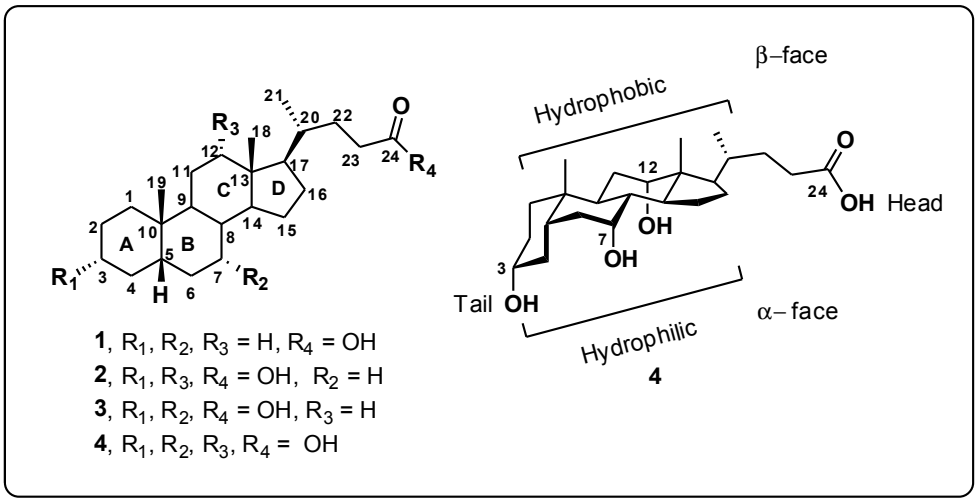


Figure 2: Chemical structures of different Bile acids.

Some bile acids in lower vertebrates, known as *allo*-bile acids, are flat because of an A/B *trans*-fusion (5α-stereochemistry). The most abundant mammalian bile acids are hydroxy derivatives of cholanoic acid (5β-cholan-24-oic acid), (Figure 2**,** compound **1**).[1]

The human bile acid pool consists mainly (~90%) of cholic acid, **4** (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid), chenodeoxycholic acid, **3** (3α,7α-dihydroxy-5β-cholan-24-oic acid), and deoxycholic acid, **2** (3α,12α-dihydroxy-5β-cholan-24-oic acid). The bile acid nucleus possesses angular methyl groups C-18 and C-19 on the convex hydrophobic *β*-face and the hydroxyl groups on the concave hydrophilic *α*-face which makes these compounds facially amphipathic.[3,4]

Bile acid based conjugates

During last decade number of reviews on bile acids and other steroidal compounds as architectural components in diverse areas of chemistry are available in the literature.[3-16] These include supramolecular host-guest systems, molecular and ionic receptors, chiral dendritic species, artificial light harvesting systems, foldamers/protein mimics, low mass organo/hydrogelators, templates/chiral auxiliaries for asymmetric synthesis, drug transport systems etc. Bile acids have been considered very useful in the preparation of new pharmaceutical drugs because of their inherent chemical and biological properties.[11]

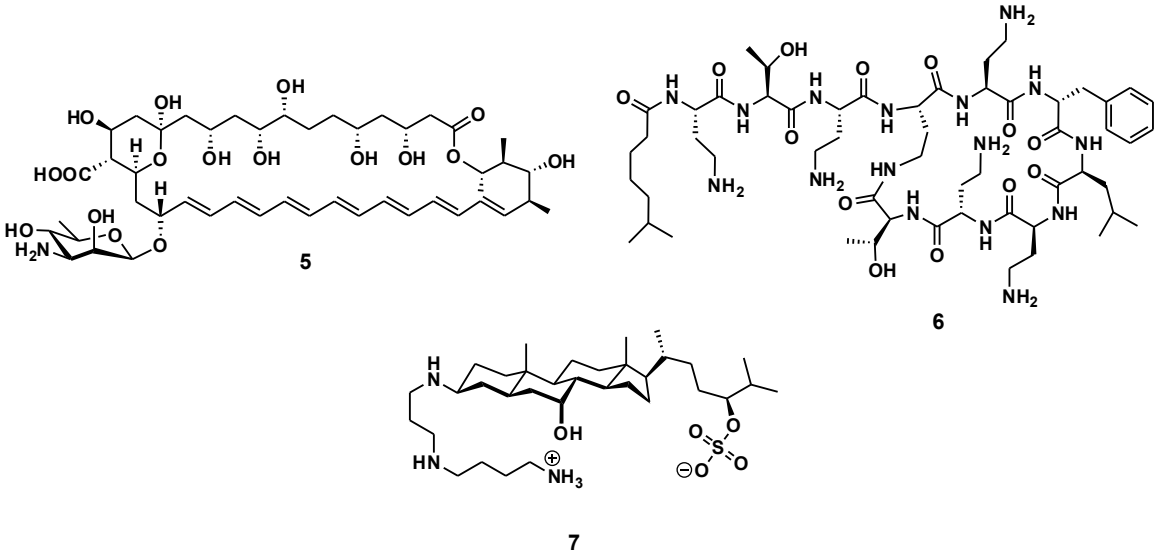


Figure 3. Amphotericin B **5**, polymyxin B **6** and squalamine in salt bridged cyclic form **7**.

They are pharmacologically interesting as potential carriers of liver specific drugs, absorption enhancers and cholesterol lowering agents.[13] A common feature of bile acid derived antimicrobials is their potential to exhibit facially amphiphilic nature, due to polar

hydroxyl groups on one face and nonpolar hydrophobic methyl group on the other.[17] This type of amphiphilicity can also be exhibited by polyene macrolide amphotericin B **5**, peptide antimicrobial agent polymixin B **6**, and squalamine in the cyclic form **7** (Figure 3) which functions as an inophores.[18]

The treatment of chronic diseases in most cases involves long-term use of drugs. For this a site-specific drug action without adverse side effects and noninvasive, preferably oral administration of drugs is necessary. The physiology of bile transport exclusively involves only liver and small intestine and hence use of bile acid as putative shuttles of pharmaceuticals should be ideal. Current research efforts are focused on specific drug targeting to the liver and on improving the intestinal absorption of poorly absorbed drugs. Very recently use of bile acids and their derivatives in designing prodrugs capable of exploiting the enterohepatic circulation of bile acids is summarized by Sievänen.[15] Large numbers of drug bile acid conjugates have been synthesized by Kramer[3] *et al*, which include chlorambucil-bile acid conjugate **8**, peptide-bile acid conjugate **9**, HMG-CoA reductase inhibitor-bile acid conjugates **10** and **11** (Figure 4).

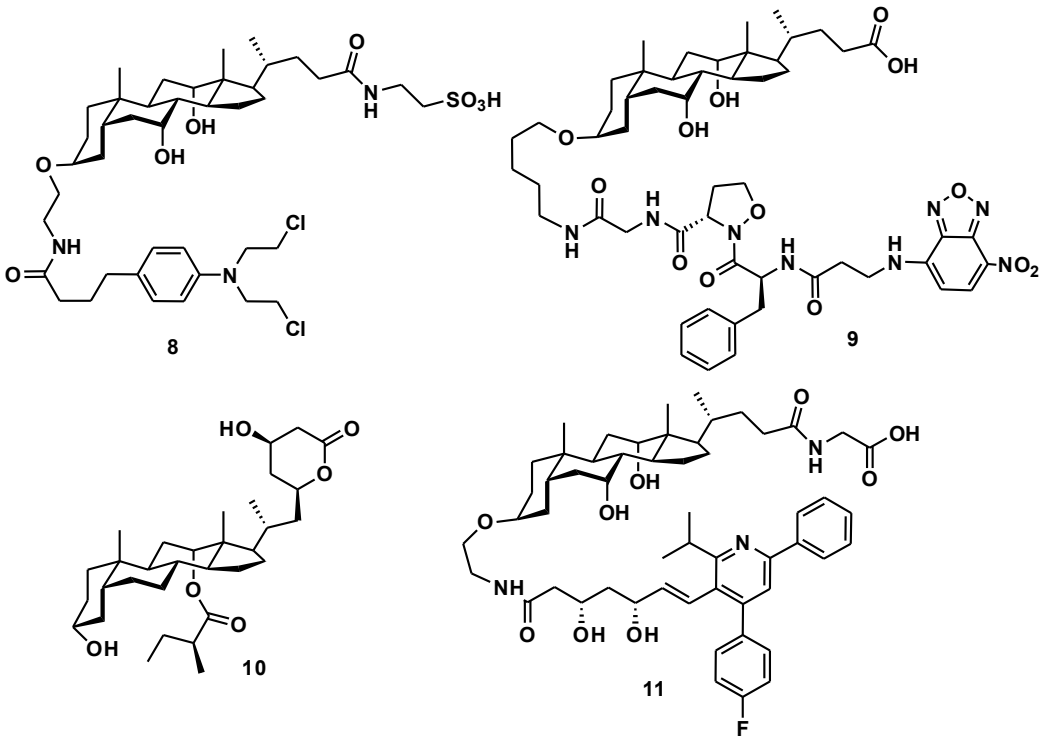


Figure 4

Synthesis of bile acid oligonucleotide conjugates 12 and 13 (Figure 5) have been reported.[19] When tested *in vivo* in rats, these conjugates resulted in an increased biliary

excretion of bile acid oligodeoxynucleotide conjugates compared to unconjugated oligodeoxynucleotides.

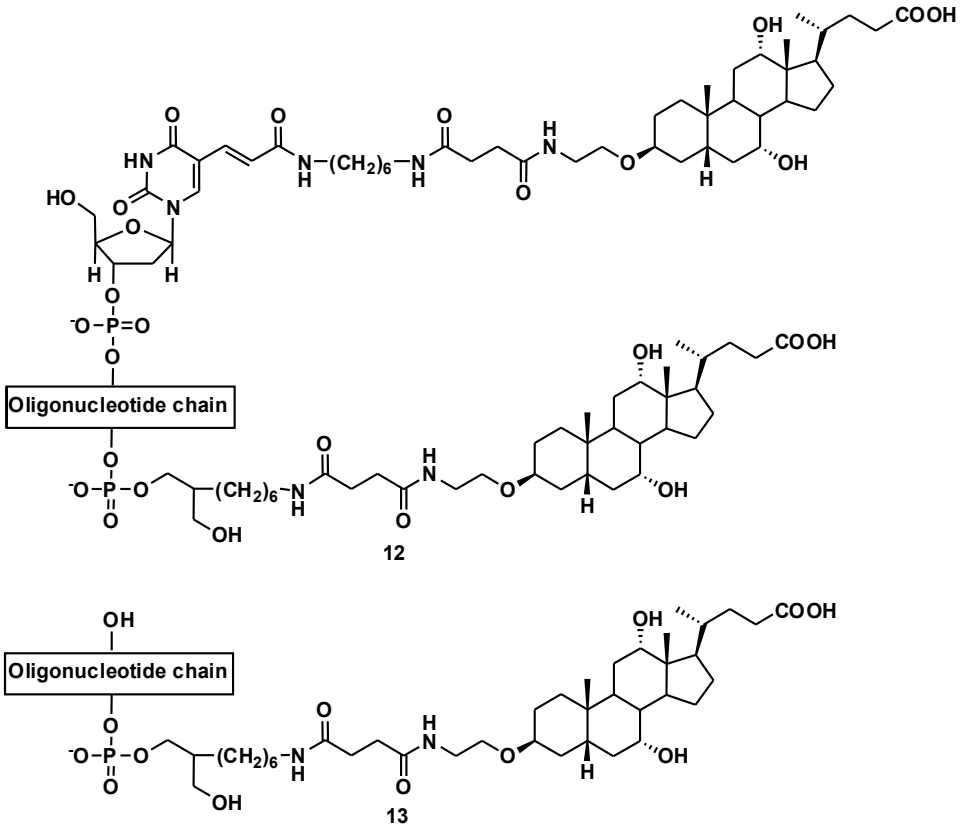


Figure 5

Ganesh and co-workers at National Chemical Laboratory[20] synthesized several oligonucleotides carrying the deoxycholic acid moiety by substituting 5-amido-(7-deoxycholic)-2′ deoxyuridine **14** (Figure 6) for thymidine at predetermined positions in the oligonucleotide sequences. The enhancement in the stability of derived triplexes was observed due to the incorporation of deoxycholic acid to nucleobase deoxyuridine in the third strand of DNA triplex.

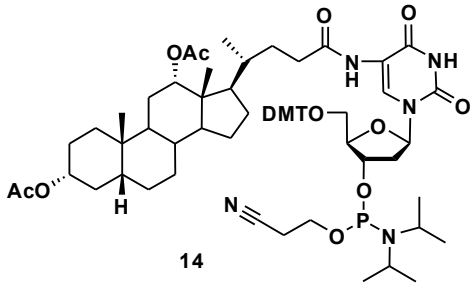
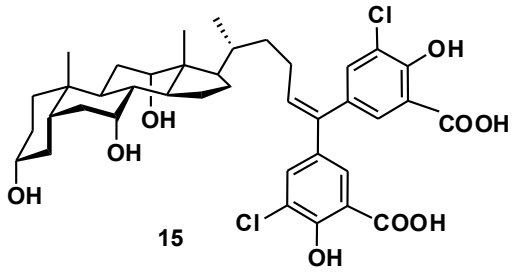


Figure 6

Cosalane is an inhibitor of HIV replication with activity against both HIV-1 and HIV-2. The poor oral availability of cosalane is mainly attributed to its poor permeation

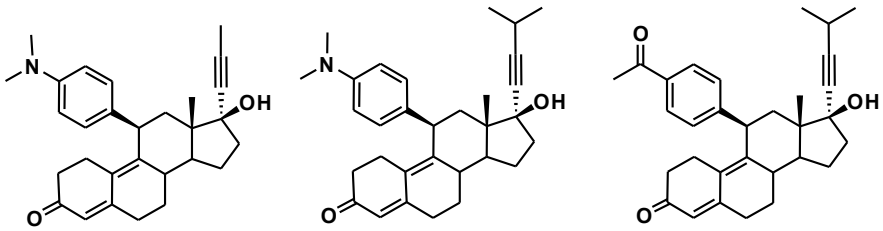
across the intestinal epithelium due to its very high lipophilicity and membrane interacting nature. With the aim to retain the anti HIV activity and to enhance bioavailibility of cosalane, Cushman *et al*. have synthesized[21] cosalane-bile acid conjugate **15** (Figure 7). Unfortunately this conjugate was found to be less potent than cosalane.



15

Figure 7

In 1980, the French pharmaceutical company Roussel-Uclaf announced the discovery[22] of RU-486, now known by the generic name mifepristone **16** (Figure 8). This was the first antiprogestin to be developed. Mifepristone **16**, when used in combination with prostaglandin, effectively and safely terminates early pregnancies. From the SAR,[23] two new analogues, **17** and **18**, of mifepristone were designed. The syntheses of these two analogues in eleven steps[24] through modified synthetic sequences and improved procedures starting from (+)-estrone has been reported earlier from our laboratory. In comparison with mifepristone **16**, the relative binding affinities of compound **18** for the progesterone receptor was found to be more, whereas that of compound **17** was less (Figure 8).



16 17 18

Figure 8

Glycemic control depends on a precise match between glucose inputs and outputs. Any disturbances in this balance can result in hypo or hyperglycemia. Glucocorticoid receptor (GR) antagonism has been validated as a strategy for regulating hepatic glucose output (HGO). Mifepristone (RU-486) **16** has been used for this validation study.

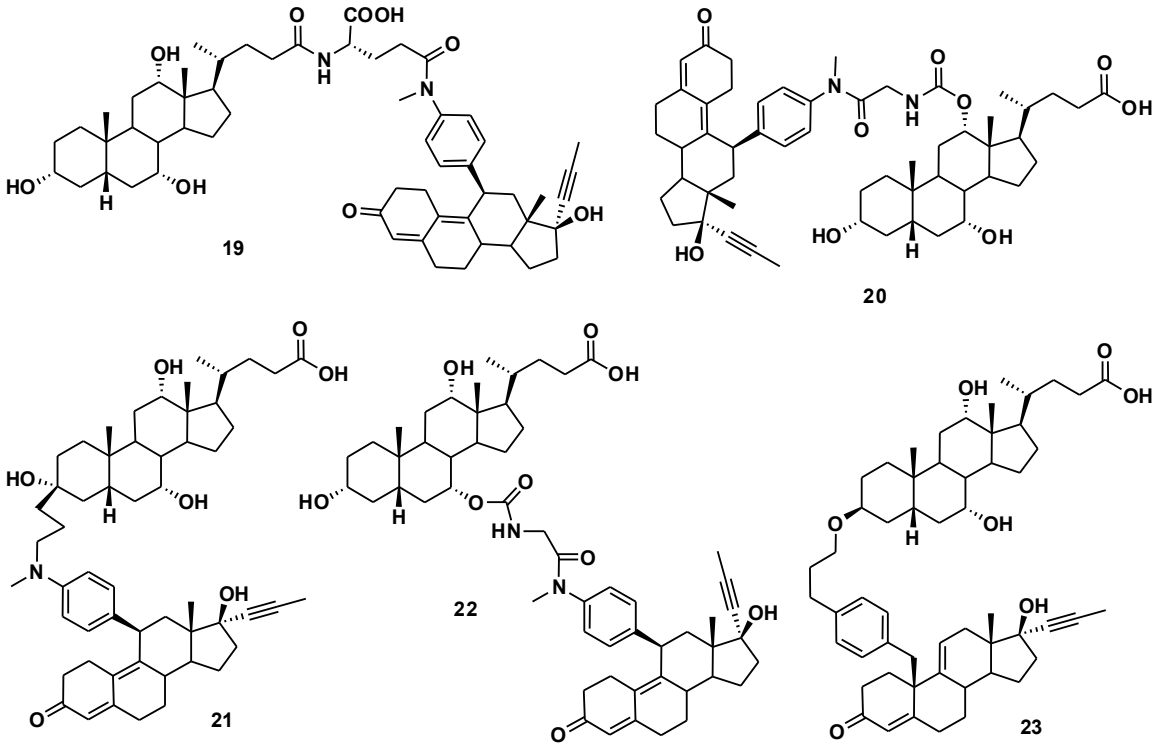


Figure 9

Long-term systematic GR antagonism is not a viable approach for the treatment of type 2 diabetes. A liver specific derivative of mifepristone would be expected to decrease HGO and improve glucose metabolism without the risk of side effects. With this assumption Geldern and coworkers have synthesized[25] number of bile acid conjugates *e.g.* **19**-**22** (Figure 9) of mifepristone **16** at different positions of bile acid using linkers to provide novel drugs for type 2 diabetes. They have also synthesized bile acid-RU-43044 conjugate **23**, which is a selective GR antagonist.

Bile acid conjugates, specifically lithocholic acid conjugates, interact with muscarinic receptors on gastric chief cells. Structural similarities between acetylcholine **24** and lithocholyltaurine **25** (Figure 10) suggested a potential molecular basis for their interaction with the same receptor. Based on this information Cheng *et al* synthesized a hybrid molecule consisting of the steroid nucleus of the lithocholyltaurine and the choline moiety of acetylcholine.[26] The new molecule, lithocholylcholine **26** inhibited binding of a cholinergic radioligand to Chinese hamster ovary cells. The bioactivity data suggest that these hybrid molecules may be used to develop selective musacrinic receptor ligands.

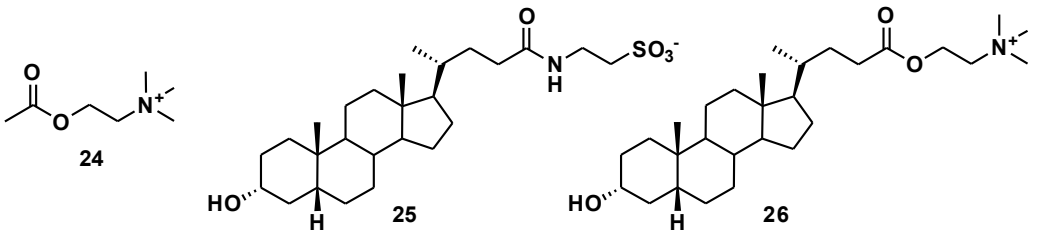


Figure 10

A series of sulfonamides incorporating bile acid moieties as potent carbonic anhydrase inhibitors have been reported.[27] Some of the most active derivatives **27** and **28** (Figure 11), incorporating 1,3,4-thiadiazole-2-sulfonamide or benzothiazole-2-sulfonamide functionalities in their molecules, showed excellent affinity for several isozymes of carbonic anhydrase.

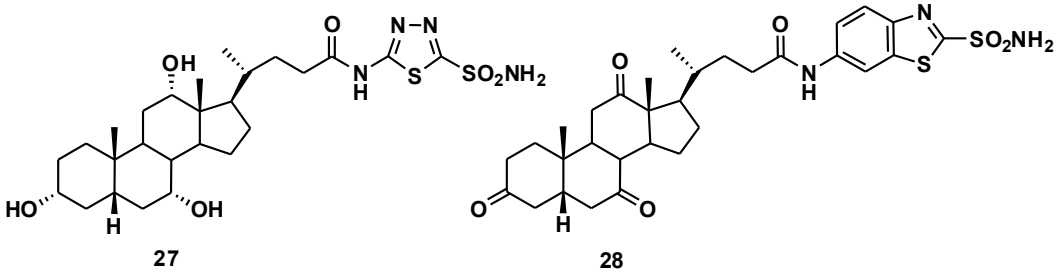


Figure 11

It has been shown that bile acids can be used as carrier units for preparation of MRI (magnetic resonance imaging) contrast agents, which enter hepatocycle by means of active transport mechanism.[28] A series of structurally different gadolinium conjugates incorporating a bile acid moiety have been prepared. Polyaminopolycarboxylic acids such as DTPA (diethylenetriamine-pentaacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) have been selected as chelating subunits for the Gd (III) ion. These conjugates *e.g.* **29**, **30** (Figure 12) showed high biliary elimination as well as good tolerabilites.

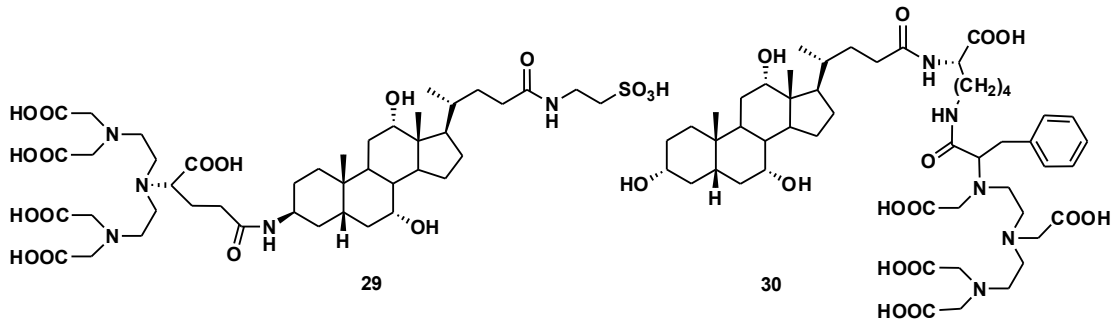


Figure 12

Aromatic heterocycles are widely used in the pharmaceutical industry, for example as anti inflammatory, antimicrobial and anticancer drugs. Based on this concept there are reports on synthesis of lithocholic acid-pyrrole conjugates,[29] corticosterone-imidazole-bis(guanidinium) conjugates[30] and lanosterol-imidazole conjugates.[31] Novel pyrazole fused bile acids were conjugated with anti-inflammatory drugs such as neproxen and drug surrogate.[32] These annulated pyrazoles and their drug conjugates **31** and **32** (Figure 13) showed good affinity for the human liver bile acid transporter as compared to human ileal bile acid transporter. Potentially such annulated pyrazoles can be used as shuttles for drug targeting.

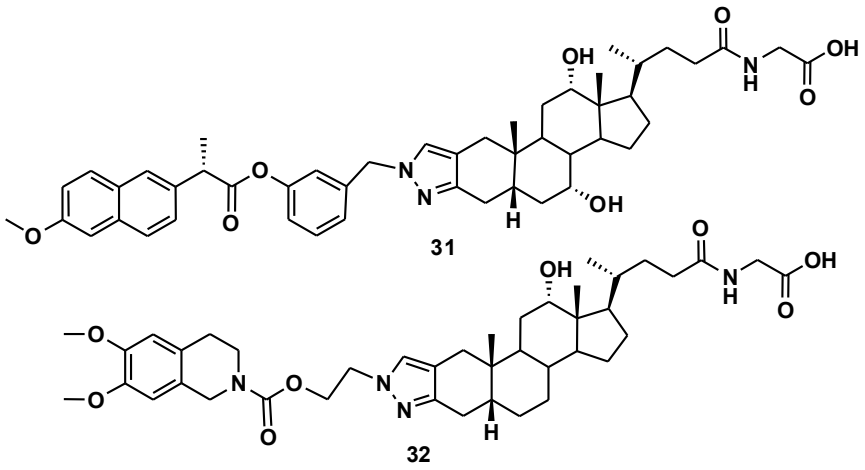


Figure 13

The 1,2,4,5-tetraoxacyclohexane (tetraoxane) moiety became an increasingly interesting pharmacophore since its antimalarial activity was found to be very similar to that of 1,2,4-trioxanes such as naturally occurring artemisinin. Solaja and his group has

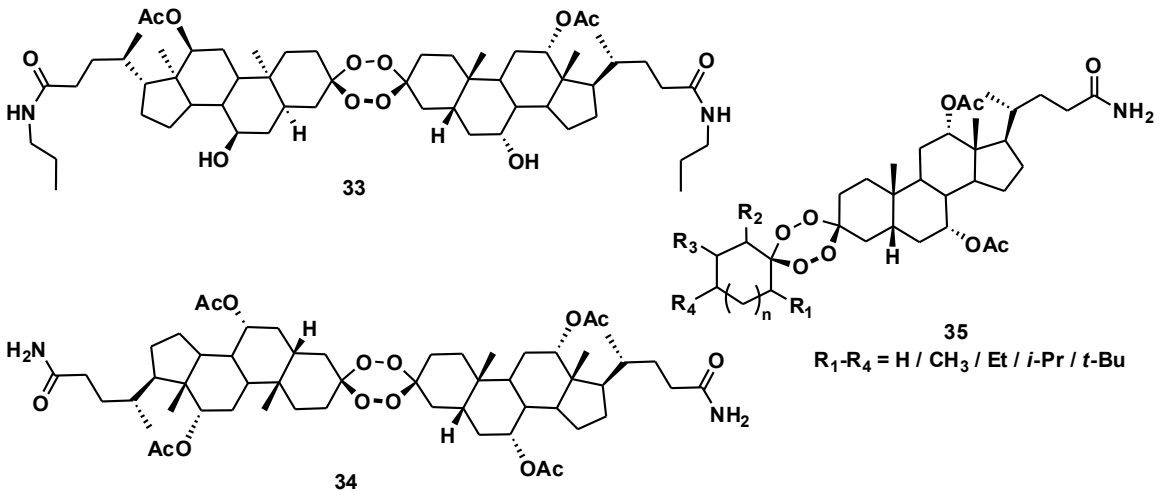


Figure 14

synthesized[33] cholic acid-derived 1,2,4,5-tetraoxanes **33**, **34** and mixed 1,2,4,5-tetraoxanes such as **35** (Figure 14) in order to explore the influence of steroid carrier on its antimalarial, antiproliferative and antimicrobial activities.

Various reports on bile acid based conjugates are available in the literature e.g. peptide-conjugates,[34a] polyamine-conjugates,[34b] insulin-deoxycholic acid conjugate, [34c] bile acid-polyamine-nucleoside conjugates,[34d] amino acid conjugates[34e, 34f] and series of bile acid conjugates of a nonsteroidal glucocorticoid receptor modulator were also revealed in the literature. [34g]

Recently there are reports from our laboratory on the synthesis of bile acid conjugates *e.g.* **36**[35] from chiral amino alcohols based on a broad-spectrum antibiotic chloramphenicol and a novel bile acid-fluconazole conjugates *e.g.* **37** and **38** (Figure 15) *via* Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition reaction.[36] These conjugates showed antibacterial as well as antifungal activities. The synthesis of bile acid polyamide conjugates **39**[37] has been recently reported by our group.

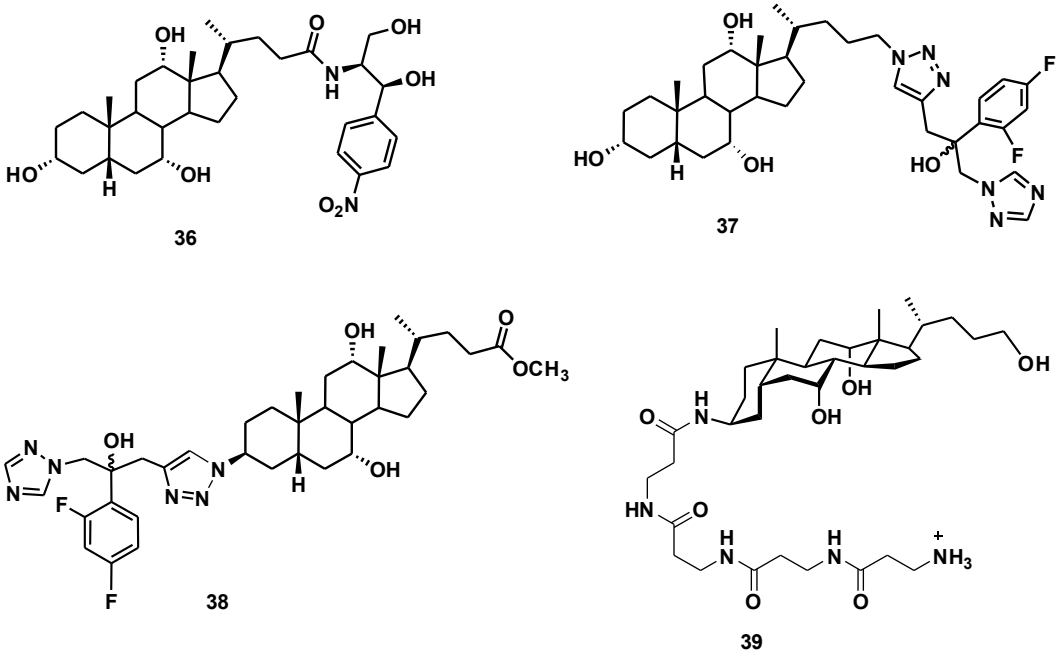


Figure 15

Steroidal β-lactams

In the world of hybrid molecules steroid-β-lactam conjugates are known for long. Nature synthesizes Pachystermine-A **40** and Pachystermine-B **41** (Figure 16)**,** a hybrid molecules which are adduct of a steroid and β-lactam ring (pharmacophore of several potent antibacterial agents). Pachystermine-A **40** and Pachystermine-B **41** are naturally

occurring steroidal β-lactams found in the buxaceous plant *Pachysandra terminalis*.[38] They are novel type of steroidal alkaloid carrying a β-lactam ring system. Similar steroidal alkaloids **42** and **43** (Figure 16) carrying a β-lactam ring system were isolated from *Pachysandrs procumbens*, using a bioassay guided fractionation based on inhibiton of ^{3}H-tamoxifen binding at the antiestrogen binding site.[39] Steroidal β-lactams **42** and **43** demonstrated significant activity as antiestrogen binding site inhibitory agents.

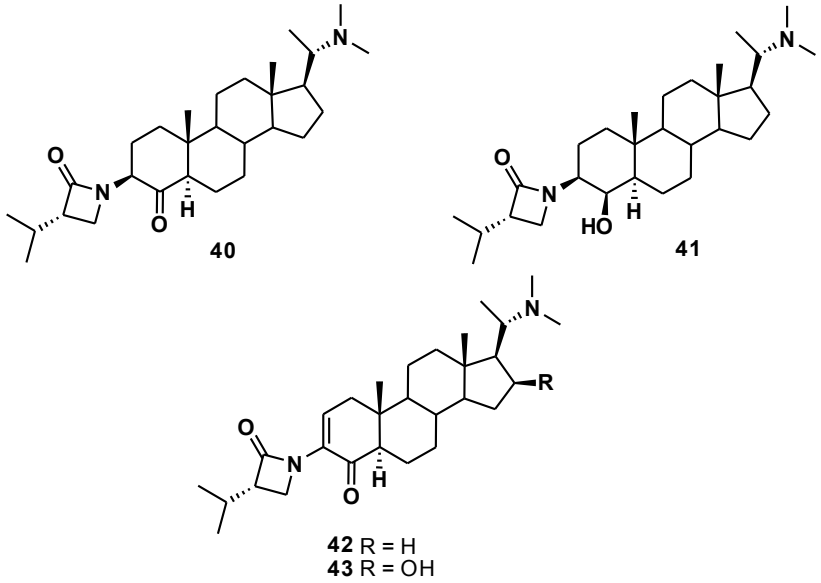


Figure 16

At present there are only two reports for synthetically prepared steroidal β-lactams. Bose and co-workers[40] in 1967 have prepared analogue **44** (Figure 17) of steroidal alkaloid Pachystermine-A by the cyclization of the β-chloroamide from 3α-aminocholestane. Ugi and co-workers[41] in 1995 have shown a simple one-pot synthesis of a steroidal β-lactam **45** (Figure 17) *via* the building block approach using four-component reaction, in which they have constructed β-lactam moiety on the side chain of dehydrocholic acid.

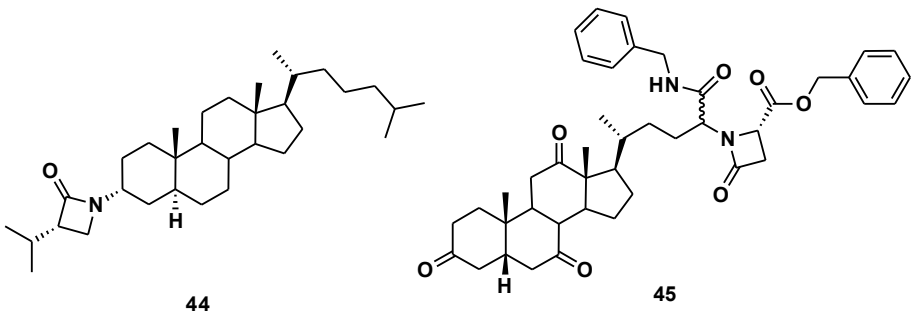


Figure 17

Section A

Design, Synthesis and Antimicrobial Activity of β-Lactam-Bile Acid Conjugates Linked via 1,2,3-Triazole.

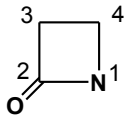
1.A.1 Abstract

Synthesis of series of novel 1,2,3-triazole linked β-lactam-bile acid conjugates **79-86** using intermolecular 1,3-dipolar cycloaddition reaction of azido β-lactam and terminal alkyne of bile acids in the presence of Cu(I) catalyst (click chemistry) have been realized in very high yields. The azido β-lactams were prepared by using the ketene-imine cycloaddition (Staudinger) reaction. All the synthesized compounds were evaluated in vitro for their antifungal and antibacterial activities against a wide variety of microorganisms (Gram negative bacteria, Gram positive bacteria and fungi). Most of the compounds exhibited significant antifungal and moderate antibacterial activity against all the tested strains.

1.A.2 Introduction

β-Lactam antibiotics

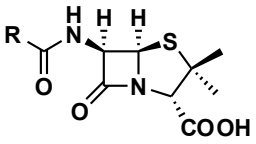
β-Lactams are a large class of antibiotics characterized by the presence of an azetidine-2-one ring (Figure 18), which is the core of biological activity. The unique structural feature and chemotherapeutic properties of β-lactam antibiotics continue to attract the attention of synthetic chemists, as much for their pharmaceutical value and as they provide variety of synthetic challenges. Although the first synthesis of β-lactam ring was reported way back in 1907 by Staudinger,[42] β-lactams as a class acquired immense importance only after the discovery of penicillin by Fleming[43] in 1928 and its structural confirmation by X-ray crystallography,[44] which unambiguously confirmed the presence of 4-membered amide ring (β-lactam). The azetidin-2-one ring was identified as the key structural unit responsible for the antibiotic activity.



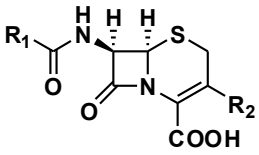
Azetidine-2-one
(β-Lactam ring)

Figure 18

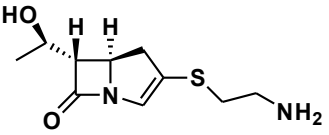
The azetidine-2-one (β-lactam) ring system is a common structural feature of a number of broad spectrum β-lactam antibiotics, like penicillins **46**, cephalosporins **47**, carbapenems **48**, nocardicins **49** and monobactams **50** (Figure 19) , which have been widely used as chemotherapeutic agents for treating microbial diseases. It also shows many other interesting biological properties, such as cholesterol absorption inhibitors,[45] human cytomegalovirus protease inhibitors,[46] thrombin inhibitors,[47] antihyperglycemic,[48] anti-tumour,[49] anti-HIV,[50] anti-inflammatory, analgesic activities[51] and serine-dependent enzyme inhibitors.[52]



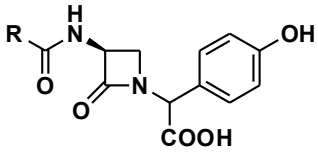
Penicillin **46**



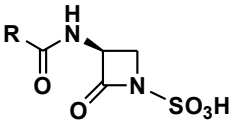
Cephalosporin **47**



Carbapenem **48**



Nocardicin **49**



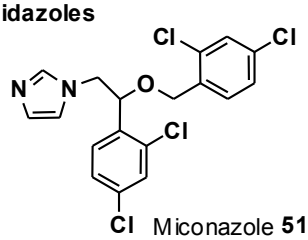
Monobactam **50**

Figure 19

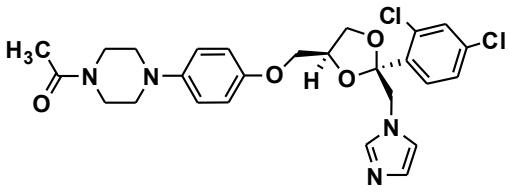
Azole antifungals

Azoles are the largest class of antifungal agents in clinical use.[53] Miconazole **51**, ketoconazole **52**, clotrimazole **53** are the topical agents and fluconazole **54** and itraconazole **55** (Figure 20) are useful in the treatment of systemic mycoses.

Imidazoles



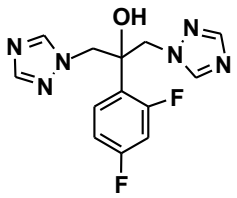
Miconazole **51**



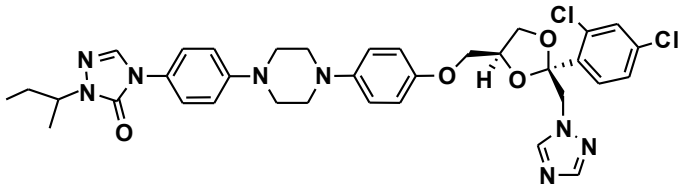
Ketoconazole **52**

Clotrimazole **53**

Triazoles



Fluconazole **54**

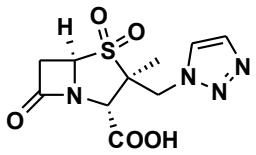


Itraconazole **55**

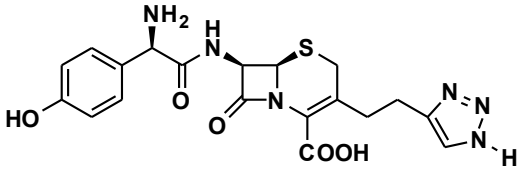
Figure 20

1,2,3-Triazole-β-lactam conjugates

1,2,3-triazole moiety in combination with the β-lactam ring system is present in a number of drugs such as the β-lactam antibiotic Tazobactam **56** and the cephalosporine Cefatrizine **57** (Figure 22).



Tazobactam **56**



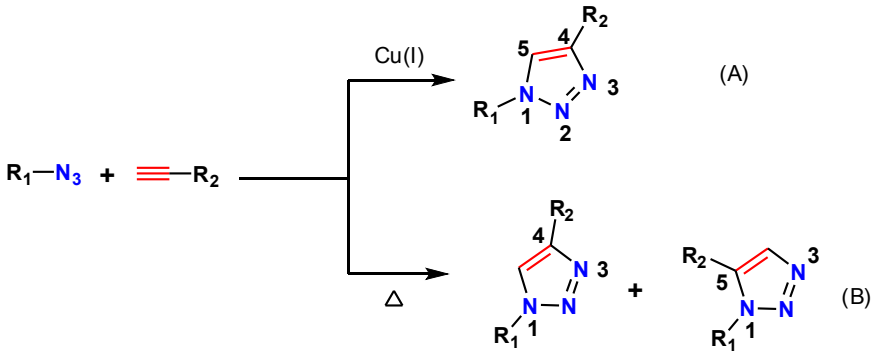
Cefatrizine **57**

Figure 22

1,2,3-Triazole moieties are attractive connecting units, as they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and solubility.[56] 1,2,3-Triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole unit shows diverse biological activities such as antibacterial,[57a] herbicidal, fungicidal,[57b] selective β_3 adrenergic receptor inhibition,[57c] anti-platelet activity,[57d] antiallergic,[57e] and anti-HIV.[57f, g]

Click chemistry

The 1,3-dipolar cycloaddition reaction of a 1,3-dipole to a dipolarophile (i.e. an acetylene or alkene) for the synthesis of five membered heterocycles are well known transformations in synthetic organic chemistry.[58] This reaction gives rise to two regioisomers namely 1,4 and 1,5-disubstituted 1,2,3-triazole products in 1:1 ratio. Recently, Sharpless[59] and Meldal[60] groups have reported the dramatic rate enhancement (up to 10^7 times) and improved regioselectivity of the Huisgen 1,3- dipolar cycloaddition reaction of an organic azide and terminal acetylene to afford, regiospecifically, the 1,4-disubstituted, 1,2,3-triazole in the presence of Cu(I) catalyst (Scheme 1).

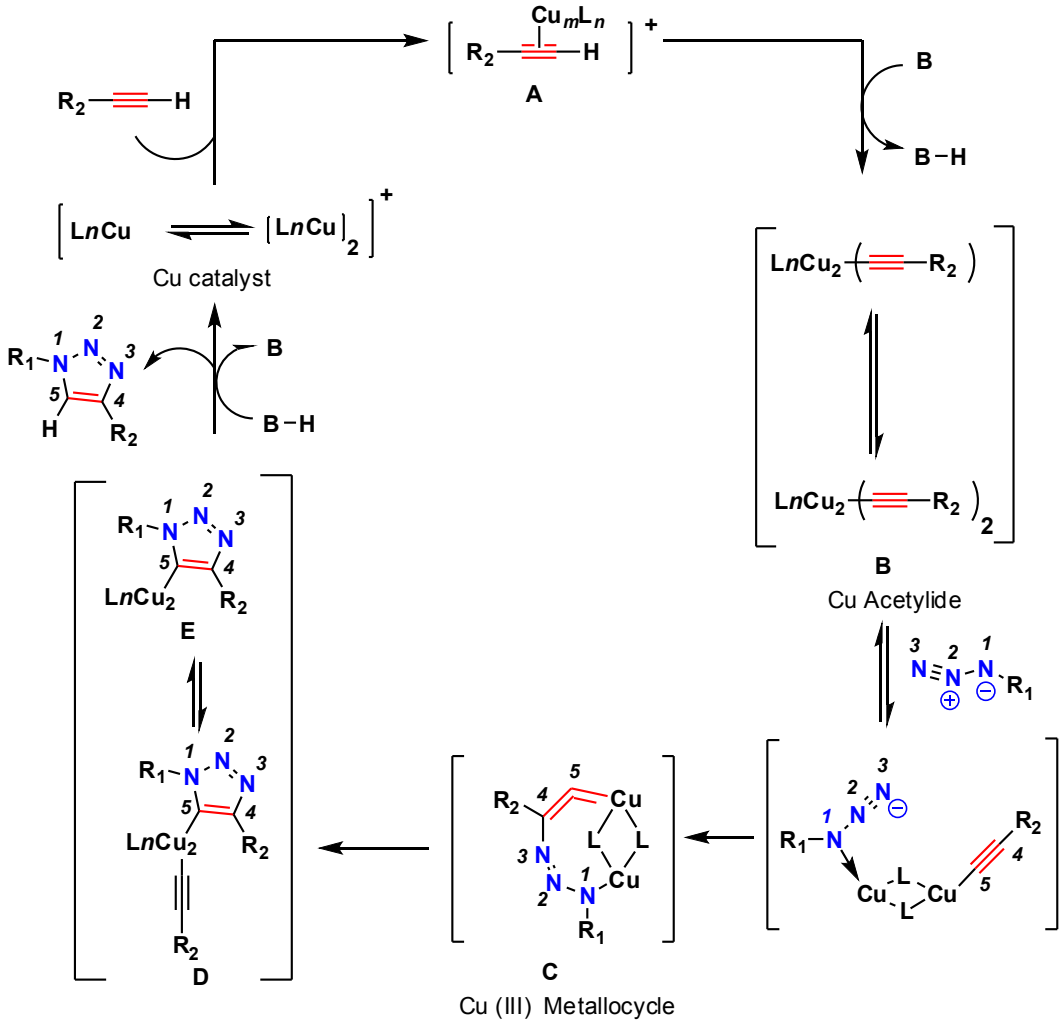


Scheme 1: 1,3-dipolar cycloaddition between organic azides and terminal alkynes

The Cu (I)-catalyzed 1,3- dipolar cycloaddition reaction has successfully fulfilled the requirement of "click chemistry" as prescribed by Sharpless and within the past few years has become a premier component of synthetic organic chemistry.[61]

The classical non-catalyzed process proceeds by concerted mechanism under thermal conditions to afford a mixture 1,4- and 1,5-disubstituted, 1,2,3-triazole regioisomers. The relative proportion of regioisomers and rate can be predicted from electronic and steric effects.[62]

The Cu(I)-catalysed ("click") process has been postulated to occur by a step wise mechanism on the basis of recent thermal and kinetic studies.[62] Substantial rate increase of the Cu(I)-catalysed process in the aqueous solvents is rationalized in terms of stepwise process which lowers the activation barrier relative to that of the non-catalysed process by



Scheme 2: Outline of plausible mechanisms for the Cu(I) catalyzed reaction between organic azides and terminal alkynes.

as much as 11.8 kcal/mol.[63, 57a] The proposed catalytic cycle involves several postulated and transient Cu(I)-acetylide complexes, starting with complexation of the alkyne to the Cu(I) metal centre to form a Cu(I)-alkyn π–complex (**A**) (Scheme 2).[64]

The enhanced reaction rate in water relative to organic solvents can be rationalized in terms of the endothermic ligand dissociation in organic media, for example acetonitrile (endothermic by 0.9 kcal mol^{-1}) relative to water (exothermic by 11.8 kcal mol^{-1}).[64] The formation of the Cu(I) acetylide complexes is also water-assisted, since water lowers the *p*Ka of the acetylene C-H by 9.8 *p*Ka units. Formation of the Cu(I)-acetylide species allows for subsequent ligand displacement with azide and results in a dimeric copper species (**B**). Complexation with azide activates it towords nucleophilic attack at the N-3 with the acetylide C-4 (The numbering is given according to triazole nomenclature). The resulting metallocycle (**C**) undergoes facile ring contraction *via* transannular association of the N-1 lone pair with the C5-Cu π* orbital to give the copper-triazole complex (**D**). Protonation of the triazole species, possibly with water and disassociation of the labile copper complex affords the 1,4-disubstituted 1,2,3-trazole (**E**), thus, regenerating the catalyst and ending the cycle.

Within a short time-frame, click chemistry has proven to be of remarkable utility and broad scope, not only in organic synthesis, but in chemical biology and drug discovery.[65] Azides and acetylenes are by definition kinetically stable entities possessing high built-in energy and are tolerant to a wide range of synthetic conditions.[66] Click chemistry is highly modular and simplifies difficult syntheses, thus, enabling a more cost-effective and efficient surveillance of structural space. The biocompatibility of the reaction, tolerance towards a broad range of *p*H and relative inertness of acetylenes and azides within highly functionalized biological milieus has allowed click chemistry to become a viable bioconjugation strategy for labeling biomolecules and for *in situ* lead discovery applications.[67] The 1,2,3-triazole moiety is a potential pharmacophore owing to its moderate dipole character and rigidity and can therefore be readily incorporated into a design strategy, rather than used as passive linker between two respective fragments of structural space.[68]

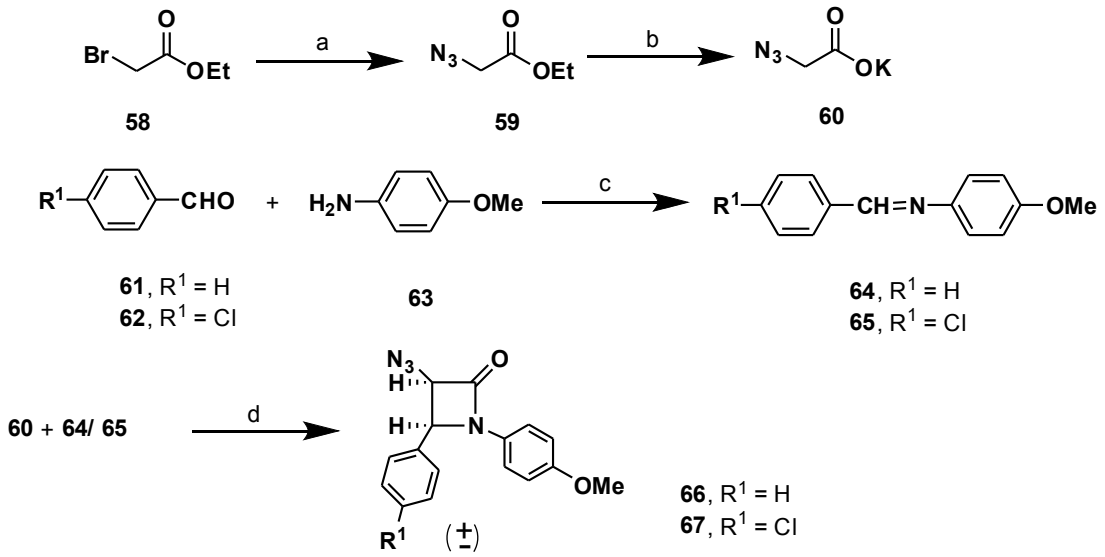
1.A.3 Present work

1.A.3.1 Objective

Literature survey reveals that bile acid transporters have been shown to accept and carry a variety of analogues that are derivatized at different positions of bile acids. 1,2,3-triazole moiety shows diverse biological activities and is present in combination of the β-lactam ring system in a numerous drugs. Based on the above-mentioned observations and in continuation of our recent work on bile acids,[35,69] we have designed and synthesized 1,2,3-triazole linked β-lactam-cholic acid/deoxycholic acid conjugates **74-81**. These bioconjugates can play a duel role. Azole and β-lactam derivative can act as an antimicrobial agent and bile acid backbone can transport whole drug at the appropriate site of action.

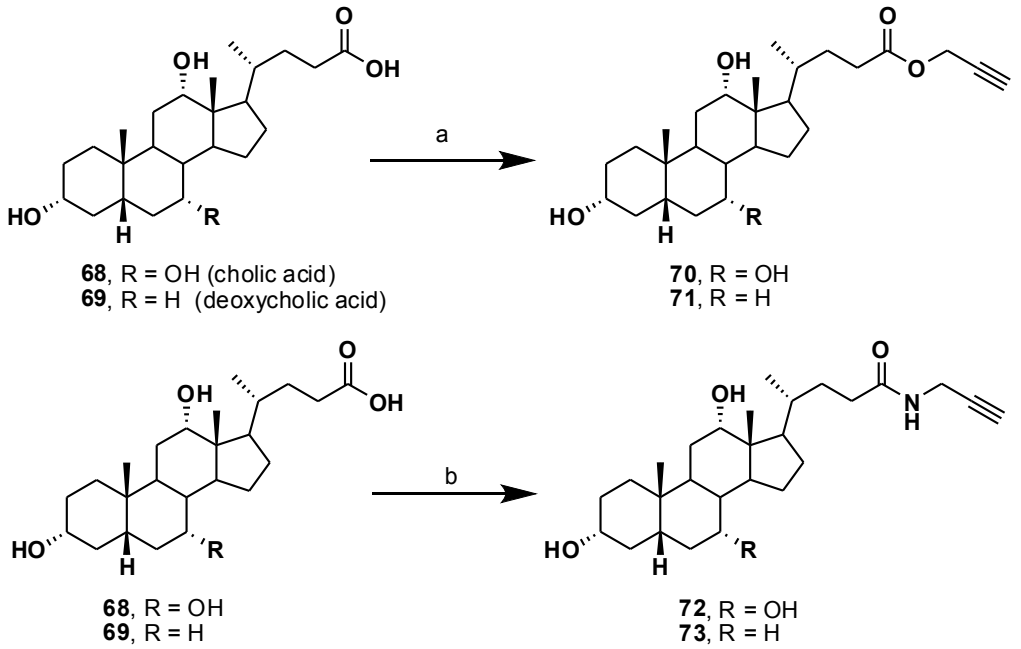
1.A.3.2 Results and Discussion

Target molecules **74-81** were synthesized using 1,3-dipolar cycloaddition reaction of β-lactams containing azide **66** and **67** and bile acids containing terminal alkyne **70-73**, in the presence of Cu(I) catalyst (click chemistry). Accordingly, the synthesis of azido β-lactams **66** and **67** was started from ethyl bromoacetate **58** (Scheme 3). Compound **58** on treatment with sodium azide afforded ethyl azidoacetate **59**. The formation of azido ester **59** was confirmed from its IR spectrum, which showed a strong absorption band at 2109 cm^{-1} due to azide group and at 1745 cm^{-1} due to the carbonyl carbon (-C=O) group. Subsequent saponification of ester functionality of compound **59** with KOH in methanol gave the potassium salt of azidoacetic acid **60** with 92% overall yield in two steps. The azido β-lactams **66** and **67** were prepared by using the ketene-imine cycloaddition (Staudinger)[70] reaction of **60** and imines **64** or **65** (prepared from the aldehydes **61** or **62** with the amine **63** in excellent yields) in the presence of triphosgene and triethylamine in anhydrous dichloromethane with 81% and 77% yields, respectively. The formation of azido β-lactam **66** was confirmed from its ^1H NMR spectrum, which showed characteristic signals at δ 5.29 (d, J = 5.3 Hz, 1H), at 5.02 (d, J = 5.3 Hz, 1H), corresponding to methine protons of β-lactam ring. Further, its IR spectrum showed absorption band at 2115 cm^{-1} corresponding to the azide group and at 1751 cm^{-1} corresponding to the carbonyl group (C=O) of β-lactam in **66**. Compounds **66** and **67** were obtained as racemic mixture.



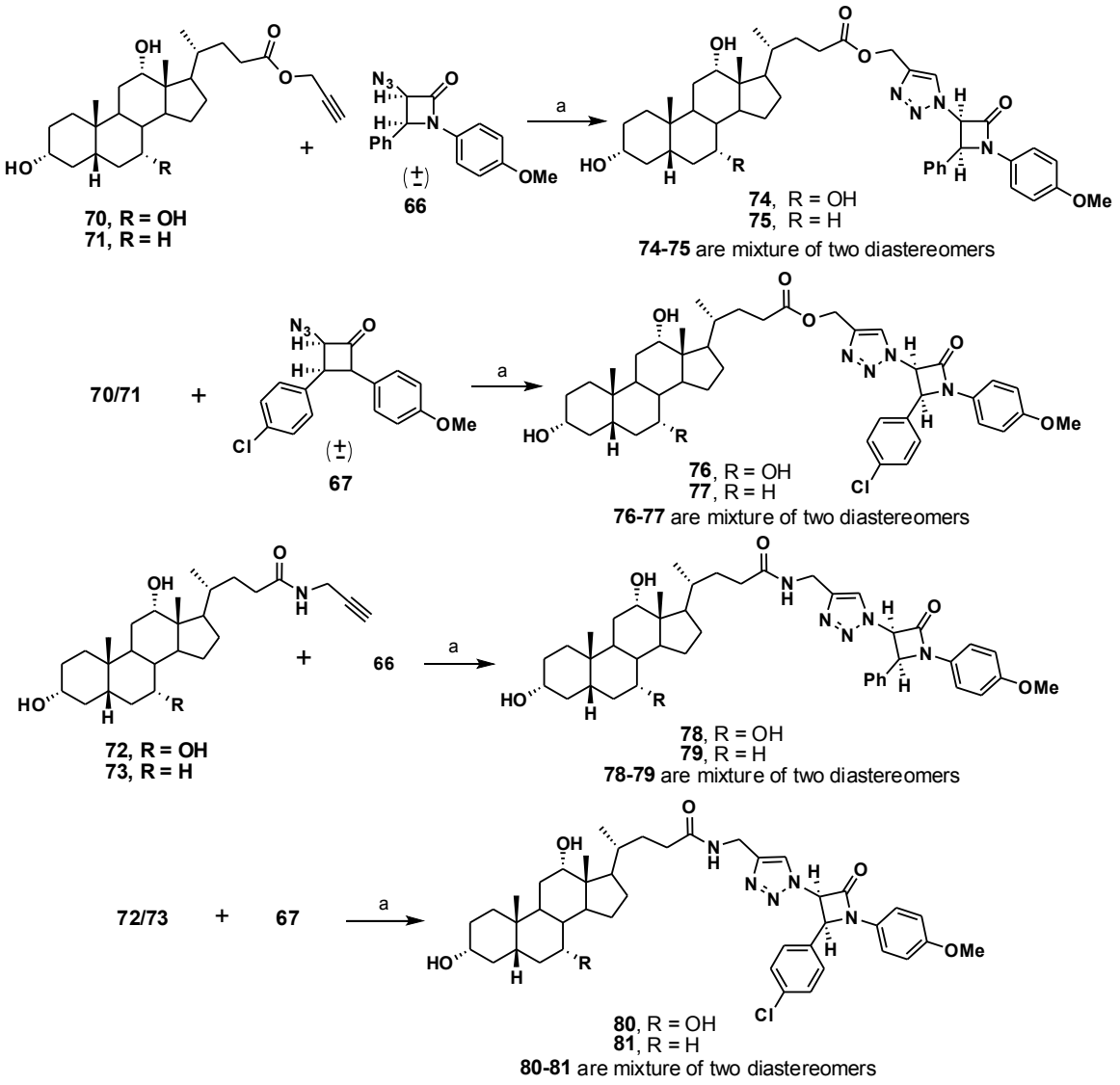
Scheme 3. Reagent and conditions: (a) NaN_3, Bu_4NBr, CH_2Cl_2/H_2O (1:1), 25 °C, 36 h, 98%; (b) KOH, MeOH, 25 °C, 4 h, 94%; (c) anhydrous $MgSO_4$, CH_2Cl_2, 25 °C, 12 h, **64** (97%) and **65 (**95%); (d) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, **66** (81%) and **67 (**77%**)**.

Propargyl esters **70** and **71** were prepared by coupling propargyl alcohol with cholic acid **68** or deoxycholic acid **69** using EDC·HCl [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] as coupling agent in DMF at 0-25 °C with 88% and 90% yields respectively (Scheme 4). The ^{1}H NMR spectrum of **71** displayed a characteristic signals at δ 4.68 (d, J = 2.2 Hz, 2H) correspondin to methylene protons (-$COOCH_2$-) and at δ 2.47 (t, J = 2.2 Hz, 1H) due to acetylene –CH. The IR spectrum of **71** showed hydroxyl absorption at 3382 cm^{-1} and ester carbonyl at 1737 cm^{-1}. Using similar reaction conditions, amides **72** and **73** containing terminal alkyne functionality were prepared by coupling propargyl amine with cholic acid **68** and deoxycholic acid **69** in excellent yields.



Scheme 4. Reagent and conditions: (a) EDC·HCl, HOBt, Propargyl alcohol, DMF, 0-25 °C, 12 h, 88% for **70** and 90% for **71;** (b) EDC·HCl, HOBt, Propargyl amine hydrochloride, Et_3N, DMF, 0-25 °C, 11 h, 89% for **72** and 92% for **73**.

Our next target was to synthesise 1,2,3-triazole linked β-lactam-bile acid conjugates **74-81**. The cycloaddition reaction of propargyl esters **70/71** with azido β-lactams **66** in the presence of Cu(I) catalyst (click chemistry)[36] under microwave irradiation furnished diastereomeric mixture of novel conjugates **74** and **75** in excellent yields (Scheme 5). The ^{1}H NMR spectrum of conjugate **75** showed a characteristic signals at δ 7.41 (s) due to triazole proton, two doublets at δ 6.38, 5.63 due to β-lactam ring protons and singlet at δ 3.79 corresponding to -OMe. It also shows peaks at δ 3.96 (bs) and at δ 3.61 (m) due to the C12α and C3α methine protons respectively from bile acid part. The formation of conjugate **75** was also confirmed from it ^{13}C NMR spectrum, which showed two typical signals at δ 142.5 and 123.8 for triazole carbons in addition to carbonyl carbon and aromatic carbon signals. In a similar way, exposure of propargyl esters **70/71** with azido β-lactam **67** and propargyl amides **72/73** with azido β-lactams **66/67** afforded diastereomeric mixture of hitherto unknown compounds **76-81** with 95-97% yields. The combination of racemic core **66** and **67** with optically pure **70-73** afforded diastereomeric mixture of triazole linked β-lactam bile acid conjugates **74-81** in equal amounts. These diastereomers were inseparable by flash column chromatography and also by crystallization. All the compounds **74-81** were fully characterized by spectroscopic data.



Scheme 5. Reagent and conditions: (a) Sodium ascorbate, $CuSO_4.5H_2O$, DMF/H_2O (7:3), microwave (385 watt), 5 min, 95-97%.

1.A.4 Bioevaluation

All the newly synthesized azido β-lactams **66**, **67**, steroidal alkynes **70-73** and 1,2,3-triazole linked β-lactam-bile acid conjugates **74-81** were tested *in vitro* for antifungal and antibacterial activity. The antifungal activity was tested using NCL isolate fungal strains *Candida albicans*, *Cryptococcous neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). Most of the pathogen fungi *viz C. albicans* are dimorphic in nature. However their use as model faces number of problems of slow growth rate and difficulties in getting synchronous growth.[71] Therefore nonpathogenic dimorphic fungus

Table 1. *In vitro* antimicrobial activity of compounds **66, 67** and **70-81.**

Comp.	Inhibitory concentration (μg/mL)													
	Fungal Strains										Bacterial Strains			
	CA		CN		BP		YL		FO		EC		SA	
	MIC[a]	IC_{50}[b]	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
66	>128	64	>128	>128	>128	>128	>128	128	>128	64	>128	64	>128	>128
67	>128	64	>128	64	>128	>128	>128	>128	>128	64	>128	128	>128	>128
70	>128	128	>128	>128	>128	>128	>128	64	>128	128	>128	128	>128	128
71	128	32	>128	64	>128	128	>128	128	>128	64	>128	128	>128	128
72	>128	>128	>128	>128	>128	>128	>128	64	>128	128	>128	128	>128	64
73	>128	64	>128	128	>128	128	>128	>128	>128	64	>128	>128	>128	128
74	64	8	32	16	32	8	64	16	64	32	64	32	32	16
75	128	64	>128	32	32	16	4	2	16	8	32	8	>128	64
76	128	16	32	8	32	17	8	4	>128	64	16	8	32	16
77	32	8	32	8	>128	32	64	32	16	8	64	32	128	32
78	32	16	128	16	64	32	16	4	32	16	32	16	32	16
79	32	16	32	8	64	32	128	32	64	16	16	8	128	32
80	64	32	32	8	32	8	16	16	128	32	32	16	64	32
81	16	8	64	16	8	4	32	8	32	8	64	32	>128	64
Amp	2	0.5	16	8	16	8	16	8	16	8	-	-	-	-
Flu	32	4	32	16	32	16	64	32	8	4	-	-	-	-
Tetr	-	-	-	-	-	-	-	-	-	-	8	4	16	8
Am	-	-	-	-	-	-	-	-	-	-	2	1	16	4

CA**,** *C. albicans* (NCL1); CN**,** *C. neoformans* (NCL2); BP**,** *B. poitrasii* (NCL3); YL**,** *Y. lipolytica* (NCL4); FO**,** *F. oxysporum* (NCL5); EC**,** *E. coli* (NCIM No.2574); SA**,** *S. aureus* (NCIM No.2122). Amp, Amphotericin B; Flu, Fluconazole; Tetr, Tetracycline; Am, Ampicillin

[a]MIC (Minimum inhibitory concentration) was determined as 90% inhibition of growth with respect to the growth control.

[b]IC_{50} was the concentration at which 50% growth inhibition was observed.

Negative control, DMSO, No inhibition.

B. poitrasii was used as a model which exhibits a rapid and simple one step process of yeast-mycelium transition in response to temperature and/or glucose change.[72] The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The MIC and IC_{50} values were determined using standard broth microdilution technique described by NCCLS.[73] In comparison with the antimicrobial activity, amphotericin B and

fluconazole were used as the reference antifungal agents, while tetracycline and ampicillin were used as the reference antibacterial agents. All the biological data of the tested compounds are depicted in Table 1 as MIC and IC_{50} values.

From the biological data (Table 1), it was observed that azido β-lactams **66**, **67** and steroidal alkynes **70-73** were almost inactive against all the tested strains. The MIC value for all these compounds were >128 μg/mL. As seen in Table 1 most of the β-lactam-bile acid conjugates **74-81** generally showed potent antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compounds **77-79** and **81** was higher or comparable to that of fluconazole against *C. albicans* with MIC value of 16-32 μg/mL. The compounds **74**, **76**, **77**, **79** and **80** showed good antifungal activity against *C. neoformans* having MIC value of 32 μg/mL comparable to that of reference drug fluconazole. However, the growth inhibitory activity of compound **81** was more potent than the reference drug amphotericin B and fluconazole against *B. poitrasii,* also the compounds **74-76** and **80** showed significant activity against *B. poitrasii* with MIC value of 32 μg/mL. *Y. lipolytica* was adversely affected by **75**, **76**, **78**, **80** and **81**, and in particular, **75** was the most potent with a low MIC value of 4 μg/mL. The compounds **75** and **77** showed significant inhibitory effect with MIC value of 16 μg/mL comparable to that of amphotericin B against *F. oxysporum*. Further more compounds **76** and **79** showed good antibacterial activity against *E. coli* having MIC value of 16 μg/mL. The compounds **74**, **76**, **78** and **80** derived from cholic acid having 7-hydroxy showed moderate antibacterial activity against *S. aureus*. However, the compounds **75**, **77**, **79**, and **81** derived from deoxycholic acid with the absence of 7-hydroxy were less active against *S. aureus* with MIC value of ≥128 μg/mL. From the overall activity results, it was observed that the ester or amide linkage and chloro substituent on phenyl ring of β-lactam part, did not affect the activity of the compounds.

1.A.5 Conclusion

A series of novel 1,2,3-triazole linked β-lactam-bile acid conjugates were synthesized using Cu(I) catalysed cycloaddition reaction of azido β-lactams and terminal alkynes derived from cholic acid/deoxycholic acid in excellent yields and their antimicrobial activities were evaluated. Compounds **74-81** demonstrated potent antimicrobial activity against all the strains tested. The compound **81** showed very good antifungal activity with

MIC value of 16 μg/mL against *C. albicans* and 8 μg/mL against *B. poitrasii*. In particular, compound **75** exhibited the maximum activity with MIC values of 4 μg/mL against *Y. lipolytica*. Additionally, only the compounds **74**, **76**, **78** and **80** derived from cholic acid were moderately active against *S. aureus*. This is the first report of synthesis and biological activity of the triazole linked β-lactam-bile acid conjugates.

1.A.6 Experimental

General procedure for synthesis of β-lactams 66 and 67

A solution of triphosgene (0.296 g, 1 mmol), in anhydrous CH_2Cl_2 (15 mL), was added slowly to a mixture of potassium salt of azidoacetic acid **60** (2 mmol), imine **64/65** (2 mmol) and triethylamine (0.84 mL, 6 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 ºC. After the addition, the reaction mixture was allowed to warm up to room temperature (28 ºC) and stirred for 15 h. The reaction mixture was then washed with water (20 mL), saturated sodium bicarbonate solution (2×15 mL) and brine (15 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated to get crude product, which was purified by column chromatography to give pure β-lactams **66** and **67**.

3-Azido-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (66):

White solid, Yield: 81%; Mp: 113-114 ºC; IR ($CHCl_3$, cm^{-1}) 2115 (N_3), 1751 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.25-7.44 (m, 7H, Ar-H), 6.77-6.85 (m, 2H, Ar-H), 5.29 (d, *J* = 5.3, 1H), 5.02 (d, *J* = 5.3, 1H), 3.76 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 160.7, 156.5, 132.6, 130.1, 129.0, 128.8, 127.4, 118.7, 114.3, 67.3, 60.6, 55.3; MS (LCMS) m/z: 317 (M + Na); Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.52; H, 5.03; N, 19.34.

3-Azido-4-(4-chlorophenyl)-1-(4-methoxyphenyl)-azetidin-2-one (67):

White solid, Yield: 77%; Mp: 117-118 ºC; IR ($CHCl_3$, cm^{-1}) 2117 (N_3), 1758 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.21-7.42 (m, 6H, Ar-H), 6.78-6.86 (m, 2H, Ar-H), 5.27 (d, *J* = 5.3, 1H), 5.05 (d, *J* = 5.3, 1H), 3.77 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 60.5, 156.6, 132.6, 134.9, 131.2, 129.8, 129.0, 128.8, 118.6, 114.3, 67.3, 60.0, 55.3; MS (LCMS) m/z 351.11 (M + Na); Anal. Calcd for $C_{16}H_{13}N_4O_2$: C, 58.45; H, 3.99; N, 17.04. Found: C, 58.31; H, 3.96; N, 16.67.

General procedure for synthesis of alkyne compounds70-73.

EDC·HCl (1.5 equiv) and HOBt (1-hydroxy benzotriazole) (0.5 equiv) were added to a solution of cholic acid **68**/deoxycholic acid **69** (1 equiv) and popargyl alcohol/propargyl amine (1.1 equiv) in dry DMF (5 mL) under argon at 0 ºC. After the addition, the reaction mixture was allowed to warm up to 28 ºC and stirred for 12 h. The reaction was quenched by adding crushed ice and extracted three times with ethyl acetate. The combined extracts were washed with water and brine, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The resulting crude product was purified by column chromatography to give pure alkyne compounds **70-73**.

Propargyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (70):

White powder, Yield: 88%; Mp: 112-114 ºC; $[\alpha]_D^{27}$ +26.15 (c 1.3, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3396, 3307, 1737; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.68 (d, *J* = 2.5 Hz, 2H, OCH_2), 3.96 (bs, 1H, CH-12), 3.84 (bs, 1H, CH-7), 3.45 (m, 1H, CH-3), 2.47 (t, *J* = 2.5 Hz, 1H, alkyne CH), 0.98 (d, *J* = 5.8 Hz, 3H, CH_3-21), 0.89 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4, 77.7, 74.7, 73.0, 71.7, 68.3, 51.6, 46.8, 46.3, 41.4, 39.3, 35.1, 34.7, 30.9, 30.6, 30.2, 29.6, 28.0, 27.4, 26.1, 23.1, 22.3, 17.2, 12.3; MS (LCMS) *m/z*: 469 (M + Na); Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48; Found: C, 72.25; H, 9.23.

Propargyl 3α,12α-dihydroxy-5β-cholan-24-oate (71):

White powder, Yield: 90%; Mp: 160-161 ºC; $[\alpha]_D^{27}$ +43.5 (c 1.5, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3382, 3307, 1737; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.68 (d, *J* = 2.2 Hz, 2H, OCH_2), 3.98 (bs, 1H, CH-12), 3.55-3.64 (m, 1H, CH-3), 2.47 (t, *J* = 2.2 Hz, 1H, alkyne CH), 0.97 (d, *J*= 5.9 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2, 77.7, 74.6, 72.9, 71.5, 51.6, 48.1, 47.1, 46.4, 42.0, 36.3, 35.9, 35.2, 35.1, 34.0, 33.5, 30.9, 30.7, 30.2, 28.5, 27.4, 27.1, 26.06, 23.6, 22.9, 17.1, 12.6; MS (LCMS) *m/z*: 453.14 (M + Na); Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83; Found: C, 74.97; H, 9.62.

N-Propargyl-3α,7α,12α-trihydroxy-5β-cholan-24-amide (72):

White powder, Yield: 89%; Mp: 276-278 ºC; $[\alpha]_D^{27}$ +22.9 (c 1.31, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3309, 1652; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.43 (t, *J* = 5.2 Hz, 1H, CONH), 4.02-4.06 (m, 2H, NH-CH_2), 3.97 (bs, 1H, CH-12), 3.84 (bs, 1H, CH-7), 3.38-3.51 (m, 1H,

CH-3), 2.84 (s, 3H, OH), 2.24 (t, *J* = 2.5 Hz, 1H, alkyne CH), 0.99 (d, *J* = 5.8 Hz, 3H, CH_3-21), 0.89 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.2, 80.0, 73.1, 71.8, 71.1, 68.4, 46.2, 41.4, 39.3, 35.3, 35.2, 34.7, 34.6, 32.4, 31.4, 30.2, 28.9, 28.0, 27.5, 26.2, 23.2, 22.4, 17.4, 12.4; MS (LCMS) *m/z*: 446.19 (M + 1), 468.16 (M + Na); Anal. Calcd for $C_{27}H_{43}NO_4$: C, 72.77; H, 9.73; N, 3.14; Found: C, 72.93; H, 9.51; N, 2.82.

N-Propargyl-3α,12α-dihydroxy-5β-cholan-24-amide (73):

White powder, Yield: 92%; Mp: 184 ºC; $[\alpha]_D^{27}$ +51.76 (c 0.97, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3475, 3446, 3238, 3209, 1633; ^1H NMR ($CDCl_3$ + $DMSOD_6$, 200 MHz) δ 6.80 (bs, 1H, CONH), 3.88-3.92 (m, 2H, NH-CH_2), 3.85 (bs, 1H, CH-12), 3.41-3.55 (m, 1H, CH-3), 2.14 (t, *J* = 2.5 Hz, 1H, alkyne CH), 0.87 (d, *J* = 6.1 Hz, 3H, CH_3-21), 0.79 (s, 3H, CH_3-19), 0.56 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$ + $DMSOD_6$, 50 MHz) δ 174.5, 79.3, 72.7, 71.0, 70.7, 47.7, 46.3, 46.0, 41.8, 35.6, 35.0, 34.9, 33.8, 33.2, 32.3, 31.2, 29.4, 28.4, 28.2, 27.2, 26.8, 25.8, 23.4, 22.7, 16.6, 12.3; MS (LCMS) *m/z*: 430.21 (M + 1), 452.19 (M + Na); Anal. Calcd for $C_{27}H_{43}NO_3$: C, 75.48; H, 10.09; N, 3.26; Found: C, 75.10; H, 9.87; N, 3.45.

General procedure for synthesis of β-lactam-bile acid conjugates74-81.

The alkynes of cholic acid/deoxycholic acid (1 equiv) and the azido β-lactams **66** or **67** (1 equiv) were dissolved in DMF/H_2O 7:3 (10 mL). To this solution $CuSO_4 \cdot 5H_2O$ (0.05 equiv) and sodium ascorbate (0.5 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 385 watt. It was then cooled to room temperature, quenched with crushed ice and extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous sodium sulphate. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography to obtain β-lactam-bile acid conjugates **74-81**.

Triazole linked β-lactam-cholic acid conjugate (74):

White solid, Yield: 95%; Mp: 127-128 ºC; IR ($CHCl_3$, cm^{-1}) 3386, 1758, 1730; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35-7.47 (m, 3H, Ar-H), 7.12-7.19 (m, 5H, Ar-H), 6.87 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.37-6.43 (m, 1H), 5.63 (d, *J* = 3.0 Hz, 1H), 4.95 (bs, 2H, -OCH_2), 3.95 (bs, 1H, CH-12), 3.84 (bs, 1H, CH-7), 3.79 (s, 3H, -OCH_3), 3.45 (bs, 1H, CH-3), 0.96 (bs,

3H, CH_3-21), 0.89 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.5, 158.1, 156.7, 142.7, 131.0, 129.8, 128.7, 128.4, 126.4, 124.0, 118.7, 114.3, 72.7, 71.5, 68.1, 67.6, 60.8, 56.6, 55.2, 46.6, 46.1, 41.3, 39.2, 35.0, 34.5, 30.8, 30.4, 30.1, 27.9, 27.3, 26.0, 23.0, 22.2, 17.0, 12.2; MS (LCMS) *m/z*: 741 (M + 1), 763 (M + Na); Anal. Calcd for $C_{43}H_{56}N_4O_7$: C, 69.70; H, 7.62; N, 7.56; Found: C, 69.43; H, 7.87; N, 7.22.

Triazole linked β-lactam-deoxycholic acid conjugate (75):

White solid, Yield: 96%; Mp: 120-123 ºC; IR ($CHCl_3$, cm^{-1}) 3446, 1758, 1733; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.35-7.41 (m, 3H, Ar-H), 7.11-7.21 (m, 5H, Ar-H), 6.87 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.38 (d, *J* = 5.4 Hz, 1H), 5.63 (d, *J* = 5.4 Hz, 1H), 4.95 (q, *J* = 12.9, 16.5 Hz, 2H, -OCH_2), 3.96 (bs, 1H, CH-12), 3.79 (s, 3H, -OCH_3), 3.54-3.69 (m, 1H, CH-3), 2.11-2.34 (m, 2H, -CH_2CO), 0.95 (d, *J* = 5.8 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.6, 158.1, 156.8, 142.5, 131.1, 129.8, 128.8, 128.5, 126.5, 123.8, 118.7, 114.4, 72.8, 71.4, 67.5, 60.9, 56.7, 55.3, 48.0, 46.9, 46.3, 41.9, 36.2, 35.8, 35.1, 35.0, 34.9, 33.9, 33.4, 30.9, 30.8, 30.5, 30.2, 28.5, 27.3, 27.0, 26.0, 23.5, 23.0, 17.1, 12.5; MS (LCMS) *m/z*: 725.22 (M + 1), 747.13 (M + Na); Anal. Calcd for $C_{43}H_{56}N_4O_6$: C, 71.24; H, 7.79; N, 7.73; Found: C, 71.15; H, 7.57; N, 7.82.

Triazole linked β-lactam-cholic acid conjugate (76):

White solid, Yield: 97%; Mp: 133-134 ºC; IR ($CHCl_3$, cm^{-1}) 3415, 1760, 1731; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.05-7.50 (m, 7H, Ar-H), 6.87 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.39 (dd, *J* = 5.4, 9.9 Hz, 1H), 5.60 (d, *J* = 5.3 Hz, 1H), 5.99 (q, *J* = 13.8, 17.3 Hz, 2H, -OCH_2), 3.95 (bs, 1H, CH-12), 3.85 (bs, 1H, CH-7), 3.79 (s, 3H, -OCH_3), 3.37-3.51 (m, 1H, CH-3), 0.95 (d, *J* = 4.3 Hz, 3H, CH_3-21), 0.89 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 157.9, 157.0, 142.8, 134.7, 129.8, 129.7, 128.8, 128.0, 124.1, 118.7, 114.5, 72.8, 71.7, 68.2, 67.5, 60.5, 56.8, 55.4, 46.8, 46.3, 41.5, 41.4, 39.4, 35.2, 35.1, 34.6, 34.5, 30.9, 30.8, 30.6, 30.3, 28.1, 27.4, 26.2, 23.1, 22.3, 17.2, 12.3; MS (LCMS) *m/z*: 797.22 (M + Na); Anal. Calcd for $C_{43}H_{55}ClN_4O_7$: C, 66.61; H, 7.15; N, 7.23; Found: C, 66.43; H, 7.37; N, 7.61.

Triazole linked β-lactam-deoxycholic acid conjugate (77):

White solid, Yield: 96%; Mp: 123-125 ºC; IR ($CHCl_3$, cm^{-1}) 3425, 1758, 1731; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.49 (s, 1H, Ar-H), 7.29-7.37 (m, 2H, Ar-H), 7.05-7.19 (m, 4H, Ar-H), 6.88 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.36 (d, *J* = 5.4 Hz, 1H), 5.6 (d, *J* = 5.4 Hz, 1H), 5.0 (q, *J* = 12.9, 17.8 Hz, 2H, -OCH_2), 3.97 (bs, 1H, CH-12), 3.79 (s, 3H, -OCH_3), 3.53-3.67 (m, 1H, CH-3), 2.18-2.36 (m, 2H, -CH_2CO), 0.94 (d, *J* = 5.3 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.8, 157.9, 157.0, 142.8, 134.9, 129.7, 129.6, 128.9, 128.0, 118.7, 114.5, 72.9, 71.6, 67.5, 60.5, 56.8, 55.4, 48.1, 47.1, 46.4, 41.9, 36.4, 35.9, 35.1, 35.04, 35.0, 34.0, 33.5, 30.9, 30.8, 30.6, 30.3, 28.5, 27.4, 27.0, 26.0, 23.5, 23.0, 17.2, 12.6; MS (LCMS) *m/z*: 759.62 (M + 1), 781.67 (M + Na); Anal. Calcd for $C_{43}H_{55}ClN_4O_6$: C, 68.01; H, 7.30; N, 7.38; Found: C, 67.80; H, 7.65; N, 7.62.

Triazole linked β-lactam-cholic acid conjugate (78):

White solid, Yield: 95%; Mp: 153-154 ºC; IR ($CHCl_3$, cm^{-1}) 3396, 1757, 1650; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.16-7.41 (m, 8H, Ar-H), 6.86 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.35 (bs, 1H, unresolved splitting), 5.62 (bs, 1H, unresolved splitting), 4.22 (bs, 2H, -OCH_2), 3.94 (bs, 1H, CH-12), 3.84 (bs, 1H, CH-7), 3.78 (s, 3H, -OCH_3), 3.42 (bs, 1H, CH-3), 2.77 (bs, 3H, -OH), 2.16 (bs, 2H, -CH_2CO), 0.96 (bs, 3H, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9, 158.5, 158.4, 156.9, 145.0, 131.2, 130.0, 128.8, 128.5, 126.7, 123.2, 118.9, 114.5, 72.9, 71.7, 68.2, 67.8, 61.1, 55.4, 46.3, 41.5, 39.5, 35.3, 34.64, 34.57, 34.3, 32.6, 31.4, 30.3, 28.1, 27.5, 26.3, 23.1, 22.4, 17.4, 12.3; MS (LCMS) *m/z*: 740.63 (M + 1), 762.62 (M + Na); Anal. Calcd for $C_{43}H_{57}N_5O_6$: C, 69.80; H, 7.76; N, 9.46; Found: C, 69.96; H, 7.39; N, 9.50.

Triazole linked β-lactam-deoxycholic acid conjugate (79):

White solid, Yield: 96%; Mp: 140-141 ºC; IR ($CHCl_3$, cm^{-1}) 3375, 1758, 1660; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34-7.38 (m, 3H, Ar-H), 7.17 (bs, 5H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.35 (bs, 1H, unresolved splitting), 6.18 (bs, 1H, -NH), 5.62 (d, *J* = 4.9 Hz, 1H), 4.24 (bs, 2H, -OCH_2), 3.96 (bs, 1H, CH-12), 3.79 (s, 3H, -OCH_3), 3.52-3.68 (m, 1H, CH-3), 0.95 (d, *J* = 5.7 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 158.3, 156.9, 144.9, 131.2, 130.0, 128.8, 128.5, 126.6, 118.8, 114.5, 72.9, 71.4, 67.7, 61.1, 55.3, 48.0, 46.7, 46.4, 42.0, 36.3, 35.9, 35.2, 34.2,

34.0, 33.5, 32.94, 32.84, 31.38, 31.33, 30.3,28.5, 27.4, 27.1, 26.1, 23.6, 23.0, 17.3, 12.6; MS (LCMS) *m/z*: 724.45 (M + 1), 746.41 (M + Na); Anal. Calcd for $C_{43}H_{57}N_5O_5$: C, 71.34; H, 7.94; N, 9.67; Found: C, 71.04; H, 7.82; N, 9.93.

Triazole linked β-lactam-cholic acid conjugate (80):

White solid, Yield: 96%; Mp: 178-179 ºC; IR ($CHCl_3$, cm^{-1}) 3388, 1764, 1652; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.33-7.56 (m, 3H, Ar-H), 7.12 (bs, 4H, Ar-H), 6.85 (d, *J* = 8.3 Hz, 2H, Ar-H), 6.34 (bs, 1H, unresolved splitting), 5.60 (bs, 1H, unresolved splitting), 4.26 (bs, 2H, -OCH_2), 3.95 (bs, 1H, CH-12), 3.83 (bs, 1H, CH-7), 3.78 (s, 3H, -OCH_3), 3.41 (bs, 1H, CH-3), 2.19 (bs, 2H, -CH_2CO), 0.95 (bs, 3H, CH_3-21), 0.89 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 158.2, 156.9, 145.1, 134.8, 129.8, 128.7, 128.3, 123.4, 118.8, 114.5, 73.0, 71.7, 68.2, 67.6, 60.6, 55.4, 46.3, 41.5, 39.4, 35.3, 34.7, 32.5, 31.4, 30.2, 29.6, 28.0, 27.5, 26.3, 23.2, 22.4, 17.4, 12.3; MS (LCMS) *m/z*: 796.71 (M + Na); Anal. Calcd for $C_{43}H_{56}ClN_5O_6$: C, 66.69; H, 7.29; N, 9.04; Found: C, 66.87; H, 7.61; N, 8.85.

Triazole linked β-lactam-deoxycholic acid conjugate (81):

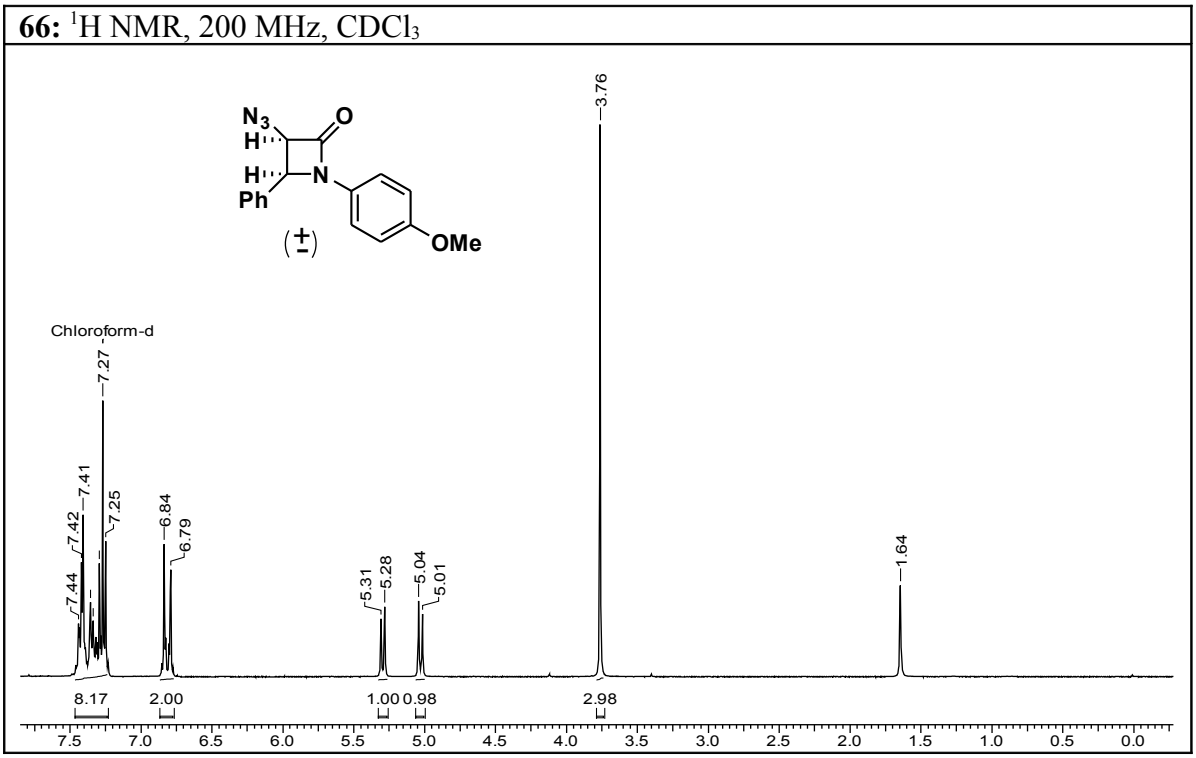
White solid, Yield: 95%; Mp: 144-145 ºC; IR ($CHCl_3$, cm^{-1}) 3373, 1758, 1658; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30-7.59 (m, 3H, Ar-H), 7.09-7.16 (m, 4H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.33 (bs, 1H, unresolved splitting), 6.17 (bs, 1H, -NH), 5.58 (bs, 1H, unresolved splitting), 4.32 (bs, 2H, -OCH_2), 3.96 (bs, 1H, CH-12), 3.79 (s, 3H, -OCH_3), 3.61 (bs, 1H, CH-3), 2.04-2.20 (m, 2H, -CH_2CO), 0.95 (d, *J* = 5.5 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.9, 158.1, 156.9, 134.8, 129.7, 127.7, 128.6, 128.2, 118.6, 114.5, 72.9, 71.5, 67.9, 60.4, 55.4, 48.0, 46.7, 46.4, 42.0, 36.3, 35.9, 35.3, 35.2, 34.0, 33.4, 32.9, 31.4, 30.3, 28.5, 27.5, 27.1, 26.1, 23.6, 23.0, 17.0, 12.6; MS (LCMS) *m/z*: 780.23 (M + Na); Anal. Calcd for $C_{43}H_{56}ClN_5O_5$: C, 68.10; H, 7.44; N, 9.23; Found: C, 68.18; H, 7.13; N, 9.19.

Antimicrobial assay: In *vitro* antifungal and antibacterial activity of the newly synthesized compounds were studied against the NCL isolate fungal strains viz., *C. albicans* (NCL1), *C. neoformans* (NCL2), *B.* poitrasii (NCL3), *Y. lipolytica* (NCL4), *F. oxysporum* (NCL5) strains and bacterial strains *E. coli* (NCIM No.2574), and *S. aureus* (NCIM No.2122) respectively to find out MIC (Minimum Inhibitory Concentration) and

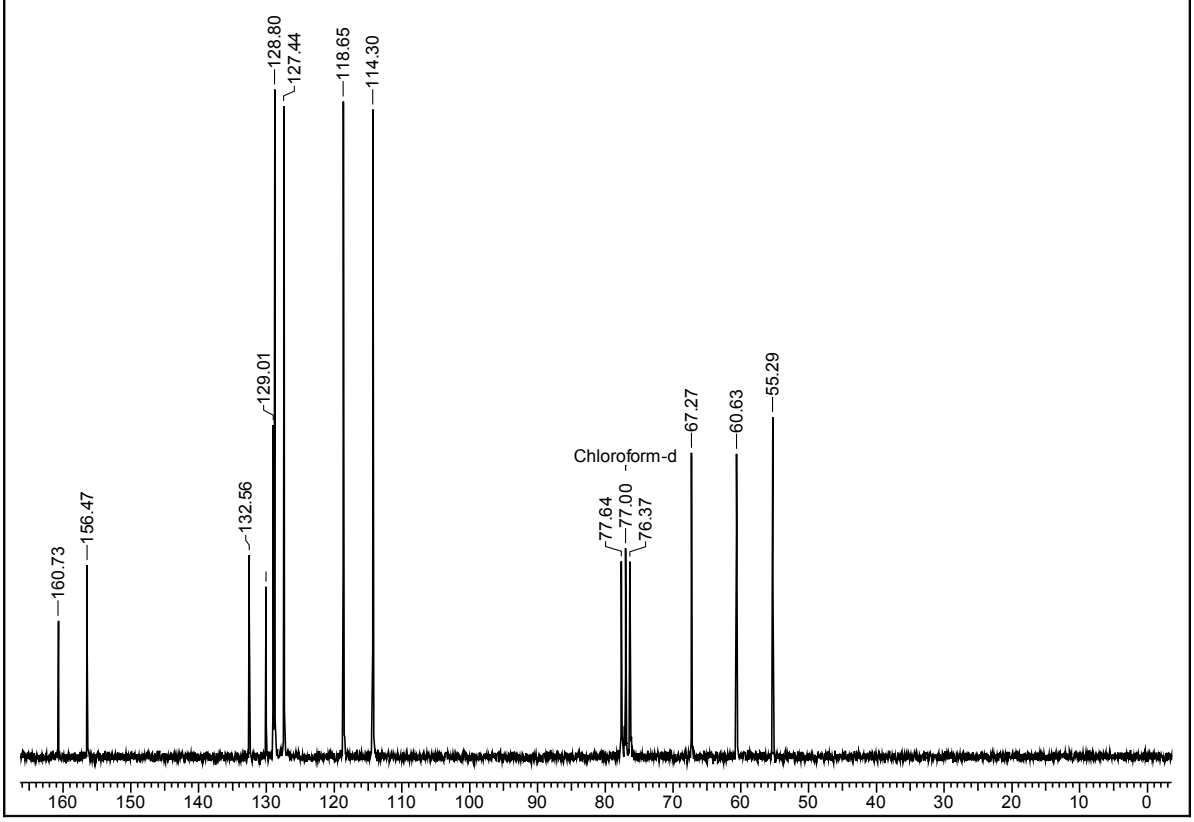
IC_{50} (50%, Inhibition of Growth). All the experiments were done in triplicate under similar experimental conditions. MIC and IC_{50} of the synthesized compounds were determined according to standard broth microdilution technique as per NCCLS guidelines.[73] Testing was performed in U bottom 96 well tissue culture plates in YPG, PDA for fungal strains and NA for bacterial strains. The concentration range of tested compounds and standard was 128-0.25 μg/ml. The plates were incubated at 28 °C for all the microorganisms except at 37 °C for *B. poitrasii*, absorbance at 600 nm was recorded to assess the inhibition of cell growth after 24 h for *B. poitrasii* and *Y. lipolytica*, 48 h for *C. albicans* and *F. oxysporum*, 72 h for *C. neoformans* and 24 h for bacterial cultures. MIC was determined as 90% inhibition of growth with respect to the growth control and IC_{50} was the concentration at which 50% growth inhibition was observed.

1.A.7 Selected Spectra

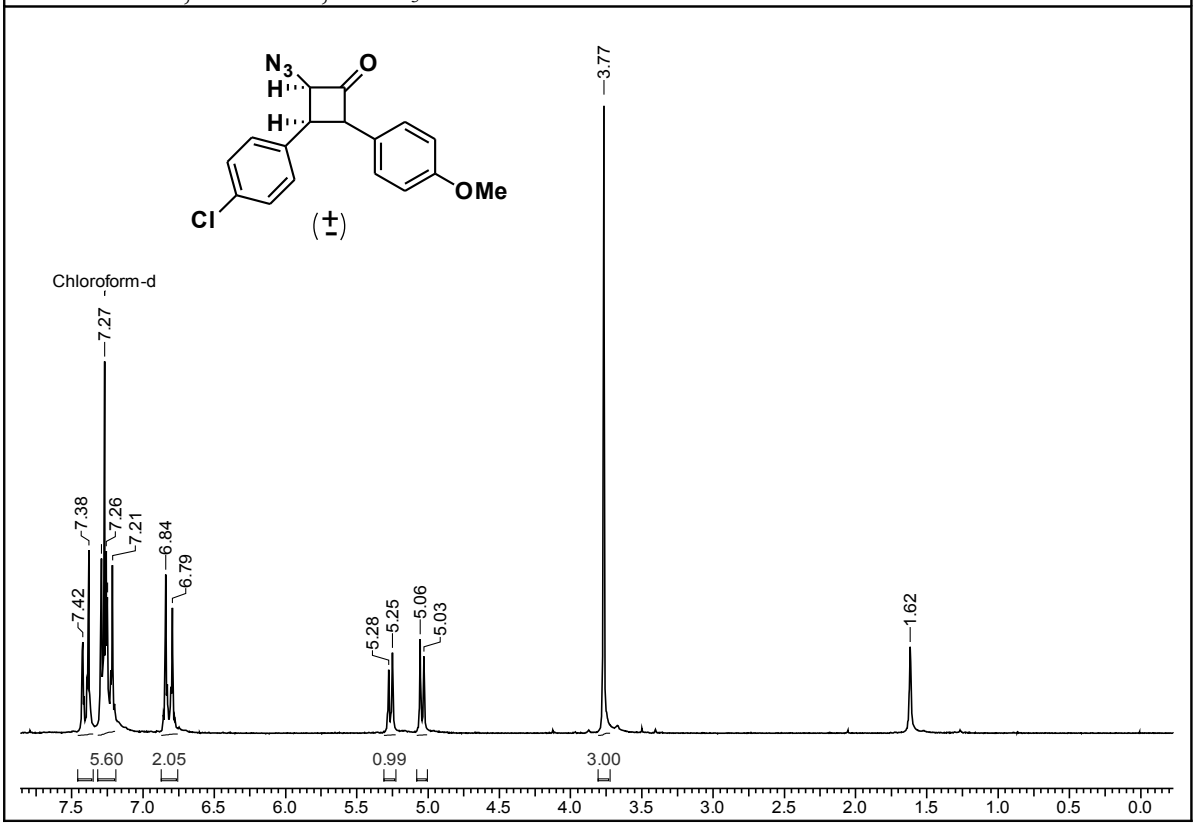
66: ^{1}H NMR, 200 MHz, $CDCl_3$



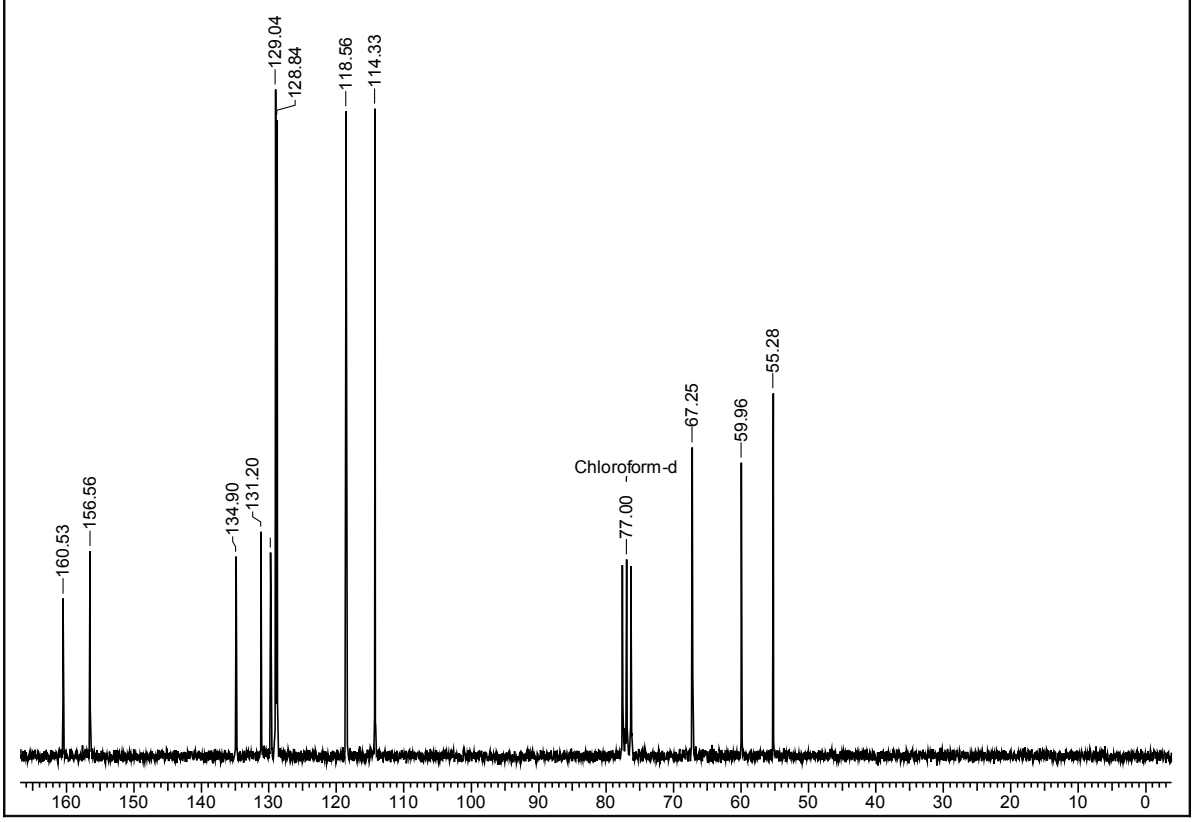
66: ^{13}C NMR, 50 MHz, $CDCl_3$



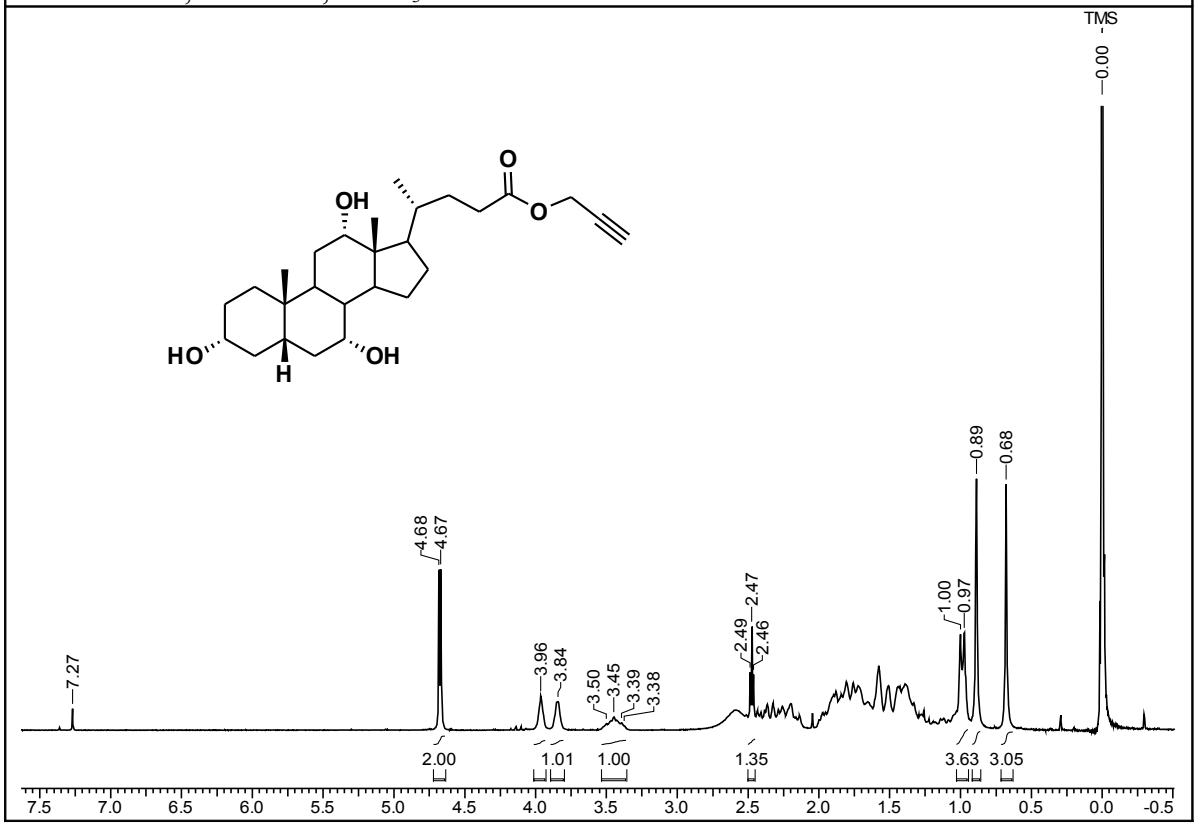
67: ^{1}H NMR, 200 MHz, $CDCl_3$



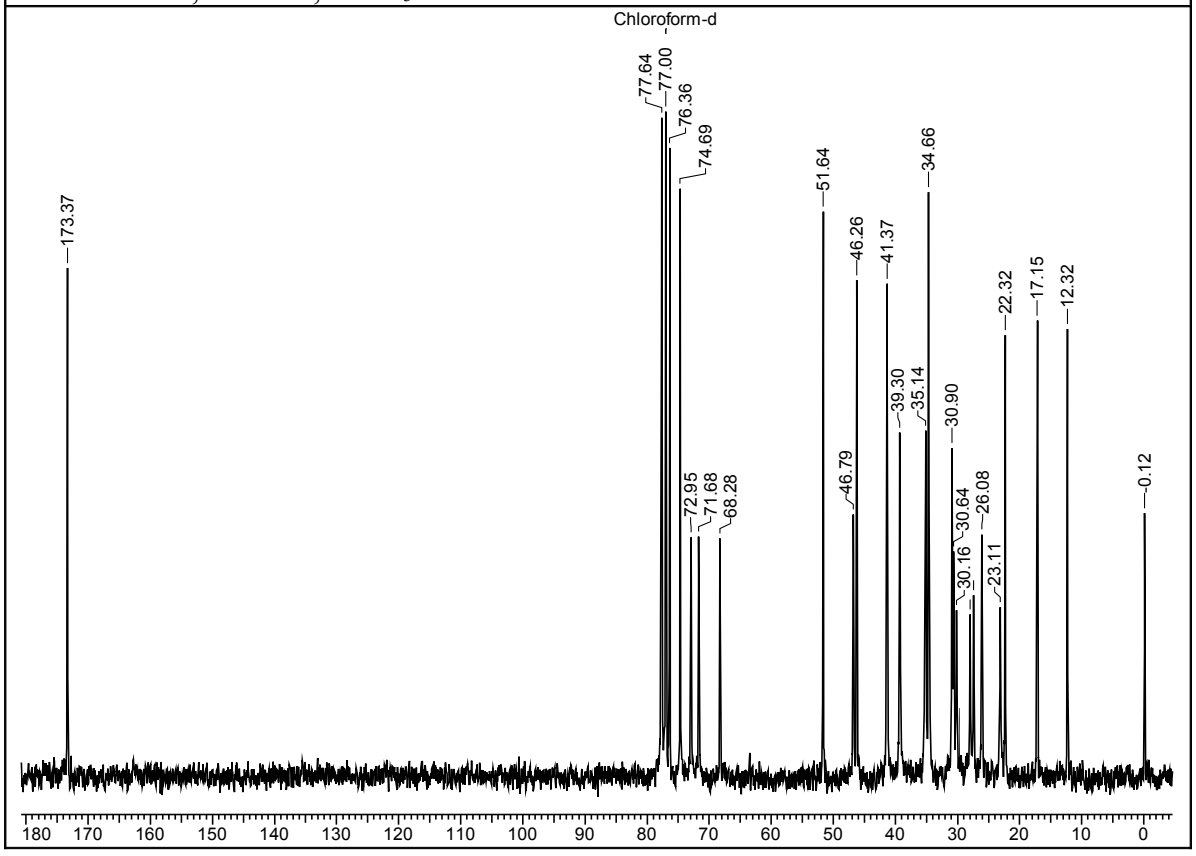
67: ^{13}C NMR, 50 MHz, $CDCl_3$



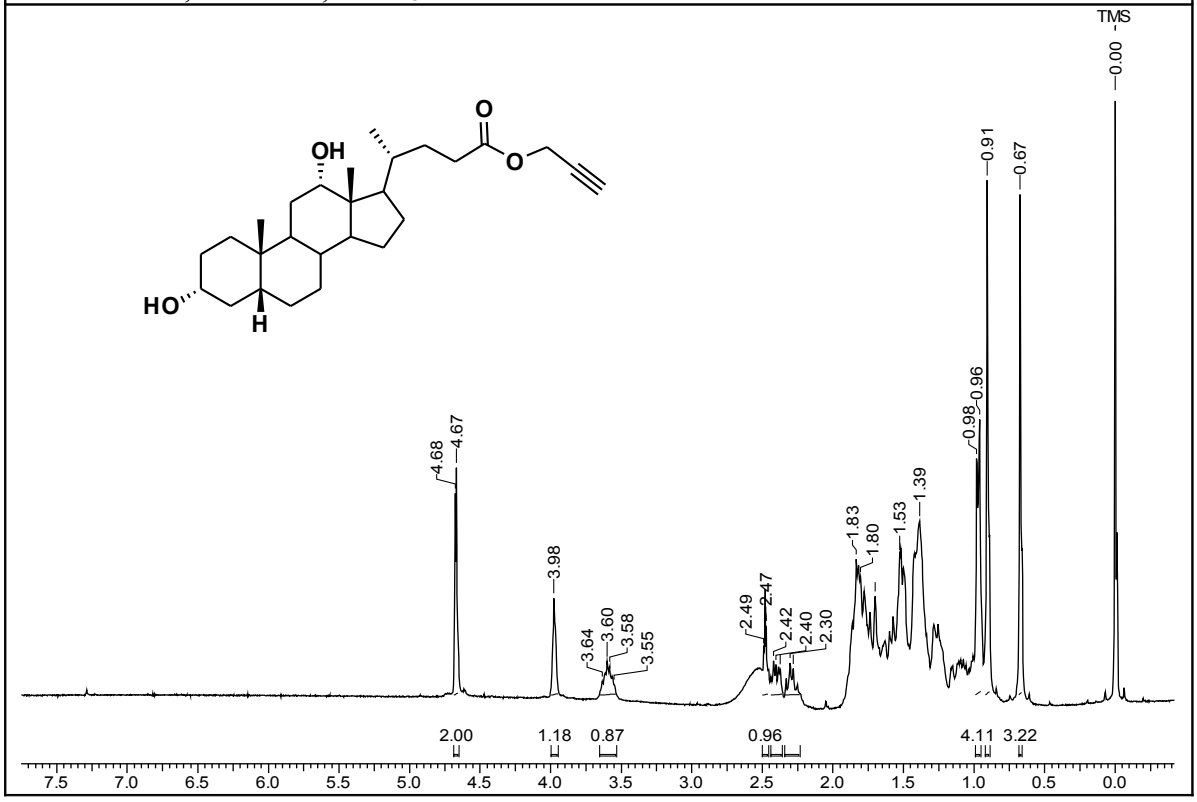
70: ^{1}H NMR, 200 MHz, $CDCl_3$



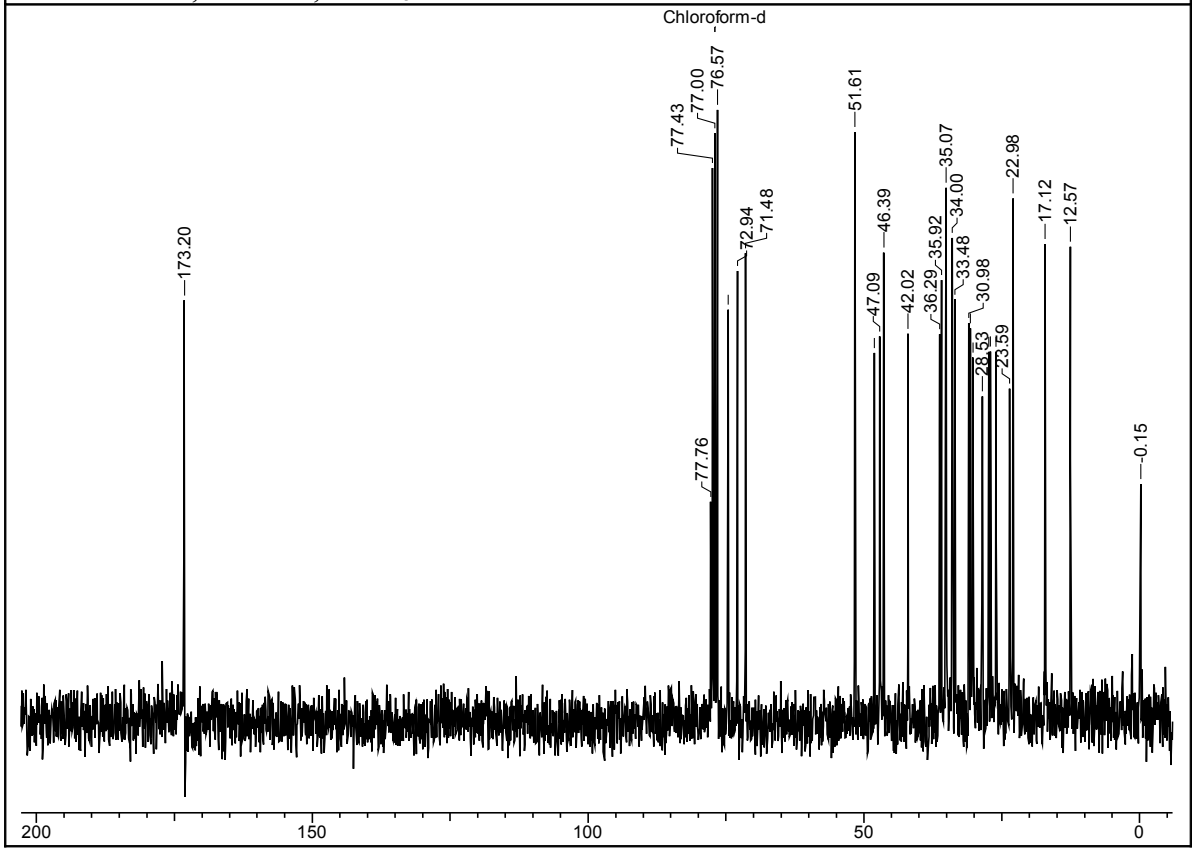
70: ^{13}C NMR, 50 MHz, $CDCl_3$



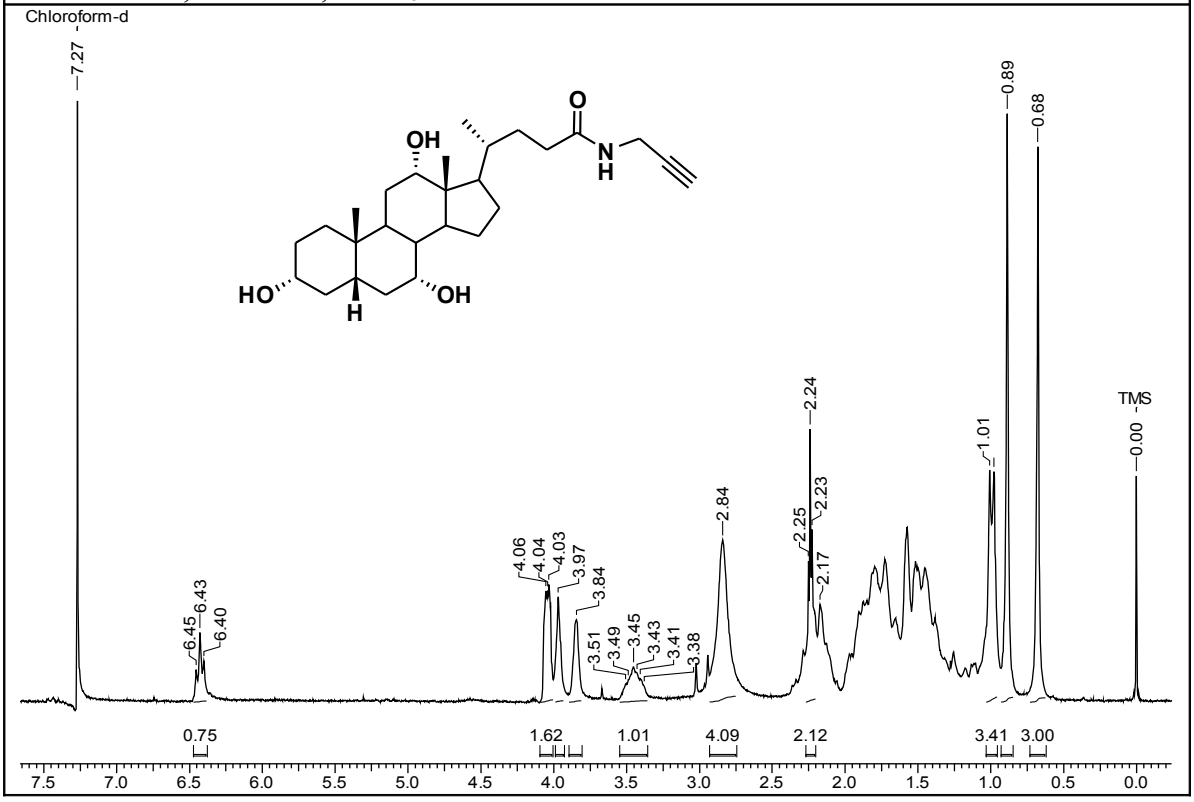
71: ^{1}H NMR, 200 MHz, $CDCl_3$



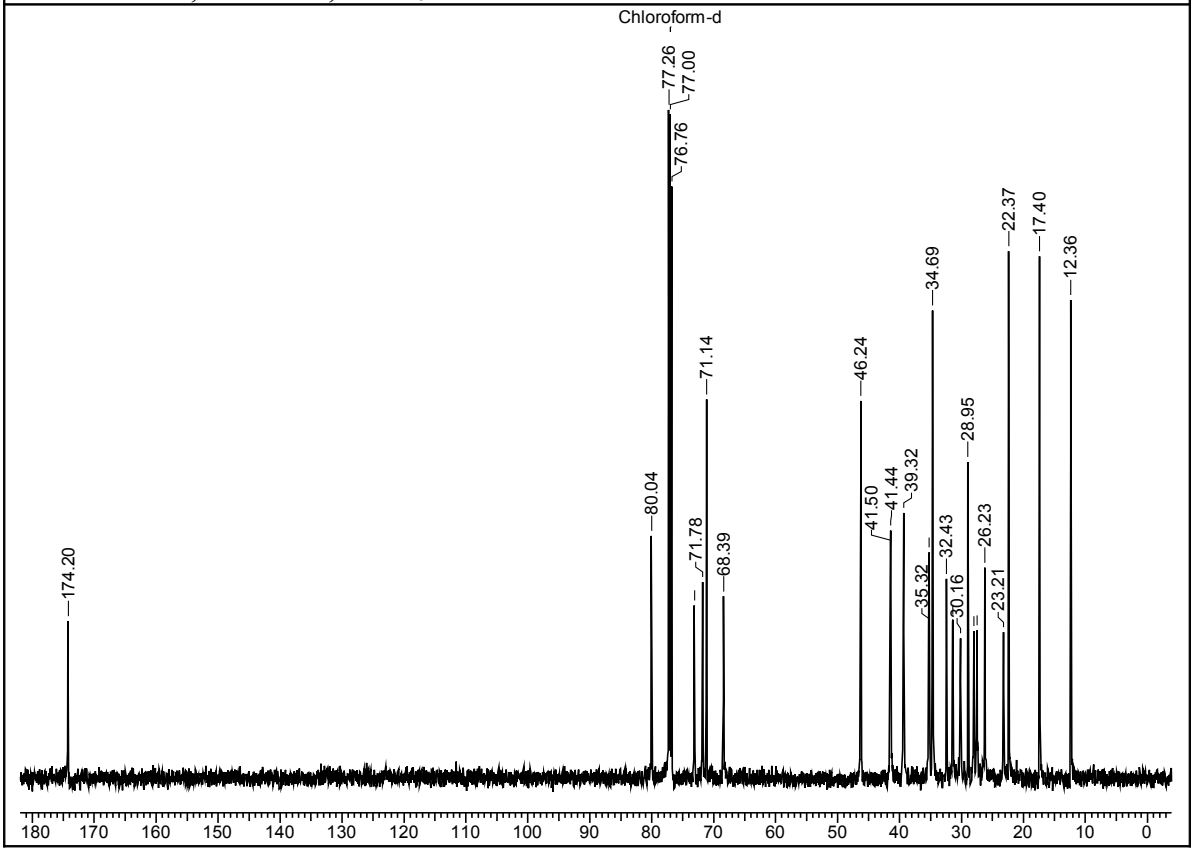
71: ^{13}C NMR, 75 MHz, $CDCl_3$



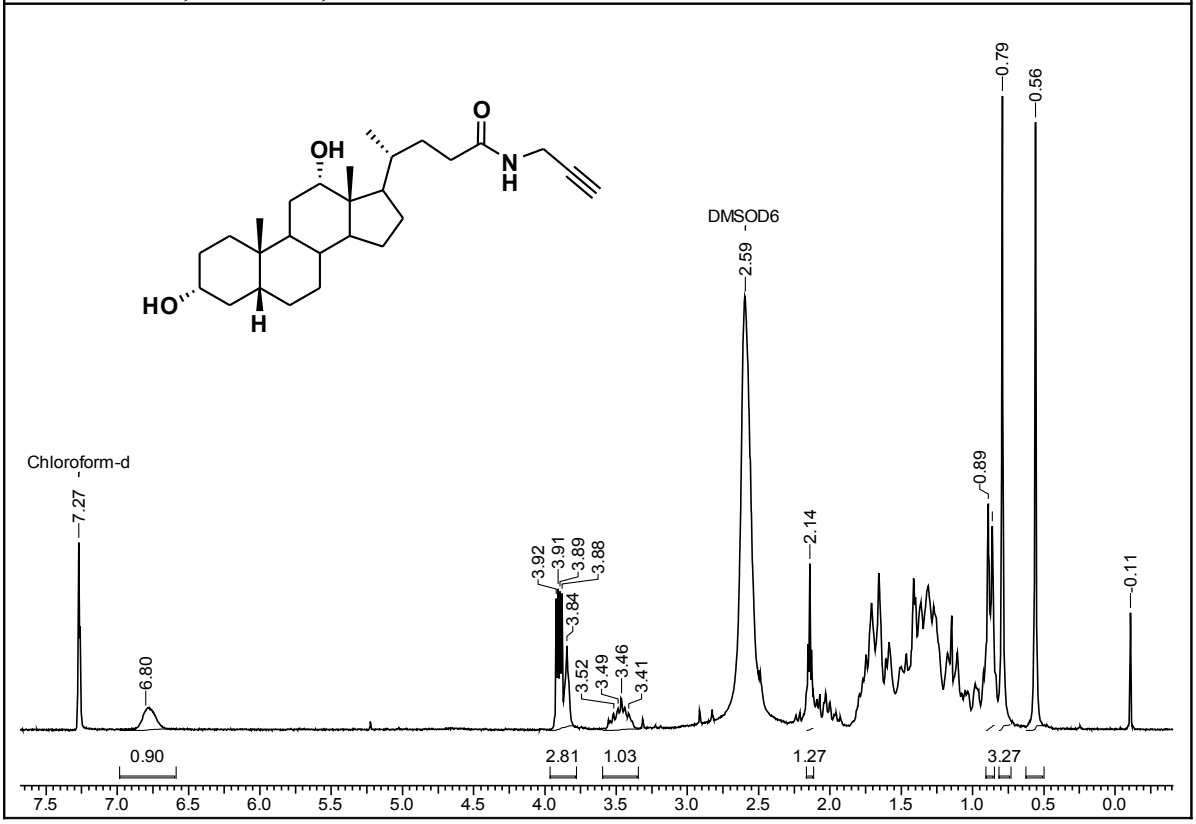
72: ^{1}H NMR, 200 MHz, $CDCl_3$



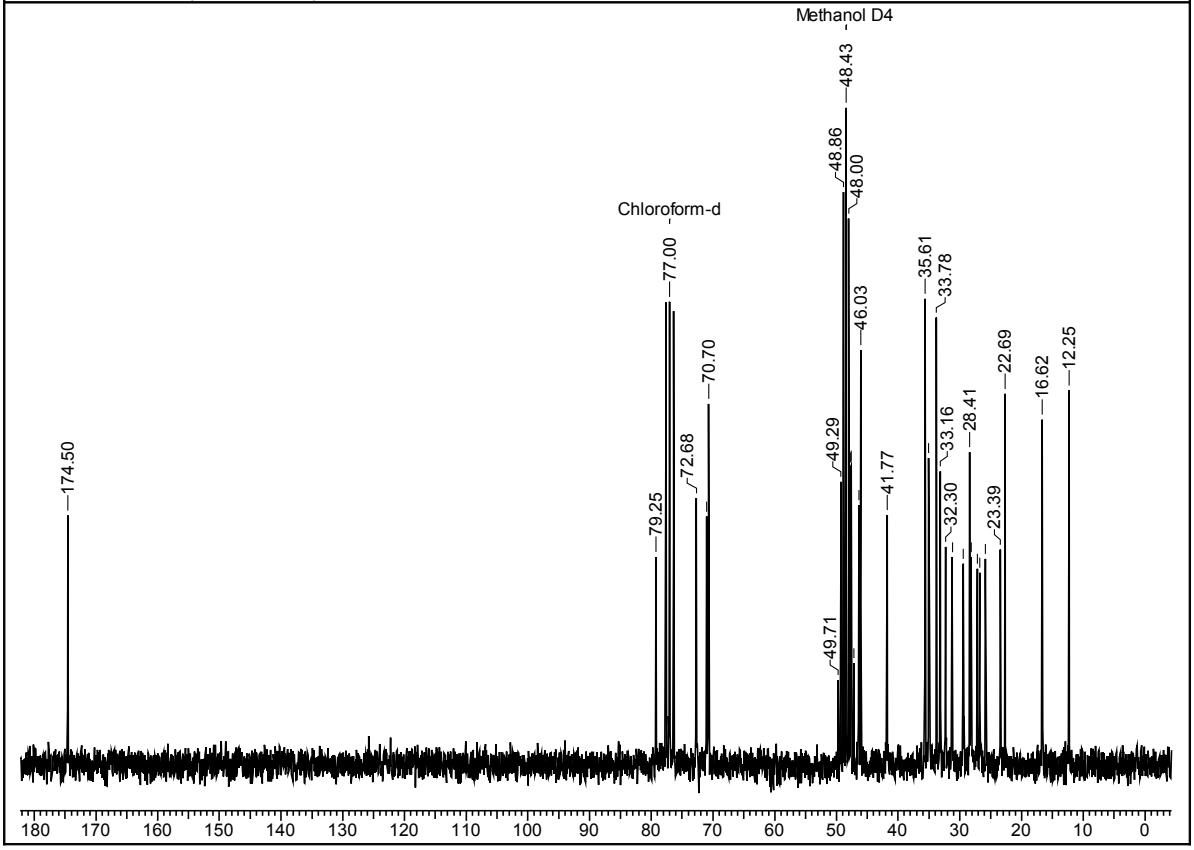
72: ^{13}C NMR, 125 MHz, $CDCl_3$



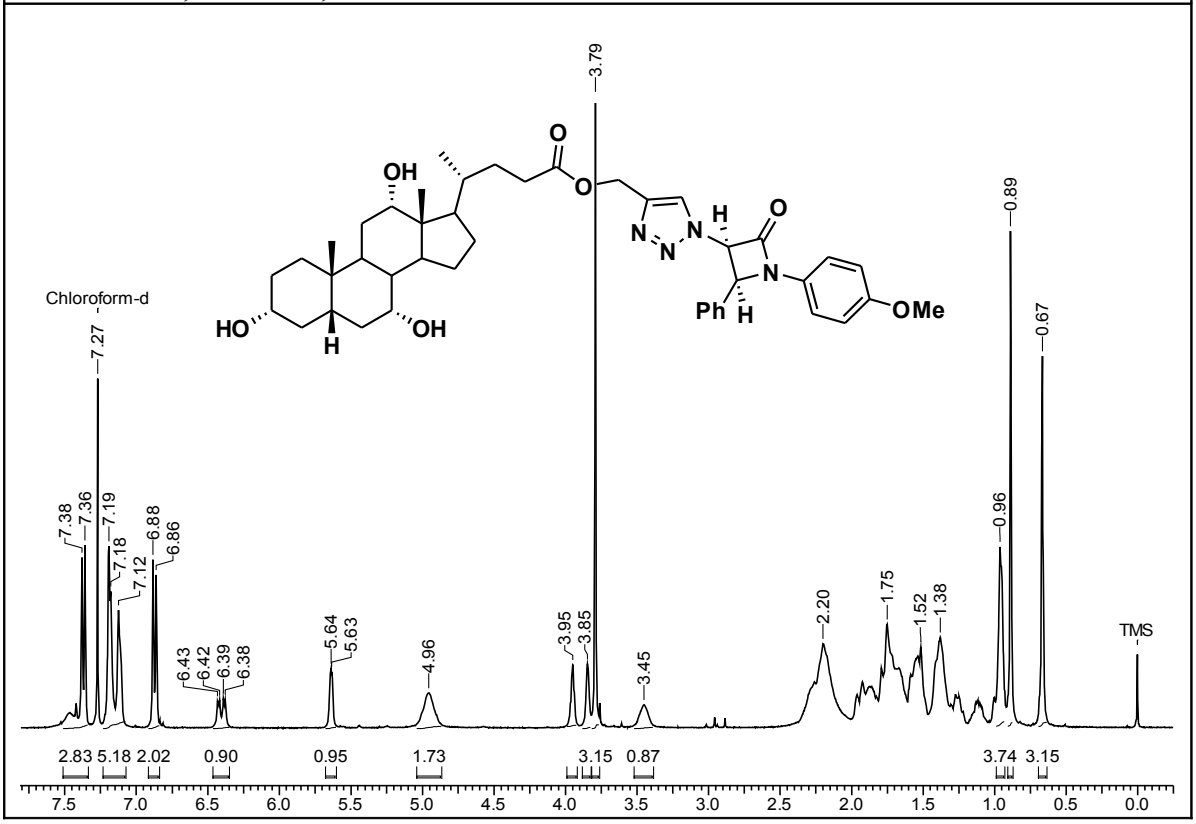
73: ^{1}H NMR, 200 MHz, $CDCl_3$ + $DMSOD_6$



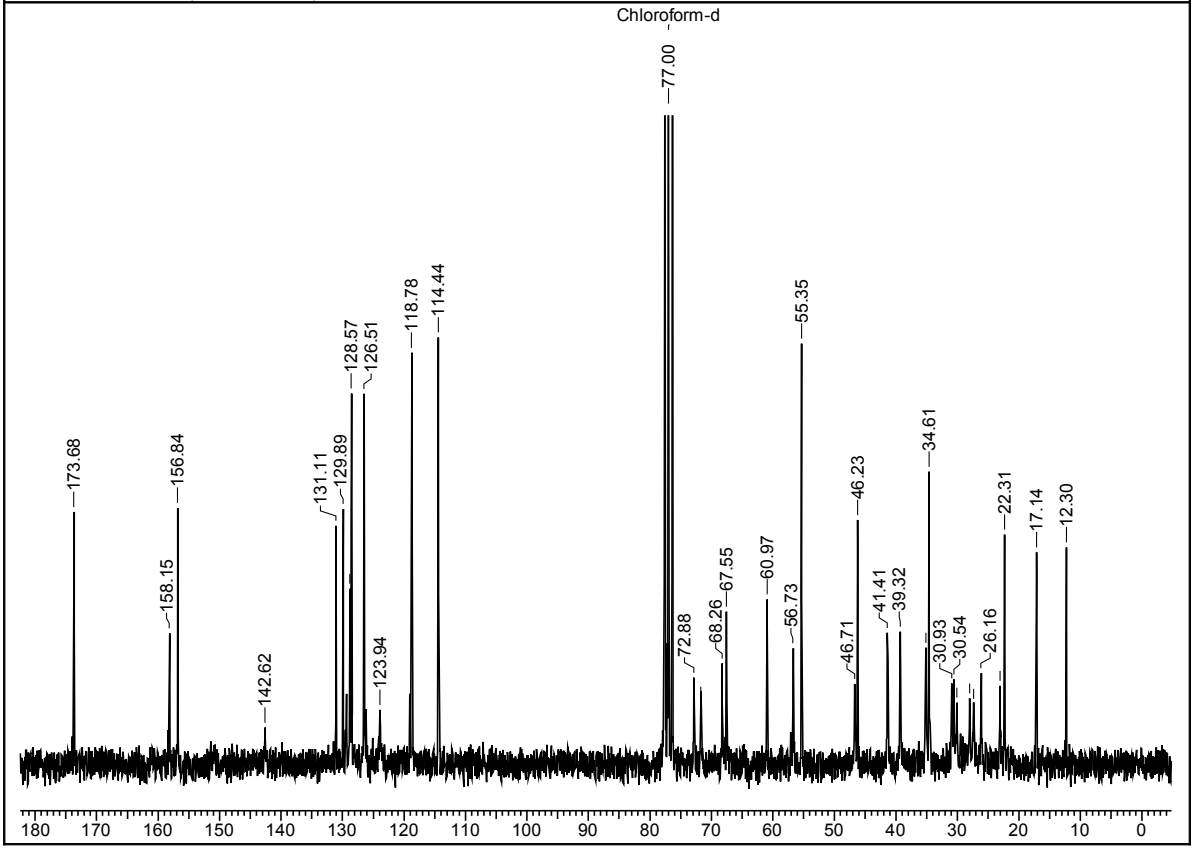
73: ^{13}C NMR, 50 MHz, $CDCl_3$ + CD_3OD



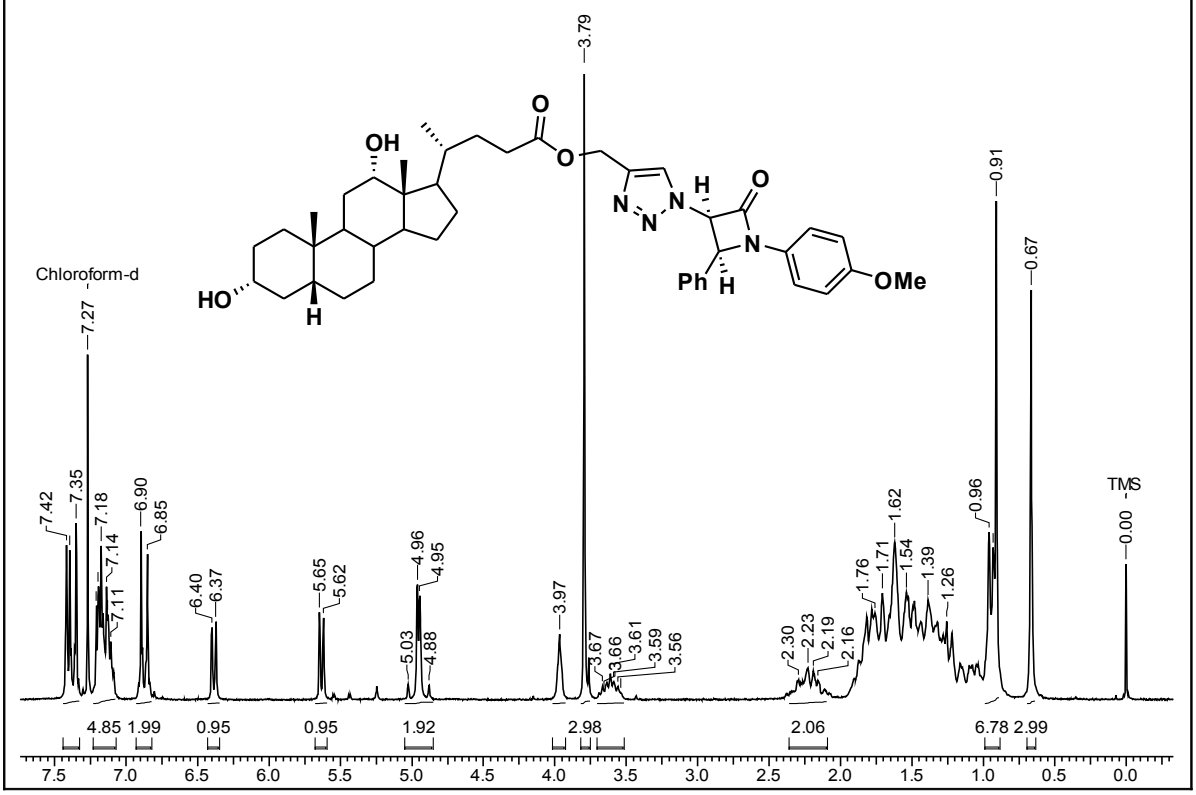
74: ^{1}H NMR, 400 MHz, $CDCl_3$



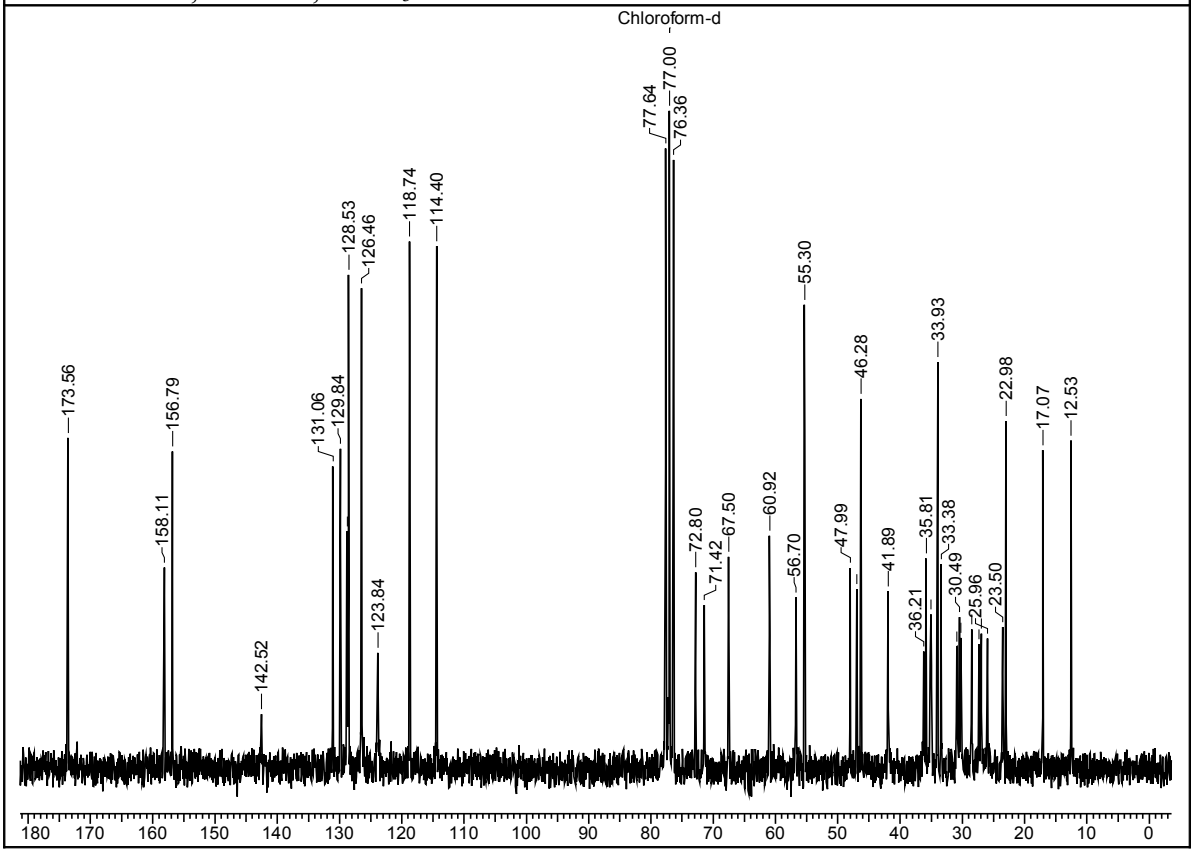
74: ^{13}C NMR, 50 MHz, $CDCl_3$



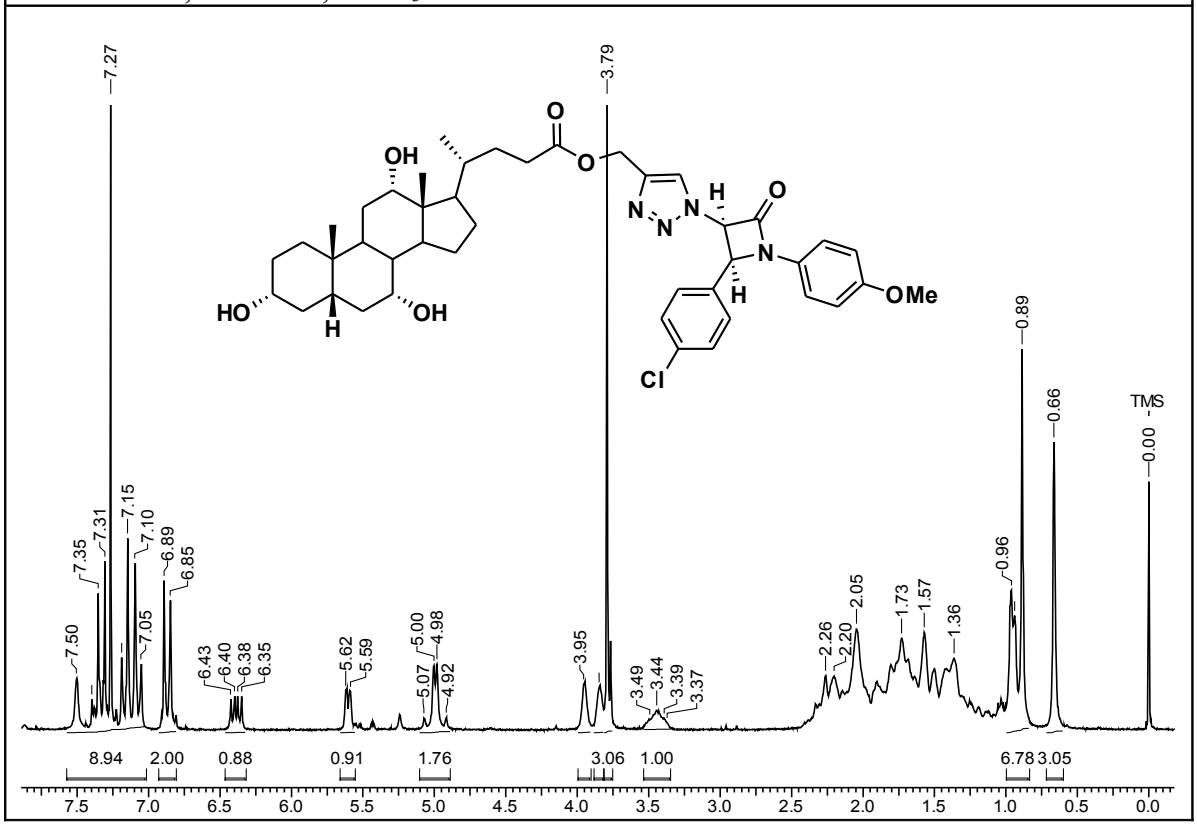
75: ^{1}H NMR, 200 MHz, $CDCl_3$



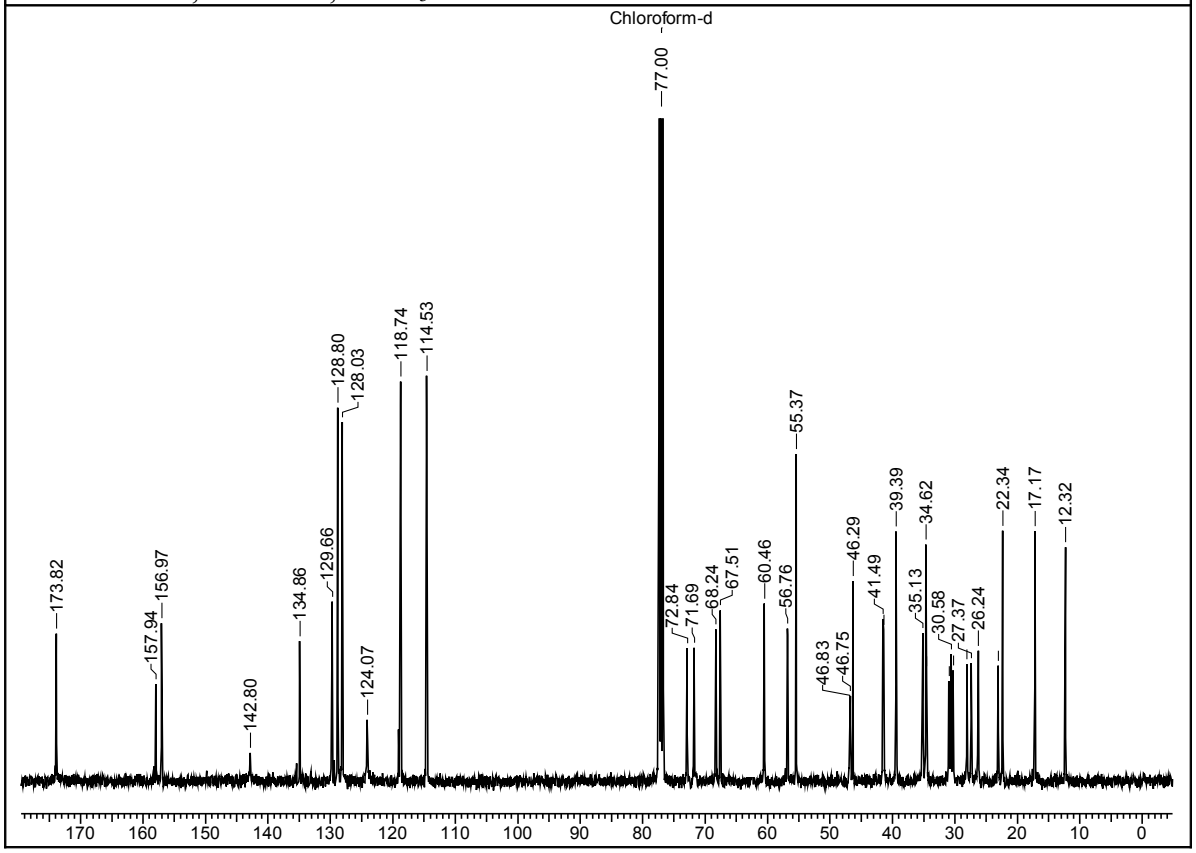
75: ^{13}C NMR, 50 MHz, $CDCl_3$



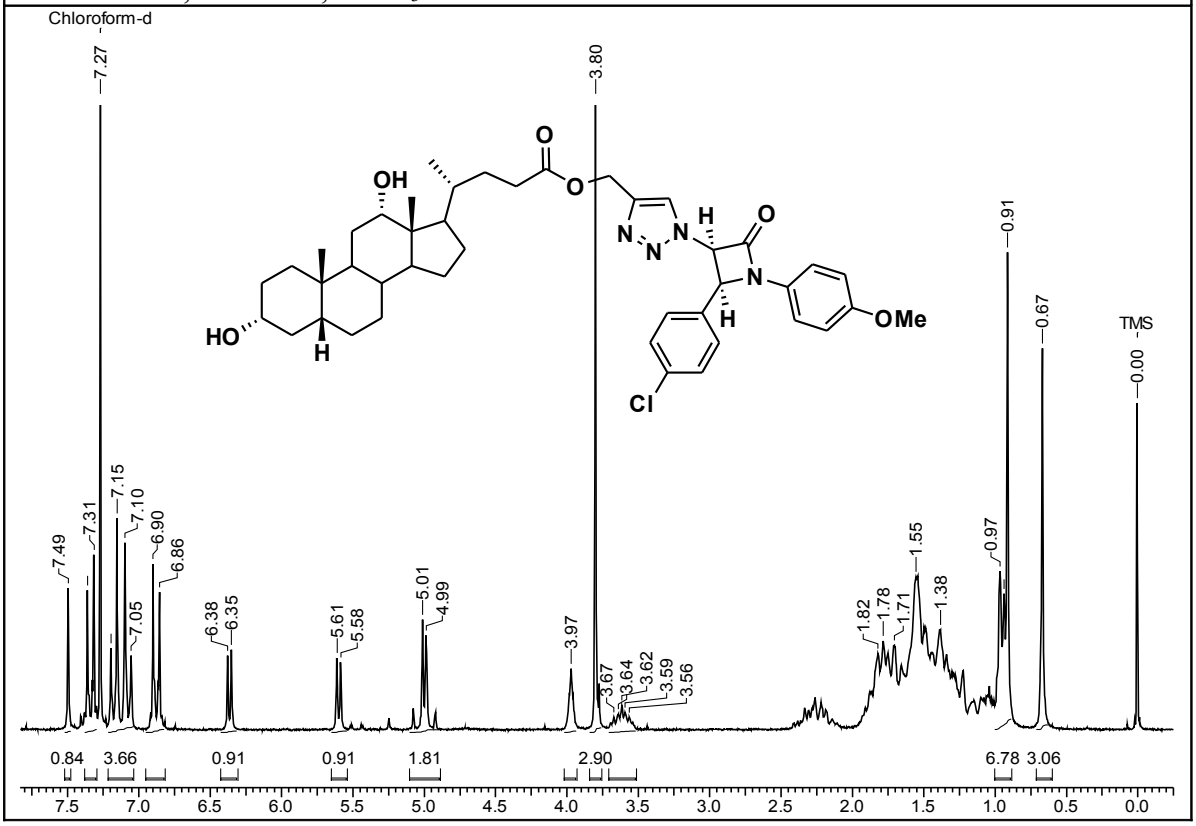
76: ^{1}H NMR, 200 MHz, $CDCl_3$



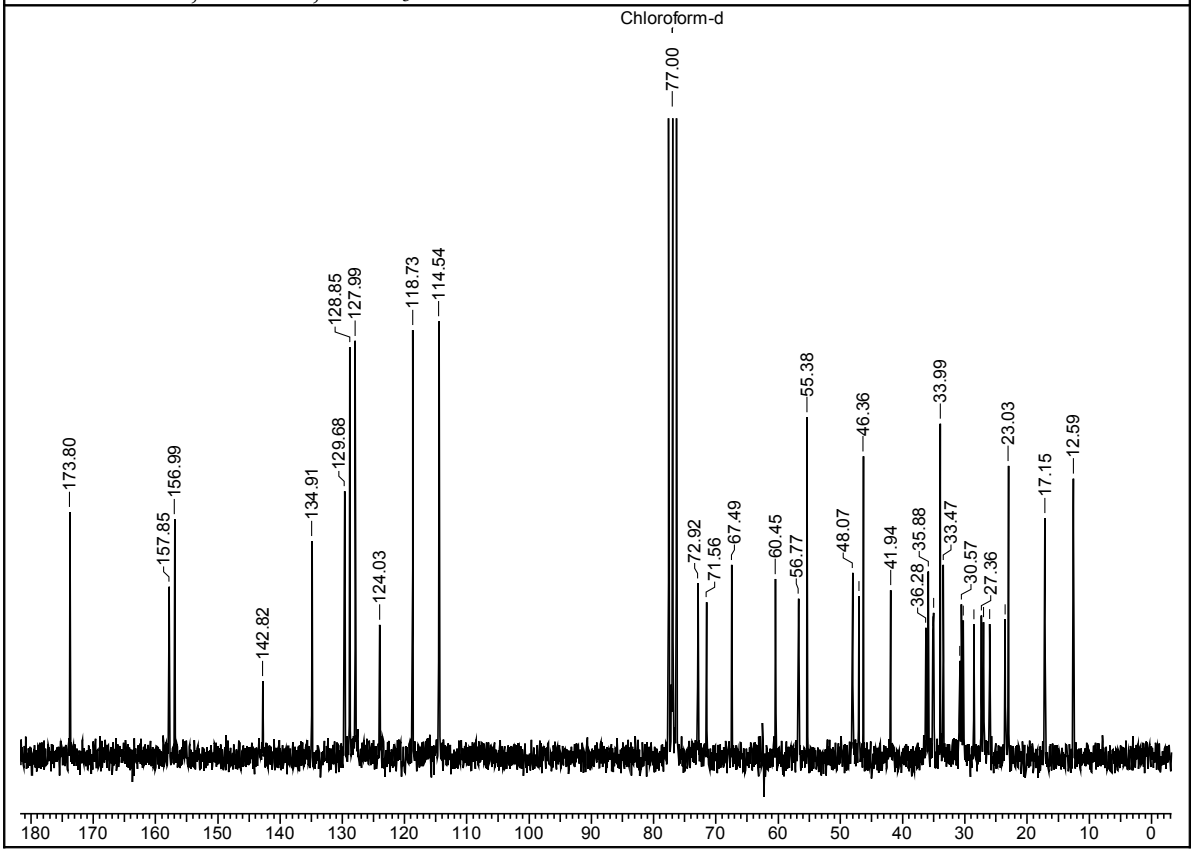
76: ^{13}C NMR, 100 MHz, $CDCl_3$



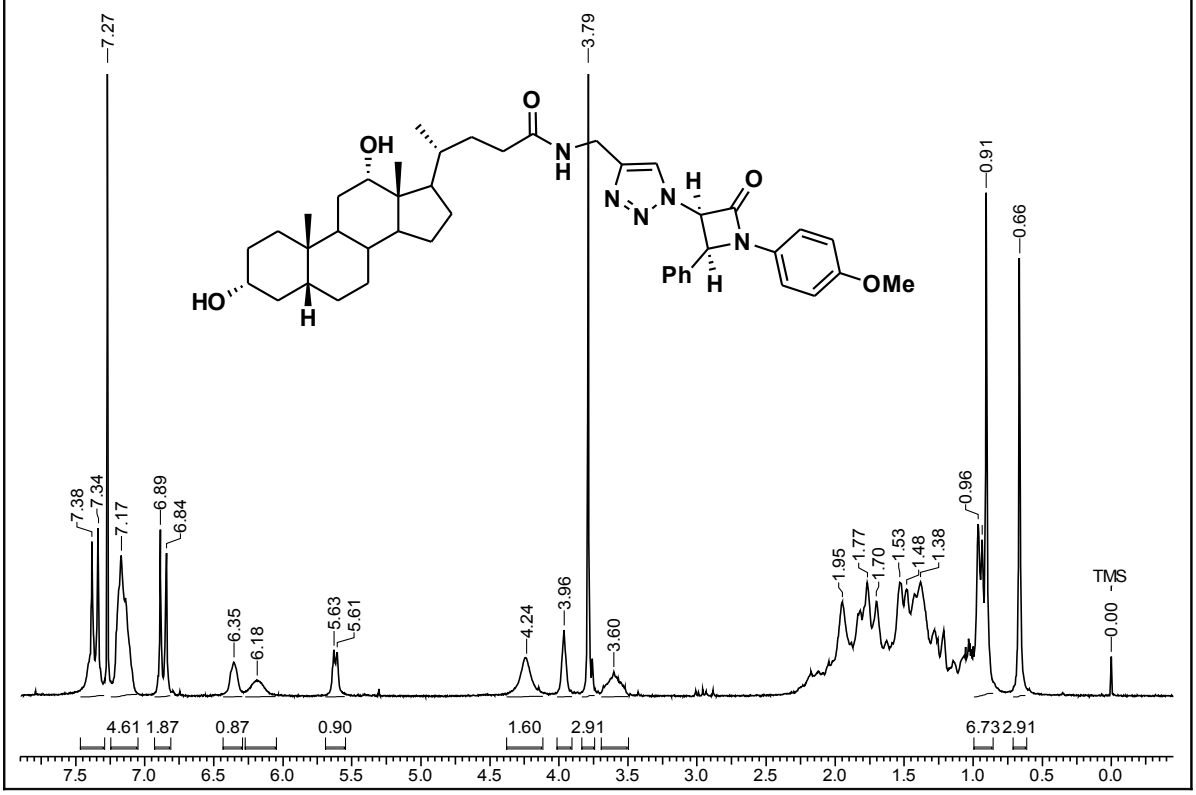
77: ^{1}H NMR, 200 MHz, $CDCl_3$



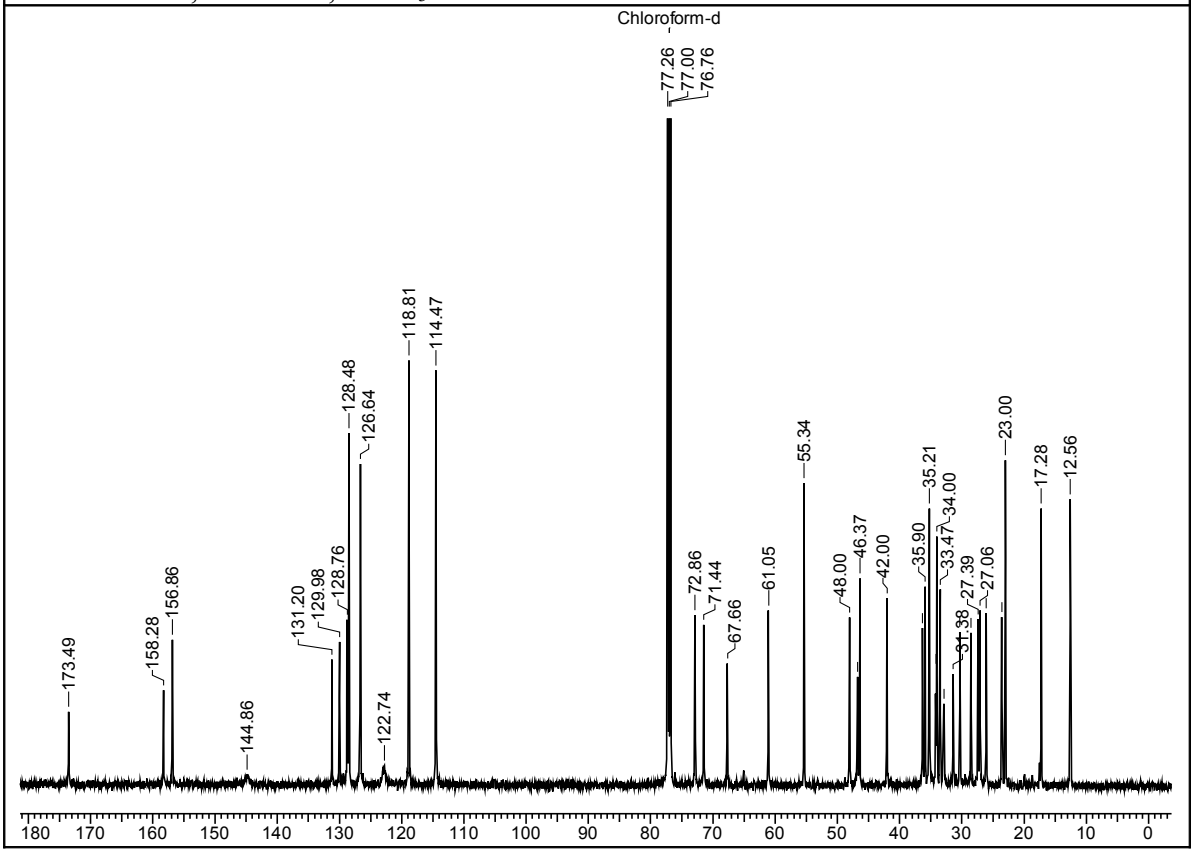
77: ^{13}C NMR, 50 MHz, $CDCl_3$



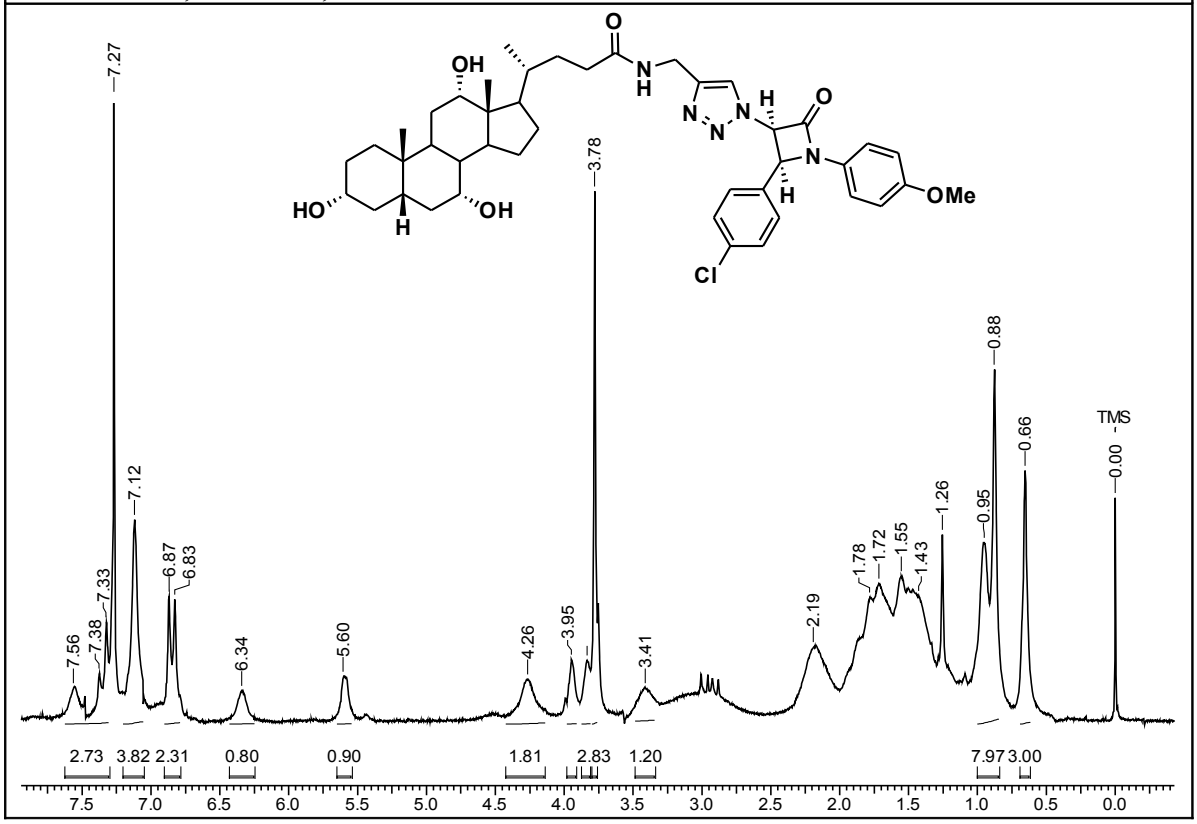
79: ^{1}H NMR, 200 MHz, $CDCl_3$



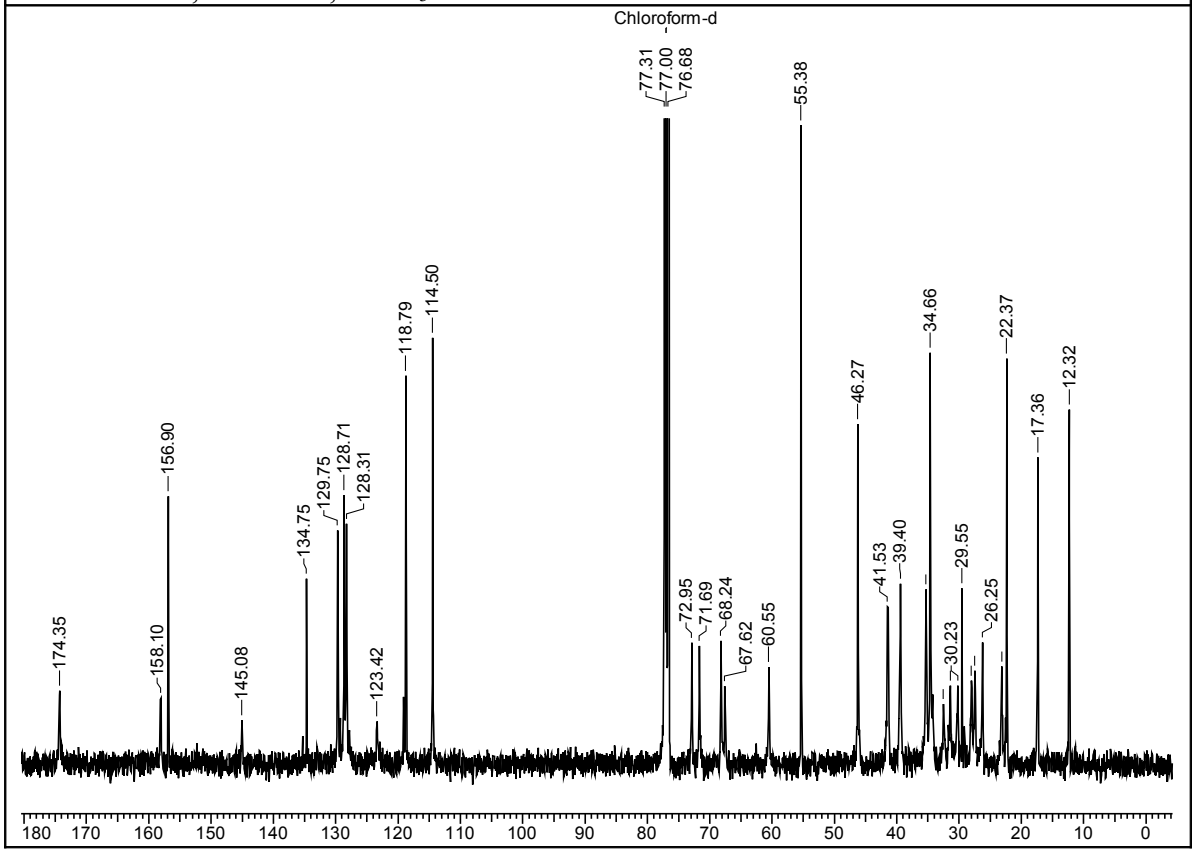
79: ^{13}C NMR, 125 MHz, $CDCl_3$



80: ^{1}H NMR, 200 MHz, $CDCl_3$



80: ^{13}C NMR, 100 MHz, $CDCl_3$



Section B

Design and Synthesis of Bile Acid-β-Lactam Conjugates Using Amide and Ether Linkage, Study Their Biological Activity

1.B.1 Abstract

The synthesis of 6-aminopenicillanic acid-bile acid conjugates, ampicillin-bile acid drug conjugates and several other bile acid-β-lactam conjugates, linked together with amide bond have been achieved in very good yields. In addition to this, we have also synthesized ether linked bile acid-β-lactam conjugates. The synthesized molecules are under biological screening for their antibacterial activity.

1.B.2 Introduction

Bile acids have been found to be attractive tool in designing pharmacological hybrid molecules and prodrugs because of its natural amphiphilic nature, rigid steroidal backbone, availability, and low cost.[15] These molecules have tremendous transport capacity and organ specificity of enterohepatic circulation.[74] They also have versatile derivatization possibilities, improving intestinal absorption ability and increasing metabolic stability of pharmaceuticals. The chemistry and biochemistry of this natural product is extensively studied and utilized in the development of various drugs, especially for hormonal imbalance, for the treatment of infections and cancer as well as inflammation.[13] However, the number of steroidal natural products is limited, where as millions of hybrids as conjugates of steroids can be prepared. During the past two decades design of such entities has been receiving increasing attention. This new approach seems to be very promising in the development of lead molecules, which can be used for combating diseases caused by bacteria and fungi that develop resistance due to indiscriminate use of antibiotics. The medicinal applications are based on the fact that the biological activity of the several new hybrids exceeds that of the parent compounds. Current research efforts are focused on specific drug targeting to the liver and on improving the intestinal absorption of poorly absorbed drugs. Several bile acid-drug conjugates, therefore have been synthesized[3] by Kramer and his coworkers, and studied their biological properties. The information about the bile acid conjugates having pharmacological applications was briefly summarized in the introductory part of this chapter.

1.B.3 Present work

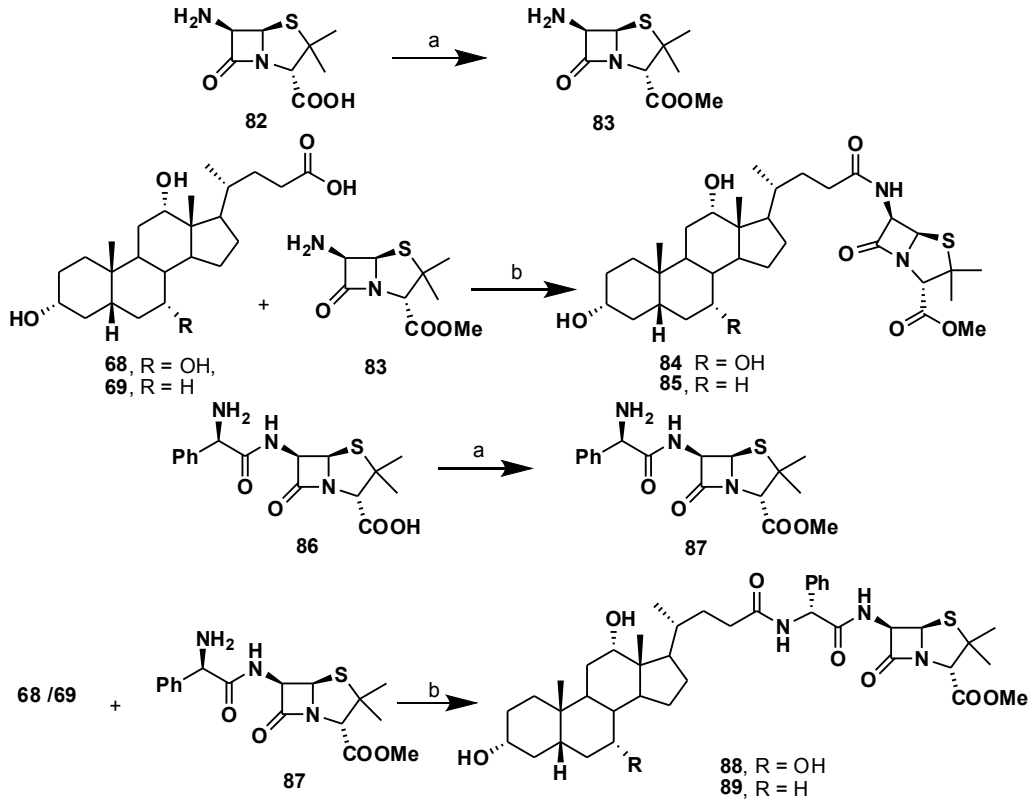
1.B.3.1 Objective

β-Lactams are a large class of antibiotics characterized by the presence of an azetidine-2-one ring, which is the core of biological activity. The azetidine-2-one (β-lactam) ring system is a common structural feature of a number of broad spectrum β-lactam antibiotics, like penicillins, cephalosporins, carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutic agents for treating microbial diseases.[75] However, microorganisms have built up resistance against the most

traditional β-lactam antibiotics due to the wide-spread overuse of antibiotics. Therefore, the phenomenon of bacterial resistance forces the continuous modification of structure of known active compounds and the development of new ones. Based on the above-mentioned observations we focused our attention on design and synthesis of new biconjugates of bile acids having amphiphilic nature and β-lactam derivatives as a pharmacophore, linked together with amide bond. We synthesized ampicillin bile acid drug conjugates and several other bile acid-β-lactam conjugates.

1.A.3.2 Results and Discussion

6-aminopenicillanic acid **82** (purchased from Lancaster chemicals) was converted to its methyl ester **83** by treating with diazomethane using literature method (Scheme 6).[76] The amine functionality of compound **83** was coupled with the cholic acid **68** and

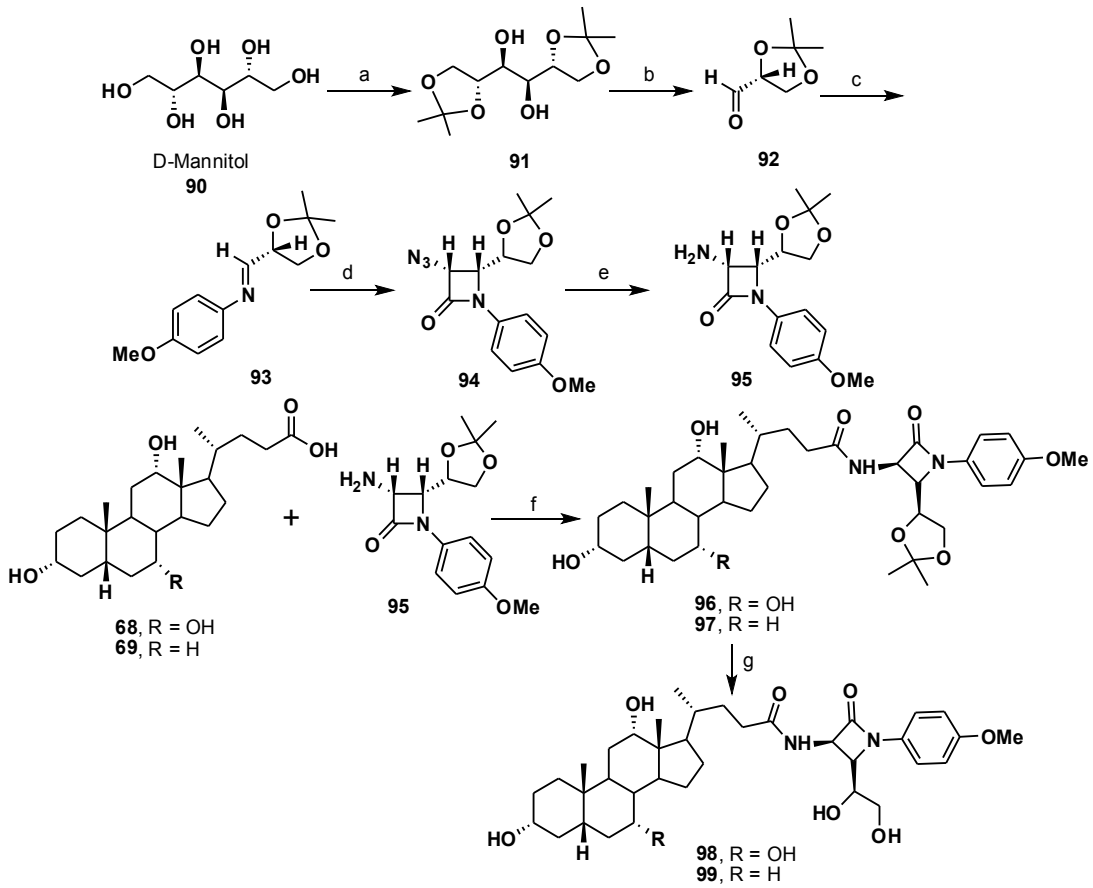


Scheme: 6 Reagent and conditions: (a) Diazomethane, Et_2O/CH_2Cl_2, 15 h, 95-96%; (b) EDC·HCl, HOBt, DMF, 0-25 °C, 11 h, 90-95%.

deoxycholic acid **69** using EDC·HCl [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] as coupling agent in DMF at 0-25 °C afforded the 6-aminopenicillanic acid-bile acid conjugate molecules **84** and **85** in very good yields. The formation of conjugate **85** was confirmed from its ^{1}H NMR spectrum, which showed characteristic

doublet at δ 6.34 due to amide proton (CONH), doublet of doublet at δ 5.72 and doublet at δ 5.54 due to β-lactam methine protons (-CH-). The CH-12 and CH-3 methine protons (-CH-OH) from deoxycholic acid part were observed at δ 3.98 (bs) and 3.56-3.67 (m) respectively. Further, its IR spectrum showed strong absorption band at 1782 cm^{-1}, 1745 cm^{-1}, and 1670 cm^{-1} due to β-lactam carbonyl, ester carbonyl and amide carbonyl groups respectively. The ampicillin **86** (gift from Hindustan Antibiotics, Pune, India) was treated with diazomethane in diethyl ether and dichloromethane to give the methyl ester of ampicillin **87**. Methyl ester **87** was coupled with cholic acid/deoxycholic acid (**68/69**) using EDC·HCl as coupling agent to obtained bile acid-ampicillin drug conjugates **88** and **89** in very good yields.

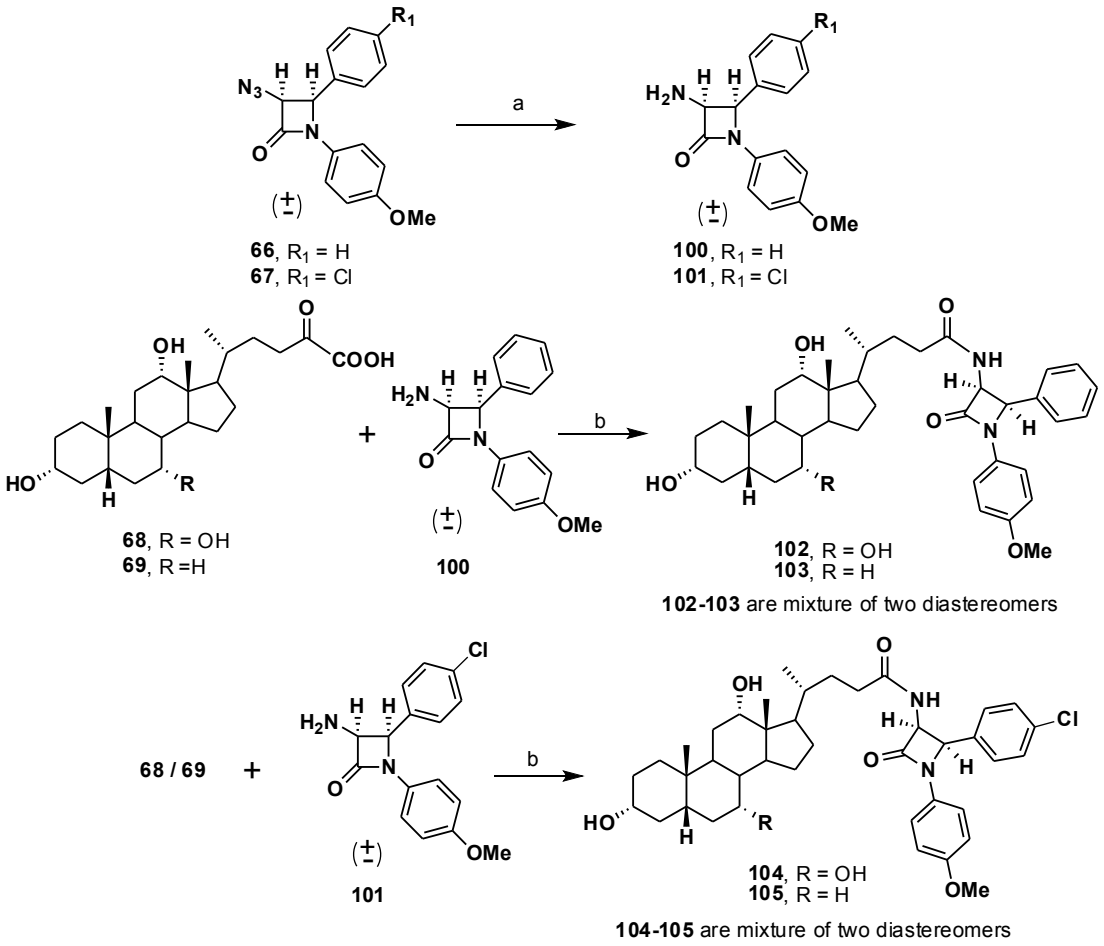
The chiral amino β-lactam **95** was prepared from D-glyceraldehyde acetonide **92** following literature procedure[77] and D-glyceraldehyde acetonide **92** was synthesized from D-mannitol **90** using literature method[78] (Scheme 7). D-mannitol **90** was protected as its



Scheme: 7 Reagent and conditions: (a) *p*-TSA, 2,2-dimethoxypropane, DMSO, 25 °C, 16 h. (b) SiO_2·$NaIO_4$, CH_2Cl_2, 0 °C, 30 min; (c) 4-methoxyaniline, Et_2O, 3 h; (d) triphosgene, Et_3N, CH_2Cl_2, 15 h; (e) EtOAc, H_2/Pd-C, 3 h. (f) EDC·HCl, HOBt, DMF, 0-25 °C, 11 h, 90-94%; (g) *p*-TSA, THF-H_2O, reflux, 24 h, 92-94%.

diacetonide **91** followed by the cleavage of diol using sodium metaperiodate afforded optically pure D-glyceraldehyde acetonide **92**. The aldehyde **92** on treatment with 4-methoxyaniline in ether gave the Schiff base **93**. The imine **93** was treated with potassium azidoacetate **60** (Chapter 1, Section A) in the presence of triphosgene and triethylamine in dichloromethane to afford β-lactam **94**, which on reduction of azide group gave the amino β-lactam **95**. The amino β-lactam **95** thus formed was coupled with the cholic acid/deoxycholic acid (**68**/**69**) using EDC·HCl as coupling agent in DMF at 0-25 °C to get the coupled products **96** and **97** (Scheme 7)**.** Deprotection of acetonide group in compounds **96** and **97** on treatment with *p*-TSA in THF-H_2O at reflux condition for 24 h furnished β-lactam conjugates **98** and **99** in good yield.

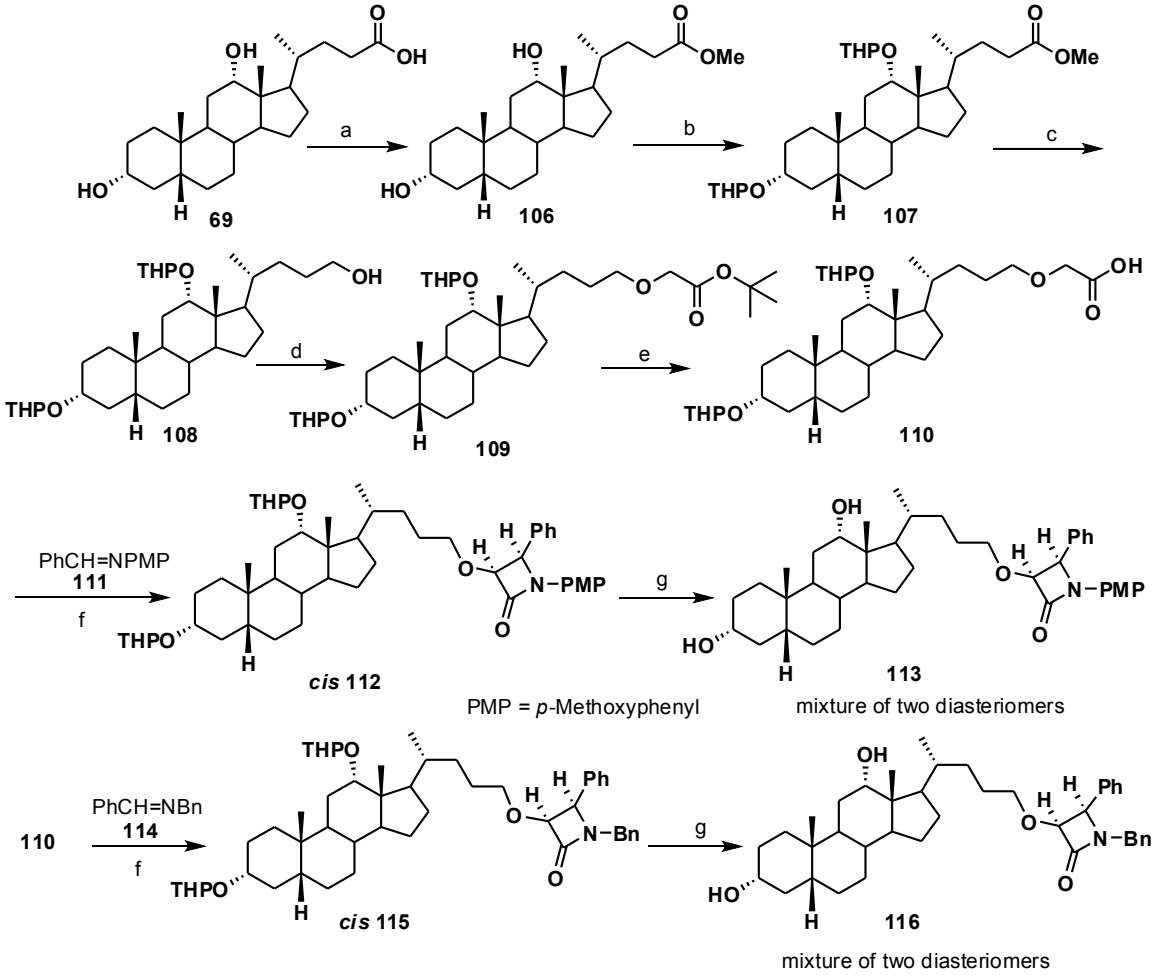
The bile acid-β-lactam conjugate molecules **102-105** were synthesized by coupling of amino β-lactams **100** and **101** with cholic acid **68** and deoxycholic acid **69.** Accordingly**,** the synthesis of amino β-lactams **100** and **101** were achieved by the reduction of azide functionality of earlier synthesized β-lactams **66** and **67** (chapter 1, section A) with Raney nickel in hydrogen atmosphere (Scheme 8). The disappearance of strong absorption band at 2115 cm^{-1} due to azide group and presence of peaks at 3452 cm^{-1}, 1741 cm^{-1} due to amine and β-lactam carbonyl groups in IR spectrum confirms the formation of **100**. There was no appreciable change in ^{1}H NMR spectra of azido β-lactam **66** and amino β-lactam **100**. However its ^{13}C NMR spectrum showed a slight upfield shift for C-NH_2 carbon *i.e.* from δ 67.3 to δ 63.6. The amino β-lactam **100** was coupled with cholic acid **68** and deoxycholic acid **69** using EDC·HCl as coupling agent in DMF at 0-25 °C obtained the diasteriomeric mixture of cholic acid/deoxycholic acid β-lactam conjugates **102-103** in very good yields. In a similar way, the coupling of amino β-lactam **101** with cholic acid **68** and deoxycholic acid **69** afforded diasteriomeric mixture of conjugates **104-105**. All the conjugates **84**, **85**, **88**, **89**, **96-99** and **102-105** were fully characterized by spectroscopic data.



Scheme: 8 Reagent and conditions: (a) Raney nickel, EtOAc, hydrogen atmosphere, 3 h, 95-96%; (b) EDC·HCl, HOBt, DMF, 0-25 °C, 12 h, 92-96%.

Our next target was to synthesise bile acid β-lactam conjugates **113** and **116** using ether linkage. We have synthesized ester and amide linked β-lactam-cholic acid/deoxycholic acid conjugates and studied their bioactivity, There is no report in the literature on the synthesis and bioactivity data of β-lactam-deoxycholic acid conjugate and we have undertaken this. The synthesis of conjugates molecules **113** and **116** were depicted in Scheme 9. Deoxycholic acid **69** in methanol was treated with catalytic amount of *p*-TSA to obtain its methyl ester **106**. Both the hydroxy groups in compound **106** were protected as THP ether by using dihydropyrane (DHP) and catalytic amount of PPTS (pyridinium *para*-tolune sulfonate) afforded compound **107**. Subsequently, reduction of ester moiety in **107** with $LiAlH_4$ provided C-24 alcohol **108**. Absence of signal due to methyl protons (COOMe) in ^1H NMR spectrum of **108** and disappearance of carbonyl absorption band in IR spectrum confirms its formation. The alcohol **108** was treated with *tert*-butyl bromoacetate and 50% aq. NaOH in benzene[79] to give the corresponding *tert*-

butyl ester **109.** The ^{1}H NMR spectrum of **109** shows typical singlet at δ 1.48 due to tert-butyl methyl protons. Its IR spectrum showed strong absorption band at 1743 cm^{-1} due to presence of ester carbonyl group. Hydrolysis of ester functionality of compound **109** with LiOH in methanol-water gave the acid **110**. The acid **110** was subjected to keten-imine cycloaddition (Staudinger) reaction[70] with imine **111** in the presence of triphosgene and triethylamine in anhydrous dichloromethane furnished *cis*-β-lactam **112**.



Scheme: 9 Reagent and conditions: (a) *p*-TSA, MeOH, 22 h, 25 °C, 96%; (b) DHP, PPTS, CH_2Cl_2, 10 h, 25 °C, 93%; (c) $LiAlH_4$, THF, 1 h, 25 °C, 86%. (d) *tert*-butyl bromoacetate, 50% aq. NaOH, benzene, 1 h, 10 °C, 61%; (e) LiOH, MeOH-H_2O, 26 h, 30 °C, 95%; (f) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, **112** (45%) or **115** (48%); (g) *p*-TSA, MeOH, 30 °C, 5 h, 96-97%.

The formation of *cis*-β-lactam **112** was confirmed from its ^{1}H NMR spectrum, which showed characteristic two doublets at δ 5.15 and at δ 4.88 due to methine protons of azetidinone ring with coupling constant J = 4.5 Hz for the *cis*-isomer. Further, its IR spectrum showed strong absorption band at 1747 cm^{-1} corresponding to the carbonyl

group (C=O) of β-lactam in **112**. Finally, the deprotection of THP group led to targeted ether linked bile acid β-lactam conjugate **113** as mixture of two diasteriomers in 97% yield. The ^{1}H NMR of **113** showed two doublets at δ 0.82 and at δ 0.80 due to the C-21 methyl protons because of mixture of diastereomers. In a similar way, keten-imine cycloaddition (Staudinger) reaction of acid **110** and imine **114** afforded *cis*-β-lactam **115**. Subsequent deprotection of THP ether **115** gave the deoxycholic acid β-lactam conjugate **116**. The compound **116** was characterized using IR, ^{1}H NMR and ^{13}C NMR spectroscopic data.

1.B.4 Bioevaluation

All the synthesized amide and ether linked bile acid-β-lactam conjugates **84**, **85**, **88**, **89**, **98**, **99**, **102-105**, **113**, and **116** are under preliminary biological evaluation to study their antibacterial activity.

1.B.5 Conclusion

In conclusion, we have synthesized 6-aminopenicillanic acid-bile acid conjugates, ampicillin-bile acid drug conjugates and several other bile acid-β-lactam conjugates, linked together with amid bond in good yields. In addition to this, the synthesis of ether linked bile acid-β-lactam conjugates have also been realized. The synthesized molecules are under biological screening for their antibacterial activity.

1.B.6 Experimental

Methyl ester of 6-aminopenicillic acid (83):

To the solution of 6-aminopenicillanic acid **82** (0.432 g, 2 mmol), in dichloromethane (8 mL) under argon atmosphere was added excess diazomethane (generated from *N*-methyl-*N*-nitrosourea and 50% aq. KOH) in ether (8 mL) at 25 ºC. The reaction mixture was stirred for 18 h. Evaporation of the solvent under reduced pressure gave the methyl ester of 6-amino-penicillanic acid **83** in 96% yield. IR ($CHCl_3$, cm^{-1}) 3333, 1784, 1745; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.52 (d, J = 4.3 Hz, 1H), 4.59 (d, J = 4.3 Hz, 1H), 4.41 (s, 1H), 3.78 (s, 3H), 1.80 (bs, 2H, NH_2), 1.65 (s, 3H), 1.50 (s, 3H).

Cholic acid-β-lactam conjugate (84):

Cholic acid **68** (0.408 g, 1 mmol) and methyl ester of 6-amino-penicillanic acid **83** (0.230 g, 1 mmol) were dissolved in dry DMF (7 mL) under an argon atmosphere and the solution was cooled to 0 ºC. HOBt (0.068 g, 0.5 mmol) and EDC·HCl (0.287 g, 1.5 mmol) were added and stirring was continued for 30 min. The reaction mixture was allowed to warm to room temperature and was stirred further for 10 h. The reaction was quenched by adding crushed ice and extracted three times with ethyl acetate. The combined extracts were washed with water and brine, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using MeOH/CH_2Cl_2 (4:96) as an eluent to afford compound **84** (0.595 g, 95 %) as white powder. Mp: 129-131 ºC; $[\alpha]_D^{27}$ +153.12 (c 2.56, MeOH); IR ($CHCl_3$, cm^{-1}) 3421, 1782, 1747, 1672; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.61 (d, *J* = 8.8 Hz, 1H, CONH), 5.73 (dd, *J* = 4.1, 8.8 Hz, 1H), 5.55 (d, *J* = 4.1 Hz, 1H), 4.43 (s, 1H), 3.97 (bs, 1H, CH-12), 3.84 (bs, 1H, CH-7), 3.78 (s, 3H, COOMe), 3.47-3.41 (m, 1H, CH-3), 1.67 (s, 3H), 1.50 (s, 3H), 0.97 (d, *J* = 6.1 Hz, 3H, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-81); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.6, 174.7, 169.8, 73.9, 72.8, 71.6, 69.1, 69.0, 65.5, 60.3, 52.9, 48.0, 47.4, 43.1, 42.9, 40.9, 40.4, 36.8, 36.5, 35.8, 33.3, 33.1, 31.5, 31.1, 29.5, 28.7, 27.8, 27.4, 24.2, 23.2, 17.7, 13.1; MS (LCMS) *m/z*: 643.81 (M + Na); Anal. Calcd for $C_{33}H_{52}N_2O_7S$: C, 63.84; H, 8.44; N, 4.51; S, 5.16; Found: C, 63.47; H, 8.70; N, 4.77; S, 4.86.

Deoxycholic acid-β-lactam conjugate (85):

Compound **85** was synthesized from deoxycholic acid **69** and methyl ester of 6-amino-penicillanic acid **83** using the procedure reported for compound **84**.

White solid, Yield: 93%; Mp: 110-111 ºC; $[\alpha]_D^{27}$ +159.88 (c 1.38, MeOH); IR ($CHCl_3$, cm^{-1}) 3348, 1782, 1745, 1670; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.34 (d, *J* = 9.0 Hz, 1H, CONH), 5.72 (dd, *J* = 4.2, 9.0 Hz, 1H), 5.54 (d, *J* = 4.2 Hz, 1H), 4.43 (s, 1H), 3.98 (bs, 1H, CH-12), 3.78 (s, 3H, -$COOCH_3$), 3.67-3.56 (m, 1H, CH-3), 1.67 (s, 3H), 1.50 (s, 3H), 0.97 (d, *J* = 6 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-81); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 173.2, 168.2, 73.0, 71.6, 70.5, 68.0, 64.6, 58.4, 52.4, 48.1, 46.8, 46.4, 42.0, 36.3, 35.9, 35.2, 35.1, 34.1, 33.5, 32.8, 31.1, 30.3, 28.6, 27.4, 27.1, 26.1,

23.6, 23.1, 17.3, 12.7; MS (LCMS) *m/z*: 627.53 (M + Na); Anal. Calcd for $C_{33}H_{52}N_2O_6S$: C, 65.53; H, 8.67; N, 4.63; S, 5.30; Found: C, 65.25; H, 8.47; N, 4.58; S, 5.02.

Methyl ester of ampicillin (87):

Compound **87** has been synthesized from ampicillin **86** following the experimental procedure described for the compound **83.**

Gummy material, Yield: 95%; IR ($CHCl_3$, cm^{-1}) 3336, 1786, 1745, 1681; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.06 (d, *J* = 9.2 Hz, 1H, CONH), 7.40-7.33 (m, 5H), 5.68 (dd, *J* = 4.2, 9.2 Hz, 1H), 5.55 (d, *J* = 4.2 Hz, 1H), 4.57 (s, 1H), 4.46 (s, 1H), 3.78 (s, 3H, COOMe), 1.80 (bs, 2H, NH_2), 1.66 (s, 3H), 1.50 (s, 3H); MS (LCMS) *m/z*: 364.45 (M + H), 386.44 (M + Na).

Cholic acid-β-lactam conjugate (88):

Conjugate **88** was synthesized from cholic acid **68** and methyl ester of ampicillin **87** following the experimental procedure described for the compound **84**.

White solid, Yield: 90%; Mp: 156-158 ºC; $[\alpha]_D^{27}$ +104.22 (c 1.52, MeOH); IR ($CHCl_3$, cm^{-1}) 3415, 3346, 1782, 1747, 1650; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41-7.29 (m, 5H), 5.71 (d, *J* = 7.3 Hz, 1H), 5.60 (dd, *J* = 4.2, 8.3 Hz, 1H), 5.45 (d, *J* = 4.2 Hz, 1H), 4.42 (s, 1H), 3.94 (s, 1H, CH-12), 3.81 (bs, 1H, CH-7), 3.76 (s, 3H, COOMe), 3.48-3.34 (m, 1H, CH-3), 1.53 (s, 3H), 1.42 (s, 3H), 0.97 (d, *J* = 5.7 Hz, 3H, CH_3-21), 0.87 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.1, 174.2, 172.3, 169.7, 138.5, 129.7, 129.2, 128.7, 73.9, 72.8, 71.5, 67.0, 65.4, 60.2, 58.0, 52.9, 48.0, 47.4, 43.1, 42.9, 40.9, 40.4, 36.7, 36.5, 35.8, 33.6, 33.0, 31.5, 31.1, 29.5, 28.6, 27.8, 27.2, 24.2, 23.2, 17.8, 13.1; MS (LCMS) *m/z*: 776.65 (M + Na); Anal. Calcd for $C_{41}H_{59}N_3O_8S$: C, 65.31; H, 7.89; N, 5.57; S, 4.25; Found: C, 65.63; H, 7.61; N, 5.85; S, 4.01.

Deoxycholic acid-β-lactam conjugate (89):

Conjugate **89** was synthesized from deoxycholic acid **69** and methyl ester of ampicillin **87** following the experimental procedure described for the compound **84**.

White powder, Yield: 91%; Mp: 143-145 ºC; $[\alpha]_D^{27}$ +104.78 (c 1.98, MeOH); IR ($CHCl_3$, cm^{-1}) 3415, 3336, 1782, 1747, 1650; ^1H NMR (CD_3OD, 500 MHz) δ 7.45-7.30 (m, 5H),

5.65 (s, 1H), 5.55 (d, *J* = 4.1 Hz, 1H), 5.47 (d, *J* = 4.1 Hz, 1H), 4.43 (s, 1H), 3.95 (s, 1H, CH-12), 3.76 (s, 3H, COOMe), 3.56-3.51 (m, 1H, CH-3), 2.40-2.35 (m, 1H), 2.27-2.21 (m, 1H), 1.55 (s, 3H), 1.41 (s, 3H), 1.02 (d, *J* = 5.8 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.1, 174.2, 172.3, 169.7, 138.5, 129.7, 129.2, 128.7, 73.9, 72.5, 71.5, 68.9, 65.4, 60.2, 58.0, 52.9, 48.0, 47.5, 43.5, 37.4, 37.1, 36.7, 36.4, 35.3, 34.7, 33.6, 33.0, 31.5, 31.0, 29.8, 28.6, 28.4, 27.4, 27.3, 24.9, 23.8, 17.7, 13.3; MS (LCMS) *m/z*: 738.54 $(M + H)^+$, 760.66 (M + Na); Anal. Calcd for $C_{41}H_{59}N_3O_7S$: C, 66.73; H, 8.06; N, 5.69; S, 4.34; Found: C, 66.53; H, 7.77; N, 5.85; S, 4.01.

(3*R*,4*R*)-*cis*-1-(*p*-Anisyl)-3-amino-4-[(*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]azetidin-2-one (95):

Mp: 168 ºC; $[\alpha]_D^{27}$ +80.4 (c 0.5, MeOH) {lit.[77a] $[\alpha]_D^{26}$ +81.9 (c 0.5, MeOH)}; IR ($CHCl_3$, cm^{-1}) 3413, 1735; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.55 (d, *J* = 9.2 Hz, 2H), 6.86 (d, *J* = 9.2 Hz, 2H), 4.39-4.17 (m, 4H), 3.91-3.82 (m, 1H), 3.79 (s, 3H, OMe), 1.74 (s, 2H, NH_2), 1.43 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.4, 156.2, 131.2, 119.6, 113.9, 109.6, 76.4, 66.8, 61.1, 60.4, 55.3, 26.4, 25.0; MS (LCMS) *m/z*: 293.41 (M + H), 315.41 (M + Na); Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58; Found: C, 61.98; H, 6.53; N, 9.27.

Cholic acid-β-lactam conjugate (96):

Compound **96** was synthesized from cholic acid **68** and 3-amino β-lactam **95** following the experimental procedure described for the compound **84**.

White solid, Yield: 90%; Mp: 232-234 ºC; $[\alpha]_D^{28}$ +40 (c 1.655, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3421, 3321, 1745, 1666; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.76 (d, *J* = 9.4 Hz, 1H, CONH), 7.48 (d, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 8.7 Hz, 2H), 5.56-5.44 (m, 1H), 4.39-4.27 (m, 2H), 4.09-3.98 (m, 2H), 3.85 (bs, 1H, CH-7), 3.77 (s, 1H), 3.75 (s, 3H, OMe), 3.48-3.39 (m, 1H, CH-3), 1.43 (s, 3H), 1.33 (s, 3H), 1.01 (d, *J* = 4.3 Hz, 3H, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.8, 166.1, 158.1, 132.4, 121.1, 114.9, 111.1, 77.8, 73.9, 72.8, 69.0, 67.4, 63.2, 57.3, 55.9, 47.9, 47.4, 43.1, 42.9, 40.9, 40.4, 36.8, 36.5, 35.8, 33.7, 32.9, 31.1, 29.53, 28.7, 27.8, 26.8, 25.3, 24.2, 23.2,

17.7, 13.1; MS (LCMS) *m/z*: 705.60 (M + Na); Anal. Calcd for $C_{39}H_{58}N_2O_8$: C, 68.59; H, 8.56; N, 4.10; Found: C, 68.83; H, 8.39; N, 4.02.

Deoxycholic acid-β-lactam conjugate (97):

Compound **97** was synthesized from deoxycholic acid **69** and 3-amino β-lactam **95** following the experimental procedure described for the compound **84**.
White solid, Yield: 94%; Mp: 140-143 ºC; $[\alpha]_D^{27}$ +27.69 (c 1.3, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3413, 1741, 1658; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.46 (d, *J* = 9.1 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 1H, CONH), 5.60-5.51 (m, 1H), 4.38-4.27 (m, 2H), 4.09-3.99 (m, 2H), 3.80-3.78 (m, 1H), 3.76 (s, 3H, OMe), 3.65-3.55 (m, 1H, CH-3), 1.40 (s, 3H), 1.34 (s, 3H), 1.00 (d, *J* = 5.3 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.2, 166.1, 158.1, 132.3, 121.1, 114.9, 111.0, 77.8, 73.9, 72.4, 67.4, 63.2, 57.3, 55.9, 48.0, 47.5, 43.5, 37.4, 37.2, 36.8, 36.4, 35.2, 34.7, 33.7, 32.9, 31.0, 29.9, 28.7, 28.4, 27.4, 26.8, 25.4, 24.9, 23.8, 17.7, 13.3; MS (LCMS) *m/z*: 667.67 (M + H), 689.69 (M + Na); Anal. Calcd for $C_{39}H_{58}N_2O_7$: C, 70.24; H, 8.77; N, 4.20; Found: C, 70.04; H, 8.46; N, 4.52.

Cholic acid-β-lactam conjugate (98):

A mixture of conjugate **96** (0.205 g, 0.3 mmol) and *p*-toluenesulphonic acid monohydrate (0.017 g, 0.09 mmol) in THF (6 mL) and water (2 mL) was refluxed for 24 h. After completion of reaction (TLC), the reaction mixture was neutralized with $NaHCO_3$ and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (25 mL) and the organic layer was washed with saturated brine solution (10 mL) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to afford the crude compound **98**. Silica gel column chromatography purification (MeOH/ CH_2Cl_2, 5:95) of the crude product gave pure **98** (0.177 g, 92%) as a white solid. Mp: 160-162 ºC; $[\alpha]_D^{27}$ +58.66 (c 1.5, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3362, 1770, 1651; ^1H NMR (CD_3OD, 400 MHz) δ 6.76 (d, *J* = 8.8 Hz, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 4.81 (d, *J* = 7.8 Hz, 1H), 4.75 (d, *J* = 10.5 Hz, 1H), 4.59-4.55 (m, 1H), 3.91 (bs, 1H, CH-12), 3.84-3.78 (m, 3H), 3.72 (s, 3H, OMe), 3.41-3.37 (m, 1H, CH-3), 0.99 (d, *J* = 6.5 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 177.2, 175.1, 154.0, 142.7, 116.0, 115.6, 81.0, 74.0, 72.8, 69.0, 61.3, 58.6, 56.2, 55.9, 48.0, 47.4, 43.1, 42.9, 40.9, 40.4, 36.5, 35.9, 33.9, 33.0, 31.1, 29.5, 28.6, 27.8, 24.2, 23.1, 17.7, 13.0; MS

(LCMS) *m/z*: 643.72 (M + H), 665.69 (M + Na); Anal. Calcd for $C_{36}H_{54}N_2O_8$: C, 67.26; H, 8.47; N, 4.36; Found: C, 67.31; H, 8.24; N, 4.18.

Deoxycholic acid-β-lactam conjugate (99):

Conjugate **99** has been synthesized from compound **97** following the experimental procedure described for the compound **98**.

White solid, Yield: 94%; Mp: 143-145 ºC; $[\alpha]_D^{28}$ +65.45 (c 0.825, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3365, 1759, 1649; ^1H NMR (CD_3OD, 400 MHz) δ 6.75 (d, *J* = 9.0 Hz, 2H), 6.65 (d, *J* = 9.0 Hz, 2H), 4.79 (d, *J* = 7.8 Hz, 1H), 4.72 (d, *J* = 10.8 Hz, 1H), 4.58-4.53 (m, 1H), 3.90 (bs, 1H, CH-12), 3.82-3.76 (m, 2H), 3.70 (s, 3H, OMe), 3.54-3.49 (m, 1H, CH-3), 0.97 (d, *J* = 6.3 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.59 (s, 3H, CH_3-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 177.2, 175.1, 154.0, 142.7, 116.0, 115.6, 81.0, 74.0, 72.5, 61.3, 58.6, 56.1, 55.9, 49.2, 48.1, 47.5, 43.6, 37.4, 37.2, 2 X 36.4, 35.3, 35.8, 33.8, 33.0, 31.0, 29.8, 28.6, 28.4, 27.4, 24.8, 23.7, 17.7, 13.2; MS (LCMS) *m/z*: 627.89 (M + H), 649.87 (M + Na); Anal. Calcd for $C_{36}H_{54}N_2O_7$: C, 68.98; H, 8.68; N, 4.47; Found: C, 68.60; H, 8.59; N, 4.67.

3-Amino-1-(4-methoxyphenyl)-4-phenylazetidine-2-one (100):

The solution of azido β-lactam **66** (0.5 g, 1.7 mmol) in methanol (10 mL) was treated with Raney nickel (2 g, excess) under hydrogen atmosphere (1 atm of H_2) for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was filtered through Celite pad and concentrated under reduced pressure. The crude product was then purified by column chromatography over silica gel ($MeOH/CH_2Cl_2$, 1:99) to get pure compound **100** as yellowish solid (0.432 g, 95%). Mp: 157-158 ºC; IR ($CHCl_3$, cm^{-1}) 3452, 1741; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42-7.22 (m, 7H), 6.80 (d, *J* = 9.0 Hz, 2H), 5.23 (d, *J* = 5.5 Hz, 1H), 4.60 (d, *J* = 5.5 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.2, 156.0, 134.3, 130.8, 128.9, 128.3, 126.9, 118.5, 114.2, 63.6, 62.0, 55.2; MS (LCMS) *m/z*: 269.22 $(M + H)^+$, 291.21 (M + Na); Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44; Found: C, 71.89; H, 5.71; N, 10.24.

3-Amino-4-(4-chlorophenyl)-1-(4-methoxyphenyl)azetidine-2-one (101):

Compound **101** has been synthesized from azido β-lactam **67** following the experimental procedure described for the compound **100**.

Yellowish solid; Yield 96%; Mp: 142-143 ºC; IR ($CHCl_3$, cm^{-1}) 3398, 1743; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41-7.18 (m, 6H), 6.80 (d, *J* = 9.1 Hz, 2H), 5.21 (d, *J* = 5.4 Hz, 1H), 4.61 (d, *J* = 5.4 Hz, 1H), 3.76 (s, 3H), 1.51 (bs, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.1, 156.1, 134.3, 132.9, 130.5, 129.2, 128.3, 118.5, 114.3, 63.6, 61.5, 55.3; MS (LCMS) *m/z*: 303.29 (M + 1), 325.28 (M + Na); Anal. Calcd for $C_{16}H_{15}ClN_2O_2$: C, 63.47; H, 4.99; N, 9.25; Found: C, 63.16; H, 5.22; N, 9.37.

Cholic acid-β-lactam conjugate (102):

Conjugate **102** was synthesized from cholic acid **68** and 3-amino β-lactam **100** using the experimental procedure described for the compound **84**.

White solid; Yield: 93%; Mp: 157-158 ºC; IR ($CHCl_3$, cm^{-1}) 3431, 1737, 1666; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.37-7.19 (m, 7H), 6.83-6.77 (m, 2H), 5.69-5.59 (m, 1H), 5.35 (d, *J* = 5.1 Hz, 1H), 3.88 (bs, 1H, CH-12), 3.83 (bs, 1H, CH-7), 3.75 (s, 3H, OMe), 3.46-3.35 (m, 1H, CH-3), 0.87 (s, 3H, CH_3-19), 0.75 (d, *J* = 5.6 Hz, 3H, CH_3-21), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2, 163.9, 156.3, 133.6, 130.6, 128.6, 128.3, 127.1, 127.0, 118.6, 114.3, 73.0, 71.7, 68.3, 61.5, 61.3, 59.8, 55.3, 46.4, 46.3, 46.2, 41.4, 39.5, 39.4, 35.3, 35.1, 34.7, 32.3, 31.2, 30.3, 28.0, 27.4, 26.2, 23.2, 22.3, 17.2, 17.1, 12.4; MS (LCMS) *m/z*: 681.58 (M + Na); Anal. Calcd for $C_{40}H_{54}N_2O_6$: C, 72.92; H, 8.26; N, 4.25; Found: C, 72.67; H, 8.40; N, 4.66.

Deoxycholic acid-β-lactam conjugate (103):

Conjugate **103** was synthesized from deoxycholic acid **69** and 3-amino β-lactam **100** following the experimental procedure described for the compound **84**.

White solid; Yield: 96%; Mp: 147 ºC; IR ($CHCl_3$, cm^{-1}) 3452, 1741, 1664; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.37-7.20 (m, 7H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.10, 5.98 (Two d, *J* = 8.3, 8.5 Hz, 1H, CONH), 5.70-5.62 (m, 1H), 5.35 (d, *J* = 5.2 Hz, 1H), 3.91 (bs, 1H, CH-12), 3.76 (s, 3H, OMe), 3.66-3.55 (m, 1H, CH-3), 0.90 (s, 3H, CH_3-19), 0.82, 0.76 (Two d, *J* = 6.6, 6.2 Hz, 3H, CH_3-21), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0, 173.8, 163.9, 163.8, 156.3, 133.6, 130.5, 128.5, 128.3, 128.2, 127.0, 118.6, 114.3,

72.8, 71.4, 2 X 61.4, 59.7, 55.3, 47.9, 47.8, 46.8, 46.5, 46.3, 46.4, 42.0, 36.3, 35.9, 35.2, 35.1, 35.0, 34.0, 33.4, 33.3, 32.5, 31.1, 30.2, 28.5, 28.3, 27.4, 27.3, 27.1, 26.1, 23.6, 23.0, 17.1, 17.0, 12.6 ; MS (LCMS) *m/z*: 643.56 $(M + H)^+$, 665.64 (M + Na); Anal. Calcd for $C_{40}H_{54}N_2O_5$: C, 74.73; H, 8.47; N, 4.36; Found: C, 74.46; H, 8.59; N, 4.41.

Cholic acid-β-lactam conjugate (104):

Conjugate **104** was synthesized from cholic acid **68** and 3-amino β-lactam **101** following the experimental procedure described for the compound **84**.

White solid; Yield: 92%; Mp: 175-177 ºC; IR ($CHCl_3$, cm^{-1}) 3436, 1745, 1666; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34-7.19 (m, 6H), 6.80 (d, *J* = 9.0 Hz, 2H), 5.62 (dd, *J* = 5.2, 8.3 Hz, 1H), 5.33 (d, *J* = 5.1 Hz, 1H), 3.89 (bs, 1H, CH-12), 3.83 (bs, 1H, CH-7), 3.75 (s, 3H, OMe), 3.40 (bs, 1H. CH-3), 0.87 (s, 3H, CH_3-19), 0.73 (d, *J* = 5.3 Hz, 3H, CH_3-21), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 174.6, 163.6, 156.4, 134.1, 132.1, 130.2, 128.7, 128.6, 118.5, 114.3, 72.9, 71.5, 68.2, 61.0, 59.9, 59.8, 55.3, 46.3, 46.1, 41.3, 39.2, 35.1, 35.0, 34.6, 34.4, 32.0, 31.2, 29.9, 27.8, 27.3, 26.1, 23.1, 22.2, 16.8, 12.2; MS (LCMS) *m/z*: 715.74 (M + Na); Anal. Calcd for $C_{40}H_{53}ClN_2O_6$: C, 69.29; H, 7.71; N, 4.04; Found: C, 69.01; H, 7.98; N, 4.27.

Deoxycholic acid-β-lactam conjugate (105):

Conjugate **105** was synthesized from deoxycholic acid **69** and 3-amino β-lactam **101** following the experimental procedure described for the compound **84**.

White solid; Yield: 95%; Mp: 150-153 ºC; IR ($CHCl_3$, cm^{-1}) 3434, 3323, 1745, 1666; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.35-7.15 (m, 6H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.36, 6.27 (Two d, *J* = 8.3, 8.1 Hz, 1H, CONH), 5.64-5.56 (m, 1H), 5.32 (d, *J* = 5.1 Hz, 1H), 3.92 (bs, 1H, CH-12), 3.76 (s, 3H, OMe), 3.66-3.55 (m, 1H, CH-3), 0.90 (s, 3H, CH_3-19), 0.84, 0.78 (Two d, *J* = 6.4, 6.3 Hz, 3H, CH_3-21), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2, 174.0, 2 X 163.7, 156.4, 134.1, 132.1, 130.2, 128.7, 128.6, 118.5, 114.3, 72.9, 72.8, 71.4, 61.0, 60.9, 59.8, 55.3, 47.8, 47.7, 46.9, 46.4, 46.3, 41.9, 36.2, 35.8, 35.2, 35.0, 34.0, 33.4, 33.3, 32.5, 32.3, 31.3, 31.2, 30.2, 28.4, 28.2, 27.4, 27.2, 27.1, 26.1, 23.6, 23.0, 17.0, 16.8, 12.5; MS (LCMS) *m/z*: 699.64 (M + Na); Anal. Calcd for $C_{40}H_{53}ClN_2O_5$: C, 70.93; H, 7.89; N, 4.14; Found: C, 71.25; H, 8.26; N, 4.42.

Methyl 3α,12α-dihydroxy-5β-cholane-24-oate (106):

A mixture of deoxycholic acid **69** (5.096 g, 13 mmol) and *p*-TSA monohydrate (0.496 g, 2.6 mmol) in dry methanol were stirred for 22 h at 25 ºC. After the completion of reaction (TLC), solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water and brine, dried over anhydrous sodium sulphate and concentrated to get methyl ester **106** (5.066 g, 96%) as a white solid; Mp: 82-105 ºC (lit.[80] mp 70-108 ºC); IR ($CHCl_3$, cm^{-1}): 3417, 1728; ^1H NMR ($CDCl_3$, 500 MHz): δ 3.98 (bs, 1H, CH-12), 3.66 (s, 3H, COOMe), 3.64-3.59 (m, 1H, CH-3), 0.98 (d, J = 6.4 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18) ; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.7, 72.9, 71.4, 51.4, 48.0, 47.0, 46.3, 42.0, 36.2, 35.9, 35.2, 34.0, 33.4, 31.0, 30.8, 30.1, 29.6, 28.4, 27.4, 27.1, 26.0, 23.6, 23.0, 17.1, 12.6.

Methyl 3α,12α-(ditetrahydropyranyloxy)-5β-cholane-24-oate (107):

To a solution of methyl ester of deoxycholic acid **106** (3.248 g, 8 mmol) and 3,4-dihydro-2*H*-pyrane (1.8 mL, 20 mmol) in dichloromethane (25 mL) was added PPTS (0.401 g, 1.6 mmol) under nitrogen atmosphere and reaction was strirred at 25 ºC for 10 h. The reaction was quenched with water and extracted with dichloromethane (3 X 20 mL). The organic extract was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. Silica gel column chromatography of the crude product using ethyl acetate/ pet ether (7:93) as eluent provided **107** (4.27 g, 93%) as a colorless thick liquid. IR ($CHCl_3$, cm^{-1}): 3417, 1728; ^1H NMR ($CDCl_3$, 200 MHz): δ 4.71-4.60 (m, 2H), 4.01-3.86 (m, 2H), 3.74 (bs, 1H), 3.66 (s, 3H, OMe), 3.61-3.44 (m, 3H), 2.44-2.17 (m, 2H), 0.92-0.87 (m, 6H, CH_3-21, CH_3-19), 0.70, 0.66 (s, 3H, CH_3-18); MS (LCMS) *m/z*: 597.63 (M + Na).

3α,12α-(ditetrahydropyranyloxy)-5β-cholane-24-ol (108):

To a stirred suspention of $LiAlH_4$ (0.266g, 7mmol) in dry THF (20 mL), compound **107** (2.012 g, 3.5 mmol) in dry THF (20 mL) was added dropwise at 25 ºC. After 1 h cold water was added to the ice cooled reaction mixture. It was filtered through celite and residue was washed with THF (40 mL). Solvent was evaporated under reduced pressure and it was extracted with ethyl acetate (3 x 50 mL). Extract was washed with water and

brine, dried over anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product which was purified by column chromatography on silica gel (EtOAc/pet ether, 20:80) to get compound **108** (1.646 g, 86%) gummy material, IR ($CHCl_3$, cm^{-1}): 3429; ^1H NMR ($CDCl_3$, 200 MHz): δ 4.71-4.58 (m, 2H), 4.0-3.86 (m, 2H), 3.75 (bs, 1H), 3.64-3.44 (m, 5H), 1.01 (d, *J* = 5.6 Hz, 3H, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.71, 0.67 (two s, 3H, CH_3-18); MS (LCMS) *m/z*: 569.35 (M + Na).

Compound (109):

A solution of compound **108** (1.641 g, 3 mmol) in benzene (7 mL), 50% aqueous NaOH (5 mL) and Bu_4NHSO_4 (0.508 g, 1.5 mmol) were stirred vigorously at 10 ºC. *t*-Butyl bromoacetate (0.66 mL, 4.5 mmol) was added dropwise rapidly, and stirring was continued for 1 h. Water (10 mL) and diethyl ether (30 mL) were then added, the mixture was stirred for 10 min. and extracted with diethyl ether (2 X 30 mL). The combined organic layers were washed with water and brine, dried with anhydrous Na_2SO_4 and concentrated under *vacuo*. The residue was purified by flash column chromatography over silica gel (EtOAc/pet ether, 7:93) to afford *t*-butyl ester **109** (1.211 g, 61%) as a thick oil, IR ($CHCl_3$, cm^{-1}): 1743; ^1H NMR ($CDCl_3$, 200 MHz): δ 4.71-4.60 (m, 2H), 3.99-3.90 (m, 5H), 3.75 (bs, 1H), 3.51-3.45 (m, 4H), 1.48 (s, 9H), 1.00 (d, *J* = 6.6 Hz, 3H, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.70, 0.66 (Two s, 3H, CH_3-18); MS (LCMS) *m/z*: 683.40 (M + Na).

Compound (110):

To a solution of ester compound **109** (1.95 g, 2.96 mmol) in methanol (10 mL) and water (4 mL) was added LiOH:H_2O (0.372 g, 8.88 mmol) at 30 ºC. The reaction mixture was stirred for 26 h and solvent was evaporated under reduced pressure to near dryness. The residue was dissolved in MeOH/H_2O (3:17) and passed through short pad of silica gel using same solvent system to obtained acid **110** (1.695 g, 95%) as a gummy material, IR ($CHCl_3$, cm^{-1}): 3419, 1733; ^1H NMR ($CDCl_3$, 200 MHz): δ 4.72-4.58 (m, 2H), 4.05-3.87 (m, 4H), 3.74 (bs, 1H), 3.62-3.45 (m, 5H), 1.00 (d, *J* = 6.4 Hz, 3H, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.70, 0.66 (Two s, 3H, CH_3-18); MS (LCMS) *m/z*: 627.33 (M + Na).

Compound (112):

A solution of triphosgene (0.082 g, 0.28 mmol), in anhydrous CH_2Cl_2 (6 mL), was added slowly to a mixture of acid **110** (0.335 g, 0.55 mmol), imine **111** (0.116 g, 0.55 mmol) and triethylamine (0.23 mL, 1.65 mmol) in anhydrous CH_2Cl_2 (8 mL) at 0 ºC. After the addition, the reaction mixture was allowed to warm up to room temperature (28 ºC) and stirred for 15 h. The reaction mixture was then washed with water (10 mL), saturated sodium bicarbonate solution (2×10 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to get crude product, which was purified by flash column chromatography over silica gel (EtOAc/pet ether, 5:95) to get pure β-lactam **112** (0.198 g, 45%) as a thick oil, IR ($CHCl_3$, cm^{-1}): 1747; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.37-7.25 (m, 7H), 6.77 (d, *J* = 9.0 Hz, 2H), 5.15 (d, *J* = 4.5 Hz, 1H), 4.88 (d, *J* = 4.5 Hz, 1H), 4.70-4.53 (m, 2H), 3.95-3.89 (m, 2H), 3.73 (s, 3H, OMe), 3.69-3.37 (m, 5H), 3.09-3.00 (m, 1H), 0.88 (s, 3H, CH_3-19), 0.75-0.70 (m, 3H, CH_3-21), 0.63, 0.59 (Two S, 3H, CH_3-18); MS (LCMS) *m/z*: 820.30 (M + Na).

Ether linked deoxycholic acid-β-lactam conjugate (113):

To a solution of compound **112** (0.255 g, 0.32 mmol) in methanol (5 mL) was added *p*-TSA (0.018 g, 0.096 mmol) at 30 °C. The reaction mixture was stirred for 5 h and solvent was evaporated under reduced pressure to dryness. The residue was dissolved in ethyl acetate washed with water, brine and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/pet ether (30:70) as eluent to give **113** (0.195 g, 97%) as white solid. Mp: 105-107 ºC; IR ($CHCl_3$, cm^{-1}) 3431, 1747; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.37-7.25 (m, 7H), 6.78 (d, *J* = 9.1 Hz, 2H), 5.16 (d, *J* = 4.5 Hz, 1H), 4.88 (d, *J* = 4.5 Hz, 1H), 3.94 (bs, 1H, CH-12), 3.74 (s, 3H, OMe), 3.67-3.56 (m, 1H, CH-3), 3.43-3.38 (m, 1H, -OCH_2), 3.10-2.99 (m, 1H, -OCH_2), 0.90 (s, 3H, CH_3-19), 0.82, 0.80 (Two d, *J* = 6.2, 6.3 Hz, 3H, CH_3-21), 062 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.9, 156.2, 133.5, 130.6, 128.4, 128.3, 128.0, 118.7, 114.2, 83.6, 73.0, 71.6, 71.5, 71.3, 62.1, 55.3, 48.1, 2 X 47.3, 46.3, 42.0, 36.3, 35.9, 35.2, 35.0, 34.0, 33.5, 31.5, 31.4, 30.3, 28.4, 27.4, 27.1, 26.1, 2 X 25.9, 23.5, 23.0, 17.4, 17.3, 12.6; MS (LCMS) *m/z*: 652.79 (M + Na); Anal. Calcd for $C_{40}H_{55}NO_5$: C, 76.27; H, 8.80; N, 2.22; Found: C, 76.61; H, 8.65; N, 2.15.

Compound (115):

Compound **115** was synthesized from acid **110** and imine **114** following the experimental procedure described for the compound **112**.

Gummy material, Yield 48%**;** IR ($CHCl_3$, cm^{-1}): 1751; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.30-7.20 (m, 8H), 7.08-7.03 (m, 2H), 4.90-4.87 (m, 1H), 4.77 (d, *J* = 14.8 Hz, 1H, NCH_2Ph), 4.67 (d, *J* = 4.3 Hz, 1H), 4.48 (d, *J* = 4.3 Hz, 1H), 3.90-3.82 (m, 2H), 3.74 (d, J = 14.8 Hz, 1H, NCH_2Ph), 3.62-3.39 (m, 3H), 3.28-3.20 (m, 1H), 2.92-2.80 (m, 1H), 0.82 (s, 3H, CH_3-19), 0.54 (s, 3H, CH_3-18); MS (LCMS) *m/z*: 805.01 (M + Na);

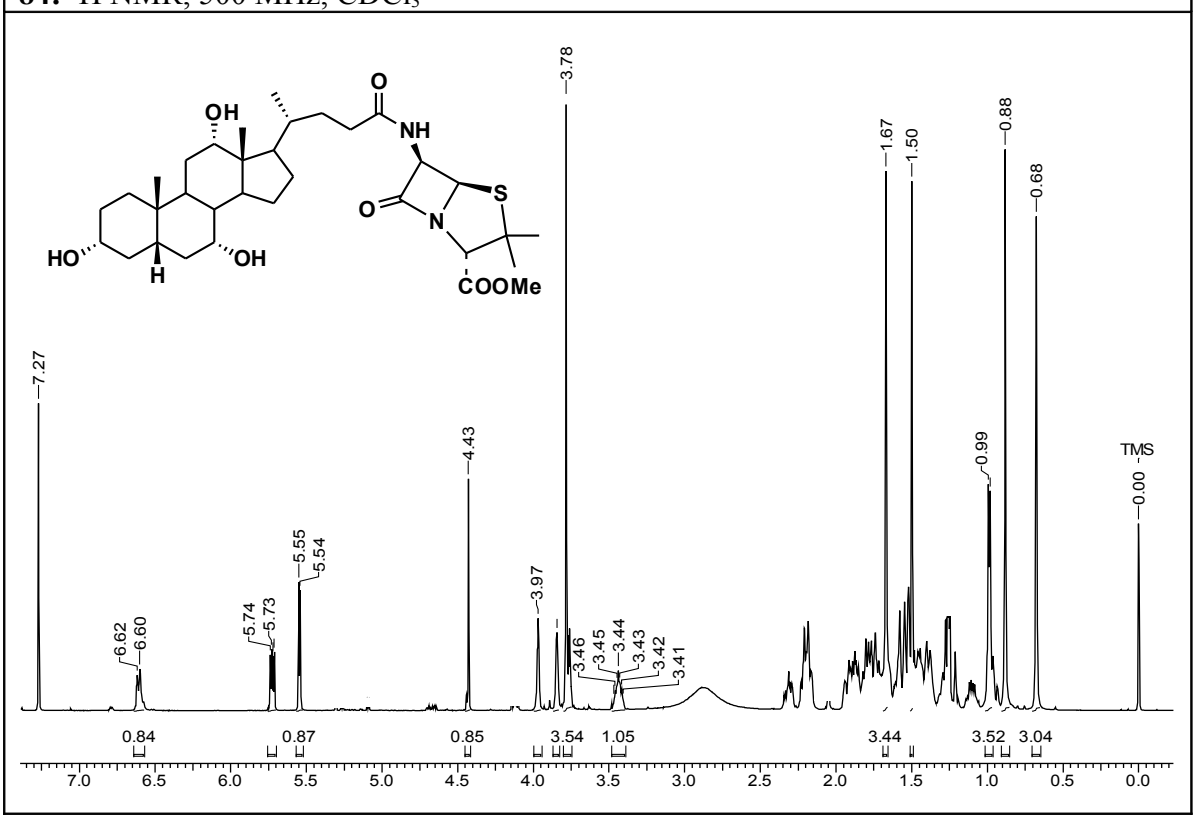
Ether linked deoxycholic acid-β-lactam conjugate (116):

Compound **116** has been synthesized from **115** following the experimental procedure described for the compound **113**.

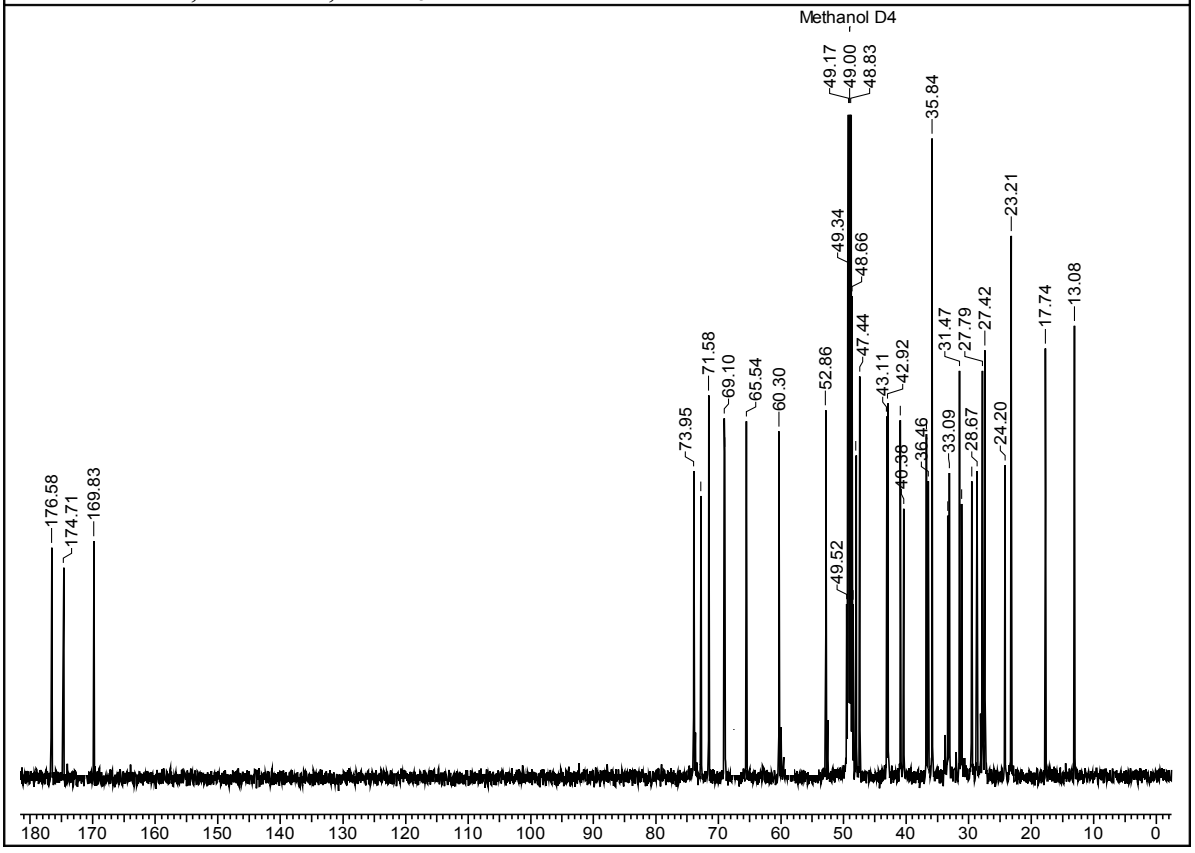
White solid; Yield 96%; Mp: 79-80 ºC; IR ($CHCl_3$, cm^{-1}) 3437, 1755; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.39-7.28 (m, 8H), 7.15-7.11 (m, 2H), 4.85 (d, *J* = 14.7 Hz, 1H, NCH_2Ph), 4.74 (d, *J* = 4.3 Hz, 1H), 4.55 (d, *J* = 4.3 Hz, 1H), 3.93 (bs, 1H, CH-12), 3.82 (d, *J* = 14.7 Hz, 1H, NCH_2Ph), 3.68-3.54 (m, 1H, CH-3), 3.38-3.26 (m, 1H), 3.00-2.87 (m, 1H), 0.90 (s, 3H, CH_3-19), 0.81, 0.78 (Two d, *J* = 6.2, 6.3 Hz, 3H, CH_3-21), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.0, 134.9, 133.5, 128.7, 128.5, 128.4, 128.2, 127.7, 84.6, 73.1, 71.7, 71.3, 71.2, 61.3, 48.1, 47.4, 47.3, 46.3, 43.8, 42.0, 36.3, 36.0, 35.2, 35.0, 34.1, 33.5, 31.6, 31.5, 30.4, 28.4, 27.4, 27.1, 26.1, 25.9, 25.8, 23.6, 23.1, 2 X 17.4, 12.6; MS (LCMS) *m/z*: 614.40 (M + H), 636.45 (M + Na); Anal. Calcd for $C_{40}H_{55}NO_4$: C, 78.26; H, 9.03; N 2.28; Found: C, 78.05; H, 9.33; N, 2.11.

1.B.7 Selected Spectra

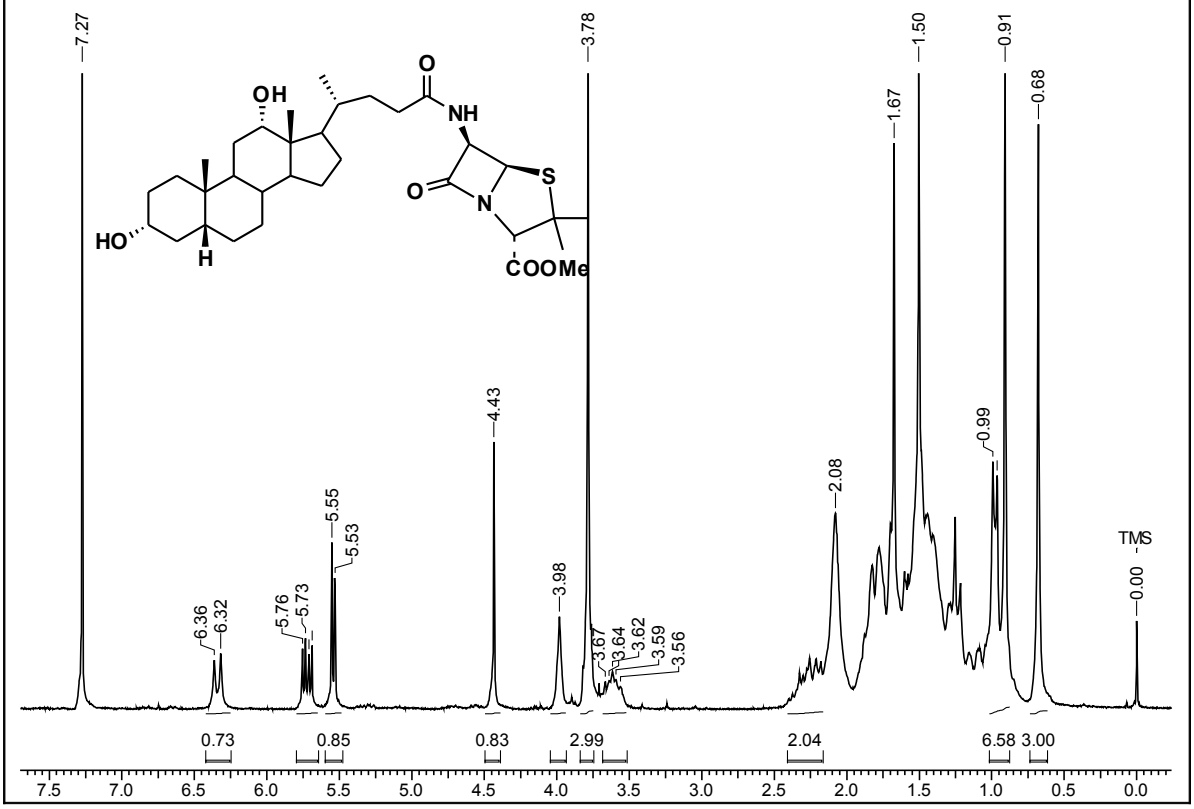
84: ^{1}H NMR, 500 MHz, $CDCl_3$



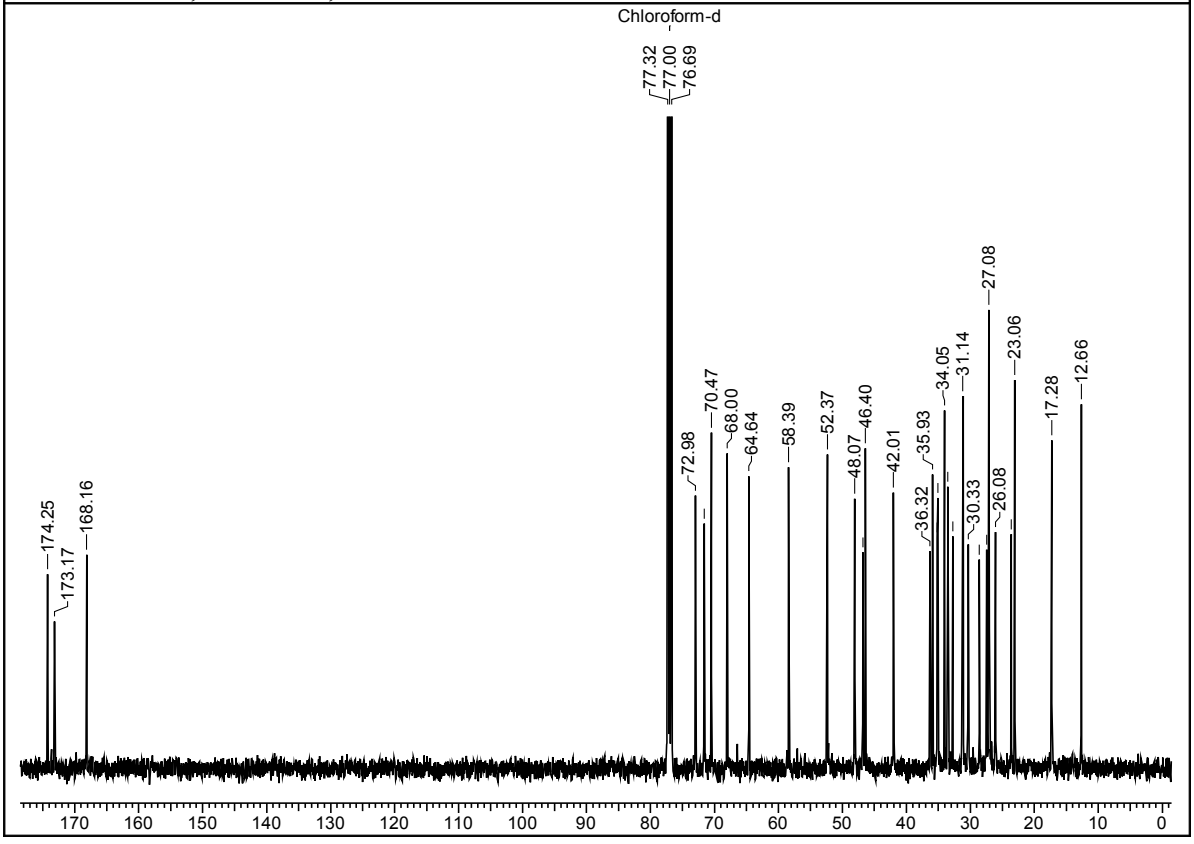
84: ^{13}C NMR, 125 MHz, $CDCl_3$



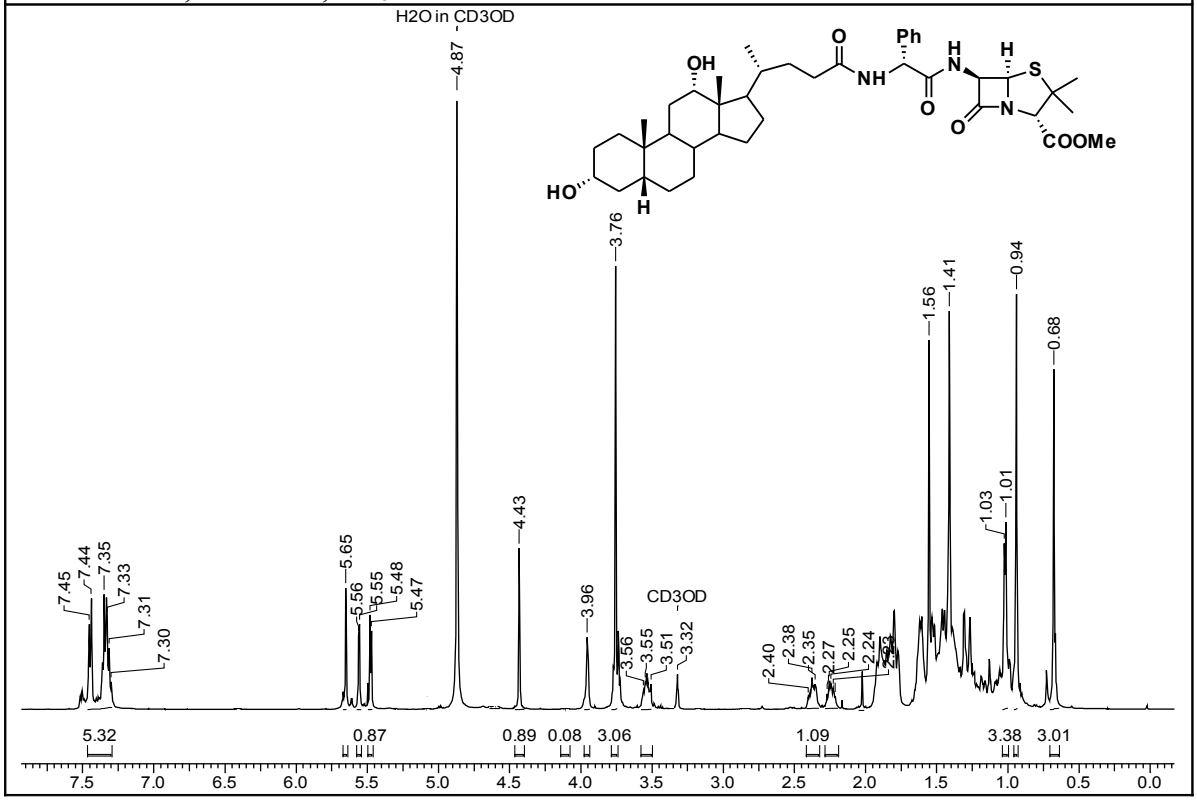
85: ^{1}H NMR, 200 MHz, $CDCl_3$



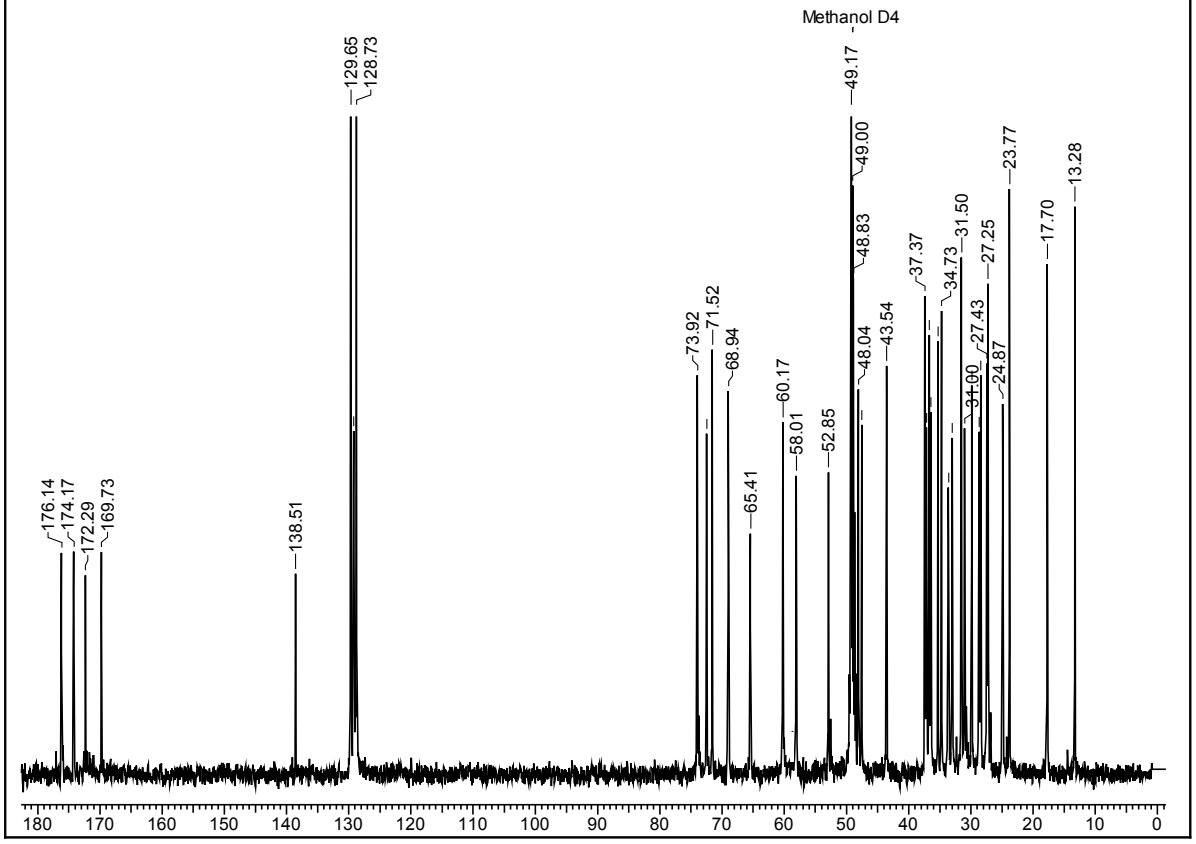
85: ^{13}C NMR, 100 MHz, $CDCl_3$



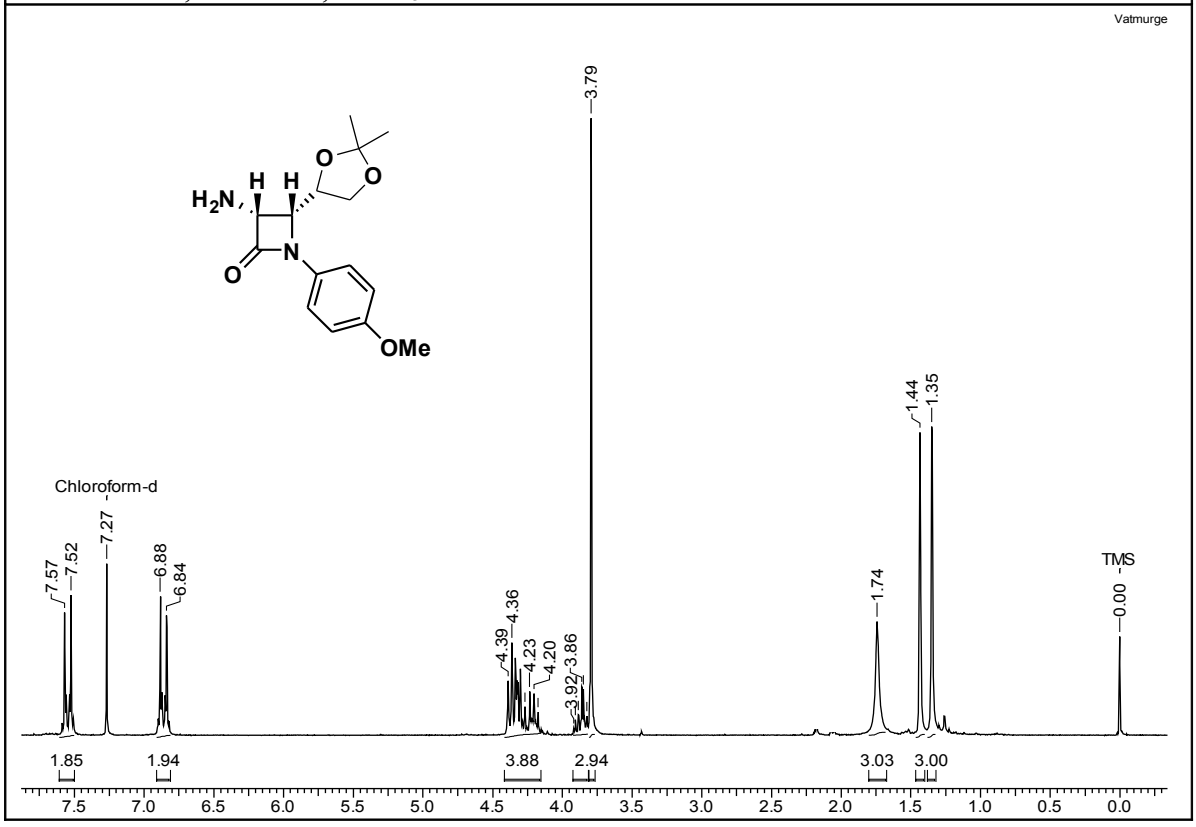
89: ^{1}H NMR, 500 MHz, CD_3OD



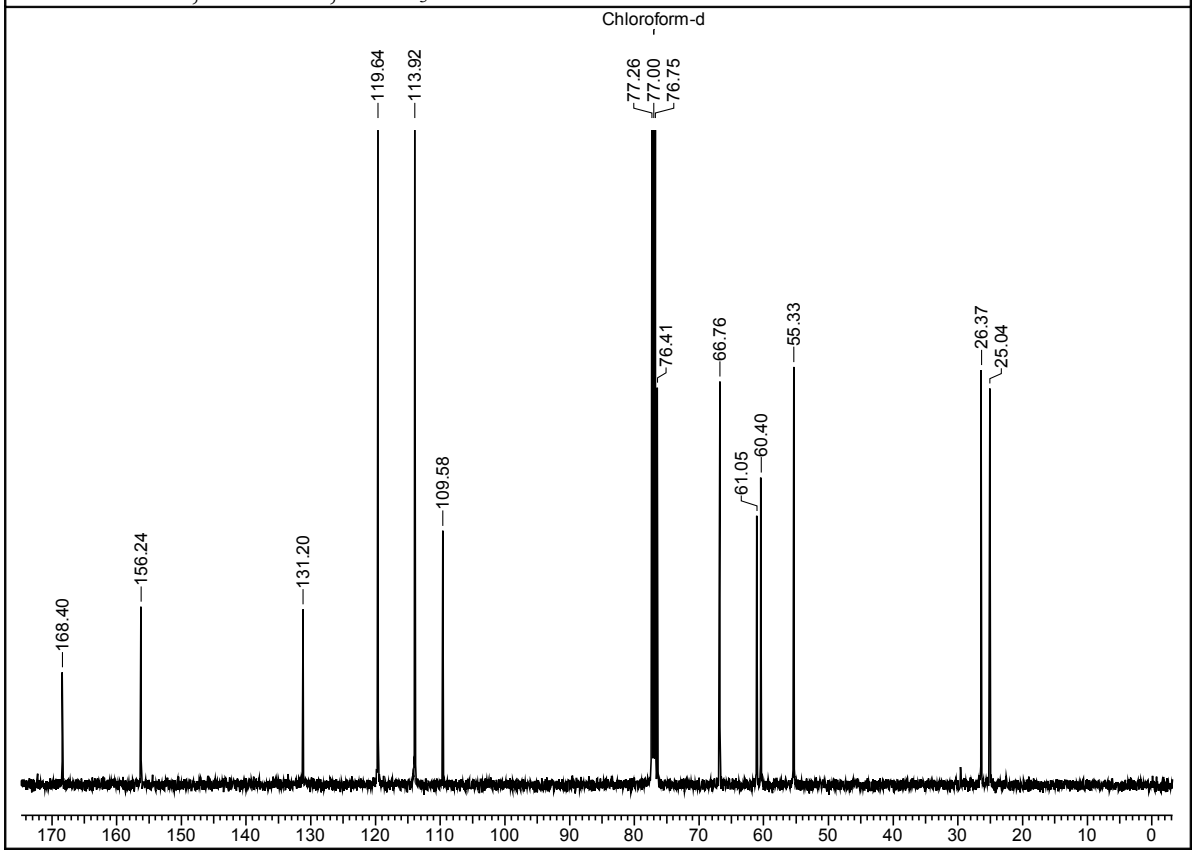
89: ^{13}C NMR, 125 MHz, CD_3OD



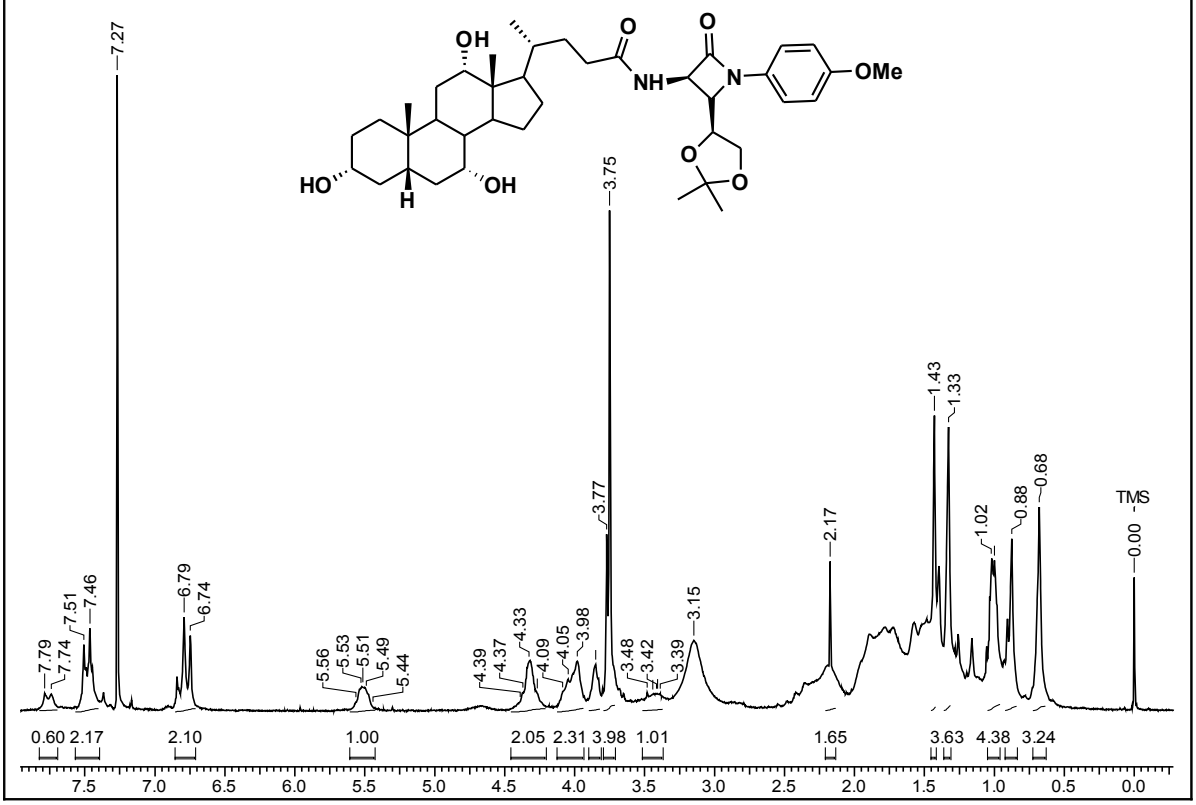
95: ^{1}H NMR, 200 MHz, $CDCl_3$



95: ^{13}C NMR, 125 MHz, $CDCl_3$



96: ^{1}H NMR, 200 MHz, $CDCl_3$



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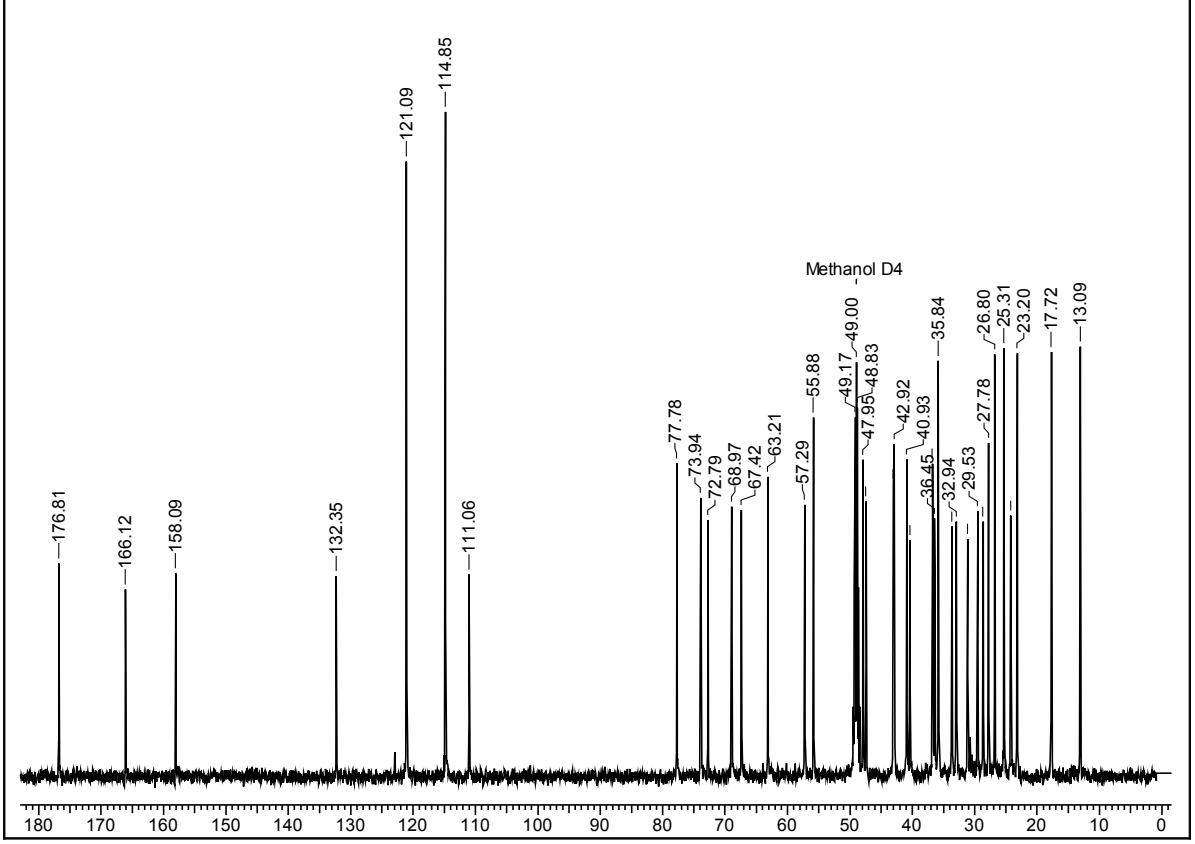
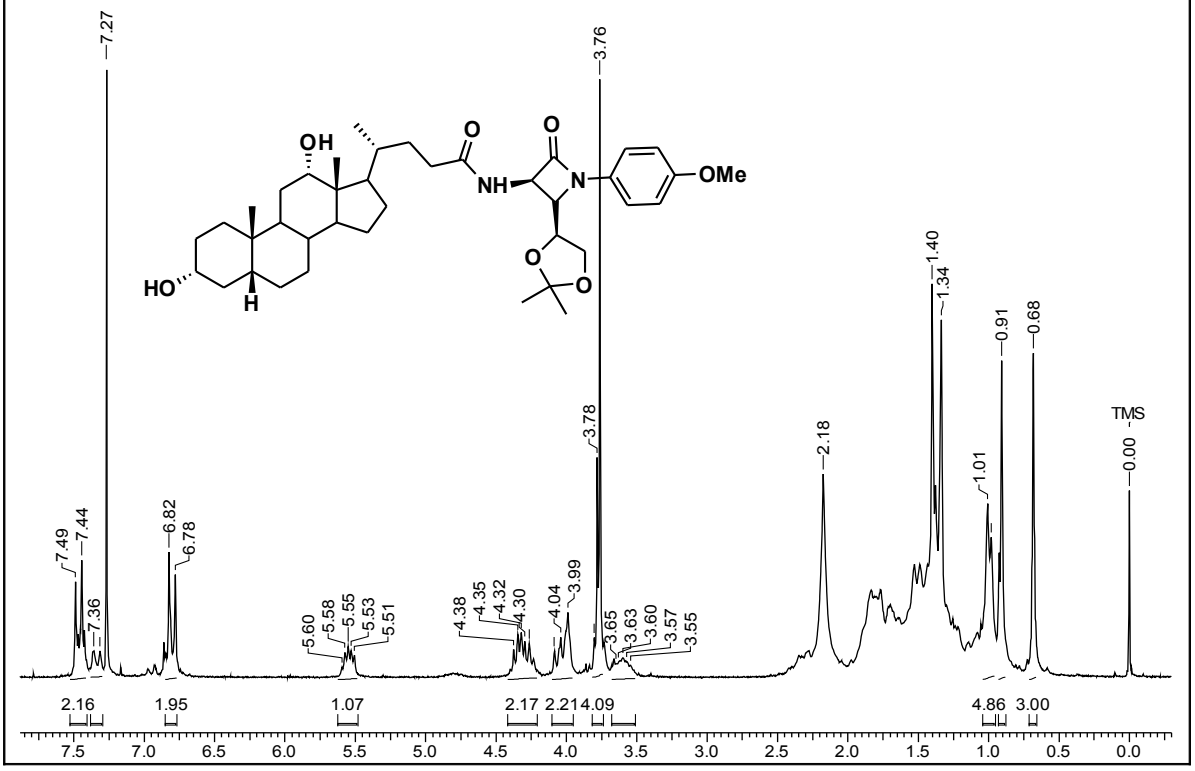
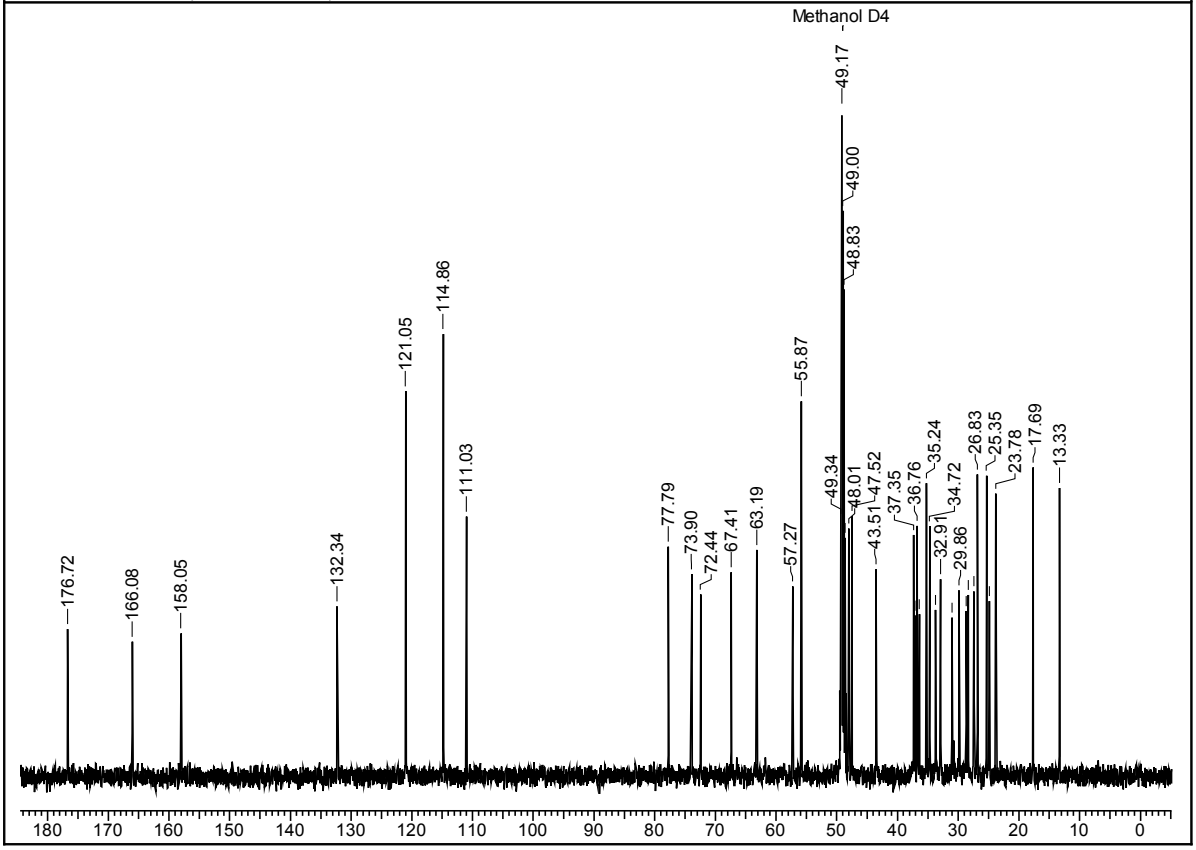


97: ^{1}H NMR, 200 MHz, $CDCl_3$



97: ^{13}C NMR, 125 MHz, CD_3OD



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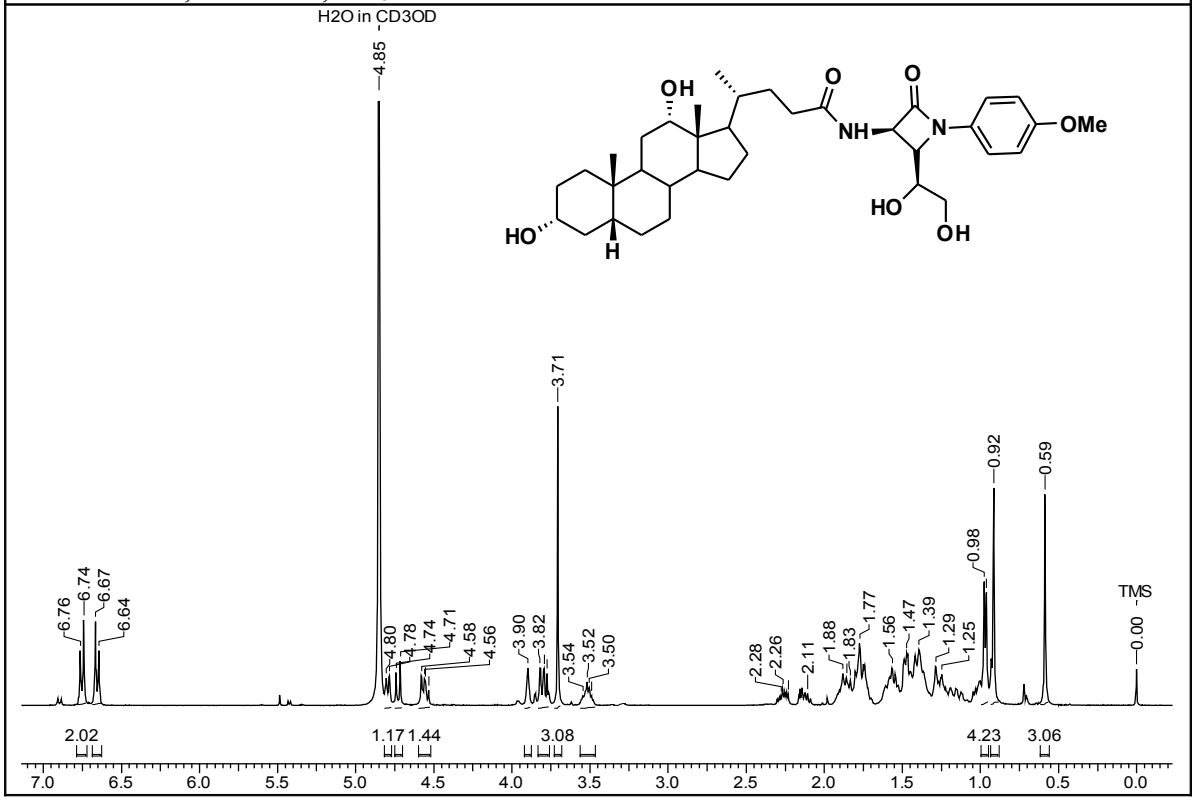

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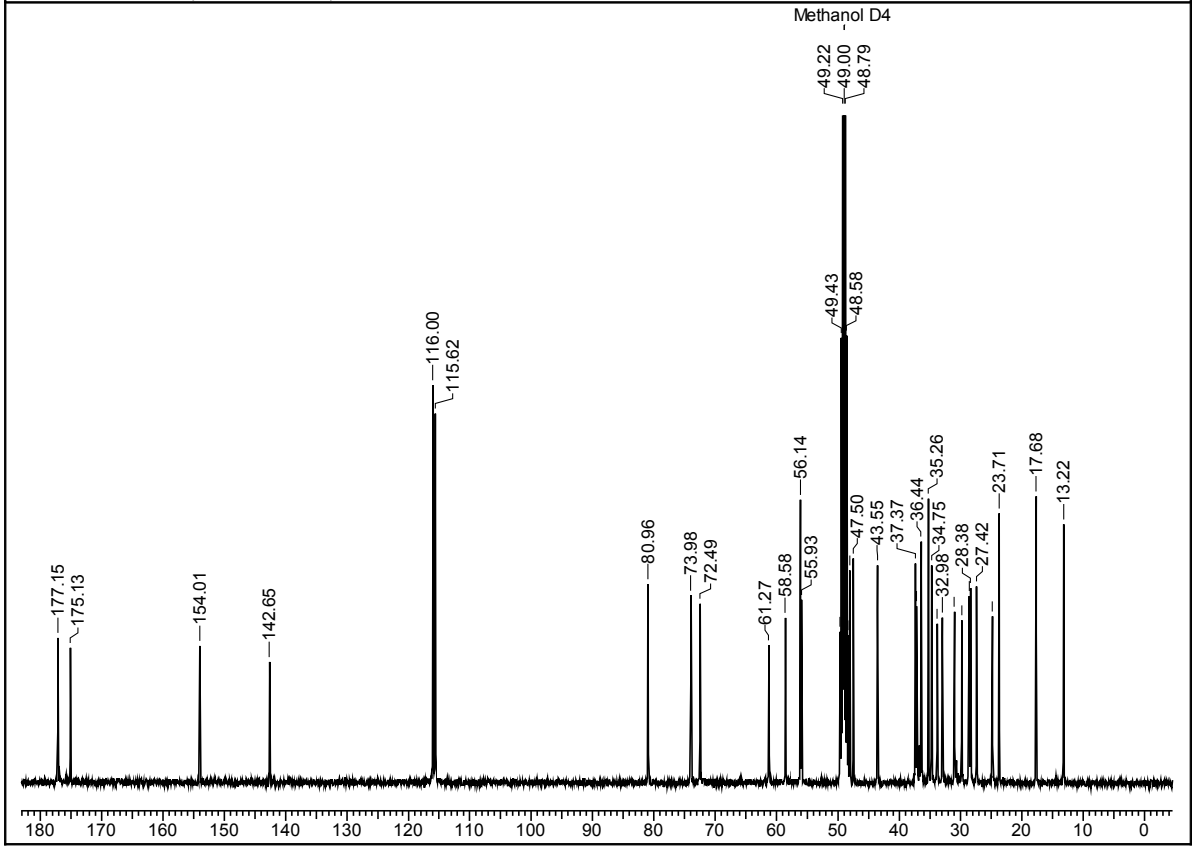

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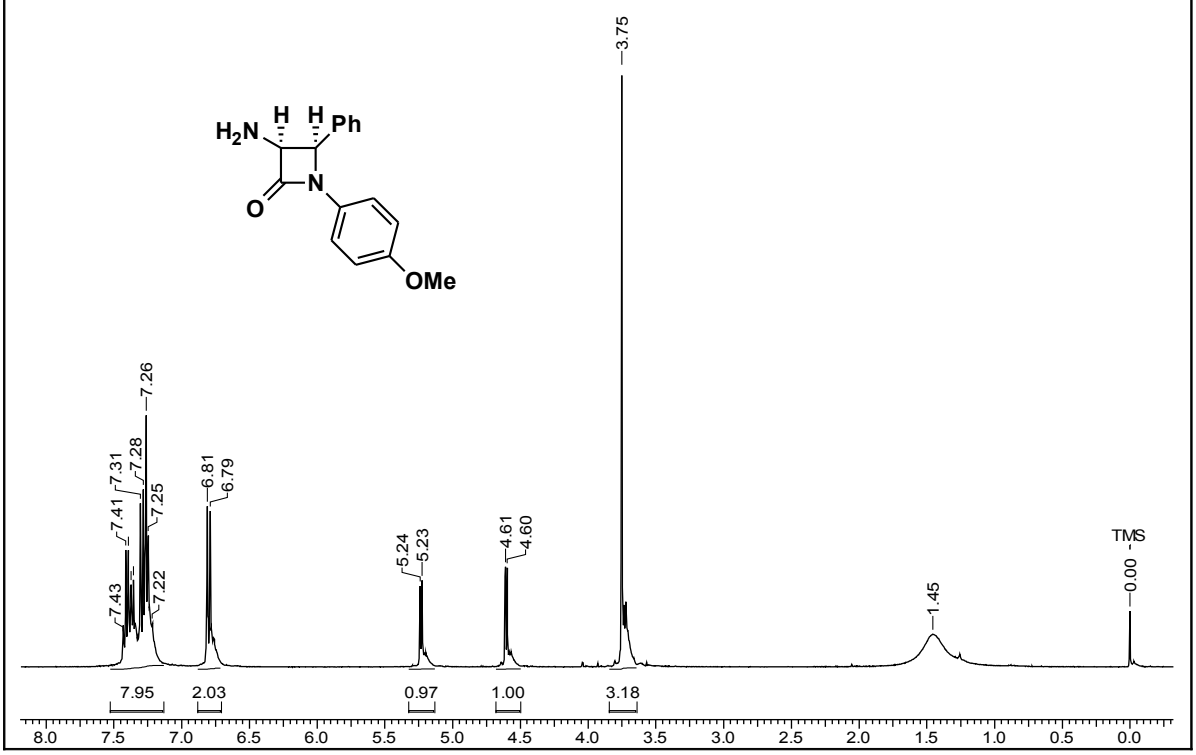

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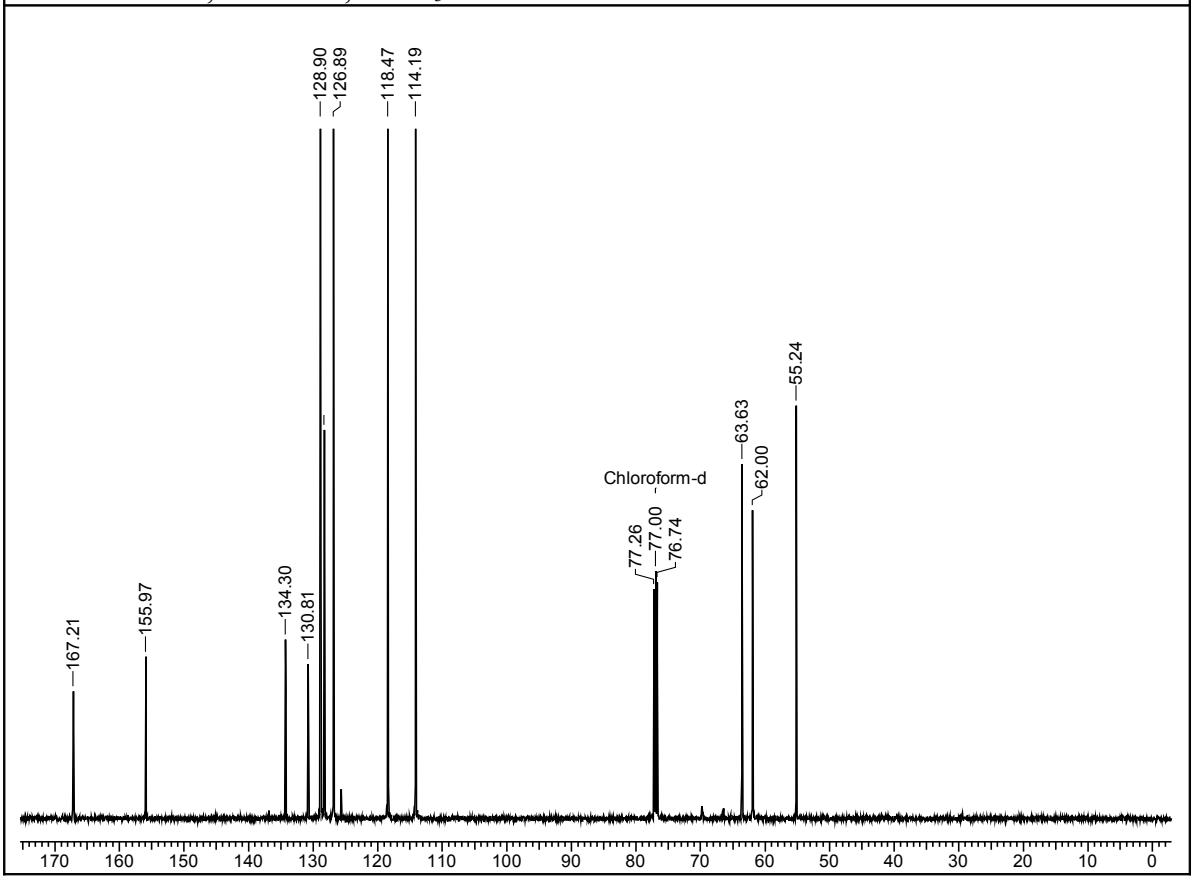

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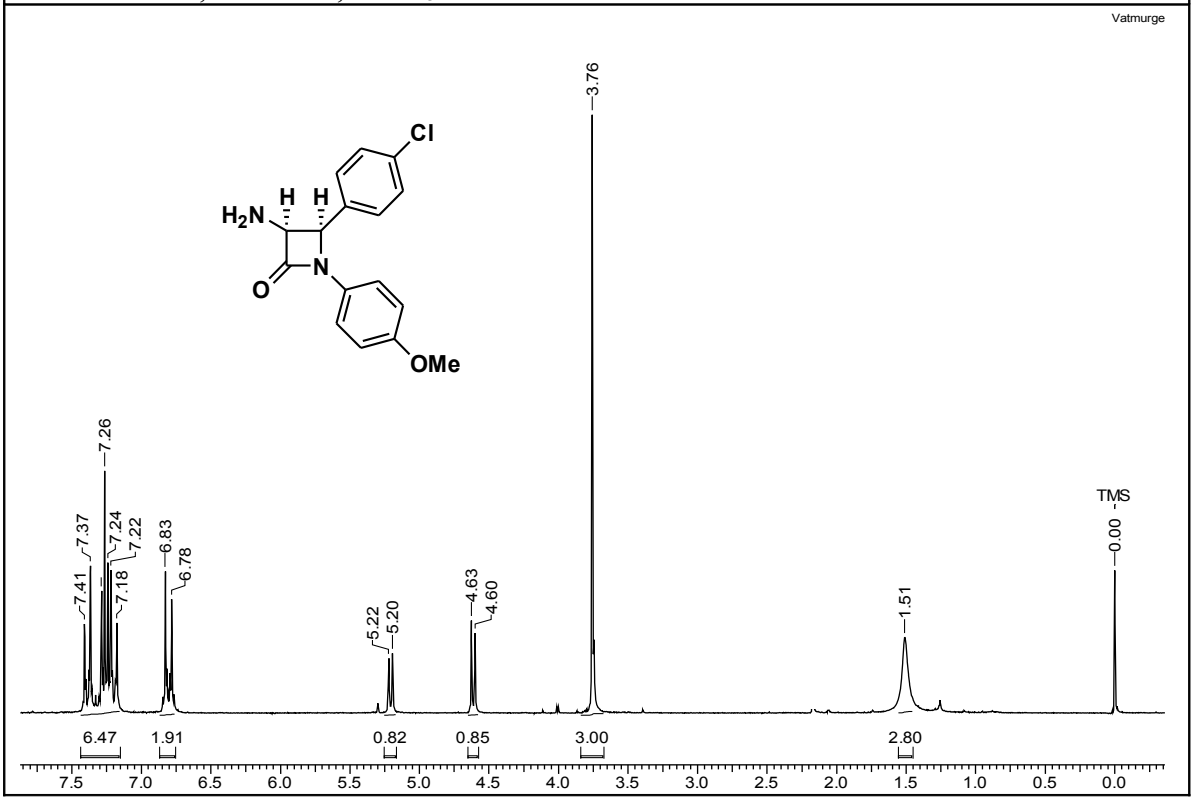

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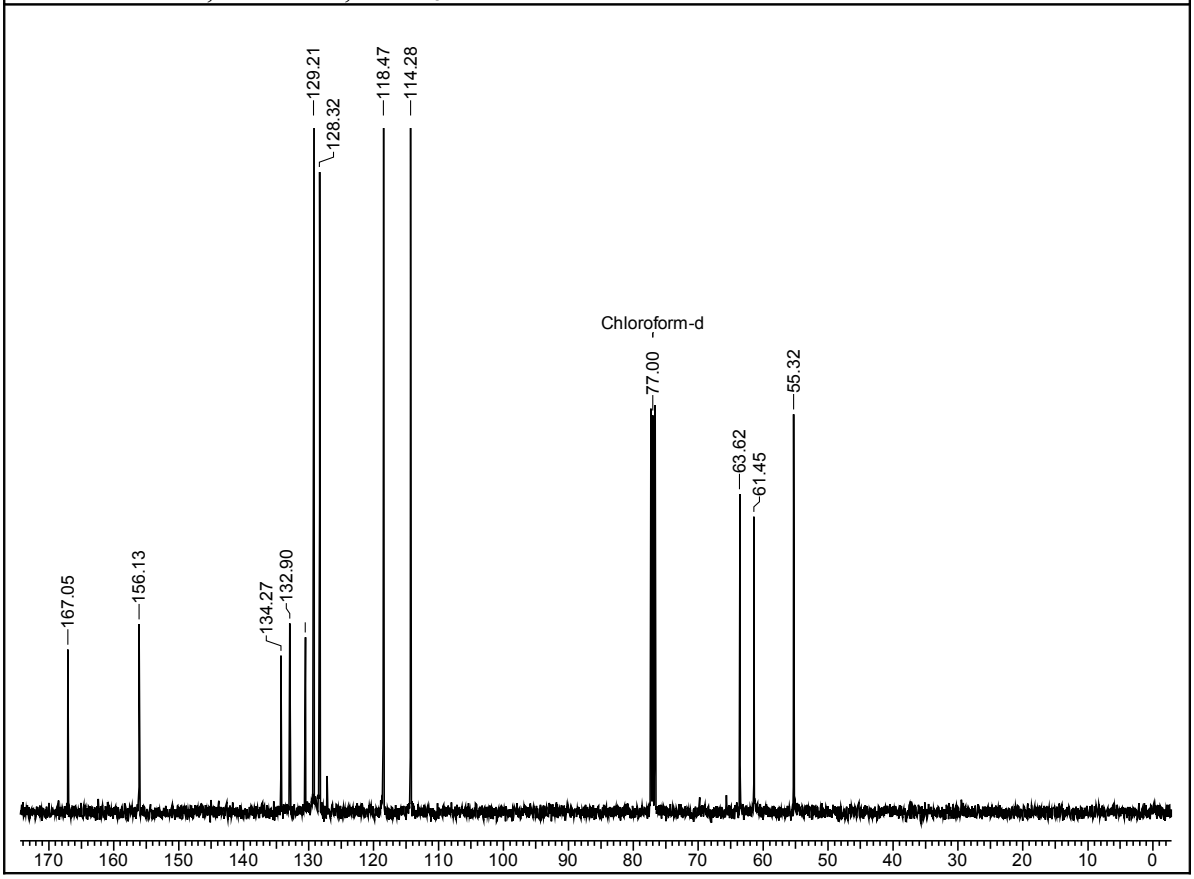
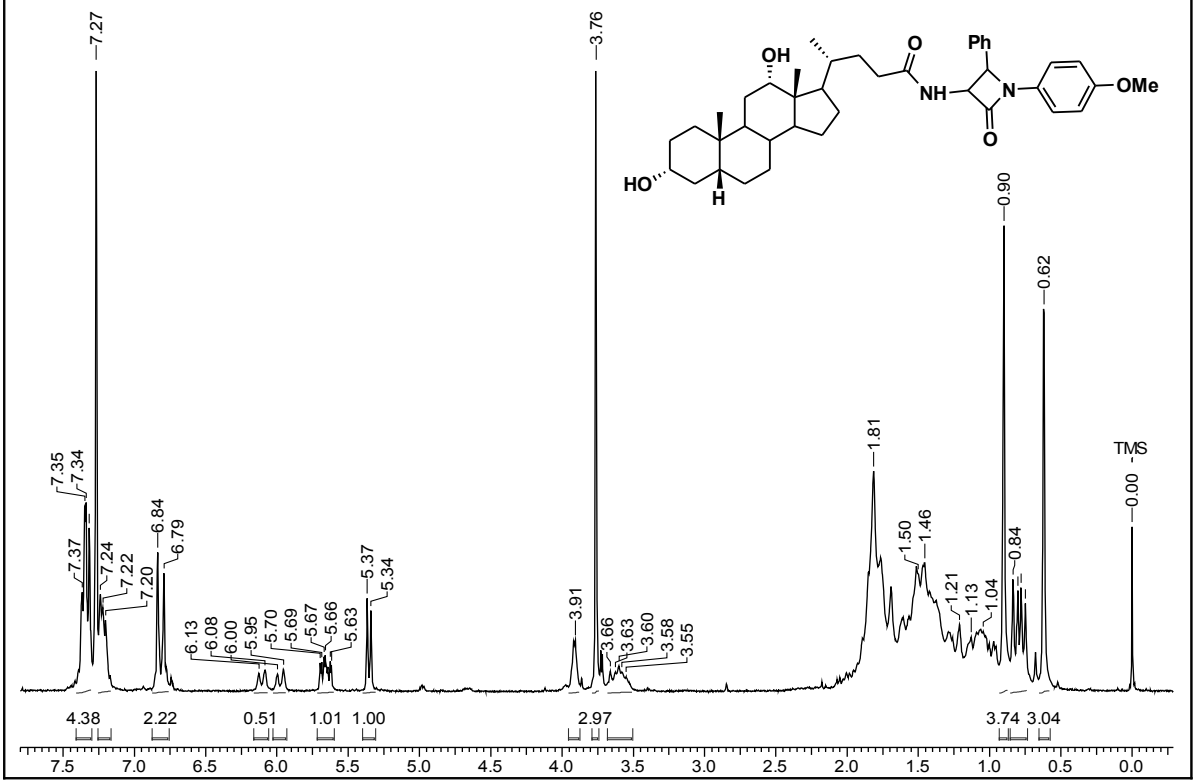


103: ^{1}H NMR, 200 MHz, $CDCl_3$



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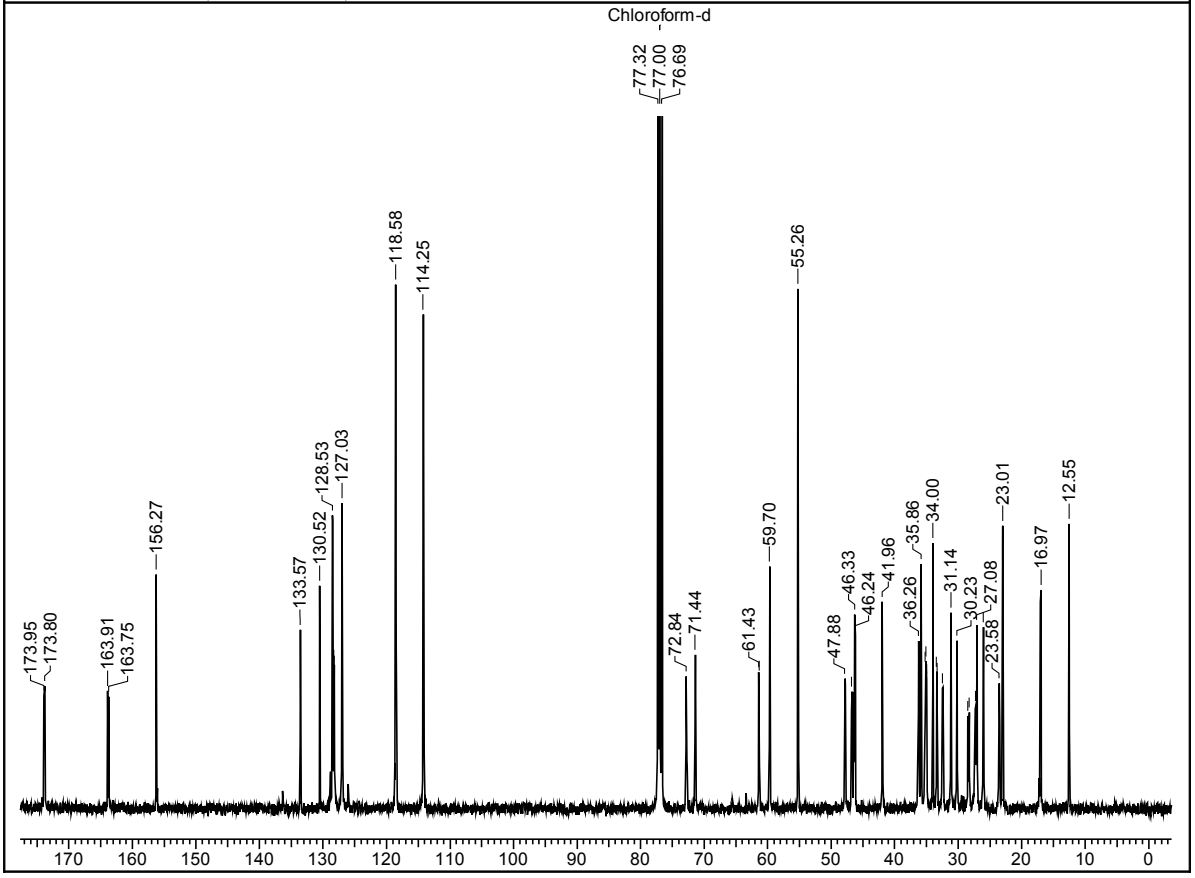

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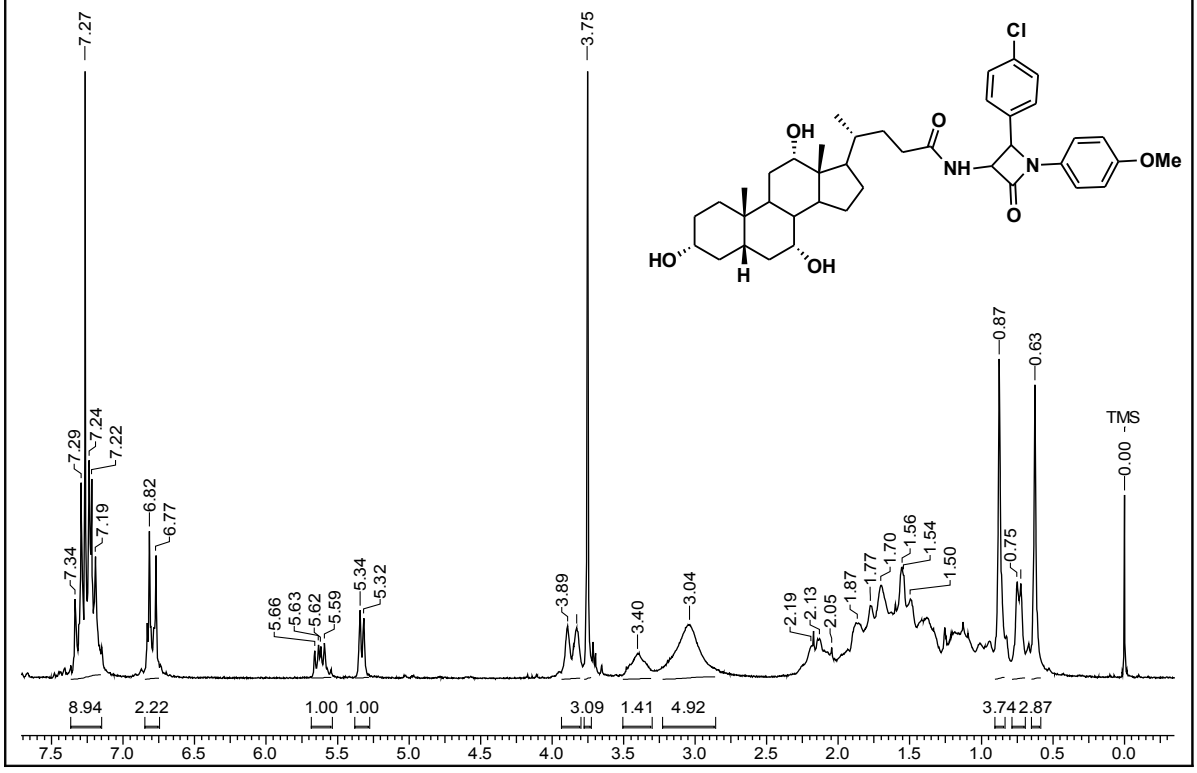

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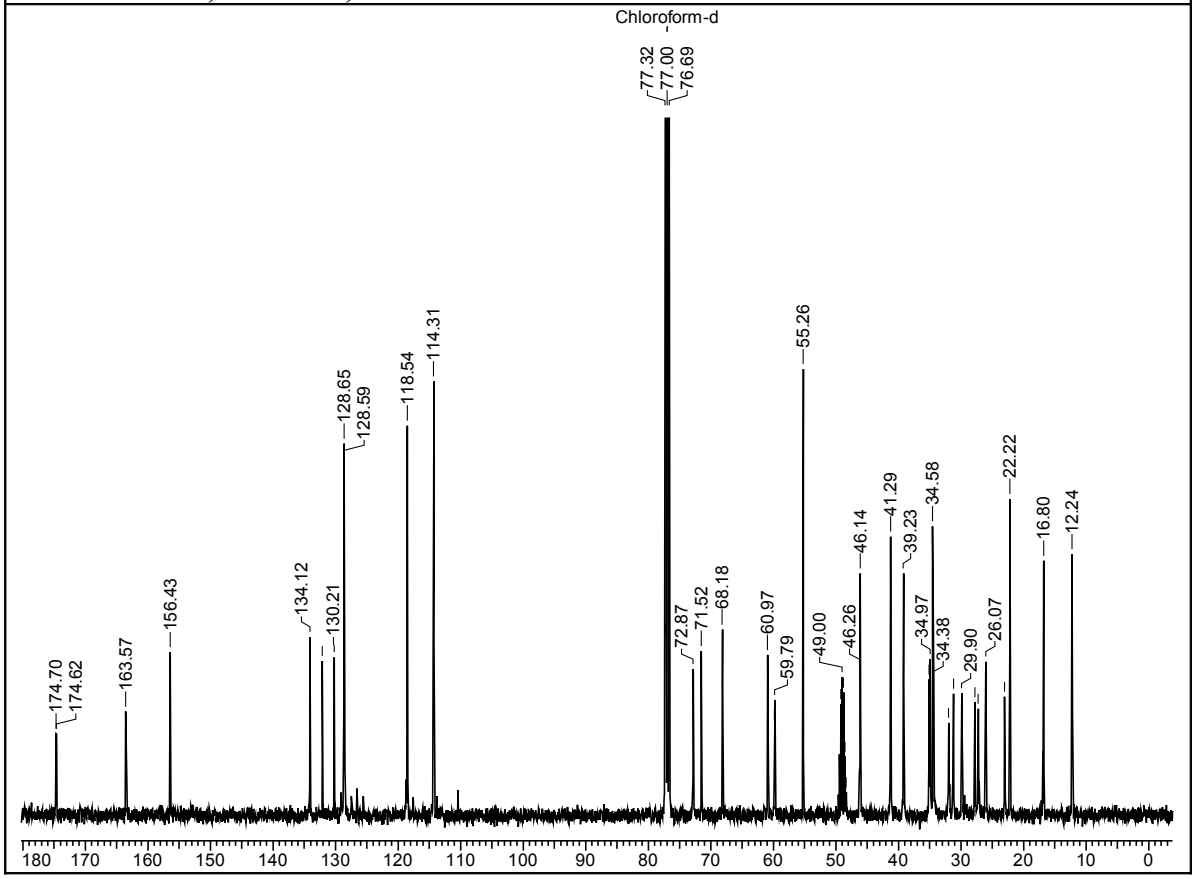

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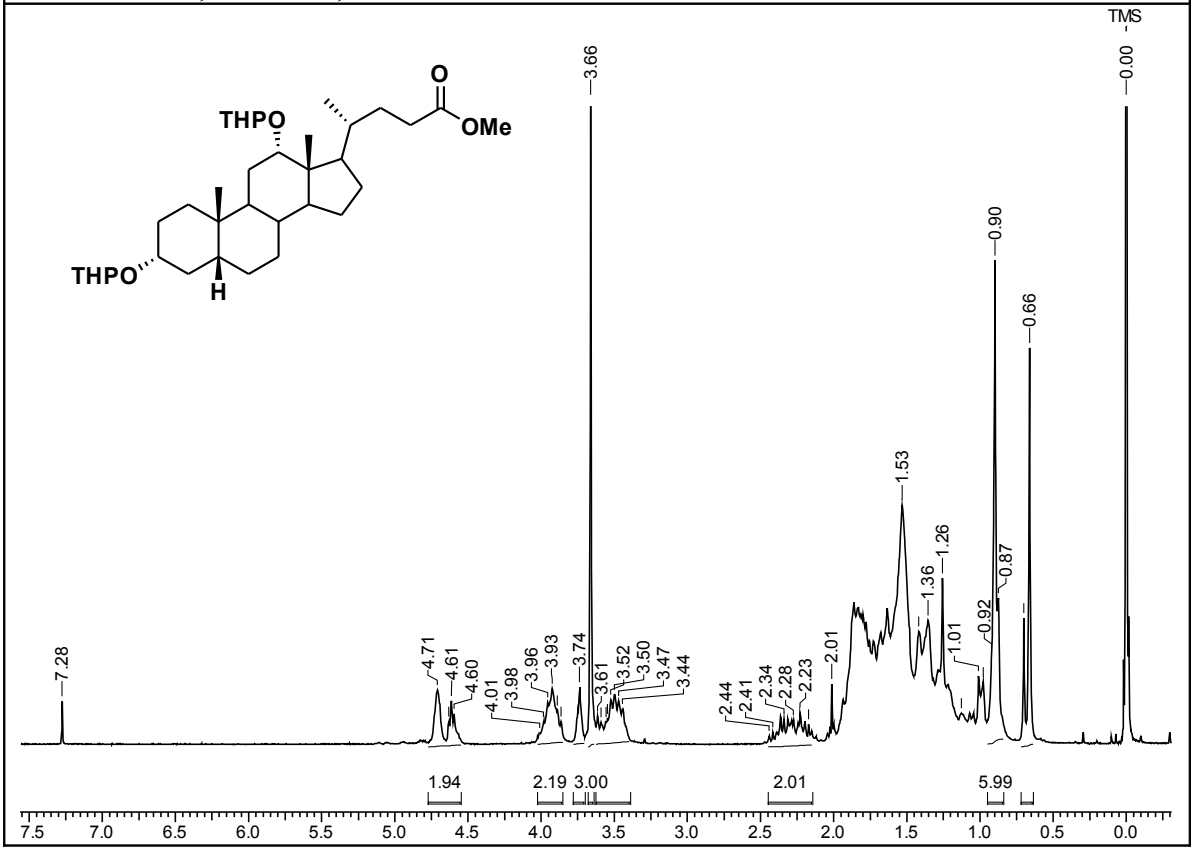

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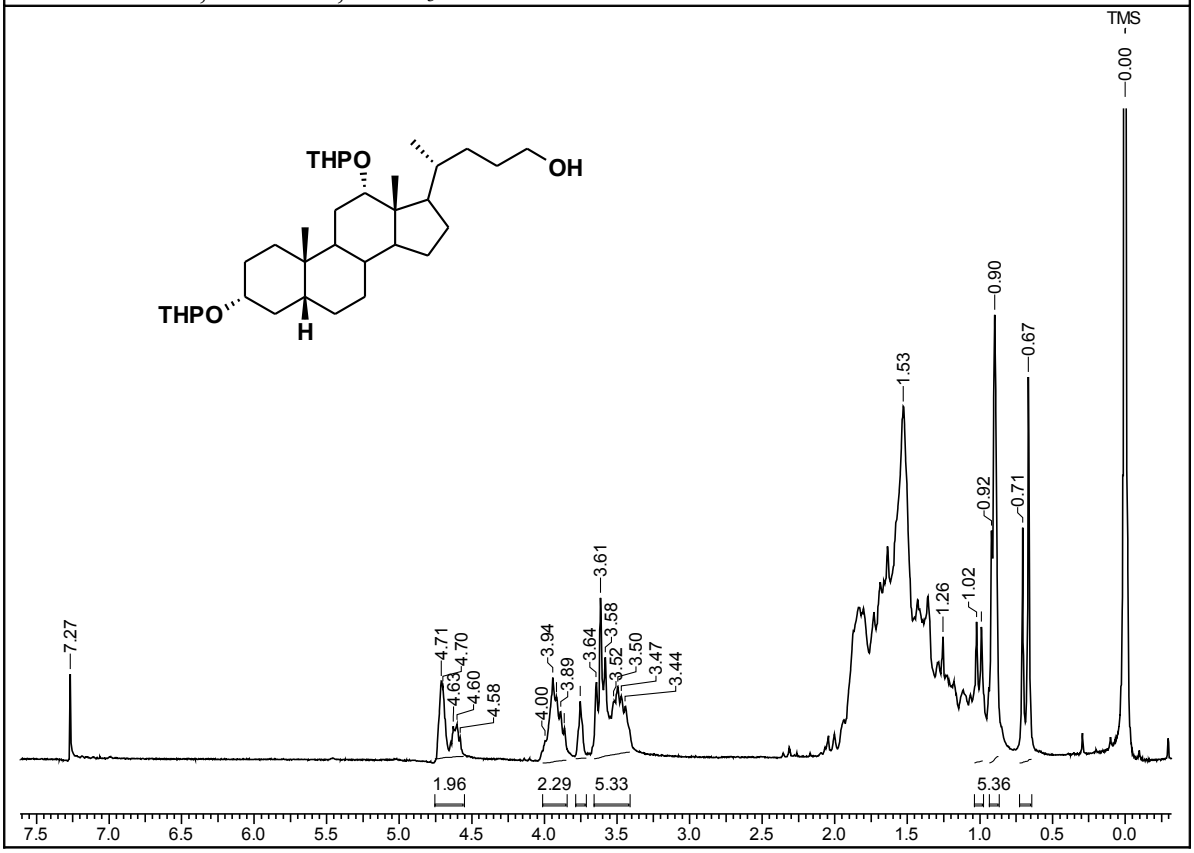

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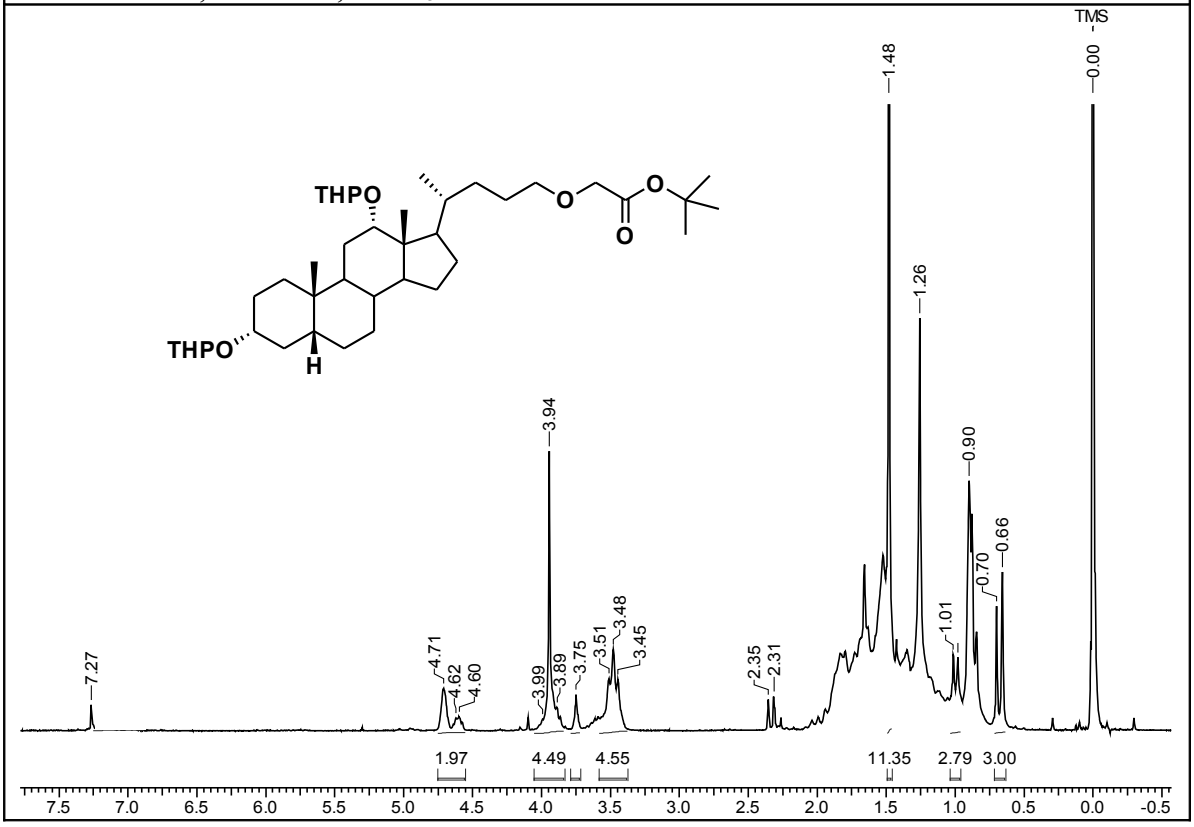
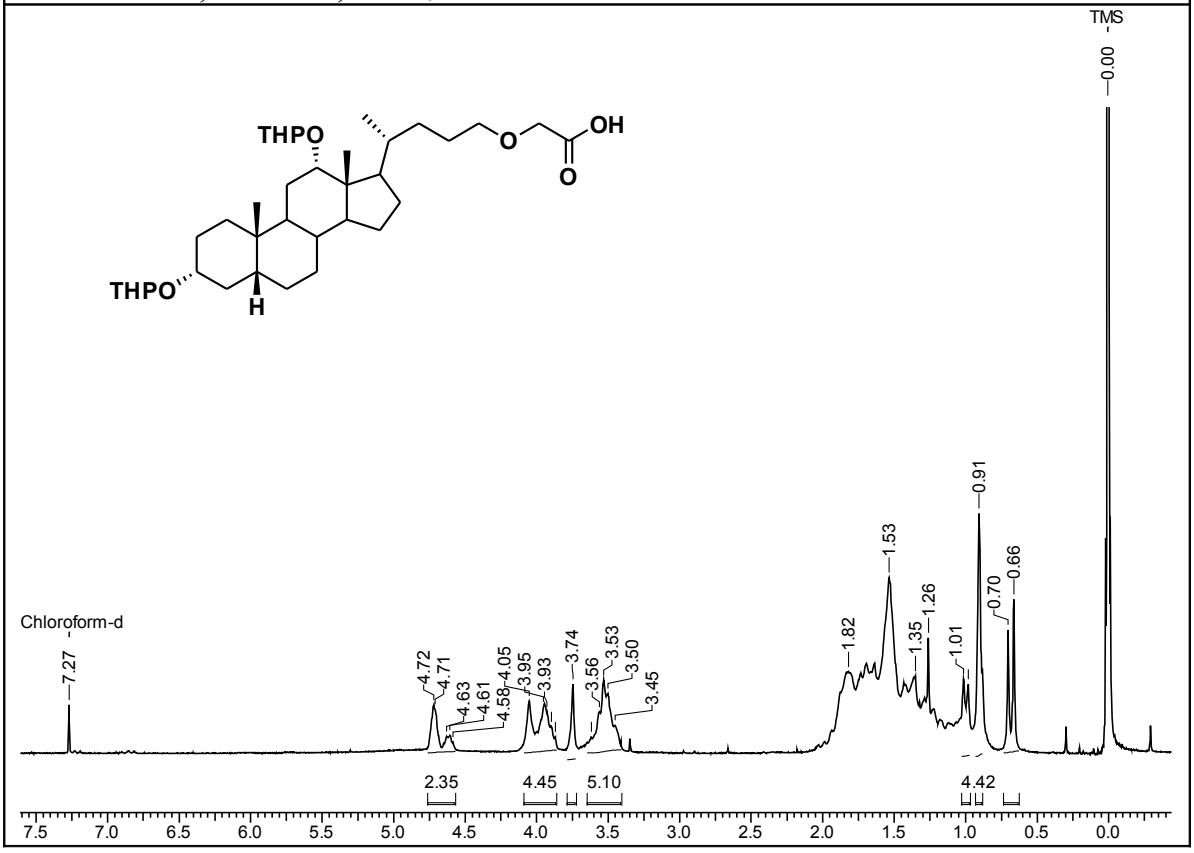
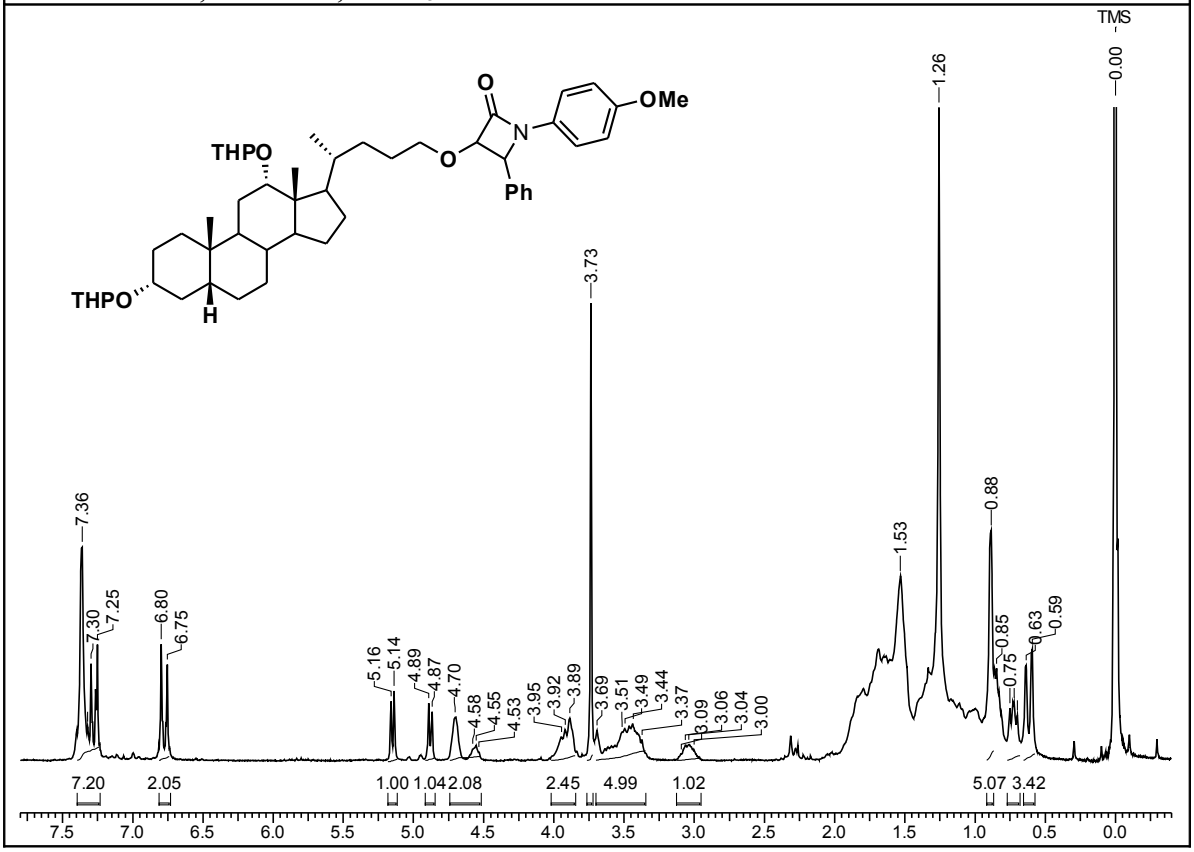


110: ^{1}H NMR, 200 MHz, $CDCl_3$



112: ^{1}H NMR, 200 MHz, $CDCl_3$



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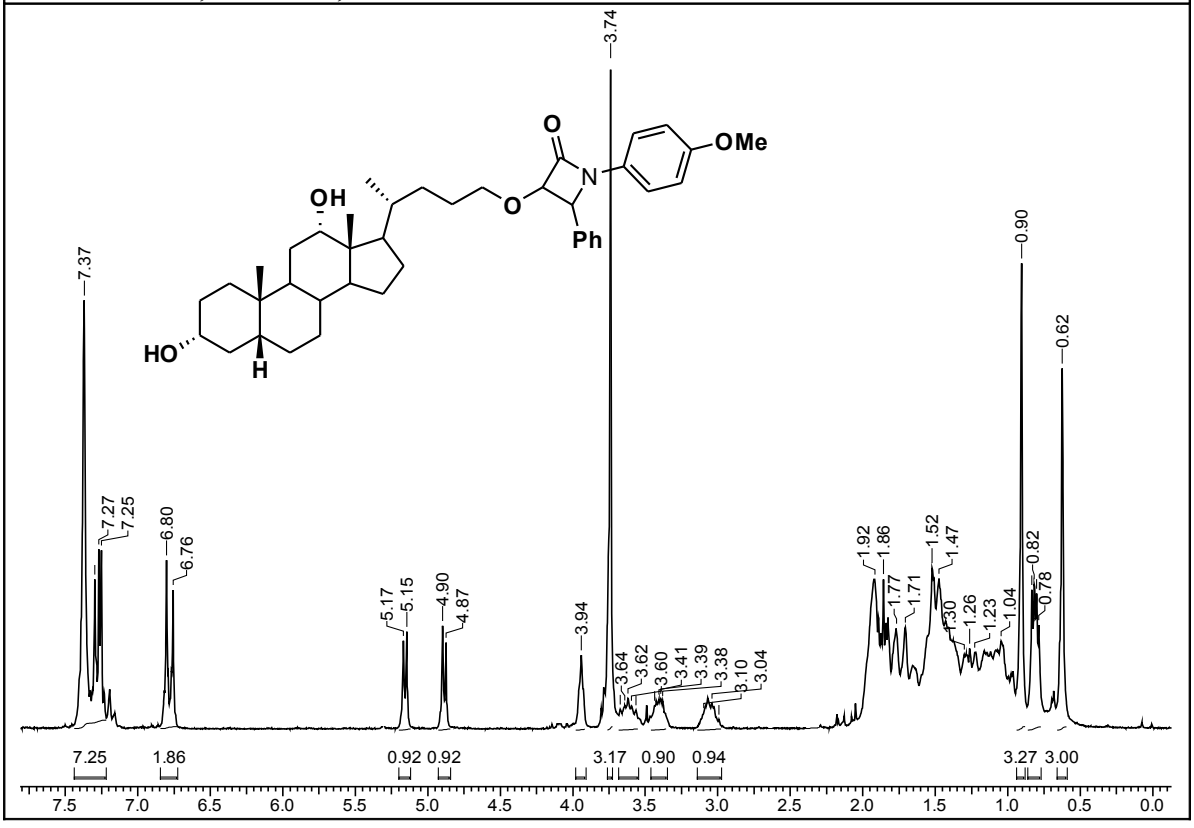
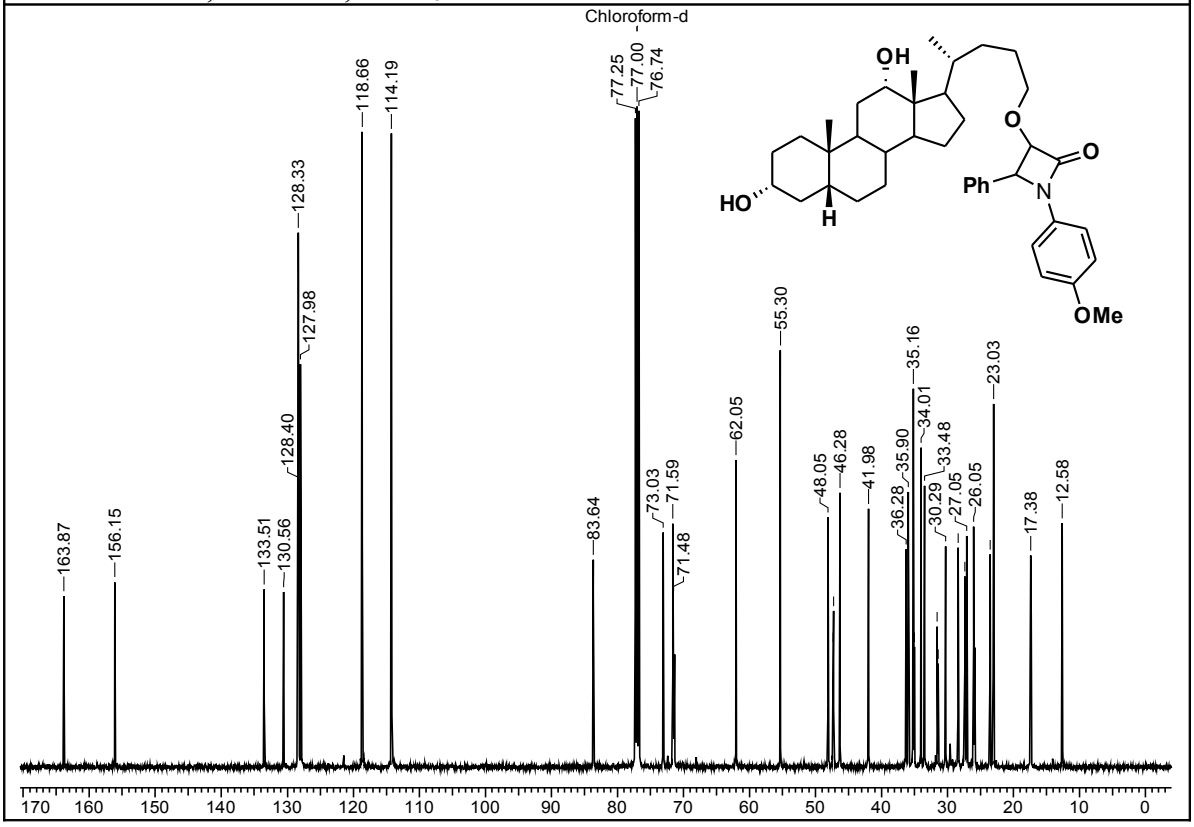
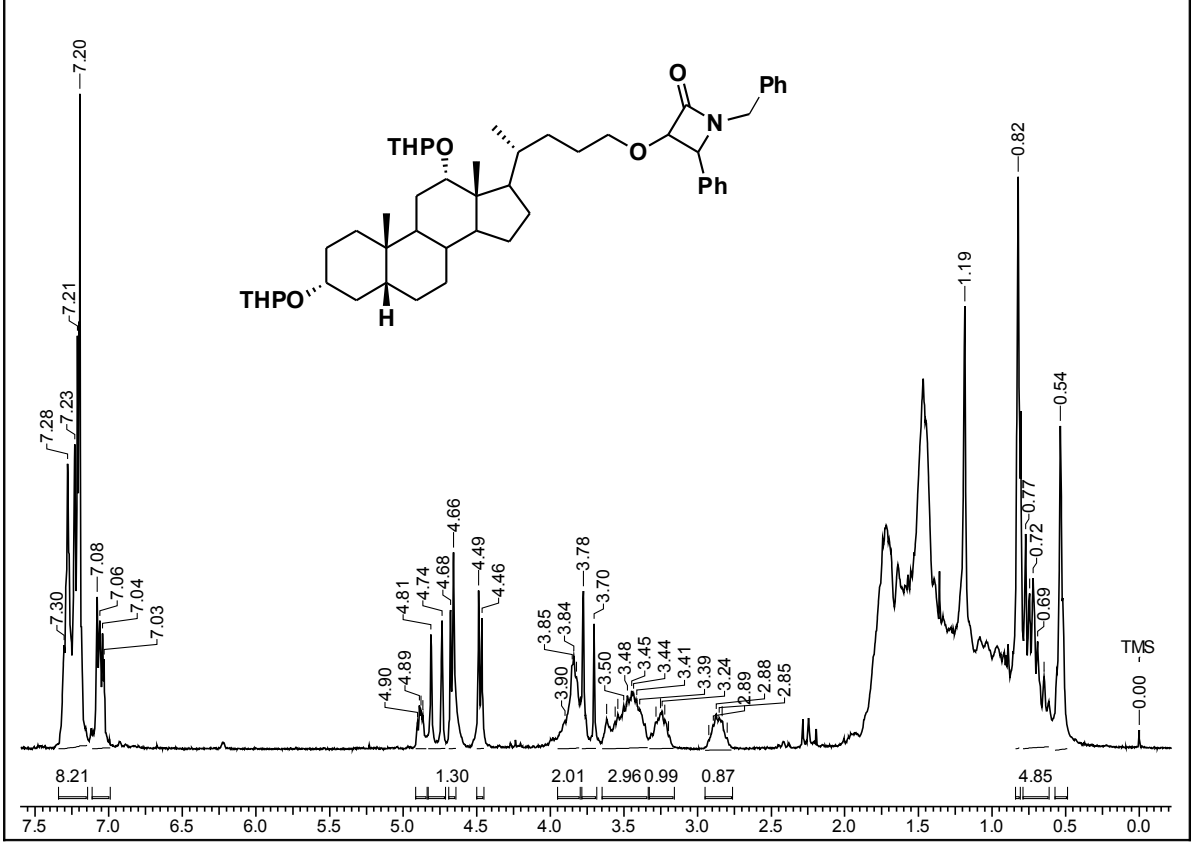


113: ^{13}C NMR, 125 MHz, $CDCl_3$



115: ^{1}H NMR, 200 MHz, $CDCl_3$



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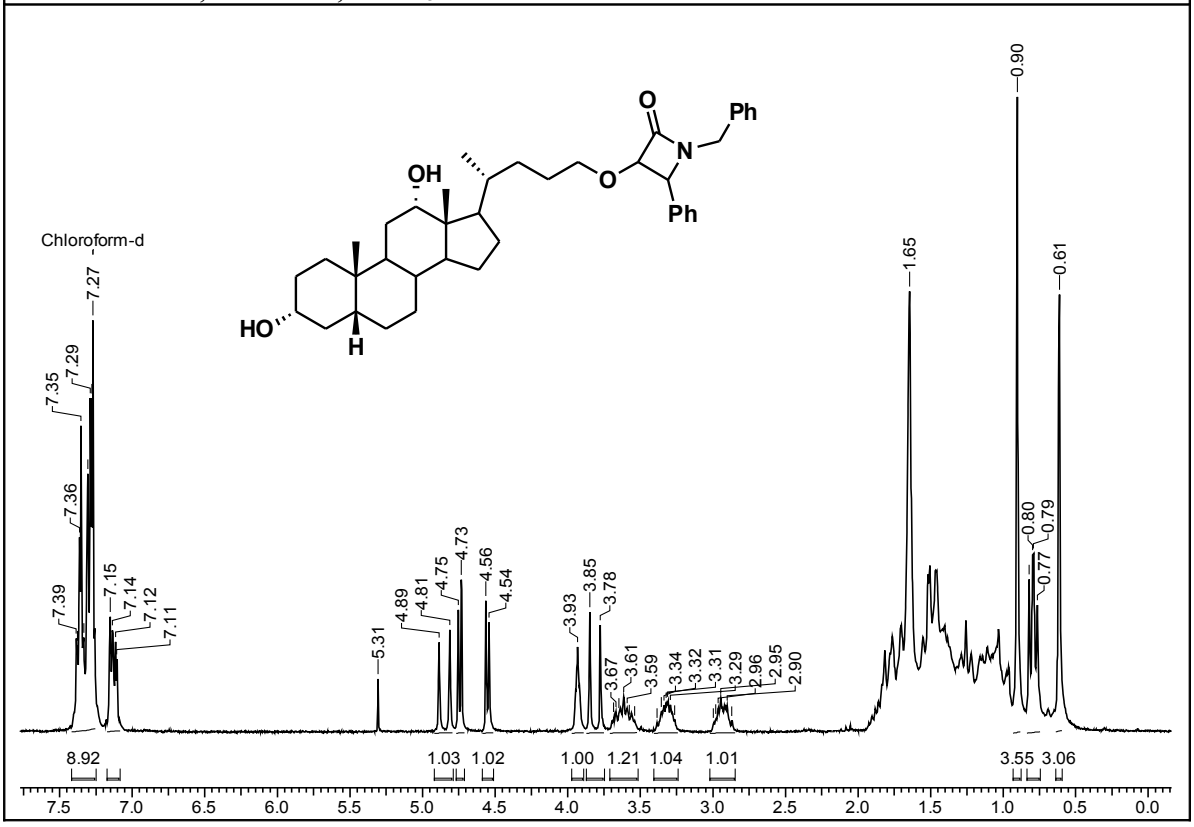
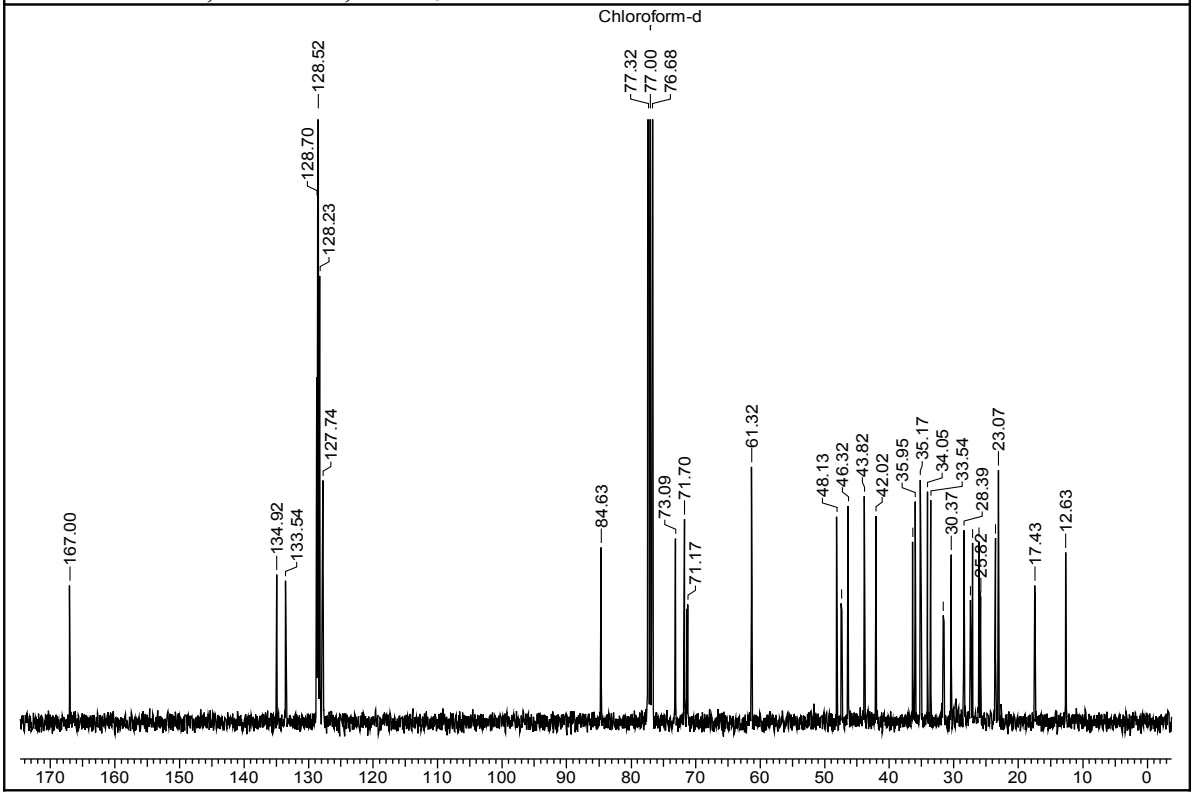


116: ^{13}C NMR, 100 MHz, $CDCl_3$



Section C

Design, Synthesis and Biological Evaluation of Bile Acid Dimers Linked with 1,2,3-Triazole and bis-β-Lactam

1.C.1 Abstract

We report herein the synthesis and biological evaluation of bile acid dimers **153-160** linked through 1,2,3-triazole and bis-β-lactam. The dimers **153-160** were synthesized using 1,3-dipolar cycloaddition reaction of diazido bis-β-lactams **151, 152** and terminal alkynes **75-78** derived from cholic acid/deoxycholic acid in the presence of Cu(I) catalyst (click chemistry). These novel molecules were evaluated in vitro for their antifungal and antibacterial activity. Most of the compounds exhibited significant antifungal as well as antibacterial activity against all the tested fungal and bacterial strains. Moreover, their in vitro cytotoxicities towards HEK-293 and MCF-7 cells were also established.

1.C.2 Introduction

Since the discovery of Japindine **117** (Figure 23), the first example of novel sulphur containing dimeric alkaloid isolated from the root-bark of *Chonemorpha macrophylla*,[81] several examples of bis(steroid) derivatives have appeared in the literature.

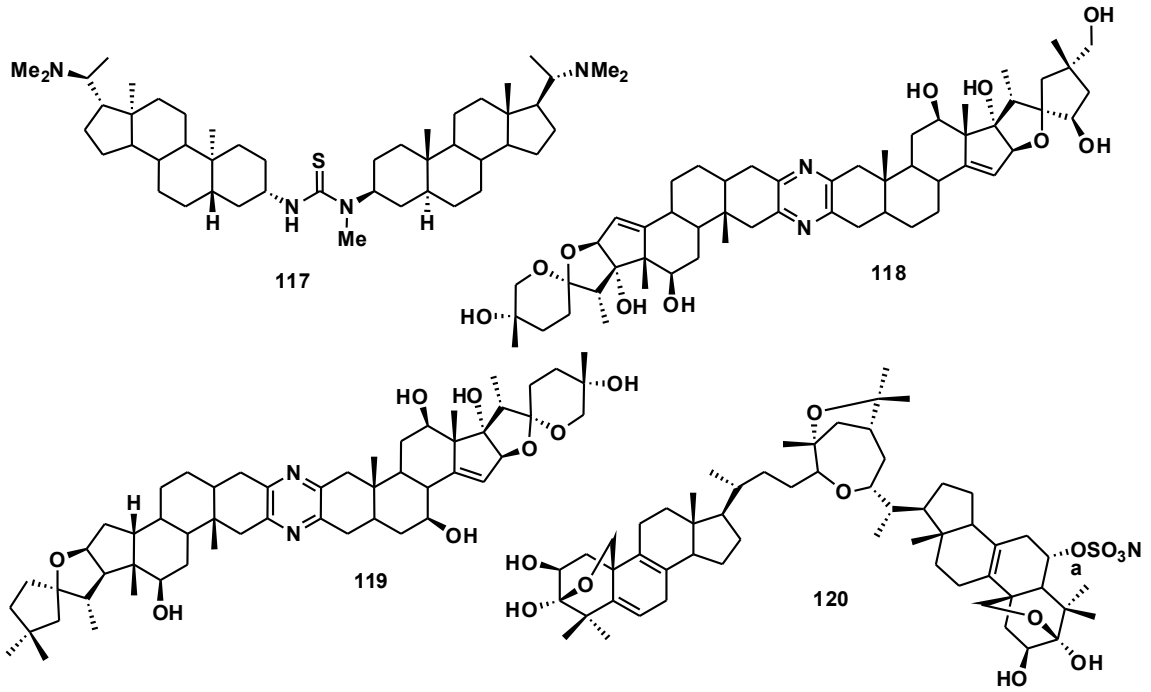


Figure 23

These dimeric and oligomeric steroids possess interesting micellar, detergent, and liquid crystal properties and many of them led to enhance pharmacological activities.[6] Among these the most pertinent with regard to their extraordinary biological activities are cephalostatins **118** and ritterazines **119** (Figure 23).[82] Cephalostatins are a group of complex steroidal pyrazine alkaloids that were isolated from the marine worm *Cephalodiscus gilchrist*.[83] They are powerful cytotoxins against the PS cell line (ED_{50} 10^{-7}-10^{-9} μg/mL) and therefore have potential applications as antitumor agents. However, they are rare marine natural products and are available in only small amounts. Heathcock and co-workers[84] for the first time achieved the synthesis of various analogs of cephalostatins and biological activities of these unsymmetrical bis-steroidal pyrazines was evaluated in the National Cancer Institute's new *in vitro* disease oriented antitumor screen. Recently Haak and co-workers have reported a simple biomimetic route to nonsymmetric pyrazines.[85] Fuchs *et al*[86] reported the first total synthesis of cephalostatin. The dimeric steroidal alkaloid ritterazines **119** are closely related to cephalostatin **118**.

Seketsu and co-workers have examined the cytotoxicity of ritterazine derivatives.[87] Their studies showed that ritterazine A **119** which contains the highest number of hydroxyl groups is the most potent. The activity decreases with decreasing the number of hydroxyl groups. Isolation of crellastatin A **120** (Figure 23)[88] from Vanuatu marine sponge *Crella sp* is the first example of a dimeric steroid connected through its side chains. Crellastatins exhibit *in vitro* antitumor activity against human bronchopulmonary non-small-cell lung carcinoma cell lines (NSCLC) with IC_{50} values in the range of 2-10 μg/mL.

Adopting the concept of dimeric steroids from nature several groups have synthesized various dimers using bile acids, as they are imperative building blocks for the synthesis of dimers, oligomers and colaphanes due to their rigid framework with multiple chiral centers. Such molecules show a wide range of potential applications in supramolecular as well as pharmacological fields.[6]

Kobuke *et al*. have synthesized bile acid based transmembrane ion channels **121** and **122** (Figure 24) by linking two units of amphiphilic cholic acid methyl ethers by biscarbamate.[89] Both the compounds **121** and **122** showed stable single ion channel currents, when incorporated into a planar bilayer membrane. They have synthesized voltage-dependent artificial ion channels **123** and **124** (Figure 24)[90] using cholic acid derivatives, connected through a *m*-xylylene dicarbamate unit at 3-hydroxyl groups. Asymmetries were introduced by terminal hydrophilic groups, carboxylic acid and phosphoric acid for **123** and hydroxyl and carboxylic acid for **124**. They found that these head groups dissociate easily under basic conditions. Compounds **123** and **124** are the first stable single ion channels having a rectification property except peptidic channels. Recently Kobuke and co-workers also synthesized bischolic acid derivatives **125** (Figure 24) linked by *m*-xylylene dicarbamate unit at 3-3'-position and examined their single ion channel properties.[91]

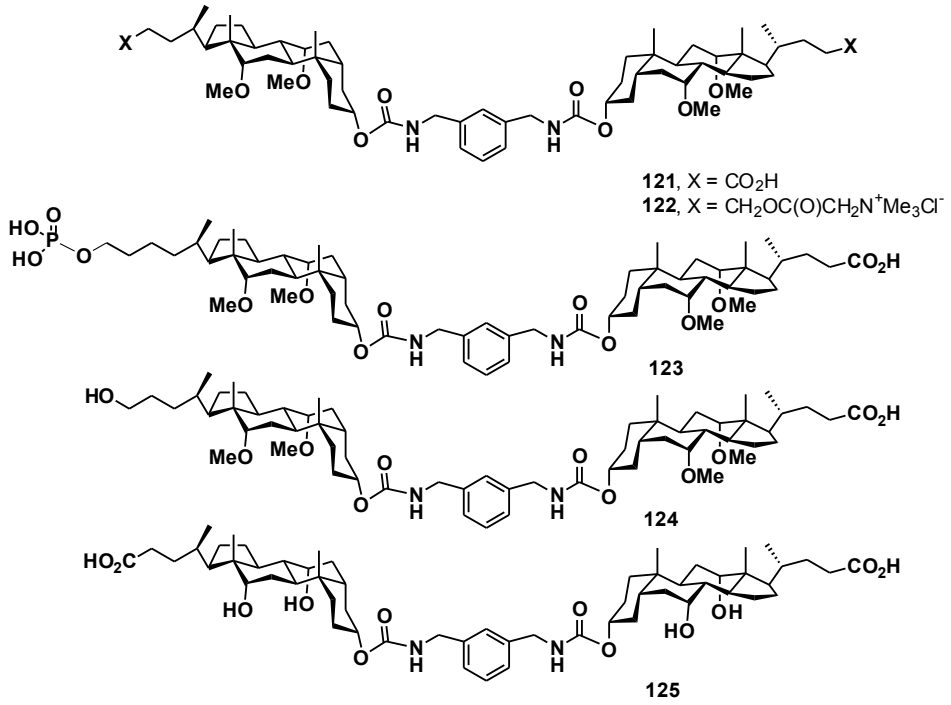


Figure 24

Bile acid based dimers and oligomers have potential applications in the area of drug design and delivery. Janout and co-workers[92] synthesized compound **126** (Figure 25). This type of molecular umbrella can cover an attached agent and shield it from an incompatible environment. Regen and co-workers synthesized[93] persulfated molecular umbrellas **127** as anti-HIV and anti-HSV agents based on the facts that, (i) a di-walled molecular umbrella, bearing three sulfate groups on each of two cholyl moieties, is capable of crossing phospholipid bilayers and (ii) anionic polymers such as dextran/dextrin sulfate and cellulose sulfate are known to inhibit cellular binding of HIV and HSV by competing for viral envelope glycoproteins. Based on the similar concepts, chlorambucil, aromatic nitrogen mustard, has been conjugated to putrescine and spermidine based scaffolds bearing one, two and four persulfated cholic acid units. The Conjugate **128** (Figure 25) bearing two sterols show improved hydrolytic stability and water solubility relative to chlorambucil. Synthesis of a series of molecular umbrella conjugates, derived from cholic acid, deoxycholic acid, spermidine, lysine, and 5-mercapto-2-nitrobenzoic acid have been reported which are capable of transporting an attached 16-mer oligonucleotide (S-dT16) across liposomal membranes.[94]

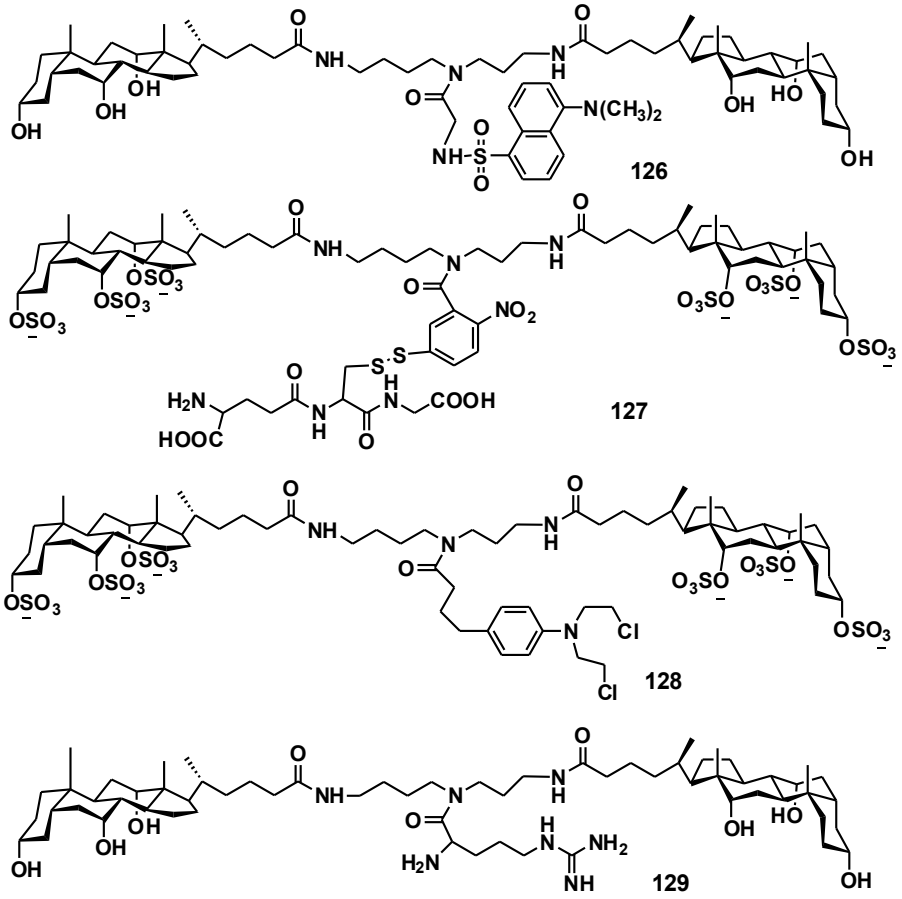


Figure 25

Burrows and Sauter reported synthesis and conformational studies of a new host system **130** (Figure 26) incorporating two molecules of cholic acid linked by a rigid diamine.[95] Proton NMR studies indicated that the compounds exist in a rigid conformation with the steroid hydroxyl groups intramolecularly hydrogen bonded. Heat or addition of methanol leads to conformational isomerism due to insertion of methanol into the cavity. Later, they reported an unusual example of binding of a carbohydrate derivative (amyl glucoside) to a synthetic molecular receptor **131**[96] and DNA binding of steroidal tetramine dimer **132**.[97] McKenna *et al*. synthesized head-to-head dimers **133** and **134** (Figure 26) of cholic acid with a linker at C-24. This type of dimers were found to solubilise perylene in aqueous solution without micelle formation.[98]

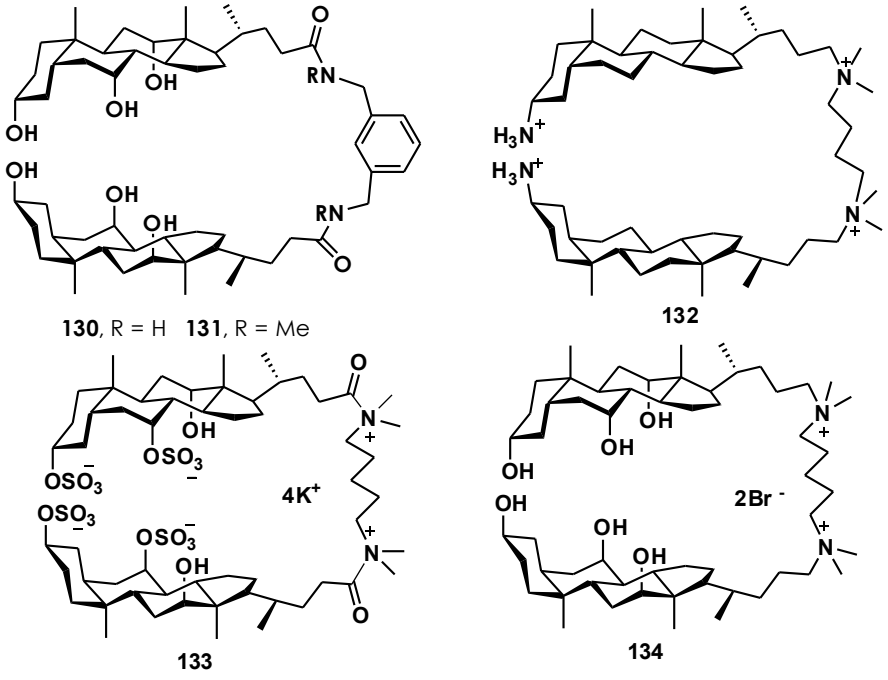


Figure 26

Wess and co-workers synthesized various bile acid dimers and trimers such as **135** and **136** (Figure 27), as bile acid reabsorption inhibitors for potential use in hypercholesterolemia. The interaction of these compounds with the specific ileal bile acid transport system was studied by inhibition of Na^+-dependent taurocholate uptake into ileal brush border membrane vesicles. Compounds **135** and **136** showed strong inhibition. These compounds were further characterized pharmacologically by *in situ* ileal perfusion experiments in rats.[99]

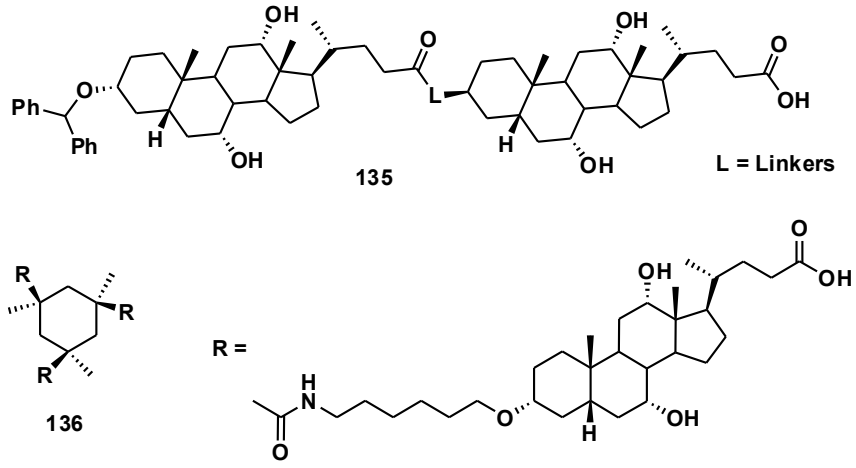


Figure 27

Zhu and co-workers[100] have reported the synthesis of 3α-dimers **137, 138** (Figure 28) of lithocholic acid and cholic acid by forming ester linkages between the C-3 hydroxyl group of bile acids and dicarboxylic acids of different lengths. Also they synthesized the 3β-dimer **139** of lithocholic acid by linking the C-3 positions of two acid units with diethylene glycol by the formation of ether linkages. These dimers have been used in the synthesis of new biodegradable polymers. Later on the synthesis of a sodium cholate dimer **140** by linking two cholic acid molecules via a spacer has been reported by the same group, the synthesized sodium cholate dimer **140** can facilitate the micellization of bile salts.[101]

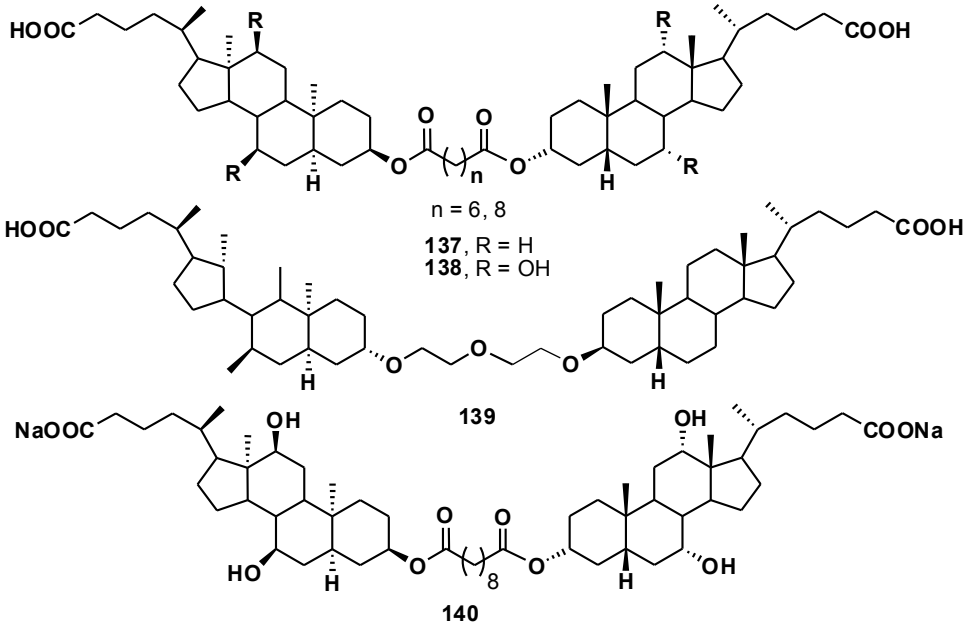


Figure 28

More recently, Yang Li *et al.* have synthesized dimeric bile acid-amino acid conjugates **141-144** (Figure 29) as the mimic of cyclic peptide. These compound shows antitumor activity against human breast cancer cell MCF-7.[102]

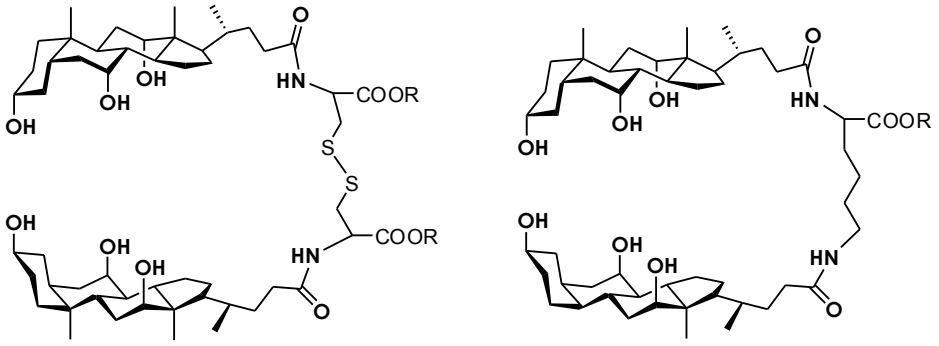


Figure 29

Taking advantage of the amphiphilic topology of bile acids, our group has reported the synthesis of various cholic acids, deoxycholic acid dimers using different linkers e.g. **145-146** (Figure 30).[103] These dimers were found to posses antifungal and antiproliferative activity. Synthesis of novel bile acid dimers **147**, **148** containing 1,2,3-triazole as a linker using click chemistry have been very recently reported form our laboratory.[104] Micellar properties of these molecules were investigated through hydrophilic dye solubilisation studies in non polar media.[105]

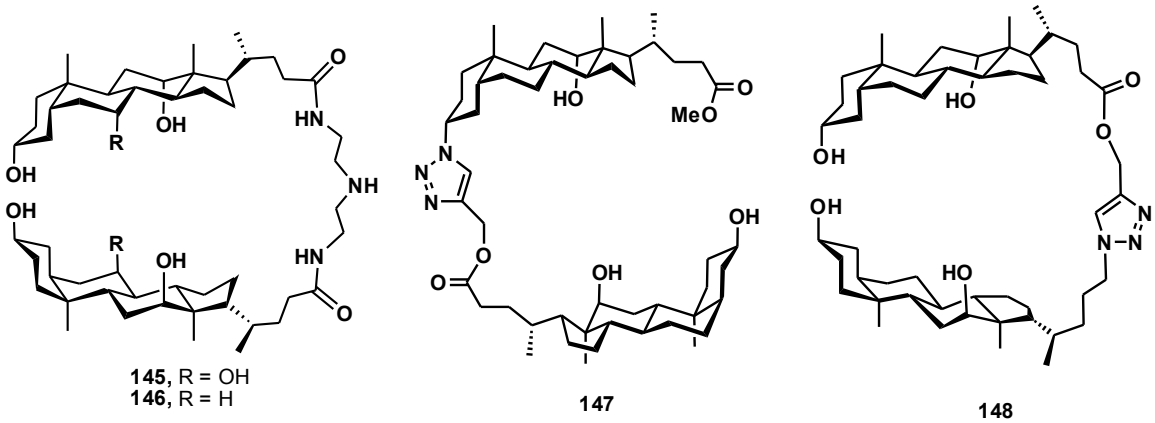


Figure 30

Bile acid dimers also act as artificial ionophores and are found to be potential receptors for neutral molecules or metal cations.[106] There are several reports[107-109] on the synthesis of bile acid dimers and their conversion to cyclophanes. Syntheses of cleft-type bile acid derivatives[110] have been reported in the literature. Dimers at other positions than C-3 and C-24, also have been rarely reported.[111]

1.C.3 Present work

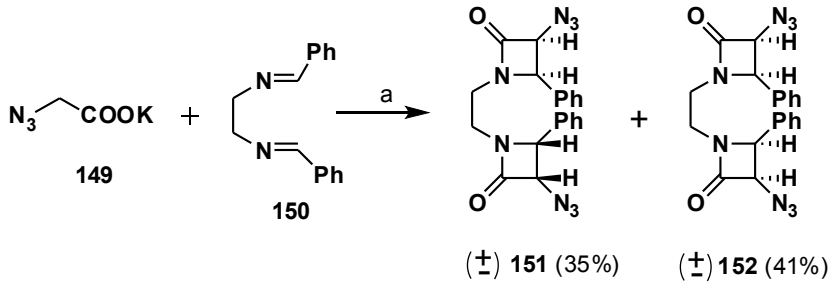
1.C.3.1 Objective

As can be seen from the above discussion, there are several reports on synthesis of bile acid dimers using various linkers. These molecules have vast applications in various fields of chemistry and medicine. Based on the above mentioned observations and in continuation of our work on bile acid dimers, we have designed and synthesized eight novel cholic acid and deoxycholic acid dimers **153-160** using both 1,2,3-triazole and bis-β-lactam as a linker and studied their antimicrobial and cytotoxic activity. Herein for the first time we are using two unique pharmacophore units such as 1,2,3-triazole and β-lactam as a linker for the synthesis of bile acid dimers. The information about the application of 1,2,3-triazole and β-lactam were briefly summarized in the introductory part of this chapter.

1.C.3.2 Results and Discussion

Cu^{I}-catalysed variant of the Huisgen 1,3-dipolar cycloaddition of azide and alkynes to afford regioselectively 1,4-disubstituted 1,2,3-triazoles with such efficiency and scope that the transformation has been described as "click" chemistry.[112] This unique transformation was independently discovered in 2002 by two group one lead by Sharpless[59] in USA and the other in Denmark by Meldal[60]. Accordingly, our target molecules **153-160** were synthesized using 1,3-dipolar cycloaddition reaction of bis-β-lactams **151, 152** containing azide and cholic acids **75, 77** and deoxycholic acids **76, 78** containing terminal alkyne (earlier synthesized in Chapter 1, Section A), in the presence of Cu(I) catalyst (click chemistry). Diazido bis-β-lactams **151** and **152** were prepared by the cycloaddition reaction (Staudinger)[70] using imine **150** and ketene derived from **149**. Thus, treatment of potassium salt of azidoacetic acid **149** and imine **150** (prepared by using literature[113] procedure) in the presence of triphosgene and triethylamine in anhydrous dichloromethane afforded the diastereomeric mixture of two diazido bis-β-lactams (Scheme 10). The mixture on careful flash column chromatographic separation on silica gel furnished diastereomeric compounds **151** and **152** in 35% and 41% yields respectively. The C_2-symmetric structure for bis-β-lactam **151** was assigned from ^{1}H NMR spectral analysis. The ^{1}H NMR spectra of the compound **151** showed two doublets at δ 2.86 and 3.86 with geminal coupling of 11 Hz for the protons of the methylene

groups joining two β-lactam rings. The *meso* structure was assigned to the other diastereomer **152** as the ^{1}H NMR spectra of this compound showed two multiplets at δ 3.11 and 3.60 due to non-equivalence of two methylenes joining two β-lactam rings. The structures of compounds **151** and **152** have been further confirmed unambiguously by X-ray crystal analysis (Figure 31).



Scheme 10. Reagent and conditions: (a) Triphosgene, Et_3N, CH_2Cl_2, 0-25 °C, 15 h, (35% for **151** and 41% for **152**).

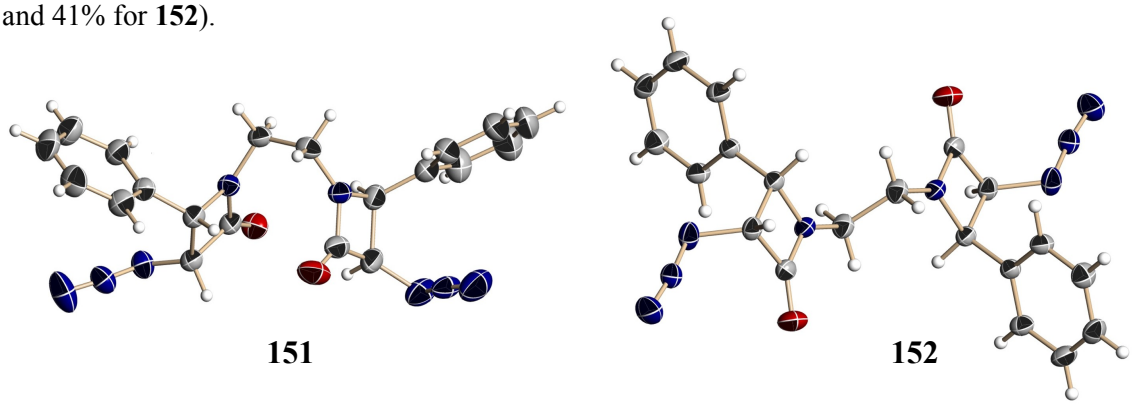
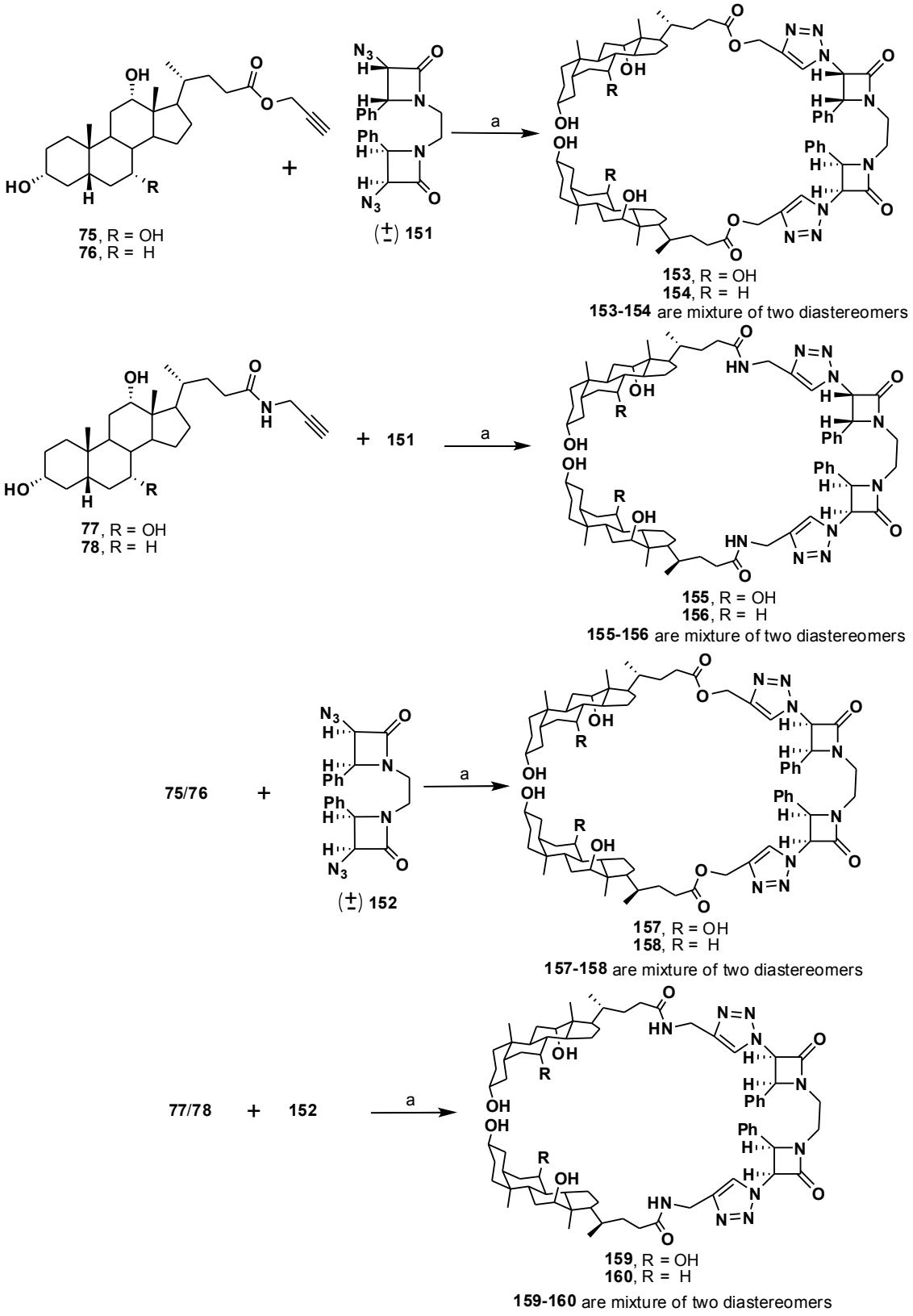


Figure 31. ORTEP view of compound **151** and **152**

Our next goal was to synthesise targeted cholic acid/deoxycholic acid dimers **153-160** linked with 1,2,3-triazole and bis-β-lactam. Reaction of propargyl ester **76** and bis-β-lactams **152** in *t*-BuOH/H_2O (7:3) with $CuSO_4{\cdot}5H_2O$, sodium ascorbate (click chemistry) at 70 °C for 32 h afforded the deoxycholic acid dimer linked with 1,2,3-triazole and bis-β-lactam **158** in 81% yield (Scheme 11). The formation of the dimer **158** was confirmed by ^{1}H-NMR which showed characteristic signals at δ 7.62 (s, 2H) due to triazole protons, at δ 5.0 (s, 4H) corresponding to the methylene protons (CH_2-O). Also, ^{13}C NMR showed characteristic signals at δ 173.6, 163.1, 124.5 and 124.1 corresponding to the ester carbonyl, β-lactam carbonyl and two triazole carbons respectively. Further its IR spectrum shows absorption bands at 3442 cm^{-1} due to hydroxy group, at 1766 cm^{-1} and 1731 cm^{-1} due to β-lactam carbonyl and ester carbonyl group. Finally the formation of dimer **158** was confirmed by mass spectral analysis {MS (LCMS) *m/z* 1285.2 $[M+Na]^+$}.



Scheme 11. Reagent and conditions: (a) Sodium ascorbate, $CuSO_4{\cdot}5H_2O$, DMF/H_2O (7:3), microwave (385 watt), 5 min, 92-95%.

The cycloaddition reaction of propargyl esters **75**, **76** and amides **77, 78** derived from cholic acid/deoxycholic acid with diazido bis-β-lactam **151** in DMF/H_2O (7:3) and $CuSO_4{\cdot}5H_2O$, sodium ascorbate under microwave[112] irradiation for five minutes furnished diasteriomeric mixture of novel dimers **153-156** in 93-95% yields. It has been well established that reaction under microwave condition substantially decreased[114] the reaction time down to few minutes as compared to several hours refluxing under thermal conditions. In a similar way, microwave irradiation of propargyl esters **75**, **76** and amides **77, 78** with azido bis-β-lactams **152** afforded diasteriomeric mixture of dimeric compounds **157-160** in 92 to 94% yields. The combination of racemic core **151** and **152** with optically pure **75-78** afforded diastereomeric mixture of dimers **153-160** in equal amounts. These diastereomers **153-160** were inseparable by flash column chromatography and also by crystallization. All the dimers **153-160** were fully characterized by spectroscopic data.

1.C.4 Bioevaluation

Antimicrobial Activity. All the newly synthesized dimers **153-160** were tested *in vitro* for antifungal and antibacterial activity. The antifungal activity was tested using fungal strains *Candida albicans, Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The MIC and IC_{50} values were determined using standard broth microdilution technique described by NCCLS.[73] In comparison with the antimicrobial activity, amphotericin B and fluconazole were used as the reference antifungal agents, while tetracycline and ampicillin were used as the reference antibacterial agents. All the biological data of the tested compounds are depicted in Table 1 as MIC and IC_{50} values.

As seen in Table 1 most of the synthesised dimers **153-160** showed moderate to good antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compounds **155** and **159** was higher to that of fluconazole against *C. albicans* with MIC value of 16-25 μg/mL. The compounds **155** and **157-160** showed good antifungal activity against *C. neoformans* having MIC value of 16-36 μg/mL higher

Table 1. *In vitro* antimicrobial activity of compounds **153-160.**

Comp.	Inhibitory concentration (μg/mL)													
	Fungal Strains										Bacterial Strains			
	CA		CN		BP		YL		FO		EC		SA	
	MIC[a]	IC_{50}[b]	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
153	32	12	51	11	17	2	>128	32	19	9	16	10	47	11
154	64	33	>128	38	44	10	14	8	16	9	>128	57	37	17
155	16	8	18	5	32	21	35	16	25	8	22	9	16	7
156	>128	35	>128	43	43	9	>128	64	75	42	67	28	>128	53
157	85	17	17	4	94	36	10	6	64	42	19	7	32	16
158	64	41	32	4	41	12	8	4	31	10	47	19	64	29
159	25	10	36	11	12	4	16	4	27	8	80	29	77	22
160	67	29	16	6	41	15	17	8	32	10	18	11	>128	64
Amp	2	0.5	27	10	23	8	15	9	16	8	-	-	-	-
Flue	28	4	43	22	39	17	78	36	8	4	-	-	-	-
Tetr	-	-	-	-	-	-	-	-	-	-	9	3	16	8
Am	-	-	-	-	-	-	-	-	-	-	2	1	14	4

CA**,** *C. albicans* (values were recorded after 48 h); CN**,** *C. neoformans* (values were recorded after 72 h); BP**,** *B. poitrasii*; YL**,** *Y. lipolytica* (values were recorded after 24 h); FO**,** *F. oxysporum* (values were recorded after 48 h); EC**,** *E. coli*; SA**,** *S. aureus* (values were recorded after 24 h). Amp, Amphotericin B; Flu, Fluconazole; Tetr, Tetracycline; Am, Ampicillin

[a]MIC (Minimum inhibitory concentration) was determined as 90% inhibition of growth with respect to the growth control.

[b]IC_{50} was the concentration at which 50% growth inhibition was observed.

Negative control, DMSO (2.5% v/v), No inhibition. "-" Not tested

or comparable to that of reference drug fluconazole. However, all the compounds **153-160** except **157** showed significant growth inhibitory activity against *B. poitrasii. Y. lipolytica* was adversely affected by **154**, **155** and **157-160**, and in particular, **157** and **158** was the most potent with a low MIC value of 8-10 μg/mL. The compound **154** showed significant inhibitory effect with MIC value of 16 μg/mL comparable to that of amphotericin B against *F. oxysporum*, where as compounds **153**, **155** and **159** also showed promising activity against *F. oxysporum*. Furthermore, among the dimers **153-160** none of the compound showed more or comparable antibacterial activity against *E.*

coli than tetracycline or ampicillin, the compounds **153**, **155, 157** and **160** were found to be moderately active with a MIC value of 16-22 μg/mL. However, only the compound **155** showed the comparable activity to that of tetracycline and ampicillin against *S. aureus* with MIC value of 16 μg/mL. From the overall activity results, it was observed that the ester or amide linkage did not affect the activity of the compounds.

Table 2. Cytotoxicity of compounds **153-160**

Compound	IC_{50} (μM)	
	HEK293	MCF-7
153	10	80
154	>1000	>1000
155	100	>1000
156	500	>1000
157	500	1000
158	>1000	>1000
159	100	>1000
160	>1000	>1000

Cytotoxic Activity. The cytotoxicity of all the dimers **153-160** was assessed *in vitro* against human embryonic kidney (HEK293) and human mammary adenocarcinoma (MCF-7) cell lines. Cholic acid and deoxycholic acid derivatives are known to promote proliferation and metastasis of cells of cancer origin and inhibit the proliferation of cells of non-cancer origin.[115] Hence we tested the cytotoxicity of the synthesized compounds in two different cell lines, one of cancer origin and the other of non-cancer origin. HEK293 cells are normal human embryonic kidney cells where as MCF-7 cells are human breast cancer derived. The cytotoxicity of the compounds **153-160** against HEK293 and MCF-7 cells was evaluated using MTT assay.[116] The observed IC_{50} values of all the evaluated compounds are shown in Table 2. Among them, compound **153** was most toxic to both the cell lines with IC_{50} values of 10 and 80 μM and rest of the compounds did not show any cytotoxicity up to 500 μM concentration to both the cell lines. Furthermore, it was observed that compounds **154**, **155**, **158** and **160** enhanced the proliferation of MCF-7 cells and not HEK293 cells.

1.C.5 Conclusion

A series of novel bile acid dimers **153-160** linked through 1,2,3-triazole and bis-β-lactam have been synthesized using Cu(I) catalysed cycloaddition reaction (click chemistry) of diazido bis-β-lactams **151-152** and terminal alkynes **75-78** derived from cholic acid/deoxycholic acid in excellent yield. These novel dimers **153-160** were evaluated for antifungal as well as antibacterial and cytotoxic activities. Most of the compounds demonstrated potent antimicrobial activity against all the strains tested. Among them, compounds **155** and **159** showed significant antifungal activity against human pathogen, *C. albicans* and compounds **155**, **157** and **160** showed appreciable antifungal activity against *C. neoformans* than reference drug fluconazole. In case of plant pathogen, *F. oxysporum* the compound **154** showed comparable inhibitory activity to amphotericin B. In particular, compounds **157** and **158** exhibited the most significant activity against *Y. lipolitica* with MIC value of 8-10 μg/mL. Compounds **153**, **155** and **157** derived from cholic acid were moderately active against *E. coli*. Additionally, only compound **155** exhibited comparable activity against *S. aureus* to that of reference drugs. Furthermore, except compound **153**, all other compounds (**154-160**) did not show any significant cytotoxicity to the tested cell lines. The synthesis of bile acid dimers linked with two pharmacophores, 1,2,3-triazole and β-lactam can open as new horizon for the control of human and plant pathogens.

1.C.6 Experimental

Procedure for Synthesis of β-Lactams (151) and (152):

A solution of triphosgene (0.741 g, 2.5 mmol), in anhydrous CH_2Cl_2 (20 mL), was added slowly to a mixture of potassium salt of azidoacetic acid **149** (0.695 g, 5 mmol), imine **150** (0.472 g, 2 mmol) and triethylamine (2 mL, 15 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 ºC. After the addition, the reaction mixture was allowed to warm up to room temperature (25 ºC) and stirred for 15 h. The reaction mixture was then washed with water (25 mL), saturated sodium bicarbonate solution (2×20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated to get the crude product of diasteriomeric mixture. Flash coloumn chromatographic purification over silica gel using ethyl acetate-petroleum ether (12:88) as an eluent afforded pure β-lactam

151 (0.281 g, 35%). On further elution with same solvent system yielded pure β-lactam **152** (0.329 g, 41%).

1,2-Bis[3′-azido-4′-phenylazetidin-2′-one-1′-yl]ethane (151):

Mp: 123-124 ºC; IR ($CHCl_3$, cm^{-1}) 2115 (N_3), 1762 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.46 -7.21 (m, 10H, Ar-H), 5.17 (d, *J* = 5.1, 2H), 4.91 (d, *J* = 5.1, 2H), 3.84 (d, *J* = 11.2, 2H), 2.86 (d, *J* = 11.2, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.8, 132.6, 129.1, 128.8, 127.7, 68.6, 59.9, 37.8. MS (LCMS) *m/z*: 425 (M + Na); Anal. Calcd for $C_{20}H_{18}N_8O_2$: C, 59.69; H, 4.51; N, 27.85. Found: C, 59.87; H, 4.62; N, 28.09.

1-[3′-azido-4′-phenylazetidin-2′-one-1′-yl]-2-[3″-azido-4″-phenylazetidin-2″-one-1″-yl]ethane (152):

Mp: 162-163 ºC; IR ($CHCl_3$, cm^{-1}) 2115 (N_3), 1762 (CO); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.48-7.28 (m, 10H, Ar-H), 4.79 (d, *J* = 4.9, 2H), 4.74 (d, *J* = 4.9, 2H), 3.55-3.69 (m, 2H), 3.04-3.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 164.7, 132.3, 129.3, 128.9, 127.9, 68.1, 60.9, 38.6. MS (LCMS) *m/z*: 425 (M + Na); Anal. Calcd for $C_{20}H_{18}N_8O_2$: C, 59.69; H, H, 4.51; N, 27.85. Found: C, 59.57; H, 4.29; N, 28.13.

X-ray crystal structure determination for (151) and (152):

Single crystal of compounds **151** and **152** were obtained from ethyl acetate –light petroleum ether mixture (30:70**)**. X-ray diffraction data were collected on a Bruker SMART APEX CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at room temperature (297 K).

Crystallographic data for (151): ($C_{20}H_{18}N_8O_2$): *M* = 402.42, Crystal dimensions 0.58 x 0.32 x 0.17 mm^3, monoclinic, space group *P 2₁/c*, *a* = 13.838(11), *b* = 6.873(6), *c* = 22.068(19) Å, β = 96.710(15)°; *V* = 2085(3) $Å^3$; *Z* = 4; ρ_{calcd} = 1.282 gcm^{-3}, μ (Mo-K_α) = 0.089 mm^{-1}, *F(000)* = 840, $2\theta_{max}$ = 50.00°, 9849 reflections collected, 3667 unique, 2786 observed ($I > 2\sigma(I)$) reflections, 298 refined parameters, *R* value 0.0541, *wR*2 = 0.1327 (all data *R* = 0.0699, *wR*2 = 0.1433), S = 1.068, minimum and maximum transmission 0.9503 and 0.9851; maximum and minimum residual electron densities 0.155 and –0.142 e $Å^{-3}$.

Crystallographic data for (152): ($C_{20}H_{18}N_8O_2$): M = 402.42, Crystal dimensions 0.78 x 0.15 x 0.13 mm^3, monoclinic, space group $P\ 2_1/c$, a = 13.196(8), b = 5.751(4), c = 13.632(8) Å, β = 109.453(9)°; V = 975.4(10) $Å^3$; Z = 2; ρ_{calcd} = 1.370 gcm^{-3}, μ (Mo-K_α) = 0.095 mm^{-1}, *F(000)* = 420, $2\theta_{max}$ = 50.00°, 8813 reflections collected, 1715 unique, 1538 observed ($I > 2\sigma(I)$) reflections, 136 refined parameters, R value 0.0359, $wR2$ = 0.0896 (all data R = 0.0399, $wR2$ = 0.0930), S = 1.070, minimum and maximum transmission 0.9296 and 0.9878; maximum and minimum residual electron densities 0.149 and –0.128 e $Å^{-3}$.

All the data were corrected for Lorentzian, polarization and absorption effects using Bruker's SAINT and SADABS programs. SHELX-97 (G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997) was used for structure solution and full-matrix least-squares refinement on F^2. Hydrogen atoms for both the structures were placed in a geometrically idealized position with C-H =0.93 Å (for Ar-H), C-H= 0.98 Å (for methyne-H) and C-H = 0.97 Å (for methylene-H) and constrained to ride on their parent atoms with $U_{iso}(H) = 1.2U_{eq}(C)$.

General Procedure for the Synthesis of Dimers (153-160):

The alkynes of cholic acid/deoxycholic acid **75-78** (1 equiv) and the diazido bis-β-lactams **151** or **152** (0.5 equiv) were dissolved in DMF/H_2O 7:3 (10 mL). To this solution $CuSO_4 \cdot 5H_2O$ (0.05 equiv) and sodium ascorbate (0.5 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 385 watt. It was then cooled to room temperature, quenched with crushed ice and extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous sodium sulphate. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography to obtain dimers **153-160**.

Dimer (153): White solid, Yield 93%; Mp: 167-168 ºC; IR ($CHCl_3$, cm^{-1}): 3411, 1766, 1731; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50 (s, 1H, triazole-H), 7.47 (s, 1H, triazole-H), 7.21 (bs, 10H, Ar-H), 6.16 (d, J = 4.8, Hz, 1H), 6.13 (d, J = 5.0, Hz, 1H), 5.56 (d, J = 4.8 Hz, 2H), 4.97 (s, 4H, -OCH_2), 4.11 (t, J = 9.3 Hz, 2H), 3.94 (bs, 2H, CH-12), 3.84 (d, J = 13.6 Hz, 2H, CH-7), 3.40 (bs, 2H, CH-3,), 3.13 (t, J = 11.8 Hz, 2H), 0.94 (s, 6H, CH_3-19), 0.86 (d, J = 7.8 Hz, 6H, CH_3-21), 0.64 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 163.4, 142.4, 130.9, 128.8, 128.4, 126.6, 124.0, 72.6, 71.3, 68.7, 67.9, 60.4, 56.5,

46.4, 46.0, 41.1, 39.0, 38.9, 38.6, 34.9, 34.4, 34.2, 30.7, 30.3, 29.6, 27.7, 27.2, 25.9, 22.8, 22.1, 16.8, 12.1; MS (LCMS) *m/z*: 1295 M + 1), 1318 (M + Na); Anal. Calcd for $C_{74}H_{102}N_8O_{12}$: C, 68.60; H, H, 7.94; N, 8.65. Found: C, 68.45; H, 7.71; N, 8.89.

Dimer (154): White solid, Yield 95%; Mp: 157-159 ºC; IR ($CHCl_3$, cm^{-1}): 3434, 1768, 1730; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.43 (s, 2H, Ar-H), 7.20-7.25 (m, 10H, Ar-H), 6.14 (d, *J* = 5.1 Hz, 2H), 5.55 (d, *J* = 5.1 Hz, 2H), 4.97 (s, 4H, -OCH_2), 4.12 (d, *J* = 11.1 Hz, 2H), 3.97 (bs, 1H, CH-12), 3.66-3.55 (m, 2H, CH-3), 3.13 (d, *J* = 11.3 Hz, 2H), 2.35-2.16 (m, 4H, -CH_2CO), 0.94 (d, *J* = 5.8 Hz, 6H, CH_3-21), 0.91 (s, 6H, CH_3-19), 0.66 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.7, 163.6, 142.7, 131.2, 129.1, 128.7, 126.8, 123.9, 72.9, 71.5, 69.1, 60.6, 56.9, 48.1, 47.0, 46.4, 42.0, 38.7, 36.3, 35.9, 35.2, 35.0, 34.0, 33.5, 31.0, 30.9, 30.6, 30.3, 28.6, 27.4, 27.0, 26.1, 23.6, 23.0, 17.2, 12.6; MS (LCMS) *m/z*: 1286 (M + Na); Anal. Calcd for $C_{74}H_{102}N_8O_{10}$: C, 70.34; H, 8.14; N, 8.87. Found: C, 70.07; H, 7.94; N, 9.02.

Dimer (155): White solid, Yield 93%; Mp: 195-196 ºC; IR (nujol, cm^{-1}): 3369, 1758, 1650; ^1H NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ 7.53 (s, 2H, triazole-H), 7.21 (bs, 10H, Ar-H), 6.12 (bs, 2H, unresolved splitting), 5.55 (s, 2H, unresolved splitting), 4.19 (s, 4H, -$NHCH_2$), 4.08 (t, *J* = 9.8 Hz, 2H, N-CH_2), 3.94 (bs, 2H, CH-12), 3.83 (bs, 2H, CH-7), 3.42-3.37 (m, 2H, CH-3,), 3.17-3.14 (m, 2H, N-CH_2), 0.96 (d, *J* = 5.5 Hz, 6H, CH_3-21), 0.89 (s, 6H, CH_3-19), 0.66 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6, 163.5, 144.7, 130.8, 128.7, 128.2, 126.7, 123.2, 72.6, 71.2, 68.7, 67.8, 60.6, 46.1, 45.9, 41.2, 41.1, 39.0, 38.7, 38.6, 35.0, 34.9, 34.3, 34.1, 33.8, 32.3, 31.2, 29.4, 27.6, 27.1, 25.9, 22.8, 22.0, 16.7, 12.0; MS (LCMS) *m/z*: 1316 (M + Na); Anal. Calcd for $C_{74}H_{102}N_8O_{12}$: C, 68.70; H, 8.10; N, 10.83. Found: C, 68.41; H, 8.23; N, 10.66.

Dimer (156): White solid, Yield 94%; Mp 177-178 ºC; IR ($CHCl_3$, cm^{-1}) 3392, 1766, 1658; ^1H NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ 7.21 (bs, 10H, Ar-H), 6.21 (bs, 2H, unresolved splitting), 5.56 (bs, 2H, unresolved splitting), 4.10 (s, 4H, -$NHCH_2$), 3.96 (s, 2H, CH-12), 3.58 (bs, 2H, CH-3), 3.14-3.00 (m, 4H, -NCH_2), 0.96 (bs, 6H, CH_3-21), 0.90 (s, 6H, CH_3-19), 0.67 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$ + CD_3OD 125 MHz): δ 163.7, 130.8, 128.8, 128.3, 126.7, 72.6, 71.0, 70.0, 59.9, 47.7, 46.4, 46.1, 41.7, 38.5, 35.7, 35.6, 35.1, 34.9, 33.8, 33.1, 31.2, 29.5, 28.2, 27.2, 26.8, 25.8, 23.4, 22.7, 16.8, 12.3; MS

(LCMS) *m/z*: 1284 (M + Na), 1285 (M + Na + 1); Anal. Calcd for $C_{74}H_{104}N_{10}O_8$: C, 70.45; H, 8.31; N, 11.10. Found: C, 70.37; H, 8.08; N, 11.29.

Dimer (157): White powder, Yield 95%; Mp: 173-175 ºC; IR ($CHCl_3$, cm^{-1}) 3454, 1770, 1731; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.67 (s, 1H, triazole-H), 7.49 (s, 1H, triazole-H), 7.30-7.27 (m, 10H, Ar-H), 6.23-6.16 (m, 2H), 5.38 (bs, 2H, unresolved splitting), 5.0 (bs, 4H, $-OCH_2$,), 3.95 (bs, 2H, CH-12), 3.84 (bs, 2H, CH-7), 3.76-3.47 (m, 6H, 2 x CH-3, 2 x $-NCH_2$), 0.95 (bs, 6H, CH_3-21), 0.88 (s, 6H, CH_3-19), 0.67 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.8, 163.2, 131.1, 129.2, 128.6,127.0, 72.7, 71.4, 68.7, 68.1, 61.7, 56.7, 2 X 46.6, 46.1, 41.3, 40.3, 39.2, 35.1, 34.5, 34.3, 30.8, 30.5, 29.8, 27.8, 27.3, 26.0, 22.8, 22.2, 17.0, 12.2; MS (LCMS) *m/z*: 1317(M + Na); Anal. Calcd for $C_{74}H_{102}N_8O_{12}$: C, 68.60; H, 7.94; N, 8.65. Found: C, 68.74; H, 7.83; N, 8.57.

Dimer (158): White solid, Yield 94%; Mp 145-146 ºC; IR ($CHCl_3$, cm^{-1}) 3442, 1766, 1731; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.62 (s, 2H, triazole-H), 7.32-7.22 (m, 10H, Ar-H), 6.15-6.13 (m, 2H), 5.37-5.33 (m, 2H), 5.0 (s, 4H, $-OCH_2$), 3.97 (bs, 1H, CH-12), 3.83-3.43 (m, 6H, 2 x CH-3, 2 x $-NCH_2$), 0.95 (bs, 6H, CH_3-21), 0.91 (s, 6H, CH_3-19), 0.67 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 163.1, 124.5, 131.2, 129.1, 128.6, 127.0, 124.1, 72.3, 71.3, 68.7, 61.8, 56.8, 47.9, 46.8, 46.3, 41.9, 40.3, 36.2, 35.8, 35.1, 35.0, 33.9, 33.3, 30.8, 30.5, 30.1, 28.5, 27.3, 27.0, 26.0, 23.5, 22.9, 17.0, 12.5; MS (LCMS) *m/z*: 1285 (M + Na); Anal. Calcd for $C_{74}H_{102}N_8O_{10}$: C, 70.34; H, 8.14; N, 8.87. Found: C, 70.26; H, 8.11; N, 8.96.

Dimer (159): White solid, Yield 93%; Mp: 199-200 ºC; IR ($CHCl_3$, cm^{-1}): 3500, 1758, 1650; ^1H NMR ($CDCl_3$ + $DMSOD_6$, 400 MHz) δ 7.60 (s, 1H, triazole-H), 7.51 (s, 1H, triazole-H), 7.27-7.25 (m, 10H, Ar-H), 6.17-6.13 (m, 2H), 5.38-5.35 (m, 2H), 4.21 (bs, 4H, $-NHCH_2$), 3.90 (bs, 1H, CH-12), 3.82-3.77 (m, 4H, 2 x CH-7, N-CH_2), 3.48-3.43 (m, 2H, CH-3), 3.39-3.33 (m, 2H, N-CH_2), 0.98 (d, J = 5 Hz, 6H, CH_3-21), 0.88 (s, 6H, CH_3-19), 0.66 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$ + CD_3OD, 125 MHz): δ 174.5, 163.2, 144.6, 131.1, 129.0, 128.4, 127.0, 123.2, 72.7, 71.3, 68.5, 67.9, 61.6, 46.0, 41.3, 39.9, 39.1, 35.1, 34.5, 34.3, 32.3, 31.3, 29.7, 29.4, 27.8, 27.2, 26.0, 22.9, 22.2, 17.0, 12.1; MS (LCMS) *m/z*: 1316 (M + Na); Anal. Calcd for $C_{74}H_{104}N_{10}O_{10}$: C, 68.70; H, 8.10; N, 10.83. Found: C, 68.46; H, 8.31; N, 10.54.

Dimer (160): White solid, Yield 94%; Mp: 180-181 ºC; IR ($CHCl_3$, cm^{-1}) 3373, 1768, 1658; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.58 (s, 1H, triazole-H), 7.47 (s, 1H, triazole-H), 7.27-7.24 (m, 10H, Ar-H), 6.72-6.63 (m, 2H, -NH), 6.12 (bs, 2H, unresolved spliting), 5.34 (bs, 2H, unresolved spliting), 4.27 (bs, 4H, -$NHCH_2$), 3.96 (bs, 1H, CH-12), 3.77 (bs, 2H, N-CH_2), 3.59-3.45 (m, 4H, 2 x CH-3, N-CH_2), 0.95 (bs, 6H, CH_3-21), 0.91 (s, 6H, CH_3-19), 0.67 (s, 6H, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.3, 163.0, 144.7, 131.0, 128.9, 128.3, 126.9, 123.1, 72.6, 71.0, 68.6, 61.4, 47.6, 46.3, 46.1, 41.7, 39.9, 35.8, 35.6, 35.1, 33.8, 33.1, 31.2, 29.7, 28.2, 27.2, 26.9, 25.9, 23.4, 22.8, 16.9, 12.3; MS (LCMS) *m/z*: 1284 (M + Na); Anal. Calcd for $C_{74}H_{104}N_{10}O_8$: C, 70.45; H, 8.31; N, 11.10. Found: C, 70.61; H, 8.15; N, 11.22.

Antimicrobial Activity: Materials and Methods:

Human pathogens *C. albicans* and *C. neoformans*; saprophytes *B. poitrasii* and *Y. lipolytica* were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants. *F. oxysporum* (plant pathogen) was maintained on PDA (potato, 20%; dextrose, 2%) agar slants at 28 ºC. *E. coli* and *S. aureus* were maintained on nutrient agar (NA; beef extract, 0.3%; peptone, 0.5%; sodium chloride, 0.5%) agar slants. Strains of *C. albicans, C. neoformans, Y. lipolytica* and *B. poitrasii* were inoculated in YPG broth. *C. albicans, C. neoformans* and *Y. lipolytica* were incubated at 28 ºC where as *B. poitrasii* was incubated at 37 ºC for 24 h. *F. oxysporum* was inoculated in potato dextrose and incubated at 28 ºC for 48 h whereas bacterial strains *E. coli* and *S. aureus* in NA broth for 24 h. Compounds **13-20** were solubilized in DMSO (2.5% v/v) and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, fluconazole, tetracycline and ampicillin were also dissolved in DMSO, and were used as a positive control.

MIC and IC_{50} Determination:

In vitro antifungal and antibacterial activity of the newly synthesized compounds were studied against the fungal strains viz., *C. albicans*, *C. neoformans*, *B. poitrasii*, *Y. lipolytica, F. oxysporum* strains and bacterial strains *E. coli*, and *S. aureus*, respectively to find out MIC (Minimum Inhibitory Concentration) and IC_{50} (50%, Inhibition of growth). All the experiments were done in triplicate under similar experimental conditions. MIC and IC_{50} of the synthesized compounds were determined according to standard broth microdilution technique as per NCCLS guidelines. Testing was performed in U bottom 96

well tissue culture plates in YPG, PD broth for fungal strains and Nutrient broth for bacterial strains. The concentration range of tested compounds and standard was 0.25 to 128 μg/mL. The plates were incubated at 28 °C for all the microorganisms except at 37 °C for *B. poitrasii*, absorbance at 600 nm were recorded to assess the inhibition of cell growth after 24 h for *B. poitrasii* and *Y. lipolytica*, 48 h for *C. albicans* and *F. oxysporum*, 72 h for *C. neoformans* and 24 h for bacterial cultures. MIC was determined as 90% inhibition of growth with respect to the growth control and IC_{50} was the concentration at which 50% growth inhibition was observed.

Antiproleferative Activity. Materials and Methods: Cell culture.

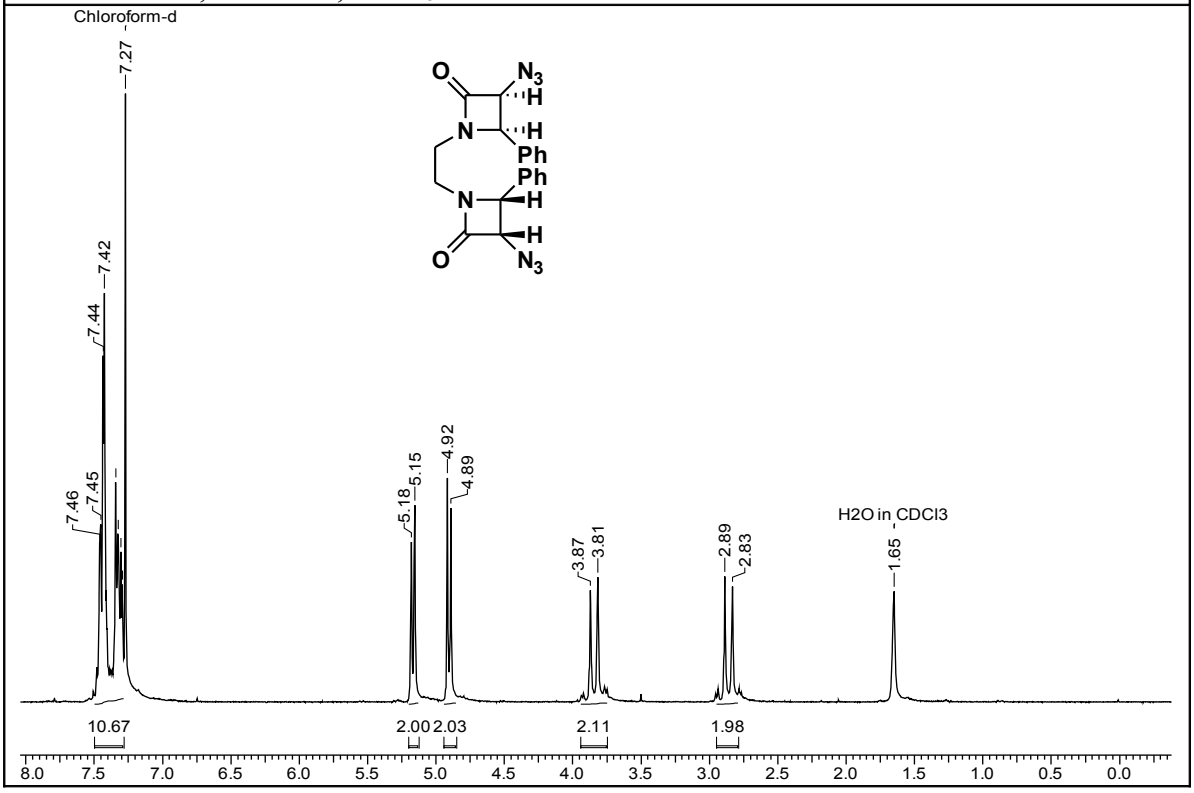
Human embryonic kidney (HEK293) and human mammary adenocarcinoma (MCF-7) cell lines were grown in a monolayer in nutrient media DMEM supplemented with fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) (all from Invitrogen Life Technologies, MD). The cells were grown at 37 °C in presence of 5% CO_2.

MTT Cell Proliferation Assay.

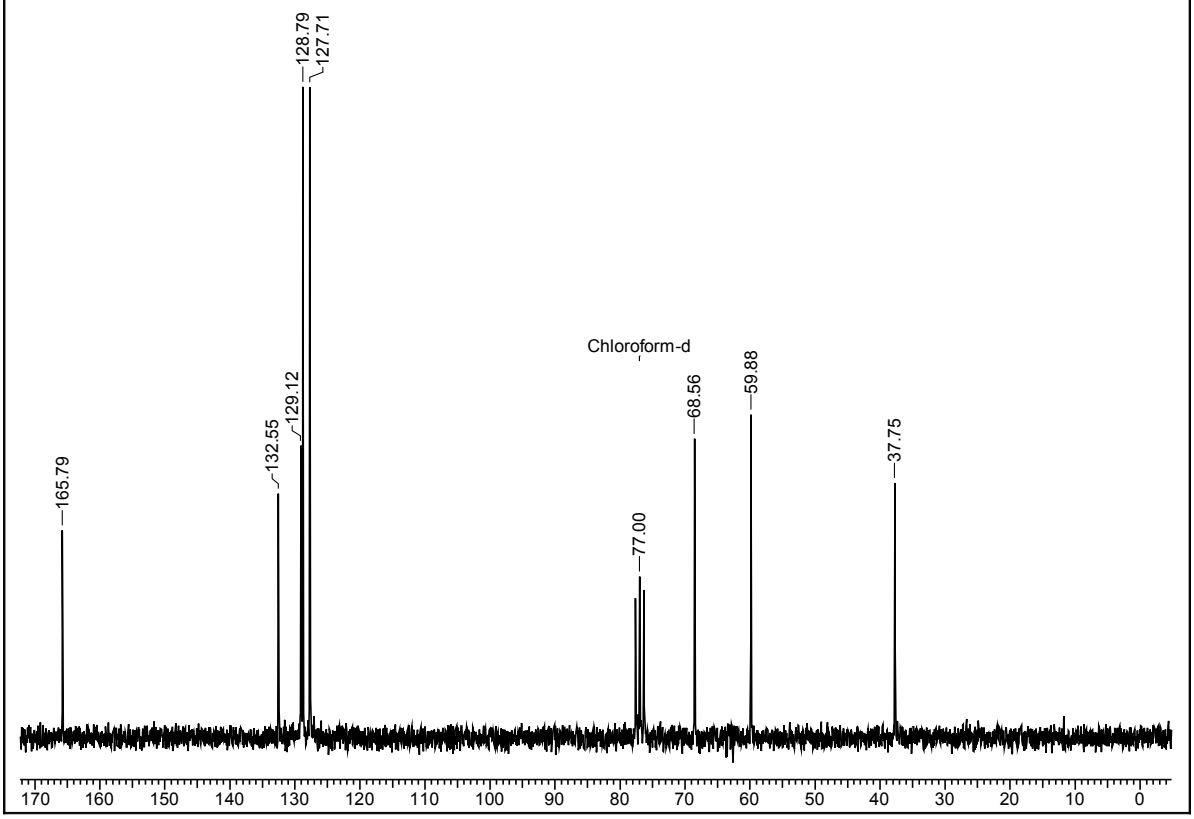
HEK293 and MCF-7 cells were plated at a density of 10^4 cells per well in 96-well tissue culture plates. Cells were allowed to adhere for 24 h at 37 °C. Stock solutions of all the compounds were prepared in DMSO at a concentration of 10 mM and diluted to the required concentration. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved (5 mg/mL) in DMEM (without phenol red) and filtered through a 0.22 μm filter before use. The cells were treated with various concentrations (0, 1, 10, 100 and 1000 μM) of compounds dissolved in DMSO for additional 48 h, in triplicate. In the control wells, nutrient medium with a corresponding concentration of DMSO only was added to the cells. Thereafter, the drug containing medium was replaced with 50 μL media containing 1 mg/mL MTT and incubated for 4 h at 37 °C. Medium was then aspirated off from the formazan crystals, which were then solubilized in 100 μL of acidified isopropanol. The optical density was read on a microplate reader at 570 nm using 630 nm as a reference filter against a blank prepared from cell free wells. Absorbance given by cells treated with the carrier DMSO alone was taken as 100% cell growth. All assays were performed in triplicates.

1.C.7 Selected Spectra

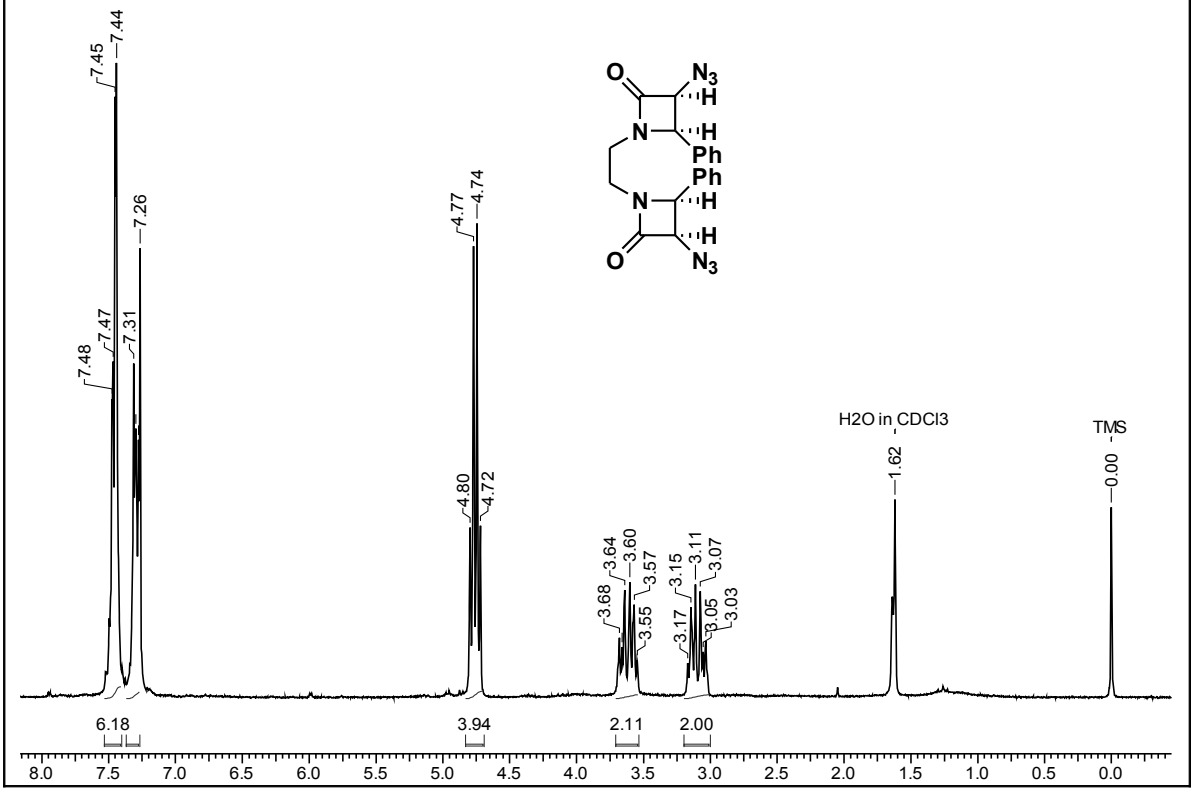
151: ^{1}H NMR, 200 MHz, $CDCl_3$



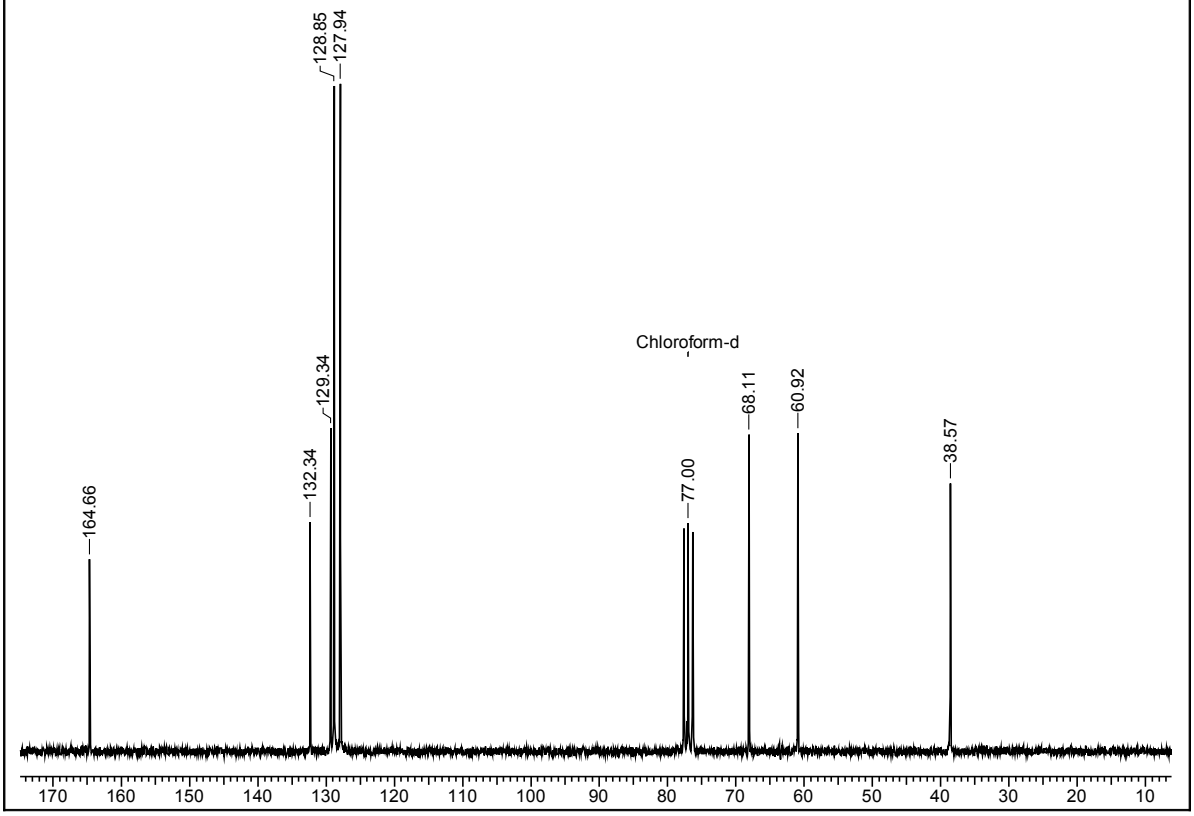
151: ^{13}C NMR, 50 MHz, $CDCl_3$



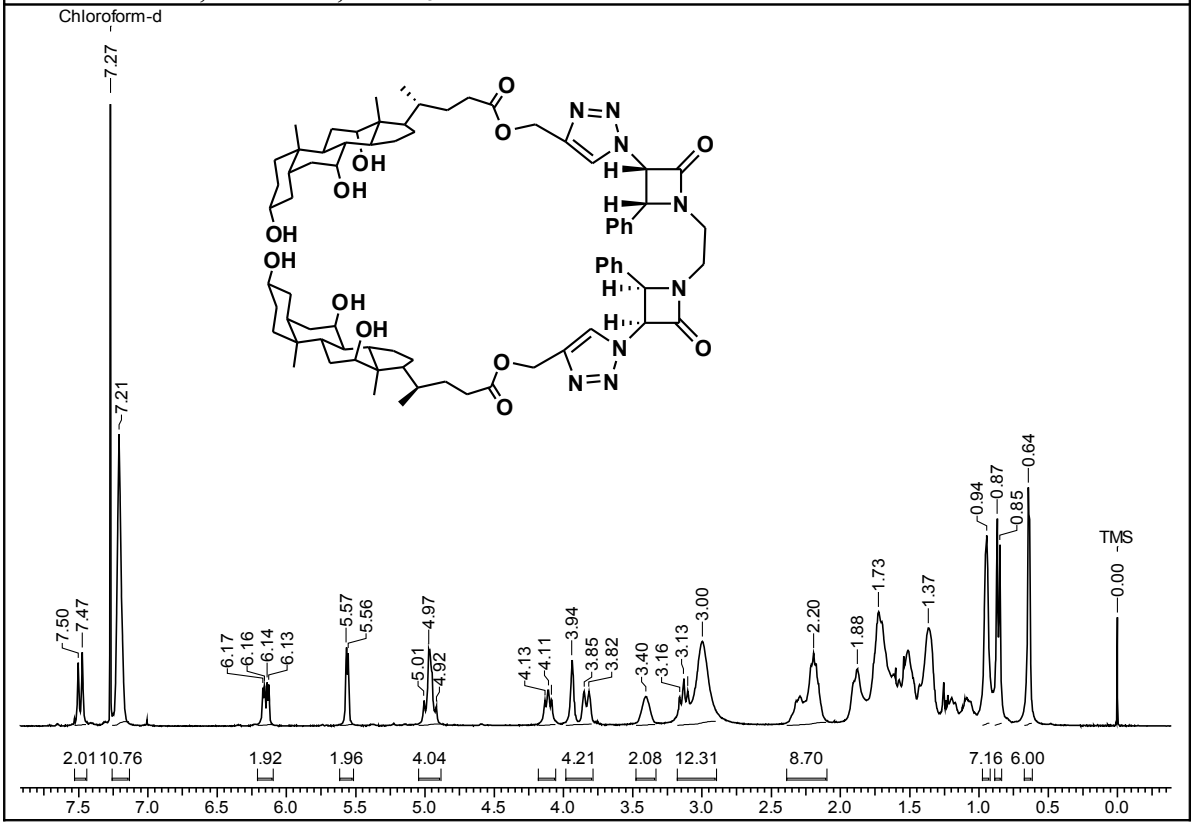
152: ^{1}H NMR, 200 MHz, $CDCl_3$



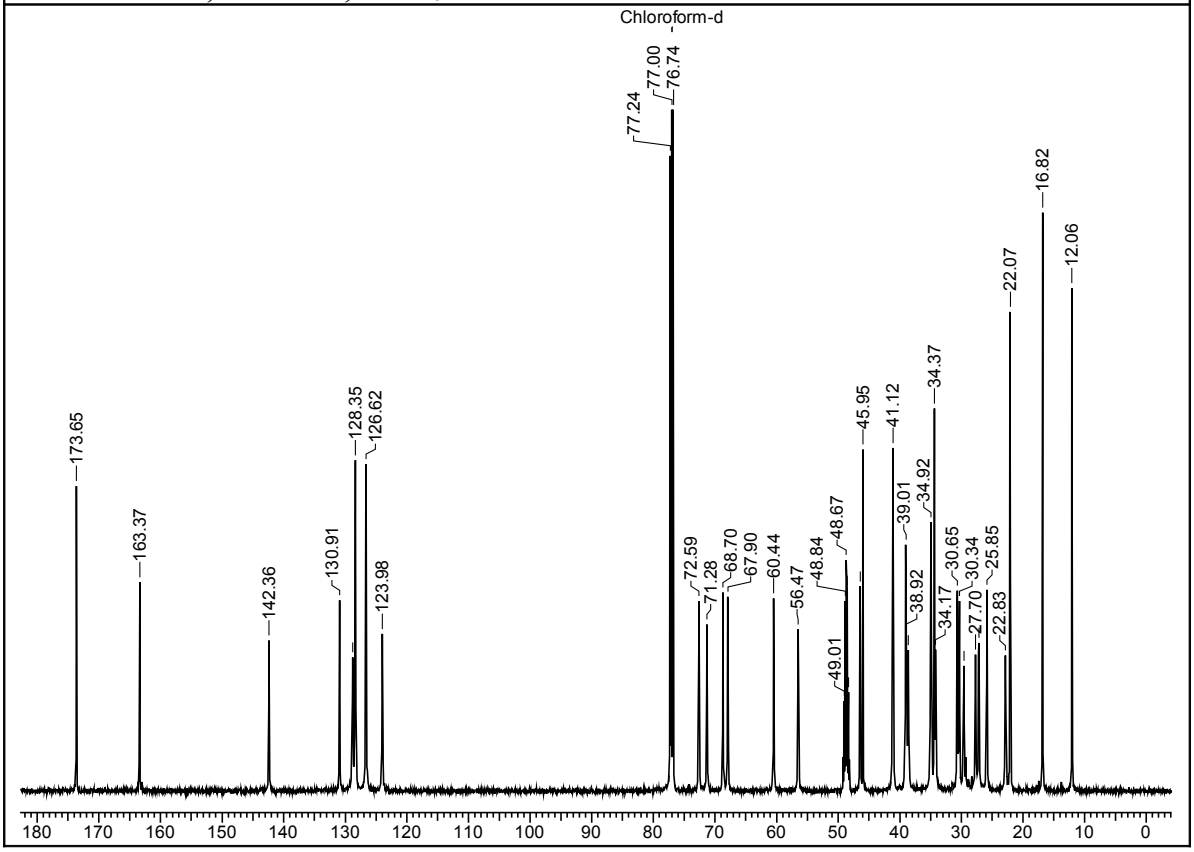
152: ^{13}C NMR, 50 MHz, $CDCl_3$



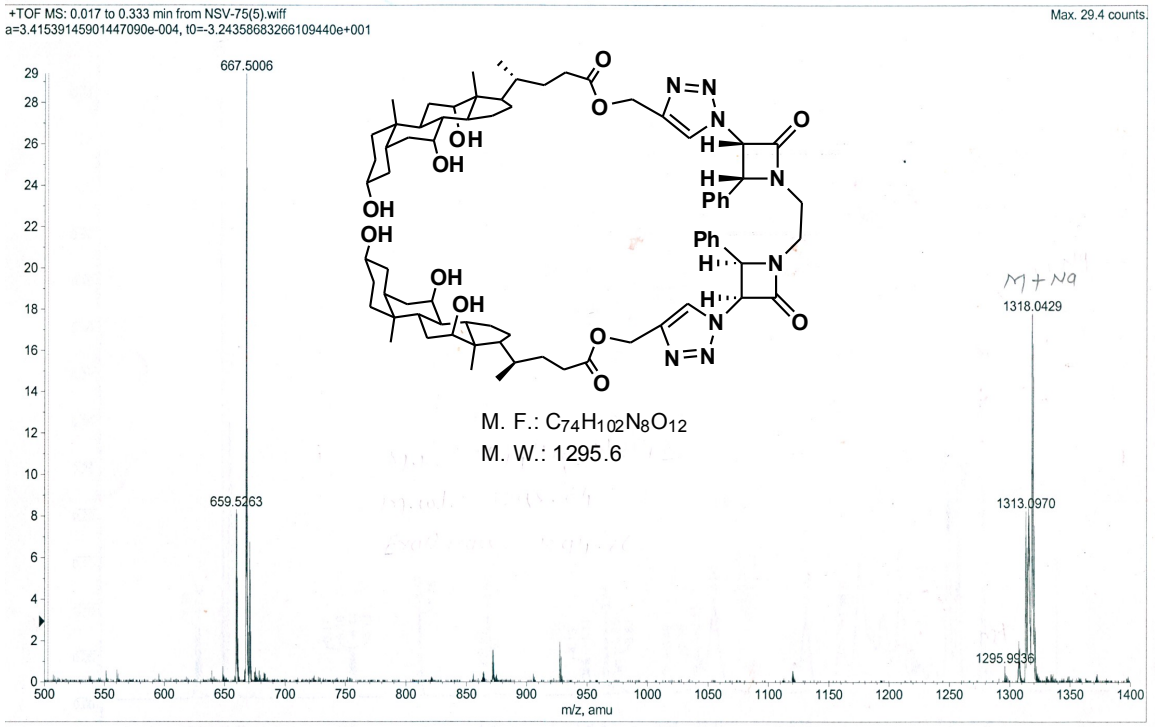
153: ^{1}H NMR, 400 MHz, $CDCl_3$



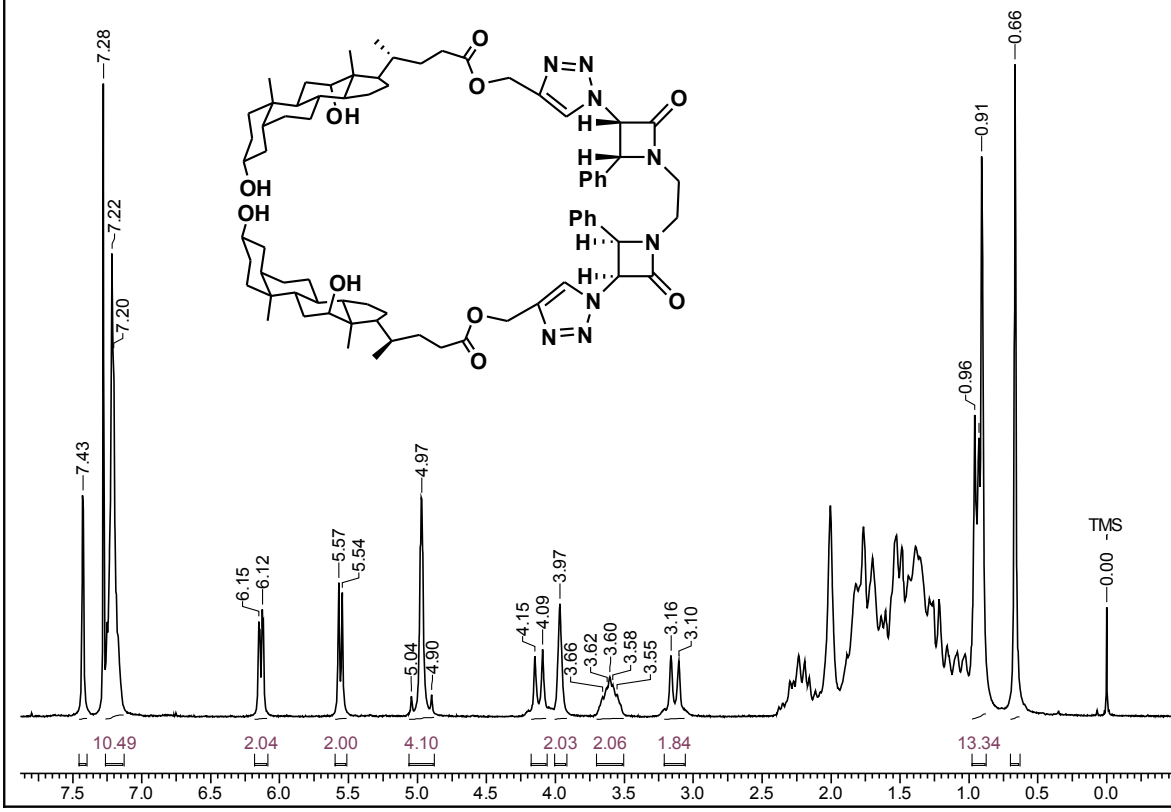
153: ^{13}C NMR, 125 MHz, $CDCl_3$



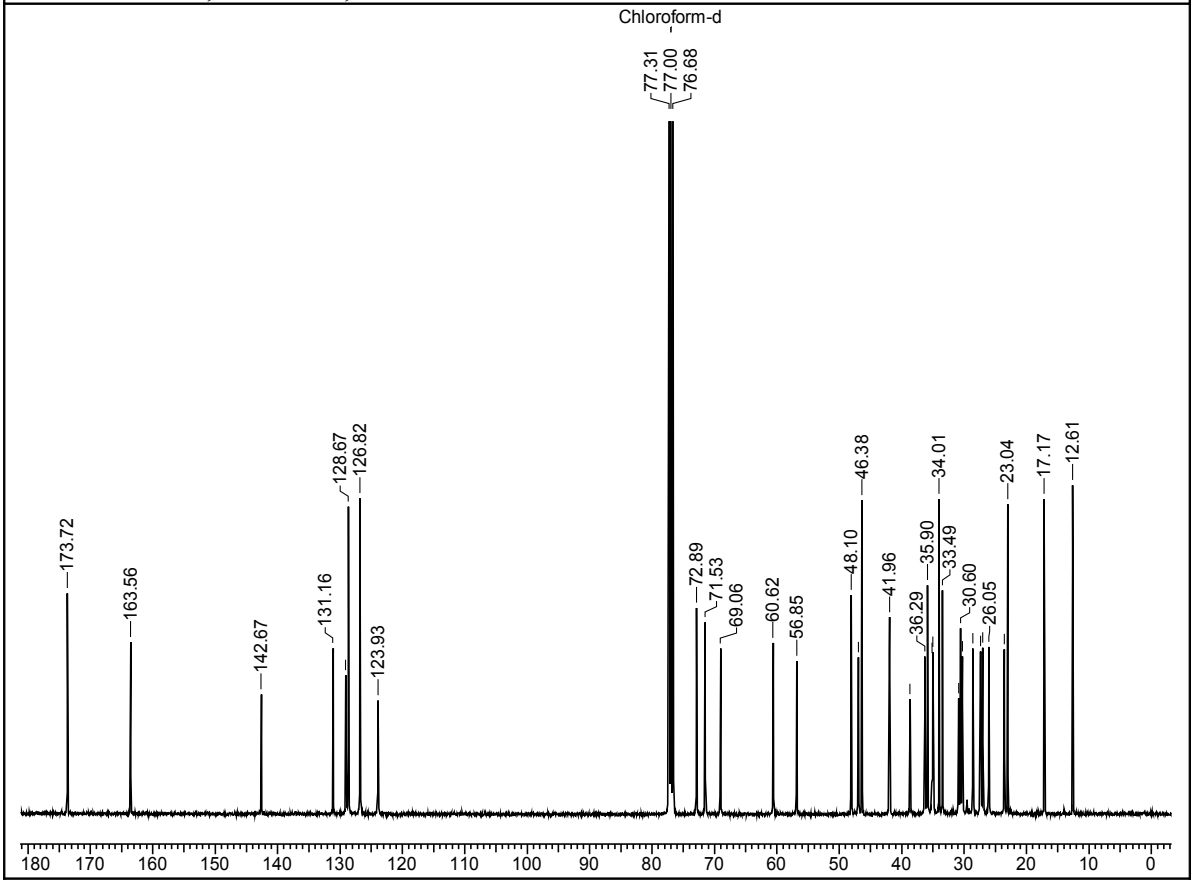
153: MS (LCMS)



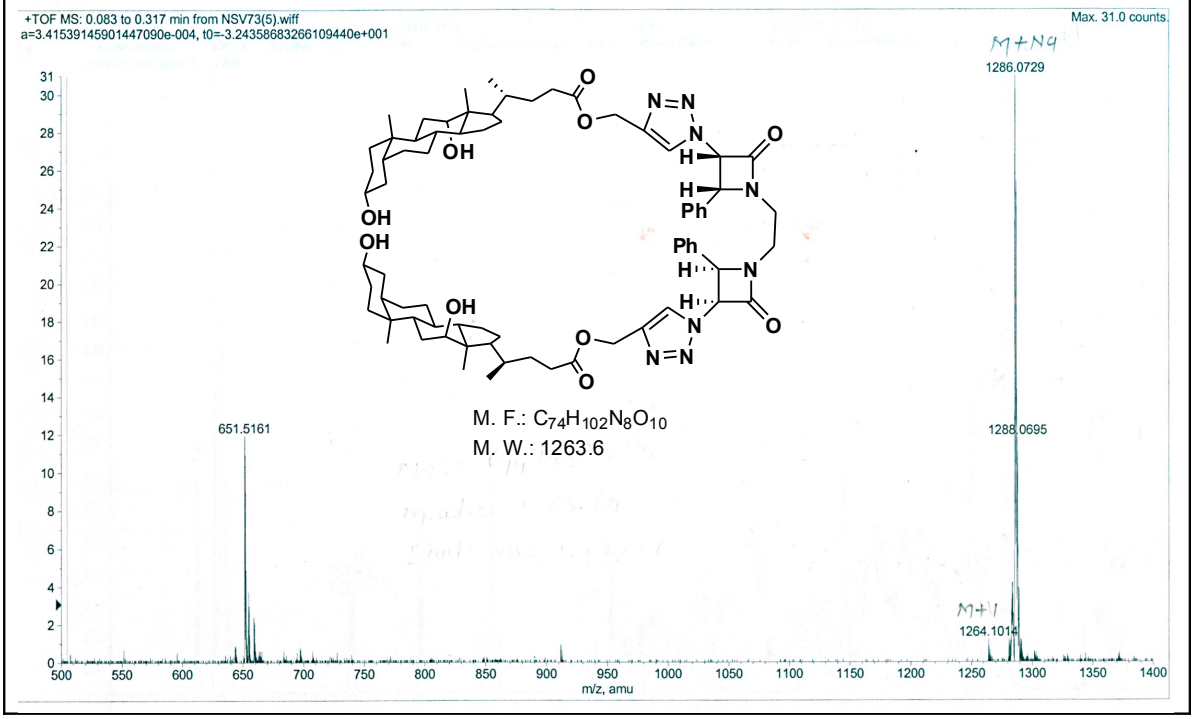
154: ^{1}H NMR, 200 MHz, $CDCl_3$



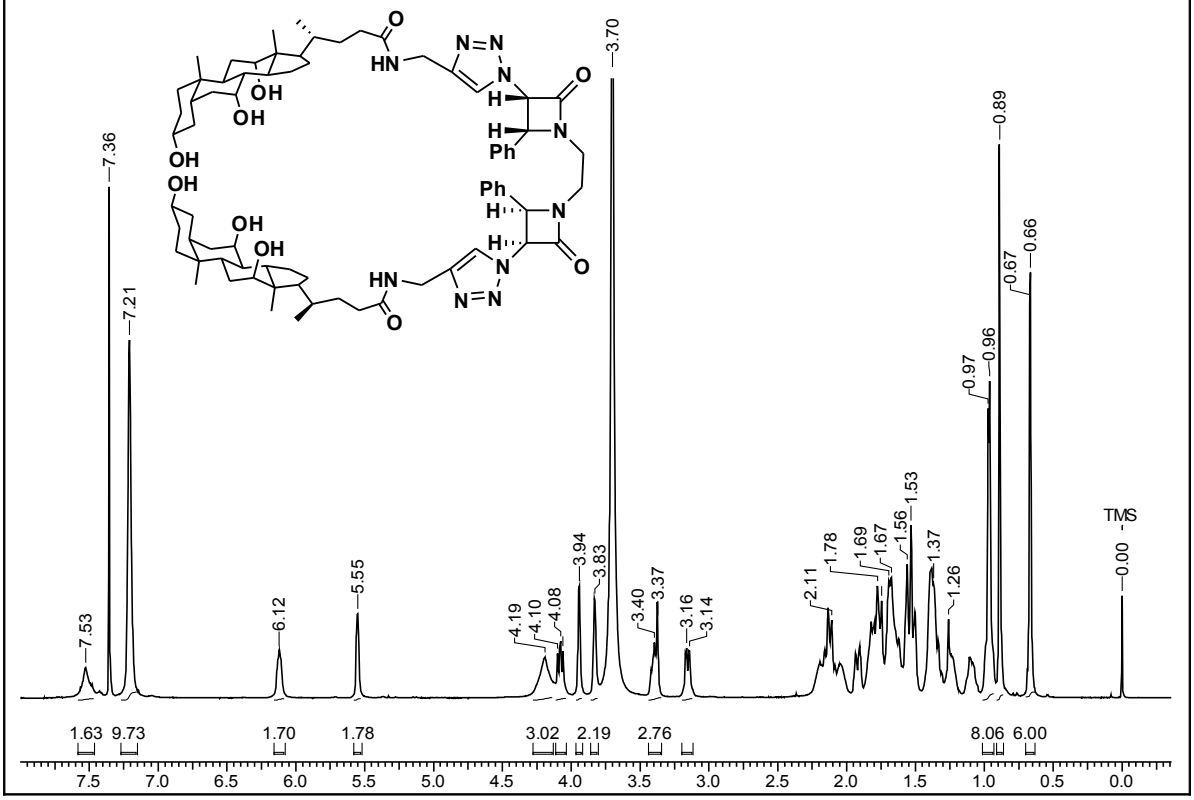
154: ^{13}C NMR, 100 MHz, $CDCl_3$



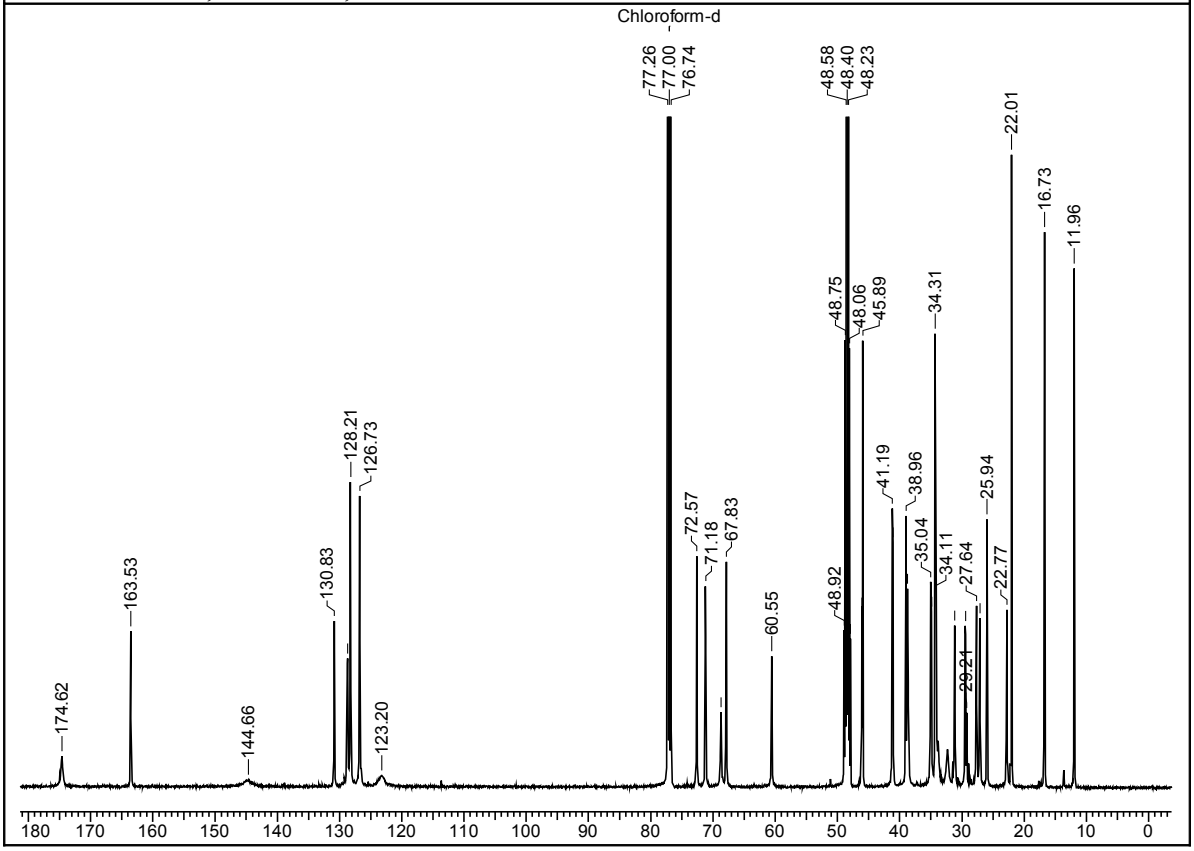
154: MS (LCMS)



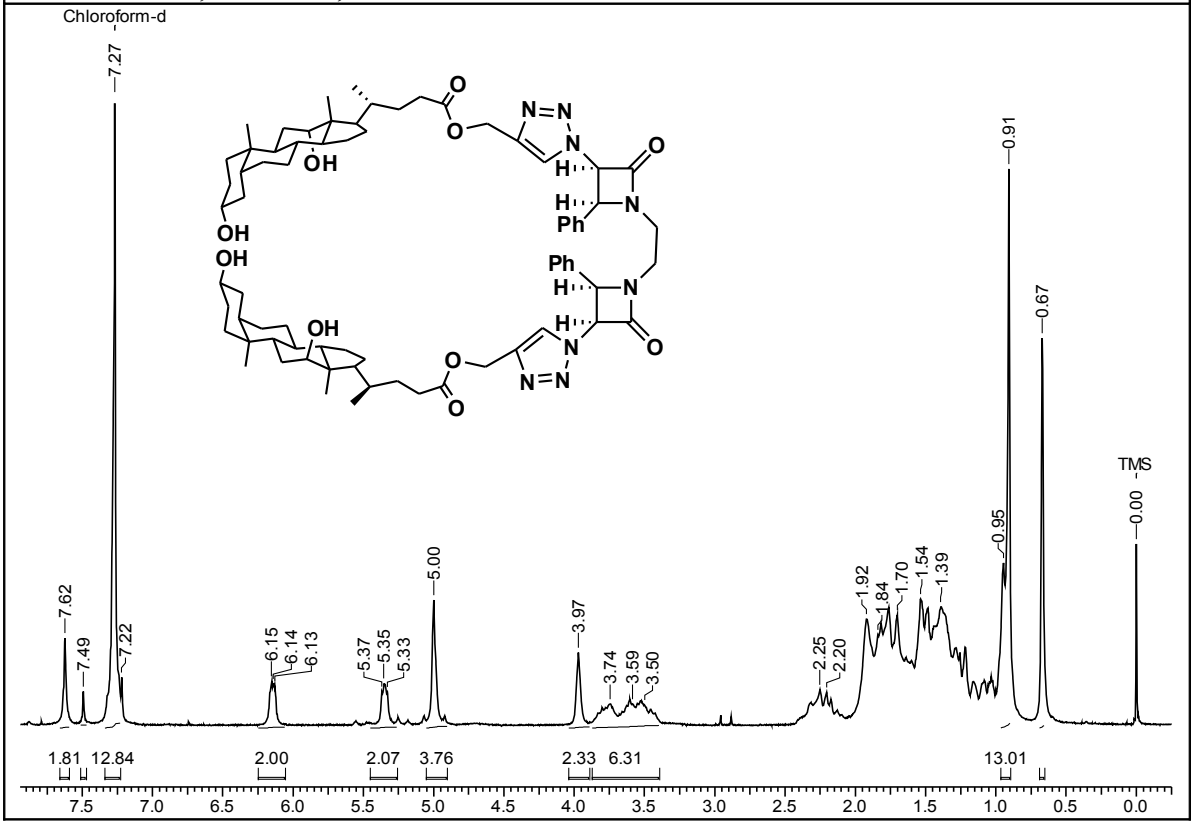
155: ^{1}H NMR, 500 MHz, $CDCl_3$ + CD_3OD



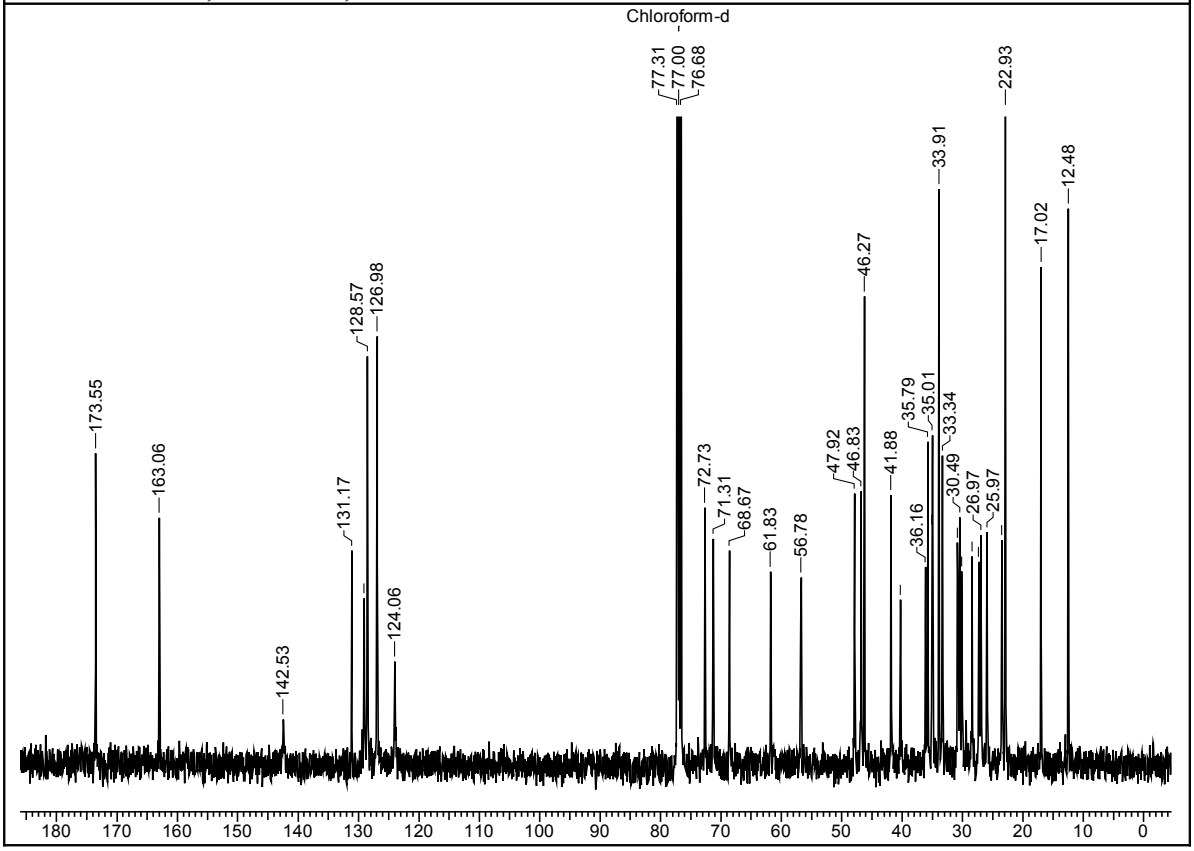
155: ^{13}C NMR, 125 MHz, $CDCl_3$ + CD_3OD



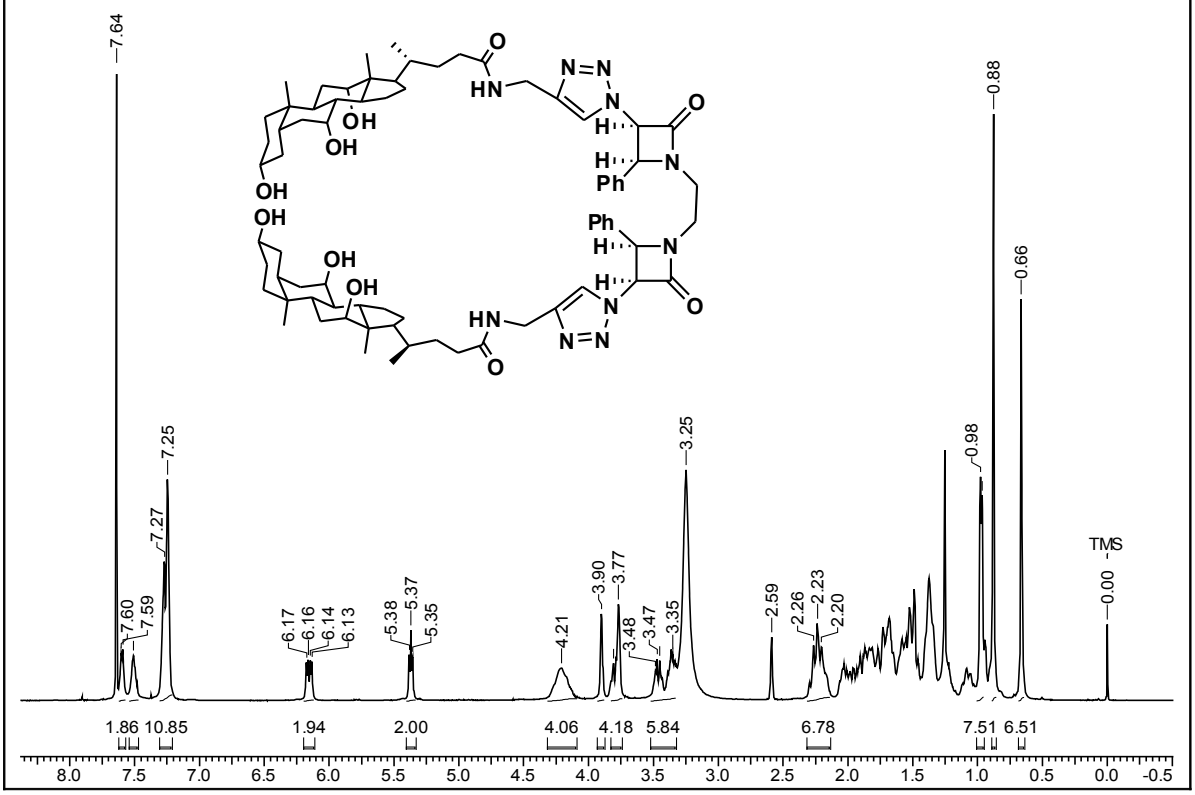
158: ^{1}H NMR, 200 MHz, $CDCl_3$



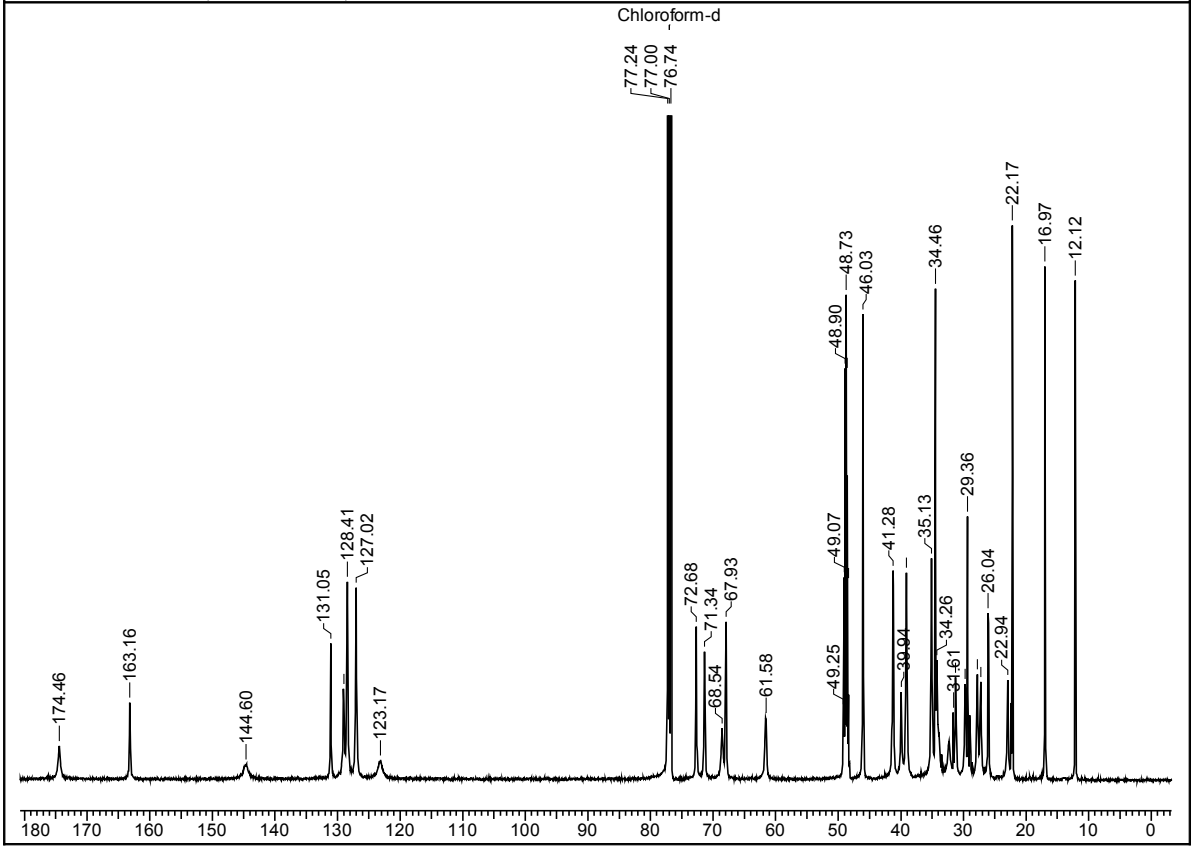
158: ^{13}C NMR, 100 MHz, $CDCl_3$



159: ^{1}H NMR, 400 MHz, $CDCl_3$ + $DMSOD_6$



159: ^{13}C NMR, 125 MHz, $CDCl_3$ + CD_3OD



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Chapter 2

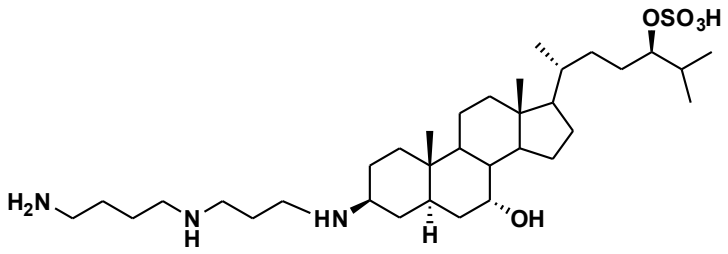
Studies Directed Towards the Synthesis of Squalamine

2.1 Abstract

A stereoselective formal synthesis of squalamine **1** was accomplished from readily available 16-dehydropregnenelone acetate (16-DPA) following ene reaction as one of the key steps.

2.2 Introduction

Squalamine **1** (Figure 1) is the first sterol-spermidine conjugate that has been isolated by Zasloff and co-workers from the stomach of the dogfish shark, *Squalus acanthias* in 1993.[1] Squalamine displays potent antimicrobial activitiy against gram-negative bacteria, gram-positive bacteria, and fungi. However, this unusual natural product found to possess antiangiogenic and antitumor activity[2] and was developed as a new chemotherapeutic approach in the treatment of late stage lung cancer and ovarian cancer.[3] The novel structural features and remarkable antitumer activity made squalamine highly attractive to researchers around the world.



1

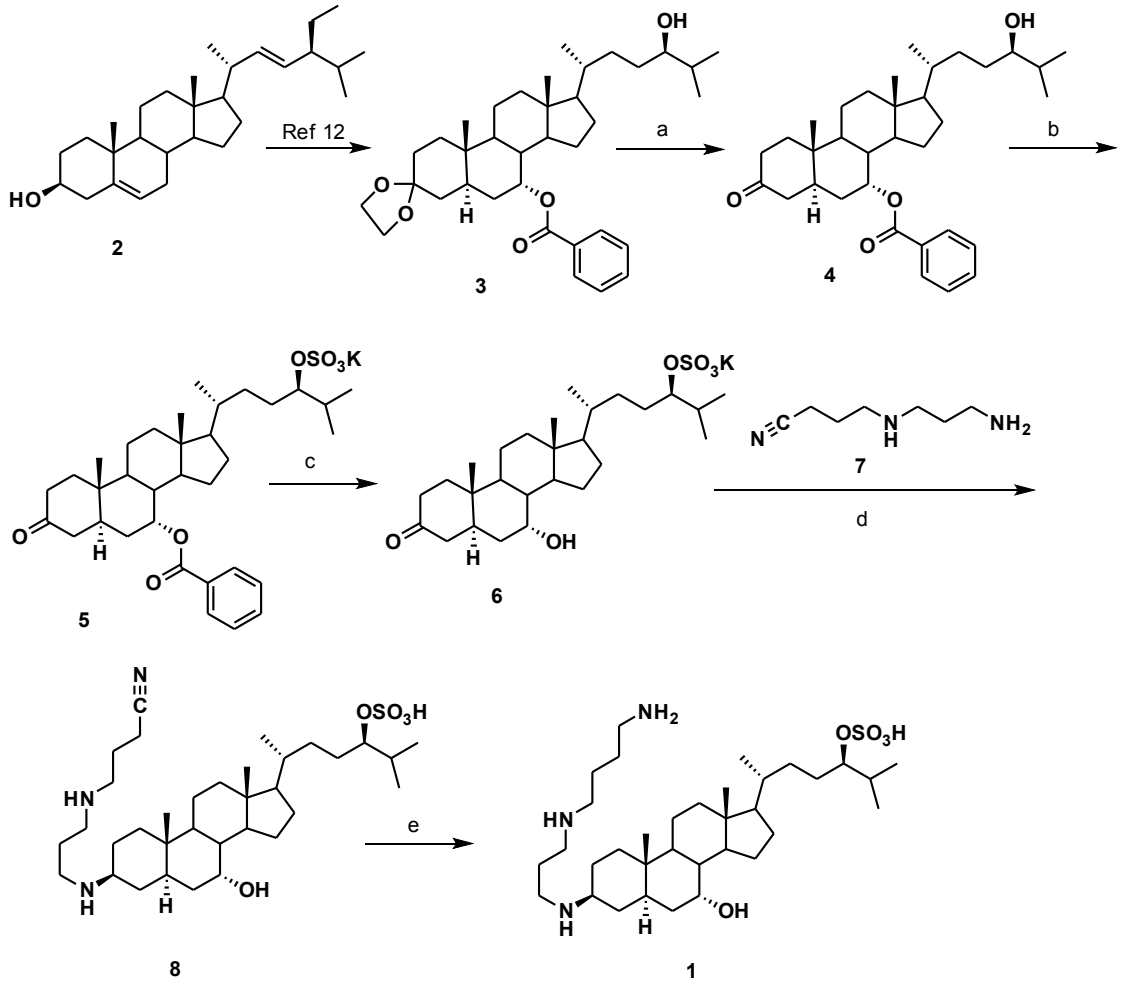
Figure 1.

2.3 Review of literature

Various methods for the synthesis of Squalamine **1** have been documented in the literature.[4-11] Some of the recent syntheses of Squalamine **1** are described below.

Kinney, W. A. *et al.* (1998)[7]

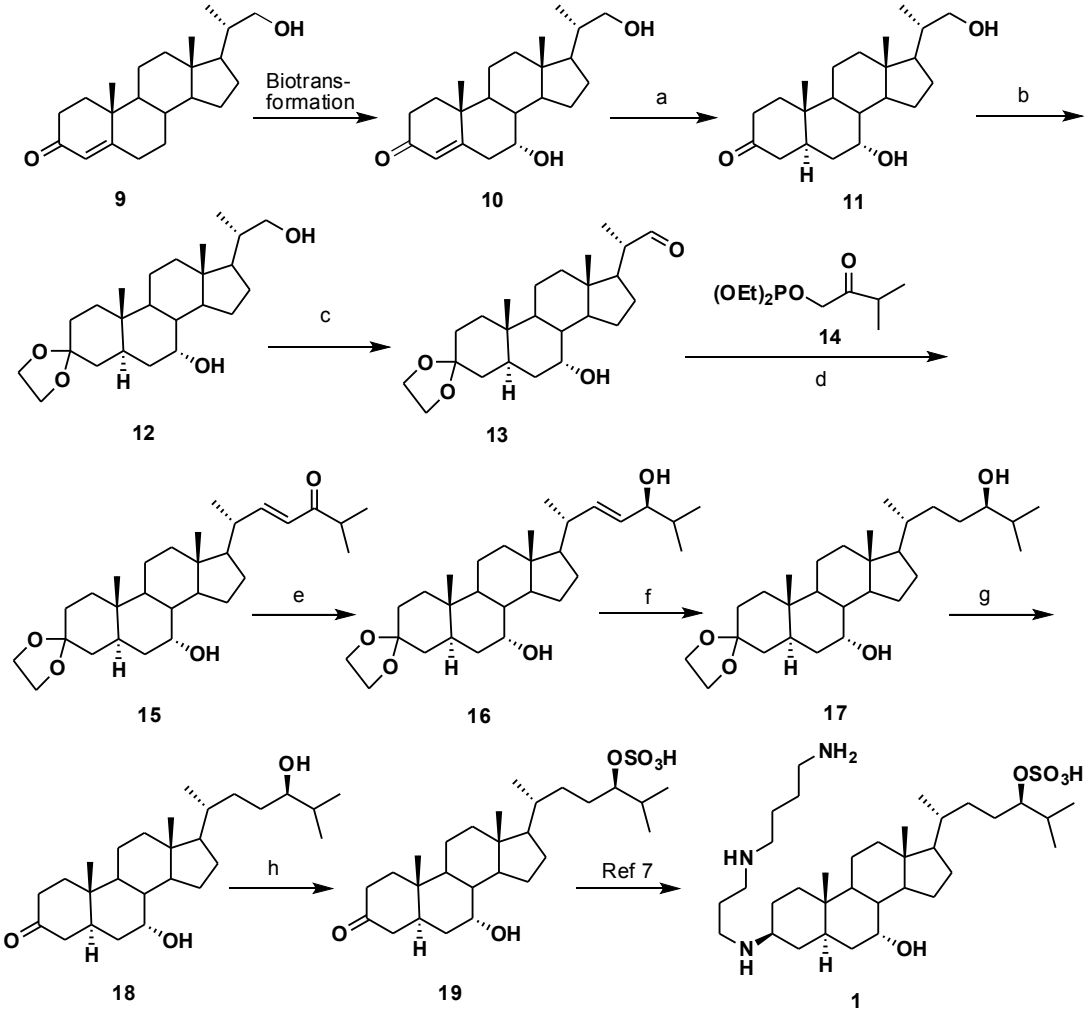
William Kinney and co-workers reported the synthesis of squalamine **1** from the intermediate **3** (Scheme 1). The compound **3** was synthesized from the stigmasterol **2** in 10 steps following literature procedure.[12] Intermediate **3** on acid hydrolysis utilizing Amberlyst 15 ion-exchange resin as catalyst gave the free ketonic compound **4**. The ketone **4** was treated with sulfer trioxed-pyridine complex to afford compound **5**. Cleavage of the benzoate at C-7 with potassium hydroxide in methanol furnished the hydroxy ketone **6**. Compound **6** on reductive amination with the masked spermidine reagent **7** using trimethyl orthoformate and sodium borohydride at -78 °C afforded nitrile **8**. The compound **8** was hydrogenated at low P^H (1-2) using platinum oxide as catalyst to yield squalamine **1**.



Scheme: 1 Reagents and conditions: (a) Amberlyst 15 ion exchange resin, acetone. (b) SO_3·py, 80 °C, 77% (after 2 steps). (c) KOH, MeOH, reflux, 90%. (d) trimethyl orthoformate, $NaBH_4$, MeOH, -78 °C (e) PtO_2 (40 psi), ethanol, TFA to P^H = 2, 60%.

Kinney, W. A. *et al.* (2000)[8]

William Kinney and co-workers reported the one more short formal synthesis of squalamine **1** utilizing the biotransformation product **10**, which is available in one step from commercially available 3-keto-23,24-bisnorchol-4-en-22-ol (**9**) (Scheme 2). The reduction of double bond in α,β-unsaturated ketone **10** with lithium in ammonia gave the trans AB-ring junction product **11**. Ketalization of **11** was performed utilizing ethylene glycol in chlorotrimethylsilane, which on selective oxidation of the C-22 alcohol with bleach and TEMPO as catalyst afforded aldehyde **13**. The aldehyde **13** was treated with Wadsworth-Emmons reagent **14** to get enone **15**, which was subjected to stereoselective

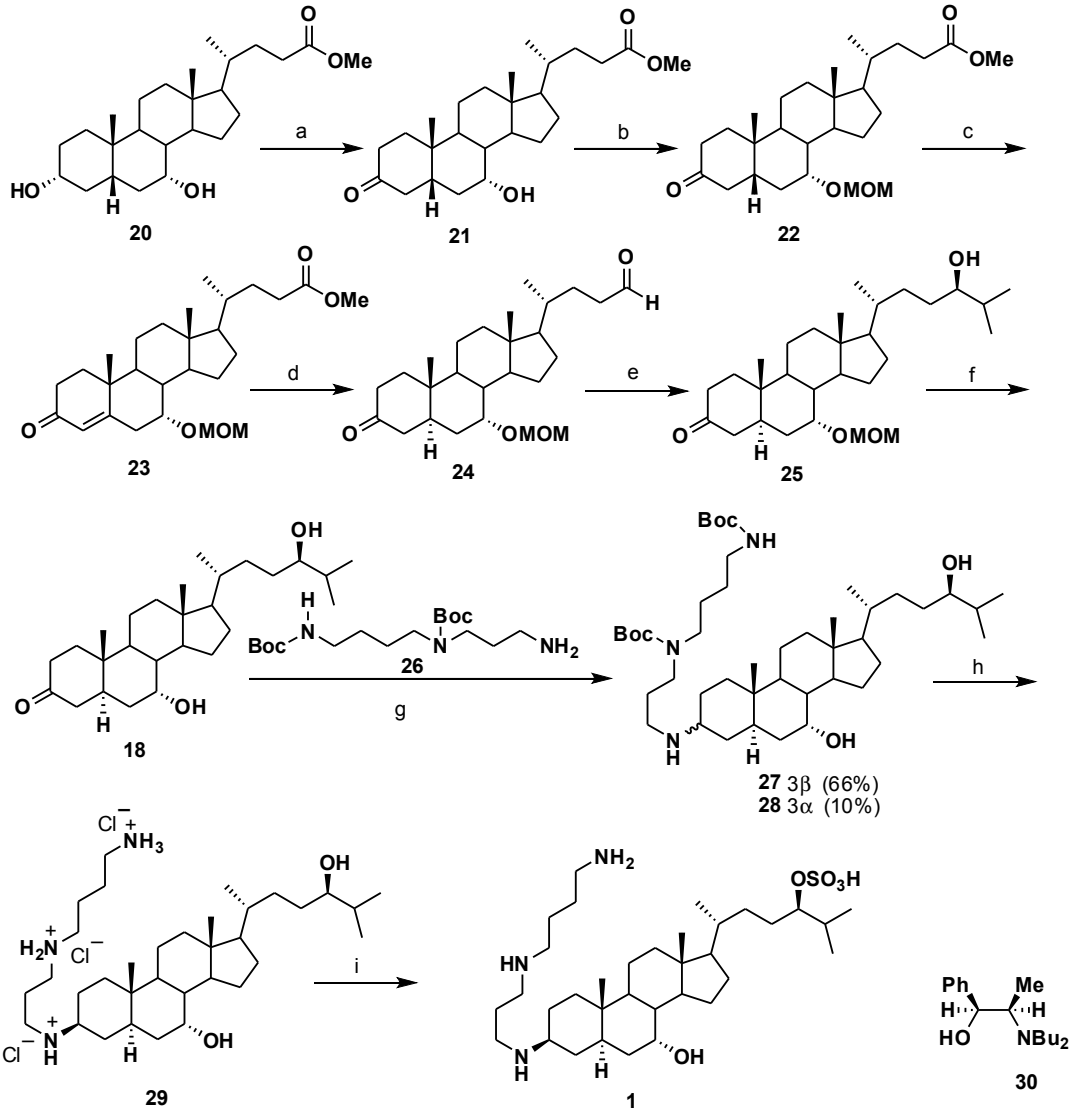


Scheme: 2 Reagents and conditions: (a) Li, NH_3, THF, 71%. (b) TMS-Cl, ethylene glycol, 84%. (c) NaOCl, TEMPO, NaBr, CH_2Cl_2, 98%. (d) *t*-BuONa, THF, 82%. (e) (*R*)-MeCBS, BH_3·THF, THF, Tolune, 80%. (f) Et_3N, tolune, 10% Pt/C, H_2 (50 psi), 92%. (g) *p*-TsOH, water, acetone, 89%. (h) SO_3·py (1.05 equiv), pyridine, 77%.

reduction with borane and (*R*)-MeCBS to yield allylic alcohol **16** in good yield. The **16** on hydrogenation in presence of 10% Pt/C followed by deprotection of the ketal afforded C-7,24-dihydroxy compound **18**. Selective sulfation was accomplished successfully by treating diol **18** with small excess (5%) of sulfur trioxide-pyridine complex to furnish **19** in good yield. Transformation from **19** to the target molecule **1** is known in the literature.[7]

Zhou, W-S. *et al.* (2003)[10]

Wei-Shan Zhou and co-workers accomplished the stereoselective synthesis of squalamine **1** in nine steps from methyl chenodeoxycholanate **20** (Scheme 3). The synthesis featured, improved dehydrogenation of **22** followed by conjugate reduction of **23** to construct the *trans* AB-ring system and efficient asymmetric isopropylation of aldehyde **24** to

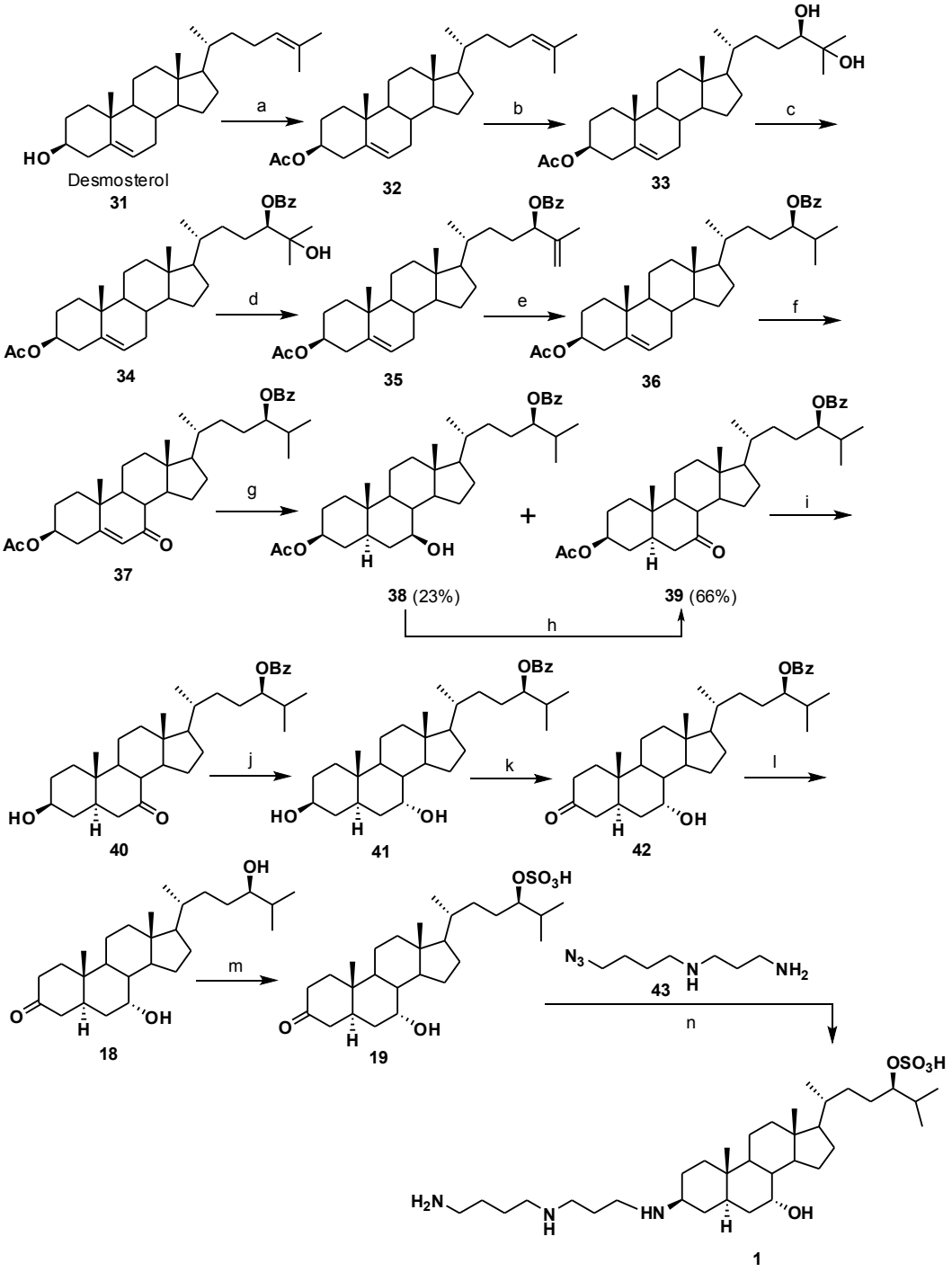


Scheme: 3 Reagents and conditions: (a) Ag_2CO_3 on Celite, toluene, reflux, 92%; (b) MOMCl, *i*Pr_2NEt, cat. NaI, CH_2Cl_2, reflux, 91%; (c) 2.0 equiv of IBX, 30 mol % of TFA, DMSO, rt, 24 h, 87%; (d) Li, ammonia, THF, -78 °C for 1 h then quenching with anhydrous NH_4Cl, 73%; (e) 20 mol % of ligand **30**, 2.2 equiv of *i*Pr_2Zn, toluene, 0 °C, 4 h, 84% yield, 99% de; (f) PPTS, *t*BuOH, reflux, 96%. (g) i) 3Å sieves, MeOH. ii) $NaBH_4$, MeOH. (h) HCl, MeOH, 91%. (i) SO_3·py, pyridine, 55%.

introduce the C-24*R*-hydroxyl group. The methyl chenodeoxycholanate **20** was selectively oxidized to the 7α-hydroxy-3-one **21** in very good yield, using freshly prepared silver carbonate on celite followed by protection of 7-hydroxy group using MOM-chloride and *N*,*N*-diisopropylethylamine afforded 7α-methoxy-methyl ether **22**. Compound **22** on dehydrogenation with IBX in the presence of TFA obtained the desired α,β-unsaturated carbonyl compound **23** which on treatment with lithium in liquide ammonia at -78 °C followed by quenching with anhydrous NH_4Cl delivered aldehydes **24**. The two functional groups were successfully transformed in only one step. The asymmetric addition reaction of the aldehyde **24** with diisopropylzinc afforded the isopropylated adduct **25** in the presence of 20 mol% of ligand **30**. Removal of the 7α-MOM protecting group of **25** with PPTS gave **18** which was subjected to reductive amination with the protected spermidine **26** furnished a mixture of aminosterols **27** and **28**. These were separated with flash chromatography with silica gel to give the 3β-aminosterol **27** (66%) and the 3α-aminosterol **28** (10%). Removal of the Boc protecting groups of **27** by treatment with HCl in MeOH gave **29** as the hydrochloride salt in very good yield, which without further purification was treated with sulfur trioxide-pyridine complex in pyridine to afford squalamine **1**.

Takeuchi, S. *et al.* (2003)[11]

Seiji Takeuchi and co-workers reported the synthesis of squalamine **1** from desmosterol **31** (Scheme 4). The synthesis commences with the acetylation of of C-3-OH of desmosterol followed by Sharpless asymmetric dihydroxylation gave the dihydroxy compound **33**. The C-24-OH was selectively protected using benzoyl chloride in pyridine to get **34** followed by treatment with $POCl_3$ and pyridine afforded the C-26 hydroxy eliminated product **35**. The chemoselective reduction of double bond between C-25 and C-26 of the compound **35** over 10% Pd-C gave the saturated (24*R*)-compound **36**. The allylic oxidation of compound **36** using the hydroxyphthalimide-catalysed air oxidation method provided 7-oxo compound **37** which was subjected to hydrogenation using Adams catalyst furnished the 7-keto compound **39** (66%) together with 7β-hydroxy compound **38** (23%). The **38** was easily transformed into the **39** by PCC oxidation.



Scheme: 4 Reagents and conditions: (a) Ac_2O, Pyridine. (b) AD-mix-β, t-BuOH-H_2O. (c) BzCl, Pyridine, 93%. (d) $POCl_3$, Pyridine, 85%. (e) 10% Pd-C, EtOAc, 63%. (f) i) Air, HO-phthalimide; ii) Ac_2O-pyridine, 64%. (g) H_2, PtO_2, EtOAc. (h) PCC, CH_2Cl_2, 92%. (i) K_2CO_3, MeOH-$CHCl_3$, 94%. (j) K-Selectride, THF, 77%. (k) Ag_2CO_3-Celite, Toluene, quant. (l) KOH, $HO(CH_2)OH$, 80%. (m) SO_3-py, Pyridine, 77%. (n) i) NaOMe, **43,** MS 3Å, MeOH, $NaBH_3CN$; iii) H_2, PtO_2, 60%.

Hydrolysis of acetyl group of **39** with K_2CO_3 in methanol followed by stereoselective reduction of the 7-keto group using K-selectride afforded the desired 7α-alcohol **41**. The 3β-hydroxyl group of **41** was selectively oxidized with silver carbonate on Celite to give the 3-keto compound **42** almost quantitatively. The benzoyl group of **42** was saponified with KOH in ethylene glycol to give **18** which on treatment with sulfur trioxide-pyridine complex afforded 24-*O*-sulfate derivative **19**. Finally, compound **19** on reductive amination with spermidine precursor **43** using $NaCNBH_3$ followed by reduction of azide group using PtO_2 furnished squalamine **1**.

2.4 Present work:

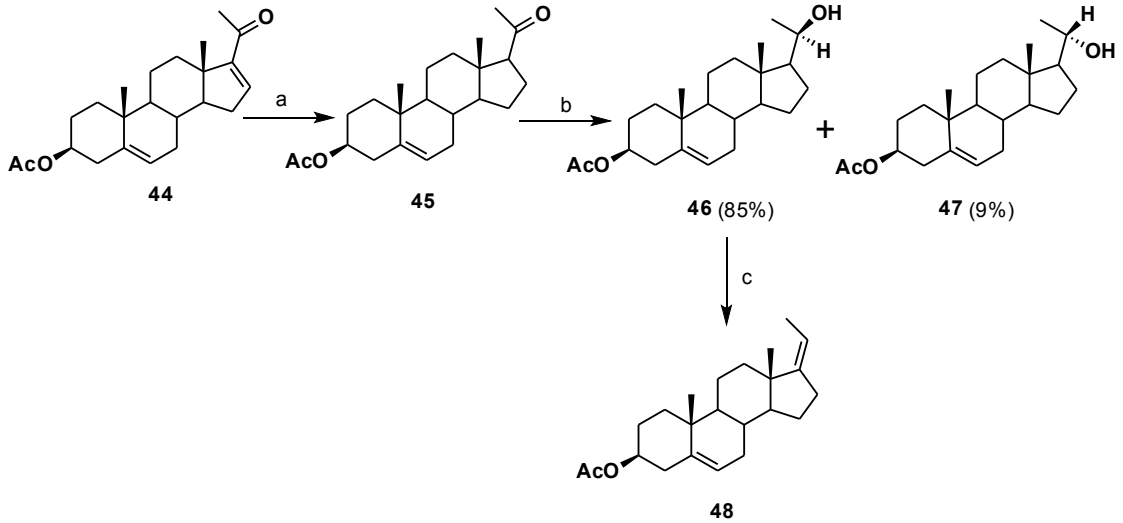
2.4.1 Objective

The squalamine (**1**) has been a synthetic target of immense interest due to its potent antiangiogenic and antitumor activity. It has also demonstrated significant antimicrobial activity against gram-positive bacteria, gram-negative bacteria and fungi. Despite numerous strategies for the synthesis of squalamine, the interest in the new and efficient methods of synthesis remains unabated. In an ongoing research programme towards the synthesis of the various biologically active compounds starting from 16 DPA,[13] we have developed a new route for the formal synthesis of squalamine.

2.4.2 Results and Discussion:

Synthesis of steroidal fragment 48

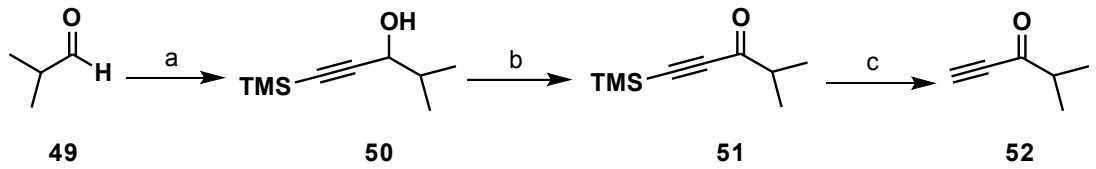
Our synthesis of squalamine **1** started from commercially available 16-dehydropregnenelone acetate (16-DPA) **44**. The 16-DPA **44** was subjected to chemoselective hydrogenation with 10% palladium on charcoal in ethyl acetate to give the saturated ketone **45** in excellent yield (Scheme 5). The reduction of ketone **45** with $NaBH_4$ in MeOH/THF (3:1) afforded epimeric mixture of alcohols **46** and **47**. These were separated using flash coloumn chromatography with silica gel to give the C-20*R* alcohol **46** in 85% yield and C-20*S* alcohol **47** in 9% yield. The dehydration of tertiary alcohol **46** with phosphorous oxychloride and pyridine furnished the Z olefine **48** in 89% yield.[13a]



Scheme: 5 Reagents and conditions: (a) 10% Pd/C, H_2, EtOAc, 45 psi, 30 ºC, 12 h, 98%; (b) $NaBH_4$, MeOH/THF, 0-25 °C, 2 h, **46** (85%) and **47** (9%); (c) $POCl_3$, pyridine, 0-27 °C, 30 h, 89%.

Synthesis of side chain fragment 52

The synthesis of side chain fragment of squalamine was started from isobutyraldehyde **49**. The treatment of aldehyde **49** with TMS-acetylene in the presence of *n*-BuLi at -78 °C obtained the propargylic alcohol **50** in 93% yield (Scheme 6). The formation of propargylic alcohol **50** was confirmed from its ^{1}H NMR spectrum, which showed characteristic signals at δ 4.15-4.17 (m, 1H) corresponding to methine proton (-CH-OH) and signal at δ 0.18 (s, 9H) due to (-$SiCH_3$) protons. Its ^{13}C NMR spectrum showed a signal at δ 68.0 corresponding to the methine carbon (-CHOH) and at δ 89.3 and 105.7 due to the alkyne carbons. The alcohol **50** was then subjected to PCC (pyridinium chlorochromate) oxidation in dichloromethane to furnish the propargylic ketone **51** in 97% yield. The disappearance of signals corresponding to methine proton (-CH-OH) in the ^{1}H NMR spectrum of **51** and a characteristic signal at δ 191.8 corresponding to carbonyl carbon in its ^{13}C NMR confirmed the formation of propargylic ketone **51**.



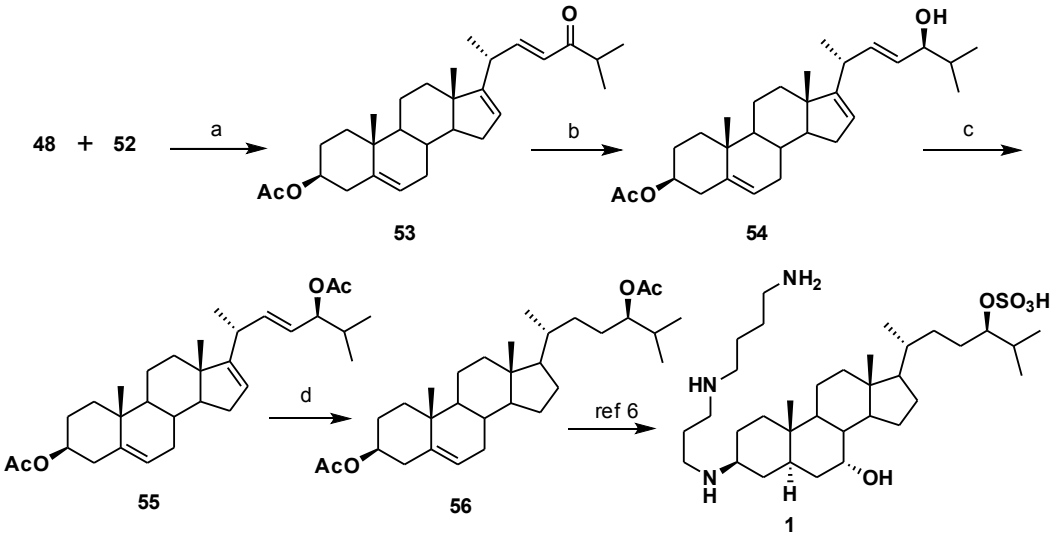
Scheme: 6 Reagent and conditions: (a) TMS-acetylene, *n*-BuLi, THF, - 78 to 25 °C, 6 h, 93%; (b) PCC, molecular sieves, CH_2Cl_2, 0-25 °C, 4 h, 96%; (c) NaF, *n*-Bu_4NBr, CH_2Cl_2/H_2O, 7 h, 72%.

The formation of propargylic ketone was also confirmed from the IR spectrum of **51**, which showed a strong absorption band at 1676 cm^{-1} due to the carbonyl carbon (-C=O) group. Phase-transfer catalysed removal of TMS group of **51** with NaF and *n*-Bu_4NBr afforded ethynyl ketone **52**.[14] The formation of ethynyl ketone **52** was confirmed by its ^{1}H NMR spectrum, which showed disappearance of signal due to (-$SiCH_3$) protons and observed a characteristic signal at δ 3.25 corresponding to alkyne protons (H-C≡C-).

Formal synthesis of squalamine 1

The ene reaction between (Z)-3β-acetoxypregna-5,17-diene **48** and ethynyl ketone **52** using $EtAlCl_2$, which is used as the Lewis acid in many cases,[15] was unsuccessful. Treatment of steroidal Z olefine **48** with ethynyl ketone **52** in the presence of borontrifluoride etherate in dichloromethane afforded α,β-unsaturated ketone **53** in 74% yield with natural steroid configuration at C-20 (Scheme 7). It is well known in the

literature that, the ene reaction of (17*Z*)-ethylidene steroids with various enophiles furnished the product with natural steroid configuration at C-20.[15,16] The ^{1}H NMR spectrum of **53** showed signals at δ 6.80 (dd, *J* = 7.8, 15.8, 1H), at δ 6.14 (d, J = 15.8, 1H) and at δ 5.42-5.38 (m, 2H) for olefinic protons. Its ^{13}C NMR spectrum displayed two characteristic signals at δ 170.4 and 204.3 for acetate carbonyl carbon (-C=O) and α,β-unsaturated carbonyl carbon (-C=O) respectively, along with the signals at δ 122.4, 123.9, 126.3, 139.9, 151.4, 157.1 for olefinic carbons. Further, its IR spectrum showed absorption band at 1732 cm^{-1} corresponding to the acetate carbonyl group and 1693 cm^{-1} corresponding to the α,β-unsaturated carbonyl group in **53**. The stereoselective reduction of ketone **53** by using (*R*)-MeCBS[8] reagent in presence of $BH_3.Me_2S$ in THF furnished the allylic alcohol **54** in 87% yield with 95% de. The configuration for newly formed stereocenter at C-24 carbon in compound **54** has been assigned as 24*S* according to the literature precedences.[8,12,17] The IR spectrum of **54** showed hydroxyl absorption at 3443 cm^{-1} and acetate carbonyl at 1722 cm^{-1}. The ^{1}H NMR spectrum of **54** displayed a characteristic multiplet at δ 3.82 correspondin to methine proton (-CH-OH) of allylic alcohol. In its ^{13}C NMR spectrum disappearance of signal corresponding to α,β-unsaturated carbonyl carbon and appearance of signal at δ 77.8 for methine carbon (-CH-OH) confirms the formation of allylic alcohol.



Scheme: 7 Reagents and conditions: (a) $BF_3.O_2Et_2$, CH_2Cl_2, 0-25 °C, 7 h, 74%; (b) (*R*)-MeCBS, $BH_3{\cdot}Me_2S$, THF, -30 °C, 0.5 h, 87%; (c) Ac_2O, DMAP, pyridine, 25 °C, 14 h, 96%; (d) Pt/C, H_2 (1 atm), EtOAc, 9 h, 94%.

Acetylation of C-24 hydroxy group in compound **54** using acetic anhydride and catalytic amount of DMAP resulted diacetate compound **55** in 96% yield. The presence of two singlet at δ 2.06 and 2.04 due to acetate methyl protons and downfield chemical shift for C-24 methine proton *i.e.* from δ 3.82 (t, 1H) to 5.02 (t, 1H) in ^{1}H NMR spectrum confirmed the formation of diacetate compound **55**. Its ^{13}C NMR displayed characteristic signals at δ 170.5 and 170.4 due to two actetate carbonyl carbons. The compound **55** on chemoselective reduction of C-16(17) and C-22(23) double bond with Pt/C and H_2 (1 atm) in ethyl acetate furnished the targeted compound **56** in 94% yield. The ^{1}H NMR spectrum of **56** displayed signals at δ 5.38 due to the ring olefine, which accounted for only one proton. The formation of **56** was further confirmed from its ^{13}C NMR spectrum, which exhibited characteristic signals at δ 171.0, 170.5 for acetate carbonyl carbons and at δ 139.5, 122.6 due to olefinic carbons. Transformation of the compound **56** to squalamine **1** is well documented in literature[6] and this constitute a formal total synthesis of squalamine starting from readily available 16-dehydropregnenelone acetate **44**.

2.5 Conclusion

In conclusion, we have achieved the stereoselective formal synthesis of squalamine (**1**), from commercially available 16-dehydropregnenelone acetate (16-DPA) by using ene reaction as one of the key steps. The C-24 hydroxyl group with required stereochemistry was introduced by employing the CBS reduction of C-24 ketone. Good yields, simple procedures and easy availability of starting materials render our approach an attractive method for the synthesis of squalamine **1**.

2.6 Experimental:

3β-Acetoxy-pregna-5-en-20-one (45):

To a solution of 16-dehydropregnenolone acetate **44** (3.56 g, 10 mmol) in ethyl acetate (100 mL) was added Pd-C catalyst (0.36 g, 10%) and hydrogenation was carried out at 45 psi pressure at room temperature (30 °C) for 10 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to obtain 3β-Acetoxy-pregna-5-en-20-one **45** (3.50 g, 98%) as a pale yellowish solid. Mp: 145-146 °C. IR (nujol, cm^{-1}): 1730 (CH_3COO), 1708 ($COCH_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.38 (d, J = 5 Hz, 1H), 4.61 (m, 1H), 2.54 (t, J = 9.0 Hz, 1H), 2.13 (s, 3H, $COCH_3$), 2.04 (s, 3H, $OCOCH_3$), 1.02 (s,

3H, CH_3-19), 0.63 (s, 3H, CH_3-18). ^{13}C NMR (50 MHz, $CDCl_3$) δ 209.2, 170.3, 139.5, 122.2, 73.6, 63.5, 56.7, 49.7, 43.8, 38.6, 37.9, 36.9, 36.4, 31.7, 31.6, 31.4, 29.6, 27.6, 24.3, 22. 7, 21.2, 20.9, 19.1, 13.1.

3β-Acetoxy-20(*R*)-hydroxy-pregna-5-en (46) and 3β-Acetoxy-20(*S*)-hydroxy-pregna-5-en (47):[18]

To a solutionof **45** (1.074 g, 3 mmol) in 16 mL MeOH/THF (3:1) was added sodium borohydride (0.228 g, 6 mmol) at 0°C. The reaction mixture was stirred for 30 min. at 0°C and 2 h at 25°C. The reaction was quenched with 1 N HCl and methanol was removed under reduced pressure. The reaction mixture was extracted with ethyl acetate (3 x 50 mL), and combined organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtained epimeric mixture of **46** and **47**. Flash column chromatographic purification over silica gel using EtOAc/pet ether (12:88) as an eluent afforded pure alcohol **46** (0.918 g, 85%). On further elution with same solvent system yielded **47** (0.097 g, 9%).

3β-Acetoxy-20(*R*)-hydroxy-pregna-5-en (46):

Mp: 161-163 ºC; $[\alpha]_D^{26}$ –69.33 (c 1.86, $CHCl_3$) {lit.,[18] 163.5-165.5 ºC; $[\alpha]_D$ –74}; IR ($CHCl_3$, cm^{-1}) 3562, 1724; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.38 (d, *J* = 5 Hz, 1H), 4.69-4.50 (m, 1H), 3.81-3.67 (m, 1H), 2.04 (s, 1H, CH_3COO), 1.15 (d, *J* = 6, Hz, 3H, 21-CH_3), 1.03 (s, 3H, 19-CH_3), 0.77 (s, 3H, 18-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.4, 139.6, 122.4, 73.8, 70.3, 58.3, 56.0, 49.9, 42.1, 39.7, 38.0, 36.9, 36.5, 31.8, 31.6, 27.6, 25.5, 24.4, 23.6, 21.3, 20.8, 19.2, 12.2. MS (LCMS) *m/z*: 383.34 (M + Na); Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06; Found: C, 76.38; H, 10.33.

3β-Acetoxy-20(*S*)-hydroxy-pregna-5-en (47):

Mp 138-140 ºC; $[\alpha]_D^{26}$ -55.88 (c 1.36, $CHCl_3$) {lit.,[18] 139-141 ºC; $[\alpha]_D$ –62}; IR ($CHCl_3$, cm^{-1}) 3502, 1730; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.38 (d, *J* = 4.8 Hz, 1H), 4.69-4.52 (m, 1H), 3.78-3.65 (m, 1H), 2.04 (s, 1H, CH_3COO), 1.23 (d, *J* = 6.2, Hz, 3H, 21-CH_3), 1.02 (s, 3H, 19-CH_3), 0.68 (s, 3H, 18-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.4, 139.5, 122.4, 73.8, 70.1, 58.2, 56.3, 49.8, 41.4, 38.6, 37.9, 36.8, 36.4, 31.7, 31.3, 27.6, 25.7, 24.1, 23.4, 21.3, 20.6, 19.1, 12.3; Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06; Found: C, 76.38; H, 10.33.

(*Z*)-3β-Acetoxy-pregna-5,17(20)-diene (48):[19]

To a solution of alcohol **46** (3.2 g, 8.89 mmol) in dry pyridine (20 mL) was added $POCl_3$, (3.4 mL, 35.56 mmol) at 0 °C. The reaction mixture was stirred at 0°C for 10 min. and at 25 °C for 30 h. The reaction mixture was poured into ice water and extracted with ethyl acetate (3 x 60 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated under vacuo. The crude product was purified by column chromatography on silica gel (EtOAc/pet ether, 3:97) to get pure **48** (2.706 g, 86%) as a white solid. Mp: 94-96 ºC; $[\alpha]_D^{27}$ -62.67 (c 2.27, $CHCl_3$) {lit.,[19] 96-98 ºC; $[\alpha]_D$ –65 (c 2.52, $CHCl_3$)}; IR ($CHCl_3$, cm^{-1}) 1734; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.39 (d, *J* = 4.7 Hz, 1H, CH-6), 5.20-5.10 (m, 1H, CH-20), 4.69-4.53 (m, 1H, CH-3), 2.04 (s, 3H, CH_3COO), 1.66 (d, *J* = 7.2, 3H, CH_3-21), 1.04 (s, 3H, CH_3-19), 0.90 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.4, 150.0, 139.6, 122.4, 113.5, 73.8, 56.5, 50.0, 44.0, 38.0, 36.9, 36.6, 31.6, 31.4, 31.3, 27.7, 24.4, 21.4, 21.1, 19.2, 16.5, 13.1; MS (LCMS) *m/z*: 365.41 (M + Na); Anal. Calcd for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01; Found: C, 80.15; H, 10.43.

4-methyl-1-(trimethylsilyl)pent-1-yn-3-ol (50):

To a solution of trimethylsilylacetylene (6.1 mL, 43.2 mmol) in dry THF (70 mL) under nitrogen atmosphere at -78 °C, was added dropwise *n*-butyllithium (1.6 M in hexane, 22.2 mL, 36 mmol). After 30 min. of stirring, a solution of isobutyraldehyde (3.26 mL, 36 mmol) in THF was slowly added. The resulting mixture was stirred at -78 °C for 1 h and then slowly warmed up to room temperature and stirred for another 6 h. It was then quenched with saturated NH_4Cl. The aqueous layer was extracted with Et_2O (2 x 150 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to get compound **50** (5.75 g, 94%), which was used without further purification in the next step. IR (Neat, cm^{-1}) 3381; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.16 (d, *J* = 5.2 Hz, 1H), 1.95-1.82 (m, 1H), 1.02 (d, *J* = 3.3 Hz, 3H), 0.99 (d, *J* = 3.3, 3H), 0.18 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 150.7, 89.8, 68.1, 34.2, 18.0, 17.3, -0.20, MS (LCMS) *m/z*: 193.12 (M + Na).

4-methyl-1-(trimethylsilyl)pent-1-yn-3-one (51):

To a suspension of alcohol **50** (4.08 g, 24 mmol) and crushed molecular sieves in CH_2Cl_2 (80 ml) was added PCC (9.29 g, 43.2 mmol) at 0 ºC. The resulting suspension was stirred at 25 ºC for 4 h. After completion of reaction (TLC), the solvent was removed under

reduced pressure. The residue was then dissolved in diethyl ether and passed through the short pad of silica gel. The solvent was removed under reduced pressure to obtain the pure ketone **51** (3.870 g, 96% yield). IR ($CHCl_3$, cm^{-1}) 1676 (-C=O); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.72-2.58 (m, 1H), 1.20 (d, *J* = 6.9 Hz, 6H), 0.025 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 191.8, 100.8, 98.6, 42.7, 17.8, -0.8; MS (LCMS) *m/z*: 191.27 (M + Na).

4-methylpent-1-yn-3-one (52):

The ketone **51** (4 g, 23.8 mmol) in CH_2Cl_2 (50 mL) was stirred vigorously overnight with an aqueous solution of NaF (2 g, 74.6 mmol) and NBu_4Br (1.53 g, 4.76 mmol). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 70 mL). The combined organic extracts were dried with Na_2SO_4 and the solvent was removed *in vacuo* to afford ethynylketone **52** (1.65 g, 72%), which was used without further purification for next reaction. IR (Neat, cm^{-1}) 2075, 1681; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.25 (s, 1H, H-C≡C), 2.68 (septet, *J* = 6.9 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 6H).

3β-Acetoxy-cholest-5,16,22-triene-24-one (53):

To the solution of **48** (0.5 g, 1.53 mmol) and ethynylketone **52** (0.294 g, 3.06 mmol) in dry CH_2Cl_2 (15 mL) was added $BF_3{\cdot}OEt_2$ (0.18 mL, 1.53 mmol) at 0 °C. The reaction mixture was stirred at 25 °C for 7 h, quenched with water and extracted with CH_2Cl_2. The organic layer was washed with brine and dried with Na_2SO_4. The solvent was concentrated in vacuo and the residue was purified on flash column chromatography over silica gel (EtOAc/pet ether, 3:97) to furnish **53** (0.473 g, 74%) as a pale yellowish solid.
Mp: 87-88 ºC; $[\alpha]_D^{26}$ -14.74 (c 1.9, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 1732 (CH_3COO), 1693 (-CO, α,β-unsaturated carbonyl); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.82 (dd, *J* = 7.8, 15.8 Hz, 1H, CH-22), 6.15 (d, *J* = 15.8 Hz, 1H, CH-23), 5.42-5.38 (m, 2H), 4.69-4.53 (m, 1H, CH-3), 3.11-3.0 (m, 1H, CH-20), 2.87 (septet, *J* = 6.8 Hz, 1H), 2.04 (s, 3H, CH_3COO), 1.20 (d, *J* = 7.0 Hz, CH_3-21), 1.13 (s, 3H), 1.10 (s, 3H), 1.05 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 204.3, 170.4, 157.0, 151.3, 139.7, 126.1, 123.8, 122.2, 73.7, 57.1, 50.4, 46.9, 38.1, 37.9, 36.8, 36.6, 35.5, 34.6, 31.4, 31.0, 30.2, 27.6, 21.3, 20.5, 19.5, 19.1, 18.4, 16.3; MS (LCMS) *m/z*: 461.33 (M + Na); Anal. Calcd for $C_{29}H_{42}O_3$: C, 79.41; H, 9.65; Found: C, 79.78; H, 10.30.

3β-Acetoxy-24(*S*)-hydroxycholest-5,16,22-triene (54):

To a solution of (*R*)-MeCBS reagent (1 M in toluene, 0.68 mL, 0.68 mmol) in THF (3 mL) was added $BH_3{\cdot}Me_2S$ complex (10 M in tetrahydrofuran, 0.14 mL, 1.36 mmol) and stirred for 1.5 h at 25 °C. The reaction mixture was cooled to -30 °C and solution of ketone **53** (0.6 g, 1.36 mmol) in THF (6 mL) was slowly added. The resulting reaction mixture was stirred at -30 °C for another 1 h. The reaction was quenched with 1 N hydrochloric acid, and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic extract were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (EtOAc/pet ether, 6:94) to afford **54** (0.524 g, 87%) as white solid. Mp: 125-127 ºC; $[\alpha]_D^{27}$ -37.24 (c 2, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 1722 (CH_3COO); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.62 (dd, *J* = 7.8, 15.3 Hz, 1H), 5.47 (dd, *J* = 7.1, 15.3 Hz, 1H), 5.40-5.37 (m, 2H), 4.65-4.57 (m, 1H, CH-3), 3.82 (t, *J* = 6.3 Hz, 1H, CH-24), 2.92-2.85 (m, 1H, CH-20), 2.04 (s, 3H, CH_3COO), 1.14 (d, *J* = 6.8 Hz, 3H, CH_3-21), 1.06 (s, 3H, CH_3-19), 0.93 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.80 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 159.0, 139.8, 137.9, 129.1, 122.4, 122.2, 77.8, 73.8, 57.3, 50.5, 46.9, 38.1, 36.8, 36.7, 35.5, 34.8, 33.9, 31.5, 31.0, 30.3, 27.7, 21.3, 2 X 20.6, 19.2, 18.2, 18.0,16.3; MS (LCMS) *m/z*: 463.47 (M + Na); Anal. Calcd for $C_{29}H_{44}O_3$: C, 79.04; H, 10.06; Found: C, 78.78; H, 10.19.

3β,24(*S*)-Diacetoxy-cholest-5,16,22-triene (55):

To the solution of alcohol **54** (0.15 g, 0.34 mmol) in pyridine (3 mL) was added acetic anhydride (1 mL, 30 mmol) and DMAP (0.009 g, 0.07 mmol). The reaction mixture was stirred at 27 °C for 10 h and pyridine was evaporated under reduced pressure. The residue was dissolved in ethyl acetate washed with water and brine, dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to afford crude product which was purified by column chromatography on silica gel (EtOAc/pet ether, 3:97) to get compound **55** (0.157 g, 96%). Mp; 121-122 ºC; $[\alpha]_D^{26}$ -48.65 (c 1.11, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 1734; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.66 (dd, *J* = 7.5, 15.4 Hz, 1H), 5.42-5.31 (m, 3H), 5.02 (t, *J* = 6.7 Hz, 1H, CH-24), 4.69-4.53 (m, 1H, CH-3), 2.93-2.79 (m, 1H, CH-20), 2.33 (d, *J* = 7.7 Hz, 2H), 2.06 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO), 1.12 (d, *J* = 6.8 Hz, 3H, CH_3-21), 1.05 (3, 3H, CH_3-19), 0.91 (d, *J* = 2.8 Hz, 3H), 0.87 (d, *J* = 2.8, 3H), 0.78 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 170.4, 158.7,

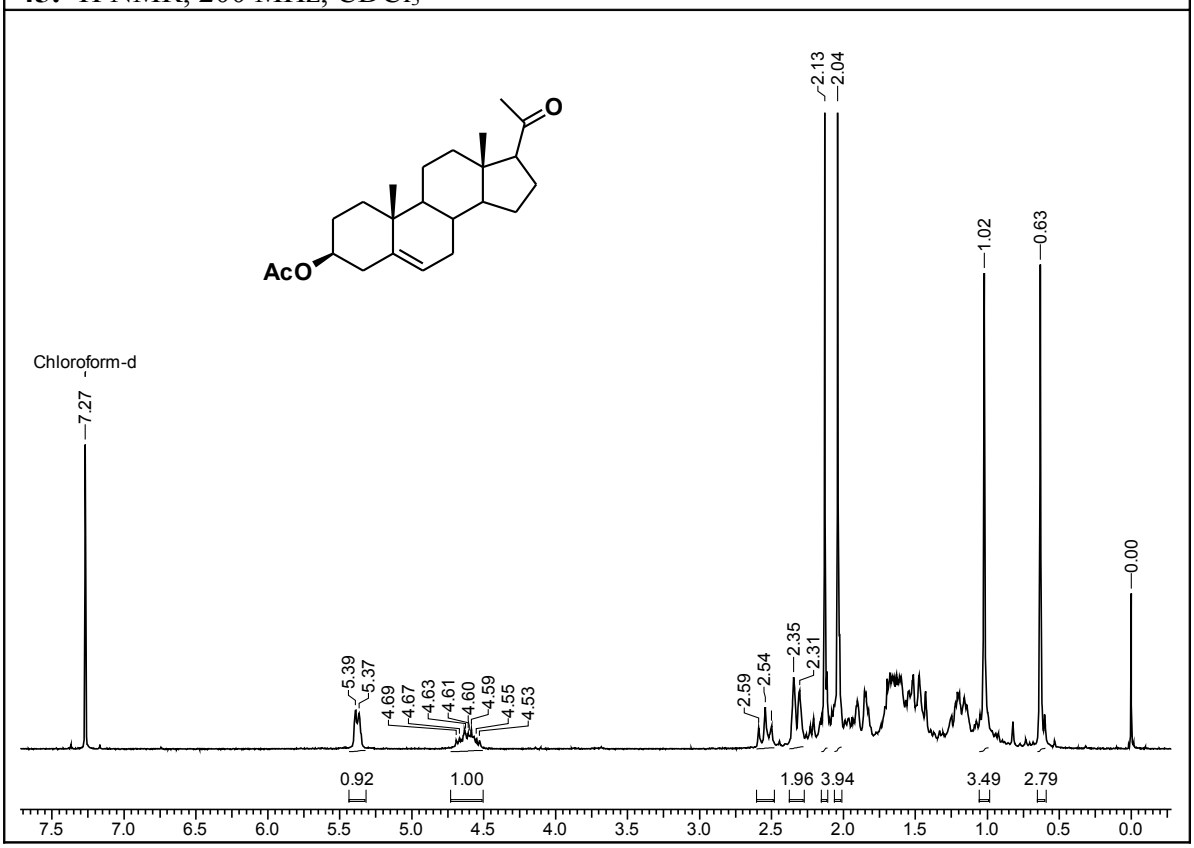
139.9, 124.5, 122.5, 79.2, 73.9, 57.3, 50.5, 46.9, 38.1, 36.9, 36.8, 35.5, 34.8, 32.2, 31.5, 31.0, 30.3, 27.7, 21.4, 21.3, 20.6, 20.3, 19.2, 18.2, 18.1, 16.3; MS (LCMS) *m/z*: 505.43 (M + Na); Anal. Calcd for $C_{31}H_{46}O_4$: C, 77.14; H, 9.61; Found: C, 77.42; H, 9.39

3β,24(*S*)-Diacetoxy-cholest-5-ene (56):

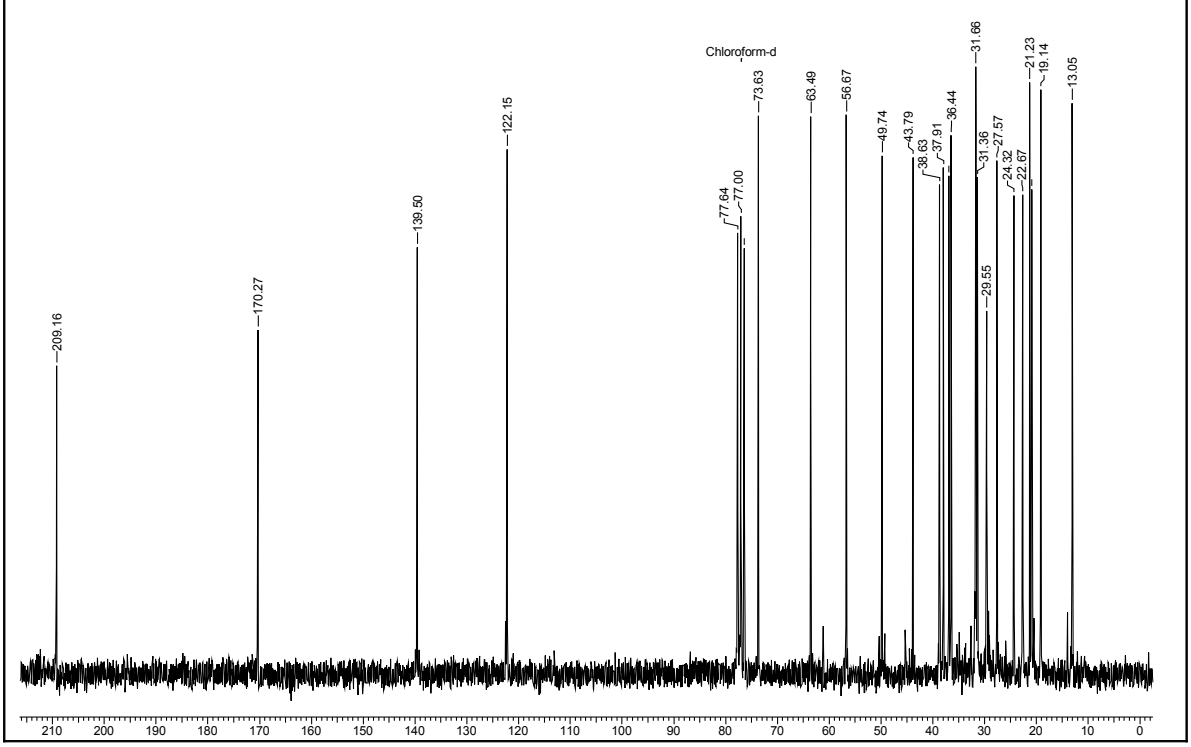
To a solution of **55** (0.05 g, 0.1 mmol) in ethyl acetate (6 mL) was added 10% platinum on carbon (0.008 g). The reaction mixture was then stirred in the hydrogen atmosphere (1 atm. of H_2) for 9 h. The reaction mixture was filtered through celite pad, washed with ethyl acetate/pet ether (1:1) mixture and concentrated to near dryness. The crude product was then purified by silica gel column chromatography (EtOAc/pet ether, 3:97) to afford pure **56** (0.047 g, 94%). Mp: 80-81 ºC; $[\alpha]_D^{26}$ -40.71 (c 1.13, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 1728; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.38 (d, *J* = 4.3 Hz, 1H), 4.74-4.52 (m, 2H, CH-3 + CH-24), 2.32 (d, *J* = 7.8 Hz, 2H), 2.05 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 1.02 (s, 3H, CH-19), 0.93-0.85 (m, 9H), 0.67 (s, 3H, CH-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0, 170.5, 139.5, 122.6, 78.6, 73.9, 56.6, 55.7, 49.9, 42.2, 38.0, 36.9, 36.5, 35.3, 31.8, 31.7, 31.4, 31.3, 28.0, 27.7, 27.2, 24.2, 21.4, 21.1, 20.9, 19.2, 18.5, 17.7, 11.8; MS (LCMS) *m/z*: 509.37(M + Na); Anal. Calcd for $C_{31}H_{50}O_4$: C, 76.50; H, 10.35; Found: C, 76.16; H, 10.54.

2.7 Selected Spectra

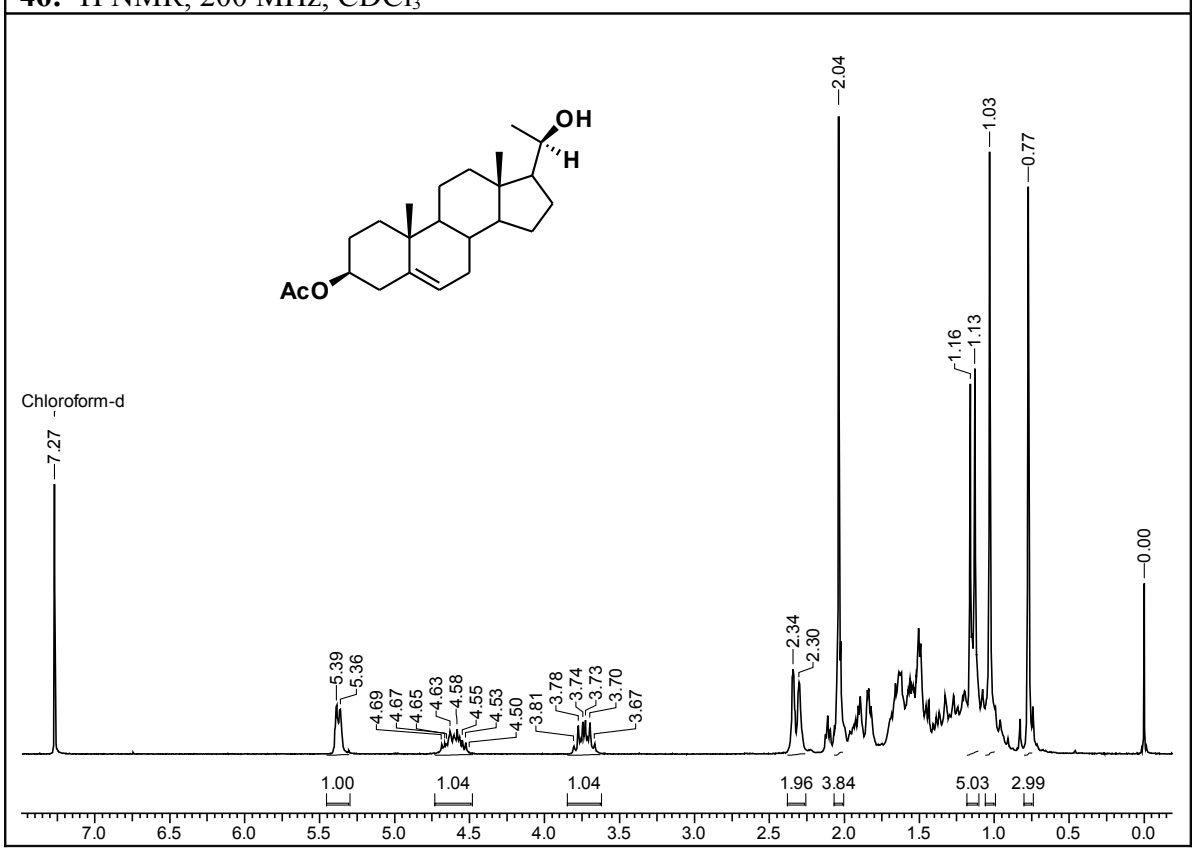
45: ^{1}H NMR, 200 MHz, $CDCl_3$



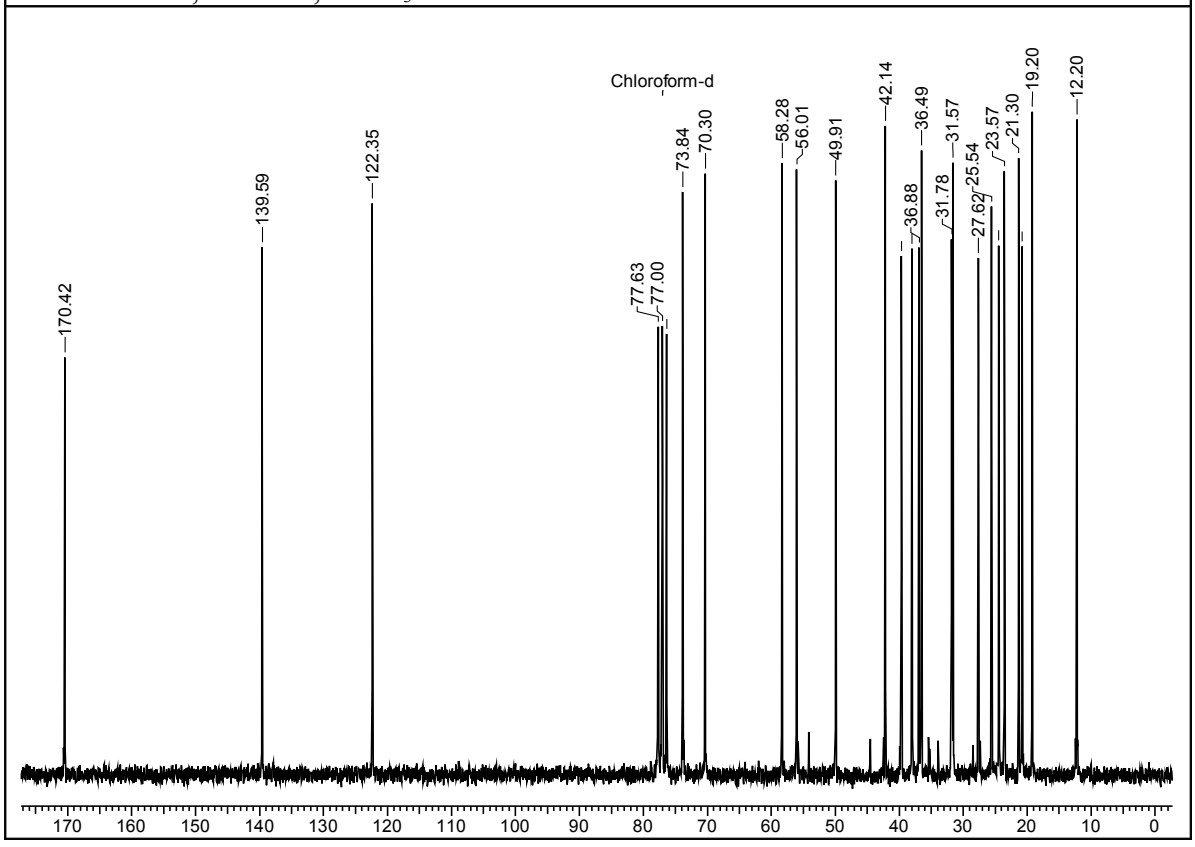
45: ^{13}C NMR, 50 MHz, $CDCl_3$



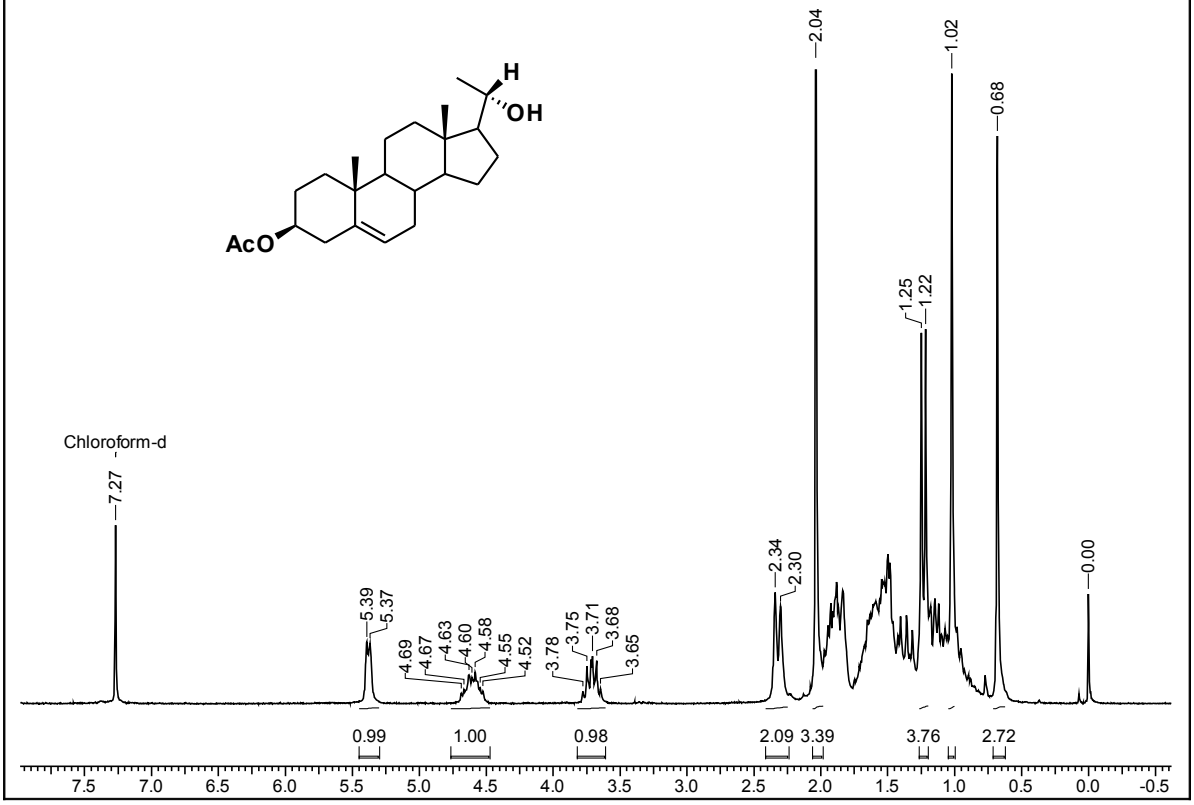
46: ^{1}H NMR, 200 MHz, $CDCl_3$



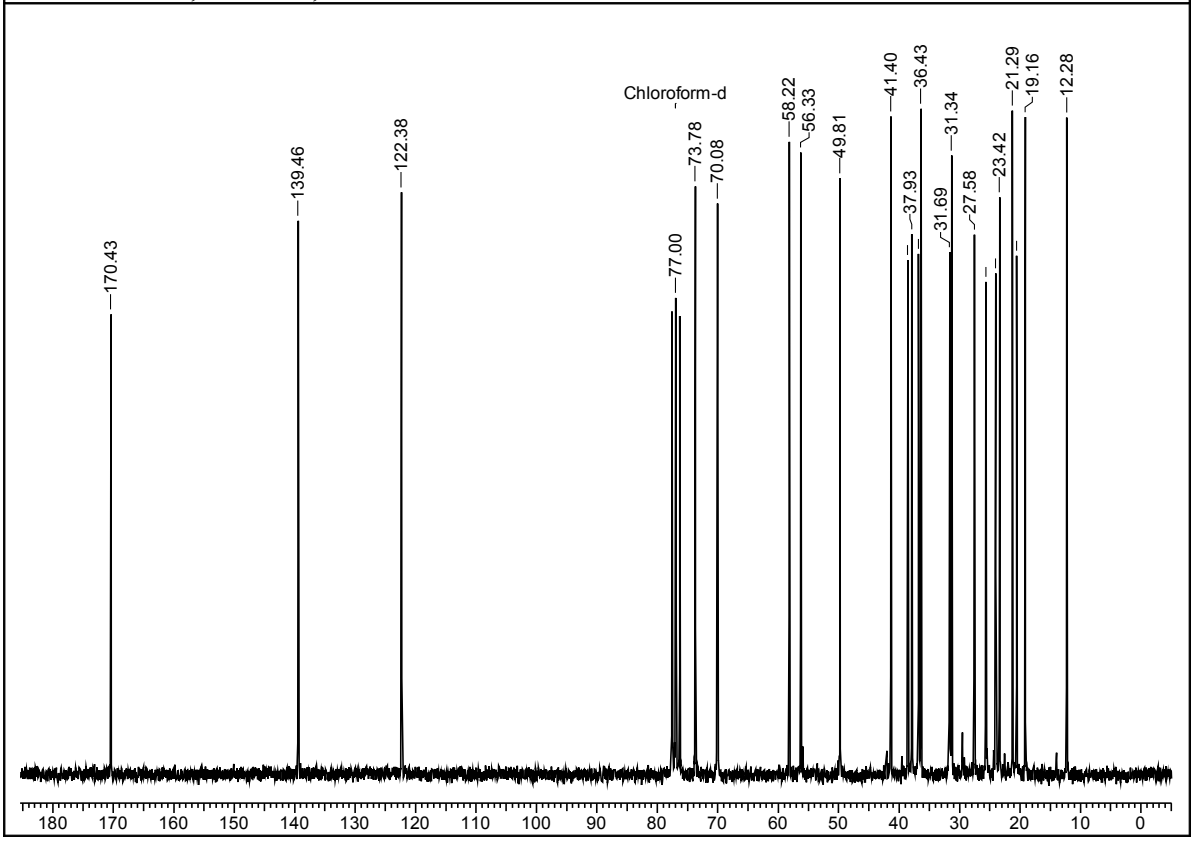
46: ^{13}C NMR, 50 MHz, $CDCl_3$



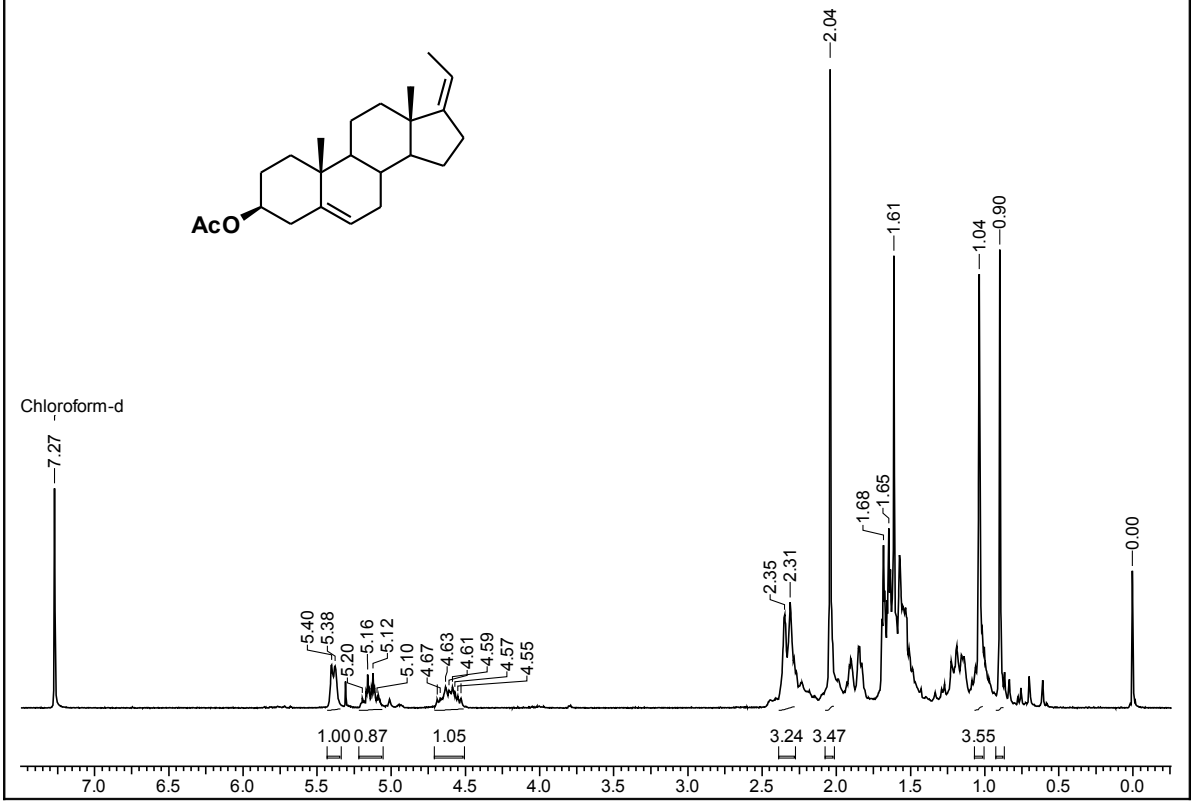
47: ^{1}H NMR, 200 MHz, $CDCl_3$



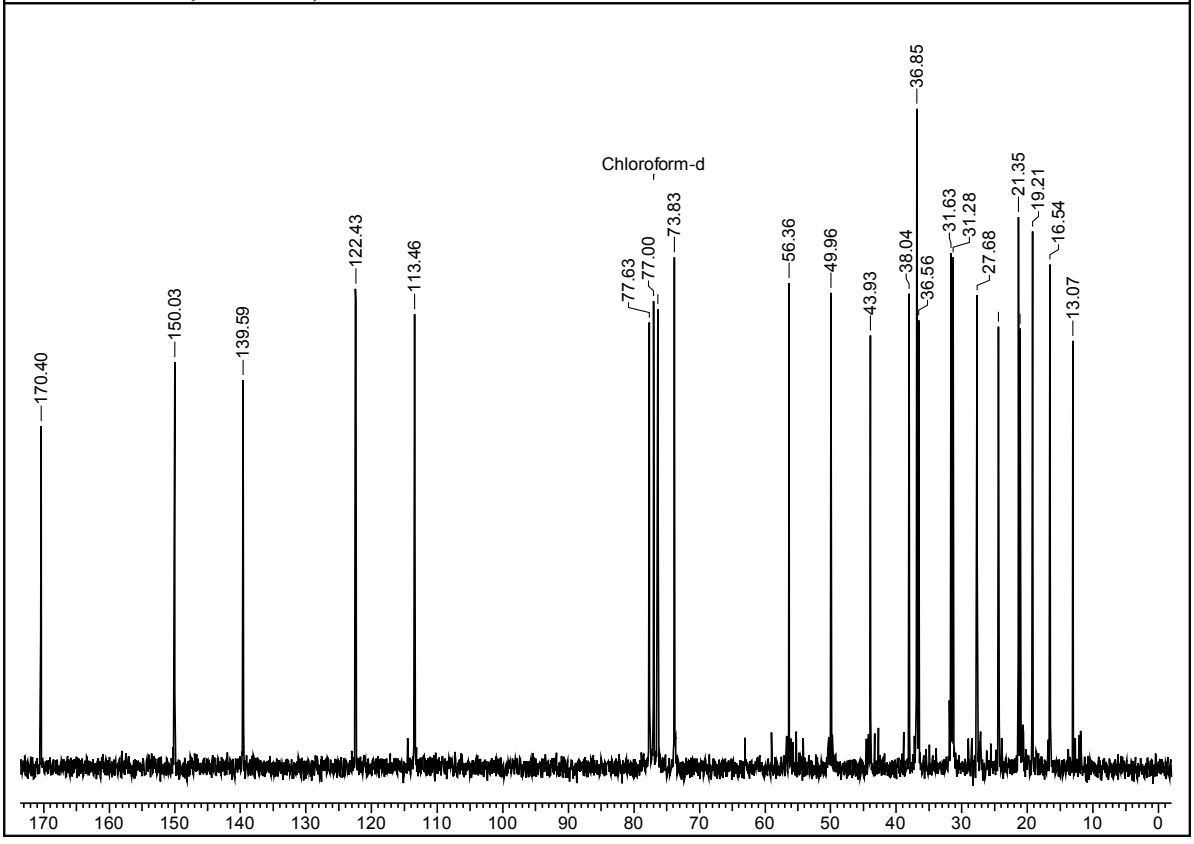
47: ^{13}C NMR, 50 MHz, $CDCl_3$



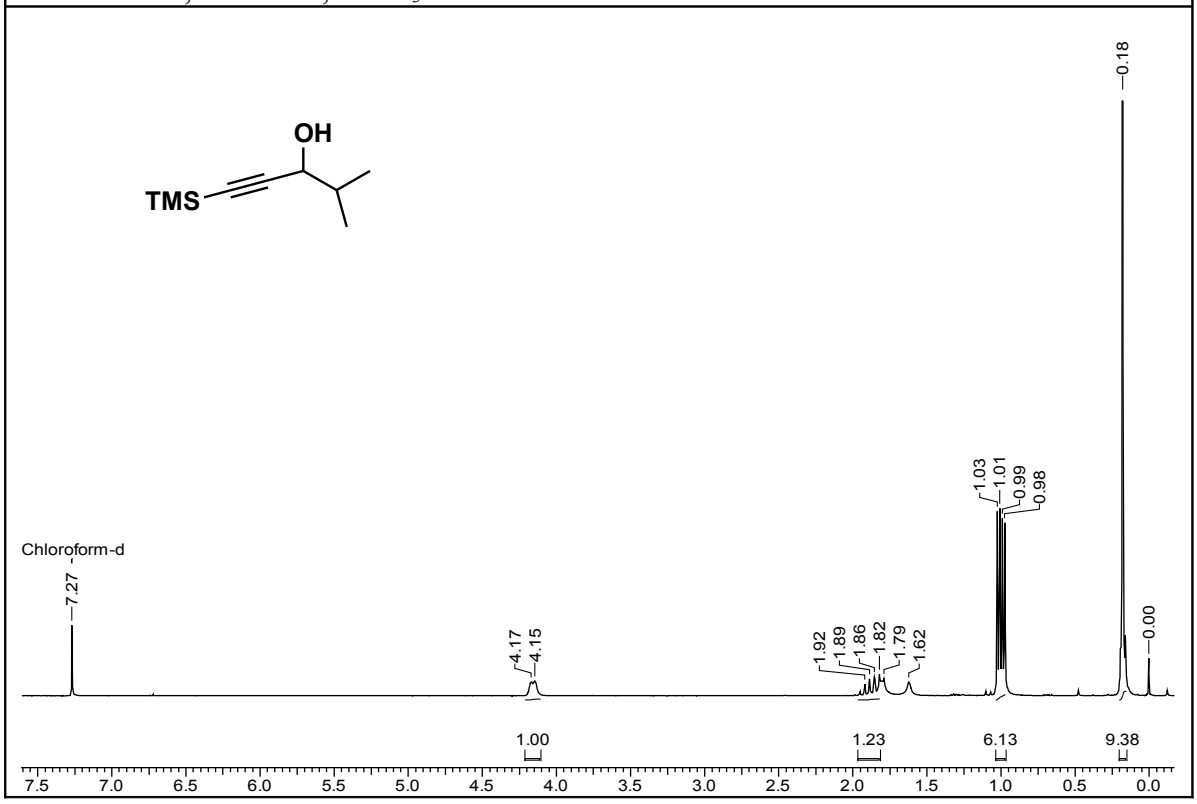
48: ^{1}H NMR, 200 MHz, $CDCl_3$



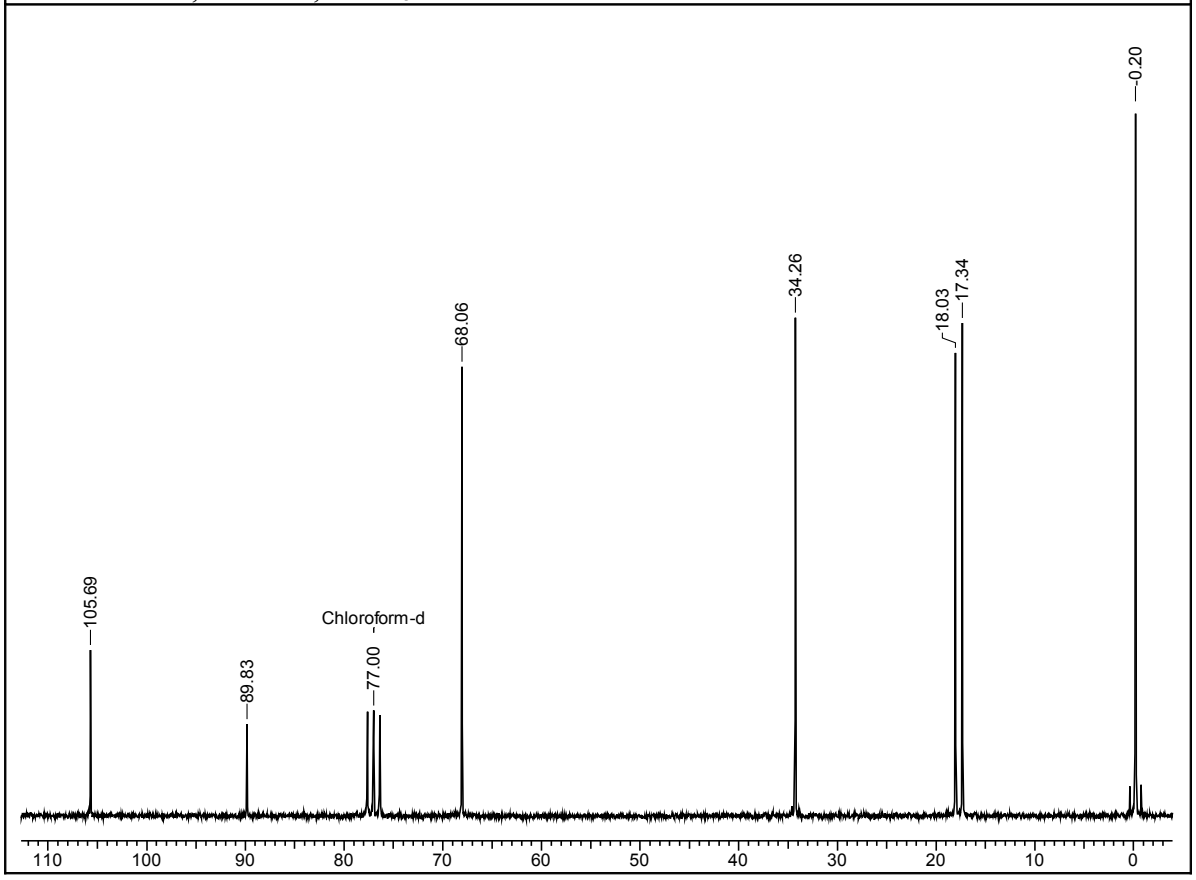
48: ^{13}C NMR, 50 MHz, $CDCl_3$



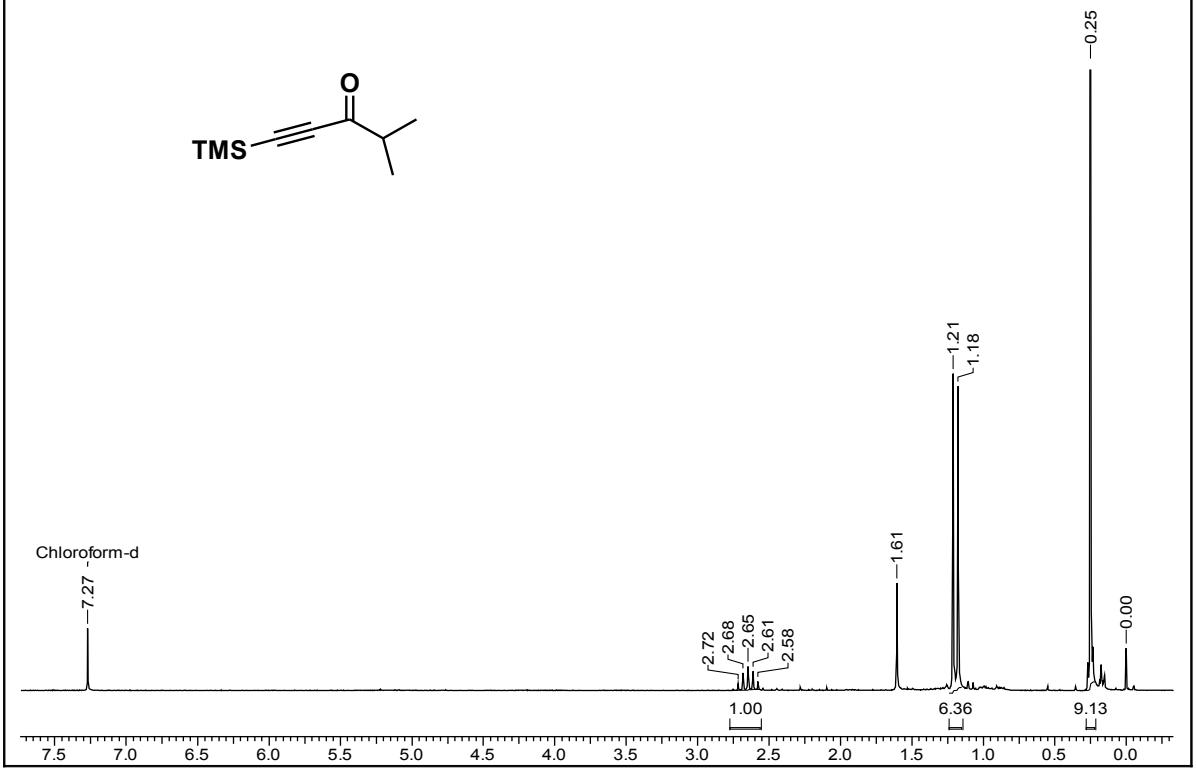
50: ^{1}H NMR, 200 MHz, $CDCl_3$



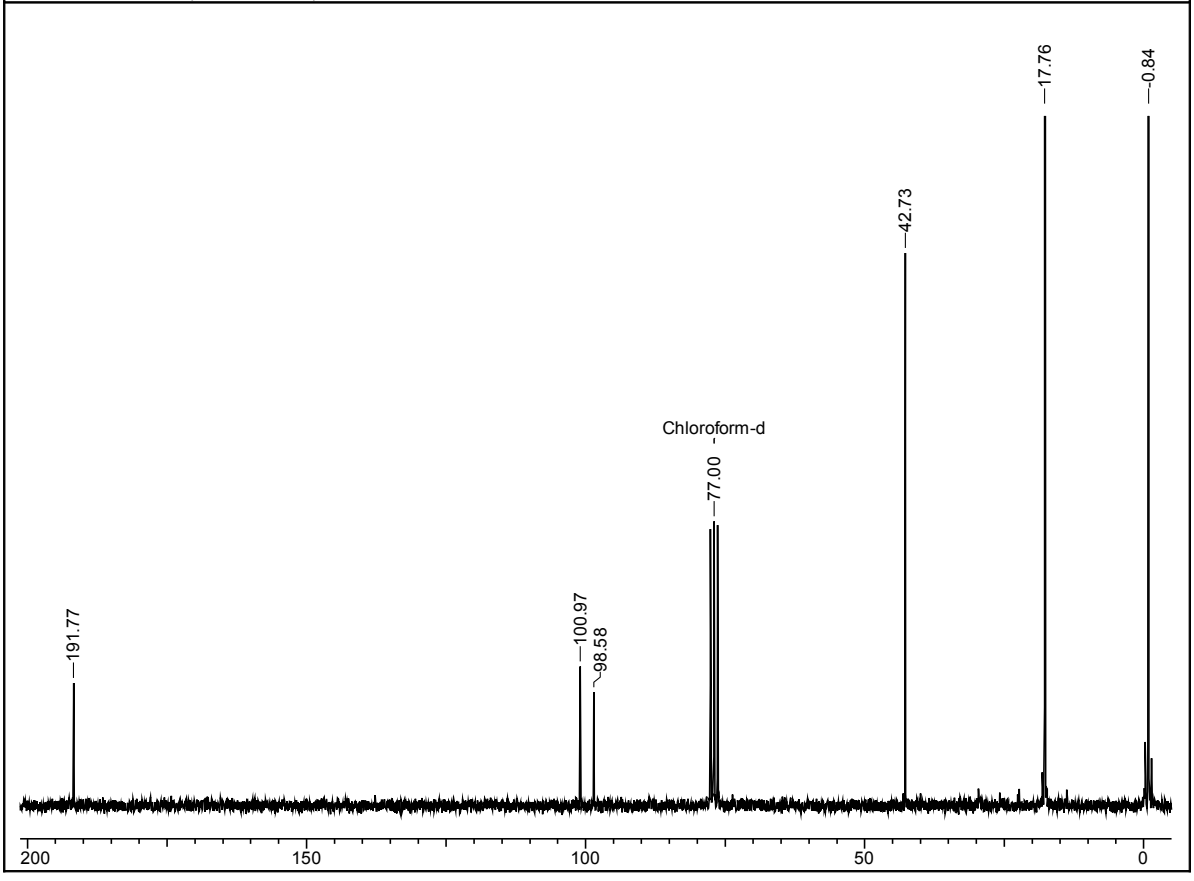
50: ^{13}C NMR, 50 MHz, $CDCl_3$



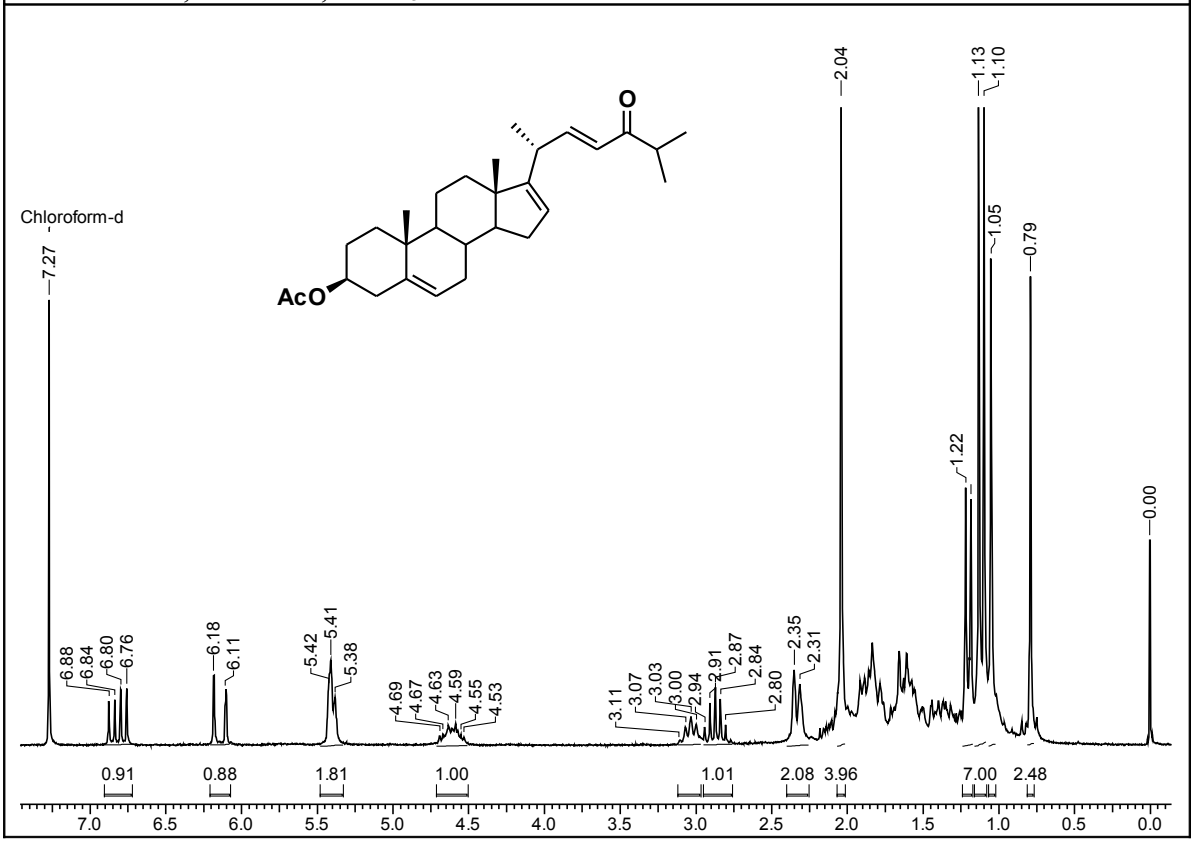
51: ^{1}H NMR, 200 MHz, $CDCl_3$



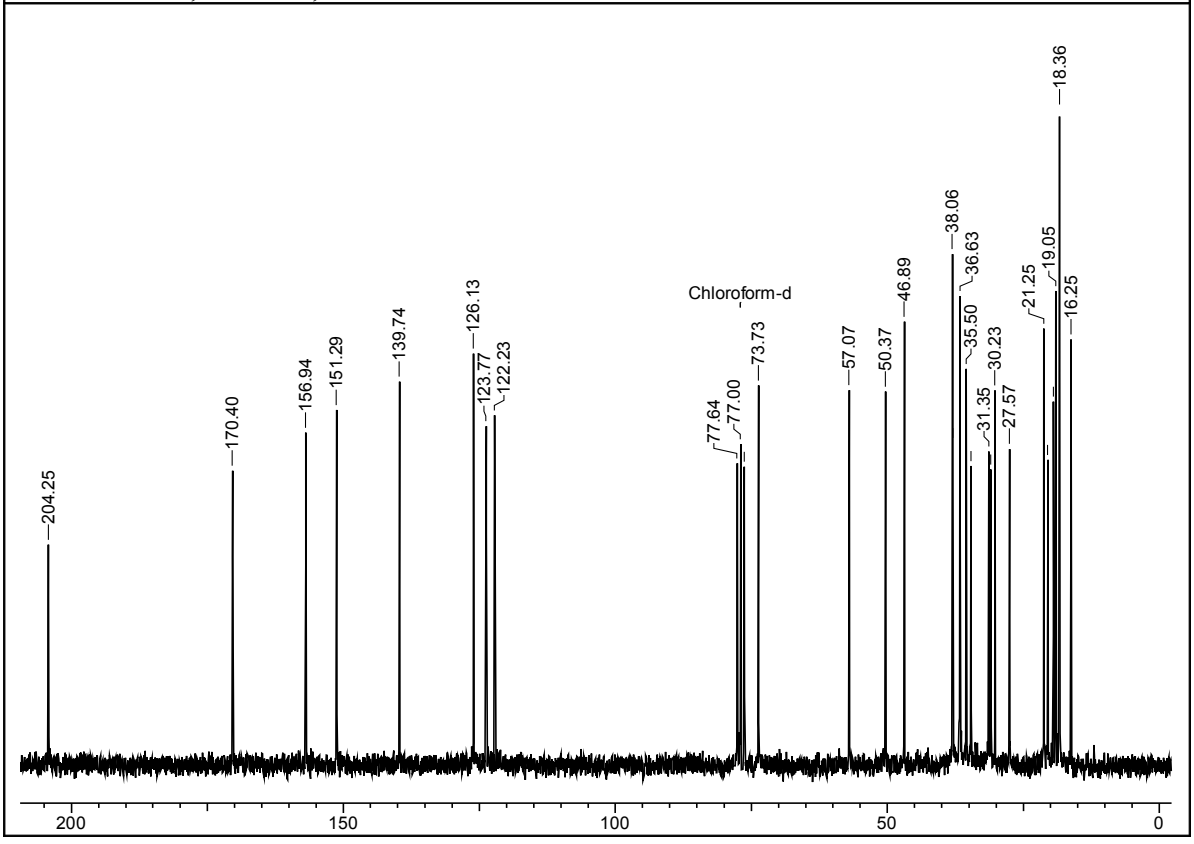
51: ^{13}C NMR, 50 MHz, $CDCl_3$



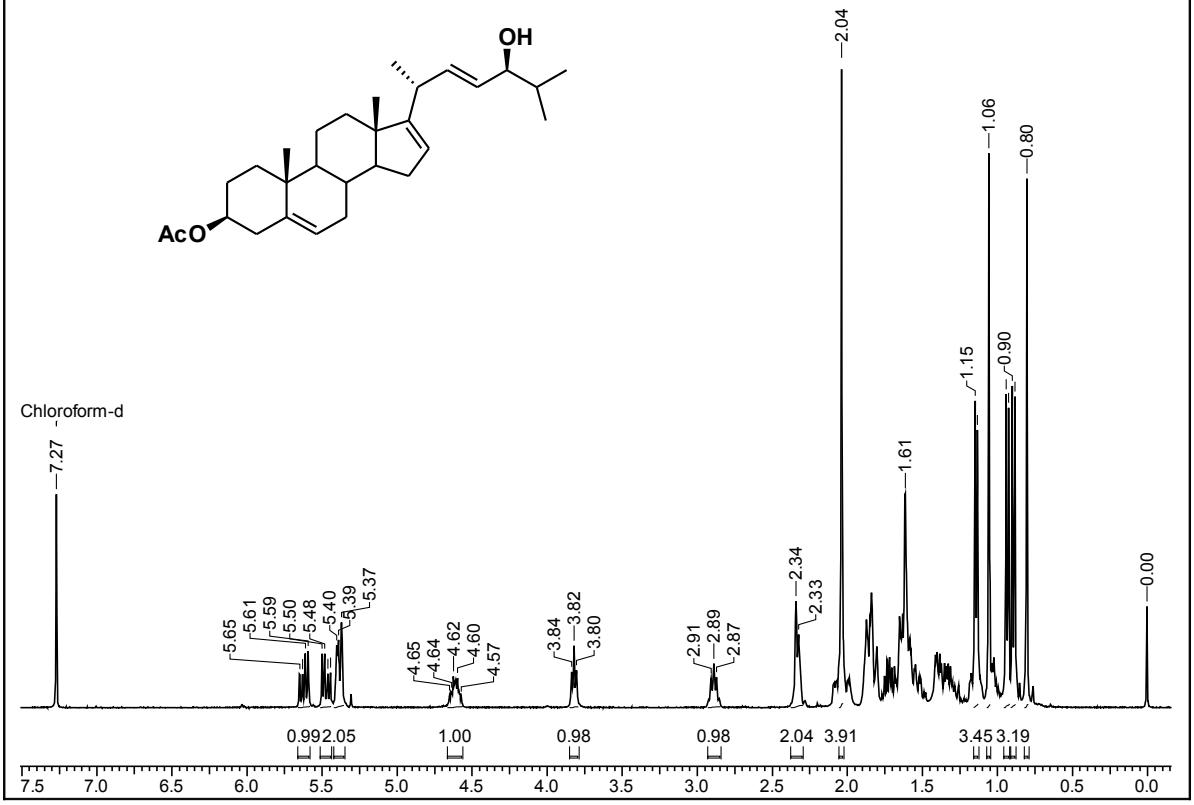
53: ^{1}H NMR, 200 MHz, $CDCl_3$



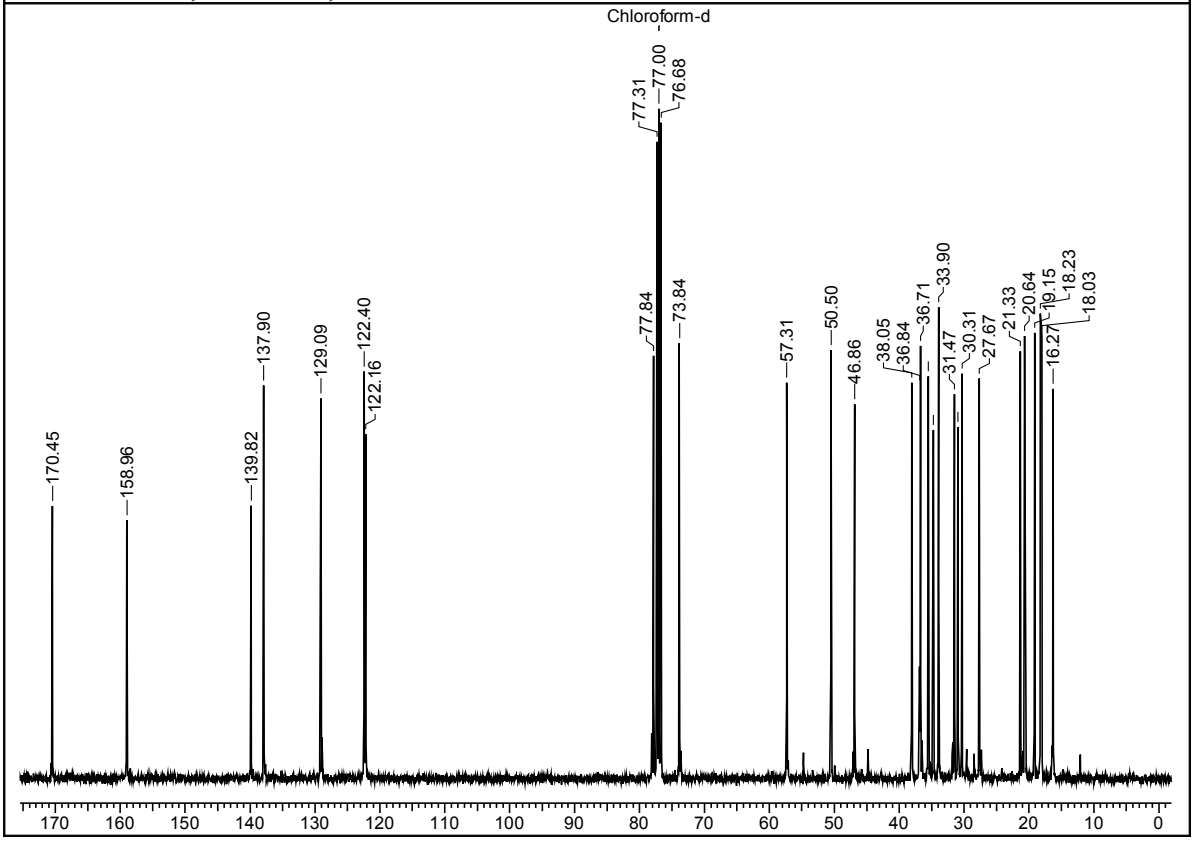
53: ^{13}C NMR, 50 MHz, $CDCl_3$



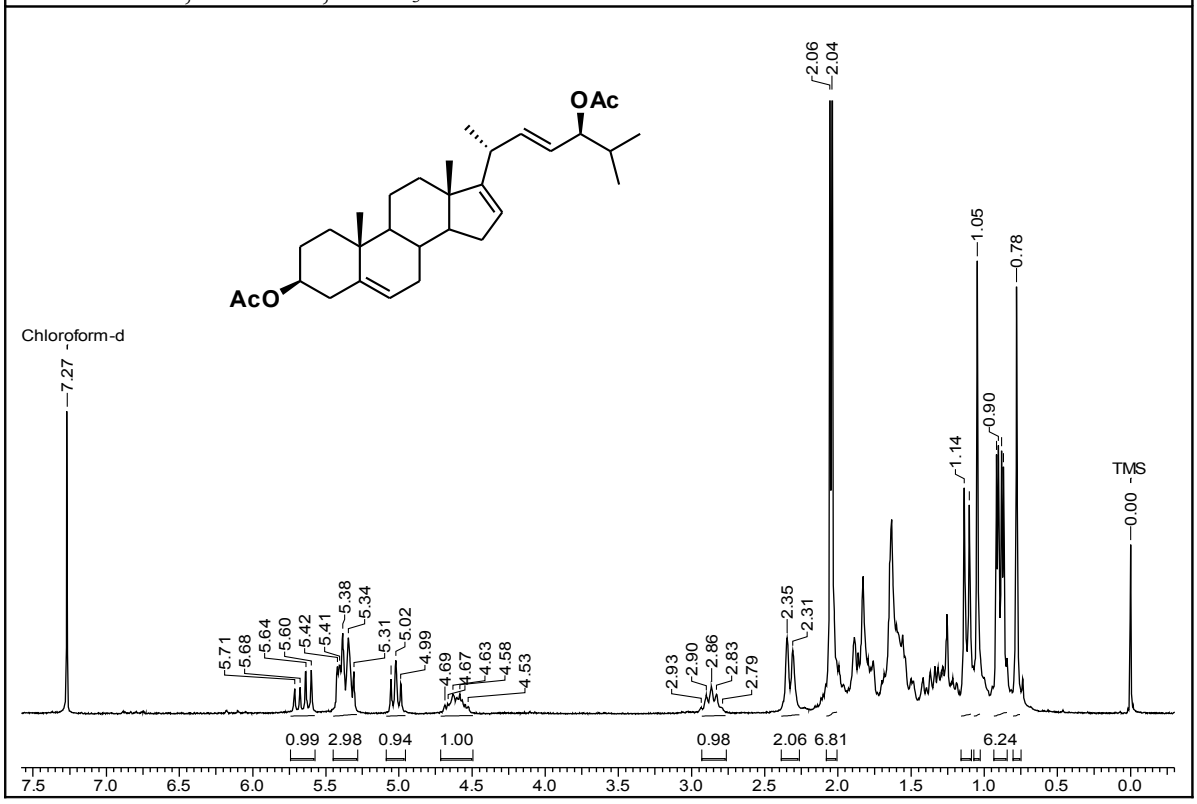
54: ^{1}H NMR, 400 MHz, $CDCl_3$



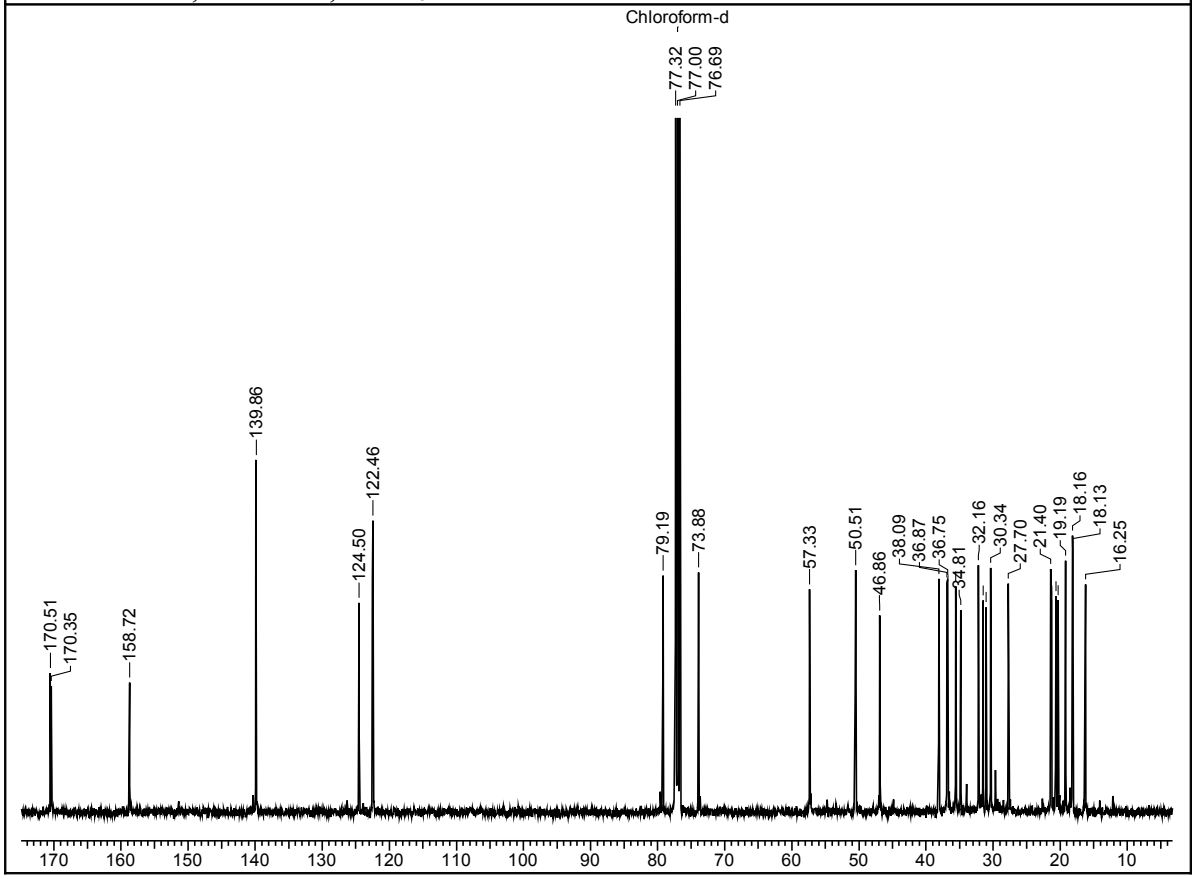
54: ^{13}C NMR, 100 MHz, $CDCl_3$



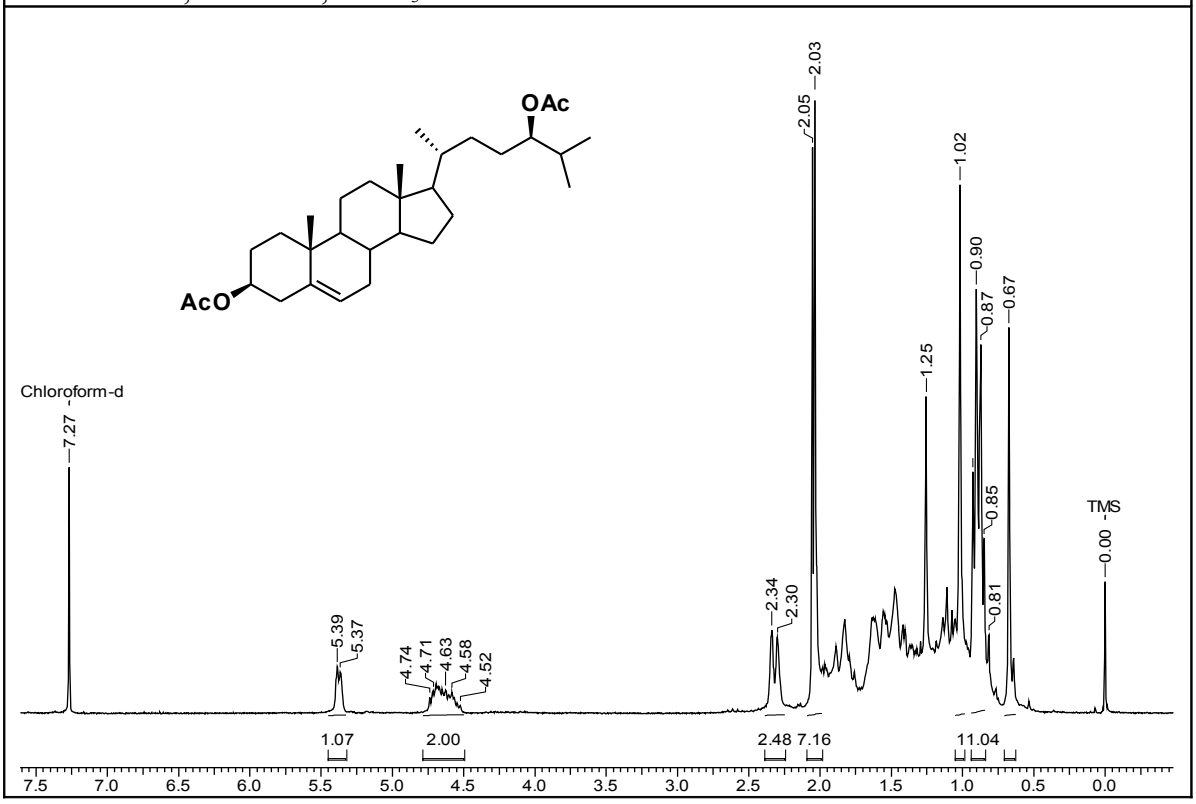
55: ^{1}H NMR, 200 MHz, $CDCl_3$



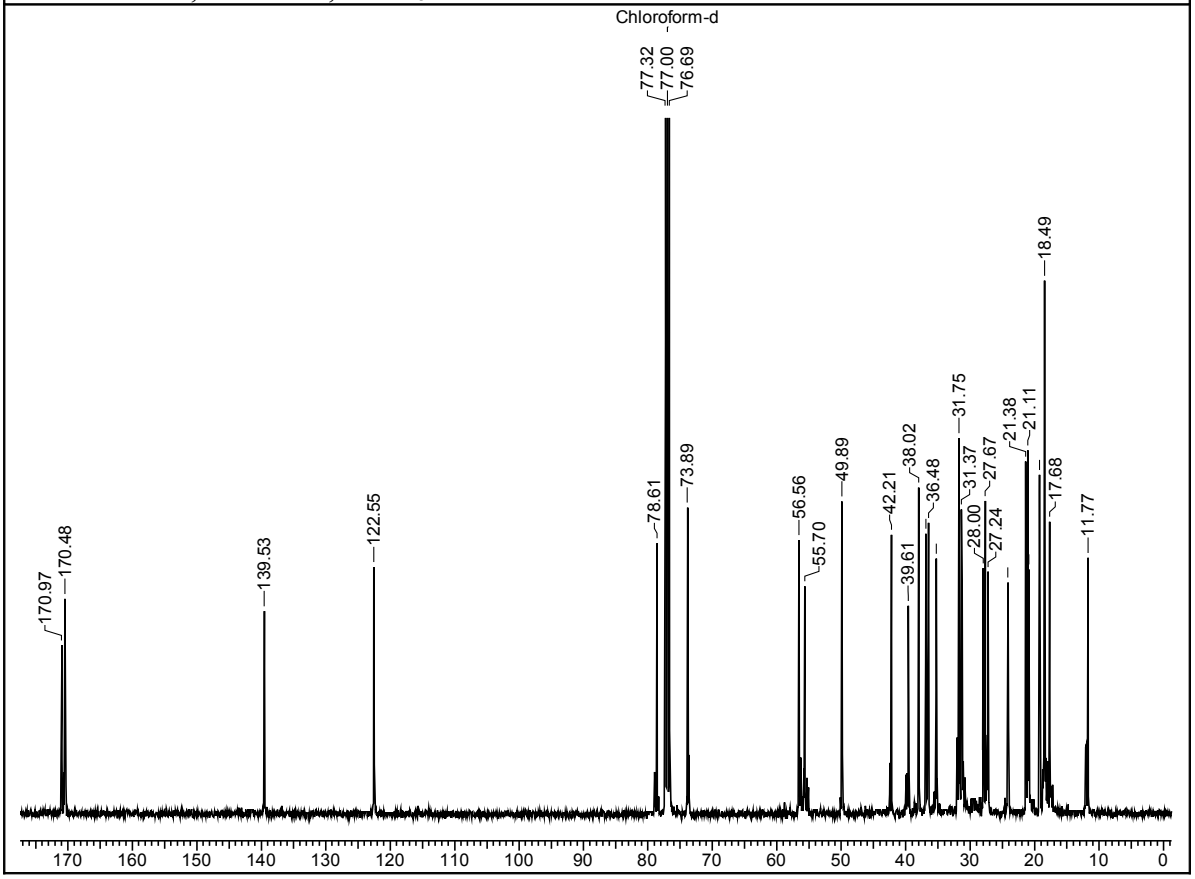
55: ^{13}C NMR, 100 MHz, $CDCl_3$



56: ^{1}H NMR, 200 MHz, $CDCl_3$



56: ^{13}C NMR, 100 MHz, $CDCl_3$



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Chapter 3

Enantioselective Reduction of Prochiral Ketones Using Chiral Amino Alcohols

3.1 Abstract

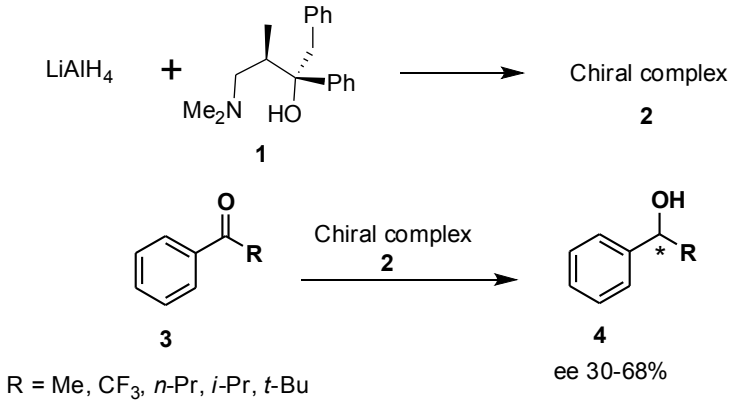
Six new chiral 1,2-amino alcohol derivatives have been synthesized starting from (1*R*,2*R*)-1-phenyl-2-aminopropane-1,3-diol. Asymmetric reduction of aryl ketones with *in situ* generated oxazaborolidine from these amino alcohol derivatives and $BH_3.Me_2S$ afforded secondary alcohols with good yield and moderate to high enantiomeric excess.

3.2 Introduction

The design of an asymmetric transformation reaction is a great challenge in organic chemistry, in particular, the development of enantioselective homogeneous catalysts in which an optically active ligand can induce asymmetry for a given reaction. Among the various asymmetric reactions enantioselective reduction of prochiral ketones to optically active alcohols have achieved great interest,[1] since optically active alcohols are valuable chiral building blocks for the synthesis of natural products and bioactive compounds.[2] In view of the enormous utility of optically active alcohols, numerous methods for the enantioselective reduction of carbonyl compounds by using mixture of $LiAlH_4$, $NaBH_4$, or BH_3·THF with chiral 1,2-diols, 1,2-amino alcohols, or 1,2-diamines have been studied.[3] Some of these methods are briefly discussed below.

Mosher approach[4]

Mosher *et al.* have reported the stereoselective reduction of aromatic ketones by a chiral reagent prepared from **(***2S,3R*)-4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol (Darvon alcohol) **1** and lithium aluminium hydride (Scheme 1). Chiral secondary alcohols were obtained with substantial enantiomeric purities.

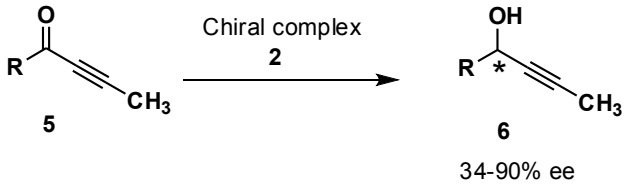


Scheme 1

Cohen approach[5]

Later on Cohen *et al.* have used the same chiral complex **2** (Scheme 1) derived from Darvon alcohol **1** and lithium aluminium hydride for the asymmetric reduction of α,β-acetylenic ketones (Scheme 2). The alcohols were obtained in 34-90% enantiomeric excess. Also this group has synthesized the different analogues of **1** and used for the

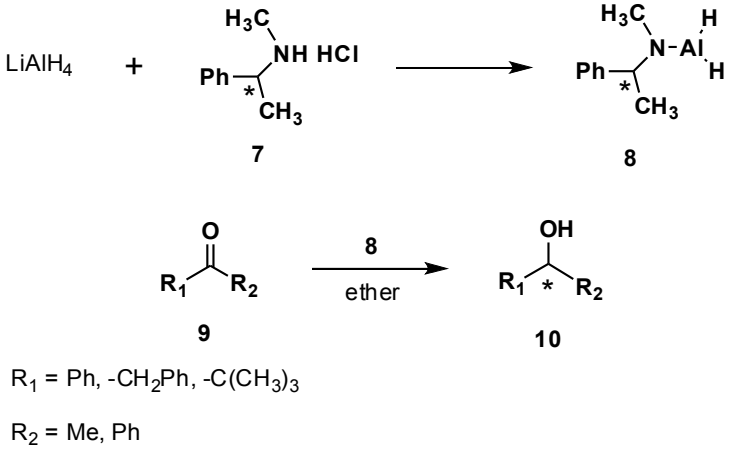
asymmetric reduction of α,β-acetylenic ketones with the complex of lithium aluminium hydride.



Scheme 2

Giongo approach[6]

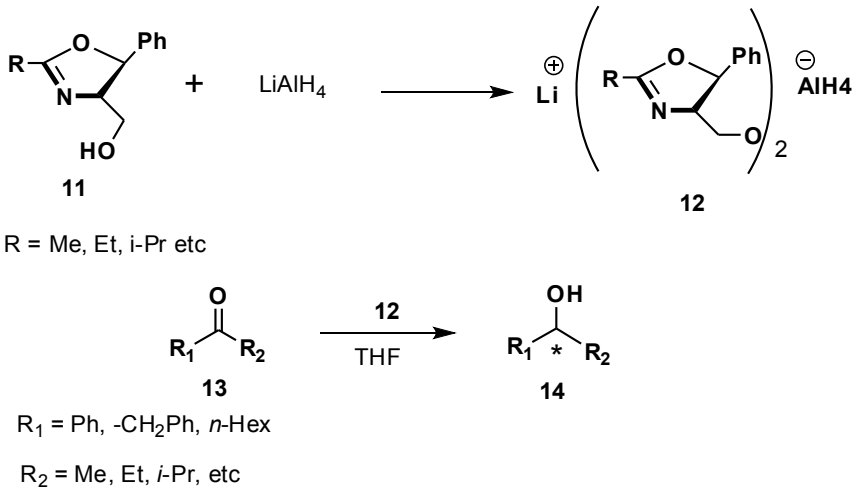
Giongo *et al.* have described the synthesis of dialkylaminoalane complex **8** by using (-)-*N*-methyl-*N*-(1-phenylethyl)amine·HCl **7** and $LiAlH_4$. The asymmetric reduction of prochiral ketones attempted by dialkylaminoalane complex **8** gave the secondary alcohols **10** in 15-84% optical yields (Scheme 3).



Scheme 3

Mayers approach[7]

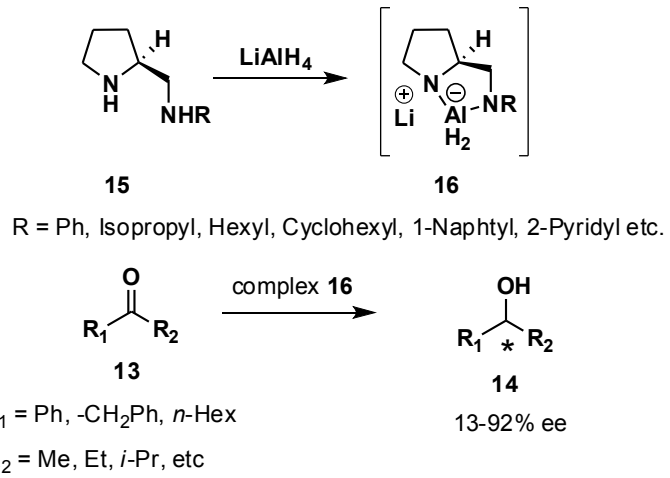
Several chiral oxazolines **11** were prepared by Mayers *et al.* in order to assess for the asymmetric reduction of various ketones. The oxazoline-hydride reagent **12** obtained from oxazolines **11** and $LiAlH_4$ were employed for the asymmetric reduction of various prochiral ketones **13** to its chiral secondary alcohols **14**. The alcohols were obtained upto 65% enantiomeric excess with 80-90% recovery of oxazoline **11** (Scheme 4).



Scheme 4

Asami approach[8]

Asymmetric reduction of prochiral ketones have been carried out by Asami and co-workers with various hydride reagents prepared from lithium aluminium hydride and (S)-2-(*N*-substituted aminomethyl)pyrolidines **15**, derived easily from commercially available L-proline. Reduction of prochiral ketones **13** using chiral complex **16** gave the optically active alcohols **14** in 13-92% enantiomeric excess (Scheme 5).

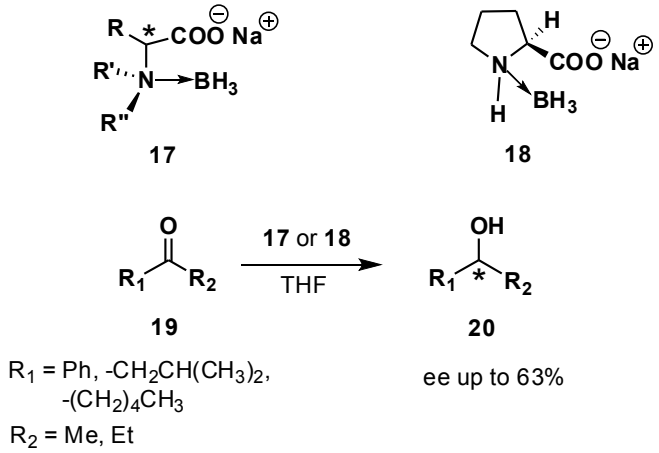


Scheme 5

Umino approach[9]

Umino *et al* utilized the chiral Na salts of α-amino acid-borane complexes **17**, which can be prepared from equimolar amounts of sodium borohydride and optically active α-amino acids at room temperature. Sodium prolinate-borane complexes **18** were found to be the

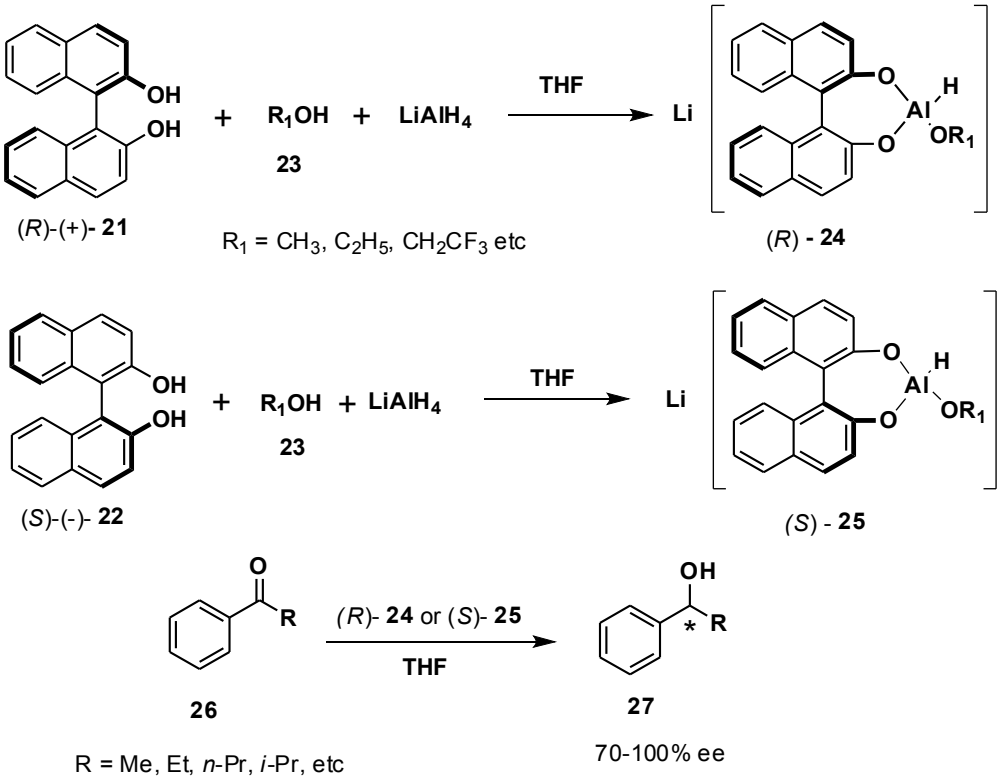
best reagents for the reduction of various prochiral ketones **19** giving chiral secondary alcohols with optical yields as high as 63% and chemical yields of 66-92% (Scheme 6).



Scheme 6

Noyori approach[10]

Noyori *et al.* have developed a new chiral hydride reagent, BINAL-H **24** or **25** by the combination of lithium aluminium hydride with equimolar amounts of (*R*)- or (*S*)-2,2′dihydroxy-1,1′-binaphthyl **21** or **22** and a simple alcohol (Scheme 7). This reducing agent exhibits very high enantioselectivity in the reduction of prochiral alkyl phenyl ketones. The reduction of prochiral alkyl phenyl ketons **26** by using BINAL-H **24** or **25** furnished the alcohols **27** in 70-100% enantiomeric excess. Later[11] on they utilized BINAL-H for the reduction of variety of structurally diverse unsaturated carbonyl compounds such as aromatic ketones, acetylenic ketones and olefinic ketones etc. In all the cases alcohols were obtained in high optical purity.



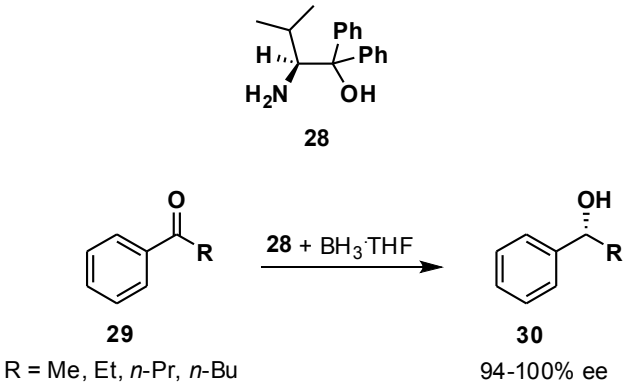
Scheme 7

Although good levels of enantioselectivity have been reported for some of these early observed studies, these reducing systems have seen very limited use in synthesis. This is because the reactive species are generally unknown, insoluble in many cases and there has been no reliable information on the mechanistic basis for enantioselectivity. The Noyori reagent BINAL-H which is made from (*S*)- or (*R*)-BINOL, $LiAlH_4$, and ethanol seems to be structurally better defined and also has very high enantioselectivity with certain substrates. The high cost and marginal practicability are factors which detract from the usefullness of this reducing system. Stoichiometric amount of reagent was used for the reduction in all the above mentioned approaches.

Itsuno approach[12]

Itsuno and co-workers have carried out pioneering work on the asymmetric reduction of prochiral aromatic ketones with the reagent prepared from (*S*)-(-)-2-amino-3-methyl-1,1-diphenylbutane-1-ol **28** and borane (Scheme 8). The asymmetric reduction of aromatic ketones **29** with *in-situ* generate **28**-borane complex proceeded in a highly sterioselective manner, giving the alcohols **30** in 94-100% enantiomeric excess with nearly 100% chemical yield. Itsuno have used the amino alcohol **28** in stoichiometric amount for the

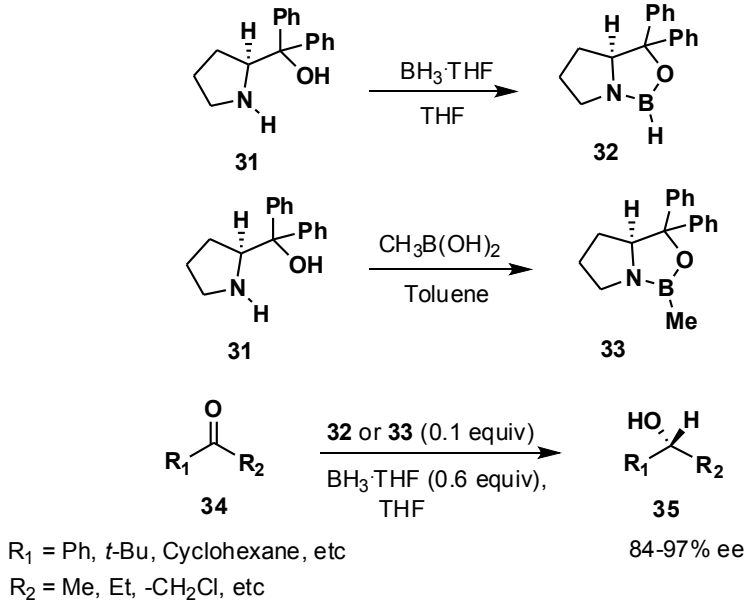
reduction of ketones. The dependence of the enantioselectivity of this process on the ratio of BH_3 to amino alcohol, the quantity of hydride present in the reagent, and the steric bulk of the ketone substituents was studied. However, no mechanistic rationale was proposed by Itsuno *et al.* for this very high enantioselectivity.



Scheme 8

Corey approach[13,14]

The catalytic behaviour of the more sterically hindered oxazaborolidine based on (*S*)-(-)-2-(diphenylhydroxymethyl)pyrrolidine **31** (prepared from L-proline) was introduced in borane reduction of ketones by Corey and co-workers.[13] Treatment of amino alcohol **31** with BH_3·THF gave oxazaborolidine **32** (CBS catalyst named after Corey, Bakshi, Shibata), which was characterized by ^{1}H NMR and ^{11}B NMR spectroscopy and mass spectrometry (EI) (Scheme 9). The methyl substituted oxazaborolidine **33**[14] was prepared from amino alcohol **31** and methylboronic acid and characterized by ^{1}H NMR and ^{11}B NMR spectroscopy and mass spectrometry (EI) (Scheme 9). The reduction of variety of prochiral ketones have been carried out using catalytic amount of oxazaborolidines **32** and **33** to get the alcohols in a very high enantiomeric excess (84-97%) and in quantitative yields. The reduction of ketones with methyl substituted oxazaborolidine **33** as the catalyst frequently resulted in appreciably higher enantioselectivity than with **32**. Additionally, catalyst **32** is extremely air and moisture-sensitive, and its formation requires extended heating with BH_3 under increased pressure. On the other hand, catalyst **33** is less sensitive and can be stored in closed containers, weighed or transferred in air, and is readily formed by heating **31** and methylboronic acid at reflux in toluene for 4 h with removal of water.



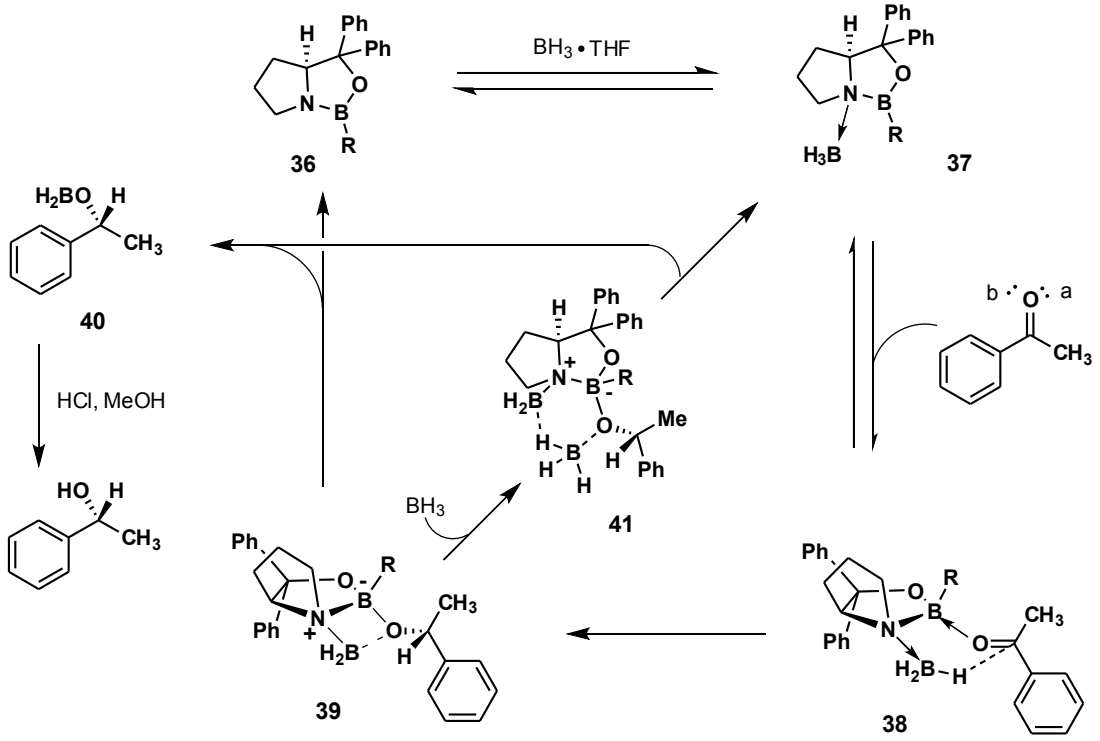
Scheme 9

Mechanism of oxazaborolidine reduction

Mechanism of the oxazaborolidine catalysed reduction of prochiral ketones to its chiral alcohol is studied by Corey and co-workers.[15] The general mechanistic model which was developed for reduction of ketones with oxazaborolidine catalyst **32** or **33** (and analogous catalysts) is outlined in Scheme 10. The mechanistic model explains (i) the absolute stereochemistry of the reduction, (ii) the outstanding enantioselectivity obtained for the reduction, (iii) the exceptional rate enhancement of the reduction, and (iv) the turnover of the catalyst.

The initial step in the pathway is the rapid (and probably reversible) coordination of BH_3 to the Lewis basic nitrogen atom on the α face of oxazaborolidine **36** to form the cis-fused oxazaborolidine·BH_3 complex **37**. Unambiguous support for the initial step comes from the observation by ^{11}B NMR spectroscopy[13] that **36** and BH_3·THF form the 1:1 complex *B*-H-**37** (**37**, R = H) and the fact that crystalline *B*-Me-**37** (**37**, R = Me) can be isolated and structurally defined by single-crystal X-ray diffraction analysis.[16] The coordination of the electrophilic BH_3 to the nitrogen atom of **36** serves to activate BH_3 as a hydride donor and also to increase strongly the Lewis acidity of the endocyclic boron atom. The strongly Lewis acidic complex **37** then readily binds to the ketonic substrate, for example acetophenone, at the more sterically accessible electron lone pair (a in the case of acetophenone) and cis to the vicinal BH_3 group as shown in **38**. This manner of

binding minimizes unfavorable steric interactions between the oxazaborolidine and the ketone, and aligns the electronically deficient carbonyl carbon atom and the coordinated



Scheme 10. Proposed mechanism for the catalytic enantioselective reduction of ketones.

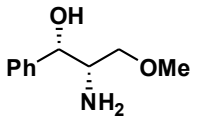
BH_3 for stereoelectronically favorable, face-selective hydride transfer via a six-membered transition state to form the reduction product **39**. Thus, the rate enhancement for the oxazaborolidine-catalyzed reduction is due to the activation of the stoichiometric reducing agent BH_3 by coordination with the Lewis basic nitrogen atom of **36** with simultaneous intensification of the Lewis acidity of the boron atom in the heterocycle for coordination to the ketone. This leads to subsequent enthalpically and entropically favorable faceselective intramolecular hydride transfer.[17]

Dissociation of the reduction product from **39** to regenerate the oxazaborolidine catalyst may occur by two different pathways: (a) reaction of the alkoxide ligand attached to the endocyclic boron atom with the adjacent boron atom of **39** to regenerate **36** and form the borinate **40** by cycloelimination;[18] or (b) by the addition of BH_3 to **39** to form a sixmembered BH_3-bridged species **41**, which decomposes to produce the catalyst-BH_3 complex **37** and borinate **40**.[19,20]

3.3 Present work

3.3.1 Objective

From the above discussion it has been observed that enantioselective oxazaborolidine catalysed borane reduction of prochiral ketones to chiral secondary alcohols provides convenient access to a wide variety of optically active secondary alcohols. We thought of synthesizing different amino alcohol derivatives from the commercially available and inexpensive chiral amino alcohol, which can be used for the reduction of prochiral ketone in combination with borane. In 1987 Mandal[21] carried out asymmetric reduction of alkyl aryl ketones with borane-methyl sulfide and the reagent prepared from (1*S*,2*S*)-(+)-2-amino-3-methoxy-1-phenyl-1-propanol **42** (Figure 1), which is the antipode of **48** with the primary alcohol at C-3 protected as methyl ether. They obtained the corresponding secondary alcohols in 30-60% enantiomeric excess. Mandal's work arises our inquisitiveness of protecting the primary alcohol at C-3 with a bulkier group and to determine the ee of the derived secondary alcohols. Very recently Chen *et al.*[22] have carried out enantioselective reduction of a *meso*-cyclic imide to its cyclic n-acyl aminol in excellent ee. This is mediated by a chiral oxazaborolidine catalyst derived from (1*S*,2*S*)-(+)-threo-1-(4-nitrophenyl)-2-amino-3-triphenylmethoxypropanol **43**. The catalyst has the primary alcohol at C-3 which is protected as *O*-triphenylmethyl ether and there is a *p*-nitrophenyl moiety at C-1 position. Chen's observation gave us the impetus to synthesise similar type of chiral catalyst in which the primary alcohol at C-3 are protected as *O-tert*-butyldiphenylsilyl (TBDPS) ether **49,** *O-tert*-butyldimethylsilyl (TBDMS) ether **50** and to



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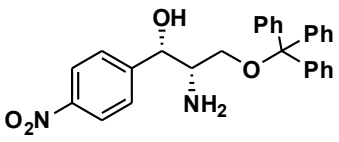

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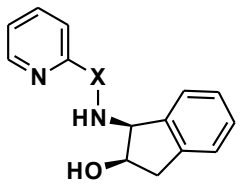

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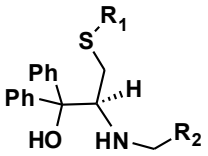
$X = CH_2, CO, SO_2$



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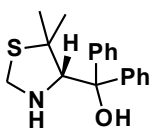
$R_1 = CH_3$, *i*-Pr

$R_2 = CH_3$, Et, *n*-Pr



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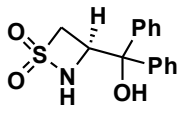


47

Figure 1

use these bulky ethers for the enantioselective reduction of prochiral ketones (Figure 2). Again, for good optical yield tertiary hydroxy[15] functionality is essential and we resorted to synthesise C-1 tertiary alcohols **51** and **52** and utilized these optically active amino alcohols for asymmetric reduction of ketones. Sibi et al used[23] borane and 10 mol% of an oxazaborolidine, derived from cis-2-amino indanol ligand **44** (Figure 1). The primary amino group of the parent amino indanol was modified to secondary amino group with 2-pyridylmethyl, 2-pyridylcarbonyl and with 2-pyridylsulfonyl moiety. These bifunctional ligands have been evaluated in the reduction of prochiral ketones with high chemical efficiency and fair to high enantioselectivity. Again, Martens and co-workers[24] have developed acyclic and cyclic secondary amino alcohols **45-47** for enantiocontrolled catalytic reduction of alkyl aryl ketones in good enantiomeric excess. The work of Sibi[23], Martens[24] and others[25] lead us to transform the C-2 primary amine to its secondary amino compound **53** and to observe the enantioselectivity for the reduction of ketones.

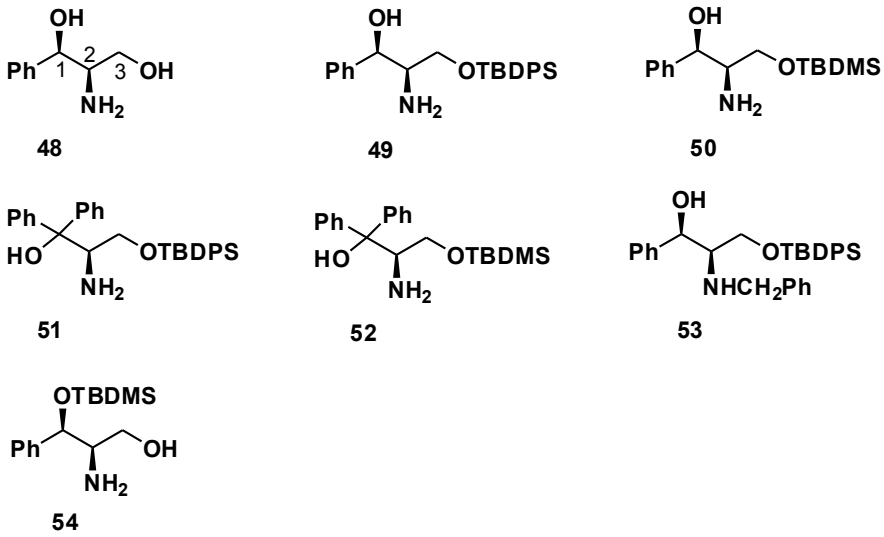


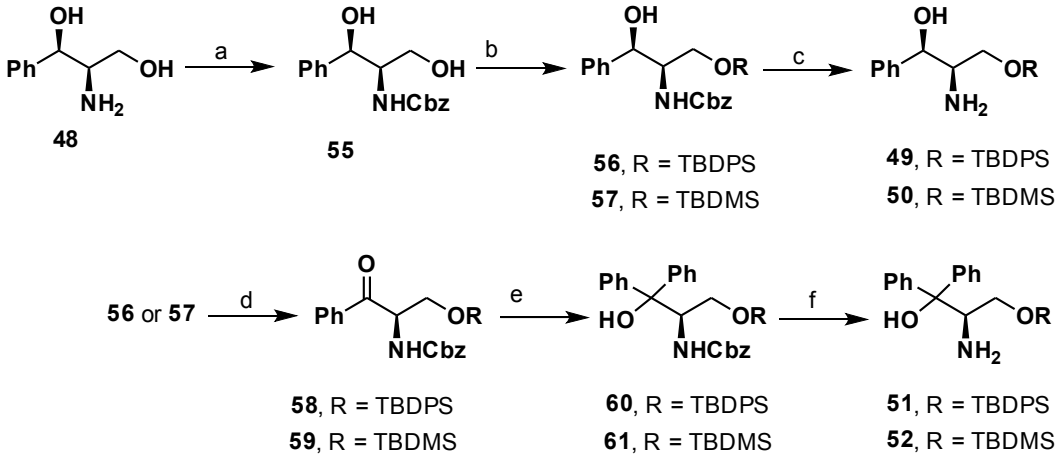
Figure 2. Amino alcohols **49-54** synthesised from **48**

It would also be highly interesting and thought provoking to build the oxazaborolidine with the primary alcohol at C-3 and amino group at C-2 position. For this the secondary C-1 hydroxy group was protected as TBDMS ether to afford compound **54** and applied this amino alcohol for the asymmetric borane reduction of alkyl aryl ketones. We would like to report here the syntheses of six new chiral amino alcohols **49-54**, their use for the enantioselective reduction of aryl ketones and the optical yields of the derived secondary alcohols.

3.3.2 Results and Discussion

Commercially available[26] and inexpensive chiral amino alcohol (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **48**, a precursor for the preparation of chloramphenicol has been utilized by us for the synthesis of all the six new chiral auxiliaries. The amino alcohols **49** and **50** have been prepared from (1*R*,2*R*)-1-phenyl-2-amino-1,3-propanediol **48** (Scheme 11**)**. Amino group in compound **48** was protected as carbamate by treating it with benzyl chloroformate in presence of sodium carbonate in water-dioxane to get amino protected compound **55** in quantitative yield. The ^{1}H NMR spectrum of **55** showed characteristic signals at δ 7.31-7.29 (m, 10H) due to the aromatic protons and at δ 4.96 (s, 2H) corresponding to methylene protons ($PhCH_2OCO$) of carbamate group. Also its ^{13}C NMR spectrum displayed typical signals at δ 156.9 for carbamate carbonyl carbon (-C=O). Further, its IR spectrum showed strong absorption band at 1697 cm^{-1} corresponding to the carbamate carbonyl group. On treatment of **55** with *tert*-butyldiphenylsilyl chloride (1.1 equivalents) and imidazole in DMF afforded primary hydroxy protected TBDPS ether **56** in 92% yield. In a similar way primary hydroxy protected TBDMS ether **57** was prepared from 55 and *tert*-butyldimethylsilyl chloride. Deprotection of carbamate group in **56** and **57** by hydrogenation using 15% Pd-C in methanol gave the required amino alcohol derivatives **49** and **50** in excellent yields. The disappearance of signals corresponding to methylene proton ($PhCH_2OCO$) in the ^{1}H NMR spectrum of **49** confirmed the formation of amino alcohol. The formation of amino alcohol **49** was also confirmed by its ^{13}C NMR, IR and mass spectrum analysis. Compounds **51** and **52** with *tert*-alcohol functionality were synthesized from the primary amino and primary hydroxy protected alcohols **56** and **57**. The secondary hydroxy group in compound **56** and **57** were subjected to oxidation with 2-iodoxybenzoic acid (IBX)[27] in ethyl acetate to afford ketones **58** and **59** in very good yield. The appearance of peak at δ 197.0 in ^{13}C NMR spectrum of **59** corresponding to benzylic carbonyl carbon and disappearance of peak due to benzylic hydroxy carbon confirms the formation of ketone **59**. Its IR spectrum showed sharp absorption band at 1716 cm^{-1} and 1689 cm^{-1} due to benzylic carbonyl and carbamate carbonyl group. The ketone **58** on treatment with an excess of Grignard reagent prepared from bromobenzene in THF furnished the tertiary alcohol **60** in 84% yield. The presence of signal at δ 81.2 due to tertiary hydroxy carbon and absence of signal corresponding to benzylic carbonyl carbon in ^{13}C NMR spectrum of **60** confirmed the formation of tertiary alcohol. Using similar reaction condition tertiary alcohol **61** was obtained in 87% yield from ketone **59**.

Lastly the deprotection of carbamate group in **60** and **61** by catalytic hydrogenation using Pd-C gave the required amino alcohols **51** and **52** respectively.

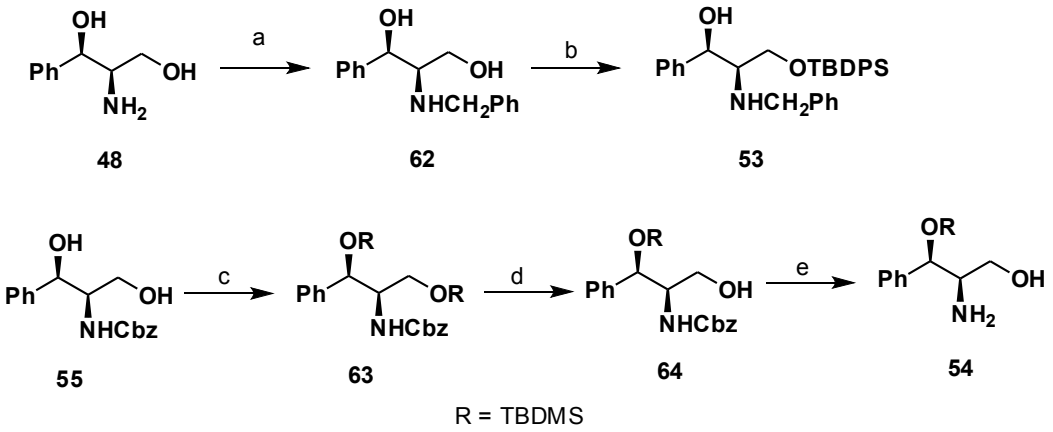


Scheme 11. Reagents and conditions: (a) $PhCH_2OCOCl$, Na_2CO_3, water-dioxane, 30 °C, 2 h, 99%; (b) Compound-**56**: TBDPSCl, imidazole, DMF, 30 °C, 10 min., 92%; Compound-**57**: TBDMSCl, imidazole, DMF, 25 °C, 0.5 h, 90%; (c) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, 98% for **49** and 99% for **50**; (d) IBX, EtOAc, reflux, 5 h, 98% for **58** and 97% for **59**; (e) Bromobenzene, Mg, THF, 25 °C, 1 h, 84% for **60** and 87% for **61**; (f) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, 99% for **51** and **52**.

Secondary amino alcohol **53** has been synthesized from amino diol **48** (Scheme 12). Compound **48** was converted into an imine with benzaldehyde which was reduced with sodium borohydride to give N-benzylated compound **62** with an overall yield of 76% in two steps. Primary hydroxy group in compound **62** was selectively converted into *tert*-butyldiphenylsilyl ether **53** in 83% yield.

Protection of secondary hydroxy group at C-1 in compound **54** has been carried out successfully as shown in Scheme 12. Initially both the primary and secondary hydroxy group in compound **55** was protected with TBDMSCl (2.4 equivalent) and imidazole in DMF to furnish compound **63** in excellent yield. The formation of **63** was confirmed from its ^{1}H NMR spectrum, which showed characteristic two singlets at δ 0.93 and 0.89 corresponding to methyl protons of two tertiary butyl group [$-C(CH_3)_3$] and four singlets at δ 0.09, 0.07, 0.04 and -0.15 are due ($-SiCH_3$) protons. Selective deprotection of primary O-TBDMS group with camphorsulfonic acid (CSA)[28] in methanol afforded alcohol **64** in 96% yield. Deprotection of carbamate group in **64** by catalytic hydrogenation gave amino alcohol **54** in 98% yield. The disappearance of signals corresponding to methylene protons ($PhCH_2OCO-$) in ^{1}H NMR spectrum and the

presence of only four peaks at δ 140.0, 128.4, 128.2 and 126.7 in aromatic region and absence of peak corresponding to carbamate carbonyl carbon in ^{13}C NMR spectrum confirmed the formation of amino alcohol **54**.



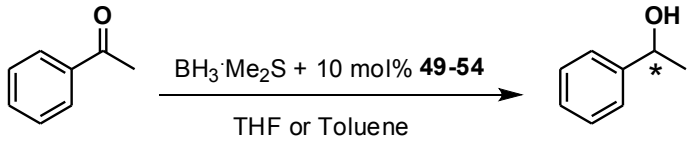
Scheme 12. Reagents and conditions: (a) (i) PhCHO, anhydrous $MgSO_4$, DCM/MeOH (3:1), reflux, 2 h; (ii) $NaBH_4$, MeOH, 0-25 °C, 3 h, 76% in two steps; (b) TBDPSCl, imidazole, DMF, 25 °C, 0.5 h, 83%; (c) TBDMSCl, imidazole, DMF, 30 °C, 13 h, 99%; (d) CSA, MeOH, 10 min., 20 °C, 96%; (e) H_2/Pd-C, MeOH, 30 °C, 10 h, 75 psi, 98%.

It is well known[15, 25] that the temperature and amount of catalyst have important effect on the enantioselectivity of the reduction. The application of catalysts **49-54** to the reduction of acetophenone was investigated with respect to temperature and catalyst concentration. The results are summerised in table 1 and table 2.

For the study of temperature effect, catalyst concentration was fixed (10 mol%) for all reactions. As shown in table 1, catalyst **49** at 50 °C in THF (entry 3) resulted 81% ee. Neither higher (65 °C, reflux temperature of THF), nor lower temperature (25 °C or 0 °C) was beneficial to the catalytic activities. The reduction by using catalyst **50** at 50 °C lead to low ee (62%, table 1, entry 11) as compare to catalyst **49** and it may be due to decrease in the bulk of primary alcohol protecting group. The best results (88% ee) were obtained for the reduction of acetophenone with catalyst **51** and **52** at 50 °C (table 1, entry 15 and 23). We have also used toluene as solvent for enantioselective reduction of acetophenone with catalyst **49** and **51** (table 1, entry 5-8 and 17-20). The ee obtained of the secondary alcohol at temperatures 25 °C, 50 °C, 75 °C and 100 °C in toluene is less as compare to THF. This may be caused by the polarity difference between THF and toluene. It should be noted that negligible asymmetric induction was observed with catalyst **53**. This may be a result of the steric repulsion of N-benzyl of the

oxazaborolidine, which prevents approach of the ketone towards borane and favors direct reduction by BH_3 (good isolated yield). In case of catalyst **54**, maximum enantioselectivity (63% ee) was obtained at 25 °C.

Table 1. Effect of temperature on enantioselectivity of reduction



Entry	Catalyst	Temp. °C	Time in min.	ee (%)[C]	Isolated yield(%)	Configuration
1	**49**	0	65[B]	11	78	S
2	**49**	25	30	46	88	S
3	**49**	50	5	81	89	S
4	**49**	65	4	79	89	S
5	**49**	25[A]	55	8	88	S
6	**49**	50[A]	10	14	89	S
7	**49**	75[A]	5	11	88	S
8	**49**	100[A]	3	9	85	S
9	**50**	0	67[B]	7	83	S
10	**50**	25	30	49	90	S
11	**50**	50	5	62	89	S
12	**50**	65	4	60	90	S
13	**51**	0	15[B]	63	81	S
14	**51**	25	35	80	90	S
15	**51**	50	5	88	89	S
16	**51**	65	4	87	87	S
17	**51**	25[A]	50	40	89	S
18	**51**	50[A]	10	67	89	S
19	**51**	75[A]	5	60	88	S
20	**51**	100[A]	2	51	86	S
21	**52**	0	28[B]	30	84	S
22	**52**	25	30	80	89	S
23	**52**	50	5	88	90	S
24	**52**	65	4	86	89	S
25	**53**	0	25[B]	4	81	S
26	**53**	25	30	7	87	S
27	**53**	50	5	7	86	S
28	**53**	65	4	2	87	S
29	**54**	0	17[B]	42	85	R
30	**54**	25	30	63	92	R
31	**54**	50	5	53	91	R

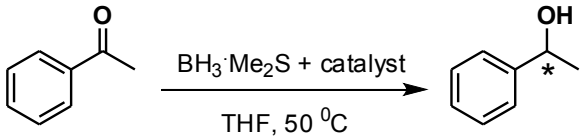
[A]Toluene is used as solvent

[B]Time in hours

[C]Determined by comparison with standard specific rotation for (*S*)-1-phenylethanol $[\alpha]_D^{20}$ -45.50 (c 2.0, MeOH).[29]

Having studied the optimum temperature, attention was turned towards the optimum concentration of the catalyst. All the reactions were carried out at optimized temperature 50 °C (for catalyst **54** at 25 °C) to study the effect of catalyst concentration on enantioselectivity. As shown in table 2, increasing the amount of catalyst from 2 to 10 mol%, the enantioselectivity of reactions improves. However, no obvious change in enantioselectivity was observed on increasing the catalyst concentration to 20 mol%. Therefore, the best experimental condition found for asymmetric reduction of acetophenone required the use of 10 mol% of catalyst at 50 °C (25 °C in case of catalyst **54**). It is worth mentioning here that loading of 7.5% and 10% of catalysts **49** and **54**, there is no significant change of optical and chemical yields (1-2% change), hence lower catalyst loading of 7.5 mole% can also be used.

Table 2. Effect of catalyst concentration on the enantioselectivity of reduction

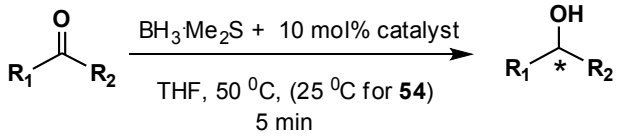


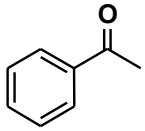
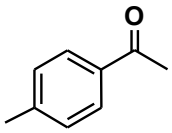
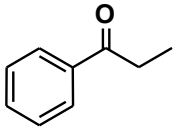
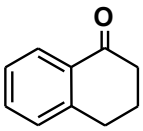
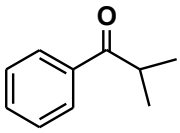
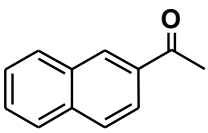
Entry	Catalyst	Catalyst mol%	ee(%)[A]	Isolated yield (%)	Configuration
1	**49**	2	67	88	S
2	**49**	5	75	90	S
3	**49**	7.5	79	90	S
4	**49**	10	81	89	S
5	**49**	20	81	88	S
6	**51**	2	45	90	S
7	**51**	5	72	92	S
8	**51**	7.5	80	91	S
9	**51**	10	88	89	S
10	**51**	20	88	88	S
11	**54**	2	48	91	R
12	**54**	5	58	89	R
13	**54**	7.5	61	90	R
14	**54**	10	63	92	R
15	**54**	20	63	90	R

[A]Determined by comparison with standard specific rotation value.

The reduction of a variety of prochiral ketones was then examined using the catalysts derived from chiral amino alcohols **49-54** under optimized temperature and catalyst concentration. The results are summerised in table 3.

Table 3. Asymmetric reduction of prochiral ketones catalysed by **49-54**



Entry	Ketone	Catalyst	ee (%)[A]	Isolated yield (%)[b]	Configuration
1		**49**	81	88	S
2		**50**	62	89	S
3		**51**	88	89	S
4		**52**	88	90	S
5		**53**	7	86	S
6		**54**	63	92	R
7		**49**	72	91	S
8		**50**	63	87	S
9		**51**	75	90	S
10		**52**	83	92	S
11		**53**	2	89	S
12		**54**	62	92	R
13		**49**	71	89	S
14		**50**	69	91	S
15		**51**	86	91	S
16		**52**	88	89	S
17		**53**	4	90	S
18		**54**	69	92	R
19		**49**	71	88	S
20		**50**	51	90	S
21		**51**	59	90	S
22		**52**	87	91	S
23		**53**	5	89	S
24		**54**	57	90	R
25		**49**	46	87	R
26		**50**	54	90	R
27		**51**	43	86	R
28		**52**	46	86	R
29		**53**	2	89	R
30		**54**	57	88	S
31		**49**	36	90	S
32		**50**	70	93	S
33		**51**	92	93	S
34		**52**	94	92	S
35		**53**	1	91	S
36		**54**	74	92	R

[A]Determined by comparison with standard specific rotations, for (*S*)-1-(4-methylphenyl) ethanol $[\alpha]_D^{25}$ -57.3 (c 0.190, $CHCl_3$),[30] for (*S*)-1-Phenyl propanol $[\alpha]_D^{25}$ - 45.45 (c 5.15, $CHCl_3$),[31] for (*S*)-1,2,3,4-tetrahydro-naphth-1-ol $[\alpha]_D^{25}$ +32.65 (c 2.5, $CHCl_3$),[32] for (*R*)-2-methyl-1-phenylpropanol $[\alpha]_D^{25}$ +47.7 (c 6.8, diethyl ether),[33] for (*R*)-1-(naphth-2-yl)-ethan-1-ol $[\alpha]_D^{25}$ +55.8 (c 4.8, $CHCl_3$).[34]

The level of asymmetric induction was found to be influenced by the nature of substrate and catalyst. As seen from table 3, asymmetric reduction of aromatic ketones with chiral amino alcohols gave fair to excellent enantiomeric excess (36 to 94%) except amino alcohol **53**. Amino alcohol **53** gave very poor enantiomeric excess in all the cases. As expected reduction with the bulky primary hydroxy protected ether at C-3 and 2-amino-1-diphenyl alcohols **51** and **52** proceeds with very good to excellent optical yields 86 to 94% (table 3, entry 3, 4, 15, 16, 22, 33, 34). Reduction of ketones with the oxazaborolidine formed with the C-1 hydroxy protected amino diol **54** yielded the secondary alcohols in moderate ee (57 to 74%) with opposite configuration in respect to all other catalyst.

Steric bulk around the prochiral carbonyl group plays an important role in the enantiomeric discrimination, as revealed by low enantiomeric excess observed in the reduction of isopropylphenyl ketone with all the six catalyst (table 3, entry 25-30).

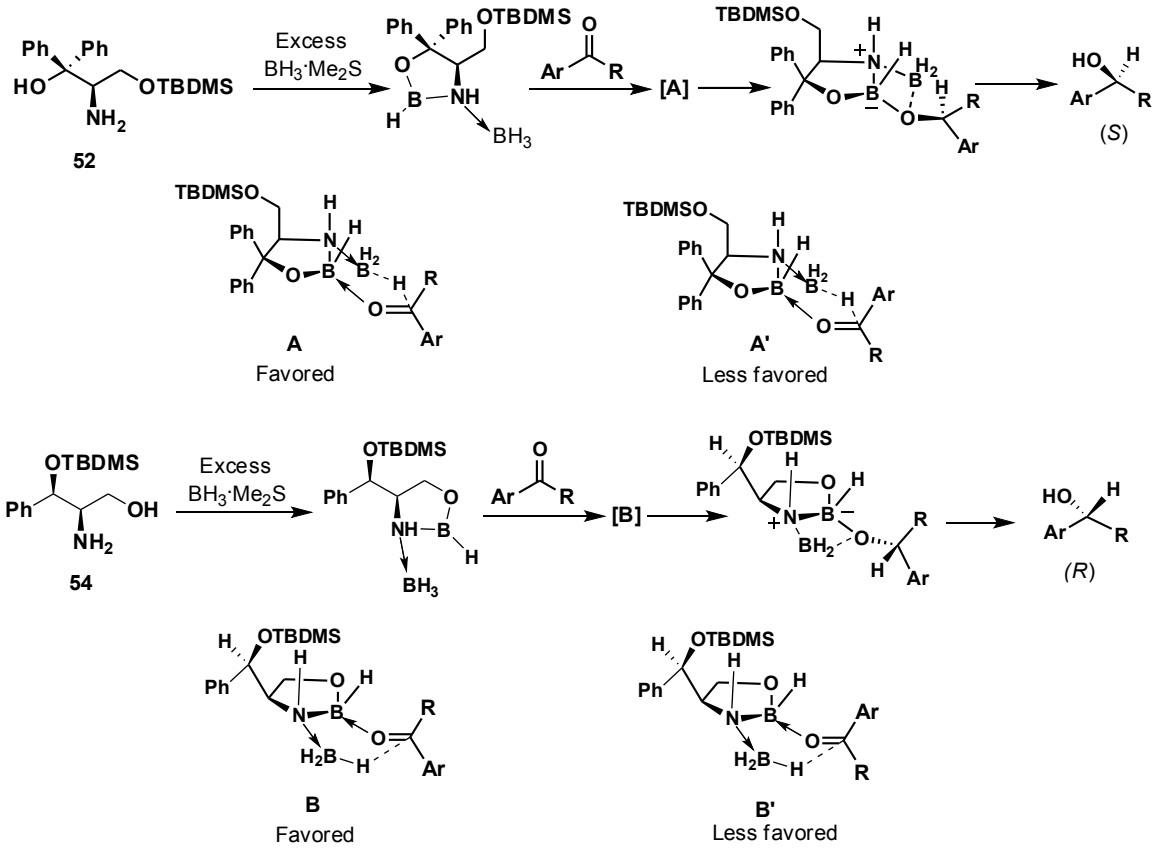


Figure 3. Proposed models for oxazaborolidine reduction.

From the proposed model (Figure 3) the configuration of the obtained chiral alcohol can be deduced. However, catalyst **54** and isopropyl phenyl ketone gave the (*S*) configuration and all other catalyst **49-53** with isopropyl phenyl ketone gave (*R*)

configuration. These results are of some concern to us. This may be due to the isopropyl group in isopropyl phenyl ketone that predominates over phenyl and changes the favored transition state to less favored transition state.

3.4 Conclusion

Six new chiral amino alcohols **49-54** have been synthesised from readily available (1*R*,2*R*)-1-phenyl-2-aminopropane-1,3-diol **48** in excellent yields. The structures of these amino alcohols **49-54** have been fully characterized and used for the enantioselective reduction of aromatic ketones. The secondary alcohols were obtained in fair to excellent enantiomeric excess (up to 94%) for the reduction of all the aromatic ketones. The *tert*-alcohol at C-1 with bulky O-protected group at C-3 (**51** and **52**) gave uniformly encouraging results in all the cases. Moderate ee was observed with the C-3 primary alcohol **54**. As the reactions are rapid and high yielding, these chiral amino alcohols may be used as an alternative to the existing amino alcohols.

3.5 Experimental

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyloxycarbonyl)-1-phenylpropane-1,3-diol (55):

To the solution of (1*R*, 2*R*)-1-phenyl-2-aminopropane-1,3-diol **48** (1.002 g, 6 mmol) in water-dioxane (20 mL, 1:1) was added sodium carbonate (0.320 g, 3 mmol). The reaction mixture was stirred for 10 min. at 30 °C. To this mixture benzyl chloroformate (0.95 ml, 6.6 mmol) was added and the reaction mixture was stirred at 30 °C for 2 h. Dioxane was removed under reduced pressure and 5 ml water was added, the reaction mixture was extracted with ethyl acetate (3 x 50 mL). The ethyl acetate extract was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford compound **55** as a white solid. The crude product was chromatographed on silica gel (methanol/dichloromethane, 5:95) to give pure product **55** (1.780 g, 99%), Mp: 105-106°C (ethyl acetate/petroleum ether); $[\alpha]_D^{26}$ -68.12 (c 0.69, $CHCl_3$); IR (nujol, cm^{-1}) 3429, 1667; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.31-7.29 (m, 10H, ArH), 5.55 (d, J = 6 Hz, 1H, NH, exchangeable with D_2O), 4.96 (s, 3H, Ph-CH_2-O-CO, Ph-CH-OH), 3.88-3.84 (m, 1H, CH-NHCbz), 3.75-3.67 (m, 2H, CH_2-O), ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.9, 141.0, 136.3, 128.4, 128.3, 128.0, 127.9, 127.8, 126.0, 73.7, 66.9, 63.5, 57.6; MS

(LCMS) *m/z*: 302.05 (M + 1), 319.05 (M + H_2O); Anal. Calcd for $C_{17}H_{19}O_4N$: C, 67.78; H, 6.35; N, 4.65; Found: C, 67.39; H, 6.30; N, 4.78.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldiphenylsilyl)-1-phenylpropane-1,3-diol (56):

Compound **55** (3.01 g, 10 mmol) and imidazole (1.7 g, 25 mmol) were dissolved in dry DMF (8 mL). To this solution *tert*-butyldiphenylsilyl chloride (2.86 mL, 11 mmol) was added and the reaction mixture was stirred at 30 °C for 10 minutes. Ice pieces were added and the reaction mixture was extracted with diethyl ether (3 x 60 mL). Organic phase was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by coloumn chromatography over silica gel using ethyl acetate/petroleum ether (12:88) as an eluent to get compound **56** as gum (4.964 g, 92%). $[\alpha]_D^{26}$ -32.38 (c 1.05, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3436, 1718; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.58-7.21 (m, 20H, Ar-H), 5.33 (d, *J* = 9.4 Hz, 1H, -NHCbz, exchangeable with D_2O), 5.04-5.01 (m, 1H, Ph-CH-OH), 4.93 (s, 2H, Ph-CH_2-OCO), 3.89-3.73 (m, 3H, CH-NHCbz, CH_2-O-Si), 3.17 (s, 1H, -OH), 1.02 (s, 9H, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.4, 141.0, 136.3, 135.4, 132.5, 129.9, 128.3, 128.2, 127.8, 127.5, 126.0, 66.6, 64.7, 57.3, 26.8, 19.1; MS (MALDI-TOF) *m/z*: 562.37 (M + Na); Anal. Calcd for $C_{33}H_{37}NO_4Si$: C; 73.43; H, 6.91; N, 2.59; Found: C, 73.09; H, 6.93; N, 2.75.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (57):

Compound **55** (1.505 g, 5 mmol) and imidazole (0.85 g, 12.5 mmol) were dissolved in dry DMF (3.5 mL). To this solution *tert*-butyldimethylsilyl chloride (0.9 g, 6 mmol) was added and the reaction mixture was stirred at 25 °C for 0.5 h. Ice pieces were added and the reaction mixture was extracted with diethyl ether (3 x 50 mL). Organic phase was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by coloumn chromatography over silica gel using ethyl acetate/petroleum ether (8:92) as an eluent to get compound **57** as a white solid, (1.877 g, 90%). Mp: 60-61°C (ethyl acetate/petroleum ether); $[\alpha]_D^{27}$ -60.16 (c 0.96, $CHCl_3$); IR (nujol, cm^{-1}) 3425, 1701; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37-7.26 (m, 10H, Ar-H), 5.42 (d, *J* = 6.9 Hz, 1H, NH-Cbz), 5.05-4.99 (m, 3H, Ph-CH-OH,

Ph-CH_2-OCO), 4.72 (bs, -1H, OH), 3.88-3.79 (m, 3H, CH_2-OSi, CH-NHCbz), 0.92 (s, 9H, -$C(CH_3)_3$), 0.07 (s, 6H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.5, 141.1, 136.5, 128.4, 128.3, 128.0, 127.9, 127.6, 126.0, 74.3, 66.7, 64.8, 57.0, 25.8, 18.2, -5.6; MS (LCMS) *m/z*: 416.05 (M + 1); Anal. Calcd for $C_{23}H_{33}NO_4Si$: C, 66.50; H, 8.00; N, 3.37; Found: C, 66.52; H, 8.41; N, 3.33.

(1*R*,2*R*)-(-)-2-Amino-3-*O*-(*tert*-butyldiphenylsilyl)-1-phenylpropane-1,3-diol (49):
To the solution of **56** (1.5 g, 12.8 mmol) in methanol (25 mL) was added Pd-C catalyst (0.150 g, 10%) and hydrogenation was carried out using a Parr apparatus at 75 psi pressure, at 30 °C temperature for 10 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to obtain (1*R*,2*R*)-1-phenyl-2-amino-3-*O*-*tert*-butyldiphenylsilyl-1,3-propanediol **49** (1.109 g, 98%) as gummy material. $[\alpha]_D^{25}$ -11.23 (c 1.25, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3396; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.63-7.57 (m, 5H, Ar-H), 7.40-7.27 (m, 10H, Ph-Si-Ph), 4.60 (d, *J* = 5.5 Hz, 1H, O-CH-Ph), 3.68-3.53 (m, 2H, CH_2-O-Si), 2.96-2.88 (m, 1H, CH-NH_2), 1.05 (s, 9H, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 142.3, 135.4, 133.0, 132.9, 129.6, 128.1, 127.6, 127.2, 126.2, 73.4, 65.5, 58.5, 26.8, 19.1; MS (MALDI-TOF) *m/z*: 406.38 (M+1); Anal. Calcd for $C_{25}H_{31}NO_2Si$: C, 74.03; H, 7.70; N 3.45; Found: C, 74.32; H, 7.89; N, 3.17

(1*R*,2*R*)-(-)-2-Amino-3-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (50):
Compound **50** has been synthesized following the experimental procedure described for the compound **49**. Gummy material, 99% yield. $[\alpha]_D^{26}$ -20.63 (c 2, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3371 1577; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.26 (m, 5H, Ar-H), 4.64 (d, *J* = 4.4 Hz, 1H, Ph-CH-OH), 3.64 (dd, *J* = 9.5, 3.7 Hz, 1H, CH_2-O-Si), 3.57 (dd, *J* = 9.5, 3.7 Hz, 1H, CH_2-O-Si), 2.96-2.93 (m, 1H, CH-NH_2), 2.29 (bs, 3H, OH+NH_2, exchangeable with D_2O), 0.91 (s, 9H, -$C(CH_3)_3$), 0.06 (s, 6H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.1, 128.2, 127.4, 126.3, 73.6, 64.2, 58.4, 25.7, 18.0, -5.7. MS (MALDI-TOF) *m/z*: 282.51 (M+1); Anal. Calcd for $C_{15}H_{27}NO_2Si$: C, 64.01; H, 9.67; N, 4.98; Found: C, 64.13; H, 9.58; N, 5.08.

(2*R*)-2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldiphenylsilyl)-1-oxo-1-phenylpropane-3-ol (58):

To the solution of carbamate alcohol **56** (10 g, 18.53 mmol) in ethyl acetate (60 ml) was added o-iodoxybenzoic acid (15.56 g, 55.58 mmol). The reaction mixture was refluxed for 5 h and then it was filtered. The filtrate was concentrated under reduced pressure. The crude material was chromatographed on silica gel (ethyl acetate/petroleum ether, 5:95) to get pure compound **58** (9.803 g, 98%) as thick oil. $[\alpha]_D^{25}$ -42.05 (c 1.28, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3423, 1718, 1685; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.92 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.65-7.15 (m, 18H, Ar-H), 6.05 (d, *J* = 8 Hz, 1H, NH-Cbz), 5.45-5.38 (m, 1H, CH-NHCbz), 5.13 (s, 2H, $PhCH_2$-OCO), 3.99 (d, *J* = 3.67 Hz, 2H, CH_2-O-Si), 0.90 (s, 9H, $-C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.1, 155.7, 136.3, 135.4, 134.9, 133.4, 132.5, 132.43 129.7, 129.6, 128.6, 128.5, 128.4, 128.0, 127.9, 127.6, 127.52, 66.7, 64.9, 57.4, 26.4, 19.0; MS (LCMS) *m/z*: 538.73 (M + 1); Anal. Calcd for $C_{33}H_{35}NO_4Si$: C, 73.71; H, 6.56; N, 2.60; Found: C, 73.57; H, 6.90; N, 2.76.

(2*R*) -2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl) -1-oxo-1-phenyl propane-3-ol (59):

Compound **59** has been synthesized following the experimental procedure described for the compound **58.** Colourless oil, 97% yield. $[\alpha]_D^{26}$ -36.43 (c 2.64, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3427, 1716, 1689; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.94 (d, *J* = 6 Hz, 2H, Ar-H), 7.61-7.34 (m, 8H, Ar-H), 5.98 (d, *J* = 7.7 Hz, 1H, NH-Cbz), 5.44-5.36 (m, 1H, CH-NHCbz), 5.15 (s, 2H, Ph-CH_2-OCO), 4.02-3.90 (m, 2H, CH_2-O-Si), 0.75 (s, 9H, $-C(CH_3)_3$), -0.13 (s, 3H, Si-CH_3), -0.17 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 197.0, 155.7, 136.3, 135.1, 133.4, 128.5, 128.5, 128.4, 128.0, 127.9, 66.8, 66.1, 64.1, 57.6, 25.5, 17.9, -5.9, -6.0; MS (LCMS) *m/z*: 414.44 (M + 1), 436.35 (M + Na); Anal. Calcd for $C_{23}H_{31}NO_4Si$: C, 66.79; H, 7.56; N, 3.39; Found: C, 67.31; H, 7.33; N, 3.47.

(2*R*)-2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldiphenylsilyl)-1,1-diphenyl propane-1,3-diol (60):

Magnesium turnings (2.5 g, 103 mmol) were placed in a flame dried two-necked RB flask with a reflux condenser attached. The flask was cooled to room temperature by flushing argon gas. Dry THF (50 ml) was added by syringe under argon atmosphere. Freshly distilled bromobenzene (5.41 mL, 51.45 mmol) was added at such a rate that mild reflux

of THF resulted. The reaction between bromobenzene and magnesium is exothermic and dropwise addition of bromobenzene took 15 min. The contents were further stirred for 15 min. The N-carbamate ketone **58** (9.258 g, 17.15 mmol) in dry THF (30 mL) was added drop wise via syringe in 20 min. at 25 °C. The whole reaction mixture was further stirred at 25 °C for 1 h. The reaction was quenched with saturated ammonium chloride. THF was removed completely and the residue was extracted with ethyl acetate (3 x 100 mL). The combined extract was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude material was purified on flash silica gel with a mixture of ethyl acetate/petroleum ether (4:96) to get white solid **60** (8.898 g) in 84% yield. Mp: 58 °C (dichloromethane/petroleum ether). $[\alpha]_D^{27}$ +9 (c 1.26, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3438, 1712; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.61-7.20 (m, 25H, Ar-H), 5.90 (d, *J* = 8.6 Hz, 1H, NH-Cbz), 5.33 (s, 1H, OH), 5.05 (s, 2H, $PhCH_2$-OCO), 4.77-4.73 (m, 1H, CH-NHCbz), 3.85 (d, *J* = 1.4 Hz, 2H, CH_2-O-Si), 1.01 (s, 9H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 155.9, 145.5, 144.2, 136.5, 135.4, 135.1, 131.5, 131.3, 130.0, 129.8, 128.5, 128.4, 128.2, 127.9, 127.7, 127.6, 126.9, 126.8, 125.1, 81.2, 66.5, 65.6, 55.5, 26.6, 18.9; MS (LCMS) *m/z*: 638.50 (M + Na); Anal. Calcd for $C_{39}H_{41}NO_4Si$: C, 76.06; H, 6.71; N, 2.27; (Found: C, 75.92; H, 7.03; N, 2.45.

(2*R*)-2-Amino-(*N*-benzyloxycarbonyl)-3-*O*-(*tert*-butyldimethylsilyl)-1,1-diphenylpropane-1,3-diol (61):

Compound **61** has been synthesized following the experimental procedure described for the compound **60**. Thick oil, 87% yield. $[\alpha]_D^{26}$ 34.64 (c 2.42, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3434, 1710; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.53-7.20 (m, 15H, Ar-H), 5.75 (d, *J* = 8.7 Hz, 1H, NH-Cbz), 5.08 (s, 2H, Ph-CH_2-OCO), 4.74-4.69 (m, 1H, CH-NHCbz), 3.90 (dd, *J* = 10.6, 2.2 Hz, 1H, CH_2-O-Si), 3.72 (dd, *J* = 10.6, 2.2 Hz, 1H, CH_2-O-Si), 0.87 (s, 9H, -$C(CH_3)_3$), -0.03 (s, 3H, Si-CH_3), -0.10 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.0, 145.4, 144.3, 136.5, 128.3, 128.3, 128.2, 127.8, 127.5, 126.8, 126.7, 125.1, 124.88, 81.1, 66.4, 64.6, 55.1, 25.6, 17.9, -6.0, -6.1; MS (LCMS) *m/z*: 514.63 (M + Na); Anal. Calcd for $C_{29}H_{37}NO_4Si$: C, 70.84; H, 7.58; N, 2.85; Found: C, 71.10; H, 7.15; N, 2.96.

(2*R*)-2-Amino-3-*O*-(*tert*-butyldiphenylsilyl)-1,1-diphenylpropane-1,3-diol (51):

Compound **51** was prepared as the same approach to **49**. Gummy material, 99% yield. $[\alpha]_D^{26}$ 39.42 (c 1.37, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3448; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.62-

7.14 (m, 20H, Ar-H), 4.80 (s, 1H, D_2O exchangable), 3.96-3.91 (m, 1H, CH-NH_2), 3.73 (dd, *J* = 10.4, 6.6 Hz, 1H, CH_2-O-Si), 3.62 (dd, *J* = 10.4, 3.2 Hz, 1H, CH_2-O-Si), 1.04 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.6, 146.7, 135.5, 135.3, 132.7, 132.7, 129.8, 129.7, 128.4, 128.1, 127.7, 127.7, 126.6, 126.4, 125.4, 125.0, 78.6, 65.3, 57.5, 26.8, 19.1; MS (LCMS) *m/z*: 482.87 (M + 1); Anal. Calcd for $C_{31}H_{35}NO_2Si$: C, 77.30; H, 7.32; N, 2.91; (Found: C, 77.42; H, 7.32; N, 2.97.

(2*R*)-2-Amino-3-*O*-(*tert*-butyldimethylsilyl)-1,1-diphenylpropane-1,3-diol (52):

Compound **52** has been synthesized following the experimental procedure described for the compound **50**. Gummy material, 99% yield. $[\alpha]_D^{26}$ 79.23 (c 2.6, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3438, 3400; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.61-7.46 (m, 4H, Ar-H), 7.36-7.16 (m, 6H, Ar-H), 3.92 (t, *J* = 9.1 Hz, 1H, CH-NH_2), 3.60 (d, *J* = 4.6 Hz, 2H, CH_2-O-Si), 0.86 (s, 9H, -$C(CH_3)_3$), -0.02 (s, 3H, Si-CH_3), -0.05 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 146.0, 145.0, 128.4, 128.1, 126.6, 126.5, 125.5, 125.0, 79.1, 64.5, 57.1, 25.7, 18.0, -5.7, -5.8; MS (LCMS) *m/z*: 359.14 (M + 2); Anal. Calcd for $C_{21}H_{31}NO_2Si$: C, 70.54; H, 8.74; N, 3.92; Found: C, 70.70; H, 8.84; N, 3.86.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyl)-1-phenylpropane-1,3-diol (62):

(1*R*,2*R*)-1-phenyl-2-aminopropane-1,3-diol **48** (1.67 g, 10 mmol) was dissolved in 20 mL dichloromethane-methanol (4:1), in which benzaldehyde (1.11 mL, 11 mmol) and anhydrous $MgSO_4$ (1.5 g) were added. The reaction mixture was refluxed for 2 h, filtered through celite bed and concentrated under reduced pressure to afford crude imine product (2.27, g). The solution of crude imine in methanol (15 mL) was cooled (0 °C) and to it, $NaBH_4$ (0.637 g, 20 mmol) was added. The reaction mixture was then allowed to warm up to 30 °C and stirred further for 3 h. The methanol was evaporated under reduced pressure and the product was dissolved in dichloromethane (20 mL), 2N HCl (10 mL) was added and it was stirred for 10 minute. The reaction mixture was neutralized by using aq. Na_2CO_3, extracted with dichloromethane (3 x 30 mL). The organic extract was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to get crude product **62**. This was purified by column chromatography on silica gel (methanol/dichloromethane, 5:95) to give yellowish solid **62** (1.955 g, 76%). Mp: 75-76°C (ethyl acetate/petroleum ether). $[\alpha]_D^{27}$ -87.48 (c 2, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3380; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.37-7.24 (m, 10H, Ar-

H), 4.65 (d, *J* = 7.4 Hz, 1H, Ph-CH-OH), 3.80 (d, *J* = 12.9 Hz, 1H, NH-CH_2-Ph), 3.67 (d, *J* = 12 Hz, 1H, NH-CH_2-Ph), 3.66 (dd, *J* = 11.3, 3.9 Hz, 1H, CH_2-OH), 3.38 (dd, *J* = 11.0, 3.5 Hz, 1H, CH_2-OH), 2.84-2.76 (m, 1H, CH-NH), 2.55 (bs, 3H, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.3, 141.2, 127.5, 127.0, 126.8, 126.1, 126.1, 125.4, 70.3, 63.2, 57.5, 49.6; MS (MALDI-TOF) *m/z*: 257.34 (M+1); Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44; Found C, 74.66; H, 7.97; N, 5.38.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyl)-3- -(*tert*-butyldiphenylsilyl)-1-phenyl propane-1,3-diol (53):

To the solution of **62** (0.877 g, 3.4 mmol) and imidazole (0.578, 8.5 mmol) in dry DMF (5 mL) was added *tert*-butyldiphenylsilyl chloride (0.97 mL, 3.74 mmol) at 25 °C and reaction mixture stirred for 0.5 h. The reaction was quenched by crushed ice and extracted by using diethyl ether (3 x 40 mL), organic layer was washed with water, brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was purified by silica gel column with a mixture of ethyl acetate/petroleum ether (2:98) as an eluent to give compound **53** as colourless oil (1.452 g, 86%). $[\alpha]_D^{25}$ -46.17 (c 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3421; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.62-7.24 (m, 20H, Ar-H), 4.61 (d, *J* = 7.8 Hz, 1H, Ph-CH-OH), 3.77-3.42 (m, 4H, CH_2-O-Si, NH-CH_2-Ph), 2.70-2.63 (m, 1H, CH-NH), 1.08 (s, 9H, -$C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.8, 138.0, 135.5, 132.9, 132.8, 129.8, 128.5, 128.2, 127.7, 127.5, 127.4, 126.8, 71.9, 64.6, 60.9, 51.2, 26.9, 19.2; MS (MALDI-TOF) *m/z*: 496.05 (M+1); Anal. Calcd for $C_{32}H_{37}NO_2Si$: C, 77.53; H, 7.52; N, 2.82; Found C, 77.27; H, 7.76; N, 2.55.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyloxycarbonyl)-1,3-*O*-(di-*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (63):

Compound **55** (5.117 g, 17 mmol) and imidazole (5.780 g, 85 mmol) were dissolved in dry DMF (18 mL). To this solution *tert*-butyldimethylsilyl chloride (6.120 g, 40.8 mmol) was added and the reaction mixture was stirred at 30 °C for 13 h. Ice pieces were added and the reaction mixture was extracted with diethyl ether (3 x 70 mL). Organic phase was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified on silica gel by column chromatography using ethyl acetate-petroleum ether (4:96) as an eluent to get compound **63** as colorless oil (8.908 g, 99%). $[\alpha]_D^{25}$ -45.57 (c 1.62, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3446,

1726; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.31-7.25 (m, 10H, Ar-H), 5.12 (d, *J* = 8.8 Hz, 1H, NH-Cbz), 5.04 (d, *J* = 2.9 Hz, 1H, Ph-CH), 5.00 (s, 2H, Ph-CH_2-OCO), 3.76-3.67 (m, 1H, CH-NHCbz), 3.57 (d, *J* = 6.3 Hz, 2H, CH_2-O-Si), 0.93 (s, 9H, -$C(CH_3)_3$), 0.89 (s, 9H, -$C(CH_3)_3$), 0.09 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3), 0.04 (s, 3H, Si-CH_3), -0.15 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.0, 142.2, 136.6, 128.4, 128.0, 127.3, 126.2, 71.7, 66.5, 61.6, 59.0, 25.8, 18.1, 18.1, -5.5, -4.7, -5.3, -5.3; MS (LCMS) *m/z*: 530.46 (M + 1), 552.45 (M + Na); Anal. Calcd for $C_{29}H_{47}NO_4Si_2$: C, 65.74; H, 8.94; N, 2.64; Found: C, 65.71; H, 8.70; N, 2.97.

(1*R*,2*R*)-(-)-2-Amino-(*N*-benzyloxycarbonyl)-1-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (64):

To a solution of **63** (10.255 g, 19.35 mmol) in methanol was added camphorsulphonic acid (4.495 g, 19.35 mmol) and the reaction mixture was stirred for 10 min. at 20 °C. Ice pieces was added in the reaction mixture and methanol was evaporated on rotary evaporator. Then it was extracted with ethyl acetate (3 x 60 mL). The combined extracts were washed with water and brine, dried over anhydrous sodium sulphate, and concentrated on the rotary evaporator. The crude product was chromatographed on silica gel by ethyl acetate/petroleum ether (30:70) as an eluent to get compound **64** as colorless oil (7.716 g, 96%). $[\alpha]_D^{25}$ -46.65 (c 2.92, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3434, 1708; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.33-7.31 (m, 10H, Ar-H), 5.20 (d, *J* = 8.1 Hz, 1H, NH-Cbz), 5.02 (s, 2H, Ph-CH_2-OCO), 4.97 (d, *J* = 3.4 Hz, 1H, Ph-CH-O), 3.89-3.77 (m, 1H, CH-NHCbz), 3.70 (d, *J* = 5.8 Hz, 2H, CH_2-OH), 0.91 (s, 9H, -$C(CH_3)_3$), 0.06 (s, 3H, Si-CH_3), -0.14 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5, 141.5, 136.3, 128.33 128.0, 127.9, 127.4, 126.2, 72.9, 66.6, 62.3, 59.1, 25.7, 18.0, -4.8, -5.4. MS (LCMS) *m/z*: 416.26 (M + 1), 438.24 (M + Na); Anal. Calcd for $C_{23}H_{33}NO_4Si$: C, 66.47; H, 8.00; N, 3.37; Found: C, 66.23; H, 7.87; N, 3.56.

(1*R*,2*R*)-(-)-2-Amino-1-*O*-(*tert*-butyldimethylsilyl)-1-phenylpropane-1,3-diol (54):

Compound **54** has been synthesized following the experimental procedure described for the compound **49**. Yield, 98%. Mp: 80 °C (ethyl acetate-petroleum ether) $[\alpha]_D^{25}$ -60.71 (c 1.12, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3402; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.32 (s, 5H, Ar-H), 5.45 (bs, D_2O exchangeable), 4.80 (d, *J* = 7.5 Hz, 1H, Ph-CH), 3.63 (dd, *J* = 11.9, 3.3 Hz, 1H, CH_2-OH), 3.52 (dd, *J* = 11.9, 6.8 Hz, 1H, CH_2-OH), 3.36-3.27 (m, 1H, CH-NH_2),

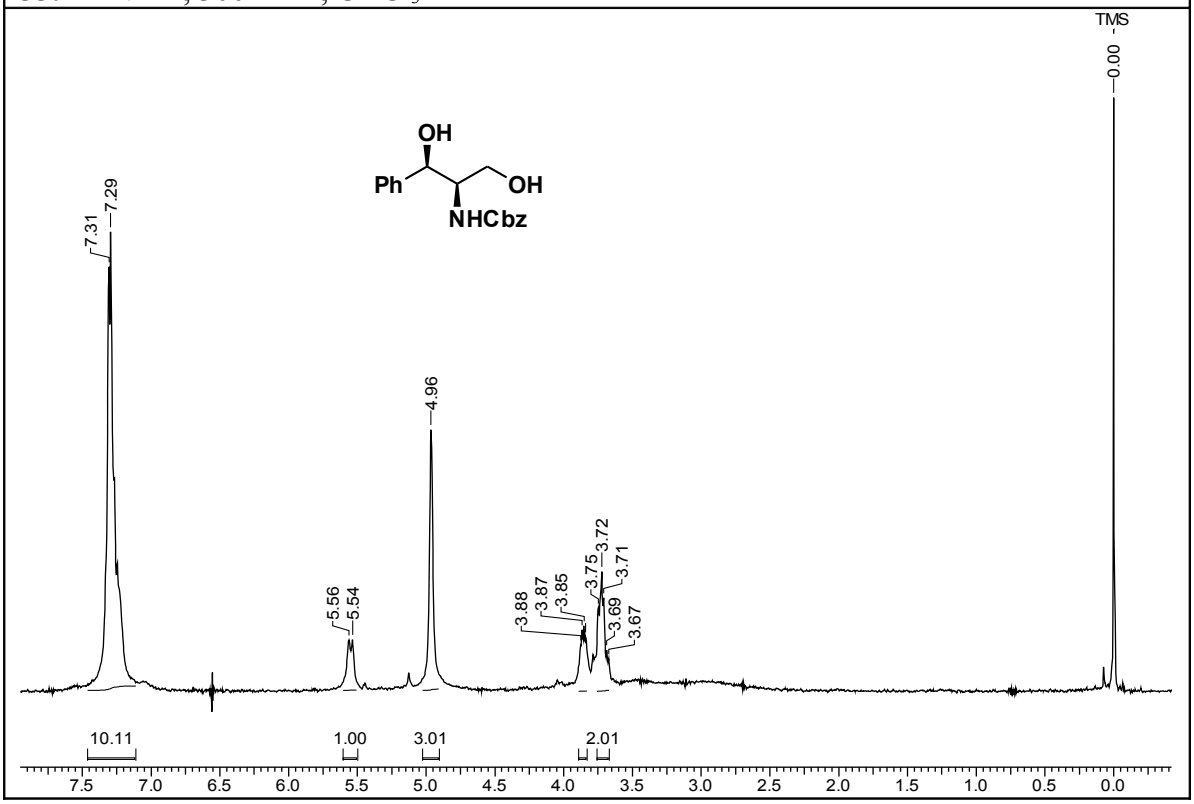
0.86 (s, 9H, -C(CH_3)$_3$), 0.09 (s, 3H, Si-CH_3), -0.23 (s, 3H, Si-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.0, 128.4, 128.2, 126.7, 73.4, 59.8, 59.7, 59.7, 25.7, 17.8, -4.7, -4.9; MS (LCMS) *m/z*: 282.41 (M + 1); Anal. Calcd for $C_{15}H_{27}NO_2Si$: C, 64.01; H, 9.67; N, 4.98; Found: C, 64.27; H, 9.64; N, 5.18.

General procedure for asymmetric reduction of ketones

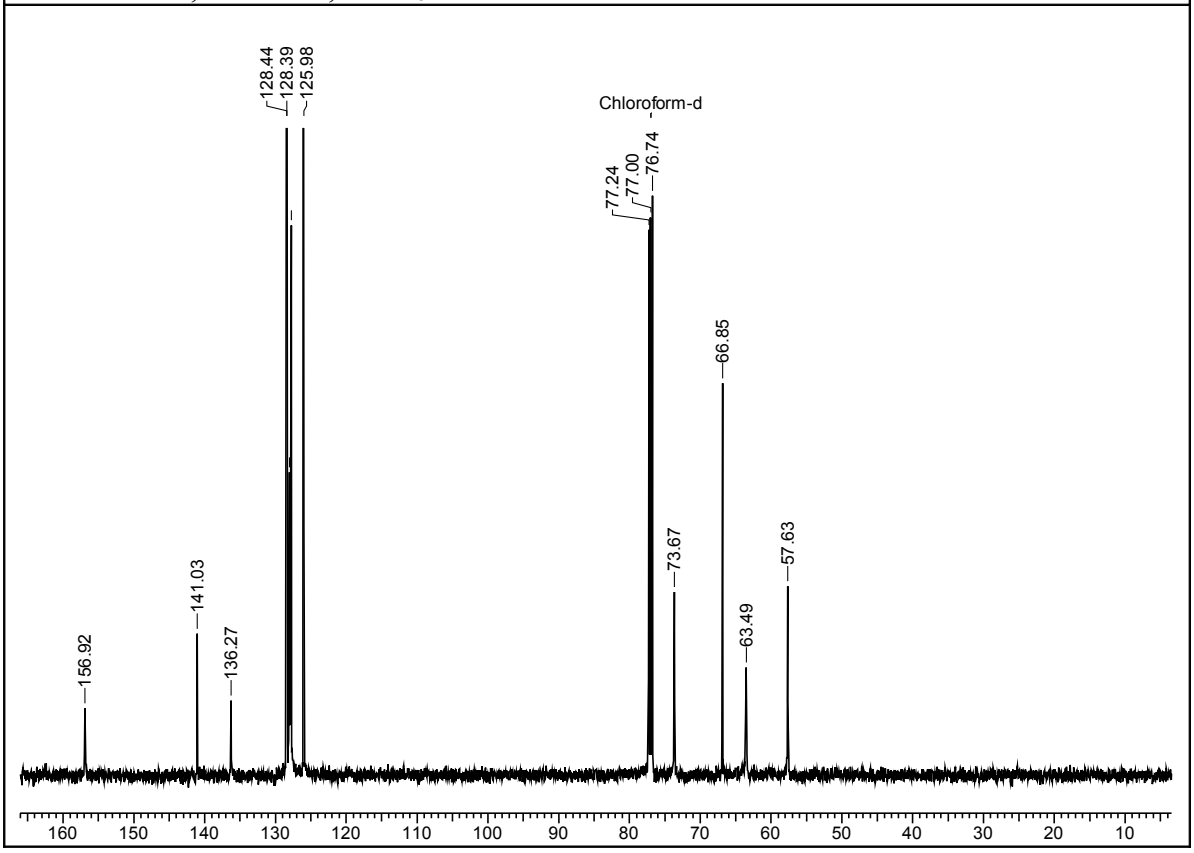
To the solution of amino alcohol **49-54** (0.2 mmol) in dry THF (3 mL) was added 10 M borane-dimethyl sulfide complex (0.23 mL, 2.3 mmol) and the reaction mixture was stirred under argon atmosphere at 25 °C for 10 min. Reaction temperature was increased to 50 °C and to that, solution of ketone (2 mmol) in dry THF (3 mL) was added dropwise over 15 min. The reaction mixture was then stirred further for 5 min. The reaction was quenched by adding 2M HCl (5 mL) and extracted with diethyl ether (3 x 20 mL). The organic layer was washed with water and brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was purified on a silica gel column chromatography with ethyl acetate/petroleum ether as an eluent to give chiral secondary alcohols.

3.6 Selected Spectra

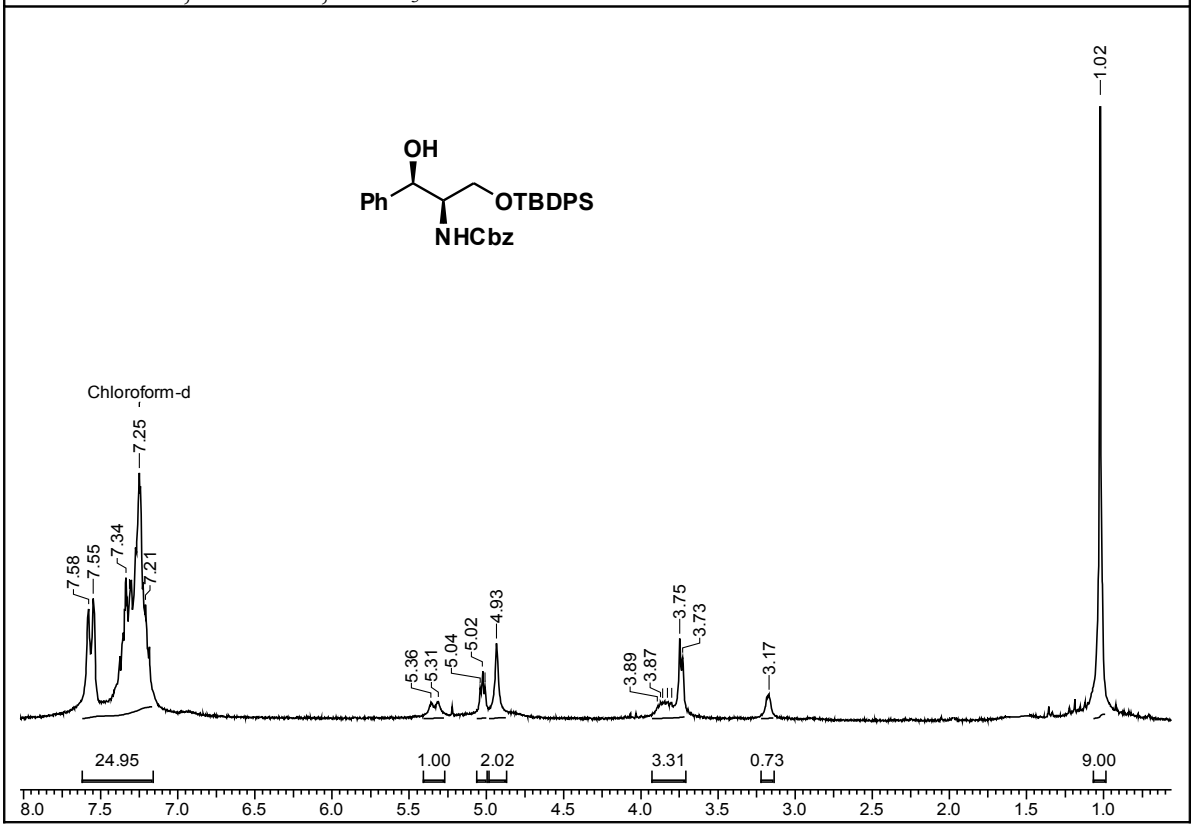
55: ^{1}H NMR, 300 MHz, $CDCl_3$



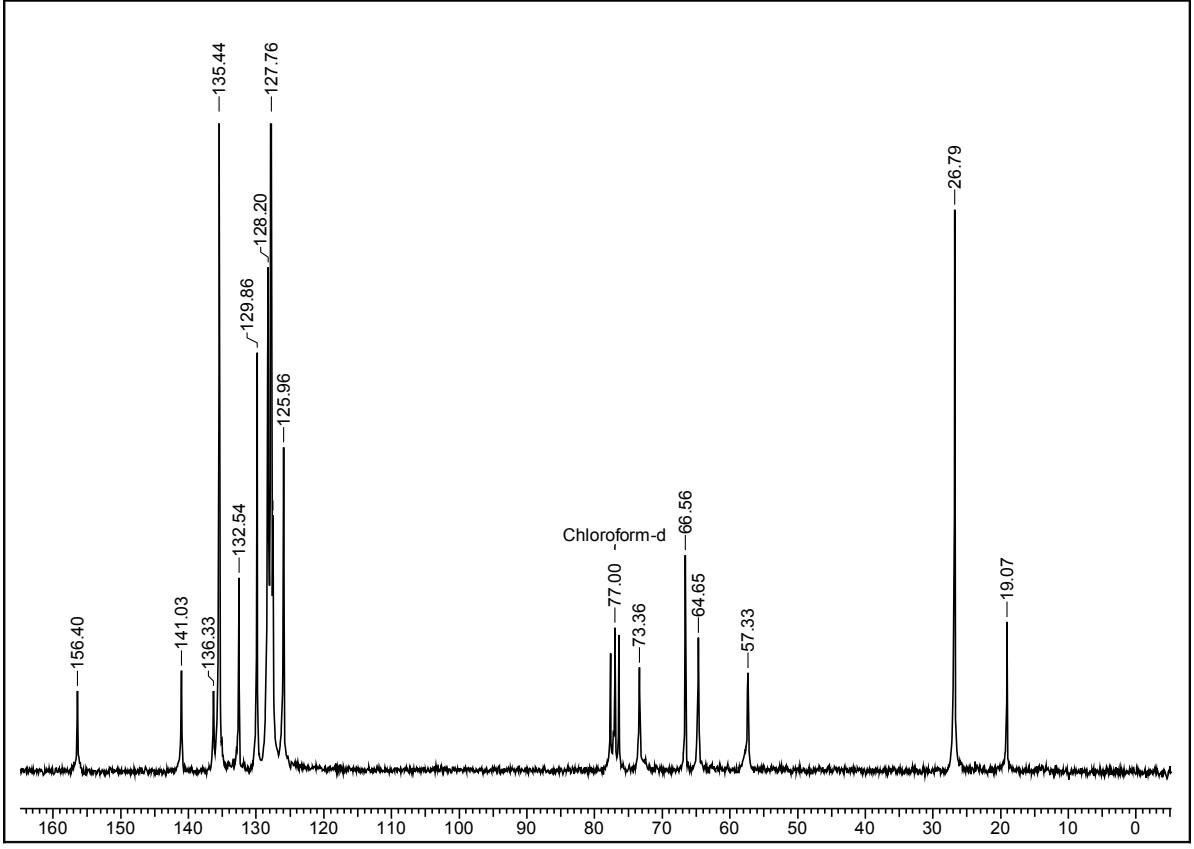
55: ^{13}C NMR, 125 MHz, $CDCl_3$



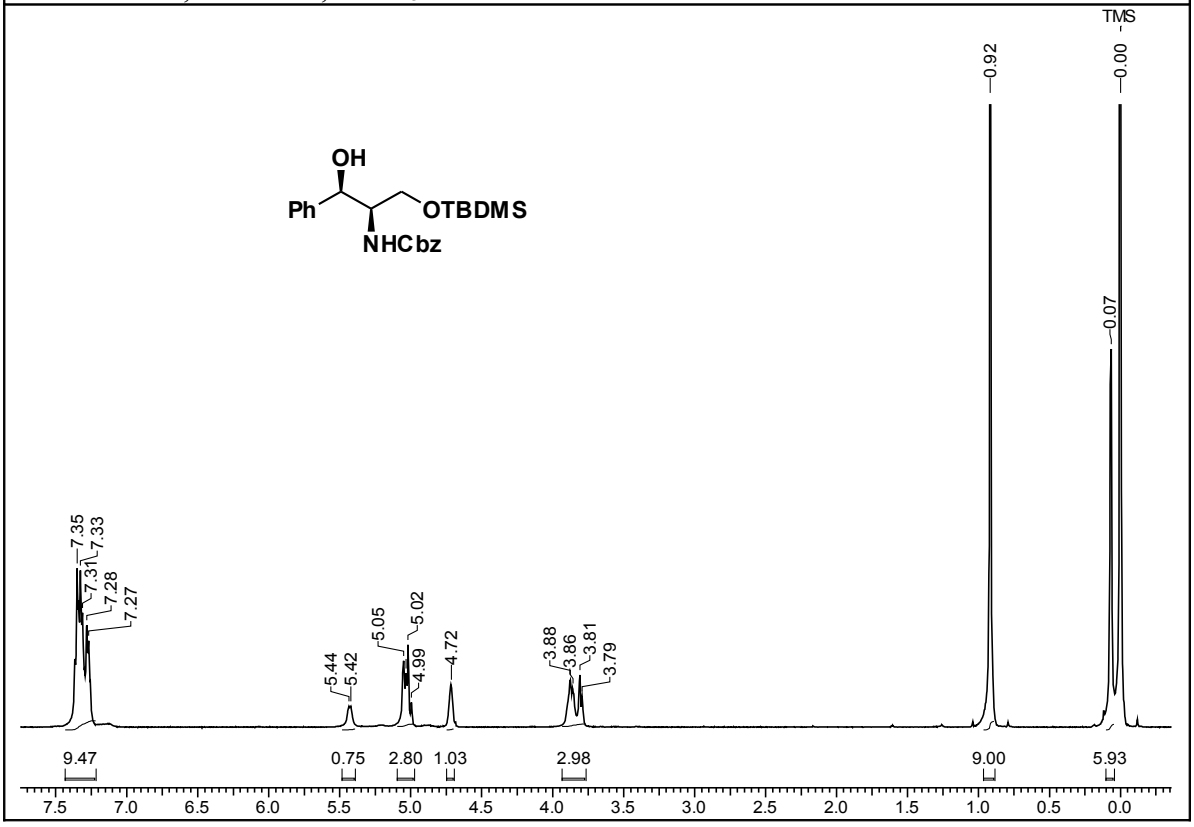
56: ^{1}H NMR, 200 MHz, $CDCl_3$



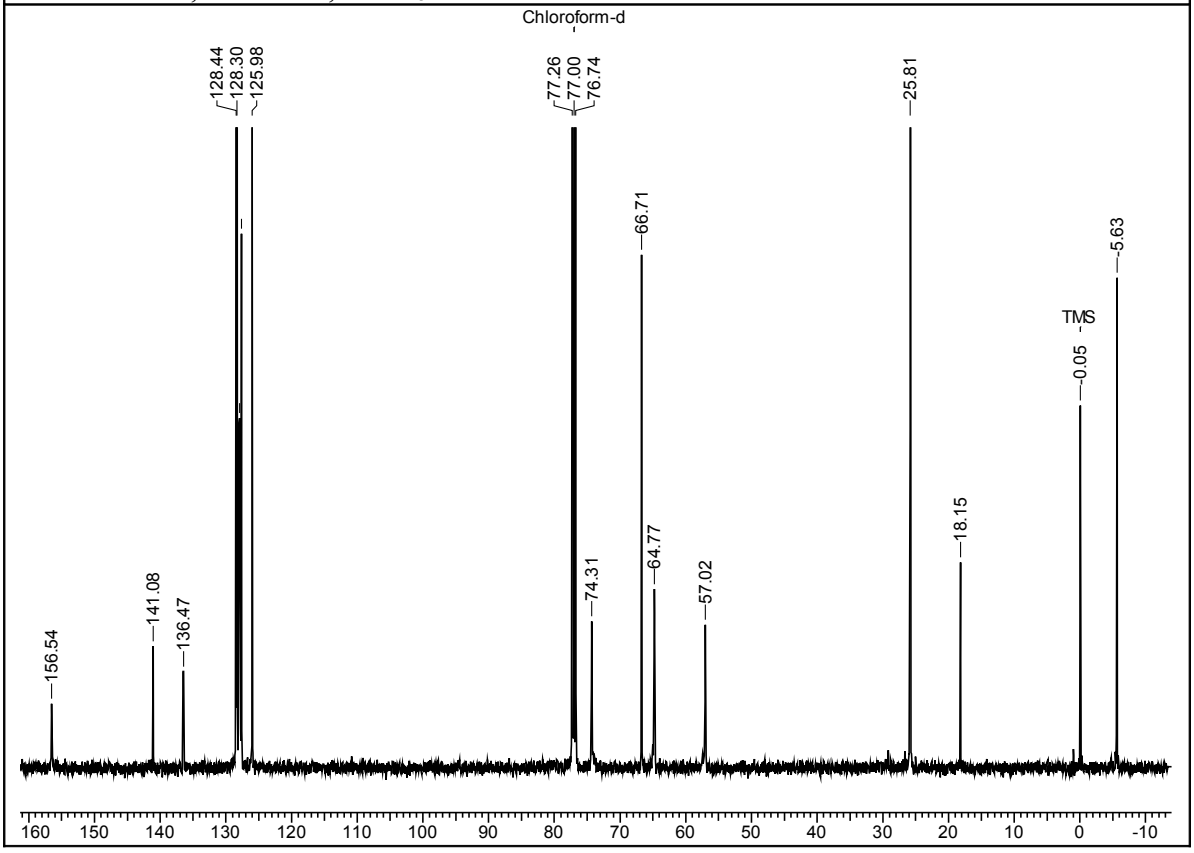
56: ^{13}C NMR, 50 MHz, $CDCl_3$



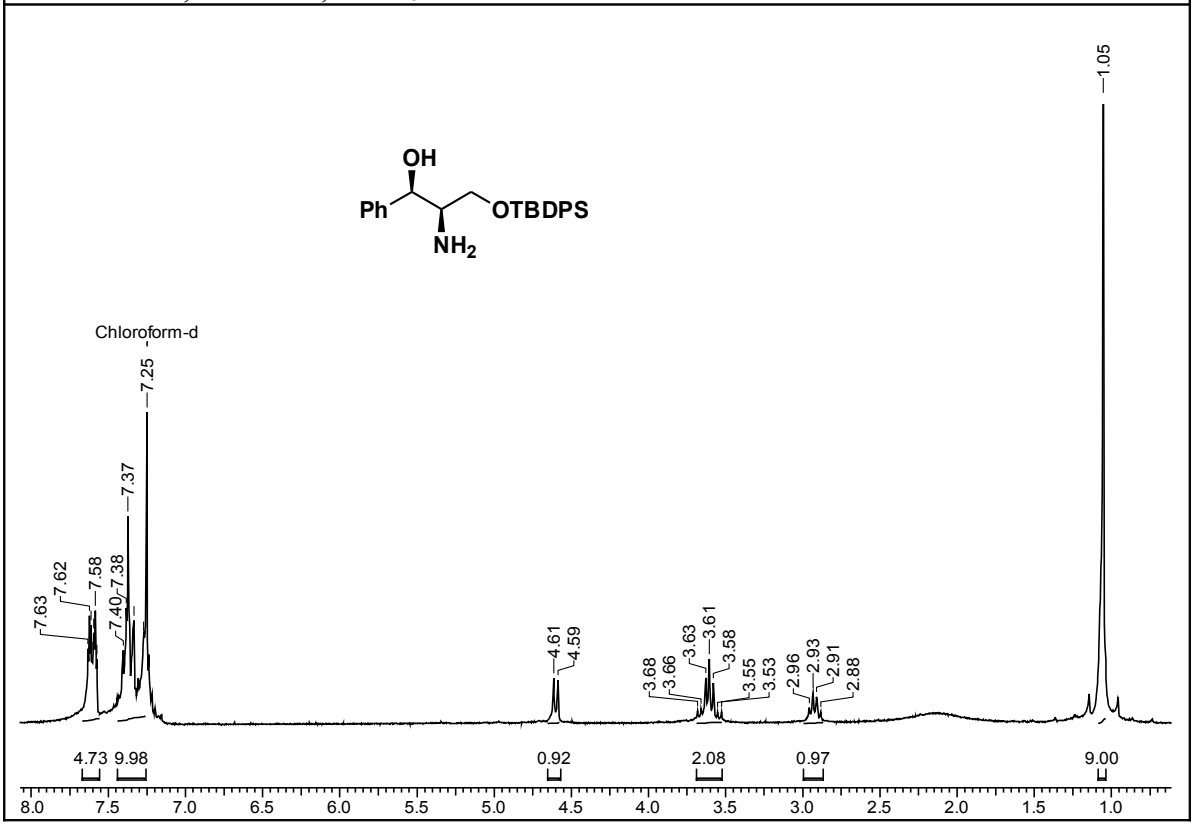
57: ^{1}H NMR, 500 MHz, $CDCl_3$



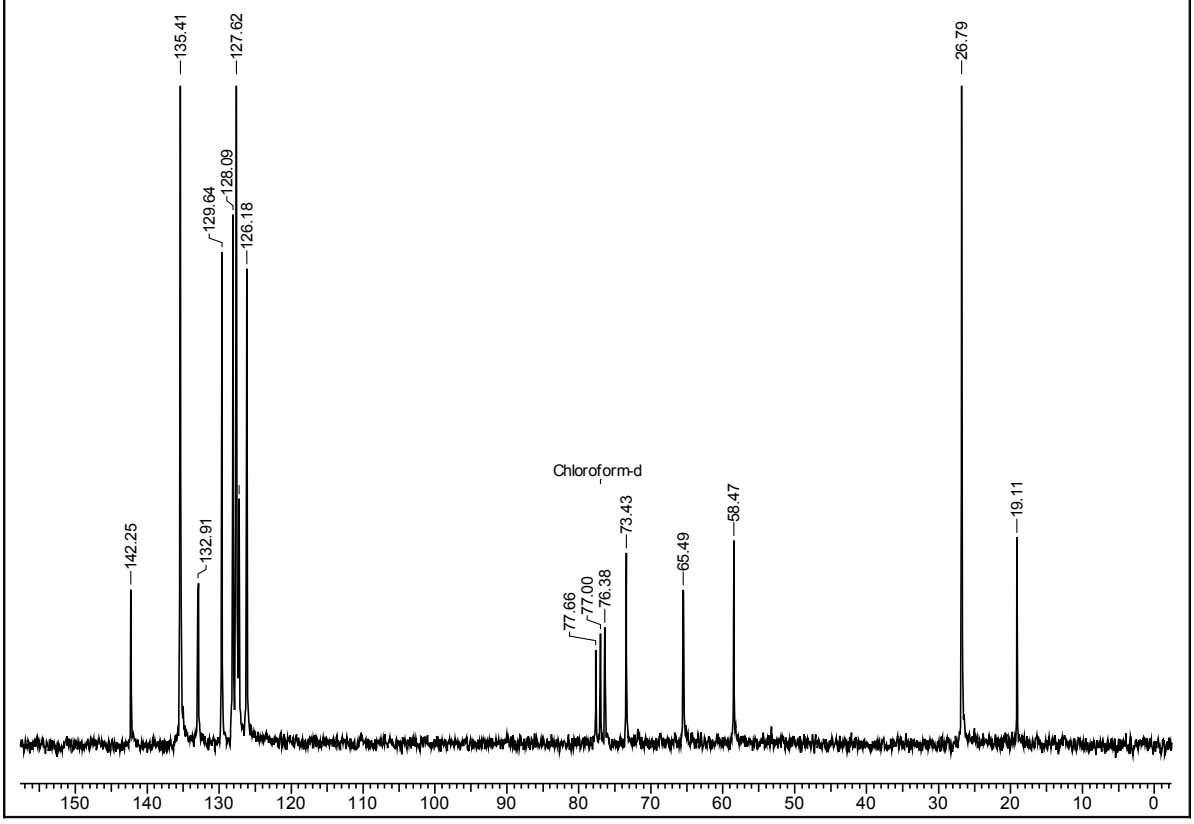
57: ^{13}C NMR, 125 MHz, $CDCl_3$



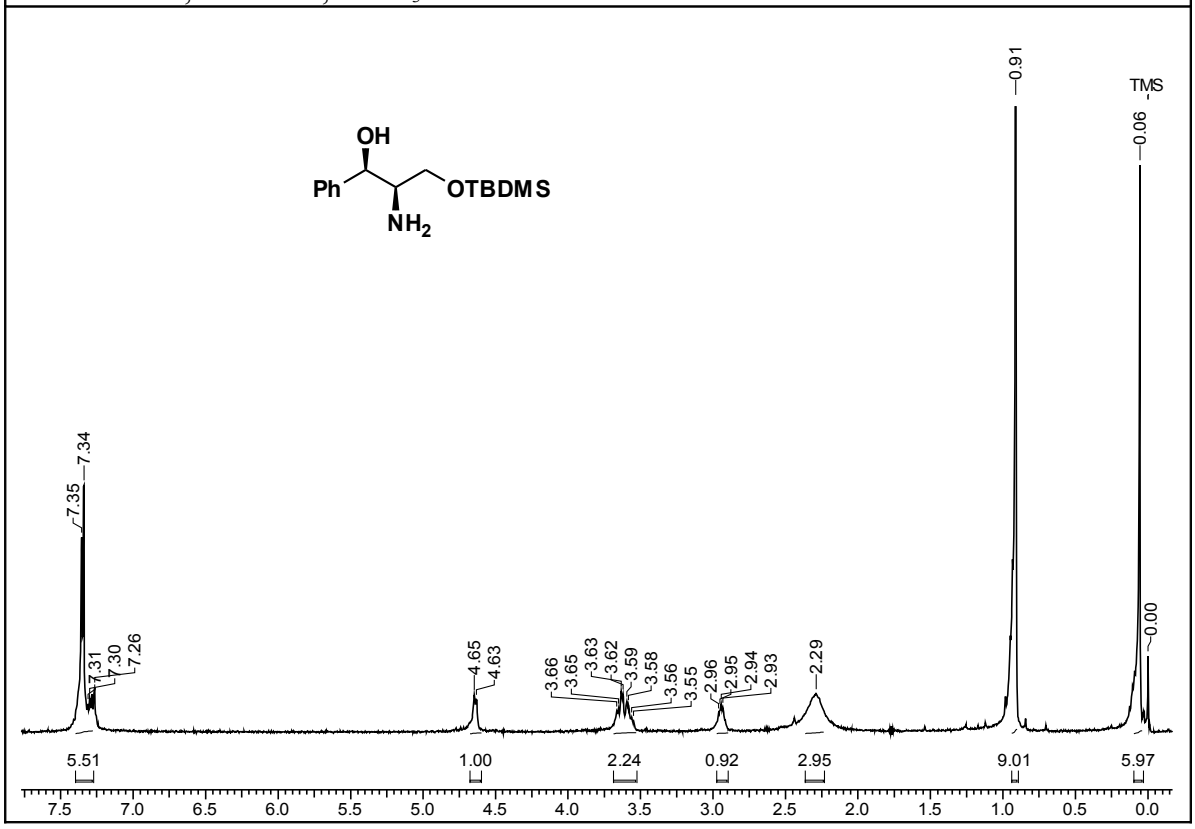
49: ^{1}H NMR, 200 MHz, $CDCl_3$



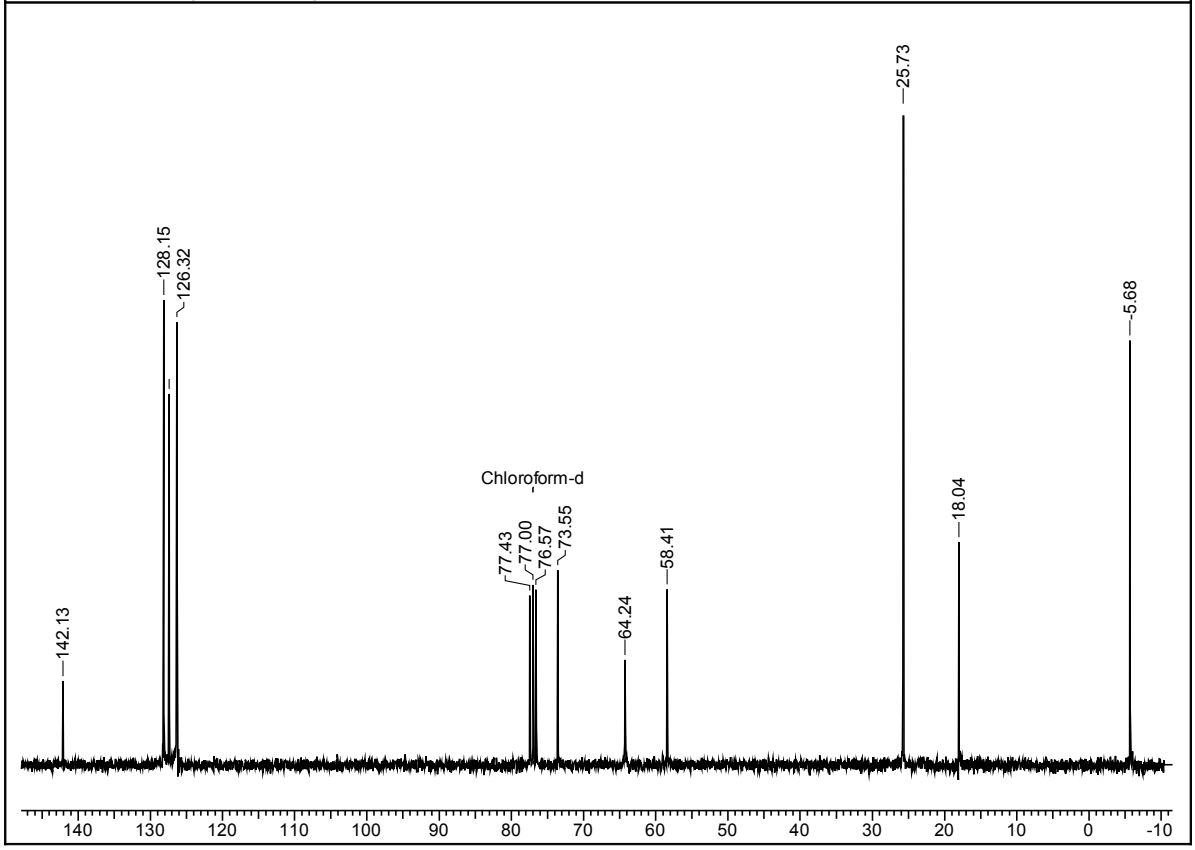
49: ^{13}C NMR, 50 MHz, $CDCl_3$



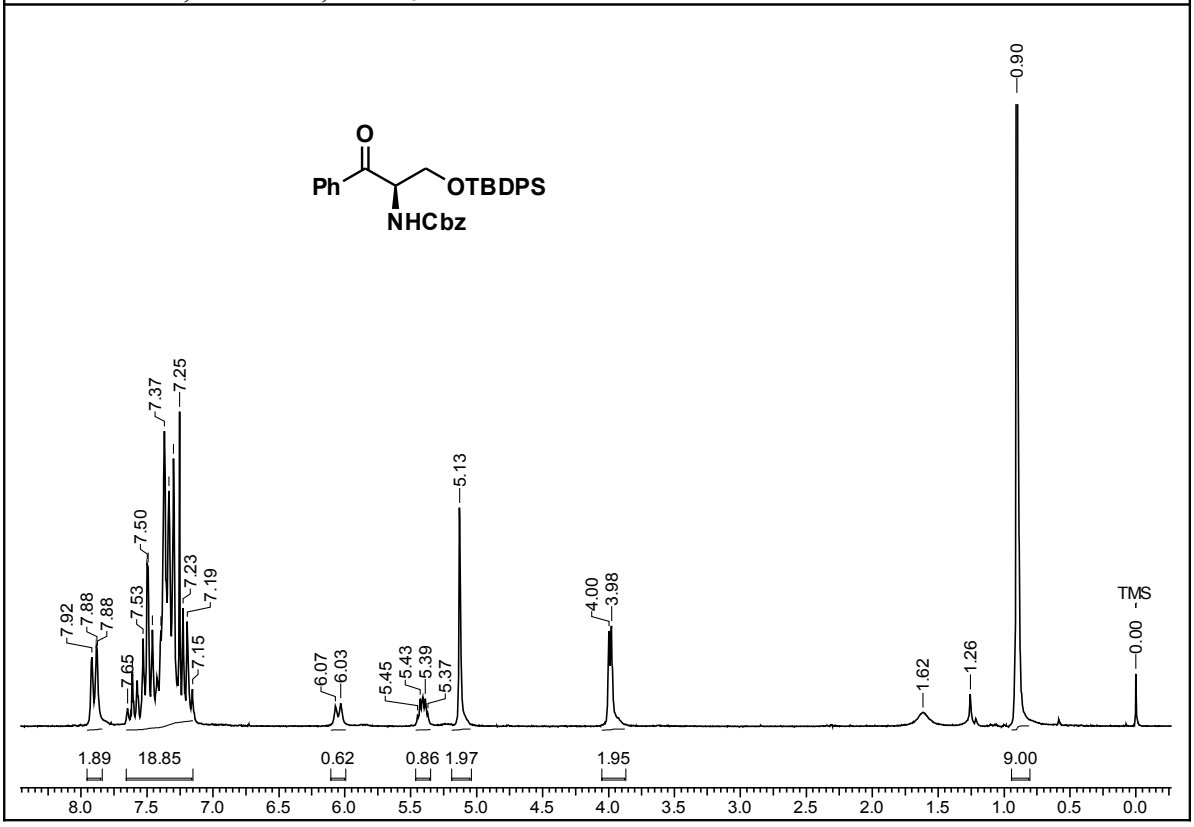
50: ^{1}H NMR, 300 MHz, $CDCl_3$



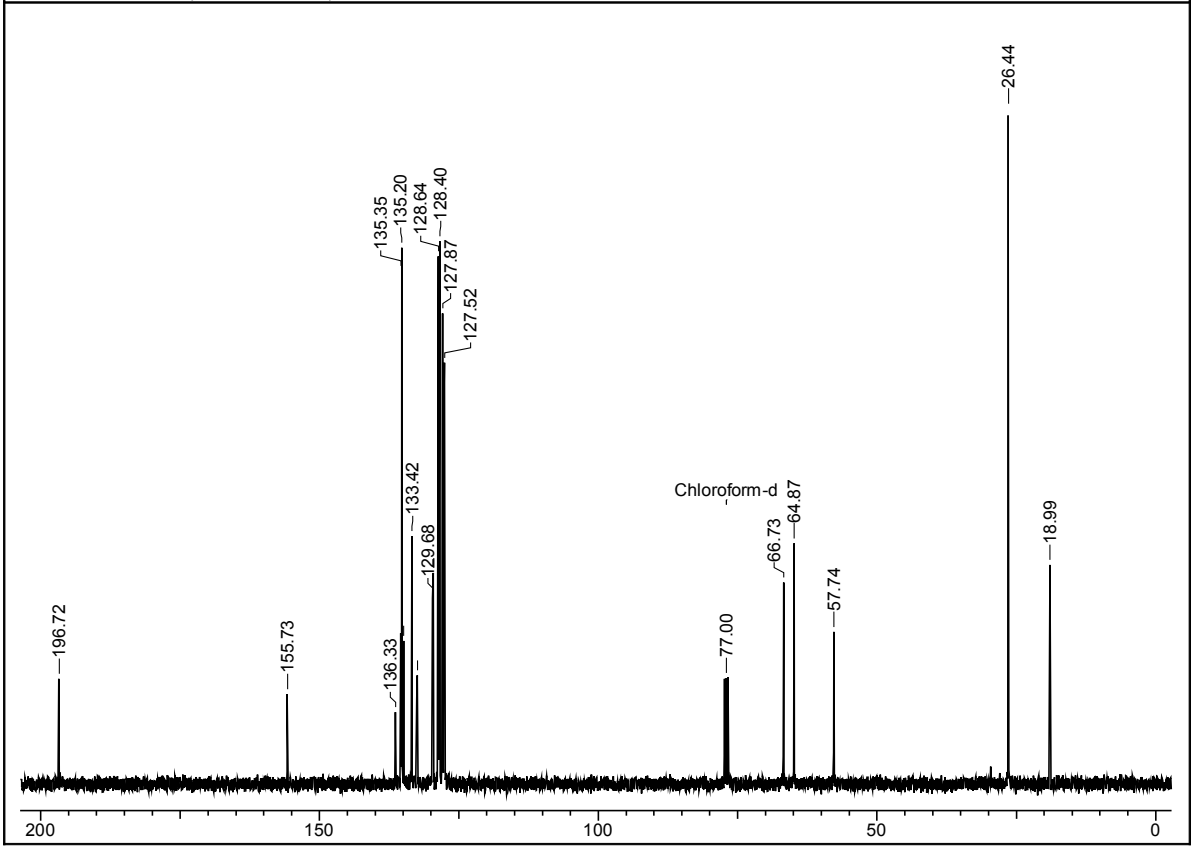
50: ^{13}C NMR, 75 MHz, $CDCl_3$



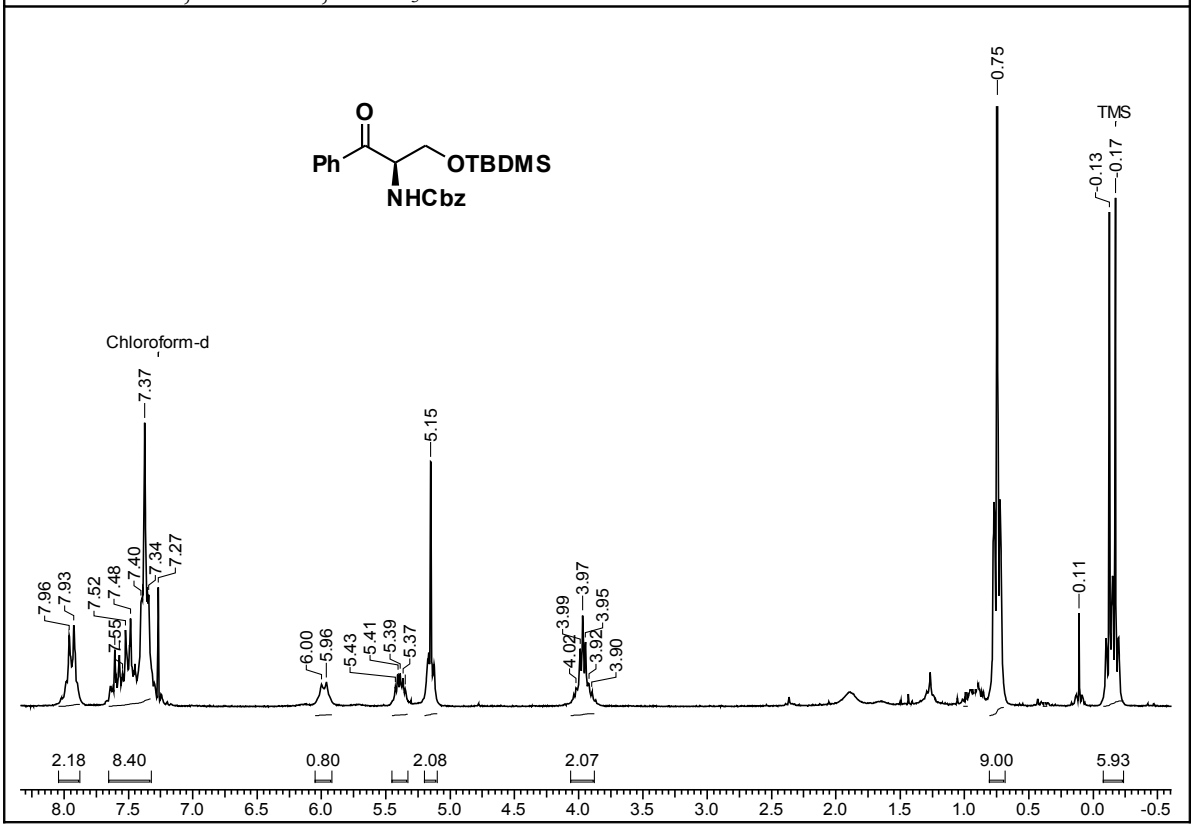
58: ^{1}H NMR, 200 MHz, $CDCl_3$



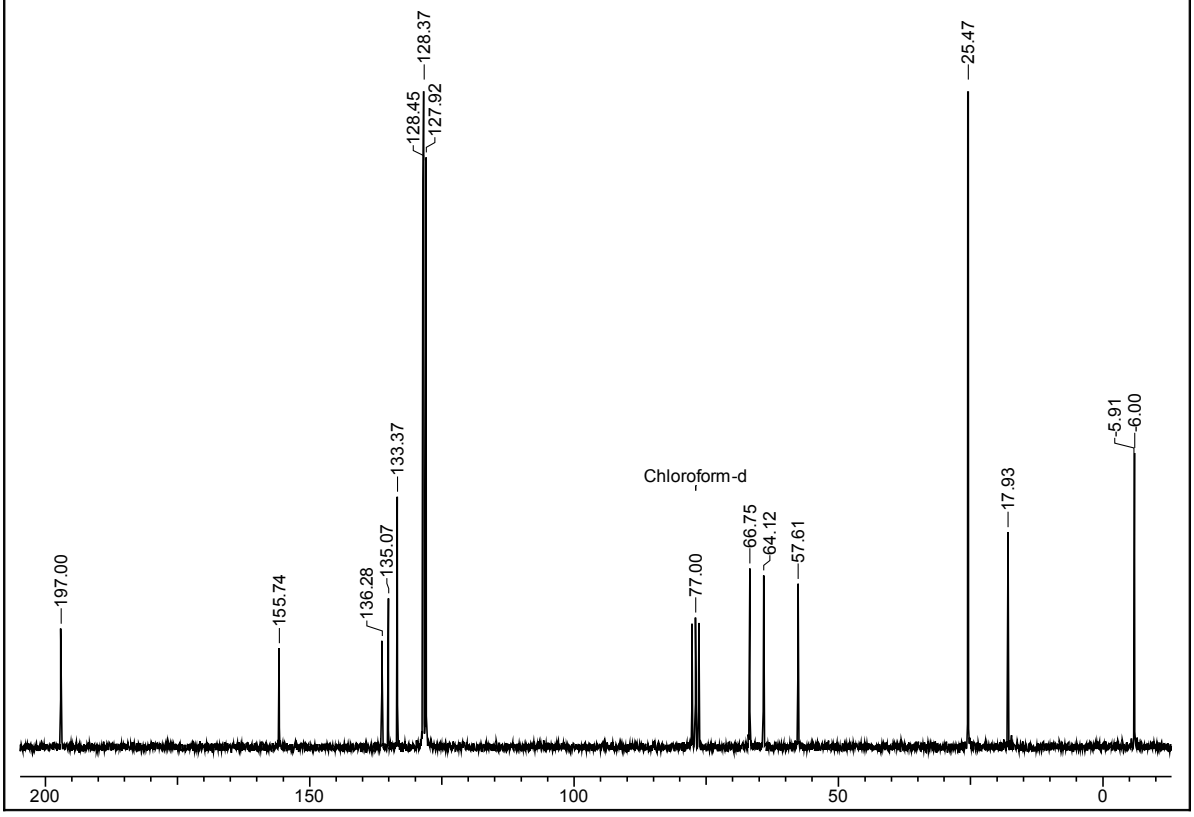
58: ^{13}C NMR, 100 MHz, $CDCl_3$



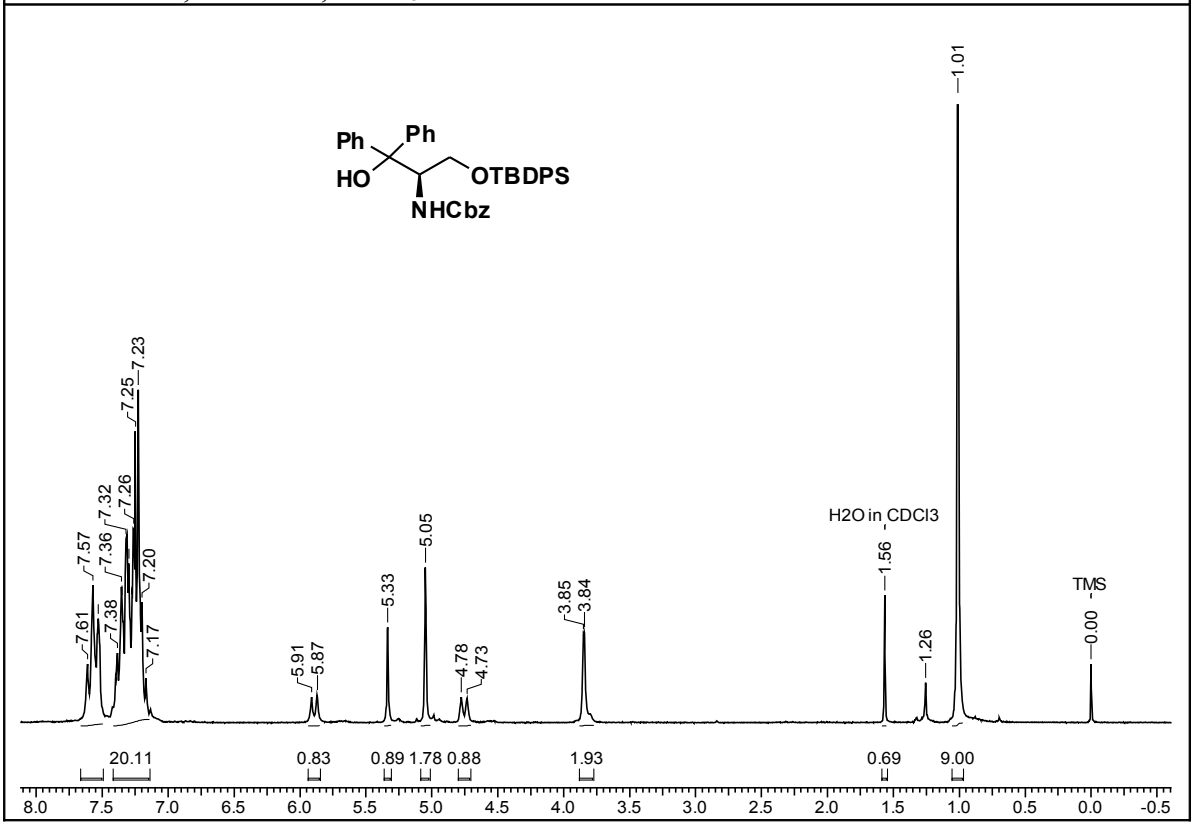
59: ^{1}H NMR, 200 MHz, $CDCl_3$



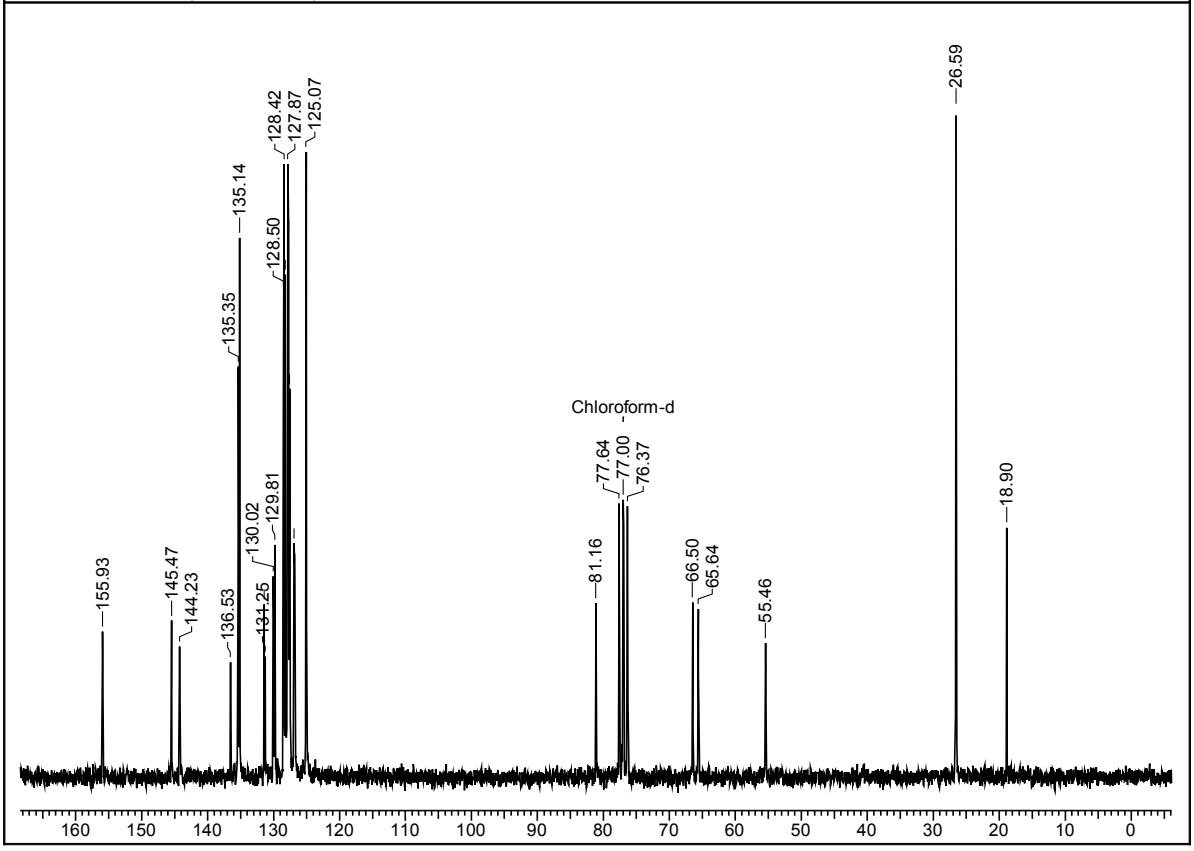
59: ^{13}C NMR, 50 MHz, $CDCl_3$



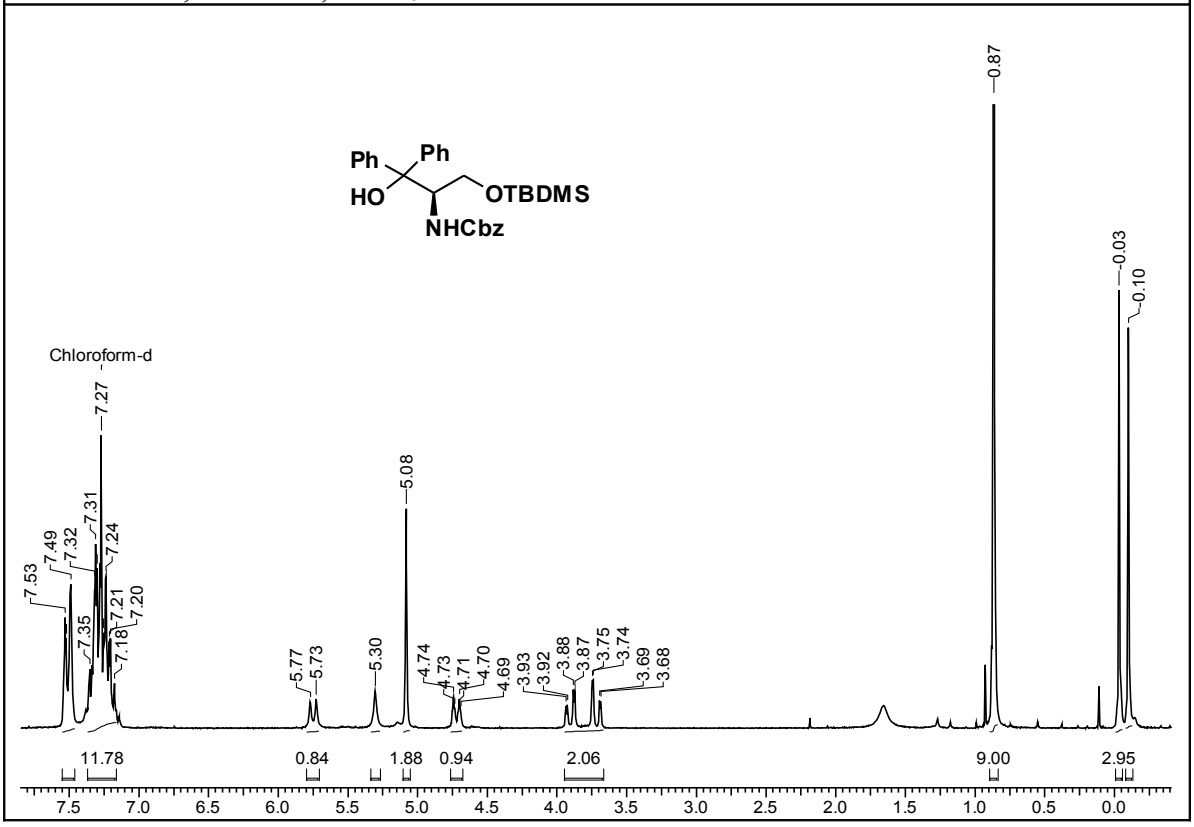
60: ^{1}H NMR, 200 MHz, $CDCl_3$



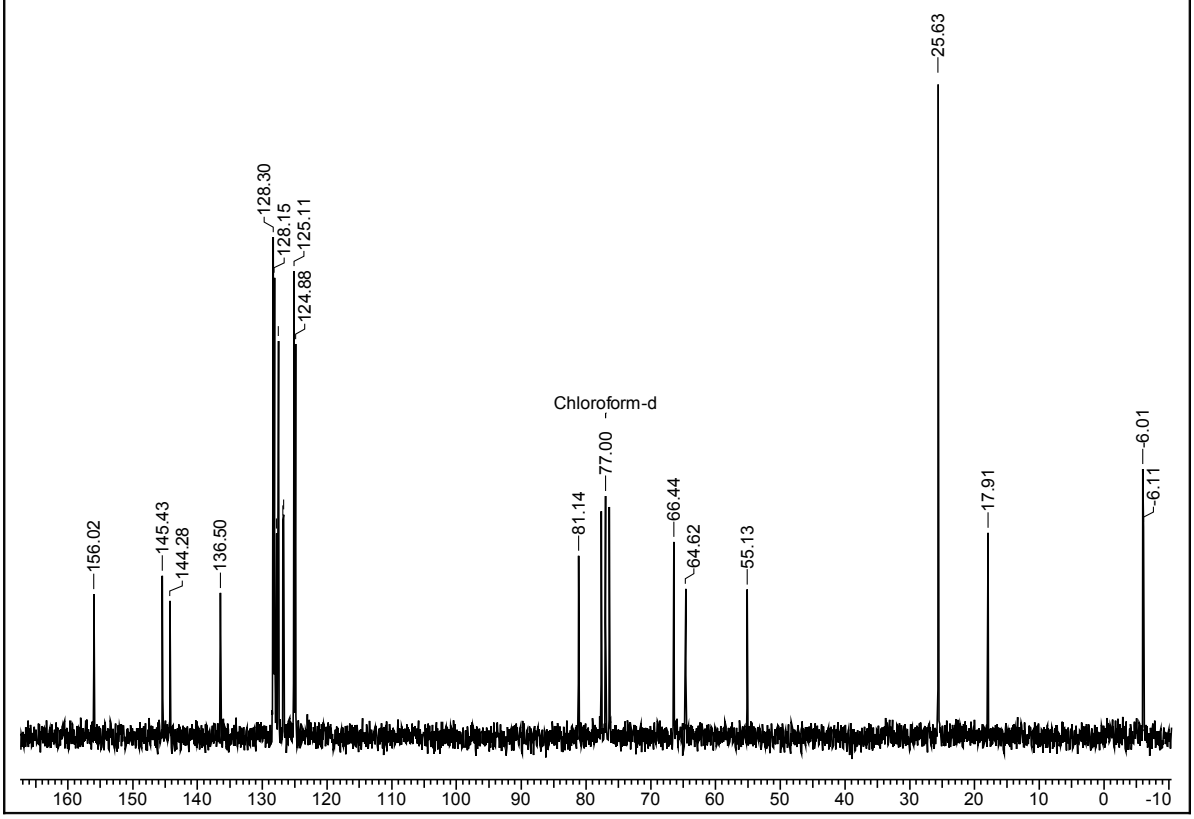
60: ^{13}C NMR, 50 MHz, $CDCl_3$



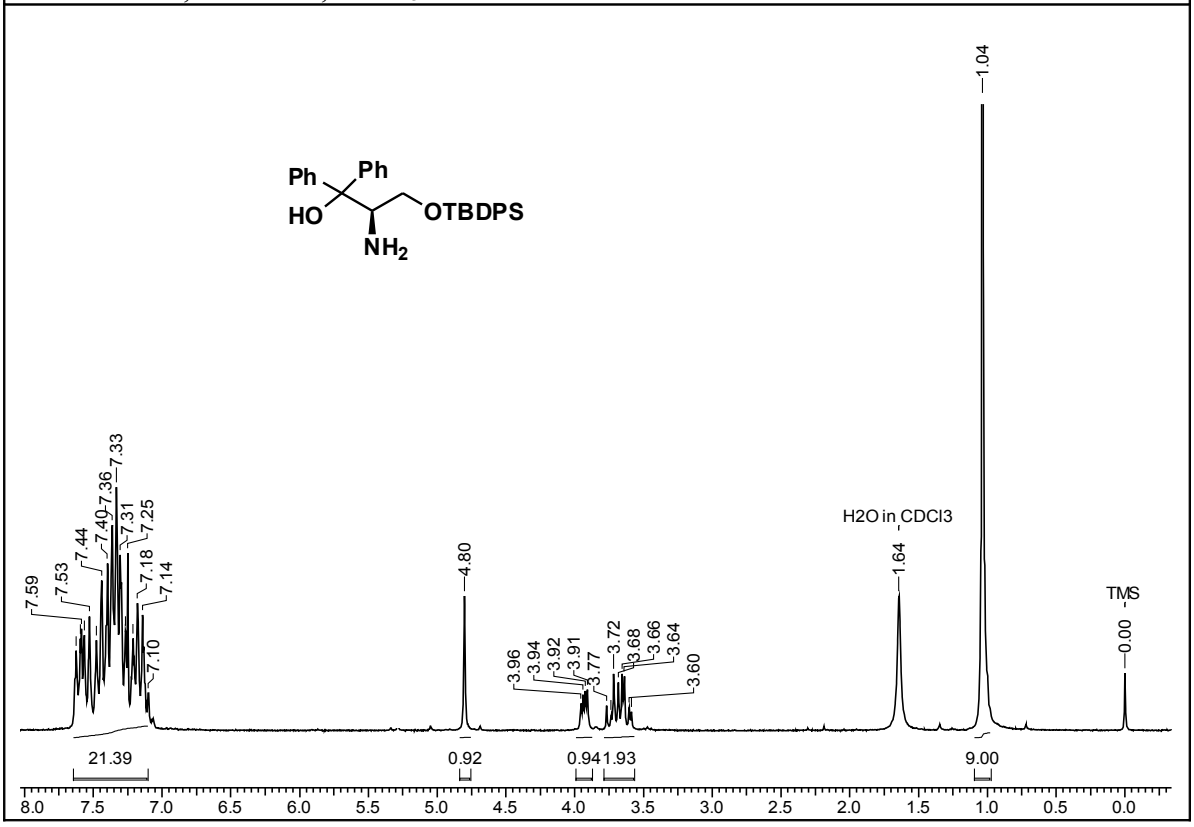
61: ^{1}H NMR, 200 MHz, $CDCl_3$



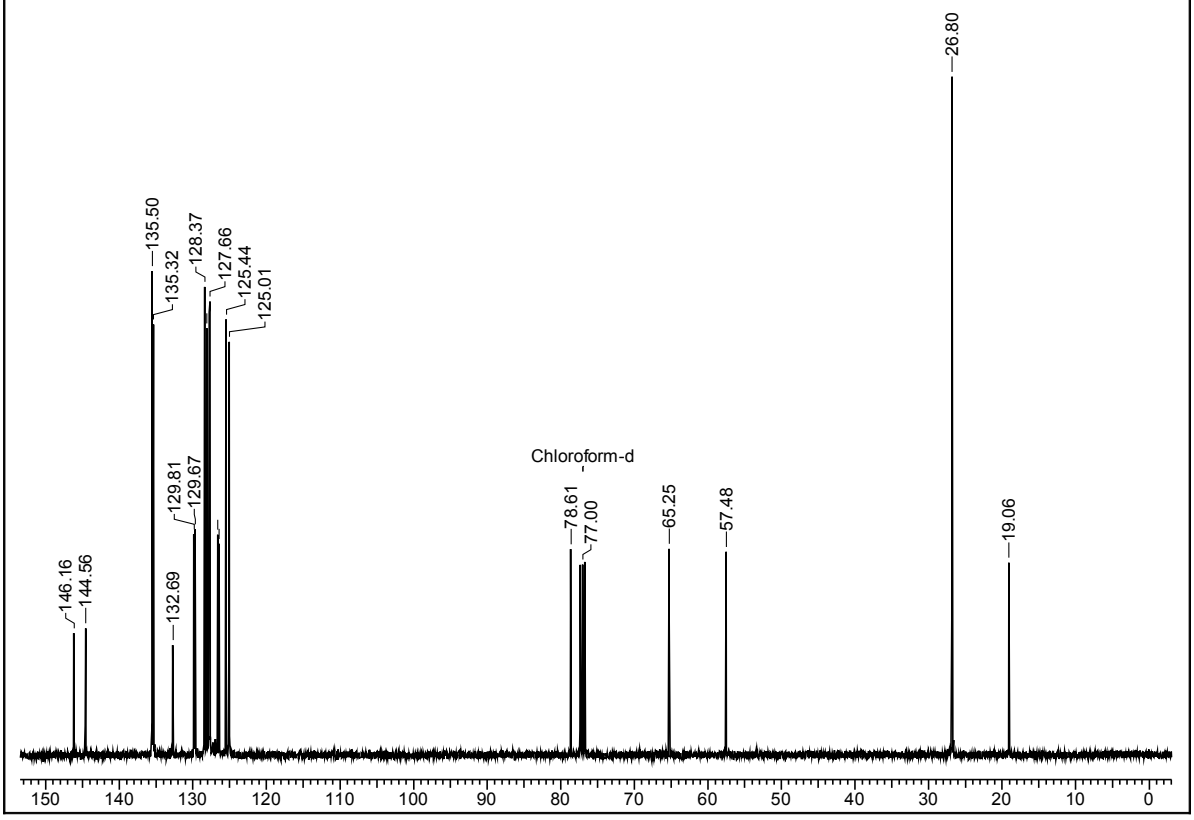
61: ^{13}C NMR, 50 MHz, $CDCl_3$



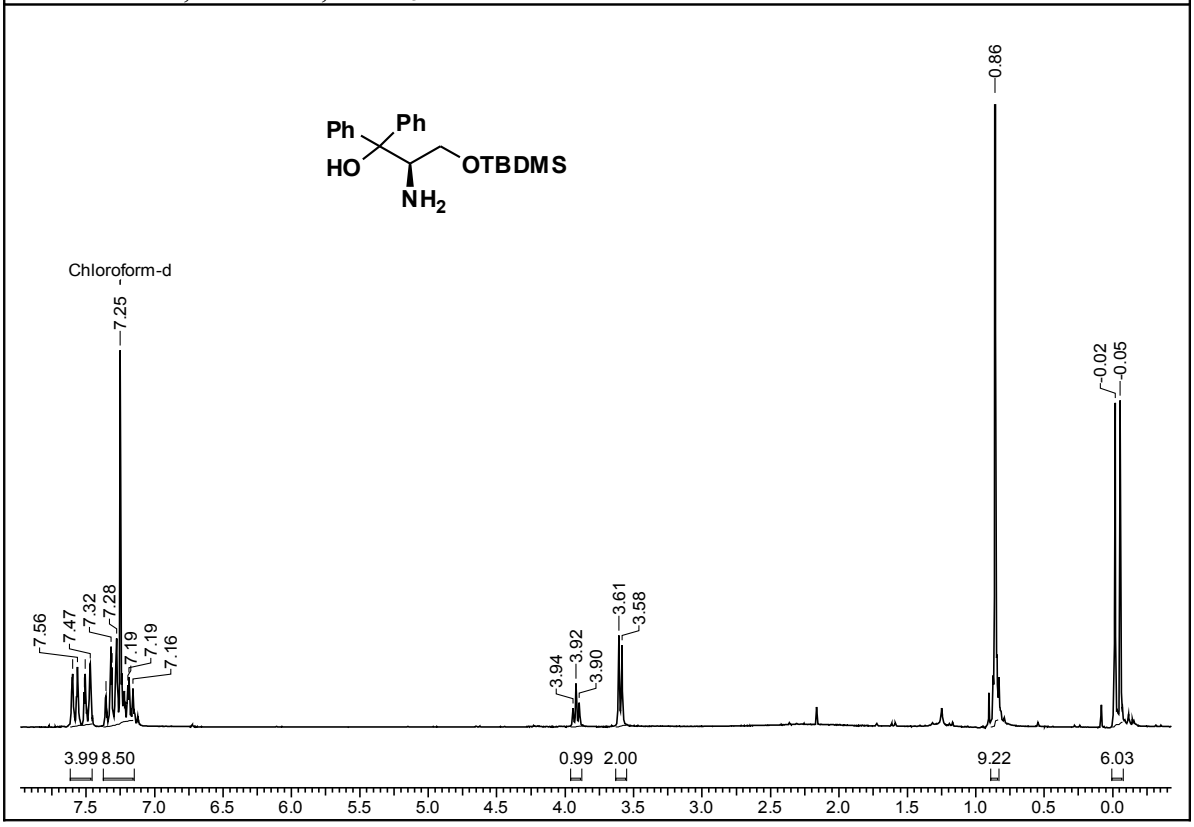
51: ^{1}H NMR, 200 MHz, $CDCl_3$



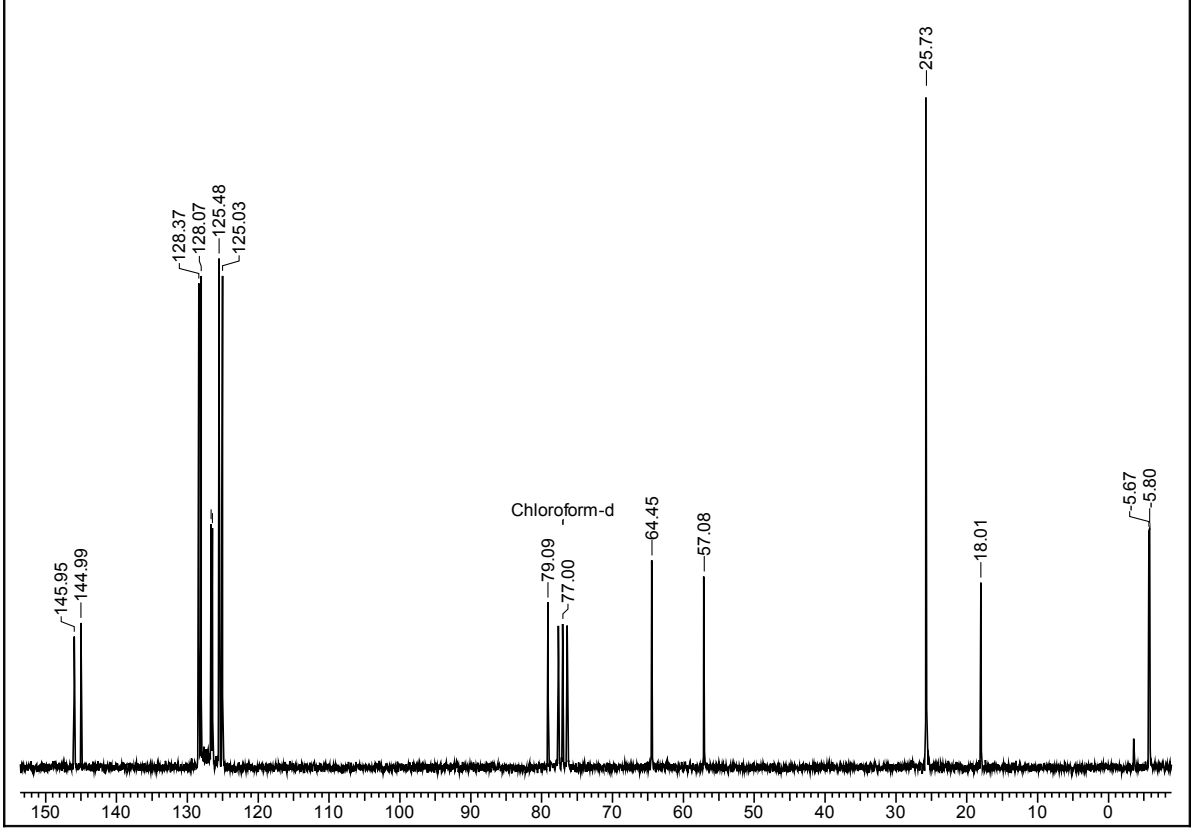
51: ^{13}C NMR, 100 MHz, $CDCl_3$



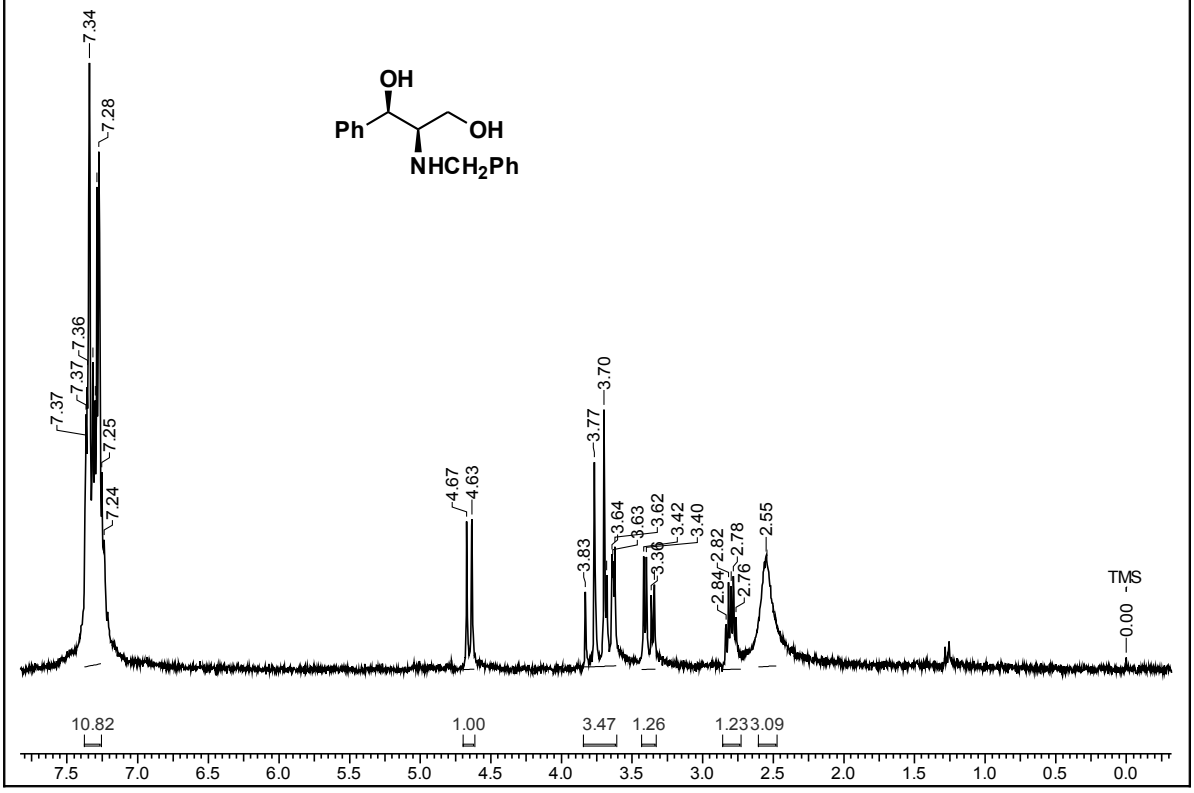
52: ^{1}H NMR, 200 MHz, $CDCl_3$



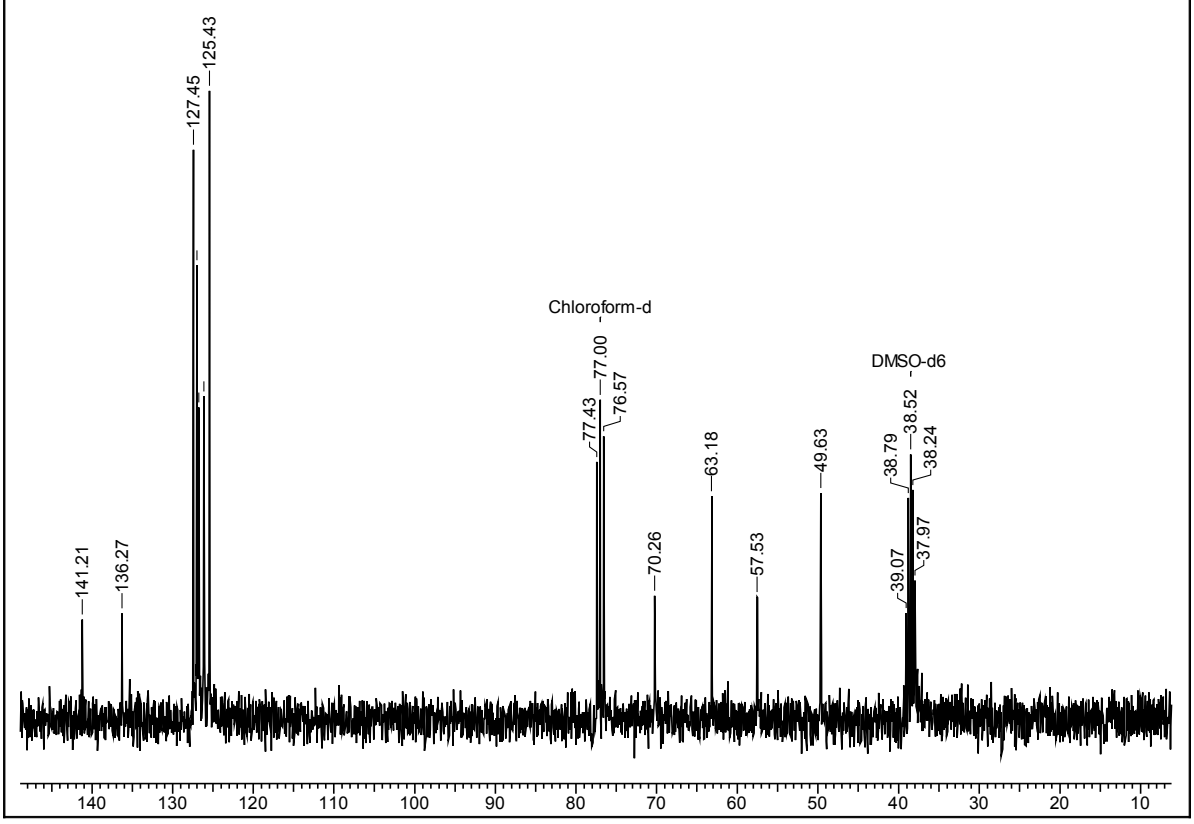
52: ^{13}C NMR, 50 MHz, $CDCl_3$



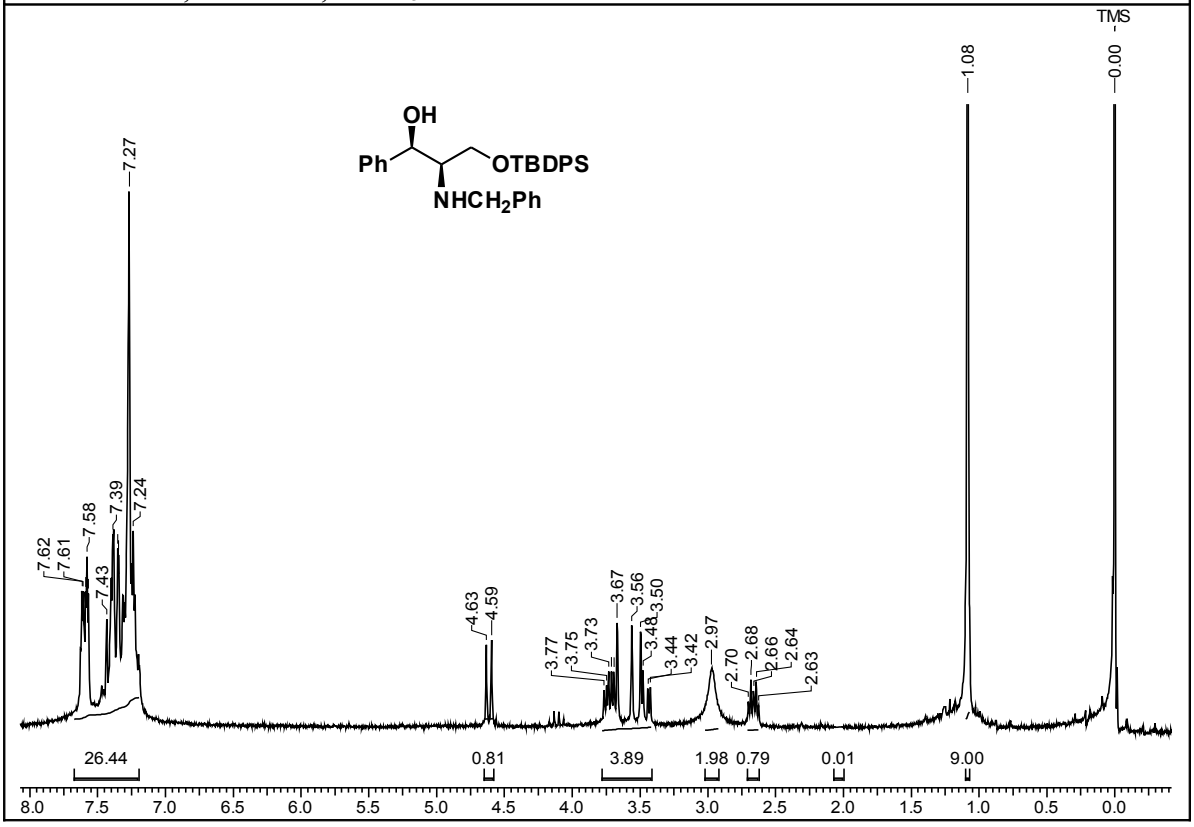
62: ^{1}H NMR, 200 MHz, $CDCl_3$



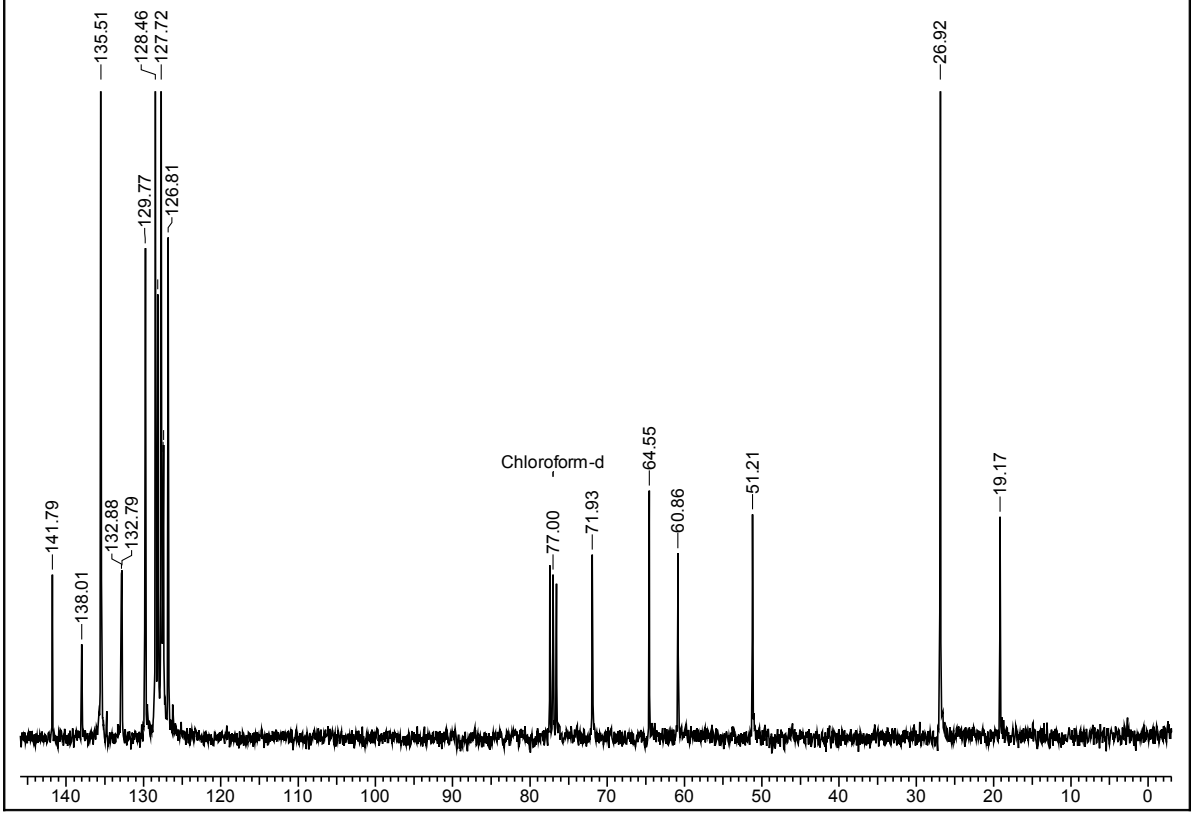
62: ^{13}C NMR, 75 MHz, $CDCl_3$



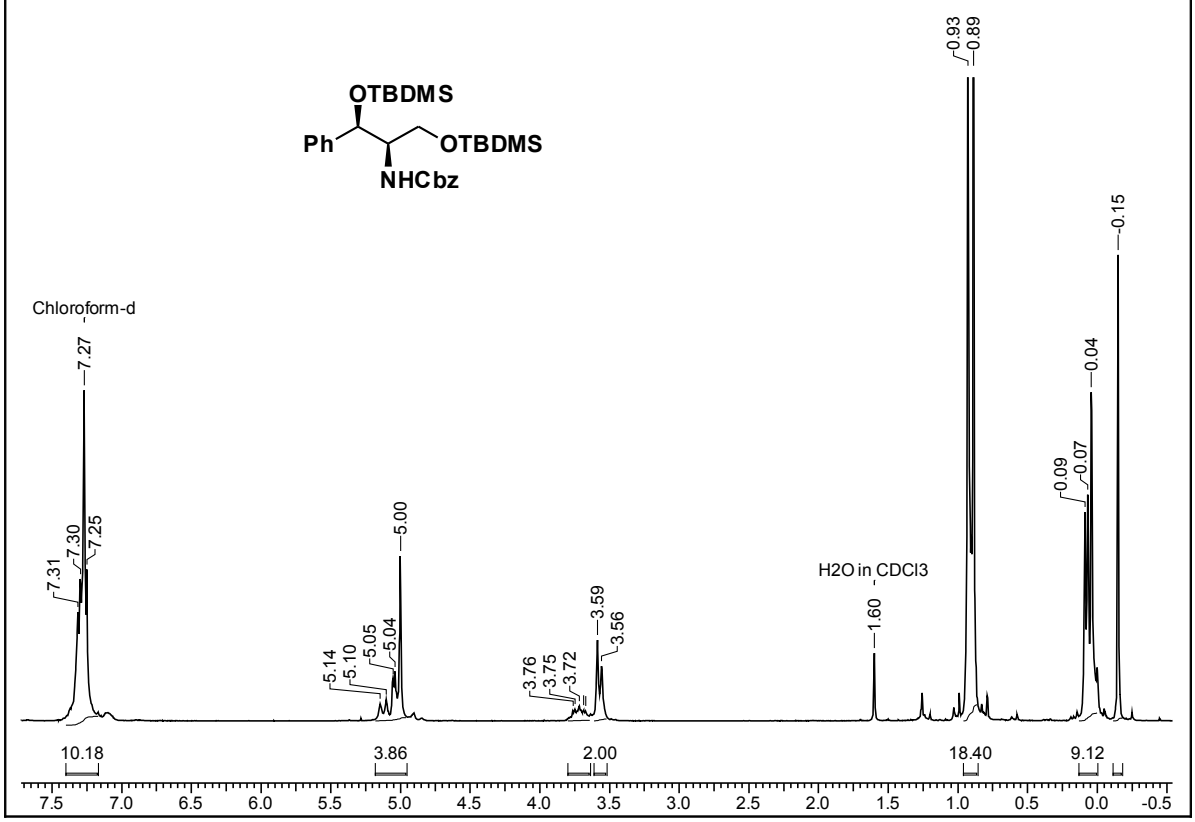
53: ^{1}H NMR, 200 MHz, $CDCl_3$



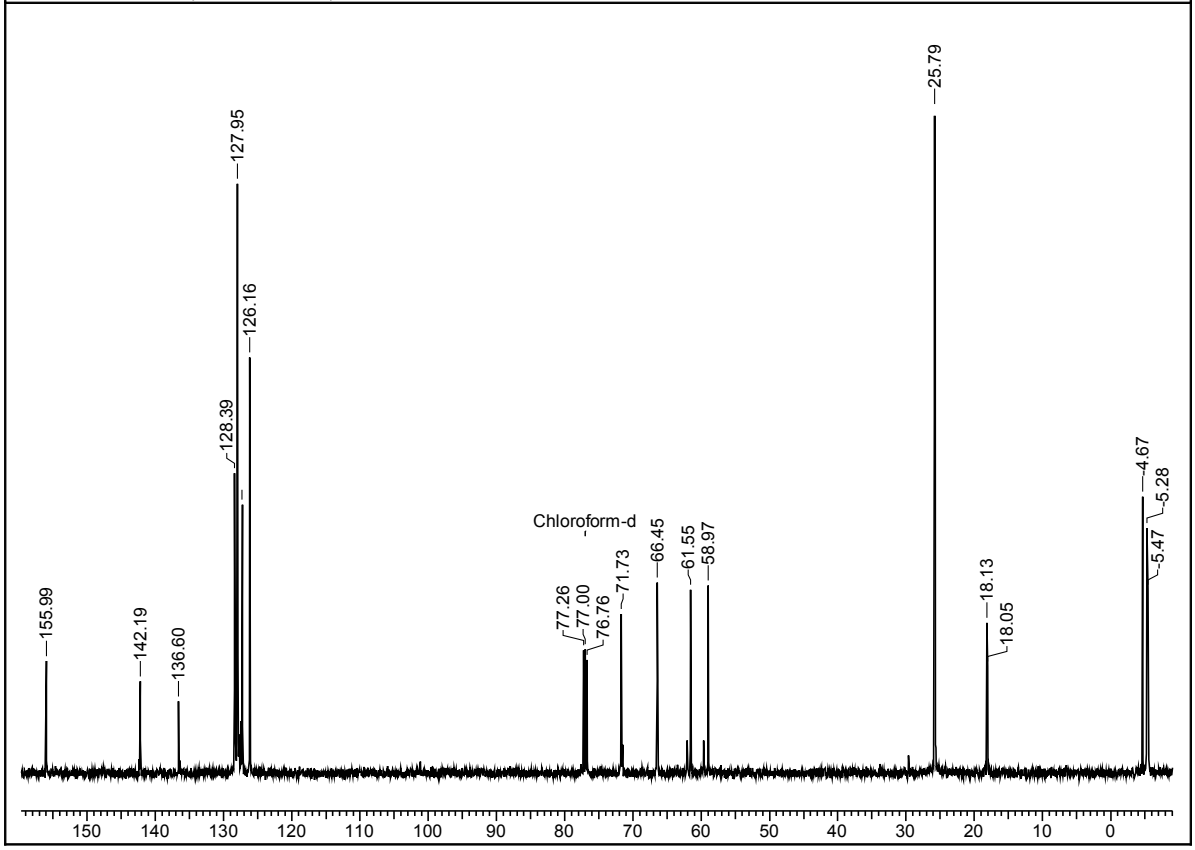
53: ^{13}C NMR, 50 MHz, $CDCl_3$



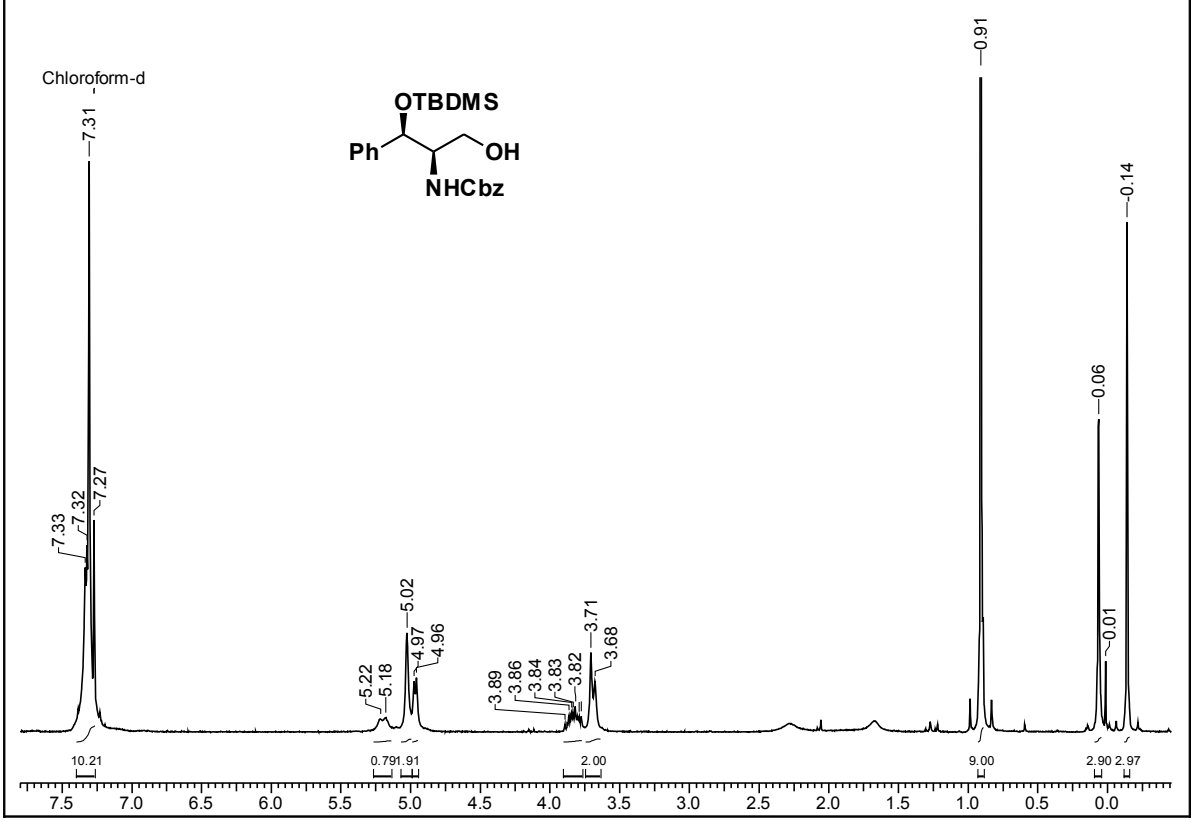
63: ^{1}H NMR, 200 MHz, $CDCl_3$



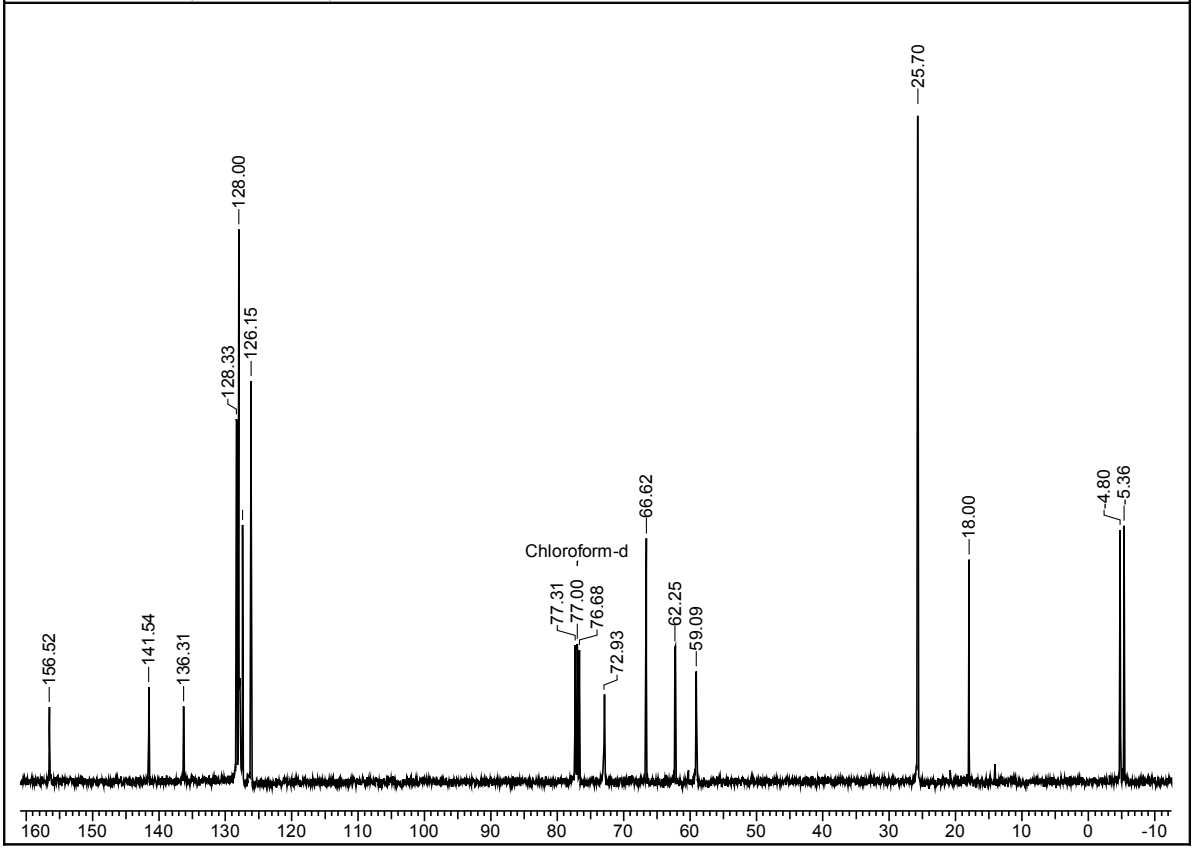
63: ^{13}C NMR, 125 MHz, $CDCl_3$



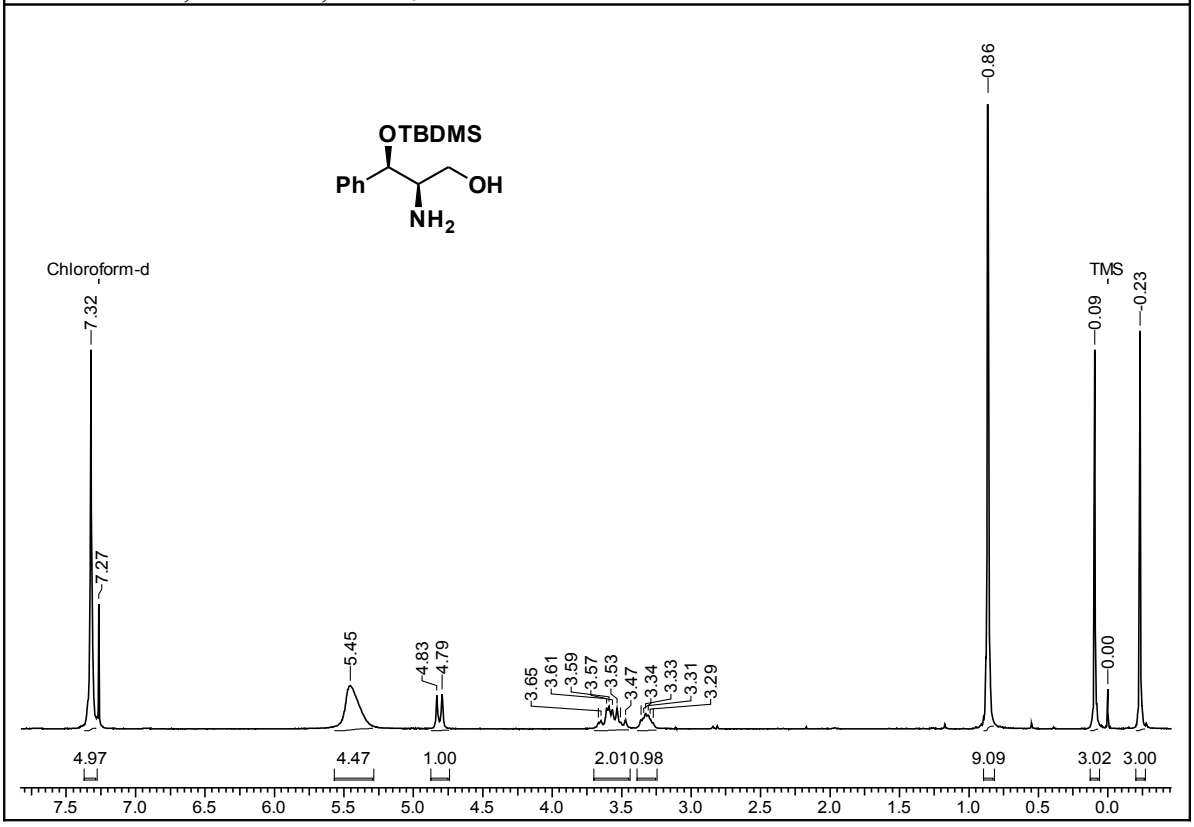
64: ^{1}H NMR, 200 MHz, $CDCl_3$



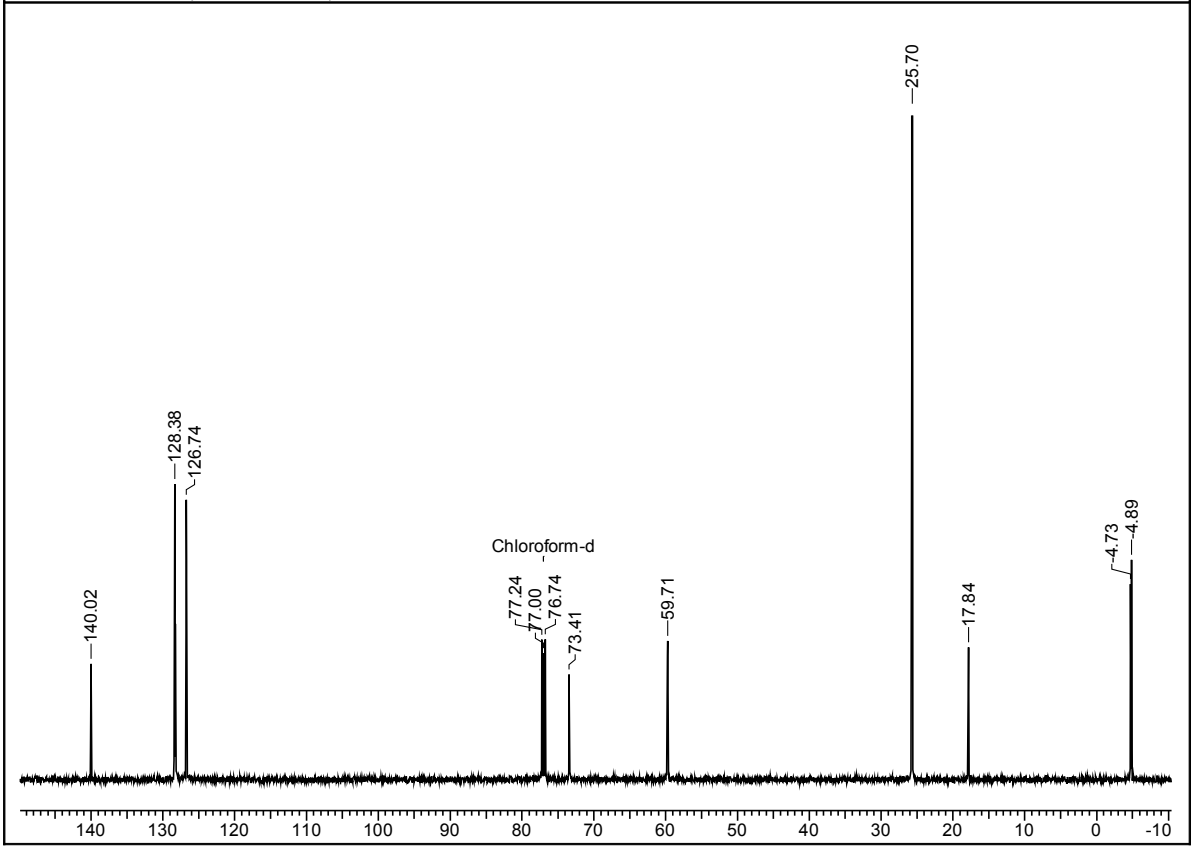
64: ^{13}C NMR, 100 MHz, $CDCl_3$



54: ^{1}H NMR, 200 MHz, $CDCl_3$



54: ^{13}C NMR, 125 MHz, $CDCl_3$



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List of Publication

1. "Synthesis and biological evaluation of bile acid dimers linked with 1,2,3-triazole and bis-β-lactam"
   Namdev S. Vatmurge, Braja G. Hazra, Vandana S. Pore, Fazal Shirazi, Mukund V. Deshpande, Sreenath Kadreppa, Samit Chattopadhyay and Rajesh G. Gonnade *Org. Biomol. Chem.* **2008**, *6*, 3823–3830.

2. "Synthesis and antimicrobial activity of β-lactam–bile acid conjugates linked via triazole"
   Namdev S. Vatmurge, Braja G. Hazra, Vandana S. Pore, Fazal Shirazi, Pradnya S. Chavan and Mukund V. Deshpande *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2043–2047.

3. "Syntheses of 1,2-Amino Alcohols and Their Applications for Oxazaborolidine Catalyzed Enantioselective Reduction of Aromatic Ketones"
   Namdev S. Vatmurge, Braja G. Hazra, and Vandana S. Pore *Aust. J. Chem.* **2007**, *60*, 196–204.

4. "Formal synthesis of squalamine from 16-dehydropregnenelone acetate (16-DPA)"
   Namdev S. Vatmurge and Braja G. Hazra (*to be communicated*)

Poster presentations / Symposia attended

1. Attended "4th INSA-KOSEF Symposium in Organic Chemistry. Contemporary organic chemistry and its future directions." National Chemical Laboratory, Pune India, January, **2009**.

2. "Design and Synthesis of Steroidal β-Lactams" **Namdev S. Vatmurge,** Vandana S. Pore, Braja G. Hazra. Poster presented at International Symposium on Advances in Organic Chemistry (INSOC 2006), Kottayam, Kerala, January, **2006**.

3. "β-Lactam on Bile Acid Side Chain" **Namdev S. Vatmurge,** Vandana S. Pore, Braja G. Hazra. Poster presented at 7th National Symposium in Chemistry (NSC-7), IACS Kolkata, India, February **2005**.

4. "Asymmetric Reduction of Prochiral Ketones Using New Chiral Amino Alcohols" **Namdev S. Vatmurge,** Vandana S. Pore, Braja G. Hazra. Poster presented at 6th National Symposium in Chemistry (NSC-6), IIT Kanpur, India, February **2004**.

5. Attended "Post NOST mini symposium in organic chemistry 2003", National Chemical Laboratory, Pune India, **2003**.

ERRATUM